Chapter 5

Building Molecular Models of DNA, Protein, and Lipids

Anne L. Cordon and Neil A. Straus

Department of Botany
University of Toronto
Toronto, Ontario M5A 3B2

Anne is a Senior Tutor cross-appointed to the Departments of Botany and Zoology. She received her B.S. (1967) from McGill University, Quebec. Anne is coordinator of the second-year cell and molecular biology course and previously was a coordinator in the introductory biology course. Her interests include instructional development, TA training, and high school liaison for teachers and students.

Neil received his B.S. (1966), M.S. (1967), and Ph.D. (1970) from the University of Toronto. Following his Ph.D., he spent 2 years of post-doctoral research at the Department of Terrestrial Magnetism, Carnegie Institution of Washington, Washington, D.C. He joined the University of Toronto in 1972 and now he is a Professor in the Department of Botany. Over the years he has coordinated and taught in a series of large introductory molecular cell biology courses at the second-year level. He has taught cell biology, genetics, and molecular genetics at various levels from first year to graduate, and coordinated and lectured in courses for non-science students.

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Contents

Introduction

The molecular model laboratory exercises presented in this chapter are the first labs in our second-year introductory course in cell and molecular biology, BIO250. We start with DNA models followed by protein and then lipid models. The lipid lab is the shortest and is easily finished in 1.5 hours. We have used the remaining time to review concepts and interrelationships of the three types of molecules. We have also successfully combined the protein and lipid labs into one 3-hour session. Two 3-hour model labs in a row seem to hold the students' attention better than three. By varying the introduction and the method of evaluation, these labs may be used at any level from first year through graduate studies. The required course textbook is *Molecular Cell Biology* by Darnell et al. (1990). Instead of redrawing common molecular structures in the Student Outline we sometimes refer students to their text; for example, for the 20 amino acids.

These exercises work well for large classes. BIO250, with an enrolment of about 750 students, is a core course requirement for all students in biology, botany, zoology, as well as for programmes in the basic medical sciences. Laboratories for BIO250 were chosen by the lecture team to compliment and reinforce their lecture material. Labs are 3 hours long and run at 10 times over 2 weeks with up to 90 students per time. A student attends 12 labs on alternate weeks throughout the term. Each lab is worth 1% of their final mark. The 17 teaching assistants (TAs) teach two groups of 20–24 students. For students to derive the maximum benefit from these model labs, the TAs need to be thoroughly briefed. Our TAs are graduate students in molecular or cell biology. In addition, we hold a mandatory 2-hour training session for TAs at the beginning of each new lab exercise. The current lecturer runs these sessions and each TA does the lab and participates in a general discussion. One and a half technicians prepare and maintain these model lab exercises.

The molecular model exercises are evaluated by a brief written quiz (0.5%) and an oral assessment (0.5%) of the student by the TA. The atmosphere in the labs is supportive — if students can't answer the TA's oral question they are encouraged to try again later in the lab. The TAs make up their own oral and written quizzes from a bank of questions and a marking guideline. Most students receive full marks for the oral component. The DNA quiz is at the end of the lab, while the protein and lipid quizzes are given at the start. Frequently TAs have a “Jeopardy”-like game in the third lab to review the concepts from all three labs. Appendix A contains the bank of questions used for these labs.

Notes for the Instructor

The model system used in this series of laboratories is called *Orbit* and is manufactured by Cochranes of Oxford Ltd. (Leafield, Oxford OX8 5NY, England; telephone: (099387) 641; telex: 83147 O.R.-G ref. Orbit). Several different types of systems were tried and rejected before selecting the Cochranes model. Cochranes' pieces are durable and accurate. The size is good: not

too large and unmanageable nor too small to handle. Unlike space filling systems, internal as well as surface characteristics of molecules are visible.

We order directly from the manufacturer rather than incurring the cost of a local supplier. The table in Appendix B summarizes the specifications of the parts for each DNA base pair, protein α-helix, and phospholipid model described in the Student Outline.

We order individual pieces in bulk rather than classroom sets; not only does bulk ordering of specific pieces save money, you may order what you need and avoid getting unnecessary pieces. To calculate how much to order, consult Appendix B, which lists the number of component pieces *per student pair* (or individual student if you prefer) for each type of model: first, multiply by the number of student pairs at a given lab time, then, multiply by a factor (e.g., two) to allow for loss, to give you the minimum number of pieces to order. If your lab times are close together, order enough so that you can set up more than one lab at a time. The model pieces are recycled from group to group but the model pieces (in labeled containers) should be checked between labs to correct sorting errors by previous students. The atom pieces are extremely durable; however, the bonds split and need replacing after several uses so order extras, particularly of the 2 cm green pieces.

We strongly suggest that you colour code certain pieces. Use coloured dots to sort pieces of similar bond angels or lengths. In addition, the color code helps to quickly assess the accuracy of the student's models; for example, distinguishing between bond angles of 108° and 120° for the carbon and nitrogen molecules in the purine rings is difficult yet critical; also the green 2 cm covalent bond is difficult to distinguish from the green 2.5 cm bond used with phosphorus.

Note: Cochranes does not sell a two-prong nitrogen molecule with 108/252 (denoted as * in the table in Appendix B). We order the three-prong 108/126/126 and cut off the prong between the 126° angles (denoted as ** in Appendix B). Alternatively, order the two-prong nitrogen with 110/250.

Student Outline

Laboratory 1: Building a DNA Molecule

Introduction

DNA is a large double-stranded polymer that is composed of only four different monomers called nucleotides. A nucleotide has three components: a phosphate group, a five-carbon sugar molecule (deoxyribose), and an organic base. The organic base is either adenine, guanine, thymine, or cytosine (abbreviated A, G, T, and C respectively). Nucleotides are linked by phosphodiester bonds: the hydroxyl group attached to the 3′ carbon of a sugar forms an ester bond to the phosphate attached to the 5′ carbon of a sugar on another nucleotide, releasing a molecule of water; the A,T C, or G base is a side group on a sugar-phosphate backbone.

The natural state of DNA is a double-stranded helix. The A-T and G-C base-pair complementarity is a consequence of the size, shape, and chemical composition of the bases. Two types of forces stabilize the DNA helix: *hydrogen* bonds between bases (adenine pairs with thymine by two hydrogen bonds, guanine pairs with cytosine by three hydrogen bonds); and *Van der Waals* forces from stacking interactions between base pairs. Ionic interactions between backbone phosphates and cellular cations or basic proteins shield the negative charges on the phosphates, negating the electrostatic repulsion between the two backbones.

Although DNA has been shown to exist in three helical configurations (A-DNA, B-DNA, and Z-DNA), B-DNA, which is a right-handed helix, is the form normally found in living tissue. In this laboratory, you will build a molecular model of B-DNA and look at stereographic pictures of all three molecular forms of DNA: A-DNA, B-DNA, and Z-DNA.

Procedure

The Molecular Kit

You will work with a partner to assemble either an A-T or G-C base pair. Each bench has a set of labeled containers with the various representative model atoms and bonds. Following either Table 5.1 or 5.2 (in Appendix C), *very carefully* count out the exact number of pieces and store them in the labeled egg carton provided. Note that the egg cartons are labeled either A-T or G-C indicating which base pair you and your partner should construct! Each bench is provided with an equal number of A-T and G-C labeled egg cartons so there will be an equal number of both types of base pairs constructed. The following is an explanation of model pieces:

1. The scale of this kit is 10^{-10} m (1 Angstrom) = 2 cm.
2. Atoms are represented by different coloured spheres with prongs protruding from their sides to indicate location and specific angles of bonds. Hydrogen is not represented by a sphere but its presence in a molecule is indicated by an unoccupied prong on one of the other atoms.
3. Bonds are represented by straws and pegs:
   (a) *Single covalent* bonds are GREEN straws of two lengths: 2 cm and 2.5 cm. *Note that the 2.5 cm straw is used only for phosphorus bonds because phosphorous is a large atom.*
   (b) *Double covalent* bonds are represented by a pair of short WHITE pegs which you will connect with a 2 cm green straw.
   (c) *Hydrogen* bonds are long WHITE straws (5 cm). The length of the white straw represents both the hydrogen bond and hydrogen atom involved in the hydrogen bond.

Step 1: Construction of Deoxyribose with Phosphate Group (Figure 5.1)

1. Construct the sugar ring using tetrahedral carbons: join four tetrahedral carbons (connect with single covalent bonds, i.e., 2 cm green straws) and join carbon 1 to carbon 4 with an oxygen atom.
2. Now hold carbon 1 in your left hand and carbon 4 in your right hand with the *oxygen facing upwards*:
   (a) Attach a green straw to carbon 1 (where the -OH group would be attached) so that it orients *away* from you.
   (b) Attach a two-pronged oxygen to carbon 3 so that it orients *towards* you.
   (c) Attach a tetrahedral carbon (carbon 5) to carbon 4 so that it orients *away* from you.
3. Phosphate group: Attach a two-pronged oxygen to carbon 5 and then join a phosphorous to this oxygen with a 2.5 cm bond. Add two single-pronged oxygens and one two-pronged oxygen to the phosphorous with 2.5 cm bonds.

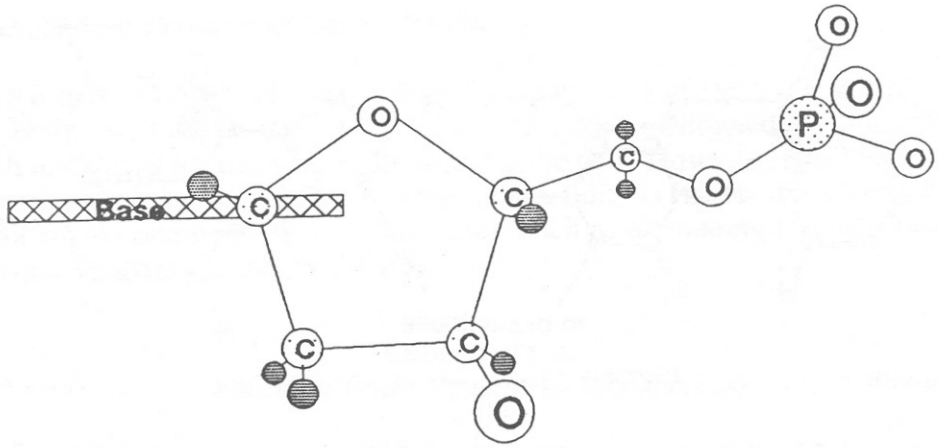

Figure 5.1. Deoxyribose phosphate.

4. The actual three-dimensional structure of deoxyribose in DNA has a slight pucker at carbon 2. To create this pucker, hold carbon 1 in your left hand and carbon 4 in your right hand so that carbon 5 is oriented away from you and gently push carbon 2 away from you. Try to retain this pucker when you construct the DNA double-helix.

Step 2: Construction of Base

Refer to Figure 5.2 to construct your base pair: either adenine and thymine, or guanine and cytosine:

1. Bond angles: You will use planar carbons and planar nitrogens for the rings. However, note that the internal bond angle (C-N or C-C) of the smaller purine ring is 108° while the internal bond angles in the pyrimidine ring and the larger purine ring are 120°. *Be careful when you attach the two carbons that connect the large and small purine rings; the 120° angle must be oriented into the large ring and the 108° angle must be oriented into the small ring.*
2. To form a double bond join two white single-pronged pegs with a 2 cm straw and then snap this structure into place so that it joins the atoms forming the double bond.
3. Oxygens involved in hydrogen bond formation are two-pronged oxygens; the oxygen of thymine that is not involved in hydrogen bond formation is a single-pronged oxygen.

Step 3: Assembly of Nucleotide

Attach the sugar to the base: the carbon 1 of the sugar is attached to the nitrogen 9 of a purine or the nitrogen 1 of a pyrimidine with the release of water.

Step 4: Assembly of Base Pair

1. Join the purine and pyrimidine bases together with hydrogen bonds and lay the base pair down on the lab bench top. *Note the position of attachment of the base pair to the sugar phosphate*

backbones. If your connection is correct, one of your sugar rings should be inverted relative to the other.

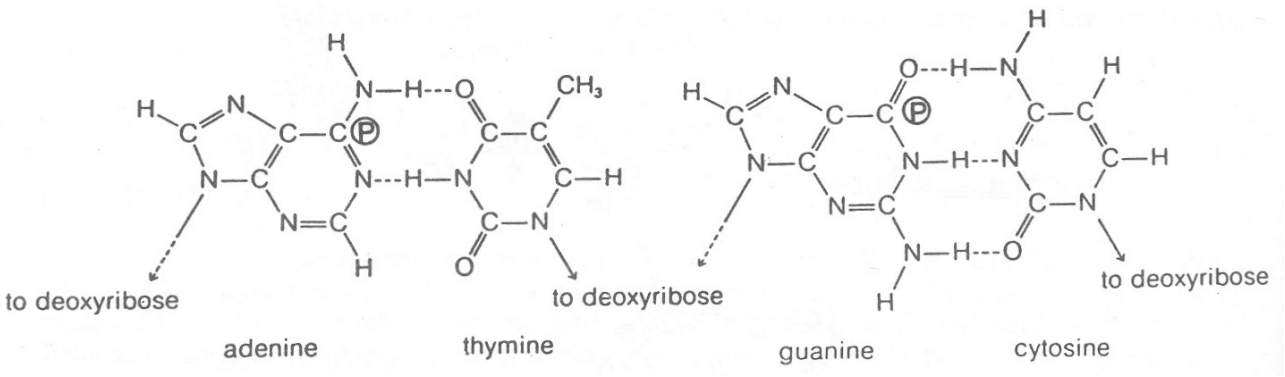

Figure 5.2. Base pairs: adenine-thymine (*left*), guanine-cytosine (*right*).

2. Even at this stage of assembly it should be apparent where the *major and minor grooves* are: Identify the position of carbon 1 of both of the deoxyribose sugars relative to the base pair on the bench and then trace two arcs, each of which start and end at these carbons and go around one side of the base pair. The larger arc represents the position of the major groove and the smaller arc represents the position of the minor groove.
3. Show the nucleotide pair to your TA. Point out the positions of the major and minor grooves and identify the atoms that may potentially form hydrogen bonds, indicating for each whether they would be a *receiver* or *donor.*

Step 5: Assembling the DNA Double-Helix

You are now ready to assemble the nucleotide pair into a class-constructed DNA model:

1. Take your nucleotide pair to the stand at the front of the lab. Detach the hydrogen bonds and reattach them after you have laid the base on the platform.
2. One of the sugar-phosphate backbones should run 5′ to 3′ and the other should run 3′ to 5′. Before you attach one phospho-sugar to the next, remove the two-pronged oxygens from the each of the 5′ phosphate groups. Create a phosphodiester bridge between your nucleotides and the next ones in the growing helix by attaching the free 3′ oxygens to the open bond of the 5′ phosphates.
3. Make sure you have retained the C_2 pucker of the sugar rings. Identify the major and minor grooves and reassure yourself that, when you are looking at your base pair, these grooves occupy the positions you have identified for your TA.
4. Note the different potential hydrogen bond patterns that are possible in the major and minor grooves on the three-dimensional model.

Step 6: Stereographic Pictures of DNA Structures

Examine the stereo photographs of the three different forms of DNA (Dickerson, 1983) and note the direction of the twist of the backbone. In this lab, you constructed a model of the B-helix of DNA. It is felt that this is the form generally found in the cell. However, structural analyses indicate that DNA fibers assume the A-helix form when the humidity is below 75% during X-ray crystallography. Under some conditions, special sequences, such as alternating CGCG, form a left-handed helix with a zigzag backbone, this is Z-DNA.

Laboratory 2:
Building Basic Protein Configurations

Introduction

Proteins form the basic machinery of the cell, performing a variety of functions essential to life. They are the enzymes that catalyze the metabolic reactions in the cell. They form both intra- and extra-cellular skeletal structures. They form pore structures in cellular membranes that control the entry and exit of molecules between the cell and its environment. As you might expect, molecules with such diverse functions can have very different chemical and physical properties. This incredible molecular variation is achieved by building these linear polymers out of 20 different amino acids. The amino acids are connected together by peptide bonds to form a long polypeptide chain that is folded back on itself to assume a specific three-dimensional shape.

During this laboratory, you are expected to become familiar with the basic polypeptide molecular configurations that contribute to the three-dimensional shape of most proteins. One configuration is the α-helix; also familiarize yourself with the demonstration model of a β-pleated sheet, the other major protein configuration.

You will be building an **amphipathic polypeptide** of the sequence KEFLSIVDNIYA; each of these letter refers to an amino acid described later. When this polypeptide is arranged in an α-helical structure take note of clusters of hydrophobic and hydrophilic amino acids.

Procedure

The Molecular Kit

You will work with a partner to assemble an α-helix. As in Lab 1 you will count out the model pieces you need from the labeled containers on the bench and transfer them to an egg carton. The sections of the container have been pre-labeled with the names of the pieces. Many of the "atoms" and bonds are the same as you used to construct the DNA molecule. What are the differences? (Compare Tables 5.1 to 5.3 in Appendix C.)

Reminders from Lab 1:

1. Be very careful to select the correct pieces and count the number of each accurately to avoid frustration later! See Table 5.3 (in Appendix C).
2. The scale of the model pieces is 10^{-10} m = 2 cm.
3. Atoms are different coloured spheres with prongs protruding from the sides representing the number and *angle* of covalent bonds.

(a) The two types of planar carbon atoms (120 and 114) are particularly tricky to distinguish because the bond angles are quite similar.

(b) Planar carbon (114°) and nitrogen (114°) are involved in peptide bond formation. *Make sure the 114° angles are located in the correct position when forming the peptide bonds.*

(c) *Hydrogen* is represented by a vacant prong.

4. Bonds are represented by coloured straws: A single covalent bond is a 2.0 cm green straw. A double covalent bond is represented by a single bond plus two short white pegs connected by another 2.0 cm green straw. Hydrogen bonds are 5.0 cm white straws (this bond length includes both the hydrogen and the hydrogen bond).

Step 1: Assemble 12 L-Amino Acids

One partner should assemble 12 core amino acids while the other partner assembles the specific side groups described below.

The central tetrahedral carbon of all amino acids except glycine are attached to four different chemical groups. This leads to two optical isomers for each amino acid: the **D** and **L** isomers (Figure 2-3 in Darnell et al., 1990). In living cells only the L-amino acids are used in translation to build proteins. Since the final three-dimensional structure depends on which direction the various groups assume in space, you must be meticulous in constructing each amino acid and *only build the L isomers.*

Build each of the 12 amino acids in the same way:

1. Hold a tetrahedral carbon with one prong in your left hand and one prong in your right so that the remaining two prongs are pointing away from you.

2. Attach a planar nitrogen to the left-handed carbon prong so the 114° bond angle is adjacent to the point of attachment.

3. Leave the right-handed prong of the tetrahedral carbon empty because this is where hydrogen is covalently attached to the tetrahedral carbon.

4. Next attach a planar 114° carbon to the top prong of the tetrahedral carbon that is pointed away from you; the 114° angle is beside the bond joining it to the central carbon.

5. Now, attach a two-pronged 180° oxygen atom to the planar carbon so that it is opposite the 114° angle; form a double bond between the oxygen and the planar carbon. *The open prong of the oxygen will be involved in hydrogen bond formation.* The R-groups of the different amino acids will be attached to the lower prong that is directed away from you. *Add a 2 cm green straw to indicate the position of the R-group attachment.*

In summary, the carboxyl carbon (a planar atomic center) is constructed so the 114° angle is directly opposite the double-bonded oxygen atom; the 114° angle of the nitrogen atom is between the central carbon and one of the hydrogens that is bonded to the nitrogen. The double-bonded oxygen is a two-pronged 180° atomic center for the helical structures because the second prong is aimed in the right direction to form the hydrogen bonds of these molecules.

Assemble the Amino Acids Side Groups

Complete the side groups for each of the following 12 amino acids (see Figure 2-2 of Darnell et al., 1990):

K (lysine, positively charged): Attach a chain of four tetrahedral carbons with a tetrahedral nitrogen on the distal end to the lower prong of the central carbon of an amino acid core structure.

E (glutamate, negatively charged): Attach (1) two tetrahedral carbons to the lower prong of the central carbon of an amino acid core structure, (2) a planar 114° carbon to the end of this two-carbon chain, so that the 114° angle is adjacent to the bond you just formed, and (3) two single-pronged oxygens to the planar carbon and form a double bond opposite the 114° angle.

F (phenylalanine, hydrophobic): Attach (1) one tetrahedral carbon to the lower prong of the central carbon of an amino acid core structure, and (2) a ring of six 120° planar carbons containing three double bonds to this tetrahedral carbon.

L (leucine, hydrophobic): Attach (1) a chain of three tetrahedral carbons to the lower prong of the central carbon of an amino acid core structure, and (2) another tetrahedral carbon to the second carbon of the chain.

S (serine, hydrophilic): Attach (1) one tetrahedral carbon to the lower prong of the central carbon of an amino acid core structure, and (2) attach a two-prong 110° oxygen to this side-chain carbon.

I (isoleucine, hydrophobic): Attach (1) a chain of three tetrahedral carbons to the lower prong of the central carbon of an amino acid core structure, and (2) a single tetrahedral carbon to the side-chain carbon closest to the amino acid backbone, such that when you hold the amino acid backbone in your left hand and the last two carbons of the side chain in your right hand, the added carbon is directed down and away from you.

V (valine, hydrophobic): Attach (1) a chain of two tetrahedral carbons to the lower prong of the central carbon of an amino acid core structure, and (2) another tetrahedral carbon to the side-chain carbon that is closest to the amino acid backbone.

D (aspartate, negatively charged): Attach (1) a tetrahedral carbon to the lower prong of the central carbon of an amino acid core structure, (2) a planar 114° carbon to this tetrahedral carbon with the 114° angle adjacent to the attaching bond, and (3) two single-pronged oxygens to the planar carbon and form a double bond opposite the 114° angle.

N (asparagine, hydrophilic): Attach (1) a tetrahedral carbon to the lower prong of the central carbon of an amino acid core structure, (2) a planar 114° carbon to this tetrahedral carbon with the 114° angle adjacent to the attaching bond, (3) a single-pronged oxygen opposite the 114° angle and form a double bond, and (4) attach a 114° planar nitrogen to the side-chain planar carbon so that the 114° angle is opposite to the carbon.

I (isoleucine, hydrophobic): Make another isoleucine as described above.

Y (tyrosine, hydrophobic): Make another phenylalanine and turn it into a tyrosine by adding a two-pronged 110° oxygen to the aromatic carbon that is on the other side of the ring opposite the point of attachment to the backbone amino acid.

A (alanine, hydrophobic): Attach a tetrahedral carbon to the lower prong of an amino acid core structure.

Step 2: Assemble the α-Helix

The Peptide Bond

The peptide bond joins the carboxyl carbon of one amino acid to the nitrogen of the next amino acid. During the formation of the peptide bond an H atom is lost from the nitrogen and an OH is removed from the carboxyl group. Because the carbon-nitrogen bond of the peptide bond forms a partial double bond, which it takes from the adjacent carbon oxygen double bond, the peptide bond forms a plane that includes the double-bonded oxygen, both central carbons of the adjacent amino acids and a hydrogen atom (Figure 2-4 in Darnell et al., 1990). Try to preserve these planes as you build the polypeptide.

Assembling the α-Helix

1. Construct a peptide bond between the *carboxyl* carbon of K and the *nitrogen* of E such that the 114° angle of nitrogen atom is opposite the carboxyl carbon; note the planar properties of the peptide bond that were described above. *The K is on the N-terminal end of the polypeptide*.
2. Continue by joining E to F, making sure that the 114° angle of nitrogen (in the peptide bond) is opposite the carboxyl carbon of the next amino acid.
3. Then, using the same procedure, join F to L and L to S trying to envision a right-handed helix with K at the lower end. Form a hydrogen bond between the backbone nitrogen of S and the backbone oxygen of K with a 5.0 cm white straw.
4. Show your TA your polypeptide.
5. Next, form a peptide bond between S and I; then, form a hydrogen bond between the backbone nitrogen of I and the backbone oxygen of E.
6. Add V to the chain and hydrogen bond it to F.
7. Add D to the chain and hydrogen bond it to L.
8. Add N to the chain and hydrogen bond it to S.
9. Add I to the chain and hydrogen bond it to the previous I.
10. Add Y to the growing chain and hydrogen bond it to V.
11. Finally, add A to the chain and hydrogen bond it to D.
12. This short stretch of α-helix is complete. Count the number of amino acids that are required for one complete turn. Hold it up and identify the hydrophobic and hydrophilic sides.
13. Show the structure to your TA for grading. Be prepared to describe why your α-helix is right-handed, demonstrate why it is an amphipathic helix, and answer other basic questions on the structure of the molecule.

Laboratory 3: Building Membrane Lipids

Introduction

Membranes are created by the hydrophobic interaction of two lipid layers. They form water-impermeable barriers within the cell and surround the cell to form a barrier that separates the cytoplasm from the external environment. They are the sites of hydrophobic reactions, and of both photosynthetic and respiratory electron transport.

During this laboratory you are expected to become familiar with the basic molecular structure of membranes. You will build a phospholipid and arrange a number of lipids into a membrane. You will also study the relationship between hydrophobic protein structures and membranes to acquire an understanding of the interactions between proteins and lipids. These interactions create pores in membranes and form other structural and reactive complexes that are important components of living membranes.

The Molecular Kit

You will be working individually in this lab. Assemble a lipid molecular kit: count out the requisite number of pieces according to Table 5.4 (in Appendix C) and store them in the labeled egg carton as you did for Labs 1 and 2. Review the descriptions of the models from Labs 1 and 2. *Which atoms and bonds are also found in DNA? In protein? Which are unique to DNA? To protein? To lipids?*

Basic Lipid Structure

Different membrane lipids have the same basic structure. Two fatty acids form ester links to the first two carbons of glycerol and the third carbon of glycerol is attached to a hydrophilic group. In this lab we will build a phospholipid, 1-oleoyl-2-linoleoyl-phosphatidylethanolamine as shown in Figure 5.3.

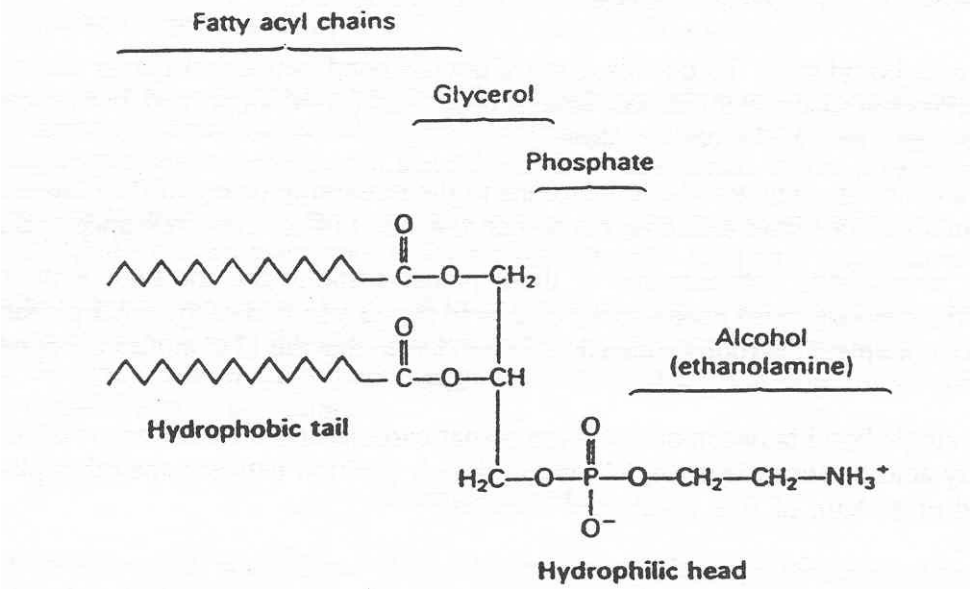

Figure 5.3. The phospholipid, 1-oleoyl-2-linoleoyl-phosphatidylethanolamine.

Glycerol

First construct the glycerol backbone by attaching three tetrahedral carbons together. Add a two-pronged 110° oxygen atom to each carbon of the chain.

Fatty Acids

The fatty acids of membrane lipids can differ from each other in length and the degree of saturation. Unsaturated fatty acids contain double bonds present, in their carbon backbone. The more double bonds, the greater the fluidity of membranes. In this lab you will build two different, unsaturated, 18-carbon fatty acids: *oleic acid* and *linoleic acid*.

Assembling Oleic Acid

Oleic acid: $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$

1. Create a carboxyl end. To do this form a double bond between a planar carbon and a single-pronged oxygen such that the double bond is opposite the 114° angle, and then attach a two-pronged 110° oxygen atom to the carbon atom.
2. Attach a chain of seven tetrahedral carbons to the remaining prong of the carboxyl carbon. Join two planar carbons with a double bond such that the 114° angles are opposite the double bond.
3. Form a single bond between one of these planar carbons and the end of the chain of seven tetrahedral carbons. Then form a single bond in the *cis* position (on the same side of the double bond) between the other planar carbon and one end of a chain of eight tetrahedral carbons.

Assembling Linoleic Acid

Linoleic acid: $CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$

1. Create a carboxyl end. To do this, form a double bond between a planar carbon and a single-pronged oxygen such that the double bond is opposite the 114° angle, and then attach a two-pronged 110° oxygen atom to the carbon atom.
2. Attach a chain of seven tetrahedral carbons to the remaining prong of the carboxyl carbon. Join two planar carbons with a double bond such that the 114° angles are opposite the double bond.
3. Form a single bond between one of these planar carbons and the end of the chain of seven tetrahedral carbons. Add a tetrahedral carbon to the *cis* position of the other planar carbon. Then, join two more planar carbons with a double bond such that the 114° angles are opposite the double bond.
4. Form a single bond between one of these planar carbons and the end carbon of the chain. Finish this fatty acid by forming a single bond in the *cis* position between the other planar carbon and one end of a chain of five tetrahedral carbons.

Diacyl Glycerol

In diacyl glycerol, oleic acid and linoleic acid are joined to the first two carbons of glycerol by ester linkages. To form one of the ester linkages, remove the two-pronged oxygen from the carboxyl group of oleic acid and then use this site to attach the fatty acid to the oxygen of the first carbon of glycerol. Repeat this process to form an ester linkage between linoleic acid and the second carbon of glycerol.

Polar Groups on the Third Carbon of Glycerol

The majority of biological membranes are composed of phospholipids; however, the internal membranes of the chloroplast are glycolipids. These lipids do not have a phosphate group but instead have one or more sugar groups as the hydrophilic component of the lipid. In phospholipids, phosphate is attached to the third carbon of glycerol by an ester linkage; other groups are generally attached to the phosphate. One of these groups is ethanolamine.

1. To complete the phospholipid construction, attach a phosphorus to the oxygen on the third carbon of glycerol by using a 2.5 cm bond. Use 2.5 cm straws to add two one-pronged oxygens and one two-pronged 110° oxygen to the phosphorus.
2. Add ethanolamine to the phosphate by connecting the terminal carbon of a chain of two tetrahedral carbons and a tetrahedral nitrogen to the open oxygen prong. *Note that you have created a polar molecule with a long hydrophobic end and a hydrophilic end.*
3. Show your molecule to your TA and identify the hydrophilic and hydrophobic areas. Be prepared to point out the ester bonds and to explain what a *cis* bond is.

Membrane Structure

Membranes are composed of a lipid bilayer with a thickness of 40–50 10^{-10} m. Remember, the scale of the molecular kit you are using is 10^{-10} m = 2 cm in the model.

1. When other students have completed building their lipids, use eight or more lipids to form a lipid bilayer with the proper dimensions. Biological membranes contain both lipids and proteins. Only proteins with hydrophobic surfaces can span the hydrophobic areas of the membrane.
2. Place a stretch of hydrophobic α-helix protein across the membrane and count out the number of amino acids that must be hydrophobic for a single α-helix to span a membrane.
3. If a molecular geneticist finds this length of hydrophobic amino acids in a protein that he or she is studying, he or she will note that this is a potential area for membrane association. Remember in your last lab you built an amphipathic α-helix — such α-helixes could combine to form a hydrophilic channel across a membrane.

Literature Cited

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APPENDIX A
Quiz Question Bank

Answer sheets are distributed to students with the diagrams of the A-T and G-C base pairs and the deoxyribose from their lab outline with certain information deleted. Students label various structures, indicate bond angles, etc., directly on this sheet. Students are reminded to clearly indicate the question number next to their answer.

Laboratory 1: Building a DNA Molecule

1. Circle the atom on the [choose one of: adenine/guanine/cytosine/thymine] base which is the attachment site for the deoxyribose.
2. Number the carbon atoms on the deoxyribose.
3. Clearly label the [major or minor] groove on the [adenine-thymine or cytosine-guanine] base pair.
4. Label the potential donors (D) and receivers (R) in the [major or minor] groove on the [adenine-thymine or guanine-cytosine] base pair.
5. What is the difference between a nucleotide and a nucleoside?
6. What forces contribute to the stability of the DNA helix?
   [(a) *Ionic* interactions between backbone phosphates and cytoplasmic ions. (b) *H-bonds* between bases in the base pair. (c) Stacking interactions (*Van der Waals*) between base pairs.]
7. Describe three major differences between DNA and RNA structure.
   [DNA is double-stranded, RNA is usually single but may by double; 2′-OH in ribose;U instead of T in RNA]
8. Why is the melting temperature of dsDNA higher in a saline solution than in pure water?

Laboratory 2: Building Basic Protein Configurations

1. Draw and label the two stereoisomers of the amino acid alanine, NH_2CHCH_3COOH. Which isomer is normally found in nature?
2. Indicate on your answer sheet (P) the first three peptide bonds on the polypeptide chain.
3. Draw and label the basic structure of all amino acids. Indicate which is the *alpha* carbon.
4. On the answer sheet with the amino acids, label each group as hydrophillic or hydrophobic. In a sentence explain the reason for your designation.
5. Explain what an amphipathic helix is.
6. Label each of the polypeptide chains on your answer sheet as either α-helix or β-pleated sheet and *briefly* explain.
7. In living cells β-pleated sheets are (choose one): (a) always antiparallel, (b) always parallel, or (c) either always antiparallel or always parallel.
8. In living cells, are all proteins *amphipathic*? Explain.
9. Label and describe the C-terminal and N-terminal in a polypeptide chain.
10. Compare and contrast the relative orientations of oxygen and R-groups in the α-helix versus the β-sheet.
11. What is a B-barrel? How might a B-barrel form a pore in the membrane?
12. Explain what is meant by *parallel* and *anti-parallel* strands in DNA and in protein.

Laboratory 3: Building Membrane Lipids

1. What is the difference between saturated and unsaturated fatty acids?
2. Explain why the degree of unsaturation affects how solid/fluid a fatty acid is at room temperature.
3. Describe/label the hydrophobic/hydrophilic portions of lipid a molecule.
4. What is a liposome?

APPENDIX B
Specifications for Model Pieces
(See Notes for the Instructor)

Model piece	Critical **angle**/dimension	Number of pieces			
		DNA		Protein	Lipid
		G-C	A-T		
Carbon (black)					
Tetrahedral		10	11	39	33
3-prong	**108**/126/126	1	1		
3-prong	**108/120**/132	2	2		
3-prong	**120**/120/120	6	6	12	
3-prong	**114**/123/123			15	8
Oxygen (red)					
1-prong		4	5	5	4
2-prong	**110**/250	10	9	2	6
2-prong	**180**/180			12	
Nitrogen (blue)					
Tetrahedral				1	1
2-prong*	**108**/252	1	1		
2-prong	**120**/240	1	1		
3-prong**	**108**/126/126	1	1		
3-prong	**114**/123/123			13	
3-prong	**120**/120/120	5	4		
Phosphorus (purple)					
Tetrahedral		2			1
Bonds (green and white straws, and pegs)					
Single covalent	green: 2 cm	45	45	121	51
Phosphate single covalent	green: 2.5 cm	8	8		4
Double covalent	white pegs (type x)	14	14	42	10
Hydrogen	white: 5 cm	3	2	8	

* Nitrogen 108/252 is not available; order 108/126/126** and cut off prong between 126/126.

APPENDIX C
Pieces Required for Model Kits

Note: The following tables should appear within the Student Outline. (They appear in an appendix in this chapter to simplify typesetting.)

Table 5.1. Kit for guanine-cytosine pair.

Model piece	Critical **angle**/dimension	# of pieces
Carbon (black)		
Tetrahedral		10
Planar 3-prong	**108**/126/126	1
Planar 3-prong	**108/120**/132	2
Planar 3-prong	**120**/120/120	6
Oxygen (red)		
Planar 1-prong		4
Planar 2-prong	**110**/250	10
Nitrogen (blue)		
Planar 2-prong	**108**/252	1
Planar 2-prong	**120**/240	1
Planar 3-prong	**108**/126/126	1
Planar 3-prong	**120**/120/120	5
Phosphorus (purple)		
Tetrahedral		2
Bonds (green and white straws, and pegs)		
Single covalent	green: 2 cm	45
Phosphate single covalent	green: 2.5 cm	8
Double covalent	white pegs	14
Hydrogen	white: 5 cm	3

Table 5.2. Kit for adenine-thymine pair.

Model piece	Critical **angle**/dimension	# of pieces
Carbon (black)		
Tetrahedral		11
Planar 3-prong	**108**/126/126	1
Planar 3-prong	**108/120**/132	2
Planar 3-prong	**120**/120/120	6
Oxygen (red)		
Planar 1-prong		5
Planar 2-prong	**110**/250	9
Nitrogen (blue)		
Planar 2-prong	**108**/252	1
Planar 2-prong	**120**/240	1
Planar 3-prong	**108**/126/126	1
Planar 3-prong	**120**/120/120	4
Phosphorus (purple)		
Tetrahedral		2
Bonds (green and white straws, and pegs)		
Single covalent	green: 2 cm	45
Phosphate single covalent	green: 2.5 cm	8
Double covalent	white pegs	14
Hydrogen	white: 5 cm	2

Table 5.3. Kit for α-helix protein.

Model piece	Critical **angle**/dimension	# of pieces
Carbon (black)		
Tetrahedral		39
Planar 3-prong	**114**/123/123	15
Planar 3-prong	**120**/120/120	12
Oxygen (red)		
Planar 1-prong		5
Planar 2-prong	**110**/250	2
Planar 2-prong	**180**/180	12
Nitrogen (blue)		
Tetrahedral		1
Planar 3-prong	**114**/123/123	13
Bonds (green and white straws, and pegs)		
Single covalent	green: 2 cm	121
Double covalent	white pegs	42
Hydrogen	white: 5 cm	8

Table 5.4. Lipid kit for 1-oleoyl-2-linoleoyl-phosphatidylethanolamine.

Model piece	Critical **angle**/dimension	# of pieces
Carbon (black)		
Tetrahedral		33
Planar 3-prong	**114**/123/123	8
Oxygen (red)		
Planar 1-prong		4
Planar 2-prong	**110**/250	6
Nitrogen (blue)		
Tetrahedral		1
Phosphorus (purple)		
Tetrahedral		1
Bonds (green and white straws, and pegs)		
Single covalent	green: 2 cm	51
Phosphate single covalent	green: 2.5 cm	4
Double covalent	white pegs	10