Course content: MCB501, Imlay section

This course is required of first-year MCB Ph.D. students. Graduate students from other units may also enroll. Students who take this class should have studied biochemistry previously; the goal of this course is to inspect the physical processes of biology at a higher level. Nevertheless, most topics begin with a summary of the foundational material to help those who have forgotten it.

Course topics.

1. Genomics.

What processes are necessary for the viability of a cell? What cell processes are conserved among organisms, and which differ?

2. Thermodynamics in biology: Enthalpy and entropy.

1. Enthalpy, types of bonding interactions, and the first law of thermodynamics.
2. What is "heat"? How is orbital energy converted to molecular motion? Predictions of ΔH from standard bond energies.
3. Why are exothermic reactions reversible? Collision theory reveals the concept of entropy.
4. Entropy, spatial organization, and substrate flow down concentration gradients. Statistical mechanics approach; transmembrane gradients as an example.
5. How can entropy drive an endothermic reaction? Derivation of the concept of free energy.

3. Thermodynamics in biology: free energy.

6. Relation between free energy and equilibrium constants.
7. Determination of free energy from experimental data.
8. Cellular metabolite concentrations and the impact of positive free-energy values.

4. Bioenergetics: fermentation pathways.

1. Predicting free-energy demand/release. Rule of thumb for predicting reaction equilibria.
2. Rationalization of free energy. Considerations of bond strength, resonance, and product clearance during substrate oxidation. Introduction to redox states.
3. Refresher on enzymic catalysis. Statistical-mechanics explanation of why enzymes reduce free energies without affecting equilibria.

5. Pathway analysis (glycolysis).

1. Glycolysis: quick review of pathway.
2. Why doesn't the cell use alternative routes?
3. Mechanisms of glycolytic enzymes: How enzymes work.
4. The original purpose of the glycolytic pathway was—biosynthesis?
5. The rate of intracellular chemistry.

6. Redox balances in fermentations.

1. Calculating redox balances--the raison d'etre of fermentations.
2. Pyruvate:formate lyase, a free-radical enzyme.

7. Pyruvate dehydrogenase and the TCA cycle: further analysis of pathway structure and enzyme mechanisms.

1. Pathway strategy: labilization of carboxylates.
2. Enzyme mechanisms; structure/function analyses.
3. Cofactor functions: thiamine, flavins, lipoate, pantothenate.

8. Predicting pathways.

One should be able to predict many catabolic and biosynthetic pathways, given only the identity of the starting or end product.

9. How do new enzymes and pathways evolve?

How do multi-step pathways evolve? What are enzyme superfamilies?

10. Branch-point kinetics.

1. Review: Michaelis-Menten kinetics, allostery.
2. Mathematical solution of simultaneous equations to demonstrate the robustness of flux controls at a branch point (e.g., acetyl-CoA) in central metabolism.
3. Isotopomer analysis to experimentally determine fluxes at branch points.

11. "Difficult" chemistry.

Why are some pathways so long? Why does nature sometimes resort to free-radical-based enzyme mechanisms?

12. Biosynthesis.

1. Precursor metabolites and global carbon routing. The virtue of metabolism as a network rather than as an aggregation of linear pathways.
2. Analysis of pathways: methionine and valine as examples. Precursor metabolites, thermodynamic constraints, and standard reaction types are emphasized.

13. Analysis and re-engineering of a biosynthetic pathway.

Dissection of a biosynthetic pathway via mutant studies, radiotracer experiments, enzyme studies, crossfeeding experiments. Kinetic controls, futile cycling, and subtrate channeling.

14. Energy production by electron-transfer reactions: thermodynamics of redox reactions.

1. Review of reduction potentials.
2. Chemical characteristics that determine the Eo' of redox-active molecules.
3. Mathematical interconversion of reduction potentials, free energy, and equilibria.
4. Proton gradients, electrical potentials, and protonmotive force. Measurements.
5. Measurements of coupling numbers. Calculations of theoretical maxima.
6. Physical basis of mitochondrial respiratory control.

7. Measurement of intracellular volume (necessary for bioenergetics calculations).
8. Thermodynamic solutions: ATPase, ion transporters.

15. Structure of the mitochondrial respiratory chain.

1. Chain structure.
2. Rationalizing redox potentials and coupling sites.
3. Experimental measurements of proton coupling values.
4. Understanding the effects of inhibitors.

16. Respiratory puzzles.

1. How does glycolysis continue if the membrane potential is large?
2. Should the coupling number of the ATPase reflect the likely membrane potential?
3. How can organisms in acidic environments avoid acidification by the ATPase?
4. How can organisms in an alkaline environment establish a membrane potential?
5. How can organisms generate NADPH when they grow on substrates that are less reducing than NADPH?

17. How do new bioenergetic systems evolve?

18. Structure/function analysis of an electron-transfer enzyme: succinate dehydrogenase.

Techniques: Biochemical assays, protein purification, mutant hunts, prediction of phenotypes, electron-paramagnetic resonance spectroscopy, redox titrations, etc.

19. Structure/function analyses of two proton-translocating enzymes: the bc1 complex and bacteriorhodopsin.

The two best-understood proton-translocating enzymes, one using a Q cycle, and the other using a photochemically-driven pump. Complex structures, flash kinetics, and mutant phenotypes are discussed for both enzymes.

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Two exams, with open notes. Scores from my section of the class are averaged with those from Raven Huang's section of the class, prior to assignment of a final grade. Homework is checked for completion but not graded.

Office hours: 2-3 pm, Friday; by appointment; or drop by my office (B303, CLSL).

If exam conflicts occur, please contact me at least one week in advance.