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Guided-Inquiry in Biochemistry Laboratory Course Improves Lab Math Skills

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ABSTRACT

Biochemistry graduates pursuing research-related careers must master basic quantitative skills. Laboratory courses present students opportunities to practice lab math skills such as dilution and solution calculations. Employers and researchers have reported inadequate math skills among bioscience graduates and there is a need to evaluate the effectiveness of laboratory teaching approaches in increasing students' lab math skills. In this three-year study, we examined the impact of guided-inquiry learning on students' ability to perform laboratory calculations required for experimental design. An upper-level undergraduate biochemistry laboratory course was divided into sections taught using an inquiry approach where students design their own experiments or a cookbook approach where protocols are provided. We wrote a Lab Math Test to measure students' lab math skills and administered this test as pre- and post-assessment to students in all sections. Students' lab math skills significantly improved from pre- to posttest scores for inquiry sections (1.18 ± 0.25 (SE) to 4.22 ± 0.37 (SE)) compared to cookbook sections (1.10 ± 0.18 (SE) to 2.89 ± 0.25 (SE)), suggesting that the inquiry approach was more effective in increasing students' lab math skills. Data showed significantly higher long-term gains for students in inquiry sections during a project-based research experience in the subsequent course. Inquiry learning can lead to a more engaging laboratory course experience and also have the positive side effect of increasing students' basic lab math skills.

KEYWORDS: Upper-division undergraduate, biochemistry laboratory, inquiry-based/discovery learning, lab math skills, lab calculations, experimental design, chemistry education and research

MONTCLAIR STATE UNIVERSITY

Guided-Inquiry in Biochemistry Laboratory Course Improves Lab Math Skills

by

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INTRODUCTION

Undergraduate biochemistry students who wish to pursue research careers need to master basic lab math skills. Laboratory courses provide natural opportunities to practice these skills, including dilution calculations and calculations to prepare solutions for an experiment. Biochemistry faculty need to develop and investigate biochemistry laboratory learning environments that cultivate strong lab math skills. The importance of quantitative and laboratory math skills has surfaced in surveys of researchers, employers, and faculty[1-3] and in the American Society of Biochemistry and Molecular Biology's (ASBMB) undergraduate curriculum recommendations.[4] Highly-ranked skills include the ability to perform basic mathematical manipulations (e.g., unit conversions, solution calculations, dilutions and serial dilutions)[2], interpret experimental data, design and conduct experiments, understand basic statistics,[1, 3] and possess good "quantitative" skills such as the ability to prepare reagents for experiments.[4] Most employers (~80 %) expect new hires to be equipped with analytical and quantitative skills, written and oral communication skills, and problem-solving skills and program approval standards of the American Chemical Society call for such skills to be taught and assessed.[5]

Despite strong agreement on the importance of quantitative skills, numeracy and computational skills are considered a positive 'development deficit'.[6] Findings by Koenig suggest that < 10 % of bioscience graduates feel well-prepared with basic mathematical skills and 20 % do not feel prepared at all.[7] The Business Council surveys of CEO's and U.S. Bureau of Labor Statistics concluded that only 18 % of the new entrants showed excellent basic math skills while 13 % were deficient.[8] Furthermore, a study found that 25 % of first-year medical students had difficulty performing basic mathematical

manipulations and struggled to interpret medical data on a three-question numeracy scale assessment.[9]

In response to increasing deficit in students' basic math skills, The *BIO2010* report,[10] Howard Hughes Medical Institute (HHMI) sponsored report on the *Scientific Foundations for Future Physicians,*[11] and the *Vision and Change* report[12] suggested a revised life science curriculum with an increased emphasis on mathematics and physical sciences. The National Research Council's new recommendations have led to efforts in blending quantitative skills into biology classroom curriculum.[13-16] This reform must now be woven into the laboratory and biochemistry settings. According to Kirschner, a laboratory course is the proper platform to teach skills that practicing scientists and professionals most commonly use.[17] Reid and Shah noted that the original reason for the development of laboratory courses was the need to produce skilled technicians for industry and highly competent workers for research laboratories.[18]

There is a need to develop and evaluate learning environments that stimulate growth in students' lab math skills. A recent study adapted peer learning as a tool to strengthen math skills in an introductory chemistry lab.[19] Peer learning resulted in larger math gains when two students of dissimilar math abilities were paired. Here we investigate the effect of guided-inquiry approaches on lab math skills in an upper-level undergraduate biochemistry laboratory course. Guided-inquiry can be an active-learning experience as students take responsibility for their learning while instructors facilitate student learning.[20] Research suggests that inquiry-based learning environments improve experimental design ability,[21-22] science process skills,[23-25] information fluency skills,[26] understanding of statistical analysis,[27] and provide students authentic research

experiences.[28] We now want to know whether guided-inquiry learning can also be used as a tool to increase students' laboratory math abilities. We focused on math skills that biochemists routinely apply in their practice, including solution calculations, dilution calculations, unit conversions and calculations needed to set up biochemical assays.[20, 22, 29-30] We were not able to identify an existing instrument to measure students' lab math skills in the context of experimental design and thus wrote an assessment, which we refer to as "Lab Math Test", to assess specific biochemistry laboratory math skills (Appendix A in Supporting Information). The assessment tool has some similarities to the assessment tools designed by Kirton and coworkers who developed a series of assessments called Structured Chemistry Examinations designed to develop and assess general laboratory-based competencies.[29] Our assessment can be administered to all students simultaneously because it does not require a facilitator to grade individual students.

We hypothesized that guided-inquiry learning would increase students' laboratory math abilities more than traditional "cookbook" approaches. To test this hypothesis, we conducted a comparison study between the two learning environments in an introductory biochemistry laboratory course, *Experimental Biochemistry I*. We revised the existing curriculum and converted two sections of *Experimental Biochemistry I* from traditional to guided-inquiry learning environments ("inquiry" sections). The remaining sections continued employing the traditional "cookbook" learning approach. To assess students' lab math competencies, we created a Lab Math Test that we administered as pre- and post-assessment in all four sections of *Experimental Biochemistry I*. We administered a delayed posttest at the end of the spring semester course, *Experimental Biochemistry II,*

to investigate long-term gains. Research questions that guided this study were: 1.) Does replacing traditional laboratory experiments with inquiry modules affect students' lab math ability in a biochemistry laboratory course? and 2.) Does inquiry-style instruction prime the students for higher lab math gains during the subsequent laboratory course?

METHODS

Participants

We conducted this study over three years (Figure 1). For the first year we included in the study undergraduate students enrolled in *Experimental Biochemistry I*, a course offered in the fall semester at Montclair State University (MSU). For the second and third year, we included students enrolled in *Experimental Biochemistry I* (fall) and *Experimental Biochemistry II* (spring). Students in both courses were juniors and seniors majoring in Biochemistry, Chemistry, Molecular Biology, or Biology. Each fall, four sections of *Experimental Biochemistry I* were offered and included in this study. During fall semesters 1 and 2, two sections of *Experimental Biochemistry I* were randomly chosen to be taught using the inquiry approach and two sections using the cookbook approach (Figure 1). Students selected their sections randomly or based on their personal academic and work schedules and did not know which section would be taught in which style. During Fall 3, all sections of *Experimental Biochemistry I* were taught using the inquiry approach. There were four instructors teaching the sections of *Experimental Biochemistry I* during the three years of study (Figure 1). For the spring semesters, two sections of *Experimental Biochemistry II* were offered, and both were taught by the same instructor using a project-based approach where students designed and conducted small research

projects in groups. Students from both inquiry and cookbook sections of *Experimental Biochemistry I* could register for the continuation course. The assessor invited all enrolled students to take part in the study with an in-person plea. To seek genuine voluntary participation, the instructor was not involved in administering the Lab Math Test. Participants remained anonymous on the assessment by using a unique code, through which their pre- and post-assessments were matched. Our study included 191 participants across three academic years; details are shown in Figure 1. The MSU Internal Review Board approved the study (Protocol # 001272).

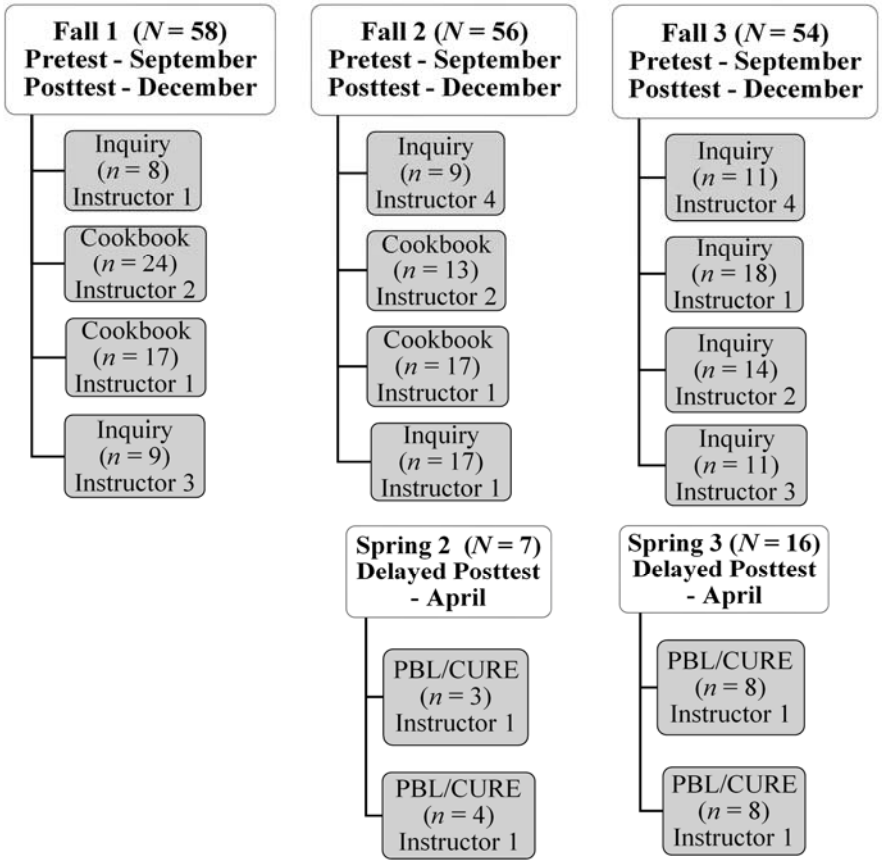

Figure 1. Project experimental design. The study was conducted over three academic years. Four sections of *Experimental Biochemistry I* were offered each fall. Two sections of *Experimental Biochemistry II* were included in the study during the spring semesters of academic years 2 and 3. For each section, the figure indicates the instructor (1, 2, 3, or 4) and whether inquiry or cookbook learning environment was used. All sections of *Experimental Biochemistry II* were taught using a project-based learning (PBL) approach in the context of a course-based undergraduate research experience (CURE). *N*-values refer to the total population included in the analysis each semester and *n*-values refer to the students belonging to each section for which data was analyzed

Experimental Design

We designed a mixed method quasi-experimental study that involved a pedagogical intervention to investigate our hypothesis. To compare the learning outcomes from inquiry and cookbook environments, two sections of *Experimental Biochemistry I* were

taught using inquiry modules and the other two sections using cookbook modules. The differences between the inquiry and cookbook modules were described previously in detail.[22] Briefly, cookbook modules provide students detailed experimental protocols while inquiry modules require students to design their own experimental protocols to answer a question provided in the module. After two years of a controlled study, the instructors began to observe higher scores for a common final in the inquiry sections and decided that it was no longer ethical to continue exposing students to the cookbook environment. For the last fall semester of the study (Fall 3), all four sections were taught using the inquiry approach. Since then all sections have been taught using the inquiry modules. The spring semester *Experimental Biochemistry II* was taught using a project-based learning approach, which offered students a course-based research experience,[31] where students designed and conducted research projects in groups. All sections of *Experimental Biochemistry II* employed the same teaching approach and were taught by the same instructor.

All sections of *Experimental Biochemistry I* followed the same curriculum with the difference that students in the inquiry environment were not given step-by-step protocols. Rather, they were given a goal or question, relevant background information, and advice on how to design an experiment to meet the goal or answer the question. Students in the cookbook sections were provided a step-by-step experimental protocol for each week's lab session. To infuse mathematical problem-solving in *Experimental Biochemistry I*, we designed a set of pre-lab Math Moment exercises, which were included in all modules (Appendices C and D in Supporting Information). Math moment exercises included laboratory math calculations to prepare students for the experiment or

the experimental design (for students in inquiry environment). Students from both cohorts completed the Math Moment questions before coming to lab. One difference between the two groups was that students in the inquiry groups performed extra calculations in addition to the Math Moments when designing their experimental protocol for the week. For example, inquiry module Eight required students to make seven serial dilutions from a given 500 nM trimethoprim stock solution (Appendix C in Supporting Information), while cookbook module Eight provided step-by-step directions to make the dilutions (Appendix D in Supporting Information). These modules are freely available on the project website (http://www.montclair.edu/csam/nsf-tues-grant/).

Assessing Student Lab Math Skills

We created a Lab Math Test, a six-item outcome assessment (Appendix A in Supporting Information) to measure the improvement in students' lab math skills (Table 1). The Lab Math Test was designed to mimic a situation where students do basic laboratory calculations to plan out an experiment. We consulted four biochemistry faculty members, who provided input on selecting the specific skills that students were tested on. The Lab Math Test provided students a scenario to which they applied mathematical concepts described in Table 1. Students were not notified in advance about the Lab Math Test and their scores were not used to calculate the final grades. On the testing day, students were given the option to take the Lab Math Test or complete an alternate assignment.

The Lab Math Test was administered to all four sections of *Experimental Biochemistry I.* Students received the pretest at the beginning of September and posttest during mid-December and the delayed posttest was given to both sections of

Experimental Biochemistry II in late April. The goal was to determine which environment resulted in larger gains in basic lab math skills. The pretest and posttest were identical except that we used different values for volumes and concentrations. The implementation of the delayed posttest towards the end of spring semester in *Experimental Biochemistry II* enabled us to monitor the longer-term impact of participation in the inquiry versus the cookbook sections of *Experimental Biochemistry I*.

Table 1. List of skills assessed through the individual rubric items in the Lab Math Test. Each rubric item was worth two points and the total possible score was 12 points. The test assessed the ability to comprehend and apply mathematical skills to an experimental scenario.

Rubric Item #	Learning objectives tested The ability to…
1.	Choose a set of numbers to represent a given range of concentrations.
2.	Perform dilution calculations using $C_1V_1 = C_2V_2$ by identifying three of the four given variables from an experimental scenario (solving for V_1)
3.	Perform dilution calculations using $C_1V_1 = C_2V_2$ by identifying three of the four given variables from an experimental scenario (solving for V_1)
4.	Perform dilution calculations to determine V_1 using $C_1V_1 = C_2V_2$ based on a range of C2 values and using information from the experimental scenario
5.	Perform unit conversions
6.	Perform a solution calculation using information provided in an experimental scenario (determine V_2)

Data Preparation and Analysis

We included the pre- and posttest scores for all students who completed both tests and consented to be part of the study in the analysis. We did not include scores for students who were absent for pre- or posttest or left one or both tests blank in the analysis. Three independent raters (undergraduate and graduate teaching assistants) scored the assessment based on a rubric we created to score the Lab Math Test (Appendix B in Supporting Information).

We analyzed students' Lab Math Test scores using mixed-model analysis of variance (ANOVA) on IBM SPSS Statistics version 24.0. ANOVA can be used to identify significant differences in means when comparing two or more groups.[32] We used a repeated-measures ANOVA to compare the means of pre-, post-, and delayed posttest scores of a fixed population to measure the significance of time as a variable and to analyze long-term effects. We used a two-way ANOVA to make multiple comparisons of means and detect interactions, such as the effect of condition (inquiry, cookbook) on students' pre- versus posttest scores. We used the Intraclass Correlation Model 3 (average measures) and found the inter-rater reliability to be excellent (Intraclass Correlation Coefficient 99 %). Here we present the data as mean ± standard errors and consider a *p*-value less than 0.05 significant.

RESULTS AND DISCUSSION

We conducted a one-way ANOVA on pretest scores to ensure that randomly distributing students to inquiry and cookbook groups for Fall 1 and 2 did not result in significant, unintended differences in lab math skills between the two groups. The results showed that students' pretest scores did not significantly differ between inquiry (1.18 ± 0.25 (SE)) and cookbook groups (1.10 ± 0.18 (SE)), F (1, 340) = 0.07, p = 0.791, suggesting that on average students in both groups entered with similar levels of prior lab math skills.

Lab Math Skill Gains in Inquiry and Cookbook Sections

Our hypothesis was that learning through a guided-inquiry approach increases students' laboratory math skills more than a traditional cookbook approach. We tested this

hypothesis by conducting a two-way ANOVA on the inquiry and cookbook groups' Lab Math Test scores. We used scores from Fall 1 and 2 in the analysis because both inquiry and cookbook approaches were implemented in *Experimental Biochemistry I* during these two semesters and were able to use this data to compare outcomes between the two learning environments (Figure 1). The posttest math scores for Fall 1 and 2 were significantly higher than the pretest scores for both groups, F (1,680) = 84.05, $p < 0.001$, $\eta^2 = 0.110$ (Figure 2). Students from both groups practiced laboratory calculations through "Math Moment" exercises that we had incorporated in both inquiry and cookbook modules (Appendices C and D in Supporting Information). Scores increased from 1.18 ± 0.25 (SE) to 4.22 ± 0.37 (SE) (pre- to posttest) and 1.10 ± 0.18 (SE) to 2.89 ± 0.25 (SE) for inquiry and cookbook groups, respectively. A 2 x 2 (Time [pretest, posttest] x Condition [inquiry, cookbook]) ANOVA reported a significant interaction between time and condition (F (1,680) = 5.634, $p = 0.018$, $\eta^2 = 0.008$). Students in the inquiry section had more opportunities to practice lab math because they had to perform calculations while designing experiments. As predicted, students in inquiry sections experienced larger gains from pre- to posttest scores compared to cookbook sections, suggesting that the inquiry approach was more effective in increasing students' lab math skills.

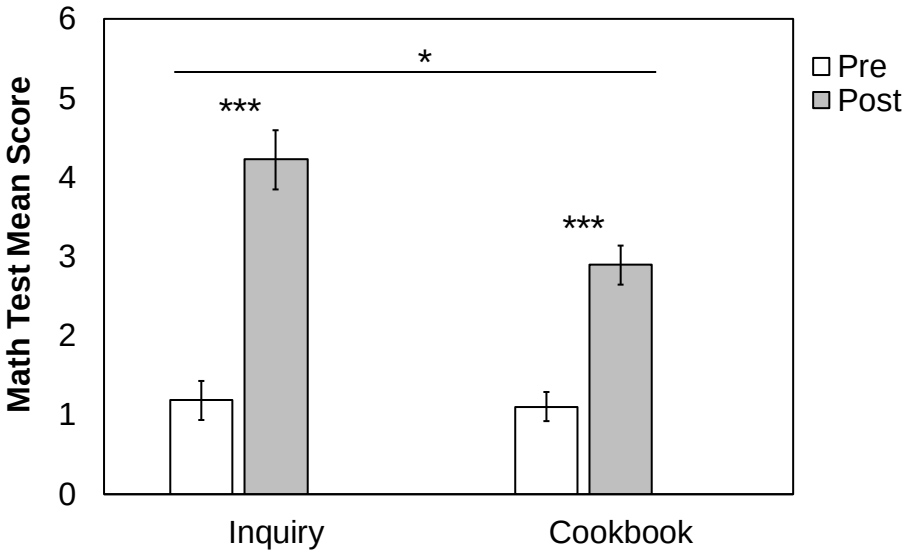

Figure 2. Student gains for pre- and posttest are shown for inquiry (n = 43) and cookbook (n = 71) sections for Fall 1 and Fall 2. A significant increase from pre- to posttest scores was reported for inquiry (F (1,256) = 46.703, $p < 0.001$, $\eta^2 = 0.154$) and cookbook (F (1,424) = 33.531, $p < 0.001$, $\eta^2 = 0.073$) sections. There was also an interaction effect between time (pre- and posttest) and condition (inquiry, cookbook), F (1,680) = 5.634, $p = 0.018$, $\eta^2 = 0.008$. The bars represent mean score and error bars represent standard errors. * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$.

Individual Lab Math Test Rubric Items

To investigate improvement in specific skills measured by the Lab Math Test (Table 1), we analyzed the differences between pre- and posttest scores for individual rubric items using a two-way ANOVA. The analysis revealed a significant improvement in pre- to posttest scores for all rubric items for inquiry and cookbook labs (Figure 3), the statistical values are listed in Table 2. Inquiry labs reported improvement in pre- to posttest scores with relatively large effect sizes $\eta^2 > 0.07$ for all rubric items (Table 2). The difference between pre- and posttest scores for each rubric item was higher for inquiry sections compared to cookbook sections. We observed a statistically significant interaction

between the pre- and posttest scores and the type of condition (inquiry vs. cookbook) for rubric items 2 and 3, F (1,680) = 6.739, p = 0.01, η^2 = 0.016 and F (1,680) = 10.72, p = 0.001, η^2 = 0.016, respectively. Rubric items 2 and 3 tested the ability to perform basic dilution calculations, which is an essential biochemistry laboratory proficiency (Table 1).[30] Students had higher learning gains in dilution calculations in the inquiry environment, perhaps because they practiced these types of calculations as part of experimental design.

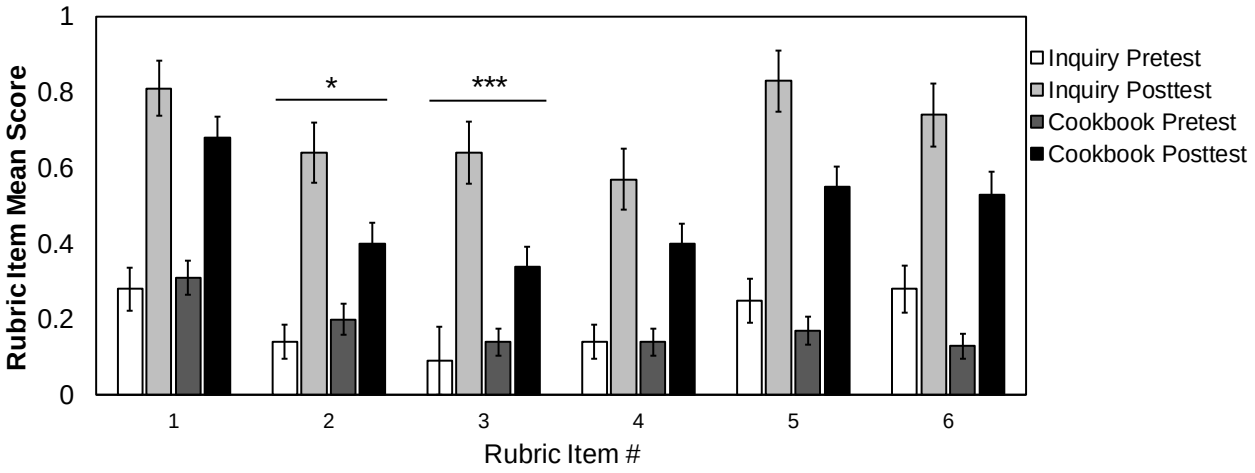

Figure 3. Student gains for individual rubric items are shown for inquiry ($n = 43$) and cookbook ($n = 71$) sections for Fall 1 and 2. An interaction between time (pre-, posttest) and condition (inquiry, cookbook) was reported for rubric item 2 (F (1,680) = 6.739, p = 0.01, $\eta^2 = 0.016$) and rubric item 3 (F (1,680) = 10.72, $p = 0.001$, $\eta^2 = 0.016$). Note: For all six rubric items, the difference between pre- and posttest for both inquiry and cookbook sections were significant, values are listed in Table 2. The bars represent mean score and error bars represent standard errors. * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$.

Table 2. ANOVA results indicating significant improvements in pre- to posttest scores for inquiry and cookbook sections for individual rubric items are shown for Fall 1 and 2 combined.

Rubric Item	Inquiry			Cookbook		
	F	p	η^2	F	p	η^2
1	F (1,256) = 32.747	< 0.001	0.113	F (1,424) = 25.817	< 0.001	0.057
2	F (1,256) = 29.163	< 0.001	0.102	F (1,424) = 8.697	0.003	0.020
3	F (1,256) = 36.079	< 0.001	0.124	F (1,424) = 10.362	0.001	0.024
4	F (1,256) = 22.380	< 0.001	0.080	F (1,424) = 15.942	< 0.001	0.036
5	F (1,256) = 33.969	< 0.001	0.117	F (1,424) = 29.596	< 0.001	0.065
6	F (1,256) = 20.010	< 0.001	0.072	F (1,424) = 33.217	< 0.001	0.073

Long-Term Lab Math Gains – Delayed Posttest

To investigate possible longer-term gains or decay in lab math scores for both learning groups, we gave students the Lab Math Test again in the follow-up course, *Experimental*

Biochemistry II, approximately four months after they had taken the posttest during Fall 2. Only a subset of students from *Experimental Biochemistry I* took the subsequent course. *Experimental Biochemistry II* was a project-based course where groups of students designed and conducted research projects using skills they had learned in *Experimental Biochemistry I*, as a class-based undergraduate research experience.[31, 33] We used data from Year 2 because it was the only year when the delayed posttest test was administered to students who originated from both inquiry and cookbook cohorts. We performed a repeated measures ANOVA for Year 2 (Fall 2 and Spring 2) using data from students that took all three tests (pre-, post- and delayed-posttests) for both inquiry and cookbook sections (Figure 4). The mean test scores for the inquiry and cookbook groups were 1.90 ± 0.83 (SE) and 4.52 ± 0.97 (SE) for pretest, 5.52 ± 0.97 (SE) and 4.86 ± 1.04 (SE) for posttest and 8.10 ± 0.92 (SE) and 6.19 ± 1.16 (SE) for delayed-posttest, respectively. A repeated measures analysis revealed a significant increase in the mean scores for inquiry sections, F (1,20) = 44.310, $p < 0.001$, $\eta^2 = 0.6890$, with time but no significant increase for the cookbook sections, $p = 0.300$. The analysis also revealed an interaction between time and condition, F (1,40) = 6.166, $p = 0.017$, $\eta^2 = 0.134$, suggesting that Lab Math Test scores of students in inquiry sections increased significantly more than students in cookbook sections.

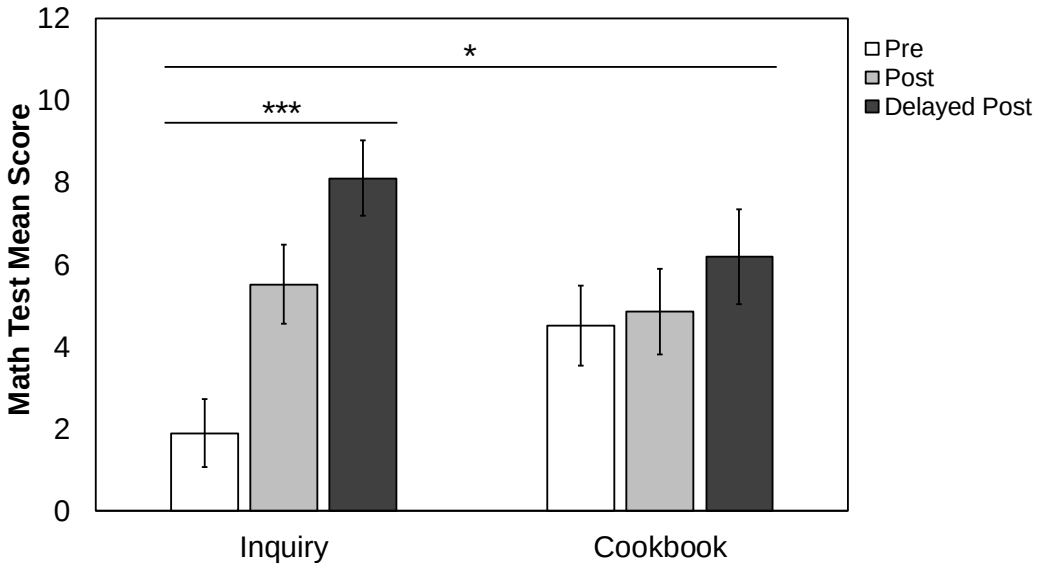

Figure 4. Student gains for pre-, post-, and delayed posttest are shown for inquiry ($n = 7$) and cookbook ($n = 7$) sections for Year 2 (Fall 2 and Spring 2). There was a significant improvement in mean scores for inquiry sections (F (1,20) = 44.310, $p < 0.001$, $\eta^2 = 0.689$), while the improvement was insignificant for cookbook sections. An interaction between time and condition was also reported, F (1,40) = 6.166, $p = 0.017$, $\eta^2 = 0.134$. The bars represent mean score and error bars represent standard errors. * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$.

After a four-month gap between posttest (5.52 ± 0.97 (SE)) and delayed-posttest (8.10 ± 0.92 (SE)), the average scores of students in inquiry sections were higher for the latter test. We find it interesting that students who originated from inquiry sections had significantly larger lab math gains during the second semester course (F (1,20) = 10.885, $p = 0.004$, $\eta^2 = 0.352$) when comparing the difference between post- and delayed posttest, compared to students who had the cookbook version of the first course ($p = 0.355$). It is possible that exposure to inquiry modules and designing experiments primed students to gain more lab math skills during the second semester class-based undergraduate research experience. These findings agree with those from another study, which found that

students exposed to inquiry learning environments transition smoothly to a research-based laboratory setting, as components of these teaching models are similar.[34] Students in a focus group commented that transitioning to a class-based undergraduate research experience was easier for students coming from an inquiry background than those coming from a cookbook section. Students coming from an inquiry background were comfortable and accustomed to designing experiments and making their own decisions, while students from cookbook background found this environment unsettling in the beginning .[22]

Bunce and coworkers concluded that within 48 hours after taking a test, a significant decrease is observed in student knowledge.[35] The effects of the decay can be remediated through multiple opportunities to practice and apply knowledge. Students in both inquiry and cookbook sections were assigned weekly lab math exercises called "Math Moments". Students in the inquiry sections had additional practice in lab math because they had to do calculations while designing experiments (Appendix C in Supporting Information). Frequent practice of applying lab math skills to experimental design may have contributed to significant long-term gains of students who were exposed to inquiry compared to cookbook learning.

Iterative Improvement and Modification of Inquiry Modules

We made minor changes to the inquiry modules and their implementation from year one to year three as previously described.[22] To improve student experience, the instructor explained the value of experimental design ability and understanding the research process in research positions post-graduation. The instructor also worked to provide students more support and encouragement by allowing students to do the first experimental design

in class where she/he can help them. The modules were further altered by incorporating intuitive introductions that explain laboratory concepts using everyday analogies. Further, math problems added to the modules were modified to more concretely relate to the calculations that students must perform during experimental design. Finally, lists of "pit-falls" were included into the modules to address common misconceptions for both the experimental design and the lab itself (Appendix C in Supporting Information).

We measured the changes from pre- to posttest scores for inquiry sections of Fall 1, 2 and 3 using a two-way ANOVA (Figure 5). For Fall 1, the performance on the Lab Math Test improved by 1.62 (posttest – pretest) points from pretest (1.16 ± 0.42 (SE)) to posttest (2.78 ± 0.58 (SE)), F (1,100) = 5.166, p = 0.025, η^2 = 0.049, Fall 2 scores improved by 3.98 points from pretest (1.19 ± 0.FF30 (SE)) to posttest (5.17 ± 0.46 (SE)), F (1,154) = 52.735, $p < 0.001$, η^2 = 0.255, and Fall 3 scores improved by 3.47 points from pretest (3.01 ± 0.30 (SE)) to posttest (6.48 ± 0.36 (SE)), F (1,322) = 54.806, $p <$ 0.001, η^2 = 0.145. A multiple comparison by Bonferroni post hoc correction revealed significant differences in pre- and posttest scores between Fall 1 and 2 (p = 0.047), Fall 2 and 3 ($p < 0.001$) and Fall 1 and 3 ($p < 0.001$). This analysis indicates that changes to inquiry modules were helpful and/or the modules were implemented in a more effective manner during years 2 and 3 compared to year 1.

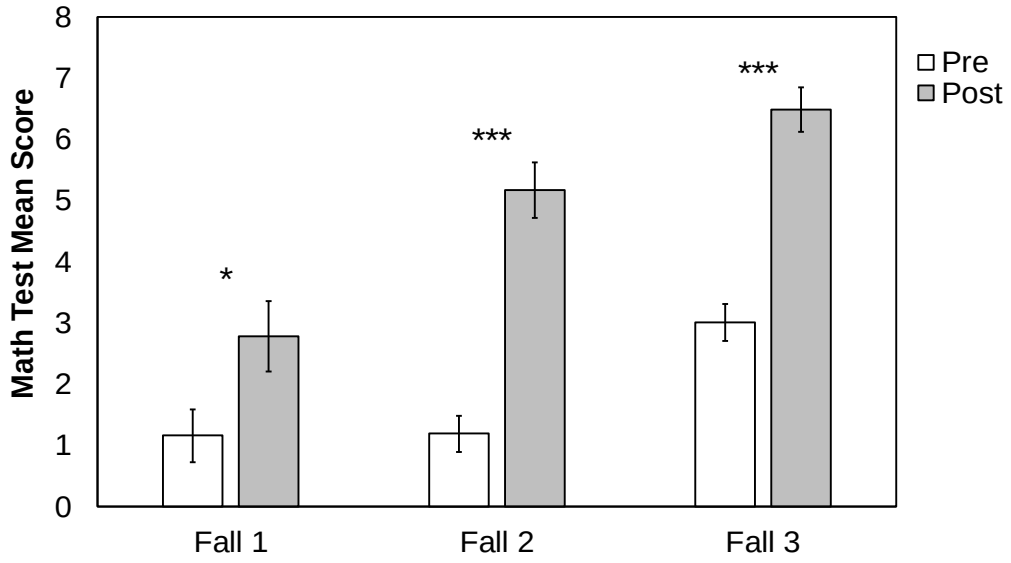

Figure 5. Pre- and posttest scores are shown for inquiry sections for Fall 1 (n = 17), Fall 2 (n = 26) and Fall 3 (n = 54). The difference in pre- to posttest score was statistically significant for Fall 1 (F (1,100) = 5.166, p = 0.025, η^2 = 0.049), Fall 2 (F (1,154) = 52.735, $p < 0.001$, η^2 = 0.255), and Fall 3 (F (1,322) = 54.806, $p < 0.001$, η^2 = 0.145). A Bonferroni post hoc correction revealed significant differences in pre- and posttest scores between Fall 1 and 2 (p = 0.047), Fall 2 and 3 ($p < 0.001$) and Fall 1 and 3 ($p < 0.001$). The bars represent mean scores and error bars represent standard errors. Statistical significance was determined using F-test. * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$.

Effect of Instructor on Student Performance

We evaluated the magnitude of the instructor effect using a two-way ANOVA. The analysis was performed separately for inquiry and cookbook sections, bearing in mind the different levels of instructor involvement in these two learning environments. In cookbook labs, the instructors were readily available to provide assistance, but student dependence on instructors was minimal due to the availability of a step-by-step experiment protocol. In inquiry labs, students were encouraged to think critically and solve problems using inquiry but may have been more depended on instructors for

guidance and coaching. The analysis reported no interaction between the pre-/posttest scores and cookbook instructors or inquiry section instructors, suggesting that instructor was not a main factor responsible for student performance on the Lab Math Test.

CONCLUSIONS AND IMPLICATIONS

Professionals collectively acknowledge the importance of learning laboratory math skills for a successful career and yet there is little research that explores teaching models that increase the learning of basic quantitative skills in biochemistry laboratory classes. Instructors may view these skills as basic and expect students to come well prepared as they enter college, yet we know this is not the case. In this study, we found that guided-inquiry learning effectively enhanced students' lab math ability compared to traditional cookbook learning. In a prior study, guided-inquiry learning was also proven effective in increasing students' experimental design ability and the increase in basic lab math skills can be viewed as a positive side-effect.[22] Inquiry learning in the laboratory can take many forms and the details for our implantation have been previously published.[22] In our modules, the inquiry approach provided students additional opportunities to practice lab math as part of designing and troubleshooting experiments. Our results cannot be used to identify the mechanism by which inquiry modules increase lab math skills but they do imply that traditional laboratory learning approach does not always optimally serve to improve students' lab math skills or experimental design ability.

The lack of validated assessment tools to investigate lab math skill gains, especially in the context of experimental design, encouraged us to write the Lab Math Test in consultation with four biochemistry faculty members. We created an assessment

that tested students' ability to do lab math in the context of setting up a biochemical assay. We hope that our findings encourage others to create new assessment instruments and systematically investigate other strategies that increase students' lab math skills in the biochemistry laboratory setting, without sacrificing other laboratory skills. A study employing a control group is useful for investigating math gains because science majors typically take multiple undergraduate courses each semester that may affect their math skills. A comparison of an intervention and a control group makes it possible to investigate the effectiveness of a teaching approach within a particular course. Findings from such studies can benefit both instructors, who can choose effective strategies to teach, and students, who can achieve higher gains when exposed to more effective learning environments.

There are limitations that must be taken into account when considering our findings. First, this study was conducted in one university in two biochemistry laboratory courses. To conclude that inquiry learning more effectively supports lab math learning in different settings, we would need to replicate this study in different types of institutions with diverse student populations. There are interesting opportunities to do so as the inquiry approach can be implemented on existing laboratory curricula in different courses using many types of experiments. Second, our findings are specific to the modules we created for this study using a common theme, dihydrofolate reductase (available at http://www.montclair.edu/csam/nsf-tues-grant/). To generalize our findings to other inquiry experiences, results of other inquiry style sequences for a laboratory would need to be created and studied. Third, the population that took the delayed posttest was small. The findings, however, are statistically significant and interestingly suggest that inquiry

learning in the fall primed students for greater improvement in lab math skills during the class-based research experience in the spring. The two-semester model with a guided-inquiry experience followed by a CURE with overlapping experimental methods may be of interest to faculty who are interested in offering students supported research experiences in a classroom environment. Fourth, it is possible that just the act of taking the assessment three times (with different numbers) resulted in increased scores. Nevertheless, this "learning from the test" phenomenon would not explain the statistically significant difference between the inquiry and cookbook groups. Finally, the students in this study were upperclassmen who came from various mathematical backgrounds and were simultaneously enrolled in other math and/or science courses, which may have influenced their performance on the Lab Math Test.

It would be interesting to further investigate the priming effect we observed in this study, where students from inquiry sections experienced larger gains in the class-based research experience compared to students from cookbook sections. We do not know the mechanism that resulted in the higher learning gains for students who had been exposed to the inquiry experience and whether the increases in skills were specific to participation in *Experimental Biochemistry II*. It would be interesting to assess student learning gains from both environments who did or did not proceed to *Experimental Biochemistry II.* Lab math may not be the first skill that comes to mind when we think about inquiry learning, which is often associated with improved ability to design and trouble-shoot experiments. Yet these data suggest that inquiry learning, which tends to better engage students in the science laboratory setting,[22] also has the desirable side effect of improving students' lab math skills.

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SUPPORTING INFORMATION

Appendix A: Lab Math Test

CODE: _____

You are running an experiment, and, in the experiment, you have four components (all liquids): NADPH, DHF, DHFR, and Buffer. You are examining the effect of the concentration of DHF on the experimental result. You need to examine 8 different concentrations between 0 and 100 μM of the compound DHF. A 200 μL total volume will be used for each experiment. Concentrations in the assay (final concentrations) must be NADPH (100 μM) and DHFR (0.08 μM). If you choose to make an intermediate dilution of DHF, you must indicate the concentration of the intermediate stock and describe how you would make it. Given this information, design the composition of the solutions in a 200 μL total volume for the 8 experiments and fill the table below accordingly.

You are provided the following solutions.

NADPH (2 mM)

DHF (2.9 mM)

DHFR (1.2 μM)

Buffer

Final Concentration of DHF in Cuvette (μM)	**Volume of NADPH (μL)**	**Volume of DHFR (μL)**	**Volume of DHF (μL)**	**DHF stock concentration (μM) used.**	**Volume of buffer (μL)**

Appendix B: Grading Rubric for Lab Math Test

Lab Math Test Scoring Rubric and Training Manual

1st column
If student writes down a range of reasonable numbers, give them 1 point.
If the range is correct based on the question, they get 2 points. To get 2 points, they must cover >70% of the range and five or more numbers are within the range. *Note: If there are several points in the correct range, and a few outside the range, they can still get 2 points.*

2nd column
If they get the correct answer (10 for all rows), they get 2 points. There is no partial credit.

3rd column
If they get the correct answer, then they get 2 points (13.3). There is no partial credit.

5th column (GRADE 5th column (item 5) before 4th column (item 4))
If they write 2900 here, give them 2 points. If 2.9 but no units, give them1 point.

If different from 2900, if they describe (even briefly) the dilution they chose, and it makes some sense, give them 2 points. If they do not describe the dilution at all, give them 0. *GRADE this before column 4.*

4th column
Calculate whether their answer is correct for first, 4th and last row. Base this grade on column 1 and 5 answers. If all are correct, then assign 2 points. If some are correct but others not, give 1 point.
Note: column four can be right even if they get 0 points for column 5.

6th column
Calculate to determine whether their answer is correct for first, 4th and last row. If yes, then give them 2 points. If some are correct, then give them 1 point. If none are correct, then give them 0.
Note: They can also do a range of DHF stock concentrations and add the same DHF volume each time and get 2 points as long as their calculation is correct.

Appendix C: Sample Inquiry Module

Module 8. Effect of the DHFR inhibitor Trimethoprim on DHFR catalytic activity. Inquiry version

1. Introduction

The activity of the enzyme DHFR can be inhibited by binding of a specific small molecule called trimethoprim (TMP). TMP is a competitive inhibitor and it blocks the active site, preventing the substrate DHF from binding. The more TMP you add to the reaction, the lower the enzyme activity is. TMP is like the glue in a lock that blocks the key from being inserted.

At very high TMP concentrations, the enzyme activity will be so low that there is no detectable decrease in the absorbance at 340 nm over time when DHFR is mixed with NADPH and DHF. On the other hand, at very low concentrations of TMP, the data looks similar to what was observed in the absence of inhibitor, i.e. there is full enzyme activity.

- The activity in the absence of inhibitor is considered 100% activity.
- The activity in the presence of a very high concentration of inhibitor is considered to be 0% activity (essentially inactive).
- The concentration at which we see 50 % activity (midpoint between 0% and 100 % activity) is called IC50, the half maximal Inhibitory Concentration.

The IC50 is a measure of the effectiveness of a substance in inhibiting a specific enzyme catalyzed reaction. It indicates how much of a particular inhibitor is needed to inhibit a given reaction by half.

Please bring a memory stick where your collected data can be stored! The data can be shared later between all lab members via email or everyone can save the data to their own memory stick. Please delete your data from the computer in the lab once you save it on your memory stick.

2. Purpose of the lab

To plan and conduct an experiment to examine the effect of the inhibitor TMP on the rate of the DHFR catalyzed reaction (DHF + DHFR + NADPH → THF + DHFR + NADP+). You will use the resulting data to determine the IC50 of TMP for DHFR.

Your assignment is to plan and conduct an experiment to examine the effect of TMP (inhibitor) concentration on the time dependence of the DHFR catalyzed reaction. You will determine an IC50 value for the inhibitor

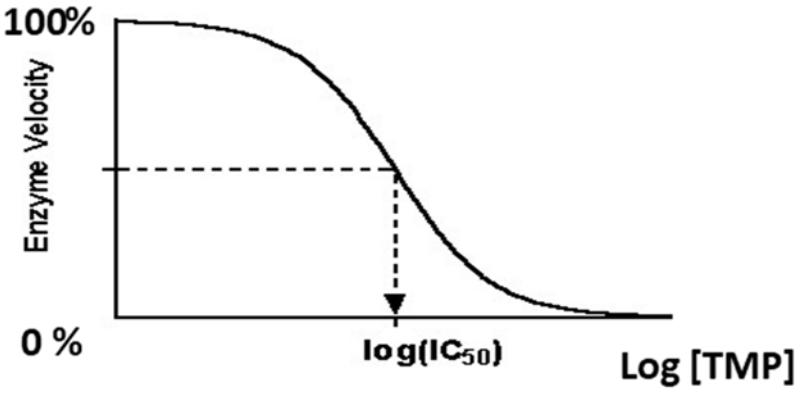
3. Agenda for the Day

- You must have worked through the Math Moment problems before coming to class. Upon entry, each student shows their work to the instructor to receive points.

- You must have prepared a protocol for the experiment, there will be time for last minute questions.
- Instructor presentation on measuring inhibition of catalytic activity.
- In class, groups review Math Moment Problems to prepare for experimental design. Each student hands in their experimental protocol.
- Conduct the experiment in groups. Each student individually records data in their personal laboratory notebook.
  Clean up

4. Background

- Useful information can be found in Chapter 8B. Please, read this section.
- We are studying the same enzyme as in Module 7 (DHFR); review Module 7 if needed. Here we study the effect of a DHFR inhibitor, Trimethoprim (TMP), on the rate of the reaction and determine its IC50.
- The assay will be conducted in a microtiterplate. Different wells will be identical reactions except that each one will have a different TMP (inhibitor) concentration.
- A solution called ENZYMEMIX is provided that contains buffer, NADPH (118 μM) and the DHFR enzyme (77nM).
- DHF is provided at a concentration of 667 μM. It is a suspension and you must mix it before adding it to the wells. DHF is light sensitive and must be protected from light as much as possible during the experiment (use aluminum foil for this purpose).
- The IC50 is the inhibitor concentration at which the initial reaction velocity is half the maximal value. To determine IC50, you can simply plot the slopes of the initial velocity data on the Y-AXIS against the log[TMP] values in the respective wells on the X-AXIS. This graph should give a "backwards S" shape. Inspect the graph to estimate the concentration of TMP that gives half maximal slope on the Y-axis.



5. Math Moment

1. Look at the serial dilution table below. Fill in the final concentrations.

Dilution		Volume of buffer to add (µL)	Total volume (µL)	Final concentration of TMP (nM)
D1	100 µL of TMP stock (500 nM)	0	100	
D2	50 µL of D1	50	100	
D3	50 µL of D2	50	100	
D4	50 µL of D3	50	100	
D5	50 µL of D4	50	100	
D6	50 µL of D5	50	100	
D7	50 µL of D6	50	100	

2. Look at the data above. If 15 µL of each dilution was added to a well and the final well volume was 200 µL, what are the final concentrations in the wells? Note: D1 solution went to well A1, D2 to well A2 etc. Please provide final well concentrations of TMP in the table below:

Dilution	Final TMP Concentration in Well (nM)
D1	
D2	
D3	
D4	
D5	
D6	
D7	

3. How would you set up a serial dilution scheme in 7 Eppendorf tubes to cover the range 500 nM to 8 nM starting with a 500 nM stock of TMP? Please show your dilution scheme.

4. How would set up a serial dilution to cover a concentration range from approximately 37 nM to 0.6 nM of TMP in an assay (in the well) having a total volume of 200 µL of which 15 µL is TMP solution? You are provided a 500 nM TMP stock solution.

6. Supplies Provided

- Ice buckets and ice
- Micropipetters and tips
- Multichannel pipettor
- 96-well microtiter plates
- Eppendorf tubes
- UV-VIS plate reader
- Buffer (40 mM HEPES at pH 6.8)
- ENZYMEMIX solution containing DHFR enzyme at 77 nM and NAPDH at 118 µM in 40 mM Hepes pH 6.8
- DHF solution at 667 µM
- Trimethoprim stock at 500 nM
- Aluminum foil

7. Advice for experimental design

- The final assay volume in the microtiter plate is 200 µL.
- You will be provided with 40 mM HEPES buffer at pH 6.8.
- You will be provided with a ENZYMEMIX solution that contains DHFR at a concentration of 77 nM and NADPH at 118 µM. You will add 170 µL of this solution to each well to obtain the desired concentrations of NADPH and enzyme (DHFR).
- You will set up an assay to determine the IC50 of TMP. Use the following concentrations in the assay well: 100 µM NADPH, 100 µM DHF, 65.5 nM DHFR (enzyme) in 40 mM Hepes, pH 6.8.
- You will be provided with a 500 nM TMP stock solution. You will make seven serial dilutions of this solution. The concentrations will range between 500 nM and 7.8 nM. Note that these are the dilutions in the Eppendorf tubes, not in the wells. You must calculate the concentrations of TMP in the wells based on the volumes used.
- When making the serial dilutions, mix each dilution before making the next one. You will have a total of 7 solutions with different TMP concentrations.
- You will be provided 200 µL DHF solution at 667 µM. You will add 15 µL of this solution to each well. Note, mix everything else together first and add the DHF last to initiate the reaction when ready to read the plate.

- Read your assay plate in the plate reader at a wavelength of 340 nm.
- Check your activity vs. inhibitor concentration right when you get the data and be prepared to repeat experiment with a different set of inhibitor concentrations. BRING A COMPUTER.
- Think about the controls you will need for your assay. For example, you will need a control to determine what happens when there is enzyme and substrate but no inhibitor in the well (this would give you 100% activity). For this use buffer instead of TMP.
- For your protocol, you must provide the exact volumes of each dilution, what you will add to the wells etc. Additionally, you must calculate the total volumes of each solution you need for your protocol. Multiply this volume by 1.25 to make sure you prepare enough of each solution.

8. Common Mistakes and Some Advice

- Remember good pipetting technique. Use the correct pipettor (P20, P200, or P1000) for the volume you measuring. Please, handle pipettors gently.
- In solution, DHF is a suspension. If the suspension is left sitting for a while the solid and liquid phases separate.
    - Therefore, before you use DHF, mix it vigorously.
- DHF is sensitive to light, keep it covered with foil when not using it.
- MAKE SURE TO LABEL TUBES AND RECORD THE WELLS.
- Be careful with pipetting. If too forcefully pipetted, reagents may splash into other wells.

9. Vocabulary

Catalytic activity, inhibitor, IC50

10. Safety

You must wear safety glasses when conducting the experiment. You must never eat or drink in the laboratory. You will need to wait in line to use the plate reader. Please, be patient when waiting for your turn to use the plate shaker and plate reader. Be gentle with the plate reader and plate shaker, these are delicate instruments. Any observed violations of these rules will result in lower final grade and/or removal from the lab. These safety items are solely the responsibility of the student.

11. Clean up

For clean-up, return the remaining original solution to the instructor. Discard dilutions in the sink and eppendorf tubes in the regular trash. Do not throw anything in the biohazard

waster. Wash 96-well plates and leave them at the sink to dry. Mark the well that you used with a marker. Return pipettors in the correct boxes, the last person puts the boxes in the cabinet in the back of the lab. Dry your ice buckets and place them back in the cabinet. Place all other items where you got them from. Make sure they are clean. Leave your bench ready for the next class to start working.

12. Homework

Data from modules 7 and 8 will combined into a formal lab report (one per group). For module 7, prepare a graph that shows the absorbance vs. time data for each of the enzyme concentration. Be sure to label the axes. When analyzing data from module 7, you should be able to use a few of your slopes (derived from a few wells) to calculate kcat. The ones that finished too quickly or too slowly will not be useful. Remember to use the initial slope (initial linear decrease) to obtain your slope (Δabsorbance/Δtime). Then convert it to Δ[DHF]/Δtime. Finally, divide by [E] to determine kcat.

For Module 8, you will need to plot the slope of your (Δabsorbance/Δtime) on the Y-AXIS against the log[TMP] on the X-AXIS. You will then need to determine the IC50 value by identifying the concentration of TMP that gives you ½ of the maximum velocity (slope). This value will be your IC50. Note that your data on the Y-AXIS will span from 100% activity (no inhibitor) to 0 % activity (slope of about 0). Remember to take the antilog before reporting your concentration. Pay attention to the units on your X-axis when reporting your value.

Appendix D: Sample Cookbook Module

Module 8. Effect of the DHFR inhibitor Trimethoprim on DHFR catalytic activity. Cookbook version

1. Introduction

The activity of the enzyme DHFR can be inhibited by binding of a specific small molecule called trimethoprim (TMP). TMP is a competitive inhibitor and it blocks the active site, preventing the substrate DHF from binding. The more TMP you add to the reaction, the lower the enzyme activity is. TMP is like the glue in a lock that blocks the key from being inserted.

At very high TMP concentrations, the enzyme activity will be so low that there is no detectable decrease in the absorbance at 340 nm over time when DHFR is mixed with NADPH and DHF. On the other hand, at very low concentrations of TMP, the data looks similar to what was observed in the absence of inhibitor, i.e. there is full enzyme activity.

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The IC50 is a measure of the effectiveness of a substance in inhibiting a specific enzyme catalyzed reaction. It indicates how much of a particular inhibitor is needed to inhibit a given reaction by half.

Please bring a memory stick where your collected data can be stored! The data can be shared later between all lab members via email or everyone can save the data to their own memory stick. Please delete your data from the computer in the lab once you save it on your memory stick.

2. Purpose of the lab

To plan and conduct an experiment to examine the effect of the inhibitor TMP on the rate of the DHFR catalyzed reaction (DHF + DHFR + NADPH → THF + DHFR + NADP+). You will use the resulting data to determine the IC50 of TMP for DHFR.

Your assignment is to examine the effect of TMP (inhibitor) concentration on the time dependence of the DHFR catalyzed reaction. You will determine an IC50 value for the inhibitor.

3. Agenda for the Day

- You must have worked through the Math Moment problems before coming to class. Upon entry, each student shows their work to the instructor to receive points.
- Instructor presentation on measuring inhibition of catalytic activity.

- In class, groups review Math Moment Problems.
- Conduct the experiment in groups. Each student individually records data in their personal laboratory notebook.
- Clean up

4. Background

- Useful information can be found in Chapter 8B. Please, read this section.
- We are studying the same enzyme as in Module 7 (DHFR); review Module 7 if needed. Here we study the effect of a DHFR inhibitor, Trimethoprim (TMP), on the rate of the reaction and determine its IC50.
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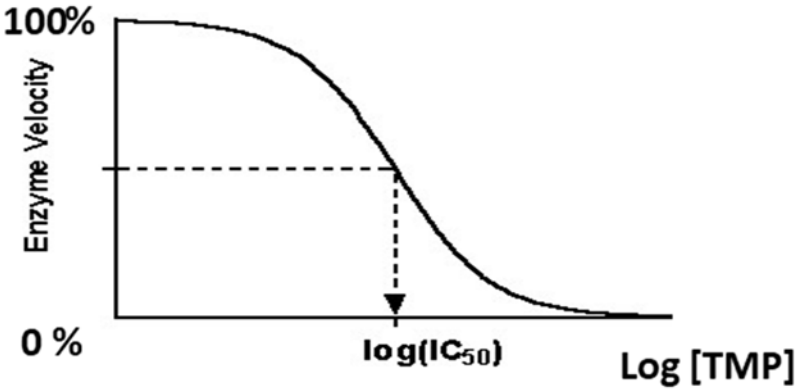
- A solution called ENZYMEMIX is provided that contains buffer, NADPH (118 μM) and the DHFR enzyme (77nM).
- DHF is provided at a concentration of 667 μM. It is a suspension and you must mix it before adding it to the wells. DHF is light sensitive and must be protected from light as much as possible during the experiment (use aluminum foil for this purpose).
- The IC50 is the inhibitor concentration at which the initial reaction velocity is half the maximal value. To determine IC50, you can simply plot the slopes of the initial velocity data on the Y-AXIS against the log[TMP] values in the respective wells on the X-AXIS. This graph should give a "backwards S" shape. Inspect the graph to estimate the concentration of TMP that gives half maximal slope on the Y-axis.



5. **Math Moment**

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D4	50 µL of D3	50	100	
D5	50 µL of D4	50	100	
D6	50 µL of D5	50	100	
D7	50 µL of D6	50	100	

2. Look at the data above. If 15 µL of each dilution was added to a well and the final well volume was 200 µL, what are the final concentrations in the wells? Note: D1 solution went to well A1, D2 to well A2 etc. Please provide final well concentrations of TMP in the table below:

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D5	
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3. How would you set up a serial dilution scheme in 7 Eppendorf tubes to cover the range 500 nM to 8 nM starting with a 500 nM stock of TMP? Please show your dilution scheme.

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6. Supplies Provided

- Ice buckets and ice
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- Multichannel pipettor
- 96-well microtiter plates
- Eppendorf tubes
- UV-VIS plate reader
- Buffer (40 mM HEPES at pH 6.8)
- ENZYMEMIX solution containing DHFR enzyme at 77 nM and NAPDH at 118 µM in 40 mM Hepes pH 6.8 buffer
- DHF solution at 667 µM
- Trimethoprim stock at 500 nM
- Aluminum foil

7. Experimental Protocol

1. Label 8 tubes D1 – D7.

2. Make serial dilutions of the trimethoprim stock in small Eppendorf tubes as shown in the table below. Mix each dilution vigorously before making the next dilution. For example, mix D2 vigorously before making D3. Fill in the final concentrations in the table below.

Dilution		Volume of buffer to add (µL)	Total volume (µL)	Final TMP concentration (nM)
D1	100 µL of TMP stock (500 nM)	0	100	
D2	50 µL of D1	50	100	
D3	50 µL of D2	50	100	
D4	50 µL of D3	50	100	
D5	50 µL of D4	50	100	
D6	50 µL of D5	50	100	
D7	50 µL of D6	50	100	

3. Mix the ENZYMEMIX (provided). Place 170 uL of ENYMEMIX in wells A1 – A8 in a microtiter plate.

4. Add 15 µL of buffer to well A8. This is your "no inhibitor" control.

5. Add 15 µL solution D7 to well A7. Add 15 µL solution D6 to well A6.
   Add 15 µL solution D5 to well A5.
   Add 15 µL solution D4 to well A4.
   Add 15 µL solution D3 to well A3.
   Add 15 µL solution D2 to well A2.
   Add 15 µL solution D1 to well A1.

Wait 1-2 minutes.

6. Add 40 µL of the provided solution of DHF (667 µM) to wells B1 – B8 (Not experimental wells). Mix DHF solution each time before you pipet from it.

7. You must do the next steps (up to when you hit read on the plate reader) as quickly as you can because the reaction will start when you add substrate (DHF). Go to the instrument, make sure all settings are ready to go. Then place plate on the tray. Only then do the following. Use a multichannel pipettor to gently mix the solution in the wells B1-B9 and then transfer 15 µL of solutions (from wells B1-B8 to wells A1-A8).

8. Read the plate (10 minutes).

9. Look at your data. Be prepared to repeat the experiment if necessary.

8. Common Mistakes and Some Advice

- Remember good pipetting technique. Use the correct pipettor (P20, P200, or P1000) for the volume you measuring. Please, handle pipettors gently.
- In solution, DHF is a suspension. If the suspension is left sitting for a while the solid and liquid phases separate.
  Therefore, before you use DHF, mix it vigorously.
- DHF is sensitive to light, keep it covered with foil when not using it.
- MAKE SURE TO LABEL TUBES AND RECORD THE WELLS.
- Be careful with pipetting. If too forcefully pipetted, reagents may splash into other wells.

9. Vocabulary

Catalytic activity, inhibitor, IC50

10. Safety

You must wear safety glasses when conducting the experiment. You must never eat or drink in the laboratory. You will need to wait in line to use the plate reader. Please, be patient when waiting for your turn to use the plate shaker and plate reader. Be gentle with the plate reader and plate shaker, these are delicate instruments. Any observed violations of these rules will result in lower final grade and/or removal from the lab. These safety items are solely the responsibility of the student.

11. Clean up

For clean-up, return the remaining original solution to the instructor. Discard dilutions in the sink and eppendorf tubes in the regular trash. Do not throw anything in the biohazard waster. Wash 96-well plates and leave them at the sink to dry. Mark the well that you used with a marker. Return pipettors in the correct boxes, the last person puts the boxes in the cabinet in the back of the lab. Dry your ice buckets and place them back in the cabinet. Place all other items where you got them from. Make sure they are clean. Leave your bench ready for the next class to start working.

12. Homework

Data from modules 7 and 8 will combined into a formal lab report (one per group). When analyzing data from module 7, you should be able to use a few of your slopes (derived from a few wells) to calculate kcat. The ones that finished too quickly or too slowly will not be useful. Remember to use the initial slope (initial linear decrease) to obtain your slope (Δabsorbance/Δtime). Then convert it to Δ[DHF]/Δtime. Finally, divide by [E] to determine kcat.

For Module 8, you will need to plot the slope of your (Δabsorbance/Δtime) on the Y-AXIS against the log[TMP] on the X-AXIS. You will then need to determine the IC50 value by identifying the concentration of TMP that gives you ½ of the maximum velocity (slope). This value will be your IC_{50}. Note that your data on the Y-AXIS will span from 100% activity (no inhibitor) to 0 % activity (slope of about 0). Remember to take the antilog before reporting your concentration. Pay attention to the units on your X-axis when reporting your value.