Class 1 – Thermodynamics


In the first class, we review some basic chemistry as it applies to macromolecular structure and function. The themes that appear in this first class will reappear throughout the course. Make every effort to grapple with them now, but also realize that we will continue to return to these topics and that your understanding of them will deepen over the course of the semester.
Class 2 – Principles of Protein Structure

Branden & Tooze, Introduction to Protein Structure, 2nd ed., Chapters 1-2.

We will devote this class to learning (or re-learning) the principles of protein structure from the ground up. The reading in Branden & Tooze forms a lucid introduction to this topic.

What are the structures, one-letter codes, and side chain pKa's for the amino acids? If you have not already done so, you need to commit these to memory: they’re an indispensable part of the language of biology. What are the salient features of the different regular secondary structures observed in proteins?
Class 3 – X-ray Crystallography

Branden & Tooze, Chapters 3-5, pp. 35-55 and 67-70.

In addition, read the relevant sections of at least one of the following:

[Easy] Branden & Tooze, Chapter 18.

[Moderate] D. Eisenberg and D. Crothers, Physical Chemistry with Applications to the Life Sciences (Benjamin/Cummings, 1979). An advanced undergraduate textbook with good chapters (16 & 17) on symmetry and crystallography.


[Rigorous] C.R. Cantor and P.R. Schimmel, Biophysical Chemistry (W.H. Freeman, 1980). Chapter 14 is an excellent introduction to crystallography at a reasonably high level.

Chapters 3-5 take up where the previous class left off, explaining the architectural principles of protein structure with both examples and useful generalizations. Read this not in order to commit it to memory but rather to begin gaining an appreciation for protein architecture. You will find such an appreciation to be invaluable as the course goes along.

This class will be mainly a lecture about protein crystallography, in which the goal is to demonstrate how collections of atoms generate patterns of diffracted X-ray spots, and how the intensities of these spots tell an X-ray crystallographer about the structural arrangement of the atoms. The presentation will be mathematically simple, but should give you a good intuitive grasp of the basic principle involved. We will also consider a few of the general principles involved in structure determination by nuclear magnetic resonance (NMR) spectroscopy.

Some questions to consider: How can you evaluate the quality (reliability) of structural models? What use might you make of them? What are the unique strengths and weaknesses of NMR and X-ray crystallography in determining macromolecular structures? These questions will continue to come up during the course as we consider the interrelationships between protein structure and function.
Class 4 – Protein Stability


The forces that stabilize protein structures – the hydrophobic effect, salt bridges, van der Waals interactions, etc. – play key roles in most if not all noncovalent intramolecular and intermolecular interactions. As you read these papers, consider how the authors adopt both qualitative and quantitative approaches, bringing together the thermodynamic and structural ideas we've been discussing.

The first half of the class discussion will concern the Lim & Sauer paper, which tries to assess the importance of various features of the hydrophobic core to protein stability. The second half of the class will encompass both the short paper by Chothia and the longer one by Eriksson et al. Chothia finds a quantitative relationship between the size of a hydrophobic residue and its hydrophobicity. Pay attention to how these quantities (i.e. size and hydrophobicity) are estimated. As you will see, Eriksson et al. can be viewed as an attempt to confirm Chothia's relationship for proteins.

As you read the papers for this class, think about the different types of forces and interactions that might stabilize native protein structures. Does water play a key role in protein stability? What do you think of the suggestion that the pattern of hydrophobic and hydrophilic residues in a protein chain, rather than the precise identity of those residues, determines its structure? What do you think about the idea that the native protein structure is determined by the fitting together of the hydrophobic core residues in a manner analogous to a three-dimensional jigsaw puzzle?

Special Note about Stereo: The Eriksson et al. paper, and some later ones, make use of stereo diagrams. Stereo diagrams are immensely helpful in visualizing three-dimensional objects. Figures that seem uninterpretable without stereo often become easy to see and informative with it. Therefore, don't be lazy – be sure to view stereo figures in these papers and future ones using stereo! Some people can see in stereo without extra help, but almost everyone can do it with the special viewers provided. The viewers allow each eye to focus on the appropriate image, and under uniform lighting conditions give a very nice stereo effect. If you have any difficulties, we will be happy to give you advice – just ask!
Class 5 – Ligand Binding

Branden & Tooze, Chapter 13.


The same noncovalent forces that stabilize the structures of individual proteins (van der Waals interactions, H-bonds, the hydrophobic effect, etc.) reappear throughout biology. Proteins use these forces to interact with one another, passing information or collaborating to carry out some key function. Enzymes bind substrates and small molecule regulators, nucleic-acid binding proteins bind DNA and RNA, many proteins interact with membranes. All of these fundamental biological events utilize the same small toolkit of non-covalent forces.

As you read Cunningham & Wells, try to answer the following questions: How do the authors calculate rate constants? What is $K_d$ and why do the authors choose to quote it? What is $\Delta \Delta G$ (in plain English)? What residues affect the on-rate? Why? What values do the authors obtain for the energy of a salt-bridge? a hydrogen bond? burying a $-\text{CH}_2$ group? Does the X-ray structure of a protein tell us everything we could want to know about its function?

One important goal is to develop a quantitative framework for considering binding. It takes some time and effort to develop a good sense for how to think about binding experiments. We will thus tackle the problem both in class and in the problem set. This effort is worthwhile. In addition to tying together key themes in the course, a clear understanding of binding reactions will help you conduct your own experiments and critically evaluate the experiments of others.

Note: The reading in Branden and Tooze is particularly worthwhile. It introduces the biological and structural context not only for the growth hormone receptor that Cunningham & Wells investigate, but also for several of the proteins we will discuss in upcoming classes.
Class 6 – Enzymes I: Principles of Catalysis

Branden & Tooze, Chapter 11.


Here, we begin a consideration of proteins as active agents in effecting biochemical transformations. A quantitative framework for handling enzyme reactions will be presented in class, building on the classic conceptualization by Pauling. Be sure you understand what Pauling proposed. Our previous work on binding will pay off here, because enzymes work by binding tightly to transition states using the same non-covalent forces with which you have already become familiar.

In addition to developing the equations used to describe enzyme reactions, we will discuss some of the reaction mechanisms described by Branden & Tooze, beginning with the catalytic triad employed by serine proteases. Be sure you understand the basic chemistry at work here. Use this framework in reading the paper by Carter & Wells. Why does the triple alanine mutant display residual activity? What does this work suggest about the evolution of the catalytic triad?
Class 7 – Enzymes II: Mechanism

Branden & Tooze, Chapter 13.


This class concerns Ras, the flagship member of a huge family of proteins involved in signal transduction and many other cellular processes. Come to class familiar with the meaning of the term "G protein", the differences between large heterotrimeric G proteins and small G proteins like Ras, and the so-called G protein cycle involving GAPs and GEFs. Make sure you don't miss the connection between G proteins and the human growth hormone receptor we studied in Class 5.

The paper by Milburn et al. is dominated by X-ray crystal structures, and provides a striking example of how atomic-resolution structure guides attempts to understand molecular function and regulation. Be sure to pay some attention to the methodology. What use can you make of information like R-factors, B-factors, etc.?

As you read Maegley et al., strive to understand the difference between dissociative and associative mechanisms for Ras-catalyzed GTP hydrolysis. Be sure you follow the authors' logic in concluding that negative charge does not accumulate on the γ-phosphoryl group in a dissociative transition state. Why might an arginine side chain provided by Ras-GAP enhance the catalytic activity of Ras? Although some of the detailed chemistry in this paper may elude you, and you shouldn't worry about that overmuch, do be sure you understand Scheme I and Fig. 1. It may help you to know that there is a typographical error in Scheme I (what is it?).
Class 8 – Enzymes III: Regulation and Allostery


Regulation is fundamental to the appropriate functioning of enzymes in the cell. Nowhere is this more apparent than for the enzymes, such as G proteins and kinases, involved in signaling processes. Cells could (and do) regulate the activity of enzymes in many different ways, a few of which we will consider today. Be sure to consult an undergraduate biochemistry textbook to remind yourself of the meaning of the terms allostery, cooperativity, and Hill coefficient.

The binding of RasGAP to Ras has a profound influence on its catalytic activity. Scheffzek et al. consider various possible mechanisms by which GAP proteins might accelerate GTP hydrolysis. Several striking answers emerge from their structural studies. Be sure that you understand what they did - the proteins they used as well as the bound ligand(s) and their relationship to the enzymatic reaction under consideration. What is the affinity of the Ras-RasGAP interaction? Are there major conformational changes that accompany GAP binding to Ras? What effect does GAP binding have on switch 2? Now why do you think Gly12 mutations are oncogenic? What catalytically important residue does GAP supply? Do these authors reach the same conclusion as Meagley et al. regarding the nature of the transition state? If not, what do you think about the discrepancy?
Class 9 – Enzymes IV: ATP Synthase


Today we will discuss what may be the world’s smallest, and one of its most elegant, machines: the ATP synthase. You will want to do a few pages of background reading in any biochemistry textbook to remind yourself how the ATP synthase uses the proton gradient across the mitochondrial membrane to provide the energy to produce ATP from ADP and inorganic phosphate. The focus of this class will be on the ways in which high-resolution structure can play an enormous role in suggesting biological mechanisms. Be sure you follow the argument in Abrahams et al. for rotational catalysis, and how the static structure relates to the different states of each subunit. How much cooperativity is there among the three catalytic sites in the enzyme? Does rotational catalysis seem too far out? Do you believe that the observations by Noji et al. prove the model? What further observations would be informative? Finally, be sure to save a little time to think about bacterial flagella (why? you tell me!).
Class 10 – Membrane Structure and Organization


Membranes and membrane proteins are of fundamental importance in cell biology. All cells and many viruses are surrounded by membranes. Likewise, the compartments within eukaryotic cells are also defined by membranes. Today we will introduce some fundamental features of membranes and membrane proteins. Note, however, that this is not the first (or the last!) time membranes come up in MOL 504. As we discussed in connection with the F₁Fₒ ATPase, proton gradients across membranes store much of the energy released by the controlled oxidation of NADH. Large multisubunit protein complexes pump protons across the membrane to generate this gradient. These protein complexes exemplify a common function of membrane proteins: many allow, or promote, the passage of molecules or ions from one side of the membrane to the other. We will explore these ideas further in classes 12 and 14, where we consider channel proteins that allow potassium ions and newly-synthesized proteins to traverse membranes. Other membrane proteins are involved in the trafficking of vesicles among intracellular compartments, and we will touch on this topic in class 11 and return for a closer look in classes 17 and 18.

Memorize the structures of cholesterol and of the phospholipids in Fig. 10-12 of Alberts. Consider the noncovalent forces that account for the structure and stability of phospholipid bilayers. Think about the energetics (and hence the rate) of “flip-flop” of phospholipids from one leaflet of the bilayer to the other and how it might compare to the rate of diffusion within a leaflet. How do detergents solubilize membrane proteins? How might you determine the three-dimensional structure of a membrane protein? Could you use X-ray crystallography?

What is the basic problem Rothman and Kennedy set out to examine? What is a pulse-chase experiment, and how is it used here? Can you think of other applications for this powerful tool? What can account for the rapid kinetics of transmembrane movement? What properties would a protein ‘flippase’ have? Can you think of an approach for isolating and identifying such a protein, if it exists?
Class 11 – Protein Purification


Alberts et al., Chapter 13, pp. 711-715.


Today, we will discuss principles of protein purification. If this topic is entirely unfamiliar to you, read the relevant section in any undergraduate biochemistry textbook before you begin the assigned reading. Please remind yourself of the physical basis for major techniques in protein purification, including measurement of protein concentration, separation by precipitation, separation by adsorption (ion exchange, hydrophobic interaction, and affinity chromatographies), and separation in solution (gel filtration and electrophoretic methods). Is a good yield or a good enrichment more crucial for the success of a purification procedure?

Classically, the basis for purifying a novel protein has been an assay that detects the presence of the protein by measuring its activity. As an example of how powerful this approach can be, we will discuss work initiated in the 1980’s by Rothman and colleagues, who bravely decided to purify proteins required for intracellular trafficking. Their cell-free assay, which took five years to develop, is described in Panel 13-1 of Alberts et al., and in far greater depth in Balch et al. Make sure you come to class understanding the basis for the Rothman group’s elegant Golgi trafficking assay.

Block et al. put the assay to work in order to purify a novel protein, NSF. As you read this paper, consider that four years of hard work separated the development of the assay (Balch et al.) from the purification of the first protein that stimulates it (Block et al.) – why do you think this was so difficult? What roles do NEM, DTT, and ATP play in the purification of NSF? How do the authors turn the Golgi transport assay into an NSF assay? How do they seek to prove that NSF has a role in intracellular trafficking? What other approaches might convince a skeptic that NSF is a genuine trafficking factor?
Class 12 - Ion Channels

Alberts et al., Chapter 11, pp. 615-619 and 631-649.


Ion channels allow ions to flow through the otherwise-impermeable barrier presented by the plasma membrane of a cell. Their importance in biology for signaling and the functioning of the nervous system is impossible to overstate (like G proteins, as you may recall). Furthermore, studying the first ion channel of known high-resolution structure will allow us to discuss principles of membrane protein structure.

The reading in Alberts provides an excellent introduction to ion channels and some of their salient functions in biology. It also introduces patch clamping, a premier technique that has allowed researchers for the past twenty years to visualize the function of single molecules. You will appreciate that few other techniques afford single-molecule sensitivity.

We will also discuss in detail the structure and function of the potassium channel protein KcsA. This structure truly represents a landmark in molecular biology. Don't miss the opportunity to spend time reading this paper carefully and appreciating the elegant design of this exquisitely specific pore. You must use a stereo viewer to get anything out of many of the most important figures. Let me say this again. You must use a stereo viewer to get anything out of many of the most important figures. OK? In addition, I strongly recommend that you download the structure file (1BL8.PDB) and view it using Deep View as you read the paper.
Class 13 – Translation and the Ribosome

Alberts et al., Chapter 6, pp. 337-339, 342-351, and 354.


Arguably nature’s most impressive macromolecular machine, the ribosome is a factory for synthesizing polypeptides. The bacterial ribosome is composed of three RNA molecules and more than 50 proteins. A towering achievement of the last five years has been the determination of the x-ray structures of both its large and small subunits. Read Alberts to solidify your understanding of the basic mechanics of translation and refamiliarize yourself with some of the key terminology before plunging into the two research articles. Of course, if you have forgotten all about nucleic acid structure, you will want to review that too.

Read Nisson et al. to understand how x-ray crystallographic analysis was used to propose a detailed mechanism for catalysis. They hypothesize that the adenine-2486 base, which is 100% conserved among ribosomes in all organisms, plays a critical role in catalysis. Specifically, Nisson et al. suggest that A2486 acts as a general acid-base catalyst, first deprotonating the amine and later protonating the ribose reaction product. Such a role is somewhat surprising, because the N-3 on the adenine base has a $pK_a$ below 3.5. Be sure you understand the issue here, and the argument that Nisson et al. construct to provide a justification. What aspects of the proposed mechanism resemble that of serine proteases? Why do you suppose nature uses RNA instead of protein to catalyze protein synthesis?

Every good hypothesis deserves a decisive experimental test. If Nisson et al. are right about A2486, its mutation should abolish ribosome activity. What is difficult about carrying out this experiment? How do Polacek et al. manage it? What do they find? (Do you suppose that Steitz and Moore viewed this result as decisive?) What conclusions do you draw about the mechanism of ribosomal protein synthesis?
Class 14 – Translocation Across the ER Membrane

Alberts et al., Chapter 12, pp. 659-669 and 689-702.


Today, we will consider translocation of something far larger than a $K^+$ ion across the membrane. The essential problem, as Blobel and Dobberstein put it in their classic 1975 paper, is that “biological membranes present a diffusion barrier for macromolecules such as proteins, but transfer of a large number of specific proteins across membranes is an important physiological activity in virtually all cells” [Blobel, G. & Dobberstein, B. (1975) J. Cell Biol. 67, 835-851]. It will be assumed that you know essentially how translation works, but if not, you need to find out (for example, by consulting Alberts et al., Chapter 6, pp. 335-365).

Simon and Blobel use direct electrophysiological methods to observe protein conducting channels in the membrane of the endoplasmic reticulum (ER). Be sure you understand their experiment, including the role of puromycin. What controls do the authors use? In what ways might the opening and closing of the pore be linked to the presence of a polypeptide in the channel, thus maintaining the seal that prevents ions and other molecules from leaking across the ER membrane? Do the data prove that the ribosome falling off closes the channel? What sorts of methods would you use to characterize the channel further? If you were able to determine its three-dimensional structure at high resolution, what might it look like?

Görlich and Rapoport devise an in vitro system that reconstitutes protein translocation. We will go through the figures in some detail, so be prepared to discuss methods, controls, and conclusions. What stimulated the authors’ interest in Sec61p? What factors appear to be most important in making this system work? What components are necessary (be sure you understand what they are)? How do they deal with the possibility of cross-contamination? Does this work exclude the possibility that other factors are required in vivo (and what does it mean for a factor to be ‘required in vivo’)? Do Görlich and Rapoport reach the same conclusion as Simon and Blobel regarding whether the channel is proteinaceous? What lines of evidence support the authors’ contention that they have ‘faithfully reproduced’ protein translocation in their reconstituted system? If the system has been faithfully reproduced, to what further uses might you put it?
Class 15 – Protein Folding in the Cell (Prof. Rye)


We will open our discussion of intracellular protein folding by revisiting why protein structures are stable. These considerations lead to the observation, first formulated by Christian Anfinsen, that the three-dimensional structure of a protein is determined by the relative thermodynamic stabilities of the folded and unfolded states of the protein. Therefore, the native three-dimensional structure of a protein is fully specified by the protein’s amino acid sequence. Anfinsen’s simple and elegant demonstration of the thermodynamic control of protein folding does not, however, provide a specific mechanism to explain how proteins fold, and we will consider some of the possibilities, including folding pathways and landscapes. Much recent work has demonstrated that the kinetics of protein folding can completely dominate, and even block, the folding of a protein, despite the overall thermodynamic driving force that pushes folding forward. The kinetic control of a folding reaction is the subject of the first paper by Baker et al. As you read this paper, consider why nature would select for large free energy barriers that slow down protein folding.

The kinetic aspects of protein folding are an enormous problem for proteins, especially on the interior of a cell. One of the consequences of slow protein folding is the population of intermediate folding states that are highly prone to aggregation. Why would a folding intermediate be prone to aggregation? Why is this particularly a problem on the interior of a cell? In order to deal with these problems, cells have evolved a network of specific machines known as molecular chaperones that are designed to prevent and correct the error-prone folding of cellular proteins. We will examine how molecular chaperones facilitate protein folding by examining one of the central components of the network of cellular chaperones, the Hsp60s (also known as chaperonins). This family of protein folding machines is one of the best characterized of the molecular chaperones. We will first review why certain proteins require molecular chaperones to fold. We will then focus our discussion on the structure, function, and mechanism of protein folding mediated by the E. coli chaperonin, GroEL.

GroEL is an elegant molecular machine that brings together many of the aspects of macromolecular biology that you have seen in previous lectures, so be prepared to think about molecular allostery and enzyme catalysis. Given what you know about enzyme catalysis, how might GroEL facilitate protein folding? Be sure to take into account the fact that GroEL is a general protein-folding machine that can function on a diverse set of proteins.
Class 16 – Intracellular Proteolysis (Prof. Rye)


In this class, we will examine the ultimate end of protein quality control and dynamics: intracellular proteolysis. Cells use a wide variety of systems to tag proteins for degradation and an equally broad array of molecular machines to carry out the degradation of these marked proteins. These proteolytic systems are used not only to rid the cell of damaged proteins, but also to control many aspects of basic cellular function. As you read the papers assigned for this class, consider why cells would spend so much energy to build and run such complex machines to carry out a chemical reaction that is intrinsically spontaneous (amide hydrolysis). Does anything about this question strike you as familiar when you think about chaperone-mediated folding? Why is the paper by Weber-Ban et al. so significant? In what way does the paper by Kenniston et al. extend or enhance what was shown by Weber-Ban et al? What is the role of local protein structure in ATP-driven proteolysis?
Class 17 – Intracellular Traffic I: Vesicle Budding

Alberts et al., Chapter 13, pp. 711-720 and 726-731.

Barlowe, C. et al. (1994) COPII: A membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell 77, 895-907.


Today we begin our discussion of vesicle trafficking by considering how the regulated assembly of coat proteins drives the budding of vesicles containing particular cargo. Chapter 13 of Alberts et al. contains an excellent introduction to this topic, and those with an interest in intracellular transport would be well advised to read the entire chapter. Otherwise, the selected background readings will provide good background and context for the specific readings.

As you study Barlowe et al., be sure you understand in practical working detail the basis for the vesicle budding assay. Key aspects of the assay are described briefly in this paper, but if the principles seem obscure to you, you may also wish to consult Rexach, M.F. and Schekman, R.W. (1991) J. Cell Biol. 114, 219-229. You should also understand how they measure the vesicles’ fusion competence (and, as always, why they were interested). You need to know the normal maturation pathway of gpαf so that you can fully appreciate its utility in these experiments. What accounts for the requirement for GTP? What role(s) do non-hydrolyzable analogs play in the experiments? What proteins are found in the budded vesicles, and what role may they play in trafficking overall? What about ER resident proteins? What can we conclude about the relative roles of COPI and COPII? What other coat(s) are involved in vesicle trafficking in eukaryotic cells?

Belden & Barlowe address one of the most interesting questions regarding trafficking: cargo selection. The fidelity of any transport system depends critically on the accurate packaging of cargo. (The second obvious requirement is for the accurate delivery of cargo to the proper destination, but we defer this question until next class.) How did the authors become interested in Erv29p? What in vitro experiments demonstrate its involvement in gpαf packaging? What are the key control experiments? If Erv29p is so important, why isn’t it an essential protein? How do the authors study its in vivo function (aren’t you glad you already know what pulse-chase experiments are!)? What evidence do the authors produce for a direct physical interaction between Erv29p and gpαf? How convinced are you, and why (or why not)? What is the potential functional significance of the dilysine motif present on Erv29p?
Class 18 – Intracellular Traffic II: Vesicle Fusion

Alberts et al., Chapter 8, pp. 489-491 and Chapter 13, pp. 720-726 and 757-765.


Today we will focus on SNAP receptors, also known as SNAREs. SNAREs are found on every cellular membrane and are central to the docking and fusion of intracellular transport vesicles. The first paper presents the discovery of SNARE proteins. Of all Jim Rothman’s many publications, this one is the most often cited. The second paper presents an interesting, yet still somewhat controversial, in vitro assay for SNARE-mediated vesicle fusion.

As you read Söllner et al., recall the original Rothman assay and the way it was used to purify NSF. Subsequent work showed that NSF binds membrane receptors through adaptor proteins called SNAPs. With this background, Rothman and colleagues went after the membrane receptors and came up with a fantastic result. Be sure you understand the logic behind Fig. 1 and the purpose of each lane in Fig. 2. How did the authors proceed from ‘bands on a gel’ to the identification of syntaxin, SNAP-25, and VAMP/syntaptobrevin? What corroborating evidence suggests that these proteins indeed are important for vesicle trafficking and fusion? When you have finished the paper, try to fit in the additional fact (discovered soon after) that NSF/SNAP acts as an ATP-dependent chaperone in the disassembly of SNARE complexes.

Weber et al. develop a fluorescence-based assay for the detection of SNARE-dependent membrane fusion. Obviously, you will want to be very clear on the principles underlying this assay, on the controls the authors use, and on the conclusions they reach. We will also discuss (as have critics) the potential shortcomings of this approach, the controls that perhaps should have been inncluded, and the discrepancies between the in vitro reconstituted system and the “real thing” – that is, neurotransmitter release by synaptic vesicles. Think about what sort of experiments might be used to carry on from those shown in this paper. For example, can you think of experiments that would address the hypothesis that SNAREs are responsible for the specificity observed in cellular membrane fusion reactions?
Class 19 – Cell Communication I: G-Protein-Linked Receptors

Alberts et al., Chapter 15 (pp. 831-871).


We begin our consideration of signal transduction, the process by which cells receive and interpret signals from their surroundings. Cell communications is one of the largest and most important topics in biology, and we will not be able to cover it comprehensively. Note, however, that you have already encountered signal transduction molecules: extracellular signals (human growth factor, acetylcholine) and their cell surface receptors, as well as intracellular signals (Ras and Sar1) and their allosteric regulators (Ras-GAP, Sec12, and Sec23). The reading in Alberts provides a well thought out introduction to the topic including both general principles and the salient features of important representative systems. Although you may not remember all the details, you should nonetheless make sure you read this for understanding. Some concepts, like modular binding domains (e.g. SH2 and SH3) and scaffold proteins, will be important for the next class.

Cerione et al. describe the reconstitution of the famous β-adrenergic receptor into frog red blood cells. These cells lack functional β-adrenergic receptors, but can nonetheless be programmed to respond to isoprenaline (a β-adrenergic receptor agonist) by the addition of β-adrenergic receptors from three different species. Know what signaling agonists and antagonists are. Be sure you understand in general terms what the β-adrenergic receptor pathway is, how the receptor is purified, how its activity is monitored, how it is reconstituted into erythrocyte cells, and how the catecholamine responsiveness of the recipient cells is measured. Note too any control experiments and their purpose. Beyond the conclusions reached by Cerione et al., what additional uses might this reconstitution system be put to? Based on later research, what do you know about the structure of the β-adrenergic receptor, and what experimental findings underpin your knowledge?
Class 20 – Cell Communication II: Enzyme-Linked Receptors

Alberts et al., Chapter 15, pp. 871-884.


Today we continue our discussion of cell communication. One very important class of cell surface receptors is the receptor tyrosine kinase (RTK) family. We will discuss a representative of the RTK family, the epidermal growth factor (EGF) receptor, and attempt to map out the early steps in the pathway linking its activation to downstream kinase cascades. One critical connection in the pathway is GRB2, a protein discovered by Lowenstein et al. Another critical link is the so-called SOS protein, recruited to the cell membrane by GRB2 in order to act as a GTP-exchange factor (GEF) for Ras. You are already aware that GEF proteins activate small GTP-binding proteins like Ras (think Sar1 activation by Sec12), and that the activation of Ras is a key event in coupling extracellular signals to intracellular responses. We will discuss how GRB2 employs a modular domain organization to bind both activated EGF receptors and SOS. What are the salient features of SH2 and SH3 domains? What are advantages (and disadvantages) of ‘modularity’?

Downstream of Ras lies the so-called MAP-kinase pathway. Yeast MAP-kinase pathways have been intensively studied. An intriguing idea mentioned in Alberts et al. is the possibility that kinases that act sequentially are physically associated by means of ‘scaffold’ proteins. What problem would this solve? Park et al. adopt an unconventional strategy for further exploring this possibility. Be sure you understand what issues they sought to examine, what strategy they employed, and what conclusions they reached. Do you agree with their interpretations of their results?
Class 21 – Cell Communication III: Networks and Bistability


Once signal transduction pathways are put together into networks, a host of fascinating properties emerge. Physicists, chemists, computer scientists, mathematicians, and engineers are joining with biologists to devise powerful tools, both experimental and theoretical, to analyze such systems. We will touch on one of many possible topics in this area today, discussing biochemical networks that give rise to bistability in cell fate decisions.

Koshland provides the motivation for reviewing essential concepts: Hill coefficients, ultrasensitivity, and "Michaelis-Menten sensitivity". Ferrell and Machleder ask whether the design of a biochemical network can give rise to the ultrasensitive response needed for a cell fate decision. Although the signaling network triggered in Xenopus oocytes by progesterone involves 30-plus molecules, the components in Fig. 3A are sufficient for understanding this paper. Study Figs. 1B and 1C to be sure you understand why single cell measurements are powerful in comparison to measurements of population averages. Be sure every panel of Fig. 1 makes sense to you. Then consider the impact of adding cycloheximide. What does this imply, and what types of models are ruled out?

Xiong and Ferrell consider the irreversibility of the oocyte maturation process. Know what "reversibility", "bistability", and "hysteresis" mean. How do the authors show that the underlying biochemical responses are irreversible? What is the point of their ΔRaf:ER experiments? What experimental perturbations convert this system into a reversible one? Consider the statement: "When the positive feedback is perturbed, the cell does the unthinkable and 'dedifferentiates', reverting to characteristics of the immature egg." Do you agree?

Ferrell and Xiong is a lucid introduction to bistability in particular and computational biology in general, written at the level of MOL 504.
Class 22 – Cytoskeleton I: Self Assembly and Dynamic Structure

Alberts et al., Chapter 16, pp. 907-923, 930-931.


We begin our study of the cytoskeleton by focusing on microtubules, the filaments that (in the words of Alberts et al.) “determine the positions of membrane-enclosed organelles and direct intracellular transport.” Microtubules exhibit a striking behavior called ‘dynamic instability’ that has interested biophysicists and cell biologists alike for at least twenty years.

Mitchison and Kirschner first invoked the idea of dynamic instability to explain the behavior they observed for their newly-purified centrosomes in the presence of tubulin. These back-to-back Nature articles (best viewed as one longish article) changed the way scientists thought about filamentous proteins. The authors found that some microtubules can be shrinking at the same time that others are getting longer. This startlingly unexpected behavior did not even depend on the presence of the centrosome! We will discuss dynamic instability in some depth.

To follow the discussion, you will need to come to class familiar with the general structure of tubulin and microtubules (e.g. their plus and minus ends, etc.). Why is the free monomer concentration at which no net assembly or disassembly occurs called the ‘steady state concentration’, not \( C_c \)? According to the first paper, what is this concentration for free microtubules? How do the authors reach this conclusion? Pay particular attention to the key experiment shown in Fig. 6 (p. 236). What accounts for microtubule loss? What results argue for depolymerization? Why must conversion between growing and shrinking be slow? What if it were very fast? For the second paper, be prepared to discuss Figures 1 and 3 in detail.
Class 23 – Cytoskeleton II: Regulation of Cytoskeletal Filaments

Alberts et al., Chapter 16, pp. 923-948.


We begin by considering how microtubules are organized within the cell. γ-tubulin was discovered in 1989 and appears to be ubiquitous in eukaryotes. Many lines of evidence suggested that γ-tubulin is essential for microtubule assembly from microtubule organizing centers. What do the studies of Zheng et al. contribute to our understanding of these nucleation sites? What lines of evidence convince you that the γ-tubulin ring complex is the microtubule-nucleating factor in the pericentriolar material? What model do the authors propose? Is it important that the nucleation site be an ‘offset’ ring rather than a flat ring?

We will next shift our focus to the organization, function, and dynamics of actin structures within the cell. From the reading in Alberts et al., you will have begun to appreciate the general division of labor between microtubules, actin filaments, and intermediate filaments. To participate in this discussion you will want and need to know about filopodia, lamellipodia, membrane ruffles, rho, rac, Cdc42, profilin, cofilin, capping protein, WASP/Scar proteins, phalloidin, and most especially the extraordinary Arp2/3 complex.

Blanchon et al. reconstitute branched actin networks using purified components. How do the different concentrations of Arp2/3 influence branching? What are the influences of actin-bound nucleotides, and how do phalloidin and beryllium fluoride affect the system? What effect does capping protein have and why? What is an elastic Brownian rachet model? Are the observable physical properties of the dendritic networks compatible with such a model? We will discuss the steps in the model presented in Fig. 3. Within the context of this model, how might cells regulate actin-based motility?

Medalia et al. serves as a progress report on the current state of electron microscopy as a tool for studying cell biology. Be sure you understand what cryoelectron tomography is. What are its capabilities, strengths, and limitations (relative, obviously, to other comparable methodologies)? Compare these results with those of the preceding paper.
Class 24 – Integrins

Alberts et al., Chapter 19, pp. 1090-1091, 1103-1106, and 1113-1118.


We will begin our final class by discussing the famous RGD (Arg-Gly-Asp) sequence first discovered in fibronectin. This sequence is shared by a number of extracellular proteins including the blood clotting factors fibrinogen and vitronectin. We will discuss the biochemical reasoning that led to the identification of this signal. RGD (or RGDS) is surprisingly short to serve as a recognition sequence for receptor proteins. What concerns does this raise? Are you convinced by the authors’ experimental results? If so, how can your concerns be addressed?

Next we turn to a more recent masterpiece, a combined structural and biochemical study of the integrin receptors that bind extracellular matrix. Unless you have studied integrins in previous classes, you will want to look at Alberts to get oriented and to place the work of Takagi et al. into context. This paper gets to the heart of integrin function, and the ways in which it might mediate signaling in both directions across the plasma membrane. Take the time to read the paper carefully – it is dense, but rewarding. Think particularly hard about the electron microscopy. Know where all the images in Fig. 2 come from, why certain portions of the molecule are “invisible” in some images, and what the numbers below each small image signify. How do the clasped and unclasped molecules differ, and how do the authors propose that unclumping is related to signaling? How are the images in Fig. 3B, D, and F generated? Recall how gel filtration and plasmon resonance work – how are they used in this context to provide additional evidence for the three proposed conformations of the integrin heterodimer? What is the logic of the designed disulfide experiment? Are the controls convincing? Going back to an issue we discussed in Class 3, what does this work imply about the possibility that lattice contacts may affect the structures observed by X-ray crystallography? What strategy do the authors propose for developing new types of drugs that interfere with integrin function?