The Biophysicist

Instructional Design for an Undergraduate Laboratory Course in Molecular Biophysics

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ABSTRACT In this article, an approach to teaching molecular biophysics is described. The organization and course content has been carefully chosen and curated so that fundamental ideas in molecular biophysics can be taught effectively to upper classmen in higher education. Three general topic areas are introduced along with accompanying experiments that illustrate major principles related to each topic area. This article outlines an approach to organizing chosen course material and suggests multiple teaching activities within each major topic area: thermodynamics, kinetics, and structural biology. Subtopics are presented along with suggested laboratory experiments. The experiments are outlined in a way that they can be readily adopted by educators teaching a biophysical chemistry lab. The accompaniment of workshop exercises as an additional teaching modality is a component of the course intended to enhance the development of important problem-solving skills and comprehension of new content. Finally, a reflection on student feedback and course outcomes along with targeted learning goals is discussed.

KEY WORDS calorimetry; kinetics; structure; instructional design; undergraduate laboratory; problem-based learning

I. INTRODUCTION

Biophysics, the study of the physical principles that underlie biological processes, is a critical component to biochemistry education at many institutions of higher education. Like most undergraduate courses in chemistry, it serves to lay the foundation in the physical chemistry realm of the core undergraduate curriculum. Biophysical chemistry is often instructed as both a lecture and laboratory-based course, the latter focusing mostly on key experimental methods and instrumentation used by biophysicists. In this article, the instructional design aspects of teaching biophysics to an undergraduate class is described along with a set of practical experiments aimed at targeting the following learning goals: (a) gain a basic understanding of biophysical principles used to study biochemical phenomena, (b) gain practical laboratory skills and familiarity with instrumentation, including keeping a good notebook, (c) develop technical writing skills through the completion and evaluation of full-length reports, and (d) successfully complete an independent discovery-based project at the end of the course.

This course may be introduced to undergraduate upperclassman during the third and fourth years of formal coursework. It is organized into 3 distinct sections, all of which encompass major areas of

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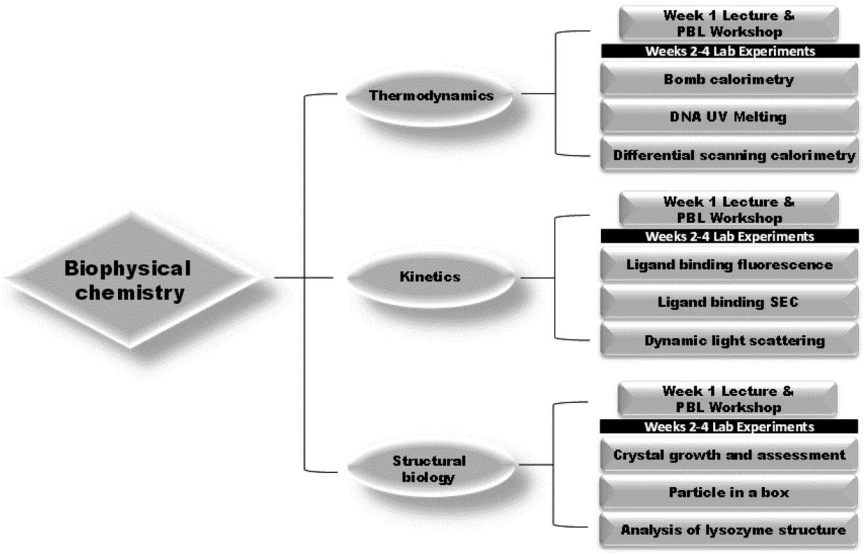

Fig 1. Schematic overview of course design and implementation for undergraduate-level laboratory course in biophysics. The content for each module is presented over the span of 4 wk beginning with a problem-based learning workshop in week 1 followed by subsequent experiments in the following 3 wk (weeks 2–4). Groups of students rotate through each of the experiments in weeks 2–4 until all have completed every experiment in the module. Each module—thermodynamics, kinetics, structural biology, and the workshop—requires a total of 4 wk to complete. One module is completed before proceeding to the next.

biophysical interest with related laboratory activities described. The use of workshops is discussed as a teaching tool focusing on the organization and implementation of lab activities rather than specifically on the content. The laboratory activities should facilitate and direct the workshop content, and they can be adapted depending on the planned laboratory activities. The 3 major topics can be presented in any way the instructor chooses. This article brings together experiments sampled from curricula, such as chemistry and physics, in a way that combines classic experimental techniques with a unique multimodal instructional approach that includes modern pedagogical practices.

II. SCIENTIFIC AND PEDAGOGICAL BACKGROUND

Topics in biophysics can vary broadly. To facilitate the organization and instruction of all the material, 3 primary teaching activities are discussed here: workshops, laboratory exercises, and a discovery-based project. All are intended to help students progress through the course toward defined learning goals (above). Workshops and laboratory exercises are both integral components used to instruct the material in each major topic area. Participation and successful completion of these activities ensures students develop the required skills, shaped by defined learning goals, to succeed.

The course is presented in 3 major defined sections that include modern and traditional lab experiments. Workshops are used as an additional teaching activity designed to introduce new topics for each section. The workshop activities focus on practicing problem solving with the use of biophysical principles presented in a lecture at the beginning of the workshop, and are synonymous with problem-based learning exercises. The benefits of problem-based learning have been studied and investigated in different curricula since the 1960s. The workshops combine elements of many different interactive learning models, including group-based learning, but here the benefits of problem-based learning aspects are emphasized (1).

Figure 1 outlines an overview of the course design illustrating how the workshops and experiments are combined to organize the delivery of course content and ensure that

important concepts are linked to the relevant experiments.

A discovery-based project is completed at the end of the course after the workshops and laboratory exercises have been completed (not included in Fig 1). Discovery-based learning is another form of interactive learning wherein the teacher or instructor assumes a more passive role while the responsibility of learning is shifted to the student (2). As a final culminating assessment, it serves the purpose of determining whether students have retained the knowledge and skills learned throughout the course and gauges whether they can apply them to new ideas.

III. METHODS

A. Workshops

Workshops begin with a lecture presentation introducing new topics from all experiments that will be carried out in the following weeks (Fig 1). Workshop lectures are similar to the traditional lecture-based format in which the experimental procedures are presented and discussed in detail, including background theoretical concepts. Data analysis is also discussed, with examples illustrating how experimental analyses should be carried out. After the lecture presentation, the workshop assignment is distributed and students work with one another to complete it during the lab period. The exercises are intended to be problem-solving group-based activities. Problems include hypothetical scenarios that require students to apply the learned concepts to solve problems successfully. The goal is to provide an effective way for students to practice solving theoretical equations and to teach them how to apply the equations to problem-based scenarios. The teaching assistant and instructor circulate throughout the class period providing guidance as students complete the assignment. By posing certain questions to students during the class period, this can further stimulate and facilitate discussions. This practice is consistent with the inquiry-based student-centered learning model (3). Exercises include problems and calculations that students are expected to apply in their lab reports. Experimental troubleshooting questions can be included to encourage students to think about issues that might arise and how this can affect their data and interpretation of results. Workshops are an effective way of integrating problem-based learning (1, 4, 5).

In this course, workshops are the first introduction to many of the important concepts required to master the material. They provide a critical point at which students either master these concepts or struggle with them. For those who struggle, the workshops are an effective way of providing more hands-on assistance so that by the time students leave the lab period, they have a sufficient understanding. Furthermore, the graded workshop assignments are returned, providing a reference for students to revisit and study data analysis practices so they can incorporate them into their lab reports the following weeks. In addition to the workshops presented here, additional exercises have also been used to familiarize students with other important skills that they use throughout the course (e.g., error analysis and referencing the literature in lab report writing). Additional workshops are not described here because they are not directly related to the biophysical subject matter presented.

B. Teaching module 1: thermodynamics

Thermodynamics is the study of energy between a system and its surroundings. In biophysics, the system under study is a biological system such as proteins, whole cells, lipids, or other biological material. A thermodynamic analysis can be used as a way to study the types of intramolecular interactions that can help stabilize a protein if coupled, for example, to a rigorous amino acid substitution such as alanine scanning (6, 7). Calorimetry is one method used to measure energy transfer in the form of heat released to the surroundings during combustion, denaturation, and ligand binding and is used experimentally to determine thermodynamic parameters of a system. Spectroscopic methods can also be employed

to monitor chromogenic shifts in absorption to track phenomena such as secondary structure formation of oligonucleotides or solvent exposure of amino acid residues such as tryptophan and tyrosine, which can be a useful measure to probe local chemical environments within proteins (8–11). The following set of experiments uses these methods to investigate the heat of combustion of foods, the thermodynamics underlying DNA oligonucleotide hybridization, and protein denaturation.

1. Bomb calorimetry and differential scanning calorimetry

In calorimetry, the heat (q) released by a system to its surroundings as a result of chemical and physical phenomena is measured and used to draw conclusions about a system under study. By measuring this heat exchange, other thermodynamic properties such as enthalpy (ΔH) and entropy (ΔS) can be determined, which ultimately allows one to determine the properties that are important in stabilizing or driving the reactions in a given system and the overall potential of a given process through the relationship between these parameters along with free energy ΔG. Applied to nutrition, calorimetry can be used to measure the energy content, kilocalories (Calories), of different foods. An earlier report of a similar experiment involved the use of exploring the energy content of pizza to analyze the nutritional content (12). However, when a chemically defined food item is used, such as a sugar cube, the data can be used to compare and contrast the information reported on a standard nutrition label. For this experiment, a standard bomb calorimeter can be used along with a food sample. The food sample is carefully situated inside the bomb, which is sealed, filled with oxygen, and subsequently placed inside a water bath (at constant volume). The sample is ignited, and afterwards, the temperature of the surrounding water reservoir (surroundings) is monitored until there is no longer an appreciable change. With the change in temperature and the known calorimeter constant, previously determined by combusting a sample with a known enthalpy (benzoic acid, −3226 kJ/mol), ΔH can be determined (13). This experiment can be carried out with commercial food samples such as sugar (sucrose), candies, or other food items with a sufficient caloric content. For these experiments, it is recommended that foods richer in caloric density be used so that a sufficient temperature change can be observed and recorded.

From the constant volume conditions described above, the following is accepted to be true,

$$\Delta U_v = q_{sys} \quad (1)$$

where ΔU_v is the internal energy of the system at constant volume and q_{sys} is the heat given off by the system at constant volume. Because the change in pressure under these conditions is also considered to be negligible, it can safely be assumed that the internal energy of the system is approximately equal to the enthalpy: $\Delta U = \Delta H$. In a bomb calorimeter, q_{sys} is measured and thus can be used to calculate ΔH on a per mole basis for a chemically defined food item such as sucrose or on a per mass basis for artificial foods for which the chemical content is not specifically defined (e.g., candy).

Because the bomb calorimeter is isolated from the rest of the universe, the reactants constitute the entire system, and the rest of the calorimeter (water bath) is defined as the surroundings. When combustion takes place, the heat released q_{sys} is subsequently absorbed by the surroundings C_{surr} and can be determined by

$$q_{sys} = C_{surr} \times \Delta T \quad (2)$$

where C_{surr} is the heat capacity of the surroundings or calorimeter constant, previously determined with benzoic acid, and ΔT is the temperature change of the water bath. If the range of ΔT is small (3–5 °C), it can be assumed that C_{surr} is nearly independent of temperature (14, 15).

Differential scanning calorimetry (DSC) is a method that can be used to measure the thermodynamic stability of a protein such as lysozyme. Lysozyme is a relatively small protein, 14.3 kDa, with a reported temperature transition peak, T_m, at approximately 73 °C (the temper-

ature at which the protein is thermally denatured). The denaturation process for lysozyme is reversible, making lysozyme highly stable relative to other proteins and especially well-suited to this experiment. This lab can be easily adapted to illustrate how factors such as pH or salt content can affect the thermal stability of lysozyme. The experiment and sample preparation are straightforward when the protein, mutants, or both are made ready ahead of time, and the analysis illustrates important thermodynamic considerations related to protein folding and unfolding at a specified or experimentally determined temperature, T_m. From the T_m and the area under the curve (both of which can be extracted from the software program used to operate the DSC, e.g., VP-DSC MicroCal (Malvern Panalytical, Westborough, MA) equipped with Origin Lab by Malvern Panalytical. other thermodynamic parameters can be calculated and conclusions can be drawn about the relative stability of lysozyme and related physiological implications (Fig 2) (16).

A typical DSC melting curve for lysozyme at pH $\sim$ 5.0 may look similar to Figure 3. Under these conditions lysozyme exhibits a 2-state model for unfolding reflected by the morphology (width of the temperature transition) and the number of peaks displayed in the DSC thermogram (16–18).

At the transition temperature, or T_m, it can safely be assumed that $[\text{protein}_{\text{natured}}] = [\text{protein}_{\text{denatured}}]$; therefore, the denatured protein is approximately 50% by mole. The free energy, ΔG, for the denaturation (u = unfolding) is related to temperature T, enthalpy ΔH (area under the curve), and entropy of denaturation ΔS by the equation:

$$\Delta G^{\circ}_{u} = \Delta H^{\circ}_{u} - T\Delta S^{\circ}_{u} \qquad (3)$$

By Eq. 3, temperature and enthalpy are known or measured, and entropy can be calculated at T_m where $\Delta G = 0$. The heat capacity, C_p, is defined over a temperature range and can be expressed as:

$$C_p = (dH/dT)_p \qquad (4)$$

It is worth noting that the ΔC_p values for the denaturation of proteins compared with oligonucleotides are distinctly different from one another (Fig 2). The molecular forces that give rise to these differences are significant and should be highlighted.

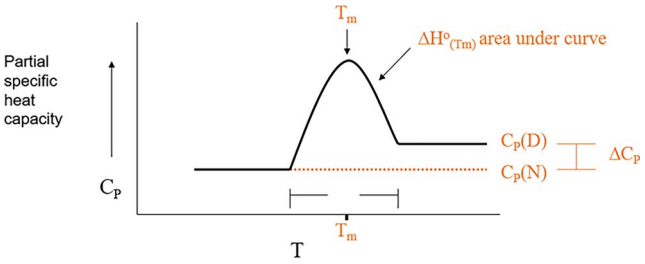

Fig 2. Schematic diagram of a typical DSC thermogram, the thermodynamic parameters that can be extracted, and their relationship to one another. T_m is the melting temperature, and C_p is the heat capacity in the natured (N) and denatured (D) forms of the protein. ΔH is the enthalpy of the thermal denaturation process.

2. Ultraviolet spectroscopic analysis of DNA melting

To explore nucleotide interactions and the interactions that give rise to secondary structure, one powerful approach is the use of inherent chromogenic shifts in ultraviolet absorbance at various temperatures to track the denaturation of complementary nucleotide strands. Nucleotides can adopt various secondary structures, such as hairpin loops and non–self-complementary and complementary structures, to name a few. Compared with their single-stranded counterparts, double-stranded DNA and RNA have an attenuated absorbance intensity at 260 nm, the typical absorbance wavelength for nucleotides. When applying heat to double-stranded DNA/RNA, the absorbance at 260 nm increases in intensity. This change can be tracked and used to calculate thermodynamic properties depending on the type of predicted secondary structure 2 single-stranded pieces of DNA can form: self-complementary versus non–self-complementary (where [strand A] = [strand B]). The thermodynamic properties underlying this interaction can be determined both experimentally and theoretically. After experimentally determining the thermodynamic parameters for this process, it can be compared by the nearest neighbor prediction method, which is based on theoretical calculations established from previous experiments (19–21). In the lab,

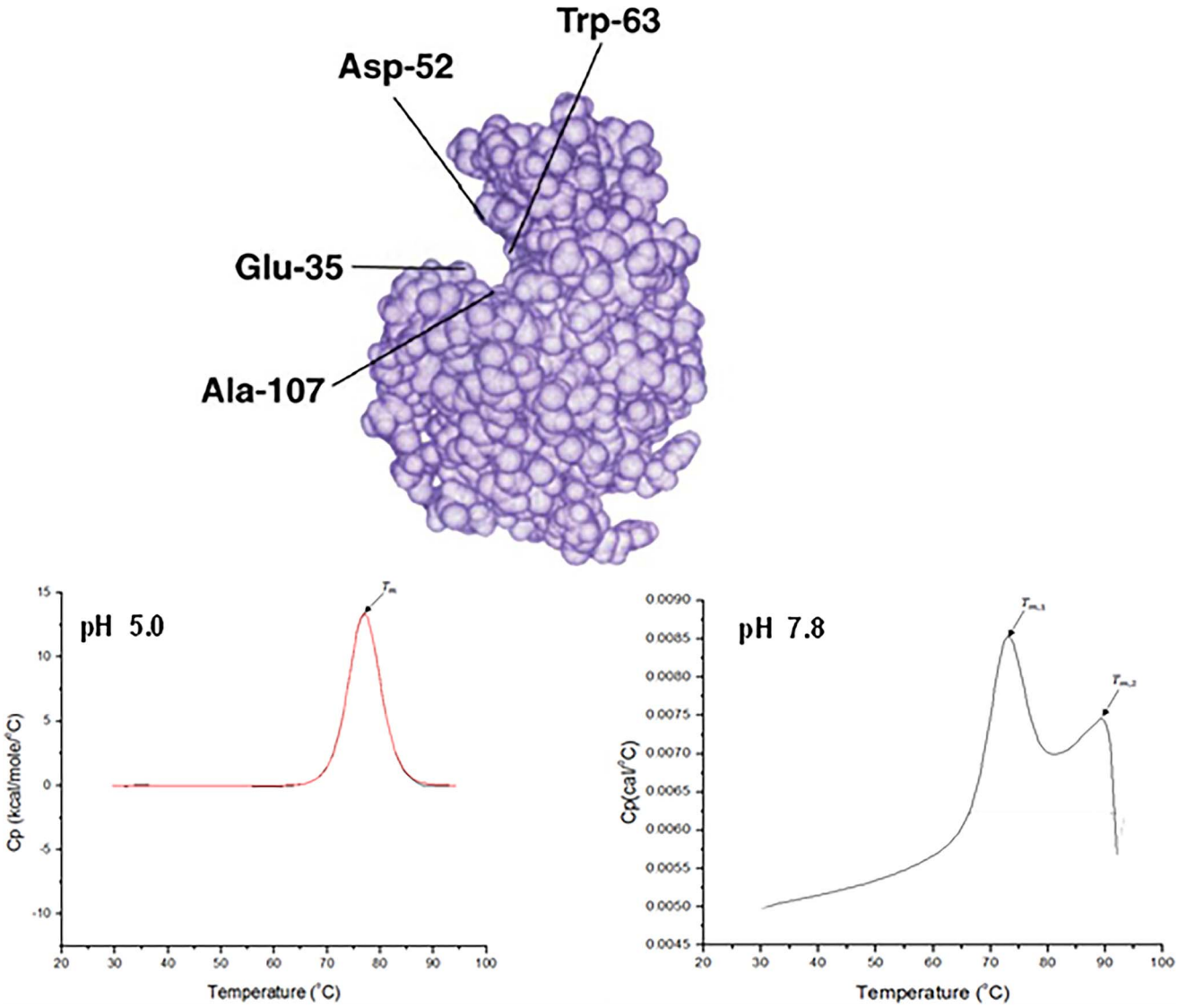

Fig 3. Figure from a biophysical chemistry lab student report comparing lysozyme denaturation at pH 5 (left) and 7.8 (right). A PyMOL illustration of lysozyme shows the residues situated at the active site. This comparison illustrates the importance of pH on the thermodynamic behavior of proteins and how thermal stability of lysozyme can vary depending on conditions. The appearance of a distinct second temperature transition T_m appears in the sample prepared at pH 7.8, compared with a single transition T_m at pH 5.0.

students work with small complementary DNA oligonucleotide sequences (heptamers). The solutions of double-stranded DNA are gradually heated to denature the 2 strands and the temperature at which this occurs is recorded by tracking the change in the absorbance intensity at 260 nm with a standard benchtop spectrophotometer equipped with a Peltier temperature controller. A plot of absorbance versus temperature can be generated from the raw absorbance data and the T_m can be used to determine other thermodynamic parameters (22–25).

The general equation for free energy is expressed by

$$\Delta G^{o} = \Delta H^{o} - T\Delta S^{o} = -RT \ln K_{eq} \qquad (5)$$

where $R = 1.987\ K^{-1}\ mol^{-1}$ and K_{eq} represents the equilibrium constant for the denaturation process. For self-complementary strands, $K_{50} = 1/[C_T]$. Therefore, when $T = T_m$, then $K_{eq} = K_{50} = 4/[C_T]$ and gives (26):

$$\Delta H - T_m \Delta S = -RT_m \ln(4/[C_T]) \qquad (6)$$

Experimental values can subsequently be compared with theoretical values (27). This

exercise can also be extrapolated to other types of DNA strands, adapted to different lengths of DNA or RNA, and used to show differences in thermal stability for GC-rich versus AT-rich strands. Reagents are readily available and can be easily prepared. Oligonucleotides can also be commercially synthesized on a milligram scale from multiple commercial sources (e.g., Integrated DNA Technologies, IDT, Eurofins, Thermo Fisher Scientific).

C. Teaching module 2: kinetics

The second topic area introduced in the course is kinetics. Here, the binding properties of different ligands with bovine serum albumin (BSA) are investigated by a variety of experimental approaches to capture the data. The principle behind each of the 3 experiments is similar, as is the data analysis. Students are exposed to a breadth of experimental techniques with the use of materials that are readily available and easy to prepare. Students learn technical skills such as fluorescence and absorbance spectroscopy along with size-exclusion chromatography. A dynamic light scattering experiment can also be included; however, it is not discussed in detail (28). The following sections outline exercises that have been implemented.

1. Binding kinetics of 8-anilinonaphthalene-1-sulfonic acid with bovine serum albumin (BSA)

In this exercise, 8-anilinonaphthalene-1-sulfonic acid (ANS) is a fluorescent molecule typically used in biochemistry to assess the hydrophobic content of a protein. ANS alone has very weak fluorescence with an emission maximum λ_{max} near 500 nm when excited at 380 nm. When ANS binds to BSA, its fluorescence increases dramatically and the λ_{max} shifts to 470–480 nm. This shift in wavelength and relative fluorescence intensity can be used to track ANS binding with BSA by slowly titrating a solution of BSA into a solution of ANS at a fixed concentration. The resulting ratio of fluorescence signal intensity F_r is determined from measurements of the initial fluorescence signal intensity F_i and final fluorescence signal intensity F_f, which has been corrected (corr) for the change in volume on adding BSA by incorporating F_o from the original fluorescence signal intensity (Eq. 7):

$$F_r = \frac{(F_i - F_o)_{corr}}{(F_f - F_o)_{corr}} \quad (7)$$

The equation for a simple binding isotherm,

$$F_r = \frac{NK_A[BSA_{free}]}{1 + K_A[BSA_{free}]} \quad (8)$$

can be used to describe the relationship between the measured fluorescence intensity and concentration of BSA, where K_A is the association constant for ANS and BSA, N are the number of binding sites, F_r is the ratio of the corrected fluorescence signal before and after binding, and [BSA] is the solution concentration. A graphical representation of the data allows one to extract K_A and determine N. Furthermore, diagnostic tools such as a Scatchard plot are used to extract even more information about the nature of binding, such as cooperativity (negative or positive), and the nature of the binding sites in BSA (equivalent or not). Data analysis with a Scatchard plot is described by Healey (29) along with an alternative experiment measuring fluorescence of ligand–DNA binding.

2. Determining binding sites of bromophenol blue with BSA

A similar approach can be used to determine the number of binding sites of bromophenol blue (BB) on BSA. Instead of using a fluorescence signal to detect the level of binding, the absorbance of BB (590 nm) is used to track ligand binding. Here, the bound ligand, BB, is physically separated from the free ligand by size-exclusion chromatography. The BSA-bound BB complex elutes before the free ligand with the use of a Sephadex G15 resin to separate the two after they have been incubated together for a period of time. By preparing a series of samples with known concentrations of BSA and BB at different ratios with respect to one another, the amount of ligand bound to BSA can be mathematically determined and a binding isotherm generated with the calculated values for bound ligand versus known quantities of free BB ligand.

Employing similar principles above, kinetic parameters can be extracted and the nature of the ligand–BSA interaction assessed. This exercise demonstrates the practical importance of size-exclusion chromatography and its separation principles while illustrating how binding kinetics can be used to investigate ligand–protein binding interactions (30–34).

D. Teaching module 3: structural biology

In this section, wet lab techniques along with basic computational methods are used together to investigate the theory underlying some very commonly used experimental approaches in structural biology: x-ray crystallography and molecular spectroscopy. Lysozyme is a great example for these studies because it can be easily crystallized with minimal equipment and reagents, and the crystal structure data is readily available from the RCSB Protein Data Bank (PDB) for analysis (35, 36). With programs such as PyMOL (version 2.5, Schrödinger, New York), key aspects of lysozyme structure can be analyzed and highlighted. Beta-carotene and lutein, both molecules with extensive conjugated pi systems, can be readily extracted from food items and their wavelength maxima determined spectroscopically. The following describes a set of exercises designed to be carried out over a 2 to 3-wk period and can readily be incorporated into the lab course.

1. Crystallizing lysozyme for structural analysis

In this exercise students use the vapor diffusion method to prepare crystals of lysozyme. Although a number of methods can be used to prepare high-quality crystals, here students learn about different parameters that can affect protein crystal growth and how these parameters can be tested simultaneously in a relatively systematic approach. Each group of students sets up a "crystal box" with a cell culture plate—24-well plates work well—in which they can test 24 different conditions to determine which conditions yield the best crystals. Altering parameters such as pH and salt concentration over a range of values gives students a chance to generate a multitude of conditions, all of which can be sampled in their crystal box (37, 38). In practice, a pH range of 4.3–4.9 has worked best with a sodium acetate buffer. Salt concentrations can vary from 0.5 to 2.0 M in the reservoir. Protein concentrations ranging from 20 to 50 mg/mL have been used successfully. A small quantity, 2–5 μL, of the prepared protein solutions are then spotted on the lid of the 24-well plate. These solutions should match the pH and buffer concentration of the reservoir, with a higher salt content in the reservoir. The plate and lid are carefully sealed and incubated at constant temperature (21–25 °C).

After 1–2 wk, the crystals can be evaluated with a standard benchtop light microscope, with which students can determine the conditions that produced the best crystals. Lysozyme crystals can range in morphology and shape. To differentiate between salt and protein crystals, subsequent experimentation can be carried out to verify. Typically, lysozyme crystals are tetragonal, whereas sodium chloride produces cubic lattice structures (39–42). A simple flame test can further verify protein from salt crystals, because salt crystals can withstand the intense flame of a Bunsen burner when passed through (43). The exercise is also a great way to get students to investigate the literature to compare their crystals with what has been reported and to compare their growth conditions (44).

2. Computational tools to analyze protein crystal structure

After students have obtained and analyzed the morphology and physical properties of crystals from their growth experiment, the next phase of the exercise encompasses an analysis by guided approach to begin understanding the kinds of questions structural biologists think about when extracting information from their crystal structure data. If one is fortunate enough to have access to an x-ray crystallography facility, one might be able to generate his or her own data; however, the use of PyMOL (or other) and crystal structure data deposited in the PDB is an alternative approach. In this exercise, students are introduced to PyMOL and begin learning how to execute commands to carry out different functions related to the

types of questions that concern structural biologists. Such questions include highlighting important features of lysozyme structure, such as its catalytic residues; elements of secondary, tertiary, and quaternary structure; and the distances between residues. Although it is easy enough to spend several class periods learning the nuances of the program, this exercise is intended to introduce students to more of its advanced features to encourage them to explore more of the capabilities of the program. Students can download an educational version of the program, or an institutional site license can be purchased. Published tutorials are readily available from which additional exercises can be adapted (45, 46). PyMOL allows students to explore protein structures at atomic resolution, providing a platform for a broad range of possibilities for structural analysis assignments. Coupled with crystallization techniques at the bench, this exercise approaches the subject of introductory structural biology, facilitating reasoning capabilities based on a previous foundation of biochemical knowledge.

3. A particle-in-a-box approach to studying interactions of light with matter

This exercise demonstrates how theory in spectroscopy and experimentation can be used to solidify the understanding of quantum theory of subatomic particles.

The use of simple pigment molecules such as lutein and beta-carotene illustrates this idea. These 2 compounds can be readily extracted from vegetables such as spinach and carrots with a few milliliters of 200-proof ethanol. Standards are also used to establish the wavelength maxima for each compound and to verify that the compounds extracted are in fact lutein and beta-carotene. Next, a wavelength scan is carried out, and the λ_{max} is recorded. The experiment described here is simplistic and can be carried out without the aid of a computational math program such as Maple (Maplesoft, Waterloo, ON, Canada), although incorporating this type of program for these problems is encouraged (47).

The value of λ_{max} can be obtained in this experiment and corroborated with theoretical values calculated with the assistance of the software program, Spartan (version 8.0.6, Wavefunction Inc., Tokyo, Japan) (48–50). With this program, students can import structure files for each molecule and then calculate bond lengths and cumulatively determine the length of the theoretical "box," or linear molecule. From the calculated length L of the box and theoretical principles of quantum mechanics introduced in the prelab lecture (workshop, Eq. 9), the wavelength of light λ required to initiate an electron transition Δn can be determined from the energy values E calculated for the transition,

$$\Delta E = \frac{\left(n_f^2 - n_i^2\right)h^2}{8\ mL^2} \qquad (9)$$

where n is the quantum energy level occupied by an electron, h is Planck's constant, L is the length of the box, and m is the mass of an electron. Conversely, measurements can be used to calculate the theoretical box length of lutein and beta-carotene and be compared to their calculated values by λ_{max} and Eq. 10,

$$E = h\nu = hc/\lambda \qquad (10)$$

where c is the speed of light and ν is the frequency associated with the wavelength of light absorbed. Several things should become clear as students work through this exercise. Molecules do not always behave, in theory, the way they are often expected to, and theoretical models are only as good as the factors used to determine them. Sometimes other important considerations give rise to discrepancies in models compared with experimentally determined values. Other compounds can be readily adapted to this exercise as well (51).

E. Bringing it all together: discovery-based learning project

At the end of the course a final discovery-based learning project is assigned to solidify all of the concepts introduced and learned throughout the course. Students are required to complete these projects independently by assigning them primary responsibility for their own work. Where the workshops are intended

to be a primer that prepares students for the upcoming laboratory experiments, the discovery-based project serves as a final assessment and provides a means of examining student learning outcomes in the course. The discovery-based project accomplishes this because it requires students to use the conceptual and theoretical knowledge acquired from the workshops and laboratory experiments to formulate their own testable hypothesis. Rather than following a defined procedure, as they do for the experiments, here they must think about the steps necessary to test their own hypothesis successfully. This assignment allows the instructor to determine whether students have understood the concepts taught and if they can apply them. Before coming to lab, students either submit a written procedure for approval or they write up their own procedure in a lab notebook. After it is approved, it can be carried out. Students submit a lab report the same as before. However, in addition to the analysis, they compare their results with those that they previously collected. Again, this exercise demonstrates whether students can apply what they learned by expanding on concepts introduced earlier in the course. The discovery aspect of the assignment often means no control set of data is readily available to which they compare their results, so new phenomena may be discovered.

The discovery-based assignment was introduced in Fall 2019 for the first time. The students selected experiments from a list based on similar experimental conditions and reagents described in the original lab exercises. For example, in the bomb calorimetry experiments, different food sources were investigated and the results were compared with the original results. Another example was the investigation of other proteins and their thermal denaturation by DSC. Some students also chose to investigate different experimental conditions for the denaturation of lysozyme. At pH 5.0, the original experimental conditions, lysozyme exhibits a 2-state unfolding process, whereas at physiological pH, 7.6–7.8, an additional transition state T_m appears in the thermogram (Fig 3). Other proteins such as catalase, BSA, hemoglobin, and lactate dehydrogenase showed markedly different denaturation states on DSC thermograms when compared with lysozyme (not shown).

After completing the discovery-based learning project, students submit a lab report the following week describing their experiments and comparing their results to those they obtained from the original experiment. They are evaluated according to the same criteria as for their laboratory reports. Figure 4 shows a sample rubric and provides criteria on the basis of which students are evaluated in their lab reports. One alternative to the discovery-based learning assignment is a mini–research presentation on a biophysical technique that was not previously introduced in class. Here, topics can be assigned to groups of students whose responsibility is to research new and novel biophysical methods. Each group of students presents on their topic and their peers evaluate them. This active learning approach allows for further introduction to biophysical methods in a way that engages the whole class and gives the instructor and students an opportunity to explore new methods.

IV. RESULTS AND DISCUSSION

An undergraduate-level biophysics lab course is described along with a course overview, intended learning objectives, and teaching considerations related to the design and implementation of each experiment. The course curriculum outlined herein organizes the instruction of the material into 3 distinct modules. Experiments were chosen for both their modern relevance in the field and their ability to illustrate basic core principles. In each section, techniques and general procedural overviews are highlighted along with suggested adaptations. Throughout the course, students work together cooperatively in groups of 3 to 5 for most of the experimental exercises and workshops. This approach to cooperative group learning has been effective, with all individuals participating and contributing nearly equivalently during the lab and when writing up their reports.

Laboratory Report Guidelines and General Grading Rubric (100 points total):

Overall Evaluation (5 points)

*These points are discretionary and can include things like grammar and/or diction in your writing

Cover Page/Abstract (5 points) (1-2 pages)

- (2 point) A title page is present at the beginning of the report with title, name, lab partner's names, date of the experiment performed
- (2 points) A focused abstract with a statement of what was done and the important results is included.
- (1 points) Abstract is 150 – 250 words

Introduction (20 points) (2 pages minimum, 3 pages maximum)

- (5 points) The introduction is clear, focused, and ordered.
- (5 points) The reader is able to follow and understand all information required for the experiment
- (5 points) In the introduction, all equations, figures, and referencing are numbered and follow the required formats
- (5 points) The introduction has a clear objectives statement that accurately reflects the purpose of the lab and a logical, testable hypothesis.

Experimental Methods (5 points) (1-2 pages)

- (2 points) Important equipment, chemicals, etc. are included. This includes the names of manufacturers and other relevant information
- (3 points) A brief but clear description of the experiment is given (with schematic if necessary). This information should define the task without unnecessary detail as followed in examples provided

Results (20 points) (2 page minimum, 5 page maximum)

- (5 points) All required data processing or analysis is completed
- (5 points) Figures: All processed data are presented in an organized way, with proper figure number, captions, and labels
- (5 points) A logical presentation of results is provided with proper linkage to previous relevant tables, equations and figures in the narratives in this section
- (5 points) In the narrative of this section, the results are reported with proper units, reasonable significant figures and uncertainty where appropriate.

Discussion (35 points) (1 page minimum, 4 page maximum)

- (7 points) The discussion includes interpretation of experimental results with fundamental chemical principles
- (20 points) Post lab questions

References (5 points) (1-2 pages)

- (3 points) A list of references is provided after the discussion in the appropriate ACS format
- (2 points) A sufficient number of references have been cited (min. 5)

Appendix (5 points) (1+ pages)

- (3 points) Copy of all lab notebook pages (signed by your instructor or TA) are included
- (2 points) Supplemental results (if applicable). *E.g.* sample calculations.

Fig 4. Sample grading rubric for student lab reports and independent discovery-based project.

A. Assessment of student success and learning outcomes

Students are evaluated primarily through participation in workshops, completion of lab experiments, and subsequent submission of full lab reports. Toward this goal, workshops are intended to provide hands-on guidance for students as they familiarize themselves with new concepts through a problem-based learning teaching methodology. Most students score well, >85%, on these exercises, provided they participate and work with their partners in groups. The primary mode of evaluation of learning outcomes is through the submission and grading of full-length laboratory reports. A detailed grading rubric is outlined in the course syllabus and requires that all major sections of a lab report or manuscript be included in the format (Fig 4). A copy of laboratory notebook pages is included at the end of the report so that students can be evaluated on their lab notebook–keeping skills along with their technical writing and data analysis abilities. The discovery-based project is carried out and

graded in a similar way to a typical lab experiment. The major difference is that students work entirely by themselves to develop a working hypothesis and experiment based on biophysical principles and the experimental techniques learned earlier in the course. This exercise is meant to assess students' ability to work independently in the lab, generate a testable hypothesis before coming to lab, and test that hypothesis during lab with an outlined and detailed experimental procedure prepared beforehand and approved by the instructor. The students collect their data and analyze it in the same way they did throughout the semester. The goal is to determine whether the targeted learning outcomes were met. All of these skills contribute to the practical learning outcomes in the course, and the analysis and lab report demonstrate students' ability to connect biophysical concepts they learned in workshops and previous experiments with their own independent project. The same grading rubric is followed for all lab reports to maintain consistency and objectivity in grading.

A look at the aggregated data from student enrollments beginning in 2017 through 2019 shows the collective grade distribution (Fig 5). In this course, student success (A–C) correlated with full participation and completion of assignments. One advantage of incorporating different teaching activities is the multiple modes of assessment that can be used to evaluate students across a range of learning styles.

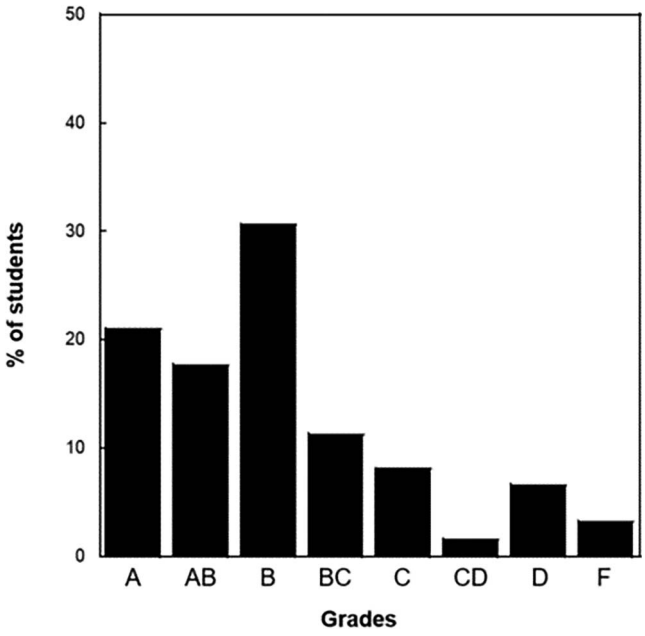

Fig 5. Aggregated grade distribution of students enrolled in the biophysical chemistry lab from 2017 through 2019. A total of 62 students were enrolled in the course during this 2-yr period of time and does not include students who withdrew or were assigned an incomplete.

B. Student feedback

On the basis of student feedback from course evaluations, workshops received a favorable response, in general. Students found the workshops and associated lectures to help introduce and explain the laboratory related concepts, which is reflected by student comments below. In some cases, students who scored average on lab reports, 72%–82%, had a substantial increase in their overall course performance (final score) with the help of the workshops. Feedback from students in response to the question, "State one or two items that you liked most about this faculty member's handling of the lab" included statements like, "The workshops were helpful" and "The prepared slides and workshops that aided in the understanding of concepts."

V. CONCLUSION

Biophysics continues to evolve and grow as a discipline. An undergraduate lab course curriculum in biophysical chemistry is presented with a breadth of topics that introduces students to the field of biophysics for the first time. The nature of the exercises described is intended to build a proficient base of general knowledge in the subject through several teaching modalities. The combination of exercises and workshop assignments presented in the curriculum demonstrates what is believed to be an effective approach to teaching biophysics to a primarily upper-level undergraduate class. The novelty of the approach is the combination of multiple modes of instruction (i.e., workshops, exercises, and independent discovery-based projects), which provides more than one mode of content delivery and student evaluation. The

rationale is that the workshops serve as an integral part of the instruction, facilitating it as a standalone course that can be cotaught either in the same semester as the corresponding biophysics lecture course or in noncoinciding semesters. Either way, student success in the lab course is not and should not be dependent on the lecture course. It should be further highlighted that this work is best considered for a learning environment where undergraduate education is the primary mission of the institution and acknowledge that at a research institution the learning objectives may differ.

AUTHOR CONTRIBUTIONS

MDR wrote and edited the manuscript; conceptualized the collective analysis of workshops, exercises, and experiments and their roles in biophysics curricula; and carried out pedagogical assessment and data analysis.

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