KEY COURSE OFFERING INFORMATION:

Institution name

University of Wisconsin-Madison

Subject and catalog number

BMOLCHEM 700

Course title

Practical Biophysics

Number of credits

3

Course designations and attributes

Grad 50% - Counts toward 50% graduate coursework requirement

Course Description

Biomolecular Chemistry 700 is a graduate-level course that connects physical biochemical principles to modern biophysical approaches in a hands-on practical course. The course starts with a refresher on thermodynamics and kinetics then moves into five distinct modules, each focusing on a single methodology. Each module follows the same format that utilizes the "tell me, show me, let me try" instructional design learning model. First, the instructors will lead a lecture that introduces the theory underpinning the method along with its strengths and limitations and will provide a deep dive into the instrumentation used for each method. Second, the class will critique a paper or papers that use the method to better understand its utility and limitations, and how generated data are interpreted. Third, the class will directly engage in the method using instrumentation available in the UW-Madison Biophysics Instrumentation Facility to measure biophysical data for model reactions and, when possible, experimental systems from their research labs. Finally, students will interpret the collected data to reveal biophysical insights into the reaction being investigated. After completion of the modules, students will integrate the knowledge that they have gained by writing a grant aim in which they will propose multiple methods studied in the course to support a novel biophysical study.

The course will be presented in two class periods each week during the fall semester and is designed to help students think critically about experimental design. The expectation is that students will work on course learning activities (reading, writing, problem sets, studying) for 2-3 hours outside of the classroom for every lecture period.

Requisites

Graduate/professional standing

Instructional mode

Face-to-face

Course learning outcomes

Upon completion of this course, students will be able to:

- Describe the strengths and weaknesses of several biophysical methods and use that knowledge to design biophysical experiments for their research.
- Explain the fundamentals of thermodynamics, kinetics, and fluorescence and practically apply this knowledge to modern research problems.
- Use data interpretation skills to interpret results from several biophysical instruments.
- Integrate their knowledge of biophysical methods in the form of an experimental plan within a grant application.

How credit hours are met by the course

The course will meet twice each week for 75 minutes of direct faculty instruction, with an estimated six hours of work outside of class for students.

INSTRUCTOR-TO-STUDENT COMMUNICATION:

Laboratory sessions

The course will include classroom and laboratory components. For the labs, students will work as teams in the UW-Madison Biophysics Instrumentation Facility to carry out experiments using state-of-the-art biophysical equipment.

Required textbooks, software, and other course material

There is no required textbook. Course materials, including any course notes, slide decks, papers, etc, will be provided to students electronically. Students will need to get a license to use Prism 10 for data analysis (available from UW-Madison DoIT).

Indication of time devoted to individual topics via academic activities

September 5 - Course introduction, expectations, general chemical equilibrium principles

September 10 - Equilibrium constants, Gibb's free energy, enthalpy, entropy, protein folding stability
September 12 - Cooperativity in molecular interactions, Hill plots

September 17 - Introduction to kinetics, rate constants, reaction order, Arrhenius equation
September 19 - Bimolecular interactions, dissociation/association rates, half times, non-ideal cases

September 24 - Binding specificity and summary/integration of thermodynamics and kinetics lectures
September 26 - Circular Dichroism and protein stability – introduction to method and instrumentation

October 1 - Circular Dichroism and protein stability – review of example papers
October 3 - Circular Dichroism and protein stability – experimental measurements in BIF

October 8 - Circular Dichroism and protein stability – report out on results of measurements
October 10 - Fluorescence Polarization/Anisotropy – introduction to method and instrumentation

October 15 - Fluorescence Polarization/Anisotropy – review of example papers
October 17 - Fluorescence Polarization/Anisotropy – experimental measurements in BIF

October 22 - Fluorescence Polarization/Anisotropy – report out on results of measurements
October 24 - Isothermal Titration Calorimetry – introduction to method and instrumentation

October 29 - Isothermal Titration Calorimetry – review of example papers
October 31 - Isothermal Titration Calorimetry – experimental measurements in BIF

November 5 - Isothermal Titration Calorimetry – report out on results of measurements
November 7 - Bio-layer Interferometry – introduction to method and instrumentation

November 12 - Bio-layer Interferometry – review of example papers
November 14 - Bio-layer Interferometry – experimental measurements in BIF

November 19 - Bio-layer Interferometry – report out on results of measurements
November 21 - Mass photometry – introduction to method and instrumentation

November 26 - Mass photometry – review of example papers
November 28 - No class – Thanksgiving holiday

December 3 - Mass photometry – experimental measurements in BIF
December 5 - Mass photometry – report out on results of measurements

December 10 – Integration and comparison of all methods explored in the course

Representative list of readings

For each method, students will read scholarly work that demonstrates how the methods are applied, how data are interpreted, and how results from the method inform our understanding of biological phenomena.

CD:

Kelly, Jess, & Price (2005) "How to study proteins by circular dichroism" *BBA* **1751**, 119-39.

Rashke & Marqusee (1997) "The kinetic folding intermediate of ribonuclease H resembles the acid molten globule a partially unfolded molecules detected under native conditions" *Nature Structural Biology* **4**, 298-304.

FP:

Moerke, N. J. (2009) Fluorescence Polarization (FP) Assays for Monitoring Peptide-Protein or Nucleic Acid-Protein Binding. *Curr. Protoc. Chem. Biol.* **1**, 1–15.

Xu, H. Q., Zhang, A. H., Auclair, C. & Xi, X. G. Simultaneously monitoring DNA binding and helicase-catalyzed DNA unwinding by fluorescence polarization. *Nucleic Acids Res.* **31**, e70 (2003).

Yoo, H. & Drummond, D. A. Using fluorescence anisotropy to monitor chaperone dispersal of RNA-binding protein condensates. *STAR Protoc.* **3**, 101409 (2022).

ITC:

Freyer & Lewis (2008) “Isothermal titration calorimetry: experimental design, data analysis, and probing macromolecule/ligand binding and kinetic interactions” *Methods in Cell Biology* **84**, 79-113.

Wiseman, Williston & Brandt (1989) “Rapid measurement of binding constants using a new titration calorimeter” *Analytical Biochemistry* **179**, 131-137.

Shinn, Kozlov, Nguyen, Bujalowski * Lohman (2019) “Are the intrinsically disordered linkers involved in SSB binding to accessory proteins?” *Nucleic Acids Research* **47**, 8581-8594.

BLI:

Kumaraswamy, S. & Tobias, R. (2015) Label-Free Kinetic Analysis of an Antibody–Antigen Interaction Using Biolayer Interferometry. in *Protein-Protein Interactions: Methods and Applications* (eds. Meyerkord, C. L. & Fu, H.) 165–182.

Sultana, A. & Lee, J. E. (2015) Measuring Protein-Protein and Protein-Nucleic Acid Interactions by Biolayer Interferometry. *Curr. Protoc. Protein Sci.* **79**, 19.25.1-19.25.26.

Dzimianski, J. V. et al. (2020) Rapid and sensitive detection of SARS-CoV-2 antibodies by biolayer interferometry. *Sci. Rep.* **10**, 21738.

MP:

Young et al (2018) “Quantitative mass imaging of single biological macromolecules” *Science* **360**, 423-427.

Hernandez et al (2022) “Residues located in the primase domain of the bacteriophage T7 primase-helicase are essential for loading the hexameric complex onto DNA” *Journal of Biological Chemistry* **298**, 101996.

Homework and other assignments

For each method, one team comprised of a subset of students in the class will perform the experiment using instrumentation in BIF on the “experimental measurements” day, and their data will be provided to all students in the class. All other students will observe. The team that carried out the experiment will prepare a presentation for the following class to report their results. All other students will hand in the results of their interpretation of the data collected during the “experimental measurement” day prior to the next class period. Reports will be scored 0-10 points and late reports will not be accepted.

At the conclusion of the course, each student will prepare an aim for an NIH-style grant proposing a biophysical analysis of their choosing. Multiple methods described in the course must be employed in the aim. Aims should be ~2 pages in length and should include a very brief description of the significance of the proposed research and an in-depth description of the experimental approach. Consideration of the strengths/weaknesses of approaches should be included. Expected results and possible pitfalls should also be included.

Grading

Grades will be based on data interpretation exercises, in-class participation, and a final written proposal.

Your grade will depend on what percentage you earn: A=92-100%, AB 87-91.9%, B=82-86.9%, BC=77-81.9, C=70-76.9%, D=60-69.9%, F<60%.

Assessment	**Total Points**
Data interpretation exercises Written or oral reports interpreting the data generated in each module will be evaluated. 10 points per module.	50
In-class participation Attendance will count for 50% of the participation grade. The remainder will be based on in-class engagement through questions and other active participation.	10
Final proposal Students will write a grant aim that proposes use of multiple methods covered in the course.	40
Total	100

Attendance is mandatory and counts for half of the in-class participation score. Absences must be excused in advance by the instructor or as soon as possible if due to illness.

Canvas course URL

TBD

Meeting time and location

TBD (presuming T/R for 75 minutes each)

Instructor titles, names, and contact information

Assistant Professor Andrea Putnam aaputnam@wisc.edu
Assistant Professor Kavi Mehta kmehta@wisc.edu
Professor James Keck jlkeck@wisc.edu

Instructor availability

Instructors will hold weekly office hours (day/time TBD)

ACADEMIC STATEMENTS AND POLICIES:

Rules, rights, and responsibility

- See the Guide's Rules, Rights and Responsibilities

Academic integrity

By enrolling in this course, each student assumes the responsibilities of an active participant in UW-Madison's community of scholars in which everyone's academic work and behavior are held to the highest academic integrity standards. Academic misconduct compromises the integrity of the university. Cheating, fabrication, plagiarism, unauthorized collaboration, and helping others commit these acts are examples of academic misconduct, which can result in disciplinary action. This includes but is not limited to failure on the assignment/course, disciplinary probation, or suspension. Substantial or repeated cases of misconduct will be forwarded to the Office of Student Conduct & Community Standards for additional review. For more information, refer to studentconduct.wiscweb.wisc.edu/academic-integrity/.

Accommodations for students with disabilities

McBurney Disability Resource Center syllabus statement: “The University of Wisconsin-Madison supports the right of all enrolled students to a full and equal educational opportunity. The Americans with Disabilities Act (ADA), Wisconsin State Statute (36.12), and UW-Madison policy (Faculty Document 1071) require that students with disabilities be reasonably accommodated in instruction and campus life. Reasonable accommodations for students with disabilities is a shared faculty and student responsibility. Students are expected to inform faculty of their need for instructional accommodations by the end of the third week of the semester, or as soon as possible after a disability has been incurred or recognized. Faculty will work either directly with the student or in coordination with the McBurney Center to identify and provide reasonable instructional accommodations. Disability information, including instructional accommodations as part of a student's educational record, is confidential and protected under FERPA.” http://mcburney.wisc.edu/facstaffother/faculty/syllabus.php

Diversity and inclusion

Institutional statement on diversity: “Diversity is a source of strength, creativity, and innovation for UW-Madison. We value the contributions of each person and respect the profound ways their identity, culture, background, experience, status, abilities, and opinion enrich the university community. We commit ourselves to the pursuit of excellence in teaching, research, outreach, and diversity as inextricably linked goals.

The University of Wisconsin-Madison fulfills its public mission by creating a welcoming and inclusive community for people from every background – people who as students, faculty, and staff serve Wisconsin and the world.” https://diversity.wisc.edu/