2014

Extending Students' Practice of Metacognitive Regulation Strategies in the Undergraduate Chemistry Laboratory and Investigation of Pb2+ Binding to Calmodulin with Circular Dichroism and Molecular Dynamics Modeling

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**Recommended Citation**

Van Opstal, Mary Twist, 'Extending Students' Practice of Metacognitive Regulation Strategies in the Undergraduate Chemistry Laboratory and Investigation of Pb2+ Binding to Calmodulin with Circular Dichroism and Molecular Dynamics Modeling' (2014). *Dissertations*. Paper 1309.  
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LOYOLA UNIVERSITY CHICAGO

EXTENDING STUDENTS’ PRACTICE OF METACOGNITIVE REGULATION STRATEGIES IN THE UNDERGRADUATE CHEMISTRY LABORATORY

AND

INVESTIGATION OF Pb2+ BINDING TO CALMODULIN WITH CIRCULAR DICHROISM AND MOLECULAR DYNAMICS MODELING

A DISSERTATION SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

PROGRAM IN CHEMISTRY

BY
MARY TWIST VAN OPSTAL
CHICAGO, IL
DECEMBER 2014
ACKNOWLEDGMENTS

I would like to thank all of the people who made my research experience possible. My advisors Patrick Daubenmire and Alanah Fitch, they not only supported me in my research at all times, but also gave me the reins to manage my research and implement new instructional strategies into the learning laboratory. They allowed me to have experience as a teacher in both the classroom and lab when I guest taught lecture and ran the laboratory as the head TA. Patrick and Alanah guided me in my ideas and helped to balance my two projects in education and bench work chemistry. Patrick introduced me to many chemistry education colleagues, and he encouraged me to attend conferences to present my research and run workshops. I know I have a great network of colleagues that I can use after graduation and in my career. Both advisors supported me in my work life balance and through the birth of both my children while researching. Additionally, I would like to thank my committee member, Duarte Mota de Freitas for the guidance in my biochemical research study. When I decided to become involved in an additional research project on lead binding to calmodulin through molecular dynamics, Ken Olsen provided support and ideas in the completion of that project. I had several undergraduates assist me in my research. I thank them for the time spent running experiments and coding interviews. I could not have done the research without my research participants! I thank them for their wonderful words and insights during interviews.
I would also like to thank the Loyola University Chicago Chemistry Department and the Graduate School for the teaching fellowships and tuition scholarships that allowed me to attend Loyola and complete my research.

Finally, I would like to thank my family who have supported me through the long hours, stressful times and the exciting results and projects I’ve completed. I thank my parents for supporting me from the very first time I became interested in chemistry, making science projects at home a part of my life, and instilling hard work in me. Thank you to Tami van Opstal and Barbara Twist for editing. Thank you to my husband, Chris, who has always been interested in my research and supported me every step of the way, and to my children, Marius and Kai for their smiling faces and laughter when I needed it.
To my mother
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**LIST OF ABBREVIATIONS**

**Chemistry Education Study**

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACS</td>
<td>American Chemical Society</td>
</tr>
<tr>
<td>CIC</td>
<td>Confidence in Chemistry</td>
</tr>
<tr>
<td>DBER</td>
<td>Disciplined Based Education Research</td>
</tr>
<tr>
<td>GALT</td>
<td>Group Assessment of Logical Thinking</td>
</tr>
<tr>
<td>MCAI</td>
<td>Metacognitive Activities Inventory</td>
</tr>
<tr>
<td>NRC</td>
<td>National Research Council</td>
</tr>
<tr>
<td>POGIL</td>
<td>Process Oriented Guided Inquiry Learning</td>
</tr>
<tr>
<td>STEM</td>
<td>Science Technology Engineering Mathematics</td>
</tr>
<tr>
<td>SWH</td>
<td>Science Writing Heuristic</td>
</tr>
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</table>

**Lead Calmodulin Interaction Study**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Apo</td>
<td>Protein with no metal ions bound</td>
</tr>
<tr>
<td>“as-is”</td>
<td>CaM protein sample contains one mole of Ca or less per mole of protein</td>
</tr>
<tr>
<td>Ca/Ca^{2+}</td>
<td>Calcium/calcium ion</td>
</tr>
<tr>
<td>Ca-CaM</td>
<td>Calcium bound calmodulin</td>
</tr>
<tr>
<td>CaM</td>
<td>Calmodulin</td>
</tr>
<tr>
<td>CD</td>
<td>Circular dichroism</td>
</tr>
<tr>
<td>EFI-IV</td>
<td>Canonical binding sites on CaM</td>
</tr>
<tr>
<td>Holo</td>
<td>Protein with metal ions bound</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>$K_d$</td>
<td>Dissociation constant</td>
</tr>
<tr>
<td>MD</td>
<td>Molecular Dynamics</td>
</tr>
<tr>
<td>ME</td>
<td>Molar Ellipticity (unit of CD signal)</td>
</tr>
<tr>
<td>MLCK</td>
<td>Myosin Light Chain Kinase</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>Pb/Pb$^{2+}$</td>
<td>Lead/lead ion</td>
</tr>
<tr>
<td>Pb-CaM</td>
<td>Lead bound calmodulin</td>
</tr>
<tr>
<td>PDB</td>
<td>Protein Data Bank</td>
</tr>
<tr>
<td>RMSD</td>
<td>Root mean square deviation</td>
</tr>
<tr>
<td>RMSF</td>
<td>Root mean square fluctuation</td>
</tr>
<tr>
<td>VMD</td>
<td>Visual Molecular Dynamics</td>
</tr>
<tr>
<td>XRC</td>
<td>X-Ray Crystallography</td>
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CHAPTER ONE
INTRODUCTION

Structure of Dissertation

With the recent addition of chemistry education to the graduate program in chemistry at Loyola, I had the opportunity to explore both chemistry education and bench work chemistry in my doctoral research. The resulting dissertation is composed of two studies, the main study in chemistry education and the second study in bench and computational biochemistry. The chemistry education study is presented first. Following this section in chapter one, the introduction, research goals and research questions are described. Chapters two through five fully describe the research on students’ metacognitive strategy use in the general chemistry laboratory. Three chapters follow this study that describe the interaction of the toxic metal lead, Pb^{2+}, with calmodulin (CaM), a calcium binding protein. Chapter six provides an introduction to the area of research. Chapter seven describes the Pb-CaM interaction through the circular dichroism technique. Chapter eight describes a molecular dynamics modeling study to predict the binding sites of Pb on CaM as well as its effects on the structure of CaM.

Chemistry Education Study

Reasons for Research

Recently, the Discipline Based Education Research (DBER) Report from the National Research Council (2012) emphasized that metacognitive strategies be
incorporated into the teaching of STEM disciplines including the chemistry laboratory because these strategies are essential for greater learning of science concepts and skills. In addition, a few studies suggest that undergraduate students use few metacognitive strategies when learning (Karpicke, Butler, & Roediger III, 2009; McCrindle & Christensen, 1995) or are not aware of their strategy use. It is beneficial for students to use and be aware of their regulation strategies because people with greater use of metacognition have significantly improved learning and understanding of content because they are more able to recognize gaps in their knowledge and can better plan and monitor skill development during the learning process (Garner & Alexander, 1989; Kuhn, 1989; Schraw & Dennison, 1994). Recent research showed that students have improved regulation strategies when metacognitive strategies were integrated into the curriculum (Kuhn, 2000; Mathabathe & Potgieter, 2014; Rickey & Stacy, 2000; Schraw, Crippen, & Hartley, 2006).

The chemistry learning laboratory is one environment that we hope students are learning science concepts and skills. Yet there is little research to date that suggests that students are actually learning chemistry concepts in lab especially in the common traditional “cookbook” labs that most students experience at the undergraduate level (Hofstein & Lunetta, 2004). To date there has been significant research to improve the state of laboratory curriculums by using different instructional strategies that promote conceptual learning and science skills. Recently developed instructional strategies include guided-inquiry labs (Science Writing Heuristic) (Greenbowe & Hand, 2005), problem-based laboratories (Argument-Driven Inquiry) (Walker, Sampson, & Zimmerman, 2011),
research-based labs (Kelly & Finlayson, 2007), and cooperative learning environments (Cooper & Kerns, 2006). These are all methods that have been shown to improve students’ chemistry knowledge and skills more than traditional labs (Russell & Weaver, 2011).

**Research Goals**

Further research may elucidate whether these methods of instruction in the lab provide environments for students to practice metacognitive regulation strategies. There is research to support that students use metacognitive strategies in cooperative and inquiry labs (Kipnis & Hofstein, 2008; Sandi-Urena, Cooper, & Stevens, 2012). Our research sought to first understand how students naturally used metacognitive regulation strategies while performing experiments in one of the recently developed instructional environments described above, the Science Writing Heuristic. We explored the types of regulation strategies that students used to solve open-ended problems and how they described their strategy use when engaged in a collaborative inquiry environment (Science Writing Heuristic) in the undergraduate chemistry laboratory.

**Qualitative Research Questions**

- How does the process by which students solve and report on open-ended laboratory problems reveal use of metacognitive regulation strategies?
- Does the type of laboratory instructional environment (SWH vs. Traditional) that students regularly experience elicit any differences in students’ use of metacognitive regulation strategies while solving open-ended laboratory problems?
CHAPTER TWO

LITERATURE REVIEW

Constructing Learning Opportunities

The chemistry laboratory in educational settings has long been considered to be essential for conceptual learning (Berry, Mulhall, Gunstone, & Loughran, 1999; Cooper & Kerns, 2006; Lloyd, 1992; Poock, Burke, Greenbowe, & Hand, 2007). However, in a review of the instructional practices, goals and learning outcomes in the chemistry laboratory over two decades, little evidence was found that the laboratory impacts students’ learning of chemistry (Hofstein & Lunetta, 2004). Students may learn technical skills through the rote verification labs, but little to no learning of chemistry concepts occurs. There is no doubt that technical skills are an essential component of the hands-on lab experience, but they are not the only goal of laboratory learning. Students in most traditional verification laboratory classrooms are not provided with an environment in which problem-solving or critical thinking skills are required, thus, they display a lack of conceptual learning. If we are to take advantage of the chemistry laboratory as an essential part of students’ learning of chemistry concepts, then it is important to understand how people learn. This understanding may help us shape laboratory instruction that is aligned to the key principles of how people learn (National Research Council (U.S.), 2000).
Students bring preconceptions (prior knowledge) whether right or wrong to any class. Research supports that when teachers engage the students to discover their current ideas, students’ misconceptions can be revealed, and instruction can be shaped to address those misconceptions (Teichert & Stacy, 2002). Therefore, frequent assessment is essential to ensure that students are resolving their misconceptions and retaining the new subject matter. Secondly, students learn by seeing patterns of meaningful information and by chunking information in relationship with big ideas. Using these patterns and chunks, students build and integrate new information into an organized conceptual framework that promotes long-term learning and conceptual understanding (Perkins & Salomon, 1989). Subject matter that is taught within a context promotes transfer of knowledge to new situations and quick retrieval of the information. Teachers must be very knowledgeable of their subject matter so they can both teach students the material and help students to successfully integrate this new information into their conceptual framework. Teachers also can assist students by providing an environment where learning for understanding, as opposed to mere memorization of facts and or skills, is promoted. Teacher-centered learning situations such as a lecture where students have no opportunities for active engagement, may be established with the intention of promoting learning for understanding, yet students in these learning settings only achieve memorization of facts provided by the instructor. This misalignment in student learning and teaching goals may be remedied by incorporating opportunities for student-centered learning in which students are actively engaged with concepts and can integrate these concepts into their mental frameworks (Bunce, 2009). Finally, students who have
metacognitive strategies can take control of their learning and improve their academic performance. Within a knowledge-centered and students-centered environment, teachers can support students’ practice of metacognitive strategies by modeling and integrating metacognitive strategies into the curriculum (National Research Council (U.S.), 2000; Schraw, 1998).

**Metacognition**

Several studies in the STEM disciplines have shown that people with greater use of metacognition have significantly improved learning and understanding of content because they are more able to recognize gaps in their knowledge and can better plan and monitor skill development during the learning and problem-solving process (Kuhn, 2000; Mathabathe & Potgieter, 2014; Schraw, Crippen, & Hartley, 2006; Veenman, Kok, & Blöte, 2005). Flavell (1979) was the first to use the term “metacognition” which he described as cognitive monitoring. Metacognition is the knowledge of and ability to understand and to self-monitor the cognitive strategies used while learning (Flavell, 1979; Schraw & Dennison, 1994).

![Figure 1. Model of metacognition as defined by Schraw (Kipnis & Hofstein, 2008)](image_url)
It is described as an internal conversation with one’s self about how one is learning. In general, metacognition consists of two components: knowledge of cognition and regulation of cognition (Brown, 1987; Georghiades, 2004).

Knowledge of cognition (metacognitive knowledge) has three components: (1) declarative, which is knowledge about one’s personal learning characteristics; (2) procedural, which is knowledge about how to perform a task; and (3) conditional knowledge, which is knowledge about when and why to select strategies to perform a task (Schraw & Moshman, 1995). An example of a task that requires metacognitive knowledge is students working to solve an open-ended lab problem. In a laboratory course, students are provided with a scenario in which they are asked to determine the concentration of a metal ion in rainwater. First, they might recognize what knowledge they have or lack to solve the problem. The students may have had previous experience or knowledge in metal ion analysis and know to use a calibration curve of known concentrations of the metal ion with spectrophotometry to plan their procedure. During the data analysis, they might recognize that in order to properly analyze data, they should graph the relationship between the absorbance and concentration of the metal ion to determine a linear equation to calculate the unknown ion concentration.

The second branch of metacognitive thought and the focus of this research is regulation of cognition (metacognitive skillfulness). It is a skill set including the planning, monitoring and evaluating of cognitive strategies that allows students to control and monitor their learning to complete a task. Planning is the step in which students think about how they will accomplish a task, what relevant previous knowledge they may have,
and what gaps in their knowledge may exist (Brown, 1987). Students’ abilities to plan may affect their performance on a task (Pintrich & De Groot, 1990). For example, when preparing to run an experiment to determine the freezing point depression of a solution, a student who does not consider the goals of the experiment and techniques used in lab may not acquire enough temperature data to adequately calculate the freezing point depression. When monitoring, students self-assess their knowledge about the task as well as the strategies they use to perform the task. In running a lab, for example, students may ask themselves questions and compare results with a partner to ensure that their data make sense. Finally, evaluating is the step in which students reflect on the strategies and goals they used to complete the task and whether they could have used different strategies to better complete the task. During this step, for example, students may reflect on their procedure for completing an experiment. They might think about what they learned and how they could improve their procedure to take more accurate and precise data if they performed the lab again.

Metacognitive knowledge and regulation strategies are innate in humans and essentially intertwined. Metacognitive knowledge is late developing in humans, but once it is developed, it is stable and people are able to reflect on and discuss this knowledge (Brown, 1987). Regulation strategies, unlike metacognitive knowledge, develop earlier, but may not be stable nor may not be recognized by the people using the strategies (Brown, 1987). As children, metacognitive processes increase naturally to a degree, such as knowledge about one’s memory abilities (Garner & Alexander, 1989). However, when moving into adolescence, people will develop further metacognition only if it becomes
necessary to do so. For example, adolescent students may be able use certain cognitive strategies such as asking questions about a problem in order to learn more information while still lacking the ability to monitor that they are actually learning the new information unless the learning environment requires in some way that they do so. Research supports that as students enter adolescence they need to be taught how to recognize their memory capabilities and how to monitor their learning to become better problem solvers (Veenman, Elshout, & Meijer, 1997). Often students are not explicitly taught these strategies, which means that they may not recognize these processes even exist. Teaching students that they can know about and regulate their learning by providing an environment that encourages them to do so can be helpful in moving students from thinking like novices to thinking more like experts (Sternberg, 1998).

Experts exhibit high levels of metacognitive knowledge and skill use when compared to novices because they have well-organized mental frameworks that recognize when their current level of understanding is insufficient and what they need to do to close that gap in understanding (Chi, Feltovich, & Glaser, 1981; Sternberg, 1998). Through metacognition, experts are also better able to transfer strategies and knowledge to new learning situations (Palinscar & Brown, 1984; Scardamalia & Bereiter, 1994). The development of metacognitive strategies by learners is essential to their learning because it leads to greater independence and self-regulation of learning, which in turn builds a foundation for efficient and lifelong learning (Veenman et al., 2005). Metacognitive strategies are considered by some to be mostly domain (topic) specific (White & Frederiksen, 1998) although others contend that they are general strategies that can be
used across many domains (Schraw, 1998). Although one may be knowledgeable of one’s metacognitive knowledge and skillfulness and can apply these skills in various domains, it is likely that different forms of metacognitive strategies are necessary when solving a chemistry problem or reviewing a book for a literature class. The type of context or environment, such as learning in an online environment, a lecture or a research-based lab may also affect the types of metacognitive strategies students use. Continued research on how metacognitive strategies are learned and used in specific learning environments is necessary.

**Supporting Metacognitive Strategy Use in the General Chemistry Laboratory**

Several studies have identified that chemistry students lack metacognitive use and awareness in laboratory classrooms (Georghiades, 2004; Haidar & Al Naqabi, 2008; Rickey & Stacy, 2000). However, other studies show that providing an environment in which metacognitive strategy use is supported improves metacognitive strategy use and awareness in chemistry students (Case, Gunstone, & Lewis, 2001; Kipnis & Hofstein, 2008; Sandi-Urena S., Cooper M.M., & Stevens R.H., 2011; Sandi-Urena, Cooper, & Stevens, 2012; White & Frederiksen, 1998). These studies have identified characteristics of instruction that support metacognitive strategy use. They include: (1) reflective prompting, (2) a supportive social environment and (3) inquiry-based approaches to instruction.

**Reflective Prompting**

Lin (2001) provides two frameworks for promoting metacognitive strategy use in students: strategy training and social environment. Reflective prompting, a type of
strategy training, by the teacher or the structuring of lab activity is a common way to encourage students to self-assess their knowledge and learning. One research study assessed students’ learning through activity and self-monitoring prompts. Secondary school students performed week-long science projects where they collected data from the internet in an online environment called the Knowledge Integration Environment. The environment was designed to encourage deep understanding of concepts as opposed to compiling scientific facts. The study found that using self-monitoring reflective prompts (“To do a good job critiquing, we need to…?”) promoted students’ integration of knowledge into science projects more than activity prompts (“Claim 1 should say…”). When explaining a concept, the students who received reflective prompts compared to activity prompts made more connections to outside knowledge in addition to general science principles learned in class (Davis & Linn, 2000). Davis (2003) also found that students understood such concepts as thermal equilibrium significantly more if they were provided with generic reflective prompts (“What are you thinking right now?”) compared to specific reflective prompts (“To do a good job critiquing, we need to…?”) before and after the activity they performed. Students can monitor and integrate knowledge more effectively with properly timed and directed reflective prompts (Bielaczyc, Pirolli, & Brown, 1995; Davis, 2003; Ge & Land, 2003; Lin & Lehman, 1999).

Another form of strategy training is informed training where students learn to use metacognitive strategies. Students are not only provided with strategies, but they also learn how these strategies might be useful to their learning. When students were provided with metacognitive prompts and discussed the relationship between the metacognitive
prompts and their learning, it helped them better understand why they were using the strategies (Bielaczyce et al., 1995). One study in math instruction found that students who were provided explicit self-addressed prompts in all problem-solving activities in an a program called IMPROVE not only outperformed their fellow students who did not receive these self-addressed prompts, but also reported using more metacognitive regulation strategies (Mevarech 2008). Providing students with instances to reflect and understand the benefits of metacognitive strategies may assist them in learning to ask themselves questions to monitor and evaluate their learning.

**Collaborative Learning**

In the second framework, a social environment where students feel that they can acknowledge what they don’t know, play a useful role for their peers, and personally reflect on their work supports metacognitive strategy use (Lin, 2001). In a learning environment, this can be described as cooperative learning in which students actively participate in the learning process through peer interactions. There are five essential characteristics of cooperative learning that promote learning: (1) common goal among students within the group, (2) accountability of each individual to the group, (3) support system to build social skills, (4) communication-skill learning for all group members, and (5) evaluation of the group’s learning and processes (Johnson & Johnson, 1999).

Research supports that students in these types of environments demonstrate stronger content knowledge (Hein, 2012) and better problem-solving skills (Ge & Land, 2003; Sandi-Urena et al., 2012) than students in passive learning environments. Sandi-Urena et al., (2011) found that the instructional interventions that included peer interactions and
reflective prompting improved metacognitive awareness and, in turn, improved problem-solving ability and conceptual understanding. In labs that were instructed using the Science Writing Heuristic (SWH) (Rudd, Greenbowe, Hand, & Legg, 2001), an inquiry-based instructional approach, students had the opportunity to collaborate to share knowledge and to use skills to better understand the content. Proper implementation of SWH labs has shown significant improvement in students’ chemical knowledge understanding (Akkus, Gunel, & Hand, 2007; Greenbowe, Rudd, & Hand, 2007; Kingir, Geban, & Gunel, 2012) and critical thinking skills (Gupta, Burke, Mehta, & Greenbowe, 2014).

In collaborative learning situations, students use their peers to regulate learning processes and construct knowledge. Collaborative learning is a valuable learning process; however, it is essential that students take an individual active part in their learning (Hodson & Hodson, 1998). Regulation strategies may be part of the social learning processes that students experience while collaborating. While developing individually as chemistry learners, students may internalize these strategies they used with their peers to strengthen their own learning processes (Kuhn, 2000).

**Inquiry-Based Pedagogy**

Inquiry labs engage students more than “verification” style labs, and students demonstrate greater conceptual understanding of subject matter and improved scientific reasoning abilities in inquiry-based learning environments (Cacciatore & Sevian, 2006; Hofstein & Lunetta, 2004; Rudd et al., 2001; Russell & Weaver, 2011; Sampson, Grooms, & Walker, 2011). Inquiry is an effective approach to learning because it is a
process where active learning by the students is emphasized to achieve content understanding through scientific skills and critical thinking (National Research Council (U.S.), 2000). An inquiry environment is student-centered, which means that a classroom or laboratory is more discussion or activity-oriented rather than centered upon lecture or the sort of laboratory class where students follow predetermined, step-by-step instructions to verify concepts presented in lecture. Knowledge creation and concept learning is not likely to occur in these passive learning settings because students are not actively engaged in learning (Hofstein & Lunetta, 2004; Rickey & Stacy, 2000).

An approach called the learning cycle demonstrates the active learning process where students construct new knowledge based on previous knowledge and experience (Lawson & National Association for Research in Science Teaching, 1989). First, students explore a concept by assimilating the information from the environment around them. This is followed by concept invention where students make sense of the concept based on what they already know. Finally in concept application, students integrate and organize the new concept into their pre-existing knowledge and mental structure (Abraham, 2011). Iterations of this learning cycle provide students with multiple opportunities to deepen their understanding.

An example of the learning cycle in the laboratory might be when students are asked to confirm that identify of their product as carbon dioxide from the reaction of calcium hydroxide and hydrochloric acid. Students may use the information from the prompt as well as equipment in the lab and information they find in their textbook, internet resources and their peers to explore the concept. Then students may move onto
making sense of the concept of confirming a product through previous knowledge about
the properties of carbon dioxide or experience with similar experiments to come up with
confirmation tests. Once students have acquired results that confirm or disconfirm their
product of carbon dioxide, they will likely integrate the knowledge they gained from
experiment into their mental framework. The new information gained might be organized
into different parts of their frameworks about identifying gases, or confirmation testing.
Through more trials of the experiment, peer discussion, or writing a report, students have
the opportunity to go through more iterations of the learning cycle to integrate the
information about confirming the identity of a reaction product.

Inquiry-based practices provide opportunities for students to build knowledge by
asking questions and determining what data needs to be collected as well as by learning
how it is collected rather than being told the information and confirming a stated idea.
Students might use skills such as observing, predicting, collecting and analyzing data,
and formulating conclusions to learn a new concept (Leonard & Penick, 2009). By
practicing such skills, students are likely to use metacognitive processes because they
have to plan, monitor, and evaluate their skills to make sure that they draw sound
conclusions from the data.

Several chemistry education studies support the idea that inquiry-based
instruction improves students’ use of metacognitive strategies (Haidar & Al Naqabi,
2008; Kipnis & Hofstein, 2008; Rickey & Stacy, 2000; Sandi-Urena S. et al., 2011;
Sandi-Urena et al., 2012). Kipnis and Hofstein (2008) investigated metacognitive use and
awareness in the laboratory while conducting open inquiry-based experiments. Their
results supported that inquiry experiments provided opportunities for students to use their metacognitive strategies and knowledge. Inquiry labs provide a strong base for supporting metacognitive strategies because of their structure and the guidance by the instructor, who assists students in using metacognitive strategies by asking questions as well as by guiding and promoting peer interactions.

**Instructional Strategies for Metacognitive Strategy Practice**

These results emphasize the importance not only of metacognition for learning chemistry concepts but also of providing an environment in which students can practice metacognitive regulation strategies. It is proposed that if teachers want their students to better understand chemistry, it is necessary to teach them metacognitive strategies (Rickey & Stacy, 2000). In recent years, research has provided several instructional strategies for laboratory environments that are inquiry-based, collaborative and reflective experiences for students. Several of these instructional strategies --, the MORE Thinking Frame, Argument Driven Inquiry (ADI), and Science Writing Heuristic (SWH) -- claim to promote metacognitive strategy use (Hand, Hohenshell, & Prain, 2004; Tien, Teichert, & Rickey, 2007; Walker, Sampson, & Zimmerman, 2011). The MORE Thinking Frame prompts students to think more like scientists in the laboratory through a process of reflection on their own ideas in order to understand experimental evidence. Students in this environment (1) Model (2) Observe (3) Reflect and (4) Explain during their laboratory experiments. This framework likely affords them opportunities to practice some metacognitive strategies. Argument Driven Inquiry sets up students to determine their own procedure, gather their own data and form an argument. The essential pieces to
ADI are the stage in which students establish an argument to support their claim with their experimental evidence and the stage in which they have the chance to peer review one another’s work. Therefore, ADI also affords students opportunities to practice metacognitive strategies. The SWH is described in detail below because it was the instructional strategy examined in this study with respect to students’ use of metacognitive strategies during their laboratory experience.

The Science Writing Heuristic

The SWH is an inquiry-based instructional strategy for the learning laboratory that is based on a student-centered and knowledge-centered learning environment. The students begin the lab by asking a question that will help them to focus on a goal for the lab. This is followed by a collaborative effort to plan the procedure. Students then take data and observations while running the experiment. The experimental section is followed by a whole-class discussion in which students examine the data, its trends, patterns and errors.

Table 1. Comparison of traditional report format to SWH template (Burke, Greenbowe, & Hand, 2006)

<table>
<thead>
<tr>
<th>Traditional Report Format</th>
<th>SWH Report and Lab Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title, Purpose</td>
<td>Beginning Questions: What are my questions?</td>
</tr>
<tr>
<td>Outline of Procedure</td>
<td>Tests - What do I do?</td>
</tr>
<tr>
<td>Data and Observations</td>
<td>Observations – What can I see?</td>
</tr>
<tr>
<td>Discussion</td>
<td>Claims - What can I claim?</td>
</tr>
<tr>
<td></td>
<td>Evidence – How do I know?</td>
</tr>
<tr>
<td>Balanced equations,</td>
<td>Reflections – How do my ideas compare with other ideas? How have my ideas changed?</td>
</tr>
<tr>
<td>calculations, and graphs</td>
<td></td>
</tr>
</tbody>
</table>
All students write lab reports that are structured as in Table 1 where they answer their beginning question with a claim that is supported by evidence from the data they collected. They write a reflection about how the conclusions they draw from their data agrees with their previous knowledge and connect to other scientific knowledge (Keys, Hand, Prain, & Collins, 1999).

Several guidelines are essential for proper implementation. It is important that students work through determining questions, tests, and data analysis in a collaborative manner with their peers. When reporting, students need to make connections between the beginning questions, the procedure performed and the claims and evidence section. The instructor serves as a facilitator to the students during the experiment to guide students to ask productive questions, model thought processes, and encourage peer interaction (Burke et al., 2006). A facilitator provides enough guidance to keep students on the right track and to ensure that they make progress in conceptual understanding, but not so much guidance that they tell the students exactly what to do.

The SWH provides a supportive environment for promoting metacognitive strategy use because the inquiry-based approach is constructed on a set of questions that prompt thinking in a collaborative, student-centered environment (Akkus et al., 2007). The structure prompts students at each step with questions about what knowledge is necessary to perform the lab, how they will perform the lab, and what they learned from the lab (Akkus et al., 2007; Keys et al., 1999; Poock et al., 2007). It requires a level of problem-solving surpassing the simple verification of concepts by asking students to
work together to determine the best path to get good data and by requiring them to argue how the data collected in lab support the claim(s) they make (Burke et al., 2006).

Through the steps of the SWH, students are encouraged to use metacognitive strategies. The students begin the lab by asking a question that will help them to focus on a goal for the lab. In order to prepare a beginning question and procedure for the lab, they must plan and consider the goals of the lab, as well as the time and resources necessary to complete the experiment. If students do not prepare well before lab, there is an opportunity during the pre-lab class discussion for students to consider strategies and goals and to plan parts of the procedure with their peers before the experiment begins. The SWH structure also supports students’ monitoring of strategies during data collection and observation because of the consistent questions asked throughout the lab procedure in order to encourage students to review and compare their data with others as the experiment is performed. This step is followed by a post-lab discussion of the data, its trends, patterns and errors. In the post-lab discussion, there is a further opportunity for students to monitor their comprehension of the data and to evaluate how well they performed the lab.

Through writing a lab report structured on the SWH prompts, students are again encouraged to assess their knowledge as they are prompted to write claims that can be supported by their collected data (Keys et al., 1999). The final section of the report prompts students to reflect on and evaluate how they did the experiment. In addition, they are asked to identify what they learned during the experiment that relates to other science content outside the laboratory. Akkus et al., (2007) describe the SWH as a
“metacognitive support” that “prompts student reasoning about data.” The SWH affords students opportunities to use metacognitive strategies through the inquiry nature of lab, the supportive social environment and consistent reflective prompts during lab and report writing. The SWH provides metacognitive strategy practice for students in each experiment which supports the repetition necessary for students to build heuristics into their learning processes as experts do (National Research Council (U.S.), 2000).

**A Need for Metacognitive Strategy Practice to Solve Open-Ended Problems**

Guided instructional labs may allow transfer of skills to more unstructured situations. Kapa (2007) found that students who were provided metacognitive support mechanisms (MSM) were better able to transfer ability from structured problems to open-ended problems in a computerized math environment. Open-ended problems are ill-structured problems in that they may have a vague goal and multiple solutions or paths to a solution (Jonassen, 1997). In comparison to traditional lab procedures and guided-inquiry labs, open-ended problems are almost completely student-centered. Open-ended problems provide an initial prompt, but students are not only required to come up with their own procedure, but they must also form their own argument from their data and use outside resources to support their argument. The results are left open to the students but are known to the instructor (Domin, 1999). A guided-inquiry lab such as the SWH provides some or all of the procedure, and students are guided how to formulate claims and evidence in order to set up an arguments. Open-ended problems can provide students with opportunities not often found in traditional lab instruction for creativity, data ownership, and problem-solving (Domin, 1999).
Metacognitive regulation strategies help a student to solve open-ended problems, and research shows that one’s ability to solve this type of ill-structured problem is affected by one’s metacognitive processes (Ge & Land, 2003). Students require greater use of metacognitive strategies to solve open-ended problems as compared to completion of a structured problem (or traditional experiment) (Jonassen, 1997; Shin, Jonassen, & McGee, 2003). In astronomy, Shin et al., (2003) found that students’ ill-structured problem-solving skills could be predicted by domain knowledge, justification skills, science attitudes and planning and monitoring metacognitive regulation strategies. Students who were provided with reflective prompts to justify their reasoning were better able to solve ill-structured problems in computer simulated biology experiments than students who received prompts to justify based on rules, emotions or no prompt (Lin & Lehman, 1999). Because many students lack sufficient metacognitive regulation strategies to solve these types of problems, integrating practice of metacognitive strategies into the laboratory environment may be beneficial to students when solving unstructured problems.

Writing and Metacognitive Strategy Use

A critical component to most instructional environments that is likely to support metacognitive strategy practice is writing. In the SWH, it is not only the laboratory experience that provides students a learning experience, but also the time they spend writing the report in which they form an argument that supports their experimental data. The SWH is founded on the theory of writing-to-learn where students learn through the discussion with peers about the scientific language they will use to write (Hohenshell &
Hand, 2006). Writing affords students opportunities for deeper thinking, making connections between their previous knowledge and the new knowledge they are learning, and seeing the possible gaps in their current knowledge (Emig, 1977; Wallace, Hand, & Prain, 2004). The SWH report was built on the model of writing called the knowledge-transforming framework. This model views writing as problem solving. Students learn to connect the data from the experiment as evidence to the claims they make through writing. By writing, students reflect on the meaning of the data and they communicate that meaning to an audience through rhetoric (Bereiter & Scardamalia, 1987). In this way, writing can promote conceptual understanding (Wallace et al., 2004). A meta-analysis of six studies that each looked at the relationship of the use of SWH as a learning tool to gains in conceptual understanding showed significant gains in conceptual understanding for students who wrote reports using the SWH as compared to students who used traditional writing strategies including writing chapter summaries or traditional lab reports (table 1) or answering post-lab questions (Gunel, Hand, & Prain, 2007).

Writing can also promote metacognitive thought. Metacognitive thought is not only an end goal, but it is also part of the writing process. For example, setting goals before and during writing assisted students’ construction of scientific explanations (Klein, 2004). Wallace, Hand and Prain (2004) suggest that students must learn metacognitive strategies to get the most out of writing. Although, writing can initiate metacognitive thought, it does not always do so. One research study found that students who were asked to describe their process of writing short essays in a biology class used very few metacognitive strategies while writing. It was clear that just writing does not mean
students use metacognitive strategies or have awareness. This suggests that we may need to be explicit in teaching students about their metacognitive capabilities (Armstrong, Wallace, & Chang, 2008). McCrindle and Christensen (1995) asked some students to write journal entries to reflect on their process of writing and what they learned. They found that students who reflected using the journals while writing scientific reports used more metacognitive strategies than students who wrote a traditional report, even though both groups, when asked, believed that metacognitive strategies were important to use while writing. Writing as a learning process is linked to metacognitive thought. This relationship can shape students’ laboratory experience and affect what they do because of that experience.

**Summary**

To provide students with an environment that supports their conceptual learning, an understanding of how people best learn must be gained. People who have greater metacognitive skill are better able to know what they don’t know, and manage their learning. Recent guidelines from the Disciplined Based Education Research community describe the importance of metacognitive strategies in science learning and recommend that metacognitive strategies be integrated into the learning laboratory (National Research Council (U.S.), 2012). Research supports that a laboratory environment with properly timed and directed reflective prompts, collaborative learning, and inquiry-based pedagogy is likely to support the practice of metacognitive regulation strategies. The SWH as an instructional strategy and report-writing template is framed by these three
essential components and may afford students the opportunity to practice metacognitive regulation strategies during weekly laboratory instruction.
CHAPTER THREE

EXPERIMENTAL DESIGN

Methods

This research study used a mixed methods experimental design to answer the following research questions:

- How does the process by which students solve and report on open-ended laboratory problems reveal use of metacognitive regulation strategies?
- Does the type of laboratory instructional environment (SWH vs. Traditional) that students regularly experience elicit any differences in students’ use of metacognitive regulation strategies while solving open-ended laboratory problems?

The primary method of data collection was qualitative while quantitative data were collected to triangulate results obtained in the study (Creswell, 2009). Qualitative methods were chosen because the research goals were to understand how and when students used metacognitive strategies in the chemistry laboratory as well to explore any differences between students in a collaborative, reflective and inquiry-based instructional environment (SWH) and a traditional “cookbook” lab environment. To gain insight into this phenomenon, quantitative surveys or exams would not adequately provide students’ descriptions and perceptions of this experience in the lab. The methods are supported by basic qualitative research protocols. Merriam (2009) describes this type of research as
focusing on the meaning, understanding, or process of the studied phenomenon, and how the participants might perceive it.

The following chapter describes the methods for obtaining participants, and the instructional environments. These descriptions are followed by the data collected: surveys, exams, and students’ words and thoughts through interviews. Finally, the data analysis process for the assessments and interview data are fully described.

Participants

Students from a declared chemistry majors course at a private, research university were asked to participate in the semester long study (The Carnegie Foundation for the Advancement of Teaching, 2010). The chemistry majors course was chosen as the site of the study because it limited the type of students to all chemistry and biochemistry majors. The students all took the same lecture course that was aligned with the lab content. Most students also took the same general chemistry course in the previous semester. These boundaries helped to reduce variability in the participant population. It was necessary to reduce variability so that the results on metacognitive strategies could be compared between students. Following IRB protocol, all students who volunteered to participate in the study signed a consent form (see Appendix A). To protect students’ identities, all students’ names were converted to codes and pseudonyms by which they were identified in data results. The population in the course in spring 2012 and 2013 was 114 students. Of the 114 students, a total of 62 students consented to participate. Thirty-five students in the non-SWH group received a traditional laboratory instruction (Spring 2012) and 27 students received laboratory instruction with the SWH (Spring 2013). The majority of the
students (86% of non-SWH group and 92% of SWH group) were first year undergraduate students at the university. In the non-SWH group, 49% of the students were female, and in the SWH group, 48% were female. Participants from both non-SWH and SWH groups took each exam and survey.

**Instructional Environment**

In the spring of 2012, students in the non-SWH group experienced a traditional instructional strategy in their weekly laboratory meetings. Students were provided with a published laboratory manual of experiments (Nelson, Kemp, & Stoltzfus, 2011) that contained step-by-step procedures. They performed experiments with a lab partner. Students individually filled out report sheets in their lab manuals with the data obtained from lab and answered pre- and post-laboratory questions. In this laboratory format, students followed a preset procedure to verify a concept or outcome that they learned in lecture.

In the spring of 2013, students were instructed using the SWH (Burke & Greenbowe, 2012). Each weekly lab session began with a student discussion about testable questions to be investigated during the experiment. The teaching assistants (TAs) guided the discussion with prompts when students were not sure how to proceed with beginning questions or procedures. After reviewing necessary protocols, students worked in groups of three or four and as a whole class to determine how the data would be collected. They ran their experiments while TAs monitored student progress by asking guiding questions when needed and encouraging peer discussion about the data being collected. The lab ended with a student-led discussion to compare data results and discuss
trends or patterns evident in the data. The TAs guided the discussion when it was not productive. Each student wrote a lab report using the SWH format (Chapter 2, Table 1).

The SWH as the instructional environment was used instead of ADI or the MORE thinking framework for several reasons. The SWH is a well-established instructional strategy that has been used for over a decade not only at the undergraduate level, but also in secondary level schools. There is a large volume of literature available that describes effects of the instruction on student’s learning, conceptual understanding and writing abilities. In addition to the research literature available, there are several articles and handouts that explain how to properly implement the instructional strategy. Lab manuals with SWH experiments are also available for use.

The topics for the experiments covered qualitative analysis, acid base reactions, kinetics, solubility, and equilibrium. The experimental procedures were similar and sometimes identical for both groups. Several of the verification lab procedures were re-written by the researcher as SWH experimental procedures. An example of an SWH lab procedure is found in appendix B. Other SWH experiments obtained through Burke and Greenbowe (2012) were matched to topics of the verification lab procedures.

**Inclusion of metacognitive strategies in the SWH environment.** In the SWH students’ lab manual, a two-page explanation of metacognitive strategies (see appendix C), their relationship to the SWH, and how they might be useful while the student was in lab was included. The SWH students received a short presentation at the beginning of the semester on metacognitive strategies and the relationship to the SWH and were reminded half way through the semester about metacognitive strategies. These presentations showed students how metacognitive strategies might be supported by their lab
experience. The sessions were not explicit that students were required to use these strategies, but rather provide students with information about the strategies. Identification and discussion of metacognitive strategies with students might help them better understand why they are using the strategies and how these strategies may benefit their learning (Bielaczyc, Pirolli, & Brown, 1995).

**Open-Ended Laboratory Problems**

Students in both groups performed the same five open-ended laboratory problems called ChemPossible experiments (Table 2) in order to establish events in common between the two weekly instructional environments every few weeks during the semester. The problems were modified from ACS small-scale experiments where students were provided a short prompt for the investigation, as well as a list of suggested materials to be used in the experiment (Silberman, 1994).

<table>
<thead>
<tr>
<th><strong>ChemPossible Experiment</strong></th>
<th><strong>Topic</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inkredible: Identification which two inks come from the same pen</td>
<td>Qualitative Analysis</td>
</tr>
<tr>
<td>The Great White Chemical Way: Identify five unknown household chemicals</td>
<td>Qualitative Analysis</td>
</tr>
<tr>
<td>Phun in pHinding pKa: Determine the pKa of an unknown weak acid</td>
<td>Acid-Base reactions</td>
</tr>
<tr>
<td>The Need for Speed: Determine the best catalyst to decompose hydrogen peroxide</td>
<td>Kinetics</td>
</tr>
<tr>
<td>Drip, Drip, Drip: Determine how physical properties of liquids affect the accuracy of a beral pipet measurements</td>
<td>Qualitative Analysis</td>
</tr>
</tbody>
</table>

Before each open-ended lab problem, an experiment designed to provide students with base knowledge and techniques (SWH or traditional) was conducted. Students
conducted their approved self-designed experiment to solve the lab problem and wrote a lab report. Neither group was provided with explicit instruction about collaboration or how to prepare the procedure. TA’s were available to answer questions and provide guidance while students planned and conducted their experiments. An example is found in appendix D. These open-ended problems provided an opportunity for students to plan, conduct and report on an experiment. The students’ work in completing these problems was the primary source of data collection.

**Data Collection**

Several types of data were collected in order to gain insight into students’ perceptions of their awareness and use of metacognitive regulation. Self-report surveys and interviews provided information on students’ perceptions of their use of metacognition. Actual metacognition cannot be measured with tests because it is an internal process. However, perceptions of metacognition can be inferred through self-report of activities that reflect one’s awareness and use of metacognitive strategies. (Meijer, Veenman, & van Hout-Wolters, 2006).

**Assessments**

Three surveys were administered to the participating students before the study. A demographic survey was used to obtain students’ gender, year in school, and chemistry classes taken previously. The Group Assessment of Logical Thinking (GALT) (Roadrangka, Yeany, & Padilla, 1983) was given as a pre-assessment to assess logical thinking ability, which correlates with academic achievement in chemistry (Bunce & Hutchinson, 1993). Logical thinking ability was categorized on a scale out of 11 as low (<5), medium (6-8) and high (>9) ability based on previous work (Daubenniere, 2004).
The Metacognitive Activities Inventory (MCAI) was administered as a baseline for self-report of habitual metacognitive strategy use during problem-solving (Cooper & Sandi-Urena, 2009). It was also given as a post-survey at the end of the semester to measure any changes over the semester. The MCAI was designed specifically for use in the chemistry classroom or laboratory for problem-solving. The Confidence in Chemistry (CIC) was given as a pre- and post-survey to measure a student’s confidence in completing the chemistry course (The LEAD Center, 1996). See appendix E for MCAI and CIC. In order to assess students’ content knowledge in general chemistry, standardized exams were given. The ACS First and Second Term General Chemistry Paired Questions exams were chosen based their use in a previous study on the implementation of SWH. ACS content exams were used to determine if SWH had an effect on conceptual understanding in lecture (Greenbowe & Hand, 2005). The first term ACS exam was given at the beginning of the course as a baseline measure to assess students’ first semester chemistry content knowledge (ACS Exams Institute, 2005). This type of exam pairs algorithmic and conceptual questions for each of the topics on the exam (ACS Exams Institute, 2005). To measure content knowledge at the end of the second semester, the similarly structured ACS Second Term General Chemistry Paired Questions Exam was administered (ACS Exams Institute, 2007). The ACS exam scores were converted to normed percentiles (ACS Examinations Institute, 2013) for comparison between the two exams.

**Interviews**

Interviews were chosen as the main source of data because it afforded access to students’ perceptions and descriptions of their metacognitive strategy use. Interviews have been used in previous research to study students’ description of their metacognitive
strategies (McCrindle & Christensen, 1995). Nine students from the non-SWH group and ten students from the SWH group were interviewed twice during each 15-week spring semester by the primary researcher. The students were chosen using a stratified, random sampling based on the GALT scores. At least three students were randomly chosen from each GALT category. Because the GALT generally correlates with academic achievement, this sampling would provide perspectives of participants at all levels of academic achievement. The interview protocol was semi-structured, which provided a pre-determined set of structured and open-ended questions but allowed for opportunities for the interviewer to ask for clarification or further information in follow-up questions (Herrington & Daubenmire, in press). An open-ended question such as “How do you think you are learning in lab?” provided an opportunity for the student to talk about any number of factors that they used to learn in the lab. A structured question such as “How much time do you set aside to prepare for lab?” provided a direct response about the amount of time students spent planning for the lab, but it still allowed students to answer in greater depth about lab preparation if they wanted to.

Interview questions were built around the constructs of metacognitive regulation strategies and learning perceptions. The main goal of the interviews was to have students explain the steps and strategies they used to plan, conduct, and report on their open-ended lab problem. In addition to students’ descriptions of how they solved open-ended lab problems, the protocols allowed students to describe their experience in lab, their perceptions of their learning, and their reflections about their strategies for problem-solving changed throughout the semester. The first interview was four weeks into the semester, after students had performed two weekly labs and two open-ended lab
problems. Students were asked about how they learned in lab as well as the steps and strategies they used to perform the lab problems and write the lab reports. The second interview occurred one to two weeks before the end of the semester. These interviews focused on how students perceived their progress over the semester as well as how conducting the open-ended experiments and their weekly lab experiments helped them learn. Students in both groups were asked the same interview questions, although SWH students were also asked directly about their metacognitive strategies practice at the end of each interview. Interviews ranged from fifteen minutes to one hour and averaged about 30-35 minutes. The interview protocol is found in appendix F.

**Lab Reports**

Students in both groups wrote full-length lab reports for each open-ended laboratory problem. The reports were graded on a rubric that identified students’ proficiency to report on scientific data. The reliable and verified “universal” lab rubric was specifically developed to assess students’ scientific reasoning skills while writing (Timmerman, Strickland, Johnson, & Payne, 2011). The rubric was adjusted slightly to fit the needs of the ChemPossible lab problems; however Timmerman et al, (2011) allowed that sections of the rubric may be dropped, and the rubric is still reliable. The total points possible were 36 points based on the twelve categories of the rubric. Students received a proficiency score as seen in Table 3.

Table 3. Proficiency scale for grading lab problem reports

<table>
<thead>
<tr>
<th>Proficiency Level</th>
<th>Novice</th>
<th>Low Intermediate</th>
<th>Intermediate</th>
<th>High Intermediate</th>
<th>Proficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>0-12</td>
<td>13-18</td>
<td>19-24</td>
<td>25-31</td>
<td>32-36</td>
</tr>
</tbody>
</table>
TAs were trained on the rubric to grade all reports. If there was a discrepancy in the grade, the TAs and professor worked to resolve the discrepancy and reach consensus about the grade. The rubric is found in appendix G. The average of all completed lab reports was used for analysis.

**Qualitative Data Analysis**

**Coding**

The qualitative data was collected and analyzed based on basic qualitative research protocols. Merriam (2009) describes basic qualitative research as a way to examine the meaning, understanding or process of a phenomenon, and how participants perceive that phenomenon. Interviews were used to gain access to students’ perceptions and descriptions of their metacognitive strategy use. The process by which the interviews transcripts were analyzed was systematic, iterative, and, initially inductive (Merriam, 2009). The qualitative analysis program NVivo 9.2 (QSR International Pty Ltd., 2013) was used in all analyses. First, a researcher transcribed all interview audio. Important phrases, themes, and patterns were initially parsed from one or two transcripts and identified as categories through open coding. Categories were determined by the data and the theory that supported the study. The number of categories in the first round of coding was large, about thirty. The constant comparison method was used to ensure that the categories matched the data in all transcripts. As more documents were analyzed, categories were grouped together conceptually. The process of data analysis became deductive as more transcripts were parsed for the already defined categories. If codes did not match data in all documents, then re-coding of previous data occurred. The categories can be named according to participants’ words, the researchers’ thoughts or theory.
Categories in this research were named according to theory and the participants’ words. The coding process was iterative and it required revision and many rounds of coding to ensure that the categories were saturated with all the essential data fitting into a category. At the conclusion of data analysis, there were between four to seven large categories. It was important that these categories were mutually exclusive and no coded phrases fit into more than one category. The categories were also specific to the data coded and were at the same level of abstraction (Merriam, 2009). In this research, codes were initially based on the instances where metacognitive regulation occurred. In a sense, these codes were predetermined and allowed for a more systematic analysis of the data; however it was essential that data were not forced into these codes. If the data did not fit into the predetermined codes, then new codes were formed.

Each transcript went through an iterative consensus coding process and was coded by at least two coders. On the first few transcripts, the researchers coded together to understand the characteristics of the code and agreed on the how to label the data (Creswell, 2009). The two coders coded all data. The agreement between the coders was measured using the Kappa coefficient. Cohen’s Kappa accounts for not only the agreement between the coders, but also the likelihood of agreement occurring by chance between the coders. NVivo suggests that above 0.75 is excellent agreement between coders (Landis & Koch, 1977). The Kappa coefficient calculated by NVivo for coder agreement of all codes in the study was 0.80. In the thirty-eight interviews, coding was performed until no more instances of metacognitive strategies and themes were found in the data. Theses iterations of coding led to saturation of the codes (Creswell, 2009). The constant comparison method was used to ensure that the categories matched the data in
all documents. If codes did not match data in all documents, then re-coding of previous data occurred (Merriam, 2009). For example, if a category was found to contain several types of monitoring, then the codes in that category in all transcripts were re-coded to fit into more specific categories. This ensured not only that the phrases coded in each category were specific, but also that they also helped to answer the research questions. Sometimes, codes were eliminated because they became redundant or did not add new information to answer the research questions.

In order to communicate findings; the data was represented in a way that appropriately answered the research questions. To compare categories of strategy use between groups, the number of students who described using a specific strategy was counted (Miles & Huberman, 1994). For example, under “draws on previous knowledge and experience”, all nine non-SWH students and all ten SWH students described this metacognitive strategy. These values were converted to percentages to compare between the groups because the sample sizes were not equal. Therefore, 100% of the non-SWH and 100% of the SWH students described using this strategy. Individual students may have described this strategy more than once in their interview; however, each student was counted as either using the strategy or not using the strategy. These frequencies allowed for comparison of individual and peer use between the groups. In this way, the interview data was represented through actual quotes from individual students as well as a more general view of the overall frequency of strategy use for all students who were interviewed. This provided a way to compare metacognitive strategy use in the two instructional environments.
Validity and Reliability

To ensure validity and reliability of the data and the analysis, several types of data were measured to show different perspectives about the same phenomenon. It was essential to include as much information from a variety of sources to describe the phenomenon (Creswell, 2009). The data collected, the theory to support the research, and the methods to analyze the data were triangulated in order to ensure internal validity and reliability of the results (Maxwell, 2005). Validity of the data was also accounted through a rich and thick description of the themes and patterns represented in the codes. Actual student quotes in the results section provided this rich description of the phenomenon. Negative cases were noted to ensure results represented the themes appropriately and were not overstated.

Quantitative Data Analysis

Quantitative data were analyzed using SPSS 21.0 (IBM corporation, 2012) and Microsoft Excel. The threshold value for significance was set at 0.05 for all analyses (Howell, 2010). Each survey was tested for reliability for each group (alpha >0.7) (Cronbach, 1951). Effect sizes used in this study were based on Cohen’s definition of effect size (1992) for small (0.1), medium (0.25), large (0.4) (1992) for ANOVA tests, and small (0.2), medium (0.5), large (0.8) for t-tests. Data that were analyzed included: the GALT, CIC, MCAI surveys, ACS exams, and the lab report scores. Scores were converted to percentages for all analyses. Bivariate Pearson correlations were also performed on MCAI, ACS scores and course grades for both groups. Guidelines suggest any correlation between 0 and 0.3 is weak to non-existent in social science. Only
correlations above 0.3 (moderate) were considered for analysis. This provided information about the how the variables were related to each other.

**Power Analysis**

A mixed model repeated measures ANOVA design was to be used to determine if there were statistically significant differences between non-SWH and SWH groups as well as within the groups themselves for MCAI survey and the ACS exams. The assumptions for repeated measures ANOVA of independence, normality, homogeneity of variance, and homogeneity of intercorrelations (sphericity) were met. However, a power analysis using GPower3.1 suggested that the power of the study was too low (< .80) using the mixed model ANOVA test because of the small sample size and effect size (Faul, Erdfelder, Lang, & Buchner, 2007). Power in the statistical sense, predicts the probability that a test will correctly reject the null hypothesis. A lower power value, between 1 and 0, increases the likelihood that a type II error may occur and that the null hypothesis was accepted when it should have been rejected (Cohen, 1992). For example, a test found that the difference between two groups’ exam scores was significant ($p$ is less than 0.05), and the power was 0.4. A power value of 0.4 indicated there was a 40% probability of correctly rejecting the null hypothesis that the two groups’ scores were equal. The probability of a type II error was 60%. If low power is calculated from a statistical test, caution should be taken when interpreting the statistical results.

Independent T-tests and non-parametric tests on assessment data were also eliminated because of small sample size and low power.

In GPower 3.1, an apriori power analysis was run to estimate the sample sizes required for both a repeated measures ANOVA between factors and within factors (see
Appendix H). These are tests for differences between the two groups (between factors) and differences within an individual in a group (within factors) over time, respectively. To estimate sample size for two effect sizes, 0.25 and 0.4 (medium and large), the following parameters were used: power = 0.8, alpha = 0.1, correlation between measures = 0.5, and sphericity = 1. For between factors (between two groups) in repeated measures ANOVA, GPower3.1 calculated the sample sizes for a medium and a large effect size as 76 and 32, respectively. For within factors, the sample sizes were 28 and 12 for medium and large effect sizes, respectively. The study sample size was 62, if the effect sizes were larger than 0.4 (large effect size), then effectively there was enough power to adequately draw statistical conclusions.

For the MCAI scores, neither effect size (within: 0.17 and between: 0.13) was larger than 0.25, thus resulting in a power value below 0.8. For the ACS scores, the between factor (group) effect size of 0.12 was less than 0.25 as well. If any differences were anticipated, the statistical results should be interpreted with caution because of the increased likelihood that the null hypothesis was incorrectly rejected when it should have been accepted. The within factor for the ACS exam scores could be interpreted because the effect size of 0.6 for both groups was greater than 0.4 (large effect size) thus a power value of larger than 0.8. The results of this test could be interpreted with some safety that the likelihood of having a Type II error was less than 20%.

**Equivalency Test**

Information from the power analysis suggested that a different method other than ANOVA or t-tests should be used to compare the SWH and non-SWH groups at the beginning and end of the study. For both ACS and MCAI scores, the null hypotheses
were that the non-SWH and SWH groups’ means for pre-assessment scores were equal and post-assessment scores were equal. The method of two one-sided t-tests was used in place of the repeated measures and independent t-tests because it is an appropriate method to establish equivalence between the groups. The method has been used in the pharmaceutical industry (Schuirmann, 1987), and it is a way in which to cautiously determine equivalence when t-tests are not appropriate. This method has been used several times in education research when conventional t-tests could not provide equivalent measures between two groups (Grove & Bretz, 2007; Lewis & Lewis, 2005a).

Two one-sided t-tests used in conjunction allow an interval \( (\Theta_1, \Theta_2) \) to be set up. Essentially, \( \Theta_1 \) is equal to \(-\Theta_2\). The values for the interval can be calculated in several ways. One way is to multiply the mean score of the control group by 0.2 to determine \( \Theta_1 \), and \( \Theta_2 \) (Schuirmann, 1987). Although a more common way in chemistry education is to calculate the interval with a small effect size (0.2 as described by Cohen, 1992) and the standard deviation of the groups with the following equation (Lewis & Lewis, 2005a):

\[
    d = \frac{\mu_{exp} - \mu_{cont}}{\sigma}
\]

where \( d \) is the effect size, \( \sigma \) is the standard deviation for either group, and \( \mu_{exp} - \mu_{cont} \) is the difference in means. The interval is the value for the difference in means. Outside of the interval \( (\Theta_1, \Theta_2) \) are the two null hypotheses. This first null hypothesis is “the difference in the means \( (\mu_{exp} - \mu_{cont}) \) is less than \( \Theta_1 \)”.

The second null hypothesis is “the difference in the means \( (\mu_{exp} - \mu_{cont}) \) is greater than \( \Theta_2 \)”.

Two groups are equivalent (the alternative hypothesis is accepted) if the difference in means \( (\mu_{exp} - \mu_{cont}) \) falls within the interval \( (\Theta_1, \Theta_2) \).

Both null hypotheses must be rejected before equivalence can be established (Lewis & Lewis, 2005a).
\[ t_1 = \frac{(x_{\text{exp}} - x_{\text{control}}) - \theta_1}{s_p \sqrt{\frac{1}{N_{\text{exp}}} + \frac{1}{N_{\text{control}}}}} \geq t_{1-a(\nu)} \] (2)

\[ t_2 = \frac{(\theta_2 - (x_{\text{exp}} - x_{\text{control}}))}{s_p \sqrt{\frac{1}{N_{\text{exp}}} + \frac{1}{N_{\text{control}}}}} \geq t_{1-a(\nu)} \] (3)

In equations 2 and 3, \( x \) is the group mean, \( N \) is the sample size, and \( s_p \) is the pooled standard deviation of the means.

The interval was determined in this study by the method of multiplying the mean by 0.2 (Schuirmann, 1987). An effect size of 0.2 similar to the Lewis and Lewis study (2005a) was too conservative to calculate the interval because there was a large standard deviation for each of the surveys and exams, and a small sample size. The \( \alpha \)-level used for the analysis was 0.10, which gave a \( t \)-value of 1.29 (Howell, 2010). If the calculated \( t \)-values for both tests are greater than the \( t \)-value for \( \alpha \) (1.29) than the two groups scores are equivalent. Microsoft Excel was used to perform all equivalency tests for GALT, MCAI, and 1\textsuperscript{st} and 2\textsuperscript{nd} term ACS exams.

**Summary**

This chapter described the experimental design that was used to investigate students’ practice of metacognitive regulation strategies through surveys, exams and interviews. Chemistry majors who participated in the study experienced either an SWH or traditional instructional environment. Students took a metacognitive assessment as well as a chemistry content exam to determine their strategy use and content knowledge. The assessments were analyzed for any differences between groups and over the course of the study. These results were built-in to triangulate results from the analysis of in-depth interviews. The interview data was analyzed using a coding scheme that first
identified when students used metacognitive regulation strategies during open-ended problems. Second, the coding scheme identified and helped to describe how students used the strategies and what supported their use of the strategies when solving their open-ended problems. The analysis with the coding scheme also delineated between metacognitive strategy use in the two instructional environments. The experimental design allowed for in-depth qualitative data and quantitative assessment scores to be combined in order to answer the research questions.
CHAPTER FOUR

RESULTS

The following chapter presents and describes the assessment and interview data that was collected and analyzed to answer the two research questions:

- How does the process by which students solve and report on open-ended laboratory problems reveal use of metacognitive regulation strategies?
- Does the type of laboratory instructional environment (SWH vs. Traditional) that students regularly experience elicit any differences in students’ use of metacognitive regulation strategies while solving open-ended laboratory problems?

The assessment data is presented first to provide information about all students who participated in the study. The survey and exam results provide not only a look at their regulation strategy use, but also their content knowledge from ACS exams and their confidence in passing the course. This provides context for the students who were interviewed and whose words richly describe their metacognitive experience in lab. The process by which coding framework was formed is explained. This is followed by the results of students’ interviews and the themes that emerged from the coding.
Assessment Results

Baseline Results

The pre-study assessments, the GALT, MCAI, and 1st term ACS exam were analyzed to determine whether the SWH and non-SWH were equivalent in logical thinking, metacognitive strategies use and content knowledge upon entering the course and study. The assessments were analyzed using two one-sided t-tests (Lewis & Lewis, 2005) to test for equivalency between groups. Results in Table 4 show that students in both SWH and non-SWH groups entering the study were equivalent on scores for the GALT, MCAI and ACS 1st term exam scores.

Table 4. Results of the equivalence tests for the baseline assessments

<table>
<thead>
<tr>
<th>Assessment</th>
<th>X_{\text{non-SWH}}^{(N=35)}</th>
<th>X_{\text{SWH}}^{(N=27)}</th>
<th>Interval</th>
<th>t_{1}^{c}</th>
<th>t_{2}^{c}</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-GALT score</td>
<td>.813</td>
<td>.761</td>
<td>(-.163, .163)</td>
<td>4.34</td>
<td>2.91</td>
<td>Equivalent</td>
</tr>
<tr>
<td>ACS 1st term Exam</td>
<td>.640</td>
<td>.688</td>
<td>(-.128, .128)</td>
<td>2.36</td>
<td>1.43</td>
<td>Equivalent</td>
</tr>
<tr>
<td>Pre-MCAI score</td>
<td>.796</td>
<td>.820</td>
<td>(-.159, .159)</td>
<td>8.99</td>
<td>7.65</td>
<td>Equivalent</td>
</tr>
</tbody>
</table>

Note: N (non-SWH) is 34 for Pre-MCAI score, the critical value for these scores is t_{0.10} = 1.29, mean values are shown as decimals not percentages.

Logical thinking categories. Students fell into three categories: high (> 9, medium (6-8) and low (< 5) based on their score on the GALT. In the non-SWH group, 20% of students were in the low category, 37% in the medium category, and 43% in the high category. In the SWH group, 30% of students were in the low category, 37% in the medium category, and 33% in the high category.
Post-Assessment Results

Confidence in passing the course. The Confidence in Chemistry survey was also given to students at the beginning and end of the semester. The results in table 5 indicated that students’ confidence in their ability to succeed in the chemistry course did not change throughout each respective semester.

Table 5. Average and standard deviation of CIC pre- and post-scores for non-SWH and SWH students

<table>
<thead>
<tr>
<th></th>
<th>Non-SWH</th>
<th></th>
<th>SWH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Pre-CIC mean score</td>
<td>78.9</td>
<td>21.9</td>
<td>87.7</td>
<td>16.3</td>
</tr>
<tr>
<td>Post-CIC mean score</td>
<td>80.1</td>
<td>22.9</td>
<td>84.8</td>
<td>11.1</td>
</tr>
<tr>
<td>Delta</td>
<td>1.2</td>
<td></td>
<td>-2.9</td>
<td></td>
</tr>
</tbody>
</table>

Lab report scores. The lab report scores for both groups are provided in Table 6. The average of both groups was 85%. The number of students who turned in lab reports decreased over the semester because students were required to turn in a minimum of three reports. Thus, several students who received high grades on the first three reports elected not to turn in the later reports.

Table 6. Average and standard deviation of lab report scores for non-SWH and SWH students

<table>
<thead>
<tr>
<th>Lab Report #</th>
<th>Non-SWH</th>
<th></th>
<th>SWH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>M (%)</td>
<td>SD (%)</td>
<td>N</td>
<td>M (%)</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>81.6</td>
<td>9.4</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>86.8</td>
<td>5.5</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>82.6</td>
<td>15.9</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>88.8</td>
<td>6.2</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>85.7</td>
<td>16.5</td>
<td>17</td>
</tr>
</tbody>
</table>

Metacognitive strategy use and content knowledge. Both the ACS and MCAI were given as post-surveys. The MCAI was given to students as a post-survey to measure
whether students had changed in metacognitive strategy use over the semester (course of
the study), and whether there was a difference between instructional environments. The 2nd Term ACS exam was also given to see if a change in content knowledge within
students and between the instructional environments. The 1st term and 2nd term ACS
exams were scaled on a normed percentage that allowed comparison of the two exams
even though the exams covered different content. Post-assessment results in Table 7 from
the two one-sided t-tests also showed that students in both groups were equivalent on
their use of metacognitive strategies and on chemistry content knowledge.

Table 7. Results of the equivalence tests for the post-assessments

<table>
<thead>
<tr>
<th>Assessment</th>
<th>$X_{\text{non-SWH}}$</th>
<th>$X_{\text{SWH}}$</th>
<th>Interval</th>
<th>$t_1$</th>
<th>$t_2$</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS 2nd term Exam</td>
<td>.776</td>
<td>.824</td>
<td>(−.155, .155)</td>
<td>2.48</td>
<td>1.67</td>
<td>Equivalent</td>
</tr>
<tr>
<td>Post-MCAI</td>
<td>.841</td>
<td>.806</td>
<td>(−.168, .168)</td>
<td>6.97</td>
<td>5.48</td>
<td>Equivalent</td>
</tr>
</tbody>
</table>

Note: N (non-SWH) is 33 for Pre-MCAI score, the critical value for these scores is $t_{\alpha 0.10} = 1.29$, mean scores are shown as decimals not percentages.

These results suggested that the instructional environments in the laboratory either did
not affect content knowledge and self-reported metacognitive strategies use or any
differences could not be detected with these assessment instruments. Please see
experimental design chapter for explanation on the decision to use two one-sided t-tests.

The change over the semester for students from pre-to post-assessment is shown
as delta for both MCAI and ACS scores (Tables 8 and 9, respectively). The pre-study
average MCAI score for each group was around 80%. The change from pre-to post-
decreased 1.4% for the SWH group, and it increased in the non-SWH group by 4.3%. If a
difference between pre and post scores existed, it would likely not be significant because
of the small effect size and small sample size indicating low power (less than 0.8). Power
values below 0.8 call for cautious interpretation of the results. Thus the change from pre-to post-MCAI was not tested for significance.

Table 8. Average and standard deviation of MCAI pre- and post-scores for non-SWH and SWH students

<table>
<thead>
<tr>
<th></th>
<th>Non-SWH</th>
<th></th>
<th>SWH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$M$</td>
<td>$SD$</td>
<td>$M$</td>
<td>$SD$</td>
</tr>
<tr>
<td>Pre-MCAI score</td>
<td>79.9</td>
<td>7.5</td>
<td>82.0</td>
<td>7.1</td>
</tr>
<tr>
<td>Post-MCAI score</td>
<td>84.2</td>
<td>9.7</td>
<td>80.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Delta</td>
<td>4.3</td>
<td>-1.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The change over the semester for the ACS exam for both groups is shown in Table 9. Their change from pre to post exam was parallel.

Table 9. Average and standard deviation for ACS 1st and 2nd term general chemistry exams for non-SWH and SWH students

<table>
<thead>
<tr>
<th></th>
<th>Non-SWH</th>
<th></th>
<th>SWH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$M$</td>
<td>$SD$</td>
<td>$M$</td>
<td>$SD$</td>
</tr>
<tr>
<td>1st Term ACS score</td>
<td>64.0</td>
<td>26.3</td>
<td>68.8</td>
<td>17.8</td>
</tr>
<tr>
<td>2nd Term ACS score</td>
<td>77.6</td>
<td>25.8</td>
<td>82.4</td>
<td>19.9</td>
</tr>
<tr>
<td>Delta</td>
<td>13.6</td>
<td></td>
<td>13.6</td>
<td></td>
</tr>
</tbody>
</table>

Because the calculated effect size was above the suggested threshold of 0.4, a large effect size, from the power analysis (see experimental design chapter) this change for both groups can be considered significant. A mixed model ANOVA was run to determine if there was a difference from pre-to post. The main effect for within an individual in either group indicated that there was a significant change in score from pre-to post, Wilk’s $\Lambda=.723$, $F(1,60)=22.98, p=.001$. A post hoc ANOVA showed that both groups significantly increased their ACS score: SWH--Wilk’s $\Lambda=.727$, $F(1,26)=9.77, p=.004$, $d=0.6$ and non-SWH --Wilk’s $\Lambda=.714$, $F(1,34)=13.61, p=.001$, $d=.62$. 
Students were equivalent on MCAI scores. Both of these results
differences Although no differences were detected between SWH and non-SWH
groups on the MCAI and ACS exams, SWH and non-SWH students described very
different metacognitive experiences while solving open-ended problems in the
laboratory. The lack of difference between groups on these variables suggests that
either the assessment instruments used could not detect the behavioral differences
that students actually experienced in the laboratory or the effects of the
instructional environment might be delayed and cannot be detected yet with these
instruments.

**Correlations**

Pearson correlations were run to provide information about the students. All
assumptions for the test were met including that the variables had a linear relationship,
that there were no significant outliers and that data were approximately normally
distributed. A student’s post-MCAI score in Table 10 was correlated with his/her final
grade in class and post-ACS exam score. The SWH group had a higher correlation of
0.492 between the ACS score and the post-MCAI score than 0.393 of the non-SWH
students. Students who performed better in class generally had a higher metacognitive
inventory score. No further analyses on the relationship between the MCAI score and 2\textsuperscript{nd}
Term ACS scores were run.
Table 10. Correlation matrix

<table>
<thead>
<tr>
<th></th>
<th>2nd Term ACS score</th>
<th>Final course grade</th>
<th>post-MCAI score</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-SWH 2nd Term ACS score</td>
<td>Pearson Correlation</td>
<td>1</td>
<td>.902**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.050</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Final course grade</td>
<td>Pearson Correlation</td>
<td>1</td>
<td>.395*</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td>.034</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Post-MCAI</td>
<td>Pearson Correlation</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>SWH 2nd Term ACS score</td>
<td>Pearson Correlation</td>
<td>1</td>
<td>.710**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Final course grade</td>
<td>Pearson Correlation</td>
<td>1</td>
<td>.495**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td>.034</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Post-MCAI</td>
<td>Pearson Correlation</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Interview Results

To address the research questions posed, students in each group were interviewed about their experiences of learning in the laboratory setting. The interviews were not only designed to identify when students used metacognitive strategies while solving open-ended problems, but also how students used these regulation strategies to complete their experiments and how students in the two instructional environments compared in their metacognitive strategy use. First, the coding framework that was created to analyze the
interviews is explained. The coding framework is followed by students’ descriptions of their strategy use and the overarching themes that were identified during coding.

**The Coding Framework**

All interview transcripts were coded using this coding framework. The initial categories were coded as instances in which students described using strategies (Table 11) that were consistent with planning, monitoring and evaluating (Schraw & Moshman, 1995). Previous research used a similar coding process to analyzing children’s metacognitive strategy use while writing in collaborative groups (Larkin, 2009). First a metacognitive phrase was identified:

> And then I had my research I did at home, I told you that I looked up stuff of how does this react with that, and why does it happen, and everything. So I kind of had an idea [about how to run the experiment].

In the first round of coding, this statement was coded as planning. As more transcripts were coded, it became apparent that students described actions consistent with several types of metacognitive strategies in each category of planning, monitoring and evaluation. The phrase was later recoded as “researches information while planning experiment” as a more defined code within planning. Because of these data and following prior studies in this area (Brown, 1987; Meijer, Veenman, & van Hout-Wolters, 2006; Schraw & Dennison, 1994), further codes within each regulation category were identified. Additional examples include “monitors for knowledge to check understanding” and “monitors for execution of procedure” under the general category of monitoring (Table 11). All transcripts including initial transcripts were coded with the detailed categories.
Table 11. Coding scheme for metacognitive regulation strategy use individually and with peers in student interviews

<table>
<thead>
<tr>
<th>Phase in lab problem</th>
<th>Code</th>
<th>General metacognitive strategy code</th>
<th>Operational definition of metacognitive regulation strategies (Schraw &amp; Dennison, 1994)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plan experiment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(before lab)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Draws on previous knowledge and experience</td>
<td>Plan</td>
<td></td>
<td>Allocate resources prior to learning/procedural knowledge</td>
</tr>
<tr>
<td>Researches information while planning experiment</td>
<td>Plan</td>
<td></td>
<td>Allocate resources prior to learning</td>
</tr>
<tr>
<td>Plans Lab Problems procedure</td>
<td>Plan</td>
<td></td>
<td>Mentally prepares for conducting lab procedure, reads, organizes for lab and notebook set up, information management strategies</td>
</tr>
<tr>
<td><strong>Conduct Experiment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(during lab)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monitors for knowledge to check understanding</td>
<td>Monitor</td>
<td></td>
<td>Comprehension monitoring</td>
</tr>
<tr>
<td>Monitors to execute procedure</td>
<td>Monitor</td>
<td></td>
<td>Comprehension monitoring</td>
</tr>
<tr>
<td>Makes error correction during experiment</td>
<td>Monitor</td>
<td></td>
<td>Debugging (error detection) strategies</td>
</tr>
<tr>
<td>Compare data results</td>
<td>Monitor</td>
<td></td>
<td>Comprehension monitoring/debugging strategies</td>
</tr>
<tr>
<td><strong>Data analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(during and after lab)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data analysis - planning</td>
<td>Plan</td>
<td></td>
<td>Consider all options to solve problem, reflects on whether goals were accomplished, reflects on better/easier way to solve problem</td>
</tr>
<tr>
<td>Data analysis - monitoring</td>
<td>Monitor</td>
<td></td>
<td>Through writing, reflects on whether goals were accomplished, reflects on better/easier way to solve problem</td>
</tr>
<tr>
<td>Data analysis - evaluation</td>
<td>Evaluate</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Evaluate experiment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(after lab)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluate strategies used in experiment</td>
<td>Evaluate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluates own thinking process through writing</td>
<td>Evaluate</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Write Report</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(after lab)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outlines before writing report</td>
<td>Plan</td>
<td></td>
<td>Set goals, ask questions about material, organizes material and knowledge in order to write</td>
</tr>
<tr>
<td>Monitors while writing</td>
<td>Monitor</td>
<td></td>
<td>Ask self questions, check my comprehension, review to understand relationships</td>
</tr>
<tr>
<td>Revises/Reviews lab report</td>
<td>Monitor</td>
<td></td>
<td>Translate new information into own words, debugging strategies, ask others for help</td>
</tr>
</tbody>
</table>
The codes were then grouped into five phases that occurred while students solved the open-ended lab experiment: plan the experiment, conduct the experiment, perform the data analysis, write lab report, and evaluate the experiment. The phases helped not only to define the process that students used to solve the open-ended problems but also helped to separate the metacognitive strategies students might be using in the laboratory during the experiment from those used during report writing about the experiment. As coding into the second round continued, it became apparent that there was a difference in how students described their metacognitive strategy use by themselves or with their peers. Students’ use of peer interactions to support their metacognitive strategies use was coded in addition to students’ use of metacognitive strategies individually. The use of peer interactions as support was identified as an overarching theme.

Students’ perception of how the weekly labs (SWH or traditional) scaffolded their solving of the open-ended laboratory problems was another theme that emerged from the coding process. Statements in which students discussed the structure of their weekly lab (SWH or traditional) and related them to solving their open-ended problems were coded as seen in Table 12.

Table 12. Coding scheme for structure of regular instructional lab (SWH or traditional) and relationship to metacognitive strategy use

<table>
<thead>
<tr>
<th>Code</th>
<th>General metacognitive strategy code</th>
<th>Operational definition of metacognitive regulation strategies (Schraw &amp; Dennison, 1994)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makes me ask guiding questions</td>
<td>Plan/ Monitor/ Evaluate Plan/ Monitor/ Evaluate Plan/ Monitor/ Evaluate</td>
<td>Information management strategies</td>
</tr>
<tr>
<td>Structures my thinking process</td>
<td>Plan/ Monitor/ Evaluate Plan/ Monitor/ Evaluate Plan/ Monitor/ Evaluate</td>
<td>Information management strategies</td>
</tr>
<tr>
<td>Provides structure for conducting and writing my lab problem</td>
<td>Plan/ Monitor/ Evaluate Plan/ Monitor/ Evaluate Plan/ Monitor/ Evaluate</td>
<td>Information management strategies</td>
</tr>
</tbody>
</table>
Many of the statements made related to how their metacognitive regulation strategies were supported. For example, SWH students often stated that the SWH labs helped them to ask questions while they performed the open-ended problems. An example statement is: “SWH gets you thinking more about the actual lab, of what happened, so it asks you more engaging questions in the rubric.” These instances were coded as “makes me ask guiding questions.” In order to describe students’ experience in the lab, phrases that compared their current lab experience to previous lab experiences as well as how weekly labs compared to solving open-ended problems were coded. These codes provided a richer description of metacognitive use in the instructional setting.

Students’ Strategy Use Revealed Through Process of Solving Open-Ended Problems

Planning the experiment. When asked what steps and strategies they used to conduct the experiments, students in both groups consistently began that they always did some planning before they performed their lab problem experiment. This planning phase occurred prior to or during the first half hour of the lab period. The amount of time for planning generally depended on whether students felt they had a procedure prepared that would accomplish their goal. When students in both groups were asked, “How much time do you set aside to prepare for lab?” they responded in several ways. The first way was a general way to plan. This was coded as “planning lab problem”.

Josie (non-SWH):

I'll definitely sit down and spend at least fifteen minutes on, okay, what's this lab asking me, like what other additional materials do I think I need, what information do I need, like maybe for my introduction or something like that, and that will help me do the lab.
Thomas (SWH)

For a lab report [compared to an SWH report], it’s a little bit shorter because there is usually a lot less variables, but you have to design the whole experiment yourself. And as long as I have a basic idea of what’s the properties I need to apply in this lab, how they work, then I’m, I try to put something together, but I always leave room open for another step I could have forgotten, or a better method.

A second way in which students planned was through looking up information about how to run the experiment, different ways to complete the procedure, and concepts they might use in the experiment.

Olivia (non-SWH):

If we get a lab problem, I'll sit there, and I'll do research for an hour or two. I'll just go on the internet and see what I can find, like, flip through my book, read those sections, so I have a really thorough understanding of what is even going on. Like, what I'm even looking for before I can even set up an experiment.

Abby (SWH):

And then I had my research I did at home, I told you that I looked up stuff of how does this react with that, and why does it happen, and everything. So I kind of had an idea.

The third mode of planning was to draw on previous knowledge and experience from high school, chemistry knowledge or other outside knowledge.

Charles (non-SWH)

*laugh* Like this sounds really hard, I don't know. How am I gonna know what these things are, but when I think about it I've done similar things in lab before that helped me.

Sophie (SWH)

I knew baking soda and vinegar. They are just like volcanoes and just like when you were little and stuff you react that and you know your teachers would show you that.
Students interviewed described very specifically the steps they took to prepare for the lab. At a minimum, they read the lab problem prompt before arriving in lab. Like the students mentioned above, 90% of the students used planning regulation strategies which included thinking of several ways to solve the problem, choosing the best procedure, and asking themselves questions about the lab before it began based on their previous knowledge and what they researched before lab.

**Conducting the experiment.** When asked how they conducted their experiment, students, unlike in the planning stage, spent little time describing their actual process or steps for completing the lab. No more than half the students talked about monitoring themselves during lab. One non-SWH student and three SWH students described asking themselves questions about whether they understand the concepts in the experiment.

Lydia, from the SWH group, when asked, “How are you learning in lab?” said:

> So even if I get stuck, I know, like, I can look through it in my head, that this is what we did like the rates [of reaction] problems. It [the lab problem] was like the rates lab, which was the same week as the lecture lab. So that helps.

Not only does Lydia stop to check her comprehension, but she also relates it back to previous knowledge in order to find relationships in the data and the lab procedure.

Students spent more time describing whether they completed the experimental procedure correctly. Almost half the students in each group talked about checking their procedure.

Sam (non-SWH)

> I sort of have a hard time just reading the procedure and taking it all in like that. So I sort of make mental steps for myself. I don't really write down the steps and whatnot but I sort of use them.
Thomas (SWH)

So I have to, no matter how many times I read the procedure, I always reread it as I’m doing the lab because it’s so crucial. If you forget one thing, so I constantly keep make sure I’m doing the procedures right. Right technique.

For Sam and Thomas, they both talk about making sure that they had completed each step by re-reading or making mental notes. Some students used checklists as well. Another area where students regulated themselves was through monitoring strategies in which they recognized errors that occurred and corrected them while performing the experiment. This was the most common form of monitoring that students in each group performed. Some students returned to planning if they recognized that they were not performing the experiment well enough to obtain useful data. When asked, “How did you approach this specific lab problem?” Henry and Olivia responded:

Henry (SWH)

I thought I was gonna be using the well plates, but that was a little, a lot, way too small of a thing to actually see anything. And so I went with test tubes and then test tube holders, so I would have, um, half of it in one test tube, half of it in other, and right by each other.

Olivia (non-SWH)

And I, and at first I think it was going to do one gram per one milliliter of the hydrogen peroxide, and it just didn't work. It just didn't work. I think I was, or I did, *sigh*, I think I added it directly to the hydrogen peroxide and [inaudible].

This indicates that Henry and Olivia used a monitoring strategy; one that resulted in a change to their current procedure because they realized that would not produce useful data with it. Monitoring while performing the lab was not as commonly described as
planning procedures were, yet students in both groups provided evidence that they used monitoring strategies while running the experiment by themselves.

**Perform data analysis.** Students generally completed their data analysis for the experiment after lab. Almost every student mentioned performing data analysis, although few students described their whole process of completing this phase of the experiment. Students may not have viewed data analysis as a part of the experimental process, thus not describing it when asked about their steps and strategies. All the students interviewed in SWH and 80% of non-SWH students described their data analysis phase. Within data analysis, fewer than half the students in both groups mentioned using planning and evaluation strategies. Yet, 90% of the SWH students mentioned monitoring themselves during data analysis while 67% of non-SWH students used monitoring strategies. Holly only talked about data analysis when asked how she wrote her lab report. She always did data analysis last because it was hard for her.

Holly (non-SWH)

> Because [data analysis] is the hardest part for me. And I feel if I can get all the other information into my head so it can start meshing, that in the end I'll have been thinking about it subconsciously so that I can bring it

Lydia (SWH) talked about planning her data analysis first when asked about the steps and strategies she used to complete her lab problem,

> I just know that after the class, I spent a lot of time, like, reading about each of the powders, and like how they should react, and like what results should have been expected. And then I went through and I compared it to my data, like, ‘cause like I labeled each one A, B, C, D.
She talks about data analysis again when asked, “What were the steps you took to write up your report?” She explained how she monitored her data analysis as she identified each of the five unknown powders in solving the lab problem:

I’m the type of person that matches things up, like, if they are spread out. So I, like, spread my data tables out… And I read through the book and then, like, I started with baking soda. And then I’ll, like, write down the qualities and the characteristics of baking soda. And then I’ll go through my data and see which ones match up. And then I’ll be, like, okay, so this could be a possible baking soda. And then I’ll do it for each of the Alka Seltzer, chalk, vitamin c…

Lydia used planning and monitoring strategies during data analysis. For example, she used planning when she mentioned that she looked up information in her book to figure out how to analyze the different powders. She used monitoring strategies to match up her experimental data with the information she found in the book.

Only two students in either group discussed evaluating their data analysis. When asked how he approached the lab problem, Magnus, an SWH student, talked of the reactions that he thought might occur, and then the reactions that occurred in the experiment and how they did not match up. He said, “Just like the categories, once I put them in, like they couldn’t all fit in, like the results didn’t happen like I thought they would.” Even though both groups described data analysis, SWH students spent more time in their interviews more clearly describing how they analyzed their data than non-SWH students.

**Evaluate experiment.** Evaluation of strategies was present in at least half of students’ description of problem-solving. Students evaluated their experience in two ways. The most common way was a reflection and evaluation on their experience across the semester. Students were also reflective in the open-ended lab problem process when
they evaluated the strategies they used to complete their experiment for the lab problem.

When asked, “Have your problem-solving strategies changed over the semester?” Liam, from the non-SWH group, shared that:

For the last lab, I walked out of there, and I figured out the fastest way to do it in my head at least. And I was just like, oh, I could have done it like this. And I could have finished the whole entire thing in an hour.

Liam described that he could have run the experiment an entirely different way upon leaving. Magnus, an SWH student, was more specific about strategies he felt he could have changed in evaluating his strategies in lab, and he responded:

I mean I wish I had observed better ‘cause I didn’t really take. Like when labeling each mystery powder, like just saying which one reacts more violently with vinegar, per se. Um. It didn’t really help just knowing small, medium, or large for me.

Reflecting on the strategies they used to complete the experiment suggests that they were aware of how they performed the procedure and what they could have done to improve their experimental procedure. About two-thirds of the students in both groups described evaluating the strategies they used to complete the lab experiment.

Many students also felt that they were able to evaluate their experiment through the process of writing their lab report. When asked about how they thought writing helped them learn, many students explained it as a way for them to see their own thinking process; to know what they do not understand.

Alex (SWH)

It allows you to, it basically allows you to organize your thoughts. And it helps, in the lab report you’re basically explaining the concept, and that’s how you know if you really understand.
Josie (non-SWH)

I think getting it all down, getting all my thoughts down, really, it's making me go through my thought process. It's making me, okay, here is one part, here is another part, and how do all these different parts connect?

These students talked about how writing a lab report makes them understand what happened in the experiment. Writing helped them to know if they really evaluated whether they understood what happened in the experiment.

**Write lab report.** Once the experiment was completed, students wrote a lab report. All students were asked how they wrote their lab report. When students talked about planning, the most common planning strategy was outlining their lab report. Many students said they made outlines or physically wrote out their report before typing up the final copy. Most students did not write the lab report from beginning to end rather they wrote the hardest or easiest part first and progressed in that order. This required the planning stage.

Holly (non-SWH)

I'll open up two word documents and one word document will have like everything the way it is supposed to look like and then I'll have another one where like okay I want to say this somewhere and I want to say this somewhere and oh and then I'll have some idea and I'll like type that out and then I'll be like okay now on the actual paper.

Alex (SWH)

The only difference is, before I do my formal lab reports, I write them down like I'm doing a science writing heuristic, and then I polish it once I'm typing it. Instead of science writing heuristic, it's a little more lenient, so I can literally write in the notebook, cross things out.

Both Holly and Alex used information management strategies in which they organized their reports to ensure they talked about everything. Holly used a strategy likely from
previous experience in writing papers. Alex wrote his reports just like his weekly reports in SWH form. He found support from his regular lab experience.

Monitoring as a metacognitive strategy was the least common described strategy when students talked about their lab reports. Only about 50% of SWH students and fewer than 25% of non-SWH students described monitoring while writing. This is similar to conducting the experiment where students practiced planning and evaluation more often than monitoring. Alex (SWH) said: “When I’m writing I see that something is wrong here and I try to figure out what would be going wrong. Just like teaching yourself almost.” Josie (non-SWH) described monitoring in a similar manner:

And then I kinda try to switch to wording around or try to change whatever they're trying to ask so it can fit my lab report and then I kinda ask myself the same questions.

Both students make it clear that they asked themselves questions while writing to make sure that they were considering all options while writing about the experiment, checking their comprehension and how well they are learning the information.

Students revised and reviewed their lab reports before turning them in. Monitoring and evaluating strategies were both included in students descriptions of their revision process. Essentially all the students in both groups went through this process of revising. The review varied widely from quickly skimming the paper for format and grammar to students who spent time making sure that their overall argument made sense. Emma (SWH) said: “I’ll even check my concepts, too. I’ll make sure that everything that I’ve concluded was actually consistent with my data and everything. Charles (non-SWH) said:

Besides proofreading I make sure that everything makes sense I guess and
that I didn't say anything like that isn't true or I don't know. Using wrong terms and things like that. And making sure that I incorporated everything that I did.

Charles and Emma thoroughly checked their lab reports. Most students did grammar checks and quick skimming. Although most recognized that they should be reviewing their reports more, many cited not having enough time or they felt that the paper was good enough for the grade. Even though these students did not show in-depth use of regulation strategies, by suggesting that they needed to improve showed that they were aware of the monitoring and evaluation strategies while they wrote and reflected.

**Summary.** Students in both groups described using metacognitive regulation strategies by themselves while solving the lab problems. Their descriptions of solving their open-ended problems revealed that all students used metacognition regulation strategies to some degree. Overall, SWH and non-SWH students’ individual use of regulation strategies in all categories was to about the same degree. When frequencies of strategies are compared, students used planning to a greater degree than either monitoring or evaluating during their experiment. These results are presented in Table 13 in the middle column. In addition to using regulation strategies individually, students also used their peers to support their strategy use. The next section describes these results.

**Students Experience Differential Support from Peers**

A theme that emerged and differentiated the SWH from the non-SWH group was the use of peers to support metacognitive strategies while solving open-ended laboratory problems. When students were asked about the steps and strategies they used to perform and to report on lab experiments, students acknowledged that feedback from peers helped them gain more confidence to conduct the experiments. At least two-thirds of students in
both groups identified the usefulness of peers when planning their experiments (Table 13, right column). The SWH group reported using more peer collaboration when monitoring to check understanding of their lab experiment, comparing their data results, and conducting data analysis when compared with the non-SWH group. Only one SWH student described using peers to evaluate the strategies they used to solve their open-ended lab problem.

**Plan experiment.** Table 13 shows that over half of the interviewed students in both groups used peers to plan lab procedures and check how to perform procedures. Sam (non-SWH) and Emma (SWH) used peer collaboration to plan their lab problems. When asked, “What in the lab helps you learn?” they said:

Sam (non-SWH)

Someone to bounce ideas and things off of, we all sort of looked at [the lab problem] and were, like, we don't know what we're going to do and then we talked and came up with some ideas just through talking. I feel like that was probably the most valuable way of [planning].

Emma (SWH)

I was with Diana and Thomas and a couple of other people, and we bounced ideas off each other, and made sure we had an experiment for every [unknown powder] that would identify every substance. I mean, a couple of them got shut down, but *laughing* it’s okay.

Sam and Emma used the planning strategy to think of several ways to solve a problem and chose the best one with their peers. Other students worked with peers to ask questions about the lab before it began.
Table 13. Individual and peer use of metacognitive strategies while solving open-ended lab problems

<table>
<thead>
<tr>
<th>Phase of lab problem</th>
<th>Specific metacognitive regulation strategy</th>
<th>Students reporting use of strategy individually</th>
<th>Students reporting use of strategy with peers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-SWH Group (N=9)</td>
<td>SWH Group (N=10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-SWH Group (N=9)</td>
<td>SWH Group (N=10)</td>
</tr>
<tr>
<td>Plan Experiment</td>
<td>Plans lab procedure</td>
<td>89%</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>Draws on previous knowledge and experience</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Researches information for experiment</td>
<td>67%</td>
<td>50%</td>
</tr>
<tr>
<td>Conduct Experiment</td>
<td>Makes error correction during experiment</td>
<td>56%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>Monitors to perform procedure</td>
<td>44%</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>Monitors to check understanding</td>
<td>11%</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>Compares data results</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22%</td>
<td>70%</td>
</tr>
<tr>
<td>Perform Data Analysis</td>
<td>Plans during data analysis</td>
<td>44%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>Monitors during data analysis</td>
<td>67%</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>Evaluates during data analysis</td>
<td>22%</td>
<td>20%</td>
</tr>
<tr>
<td>Evaluate Experiment</td>
<td>Evaluates strategies</td>
<td>67%</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>Evaluates thought process through writing</td>
<td>56%</td>
<td>70%</td>
</tr>
<tr>
<td>Write Lab Report</td>
<td>Outlines before writing report</td>
<td>78%</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>Monitors while writing</td>
<td>22%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>Revises/Reviews lab report</td>
<td>100%</td>
<td>80%</td>
</tr>
</tbody>
</table>


While students conducted their open-ended experiments, they used monitoring strategies with their peers that included checking their understanding of the procedure and the chemistry content of the lab.

**Conduct experiment.** Similar to regulation strategies used individually, students checked their understanding and execution of procedure through their peers and by making error corrections during lab. Both Josie (non-SWH) and Abby (SWH) used their peers to ensure that they performed their experiment correctly:

**Abby (SWH)**

Well, I feel it helps me a little because I’m obviously talking to another person and then there is, like, they have ideas that I don’t have or I have ideas maybe that they didn’t think of. So we can, like, work things out like that. Also, it helps, maybe by myself, I can’t get something done, so I can ask them, hey, I need your help to do this.

**Josie (non-SWH)**

If all else fails I'll go to someone, you know, try to see if anyone else is getting what they're doing. I was so afraid of people saying, no that's not right, you're going about it all wrong, I was always afraid of that, but now it's just I'd rather hear their opinions and figure out that I'm wrong then do the lab all wrong and have to come back and do it again.

Students in the SWH lab collaborated with their peers to a greater degree (80% compared to 56%) to monitor themselves and to check their understanding of the chemistry concepts while performing the lab. Students in both groups used their peers extensively to check their understanding (more than half), whereas less than a third of students described using the strategy individually. It is clear that students in both groups perceive their peers as useful to their learning. Ellen (SWH) felt that: “The only other resource you have is the other people doing the lab with you, so, you know, just have to figure out together. I think it’s the best
way.” Ellen viewed her peers as her only resources while running the experiment. Holly (non-SWH) used her peers to understand new material and to check to make sure she understood the material.

If people have questions and if I can answer them, then it's like OK, I know this because I explained it to them. But sometimes it's like I'll be listening to people and like shoot, I don't know that either. It's like I'll look down at my answer and I'm like, this doesn't feel right.

They helped her to ask her own questions about what she did not understand. Students also used their peers to make corrections in their experiments. In this monitoring strategy, there is an opposite trend in use of peers. More non-SWH students used peers to make corrections (44%) compared to one SWH student. Ethan (non-SWH), for examples, said: “We had to revise our whole procedure and do it over after the first few trials when we saw that it wasn't working.” Peter (non-SWH), had a similar experience when planning to mix his unknown samples with vinegar:

As we went through, we almost, um, we were initially going to do the 0.5 grams samples like you said, and we changed it to point-one because they were too big for the reaction well. If we would have put the vinegar in one of the ones that bubbled up it would have spilled over into the other containers and contaminated the rest of those

These students used debugging strategies in which they monitored for errors and sought to correct these errors while still performing the lab. Some debugging strategies include asking others for help, re-evaluating their assumptions about the experiment, and changing strategies when they did not understand the experiment or the strategy did not work.

Throughout the interviews, when describing their experiments, many students used the plural “we” instead of the singular “I” even though students ran the experiments
individually. It was common for students to plan and run the same experiment as many of their peers. Students interviewed described that it afforded them the chance to discuss problems or compare data with their peers more easily.

Each of the monitoring strategies above was prevalent at both the individual level and the group level. Comparing data results was the only monitoring strategy that came strictly from peer interactions. Only 22% of non-SWH students described any sort of peer comparison of data results during lab whereas at least 70% of SWH students described comparing data results with peers.

Ethan (non-SWH)

I mean for collaboration effort also, there's someone to compare off of, so especially when you're unsure about something you could say: "oh all nine people around me are getting the similar results", or I'm getting something totally different, maybe I messed up somewhere.

Alex (SWH)

And we repeated some of them just to make sure that they were similar results so there was not a one-time error or anything like that. So we made sure we had the same results and … we pieced together what our unknowns were. The vitamin C was a dead give-away, that was a little bit orange. So it is okay. That’s your unknown B, this one’s my unknown D. Okay. And then we just matched them up like that, which ones reacted with water, and then we said, okay, what do you think they are, what I think they are, and we compared the results, and said, okay, we all had the same thing. And many other people had the same exact answers as well.

SWH students continued to use peers to support metacognitive strategies by reviewing with their peers whether or not their data made sense. The data comparison not only helped students check their understanding of the data, but it also helped them check for any errors in their procedure that may have affected their data. Data comparison
provided students with opportunities to re-run their experiment if their data did not match their peers’ data.

**Perform data analysis.** As the students moved into the data analysis stage, the frequency of peer use decreased for both groups, but 30% of SWH students still reported using peers during data analysis whereas non-SWH students did not report any use of peers. For example, Henry from the SWH group, when asked to describe the steps or strategies he took to complete his lab report, responded with: “Then I’ll go through my procedure, write that out, and then analyze the data with people. Just to get that extra, like, point of view and seeing how their [data] is affected.” Lydia from the SWH group, made it clear that talking about data was common: “Oh, usually, then a bunch of us just get together and we talk about it after lab.” Most students used peers as a way to check that they had performed the data analysis correctly and had similar results. It was not apparent whether students used peers to evaluate their procedures and strategies used to perform the lab experiment, such as asking questions about whether they had considered all the ways to solve the lab problem. Regulation strategy use was less common with peers than individually for most students.

**Evaluate experiment.** Only one student described that she used peers to reflect on the strategies she used in lab.

**Abby (SWH)**

Some people in our chemistry lab, they always do things differently. And talking to them gives you more ideas about how you could have done the lab or what, if you were to repeat it, what could you do to make it better?

She found her peers useful after her experience in lab. They helped her to see the different ways she could have performed the lab to take better data. The degree of peer
support decreased through the phases of the lab problem. More students used peers for planning and some monitoring, whereas few students described data analysis or evaluation with their peers.

**Write lab report.** Few students described writing their lab reports together, and only two or three students from each group described having peers review their paper before submission.

Jane (non-SWH)

I'll have someone read it again just, I remember I was at the IC last time and I just met my friend and I was like ‘here is my laptop, just read it, ‘am I missing anything? If you have to put any notes just do it.’ I'll do it [peer review], get that out of the way really quick.

Henry (SWH)

And I was collaborating with another student on my floor, and so we helped each other out on spots we needed helping. We read it back to them, and [ask] does this make sense?

Jane used her peer to do a quick check on organization, formatting and “does it sound okay?” It does not appear that she did much monitoring or evaluating or that she got much out of this process. Henry on the other hand, used his peer to help with his understanding of the experiment. They were in a conversation that prompted each to ask questions and evaluate whether they completed the experiment and report successfully.

**Students’ perception of their peers.** In both groups, students’ overall perception of their peers was positive. Peers provided support for new ideas, knowledge, and confidence to complete the experiment. Liam, a non-SWH student, felt that “in the lab situation, I like it because there's a group there if you need them.” Henry, an SWH student, said:
Working in a team really helps me out because, like, I can only think of so many things and somebody may think of completely different things I looked over. The most learning comes before the lab, when we are in a group and talking, and bouncing back ideas. Just helps give everybody and me especially, a new perspective because going into it, you only see it one way, like, oh, that’s the way I would do it, but then, oh, there might be a different way that’s better. So. I think that’s where most of my learning comes from, for me at least.

Even though peers generally were helpful, sometimes they became a hindrance especially if groups of students were on the wrong track or did not understand concepts behind the experiment. Peter, a non-SWH student struggled with this in several labs:

The other kids around you are barriers, although they help you because; well when they, if they get something wrong and they try helping you get stuff, [do something] to something, and then you get it wrong. That happened with our last lab.

**Summary.** Students in both groups used peers while performing their open-ended problems even though the instructors did not instruct students to work together during those sessions. This theme not only helped to describe when student used metacognitive strategies but also how they used these strategies through their peers. Finally, it portrays a difference between the SWH and non-SWH instructional environments. Students in the SWH group used peers in more phases of the experiment, specifically checking understanding, comparing data results, performing data analysis and evaluating strategies than the non-SWH students.

**Comparison of Individual and Peer Use of Metacognitive Strategies**

In general, students used metacognitive strategies to about the same degree. However, there are a few metacognitive strategies that are prominently described in both SWH students and non-SWH students. Table 14 describes five metacognitive strategies that were coded for both individual use and peer use. It shows the number of students
who only used the strategy individually, who only used the strategy with peers, those who used the strategy with both, and then the number of students who mentioned neither peer nor individual use of the strategy.

Under planning, it was interesting that most students in both groups planned individually and with peers. However, SWH students did this more often. Another strategy that signified a difference between the groups was checking their understanding. All SWH students used this strategy, and they almost exclusively used peers in this phase. Almost half of non-SWH students did not mention checking their understanding at all. This may be attributed to the focus in the lab instruction. SWH focused heavily on understanding the data collected whereas the traditional format was focused more on data collection and calculation practice.

Table 14. Comparison for non-SWH and SWH students’ individual and peer use within five metacognitive strategies

<table>
<thead>
<tr>
<th>Regulation strategy</th>
<th>Group</th>
<th>Type of strategy use</th>
<th>Individually</th>
<th>With Peers</th>
<th>Both</th>
<th>Neither</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plan lab problem</td>
<td>non-SWH</td>
<td>33%</td>
<td>11%</td>
<td>56%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWH</td>
<td>10%</td>
<td>0%</td>
<td>80%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Makes error correction</td>
<td>non-SWH</td>
<td>22%</td>
<td>11%</td>
<td>33%</td>
<td>33%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWH</td>
<td>40%</td>
<td>0%</td>
<td>10%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Monitors to execute procedure</td>
<td>non-SWH</td>
<td>33%</td>
<td>33%</td>
<td>11%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWH</td>
<td>30%</td>
<td>20%</td>
<td>10%</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>Monitors to check understanding</td>
<td>non-SWH</td>
<td>0%</td>
<td>44%</td>
<td>11%</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWH</td>
<td>20%</td>
<td>70%</td>
<td>10%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Evaluates strategies</td>
<td>non-SWH</td>
<td>67%</td>
<td>0%</td>
<td>0%</td>
<td>33%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWH</td>
<td>50%</td>
<td>10%</td>
<td>0%</td>
<td>40%</td>
<td></td>
</tr>
</tbody>
</table>

Note: percentages are based on actual number of students who reported this strategy. Non-SWH N=9, SWH N=10

When comparing individual and peer use, there is an opposite trend in the codes “makes error correction” and “monitors to execute procedure”. SWH students did not
describe error correction or monitoring to check they are performing the experiment correctly as much as non-SWH students. It is possible that SWH students did not use these strategies because they felt it was unnecessary. As SWH students planned individually and together, it is possible that since they had a better prepared procedure, they did not need to check on their execution of the experiment nor did they run into as many errors during the experiment. Perhaps in this way, they had more time to focus on understanding the data. Evaluation was the only strategy in which both groups exclusively practiced this individually or not at all. It is clear that both groups used strategies to various degrees, with and without peers.

**Students Experience Differential Support from Structure of Weekly Instructional Labs**

Students’ responses about metacognitive strategy use during open-ended lab problems were affected by their weekly experiences with the instructional format (SWH or non-SWH). This theme reveals a difference between SWH and non-SWH instructional environments and students’ experience of metacognitive strategy use. Students were asked in the interview “How do you think the weekly [SWH or regular] lab experiments prepare you to solve and report on the open-ended lab problems?” The non-SWH and SWH groups did not describe similar experiences when asked whether their weekly instructional labs (non-SWH or SWH) supported them solving the open-ended problems. It became clear from the interviews that the weekly SWH lab experiments provided students with more effective opportunities to transfer metacognitive regulation strategies from the weekly labs to the open-ended lab problems than the non-SWH weekly experiments. SWH students described that the lab problems were comparable to their SWH experiments, whereas the non-SWH group found little to no connection between
their weekly traditional labs and the open-ended problems. Charles (non-SWH) replied to
the above question with:

I would say it [the weekly experiment] gives me, like, a general direction
of where to go with the lab problems. How to start, I guess, and how to
approach coming up with a procedure. But other than that I think they're
kind of different, so I don't know. I kind of have trouble seeing any direct
correlation between them.

Charles found that his weekly lab experience did not support or prompt him to think
about how to solve the open-ended problems. Sam, a non-SWH student who had
performed open-ended lab problems in high school, found the lab problems easy and
thought that the scientific method in general provided structure. He responded to the
same interview question with:

I guess just through all of my, like, chemistry experiences, even high
school, there is a set structure that everything is supposed to [go through],
you make the procedure and then you make a hypothesis, then you make a
procedure, and then you perform it. So, like, that structure is still the
expectation in the lab problems. And I mean that's the proven scientific
method.

Students in the non-SWH group who may have not had as much experience with lab
problems from prior courses as Sam felt lost while performing the open-ended lab
problems. Some cited that they always needed examples to start something. With respect
to the open-ended problems compared to the traditional weekly labs, Holly said, “Yeah,
my senses are overloaded, and I don't know where to begin.” She struggled to find
support for the open-ended problems from her weekly labs,

I think they show good examples of how the lab, the write-up should be
setup. So, I try to draw from that. And it gives you more experience for
when you have like to setup a problem or know like when the um, like
whether to use like the reaction plates or like the test tubes, and that’s
about it.
Like many other non-SWH students and as Holly and Charles mentioned, the traditional instructional environment did not provide support for how to think about solving the problem. Contrarily, SWH students felt that the weekly SWH labs provided support to solve their open-ended lab problems. Often they cited that SWH labs and lab problems were about the same difficulty, and sometimes lab problems were easier than the SWH labs.

**SWH provides structure for thinking process.** SWH students thought SWH labs provided both writing and thinking frameworks for solving the open-ended lab problems. Answering the question, “How do the SWH labs help you to prepare for the lab problems?” Sophie, an SWH student, shared that:

[An SWH lab experiment] helps me to process things and think through things. Like, if I can’t deal with certain things. Like, with the phenolphthalein, I just couldn’t just put it with the solids. You have to dissolve it with water. It helps me think more, if we do the group discussions or just thinking.

The SWH instructional format provided Sophie with instances in which she described using metacognitive strategies, including monitoring her knowledge and correcting for errors that occurred in lab. Thomas explained that the repetition of the SWH labs helped him to know what to think about when performing and reporting on the lab problems. “You just, you know what things to think about, or what things to write about from doing so many SWHs.” Each step of the SWH was repeated each week in lab. Students were reminded in lab how to set up an experiment, and the report template prompted students while planning and writing. This consistency is a crucial component of the SWH that helped students to see this instructional framework as the natural way to think in lab.

**SWH encourages self-questioning.** Forty percent of the SWH students found
that the prompting from the SWH made them keep in mind questions to ask while they performed their open-ended lab problems. Lydia, an SWH student, stated: “In the SWH lab, we were told to ask these questions, to think about these concepts. So, it’s like, maybe I should keep that in mind, what concepts are related to this, and what questions I should ask.” This structure of prompting encouraged students not only to ask questions about the lab before they began the experiment as a planning regulation strategy but also to check that they understood what was going on while running the experiment as a monitoring strategy.

William (SWH)

Question everything. I mean I, it’s kind of a fundamental idea behind the SWHs. So at least it made me pick up, just question absolutely every single move that you make. Whether it’d be if you pour something into a test tube, you don’t know if it’s sturdy or not. Or if a method you have been using is applicable to planning other results that you need.

William clearly describes that the SWH format made him question everything.

Essentially, the SWH afforded him the opportunity to ask himself questions throughout an entire experiment.

**SWH provides a framework to conduct and report on an experiment.** SWH students also found the actual format for the SWH labs to be useful while performing the lab. The structure of the beginning question, claims with evidence, and reflection provided a base on which they could build their open-ended lab experiments.

Ellen (SWH)

I feel like the writing heuristics kind of are more guiding you, so it’s nice to write those labs further, a little bit more guided and structured. And then be able to use that as a, uh, template for the formal labs. I think that’s helpful.
Sophie (SWH)

I pretty much think they [SWH and lab problem] are the same. That’s how I feel on it. The lab reports require more rigorous info in them, so it takes more time. But I think they are the same.

It was likely that SWH students were applying the organizational structure, the actual heuristic, from the SWH to their open-ended lab problems. The fact that the SWH prompts for data analysis may provide support for why SWH students described using more regulation strategies in solving their lab problems especially in the data analysis section (see Table 13). SWH students perceived that the SWH had them think in a more organized way that assisted them when solving the lab problems. Non-SWH students felt that their weekly labs did not provide such parallel structure. The SWH students made many connections between the structure of the SWH, metacognitive strategy use and how it prepared and guided them to solve open-ended lab problems. Students in the traditional lab did not describe having an instructional environment that supported their metacognitive strategy practice. They provided little, if any description of transfer of their work in weekly labs to the open-ended lab problems.

**Students’ perceptions of open-ended problems.** Students in both non-SWH and SWH labs had traditional worksheet-based experiments in the first semester chemistry laboratory. This often brought up comparisons between what students experienced in the second semester lab as compared to first semester. Both SWH and non-SWH students described differences. Henry and Alex responded to, “How do you compare what you did this semester with your first semester lab?” with:

Henry (SWH)

Not really because a lot of it [first semester lab] was just worksheet-
based, and so, they just told us to do this and then you’re just supposed to write your observations. Like, I mean you didn’t really have to think about anything that much, and this [SWH lab] is more, this is making thinking more like a second nature.

Alex (SWH)

No, it didn’t. So. It may have been easier, but this actually gets you to think more and you learn it. You actually learn it ‘cause you know ‘cause you gotta think about what you’re actually doing, what concept you learned in order to carry out the experiment.

Lab has become more work for Henry and Alex, but they feel that they are learning how to think in the SWH lab. Non-SWH students also felt that this lab experience was different than the previous semester. Even though they still had worksheet-based labs, the open-ended problems clearly had an effect on most students.

Jane (non-SWH)

Instead of just here mix this, mix this, you have to know what's going on there. And the lab write-ups too where you have to make the connections to the world knowledge like, it ties it all together for you. Instead of just reading off a procedure and it's telling you what to do, I think you get more out of [the lab problem] when you have to come up with your own procedure and organization. It's more professional.

All non-SWH students found that completing the open-ended problems were beneficial to their learning even when they were more difficult than the weekly problems. They also felt they enjoyed the problems more.

**Summary**

The results presented in this chapter provided a deeper look into how and when students used metacognitive strategies while solving open-ended problems. First the assessment results provided a general picture of the chemistry majors in the research study. Both groups significantly increased their content knowledge over the semester, and
there was no difference in the MCAI scores, a survey for habitual metacognitive regulation strategy use. The interviews, however, showed true differences between the two groups. Students in both groups followed the same pattern of metacognitive strategy with the open-ended problems they solved. The strategies were organized into the phases of planning, conducting the experiment, performing data analysis, evaluating the experiment, and writing the lab report. Students in both groups used metacognitive strategies individually to about the same degree. When strategies were compared, all students used planning to a greater degree than either monitoring or evaluating while solving their open-ended lab problem.

Differences arose in students’ responses as they continued to describe different avenues to plan, conduct and evaluate their experiment. The two ways in which the non-SWH and SWH differed were the degree of peer use while practicing metacognitive strategies, and how they perceived their weekly lab experiments (SWH or traditional) to affect their metacognitive strategy use. Both groups used peers to practice planning and monitoring regulation strategies; however, SWH students used peers to a greater degree when trying to understand the concepts and analyze the data from the problems. This difference suggests that SWH students used peers for sense-making of the data, a higher order thinking level, whereas non-SWH students used peers to make sure they performed the experiment correctly, which, while important, is a lower order thinking level. Finally, SWH students found that their weekly SWH template acted as a framework for their thinking process as well as a template to prepare for their open-ended lab problems. These results suggest that the instructional environment has an impact on students’ described metacognitive regulation strategy practice while solving open-ended problems.
CHAPTER FIVE

DISCUSSION

The following chapter discusses how the interview and survey data results that were obtained and interpreted answer the two research questions:

- How does the process by which students solve and report on open-ended laboratory problems reveal use of metacognitive regulation strategies?
- Does the type of laboratory instructional environment (SWH vs. Traditional) that students regularly experience elicit any differences in students’ use of metacognitive regulation strategies while solving open-ended laboratory problems?

The discussion addresses the findings for both questions. First, the process by which the students solved the open-ended problems is discussed. This is followed by an exploration of similarities found between the two groups descriptions of strategy use and lab experience. The assessment results are discussed to describe why the two groups may be similar. Finally, the key differences in the two groups’ strategy use and support of their strategy use is discussed through interviews. The type of weekly instructional environment (SWH and non-SWH) elicits qualitative differences in the types and degrees of use of these regulation strategies.
Similarities between SWH and Non-SWH Students

Research shows that while undergraduate students have some natural metacognitive ability (Garner & Alexander, 1989) and also demonstrate some metacognitive strategy use, it is less than they could be using. In two studies, students often had opportunities to employ metacognitive strategies to a greater degree but did not act on the opportunities (Armstrong, Wallace, & Chang, 2008; Kung & Linder, 2007). Clearly, students use metacognitive strategies although they may or may not be aware of these strategies. The interviews revealed that most SWH and non-SWH students practiced metacognitive strategies including planning, monitoring and evaluating by themselves. These strategies were described in five phases: (1) plan experiment, (2) conduct experiment, (3) analyze data, (4) evaluate experiment, and (5) write report. Generally, students practiced planning strategies more than monitoring or evaluating in conducting and reporting on their experiment.

MCAI Results

It was hypothesized that students in the SWH group might have higher MCAI scores after completion of the semester. It was reasoned that students would be implicitly experiencing metacognitive regulation practice during SWH labs because the instructional environment was inquiry-based, collaborative with reflective prompting. These three characteristics have been shown to support metacognitive strategy practice. SWH students were also provided with two opportunities to learn about how metacognitive strategies were related to the SWH and how these strategies might be useful to them. Such identification and discussion of metacognitive strategies with
students might help them better understand why they are using the strategies (Bielaczyc, Pirolli, & Brown, 1995).

Yet, based on the MCAI results, students in the SWH were equivalent to non-SWH students on self-reported strategy use at the end of the course. There are several factors that might explain the lack of difference between groups. The MCAI asks students only about their individual use of strategies (Cooper & Sandi-Urena, 2009) which align with the interview results where students in both groups described using the same pattern of strategies and approximately the same number of strategies individually. Students in both groups started with high pre-study scores around 80%. In two previous research studies, the MCAI scores of first semester general chemistry students were around 75% (Cooper & Sandi-Urena, 2009; Sandi-Urena S., Cooper M.M., & Stevens R.H., 2011). The average of MCAI scores for first-year graduate students was about 80% (Cooper & Sandi-Urena, 2009). The students in this study had scores similar to first-year graduate students rather than general chemistry students. As a higher inventory score indicates greater metacognitive strategy use, it is likely that the chemistry majors in this study were already highly metacognitive as defined by the inventory. The high pre-score may cause a potential ceiling effect where students are not likely to report a much higher inventory score. Thus the change from pre- to post- may be too small.

It is also possible that the MCAI could not detect the differences in behaviors that the students described in the interviews because the effect of the instructional environment may be delayed or students needed to experience the environment longer to report any difference in their strategy use. Finally, students may not report a difference in
metacognitive behavior on this survey because of their lack of awareness of the strategies. Piaget’s theory of regulation suggests that using regulation strategies may not be always be a conscious experience. Therefore, it is also possible that students use strategies but they are not aware of the strategies enough to recognize their use of them (Brown, 1987) or rate them on a survey.

**Correlation between ACS scores and MCAI scores.** The results showed that students’ self-reported regulation strategy use, the MCAI results, was moderately correlated with their final ACS exam score and with final course grade. A higher post-ACS exam score generally indicated a higher post-MCAI score. Previous research has shown that metacognitive abilities are moderately correlated with learning performance and a student’s grade in the course (Cooper & Sandi-Urena, 2009; Veenman, Kok, & Blöte, 2005). The SWH group had a higher correlation value between both the MCAI and ACS scores and MCAI and final course grade than the non-SWH group.

**Metacognitive strategy use and content knowledge.** The results showed that groups were equivalent on the pre-and post-ACS exam scores. Students in both groups also increased equally from pre- to post-score. It was reasoned that students in the SWH group might have higher scores based on previous research from Greenbowe and Hand (2005). They found that students who participated in an SWH lab and a lecture course had significantly higher scores on the 1st term ACS exam than non-SWH students in the lecture course. This research approach varied from the Greenbowe and Hand (2005) study in two ways. SWH was only taught second semester and the second semester lecture course for each group was taught using the POGIL method by the same professor,
not through traditional lecture as in the SWH comparison study. Although SWH had an effect on content knowledge in the comparison study, the POGIL method in the lecture course may have overshadowed any differences in the study. When compared with lecture, research supports that POGIL significantly increases content knowledge scores on algorithmic and conceptual exam questions (Daubenmire & Bunce, 2008; Lewis & Lewis, 2005b; Straumanis & Simons, 2008). Both groups exhibited the same pattern of statistically significant growth in scores from the 1st term ACS exam to the 2nd term ACS exam (Table 9). These results suggest that the difference in laboratory environment did not affect students’ content knowledge as measured by the ACS exams while in the study.

**Writing and Metacognitive Strategy Use**

During the writing phase, students in both groups used metacognitive strategies to about the same degree. When asked how writing helped them learn, most students felt that writing was useful particularly in a reflective manner such that they could see what they knew and did not know. Writing helped them see the gaps in their knowledge, after which they could take steps to close those gaps (Emig, 1977; Klein, 1999). Students in the SWH group described more often that they monitored themselves while writing. This may be attributed to the focus in the SWH template on checking that one’s argument makes sense. The interview results have some agreement with previous research that showed that students who were provided with instances to practice metacognitive strategy use while writing used more metacognitive strategies (McCrindle & Christensen, 1995). Students in this study wrote the lab report in the same style based on the same
rubric. During instruction, the TAs did not show students how writing their weekly lab reports might support them to write their open-ended lab reports. Both groups scored an average of 85% on their five lab reports. A recent pilot research study found that students who experienced an SWH style lab compared to a traditional lab scored no differently when asked to write a formal summary lab report in which they had to support an argument with their data. However, SWH students were significantly more likely to receive a higher score on the argument in the report (Cronje, Murray, Rohlinger, & Wellnitz, 2013). Although, the arguments in the lab reports were not scored separately in this study, SWH students may be able to better formulate their data and support their argument properly with the evidence. The ability to support an argument correlates with metacognitive strategies use (Klein, 2004).

**Differences between SWH and Non-SWH Students**

Although the assessments and report writing did not indicate differences between the SWH and non-SWH groups, the interview results provided two substantial differences in the students’ choice to use metacognitive regulation strategies. The differences that arose between the instructional environments involved peer support and support from the structure of weekly laboratory experiences for metacognitive strategy use. These differences indicated behaviors that SWH students tended to practice more metacognitive strategies while conducting and reporting on their open-ended problems than non-SWH students. The type of instructional environment that students experience can shape students’ behavior and practice of metacognitive regulation strategies.
Peers Support Practice of Metacognitive Strategies

The first distinguishing feature between the two instructional environments is that students in the SWH group described using their peers for regulation activities to a greater degree than the non-SWH group. The finding that students employ peers for metacognitive strategy use is supported in other research as well (Sandi-Urena S. et al., 2011; Sandi-Urena, Cooper, & Stevens, 2012). Their research found that when students worked in groups to solve ill-structured problems, students not only used metacognitive regulation strategies, but also reported more strategy use over the intervention period.

This research expands on these results to describe how students used their peers during the phases of solving the open-ended lab problems (Table 12) to support their strategy use. Even though both groups used peers to about the same degree in planning and checking that they were performing the procedure correctly, SWH students used peers to support metacognitive regulation to a greater degree to check their understanding of the experiment, to compare data and to perform data analysis. Students sought out different points of view from peers to support their strategy use while conducting the experiment. These results are supported by Grimberg (2007) in her analysis of students’ reflective actions in an SWH environment.

By checking with their peers about procedural steps, both non-SWH and SWH students monitored their procedures in order to get reliable data for analysis. For the non-SWH students, the use of peer support seemed to stop there. They did not use their peers much to foster understanding for why they were doing the experiment. SWH students, on the other hand, used their peers to monitor and to help them understand what was
happening in the experiment and how this knowledge might be used for data analysis.

Kung and Linder (2007) observed students’ metacognitive behavior in a physics lab. They found that students made metacognitive statements, but students did not always act on those statements. Students who did act on their statements appeared to participate in more sense-making by discussing the concepts and data from the experiment. SWH students may be experiencing more of this sense-making while conducting the experiment and performing data analysis. They spent more time on higher order thinking. The instances in which students compared data with peers while conducting the experiment informed decisions about running more trials and helped them check whether they had consistent results.

Only SWH students reported using their peers outside instructional time in order to gather another viewpoint on their data as a way to support their data evaluation. Again, the instructional setting may have influenced this decision. SWH students worked together to form beginning questions, decide on procedures, and compare data results during weekly lab instruction. In addition to using their peers to perform data analysis, students elected to use their peers while they planned for the experiment and wrote lab reports. Each of these instances was an unstructured situation outside of the laboratory time. Students who use peers to practice regulation strategies may be able to more easily integrate these strategies into their own learning framework and use them effectively (Hodson & Hodson, 1998). Working with peers also provided students instances where they used metacognitive strategies socially which may allow for eventual internal use of
these strategies by the individual students (Kuhn, 2000). The elective use of peers likely pushed students to gain more expert-like skills while solving the open-ended problems.

Non-SWH students did not describe any use of peers when performing data analysis or evaluation although some described use of peers while report writing. The non-SWH “traditional” labs in this study did not explicitly prompt students to work together to plan, monitor, or evaluate their experiments. This may explain why students in non-SWH, traditional lab courses used peers less during metacognitive regulation. It is not part of their regular practice to use peers. Even though non-SWH students did not describe collaborating with peers much during laboratory time and very little outside of the laboratory time, their overall perception of using their peers was positive which is shown in other studies (Cooper & Kerns, 2006).

**SWH Supports Practice of Metacognitive Strategies**

The second distinguishing feature between instructional settings was students’ descriptions of how their weekly (SWH or traditional) labs helped them to structure their open-ended lab problems. Overall, SWH students indicated that their regulation strategy use was impacted by the structure of the SWH when solving open-ended lab problems. The SWH supported them through the prompting from the SWH template as well as the inquiry-based pedagogy. They felt that the SWH provided them a template to prepare open-ended problems as well as a scaffold for how to think and process knowledge. These results are in agreement with two other studies on SWH instruction in secondary school age students (Grimberg, 2007; Hohenshell & Hand, 2006). Both studies maintained that students demonstrated behaviors consistent with metacognitive
awareness and strategy use when prompted by the reflective statements in their SWH labs. When students were asked in the interview about how their weekly lab supported their open-ended problem-solving, only SWH students responded that the prompting from their weekly labs helped them solve their open-ended problems. A recent study found that when students used the SWH template for report writing, their use of reflective statements was greater in guided inquiry labs compared to verification style labs (Xu & Talanquer, 2013).

These findings confirm and expand earlier research that shows inquiry-based labs provide opportunities for students to use metacognitive strategies during laboratory (Kipnis & Hofstein, 2008). Through interviews and observations, they found that students elicited metacognitive knowledge awareness and general regulation strategies while performing inquiry-based labs. SWH students tended to describe that their weekly lab instruction was teaching them ways to think: teaching them how to ask themselves questions, how to plan an experiment and how to frame their thinking, which are all metacognitive processes. Non-SWH student identified few and in some cases, none of these types of relationships between their weekly instruction and open-ended problems. The SWH students’ use of metacognitive regulation strategies during lab time and outside of lab may suggest that they are learning how to better integrate and organize knowledge into their long-term learning structures.

SWH students’ greater use of metacognitive regulation strategies allowed them to feel more prepared to solve their open-ended lab problems. Non-SWH students identified that they enjoyed solving the problems but did not always feel prepared or that they “felt
more lost.” These results agree with research that students in inquiry-based labs perceive that they learn more and enjoy the open-ended problems more than traditional forms of instructional labs (Berg, Bergendahl, Lundberg, & Tibell, 2003). By providing an environment with inquiry strategies, effective prompting, and productive peer interactions, instruction can strengthen and deepen students’ use of metacognitive regulation strategies.

**Conclusion**

There have been several research studies on metacognition in the lab setting. Some have focused on whether students are using general metacognitive strategies. Others have identified whether an intervention might impact a student’s practice (Case, Gunstone, & Lewis, 2001; Kipnis & Hofstein, 2008; Sandi-Urena et al., 2012). This study moves this area of research forward by going beyond simply identifying that students are using planning, monitoring and evaluating strategies in the lab environment. It first examined when and how students used specific metacognitive regulation strategies as identified by Schraw and Dennsion (1994) under the general strategies of planning, monitoring and evaluation. The research then explored how an environment that supports metacognitive strategy practice through inquiry-based pedagogy, reflective prompting and peer collaboration affected students’ practice of these strategies while solving open-ended problems.

Overall, both groups described practicing regulation strategies individually. They described using planning more than monitoring or evaluation strategies when conducting and reporting on their open-ended lab problems. This was supported by survey and
interview evidence. The differences in the two environments supported by student
interviews suggest that the SWH environment allowed students greater practice of
metacognitive strategies. Through the support of their peers and the structure of their
weekly lab instruction, SWH students described more metacognitive regulation
strategies. Strategies that were predominately used by SWH students included checking
understanding of concepts and data comparison during the experiment. This indicated
that they likely spent more time making sense of their data. SWH group also performed
data analysis with their peers. On the other hand, non-SWH students used many
metacognitive strategies individually; however they found less support through their
peers and almost no support for their practice through weekly lab instruction. This
research not only provided a lens into students’ descriptions of their regulation strategy
practices in the laboratory, but it also supported that the way that a laboratory
environment is arranged can affect these regulation strategy practices and their transfer to
new situations.

These results may be useful for instructional purposes to those instructors who are
interested in adding a focus on metacognitive strategies to their instruction. The SWH is a
well-established instructional strategy and can be implemented as is or provide a template
for an instructor to build an instructional environment that is collaborative, inquiry-based
with reflective prompting where metacognitive strategy use is encouraged.
Limitations

Reflexivity as a Qualitative Researcher

The importance of understanding the limitations of one’s research is necessary in qualitative research. I as the researcher was the main instrument for data collection. Not only did I collect the data, but I was also part of the data in the interview process and through my presence as the teaching assistant in the laboratory. I interacted with the students outside of the research study on a weekly basis during the period of the study. As a TA, I did not grade any student’s assignments that participated in the study. As a researcher, I consistently reflected how my bias and experiences might affect the data and analysis.

In the study, I built in components that would strengthen the validity. As a researcher I was a part of all the laboratory sessions. In this way, I was able to understand the context in which students described their strategy use. I also kept notes after each session for me to record what I saw happen during lab and think about how it might affect the research. When planning the study, I included several types of data collection, including interviews and surveys. For the interview process, students were randomly chosen.

As a TA, I had a desire for the students to succeed in both traditional and SWH lab formats. I had to actively monitor that I was teaching according to each style. To ensure that I did not cross-teach these methods, the traditional format section was taught the spring before the SWH section. I also worked with a TA who not only taught both
sections to reduce instructor variability, but also was aware of the research but not aware of the research questions.

My perspective as a qualitative researcher is not to remove my bias and assumptions from my research but allow readers to interpret my research with an understanding of these biases and perspectives. Maxwell’s quote more eloquently describes my perspective as a qualitative researcher:

The reason for making your perspective, biases, assumptions clear to the reader is not to eliminate “variance between researchers in values and expectations they bring to the study, but with the understanding how a particular researcher’s values and expectations influence the conduct and conclusions of the study” (Maxwell, 2005, pg. 108).

Unlike a single data point in quantitative research, qualitative research with human participants is “holistic, multidimensional, and ever-changing” (Merriam, 2009). A researcher who is involved and close to the data and participants can describe a more realistic picture of the phenomenon studied.

**Experimental Design**

SWH was implemented for one semester, and research supports that one semester of a new environment may not provide lasting support for students in changing their learning or skill use in subsequent learning situations (Engelbrecht, Harding, & Du Preez, 2007). Even though change in students’ conceptual understanding and learning performance has been detected in previous studies, little change in metacognitive strategy use was reported by students in the MCAI in this study. Perhaps one semester of the instructional environment was not enough for change to be evident through these self-report surveys. It is also possible that the types of differences seen in the students’
metacognitive behavior could not be detected with this instrument. A survey in which students are asked about how their metacognitive strategies are supported may provide a better measure for these regulation behaviors that students described during lab time. Students pre-scores were high on the MCAI which suggests that change might not be detected because of a ceiling effect. The data provided a lens into students’ use of metacognitive strategies during this specific laboratory experience. It did not provide a latitudinal or longitudinal view on whether students might continue to use these strategies in other chemistry laboratory courses.

The students selected for the study were declared chemistry majors who generally came into the university courses with a considerable high school background in chemistry, i.e. Advanced Placement or honors high school chemistry. Most participants, though, had no experience in solving open-ended problems, and many had little high school laboratory experience. The type of students chosen reduces the ability to generalize to other types of students taking chemistry such as non-majors, yet this study suggests that students of all levels of academic achievement use some degree of metacognitive strategies, and students in the SWH environment generally used more of those strategies.

Data Analysis

As data analysis occurred, it became clear that the interviews would provide the bulk of the data. Survey and assessment data were more difficult to interpret because of small sample sizes. The anticipated size of the study was about twice as many students than the number who actually participated in the study. The small sample size made it
difficult to perform statistical tests on the data for generalization beyond the chemistry majors course. Even though the MCAI surveys were given in the laboratory setting, the lab and lecture class were considered one course. When answering the survey, students may have thought of the strategies they used to answer chemistry problems in class instead of or in addition to the problems in lab. This study was specifically interested in the strategies students used while in the lab and performing the open-ended problems. In future work, it may be beneficial to give the students surveys immediately before and after the open-ended lab problems. The ACS exams selected also targeted content that is more aligned with lecture sections than with laboratory courses. The ACS exam was also a section of the final exam in the course and was heavily weighted. Final exam stress may have been a variable that was not accounted for in the study.

**Future Research**

This research study was completed during the second semester of a chemistry course. To date, data has been collected in a secondary study for a full year of general chemistry lab. This will provide a longitudinal picture of students’ metacognitive strategy use in their general chemistry laboratory experience. It will also provide opportunities to investigate further students’ strategy use during report writing. The sample size provided excellent qualitative data for support; however, if generalization to other chemistry students is desirable, a larger sample size is necessary for statistical analysis of the MCAI and ACS exams. Since the population only consisted of chemistry majors, there is an interest in how different types of students, for example non-majors or students who took
less chemistry in high school, and their strategy use are affected by an SWH environment.

This study found that the qualitative differences of students’ behaviors to use peers and their weekly laboratory labs to support their metacognitive strategy practice were not detected in the quantitative assessments. These results indicate that further research on instruments that might characterize students’ differences in regulation behavior is necessary. An instrument may need to be developed that can characterize students’ strategy use through the supports (peers, lab structure) in the laboratory. In addition, the MCAI provides information about the level of students’ regulation strategy use. It may be useful to test the instrument in a variety of laboratory environments and with a variety of students to determine a normed standard to which instructors could compare their students. The students’ scores in this study were found to be about 5% higher than those reported in earlier research.

It is desirable for students to not only learn and use metacognitive strategies in their learning, but also continue to use those strategies in subsequent learning situations. If the SWH supports practice of metacognitive strategies, further research on students’ use of metacognitive strategies in subsequent laboratory classes, regardless or even in spite of instructional approaches would be useful. Other instructional strategies such as ADI and the MORE thinking frame are likely to support transfer of metacognitive strategy use like the SWH. A comparison of the impact of these instructional strategies on strategy use may provide further understanding of the characteristics that support strategy use. This research only looked at students regulation strategy use. Because
metacognitive knowledge is often intertwined with metacognitive strategy use (skillfulness), there is a need to understand how students are aware of their knowledge of their metacognitive processes during a lab situation.
CHAPTER SIX
INTRODUCTION FOR STUDIES ON THE Pb-CAM BINDING INTERACTION

Calmodulin

Research to investigate binding, structure, and function of calmodulin (CaM), a common calcium binding protein, with metal ions is extensive. The binding of calcium, physiological ions like magnesium, and toxic metals to CaM has been investigated through several techniques including circular dichroism (CD), fluorescence spectroscopy, Nuclear Magnetic Resonance (NMR), and binding assays (Kirberger, Wong, Jiang, & Yang, 2013; Martin & Bayley, 1986; Ouyang & Vogel, 1998; Shirran & Barran, 2009). CaM is not only heat and pH resilient (Blumenthal & Stull, 1982; Brzeska, Venyaminov, Grabarek, & Drabikowski, 1983), but it is also relatively inexpensive and commercially available. It is commonly used as a model for calcium binding proteins. Calmodulin is part of the calcium binding protein (CaBP) superfamily. As an essential calcium binding protein, CaM assists other proteins that lack the ability to bind calcium. It regulates calcium for muscle contraction, memory, nerve growth, immune response and signal transducing. It is one of the most highly conserved eukaryotic proteins with 90% sequence similarity between multicellular eukaryotes (Celio, Pauls, & Schwaller, 1996). CaM is a small 17 kD dumbbell shaped protein with a 148 residue sequence. It contains two domains connected by a flexible central linker.
Within the two domains sit two EF hands each. The EF hands are 30 residue long helix-loop-helix motifs. The middle loop of twelve residues binds calcium in a pentagonal bipyramidal geometry (Finn & Forsén, 1995; Kirberger & Yang, 2008). In the N-domain are the canonical binding sites EFI and EFII, and in the C-domain are EFIII and EFIV.

Calmodulin binds calcium when the resting cell concentration of calcium, about 100 nM, increases to about 10 μM. Calcium binding occurs cooperatively first at the EFI (residues 20-32) and EFII (residues 56-68) sites in the N-domain followed by the EFIII (residues 93-105) and EFIV (residues 129-141) in the C-domain (Celio et al., 1996). The dissociation constants of calcium to CaM are 11 μM for the N-domain and 2 μM for the C-domain (Kirberger et al., 2013), which shows that C-domain binds Ca^{2+} more tightly than the N-domain. These dissociation constants are dependent on ionic strength, pH and temperature (Van Eldik & Watterson, 1998). The binding of calcium allows calmodulin to
bind target proteins such as myosin light chain kinase (MLCK) in the central pocket. Figure 2 shows the transition from a saturated calcium structure to the globular structure bound to a target peptide of MLCK. The central linker bends for target protein activation (Figure 2b) (Finn et al., 1995) CaM activates about 100 target proteins and enzymes. Several common calmodulin binding proteins include the aforementioned smooth muscle MLCK used for calcium signaling in muscle tissue, phosphorylase kinase used in glycogen metabolism, nitric oxide synthase for signaling in the synthesis of nitric oxide (Celio et al., 1996).

**Lead Toxicity**

The importance of understanding the effect of lead on a biological system is essential. Lead targets the central nervous system and soft tissues, and it is stored in the bones. It commonly displaces many essential metals in the body including calcium, zinc, and magnesium. Lead (Pb$^{2+}$) is similar in ionic radius and charge to Ca$^{2+}$, 1.20 Å vs. 0.99 Å, respectively (Lide, 2004). Not only can Pb$^{2+}$ gain entrance to the cell via Ca$^{2+}$ voltage channels, but it can also improperly activate or deactivate calcium binding proteins (Chao, Bu, & Cheung, 1995; Kern, Wisniewski, Cabell, & Audesirk, 2000; Ouyang & Vogel, 1998). Symptoms of lead poisoning include memory loss, anemia, bone loss, elevated blood pressure, and neurobehavioral and learning problems (Bridges & Zalups, 2005). There are several ways in which lead enters the body, most readily through inhalation and ingestion. Some Pb$^{2+}$ is excreted; however, much of it is stored in the bone where it easily displaces calcium in osteocalcin (Dowd, Rosen, Mints, & Gundberg, 2001). The most vulnerable populations of lead poisoning are children, pregnant women
and occupational workers. Lead poisoning increases in persons with a lower dietary intake of calcium (Bridges & Zalups, 2005). Calcium deficiency is commonly found in children, immigrants and the elderly. A common method to measure lead in the body is through blood lead levels (BLL). The average BLL of an adult is 1.2 µg/dL (Centers for Disease Control and Prevention, 2013). Lead poisoning in children is defined at 5 µg/dL and in adults at 25 µg/dL (Centers for Disease Control and Prevention, 2014). As of 2012, 0.62% of children in the US had BLL that exceeded the 10 µg/dL limit down from 3.96% in 2000 (Centers for Disease Control and Prevention, 2012). Lead poisoning can be quickly remediated by screening children and adults for high BLL. Removing lead paint from older homes and cleaning up environmental sites can also reduce lead poisoning.

**Pb-CaM Interaction**

Although CaM is a calcium binding protein, it also very easily binds lead. Lead binds and activates calmodulin in a similar fashion to calcium. The dissociation constants of Pb\(^{2+}\) were determined through titrations via fluorescence spectroscopy (Kirberger et al., 2013). In table 15, the dissociation constants for Pb\(^{2+}\) and Ca\(^{2+}\) are compared. Pb\(^{2+}\) binds more tightly than Ca\(^{2+}\) in both domains.

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>Activation of MLCK</th>
<th>(K_d) at C-terminus</th>
<th>(K_d) at N-terminus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>5 µM</td>
<td>2.0 µM</td>
<td>11.5 µM</td>
</tr>
<tr>
<td>Lead</td>
<td>10 µM</td>
<td>0.7 µM</td>
<td>1.4 µM</td>
</tr>
</tbody>
</table>

Pb\(^{2+}\) is also able to displace Ca\(^{2+}\) in at least the C-domain according to the loss of intensity in fluorescence data (Kirberger et al., 2013). Previous research with ESI Mass Spectrometry showed that Pb\(^{2+}\) displaced Ca\(^{2+}\) seen through the loss of Ca\(^{2+}\) peaks when Pb\(^{2+}\) was added to the protein (Shirran & Barran, 2009). That study also found that Pb\(^{2+}\)
can bind to apo-CaM although only at very high concentrations, greater than six moles Pb$^{2+}$ to one mole CaM.

Despite the stronger dissociation constant of Pb$^{2+}$ to CaM, maximum activation of MLCK occurs at larger concentrations of Pb$^{2+}$, 10 μM in comparison to calcium at 5 μM (Chao et al., 1995). Pb$^{2+}$ has a biphasic effect of activating CaM followed by inactivation of CaM at large concentrations greater than 10 μM. Lead toxicity is likely an effect of the interaction of Pb$^{2+}$ to bind to CaM. Although research shows that Pb$^{2+}$ binds to and is able to both activate and inactivate CaM, it is not clear how Pb$^{2+}$ causes CaM to stop binding target proteins.

Beyond the canonical binding sites found at the EF hands, research has described auxiliary sites for both Ca$^{2+}$ (Milos, Schaer, Comte, & Cox, 1989) and Pb$^{2+}$ on the protein surface (Kirberger et al., 2013; Wilson & Brunger, 2003). Sites beyond the EF hands for Ca$^{2+}$ have not been well identified in crystal structures. In the case of lead, there are two X-ray crystallographic structures (PDB 2v01 and 1n0y, Figure 3) of Pb-CaM.

Figure 3. Pb-CaM in 2v01 and 1n0y XRC structures (Kursula & Majava, 2007; Wilson & Brunger, 2003)
Both structures show Pb\(^{2+}\) bound to all four EF hand canonical sites as well as several other areas on the protein (7 additional sites on 1n0y and 4 additional sites on 2v01) (Kursula & Majava, 2007; Wilson & Brunger, 2003).

Through an analysis of all Pb\(^{2+}\) bound structures in the Protein Data Bank (PDB), Kirberger and Yang (2008) conclude that Pb\(^{2+}\) is likely to bind opportunistically at any site that has a negative charge such as oxygen containing residues in addition to displacing the calcium ions at the canonical sites (EF hands). One specific area where Pb\(^{2+}\) ions might bind opportunistically is the central linker. This may be why Pb\(^{2+}\) eventually inhibits the ability of CaM to activate the target proteins (Kirberger et al., 2013).

**Binding Characteristics of Pb\(^{2+}\)**

Lead has several similar characteristics to calcium including a 2+ charge and similar ionic radii. Lead can also bind with the same coordination number as calcium (seven bonds) (Shimoni-Livny, Glusker, & Bock, 1998). Gourlaouen and Parisel’s (2007) modeling results show that the coordination geometry is the same. Lead has, however, different orientation and bond angles due to a lone pair. In lone pair theory, the 6s orbital is envisioned as contracting which requires an increase in energy to interact with or remove that lone pair (Shimoni-Livny et al., 1998). Lead contains a stable lone pair of electrons in the 6s orbital that does not participate in binding except in specific tight quarters. In some lead oxides mixing of the lead 6s orbitals to oxygen p orbitals is observed (Payne et al., 2007).
Figure 4. An electron density model of Pb\(^{2+}\) binding to CaM (Gourlaouen & Parisel, 2007)

Lead binds with both holodirected and hemidirected character in molecules. A holodirected coordination suggests that the Pb\(^{2+}\) bonds are evenly situated within a sphere, whereas a hemidirected coordination of Pb\(^{2+}\) leaves a defined gap in part of the coordination sphere. In Figure 4, lead binds in a holodirected coordination within the EF hands of calmodulin (Gourlaouen & Parisel, 2007) The “lone pair” on Pb\(^{2+}\) requires more space and slightly distorts the shape at the binding site. The shape of the binding site is also similar to that of Ca\(^{2+}\) except for the decrease in the axial angle where the lone pair of lead sticks out. This causes a change in how lead sits in the binding site; however, it is subtle enough that calmodulin continues to activate other proteins with lead instead of calcium. It may also contribute to the ability of lead to bind to other areas of calmodulin than the EF hands.
CHAPTER SEVEN

STUDY 1: EFFECT OF Pb$^{2+}$ BINDING ON CAM SECONDARY STRUCTURE THROUGH CIRCULAR DICHROISM

Introduction

The unique abilities of Pb$^{2+}$ to bind CaM more strongly than Ca$^{2+}$, the biphasic activation, and opportunistic binding on the protein surface suggest that Pb$^{2+}$ is likely to have an effect on the structure of CaM, specifically the secondary structure. CaM is considered to be mostly α-helix (56.8%) (Lees, Miles, Wien, & Wallace, 2006) with minimal β-sheet structure. The α-helix and β-sheet structures are formed due to peptide bonds that create chiral structures (Figure 5). Those structures can be probed via circular dichroism (CD) because the peptide bonds act as chromophores that absorb light in the far UV region (260-190 nm) of the spectrum.

Figure 5. α-helix and β-sheet structures and peptide bond structure (Beta sheet structure.2014; An alpha-helix.2014)
The angular differences in the peptide bonds caused by the three different types of structure (α-helix, β-sheet and random coil) cause changes in the intensity and location of the CD peaks. Figure 6 shows the typical and theoretical CD UV response of the three “pure” secondary structures. As can be observed, there are shifts in wavelength, intensity, and sign of the CD signal.

Figure 6. Theoretical CD signal for pure α-helix, β-sheet and random coil. Signal peaks and electron transitions are defined. Pure α-helix: positive (\(\pi \to \pi^*\)) perpendicular at 190 nm and negative (\(\pi \to \pi^*\)) parallel at 208 nm caused by exciton coupling and negative at 222 nm (\(n \to \pi^*\)). Pure β-sheet: negative at 218 nm (\(\pi \to \pi^*\)), positive at 196 nm (\(n \to \pi^*\)). Pure random coil: positive at 212 nm (\(\pi \to \pi^*\)), negative at 195 nm (\(n \to \pi^*\)). (Wallace & Janes, 2009).

In practice, a composite signal is obtained and deconvoluted via one of several major analysis algorithm packages including CDSSTR, CONTINLL, VARSLC, or SELCON3 (Whitmore & Wallace, 2008). Circular dichroism is useful for analysis of proteins.
because the technique is very sensitive to protein structure and dynamics. Because CaM is mostly α-helix, the changes in signal at the two peaks (208 nm and 222 nm) are of interest. Changes in negative peaks at 208 nm and 222 nm are indicators for secondary structure change, generally, a loss or gain in α-helical content.

**Research Goals**

Circular dichroism has been used to identify secondary structure changes within CaM when bound with Ca$^{2+}$, toxic metals or target peptides (Martin & Bayley, 1986; Maune, Beckingham, Martin, & Bayley, 1992). Cadmium, a toxic metal, bound to CaM showed no secondary structure change in CD spectra when compared to calcium (Martin & Bayley, 1986). Pb$^{2+}$ is also a very toxic metal, and has not been described using CD. The primary goals of the study were to determine: (1) whether the addition of Pb$^{2+}$ to CaM caused a change in secondary structure that could be detected using CD, and (2) if a change occurred, the minimum amount of Pb$^{2+}$ that was needed for the change to occur.

**Method**

**Materials**

Lyophilized CaM (from bovine brain) was purchased from Ocean Biologics (Seattle, WA). All CaM samples contained about one mole of Ca$^{2+}$ per mole of protein unless dialyzed to the apo-form (no metal bound) (Bauman, 2012). These samples are referred to as the “as-is” form. The following chemicals were used: Lead nitrate (Pb(NO$_3$)$_2$), Calcium nitrate (Ca(NO$_3$)$_2$), Tris-hydrochloride buffer (Tris-HCl), sodium ethylenediaminetetraacetic acid (EDTA), Guanidinium chloride (GuHCl) and Chelex (BioRad). Nanopure water was used for all solutions. The buffer was 20 mM Tris-HCl with pH 7.6. At this pH, CaM activates its target proteins (Blumenthal & Stull, 1982).
Trace metal ions were removed from the buffer solutions and water with Chelex beads in a column or batch method (Bio-rad Technologies). The CaM was stored at -20°C in filtered nanopure water and dialyzed into 20 mM Tris-HCl before use. Glassware and plasticware were rinsed in 1 M hydrochloric acid and 1 M EDTA to remove trace metals.

**Protein concentration.** Protein concentration was measured using a spectrophotometer with a quartz cuvette (Hellma Optics, Plainview, NY) of 1 cm pathlength and 20 mM Tris-HCl buffer as the baseline. The measurement was made at 280 nm where CaM has an optical density of 0.2 cm$^{-1}$ for 1 mg/ml concentration (Bauman, 2012). For concentration calculations, this value was converted to the molar extinction coefficient of 3338 M$^{-1}$ cm$^{-1}$.

**Circular Dichroism Instrument Parameters**

Spectra were taken with an Olis DSM CD spectrophotometer (Olis, Inc., Bogart, CA). The method for calculating the CD spectra is known as digital subtraction (DSM) where the raw data for the difference in absorbance ($A_{\text{left}}$ and $A_{\text{right}}$) is measured directly. The quartz cylindrical cell (Hellma Optics, Plainview, NY) had a pathlength of 10 mm and a volume of 2500 uL. CD spectra were recorded from 260-190 nm in the far UV in 5 mM Tris-HCl buffer. A low concentration of buffer was used to reduce interfering absorbance of the buffer below 210 nm. The number of scans ranged from one to three. The bandwidth was 0.5 nm. Protein concentrations between 0.015 and 0.04 mg/ml were used for all analyses. Even though data was taken between 200 nm and 190 nm, it was not often used because of the signal interference from the buffer absorbance.
**Titration Experiments**

Titrations with Ca(NO$_3$)$_2$ and Pb(NO$_3$)$_2$, separately, were performed to determine effects on CD signal. The lowest concentration used was 100 pM, which is lower than the cellular level concentrations of Ca$^{2+}$ and Pb$^{2+}$ concentrations, 10 µM and 100 nM respectively (Godwin, 2001). The highest concentration used was 40 µM for approximately a 0.02 mg/ml CaM sample. Titrations were performed by adding 5 mM Pb$^{2+}$ or Ca$^{2+}$ solution in increments of 1 µM to 10 µM to the CaM sample. The titrations were added directly to the CaM sample in the cuvette. The cuvette was only removed to mix the solution, and then placed back in the sample compartment for measurement.

**Denaturation Experiments**

**Chemical denaturation.** Pb$^{2+}$ and Ca$^{2+}$ saturated samples of CaM were denatured on the OLIS instrument at 222 nm at 25 °C. Guanidinium chloride (GuHCl) was used as the denaturant. The molarity of the denaturant was determined using both refractive index and by weight (Shirley, 1995). It is suggested that urea is a more appropriate denaturant to use because it has fewer complex interactions with calmodulin. However, these interactions are minimized when calmodulin is saturated with ions such as calcium (Masino, Martin, & Bayley, 2000). GuHCl was used because it does not absorb at the wavelength 222 nm where the data points were taken (Shirley, 1995). Signal at 208 nm was not taken because GuHCl absorbs significantly at this wavelength. Two stock solutions were prepared: (1) 5 mM Tris-HCl buffer, pH 7.6 with Pb-CaM or Ca-CaM, and (2) 6 M GuHCl denaturant with Pb-CaM or Ca-CaM. Protein concentrations ranged from 0.02 mg/ml to 0.03 mg/ml for all samples. About 2 mM of Ca$^{2+}$ or Pb$^{2+}$ was added to each sample to saturate it. This value was calculated from the results of the titration.
experiments when the change in spectra stopped for Pb-CaM. Stock solutions 1 and 2 were combined to prepare samples that contained no denaturant to 6 M denaturant in increments of 0.20 M and 0.50 M. Samples sat for at least 15 minutes at room temperature before analysis to allow for denaturation to occur. Each sample was analyzed in triplicate via CD. Each sample was added and removed from the cuvette using a pipette. The cuvette was not removed from the sample chamber in order to decrease fluctuation in baseline.

**Thermal Denaturation.** Calmodulin was studied with far-UV CD at 208 nm and 222 nm in 5 mM Tris-HCl, pH 7.6. CaM samples were prepared in 5 mM Tris-HCl, and were saturated with 2mM Ca$^{2+}$ or Pb$^{2+}$ each similar to chemical denaturation. The samples were heated from 20°C to 90 °C at an interval of 5 °C /min. The sample temperature was measured with the sample holder jacket. For reversibility, the samples were cooled to the initial temperature of 25 °C. Each sample obtained its initial signal.

**Data Analysis**

The acquired CD data was automatically subtracted from the baseline and digitally filtered using a seven data point smoothing process using the Savitsky-Golay algorithm in the OLIS software. All spectra were exported as ASCII files and imported into Microsoft EXCEL. The signal in millidegrees ($\theta$) was converted to molar ellipticity [$\theta$]$_{mrw}$ in order to account for the concentration of each sample:

$$[$\theta]$$_{mrw} = \frac{(signal(\theta) \cdot MW_{mrw})}{10 \cdot concn \cdot pathlength}$$  \hspace{1cm} (4)

In equation 4, the signal units are millidegrees and the mean residue weight (MW$_{mrw}$) is 112.7 kDa per residue. This was calculated from protein weight and the number of amino
acid residues (16686 kDa/148 residues) (UniProt Consortium, 2014). The units for the concentration of the sample are mg/ml, and the pathlength units are cm. The units for the signal are molar ellipticity, degrees*cm²/dmol. All comparisons of CD signal data were in units of molar ellipticity (ME).

Secondary structure fractions were determined through the CDSSTR program (Sreerama & Woody, 2000) available on the Dichroweb website (Whitmore & Wallace, 2008). The CDSSTR program analyzes CD spectra through the singular value decomposition algorithm. It provides six types of structure. α-helix and β-sheets fall into two types of categories, regular and distorted followed by categories for turns and unordered structure (commonly referred to as random coil). The reference data set SP175 that was used for the CDSSTR analysis contains a CaM structure (Lees et al., 2006). According to the protein circular dichroism databank (PCDDB), CaM contains 56.8% α-helix. This percentage was determined using DSSP, an algorithm that calculates the most likely secondary structure based on the 3D structure of the protein (the crystal structure) (Joosten et al., 2011). The 3D structure (PDB 1LIN) was fitted to the CD spectrum of CaM (Whitmore L et al., 2011).

T-tests (two-tailed) were used to test the significance of the signal change at 208 and 222 nm and the ratio $\theta_{208}/\theta_{222}$ for Ca²⁺ and Pb²⁺ titrations to CaM. The Microsoft Excel Data Analysis package was used for all calculations. If the significance value ($p$) was less than 0.05, then the change in signal was statistically significant.

**Chemical Denaturation.** The overall stability of a protein was determined by the difference in free energy between the folded and unfolded state. A two-state folding mechanism was used to calculate the free energy (Shirley, 1995):
In order to calculate the free energy of the protein, the following equations were used:

\[ f_U = \frac{(y_F - y)}{(y_F - y_U)} \] \hspace{1cm} (5)

\[ K = \frac{f_U}{f_F} = f_U / (1 - f_U) = \frac{(y_F - y)}{(y - y_U)} \] \hspace{1cm} (6)

\[ \Delta G = -RT \ln(K) = -RT \ln[(y_F - y)/(y - y_U)] \] \hspace{1cm} (7)

First the amount of protein unfolded in the presence of denaturant is calculated using equation 5. The amount of protein unfolded is represented by \( f_U \), and \( y \) represents the observed CD signal at 222 nm for the protein fully folded \( (y_F) \) and unfolded \( (y_U) \) and for each concentration of guanidine hydrochloride. In equation 6, \( f_F \) represents the amount of protein folded. The equilibrium constant \( K \) can be calculated by equation 6. Finally \( \Delta G \) is calculated with equation 7. The temperature used was 298 K and the \( R \) value was 1.987 cal/mol*K. To determine the stability of each sample, the data points from the transition area on the curve are graphed, and a line of best fit is calculated. The line of best fit is represented by equation 8:

\[ \Delta G = \Delta G_{H_2O} - m[D] \] \hspace{1cm} (8)

\[ [D]_{1/2} = \frac{\Delta \Delta G_{H_2O}}{m} \] \hspace{1cm} (9)

The denaturant concentration is \([D]\), and \( m \) is slope. By extrapolation back to the y-axis, the equation provides the free energy with no denaturant \( (\Delta G_{H_2O}) \). A larger free energy suggests a more stable protein. The point at which half the protein is unfolded with denaturant \([D]_{1/2}\) is calculated using equation 9.

Previous research indicates that the unfolding of calmodulin follows a two-state mechanism (Moosavi-Movahedi, Naderi, & Farzami, 1994). However, more recently, it
is suggested to be three-state where the intermediate is the C-terminus unfolding followed by the N-terminus (Masino et al., 2000; Protasevich et al., 1997). Other research suggests that calmodulin follows a two-state folding in its holo form and a three-state folding in its apo form (Wang, Liang, Czader, Waxham, & Cheung, 2011). Data was only analyzed using a two-state mechanism based on the suggestion of Wang et al., (2011).

Results

Conformational Stability of CaM

Thermal denaturation was performed to compare Pb-saturated CaM and Ca-saturated CaM. Data were taken at 208 nm and 222 nm in the far-UV CD spectrum. Figure 7 indicates that a loss in signal occurred at 222 nm in both samples. A loss of signal was also seen at 208 nm for both samples (data not shown). The change in signal at 222 nm was greater than 208 nm. The relative signal change between 222 and 208 nm might be explained with reference to Figure 7. The change in the signal at 208 is primarily due to loss of the n →π* negative peak with no anticipated corresponding increase from the random coil signal. The signal change at 222 nm arises due to both loss in negative going signal from the n →π* associated with the α-helix and an increase in the positive n →π* signal associated with the random coil. In practice, however, the relative magnitude of the 208 and 222 peaks is frequently observed to remain constant, as will be discussed below.

Neither sample unfolded completely. The change in signal was less than 7500 molar ellipticity (ME) units when heated to 90 °C. This signal is just more than half of the signal loss (12000 ME) for complete denaturation as seen in the chemical denaturation in Figure 7. Melting temperatures (t_m) for the thermal denatured samples
was not determined, because the samples did not denature completely. The melting temperature for Ca-CaM has been shown to be greater than 90 °C (Brzeska, Venyaminov, Grabarek, & Drabikowski, 1983). This supports why neither sample, Ca-CaM and Pb-CaM, denatured by 90 °C. It also indicates that Ca-CaM and Pb-CaM denatured in a similar fashion.

Figure 7. Thermal denaturation and chemical denaturation for Pb-CaM and Ca-CaM at 222 nm. Thermal denaturation runs are an average of 2 runs each. Chemical denaturation runs are an average of 3 runs each.

Chemical denaturation was also used to compare the stability of calmodulin when saturated with lead or calcium. In the case of chemical denaturation, only the 222 nm signal was detected. As a result, it is not known whether the 222 nm response was greater than the 208 nm response as observed in the thermal denaturation. The samples in the chemical denaturation were completely unfolded by 4.5 M GuHCl. Ca-CaM was more
stable than Pb-CaM. Table 16 shows that the $\Delta G_{H_2O}$ of 2.33 kcal/mol for Ca-CaM was larger than 2.15 kcal/mol of Pb-CaM.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Delta G_{H_2O}$ (kcal/mol)</th>
<th>$[D]_{1/2}$ (M)</th>
<th>$m$ (kcal/mol*M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca-CaM</td>
<td>2.33</td>
<td>2.36</td>
<td>-0.986</td>
</tr>
<tr>
<td>Pb-CaM</td>
<td>2.15</td>
<td>2.29</td>
<td>-0.939</td>
</tr>
</tbody>
</table>

A larger change in free energy indicated more stability in the structure. It was less likely to denature in the presence of Ca$^{2+}$ than Pb$^{2+}$. The amount of denaturant $[D]_{1/2}$ it took to unfold the protein also indicated that the Ca-CaM was more stable than Pb-CaM.

**Pb-CaM vs. Ca-CaM**

The thermal and chemical denaturation data indicated similarities in conformational stability between the Ca-CaM and Pb-CaM ion-protein complexes. The titrimetric CD data, however, suggest that there were differences between the two structures. Titrations of Ca$^{2+}$ and Pb$^{2+}$ were performed separately to compare the effects of the ion on the CaM structure. For analysis, the two peaks of importance were 208 nm and 222 nm. The peak around 190 nm was not used because the signal was too noisy from absorption of the buffer and oxygen. Results from the addition of Pb$^{2+}$ to saturate CaM (Figure 8) compared to Ca$^{2+}$ saturated CaM (Figure 9) show clear differences in the 208 nm signal.

As discussed, an earlier inspection of the CD signals for pure $\alpha$-helix and random coil structures suggest that the 222 nm peak should be more sensitive to changes than the 208 nm peak. In practice, both peaks change in a similar fashion with the relative magnitude remaining constant. In these current data, it is clear that both peaks did not change equally in the Pb-CaM.
Figure 8. CD signal of initial and final Pb$^{2+}$ titration additions to CaM. Pb$^{2+}$ concentrations 0 µM – 12 µM to 0.03 mg/ml “as-is” CaM in 5 mM Tris HCL buffer, pH 7.6

Figure 9. CD signal of initial and final Ca$^{2+}$ titration additions to CaM. Ca$^{2+}$ concentrations 0 µM – 12 µM to 0.03 mg/ml “as-is” CaM in 5 mM Tris HCL buffer, pH 7.6
A ratio of the signal peaks ($\theta_{208}/\theta_{222}$) in molar ellipticity for each CD spectrum produced was calculated for three samples: “as-is” CaM, Pb-CaM and Ca-CaM. The ratio of signals at 208 nm and 222 nm provided information about how the two peaks change in relationship to each other. Ca-CaM and Pb-CaM were saturated with 12 µM Ca$^{2+}$ or Pb$^{2+}$. This concentration was chosen for saturation because it is proposed that ions may bind to CaM at other auxiliary sites than the four EF hand canonical sites (Milos, Schaer, Comte, & Cox, 1989). Thus more than four moles of Pb$^{2+}$ or Ca$^{2+}$ to one mole of protein were required to saturate the canonical sites and any other possible sites on the protein.

The signal at 208 nm was divided by the signal at 222 nm (Figures 8 and 9). In previous research, Sun, Yin, Coffeen, Shea, & Squier, (2001) determined a ratio of 1.03 for CaM saturated with Ca$^{2+}$. In Table 17, the ratio for Pb-CaM (0.88) was much smaller than Ca-CaM (1.01) and the “as-is” CaM sample (1.02). The difference was statistically significant indicated by a t-test. These results indicated that the CD signal of CaM significantly changed when Pb$^{2+}$ was titrated into “as-is” CaM. However, the signal did not change when Ca$^{2+}$ was titrated into to “as-is” CaM.

### Table 17. Paired T-test of $\theta_{208}/\theta_{222}$ ratios for the CD signal of Pb-CaM and Ca-CaM

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean of $\theta_{208}/\theta_{222}$ ratio$^a$</th>
<th>df</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>“as-is” CaM</td>
<td>1.02 (.02)$^b$</td>
<td>3</td>
<td>1.28</td>
<td>.289</td>
</tr>
<tr>
<td>Ca-CaM$^c$</td>
<td>1.01 (.02)</td>
<td>6</td>
<td>18.03</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>Pb-CaM$^c$</td>
<td>.88 (.02)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Each sample was compared against CD spectra with no additions of Ca$^{2+}$ or Pb$^{2+}$, “as-is” CaM. The variation in the “as-is” sample values is caused by the slight variation in the CD signals. $^b$The ratio for CaM is an average of all samples before Ca$^{2+}$ or Pb$^{2+}$ was added to saturate the sample. $^c$samples were saturated with 12 µM Pb$^{2+}$ or Ca$^{2+}$. *statistically significant at p<0.05 standard deviations are in parentheses

The difference in signal from Figures 8 and 9 are compared in Figure 10. The signal change was calculated by subtracting the titrated signal for the titration of Ca$^{2+}$ and Pb$^{2+}$ to “as-is” CaM from the sample signal before any titration was made. It is evident
that the addition of Pb²⁺ to CaM caused a greater change in the CD signal for the 208 nm peak than the Ca²⁺ titration. The change was almost immediate for Pb²⁺ at 1 µM at 208 nm. The signal at 222 nm stayed relatively constant for both Pb²⁺ and Ca²⁺ titrations.

Figure 10. Change in CD signal at 208 nm and 222 nm for Ca²⁺ and Pb²⁺ titrations to CaM from 0 µM to 12 µM. The Pb²⁺ titration is an average of four trials. The Ca²⁺ titration is an average of two trials. A more positive ME indicates that a greater change occurred from the “as-is” CaM sample.

**Analysis of Secondary Structure**

A change in CD signal indicates a change in secondary structure. For example, a less negative signal at 208 nm and 222 nm likely indicates loss of α-helical structure. To determine whether the helical content of CaM decreased with the addition of Pb²⁺, Pb²⁺ and Ca²⁺ saturated CaM sample spectra were analyzed with the CDSSTR analysis method on DiChroweb (Whitmore & Wallace, 2008). The current work compares well with previously calculated data seen in Table 18. The average calculated helical content
for "as-is" CaM was 57.1% and the average random coil content was 18.5% while literature reports 56.8% and 18.9%, respectively (Lees et al., 2006).

Table 18. CDSSTR analysis results of secondary structure for CaM

<table>
<thead>
<tr>
<th>Form of CaM</th>
<th>Total α-helical content (%)</th>
<th>Normal α-helical content (%)</th>
<th>Random coil content (%)</th>
<th>Author and algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaM (apo)</td>
<td>38-40&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>Martin and Bayley (1986), CONTINLL</td>
</tr>
<tr>
<td>Ca-CaM</td>
<td>45-48&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaM</td>
<td>56.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.9</td>
<td></td>
<td>Lees et al., (2006) DSSP</td>
</tr>
<tr>
<td>&quot;as-is&quot;-CaM</td>
<td>57.1 (5.3)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>41.2 (4.0)</td>
<td>18.5 (2.3)</td>
<td>Current Work, CDSSTR</td>
</tr>
<tr>
<td>Ca-CaM&lt;sup&gt;e&lt;/sup&gt;</td>
<td>59.4 (3.8)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.3 (4.9)</td>
<td>18.2 (3.5)</td>
<td></td>
</tr>
<tr>
<td>Pb-CaM&lt;sup&gt;e&lt;/sup&gt;</td>
<td>52.9 (6.4)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>37.8 (2.9)</td>
<td>20.5 (2.6)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>standard deviations in parentheses. <sup>b</sup> CONTINLL calculates only one type of helix. <sup>c</sup>DSSP calculates all regular helix based on the crystal structure. <sup>d</sup>CDSSTR calculates the total helical content which contains both regular and distorted helices. Total secondary structure sums to 1. <sup>e</sup>CaM was saturated with 12 µM Ca<sup>2+</sup> or Pb<sup>2+</sup>.

The difference in helical content percentages found between studies may be attributed to the use of different deconvolution processes, reference data sets to calculate the secondary structure, and the quality and concentration of the sample. In the current study the difference between Ca-CaM and Pb-CaM was 59.4% versus 52.9%. In Table 19, the data indicated that the percentage of Pb-CaM helical content was significantly different than Ca-CaM and "as-is" CaM.

Table 19. T-test results for comparison of helical content in titrated samples of Ca-CaM and Pb-CaM

<table>
<thead>
<tr>
<th></th>
<th>p-value</th>
<th>t-value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca-CaM vs Pb-CaM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.01*</td>
<td>2.144</td>
<td>20</td>
</tr>
<tr>
<td>Ca-CaM vs &quot;as-is&quot; CaM</td>
<td>.38</td>
<td>2.093</td>
<td>19</td>
</tr>
<tr>
<td>Pb-CaM vs &quot;as-is&quot;CaM</td>
<td>.04*</td>
<td>2.080</td>
<td>21</td>
</tr>
</tbody>
</table>

<sup>*</sup>p<0.05 is statistically significant. <sup>a</sup>Equal variances were not assumed.
This data supported the decrease in signal at the 208 nm peak, and the statistically significant difference in the $\theta_{208}/\theta_{222}$ ratio for Pb-CaM when compared to Ca-CaM. Overall, the CD spectral data suggested that there may be a structural change or a rearrangement occurring in Pb-CaM that was not present in Ca-CaM.

**Discussion**

There is considerable research on the biphasic effects of Pb$^{2+}$ on the activation of CaM (Chao, Bu, & Cheung, 1995; Kern, Wisniewski, Cabell, & Audesirk, 2000). Yet it is still not understood how Pb$^{2+}$ might cause these effects on CaM. One hypothesis is that Pb$^{2+}$ binds opportunistically to the areas of the protein other than the canonical EF binding sites based on negative charge density (Kirberger, Wong, Jiang, & Yang, 2013). Although CD spectral data did not provide binding sites for the Pb$^{2+}$ ions, changes present in the CD spectral data suggest that the secondary structure of CaM was affected differently by the binding of Pb$^{2+}$ ions to the protein than the binding of Ca$^{2+}$ ions.

The thermal denaturation data of Pb-CaM compared to Ca-CaM (Figure 7) indicated that there was little difference in the unfolding of the protein. When saturated with either metal, the melting temperature was above 80 °C, which has been presented previously in research (Browne, Strom, Martin, & Bayley, 1997; Brzeska et al., 1983). This indicated little change occurred in the protein. The chemical denaturation data provided additional support that the change is not dramatic, yet that the Ca-CaM has a slightly more stable conformation than Pb-CaM.

Titrimetric CD data showed the greatest difference between Ca-CaM and Pb-CaM. CD spectral data depicted a greater change in signal at the 208 nm negative peak with no change in the 222 nm negative peak (Figure 8). This change occurred only when
“as-is” CaM was titrated with Pb$^{2+}$ as compared to Ca$^{2+}$. Titrimetric CD data with cadmium, zinc and tin were completed as well (data not shown), and no change in CD spectra was evident when compared with Ca-CaM. The $\theta_{208}/\theta_{222}$ ratio of peaks for Pb-CaM (0.88) was determined to be significantly different than either Ca-CaM (1.01) or “as-is” CaM (1.02). A ratio value of one indicates that the peak at 208 nm has the same signal as the peak at 222 nm and the protein contains mostly helical content. For ratios that are significantly less than one as seen in the Pb-CaM samples, causes are less understood (Paulucci et al., 2002). Libante, Thion and Lane (2001), in a study of the binding of ATP to SopA proposed that a loss of signal at 208 nm indicated a decrease in the proportion of $\alpha$-helical structure to $\beta$-sheet structure. The smaller ratio has been attributed to more random coil, interaction of helices, and rearrangement of helices (Lawton et al., 2002; Woolley & Wallace, 1993).

The present data exhibited an increase (less negative ME) in the CD spectrum (Figure 7) at 208 nm when Pb$^{2+}$ was bound to CaM, and this may indicate more random coil structure, which has a negative CD signal at 195 nm. This is supported by the analysis from the CDSSTR deconvolution program in Table 18. The results showed that as $\alpha$-helical structure decreased in Pb-CaM by 4.2%, there was a 2% increase in random coil structure when compared to “as-is” CaM. Ca-CaM showed an increase of 2.3% in $\alpha$-helical structure. The data in Table 19 indicated that $\alpha$-helical structure was significantly less in Pb-CaM than in Ca-CaM. NMR and crystal structure data support an increase in random coil structure (Kirberger et al., 2013; Wilson & Brunger, 2003). The change in random coil structure may occur outside of the EF hands in areas closer to the linker (residues 76-84). The crystal structure 1n0y in Figure 3 (Chapter 6) shows seven
additional Pb\(^{2+}\) ions bound to sites outside of the EF hands closer to the linker region (Wilson & Brunger, 2003). The theory of opportunistic binding proposes that Pb\(^{2+}\) ions bound in areas of negative charge on CaM. The linker region contains several glutamic acid and aspartic acid residues that commonly bind Pb\(^{2+}\) ions. The linker region may have more random coil if Pb\(^{2+}\) ions are bound in that region similar to the binding of target proteins (Kirberger et al., 2013).

Recently with the use of NMR data, research showed that Pb\(^{2+}\) binding to CaM may cause a conformational change that is similar to a target protein binding to calmodulin (Kirberger et al., 2013). When binding a target protein, the conformation of CaM becomes closed and the domains are closer together with a bend in the linker. Areas of the protein become hydrophobic. The hydrophobicity of the environment around the protein can affect the ratio of \(\frac{\theta_{208}}{\theta_{222}}\) (Rodger & Nordén, 1997). Paulucci et al., (2002), proposed that solvent effects or conformational changes in helices would affect the ratio. Several studies note that the addition of an organic solvent trifluoroethanol (TFE) increased helical content (Bayley, Martin, & Jones, 1988; Dunlap, Kirk, Pena, Yoder, & Creamer, 2013) and the ratio of \(\frac{\theta_{208}}{\theta_{222}}\) (Paulucci et al., 2002). Theoretical modeling of the 208 nm transition suggested that interaction of helices contributed to the conformational change (Bode & Applequist, 1997). These previous results taken together with the current results indicate that there was a loss of helical structure in Pb-CaM corresponding with an increase in a more hydrophobic environment around the remaining helices.


**Conclusion**

Changes in CD signal were observed when less than 1 µM Pb$^{2+}$ bound with CaM to saturated concentrations of Pb$^{2+}$. The CD signal and deconvolution data supported that α-helical content decreased as random coil content increased when Pb$^{2+}$ bound to CaM. These results provided further support that Pb$^{2+}$ has unique characteristics when interacting with CaM when compared with other metals including Ca$^{2+}$, Cd$^{2+}$, or Mg$^{2+}$. Although, CD is an excellent technique to probe the secondary structure, further research is necessary to understand why the signal change occurred. Experiments to show why the Pb-CaM gave a small ratio of $\theta_{208}/\theta_{222}$ when compared to Ca-CaM are needed. Further data to support the small ratio may come from studies on the hydrophobic areas of the protein, how the helices on the protein change in relationship to each other, and examination of the tertiary structure of CaM. Molecular dynamics studies may provide more information on the increase in random coil, where it occurs on the protein, and if it is associated with an increase in the hydrophobic domains.
CHAPTER EIGHT

STUDY 2: MOLECULAR DYNAMICS MODELING OF Pb$^{2+}$ BINDING TO CaM

Introduction

There has been considerable research to understand what happens when lead binds to calmodulin (CaM) as well as research to understand where Pb$^{2+}$ binds on CaM. To date, the interaction of Pb$^{2+}$ and CaM has not been explored through molecular dynamics simulations. Most simulations have been used to determine how Ca$^{2+}$ and other ions including magnesium, strontium and lanthanum bind to CaM (Lepšík & Field, 2007) as well as the effects of binding on the conformation of the CaM structure (Fiorin, Biekofsky, Pastore, & Carloni, 2005; Komeiji, Ueno, & Uebayasi, 2002; van der Spoel, de Groot, Hayward, Berendsen, & Vogel, 1996). Generally the interest has been in the conformation of calmodulin in solution (Shepherd & Vogel, 2004; Wriggers, Mehler, Pitici, Weinstein, & Schulten, 1998) because the crystal structures do not accurately depict the flexible nature of the central linker (Kuboniwa et al., 1995). Molecular dynamics modeling was used to predict the location of Pb$^{2+}$ binding sites on CaM and to investigate how Pb$^{2+}$ binds to CaM.

The technique that was used in this study allowed for the CaM protein structure to interact with randomly placed Pb$^{2+}$ ions in the simulation water box.
Pang et al., (2013) used fragment homology modeling and molecular dynamics to predict Ca\textsuperscript{2+} binding sites on CaM. That study randomly placed about ten Ca\textsuperscript{2+} ions around individual binding site from calcium binding proteins in the simulation. They were able to correctly predict Ca\textsuperscript{2+} binding sites about 87.7% of the time, although they found that it was essential to place the Ca\textsuperscript{2+} ions in energy minimized areas around the binding sites to accurately predict the sites. Another study predicted the Ca\textsuperscript{2+} binding sites on calcium binding protein including CaM by using the MUG\textsuperscript{SR} algorithm with side chain torsional rotation (Wang et al., 2010). The MUG\textsuperscript{SR} algorithm finds oxygen clusters and then calculates a calcium center near those groups based on certain filters. If sites are eliminated the side chains are rotated to determine if they might fit a calcium center. This method had 78% accuracy with three correct residue hits within a site. Another study has predicted Ca\textsuperscript{2+} binding sites on calcium binding proteins by using the carbon atoms near the oxygen atoms that bind Ca\textsuperscript{2+} with high accuracy (Zhao et al., 2012). These simulations and algorithms have successfully predicted binding sites for calcium on calcium binding proteins.

**Research Goals**

The goals in the molecular dynamics study were: (1) to predict Pb\textsuperscript{2+} binding sites on CaM and the degree of accuracy in binding, (2) to gain insight into how Pb\textsuperscript{2+} binds to CaM, and (3) to investigate how Pb\textsuperscript{2+} binding affects the flexibility and structure of CaM.

**Methods**

The protein structure for holo Pb-CaM (PDB: 2v01; Kursula & Majava, 2007) was acquired from the Protein Data Bank (Berman et al., 2000). A charge was added to
His residue 107 HSE visually after assessing its interactions with surrounding carboxylates. The molecule was prepared for the simulation using the molecular graphics program, VMD 1.9.1 (Humphrey, Dalke, & Schulten, 1996). The 2v01 protein structure and 100 randomly added Pb$^{2+}$ ions were placed in a TIP3+ water box that extended 20 Å past the protein. The total number of atoms in the box was about 370,000. The charge in the simulation was neutralized with 172 sodium ions and 348 chloride ions. In the protein structure (2v01), the eight Pb$^{2+}$ ions in the original structure were removed.

The equilibration procedure involved energy minimization with and without restraints on the protein coordinates (3000 steps each) with a conjugated gradient algorithm using the CHARMM27 force field (MacKerell et al., 1998) and the NAMD molecular dynamics (MD) program (Phillips et al., 2005). This was followed by slow heating from 10 to 310 K (30,000 steps), and then pressure and temperature equilibration using a Langevin piston (10,000 steps). Finally, unrestrained dynamics for 30,000 steps were done before data acquisition. The time step was 2 fs. Every 150th step was saved in the trajectory for analysis. Periodic boundary conditions were used. For long-range electrostatic potential, the Particle Mesh Ewald technique was applied. The cutoffs for nonbonding (van der Waals and electrostatic) interactions were 12 Å. The switch distance was 10 Å, and 1.0 1–4 scaling factor was used. This process constitutes equilibration of the structure. A production MD simulation was then run for 60 ns using the same parameters. Due to the size of the box, it took about 2 days per nanosecond. A reference simulation was run for 10 ns using the same procedure with the structure of 2v01 with the eight Pb$^{2+}$ and one Ca$^{2+}$ ions retained.
All diagrams were generated using VMD 1.9.1. Visual analysis of results was performed using VMD. Each interaction between a Pb\textsuperscript{2+} ion and residue was recorded. A bonding interaction was considered to occur at distances less than three angstroms between the Pb\textsuperscript{2+} ion and an atom of the protein. Data included the residue, the type of atom on the residue with which Pb\textsuperscript{2+} interacted, and the time that interaction lasted. An interaction longer than 5 ns was considered to be permanent in the simulation. Interactions between 1 ns and 5 ns were considered transient binding sites. All interactions less than 1 ns were not considered for analysis for two reasons. Some Pb\textsuperscript{2+} ions were expected to be near the protein yet the electrostatic interaction for these Pb\textsuperscript{2+} ions with residues would be too small for the binding to be permanent. A second reason was some of these sites were not oxygen atoms, but hydrogen or carbon atoms that were unlikely to permanently bind a Pb\textsuperscript{2+} ion. Non-bonding electrostatic and van der Waals interaction energies were calculated for each Pb\textsuperscript{2+} that bound to the protein using NAMD.

Root Mean Standard Deviation (RMSD) of the protein and Root Mean Square Fluctuation (RMSF) per residue of the alpha carbons on the main chain were calculated for the protein. RMSD is the standard deviation of the protein conformation from a reference conformation for each time step in the simulation. In this simulation, the reference simulation is the protein in the first time step of the simulation. RMSF values are the standard deviations of the RMSD values over the time of the simulation. RMSF accounts for the magnitude of change (fluctuation) in each residue when compared to the average change of that residue.

The resulting Pb-CaM structure (based on 2v01) from the simulation was compared to the two X-ray crystallography (XRC) structures of Pb-CaM (2v01 and 1n0y)
available in the PDB. The binding sites in these two structures, 1n0y and 2v01, were determined by measuring distances between a Pb\(^{2+}\) ion and residues using VMD. Again only interactions less than or equal to 3.0 Å was considered to be bound.

**Results**

**Permanent Binding vs. Transient Binding**

Pb\(^{2+}\) ions bound at sites both permanently and transiently. The time in which the Pb\(^{2+}\) ions spent within 3 Å of a residue initially determined whether the site was permanent or transient. An analysis of the non-bonding interaction energies (electrostatic and van der Waals) of the Pb\(^{2+}\) ions provided detailed information on the type of binding. The electrostatic interaction energy is displayed for three Pb\(^{2+}\) ions that bound over the course of the simulation. Only electrostatic interaction energy is shown because the calculated van der Waals was insignificant compared to the electrostatic energy (see appendix I). Electrostatic forces are likely to dominate because the Pb\(^{2+}\) ion holds a 2\(^{+}\) charge, and the oxygen atoms to which a Pb\(^{2+}\) ion might bind hold a partial negative charge. Table 20 displays the characteristics of three example bound Pb\(^{2+}\) ions.

**Table 20. Characteristics for three example Pb\(^{2+}\) ions that bound to CaM**

<table>
<thead>
<tr>
<th>Pb(^{2+}) ion Code</th>
<th>Residues (Time in simulation)</th>
<th>Region</th>
<th>Type of bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>1H</td>
<td>Glu 84 (6.8 ns) Asp 80 (31.3 ns) Glu 7 (55.8 ns)</td>
<td>Linker</td>
<td>Permanent</td>
</tr>
<tr>
<td>3H</td>
<td>Lys 21 (9.9 ns for 1.8 ns on protein)</td>
<td>EFI</td>
<td>Transient</td>
</tr>
<tr>
<td>12H</td>
<td>Asp 131 (33.5 ns) Asp133 (34.9 ns)</td>
<td>EFIV</td>
<td>Permanent</td>
</tr>
</tbody>
</table>
The two distinctions between permanent and transient sites are presented in Figure 11. First, the electrostatic interaction energy for transient binding was less negative than \(-300\) kcal/mol for an interaction. The second difference was the interaction energy returned to zero for a \(\text{Pb}^{2+}\) ion transiently binding, like \(3\text{H}\). Permanent binding \(\text{Pb}^{2+}\) ions, \(1\text{H}\) and \(12\text{H}\), increased in energy as more bonds were made.

![Figure 11](image.png)

Figure 11. Electrostatic interaction energy for comparison of one transient (3H) and two permanent (1H and 12H) \(\text{Pb}^{2+}\) binding sites. 3H binds in EFI, 12H binds in EFIV and 1H binds in the linker region. The line at \(-300\) kcal/mol is a cut-off for transient binding sites.

3H had an initial binding energy of \(-205.3\) kcal/mol. It was, however, unable to capitalize on the initial binding energy. It was transiently bound to Lys 21 found in EFI from 9.9 ns to 11.7 ns. The common residues to which \(\text{Pb}^{2+}\) binds in this EF hand are Asp 20, Asp 22, Asp 24, Thr 26 and Glu 31, and the transient binding occurred between two of these residues. It was not until 43 ns that another \(\text{Pb}^{2+}\) ion permanently bound to Asp 20, Thr 26 and Glu 31 at this site. It is possible that the hand was not properly formed to allow the \(\text{Pb}^{2+}\) to easily bind until more changes occurred in the structure of the protein.
A permanent site tended to have a more negative initial interaction energy. Consider, the Pb\(^{2+}\) ion, 1H. This was the third Pb\(^{2+}\) ion in the simulation to bind after one each in EFI and EFIV. 1H bound Glu 84 in a bidentate fashion quickly at -303 kcal/mol and -429 kcal/mol. The interaction energy decreased to -680 kcal/mol when Asp 80 bound. These first two events occurred in the linker region (Met 76 to Glu 84). In the simulation, the binding occurred very quickly starting at 6.8 ns with Glu 84. The Pb\(^{2+}\) ion later bound Asp 80 at 31.3 ns. It is likely that the large time difference between these two residues is due to conformation. The Pb\(^{2+}\) ion may not have been close enough to any other residue until the linker was bent more (see next section). Finally, it bound Glu 7 close to the end of the simulation at 55.8 ns dropping the interaction energy to -824 kcal/mol. The residue Glu 7 is not in the linker region. It is outside of EFI near the C-terminus. This bond could only have occurred because of a conformational change in the protein. The C-terminus was close enough to the linker to have bound the Pb\(^{2+}\) ion, 1H.

Although permanent sites generally had larger negative energy values, this was not always the case. Consider the case of Pb\(^{2+}\) ion, 12H. 12H bound Asp 131 at 33.5 ns with a much lower initial energy of -203 kcal/mol, yet within 1 ns, Asp 133 bound with -334 kcal/mol and finished binding the other oxygen on Asp 131 with -506 kcal/mol at 37.1 ns. These residues bound in quick succession for a dramatic decrease in electrostatic interaction energy. It appears that some Pb\(^{2+}\) ions such as 12H wait until a suitable binding spot opens up before permanent binding occurs.

**Peptide Residues Binding Pb\(^{2+}\) on CaM**

Each of the canonical [EF hands] sites contains a number of residues that contribute to binding. Consequently, both sites and residues within those sites
characterized binding. The results showed that Pb$^{2+}$ ions bound in several sites in addition to the canonical sites. Over the course of 60 ns simulation, 16 Pb$^{2+}$ ions of the 100 Pb$^{2+}$ ions in the water box interacted with 26 residues on the protein. Many of the Pb$^{2+}$ ions bound to more than one residue on the protein. A total of 12 Pb$^{2+}$ ions were permanently bound to 12 sites. Within those sites, a total of 21 residues were bound. It was found that 14 residues (58%) bound to seven Pb$^{2+}$ ions matched with the XRC structures, 1n0y and 2v01. Seven residues sites permanently bound with five Pb$^{2+}$ ions (27%) not found in the XRC structures. There were four transient sites each with one Pb$^{2+}$ bound (15%) each not confirmed with XRC structure data.

The residues that do not match XRC structural data were not unusual. Most unconfirmed residues were either Glu or Asp on the protein generally just outside of a canonical EF binding site. The area that had the largest concentration of unconfirmed residues binding was in EFIV from Asp 118 to Ala 128 where one Pb$^{2+}$ ion bound at Glu 119, and one at Glu 123 and Glu 127. A Pb$^{2+}$ ion also bound outside of EFII at Asp 50 and Glu 54.

The most common residues bound to Pb$^{2+}$ ions were Glu and Asp followed by Asn and Gln, Thr, Met, Lys and Ser indicated in Table 21. The order represented is supported by a previous PDB analysis of the most common Pb$^{2+}$ binding sites to side chain residues with available oxygen atoms (see last column in Table 21) (Kirberger & Yang, 2008). The confirmed sites had a larger number of Asp residues bound than Glu residues. In the unconfirmed binding sites, there was a greater number of Glu residues over Asp residues. The transient sites consisted of residues that do not commonly bind with Pb$^{2+}$ (Gln 143, Asn 42, Ser 17, and Lys 21). These residues were either just outside
of EF hand binding sites or in an EF binding site but not associated with the residues that are commonly bound to Pb$^{2+}$. The percentage of residues that permanently bound to Pb$^{2+}$ that were found in the canonical hands was 36%.

Table 21. The type of residues bound to Pb$^{2+}$ ions

<table>
<thead>
<tr>
<th>name of residue</th>
<th>total binding residues</th>
<th>confirmed binding residues</th>
<th>unconfirmed binding residues</th>
<th>transient binding residues</th>
<th>Literature (Kirberger &amp; Yang, 2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glutamic acid</td>
<td>38% (10)</td>
<td>40% (6)</td>
<td>57% (4)</td>
<td></td>
<td>63%</td>
</tr>
<tr>
<td>aspartic acid</td>
<td>35% (9)</td>
<td>47% (7)</td>
<td>29% (2)</td>
<td></td>
<td>33%</td>
</tr>
<tr>
<td>asparagine</td>
<td>8% (2)</td>
<td>7% (1)</td>
<td>25% (1)</td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td>glutamine</td>
<td>4% (1)</td>
<td>7% (1)</td>
<td>25% (1)</td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>threonine</td>
<td>4% (1)</td>
<td>7% (1)</td>
<td></td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>methionine</td>
<td>4% (1)</td>
<td></td>
<td>14 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lysine</td>
<td>4% (1)</td>
<td></td>
<td>25% (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>serine</td>
<td>4% (1)</td>
<td></td>
<td>25% (1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes. # of residues bound to a Pb$^{2+}$ ion are in parentheses

All the residues to which Pb$^{2+}$ bound contained oxygen atoms as main chain carbonyls or side chain carbonyl functional groups. Majority (86%) of the bonds to oxygen atoms were bidentate. For example, a lead ion might bind to three oxygen atoms on three residues, but also have six bonds because of the bidentate nature. Kirberger and Yang (2008) estimated 78% of Pb$^{2+}$ ions bound to oxygen atoms in PDB structures was bidentate.

Prediction of Pb$^{2+}$ Binding Sites with Simulation

Two XRC structures exist for Pb-CaM in the Protein Data Bank, 1n0y and 2v01. The simulation results were compared with these two structures to determine whether the MD simulation predicted Pb$^{2+}$ binding sites accurately. These results showed that Pb$^{2+}$ ions were bound to four of the seven confirmed sites in 2v01 (57%), and eight of eleven
sites in 1n0y structure (73%). The simulation matched four (EFI, EFII, EFIV and Glu 14) of the five common sites represented in both structures. In the time frame of the simulation, the canonical binding site of EFIII was not filled by a Pb\(^{2+}\) ion although it was bound in both XRC structures. A Pb\(^{2+}\) ion bound at Glu 87 which is in the EFIII region but closer to the linker region than the EFIII canonical binding site. Both XRC structures contain Pb\(^{2+}\) binding sites that are unique. The 2v01 structure has two Pb\(^{2+}\) ions that are bound at Asp 118 and Asp 122. The simulation showed two Pb\(^{2+}\) ions bound to Glu 119 and Glu 123 instead of Asp 118 and Asp 122. The simulation matched with the 1n0y structure for three Pb\(^{2+}\) bound to Asp 80, Glu 83, and Glu 84 in the linker region and an additional Pb\(^{2+}\) bound to Glu 7 outside of the EFI binding region.

Table 22. Percentage of residues in confirmed sites matching XRC structures

<table>
<thead>
<tr>
<th>XRC structure</th>
<th>Confirmed residues matches</th>
<th>Total residue sites on XRC structure</th>
<th>% of confirmed residue /total residue sites on XRC structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1n0y</td>
<td>15</td>
<td>32</td>
<td>47%</td>
</tr>
<tr>
<td>2v01</td>
<td>8</td>
<td>24</td>
<td>33%</td>
</tr>
</tbody>
</table>

The residues within all sites that Pb\(^{2+}\) ions bound were compared. Table 22 shows that the simulation matched 47% of residues within the eight confirmed binding sites in the 1n0y structure. In the 2v01 structure, 33% of the residues within the four binding sites matched.

Order, Binding, and Conformational Change

The manner in which total Pb\(^{2+}\) binds to CaM is described in this section. Figure 12 displays how the total number of Pb\(^{2+}\) bound changed over the time of the simulation. The number of Pb\(^{2+}\) ions bound to CaM increased steadily up to seven for the first 30 ns.
In the same time frame, the protein structure changed minimally from 4 to 6 Å, according to RMSD data. The RMSD is the change in the overall protein structure for each time point as compared to the first time point in the simulation.

A plateau from 30 – 42 ns ensured at seven Pb$^{2+}$ bound. In the first part (30-35 ns) of that time that structure of the protein is seen to undergo a major conformational change according to the RMSD change from 6 to 12 Å. From 35 ns to the end of the binding plateau (41 ns) little structural change is observed. At 42 ns, the protein relaxes slightly (RMSD change from 12 to 10 Å) at which point, four Pb$^{2+}$ are bound in rapid succession for a total of eleven Pb$^{2+}$. The binding initiates more structural change with the RMSD rising from 10 to 13 Å. A dramatic increase in Pb$^{2+}$ bound from seven to eleven occurred at 42 ns. By 50 ns, the number of Pb$^{2+}$ bound was 12 and remained at 12 until the end of the simulation at 60 ns.

Figure 12 showed that Pb$^{2+}$ ions bind to regions of CaM (N-domain, C-domain and linker) in the following order: EFII (N), EFIV (C), linker, EFII (N), EFI (N), linker, EFIV (C), EFI (N), Linker, EFIV (C), EFIV (C). No binding occurred in the canonical EFIII hand although one Pb$^{2+}$ was bound just outside the linker region in EFIII at Glu 87 initiating the rapid binding of four Pb$^{2+}$ including itself. The sequence may be random, but it is interesting to note that the sequence is consistent with literature about binding occurring primarily in the N-domain and ending primarily in C-domain (Celio, Pauls, & Schwaller, 1996; Kirberger, Wong, Jiang, & Yang, 2013).
Figure 12. a. Total Pb$^{2+}$ bound to CaM, b. RMSD of the Pb-CaM structure during simulation. Each vertical line indicates a new Pb$^{2+}$ ion that bound to a site during the simulation. N-domain: EFI: 8 – 43, EFII: 44 – 75, Linker: 76 – 84, C-domain: EFIII: 85 – 116, EFIV: 117 – 148. The Pb-CaM structure in pictures at 30 ns and 40 ns in simulation. C-terminus starts on upper right of protein structure.

In an effort to further understand how Pb$^{2+}$ may affect the structure of CaM, the flexibility of the protein was analyzed. The RMSF is the change in each residue as
compared to the average fluctuations for that residue over the time frame of the
simulation. Figure 13 shows how each residue over the course of the simulation was
affected by Pb\textsuperscript{2+} binding. The structure in the reference simulation showed little change
in flexibility of the chain over the course of 10 ns. In the 60 ns simulation, the flexibility
of the residue chain changed in several regions of the protein as shown by increased
RSMF values. In Figure 13, the greatest change occurred in EFIV, EFI, and between EFI
and EFII. There was less change in the linker and EFIII. It was anticipated that the
change in flexibility would occur in all the regions in which Pb\textsuperscript{2+} ions bound. The RSMF
data suggested that the flexibility of EFIV changed significantly during the simulation.

![Figure 13. RMSF of Pb-CaM structure in the 60 ns simulation and 10 ns reference simulation. Binding sites: EFI: Asp 20 to Leu 32, EFII: Asp 56 to Phe 68, Linker: Met 76 to Glu 84, EFIII: Asp 93 to Leu 105, EFIV: Asp 129 to Phe 141.]

The number of Pb\textsuperscript{2+} ions at the end of the simulation around the EFIV binding site
was five although only one was bound in the canonical binding site. These ions that
bound may have decreased the flexibility of EFIV over the simulation.
The region between EFI and EFII had one Pb$^{2+}$ ion bound. The change in flexibility in this region may be attributed to the Pb$^{2+}$ ion bound that bound residue Asp 50 at 1.4 ns, or it may be caused by a Pb$^{2+}$ ion that first bound Asp 58 in EFII at 11.1 ns followed by a Pb$^{2+}$ ion that first bound Asp 20 at 43.3 ns. According to Figure 13, there is greater change in the RSMF of residues in the N-domain (EFI and EFII) than the C-domain (EFIII and EFIV). This indicated that the N-domain had more flexibility than the C-domain over the course of the simulation.

**Discussion**

This research with the Pb-CaM complex through molecular simulations provided the very first simulation of the Pb-CaM interaction and described where and how Pb$^{2+}$ bound to CaM and how it affected the flexibility of the protein structure. In addition, the simulation provided a new method in which binding sites may be predicted using molecular dynamics.

The simulation was able to predict known binding sites of Pb$^{2+}$ ions to CaM in both XRC structures. It extends research in the area of prediction modeling of binding sites in calcium binding proteins. To date most of prediction modeling has revolved around calcium binding in calcium binding proteins (Pang et al., 2013; Wang et al., 2010; Zhao et al., 2012). There is some previous research that predicted Pb$^{2+}$ binding to osteocalcin with genetic algorithm molecular dynamics. That study predicted that Pb$^{2+}$ ions bound to oxygen atoms on Gla residues, which was supported by NMR and CD data (Dowd, Li, & Gundberg, 2008). This method in this study was similar to Pang et al., (2013) in which they randomly placed Ca$^{2+}$ ions around binding sites in eighteen crystal structures to predict the binding sites and residues within those sites. They found 87.8%
accuracy with the binding sites and 71.1% with residues within those sites when compared to the crystal structures. The method in this study was 57% accurate with the 2v01 XRC structure and 73% accurate with the 1n0y structure for binding sites and 47% and 33%, respectively for residues within those binding sites. Although, the accuracy is lower, this is likely because Pb\(^{2+}\) ions were placed randomly in the water box with the entire protein rather than near specific binding sites like in the work of Pang et al. This method shows promise as a simple method to predict binding sites with ligands on a protein.

The binding sites and the specific residues to which Pb\(^{2+}\) ions bound matched XRC structural data. The simulation matched three of the four canonical hand sites to which Pb\(^{2+}\) ions are known to bind (Kirberger et al., 2013). The EFIII canonical binding site never bound a Pb\(^{2+}\) ion in contrast to XRC structural data. However, a deeper analysis of the occupancy in the crystal structure sites for 1n0y shows a much lower occupancy of 0.3 than the unity occupancy of the remaining EF hands (Wilson & Brunger, 2003). Other previous research indicated issues with metal binding in EFIII. In the analysis of the solution structure of 1cfd, apo-CaM, the averaging process for the C-domain and specifically EFIII provided poor and hard to interpret NMR NOE data (Kuboniwa et al., 1995).

There were sites found in the simulation that were unique to either 2v01 or 1n0y XRC structure. When compared to the 2v01 structure, there were two residues in the simulation bound to Glu 119 and Glu 123 instead of Asp 118 and Asp 122 in the 2v01 XRC structure. There are several reasons why the bound residues in the simulation do not exactly match those in the 2v01 structure. First, Pb\(^{2+}\) is more attracted to Glu (40%) over
Asp (20%) as indicated in the Protein Data Bank analysis of atoms that most commonly bind to Pb\(^{2+}\) (Kirberger & Yang, 2008). Secondly, Glu is longer than Asp and may contribute to Pb\(^{2+}\) interacting with Glu first if the conformation of the protein is more closed in this region of the protein. Finally, the simulation allows for a moving protein whereas a crystal structure may have packing forces that drive the Pb\(^{2+}\) ions to interact differently than they might in solution. Other simulations of Ca-CaM have suggested that crystal packing forces contributed to the differences between modeling and crystal structures (Fiorin et al., 2005; Wriggers et al., 1998).

Another area where the simulation showed unique binding sites to one of the XRC structures was the linker region found in the 1n0y structure. It is proposed that Pb\(^{2+}\) ions bind in the linker region (Met 76 to Glu 84). The linker region contains three Glu and two Asp residues within the nine residues. The data not only support a binding site in the linker, but also suggest that there may be at least two Pb\(^{2+}\) binding sites. Previous NMR research indicated dramatic changes in the linker region when Pb\(^{2+}\) was added to Ca-CaM. That study attributed the dramatic changes at Asp 78, Asp 80, Ser 81, Glu 82, Glu 83 and Glu 84 as an addition of a binding site in the linker region (Kirberger et al., 2013). In addition, previous research suggested a metal binding site in the linker region based on the disappearance of NMR signals (Bertini, Gelis, Katsaros, Luchinat, & Provenzani, 2003). This is strengthened by data from the XRC structure of 1n0y in which three Pb\(^{2+}\) ions were bound at 76, 80, and 82 and 83, 87 (Wilson & Brunger, 2003). The three Pb\(^{2+}\) binding sites in the simulation were at residues 78, 80, 83, and 84. In the 2v01 structure, although a Pb\(^{2+}\) was not bound in the linker, there was a Ca\(^{2+}\) ion bound at residues 74 and 78 (Kursula & Majava, 2007). These data not only confirmed earlier
work that metal ions can bind to auxiliary sites on CaM outside the canonical EF hands (Milos, Schaer, Comte, & Cox, 1989), but they also supported the theory of opportunistic binding of Pb$^{2+}$ to CaM. Opportunistic binding does not displace an ion, but binds through electrostatic interactions with residues with oxygen atoms like Glu and Asp (Kirberger et al., 2013).

Other previous data show that there are significant changes between residues Thr
117 and Arg 126 (Kirberger et al., 2013; Kursula & Majava, 2007). The RMSF data in
Figure 13 indicated that there is greater flexibility in this area for Pb-CaM. RMSF data
also suggested that the linker region (Met 76 to Glu 84) was flexible. Kuboniowa et al.,
(1995) proposed that the linker was highly flexible in solution in the analysis of the apo-
CaM. In the analysis of the crystal structure of 1n0y, the electron density of the linker
could not be determined, which suggested that it is flexible (Wilson & Brunger, 2003).
Other research using simulations of Ca-CaM found that the linker bent and became less
α-helical (Wriggers et al., 1998; Yang, Jas, & Kuczera, 2004). The simulation data as
seen in Figure 12 showed that the linker changed and bent bringing the two domains
closer together. This again supports the theory of opportunistic binding where Pb$^{2+}$ ions
bind to close off the linker region where target proteins should bind.

Pb$^{2+}$ ion binding to CaM affected the structure and stability. The RMSF data in
Figure 13 showed that the C-domain was more stable than the N-domain. A more stable
protein is indicated by less change in the fluctuations for residues. Computational
modeling with Ca-CaM presented that the N-domain was less stable than the C-domain
(Shepherd & Vogel, 2004). Chemical and thermal denaturation of the Ca$^{2+}$ bound
domains with fluorescence and CD also provided evidence that the C-domain was more stable (Masino, Martin, & Bayley, 2000).

Tyrosine fluorescence and NMR chemical shifts from previous research provided evidence that Pb\(^{2+}\) displaces Ca\(^{2+}\) in EFI and EFII; however Pb\(^{2+}\) does not displace Ca\(^{2+}\) in EFIII or EFIV (Kirberger et al., 2013). The simulation data support the binding in EFI and EFII, however, Pb\(^{2+}\) bound in EFIV as well. The simulation involved a holo protein (Pb-CaM) that had original Pb\(^{2+}\) ions removed before the addition of free Pb\(^{2+}\) ions to the water box. The removal of the original Pb\(^{2+}\) ions allowed the protein structure more flexibility as seen in Figure 13 perhaps making the structure more similar to the apo-CaM. NMR data support that in apo-CaM, a Pb\(^{2+}\) ion binds to EFIV first, followed by binding at EFI, EFII and EFIII (Kirberger et al., 2013).

**Conclusion**

Often experimental studies have had problems with Pb\(^{2+}\) precipitating at larger concentrations (Kirberger et al., 2013; Ouyang & Vogel, 1998; Shirran & Barran, 2009), this research provided a new way to look at the Pb-CaM interaction without those experimental issues in molecular dynamics modeling. The results not only support previous research that proposed that Pb\(^{2+}\) ions bound outside of the canonical hands, but it also described how Pb\(^{2+}\) bound to CaM and how it affected the flexibility of the protein structure. The research supported the theory of opportunistic binding in which Pb\(^{2+}\) ions bound to the linker and auxiliary sites and may inhibit CaM from binding with target proteins and contributing to Pb\(^{2+}\) toxicity.

Limitations to the research include the comparison of simulation data with that of experimental Pb\(^{2+}\) ion displacement data. Although the simulation did not show
displacement of an ion by Pb, the results of binding sites and order of binding are relatively consistent. The time scale of the simulation was 60 ns. This is a long simulation, but many of the sites with Pb$^{2+}$ bound only had one or two residues bound to the Pb$^{2+}$ atom. Analysis of the XRC structures suggests that four to six bonds are available. A longer simulation would likely increase the accuracy of the prediction results.

**Future Research**

With the current simulation data, code will be written to monitor the hydrophobicity changes near the linker and in the EF hands with time, similar to the data obtained for the RSMD. Further more the amount of the α-helix present will be obtained by running the structural (PDB) coordinates from the simulation through secondary structure analysis data packages like DSSP in a manner similar to that used in analyzing the CD data. This will help to validate and confirm the changes in α-helix content observed in CD. To date, a second study simulating Pb$^{2+}$ binding to apo-CaM has been performed. Although most CaM in the cell has some amount of calcium in it, how Pb$^{2+}$ ions bind to the apo form may suggest why Pb$^{2+}$ binds to certain auxiliary sites. Future research would seek to answer whether the major conformation change around 31 ns occurs in Ca-CaM compared to Pb-CaM, and whether the same type of binding triggers the change in conformation. This could be achieved by running a simulation with Ca$^{2+}$ similar to that of Pb$^{2+}$. Additional simulations of thermal denaturation of Ca-CaM and Pb-CaM may be performed to compare with experimental data from thermal denaturation using circular dichroism. These data would provide the stability and conformational changes that occur with Pb-CaM.
CONSENT TO PARTICIPATE IN RESEARCH

Project Title: A study of metacognitive awareness and regulation of chemistry majors through the use of the Science Writing Heuristic as an explicit metacognitive strategy

Researcher(s): Mary van Opstal

Faculty Sponsor: Dr. Patrick L. Daubenmire

Introduction:
You are being asked to volunteer to take part in a research study being conducted by Mary van Opstal for a dissertation under the supervision of Dr. Patrick L. Daubenmire in the Department of Chemistry at Loyola University of Chicago.

You are being asked to participate because you enrolled in the spring section of Basic Inorganic Chemistry CHEM 106 lecture and laboratory, and you are a declared chemistry major.

The research contains two groups: spring 2012 students are the control group and students in spring 2013 will be the treatment group. The spring 2012 student responses will serve as baseline data for comparative analysis to student responses in the spring 2013 courses who will receive instruction using a teaching tool called the Science Writing Heuristic.

Please read this form carefully. You will have the opportunity to ask any questions or request clarification before deciding whether or not you choose to participate in the study.

Purpose:
The purpose of this study is to learn whether direct instruction of a specific strategy for learning in the laboratory affects how students monitor their own learning in the laboratory setting. We will compare descriptions and identifications of students’ regulation and awareness of learning between students who utilize the science writing heuristic as an explicit strategy and those who do not utilize this tool.

Procedures:
In order to collect data to address the research questions and hypotheses, we are asking your permission to use your responses from class activities. Specifically, these class activities include:

- Two tests at the beginning of the semester that assess your current level of knowledge and ability. The first is the American Chemical Society end of first semester general chemistry multiple-choice exam. The second is the Group Assessment of Logical Thinking (GALT). The GALT is an 11-item assessment of logical reasoning skills.
- Three surveys at: (1) a demographic survey, (2) survey on your confidence in chemistry, and (3) survey about your awareness and level of and use of learning strategies.
• American Chemical Society 2nd semester general chemistry exam at the end of the semester
• Written laboratory reports for each laboratory problem.

In addition to these class activities, you may be asked to participate in a set of interviews. Participants will randomly be chosen for interviews. The interview will focus on questions about your learning experience in the lab and information about how you wrote your lab reports. Interviews will last approximately 30-45 minutes, and they will take place at two different points throughout the semester. The interviews will be audio recorded.

Finally, if you choose to allow these data to be used in the research study, we will ask you at the end of the following fall semester to respond to an online survey, similar to the one you will take at the beginning and end of this semester.

Risks/Benefits:
There are no foreseeable risks involved in participating in this research beyond those experienced in everyday life as part of your work as an undergraduate student in chemistry

There may be no direct benefits to you for participating, but the results may lead to additional information about how to enhance teaching and learning in the chemistry laboratory.

Confidentiality:
All data collected from you will be coded so that any identifying information remains confidential. Student names and coded numbers will be stored in a secure location that is password protected. Any reports of results of this study will use coded information or pseudonyms regarding student responses.

Voluntary Participation:
Participation in this study is voluntary and has no influence on your grade in the course. Mary van Opstal, who will recruit you and also be your TA will not participate in grading of your exams or laboratory reports and will not participate in any grade assignment for you in the course. Additionally, in order to protect the relationship of students with course instructors, the list of students who choose to participate in the research will be kept confidential (known only to Mary van Opstal) until the course is completed and grades have been submitted. If you do not want to be in this study, you do not have to participate. Even if you decide to participate, you are free not to answer any question or to withdraw from participation at any time without penalty. Your choice to not participate means that data collected that involves you will not be included in the research analysis.
Compensation:
If you are asked to participate in an interview and complete the set of interviews, you will be entered into a drawing to receive a $50 iTunes gift card.

Contacts and Questions:
If you have questions about this research study, please feel free to contact:
Mary van Opstal at mtwist@luc.edu or 616.706.7713 or
Patrick Daubenmire at pdauben@luc.edu or 773.508.8248

If you have questions about your rights as a research participant, you may contact the Loyola University Office of Research Services at (773) 508-2689.

Statement of Consent:
Your signature below indicates that you have read the information provided above, have had an opportunity to ask questions, and agree to participate in this research study. You will be given a copy of this form to keep for your records.

_________________________     __________________     __________________
Participant’s Signature        Printed name        Date

_______________________________     __________________
Researcher’s Signature          Date
APPENDIX B

SAMPLE SWH LAB EXPERIMENT
SWH Lab: Spectrophotometry and Equilibrium

Prepared by: Mary van Opstal

Introduction: Similar to the cobalt equilibrium lab last week, we can see the reaction between iron nitrate and sodium thiocyanate as the color of the solution changes. With the use of spectrophotometry and the knowledge of the proportional relationship between absorbance and concentration (Beer’s Law $A=εlc$), you will calculate the equilibrium constant of for this chemical reaction.

Safety:
Please wear your goggles at all times and gloves while working directly with reagents. Be careful when working with any chemical: acid, base, solid or liquid. None of these should make contact with your skin or eyes.

Chemicals
2.00*10^{-1} M Iron nitrate, Fe(NO_3)_3
2.00*10^{-3} M Sodium thiocyanate NaSCN
0.1M Nitric acid HNO_3

Equipment
50.00mL volumetric flasks
Thermometer
10.00mL graduated pipet
5.00mL serological pipet
small beakers
test tubes (medium-large)
Spectrovis unit (with directions)
Cuvette

Fe(NO_3)_3 + NaSCN $\leftrightarrow$ NaNO_3 + FeNCS^{2+}

You will first generate a calibration curve of standard solutions of FeNCS^{2+} within known concentrations. From this data, you will be able to determine the concentrations of FeNCS^{2+} solutions you make in Part B. Finally you will calculate the equilibrium constant (K_{eq}) for the reaction above.

Techniques:
Volumetric and serological pipet with bulbs
Volumetric flasks

Procedure:
Work Strategies: You and your group (of 4) will prepare six known concentrations of FeNCS^{2+}. These are known as standard solutions. How will you ensure replication of each concentration? How will you determine concentrations? Each pair (within a group) will run standard solution on their own spectrophotometer. For Part B, you and your group (of 4) will prepare six additional solutions of FeSCN^{2+} in Part B to determine the equilibrium constant of the above reaction. Each pair will then run each solution on their own spectrophotometer. How will you ensure replication?

Part A. Preparation of a calibration curve
You will need to prepare 0M and 2.00*10^{-4} M FeNCS^{2+}, as well as four concentrations of FeNCS^{2+}, between 0M and 2.00*10^{-3} M FeNCS^{2+} concentrations. What concentrations will you make up?
To calculate the volume of NaSCN to add to each volumetric flask, you will need the molarity of the stock solution 2.00*10^{-3} \text{ M} \text{ NaSCN} and the total volume of the final solution, which is 50.00mL.

What equation can you use to determine the concentration of NaSCN to add?

In order to prepare these six known standards, obtain about 30mL of iron nitrate (2.00*10^{-1} \text{ M} \text{ Fe(NO}_3)_3) from the stock solution in the hood along with 10mL of NaSCN in separate small beakers. Add 10.00mL (using a volumetric pipette) of 2.00*10^{-1} \text{ M} \text{ Fe(NO}_3)_3 to each 50.00mL volumetric flask, then add the calculated volume of NaSCN to its respective volumetric flask. (One solution should contain no NaSCN.) Finally you will add enough 0.1M nitric acid to each volumetric flask to fill it up to the line on the neck of the flask.

*Each solution contains excess \text{ Fe(NO}_3)_3 so that the reaction between \text{ Fe(NO}_3)_3 and NaSCN is pushed almost to completion. LeChatlier’s principle is quite useful here! What is the product that causes the solution to be colored?

In order to measure the absorbance of each standard solution for your calibration curve, obtain all six solutions. Set up your spectrophotis unit following the instructions provided. Take a blank. The blank is your first solution that contains only \text{ Fe(NO}_3)_3. What is the concentration of the \text{ Fe(NO}_3)_3 in this sample? Why do we take a blank when running a spectrophotometer?

Once you have calibrated your spectrophotis with your blank. You will take a spectrum of each solution. Record the absorbance (y-axis) at the maximum peak as well as the wavelength (x-axis) at which that peak occurred for each concentration.

Figure 1. Example UV-vis Spectrum

Take a minute and look over your data. As concentration of \text{ FeNCS}^{2+} increases, what do the absorbance values do? Increase? Decrease? If your data does not follow Beer’s Law, you may need to remake a solution and test it again. Talk with the groups around you about your calibration curves. Are they similar?

Once you have taken the appropriate data, you will prepare a graph of Absorbance (y-axis) vs. concentration of \text{ FeNCS}^{2+} (x-axis) on Microsoft Excel and determine the line of best fit. This should follow the general equation

\[ Y = mX + b \]

where \textbf{X} is concentration and \textbf{Y} is absorbance.
Part B. Determine an Equilibrium Constant for the reaction between Fe(NO_3)_3 and NaSCN.

As a group, in six medium-large dry test tubes prepare solutions of 5.0mL of 0.002M Fe(NO_3)_3. **You need to dilute the stock solution of iron nitrate to obtain this concentration of 0.002M** and increasing amounts of 0.002M NaSCN from 0.0 mL to 5.0 mL. You may need to add additional 0.1M HNO_3 so each test tube contains a total volume of 10.0mL. The first test tube should contain only Fe(NO_3)_3. The following test tubes (2-5) should contain known amounts of 0.002M NaSCN between 0.0mL and 5.0mL.

**Equilibrium constants are temperature dependent. Take the temperature of one of your solutions.**

On completion of your samples, take a spectrum of each sample record the absorbance (y-axis) at the maximum peak as well as the wavelength (nm) at which the peak occurred.

Does your data make sense? As the concentration of NaSCN is increased, what happens with the absorbance?

Check with groups around you to ensure similar results.

**Disposal: Please dispose of all samples into the containers labeled Iron Thiocyanate (FeNCS^{2+}) Excess.**

Analysis

Please show appropriate graphs or tables for the data you collected.

Use your collected data to determine the equilibrium constant, include the following:

- Net ionic equation for the equilibrium
- Equilibrium constant expression
- Initial concentrations of Fe^{3+} and SCN^-
- Equilibrium concentrations of FeNCS^{2+}, Fe^{3+} and SCN^-.
- Absorbance for each solution
- K_eq for each solution

- What is the average K_eq?
- Please show standard deviation for the calculated average K_eq and relative standard deviation
- \( \text{RSD} = \frac{\text{standard deviation (s)}}{\text{mean of your value (x)}} \)
- What does the RSD tell you about your precision during the experiment?
- Compare your average K_eq with a literature value and calculate absolute percent error.
  - \( \text{Relative % error} = \frac{\text{experimental value - true value}}{\text{true value}} \times 100 \)
- What does the relative percent error say about the accuracy of your value (K_eq)?
- How does your data compare with other groups in the lab?
Postlab questions

1. How would the accuracy of your determined $K_{eq}$ change if all your volume measurements were made with graduated cylinders rather than volumetric (or serological) pipets?

2. If all the SCN$^-$ was not converted completely to FeNCS$^{2+}$ when the calibration curve was prepared, would this raise or lower the value of $K_{eq}$? Explain.

3. Use the mean value you determined for $K_{eq}$ to calculate the SCN$^-$ concentration in a solution where the initial Fe concentration is 4.00x10$^{-2}$ and the initial SCN$^-$ concentration is 1.00x10$^{-3}$M. Is all of the SCN$^-$ in the form of FeNCS$^{2+}$?

4. Does the resolute of calculation in question 3 justify your original assumption that all of the SCN$^-$ is in the form of FeNCS$^{2+}$?

5. Based upon your answer to question 4, is your measured value of $K_{eq}$ too high or too low?
APPENDIX C

METACOGNITIVE STRATEGIES PAGE IN LAB MANUAL
Metacognition

How do you know that you know something? Is this an important question to ask yourself as you are learning new things? An inquiry based learning environment gives you the opportunities to explore information, think critically, develop new ideas, solve problems, and check those ideas in new situations. Learning scientists (cognitive psychologists) call this cognition. Obviously these are key components to learning something. Additional key processes in learning are your motivation (which includes how confident you are in your ability to learn), and tracking how well you are learning something. This tracking process is what scientists call metacognition. (You will become more familiar with the prefix meta as you continue your study in chemistry!) It helps you monitor and regulate how well you are learning something. There are two areas within metacognition: knowledge of cognition and regulation of cognition.

Knowledge of cognition:

- **Declarative**: You know about your learning processes and beliefs and what factors affect your learning performance.
- **Procedural**: You know which strategies you use to accomplish a task.
- **Conditional**: You know when and why you apply certain strategies to accomplish a task.

Regulation of cognition

- **Planning**: You prepare goals and strategies to accomplish your task.
- **Monitoring**: You ask yourself questions to make sure you understand what you are learning.
- **Evaluation**: You reflect on the skills you’ve used to accomplish your task, the goals you set, and what you have learned during lab.
During beginning questions, you will use procedural knowledge to know how to ask questions, and planning to think about what you know and what the experiment is about before you ask a question.

To perform tests, you will use your planning skills and have goals about how you will run your experiment.

When you take data, you will need to use your monitoring skills to recognize whether the date makes sense.

To back up your claim with appropriate evidence, you will use your monitoring skills to check that your data makes sense.

During reflection and peer comparison, you will use evaluation to look back on what you learned, what changes can be made to the experiment, and errors that can be avoided.
APPENDIX D

SAMPLE OPEN-ENDED LAB PROBLEM
MISSION CHEMPOSSIBLE: Laboratory Problem #04
The Great White (Chemical) Way!

Description of the Problem
Your mission, should you choose to accept it, is to design and conduct a laboratory experiment that supports clear distinctions and identification of five white powders.

Objective
The problem is designed for you to apply qualitative analysis in order to identify some common household substances.

Sample Material
The five white powders that you will be identifying are:
- Chalk (CaSO₄ & CaCO₃)
- Alka-Seltzer ® (C₆H₈O₇ & NaHCO₃)
- Washing Soda (Na₂CO₃)
- Baking Soda (NaHCO₃)
- Vitamin C (C₆H₈O₆)

Test Chemicals and Equipment Available
- Vinegar
- Water
- Phenolphthalein solution

Equipment in your drawer
- Weigh boats (please clean and return for recycled use!)

Experimental Design
Consider and create an experiment that is able to make clear distinctions among the five powders. For full identification you may need to consult additional resources to help you align the results of the tests you perform with characteristic tests for the compounds in these powders. In performing your tests, please be aware that only 1.5 g of each powder is available per student in the lab. Please consider using small amounts (<0.5 g or ½ teaspoon) of your test materials. Before you may perform your experiment, your design must be approved by the instructor or a teaching assistant.
APPENDIX E

MCAI AND CIC SURVEYS
Part A. Metacognitive Activities Inventory – Reference: Cooper, M. M.; Sandi-Urena, S. Design and Validation of an Instrument to Assess Metacognitive Skillfulness in Chemistry Problem Solving. *J. Chem. Educ.* 2009, 86, 240-245. This survey asks you about the strategies and steps you use to solve problems, complete tasks or study. Remember there are no right or wrong answers. Please circle the number that best represents you. The evaluation scale is listed below:

Evaluation Scale: (5) strongly agree (4) agree (3) Neutral (2) disagree (1) strongly disagree

1. I read the statement of a problem carefully to fully understand it and determine what the goal is.  5 4 3 2 1

2. When I do assigned problems, I try to learn more about the concepts so that I can apply this knowledge to test problems.  5 4 3 2 1

3. I sort the information in the statement and determine what is relevant.  5 4 3 2 1

4. Once a result is obtained, I check to see that it agrees with what I expected.  5 4 3 2 1

5. I try to relate unfamiliar problems with previous situations or problems solved.  5 4 3 2 1

6. I try to determine the form in which the answer or product will be expressed.  5 4 3 2 1

7. If a problem involves several calculations, I make those calculations separately and check the intermediate results.  5 4 3 2 1

8. I clearly identify the goal of a problem (the unknown variable to solve for or the concept to be defined) before attempting a solution.  5 4 3 2 1

9. I consider what information needed might not be given in the statement of the problem.  5 4 3 2 1

10. I try to double-check everything: my understanding of the problem, calculations, units, etc.  5 4 3 2 1

11. I use graphic organizers (diagrams, flow-charts, etc) to better understand problems.  5 4 3 2 1

12. I experience moments of insight or creativity while solving problems.  5 4 3 2 1

13. I jot down things I know that might help me solve a problem, before attempting a solution.  5 4 3 2 1
14. I find important relations amongst the quantities, factors or concepts involved before trying a solution.

15. I make sure that my solution actually answers the question.

16. I plan how to solve a problem before I actually start solving it (even if it is a brief mental plan).

17. I reflect upon things I know that are relevant to a problem.

18. I analyze the steps of my plan and the appropriateness of each step.

19. I attempt to break down the problem to find the starting point.

20. I spend little time on problems for which I do not already have a set of solving rules or that I have not been taught before.

21. When I solve problems, I omit thinking of concepts before attempting a solution.

22. Once I know how to solve a type of problem, I put no more time in understanding the concepts involved.

23. I do not check that the answer makes sense.

24. If I do not know exactly how to solve a problem, I immediately try to guess the answer.

25. I start solving problems without having to read all the details of the statement.

26. I spend little time on problems I am not sure I can solve.

27. When practicing, if a problem takes several attempts and I cannot get it right, I get someone to do it for me and I try to memorize the procedure.
Part B. Confidence in Chemistry Survey

Reference: From beginning of semester college chemistry course survey, The LEAD Center – University of Wisconsin Madison)

From the statements listed below, please assess your CONFIDENCE level in the areas in attending this course. Please circle the number that best represents your degree of confidence. Scale is 5 – high confidence, 1 – low confidence.

<table>
<thead>
<tr>
<th>Confidence in your ability to…</th>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Understand key concepts in chemistry</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>2. Solve chemistry problems</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>3. Understand the chemistry of lab experiments</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>4. Perform lab experiments</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>5. Apply your knowledge of chemistry to the real world</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>6. Understand science topics other than chemistry</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>7. Succeed in this chemistry course</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>8. Succeed in a chemistry related discipline</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>9. Reach understanding of key concepts in chemistry be working alone.</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>10. Reach understanding of key concepts by working in a group</td>
<td>5 4 3 2 1</td>
<td></td>
</tr>
<tr>
<td>11. What grade do you expect in this course?</td>
<td>A B C D F</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX F

INTERVIEW PROTOCOLS
Interview Protocol

First Interview (Occurred about four weeks into semester, written two lab reports at this point)
“P” indicates probing question asked if student answers too briefly

Part 1: Learning experience in the laboratory
1. What steps or strategies do you take to get through lab experiments?
   a. P: Can you be specific? (Lab problem or regular experiment)
2. How do you think you are learning during lab?
   a. How is your learning supported while running experiments in the laboratory?
   b. P: What in the lab helps you learn?
   c. P: Factors (Environment, Peer interactions, personal factors)
   d. Are there any barriers or obstacles you experience while learning in lab? If so, can you describe them?
      a. How do you get over them?
3. How do you know (when) you’ve learned something new in a lab experiment (or the lab in general)?
   a. P: Feelings, conscious, change, connections,
4. How do you think the regular lab experiments support/prepare you to solve and report (write) the Chempossible lab problems? (for example the five white powders)

Part 2: Student Explanation of Lab write up (Lab report will be used for this part of the interview from the lab problem: The great white chemical way).
5. How did you approach the problem (The great white chemical way) in this lab?
6. What were the steps you took to write up your lab report?
   a. Tell me your thought process.
   b. Can you describe the final steps you took just before you handed the report in?
   c. Why did you organize your report and data in this format
      a. P: If missing pieces… why did you not include these parts?
7. How long did it take you to create and write the report? What were your feelings about writing the report?
8. What sources did you use to complete your lab report?
   a. Is there anything you would change about your approach to writing or setting up the laboratory experiment for your next Chempossible problem?
9. Describe how you think writing these reports helps you learn.

As asked at the end of the SWH group 1st interview. Not used in analysis.
10. We talked about metacognition at the beginning of the semester. Can you tell me a little about how you think MC is useful to you and whether you are using it in the lab?
Second Interview (Occurred 1 week before the end of the semester. (Written four lab reports at this point)

Part 1: Learning experience in the laboratory
1. How do you think you are learning during lab? How do you learn
   a. What is the best way for you to learn new information?
2. If you are learning about catalysts, how do you know when you really know the information; learned the essential ideas, for a test?
3. How much time do you set aside to prepare for lab?
4. Reflecting back on lab, what have you excelled at, and what have you struggled with?
5. What makes you excited about chemistry?

Part 2: Student Explanation of Lab write up (Lab report will be used for this part of the interview)
6. After writing four lab reports, have your steps to writing the report changed in any way? If so, how??
7. These are the changes you suggested that you might change about how you write your next lab report during your first interview, how did you incorporate them into your more recent lab reports. (if they don’t answer this question previously)
8. You have completed your last lab, take me through the timeline and organization for writing your lab report that is due next week. (Student may be in the process of writing it)
9. Describe what you have learned from writing four lab reports.
10. How has writing lab reports affected your learning in lab?
11. How do you think writing lab reports will help you in future lab classes?
12. How do you compare your learning processes between writing reports for the Chempossible problems and completing the data and analysis from the workbook labs?
13. How do you think writing helps you learn?

Asked at the end of the SWH Group 2nd interview.
14. How has using the SWH changed the way you think and learn in lab? How have you benefited from using the SWH in lab?
15. How has your use of metacognitive strategies changed over the semester including Planning, Monitoring and Evaluation? (Not used in analysis)
APPENDIX G

LAB REPORT SCORING RUBRIC
## Laboratory Problem Report Scoring Rubric

Student Name: _______________________

<table>
<thead>
<tr>
<th>Section</th>
<th>Criteria</th>
<th>Guiding Question(s)</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>Context</td>
<td>A clear sense of why this knowledge may be of interest to a broad audience or is useful in solving the lab problem.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The writer provides some relevant context for the research questions or problem.</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The writer provides only vague or generic references to the broader context of chemistry that involves this problem.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The importance of the context for the research problem is not addressed.</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Accuracy &amp; Relevance</td>
<td>Has the appropriate level of specificity to support why this context fits the research design and how error is minimized under these parameters.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Has a good level of specificity as described above, but contains a minor omission, inaccuracy, or incomplete connection.</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td>Omits important information and is overly narrow or overly general so that only a loose connection of relevance is established between the context and the experiment.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Omits critical information and is too disjointed to make a clear connection of context to relevance.</td>
<td>0</td>
</tr>
<tr>
<td>Questions &amp; Hypothesis/es</td>
<td>Testable</td>
<td>A comprehensive and appropriate set of questions and/or hypothesis/es are stated that can distinguish among multiple major factors or potential explanations for the phenomena at hand.</td>
<td>3</td>
</tr>
<tr>
<td>Section</td>
<td>Criteria</td>
<td>Guiding Question(s)</td>
<td>Points</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
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<tr>
<td></td>
<td></td>
<td>A limited set of questions and/or hypothesis/es are provided, but do address more than one potential mechanism, explanation, or factors for the topic.</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Questions or hypothesis/es are stated but are too vague or and are not clearly testable.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None indicated.</td>
<td>0</td>
</tr>
<tr>
<td>Methods</td>
<td>Controls &amp; Replication</td>
<td>Controls consider a comprehensive and complete set of relevant factors and have become methods of differentiating between multiple hypotheses. Replication is robust.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls take most factors into account. Replication is appropriate</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls consider only one major relevant factor. Replication is modest.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls and/or replication are nonexistent.</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Experimental Design</td>
<td>Describes how established techniques are used, refined, or modified in this design, is appropriate for addressing the questions and/or hypothesis/es, is clearly explained, and conveys processes and/or protocols for minimizing error when conducting the experiment.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Missing one of the elements described for 3 points.</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td>Missing two or more of the elements described for 3 points.</td>
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<tr>
<td></td>
<td></td>
<td>Inappropriate, poorly explained and/or indecipherable.</td>
<td>0</td>
</tr>
<tr>
<td>Results</td>
<td>Data Selection &amp; Analysis</td>
<td>Data are relevant, accurate, and comprehensive. Reader can fully evaluate validity of writer’s conclusions and assumptions.</td>
<td>3</td>
</tr>
<tr>
<td>Section</td>
<td>Criteria</td>
<td>Guiding Question(s)</td>
<td>Points</td>
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<tr>
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<tr>
<td>Data Presentation</td>
<td>Data are relevant, accurate, and complete with any gaps being minor. Reader can fully evaluate whether the hypotheses were legitimately supported or rejected with the data provided.</td>
<td>2</td>
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<td></td>
<td>At least one relevant dataset per hypothesis is provided but some necessary data are missing or inaccurate. Reader can satisfactorily evaluate some but not all of the writer’s conclusions.</td>
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<td></td>
<td>Data are incomplete and/or haphazard to provide a reasonable basis for testing the hypothesis. Uses a format or graph type which highlights the relationships between the data points or other relevant aspects of the data. Has informative, concise, and complete captions. Contains no mistakes.</td>
<td>0</td>
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<td></td>
<td>Graph types or table formats are appropriate for data. Includes captions that are at least somewhat useful. Contains only minor mistakes that do not interfere with the reader’s understanding.</td>
<td>3</td>
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<td></td>
<td>Is technically correct but inappropriate format prevents the reader from deriving meaning or using it. Captions are missing or inadequate. Contains noticeable errors but the reader is still able to derive some meaning from each figure.</td>
<td>2</td>
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<td></td>
<td>Is inappropriate with captions that are confusing or indecipherable. Is missing labels and/or units which prevent the reader from being able to derive any useful information from the graph.</td>
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<td>Section</td>
<td>Criteria</td>
<td>Guiding Question(s)</td>
<td>Points</td>
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<td>------------------------</td>
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<tr>
<td>Conclusions</td>
<td>Conclusions are completely justified by data. Connections between the hypothesis, data, and conclusions are comprehensive and persuasive. Conclusions address or logically refute or explain conflicting data.</td>
<td></td>
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<td></td>
<td>Conclusions are clearly and logically drawn from and bounded by the data provided with no gaps in logic. A reasonable clear chain of logic from hypothesis to data to conclusion is made. Conclusions attempt to discuss or explain conflicting or missing data.</td>
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<td></td>
<td>Conclusions have some direct basis in the data, but may contain some gaps in logic or data or are overly broad. Connections between hypothesis, data, and conclusion are present but weak. Conflicting or missing data are poorly addressed.</td>
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<td></td>
<td>Conclusions have little or no basis in data provided. Connections between hypothesis, data and conclusions are nonexistent, limited, vague or otherwise insufficient to allow reasonable evaluation of their merit. Conflicting data are not addressed.</td>
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</tr>
<tr>
<td>Alternative Explanations</td>
<td>Have become a suite of interrelated hypotheses that are explicitly tested with data. Discussion and analysis of alternatives is based on data, complete and persuasive with a single clearly supported explanation remaining by the end of the discussion.</td>
<td></td>
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<td></td>
<td>Are tested as hypotheses; those not tested are reasonably evaluated in the discussion section. Discussion of alternatives is reasonably complete, uses data where possible and results in at least some alternatives being persuasively dismissed.</td>
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<td>Section</td>
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<td>Guiding Question(s)</td>
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<td>Are provided in the discussion only. May include some trivial or irrelevant alternative. Discussion addresses some but not all of the alternatives in a reasonable way.</td>
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<td>Are not provided, are trivial or irrelevant, and/or are mentioned but not discussed or eliminated.</td>
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<td>Are presented as factors modifying the author’s conclusions. Conclusions take these limitations into account.</td>
<td>3</td>
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<td></td>
<td>Are relevant, but not addressed in a comprehensive way. Conclusions fail to address or overstep the bounds indicated by the limitations.</td>
<td>2</td>
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<td>Are discussed in a trivial way (e.g. “human error” is the major limitation invoked).</td>
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<td></td>
<td></td>
<td>Are not discussed.</td>
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<tr>
<td>Limitations of design</td>
<td></td>
<td>Is salient, plausible, and insightful.</td>
<td>3</td>
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<td></td>
<td></td>
<td>Is useful, but indicate incomplete or insufficient connections.</td>
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<td>Is vague or implausible.</td>
<td>1</td>
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<td></td>
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<td>Is not addressed.</td>
<td>0</td>
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<tr>
<td>Connection to other knowledge</td>
<td></td>
<td>A clear organizational strategy is present with a logical progression of ideas. There is evidence of an active planning for presenting information; this paper is easier to read than most. Word usage facilitates reader’s understanding. No more than two mistakes are made in grammar and spelling</td>
<td>3</td>
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<tr>
<td></td>
<td></td>
<td>A clear organizational strategy is present with a logical progression of ideas. Word usage is accurate and aids the reader’s understanding. More than two but less than five grammar and spelling mistakes are made.</td>
<td>2</td>
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<tr>
<td>Section</td>
<td>Criteria</td>
<td>Guiding Question(s)</td>
<td>Points</td>
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<td>There is some evidence of an organizational strategy though it may have gaps or repetitions. General word usage is appropriate. Multiple mistakes are made in grammar and spelling, but errors do not hinder the meaning of the paper.</td>
<td>1</td>
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<tr>
<td></td>
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<td>Information is presented in a haphazard way. Word usage is confused or incorrect. Multiple grammar and spelling errors detract from the meaning of the paper.</td>
<td>0</td>
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</tbody>
</table>

Additional comments from the grader:

Total Score: ___________/36
APPENDIX H

GPOWER3.1 ANALYSIS
## Power Analysis for MCAI and ACS measures

### F tests - ANOVA: Repeated measures, within factors

**Analysis:** A priori: Compute required sample size  
**Input:**  
- Effect size $f = 0.4$  
- $\alpha$ err prob $= 0.1$  
- Power (1-$\beta$ err prob) $= 0.8$  
- Number of groups $= 2$  
- Number of measurements $= 2$  
- Corr among rep measures $= 0.5$  
- Nonsphericity correction $\varepsilon = 1$  

**Output:**  
- Noncentrality parameter $\lambda = 7.680000$  
- Critical $F = 3.2850153$  
- Numerator df $= 1.0000000$  
- Denominator df $= 10.0000000$  
- Total sample size $= 12$  
- Actual power $= 0.8243440$

### F tests - ANOVA: Repeated measures, within factors

**Analysis:** A priori: Compute required sample size  
**Input:**  
- Effect size $f = 0.25$  
- $\alpha$ err prob $= 0.1$  
- Power (1-$\beta$ err prob) $= 0.8$  
- Number of groups $= 2$  
- Number of measurements $= 2$  
- Corr among rep measures $= 0.5$  
- Nonsphericity correction $\varepsilon = 1$  

**Output:**  
- Noncentrality parameter $\lambda = 7.000000$  
- Critical $F = 2.9091325$  
- Numerator df $= 1.0000000$  
- Denominator df $= 26.0000000$  
- Total sample size $= 28$  
- Actual power $= 0.8240978$

### F tests - ANOVA: Repeated measures, between factors

**Analysis:** A priori: Compute required sample size  
**Input:**  
- Effect size $f = 0.4$  
- $\alpha$ err prob $= 0.1$  
- Power (1-$\beta$ err prob) $= 0.8$  
- Number of groups $= 2$  
- Number of measurements $= 4$
\textbf{Output:} Noncentrality parameter $\lambda = 6.6560000$

Critical F $= 2.9271175$
Numerator df $= 1.0000000$
Denominator df $= 24.0000000$
Total sample size $= 26$
Actual power $= 0.8054668$

\textbf{F tests - ANOVA:} Repeated measures, between factors

\textbf{Analysis:} A priori: Compute required sample size

\textbf{Input:} Effect size f $= 0.25$
$\alpha$ err prob $= 0.1$
Power (1-\beta err prob) $= .8$
Number of groups $= 2$
Number of measurements $= 4$
Corr among rep measures $= 0.5$

\textbf{Output:} Noncentrality parameter $\lambda = 6.4000000$
Critical F $= 2.7882459$
Numerator df $= 1.0000000$
Denominator df $= 62.0000000$
Total sample size $= 64$
Actual power $= 0.8043510$
APPENDIX I

ELECTROSTATIC AND VAN DER WAALS INTERACTION ENERGY GRAPH
Figure 15. Electrostatic and van der Waals Interaction energy for Pb ion 1H. Bound to Asp 80, Asp 84, and Glu 7.
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VITA

Mary Twist van Opstal was born in Ada, Michigan. Before attending Loyola University Chicago, she attended Hillsdale College and completed a Bachelor of Science degree in Chemistry, with Highest Distinction, in 2008.

While at Loyola, Dr. van Opstal received a teaching fellowship from 2009 to 2013. She was a research assistant on a NSF grant-funded project from 2013-2014 that introduced and encouraged high school students to use environment friendly behaviors and learn about sustainability of the environment. In addition to teaching general chemistry and instrumental analysis laboratories, Mary implemented a well-established inquiry and collaborative method of instruction, the Science Writing Heuristic, in the chemistry majors two-semester general chemistry course. Dr. van Opstal was also a representative from the chemistry department to the Graduate Student Organization from 2010-2012.