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Moving College Students to a Better Understanding of Substrate Specificity of Enzymes Through Utilizing Multimedia Pre-Training and an Interactive Enzyme Model

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MOVING COLLEGE STUDENTS TO A BETTER UNDERSTANDING OF SUBSTRATE SPECIFICITY OF ENZYMES THROUGH UTILIZING MULTIMEDIA PRE-TRAINING AND AN INTERACTIVE ENZYME MODEL

by

Mounir R. Saleh

Abstract of a Dissertation
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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ABSTRACT

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Scientists' progress in understanding enzyme specificity uncovered a complex natural phenomenon. However, not all of the currently available biology textbooks seem to be up to date on this progress. Students' understanding of how enzymes work is a core requirement in biochemistry and biology tertiary education. Nevertheless, current pre-college science education does not provide students with enough biochemical background to enable them to understand complex material such as this. To bridge this gap, a multimedia pre-training presentation was prepared to fuel the learner's prior knowledge with discrete facts necessary to understand the presented concept. This treatment is also known to manage intrinsic cognitive load during the learning process. An interactive instructional enzyme model was also built to motivate students to learn about substrate specificity of enzymes. Upon testing the effect of this combined treatment on 111 college students, desirable learning outcomes were found in terms of cognitive load, motivation, and achievement. The multimedia pre-training group reported significantly less intrinsic cognitive load, higher motivation, and demonstrated higher transfer performance than the control and post-training groups. In this study, a statistical mediation model is also proposed to explain how cognitive load and motivation work in concert to foster learning from multimedia pre-training. This type of research goes beyond simple forms of "what works" to a deeper understanding of "how it works," thus enabling informed decisions for multimedia

instructional design. Multimedia learning plays multiple roles in science education. Therefore, science learners would be some of the first to benefit from improving multimedia instructional design. Accordingly, complex scientific phenomena can be introduced to college students in a motivating, informative, and cognitively efficient learning environment.

It might be said

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May 2015

DEDICATION

I dedicate Chapter I to Mom who wrote down my first words.

I dedicate Chapter II to Dad who used to call me past 2:00 A.M.

to ask me about my progress. Yes Dad, I’m done!

I dedicate Chapter III to my two brothers. Together, the neighbourhood

named us the pride of lions.

I will save Chapter IV for the first person to call me daddy.

Finally, I dedicate Chapter V to my wife, *Amani*, with whom

I wish to conclude the last chapter of my life...

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It took a village to finish this project. Below is only some of the support I received:

- Dr. Sherry Herron believed in me as a burgeoning researcher and made sure to employ every available resource to remove obstacles, including a grant to build the beta prototype of my interactive model.
- Dr. Kyna Shelley closely monitored the literature review process and consistently supported me in word and action. She used to answer my emails on weekends and adapt her busy schedule for our meetings.
- Dr. Kristy Daniel walked me through the pilot study and made sure to expose my talents in international conferences. Distinguished researchers found their way to my poster.
- Dr. Robert Bateman devoted his time and expertise to reword Items in my assessment instruments.
- Dr. David Uttal agreed to volunteer as an external committee member, just for the sake of extending knowledge.
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- Dr. Sherry Herron, Dr. Kristy Daniel, Dr. Kyna Shelley, and Dr. Sheila Hendry face-validated the assessment instruments.
- Dr. Sherry Herron, Dr. Angela Bruni, Dr. Mary Gilmore, Ms. Kathryn Morris, and Dr. Mary Makey each devoted a session of their classes for me to interview their students.

- Mr. Wassim Sidani prepared the static illustrations that appear in the pilot study, worksheet, and assessment instruments. Flash® animations used in the pilot study and those used in the multimedia presentation are all his work. The stickers on the interactive model are his as well.
- Ms. Amani Jamal transcribed all of the participants' responses in the pilot study. She also served as one of the two graders of Item 7 in the transfer test.
- Ms. Celia Young offered administrative and logistic support that was way beyond expectations.
- Dr. Anton Netchaev designed an early version of the electronic circuit and wrote a functional code for the alpha prototype. Mr. Michael McDonald built the beta prototype. Mr. Daniel Garcia taught me how to fix the circuit in the beta prototype.

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CHAPTER I

INTRODUCTION

Scientists' progress in understanding enzyme specificity uncovered a complex natural phenomenon. However, not all of the currently available biology textbooks seem to be up to date on this progress. A good example from biochemistry is the replacement of the old "lock and key" model of enzyme specificity (Fischer, 1894) by the "induced fit" model (Koshland, 1958). However, a quick search on the concept of enzyme specificity in some of the widely used high school biology textbooks revealed inconsistent representations of our current understanding of how enzymes work. Out of the twelve examined textbooks[1], 83.3% failed to simultaneously cover the basic elements of the concept, (1) conformational change and (2) chemical interaction between the substrate and catalytic amino acids, Figure 1.

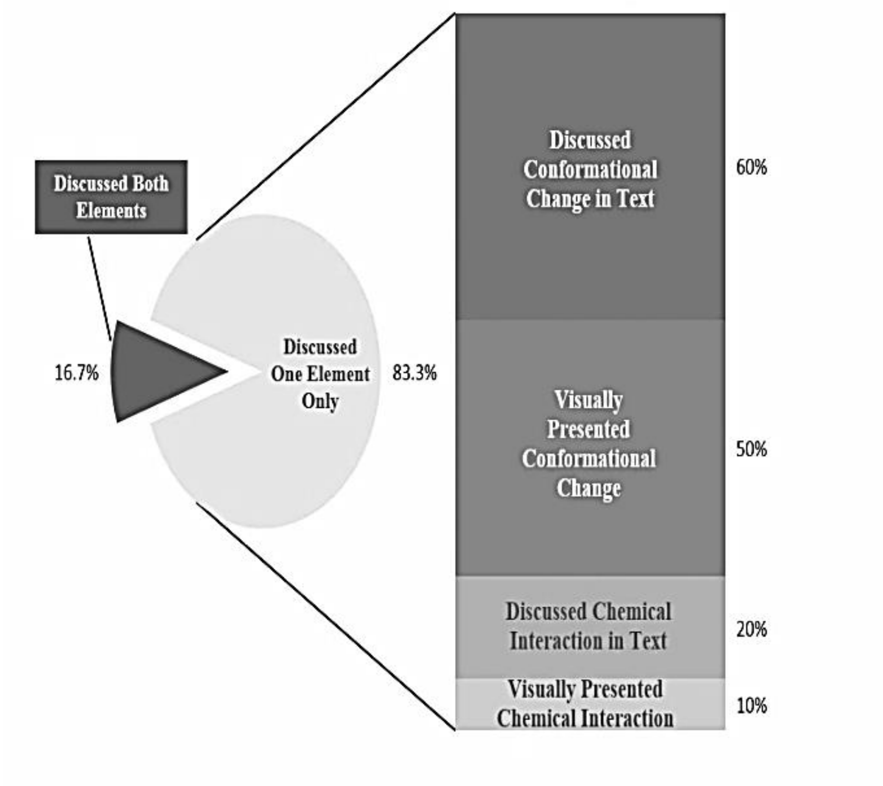

Figure 1. Bar of a pie chart showing inconsistency in concept representation.

[1]See the list of checked textbooks in Appendix A.

Particularly, only 60% covered the role of conformational change in text while 50% visually represented it. Also, a mere 20% of sampled textbooks touched on the chemical basis of the phenomenon while 10% visually represented the chemical interaction. This is not to mention the complete absence of other concept elements like binding affinity and enzyme reactivity which may be argued to be only relevant to upper college level education.

Having known this, it is not surprising that only one of the eight senior college students interviewed in a pilot study seemed to have heard about the “induced fit” model in previous biology courses (Saleh, Halverson, & Gearity, 2013; see Appendix C). Mind that this concept is directly related to understanding other important areas in science, such as how some pharmaceutical drugs work on certain life-threatening diseases and the role of some mutations in causing inherited metabolic disorders, let alone comprehending other biological mechanisms at the molecular level (e.g. ligand-receptor specificity).

Nevertheless, addressing this problem is a twofold challenge. First, the old concept is easy to comprehend partly because it is represented through a very familiar example, “lock and key.” On the other hand, the new concept describes enzyme specificity as a complex process with various interacting parts that cannot be learned in isolation (Pollock, Chandler, & Sweller, 2002). As a consequence, such complex material may impose high cognitive load on the learner’s working memory (Marcus, Cooper, & Sweller, 1996; Sweller & Chandler, 1994).

Second, for the “lock and key” model, a visual aid ─if any─ has to be static at most for students to grasp the idea of “how enzymes work.” However, the current model defines enzymes as dynamic entities. This entails another source of cognitive load necessary to mentally visualize the appropriate model (Schönborn & Anderson, 2006).

For the purpose of meeting the first challenge, literature that deals with learning complex material was critically reviewed. Accordingly, the presented concept was first segmented into sets of experiments to ensure a guided inquiry approach that is known to save the learner from going through fruitless solution procedures (Kirschner, Sweller, & Clark, 2006). A slightly modified version of multimedia pre-training was then implemented following an experimental approach (Mayer, Mautone, & Mathias, 2002). Description and reason for this modification will be described later in this chapter. Multimedia pre-training fuels learner's knowledge with discrete facts necessary to understand targeted conceptual knowledge. It was particularly selected because current pre-college science education does not provide students with enough biochemical background that qualifies them to understand complex material such as enzyme specificity (NGSS, 2013).

To meet the second challenge, an interactive physical model was built along with several pieces to represent the enzyme and its substrates, see Figure 10. This model, along with the worksheet, stood as an instructional kit meant to further help students understand the concept with minimum cognitive load (Vekiri, 2002), and be motivated to learn about it (Mayer, 2009).

Statement of the Problem

It might be challenging to mass communicate with textbook publishers referring to the inconsistency in representing this concept and its potential influence on the progress of tertiary biochemical education; not to judge the biochemical foundation offered in secondary education. Alternatively, implementation of effective instructional techniques, such as multimedia pre-training, may prove invaluable tools to fill in such educational gaps. In the original version of multimedia pre-training principle, instruction is split into two stages (Mayer, Mathias, & Wetzell, 2002). In the first stage, the learner constructs a set of models corresponding to the major components of the studied system (component models). In the second stage, the learner builds a causal model based on the set of constructed component models. A causal model is basically a cause-and-effect chain of events where a change in the state of one component causes a “principle-based” change in the state of another component and so on. Most of the known pre-training studies have shown significant differences between treated and control groups with high effect sizes (Clarke, Ayres, & Sweller, 2005; Kester, Kirschner, & van Merriënboer, 2004; Kester,Kirschner, & van Merriënboer,2006; Mayer et al., 2002; Mayer, Mautone, & Prothero, 2002; Pollock et al., 2002). These desirable effects were true for learners with, (1) low domain-specific knowledge studying (2) complex material. The two boundary conditions should not pose a problem, in this regard, when trying to teach high school graduates (low prior knowledge) biochemical concepts such as substrate specificity of enzymes[2] (complex material). However, no known research in biochemical education is available in this regard.

Additionally, there seem to be some other limitations to pre-training that put off its effectiveness in certain cases (Ayres, 2006; Plass, Chun, Mayer, & Leutner, 2003). In Plass et al.’s (2003) study for example, pre-training was provided in a second language multimedia lesson in the form of annotations of key terms. Results related to text comprehension showed that only some students benefitted from pre-training. Another example comes from Ayres’ (2006) study in which students were provided with pre-training prior to learning a mathematical domain. In the pre-

[2]There are four distinct types of enzyme specificity: absolute (aka substrate), group, linkage, and stereochemical specificity. In this study, only substrate specificity is explained.

training session, students progressed from part-tasks (isolated elements) to whole-tasks (simple-to-complex). Surprisingly, this approach proved to be ineffective regardless of students' prior knowledge. On the other hand, Musallam (2010) used a very similar approach to Ayres' (2006) and reported opposite results. In Mussallam's study (2010), both techniques (part-task & whole-task) were used in the pre-training episode prior to teaching a lesson on chemical equilibrium. This treatment resulted in lower ratings of mental effort accompanied with high transfer performance. Considering results of these three studies from different domains of knowledge (Language, Math, & Chemistry), and knowing that pre-training is meant to meet the complexity of the presented material, brings the discussion that complexity may arise from factors other than element interactivity; which has repeatedly been reported to constitute complex material (Sweller & Chandler, 1994). Therefore, besides reducing element interactivity[3], the decision to be made for how to introduce pre-training prior to teaching biochemical concepts (e.g. substrate specificity) must take into account the nature of the presented material. For instance, all of the known science-related multimedia pre-training experiments were based on lessons involving mechanical systems (Mayer, Mathias, & Wetzell; 2002) or geological features (Mayer, Mautone, & Prothero; 2002). Substrate specificity does share these systems/features some of their aspects in a sense that all involve mechanical/kinetic parameters as well as the need for visual aids. However, overlooking chemical processes driving the kinetics in substrate specificity is an oversimplification of the phenomenon. Also, disregarding the learner's need to mentally shift between levels of visual representations of these processes might pose another problem (Shönborn & Bögeholz, 2013). In substrate specificity, the learner has to visualize available functional groups in the active site of

[3]The concept of element interactivity is discussed in details in Chapter II

the enzyme (one level of representation) as well as imagine possible chemical interactions among these functional groups (another level of representation) to conclude the kinetics of the process. Therefore, a modified version of multimedia pre-training is proposed in this study where underlying processes of the presented phenomenon and their associated terms are explained and visually presented.Associated terms are included because studying these processes obviously requires applying knowledge of their related terms (Shönborn & Bögeholz, 2009).

Purpose of the Study

This research has three objectives. First, it investigates whether the proposed version of multimedia pre-training helps college students better understand the concept of substrate specificity of enzymes, reflected by improved transfer performance. Second, it examines the influence of the instructional kit on motivating students to learn about this concept. Third, it aims at explaining the process through which both treatments, pre-training and the instructional kit, affect/influence transfer performance through manipulation of prior factual knowledge and/or cognitive load.

The overarching hypothesis in this study is formulated as such, "if the pre-training group was able to build component models and attend to the underlying processes and terms before engaging in building a causal model for how substrate specificity works, then they will outperform the no pre-training group in a transfer test."

Because cognitive load and motivation are theorized to play significant roles in multimedia learning (Mayer, 2011), corresponding variables were also measured and tested as putative mediators of the process at work. These variables included intrinsic and germane cognitive load as well as motivation to learn the presented material through the instructional kit. Particularly, this research aims at answering the following questions:

1. What is the overall difference, if any, in retention and transfer performance among the three groups (pre-training, no pre-training, and post-training)?

2. What is the difference, if any, in transfer performance among the three groups based on the cognitive dimension of revised Bloom's taxonomy?

3. What is the difference, if any, in extraneous, intrinsic, and germane cognitive loads among the three groups?

4. What is the motivational level of students to learn about substrate specificity through the instructional kit?

5. Does intrinsic cognitive load mediate the effect of pre-training on transfer performance?

6. Does prior factual knowledge mediate the effect of pre-training on transfer performance?

7. Does intrinsic cognitive load mediate the effect of pre-training on transfer performance in parallel with prior factual knowledge?

8. Does prior factual knowledge, intrinsic, and germane cognitive loads mediate the effect of pre-training on transfer performance in series?

9. Does germane cognitive load mediate the influence of motivation on transfer performance?

Implications of the Study

Theoretical Implications. Multimedia instructional principles are still under development (Mayer, 2010a). In a relatively recent publication (Mayer, 2011), additional principles that did not appear in earlier review books were discussed (Mayer, 2001, 2009). The boundary conditions of each principle (when and for whom the principle works) are also a recent addition to multimedia learning (Mayer, 2009). Therefore, the proposed study on multimedia pre-training principle is expected to be welcome and appreciated as “more work is needed on how best to create effective pre-training experiences” (Mayer, 2009, p. 199).

Literature on multimedia learning is replete with studies showing how multimedia instructional principles foster knowledge transfer through cognitive load manipulation. It also includes massive reports on motivation and achievement (McGill, 2012). Very few studies, though, suggest theoretical models for how cognitive load and motivation combined have their impact on learning from multimedia (Moreno & Mayer, 2007). Additionally, no known research provides empirical evidence on how the interaction of both relates to knowledge transfer (Mayer, 2011). Bridging the gap between these two heaps in literature might provide insights for advancing instructional design. The mediation model[4] proposed in this study might constitute a precursor of the bridge that would link the two subfields. A review of this study and the like would then support/refine this current model which would eventually extend some multimedia learning theories, such as the influential cognitive theory of multimedia learning (Mayer, 2002).

Practical Implications. Science learners should be the first to benefit from enhancing multimedia instructional design as multimedia learning is known to play multiple roles in science education (Chiu & Wu, 2009).The introduced scientific concept describes an important phenomenon about enzymes which represent the core of biochemical education at the high school and college levels. The prepared instructional kit can be used as a supplemental instructional material to keep the science curriculum updated to the latest scientific discoveries (Johnson,

[4]A statistical term for a model explaining the process through which variable *X* (e.g. multimedia pre-training/instructional kit) affect variable *Y* (e.g. transfer performance) through some other variable(s) M_i(e.g. prior factual knowledge, cognitive load).

2008).Findings of this study could be quite applicable when explaining specificity in other contexts such as receptor-ligand interactions in signal transduction pathways and antigen-antibody interactions in humoral immune responses.

List of Definitions

The following terms are defined as they are used in this study.

Active site. “Only a certain region of the enzyme, called the active site, binds to the substrate. The active site is a groove or pocket formed by the folding pattern of the protein” (Mansur et al., 2015).

Amino acid. “Any of a group of organic molecules that consist of a basic amino group ($-NH_2$), an acidic carboxyl group ($-COOH$), and an organic *R* group (or side chain) that is unique to each amino acid” (Reddy, 2014).

Analyze. “break material into constituent parts and determine how parts are related to one another and to an overall structure or purpose” (Anderson et al., 2001, p. 31).

Catalyst. “Any substance that increases the rate of a reaction without itself being consumed” (Mansur et al., 2015).

Catalysis. “The modification of the rate of a chemical reaction” (Taylor, 2015).

Catalytic amino acids. Amino acids in the active site of an enzyme that are involved in catalysis.

Cellular respiration. “the process by which organisms combine oxygen with foodstuff molecules, diverting the chemical energy in these substances into life-sustaining processes and discarding, as waste products, carbon dioxide and water” (Mansur et al., 2015).

Conceptual knowledge. “is knowledge of more complex, organized knowledge forms. It includes knowledge of …theories, models, and structures” (Anderson et al., 2001, p. 27).

Create. “put elements together to form a coherent or functional whole; reorganize elements into a new pattern or structure” (Anderson et al., 2001, p. 31).

Enzyme. “A substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process” (Mansur et al., 2015).

Evaluate. “Make judgments based on criteria and standards” (Anderson et al., 2001, p. 31).

Explain. “occurs when a student is able to construct and use a cause-and-effect [causal] model of a system” (Anderson et al., 2001, p. 75).

Extraneous (extrinsic) cognitive load. Extraneous load is caused by the instructional design and the conditions of the learning environment (Ayres, 2006).

Factual Knowledge. “is knowledge of discrete, isolated content elements” (Anderson et al., 2001, p. 27).

Germane (generative) cognitive load. Germane load is the cognitive load required for “schema formation and automation” (Ayres, 2006, p. 287).

Glycolysis. “Sequence of 10 chemical reactions taking place in most cells that breaks down glucose, releasing energy that is then captured and stored in ATP” (Mansur et al., 2015).

Intrinsic (essential) cognitive load. Intrinsic load originates from the inherent nature of the presented material (van Merriënboer & Sweller, 2005).

Meaningful learning. “Meaningful learning occurs when students build the knowledge and cognitive processes needed for successful problem solving” (Mayer, 2002, p. 227).

Multimedia learning. Multimedia learning refers to learning from presented words and pictures (Mayer, 2009).

Photosynthesis. “the process by which green plants and certain other organisms transform light energy into chemical energy” (Lambers, 2015).

Remember. “retrieve relevant knowledge from long-term memory.” (Anderson et al., 2001, p. 31)

Rote learning. Rote learning occurs when the learner can recall almost all of the facts presented in a lesson but cannot use them to solve a problem (Mayer, 2002).

Schemas. Schemas are cognitive constructs that organize information elements (Pollock et al., 2002).

Substrate. “the substance (substrate) upon which an enzyme acts to form a product” (Mansur et al., 2015).

Understand: “construct meaning from instructional messages” (Anderson et al., 2001, p. 31).

Delimitations

In this section, study boundaries are set through explaining reasons for why certain theories, conceptual frameworks, participants, and methodological procedures were not employed although they might have been applicable.

Recall that, in the pre-training episode, terms related to the presented underlying processes were also explained. This approach may be viewed as an application of the conceptual framework proposed by Shönborn and Bögeholz (2013) who identified four hierarchical levels of biological knowledge; biological terms, biological concepts, underlying biological principles, and biological fundamentals. However, this framework was not adopted as its horizontal and vertical translations[5] exceed the scope of this study. Mind that the only translations involved here are those between terms and processes. As for visual representations, only two (symbolic & submicro) of the four levels (symbolic, submicro, micro, & macro) in biological education are utilized[6] (Tsui & Treagust, 2013). Consequently, the proposed cube model by Tsui and Treagust (2013) for learning biology with multiple external representations was also not employed. Nevertheless, consideration of excluded levels, whether organizational or representational, in future research might be quite interesting especially that earlier examination of Shönborn's and Bögeholz's (2013) framework did not involve interdisciplinary domains of knowledge such as biochemistry.

One of the three types of cognitive load measured in this study is germane cognitive load, which is caused by learner's motivation to make sense of the presented material (Mayer, 2009). In multimedia learning, learner's motivation is often boosted through using a human voice, rather than a machine voice, and/or a conversational style, rather than a formal style (Mayer, 2009). This type of motivation is meant to grasp the learner's attention and is based on the social agency theory (Atkinson, Mayer, & Merill, 2005). Accordingly, phrases such as, "this chemical reaction takes place in *our* body" were used during the pre-training episode. Additionally, a human-to-human communication, where conversation was a de facto of the guided inquiry approach, was maintained in the main lesson. However, an actual score for motivation was needed to meet some of the study objectives. Accordingly, the

[5]Vertical translation involves moving back and forth between the four levels of biological organization. Horizontal translation however, involves moving from one representation to another at the same level of biological organization (Shonborn & Bögeholz, 2013).

[6] Description of visual representations is detailed in chapter III.

instructional material motivation survey IMMS was utilized. IMMS is based on Keller's ARCS model of motivation, which counts for attention, relevance, confidence, and satisfaction with the instructional material (Keller, 2010). Therefore, instead of *assuming* increased attention provided by the social agency theory, IMMS scale provides a *score* for attention, perceived relevance, confidence to interact with, and satisfaction with the instructional material.

A substantial portion of participants in multimedia learning research are non-science students who are asked to learn about scientific topics beyond their area of expertise/interest (see de Jong, 2010). This limitation raises concerns about the external validity of research outcomes, i.e. generalizing inferences to include students of science learners. Therefore, only college students pursuing careers in science or majors that require at least a general biology course were included in this study. This course was chosen, in particular, as a prerequisite because understanding the presented concept, substrate specificity of enzymes, required some basic knowledge of biology.

Another type of cognitive load measured in this study is extraneous cognitive load, which results from poor instructional design and conditions of the learning environment (Ayres, 2006). This construct was measured through subjective difficulty rating of the learning environment and format of the learning material (Cierniak, Sheiter, & Gerjets, 2009). Extraneous cognitive load can rather be objectively measured through asking the learner to respond to a secondary task, by pressing on the space bar, so as to record their response time (DeLeeuw & Mayer, 2008). Learners experiencing higher extraneous load are assumed to take longer time to respond (in milliseconds). However, the problem with this measurement is that it requires devoting unnecessary cognitive resources to the secondary task (e.g. color change in the screen) while attending to the primary task, i.e. learning the material. It is for this reason that the secondary task approach was not employed. To conciliate for the inherent subjectivity in self rating, extraneous cognitive load was measured four times throughout the experiment.

Limitations

The results of this study are limited to college students enrolled in either of two southern universities or a southern community college. Participants were, on average, novice learners in the presented domain of knowledge. Therefore, study inferences do not necessarily apply to learners of high prior knowledge. Matter of fact, opposite results may be obtained in terms of certain research outcomes (Kalyuga, 2007). Descriptive statistics of study participants revealed prevalence of certain gender and ethnicity. These limitations may entail replicating the study with different demographic distributions, especially that differential learning outcomes were detected based on gender and race.

CHAPTER II

LITERATURE REVIEW

Introduction

This review considers several instructional principles meant to facilitate learning complex material and highlights an untouched issue associated with one of these principles, the multimedia pre-training principle, especially when it comes to learning biochemical concepts such as the current model of substrate specificity of enzymes (Johnson, 2008). Cognitive Theory of Multimedia Learning CTML and Cognitive Load Theory CLT are critically reviewed because of their shared triarchic model of cognitive load which plays a significant role in learning complex material (de Jong, 2010). A detailed analysis on the nature, source(s), and corresponding instructional principles for each type of the three cognitive loads (extraneous, intrinsic, and germane) is also carried out.

The driving assumption in instructional principles meant for learning complex material is that its constituting elements cannot be learned in isolation because they naturally interact (Pollock et al., 2002). For instance, one can separately learn what is a conformational change, a catalytic amino acid, or study the chemical structure of a certain substrate. However, to understand substrate specificity, they need to simultaneously learn that binding of a substrate, with a specific chemical structure, to its enzyme drives the latter to undergo the proper conformational change necessary to align the various catalytic amino acid residues along with this substrate. In other words, they need to simultaneously consider the various elements and their possible interactions. As a consequence, such material imposes high cognitive demands on the learner's working memory (Marcus, Cooper, & Sweller, 1996; Sweller & Chandler, 1994).

Besides being highly interactive, concepts such as substrate specificity necessitate the use for visual tools to help learners construct targeted mental models (Schönborn & Anderson, 2006). Mind that substrate specificity involves enzymes which are submicroscopic and dynamic entities. It also involves considering forms of interaction between a substrate and the enzyme's catalytic amino acids, which are often represented by conventional symbols. This means that, to understand this concept, the learner has to mentally shift between levels of representations (submicro and symbolic) that can also be cognitively demanding (Bayrhuber, Hauber, & Kull, 2010). Therefore, explicit visual representation of these levels during instruction might be effective (Shönborn & Bögeholz, 2013).

However, Mayer (1997) stresses that these visual aids are of no assistance in the absence of well-designed instructional principles which is a central dogma of the cognitive theory of multimedia learning. Given that one aim of this work is to improve college students' understanding of substrate specificity, a modified version of a multimedia instructional principle, pre-training principle, is proposed to meet the complexity of this concept and the like.

A Critical Review of the Cognitive Theory of Multimedia Learning and Cognitive Load Theory

Cognitive Theory of Multimedia Learning

Multimedia learning refers to learning from presented words and pictures (Mayer, 2009). The cognitive theory of multimedia learning CTML explains how people construct mental models from these words and pictures. According to CTML, the learner actively attempts to make sense of the presented material and ultimately constructs new knowledge after integrating it with prior knowledge (Mayer, Moreno, Boire, & Vagge, 1999). In this sense, CTML has a constructivist orientation to learning. However, most of CTML's foundation draws from mainstream cognitive theories like the model of working memory (Baddeley & Hitch, 1974), dual-coding theory (Paivio, 1986), and cognitive load theory (Sweller, 1988). Therefore, a cognitive-constructivist orientation better describes CTML.

The current version of CTML (Mayer, 2010a) is based on three elements/assumptions: (1) humans engage in active cognitive processes to make sense of the presented material (active-processing), (2) they possess separate channels for processing visual and auditory information (dual-channel), and (3) they can process a limited amount of information in each channel at one time (limited-capacity).

The active-processing assumption, originally taken from Wittrock's generative theory (1974), states that the learner constructs knowledge through actively selecting relevant information, building internal and external connections, and integrating new knowledge with existing knowledge (Lewis & Mayer, 1987; Mayer, 1982, 1984, 1985).

The dual-channel assumption states that people possess separate channels for processing visual and auditory information (Mayer & Anderson, 1991, 1992). This is consistent with Paivio's (1986) dual-coding theory that distinguishes between the cognitive verbal and non-verbal coding systems. It is also consistent with Baddeley's original model (1986) of working memory which proposes two subcomponents: the visuo-spatial sketchpad that maintains and manipulates visual images and the phonological loop which stores and rehearses verbal information.

Combining these two assumptions (active-processing and dual-channel) extends one piece of the first assumption (building internal and external connections). It does so through introducing the thesis that humans construct two major types of connections in multimedia situations: (1) verbal and visual *representational* connections, previously called internal connections (Mayer, 1985), and (2) *referential* connections between verbal and visual representations, formerly called external connections (Mayer, 1985).

This extension of the theory is of ample importance as it has both theoretical and practical implications. Practically, Mayer and Anderson (1991, 1992) found that learner's performance on retention tests depends on their constructed *representational* connections while their performance on problem-solving tests depends on their constructed *referential* connections. Theoretically, this extension emphasizes the role of the limitation of working memory (WM) when meaningful learning, rather than rote learning, is the goal of instruction. The relationship between these connections and the limitation of WM may be demonstrated by the results of a study by Kirschner, Paas, and Kirschner (2009). In this study, a favorable relationship between transfer test performance and mental effort was found for collaborating group learners indicating construction of *referential* connections. A favorable relationship was also found between retention test performance and mental effort for individual learners (Kirschner et al., 2009) indicating mere construction of *representational* connections. In terms of WM capacity, group learners in this study were apparently able to make use of each other's processing capacities through sharing individually processed information elements with each other. Individual learners however, relying on their own WM capacity, would only focus on remembering information elements instead of relating them to each other so as to construct the *referential* connections made by group learners.

The limited-capacity assumption can be explained in the context of Wittrock's generative theory (Mayer, Steinhoff, Bower, & Mars, 1995), Paivio's dual coding theory (Mayer & Sims, 1994), Baddeley's model (1986) of working memory, or probably best in the context of Sweller's (1988) cognitive load theory (DeLeeuw & Mayer, 2008; Mayer, 1997, 2001, 2002b, 2003, 2005, 2009, 2010b, 2011; Mayer, Bove, Bryman, Mars, & Tapangco,1996; Mayer et al., 1999; Mayer, Heiser, & Lonn, 2001; Mayer & Moreno, 2002, 2003). Matter of fact, the limited-capacity assumption is one of the four interrelated assumptions of CLT (Pollock et al., 2002). These assumptions are described below based on the observation that current literature about learning complex material heavily relies on them. Additionally, the proposed modification of CTML's pre-training instructional principle partially rests on these same assumptions.

Cognitive Load Theory

CLT states that: (1) humans possess a limited WM and a (2) capacious long-term memory; (3) *schemas* (cognitive constructs that organize information elements) held in long-term memory are used to structure knowledge in a way that requires less WM; and (4) automation allows schemas to be processed automatically rather than consciously in WM, thus reducing WM load (Pollock, Chandler, & Sweller, 2002). Consequently, with expertise, even highly interacting elements of information can be dealt with as *one* element in WM (van Merriënboer & Sweller, 2005).

CLT researchers distinguish between three sources of WM loads: extraneous, intrinsic, and germane cognitive loads (Sweller, van Merriënboer, & Paas, 1998; Paas, Tuovinen, Tabbers, & van Gerven, 2003). Extraneous cognitive load is caused by instructional design and conditions of the learning environment (Ayres, 2006). Intrinsic cognitive load in turn originates from the inherent nature of the presented material (van Merriënboer & Sweller, 2005). Germane cognitive load is required for schema formation and automation (Ayres, 2006).

These three types of cognitive load do echo in CTML literature. DeLeeuw and Mayer (2008) showed that there are three measurable types of WM load: extraneous (*or extrinsic*), intrinsic (*or essential*), and germane (*or generative*) loads. These cognitive loads currently represent the core of CTML as Mayer (2009) considers them the “organizing framework” of the theory. Although CTML apparently reverberates CLT in this regard, CTML still holds a better fit to deal with learning abstract concepts with dynamic elements such as enzyme specificity because it explicitly accounts for cognitive processes related to visual information. For instance, CTML incorporates into its model processes like selecting visual information, organizing images into pictorial models, and integrating pictorial models with prior knowledge along with verbal models (Mayer & Moreno, 2003).

In summary, CTML is based on three assumptions (Mayer et al., 1996): active-processing, dual-channel, and limited-capacity assumptions. These assumptions are the basis of five cognitive processes that are assumed to take place during multimedia learning: selecting words, selecting pictures, organizing words, organizing images, and integrating new knowledge with prior knowledge. These processes in turn result in three kinds of cognitive load: extraneous, intrinsic, and germane loads. DeLeeuw and Mayer (2008) place these loads in a triarchic model of cognitive load. This model states that there are three possible threats to the five cognitive processes: too much extraneous load that may hinder all of the five processes; too much intrinsic load that may hinder “selecting and organizing” verbal and pictorial information; and too little germane load that may hinder “organizing and integrating” selected verbal/pictorial information (Mayer, 2011). Hence, the ultimate goal of instructional principles/strategies described in the next section is to identify the sources of each type of load and eventually reduce unnecessary extraneous load, manage intrinsic load, and/or foster germane load.

The Three Types of Cognitive Load

As stated earlier, the triarchic model of cognitive load is a common framework in both CTML and CLT literature (DeLeeuw & Mayer, 2008, Sweller et al., 1998). This is one reason why researchers from both fields thoroughly cite each other. Nevertheless, there are important differences between the two fields that make merging them a catalyst for future research. First, all CTML's work is based on learning with words and pictures while CLT discusses learning with visuals only in two of its seven themes (Ayres & van Gog, 2009). Second, CLT essentially runs its experiments on different content areas than CTML; see (Mayer, 2009) for a comprehensive review. Third, the majority of CTML's results are based on very short lab-based learning sessions (de Jong, 2010). Therefore, a critical review of both literatures is necessary if effective instructional principles for a wide range of content areas are to be discovered in real learning conditions rather than within a laboratory environment.

Extraneous Cognitive Load

The majority of instructional principles/strategies in CTML and CLT focus on reducing extraneous cognitive load (Atkinson, Derry, Renkl & Wortham, 2000; Ayres, 1993, 2006; Ayres & Sweller, 2005; Cerpa, Chandler, & Sweller, 1996; Chandler & Sweller, 1992; Kester, Kirschner, van Merriënboer,& Baumer,2001; Kester et al., 2004; Lowe, 1999; Mayer, 2009; Paas &van Merriënboer, 1994; Renkl, Stark, Gruber, & Mandl, 1998; Renkl, Hilbert, & Schworm, 2009; Rourke & Sweller, 2009; Sweller, 1993, 1989; Sweller & Chandler, 1991; van Merriënboer, Kirschner, & Kester, 2003; Wirth, Künsting, & Leutner, 2009). What made this type of cognitive load a focal point of research is the general consensus that extraneous load does not contribute to learning and that it can be avoided by proper instructional design (van Merriënboer & Sweller, 2005).

Various sources of extraneous load are pointed out in literature. One thoroughly studied source is the split-attention effect which refers to separately presenting corresponding elements of information that need to be mentally integrated (Cerpa et al., 1996; Mayer, 2009). In this case, the learner has to use cognitive resources to search for one element while holding the other in their working memory. Two types of split-attention may occur in multimedia learning as a result of spatially and/or temporally splitting elements. In a spatial split, related elements (e.g. words and their corresponding visuals) are presented far from each other on the same page/screen (Mayer, 2009) or even on multiple delivery systems (e.g. a paper manual and a computer screen) (Cerpa et al., 1996). In a temporal split, related elements are simply separated in time (Mayer, 2009; Ayres & Sweller, 2005; Lowe, 1999). Both CTML and CLT researchers call for integration/contiguity instructional principles based on empirical research (see a comprehensive list of experiments on Appendix B in Dacosta, 2008).

An extension of the split-attention effect is “redundancy” in the presented material. Examples of redundancy include providing the learner with the same material, printed in different media (Cerpa et al., 1996) or through pictures, narration, and printed text (Mayer, 2009). In the first example, extraneous load can be pertained to the same reasons leading to split-attention effect. In the second example, extraneous load is rooted to two unnecessary processes (Mayer, 2009): the learner has to scan between printed words and visuals thus overloading the visual channel as well as spend mental effort trying to match printed and spoken words (see a comprehensive list of experiments on Appendix B in Dacosta, 2008).

So far, discussed sources of extraneous load relate to the nature of the presented material itself, i.e. being split or redundant. However, sometimes extraneous load results from: (1) the characteristics of the learner (given a baseline of complexity in the material), or (2) an interaction between the nature of the presented material and the characteristics of the learner. An example of the first case is when learners with no domain-specific schemas are asked to solve conventional problems (Sweller, 1993). Extraneous load originates here from the fact that such learners may need to go through several fruitless solution procedures. CLT researchers advocated a number of strategies to counter this type of load through asking the learner to solve goal-free problems or start with worked examples before they move on to solving incomplete problems and finally work on conventional problems (Atkinson et al., 2000; Ayres, 1993; van Merriënboer et al., 2003; Sweller, 1993). Two examples of the second case (interaction) can be found in CTML literature. Extraneous load may result when the presented material is incoherent (contains interesting but irrelevant words and pictures) and the learner has low domain knowledge or low working-memory capacity (see coherence principle in Appendix B in Dacosta, 2008; Mayer, 2009). Another example of the second case is when the learner has low reading skills and the lesson is disorganized (see Appendix B in Dacosta, 2008; Mayer, 2009). In this case, Mayer (2009) recommends applying the signalling principle which theoretically aids the learner to build connections between key elements of the multimedia lesson (see discussion about referential connections above).

It is worth mentioning that the abovementioned sources of extraneous load and their corresponding instructional principles function under boundary conditions defined by the current theoretical understanding of how we process information (Mayer, 2009). Considering these principles as universal non-interacting rules may lead to viewing the results of several experiments as conceptual threats to CTML when this is not the case (Kalyuga, Chandler, & Sweller, 2004; Mayer & Johnson, 2008; Moreno & Mayer, 2002). For instance, Mayer and Johnson (2008) reported better retention performance if redundant short words, along with identical narrated words, were placed near related diagrams. Although this result may seem contradictory to the redundancy principle, it still applies to the overarching theory. Mind that the added text was shortened (see coherence principle), put next to related diagrams (see spatial contiguity principle), and represented only the key elements of the lesson (see signalling principle). Therefore, given that three principles of extraneous load were applied, the redundancy effect was diluted as the *overall* extraneous cognitive load was reduced allowing for further processing.

Germane Cognitive Load

Germane load results from cognitive processing that is aimed at making sense of the presented material. More specifically, it corresponds to organizing selected material from the instructional message and integrating it with prior knowledge so as to construct new schemata (Mayer, 2009, 2011; Sweller et al., 1998; van Merriënboer et al., 2006).

For CTML, this type of load is dependent on the learner's level of motivation. Put another way, if the learner lacks motivation then they may not engage in germane load even if they still have WM capacity available after engaging in extraneous and intrinsic load (Mayer, 2009, 2011). This is why instructional strategies aimed at reducing extraneous and intrinsic load should be tied to strategies that foster germane cognitive load (van Merriënboer et al., 2006).

Three effective instructional principles appear in CTML literature: personalization, voice (Mayer, 2009, 2010), and multimedia principles (Mayer, 2009). The personalization principle calls for presenting words in a conversational style rather than a formal one. The voice principle calls for using a human voice instead of a machine voice (in case of e-Learning for example). The theoretical rationale behind these two principles is that learners are likely to perceive the instructor as a conversational or social partner, and therefore, would be motivated to cognitively engage in the learning session (Mayer, 2009, p. 242) (see Dacosta (2008) for a comprehensive list of experiments on both principles). The multimedia principle states that people (especially novice learners) learn better when the material is presented in words and pictures rather than in words alone (Mayer, 2009, p.223). The theoretical rationale of this principle is that learners processing a multimedia message are more likely to build verbal and pictorial representations as well as referential connections between these representations. Although this explanation is concordant with CTML foundation, it is not clear how the multimedia principle fosters germane load from a “motivational” point of view. Add that not all pictures are of the same pedagogical quality (Vekiri, 2002), different pictures belong to different categories that serve distinct objectives (Levin & Mayer, 1993), and multiple representations of the same concept serve different pedagogical functions (Ainsworth, 1999), let alone the role of prior knowledge of the learner who is interacting with these representations (Seufert, 2003). Perhaps, this is why Mayer (2010) later dropped this approach from his list of instructional principles and processed it as a component of every other instructional principle of multimedia learning.

CLT researchers also offer a set of strategies to foster germane load (Atkinson & Renkl, 2007; de Croock, van Merriënboer, & Paas, 1998; Paas & van Gog, 2006;

Sweller et al., 1998; van Merrinboer & Sweller, 2005). Predominantly, their approaches are based on increasing variability in practice problems (de Croock et al., 1998; Sweller et al., 1998) or on self-explanation prompts while studying worked examples (Atkinson & Renkl, 2007).

CLT literature does not seem to have a clear identification of the source(s) of germane load (see review by Ayres & van Gog, 2009). Additionally, the nature of this type of load does not sound to be clearly identified in this field (de Jong, 2010). Kalyuga (2007, p.527) for instance, states that certain instructional techniques (e.g. self-explanation prompts) aimed at fostering germane load “could effectively become a form of extraneous load” if WM capacity is exceeded. This quote and the like (Paas et al., 2004, pp. 2-3) in CLT literature reset the question of what distinguishes germane load from the other two types of cognitive load (see de Jong, 2010 for an extensive discussion).

Until this question is answered, Mayer’s reference to the role of motivation in fostering germane cognitive load remains the best haven for further research in this regard. The personalization and voice principles come in agreement with the social agency theory; a motivation theory that advocates using social cues to keep the attention of the learner (Pintrich & Schunk, 2002). Kellers’ model of motivation (2010) may even be a better fit as it counts for leaner’s attention, perceived relevance, confidence, and satisfaction ARCS with the material used to deliver the instructional message.

Intrinsic Cognitive Load

Intrinsic cognitive load pertains to cognitive load that the learner experiences upon selecting information from the presented material and initially organizing it in their working memory (Mayer, 2011). This load becomes a burden on WM when the material is complex enough to hinder further processing of information (organizing and integrating it with prior knowledge). The outcome of instruction in this case would be rote learning, as indicated by exclusive high retention performance, rather than meaningful learning, as indicated by high transfer performance (Mayer, 2002). This scenario can best be demonstrated based on a study mentioned earlier (Kirschner et al., 2009) in which group learners were able to engage in deep processing reflected by high transfer efficiency while individual learners were only able to engage in initial processing reflected by high retention efficiency.

A central question here is: what defines a "complex" material? In CLT, a complex material has high element interactivity regardless of the number of isolated elements (Sweller, 1998; Sweller et al., 1994). In CTML terms, complex material is the "material that consists of many steps and underlying processes" (Mayer, 2009, p. 80). A literal analysis to this definition makes CTML sounds like it does count the number of isolated elements (number of steps) as adding to complexity. Nevertheless, all the introduced concepts in CTML lessons are presented as cause-and-effect chain of events (e.g. car braking system, air pumps, etc.). Hence, these steps are naturally interacting.

Aside from interactivity, Mayer suggests that intrinsic overload may result when the learner is unfamiliar with the complex material (Mayer, 2009, 2011). Again, this may open the discussion that unfamiliarity is a characteristic of the learner – not the material – and therefore cannot be considered as innate or *intrinsic*. Well, note that the entire concept of element interactivity is based on the extent of acquired schemas by a given learner and as mentioned earlier, highly interacting elements of information can be dealt with as one element upon schema acquisition and automation (van Merriënboer & Sweller, 2005). Therefore, the notion of element interactivity is a relative measure depending on the learner's background rather than an absolute one.

In any case, element interactivity does not seem to solely define what constitutes a complex material (de Jong, 2010). For instance, Klahr and Robinson (1981) reported that children experienced more difficulty solving nontraditional Tower of Hanoi problems although the required number of moves as well as the number of pegs and disks (interacting elements) is the same. This leads to the hypothesis that some domains of knowledge are complex regardless of the degree of interactivity. Take biochemistry and biology for example. Both disciplines are "hierarchically organized and nested domains of knowledge" (Treagust & Tsui, 2013, p. 10) where the learner has to move back and forth from the symbolic level (e.g. what is the mechanism of glycolysis) to, the submicro level (which enzymes and other molecules are involved), the micro level (where in the cell glycolysis is taking place), and the macro level (how does glycolysis relate to the entire energy profile of tissues within organs, systems, organisms, etc.) in order to understand this complex material; let alone the need for understanding the interrelationships within and between the nested systems (cellular respiration and photosynthesis) (Schwartz & Brown, 2013). Having this been said, it is obvious now that our understanding of what constitutes a complex material is still a growing body of knowledge. Nevertheless, and contrary to earlier beliefs that intrinsic load is unalterable as it is innate to the material (Sweller et al., 1998), educational researchers were lately able to offer several instructional principles/strategies that successfully managed this type of cognitive load. Below is a discussion of a representative sample.

Four types of sequencing techniques appear in CLT literature: whole-task, part-whole task, whole-part task, and part-task sequencing (Pollock et al., 2002; van

Merriënboer et al., 2003; van Merriënboer et al., 2006; van Merriënboer & Sweller, 2005). In the whole-task approach (novice) learners practice on the simplest form of the whole task and progress toward more complex forms of the same task (van Merriënboer et al., 2003). However, in part-whole task sequencing (one form of pre-training), students are exposed to slightly interacting elements before dealing with the whole interactivity at a time (van Merriënboer et al., 2006). Conversely, in whole-part task sequencing, students have to deal with the full complexity of the material right from the beginning but still have to be restricted to subsets of interacting elements (van Merriënboer et al., 2006). This latter approach is basically reducing extraneous load along with intrinsic load since the learner's attention is focused on elements that represent correct solution steps only. The fourth technique, part-task sequencing, breaks interacting elements into *isolated* ones followed by a full account of all the interacting elements (Pollock et al., 2002; van Merriënboer & Sweller, 2005).

Pollock et al.'s (2002) study on part-task sequencing (another form of pre-training) is quite enriching to the current discussion as it uses multimedia instruction (mainly a CTML area of interest) and analyzes its results in the context of CLT. In their experiment, the course included a two-phase multimedia lesson for two different groups of learners. One group (pre-training) received part-task sequencing with a focus on the components of an electrical system (isolated elements condition) followed by an explanation of how all the components interact in this system (interacting elements condition). The other group (no pre-training) however, focused on how all the components interact in both phases (interacting elements condition). Upon testing on how the components work together, the part-task sequencing group demonstrated higher performance than the other group. These results are multifaceted. First, they show empirical evidence against the hypothesis that part-task sequencing works better for tasks where internal components have a low degree of interdependency (Naylor & Brigs, 1963). It goes without saying that components of an electrical system function in a network with complete interdependency. Second, these results support the *cognitive load reduction* perspective through CLT's part-task sequencing strategy and CTML's pre-training principle as opposed to the *additional instruction* perspective (Mayer et al., 2002). Mind that the no pre-training group took the lesson twice and still scored worse! Third, these results bring attention to the possible effectiveness of part-task sequencing in helping the learner construct and automate simple schemas in a way that it would facilitate construction of the ultimate schemata (van Merriënboer at al., 2005). For example, if the learner receives pre-training on the functional role of the various catalytic amino acid residues (automation of isolated elements), then they are more likely to guess the possible outcomes of replacing one amino acid by another within the active site of an enzyme in terms of its substrate specificity (target schemata).

Another group of researchers suggested a unique approach to managing intrinsic load: molar versus modular problem solution procedures (Gerjets, Scheiter, & Catrambone, 2004; Gerjerts, Scheiter, & Catrambone, 2006). A "molar" view of problem-solving focuses on identifying problem categories and their associated overall solution procedures. In a "modular" view, complex solution procedures are broken down into smaller meaningful procedures. The modular view is inspired, but very different, from the part-whole task and whole-task sequencing techniques (see above). It is different from the former because it calls for solving the whole task right from the beginning. It is also different from the latter because it does not alter the difficulty of the subtasks. Rather, it uses the same example problems for both modular and molar solution procedures (Gerjets et al., 2004). This "modular" approach to complex material demonstrated superiority to almost all of the discussed instructional strategies in that learners with low and high prior knowledge benefitted from it (Gerjets et al., 2004). Nevertheless, Gerjets et al. (2004) acknowledge that this approach seems to be restricted to specific areas in learning mathematics (e.g. probability problems).

Three principles are recommended in CTML literature to manage intrinsic cognitive load (Dacosta, 2008; Mayer, 2009, 2011): segmenting, modality, and pre-training principles. Segmenting a lesson means breaking it down into sequential parts while keeping the pace under the learner's control. Theoretically, the learner would have enough time to intellectually assimilate each segment to the previous ones before moving on with the rest of the lesson (Mayer, 2009). In this regard, segmenting is comparable to modular worked examples in which complex solutions are broken down into smaller meaningful solution elements (Gerjets et al., 2004). The modality principle states that complex material is better presented in pictures and *spoken* words rather than pictures and *printed* words (Low & Sweller, 2005; Mayer, 2009; Mousavi, Low & Sweller, 1995). The fundamental concept behind this principle is the dual-channel assumption of CTML; i.e. upon replacing printed text by spoken one, the words are off-loaded from the visual channel onto the verbal channel allowing further processing of information. In the pre-training principle, instruction is split into two stages. In the first stage, the learner constructs a set of models corresponding to the major components of the studied system (component models). In a component model, a major part is perceived as a unit that holds a name and exists in defined states. In the second stage, the learner builds a causal model based on the set of constructed component models. A causal model is basically a cause-and-effect chain of events where a change in the state of one component causes a principle-based change in the state of another component and so on. By splitting the processes of building both models at the same time, the learner is likely to reserve some space in their working memory for generative processing (germane load).

Empirical evidence for the effectiveness of this form of pre-training comes from all of the known experiments conducted by Mayer and his colleagues (Mayer et al., 2002; Mayer et al., 2002; Pollock et al., 2002). Different versions of pre-training have also shown desirable effects (Clarke et al., 2005; Kester et al., 2004, 2006). In Clarke et al.'s (2005) study for example, pre-training was a practice session on the key features of a spreadsheet prior to a multimedia math lesson on functions. Kester et al.'s (2004) pre-training however was a list of definitions of key terms for a formula in a statistical technique. Although the majority of the known pre-training experiments show desirable learning outcomes, some concerns about this instructional technique still need to be resolved. These concerns are discussed in the Problem Statement section.

Theoretical Framework

The Triarchic Model of Cognitive Load: Three Scenarios of Multimedia Learning

The essence of multimedia instruction grounded in the triarchic model of cognitive load is meeting the limitations of the learner's cognitive capacity through reducing extraneous load, managing intrinsic load, and/or fostering germane load. The decision for which of the three actions to take, however, depends on the given scenario. For instance, if the learner got engaged in too much extraneous processing (extraneous overload) and probably a bit of intrinsic load which exceed their cognitive capacity, then they will not be able to engage in the amount of intrinsic and germane loads necessary to learn the presented material, see Figure 2 (Mayer, 2011). In this case, an appropriate action would be reducing or ultimately eliminating the sources of extraneous cognitive load.

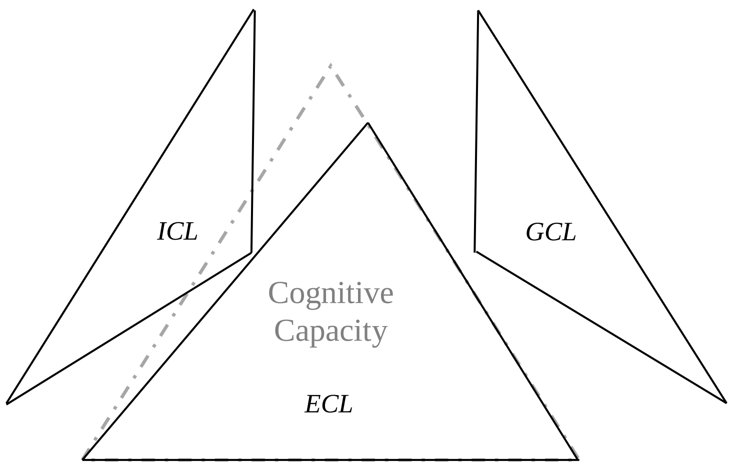

Figure 2. A conceptual diagram showing a learning scenario where too much extraneous cognitive load is hindering engagement in necessary intrinsic and germane cognitive loads. Key: ECL=extraneous cognitive load, ICL=intrinsic cognitive load, GCL=germane cognitive load.

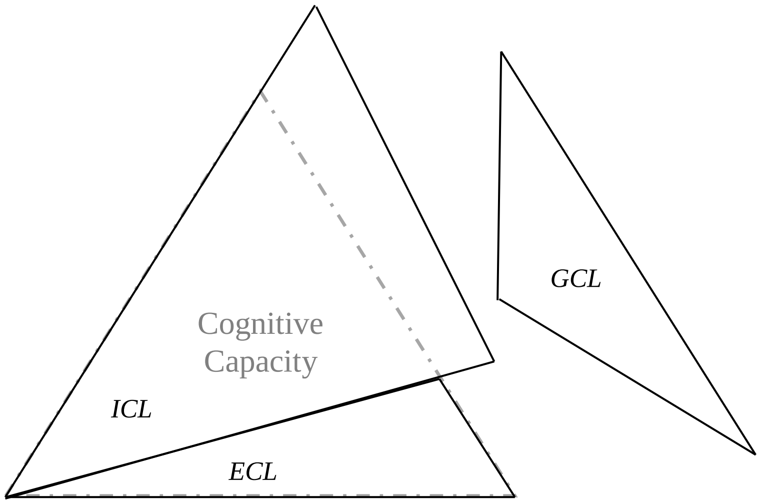

Figure 3. A conceptual diagram showing a learning scenario where too much intrinsic cognitive load is hindering engagement in necessary germane cognitive load. Key: ECL=extraneous cognitive load, ICL=intrinsic cognitive load, GCL=germane cognitive load.

Even after reducing extraneous load, intrinsic cognitive load required by some material might be too demanding that it exceeds the learner's cognitive capacity, see Figure 3 (Mayer, 2011). Consequently, the learner would not be able to employ the necessary amount of intrinsic load to learn the presented material; let alone engaging in germane cognitive load. In this scenario, a proper approach would be managing intrinsic load so as to minimize its burden on the learner's cognitive capacity and ultimately saving room for germane load.

The third scenario takes place when the learner has enough cognitive capacity even after engaging in extraneous and intrinsic loads but they are not motivated to efficiently utilize this remaining capacity, see Figure 4. In this case, rote learning takes place where the learner might remember the presented concepts without making sense of them (Mayer, 2011). The instructional objective in this case would be fostering germane cognitive load.

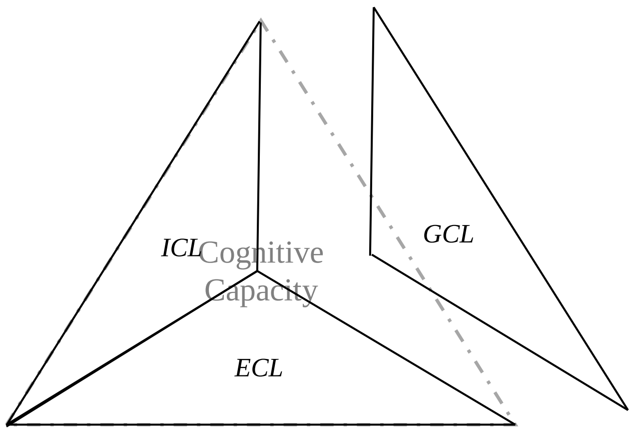

Figure 4. A conceptual diagram showing a learning scenario where too little germane cognitive load is taking place. Key: ECL=extraneous cognitive load, ICL=intrinsic cognitive load, GCL=germane cognitive load.

Assessing the Learning Outcome of Multimedia Instruction

Once limitations of the learner's cognitive capacity are met, the structure of the learner's knowledge (constructed schemata) should be analyzed to assess the effectiveness of the instructional approach. A common strategy to do so in CTML literature is to quantitatively compare the number of correct responses between experimental and control groups (see a comprehensive list of CTML experiments on Appendix B in Dacosta, 2008).Though being an accurate measure, this strategy does not provide specific guidance for how to *re*design instruction upon undesirable learning outcomes. Additionally, this assessment practice has dictated compromise in meta-analysis studies. In Mayer's review book (2009) for instance, effect sizes of treatments from studies grounded in CLT were compared to those of CTML regardless of the fact that many CLT studies focused on retention whereas CTML's aimed at fostering transfer (Mayer, 2009). Mind that retention and transfer are products of different types of cognitive processing. In particular, retention performance reflects whether the learner has engaged in intrinsic cognitive load while transfer performance mainly reflects engagement in germane cognitive load (Kirschner, Paas, & Kirschner, 2009).

Moreover, this common approach to assessment does not seem to have enough power to discern differences among versions of the same instructional principle. Take the pre-training principle for example. There is one known version of pre-training in CTML literature (Mayer, 2009) and four versions ―aka sequencing techniques― in CLT literature (van Merriënboer, Kirschner, & Kester, 2003). Comparison of effect sizes for experiments related to both theories, CLT and CTML, showed similar scores despite differences in theoretical rationales among these treatments (Mayer, 2009). However, deeper analysis of learners' responses based on a descriptive framework might prove one version of pre-training superior to another.

The framework provided in the cognitive dimension of revised Bloom's taxonomy has this descriptive power embedded in its six hierarchical cognitive orders

(Anderson et al., 2001). This framework does not only highlight engagement in intrinsic and/or germane loads. Rather, it demonstrates the depth of germane load the learner might have engaged in. This is reflected by the ability of the learner to answer a continuum of transfer Items ranging from simply "*Understanding*" a concept up to "*Creating*" a novel solution to a problem. Therefore, assessment based on this framework would provide higher resolution to compare different versions of the same instructional principle, *re*design instruction in light of cognitive order analysis, and ultimately contribute to meta-analysis with fewer compromises through comparison of retention-to-retention and transfer-to-transfer performances.

Pre-training as a Multimedia Instructional Technique

The choice of the pre-training principle as an instructional technique in this study partially originates from the traditional constructivist perspective that learner's prior knowledge is a major factor shaping the structure of constructed schemata (Ausubel, 1986). From this standpoint, a pre-training episode preceding the main lesson, where relevant discrete facts are presented, is assumed to increase learner's prior factual knowledge necessary to construct the desired schemata/mental models (Mayer et al., 2002).

From CLT's and CTML's cognitive constructivist perspective, constructing mental models in a domain such as modern biochemistry tends to be cognitively demanding given the complexity of this material. Recall that CLT researchers define complex material as one that consists of six or more interacting elements (Sweller & Chandler, 1994). The concept of substrate specificity comprises eight of these — assumed for a novice learner in the field. It states that: (1) Binding of a substrate with (2) a specific chemical structure (3) drives the enzyme to (4) undergo the (5) proper conformational change necessary to (6) align the (7) various catalytic amino acid residues (8) along with this substrate. These several steps along with their underlying processes such as binding affinity of the substrate to the enzyme and enzyme reactivity toward the substrate collectively define this concept as fairly complex by CTML researchers as well. CTML considers a lesson as “so complex” if it is “consisting of many steps and underlying processes” (Mayer, 2009, p. 80). Hence, the inherent complexity of this domain of knowledge is likely to put the learner in the second learning scenario described above. Consequently, CTML pre-training would be an effective instructional technique to foster transfer of this knowledge.

Original version of multimedia pre-training. From CTML’s standpoint, the learner separately constructs models of major components in the system during the pre-training episode. To build a component model, the learner is introduced to the names of major components and the various states that each of these components can be in (Mayer, Mathias, & Wetzel, 2002). In the process of substrate specificity, the major components are the substrates (e.g. glucose and ATP which can be phosphorylated or dephosphorylated), the enzymes’ reactive catalytic amino acid(s) (e.g. aspartate which can be charged or uncharged), and stabilizing catalytic amino acids (which can be interacting with the transition state or not).

In the main lesson, the learner constructs a causal model out of the built component models. With the causal model constructed, the learner can relate the state change of a component to that of another in a principled cause-and-effect sequence of events (Mayer et al., 2002). In substrate specificity, the causal model runs in this sequence: binding of proper substrates to the active site of the enzyme, causes the enzyme to undergo a proper conformational change, which causes substrates and catalytic amino acids to align, which causes the chemical reaction to take place at a desired rate.

Proposed version of multimedia pre-training. This approach to pre-training seems sufficient to facilitate some transfer tasks. For example, if the problem required replacing the natural substrate with one of its analogues, then the causal model described earlier would simply run as follows: binding of improper substrates to the active site of the enzyme, causes the enzyme to undergo an improper conformational change, which causes substrates and catalytic amino acids to misalign, which causes the chemical reaction to take place at an undesired rate, if not all. Relating to the car brake example in Mayer et al. (2002), the transfer task just described is similar to a problem asking about the consequences of replacing the brake shoe with one that has a different (say smaller) surface contact with the brake drum. The causal model described in this study would be applied as follows, "the car's brake is pushed down … which causes the brake shoe to move forward, which will cause the brake drum to be pressed against [less efficiently], which will [take longer for] the wheel to slow down or stop" (p. 147). Nevertheless, if this same problem were to ask about the consequences of replacing the brake shoe by another with a different contact material with the brake drum, then running the causal model would require comprehension of the underlying process that stops the wheel, i.e. the friction between the shoe and the drum which converts kinetic energy of the car to thermal energy. In such a transfer task, the learner needs to select relevant frictional properties of the given contact material, such as ability to recover quickly from increased temperature, to determine how to run the causal model. Theoretically speaking, the learner has to move back and forth along the hierarchical levels of organization in the braking system from the symbolic level (switching between forms of energy), the submicro level (molecules constituting the contact material), to the macro level (bringing the wheel to a stop) to be able to properly run the causal model (Treagust & Tsui, 2013). Therefore, extending component model pre-training to include understanding of the underlying processes (symbolic level) and the associated terms (submicro level) of the targeted causal model would facilitate such transfer tasks (Schönborn & Bögeholz, 2013).

This proposed extension to component model pre-training has a two-fold benefit. First, it accounts for the hierarchical organization of some domains of knowledge such as biology and biochemistry (Treagust & Tsui, 2013). Second, it bridges the gap between CTML's definition of a complex material; "that consists of many steps and *underlying processes*" (Mayer, 2009, p. 80) and the current approach to pre-training through extending the latter to account for the role of "*underlying processes*."

To put this into play, we can refer back to the substrate specificity lesson. Recall that the transfer task asked about the consequences of replacing the natural substrate by an analogue. However, if the problem were to ask about the consequences of replacing a catalytic amino acid by another (case of a point mutation), then running the causal model would require comprehension of the underlying processes that determine substrate specificity, i.e. the interaction between the substrate and the amino acid which may contribute to reactivity and/or binding affinity. In this task, the learner needs to select relevant chemical properties of the given amino acid, such as charge and polarity of its side chain, to determine how to run the causal model. In other words, the learner has to move back and forth along the hierarchical levels of organization in this biochemical phenomenon between the symbolic level (forms of chemical interaction) and the submicro level (functional groups present in the active site of the enzyme) to be able to properly run the causal model. Obviously, this mental shifting between levels of representation is cognitively demanding (Bayrhuber et al., 2010) and requires instructional support to be effective

(Shönborn & Bögeholz, 2013). Therefore, explicit multimedia presentation of these levels during the pre-training episode would help initiate the shifting process.

To summarize, a modified version of multimedia pre-training that goes beyond what is done by Mayer, Mautone, and Prothero (2002) or Mayer, Mathias, and Wetzel (2002) is explored in this study. Technically speaking, the proposed form of pre-training extends the 3-step approach to component model pre-training (Mayer, Mathias, and Wetzel, 2002) to include a fourth step where underlying processes of the presented phenomenon (substrate specificity) and their associated terms are explained.

Aligning the Three lenses of the Theoretical Framework

The triarchic model of cognitive load is the organizing framework for *learning* from multimedia (Mayer, 2009). The pre-training principle is an *instructional* method grounded in this framework (Sorden, 2012). The cognitive dimension of revised Bloom's taxonomy is chosen as an *assessment* framework for learning from pre-training (Anderson et al, 2002). These three lenses of the theoretical framework, *learning*, *instruction*, and *assessment*, belong to the same cognitive-constructivist perspective. Therefore, aligning these lenses should help see more clearly the array of options for how to tailor instructional approaches needed to meet the complexity of scientific concepts such as substrate specificity of enzymes.

CHAPTER III

METHODOLOGY

In this chapter, the overarching hypothesis and research questions are stated. Also, research design, materials design, methodological framework, sampling and experimental procedures, data collection instruments, and data analysis procedures are discussed.

Overarching Hypothesis and Research Questions

The leading hypothesis in this study is formulated as such: if the pre-training group was able to build component models and attend to the underlying processes and terms before engaging in building a causal model for how substrate specificity works, then they will outperform the no pre-training group in a transfer test. A competing hypothesis however, may state that: if the pre-training group received additional instruction in the form of pre-training to learn how substrate specificity works, then they will outperform the no pre-training group in a transfer test. To refute the second hypothesis, a post-training group was included in which participants received the same amount of instruction received by the pre-training group but in a reversed order. Since multimedia pre-training is thought to foster transfer through cognitive load manipulation (Mayer et al., 2002), and motivation is also theorized to play some role in this process (Mayer, 2011), corresponding variables were measured and tested as putative mediators of the process at work. These variables included intrinsic and germane cognitive load as well as motivation to learn the presented material through the instructional kit.

Particularly, this research aims at answering the following questions:

1. What is the overall difference, if any, in retention and transfer performance among the three groups (pre-training, no pre-training, and post-training)?

2. What is the difference, if any, in transfer performance among the three groups based on the cognitive dimension of revised Bloom's taxonomy?

3. What is the difference, if any, in extraneous, intrinsic, and germane cognitive loads among the three groups?

4. What is the motivational level of students to learn about substrate specificity through the instructional kit?

5. Does intrinsic cognitive load mediate the effect of pre-training on transfer performance?

6. Does prior factual knowledge mediate the effect of pre-training on transfer performance?

7. Does intrinsic cognitive load mediate the effect of pre-training on transfer performance in parallel with prior factual knowledge?

8. Does prior factual knowledge, intrinsic, and germane cognitive loads mediate the effect of pre-training on transfer performance in series?

9. Does germane cognitive load mediate the influence of motivation on transfer performance?

Research Design

Variables

Independent variable. Except for research questions 4 and 9, this study follows an experimental approach. One hundred eleven participants were randomly assigned to one of three groups (n=37 in each group): no pre-training (X=0), pre-training (X=1), or post-training (X=2). Therefore, the independent variable in this experiment is the assigned group (X=0, 1, or 2). For research questions RQ.4 and RQ.9, a correlational approach is followed because all participants received the same treatment. For RQ.4, the predictor/independent variable is the usage of the instructional kit. For RQ.9, the predictor is the motivational level of the participant.

Dependent Variables. In the first research question RQ.1, the dependent variables are retention and transfer performance. In RQ.2, five dependent variables are measured. Each of the 5 variables represent transfer performance is in terms of a specific cognitive order in revised Bloom's taxonomy. These orders are, understanding, analyzing, applying, evaluating, and creating. RQ.3 involves three dependent variables. These are extraneous, intrinsic, and germane cognitive loads. RQ.4 probes the motivational level of participants. In RQ.5 through RQ.9, the dependent variable is transfer performance. Figure 5 highlights the variables of the experiment.

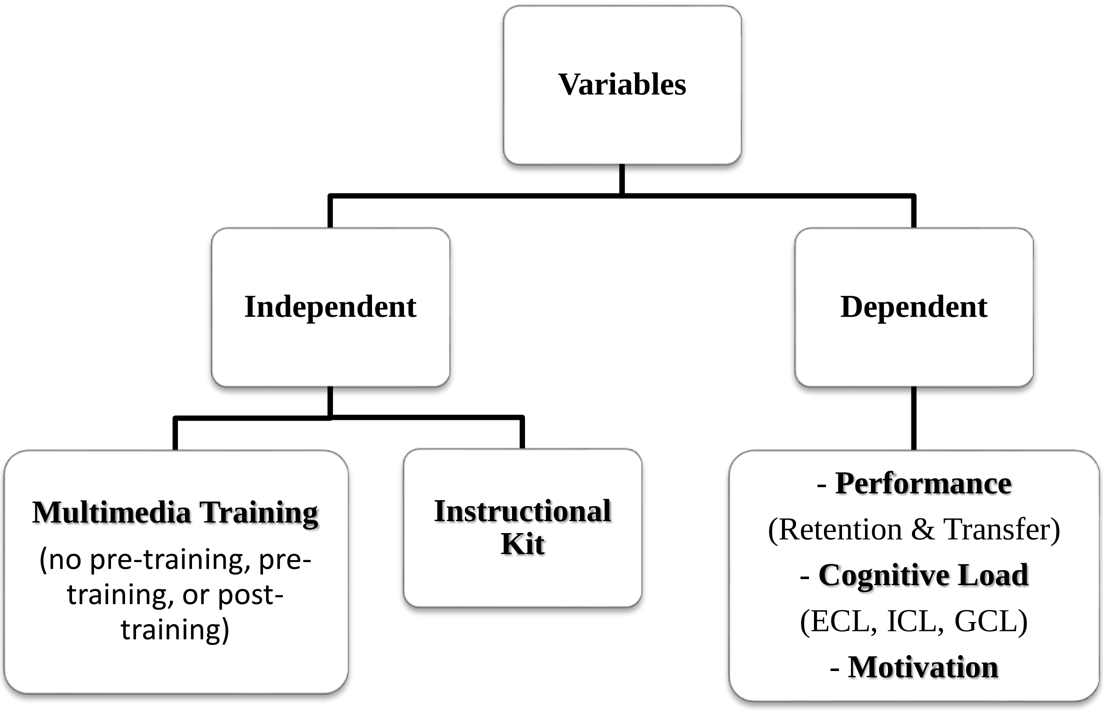

Figure 5. A hierarchical diagram highlighting variables of the experiment. Key: ECL=extraneous Cognitive Load; ICL=intrinsic cognitive load, GCL=germane cognitive load.

Methodological Framework

Working within the triarchic model of cognitive load. As stated earlier, pre-training is a treatment meant to foster transfer performance through reducing intrinsic cognitive load (Mayer, 2009). However, with the known limitation of working memory (Baddeley & Hitch, 1974), special attention is given to extraneous and germane cognitive loads since the proposed treatment is theorized to function within a triarchic model of cognitive load (DeLeeuw & Mayer, 2008).

Germane Cognitive Load. Research recommends fostering germane cognitive load along with reducing intrinsic cognitive load (van Merriënboer, Kester, & Paas, 2006). Consequently, two measures were taken. An interactive physical model was designed and used to motivate participants to learn about the presented concept based on the notion that motivation causes germane cognitive load (Mayer, 2009). Such a potentially motivating instructional tool would also "provide more valid measurements of cognitive load" (DeLeeuw & Mayer, 2008, p. 231). Another important measure is the guided inquiry approach to the main lesson (see worksheet in Appendix D). This approach is followed because it is known to prime germane cognitive load without engaging in extraneous cognitive load — compared to open discovery (Kirschner, Sweller, & Clark, 2006).

Extraneous Cognitive Load. Several measures were also taken to keep extraneous cognitive load controlled at low levels. In the worksheet for instance, the learner has to fill in the blanks to formulate hypotheses and draw out conclusions/deductions, instead of composing hypotheses and conclusions themselves (de Jong, 2011). This tactic eliminates any possible extraneous load caused by potential lack of inquiry skills which is a confounding variable that does not contribute to learning content knowledge. To further reduce extraneous cognitive load, "*Hints"* were associated with conclusion/deduction questions to help students identify the relevant variables in each experiment (de Jong, 2011). Notice that these *Hints* are solely meant to highlight important experimental observations but never guarantee correct answers. Hence, they should not be viewed as aids of schema acquisition and therefore tampering with germane load as feedback does (Ayres, 2006). Instead, these *Hints* are one application of the signaling principle which reduces extraneous load by highlighting the important parts of an instructional message (Mayer, 2009).

Materials Design

Multimedia Pre-training Presentation

To produce the pre-training presentation, the3-step approach described in Mayer et al. (2002) was followed with an additional step described below. The first step is to decompose the presented system into "functionally meaningful units" (Mayer et al., 2002, p. 148). That is, the degree of decomposition enables the learner to describe a cause-and-effect relationship among the components of the system. In substrate specificity, the major components that were highlighted are the enzyme's catalytic amino acids (both reactive and stabilizing), and its substrates.

In the second step, each component is named and highlighted, Figure 6. The three methods described in Mayer et al. (2002) were followed: (a) the component is *labelled* by either drawing an arrow to the component from its name (e.g. substrates) or by inserting a caption close to it (e.g. glucose, ATP, and enzyme); (b) the component is *highlighted* in a translucent oval while its name is presented in a caption within the oval (e.g. catalytic amino acids); (c) the component is *concretized* by using a unique color, such as yellow for the enzyme, white and blue for catalytic amino acids, and red for the substrates (the green circle in ATP represents the terminal phosphate that will later transfer to glucose). The states of each component are also described during the narrated animation in separate slides as shown in Figure 6. Note that, at this stage, levels of submicroscopic representations are switched between ‘cartoon diagrams’ to ‘abstract line drawing diagrams’ of substrates and catalytic amino acids in order for students to easily visualize the underlying chemical processes taking place between these two components (Newberry, 2002).

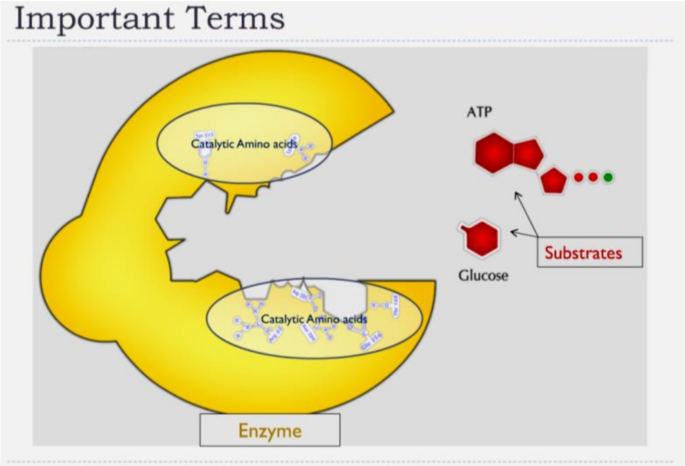

Figure 6. A screenshot of the multimedia pre-training episode demonstrating how interacting components were highlighted.

In the third step, the state changes for each component are elaborated. Building on the general state changes in the enzyme previously described in spoken words, see Figure 7, a narrated animation was provided to show the enzyme in three different conditions: one where the enzyme snaps all the way down and bounces back at a desired rate (proper conformational change), another condition where the enzyme snaps half way down and bounces back (improper conformational change), and a third condition where the enzyme undergoes the same state changes as in the first condition but at a faster rate (improper conformational change), Figure 8.

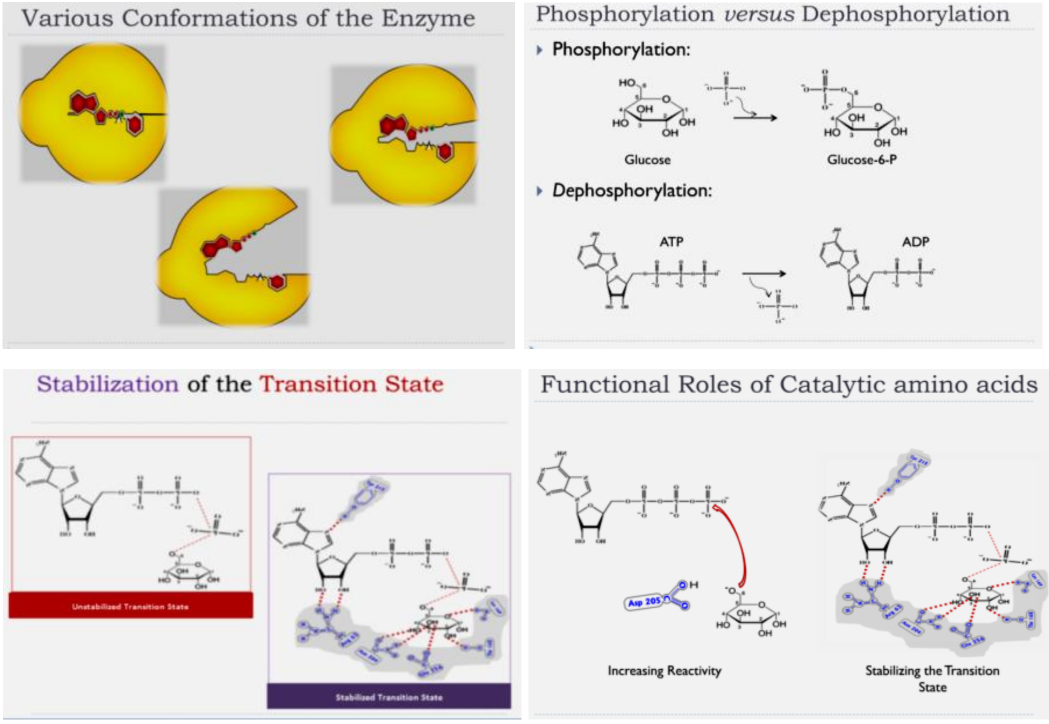

Figure 7. Four screenshots of the multimedia pre-training episode showing state changes of interacting components. The upper left slide demonstrates different states of the enzyme which can be closed, opened, or partially closed/opened. The upper right slide shows the states of molecules interacting with the enzyme. These are glucose and the nucleotide which can be phosphorylated or dephosphorylated. The lower left slide represents the transition state which can be stabilized or not. The lower right slide shows a reactive catalytic amino acid (left), which can be oxidized or reduced by abstracting a hydrogen atom from glucose. This same slide also shows stabilizing catalytic amino acids (right), which can be interacting with the transition state (symbolized by dotted lines) or not.

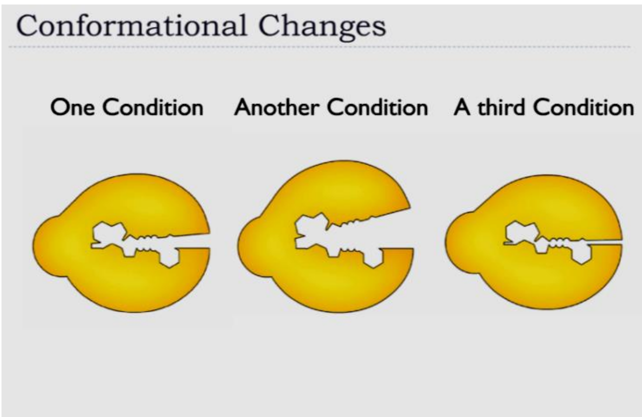

Figure 8. A screenshot of the multimedia pre-training episode showing a slide animating the enzyme in three different conditions, i.e. state changes.

These enzyme's state changes are dictated by underlying processes among the major components. Hence, in the fourth step, these processes and their associated terms are explained. In substrate specificity, represented processes are the chemical interactions between substrates and catalytic amino acids. Although these interactions ultimately filter down to reactivity and binding affinity, the number of these processes and their associated terms can be overwhelming. Therefore, each process is cut into small meaningful units as is done in the first step. For instance, participants learn that in a chemical reaction one substance turns into another substance by passing through an intermediate molecule. This symbolic representation of the chemical reaction (e.g. $A+B \rightarrow X \rightarrow C + D$) is followed by submicroscopic (structural) representations of associated terms such as reactants (e.g. glucose and adenosine triphosphate), products (e.g. glucose-6-phosphate and adenosine diphosphate), transition state intermediate, and functional groups of reacting molecules (e.g. hydroxyl and phosphate groups in a phosphotransferase reaction). Participants also learn that a catalytic amino acid might be reactive and/or stabilizing to a molecule occupying the active site of the enzyme. The symbolic representation of substrate-catalytic amino acid interaction (e.g. an arrow depicting a nucleophilic attack, or a dotted line depicting stabilization by H-bonds) includes structural representation of terms such as substrates, active site, catalytic amino acids, and stabilized transition state.

The multimedia presentation was produced with iSpring Pro7 and spanned 7-minutes. In the lower left corner of the screen, there was a play icon that flashed when the narration in each slide was completed. In the same corner, there were forward and backward icons for the participant to navigate within the slide. The multimedia presentation was programmed in a way that enabled rewinding a slide but never skipping one. Figure 9 below shows a selected frame from the presentation.

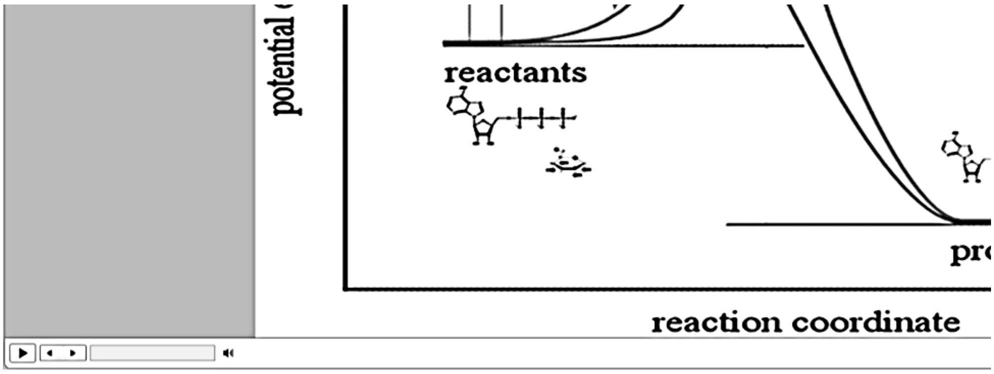

Figure 9. A cropped screenshot of the multimedia pre-training episode showing navigating icons used by students to control the multimedia presentation.

The presentation consisted of 11 slides of narrated animations (Appendix B). The first slide began with the definition of a chemical reaction and gave the process of glucose phosphorylation as an example. As stated earlier, the symbolic representation of the process in the form of a chemical equation was followed by a submicroscopic representation of the reactants, their functional groups, and the products. The second slide furthered explanation of the same chemical reaction by introducing the concept of the transition state. In this slide, the transition state — which forms during glucose phosphorylation — is structurally represented within the chemical equation. In the third slide, the role of enzymes in stabilizing the transition state is shown in a graph representing the energy profile of the reaction. Reactants, transition state, and products of the chemical reaction were embedded in the graph. In the fourth slide, the transition state is represented in both states, stabilized/unstabilized. The stabilized state is shown interacting with surrounding catalytic amino acids, where the interaction is symbolized with dotted lines. The fifth slide highlights the consequence of this interaction with the stabilized transition state located at a lower energy barrier within the energy profile of the reaction. In sixth seventh and eighth slides, possible chemical interactions between a catalytic amino acid and a substrate/transition state are discussed. The interaction between a reactive catalytic amino acid (e.g. aspartate) and glucose for example is symbolized by abstraction of a hydrogen atom from glucose by aspartate rendering glucose negatively charged (the −*ve* sign appeared on glucose indicating increased reactivity). Increased reactivity of glucose is then symbolized by an arrow pointing from glucose to phosphorus in the terminal phosphate of ATP. Submicroscopic representation of substrates, catalytic amino acids, enzyme's active site, and transition state in these three slides shifted from 'cartoon diagrams' to 'abstract line drawing diagrams' when representation of atomic interactions was needed. In the ninth slide, cartoon diagrams represented the enzyme in three different conformations (i.e. states). The tenth slide included a narrated animation of possible conformational changes (state changes) of the enzyme in different conditions. The final slide represented the state changes of the molecules interacting with the enzyme such as glucose and ATP, which can be phosphorylated or dephosphorylated. (De)Phosphorylation processes were each symbolized in chemical equations.

The Worksheet

The guided inquiry lesson spanned 30-33 minutes and was based on a paper-based worksheet that consisted of five experiments. In the first experiment, students observed how the wild-type enzyme reacts to its natural substrates through changing its shape to get them aligned and close enough to react. The aim of this experiment was to explain the role of conformational change in aligning substrates thus speeding up the chemical reaction. To emphasize the role of catalytic amino acids in this process, the second experiment dealt with a mutant form of the enzyme in which the reactive catalytic amino acid residue was replaced by a non-reactive one. Students noted that the enzyme would undergo the same conformational change as in the first experiment but still *no* reaction. This instructional step was important to emphasize the role of underlying chemical processes in the causal model that is being constructed. The third set of experiments demonstrated the possible reactions of the enzyme toward substrate analogues. Students here noticed how the enzyme reacted differently by undergoing an improper conformational change that failed to align the bound substrates along with the reactive amino acid. They also learned that, in few cases, the enzyme may react in an undesirable rate of conformational change. This helped students realize that enzyme specificity is a kinetic property of the enzyme; an objective that was covered in the last two experiments. In these final experiments, students learned that enzyme specificity mainly depends on kinetic parameters like *binding affinity* of substrates to their enzyme and *reactivity* of the enzyme toward these substrates. For binding affinity, students counted the number of possible H-bonds that can form between a natural substrate and its surrounding catalytic amino acids. They did the same with a substrate analogue to examine which is more attracted to the enzyme, thus increasing the chances for the *desired* chemical reaction to take place. In the last experiment, the instructor demonstrated how the enzyme reacts with almost all of the natural substrate molecules while reacting to very few of the analogue. The fact that the enzyme reacts with an analogue, though at a very low rate, helped students appreciate that enzyme specificity is more than a simple all-or-none phenomenon. Rather, they come to the conclusion that enzyme specificity is a complex phenomenon defined by underlying chemical processes and variables. More specifically, they learn how (1) a *conformational change* facilitates (2) the *interaction* between the substrates and catalytic amino acids which in turn is directly connected to (3) the *binding affinity* of these substrates to these same amino acids as well as (4) the *reactivity* of the enzyme toward these substrates. Notice here the need to shift between levels of representation from submicroscopic (conformational change of the entire enzyme), to even more submicroscopic (interactions at the level of molecules present in the active site of the enzyme), to concepts represented in symbols (e.g. the number of H-bonds representing the strength of binding affinity). Visual representations embedded in this worksheet are exact copies of those used in the pre-training presentation.

The Interactive Physical Model

The interactive physical model used with the worksheet represented the enzyme along with different pieces corresponding to different substrates. The different components were highlighted following the concretization method used to produce the pre-training presentation (see step-2 above). Addition of interesting, but irrelevant features (e.g. smiley eyes), to the model were intentionally avoided assuming that they may be detrimental to the learning process (Lehman et al., 2007), let alone that otherwise the model would have been perceived as too juvenile by college students, Figure 10.

Figure 10. Photograph of the interactive physical model. (Mounir R. Saleh. Instructional enzyme model. U.S. patent application No.: 29508018. November 1, 2014)

A removable metallic strip was designed to represent the reactive catalytic amino acid. This was planned to emphasize the role of chemical interactions underlying the phenomenon of substrate specificity since only one out of eight students in a pilot study mentioned the role of the catalytic group in their responses (Appendix C). The model was also designed to be dynamic because, in the same pilot study, students exposed to dynamic representations demonstrated deeper understanding of the concept than those treated with static representations (Appendix C).

Assessment Instruments

All of the 4 assessment instruments, pre-test (1) and (2) and post-test (1) and (2), were paper-based. Figures and tables were duplicated as much as needed to avoid the split attention effect that may result from flipping pages back and forth while trying to generate solutions to presented problems because this would otherwise increase extraneous cognitive load; an undesired confounding variable (Chandler & Sweller, 1991).

Procedures

Sampling Procedure

After obtaining approval from the Institutional Review Board at The University of Southern Mississippi USM (Appendixes E and F), one hundred eleven college students were recruited from two southern universities and a southern community college to participate in the study by sending announcements through the schools' mail-out or through instructors. Participants obtained raffle tickets, the numbers on which were used on all of their responses to maintain anonymity. Duplicates of obtained tickets were used to run 4 raffles on $200-worth tablets (*as an incentive for participation*). Each session took about 1.5 hours.

Experimental Procedure

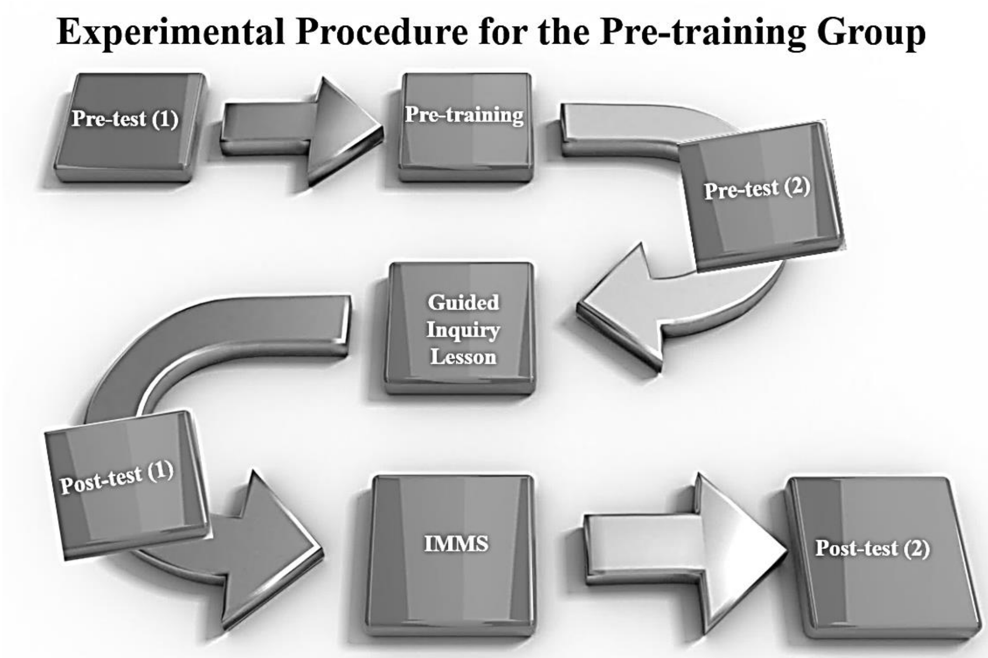

Figure 11. Chain diagram showing the experimental procedure for the pre-training group. Key: IMMS=Instructional Materials Motivation Survey.

Multiple learning sessions were held depending on availability of participants, which were treated and tested in groups of up to twenty three per session. Fidelity of treatment was maintained through utilizing a recorded multimedia presentation, during the pre/post-training episode, and a fully structured Question-and-Answer worksheet for the guided inquiry lesson. Learning sessions were held in laboratories equipped with Macintosh/Microsoft computers with earphones and 32/19-inch (81/48-cm) monitors.

Participants were randomly assigned to one of three groups by asking them to pick up any computer in the laboratory. In front of each computer, there was a package of papers consisting of the worksheet and all assessment instruments. An individual participant would know their assigned group from the color of the paper clip that was holding the package. The color code was uncovered after all participants have chosen their spots.

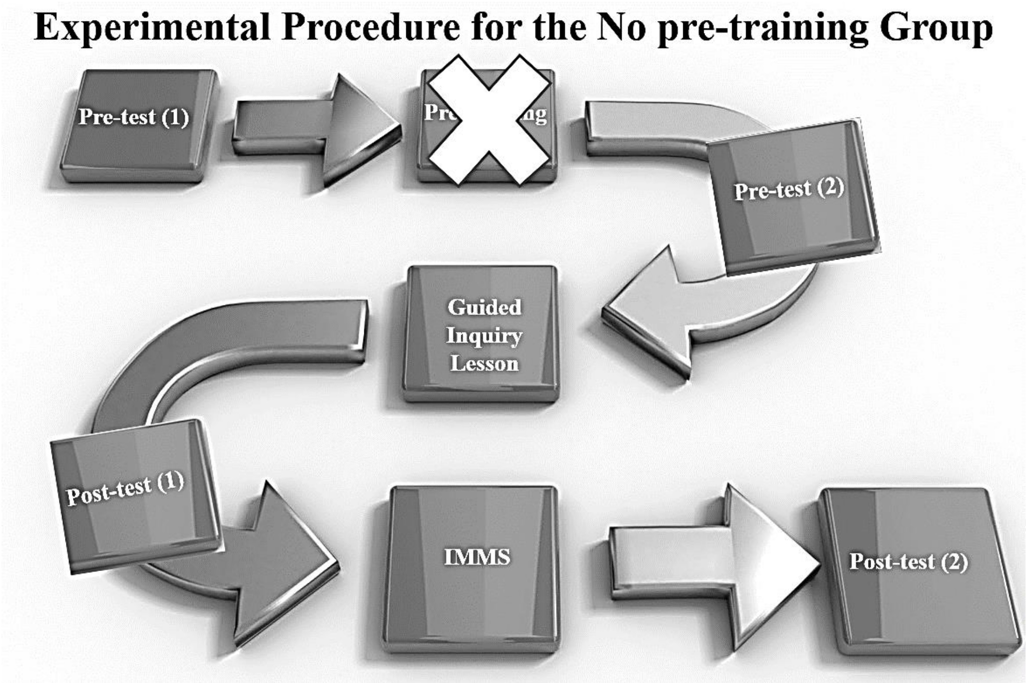

Figure 12. Chain diagram showing the experimental procedure for the no pre-training group. Key: IMMS=Instructional Materials Motivation Survey.

Figures 11, 12, and 13 summarize the experimental flow for the pre-training, no pre-training, and post-training groups respectively. After reading the consent form (Appendix G), all participants took pre-test (1) (Appendix H) to assess their general knowledge about substrate specificity. Afterwards, participants in the pre-training group received the computer-based multimedia presentation, whereas participants in the other two groups received no pre-training. Then, all participants took another pre-test (2) to assess their prior factual knowledge related to the concept to be presented afterwards (Appendix I). Thereafter, all groups attended the guided inquiry lesson on substrate specificity based on the instructional kit. After instruction, participants in the post-training group received the same multimedia presentation presented to the pre-training group before instruction, whereas participants in the other two groups received no further training. Then, all participants sequentially took, post-test (1) (Appendix J) and post-test (2) (Appendix K) which assessed their retention and transfer abilities respectively, an Instructional Materials Motivation Scale IMMS[7](Appendix M) that measured their motivation to learn the concept (Keller, 2010), followed by a short demographic survey (Appendix N).

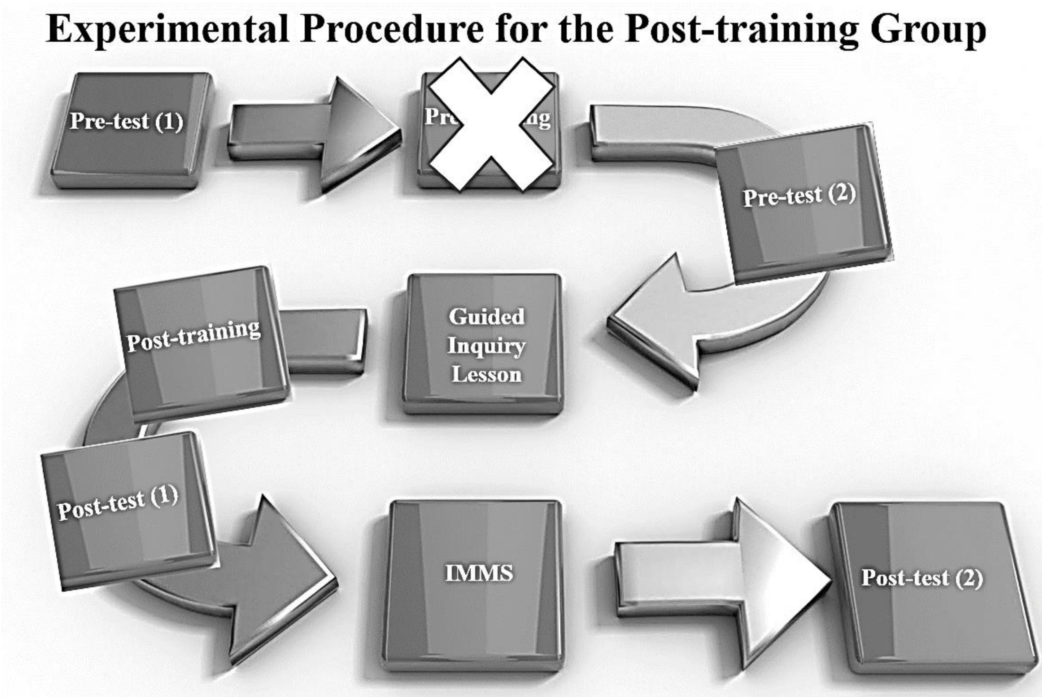

Figure 13. Chain diagram showing the experimental procedure for the post-training group. Key: IMMS=Instructional Materials Motivation Survey.

Intrinsic cognitive load was assessed during instruction and assessment through self-rating of mental effort (Paas &van Merriënboer, 1993). Germane and extraneous cognitive loads were measured right after instruction, post-test (1), and post-test (2), through difficulty rating of the studied concept and the learning environment respectively (Swaak & De Jong, 2001). Cognitive load surveys were embedded in the worksheet and assessment instruments as described later in this chapter.

[7]Appendix L shows an Email from the author of this survey (Keller, 2010) who approved using the adapted form, see Appendix M

Instrumentation

Knowledge Assessment Instruments

All of the knowledge assessment instruments were prepared and checked for validity and reliability. These instruments are pre-test (1), pre-test (2), post-test (1), and post-test (2). Each test Item is worth one point of the total score of a test except for Item 7 of post-test (2) which was partially credited. Reliability of all assessment instruments was measured by computing Cronbach's alpha score of internal consistency excluding Item 7. Reliability of the latter was measured through computing intra-class correlation coefficient. Validity measures are discussed later in this section.

Pre-test (1) consists of 6 multiple choice Items MCI assessing prior knowledge about the concept of substrate specificity. Post-test (1) is identical to pre-test (1). Validity of these two instruments is discussed along with post-test (2).

Pre-test (2) consists of 16 MCI assessing learner's knowledge of major components, underlying processes, and associated terms to substrate specificity. Content of this instrument is aligned with that in the pre-training presentation. Three science educators examined this alignment along with the clarity of each test Item. Since the knowledge assessed in this instrument comprised discrete facts prior to instruction, this instrument stood as a measure of prior factual knowledge.

Post-test (2) consists of 18 MCI and one free-response Item (Item 7). The first 2 Items of this instrument are illustrative questions and were not counted for scoring purposes. According to the knowledge dimension of revised Bloom's taxonomy, post-test (1) and post-test (2) stood as retention and transfer tests respectively. According to the cognitive dimension, Items constituting both tests covered the six cognitive orders as detailed in Table 1 and described thereafter.

Table 1

Item Specification Grid

Test	Cognitive Order					
Section	Remember	Understand	Apply	Analyze	Evaluate	Create
Retention	1, 2.a-d, 3	—	—	—	—	—
Transfer*	—	3, 4, 5, 6	17, 18,	8, 9, 10,	13, 14, 15,	7

*Items 1 and 2 in transfer test are only illustrative and hence were not rated.

Remember. This section involved retrieving concept elements presented during instruction in order to compare it with presented multiple choices in the test (Anderson et al., 2001).

Understand. In this set of questions, students had to use their constructed causal model to explain how a change in the chemical structure of the substrate would affect enzyme reactivity or substrate affinity (Anderson et al., 2001).

Apply. Here, students had to choose the most effective procedure to modify enzyme reactivity or substrate affinity (Anderson et al., 2001).

Analyze. In this set of Items, students were given a set of experimental results and asked to choose the most relevant result to determine the contribution of an amino acid in specificity (Anderson et al., 2001).

Evaluate. At this point, students had to evaluate a set of claims formulated based on a given set of experimental observations (Anderson et al., 2001).

Create. In this question, students were asked to describe a solution plan for a given problem (Anderson et al., 2001). Particularly, they had to design an artificial substrate with higher affinity to the enzyme but still receive no enzyme reactivity.

Validity Measures

Face validity. Post-tests (1) and (2) were examined for face, content, and discriminant validity as well as for Item performance. To obtain face validity for the cognitive process dimension, four science educators and an educational psychologist examined each test Item as it pertains to one of the six cognitive orders in revised Bloom's taxonomy.

Concept Objectives
Retention Section (Test Items 1, 2.a-d, 3):
To test if the students have *remembered* the following concepts from class discussion:

R1. Enzymes are specific in their action.

R2. Binding of the substrate to its enzyme induces the enzyme to undergo a *conformational change* so as to fit the substrate thus catalyzing the chemical reaction

R3. A substrate that is analogous to the natural substrate of an enzyme is *likely* to:

- R3a. Have a *lower* binding affinity to the enzyme
- R3b. Induce the enzyme to undergo a *different* conformational change
- R3c. Cause the enzyme to function at a *lower* reactivity compared to the natural substrate
- R3d. Have an *improper* orientation with the catalytic amino acids and yield *no* products

Transfer Section (Test Items 1-19):
To test if the students are able to *transfer* (use) the following concepts:

T1. Main factors affecting enzyme specificity are:

- T1a. The *binding affinity* of the enzyme for a substrate
- T1b. The *reactivity* of the enzyme toward a substrate

T2. Undergoing a *proper* conformational change is necessary for the enzyme to appropriately *align* the substrate with the catalytic amino acids

T3. Catalytic amino acids are responsible for *stabilization of the transition state* of the substrate (i.e. increasing its binding affinity) and/or *increasing the reactivity* of the substrate

Figure 14. List of concept objectives for post-test (1) (Retention Section) and post-test (2) (Transfer Section).

Content validity. A professor emeritus in biochemistry was first asked to examine the clarity of each Item and whether test Items are aligned with the objectives listed in Figure 14. He also examined whether the presented data in the test resemble those that might be obtained in real experiments. After making the necessary edits recommended by this subject matter expert, seven faculty members —five biochemists and two chemists— from different universities were asked to rate each Item as *essential*, *useful but not essential*, or *not necessary*.

Discriminant validity. Recall that these two tests were planned to distinguish between students whose prior knowledge was expected to differ as a result of the pre-training/no pre-training treatment. Accordingly, these instruments were checked for their ability to discriminate between science and non-science students. It was theorized that a valid instrument assessing knowledge/understanding of a scientific concept at the tertiary level education (enzyme specificity) should discriminate between the two groups because, presumably, they possess different prior scientific knowledge. More precisely, it was theorized that a valid construct assessing content knowledge "*Remember*" of science and non-science students before instruction should discriminate between the two groups but not necessarily after instruction. Also, a cognitive construct assessing students' transfer performance "*Understand* through *Create*" after instruction should discriminate between science and non-science students; especially if embedded in a highly scientific context such as the one on hand.

Item analysis. Computation of difficulty and discrimination indices helped analyze poorly performing Items. Item difficulty index p represents the proportion of test takers who answered the Item correctly. Mathematically, p can range from 0 (none of the test takers answered the Item correctly) to 1 (all test takers answered the Item correctly). Generally, difficulty values below 0.2 are considered very difficult Items, and values above 0.9 are considered very easy Items (Chang et al., 2011). Such Items do not provide valuable information about students' abilities. Discrimination index D demonstrates how well the Item serves to distinguish between test takers based on either an internal or external criterion. For reliability measures, D is computed based on an internal criterion such as total test scores (Aiken, 2003). For validity measures however, *D* is computed based on an external criterion which, in this case, is the major of the participant — science *versus* non-science students (Aiken, 2003). Mathematically, *D* can range from -1 (e.g. all non-science students answered the Item correctly but none of science students did) to +1 (e.g. all science students answered the Item correctly but none of non-science students did). In general, *D* value of 0.2 and above is acceptable. For standardized tests however, *D* value of 0.3 and above is desirable (Doran, 1980). A universal framework for analyzing *D* does not seem to exist though. For instance, Brown and Abeywickrama (2004, p. 303) state that, “no absolute rule governs the establishment of acceptable and non-acceptable [*D*] indices.” Yet, with difficulty and discrimination indices being inherently related, *D* values might be interpreted along with corresponding *p* values for each Item. Brennan (1972) provides the following criteria for this analysis: (a) Items that discriminate negatively are clearly unacceptable because the lower group outperformed the upper group, (b) Items that discriminate positively are acceptable if the criterion is to differentiate between the two groups, (c) a non-discriminating Item with low *p* value is not ideal because it is too difficult for both groups, and (d) a non-discriminating Item with high *p* value is acceptable because both groups are passing the Item.

Item 7. Validity and reliability of Item 7 were studied separately from other Items because it is the only free response, partially credited question. Students were asked to explain their solution plan to a given problem both in words and in drawings in order to maintain cross-data validity checks (Patton, 2002). To help reduce potential bias, Item 7 was graded by two independent raters based on a predefined rubric (see Figure 15) and intra-class correlation coefficient was computed under the "absolute agreement" condition.

Rubric for Item 7

Increasing binding affinity:

Any modifications in the structure of the substrate that would increase the number of non-covalent bonds between the enzyme and the substrate are acceptable. This includes but is not limited to:

- Introduction of an additional attractive group on the substrate that would interact with the free rightmost group on the enzyme
- Introduction of additional attractive group(s) that would result in formation of more bonds between a given group on the enzyme and the introduced one(s) along with the already existing bonds that this given group is forming.

Reducing reactivity:

Any modifications in the structure of the substrate that would block/weaken the interaction of the catalytic group with the bond to be broken are acceptable. This includes but is not limited to:

- Displacement of the bond to be broken in a way that prevents access of the catalytic group by any alternative forms of conformational change.
- Replacement of the bond to be broken by another that is nonreactive to the catalytic group.

Figure 15. Rubric for Item 7 in post-test (2).

Cognitive Load Assessment Instruments: Measuring the three types of cognitive load

Measuring intrinsic cognitive load. During the lesson, the learner was asked to rate their level of mental effort at eight points throughout the worksheet on a scale of 9-points ranging from 1 (*extremely low mental effort*) to 9 (*extremely high mental effort*). This single-item survey has been frequently implemented in multimedia research (Ayres, 2006; DeLeeuw & Mayer, 2008; Paas & van Merriënboer, 1993, 1994) and is experimentally validated to be sensitive to changes in intrinsic cognitive load (DeLeeuw & Mayer, 2008). In this survey, the learner is asked the following question: "*Please rate your level of mental effort on this part of the lesson.*" Four points in the worksheet were identified to be with the lowest complexity. At these points, the learner has only to report one/two observations in the experiment (just before rating their mental effort). Four other points were identified to be with the highest complexity. At these particular points, the learner has to draw out a conclusion/deduction based on their observations from previous experiments.

Measurement of mental effort is repeated several times to increase accuracy as recommended by a critical review (deJong, 2010). Cronbach's alpha was computed to investigate internal reliability of mental effort rating at both lowest and highest complexity points. The mean score of the eight points represent overall intrinsic cognitive load during the entire period of learning. Similarly, the 2 ratings of mental effort in post-test (1) and 9 ratings in post-test (2) were all averaged to calculate the overall intrinsic cognitive load during the entire period of assessment. Here, the learner is asked the following question: "*Please rate your level of mental effort on this part of the test."* The labels and anchor terms of this scale are shown below:

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

Measuring germane cognitive load. Immediately after the lesson, the learner was asked to make a retrospective rating of the lesson's *difficulty* through using a 9-point scale ranging from 1 (*extremely easy*) to 9 (*extremely difficult*). Although ratings of *mental effort* and *difficulty* may sound to measure the same construct, van Gog and Paas (2008, p. 23) have shown that ''…the outcomes of the *effort* and *difficulty* questions in the [instructional] efficiency formula are completely opposite.'' This could especially be true when the learner finds the lesson extremely difficult and therefore gives up spending any mental effort to understand it. Additionally, this single-item survey has been used by a number of studies (DeLeeuw & Mayer, 2008; Kalyuga, Chandler, & Sweller, 1999; Mayer & Chandler, 2001) and is experimentally validated to be sensitive to differences in transfer performance which is an indication of germane cognitive load (DeLeeuw & Mayer, 2008). Right after the lesson, the learner was asked the following question: "*Please indicate how difficult this lesson* *was*.” The same survey is repeated immediately after each of pre-test (1), post-test (1), and post-test (2) for the same reason. Right after each test, the learner was asked the following question: “*Please indicate how difficult this test was*.” The mean score of the four points represent overall germane cognitive load. The labels and anchor terms of this scale are shown below:

1	2	3	4	5	6	7	8	9
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

Measuring extraneous cognitive load. Immediately after the difficulty rating survey, extraneous cognitive load was monitored through asking the learner the following question: “*Please indicate how difficult it is to work in this learning environment.*” The number of points, labels, and anchor terms are identical to that of the difficulty rating. This single-item survey has been used by a number of studies (Cierniak et al., 2009; Kalyuga et al., 1998; Mussallam, 2010) and it is used here only to assure that extraneous cognitive load is controlled at low levels throughout the lesson, pre-test (1), post-test (1), and post-test (2).

Motivation Assessment Instrument

Motivation level associated with using the instructional kit was measured by utilizing the Instructional Materials Motivation Scale (IMMS) constructed by Keller (1987). This scale addresses the following constructs of motivation: attention, relevance, confidence, and satisfaction. The validity and reliability of this scale has been established at the college-level setting by Huang et al. (2006). This 5 minutes instrument, which was administered after post-test (2), consists of 26 questions divided into subsections of eight items on attention, six items on relevance, six items on confidence, and six items on satisfaction (Appendix L). The learner was asked to rate each question based on the following Likert scale: 1=Strongly disagree, 2=Disagree, 3=Neither disagree nor agree, 4=Agree, 5=Strongly agree. IMMS total score was used in this study to assess student motivation to learn about substrate specificity through the previously described instructional kit. Figure 16 summarizes the above discussion.

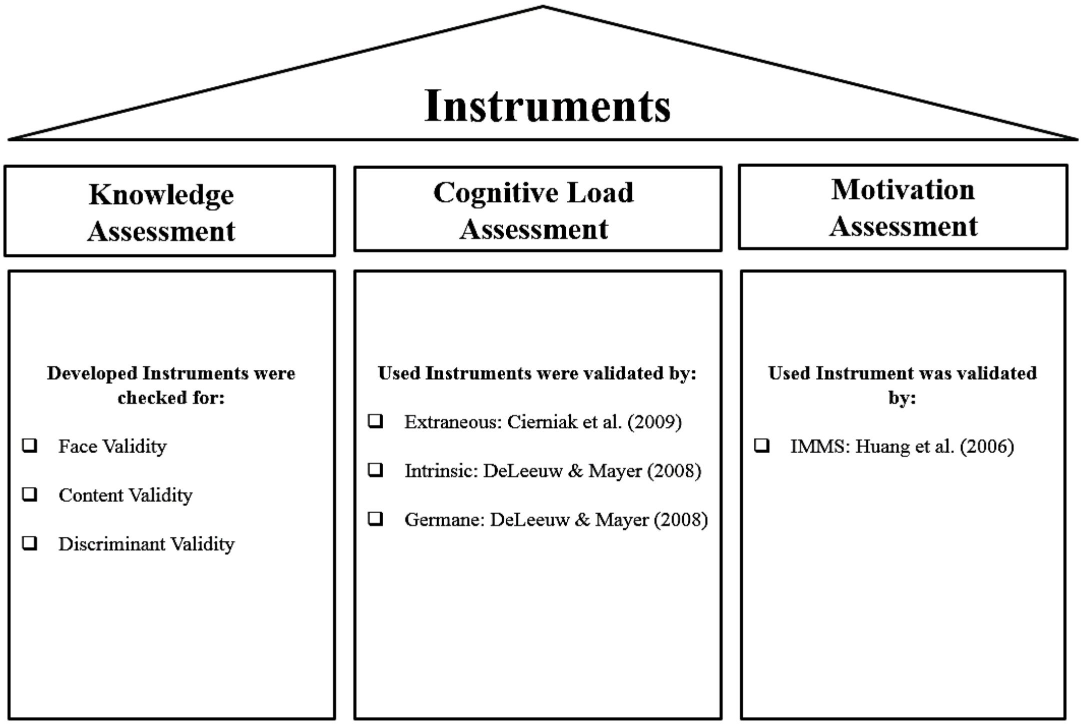

Figure 16. Diagram summarizing how/by whom assessment instruments were validated.

Data Analysis

Outliers and Missing Data Analysis

Out of the 13,320 data points, 17 (0.12%) outliers were identified based on the Hoaglin, Iglewics, and Tukey's labelling rule (1987) and were trimmed and treated as missing data. Afterwards, Little's Missing Completely At Random (MCAR) test was run to make sure that missing data is not missing in a systematic pattern. This test was followed by the modern multiple imputations MI technique which was performed on

IBM SPSS 19 software with 5 imputations, each running for 10 iterations while taking into account 119 variables. Markovski and Monte Carlo method was followed since Little's MCAR test demonstrated random patterns of missing data. MI technique was preferred over traditional methods because it helps avoid limitations such as statistical power inflation caused by mean substitution (Allison, 2001), loss in statistical power caused by listwise deletions, and negative R^2 values caused by pairwise deletions (Field, 2009).

Discriminant Validity Analysis

Independent t-tests were conducted to validate pre-test (1) and post-test (1) for their ability to distinguish between science (n=48) and non-science (n=63) students. Controlling for post-test (1) scores, one-way ANCOVA was conducted to test discriminant ability of post-test (2) based on the notion that ability to transfer knowledge is inherently related to the amount of acquired knowledge in the first place, which was measured via post-test (1). F-tests were conducted for the cognitive orders "*Remember*, *Apply*, *Analyze*, and *Evaluate*" to test the difference between the two groups. Wilcoxon rank-sum tests were conducted for cognitive orders "*Understand* and *Create*" because F-test assumptions of normal distribution were not met and sample sizes were not equal.

Answering Research Questions 1-9

R.Q.1. One-way analyses of covariance ANCOVA's were conducted on each test score, post-test (1) and post-test (2), with treatment group as the independent variable and IMMS score as the covariate. Pairwise comparison (LSD) was conducted to study differences among individual groups (p<.05).

R.Q.2. One-way analyses of variance ANOVA's were conducted on each of the 5 cognitive orders, "*Understand* through *Create*," with treatment group as the independent variable. Tukey's (HSD) test was conducted for pairwise comparisons among the three groups.

R.Q.3. One-way ANOVA's were conducted on extraneous and intrinsic cognitive load ratings with treatment group as the independent variable. For intrinsic cognitive load, Tukey's HSD test was also conducted because the three groups significantly differed from each other. One-way ANCOVA was conducted on germane cognitive load rating with IMMS (motivation) score as the covariate as IMMS score was found to predict germane cognitive load.

*R.Q.4.*Simple computation of the mean and standard deviation of total IMMS score was performed.

R.Q.5-9. Answers to these questions were generated via PROCESS version 2.13, which is a computational tool that was installed as a custom dialog into IBM SPSS 19 software's Analyze menu. PROCESS is written by Andrew Hayes and its documentation is available in (see Appendix A in Hayes, 2013). Among other functions, PROCESS carries out regression-based mediation analysis needed to answer research questions similar to R.Q.5 through R.Q.9. This tool was used to generate all of the reported model coefficients, standard errors, *t*-test values, *p*-values, and bias-corrected confidence intervals with 10,000 bootstraps based on ordinary least squares OLS regression analysis (since all of the studied dependent variables are continuous). This is true for all of the reported direct and indirect effects of both simple and multiple mediator models described in Chapter IV. The current version of PROCESS can analyze 76 mediation, moderation, and conditional process models. To answer R.Q. 5, 6, 7, and 9, model=4 was utilized because it "is used for both simple mediation and parallel multiple mediator models" (Hayes, 2013, pp. 132-134). For

R.Q.8, model=6 was used because it "tells PROCESS this is a serial multiple mediator model" (Hayes, 2013, p. 151).

Answers to R.Q. 5-9 were generated within the following analytical framework: First, variables of studied models should be correlated in the first place before establishing causation. Second, for variable *X* to cause variable *Y*, *X* should happen first. Third, competing alternative explanations should be entertained before claiming a causal relationship between the two variables. Fourth, possible confounding variables to the studied relationship should be controlled.

CHAPTER IV

RESULTS

As detailed below, this research met its three objectives. First, it demonstrated that the proposed version of multimedia pre-training did help college students better understand the concept of substrate specificity of enzymes, reflected by improved transfer performance. Second, it showed that the used instructional kit motivated students to learn about this concept.Third, it offered an explanation for the process through which both treatments, pre-training and the instructional kit, affect/influence transfer performance through manipulation of prior factual knowledge and the two types of cognitive load, intrinsic and germane.

In this chapter, results of missing values analysis and demographic statistics open the discussion. Validation of developed assessment instruments is established before discussing answers to the 9 Research Questions R.Q.'s. Reliability of scores is provided along with answers to these questions.

Missing Values

Overall summary of missing values showed a non-threatening percentage, 2.66%. Except for SAT (98.2%) and science ACT scores (44.1%), percentage of missing values per variable ranged from 0% to 19.8% with a median of 0.9%. Therefore, only SAT and science ACT scores were excluded from MI procedure. These two variables were not used in any sort of analysis.

Demographic Statistics

One hundred eleven college students participated in this study. Forty eight participants were science students (biology, 43; biochemistry, 5) and 63 were non-science students pursuing majors that require at least a general biology course (medical technology, nursing, and nutrition). The majority of participants were 18-24 years of age (85.26%), females (67.7%), white/Caucasian (70.3%), fully enrolled college students (88.3%), and native English speakers (99.1%). Completed years of college education ranged from zero (freshman) to six (graduate) with a median of 3 years of college (junior).Students were equally distributed in the pre-training, no pre-training, and post-training groups (*n*=37 in each group). Demographic distribution of students was similar across the three groups based on age, gender, ethnicity, language, years of college education, college enrolment, GPA, major, and number of taken biochemistry courses, Table 2. Survey scores of basic computer skills were high (α=.831), 28.42±2.625 (max. score=30), and similar between the two groups that used the computer, Table 2. To understand reported statistics in Table 2, codes for variables appearing in this table are listed below:

- age (1, 18yrs or younger; 2, 19-24yrs; 3, 25-29yrs; 4, 30-44yrs; 5, 45yrs or older),
- gender (1, female; 2, male),
- ethnicity (1, Asian; 2, African American/Black; 3, Caucasian/White; 4, Hispanic/Latino; 5, Native American; 6, Pacific Islander; 7, Other),
- language (1, English; 2, Spanish; 3, Other),
- years of college education (0, high school; 1, freshman; 2, sophomore; 3, junior; 4, senior; 5, bachelor; 6, graduate),
- college enrolment (1, full-time; 2, part-time),
- GPA (1, less than 3.0; 2, 3.0-3.3; 3, 3.4-3.6; 4, 3.7-3.9; 5, above 3.9),
- major (1, science; 2, non-science), and
- number of taken biochemistry courses (0, none; 1, a single course; 2, two courses)

Prior knowledge, self-rating of mental effort, rated difficulty of the learning environment, as well as perceived difficulty of test content, were all measured via pre-test (1) and revealed homogeneity among the three groups in these terms, Table 2. On average, pre-test scores (α=.509) demonstrated that study participants were learners of low prior knowledge, 2.14±1.346 (max. score=5). Referring to anchor terms of mental effort and difficulty rating scales, participants reported medium mental effort, 5.02±1.396, moderately easy learning environment, 2.97±1.872, and neither easy nor difficult test content, 5.31±1.762 (range=1-9).

Table 2

Demographic Distribution of Participants Among the Three Groups

Demographics	Mean±*SD*	ANOVA
Age	1.98±.726	$F(2, 108)=.628$, *ns*
Gender	1.32±.470	$F(2, 108)=.362$, *ns*
Ethnicity	2.81±.684	$F(2, 108)=.229$, *ns*
Language	1.01±.095	$F(2, 108)=1.000$, *ns*
College Education	2.93±1.762	$F(2, 108)=2.527$, *ns*
GPA	2.68±1.129	$F(2, 108)=.756$, *ns*
Enrolment	1.12±.468	$F(2, 108)=1.976$, *ns*
Major	1.57±.498	$F(2, 108)=2.968$, *ns*
Number of Taken Biochemistry Courses	.65±.860	$F(2, 108)=1.362$, *ns*
Basic Computer Skills	28.42±2.625	$t(46.895)=1.835$, *ns*
Prior Knowledge	2.14±1.346	$F(2, 108)=2.205$, *ns*
Rating of Mental Effort	5.02±1.396	$F(2, 108)=.788$, *ns*
Difficulty Rating of Learning Environment	2.97±1.872	$F(2, 108)=.679$, *ns*
Difficulty Rating of Test Content	5.31±1.762	$F(2, 108)=2.525$, *ns*

Note. SD=standard deviation, *ns*= not statistically significant, $p>.05$

Validation of Knowledge Assessment Instruments

Face Validity

All of the five examiners arrived to an agreement on every test Item except a single Item which was eliminated from the final version of the instrument (see Table 1 in Chapter III).

Content Validity

As stated earlier, seven faculty members —five biochemists and two chemists— from different universities rated each Item as *essential E*, *useful but not essential U*, or *not necessary NS*, Table 3. All test Items were included in the version distributed to the sample of students because 85.7-100% of the responses deemed each Item as *essential/useful but not essential* E/U to assess knowledge and/or understanding of enzyme specificity. These responses are considered valuable to other researchers who may use this instrument in the future because 92% of the Items received 100% E/U rating.

Table 3

Subject Matter Expert Rating of Item Importance

	Test Item	Met Objective	Subject Matter Expert Rating			%E/U
			E	U	NS	
Retention Section	1.	R1	7	0	0	100
	2.a	R3	6	1	0	100
	2.b	R3.b	5	2	0	100
	2.c	R3.c	4	3	0	100
	2.d	R3.d	4	2	1	85.7
	3	R2	6	1	0	100
Transfer Section	1*	Illustrative	3	2	2	—
	2*	Illustrative	3	2	2	—
	3	T1.a	5	2	0	100
	4	T1.a	4	3	0	100
	5	T1.b, T2	5	2	0	100
	6	T1.b, T2	4	3	0	100
	7	T1.a, T1.b, T2	4	3	0	100
	8	T1.b	6	1	0	100
	9	T1.a	6	1	0	100
	10	T1.a	6	1	0	100
	11	T1.b	6	1	0	100
	12	T1.a	6	0	1	85.7

Table 3 (continued).

	Test Item	Met Objective	Subject Matter Expert Rating			%E/U
			E	U	NS	
Transfer Section	13	T1.a, T1.b	5	2	0	100
	14	T1.a, T1.b	5	2	0	100
	15	T1.a, T1.b	5	2	0	100
	16	T1.a, T1.b	5	2	0	100
	17	T3	6	1	0	100
	18	T3	5	2	0	100
	19	T3	6	1	0	100
	20	T3	6	1	0	100
	21	T3	5	2	0	100

Note. E=essential, U=useful but not essential, NS=not necessary.

*Denotes that Item is illustrative and hence is not rated.

Discriminant Validity

Overall test. Controlling for the significant difference in pre-test scores (α=.509), $t(109)=4.69$, $p<.001$, One-Way ANCOVA demonstrated that participant's major (science *versus* non-science) had a significant effect on their overall test score (α=.740) with science students scoring higher than their non-science counterparts, $F(2, 108)=11.45$, $p=.001$. The observed power of this test was .918 which indicates that a Type I error is unlikely. Therefore, this assessment instrument satisfies the discriminant validity check because it can distinguish between groups that it theoretically should distinguish between.

*Retention section (pre/post-test (1)).*To validate the answer to R.Q.1, discriminant validity of the test section assessing content knowledge "*Remember*" of science and non-science students was examined. As theorized, science students scored higher than their non-science counterparts before instruction (α=.509), $F(1, 109)= 21.99$, $p<.001$. Similarly, they did so after instruction (α=.604), $F(1, 108)= 14.16$, $p<.001$.Therefore, this cognitive construct satisfies the discriminant validity check. However, poor reliability of pre-test (1) scores is acknowledged (α=.509).

*Transfer section (post-test (2)).*To validate answers to research questions R.Q.1, 2, and 5-9, discriminant validity of the entire transfer section as well as each of its cognitive orders "*Understand* through *Create"* were examined. Given that ability to transfer knowledge is inherently related to the amount of acquired knowledge in the first place, participants' scores on retention were used as a covariate while testing transfer performance because of the obtained significant difference in retention (see the previous paragraph). The choice for this measure is statistically supported by the fact that scores on retention significantly predicted transfer scores, β=1.23, $t(108)=6.23$, $p<.001$. Retained knowledge after instruction explained a significant portion of the variance in transfer test scores, $R^2=.265$, $F(1, 108)=38.84$, $p<.001$.

Science students demonstrated significantly higher transfer performance than non-science students with a participant's major explaining almost 32% of the variance in transfer performance (α=.706), $R^2=.319$, $F(2, 107)=24.69$, $p<.001$. Therefore, this cognitive construct satisfies the discriminant validity check because it can discriminate between both groups. Again, the transfer section involved '*Understand* through *Create'* Items that are discussed in details below.

Understand. In this set of questions, science students demonstrated higher understanding of the presented concept than non-science students (W=3,118, $p=.008$).

Apply. Here, science students were better able to apply scientific information to a real experimental problem than non-science students $F(2, 107)=17.27$, $p<.001$.

Analyze. In this set of Items, a participant's major had a significant effect on their ability to analyze the results of scientific experiments presented in the test with science students scoring higher than their non-science counterparts $F(2, 107)=6.25$, $p=.003$.

Evaluate. In this cognitive order, participants of science majors showed better ability to critique the given hypotheses than their non-science counterparts $F(2, 107)=3.09$, $p<.05$. Despite being statistically significant, this difference may not be considered as practically so with a Cohen's *d* of as low as .21. This low effect size can be attributable to the fact that non-science students in this sample were medical technologists, nurses, and nutritionists who are trained to evaluate data-driven claims based on defined criteria. For example, they are used to evaluate claims such as "diabetes *may contribute* to dehydration" based on relevant facts such as "diabetic patients experience excessive urination." Therefore, it might be helpful to test this cognitive dimension on a sample with different non-science majors.

Create. In this question, science students were better able to conceive a novel solution to the given scientific problem than non-science students (W=3,159, $p=.008$).

Item Analysis

As stated earlier, difficulty values are recommended not to exceed the range of [0.2-0.9] (Chang et al., 2011). As displayed in the second leftmost column of Table 4, difficulty values for the entire test Items fell within this range and therefore were neither too easy nor too difficult for the sampled students.

As a reliability measure, *D* was computed based on overall test scores (Aiken, 2003). This is shown on the rightmost column of Table 4 and shows that all Items are discriminating positively except for Item 1 and Item 16 which were dropped from the test. As a validity measure however, *D* was computed based on the major of the participant — science *versus* non-science students (Aiken, 2003). This is shown on the second rightmost column of Table 4. Based on this measure, combined with Brennan's criteria described earlier, Items 1.b and 18 are considered unacceptable because they are negatively discriminating Items ($D<0.0$). Items 1.c, 1.d, 5, 11, 17, 19, and 20 are acceptable because they are positively discriminating Items (*D*>0.2). Items 9, 12, and 21 are not ideal because they are non-discriminating with low *p* values (*p*<0.5, *D*<0.2). The rest of the Items are acceptable because they are non-discriminating with high *p* values (*p*>0.5, *D*<0.2). Based on this Item analysis, all of test Items were retained except for Item 1, 2.b, 16, and Item 18, which are highlighted by an asterisk in Table 4.

Table 4

Item Difficulty and Item Discrimination Indices for Both Sections of the Test

Retention Section					
Item	*p*	*p* for sciences [a]	*p* for non-sciences [b]	*D* [c]	*D'* [d]
1*	0.36	0.48	0.27	0.21	-0.14
2.a	0.55	0.65	0.47	0.18	0.59
2.b*	0.76	0.71	0.81	-0.10	0.24
2.c	0.62	0.77	0.50	0.27	0.55
2.d	0.43	0.58	0.31	0.28	0.66
3	0.61	0.71	0.53	0.18	0.41
Transfer Section					
Item	*p*	*p* for sciences [a]	*p* for non-sciences [b]	*D* [c]	*D'* [d]
3	0.81	0.88	0.76	0.11	0.34
4	0.81	0.85	0.78	0.08	0.48
5	0.68	0.81	0.57	0.24	0.62
6	0.74	0.79	0.70	0.09	0.55
7	0.22	0.88	0.71	0.16	0.34
8	0.90	0.31	0.14	0.17	0.47
9	0.78	0.94	0.87	0.06	0.21
10	0.61	0.65	0.59	0.06	0.34
11	0.64	0.77	0.54	0.23	0.41

Table 4 (continued).

Transfer Section					
Item	*p*	*p* for sciences [a]	*p* for non-sciences [b]	*D* [c]	*D'* [d]
12	0.35	0.40	0.32	0.08	0.24
13	0.62	0.67	0.59	0.08	0.55
14	0.54	0.56	0.52	0.04	0.41
15	0.68	0.75	0.63	0.12	0.59
16*	0.29	0.33	0.25	0.08	-0.07
17	0.38	0.58	0.22	0.36	0.55
18*	0.28	0.25	0.30	-0.05	0.28
19	0.42	0.56	0.32	0.25	0.69
20	0.49	0.73	0.30	0.43	0.69
21	0.41	0.44	0.38	0.06	0.45

[a] Difficulty index computed just for sample of science students.

[b] Difficulty index computed just for sample of non-science students.

[c] Discrimination index based on the external criterion (participant's major)

[d] Discrimination index based on the internal criterion (overall test score)

[*] Unacceptable Item

Item 7. Validity and reliability of Item 7, the only free response question, are discussed in this section. Recall that students were asked to design an artificial substrate with higher affinity to the enzyme but still receive no enzyme reactivity. They were required to explain their solution plan both in words and in drawings. One students' response is presented below to demonstrate how their drawings were used to validate interpreting their verbal responses. Participant_465305: "You could add another bonding site that interrupts the site of the bond to be broken. Interrupts as in stops it from fully closing on it." By referring their verbal response to the drawing in Figure 17, one can tell that this participant conceived the solution through two structural modifications to the natural substrate. To increase binding affinity, they added a negatively charged group to the rightmost side of the substrate “add another bonding site” to utilize the free positive charge on the enzyme. Alongside, they moved the bond to be broken away from the catalytic group to “stop[s] [the bond] from fully closing on [the catalytic group].” This is evident from the up-down open headed arrow in the drawing. This modification is expected to reduce enzyme reactivity toward the substrate.

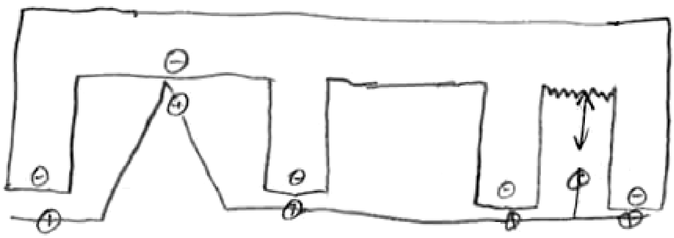

Figure 17. A drawing by one of the participants in partial response to Item 7. They added a negatively charged group to the rightmost side of the substrate as well as raised the bond-to-be-broken away from the catalytic amino group.

To help reduce potential bias, Item 7 was graded by two independent raters based on a predefined rubric (see Figure 16 in Chapter III) and examination of absolute agreement resulted in a high intra-class correlation coefficient (.983).

To wrap up, these knowledge assessment instruments fulfilled content and face validity. More importantly it satisfied discriminant validity checks for a sample of science and non-science students. It, therefore, is a valid measure for assessing potential differential effects of CTML instructional interventions such as multimedia pre-training on student populations that differ on their prior knowledge, either initially or as a result of pre-training. Imputation of missing values permitted answering research questions R.Q. 5-9 with a complete dataset.

R.Q.1: What is the difference, if any, in retention and transfer performance among the three groups?

Table 5

Mean Retention, Transfer, and Standard Deviations for the Three Groups

	Retention		Transfer	
Group	Mean	*SD*	Mean	*SD*
Pre-training	3.45	1.37	11.47*	2.79
No pre-training	2.71	1.54	9.27	3.59
Post-training	2.72	1.41	8.44	2.31

Note.SD=standard deviation

* Asterisk denotes that the pre-training group outperformed the other two groups, $p<.05$.

Table 5 shows the means and standard deviations for retention (post-test (1)) and transfer (post-test (2)) tests for each group. One-Way ANOVA's were conducted on each test score with treatment group as the independent variable and IMMS score as the covariate. Motivation to learn about the presented material through the physical model (IMMS score) was observed to be significantly different among the three groups, $F(2, 108)=5.628$, $p=.005$, and correlated with retention ($r=.280$, $p=.003$) and transfer ($r=.528$, $p<.001$). Hence it was controlled to improve sensitivity of the *F*-test. The groups did not differ on retention test score ($\alpha=.604$), $F(2, 106)=1.654, p>.05$. However, they differed significantly on their transfer test scores ($\alpha=.706$), $F(2, 106)=5.197$, $p=.007$, with pairwise comparison (LSD) revealing that the pre-training group outperformed the other two groups ($p<.05$) which did not differ from each other ($p>.05$). In other words, the pre-training treatment improved college students' transfer of their acquired knowledge of substrate specificity to novel situations. Effect sizes were .525 for retention and 1.183 for transfer (Cohen's *d*, based on comparing the pre-training and post-training groups).

Additional Analysis Related to R.Q.1

Although the three groups did not differ on retention performance, an overall significant increase in test scores was shown from paired sample *t-test* indicating a successful treatment in terms of conceptual knowledge gain, $t(110)=-6.242$, $p<.001$. Also, participants in the pre-training group demonstrated higher knowledge of major components, underlying processes, and terms related to the targeted causal model than the other two groups ($p<.001$) which did not differ from each other ($p=.543$), $F(2, 108)=21.215$, $p<.001$. This outperformance for the pre-training group is reflected by pre-test (2) scores ($\alpha=.752$) taken right after the pre-training episode and before the main lesson.

R.Q.2: What is the difference, if any, in transfer performance among the three groups based on the cognitive dimension of revised Bloom's taxonomy?

Table 6

Means and Standard Deviations of Cognitive Orders for the Three Groups

Group		Cognitive Dimension of Transfer Performance				
		Understand	*Apply*	*Analyze*	*Evaluate*	*Create*
Pre-training	Mean	3.70*	2.22‡	3.11	2.13	.32
	SD	.66	1.36	.99	1.00	.38
No pre-training	Mean	2.76	1.65	2.92	1.76	.19
	SD	1.26	1.38	1.06	1.06	.32
Post-training	Mean	2.65	1.22	2.78	1.65	.15
	SD	1.13	1.06	1.06	.95	.31

Note.SD=standard deviation

** Asterisk denotes that the pre-training group outperformed the other two groups, $p<.05$.

‡ Asterisk denotes that the pre-training group outperformed one of the other two groups, $p<.05$.

From left to right, Table 6 shows the means and standard deviations of scores on *Understand*, *Apply*, *Analyze*, *Evaluate*, and *Create* test Items for each group. One-Way ANOVA's were conducted on each of the 5 cognitive orders (*see Table 1 in chapter III*) with treatment group as the independent variable. Performance of the three groups differed significantly on *Understand* Items (α=.592), $F(2, 108)=11.294$, $p<.001$. Tukey's test revealed that the pre-training group outperformed the other two groups ($p\leq.001$) which did not differ from each other ($p>.05$). In other words, the pre-training treatment moved college students to a better understanding of substrate specificity. Performance of the pre-training group was also better than the post-training group on *Apply* Items (p=.010) but did not differ from performance of the control group (p=.138), $F(2, 108)=5.755$, $p<.01$. It is worth mentioning here that, unlike other test Items, *Apply* Items required knowledge of possible interactions between molecules of different polarity and/or charge, which are concepts covered in high school chemistry. Therefore, differential preparedness to college education, reflected by total ACT scores, might have influenced performance on these particular Items. This hypothesis is supported by the observation that participants in the pre-training group reported significantly higher ACT scores than the post-training group (p=.010) but not different from those in the control group (p=.123), $F(2, 108)=4.611$, $p<.05$. Running the F-test again, while controlling for total ACT scores, demonstrated that the pre-training treatment did not help college students better apply their understanding of substrate specificity (α=.679), $F(2, 107)=2.744$, $p>.05$. This finding replicated for performance on *Analyze* (α=.564; $F(2, 108)$=. 910, $p>.05$), *Evaluate* (α=.616; $F(2, 108)=2.380$, $p>.05$), and *Create* Items (ICC=.983; $F(2, 108)=2.317$, $p>.05$).

R.Q.3: What is the difference, if any, in extraneous, intrinsic, and germane cognitive loads among the three groups?

Table 7

Mean Extraneous, Intrinsic, and Germane Cognitive Load for the Three Groups

	Extraneous		Intrinsic		Germane	
Group	Mean	*SD*	Mean	*SD*	Mean	*SD*
Pre-training	2.73	1.27	3.91*	1.24	5.27	1.29
No pre-training	3.21	1.72	4.72	1.43	6.14	1.47
Post-training	2.88	1.83	4.65	1.15	5.80	1.16

Note.*SD*=standard deviation

* Asterisk denotes that the pre-training group outperformed the other two groups, $p<.05$.

Table 7 shows the means and standard deviations of self-ratings on extraneous, intrinsic, and germane cognitive loads for each group. One-Way ANOVA's were conducted on extraneous and intrinsic cognitive load ratings with treatment group as the independent variable. The three groups did not differ on their rating of extraneous cognitive load (α=.922), $F(2, 108)=.840$, $p>.05$. However, they significantly differed on their rating of intrinsic cognitive load (α=.887), $F(2, 108)=4.585$, $p=.012$. Pairwise comparison (Tukey HSD) revealed that the pre-training group outperformed the other two groups ($p<.05$), which did not differ from each other ($p>.05$). In other words, the pre-training treatment helped college students to process the concept of substrate specificity with less mental effort. It also helped them process assessment questions with less mental effort compared to the other groups[8] (α=.894), $F(2, 108)=4.903$, $p=.009$. One-Way ANCOVA was conducted on rating of

[8]Difference in intrinsic cognitive load during *assessment* is considered an ancillary finding in this research. However, this outcome is of high interest to researchers who study performance efficiency (Paas et al., 2003; Paas & van Merriënboer, 1993). Please note that discussion of intrinsic cognitive load throughout this work pertains to that measured during *instruction*, unless specified otherwise.

germane cognitive load with IMMS (motivation) score as the covariate as it significantly predicted rating on germane cognitive load, β=-.025, t(109)=-2.79, p=.006. This observation is theoretically supported by the notion that germane cognitive load is “caused by the motivation of the learner” (Mayer, 2009, p.80). Hence, controlling for motivation, the three groups did not differ on their rating of germane cognitive load (α=.829), $F(2, 108)=2.892$, $p>.05$. Effect sizes were .095 for extraneous, .432 for germane, and .619 for intrinsic cognitive loads (Cohen’s *d*, based on comparing the pre-training and no post-training groups).

R.Q.4: What is the motivational level of students to learn about substrate specificity through the instructional kit?

Reliability scores for IMMS survey are summarized in Table 8. Cronbach’s α coefficient values for the entire scale as well as all of its four subscales were above 0.7.

Table 8

Reliability Scores of Instructional Material Motivation Survey (IMMS)

	Subscales				Entire Scale
	Attention	Relevance	Confidence	Satisfaction	
Score	.834	.798	.765	.804	.932

Results obtained from IMMS scale showed high overall motivation (4.100±.537) as well as high scores in all of the constituting subscales, attention (4.370±.503), relevance (4.101±.660), confidence (3.910±.635), and satisfaction (4.004±.654). Collectively, these results suggest that students were motivated to learn about enzyme specificity through using the instructional kit (4.1*out of* 5). Specifically, this material helped them stay engaged in the learning process (4.4 *out of* 5) as they perceived it to be relevant (4.1 *out of* 5), easy to use (3.9 *out of* 5), and enjoyable (4.0 *out of* 5). These results are displayed in Figure 18.

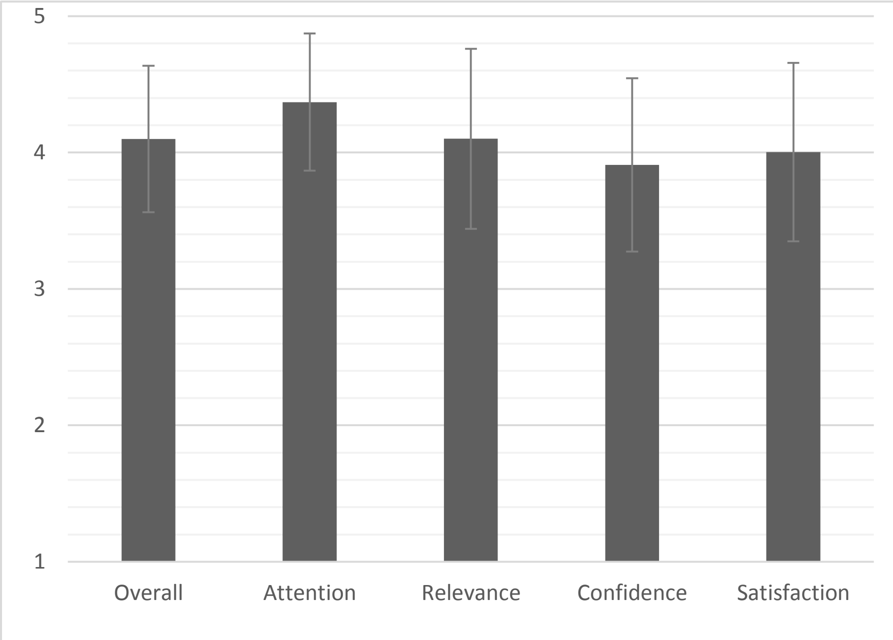

Figure 18. A bar graph showing overall motivation (mean ± standard deviation) as well as reported levels of attention, relevance, confidence, and satisfaction with the instructional kit.

Despite that all groups received this same treatment, a significant difference in their motivational levels was observed based on One-Way ANOVA, $F(2, 108)=6.029$, $p=.005$. It might be tempting to claim that pre-training treatment increased learner's motivation to learn about the presented concept since the pre-training group was more motivated than the other two groups ($.05<p<.10$). Yet, regression analysis demonstrated that pre-training does not predict motivation, $\beta=-1.709$, $t(109)=-1.045$, $p=.298$. Therefore, a spurious correlation between pre-training and motivation might be in action. Further discussion will follow in Chapter V.

Proposing an Explanation for How Multimedia Pre-training is Fostering Transfer Performance

So far, discussion revolved around whether pre-training positively affects transfer. The question that follows is “how” pre-training exerts this effect on transfer. In other words, what are the intervening variable(s) ─mediators─ that explain(s) this effect. For example, we previously found evidence that participants in the pre-training group reported less intrinsic cognitive load than the no pre-training group, $t(72)=-2.615$, $p=.011$. This reduction in intrinsic cognitive load, in turn led to increased transfer performance (controlling for no/pre-training effect), $\beta=-.398$, $t(71)=-3.706$, $p<.001$. However, does this follow that intrinsic cognitive load is a true mediator in this process? If so, are there other mediators that can be detected under the given experimental conditions? If so, are they running in series and/or in parallel with this mediator? A set of mediation analyses were conducted to answer these questions.

Causal order is entertained in this analysis since a cause-effect association entails that a cause temporally precedes its effect. For example, a logical direction of causal flow in this experiment would run from multimedia training (*X*) to reduction in intrinsic cognitive load (*M*) to higher transfer performance (*Y*). This sensible flow demands excluding participants in the post-training group from this particular analysis as the direction would otherwise be, *M* to *X* to *Y*; which is arguably counterintuitive. Therefore, only participants in the pre-training and control group are included in this analysis, $n_{Total}=74$.

R.Q.5: Does intrinsic cognitive load mediate the effect of pre-training on transfer performance?

Model-1: A Simple Mediator Model

Recall that the driving hypothesis in this study is that pre-training on components, underlying processes, and terms related to substrate specificity would prompt learners to construct component models before engaging in construction of the casual model during instruction. Consequently, and relative to a control group, the pre-training group would have the advantage of using freed space in working memory (reflected on reduced intrinsic cognitive load) to engage in further integration of the instructional message with prior knowledge (reflected on increased transfer performance).

Table 9

Descriptive Statistics for Ratings of ICL and Transfer Test Scores in the Two Conditions

Condition		*M* ICL	*Y* Transfer
Pre-training (*X*=1)	Mean	3.908	11.474
	SD	1.238	2.795
No pre-training (*X*=0)	Mean	4.722	9.270
	SD	1.430	3.599
	Mean	4.315	10.372
	SD	1.390	3.387

Note. *M*=putative mediator, *Y*=outcome, ICL=intrinsic cognitive load, *SD*=standard deviation

Descriptive statistics for each of the two variables, intrinsic cognitive load and transfer test scores, in the two conditions are illustrated in Table 9. Results of regression analysis are summarized in Table 10, and regression coefficients are superimposed on a statistical model presented in Figure 19. The influence of motivation on transfer is statistically controlled to obtain better estimates of the models' coefficients. This measure is taken because motivation was found to be a significant predictor of transfer, β=.139, $t(72)=5.998$, $p<.001$.

The *a* coefficient indicates that two learners that differed by one unit on multimedia training (0, control; 1, pre-training) are estimated to differ by *a*=-6.506 units on intrinsic cognitive load. So, those assigned to pre-training group are 6.506 units lower on intrinsic cognitive load than those assigned to the no pre-training group.

Table 10

Coefficients of a Simple Mediation Model (model-1)

		Consequent						
		M (ICL)				*Y* (Transfer)		
Antecedent		Coeff.	*SE*	*p*		Coeff.	*SE*	*p*
X (Group)	*a*	-6.506	2.488	.011	c'	0.977	0.650	.137
M (ICL)		—	—	—	*b*	-0.074	0.031	.019
C (MOT)		—	—	—	*f*	0.105	0.024	< .001
Constant	i_1	44.278	3.934	< .001	i_2	-0.215	3.263	.947
		R^2= 0.087 $F(1, 72) = 6.836, p = .011$				R^2= 0.417 $F(3, 70) = 16.719, p < .001$		

Note. M=putative mediator, ICL=intrinsic cognitive load, *Y*=outcome, Coeff.=coefficient, *SE*=standard error, MOT= motivation

The *b* coefficient means that two learners assigned to the same group, but differ by one unit on intrinsic cognitive load are estimated to differ by *b*=-.074 units on transfer test score. The negative sign of *b* means that those relatively low on intrinsic cognitive load are estimated to score higher on the transfer test. The product of multiplying the two coefficients, *a* and *b*, yields the indirect effect of pre-training on transfer through intrinsic cognitive load, *ab*=.483. So, relative to the control group, those who are assigned to pre-training were, on average, .483 units higher on their transfer test score as a result of reduction in intrinsic cognitive load.

The direct effect is the portion of the effect of pre-training on transfer that is unexplained by intrinsic cognitive load and is estimated as *c'*=.977. This means that two learners assigned to different groups but are equal on intrinsic cognitive load are estimated to differ by .977 units on their transfer test score.

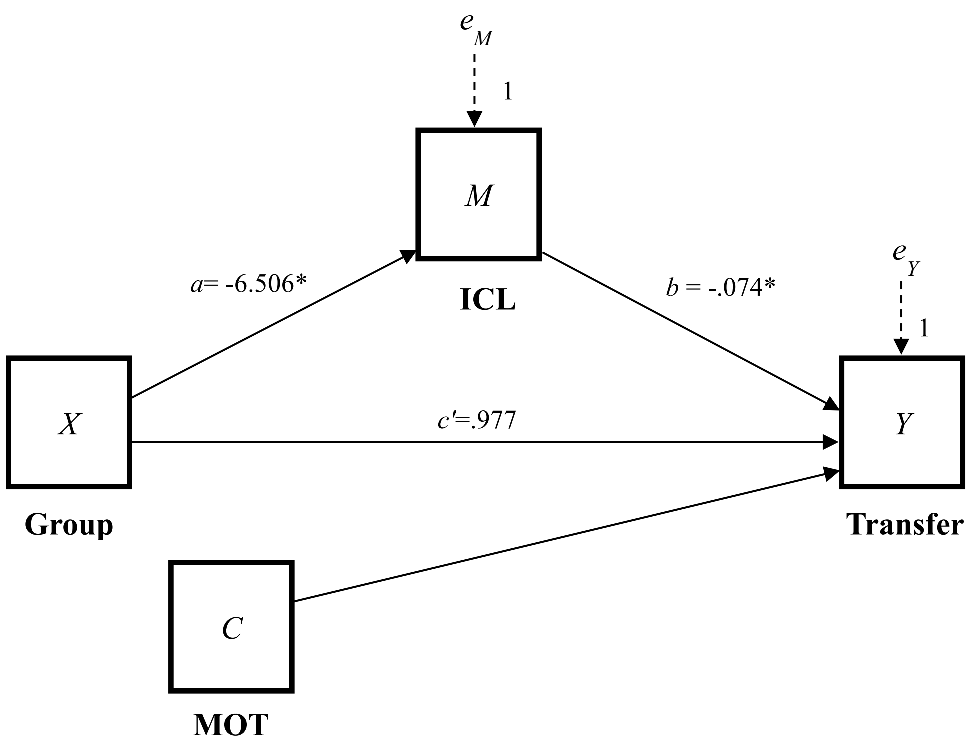

Figure 19. A statistical diagram of a simple mediation model (model-1) proposing an explanation for how pre-training might be affecting transfer performance. Key: *X*=independent variable; *M*=putative mediator; *Y*=outcome; *C*=controlled variable; e_M & e_Y=errors in estimation of *M* and *Y* respectively; *a*, *b*, &*c'*=regression coefficients; ICL=intrinsic cognitive load; MOT=motivation. Asterisk denotes significance at $p<.05$.

The direct effect was not significant though, *c'*=.977, $t(72)=1.503$, $p>.05$. This *p*-value means that the true value of the direct effect can be zero within a 95% confidence interval (CI) that is estimated to range from -.3191 to 2.2724. As with the direct effect, significance of the indirect effect can be inferred by deriving a *p*-value for a given null hypothesis (normal theory approach) or through generating an estimate of a confidence interval. Nevertheless, estimates of 95% bias-corrected (bc)

10,000 bootstrap confidence intervals were solely utilized to test significance of *in*direct effects all through this analysis based on the notion that, “bootstrap confidence intervals [...] yield inferences that are more likely to be accurate than when the normal theory approach is used” (Hayes, 2013, p. 106). Hayes’ book (2013, pp. 102-116) can be consulted for a detailed discussion on why it is recommended to rely on this method in search for real indirect effects. The indirect effect of pre-training on transfer through intrinsic cognitive load was statistically significant as the 95% bc bootstrap CI for the true product ${}_{T}ab$ is estimated to range from .0893 to 1.2672. Since the entire interval lies above zero, it can be claimed that the effect of pre-training on transfer through intrinsic cognitive load is positive.

The total effect of the treatment on transfer is estimated as, *c*=1.3231. This tells us that learners who received pre-training, on average, scored 1.3231 units higher on transfer test than those who did not receive pre-training. The total effect of pre-training on transfer was statistically significant, *c*=1.323, *t*(72)=2.022, *p*=.047. The following conceptual diagram, Figure 20, summarizes the discussion in this section.

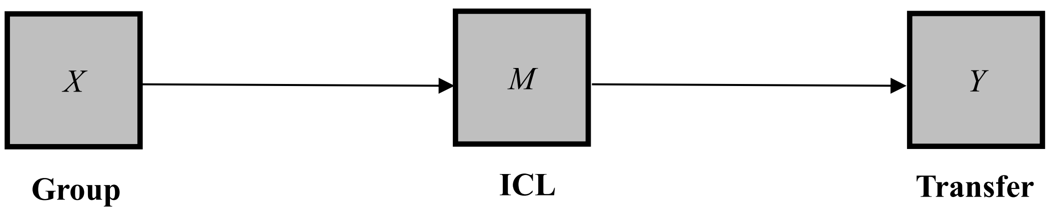

Figure 20. A conceptual diagram of a simple mediation model (model-1) proposing an explanation for how pre-training might be affecting transfer performance. Key: *X*=independent variable; *M*=putative mediator; *Y*=outcome; ICL=intrinsic cognitive load.

Again, the direct effect of pre-training on transfer was not significant (-.3191 to 2.2724). This might be tempting to celebrate discovery of the entire mechanism through which pre-training fosters transfer as this would mean that association between the two variables is entirely accounted for by the indirect effect just described. Nevertheless, Rucker et al. (2011) and Hayes (2013) argue that this is an empty reasoning “that should be abandoned” because it is “too sample-size-dependent” and has no theoretical value (Hayes, 2013, p. 172). Along these lines, the knowledge dimension of the pre-training treatment may also be mediating some of the effect of pre-training on transfer performance. Recall that we previously found evidence that participants in the pre-training group demonstrated higher prior factual knowledge than the no pre-training group, $t(72)=-4.849$, $p<.001$. Increase in prior factual knowledge, in turn led to increased transfer performance (controlling for no/pre-training effect), $\beta=.667$, $t(71)=6.572$, $p<.001$. Analysis supporting this claim is described below.

R.Q.6: Does prior factual knowledge mediate the effect of pre-training on transfer performance?

Model-2: A Simple Mediator Model

Descriptive statistics for each of the two variables, pre-test (2) score (measuring prior factual knowledge) and transfer test score, in the two conditions are illustrated in Table 11. Results of regression analysis are summarized in Table 12, and regression coefficients are superimposed on a statistical model presented in Figure 21. The influence of motivation on transfer is statistically controlled for the same reason described earlier.

Table 11

Descriptive Statistics for Prior Factual Knowledge (PFK) and Transfer Test Scores in the Two Conditions

Condition		*M* PFK	*Y* Transfer
Pre-training (*X*=1)	Mean	12.243	11.474
	SD	2.639	2.795

Table 11 (continued).

Condition		*M* PFK	*Y* Transfer
No pre-training (X=0)	Mean	8.892	9.270
	SD	3.273	3.599
	Mean	10.568	10.372
	SD	3.400	3.387

Note. M=putative mediator, *Y*=outcome, *SD*=standard deviation

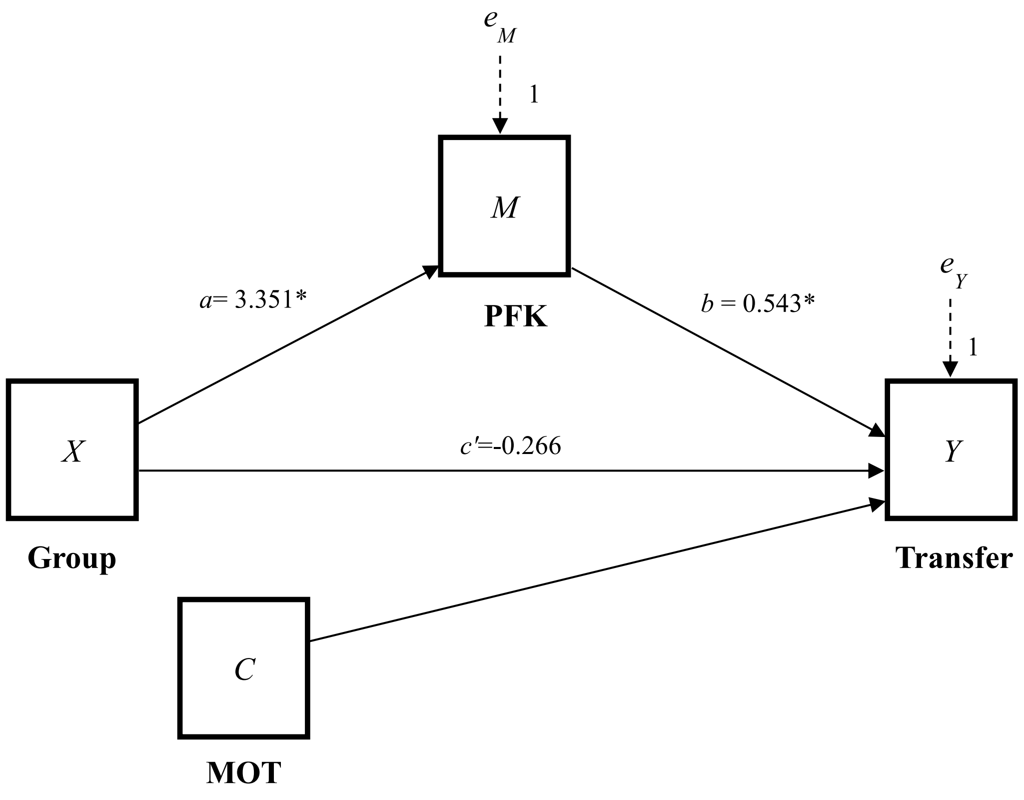

Figure 21. A statistical diagram of a simple mediation model (model-2) proposing an explanation for how pre-training might be affecting transfer performance. Key: X=independent variable; M=putative mediator; Y=outcome; C=controlled variable; e_M & e_Y=errors in estimation of M and Y respectively; a, b, &c'=regression coefficients; PFK=prior factual knowledge; MOT=motivation. Asterisk denotes significance at p<.05.

The *a* coefficient indicates that two learners that differed by one unit on multimedia training (0, control; 1, pre-training) are estimated to differ by a=3.351

units on prior factual knowledge. So, those assigned to pre-training group are 3.351 units higher on prior factual knowledge than those assigned to the no pre-training group. The *b* coefficient means that two learners assigned to the same group, but differ by one unit on prior factual knowledge are estimated to differ by *b*=.543 units on transfer test score. The positive sign of *b* means that those relatively high on prior factual knowledge are estimated to score higher on the transfer test.

The product of multiplying the two coefficients, *a* and *b*, yields the indirect effect of pre-training on transfer through prior factual knowledge, *ab*=1.821. So, relative to the control group, those who are assigned to pre-training were, on average, 1.821 units higher on their transfer test score as a result of increased prior factual knowledge.

The direct effect is estimated as *c'*=-.266. This means that two learners assigned to different groups but are equal on prior factual knowledge are estimated to differ by .266 units on their transfer test scores. However, the direct effect was not significant, *c'*=-.266, *t*(72)=-.440, *p*>.05.

Table 12

Coefficients of a Simple Mediator Model (model-2)

		Consequent						
		M (PFK)				*Y* (Transfer)		
Antecedent		Coeff.	*SE*	*p*		Coeff.	*SE*	*p*
X (Group)	*a*	3.351	0.691	< .001	c'	-0.266	0.605	.661
M (PFK)		—	—	—	*b*	0.543	0.093	< .001
C (MOT)		—	—	—	*f*	0.094	0.020	< .001
Constant	i_1	5.540	1.093	< .001	i_2	-5.441	2.511	.034
		R^2= 0.246				R^2= 0.576		
		$F(1, 72) = 23.509, p < .001$				$F(3, 70) = 31.670, p < .001$		

Note. M=putative mediator, PFK=prior factual knowledge, *Y*=outcome, Coeff.=coefficient, *SE*=standard error, MOT= motivation

The indirect effect of pre-training on transfer through prior factual knowledge was statistically significant as the 95% bc bootstrap CI for the true product $_{T}ab$ is estimated to range from 1.0002 to 3.0396. Since the entire interval lies above zero, we can claim that the effect of pre-training on transfer through prior factual knowledge is positive.

The total effect of this treatment on transfer is estimated as, c=1.323, and is statistically significant, $t(72)=2.022$, $p=.047$. Exactly like the previous model, this model tells us that learners who received pre-training, on average, scored 1.323 units higher on the transfer test than those who did not receive pre-training. The following conceptual diagrams summarize the discussion held so far, Figure 21.

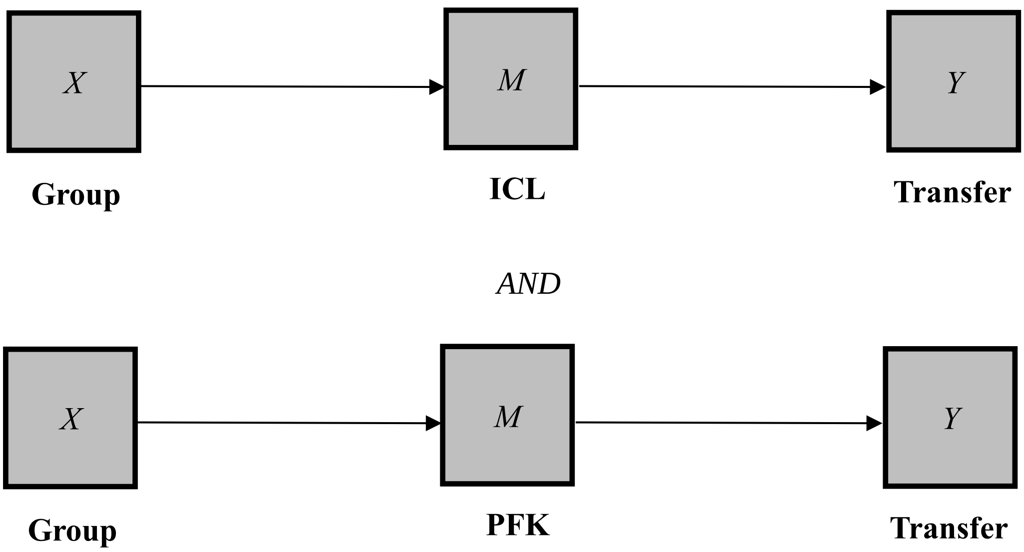

*Figure 21.*A conceptual diagram of two simple mediation models (model-1 & model-2) proposing an explanation for how pre-training might be affecting transfer performance. Key: *X*=independent variable; *M*=putative mediator; *Y*=outcome; ICL=intrinsic cognitive load, PFK=prior factual knowledge.

The Two Models: Separate or Combined?

So, both prior factual knowledge and intrinsic cognitive load were found to mediate the effect of pre-training on transfer. A sensible question that follows is whether one would obtain better estimates if both mediators are included in a single model (Von Hippel et al., 2011) or if they were kept in separate models (Gibbs, Ellison, & Lai, 2011). Perhaps, studying the process both ways can be quite informative (Hayes, 2013).

R.Q.7: Does intrinsic cognitive load mediate the effect of pre-training on transfer performance in parallel with prior factual knowledge?

Model-3: A Parallel Multiple Mediator

Results of regression analysis are summarized in Table 13, and regression coefficients are superimposed on a statistical model presented in Figure 22. Again, influence of motivation on transfer was controlled.

Table 13

Coefficients of a Parallel Multiple Mediator Model (model-3)

		Consequent										
		M_1 (PFK)				M_2 (ICL)				*Y* (Transfer)		
Antecedent		Coeff.	*SE*	*p*		Coeff.	*SE*	*p*		Coeff.	*SE*	*p*
X (Group)	a_1	3.351	0.691	< .001	a_2	-6.506	2.488	.011	c'	-0.303	0.607	.619
M_1 (PFK)		—	—	—		—	—	—	b_1	0.512	0.098	< .001
M_2 (ICL)		—	—	—		—	—	—	b_2	-0.027	0.028	.329
C (MOT)		—	—	—		—	—	—	f	0.088	0.021	< .001
Constant	i_{M1}	5.540	1.093	< .001	i_{M2}	44.279	3.934	< .001	i_Y	-3.245	2.845	.258
		R^2=0.246 F (1,72)=23.509, p<.001				R^2=0.087 F (1,72)=6.836,				R^2=0.582 F (4,69)=23.984,		

Note. $M_{1/2}$=putative mediator, PFK=prior factual knowledge, ICL=intrinsic cognitive load, *Y*=outcome, Coeff.=coefficient, *SE*=standard error, MOT= motivation

The specific indirect effect of pre-training on transfer through prior factual knowledge (*X* to M_1 to *Y*) is estimated as a_1b_1=3.351(.512) =1.717. This specific indirect effect can be claimed as significantly positive because its bootstrap confidence interval is completely above zero (.9182 to 2.9536). The specific indirect effect of pre-training on transfer through intrinsic cognitive load (*X* to M_2 to *Y*) is estimated as, a_2b_2=-6.506(-.027) =.178, and cannot be claimed as significant as its bootstrap confidence interval straddles zero (-.1247 to .6754). The direct effect is estimated as insignificant, *c'*=-.303, *t*(72)=-.499, *p*>.05. Estimate of the total effect did not differ from the previous two models.

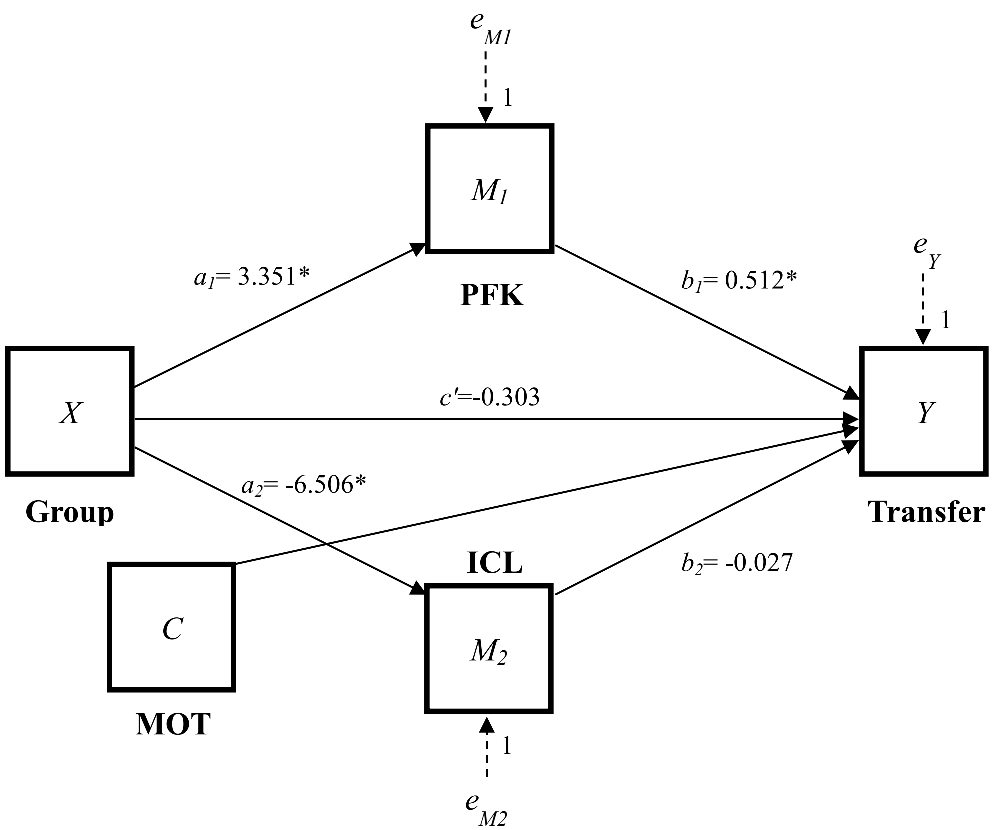

Figure 22. A statistical diagram of a parallel mediation model (model-3) proposing an explanation for how pre-training might be affecting transfer performance. Key: *X*=independent variable; M_1& M_2=putative mediators; *Y*=outcome; *C*=controlled variable; e_{M1}, e_{M2} & e_Y=errors in estimation of M_1, M_2, and *Y* respectively; a_1, b_1, a_2, b_2, &*c'*=regression coefficients; PFK=prior factual knowledge; MOT=motivation; ICL=intrinsic cognitive load. Asterisk denotes significance at *p*<.05.

Based on these statistics, the notion that intrinsic cognitive load mediates the effect of pre-training on transfer is no more supported when both mediators are included in a single model (a_2b_2 can be zero; -.1247 to .6754). This finding drew attention to two theoretical issues in the current model. First, proposing that both putative mediators run solely in parallel means that the two share nothing more than their common cause (pre-training), which is not the case ($r_{partial}$=-.388, p=.001). Additionally, it is known that prior knowledge shapes the cognitive process of knowledge construction (Ausubel, 1968). Therefore, one direction in the causal flow would be that increased prior factual knowledge (M_1) reduces intrinsic cognitive load (M_2) — which puts the two variables in series rather than in parallel.

Second, just because intrinsic cognitive load was found to be mediating the effect of pre-training on transfer in model-1 does not necessarily mean that reduction in intrinsic cognitive load causes increase in transfer performance. A non-causal alternative explanation could be that intrinsic cognitive load is related to transfer because it is correlated with germane (generative) cognitive load, r=.745, p<.001, that might be the authentic variable transmitting the influence of intrinsic cognitive load on transfer. This proposed explanation is theoretically supported by the notion that freed space in working memory, resulting from reduction in intrinsic cognitive load, may only be utilized for rote memorization rather than generative meaningful learning; a condition known as generative underutilization (Mayer, 2011). Hence, the mechanism that might be at work here is that pre-training (X) increases prior factual knowledge (M_1) which reduces intrinsic cognitive load (M_2) which enables engagement in germane cognitive load (M_3) which in turn causes increased transfer performance (Y), Figure 23.

In other words, the process may better be explained through a serial mediator model (X to M_1 to M_2 to M_3 to Y) rather than the parallel mediator model just examined (X to M_1 and M_2 to Y). Mind that this reasoning does not reject the possibility that increased prior factual knowledge would still retain some of its isolated influence on transfer performance (X to M_1 to Y) aside from doing so through cognitive load manipulation (*X* to M_1 to M_2 to M_3 to *Y*). However, it does reject the isolated influence of intrinsic cognitive load on transfer performance (*X* to M_2 to *Y*).

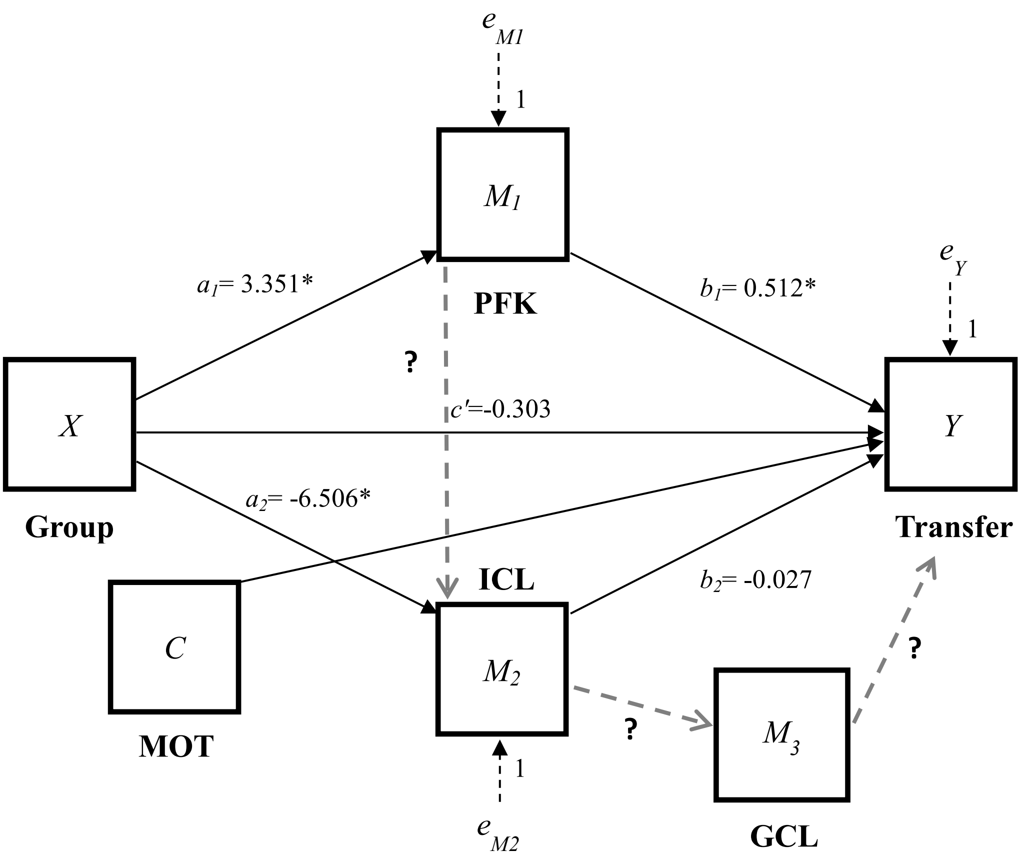

Figure 23. An alternative statistical diagram proposing an explanation for how pre-training might be affecting transfer performance. Key: *X*=independent variable; M_1& M_2=putative mediators; *Y*=outcome; *C*=controlled variable; e_{M1}, e_{M2} & e_Y=errors in estimation of M_1, M_2, and *Y* respectively; a_1, b_1, a_2, b_2, & *c'*=regression coefficients; PFK=prior factual knowledge; MOT=motivation; ICL=intrinsic cognitive load. Asterisk denotes significance at p<.05.

R.Q.8: Does prior factual knowledge, intrinsic, and germane cognitive loads mediate the effect of pre-training on transfer performance in series?

Model-4: Serial Multiple Mediator Model

Table 14

Descriptive Statistics for PFK, ICL, GCL, and Transfer Test Scores in the Two Conditions

Condition		M_1 PFK	M_2 ICL	M_3 GCL	Y Transfer
Pre-training (*X*=1)	Mean	12.243	3.908	5.286	11.474
	SD	2.639	1.238	1.274	2.795
No pre-training (*X*=0)	Mean	8.892	4.722	6.179	9.270
	SD	3.273	1.430	1.433	3.599
	Mean	10.568	4.315	5.732	10.372
	SD	3.400	1.390	1.420	3.387

Note. $M_{1/2/3}$=putative mediator, *Y*=outcome, PFK=prior factual knowledge, ICL=intrinsic cognitive load, GCL=germane cognitive load, *SD*=standard deviation

Descriptive statistics for each of the four variables, prior factual knowledge, intrinsic cognitive load, germane cognitive load, and transfer test scores, in the two conditions (pre-training *versus* no pre-training) are illustrated in Table 14. Results of regression analysis are summarized in Table 15, and regression coefficients are superimposed on a statistical model presented in Figure 24. Since motivation is theorized and found to influence one of the putative mediators (germane cognitive load, β=-.032, $t(72)$=-2.831, p=.006) as well as the outcome (transfer, β=.139, $t(72)$=5.998, p<.001), it was included as a covariate of all of the *M*'s as well as *Y* in this model as recommended by (Hayes, 2013).

As illustrated in the statistical model (Figure 24), there are 7 possible indirect paths between pre-training and transfer given the order of the three putative mediators (M_1 to M_2 to M_3).

These seven paths are keyed below as Ind_i and the significance of each is summarized in Table 16:

Ind_1: pre-training (X) → prior factual knowledge (M_1) → transfer (Y)

Ind$_2$: pre-training (X) → prior factual knowledge (M_1) → intrinsic cognitive load (M_2) → transfer (Y)

Ind$_3$: pre-training (X) → prior factual knowledge (M_1) → germane cognitive load (M_3) → transfer (Y)

Ind$_4$: pre-training (X) → prior factual knowledge (M_1) → intrinsic cognitive load (M_2) → germane cognitive load (M_3) → transfer (Y)

Ind$_5$: pre-training (X) → intrinsic cognitive load (M_2) → transfer (Y)

Ind$_6$: pre-training (X) → intrinsic cognitive load (M_2) → germane cognitive load (M_3) → transfer (Y)

Ind$_7$: pre-training (X) → germane cognitive load (M_3) → transfer (Y)

Ind$_1$: pre-training(□) → prior factual knowledge (M_1)

Table 15

Coefficients of a Serial Multiple Mediator Model (model-4)

	Consequent															
		M_1 (PFK)				M_2 (ICL)				M_3 (GCL)				Y (Transfer)		
Antecedent		Coeff.	*SE*	*p*		Coeff.	*SE*	*p*		Coeff.	*SE*	*p*		Coeff.	*SE*	*p*
X (Group)	a_1	2.925	0.689	< .001	a_2	-1.336	2.596	.608	a_3	-0.226	0.259	.384	c'	-0.465	0.585	.430
M_1 (PFK)		—	—	—	d_{21}	-1.138	0.399	.006	d_{31}	-0.027	0.042	.518	b_1	0.493	0.095	< .001
M_2 (ICL)		—	—	—		—	—	—	d_{32}	0.088	0.012	< .001	b_2	0.035	0.036	.329
M_3 (GCL)		—	—	—		—	—	—		—	—	—	b_3	-0.713	0.271	.010
C (MOT)	f	0.061	0.025	.015	g	-0.195	0.086	.027	h	-0.001	.009	.950	j	0.088	0.020	< .001
Constant	i_{M1}	-0.516	2.643	.846	i_{M2}	69.279	8.820	< .001	i_{M3}	3.388	1.213	.007	i_Y	-0.829	2.881	.774
		R^2=0.307 F (2,71)=15.736, p<.001				R^2=0.277 F (3,70)=8.929, p<.001				R^2=0.568 F (4,69)=22.642, p<.001				R^2=0.620 F (5,68)=22.217, p<.001		

Note. $M_{1/2/3}$=putative mediator, PFK=prior factual knowledge, ICL=intrinsic cognitive load, GCL=germane cognitive load, Y=outcome, Coeff.=coefficient, *SE*=standard error, MOT= motivation

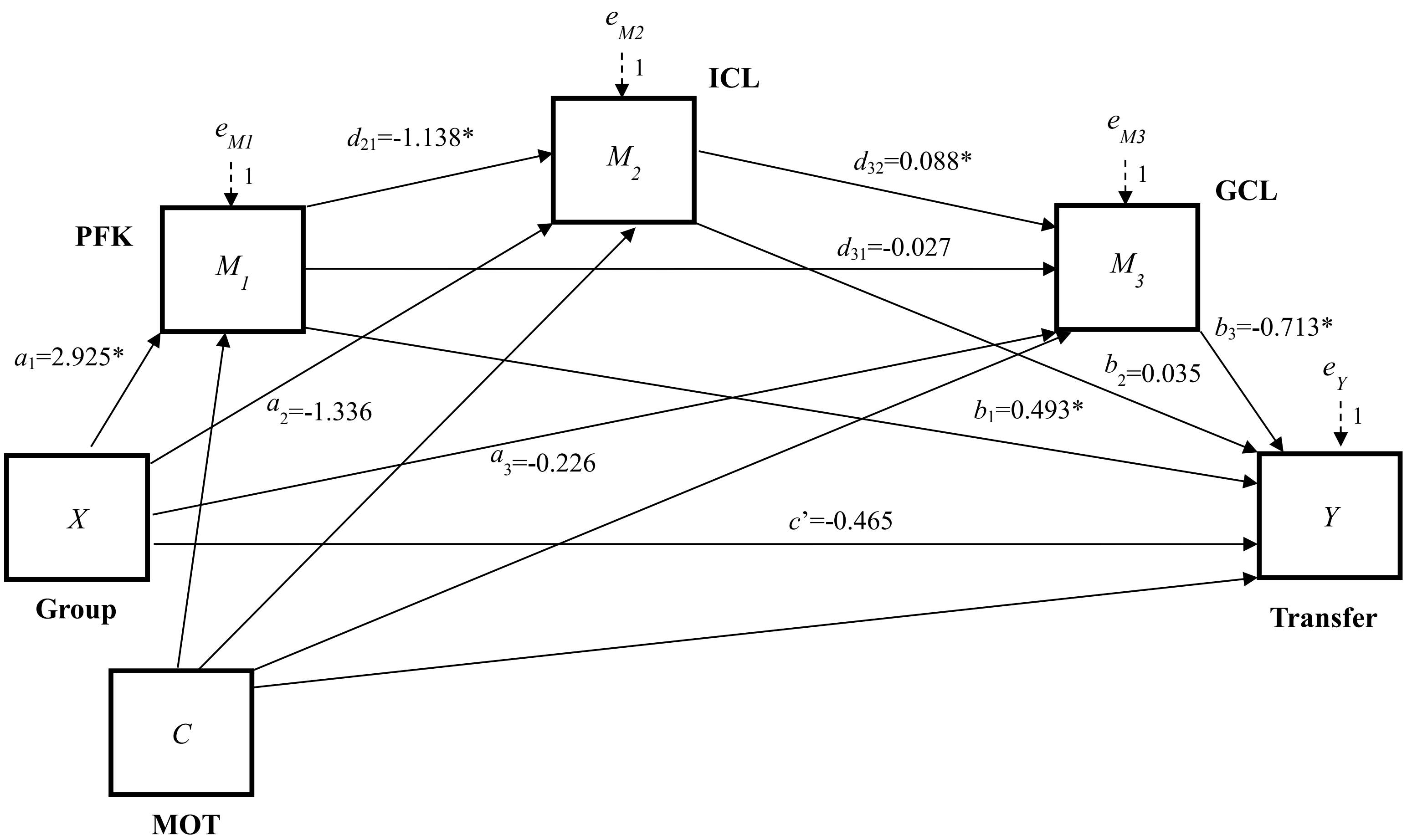

Figure 24. A statistical diagram of a serial mediation model (model-4) proposing an explanation for how pre-training might be affecting transfer performance. Key: X=independent variable; M_i=putative mediator; Y=outcome; C=controlled variable; $e_{Mi,/Y}$=errors in estimation; a_j, b_k, dkj & c'=regression coefficients; PFK=prior factual knowledge; MOT=motivation; ICL=intrinsic cognitive load, GCL=germane cognitive load. Asterisk denotes significance at $p<.05$.

Table 16

Possible Indirect Effects between Pre-training and Transfer through PFK, ICL, and GCL

Path	Effect	Boot *SE*	Boot LLCI	Boot ULCI
Ind_1	1.4415*	.4987	.6613	2.6548
Ind_2	-.1172	.1324	-.4721	.0920
Ind_3	.0568	.1099	-.0767	.3727
Ind_4	.2086*	.1213	. 0592	.5849
Ind_5	-.0471	.1394	-.5154	.1088
Ind_6	.0837	.1700	-.1999	.4899
Ind_7	.1615	.2005	-.1480	.6618
Total	1.7878	.5244	.8853	2.9498

Note. PFK=prior factual knowledge, ICL=intrinsic cognitive load, GCL=germane cognitive load,

LLCI=lower limit of confidence interval, ULCI=upper limit of confidence interval

* Asterisk denotes significant indirect effect.

Consistent with the above reasoning, the two proposed indirect effects (denoted $Ind_1$and Ind_4) were statistically supported, Table 16. Statistical significance of path:

- (Ind_1) means that prior factual knowledge mediates some portion of the effect of pre-training on transfer in isolation from cognitive load manipulations.
- (Ind_4) denotes that prior factual knowledge mediates some other portion of the effect of pre-training on transfer through cognitive load manipulations.

In further support, all of the other alternative sequences could not be held true as their corresponding bootstrap confidence intervals contained zero as a possible value of their true effect, Table 16. Statistical insignificance of path:

- (Ind_2) adds evidence to the argument that intrinsic cognitive load does not exert a direct influence on transfer, see (Ind_5).
- (Ind_3) indicates that prior factual knowledge does not influence germane cognitive load regardless of intrinsic cognitive load.
- (Ind_5) is consistent with the above discussion that intrinsic cognitive load does not directly influence transfer.
- (Ind_6) shows that cognitive load manipulation does not mediate the effect of pre-training on transfer in isolation from prior factual knowledge.
- (Ind_7) demonstrates that pre-training does not exert direct influence on germane cognitive load.

Based on this body of evidence, the studied process taking place can be described through two indirect paths. The first path (Ind_1) is the specific indirect effect of pre-training on transfer through prior factual knowledge (X to M_1 to Y), estimated as a_1b_1=2.925(.493)= 1.441 and shown in Table 16. This specific indirect effect can be claimed as significantly positive because its bootstrap confidence interval is completely above zero (.6613 to 2.6548). Those learners assigned to the pre-training group acquired more prior factual knowledge before instruction (a_1 is positive), and this increased prior knowledge translated into higher transfer performance (b_1 is positive) independent of intrinsic and germane cognitive load variations.

The second indirect path (Ind_4) is the specific indirect effect of pre-training on transfer through prior factual knowledge, intrinsic cognitive load, and germane cognitive load in serial, with prior factual knowledge modelled as affecting intrinsic cognitive load which influenced germane cognitive load, which in turn influenced transfer performance (*X* to M_1 to M_2 to M_3 to *Y*). This specific indirect effect is estimated as $a_1d_{21}d_{32}b_3$=2.925(-1.138).088(-.713)=.209 and can be interpreted as significantly positive since its bootstrap confidence interval is entirely above zero (.0592 to .5849). Relative to those assigned to the no pre-training group, those in the pre-training group acquired more prior factual knowledge before instruction (a_1 is positive), which reduced intrinsic cognitive load during instruction (d_{21} is negative) which enabled increased engagement in germane cognitive load (led learners to perceive the concept as less difficult; d_{32} is positive) which translated into better transfer performance (less difficulty translated into higher transfer; b_3 is negative).

The direct effect of pre-training on transfer is estimated as *c'*=-.465. This means that two learners assigned to different groups but are equal on prior factual knowledge, intrinsic and germane cognitive loads are estimated to differ by .465 units on their transfer test scores. Nevertheless, the direct effect was not significant, *c'*=-.465, $t(72)$=-.794, p>.05. On the contrary, the total indirect effect was statistically significant and estimated as 1.7878 (.8853 to 2.9498), Table 16.

The total effect of this model is estimated as *c*=1.323, $t(72)$=2.0219, p=.047, which cleanly sums up the total indirect effect and direct effect, 1.7878+(-.465)= 1.323. Interestingly, this estimate is consistent with the difference in estimated marginal means (after partialing out motivation) of transfer test scores between the pre-training group (11.034) and the no pre-training group (9.710), 11.034 - 9.710= 1.324. Again, this total effect means that learners who received pre-training, on average, scored 1.323 units higher on the transfer test than those who did not receive pre-training. The conceptual diagram in Figure 24 summarizes the abovementioned discussion.

Interestingly, the specific indirect effect of pre-training on transfer solely through prior factual knowledge (Ind_1) is statistically significant from the specific indirect effect of pre-training on transfer through prior factual knowledge, intrinsic, and germane cognitive load in serial (Ind_4), as the 95% bc bootstrap confidence interval for this difference is above zero, $a_1b_1 – a_1d_{21}d_{32}b_3$ =1.2329 (.4463 to 2.4720). Since both specific indirect effects have the same sign, it can be interpreted that pre-training has a greater effect on transfer through prior factual knowledge in isolation rather than it does through cognitive load manipulation translated through prior factual knowledge. Nevertheless, it is worth mentioning that including the effect of cognitive load manipulation in the model significantly improves its predictive power to explain an additional 6.8% of the variance in transfer test score (R^2_{change}=.068) with a significant *F* change of, $F(2, 69)$=4.839, p=.011.

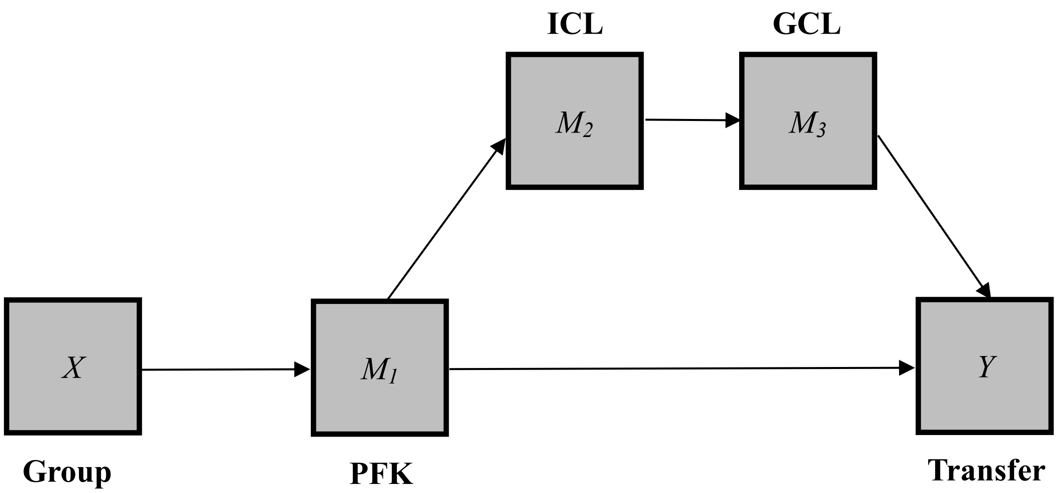

Figure 24. A conceptual diagram of a serial multiple mediation model (model-4) proposing an explanation for how pre-training might be affecting transfer performance. Key: *X*=independent variable; M_i=putative mediator; *Y*=outcome; PFK=prior factual knowledge; ICL=intrinsic cognitive load, GCL=germane cognitive load.

R.Q.9: Does germane cognitive load mediate the effect of motivation on transfer performance?

Model-5: A Simple Mediator Model

Recall from previous discussion that reduction in intrinsic cognitive load theoretically frees some space in working memory so that the learner can afford engaging in germane cognitive load and consequently perform better in a transfer test. This is consistent with the model proposed above. However, affording to engage in germane cognitive load does not necessarily mean that the learner is motivated to do so (Mayer, 2011). In this sense, motivation might be moderating the effect of intrinsic cognitive load on transfer through germane cognitive load. Nevertheless, this explanation is rejected as the interaction between motivation and intrinsic cognitive load did not predict engagement in germane cognitive load, β=-.0007, $t(72)$=-.280, p>.05. This result highlights the underestimation of such reasoning to the effect of motivation on germane cognitive load since the former is theorized to cause the latter (Mayer, 2009) rather than simply moderate the effect of another cause on it. Therefore, an alternative explanation would be that increased motivation (*X*) causes engagement in germane cognitive load (*M*) which in turn influences transfer (*Y*) above and beyond the effect of pre-training on both, germane cognitive load and transfer performance. This explanation is supported by the following analysis.

Table 17

Coefficients of a Simple Mediator Model (model-5)

		Consequent						
		M (GCL)				*Y* (Transfer)		
Antecedent		Coeff.	*SE*	*p*		Coeff.	*SE*	*p*
X (MOT)	*a*	-0.099	0.046	.036	c'	0.107	0.022	< .001
M (GCL)		—	—	—	*b*	-0.199	0.055	< .001
C (Group)	*f*	-2.818	1.297	.033	*g*	0.761	0.625	.230
Constant	i_1	37.861	4.976	< .001	i_2	2.114	3.129	.501

Table 17 (continued).

	Consequent	
	M (GCL)	*Y* (Transfer)
	R^2= 0.148 *F*(2, 71) = 6.187, *p*=.003	R^2= 0.468 *F*(3, 70) = 20.534, *p* < .001

Note. M=putative mediator, GCL=germane cognitive load, *Y*=outcome, Coeff.=coefficient, *SE*=standard error, MOT= motivation

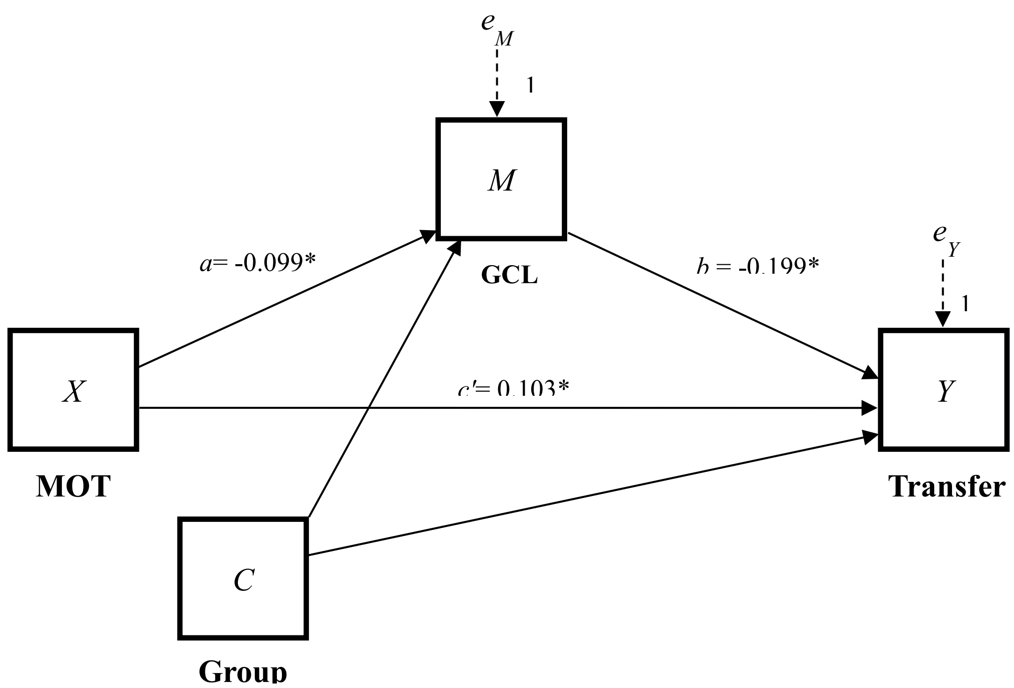

Figure 25. A statistical diagram of a simple mediation model (model-5) proposing an explanation for how motivation might be affecting transfer performance. Key: *X*=independent variable; *M*=putative mediator; *Y*=outcome; *C*=controlled variable; e_M & e_Y=errors in estimation of *M* and *Y* respectively; *a*, *b*, & *c'*=regression coefficients; MOT=motivation; GCL=germane cognitive load. Asterisk denotes significance at *p*<.05.

Results of regression analysis are summarized in Table 17. Again, the effects of pre-training on germane cognitive load and transfer were partialed out to obtain better estimates of the process. The proposed mediator, germane cognitive load, is regressed on motivation to produce the *a* coefficient. Transfer test score is regressed on both germane cognitive load and motivation to produce the *b* and *c'* coefficients respectively. Regression coefficients are superimposed on a statistical model presented in Figure 25. The product of multiplying the two coefficients, *a* and *b*, yields the indirect effect of motivation on transfer through germane cognitive load controlling for pre-training effects, *ab*=-.099(-.199)=.0198. This indirect effect of .0198 means that two learners who differ by one unit on motivation, but are assigned to the same group (pre-training/no pre-training), are estimated to differ by .0198 units in their transfer test scores as a result of the tendency for those who are more motivated to engage in germane cognitive load which in turn translates into high transfer performance. This path is statistically significant since the 95% bootstrap confidence interval is completely above zero (.0034 to .0481).

The direct effect of motivation, *c'*=.107, is the estimated difference in transfer test scores between two learners experiencing the same level of germane cognitive load but who differ by one unit in motivation. *c'* is positive, meaning that the learner being more motivated but who is equal on germane cognitive load is estimated to score higher on the test by .107 units. This direct effect is statistically different from zero, *c'*=.107, $t(71)=4.805$, $p<.0001$. The total effect of motivation on transfer is estimated to be *c*=.127 and is also statistically significant, *c*=.138, $t(72)=5.433$, $p<.0001$. This tells us that two learners who differed by one unit in motivation are estimated to differ by .127 in their test scores with the more motivated learner scoring higher (*c* is positive).

It is worth mentioning that inclusion of the effect of motivation in the model significantly improves its predictive power to explain an additional 11% of the variance in transfer test score ($R^2_{change}=.011$) with a significant *F* change of, $F(1, 68)=19.798$, $p<.001$. Collectively, included variables in the model explain an impressive 62.3% of the variance in transfer test scores, $R^2=.623$. The two conceptual diagrams in Figure 26 below sum up the entire discussion of how pre-training and motivation are thought to influence transfer performance.

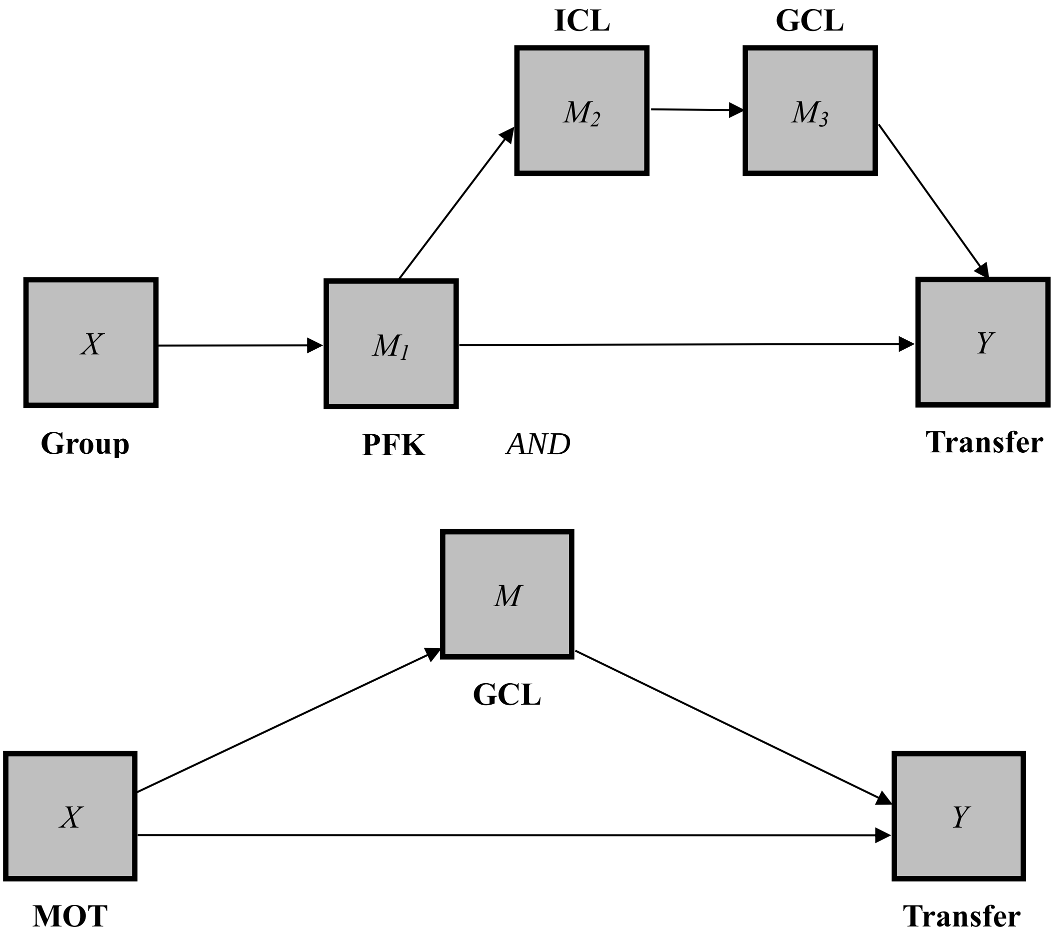

Figure 26. A conceptual diagram of two separate mediation models (up: model-4, down: model-5) proposing an explanation for how pre-training and motivation might be affecting transfer performance. Key: *X*=independent variable; M_i=putative mediator; *Y*=outcome; PFK=prior factual knowledge; ICL=intrinsic cognitive load, GCL=germane cognitive load; MOT=motivation.

The Two Models: Separate or Combined?

So, germane cognitive load was found to mediate some of the effect of pre-training *and* motivation on transfer performance. A normal question that follows is whether germane cognitive load can link the two models to explain the single process of *how* both treatments are fostering transfer, Figure 27.

However, this means that model-5 needs to be tested again while controlling for the effect of intrinsic cognitive load on germane cognitive load as well as controlling for the effect of prior factual knowledge on transfer. While there might not be a problem with the latter, controlling the effect of intrinsic on germane cognitive load is likely to bring up one of the standard concerns in multiple linear models where correlated variables are involved, r=.745, p<.001[9].

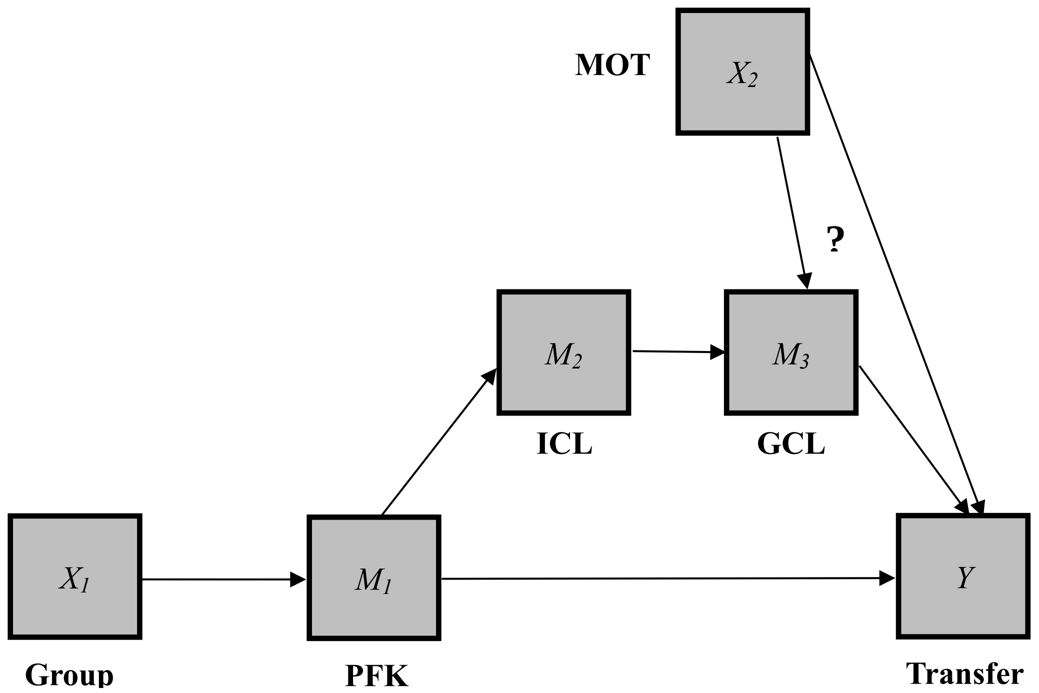

Figure 27. A conceptual diagram of two integrated mediation models (up: model-4, down: model-5) proposing an explanation for how pre-training and motivation might be affecting transfer performance. Key: *X*=independent variable; M_i=putative mediator; *Y*=outcome; PFK=prior factual knowledge; ICL=intrinsic cognitive load, GCL=germane cognitive load; MOT=motivation.

To demonstrate the significant power reduction that this correlation offers to the integrated model, one can utilize G*Power[10] to estimate the sample size needed to run the analysis first by controlling solely for the pre-training effect (case of model-5), second by controlling for the effect of intrinsic on germane cognitive load, and third

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[9] mean-centering intrinsic and germane cognitive load scores did not solve the problem, r=.750, p<.001.
[10] G*Power 3.1.9.2 was used to estimate sample sizes under the following conditions: two tails, α err prob.=.05, power 1-βerr prob.=.80

by controlling for both intrinsic cognitive load and prior factual knowledge (case of integrated model; Figure 27).

Table 18

Portion of Variance in Transfer Performance (partial R^2) Explained by GCL in Each Case

			Change Statistics				
Model	R^2	*SE*	R^2 Change	*F* Change	df_1	df_2	*p*-value
First Case (*controlling for Group & MOT*)							
1	.369[a]	2.726	.369	20.801	2	71	.000
2	.468[b]	2.522	.099*	12.980	1	70	.001
Second Case (*controlling for ICL & MOT*)							
1	.399[c]	2.663	.399	23.531	2	71	.000
2	.457[d]	2.548	.058*	7.505	1	70	.008
Third Case (*controlling for PFK, ICL, & MOT*)							
1	.580[e]	2.241	.580	32.241	3	70	.000
2	.620[f]	2.148	.039*	7.140	1	69	.009

Note. MOT= motivation, ICL=intrinsic cognitive load, PFK=prior factual knowledge, GCL=germane cognitive load.

* Denotes partial R^2 of GCL

Dependent variable: Transfer performance

a. Independent variables: Group, MOT b. Independent variables: Group, MOT, GCL

c. Independent variables: ICL, MOT d. Independent variables: ICL, MOT, GCL

e. Independent variables: PFK, ICL, MOT f. Independent variables: PFK, ICL, MOT, GCL

Table 18 labels partial R^2score of germane cognitive load by an asterisk. This score reflects the portion of variance in transfer performance explained by germane cognitive load in each of the three cases. Upon plugging in each of these three scores into G*Power, estimate of the sample size needed for the: first case is 74 students which is exactly what we have (model-5), second case is 130 students which demonstrates expected power reduction, and 196 for the proposed integrated model, Figure 27. Given that the present sample is short of 122 students (196-74), this final question remains unanswered.

CHAPTER V

DISCUSSION

An Overview

Before diving into details, remember that the pre-training group demonstrated better transfer performance than the no pre-training group. This provides evidence that the proposed form of multimedia pre-training moved college students to a better understanding of substrate specificity of enzymes. This piece of research outcome offers equal credit to the two competing hypotheses though, since both postulate that the pre-training group "will outperform the no pre-training group in a transfer test." Put another way, it neither supports the knowledge construction perspective, which is the basis of the leading hypothesis[11], nor the additional instruction perspective, which is the basis of the competing hypothesis[12]. Here lies the importance of another piece of outcome, which is transfer performance by the post-training group. Since the post-training group did not demonstrate better transfer performance than the no pre-training group, although they received the same amount of instruction as the pre-training group, the competing hypothesis is rejected and the corresponding additional instruction perspective is unfavored.

The leading hypothesis holds the assumption that the pre-training experience increases learner's prior factual knowledge related to the presented material, which aids the knowledge construction process (Mayer et al., 2002). This, in part, is supported by the finding that the pre-training group scored significantly higher in pre-test (2)[13] than the other two groups. It also is supported by the fact that prior factual knowledge was found to mediate some of the effect of pre-training on transfer

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[11] If the pre-training group was able to build component models and attend to the underlying processes and terms before engaging in building a causal model for how substrate specificity works, then they will outperform the no pre-training group in a transfer test.

[12] If the pre-training group received additional instruction in the form of pre-training to learn how substrate specificity works, then they will outperform the no pre-training group in a transfer test.

[13] Pre-test (2) measured prior factual knowledge

performance. All of this evidence lends support to the constructivist perspective of multimedia pre-training.

Nevertheless, this treatment also has a cognitive component expressed in terms of cognitive load. This is demonstrated by the significantly lower mental effort ratings by the pre-training group compared to the other two groups. The proposed version of multimedia pre-training is thought to exert this effect in two ways. First, upon building component models before engagement in building the causal model, some of the cognitive demands imposed by the presented material are shifted to the pre-training episode thus offloading the working memory during the main lesson (Mayer, 2009). Second, visual representation of the underlying processes and associated terms are theorized to help the learner mentally shift between levels of representation with less cognitive demands (Shönborn & Bögeholz, 2013). Which of the two ways is more effective in reducing intrinsic cognitive load remains an interesting question to be answered.

Either way, reported mental effort reduction, along with increased prior factual knowledge, suggest that a cognitive-constructivist perspective to multimedia pre-training is a better fit to this treatment than the traditional constructivist perspective (Ausubel, 1986). It is through this perspective; research questions in this study were set, answered, and discussed.

Discussion Related to Research Question.1, R.Q.1

Recall that the pre-training group did not outperform the other groups in the retention test. A similar result was obtained in one of the three experiments conducted by Mayer et al. (2002). Such a result is not surprising because multimedia pre-training is an instructional technique meant for fostering transfer rather than retention performance (Mayer et al., 2002). Nevertheless, type II error is likely here because the observed power was as low as .337.

As stated earlier, the pre-training group significantly outperformed the other two groups in the transfer test with a high effect size of 1.18. The statistical significance of this result is consistent with that of eleven known multimedia pre-training experiments conducted by other researchers, Table 19. The practical significance is also consistent with the median effect size of these studies, .94.

Table 19

Experiments on Multimedia Pre-training Principle

Experiment	Effect Size
Eitel, Scheiter, & Schuler (2013)	1.37
Clarke, Ayres, & Sweller (2005, experiment1a)	1.87
Kester, Kirshner, &van Merriënboer (2006)	0.72
Mayer, Mathias, & Wetzell (2002, experiment 1)	0.79
Mayer, Mathias, & Wetzell (2002, experiment 2)	0.92
Mayer, Mathias, & Wetzell (2002, experiment 3)	1.00
Mayer, Mautone, & Prothero (2002, experiment 2)	0.57
Mayer, Mautone, & Prothero (2002, experiment 3)	0.85
Musallam (2010)	0.94
Pollock, Chandler, & Sweller (2002, experiment 1)	1.22
Pollock, Chandler, & Sweller (2002, experiment 2)	1.15
Median	0.94

Note. Experiments conducted on learners of high prior knowledge/skills were excluded.

Discussion Related to R.Q.2

Students in the pre-training group showed better performance on the set of *Understand* Items than the other two groups. Theoretically, the pre-training group were better able to use their constructed causal model to explain how a change in the chemical structure of the substrate would affect substrate affinity or enzyme reactivity. In some of these Items, students had to count the number of electrostatic bonds that might form between the transition state and the active site of the enzyme to determine substrate affinity. In some other Items, they had to examine proximity of the catalytic group from the bond-to-be-broken to determine enzyme reactivity. Obviously, this type of transfer required comprehension of the underlying processes that determine substrate specificity. These results lend support to the proposed version of multimedia pre-training, which suggests explicit representation of underlying processes and associated terms. However, the claimed superiority of the proposed version over the original one should be validated through empirical comparisons.

In brief, the pre-training group demonstrated deeper understanding of substrate specificity. Nevertheless, this finding did not hold for the remaining four cognitive orders. That is, the pre-training group was not able to better apply what they learned, analyze given results, evaluate stated claims, or create more solutions than the other two groups. These results may be referred to three possible causes: (1) issues with the data-generating instrument, (2) differences in cognitive skills between-group learners, and/or (3) working memory capacity. The first putative cause is rejected because the developed assessment instrument satisfied discriminant validity checks for each cognitive order and its scores were found reliable (α=.706). The second possible cause might have been in action if participants of the other two groups were pursuing majors that require practicing certain cognitive skills (e.g. evaluating scientific claims) while those of the pre-training group were not. However, demographic analysis revealed even distribution of majors among the three groups (see chapter IV). The third cause is in line with the cognitive-constructivist perspective of multimedia pre-training. Let us use the set of *Apply* Items to discuss why working memory capacity could be the cause.

Recall that in these Items, students had to choose the most effective procedure to modify enzyme reactivity or substrate affinity. This task required knowledge of possible interactions between molecules of different polarity and/or charge. It also required knowledge of the side chains of amino acids mentioned in the problem. Bearing this in mind, all of this information was provided in the test. However, consciously processing this information while trying to solve the given problem appears to be demanding enough to overload the learner's working memory[14]. This scenario explains why participants reported significantly higher mental effort and difficulty ratings to *Apply* Items than to the entire test, $t(110)=11.95$, $p<.001$ and $t(110)=8.10$, $p<.001$ respectively.Therefore, the learner might have needed to cognitively *automate* the characteristics of these amino acidsbefore hand (van Merriënboer & Sweller, 2005)— say through a drag-and-drop exercise within the pre-training episode, Figure 28.

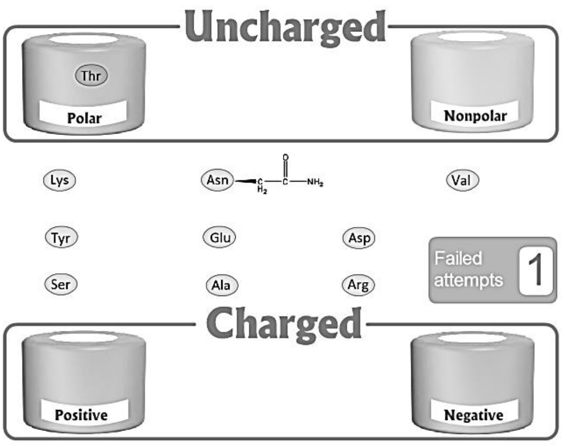

Figure 28. A screenshot of a drag-and-drop exercise that might have been added to the multimedia pre-training episode.

This automation would have allowed the side chains of amino acids to be processed *automatically* rather than consciously in working memory, thus reducing

[14]Especially that study participants were learners of low prior knowledge (see Chapter IV).

cognitive load (Pollock, Chandler, & Sweller, 2002). Empirical testing of this analysis would reveal how such cognitive automation may influence transfer performance at this cognitive order.

Discussion Related to R.Q.3

The three groups did not differ on extraneous and germane cognitive load. Yet, the pre-training group reported less intrinsic cognitive load than the other two groups during both, instruction and assessment. These results are in line with the theoretical foundation of multimedia pre-training principle (Mayer, 2009): Intrinsic cognitive load relates to the complexity of the presented material, which was managed through moving some cognitive demands to a pre-training episode.

The only known pre-training study that measured all of the three types of cognitive load is Musallam's dissertation (2010). Ratings of extraneous load in his study also did not differ between the pre-training and the no pre-training groups. However, an "unexpected significant difference in germane cognitive load" rating was found between the two groups (Musallam, 2010, p. 87). As for intrinsic cognitive load, Musallam measured it only during assessment and his results are in agreement with the corresponding results in this study. Kester et al. (2006) however, measured intrinsic cognitive load during both, practice and assessment. Again, ratings of intrinsic cognitive load during practice are consistent with those obtained this study. On the other hand, ratings measured during assessment did not show a significant difference between groups, which is contrary to what is obtained here (Kester et al., 2006). In any case, and as stated in chapter IV, changes in intrinsic cognitive load during *assessment* are not a primary interest in this project since performance efficiency ($\frac{\text{test score}}{\text{mental effort score}}$) is not a question to be answered (Kalyuga & Sweller, 2005).

In terms of practical significance, the proposed treatment revealed a medium effect size on intrinsic load reduction (.62) whereas Kester et al.'s (2006) showed a small effect size (.35). More studies are needed before being able to discuss this practical inconsistency.

Discussion Related to R.Q.4

High motivation scores reported by study participants indicate that the developed instructional kit promoted motivation to learn about substrate specificity of enzymes. Promoting motivation was important as it helped in fostering germane cognitive load, $\beta=-.025$, $t(109)=-2.79$, $p=.006$. This step was taken based on the notion that treatments aimed at reducing intrinsic cognitive load (e.g. pre-training) should be balanced with those that foster germane cognitive load (van Merriënboer et al., 2006).

Recall from Chapter IV that the pre-training group was, unexpectedly, more motivated than the other two groups ($.05<p<.10$), $F(2, 108)=6.029, p=.005$. Yet, regression analysis demonstrated that pre-training does not predict motivation, $\beta=-1.709$, $t(109)=-1.045$, $p=.298$. Additionally, IMMS scale was worded to measure motivation about learning the material through the instructional kit and has nothing to do with the pre-training experience. Therefore, an epiphenomenal process might be causing this spurious correlation between pre-training and motivation. One possible explanation comes from the way in which intrinsic and germane cognitive loads are measured. Mind that there is a strong correlation between mental effort rating (measuring intrinsic load) and difficulty rating (measuring germane load), $r=.698$, $p<.001$. Therefore, a measure taken to reduce mental effort (pre-training) may also influence perceived difficulty[15]. Since perceived difficulty is also correlated with

[15]This drawback of the named instruments should not be perceived as a serious threat to their validity. Matter of fact, the observed correlation reflects convergence validity since the corresponding constructs, intrinsic and germane cognitive loads, are

motivation, *r*=-.259, *p*=.006, students perceiving the material as less difficult would consequently be more motivated to learn about it (Hom & Maxwell, 1983).

Discussion Related to R.Q.5-9

The first mediation model showed that intrinsic cognitive load reduction mediates the effect of pre-training on transfer. This supports a cognitive perspective to multimedia pre-training. The second model revealed that increase in prior factual knowledge also mediates the effect of pre-training on transfer. This rather supports a constructivist perspective to the same treatment. Does this follow that both perspectives are supported? The third and fourth models answer this question.

In the third model, the mediation effect of intrinsic cognitive load disappeared after factoring in prior factual knowledge. This hinted to two possible scenarios. First, the two variables (intrinsic load and prior knowledge) could be conceptually related especially that they remained correlated after controlling for their common cause; i.e. pre-training. Second, intrinsic cognitive load reduction, by itself, might not be enough to explain how pre-training improves transfer performance through cognitive load manipulation. Analysis of the fourth model suggested that both scenarios were taking place. In this model, intrinsic cognitive load was found to play a mediation role *only* through germane cognitive load, which is consistent with the second scenario. Additionally, the mediation role played by intrinsic and germane loads was not in isolation from prior factual knowledge, which is in line with the first scenario. Rather, the only statistically supported track in which cognitive load is involved was as follows: Pre-training (X) increases prior factual knowledge (M_1) which reduces intrinsic cognitive load (M_2) which enables engagement in germane cognitive load

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theoretically related. In multimedia learning, intrinsic cognitive load is the load resulting from cognitively selecting words and pictures from the instructional message as well as from initially organizing them into verbal and pictorial models (Mayer, 2011). Germane cognitive load is a consequent of further organizing these cognitive models and integrating them with prior knowledge (Mayer, 2011). Hence, both types of cognitive load share some common source, which is organizing cognitive models. Accordingly, instruments measuring these two constructs should be correlated.

(M_3) which in turn causes increased transfer performance (Y), (X to M_1 to M_2 to M_3 to Y).

These findings have two important theoretical implications. First, a cognitive-constructivist perspective to multimedia pre-training is supported again because cognitive load manipulation mediated the effect of pre-training on transfer *along* with variation in prior factual knowledge. Second, involvement of these three particular variables (prior knowledge, intrinsic and germane loads) in the process is consistent with CTML's explanation of how people learn from multimedia learning. According to CTML, the learner selects words and pictures from a multimedia message and initially organizes them into corresponding verbal and pictorial models (Mayer, 2011). These cognitive steps result in intrinsic cognitive load, and obviously are not enough to promote transfer because they have not yet been integrated. If enough working memory is left after engaging in intrinsic cognitive load, then the learner may further organize these verbal and pictorial models as well as integrate them with prior knowledge (Mayer, 2011). These further steps lead to germane cognitive load, which translates into higher transfer performance. Mind that these steps involve integration of prior knowledge which may explain why cognitive load manipulation could not mediate the effect of pre-training on transfer in isolation from prior factual knowledge.

The fifth model supported the notion that germane cognitive load is caused by motivation since the former mediated the effect of the latter on transfer (Mayer, 2009). However, a significant portion of the effect of motivation on transfer could not be explained by germane cognitive load. This finding is consistent with some earlier conceptual models proposed by Moreno and Mayer (2007) and Mayer (2011) who thought that motivation regulates the abovementioned cognitive steps through a mechanism that is still to be explained. The type of motivation that is often applied in multimedia learning is based on social agency theory (Atkinson, Mayer, & Merill, 2005). Motivation scores appearing in this study are based on ARCS model of motivation for reasons explained in chapter I (Keller, 2010). It would also be interesting to try other techniques that would increase motivation based on interest, self-beliefs, and/or goals (Pintrich & Schunk, 2002).

Process Inferences

So far, we discussed the role of prior knowledge, cognitive load, and motivation in learning from multimedia pre-training and the instructional kit. However, studying the degree of importance of each of the three variables might be informative for instructional design. As stated earlier, the mediation role of prior knowledge was statistically significant from that of cognitive load, which emphasizes the notion that, “prior knowledge is the single most important individual difference dimension in instructional design” (Mayer, 2009, p. 193). Certainly, this does not imply ignoring the limitation of working memory capacity; especially that cognitive load manipulation explained a significant 6.8% of the variance in transfer performance. Motivation, in turn, explained an additional 11%, some of which was through fostering germane cognitive load. This percentage (11%)sounds reasonable for the assumed role of motivation as a regulator of cognitive engagement (Moreno, Mayer, 2007). Collectively, the three variables explained 62.3% of the variance in transfer performance, which is convincing enough to take them all into account when designing multimedia pre-training.

Limitations

Two of the three experimental groups, control and pre-training groups, took the retention test right after instruction. The third group, post-training group, had to listen to the 7-minute multimedia presentation before taking the test. Therefore, it would be useful to replicate the study while controlling for the time between instruction and the test to avoid longer retention time for the post-training group. Also, the addition of a fourth group that receives the original version of multimedia pre-training, would have enabled comparison of learning outcomes between the original and proposed versions.

Contrary to all of the instruments used in this study, scores generated by pre-test (1) had poor internal consistency (Cronbach's α=.509). It is worth mentioning though that these scores were not used to answer any of the nine research questions.

Answers to some research questions involved linear regression analysis. Although quite useful, one drawback of this technique is that it loses predictive power when highly correlated independent variables are co-factored in the regression equation. It is for this reason, the last research question was not completely answered, given a sample size of seventy four participants[16].

Finally, the majority of participants were females (67.7%) and white/Caucasians (70.3%). It would be informative to replicate the study with different gender and ethnicity distributions especially that retention test scores were found statistically different based on gender, $F(1, 108)=6.060$, $p=.015$, while transfer test scores were different based on ethnicity, $F(4, 105)=2.845$, $p=.028$. In terms of college preparedness, based on composite ACT scores, research findings cannot be generalized to U.S. college students population[17] because sampled students reported

[16] Total sample size is one hundred eleven participants but those involved in analysis corresponding to this research question are seventy four.
[17] Population data is retrieved from 2014 National and State Scores report published online by ACT®; www.act.org

better college preparedness than the general population, z[18]=-3.828, $p<.001$. Median effect size of this difference between the studied sample and students in U.S. states requiring 100% participation in this test was also not negligible, .656[19] (based on Cohen's *d* effect size). Therefore, it would also be helpful to replicate the study while recruiting participants from less selective universities than the ones involved in this project.

Conclusion

This study demonstrated the potential role of multimedia pre-training in moving college students to a better understanding of complex scientific concepts such as substrate specificity of enzymes. It also demonstrated how utilizing visual models, such as the developed instructional kit, can promote motivation to learn about scientific phenomena such as substrate specificity.

A general overview of the obtained results shows consistency with the assumptions described in the theoretical framework. For instance, participants' ratings of the three types of cognitive load came in agreement with the proposed learning framework, which states that complexity of substrate specificity would put the learner in the second learning scenario through imposing too much intrinsic cognitive load, Figure 3. The no pre-training and post-training groups reported significantly higher intrinsic cognitive load than the pre-training group, but were similar to the latter on extraneous and germane cognitive loads. Aside from being consistent with the learning framework, these results also show that the proposed multimedia pre-training was accurately designed to meet the complexity of the presented material without interfering with the other two types of cognitive load.

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[18]Based on Wlicoxon Signed Ranks Test

[19]U.S. State (Cohen's *d*): CO (.526), IL (.494), KY(.695), LA (.874), MI (.645), MS (.943), MT (.580), NC (.898), ND (.571), TN (.707), UT (.502), and WY (.667).

Knowledge assessment further supported this analysis. The no pre-training and post-training groups scored significantly lower in the transfer test than the pre-training group, but did not differ from the latter in the retention test. Again, this is consistent with the assumption that substrate specificity was complex enough to overload the learner's working memory, thus hindering further processing necessary to promote transfer.

The complexity of substrate specificity is thought to be met during the pre-training episode through explicit representation of the underlying processes and terms related to substrate specificity (Shönborn & Bögeholz, 2013) as well as representation of the major components of the studied system (Mayer et al., 2002). This is evident from the fact that the pre-training group demonstrated higher knowledge of major components, underlying processes, and terms than the other two groups. Additionally, this type of knowledge (prior factual knowledge) significantly mediated the effect of the proposed pre-training on transfer performance.

Utilizing the cognitive dimension of revised Bloom's taxonomy in knowledge assessment helped diagnose some undesirable outcomes (Anderson et al., 2001). Although the proposed version of pre-training promoted deeper understanding of substrate specificity, it did not enhance transfer performance at the higher cognitive orders (*Apply*, *Analyze*, *Evaluate*, and *Create*). These results support the assumption that the utilized assessment framework provides enough resolution to highlight pitfalls in instructional design. It also shows how this framework provides guidance for how to redesign instruction based on cognitive order analysis. For instance, analysis of students' performance on *Apply* Items suggests inclusion of CLT part-task technique in the pre-training presentation; during which time, students have the opportunity to automate the functional roles of amino acids prior to instruction rather than consciously processing them during assessment (see discussion section related to R.Q.2).

Mediation analysis highlighted the interrelationship between motivation, cognitive and knowledge dimensions while learning from multimedia pre-training. On the one hand, it did so through emphasizing the connection between knowledge and cognitive dimensions. On the other, it demonstrated how motivation can regulate multimedia learning through the cognitive dimension. Combined, the three dimensions explained over 60% of the variance in transfer test scores.

Such type of studies goes beyond simple forms of "*what works*" to a deeper understanding of "*how it works,*" thus enabling informed decisions for multimedia instructional design and redesign. Accordingly, complex scientific phenomena can be introduced to college students in a motivating, informative, and cognitively efficient learning environment.

APPENDIX A

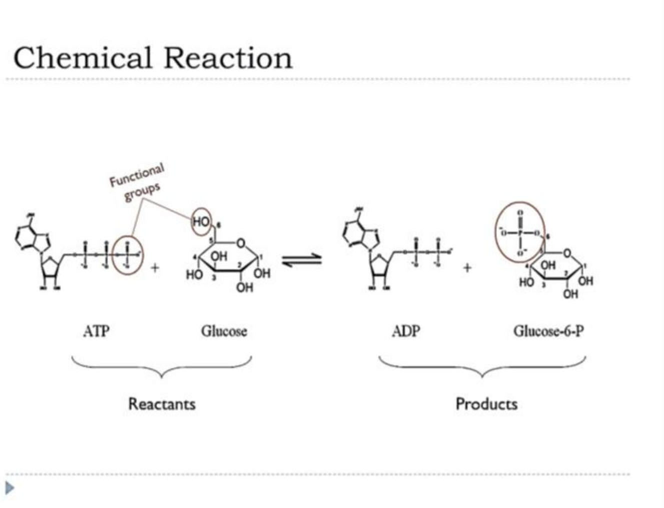
LIST OF CHECKED TEXTBOOKS

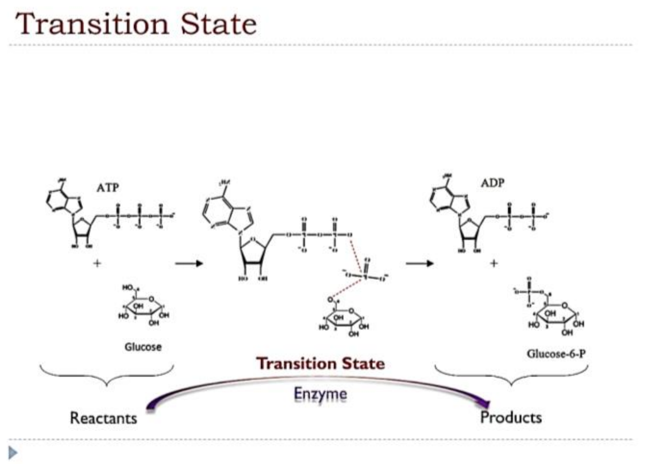
		Concept Elements			
		Conformational Change		Chemical interaction between substrates and catalytic amino acids	
		Text	Figures	Text	Figures
Publisher (Year)/ISBN-10	Cengage Learning (2015)/1-285-43182-0	√	√	×	×
	Pearson (2014)/0-321-82171-8	×	×	×	×
	W.H. Freeman and Company (2013)/1-4641-0243-0	×	×	√	×
	Pearson (2013)/0-321-77260-1	√	×	√	×
	Benjamin Cummings (2012)/0-321-70167-4	√	√	×	×
	Pearson (2011)/0-13-369347-3	√	×	×	×
	Glencoe (2011)/0-07-894926-2	√	×	×	×
	Pearson/Benjamin Cummings (2008)/0-13-224950-2	√	√	√	×
	Glencoe/McGraw-Hill (2006)/0-07-866428-4	√	√	×	×
	Kendall/Hunt (2003)/0-7872-8685-0	×	√	×	×
	Kendall/Hunt (2002)/0-7872-7526-3	√	√	×	×
	W.H. Freeman and Company (2001)/0-7167-3873-2	×	×	√	√

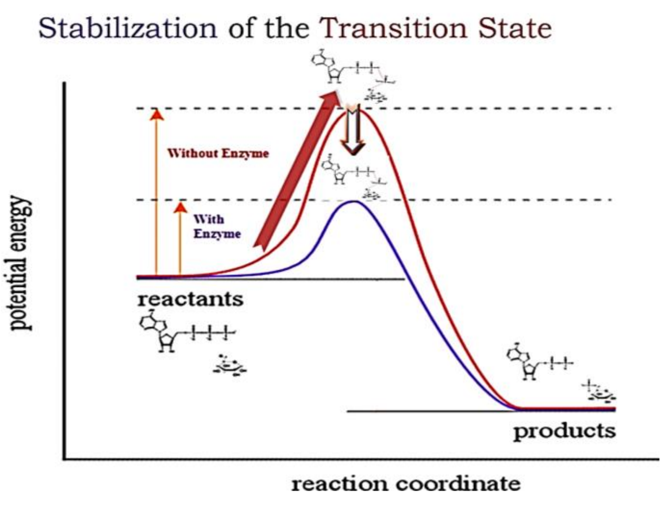
√: concept element is described; ×: concept element is not described

APPENDIX B

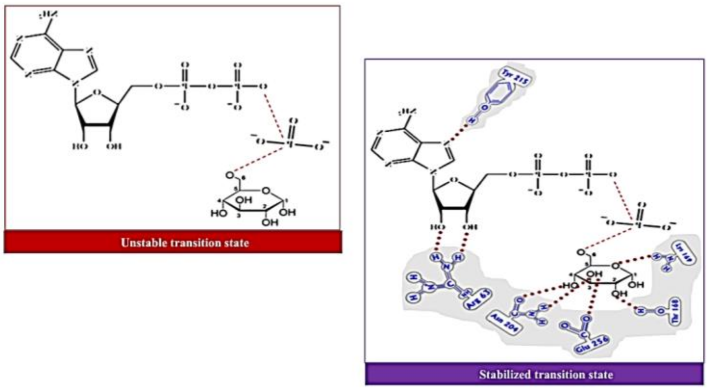
MULTIMEDIA PRE-TRAINING SLIDES



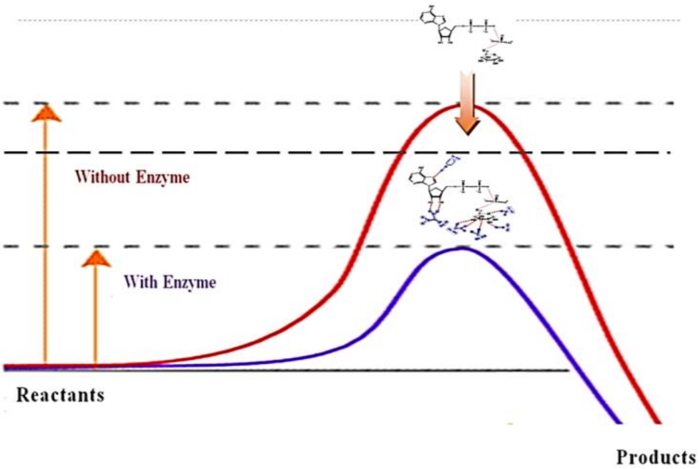




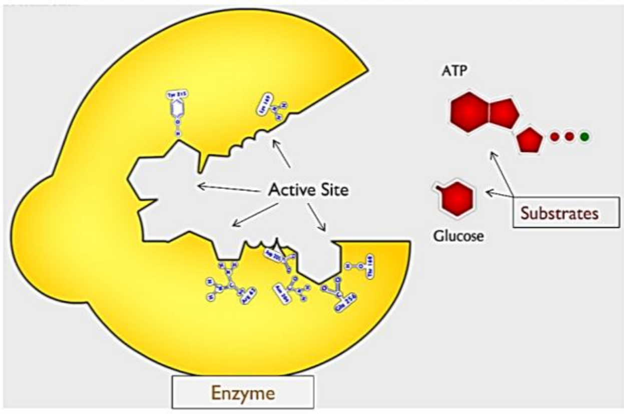
Stabilization of the Transition State



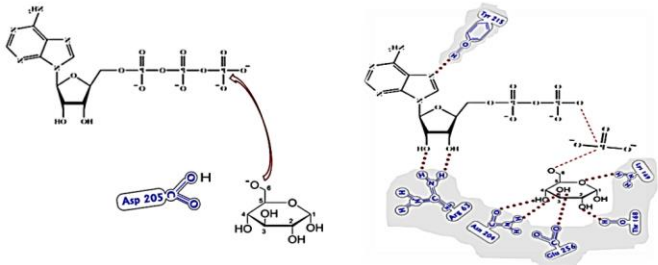
Stabilization of the Transition State



Important Terms



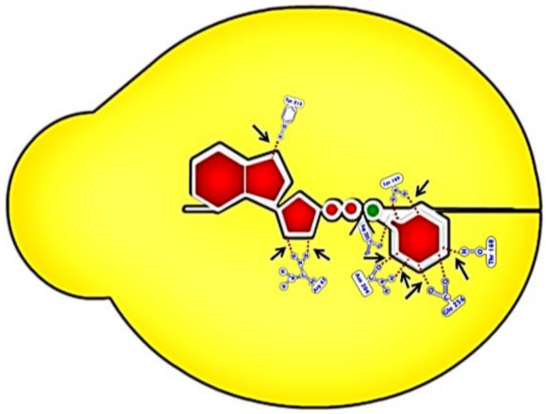
Functional Roles of Catalytic amino acids



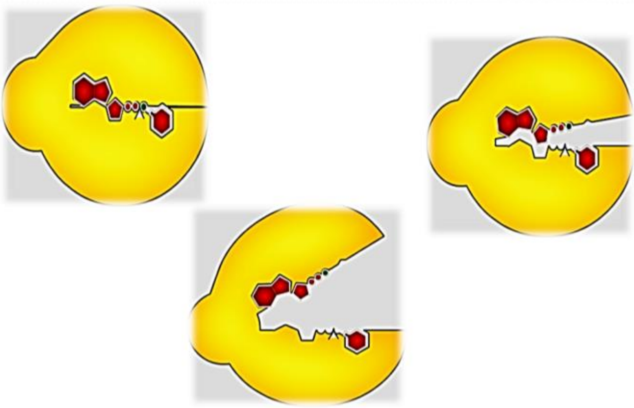
Increasing Reactivity

Stabilizing the Transition State

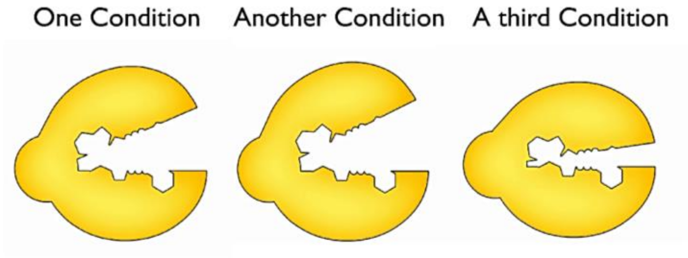
Binding Affinity



Various Conformations of the Enzyme

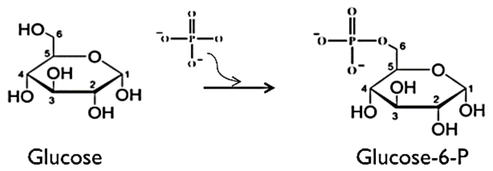


Conformational Changes

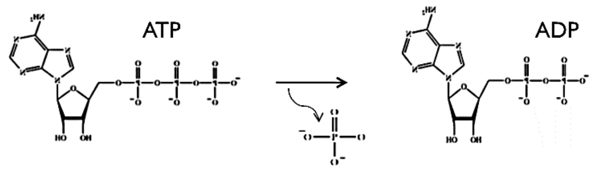


Phosphorylation *versus* Dephosphorylation

- Phosphorylation:



- *Dephosphorylation:*



APPENDIX C

PILOT STUDY

Moving Students to a Better Understanding of Enzyme Specificity

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Abstract

Students often struggle with understanding enzymatic reactions. One reason for students' confusion stems from the traditional instruction of the inaccurate "Lock and Key" model of enzyme specificity. However, proper understanding of this concept is connected to the students' understandings of other biological concepts. To address this problem, we developed a lesson based on a more scientifically accurate model; the "Induced Fit" model. We also supported this lesson with either of the two visual representations, static or dynamic, to compare the influence of each representation on understanding the concept. We used pre/post-tests, interviews, collected artifacts, and administered a follow-up content exam from eight senior students of the school of Human Performance and Recreation and compared them to fifteen uninstructed students at a research-intensive university. Upon analysis, we identified a positive influence of both representations on developing knowledge about the "Induced Fit" model. Furthermore, both representations helped in retaining more information about this concept as compared to controls. However, students exposed to dynamic representations demonstrated deeper understanding of the "Induced Fit" model.Therefore, we recommend a consistent representation of the "Induced Fit" model of enzyme specificity. Nevertheless, further research is needed before complete adoption of this concept by teachers and textbook publishers.

<u>Subject/Problem</u>: One of the main aspects of the nature of science is tentativeness of scientific knowledge. A good example from biochemistry is the replacement of the old "Lock and Key" model (Fischer, 1894) of enzyme specificity by the new "Induced Fit" model (Koshland, 1958). However, the representation of the "Lock and Key" model still persists in science textbooks and instruction. This persistence could be because the old model represents the concept through a concrete example; the lock and the key. Another reason would be the lack of consensus about the best practice of visual representations in the field of science education especially when it comes to representing abstract and dynamic models like the "Induced Fit" model (Morrison and Tversky, 2001; Sanger, 2000; Williamson, 1995). Therefore, the objective of this project is to study the influence of two different visual modes of representing the "Induced Fit" model on developing proper understanding of enzyme specificity.

Theoretical Framework: Learners often construct their own understandings based on their personal experiences (Duit & Treagust, 2003). For example, the learner may maintain the old model because it represents the enzyme as a known entity with a "fixed" shape; the "Lock" and its own specific "key." Eventually, based on the learner's personal experience, the enzyme is "specific" in action because it has a "fixed" shape. This case coincides with Strike's and Posner's revisionist theory of conceptual change (1992) that ordinary language analogues of scientific terms may structure the learner's perception of scientific concepts and eventually hinder the development of new knowledge. Given the simplicity of the "Lock and Key" representation and the challenging dynamics of the "Induced Fit" model, we also expect students to face difficulties in developing a full understanding of the "Induced Fit" model especially those with limited spatial intelligence (Gardner, 1983). Cook (2006) suggested that visual representations enhance learning and facilitate developing connections between new and prior knowledge. Yet, little research has been conducted on the role of visualization in biology education (Gilbert, 2005b), particularly when it comes to understanding dynamic three-dimensional entities like enzymes under action. Therefore, the theoretical framework that drives our study is knowledge development with visualization.

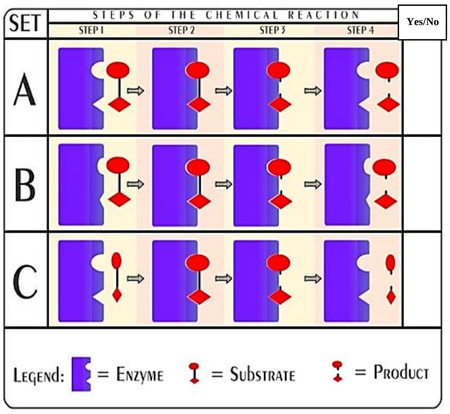
Literature Review: One reason behind resistance against developing new knowledge is the high degree of satisfaction of learners with their own existing conceptions (Posner et al., 1982). Given that learning is a cognitive activity, learners must establish a cognitive dissonance about their own existing preconceptions before changing them. Cognitive dissonance could be achieved by knowing that these concepts are incapable of explaining all the related scientific phenomena. Once dissatisfaction with these preconceptions is established, learners become motivated to accept scientific concepts. For this to take place, they have to be able to perceive the plausibility or the capacity of the new concepts to solve problems that the old ones could not (Posner et al., 1982). For example, in our case, the current "Induced Fit" model can explain why water molecules entering the active site of the enzyme Hexokinase cannot hydrolyze ATP (Koshland, 1958); an explanation that cannot be offered by the old "Lock and Key" model. The transitional phase from old to new constructs turns out to be a truly difficult task with some topics that need internal cognitive representation of abstract ideas (Palmer, 1978). This leads us to think about the importance of representations in the context of learning abstract scientific processes like enzymatic activity. However, the effects of various modes of representation in science education appear to be inconsistent in literature (Morrison and Tversky, 2001; Sanger, 2000; Williamson, 1995). For example, Morrison and Tversky (2001) argued that animated graphics "did not further increase effectiveness." In contrast, Williamson and Abraham (1995) showed that computer animations revealed increased understanding of a concept. A third investigation used computer animations combined with instruction based on Posner's conceptual theory and credited the increased understanding to the implemented mode of instruction (Sanger, 2000). Therefore, given the complexity of the process of learning on the one hand and the controversy of reports about visualization on the other hand, we believe that the efficiency of any mode of representation has to be studied in light of the implementation of our current knowledge about how people learn. Further, it is difficult to find any research that empirically examines the usefulness of different modes of representations of the same topic (e.g. enzyme specificity) while implementing the current theories of knowledge development as a platform for building new concepts. Addressing this gap in literature is the objective of our study.

Purpose/Research Questions: The students' proper understanding of how enzymes work is connected to their understanding of other biological concepts like the effect of mutations on the activity of enzymes. Therefore, we believe that understanding the new model of enzyme specificity, the "Induced Fit" model, should be the foundation on which further scientific concepts are built. For this purpose, we studied the influence of static and dynamic visual representations of enzyme activity on developing proper understanding and retention of this concept. Specifically, we attempted answering the following research questions: How does each of the two representations influence the dissatisfaction of learners with their own preconceptions about enzyme specificity? How does each of the two representations influence building proper knowledge about the "Induced Fit" model? How does each of the two representations influence the proper understanding of the "Induced Fit" model? And how does each of the two representations influence retention of the new knowledge?

Research Design/Methods: Twenty senior students from the School of Human Performance and Recreation at a southern university voluntarily participated in our study. One student stood as a pilot participant, fifteen stood as controls as they did not receive any instruction, four received instruction with static representations, and four received instruction with dynamic representations. To answer our research questions, we employed identical multiple-choice pre- & post-tests to evaluate changes in knowledge about the introduced concept. We also utilized two other qualitative tools, interviews and collected artifacts, to perform cross-data consistency checks among the four tools (Patton, 2002). To measure the influence of each mode of representation on information retention, we administered a follow-up content exam to all our participants after an average of six weeks from instruction. We then, utilized the pre/post-tests, interview transcripts, and collected artifacts to generate individual profiles for the instructed students. Finally, we performed an inductive analysis approach to study the scientific accuracy of student's responses on the content exam. This qualitative methodology helped us hypothesize which of the two chosen modes of visual representations better influenced the understanding of the "Induced Fit" model of enzyme specificity. It also helped us examine how these two representations influenced information retention by the instructed students as compared to the controls.

Findings: The findings of this study show how both static and dynamic representations, when combined with proper instructional design, positively influence students' ability to build proper knowledge about enzyme specificity. Prior to instruction, all the students' responses to questions in the pre-test and oral responses before the explanation phase showed their satisfaction with the old "Lock and Key" model. For example, Adam (participants' names are pseudonyms) described the "Lock and Key" model as "*accurate*" simply because it tells him that enzymes act only on specific substrates, "From what I learned and exercised in Physiology, you have an enzyme that can act on certain substrates but does not act on others." Adam's reference to previous instruction to defend his ideas about enzymes was not the only case. Most participants recalled learning about the "Lock and Key" model in previous Biology courses. However, some participants held other conceptions parallel to the "Lock and Key" model. One student, Sophia, had some preliminary ideas about the

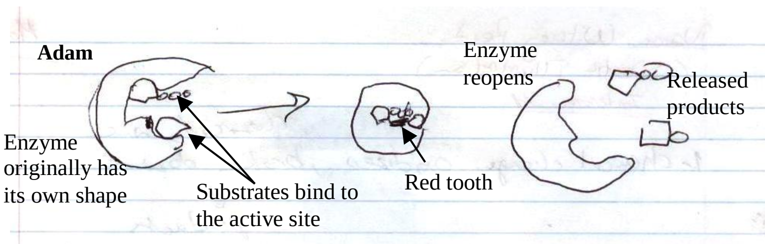
"Induced Fit" model and seemed to accept both models. Alexander, in turn, even held the conception that "a substrate changes it shape to be acted on by a specific enzyme." However, after instruction, all participants (8/8) expressed dissatisfaction with their prior knowledge. Adam, for example, explained his new stance about the concept as follows, "The "Lock and Key" model assumes that the enzyme stays the same and the substrate has to fit into it. And they have evidence, I mean pictures, that show that the enzyme does *does*[emphasis] change its shape." Adam's response not only suggests cognitive dissonance with the "Lock and Key" model, but also the ability to build minimal knowledge about the current model.



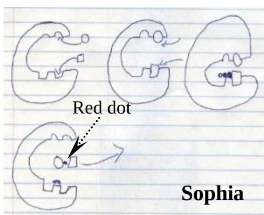
Alexander as well abandoned his previous misconceptions. For instance, when we asked him about his thoughts about set A (see right) he replied, "The enzyme will have to change in order for the reaction to take place because otherwise the two substrates would not meet with the catalytic group. So, no reaction." And when we asked him about (set C) he said; "I don't think C would work just because the substrate would change." These responses indicate his dissatisfaction with the "Lock and Key" model (represented in set A) and his disregard of the notion of a flexible substrate (represented in set C).

To see whether both visual groups of students were able to build proper knowledge about the new model, we asked them to explain how enzymes work; both in words and in drawings. All their verbal responses suggested their acquisition of the concept of the "Induced Fit" model through reciting the proper enzymatic mechanism of action. For example, when we asked Olivia how enzymes work, she answered:

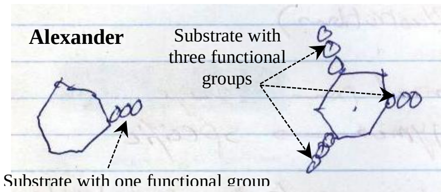
> The enzyme originally had its own shape, therefore substrates will bind to the active site that they are congruent with, at this time the enzyme closes to align the substrates and must align over the red tooth so that it can catalyze and change the substrates into a new product, and then that's when the enzyme reopens to let out the new product.



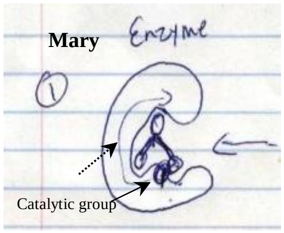
It is obvious here that Olivia was able to retain the importance of the catalytic group (red tooth); a core feature of the new model. In addition to mentioning it throughout the interview, she brought up its role while describing Set Awhere the illustration does <u>not</u> show the red tooth "Step 3: the substrate is, it is catalyzed by the red tooth. Step 4, the product is released." All but one (3/4) of the participants' drawings were clear enough to illustrate their proper understanding of the mechanism of enzyme specificity. Adam's drawing above stands as one example of how they visualized the process.



In contrast to all other students whom we exposed to the static representations, Sophia's drawing uncovered a misconception that she probably developed during the explanation phase. It seemed to us that she was mistaking the transferred phosphate group to glucose for the catalytic group of the enzyme (see right) and therefore believed that enzymes are consumed by the end of the reaction. Sophia later confirmed her acquisition of this misconception while describing her drawing, “That’s the red dot you’re talking about…I think the dot goes with them.” This developed misconception did not replicate with the students exposed to dynamic representations.



Before claiming that our participants have really developed proper understanding of enzyme specificity, we challenged their ability to use the new knowledge by asking them what would happen if the enzyme was exposed to two comparable substrates in size/shape. To answer this question, they first have to realize the importance of the chemical composition of the alternative substrate and second relate this composition to the catalytic group of the enzyme. All participants of both visual groups gave responses that suggested their ability to apply the new knowledge. For instance, in the post-test, Leo wrote, “It [reaction] will be slower or not at all.” However, only one of the students exposed to the static representations was able to relate this enzymatic behavior to the chemical composition of the substrate. Alexander: “If the Lock and Key were correct, you could use this [pointing on the left substrate with one functional group] or this [pointing on the right one with three functional groups] and it would give you *the same thing* because this shape and this shape are the same.” Yet, he was still unable to relate this enzymatic behavior to the catalytic group of the enzyme.



In contrast, all students exposed to the dynamic representations gave importance to the chemical composition of the substrate “Although shape is a factor, it’s not just about [the] shape, *it’s gotta be the specific chemical make-up*.” Nevertheless, only one of these students, Mary, was able to relate this chemical composition to the catalytic group. She interpreted her response in the post-test “They [the two comparable substrates] must possess the right chemical make-up” by relating it to the catalytic group as she pointed to her drawing and said, “It doesn’t mean it’s gonna work because *this* still may not say that [the substrate] is okay.” Mary’s drawing on the right embodies how her responses revolved around the catalytic group. It shows the catalytic group (solid arrow) sending a signal (dashed arrow) to the upper jaw of the enzyme to snap down once the substrate with the right chemical make-up binds to the active site of the enzyme.

Chart-A: **% Students Possessing Each Notion (Static vs. Dynamic)**

	Controls	**Static Rep.**	**Dynamic Rep.**
1st notion	13.3% students	50% students	100% students
2nd notion	13.3% students	50% students	75% students
3rd notion	0% students	25% students	25% student

In the follow-up content exam, we found that the instructed students were able to retain one or more of three notions: (1) the concept of an “Induced” fit between the enzyme and its substrate, (2) the possible enzymatic behavior with comparable substrates, (3) and the role of the catalytic group in the reaction. We also compared their responses to fifteen uninstructed students (controls). Only 2/15 (13.3%) of the controls seemed to know about the first and second notions. None of these controls possessed the third notion (0%). Regarding the instructed students, 2/4 (50%) of the exposed students to static representations (Static Rep.) retained the first notion as opposed to 4/4 (100%) of those exposed to the dynamic representations (Dynamic Rep.). Also, a lower percentage (50%) of the Static Rep. recalled the second notion as opposed to (75%) of the Dynamic Rep. However, only (25%) of both the Static Rep. and Dynamic Rep. remembered the third notion (see Chart-A).

Conclusions:This study investigated how students understood and retained the current concept of enzyme specificity as we used two different modes of visual representations; static and dynamic. We found that both representations helped lead students to dissatisfaction with their preconceptions. Also, both representations positively influenced knowledge development about the “Induced Fit” model. Both representations as well helped in retaining information about this concept (25%-100%) as compared to controls (13.3%). The dynamic representations in turn, helped overcome developed misconceptions through instruction with static representations. Students exposed to dynamic representations demonstrated deeper understanding of the “Induced Fit” model. Mind that almost all the students (4/5) that mentioned the importance of the chemical composition of the substrate and the only one that related this to the catalytic group were exposed to dynamic representations. These representations also helped increase retention of information as compared to the static ones. All these findings lead to the postulation that the dynamic representations stood for these students as an extension to their spatial intelligence abilities (Hegarty and Kriz, 2008) which, in turn, saved more space in their working memory. Consequently, with this saved room, the dynamic representations enabled them to achieve the reported deeper understanding of enzyme specificity and the increased retention of information as compared to the findings from the static representations (Bransford, 2000).

Implications & Contributions:The findings of this study stand as a model to instructors for how to challenge long-established inadequate conceptions. Though the old “Lock and Key” model is a simple explanation of enzyme specificity (Driver, 1983) and it does not require high levels of spatial intelligence as compared to the dynamic “Induced Fit” model (Gardner, 1983), the visual representations used in our lesson stood as tools to build proper knowledge of the new concept. The dynamic representations in particular, enabled proper understanding and increased retention as well. The students’ understanding of how enzymes work is connected to their understanding of other biological concepts. Therefore, based on our findings, we recommend a consistent representation of the “Induced Fit” model of enzyme specificity. However, further research is needed before complete adoption of this concept by classroom teachers and Biology textbook publishers.

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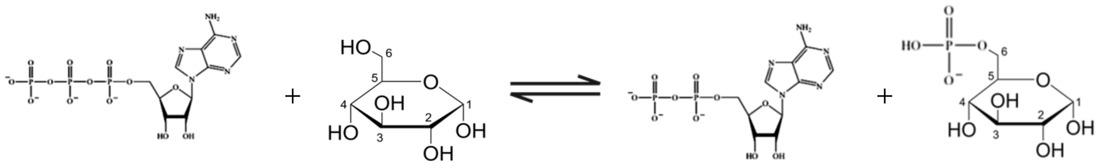
APPENDIX D

WORKSHEET

Code: ____________ Group: ______

Experiment-1:

Consider the following chemical reaction:



ATP Glucose ADP Glucose-6-P

Substrates Products

- Step-1: Put Glucose and ATP in their binding sites within the enzyme.
- Step-2: Push the button.

1- What happened to the shape of the enzyme?

____

2- How did this change affect the positioning of Glucose and ATP with respect to each other?

____

3- What happened to the enzyme afterwards (after it snapped down)?

____

4- What happened to Glucose? What happened to ATP?

____

Please rate your level of mental effort on this part of the lesson:
(Think of mental effort as how "hard" you had to think to answer the previous question)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

5- Is this likely to happen if the enzyme's shape did not change (as it did here)? Why?

____

Conclusion-1:

6- **What do you conclude from this experiment?**

The enzyme has to undergo a ______________ change to ______ the substrates, thus catalyzing the chemical reaction.

Experiment-2:

— Step-1: Replace the catalytic amino acid Aspartate (Asp-205) by Alanine.
— Step-2: Repeat steps 1-3 of Experiment-1.

Aspartate

Alanine

1- What happened to the shape of the enzyme?

____

2- How did this change affect the positioning of Glucose and ATP with respect to each other?

____

3- What happened to the enzyme afterwards (After it snapped down)?

____

4- What happened to Glucose? What happened to ATP?

____

Please rate your level of mental effort on this part of the lesson:
(Think of mental effort as how "hard" you had to think to answer the previous question)

1	2	3	4	5	6 7	8	9	
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

5- Compare the result of this experiment to that of experiment-1.

____

6- Explain the reason for getting different results.

____

Conclusion-2:

7- What do you conclude from this experiment?

> The presence of the **proper** _______________ is essential for the chemical reaction to be catalyzed by the enzyme.

Deduction-1:
How does the enzyme catalyze the chemical reaction?
(Hint: Review answers to conclusions-1&2)

> To catalyze the chemical reaction, the enzyme undergoes a _____________ change to _____ the substrates along with the _____________________.

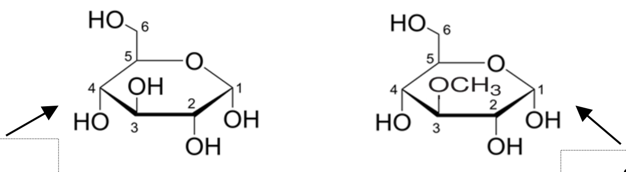
Please rate your level of mental effort on this part of the lesson:
(Think of mental effort as how "hard" you had to think to answer the previous question)

1	2	3		4	5	6 7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

---

Experiment-3a:

Glucose 3-methyl Glucose



Original Substrate

Substrate Analogue-1

1- Compare the shape of both molecules.

____

2- Contrast the chemical structure between the two molecules.

____

- Step-1: Put 3-methyl Glucose and ATP in their binding sites within the enzyme.
- Step-2: Push the button.

3- What happened to the shape of the enzyme?

____

Please rate your level of mental effort on this part of the lesson:

(Think of mental effort as how "hard" you had to think to answer the previous question)

1 2 3 4 5 6 7 8 9

Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

4- Compare the performance of the enzyme with Glucose (from Experiment-1) to that with 3-methyl Glucose.

____

5- How did this performance affect the positioning of 3-methyl Glucose and ATP with respect to each other?

____

6- How did this change affect the positioning of 3-methyl Glucose and ATP with respect to the reactive catalytic amino acid?

____

7- What happened to 3-methyl Glucose? What happened to ATP?

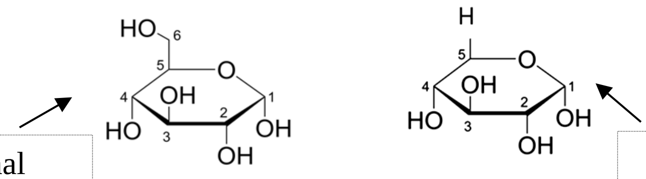
____

8- Formulate a hypothesis explaining your observations from this experiment.
(Hint: Review answer to question-2)

If a molecule has a ________ chemical structure than that of the original substrate, then it (will/will not induce) the enzyme to catalyze the chemical

Experiment-3b:

Glucose Xylose



Original Substrate

Substrate Analogue-2

9- Compare the shape of both substrates.

10- Contrast the chemical structure between the two substrates.

- Step-1: Put Xylose and ATP in their binding sites within the enzyme.
- Step-3: Push the button.

11- What happened to the shape of the enzyme?

12- Compare the performance of the enzyme with Glucose (from Experiment-1) to that with Xylose.

13- How did this performance affect the positioning of Xylose and ATP with respect to each other?

14- How did this change affect the positioning of Xylose and ATP with respect to the reactive catalytic amino acid?

15- What happened to ATP? What happened to Xylose?

Please rate your level of mental effort on this part of the lesson:

(Think of mental effort as how "hard" you had to think to answer the previous question)

1 2 3 4 5 6 7 8 9

Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

16- Did the enzyme catalyze the *desired* chemical reaction, i.e. did it transfer the phosphate group to the available substrate (Xylose)?

Conclusion-3:

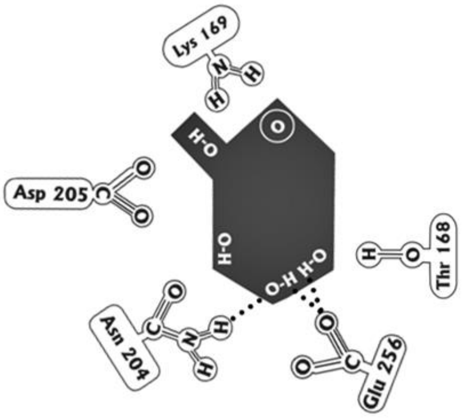
17- What can you conclude from these two experiments?
(Hint: Review your answer to questions-8 based on the new observations)

Substrates with different chemical structures than that of the original substrate (will/will not induce) the enzyme to catalyze the _________ chemical reaction.

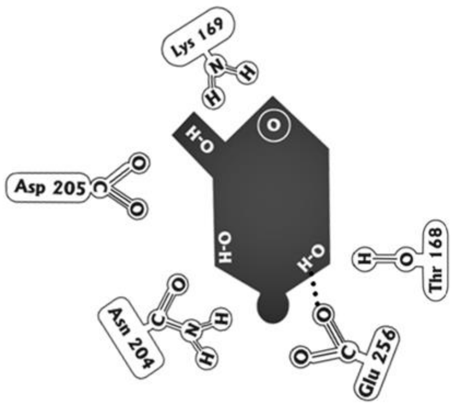
Please rate your level of mental effort on this part of the lesson:
(Think of mental effort as how "hard" you had to think to answer the previous question)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

Experiment-4:



Glucose



3-methyl Glucose

— Step-1: Count the number of possible H-bonds forming between Glucose and the catalytic amino acid residues within the active site of the enzyme. Record it here: _____.

— Step-2: Count the possible number of H-bonds forming between 3-methyl Glucose and the catalytic amino acid residues within the active site of the enzyme. Record it here: _____.

1. Which of the two substrates do you think would have a *higher* ***binding affinity*** to the enzyme? Why?

   ____

2. The transition state of which substrate do you think would be *more stabilized* by these stabilizing catalytic amino acids?

   ____

Deduction-2:

So far, what does it normally take for an enzyme to react with a substrate?
(Hint: Review answers to deduction-1and experiment-4)

- The _________________ of the substrate to the enzyme.
- The alignment of the *substrate* along with the __________________ brought by a _______ conformational change.

Please rate your level of mental effort on this part of the lesson:
(Think of mental effort as how "hard" you had to think to answer the previous question)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

Experiment-5:

- Step-1: Take *10* Glucose molecules with one ATP molecule.
- Step-2: Put ATP and one Glucose molecule in their binding sites within the enzyme.
- Step-3: Push the button.
- Step-4: Record what happens.
- Step-5: Repeat steps (1) through (5) with the rest of Glucose molecules.

2- With how many Glucose molecules the enzyme reacted properly? Calculate the percentage.

___

- Step-6: Now, take *10* 3-methyl Glucose molecules with one ATP molecule.
- Step-7: Repeat steps (1) through (5) with all of 3-methyl Glucose molecules.

3- With how many 3-methyl Glucose molecules the enzyme reacted properly? Calculate the percentage.

___

4- Toward which of the two substrates do you think the enzyme has *more* ***reactivity***?

___

Deduction-3:

Which parameters would you look at when measuring the specificity of an enzyme toward a substrate?
(Hint: Review your answers to experiment-4 and experiment-5)

The parameters are ___________________ and ___________________.

Please rate your level of mental effort on this part of the lesson:
(Think of mental effort as how "hard" you had to think to answer the previous question)

1 2 3 4 5 6 7 8 9

○	○	○	○	○	○	○	○	○
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

....

Please answer the two survey questions below:

1- **Please indicate** *how difficult* **this** *lesson* **was by checking the appropriate answer:**

1 2 3 4 5 6 7 8 9

○	○	○	○	○	○	○	○	○
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

Please take one minute to read the instructions for the following question:

The following question **does NOT relate to the concept of enzyme specificity**. "Learning environment" refers to the classroom atmosphere, how easy it is for you to understand the *format* of the test, and other factors that relate ***not*** *to the concept of enzyme specificity*, but to the *materials* you are using and the *environment* you are in.

2- **Please indicate** *how difficult* **it is to work in this** *learning environment* **by checking the appropriate answer:**

1 2 3 4 5 6 7 8 9

○	○	○	○	○	○	○	○	○
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

APPENDIX E

IRB APPROVAL LETTER (1)

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI.

INSTITUTIONAL REVIEW BOARD
118 College Drive #5147 | Hattiesburg, MS 39406-0001
Phone: 601.266.5997 | Fax: 601.266.4377 | www.usm.edu/research/institutional-review-board

NOTICE OF COMMITTEE ACTION

The project has been reviewed by The University of Southern Mississippi Institutional Review Board in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services (45 CFR Part 46), and university guidelines to ensure adherence to the following criteria:

- The risks to subjects are minimized.
- The risks to subjects are reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered regarding risks to subjects must be reported immediately, but not later than 10 days following the event. This should be reported to the IRB Office via the "Adverse Effect Report Form".
- If approved, the maximum period of approval is limited to twelve months. Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: **14032711**
PROJECT TITLE: **Moving College Students to a Better Understanding of Substrate Specificity of Enzymes through combining Part-task and Pre-training techniques**
PROJECT TYPE: **New Project**
RESEARCHER(S): **Mounir Saleh**
COLLEGE/DIVISION: **College of Science and Technology**
DEPARTMENT: **Center for Science and Math Education**
FUNDING AGENCY/SPONSOR: **N/A**
IRB COMMITTEE ACTION: **Exempt Review Approval**
PERIOD OF APPROVAL: **04/04/2014 to 04/03/2015**

Lawrence A. Hosman, Ph.D.
Institutional Review Board

APPENDIX F

IRB APPROVAL LETTER (2)

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI.

INSTITUTIONAL REVIEW BOARD
118 College Drive #5147 | Hattiesburg, MS 39406-0001
Phone: 601.266.5997 | Fax: 601.266.4377 | www.usm.edu/research/institutional-review-board

NOTICE OF COMMITTEE ACTION

The project has been reviewed by The University of Southern Mississippi Institutional Review Board in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services (45 CFR Part 46), and university guidelines to ensure adherence to the following criteria:

- The risks to subjects are minimized.
- The risks to subjects are reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered regarding risks to subjects must be reported immediately, but not later than 10 days following the event. This should be reported to the IRB Office via the "Adverse Effect Report Form".
- If approved, the maximum period of approval is limited to twelve months.
  Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: **CH-B14032711**
PROJECT TITLE: **Moving College Students to a Better Understanding of Substrate Specificity of Enzymes through combining Part-task and Pre-training techniques**
PROJECT TYPE: **Change to a Previously Approved Project**
RESEARCHER(S): **Mounir Saleh**
COLLEGE/DIVISION: **College of Science and Technology**
DEPARTMENT: **Center for Science and Math Education**
FUNDING AGENCY/SPONSOR: **N/A**
IRB COMMITTEE ACTION: **Exempt Review Approval**
PERIOD OF APPROVAL: **05/13/2014 to 05/12/2015**

Lawrence A. Hosman, Ph.D.
Institutional Review Board

APPENDIX G

CONSENT FORM

The primary purpose of this study is to promote proper knowledge about how enzymes work. I will ask you to participate in a single 30 minutes lesson about the current model of enzyme specificity. You will also be asked to participate in some evaluation measurements for this study. I will ask you to complete two pre-tests, a short survey, and watch a short multimedia presentation prior to presentation of the lesson, and I will then ask you to participate in two post-tests and another 2-page survey to see what you have learned from the lesson and how did you find it. A short demographic survey will conclude the whole 1.5-hr session. You may choose not to participate. Everything is completely voluntary.

Your potential benefit is winning a tablet. A raffle will be run by the end of this session. There are no foreseeable psychological or physical risks expected as a result of participating in this study. You may voluntarily withdraw from the study at any time during the process **without** penalty. You are guaranteed confidentiality as you are using the number in the raffle ticket you have just received in all of your responses. Your participation in this study is entirely voluntary. You may decline to answer any questions that make you uncomfortable. All information gathered will be kept confidential. All returned evaluation tools will be destroyed when the study is completed. These tools are your responses to the pre/post-tests and surveys as well as questions asked in the worksheet.

This study and this consent form has been reviewed by the Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations. Any questions or concerns about participation in this research should be directed to the Chair of the Institutional Review Board at The University of Southern Mississippi, 118 College Drive # 5147, Hattiesburg, MS 39406, (601)-266-6820.

By completing the pre/post-tests and surveys, you are giving consent to participate in this study.

APPENDIX H

PRE-TEST (1)

Code: ___________ Group: _____

1. Which of the following statements best describes how substrates bind to enzymes? *(1 pt)*

A. *Any* substrate can bind to *any* enzyme to be acted on
B. Enzymes normally bind to *specific* substrates
C. *Both* the enzyme and its substrate have *fixed* shapes that fit into one another
D. Other (explain): _________

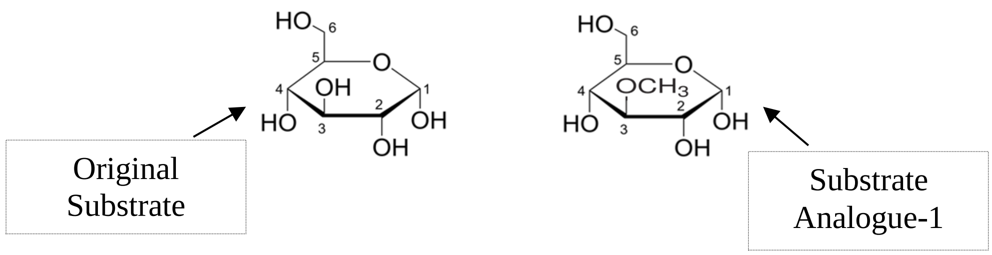
*Please rate your level of mental effort on question (**1**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

2. Glucose is the original substrate for the enzyme Hexokinase. 3-methyl Glucose is an analogue to Glucose. See both substrates below:

Glucose 3-methyl Glucose



If 3-methyl Glucose was added to Hexokinase, then it: *(1 pt each)*

a. Is *likely* to bind to Hexokinase with a/the ________ *affinity*.
   A. same
   B. higher
   C. lower
   D. Other (explain): _________

b. *Might* induce Hexokinase to undergo a/the _________.
   A. same conformational change
   B. different conformational change

C. same rate of conformational change
D. None of the above

c. *Might* cause Hexokinase to function at a/the _______ *reactivity*.
A. same
B. higher
C. lower
D. Other (Specify): _________

d. Is *likely* to have an/the ______ orientation with the catalytic amino acids and yield (the) ______ products.
A. proper, same
B. proper, different
C. improper, same
D. improper, no

3. ATP and Glucose are two substrates that react with the enzyme Hexokinase. During the course of this particular chemical reaction, Hexokinase produces Glucose-6-phosphate by using ATP to phosphorylate Glucose. Arrange the following into the correct sequence of events in this chemical reaction: *(1 pt)*

1. The binding of ATP and Glucose induces the enzyme to fit these substrates
2. The enzyme restores its original shape and the products are released
3. ATP and Glucose bind to their specific positions in the active site of the enzyme
4. The enzyme generates products by using ATP to phosphorylate Glucose

A. 1, 3, 4, 2
B. 3, 1, 4, 2
C. 3, 1, 2, 4
D. 4, 2, 3, 1

Please rate your level of mental effort on question (**3**):

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

Please answer the two survey questions below:

3- **Please indicate** *how difficult* **this** *test* **was by checking the appropriate answer:**

○	○	○	○	○	○	○	○	○
1	2	3	4	5	6	7	8	9
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

Please take one minute to read the instructions for the following question:

The following question **does NOT relate to the concept of enzyme specificity**. "Learning environment" refers to the classroom atmosphere, how easy it is for you to understand the *format* of the test, and other factors that relate ***not*** *to the concept of enzyme specificity*, but to the *materials* you are using and the *environment* you are in.

4- **Please indicate** *how difficult* **it is to work in this** *learning environment* **by checking the appropriate answer:**

○	○	○	○	○	○	○	○	○
1	2	3	4	5	6	7	8	9
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

END OF TEST

APPENDIX I

PRE-TEST (2)

Code: ____________ Group: ______

Circle the correct choice for each of the statements below:

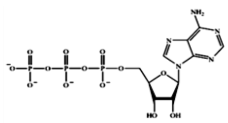
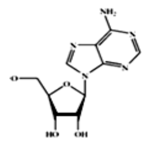
1. You can tell if a chemical reaction has occurred because it always produces:
   A. A different substance
   B. Reactants
   C. A change of state
   D. Water

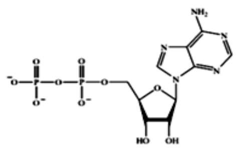
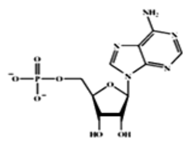
2. Transition state theory:
   A. Explains the transformation of reactants to products via the transition state
   B. Studies energy minima that occur between reactants and products
   C. Assumes equal concentrations of transition states and reactants
   D. All of these answers

3. Which of the following statements most accurately describes the energy of the transition state?
   A. The transition state is lower than the energy of the reactants but higher than the energy of the products
   B. The transition state is higher than the energy of both the reactants and the products
   C. The transition state is lower than the energy of both the reactants and the products
   D. The transition state is higher than the energy of the reactants but lower than the energy of the products

4. The *reactants* in an enzymatic reaction are called the *substrates* for that enzyme:
   A. True
   B. False
   C. I don't know

5. Which of the following molecules represent ATP:

A.  C. 

B. ). 

6. What molecules will form when ATP is broken down?
   A. Just ADP
   B. ADP + phosphate
   C. Just phosphate
   D. AMP + two phosphates

7. Which of the following statements is correct about enzymes?
   A. Enzymes slow down chemical reactions
   B. Not all reactions in a cell require enzymes
   C. Enzymes are considered as catalysts of chemical reactions
   D. Enzymes are not specific in their actions

8. All of the following statements are true about enzymes EXCEPT?

A. Enzymes bind with their substrates in such a way that the reaction can occur more readily
B. Enzymes raise the energy requirements of a chemical reaction
C. Enzymes bring together particular molecules and cause them to react together
D. Enzymes can be used over and over again

9. The ________ is the portion of an enzyme where the substrates bind in such a way that they are oriented to react.

A. Inhibitory site
B. Active site
C. Enzyme-substrate complex
D. Coenzyme

10. H-bonding occurs when ________:

A. A hydrogen atom forms a covalent bond with a slightly negative atom (e.g. H–O, H–N, etc)
B. A hydrogen atom forms a covalent bond with another hydrogen atom (e.g. H–H)
C. A hydrogen atom bonded to a slightly negative atom forms a non-covalent bond with another slightly negative atom (e.g. O–H·····O, O–H·····N, etc)
D. A hydrogen atom bonded to a slightly negative atom forms a non-covalent bond with a positive ion (e.g. O–H·····Na^+)

11. One way to represent the hydroxyl group is:

A. CO_2
B. H_2O
C. -OH
D. CH_3

12. A functional group is:

A. The part of an organic compound where chemical reactions take place
B. A hydroxyl group or a phosphate group
C. Made of atoms such as oxygen, hydrogen, nitrogen, phosphorus, and sulfur
D. All answers are correct

13. The group that is unique in each amino acid is the:

A. R-group
B. Amino group
C. Carboxyl group
D. None of these

14. The catalytic amino acids within the active site of an enzyme:

A. May increase the reactivity of the substrates
B. Help stabilize the transition state
C. All of the above
D. None of the above

15. The “binding affinity” of a substrate is the strength with which the substrate binds to an enzyme:

A. True
B. False
C. I don’t know

16. Phosphorylation of a compound is:

A. The removal of a phosphate group from that compound
B. The addition of a phosphate group to that compound
C. Never coupled with dephosphorylation of another compound
D. Connecting this compound with another through a phosphate linkage

17. A “conformational change” is the change of a protein from a primary to a secondary structure and vice versa:

A. True
B. False
C. I don’t know

END OF TEST

APPENDIX J

POST-TEST (1)

Code: ___________ Group: _____

4. Which of the following statements best describes how substrates bind to enzymes? *(1 pt)*

E. *Any* substrate can bind to *any* enzyme to be acted on
F. Enzymes normally bind to *specific* substrates
G. *Both* the enzyme and its substrate have *fixed* shapes that fit into one another
H. Other (explain): _________

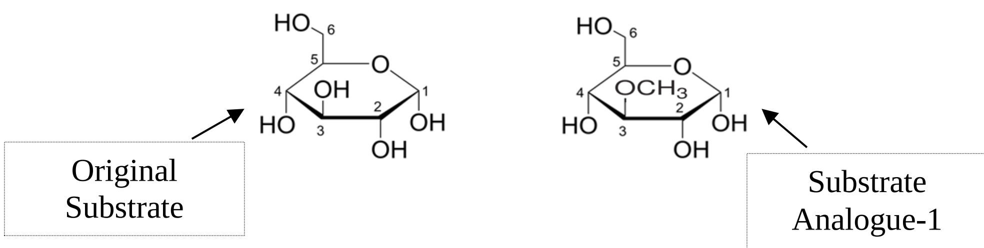
Please rate your level of mental effort on question **(1)***:*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

5. Glucose is the original substrate for the enzyme Hexokinase. 3-methyl Glucose is an analogue to Glucose. See both substrates below:

Glucose 3-methyl Glucose



If 3-methyl Glucose was added to Hexokinase, then it: *(1 pt each)*

e. Is *likely* to bind to Hexokinase with a/the ________ *affinity*.
E. same
F. higher
G. lower
H. Other (explain): _________

f. *Might* induce Hexokinase to undergo a/the _________.
   E. same conformational change
   F. different conformational change
   G. same rate of conformational change
   H. None of the above

g. *Might* cause Hexokinase to function at a/the _______ *reactivity*.
   E. same
   F. higher
   G. lower
   H. Other (Specify): _________

h. Is *likely* to have an/the ______ orientation with the catalytic amino acids and yield (the) ______ products.
   E. proper, same
   F. proper, different
   G. improper, same
   H. improper, no

6. ATP and Glucose are two substrates that react with the enzyme Hexokinase. During the course of this particular chemical reaction, Hexokinase produces Glucose-6-phosphate by using ATP to phosphorylate Glucose. Arrange the following into the correct sequence of events in this chemical reaction: *(1 pt)*

1. The binding of ATP and Glucose induces the enzyme to fit these substrates
2. The enzyme restores its original shape and the products are released
3. ATP and Glucose bind to their specific positions in the active site of the enzyme
4. The enzyme generates products by using ATP to phosphorylate Glucose

   E. 1, 3, 4, 2
   F. 3, 1, 4, 2
   G. 3, 1, 2, 4
   H. 4, 2, 3, 1

Please rate your level of mental effort on question (**3**):

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

Please answer the two survey questions below:

5- Please indicate *how difficult* **this** *test* **was by checking the appropriate answer:**

○	○	○	○	○	○	○	○	○
1	2	3	4	5	6	7	8	9
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

Please take one minute to read the instructions for the following question:

The following question **does NOT relate to the concept of enzyme specificity**. "Learning environment" refers to the classroom atmosphere, how easy it is for you to understand the *format* of the test, and other factors that relate ***not** to the concept of enzyme specificity*, but to the *materials* you are using and the *environment* you are in.

6- Please indicate *how difficult* **it is to work in this** *learning environment* **by checking the appropriate answer:**

○	○	○	○	○	○	○	○	○
1	2	3	4	5	6	7	8	9
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

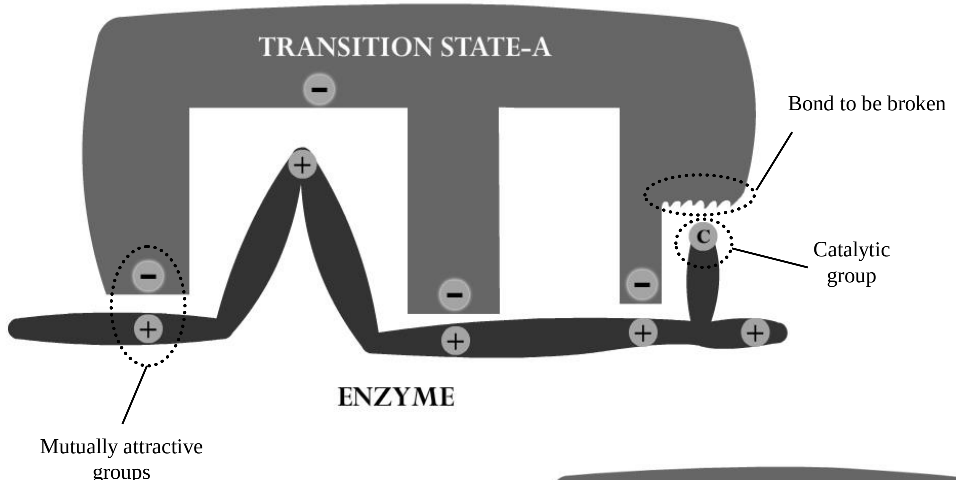
END OF TEST

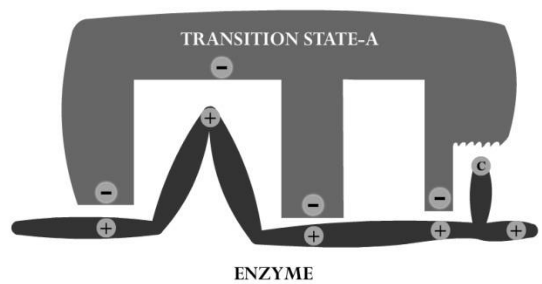
APPENDIX K

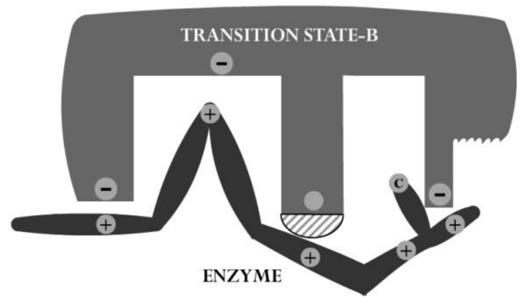
POST-TEST (2)

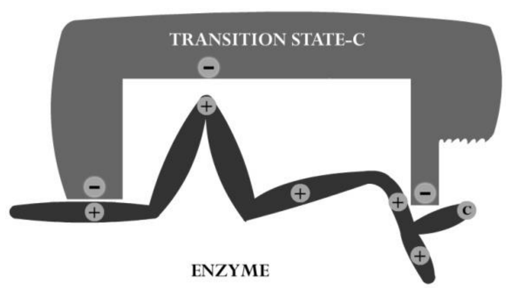
Code: ___________ Group: _____

The set of pictures below shows the interaction of an enzyme with transition states (TS) of three similar substrates. TS-A corresponds to the transition state of the natural substrate. Please read the following annotated illustration before you proceed:









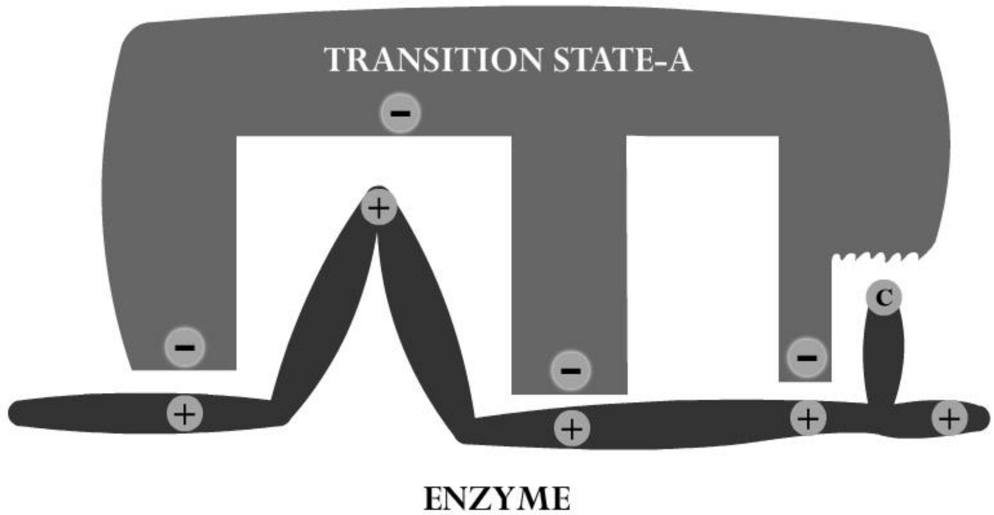
1. How many attractive groups (circled pluses) does the enzyme have? *(No pts)*

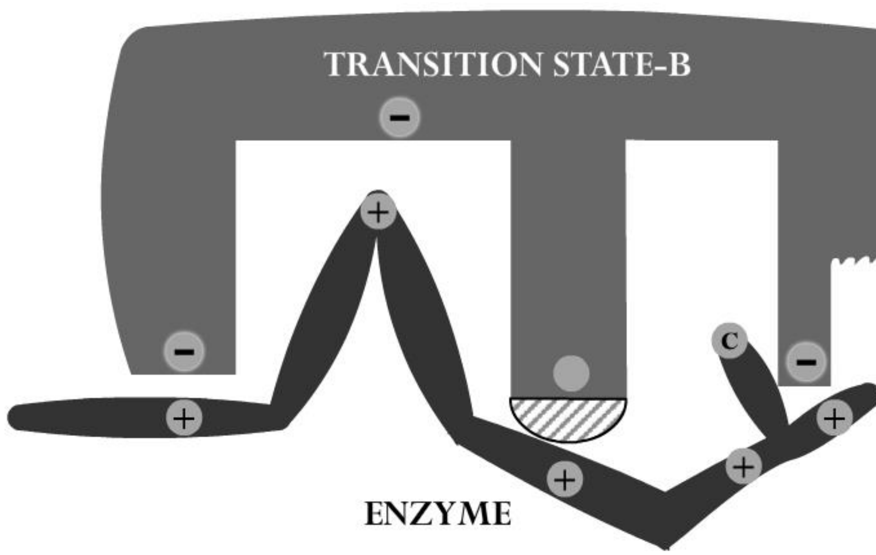
   Answer: ___

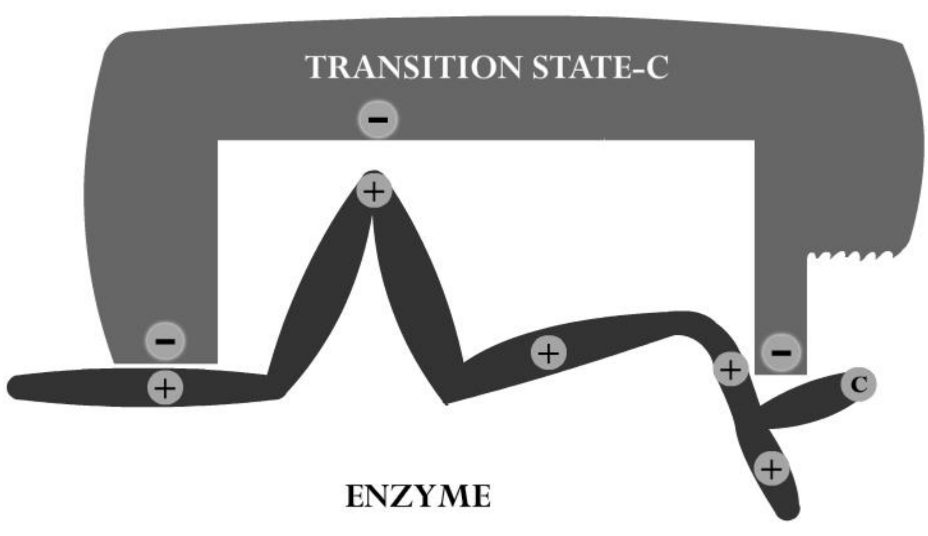
2. How many attractive groups (circled minuses) does ***each*** transition state have? *(No pts)*

   Answer: TS-A= __, TS-B= __, TS-C=__.

3. Transition state-B (TS-B) has an additional part on its middle piece which makes it different from transition state-A. This difference would ______ the *binding affinity* of TS-B to the enzyme: *(1 pt)*
   A. not affect
   B. increase
   C. decrease
   D. cannot be determined


TRANSITION STATE-A
C
ENZYME


TRANSITION STATE-B
C
ENZYME


TRANSITION STATE-C
C
ENZYME

4. Transition state-C (TS-C) differs from transition state-A by missing a middle piece. This difference would ______ the *binding affinity* of TS-C to the enzyme:*(1 pt)*

A. not affect
B. increase
C. decrease
D. cannot be determined

*Please rate your level of mental effort on question (**4**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

5. The enzyme has a different conformation with transition state-B (TS-B) than with transition state-A. This difference would ______ the *reactivity* of the enzyme to TS-B:*(1 pt)*

A. not affect
B. increase
C. decrease
D. cannot be determined

6. The enzyme has a different conformation with transition state-C (TS-C) than with transition state-A. This difference would ______ the *reactivity* of the enzyme to TS-C:*(1 pt)*

A. not affect
B. increase
C. decrease
D. cannot be determined

*Please rate your level of mental effort on question (**6**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

7. (**a**) What would you do if you were to design an artificial transition state that would:

- bind *tighter* to the enzyme than transition state-A
  AND
- *still* the enzyme *does not react* with it? *(2x0.5 pt)*

Answer:

____
____
____
____
____.

*Please rate your level of mental effort on question (**7**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

(**b**) Draw the artificial transition state that you just designed as it interacts with the enzyme.

Answer:

Phosphorylation of Acetate by ATP is catalyzed by the enzyme Acetate Kinase (ACK).

Suppose that you performed a series of experiments to determine the catalytic role of 4 amino acids within the active site of ACK with regard to ATP. See the obtained results below:

Note: K_{cat} reflects the ***reactivity*** of an enzyme toward a substrate.

K_m often reflects the ***binding affinity*** of an enzyme for a substrate.

Experiment	Obtained Results
1. Replacement of Asn-148 by Alanine	Experiment-1 A. K_{cat} dramatically changed B. K_m did *not significantly* change
2. Replacement of Ser-10 by Alanine	Experiment-2 A. K_{cat} did *not significantly* change B. K_m dramatically changed
3. Replacement of Lys-14 by Alanine	Experiment-3 A. K_{cat} did *not significantly* change B. K_m did *not significantly* change
4. Replacement of Lys-28 by Alanine	Experiment-4 A. K_{cat} did *not significantly* change B. *Non-significant* variations in *conformational change*

Asn-148: Asparagine at position 148Ser-10: Serine at position 10
Lys-14: Lysine at position 14Lys-28: Lysine at position 28

Referring to the Obtained Results in the table above:

Complete statements **1**through **5** by filling the blank with the correct choice: *(1 pt each)*

A. See table
B. See table
C. Not enough data
D. Other (Specify!)

8. Based on result-___ from Experiment-1, Asn-148 highly contributes to *reactivity* toward ATP
9. Based on result-___ from Experiment-2, Ser-10 is important for the *binding affinity* of ATP
10. Based on result-___from Experiment-1, Asn-148 is **not** important for the *binding affinity* of ATP

*Please rate your level of mental effort on question (**10**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

The table below is a <u>duplicate</u> to the previous table

Note: $\boldsymbol{K_{cat}}$ reflects the ***reactivity*** of an enzyme toward a substrate.

$\boldsymbol{K_m}$ often reflects the ***binding affinity*** of an enzyme for a substrate.

Experiment	**Obtained Results**
1. Replacement of Asn-148 by Alanine	Experiment-1 A. K_{cat} dramatically changed B. K_m did *not significantly* change
2. Replacement of Ser-10 by Alanine	Experiment-2 A. K_{cat} did *not significantly* change B. K_m dramatically changed
3. Replacement of Lys-14 by Alanine	Experiment-3 A. K_{cat} did *not significantly* change B. K_m did *not significantly* change
4. Replacement of Lys-28 by Alanine	Experiment-4 A. K_{cat} did *not significantly* change B. *Non-significant* variations in *conformational change*

Asn-148: Asparagine at position 148Ser-10: Serine at position 10
Lys-14: Lysine at position 14Lys-28: Lysine at position 28

A. See table
B. See table
C. Not enough data
D. Other (Specify!)

11. Based on result-___from Experiment-3, Lys-14 does **not** contribute to *reactivity* toward ATP
12. The contribution of Lys-28 to the *binding affinity* of ATP can be analyzed based on result- ____ from Experiment-4

*Please rate your level of mental effort on question (**12**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

The table below shows the <u>same obtained results</u> as in the previous table but in a <u>different format</u>

Note: K_{cat} reflects the ***reactivity*** of an enzyme toward a substrate.

K_m often reflects the ***binding affinity*** of an enzyme for a substrate.

Experiment	Obtained Results
1. Replacement of Asn-148 by Alanine	K_{cat} dramatically changed K_m did *not significantly* change
2. Replacement of Ser-10 by Alanine	K_{cat} did *not significantly* change K_m dramatically changed
3. Replacement of Lys-14 by Alanine	K_{cat} did *not significantly* change K_m did *not significantly* change
4. Replacement of Lys-28 by Alanine	K_{cat} did *not significantly* change *Non-significant* variations in *conformational change*

Asn-148: Asparagine at position 148Ser-10: Serine at position 10
Lys-14: Lysine at position 14Lys-28: Lysine at position 28

Referring to the table above:

Complete statements **6** through **9** by filling the blank with the correct choice: *(1 pt each)*

A. Contributes B. does not contribute
B. C. not enough data D. Other (Specify!)

13. Ser-10 ____to ACK specificity toward ATP
14. Asn-148____to ACK specificity toward ATP

*Please rate your level of mental effort on question (**14**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

15. Lys-14____ to ACK specificity toward ATP
16. Lys-28____ to ACK specificity toward ATP

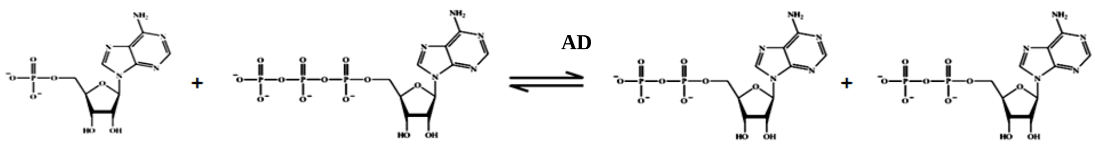
*Please rate your level of mental effort on question (**16**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

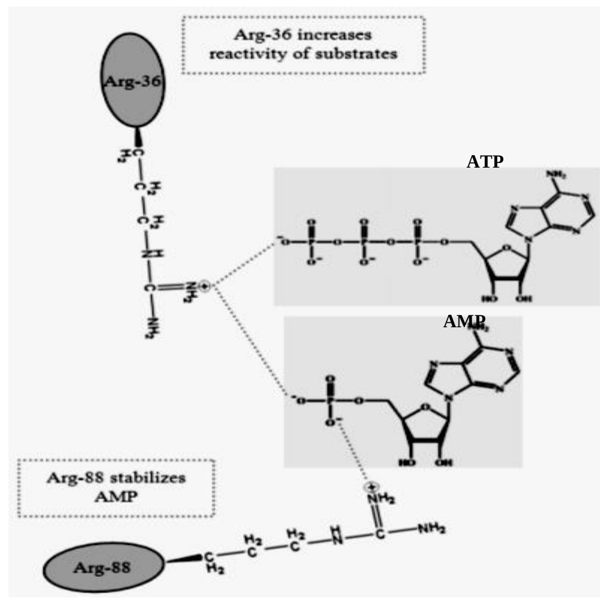
Adenylate Kinase (ADK) phosphorylates AMP through transferring the terminal phosphate of ATP to AMP.

Below is the chemical reaction:



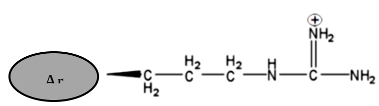
AMP ATP ADP ADP

ADK has several *positively* charged Arginine (Arg) residues in its active site. Arg-88 is responsible for *binding* to AMP offering it stabilization(see adjacent figure). Yet, Arg-36 increases the *reactivity* of ATP and AMP through simultaneously binding to both substrates (see the same figure).

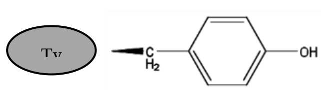


Consider the following amino acids as well as the footnote below to answer the following questions[20]:

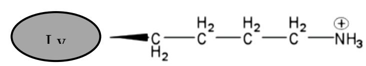
Arginine



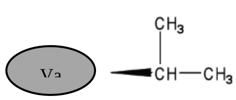
Tyrosine



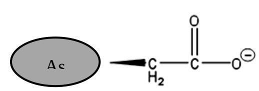
Lysine



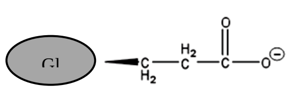
Valine



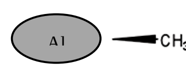
Aspartate



Glutamate



Alanine



Which of the following procedures would you consider the <u>most effective</u> if you wanted to:*(1 pt each)*

17. Significantly *reduce* the *binding affinity* of AMP to the enzyme?
    A. Replace Arg-88 with Glutamate

[20]Molecules of opposite charges can interact with one another. A polar amino acid has a polar group within its side chain (e.g. –OH, C=O group). A polar amino acid can interact with polar molecules whether they are charged or uncharged. A non-polar amino acid is made entirely from -CH_n group(s) and can only interact with non-polar molecules.

B. Replace Arg-88 with Lysine
C. Replace Arg-36 with Tyrosine
D. Replace Arg-36 with Lysine

18. Substitute Arg-88 with an amino acid that can *still* interact with AMP?
A. Replace Arg-88 with Valine
B. Replace Arg-88 with Alanine
C. Replace Arg-88 with Tyrosine
D. None of the above

19. Significantly *reduce* the *reactivity* of the enzyme to the substrates?
A. Replace Arg-88 with Lysine
B. Replace Arg-36 with Valine
C. Replace Arg-36 with Lysine
D. Replace Arg-88 with Tyrosine

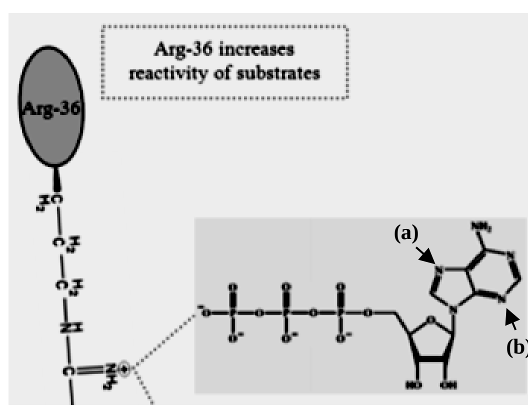
*Please rate your level of mental effort on question (**19**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

20. Substitute Arg-36 with an amino acid that can *still* interact with ATP and AMP?
A. Replace Arg-36 with Alanine
B. Replace Arg-36 with Aspartate
C. Replace Arg-36 with Valine
D. Replace Arg-36 with Lysine

21. *Increase* the *binding affinity* of ATP?
A. Introduce Alanine at *either* positions (a) or (b)
B. Introduce Tyrosine at *either* positions (a) or (b)
C. Introduce Tyrosine at *both* positions (a) and (b)
D. Introduce Alanine at *both* positions (a) and (b)



*Please rate your level of mental effort on question (**21**):*

(Think of mental effort as how "hard" you had to think to answer these questions)

1	2	3	4	5	6	7	8	9
Extremely Low mental effort	Low	Moderately Low	Slightly Low	Medium mental effort	Slightly High	Moderately High	High	Extremely High mental effort

Please answer the three survey questions below:

Please indicate *how difficult* **the** *last 5 questions* **were by checking the appropriate answer:**

1	2	3	4	5	6	7	8	9
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

Please indicate *how difficult* **the** *Overall test* **was by checking the appropriate answer:**

1	2	3	4	5	6	7	8	9
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

Please take one minute to read the instructions for the following question:

The following question **does NOT relate to the concept of enzyme specificity**. "Learning environment" refers to the classroom atmosphere, how easy it is for you to understand the *format* of the test, and other factors that relate ***not** to the concept of enzyme specificity*, but to the *materials* you are using and the *environment* you are in.

Please indicate *how difficult* **it is to work in this** *learning environment* **by checking the appropriate answer:**

1	2	3	4	5	6	7	8	9
Extremely Easy	Easy	Moderately Easy	Slightly Easy	Not Easy & Not Difficult	Slightly Difficult	Moderately Difficult	Difficult	Extremely Difficult

END OF TEST

APPENDIX K

LETTER OF SUPPORT FROM THE AUTHOR OF IMMS

From: John Keller<jkellersan@gmail.com>
To: Mounir Saleh<mounir.saleh@usm.edu>

Dear Mounir,

Please be advised that you may use the Instructional Materials Motivation Survey (IMMS) in your research. There is no fee for using the instrument.

Best wishes for a successful study!

Sincerely,

John K.

John M. Keller, Ph.D.
Professor Emeritus
Educational Psychology and Learning Systems
Florida State University
9705 Waters Meet Drive
Tallahassee, FL 32312-3746
Phone: 850-294-3908

*Official ARCS Model Website:*http://arcsmodel.com.
Keller, J.M. (2010), ***Motivational Design for Learning and Performance: The ARCS Model Approach***. New York: Springer. Now available in English, Japanese, and Korean.

APPENDIX L

INSTRUCTIONAL MATERIALS MOTIVATION SURVEY

Code : ___________ Group: ____

Please answer the following questions in relation to your experience in the learning session you have just completed. These questions relate to the *thoughts and feelings* you may have experienced during the session. There are no right or wrong answers. Draw a circle on the number that indicates how much you agree or disagree with each statement. If you are uncertain of or neutral about your response, you may always select "Neither Agree or Disagree"

	Strongly Disagree	Disagree	Neither Agree or Disagree	Agree	Strongly Agree
Attention					
1. There was something interesting about the Enzyme Model that got my attention.	1	2	3	4	5
2. This Enzyme Model is eye-catching.	1	2	3	4	5
3. The Enzyme Model helped to hold my attention.	1	2	3	4	5
4. The design of the Enzyme Model looks appealing.	1	2	3	4	5
5. This Enzyme Model stimulated my curiosity.	1	2	3	4	5
6. The Enzyme Model helped me learn some important things about enzyme specificity.	1	2	3	4	5
7. The variety of pieces that came with the Enzyme Model helped keep my attention.	1	2	3	4	5
8. The worksheet associated with the Enzyme Model was appropriate to understand about enzyme specificity.	1	2	3	4	5

	Strongly Disagree	Disagree	Neither Agree or Disagree	Agree	Strongly Agree
Relevance					
1. It is clear to me how the Enzyme Model is related to the concept of enzyme specificity.	1	2	3	4	5
2. Completing this session successfully was important to me.	1	2	3	4	5
3. The content in the worksheet associated with the Enzyme Model conveys the impression that it is worth knowing.	1	2	3	4	5
4. The concept of enzyme specificity was relevant to my major because I need to know about how	1	2	3	4	5

enzymes work.					
5. I could relate the content of the worksheet associated with the Enzyme Model to my own coursework.	1	2	3	4	5
6. The content of the worksheet which comes with the Enzyme Model will be useful to me.	1	2	3	4	5

	Strongly Disagree	**Disagree**	**Neither Agree or Disagree**	**Agree**	**Strongly Agree**
Confidence					
1. When I first looked at the Enzyme Model, I had the impression that it would be easy to understand enzyme specificity through using it.	1	2	3	4	5
2. The concept of enzyme specificity was easier to understand through the Enzyme Model than I thought it would be.	1	2	3	4	5
3. I felt confident that I knew what I was supposed to learn from this learning session while using the Enzyme Model.	1	2	3	4	5
4. I was able to pick out and remember the important points.	1	2	3	4	5
5. After attending this learning session, I was confident that I would be able to do well in the post-test.	1	2	3	4	5
6. As I went through the worksheet, I was confident that I could learn the content.	1	2	3	4	5

	Strongly Disagree	**Disagree**	**Neither Agree or Disagree**	**Agree**	**Strongly Agree**
Satisfaction					
1. Completing this learning session, with the Enzyme Model, gave me a satisfying feeling of accomplishment.	1	2	3	4	5
2. I enjoyed the Enzyme Model so much that I would like to play with it myself.	1	2	3	4	5
3. I really enjoyed the Enzyme Model being used in class.	1	2	3	4	5
4. It was a pleasure to work on such a well-designed worksheet.	1	2	3	4	5
5. It felt good to successfully complete this learning session.	1	2	3	4	5
6. It was a pleasure to work on such a well-designed Model.	1	2	3	4	5

APPENDIX M

DEMOGRAPHIC SURVEY

Code: ____________ Group: _____

Please take a few minutes to complete this short survey. All individual responses are ***anonymous*** and there is no intent to identify individual respondents. Only the consolidated results will be analyzed.

1. Age: In years
   - ☐ 18 or younger
   - ☐ 19-24
   - ☐ 25-34
   - ☐ 35-44
   - ☐ 45 or older

2. Gender: What is your sex?
   - ☐ Female
   - ☐ Male

3. Education: What is the level of school you have completed?
   - ☐ High School
   - ☐ 1 year of College
   - ☐ 2 years of College
   - ☐ 3 years of College
   - ☐ 4 years of College
   - ☐ Bachelors Degree
   - ☐ Graduate Degree

4. Ethnicity: How do you classify yourself?
   - ☐ Asian
   - ☐ African American/Black
   - ☐ Caucasian/White
   - ☐ Hispanic/Latino
   - ☐ Native American
   - ☐ Other (Please Specify) _______________________

5. Language: What is your primary language?
   - ☐ English
   - ☐ Other(Please Specify) ________

6. Major: What is your major?
   - ☐ Biology
   - ☐ Biochemistry

☐ Other(Please Specify) ____________

7. Background: Please check the courses that you took/are taking:
   - ☐ Principles of Biochemistry
   - ☐ Biochemistry I (Structure & Catalysis)
   - ☐ Biochemistry II (Bioenergetics & Metabolism)
   - ☐ Biochemistry III (Information Pathways)
   - ☐ Analytical Biochemistry

8. ACT Score:

   Total Score: ____ Science Test Score: ____

   If you have not taken the ACT, please provide your SAT score below

9. SAT Score:

   Total Score: ____ Subject: _____

10. GPA: Which of the following best describes your current GPA?
    - ☐ Less than 3.0
    - ☐ 3.0-3.3
    - ☐ 3.4-3.6
    - ☐ 3.7-3.9
    - ☐ More than 3.9

11. Enrollment: Are you a full student?
    - ☐ Yes
    - ☐ No

12. Computer Basic Skills: Please rate the following from Low to High, 1-5
    ___ How comfortable are you with computers?
    ___ I am comfortable with the basics of the Windows operating system
    ___ I know how to start up a software application and to close it.
    ___ I understand how to minimize and maximize applications in Windows.
    ___ I know how to minimize multiple open applications on the task bar at the bottom of the screen and reopen them at any time.
    ___ I understand how to resize application windows and move them anywhere on the screen.

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