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WHAT CAN BIOCHEMISTRY STUDENTS LEARN ABOUT PROTEIN TRANSLATION? USING VARIATION THEORY TO EXPLORE THE SPACE OF LEARNING CREATED BY SOME COMMON EXTERNAL REPRESENTATIONS

by

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A dissertation submitted in partial fulfillment
of the requirements for the

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THE GRADUATE COLLEGE

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Thomas J. Bussey

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What Can Biochemistry Students Learn About Protein Translation? Using Variation Theory to Explore the Space of Learning Created by Some Common External Representations

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May 2013

ABSTRACT

What Can Biochemistry Students Learn About Protein Translation? Using Variation Theory to Explore the Space of Learning Created by Some Common External Representations

by

Thomas J. Bussey

Dr. MaryKay Orgill, Advisory Committee Chair
Associate Professor of Chemistry
University of Nevada, Las Vegas

Biochemistry education relies heavily on students' ability to visualize abstract cellular and molecular processes, mechanisms, and components. As such, biochemistry educators often turn to external representations to provide tangible, working models from which students' internal representations (mental models) can be constructed, evaluated, and revised. However, prior research has shown that, while potentially beneficial, external representations can also lead to alternative student conceptions.

Considering the breadth of biochemical phenomena, protein translation has been identified as an essential biochemical process and can subsequently be considered a fundamental concept for biochemistry students to learn. External representations of translation range from static diagrams to dynamic animations, from simplistic, stylized illustrations to more complex, realistic presentations. In order to explore the potential for student learning about protein translation from some common external representations of translation, I used variation theory. Variation theory offers a theoretical framework from which to explore what is intended for students to learn, what is possible for students to learn, and what students actually learn about an object of learning, e.g., protein translation.

The goals of this project were threefold. First, I wanted to identify instructors' intentions for student learning about protein translation. From a phenomenographic analysis of instructor interviews, I was able to determine the critical features instructors felt their students should be learning. Second, I wanted to determine which features of protein translation were possible for students to learn from some common external representations of the process. From a variation analysis of the three representations shown to students, I was able to describe the possible combinations of features enacted by the sequential viewing of pairs of representations. Third, I wanted to identify what students actually learned about protein translation by viewing these external representations. From a phenomenographic analysis of student interviews, I was able to describe changes between students prior lived object of learning and their post lived object of learning.

Based on the findings from this project, I can conclude that variation can be used to cue students to notice particular features of an external representation. Additionally, students' prior knowledge and, potentially, the intended objects of learning from previous instructors can also affect what students can learn from a representation. Finally, further study is needed to identify the extent to which mode and level of abstraction of an external representation affect student learning outcomes.

ACKNOWLEGEMENTS

This has been a long and arduous process. I would be remiss if I did not acknowledge all of the family, friends, colleagues, and mentors who have helped to ensure that I was able to complete this phase in my life. I would first like to thank my partner, Carlos Ramirez, for your love, patience, support, and humor. You have been understanding and encouraging throughout this process. You have allowed me to take the time to do what I needed to do and to focus when I needed to focus. You have distracted me when I needed to be distracted (and sometimes when I did not). But most of all, you have loved me, and for that, I am grateful.

I would like to thank my family for your encouragement and for instilling in me a sense of ambition and inquisitiveness. My mom has always been my cheerleader. Without your continual support, I would not have been able to pursue my dreams. My dad has always told me to "do what you love." For that, I would like to thank you. You have given me the freedom to do what I wanted to do and be who I wanted to be. And to my sister, you have provided me with friendship and a willing student since we were little kids. You have allowed me to teach you, and because of that, I have learned so much from you. Thank you for your enduring love and support.

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contribution to the development and refinement of the coding scheme used in this project. I would also like to thank Dr. Sonia Underwood for your support, encouragement, and friendship over the past several years.

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Lastly, I would like to thank my advisor and mentor, Dr. MaryKay Orgill, for your wisdom and insight, encouragement and perseverance. Over the past many years, you have guided me to think beyond what I knew, work harder than I thought possible, and to become more than what I imagined for myself. Thank you for letting me take my journey through this process, for guiding me when I needed help, and for giving me freedom to pursue my interests. Thank you for being my teacher, my mentor, and my friend.

TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGEMENTS v

LIST OF TABLES xiii

LIST OF FIGURES xiv

CHAPTER 1 INTRODUCTION 1
- Project Rationale 1
  - The Importance of Translation 2
- Guiding Research Questions 6

CHAPTER 2 VISUAL LITERACY IN BIOCHEMISTRY EDUCATION 8
- Art, Science, and Knowing 8
- Terminology of Visual Literacy 12
- What is Visual Literacy? 14
- External Representations in Science 16
  - External Representations and Prior Knowledge 18
  - Modes of External Representations 22
  - Level of Abstraction of the Representation 25
- Potential Outcomes of Using External Representations 26
  - Potential Benefits of Using External Representations 26
  - Potential Negative Results of Using External Representations 28
- Visual Literacy and External Representations in Biochemistry Education 32
  - The Role of Translation in Biochemistry Education 35
    - External Representations of Protein Translation 37

CHAPTER 3 VARIATION THEORY 39
- Theoretical Framework 39
  - Aims of Variation Theory 40
- Key Concepts of Variation Theory 41
  - Awareness, Discernment, and Simultaneity 41
  - The Object of Learning 43
    - The Intended Object of Learning 44
    - The Enacted Object of Learning 44
    - The Lived Object of Learning 45
  - Critical Features 47
  - Perception and Variation 47
  - Significant Patterns of Variation 48
- Methods and Assumptions of Variation Theory 49
  - The Intended Object of Learning 50
  - The Enacted Object of Learning 50
  - The Lived Object of Learning 51

Data Analysis 52
Rationalization for Using Variation Theory in this Project 53
Modifications to Variation Theory in the Project 55

CHAPTER 4 METHODOLOGY 57
Research Questions 57
Research Design 57
The Intended Object of Learning 59
Participants 59
External Representations 61
Basic Criteria for External Representations Used in this Study 61
Representational Mode 62
Level of Abstraction 63
Selection of External Representations 63
Static External Representations of Translation 65
Stylized, Dynamic External Representations of Translation 69
Realistic, Dynamic External Representations of Translation 72
Instructor Interview Protocol 74
Data Collection and Analysis 79
The Enacted Object of Learning 79
External Representations 79
Data Analysis 81
Variation Analysis of Dynamic External Representations (VADER) 83
Application of the VADER Methodology 85
Application of the VADER Method to the Current Study 93
The Lived Object of Learning 94
Participants 94
External Representations 96
Student Interview Protocol 96
Data Collection and Analysis 99

CHAPTER 5 ANALYSIS, RESULTS, AND DISCUSSION OF RESEARCH QUESTION 1 101
The Intended Object of Learning: Chapter Overview 101
Instructors' Perception of the Importance of Protein Translation in Biochemistry Education 102
Critical Features of Protein Translation 104
Instructor Responses Prior to Viewing the External Representations .106
Common Features 108
The Ribosome 108
The mRNA 109

The tRNA 110
Interaction Features 110
Other Features 111
Instructor Responses to Common External Representations of Protein Translation 113
Common Features 114
Component Features 115
Ribosome-related Features 115
mRNA-related Features 117
tRNA-related Features 118
Additional Component Features 119
Interaction Features 120
Peptide bond Formation Interaction Feature 121
Codon/Anti-codon Interaction Feature 123
Determination of Critical Features 124
Primary Critical Features 126
Secondary Critical Features 128
Tertiary Critical Features 133
Discussion and Conclusions 135

CHAPTER 6 ANALYSIS, RESULTS, AND DISCUSSION OF RESEARCH QUESTION 2 138
The Enacted Object of Learning: Chapter Overview 138
Selection of Preferred External Representations 139
Selection of a Static External Representations 140
Selection of a Stylized, Dynamic External Representations 142
Selection of a Realistic, Dynamic External Representations 143
Instructor Perceptions of the Types of Variation Between Features 146
Color 146
Shape 147
Other Types of Variation Used for Visual Cueing 149
Changes in Relative Position 150
Changes in Relative Size 151
Changes in Labeling 152
Identification of Non-critical Features 153
Variation Analysis of Dynamic External Representations (VADER) 158
Variation Analysis of External Representation #2 159
Variation Analysis of External Representation #3 163
Variation Analysis of External Representation #6 166
Comparison of the Three Selected External Representations 169
Summary of the Three Selected External Representations 174
The Influence of the Pairwise Viewing of External Representations 175
Enacted Object of Learning Created by External Representations #2 and #3 176
Enacted Object of Learning Created by External Representations #2 and #6 178

Enacted Object of Learning Created by External Representations #3 and #6 179
Chapter Summary 180

CHAPTER 7 ANALYSIS, RESULTS, AND DISCUSSION OF RESEARCH QUESTION 3 182
The Lived Object of Learning: Chapter Overview 182
Do External Representations of Protein Translation Create a Space of Learning? 183
Do Students Notice Variation 187
Prior Lived Object of Learning 195
Students' General Prior Knowledge 199
The Use of Prior Knowledge to Determine if Learning Occurred 202
The Potential Impact of Prior Instructor's Intentions for Student Learning 202
Post Lived Object of Learning 204
The Influence of Prior Knowledge 204
Higher Levels of Students' Prior Knowledge 204
Intermediate Levels of Students' Prior Knowledge 209
Lower Levels of Students' Prior Knowledge 212
The Influence of the Enacted Object of Learning 214
Features of External Representation #2 Noticed by Students 216
Ribosomal Subunit Naming 216
Peptide Bond Formation Reaction 219
Features of External Representation #3 Noticed by Students 223
Shine-Dalgarno Sequence 224
Initiation Complex Formation 228
Role of the Release Factors in the Termination Stage 231
Features of External Representation #6 Noticed by Students 234
Dynamic Movement 234
Reaction Kinetics 236
Cellular Environment 237
The Influence of Presentation Order 240
Chapter Summary 243

CHAPTER 8 CONCLUSIONS AND IMPLICATIONS 244
Project Overview 244
Instructor Considerations For Selecting an External Representation to Depict an Object of Learning 244
The Importance of Alignment 245
The Influence of Variation 247
The Influence of Students' Prior Knowledge 250
The Potential Influence of Mode 254
The Potential Influence of Abstraction 259
Future Work 261
Future Methodological Development 262

Project Limitations 264
Conclusion 265

APPENDIX A: COPYRIGHT PERMISSIONS FOR ARTWORK, FIGURES, AND ANIMATIONS 268

APPENDIX B: INSTRUCTOR INTERVIEW GIUDE 286

APPENDIX C: STUDENT INTERVIEW GUIDE 290

APPENDIX D: DEFINITION OF CODES 293

APPENDIX E: INITIAL CODED FEATURES OF PROTEIN TRANSALTION FROM INSTRUCTOR INTERVIEWS 298

APPENDIX F: FINAL CODED FEATURES OF PROTEIN TRANSALTION FROM INSTRUCTOR INTERVIEWS 300

APPENDIX G: CATEGORIZATION OF CRITICAL FEATURES OF PROTEIN TRANSLATION 302

APPENDIX H: FRAME SEQUENCE OF EXTERNAL REPRESENTATION #2 304

APPENDIX I: VADER PLOT OF EXTERNAL REPRESENTATION #2 306

APPENDIX J: AVERAGE CUEING OF EXTERNAL REPRESENTATION #2 314

APPENDIX K: FRAME SEQUENCE OF EXTERNAL REPRESENTATION #3 316

APPENDIX L: VADER PLOT OF EXTERNAL REPRESENTATION #3 324

APPENDIX M: AVERAGE CUEING OF EXTERNAL REPRESENTATION #3 344

APPENDIX N: FRAME SEQUENCE OF EXTERNAL REPRESENTATION #6 346

APPENDIX O: VADER PLOT OF EXTERNAL REPRESENTATION #6 356

APPENDIX P: AVERAGE CUEING OF EXTERNAL REPRESENTATION #6 384

APPENDIX Q: COMPARISON OF THE AVERAGE CUEING OF THE SELECTED EXTERNAL REPRESENTATIONS 386

APPENDIX R: COMPARISON OF EXTERNAL REPRESENTATIONS #2 AND #3 388

APPENDIX S: COMPARISON OF EXTERNAL REPRESENTATIONS #2 AND #6 390

APPENDIX T: COMPARISON OF EXTERNAL REPRESENTATIONS #3 AND #6 392

APPENDIX U: GROUP COMPARISON OF LEARNED FEATURES 394

BIBLIOGRAPHY 396

VITA 407

LIST OF TABLES

Table 1: Enacted Objects of Learning 82

Table 2: Example of VADER coding and plot 92

Table 3: Primary Critical Features of Protein Translation.....127

Table 4: Secondary Critical Features of Protein Translation.....128

Table 5: Tertiary Critical Features of Protein Translation.....133

Table 6: Student Groupings 183

LIST OF FIGURES

Figure 1: Flow chart of the Central Dogma 2

Figure 2: Student drawing of protein translation during pilot study.....5

Figure 3: Textbook illustration of protein translation. 5

Figure 4: Claude Monet, Water Lillies, 1906. 10

Figure 5: Mary Cassatt, The Child's Bath (The Bath), 1893.....10

Figure 6: Representational quadrants.....34

Figure 7: The objects of learning within variation theory. 46

Figure 8: External Representation #1. 68

Figure 9: External Representation #2. 68

Figure 10: External Representation #3. 71

Figure 11: External Representation #4. 71

Figure 12: External Representation #5. 73

Figure 13: External Representation #6. 73

Figure 14: Example of a frame series used to conduct VADER coding 91

Figure 15: Frame 1 of External Representation #1 116

Figure 16: Frame 1 of External Representation #2. 116

Figure 17: Frame 24 of External Representation #4. 118

Figure 18: External Representation #4, Frame 82. 148

Figure 19: External Representation #3: Frames 7 - 9. 151

Figure 20: Labeling in External Representation #2 frame 3 and External Representation #3 Frame 10. 153

Figure 21: Identification of the stop codon nucleotide sequence in External Representation #3 frame 34. 154

Figure 22: Identification of amino acid interaction in External Representation #3 frames 29 and 30. 155

Figure 23: Identification of the initial tRNA/ribosome/mRNA feature in External Representation #2 frame 1 and External Representation #3 frame 5.....156

Figure 24: Identification of hydrogen bonding (mRNA/ribosome) in External Representation #3 frame 12. 157

Figure 25: Identification of the cellular environment (cytoplasm) in External Representation #6 frame 12. 158

Figure 26: VADER plot of External Representation #2. 162

Figure 27: Comparison of VADER plots for ER2, ER3, and ER6. 172

Figure 28: Unit comparison of VADER plots. 174

Figure 29: Student 19(C), Initial drawing. 185

Figure 30: Student 19(C), Final drawing. 186

Figure 31: Student 18(D): Initial drawing showing the mRNA movement relative to the stationary ribosome. 189

Figure 32: Student 18(D), Initial drawing with the depiction of the "GFP" showing the direction it moves the mRNA 190

Figure 33: Frame 6 of External Representation #2 indicating the direction of ribosome movement. 191

Figure 34: Student 18(D), Final drawing indicating the ribosomal movement relative to a stationary mRNA. 193

Figure 35: A.) Student 20(A), Initial drawing. B.) Textbook image from Berg, et. al. (2007, p. 872) depicting protein translation. 196

Figure 36: Student 5(F), Initial drawing with the tRNA represented as a labeled oval. 197

Figure 37: Student 5(F), Initial drawing of the cloverleaf structure of tRNA. 197

Figure 38: Student 8(B), Initial drawing of peptide bond formation (incorrect). 200

Figure 39: Student 7(C), Initial drawing of the first step in the mechanism of peptide bond formation 206

Figure 40: Student 7(C), Initial drawing of the second step in the mechanism of peptide bond formation. 207

Figure 41: Student 7(C), Initial drawing of the third step in the mechanism of peptide bond formation. 208

Figure 42: Frame 1 of External Representation #2. 209

Figure 43: Frame 17 of External Representation #3 211

Figure 44: Student 4(A), Initial drawing. 217

Figure 45: Student 4(A), Final drawing. 218

Figure 46: Student 12(D), Initial drawing. 219

Figure 47: Student 12(D), Final drawing 221

Figure 48: Student 8(B), Initial drawing of the mechanism of peptide bond formation. 222

Figure 49: Student 8(B), Final drawing of the mechanism of peptide bond formation. 223

Figure 50: Student 25(B), Initial drawing. 225

Figure 51: Student 25(B), Final drawing 227

Figure 52: Student 26(B), Initial drawing. 229

Figure 53: Student 26(B), Final drawing. 230

Figure 54: Student 16(A): Initial drawing. 232

Figure 55: Student 16(A), Final drawing. 233

Figure 56: Frame 37 from External Representation #3. 233

Figure 57: Student 30(C): Initial drawing. 235

Figure 58: Student 30(C), Final drawing. 236

Figure 59: Student 18(D), Initial drawing. 237

Figure 60: Student 18(D), Final drawing. 239

Figure 61: Frame 4 from External Representation #6. 239

Figure 62: The influence of previous instruction on students' lived object of learning.. 252

CHAPTER 1

INTRODUCTION

Project Rationale

I began this project with an interest in identifying the core concepts in biochemistry. I first became interested in biochemistry as an undergraduate at the University of Wisconsin - Madison. Biochemistry appealed to me because I felt it combined the real-world applicability of biology with the rules and structure of chemistry. The biological aspect gave my study of biochemistry purpose in that what I was learning was fundamental to understanding how life works. The chemical aspect gave my studies a predictable framework through which I was able to organize my understanding of biochemistry. Thus as a motivated student, I was particularly focused on distilling biochemistry into the fundamental concepts I needed to know in order to be a successful in that arena.

After graduating, I began teaching high school science. It was then that my interests shifted from what I could learn about biochemistry to what my students should learn about biochemistry. As both a biology and chemistry teacher, I used biochemistry to elaborate on concepts in both contexts. Within biology, biochemistry allowed me to explain biological phenomena in greater detail. Within chemistry, biochemistry allowed me to show personal, human applications of the chemistry we were learning in class. It is through my teaching experience that I became interested in how to *best* present biochemical concepts to students.

The Importance of Translation

As a biology teacher, I was particularly interested in how to explain the biochemical processes of the central dogma, i.e., DNA replication, RNA transcription, and protein translation, to my students because I found that these were key concepts necessary to foster students' understanding of other biochemical process and phenomena such as genetics. In fact, the central dogma has been identified as an important aspect of the biochemistry curriculum (Bell, 2001) and a basic biological principle (Cloud-Hansen, Kuehner, Tong, Miller, & Handelsman, 2008; Miskowski, Howard, Abler, & Grundwald, 2007). However, a preliminary study of biotechnology and microbiology students found that only "45% [of the students] could explain the central dogma in the form of a correct flow chart" (similar to Figure 1) (Chattopadhyay & Mahajan, 2004, p.19).

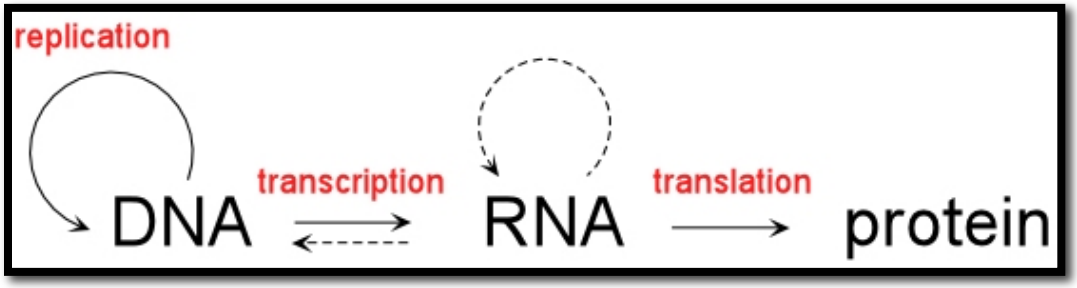


Figure 1. Flow chart of the Central Dogma (Cronk, 2010).

I continued to pursue my interest in how students understood biochemistry and, specifically, concepts of the central dogma as began my graduate work. Among the processes of the central dogma, I found that translation, specifically, has been identified as an essential biochemical process of "central importance" (Nelson and Cox, 2000, p.1020). Cellular structure and function, enzymatics, metabolic pathways, and signal transduction all require proteins and are reliant on protein translation. It therefore stands to reason that the biochemical processes and mechanisms of translation are fundamental

learning objectives for biochemistry education (Anderson, Mitchell, & Osgood, 2005; Bell, 2001; Cody & Treagust, 1977; Dods, 1996; Fisher, 1985; Tsui & Treagust, 2004). However, Klymkowsky (2007) notes that "[i]t is common to find that students lack an accurate and confident understanding of basic biological concepts, such as […] translation […]" (pp. 190-191). For example, Fisher (1985) identified the misconception held by some students that amino acids are the products of translation. Moreover, Rotbain, Marbach-Ad, and Stavy (2006) note that protein translation is "consistently cited as [one of] the most difficult components of biology to learn" (p. 501).

Although protein translation has been identified as an important yet difficult topic for students to learn, little educational research regarding students' understanding of process has been reported. I found that prior research related to student understanding of the processes of the central dogma has focused primarily on the broader topic of genetics. For example, The Students' and Teachers' Conceptions and Science Education Bibliography (STCSE) has identified approximately 70 studies that focus specifically on genetics (Duit, 2009). Similarly, the Conceptual And Reasoning Difficulties in science, mathematics, and technology website (CARD) contains numerous entries pertaining to genetics concepts (Anderson & McKenzie, 2002). Many of these studies focus on students' understanding of DNA as the primary genetic material (e.g. Marbach-Ad, 2001). Additionally, several genetics concept inventories—multiple-choice assessments of student knowledge of genetics concepts—address concepts such as DNA replication and RNA transcription (Bowling, *et al.*, 2008; Shi, *et al.*, 2010; Smith, Wood, & Knight, 2008); there is not, however, a concept inventory that focuses exclusively on protein translation. Therefore, I decided to design and carry out my own research project to

investigate students' understandings of protein translation. I selected the topic of translation in order to address (a) the void in the research literature and (b) the biochemical importance of this metabolic pathway.

In the spring of 2008, I conducted a pilot study in which I surveyed ninety-three second semester biochemistry students. Students were asked to verbally or visually explain the process of protein translation. In analyzing student responses, I was struck by their use of diagrammatic external representations. Many students were able to reproduce images (Figure 2) that were strikingly similar to images found in biochemistry textbooks (Figure 3). However, upon closer inspection, I began to notice that the student drawings were often highly simplified. Important components such as elongation factors, strand polarity, and indications of the dynamic nature of the process were often not included in their representations.

I began to wonder if this was a result of the simplistic questions I had asked students or if there were some inherent limitations to students' understanding of the topic or their use of visual representations to explain it. Subsequently, I began to wonder if students were only paying attention to certain aspects of the representations presented to them in class. If so, which ones and why? As a teacher, I had often used external representations, including models, illustrations, diagrams, and animations, to present information, including protein translation, to my students, but I wondered which representation was *best* for promoting student understanding of the material? Depending on the content and level of the student, perhaps some external representations are better at promoting student understanding than others. It is from these experiences and with these musings that I undertook this project to further explore students' and teachers' use of

external representations of protein translation and how students use those representations to make sense of the biochemical information presented to them.

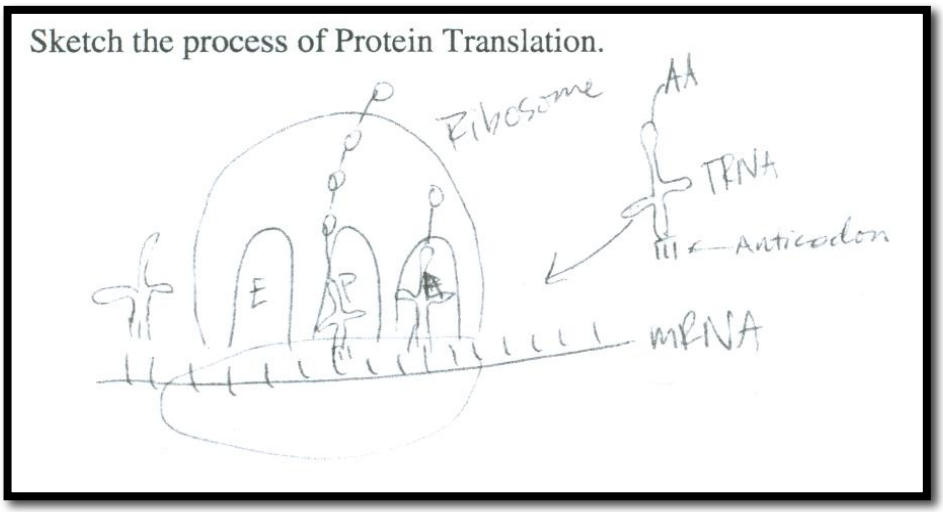


Figure 2. Student drawing of protein translation during pilot study.

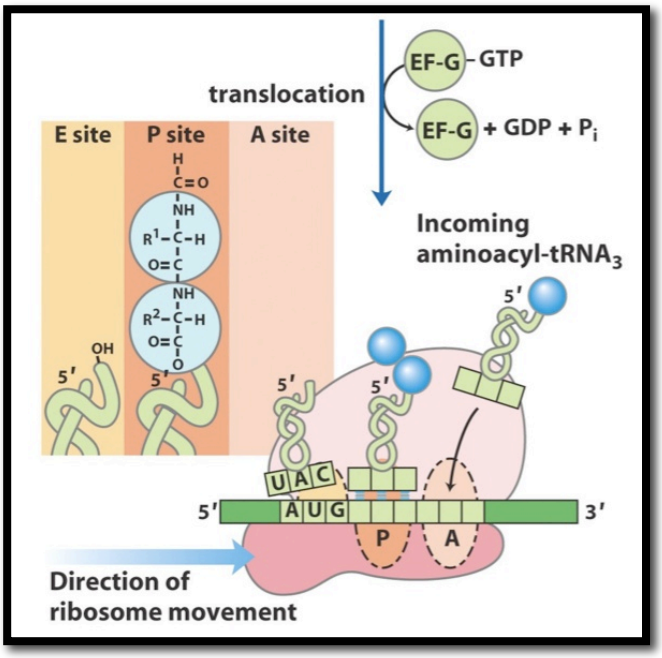


Figure 3. Textbook illustration of protein translation. Note: This image specifically depicts translocation during the elongation stage of protein translation. Image taken from Nelson and Cox (2008, p. 1093).

Guiding Research Questions

In developing research questions for this project, I reflected back on my almost nine years of high school and college teaching experience. As an educator, my focus had been two-fold: what should my students learn about subject X, and what did they actually learn about said subject? However, in transitioning from an educator to a researcher, a third question has emerged: what is possible for students to learn about subject X? Accordingly, I developed the following three questions to guide my research regarding external visualization of protein translation.

1.) What do instructors of second semester biochemistry students intend for their students to learn from external representations of translation?

2.) What is possible for second semester biochemistry students to learn from external representations of translation?

3.) What do second semester biochemistry students learn from external representations of translation?

These questions have been informed by the literature on variation theory (see Chapter 3). Often what educators intend for their students to learn (the "intended object of learning") is not possible, in whole or in part, for them to learn. This may be due to a variety of reasons. Perhaps what is intended for students to learn is never presented to the students, i.e., an external representation does not contain a particular component. For example, if a driving instructor intends for students to learn that stop signs are red but textbook images of stop signs are printed in black and white, students cannot learn that stop signs are red from those images. Thus, it is what is actually presented to students (the "enacted object of learning") that defines what is possible for students to learn; and it

is that which is possible for students to learn in addition to students' prior knowledge that governs what the students actually learned (the "lived object of learning").

In this project, I examined the possibilities for student learning about protein translation from external representations. I used variation theory as my theoretical framework in order to distinguish between what students should learn from the perspective of the instructors, what students can learn as afforded by the selected external representations, and what students actually learn as described by the students. From these three perspectives, I hoped to learn which external representations promote a student understanding of protein translation that best aligns with instructors' intentions for student learning about translation.

CHAPTER 2

VISUAL LITERACY IN BIOCHEMISTRY EDUCATION

Art, Science, and Knowing

> All men by nature desire to know. An indication of this is the delight we take in our senses; […] and above all others the sense of sight. For not only with a view to action, but even when we are not going to do anything, we prefer seeing (one might say) to everything else. The reason is that this, most of all the senses, makes us know and brings to light many differences between things. (Aristotle, 2001, p. 689, as cited in Santas & Eaker, 2009, p. 164)

The advent of technology, specifically television and the Internet, have solidified the primacy of images within the Zeitgeist of the Information Age (Santas & Eaker, 2009). Subsequently, the sentiments of Aristotle are echoed in the current vernacular: "A picture is worth a thousand words" and "Seeing is believing."

Humans have long recognized the power of images to convey ideas (Abel, 1980). Discussions of art, for example, often revolve around the meaning evoked by a piece. UC Berkeley Professor of English Elizabeth Abel elaborates upon this idea while discussing the French Romantic artist Delacroix. Abel (1980) claims that "[g]ood painting […] is not reducible to a statement; rather, it expresses and evokes a state of mind indirectly through the interplay of all its parts" (p. 46). She goes on to note that the artistic image "becomes an expressive object rather than a referential statement" (Abel, 1980, p. 46). The question then becomes, if an image is expressive rather than referential, what is being expressed?

One of my favorite places to visit is the Art Institute of Chicago. I cannot help but get excited as I pass by the patinated bronze lions and walk into the galleries. The imagery on display conjures up a wealth of thoughts, emotions, and responses. Claude Monet's 1906 *Water Lillies* (Figure 4) reminds me of the lake on our family farm

(Copyright permission for all images and animations used in this project can be found in Appendix A). The warm summer breeze, the tranquility of the still water, the smell of damp soil, the serenity of this scene plays out in my mind every time I see this painting. Mary Cassatt's 1893 *The Child's Bath* (Figure 5), however, reminds me of my grandmother. A print of this painting hangs in my grandparents' living room. As a child, I remember looking at this image. The mother and child dynamic; the soft, firm, knowing hand; the quite dignity of daily life reminds me of my grandmother. Although these two paintings invoke certain memories and meaning for me, these are not universal experiences or interpretations of these images.

As a theory of learning, constructivism posits that new knowledge, as experienced and perceived by the learner, is integrated with the learner's prior knowledge to "construct" a new understanding (Ferguson, 2007). Dewey (1934) notes that "[art] intercepts every shade of expressiveness found in objects and orders them in a new experience of life" (p. 104). In this way, I would assert that art, i.e., imagery, can be viewed as a "new experience" and can thus be used to convey new meaning and subsequent knowledge to students. While prior experience and subsequent meaning of imagery is unique to the individual, socially negotiated norms, customs, and interactions help to define common meaning of shared experiences. Discussions of the implicit and explicit meaning of an image and the limitations of the image can generate a collective understanding of what is meant (Ferguson, 2007).

Educators have long relied on images, diagrams, models, and other representations to illustrate a collective, common meaning of specific content (Pinar, Reynolds, Slattery, & Taubman, 2004). As a high school science teacher, I often found

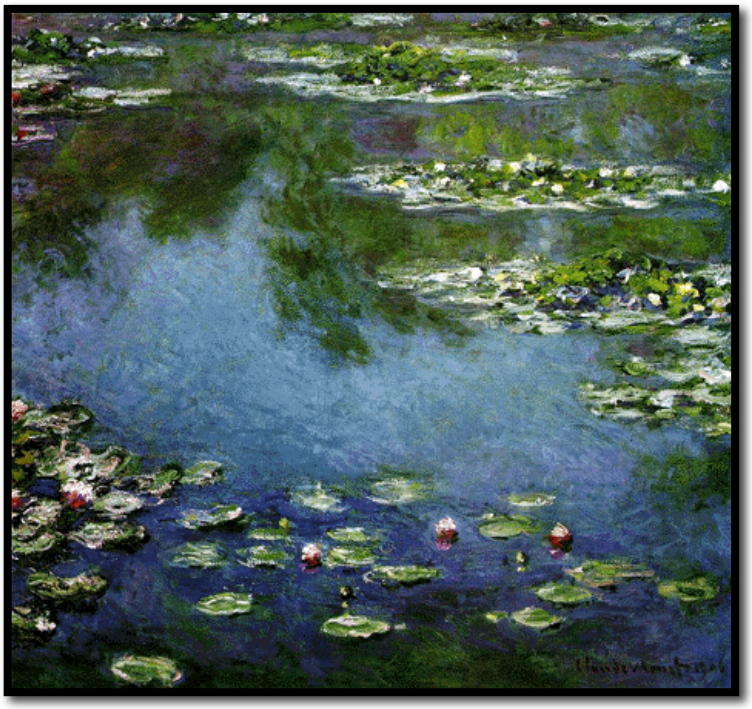


Figure 4. Claude Monet, *Water Lillies*, 1906, Art Institute of Chicago.

Figure 5. Mary Cassatt, *The Child's Bath* (*The Bath*), 1893, Art Institute of Chicago.

myself relying on the images of science found in textbooks and supplemental teacher resources to convey fundamental ideas to my students. Artists' renderings of cellular components and activities, or molecular structures and reactions were necessary components of my teaching. However, I quickly learned that imagery posed a potential obstacle to learning for my students. For example, I used a series of images to show my chemistry students the developments in atomic theory from Democritus through the Bohr model. However, when I asked them to explain each model, they conveyed a variety of understandings. For example, one student offered an fairly accurate explanation of negative electrons orbiting the positive nucleus while another student suggested that electrons "orbited the nucleus because of gravity" in reference to the Bohr model of an atom. This student used their knowledge of planetary motion to explain atomic structure. Clearly, students may still develop varied understandings of a shared image based on variations in their level of prior knowledge of the material (e.g., Cook, 2006, 2008).

The variation in previous experiences is not the only factor influencing students' varied interpretations and understandings of images. Students are also increasingly experiencing variation in the way in which information is presented. In step with the development of new technologies, the use of educational imagery—imagery used in an educational context to convey information—has grown exponentially (Schörnborn & Anderson, 2006). A 1993 study of science textbooks found that nearly 55% of the printed page was composed of illustrations (Mayer, 1993), thereby making illustrations a significant instructional medium through which information, in particular science information, is conveyed to students. In 1997, Kozma and Russell described the modern chemistry classroom, noting that "classroom whiteboards and modern chemistry

textbooks are filled with diagrams, charts, graphs, equations, and formulas along with words, photographs, and illustrations. Increasingly, newer educational technologies—video and computers—are also being used in the classroom to represent chemical phenomena in new ways" (p. 950). However, this growth in educational imagery is not limited to chemistry. In fact, The New York Times recently featured a video in its online edition entitled "The Animators of Life" describing how movie animation technology has fostered the development of molecular animations of biological phenomena (Olsen, 2010). However, as with any instructional strategy, images—animations or illustrations—are only as useful as students' ability to understand them. Thus, if we are to successfully use imagery and the technology that facilitates this imagery in the classroom, it is essential that students have the visual literacy necessary to decode and make sense of the visual information.

Terminology of Visual Literacy

Before we go too much further, it seems appropriate to pause and define how I will be using some of the common terminology of visual literacy. To begin, *visual literacy* refers to the ability to understand and create visual representations of conceptual and/or physical objects or phenomena. This term is often used in contrast to *verbal literacy* or, simply, *literacy* (Avgerinou & Ericson, 1997). Just as *verbal literacy* indicates the ability to read and write words, *visual literacy* implies the ability to read (make meaning from) and write (draw or construct) images (Schörnborn & Anderson, 2006).

As visual literacy centers on the use and meanings of images, much of the discussion within this area concerns the nature of the imagery in question. While the term *image* can be used to refer to a physical, visible artifact such as a photo or illustration, it

can also be used to refer to a mental construct such as an imagined image as created by and held in the mind's eye. It should also be noted that an image is separate and distinct from the physical object or phenomenon of which it represents. For greater clarity, I will use the term *representation* rather than image as the term representation implies that the visual imagery in question may not bare a one-to-one correlation to that which is being represented but is, instead, meant to depict some aspect or collection of features of the object or process in question as understood by the representational designer. A representation may simplify, exaggerate, or negate components, conditions, or relationships in order to convey an intended meaning (Mayer, 2003).

Just as the term *image* can mean both a physical or imagined image, the term *representation* can be parsed out in this way. Glasgow, Narayanan, and Chandrasekaran (1995) note that representations can be classified as external or internal. *Internal representations*, often referred to as *mental models* (Johnson-Laird, 1983), are conceptualizations by the individual of a perceived external reality. Internal representations may vary from robust schema, or mental frameworks, to transient, on-the-fly conceptualizations. *External representations*, on the other hand, are representations external to the mind. From this point forward, I will use the terms *representation* and *external representation* interchangeably as this project will focus specifically on external representations of protein translation. Internal representations—i.e., mental models—will be specifically identified as such.

External representations are depictions of an object or phenomenon as conveyed by a designer through some type of medium. External representations include, but are not limited to: physical, three-dimensional models; videos; computer simulations;

animations; photographs; illustrations; diagrams; flowcharts; graphs; and equations (Schörnborn & Anderson, 2006). External representations can be further classified as being in one of three different *modes*. The representational mode refers to the type or form of the external representation. External representational modes are static, dynamic, or multimedia (Schörnborn & Anderson, 2006). A *static* representation lacks movement, such as a picture found in a textbook. A *dynamic* representation contains movement, such as an animation. For the purposes of this project, I will be focusing on static and dynamic representations as *multimedia* representations contain both visual and verbal cues, such as a video with narration. The introduction of verbal information within multimedia representations presents a confounding variable. As my research interests center on students' understanding of visual information, I will restrict my use of external representations to those presenting only visual cues. Specifically, I will be focusing on static illustrations and dynamic animations of protein translation. The following discussion of visual literacy will utilize the previously defined terminology.

What is Visual Literacy?

Historically, education has relied on written and spoken language as the custodian and conveyor of knowledge (Pinar *et al.,* 2004). Traditional curricula have centered on the basic tenants of reading and writing (and arithmetic), and current education standards and assessments indicate the primacy of these skills (Lewis, 2008; Cassell, 2004). Consequently, reading and writing, often referred to as verbal literacy (Huffaker, 2004), have received significant attention within the educational and research communities (Stokes, 2002). A great deal of educational capital has been spent developing students'

verbal literacy, including their ability to read and write language and the ability to think, learn, listen, and express oneself in terms of words (Stokes, 2002).

Similarly, science education has followed suit. The National Science Education Standards (NRC, 1996) identifies scientific literacy as an analogous set of skills.

> Scientific literacy means that a person can ask, find, or determine answers to questions derived from curiosity about everyday experiences. It means that a person has the ability to describe, explain, and predict natural phenomena. Scientific literacy entails being able to read with understanding articles about science in the popular press and to engage in social conversation about the validity of the conclusions. Scientific literacy implies that a person can identify scientific issues underlying national and local decisions and express positions that are scientifically and technologically informed. (p. 22)

I would note that, as stated, science literacy is framed solely in terms of verbal skills. There is no mention of visual literacy or elements of visual literacy. However, the language of science is not restricted to conventional modes of verbal literacy, text and speech. Science is often expressed as visual representations: graphs, flow charts, models, and pictures (Trumbo, 1999; Wellington & Osborne, 2001); and scientists often use representations in their work to, *inter alia*, present data or visualize a phenomenon. Illustrations allow for the diagrammatic representation of complex relationships and processes that may not be easily expressed in words. As such, the ability of students to comprehend, evaluate, and construct visual representations in science is necessary skill (Stanely, 1996).

Schönborn and Anderson (2006) note that "the ability to read (understand or make sense of) and write (draw) […] including the ability to think, learn, and express oneself in terms of images" is defined by educational researchers as *visual literacy* (p. 97). Visual literacy can promote the development of knowledge that students may not be able to acquire from text alone (Mayer, Bove, Bryman, Mars, & Tapangco, 1996). For example,

Mayer and colleagues (1996) describe a series of experiments in which participants were asked to explain a scientific system—the generation of lightning—and use their explanation to solve problems. Participants were given a lengthy text description, a text summary, or a summary that included visuals. Mayer *et al*. (1996) found that the summary with visuals was the most effective at promoting scientifically accurate student understanding.

While verbal literacy is an essential educational skill as denoted by educational policy statements and curriculum documents, visual literacy is a necessary component of science education and essential to the practice of science (Trumbo, 1999). Students' visual literacy in science is affected by a variety of factors. Thus, it is important to understand the types of images students are exposed to in the science classroom, as well as the factors that affect students' abilities to learn from them.

External Representations in Science

Cognitive psychologists distinguish external representations from internal representations, i.e., mental models, which are representations constructed in the mind of the individual (Schönborn & Anderson, 2006). External representations are defined as visualization tools that represent phenomena that occur in the external world, external to the mind (Schönborn & Anderson, 2006). External representations in science range from symbolic representations of chemical equations to graphical diagrams of empirical data, from static images to dynamic animations, from simplistic illustrations to complex multimedia presentations. External representations have the potential to make the unseen, seen; they can depict intricate relationships or deconstruct complicated processes; they can often negate the constraints of size, time, and space.

> Scientific images do not, of course, aim to record what is visible, their purpose is to make visible. This applies to the ordinary enlargement as well as to the miracle of the electron-scanning microscope which has enabled scientists to answer so many questions—always presupposing that they know the specifications of the instrument, its magnification, power of resolution, and so on. (Gombrich, 1980, p. 185)

External representations have been shown to be an extremely efficient means of communication "if the viewer understands the rules of construction" (Perino, 2001, p. 16). A photograph, for example, is an image in the same way a great painting is an image; they are both subject to distortion and interpretation and separate from the physical reality they are meant to capture, depict, or evoke. A photograph is not necessarily more realistic than a painting of the same subject. Certainly, there are different constraints to the media used in photography and painting; however, the construction of the camera, the properties of photosensitive material, and the conditions of exposure govern the creation of the photograph. If the time of exposure is lengthened, the characteristics of the photograph are changed. If the lens of the camera is changed, the image is changed. If the position of the camera is changed, the image is changed. If the film is altered, the image is changed. If the film is not developed in a certain way, the image is changed. However, if you understand the constraints of the medium, you are able to draw conclusions from the image (Gombrich, 1980).

Scientists have used instrumentation to create artifacts—external representations—and make observations of phenomena that could otherwise not be recorded or observed. Similar to a photograph, the external representations of scientific instrumentation aim to capture some aspect(s) of an object or phenomenon as filtered through the instrument and presented in a chosen medium and format. In the same way that an electron micrograph creates a representation of a molecule, thereby making it

visible to a scientist, external representations can be used in an educational setting to make non-experiential scientific concepts visible to a student. However, a teacher's ability to effectively use external representations and students' ability to understand and make meaning from those representations can be affected by an array of factors, such as students' prior knowledge, the type (mode) of representation, and the level of abstraction presented in the representation (Cook, 2006; Cook, Wiebe, Carter, 2008; Schönborn & Anderson, 2006).

External Representations and Prior Knowledge

According to constructivist theories of knowledge, individuals interact with and interpret experiential knowledge through internalized socio-cultural standards and norms in an effort to construct meaning (Ferguson, 2007). However, each individual encounters a unique set of experiences as they navigate through the world. This history of novel experiences creates a unique cognitive framework and guides the perception and integration of new knowledge within the individual. Thus, prior knowledge is an important element in the construction of conceptual knowledge (Novak, 1990).

As individuals *read* external representations, they use their prior knowledge in two ways. First, prior knowledge is used to identify relevant information within the external representation. Second, prior knowledge is added to the information gleaned from the external representation to construct a more coherent meaning of the depicted concept. In this way, individuals are able to construct a working mental model of the concept depicted by the external representation by integrating elements of the external representation with their prior knowledge (Braune & Foshay, 1983).

Several studies have explored the influence of prior knowledge on students' development of visual literacy skills (Kozma & Russell, 1997; Cook, 2006; Cook, *et al.,* 2008; Schnotz & Lowe, 2003; Lowe, 1996, 2003, 2004). The majority of these studies have focused on the differences between the visual literacy skills of *novices* and those of *experts*. I would note that there is a variety of literature about the influence of students' prior knowledge and expert/novice differences in visual literacy skills, knowledge structures, and learning. However, for the purposes of this project, I have chosen to restrict the literature discussed here to that pertaining specifically to students' use and understanding of external representations of scientific phenomena. I chose to focus the review of the literature in this way to reflect the nature of this project and to discuss the specific role of prior knowledge on students' use and understanding of scientific content presented as external representations.

Novice learners are generally assumed to have less prior knowledge than experts for use in constructing mental representations from instructional materials (Johnson & Lawson, 1998). Additionally, it is thought that what knowledge they do have is not interconnected or hierarchically organized enough to allow for proficient interpretation and integration of new information (Johnson & Lawson, 1998). As a result, novice learners tend to focus on superficial aspects of external representations (Heyworth, 1999; Lowe, 2003, 2004); and the mental models they develop tend to be overly simplistic (Snyder, 2000). For example, Lowe (2004) asked 12 undergraduate students with no "specialized knowledge of meteorology," i.e., novice learners, to answer a series of questions about an animated weather map. He found that students "tended to use low level strategies that addressed isolated spatial and temporal aspects of the animation to

the neglect of more inclusive dimensions" (p. 270). That is to say, the students tended to focus on specific features of the external representation rather than the cumulative meaning of those features. As a result, the novices in Lowe's study were unable to make meaningful meteorological predictions based on their superficial and piecemeal approach to understanding the representation.

Experts, on the other hand, have more prior knowledge than novices; but, even more importantly, their knowledge is more highly organized. Their intricate network of prior knowledge and depth of experiences allows expert learners to better relate their mental models to generally agreed upon scientific principles (Geelan, 1997). Because their understanding is not simply superficial and descriptive, experts develop deeper and more meaningful comprehension of material as compared to novices (Snyder, 2000). For example, Synder (2000) asked nine university physic professors, i.e., expert learners, to complete a card-sort activity in order to explore their understanding of models of mechanics. She found that the experts categorized the concepts according to their model- and theory-based attributes, thereby describing a coherent view of the concepts (Synder, 2000). Similarly, a recent study by Stieff, Hegarty, and Dixon (2010) found that students with "more experience in [a] discipline display [problem solving] strategies similar to those seen among experts" (p. 124). This suggests that students with more experience in a discipline are becoming more like experts in their thinking. However, which experiences are essential in developing expertise?

A more fundamental question may be how do novices become experts? More to the point, how do we learn? Marton and Booth (1997) note that Plato and Socrates explored this question. Meno, a student of Socrates, asks "How can you search for

something when you do not know what it is? You do not know what to look for, and if you were to come across it you would not recognize it as what you are looking for" (cited in Marton & Booth, 1997, p. 2). Experts are experts because they have the ability to quickly identify what information to attend to based on prior knowledge and experience. Novices, on the other hand, are novices because they do not have the ability, the prior knowledge, or the prior experience to discern which information is important and which is not. Marton and Booth (1997) note "[t]he surprising answer of Plato—or Socrates—to the question 'How do we gain knowledge about the world?' is that we cannot gain knowledge about the world. Learning is impossible. The paradox lies in the observation that we certainly do learn!" (p. 2).

The notable observation of Plato and Socrates is that novices do not know what they are looking for. In order for novices to attend to the appropriate information, they must be made aware of it. It is here that external representations present the ability to significantly influence student learning (Lindgren & Schwartz, 2009). External representations have the potential to cue students to discern particular pieces of information. Some studies have shown that the shape, color, or orientation of components within the external representation (e.g., Koch & Ullman, 1985; Patrick, Carter, & Wiebe, 2005), the use of arrows (e.g., Hommel, Pratt, Colzato, & Godijn, 2001), supporting text (e.g., Mayer *et al.,* 1996), or the mode of presentation (e.g., Lewalter, 2003) can all be used to direct students' attention to particular aspects of the representation. For example, Lewalter (2003) explored the effect of mode of representation, static vs. dynamic, on student comprehension of optics. Sixty undergraduates were divided into either a control group—with no illustrations—or one of two experimental groups—one with static

illustrations and one with dynamic illustrations. Lewalter found that dynamic visuals increased student performance on problem-solving and comprehension tasks. However, the study did not show that dynamic visuals were able to cue students to the dynamic aspects of the content. To get students to notice the dynamic aspects of the content, Lewalter speculates that "arrows and series of frames … may be sufficient for students to acquire factual knowledge in this case" (2003, p. 187). Patrick and colleagues claim that "[v]isual representations should purposefully use cueing strategies to attract attention and influence what information learners will extract" (2005, p. 364). Thus, if an external representation can be designed in a certain way, students can potentially be cued to attend to information in an expert-like manner.

Modes of External Representations

As students interact with external representations, there are many variables that may affect students' understanding of the content depicted by the representation. Schönborn, Anderson, and Grayson (2002) note that

> there are at least three factors affecting the ability of students to interpret [an external representation], the ability of the students to reason with the diagram, students' understanding (or lack thereof) of the concepts of relevance to the diagram, and the mode in which the desired phenomenon is represented (p.96).

While students' prior knowledge, subsequent understanding of the concepts, and reasoning abilities may significantly influence the potential for learning from the students' end, the teacher may also influence learning by selecting the way in which information is presented in the external representation, such as the representational mode. For example in the 2002 study by Schönborn, Anderson, and Grayson cited above, the authors explored students' understanding of and ability to reason with textbook illustrations of Immunoglobin G (IgG). They concluded that "in many cases, the students

are focusing on surface-level features of the diagram when extracting meaning from it" (p. 95). This attention to surface features of the mode of the representations, such as color or shape, may cause students to gloss over the deeper meaning of the representation.

Schönborn and Anderson (2006) have identified three modes of representation: static, dynamic, and multimedia modes. Mayer and Moreno (2003) note that "pictures can be static (e.g., illustrations, graphs, charts, photos, or maps) or dynamic (e.g., animation, video, or interactive illustration)" (p. 43). Thus, the static and dynamic modes are solely visual modes of representation. The multimedia modality is distinguished as an integration of visual and verbal information, such as a narrated video (Mayer & Moreno, 2003).

Graphic designers, textbook publishers, researchers, and instructors (all of whom I will refer to as the *teacher* for their role in providing instructional materials to *students*, i.e., anyone in a position to learn from those materials) have a significant element of control over the way in which information is presented to students. As described previously, a considerable amount of research has shown that the nature of the external representations influences student learning. For example, Patrick and colleagues (2005) demonstrated that students attend to different features of an external representation depending on the nature of the representation. They used eye tracking to identify which representational features students were focusing on when they were shown either "simple" 2D graphics or "rich" 3D graphics of DNA replication (Patrick *et al.*, 2005, p. 353). Patrick and colleagues found that the twisting shape depicted in the 2D representation provided participants with more information about the helical shape of the DNA double helix as opposed to the 3D graphic. However, the "more realistic shapes"

used to depict the enzymes in the 3D graphic were preferred by participants over the geometric shapes of the 2D graphic (Patrick *et al.*, 2005, p. 363). While students might prefer information-rich graphics, that does not mean that they learn more or better from them. In fact, Mayer and colleagues document the potential for cognitive overload based on the nature of the external representations (see *Potential Negative Results of Using External Representations* below for a more detailed discussion of cognitive overload) (Mayer 1997; Mayer & Johnson, 2008; Mayer & Moreno, 1998, 2002, 2003; Mayer, Heiser, & Lonn, 2001; Mayer Mautone, & Prothero, 2002; Mayer, Hegarty, Mayer, & Campbell, 2005).

For the purposes of this research study, I focused only on the static and dynamic external representational modes. Noting Schönborn and Anderson's (2006) call to develop biochemistry students' visual literacy, I found that a singular focus on the visual modes of representation was the most appropriate for this study. Lowe (2003) observes that "in recent years there has been a growing trend across a range of media to use highly illustrated materials for instruction rather than relying on largely text-based presentations of information" (p.157). The inclusion of the multimedia mode into this study would introduce confounding variables, i.e., text or narration. Additionally, multimedia learning has been addressed a length by Mayer and colleagues (Mayer 1997, 2001, 2003; Mayer & Chandler, 2001; Mayer & Johnson, 2008; Mayer & Moreno, 1998, 2002, 2003; Mayer & Sims, 1994; Mayer, Dow, & Mayer, 2003; Mayer, Heiser, & Lonn, 2001; Mayer, Mautone, & Prothero, 2002; Mayer, Hegarty, Mayer, & Campbell, 2005). However, there is a lack of research to support the prevalent assumption that animations are superior to

static graphics (Lowe, 2003). Thus, further investigation into the pedagogical implications of static vs. dynamic external representations is warranted.

Level of Abstraction of the Representation

In addition to the mode of representation, Schönborn and Anderson (2006) also identify the level of abstraction of a representation as a factor that may influence students understanding of the content depicted by the representation. The level of abstraction refers to the degree of realism depicted in the representation. For example, a drawing of a flower might be considered less *realistic* as compared to a photo of a flower. In this way, realism is a measure of how similar a representation is to the actual object it is meant to depict (Rieber, 1994). Schönborn and Anderson (2006) note that "biochemists make use of a wide range of [external representations…] which can be placed on a continuum from abstract, to more stylized, to more realistic representations of phenomena" (p. 95). Schönborn and Anderson (2006) go on to speculate that students' ability to translate between representations of varying levels of abstraction may not be well developed, thereby making it difficult for students to decode the information presented in representations of biochemical phenomena at varying levels of abstraction.

As part of a larger meta-analysis, Höffler and Leutner (2007) examined the role of the level of abstraction in promoting learning from dynamic and static representations. They concluded that the level of abstraction of an animation was correlated with student learning outcomes. They determined that highly realistic animations supported student learning more than did less realistic animations or static representations. This contradicted a review of the literature by Tversky, Morrison, and Betrancourt (2002) on the role of animations in teaching complex systems, including biological systems.

Tversky *et al.* (2002) concluded that "animations should lean toward the schematic and away from the realistic" (p. 258). Thus the question remains, what role—if any—does the level of abstraction play in determining the effectiveness of an external representation to promote student learning?

Potential Outcomes of Using External Representations

There are many potential results–both positive and negative–of using external representations to present information to students. Several studies advocate the use of external representations as a preferred instructional strategy to convey information and to enhance student understanding of the content (e.g., Schönborn & Anderson, 2006). However, it has also been shown that students may develop alternative conceptions from external representations (e.g., Lowe, 1996). Additionally, external representations may present students with too much information, leading to cognitive overload (e.g., Schnotz, Böckheler, & Grzondziel, 1999). Thus, it is important to understand not only how external representations may be beneficial to students but also how they might be harmful, as well as potential ways to proactively address any potential harmful effects of using external representations in an educational context.

Potential Benefits of Using External Representations

External representations have gained wide use as a pedagogical tool as of late (Lowe, 2003). Within biochemistry "this is reflected by the exponential growth over the years in the number and range of visualization tools now available to the biochemist for teaching, learning, and research" (Schönborn & Anderson, 2006, p. 94). However, what are the potential benefits of using external representations? Ausburn and Ausburn (1978)

observed six potential benefits of student instruction in visual literacy using external representations.

> 1. [An] increase in all kinds of verbal skills,
> 2. [An] improved self-expression and ordering of ideas,
> 3. [An] increase in student motivation and interest in subjects of all types and at all levels,
> 4. [The ability to 'reach'] students not being reached in traditional ways. Students such as the educationally disadvantaged, the truant, the socially underprivileged, the emotionally disturbed, the intellectually handicapped, the ethnic and bilinguals, the dyslexic, the deaf, those with speech pathology problems—all respond and have been helped in terms of both interest and achievement,
> 5. [An] improved image of self and relationship to the world,
> 6. [An] improved self-reliance, independence, and confidence. (p. 295)

In additional to the conventional wisdom that external representations can enhance student learning (Lowe, 2003), the science education research literature has identified several ways in which external representations may be beneficial. Winn (1991) summarizes a collection of research on static external representations, specifically maps and diagrams, noting that these external representations are "well-suited to illustrate inter-component relationships and sequences" (p. 240). Winn (1991) goes on to acknowledge that external representations can be used to integrate new knowledge with prior knowledge and make abstract content more coherent. Similarly, Harrison and Treagust (2000) note that external representations can be a valuable tool for students to construct new or more intricate knowledge. Their study examined a high school student's understanding of an atom over the course of instruction, which included exposure to a variety of external representations. They found that following instruction, the student displayed an understanding of atomic and molecular models that was much more consistent with scientifically accepted conceptions as compared to the student's prior non-scientific conceptions (Harrison & Treagust, 2000).

Bauer and Johnson-Laird (1993) discuss how the use of diagrams can improve students' reasoning abilities. They asked 48 undergraduate students to answer a series of questions about either people and places or electrical circuits. Some students were asked questions verbally while other were asked diagrammatically. Bauer and Johnson-Laird (1993) found that the participants shown the diagrams were better able to answer the questions correctly, regardless of the domain (people and places or circuitry). They concluded that "certain diagrams can help individuals to reason more rapidly and more accurately" (Bauer & Johnson-Laird, 1993, p. 378). As such, external representations can be an extremely beneficial resource to allow students to develop their conceptual knowledge and reasoning abilities.

External representations can also play a much more practical role in science education. Linn, Davis, and Eylon (2004) describe four basic tenants or *metaprinciples* to scaffold knowledge integration in science education: 1.) make science accessible, 2.) make thinking visible, 3.) help students learn from others, and 4.) promote autonomy and lifelong learning. Of these four tenants, external representations address at least two of them: making science *accessible* and making science *visible.* For example, Kozma and Russell (1997) note that "the expansion of the universe, tectonic plate drift, evolution of species, and molecular structure and reactivity are all scientific phenomena that are not available to direct experience" (p. 949) External representations can provide a way to make science topics like these accessible and visible.

Potential Negative Results of Using External Representations

While external representations can be an important and beneficial part of science education, they can also have negative effects on students' learning. For example, they

might lead to the development of alternative, non-scientific conceptions. As discussed previously, prior knowledge is an important factor in determining what students can learn. Lowe (1996) describes how meteorologists and non-meteorologists come to understand a static weather map differently. Lowe concludes that differences in content knowledge lead to differences in understanding of a content-related representation. He goes on to suggest that the representational design of the diagram "implies [that] viewers possess appropriate background knowledge concerning the depicted situation" (Lowe, 1996, p. 377). If viewers fail to posses the "appropriate background knowledge," it is not simply that they will not be able to understand the representation; instead, viewers can develop alternative understandings of what is being depicted in the representation.

Harrison and Treagust (1996) interviewed 48 secondary students regarding their understandings of atoms and molecules. As part of the study, students were shown six different diagrams of atoms and asked to rank them according to how closely the representations aligned with their understanding of an atom. Harrison and Treagust (1996) suggest that students may hold alternative conceptions of atoms due to inaccurate decoding and interpretation of external representations. Similar to Lowe (1996), Harrison and Treagust suggest that students may not have sufficient knowledge of the topic, which results in their inability to "appropriately" understand what information is being presented in the representation and the subsequent formation of alternative conceptions based on the representation.

Lowe (1996, 2003) and Kozma and Russell (1997) offer another possibility as to why students may develop alternative conceptions based on their experience of an external representation: they focus on surface features. In Lowe's study of

meteorologists' (expert) and non-meteorologists' (novice) interpretations of a static weather map, he describes the non-meteorologists' explanation of what was represented by the weather map, noting that they focused almost exclusively on the surface features of the diagram as opposed to the most meteorologically relevant details. In 2003, Lowe found a similar result using an animated weather map, noting that "the animation did not appear to be effective in making subjects any more sensitive to these less obvious dynamics aspects, despite the fact that they were explicitly depicted in the animation and a high degree of user interaction was provided for" (p. 173).

Kozma and Russell (1997) also found that novices tend to focus on the surface features of a representation instead of on the most conceptually important features. They asked chemists (expert) and undergraduate chemistry students (novice) to complete a card-sort in which they were to arrange various chemistry representations into "meaningful" groups. Each card corresponded to a computer display showing an equation, a graph, an animation, or a video segment related to chemistry. Kozma and Russell found that the "experts formed their groups around concepts and principles in the domain," whereas, "[n]ovices made smaller groups and were more likely to give reasons for these groupings that merely described the common surfaces" of the representations (1997, p. 960). They concluded that "the chemical understanding of novices was bound to the common surface features of the representations" (Kozma & Russell, 1997, p. 960). Therefore, because students focus on the surface features they may develop the inaccurate understanding from a representation or one that differs from what is intended by the teacher. This may be due to students' inability to distinguish between surface features of a representation and meaningful features of that representation.

A second potential negative result of using external representations is that students may experience cognitive overload. The idea of cognitive overload is based on Cognitive Load Theory. Ayres and van Gog (2009) describe Cognitive Load Theory as "a model of human cognitive architecture that assumes that working memory […] is very limited in terms of being able to store and process information […], whereas long-term memory […] has a vast capacity, able to store an almost limitless amount of information" (p. 253). Information is thought to enter the human memory system through working memory before potentially being stored in long-term memory. However, if incoming information overwhelms the limited capacity of working memory, that information may not be able to processed and will not be stored in long-term memory.

Several studies have linked the use of external representations to cognitive overload. Chandler and Sweller (1991) describe a series of experiments in which students were shown one of two types of diagrams. The first type of diagram contained a block of text describing the diagram followed by the diagram. The second type of diagram integrated the text into the diagram such that each part of the diagram was labeled and described within the diagram. Chandler and Sweller (1991) found that the students shown the integrated diagrams outperformed students shown the diagram with the separated information. To explain this observation, they describe what they call the split-attention effect. They propose that the integrated diagram reduced the amount of cognitive resources required for students to pair up the appropriate information from the text with the appropriate part of the diagram to make meaning out of the representation. According to this theory, If student attention is split between the text and the diagram, they are more apt to experience cognitive overload and less likely to understand the information

presented in the representation. Thus, the design of the external representation may cause students to fail to understand the representation due to cognitive overload.

Another issue related to cognitive overload is the amount of information presented to students in a representation. Some studies suggest that animations may be more likely to cause cognitive overload because significantly more information is presented to students as compared to a static representation or text alone. For example, Schnotz, Böckheler, and Grzondziel (1999) describe a study in which 12 pairs of university students were presented with a learning environment containing either animated or static representations of circumnavigation. The study showed that students who were shown the animated pictures did worse (as measured by a pre-test, post-test evaluation) than students who were shown the static pictures. Schnotz and colleagues (1999) conclude that "although animated pictures may provide external support for mental simulations, they do not appear to be generally beneficial for learning, because they can prevent individuals from performing relevant cognitive processes" (p. 245). Therefore, the mode and content of a representation may lead to cognitive overload.

Although external representations have the potential to cause cognitive overload and/or the development of alternative, non-scientific conception in students, the potential for improved student learning and reasoning and the ability to make science accessible and visible to students means that external representations can be powerful instructional tools if we understand how to best use them.

Visual Literacy and External Representations in Biochemistry Education

Johnstone (1993) describes chemical phenomenon as being represented at three different levels: the macroscopic, the sub-microscopic, or the symbolic levels.

Johnstone's triplet is often used to describe and compare the visible (macroscopic) phenomena of chemistry, the invisible (sub-microscopic or particulate) phenomena of chemistry, and the attempt to depict (symbolic) the phenomena of chemistry (Figure 6). The symbolic level includes representations of macroscopic components or phenomena, such as an illustration of a glass of water, as well as particulate components or phenomena, such as a space-filling model of a water molecule. In addition to depict a range of sizes, representations may also fall on a continuum of abstraction as described earlier (see Chapter 2, Level of Abstraction of the Representation). On the macroscopic level, a photo of a glass of water could be considered more realistic than the text "A glass of water." On the sub-microscopic level, a chemical formula of water (H_2O) could be considered more abstract than a space-filling model of a water molecule because the space-filling model attempts to depict the general structure of the molecule while the chemical formula does not (Figure 6).

Representations at all levels of Johnstone's triplet have long been a part of chemistry and chemistry education. Similarly, the range of representations have also had a place in biochemistry and biochemistry education. The physical artifacts upon which biochemistry is based vary from representations of macroscopic constituents (e.g., Western Blots) to representations of microscopic components (e.g., electron micrographs) to representations of molecular configurations (e.g., protein modeling programs) (Schönborn & Anderson, 2006). In order to explore and explain biochemical and cellular phenomena, biochemists rely on external representations to facilitate conceptualization and instruction of processes at all levels of organization, i.e., macroscopic to sub-microscopic and abstract to realistic (Schönborn & Anderson, 2006, 2010).

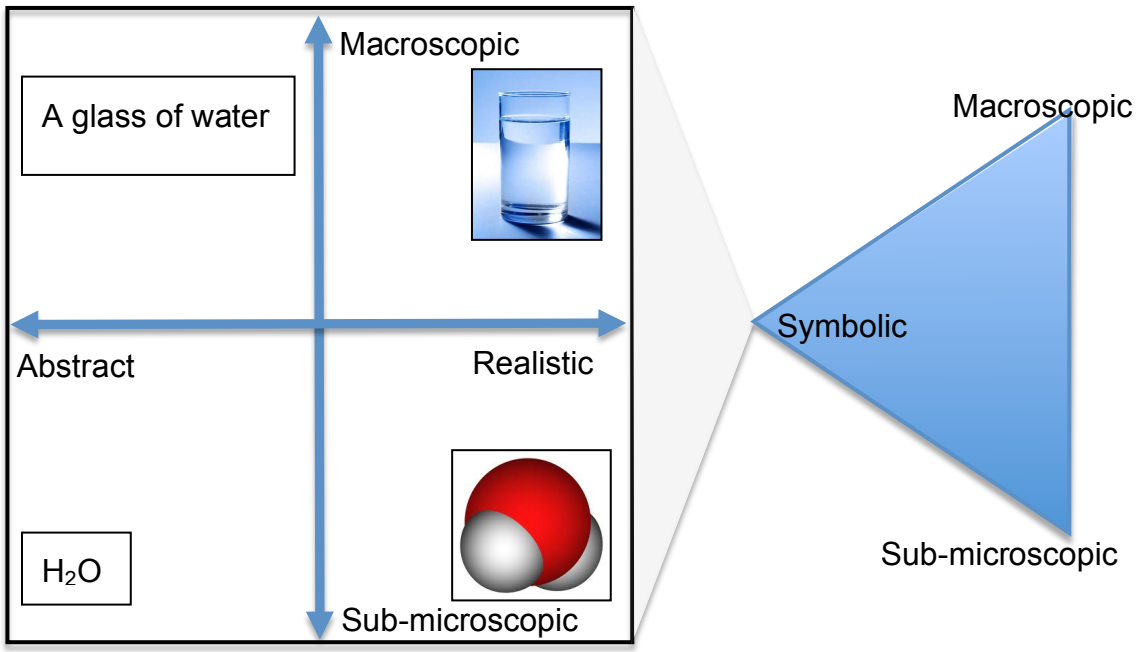


Figure 6. Representational quadrants. Note: this representation of symbolic phenomena with reference to the levels of chemical phenomena has been modified from the model proposed by Johnstone (1993).

Biochemists demonstrate expert visual literacy by decoding, evaluating, interpreting, manipulating, and constructing external representations (Schönborn & Anderson, 2010). They also display other cognitive skills related to visual literacy, including the ability to translate between multiple external representations and between the various levels of organization, as well as the ability to "visualize orders of magnitude, relative size, and scale" (Schönborn & Anderson, 2010, p.349). Clearly, the ability to construct meaning from visual representations (visual literacy) is a necessary skill for biochemists. It is also a necessary skill for biochemistry students, who will be presented with a large number of representations over the course of their educational careers. However, just as verbal literacy is not innate or intuitive to students nor is visual literacy.

Thus, Avgerinou & Ericson (1997) argue that this skill must be "identified and taught" to biochemistry students (p. 288).

The Role of Translation in Biochemistry Education

Duit (2009) has identified some 8400 scholarly works regarding students' and teachers' conceptions and science education. Among them are several studies regarding student understandings (and misunderstandings) of various molecular life science topics. Specifically, the "central dogma" of molecular biology has been identified as an important aspect of the biochemistry curriculum (Bell, 2001) and a basic biological principle (Cloud-Hansen *et al.*, 2008).

> The Central Dogma. This states that once 'information' has passed into protein it cannot get out again. In more detail, the transfer of information from nucleic acid to protein may be possible, but transfer from protein to protein, or from protein to nucleic acid is impossible. Information means here the precise determination of sequence, either of bases in the nucleic acid or of amino-acid residues in the protein. (Crick, 1957, as cited in Thieffry & Sarkar, 1998, p. 312)

Francis Crick first published his description of the *Central Dogma* in 1958 (Thieffry & Sarkar, 1998). His original account of the central dogma described a singular direction of expression of genetic information (from DNA to RNA to proteins). Specifically the term *dogma* denotes an authoritative set of principles. Stegmann (2005) notes that "although the 'dogma' has been repeatedly and severely criticized […] it is still portrayed as a fundamental biological principle in current textbooks (e.g., Nelson & Cox, 2000)" (pp. 430-431). Additionally in 1990, a study of college-level biology textbook found that the underlying progression of protein synthesis from DNA to RNA to protein remains virtually unchanged in textbooks from 1960s through the subsequent thirty years (Gaster, 1990); although the term *central dogma* has been replaced by *protein synthesis*.

To appreciate the central importance of protein synthesis, consider the cellular resources devoted to this process. Protein synthesis can account for up to 90% of the chemical energy used by the cell for all biosynthetic reactions. The 20,000 ribosomes, 100,000 related protein factors and enzymes, and 200,000 tRNAs used in protein synthesis reactions in a typical bacterial cell can account for more than 35% of the cell's dry weight (Nelson and Cox, 2000).

Cellular structure and function, enzymatics, metabolic pathways, and signal transduction all require proteins and are reliant on protein synthesis. In 1977, Cody and Treagust described a biochemistry course in which amino acids, proteins, and enzymes were three of the six topics covered. Almost two decades later, Dods (1996) described a problem-based learning biochemistry course and identifies amino acids, proteins, enzymes, and hormones as four of the six topics covered by the course; and in 2005, Anderson, Mitchell, and Osgood compare the content of conventional and cooperative learning curricula, noting proteins and protein synthesis as elements of both. Thus, protein synthesis is not only an important process in the cell, but a topic that receives much attention in biochemistry courses.

Protein synthesis can be divided into several distinct processes, including DNA replication (the process of generating new copies of DNA molecules from the information encoded in another molecule of DNA), transcription (the process of generating RNA molecules from the information encoded in a molecule of DNA), and translation (the process of generating protein molecules from the information encoded in an RNA molecule). It would be possible to design research studies that examine students' understandings of each of these individual processes; however, I have chosen, for the

purposes of this study, to focus on the process of protein translation because it is an essential biochemical process of "central importance" (Nelson and Cox, 2000, p.1020), yet is "consistently cited as [one of] the most difficult components of biology to learn" (Rotbain *et al.*, 2006, p. 501). Moreover, there is little educational research regarding students' understandings of this topic. Therefore, I selected the topic of translation in order to address (a) the void in the research literature and (b) the biochemical importance of this metabolic pathway.

External representations of protein translation. In order to convey the structure and function of proteins and their associated biochemical pathways, including protein translation, graphic designers often rely on external representations.

> The complexity of proteins with thousands of atoms presents a challenge for the depiction of their structure. Several different types of representations are used to portray proteins, each with its own strengths and weaknesses. [These types include] space-filling models, ball-and-stick models, backbone models, and ribbon diagrams. (Berg, Tymoczko, & Stryer, 2007, p. 61)

The variety of external representations and the "range of symbolism" used to represent biochemical phenomena pose an obstacle to biochemistry students (Schönborn & Anderson, 2006). For example, Schönborn, Anderson, and Grayson (2002) asked 151 students, most of whom were second year biochemistry students, to explain a textbook diagram of immunoglobin G (IgG) protein. They assessed student understanding of the diagram and its content through a series of written probes. Subsequently, ten participants volunteered to be interviewed. The authors conclude that students displayed several difficulties in understanding the diagram. They note that a possible source of student difficulties "is the fact that biochemistry textbooks often use more than one convention to represent a single structural feature of a molecule […]" (Schönborn, Anderson, &

Grayson, 2002, p. 96). They observe that the diagram of IgG they showed to students used a black line to represent a disulfide bond, whereas, other diagrams use a yellow line or –S–S– to represent a disulfide bond. In addition to representing the disulfide bond, the authors note that straight black lines were also used to represent the polypeptide chains of IgG. They conclude that one of the factors contributing to student difficulties in understand the external representation was students' inability to decipher the symbols used to depict the structural features of the IgG protein.

While some studies have explored students' understanding of external representations of protein structure, I have not been able to find any study that looks at students' understanding of external representations of protein translation. Even though protein translation is an important foundational biochemical concept, much of the educational research in the area of molecular life science has focused on genetics (Tibell & Rundgren, 2010). Subsequently, students' understanding of the components and processes of protein translation have not been explored in much detail in the current life science education literature. It should also be noted that a 2005 study by McClean and colleagues did explore the effectiveness of animations of molecular and cellular processes including, but not limited to, translation. They conclude that "animation improves student learning" (p. 177) yet they make no mention of specific student understanding of translation or the animation of translation. Thus, due to the lack of research on this topic and the biochemical importance of protein translation, I have chosen to explore how students' understanding of translation is influenced by external representations of this metabolic pathway.

CHAPTER 3

VARIATION THEORY

Theoretical Framework

> A theoretical framework is a system of ideas, aims, goals, theories, and assumptions about knowledge; about how research should be carried out; and about how research should be reported that influences what kind of experiments can be carried out and the type of data that result from these experiments […] Because a theoretical framework has great influence on the design, data collection, and data analysis of qualitative studies, each qualitative researcher must make explicit the framework he or she has chosen for a particular study. (Orgill & Bodner, 2007, p vii)

In heeding the advice of Orgill and Bodner (2007), I have chosen variation theory as my theoretical framework for this research project. Variation theory follows from the phenomenographic research tradition (Runesson, 2005). Phenomenography grew out a series of empirical research studies conducted by a Swedish research group in the 1970s in an attempt to answer the questions "1.) What does it mean, that some people are better learners than others?; and 2.) Why are some people better at learning than others?" (Pang, 2003, p.146). Marton (1981) asserts that there are a limited number of qualitatively unique ways in which different people experience the same phenomenon. Thus, the objective of phenomenographic research is to identify the variation in experience a particular group of people has of a particular phenomenon (Stamouli & Huggard, 2007).

Variation theory, sometimes referred to as "new phenomenography," reflects a shift within the phenomenographic research tradition (Tan, 2009). Instead of identifying the range of variation in experience of a phenomenon, variation theory looks at *why* people experience a phenomenon differently in the first place, i.e., what causes the variation in perception of a phenomenon? For any given phenomenon, there are many different aspects to which an individual could pay attention. The individual's experience

with a given phenomenon depends on the particular set of aspects to which they attend. In order to experience a phenomenon in a particular way, an individual discerns and assigns meaning to certain aspects of that phenomenon. "The aspects of the phenomenon and the relationships between them that are discerned and simultaneously present in the individual's focal awareness define the individual's way of experiencing the phenomenon" (Marton & Booth, 1997, p.101).

How we experience a phenomenon depends on which aspects of the phenomenon are held in our focal awareness. Cognitive research has shown that the human brain is only capable of processing a limited amount of information at any given time (Miller, 1956). Therefore, in experiencing a phenomenon, we are only able to attend to certain aspects of the phenomenon. Marton and Booth (1997) note that "[i]f we consider an individual at any instant, he or she is aware of […] certain aspects of reality focally while other things have receded to the background" (p.108). Thus, if we want people to experience a phenomenon in a particular way, we need them to attend to certain critical features of the phenomenon.

Aims of Variation Theory

To better understand what something IS, we need to be able to contrast it with what it IS NOT. Thus, in order to discern some aspect of a phenomenon, an individual must experience variation in that aspect. This experience of variation allows the learner to create meaning for the phenomenon. For example, when I was a child, I would help my mom pick out ripe fruits and vegetables at the grocery store. Each fruit and vegetable had its own characteristics that I was taught to use to judge whether or not a particular item was "ripe." Peaches were judged based on their smell. In order to discern the

"peachyness" of the smell, I had to experience the smell of unripened peach, a ripe peach, and an over-ripened peach. In experiencing variation in the smell of a peach, I was able to give meaning to the concept of a "ripe peach."

The aim of education is to facilitate students' learning, to develop students' capabilities in various situations, and to provide strategies to solve varying problems and respond appropriately to constraints within the educational environment (Bowden & Marton, 1998). The question remains, how do we as educators (as researchers, teachers, and/or instructional designers) facilitate students' experience with the instructional material in such a way as to allow them to perceive the appropriate and necessary characteristics of the material, i.e., the critical features? In order get students to focus on certain critical features of a given phenomenon, we need to draw their attention to those critical features. One way of drawing their attention is to vary those critical features. Thus, according to variation theory, in order to help students learn about a given concept in a specific way, we must allow the students to experience variation in certain critical features of that phenomenon.

Key Concepts of Variation Theory

Awareness, Discernment, and Simultaneity

Marton and Booth (1997) ask "what does it mean to experience something in a certain way?" (p. 86). Because of limited cognitive capacity, we are unable to notice all aspects of an experience at all times (Miller, 1956). Instead, Gurwitsch (1964) notes that some aspects are brought in the foreground of our awareness while other aspects recede into the background. The particular features brought into *focal awareness* form the basis of the subsequent construction of knowledge for that experience (Marton & Booth, 1997).

"[Q]ualitatively different ways of experiencing something can be understood in terms of differences in the structure and organization of awareness at a particular moment" (Marton & Booth, 1997, p. 100). The educational challenge lies in directing students to focus their awareness on those aspects deemed critical for experiencing something in a manner that promotes learning.

In order for an individual to be aware of certain aspects of a phenomenon, those aspects must first be discerned from their environment. "To experience something is to discern parts and the whole, aspects and relations" (Bowden & Marton, 1998, p. 33). *Discernment* is, therefore, the ability to hold an aspect in focal awareness and contrast it with its environment. By discerning a particular aspect of a phenomenon, one is acknowledging the existence of the environment and the experienced variation between the aspect and its environment (Marton & Booth, 1997). Thus, "variation is a necessary condition for effective discernment" (Bowden & Marton, 1998, p. 35). This experience of variation between the aspect and its environment allows for the perception of that aspect. It should be noted that Marton and Tsui (2004) make a clear distinction between "discernment and being told" (p. 10). The context of experienced variation holds a great deal of information. *Discernment* is a product of direct experience while *being told* is non-contextualized and, therefore, lacks a great deal of meaning and significance.

It is not enough for a learner to be aware of and discern a single feature of a given phenomenon. In order to truly understand a phenomenon, learners must be simultaneously aware of multiple features of the phenomenon and able to discern the phenomenon from its environment. In other words, in developing an understanding of a concept, it is not enough to be aware of individual features of a given phenomenon at

discrete moments in time. Learners must be *simultaneously* aware of multiple critical features of a concept.

Based on experienced variation and prior knowledge, several aspects of a given phenomenon may be discerned (Marton and Tsui, 2004). However, cognitive load limits our ability for simultaneous focal awareness. Thus, "the aspects of the phenomenon and the relationships between them that are discerned and simultaneously present in the individual's focal awareness define the individual's way of experiencing the phenomenon" (Marton and Booth, 1997, p. 101). As such, two individuals who experience the same phenomenon may come to understand that phenomenon differently if they are aware of and simultaneously discern different aspects of that phenomenon. In the classroom, two students may experience the same lesson yet come to different understandings of a given concept.

The Object of Learning

The act and/or pursuit of learning implies that there is something to be learned. Marton and Booth (1997) describe this something as the *object of learning*, i.e., what is to be learned by the student. The object of learning is the central focus of variation theory (Runesson, 2005). As the learning process involves both the teacher and the student and the research process involves a researcher, we can follow the object of learning from the teacher to the student as described by the researcher. Variation theory examines the object of learning from three different perspectives, each of which will be described below: the *intended object of learning* (the teacher), the *enacted object of learning* (the researcher), and the *lived object of learning* (the student).

The intended object of learning. The teacher's perspective of the object of learning is referred to as the *intended object of learning* (see Figure 7). In developing curricular and instructional materials, the teacher (note an inclusive use of the word teacher to encapsulate classroom instructors, curriculum designers, textbook publishers, and graphic designers) *intends* for students to learn particular concepts in particular ways (Marton & Tsui, 2004). This intention manifests in the selection, organization, and preparation of curricular and instructional materials.

The enacted object of learning. The act of learning, however, is not defined solely by the teachers' intentions. Instead, it is co-constructed though the interaction between the student and the teacher (see Figure 7) (Runesson, 2005). The *enacted object of learning* constitutes what is actually presented to students and constrains the possibilities of experience and, subsequently, learning. For example, a math teacher might intend for students to learn about fractions, but if students are only ever presented with problems containing whole numbers, then they never experience fractions and, subsequently, cannot learn about them. It should be noted that "no conditions of learning ever cause learning. They only make it possible for learners to learn certain things" (Marton & Tsui, 2004, pp. 22-23). Thus, the possibility of learning created by the enacted object of learning constitutes a *space of learning* for students (Marton & Tsui, 2004).

Most commonly, the space of learning is described in the context of a classroom (Marton & Tsui, 2004). However, I propose that the learning environment created by any forum (i.e., classroom, textbook, external representation) has the potential to comprise the enacted object of learning. For example, as a conduit for the intended object of learning and an interface for presenting what is possible for students to learn, an external

representation constitutes a powerful space of learning. In Chapter 2, I have discussed how external representations can be beneficial for learning. As a forum for promoting learning, the representation can be seen to enact the possibilities of learning, and, therefore, can be defined as the enacted object of learning.

The lived object of learning. Lastly, the students' perspective of the object of learning is referred to in variation theory as the *lived object of learning* (Figure 7). Marton and Tsui (2004) identify the lived object of learning as "the way students see, understand, and make sense of the object of learning when the lesson ends and beyond" (p. 22). Students' perception of the enacted object of learning provides the basis from which students construct meaning of their experience. The lived object of learning is often the focus of educational research, i.e., what did students actually learn?

The lived object of learning is informed not only by the enacted object of learning but also by students' prior knowledge and experiences. Based on the extensive literature on expert/novice differences and the influence of prior knowledge on learning outcomes (see Chapter 2), I have distinguished between a *prior lived object of learning* and a *post lived object of learning*. I will use the term *prior* to refer to students' understanding of the object of learning before they have been exposed to a particular enacted object of learning. I will use the term *post* to refer to students' knowledge of the object of learning as expressed during and after exposure to the enacted object of learning. The prior lived object of learning comprises any prior knowledge of and experience with the object of learning. The prior lived object of learning may serve to inform students' discernment of critical features of the enacted object of learning. In the absence of a robust prior lived

object of learning, students may rely more heavily on variation within the enacted object of learning to construct their post lived object of learning.

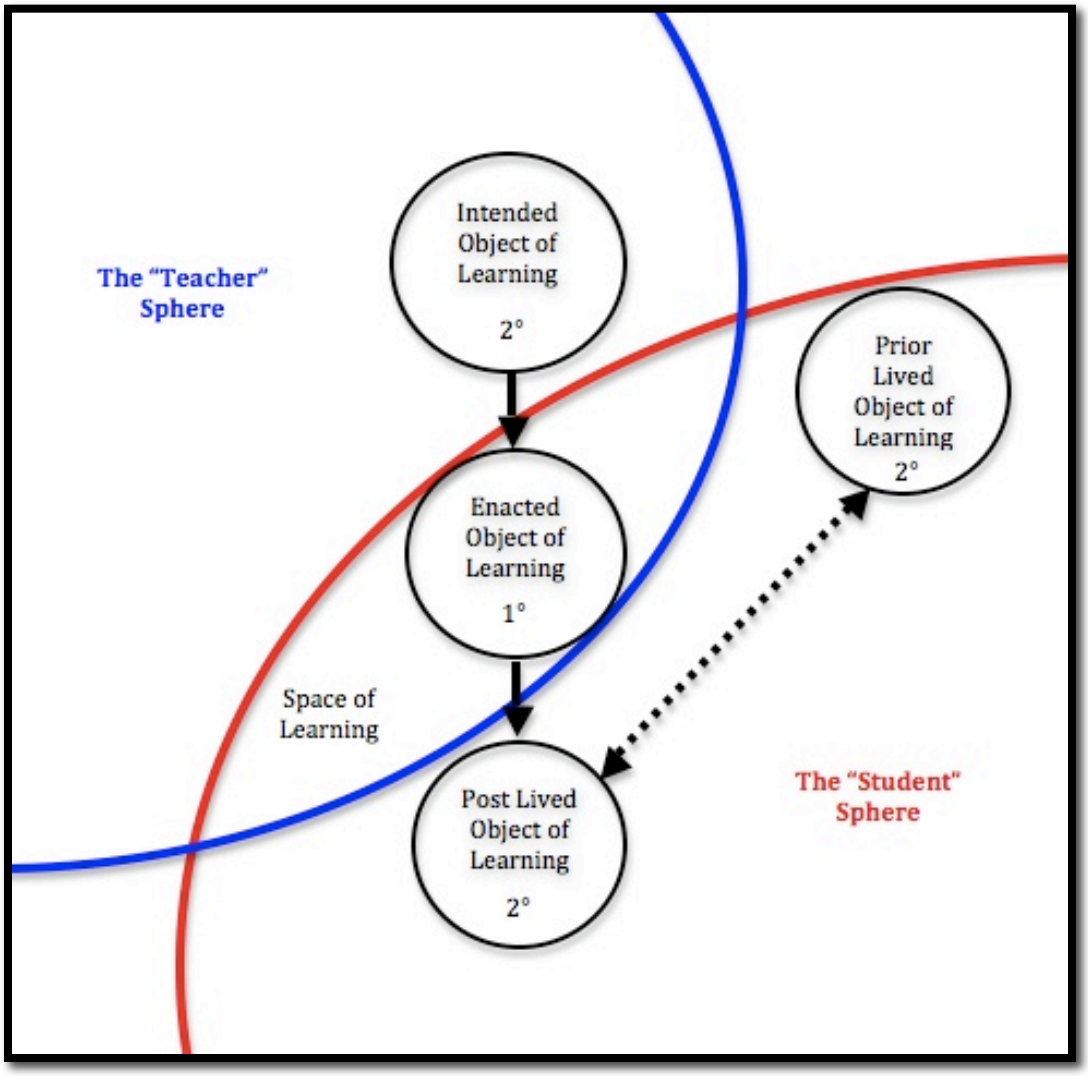


Figure 7. The objects of learning within variation theory. Note: this representation of variation theory has been modified from the model proposed by Rundgren and Tibell (2009, p. 230).

Marton and Tsui (2004) describe the lived object of learning as what students learn about some object of learning. This discussion of what has been learned by students aligns with what I have termed the post lived object of learning. However, the body of

literature on the effects of prior knowledge on the learning process suggests that it is essential to account for students' previous experience(s) with the object of learning of interest. Thus, I am interested in not only students' post lived objects of learning but also in their prior lived objects of learning.

Critical Features

From an educational perspective, the challenge becomes aligning the perceived features stimuli with the conceptually relevant and important information vital for learning. Considering the previous discussion of external representations (see Chapter 2), the perception of variation within the salient features of the representation allows students to attend to and perceive the representation in a particular way. However, the perceived features of an external representation may not correspond to the critical features necessary to promote conceptual development of a particular concept.

Marton and Tsui (2004) describe *critical features* as those necessary to distinguish one way of thinking from another; however, the term is used more often to describe the "features and conditions necessary for learning" (e.g., Rundgren & Tibell, 2009, p. 229). I will be using the term *critical features* to describe the aspects of a given phenomenon as determined by the instructor(s) that students must perceive in order to develop a scientifically accurate conception. If we, as educators, want students to understand a specific phenomenon in a particular way, then they must experience the critical features of that phenomenon in a certain way.

Perception and Variation

We are all exposed to a constant barrage of stimuli from the world around us. The ability to perceive a selected stimulus from the onslaught of others is a vital component

of learning and, more broadly, survival. Gerow and Bordens (2000) define perception as "the cognitive process of selecting, organizing and interpreting stimuli" (p. 89). With constant exposure to stimuli, our sensory systems have evolved such that we do not perceive the constancy of baseline stimuli. Researchers have found that if a subject is exposed to a new stimulus, such as a sound, that stimulus will be perceived at first; however, with constant exposure to that stimulus over time, the subject no longer attends to and perceives that stimulus (Gerow & Bordens, 2000).

"Typically, we select a few details to which we attend. A detail that captures our attention is called a salient detail" (Gerow & Bordens, 2000, p. 119). The question then becomes, which details are salient? If constant stimuli result in inattention, it stands to reason that variation of stimuli would draw attention. If a stimulus is varied, the subject will remain perceptually aware of it. Therefore, variation allows us to perceive some stimuli as important and requiring of our attention.

Significant Patterns of Variation

"According to variation theory, a phenomenon and/or its critical features are made visible in a teaching context through variation" (Orgill, 2012, p. 3392). *Contrast*, *generalization*, *separation*, and *fusion* have been defined by Marton and colleagues (e.g., Marton & Pang, 2006; Marton & Tsui, 2004) as four significant patterns of variation (Guo, Pang, Yang, and Ding, 2012; Orgill, 2012).

Contrast allows the individual to compare an object of learning or a feature of that object with something it is not. This allows the individual to create meaning for an object or feature by defining it against things that are different from it. *Generalization* allows the individual to compare similar instances of the object of learning. "To fully understand

an object of learning, the learner must experience many other examples to generalize the meaning" (Guo *et al*, 2012, p. 255). Generalizing provides learners experiences that allow them to distinguish between essential and irrelevant features. *Separation* allows the individual to discern one feature of an object of learning from other features by varying only the feature of interest while holding all other features constant. This allows the individual to experience and construct meaning for a particular feature of the object of learning, critical or otherwise, independent of other features. In this way, each part or features is separated from the whole. Lastly, *fusion* allows the individual to discern variation in several features of an object of learning simultaneously. The experience of multiply varied features facilitates the discernment of relationships between the features of an object of learning. Each part is fused together to create the whole.

Methods and Assumptions of Variation Theory

> People live in a world which they—and not only the researchers—experience. They are affected by what affects them, and not by what affects the researchers. What this boils down to […] is taking the experiences of people seriously and exploring the physical, the social, and the cultural world they experience. (Marton & Booth, 1997, p. 13)

Variation theory has traditionally relied on classroom observations and focused on examining the enacted object of learning to explore the potential for student learning. However, for the purposes of this project, a broader research methodology is required. As informed by variation theory, data must be collected to answer three different questions:

1. What did the teacher intend for students to learn? (the *intended object of learning*),
2. What is possible for the students to learn? (the *enacted object of learning*), and
3. What did students actually learn? (the *lived object of learning*).

As such, one of the primary data sources for this project is participant interviews.

> [T]he only route we have into the learner's own experience is that experience itself as expressed in words or acts. We have to ask learners what their experiences are like, watch what they do, observe what they learn and what makes them learn, analyse what learning is for them. (Marton & Booth, 1997, p. 16)

The Intended Object of Learning

The intended object of learning "consists of the concepts and their features that the teacher […] aims to communicate" (Rundgren & Tibell, 2009, p. 229). As the intended object of learning is internalized within the teacher, a retelling of the teacher's perceptions of the object of learning offers insight into the intention behind curricular and instructional design. The intended object of learning is unique to the individual and can only be expressed as "a second-order description, a description of the phenomenon as experienced" and retold by the individual (Marton & Booth, 1997, p. 163). A second-order perspective means that the information received by the researcher is expressed by another party (in this case, the teacher) instead of being observed directly by the researcher. Thus, instructor interviews were used to assess the intended object of learning.

Enacted Object of Learning

The enacted object of learning is described from a first-order perspective (Marton & Booth, 1997). "It is described by the researcher from the point of view of what is afforded to the learners" (Runesson, 2005, p.70). Researcher observations of the enacted object of learning, often the classroom, are often the primary (and sometimes only) source of data in variation theory literature (e.g., Runesson, 1999). This enactment of the object of learning is often captured as audio and video data (e.g., Ingerman, Linder, & Marshall, 2009).

In the current study, I gave students external representations of protein translation. This learning event did not occur in a classroom context, but during a research interview. Thus, it was not a classroom experience, but the external representations themselves that created possibilities for learning about protein translation. For this reason and for the purposes of this study, I defined the enacted object of learning as the possibilities of learning afforded by the external representations shown to the students.

In typical studies informed by variation theory, the enacted object of learning is described in terms of classroom events and materials that created possibilities for learning in that context. In the spirit of these studies, I, as the researcher, conducted a detailed analysis of each representation of protein translation shown to the students and the possibilities for student learning created by those representations (see Chapter 6).

The Lived Object of Learning

The lived object of learning describes what students actually learn about a given object of learning, as opposed to what their instructor intends for them to learn or what the learning environment makes possible for them to learn about that object of learning. Similar to the intended object of learning, the lived object of learning is unique to the individual and can only be expressed as a second-order description. This individual retelling of experience may come in the form of individual interview, written artifacts, or group discussions (e.g., Rundgren & Tibell, 2009).

Variation theory traditionally describes the lived object of learning as what students *have learned*. However, considering the literature on prior knowledge and the discussion of the lived object of learning in the variation theory literature, I have designated two constructs of the lived object of learning: (a) the prior lived object of

learning and (b) the post lived object of learning. Prior knowledge (the prior lived object of learning) is often assessed via a pre-test (e.g., Cook *et al.*, 2008), card-sorting tasks (e.g., Lowe, 1996), or interview prior to the experience (e.g., Schönborn & Anderson, 2009). The post lived object of learning describes the student's experience of a particular phenomenon in relation to (and as a result of) their experience of that phenomenon.

For the purposes of this project, the prior and post lived objects of learning were assessed via student interviews (see Chapter 4). The three-phase single interview technique (3P-SIT) was selected because it was designed to elicit students' prior knowledge of a topic and their reasoning with and evaluation of an external representation of the topic of interest (Schönborn & Anderson, 2009; Schönborn, Anderson, & Mnguni, 2007). Thus, student interviews were used to address both prior and post lived objects of learning with respect to selected external representations of protein translation.

Data Analysis

Data analysis within variation theory centers on the identification and tracking of critical features either across or within objects of learning. Within the intended object of learning, teachers may address several critical features of an object of learning. The teacher perspective as to what constitutes a critical feature is an important insight into an expert line of thinking. As one of the intentions of education is to progress students along the expert/novice continuum, if students can discern and be aware of the critical features identified by the teachers, then perhaps they are more likely to develop expert-like conceptual knowledge of the object of learning.

Similarly, students' identification and use of critical features offers a means of comparison between the intended object of learning and the lived object of learning (both prior and post). Additionally, assessment of students' prior and post lived objects of learning offers a measure of learning from the experienced phenomenon. Examination of the enacted object of learning (i.e., the external representation) offers a comparison or measure of alignment between the instructors' intentions for student learning.

Rationalization for Using Variation Theory in this Project

Schönborn and Anderson (2006) suggest that rigorous investigation of how external representations compare to student mental models, i.e., their internal conceptions, will allow us to identify the advantages and disadvantages of the various modes of external representations. "If we can do this, we will be in a better position to suggest what, when, why, and how a particular external representation should be used to achieving desired learning outcomes" (p. 97). Variation theory provides a uniquely well-suited framework from which to investigate external representations in biochemistry.

Variation theory has been identified as "a potentially powerful framework for examining student learning of chemistry and improving chemistry teaching and learning" (Bussey, Orgill, & Crippen, 2013, p. 16). Previous studies have used variation theory to examine K-12 teachers' professional development "learning studies" (Marton & Tsui, 2004; Orgill, 2012; Pang & Marton, 2003), as well as students' understandings of chemistry (Park, Light, Swarat, & Denise, 2009), biochemistry (Rundgren & Tibell, 2009), physics (Hekkenberg, 2012; Ingerman *et al.*, 2009; Linder, Fraser, & Pang, 2006; Ling, Chik, & Pang, 2006), nanoscience (Swarat, Light, Park, & Drane, 2011), mathematics (Mok, 2009; Runesson, 1999, 2005), computer science (Suhonen,

Thompson, Davies, & Kinshuk, 2008), economics (Pang & Marton, 2003), and educational policy (Tan, 2009).

Using variation theory, I was able to investigate a single object of learning (protein translation) from three different perspectives, i.e., those of the teacher, the student, and the researcher. From the teacher's perspective, I was able to identify both the expert mental model of protein translation as well as their understanding of what and how information should be presented to students in order to promote learning. Using this information, I was able to identify some of the advantages and disadvantages of the various external representations under investigation in this project. From the students' perspective, I was able to explore both the naïve—or more accurately, the intermediate or non-expert—mental models of protein translation as well as their understandings of what and how information is being presented via the various external representations. As students were asked to express their understanding of translation prior to and following exposure to a set of external representations, any change in student understanding was attributed to the representation.

I, as the researcher, was able to identify the features of the representation(s) that allowed students to revise their understanding of translation in a way that more closely aligns with the expert (teacher's) conception of translation. To this end, the identification and subsequent investigation of the space of learning created by the students' exposure to various external representations of translation (the enacted object of learning) allowed me to describe the critical features necessary to understand protein translation in a scientifically accurate manner (as outlined by the intended object of learning). Variation theory allows for the triangulation of the teacher, student, and research perspectives. In

this way, variation is uniquely well suited to address issues of representational and conceptual alignment in the identification of *good* biochemical representations as called for by Schönborn and Anderson (2006).

Modifications to Variation Theory in the Project

In applying the theoretical framework of variation theory to the investigation of external representations of biochemistry, specifically protein translation, I have proposed two substantive modifications to variation theory. The first modification pertains to the enacted object of learning. Variation theory has traditionally explored the enacted object of learning in the context of the classroom (e.g., Runesson, 2005). However, the prevalent use of external representations in science education, and specifically in biochemistry, warrants the expanded use of variation theory to explore the space of learning constituted by external representations.

Just as the classroom environment creates the possibility for student learning to occur, so, too, does the external representation. Certain selected information is presented to students via an external representation in the same manner a teacher would select and present information to students in a classroom. Students' ability to decode and make meaning of the information depicted in an external representation establishes what is possible for students to learn in that context. Considering the enacted object of learning to be the context within which the possibility for student learning is defined, an external representation can clearly meet this standard.

The second modification pertains to the lived object of learning. As discussed previously, there is a large literature base that has identified the importance of prior knowledge in students' ability to understand and use external representation is science

(e.g., Cook *et al.*, 2008). Previously stated versions of variation theory contained no provision to assess students' prior knowledge. Students' understanding of the object of learning has previously only been assessed following student exposure to the enacted object of learning. However, I would assert that this post-exposure measure of students' lived object of learning is not sufficient to distinguish between students' *prior* knowledge of the object of learning and their *post* lived object of learning.

As my particular research interests pertain to identifying the possibilities for students learning created by external representations of protein translation, I needed to be able to distinguish between students' prior knowledge and the learning that has occurred because of students' experience of the external representation. To do so, I assessed students' prior lived object of learning, i.e., students' prior knowledge before they were exposed to the external representation, and students' post lived object of learning, i.e., students' conceptual understanding after they were exposed to the external representation. In addressing students' lived object of learning at two points in time, I was better able to differentiate between the effects of prior knowledge and the enacted object of learning.

CHAPTER 4

METHODOLOGY

Research Questions

I examined the following research questions in this study:

1.) What do instructors of second semester biochemistry students intend for their students to learn from external representations of translation, i.e., what is the *intended object of learning*?

2.) What is possible for second semester biochemistry students to learn from external representations of translation, i.e., what is the *enacted object of learning*?

3.) What do second semester biochemistry students learn from external representations of translation, i.e., what is the change from the *prior lived object of learning* to the *post lived object of learning*?

Research Design

The object of learning for this project is the biochemical process of protein translation. Specifically, I am interested in what second semester biochemistry students can learn from external representations of translation. Consistent with the principles of variation theory and research on prior knowledge, I have investigated four separate objects of learning: 1.) the intended object of learning, 2.) the enacted object of learning, 3.) the prior lived object of learning, and 4.) the post lived object of learning.

In this project, I asked students to describe their understandings of translation before, during, and after they were shown a series of external representations (static figures and/or animations) in order to assess their prior lived object of learning of translation as well as their post lived object of learning as informed by the space of

learning created by the sequential exposure of randomly assigned pairs of external representations. For the purposes of this project, *prior* refers to students' knowledge of translation as expressed before students have been exposed to the external representations of interest, i.e., students' prior knowledge of translation. *Post* refers to students' knowledge of translation as expressed during and after exposure to the external representations of interest.

Because students' construction of knowledge is influenced both by a learning event (in this case, being exposed to particular external representations of protein translation) and their prior knowledge, I felt that it was essential to determine what students knew about protein translation before being exposed to a particular series of external representations during the student interviews. All student participants had been previously exposed to the concept of protein translation in high school biology, college-level biology, and college-level biochemistry courses. Thus, all student participants had some level of prior knowledge of the concept of protein translation. In order for me to determine if students learned from their experience with the external representations, I first needed to account for what they already knew about protein translation before being exposed to those representations.

What do I mean when I say that students *learned* from their experiences with the external representations, though? Marton, Beaty, and Dall'Alba (1993) have identified six conceptions of learning. They describe learning as 1.) increasing one's knowledge, 2.) memorizing and reproducing, 3.) applying, 4.) understanding, 5.) seeing something in a different way, and 6.) changing as a person. Within the context of this project, I will use the term *learning* to refer to seeing something in a different way. This means that

although students may have already had prior knowledge pertaining to an object of learning (in this case protein translation), if their experience with the enacted object of learning (in this case, external representations of protein translation) allows them to see the object of learning in a different way, students are learning. Thus, while students may already *know* about protein translation, their ability to recall the details about this object of learning, as described by their instructors, may be limited and cursory. If the external representations of protein translation allow students to see translation in a different way (hopefully in a more biochemically accurate way), students are *learning*. Specifically, I will measure learning as a progression of stated or depicted knowledge towards a more scientifically accurate understanding of protein translation.

The methods I used to identify the various objects of learning and to answer the research questions posed at the beginning of this chapter will be detailed in the sections that follow.

The Intended Object of Learning

Participants. The intended object of learning is defined by what teachers (in the broad sense of the word) intend for students to learn about a designated object of learning. As such, data on teacher intentions for student learning of protein translation was obtained from interviews with five biochemistry instructors from two universities. Two instructors were interviewed from a large Midwestern university, and three instructors were interview from a large Southwestern university. I have defined a biochemistry instructor to be a university faculty member who has taught at least one introductory biochemistry course.

I selected the two universities from which instructors were interviewed for three reasons: 1.) access to participants, 2.) institutional variance, and 3.) textbook alignment. While access was a notable consideration in selecting research participants, institutional variance and textbook alignment provided good measures of validity to the subsequent findings from the instructor interviews in this project. In order to identify whether a particular instructor perception was institutionally specific or more broadly shared by experts in the field, I conducted an analysis of instructor responses from each institution. Additionally, the static external representations I used in this project have been selected from the textbooks used by each university—Berg *et al.* (2007) at the Southwestern university and Nelson and Cox (2008) at the Midwestern university—for their Biochemistry II courses. Although this was not the rationale for choosing the representations (see Intended Object of Learning – External Representations in Chapter 4), the instructor familiarity with the texts provided insight into the educational and instructional value of the representations.

I used purposeful sampling to select the specific instructor participants for my study. Purposeful sampling, Creswell (2007) argues "can purposefully inform an understanding of the research problem and central phenomenon in the study" (p. 125). In this study, the central phenomenon under study was protein translation. As such, the instructors interviewed for this project all held a Ph.D. in a field related to biochemistry and had taught a college-level biochemistry course that had addressed the topic of protein translation. This was done to ensure that instructor participants had a consistent level of prior knowledge of the topic and sufficient pedagogical knowledge and experience in

order to identify what students should learn about protein translation (i.e., the intended object of learning).

This sample size of five instructors is consistent with other studies of teachers' intentions for student learning utilizing variation theory (e.g., Pang & Marton, 2003; Runesson, 1999). However, additional instructor interviews were considered until it was determined that the sample size allowed for the collected data to reach *saturation*. Saturation is defined as the point at which no new themes emerge from a data set.

> Using the constant comparative approach, the researcher attempts to "saturate" the categories – to look for instances that represent the category and to continue looking (and interviewing) until the new information obtained does not further provide insight into the category. (Creswell, 2007, p. 160)

External representations. In order to identify the intended object of learning in this study (i.e., to answer the first research question), I interviewed instructors about their understandings of protein translation, their conceptions of what students should learn about protein translation, and their perceptions of specific external representations used to teach protein translation. In this section, I will describe the external representations I used for the instructor interviews (and, later, for the student interviews), as well as my reasons for choosing these specific external representations.

Basic criteria for external representations used in this study. External representations of protein translation and the students' interactions with those external representations constitute the enacted object of learning for this study. Because there are many individual representations and types of representations that have been used to portray the process of protein translation, I needed to set criteria by which I would choose the external representations used in this study. All representations were selected from common sources. For the static representations, the top two-bestselling biochemistry

textbooks were used from which to select a representation. For the animations, published or referenced web resources were used from which to select a representation. All external representations that were selected for use in this project depicted an mRNA strand, a ribosome, and a peptide chain because Nelson and Cox (2000) identified these components as essential components of translation. Additionally, all representations depicted at least the elongation stage of translation. Although translation is divided into four stages—1.) Activation, 2.) Initiation, 3.) Elongation, and 4.) Termination (Nelson & Cox, 2000)—I was unable to identify any representation that depicted all four stages. As such, I decided to focus on representations that primarily depict aspects of the elongation stage as it is during this stage that the vast majority of the mRNA sequence is *translated* into the polypeptide sequence by the ribosome (Nelson & Cox, 2000).

Representational mode. In addition to the more basic criteria mentioned in the previous section, I also wanted to take into consideration the mode of the external representations used in this study because existing research has shown that representational mode can affect students' ability to interpret and understand external representations. As described previously, visual representations can be either static (such as images in a textbook) or dynamic (such as an animation).

Schönborn and Anderson (2006) posit that "an animated [external representation] mode might be more useful for teaching about dynamic metabolic reaction than a static one would be" (p. 96). However, they go on to note that Lowe (2003) found that "dynamic […external representations] are not always superior to static [external representations]" because students may experience cognitive overload due to "the greater amount of information that has to be processed than in the case of a static external

representations" and may become "distracted by its highly dynamic and esthetic appearance" (Schönborn & Anderson, 2006, p. 97). Because of the potential influence of representational mode on student learning from external representations, I wanted to make sure I included both static and dynamic images in my study.

Level of abstraction. The level of abstraction has also been identified as an important characteristic that affects student learning from external representations (Schönborn & Anderson, 2006). By level of abstraction, I am referring to how realistic or stylized a representation is. Dwyer (1969) found that varying the amount of "realistic detail" in an external representation affected student understanding. He notes that it is possible that the realistic external representations "distracted students from the relevant learning cues and thereby hindered their acquisition of the intended information" (Dwyer, 1969, p. 152). Again, based on this suggestion that the level of abstraction influences student learning form external representations, I wanted to include in this project representations that varied in the amount of realistic detail they portrayed.

Selection of external representations. Using the criteria described in the previous section, I chose three sets of external representations: two static illustrations, two stylized (low level of realistic detail) animations and two realistic animations to use in my study. In this section I will provide descriptions of the selected representations used in my study as well as my rationale for choosing each representation.

In the first stage of this project, I asked biochemistry instructors to evaluate three sets of external representations. Each set consisted of two external representations of a given form, i.e., two static illustrations, two stylized animations, or two realistic animations. I define a static external representation, such as a textbook illustration, as a

representation in which the representational components (the images and other visual cues such as color, highlighting and text) do not move. The selected static external representations contain dynamic indications, i.e., arrows or multiple frames indicating a time lapse; however, there is no physical movement of the image. In contrast, a dynamic external representation, such as an animation, is one in which the representational components do move.

I further distinguish a stylized external representation as one in which the representational components are highly simplified. In the context of protein translation, the term *stylized* is applied along a continuum to describe representations bearing low or no correlation to the molecular architecture of the transcriptional machinery, components, and environment. In contrast, the term *realistic* is applied to external representations in which representational components seem to replicate the natural state and condition of the object or phenomenon. In the context of protein translation, the term *realistic* is applied along a continuum to describe representations bearing a relatively high correlation to the molecular architecture of the transcriptional machinery, components, and environment.

While the terms *static* and *dynamic* can be applied in a relatively objective manner (either the components of the representation are moving or they are not as deemed by the researcher), the terms *stylized* and *realistic* are inherently subjective, as they require interpretation by the researcher to assign the degree of realism. It is for this reason that I have allied my use of the terms stylized and realistic with representational indications of molecular structure (such as ball-and-stick or space filling models): the

more indications of molecular structure and interactions, the higher the degree of realism within the representation.

With these distinctions in mind, instructors were asked to respond to representational sets containing two external representations of each type. Pairing two of the same type of representations together allowed the instructors to compare and evaluate which representation best conveyed the concept of protein translation while controlling for the mode of presentation. By *best* I mean which representation would be considered the most ideal representation for promoting student understanding of protein translation from the perspective of the instructor. From the three sets of representations, the instructors were asked to choose the three best representations (one of each type), which were then shown to the student participants during the student interviews. The selection of the best representations was meant to provide student participants with the most ideal enacted object of learning such that the ability of the external representation to cue students to notice various aspects was optimized. As one of the research goals of this project is to describe what is possible for students to learn about translation, providing students with the best possible representations, theoretically, provided the most possibilities for student learning.

Static external representations of translation. I chose static external representations from the two bestselling biochemistry textbooks on amazon.com: *Lehninger: Principles of Biochemistry* by David Nelson and Michael Cox (2008) and *Biochemistry* by Jeremy Berg, John Tymoczko, and Lubert Stryer (2007). These static external representations were chosen to characterize common external representation found in textbooks. As the two-bestselling biochemistry textbooks on amazon.com, these

texts (and subsequently their external representations) have been purchased and most likely viewed by a large number of biochemistry students. This top-selling status is reflected in the use of these textbooks at many colleges and universities, including those universities from which I selected project participants (both instructors and students). Specifically, the student participants in this project had used the Berg *et al.* (2007) textbook in their biochemistry course. Therefore, I have assumed that the students participating in this project (see Lived Object of Learning – Participants in Chapter 4) have probably been exposed to these external representations or ones similar to them.

From each of these two textbooks, I selected an external representation depicting the elongation process of protein translation (see The Intended Object of Learning – Basic criteria for selecting external representations in this study, Chapter 4). I will note that I have not described the static external representations as stylized or realistic as the only static representations of the process of translation in these textbooks have little or no indication of molecular structure. In this way, it would be appropriate to define the chosen textbook illustrations as stylized, static external representations.

The first static external representation (Figure 8) I selected comes from Berg *et al*. (2007). The primary reason I selected External Representation #1 (ER1) was because it was the only illustration of protein translation in the Berg text that depicted all of the previously described components and steps. ER1 uses color to contrast different components, parts, and processes, which provides a distinct point of variation within the representation. ER1 also contains cueing phrases as labels, such as "peptide bond formation," which potentially provide greater meaning to the images of the

representation. Lastly, ER1 is highly simplified. This will be contrasted with the second static external representation (Figure 9).

External Representation #2 (ER2) was chosen from the Nelson and Cox (2008) textbook because it contains a composite series of representations that depicts the same series of steps as are depicted in ER1, providing a degree of continuity between ER1 and ER2. ER2 also employs color and cueing phrase labels, similar to ER1 but to a greater degree. However, in contrast to ER1, ER2 contains several additional representational components. For example, ER2 includes additional molecular components, such as elongation factors, not depicted in ER1. The depiction of these additional components could cause students to expand their descriptions of the process to include these components or may cause cognitive overload. ER2 also includes magnified views of the component interactions occurring in the transfer RNA binding sites, which provides a size contrast that is not present in ER1. ER2 uses arrow symbolism similar to ER1 to indicate stepwise interactions. However, ER2 is presented as three paneled steps as opposed to one panel that includes several steps, as in ER1. The organizational layout of ER2 may allow students to attend to each step separately or may cause confusion if students are unsure of how they should read the representation.

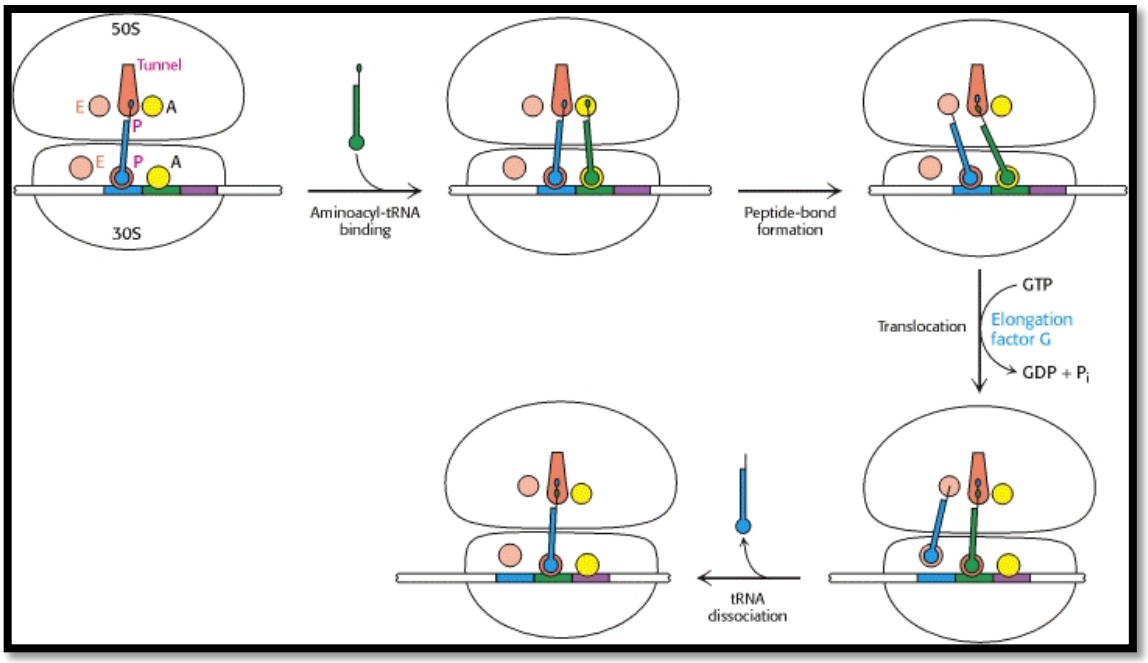


Figure 8. External Representation #1. Image taken from Berg *et al.* (2007, p. 872).

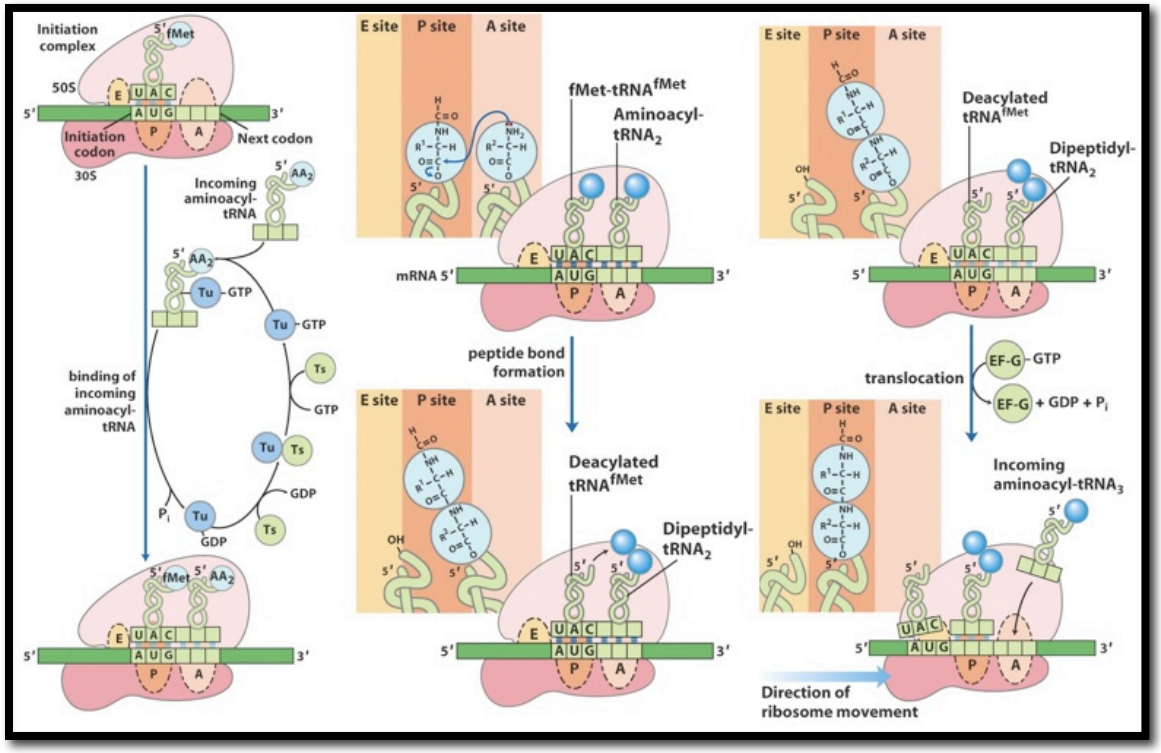


Figure 9. External Representation #2. Image compiled from Nelson and Cox (2008, pp. 1047, 1049).

Stylized, dynamic external representations of translation. In addition to the two static external representations, I selected four animations to typify dynamic external representations. I have characterized two animations as *stylized,* indicating that the shape of the components does not bear a significant correlation to the scientifically accepted molecular architecture of the components. I selected the first stylized dynamic external representation (Figure 10, http://www.biostudio.com/demo_freeman_protein_synthesis.htm) because it was produced by BioStudio, which is the company that has designed and developed science animations for the life science textbooks published by W.H. Freeman and Co. W. H. Freeman also happens to be the textbook publisher for *Biochemistry* (Berg *et al.*, 2007) and *Lehninger: Principles of Biochemistry* (Nelson & Cox, 2008). As such, this representation provides a notable comparison between instructor reactions to ER1 and ER2 to External Representation #3 (ER3). This allows for instructors to identify potential similarities in the representational design of ER1, ER 2, and ER3. As with ER1 and ER2, ER3 contains all of the previously identified translational components and steps. However, it should be noted that ER3, as with the other dynamic external representations, includes more components and steps than ER1 or ER2 because of the allowances of time and the dynamic nature of the representational mode. Because of limitations of the mode, static representations depict a single point in time or a series of discrete points in time in compilation whereas, animations allow for the ebb and flow of representational components, thereby depicting change over time and the dynamic nature of the relationships between component parts of the system.

The second stylized dynamic external representation (Figure 11, http://vcell.ndsu.nodak.edu/animations/translation/movie-flash.htm) was selected from the Virtual Cell Animation Collection from the Molecular and Cellular Biology Learning Center. Unlike ER3, External Representation #4 (ER4) was not designed as a supplemental material for a textbook. Instead, ER4 was designed specifically to introduce students to new content. Additionally, ER4 has been experimentally shown to improve student learning as compared to student learning without the animation (McClean *et al.,* 2005). ER4 may also be considered more aesthetically appealing than ER3 because of the increased quality of the computer graphics, i.e., the richer use of color and the 3-D-esque coloration of the components.

In addition to the difference in appearance between ER3 and ER4, there are other significant differences in the way protein translation is presented in the two representations. First, while the cellular components of protein translation are labeled in both representations, the labeling occurs at different points in the animation. In ER4, the components are labeled during an introductory sequence in which a definition of translation is also shown. In ER3, the components are labeled as the animation advances through the process of translation. Second, although both stylized animations present a highly simplified depiction of translation, they varied in the amount of information they presented. For example, ER3 contains more noticeable text cues, such as abbreviations for amino acids and mRNA base sequence information, than does ER4. These text cues may focus students' attention on the particular, labeled aspects of the protein translation process.

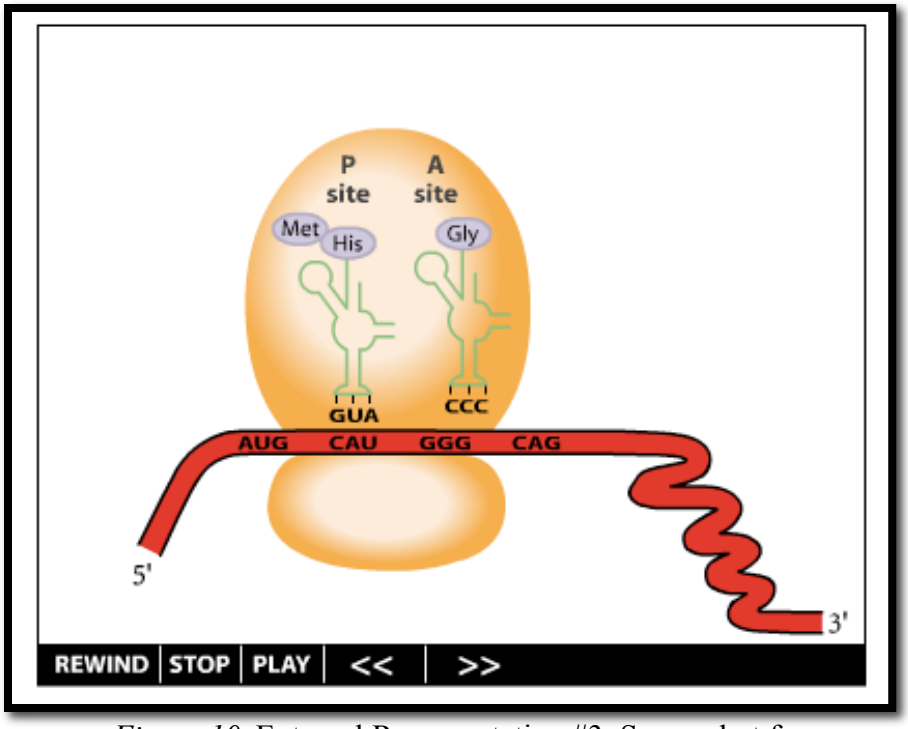


Figure 10. External Representation #3. Screenshot from http://www.biostudio.com/demo_freeman_protein_synthesis.htm.

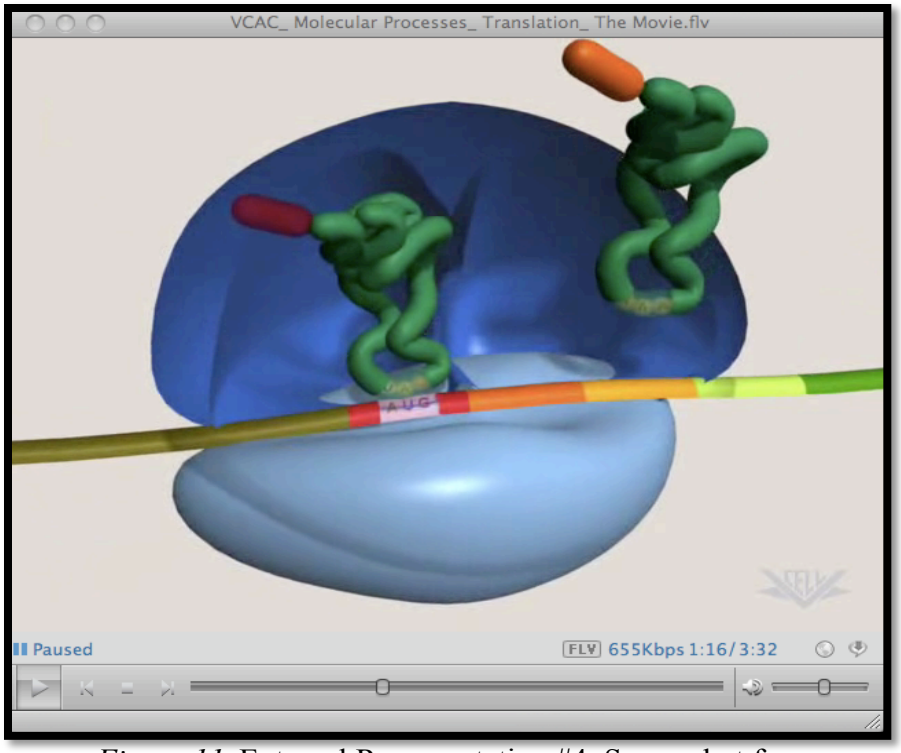


Figure 11. External Representation #4. Screenshot from http://vcell.ndsu.nodak.edu/animations/translation/movie-flash.htm.

Realistic, dynamic external representations of translation. I characterized the final two animations as *realistic*, indicating that the animation more closely depicts the molecular architecture of the components and interactions of the process (as compared to ER3 and ER4). I selected both realistic animations from MolecularMovies.org: A Portal to Cell & Molecular Animation (http://www.molecularmovies.com/), which is a web resource of molecular animations. These types of molecular animations have been described by Erik Olson, a reporter for the New York Times, as "a rapidly growing field that seeks to bring the power of cinema to biology … [by] building on decades of research and mountains of data [to recreate] in vivid detail the complex inner machinery of living cells" (Olson, 2010b). These animations were intentionally designed to depict the current scientific understanding of the molecular composition and structure of biochemical components and the interactions that occur during cellular processes such as protein translation. It is with these considerations that I characterized External Representation #5 (ER5) and External Representation #6 (ER6) as realistic as compared to ER3 and ER4.

ER5 (Figure 12, http://www.mrc-lmb.cam.ac.uk/ribo/homepage/movies/translation_bacterial.wmv) and ER6 (Figure 13, http://www.wehi.edu.au/education/wehitv/dna_central_dogma_part_2_-_translation/) both depict most of the same molecular components and process as ER3 and ER4. Similar to ER3 and ER4, ER5 and ER6 also present more steps and components than do ER1 or ER2. This increase in complexity from static to dynamic representations is well noted in the literature (e.g., Lowe, 2003). Although ER5 and ER6 are both more complex

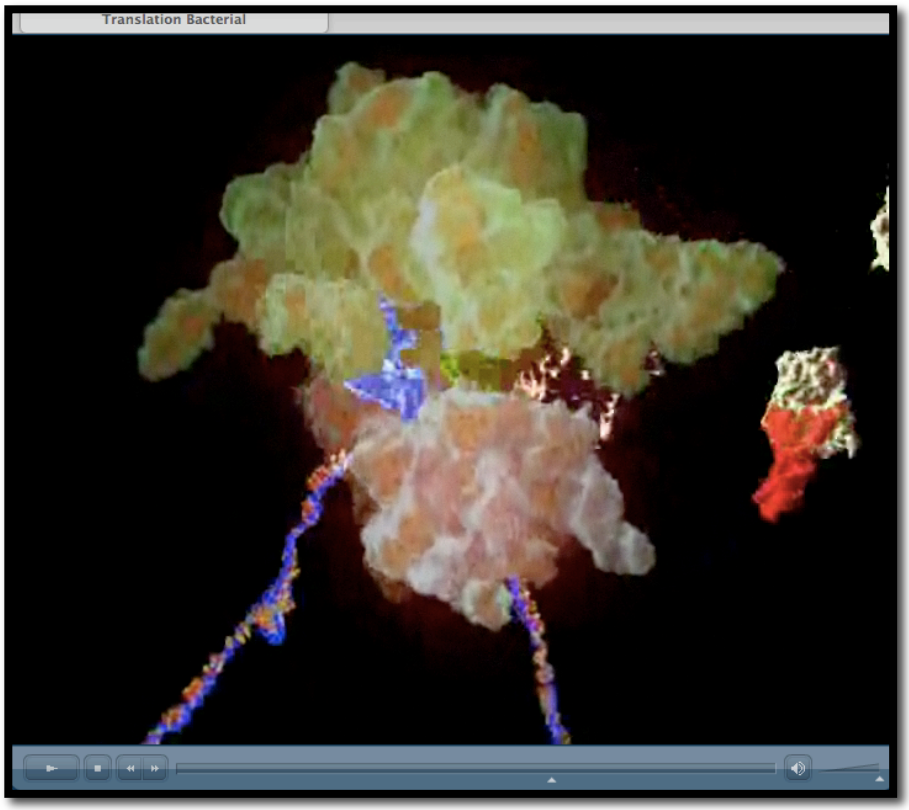


Figure 12. External Representation #5. Screenshot from http://www.mrc-lmb.cam.ac.uk/ribo/homepage/movies/translation_bacterial.wmv.

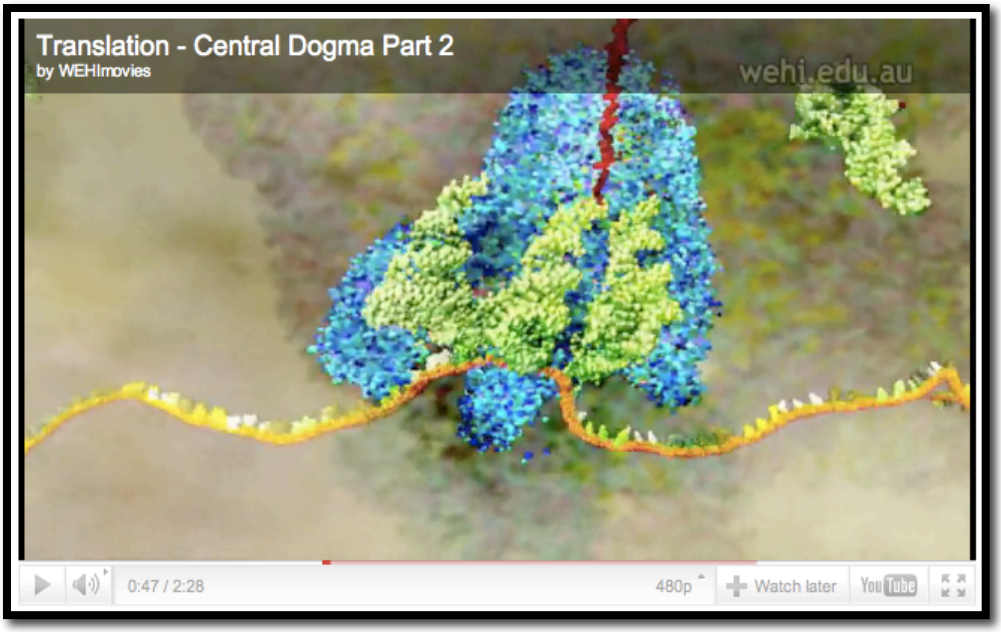


Figure 13. External Representation #6. Screenshot from http://www.wehi.edu.au/education/wehitv/dna_central_dogma_part_2_-_translation/.

than any of the other representations chosen for this study, ER5 is more simplistic than ER6 in that there are fewer components presented to the viewer in ER5 than in ER6. ER5 presents the translational components and interaction on a black background. This blank canvas presentation of translation is similar to ER1-ER4. ER6, however, depicts other features (out of focus) in the background, thereby illustrating the cellular context of translation. An additional notable difference between ER5 and ER6 is found in the labeling cues presented in ER5. ER5 uses text cues similar to ER1-E4 to label the components and steps. ER6 does not use any text cues, and was therefore used to assess whether instructors identify alphanumeric text labels as an important component of external representations.

All four dynamic external representations, ER3-ER6, were designed as multimedia external representations, meaning that they present information both visual and sound cues (Schönborn & Anderson, 2006). ER3-ER5 are presented with narration, each describing the components and processes depicted by the representations. ER6 is not narrated but does contain sounds indicating movement and component interactions. As the focus of this project is the visual literacy of the participants and the space of learning created by the visual external representations described above, ER3-ER6 were presented without auditory cues, i.e., narration and other presentation sounds were not presented to participants, so as to focus participant attention to the visual information and to eliminate potential complicating variables introduced by variations in narrated text and sound.

Instructor interview protocol. I identified instructors' intentions for learning from the external representations of protein translation through interviews. The semi-structured instructor interview protocol (see Appendix B: Instructor Interview Guide) was

adapted from Schönborn and Anderson's Three-Phase Single Interview Technique (3P-SIT) (Schönborn & Anderson, 2009; Schönborn *et al.*, 2007). The 3P-SIT model was chosen because it addresses both participant knowledge of the content (the object of learning) and their understanding external representations depicting the object of learning. During Phase 1 of 3P-SIT, Schönborn *et al.* (2007) describe using "free response probes to gather information about a participant's conceptual knowledge of a particular phenomenon of interest before exposure to any [external representation]" (p.293). The phenomenon in question is analogous to the object of learning, and the conceptual knowledge assessment before exposure establishes the extent of participants' prior knowledge of the object of learning.

Schönborn *et al.* (2007) go on to describe that

> [u]pon exposure of a [participant] to an [external representation] of interest, Phase 2 uses semi-structured probes to measure [the participant's] ability to reason with the [external representation] and with their own conceptual knowledge. In Phase 3, [participants] respond to semi-structured probes about the [external representation] of interest in order for the researcher to measure the effect of the actual mode of the representation on [participant] interpretation processes. (p.293)

While 3P-SIT was designed to "gather data on *students'* interpretation of [external representations]" (Schönborn *et al.*, 2007, p. 293), the instructor interview protocol for this project was intended to gather data on instructors' intended object of learning of translation, i.e., what do they understand about protein translation, and what do they believe students should know about this biochemical process? Subsequently, the key characteristics of protein translation and the representational features that depict those characteristics as identified by the instructors were noted as the critical features of the intended and enacted objects of learning of translation, respectively.

The instructor interview protocol was expanded from the original 3P-SIT model to include initial demographic questions followed by five phases of questions:

- Demographics
- Phase 1: The Intended Object of Learning of Translation
- Phase 2: Instructor Evaluation of the Static External Representations of Translation
- Phase 3: Instructor Evaluation of the Stylized, Dynamic External Representations of Translation
- Phase 4: Instructor Evaluation of the Realistic, Dynamic External Representations of Translation
- Phase 5: Instructor Perceptions of External Representations of Translation

General demographic questions, such as "tell me about your current research, or tell me about your teaching experience," allowed me to confirm whether or not the selected participants met the participant criteria defined above. Additionally, these questions allowed the interviewee to share information that is comfortable and familiar, thereby building rapport so that they could feel more comfortable sharing their thoughts and opinions later on in the interview.

The objective of Phase 1 was to establish what instructors think students *should* learn about translation, i.e., the intended object of learning. Note that the intended object of learning is defined completely within teacher sphere, i.e., from the instructors' perspectives (see Chapter 3, Figure 6). To this end, I asked the instructors to explain their understanding of protein translation and how they would or do go about explaining this process to students. Specifically, I asked them to identify the main idea(s) students should

come away with following instruction on this topic, i.e., the critical features of the content. Additionally, I asked the instructors to identify any potential problems students might encounter when trying to understand translation. Note that this line of questioning precedes the instructors' exposure to the external representations of translation. This was done to ensure that initial instructor responses were shaped solely by their prior content and pedagogical knowledge of protein translation. Any subsequent deviation in instructor responses following exposure to the external representation could then be attributed to the representations.

In Phase 2, I used semi-structured probes to allow instructors to evaluate the two static external representations of translation, i.e., ER1 and ER2 (Figure 8 and Figure 9, respectively). Considering both ER1 and ER2, I asked the instructors to choose which one they like best. By *best* I meant which of the two static representations would the instructor choose to use to promote student understanding of protein translation. Of the representation they did not choose, I asked the instructors to describe why they did not like it and what could be changed about the representation to make it better for promoting student understanding of protein translation. I also asked the instructors to describe what they think students could learn from and notice about that representation. I asked similar questions about the representation they did like. Furthermore, I asked them to consider what this representation would allow students to learn that would not be allowed by the other representation.

The instructors' choice of the best static representation had consequences for other parts of this study. Later on in this project, during the student interview protocol (see Appendix C: Student Interview Guide), I showed some students the best static

external representation as identified by the instructors. The static external representation of translation that was identified by the instructors as the best then defined a portion of the space of learning of the enacted object of learning of translation for those students. That means that the static representation selected as the best determined a particular range of possibilities of what students could and could not learn within that particular learning environment. Instructor evaluation of the static external representations also allowed me to identify the critical features of the enacted object of learning. These are the features instructor felt students should learn in order to develop a biochemical understanding of translation. Phases 3 and 4 utilized the same procedure as Phase 2 but with stylized (ER3 and ER4) and realistic (ER5 and ER6) dynamic external representations, respectively. I asked the instructors the same set of questions for each of these sets of external representations.

Finally in Phase 5, I explored instructors' perceptions of the effects of the mode of representation and the space of learning created by the sequencing of external representations. Of the three representations they identified as the best representation based on mode, I asked them to choose which one they would show their students. Schönborn and Anderson (2006) note that there "is an automatic pedagogical superiority that has been bestowed upon animated [external representations…] as compared with static [external representations]" (p. 97). I asked instructors to pick one "best" figure out of the three best for two reasons: 1.) I was interested in seeing whether the instructors who participated in this project felt animations are superior to static images for facilitating and supporting student learning about translation; and 2.) I was interesting in

seeing how the level of abstraction (stylized versus realistic) influenced instructor responses about the animated (dynamic) representations.

Data collection and analysis. The instructor interviews were audio-taped and transcribed verbatim. Any artifacts created by the instructor during the interview were collected. As the intention was to describe instructors' perceptions of the critical features of translation and the mode of representation, a grounded theory approach was used analyze this data. Interview transcripts and artifacts were read, reread, and iteratively coded for critical features of the conceptual knowledge of translation and mode representation, i.e., information that could be used to answer the following questions: what should students know about translation, and what should students know in order to understand external representations of translation? These critical features were used as the basis for the subsequent analysis of the enacted object of learning.

The Enacted Object of Learning

External representations. As previously mentioned, one outcome of the instructor interview was the identification of three best external representations, one of each mode. Not all instructors agreed on which representations were the best; therefore, I sought for a clear majority in order to identify the three best representations, noting objections to the majority opinion. The three best external representations—one of each mode—were incorporated into the student interview protocol (see Appendix C: Student Interview Guide).

For the purposes of this study, students' exposure to a set of external representations of protein translation constituted the enacted object of learning. Applications of variation theory often utilize observations of instruction in the classroom

as the context for the enacted object of learning (e.g., Marton & Pang, 2007). Thus, the enacted object of learning, i.e., the possibilities for student learning about the object of learning, is defined by what students are exposed to in the classroom. However, in this project, I am interested in exploring the possibilities for student learning about translation that are generated through students' exposure to external representations of translation. As such, in order to define the enacted object of learning, I will describe the possibilities for student learning afforded by exposure to the external representations.

Students were exposed to two of the best representations, each of a different modality or level of abstraction. Thus, the enacted object of learning occurs on two levels. First, each individual external representation defines a range of possible learning outcomes for students, a representational enacted object of learning. Second, the sequence of external representations to which students are exposed constitutes a gestalt enacted object of learning, in that the whole is greater than the sum of its parts (Gerow & Bordens, 2000). By this I mean that the cumulative range of possible learning outcomes is not merely the summation of the possibilities afforded by each individual representation but is greater than the sum as afforded by the variation between representations, i.e., the opportunity to compare the variation and the invariance between representations allows students to potentially notice more features than either representation could facilitate individually.

For example, by viewing a static illustration of protein translation before an animation of the same process, students might notice that the steps of translation depicted in the illustration do not occur in a clearly defined or predictable manner in the animation. If students were only shown the illustration, students might notice the

depiction of clearly defined, individual events such as peptide bond formation followed by translocation. However, if students only viewed the animation, the speed with which those events occur during the animation might cause students to not notice individual biochemical events but rather to focus on the flow of components within the system, such as the flow of aminoacyl-tRNAs into the A site of the ribosome and deacylated tRNAs out of the E site. By viewing the illustration first, students might notice the individual steps occurring during the animation that they otherwise might not have noticed. Similarly, students could gain an understanding of reaction rates and system dynamics from the animation that they might not have come to understand from the illustration alone.

If the representational order were reversed and students were shown the animation prior to the illustration, students might not come to the same understanding of translation as described previously. Because of the amount of information presented in the animation and the speed at which it is presented, students may experience cognitive overload, which would result in an incomplete understanding of the information that was depicted in the animation. Subsequent viewing of the illustration may clarify some points of the animation, but students' ability to recall the complex sequence of events depicted by the animation in order to more fully understand the illustration may lead to a wholly different student conception of translation from that of the alternative order described previously.

Data analysis. Data analysis of the enacted object of learning focused on identifying patterns of variation and invariance (Runesson, 2005) within and between the best external representations of translation. As previously described, the enacted object of learning consisted of student exposure to two of the three best representations selected by

the instructors. I chose to show students only two of the three best representations in order to minimize any confounding or complicating variables during data analysis. If all students were exposed to all three representations, student responses might have been due to exposure to an individual representation, the interaction of two representations, or the interaction of all three representations. In order to fully explore the impact of individual representations and the impact of the order of presentation of the external representations, I identified six different conditions to which students could be exposed during the interviews (Table 1):

Table 1
Enacted Objects of Learning

Group	First External Representation (Mode)	Second External Representation (Mode)
A	Static	Stylized, Dynamic
B	Stylized, Dynamic	Static
C	Static	Realistic, Dynamic
D	Realistic, Dynamic	Static
E	Stylized, Dynamic	Realistic, Dynamic
F	Realistic, Dynamic	Stylized, Dynamic

Note: The group assignments are described by the sequenced modal order of the external representations of translation selected by the instructors.

I analyzed each of the enacted objects of learning listed in Table 1 from a *first-order perspective,* meaning that I, as the researcher, used my experiences of the world in order to identify the possibilities for learning created by the representational content and sequence of the representations of translation. This is a wholly different analytical perspective as compared to the intended and lived objects of learning, which employ a

second-order perspective, i.e., a focus on people's experiences of the world, and is consistent with variation theory (Marton & Booth, 1997). A second-order perspective relies on an individual's retelling of their experience of a phenomenon whereas a first-order perspective relies on first-hand experience of a phenomenon.

Prior to conducting the instructor interviews, I examined all six external representations, i.e., ER1-ER6, individually. Based on my own understanding of protein translation, I identified the critical features I deemed present in each representation. I would note that my undergraduate training and degree is in biochemistry. This allowed me to knowledgeably analyze and identify critical features of protein translation present in the selected external representations and to define the enacted objects of learning created by each of the conditions listed in Table 1.

Following the instructor interviews—in which instructors identified, from their perspectives, the features of protein translation that are critical for developing a correct understanding of that cellular process—I reassessed my previously described enacted objects of learning for the three representations selected by the instructors as being the best representations of each mode to promote student understanding of translation. During this reassessment, I revised my original list of the critical features of protein translation by incorporating features deemed necessary or present by the instructors. The end result of the reassessment was a final list of critical features that I used to assess all three objects of learning (see Appendix F: Final Coded Features of Protein Translation from Instructor Interviews).

Variation analysis of dynamic external representations (VADER). In order to answer the research question "what is possible for second semester biochemistry students

to learn from external representations of translation?," I analyzed each of the best representations selected by the instructors in order to determine which of the previously identified critical features (see Appendix F: Final Coded Features of Protein Translation from Instructor Interviews) were present in the representations and, thus, possible for students to learn about. Additionally, for each critical feature identified in a representation, the extent or degree to which that feature was varied was also assessed. I further determined if any additional features (beyond the critical features identified by the instructors) were depicted. These features were termed *non-critical features* to distinguish them from the features identified by the instructors. I noted the presence of non-critical features because the presence of any feature (critical or non-critical) created the possibility for students to notice and learn about them, thereby defining the space of learning enacted by the representations.

A meticulous review of the literature failed to identify an applicable methodology capable of identifying 1.) whether a given critical feature of an object of learning was present in an external representation and 2.) the extent to which that critical feature is varied in the representation. Thus, I developed the Variation Analysis of Dynamic External Representations (VADER) methodology. This methodology is based on the theoretical underpinnings of variation theory. "According to variation theory, a phenomenon and/or its critical features are made visible in a teaching context through variation" (Orgill, 2012, p. 3392). Therefore, the guiding principle in the development of the VADER method was the idea that the variation of features of an external representation creates the potential for students to notice those features, which noticing creates the possibility of student learning. Features that are not varied, on the other hand,

would be noticed less by students and would, thus, influence student learning to a lesser degree than those features that are varied. Along these lines of thinking, absent features would not be noticed at all and would have no influence on student learning. Based on these assumptions, the VADER method attempts to identify which critical features are present in a given external representation and then, based on the amount of variation in the features that are present, assign a value indicating the potential of that feature to be noticed and, thus, possibly influence student learning.

There are many features that could vary in any given representation. For the purposes of this project and to develop the VADER methodology, I chose to focus on variations in position, size, and labeling.

Application of the VADER methodology. As mentioned previously, I made a list of the critical features of protein translation and external representations of protein translation, based both on my assessment of the representations and on information gleaned from the instructor interviews (see Appendix F: Final Coded Features of Protein Translation from Instructor Interviews). My first goal in analyzing the external representations was to determine whether those features were present or not. Determining the presence of any given critical feature proved to be challenging. Protein translation is a dynamic biochemical process and, as such, the components that are present and the interaction between those components changes with time. While this variation in time was an integral component in the stylized and realistic dynamic representations (the animations), it was, nonetheless, present in the static illustration of protein translation as well. Arrows served as dynamic indicators within the static external representation denoting a state of the process prior to the change in time represented by the arrow and a

state of the process after the arrow.

My second goal in analyzing the external representations in this project was to determine whether those features varied over the course of the representation. To account for the change in time and the variation associated with it, all three best representations were broken into to a series of frames. In the case of the static representation with dynamic indicators, frames were identified prior to an arrow and following an arrow. For the dynamic representations, frames were identified as screenshots taken every 2 seconds during the course of the animation. Two seconds was deemed an appropriate time span between frames as no critical feature was present on screen for less than two seconds. Thus, all features were accounted for.

Although I have named the analysis method VADER (Variation Analysis of *Dynamic* External Representations), I assert that this methodology can be applied to static representations with dynamic indicators, such as arrows. In the VADER method, dynamic external representations are analyzed as a series of static representations. Similarly, a static representation containing a series of dynamic indications denoting the passage of time over the course of the representation can be broken up into a series of "frames" (pictures separated by the dynamic indicators), which can then subsequently be analyzed using the VADER method. In this way, an external representation with dynamic indicators can be thought of and analyzed as a quasi-dynamic external representation. The same cannot be said for a static external representation depicting a single point in time. Because the VADER method compares the change in representational features from one point in time to another, both the static representation containing dynamic indicators as well as the dynamic animations can be analyzed using this methodology.

Following the division of each representation into a series of frames, I looked for variation in the external representations along three distinct aspects. The first was position. It was noted that features could vary in position from one frame to the next. Variation in position was defined as a change in the location of the feature relative to the other features present in the frame. If the feature changed position from one frame to the next, that change in location could be noticed by the viewer and cause them to focus on that feature, potentially leading to learning. A second aspect that could vary was size. Variation in size was defined as a change in the magnitude of the shape of the feature. If the feature changed size, i.e., the representation zoomed in or out on a feature, that change in magnitude could be noticed by the viewer and cause them to focus on that feature, potentially leading to learning. The final aspect of an external representation that could be varied was labeling. Labeling was defined as the application of an alphanumeric text cue used to name a feature. Variation in the presence of a label for a particular feature could be noticed by the viewer and cause them to focus on that feature, potentially leading to learning.

A coding scheme was developed in order to quantify the variation of each feature. The assumption that guided the development of the coding scheme was that highly varied features have the potential to be more noticeable by students and could, therefore, influence student learning to a greater extent than invariant or absent features. Here, I will describe the general process for assigning a value to the potential noticeability of a given critical feature. Later on, I will demonstrate this analysis process with reference to a specific external representation.

The first quantification aspect that was accounted for during the development of

the coding scheme was the identification of value to be assigned to a given feature. If a feature was present in all frames of a representation, then it could be noticed in any or all of them. However, if the feature was present but was invariant, the potential for that feature to garner attention from the viewer would fade over time. To account for the fading of invariant features, the number of frames for a given representation was used as the *high code*. This means that if there were three frames to analyze for a given representation, the initial appearance of a feature would be coded as *3*. If there were six frames, the first appearance of a feature would be coded as *6*.

Each feature was assigned a value corresponding to its presence, absence, or variation for each frame of each external representation. If the feature remained present but unchanged in the next frame in the series, then that feature would be coded as *the previous score minus one*. For example, if a feature had previously been coded as 3, a sequential invariant appearance of that feature in the following frame would be coded as 2 (3-1). If the feature had previously been coded as 6, it would next be coded as 5 (6-1). Thus, features that were present in any given frame were assigned a value, with that value decreasing over time if the feature was not varied along a particular aspect (i.e., size, labeling, or position). Because features that are not varied are less likely to be noticed by a viewer, the numbers assigned to a particular feature give an indication of the noticeability of that feature, with larger numbers indicating a larger potential for noticeability.

The common subtraction of one for invariant features means that VADER coding does not allow for equal fading of invariant features. However, if a feature is invariant yet present for a longer time, the chance of it being noticed by the viewer is greater than if

that feature were only present for a short amount of time. Thus, with more frames, an invariant feature should fade more slowly than one present in a smaller number of frames. This assumes that time is measured equally from one analysis frame to the next.

While all features that were present in an external representation were given a positive code—with the magnitude of the code an indication of how noticeable the feature is in a given frame—all absent features were coded with a *zero code*. The assumption guiding this coding decision is that if a feature is not present in a frame, then a viewer cannot notice it and, thus, cannot learn from it. It is also notable that the disappearance of a previously present feature is potentially noticeable. The drop from a scored code to a zero code would be noticeable and accounted for in a drastic change in the VADER coding.

The coding scheme for VADER not only captures if whether a given feature is absent, present, or invariant, but also if a feature changes in a given aspect from one frame to the next. A change in any feature would cause the coding of that feature to be restarted such that the code would go back to the high code.

The VADER coding scheme also accounts for the summative effects of variation across multiple aspects. If a feature changes position, size, and labeling from one frame to the next, that feature is more noticeable than a feature that only changes position. To account for the summative effect of variation across multiple aspects, I coded variation in each aspect—color, position, size, and labeling—separately before summing the score codes for each feature in each frame. These combined scores were converted into percentages by dividing each summed score by *the high score multiplied by three* (the highest possible sum) and then multiplying by one hundred.

The percentage generated from the combined scores was then used to generate a diagram that I call a VADER plot, i.e., a visualization of the presence of a feature and the extent of variation in that feature. In order to generate a VADER plot for each representation, I assigned the percentage for a given feature in each frame a color based on a grey scale gradient. Higher percentages indicated more variation in the feature. These highly varied features were shaded in a darker color. Lighter colors were used to indicate less variation, i.e., that the feature was less noticeable.

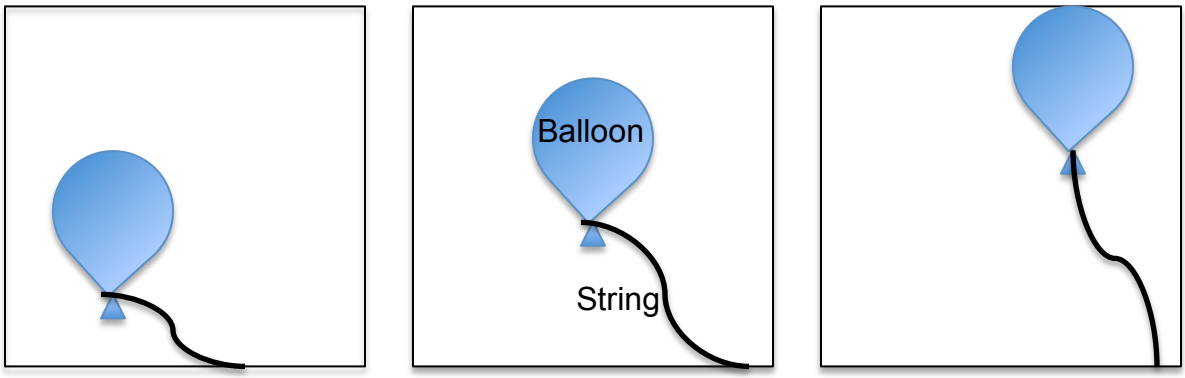
In this project, I used shape and color to identify the individual features of a representation. In other words, I assumed that color and shape determine the identity of individual features and distinguish one feature from the next. Based on these assumptions, any changes in the shape and/or color of a given feature indicate that the feature is changing identity. Because I have assumed that all features will retain their identities throughout a given representation, the VADER coding scheme does not account for changes in feature shape or color. Instead, the VADER analysis method accounts for variation in three other representational aspects: variation in relative position, variation in relative size, and variation in labeling.

I used the following questions to assign an appropriate code to each feature in each frame of a given representation:

1. Is the (aspect of variation) of (feature) present/apparent?
    a. Yes = Continue to Step 2
    b. No = Do not code (i.e., code zero)
        i. Note: the disappearance of a feature is always coded zero as it is no longer possible for a viewer to notice that feature.

2. Did the (aspect of variation) of (feature) change?
    a. Yes = highest code (i.e., the number of frames)
        i. Note: the appearance of a feature is always a change from the previous lack of that feature.
    b. No = Coded as previous code minus one (i.e., a fade in importance).

Consider the following example of a representation of a balloon containing three frames (Figure 14). In this section, I will apply the previously described coding scheme to the feature of the *balloon* depicted in this representation. I am able to identify this feature using my prior knowlegde of the shape and appearance of a balloon. In this case the balloon appears as a blue upside-down teardrop shape with a little flare at the bottom. It can be contrasted with the background which appears not colored. It can also be contrasted with the string which is different in color, shape, size, and position.



Frame 1 Frame 2 Frame 3

Figure 14. Example of a frame series used to conduct VADER coding.

I first code the feature of balloon in terms of its relative position (see Table 2). Using the procedure outlined above, I ask myself is the *relative position* of the *balloon* present/apparent? The answer is Yes, so then ask myself did the *relative position* of the *balloon* change from the previous frame? There was no previous frame, so this is a

change. Therefore, I score the feature of balloon in frame 1 with the high score for this representation. There are three frames in this particular representaion, so the high score is 3. In frame 2, the balloon changes position as compared to frame 1. I ask the same sereis of questions. Again, yes the balloon is present in frame 2 and it changed position as compared to frame 1. This change in position could lead a viewer to notice this feature. Thus, the code for position for the balloon in frame 2 is 3. Lastly, the balloon again changes position from in frame 3 as compared to frame 2. Again, this change is coded with the high score, i.e., the balloon is coded 3 for position in frame 3.

I can then code the feature of balloon in terms of size (see Table 2) by asking the same series of questions but instead of looking at the relative position of the feature, I can look at the realtive size of the feature. The initial appearance of the balloon in frame 1 is a change in size compared to not seeing it prior to frame 1. Thus, the code for size for frame 1 is coded as 3. The balloon does not change size between frames 1 and 2 making the code for size for frame 2 a 2, i.e., 3-1. Lastly, the balloon does not change size between frames 2 and 3 making the code for size for frame 3 a 1, i.e., 2-1.

Table 2
Example of VADER Coding and Plot

Aspect of variation	Feature	Frame 1	Frame 2	Frame 3
Position	Balloon	3	3	3
Size		3	2	1
Labeling		0	3	0
Combined		66.7%	88.9%	44.4%
VADER Plot				

The final coded aspect of variation is labeling (see Table 2). There is no label for the balloon in frame 1. Therefore, the code for label for frame 1 is 0. There is a label for balloon in frame 2. This is the initial appearance of the label; thus, the code for label for frame 2 is 3. Finally, the label for balloon disappears in frame 3. Therefore, the absence of a label makes the code for label in frame 3 a 0.

Codes from all three aspects are combined and divided by 9, i.e., 3 x 3 is the highest possible combined score. The combined score is then multipled by 100 to give a percentage and assigned a color from the grey scale graident based on the percentage. Based on this analysis, we can conclude that students can learn about the feature of a balloon from the representation presented in Figure 13 because the feature is present. We can also say that a viewer might notice the feature of a balloon most in frame 2 as this is when the most variation is present to cue the viewer to notice it.

When combined with analysis of other features, VADER coding and, in particular, the VADER plot can help researchers visualize which features of a given concept are present in an external representation and how much—and when—a viewer might notice them most, if at all. The extent to which certain features are noticeable will then influence what the viewer can learn about the content presented in the representaion.

Application of the VADER method to the current study. Each of the three best representations were coding using the VADER method and VADER plots were constructed for each (see Appendices I, L, and O: VADER Plots of External Representations #2, #3, and #6, respectively). Each VADER plot was analyzed individually in order to identify the affordances of each representation. Subsequently, each combination of the three representations in pairs of two were analyzed in order to

determine the affordance of the enacted object of learning created by the sequential viewing of each pair combination (see Table 1).

The Lived Object of Learning

Participants. The lived object of learning is defined by what *students* (in the broad sense of the word) learn about a designated object of learning. Accordingly, I interviewed thirty undergraduate Biochemistry II students from a large Southwestern university in order to identify the lived object of learning for this study. This number of participants is consistent with other studies using variation theory to examine student understanding developed from external representations (e.g., Rundgren & Tibell, 2009); however, additional student interviews were considered until I determined that the data set had reached saturation.

Students were recruited during the semester in which they were enrolled in a second semester biochemistry course and participated on a voluntary basis. Students were recruited in class, with the instructor's permission, following their exam that covered protein translation. The second semester biochemistry course was chosen specifically because this was the first time students had been exposed to a description of protein translation in the context of a biochemistry course. Previous discussions of protein translation in high school and/or undergraduate biology courses would have exposed students to a cursory explanation of translation from a biology perspective; however, the biochemical components and interactions of this metabolic pathway are discussed in greater detail in a second semester biochemistry course. Additionally, all students enrolled in the second semester biochemistry course had met the course prerequisites. Specifically, students had taken first semester biochemistry; and had, therefore, been

exposed to an introduction to biochemistry. Also, student participants had taken similar amounts of undergraduate coursework. This ensured that all students had been exposed to a similar level of prior instruction regarding the components and processes of protein translation. However, as students' prior knowledge of translation (prior lived object of learning) was assessed during the student interviews, I will further elaborate on the influence of prior knowledge on student learning outcomes and distinguish between this and the effects of the external representations on student learning outcomes (see Chapter 7: The Lived Object of Learning).

Student volunteers were randomly divided into one of six groups, each group consisting of at five students (Table 1). Group A was shown the static external representation followed by the stylized animation. Group B was shown the stylized animation followed by the static external representation. Group C was shown the static external representation followed by the realistic animation. Group D was shown the realistic animation followed by the static external representation. Group E was shown the stylized animation followed by the realistic animation. Group F was shown the realistic animation followed by the stylized animation. The two representations and the order in which they were shown constitute a unique enacted object of learning and provided the basis for which analysis of students' post lived object of learning was predicated. As mentioned previously, the purpose of these groups is two-fold: 1.) the groupings allowed me to determine the effect of exposure to multiple representations on students' understanding of translation, and 2.) the groupings allowed me to examine the effect of order of presentation on students' understanding of translation.

External representations. The external representations shown to the student participants during the student interviews were selected based on the instructor interviews, as described previously. These preferred representations were determined to be ER2, ER3, and ER6 (see Chapter 6: Selection of Preferred External Representations). Two of the three external representations preferred by the instructors were presented to the students as determined by the student's random group assignment. As noted earlier, all representations were shown without sound. For the purposes of this project, alphanumeric labels were considered part of the representational design as they are visual, symbolic cues used to draw viewer attention to a particular representational component. The exclusion of sound ensured that student responses were limited to their experience of the visual features of the external representations and their prior knowledge.

Student interview protocol. The semi-structured student interview protocol (see Appendix C: Student Interview Guide) was adapted from Schönborn and Anderson's Three-Phase Single Interview Technique (3P-SIT) (Schönborn, Anderson, & Mnguni, 2007; Schönborn & Anderson, 2009). The 3P-SIT model was chosen because it addresses both participant knowledge of the content (the object of learning) and their understanding of external representations. Additionally, prior research in the area of biochemistry education has utilized a similar 3P-SIT modified protocol to assess student understanding of enzyme-substrate interactions using multiple representations (Linenberger & Bretz, 2012a). While 3P-SIT was designed to "gather data on students' interpretation of [external representations]" (Schönborn *et al.*, 2007, p. 293), I expanded the student interview protocol for this project in order to assess students' prior lived object of

learning of translation as well as their post lived object of learning as informed by the space of learning created by the sequential experience of the assigned external representations. For the purposes of this project, *prior* refers to before students had been exposed to the external representations of interest, i.e., students' prior knowledge of translation. *Post* refers to students' knowledge of translation as expressed during and after exposure to the external representations of interest.

The student interview protocol has been expanded from the original 3P-SIT model to include initial demographic questions followed by six phases of questions:

- Demographics
- Phase 1: Students' Prior Knowledge of Translation (Prior Lived Object of Learning)
- Phase 2: Student Perceptions of the External Representation of Translation #1
- Phase 3: Student Evaluation of the External Representation of Translation #1
- Phase 4: Student Perceptions of the External Representation of Translation #2
- Phase 5: Student Evaluation of the External Representation of Translation #2
- Phase 6: Student Comparison of External Representations #1 and #2

General demographic questions, such as "Have you taken Biochem II?" or "What are your feelings towards biochemistry?," allowed me to confirm whether or not the selected participants had met the participant criteria defined earlier. Additionally, these questions allowed me to build rapport with the student, hopefully making the student feel more comfortable sharing their thoughts and opinions later on during the interview. The expansion from three phases to six phases was done to allow for episodic questioning related to students' prior knowledge of translation, perceptions and evaluation of one

mode/level of abstraction of external representation, perceptions and evaluation of a second mode/level of abstraction of external representation, and comparison of the two representations.

The objective of Phase 1 was to establish students' prior knowledge of translation. I used this data set to establish change in perception or focus following student exposure to the external representations, i.e., learning as a result of exposure to the enacted object of learning. Note that the lived object of learning is defined completely within student sphere, i.e., from the students' perspective (see Chapter 3, Figure 6); thus, analysis of the critical features of translation has been described from the point of view of the student. To this end, I asked the students to explain their understanding of protein translation and how they would or do go about explaining this process to another student. Specifically, I asked then to identify the main idea(s) of protein translation, i.e., the critical features of the content.

In phases 2 and 3, I explored students' perception and evaluation of the first external representation they were shown. Similarly in phases 4 and 5, I probed students' perception and evaluation of the second external representation. I asked students to talk through what they were seeing in the external representation of interest as they were seeing it. In exploring students' way of seeing a particular representation, I was able to gain an insight into potential advantages and disadvantages of presenting information to students via a particular representational design component, i.e., use of color, or representational modality. Additionally, I asked students to describe what they found confusing about the representation of interest and what changes they would make in order to improve the representation. In phases 2 – 5, I utilized a series of think-aloud cues and

semi-structured probes to elicit responses that allowed students to articulate what they were noticing about the representation and the content.

Finally in phase 6, I explored student perceptions of the effects of the mode of representation/level of abstraction and the space of learning created by the sequencing of external representations. I asked students to compare the two external representations they were shown and identify their difference and similarities, whether or not they contained the same information, and which one was easier for them to understand. Additionally, I asked them to explain which representation they would use to explain translation to another student. Finally, I asked students explain the their understanding of translation following their exposure to the external representations. This was done to establish whether the external representations afforded students the opportunity to learn in the sense that they were able to see the process of translation in a different way.

Data collection and analysis. The student interviews were audio-taped and transcribed verbatim. Any artifacts created by the student during the interview were collected. In particular, students were asked to draw their initial and final understanding of protein translation. These drawings were collected through the use of a Livescribe Echo™ Smartpen. The use of digital pen- and paper-technology has been previously used as a data collection technique within biochemistry education research (Linenberger & Bretz, 2012b). This technology allows students to draw an image as they normally would on a piece of paper. However, that image is then tracked and digitized by the smartpen to allow subsequent time stamped image analysis. I chose to integrate this technology into the student interview protocol to allow students to represent their understanding of protein translation in both images and words. While I have assumed that the instructors

have sufficient background knowledge in order to be able to speak knowledgeably about the process, students may not have a coherent understanding. Thus, by allowing students to represent their ideas in terms of both images and words, I believe students will be able to express their understanding of the process in a more complete fashion than if they had been limited to verbal expression alone. Additionally, the smartpen allows me to see if students begin to incorporate not only the content presented in the external representations into their understanding of the process but also the representational imagery as well.

As the intention of this project is to describe student' perceptions of the critical features of translation and the mode of representation, a grounded theory approach was used analyze this data. Interview transcripts and artifacts were coded for critical features of the conceptual knowledge of translation and mode/level of abstraction of the representation, i.e., what did students notice about translation, and what did student notice about representational design and modality of external representations of translation? The critical features of students' conceptual knowledge of translation before and after exposure to the external representations were compared in order to assess the degree of learning. Finally, the critical features identified by students were compared to the critical features identified by the instructors. Alignment between instructors' and students' critical features were used to assess the degree to which students hold an expert view of translation and the effect of external representations in promoting an expert view of translation. Variation in student responses was attributed to variation in the enacted object of learning unique to each student grouping.

CHAPTER 5

ANALYSIS, RESULTS, AND DISCUSSION OF RESEARCH QUESTION 1

The Intended Object of Learning: Chapter Overview

My first objective of this project was to identify biochemistry instructors' intentions for student learning. Specifically, Research Question 1 asked: What do instructors of second semester biochemistry students intend for their students to learn from external representations of translation? Based on interviews with instructors, I was able to come to several general conclusions about the instructors' intentions for the teaching of protein translation concept. First, I verified that these instructors perceive that learning about protein translation is a valuable educational objective of undergraduate level biochemistry instruction. Second, when I analyzed instructor interviews in order to determine the critical features of protein translation—i.e., those features the instructors deem critical for developing a correct understanding of protein translation concepts—I found that the instructors referred to some common critical features of protein translation more often than they referred to others. In fact, based on the instructors' comments, I was able to categorize the critical features identified by the instructors as being *primary*, *secondary*, or *tertiary* in terms of their importance for student understanding of translation. I further categorized these features by overall themes. Finally, I determined that, in general, instructors shared a common intention for student learning about protein translation and that any variation in the critical features identified by individual instructors reflected differences in individual interests or applications of the biochemical concepts underlying protein translation.

Instructors' Perception of the Importance of Protein Translation in Biochemistry Education

One of the underlying assumptions of this project is that protein translation is an important object of learning within the undergraduate-level biochemistry education curriculum; hence, biochemistry students should be learning particular critical features of this object of learning in order to develop correct understandings of it. Although limited prior literature has documented the importance of this topic (see Chapter 2), it was necessary to establish whether or not the instructor participants in this project held a similar view, as their responses to the instructor interview (see Appendix B: Instructor Interview Guide) would be based on their value of the topic of protein translation and their subsequent intentions for student learning about this object of learning.

During the interviews, all instructors identified protein translation as a component of their instruction. As articulated by Instructor 3, the instructor participants described protein translation as an important biochemical topic:

> [00:03:49.9] **Instructor 3:** [Protein translation] is obviously one of the most important concepts […] in biochemistry. It's part of the dogma, the […] biochemistry dogma, going from DNA to mRNA to […] protein, so it is very important for the students.

Instructor 5 went on to elaborate on the foundational nature of students' knowledge of protein translation, noting that many biochemistry students will need to apply this type of knowledge as they continue on in medical school or graduate school.

> [00: 12:55.9] **Instructor 5:** If I can give them that foundational overview, ah, and this includes protein synthesis, then they are going to understand some drugs are ribotoxins, and how would that work? […] So, ribotoxic stress is a really good way to treat cancer. […] If I can give them that part of it so that then they can understand, "Yeah you can't make protein bonds if you do that, and you're going to kill the ability of the cell to make proteins, and you're going to have a real problem."

Although the instructors agreed that protein translation was an important biochemical topic, they were quick to point out that the treatment of this information from a biochemical perspective was different from that of a biological perspective.

> [00:06:01.9] **Instructor 3:** The biochemistry approach is to look at things at more at the molecular level, so we are more interested in looking at […] how the actual mechanisms of this reactions work. […] I love to see this, this beautiful eh, schemes where you can actually look at individual groups and individual functional, chemical functional, you know, groups and see what they do at the molecular level […] From a molecular biology point of view, you would be looking more at eh, eh, the interactions between the different […] players, the proteins, the mRNA, but more of those interactions than the actual forces that are involved in protein translation.

In this way, biochemistry is focused on the chemical or mechanistic approach to describing the protein translation process rather than a more general overview of the interactions of components of the process as described in molecular biology.

> [00:21:31.7] **Instructor 4:** One of the things that I look for to define my course is, um, non-redundancy with their biology classes. So almost all of my students are also taking molecular biology, immunology, virology, microbiology, genetics […] whereas, this is a chemistry course and so for me, I definitely want to focus on, on chemical concepts, chemical principles, and, and all the things I think they're not going to get elsewhere.

Although biochemistry was described by the instructors as being conceptually focused on the chemical concepts and principles of the process, this is due in part to the fact that instructors may perceive this as a deficiency in the treatment of the process from a biological context. Thus, some of the instructors perceived that a biochemical presentation of the protein translation process complements and bolsters more biological presentations of protein translation to which the students have been exposed.

> [00:17:02.3] **Researcher:** You had mentioned earlier that, ah, you know, you kind of see some of your role as filling in the gaps […]. What are things you feel like you're filling in that they're not getting in [a more biology focused] context?

> [00:17:25.0] **Instructor 5:** Just greater structure. Just greater specificity of structure. For instance, um, this is definitely true for high school, but over there in biology, if they're teaching molecular biology or genetics, they're, they're more interested in information flow throughout the cell and diseases and, and how things can be disrupted; whereas, and I always tell them this, what's the prefix for this course, it's CHEM. It's chemistry. […The content is] overlapping for sure, but it's a different treatment of the area completely, and, and it should be. It's, it's, it's chemistry, so it's more of the actual specific structures and chemical reactions.

These quotations suggest that biochemistry instructors have a unique intended object of learning as compared to other instructors who may teach about protein translation. Subsequently, the critical features identified by the biochemistry instructors may be different from the features identified by instructors in a different discipline. Therefore, the results and implications discussed in this chapter and the following chapters should be considered in light of the discipline in addition to the object of learning.

Critical Features of Protein Translation

My goal in addressing Research Question 1 was to identify the features students *should* learn about protein translation, i.e., the critical features of protein translation. To do this, I coded the instructors' interviews for features that were stated or implied as being important or necessary for students to know in order to understand the process of protein translation. For example, Instructor 2 describes the ribosome as both a named feature and an implied feature, both of which were coded as *General molecule R*, referring to a description or representation of the ribosome as a general entity.

> [00:21:35.8] **Instructor 2:** You show the workings of the ribosome […], a mRNA being threaded through that thing […].

In this case, the "ribosome" is first named and was coded as General molecule R. He then goes on to refer to "that thing" to again describe the ribosome. "That thing" was also coded General molecule R. In this example, "mRNA" is also identified as a named

feature and was coded as General molecule M, referring to a description or representation of the messenger RNA as a general entity.

This coding scheme was revised using the constant comparative method throughout the coding process. Highly similar codes were collapsed until each defined code described a unique feature of protein translation. The coding scheme was validated using an external coder. The external coder was a postdoctoral researcher with expertise in biochemistry education. She coded two excerpts from the instructor interviews using the code list. She was asked to apply codes as they were defined on the code list and to identify any areas of confusion or possible new codes. Inconsistent coding and ambiguous code definitions were revised in collaboration with the external coder. A final revised code list containing an operational definition of each code was then created and can be found in Appendix D.

Near the beginning of their interviews, I asked instructors to identify the features of protein translation that were important for biochemistry students to learn and/or understand. I used the features described by the instructors to generate the previously described code list of features of protein translation.

After I asked the instructors to discuss the features of translation that they felt their students should know and understand about protein translation, I showed the instructors a series of six external representations of protein translation and asked them to respond to each representation. I then coded those responses, paying particular attention to repeated or new features of protein translation for each representation. I chose to code the instructors' responses to the external representations separate from the coding of their comments about what students should learn about protein translation to acknowledge the

potential influence of the selected representations on the instructors' perceptions of what they felt was important for students to learn. In the sections that follow, I will discuss instructors' initial responses first and separately in order to acknowledge their potential prominence in instructors' intentions for student learning. I will then discuss the instructors' critiques of the selected external representations. Finally, I will use information from the instructors' interviews to comment on the relative importance of the individual critical features for promoting student learning about protein translation.

Instructor Responses Prior to Viewing the External Representations

As noted above, I analyzed instructors' initial responses separately from their responses to the external representations in order to identify any potential influence resulting from their subsequent evaluation of selected external representations of protein translation.

Unique individual or disciplinary emphases may make some features of protein translation more or less important in promoting a particular understanding of that object of learning. Although all of the instructors who participated in my study had taught a second semester biochemistry course, they did not come from the exact same disciplinary training and do not focus on the same research areas. As such, the instructors interviewed in this study did not identify the exact same critical features in their initial descriptions of what students should learn about protein translation. However, some features were mentioned by all of the instructors as being critical to developing correct understandings of protein translation. The fact that all instructors identified these common critical features prior to their exposure to the selected representations suggests that these

common features are particularly important in promoting learning, from the instructors' perspective.

The use of the term *common* in this discussion refers to features identified by *all* five instructors. In order to distinguish common features from others, I have also identified majority features and minority features. The term *majority* refers features discussed by three or four of the instructors but not all five. The term *minority* refers to features discussed by one or two of the instructors.

All features identified during Phase 1 of the instructor interviews, i.e., prior to the instructors' evaluation of the selected external representations, are presented in Appendix E: Initial Coded Features of Protein Translation from Instructor Interviews. The coding of the instructor interviews resulted in a count pertaining to the frequency with which a given instructor referred to a particular feature. As phenomenography is not concerned with the frequency with which codes appear, I will not present that data. Instead, I will indicate if a feature was present or not present The features are organized alphabetically based on the number of instructors that referred to those features, i.e., whether they were common, majority, or minority features. Any feature mentioned by an instructor regardless of magnitude of the utterance frequency is highlighted in green. Any feature not mentioned is highlighted in red.

The absolute magnitude of the utterance frequency, i.e., how often the instructors mentioned a particular feature, was not deemed significant, and is therefore, not presented in Appendix E: Initial Coded Features of Protein Translation from Instructor Interviews. Some instructors were simply more verbose as compared to others. Additionally, an explanation may warrant a repeated reference to a specific feature in

order to indicate a relationship rather than dominance of that feature over another. For example, in the previous quotation from Instructor 2:

> [00:21:35.8] **Instructor 2:** You show the workings of the ribosome […], a mRNA being threaded through that thing […].

General molecule R was coded twice and *General molecule M* was coded once. This does not necessarily indicate that Instructor 2 thinks that the ribosome is twice as important as the mRNA. However, it does show that the ribosome is an important feature of protein translation from the perspective of this particular instructor. I identified *any* initial reference to a specific feature of protein translation, regardless of utterance frequency, as a possible critical feature of the object of learning, but did not use the magnitude of the utterance frequency as a direct measure between instructors.

Common features. Although I identified 38 unique features of protein translation from the instructors' initial responses, not all instructors referenced the same features. In fact, all five instructors initially identified only three common features: the ribosome (*General molecule R*), the mRNA (*General molecule M*), and the tRNA (*General molecule TR*).

The ribosome. The ribosome was the most highly referenced feature during Phase 1 of the Instructor Interviews. For example, Instructor 2 described the importance of student understanding of the ribosomal structure.

> [00:09:01.5] **Instructor 2:** You'd certainly have to layout the structure of the ribosome […]

The ribosome, as both a structural component of the translational machinery and as the catalytic component of the peptide bond formation reaction, was highly referenced by all

of the instructors. For example, Instructor 3 described the catalytic nature of the ribosome.

> [00:04:05.4] **Instructor 3:** The students need to understand […] the role that, that ribosomes play in, ah, in the whole process especially the ribosomal RNA, how it's actually part of the catalytic machinery […]

As indicated in the description of the ribosome by Instructor 3, many of the features identified by the instructors were described based on their "role" in the process. Therefore, the instructors' common reference to the ribosome indicates that it plays a central role in the process of protein translation. Overall, the instructors identified knowledge of the ribosome as a general entity, knowledge of the ribosome's structure, and knowledge of the ribosome's catalytic properties to be important to students' developing a correct understanding of protein translation.

The mRNA. The messenger RNA was also identified by all of the instructors as a key feature of protein translation. Instructor 3 described the role of mRNA as an important intermediary in the information-processing pathway of the Central Dogma.

> [00:04:07.1] **Instructor 3:** The students need to understand the concept of moving from DNA to mRNA to protein, and understand how the, how the cell uses different types of RNAs to, eh, eh, eh to translate the […] sleeping code of DNA into the functional code that is protein, and that includes the mRNA.

The instructors highlighted the role of mRNA in the production of "functional" proteins. Instructor 2 also identifies the mRNA as a necessary component of translation and discusses the importance of student understanding of processing of mRNA prior to translation, i.e., providing a big picture view of translation as a part of a bigger process rather than as a discrete reaction.

> [00:17:33.4] **Instructor 2:** I think, also it would be useful to show how a lot of messenger RNAs have all kinds of regulatory sequences on them.
> [00:17:42.2] **Researcher:** Mmm

> [00:17:42.9] **Instructor 2:** There are many proteins that bind to those and those affect translation.
> [00:17:45.3] **Researcher:** Right
> [00:17:45.7] **Instructor 2:** and so, I think it would be useful to let students know that, um, um messenger RNAs aren't just synthesized and, and um immediately get translated. A lot of them go through a much more complicated process before they get translated.

Instructor 2 elaborates on the information-processing context of this process, noting that as the molecular information progresses from DNA to mRNA to protein, there are regulatory processes involved as well. The messenger RNA does not enter translation solely as the product of transcription but also the product of regulatory elements that can influence and alter the protein outcome of translation.

The tRNA. This big picture application of the context and implications for protein translation is coupled with a micro-scale emphasis on the chemistry of structure as seen in instructors' descriptions of the transfer RNA. For example, Instructor 5 emphasizes the disciplinary differences in the presentation of tRNA structure to students.

> [00:18:39.5] **Instructor 5:** It's chemistry, so it's more of the actual specific structures […] and a good example is probably the transfer RNA. The classic cloverleaf structure, [a biology class] could probably show that. I show the folded structure, the three-dimensional one and try to, at times, indicate the very interesting, um, non-standard Watson-Crick base pairing.

Similar to Instructor 5, Instructor 4 notes the intricacies of transfer RNA structure when describing what students should know about protein translation.

> [00:25:45.3] **Instructor 4:** You know, the nature of the chemical linkage that connects a tRNA molecule to its cognate amino acid, it's an ester linkage. […] My guess is that's maybe something that's rather glossed over more in a, in a biology class.

Interaction features. In addition to individually referencing the ribosome, mRNA, and tRNA, instructors also refer to the interactions between these components.

For example, Instructor 4 describes the mRNA/tRNA interaction during codon/anticodon base pairing.

> [00:26:53.6] **Instructor 4:** I'm emphasizing, you know, chemical issues, a lot like, um, for example, […] the tRNA anti-condon and the messenger RNA codon as being complementary, that's reverse complement. Thinking about strand polarity very carefully.

Although all the instructors refer to the ribosome, mRNA, and tRNA in their initial responses, they do not describe the interactions between these components in a similar fashion, i.e., not all instructors address complementarity.

It is interesting to note that the common features identified by all five instructors were specific components of the process rather than chemical properties or reactions. When describing the difference between a biochemical and biological treatment of protein translation, the instructors had seemed to relegate the component parts of the process to a biological treatment of this object of learning. This may indicate that the instructors see the ribosome, mRNA, and tRNA as necessary biological prior knowledge that provides a foundation for a more chemically-focused discussion of the chemical properties, forces, or reactions involved in protein translation.

Other features. Although not all instructors described the same component interactions as being important for student learning, all instructors did describe some element of chemistry within the context of the object of learning. Most notably, three instructors described peptide bond formation as an important feature. Instructor 3 said he would describe how "the next [tRNA] would come in, how they will link together […]." More explicitly, Instructor 2 said "[y]ou'd have to lay out the mechanism of, um, peptide bind formation […]." Instructor 5 also identified peptide bond formation when asked

about the challenges of teaching protein translation, although he describes this feature as one that the students generally understand.

> [00:14:20.8] **Researcher:** Are there challenges to teaching this topic, are there things that students just don't seem to pick up on or misconceptions they seem to be operating under?
> [00:16:28.5] **Instructor 5: […]** The funny thing about it is I don't think the chemistry, you know, […] the first slot and then the peptidyl and their getting together, and forming that peptide bond, that doesn't seem to be a big issue. They do get that.

Although Instructor 5 did not view peptide bond formation as a challenging component of the object of learning, he did indicate that this was a feature that was part of his regular instruction on the topic. Thus, he identified peptide bond formation as an important feature for understanding protein translation.

Although Instructor 4 did not describe peptide bond formation in his initial description of protein translation, he did address another chemical topic, that of the codon/anti-codon interaction.

> [00:26:53.6] **Instructor 4:** I'm emphasizing, you know, chemical issues, a lot like, um, for example, […] the tRNA anti-codon and the messenger RNA codon as being complementary, that's reverse complement. Thinking about strand polarity very carefully.

Implied in this description of codon/anti-codon complementarity is the suggestion that hydrogen bond formation been the nitrogenous bases of the mRNA codon and the tRNA anti-codon loop is an important part of the protein translation process. Additionally, the statement regarding careful consideration of strand polarity refers to the directionality of nucleic acids and the corresponding phosphodiester bonds linking neighboring nucleotides. This directionality confers has implications for the resulting primary structure of a polypeptide. Both complementarity and polarity have implications for students' understanding the process and outcome of protein translation.

Beyond specific chemical properties and interactions, Instructor 1 highlights the importance of translation to the broader pursuit of science.

> [00:18:45.9] **Instructor 1:** You know, the trivial stuff is the sequence in which [protein translation] happens, the messenger binds, eh, those things are, are hardly more than rote memorization,
> [00:18:57.3] **Researcher:** MmHmm
> [00:18:58.7] **Instructor 1:** and any kid who's been through freshman biology ought to have a pretty reasonable notion about what happens first and so on, but I think what's really challenging about this is to, is to understand the energy and thermodynamics, evolution, timing,
> [00:19:16.1] **Researcher:** MmHmm
> [00:19:17.7] **Instructor 1:** uh, proofreading and, and editing these things, and when people see it that way, they realize there is actually a lot we don't understand here, so I, I think the last thing I'd say that's, that's important to learn about translation, or for that matter about anything, is that the last word hasn't been written yet. […] There are no doubt more surprises left, there is no question there are, and, uh, I think it makes biochemistry a lot more exciting to people if they realize it isn't dead. That it's still very much alive, and that there is still plenty of room for improvement.

Instructor 2 echoes a similar sentiment.

> [00:15:18.2] **Instructor 2:** It's an enormously complicated process and it's getting more complicated all the time, especially in eukaryotes.

Many of these general considerations, although not exclusive to intentions for student learning about specifically protein translation, do provide a sense that biochemistry instructors are looking to situate their instruction in a broader context using features of protein translation as examples of specific chemical properties and relationships.

Instructor Responses to Common External Representations of Protein Translation

After instructors commented about protein translation and what students should learn and know about this concept (Phase 1 of the interview), I asked them to evaluate a series of six common external representation of the process in Phases 2-4. These representations were purposefully selected to depict a range of modes and levels of abstraction (see Chapter 2). As with the instructors' responses in Phase 1 of the interview,

I analyzed their responses to Phases 2-4 for reference to features that are critical for learning about protein translation.

In Appendix F (Final Coded Features of Protein Translation from Instructor Interviews), I have listed all of the critical features of protein translation I identified in the instructors' responses to Phases 1-4 of the interviews. The features identified by the instructors before being exposed to the external representations are highlighted in yellow. These are the features from Appendix E: Initial Coded Features of Protein Translation from Instructor Interviews. I observed that many of the features mentioned initially by the instructors remained salient as they viewed the representations. For example, almost all of the common features coded in Appendix F (Final Coded Features of Protein Translation from Instructor Interviews) were mentioned initially by the instructors. This indicates that the instructors found these features to be very important in describing and subsequently understanding the process of translation. Any feature mentioned by the instructor, regardless of the magnitude of the utterance frequency, is highlighted in green. Any feature not mentioned is highlighted in red.

Common features. I identified a total of 59 features of protein translation in the instructors' responses to the external representations. Seventeen of those features were identified by all five instructors and were categorized as common features. Three of the common features in Appendix F (Final Coded Features of Protein Translation from Instructor Interviews) were the same three common features identified from instructors' initial responses in Appendix E: Initial Coded Features of Protein Translation from Instructor Interviews. This shows the ribosome, the mRNA, and the tRNA remain salient in instructors' evaluation of the external representations and are important components of

the process of translation in the view of these instructors. In fact, many of the common features identified in Appendix F (Final Coded Features of Protein Translation from Instructor Interviews) were identified as further elaborations on the three initial common features. These common features generally described a sub-component or provided a more detailed description of one of the initial common features. In the sections that follow, I will discuss these features that elaborate on the originally identified common features in more detail. Throughout this discussion I will use the term *component feature* to refer to a feature that described a specific component of translation. I will also use the term *interaction feature* to refer to a feature that describes the interaction between two or more component features.

Component features. During Phases 2-4 of the instructor interviews, the instructors elaborated on many of the common component features they had described prior to viewing the common external representations. In particular, the ribosome, mRNA, and tRNA were all described in further detail. The following sections describe the subcomponents of each of these component features as well as some additional component features that were described by the instructors.

Ribosome-related features. The initial common general reference to the ribosome was augmented by a more elaborate common description of the large and small ribosomal subunits and the A, P, and E sites of the ribosome. Instructor 3 describes how ER1 would attract students' attention to the large and small subunits (see Figure 15).

> [00:17:43.3] **Instructor 3:** [In response to ER1] I think it would draw their eyes to the two big circles, the 50S and 30S […]

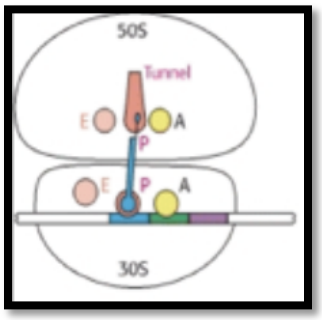


Figure 15. Frame 1 of External Representation #1.

Instructor 5 describes ER2 as being more detailed than ER1, noting that this level of detail extends to include information on the general shape of the large and small ribosomal subunits (see Figure 16).

> [00:30:51.4] **Instructor 5:** [In response to ER2] It's more highly detailed even to the point where they are trying to show the relative shapes of the small and large ribosome.

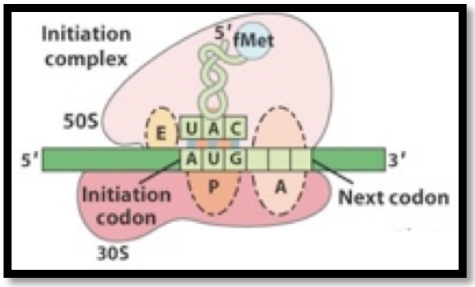


Figure 16. Frame 1 of External Representation #2.

While both Instructors 3 and 5 noted that students would be able to use the external representations to differentiate between the large and small subunits, as depicted in ER1 and ER2, Instructor 3 pointed out that students should be able to use ER1 to differentiate between the three binding sites of the ribosome.

> [00:17:02.7] **Instructor 3:** [In response to ER1] From here they can get, eh, eh, can get the different ribosomal units, you have the different sites […]

In addition to identifying features of protein translation in the external representations, instructors called attention to features that were lacking from the external

representations. For example, Instructor 2 indicates that ER1 lacks a sufficiently detailed depiction of all three binding sites.

> [00:20:01.7] **Instructor 2:** [In response to ER1] You have the number of sites, ah, I mean the number of tRNA binding sites, and, and there's a loose representation of the function. So it's ok. I think it maybe just a little too simplified.

This instructor's comment suggests that a more detailed representation of the binding sites of the ribosome would facilitate learning about protein translation more than would the more simplified representation of binding sites presented in ER1. This assertion is supported by Instructor 3.

> [00:31:30.8] **Instructor 3:** [In response to ER3] The A-site, P-site, eh, eh, are not shown very clearly.

Instructor 5 also notes that neither ER3 nor ER4 displayed all of the ribosomal binding sites.

> [00:54:40.5] **Instructor 5:** [in response to ER3 and ER4] I can't remember, but I don't think either one of them had the exit site, so that's not good.

Although the instructors identified many component features of the ribosome, the components were cited in a broader discussion of the underlying chemistry and interactions of protein translation.

mRNA-related features. As they did with the ribosome, instructors continued to address and refer to the general entity of the messenger RNA when commenting on the external representations. In particular, the codon feature was also commonly described as a more specific structural element of the mRNA. For example, Instructor 5 described how the color-coding of the codons in ER4 helped to identify that feature (see Figure 17).

> [00:52:47.6] **Instructor 5:** [in response to ER4] I did like […] showing the codons.

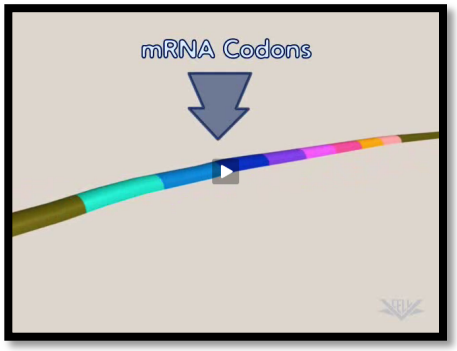


Figure 17. Frame 24 of External Representation #4.

The codon was also commonly discussed in terms of the codon/anti-codon component pair. The underlying relationship of this pairwise description hints at the hydrogen bonding occurring during codon/anticodon base pairing interaction. However, as described by Instructor 3, this pairwise description could also refer simply to the component features of the codon of the mRNA and the anti-codon of the tRNA.

> [00:19:02.8] **Instructor 3:** [in response to ER2] The thing I like about this one, though, is that it shows a better representation of tRNA […]. It shows also the anti-codon/codon, eh, eh, explicitly […].

tRNA-related features. Although, in their initial responses, instructors referred to the tRNA in more general terms, they referred to more specific subfeatures of the tRNA as they interacted with the external representations. For example, instructors commonly gave descriptions of the anti-codon loop and the P-site positioning of a tRNA engaging in peptide bond formation.

> [00:23:36.7] **Instructor 2:** [in response to ER1] I think another concept is sort of missing in [ER1], is this concept that the tRNAs, um, have a anticodon that is complementary to something in the messenger RNA.
> [00:23:49.3] **Researcher:** Mmm
> [00:23:50.0] **Instructor 2:** and it's not just random tRNA that are binding there. All of them are coded to bind in a certain order and peptide binds are setup to be created in a certain order.
> [00:24:02.3] **Researcher:** Right.

> [00:24:03.6] **Instructor 2:** and, um, I think that is a critical concept through in whatever representation that you put out there.

In this description, Instructor 2 connects four different common features: three component features—the transfer RNA, the tRNA anti-codon, and the messenger RNA—and one interaction feature, complementarity. This relationship between the features is then used to describe the formation of the primary sequence of the polypeptide. This indicates that all four features are involved in the formation of the primary sequence of the polypeptide.

Instructor 1 describes the tRNA position relative to the site where the peptide bond is formed in the ribosome. Again this indicates the interactions between individual component features.

> [00:34:43.4] **Instructor 1:** [in response to ER2] I didn't see as clearly [in ER1] as I do here that the dipeptide is attached to the tRNA that's initially in the A-site,
> [00:34:54.2] **Researcher:** Mmm
> [00:34:54.9] **Instructor 1:** and then I see something happening here, ah, in which the dipeptide-tRNA becomes associated with the P-site.
> [00:35:05.0] **Researcher:** Mmm
> [00:34:05.7] **Instructor 1:** So I see that there's been a jump that took place there.

As with the instructors' descriptions of the ribosome, the messenger RNA and transfer RNA and their associated elements are cited in a broader discussion of the underlying chemistry, i.e., the codon/anti-codon base pairing (hydrogen bonding), and the ramifications of the sequence ordering of from mRNA to polypeptide. Therefore, these components are also considered important features of protein translation.

Additional component features. In addition to the ribosome, mRNA, and tRNA, two other component features were commonly discussed during the instructor interviews: amino acids and the polypeptide chain.

> [00:52:52.1] **Instructor 5:** [in response to ER4] I think they probably could show the actual structure of the amino acid, but when they start growing the chain, still it wouldn't have been that hard, probably, but they just have these little balloons.

Given the instructors' emphasis on the chemistry underlying protein translation, it stands to reason that the product of this process, the polypeptide chain, and its component parts, the amino acids, were commonly identified and deemed critical features of protein translation.

Interaction features. When I coded the instructors' initial comments about protein translation, I found that all of the common features mentioned by all instructors were component features: they described individual components or structures that are involved in the process of protein translation. Although, in these initial responses, many instructors emphasized "the chemistry" of the protein translation process, I was unable to assign a specific interaction feature code to any of their comments because the instructors used the general term "chemistry" in referring to protein translation instead of discussing specific chemical interactions or behaviors that are involved in the process. Several aspects of the process of protein translation involve chemical forces or reactions, i.e., chemistry; but the instructors did not refer to them specifically during Phase 1 of the interview.

During the Phases 2-4 of their interviews, as they interacted with and commented on the external representations I provided, instructors were more likely to comment about specific chemical interactions and processes ("chemistry") involved in protein translation than they were during Phase 1. In fact, two specific applications of the term "chemistry"—both of which refer to specific chemical interactions—became apparent in their responses: the "chemistry" of peptide bond formation and complementarity.

Peptide bond formation interaction feature. I observed the first of these interaction features in instructors' descriptions of the "chemistry" of peptide bond formation.

> [00:31:18.6] **Instructor 5:** [In response to ER2] They actually show the chemistry, the actual, the nucleophilic attack on the, on the ester bond there from the A-site to the P-site to form a peptide bond, so that's actually good. I actually like that.

While Instructor 5 expressed approval of a depiction of peptide bond formation in ER2, other instructors expressed disapproval of a particular representation if it failed to depict the chemistry of peptide bond formation. For example, Instructor 3's reaction to ER1, which he was shown before being shown ER2, included the following statement:

> [00:17:16.8] **Instructor 3:** [In response to ER1] I think it would be hard for [students] to get the, the amino acid linkage. They are here, but not very well described, eh, the peptide bond formation. Eh, yeah, I don't like it that much.

Both Instructor 5's approval of the depiction of peptide bond formation in ER2 and Instructor 3's disapproval of the *lack* of a depiction of peptide bond formation in ER1suggests that students *should* be learning about peptide bond formation (i.e., that peptide bond formation is a *critical* feature for protein translation). Additionally, the fact that Instructor 3's comment was made before he saw ER2 (a representation with a depiction of peptide bond formation) indicates that his intention that students learn about the feature of peptide bond formation was not influenced by his having first seen the feature in a representation.

Other instructors also commented on the importance of depicting the process of peptide formation in external representations of protein translation.

> [00:22:55.9] **Instructor 2:** I think it's necessary to get some chemistry in there just so that students have a feeling for why the growing peptide ends up on the

> tRNA it ends up on. Um, if you don't understand who's attacking who, and what the reaction looks like, then it's hard to follow all that.

Instructor 2 made this comment after comparing ER1 and ER2. Similar to Instructor 5, he described the "chemistry" of peptide bond formation in ER2 as being an important feature for students to learn and, similar to Instructor 3, noted that the lack of chemistry in ER1 made that representation less beneficial for student learning.

This feature continued to be noted by instructors during the presentation of the dynamic representations.

> [00:52:08.0] **Instructor 5:** [Both ER3 and ER4] get a low grade on the formation of the peptide bond because even in this one, again, they had [the amino acid] disconnecting from the peptide transfer RNA and going to the aminoacyl one which the aminoacyl one, they, and they didn't show this at all, but there is a hybrid structure […] and then it's a nucleophilic attack from the, and they didn't show that. Actually [the amino acid] popped off and then come over, just the reverse chemistry would have to occur if that was the case. That's really misleading.

He goes on to evaluate ER3 and ER4 based on their presentation of chemistry of peptide bond formation.

> [00:56:37.0] **Instructor 5:** [ER3 and ER4] both fail. Neither one of them show a bond. Neither one of them actually show properly the site of the chemistry.

Similarly, Instructor 2 critiqued ER3 based on its depiction of peptide bond formation.

> [00:37:22.1] **Instructor 2:** [In response to the ER3] Well there's a fair amount of structural information in this one. Peptide bond formation isn't being shown. Complementarity is being shown and that's good. So the overall outline of the process is pretty well represented. Now the messenger RNA gets lined up in the, on the 30S subunit is getting represented pretty well. […]
> [00:38:18.1] **Researcher:** So overall, effective, ineffective? How would you evaluate that one?
> [00:38:22.6] **Instructor 2:** Overall, that's a fairly effective overview. Um, the chemistry isn't represented at all so seeing why those amino acids move from one to the other. That, ah,
> [00:38:37.1] **Researcher:** MmHmm

> [00:38:38.2] **Instructor 2:** Um, I'm not sure a student would really understand why that happens, but um, other than that, it's got most of the elements there for the basic process anyway.

Although Instructor 2 describes two chemical features of protein translation, complementarity and peptide bond formation, he uses the term "chemistry" to specifically mean peptide bond formation. Therefore, the common application of chemistry in the context of peptide bond formation and the integration of the common component features of the ribosome, tRNAs, amino acids, and the resulting polypeptide chain, indicate that peptide bond formation is a critical feature of protein translation.

Codon/Anti-codon interaction feature. As noted above by Instructor 2, the second common chemical feature identified by instructors was that of complementarity. In their comments, the instructors used the word "complementarity" to refer to the codon/anti-codon base pairing that occurs during protein translation. For example, Instructor 1 describes the "match" between complementary codons and anti-codons, indicating the pairing of these component features.

> [00:32:47.1] **Instructor 1:** [In response to ER2] They actually spell out the three nucleotides in the codon and anti-codon and make it clear that the positioning of the tRNA is dictated by that match.

The underlying chemical force responsible for codon/anti-coding recognition and stability is the formation of hydrogen bonds between the nitrogenous bases of the messenger RNA codon and the transfer RNA anti-codon loop. Although the term "complementarity" or the phrase "codon/anti-codon" were regularly used by the instructors, "hydrogen bonding" or "hydrogen bond formation" were not commonly described. In fact, they were almost never used to describe the interaction between a codon and an anti-codon. Instead, this chemical interaction was generally described in

terms of an interaction between the component parts (for example, "codon/anti-codon interaction" instead of "hydrogen bonding between the mRNA and tRNA"). Because all instructors referred to the codon/anti-codon base pairing that occurs during protein translation, I determined that codon/anti-codon base pairing is a critical feature of protein translation.

Ultimately, I differentiated this feature into two unique codes: *Codon/Anti-codon base pairing I* and *Codon/Anti-codon base pairing E*. The I and E designations refer to the initiation (I) and elongation (E) stages of protein translation. I decided to use different codes to differentiate between separate codon/anti-codon interactions that occur at various points in time during the dynamic process of protein translation.

Determination of Critical Features

The over-arching research question addressed in this chapter asks what students *should* learn about protein translation. Variation theory asserts that the critical features of the object of learning are those features necessary for students to learn in order to understand that object of learning in a particular way. In my analysis of the instructor interviews, I was particularly interested in the features that the instructors identified as being critical for learning about protein translation because instructors' beliefs about those critical features can influence their intentions for student learning about protein translation. Likewise, instructors' intentions for student learning may influence their identification of critical features of protein translation. In other words, while one instructor may have a certain intention for student learning and a corresponding series of critical features, another instructor may have a different intention for student learning and a different corresponding set of critical features.

Because I interviewed five instructors in this project, there was the possibility of identifying five different intentions for student learning about protein translation. This can be seen in the unique series of features identified by each instructor. All five instructors identified 17 common features of protein translation, and I identified 16 of those common features as important within instructors' intentions for student learning (I will discuss the less important common feature in the upcoming section). In addition to the 17 common features of protein translation, 42 other features of protein translation were described by at least one of the instructors. All of these features describe a variety of scientifically accurate components and interactions of protein translation. As I have defined *critical features* to be those features necessary to promote a scientifically accurate conception of protein translation, all 59 features identified from the analysis of the instructor interviews are considered critical features. However, based on my further analysis of the instructor interviews, some critical features seem to be more important than others based on how many of the instructors described the same features. If all of the instructors discussed the same feature, then I found that feature to be more important that a feature only discussed by one instructor. Therefore, I have categorized the critical features, based on their expressed importance, as *primary*, *secondary*, or *tertiary* critical features.

In the sections that follow, I will describe my reasoning for the categorization of the critical features as either *primary*, *secondary*, or *tertiary* critical features. I will use the phrases *initial common features* and *final common features* to refer to the common features of protein translation identified during Phase 1 of the interviews and the common features of protein translation identified during Phases 2-4 of the interviews, respectively.

Primary Critical Features. Although the utterance frequency was not used as a direct measure of feature importance, a comparison between the utterance frequency of common features identified from Phase 1 of the instructor interviews and common features identified from Phases 2-4 of the interviews shows that these features were repeatedly referred to during the interviews. For example, Instructor 3 initially mentions the mRNA as a general molecule seven times. By the end of the interview, he has mentioned the same feature an additional ten times. This repeated emphasis indicates a strong belief in the importance of that feature. Similar trends can be seen in the utterance frequency of the *ribosome general molecule* and *tRNA general molecule* codes. Thus, the ribosome general molecule, mRNA general molecule, and the tRNA general molecule can be clearly identified as important critical features.

Similarly, most of the other final common features were initially identified by the majority of instructors. For example, peptide bond formation was initially mentioned by Instructors 2, 3, and 5. At the conclusion of the interview, all five instructors had repeatedly identified peptide bond formation as a critical feature necessary for biochemistry students to learn. Critical features described by all five instructors at some point in the interview (Phases 1-4) were identified as *primary* critical features (Table 3) as an indication of their importance to all of the instructors. The primacy of these features across all instructors' descriptions can be seen as a strong indication of their importance for student understanding of protein translation.

As mentioned previously, I did not consider one of the seventeen common features (those identified and discussed by all five instructors) to truly be "important" to the instructors. Although the random motion of cellular components was identified as a

Table 3 *Primary Critical Features of Protein Translation*
Coded Feature
Aminoacyl (A) site
Anti-codon loop
Codon
Codon/Anticodon base pairing E
Codon/Anticodon base pairing I
Exit (E) site
General molecule M
General molecule P
General molecule R
General molecule TR
General molecule(s) AA
Large subunit
P-site tRNA
Peptide bond formation
Peptidyl (P) site
Small subunit

common feature, it was not included among the primary critical features. The categorization of this feature as commonly described was potentially inflated. Of all six representations shown to the instructors, only ER6 depicted the random motion of cellular components. All other representations showed only directed movement of translational components. In order to make a general comparison between the way in which component motion was conveyed in ER6 and the other representations, I posed a directed question to the instructors, asking them to respond to this specific feature. As such, instructors commonly described this feature, but only in response to my direct questioning. Moreover, their descriptions indicated that they felt this feature was only mildly important for learning about protein translation.

> [00:51:59.7] **Researcher:** How important do you think it is to convey this sort of random motion idea as far as biochemical processes go for student.

> [00:52:06.6] **Instructor 2:** I think you could do for just a second and get the point across.

Therefore, because this feature was first mentioned only in response to my prompt and not repeatedly or generally mentioned in the interviews, I did not consider it to be a primary critical feature.

Secondary Critical Features. While some *primary* critical features were mentioned by ALL instructors, other critical features were mentioned only by a majority of instructors. I categorized the features which were identified by three or four instructors as *secondary* critical features (Table 4).

Table 4
Secondary Critical Features of Protein Translation

Coded Feature
3' end TR
3’ end M
3’ poly A tail
5’ end M
A-site tRNA
Evolution
Exiting tRNA
General molecule(s) E
General molecule(s) RF
General process A
General process E
General process I
Incoming tRNA
Methionine
Nucleotide sequence (multiple codons)
Primary structure
Random motion of components
Reaction kinetics
Regulation
Ribosomal translocation
Shine-Dalgarno sequence
Tunnel

Included in this category was the common interaction feature pertaining to the random motion of components. As described earlier, although this feature was common to all instructors, it was prompted and not generally repeatedly described. Therefore, it was not considered to be a primary feature, yet because all instructors agreed that it was somewhat important, it was considered to be a secondary feature.

Many of these secondary features reinforced or further clarified the primary critical features. In particular, the primary component features related to the mRNA, the ribosome, the tRNA, the amino acids, and the polypeptide chain were expanded and clarified by these secondary features. For example, the nucleotide sequence of the mRNA (*Nucleotide sequence (multiple codons)*) was described by four of the five instructors. Each of these descriptions of the nucleotide sequence of the mRNA further expanded on the mRNA component feature. In the quotation below, Instructor 1 uses the term "sequence" to refer to the mRNA nucleotide sequence, discussing its relation to several other primary critical features including the ribosome.

> [00:55:54.4] **Instructor 1:** [In response to ER3] The individual players are identified with labels that, in the very early part of it where you blew up and showed the sequence and binding of the sequence to the ribosome, that was very nice.

In this quotation, he uses the term *sequence* to refer both to the mRNA as a general feature of the process and the mRNA's relationship with another primary feature, the ribosome. His comments clarify the mRNA component feature in that mRNA is not only a general component of the protein translation process but also a component that interacts with the ribosome during protein translation.

Instructor 1 goes on to note that:

> [01:05:20.0] **Instructor 1:** [ER3] had a very nice, clear representation of how the sequence of the anticodon and the codon matches up. It doesn't have a color coder, but if we know our base paring rules we see the sequences there.

In this quotation, he uses the description of the nucleotide sequence to refer not to the mRNA in general, but to the codon as a functional unit of the mRNA and to the codon/anti-codon interaction as a prominent chemical interaction of protein translation.

Although both of the preceding descriptions of the nucleotide sequence of mRNA are used in reference to other primary critical features, the specific use of the term "sequence" was seen as an acknowledgement of the additional information provided by an understanding of the nucleotide bases and their role in the process of protein translation as compared to a general reference to the mRNA. The mRNA could be represented simply as a red line, as it is in ER3, but adding the nucleotide sequence AUG CAU GGG CAG gives additional structural information about the messenger RNA. Similarly, the additional secondary features pertaining to the mRNA, ribosome, tRNA, amino acids, and polypeptide chain serve to further detail or clarify the more general corresponding component feature.

Two other component features were identified as secondary critical features: elongation factors and release factors. The majority of instructors described these two factors as general entities rather than specific factors. This indicated that although many instructors felt the need to include a discussion of these factors in the description of the process, the general nature of their inclusion and lack of common discussion warrant their classification as secondary features. Also, both features were described in terms of their role in facilitating the general process of protein translation. Rather than being a central component or interaction, these features tended to be seen as features helping to support

the overall process. For example, Instructor 5 commented on the depiction of the release factor as a good addition to the depiction of the termination of the process.

> [00:45:07.6] **Instructor 5:** [In response to ER3] We're at the end of it now. Now they're just showing the complete chain. Oh nice, now we've got the release factor coming in.

The secondary critical features also included several interaction features. Unlike the secondary component features, these interaction features generally provided new information regarding the interactions occurring at various points in time during the protein translation process, specifically during activation, initiation, and elongation. The majority reference to these general processes indicated the addition of sequence ordering information to the intentions for student learning. For example, Instructor 3 uses the phrase "the actual process" to describe the elongation stage of protein synthesis depicted in a certain portion of ER6. This seems to indicate that he is valuing certain stages of the process more than other, or perhaps he is valuing the chemistry that is occurring during those stages.

> [00:45:14.7] **Instructor 3:** [In response to ER6] They isolated that part, and I think that's appropriate because then I can explain that and say ok when, now you have the mRNA, now you have the ribosome, you have all this tRNA floating around, and then basically what they did is basically zoomed in, into the, the actual process, the mechanistic process.

Initiation, although not referred to by name in the quotation above, is identified in the sequential discussion of component interactions, "now you have the mRNA, now you have the ribosome." This stage ordering of the process is used to categorize interactions based on time, i.e., this happens first, then this, then that. Although several instructors discuss similar stage descriptions of interaction features, other instructors specifically discounted the importance of this information for a biochemical presentation of the topic.

> [00:18:45.9] **Instructor 1:** You know, the trivial stuff is the sequence in which [protein translation] happens, the messenger binds, eh, those things are, are hardly more than rote memorization,
> [00:18:57.3] **Researcher:** MmHmm
> [00:18:58.7] **Instructor 1:** and any kid who's been through freshman biology ought to have a pretty reasonable notion about what happens first and so on […]

This does not imply that students should not know the sequence of events. Instead, it implies that students should already know the sequence of events by the time they are presented with protein translation in a biochemistry course. Thus, the categorization of sequence information and the corresponding interaction features as secondary features is consistent with instructor perceptions.

Also among the secondary interaction features are three features not associated with a particular stage of protein translation: reaction kinetics, evolution, and regulation. Their secondary status is appropriate in that these features were used to address broad applications of students' knowledge of protein translation rather than their specific knowledge of the process of translation. For example, Instructor 5 integrates both regulation and evolutionary concepts in the teaching of translation.

> [00:11:20.7] **Instructor 5:** The fun part of teaching [protein translation] is I like regulation. I love talking about how things are regulated and when you're talking about biochemistry, how things are regulated like pathways of feedback and so forth and pathways that are positive loops and all that. I always tell [the students], look at the big picture. Does this make sense? And it always makes beautiful sense after several billion years of evolution. […] They get kind of an eye opening, oh yeah that just, that just, of course, that's the way it should be, it just makes sense, and when biochemistry, when it all starts to tie in like that back together, and, eh, I kind of understand the big picture, I think then we've accomplished something.

These broad applications seemed to reinforce individual instructor preferences within biochemistry and are not exclusive to the process of protein translation yet situate it

within the "big picture." Thus, while interesting, these features are deemed secondary critical features.

Tertiary Critical Features. The remaining features identified during the analysis of the instructor interviews were described infrequently and by only one or two of the instructors. These tertiary features are still deemed critical as they were explicitly identified by the instructors even if only by one of them; however, the lack of common discussion of these features indicated that they were viewed as being among the least important features of protein translation (Table 5).

Table 5
Tertiary Critical Features of Protein Translation

Coded Feature
16S rRNA
2D shape
3D shape
5' end T
5' methylated cap
E-site tRNA
EF-G
EF-Ts
EF-Tu
Energetics
General molecule(s) IF
GTPase activity of EFs
Hydrogen bonding E
Hydrogen bonding I
Nucleotide sequence (anti-codon loop)
Nucleotide sequence (start codon)
Regeneration of activated tRNAs
Secondary structure
Sequential AA
Start codon
Stop codon

Similar to the secondary features, the tertiary components features generally included more detailed descriptions of the previously identified features. The tertiary

features also included one new component feature: initiation factors. This feature was only generally mentioned by Instructor 2.

> [00:14:57.5] **Instructor 2:** I would love to see a movie like that.
> [00:14:59.6] **Researcher:** Yeah, well [laughs]
> [00:15:01.2] **Instructor 2:** And then showing all of the other pieces. Like showing, um, you know factors come and, and bind the signal sequences […]

Therefore, this feature, while scientifically accurate, is not a highly emphasized component of the process. Furthermore, the lack of a majority description of the feature warrants its tertiary categorization.

Interestingly, hydrogen bonding is included among the tertiary interaction features. This feature is notable as it is highly related to the primary feature of codon/anti-codon base pairing; however, its tertiary status indicates that instructors do not seem to emphasize the chemical nature of the base pairing interaction. Instead, the chemical focus of protein translation seems to be placed on the peptide bond formation.

In the current study, I have assumed that the features that instructors explicitly mentioned during their interviews are aligned with the intentions for student learning that are communicated most clearly in the instructors' classrooms. Whether this is a valid assumption is a question for a future study. Moreover, a future study should ensure that the features that I have identified as primary, secondary, and tertiary are aligned with instructors' perceptions of the importance of those features. It may be that what I have identified as primary, secondary, and tertiary features based on the general descriptions of translation by the instructors are not valued in the same way by the instructors. For example, it may be that biochemistry instructors do value hydrogen bond formation and intend for their students to learn about the hydrogen bonding events that occur during

translation, even though they did not mention these interactions explicitly in their interviews.

Discussion and Conclusions

I analyzed of the instructor responses to the instructor interviews and identified 59 critical features of protein translation. I categorized the critical features based on their common discussion among the instructors. I designated unprompted features addressed by all five instructors at some point during the interview as primary critical features, features addressed by a majority of instructors were designated secondary critical features; and the features addressed by a minority of instructors were designated tertiary critical features. Although I determined that the secondary and tertiary features were less emphasized by the instructors and were subsequently coded less, I still included them as critical features because at least one of the instructors identified them as important and because they indicated scientifically accurate thinking and understanding of the process of protein translation. As such, all critical features were used in the subsequent analyses of the external representations (see Chapter 6) and the student interviews (see Chapter 7).

In addition to their primary, secondary, or tertiary status, the critical features were also inter-related. Multiple features referred to the same general entity or stage of translation. For example, the large and small ribosomal subunit and the A, P, and E-sites all referred to components of the ribosome, whereas peptide bond formation and ribosomal translocation both referred to interactions that take place during the elongation stage of translation. As such, the coded features were categorized into two general themes of features: 1.) Components/Structures of Protein Translation and 2.) Interactions/Chemistry of Protein Translation. Components/Structures were identified as

features used to describe or depict individual constituents or a portion of a constituent perceived as being involved in the process of protein translation. Interaction/Chemistry were identified as features used to describe or depict relationships between two or more components of process. Some of these relationships were described or depicted as specific chemical forces or reactions while others were described as more general component interactions.

Each theme was sub-categorized into general components or stages of protein translation (see Appendix G: Categorization of Critical Features of Protein Transaltion). This resulted in the identification of eight general component parts of protein translation: the mRNA, the ribosome, the tRNAs, the amino acids, the polypeptide chain, the initiation factors, the elongation factors, and the release factors. This also resulted in the identification of three general stages of protein translation: activation, initiation, and elongation. (Although termination is generally identified as the fourth stage of protein translation, it was not among the features identified by the instructors). There was also a category created for miscellaneous features: General considerations. These miscellaneous features generally referred to interactions or concepts that were significantly broader than the topic of protein translation. However, most of these general consideration features were discussed by the instructors as they described how to place translation in a broader context.

Using the primary, secondary, and tertiary categories as well as the general features and themes, I determined that the general intended object of learning was that students should develop a functional conception of the components and sequence of events in order to understand the underlying chemistry of protein translation. It was

deemed critical that students should learn the how the component parts of the mRNA, ribosome, and tRNAs assemble to create the translational machinery. Specifically the codon/anti-codon base pairing interaction was emphasized as an important feature in understanding how the genetic message was transferred from the mRNA into the polypeptide chain. Additionally, it was identified that students should learn how the amino-acylated tRNAs interact in the various sites of the ribosome during peptide bond formation. Variation in instructors' intentions for student learning was seen outside of these general intentions and centered on a particular area of instructor interest or on the application of protein translation to a broader biochemical context.

CHAPTER 6

ANALYSIS, RESULTS, AND DISCUSSION OF RESEARCH QUESTION 2

The Enacted Object of Learning: Chapter Overview

My objective in this portion of the project was to describe the space of learning enacted during the student interviews. Specifically, Research Question 2 asked: What is possible for second semester biochemistry students to learn from external representations of translation? The enacted object of learning defines the boundaries of the space of learning, i.e., what is possible for students to learn about an object of learning.

Within this project, the enacted object of learning was defined by the series of external representations shown to the students. As described previously (see Chapter 4), I showed students two of the three *best* representations as determined from the instructor interviews. In this chapter, I will first describe the instructors' selection of the preferred representations that were then shown to the students. Then I will describe my analysis of the representations for the presence and variation of critical features for learning about protein translation. As I carried out this analysis for the critical features of protein translation, I also noted additional non-critical features that are present in the representations. (By non-critical features, I am referring to features present in the representation but not identified by the instructors. Non-critical does not imply that these features are unimportant. They are included in this discussion because they are present in one or more of the representations shown to the students and could therefore be noticed and possibly learned by the students.)

Based on the critical features identified from the instructors' intentions for student learning and the additional non-critical features identified in the selected representations,

I constructed and analyzed VADER plots in order to determine what could be learned from each representation. After discussing the possibilities for learning created by the individual representations, I will end the chapter by discussing the affordances and limitations of the paired ordering of representations.

Selection of Preferred External Representations

Overall, the instructors who were interviewed in this study noted that protein translation is an important but complicated process. The instructors expect the students in their biochemistry courses to integrate information they have learned in their previous biology and chemistry courses with knowledge about biochemical processes in order to develop a scientifically accurate understanding of protein translation.

As they discussed what students should learn about protein translation, instructors identified a large number of critical features to which students should attend (see Chapter 5 and Appendix G: Categorization of Critical Features of Protein Translation). However, cognitive research has shown that students are only able to attend to a limited number of features or aspects of a given phenomenon at a time, a fact which was realized by some of the instructors and taken into account in their selection of the *best* representation of a given mode for teaching protein translation concepts. In fact, several instructors noted that some representations were better at presenting limited amounts of information to students and/or cueing students to attend to certain features over others. For example, Instructor 2 described an idealized way of representing a particular topic by varying the amount and order of information presented to students.

> [00:27:25.3] **Instructor 2:** I mean one of things that I think biochemists really like is structures. You know, you've got all of these protein structures and they're beautiful […] and biochemists love them and the temptation is to use bazillions of them in [a] book. Um, the reality is students don't care about those so much. They

> want to know what's going on, and they want to know what information do I need to know to get through the next exam. […] If you put an elaborate structure there and you say this is the phosphorylated form [of a protein] and they can't see the phosphates, that's not a good thing. […] Now if you make a, a simpler form that sort of suggests the shape [of the protein] but allows you to highlight the phosphorylations and, or other modifications or the substrate coming in or whatever it is that you want to show, I think you're better off.

He goes on to say:

> [00:29:05.2] **Instructor 2:** I think the key thing is trying to design [a representation] so students can get the information that they're supposed to get out of a figure.

Many of the instructors shared the sentiments of Instructor 2. Some representations were viewed as being better than others because they contained many or most of the primary critical features and were thought to cue students to notice those critical features. Other representations were viewed less favorably because they either inaccurately displayed certain critical features or glossed over them all together.

In all, I presented six common external representations to the instructors: two static illustrations (ER1 and ER2), two stylized animations (ER3 and ER4), and two realistic animations (ER5 and ER6). During the course of the instructor interviews, I asked each instructor to select one of the two representations of each type that would best convey to students the features of protein translation they felt were most important. I analyzed those instructor responses in order to determine which representations were shown to students. In the sections that follow, I will discuss the instructors' reasons for selecting each preferred representation.

Selection of a Static External Representation

When asked which representation, ER1 or ER2, they would choose to use to explain the process of protein translation to biochemistry-level students, four of the five

instructors selected ER2. Instructor 2 liked the simplicity of ER1 but felt that it did not give a biochemistry-level student enough information. This is an interesting comment as both of these figures came from commonly used college-level textbooks.

> [00:19:34.1] **Instructor 2:** I think [ER1] is an ok overview for, to give students the idea that there are these repeating steps, but there's not much information actually in this image.

Instructor 2 goes on to compare ER1 with ER2.

> [00:20:43.9] **Instructor 2:** So [ER2] has all the sites in it, but it also has the chemistry.

The additional chemical information in ER2 was cited by many of the instructors as the reason ER2 was a more appropriate representation of the biochemistry of protein translation for their students. As described by Instructor 3, most of the instructors preferred the ER2 over ER1.

> [00:27:47.4] **Researcher:** If you had to show a, sort of, second semester biochem, entry–level biochem student one of these two representations, ah, which one would you choose and how would you show it to them?
> [00:27:59.7] **Instructor 3:** I would definitely choose [ER2].

Instructor 5 was the only instructor to prefer ER1 instead. His preference was ER1 was due to the simplicity of ER1. He felt that students would be too overwhelmed by the information in ER2. However, he did agree that ER2 was more biochemically accurate.

> [00:38:47.1] **Researcher:** [Referring to ER1 and ER2] Of the two representations […] which one represents the content best?
> [00:38:53.8] **Instructor 5:** Probably [ER2], because number two's got a hell of a lot more content [laughs]. I mean it really does.

Based on the general consensus among the instructors, ER2 was selected as the static external representation that was shown to students.

Selection of a Stylized, Dynamic External Representation

When asked which representation, ER3 or ER4, they would choose to use to explain the process of protein translation to biochemistry-level students, four of the five instructors selected ER3.

> [01:03:50.5] **Researcher:** If you had to compare [ER3] and [ER4] to each other, which one of those two would you have preferred to use?
> [01:03:56.4] **Instructor 1:** Oh I'd take [ER3] in a heartbeat.

Although instructors expressed a preference for ER3 over ER4 when forced to choose between the two, several instructors critiqued both ER3 and ER4 because neither includes a good depiction of peptide bond formation.

> [00:52:08.0] **Instructor 5:** [Both ER3 and ER4] get a low grade on the formation of the peptide bond because even [ER4], again, they had [the amino acid] disconnecting from the peptide transfer RNA and going to the aminoacyl one which the aminoacyl one, they, and they didn't show this at all, but there is a hybrid structure […] and then it's a nucleophilic attack from the, and they didn't show that. Actually [the amino acid] popped off and then come over, just the reverse chemistry would have to occur if that was the case. That's really misleading.

Instructor 5 goes on to note that:

> [00:56:37.0] **Instructor 5:** [ER3 and ER4] both fail. Neither one of them show a bond. Neither one of them actually show properly the site of the chemistry.

The instructors seemed to prefer ER3 because of other information presented in that representation, such as the Shine-Dalgarno sequence. ER4 was generally seen as "too much like a video game," as Instructor 3 commented. This seems to be a reference to the glossy, 3D appearance of the component representations, as noted by Instructor 3.

> [00:35:13.2] **Instructor 3:** I think [the 3D appearance] makes it distracting […] Now you're looking at how pretty, it looks too pretty. […] Students are going to be looking at the shapes and the other shapes coming in […] and out. […] That makes it too distracting.

While a majority of the instructors selected ER3 over ER4, Instructor 5 felt that ER4 was the "best" representation, citing the overall level of realism in ER4 as part of the reason for his preference of ER4 over ER3.

> [00:50:01.7] **Instructor 5:** I like the transfer RNA better here [than in ER3] because actually, it's, it's more realistic. […] The overall shape is more, more correct.

He goes on to say:

> [00:55:37.9] **Instructor 5:** I'd probably [choose ER4], just 'cuz it has a, more of a three dimensional aspect because if I'm going to do animation, that's what I want. To me, it's not going to be a learning, let's see, a learning opportunity so much as sort of a big picture environment where I can say 'this is what we think in terms of a contour relief in three dimensional structure, we kind of think this is the relationship of the players and we're trying to get things to scale and so forth.

However, he went on to say that he would not use either of these representations to teach protein translation because of their incorrect and misleading presentation of peptide bond formation.

Based on the general consensus among the instructors, ER3 was selected as the stylized, dynamic representation that was shown to students.

Selection of a Realistic, Dynamic External Representation

When asked which representation, ER5 or ER6, they would choose to use to explain the process of protein translation to biochemistry-level students, four of the five instructors selected ER6.

> [00:45:55.1] **Researcher:** Again, if you had to pick one of these two representations to show to an undergrad, undergraduate level student, which one would you choose?
> [00:45:55.1] **Instructor 3:** Oh, [ER6], definitely six.

The instructors indicated overall that both ER5 and ER6 were more complicated than the other external representations because of their realistic depictions of the

structures involved in protein translation; however, they found ER5 to be somewhat more difficult to understand than ER6. For example, Instructor 1 described the general complexity of ER5 as follows:

> [01:11:34.1] **Instructor 1:** [In response to ER5] You know if you asked me what I took away from that, um, I got the idea that its very complicated process involving a number of players, some of which were briefly identified, but none of them stuck. I don't think I could go back and name you very many of the players. I had, even though the G proteins were shown here, I didn't get any sense that this was a process in which energy was invested. I didn't get any connection between the sequence of the codons and the sequence of the amino acids. They, they, they represented the forming polypeptide chain gorgeous, but that's just distracting. A simple schematic showing one to one color matches is much more effective.

Similarly, Instructor 5 described ER5 as visually distracting.

> [01:01:48.1] **Instructor 5:** It's too much in terms of, um, it would be fine for maybe a research article […] that might work, but it, it seems to be very busy at the very beginning just setting it up, and you would have a very hard time following that. I mean I did actually, to tell you the truth.

He went on to say:

> [01:05:55.8] **Instructor 5:** I found myself just wanting to look at the contours, it was very distracting, actually. […] I couldn't follow, and I teach it [laughs].

ER6 was thought to be somewhat distracting as well but some instructors also felt that it helped to convey the general sense of the protein translation process as a whole. Instructor 2 indicated that ER6 would be useful if it were to be shown to students after they were shown a representation depicting a more schematic introduction to protein translation, such as that presented in ER2.

> [00:50:37.7] **Instructor 2:** [In response to ER6] Even with a lot going on, um, still they're not showing the chemistry, so, um, so again if you had that preview and you showed them the chemistry then this would be useful to sort of show them that, you know, all of these things are happening, you know, all together as a big coordinated thing going on.

Instructor 4 noted that the background information depicted in ER6 provided an appropriate setting for the process by placing translation in a cellular context.

> [01:18:58.9] **Instructor 4:** [In response to ER6] It does a pretty good job of conveying kind of the concept of, you know, a lot of molecules bouncing around because I know students don't really have a, a good feel for, you know, molecules in motion and the numbers of them, and, you know, the scale of those things. […] I think it's easier to look at than [ER5].

Instructor 1 provided the counter-opinion by selecting ER5.

> [01:19:45.6] **Researcher:** Is there one you would prefer to show students?
> [01:20:02.2] **Instructor 1:** You know I probably choose not to show either of them but if I had to choose between them I think that the my recollection that [ER5] at least had the occasional label to help us see where the players were, and so I think I would probably take it for that reason.

Although Instructor 1 would not have used either ER5 or ER6 to teach the concept of protein translation, when forced to choose one *best* representation of the two, he chose ER5. His selection of this representation was based on the visual cueing, e.g., the labeling, shown in ER5. He felt that the labeling might allow students to better identify the cellular components of protein translation shown in the representation, leading to a better understanding of the component interactions of the process.

Although neither ER5 nor ER6 were strongly favored by the instructors, the general consensus indicated that ER6 would be better to promote student learning than ER5. As a result of the instructors' evaluations of the six selected external representations of protein translation, ER2, ER3, and ER6 were chosen as the static; stylized, dynamic; and realistic, dynamic representations, respectively, which were then shown in sequenced pairs to the student participants during the student interviews (see Chapter 4, Chapter 7, and Appendix C).

Instructor Perceptions of the Types of Variation between Features

Variation theory describes contrast as a significant pattern of variation. Contrast allows the individual to compare an object of learning or a feature of that object with something it is not. This allows the individual to create meaning for an object or feature by defining it against things that are different from it. By identifying differences in the appearance of one feature as compared to another feature, a student would be able to differentiate between features by assigning each a unique identity based on appearance. The instructors described two aspects of variation that were used to distinguish one feature in a representation from another feature: color and shape.

Color

Color was identified by all instructors as a useful representational cue used to distinguish between features. For example, Instructor 4 notes that:

> [00:15:28.5] **Instructor 4:** It sounds a little superficial, but of course, I mean, if you're a student you know it's not. Intelligent use of color in a diagram is enormously important for understanding.

Later on in the interview, he says:

> [00:44:17.7] **Instructor 4:** If you are going to use color coding effectively […] use colors where you want a relationship to be noticed and then don't gum it up with a bunch of more colors […] that aren't really trying to tell you anything in particular.

Similarly, Instructor 3 notes that color can draw students' attention to certain features, but if it is not clear what those colors or features are meant to represent or why they are being distinguished from one another, than students may not be able to fully use differences in color to identify specific features.

> [00:17:54.4] **Instructor 3:** [In response to ER1] I think the, the color will draw their attention but I think the color scheme is gonna, gonna, eh, eh, confuse them.

Instructor 1 has had previous experience consulting on the development of figures for a biochemistry textbook. He notes that color is generally used in textbook figures to specifically identify various features of a representation and distinguish one from another even if they are closely related, as seen in the following exchange after he had viewed both ER1 and ER2.

> [00:39:07.6] **Researcher:** So a couple things that you mentioned, um, in [ER1], one of the critiques was that there was the use of too many colors, whereas in [ER2] you mentioned that the green specifically meant, um, RNA, and are there coloring cues here you picked up on or that you liked better or worse compared to the first one?
> [00:39:27.1] **Instructor 1:** Yes, so, so look, for one thing the three ribosomal sites are all, are all the same basic color but in three shades, so that they represent the same kind of thing, sites, but not the same thing. The, ah, color code to indicate the amino acid groups as they are attached to the tRNA here is carried through into the cartoon […]. One of things you can't tell by looking at a single figure is whether this is internally consistent with the others [in the textbook]. It must be. I mean it's absolutely terrible to switch colors or switch conventions. You must never do that, ever.

Shape

Shape was also cited as an indicator of feature identity within a representation. For example, Instructor 2 notes that shape, like color, can be used to distinguish between features; however, shape is only useful if it has first been defined as a particular feature.

> [00:22:15.6] **Instructor 2:** I think color and shapes help [students learn] as long as you lead into these details with an overview that sort of gives students a sense that the ribosome's a big thing. It has a shape to it, and these different icons we're using later on, you mean something. I think the shape and everything work pretty well. Um, if you don't do that, I think the shapes are worthless.

Thus, a square is just a square until you are told that a square represents a particular feature.

Instructors also noted that similarity in appearance, both shape and color, could lead to confusion as to the identity of each feature. For example in ER4, the resulting

polypeptide chain is similar, in both color and shape, to the mRNA strand (see Figure 18). Instructor 3 notes that:

> [00:35:00.4] **Instructor 3:** [In response to ER4] The protein doesn't look, it looks exactly the same as, eh, the mRNA. The same type of shape.

Instructor 4 also describes the possible confusion caused by the similarity between the representations of the protein and mRNA in ER4.

> [1:05:16.5] **Instructor 4:** At the end [of ER4] you see that the polypeptide has these color coded bits and they correspond to the messenger RNA with the same, the codons are colored, and possibly some [students] could have the misunderstanding that they were sort of similar or identical rather than correspondent.

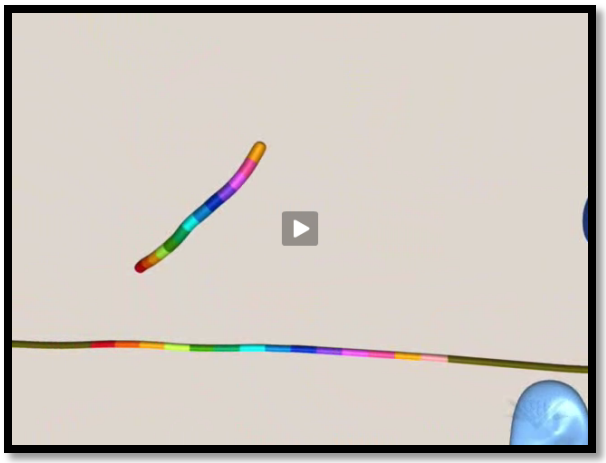


Figure 18. External Representation #4, Frame 82.

According to the instructors, students should be able to distinguish between the different components presented in representations of protein translation because those components are presented with different shapes and in different colors relative to each other. On the other hand, students should also understand that if a given component maintains the same color and shape, that component has not changed over the course of the process presented in the representation. If color and shape were to change, that would indicate that

something about that feature has changed. As described earlier by Instructor 1, "it's absolutely terrible to switch colors or switch conventions. You must never do that, ever."

Representational consistency, in color and shape, was also cited as a complicating factor when using multiple representations from different sources to teach a particular topic. For example, Instructor 3 notes that:

> [00: 26:27.4] **Instructor 3:** The problem that I've, that I'm finding with [showing a static representation followed by a dynamic representation] is that it's hard to find a static representation and a [dynamic] representation that uses the same scheme. […] Sometime I actually get schemes that are opposite, so I have to start again and that sometimes makes it more confusing [for the students].

Therefore, instructors felt that students use invariance in feature color and shape to assign feature identity and decode a representation. They expressed concern that differences in color or shape between different representations would be confusing for students. For example, would students be confused if the mRNA stand in one representation of protein translation was presented as a black string while that same mRNA strand was presented as a string consisting of sections with alternating colors? Would they know that both of those objects represent the same biochemical component?

In summary, according to the instructors, the shape and color of a given feature do not vary over the course of a biochemical representation. Therefore, I did not account for these aspects in the VADER coding method, since the VADER method focuses on aspects of representations that have the potential to change over the course of a representation.

Other Types of Variation Used for Visual Cueing

The instructors also discussed three other aspects of variation that could act as potential cues to draw viewer attention to particular features of a representation: changes

in relative position, changes in relative size, and the use of alphanumeric labeling. Because these aspects could vary in a given biochemical representation, I have chosen to account for changes in them in the VADER coding of the selected external representations.

Changes in relative position. Protein translation is a dynamic process. In order to depict the dynamics of the process, the selected external representations of protein translation show changes in the relative positions of the component features throughout the representation as indications of the movement of component parts during the various stages and of specific interactions between those component parts during the process. Several of the instructors noted this change in position of particular features. For example, Instructor 3 described the movement of the ribosome relative to the mRNA, the sequential introduction of amino acids relative to each other, and the corresponding codon/anti-codon base pairing interactions that were depicted during elongation in ER3.

> [00:30:07.9] **Instructor 3:** [In response to ER3] I like the fact, though, that, that it has more, it's, it's moving along the mRNA, so you can see one amino acid at a time, one code being deciphered at a time. […] That's actually nice. If you have multiple steps, you can see one step at a time.

Although ER3 is an animation and was identified as such because of the relative motion of depicted features, relative change in position could also be seen in the static representation, ER2. For example, Instructor 1 described the relative change in position of the dipeptidyl-tRNA from the A-site to the P-site in a series of frames in ER2 depicting ribosomal translocation (see ER2_5 and ER_6 in Appendix H: Frame Sequence of External Representation #2).

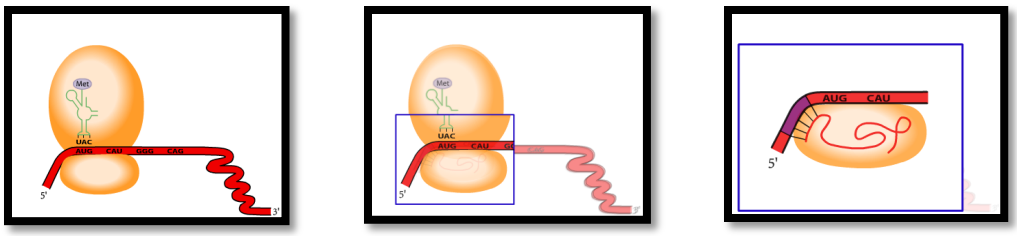
> [00:34:45.9] **Instructor 1:** [In response to ER2] I see here that the dipeptide is attached to the tRNA that is initially in the A-site, and then I see something happening here that, um, in which the dipeptide tRNA becomes associated with

> the P-site, so I see that there's been a jump that took place there, and I see that that jump was accompanied by GTP hydrolysis. That is the energy step.

Thus, change in position can serve to call attention to the relative movement and subsequent interactions of component features of a representation.

Changes in relative size. In addition to change in position, a change in the relative size of features was also noted as a possible means by which to call a viewer's attention to a particular component or interaction of components in a representation. For example, Instructor 3 responds to the change in relative size when ER3 zooms in on the alignment of the 16S rRNA of the small ribosomal subunit and the Shine-Dalgarno sequence of the mRNA (see Figure 19). He notes that the change in size would focus students' attention on the interaction of these components.

> [00:29:27.3] **Instructor 3:** [In response to ER3] The, the binding to the, to the beginning of the mRNA, it's the Shine-Dalgarno sequence, eh, eh, it's too much. There's now too much information in there, which distracts form the most important part of the system. [Students] would think this is the most important part, and now that part, which is the actual reaction of the system is not shown very well.



ER3_7 ER3_8 ER3_9

Figure 19: External Representation #3: Frames 7 - 9

Although Instructor 3 does not like that ER3 has zoomed in on the particular interaction between the Shine-Dalgarno sequence and the small ribosomal subunit, he acknowledges that this change in the size of the component features would cause students to attend to

those features and potentially notice them more after they have returned to their original size because their change in size has indicated that they are an important component of the representation. He goes on to note that the interaction he feels is more important, i.e., peptide bond formation, is glossed over in ER3. To remedy this lack of focus on the chemistry, he suggests that a second zoomed in scene be added to ER3 in order to focus students' attention on that interaction as well.

> [00:31:03.4] **Instructor 3:** [In response to ER3] I would have included, at least in the first peptide, peptide bond synthesis, a blow up showing how the reaction actually happens.

Instructor 2 makes a similar recommendation.

> [00:39:05.7] **Instructor 2:** [In response to ER3] The first time one of [the amino acids] moves over, maybe have a frame come up and, and hone in on that and show the chemistry, show why it's moving over. Um, that might be a nice thing to do. […] In terms of the basics, though, it's not bad. Um, it might be, the only things I would add is, is a frame that really showed the chemistry going on.

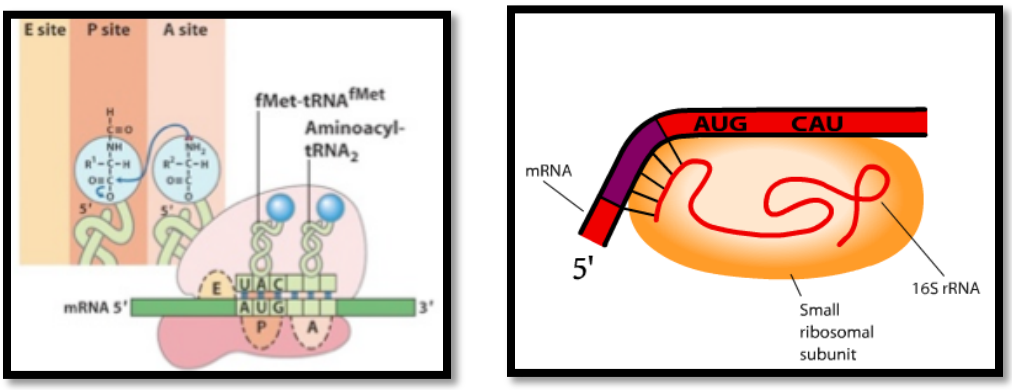
Thus, change in size was identified as an aspect of variation of the selected representations that could potentially call students' attention to particular components or interactions of protein translation.

Changes in labeling. Instructors also identified alphanumeric labels as a source of effective student cueing within external representations. For example, sequential information labels in static representations of dynamic processes, such as protein translation, can cue students to the appropriate time-lapse progress of the information presented within the representation. Instructor 1 notes that:

> [00:23:38.2] **Instructor 1:** Uh one of things that I discovered about illustrations that distinguishes good ones from bad ones is that there are lots of beautiful illustrations in textbooks that don't tell your eye where to start, and I've discovered that students really like it if a process, a complicated process like translation, is, uh, described in numbered steps, so your eyes look at this complicated piece of art and it follows, and at each point you are able to say yea I

see, I see that. I think that you can do on paper pretty effectively and to the extent that you can add that element to, um, an animation on a screen I think that is very good. Your eye just can't be every place all at the same time, and, uh the temptation with modern molecular graphics is that they're so damned beautiful that you try to show everything all at once, and it doesn't work.

Although sequential labels can be used to indicate the progression of time through or staging of the process, labels can also be used simply to indicate identity. For example, ER2 and ER3 both use labels to name specific features. The green bar in ER2 and the red bar in ER3 are both labeled "mRNA" (see Figure 20). Thus, letters and numbers were used to add additional information to or clarify information presented in some of the external representations.



ER2_3 ER3_10

Figure 20. Labeling in External Representation #2 Frame 3 and External Representation #3 Frame 10.

Identification of Non-critical Features

In their comments about the different external representations, instructors identified 59 features of protein translation that are critical for developing correct understandings of that concept (see Chapter 5). When I undertook an independent analysis of the representations, I identified six additional features of protein translation that the instructors had not mentioned. Because the instructors did not mention these

features in their comments, I did not consider them to be critical for learning about protein translation; however, I did track the presence and variation of these features cueing, as they could have been features that contributed to student learning. The first additional feature was *Nucleotide sequence (stop codon)*. This feature was identified in ER3 (Figure 21). I distinguished this feature from the previously identified feature *Stop Codon* to acknowledge the additional information provided by the presence of the nucleotide sequence. This code is in line with the nucleotide sequence codes for the start codon and multiple codons. As a further clarification of the stop codon, this feature was added to the mRNA general feature category.

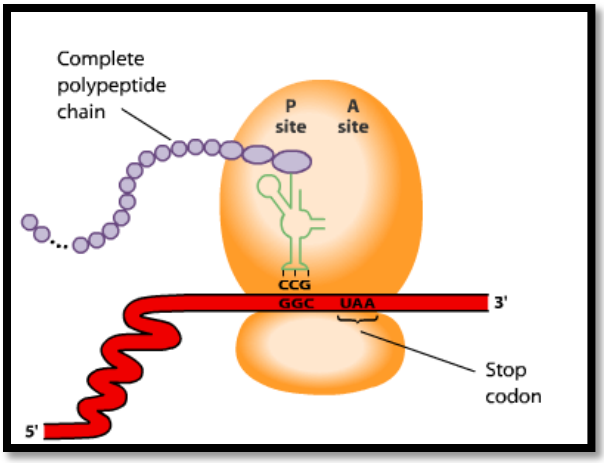
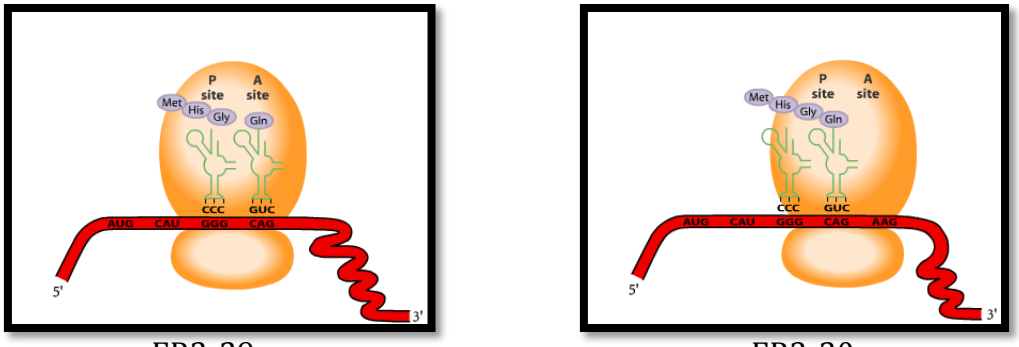


Figure 21. Identification of the stop codon nucleotide sequence in External Representation #3 frame 34.

The second additional feature was *AA/AA Interaction*, which is an indication of amino acid/amino acid interaction. During my analysis of the representations, I noticed while peptide bond formation was only depicted in ER2, amino acids were shown to be interacting with one another to form a chain of amino acids in ER3 (see Figure 22). I determined that students would not be able to learn about peptide bond formation from

this type of depiction (ER3); however, they would be able to identify that the amino acids in the A- and P-sites interact in a particular way in order to form the growing polypeptide. Thus, the additional code was added to indicate the more general depiction of amino acid interaction during peptide bond formation. As this code was a non-chemical description of the peptide bond formation feature, this feature was included in the general feature category of Elongation as an indication of the stage in which the feature was found to be present.

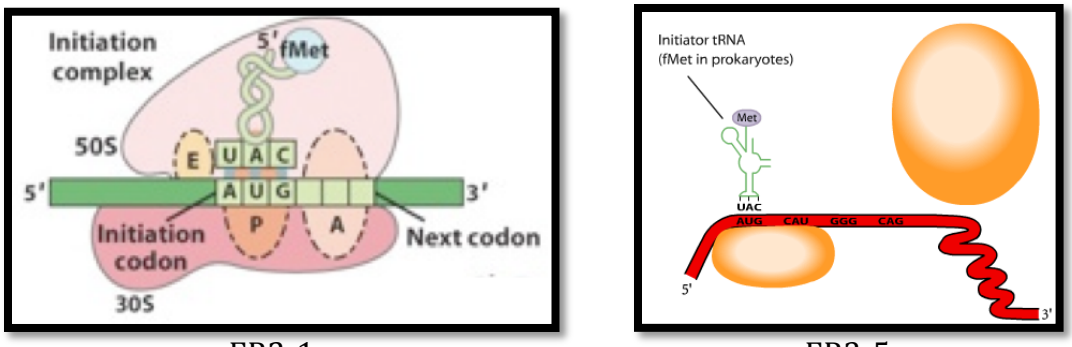


ER3_29 ER3_30

Figure 22. Identification of amino acid interaction in External Representation #3 frames 29 and 30.

The third additional feature was *Initial tRNA/ribosome/mRNA*. Instructors had previously identified the interaction between the tRNA, the ribosome, and the mRNA during elongation; however, both ER2 and ER3 depict the existence or formation of the initiation complex, i.e., the interaction between the mRNA, the small ribosomal subunit, the initiator tRNA, and the subsequent recruitment of the large ribosomal subunit that occurs specifically during the initiation stage of translation (see Figure 23). In order to distinguish the interaction between the initial tRNA and the translational machinery during initiation and subsequent tRNA/translational machinery interactions during the

elongation phase of protein translation, the code *Initial tRNA/ribosome/mRNA* was developed.



ER2_1 ER3_5

Figure 23. Identification of the initial tRNA/ribosome/mRNA feature in External Representation #2 frame 1 and External Representation #3 frame 5.

I identified a fourth non-critical feature in ER3: *Hydrogen Bonding (mRNA/Ribosome).* The instructors had previously identified the interaction between the 16S rRNA of the small ribosomal subunit and the Shine-Dalgarno sequence as part of the general initiation interaction. In ER3, this interaction is depicted; however, specific hydrogen bonds are indicated between the two components (see Figure 24). Thus, this hydrogen bonding interaction could be noticed by students and incorporated into their understanding of the process of protein translation. Both the *Initial tRNA/ribosome/mRNA* and *Hydrogen bonding (mRNA/Ribosome)* codes are related to processes that occur during the initiation stage of translation and were correspondingly added to the Initiation general feature category.

The fifth additional feature was *General process T*. Although ER2 depicted mainly elongation, ER3 and ER6 both depicted the termination stage of protein translation. The instructors had previously identified the presence of the release factor, but the termination stage of the process was not addressed. As such, *General process T*

was included in the list of codes to identify the general process by which the polypeptide is released and the translational machinery dissociates. *General process T* was designated as its own general feature of *Termination*, which is in line with the other stage designations of Activation, Initiation, and Elongation previously identified.

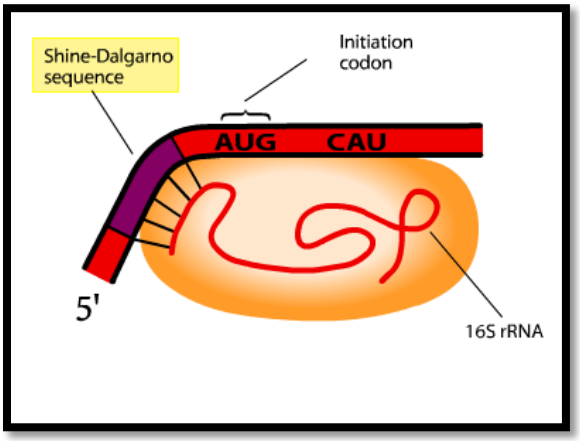


Figure 24. Identification of hydrogen bonding (mRNA/ribosome) in External Representation #3 frame 12.

The last non-critical feature I added to the code list was *Cellular environment (Cytoplasm)*. ER2 and ER3 both present the process of protein translation on a white background. However, ER6 depicts a general cellular environment in the background and the process and components of protein translation in the foreground (see Figure 25). The additional information of the cellular location and component interactions not directly involved in translation could be noticed and incorporated into students' understanding of the process. *Cellular environment (Cytoplasm)* was added to the General Considerations features as this additional information does not address either a component or stage of translation.

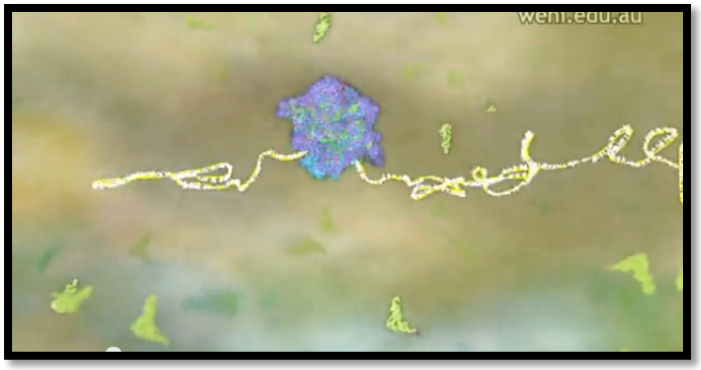


Figure 25. Identification of the cellular environment (cytoplasm) in External Representation #6 frame 12.

Variation Analysis of Dynamic External Representations (VADER)

In order to evaluate the possibilities for student learning about protein translation from the three instructor-selected representations, it was first necessary to develop an appropriate and theoretically aligned analysis method to evaluate those representations and compare them to one another. I developed the Variation Analysis of Dynamic External Representations (VADER) method in order to 1.) identify which features, critical or otherwise, were present in a given representation, and 2.) identify the relative cueing ability of features deemed present based on the amount of variation depicted in the representation as determined by changes in position, size, or labeling (see Chapter 4). By cueing ability, I am referring to the ability of change in an aspect of variation of a feature depicted in a representation to call a viewer's attention to that feature. For example, if a feature changes size, that change in feature size might cue a viewer to notice that feature. Features that change more than others may have more cueing ability and may be more noticed than features that change less or not at all.

In the following three sections pertaining to ER2, ER3, and ER6, I will describe the features that are present in each of the representations. Specifically, I will discuss the

component features, the interaction features, and then the general consideration features. I will then discuss shifts in potential cueing focus over the course of the representation. Then I provide a summary of the overall findings and focus of each particular external representation.

I will use this information in Chapter 7 to analyze which features students were able to learn from which representations. However, more generally, if instructors want their students to learn something specific about protein translation from their interactions with a representation, but that feature is not present in the representation then students are not going to be able to learn about that feature. Thus, analysis of the features provides a measure of insight into the possibilities for student learning from these representations, i.e., the enacted object of learning.

Variation Analysis of External Representation #2

Static representations, such as ER2, pose a potential problem when conducting variation analysis. Specifically, nothing is varying over time in the representation. The image is static. However, protein translation is not static. It is a dynamic cellular process. As such, static representations of dynamic processes contain dynamic indicators. In the case of ER2, change over time is indicated through the use of arrows and the relative positioning of storyboard frames within the representation. Using the head and tail of the arrows, left to right and top to bottom reading conventions, and knowledge of the process in order to determine the sequential ordering of the information presented in ER2, six frames were identified (see Appendix H: External Representation #2 Frame Series). These six frames depicted six points in time presented in the static representation. I analyzed these six frames to create a VADER plot indicating the potential cueing ability

of each feature identified as being present over the course of the frame series (see Appendix I: VADER Plot of External Representation #2). A frame is defined as a snapshot of a representation indicating a specific moment in time. Frames in the frame series are arranged chronologically.

Forty-one features were identified as being depicted in ER2 (see Appendix J: Average Cueing of External Representation #2). The presence of a feature was coded using the VADER method for possible visual cueing potential, i.e., the higher the percentage, the more that feature varied and the greater the possibility a viewer would be cued to notice it. Percent cueing potential was color-coded with black indicating 100% cueing potential, i.e., all aspects of variation changed, white indicating 0% cueing potential or the absence of that feature, and a grey scale to represent the relative cueing ability for the remainder of the cueing percentages.

I identified all sixteen of the primary critical features in ER2. I also identified several secondary features from each of the general component categories in ER2. Among the component features, the mRNA was depicted, along with individual designations of two codons, one of which was the start codon containing the AUG start codon nucleotide sequence. The mRNA also featured polarity indicators noting the relative 5' and 3' orientation of the molecule. The ribosome was depicted as a multicomponent object, with different subcomponents depicted in various shades of orange. This depiction included the large and small subunits as well as the A-, P-, and E-sites. Three unique tRNAs were identified in green, including the initiator tRNA and two subsequent tRNAs. All tRNAs depicted a loose representation of the three dimensional L-shaped structure of a tRNA and included a depiction of the anti-codon loop. Similar to

the mRNA, strand polarity was also indicated by 3' and 5' end designations. Additionally, specific orientations of the tRNAs in the A-, P-, and E-site were shown. Amino acids, including the formylmethionine on the initiator tRNA as well as two subsequent general amino acids, were depicted in blue. The polypeptide chain and its primary sequence were also depicted in ER2. The final component features identified in ER2 were the elongation factors EF-Tu, EF-Ts, and EF-G. The only general component features not depicted by ER2 were the initiation and release factors. This can be attributed to the focus of ER2 on the process of elongation.

Among the interaction features, activation and termination were not depicted in ER2. However, initiation and elongation were. Codon/anti-codon base pairing with specific indications of hydrogen bonding between the initiator tRNA and the mRNA start codon were depicted. Additionally, the general interaction of the initiation complex involving the mRNA, ribosome, and initiator tRNA was shown. In a subsequent frame of the representation, all of the features of elongation were depicted with peptide bond formation drawing the most potential cueing, i.e., that was the feature that varied the most and would potentially be noticed most by a viewer. There were no general consideration features depicted in ER2. As a result, this representation can be seen to focus primarily on the component parts of translation and the general chemistry of initiation and elongation.

The VADER plot of ER2 (Figure 26) shows the condensed nature of the presentation of features presented in ER2. Multiple features are being shown at once. Additionally, several features are being varied across multiple aspects simultaneously. There are multiple changes in position, size, and labeling for various features at the same

VADER	Plot								
Feature	ER2		Frame	1	2	3	4	5	6
	Components/Structure	mRNA	General molecule M						
			Codon						
			Nucleotide sequence (multiple codons)						
			Shine-dalgarno seuqence						
			5' end M						
			3' end M						
			3' poly A tail						
			5' methylated cap						
			Start codon						
			Stop codon						
			Nucleotide sequence (start codon)						
			Nucleotide sequence (stop codon)						
		Ribosome	General molecule(s) R						
			Large subunit						
			Small subunit						
			Aminacyl (A) site						
			Peptidyl (P) site						
			Exit (E) site						
			16S rRNA						
			Tunnel						
		tRNA	General molecule(s) T						
			Anti-codon loop						
			P-site tRNA						
			A-site tRNA						
			3' end T						
			E-site tRNA						
			3D shape						
			2D shape						
			5' end T						
			Nucleotide sequence (Anti-codon loop)						
		Amino Acids	General molecule(s) AA						
			Methionine						
			Sequential AA						
		Polypeptide chain	General molecule P						
			Primary structure						
			Secondary structure						
		Initiation Factors	General molecule(s) IF						
		Elongation Factors	General molecule(s) EF						
			EF-Tu						
			EF-Ts						
			EF-G						
		Release Factors	General molecule(s) RF						
	Interactions/Chemistry	Activation	General process A						
			Regeneration of activated tRNAs						
		Initiation	Condon/Anti-codon base pairing I						
			General process I						
			Hydrogen bonding (codon/anticodon) I						
			Hydrogen bonding (mRNA/ribosome) I						
			Initial tRNA/ribosome/mRNA						
		Elongation	Peptide bond formation						
			Condon/Anti-codon base pairing E						
			General process E						
			Incoming tRNA/ribosome/mRNA						
			Exiting tRNA/ribosome/mRNA						
			Ribosomal translocation						
			GTPase activity of EFs						
			Hydrogen bonding (codon/anticodon) E						
			AA/AA interaction						
		Termination	General process T						
	General Considerations		Reaction Kinetics						
			Evolution						
			Regulation						
			Random motion of cellular components						
			Energetics						
			Cellular Environment (Cytoplasm)						

Figure 26. VADER plot of External Representation #2

time. As a result, ER2 may be potentially cognitively overwhelming to a viewer. Frame 1 contained the fewest number of features with 23, and frame 6 contained the highest number of features with 37. An average of 31 features were depicted per frame. The features with the highest cueing potential initially are the mRNA, ribosome, and initiator tRNA. The cueing then shifts to the incoming second tRNA as assisted by the elongation

factors EF-Tu and EF-Ts. Then, the cueing focuses on those P-site and A-site tRNAs and the resulting peptide bond formation reaction. Finally, the cueing shifts to the introduction of EF-G to cause ribosomal translocation and to the introduction of a new incoming tRNA. Throughout the representation, the majority of features that are introduced remain in view, although many continue to vary, throughout the remainder of the representation. This results in a large number of features to which a viewer could attend by the end of the representation.

Variation Analysis of External Representation #3

I took screen shots ("frames") of the ER3 animation at two-second intervals, which resulted in a 43-point frame series (see Appendix K: External Representation #3 Frame Series). I then conducted a VADER analysis of ER3 and constructed a corresponding VADER plot (see Appendix L: VADER Plot of External Representation #3). I identified 41 features in ER3 (see Appendix M: Average Cueing of External Representation #3). Although many of these features are the same as those depicted in ER2, they are not all the same.

ER3 depicts 14 of the 16 primary critical features. The mRNA was depicted as a red bar with some minor indications of flexibility on the ends; however, in general, the mRNA was depicted as a stationary molecule, similar to its portrayal in the static representation, ER2. ER3 depicts a series of individual codons, including the start and stop codons with sequence information. mRNA strand polarity is also depicted. The ribosome was depicted as an orange multi-component structure, similar to the manner in which this component is presented in ER2. The large and small subunits were identified along with the A- and P-sites. There was no E-site indication in ER3. The tRNA were

identified as green lines depicting the two dimensional cloverleaf structure of the tRNA. A clear indication of the anti-codon loop with specific sequence information was identified. Additionally, the relative A- and P-site positioning of the tRNA was depicted; however, due to the lack of an E-site, the E-site position was not clearly discernable. Amino acids, including methionine and multiple sequential amino acids, were depicted as small grey ovals. Additionally, the polypeptide chain and its primary sequence were also depicted. The final component feature identified in ER3 was the release factor. The initiation factors or elongation factors were not depicted in this representation.

Three of the four stages of translation were identified in ER3. Activation of the tRNAs was not depicted. Initiation and, in particular, the assembly of the initiation complex was depicted. This included the interaction of the initiator tRNA, the small ribosomal subunit, and the messenger RNA. Specifically, the depiction of the hydrogen bond interaction between the Shine-Dalgarno sequence of the mRNA and the 16S rRNA of the small ribosomal subunit was identified. Additionally, the codon/anti-codon interaction between the start codon and the anti-codon loop of the initiator tRNA was shown.

During the depiction of elongation, amino acids were shown to interact with one another to form the growing polypeptide chain; however, peptide bond formation was not depicted. Codon/anti-codon interactions between the subsequent tRNAs and the mRNA were shown, along with the relative positioning of incoming and exiting tRNAs. Although ribosomal translocation was depicted, there was not an indication of the role of EF-G or its GTPase activity in this process. Finally, termination was shown: as the release factor entered the A-site, the polypeptide chain was released, and the translational

machinery dissociated. ER3 did not include any of the features in the General Consideration category. ER3 contained all of the primary critical features of translation except for the E-site of the ribosome and a depiction of peptide bond formation. As a result, this representation can be seen to focus primarily on the components and their general interactions and less on the underlying chemistry of translation.

Because of the size of the VADER plot for ER3, I have chosen to place it in the Appendix (see Appendix L). Please refer to that section during the following discussion. Frames 9-14 contained the fewest number of features with 11 each; and frames 21, 22, 25-27 contained the highest number of features with 28 each. An average of 21 features were depicted per frame. The VADER Plot shows four general regions of focus. Note the distinct shift in features that occurs at frame 9, around frame 14 and around frame 33. The same general components are presented from frame 1 through frame 8. During this initial scene, the mRNA, ribosome, and initiator tRNA, along with the corresponding methionine are assembling to form the initiation complex. High cueing potential can be seen for the sequence information in the codon and anti-codon along with the mRNA strand polarity and initiator amino acid.

The change in the VADER plot from frame 9 through about frame 14 reflects a scene change during the representation. More accurately, the initial scene zooms in to focus on the alignment of the mRNA and the small ribosomal subunit. The cueing focus jumps from one feature to the next as individual features are labeled in the animation. The shift in the VADER plot around frame 14 reflects the zooming out of the representation back onto the original initiation complex. This third scene, from about frame 14 to approximately frame 33, depicts the elongation process. Aminoacylated-

tRNAs enter the ribosome in the A-site and progress to the P-site as the amino acids interact to form the growing polypeptide change. The cueing potential during this scene is focused on the A- and P-site as the ribosome moves along the mRNA strand. The amino acids also have high cueing potential because of the variation in their position that occurs during this scene.

The final scene begins around frame 33 as the ribosome stops moving along the mRNA and tRNAs stop moving through the ribosome. This scene depicts the termination of the protein translation process as the release factor comes in and the polypeptide chain is released. The ribosome, mRNA, and tRNA then dissociate from one another. The polypeptide chain and the release factor both have high cuing potential during this scene. This stage-wise organization of scenes in the stylized, dynamic representation resulted in fewer features being depicted at any one time and a general spreading out of cueing events over the course of the representation.

Variation Analysis of External Representation #6

I took screen shots ("frames") of the ER6 animation at two-second intervals, which resulted in a 74-point frame series (see Appendix N: External Representation #6 Frame Series). I then conducted a VADER analysis and a corresponding VADER plot for ER6 (see Appendix O: VADER Plot of External Representation #6). I identified 24 features in ER6 (see Appendix P: Average Cueing of External Representation #6). This was significantly fewer features than I identified in either ER2 or ER3. Additionally, while ER6 contains nearly half of the number of features present in ER3, ER6 was nearly twice as long as ER3. Frames 1-3 contained the fewest number of features with 2 each,

and frames 24-28 contained the largest number of features with 20 each. An average of 9 features was depicted per frame.

ER6 depicted 10 of the 16 primary critical features. The mRNA is depicted as a yellow strand with white, yellow and green depictions of codons. The ribosome is depicted as a blue small subunit and a purple large subunit. The use of the same tonal family indicates a relationship between the subunits and distinguishes it from the mRNA. Unlike previous depictions of the ribosome in ER2 and ER3, there was no representational depiction of the three tRNA binding sites. Green tRNAs are shown floating around the ribosome/mRNA complex. The tRNAs were shown in the three dimensional L-shaped configuration with a red amino acid attached to one end. The red amino acids varying in shape depicting they various types of amino acids. The polypeptide chain was depicted and secondary structure was also shown. However, the primary structure was not clearly discernable. No translation factors were depicted in ER6.

Among the interaction features, ER6 was the first representation to depict the activation of tRNAs. Initiation was also depicted; however, the assembly of the mRNA and ribosome occurred very quickly and no initiator tRNA was depicted. The largest number of frames was focused on elongation with particular emphasis on ribosomal translocation. Termination was only partially indicated by the release of the folded polypeptide chain.

ER6 also contained depictions of two general consideration features. First, the cellular environment of the cytoplasm was depicted throughout this representation. Second, the component parts of translation, in particular the tRNAs, were shown to

interact in a somewhat random fashion. Together, these features provided a more realistic representation of the components and interactions of translation. However, the limited number of features depicted overall provided a fairly general overview of the process.

Due to the size of the VADER plot of ER6, please refer to Appendix O during the following discussion. Similar to ER3, the VADER plot of ER6 shows scene shifts as the representation progresses. I identified six unique scenes in ER6 from my analysis of the VADER plot. The first scene, depicted in frames 1 through 15, shows the cellular location of the messenger RNA as it combines with the small and large ribosomal subunits. This scene shows the relative sizes of the mRNA, ribosome, and the tRNAs and shows the general stages of initiation and elongation. Frames 16 through 22 depict the general relationship between the ribosome and the mRNA, as well as their general movement. The third scene, depicted in frames 23 through 28, shows the progression of tRNAs through the ribosome, as seen in a cut-away view. The fourth scene, depicted in frames 29 through 33, shows the entrance of the tRNAs and the threading of the mRNA through the ribosome. This scene provides a contrast between the cut-away view in order to convey the spatial arrangement of the mRNA, ribosome, and tRNAs. The fifth scene, depicted in frames 34 through 42, focuses on the tRNA/amino acid pairing. Although the specific process of aminoacylation of the tRNAs is not depicted, this scene provides the general sense that tRNAs and amino acids are paired. The final scene, depicted in frames 43 through 74, shows the growing polypeptide chain as it exits through the top of the large ribosomal subunit. As the protein emerges, it folds and changes its shape in an indication of secondary structure. Also during this scene, termination is only briefly addressed when the folded protein is released and floats out of frame.

I identified very few highly cued features in ER6. There were, however, some features that were more generally cued throughout the animation. For example, the cellular context of protein translation is depicted throughout the animation. The structure of the mRNA and codons are cued. Additionally, the structure of the large and small subunits of the ribosome and the tRNAs are generally cued. Although elongation is the only stage that is significantly cued, there are no indications of the chemistry (i.e., the reactions) involved in protein translation. Thus, this representation can be viewed as giving a general overview of the protein translation process, with an emphasis on cellular context, structural relationships, and general component interactions.

Comparison of the Three Selected External Representations

ER2, ER3, and ER6 share 17 features in common. These features are highlighted in green in Appendix Q: Comparison of the Average Cueing of the Selected External Representations. Ten of those features align with the primary critical features identified by the instructors. These features include a general depiction of the mRNA molecule as well as specific codon representations. The ribosome was depicted as a general structure with designations of the small and large ribosomal subunits. A general depiction of the transfer RNAs were also presented in all three representations. The depictions of the tRNA included the A-, P-, and E-site positioning of the tRNAs relative to the mRNA/ribosome complex and the codon/anti-codon base pairing interaction between the tRNAs and the mRNA. Although not all of the representations included depictions of the A-, P- and E- sites on the ribosome, the relative positioning of the tRNAs within the mRNA/ribosome complex implies the presence of these tRNA binding sites. A general

depiction of the amino acids and the resulting polypeptide chain were also identified in all three representations.

Eight critical features were not presented in any of the representations. These features are highlighted in red in Appendix Q: Comparison of the Average Cueing of the Selected External Representations. All of these non-depicted features were secondary or tertiary critical features. The presence of 10 of the 16 primary critical features in all three representations indicates that the representational designers' intentions were similar to those of the instructor participants. Additionally, the lack of depiction of the certain features, i.e., those features highlighted in red in Appendix Q: Comparison of the Average Cueing of the Selected External Representations, supports the original categorization of these features as secondary or tertiary features.

The remaining 38 features are depicted in one or two of the three representations. Among these features is the lack of a common depiction of two of the primary critical features, peptide bond formation reaction as well as the three tRNA ribosomal binding sites. ER2 and ER3 both depict the A- and P-sites of the ribosome. Only ER2 depicts the E-site, and ER6 does not contain a depiction of any of the binding sites. As a result, all three representations show a different depiction of these sites. The inconsistent depiction of these three primary critical features shows a lack of alignment between instructors' intentions for student learning and the enacted object of learning as constrained by these external representations.

Peptide bond formation was identified as being a primary critical feature of protein translation. In fact, instructors made particular mention of the importance of "the chemistry" in the biochemical discussion of protein translation. Despite this fact, peptide

bond formation was not specifically depicted in all three representations. Only ER2 provides a depiction of the peptide bond formation reaction. Neither ER3 nor ER6 indicate the bond formation reaction. Furthermore, ER6 does not clearly depict that amino acids interact in the formation of the polypeptide chain. Although all three representations depict the growing polypeptide chain, the biochemical mechanism underlying its formation is not consistently shown. This suggests that neither ER3 nor ER6 can provide the appropriate space of learning from which a student would be able to learn about the chemistry of protein translation.

A comparison of the VADER plots of ER2, ER3, and ER6 also reveals significant differences in the overall structure of each representation (Figure 27). Figure 27 contains a lot of information. My intention in showing this figure is to demonstrate the overall difference in appearance of these three plots. The VADER plot of ER2 shows that most features remain present over the entire representation with the addition of new features as the representation progresses. The heavy initial cueing seen in ER2 could potentially split a viewer's attention between any number of those features. ER3 and ER6, on the other hand, depict a series of more focused "scenes," as seen in the dramatic shifts in features that are present during different segments of the representation. These individual scenes show a limited number of features and then transition to a new scene in which new features or interactions are depicted. The scene progression of ER3 and ER6 generally follows the stages of translation and may help students to develop an understanding of the sequential nature of the process.

In addition to the overall structure, variations in the duration or the number of frames present in a given representation are quite notable among the three

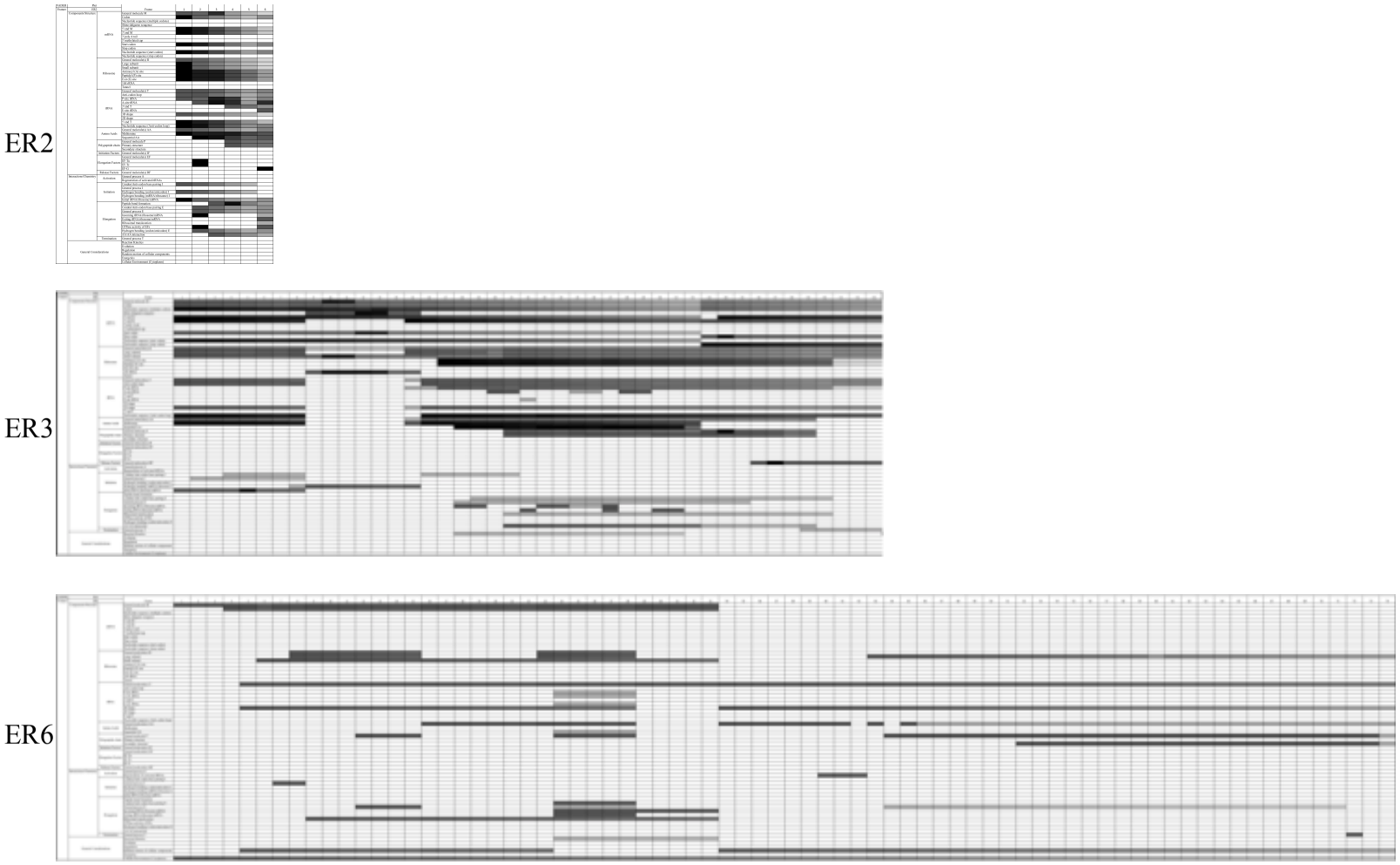


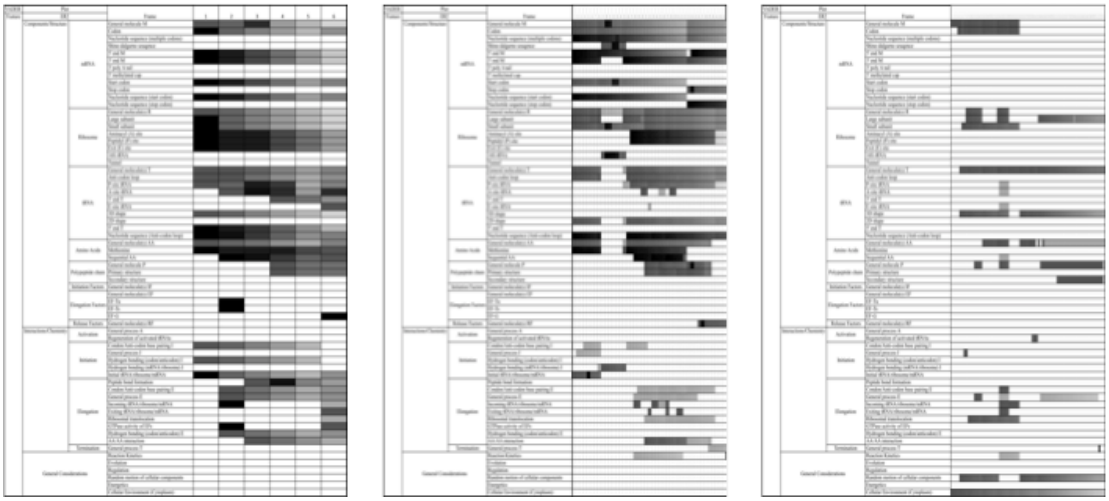
Figure 27. Comparison of VADER plots for ER2, ER3, and ER6

representations. ER2 contains only 6 frames and, thus, presents protein translation in a more compressed manner than either ER3, with 43 frames, or ER6, with 74 frames. However, the number of frames present in a given representation is not related to the number of features that can be presented in that representation. For example, both ER2 and ER3 happen to contain the same number of depicted features, 41 features each, although the number of frames for ER3 is more than seven times greater than ER2. As a result, the VADER plot of ER2 shows a large number of features being depicted simultaneously, i.e., in the same frame. In fact, an average of 31 features are depicted per frame in ER2 as compared to an average of 21 features per frame in ER3. A reduction in the number of simultaneously presented features could lead to improved cueing of selected features in one representation over another.

ER6 is significantly longer than either ER2 or ER3. However, only 24 features are depicted over the duration of ER6. The greater number of frames and the lack of depicted features is reflected in the sparsely populated VADER plot of ER6. There is also a noticeable decrease in the cueing potential of the features that are present in ER6. This is a direct result of the lack of labeling in ER6. This may be a result of the attempt to portray ER6 as a realistic representation of translation. As such, labels are not a naturally occurring feature of the process and were not included in this representation. However, because labeling is one of three aspects of variation that were coded in the VADER plots, ER6 shows a decrease in cueing potential.

The length of the frame series between ER3 and ER6 are directly comparable because they both have frames that correspond to 2-second time intervals of each animation. (All discussions of time are in reference to the viewer's elapsed time not the

amount of time that it would take for the processes depicted in the representation to occur in nature.) However, they are not directly comparable to ER2. Because ER2 is a static representation with dynamic indicators, the elapsed time depicted from one frame to the next in ER2 is not a standard measure of time. An additional comparison between the VADER plots of ER2, ER3, and ER6 was conducted in order to negate the direct measure of time, and instead, examined each representation as a unit with a discrete beginning and end (Figure 28). To construct Figure 28, the VADER Plots of ER3 and ER6 were condensed into the same width as the VADER Plot of ER2. From the unit comparison, it can be seen that potential cueing and present features are generally more dispersed in ER3 and ER6 as compared to the heavy initial cueing in ER2. The reduction in simultaneous depiction of features may help to better direct the cueing potential over the course of the representation in ER3 and ER6 as compared to ER2.



ER2 ER3 ER6

Figure 28. Unit comparison of VADER plots

Summary of the three selected external representations. Overall, a comparison between of the three representations reveals that each of them has a unique set of

depicted features. First, ER2 and ER3 show general similarities in the features presented and the aspects of variation used to create cueing potential for those features. However, ER3 depicts less of the chemistry as compared to ER2. Second, ER2 and ER6 show very little similarity in the material depicted. General component features are found in each, but ER2 shows a more detailed depiction of the chemistry as compared to ER6. Although ER6 depicts a more realistic component structure, it lacks the depiction of subcomponent features and specific chemical reactions found in ER2. Third, ER3 and ER6 show little similarity other than the basic depiction of the general components. As with ER2, ER6 depicts very little subcomponent specificity as compared to ER3. As a result, unless a viewer has detailed knowledge of the process of protein translation, very little information can be learned from ER6, whereas ER2 and ER3 may provide a better space of learning as they depict more features than ER6. Additionally, the features depicted in ER2 and ER3 are more closely aligned with the instructors' intentions for student learning, with many of those features aligning with instructors' primary critical features. On the other hand, fewer of the features depicted in ER6 align with instructors' primary critical features. Overall, ER2 provides the best, and only, representation of peptide bond formation as emphasized by the instructors and, therefore, best aligns with instructors' intentions for student learning about protein translation.

The Influence of the Pairwise Viewing of External Representations

Each representation creates a unique enacted object of learning. Moreover, the pairwise combination of representations as they are presented to students creates an expanded enacted object of learning. As none of the representations depict all of the same features, the introduction of new features by a second representation can create the

opportunity for a viewer to notice and contrast the features of the representations. Additionally, the repeated depiction of the features that a pair of representations have in common could create the opportunity for generalization as the viewer has the chance to compare similar instances of the same feature.

The combined possibilities for student learning from the viewing of two representations, regardless of the order in which those representations are presented, will be the same. Students exposed to the same two representations, regardless of order, are exposed to the same features. For example, students in Groups A and B were both exposed to ER2 and ER3. As such, both groups of students were exposed to the same set of features and have the potential to learn about any of those features. Therefore, presentation order does not limit what is possible for students to learn from the pairwise viewing of two representations (the enacted object of learning); but, as noted earlier, the presentation order may influence what students actually learn from the representations (their lived objects of learning). In this chapter, I focus on the enacted object of learning—the possibilities for student learning—by describing the features that could be noticed by students in the pairs of representations to which they were exposed. In Chapter 7, I will revisit the potential impact of the ordering of representations on students' lived objects of learning.

Enacted Object of Learning Created by External Representations #2 and #3

Students in Groups A and B were exposed to ER2 and ER3 in opposite sequence and share a common enacted object of learning. These two representations share 30 features in common. These features are highlighted in green in Appendix R: Comparison of External Representations #2 and #3. These features include the general mRNA

molecule, the codon, the 5' and 3' ends of the mRNA, the start codon, and the specific nucleotide sequence of the start codon. The ribosome is generally indicated along with the large and small subunits, and the A- and P-sites. The tRNA general molecule, the anti-codon loop and its nucleotide sequence, and the A-, P-, and E-site tRNA positions along with the amino acids, methionine, and the sequential amino acids are also depicted. The general polypeptide chain is shown along with the primary sequence. The codon/anticodon base pairing during initiation along with the initiation complex are depicted as well. Almost all of the features of elongation are depicted except the GTPase activity of the elongation factors. Lastly, a general amino acid/amino acid interaction is depicted.

Features that are only found in one of the pair are highlighted in yellow. These features might cause a potential contrast between the representations and cause the viewer to notice these features. These features include multiple codon sequences, the Shine-Dalgarno sequence, the stop codon and its sequence, the 16S rRNA, and the E-site of the ribosome. The shape and polarity of the tRNAs are shown in one representation along with the elongation factors. The chemistry is also shown in one representation. This includes the general depiction of hydrogen bonding and peptide bond formation. Termination and kinetics are also included in only one of the representations.

Overall, 16 of the features presented in one or both of the representations align with the 16 primary critical features identified by the instructors. Thus, students in Groups A and B have the potential to notice all 16 primary critical features. Features that are not found in either representation and are, therefore, not possible to be learned from the enacted object of learning created by exposure to this pair of representations are

highlighted in red. These include most of the tertiary critical features. The type of feature is identified in the Type column in Appendix R: Comparison of External Representations #2 and #3. NC in this column is used to identified non-critical features. Overall, the enacted object of learning for ER2/ER3 contains a large number of features with the component features having a higher cueing potential, in general, than the interaction features.

Enacted Object of Learning Created by External Representations #2 and #6

Students in Groups C and D were exposed to ER2 and ER6 in opposite sequence and share a common enacted object of learning. These two representations share 18 features in common. These features are highlighted in green in Appendix S: Comparison of External Representations #2 and #6. 16 of those features align with the 16 primary critical features identified by the instructors. These features include the general mRNA molecule and the codon. The ribosome is generally indicated along with the large and small subunits along tRNA general molecule and the A-, P-, and E-site tRNA positions. The amino acids, the sequential amino acids, and the general polypeptide chain are also depicted. Most of the features of elongation are depicted except peptide bond formation, the GTPase activity of the elongation factors and the amino acid/amino acid interaction.

Features that are only found in one of the pair are highlighted in yellow. The vast majority of these features are found in ER2. These features include polarity designations, the start codon and sequence, the ribosomal sites, and the general structure of the tRNA are shown. The methionine, elongation factors, peptide bond formation, and most of the features of initiation and elongation are shown only in this representation.

Features that are not found in either representation and are, therefore, not possible to be learned from the enacted object of learning created by exposure to this pair of representations are highlighted in red. These include most of the tertiary and some secondary critical features. Overall, the enacted object of learning for ER2/ER6 contains fewer features as compared to the enacted object of learning created by the ER2/ER3 pairing. Similarly, most of component features have higher cueing potential than do the interaction features, however, there is some higher cueing among the general consideration features than was seen in the ER2/ER3 pairing.

Enacted Object of Learning Created by External Representations #3 and #6

Students in Groups E and F were exposed to ER3 and ER6 in opposite sequence and share a common enacted object of learning. These two representations share 19 features in common. These features are highlighted in green in Appendix T: Comparison of External Representations #3 and #6. These features include the general mRNA molecule and the codon. The ribosome is generally indicated along with the large and small subunits along tRNA general molecule and the A-, P-, and E-site tRNA positions are shown. The amino acids, the sequential amino acids, and the general polypeptide chain are also depicted. The general process of initiation and most of the features of elongation are depicted except peptide bond formation, the GTPase activity of the elongation factors and codon/anticodon base pairing.

Features that are only found in one of the pair are highlighted in yellow. The vast majority of these features are found in ER3. These features include the Shine-Dalgarno sequence, polarity designations, the start codon, stop codon and their sequences, the ribosomal sites, and the anti-codon loop. tRNA shape is contrasting between these two

representations as well as the depictions of primary and secondary sequence. The methionine, release factors, amino acid/amino acid interaction, and most of the features of initiation and elongation are shown only in this representation.

Overall, 14 of those features align with the 16 primary critical features identified by the instructors.

Features that are not found in either representation and are, therefore, not possible to be learned from the enacted object of learning created by exposure to this pair of representations are highlighted in red. These include most of the tertiary critical features. Overall, the enacted object of learning for ER3/ER6 contains fewer features as compared to ER2/ER3 but a similar number to ER2/ER6. Similarly, most of component features have higher cueing potential than do the interaction features as in the ER2/ER6 pairing. Additionally, the cueing among the general consideration features is similar to the ER2/ER6 pairing.

Chapter Summary

In the chapter, I presented the enacted object of learning as determined by variation analysis of the three representations; ER2, ER3, and ER6. Initial discussion concerning the selection of these representation was presented followed by instructors' discussion of features of variation to which students could potentially pay attention. This discussion was presented as support for the choice of aspects of variation I monitored with the VADER method. I conducted and presented the variation analysis of each of the representations in order to identify the series of features that defined the possibilities of learning within each individual representation. From this discussion, I noted that ER2 contained the most features as well as the highest number of primary critical features.

This suggests that ER2 would be best aligned with instructors' intentions for student learning. I also discussed the enacted object of learning as created by a pair of representations. I noted that even though presentation order was varied between groups, groups that were presented with the same two representations had the same possibilities for learning, i.e., the same number of features was presented to both groups. This comparison and the alignment with the instructors' intentions for student learning will be used in Chapter 7 to compare what students actually learned with what was possible for them to learn and what was intended for them to learn.

CHAPTER 7

ANALYSIS, RESULTS, AND DISCUSSION OF RESEARCH QUESTION 3

The Lived Object of Learning: Chapter Overview

My final objective of this project was to characterize biochemistry students' learning as measured by the change in their lived object of learning. Specifically, Research Question 3 asked: What do second semester biochemistry students learn from external representations of translation? A student's post lived object of learning is individually constructed based on a student's prior knowledge and their perception of the enacted object of learning. Therefore, I identified learning as a change between a students' prior lived object of learning and their post lived object of learning.

In this chapter, I will first establish that the external representations used in this project provided the opportunity for student learning to occur. I will then describe students' prior lived object of learning, i.e., students' prior knowledge of protein translation. Finally, I will discuss what students actually learned (their post lived objects of learning) as it relates to their prior knowledge and the specific enacted object of learning to which they were exposed.

I named the students discussed in this chapter based on the order in which they were interviewed and the experimental group (see Chapter 4, Table 1) to which they were randomly assigned (see Table 6). For example, the first student that I interviewed was randomly assigned to Group E, so I named that participant Student 1(E). The second student I interviewed was randomly assigned to Group C, so I named that participant Student 2(C). Although I did not consider the order in which students were interviewed to be an important factor contributing to their lived object of learning, I did use the group

assignments in the analysis presented in this chapter as this determined the enacted object of learning experienced by each student.

Table 6
Student Groupings

Group	Student Number	Name	Group	Student Number	Name
Group A (Shown ER2 then ER3)	4	Student 4(A)	Group B (Shown ER3 the ER2)	3	Student 3(B)
	11	Student 11(A)		8	Student 8(B)
	16	Student 16(A)		14	Student 14(B)
	20	Student 20(A)		25	Student 25(B)
	23	Student 23(A)		26	Student 26(B)
Group C (Shown ER2 then ER6)	2	Student 2(C)	Group D (Shown ER6 then ER2)	6	Student 6(D)
	7	Student 7(C)		12	Student 12(D)
	15	Student 15(C)		18	Student 18(D)
	19	Student 19(C)		24	Student 24(D)
	30	Student 30(C)		27	Student 27(D)
Group E (Shown ER3 then ER6)	1	Student 1(E)	Group F (Shown ER6 then ER3)	5	Student 5(F)
	10	Student 10(E)		9	Student 9(F)
	13	Student 13(E)		17	Student 17(F)
	21	Student 21(E)		22	Student 22(F)
	29	Student 29(E)		28	Student 28(F)

Do External Representations of Protein Translation Create a Space of Learning?

One of the underlying assumptions I made when designing this project was that an external representation of protein translation (or a pair of them as in the case of this project) could constitute an enacted object of learning. Variation theory has typically been applied to classroom learning studies in which the enacted object of learning has been defined as the classroom environment (Bussey *et al.,* 2013). However, in this study, I have defined the enacted object of learning to be the possibility for learning created by the features of protein translation depicted by some common external representations. While I have argued previously that an external representation can constitute a space of learning (see Chapters 3 and 4), I have been able to use the results of the student interviews to confirm that student can, in fact, learn from viewing external

representations of an object of learning. (As a reminder, all student participants in this project had completed a biochemistry course in which protein translation was taught 2 to 4 weeks prior to these interviews.)

Take, for example, Student 19(C). She begins her initial description of protein translation, prior to viewing any representations, by stating that the tRNA will "generate" an amino acid. This description implied that the tRNA came into the ribosome/mRNA complex, and once there, the tRNA would create an amino acid.

> [00:16:08.1] **Student 19(C):** Like, ok, there's tRNA sequence that's a anti-codon where, and then the mRNA will, I think they, I think they, I don't know if they bind together, but it's like the tRNA will read the mRNA I think.
> [00:16:59.8] **Researcher:** Ok. What do you mean by read?
> [00:16:30.7] **Student 19(C):** Like, look at the three, um, like the, ah, A, U, G, C. You know what I mean? Those things? I can't remember what they're called, and will then from that form, um, like the tRNA will, there will be, like, an amino acid generated.

She then describes the amino acids as possibly coming from the ribosome.

> [00:17:10.7] **Student 19(C):** You'll go through like three bases at a time, it will read three bases at a time, and then that will spit out an amino acid.
> [00:17:19.0] **Researcher:** Where do the amino acids come from?
> [00:17:20.9] **Student 19(C):** Um, from the top of the ribosome, [laughs] I think, or wait, no. […] I'm trying to think of this from micro. Alright, so the, it comes from, I think it's the top part of the ribosome that gives, that the amino acids come out of, but I don't know how.

She was then asked to draw her understanding of the process (Figure 29). As she draws the tRNA, she states that this amino acid, which she has drawn on the top of the tRNA as a square, is not actually attached to the tRNA, but comes out of the top of the tRNA.

> [00:25:09.5] **Student 19(C):** So [the tRNA] has four domains and then this one right here is where the anti-codon three base pairs are […], and here we get protein [draws a square on the top and labels it protein]. I don't think that's connected. Or base pairs, ah, no, amino acid [re-labels the square A.A.]
> [00:25:36.0] **Researcher:** So the amino acid is on the tRNA?
> [00:25:38.4] **Student 19(C):** Yeah, well it comes out from there [draws an arrow pointing up indicating the amino acid is coming out of the tRNA], wait. I'm

trying to think of this picture that I've seen. Yeah, I think it comes from [the top of the tRNA]. I think.

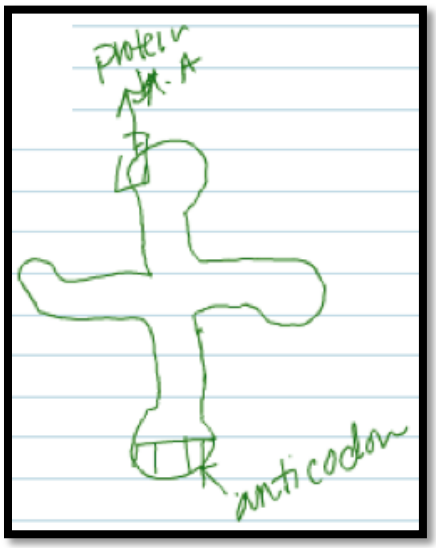


Figure 29. Student 19(C), Initial drawing

When pressed again about where the amino acid in her drawing came from, she describes that she did not think they would be floating around in the cellular environment.

[00:26:10.4] **Researcher:** So where does the amino acid come from?
[00:26:12.9] **Student 19(C):** Um, alright, amino acid, I don't think they are free floating but I could be wrong about that.

She then changes her mind.

[00:27:18.4] **Student 19(C):** I think they are free floating.

Following this discussion, Student 19(C) was then shown ER2 followed by ER6. Her response to ER2 did not seem to resolve her confusion about the relationship between the tRNA and the amino acid. However, after viewing ER6, she made the connection that the tRNA was indeed attached to the amino acid.

[00:54:28.0] **Researcher:** So tell me about [ER6].
[00:54:29.4] **Student 19(C):** So, this one has the mRNA […] and then you had the bottom part of the ribosome binded to it, and then the top part came and bind to it, and then you had all these charged, or all these RNAs, tRNAs floating around, and then you have, um, um, the charged part, um, maybe that charged part corresponds to the amino acid that's supposed to be spit out.

She then incorporates this new information into her final description of translation.

> [01:08:50.9] **Student 19(C):** We're gonna have, so, these tRNAs have amino acids attached to them, and then what's going to happen is there's gonna be some bonding that's gonna happen between these two amino acids which is gonna give us, um, [whispers] let me think, does it go on the first one in the A-site, [full voice] so the amino acid is gonna leave the tRNA from the P-site and there's gonna be two amino acids in the A-site, and then, what's gonna happen its that these, um, tRNAs are gonna move from the P and the A to the E and the P. […] This E is gonna go outside. It's gonna leave, and you're gonna another tRNA come in with an amino acid.

Although her final description is still not quite accurate, Student 19(C) does demonstrate significant improvement in her understanding of the relationship between the tRNA and amino acid in this process. Her final drawing of the process depicts a series of time points in which the tRNAs with attached amino acids enter the ribosome as the polypeptide chain grows (Figure 30). The ribosome with a long chain of amino acids then reaches a stop codon where the ribosome dissociates and the final protein folds as it the translational machinery dissociates.

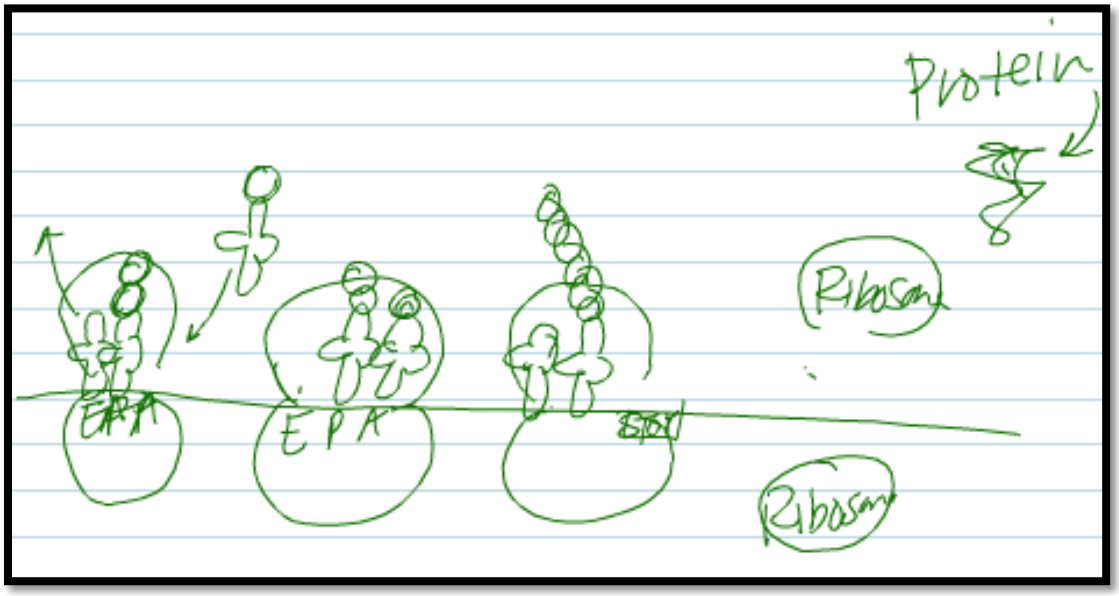


Figure 30. Student 19(C), Final drawing

I then asked her to clarify the relationship between the amino acid and the tRNA.

> [01:13:00.5] **Researcher:** So the tRNA is bringing the amino acid into the complex?
> [01:13:02.2] **Student 19(C):** Yep.
> [01:13:06.3] **Researcher:** […] You had mentioned originally that the amino acids, they are probably just floating around the outside [of the ribosome], is that still the case? Does the tRNA go out and grab those amino acids?
> [01:13:15.9] **Student 19(C):** No, because it showed in [ER6] that the tRNA, I think it was modifying its end. I think that's what the animation was trying to show by changing the shape of [the amino acid], […] so maybe, um, that's what happens. You have you amino acid that can be charged on tRNA.

In justifying her final description, she cites the information provided in ER6 as the evidence for her revised description of this portion of the process. Although she does not display a scientifically accurate understanding of how the amino acids come to be attached to the tRNA, she does develop a better understanding of the relationship between the tRNA and the amino acids as depicted by ER6. ER6, in fact, does not show tRNA synthetase or depict the esterification of the amino acids or their precursors onto their cognate tRNAs. Hence, this is not a feature that could be learned from this representation. However, ER6 does properly depict the aminoacylated-tRNA structure and its interaction with the ribosomal complex, which is learned by Student 19(C) and used to correct her prior non-scientific explanation of that feature of the process. This demonstrates that external representations do, indeed, constitute a space of learning and can be considered a viable enacted object of learning, thereby validating my initial assumption.

Do Students Notice Variation?

Another assumption I made when designing this project was that variation within and between features of a representation could cue students to notice and potentially learn

from that experienced variation. Although I had used this assumption, guided by the theoretical framework of variation theory, to design and conduct the Variation Analysis of Dynamic External Representations (VADER) method described in Chapter 6, I have been able to use the results of the student interviews to confirm that student do, in fact, notice and demonstrate learning from experienced variation of features depicted in external representations. I have found numerous examples of students who used variation in features depicted in the external representation to rationalize or justify their final understanding of translation. For example, many students initially explained or came to notice that the ribosome and the mRNA move relative to one another during the ribosomal translocation of elongation. However, I noticed that the way in which that motion was depicted potentially influenced students' description.

Motion is relative to the viewer. I noticed that the graphic designers represented the relative motion of the ribosome and the mRNA in one of two ways. One option was to hold the mRNA in a constant position within the frame of reference and move the ribosome relative to the mRNA. I noticed that ER2 and ER3 used this strategy to depict the movement of the ribosome relative to the mRNA. Another option was to hold the position of the ribosome constant within the frame of reference and move the mRNA. I found that ER6 used this strategy.

Following from variation theory, I would expect that students who saw ER6 might be more likely to notice the mRNA movements and that students who saw ER2 and/or ER3 to be more likely to notice the ribosome movement, as those are the features that are changing in reference to the viewer's position. This would illustrate a change in perspective based on the presentation of relative motion of different component features.

Student 18(D) initially describes the mRNA as moving "right to left" though the ribosome.

> [00:22:10.6] **Researcher:** How does the anti-codon and codon, how does that matching happen?
> [00:22:17.0] **Student 18(D):** So you have the, where it matches like perfectly. The anti-codon is the bottom loop of the transfer RNA.
> [00:22:25.0] **Researcher:** Ok
> [00:22:26.2] **Student 18(D):** Um, then the codon is within the messenger RNA as the messenger RNA moves from right to left and shuffles through [the ribosome].

She also depicts mRNA movement with two arrows pointing to the left under the right and left sides of her initial drawing (Figure 31).

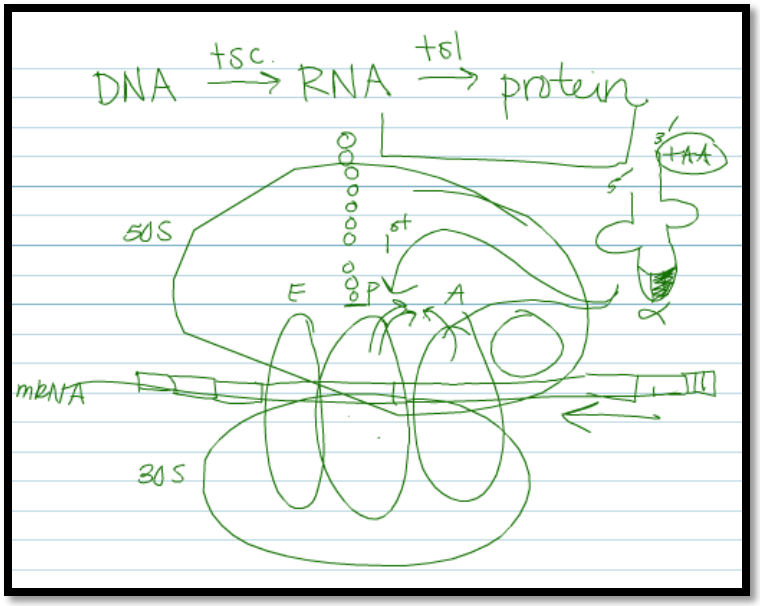


Figure 31. Student 18(D), Initial drawing showing mRNA movement relative to a stationary ribosome.

She then goes on to explain how "GFP kicks" the mRNA through the ribosome. By "GFP" I think she is describing the function of Elongation Factor-G (EF-G) rather than the green florescent protein.

> [00:27:42.4] **Researcher:** I think we covered everything. Are there any other, so we talked about the RNA the ribosome, tRNAs, amino acids, there were release factors, ah, that you mentioned

[00:27:55.2] **Student 18(D):** Disassembly
[00:27:57.1] **Researcher:** Ah, there's another one, I forget the name that you said, G
[00:28:00.3] **Student 18(D):** Oh yeah. GFP.
[00:28:02.1] **Researcher:** You were talking about kicking.
[00:28:03.2] **Student 18(D):** They are like little football, yeah.
[00:28:04.4] **Researcher:** What is that?
[00:28:04.5] **Student 18(D):** It has a big round head and a little stick and it kicks everything over, so it moves the strand through the ribosome so the next strand can be read.

By "strand," I think she is referring to the codons. She then goes on to add a depiction of "GFP" to her initial drawing to show the direction the "GFP" would move the mRNA strand (Figure 32).

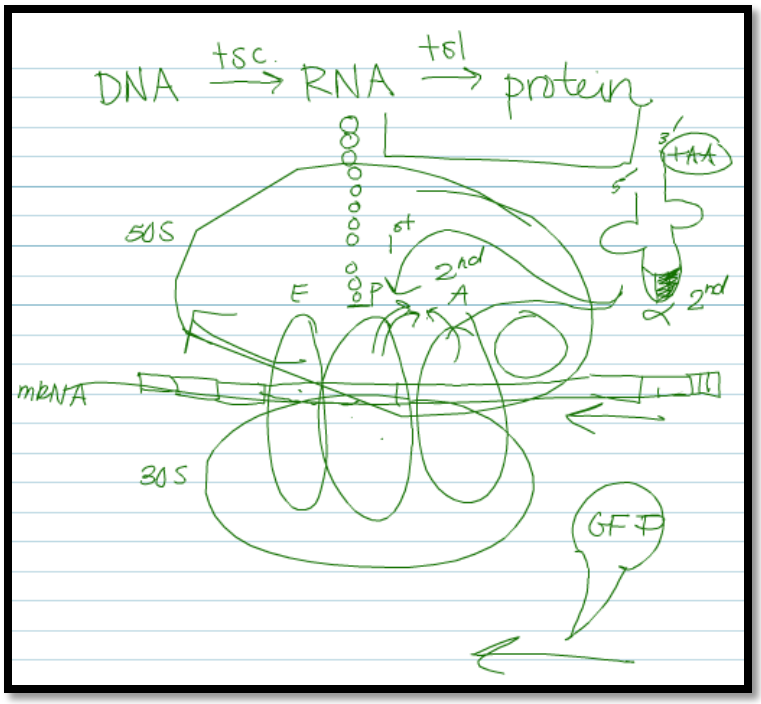


Figure 32. Student 18(D), Initial drawing with the depiction of the "GFP" showing the direction it moves the mRNA.

This student, Student 18(D), first saw ER6, a representation that depicts the mRNA movement through a stationary ribosome, consistent with her initial description of this process. She then saw ER2, a representation that depicts the ribosome movement

relative to the mRNA with an arrow indicating left to right movement (Figure 33). She makes particular note of the element of movement, or lack thereof, depicted in ER2.

> [00:56:05.2] **Student 18(D):** It doesn't show [pause] oh, here's the translocation, so the middle, yeah, and how everything is forced to move from position to position. The transfer RNA to release from the Exit site, but I don't know that this reaction showing translocation, EF-G, yeah, it shows you have hydrolysis and you go from more phosphates to less, so you're having this energy go in to it, but it doesn't necessarily emphasize visually how everything's moving. […] You just notice next that there's another transfer RNA, transfer RNA 3, the ribosome is moving one way, everything else is moving [the other] way. Um, because they put a lot of emphasis on the incoming transfer RNA binding […] but not necessarily how everything is moving beyond here's the direction [referring to the arrow].

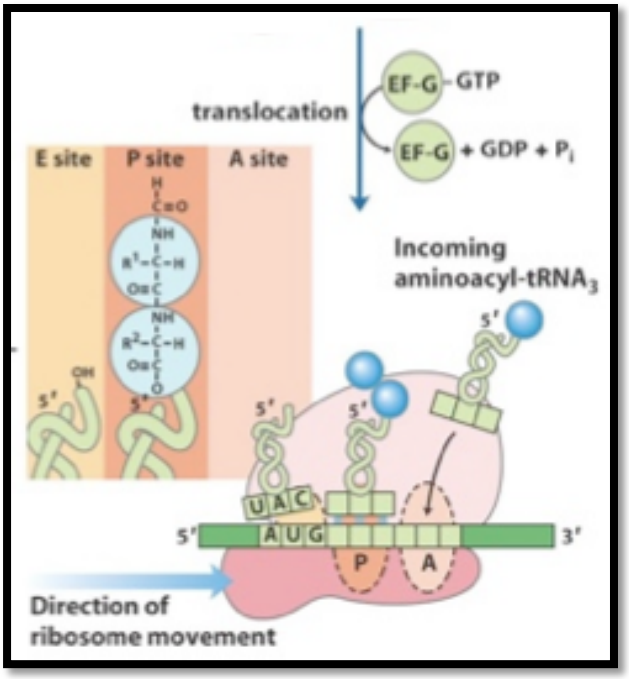


Figure 33. Frame 6 of External Representation #2 indicating the direction of ribosome movement.

In her final description, Student 18(D) alters her original description and instead adopts the ribosomal movement depicted in ER2 to describe translocation.

> [01:04:30.9] **Researcher:** Earlier, you drew for me translation. I'm going to ask you to do it for me one more time and you can make any changes that you want to based on how you drew it out for me the first time or if you want to add in different parts that you saw, or, you know, clarify how things work, this is your

> opportunity to do that. So same thing, walk me through the process, show me all the parts, how they work.
>
> [01:04:54.2] **Student 18(D):** Ok, once again, the strand comes out [of the nucleus], now we're in the cytosol, this comes through, assemble the ribosome, […], here's a GTP reaction to move the first amino acid into the exit site and out of the exit site so that the third amino acid can come in, […] add the next amino acid, move the ribosome in this direction [draw arrow from left to right in the small ribosomal subunit], next transfer RNA, third transfer RNA, fourth transfer RNA, continue to grow the peptide, […] we get to the end of it, UAA, release factor comes into the A-site, snips the peptide strand, release the peptide, disassembly.

Her final drawing also reflects this shift in the reference frame with an arrow now indicating ribosomal movement rather than mRNA movement (Figure 34).

Neither of these depictions (with reference to the movement of the mRNA or the ribosome) is incorrect. Movement is relative based on the frame of reference of the viewer. However, this exchange is notable because it demonstrates that students take more notice of the features that are varied rather than the features that are constant.

Why, then, did Student 18(D) not continue with her original explanation of movement as supported by ER6? ER2 contained an additional aspect of variation that was not found in ER6, labeling. None of the features of ER6 were presented with an alphanumeric label. I noticed that the students, in general, had a more difficult time identifying the features in ER6 than they did in ER2 and ER3, in which those same features were explicitly labeled. Perhaps the additional information provided in the label, i.e., direction of ribosome movement, altered the student's perception of which component should be depicted as moving relative to the other. Regardless of the reason why, I did find that the students generally noticed varied features, i.e., those that changed position, size, or labeling, over features that did not. The instructors also noticed these varied features and often identified them as ones that students might notice in viewing a

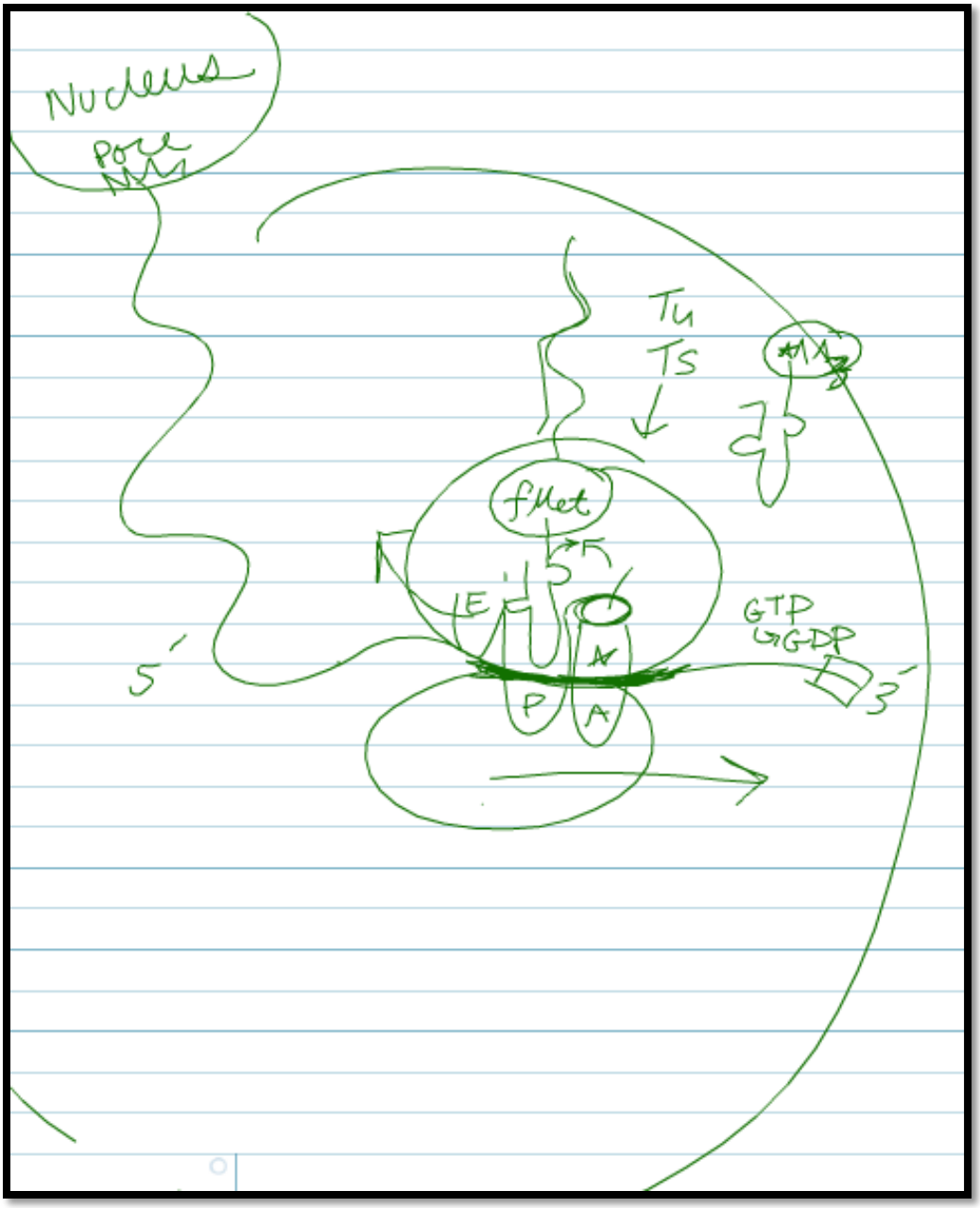


Figure 34. Student 18(D), Final drawing indicating the ribosomal movement relative to a stationary mRNA.

representation. For example, Instructor 5 describes how ER3 emphasizes the alignment of the mRNA and small ribosomal subunit through the Shine-Dalgarno sequence interaction and that this emphasis would cause students to notice this feature.

> [00:47:25.6] **Instructor 5:** The best part of [ER3], though, had nothing to do with translation itself, but was the locking of the Shine-Dalgarno sequence to the 16S ribosomal RNA in the small subunit. That's really, that was very good. Yeah, that was nicely done. That was very clear, and that, that made a lot of sense
> [00:47:42.8] **Researcher:** If a student were watching this, what do you think they would take away form it?
> [00:47:46.3] **Instructor 5:** That. They would, they would like that. It had a nice emphasis. Yeah it was very clear. They actually stalled out on it a little bit and they go 'oh yeah I see all the hydrogen bonding between them.'

In fact, students did notice the Shine-Dalgarno emphasized by ER3. Moreover, several students suggested improvements to better depict the chemistry of peptide bond formation in ER3 by adding a zoomed in scene similar to that of the Shine-Dalgarno interaction but to depict the chemistry. For example, Student 4(A) described how he preferred ER2 over ER3 because it showed more of the "actual chemistry." He then proposed the following idea to incorporate the chemistry from ER2 in ER3 (which depicts a generic interaction in which one amino acid moves next to the next amino acid with no indication of the chemical reaction).

> [00:29:38.1] **Researcher:** Do you think if you added those things that you like from [ER2] to [ER3] that that would improve the second one or would that not?
> [00:29:47.3] **Student 4(A):** Yeah, probably, um, if you were able to see the actual, like, chemistry binding, like they zoomed in and then showed that it was binding to it and then it transfers instead of just putting in the next tRNA and it just moves and then it pops off that one and move to the next one. It kind of leaves you with 'well how did that happen?'

Therefore, students do notice variations in the way features are depicted and they see directed cueing such as the zooming in or labeling of features, as in the Shine-Dalgarno interaction scene in ER3, as a way of calling there attention to particular features. These findings are consistent with variation theory and supports the VADER method which was developed to identify which features were most likely to be noticed based on the amount of variation the exhibit. Therefore, instructors should give careful consideration to how

and when features of a representation are varying. If variation might help a student to learn about a critical feature, then that variation is well utilized within the educational objectives for the representation. However, if a representation displays variation in features that are not deemed critical, then a less distracting representation might need to be sought out in order to help best direct students' attention on the features of most importance to the instructor.

Prior Lived Object of Learning

I asked all of the student participants in this project to explain and draw their understandings of protein translation prior to seeing any of the selected representations in order to assess their prior knowledge of the object of learning. Using students' initial descriptions of the process, I was able to establish that students displayed a wide range of understandings.

When I initially asked the students to describe their understandings of the process, several of them referred to their previous instruction on the topic from courses other than biochemistry. As described above, Student 19(C) referred to her instruction in translation from her microbiology class as she tried to explain the relationship between the amino acids and some of the other translational components.

> [00:17:20.9] **Student 19(C):** Um, from the top of the ribosome, [laughs] I think, or wait, no. […] I'm trying to think of this from micro. Alright, so the, it comes from, I think it's the top part of the ribosome that gives, that the amino acids come out of, but I don't know how.

Student 20(A) also refers to previous instruction about translation as he recalls the termination of the process.

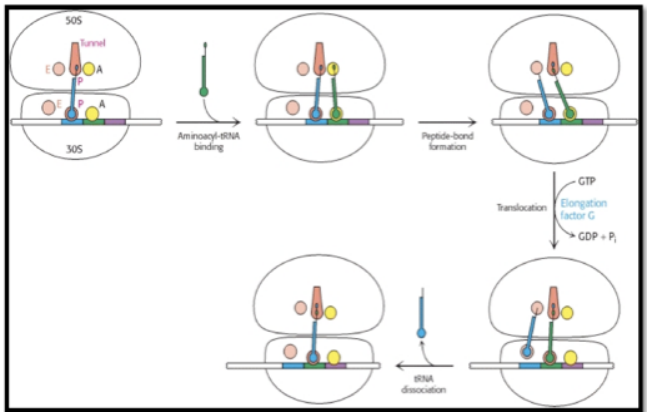
> [00:09:24.7] **Student 20(A):** I guess there's a stop codon. I'm trying to think, I don't remember how we did it in biochem but I remember in some other class

they said [the stop codon] coded for nothing, but then in biochem we said it was something that looks like a tRNA but really doesn't have anything.
[00:09:38.4] **Researcher:** Ok.
[00:09:39.1] **Student 20(A):** So when it moves into the P-site, it's really nothing. There's no amino acid to attach to [the polypeptide chain], and it kind of lets it free.
[00:09:45.5] **Researcher:** Lets what free?
[00:09:46.7] **Student 20(A):** The peptide, the polypeptide.

Additionally, some students made specific mention of the images of the components or process they had previously seen. Student 20(A) continued his previous description of translation by drawing an image (Figure 35A) he remembered from his textbook (Figure 35B).

[00:19:04.4] **Student 20(A):** The first [tRNA] is in [the ribosome]. It's happy. Maybe another one comes in. I mean you can see I'm just basically copying this photo from what I remember from the book.

A



B

Figure 35. A.) Student 20(A), Initial drawing. B.) Textbook image from Berg, *et al.* (2007, p. 872) depicting protein translation. This is the biochemistry textbook used by Student 20(A).

Student 5(F) also describes previously seen textbook images as she explains the structure of the tRNA. Her initial drawing begins with a depiction of the tRNA as an oval labeled tRNA (Figure 36).

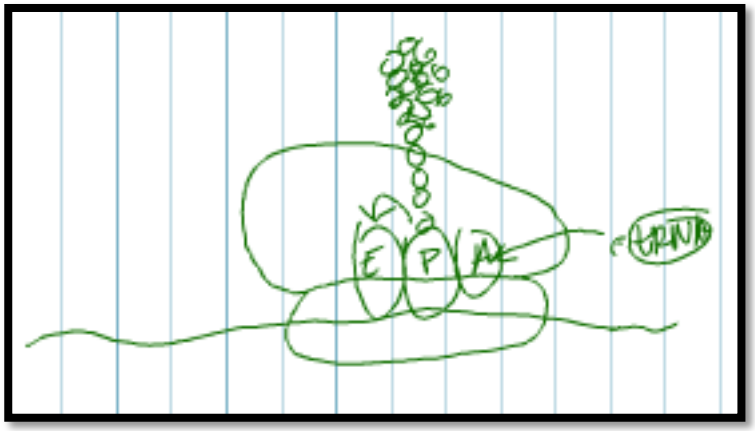


Figure 36. Student 5(F), Initial drawing with the tRNA represented as a labeled oval.

She then goes on to explain that the structure is more like a cloverleaf like the images she remembers from her textbook. She then recreates her remembered image (Figure 37).

> [00:13:47.4] **Researcher:** You have [drawn] the tRNA as kind of a circle thing. Is that what it, it looks like, or does it look different in real life?
> [00:13:54.9] **Student 5(F):** Well, in the textbook, it shows as a cloverleaf structure.
> [00:13:58.0] **Researcher:** A cloverleaf?
> [00:13:59.2] **Student 5(F):** Yeah
> [00:13:59.3] **Researcher:** Can you like maybe draw what you think that looks like?

Figure 37. Student 5(F), Initial drawing of the cloverleaf structure of tRNA.

Although not referencing her textbook, Student 23(A) also described a remembering of previously seen images of the process. As I prompted her during her

initial description of the initiation process, she tried to recall previous images she had seen in order to describe the appropriate sequence regarding the integration of the initiator tRNA during the assembly of the initiation complex.

> [00:11:42.2] **Researcher:** I just want to go back to initiation, when we're starting the process. You said that there was a start codon
> [00:11:46.1] **Student 23(A):** Yes.
> [00:11:46.5] **Researcher:** on the mRNA, right?
> [00:11:48.2] **Student 23(A):** Correct.
> [00:11:48.9] **Researcher:** And it's bringing in that methionine, right?
> [00:11:51.9] **Student 23(A):** Right
> [00:11:53.1] **Researcher:** Is the, the tRNA and the ribosome, is that already on or is that already there [on the mRNA] or when does that come in?
> [00:12:04.5] **Student 23(A):** As far as order of operation. [laughs] Ah, I'm not completely sure.
> [00:12:18.2] **Researcher:** Ok. If you had to take a guess as to when things come into the system, what, how would you think that would work?
> [00:12:25.0] **Student 23(A):** Well, I was trying to think back and think about figures that I've seen, and I, I think the ribosome is usually there when methionine is added, eh, present so, it would seem that methionine is added after the ribosome, but it could be the other way around.

During their interviews, the instructors acknowledged that students were coming to biochemistry having seen protein translation in several other classes (see Chapter 5). From the student descriptions, I can state that students also acknowledge the overlap between the material between presented in biochemistry and other biology courses. This overlap could provide both a source of scientifically accurate prior knowledge or a variety of non-scientific conceptions or gaps in knowledge that might make the learning about protein translation in a biochemical context all the more challenging. For this reason, I think that my methodological inclusion of prior knowledge into the theoretical framework of variation theory is warranted. Not only does the literature support the assertion that students' prior knowledge will affect their learning (see Chapter 2), but the

fact that the students are referring to their own learning in other classes in reference to their understanding of protein translation adds further validation to this claim.

Not only are some students referring to the content they learned in other classes, but also they are referring specifically to the representations that were used in those classes. This demonstrates the importance of external representations to student learning. Thus, it is important to understand how students are interpreting and learning from these representations (as is being explored in this project) in order to better use them to promote student learning of critical feature of an object of learning.

In the sections that follow, I will first describe the general features that students included in their initial descriptions of protein translation. I will then discuss how students' prior knowledge was used, in part, to determine the extent to which learning had occurred over the course of the student interviews. Finally, I will discuss how the level of students' prior knowledge affected their ability to learn from the external representations during the interviews.

Students' General Prior Knowledge

As seen in the selected student descriptions above, I found that the students displayed a range of prior knowledge of protein translation, some of which was scientifically accurate and some of which was not. I identified non-scientific student understandings to be both statements of non-scientific features or the absence of a statement of a scientific feature. For example, Student 8(B) knew that peptide bond formation was part of the translation process; however, he was not able to correctly explain the peptide bond formation. In his initial drawing, he shows an attack from the double bond of the carbonyl on the incorrectly charged nitrogen of the neighboring amine

group (Figure 38). This would be considered a demonstration of non-scientific knowledge of peptide bond formation. Student 5(F), on the other hand, displays an initial lack of stated knowledge of peptide bond formation. She does not mention peptide bond formation or even that amino acids would have to bind together to form the polypeptide chain during her initial description of the process. In both of these instances, Students 8(B) and 5(F) would be considered to have a non-scientific understanding of the feature of peptide bond formation. During coding of the student interviews, this non-scientific understanding was not coded. Because all codes (see Appendix D) were defined according to current scientific understanding of the component, interaction, or general concept to which they were applied, non-scientific student descriptions or depictions would not meet the definition of the codes and would, therefore, not be coded. Only scientifically accurate student descriptions or drawing were coded for the features present.

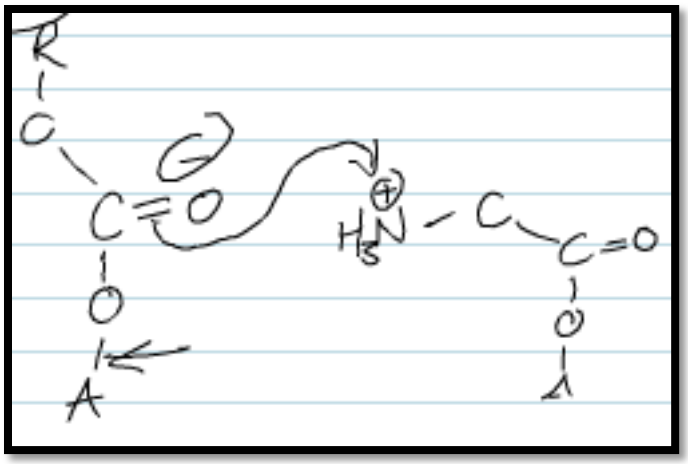


Figure 38. Student 8(B), Initial drawing of peptide bond formation (incorrect).

Many of the students were able to accurately describe the primary critical features of the mRNA; the tRNA; the ribosome, including the large and small subunits; the amino acids; and the polypeptide chain. Additionally, they could generally describe the

interaction between adjacent amino acids even though their description did not include a mechanistic explanation of peptide bond formation. Student 6(D) typifies the basic initial description offered by many of the students.

> [00:10:33.4] **Researcher:** I want to talk specifically about protein translation
> [00:10:36.7] **Student 6(D):** OK
> [00:10:37.0] **Researcher:** Um, or whatever you are comfortable calling it. So what I going to ask you to do is kind of do a brain dump for me, so I'm going to ask you to think about if you were the professor, explaining to me, the student, what I needed to know to understand the process of just protein translation […]. Where would I start, and where would I end to explain that whole process
> [00:11:02.6] **Student 6(D):** Do you want me to, ok. […] Ok, so you would need to understand that the mRNA is, is the representation that's going to allow proteins to be made. It's like the, the, ah, language that's going to describe which proteins, I mean amino acids need to be brought. So you need to know how mRNA is made. Where it comes from. Um, you need to know the way that the amino acids get brought to, well you have to know about the ribosome [laughs], and how the ribosome gets to where it needs to start on the mRNA, and then you need to know how the tRNAs are connected to the amino acids, and how the tRNAs are brought and sort of organized by the ribosome to put the amino acids together, the process or the, um, mechanics of the actual peptide bond formation, and the disassembly […] and how it gets stopped and released, I guess.

Based on the coding of the initial student responses and drawings, I determined that most students displayed a basic knowledge of the primary component features; however, they often could not accurately describe or depict the specific interactions of those components. This lack of knowledge generally centered on the sequencing of events during the various stages of the process, such as the proper formation of the initiation complex, and the chemical interactions of the process, such as the mechanism of peptide bond formation. In this way, students seem to demonstrate a decent *biology* understanding of protein translation (a component view), but that they do not have a sufficient *biochemistry* understanding of protein translation (more of an interaction view), which better aligns with biochemistry instructors' intentions for student learning.

The Use of Prior Knowledge to Determine if Learning Occurred

The purpose of coding for students' prior lived objects of learning, i.e., their prior knowledge of protein translation, is that it then allowed me to make a comparison between their prior lived objects of learning (before they saw the representations) and their post lived objects of learning (after they saw the representations). I have defined learning to be a change toward a more biochemically accurate understanding of translation as expressed by the students. In order to assess whether learning has occurred, i.e., a change in the way an object of learning is perceived, as the result the enacted object of learning, I compared each student's initial description and drawing(s) of the process with their final description and drawing(s). If students were seen to incorporate features presented in the enacted object of learning and/or correct or augment their prior knowledge to reflect a more scientific understanding of the process than they expressed at the beginning of the interview, then I concluded that learning had occurred.

The Potential Impact of Prior Instructors' Intentions for Student Learning

One of the distinctions made by more than half of the students when describing their initial understanding of translation was between prokaryotic and eukaryotic systems. During the instructor interviews, I noted that only a passing reference was made to the prokaryotic/eukaryotic distinctions of protein translation. I found that the general focus of biochemistry instructors' intentions for student learning was centered on the overarching components and their specific chemical interactions rather than on the specific naming and minor structural differences of prokaryotic and eukaryotic components. I found that students, on the other hand, generally asked initially if they should describe a prokaryotic or eukaryotic system. Student 6(D), for example, distinguished between prokaryotes and

eukaryotes while describing the process of initiation. Specifically, she addressed the differences in the mRNA sequence used to distinguish the start site during the formation of the initiation complex.

> [00:12:34.4] **Student 6(D):** The mRNA has particular sequences that are recognized, are we talking about prokaryotes or eukaryotes here? […] We'll go with prokaryotes, but understanding that eukaryotes are somewhat similar, but has more proteins in it.

I interpreted students' concern for system specificity as a possible result of their prior instruction on the topic. Students seem to have placed a high value on noting the distinctions between prokaryotic and eukaryotic metabolic processes. This may be the result of previous instructors' intentions for student learning. This is not to suggest that distinctions between prokaryotic and eukaryotic systems could not be a part of a biochemical presentation of the material; however, based on the instructor interviews, these distinctions were generally not a primary concern. The instructors' intentions for student learning were centered more prominently on the underlying chemistry of the process, which is not significantly different from cell to cell.

Overall, students demonstrated that certain features were more salient to them than others regardless of the origin of that salience. Generally, students described component features including the mRNA, the ribosome, the tRNAs, the polypeptide chain, and the amino acids. Additionally, many students described the distinctions between prokaryotic and eukaryotic systems as an important consideration when describing translation. Students seem to overlook much of the underlying chemistry of protein translation in their initial descriptions, which was the primary focus of the instructor interviews.

Post Lived Object of Learning

In research question 3, I asked what did students learn about protein translation from some common external representations depicting the process? In order to determine what students actually learned, I first determined the influence of students' prior knowledge on their final descriptions of the process. I then determined the influence of each individual representation. Finally, I described the influence of the ordered pairwise combinations of representations.

The Influence of Prior Knowledge

Bussey *et al.* (2013) assert that a student's lived object of learning is informed by both the enacted object of learning as well as by the student's prior knowledge of the object of learning. In order to determine the influence of prior knowledge on student learning from external representations of translation, I compared students' initial descriptions of translation (i.e., their prior lived objects of learning) to their post lived objects of learning. In the following sections, I will discuss how high levels of prior knowledge affected students' interpretation of the external representations and limited the possible learning from those representations. I will also discuss how lower levels of prior knowledge created a greater possibility for student learning from the external representations but also seemed to limit what was possible for them to learn from those representations.

Higher Levels of Student Prior Knowledge. A few students exhibited a significantly higher level of scientifically accurate prior knowledge. The overall level of prior knowledge was determined by comparing the number of features of protein translation initially identified by the students to the features that instructors identified as

critical features. Students who were able to accurately describe more primary critical features were determined to have a higher level of prior knowledge (see Chapter 5, Table 3). I consider students who identified all 16 primary critical features to have high levels of prior knowledge. Students who were initially able to describe more than half of the primary critical features were considered to have an intermediate level of prior knowledge. Students who were only able to describe less than half of the primary critical features were considered to have lower levels of prior knowledge.

Three of the 30 students (Student 7(B), Student 26(D), and Student 28(F)) were able to accurately describe all 16 primary critical features in their initial descriptions (see Chapter 5, Table 3). For example, Student 7(C) gave one of the most thorough initial descriptions of translation, identifying some features of translation that not even the instructors identified, such as the specific initiation and release factors.

> [00:06:14.3] **Researcher:** So what I'm going to have you do is pretend you are the professor. I am the student. I want you to explain to me the process of protein translation. Where would I start to explain to a student how the process works? Where would I end?
> [00:06:27.0] **Student 7(C):** Well, ah, well it's somewhat different for prokaryotes and eukaryotes, but the first thing we'd go over is how it is for prokaryotes. What it is, is that the two subunit will initially be separate. And to the small subunit, you'll have the IF-1, which is bound to the A-site., and then you'll have the IF-3 which is, which is, which is, ah bound in such a way that it prevents, ah, it prevents the 50S subunit from binding prematurely.
> [00:06:54.0] **Researcher:** Subunits of what?
> [00:06:55.9] **Student 7(C):** Ah, subunits of, ribosomal subunits, excuse me.
> [00:06:57.4] **Researcher:** Ok.
> [00:06:57.9] **Student 7(C):** Yes, and then the IF-2 will, will be directed by IF-1 to place the formylated methionine tRNA, right at, not just at AUG but AUG in the proper context. It has to be the right kind, and there you'll have, ah, it'll all start, and once that happens, um, ah, IF-1 and IF-3 will be ejected. They will be released, and that will allow the 50S ribosomal subunit, the large one, to come in and bind, and once that happens, the IF-2 will be bound to GTP but that will hydrolyze to GDP, and then IF-2 will leave, and then, and then transcription will start, and then after that, you'll have the EF-Tus come in and bring a novel methionine tRNA, ones other than that, and this can be methionine or anything

else. Anything except for formylmethionine tRNA, right, so that will bind in to the A-site, and then you'll have the, um, you'll have, um, the polypeptide chain, you'll have the, um, the amino acid that was there first, the formylmethionine, and that will be transferred onto the new, ah, amino acid that is brought in, and then after that, what will happen is that you'll have the elongation factor, ah, EF-G come in, and it will bind right next to the A-site, and it will push it over by one codon so the, ah, the amino acid that was, the amino acid tRNA, aminoacyl-tRNA, that was bound to the A-site will, will be shifted over to the P-site, and then the amino acid, I'm sorry, the tRNA that no longer has the amino acid will be shifted over from the P-site, and it will be released. It will be let go, and this, and this process continues until you get to a stop codon, and then you'll have a release factor, either release factor 1 or release factor 2 depending on, um, depending on if it's a UGA or a UAG. Actually you can have either release factor, if it's UAA, and then release factor 3 will help which ever release factor comes in bind to that site and that will cause the, um, the polypeptide chain to be released from the tRNA. It will go through and go off on its own, and then, um, it will cause the whole subunit, it will cause the whole ribosomal complex to come apart.

Student 7(C) continued his explanation by drawing out the a series of three steps depicting the mechanism of peptide bond formation. First he shows the lone pair of electrons on the nitrogen of the amine of the amino acid on the tRNA in the A site to the carbonyl carbon of the amino acid on the tRNA in the P-site (Figure 39).

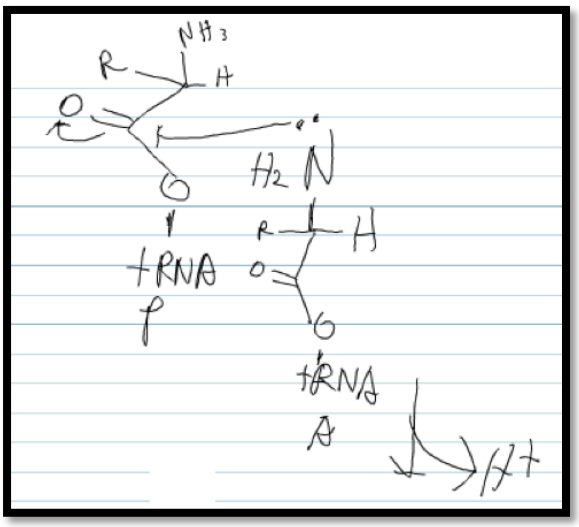


Figure 39. Student 7(C), Initial drawing of the first step in the mechanism of peptide bond formation.

He then shows the formation of the peptide bond between the nitrogen and carbon and the breaking of the bond between the carbon and the oxygen connected to the 3’ end of the tRNA (although it is not depicted in his drawing, he had earlier labeled the 3’ and 5’ ends of the tRNA) (Figure 40).

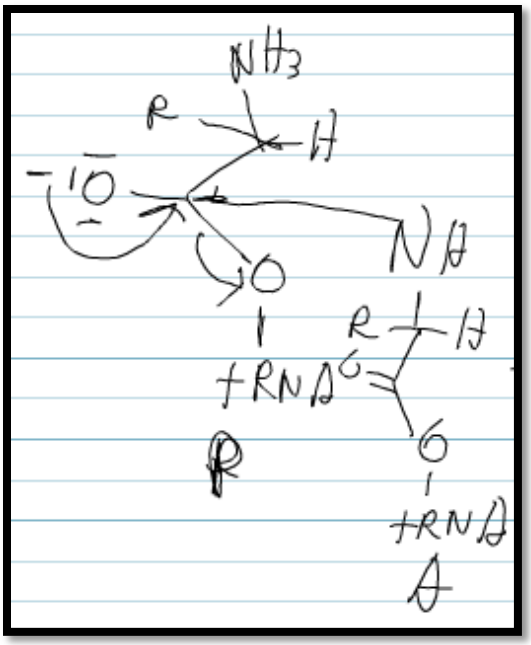


Figure 40. Student 7(C), Initial drawing of the second step in the mechanism of peptide bond formation.

He then shows the final dipeptide attached to the tRNA that is in transition between the A-site and the P-site and the deacylated tRNA with its 3’ hydroxyl in transition between the P-site and the E-site. Last (and unrelated to the specific mechanism), he depicts elongation factor G as a component involved in the translocation indicated by the tRNA site shifts along the bottom (Figure 41). Although Student 7(C) did not identify all of the critical features listed by the instructors, he did accurately identify a large number of them. This reduced the possibility that the representation would depict a feature that was different or lacking from his initial description. Therefore, high prior knowledge students

would not demonstrate learning as frequently as those with an intermediate or low level of prior knowledge, i.e., there could be a ceiling effect.

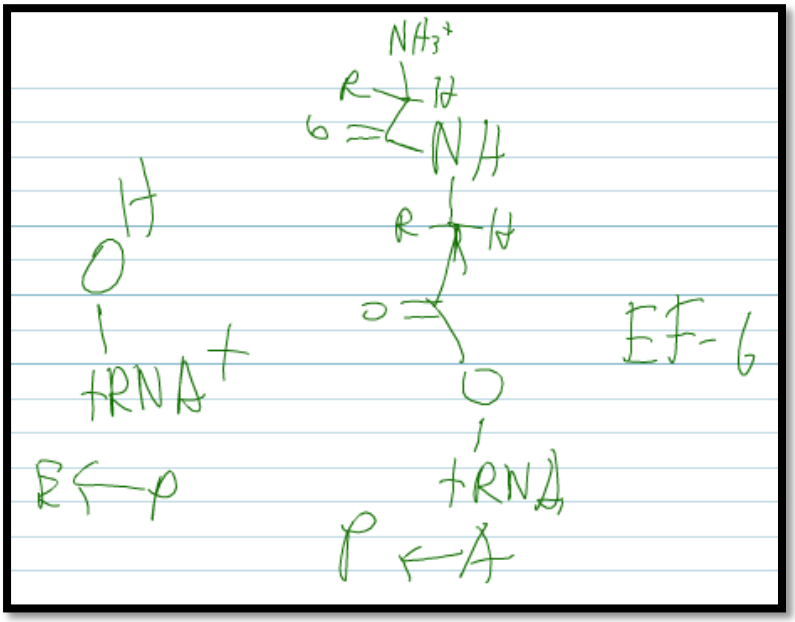


Figure 41. Student 7(C), Initial drawing of the third step in the mechanism of peptide bond formation.

Because Student 7(C) initially demonstrated a scientifically accurate understanding of peptide bond formation, he was not able to learn that information when he was shown ER2. Generally, students who displayed high levels of prior knowledge displayed a ceiling effect in that they did not demonstrate any evidence of learning from the viewing of the external representations not because that information was not there, but because they already demonstrated that they knew that material during the initial phase of the interview. In many cases, students seemed to use the representations to confirm their original understanding of the process.

Upon viewing of ER2, Student 7(C) proceeded to describe the depicted features and identified other features that he had discussed during his initial description but that were not presented in the representation.

> [00:36:29.9] **Researcher:** What I'm going to ask you to do is kind of think out loud for me and tell me […] what's going on. So here is [ER2].
> [00:36:38.9] **Student 7(C):** Alright, so, alright, so here you have the initiation complex. We have the AUG in the P-site, and of course it doesn't show it here, but like I said earlier, the AUG has to be in the proper context. So you'll have the formylated methionine coming into the P-site with the, ah, begin brought in by IF-2 of course. So it will be brought in and after that IF-1 and IF-3 will be ejected, and after the large subunit, um joins the whole complex, then IF-2, I mean the GTP on IF-2 is hydrolyzed to GDP and that will leave as well.

Of the features discussed in by Student 7(C), IF-1, IF-2, IF-3, the GTPase activity of IF-2, and the formation of the initiation complex are not depicted in ER2. Instead, the first frame of ER2 shows the fully formed initiation complex with no initiation factors present (Figure 42).

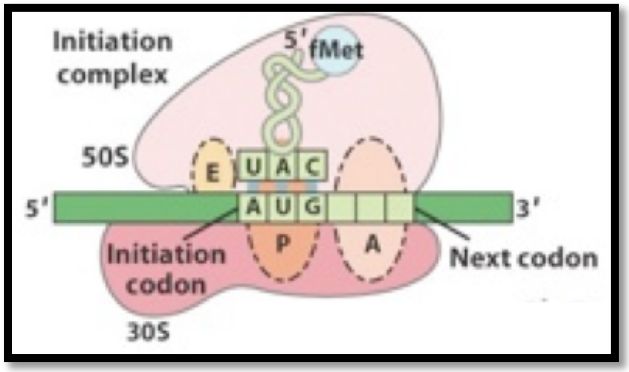


Figure 42. Frame 1 of External Representation #2

Student 7(C)'s description of non-depicted features in ER2 demonstrates that he is interpreting this representation in light of his prior knowledge and incorporating these features into his understanding of what is being depicted in ER2.

Intermediate Levels of Prior Knowledge. Students who display an intermediate amount of prior knowledge were generally able to recognize where they lacked sufficient knowledge. As a result, they were more likely to identify differences between their initial descriptions and the features presented in the external representations. They were then also more likely to include those features in their final description of the process. This

change from prior to post lived object of learning was then identified as an instance of learning. For example, during his initial description of translation, Student 25(B) describes how the process would end.

> [00:39:57.8] **Student 25(B):** The tRNA would go out [of the ribosome], leave through the E, exit-site, and then, this is the part I was telling ya I didn't know exactly if there is a release factor, or if there's anything at all that comes in, or if the mRNA just runs out [of the ribosome], but the overall [translation complex] itself, um, would dissolve or break apart.

His initial confusion about the exact component interactions during termination indicate that he does not initially have a scientific understanding of the process. However, he is aware of the deficiency in his knowledge. Perhaps this makes him more aware when, in ER3, he is shown a depiction of the release factor as it interacts with the stop codon during termination.

> [00:47:48.0] **Student 25(B):** [describing ER3] It keeps going and going until it runs into a stop codon. Oh, there it is, the release factor, and then the nascent chain leaves and goes to wherever its destination is, and then it breaks apart.

At the end of the interviewer he then incorporates the release factor into his final description.

> [01:05:32.0] **Student 25(B):** I'm assuming off of [ER3] here, at the very, very end, and after your stop codon, UAG there, that your release factor comes in and that's what ends up breaking, breaking them apart.

Although his description is still not completely accurate, he is now able to describe the role of the release factor in the termination stage of translation; and he cites ER3 has evidence of this interaction.

Other students used their prior knowledge to create new non-scientific conceptions of the depicted features. They seem to know just enough information to

confuse the issue. For example, Student 8(B) was shown ER3 in which the E-site is not labeled (Figure 43). He states that:

> [00:50:09.5] **Student 8(B):** This is eukaryotic because there's no E-site.

Later in the interview, he confirms his application of prior knowledge during the following exchange:

> [00:56:48.2] **Researcher:** When [ER3] had started and they labeled the A- and the P-sites, you had mentioned right away then that you knew this was eukaryotic because it did have that E-site, was there anything else that triggered you, or helped you figure out what they were trying to show, where it was prokaryotic, eukaryotic, that kind of stuff?
> [00:57:07.9] **Student 8(B):** Um, well the biggest thing is, ah, the P-site and A-site, um, and that's, as far as recognizing things right away off the top of your head, that's the easiest thing to recognize. Um, I think the Shine-Dalgarno might be eukaryotic only and not prokaryotic, um and also the 16S, um like the numbering of the S subunits is all different in eukaryotes than it is in prokaryotes, so, um, little things like that. Um, but the mRNA sequence would still be the same codons.
> [00:57:58.2] **Researcher:** So do eukaryotic ribosomes, they, there's no E-site at all?
> [00:58:03.2] **Student 8(B):** Um, that is correct.

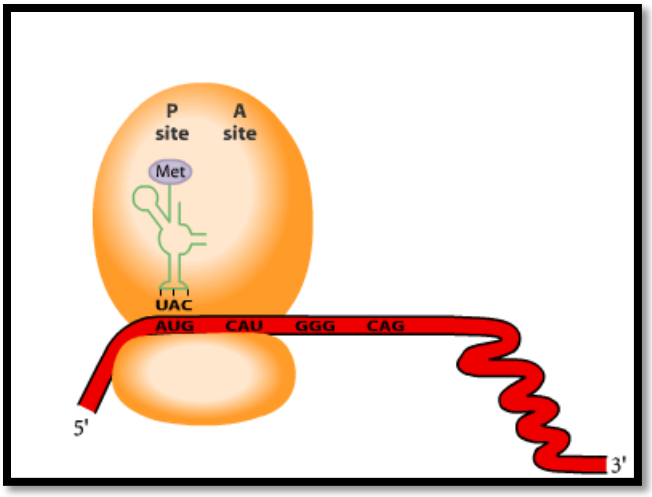


Figure 43. Frame 17 of External Representation #3

His assertion that eukaryotic ribosomes lack an E-site is not accurate; however, based on his misapplication of this piece of prior knowledge, he then misattributes other

relevant features of the ribosome structure to prokaryotic/eukaryotic distinctions. The Shine-Dalgarno sequence (a ribosomal binding site) is found in prokaryotic mRNAs; however, Student 8(B) misapplies this feature to make his case for why ER3 is depicting a eukaryotic system. He also notes that the rRNA fragments are different in size between prokaryotic and eukaryotic ribosomes. This is also true; however, he again applies the 16S rRNA, a component of the prokaryotic 30S ribosomal subunit, to a eukaryotic system. As shown by Student 25(B), an explicit awareness of a lack of knowledge may make the depiction of those features more salient to the student and may cause those students to learn from those depictions. On the other hand, as shown by Student 8(B), non-scientific prior knowledge can be misapplied and distort the meaning of the features depicted in a representation. In either case, students' prior knowledge was shown to affect their ability to learn from the representations.

Lower Levels of Student Prior Knowledge. I found that students with lower amounts of prior knowledge about protein translation were confused more easily by the physical depictions of the content or changes in the depictions within or between representations than were students with greater amounts of prior knowledge about protein translation. For example, Student 19(C) was first shown ER2 in which the tRNA is depicted in a very stylized L-shaped configuration. She was able to identify the tRNAs because they were labeled. However, she was used to seeing the tRNA depicted in the two dimensional cloverleaf configuration and expressed her dislike for the way in which the tRNA was represented in ER2.

> [00:49:47.7] **Student 19(C):** The tRNA, for me, is a little weird because I've never seen it drawn like that. I always see it drawn in that cross pattern, um, so that kind of was a little bit confusing to me.
> [00:49:59.2] **Researcher:** Why do you think they chose to draw it like that?

> [00:50:01.1] **Student 19(C):** Um, maybe it's more realistic because I'm sure it's probably not [in that cross] shape, but, um, we like to make it simpler. You know what I mean. I know [the cross shape is] not how it looks realistically, like I get that, but for me, it's a lot easier if I see that, I know that's tRNA.

She was then shown ER6 in which the tRNAs are shown in an even more realistic L-shaped configuration. This change in depiction caused Student 19(C) to initially not recognize the tRNAs.

> [00:52:30.8] **Student 19(C):** This looks like it's mRNA. That's the ribosome, and it it's going to bind to the start sequence […]. I don't really know what that is. Maybe they are protein [she says referring to the tRNAs].

It was not until she saw them interacting in the tRNA binding sites of the ribosome that she was able to identify those features as the tRNAs. As there were no labels in ER6, she was only able to identify the tRNAs based on her prior knowledge of the interaction and relative positioning of the tRNAs and the ribosome.

> [00:53:15.8] **Student 19(C):** So this is the ribosome working on the mRNA. Then you get your, oh, these are tRNAs. Oh ok, I get it.

It seems that a lower level of prior knowledge can complicate the decoding of depicted features. Student 19(C), for example, was not able to identify the tRNAs depicted in ER6 until about half way through the animation. As such, any interactions and features involving the tRNAs prior to that point would not be able to be accurately understood. If students do not have enough prior knowledge to be able to identify the basic components of the process, then they tend not to be able to learn from the features that are presented in a representation.

Overall, students' prior knowledge of protein translation was seen to be a significant influence on their ability to understand and learn from the external representations. This finding supports the inclusion of students' prior lived object of

learning into the model of variation theory used in this project. It also suggests that instructors should be aware of the level of students' prior knowledge when selecting appropriate external representations as students' prior knowledge will limit what can be learned from the representation.

The Influence of the Enacted Object of Learning

In addition to students' prior knowledge, the enacted object of learning constrains the possibilities for learning. In order to identify any potential influence of the enacted object of learning on the lived object of learning, it was first necessary to determine what students actually learned. A comparison between students' prior lived object of learning and post lived object of learning revealed that some features were more likely to be learned by students. Appendix U (Group Comparison of Learned Features) presents a summary of which features were learned. If learning was demonstrated by one or more students in a group, the feature is highlighted in green. If a feature was not learned by any students in the group, the feature is highlighted in red.

An initial survey of Appendix U (Group Comparison of Learned Features) revealed that some features, such as the Shine-Dalgarno sequence were more likely to be learned that others. Other features such as general knowledge of the mRNA were not. What I will show in the following sections is that both students' prior lived object of learning and their perceptions of their specific enacted object of learning were contributing to students' post lived object of learning.

In this study, students were exposed to one of three enacted objects of learning defined by a pair of external representations. Each representation depicted a series of features (critical or otherwise), and the combined series of features depicted by both

representations defined a particular enacted object of learning. Within each enacted object, I varied the presentation order of the representations in order to determine the if the students' understanding of the features viewed initially influenced their perception of the features viewed in a second external representations. However, whether a student saw ER2 and then ER3 as in Group A or ER3 and then ER2 as in Group B, both groups of students were exposed to the same total possible features, i.e., the same enacted object of learning, the same total possibilities for learning.

Some features were presented exclusively in one representation. Therefore, any inclusion of these features in students' final descriptions of translation could be attributed to that particular representation as long as that student did not initially describe that feature prior to viewing the representations. For example, in the case of the Shine-Dalgarno, which will be discussed in more detail later on in this section, students in Groups A, B, E, and F displayed learning of this feature. In order to learn this feature, students first had to demonstrate a lack of or inappropriate knowledge of the Shine-Dalgarno sequence. This was actually seen in all six groups. However, only ER3 contained a depiction of the Shine-Dalgarno sequence. It was noticed that only students who had been exposed to ER3 demonstrated learning about this feature (see Appendix U: Group Comparison of Learned Features). As such, I can claim that because ER3 presented students with only opportunity to potentially learning about the Shine-Dalgarno, any subsequent student learning following exposure to ER3 helped students to learn about this feature. In the following sections, I will discuss the features of the various external representations that, as a consequence of being noticed by the students,

potentially contributed to their learning. Although, I will not discuss all of the features learned by students, I will present the most learned features for each representation.

Features of external representation #2 noticed by students. Groups A, B, C and D all viewed ER2. Among the features of ER2 that, as a consequence of being noticed, contributed to student learning were the prokaryotic conventions of the ribosome (specifically the ribosomal subunit naming), the structure of the ribosome, and the mechanism of peptide bond formation.

Ribosomal subunit naming. As noted previously, students expressed a preoccupation with the prokaryotic versus eukaryotic distinctions of protein translation. Among these was the naming of the large and small ribosomal subunits. Students generally chose to describe translation in terms of prokaryotic naming conventions, i.e., using the prokaryotic terms 30S and 50S to refer to the small and large subunits rather than the eukaryotic terms 40S and 60S. However, students would occasionally use eukaryotic naming. For example, Student 4(A) named the large subunit "60S" and the small subunit "40S" during his initial description and drawing (Figure 44).

> [00:10:54.8] **Student 4(A):** [mumbles] Ok with the ribosome [draws and labels the large subunit "60S"] Ok, this is the AUG [draws the mRNA and labels the start codon], and there's the binding of the 40S [draws and labels the small subunit] which brings, I don't really know how to represent the tRNAs [laughs], but we'll just say it's like that [draws a sideways L shape and labels Met on one end].

This student was shown ER2 followed by ER3. ER2 is the only representation that labels the ribosomal subunits using the Svedberg unit, i.e., a number followed by *S* which is a measure of the rate of sedimentation and an indirect measure of size. ER2 depicts prokaryotic translation and, therefore, the large and small subunits are labeled 50S and

Figure 44. Student 4(A), Initial drawing

30S, respectively. Thus, there was a potentially noticeable variation between what Student 4(A) initially drew (a eukaryotic depiction of translation) and what he saw in ER2 (a prokaryotic depiction of translation). After seeing ER2, Student 4(A) incorporated these new prokaryotic labels along with some other prokaryotic elements, such as the Shine-Dalgarno sequence and formylated methionine, into his final description and drawing (Figure 45)

> [00:31:20.7] **Researcher:** Alright, so we're going to come back to your original drawing, and what I'm going to have you do up here on the top is recreate what you've drawn but if you want to change anything or add anything, again sort of walk me through the steps of, of protein translation.
> [00:31:32.9] **Student 4(A):** Ok, so [draws for a while in silence] let's draw the 30S and then this binds here [draws an arrow to the Shine-Dalgarno sequence] because it binds there, it recognizes the AUG start site, and that, once this binds, it basically brings in the formylmethionine, and then that complex recruits the 50S.

Although depictions of the large and small ribosomal subunits are present throughout ER2, the 30S and 50S labels are only present in the first frame. Moreover, they are two of eighteen alphanumeric labels present in frame 1 of ER2. It is possible that Student 4(A) noticed the difference between his eukaryotic ribosomal subunit labels and the prokaryotic labels shown in ER2. However, it should be noted that ER2 is a static

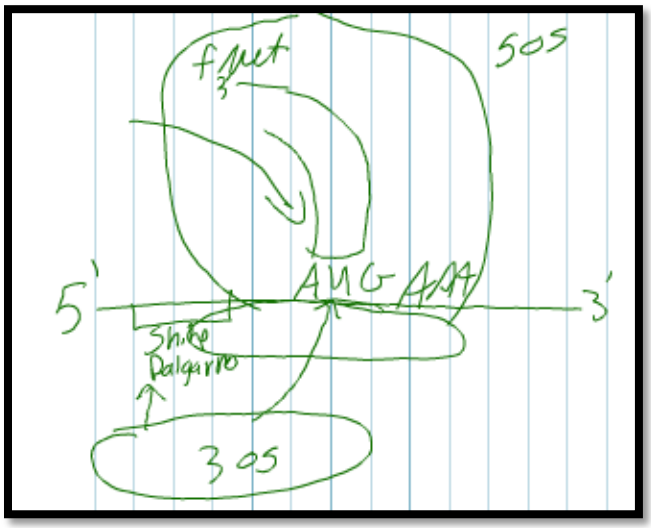


Figure 45. Student 4(A), Final drawing

representation, so even though the 30S and 50S labels only appear in the first frame, that frame is available to students during the entire viewing of the representations. One of affordances of a static diagram is that students have the ability to continually reference any portion of the representation in relation to any other. This is not true of the animations as the appearance of various features changes over time, as seen in the VADER plots of ER3 and ER6 (see Chapter 6 or Appendices L and O).

Student 4(A) may have seen the consistency between his drawing and many of the other features of ER2 as an indication that his depiction of features were mostly initially correct and that he did not need to focus on or changed those aspects of his original depiction. However, the difference in labeling between his drawing and ER2 could have created a noticeable variation between his initial description and his perception of the correct or preferred description as provided by the representation. As a result, he incorporated this new naming into his final description and drawing.

Peptide bond formation reaction. Peptide bond formation was among the primary critical features identified by the instructors and was only depicted as a reaction mechanism in ER2. This was also a highly cued feature of ER2, appearing in four of its six frames and exhibiting changes in position, size, and labeling. Based on the emphasis given to the depiction of peptide bond formation in ER2, it is reasonable that this feature was noticed by many of the students who viewed ER2. However, prior knowledge seemed to influence just thow much a student was able to learn from being exposed to that feature of the representation. For example, Student 12(D)'s initial explanation included a discussion of the fact that, during the protein translation process, amino acids bond; however, she does not provide any detail about the chemistry underlying this bonding event.

> [00:22:24.7] **Student 12(D):** Your tRNA would bring in your amino acids which would then do your bonding.

Similarly, her initial drawing provides no information on the bond formation reaction (Figure 46). She draws a final chain of amino acids, labeled AA chain, but does not

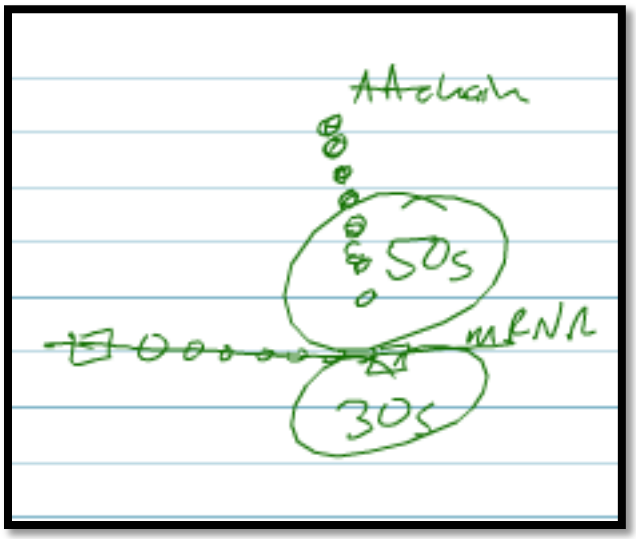


Figure 46. Student 12(D), Initial drawing

indicate any connections between the circles, which she has identified as the amino acids. Instead, her initial drawing provides a general depiction of the sequencing of amino acids but not their bonding. Her final drawing, however, is drastically altered from her initial drawing because she attempted to incorporate the peptide bonding feature into her explanation (Figure 47). Her drawing even indicates "zoom" as she replicates the features of ER2. However, even though she includes many features similar to those presented in ER2, she is still not able to completely articulate the chemical mechanism and has not drawn the mechanism other than to indicate that the N-terminus of "AA_2" attacks the C-terminus of "AA_1."

I think that her inability to articulate or represent the chemical mechanism can be, at least in part, attributed to a lack of prior knowledge of the underlying chemistry or chemical mechanism in general. She was not able to ariculate or depict a mechanism of peptide bond formation in her intial description of the process. She seems to have identified the features that are present in the zoomed-in portion of ER2 as features of importance in that she has chosen to integrate these features into her own description of protein translation. However, while she replicated the general structure of frames 2-6 of ER2—i.e., its E, P, and A site columns and the general shapes of the amino acids and tRNAs—she did not depict the chemical reaction in her drawing, even though the chemical reaction is represented in the external representation. In the following quotation, Student 12(D) attempts to describe the chemistry that occurs during protein translation.

> [01:03:18.8] **Researcher:** How do I get A_1 off of t_1 and onto t_2? I know you drew this arrow. What is that showing?
> [01:03:26.1] **Student 12(D):** I, so [A_2] is going to bind to the CO over here, and then it's going to do a release of, is it CO? And then when [A_1] binds onto the N-terminus, it's going to release an O, and I'm pretty sure that [the O] stays with the t.

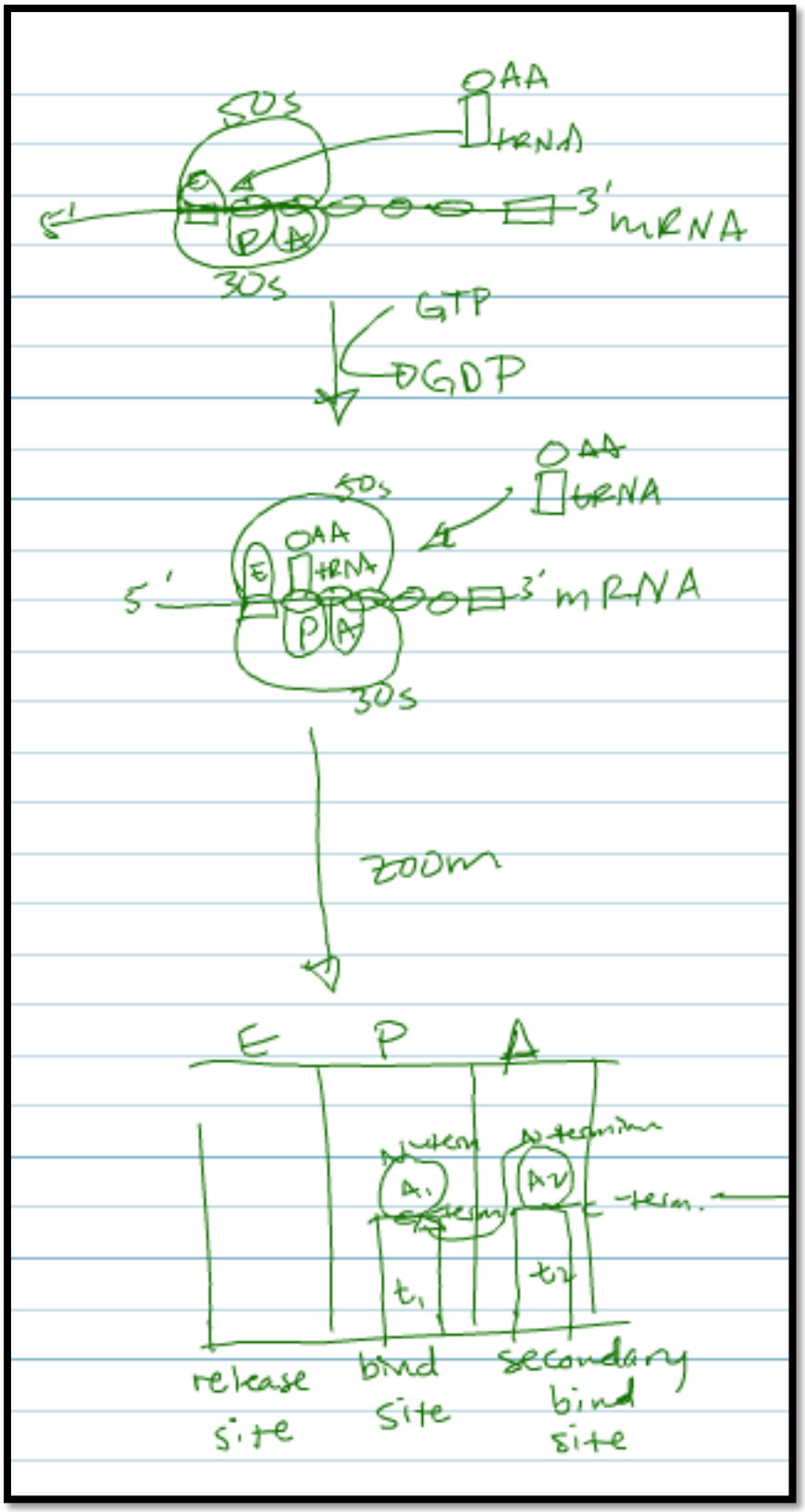


Figure 47. Student 12(D), Final drawing

[01:03:44.3] **Researcher:** So is that causing the bond to break between the A_1 and the t_1?
[01:03:47.9] **Student 12(D):** Yeah, causing that O bond and then the t would have its O and Os are generally, at least with the other electron pairs, are happy, most of the time. It would probably cause a double bond to form in the t, maybe cause a whole shift, but that would be something you would probably show more on like an in depth level.

Overall, Student 12(D)'s limited prior knowledge of the chemistry involved with peptide bond formation seems to have limited what she could learn (and, thus, what she could integrate) from ER2. Students who had higher levels of prior knowledge, on the other hand, were more able to successfully integrate the chemical mechanism from ER2 into their final descriptions of protein translation.

Student 8(B) initially recognized that peptide bond formation was an important feature of translation as evidenced by his attempt to explain the chemical mechanism involved. Very few students initially described or drew this mechanism at all. So while Student 8(B) was not able to accurately describe the mechanism, he does display an awareness of the importance the mechanism plays in the process. His initial attempt to explain the chemistry involved in the formation of the peptide bond shows some chemical knowledge (Figure 48). He is able to represent that an area of negative charge

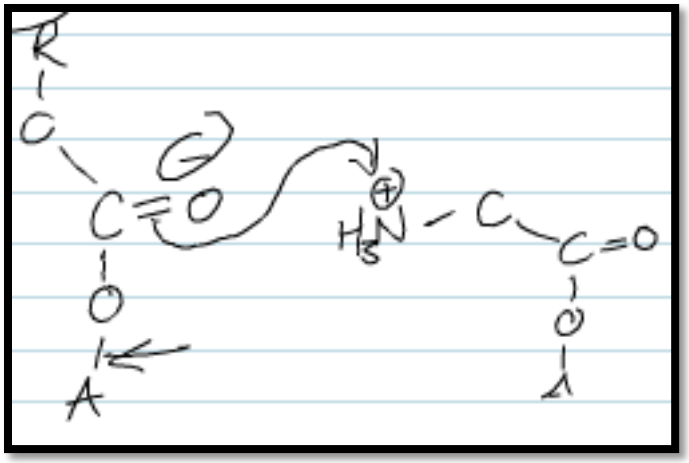


Figure 48. Student 8(B), Initial drawing of the mechanism of peptide bond formation.

would attack an area of positive charge; however, his initial drawing incorrectly shows an attack of the double bond of the carbonyl on the positively charged amine group. However, after viewing ER2, Student 8(B) replicated the chemistry depicted in that representation in his final drawing of the process (Figure 49).

Based on the comparison between the learning or lack of learning demonstrated by Students 12(D) and 8(B), I have concluded that student understanding of the features depicted in any of the representations is informed not only by their ability to identify

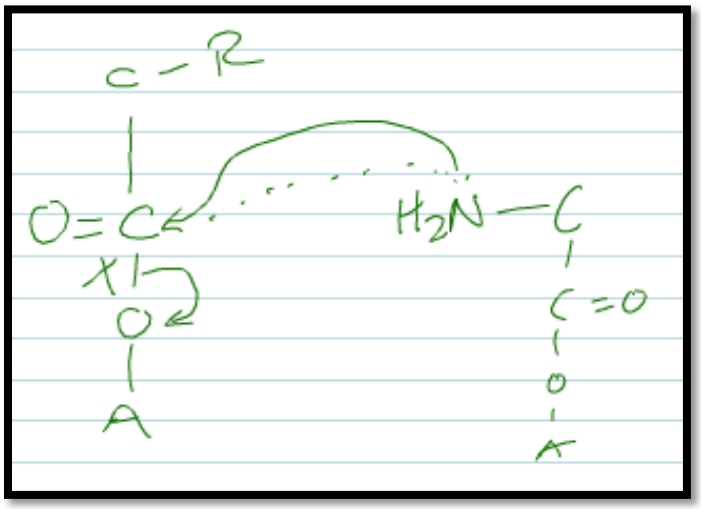


Figure 49. Student 8(B), Final drawing of the mechanism of peptide bond formation.

those features as salient but also by their ability to then decode the information presented in that feature. In this way, students' understanding of a feature, such as the peptide bond formation depicted in ER2, seems to be informed by importance they place on that feature and their ability to understand the content of the feature being depicted.

Features of external representation #3 noticed by students. Groups A, B, E and F all viewed ER3. Among the features of ER3 that, as a consequence of being noticed, contributed to student learning were the Shine-Dalgarno sequence, the formation of the initiation complex, and the role of the release factor in termination.

Shine-Dalgarno sequence. I found the depiction of the Shine-Dalgarno sequence in ER3 to be one of the most learned features from this representation. In examining the VADER Plot for ER3 in order to determine why students were focusing on this particular feature, I found that the Shine-Dalgarno sequence was a highly cued feature when it was presented in ER3. Additionally, I observed that it was presented in the absence of many other features that had previously been present. For example, initially ER3 depicts the formation of the initiation complex. The animation then zooms in on the interaction between the small ribosomal subunit and the mRNA strand. This causes a drop in the number of features depicted in the frame from an average of 19 features per frame to an average of 11 features during the presentation of the Shine-Dalgarno sequence. I determined that this drop in the demand on students' attention, combined with the increase in size as the frames zoom in on the small ribosome/mRNA interaction, could make it easier for students to notice the features, including the Shine-Dalgarno sequence, presented during this scene.

I also observed that the individual components involved in the interaction depicted in this scene were labeled. However, instead of labeling all of the features at the same time, the animation only labels three features at any one time. Those labels were individually highlighted in yellow, calling attention to each component of the interaction. Overall, I think that the high level of cueing as a result of the aspects of variation of the Shine-Dalgarno sequence presented in ER3 served to call many students' attention to that feature.

Some students demonstrated prior knowledge of the Shine-Dalgarno sequence, including it in both their initial and final descriptions of translation. However, other

students did not include the Shine-Dalgarno sequence in their initial descriptions. If a student did not include the Shine-Dalgarno sequence in their initial descriptions of protein translation and was not shown ER3, they did not include the Shine-Dalgarno sequence in their final descriptions of the process. I think this shows that these students either did not know or remember the Shine-Dalgarno sequence and its role in the prokaryotic formation of the initiation complex, or they did not see it as a salient feature of the process and, therefore, did not include it in their initial or final descriptions.

ER3 did, however, have an effect on some of the students who saw it. Some students that did not include the Shine-Dalgarno sequence in their initial description recognized it as a feature of translation and included it in their final descriptions of the process after being shown ER3. Many of these students demonstrated intermediate levels of prior knowledge. For example, Student 25(B) initially made no reference to the Shine-Dalgarno sequence in his description or drawing of initiation (Figure 50).

> [00:34:47.6] **Student 25(B):** Ok, so you have a smaller part of the ribosome down here [draws the small subunit on the bottom], which is going to be like a 30S here [labels the small subunit], and then you have a larger one up here [draws the large subunit on the top], which is like a 50S [labels the large subunit]. Um, and your mRNA is going to be through here [draws mRNA strand between large and small subunits] in this direction. What ends up happening is, ah, this is what I was saying, the initiation factors, and there's 3 or 4 of them around, and I don't remember the names [labels IF=3-4 indicating the number of initiation factors involved]. Ah, they're going to come in and bind the mRNA to this small subunit before the 50S is able to come down because if they clamp down before [referring to the subunits coming together], from what I know you wouldn't be able to have the mRNA come in.

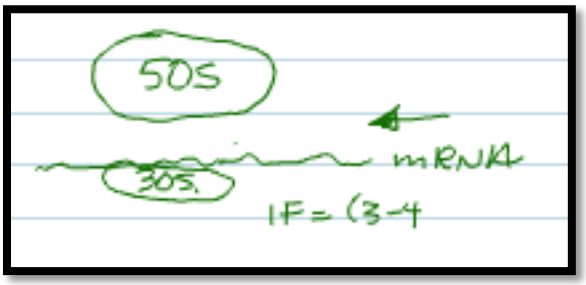


Figure 50. Student 25(B), Initial drawing

Although he mentions the binding between the mRNA and the small ribosomal subunit, he does not identify the role of the Shine-Dalgarno or other start sequence in the assembly process. However, while viewing ER3, he identifies not only the presence of the Shine-Dalgarno sequence depicted in the animation but describes its role in orienting the ribosome at the proper start site for translation to begin.

> [00:46:47.4] **Student 25(B):** [describing ER3 as it is playing] Ok, so you've the small, um, part of the ribosome that's binding to the mRNA which bends the mRNA. […] It goes to find the specific sequence, in this case the Shine-Dalgarno or start site, to know where to actually start the translation […].

This discussion of the specific role of the Shine-Dalgarno sequence in the protein translation process suggests that Student 25(B) was previously aware of this feature; however, his lack of inclusion of this feature in his initial description suggests that he did not initially find the Shine-Dalgarno sequence to be a salient feature of the process. After watching both representations presented to Group B, he does include the Shine-Dalgarno sequence in both his verbal description as well as in his drawing of initiation.

> [01:03:47.0] **Researcher:** So we're going to come back to your drawing over here. So I'm going to have you, one more time, just re-explain to me the process of translation. You can add, change, edit, keep the same anything you did before.
> [01:03:59.5] **Student 25(B):** Ok, um, I don't know. I'm pretty happy with my explanation here. […] I think the only thing that I didn't really mention was once you have the large subunit and small subunit bind or bound together, um, your mRNA has, like I said, the Shine-Dalgarno or a, or a start site, something that triggers the actual start of [translation].

Although not quite accurately, he then draws in the Shine-Dalgarno sequence as part of his final drawing to explain the alignment of the ribosome on the mRNA (Figure 51)

Of all of the features presented in ER3, the Shine-Dalgarno sequence is one of the more interesting. In frames 9 through 14, ER3 zooms in on the alignment of the mRNA and small ribosomal subunit. Among the limited number of features depicted during this

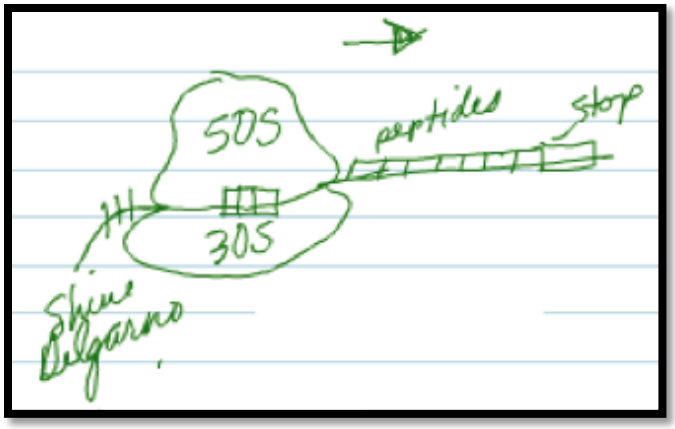


Figure 51. Student 25(B), Final drawing

scene is the Shine-Dalgarno sequence, which is highly cued in frames 12 and 13 (96.1% and 93.8%, respectively). As mentioned previously, students tended to notice the Shine-Dalgarno sequence in ER3. It may be that the way in which this feature is being depicted (i.e., the fact that the scene zooms in on and labels this interaction) is causing students to notice and incorporate this feature into their final descriptions of the process.

Interestingly, though, the Shine-Dalgarno sequence is not the only feature cued in this way during this scene. The 16S rRNA is also zoomed in on and labeled; however, no students described or drew the 16S rRNA in their final descriptions. This is interesting because the 16S rRNA is the ribosomal complement to the Shine-Dalgarno sequence, yet it is not being learned in the same way. It may be that it is not enough that both features are cued in the same way. Many students, like Student 25(B), seemed to be familiar with the Shine-Dalgarno sequence and less familiar with the 16S rRNA. Therefore, perhaps the high cueing combined with students' undeclared prior knowledge of the Shine-Dalgarno sequence is leading to the incorporation of that feature into students' final descriptions of protein translation. Further research is needed to examine why some

highly cued features, such as the Shine-Dalgarno sequence, were more frequently articulated in students' final descriptions or depicted in students' final drawings while other similarly cued features, such as the 16s rRNA, were not.

Initiation complex formation. I found that ER3 was better than ER2 or ER6 at helping students learn the appropriate order in which components interacted during the various stages of translation, especially during the initiation stage. ER2 only showed a single frame indicating that the initiation complex had formed; however, it did not include any depictions of how that complex was formed or the order in which component parts interacted during the initiation stage. ER6 depicted the binding of the small and large ribosomal subunits to the mRNA during the initiation stage, but it did not show the appropriate binding of the initiator tRNA. ER3, on the other hand, depicted the appropriate ordered joining of the mRNA, small ribosomal subunit, the initiator tRNA, and the large ribosomal subunit. It should be noted that ER3 was not completely accurate, as it did not depict the role of the initiation factors in this process. Many students initially described the initiator tRNA entering after the large and small subunit have coming together rather than before. Students without prior knowledge of the formation of the initiation complex and who were not shown ER3 generally guessed as to the sequential ordering of components in the initiation complex. For example, Student 19(C) attempts to rationalize how the initiator tRNA comes to be joined to the initiation complex.

> [01:11:58.9] **Researcher:** How do you think that that first [tRNA] gets in [to the P-site]?
> [01:12:01.7] **Student 19(C):** Um
> [01:12:03.4] **Researcher:** Because from then on out, you're talking about the tRNAs coming into the A-site.
> [01:12:07.1] **Student 19(C):** Yeah, I think that it has to already, maybe these are already connected with the, because, because the start codon is an AUG which

is a methionine, um, so maybe this large ribosome always has a tRNA with a methionine on it.
[01:12:24.6] **Researcher:** So when it comes in, it brings it?
[01:12:15.3] **Student 19(C):** Yeah, and then the other ones will come in.

Student 26(B) displays a similar lack of prior knowledge of this feature. She begins her description of initiation complex formation by noting that the first tRNA, attached to formylated methionine (fMet-tRNA) comes into the P-site after the assembly of the large and small ribosomal subunits on the mRNA (Figure 52).

[00:26:13.6] **Student 26(B):** You have the ribosome, [draws both subunit initially connected] […] so this is the 30S [labeling the small subunit] and this is the 50S [labeling the large subunit] and the whole thing is 70S. Um, and then you have like three sites in, in the ribosome. There's the exit, E-site, the P-site, and the A-site [draws and labels all three sites] and this is the mRNA [draws and labels the mRNA strand]. It goes from a 5' to 3' direction. Um, so the charged, um, say that the charged formylated methionine tRNA would come in here [draws fMet-RNA outside of the ribosome/mRNA complex] to the P-site here [draws an arrow into the P-site]. That's where the AUG would be.

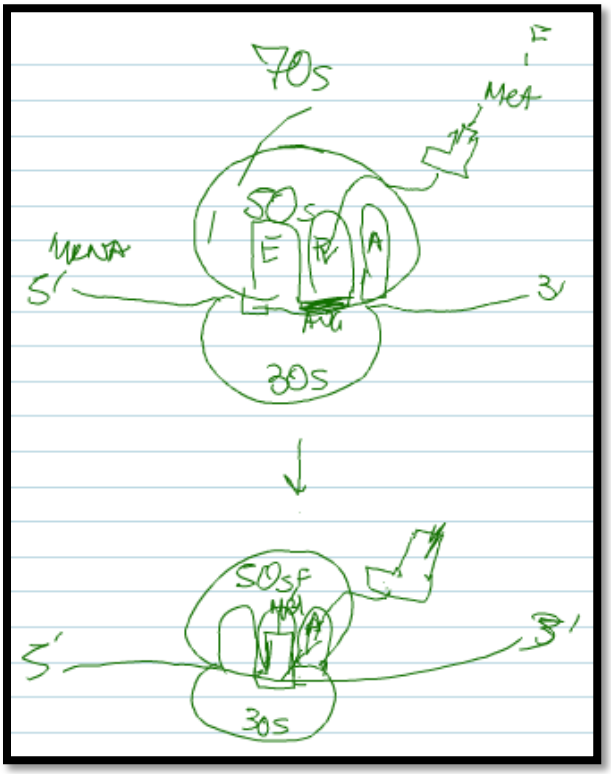


Figure 52. Student 26(B), Initial drawing

However, unlike Student 19(C), Student 26(B) was then shown ER3. After viewing ER3, she begins to recreate her original drawing but then stops and re-sequences the formation of the initiation complex in her final description of the process (Figure 53).

> [01:07:52.1] **Researcher:** So the very last thing we're going to do is come back to your drawing […], so one more time, I'm going to have you draw for me the process of translation. You can leave anything the same [as your initial drawing] that you want or add anything in if you want to do that as well, and again just kind walk me through from start to finish. […]
> [01:08:33.2] **Student 26(B):** Ok, so, um, again I guess we're going to do the general prokaryotic translation, so you have the 30S and 50S subunits on the […] mRNA, and, um, wait, so the start codon would be here AUG, in the P-site. You would still have the formylated methionine attached to the tRNA, um, and, um, there again would be initiation factors involved, oh wait. Can I cross that out?
> [01:10:21.8] **Researcher:** Yeah
> [01:10:23.3] **Student 26(B):** Ok, Forget that. So, um, there was the pre-initiation complex, right, and this was the 30S [drawing only the small subunit], and, um, then there would be the AUG here [labels AUG on mRNA strand], and then there would be the initiation factors floating, well they wouldn't be floating around, they would be actively involved and directing this formylated tRNA [draws fMet-tRNA on the mRNA strand] to its appropriate site here so it can make its correct codon/anticodon base paring. […] So, now the 50S subunit can come in and bind [draws a new frame showing the fully assembled initiation complex followed by a new incoming tRNA entering into the A-site].

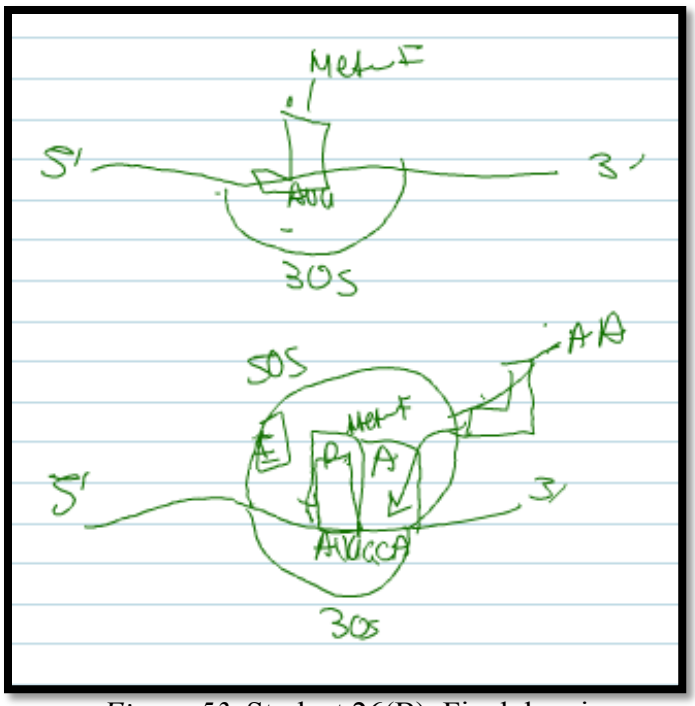


Figure 53. Student 26(B), Final drawing

The comparison between Students 19(C) and 26(B) shows that although both students begin with the same non-scientific explanation regarding the sequencing of the entry of the initiator tRNA into the initiation complex, Student 26(B) was able to correct her initial description by potentially noticing that the depiction of this process provided in ER3 was different from her original description. Thus, she changed her description to align with the perception that the representation was accurately displaying the process. Student 19(C), on the other hand, was not shown ER6; and neither ER2 nor ER6 provided the necessary information regarding when the initiator tRNA entered the initiation complex. As a result, she was unable to learn that information because she was never presented with the possibility of learning that information.

It is possible that Student 26(B) noticed the ordering information simply because it was present in the representation. However, it is also possible that the variation in the position of the components of the initiation complex could have cued Student 26(B) to notice those features more than others. Also, the initiator tRNA is labeled in ER3 once it is attached to the mRNA/small ribosomal subunit portion of the complex. This labeling occurs prior to the joining of the large ribosomal subunit. This may be an additional cue which would allow the viewer to notice the order in which features enter to form the initiation complex.

Role of the release factors in the termination stage. The release factor was a feature commonly noticed in ER3 and incorporated into students' final depictions of translation. Many students were able to initially identify that a release factor would be used to stop the translation process and release the protein during termination. However, several students acknowledged confusion over the exact sequencing of events of

termination, including the role of the release factor in this process. Specifically, they were unsure whether a tRNA first paired with the stop codon to bring in the final amino acid, followed by the arrival of the release factor, or if the release factor first interacted with the stop codon directly. Student 16(A), for example, drew an initial interaction between a tRNA and the codon and indicated that the release factor would come in afterward to terminate translation (Figure 54).

Figure 54. Student 16(A): Initial drawing

However, after watching the release factor bind to the stop codon as depicted in ER3, Student 16(A) then describes an altered final conception of termination, as demonstrated in the following exchange.

> [00:23:16.1] **Student 16(A):** [Final description] The stop codon would be in the A-site.
> [00:23:19.3] **Researcher:** So the release factor is that thing interacting with the stop codon?
> [00:23:23.3] **Student 16(A):** Yes, I believe I said in the first picture, eh, diagram that I made, um, that there was a, a new specific tRNA to interact with the stop codon, but from [seeing ER3], um, it didn't appear that a new tRNA came in and recognized it. I believe the release factor just recognized that there's a stop codon within the A-site, so it's time to come in and disassemble everything.

He then goes on to draw a final depiction of the release factor interacting directly with the stop codon (Figure 55). He even goes so far as to incorporate the iconography of the square depiction of the release factor from ER3 into his drawing (Figure 56).

Figure 55. Student 16(A), Final drawing

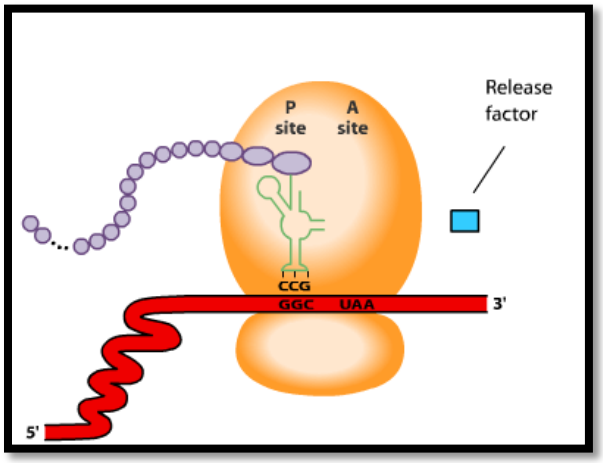


Figure 56. Frame 37 from External Representation #3

Student learning from ER3 seems to be very literal. Many students seemed to notice and learn from highly cued features such as the Shine-Dalgarno sequence or the release factor. Students then incorporated those features into their final descriptions but not always in a completely accurate manner. For example, Student 25(B) named and drew the Shine-Dalgarno sequence in his final drawing but did not completely accurately describe its role. Additionally, students' final drawings tended to include direct reference

to the stylized features depicted in ER3 such as Student 16(A)'s inclusion of the square depiction of the release factor. This may indicate that students see these representations as depicting the truth and potentially as a form to be emulated in their own drawings of the process.

Features of external representation #6 noticed by students. Groups C, D, E, and F all viewed ER6. Among the features of ER6 that, as a consequence of being noticed, contributed to student learning were the dynamic movement of the cellular components during the translation process, the kinetics of the protein translation process, and the general cellular environment in which protein translation takes place.

Dynamic movement. Similar to ER3, ER6 is a dynamic representation. As with ER3, I found that students were able to attend to the dynamic features presented in ER6 and incorporate the dynamic nature of the representation into their understanding of protein translation. For example, Student 30(C) was first shown ER2, the static representation, and then ER6. Prior to seeing either representation, she explained that the translational machinery contained a single, stationary tRNA and that the "proteins" moved in and out of this complex independently.

> [00:28:33.9] **Student 30(C):** [Drawing prior to viewing either representation] Well, the, the proteins are coming to the, to the transfer RNA and they're attaching to the protein binding site […] of the transfer RNA and it tells them exactly what, it matches them to the three nucleotides.

By "proteins" she seems to be referring to the amino acids, as she has labeled this component of her drawing "Phe" and referred to it by the name "phenylalanine" (Figure 57). Later on in the interview, however, she also refers to several of these "proteins" as "a chain of amino acids." Her confusion of the terminology aside, her conception of a

stationary tRNA and components (proteins or amino acids) that come into the tRNA is the feature I am addressing in this example.

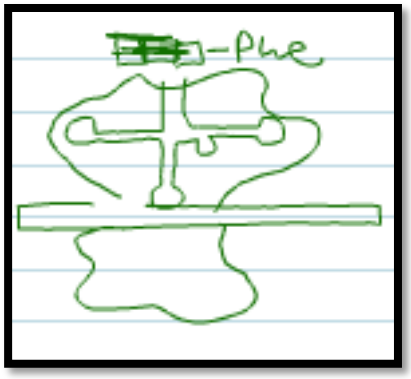


Figure 57. Student 30(C): Initial drawing

After her initial drawing of this process, she was shown ER2. Even though the representation contains depictions of tRNA movement, she does not identify that feature of translation. However, after she saw ER6, she corrected her previous error with regard to the motion of the tRNA.

> [00:59:09.6] **Student 30(C):** [While watching ER6] Yeah, so the transfer RNA doesn't stay in one place like I drew [initially].

She then incorporated this new understanding of the dynamic movement of multiple tRNA molecules through the translational machinery in her final description and drawing of the process (Figure 58). Originally, she asserted that the tRNA was bound to the mRNA and the amino acids moved to them. Her final description indicates that the charged tRNA is what moves.

> [01:06:42.1] **Student 30(C):** [Drawing after to viewing either representation] You'd have, um, the transfer RNA that comes in, and it binds to the codon that's right here, and the anti-codon is on the, on the transfer RNA, and the first, um, the first, eh, amino acid formed would be methionine or formylmethionine, um, and then when that happens you have the new incoming transfer RNA […], and that would come right next.

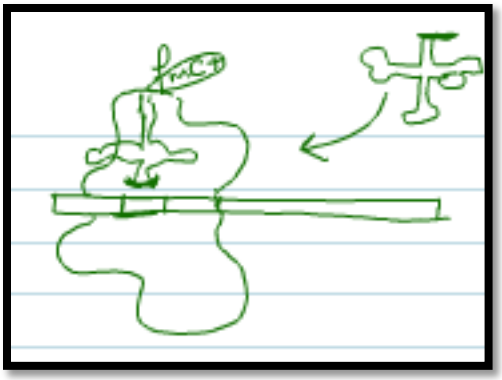


Figure 58. Student 30(C), Final drawing

Reaction kinetics. I found that students that viewed ER6 made general comments about how quickly the middle portion of the animation runs. In a span of approximately 8 seconds, 20 charged tRNAs are shown moving into the A-site, moving over to the P-site, and moving again to the E-site before the leave. The speed with which the components are moving is significantly faster than they do in the other dynamic representation, ER3. Students generally commented on this this speed as a depiction of the actual reaction kinetics (which are actually even much faster than are shown in ER6). For example, Student 18(D) notes that:

> [00:46:15.8] **Student 18(D):** It was nice to see the process, how quickly it moves and the actual movement. That you really are bringing A and P together, transferring it out. […]

I then followed up on her acknowledgement of the speed of the reaction.

> [00:49:10.3] **Researcher:** Why do you think they are showing it to you so quickly?
> [00:49:12.3] **Student 18(D):** Because that's how the cell works.

Similarly, Student 15(C) described this representation by saying:

> [00:46:02.0] **Student 15(C):** I think [ER6] does a good job of real time action.

These descriptions were notable in that students did not initially discuss kinetics.

However, ER6 seems to have elicited specific references to how fast this process actually moves. It is possible that the speed with which the animation is running is what is cueing student to notice the feature of kinetics. This is an affordance unique to dynamic representations. Although a static image such as ER2 can contain dynamic indicators such as arrows or the change in position of features from one frame to the next, the lack of physical movement may not allow students to learn about kinetics in the same way that ER6 seems to have.

Cellular environment. One of the features unique to ER6 is a depiction of the cellular environment. In both ER2 and ER3, protein translation is depicted with a white background (i.e., the translation process is shown separate from its cellular context). However, ER6 depicts protein translation as occurring within the cellular environment. Specifically, it shows the mRNA strand emerging from a nuclear pore and moving in the cytoplasm. This general cellular location was a feature noticed by several students. For example, Student 18(D) initially drew translation in a mostly accurate manner but with out any indication of the cellular environment (Figure 59). However, as she viewed ER6,

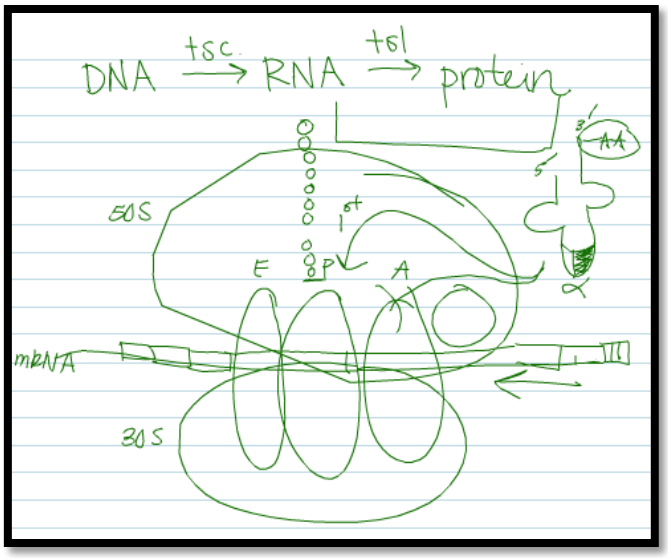


Figure 59: Student 18(D), Initial drawing

she gave the following running commentary in which she described the cytosolic location of the components and subsequent process.

> [01:05:07.9] **Student 18(D):** The strand comes out. Now we're in the cytosol. [The mRNA] comes through. Assemble the ribosome. […]

She then included this depiction of the cellular environment in her final drawing of the process (Figure 60). She even captured some of the graphic elements shown in ER6 (Figure 61).

Among the three features of ER6 that seem to have contributed most to student learning, my VADER analysis identified *kinetics* and *cellular environment* as cued features of ER6 (Chapter 6). The cellular location was a highly cued feature of ER6. It was depicted in all 74 frames. The high cueing may indicate that students are more likely to notice the cellular background and incorporate that feature into their understanding of translation. The kinetics feature was not highly cued overall because of the limited length of the scene in which it appears. Even within its scene, the only indication of kinetics was the speed of the change in position of the tRNA. Although I have previously identified change in position as an aspect of variation that could be noticed by students, it seems that the overall speed of the change in position is allowing students to notice more thanjust the charged tRNAs and mRNA which are doing the moving in ER6. The student comments above seem in imply that they inferred a realistic depiction of the rate at which the process proceeds. Perhaps students are drawn to the rapid motion and take greater note of the kinetic considerations of the translation process. Further analysis and study would be needed to confirm this claim.

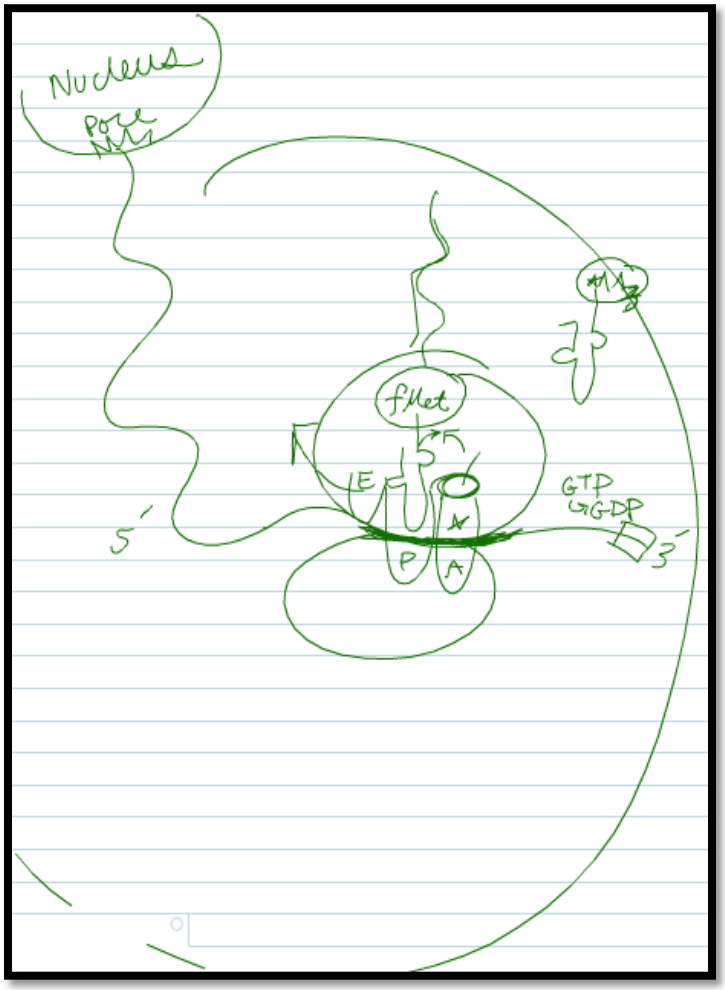


Figure 60. Student 18(D), Final drawing

Figure 61. Frame 4 from External Representation #6

The influence of presentation order. In addition to the influence of the individual representations on student understanding of translation, the pairwise viewing of the representations can be seen to direct students' attention to various features of the enacted object of learning. For example, Groups C and D were both shown the same two representations, ER2 and ER6. Group C was shown ER2 followed by ER6, while Group D was shown ER6 followed by ER2. Together, both groups have been exposed to the same series of features and, therefore, have the possibility to learn from any one of those features regardless of the presentation order. Although the presentation order does not alter the enacted object of learning (i.e., the features about which students can learn), the presentation order could potentially alter the body of knowledge possessed by the student as they successively experience each representation. Specifically, what students know and understand while viewing the first representation (what I have previously described as their prior knowledge) is different from what they know and understanding while viewing the second representation because students have the possibility of learning from the first representation. As a consequence, their perceptions of the second representation could be altered by what they have experienced and learned by interacting with the first representation.

While I was designing this project, I hypothesized that the order of presentation would be reflected in student learning outcomes. The pairwise student groupings were meant to explore this aspect of sequential student learning from multiple enacted objects of learning. Although I was not able to find much evidence to support this hypothesis, I was able to find an example of this altered understanding of the second representation, as informed by the viewing of the first representation, in the following exchange.

> [01:08:19.8] **Researcher:** So tell me about [ER6]. What did you think about it?
> [01:08:24.9] **Student 2(C):** Well it's a good thing we went over [ER2] before I looked at this because I probably wouldn't have any idea where to begin. […] The colors were fairly similar to the picture before, the still picture, so that made it, that was nice, because if they were different, that might have been confusing. Um, because it did throw me off a little bit that [the amino acids] were red instead of blue […], but knowing that, that the green squiggly looked the same as [the tRNA in ER6] and the general L structure let me assume that the reds were [amino acids] now not the blue.

Student 2(C) described how she decoded ER6 by using her knowledge of the depicted features from ER2. ER6 did not contain any labels or information other than the shape, color, and relative interactions of the components. As such, Student 2(C) described using primarily color to compare similar instances of the same features and generate an understanding of the information presented in ER6 based on the information she learned in ER2.

Part of the complication in identifying the influence of presentation order was in being able to identify a potential noticed feature in one representation that was then used by the student to explain a second feature in the second representation. In the example above, Student 2(C) commented explicitly on the fact that she drew on the use of color in ER2 to assign identify to the components of the representation. She then used similarities in the way features were depicted to assignment identities to the features in ER6, even though it was only a coincidence that similar colors were used to depict similar features in both representations (which came from different sources). Thus, it seems as though students are taking representational conventions, such as color, from one representation and are mapping them onto other representations.

Even though this project didn't provide significant evidence of the influence of presentation order on student learning, the evidence of the influence of prior knowledge

is very clear in this project. As an aspect of prior knowledge, i.e., knowledge gained prior to experiencing the second representation, the order of presentation may still affect what students learn from a series of representations. This study, as informed by variation theory, has relied on student utterances and drawings as indications of their state of knowledge at various points in time. It may be that certain features are being noticed but are not being described. Therefore, it is possible that certain features depicted in the first viewed representation are noticed but unstated and are then used by the student (either in a knowing or unknowing manner) to understand features and describe the depicted features in the second viewed representation. Furthermore, the earlier conversation of students' rememberings of prior instruction and, specifically, prior representations supports the assertion that students are using their understandings the representations they have been shown in the past to understand subsequent representations. Additionally, students' ability to decode representations is not only dependent on students' knowledge of the content but also their knowledge of the representational conventions, i.e., color or arrows as dynamic indicators.

Although I did not see much evidence of the influence of presentation order on student learning in the current study, prior research and the evidence of the influence of prior knowledge demonstrated in this project suggest that presentation of could affect student learning from the sequential presentation of multiple representations. In order to determine the influence of presentation order on learning from external representations, I propose designing representations that specifically distort a single feature from one representation to the next. A three point comparison could then be made between students' initial prior knowledge, their first post lived object of learning after

experiencing the first representation, and their second post lived object of learning after experiencing the second representation.

Chapter Summary

In this chapter, I examined students' lived object of learning in comparison to their prior knowledge and experience of a particular enacted object of learning. I found that students' prior lived object of learning had a significant impact on whether or not students could learn from their experience of an enacted object of learning. Students with high levels of prior knowledge did not display learning in the current study, as their initial stated knowledge of the translation process already included the features that were depicted in the representations. Students with intermediate and lower levels of prior knowledge students were able to display learning if they were able to notice and make appropriate meaning of the features depicted in the representations.

Overall, the features depicted in individual representations and the way those features were presented seem to have an impact on what was possible for students to learn. Students' selective attention to certain features seemed to be due to the high cueing potential of those features or the persistence of those features over the course of the representation. Finally, although limited, the current study does provide some evidence that presentation order can influence student understanding of sequentially viewed representations. Thus, further research is needed to examine and clarify the impact of one representation on the sequential viewing of another representation.

CHAPTER 8

CONCLUSIONS AND IMPLICATIONS

Project Overview

The goals of this project were threefold. First, I wanted to identify instructors' intentions for student learning about protein translation. From a phenomenographic analysis of instructor interviews, I was able to determine the critical features instructors felt their students should be learning. Second, I wanted to determine which features of protein translation were possible for students to learn from some common external representations of the process. From a variation analysis of the three representations shown to students, I was able to describe the possible combinations of features enacted by the sequential viewing of pairs of representations. Third, I wanted to identify what students actually learned about protein translation by viewing these external representations. From a phenomenographic analysis of student interviews, I was able to describe changes between students' prior lived object of learning and their post lived object of learning. In this chapter, I will discuss the utility of these findings from the point of view of an instructor, i.e., what considerations should an instructor take into account when selecting a representation of protein translation to show their students? I will also discuss the future work and limitations related to this project.

Instructor Considerations For Selecting an External Representation to Depict an Object of Learning

Based on the results of this project, I have been able to make some conclusions about the influence of a variety of factors on student learning from external representations of protein translation. I will now synthesize my analysis of the three

objects of learning in order to describe considerations that can be addressed by instructors when selecting a representation of protein translation as an educational resource. As a powerful learning environment, representations pose a great opportunity to foster and direct student learning. In this section, I will address the importance of aligning the intended and enacted object of learning in order to produce an aligned lived object of learning. I will then discuss the influence of variation and prior knowledge on students' perceptions of the external representations and on their subsequent learning from those representations. I will also discuss the potential influence of mode and level of abstraction on students' learning from the external representations. Finally, I will discuss the future work and project limitations related to this study.

The Importance of Alignment

I found that most of the students who were interviewed in this project were able to learn from at least one or the combination of two external representations. Generally, a lack of learning was seen from students with higher levels of prior knowledge as opposed to any other group of students. This was due to the fact that these students had already demonstrated a sufficient understanding of the material before viewing the representations. Students who were able to learn from the representations generally demonstrated a lack of stated prior knowledge of a feature or features of protein translation that were then depicted in one or both of the representations. As such, external representations can and should be considered an important space of learning for students as they develop a robust knowledge of protein translation. Therefore, it is important that instructors carefully select the representations that they will use to promote learning.

In selecting an appropriate representation for promoting student learning about protein translation, instructors should first consider the alignment between their intentions for student learning and the possibilities for learning created by the external representation. While students can learn from a variety of representations, not all representations depict the same features. Moreover, not all features are depicted in the same manner. Additionally, some representations may present additional, non-critical features that may distract students from noticing and learning about the critical features. Therefore, instructors should carefully consider which features are critical for students to learn and then select a representation or representations that cue students to notice those features.

In the context of this project, I found that ER2 was the only representation that depicted all 16 of those features instructors had identified as primary critical features (Chapter 5, Table 3). My analysis of the instructor interviews revealed that instructors' intentions for student learning centered on developing students' understanding of the chemistry of protein translation, and ER2 was the only representation to depict peptide bond formation. As such, I concluded that ER2 as an enacted object of learning best aligned with instructors' intended object of learning. I determined that ER3 was the representation that next best aligned with instructors' intended object of learning, depicting 14 of the 16 identified primary critical features. ER6 was aligned third best with a depiction of only 10 primary critical features. Although I have used the primary critical features in order to rank these representations, an instructor might have a specific sub-intention for a particular representation. For example, as described in Chapter 5, Instructor 5 was particularly interested in regulation.

> [00:11:20.7] **Instructor 5:** The fun part of teaching [protein translation] is I like regulation. I love talking about how things are regulated and when you're talking about biochemistry, how things are regulated like pathways of feedback and so forth and pathways that are positive loops and all that. I always tell [the students], look at the big picture. Does this make sense? And it always makes beautiful sense after several billion years of evolution. […] They get kind of an eye opening, oh yeah that just, that just, of course, that's the way it should be, it just makes sense, and when biochemistry, when it all starts to tie in like that back together, and, eh, I kind of understand the big picture, I think then we've accomplished something.

The representations this particular instructor selects may focus more on the regulatory elements of protein translation than would the representations chosen by another instructor. Because students learn from external representations, it is of the utmost importance that an instructor selects a representation that explicitly depicts their individual intentions for student learning.

The Influence of Variation

Another important consideration to take into account when selecting and using external representations as a space of learning is the influence of variation within and between features of the representation(s). Variation theory suggests that students should notice varied features, and that this noticed variation could then lead to learning. In the current study, I have demonstrated that students do indeed notice and learn from variation. Specifically, students seem to notice variation within a particular feature of a representation, between features in two different representations, and between their prior knowledge of a feature and the representational depiction of that feature.

One of the most noticed features of any of the representations used in the current study was the Shine-Dalgarno sequence of ER3. As described in the variation analysis of ER3 in Chapter 6, the Shine-Dalgarno sequence was depicted as varying in size and labeling as ER3 zoomed in on the mRNA/small ribosome alignment interaction that

occurs during the initiation stage of protein translation. In Chapter 7, I described how many students, including Student 25(B), learned from ER3 and incorporated the Shine-Dalgarno sequence into their final descriptions and/or drawings of the translation process.

> [01:03:47.0] **Researcher:** So we're going to come back to your drawing over here. So I'm going to have you, one more time, just re-explain to me the process of translation. You can add, change, edit, keep the same anything you did before.
> [01:03:59.5] **Student 25(B):** Ok, um, I don't know. I'm pretty happy with my explanation here. […] I think the only thing that I didn't really mention was once you have the large subunit and small subunit bind or bound together, um, your mRNA has, like I said, the Shine-Dalgarno or a, or a start site, something that triggers the actual start of [translation].

Both instructors and students described how the "zooming in" or variation in size was a good way to cue viewers to notice other information as well. For example, Student 4(A) suggests that adding a zoomed in view of the mechanism of peptide bond formation, similar to that shown in ER2, would improve students' understanding from ER3.

> [00:29:38.1] **Researcher:** Do you think if you added those things that you like from [ER2] to [ER3] that that would improve the second one or would that not?
> [00:29:47.3] **Student 4(A):** Yeah, probably, um, if you were able to see the actual, like, chemistry binding, like they zoomed in and then showed that it was binding to it and then it transfers instead of just putting in the next tRNA and it just moves and then it pops off that one and move to the next one. It kind of leaves you with 'well how did that happen?'

Thus, variation in position, size, and labeling, as described in the VADER coding of Chapter 6, can be effective cues to helps students to notice and potentially learn about particular features of an object of learning depicted in a representation.

In addition to variation within features, I also found that students noticed variation between the same features depicted in different representations. For example, Student 2(C) noticed that the amino acids were depicted differently in ER2 and ER6.

> [01:08:19.8] **Researcher:** So tell me about [ER6]. What did you think about it?
> [01:08:24.9] **Student 2(C):** Well it's a good thing we went over [ER2] before I looked at this because I probably wouldn't have any idea where to begin. […] The

> colors were fairly similar to the picture before, the still picture, so that made it, that was nice, because if they were different, that might have been confusing. Um, because it did throw me off a little bit that [the amino acids] were red instead of blue […], but knowing that, that the green squiggly looked the same as [the tRNA in ER6] and the general L structure let me assume that the reds were [amino acids] now not the blue.

In this case, however, the variation between representations was more of a hindrance to learning rather than a support. Student 2(C) notes that the only reason she was able to figure out that the amino acids were the same feature being depicted differently was that the tRNAs were both depicted in a similar manner, i.e., both "green squiggly" shapes.

Instructor 1 notes that multiple representations should be internally consistent.

> [00:39:27.1] **Instructor 1:** […I]t's absolutely terrible to switch colors or switch conventions. You must never do that, ever.

Thus, representational consistency is also an important consideration when using multiple representations to create a space of learning. Variation between the same or similar features of multiple representations may cause students to notice those differences and question whether the representations are depicting the same things. If the goal of an instructor is to use external representations to support or facilitate learning, then students' noticing of representational inconsistencies would be an unproductive learning outcome.

Finally, some students seemed to notice variation between their stated or depicted prior knowledge of translation and the features of the process depicted in the representations. As described above and in Chapter 7, Student 25(B) did not initially include the Shine-Dalgarno sequence in his description of translation. However, when he saw the Shine-Dalgarno sequence depicted in ER3, he recognized it as lacking from his original description and included it in his final description of the process. Thus, students' metacognitive abilities may facilitate their noticing of variation between their current

knowledge and the content being depicted in a representation. As such, it may be beneficial to support students' reflective thinking as a way of supporting their learning from external representations.

The Influence of Students' Prior Knowledge

One of the interesting trends I observed in the data was the difference in the emphasis that students and instructors placed on the distinctions between eukaryotic and prokaryotic protein translation. Instructors' intentions for student learning focused, by and large, on the chemistry of the process, in particular on the peptide bond formation and the complementarity between codon and anticodon. Many students, on the other hand, seemed overly concerned about defining the context of the process in an effort to describe the correct components. When they were first asked to explain the process of protein translation, they would ask if they should explain prokaryotic or eukaryotic translation, even though the underlying chemical reactions and interactions identified by the instructors were system independent. The following exchange with Student 25(B) illustrates the student preoccupation with the prokaryotic versus eukaryotic distinctions.

> [00:21:47.3] **Researcher:** We're going to talk about protein synthesis specifically today […]. I just going to have you tell me anything and everything you can remember about that process. What are the major components? How do they interact? What do they do? That kind of thing.
> [00:22:08.3] **Student 25(B):** Where do you want to start? Do you want to talk just translation?
> [00:21:11.5] **Researcher:** So we're going to talk just translation.
> [00:22:13.4] **Student 25(B):** Just translation. Ok.
> [00:21:14.8] **Researcher:** So wherever translation would begin until I get to a final protein. That process.
> [00:22:19.9] **Student 25(B):** Ok. This is where I feel like I've done all my cramming and hopefully it's still in there. Well I know you have different translation process for you pro, ah, prokaryotes and eukaryotes.
> [00:22:32.6] **Researcher:** Ok

[00:22:33.7] **Student 25(B):** And, ah, your prokaryote proteins, there's gonna be a large subunit and small subunit. In your eukaryotes, it's your 60 and your 40 and they come together and create an 80 S.
[00:22:46.6] **Researcher:** Ok
[00:22:47.7] **Student 25(B):** And, your prokaryotes
[00:22:49.8] **Researcher:** Large and small subunits of what?
[00:22:52.4] **Student 25(B):** Ah, that's gonna be your overall ribosome, and, ah, the smaller one in your prokaryotes I mean, you're gonna have a 50 and a 30 S and they come together and create a 60 S.
[00:23:09.6] **Researcher:** Ok. Are the numbers important or is it, do you just need to know large and small?
[00:23:14.7] **Student 25(B):** Well, your, I guess it depends on the depth you go in, but their important in the sense of, they code for different things, different proteins. Um, they're going to be broken down in to your 5Ss, your 13Ss, 16Ss depending on which part of the proteins you're actually talking about because each of those will have a specific process once the ribosome all comes together.

Additionally, many students pointed out system-specific features such as the ribosomal subunit and elongation factor naming in ER2 (Prokaryotic), the Shine-Dalgarno sequence in ER3 (Prokaryotic), or the depiction of a nucleus in ER6 (Eukaryotic). I think that this suggests that the student participants involved in this project felt that the system distinction was an important consideration when describing protein translation. I found this interesting because the instructor participants did not focus on the distinction between prokaryotic and eukaryotic systems. This is not to suggest that the differences between biological systems are entirely unimportant; however, the biochemistry instructors interviewed in this project generally described an intended object of learning that focused more on the chemistry of translation rather than the biological context of translation.

Why would the students value some information of an object of learning more than the instructors who teach this topic? In that case of protein translation, students are not learning about this phenomenon for the first time. Students have learned about protein translation in multiple high school and college courses. Both the instructors and the

students acknowledged the fact that, in college alone, protein translation is covered both in biochemistry and a variety of biological science courses, e.g., microbiology. As such, it is possible that students have been exposed to multiple different instructors' intentions for student learning about protein translation over the course of their individual educational histories. Therefore, students' prior lived object of learning has been constructed through their perceptions of a previous enacted object of learning, which was informed by their previous instructors' intended object of learning (Figure 62). Anecdotally, I informally asked three biology professors if prokaryotic and eukaryotic distinctions in translation would be emphasized in a biology course. All three professors indicated that they could be. They all said that the prokaryotic system was generally the focus of instruction but that eukaryotic differences could certainly be addressed as well.

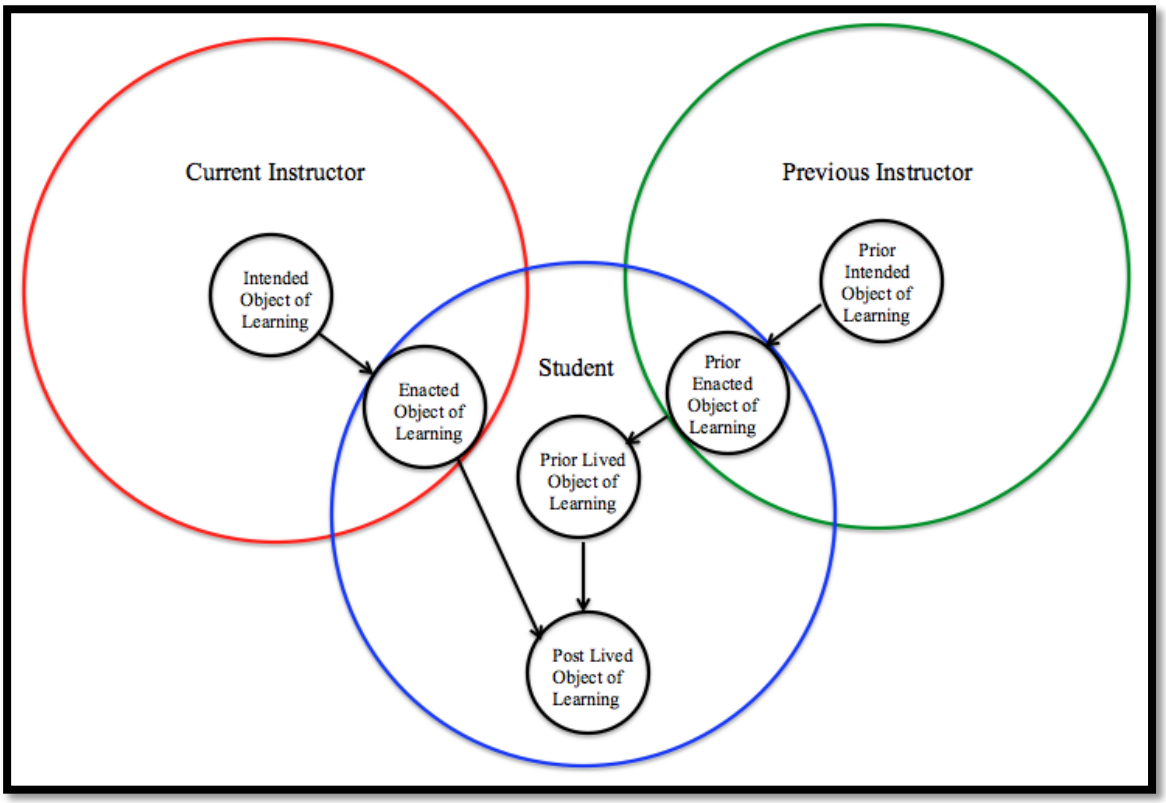


Figure 62: The influence of previous instruction on students' lived object of learning.

Previous instructors' intentions for student learning as enacted and then lived by the student inform how that student will then perceive a new space of learning. While this prior knowledge does not limit the possibilities for student learning, it does inform which features students might notice and value. A biochemistry instructor may not intend for students to learn about the prokaryotic and eukaryotic distinctions within protein translation, but a student who has previously learned to value these distinctions may be more likely to notice those features presented in a new enacted object of learning and continue to integrate them into their lived object of learning. Thus, students' prior knowledge frames the manner in which they engage and interact with a new presentation of information. Instructor 2 acknowledges that students' preference for certain features of the material, or more accurately their aversion to other aspects, is an important pedagogical consideration.

> [00:09:49.0] **Researcher:** Do you find that [students'] prior knowledge that they bring with them to class potentially influences, for better or worse, their understanding of the biochemistry aspect of [protein translation…]?
> [00:10:11.4] **Instructor 2:** Hmm, [pause] I mean, I think if you have students coming from biology, they are going to be reluctant to understand a little bit of electron pushing that's needed to understand the mechanism. […] Um, you have students coming from chemistry; they're going to be, um, kind of overwhelmed by the size and complexity of that structure. Um, so you've got to hold hands on both sides a little bit.

In biochemistry, very little material is completely new to students. Many of the concepts have been presented in prerequisite courses, and students will bring that knowledge with them into the biochemistry classroom. In this study, I found that students frequently cited previous instruction, both verbal and visual, while attempting to construct individually meaningful descriptions of protein translation. This means that biochemistry instructors must consider not only what they intend for students to learn

about topics such as protein translation, but also what students have previously learned about that topic as this may cause students to attend to some features more than others even if those features may not be seen as critical features by the current instructor. While it is certainly possible that an individual biochemistry instructor might choose to emphasize prokaryotic and eukaryotic distinctions in protein translation, it would, nonetheless, be valuable for that instructor to consider the impact of other instructors' intentions for student learning in complementary courses. This information could be used to redirect student attention to those features deemed critical to promote a particular understanding of the topic. To paraphrase one of the biology professors with whom I spoke: "I guess we should be talking to each other more."

The Potential Influence of Mode

The mode of the representation, defined in this project as static or dynamic, was a primary consideration when designing this project. Lowe (2003) described the lack of research to support the prevalent assumption that animations are superior to static graphics. Therefore, to address this gap in the literature, this project attempted to examine the effect of representational mode on student learning.

I think that the mode of the representation of ER2, i.e., static, may have distracted some students from attending to certain critical features because of the high number of simultaneously presented features. I found that, in general, students had to pause and orient themselves in terms of the order and flow of the static representation. I also think that in order to fully appreciate the depicted features, students would also have to mentally separate one frame from the next in order to perceive the temporal relationship between features and to perceive any variation in position, size, and labeling as shown in

the Frame Series and VADER Plot of ER2 (see Appendix H and Appendix I, respectively.) I think that this added mental task of temporally reading the static diagram depicting a dynamic process adds an additional cognitive factor for students to balance as they evaluate and construct meaning for the features they notice in ER2.

ER3 contained the same amount, although not the same identity, of features as ER2. However, students described ER3 as being less complicated or difficult to understand. This may be due to the specific features presented in ER2 versus ER3, or perhaps this is a result of the distribution of features over the course of the representation. While ER2, more or less, presented all features simultaneously, ER3 introduced features in a sequential manner.

The static nature of ER2 necessitated its simultaneous presentation of all features, and the presentation of a large number of features at the same time could pose a challenge for students. However, students seemed to deal with this challenge by using the arrows and general positioning as dynamic indicators to divide the representation into a series of frames. As such, they were then noticing one frame of features then the next as they described ER2. In this way, ER2 could be viewed a frame series in the same way that ER3 and ER6 were. Even still, the vast majority of features were presented simultaneously in ER2. The distributed presentation of features may have made it easier for a viewer to follow the introduction of features in ER3. ER6 presented the fewest number of features but did so in a distributed manner, similar to ER3.

Based on the analysis of the representations used in this project, it seems as though the dynamic representations could be used to cue student to notice certain features more so than the static representation could. Strategies such as the zoom in scene for the

Shine-Dalgarno sequence in ER3 showed the potential to draw students' attention to one particular depicted feature. Unfortunately, the Shine-Dalgarno sequence was not of the utmost importance as described by the instructor intentions. Thus, if the dynamic representations were re-designed to emphasize the chemistry of the process, student attention would be drawn to peptide-bond formation, one of the primary critical features of protein translation, as defined by the instructors. Both students and instructors acknowledged that the addition of an emphasized peptide-bond formation feature would improve a given representation of protein translation.

While the dynamic representations might be better able to cue students to notice certain critical features, like zooming in on a depiction of peptide bond formation as was suggested above, it was generally thought that the animations contained less information. Even though ER3 contained the same number of features as ER2, this was not noticed by the viewers. For example, Student 16(A) responds to the amount of information she perceived to be in ER3.

> [01:10:08.1] **Student 16(A):** [in response to ER3] Um, everything, it had all the basic components of, um, translation. It wasn't as detailed [as ER2] because I imagine making an animation, you can't really have the words, like long words, popping up. You don't really have time to read it.

Interestingly, she attributes the level of detail to the mode itself rather than the particular representation. This might suggest that even if there is no difference between the effect of static versus dynamic representations on student learning, student perceptions of the mode may affect how they perceive the representations; and this might affect how students learn from them.

The VADER plots for ER2, ER3, and ER6 revealed overall structural differences between the representations. I found that ER2 showed a highly condensed presentation of

its features, while ER3 and ER6 demonstrated a scene structure in which a given feature entered and/or exited the frame over the course of the representation. These structural differences may not be exclusive to mode but they may be more prominent in some modes over others. For example, it may be easier to depict a series of scenes in an animation rather than in a static image. Specifically, the number of frames in ER3 is more than 7 times the number of frames in ER2. The physical space needed to depict a static version of ER3 might be considerable. Thus, it may be a more efficient use of time and space to depict a lot of information in a shorter series of frames as was seen in the static representation. However, an economic use of time and space may not make for the best learning environment. For example, students with lower level of prior knowledge of an object of learning may not be able to fully comprehend a representation containing a large number of simultaneously depicted features of that object because they are less familiar with the components, interactions, or representational conventions used to convey that information. Therefore, instructors should consider the both the efficiency of the representation at conveying information as well as students' ability to understanding the information being depicted.

Another consideration related to the mode of the representation is the ability of the students to control the viewing event. Because the animations, at least the ones I used in this project, run at a certain speed, it might be challenging for students to process the information depicted in the dynamic representation at the speed at which it is presented. The static representation, on the other hand, gives students ownership over the viewing process. They can control where they look and how long they look there. Another potential benefit of the static representation is that it allows students the ability to look at

an earlier frame to understand features depicted in a later frame of the representation. This reflective viewing may improve students' understanding of the material presented in the static representation.

Depending on what information the instructor is trying to convey in a representation, one mode may be better for promoting learning than another. For example, if an instructor intends to show students the kinetics of translation, a dynamic animation may be more appropriate. On the other hand, if an instructor wants to show the relative orientation of the initiator tRNA in the initiation complex at a specific moment in time, perhaps a static illustration would be more appropriate.

Although I was not able to completely distinguish the specific influence of mode on student learning, I think the analysis conducted in this project has led to some interesting insights regarding the potential affordances and structures of different representational modes. Specifically, instructors should consider the number of features simultaneously presented to students. The static representation, ER2, used in this project depicted the most features per frame, while the stylized, dynamic representation, ER3, depicted the same total number of features, but far fewer per frame. Fewer simultaneously presented features may make it easier for students to learn about the features depicted in ER3 rather than those in ER2.

Another important consideration is the interactivity of the representational mode. The advantage of the static representation in this project is that it allows students to revisit and re-evaluate previously seen features. If a dynamic representation is presented to students, it may be important to allow students to control the speed of the viewing event or allow them to re-watch the animation as needed. This would allow them

additional time to evaluate and process the information being depicted in the representation.

The Potential Influence of Abstraction

The other factor that I originally considered when designing this project was the level of abstraction of the representation. However, similar to evaluations of the mode of representation, I was not able to directly compare the stylized representations with the realistic representation. Although I attempted to control for variations between representations, I found that the representations contained different amounts and types of information; and, therefore, I was not able to attribute any specific student learning outcomes to variation in the level of abstraction. However, I was able to gain some interesting insights from the instructors regarding the potential influence of level of abstraction.

The instructors generally felt that realistic representations of the content were not essential for promoting student learning about protein translation. In fact, many of them felt that the extra information presented in a more realistic representation might cue students to notice features that were not deemed important by the instructors. For example, Instructor 4 describes how the specific shapes of the component features might distract students.

> [1:09:57.7] **Instructor 4:** For a practicing scientist, a more realistic view is important but […] for a biochem class, you know, so if students visualize a ribosome as two oval subunits that come together, so what? No harm is done if they understand the process and the chemistry and further more, they all realize that they are not really these perfect ovals. They all realize that they are looking at a schematic and that the actual molecular contours are going to be not these perfect ovals so it's not even like they are under some misapprehension, you know, and for them to see it in its you know really an accurate looking shape, it's just simply not important at [the undergraduate] level.

Therefore, instructors should consider that students are not practicing biochemists. They are biochemistry students. As such, representational realism may not provide an appropriate space of learning for students at this level. Moreover, Instructor 4 goes on to explain that stylized representations may better align with biochemistry instructors' intentions for student learning.

> [1:23:54.5] **Instructor 4:** Honestly, for most biochemistry, schematic is better than realistic. I think really.
> [1:23:54.5] **Researcher:** Why do you say that?
> [1:23:54.5] **Instructor 4:** Well because, um, because the, the, the important thing is understanding the players and what they do and how they interact with each other and the chemistry that occurs because of that and the contours of the molecular features, you know, the exact shape of this and that protein is, is just less important unless you going to be, you know, making, unless you're designing site-directed mutagenesis experiments, in which case you really need to know right where the active site is, what it looks like, […] unless you're doing research, honestly, whether […] a protein […] is globular with a cleft or globular with a wide cleft or apparently almost spherical with odds and ends and nooks and crannies and no discernible features, any of those could be equally well represented by a just something that's just more stylized, you know, and there's nothing wrong with that, and then, and then of course each, in a schematic, each object can have its own characteristic shape and maybe it's a parallelogram, maybe that isn't really corresponding much to what it really is in real life, ah, but if that gives it a characteristic shape that will let you differentiate it from the other guy and keep track of it […] the precise molecular contours of those molecules is just of way less importance than what they do and how they work together and all that kind of stuff.

Thus, it is important for instructors to see students as novice biochemists, and to consider the appropriateness of the depiction of the object of learning with regard to instructors' intentions for student learning.

Interestingly, I found myself operating under the assumption that a realistic representation would potentially be more complicated by the addition of added structural information. However, the VADER analysis revealed that ER6, a realistic representation, did not include a large amount of features, critical or otherwise. This reduction in the

amount of information presented in a realistic representation as compared to the stylized representations (both static and dynamic) may be due to the fact that chemistry schematics and conventions are inherently stylized. For example, the way that we would imagine the peptide bond formation to occur through an arrow pushing mechanism is a highly stylized representation of the phenomenon. There is no arrow appearing in a realistic setting to form a bond between the amine nitrogen and the carbonyl carbon. Thus, the fact that ER6 did not present any of the chemistry of the process is potentially due, in part, to the effort to portray a realistic environment. Thus, not all of the critical features identified by instructors could be portrayed in a realistic manner. Therefore, realism may be at odds with instructors' intentions for student learning about translation and provides an additional consideration for instructors when selecting representations to promote student learning.

Future Work

In this project, I set out to describe what was possible for students to learn about the biochemistry of protein translation from some common external representations. What I found was that students were able to learn about a variety of features of protein translation from these representations but that not all of these features aligned with instructors' intended object of learning. However, there seem to be a variety of competing forces that also influenced what students could learn from the external representations, including students' prior knowledge, variation within and between representations, and potentially, mode and level of abstraction. However, further research is needed in order to determine more clearly how each of these forces potentially affects students learning.

Schönborn and Anderson (2006) have stated that the mode and level of abstraction of a representation are factors that may influence students' understanding of the content depicted in a representation. My work suggests that animations may be better than static images at distributing information throughout a representation and, therefore, may make it easier for students to notice and integrate that information into their understanding of the information depicted in the animation. My work also suggests that more realistic animations cannot always show the features instructors would like their students to see because those features are not typically depicted in a realistic way. For example, the schematics of the mechanism of peptide bond formation are not a realistic representation of what a bond actually looks like.

Because Schönborn and Anderson suggest that the mode and level of abstraction of a representation are factors that may influence students' understanding of the content depicted in a representation and because my work hints at possible influences of these two factors, in a future project, I would create or identify external representations that contain similar feature depictions but that vary only in mode and/or level of abstraction. This would allow me to better address the influence of these two factors without content disparities influencing student learning.

Future Methodological Development

In my attempts to analyze the external representations chosen for this study, I developed the Variation Analysis of Dynamic External Representations (VADER) method. This methodology was useful in that it gave me a very structured way to analyze the variation that was present in the different external representations that were shown to students in this study. Given that this study was the first attempt to use the VADER

method to analyze dynamic external representations, I feel that future studies could further develop and improve upon this method. Altogether, I have noticed a number of issues or limitations that should be resolved as the method continues to be used.

Although I think that VADER was able to depict a qualitative picture of the potential cueing created by various aspects of variation, I do not think that it was able to fully capture all of the aspects of variation and their relationships that affect students' ability to notice certain features over others or that it provided a direct measure between one representation and another. I designed the VADER method such that unchanged features would fade proportionally over the entire course of their depiction. However, it may be that once a feature has persisted unchanged for a certain amount of time, that it would the fade exponentially from a viewer's attention. Additionally, it may be that features presented earlier in the dynamic representation fade in importance over time as compared to similarly cued features presented at the end of the representation. Future research that examines the fading of features over time can be used to further improve the VADER method.

I also noticed that the VADER plots treated all features equally regardless of size relative to other features or the prominence of features found center of frame. I think that larger features might be more noticeable than smaller features, and a viewer might be more likely to notice features that are center of frame rather than features that are found around the parameter of the frame. A future VADER scoring regimen should account for these additional aspects of variation. Finally, the current regimen weights all aspects of variation equally, i.e., change in position, size, and labeling. However, it might be that

students are more likely to notice a label rather than a change in position. For example, Student 8(D) comes an inaccurate conclusion based on the lack of a label.

[00:50:09.5] **Student 8(B):** This is eukaryotic because there's no E-site.

He is referring to the fact that ER3 does not have a label for the E-site of the ribosome. He is noticing that the A and P-sites are labeled, but aside from the labels there are no other indication of the binding sites. He is, therefore, noticing the other labels more than the relative position of the tRNA which does indicate an E-site position, although it is not labeled or marked.

The weighting of individual aspects of variation in determining VADER coding should also be considered as the method improves. Although the method can certainly be improved through future development and research, I believe that, overall, in developing the VADER method, I have established a useful and structured method for analyzing and qualitatively comparing dynamic representations or static representations with dynamic indicators.

Project Limitations

I have identified several limitations to this project. As a study built on the phenomenographic tradition, there are inherent limitations to the work. In accordance with variation theory, I have assumed that participants' verbal descriptions and visual depictions represent their salient knowledge of the content. It is possible that I have not been able to fully capture instructors' intended object of learning or students' lived object of learning. However, I have been able to capture their expressed objects of learning, which is consistent with the phenomenographic tradition.

I also think that the range of student prior knowledge may have had more of an influence on student learning outcomes in this project than the representations themselves. Any future studies should look to better normalize student prior knowledge perhaps by strategically sampling and grouping students based on performance measures. I think this would ensure that any trends in the student data could more accurately be attributable to a specific enacted object of learning. However, even with the range of prior knowledge, I was still able to identify a variety of interesting findings and insight from this project and, specifically, provide evidence of learning from the representations.

With regard to the representations used in this project, I have also identified a few limitations. Even though the instructors were able to select the final three representations that were shown to students, the instructors chose from a very small, pre-selected pool of possible representations. Additionally, I have noted that the representations did not depict the same features. Any future studies should attempt to identify or create representations that contain features that might be more easily comparable in order to better assess the specific impacts of a given representation on student learning. For example, a future project could create a static image from selected frames of an animation. In this way the features are the same but the mode would be different. This would allow for a more direct comparison between representations and could be complementary to the conclusions drawn from this project. The VADER method developed in the current study would provide a good measure of feature similarity between representations in future work.

Conclusion

> [00:29:05.2] **Instructor 2:** I think the key thing is trying to design [a representation] so students can get the information that they're supposed to get out of [it].

Instructor 2 has identified the heart of this project. External representations in biochemistry, and more broadly in science, education have the potential to be powerful learning environments as long as students can notice the critical features of the object of learning. In this study, the alignment between instructors' intentions and student learning was only possible if those critical features were presented to students in the representations to which they were exposed. As such, instructors should design or select representations to specifically depict defined objects of learning to students. This design or selection should be done with explicit intentions of what it is that students should be learning from their experience of a representation in order to minimize the possibilities for student learning of non-critical features. Instructors should ask themselves: "What are the critical features of a particular object of learning, and are these presented in the representation in the manner that would allow students to notice them?"

With regard to the enacted object of learning, the space of learning created by a representation should depict an object of learning in such a way as to minimize distracting or competing critical features or to de-emphasize non-critical features. When presenting more than one representation pertaining to the same object of learning, instructors should consider representational continuity, i.e., are features presented in a similar manner across representations. Variation between the same features in different representations could lead to student difficulties in decoding and assigning meaning to depicted features.

With regard to students' lived object of learning, students' prior knowledge, and specifically prior instructors' intentions for student learning, must be carefully considered by current instructors when enacting a given object of learning. Finally, I suggest that

further work be conducted on the influence of variation, the development of the VADER methodology, and the potential influence of the mode and level of abstraction in order to more fully realize the potential for external representations to constitute a meaningful space of learning and to create a more scientifically accurate lived object of learning for students.

The current study has provided a successful model for the exploration of the space of learning and student learning outcomes created by some common external representations of protein translation. Primary, secondary, and tertiary critical features of protein translation were identified based on interviews with biochemistry instructors. The identification of the instructors' intended object of learning was then used to inform the development of the VADER coding methodology. This variation analysis was used to describe the enacted object of learning created by the pairwise presentation of external representations of protein translation. Finally, student learning was determined by comparing their stated prior knowledge of translation with their final descriptions of the process. Based on the findings from this project, I was able to provided several recommendations for instructors to consider when selecting and using external representations to promote student learning in biochemistry and, more broadly, in science.

APPENDIX A

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3 messages

Thomas Bussey <busseyt2@unlv.nevada.edu> Tue, Feb 19, 2013 at 8:41 AM
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Thom
--
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Central Dogma.tiff
29K

Cronk, Jeff <cronk@gonzaga.edu> Thu, Feb 28, 2013 at 7:02 PM
To: Thomas Bussey <busseyt2@unlv.nevada.edu>

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Kind regards,

Jeff

Jeff D. Cronk, Ph.D.
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TO: Thomas Bussey

FR: Diane Kraut, W.H. Freeman & Company/Worth Publishers

DATE: February 8, 2013

SUBJECT: Permission to use material from *Berg et al. Biochemistry, 6/e* and *Nelson/Cox Lehninger Principles of Biochemistry, 5/e*

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Diane Kraut
Permissions Assistant
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Thomas Bussey <busseyt2@unlv.nevada.edu>

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3 messages

Thomas Bussey <busseyt2@unlv.nevada.edu> Tue, Jan 29, 2013 at 2:23 PM
To: oleavey@biostudio.com

Hello,

My name is Thomas Bussey. I am a doctoral student in the Chemistry Department at the University of Nevada, Las Vegas.

I am currently working on my dissertation project. I am examining biochemistry students' understanding of the process of protein translation. In my interviews with students and teachers, I would like to use the following animation to elicit participant responses.

http://www.biostudio.com/demo_freeman_protein_synthesis.htm

Would you be willing to grant permission for me to use this animation in my dissertation?

Thank you very much for your time and consideration in this matter.

--
Thomas Bussey
Graduate Assistant
Department of Chemistry
University of Nevada, Las Vegas
(702)895-3743
busseyt2@unlv.nevada.edu

Tanya Awabdy <awabdy@biostudio.com> Mon, Feb 4, 2013 at 11:44 AM
To: Thomas Bussey <busseyt2@unlv.nevada.edu>
Cc: Mark O'Leavey <oleavey@biostudio.com>

Hi Thomas,

Yes, it's fine for you to use our protein synthesis animation for your dissertation research. I'd love to learn what comes out of your project -- possible for you to forward any publications, when you get to that stage?

Best of luck,

Tanya

Tanya Awabdy, Ph.D.
awabdy@biostudio.com

BioStudio Visual Communications, Inc.
3740 SE Taylor St.
Portland, OR 97214-4345
503 236 8686 Office
503 236 8685 Fax
415 305 0292 Cell
www.biostudio.com

From: Mark O'Leavey
Sent: Wednesday, January 30, 2013 8:42 AM
To: Tanya Awabdy
Subject: Fwd: Copyright Permission for Animation Used in Doctoral Dissertation

[Quoted text hidden]

Thomas Bussey <busseyt2@unlv.nevada.edu> Mon, Feb 4, 2013 at 11:46 AM
To: Tanya Awabdy <awabdy@biostudio.com>

Thank you very much!

I would be more than happy to send you publications that come from this project.

Thank you again.

Thom
[Quoted text hidden]

Thomas Bussey <busseyt2@unlv.nevada.edu>

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3 messages

Thomas Bussey <busseyt2@unlv.nevada.edu> Tue, Jan 29, 2013 at 2:25 PM
To: phillip.mcclean@ndsu.edu

Hello Dr. McClean,

My name is Thomas Bussey. I am a doctoral student in the Chemistry Department at the University of Nevada, Las Vegas.

I am currently working on my dissertation project. I am examining biochemistry students' understanding of the process of protein translation. In my interviews with students and teachers, I would like to use the following animation to elicit participant responses.

http://vcell.ndsu.nodak.edu/animations/translation/movie-flash.htm

Would you be willing to grant permission for me to use this animation in my dissertation?

Thank you very much for your time and consideration in this matter.

--
Thomas Bussey
Graduate Assistant
Department of Chemistry
University of Nevada, Las Vegas
(702)895-3743
busseyt2@unlv.nevada.edu

Phil <mcclean@beangenes.cws.ndsu.nodak.edu> Wed, Jan 30, 2013 at 6:36 AM
To: Thomas Bussey <busseyt2@unlv.nevada.edu>

Yes, you can use the animation. Good luck with your exam.

Phil McClean
Professor / Department of Plant Sciences
NORTH DAKOTA STATE UNIVERSITY
Fargo, ND 58102
p:701.231-8443 / f:701.231-8474 / www.ndsu.edu
[Quoted text hidden]

Thomas Bussey <busseyt2@unlv.nevada.edu> Wed, Jan 30, 2013 at 8:36 AM
To: Phil <mcclean@beangenes.cws.ndsu.nodak.edu>

Dr. McClean,

Thank you very much.

Thom
[Quoted text hidden]

Thomas Bussey <busseyt2@unlv.nevada.edu>

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3 messages

Thomas Bussey <busseyt2@unlv.nevada.edu> Tue, Jan 29, 2013 at 2:28 PM
To: ramak@mrc-lmb.cam.ac.uk

Hello Dr. Ramakrishnan,

My name is Thomas Bussey. I am a doctoral student in the Chemistry Department at the University of Nevada, Las Vegas.

I am currently working on my dissertation project. I am examining biochemistry students' understanding of the process of protein translation. In my interviews with students and teachers, I would like to use the following animation to elicit participant responses.

http://pubs.acs.org/cen/multimedia/85/ribosome/translation_bacterial.html

Would you be willing to grant permission for me to use this animation in my dissertation?

Thank you very much for your time and consideration in this matter.

--
Thomas Bussey
Graduate Assistant
Department of Chemistry
University of Nevada, Las Vegas
(702)895-3743
busseyt2@unlv.nevada.edu

V. Ramakrishnan <ramak@mrc-lmb.cam.ac.uk> Wed, Jan 30, 2013 at 1:23 AM
To: Thomas Bussey <busseyt2@unlv.nevada.edu>

Dear Thomas,
I am happy for you to use the movie as long as you don't remove the opening credit sequence. Also, I would prefer you took the movie directly from our web site, www.mrc-lmb.cam.ac.uk.ribo/ rather than the acs web site.
Yours sincerely,
Venki Ramakrishnan
[Quoted text hidden]

Thomas Bussey <busseyt2@unlv.nevada.edu> Wed, Jan 30, 2013 at 8:34 AM
To: "V. Ramakrishnan" <ramak@mrc-lmb.cam.ac.uk>

Hi Dr. Ramakrishnan,

Thank you very much. I will not remove the opening credits, and I will change the citation to reflect the animation on your website rather than the ACS site. Thanks again.

Thom
[Quoted text hidden]

Thomas Bussey <busseyt2@unlv.nevada.edu>

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3 messages

Thomas Bussey <busseyt2@unlv.nevada.edu> Tue, Jan 29, 2013 at 2:33 PM
To: wehi-tv@wehi.edu.au

Hello,

My name is Thomas Bussey. I am a doctoral student in the Chemistry Department at the University of Nevada, Las Vegas.

I am currently working on my dissertation project. I am examining biochemistry students' understanding of the process of protein translation. In my interviews with students and teachers, I would like to use the following animation from WEHI.TV to elicit participant responses.

http://www.wehi.edu.au/education/wehitv/dna_central_dogma_part_2_-_translation/

Would you be willing to grant permission for me to use this animation in my dissertation?

Thank you very much for your time and consideration in this matter.

--
Thomas Bussey
Graduate Assistant
Department of Chemistry
University of Nevada, Las Vegas
(702)895-3743
busseyt2@unlv.nevada.edu

Drew Berry <berry@wehi.edu.au> Tue, Jan 29, 2013 at 2:37 PM
To: Thomas Bussey <busseyt2@unlv.nevada.edu>

Hi Thomas

Please do use the animation. There are more versions of it on biointeractive.org for download

Best of luck

drew
[Quoted text hidden]

The information in this email is confidential and intended solely for the addressee.
You must not disclose, forward, print or use it without the permission of the sender.

Thomas Bussey <busseyt2@unlv.nevada.edu> Tue, Jan 29, 2013 at 2:39 PM
To: Drew Berry <berry@wehi.edu.au>

Drew,

Thank you very much.

Thom
--
Thomas Bussey
Graduate Assistant
Department of Chemistry
University of Nevada, Las Vegas
(702)895-3743
busseyt2@unlv.nevada.edu

[Quoted text hidden]

APPENDIX B

INSTRUCTOR INTERVIEW GUIDE

Demographics

- Tell me a little bit about yourself.
    - Where did you go to school?
    - How long have you been at (the school name)?
    - Tell me a little bit about your research. What are you currently working on?
    - Tell me about your teaching experience. What courses do you teach?

Phase 1 – Intended Object of Learning of Translation

- Today I would like to talk about translation. Some people might refer to it as protein synthesis … [long pause] … can you explain to me how the process works? … what are the essential components of the process? Feel free to draw if that makes it easier to explain. [Paper and pencil will be provided to all interviewees.]

- Do you teach (or have you taught) translation?

- If you were going to explain this process to your students, how would you go about doing so? What things would you emphasize to them so they understand the process?

- What do you see as being the main idea? What's the "big thing" students should come away with after learning about translation?

- What prior knowledge do your students need to have in order to understand translation?

- What difficulties do you think students might encounter when trying to understand this topic?

- Do you think translation is an important biochemical process for student to understand? Explain your answer.

Phase 2 – Instructor Evaluation of the Static External Representations of Translation

- I'm going to show you a series of external representations. An external representation is an image or animation, just some type of representation. I'm going to show them to you two at a time. For each set, I would like you to pick the one that you would show to your students to explain translation … so here's

#1 … and here's #2 … which one would you choose to show your students? Why did you choose # ____? What didn't you like about the other representation?

- Consider the external representation you didn't like.
    - Why didn't you like it?
    - What could students learn from that representation?
    - What things would they notice? (What would stick out to them?)
    - What would you change about this representation to make it better?

- Consider the external representation you did like.
    - Why did you like it?
    - What could students learn from this representation?
    - What things would they notice?
    - What could students learn from this representation that they wouldn't get from the other one?
    - Is there anything on this representation in particular that you don't like or think might be confusing to students?
    - What do you think this external representation is not showing? Explain your answer.
    - Consider yourself a graphic designer or textbook author. If you could change this external representation in any way, what would you do to improve it, if anything?

Phase 3 – Instructor Evaluation of the Stylized, Dynamic External Representations of Translation

- Again, for this set, I would like you to pick the one that you would show to your students to explain translation … so here's #3 … and here's #4 … which one would you choose to show your students? Why did you choose # ____? What didn't you like about the other representation?

- Consider the external representation you didn't like.
    - Why didn't you like it?
    - What could students learn from that representation?
    - What things would they notice? (What would stick out to them?)
    - What would you change about this representation to make it better?

- Consider the external representation you did like.
    - Why did you like it?
    - What could students learn from this representation?
    - What things would they notice?

- What could students learn from this representation that they wouldn't get from the other one?
- Is there anything on this representation in particular that you don't like or think might be confusing to students?
- What do you think this external representation is not showing? Explain your answer.
- Consider yourself a graphic designer or textbook author. If you could change this external representation in any way, what would you do to improve it, if anything?

Phase 4 – Instructor Evaluation of the Realistic, Dynamic External Representations of Translation

- For the last set, I would like you to again pick the one that you would show to your students to explain translation … so here's #5 … and here's #6 … which one would you choose to show your students? Why did you choose # ____? What didn't you like about the other representation?

- Consider the external representation you didn't like.

  - Why didn't you like it?
  - What could students learn from that representation?
  - What things would they notice? (What would stick out to them?)
  - What would you change about this representation to make it better?

- Consider the external representation you did like.

  - Why did you like it?
  - What could students learn from this representation?
  - What things would they notice?
  - What could students learn from this representation that they wouldn't get from the other one?
  - Is there anything on this representation in particular that you don't like or think might be confusing to students?
  - What do you think this external representation is not showing? Explain your answer.
  - Consider yourself a graphic designer or textbook author. If you could change this external representation in any way, what would you do to improve it, if anything?

Phase 5 – Instructor Perceptions of External Representations of Translation

- So you chose #s _____, _____, and _____. Having now seen all of the representations, would you change anything about your responses?

- If you were to show your students all three external representations, what order would you show them in? Why?

- Do you think order matters? In other words, do you think student's understanding would change if you changed the order in which they see them? Why or why not?

- If you had to choose only one external representation to show to your students to explain translation, which one would you choose? Why # _____?

- Do you think students' understanding would change if the type of representation changed? In other words, do you think students would understand translation differently if they were only shown an illustration or an animation, or if they were only shown a stylized representation or a realistic one? Why or why not?

- What would your ideal representation for teaching translation look like? Don't feel bound by traditional presentation formats like textbooks or powerpoint slides. If anything were possible, how would you show students protein translation?

- Do you have any final thoughts about external representations or translation? Thank you so much for your time today.

APPENDIX C

STUDENT INTERVIEW GUIDE

Demographics

- Tell me a little bit about yourself.
    - What year are you in school? (Sophomore, Junior, …)
    - What is your major?
    - What do you want to do after graduate?
    - Have you taken Biochem II? If so, when?
    - What are your feelings towards biochemistry? Why do you say that?
    - How is biochemistry similar to other science classes you have taken? How is it different?

Phase 1 – Students' Prior Knowledge of Translation (Prior Lived Object of Learning)

- Today I would like to talk about translation. Some people might refer to it as protein synthesis … [long pause] … take your time and start thinking about this process and its components and the sequence of events. Take as much time as you want, don't rush, just relax and think about it for a while [long pause] … think about everything you know about this process, what parts are involved [long pause] … slowly, let your thoughts flow … [silence]. When you are ready, go ahead and tell me what you know about translation … if you want, you can draw things too [paper and pencil will be provided to all interviewees] … [after a while] … ok, what are you thinking about now …

- If you were going to explain this process to another student, how would you go about doing so? What things would you emphasize to them so they could understand the process?

- What do you see as being the main idea? What's the "big thing" students should come away with after learning about translation?

- What do you think is the most difficult thing about learning or understanding translation?

Phases 2 – 6 – Students' Experiences of External Representations of Translation (Post Lived Object of Learning)

Phase 2 – Student Perceptions of the External Representation of Translation #1

- I'm going to show you two external representations of translation. An external representation is just a fancy way of saying an image or an animation, just some type of representation … [pause] … When you look at the external representation, I'm going to ask that you sort of think out loud for me so I know what you're

thinking. Tell me what you're seeing, what you're thinking about. Anything that's going through your mind. Don't worry about being "right." I just want to know what you know about what you're seeing.

- Go ahead and take a look at the first external representation … tell me what you're seeing.

- What can you tell me about what is happening in this external representation?

- Tell about all of the different parts you see. What are they? What do they do? How do they interact? What are the most important parts?

Phase 3 – Student Evaluation of the External Representations of Translation #1

- Is there anything on the external representation in particular that you don't understand or find confusing? … What about this do you find confusing?

- What do you think this external representation is not showing? Explain your answer.

- Do you think this is a good and clear representation? Give reasons for your answer.

- Consider yourself a graphic designer or textbook author. If you could change this external representation in any way, what would you do to improve it, if anything?

Phase 4 – Student Perceptions of the External Representation of Translation #2

- I'm going to show you the second external representation of translation. Remember to go ahead and just think out loud. Don't worry about being "right" or "wrong." I just want to know what you're seeing.

- Here's the second external representation … tell me what you're seeing.

- What can you tell me about what is happening in this external representation?

- Tell about all of the different parts you see. What are they? What do they do? How do they interact? What are the most important parts?

Phase 5 – Student Evaluation of the External Representations of Translation #2

- Is there anything on the external representation in particular that you don't understand or find confusing?

- What do you think this external representation is not showing? Explain your answer.

- Do you think this is a good and clear representation? Give reasons for your answer.

- Consider yourself a graphic designer or textbook author. If you could change this external representation in any way, what would you do to improve it, if anything?

Phase 6 – Student Comparison of External Representations #1 and #2

- Take a minute and think about the two external representations you looked at. [The student will be shown both representations at this point] What differences did you notice between the two representations? What similarities did you notice?

- Do you think both representations contained the same amount of information? Why do you say that?

- Which external representation do think was easier to understand? What made # _____ easier?

- If you were going to compare them, which representation do you think was the best? What about # _____ makes it better than # _____?

- If you were going to explain translation to another student, would you choose to show them one of these external representations?

    - If yes, which one? Why # _____? What would the student have to know before you showed them the representation? What things would you point out to them better understand the representation?
    - If no, why not? Would you show them something different? How would you get them to understand translation?

- Do you find it helpful or not to use external representations when you learn about things like translation? Why do you say that?

- Earlier you said that translation was _____ (repeat what they said in Phase 1, show any drawings they made). Do you still agree? Is there anything you would add or change?

- Do you have any final thoughts about external representations or translation? Thank you so much for your time today.

APPENDIX D

DEFINITION OF CODES

16S rRNA: A description or depiction of the ribosomal ribonucleic acid component of the small ribosomal subunit od prokaryotes.

2D shape: A description or depiction of the two dimensional cloverleaf shape of a transfer ribonucleic acid.

3′ end M: A description or depiction of the 3’ hydroxyl end of a messenger ribonucleic acid.

3′ end T: A description or depiction of the 3’ hydroxyl end of a deacylated transfer ribonucleic acid.

3′ poly A tail: A description or depiction of the polyadenylated 3’ tail of a mature eukaryotic messenger ribonucleic acid.

3D shape: A description or depiction of the three dimensional L-shape of a transfer ribonucleic acid.

5′ end M: A description or depiction of the 5’ phosphate end of a messenger ribonucleic acid.

5′ end T: A description or depiction of the 5’ phosphate end of a transfer ribonucleic acid.

5′ methylated cap: A description or depiction of the 7-methylguanosine cap at the 5’ end of a mature eukaryotic messenger ribonucleic acid.

A-site tRNA: A description or depiction of a transfer ribonucleic acid occupying the Aminoacyl (A) site of the ribosome.

AA/AA interaction: A description or depiction of two or more amino acids interacting with one another. A peptide bond does not need to be indicated.

Aminoacyl (A) site: A description or depiction of the animoacyl (A) site of the ribosome.

Anti-codon loop: A description or depiction of the anti-codon loop of a transfer ribonucleic acid. The nucleotide base sequence does not need to be indicated.

Codon: A description or depiction of a codon on the messenger ribonucleic acid. The nucleotide base sequence does not need to be indicated.

Condon/Anti-codon base pairing E: A description or depiction of the interaction between start codon of the messenger ribonucleic acid and the complementary anti-codon

on the anti-codon loop of the initiator transfer ribonucleic acid. This interaction occurs only during the initiation phase of protein translation. Hydrogen bonding does not need to be indicated.

Condon/Anti-codon base pairing I: A description or depiction of the interaction between a codon of the messenger ribonucleic acid and a complementary anti-codon on the anti-codon loop of a transfer ribonucleic acid. This interaction occurs only during the elongation phase of protein translation. Hydrogen bonding does not need to be indicated.

E-site tRNA: A description or depiction of a transfer ribonucleic acid occupying the Exit (E) site of the ribosome.

EF-G: A description or depiction of the prokaryotic elongation factor G

EF-Ts: A description or depiction of the prokaryotic elongation factor Ts

EF-Tu: A description or depiction of the prokaryotic elongation factor Tu

Energetics: A description or depiction of thermodynamic considerations of the process of protein translation.

Evolution: A description or depiction of the impact of the process of protein translation on evolution.

Exit (E) site: A description or depiction of the Exit (E) site of the ribosome.

Exiting tRNA/ribosome/mRNA: A description or depiction of the relationship between the messenger ribonucleic acid, the ribosome, and the transfer ribonucleic acid that has exited the ribosome.

General molecule M: A description or depiction of the messenger ribonucleic acid as a general entity.

General molecule P: A description or depiction of the polypeptide chain as a general entity.

General molecule(s) AA: A description or depiction of one or several amino acids as a general entity.

General molecule(s) EF: A description or depiction of one or several elongation factors as a general entity.

General molecule(s) IF: A description or depiction of one or several initiation factors as a general entity.

General molecule(s) R: A description or depiction of the ribosome as a general entity.

General molecule(s) RF: A description or depiction of one or several release factors as a general entity. The term termination factor(s) will be included in the code.

General molecule(s) T: A description or depiction of one or several transfer ribonucleic acids as a general entity.

General process A: A description or depiction of the process of activation of transfer ribonucleic acids as a general entity. This code also includes charging of transfer ribonucleic acids.

General process E: A description or depiction of the process of elongation as a general entity. This code includes indications of polypeptide chain growth.

General process I: A description or depiction of the process of initiation as a general entity.

General process T: A description or depiction of the process of termination as a general entity.

GTPase activity of EFs: A description or depiction of the process of hydrolysis of guanosine triphosphate to guanosine diphosphate and phosphate as carried out by an elongation factor as a general entity.

Hydrogen bonding (codon/anticodon) E: A description or depiction of the hydrogen bonds formed between the codon of a messenger ribonucleic acid and the anti-codon of a complementary transfer ribonucleic acid during elongation.

Hydrogen bonding (codon/anticodon) I: A description or depiction of the hydrogen bonds formed between the start codon of the messenger ribonucleic acid and the anti-codon of the initiator transfer ribonucleic acid during initiation.

Hydrogen bonding (mRNA/ribosome) I: A description or depiction of the hydrogen bonds formed between the Shine-Dalgarno sequence of prokaryotic messenger ribonucleic acid and the16S ribosomal ribonucleic acid of the small ribosomal subunit during initiation.

Incoming tRNA/ribosome/mRNA: A description or depiction of the relationship between the messenger ribonucleic acid, the ribosome, and the transfer ribonucleic acid that is about to enter the Aminoacyl (A) site the ribosome.

Initial tRNA/ribosome/mRNA: A description or depiction of the relationship between the messenger ribonucleic acid, the ribosome, and the initiator transfer ribonucleic acid during the formation of the initiation complex.

Large subunit: A description or depiction of the large ribosomal subunit.

Methionine: A description or depiction of the amino acid, methionine, of the initiator transfer ribonucleic acid in eukaryotes. This code also includes formylmethionine on the initiator transfer ribonucleic acid in prokaryotes.

Nucleotide sequence (Anti-codon loop): A description or depiction of the nucleotide sequence of the anti-codon loop of a transfer ribonucleic acid.

Nucleotide sequence (tRNA): A description or depiction of the nucleotide sequence of a transfer ribonucleic acid. This code is inclusive of the anti-codon loop and refers to the entire sequence of the transfer ribonucleic acid.

Nucleotide sequence (multiple codons): A description or depiction of the nucleotide sequence of multiple codons of the messenger ribonucleic acid. This code is inclusive of the start and stop codons and refers to a series of codon on the messenger ribonucleic acid.

Nucleotide sequence (start codon): A description or depiction of the nucleotide sequence of the start codon of the messenger ribonucleic acid (AUG).

Nucleotide sequence (stop codon): A description or depiction of the nucleotide sequence of the stop codon of the messenger ribonucleic acid.

P-site tRNA: A description or depiction of a transfer ribonucleic acid occupying the Peptidyl (P) site of the ribosome.

Peptide bond formation: A description or depiction of the nucleophilic attack by the lone pair electrons on the amine group of the amino acid of the A-site tRNA on the carbonyl carbon of the amino acid of the P-site tRNA resulting in the formation of a peptide bond. This is a mechanistic description or depiction.

Peptidyl (P) site: A description or depiction of the Peptidyl (P) site of the ribosome.

Primary structure: A description or depiction of the primary sequence of amino acids of the polypeptide chain. The identity of the amino acids does not need to be indicated.

Random motion of cellular components: A description or depiction of the random motion of cellular components in the cytoplasm. Indications of how those random motions can lead to component collisions and subsequent chemical reaction may also be included.

Reaction Kinetics: A description or depiction of the of the rate or speed of the process of protein translation.

Regeneration of activated tRNAs: A description or depiction of the process of regenerating transfer ribonucleic acids with an appropriate amino acid that corresponds to the anti-codon sequence.

Regulation: A description or depiction of various aspects of cellular regulation on the process of protein translation.

Ribosomal translocation: A description or depiction of the movement of the ribosome from one codon to the next from the start codon to the stop codon of the messenger ribonucleic acid.

Secondary structure: A description or depiction of the folding of the polypeptide chain as it interacts with the cytoplasm.

Sequential AA: A description or depiction of the amino acid attached to the incoming transfer ribonucleic acid.

Shine-Dalgarno sequence: A description or depiction of the Shine-Dalgarno region of the prokaryotic messenger ribonucleic acid located upstream of the start codon.

Small subunit: A description or depiction of the small ribosomal subunit.

Start codon: A description or depiction of the start codon of the messenger ribonucleic acid. The nucleotide sequence does not need to be indicated.

Stop codon: A description or depiction of the stop codon of the messenger ribonucleic acid. The nucleotide sequence does not need to be indicated.

Tunnel: A description or depiction of the channel through the large ribosomal subunit from the P-site to the cytoplasm though which the growing polypeptide chain exits the ribosome.

APPENDIX E

INITIAL CODED FEATURES OF PROTEIN TRANSALTION FROM INSTRUCTOR INTERVIEWS

Code Frequency Category	Coded Feature	Instructor Utterance*				
		I1	I2	I3	I4	I5
Common (All instructors)	General molecule M					
	General molecule R					
	General molecule(s) T					
Majority (3 or 4 instructors)	Aminoacyl (A) site					
	Anti-codon loop					
	Condon					
	Evolution					
	Exit (E) site					
	General molecule AA					
	General molecule P					
	Peptide bond formation					
	Peptidyl (P) site					
Minority (1 or 2 instructors)	3' end M					
	3’ end T					
	3D shape					
	5’ end M					
	A-site tRNA					
	Condon/anti-codon base pairing E					
	Condon/anti-codon base pairing I					
	Energetics					
	Exiting tRNA					
	General molecule(s) IF					
	General Process A					
	General process I					
	Incoming tRNA					
	Large subunit					
	Nucleotide sequence (multiple codons)					
	Nucleotide sequence (start codon)					
	P-site tRNA					
	Primary structure					
	Reaction kinetics					
	Regeneration of activated tRNAs					
	Regulation					
	Ribosomal translocation					
	Small subunit					
	Start codon					
	Stop codon					

	Tunnel					

Note: Green indicates that an instructor referred to a given feature. Red indicates that an instructor made no reference to a given feature.

*Instructors are abbreviated with the letter “I” and their number. For example, Instructor 1 is abbreviated as I1.

APPENDIX F

FINAL CODED FEATURES OF PROTEIN TRANSALTION FROM INSTRUCTOR INTERVIEWS

Code Frequency Category	Coded Feature	Instructor Utterance*				
		I1	I2	I3	I4	I5
Common (All 5 instructors)	Aminoacyl (A) site					
	Anti-codon loop					
	Condon					
	Condon/anti-codon base pairing E					
	Condon/anti-codon base pairing I					
	Exit (E) site					
	General molecule AA					
	General molecule M					
	General molecule P					
	General molecule R					
	General molecule(s) T					
	Large subunit					
	P-site tRNA					
	Peptide bond formation					
	Peptidyl (P) site					
	Random motion of components					
	Small subunit					
Majority (3 or 4 instructors)	3' end T					
	3' end M					
	3' poly A tail					
	5' end M					
	A-site tRNA					
	Evolution					
	Exiting tRNA					
	General molecule(s) EF					
	General molecule(s) RF					
	General process A					
	General process E					
	General process I					
	Incoming tRNA					
	Methionine					
	Nucleotide sequence (multiple codons)					
	Primary structure					
	Reaction kinetics					
	Regulation					
	Ribosomal translocation					
	Shine-Dalgarno sequence					

	Tunnel					
Minority (1 or 2 instructors)	16S rRNA					
	2D shape					
	3D shape					
	5’ end T					
	5’ methylated cap					
	E-site tRNA					
	EF-G					
	EF-Ts					
	EF-Tu					
	Energetics					
	General molecule(s) IF					
	GTPase activity of EFs					
	Hydrogen bonding (Codon/Anti-codon) E					
	Hydrogen bonding (Codon/Anti-codon) I					
	Nucleotide sequence (anti-codon loop)					
	Nucleotide sequence (start codon)					
	Regeneration of activated tRNAs					
	Secondary structure					
	Sequential AA					
	Start codon					
	Stop codon					

Note: Green indicates that an instructor referred to a given feature. Red indicates that an instructor made no reference to a given feature. Yellow indicates a feature that was mentioned prior to viewing the common external representations.

*Instructors are abbreviated with the letter “I” and their number. For example, Instructor 1 is abbreviated as I1.

APPENDIX G

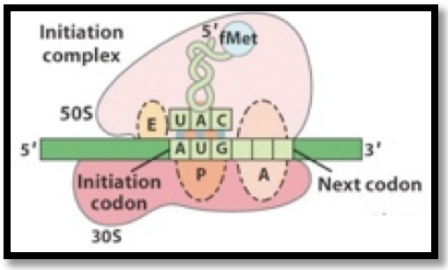
CATEGORIZATION OF CRITICAL FEATURES OF PROTEIN TRANSLATION

Theme	General Feature	Coded Feature	Type of Feature
Component/Structure	mRNA	General molecule M	1°
		Condon	1°
		Nucleotide sequence (multiple codons)	2°
		Shine-Dalgarno sequence	2°
		5’ end M	2°
		3’ end M	2°
		3’ poly A tail	2°
		5’ methylated cap	3°
		Start codon	3°
		Stop codon	3°
		Nucleotide sequence (start codon)	3°
	Ribosome	General molecule R	1°
		Large subunit	1°
		Small subunit	1°
		Aminoacyl (A) site	1°
		Peptidyl (P) site	1°
		Exit (E) site	1°
		Tunnel	2°
		16S rRNA	3°
	tRNA	General molecule(s) T	1°
		Anti-codon loop	1°
		P-site tRNA	1°
		A-site tRNA	2°
		3' end T	2°
		E-site tRNA	3°
		3D shape	3°
		2D shape	3°
		5’ end T	3°
		Nucleotide sequence (anti-codon loop)	3°
	Amino Acids	General molecule AA	1°
		Methionine	2°
		Sequential AA	3°
	Polypeptide Chain	General molecule P	1°
		Primary structure	2°
		Secondary structure	3°
	Initiation Factors	General molecule(s) IF	3°
	Elongation Factors	General molecule(s) EF	2°
		EF-Tu	3°
		EF-Ts	3°

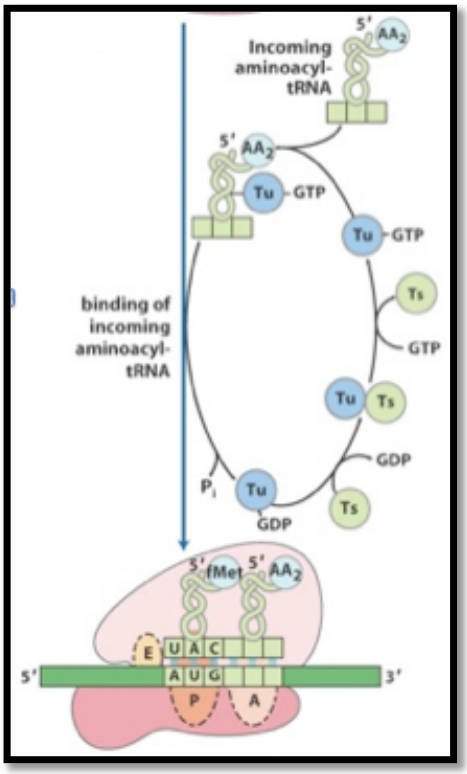
		EF-G	3°
	Release Factors	General molecule(s) RF	2°
Interactions/Chemistry	Activation	General process A	2°
		Regeneration of activated tRNAs	3°
	Initiation	Condon/anti-codon base pairing I	1°
		General process I	2°
		Hydrogen bonding (Codon/Anti-codon) I	3°
	Elongation	Peptide bond formation	1°
		Condon/anti-codon base pairing E	1°
		General process E	2°
		Incoming tRNA/ribosome/mRNA	2°
		Exiting tRNA/ribosome/mRNA	2°
		Ribosomal translocation	2°
		GTPase activity of EFs	3°
		Hydrogen bonding (Codon/Anti-codon) E	3°
General Considerations		Reaction kinetics	2°
		Evolution	2°
		Regulation	2°
		Random motion of components	2°
		Energetics	3°

APPENDIX H

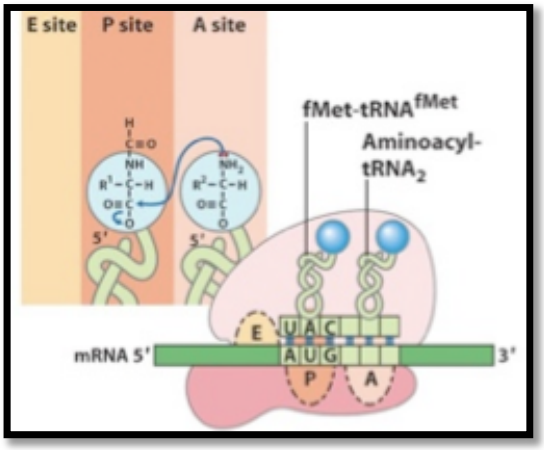
FRAME SEQUENCE OF EXTERNAL REPRESENTATION #2



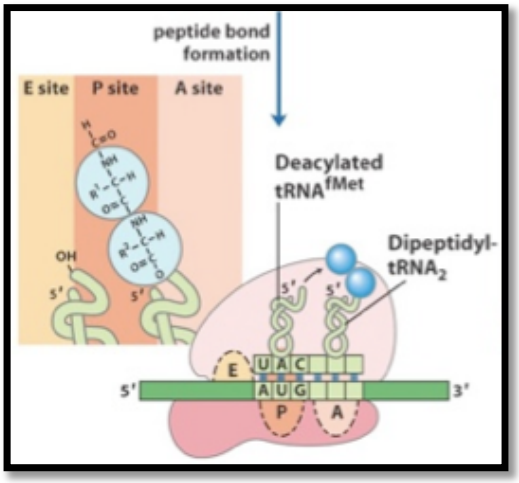
ER2_1



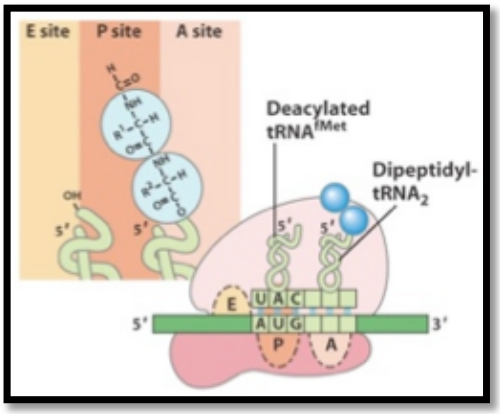
ER2_2



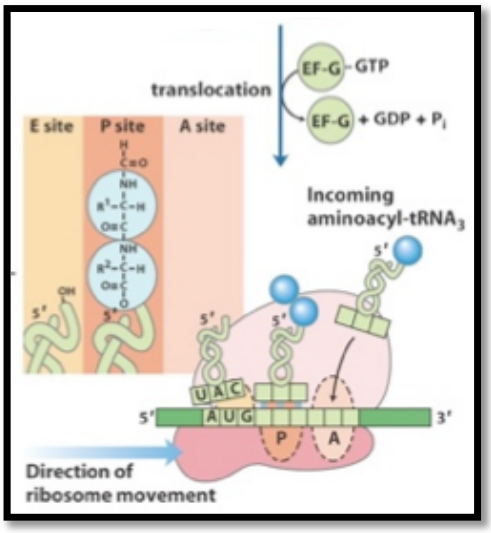
ER2_3



ER2_4


E site
P site
A site
Deacylated tRNA^{iMet}
Dipeptidyl-tRNA$_2$
5'
3'
E
P
A
U A C
A U G

ER2_5


translocation
EF-G
GTP
EF-G
+ GDP + P$_i$
E site
P site
A site
Incoming aminoacyl-tRNA$_3$
5'
3'
U A C
A U G
P
A
Direction of ribosome movement

ER2_6

APPENDIX I

VADER PLOT OF EXTERNAL REPRESENTATION #2

Plot Overview:

VADER	Plot								
Feature	ER2		Frame	1	2	3	4	5	6
	Components/Structure	mRNA	General molecule M						
			Codon						
			Nucleotide sequence (multiple codons)						
			Shine-dalgarno seuqence						
			5' end M						
			3' end M						
			3' poly A tail						
			5' methylated cap						
			Start codon						
			Stop codon						
			Nucleotide sequence (start codon)						
			Nucleotide sequence (stop codon)						
		Ribosome	General molecule(s) R						
			Large subunit						
			Small subunit						
			Aminacyl (A) site						
			Peptidyl (P) site						
			Exit (E) site						
			16S rRNA						
			Tunnel						
		tRNA	General molecule(s) T						
			Anti-codon loop						
			P-site tRNA						
			A-site tRNA						
			3' end T						
			E-site tRNA						
			3D shape						
			2D shape						
			5' end T						
			Nucleotide sequence (Anti-codon loop)						
		Amino Acids	General molecule(s) AA						
			Methionine						
			Sequential AA						
		Polypeptide chain	General molecule P						
			Primary structure						
			Secondary structure						
		Initiation Factors	General molecule(s) IF						
		Elongation Factors	General molecule(s) EF						
			EF-Tu						
			EF-Ts						
			EF-G						
		Release Factors	General molecule(s) RF						
	Interactions/Chemistry	Activation	General process A						
			Regeneration of activated tRNAs						
		Initiation	Condon/Anti-codon base pairing I						
			General process I						
			Hydrogen bonding (codon/anticodon) I						
			Hydrogen bonding (mRNA/ribosome) I						
			Initial tRNA/ribosome/mRNA						
		Elongation	Peptide bond formation						
			Condon/Anti-codon base pairing E						
			General process E						
			Incoming tRNA/ribosome/mRNA						
			Exiting tRNA/ribosome/mRNA						
			Ribosomal translocation						
			GTPase activity of EFs						
			Hydrogen bonding (codon/anticodon) E						
			AA/AA interaction						
		Termination	General process T						
	General Considerations		Reaction Kinetics						
			Evolution						
			Regulation						
			Random motion of cellular components						
			Energetics						
			Cellular Environment (Cytoplasm)						

Section Layout*:

Section 1	Section 4
Section 2	Section 5
Section 3	Section 6

*The section layout pertains to the following 6 pages. In order to reconstruct the full VADER plot for ER2, the following 6 sections should be arranged according to the section layout above.

VADER Plot ER2
Section 1

VADER	Plot				
Feature	ER2		Frame	1	2
	Components/Structure	mRNA	General molecule M	66.7%	61.1%
			Codon	100.0%	61.1%
			Nucleotide sequence (multiple codons)	0.0%	0.0%
			Shine-dalgarno seuqence	0.0%	0.0%
			5' end M	100.0%	88.9%
			3' end M	100.0%	88.9%
			3' poly A tail	0.0%	0.0%
			5' methylated cap	0.0%	0.0%
			Start codon	100.0%	88.9%
			Stop codon	0.0%	0.0%
			Nucleotide sequence (start codon)	100.0%	88.9%
			Nucleotide sequence (stop codon)	0.0%	0.0%
		Ribosome	General molecule(s) R	66.7%	61.1%
			Large subunit	100.0%	61.1%
			Small subunit	100.0%	61.1%
			Aminacyl (A) site	100.0%	88.9%
			Peptidyl (P) site	100.0%	88.9%
			Exit (E) site	100.0%	88.9%
			16S rRNA	0.0%	0.0%
			Tunnel	0.0%	0.0%
			General molecule(s) T	66.7%	66.7%
			Anti-codon loop	66.7%	66.7%
			P-site tRNA	66.7%	61.1%

VADER Plot ER2
Section 2

		tRNA	A-site tRNA	0.0%	66.7%
			3' end T	0.0%	0.0%
			E-site tRNA	0.0%	0.0%
			3D shape	66.7%	61.1%
			2D shape	0.0%	0.0%
			5' end T	100.0%	88.9%
			Nucleotide sequence (Anti-codon loop)	100.0%	94.4%
		Amino Acids	General molecule(s) AA	66.7%	61.1%
			Methionine	100.0%	88.9%
			Sequential AA	0.0%	100.0%
		Polypeptide chain	General molecule P	0.0%	0.0%
			Primary structure	0.0%	0.0%
			Secondary structure	0.0%	0.0%
		Initiation Factors	General molecule(s) IF	0.0%	0.0%
		Elongation Factors	General molecule(s) EF	0.0%	0.0%
			EF-Tu	0.0%	100.0%
			EF-Ts	0.0%	100.0%
			EF-G	0.0%	0.0%
		Release Factors	General molecule(s) RF	0.0%	0.0%
	Interactions/Chemistry	Activation	General process A	0.0%	0.0%
			Regeneration of activated tRNAs	0.0%	0.0%
		Initiation	Condon/Anti-codon base pairing I	66.7%	61.1%
			General process I	0.0%	0.0%
			Hydrogen bonding (codon/anticodon) I	66.7%	61.1%
			Hydrogen bonding (mRNA/ribosome) I	0.0%	0.0%

VADER Plot ER2
Section 3

			Initial tRNA/ribosome/mRNA	100.0%	61.1%
		Elongation	Peptide bond formation	0.0%	0.0%
			Condon/Anti-codon base pairing E	0.0%	66.7%
			General process E	0.0%	66.7%
			Incoming tRNA/ribosome/mRNA	0.0%	100.0%
			Exiting tRNA/ribosome/mRNA	0.0%	0.0%
			Ribosomal translocation	0.0%	0.0%
			GTPase activity of EFs	0.0%	100.0%
			Hydrogen bonding (codon/anticodon) E	0.0%	66.7%
			AA/AA interaction	0.0%	0.0%
		Termination	General process T	0.0%	0.0%
	General Considerations		Reaction Kinetics	0.0%	0.0%
			Evolution	0.0%	0.0%
			Regulation	0.0%	0.0%
			Random motion of cellular components	0.0%	0.0%
			Energetics	0.0%	0.0%
			Cellular Environment (Cytoplasm)	0.0%	0.0%

Features per frame	23	31

VADER Plot ER2
Section 4

3	4	5	6	Average
83.3%	38.9%	27.8%	16.7%	49.1%
50.0%	38.9%	27.8%	44.4%	53.7%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
72.2%	55.6%	38.9%	22.2%	63.0%
72.2%	55.6%	38.9%	22.2%	63.0%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
72.2%	55.6%	38.9%	50.0%	67.6%
0.0%	0.0%	0.0%	0.0%	0.0%
72.2%	55.6%	38.9%	50.0%	67.6%
0.0%	0.0%	0.0%	0.0%	0.0%
50.0%	38.9%	27.8%	16.7%	43.5%
50.0%	38.9%	27.8%	16.7%	49.1%
50.0%	38.9%	27.8%	16.7%	49.1%
88.9%	72.2%	55.6%	38.9%	74.1%
88.9%	72.2%	55.6%	38.9%	74.1%
88.9%	72.2%	55.6%	38.9%	74.1%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
61.1%	50.0%	38.9%	50.0%	55.6%
55.6%	44.4%	33.3%	44.4%	51.9%
88.9%	77.8%	33.3%	50.0%	63.0%

VADER Plot ER2
Section 5

94.4%	83.3%	38.9%	83.3%	61.1%
0.0%	66.7%	55.6%	44.4%	27.8%
0.0%	0.0%	0.0%	66.7%	11.1%
50.0%	38.9%	27.8%	16.7%	43.5%
0.0%	0.0%	0.0%	0.0%	0.0%
77.8%	61.1%	44.4%	27.8%	66.7%
77.8%	61.1%	44.4%	50.0%	71.3%
55.6%	44.4%	33.3%	50.0%	51.9%
88.9%	88.9%	72.2%	66.7%	84.3%
94.4%	77.8%	61.1%	66.7%	66.7%
0.0%	66.7%	55.6%	55.6%	29.6%
0.0%	66.7%	55.6%	55.6%	29.6%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	16.7%
0.0%	0.0%	0.0%	0.0%	16.7%
0.0%	0.0%	0.0%	100.0%	16.7%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
50.0%	38.9%	27.8%	0.0%	40.8%
0.0%	0.0%	0.0%	0.0%	0.0%
50.0%	38.9%	27.8%	0.0%	40.8%
0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER2
Section 6

50.0%	38.9%	27.8%	44.4%	53.7%
66.7%	94.4%	50.0%	38.9%	41.7%
55.6%	44.4%	33.3%	44.4%	40.7%
55.6%	44.4%	33.3%	44.4%	40.7%
0.0%	0.0%	0.0%	33.3%	22.2%
0.0%	0.0%	0.0%	66.7%	11.1%
0.0%	0.0%	0.0%	33.3%	5.6%
0.0%	0.0%	0.0%	66.7%	27.8%
55.6%	44.4%	33.3%	44.4%	40.7%
66.7%	55.6%	44.4%	33.3%	33.3%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%
				Average
30	33	33	37	31

APPENDIX J

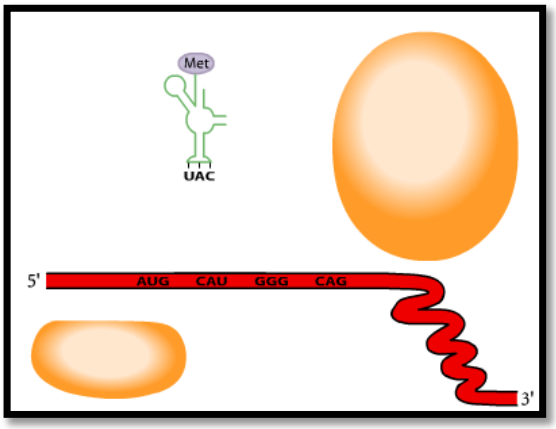
AVERAGE CUEING OF EXTERNAL REPRESENTATION #2

Theme	General Feature	Feature	Average Cueing Potential
Components/Structure	mRNA	General molecule M	49.1%
		Codon	53.7%
		Nucleotide sequence (multiple codons)	0.0%
		Shine-Dalgarno sequence	0.0%
		5' end M	63.0%
		3' end M	63.0%
		3' poly A tail	0.0%
		5' methylated cap	0.0%
		Start codon	67.6%
		Stop codon	0.0%
		Nucleotide sequence (start codon)	67.6%
		Nucleotide sequence (stop codon)	0.0%
	Ribosome	General molecule(s) R	43.5%
		Large subunit	49.1%
		Small subunit	49.1%
		Aminacyl (A) site	74.1%
		Peptidyl (P) site	74.1%
		Exit (E) site	74.1%
		16S rRNA	0.0%
		Tunnel	0.0%
	tRNA	General molecule(s) T	55.6%
		Anti-codon loop	51.9%
		P-site tRNA	63.0%
		A-site tRNA	61.1%
		3' end T	27.8%
		E-site tRNA	11.1%
		3D shape	43.5%
		2D shape	0.0%
		5' end T	66.7%
		Nucleotide sequence (Anti-codon loop)	71.3%
	Amino Acids	General molecule(s) AA	51.9%
		Methionine	84.3%
		Sequential AA	66.7%
	Polypeptide chain	General molecule P	29.6%
		Primary structure	29.6%

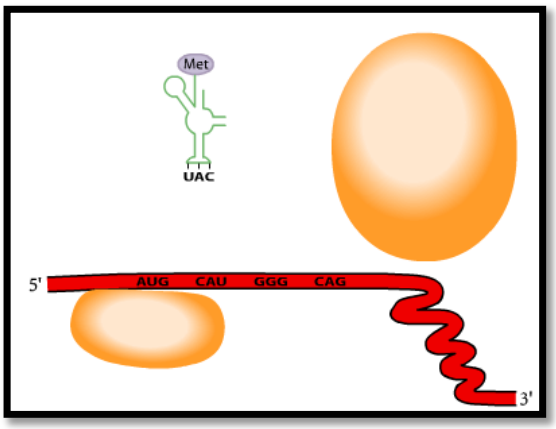
		Secondary structure	0.0%
	Initiation Factors	General molecule(s) IF	0.0%
	Elongation Factors	General molecule(s) EF	0.0%
		EF-Tu	16.7%
		EF-Ts	16.7%
		EF-G	16.7%
	Release Factors	General molecule(s) RF	0.0%
Interactions/Chemistry	Activation	General process A	0.0%
		Regeneration of activated tRNAs	0.0%
	Initiation	Condon/Anti-codon base pairing I	40.8%
		General process I	0.0%
		Hydrogen bonding (codon/anticodon) I	40.8%
		Hydrogen bonding (mRNA/ribosome) I	0.0%
		Initial tRNA/ribosome/mRNA	53.7%
	Elongation	Peptide bond formation	41.7%
		Condon/Anti-codon base pairing E	40.7%
		General process E	40.7%
		Incoming tRNA/ribosome/mRNA	22.2%
		Exiting tRNA/ribosome/mRNA	11.1%
		Ribosomal translocation	5.6%
		GTPase activity of EFs	27.8%
		Hydrogen bonding (codon/anticodon) E	40.7%
		AA/AA interaction	33.3%
	Termination	General process T	0.0%
General Considerations		Reaction kinetics	0.0%
		Evolution	0.0%
		Regulation	0.0%
		Random motion of cellular components	0.0%
		Energetics	0.0%
		Cellular environment (Cytoplasm)	0.0%

APPENDIX K

FRAME SEQUENCE OF EXTERNAL REPRESENTATION #3



ER3_1



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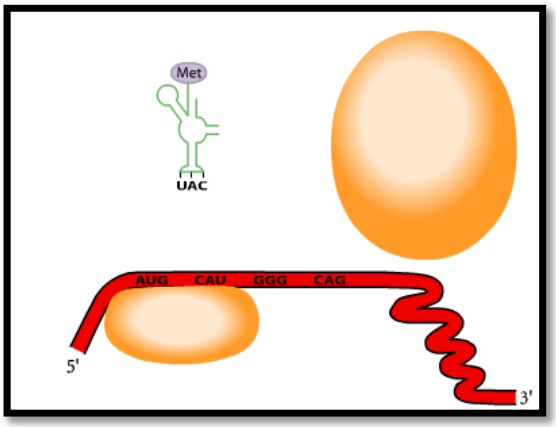

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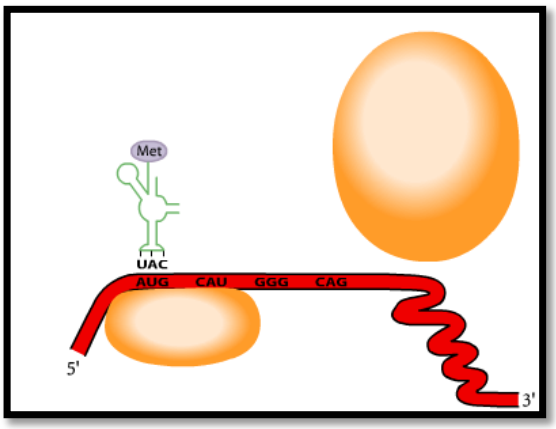

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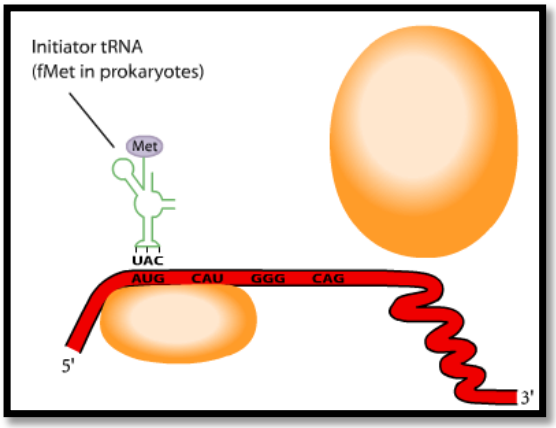

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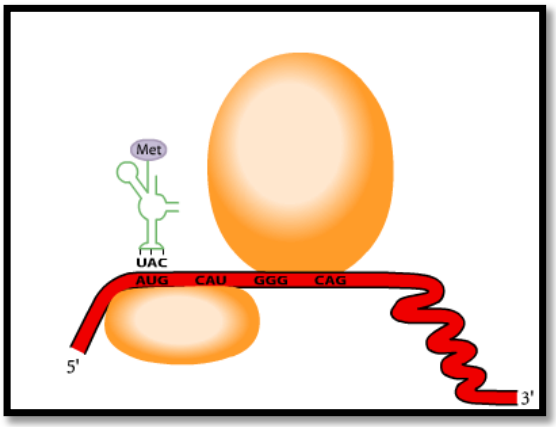

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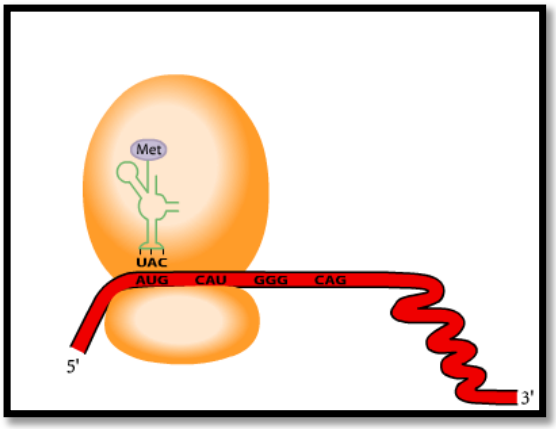

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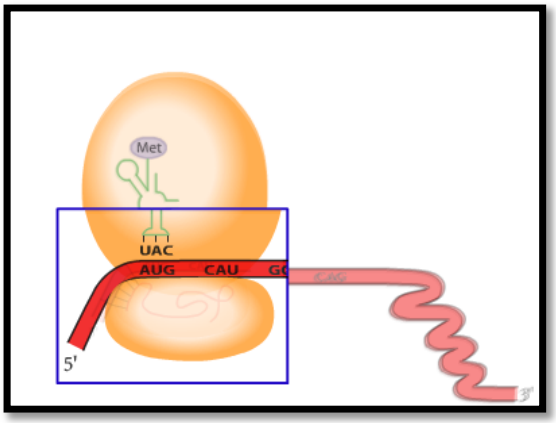

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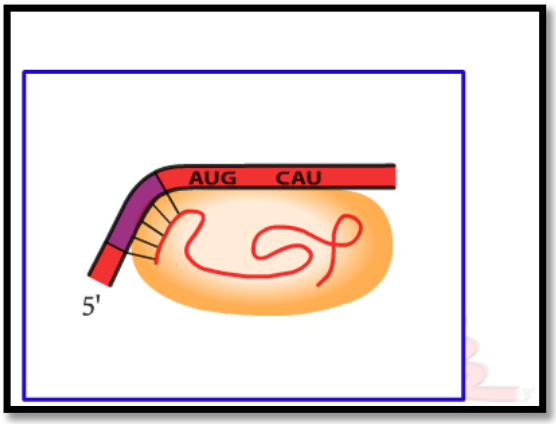

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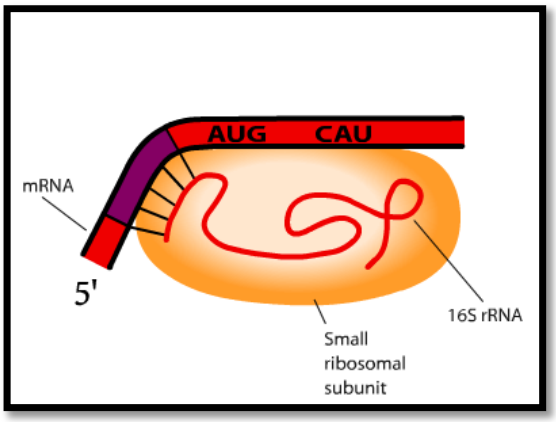

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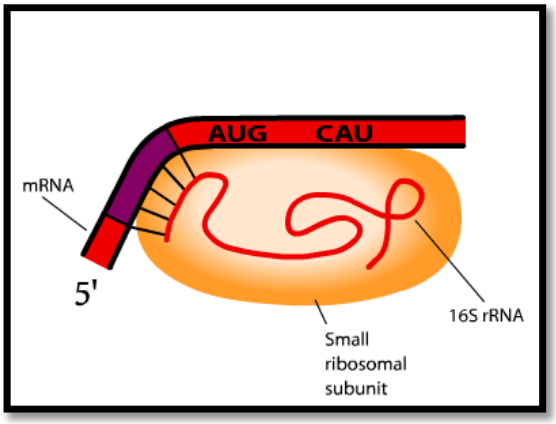

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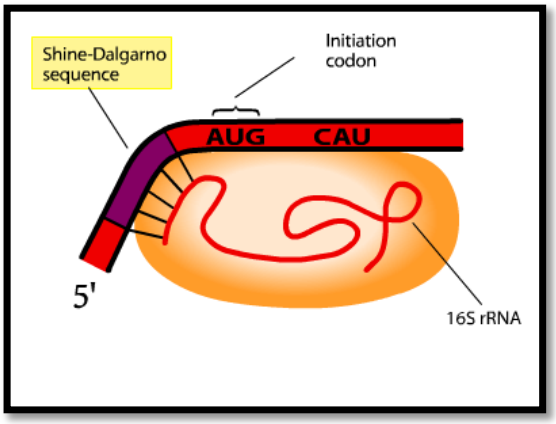

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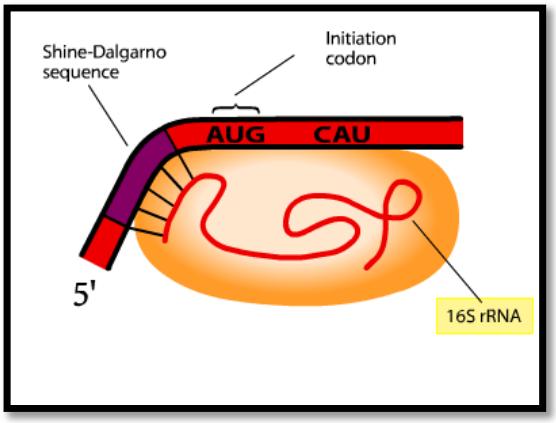

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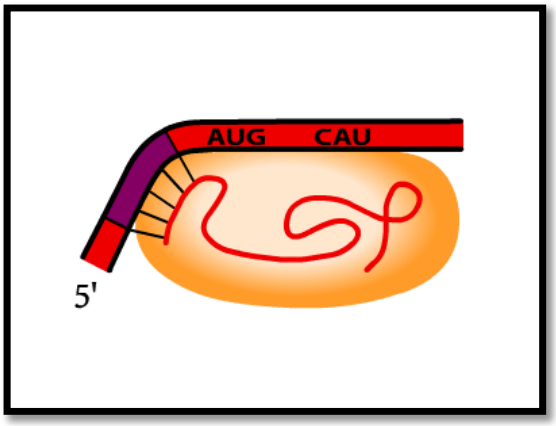

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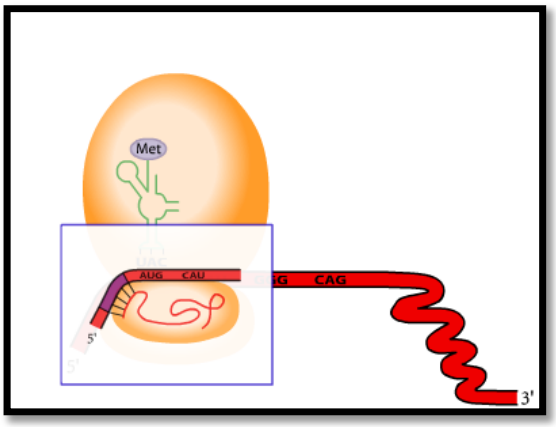

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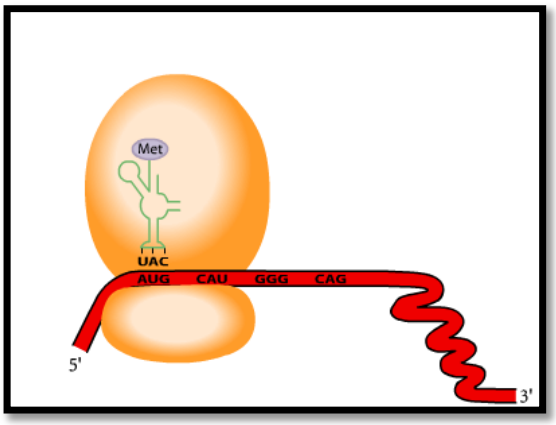

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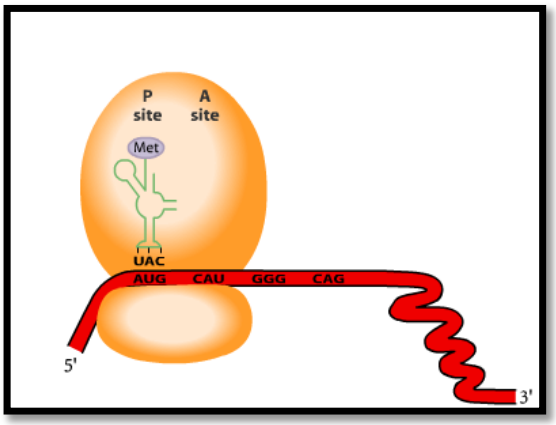

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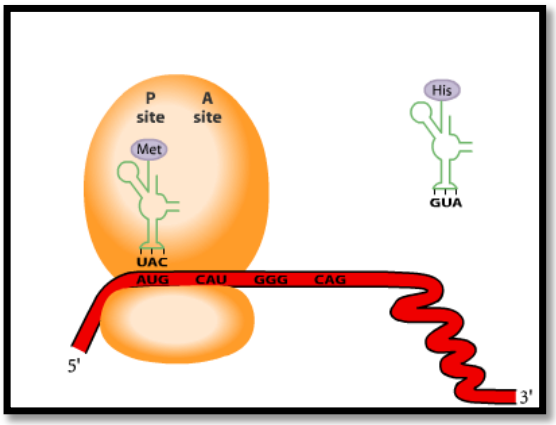

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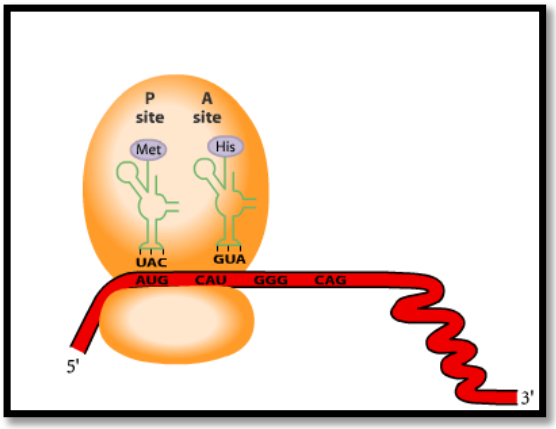

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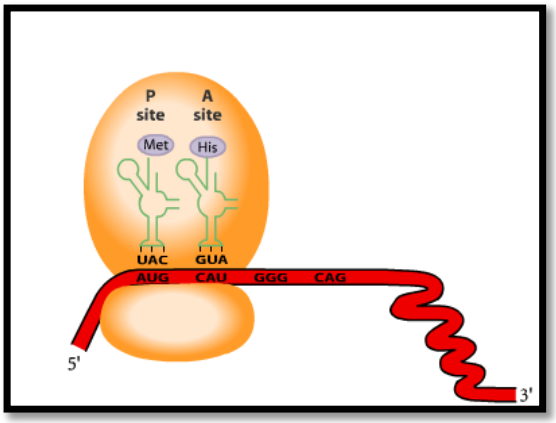

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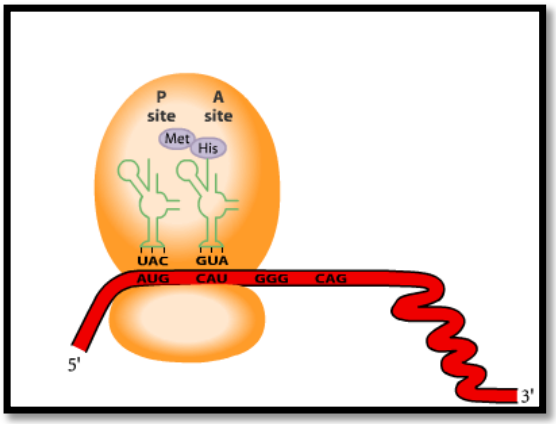

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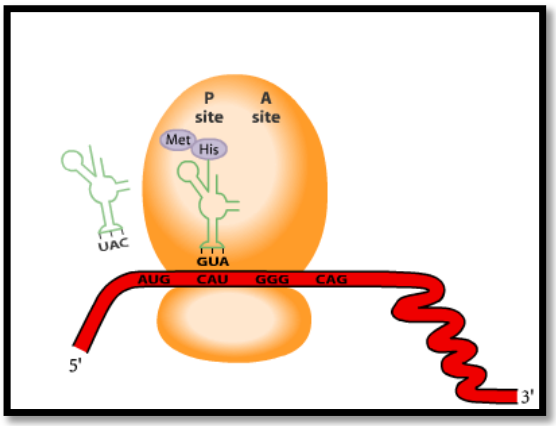

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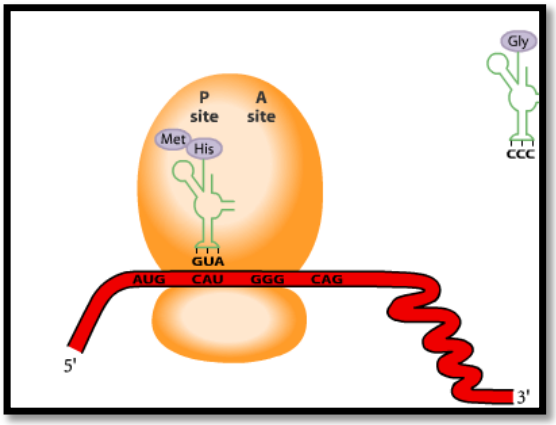

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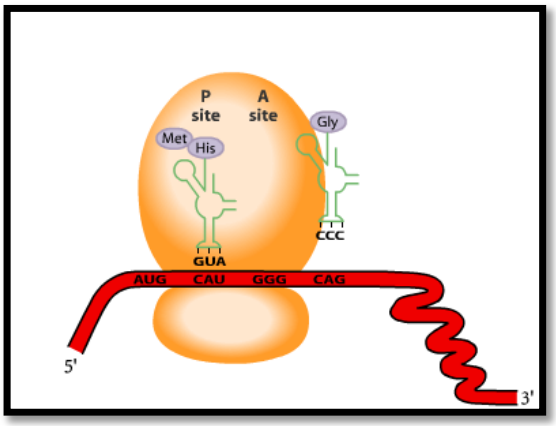

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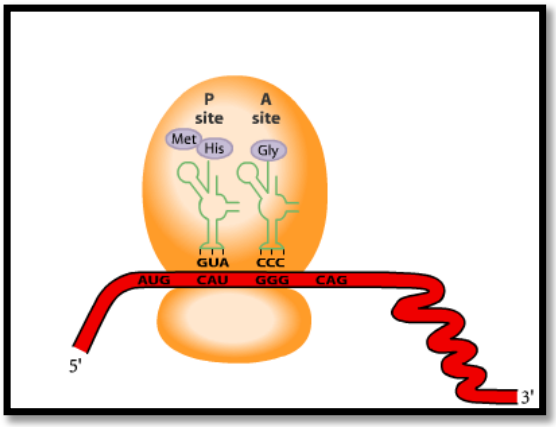

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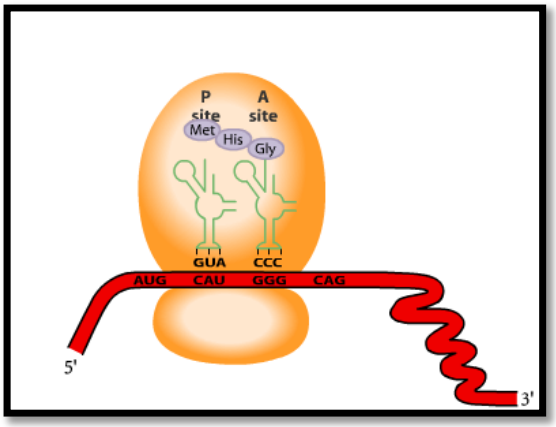

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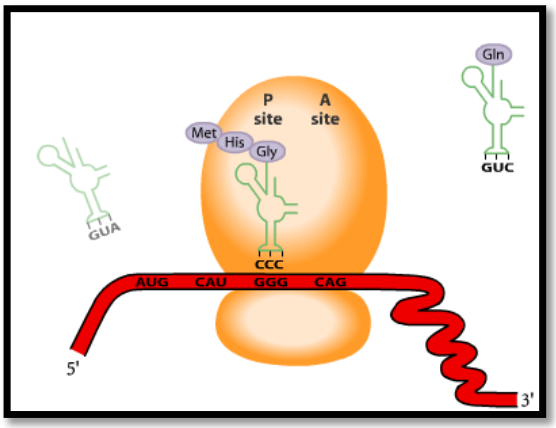

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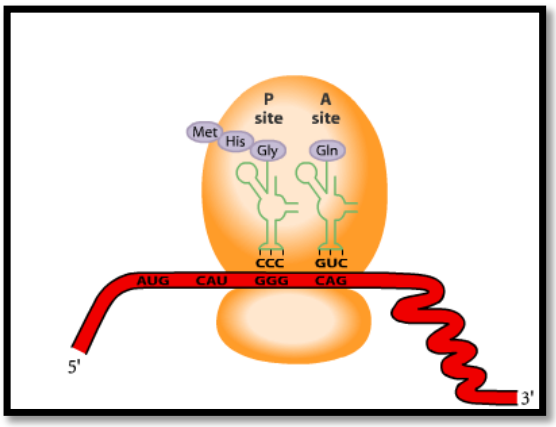

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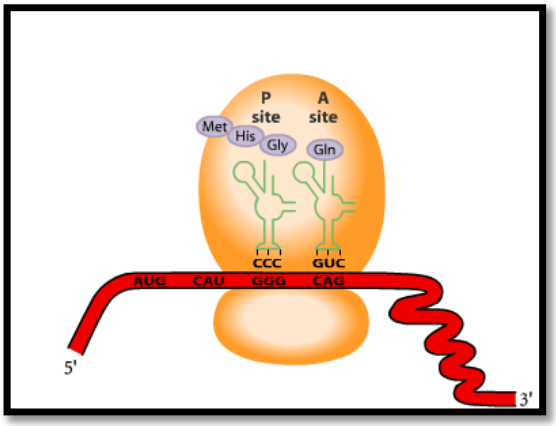

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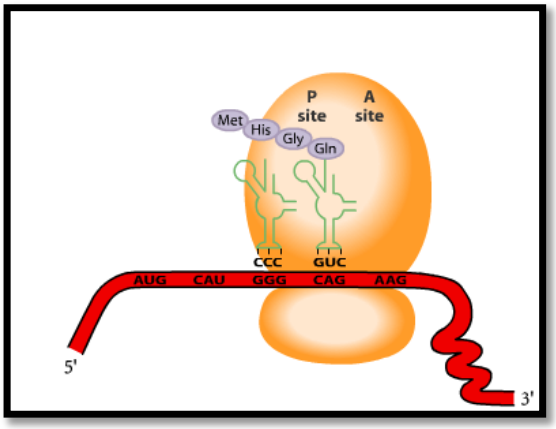

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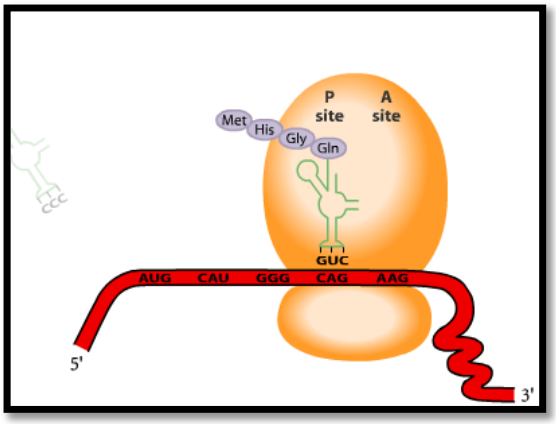

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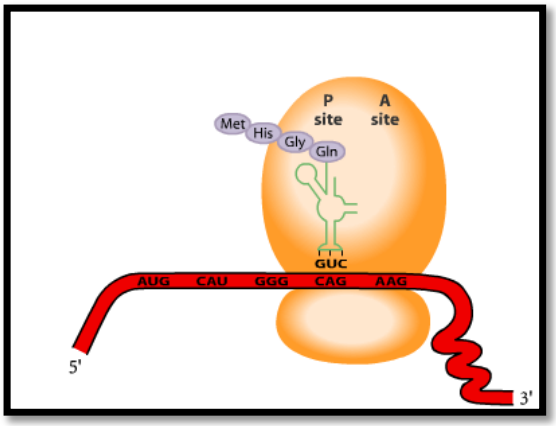

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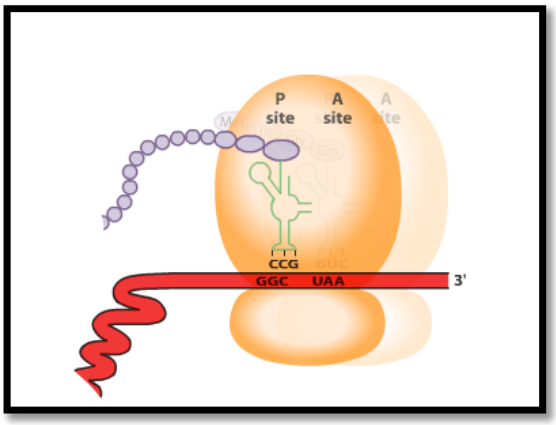

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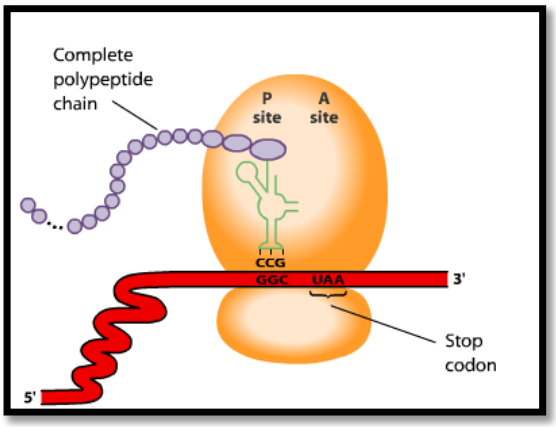

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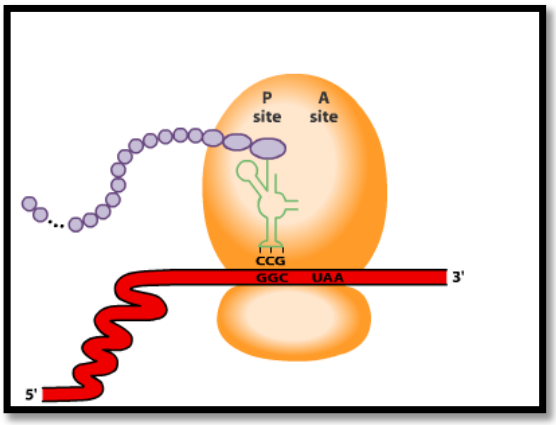

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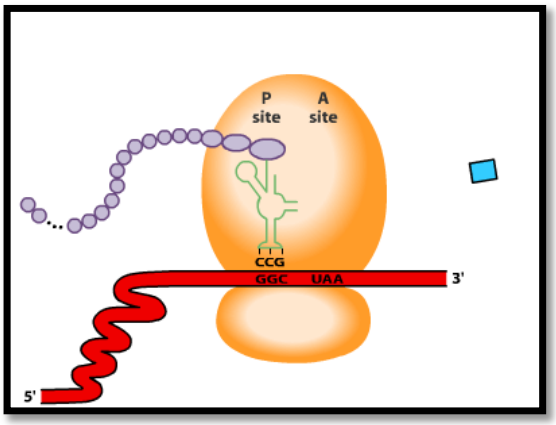

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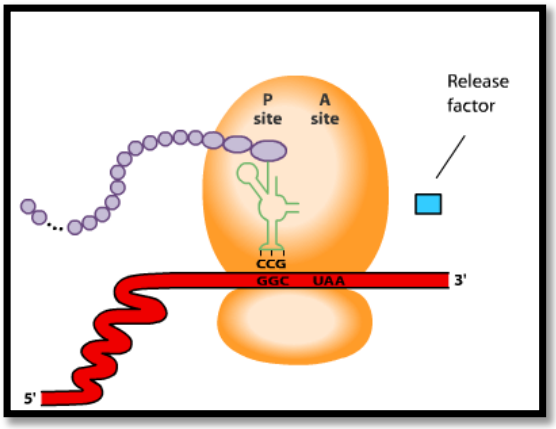

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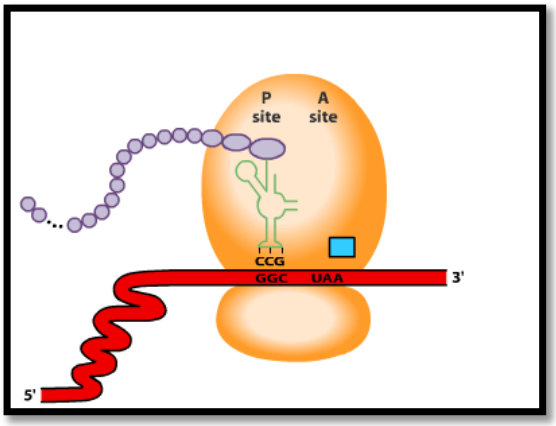
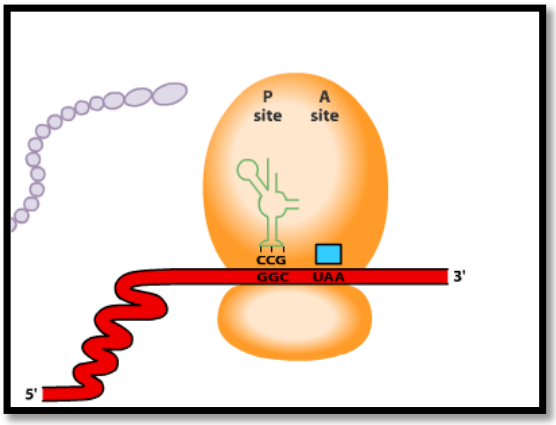


ER3_38



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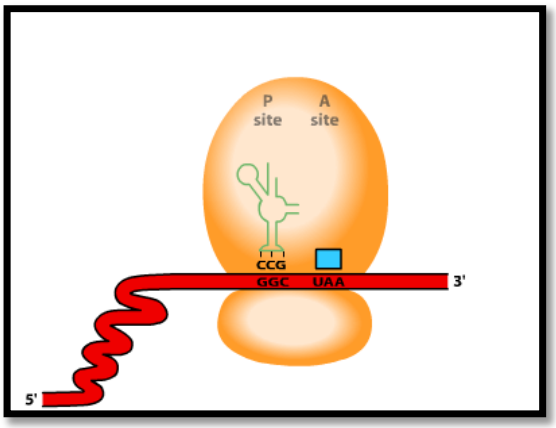

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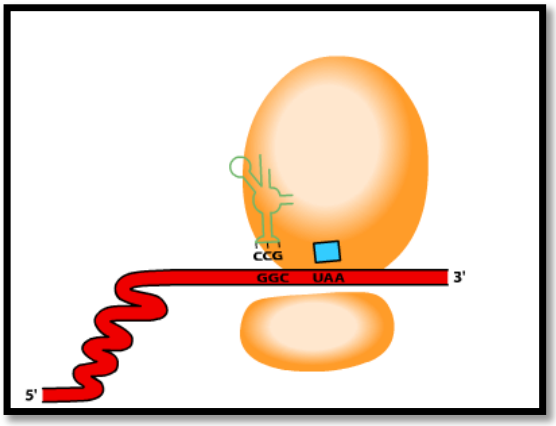
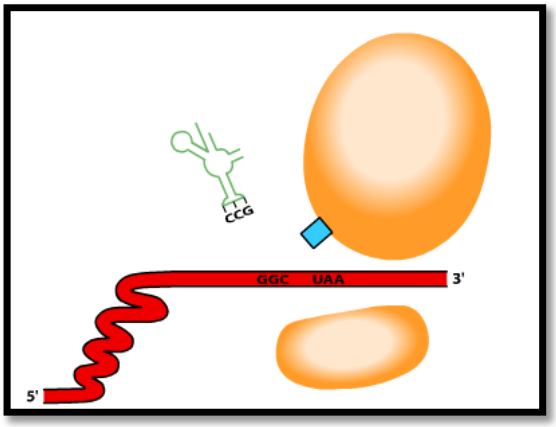
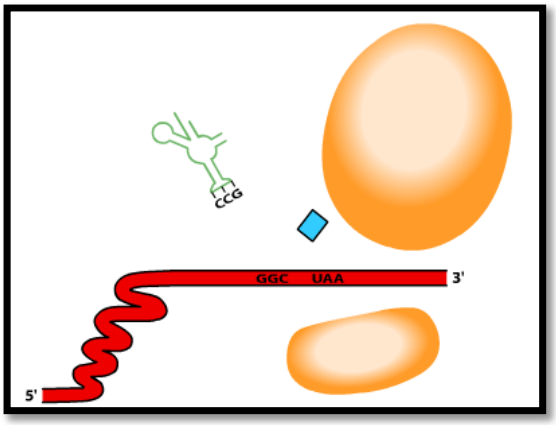


ER3_41



ER3_42

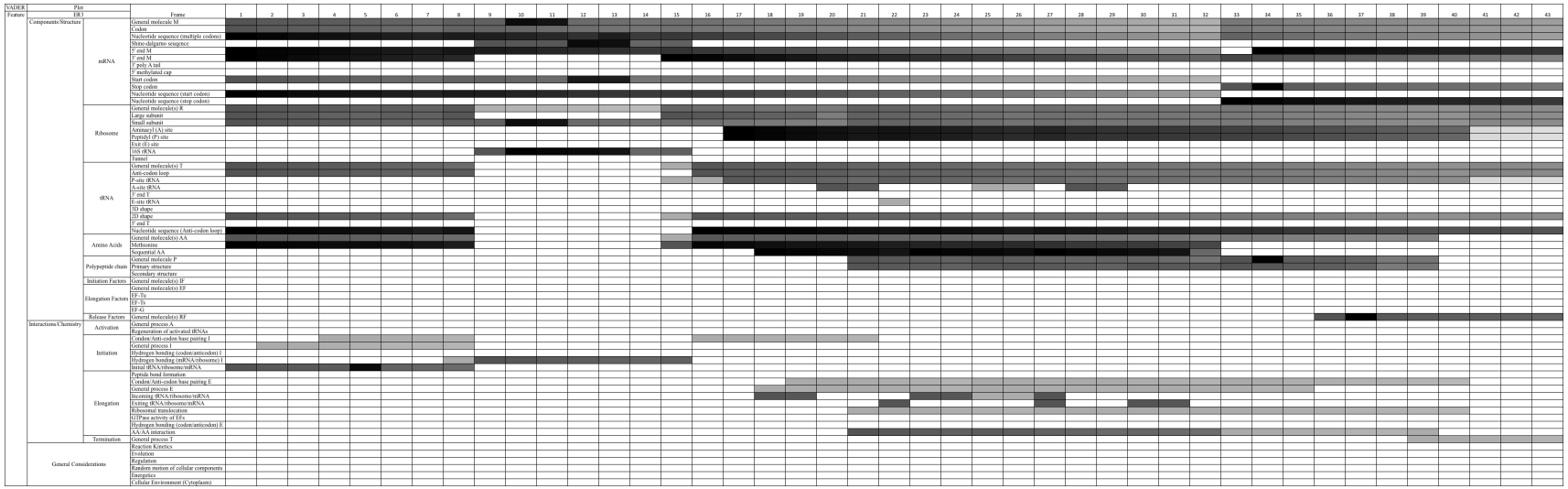


ER3_43

APPENDIX L

VADER PLOT OF EXTERNAL REPRESENTATION #3

Plot Overview:



Section Layout*:

Section 1	Section 4	Section 7	Section 10	Section 13	Section 16
Section 2	Section 5	Section 8	Section 11	Section 14	Section 17
Section 3	Section 6	Section 9	Section 12	Section 15	Section 18

*The section layout pertains to the following 18 pages. In order to reconstruct the full VADER plot for ER3, the following 18 sections should be arranged according to the section layout above.

VADER Plot ER3
Section 1

VADER	Plot				
Feature	ER3		Frame	1	2
	Components/Structure	mRNA	General molecule M	66.7%	65.1%
			Codon	66.7%	65.1%
			Nucleotide sequence (multiple codons)	100.0%	97.7%
			Shine-dalgarno seuqence	0.0%	0.0%
			5' end M	100.0%	97.7%
			3' end M	100.0%	97.7%
			3' poly A tail	0.0%	0.0%
			5' methylated cap	0.0%	0.0%
			Start codon	66.7%	65.1%
			Stop codon	0.0%	0.0%
			Nucleotide sequence (start codon)	100.0%	97.7%
			Nucleotide sequence (stop codon)	0.0%	0.0%
		Ribosome	General molecule(s) R	66.7%	65.9%
			Large subunit	66.7%	65.1%
			Small subunit	66.7%	65.9%
			Aminacyl (A) site	0.0%	0.0%
			Peptidyl (P) site	0.0%	0.0%
			Exit (E) site	0.0%	0.0%
			16S rRNA	0.0%	0.0%
			Tunnel	0.0%	0.0%
			General molecule(s) T	66.7%	65.1%
			Anti-codon loop	66.7%	65.9%
			P-site tRNA	0.0%	0.0%

VADER Plot ER3
Section 2

		tRNA	A-site tRNA	0.0%	0.0%
			3' end T	0.0%	0.0%
			E-site tRNA	0.0%	0.0%
			3D shape	0.0%	0.0%
			2D shape	66.7%	65.1%
			5' end T	0.0%	0.0%
			Nucleotide sequence (Anti-codon loop)	100.0%	98.4%
		Amino Acids	General molecule(s) AA	66.7%	65.1%
			Methionine	100.0%	97.7%
			Sequential AA	0.0%	0.0%
		Polypeptide chain	General molecule P	0.0%	0.0%
			Primary structure	0.0%	0.0%
			Secondary structure	0.0%	0.0%
		Initiation Factors	General molecule(s) IF	0.0%	0.0%
		Elongation Factors	General molecule(s) EF	0.0%	0.0%
			EF-Tu	0.0%	0.0%
			EF-Ts	0.0%	0.0%
			EF-G	0.0%	0.0%
		Release Factors	General molecule(s) RF	0.0%	0.0%
	Interactions/Chemistry	Activation	General process A	0.0%	0.0%
			Regeneration of activated tRNAs	0.0%	0.0%
		Initiation	Condon/Anti-codon base pairing I	0.0%	0.0%
			General process I	0.0%	33.3%
			Hydrogen bonding (codon/anticodon) I	0.0%	0.0%
			Hydrogen bonding (mRNA/ribosome) I	0.0%	0.0%

VADER Plot ER3
Section 3

			Initial tRNA/ribosome/mRNA	66.7%	65.1%
		Elongation	Peptide bond formation	0.0%	0.0%
			Condon/Anti-codon base pairing E	0.0%	0.0%
			General process E	0.0%	0.0%
			Incoming tRNA/ribosome/mRNA	0.0%	0.0%
			Exiting tRNA/ribosome/mRNA	0.0%	0.0%
			Ribosomal translocation	0.0%	0.0%
			GTPase activity of EFs	0.0%	0.0%
			Hydrogen bonding (codon/anticodon) E	0.0%	0.0%
			AA/AA interaction	0.0%	0.0%
		Termination	General process T	0.0%	0.0%
	General Considerations		Reaction Kinetics	0.0%	0.0%
			Evolution	0.0%	0.0%
			Regulation	0.0%	0.0%
			Random motion of cellular components	0.0%	0.0%
			Energetics	0.0%	0.0%
			Cellular Environment (Cytoplasm)	0.0%	0.0%

Features per frame	17	18

VADER Plot ER3
Section 4

3	4	5	6	7	8	9	10	11
65.1%	63.6%	62.0%	60.5%	58.9%	62.8%	62.0%	94.6%	92.2%
63.6%	62.0%	60.5%	58.9%	57.4%	61.2%	60.5%	59.7%	58.1%
95.3%	93.0%	90.7%	88.4%	86.0%	89.1%	87.6%	86.0%	83.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%	65.9%	64.3%
96.9%	94.6%	92.2%	89.9%	87.6%	90.7%	89.1%	87.6%	85.3%
95.3%	93.0%	90.7%	88.4%	86.0%	83.7%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
63.6%	62.0%	60.5%	58.9%	57.4%	61.2%	60.5%	59.7%	58.1%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
95.3%	93.0%	90.7%	88.4%	86.0%	89.1%	87.6%	86.0%	83.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
65.1%	63.6%	62.0%	62.8%	61.2%	65.1%	31.0%	30.2%	29.5%
63.6%	62.0%	60.5%	62.8%	62.0%	65.9%	0.0%	0.0%	0.0%
65.1%	63.6%	62.0%	60.5%	58.9%	62.8%	62.0%	94.6%	92.2%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%	99.2%	96.9%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
65.1%	64.3%	62.8%	61.2%	59.7%	58.1%	0.0%	0.0%	0.0%
65.1%	64.3%	62.8%	61.2%	59.7%	63.6%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER3
Section 5

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
65.1%	64.3%	62.8%	61.2%	59.7%	58.1%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
96.9%	95.3%	93.0%	90.7%	88.4%	91.5%	0.0%	0.0%	0.0%
65.1%	64.3%	62.8%	61.2%	59.7%	58.1%	0.0%	0.0%	0.0%
96.9%	95.3%	96.1%	93.8%	91.5%	89.1%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	33.3%	32.6%	31.8%	31.0%	30.2%	0.0%	0.0%	0.0%
32.6%	31.8%	31.0%	30.2%	29.5%	28.7%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	33.3%	66.7%	65.9%	64.3%

VADER Plot ER3
Section 6

65.1%	64.3%	96.1%	61.2%	59.7%	58.1%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

18	19	19	19	19	20	11	11	11

VADER Plot ER3
Section 7

12	13	14	15	16	17	18	19	20
58.1%	56.6%	55.0%	57.4%	55.8%	54.3%	52.7%	51.2%	49.6%
56.6%	55.0%	53.5%	55.8%	54.3%	52.7%	51.2%	49.6%	48.1%
81.4%	79.1%	76.7%	78.3%	76.0%	73.6%	71.3%	69.0%	66.7%
96.1%	93.8%	59.7%	62.0%	0.0%	0.0%	0.0%	0.0%	0.0%
82.9%	80.6%	78.3%	79.8%	77.5%	75.2%	72.9%	70.5%	68.2%
0.0%	0.0%	0.0%	100.0%	97.7%	95.3%	93.0%	90.7%	88.4%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
89.9%	87.6%	53.5%	55.8%	54.3%	52.7%	51.2%	49.6%	48.1%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
81.4%	79.1%	76.7%	78.3%	76.0%	73.6%	71.3%	69.0%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
28.7%	27.9%	27.1%	59.7%	58.1%	56.6%	55.0%	53.5%	51.9%
0.0%	0.0%	0.0%	66.7%	65.1%	63.6%	62.0%	60.5%	51.2%
58.1%	56.6%	55.0%	57.4%	55.8%	54.3%	52.7%	51.2%	49.6%
0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	97.7%	95.3%	93.0%
0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	97.7%	95.3%	93.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
94.6%	92.2%	59.7%	62.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	33.3%	65.9%	64.3%	64.3%	63.6%	62.8%
0.0%	0.0%	0.0%	0.0%	66.7%	65.1%	65.1%	64.3%	63.6%
0.0%	0.0%	0.0%	33.3%	32.6%	65.1%	63.6%	62.0%	60.5%

VADER Plot ER3
Section 8

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	33.3%	65.9%	64.3%	64.3%	63.6%	62.8%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	100.0%	97.7%	98.4%	96.9%	95.3%
0.0%	0.0%	0.0%	33.3%	65.9%	64.3%	64.3%	63.6%	62.8%
0.0%	0.0%	0.0%	66.7%	98.4%	96.1%	93.8%	91.5%	92.2%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	98.4%	96.9%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	33.3%	32.6%	31.8%	31.0%	30.2%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
62.8%	61.2%	59.7%	62.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER3
Section 9

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	33.3%	33.3%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	33.3%	32.6%	33.3%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%	65.9%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	33.3%	32.6%	31.8%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

11	11	11	18	18	20	23	24	24

VADER Plot ER3
Section 10

21	22	23	24	25	26	27	28	29
48.1%	46.5%	45.0%	43.4%	41.9%	40.3%	38.8%	37.2%	35.7%
46.5%	45.0%	43.4%	41.9%	40.3%	38.8%	37.2%	35.7%	34.1%
64.3%	62.0%	59.7%	57.4%	55.0%	52.7%	50.4%	48.1%	45.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
65.9%	63.6%	61.2%	58.9%	56.6%	54.3%	51.9%	49.6%	47.3%
86.0%	83.7%	81.4%	79.1%	76.7%	74.4%	72.1%	69.8%	67.4%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
46.5%	45.0%	43.4%	41.9%	40.3%	38.8%	37.2%	35.7%	34.1%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
64.3%	62.0%	59.7%	57.4%	55.0%	52.7%	50.4%	48.1%	45.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
62.0%	61.2%	59.7%	58.1%	56.6%	55.0%	57.4%	55.8%	54.3%
62.0%	61.2%	59.7%	58.1%	56.6%	55.0%	57.4%	55.8%	54.3%
62.0%	61.2%	59.7%	58.1%	56.6%	55.0%	57.4%	55.8%	54.3%
90.7%	92.2%	89.9%	87.6%	85.3%	82.9%	84.5%	82.2%	79.8%
90.7%	92.2%	89.9%	87.6%	85.3%	82.9%	84.5%	82.2%	79.8%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
61.2%	61.2%	60.5%	58.9%	57.4%	55.8%	57.4%	56.6%	55.0%
62.0%	62.0%	61.2%	60.5%	59.7%	58.1%	58.1%	57.4%	55.8%
58.9%	62.8%	61.2%	59.7%	58.1%	56.6%	58.9%	57.4%	55.8%

VADER Plot ER3
Section 11

65.1%	0.0%	0.0%	0.0%	33.3%	32.6%	0.0%	66.7%	65.1%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	33.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
61.2%	61.2%	60.5%	58.9%	57.4%	55.8%	57.4%	56.6%	55.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
93.0%	92.2%	94.6%	93.0%	91.5%	89.1%	91.5%	89.9%	87.6%
62.0%	60.5%	60.5%	59.7%	58.9%	58.1%	57.4%	56.6%	55.8%
90.7%	88.4%	86.0%	83.7%	84.5%	82.9%	80.6%	78.3%	78.3%
94.6%	92.2%	100.0%	98.4%	96.9%	94.6%	100.0%	98.4%	96.1%
66.7%	65.1%	63.6%	62.0%	63.6%	62.8%	61.2%	59.7%	60.5%
66.7%	65.1%	63.6%	62.0%	63.6%	66.7%	65.1%	63.6%	62.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
29.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER3
Section 12

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
32.6%	31.8%	31.0%	30.2%	33.3%	32.6%	31.8%	33.3%	32.6%
33.3%	32.6%	31.8%	31.0%	33.3%	33.3%	32.6%	31.8%	31.0%
0.0%	0.0%	66.7%	65.9%	33.3%	32.6%	66.7%	0.0%	0.0%
0.0%	66.7%	0.0%	0.0%	0.0%	0.0%	66.7%	0.0%	0.0%
33.3%	33.3%	32.6%	31.8%	31.0%	30.2%	33.3%	32.6%	31.8%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	65.1%	63.6%	62.0%	63.6%	62.8%	61.2%	59.7%	58.1%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
31.0%	30.2%	29.5%	28.7%	27.9%	27.1%	26.4%	25.6%	24.8%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

28	28	27	27	28	28	28	27	27

VADER Plot ER3
Section 13

30	31	32	33	34	35	36	37	38
34.1%	32.6%	31.0%	52.7%	51.9%	50.4%	48.8%	47.3%	45.7%
32.6%	31.0%	29.5%	52.7%	51.2%	49.6%	48.1%	46.5%	45.0%
43.4%	41.1%	38.8%	61.2%	58.9%	56.6%	54.3%	51.9%	49.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
45.0%	42.6%	40.3%	0.0%	100.0%	97.7%	95.3%	93.0%	90.7%
65.1%	62.8%	60.5%	72.1%	69.8%	67.4%	65.1%	62.8%	60.5%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
32.6%	31.0%	29.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	66.7%	98.4%	63.6%	62.0%	60.5%	58.9%
43.4%	41.1%	38.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	100.0%	97.7%	95.3%	93.0%	90.7%	88.4%
55.0%	54.3%	52.7%	52.7%	51.9%	50.4%	48.8%	47.3%	45.7%
55.0%	54.3%	52.7%	52.7%	51.9%	50.4%	48.8%	47.3%	45.7%
55.0%	54.3%	52.7%	52.7%	51.9%	50.4%	48.8%	47.3%	45.7%
79.8%	78.3%	76.0%	75.2%	73.6%	71.3%	69.0%	66.7%	64.3%
79.8%	78.3%	76.0%	75.2%	73.6%	71.3%	69.0%	66.7%	64.3%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
55.0%	54.3%	52.7%	52.7%	51.9%	50.4%	48.8%	47.3%	45.7%
54.3%	55.0%	53.5%	53.5%	52.7%	51.2%	49.6%	48.1%	46.5%
56.6%	55.8%	54.3%	54.3%	53.5%	51.9%	50.4%	48.8%	47.3%

VADER Plot ER3
Section 14

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
53.5%	54.3%	52.7%	52.7%	51.9%	50.4%	48.8%	47.3%	45.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
85.3%	85.3%	82.9%	82.2%	80.6%	78.3%	76.0%	73.6%	71.3%
55.0%	53.5%	51.9%	52.7%	51.9%	50.4%	48.8%	47.3%	45.7%
76.7%	74.4%	72.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
93.8%	91.5%	58.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
59.7%	58.1%	55.0%	66.7%	99.2%	64.3%	62.8%	61.2%	51.9%
66.7%	65.1%	63.6%	66.7%	65.9%	64.3%	62.8%	61.2%	51.9%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%	99.2%	65.1%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER3
Section 15

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
31.8%	31.0%	30.2%	33.3%	32.6%	31.8%	31.0%	30.2%	29.5%
33.3%	32.6%	31.8%	31.0%	30.2%	29.5%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	65.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	33.3%	32.6%	33.3%	32.6%	31.8%	31.0%	30.2%	29.5%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
59.7%	58.1%	56.6%	33.3%	32.6%	31.8%	31.0%	30.2%	29.5%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
24.0%	23.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

27	27	26	23	24	24	24	24	24

VADER Plot ER3
Section 16

39	40	41	42	43	Average
44.2%	42.6%	41.1%	39.5%	38.0%	51.6%
43.4%	41.9%	40.3%	38.8%	37.2%	48.9%
47.3%	45.0%	42.6%	40.3%	38.0%	65.9%
0.0%	0.0%	0.0%	0.0%	0.0%	11.8%
88.4%	86.0%	83.7%	81.4%	79.1%	75.1%
58.1%	55.8%	53.5%	51.2%	48.8%	66.1%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	38.9%
57.4%	57.4%	55.8%	54.3%	52.7%	16.0%
0.0%	0.0%	0.0%	0.0%	0.0%	53.2%
86.0%	85.3%	82.9%	80.6%	78.3%	22.7%
44.2%	42.6%	46.5%	45.7%	45.0%	51.7%
44.2%	42.6%	46.5%	45.7%	45.0%	48.4%
44.2%	42.6%	46.5%	45.7%	45.0%	56.9%
62.0%	59.7%	14.7%	14.0%	13.2%	46.0%
62.0%	59.7%	14.7%	14.0%	13.2%	46.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	13.3%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
44.2%	42.6%	46.5%	45.7%	45.0%	48.0%
45.0%	43.4%	47.3%	46.5%	45.7%	47.9%
45.7%	44.2%	14.7%	14.0%	13.2%	33.7%

VADER Plot ER3
Section 17

0.0%	0.0%	0.0%	0.0%	0.0%	7.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.8%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
44.2%	42.6%	46.5%	45.7%	45.0%	48.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
69.0%	66.7%	69.8%	68.2%	66.7%	72.6%
48.1%	0.0%	0.0%	0.0%	0.0%	44.0%
0.0%	0.0%	0.0%	0.0%	0.0%	52.9%
0.0%	0.0%	0.0%	0.0%	0.0%	32.8%
54.3%	0.0%	0.0%	0.0%	0.0%	27.9%
54.3%	0.0%	0.0%	0.0%	0.0%	27.9%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
64.3%	62.8%	62.8%	62.0%	61.2%	12.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	8.1%
0.0%	0.0%	0.0%	0.0%	0.0%	5.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	11.1%

VADER Plot ER3
Section 18

0.0%	0.0%	0.0%	0.0%	0.0%	12.5%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
28.7%	27.9%	0.0%	0.0%	0.0%	16.1%
0.0%	0.0%	0.0%	0.0%	0.0%	13.4%
0.0%	0.0%	0.0%	0.0%	0.0%	9.2%
0.0%	0.0%	0.0%	0.0%	0.0%	6.2%
28.7%	27.9%	0.0%	0.0%	0.0%	14.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	0.0%	0.0%	0.0%	0.0%	22.3%
33.3%	32.6%	31.8%	31.0%	30.2%	3.7%
0.0%	0.0%	0.0%	0.0%	0.0%	9.2%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
					Average
25	21	19	19	19	21

APPENDIX M

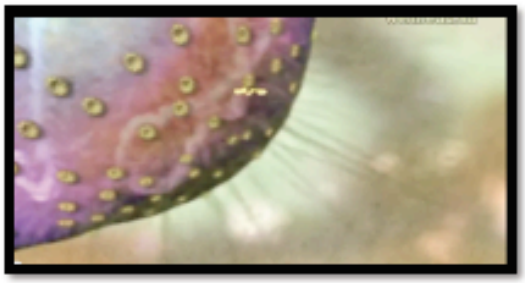
AVERAGE CUEING OF EXTERNAL REPRESENTATION #3

Theme	General Feature	Feature	Average Cueing Potential
Components/ Structure	mRNA	General molecule M	51.6%
		Codon	48.9%
		Nucleotide sequence (multiple codons)	65.9%
		Shine-Dalgarno sequence	11.8%
		5' end M	75.1%
		3' end M	66.1%
		3' poly A tail	0.0%
		5' methylated cap	0.0%
		Start codon	38.9%
		Stop codon	16.0%
		Nucleotide sequence (start codon)	53.2%
		Nucleotide sequence (stop codon)	22.7%
	Ribosome	General molecule(s) R	51.7%
		Large subunit	48.4%
		Small subunit	56.9%
		Aminacyl (A) site	46.0%
		Peptidyl (P) site	46.0%
		Exit (E) site	0.0%
		16S rRNA	13.3%
		Tunnel	0.0%
	tRNA	General molecule(s) T	48.0%
		Anti-codon loop	47.9%
		P-site tRNA	33.7%
		A-site tRNA	7.7%
		3' end T	0.0%
		E-site tRNA	0.8%
		3D shape	0.0%
		2D shape	48.0%
		5' end T	0.0%
		Nucleotide sequence (Anti-codon loop)	72.6%
	Amino Acids	General molecule(s) AA	44.0%
		Methionine	52.9%
		Sequential AA	32.8%
	Polypeptide chain	General molecule P	27.9%
		Primary structure	27.9%

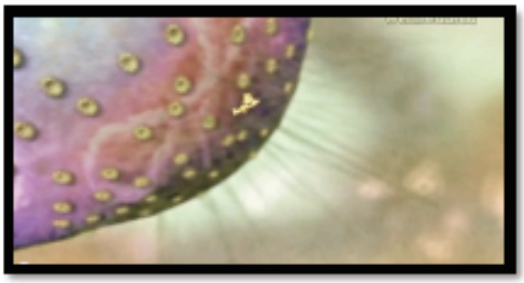
		Secondary structure	0.0%
	Initiation Factors	General molecule(s) IF	0.0%
	Elongation Factors	General molecule(s) EF	0.0%
		EF-Tu	0.0%
		EF-Ts	0.0%
		EF-G	0.0%
	Release Factors	General molecule(s) RF	12.7%
Interactions/ Chemistry	Activation	General process A	0.0%
		Regeneration of activated tRNAs	0.0%
	Initiation	Condon/Anti-codon base pairing I	8.1%
		General process I	5.0%
		Hydrogen bonding (codon/anticodon) I	0.0%
		Hydrogen bonding (mRNA/ribosome) I	11.1%
		Initial tRNA/ribosome/mRNA	12.5%
	Elongation	Peptide bond formation	0.0%
		Condon/Anti-codon base pairing E	16.1%
		General process E	13.4%
		Incoming tRNA/ribosome/mRNA	9.2%
		Exiting tRNA/ribosome/mRNA	6.2%
		Ribosomal translocation	14.7%
		GTPase activity of EFs	0.0%
		Hydrogen bonding (codon/anticodon) E	0.0%
		AA/AA interaction	22.3%
	Termination	General process T	3.7%
General Considerations		Reaction kinetics	9.2%
		Evolution	0.0%
		Regulation	0.0%
		Random motion of cellular components	0.0%
		Energetics	0.0%
		Cellular environment (Cytoplasm)	0.0%

APPENDIX N

FRAME SEQUENCE OF EXTERNAL REPRESENTATION #6



ER6_1



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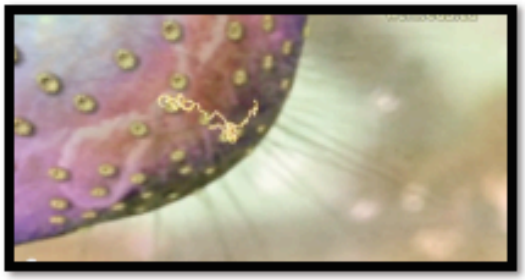
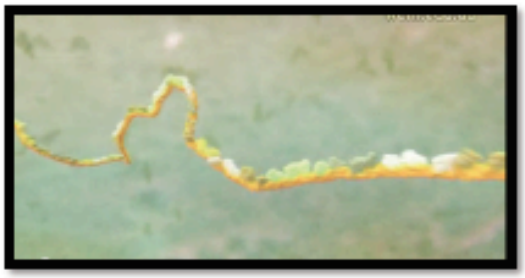


ER6_3

ER6_4



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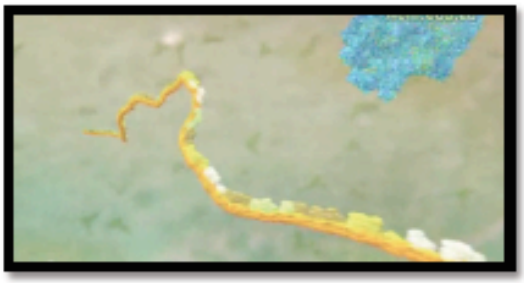

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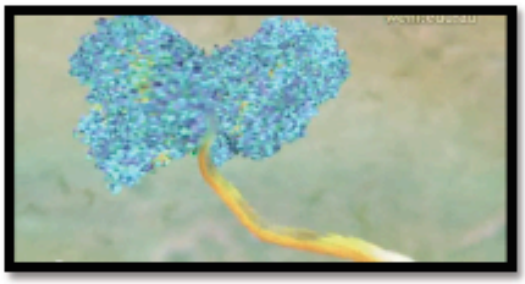

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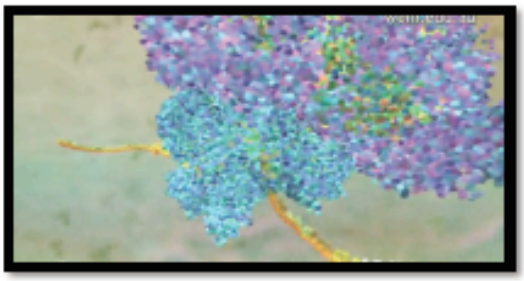

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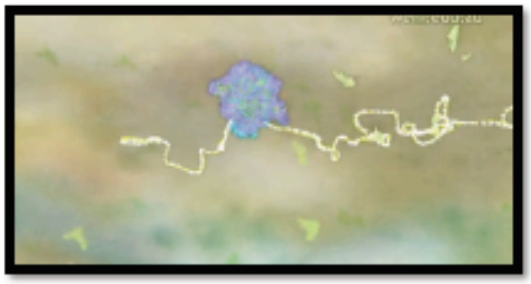

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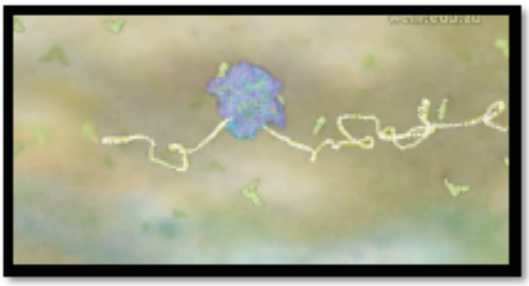

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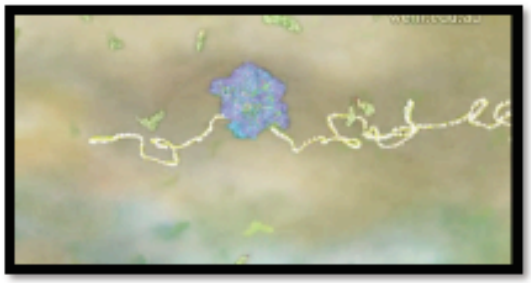

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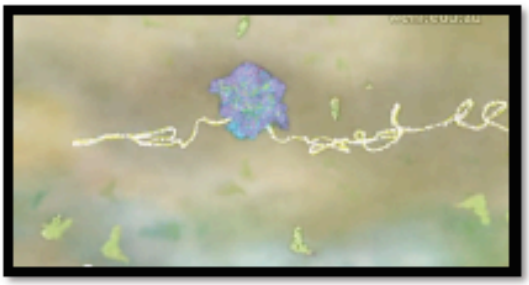

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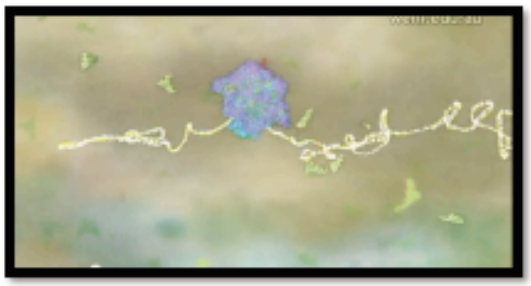

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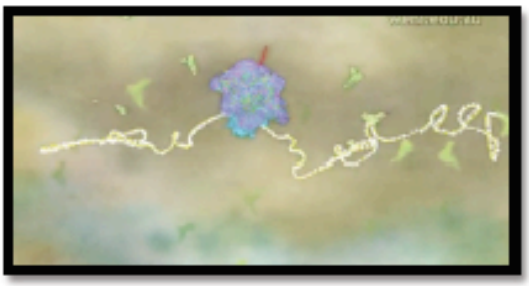

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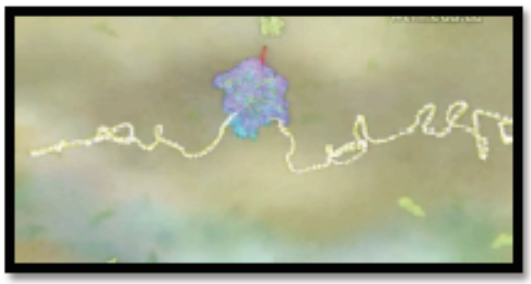

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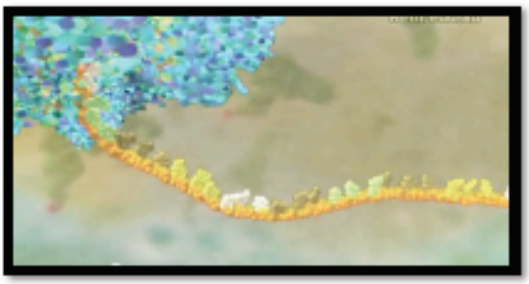

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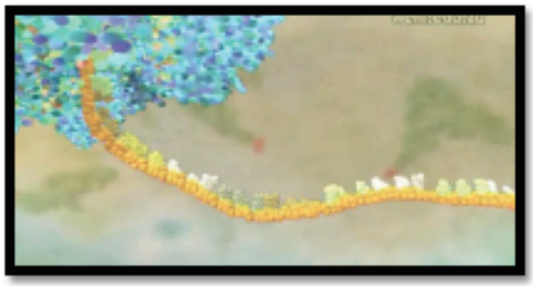

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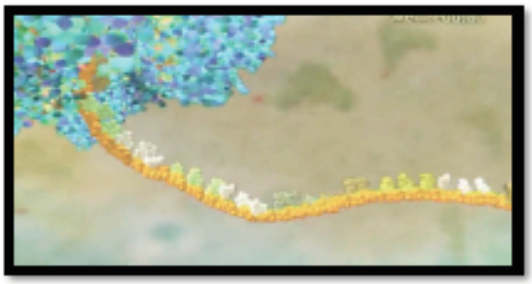

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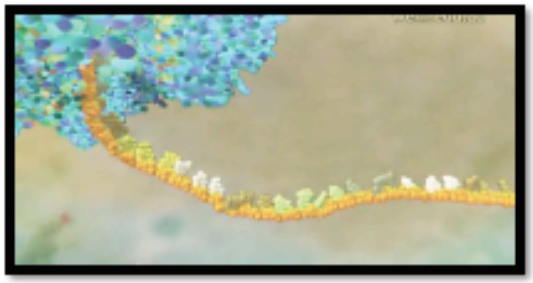

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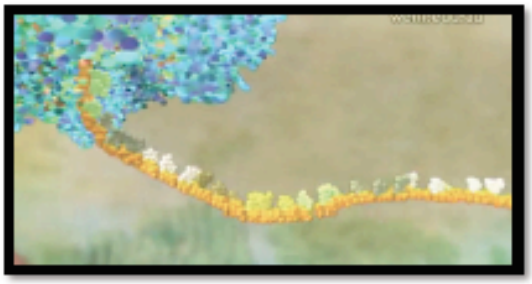

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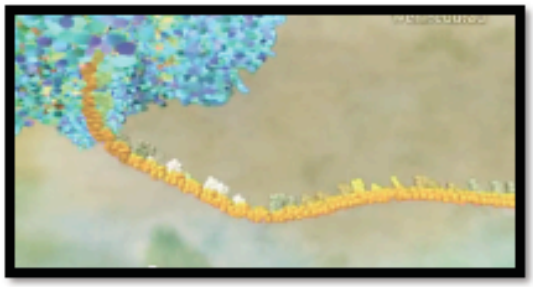

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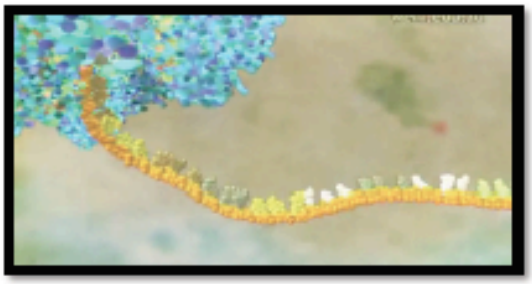

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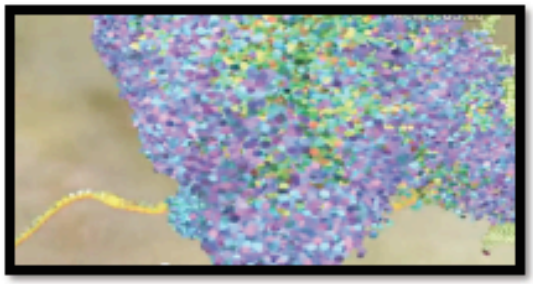

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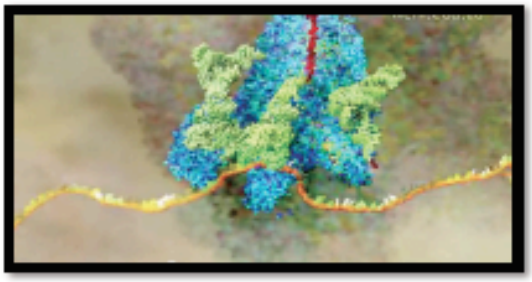

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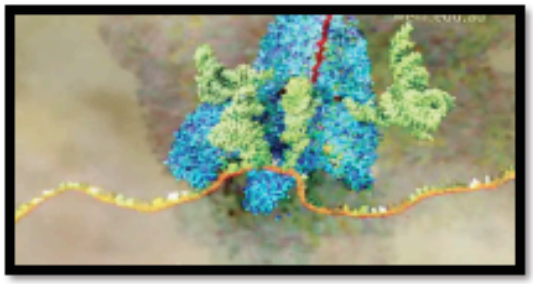

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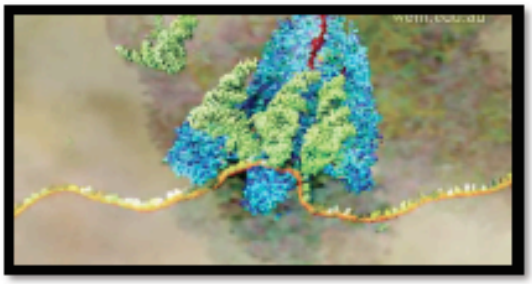

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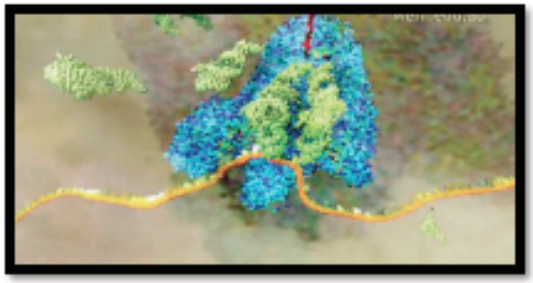

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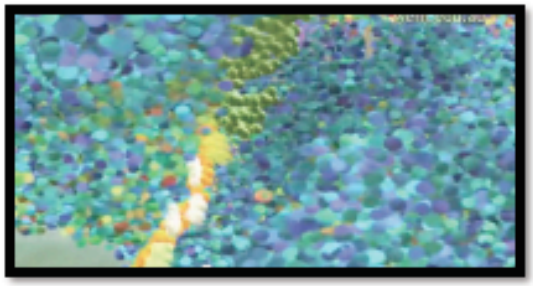

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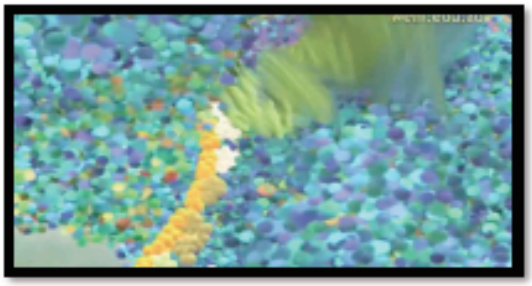

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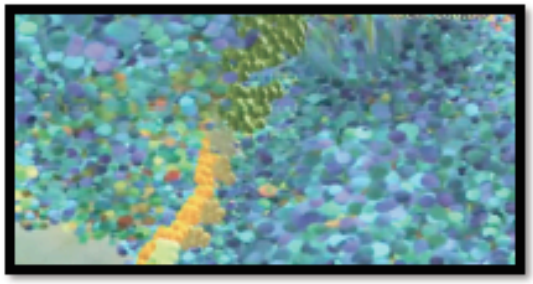

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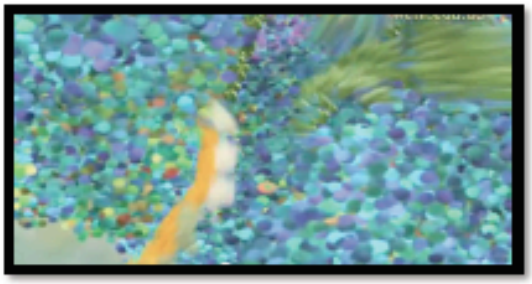

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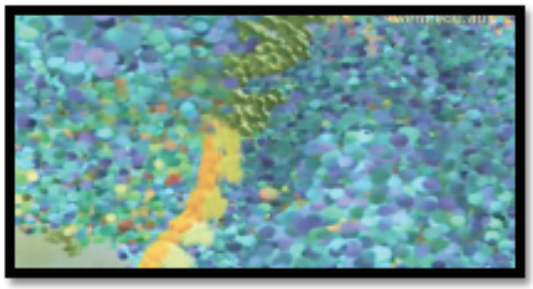

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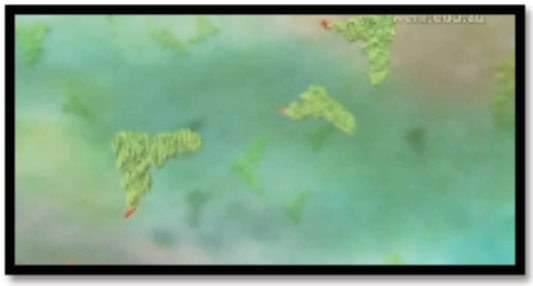
ER6_34



ER6_35

ER6_36

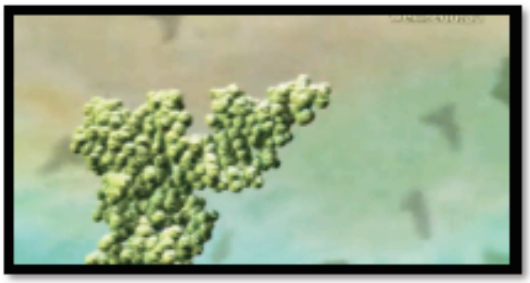
ER6_37

ER6_38

ER6_39

ER6_40

ER6_41



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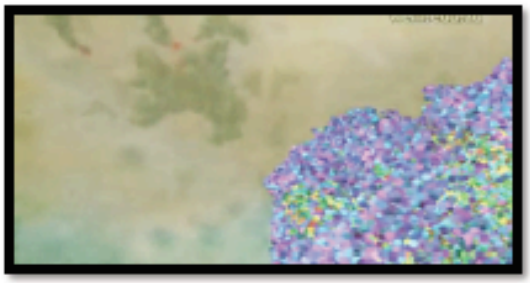

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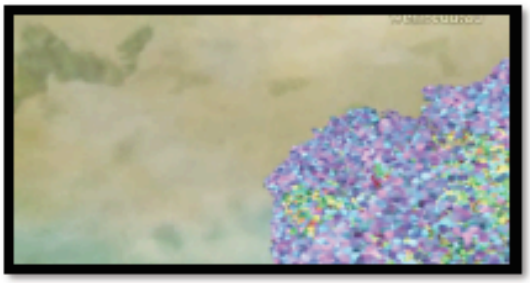

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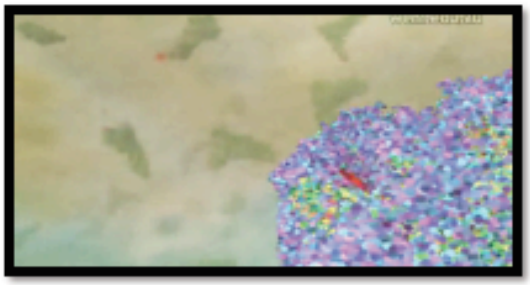

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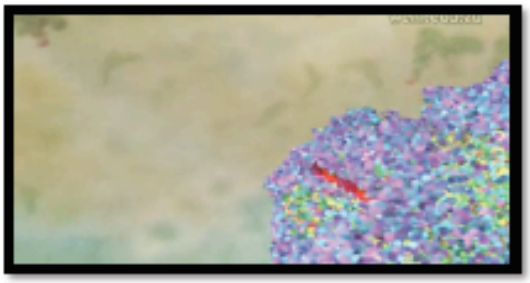
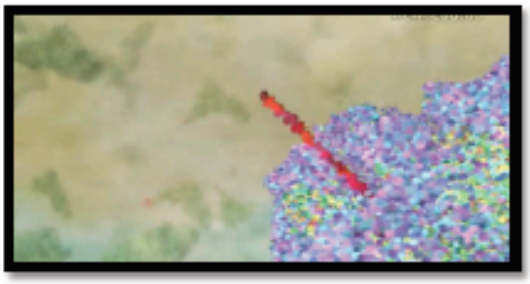


ER6_46

ER6_47



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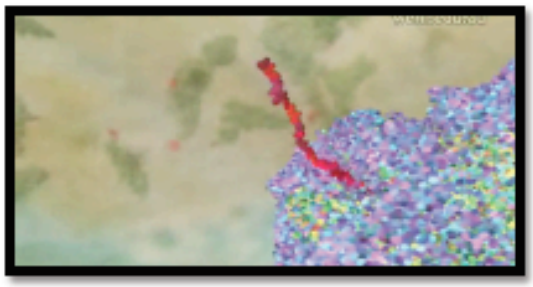

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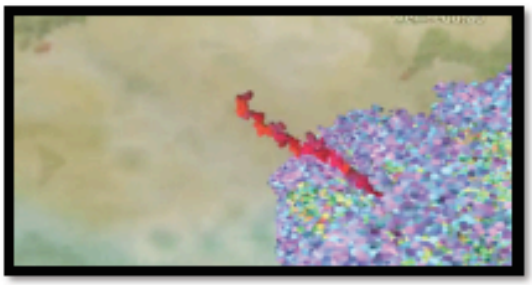

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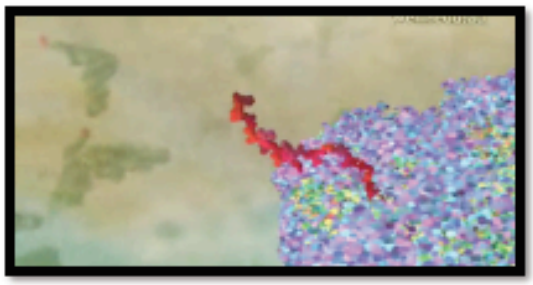

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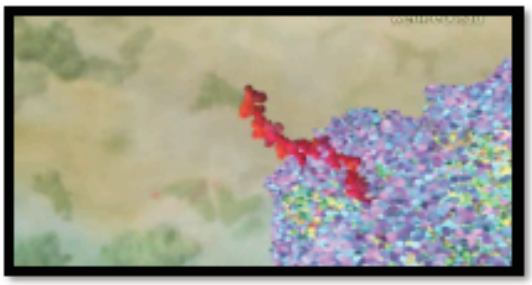

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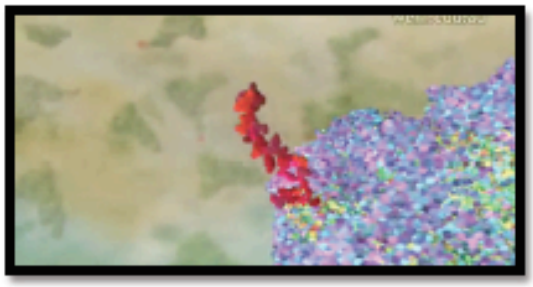

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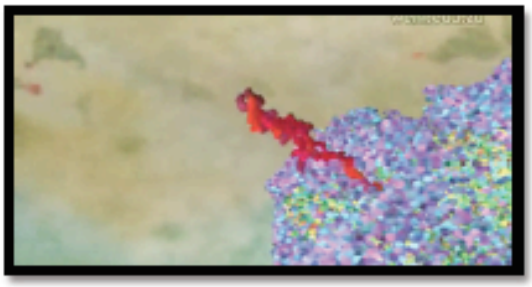

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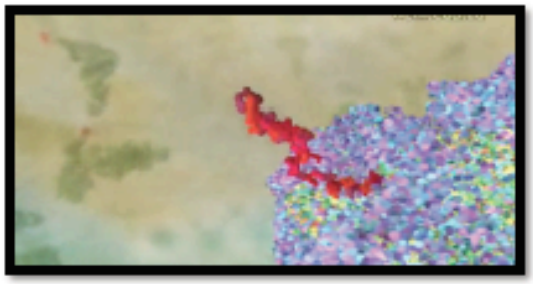

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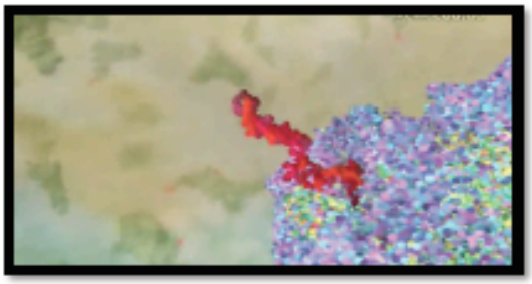

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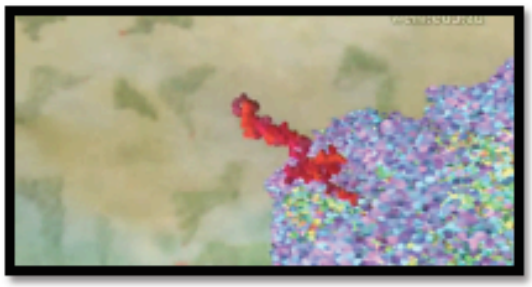

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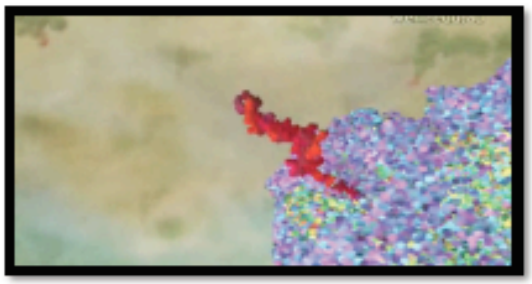

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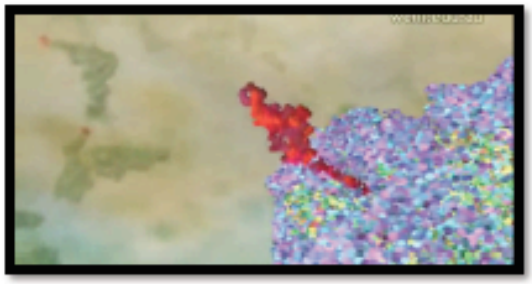

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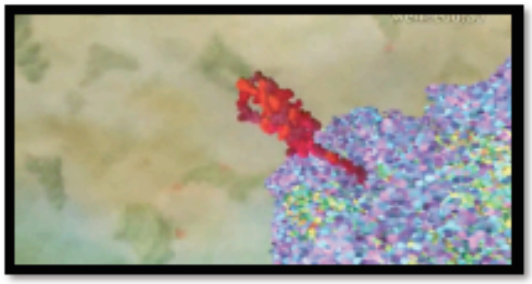

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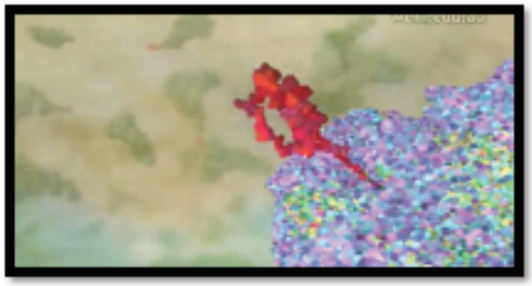

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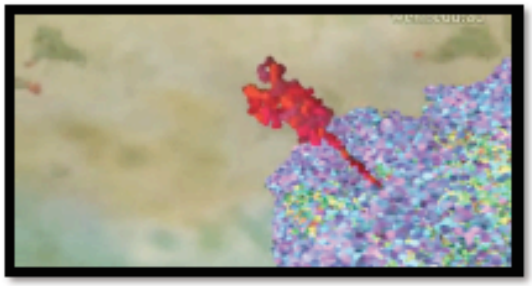
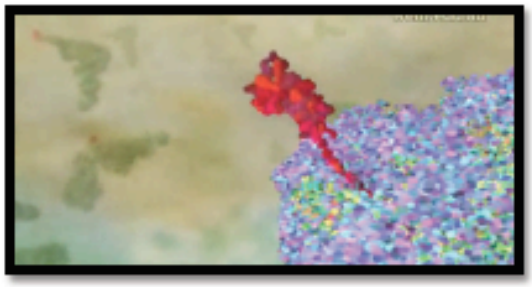
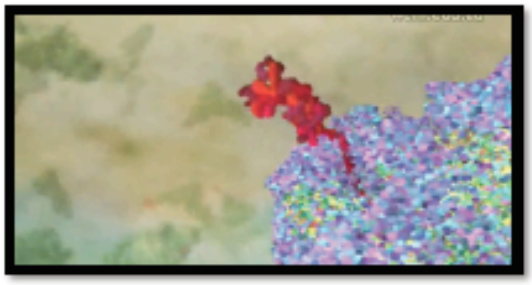


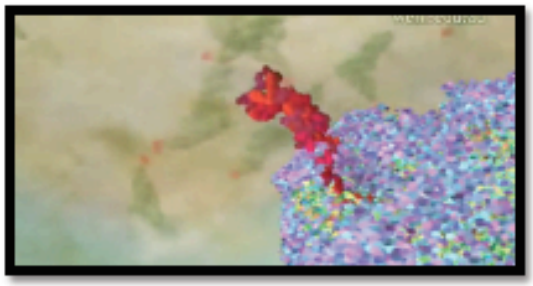
ER6_62



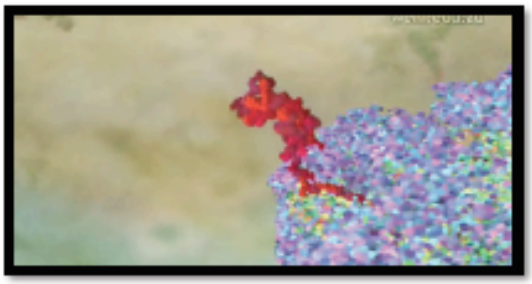
ER6_63



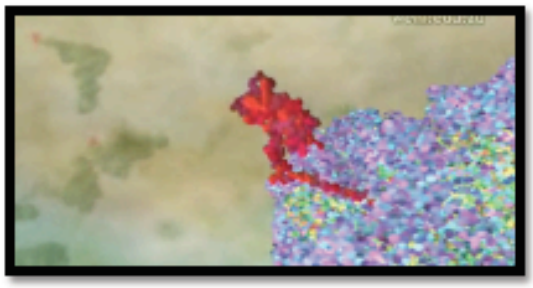
ER6_64



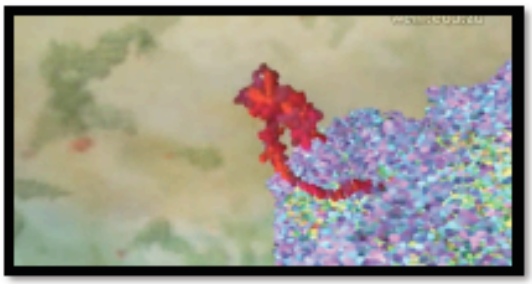
ER6_65



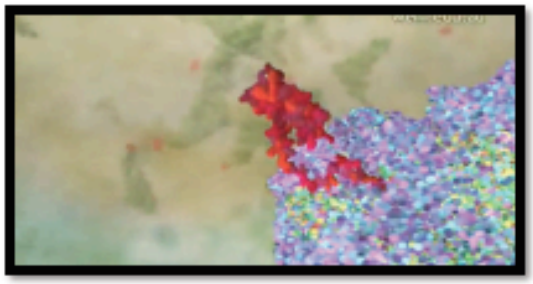
ER6_66



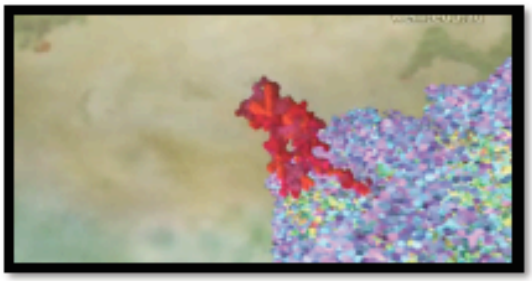
ER6_67



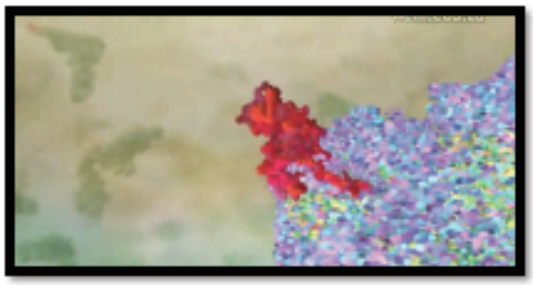
ER6_68



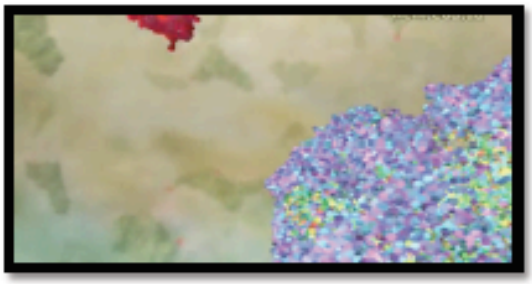
ER6_69



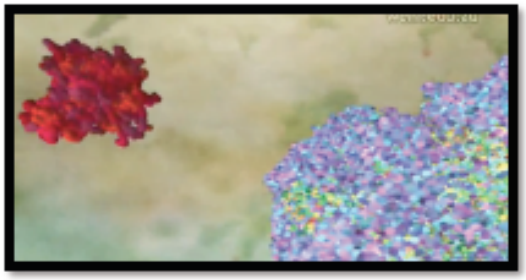
ER6_70



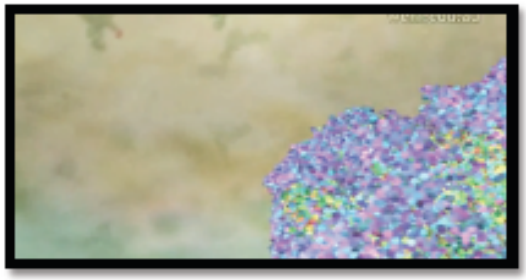
ER6_71



ER6_72



ER6_73

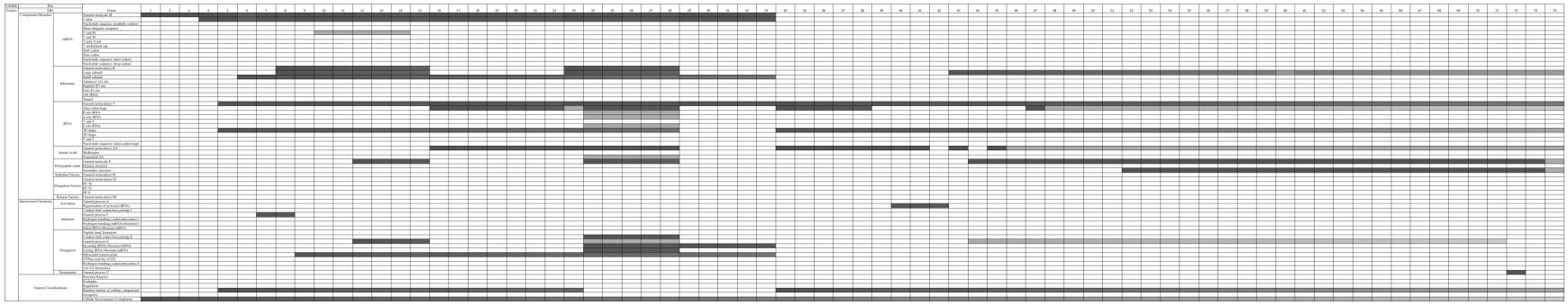


ER6_74

APPENDIX O

VADER PLOT OF EXTERNAL REPRESENTATION #6

Plot Overview:



Section Layout*:

Section 1	Section 4	Section 7	Section 10	Section 13	Section 16	Section 19	Section 22	Section 25
Section 2	Section 5	Section 8	Section 11	Section 14	Section 17	Section 20	Section 23	Section 26
Section 3	Section 6	Section 9	Section 12	Section 15	Section 18	Section 21	Section 24	Section 27

*The section layout pertains to the following 27 pages. In order to reconstruct the full VADER plot for ER6, the following 27 sections should be arranged according to the section layout above.

VADER Plot ER6
Section 1

VADER	Plot					
Feature	ER6		Frame	1	2	3
	Components/Structure	mRNA	General molecule M	66.7%	66.7%	66.7%
			Codon	0.0%	0.0%	0.0%
			Nucleotide sequence (multiple codons)	0.0%	0.0%	0.0%
			Shine-dalgarno seuqence	0.0%	0.0%	0.0%
			5' end M	0.0%	0.0%	0.0%
			3' end M	0.0%	0.0%	0.0%
			3' poly A tail	0.0%	0.0%	0.0%
			5' methylated cap	0.0%	0.0%	0.0%
			Start codon	0.0%	0.0%	0.0%
			Stop codon	0.0%	0.0%	0.0%
			Nucleotide sequence (start codon)	0.0%	0.0%	0.0%
			Nucleotide sequence (stop codon)	0.0%	0.0%	0.0%
		Ribosome	General molecule(s) R	0.0%	0.0%	0.0%
			Large subunit	0.0%	0.0%	0.0%
			Small subunit	0.0%	0.0%	0.0%
			Aminacyl (A) site	0.0%	0.0%	0.0%
			Peptidyl (P) site	0.0%	0.0%	0.0%
			Exit (E) site	0.0%	0.0%	0.0%
			16S rRNA	0.0%	0.0%	0.0%
			Tunnel	0.0%	0.0%	0.0%
			General molecule(s) T	0.0%	0.0%	0.0%
			Anti-codon loop	0.0%	0.0%	0.0%
			P-site tRNA	0.0%	0.0%	0.0%

VADER Plot ER6
Section 2

		tRNA	A-site tRNA	0.0%	0.0%	0.0%
			3' end T	0.0%	0.0%	0.0%
			E-site tRNA	0.0%	0.0%	0.0%
			3D shape	0.0%	0.0%	0.0%
			2D shape	0.0%	0.0%	0.0%
			5' end T	0.0%	0.0%	0.0%
			Nucleotide sequence (Anti-codon loop)	0.0%	0.0%	0.0%
		Amino Acids	General molecule(s) AA	0.0%	0.0%	0.0%
			Methionine	0.0%	0.0%	0.0%
			Sequential AA	0.0%	0.0%	0.0%
		Polypeptide chain	General molecule P	0.0%	0.0%	0.0%
			Primary structure	0.0%	0.0%	0.0%
			Secondary structure	0.0%	0.0%	0.0%
		Initiation Factors	General molecule(s) IF	0.0%	0.0%	0.0%
		Elongation Factors	General molecule(s) EF	0.0%	0.0%	0.0%
			EF-Tu	0.0%	0.0%	0.0%
			EF-Ts	0.0%	0.0%	0.0%
			EF-G	0.0%	0.0%	0.0%
		Release Factors	General molecule(s) RF	0.0%	0.0%	0.0%
	Interactions/Chemistry	Activation	General process A	0.0%	0.0%	0.0%
			Regeneration of activated tRNAs	0.0%	0.0%	0.0%
		Initiation	Condon/Anti-codon base pairing I	0.0%	0.0%	0.0%
			General process I	0.0%	0.0%	0.0%
			Hydrogen bonding (codon/anticodon) I	0.0%	0.0%	0.0%
			Hydrogen bonding (mRNA/ribosome) I	0.0%	0.0%	0.0%

VADER Plot ER6
Section 3

			Initial tRNA/ribosome/mRNA	0.0%	0.0%	0.0%
		Elongation	Peptide bond formation	0.0%	0.0%	0.0%
			Condon/Anti-codon base pairing E	0.0%	0.0%	0.0%
			General process E	0.0%	0.0%	0.0%
			Incoming tRNA/ribosome/mRNA	0.0%	0.0%	0.0%
			Exiting tRNA/ribosome/mRNA	0.0%	0.0%	0.0%
			Ribosomal translocation	0.0%	0.0%	0.0%
			GTPase activity of EFs	0.0%	0.0%	0.0%
			Hydrogen bonding (codon/anticodon) E	0.0%	0.0%	0.0%
			AA/AA interaction	0.0%	0.0%	0.0%
		Termination	General process T	0.0%	0.0%	0.0%
	General Considerations		Reaction Kinetics	0.0%	0.0%	0.0%
			Evolution	0.0%	0.0%	0.0%
			Regulation	0.0%	0.0%	0.0%
			Random motion of cellular components	0.0%	0.0%	0.0%
			Energetics	0.0%	0.0%	0.0%
			Cellular Environment (Cytoplasm)	66.7%	65.8%	64.9%

Features per frame	2	2	2

VADER Plot ER6
Section 4

4	5	6	7	8	9	10	11	12	13
66.7%	66.7%	66.2%	65.8%	65.3%	66.7%	66.2%	65.8%	65.3%	64.9%
66.7%	66.7%	66.2%	65.8%	65.3%	66.7%	66.2%	65.8%	65.3%	64.9%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	66.7%	66.2%	65.3%	64.4%	63.5%	62.6%
0.0%	0.0%	0.0%	0.0%	66.7%	66.2%	65.3%	64.4%	63.5%	62.6%
0.0%	0.0%	66.7%	66.7%	66.2%	66.2%	65.3%	64.4%	63.5%	62.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	66.7%	66.2%	65.8%	65.3%	66.7%	66.2%	65.8%	65.3%	64.9%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 5

| | | |

VADER Plot ER6
Section 6

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%	65.8%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	66.7%	65.8%	64.9%	64.0%	63.1%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
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0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	66.7%	65.8%	64.9%	64.0%	63.1%	62.2%	61.3%	60.4%	59.5%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
64.0%	64.9%	64.0%	63.1%	62.2%	63.1%	62.2%	61.3%	60.4%	59.5%

3	6	7	8	10	10	10	10	12	12

VADER Plot ER6
Section 7

14	15	16	17	18	19	20	21	22	23
64.4%	64.0%	66.7%	66.2%	65.8%	65.3%	64.9%	64.4%	64.0%	66.7%
64.4%	64.0%	66.7%	66.2%	65.8%	65.3%	64.9%	64.4%	64.0%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
61.7%	60.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%
61.7%	60.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%
61.7%	60.8%	63.1%	62.2%	61.3%	60.4%	59.5%	58.6%	57.7%	59.9%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
64.4%	64.0%	66.7%	66.2%	65.8%	65.3%	64.9%	64.4%	64.0%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 8

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
60.4%	59.5%	61.7%	60.8%	59.9%	59.0%	58.1%	57.2%	56.3%	58.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	66.7%	66.2%	65.8%	65.3%	64.9%	64.4%	64.0%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
65.8%	65.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 9

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
64.9%	64.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
62.2%	61.3%	63.5%	62.6%	61.7%	60.8%	59.9%	59.0%	58.1%	60.4%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
58.6%	57.7%	61.7%	60.8%	59.9%	59.0%	58.1%	57.2%	56.3%	58.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
58.6%	57.7%	59.9%	59.0%	58.1%	57.2%	56.3%	55.4%	54.5%	56.8%

12	12	9	9	9	9	9	9	9	11

VADER Plot ER6
Section 10

24	25	26	27	28	29	30	31	32	33
66.2%	65.8%	65.3%	64.9%	64.4%	66.7%	66.2%	65.8%	65.3%	64.9%
66.2%	65.8%	65.3%	64.9%	64.4%	66.7%	66.2%	65.8%	65.3%	64.9%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
65.8%	64.9%	64.0%	63.1%	62.2%	0.0%	0.0%	0.0%	0.0%	0.0%
65.8%	64.9%	64.0%	63.1%	62.2%	0.0%	0.0%	0.0%	0.0%	0.0%
59.0%	58.1%	57.2%	56.3%	55.4%	57.2%	56.3%	55.4%	54.5%	53.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.2%	65.8%	65.3%	64.9%	66.7%	66.2%	65.8%	65.3%	64.9%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	33.3%	33.3%	33.3%	33.3%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 11

33.3%	33.3%	33.3%	33.3%	33.3%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	33.3%	33.3%	33.3%	33.3%	0.0%	0.0%	0.0%	0.0%	0.0%
58.1%	57.2%	56.3%	55.4%	54.5%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.2%	65.8%	65.3%	64.9%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	33.3%	33.3%	33.3%	33.3%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	65.8%	64.9%	64.0%	63.1%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 12

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.2%	65.8%	65.3%	64.9%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	32.9%	32.4%	32.0%	31.5%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.2%	65.8%	65.3%	64.9%	66.7%	66.2%	65.8%	65.3%	64.9%
66.7%	66.2%	65.8%	65.3%	64.9%	0.0%	0.0%	0.0%	0.0%	0.0%
59.5%	58.6%	57.7%	56.8%	55.9%	57.7%	56.8%	55.9%	55.0%	54.1%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	32.9%	32.4%	32.0%	31.5%	31.1%	30.6%	30.2%	29.7%	29.3%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
55.9%	55.0%	54.1%	53.2%	52.3%	54.1%	53.2%	52.3%	51.4%	50.5%

19	19	19	19	19	7	7	7	7	7

VADER Plot ER6
Section 13

34	35	36	37	38	39	40	41	42	43
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.2%	65.8%	65.3%	64.9%	66.7%	65.8%	64.9%	64.0%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 14

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	65.8%	64.9%	64.0%	63.1%	64.4%	63.5%	62.6%	61.7%	62.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.2%	65.8%	65.3%	64.9%	66.7%	66.2%	65.8%	0.0%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%	65.8%	64.9%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 15

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	65.8%	64.9%	64.0%	63.1%	62.2%	61.3%	60.4%	59.5%	62.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
51.8%	50.9%	50.0%	49.1%	48.2%	47.3%	46.4%	45.5%	44.6%	47.7%

5	5	5	5	5	5	6	6	5	6

VADER Plot ER6
Section 16

44	45	46	47	48	49	50	51	52	53
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
65.8%	64.9%	64.0%	63.1%	62.2%	61.3%	60.4%	59.5%	58.6%	57.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.2%	65.8%	65.3%	64.9%	64.4%	64.0%	63.5%	63.1%	62.6%	62.2%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 17

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
61.7%	60.8%	59.9%	59.0%	58.1%	57.2%	56.3%	55.4%	54.5%	53.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	66.7%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 18

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	32.9%	3.2%	32.0%	31.5%	31.1%	30.6%	30.2%	29.7%	29.3%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
61.7%	60.8%	59.9%	59.0%	58.1%	57.2%	56.3%	55.4%	54.5%	53.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
46.8%	45.9%	45.0%	44.1%	43.2%	42.3%	41.4%	40.5%	39.6%	38.7%

7	8	8	8	8	8	8	8	9	9

VADER Plot ER6
Section 19

54	55	56	57	58	59	60	61	62	63
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
56.8%	55.9%	55.0%	54.1%	53.2%	52.3%	51.4%	50.5%	49.5%	48.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
61.7%	61.3%	60.8%	60.4%	59.9%	59.5%	59.0%	58.6%	58.1%	57.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 20

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
52.7%	51.8%	50.9%	50.0%	49.1%	48.2%	47.3%	46.4%	45.5%	44.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 21

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
28.8%	28.4%	27.9%	27.5%	27.0%	26.6%	26.1%	25.7%	25.2%	24.8%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
52.7%	51.8%	50.9%	50.0%	49.1%	48.2%	47.3%	46.4%	45.5%	44.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
37.8%	36.9%	36.0%	35.1%	34.2%	33.3%	32.4%	31.5%	30.6%	29.7%

9	9	9	9	9	9	9	9	9	9

VADER Plot ER6
Section 22

64	65	66	67	68	69	70	71	72	73
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
47.7%	46.8%	45.9%	45.0%	44.1%	43.2%	42.3%	41.4%	40.5%	39.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
57.2%	56.8%	56.3%	55.9%	55.4%	55.0%	54.5%	54.1%	53.6%	53.2%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 23

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
43.7%	42.8%	41.9%	41.0%	40.1%	39.2%	38.3%	37.4%	36.5%	35.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.2%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.7%	66.2%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

VADER Plot ER6
Section 24

0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
24.3%	23.9%	23.4%	23.0%	22.5%	22.1%	21.6%	21.2%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.7%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
43.7%	42.8%	41.9%	41.0%	40.1%	39.2%	38.3%	37.4%	36.5%	35.6%
0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
28.8%	27.9%	27.0%	26.1%	25.2%	24.3%	23.4%	22.5%	21.6%	20.7%

9	9	9	9	9	9	9	9	9	8

VADER Plot ER6
Section 25

74	Average
0.0%	29.3%
0.0%	26.6%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	12.1%
38.7%	34.9%
0.0%	22.8%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
52.7%	59.5%
0.0%	0.0%
0.0%	2.3%

VADER Plot ER6
Section 26

0.0%	2.3%
0.0%	0.0%
0.0%	2.3%
34.7%	48.4%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
33.3%	33.5%
0.0%	0.0%
0.0%	2.3%
33.3%	35.4%
0.0%	0.0%
33.3%	20.3%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	2.7%
0.0%	0.0%
0.0%	1.8%
0.0%	0.0%
0.0%	0.0%

VADER Plot ER6
Section 27

0.0%	0.0%
0.0%	0.0%
0.0%	4.4%
0.0%	15.6%
0.0%	8.9%
0.0%	4.4%
0.0%	20.3%
0.0%	0.0%
0.0%	0.0%
0.0%	0.0%
0.0%	0.9%
0.0%	4.2%
0.0%	0.0%
0.0%	0.0%
34.7%	44.3%
0.0%	0.0%
19.8%	46.5%
	Average
8	9

APPENDIX P

AVERAGE CUEING OF EXTERNAL REPRESENTATION #6

Theme	General Feature	Feature	Average Cueing Potential
Components/ Structure	mRNA	General molecule M	29.3%
		Codon	26.6%
		Nucleotide sequence (multiple codons)	0.0%
		Shine-Dalgarno sequence	0.0%
		5' end M	0.0%
		3' end M	0.0%
		3' poly A tail	0.0%
		5' methylated cap	0.0%
		Start codon	0.0%
		Stop codon	0.0%
		Nucleotide sequence (start codon)	0.0%
		Nucleotide sequence (stop codon)	0.0%
	Ribosome	General molecule(s) R	12.1%
		Large subunit	34.9%
		Small subunit	22.8%
		Aminacyl (A) site	0.0%
		Peptidyl (P) site	0.0%
		Exit (E) site	0.0%
		16S rRNA	0.0%
		Tunnel	0.0%
	tRNA	General molecule(s) T	59.5%
		Anti-codon loop	0.0%
		P-site tRNA	2.3%
		A-site tRNA	2.3%
		3' end T	0.0%
		E-site tRNA	2.3%
		3D shape	48.4%
		2D shape	0.0%
		5' end T	0.0%
		Nucleotide sequence (Anti-codon loop)	0.0%
	Amino Acids	General molecule(s) AA	33.5%
		Methionine	0.0%
		Sequential AA	2.3%
	Polypeptide chain	General molecule P	35.4%
		Primary structure	0.0%

		Secondary structure	20.3%
	Initiation Factors	General molecule(s) IF	0.0%
	Elongation Factors	General molecule(s) EF	0.0%
		EF-Tu	0.0%
		EF-Ts	0.0%
		EF-G	0.0%
	Release Factors	General molecule(s) RF	0.0%
Components/ Structure	mRNA	General process A	0.0%
		Regeneration of activated tRNAs	2.7%
	Initiation	Condon/Anti-codon base pairing I	0.0%
		General process I	1.8%
		Hydrogen bonding (codon/anticodon) I	0.0%
		Hydrogen bonding (mRNA/ribosome) I	0.0%
		Initial tRNA/ribosome/mRNA	0.0%
	Elongation Ribosome	Peptide bond formation	0.0%
		Condon/Anti-codon base pairing E	4.4%
		General process E	15.6%
		Incoming tRNA/ribosome/mRNA	8.9%
		Exiting tRNA/ribosome/mRNA	4.4%
		Ribosomal translocation	20.3%
		GTPase activity of EFs	0.0%
		Hydrogen bonding (codon/anticodon) E	0.0%
		AA/AA interaction	0.0%
	Termination	General process T	0.9%
General Considerations		Reaction kinetics	4.2%
		Evolution	0.0%
		Regulation	0.0%
		tRNA	44.3%
		Energetics	0.0%
		Cellular environment (Cytoplasm)	46.5%

APPENDIX Q

COMPARISON OF THE AVERAGE CUEING OF THE SELECTED EXTERNAL REPRESENTATIONS

Theme	General Feature	Feature	Average Cueing Potential		
			ER2	ER3	ER6
Components/Structure	mRNA	General molecule M	49.1%	51.6%	29.3%
		Codon	53.7%	48.9%	26.6%
		Nucleotide sequence (multiple codons)	0.0%	65.9%	0.0%
		Shine-Dalgarno sequence	0.0%	11.8%	0.0%
		5' end M	63.0%	75.1%	0.0%
		3' end M	63.0%	66.1%	0.0%
		3' poly A tail	0.0%	0.0%	0.0%
		5' methylated cap	0.0%	0.0%	0.0%
		Start codon	67.6%	38.9%	0.0%
		Stop codon	0.0%	16.0%	0.0%
		Nucleotide sequence (start codon)	67.6%	53.2%	0.0%
		Nucleotide sequence (stop codon)	0.0%	22.7%	0.0%
	Ribosome	General molecule(s) R	43.5%	51.7%	12.1%
		Large subunit	49.1%	48.4%	34.9%
		Small subunit	49.1%	56.9%	22.8%
		Aminacyl (A) site	74.1%	46.0%	0.0%
		Peptidyl (P) site	74.1%	46.0%	0.0%
		Exit (E) site	74.1%	0.0%	0.0%
		16S rRNA	0.0%	13.3%	0.0%
		Tunnel	0.0%	0.0%	0.0%
	tRNA	General molecule(s) T	55.6%	48.0%	59.5%
		Anti-codon loop	51.9%	47.9%	0.0%
		P-site tRNA	63.0%	33.7%	2.3%
		A-site tRNA	61.1%	7.7%	2.3%
		3' end T	27.8%	0.0%	0.0%
		E-site tRNA	11.1%	0.8%	2.3%
		3D shape	43.5%	0.0%	48.4%
		2D shape	0.0%	48.0%	0.0%
		5' end T	66.7%	0.0%	0.0%
		Nucleotide sequence (Anti-codon loop)	71.3%	72.6%	0.0%
	Amino Acids	General molecule(s) AA	51.9%	44.0%	33.5%
		Methionine	84.3%	52.9%	0.0%
		Sequential AA	66.7%	32.8%	2.3%
	Polypeptide chain	General molecule P	29.6%	27.9%	35.4%

		Primary structure	29.6%	27.9%	0.0%
		Secondary structure	0.0%	0.0%	20.3%
	Initiation Factors	General molecule(s) IF	0.0%	0.0%	0.0%
	Elongation Factors	General molecule(s) EF	0.0%	0.0%	0.0%
		EF-Tu	16.7%	0.0%	0.0%
		EF-Ts	16.7%	0.0%	0.0%
		EF-G	16.7%	0.0%	0.0%
	Release Factors	General molecule(s) RF	0.0%	12.7%	0.0%
Interactions/Chemistry	Activation	General process A	0.0%	0.0%	0.0%
		Regeneration of activated tRNAs	0.0%	0.0%	2.7%
	Initiation	Condon/Anti-codon base pairing I	40.8%	8.1%	0.0%
		General process I	0.0%	5.0%	1.8%
		Hydrogen bonding (codon/anticodon) I	40.8%	0.0%	0.0%
		Hydrogen bonding (mRNA/ribosome) I	0.0%	11.1%	0.0%
		Initial tRNA/ribosome/mRNA	53.7%	12.5%	0.0%
	Elongation	Peptide bond formation	41.7%	0.0%	0.0%
		Condon/Anti-codon base pairing E	40.7%	16.1%	4.4%
		General process E	40.7%	13.4%	15.6%
		Incoming tRNA/ribosome/mRNA	22.2%	9.2%	8.9%
		Exiting tRNA/ribosome/mRNA	11.1%	6.2%	4.4%
		Ribosomal translocation	5.6%	14.7%	20.3%
		GTPase activity of EFs	27.8%	0.0%	0.0%
		Hydrogen bonding (codon/anticodon) E	40.7%	0.0%	0.0%
		AA/AA interaction	33.3%	22.3%	0.0%
	Termination	General process T	0.0%	3.7%	0.9%
General Considerations		Reaction kinetics	0.0%	9.2%	4.2%
		Evolution	0.0%	0.0%	0.0%
		Regulation	0.0%	0.0%	0.0%
		Random motion of cellular components	0.0%	0.0%	44.3%
		Energetics	0.0%	0.0%	0.0%
		Cellular environment (Cytoplasm)	0.0%	0.0%	46.5%

APPENDIX R

COMPARISON OF EXTERNAL REPRESENTATIONS #2 AND #3

Theme	General Feature	Feature	Average Cuing Potential		Type
			ER2	ER3	
Components/Structure	mRNA	General molecule M	49.1%	51.6%	1°
		Codon	53.7%	48.9%	1°
		Nucleotide sequence (multiple codons)	0.0%	65.9%	2°
		Shine-Dalgarno sequence	0.0%	11.8%	2°
		5' end M	63.0%	75.1%	2°
		3' end M	63.0%	66.1%	2°
		3' poly A tail	0.0%	0.0%	2°
		5' methylated cap	0.0%	0.0%	3°
		Start codon	67.6%	38.9%	3°
		Stop codon	0.0%	16.0%	3°
		Nucleotide sequence (start codon)	67.6%	53.2%	3°
		Nucleotide sequence (stop codon)	0.0%	22.7%	NC
	Ribosome	General molecule(s) R	43.5%	51.7%	1°
		Large subunit	49.1%	48.4%	1°
		Small subunit	49.1%	56.9%	1°
		Aminacyl (A) site	74.1%	46.0%	1°
		Peptidyl (P) site	74.1%	46.0%	1°
		Exit (E) site	74.1%	0.0%	1°
		16S rRNA	0.0%	13.3%	3°
		Tunnel	0.0%	0.0%	2°
	tRNA	General molecule(s) T	55.6%	48.0%	1°
		Anti-codon loop	51.9%	47.9%	1°
		P-site tRNA	63.0%	33.7%	1°
		A-site tRNA	61.1%	7.7%	2°
		3' end T	27.8%	0.0%	2°
		E-site tRNA	11.1%	0.8%	3°
		3D shape	43.5%	0.0%	3°
		2D shape	0.0%	48.0%	3°
		5' end T	66.7%	0.0%	3°
		Nucleotide sequence (Anti-codon loop)	71.3%	72.6%	3°
	Amino Acids	General molecule(s) AA	51.9%	44.0%	1°
		Methionine	84.3%	52.9%	2°
		Sequential AA	66.7%	32.8%	3°
	Polypeptide chain	General molecule P	29.6%	27.9%	1°

		Primary structure	29.6%	27.9%	2°
		Secondary structure	0.0%	0.0%	3°
	Initiation Factors	General molecule(s) IF	0.0%	0.0%	3°
	Elongation Factors	General molecule(s) EF	0.0%	0.0%	2°
		EF-Tu	16.7%	0.0%	3°
		EF-Ts	16.7%	0.0%	3°
		EF-G	16.7%	0.0%	3°
	Release Factors	General molecule(s) RF	0.0%	12.7%	2°
Interactions/Chemistry	Activation	General process A	0.0%	0.0%	2°
		Regeneration of activated tRNAs	0.0%	0.0%	3°
	Initiation	Condon/Anti-codon base pairing I	40.8%	8.1%	1°
		General process I	0.0%	5.0%	2°
		Hydrogen bonding (codon/anticodon) I	40.8%	0.0%	3°
		Hydrogen bonding (mRNA/ribosome) I	0.0%	11.1%	NC
		Initial tRNA/ribosome/mRNA	53.7%	12.5%	NC
	Elongation	Peptide bond formation	41.7%	0.0%	1°
		Condon/Anti-codon base pairing E	40.7%	16.1%	1°
		General process E	40.7%	13.4%	2°
		Incoming tRNA/ribosome/mRNA	22.2%	9.2%	2°
		Exiting tRNA/ribosome/mRNA	11.1%	6.2%	2°
		Ribosomal translocation	5.6%	14.7%	2°
		GTPase activity of EFs	27.8%	0.0%	3°
		Hydrogen bonding (codon/anticodon) E	40.7%	0.0%	3°
		AA/AA interaction	33.3%	22.3%	NC
	Termination	General process T	0.0%	3.7%	NC
General Considerations		Reaction kinetics	0.0%	9.2%	2°
		Evolution	0.0%	0.0%	2°
		Regulation	0.0%	0.0%	2°
		Random motion of cellular components	0.0%	0.0%	2°
		Energetics	0.0%	0.0%	3°
		Cellular environment (Cytoplasm)	0.0%	0.0%	NC

APPENDIX S

COMPARISON OF EXTERNAL REPRESENTATIONS #2 AND #6

Theme	General Feature	Feature	Average Cuing Potential		Type
			ER2	ER6	
Components/Structure	mRNA	General molecule M	49.1%	29.3%	1°
		Codon	53.7%	26.6%	1°
		Nucleotide sequence (multiple codons)	0.0%	0.0%	2°
		Shine-Dalgarno sequence	0.0%	0.0%	2°
		5' end M	63.0%	0.0%	2°
		3' end M	63.0%	0.0%	2°
		3' poly A tail	0.0%	0.0%	2°
		5' methylated cap	0.0%	0.0%	3°
		Start codon	67.6%	0.0%	3°
		Stop codon	0.0%	0.0%	3°
		Nucleotide sequence (start codon)	67.6%	0.0%	3°
		Nucleotide sequence (stop codon)	0.0%	0.0%	NC
	Ribosome	General molecule(s) R	43.5%	12.1%	1°
		Large subunit	49.1%	34.9%	1°
		Small subunit	49.1%	22.8%	1°
		Aminacyl (A) site	74.1%	0.0%	1°
		Peptidyl (P) site	74.1%	0.0%	1°
		Exit (E) site	74.1%	0.0%	1°
		16S rRNA	0.0%	0.0%	3°
		Tunnel	0.0%	0.0%	2°
	tRNA	General molecule(s) T	55.6%	59.5%	1°
		Anti-codon loop	51.9%	0.0%	1°
		P-site tRNA	63.0%	2.3%	1°
		A-site tRNA	61.1%	2.3%	2°
		3' end T	27.8%	0.0%	2°
		E-site tRNA	11.1%	2.3%	3°
		3D shape	43.5%	48.4%	3°
		2D shape	0.0%	0.0%	3°
		5' end T	66.7%	0.0%	3°
		Nucleotide sequence (Anti-codon loop)	71.3%	0.0%	3°
	Amino Acids	General molecule(s) AA	51.9%	33.5%	1°
		Methionine	84.3%	0.0%	2°
		Sequential AA	66.7%	2.3%	3°
	Polypeptide chain	General molecule P	29.6%	35.4%	1°

		Primary structure	29.6%	0.0%	2°
		Secondary structure	0.0%	20.3%	3°
	Initiation Factors	General molecule(s) IF	0.0%	0.0%	3°
	Elongation Factors	General molecule(s) EF	0.0%	0.0%	2°
		EF-Tu	16.7%	0.0%	3°
		EF-Ts	16.7%	0.0%	3°
		EF-G	16.7%	0.0%	3°
	Release Factors	General molecule(s) RF	0.0%	0.0%	2°
Interactions/Chemistry	Activation	General process A	0.0%	0.0%	2°
		Regeneration of activated tRNAs	0.0%	2.7%	3°
	Initiation	Condon/Anti-codon base pairing I	40.8%	0.0%	1°
		General process I	0.0%	1.8%	2°
		Hydrogen bonding (codon/anticodon) I	40.8%	0.0%	3°
		Hydrogen bonding (mRNA/ribosome) I	0.0%	0.0%	NC
		Initial tRNA/ribosome/mRNA	53.7%	0.0%	NC
	Elongation	Peptide bond formation	41.7%	0.0%	1°
		Condon/Anti-codon base pairing E	40.7%	4.4%	1°
		General process E	40.7%	15.6%	2°
		Incoming tRNA/ribosome/mRNA	22.2%	8.9%	2°
		Exiting tRNA/ribosome/mRNA	11.1%	4.4%	2°
		Ribosomal translocation	5.6%	20.3%	2°
		GTPase activity of EFs	27.8%	0.0%	3°
		Hydrogen bonding (codon/anticodon) E	40.7%	0.0%	3°
		AA/AA interaction	33.3%	0.0%	NC
	Termination	General process T	0.0%	0.9%	NC
General Considerations		Reaction kinetics	0.0%	4.2%	2°
		Evolution	0.0%	0.0%	2°
		Regulation	0.0%	0.0%	2°
		Random motion of cellular components	0.0%	44.3%	2°
		Energetics	0.0%	0.0%	3°
		Cellular environment (Cytoplasm)	0.0%	46.5%	NC

APPENDIX T

COMPARISON OF EXTERNAL REPRESENTATIONS #3 AND #6

Theme	General Feature	Feature	Average Cuing Potential		Type
			ER3	ER6	
Components/Structure	mRNA	General molecule M	51.6%	29.3%	1°
		Codon	48.9%	26.6%	1°
		Nucleotide sequence (multiple codons)	65.9%	0.0%	2°
		Shine-Dalgarno sequence	11.8%	0.0%	2°
		5' end M	75.1%	0.0%	2°
		3' end M	66.1%	0.0%	2°
		3' poly A tail	0.0%	0.0%	2°
		5' methylated cap	0.0%	0.0%	3°
		Start codon	38.9%	0.0%	3°
		Stop codon	16.0%	0.0%	3°
		Nucleotide sequence (start codon)	53.2%	0.0%	3°
		Nucleotide sequence (stop codon)	22.7%	0.0%	NC
	Ribosome	General molecule(s) R	51.7%	12.1%	1°
		Large subunit	48.4%	34.9%	1°
		Small subunit	56.9%	22.8%	1°
		Aminacyl (A) site	46.0%	0.0%	1°
		Peptidyl (P) site	46.0%	0.0%	1°
		Exit (E) site	0.0%	0.0%	1°
		16S rRNA	13.3%	0.0%	3°
		Tunnel	0.0%	0.0%	2°
	tRNA	General molecule(s) T	48.0%	59.5%	1°
		Anti-codon loop	47.9%	0.0%	1°
		P-site tRNA	33.7%	2.3%	1°
		A-site tRNA	7.7%	2.3%	2°
		3' end T	0.0%	0.0%	2°
		E-site tRNA	0.8%	2.3%	3°
		3D shape	0.0%	48.4%	3°
		2D shape	48.0%	0.0%	3°
		5' end T	0.0%	0.0%	3°
		Nucleotide sequence (Anti-codon loop)	72.6%	0.0%	3°
	Amino Acids	General molecule(s) AA	44.0%	33.5%	1°
		Methionine	52.9%	0.0%	2°
		Sequential AA	32.8%	2.3%	3°
	Polypeptide chain	General molecule P	27.9%	35.4%	1°

		Primary structure	27.9%	0.0%	2°
		Secondary structure	0.0%	20.3%	3°
	Initiation Factors	General molecule(s) IF	0.0%	0.0%	3°
	Elongation Factors	General molecule(s) EF	0.0%	0.0%	2°
		EF-Tu	0.0%	0.0%	3°
		EF-Ts	0.0%	0.0%	3°
		EF-G	0.0%	0.0%	3°
	Release Factors	General molecule(s) RF	12.7%	0.0%	2°
Interactions/Chemistry	Activation	General process A	0.0%	0.0%	2°
		Regeneration of activated tRNAs	0.0%	2.7%	3°
	Initiation	Condon/Anti-codon base pairing I	8.1%	0.0%	1°
		General process I	5.0%	1.8%	2°
		Hydrogen bonding (codon/anticodon) I	0.0%	0.0%	3°
		Hydrogen bonding (mRNA/ribosome) I	11.1%	0.0%	NC
		Initial tRNA/ribosome/mRNA	12.5%	0.0%	NC
	Elongation	Peptide bond formation	0.0%	0.0%	1°
		Condon/Anti-codon base pairing E	16.1%	4.4%	1°
		General process E	13.4%	15.6%	2°
		Incoming tRNA/ribosome/mRNA	9.2%	8.9%	2°
		Exiting tRNA/ribosome/mRNA	6.2%	4.4%	2°
		Ribosomal translocation	14.7%	20.3%	2°
		GTPase activity of EFs	0.0%	0.0%	3°
		Hydrogen bonding (codon/anticodon) E	0.0%	0.0%	3°
		AA/AA interaction	22.3%	0.0%	NC
	Termination	General process T	3.7%	0.9%	NC
General Considerations		Reaction kinetics	9.2%	4.2%	2°
		Evolution	0.0%	0.0%	2°
		Regulation	0.0%	0.0%	2°
		Random motion of cellular components	0.0%	44.3%	2°
		Energetics	0.0%	0.0%	3°
		Cellular environment (Cytoplasm)	0.0%	46.5%	NC

APPENDIX U

GROUP COMPARISON OF LEARNED FEATURES

Theme	General Feature	Feature	Group					
			A	B	C	D	E	F
Components/Structure	mRNA	General molecule M						
		Codon						
		Nucleotide sequence (multiple codons)						
		Shine-Dalgarno sequence						
		5' end M						
		3' end M						
		3' poly A tail						
		5' methylated cap						
		Start codon						
		Stop codon						
		Nucleotide sequence (start codon)						
		Nucleotide sequence (stop codon)						
	Ribosome	General molecule(s) R						
		Large subunit						
		Small subunit						
		Aminacyl (A) site						
		Peptidyl (P) site						
		Exit (E) site						
		16S rRNA						
		Tunnel						
	tRNA	General molecule(s) T						
		Anti-codon loop						
		P-site tRNA						
		A-site tRNA						
		3' end T						
		E-site tRNA						
		3D shape						
		2D shape						
		5' end T						
		Nucleotide sequence (Anti-codon loop)						
	Amino Acids	General molecule(s) AA						
		Methionine						
		Sequential AA						
	Polypeptide chain	General molecule P						
		Primary structure						
		Secondary structure						

	Initiation Factors	General molecule(s) IF						
	Elongation Factors	General molecule(s) EF						
		EF-Tu						
		EF-Ts						
		EF-G						
	Release Factors	General molecule(s) RF						
Interactions/Chemistry	Activation	General process A						
		Regeneration of activated tRNAs						
	Initiation	Condon/Anti-codon base pairing I						
		General process I						
		Hydrogen bonding (codon/anticodon) I						
		Hydrogen bonding (mRNA/ribosome) I						
		Initial tRNA/ribosome/mRNA						
	Elongation	Peptide bond formation						
		Condon/Anti-codon base pairing E						
		General process E						
		Incoming tRNA/ribosome/mRNA						
		Exiting tRNA/ribosome/mRNA						
		Ribosomal translocation						
		GTPase activity of EFs						
		Hydrogen bonding (codon/anticodon) E						
		AA/AA interaction						
	Termination	General process T						
General Considerations		Reaction kinetics						
		Evolution						
		Regulation						
		Random motion of cellular components						
		Energetics						
		Cellular environment (Cytoplasm)						

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Dissertation Title: What Can Biochemistry Students Learn About Protein Translation? Using Variation Theory to Explore the Space of Learning Created by Some Common External Representations

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