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Article

Bi-National and Interdisciplinary Course in Enzyme Engineering*

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Higher education institutions and scientific funding agencies are emphasizing international projects that involve the integration and synergy between research groups, particularly if different disciplines are involved. Students with an education that reflects these trends will have more tools to succeed in the future, but it is challenging to provide this type of learning experience. Here we present the organization of a bi-national course with the goals to teach students protein structure/function relationships, which give them actual research experience in both computational and experimental laboratories, and engage them in an international networking experience. Two collaborative learning courses were organized at Loyola University Chicago (USA) and Universidad Nacional del Litoral (Argentina) for graduate and advanced undergraduate students. Multiple instructors at different stages in their careers gave lectures during the course and were able to interact with students on a one-on-one basis. Nearly every student from both institutions thoroughly enjoyed this approach, and they learned more about protein structure and gained important tools for their own research. We believe that this type of course design is applicable and transferable to other institutions and areas of science. We found that the combination of international networking and incorporation of actual research projects ignited the enthusiasm of students and instructors. Due to the success of these courses, we planned to incorporate them as regular series in our curriculum.

Keywords: protein structure, bioinformatics, computational chemistry, site-directed mutagenesis, international education.

Modern biochemistry is an international discipline, but education of biochemists is a local activity. To promote an international educational experience we have developed an intensive course with three main goals. First, the students learned cutting-edge topics on protein structure/function relationships, with a view of how to apply techniques for enzyme engineering. Second, students experienced actual research projects in the laboratory sessions. This may encourage and prepare them to become active members of a research group. Third, we implemented an international networking experience for both students and teachers.

The value of these objectives has been discussed in the literature [1–4], but these goals have rarely been incorporated into a single course design. While the benefits of research are obvious at the graduate level, the need for an undergraduate research experience has also been recognized. In the United States, the Boyer report on reinventing undergraduate education (http://naples.cc.sunysb.edu/Pres/boyer.nsf/TheBoyerReport.html) emphasized the importance of undergraduate involvement at research universities for increasing student retention. This is true at smaller schools as well [1, 5], but it presents some challenges due to constraints in faculty time and research facilities [5]. These studies establish that research motivates undergraduates by increasing their involvement, while also increasing the satisfaction that faculty gain through teaching [1]. Research for undergraduates has often been through independent experiences, such as the very successful Research Experiences for Undergraduates programs funded by the National Science Foundation [6]. For most schools this is not an easy option [5], however, many do an outstanding job at engaging undergraduates in independent research projects. This type of experience has been increasing at many schools in recent years [3]. Another way to approach this problem is to increase the number of research-based courses [1], which can provide a more cost-effective solution [5].

Collaborative learning laboratory courses have been used at a number of schools to involve students in a research experience, ranging from a single semester [7,

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8] to several year programs [5]. The courses can be either laboratory-only [7] or combined with lecture [8]. Large scale course-based collaborative research projects, such as the genomics education partnership (GEP) centered at Washington University in St. Louis [9], involve undergraduates and result in publication of new data. The GEP demonstrated that this approach provided results similar to individual research. Both approaches were better over a wide range of educational objectives than traditional courses without research [9].

The interaction with international experts has been shown to be beneficial for students [4], but providing an international experience for them is a more challenging goal. Cultural diversity is often addressed in terms of ethnic differences within a country like the USA [10], rather than actual international experience. To achieve this goal, a shorter-course format is more practical since faculty and students from other countries cannot normally leave their home institutions for extended periods. This format offers the possibilities of broader course selection and more student-networking in smaller programs [4].

To address these issues, we organized two courses to expose students to multiple techniques and expertise from scientists of different cultures, with diverse pedagogic styles, and who are at different stages in their careers. One goal is that students understand enzyme structure through experimental approaches and another one is to allow them to experience research from several perspectives, while giving instructors flexibility in lectures and laboratory exercises.

COURSE CONTENTS AND DESIGN

Rationale

To fulfill our main goals of learning protein structure/function relationship, experiencing actual research projects, and implementing international networking, we had to consider several logistics. We wanted to strengthen ties between two Universities (Loyola University Chicago (LUC)[1], IL, USA, and Universidad Nacional del Litoral (UNL), Santa Fe, Argentina) extending research collaborations and teaching courses for advanced undergraduate and graduate students in their fields of expertise. UNL has a strong program in plant biochemistry, whereas LUC can provide advanced computational and experimental techniques not available at UNL. For instance, the specific course content depended in part on the facilities available at the host institution. UNL had an outstanding computational teaching laboratory and continuous support staff to maintain it, but had fewer experimental laboratory resources devoted to the course. While LUC had excellent computational facilities available, it could also provide a laboratory and support for the experimental portion. Since the courses were the same length at both institutions, some of the computational experiments were not performed at LUC. Instead, these were replaced by an experimental laboratory that demonstrated mutagenesis techniques applied to an enzyme-engineering problem. To give students a broader background we combined computational and experimental laboratory experiences. We are not expecting students to become experts during the 2-week period; but they should gain enough confidence to try both approaches in their own research laboratories.

In the USA, it is common for humanities and social science undergraduates to spend a semester overseas, but it is less common in the sciences due to the sequential structure of the curriculum. Thus, this international aspect is particularly important for the LUC undergraduates. To give students a unique international experience we incorporated multiple instructors and students from different institutions. In addition, it allowed us to expand our research horizons by establishing interdisciplinary collaborations.

An important aspect of the courses was that students work on real scientific problems during the laboratory sessions rather than performing a series of well-established experiments. While this may lead to unexpected difficulties, it is extremely advantageous for the student to learn how to approach a problem in an actual research environment. Moreover, their enthusiasm and involvement significantly increases with this approach. They also gain an appreciation for research difficulties in a controlled environment where they are likely to obtain positive results in a short time. This means the course organizers must select a problem that is easy to solve using the techniques discussed. The small project should be successful, but at the same time should not be something already reported in the literature. Thus, the results will be uncertain to some extent and should encourage discussion and analysis. Based on our experience, we strongly believe that a reasonable risk factor should be introduced into the classroom with different systems.

General Organization

Two workshop-style courses were held; one was at UNL during July 2007 and the other at LUC in June 2008. These correspond to winter and summer breaks at the institutions, respectively. Both intensive courses comprised 2 weeks of full-time (7–8 hours per day) training in experimental and computational methods. The structure of the courses became one of "immersion" since students were exposed to both lectures and laboratory work without interruption.

The overall design was to alternate lectures and laboratory experiences so that students did not become overwhelmed. Lectures were given in English in both countries, and were limited to two per day and were usually 1–1.5 hours with plenty of time for discussion and questions. This allowed Argentine students to practice English, which is essential to their development towards a scientific career. At the end of the courses, students from both institutions gave oral presentations discussing how they would use information from the course in their own research.

Personnel

Two professors from LUC taught the first course at UNL with the assistance of two professors from UNL. A graduate student from LUC was an assistant in the com-

[1]The abbreviations used are: LUC, Loyola University Chicago; UNL, Universidad Nacional del Litoral; ADP-Glc PPase, ADP-glucose pyrophosphorylase.

puter laboratory section along with technical support from two part-time information technology technicians from UNL. In this course, there were 5 undergraduate and 18 graduate students from different cities of Argentina (Santa Fe, Buenos Aires, Rosario, Córdoba, and Mar del Plata). The graduate student assistant from Chicago (USA) also participated. At LUC, lectures were given by a professor and a graduate student from UNL, two professors from LUC, and an invited guest speaker from the University of Michigan. The laboratory portion was taught by a graduate student from LUC and the same graduate student from UNL. The class at LUC consisted of 12 graduate students and 3 undergraduates enrolled at the host university.

Lectures

Lectures were designed to expose students to a broad variety of computational and experimental approaches to enzymology. The aim was to select topics that would be applicable for many different areas of protein biochemistry, so each student would be able to apply some techniques to their own research. Since this course was composed of both advanced undergraduates and graduate students with a variety of backgrounds, it was necessary to briefly introduce protein structure and enzymology at the beginning of the course. These topics were followed by a discussion of structure visualization techniques, which are a critical part of protein science. Additional topics included (in the order presented) homology modeling and model verification, protein purification, saturation mutagenesis, X-ray structure determination, chemical modification, and molecular dynamics (Table I). Most of the topics presented in class were reinforced by laboratory experience, although protein purification, saturation mutagenesis, X-ray crystallography and chemical modification were not, due to the time constraints of a 2-week course. Lectures were in the form of PowerPoint slides and all course materials were made available on Blackboard (http://www.blackboard.com/). Each day, the lecture was immediately followed by both computational and experimental laboratory exercises.

Laboratory

The laboratory section of the course was designed for students to investigate a research question not answered in the scientific literature. We chose the control enzyme of the glycogen/starch biosynthetic pathway, ADP-glucose pyrophosphorylase (ADP-Glc PPase) [11, 12], because several of the instructors use it in their own research. Our goal was to use both computational and experimental approaches to determine if two residues in the active site of *Escherichia coli* ADP-Glc PPase were necessary for catalysis. There were sufficient computers available for them to perform computations; however, there were not enough resources available for each student to have their own equipment in the experimental laboratory. We rotated two groups in and out of both laboratories to involve each student in all aspects of the experimental portion.

Computational Section—The computational laboratory started immediately with exercises involving molecular visualization. To begin, students downloaded structures of ADP-Glc PPase from *Solanum tuberosum* tuber [13] and *Agrobacterium tumefaciens* [14] from the Protein Data Bank (www.rcsb.org/pdb). The corresponding PDB IDs were 1YP2, 1YP3, and 1YP4 for potato tuber and 3BRK for *A. tumefaciens*, respectively. We showed students how to use both VMD and its multiple alignment extension (www.ks.uiuc.edu/Research/vmd/; [15]), and PDB viewer (http://spdbv.vital-it.ch/; [16]) to make diagrams of these structures. Next, students learned how to make comparative "homology" models and predict the three-dimensional structure of proteins based on suitable templates. The crystal structures from potato tuber and *A. tumefaciens* were used as templates to build models of the *E. coli* enzyme. We explored the advantages and disadvantages of several alternative programs, including software such as Modeller (http://salilab.org/modeller/modeller.html; [17]) and Web-based modeling with SWISSMODEL (http://www.espasy.ch/swissmod; [16, 18]). The class was divided into two groups, and each modeled the wild-type plus a separate *E. coli* mutant protein.

Since manual refinements in the alignment are critical for successful molecular modeling, we emphasized criteria used to obtain suitable models. We demonstrated how to use several programs, including Verify3D (http://nihserver.mbi.ucla.edu/Verify_3D; [19]) and WHATCHECK (http://swift.cmbi.ru.nl/gv/whatcheck/; [20]) for model validation. Students then evaluated the quality of their individual models, and adjusted the alignment to obtain the best possible one for their proteins. PDB viewer linked to POV-Ray (www.povray.org/) was used for final visualization. Using their models, students formulated a hypothesis regarding the role of each residue in the catalysis of the enzyme. These predictions were tested by comparing the results obtained for mutant proteins in the experimental laboratory.

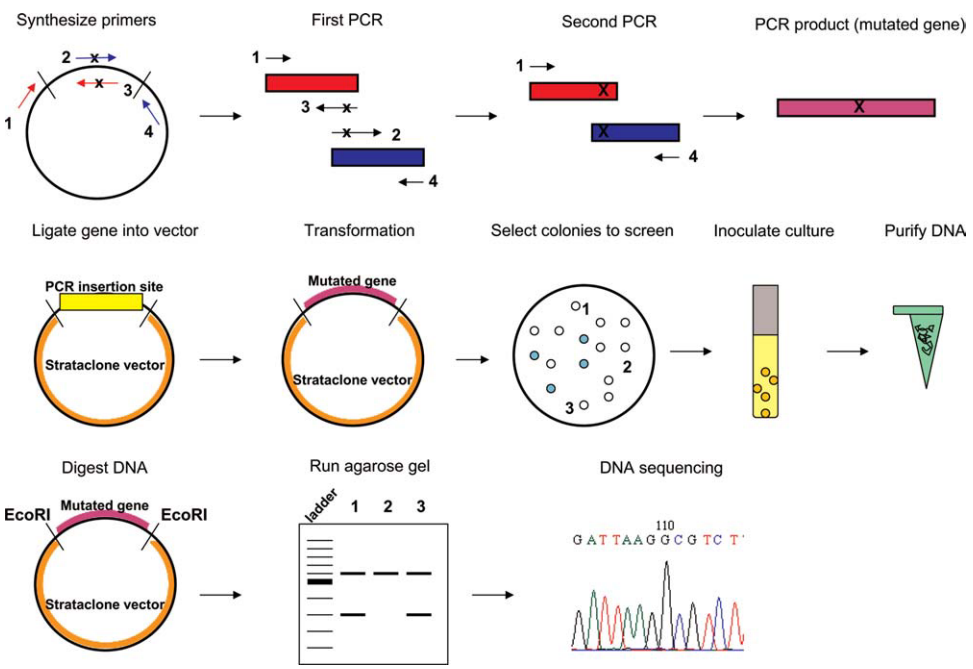
The theory and practice of molecular mechanics and its application to the dynamics of protein structures was also explained. Students learned how to perform and analyze molecular dynamics (MD) simulations using the NAMD program (http://www.ks.uiuc.edu/Research/namd/; [21]). An advanced student in the class performed energy minimizations and short simulations (1 ns) of the wild-type and mutant proteins on LUC's 48-processor cluster, and visualized the results using VMD. The students, together as a class, proposed a conclusion regarding how the flexibility of the enzyme changed in the presence or absence of the residues.

Experimental Section—On the first day of class, students were given a schedule (Table I) and an instruction manual describing general procedures for each technique. We used routine techniques from the LUC research laboratories to design the experiments. Before the course began, primers for PCR were synthesized (IDT, http://www.idtdna.com) to mutate the Arg32 and Lys42 residues to alanine. The class was divided into

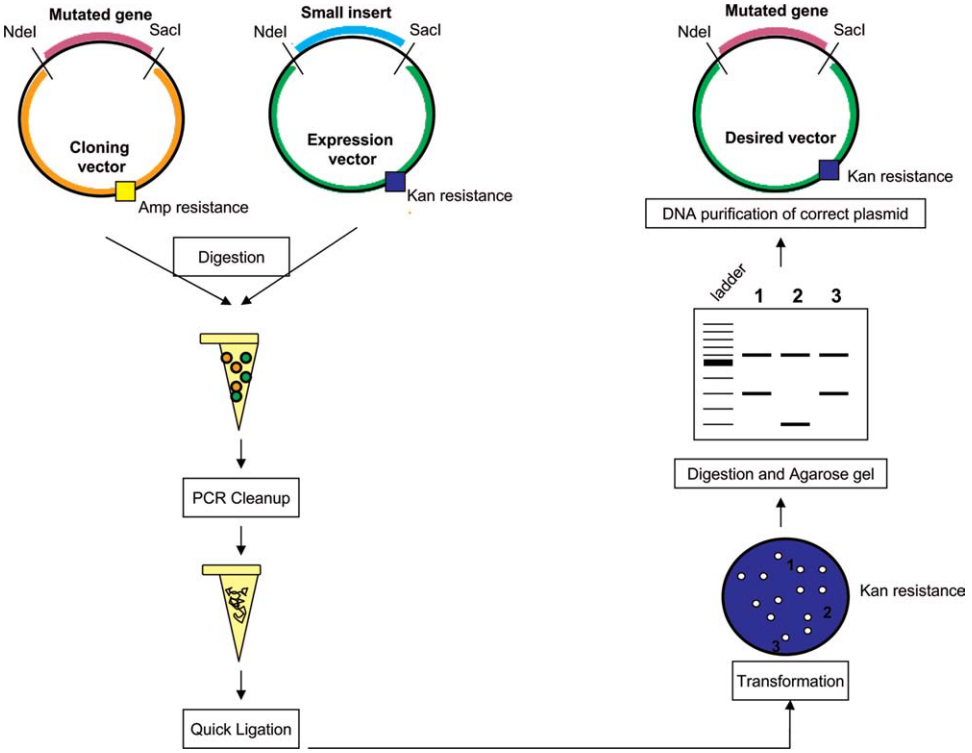
TABLE I
Schedule for 2008 workshop at LUC

	Lecture	Experimental	Computational
Day 1	Protein structure Protein visualization	PCR of 1st and 2nd fragments Run agarose gel Gel extraction PCR of whole gene product and controls	Downloading PDB files Using VMD and PDB viewer
Day 2	Basic enzymology Homology modeling	Run gel of whole gene product and controls Gel extraction Strataclone kit	PSI blast Submit Swiss model
Day 3	Protein purification Model verification	Inoculate cultures	Examine model Build two mutant proteins
Day 4	Molecular mechanics Cloning	Purify DNA Digestion overnight	Verify 3D on original model
Day 5	Energy minimization Saturation mutagenesis	Agarose gel Send DNA to sequencing Setup digestion overnight Transform *E. coli* cells	Energy minimization of mutants
Day 6	Enzyme characterization Mutagenesis	PCR-cleanup of digestion Quick-ligation Transformation of NEB turbo cells Inoculate cultures Grow, induce, add glucose Stain with iodine	Visualization of mutant proteins
Day 7	X-ray crystallography Chemical modification	Inoculate cultures Streak plates with cells	Setup molecular dynamics
Day 8	Molecular dynamics Being a graduate student in another country	Purify DNA Digestion overnight Stain cells with iodine	Comparison of computational and experimental results
Day 9	Analysis of molecular dynamics	Run agarose gel electrophoresis	Analysis of molecular dynamics
Day 10	Student presentations	N/A	N/A

two groups, and each group was assigned a different mutant. To begin, site-directed mutagenesis was performed using a PCR-based approach [22] (Scheme 1). We chose the high-fidelity Phusion DNA polymerase (New England Biolabs) for these reactions, which allowed for fewer errors and used shorter extension times than *Taq* DNA polymerase. To maximize subcloning efficiency, a commercial topoisomerase-based cloning vector (Stratagene) was used to insert the blunt PCR products for screening and sequencing. The mutated genes were subcloned (Scheme 2) into an expression vector (pMAB5, [23]) and transformed into *E. coli* AC7OR1-504 cells, which lack endogenous ADP-Glc PPase activity [24]. The proteins were expressed, and the extent of



SCHEME 1. **Site-directed mutagenesis using PCR-based approach.**



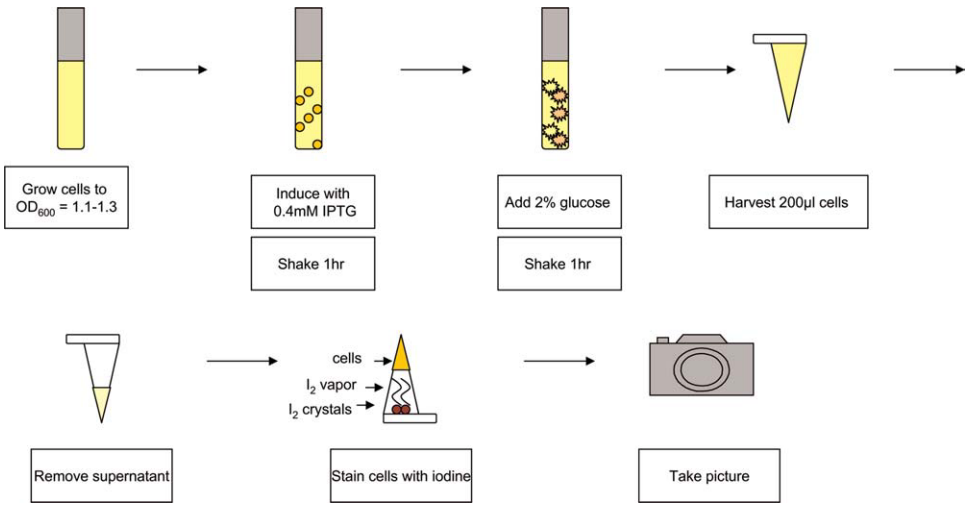
SCHEME 2. **Insertion of mutated genes into a desired expression vector.**

glycogen production of the mutant and wild-type proteins (Scheme 3) was qualitatively tested using a modified iodine staining method [25]. Based on their results, students concluded whether these two residues played a role in catalysis of the enzyme.

RESULTS

Student Evaluations

Each course was evaluated using cross sectional student surveys [26] at its completion, which provided guidance for future improvements of the course. The survey questions for the two groups were slightly different due to the evolving nature of the course. In general, both Argentine (Table II) and USA (Table II) students were very enthusiastic about both the content and the structure of these workshop-style courses. Regarding the design of the courses, all USA students (100%) thought team teaching enhanced the quality and 91% thought the intensive format was a good experience. LUC students, who have been critical of team-taught classes in other situations, liked the approach taken in this class. The vast majority of both classes agreed or strongly agreed that the courses satisfied their expectations (96% Argentine, 92% USA). Nearly all students from both courses (91% Argentine, 92% USA) felt they learned more about protein structure. When students were asked if they were exposed to important tools that would be applicable to their own research,



SCHEME 3. **Expression of protein and iodine staining of glycogen.**

TABLE II
Results of Argentina and USA Survey

	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Results of Argentina survey					
Expectations satisfied?	35%	61%	4%		
Important tools for own research?	35%	57%	9%		
Foreign Professors—Open mind to key subjects?	43%	48%	9%		
Foreign Professors—Open mind to research opportunities?	17%	48%	30%	4%	
Post Doc at Loyola?	39%	39%	22%		
Recommend this workshop?	70%	30%			
Learned more about protein structure?	91% Yes	9% No			
Results of USA survey					
Expectations satisfied?	58%	33%	8%		
Important tools for own research?	58%	17%	25%		
Foreign Professors—Open mind international aspects of research?	75%	25%			
Intensive format was a good experience?	58%	33%	8%		
Team teaching enhances quality?	75%	25%			
Recommend this workshop?	92%	8%			
Learned more about protein structure?	92% Yes	8% No			

*Percentages were rounded to the nearest whole number.

92% of Argentine students compared to 75% of USA students agreed or strongly agreed. The overall percentage was higher for Argentine students, but more USA students (cf. 58 with 35%) strongly agreed with this comment. A larger percentage of USA students were neutral compared to Argentine students for this question.

The involvement of foreign professors was considered a significant advantage for both groups (Table II). For Argentine students, the course made them more aware of foreign research opportunities, particularly at LUC, even though they are accustomed to look for them overseas. For instance, Argentine students were asked if they would be more likely to consider a post-doctoral appointment at LUC now that they were exposed to this course. Surprisingly, 78% of students agreed or strongly agreed with this statement. When asked if the inclusion of foreign professors in the course opened their minds to research opportunities around the world, 65% felt this was true. Conversely, all students (100%) in the USA felt the foreign professors opened their minds to international aspects of research. Interestingly, all students (100%) in both courses would recommend the workshop if it was available in the future. More students in the USA strongly agreed with this comment than Argentine.

The Argentine students felt there were several positive aspects of the course (Table III Argentina, July 2007). These included gaining new knowledge and tools, making new contacts with foreign professors and other people, and being exposed to issues related to their own research projects. The negative aspects of the course included little personal practice with the computers, use of Linux, lack of a timetable, and the order in which topics were presented could be improved. Their suggestions for the course in the future were to give the students a printed guide for the course, give more description of the computer programs, more publicity of the course, more personal practice with computers, more teachers, dividing the course by software, and modeling of their own proteins. Other subjects they would enjoy studying in a course like this included docking, protein interaction, how to prepare scripts, model optimization, protein-ligand interaction, and drug design.

The USA students felt positive aspects of the course (Table III USA, June 2008) included multiple professors with different expertise, learning new techniques in the laboratory and lecture at the same time, interacting with foreign researchers, and having a laboratory experience in a graduate course. Some negative aspect comments seemed to be conflicting. For instance, some students thought that too much background knowledge was expected, but others thought the class was somewhat repetitive of previous classes. Also, some students felt it was difficult to concentrate on the same topic all day, while others enjoyed the short intensive format. The students did not like to work as a team in the laboratory because they wanted to perform each technique on their own, and suggested breaking the class into smaller groups to achieve this. Although they saw the teamwork in the laboratory as a negative aspect, they had a positive opinion about the laboratory portion. To improve the course, the USA students also suggested lengthening it to cover a little more material. This was in contrast to the Argentine students who thought there was too much material presented. It seems like the amount of material presented in the USA course was more reasonable than that of the course in Argentina. Other subjects the USA students would like to study in this format were cancer biology, gene sequencing, and medicinal chemistry.

Experimental Results

The course design was flexible enough to accommodate changes in the experimental portion due to technical problems. For example, we decided to proceed with the ligation of the mutant genes into the desired expression vector before we obtained the results from the sequencing facility at the University of Chicago Cancer Research Center. Once the sequences were analyzed, we found the Arg32 to Ala mutant was correct. The Lys42 to Ala mutant was obtained, but it had unwanted mutations in the gene. At this point, we decided that both groups should work on the Arg32 mutant. Although there were problems in obtaining the desired Lys42

TABLE III
Comments from students

	Argentina, July 2007	USA, June 2008
Positive Aspects	New knowledge – tools	Multiple professors with different expertise
	New contacts with foreign Professors and other people	Learning new techniques in the laboratory and lecture at the same time
	Good relationship between lectures and practice	Interacting with foreign researchers
	Issues related with their own projects	The short, intensive format
	Many issues	Having a laboratory experience in a graduate course
	Professors were very clear	
	Good basis to go on	
Negative Aspects	Little personal practice with computers	Too much background knowledge was expected
	Use of Linux	Class was somewhat repetitive of previous classes
	Lack of a timetable	Difficult to concentrate on the same topic all day
	Little time for so many issues	Having to work as a team in the laboratory so that each student did not do everything
	Order of topics (first modeling, next dynamics)	
Suggestions	Printed guide	Lengthen the course to cover a little more material
	More description of programs	Spend more time on background material.
	More publicity (of the course)	Break the class into smaller groups for the laboratory
	More personal practice with computers	Be less repetitive of previous courses
	More teachers	
	Divide the course by software (related to the order)	
	Modeling of their own proteins	
Other Subjects	Docking	Cancer biology
	Protein interaction	Gene sequencing
	How to prepare scripts	Medicinal chemistry
	Model optimization	
	Protein-ligand interaction	
	Drug design	

mutant, students were able to experience actual difficulties in research rather than a textbook example. Additionally, they learned troubleshooting and how to strategically approach this challenge to successfully obtain the Lys42 mutant in the future. After protein expression, students determined cells carrying the enzyme with the Arg32 to Ala mutant did not stain with iodine, whereas the cells with the wild-type enzyme did (Fig. 1). Molecular modeling revealed Arg32 interacted with the substrate at the active site (Fig. 2).

DISCUSSION

The purpose of these courses was to immerse the students in the study of protein chemistry and enzymology, while also giving them an appreciation for the international nature of modern science. The immersion approach has been very successful in teaching foreign languages [27] and has occasionally been applied to teaching science [2, 28]. For a course involving instructors from overseas, this approach is especially practical because it does not require a long residence at the host institution for the visiting faculty. Since science is a very international activity, it is clearly advantageous for students to learn in an international context [29].

Cultural Aspects

In Argentina, where Spanish is the official language, the course was developed in English because it is the most widely used language in science. This is an

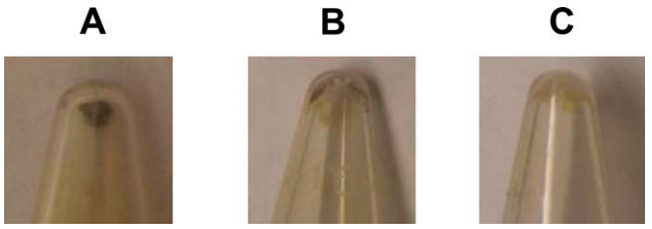


FIG. 1. **Iodine staining of glycogen for (*A*) wild-type *E. coli* ADP-glucose pyrophosphorylase (positive control), (*B*) Arg32 to Ala32 mutant *E. coli* ADP-glucose pyrophosphorylase, and (*C*) pMAB5 (negative control).**

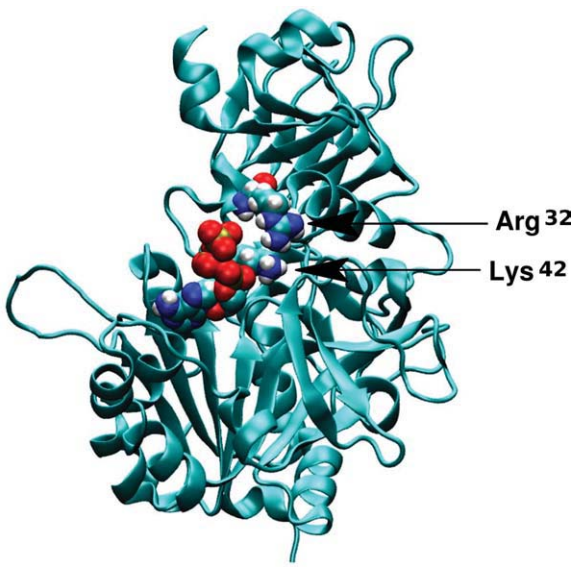


FIG. 2. **Comparative "homology" model of *E. coli* ADP-glucose pyrophosphorylase after 1 ns of molecular dynamics.** ATP was modeled in the active site.

obstacle for many non-native English speakers once they begin to publish their results. Studies in Denmark have shown that learning English is important for students due to the internationalization of science [30]. An immersion approach requires learning new topics in a foreign language, which called for an additional effort from the students. However, nearly all (96%) of students said that attending a course in English was a beneficial experience (data not shown). The feedback clearly showed that the students considered this a good opportunity to be in contact with spoken English and improve their scientific communication skills.

One of the most delightful aspects of these short courses was the lectures presented in both countries by the graduate student instructors from the visiting country. The students compared different customs intrinsic to the two countries: sports, music, foods, hobbies, and many other cultural and free-time activities. They included a discussion about the experiences of being a graduate student at their home institution. In addition, students were exposed to differences and similarities between a private Jesuit university and a free state-run university. This type of exchange was very important to the international bridge building between the students.

Outcomes

The success of the course was evaluated based on several parameters. These included practicality/feasibility of the course, student satisfaction and performance, and future collaborations established between students and amongst instructors. Student performance was evaluated based on effort, presentations about their research, and how they connected their own individual research projects to the course material. We observed that every student was able to identify applications of the techniques presented for their current or future research. This was particularly true for site-directed mutagenesis, comparative modeling and molecular graphics.

We believe that we reached the goal of increasing faculty satisfaction in teaching through the course. It has been shown that there are several advantages for faculty and students when this occurs [1]. The level of enthusiasm during these courses was extremely high, and the type of interaction between faculty and students would be difficult to match in other situations. We believe that our experimental approach with a reasonable amount of risk and a genuine research interest must have sparked the student's attention. The combination of computational and experimental techniques also helped students with different backgrounds to have a better grasp of interdisciplinary research. In addition, most of the students agreed that visualization and modeling techniques helped them learn protein structure in a way they never imagined. Therefore, we concluded some visualization techniques could be useful in regular biochemistry courses that deal with protein structure.

The American graduate student teaching in Argentina felt that the experience of being immersed in a Spanish-speaking country was beneficial for two reasons. First, the student was able to improve communication in Spanish with native speakers. Second, the student was exposed to a different culture and was able to network and establish contacts for future collaborations. The Argentine graduate student teaching in the USA was also able to network and establish contacts for collaborations. In fact, at the end of the 2008 course the two graduate student instructors were able to work together on another experimental research project.

We found that the organization of this course was not only enjoyable but also feasible for instructors, and the benefits clearly outweighed the costs. The organizational expenses were $5000 and $6000 at UNL and LUC, respectively, which were mostly airplane tickets and housing. The possibility to network with foreign students and researchers was a unique experience for everyone involved. In fact, one student at UNL applied for the LUC Ph.D. program.

Applicability

Our approach for designing these courses could be easily applicable and transferable to other institutions and areas of science. However, several factors should be considered when organizing them. First, it is important to find international partners. This may not be very difficult since many research groups either have international collaborators or know someone who does. For example, many institutions in South America may welcome exchanges. In fact, there are agencies that may fund them, such as the Argentine-Brazilian Center for Biotechnology (http://www.mincyt.gov.ar/cabbio2.htm), and this could be true for other parts of the world. Second, the partners should agree on suitable course contents that link their expertise. Third, it is necessary to find a mini research project that the students can perform during the timeframe of the course.

Computational and enzymological topics are a good match in protein science, but many other interdisciplinary interactions are possible. An enzyme with a close homologue of known three-dimensional structure allows students to build appropriate models and do rational site-directed mutagenesis. The mutations should be chosen in a way to spark a genuine interest in knowing the results, where low-risk experiments will lead to a firm conclusion. A typical example of a "low-risk medium impact" approach would be mutagenesis that has not been performed in one organism, but was previously performed in another. In addition, a simple technique should be used to evaluate the results of the mutations, such as an easy enzyme assay, bacterial screening, or a staining procedure. In our case we tried to determine whether a critical residue in a plant enzyme was also critical in a bacterial enzyme.

It was especially important for students from both countries to have access to free computational programs for two reasons. First, the techniques presented in class are more feasible for students to actually apply to their own individual projects. Second, the students have hands-on experience using the exact programs presented in class. Since the laboratory involved a small

research problem to be performed in a short period, it was very important that all of the techniques be routinely used by the instructors. Due to the intensive course format, it was necessary to develop a detailed daily schedule as well as optimize the general conditions for techniques before the class began to ensure success. Instructors must be able to anticipate the common mistakes made by beginning students and be able to quickly correct them. In addition, they should be able to fix equipment malfunctions rapidly to keep the course on schedule and complete its learning objectives within 2 weeks.

Experimental Conclusions

The students concluded that the Arg32 residue from the *E. coli* ADP-Glc PPase was important in catalysis for two reasons. First, the modeling showed this residue interacted with the substrate, which would potentially be critical to catalysis of the enzyme. Second, the cells did not stain with iodine, which is indicative of no glycogen production. The students concluded that this residue was essential to catalysis. They hypothesized that the Lys42 residue was also important for catalysis based on results of the homology modeling experiments. Students were unable to perform the protein expression for this mutant since there were unwanted mutations, but predicted there would also be little to no glycogen production.

Course Adjustments

We received several suggestions from the first year (Table III) that were implemented in the second version of the course. These included organizational issues, such as having a more explicit schedule and better handouts on the laboratory methods. Additionally, we switched from GNU/Linux to Windows® for the computer operating system, and our original choice of compatible software allowed us to do this. GNU/Linux has some advantages in terms of training for actual computational chemistry. However, this switch to Windows® allowed students to concentrate on programs and science they were learning, rather than the operating system of their computers. We also increased the number of instructors, allowing both a broader expertise for the techniques being taught and more help during the laboratory sessions.

One consistent suggestion from the Argentine students (Table III) was that docking should be added to the computational part of the course and would fit well with the enzyme-engineering theme. To stay within the 2-week framework, however, this would require dropping another topic, probably molecular dynamics. It is not clear if all of the advanced docking techniques could be taught, especially without a background in molecular dynamics. Certainly an introduction and an example of the DOCK program (http://dock.compbio.ucsf.edu/) could be added.

Differences in Student Perspectives

Even though most of the students from both courses agreed or strongly agreed with a question, the percentage of USA students who strongly agreed was higher than the percentage of Argentine students (Table II). This observation can be explained by the cultural differences in the "mental scale" of agree and strongly agree. For instance, Argentine students are more apt to use "strongly agree" as indicative of something extraordinary. In fact, our perception during the course was that the Argentine students were more enthusiastic than USA students about the topics presented. It is important to note that these types of cultural considerations should be taken into account when designing surveys if the purpose is to compare two groups from different cultures. Our purpose was to gauge the level of enthusiasm from the students and obtain feedback to improve the course. In this respect, we were satisfied with the results.

When students were asked if the course gave them important tools for their own research, there was a slightly higher fraction of USA compared to Argentine students that were neutral (25% vs. 9%, Table II). The reason for this may be that the educational approaches are different for the two countries. For instance, the USA students generally take their graduate courses at the very beginning of the program before they begin research. The Argentine students, however, begin research as they enter the program and take several short courses throughout their graduate career. Perhaps the few USA students who were neutral could not see applications because they either were at the beginning stages or not yet involved in research.

One interesting result was the Argentine student response to the question about post-doctoral opportunities at LUC. Nearly 78% of the students agreed or strongly agreed that they would now consider LUC as a possibility for this research (Table II). Perhaps, more students would consider this because they are more accustomed to looking for international post-doctoral opportunities. In fact, one of the Argentine students that attended the course was awarded a Fullbright post-doctoral fellowship to come to LUC in 2010. Interestingly, all of the USA students felt that foreign professors opened their minds to international aspects of research. Unlike the Argentine students, fewer USA students are accustomed to international research and opportunities.

Concluding Remarks

We intend to establish an annual series of courses between LUC and UNL that will enrich both institutions. Overall, the immersion collaborative approach was very successful at both universities, and we would encourage other groups to implement this approach. In fact, we think that it is very feasible to transfer our experience to other institutions. A true inter-exchange of culture and networking experiences in these courses provided a positive learning atmosphere. In addition, we observed that both students and instructors were very enthusiastic to apply the knowledge acquired to a real research problem. This enthusiasm was reflected by the amount of

information the students learned in a short period, and the dedication of the instructors.

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