MCB 293 — Biochemistry, Chemical and Structural Biology

Overall objective: To understand the chemical constituents of proteins, RNA, DNA, and other biomolecules; the general physical principles that underlie their structure and function; and experimental methods used to study these principles. We will emphasize the basic concepts and knowledge in this area that are generally assumed as working knowledge for scientists in biomedical research, and we will additional discuss state-of-the-art primary literature. You will re-encounter the principles covered in MCB 293 throughout your research career. We will also have modules throughout the course that will introduce students to modern techniques and approaches to answering biochemical questions. This includes untargeted metabolomics, protein modeling and substrate docking, and PyMOL for visualizing and presenting the structural properties of biomolecules.

Instructors:

Dr. Ryan Nett (Biolabs 3035; rnett@fas.harvard.edu; Slack or email)

Teaching Fellow:

Eric Fields (Biolabs 3036; efields@g.harvard.edu; email preferred)

Office Hours:

Ryan: Mondays, 3:00 PM (Biolabs 3035)

Eric: Fridays, 10:15 AM (in classroom, or Biolounge)

Lectures & section:

When: Monday, Wednesday, Friday 9:00-10:15 AM

Where: Biolabs 1087

Grading

Weekly P-sets: 50% (2 x 10 = 20 submissions, lowest 2 grades will be dropped)

Weekly Reading Quizzes: 10%

Student-led presentation: 15%

PyMOL final project: 15%

Participation: 10%

All course components will be graded on effort. Writing down the correct answer on a P-set without explanation is not sufficient; we're looking for a demonstration of effort and understanding. Explain your work clearly and carefully, so it may help you think clearly and carefully. In class, we ask for you to be engaged. Explain to us what questions you have – we encourage curiosity on all topics!

Please discuss forthcoming absences immediately. We will treat these case-by-case. Unexcused absences and late submissions (-10%/day) will hurt your grade.

Course website: https://canvas.harvard.edu/courses/128457

Problem sets & section

We are trying a new approach to lectures, sections and problem sets to help you absorb the material. To provide you with immediate feedback on problem sets, we will have weekly P-sets, posted a week beforehand. These P-sets can be challenging. We want you to *first try these problems on your own* (deadline on Wednesday nights), followed on Friday mornings by in-class small-group discussion of the problems on each P-set with feedback from the course staff. You will *then mark up your P-set* (in or after class) to show what you learned and resubmit your P-set by Friday night. Both submissions will be graded with equal weight. This approach can be intensive during the semester. In exchange, there is no final exam.

Each year, we add new problems. This year, we hope to base some new problems on materials from MCB faculty candidate seminars (as if you needed more reason to attend!).

Student presentations

During the last half of the semester, we will have a mix of lectures and student-led presentations/discussion of primary literature. The goal of this is to engage you with new, cutting-edge science that relates the conceptual knowledge from this course to real research. Students will work in pairs (or small groups, depending on numbers) and to lead a manuscript presentation and also generate relevant questions for the corresponding Pset; we will work with groups on developing these questions. Grading will be based on the apparent effort and quality of the presentation and Pset questions – we're mostly looking for commitment and thoughtfulness that will engage everyone in the course!

PyMOL Movie

You will design, produce and present a PyMOL-based movie illustrating a molecular system of your choice. The process will start midway through the semester, after an introduction to movie making and scripting in PyMOL. You will generate a proposed movie outline, on which you will receive feedback and suggestions. You will then hand in and present your movie to the class at the end of the semester (see schedule below).

Required & Recommended Reading

The lecture material this year will relate to the textbook *The Molecules of Life*, written by Kuriyan, Konforti, and Wemmer. This syllabus contains a description of required reading from the textbook for each week. We ask that you *do this reading before the lectures* to get the most out of the lectures and focus on those aspects that you found the most challenging. To help you assess your understanding, we will have *brief quizzes on Canvas due Sunday nights* (except before the first week of class!). Additional content (online videos, articles, etc) will be suggested, and we encourage you all to share materials that you find to be helpful!

Availability. A few copies & e-copies of the textbook are available through the Harvard library. The cost of renting the e-textbook typically comes down significantly a few weeks into the semester. To this end, we are providing you with copies of the first four chapters that we'll need. After that, please make sure to buy or rent the (e)textbook.

We expect professional conduct from all students

It may be that you already have a solid understanding of some parts of the material and not of others – this is inherent in having a cohort of graduate students with diverse academic backgrounds. We trust that you will make prudent choices about how you allocate your time and effort. However, *we expect you to fully participate in the lectures and small-group work.* Be present, for a more rewarding experience for yourself and everyone else.

Canvas & Assignment Submission

Lecture notes, assignments, and the most up-to-date version of the syllabus will be posted on Canvas. You will also submit assignments through Canvas. *Please do so by the assigned due dates*. Individual Pset work not received by the time we review it will be considered incomplete unless an exception was granted before the posted deadline.

Slack & online assistance

We will use Slack, rather than email or Canvas, for most announcements and correspondence about any other issues as they come up outside of class or office hours. *If you have a question about how to answer an*

<u>assignment</u>: **come to office hours or contact us in the #pset-help channel**. Just one rule: *<u>No lazy questions</u>*: you must describe which steps you have already taken to answering the question, or we will not help you until you do so.

To join the Slack workplace, follow this link: https://canvas.harvard.edu/courses/128457/external_tools/63266

Python & Matlab

In previous years, we used Matlab. This year we will use Python. If you need additional practice, consider working through the materials at TBD

Accommodations for students with disabilities

Students needing academic adjustments or accommodations because of a documented disability must present their Faculty Letter from the Accessible Education Office (AEO) and speak with a course instructor by the end of the second week of the term. All discussions will remain confidential, although Faculty are invited to contact AEO to discuss appropriate implementation.

Honor code and academic integrity policy

Discussion and the exchange of ideas are essential to doing academic work. We have designed a range of assignments and exercises for this course to both enhance and test your knowledge, and each has a different collaboration policy.

You are encouraged to discuss the workshop exercises and movie design and production with classmates and others, but the work you hand in must be your own writing and should reflect your thought process. Quizzes and the first round of Pset submissions each week must be your own work.

In all work, use citations and references as you would for any published documents. That is, you should cite sources, such as books, articles, and websites, that have helped you with your work using appropriate citation practices. For more information on citation, you can consult <u>The Guide to Citing in the Life Sciences.</u>

Regarding the use of Artificial Intelligence (AI) - e.g. ChatGPT - or any search engines**: the ability of new technology to provide robust answers faster does not fundamentally change the criteria of the honor code and academic integrity policy. Ultimately, ***the work you submit needs to be your own. I would view AI and other online resources in the same way as a textbook or a manuscript. You can (and in some cases should) make use of all resources at your disposal to learn and answer questions. However, simply copying and pasting from a source is highly problematic, and importantly, does not convey an understanding of the material. The goal of this course is to not just to learn fundamental concepts in biochemistry – it's also an exercise in problem solving. ***As such, we strongly discourage you to rely on AI for answering questions.*** Moreover, ***explicit copy/pasting of AI-generated material (or any other published material, for that matter) is unacceptable*** and will invoke, at the very least, a loss in points on an assignment, and potential escalation, as necessary.

Weekly schedule:

	Monday	Tuesday	Wednesday	Thursday	Friday	Weekend
week 1	**Lecture 1** 9:00 – 10:15 am Ryan's office hour: 3pm		**Lecture 2** 9:00 – 10:15 am		**Section** 9:00 – 10:15 am *Pset review* Eric's office hour: 10:15 am	**Due by Sun midnight:** Reading quiz for week 2
week 2	**Lecture 1** 9:00 – 10:15 am Ryan's office hour: 3pm		**Lecture 2** 9:00 – 10:15 am **Due at midnight:** Individual PSet for week 1		**Section** 9:00 – 10:15 am *Pset review* Eric's office hour: 10:15 am **Due at midnight:** Revised PSet for week 1	**Due by Sun midnight:** Reading quiz for week 3
week 3	**Lecture 1** 9:00 – 10:15 am Ryan's office hour: 3pm		**Lecture 2** 9:00 – 10:15 am **Due at midnight:** Individual PSet for week 2		**Section** 9:00 – 10:15 am *Pset review* Eric's office hour: 10:15 am **Due at midnight:** Revised PSet for week 2	**Due by Sun midnight:** Reading quiz for week 4

Week	Date	Topic	Assignments Due*	Instructor
	M 1/22	Introduction to 293		RN
1	W 1/24	Protein Chemistry and Structure	Favorite protein	RN
	F 1/26	PyMOL tutorial		RN/EF
	M 1/29	Nucleic Acids: Chemistry and Structure		RN
2	W 1/31	Glycans and Lipids	Wed night, Fri night	RN
	F 2/2	Pset-review 1: Protein structure		RN/EF
	M 2/5	Thermodynamics		RN
3	W 2/7	~~Intramolecular Forces & Protein Folding~~ ***canceled***	Wed night, Fri night	RN
	F 2/9	Pset-review 2: Nucleic acids, glycans, lipids		RN/EF
	M 2/12	Intramolecular Forces & Protein Folding		RN
4	W 2/14	Free energy, chemical potential, & redox chemistry	**None due this week**	RN
	F 2/16	PyMOL – basics of scripting, movies and morphs		RN/EF
	M 2/19	**PRESIDENTS DAY – no class**		-
5	W 2/21	Ligand binding & molecular recognition	**None due this week**	RN
	F 2/23	Manuscript presentation – Ryan and Eric		RN/EF
	M 2/26	Allostery		RN
6	W 2/28	Student presentation 1 – Arshia, Fardin, & Katia	Wed night, Fri night	RN
	F 3/1	Pset review 3: Thermodynamics, protein folding, free energy		RN/EF
	M 3/4	Enzyme kinetics/catalysis		RN
7	W 3/6	Student presentation 2 – Adam & Minseon	Wed night, Fri night	RN
	F 3/8	Pset review 4: Allostery & enzymes		RN/EF
	3/9-3/17	**SPRING BREAK!**	**SPRING BREAK!**	
	M 3/18	Student presentation 3 – Haoxuan & Reena		RN/EF
8	W 3/20	Biology & Biochemistry of Cell Surface glycoRNA - Ryan Flynn	Wed night, Fri night	RF
	F 3/22	Pset review 5: Metabolism		all

	M 3/25	Biochemical Basis for Metabolic Disease - Fabiola Muro-Villanueva		FMV
9	W 3/27	Student presentation 4 – Eric & Noah	Wed night, Fri night	RN
	F 3/29	Pset review 6: glycoRNAs and chemical biology		RN/EF
	M 4/1	Max Prigozhin – Biological Imaging		RN
10	W 4/3	Student presentation 5 – Aditya & Wesley	Wed night, Fri night	RN
	F 4/5	Pset review 7: Biochemical Basis for Metabolic Disease		RN/EF
	M 4/8	Natural Product Biochemistry – Colin Kim		CK
11	W 4/10	Student presentation 6 – Hisham & Isra	Wed night, Fri night	RN/EF
	F 4/12	Pset review 8: Prigozhin lecture on imaging		RN/EF
	M 4/15	Structural Biology - Jaime Martinez Grundman		JMG
12	W 4/17	Student presentation 7 – Alex & Myrthe	Wed night, Fri night	RN/EF
	F 4/19	Pset review 9: Natural Product Biochemistry		RN/EF
	4/22	PyMOL movie presentations 1		RN/EF
13	4/24	PyMOL movie presentations 2	Wed night, Fri night	RN/EF
	4/26	Pset review 10: Structural Biology		SN/EF

Week 1: Mon Jan 22
Topic: Protein Chemistry & Structure

Learning objectives

- Understand the unique properties of water
- Know the forces that contribute to molecular interactions
- To be able to navigate a protein structure and relate its structural and functional features.
- To familiarize yourself with the amino acids, the aspects in which they differ; protein secondary structure elements; and the essential features of the classification of proteins

Required Reading: Ch. 4 (paragraphs indicated in PDF)

Recommended reading: Ch. 1

Schedule:

M: overview of course syllabus & pymol installation; initial discussion of protein chemistry.

W: Protein chemistry

F: PyMOL tutorial; description of initial pymol exercise

Week 2: Mon Jan 29
Topic: Nucleic Acid, Glycan, and Lipid Chemistry and Structure

Learning objectives**:**

- Identify the building blocks of nucleic acid primary sequence (bases); secondary structure (helix types; RNA elements such as bulges, stems, loops, GNRA tetraloops).
- Be aware of DNA and RNA covalent modifications.
- Understand the geometry of canonical, wobble base pairing, and Hoogsteen base pairing and its consequences for recognition by other nucleic acid strands and proteins.
- Understand the role of pi stacking electrostatic interactions and Van der Waals interactions in the formation of double-helical structures.
- Recognize the basic elements of lipids and complex carbohydrates and the role of hydrogen bonding and the hydrophobic effect in their respective roles.
- Understand the elementary building blocks of lipids

Required prior reading: Ch. 2 (paragraphs indicated in PDF)

Recommended prior reading: Ch. 3

Schedule:

M: Nucleic acid chemistry

W: Glycans & lipids

F: Protein structure Pset (PyMOL-heavy)

Week 3: Mon Feb 5
Topic: Thermodynamics

Learning objectives

Thermodynamics

- The difference between isolated, closed, and open systems;
- Energy is conserved (heat + work in a closed system)
- Entropy summarizes the (log of) the number of possible states of a system; in an isolated system, entropy always increases.
- The hydrophobic effect is dominated by the entropy of water.
- The Boltzmann distribution of states is the distribution that maximizes the entropy of a system given its total energy.
- Understand that (if not how) the statistical and thermodynamic definitions of entropy are related, connecting molecular scale fluctuations to experimental observations (heat, temperature).
- Understand that statistical entropy can also be calculated using probabilities, rather than multiplicities of states.
- Understand how the probabilities of states of molecules and larger systems relate to their energy (the Boltzmann distribution).
- Understand that k_BT determines the relative probability of states of different energy, and that as a consequence, the average energy of a 'degree of freedom' equals 1 k_BT.

Required reading: Ch. 7 (Counting statistics and entropy); Ch.8 (Linking energy and entropy), parts A and B (sections 8.1-8.12); Ch. 6C (molecular forces), Ch. 5A (thermodynamic hypothesis); Ch.10D (protein folding free energy budget); 18A (protein folding)

Recommended reading: Ch. 6A (heat transfer); Ch. 8, part C

Schedule:

M: Thermodynamics

W: ~~Intramolecular Forces & Protein Folding~~ ***canceled***

F: Nucleic acid, glycan, & lipid Pset (PyMOL-heavy)

Week 4: Mon Feb 12
Topic: Intramolecular Forces & Protein Folding; Free Energy, Chemical Potential & Redox Chemistry; PyMOL: scripting, movies & morphs

Learning objectives:

Intramolecular Forces & Protein Folding

- Understand that for many proteins, the primary structure uniquely encodes the tertiary structure, even though an extremely large number of conformations would, in principle, be possible.
- Understand the main contributions to the free energy of folding of proteins, including the role of water.

Free Energy, Chemical Potential, & Redox Chemistry

- Free energy is the central concept that ties together our understanding of what molecules will and will not do.
- Review how free energy relates to energy and entropy, and to the direction of spontaneous change of chemical or physical processes.
- Free energy is a state function, such that changes in free energy can be calculated using fictitious transformations.
- Free energy relates to equilibrium constants through $\Delta G = -RT \log K$
- Coupling of chemical reactions to ATP hydrolysis dramatically shifts the equilibrium.
- Chemical potential is the free energy of a molecule or a mole of molecules relative to a reference state.

PyMOL: scripting, movies, & morphs

- Know how to make presentation quality PyMOL images and movies
- Become comfortable with using PyMOL to visualize macromolecular structures for probing biological questions

Required reading: Ch.10D (protein folding free energy budget); 18A (protein folding); Ch. 9 (Free Energy; 9.1-9.7, 9.12-9.16), Ch. 10 (Chemical Potential; 10.1-10.11), Ch. 11 (Redox Chemistry; 11.1-11.7)

Recommended reading: Ch. 18B (chaperones)

Schedule:

M: Intramolecular Forces & Protein Folding
W: Free Energy, Chemical Potential & Redox Chemistry
F: PyMOL: scripting, movies & morphs

Week 5: Mon Feb 19
Topic: Ligand Binding, Molecular Recognition

Learning objectives:

- Understand how binding equilibria are described (K_d)
- Understand some of the ways in which binding equilibria are studied experimentally
- Understand the difference between lock-and-key, induced fit, and conformational selection models for the interactions between ligands and protein (or between proteins)
- Understand the difference between affinity and specificity
- Understand how binding equilibria are described (K_d)
- Understand some of the ways in which binding equilibria are studied experimentally (this is kind-of treated in different parts of Ch. 12)
- Understand the difference between lock-and-key, induced fit, and conformational selection models for the interactions between ligands and protein (or between proteins)

Required reading: Ch. 12 (Thermodynamics of Molecular Recognition; 12.1-12.11, 12.15-16), Ch. 13 (Specificity of Molecular Recognition; 13.1-13.4)

Recommended reading: remainder of Ch. 12 & 13.

Schedule:

M: Holiday (no class)

W: Ligand Binding, Molecular Recognition

F: Manuscript presentation – Ryan and Eric

Week 6: Mon Feb 26
Topic: Allostery

Learning objectives**:**

- Understand the conceptual and mathematical basis for how binding of one ligand can affect binding of a second ligand
- Relate cooperativity to the function of hemoglobin as a model example of allostery
- Determine the relationship between reaction rates and concentrations.

Required reading: Ch. 14 (14.1-14.3; 14B) Ch. 15A: rate laws; Ch. 15C: catalysis, Arrhenius; 16.1-25

Recommended reading: 15B (reversibility; measurements); rest of ch. 16.

Schedule:

M: Allostery

W: Student presentation 1

F: Thermodynamics, Protein Folding, Free Energy - Pset

Week 7: Mon Mar 4
Topic: Enzyme Kinetics/Catalysis + Principles of Metabolism

Learning objectives:

- Determine the relationship between reaction rates and concentrations.
- Understand the utility of Michaelis-Menten kinetics and how to measure this.
- Be able to define the various Michaelis-Menten variables (K_m, V_{max}, K_{cat}).
- Conceptualize the mechanisms by which enzymes accelerate reactions.

Required reading: Ch. 15A: rate laws; Ch. 15C: catalysis, Arrhenius; 16.1-25

Recommended reading: 15B (reversibility; measurements); rest of Ch. 16.

Schedule:

M: Enzyme Kinetics & Catalysis
W: Student presentation 2
F: Allostery - Pset

--SPRING BREAK--

Week 8: Mon Mar 18
Topic: glycoRNAs: Discovery and Tool Development (Professor Ryan Flynn, Harvard Medical School)

Learning objectives:

- Expand on understanding of glycosylation in biochemistry and cell biology more broadly
- Understand the emerging roles of glycoRNAs in cell biology
- Learn the methods used to identify and study glycoRNAs

Required reading: Flynn et al. (2021) *Cell* 184, 3109–3124.

Recommended reading: Disney (2021) *Cell* 184 (highlight of Flynn et al.); Xie et al. (2023) bioRxiv

Schedule:

M: Student presentation 3
W: glycoRNAs – Professor Ryan Flynn
F: Enzyme kinetics/catalysis - Pset

Week 9: Mon Mar 25
Topic: Metabolic Disease and Biological Solutions (Dr. Fabiola Muro-Villanueva, Harvard University)

Learning objectives:

- Understand the basics of how gene editing tools and how they can be used to treat genetic diseases
- Learn a case study for how specific genetic mutations lead to biochemical causes of disease
- Relate aberration in metabolic or biochemical function to a macroscopic disease phenotype

Required reading: Haney et al. (2024) *Nature*. https://doi.org/10.1038/s41586-024-07185-7

Recommended reading: Wu et al (2019) *Nature*. 25, 776-783. Barr, AJ (2018) *Essays in Biochemistry*. 62, 619-642.

Schedule:

M: Metabolic/genetic disease – Dr. Fabiola Muro-Villanueva
W: Student presentation 4
F: glycoRNAs - Pset

Week 10: Mon Apr 1
Topic: Bioimaging (Professor Max Prigozhin, Harvard University)

Learning objectives:

Required reading:

Recommended reading:

Schedule:

M: Bioimaging – Professor Max Prigozhin
W: Student Presentation 5
F: Metabolic/genetic disease - Pset

Week 11: Mon Apr 8
Topic: Natural Product Biochemistry – Dr. Colin Kim (Harvard University)

Learning objectives:

Required reading:

Recommended reading:

Schedule:

- **M**: Natural Product Biochemistry – Dr. Colin Kim
- **W**: Student Presentation 6
- **F**: Bioimaging - Pset

Week 12: Mon Apr 15
Topic: Structural Biology – Dr. Jaime Martinez Grundman (Harvard University)

Learning objectives:

Required reading:

Recommended reading:

Schedule:

- **M**: Structural Biology – Dr. Jaime Martinez Grundman
- **W**: Student Presentation 7
- **F**: Natural product biochemistry - Pset

Week 13: Mon Apr 22
Topic: Student PyMOL Movie Presentations

Schedule:

- **M**: Student movies - 1
- **W**: Student movies - 2
- **F**: Structural biology - Pset

Cross reference between lectures and book

Ch. 1	1A: Interactions between molecules Skip?
	1B: Intro to nucleic acids and proteins Skip?
	1C: replication, transcription, translation Will be assumed known
Ch. 2	2A: double-helical structures of DNA, RNA NA
	2B: functional (and chemical) versatility of RNA NA
Ch 3	3A: glycans Skip?
	3B: lipids and membranes Skip?
Ch. 4	4A: Proteins: general principles Proteins 1
	4B: Backbone conformation Proteins 1
	4C: Structural motifs & secondary structure
	4D: Structural principles of membrane proteins
Ch. 5	5A: Thermodynamic hypothesis Forces & Folding
	5B. Sequence comparisons Skip
	5C: Structural variation in proteins
	5D: The evolution of modular domains
Ch. 6	6A: Thermodynamics of heat transfer (1st law) Thermo 1
	6B: Heat capacity & the Boltzmann dist Skip
	6C: Energetics of intermolecular interactions Forces & Folding

Ch. 7	7A: counting statistics Thermo 2
	7B: Entropy Thermo 2
Ch. 8	8A: Energy Distributions & Entropy
	8B: The Boltzmann Distribution
	8C: Entropy and Temperature
Ch. 9	9A: Free Energy Free Energy & Chemical Pot
	9B: Standard free energy changes Free Energy & Chemical Pot
	9C: Free Energy & Work (ATP) Free Energy & Chemical Pot

Ch. 10	10A: Chemical Potential Free Energy & Chemical Pot
	10B: Equilibrium Constants Free Energy & Chemical Pot
	10C: Acid-Base equilibria Free Energy & Chemical Pot
	10D: Free energy changes in protein folding Forces & Folding
Ch. 11	11A. Redox reactions in biology
	11B. Reduction potentials and free energy
	11C: Ion pumps and channels in neurons
	11D. Action potentials in neurons
Ch. 12	12A. Thermodynamics of binding 12B. Drug binding by proteins

Ch. 13	13A: Affinity and specificity in Mol. Recognition
	13B: [Specificity] in protein-protein interactions
	13C. Recognition of nucleic acids by proteins Wrap into NA?
Ch. 14	14A. Ultrasensitivity of Mol Response (allostery) Ultrasensitivity
	14B: Allostery in hemoglobin
Ch. 15	15A. General kinetic principles
	15B. Reversible reactions, steady state, eq.
	15C: Factors affecting rate cst (catalyst, diffusion)
Ch. 16	16A: MIchaelis-Menten kinetics
	16B: Inhibitors and other complex kinetics
	16C: Protein Enzymes
	16D: RNA enzymes Skip
Ch. 17	17A: Random walks Diffusion
	17B: Macroscopic descriptions (examples, visc.) Diffusion
	17C: Expt. measurement of diffusion
Ch. 18	18A: Protein folding Forces & Folding
	18B: Chaperones for protein folding Forces & Folding
	18C: RNA folding Skip

Ch. 19	19A: Measuring the stability of dsDNA Skip (use for Qs)
	19B: Fidelity in DNA replication Proofreading
	19C: Fidelity in translation (ribosome) Proofreading