BASIC CONCEPTS IN BIOCHEMISTRY

A STUDENT'S SURVIVAL GUIDE

Second Edition

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· P R O L O G U E ·

Basic Concepts in Biochemistry: A Student's Survival Guide is not a conventional book: It is not a review book or a textbook or a problem book. It is a book that offers help in two different ways—help in understanding the concepts of biochemistry and help in organizing your attack on the subject and minimizing the subject's attack on you.

This book presents what are often viewed as the more difficult concepts in an introductory biochemistry course and describes them in enough detail and in simple enough language to make them understandable. We surveyed first- and second-year medical students at a national student meeting asking them to list, in order, the parts of biochemistry they found most difficult to understand. The winner (or loser), by far, was integration of metabolism. Metabolic control, pH, and enzyme kinetics ran closely behind, with notable mention given to molecular biology and proteins.

Biochemistry texts and biochemistry professors are burdened with the task of presenting facts, and the enormity of this task can get in the way of explaining concepts. Since I don't feel burdened by that necessity, I've only outlined most of the facts and concentrated on concepts. My rationale is that concepts are considerably easier to remember than facts and that concepts, if appropriately mastered, can minimize the amount of material that has to be memorized—you can just figure everything out when required. In *Basic Concepts in Biochemistry*, central concepts are developed in a stepwise fashion. The simplest concepts provide a review of what might have been forgotten, and the more complex concepts present what might not have been realized.

· C O N T E N T S ·

· P R E F A C E ·

Since the first edition of this series, we have witnessed the birth of "molecular medicine," using biochemistry, cell biology, and genetics to diagnose and treat disease. Consequently, the basic sciences are becoming more important to the practice of medicine. This puts a new pressure on the student—to understand the basis of molecular medicine and the molecular sciences. I still think that it's easier to remember things that you understand, things that make sense. That's the idea behind the Basic Concepts series and that's why I have been so pleased with the expansion of the Basic Concepts series beyond Biochemistry.

The revisions in the second edition include two new chapters, "Membranes and Membrane Proteins" and "Signal Transduction Pathways." These topics are related to the explosion of new information about cell signaling and signal transduction pathways. In addition, I've added some tables of information that I think will be helpful in seeing the big picture (and remembering some of the more important details). As before, the major topics and things to remember are set off in boxes so that if you already know everything in the box, you can skip the rest of the section.

· C H A P T E R · 1 ·

WHERE TO START

INSTRUCTIONS

Read for understanding. Read only what you don't know. Organize, organize, organize.

The first page of each chapter presents an index. A title-summary box for each section presents a short summary and memory jogger intended to be helpful for review. If you already know what the boxed terms mean and feel comfortable with them, don't bother to read the text section that follows—proceed until you find a heading you don't understand, and then read till you understand. The first rule (it may not really be the first rule, but it is a rule) is not to waste time reading things you already know.

Keep on not reading the text until you find something you don't understand—then read the text till you do. The sections are generally arranged in order of increasing complexity and build on previous sections. So if you screwed up and jumped in over your head, back up a section or two. Another option is just to look at the pictures. Pictures and diagrams, if extensively annotated and carefully designed (by you), can be an enormous review aid.

WHAT DO I NEED TO KNOW?

You need to know only the things you will need later.

Medicine and biology are becoming increasingly molecular in nature, so one answer to the question is that you need to know things down to the last atom. *Everything* is not the right answer. You can't possibly learn it all. Therefore, you will have to be selective.

Another answer is that you just need to know the things on the exam. *Later* ends at the final. In reality, later may be longer than this. Try to pick out the major concepts of biochemistry as you go along. Concepts are generally easier to remember than factual details—particularly if the concepts make sense.

INSTRUCTIONS FOR USE

Understand the concepts first. Make notes. Never use a colored highlighter.

General concepts don't need to be memorized. Once you understand them, they provide a framework to hang the rest of the material on. Since they don't need to be memorized, they can be learned (or thought about) almost anywhere. To remember something, write it down. Don't just highlight it with a colored pen or pencil. Highlighting is a great way to forget to read the material.

STUDYING AND EXAMS

Organize, understand, condense, memorize.

• 1. ALWAYS REMEMBER THAT IT IS POSSIBLE TO BE A WORTHWHILE HUMAN BEING REGARDLESS OF (OR IN SPITE OF) HOW MUCH BIOCHEMISTRY YOU KNOW. This won't necessarily help you with biochemistry, but it may help you keep your sanity.

• 2. MINIMIZE THE AMOUNT OF MATERIAL THAT YOU HAVE TO MEMORIZE. If you understand a general concept, you can often figure out the specific details rather than memorize them. For example,

does phosphorylation activate or inactivate acetyl-CoA carboxylase? You could just memorize that it inactivates the enzyme. However, this would not help when it came to the phosphorylation of glycogen synthase. Try the following line of reasoning. We store energy after eating and retrieve it between meals. Storage and retrieval of energy do not happen at the same time. Protein phosphorylation generally increases when you're hungry. Since both acetyl-CoA carboxylase and glycogen synthase are involved in energy storage (fat and glucose, respectively), they will both be inactivated by phosphorylation. For just two enzymes, it might be easier to just memorize all the regulatory behaviors—but for several hundred?

• 3. ARRANGE NOTES AND STUDY TIME IN ORDER OF DECREASING IMPORTANCE. During the first (or even second and third) pass, you can't possibly learn everything biochemistry has to offer. Be selective. Learn the important (and general) things first. If you have enough gray matter and time, then pack in the details. Organize your notes the same way. For each topic (corresponding to about a chapter in most texts) write down a *short* summary of the really important concepts (no more than one to two pages). Don't write down the things that you already know, just the things you're likely to forget. Be really cryptic to save space, and use lots of diagrams. These don't have to be publication-quality diagrams; they only have to have meaning for you. The idea is to minimize the sheer volume of paper. You can't find yourself at finals time with a yellow-highlighted 1000-page text to review 2 days before the exam. An enormous amount of information can be crammed onto a diagram, and you learn a significant amount by creating diagrams. Use them extensively.

• 4. SORT OUT THE TRIVIA AND FORGET ABOUT IT. The most difficult part may be deciding what the important things actually are. After all, if you've never had biochemistry, it all sounds important (or none of it does). Use the following trivia sorter (or one of your own invention) to help with these decisions. To use this sorter, you must first set your trivia level. Your trivia level will depend on whether you just want to pass or want to excel, whether you want to devote a lot of time or a whole lot of time to biochemistry, and your prior experience. Once you set this level, make sure you know *almost everything* above this level and ignore almost everything below it. Setting your trivia level is not irreversible; the setting can be moved at any time. You should consider levels 7 to 10 as the minimal acceptable trivia level (passing). The trivia sorter shown here is generic. You can make your own depending on the exact demands of the course you're taking. Levels 21 and 22 might be too trivial for anybody to spend time learning (again, this is opinion).

TRIVIA SORTER

1. Purpose of a pathway—what's the overall function?
2. Names of molecules going into and coming out of the pathway
3. How the pathway fits in with other pathways
4. General metabolic conditions under which the pathway is stimulated or inhibited
5. Identity (by name) of control points—which steps of the pathway are regulated?
6. Identity (by name) of general regulatory molecules and the direction in which they push the metabolic pathway
7. Names of reactants and products for each regulated enzyme and each enzyme making or using ATP equivalents
8. Names of molecules in the pathway and how they're connected
9. Structural features that are important for the function of specific molecules in the pathway (this includes DNA and proteins)
10. Techniques in biochemistry, the way they work, and what they tell you
11. Molecular basis for the interactions between molecules
12. Genetic diseases and/or specific drugs that affect the pathway
13. Essential vitamins and cofactors involved in the pathway
14. pH
15. Enzyme kinetics
16. Specific molecules that inhibit or activate specific enzymes
17. Names of individual reactants and products for nonregulated steps
18. Chemical structures (ability to recognize, not draw)
19. Structures of individual reactants and products for all enzymes in pathway
20. Reaction mechanism (chemistry) for a specific enzyme
21. Cleavage specificity for proteases or restriction endonucleases
22. Molecular weights and quaternary structures

• 5. DON'T WASTE TIME ON ABSOLUTE TRIVIA UNLESS YOU HAVE THE TIME TO WASTE. It is possible to decide that something is just not worth remembering; for example, cleavage specificities of proteases or restriction endonucleases, and protein molecular weights, are

obvious choices. You can set the “too trivial to bear” level anywhere you want. You could decide that glycolysis is just not worth knowing. However, if you set your limits totally in the wrong place, you will get another chance to figure this out when you repeat the course. The trivia line is an important line to draw, so think about your specific situation and the requirements of the course before you draw it.

• C H A P T E R • 2 •

PROTEIN STRUCTURE

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Proteins start out life as a bunch of amino acids linked together in a head-to-tail fashion—the primary sequence. The one-dimensional information contained in the primary amino acid sequence of cellular proteins is enough to guide a protein into its three-dimensional structure, to determine its specificity for interaction with other molecules, to determine its ability to function as an enzyme, and to set its stability and lifetime.

AMINO ACID STRUCTURE

Remember a few of the amino acids by functional groups. The rest are hydrophobic.

Remembering something about the structures of the amino acids is just one to those basic language things that must be dealt with since it crops up over and over again—not only in protein structure but later in metabolism. You need to get to the point that when you see Asp you don't think snake but see a negative charge. Don't memorize the amino acids down to the last atom, and don't spend too much time worrying about whether glycine is polar or nonpolar. Methylene groups ($–CH_2–$) may be important, but keeping track of them on an individual basis is just too much to ask. Organize the amino acids based on the functional group of the side chain. Having an idea about functional groups of amino acids will also help when you get to the biosynthesis and catabolism of amino acids. Might as well bite the bullet early.

HYDROPHILIC (POLAR)

• **CHARGED POLAR** *Acidic* ($–COO^{\square}$) and *basic* ($–NH_3^{\square}$) amino acid side chains have a charge at neutral pH and strongly "prefer" to be on the exterior, exposed to water, rather than in the interior of the protein. The terms *acidic* and *basic* for residues may seem a little strange. Asp and Glu are called acidic amino acids, although at neutral pH in most proteins, Asp and Glu are not present in the acidic form (–COOH) but are present in the basic form ($–COO^{\square}$). So the acidic amino acids, Asp and Glu, are really bases (proton acceptors). The reason that Asp and Glu are called acidic residues is that they are such strong acids (proton donors) they have already lost their protons. Lys, Arg, and His are considered basic amino acids, even though they have a proton at neutral pH. The same argument applies: Lys, Arg, and His are such good bases (proton acceptors) that they have already picked up a proton at neutral pH.

	FUNCTIONAL GROUP		AMINO ACID
		Hydrophilic, Polar	
Acidic	Carboxylates	$—COO^{\square}$	Asp, Glu
Basic	Amines	$—NH_3^{\square}$	Lys, Arg, His
Neutral	Amides	$—CONH_2$	Asn, Gln
	Alcohols	—OH	Ser, Thr, Tyr
	Thiol	—SH	Cys
		Hydrophobic, Apolar	
Aliphatic		$—CH_2—$	Ala, Val, Leu, Ile, Met
Aromatic		C Rings	Phe, Trp, Tyr
Whatever			Pro, Gly

Charged groups are usually found on the surface of proteins. It is very difficult to remove a charged residue from the surface of a protein and place it in the hydrophobic interior, where the dielectric constant is low. On the surface of the protein, a charged residue can be solvated by water, and it is easy to separate oppositely charged ions because of the high dielectric constant of water.[1] If a charged group is found in the interior of the protein, it is usually paired with a residue of the opposite charge. This is termed a *salt bridge*.

• **NEUTRAL POLAR** These side chains are uncharged, but they have groups (–OH, –SH, NH, C═O) that can hydrogen-bond to water. In an unfolded protein, these residues are hydrogen-bonded to water. They prefer to be exposed to water, but if they are found in the protein interior they are hydrogen-bonded to other polar groups.

HYDROPHOBIC (APOLAR)

Hydrocarbons (both aromatic and aliphatic) do not have many (or any) groups that can participate in the hydrogen-bonding network of water. They're greasy and prefer to be on the interior of proteins (away from water). Note that a couple of the aromatics, Tyr and Trp, have O and N, and Met has an S, but these amino acids are still pretty hydrophobic. The hydrophobic nature usually dominates; however, the O, N, and S atoms often participate in hydrogen bonds in the interior of the protein.

INTERACTIONS

A few basic interactions are responsible for holding proteins together. The properties of water are intimately involved in these interactions.

[1] The dielectric constant is a fundamental and obscure property of matter that puts a number on how hard it is to separate charged particles or groups when they're in this material. In water, charge is easy to separate (water has a high dielectric constant). The charge distribution on water is uneven. It has a more positive end (H) and a more negative end (O) that can surround the charged group and align to balance the charge of an ion in water. This dipolar nature of water makes it easy for it to dissolve ionic material. Organic solvents like benzene or octane have a low dielectric constant and a more uniform distribution of electrons. They do not have polar regions to interact with ions. In these types of solvents, just as in the interior of a protein, it is very difficult to separate two oppositely charged residues.

WATER

Water's important. Polar amino acid chains can participate in hydrogen bonding to water, or hydrophobic side chains can interfere with it.

The properties of water dominate the way we think about the interactions of biological molecules. That's why many texts start with a lengthy, but boring, discussion of water structure, and that's why you probably do need to read it.

Basically, water is a polar molecule. The H—O bond is polarized—the H end is more positive than the O end. This polarity is reinforced by the other H—O bond. Because of the polarity difference, water is both a hydrogen-bond donor and a hydrogen-bond acceptor. The two hydrogens can each enter into hydrogen bonds with an appropriate acceptor, and the two lone pairs of electrons on oxygen can act as hydrogen-bond acceptors. Because of the multiple hydrogen-bond donor and acceptor sites, water interacts with itself. Water does two important things: It squeezes out oily stuff because the oily stuff interferes with the interaction of water with itself, and it interacts favorably with anything that can enter into its hydrogen-bonding network.

HYDROPHOBIC INTERACTION

Proteins fold in order to put as much of the greasy stuff out of contact with water as possible. This provides much of the "driving force" for protein folding, protein–protein interactions, and protein–ligand interactions (Fig. 2-1).

The *driving force* for a chemical reaction is what makes it happen. It's the interaction that contributes the most to the decrease in free energy. For protein (and DNA) folding, it's the hydrophobic interaction that provides most of the driving force. As water squeezes out the hydrophobic side chains, distant parts of the protein are brought together into a compact structure. The hydrophobic core of most globular proteins is very compact, and the pieces of the hydrophobic core must fit together rather precisely.

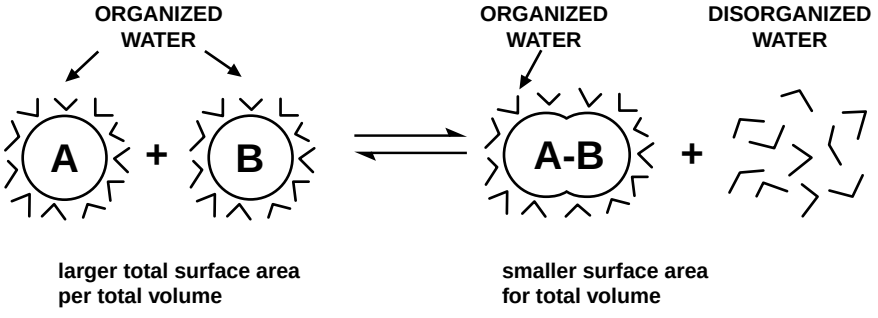


Figure 2-1 The Hydrophobic Interaction
As hydrophobic surfaces contact each other, the ordered water molecules that occupied the surfaces are liberated to go about their normal business. The increased entropy (disorder) of the water is favorable and drives (causes) the association of the hydrophobic surfaces.

Putting a hydrophobic group into water is difficult to do (unfavorable). Normally, water forms an extensive hydrogen-bonding network with itself. The water molecules are constantly on the move, breaking and making new hydrogen bonds with neighboring water molecules. Water has two hydrogen bond donors (the two H—O bonds) and two hydrogen bond acceptors (the two lone electron pairs on oxygen), so a given water molecule can make hydrogen bonds with neighboring water molecules in a large number of different ways and in a large number of different directions. When a hydrophobic molecule is dissolved in water, the water molecules next to the hydrophobic molecule can interact with other water molecules only in a direction away from the hydrophobic molecule. The water molecules in contact with the hydrophobic group become more organized. In this case, organization means restricting the number of ways that the water molecules can be arranged in space. The increased organization (restricted freedom) of water that occurs around a hydrophobic molecule represents an unfavorable decrease in the entropy of water.[2] In the absence of other factors, this increased organization (decreased entropy) of water causes hydrophobic molecules to be insoluble.

The surface area of a hydrophobic molecule determines how unfavorable the interaction between the molecule and water will be. The big-

[2] As with most desks and notebooks, disorder is the natural state. Order requires the input of energy. Reactions in which there is an increasing disorder are more favorable. Physical chemists (and sometimes others) use the word *entropy* instead of *disorder*. There's a discussion of entropy at the end of this book.

ger the surface area, the larger the number of ordered water molecules and the more unfavorable the interaction between water and the hydrophobic molecule. Bringing hydrophobic residues together minimizes the surface area directly exposed to water. Surface area depends on the square of the radius of a hydrophobic "droplet," while volume depends on the cube of the radius. By bringing two droplets together and combining their volume into a single droplet of larger radius, the surface area of the combined, larger droplet is less than that of the original two droplets. When the two droplets are joined together, some of the organized water molecules are freed to become "normal." This increased disorder (entropy) of the liberated water molecules tends to force hydrophobic molecules to associate with one another. The hydrophobic interaction provides most of the favorable interactions that hold proteins (and DNA) together. For proteins, the consequence of the hydrophobic interaction is a compact, hydrophobic core where hydrophobic side chains are in contact with each other.

VAN DER WAALS INTERACTIONS AND LONDON DISPERSION FORCES

These are very short-range interactions between atoms that occur when atoms are packed very closely to each other.

When the hydrophobic effect brings atoms very close together, van der Waals interactions and London dispersion forces, which work only over very short distances, come into play. This brings things even closer together and squeezes out the holes. The bottom line is a very compact, hydrophobic core in a protein with few holes.

HYDROGEN BONDS

Hydrogen bonding means sharing a hydrogen atom between one atom that has a hydrogen atom (donor) and another atom that has a lone pair of electrons (acceptor):

—C═O··H_2O H_2O··H—N— —C═O··H—N— H_2O··H_2O

The secondary structure observed in proteins is there to keep from losing hydrogen bonds.

A hydrogen bond is an interaction between two groups in which a weakly acidic proton is shared (not totally donated) between a group that has a proton (the donor) and a group that can accept a proton (the acceptor). Water can be both a hydrogen-bond donor and a hydrogen-bond acceptor. In an unfolded protein, the hydrogen-bond donors and acceptors make hydrogen bonds with water. Remember that the polar amino acids have groups that can form hydrogen bonds with each other and with water. The peptide bond [–C(═O)–NH–] that connects all the amino acids of a protein has a hydrogen-bond donor (NH) and a hydrogen-bond acceptor (═O). The peptide bond will form hydrogen bonds with itself (secondary structure) or with water.

Everything is just great until the hydrophobic interaction takes over. Polar peptide bonds that can form hydrogen bonds connect the amino acid side chains. Consequently, when hydrophobic residues aggregate into the interior core, they must drag the peptide bonds with them. This requires losing the hydrogen bonds that these peptide bonds have made with water. If they are not replaced by equivalent hydrogen bonds in the folded structure, this costs the protein stability. The regular structures (helix, sheet, turn) that have become known as *secondary structure* provide a way to preserve hydrogen bonding of the peptide backbone in the hydrophobic environment of the protein core by forming regular, repeating structures.

SECONDARY STRUCTURE

Secondary structure is not just hydrogen bonds.

α Helix: Right-handed helix with 3.6 amino acid residues per turn. Hydrogen bonds are formed parallel to the helix axis.

β Sheet: A parallel or antiparallel arrangement of the polypeptide chain. Hydrogen bonds are formed between the two (or more) polypeptide strands.

β Turn: A structure in which the polypeptide backbone folds back on itself. Turns are useful for connecting helices and sheets.

Secondary structure exists to provide a way to form hydrogen bonds in the interior of a protein. These structures (helix, sheet, turn) provide ways to form regular hydrogen bonds. These hydrogen bonds are just replacing those originally made with water.

As a protein folds, many hydrogen bonds to water must be broken. If these broken hydrogen bonds are replaced by hydrogen bonds within

the protein, there is no net change in the number of hydrogen bonds (Fig. 2-2). Because the actual number of hydrogen bonds does not change as the secondary structure is formed, it is often argued that hydrogen bonds don't contribute much to the stability of a protein. However, hydrogen bonds that form after the protein is already organized into the correct structure may form more stable hydrogen bonds than the ones to water. Hydrogen bonding does contribute somewhat to the overall stability of a protein; however, the hydrophobic interaction usually dominates the overall stability.

Small peptides generally do not form significant secondary structure in water (there are some that do). For small peptides that do not form stable secondary structure, there are often other favorable interactions within the peptide that stabilize the formation of the helix or sheet structure.

The stability of secondary structure is also influenced by surrounding structures (Fig. 2-3). Secondary structure may be stabilized by interactions between the side chains and by interactions of the side chains with other structures in the protein. For example, it is possible to arrange the amino acid sequence of a protein or peptide into a helix that has one face that is hydrophobic and one that is hydrophilic. The helix wheel shown in Fig. 2-3 illustrates how this is possible. View the helix as a long cylinder. The peptide backbone spirals up and around the cylinder. The

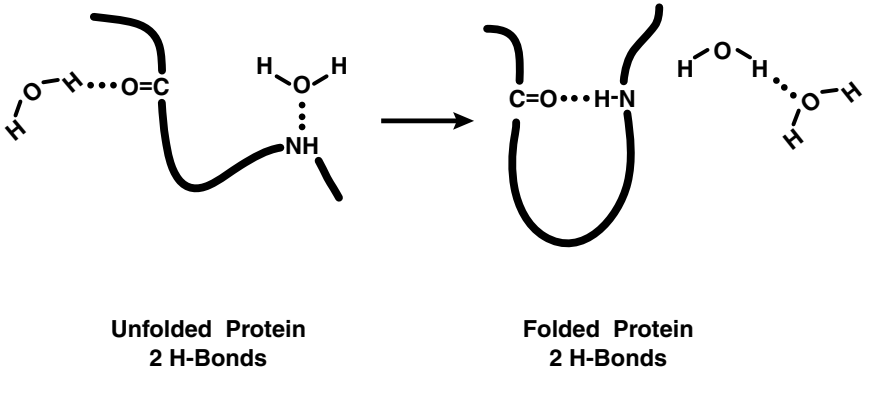
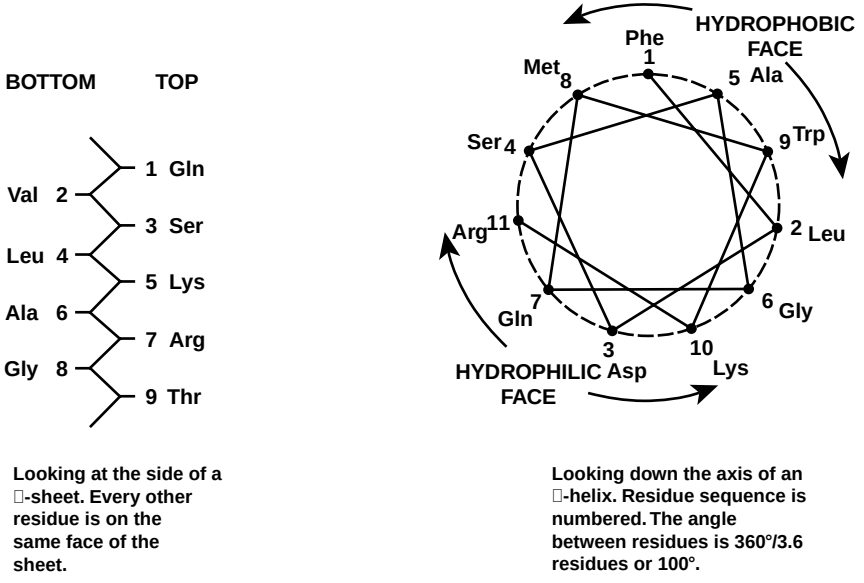


Figure 2-2 Solvation in Protein Folding
In an unfolded protein, water makes hydrogen bonds to all the donors and acceptors. As the protein folds and some polar groups find themselves inside, many of the hydrogen bonds with the solvent are replaced by hydrogen bonds between the different donors and acceptors in the protein. Because hydrogen bonds are being replaced rather than gained or lost as the protein folds, there is not a large net stabilization of the protein by the hydrogen bonds.



Looking at the side of a β-sheet. Every other residue is on the same face of the sheet.

Looking down the axis of an α-helix. Residue sequence is numbered. The angle between residues is 360°/3.6 residues or 100°.

Figure 2-3
SECONDARY-STRUCTURE STABILIZATION is not provided by just the hydrogen bonds. On the left, you're looking at a representation of a β sheet in which the amino acid side chains alternately stick up and down. If every other side chain is hydrophobic, one side of the sheet will be hydrophobic and the other side will be hydrophilic. Interaction of the hydrophobic side with a hydrophobic region on the protein will add stability to the β sheet. On the right an α helix is shown with a hydrophobic and a hydrophilic face. Again, putting the hydrophobic face (or surface) up against another hydrophobic region of the protein will stabilize the helix. In the helix representation, there is a 100° angle (360°/3.6 residues) between residues. Side chains would stick out from the side of the cylinder defined by the helix.

side chains of the amino acid residues point out from the helix. Each amino acid residue moves up the helix and around the helix at an angle of 100° (360°/turn ÷ 3.6 residues/turn = 100°/residue). What you see in Fig. 2-3 is a view looking down the helix axis. The side chains are on the side of the circle (cylinder). One surface of the helix has only hydrophobic side chains, while the other side has hydrophilic side chains. This is termed an *amphipathic* helix (or *amphiphilic*, depending on whether you're a lover or a hater). With these kinds of helices, the hydrophobic face is buried in the interior while the hydrophilic face is exposed to water on the surface. There are two ways to look at this. The formation of the helix allows it to interact in a very specific way with

the rest of the protein. Alternatively, you could suppose that the interaction with the rest of the protein allows the helix to form. These are equivalent ways to view things, and energetically it doesn't make any difference (see linked thermodynamic functions in Chap. 24 if you dare)—the result is that the presence of a hydrophobic and a hydrophilic side of a helix and a complementary hydrophobic region in the interior of the protein makes it more favorable to form a helix. Secondary structure can be stabilized by interactions with other parts of the protein.

 Sheets can also have a hydrophobic face and a hydrophilic face. The backbone of the sheet is arranged so that every other side chain points to the same side of the sheet. If the primary sequence alternates hydrophobic–hydrophilic, one surface of the sheet will be hydrophobic and the other will be hydrophilic.

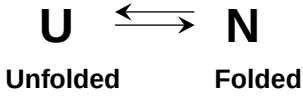
PROTEIN STABILITY

Protein stability is proportional to the free-energy difference between an unfolded protein and the native structure (Fig. 2-4).

It's a miracle that we're here at all. Most proteins are not very stable even though there are a large number of very favorable interactions that can be seen in the three-dimensional structure. The reason is that the favorable interactions are almost completely balanced by unfavorable interactions that occur when the protein folds. A reasonably small net protein stability results from a small net difference between two large numbers. There are lots of favorable interactions but also lots of unfavorable interactions.

Protein stability is just the difference in free energy between the correctly folded structure of a protein and the unfolded, denatured form. In the denatured form, the protein is unfolded, side chains and the peptide backbone are exposed to water, and the protein is conformationally mobile (moving around between a lot of different, random structures). The more stable the protein, the larger the free energy difference between the unfolded form and the native structure.

You can think about the energy difference in terms of an equilibrium constant if you want. For the folding reaction, the equilibrium constant K_{eq} [native]/[denatured] is large if the protein is stable. Proteins can be denatured (unfolded) by increasing the temperature, lowering the pH, or adding detergents, urea, or guanidine hydrochloride. Urea and guanidine hydrochloride denature proteins by increasing the solubility of the hydrophobic side chains in water. Presumably these compounds, which



$$K_{eq} = \frac{[N]}{[U]}$$

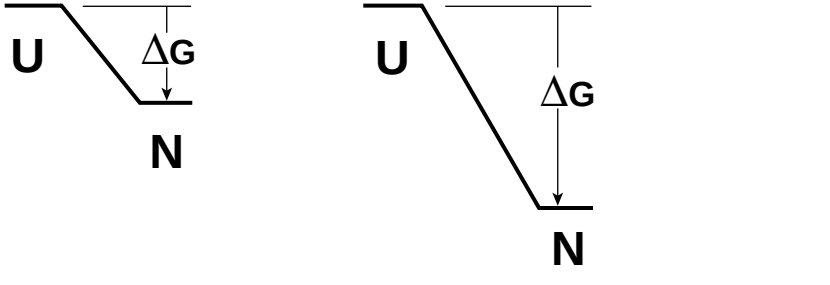


Figure 2-4
The **FREE-ENERGY CHANGE** during a reaction such as the folding of a protein is related to how big the equilibrium constant is. For reactions that are downhill and favorable, the free energy of the product is lower than that of the reactant. The change in free energy (products □ reactants) is less than zero (negative). Very downhill reactions have very large equilibrium constants.

are polar, alter water structure in some way to make it easier to dissolve hydrophobic molecules.[3]

Protein structure (and also the interactions between proteins and small molecules) is a compromise. It may be necessary to sacrifice a hydrogen bond or two in order to gain two or three hydrophobic interactions. In contrast, it may be necessary to place a hydrophobic residue in contact with water in order to pick up a few more hydrogen bonds in

[3] You may have figured out from this sentence that it's not exactly known how urea and guanidine denature proteins.

secondary structure. So it's all a compromise—a constant game of give and take. The game involves getting as many favorable interactions as you can while doing as few of the unfavorable things as possible.

FAVORABLE (GOOD) INTERACTIONS

Try to get as many of these as possible:

1. Hydrophobic interactions
2. van der Waals interactions
3. London dispersion forces
4. Hydrogen bonds
5. Charge–charge interactions

These are the favorable interactions that were discussed above. They work together to provide stabilizing interactions that hold the structure together.

UNFAVORABLE (BAD) INTERACTIONS

Avoid as many of these as possible:

1. Organizing anything into a structure (decreasing entropy)
2. Removing a polar group from water without forming a new hydrogen bond to it
3. Removing a charged group from water without putting an opposite charge nearby or putting two like charges close together
4. Leaving a hydrophobic residue in contact with water
5. Putting two atoms in the same place (steric exclusion)

There are numerous bad things (energetically speaking) that can happen when proteins fold into a three-dimensional structure. The worst thing that has to happen is that lots of covalent bonds in the protein must assume relatively fixed angles. They're no longer free to rotate as they were in the unfolded form. Protein folding requires a large loss in the conformational entropy (disorder) of the molecule. Restriction of the conformational freedom is probably the biggest unfavorable factor opposing the folding of proteins.

When a protein folds, most of the hydrophobic side chains pack into the interior. As they move into the interior, they must drag the polar amides of the polypeptide backbone with them. These backbone amides must lose contact with water and break hydrogen bonds to the solvent.[4] If these hydrogen bonds that were formed with the solvent aren't replaced by new hydrogen bonds between the different polar groups that now find themselves in the interior, there will be a net loss in the number of hydrogen bonds upon folding—this is not good. Secondary structure provides a way to allow much of the polypeptide backbone to participate in hydrogen bonds that replace the ones made with water. But then there's the odd residue that just may not be able to find a suitable hydrogen-bonding partner in the folded protein. This costs energy and costs the protein stability. The same thing happens with charged residues (although they're almost always ion-paired). By the same token, it may occasionally be necessary to leave a hydrophobic group exposed to water. It may not be possible to bury all the hydrophobic residues in the interior. If not, this is also unfavorable and destabilizes the protein. All these unfavorable interactions sum up to make the protein less stable.

Don't get the impression that proteins need to be as stable as possible and that the unfavorable interactions are necessarily bad. Proteins shouldn't live forever. A good bit of metabolism is regulated by increasing and decreasing the amount of a specific enzyme or protein that is available to catalyze a specific reaction. If a protein were too stable, it might not be possible to get rid of it when necessary.

The net result of all the favorable and unfavorable interactions is that they're almost balanced. For a 100-residue protein, it is possible to estimate roughly that the sum of all the favorable interactions that stabilize the three-dimensional, native structure is on the order of □500 kcal/mol. This comes from all the favorable hydrophobic, van der Waals, hydrogen-bonding, and electrostatic interactions in the native protein. In contrast, the sum of all the unfavorable interactions that destabilize the structure is probably near □490 kcal/mol. These come from conformational entropy losses (organization of the protein into a structure) and other unfavorable effects such as leaving a hydrophobic group exposed to water or not forming a hydrogen bond in the interior after having lost one that was made to water in the unfolded state. The net result is that the three-dimensional structure of a typical protein is only about □5 to □15 kcal/mol more stable than the denatured, structureless state.

[4] The same argument applies to polar groups on the side chains of the amino acids.

TEMPERATURE-SENSITIVE MUTATIONS

These are mutations that decrease the stability of a protein so that the denaturation temperature is near 40°C.

A single methylene group (–CH_2–) involved in a hydrophobic interaction may contribute as much as □1.5 to □2 kcal/mol to the stability of a protein that is only stable by □10 kcal/mol. A single hydrogen bond might contribute as much as □1.5 to □3.5 kcal/mol. If a mutation disrupts interactions that stabilize the protein, the protein may be made just unstable enough to denature near body (or culture) temperature. It might strike you as strange that we were talking earlier about how hydrogen bonds didn't contribute much to the net stability of proteins and now I'm telling you they contribute □1.5 to □3.5 kcal/mol. Both statements are more or less right. In the first case we were considering the folding process in which a hydrogen bond to solvent is replaced by a hydrogen bond in the folded protein—the result is a small contribution of a hydrogen bond to stability. What we're talking about now is messing up a protein by changing one amino acid for another by mutation. Here we're destroying an interaction that's present in the intact, folded protein. For any hydrogen-bonded group in the folded protein, there must be a complementary group. A donor must have an acceptor, and vice versa. Making a mutation that removes the donor of a hydrogen bond leaves the acceptor high and dry, missing a hydrogen bond. In the unfolded protein, the deserted acceptor can be accommodated by water; however, in the folded protein the loss of the donor by mutation hurts. It costs a hydrogen bond when the protein folds. The result: a loss in stability for the protein. Loss in stability means that the protein will denature at a lower temperature than before.

Temperature-sensitive mutations usually arise from a single mutation's effect on the stability of the protein. Temperature-sensitive mutations make the protein just unstable enough to unfold when the normal temperature is raised a few degrees. At normal temperatures (usually 37°C), the protein folds and is stable and active. However, at a slightly higher temperature (usually 40 to 50°C) the protein denatures (melts) and becomes inactive. The reason proteins unfold over such a narrow temperature range is that the folding process is very cooperative—each interaction depends on other interactions that depend on other interactions.

For a number of temperature-sensitive mutations it is possible to find (or make) a seond mutation in the protein that will suppress the effects

of the first mutation. For example, if the first mutation decreased the protein stability by removing a hydrogen-bond donor, a second mutation that changes the acceptor may result in a protein with two mutations that is just as stable as the native protein. The second mutation is called a *suppressor mutation.*

LIGAND-BINDING SPECIFICITY

This is also a compromise (Fig. 2-5).

The specificity of the interaction between a protein and a small molecule or another protein is also a compromise. We've just said that charge–charge and hydrogen-bond interactions don't contribute a lot to the stability of a protein because their interaction in the folded protein simply replaces their individual interaction with water. The same may be said of the interaction between an enzyme and its substrate or one protein and another. However, there is a huge amount of specificity to be gained in these kinds of interactions. For tight binding, the protein and its ligand must be complementary in every way—size, shape, charge, and hydrogen-bond donor and acceptor sites.

Both the protein and the ligand are solvated by water when they are separated. As the two surfaces interact, water is excluded, hydrogen bonds are broken and formed, hydrophobic interactions occur, and the protein and ligand stick to each other. As in protein folding and for the same reasons, the hydrophobic interaction provides much of the free energy for the association reaction, but polar groups that are removed

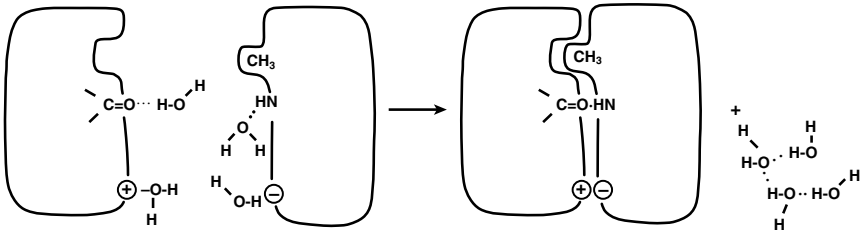


Figure 2-5
The **ASSOCIATION** of two molecules uses the same interactions that stabilize a protein's structure: hydrophobic interactions, van der Waals interactions, hydrogen bonds, and ionic interactions. To get the most out of the interaction, the two molecules must be complementary.

from water by the interaction must find suitable partners in the associated state.

Consider what happens when a nonoptimal ligand binds to the protein. The binding of this modified ligand is much weaker not because it's not the right size to fit into the protein-binding site, but because the complementary group on the protein loses a favorable interaction with water that is not replaced by an equally favorable interaction with the ligand (Fig. 2-6).

As with the formation of secondary structure, the multiple, cooperative hydrogen bonds that can be formed between the ligand and the protein may be stronger and more favorable than hydrogen bonds that the ligand might make to water. Hydrogen bonding may, in fact, make some contribution to the favorable free energy of binding of ligands to proteins.

GLOBAL CONCLUSION

Now that you understand the basis for the interactions between functional groups in water, you also understand the basis for most interactions: DNA–DNA, DNA–RNA, DNA–protein, RNA–protein, protein–protein, protein–ligand, enzyme–substrate (Get the picture?), antibody–antigen, protein–chromatography column—it's all the same stuff.

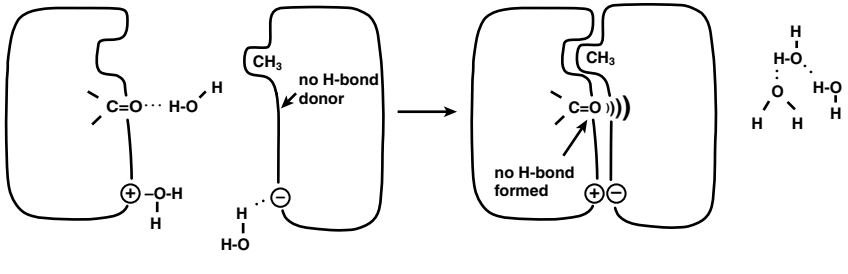


Figure 2-6
SPECIFICITY in the association of two proteins or a protein and a small molecule results from the requirement that the two interacting molecules must be complementary—complementary in charge, hydrogen bonding, and hydrophobic patches as well as shape. If any of the possible interactions are not satisfied, the strength of the interaction suffers.

• C H A P T E R • 3 •

MEMBRANES AND MEMBRANE PROTEINS

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GENERAL MEMBRANE FUNCTION

1. Separates one area of the cell from another
2. Provides a diffusion barrier
3. Concentrates membrane-associated molecules
4. Enables ion and concentration gradients

Membranes separate one part of the cell from the other. Proteins and other molecules can be localized in the membrane. Membrane localization concentrates the molecules and makes it easier for them to find each other (two-dimensional diffusion) than it is for two molecules in solution (three-dimensional diffusion). Because most molecules can't pass through the membrane by themselves, the cell machinery can create con-

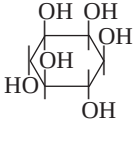
centration gradients across membranes by pumping specific molecules out of the cell and/or by allowing specific molecules into the cell. As we'll see later, these gradients are a source of energy for the cell and can be used for signaling.

MEMBRANE COMPOSITION

This includes negative phospholipids (PG, PS, PI), neutral phospholipids (PC, PE, sphingolipids), cholesterol, and asymmetric structure.

Lipids are biological molecules that are soluble in certain organic solvents (whether or not something is a lipid is operationally defined by the solubility). Lipids include a variety of molecules such as triglycerides, phospholipids, and cholesterol. The major type of lipid in membranes is the phospholipid. They're called phospholipids because they all contain a phosphate diester.

COMMON PHOSPHOLIPIDS

HEAD GROUP	STRUCTURE	LIPID NAME	ABBREVIATION
	Neutral Lipids		
Ethanolamine	$HOCH_2CH_2NH_3^{\square}$	Phosphatidylethanolamine	**PE**
Choline	$HOCH_2CH_2N^{\square}(CH_3)_3$	Phosphatidylcholine also called Lecithin	**PC**
	Acidic Lipids (negatively charged—remember the negative charge on the phosphate group)		
Serine	$HOCH_2CH(CO_2^{\square})NH_3^{\square}$	Phosphatidylserine	**PS**
Inositol		Phosphatidylinositol	**PI**
Glycerol	$HOCH_2CH(OH)CH_2OH$	Phosphatidylglycerol	**PG**

The other phospholipids that you may encounter are based on sphingosine. They are derived from serine instead of glycerol but the concept is the same. They have two long, fatty acid chains, a phosphate diester, and a choline-like charged group. This is a neutral lipid.

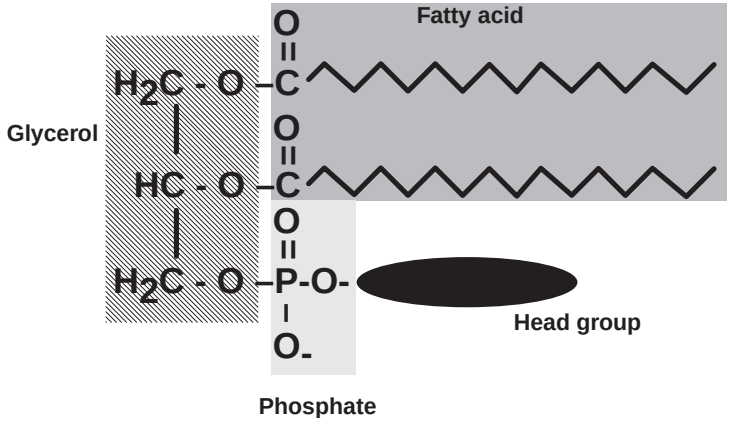
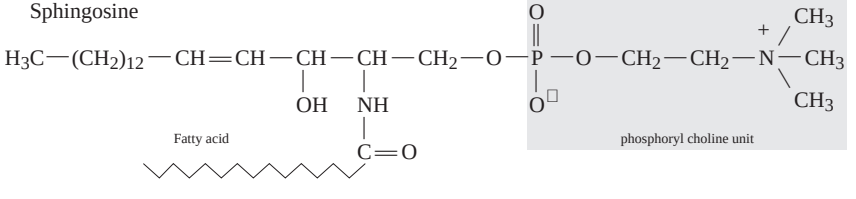


Figure 3-1 Structure of Phospholipids
The hydrophobic tail is provided by long-chain fatty acids attached to a glycerol backbone. The head group contains oxygen and may be positively charged or neutral. The name of the phospholipid is dictated by the head group. The head and tail are attached through a phosphate diester.



Sphingomyelin

Glycolipids are derived from sphingosine, but have a sugar unit, such as glucose or galactose attached instead of the choline unit. The carbohydrate can be extended to form more complex structures, including branches. The sugars point out from the cell surface and are involved in cell-cell recognition.

Cholesterol is an essential component of mammalian membranes. It is obtained from the diet or can be synthesized from acetyl-CoA.

PHOSPHOLIPID BILAYER

This consists of two layers: tails inside, heads outside. The hydrophobic part is 30 Å thick.

Phospholipids are detergents; they have a hydrophobic part (the fatty acid tail) and a hydrophilic part (the head) (Fig. 3-1). The phospholipids

associate with each other through hydrophobic interaction, forming two layers (leaflets) of phospholipid (Fig. 3-2). This buries the hydrophobic fatty acid tails and exposes the polar part (head) to water. Because of the thick layer of hydrocarbon, any molecule that may try to penetrate the bilayer must pass through this hydrophobic region. For polar molecules and ions this is very difficult because they must lose the strong interactions they make with water in order to pass through the bilayer.

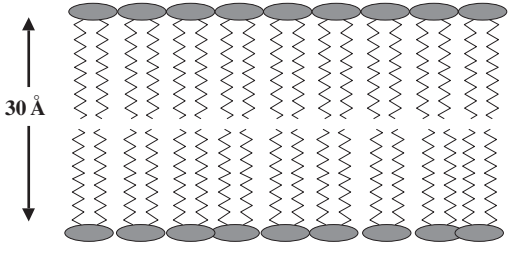


Figure 3-2
PHOSPHOLIPIDS associate to form a bilayer consisting of a hydrophobic core (phospholipid tails) and a polar surface (phospholipid heads).

MEMBRANE STRUCTURE

Membranes are asymmetric. Integral membrane proteins can't be washed off. Peripheral membrane proteins can be washed off. Membrane spanning segments and lipid modification (fatty acylation and prenylation), anchor proteins in a fluid bilayer (Singer fluid mosaic model).

The membrane establishes in and out. The membrane is asymmetric because the inner and outer leaflets can have a different lipid composition and contain different proteins (Fig. 3-3). Proteins can be associated with either side of the membrane, or they can pass through the membrane using membrane-spanning segments. The functional part of the protein can be on the cytosolic side, the external side, or even in the membrane itself. A common structure for spanning a membrane is an □-helix (but there are examples of sheets spanning a membrane). It takes about 20 amino acid residues arranged in a helix to span to a 30 Å hydrophobic interior of the bilayer.

Proteins that can be removed from membranes by washing them with salt solutions or low pH solutions (disrupts ionic interactions) are called *peripheral membrane proteins*. Proteins that cannot be removed without disrupting the membrane with detergents are called *integral*

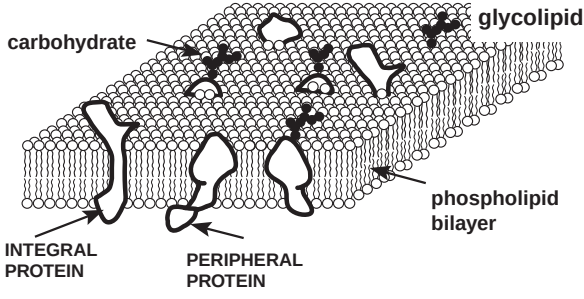


Figure 3-3
FLUID-MOSAIC MODEL of membrane structure. Proteins and lipids that are embedded in the lipid bilayer diffuse rapidly in the plane of the membrane.

membrane proteins. Remember that the distinction between integral and peripheral membrane proteins is operational rather than structural. All proteins that pass through the membrane one or more times will be integral membrane proteins, but not all integral membrane proteins will pass through the membrane—it depends on whether or not the protein can be removed by salt or low pH washes. Peripheral membrane proteins associate with the membrane or, more usually, with integral membrane proteins.

POSTTRANSLATIONAL MODIFICATION

Posttranslational modification can affect membrane association by prenylation (adding C15 or C20 unsaturated hydrocarbons) or fatty acylation (C14 or C16). Glycoproteins and glycolipids on the exterior face of the membrane have carbohydrates attached.

Some proteins can be posttranslationally modified by the addition of prenyl groups. Prenyl groups are long-chain, unsaturated hydrocarbons that are intermediates in isoprenoid synthesis. The *farnesyl* group has 15 carbons, and the *geranylgeranyl* has 20 carbons. They are attached to a cysteine residue near the end of the protein as a thiol ether (Protein-S-R). Other proteins can have a long-chain fatty acid (C14═myristoyl, C16═palmitoyl) attached to the amino terminus as an amide. These fatty acid modifications can increase the association of proteins with the membrane.

Glycoproteins and glycolipids have complex sugar residues attached. Since they are attached in the ER and Golgi compartments, the sugar

coating will point outward from the cell (will be on the outside surface of the membrane). Membrane proteins as well as phospholipids and glycolipids are embedded in the lipid bilayer and move around in the plane of the bilayer very rapidly.

MEMBRANE FLUIDITY

Increasing fluidity makes lateral diffusion faster. Fluidity increases with increased temperature, increased content of short-chain fatty acids, and increased content of *cis*-fatty acids. Cholesterol increases the fluidity of membranes that are not very fluid, but decreases the fluidity of membranes that are already fluid.

The membrane is a dynamic assembly and things are diffusing rapidly in the plane of the bilayer. The middle of the bilayer has been likened to olive oil. As with oil, cooling the lipid bilayer will cause the hydrocarbons to become more ordered (structured). The side chains pack closer to each other, and the fluidity of the membrane is lower. Things that disrupt the ability of the side chains to pack in a regular fashion make the membrane more fluid (Fig. 3-4). These include high temperature, lipids with shorter chains (□C16), and lipids with *cis*-double bonds. The shorter lipids and the *cis*-double bonds cause the occurrence of holes (packing defects).

Cholesterol has a funny effect on membrane fluidity. Because of its shape, cholesterol prevents long-chain fatty acids from packing close to each other. When cholesterol is added to a membrane composed largely

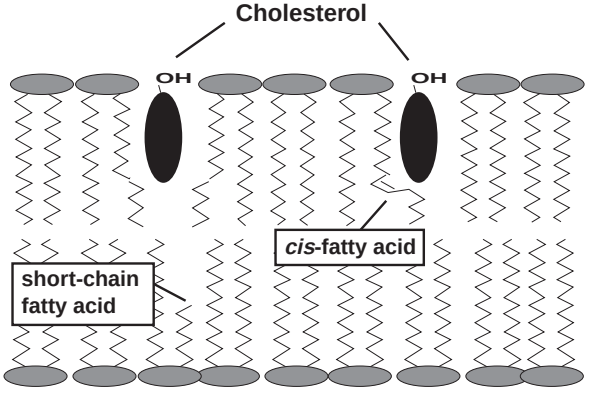


Figure 3-4
MEMBRANE FLUIDITY is regulated by altering the chain length of fatty acids, the presence of *cis*-unsaturations, and the content of cholesterol.

of saturated, long-chain fatty acids, it will cause the fluidity to increase. However, cholesterol is just the right size to pack into the defects caused by *cis*-fatty acids. In a membrane (like most mammalian membranes) that contains significant *cis*-fatty acids, adding cholesterol will cause the membrane fluidity to decrease.

DIFFUSION IN MEMBRANES

Lateral diffusion is in the plane of the membrane, and transverse (flip-flop) diffusion is perpendicular to the membrane (through the membrane). Lateral diffusion (in two dimensions) is fast, and transverse diffusion is slow (or nonexistent) except for gases (CO_2, NH_3) and hydrophobic, uncharged, small molecules (such as cholesterol)

To diffuse rapidly in the plane of the membrane (lateral diffusion), a molecule must simply move around in the lipid environment (including the polar head groups). It need not change how it interacts with phospholipids or with water since it is constantly exposed to pretty much the same environment. Lateral diffusion can be slowed (or prevented) by interactions between membrane proteins and the cellular cytoskeleton. This spatially restricts a plasma membrane protein to a localized environment.

To move through the membrane (change sides or transverse diffusion), a molecule must be able to pass through the hydrophobic portion of the lipid bilayer. For ions and proteins, this means that they must lose their interactions with water (desolvation). Because this is extremely difficult, ions and proteins do not move through membranes by themselves. Small molecules such as CO_2, NH_3 (but not NH_4^+), and water can diffuse through membranes; however, most other small molecules pass through the lipid bilayer very slowly, if at all. This permeability barrier means that cells must develop mechanisms to move molecules from one side of the membrane to the other.

MOVEMENT OF IONS AND MOLECULES ACROSS MEMBRANES

This requires the participation of a protein transporter. Molecules move spontaneously toward lower concentration (chemical gradient) and opposite charge (electrical gradient). Moving in the opposite direction requires the input of energy.

Because the cell membrane is not permeable to ions and most molecules, the cell can regulate the concentrations of things on either side of the membrane. There are two factors that influence the movement of ions and molecules through a membrane. These are the concentration gradient across the membrane (also called the "chemical potential") and the electrical potential of the membrane.

A *concentration gradient* (chemical potential) exists if the concentration of a given molecule or ion is different on the two sides of the membrane. If you punch a hole in the membrane, the concentration of the molecule will try to equalize itself on the two sides of the membrane (if it is an uncharged molecule).

Normally, cells maintain a slight excess of negative ions inside the cell. This costs energy, but you'll see it's worth it. This uneven distribution of charge across the membrane results in a *membrane potential* (or electrical potential). The membrane potential is negative, indicating that the inside of the cell is negatively charged as opposed to the outside which is positive. It has a normal value of about □0.06 V (□60 mV). As long as the membrane potential is maintained, it will affect how ions move (the movement of molecules with no charge is not sensitive to the membrane potential). Moving an ion toward the opposite charge (moving a positive ion from outside to inside the cell) will be easier than moving the ion toward the same charge.

The membrane potential and the concentration gradient can reinforce each other or they can be in opposition to each other. The total force tending to move a molecule or ion through a membrane is called the *electrochemical potential*. When the concentration gradient and the electrical potential work to oppose each other, the stronger effect wins. If someone forces you to get quantitative (this may be a physiologist rather than a biochemist), see the section on the Nernst equation at the end of the chapter.

TRANSPORT ACROSS MEMBRANES

Facilitated diffusion (channel): molecule moves down its electrochemical gradient. *Active transport (pump)*: molecule moves up its electrochemical gradient (requires energy input). Pumps use energy (usually ATP hydrolysis). $Na^{\square}$ high outside/$K^{\square}$ high inside.

Because membranes are impermeable to most molecules, you must have a transporter (a protein) in the membrane to help molecules or ions move through it (Fig. 3-5). They are also called *channels* because they behave like selective holes in the membrane. Transporters are selective

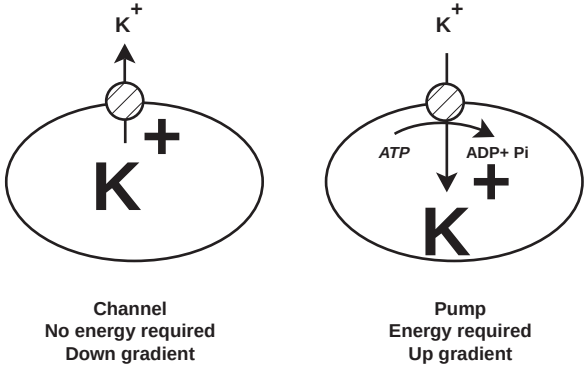


Figure 3-5
Moving from high to low concentration **(CHANNEL)** does not require the input of energy. Moving from low to high concentration **(PUMP)** does require some input of energy, usually in the form of ATP hydrolysis of another ion moving down its concentration gradient at the same time.

because they usually allow only one type of ion to pass through the membrane. For example, the calcium channel that releases calcium from the endoplasmic reticulum, releases only calcium but not other divalent cations. What distinguishes a channel from a pump is that the movement of ions through a channel does *not* require any input of energy.

Channels can be gated. This means that something causes the channel to open. For example, a ligand-gated channel opens when a specific ligand binds to a receptor in the membrane. The acetylcholine receptor is a ligand-gated sodium channel that initiates the flow of sodium into the cell (and potassium out) when the receptor binds the neurotransmitter, acetylcholine. Voltage-gated channels open and close in response to changes in the membrane potential. The acetylcholine receptor is also voltage-gated. When the membrane potential becomes positive, it opens the channel further, increasing the rate of membrane depolarization.

Pumps move ions and molecules up their electrochemical gradient. Pumps require energy, usually in the form of ATP hydrolysis. Sodium-potassium ATPase is an example of a pump. Cells maintain a higher concentration of potassium inside the cell than they do outside the cell. Sodium is maintained low inside, high outside. Sodium-potassium ATPase pumps three sodium ions from inside the cell to outside. This is the unfavorable direction—$Na^{\square}$ moves from low concentration to a higher one and against the membrane potential. At the same time, it also

pumps two potassium ions from outside the cell to inside (against the concentration gradient but with the electrical gradient). Both ion movements are unfavorable so that the transport process requires energy.

Pumps work by changing their structure and binding characteristics during the cycle of ATP binding, hydrolysis, and release of ADP and Pi. Exactly when and what triggers these changes will vary from pump to pump, but the essential feature is that there must be a cycle that moves a binding site from one side of the membrane to the other while it also changes the affinity for the ligand. When it faces outside, the sodium-binding site must have a low affinity for sodium. This allows sodium to be released where the concentration is high. The potassium-binding site will have a high affinity for potassium when it faces outside. This allows potassium to be taken up outside where the concentration is low. During the movement to the other side of the membrane (this doesn't happen at the same time for the potassium and sodium sites), the affinity for substrate changes. When it faces inside, the sodium-binding site has a high affinity so that it can take up sodium at the low concentration inside. The potassium site that faces inside has a low affinity so that it can release potassium at the high concentration inside the cell.

Ion gradients can also be used to transport other molecules. During digestion, glucose is concentrated in the intestinal epithelium (moves from low concentration outside to a high concentration inside). The energy for this process comes from coupling the transport of glucose to the cotransport of sodium from outside the cell to inside (down its concentration gradient; Fig. 3-6).

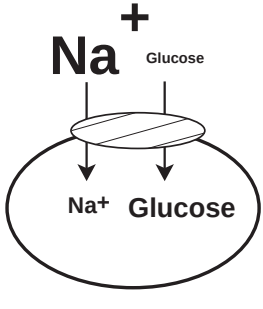


Figure 3-6
Intestinal epithelial cells concentrate **GLUCOSE** from the intestinal lumen by using the energy from moving sodium down its electrochemical gradient to provide the energy for moving glucose against (up) its concentration gradient.

THE NERNST EQUATION

$V = RT/zF \ln (C_o/C_i)$
$\Delta G_{ion} = zFV = z(23 \text{ kcal/mol/V})V$
$\Delta G_{conc} = -RT\ln (C_o/C_i) = -1.36 \log (C_o/C_i)$
$\Delta G_{total} = \Delta G_{ion} + \Delta G_{conc}$

The Nernst equation tells you quantitatively about the energetics of ions moving through membranes. There are two things you need to consider to decide which direction an ion will move spontaneously or whether an ion movement will require energy or not. The first thing to consider is the concentration difference across the membrane. There is an energy associated with maintaining this. Like other energies we've talked about, it will be a free energy. The free energy difference for having different concentrations across a membrane is the following:

$$\Delta G_{conc} = -RT\ln\left(\frac{C_{out}}{C_{in}}\right) = -1.36 \log\left(\frac{C_{out}}{C_{in}}\right)$$

Here R is the gas constant and T is the temperature (in kelvin). Like the other energetic things we've done (see Chapter 24), using R and T and converting from natural to base 10 logs, the factor in front of the concentration ratio is 1.36 kcal/mol. A concentration ratio of 10 between the outside and inside corresponds to a free energy of −1.36 kcal/mol. The sign here can be confusing—it's a convention. Having higher concentration outside than inside is considered favorable. If something moves from higher to lower concentration, that's in the favorable direction and will contribute a negative ΔG. The ΔG is independent of whether or not the molecule is charged.

If the species has a charge on it, there's another ΔG associated with moving it through a membrane.

$$\Delta G_{ion} = zFV$$

Here z is the charge, F is the faraday (23 kcal/V/mol), and V is the potential difference (voltage) across the membrane. Positive ions (z = +1) will move toward places where the charge is negative. Cells (except when they depolarize) are usually negative inside. The membrane potential is maintained by using energy (ATP hydrolysis) to move ions across the membrane. A membrane potential of −60 mV (negative inside) means that the ΔG_{ion} required to set up this gradient would be (1)*23*0.06 = −1.38 kcal/mol. This would amount to maintaining a factor of 10 difference in the concentration of one positively charged ion

(higher outside than inside). The unequal charge distribution is discussed in terms of volts and is called the *membrane potential*. In reality, multiple ions contribute to the net membrane potential.

The direction that something will move spontaneously is determined by the free energy (□G) for that movement, which is going to depend on direction. The □G for moving from out to in will be of the opposite sign of the □G for moving from in to out. When you're trying to decide which way something might move spontaneously (where □G is negative), you have to assume a direction, calculate the □G for that direction, and look at the sign. If it's negative (favorable), things will move in the direction you assumed. If it's positive, things will move in the opposite direction.

For any molecule distributed across a membrane at equilibrium, the overall □G will be the sum $\square G_{ion}$ □ $\square G_{conc}$. Thus, at equilibrium zFV □ □1.36 log (C_{out}/C_{in}). If C_{out}/C_{in} is 10, then V will be □1.36(kcal/mol)(1)/(1 □ 23 kcal/V/mol) □ □1.36/23 □ □0.06 V (or 60 mV). The conclusion is that a ratio of 10:1 for a positive ion outside the cell will create a voltage difference of □60mV (negative side). By convention, the potential difference is defined with respect to the inside—a more negative inside will have a negative membrane potential while a more positive inside will have a positive membrane potential.

There are lots of questions that can be asked about your understanding of membrane potential. One common question is to give you a membrane potential, a few concentrations of ions, and ask which way will things move spontaneously. Which way will an ion move? It will move so that the □G is □ 0 (favorable). Let's do an example. Assume that the membrane potential of the cell is □60 mV (inside). This is near the potential that the cell normally maintains and denotes that there are more negative ions inside the cell than outside. Let's also assume that the concentration of $Na^{\square}$ is 20 mM outside and 100 mM inside. Now, if we were to punch a hole in the membrane that would allow $Na^{\square}$ to move (a channel), which way would it move?

The signs of □G are dictated by what you call inside and outside, so let's try to figure out the magnitude of □G and determine the sign of □G by intuition. Because the cell is negative inside, the membrane potential would tend to cause the $Na^{\square}$ to move from outside to inside (□ likes □). If we assume that the $Na^{\square}$ would move from outside to inside, this would correspond to a $\square G_{ion}$ □ □(1)23(0.06) □ □1.38 kcal/mol. The sign would be negative because moving inside would be good (remember □ likes □). Now you have to consider the concentration gradient. Since the $Na^{\square}$ is higher inside than outside, the concentration gradient would make it harder to move the $Na^{\square}$ from outside to inside (what we assumed originally). The $\square G_{conc}$ would be positive and equal to □1.36*log(20/100) □ □1.36*log(20/100) □ □1.36(□0.7) □ □0.95 kcal/mol. The easiest thing to do here is to forget whether outside or inside is on top of the

ratio of concentrations. If you make a mistake here, all it will do is change the sign of □G, not the magnitude of the number. So be sure that you determine if each □G makes sense. If we had made that mistake here, the $\square G_{conc}$ we calculated would be □0.95 kcal/mol instead of □0.95 kcal/mol. But this must be wrong—we know that with a higher concentration inside, the $Na^{\square}$ would like to move down its concentration gradient. Since we're calculating the □G for moving from outside to inside (we assumed that), then the $\square G_{conc}$ must be positive.

We've just found that the $\square G_{ion}$ is □1.38 kcal/mol for moving $Na^{\square}$ from outside to inside and that $\square G_{conc}$ is □0.95 kca/mol. Since the total □G is negative (□1.38 □ 0.95), the $Na^{\square}$ will move in the direction we assumed (outside to inside). Even though the concentration gradient is opposing the movement, the strength of the membrane potential is sufficient to cause the ion to move up its concentration gradient. This requires energy—which in this case must come from maintaining the membrane potential. You've just seen how a pump works.

· C H A P T E R · 4 ·

DNA-RNA STRUCTURE

DNA Structure

DNA Stability

RNA Secondary Structure

DNA STRUCTURE

Double helix
A □ Adenine □ purine
T □ Thymine □ pyrimidine (DNA only)
G □ Guanine □ purine
C □ Cytosine □ pyrimidine
U □ Uracil □ pyrimidine (RNA only)
AT/GC base pairs
Antiparallel strands
Major groove–minor groove
A-, B-, and Z-DNA

The two complementary strands of the DNA double helix run in antiparallel directions (Fig. 4-1). The phosphodiester connection between individual deoxynucleotides is directional. It connects the 5□-hydroxyl group of one nucleotide with the 3□-hydroxyl group of the next nucleotide. Think of it as an arrow. If the top strand sequence is written with the 5□ end on the left (this is the conventional way), the bottom strand will have a complementary sequence, and the phosphate backbone will run in the opposite direction; the 3□ end will be on the left. The antiparallel direc-

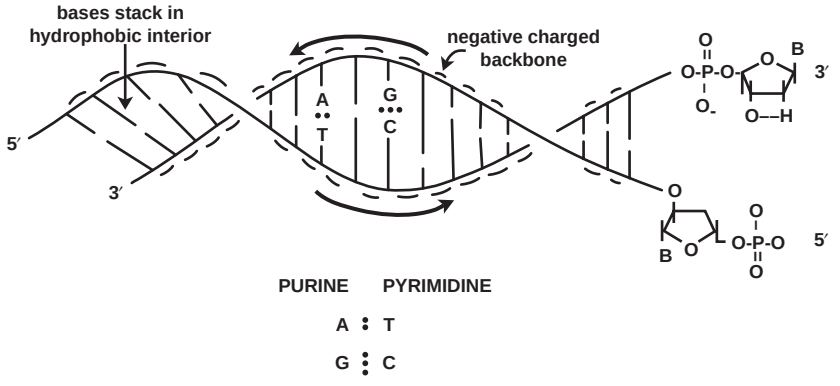


Figure 4-1 Structural Features of DNA

tionality of DNA is an important concept (i.e., it always appears on exams). Either of the two strands could be written on top (just rotate the paper by 180°), but if the DNA codes for a protein, the top strand is usually arranged so that it matches the sequence of the RNA that would be made from the DNA (see later). In Fig. 4-2, you're looking at a base pair as it would be seen from above, looking down the helix axis. The DNA double helix has two grooves—the major and the minor. If the helix were flat, the major and minor grooves would correspond to the two different flat surfaces represented by the front and back of the flat sheet. The major and minor grooves are different size because the two strands come together so that the angle between corresponding points on the phosphate backbone is not 180°. Many of the sequence-specific interactions of proteins with DNA occur along the major groove because the bases (which contain the sequence information) are more exposed along this groove.

The structures shown in Fig. 4-1 are for B-form DNA, the usual form of the molecule in solution. Different double-helical DNA structures can be formed by rotating various bonds that connect the structure. These are termed *different conformations*. The A and B conformations are both right-handed helices that differ in pitch (how much the helix rises per turn) and other molecular properties. Z-DNA is a left-handed helical form of DNA in which the phosphate backbones of the two antiparallel DNA strands are still arranged in a helix but with a more irregular appearance. The conformation of DNA (A, B, or Z) depends on the temperature and salt concentration as well as the base composition of the DNA. Z-DNA appears to be favored in certain regions of DNA in which the sequence is rich in G and C base pairs.

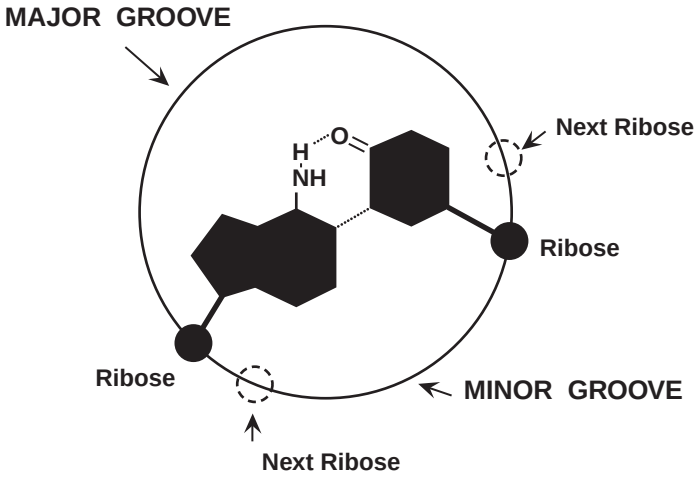


Figure 4-2
DNA has a **MAJOR AND MINOR GROOVE** because the bases attach at an angle that is not 180° apart around the axis of the helix. The major groove has more of the bases exposed. Sequence-specific interactions with DNA often occur along the major groove. Since the helix is right-handed, the next ribose shown is above the last one.

DNA STABILITY

Melting is denaturation.
Annealing is renaturation.
Hydrophobic stacking provides stability.
Intercalating agents stack between bases.

STABILITY INCREASED BY

Decreased temperature
Increased GC content (three hydrogen bonds)
Increased salt (ionic strength)

The DNA double helix is stabilized by hydrophobic interactions resulting from the individual base pairs' stacking on top of each other in the nonpolar interior of the double helix (Figs. 4-1 and 4-2). The hydrogen bonds, like the hydrogen bonds of proteins, contribute somewhat to the overall stability of the double helix but contribute greatly to the specificity for forming the correct base pairs. An incorrect base pair would not

be able to form as many hydrogen bonds as a correct base pair and would be much less stable. The hydrogen bonds of the double helix ensure that the bases are paired correctly.

The double helix can be denatured by heating (melting). Denatured DNA, like denatured protein, loses its structure, and the two strands separate. Melting of DNA is accompanied by an increase in the absorbance of UV light with a wavelength of 260 nm. This is termed *hyperchromicity* and can by used to observe DNA denaturation. DNA denaturation is reversible. When cooled under appropriate conditions, the two strands find each other, pair correctly, and reform the double helix. This is termed *annealing*.

The stability of the double helix is affected by the GC content. A GC base pair has three hydrogen bonds, while an AT base pair has only two. For this reason, sequences of DNA that are GC-rich form more stable structures than AT-rich regions.

The phosphates of the backbone, having a negative charge, tend to repel each other. This repulsion destabilizes the DNA double helix. High ionic strength (high salt concentration) shields the negatively charged phosphates from each other. This decreases the repulsion and stabilizes the double helix.

Intercalating agents are hydrophobic, planar structures that can fit between the DNA base pairs in the center of the DNA double helix. These compounds (ethidium bromide and actinomycin D are often-used examples) take up space in the helix and cause the helix to unwind a little bit by increasing the pitch. The pitch is a measure of the distance between successive base pairs.

RNA SECONDARY STRUCTURE

Stem A stretch of double-stranded RNA
Loop: A loop of RNA
Hairpin loop: A very short loop
Pseudoknot: Interaction between one secondary structure element and another part of the same RNA molecule

RNA is often depicted as a single-stranded molecule. However, in many RNA's, internal complementarity may result in secondary (and tertiary) structure in which one part of the RNA molecule forms a double-stranded region with another part of the same molecule. There are usually a number of mismatches in these structures. Names have been given to some of these structural features (Fig. 4-3).

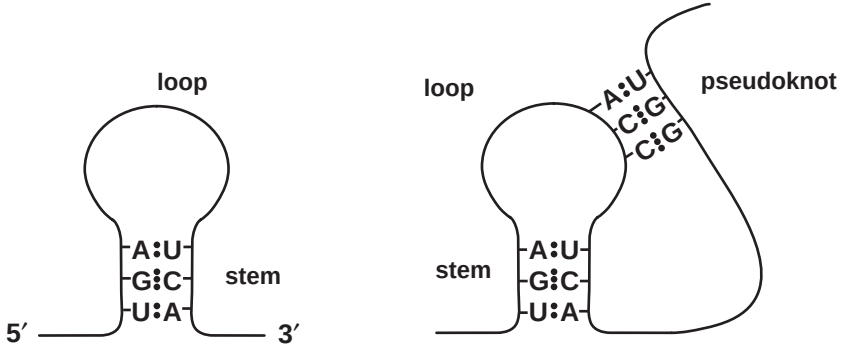


Figure 4-3 RNA Secondary Structure
A single molecule of RNA often contains segments of sequence that are complementary to each other. These complementary sequences can base-pair and form helical regions of secondary structure. Interactions between the secondary structures give RNA a significant folded, three-dimensional structure.