Structures of Biomolecules

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Experiment designed by Tim Herman, Michael Patrick, John Moore, Jeremiah Depta and Gordon Bain

Purpose of the Experiment

To learn how to interpret models of biomolecular systems.
To appreciate how different depictions of a complex system can be used to improve understanding.
To identify areas of secondary structure in biomolecules.
To learn about the importance of hydrogen bonding in biomolecules.
To build an understanding of how amino acids interact with each other and their surroundings to impart particular properties to an area of a biomolecule or the molecule as a whole.

For Your Safety

There are no safety concerns associated with this experiment.

Background

Biomolecular systems are highly complex, and an understanding of their structure has only been achieved on any level within the past 40 years. Modern instrumental and computational techniques have made it possible to solve the structures of a large number of biological molecules. Most of the progress in this area has been accomplished within the past 10 years. The complexity of biologically active molecules is such that a conventional ball and stick representation of every atom and bond in the structure is often of little use because "you can't see the forest for the trees". Also, the recurrence of common features referred to as "secondary structure" in many proteins allows some level of detail to be suppressed in a picture or model in order that the area(s) of the protein that are of the greatest interest may be visible to an observer.

In this experiment, you will work with a number of models that have been designed to allow you to "see" different features of some biomolecules. In answering the questions posed about each model and/or exercise, you should gain some insight into biomolecular chemistry and build your skills in interpreting both two and three dimensional depictions of biomolecules.

Preparing Yourself for this Experiment

You should have attended all the lectures on biochemistry and worked through the Proteins 1 and 2 and DNA 1 biomolecules tutorials delivered via WebCT.

Names of group members

_____ _____

_____ _____

_____

Experimental

Organization

There are four stations on each side of the lab. Your lab section will be divided up into four groups. You will rotate from one station to the next with the rest of your group. Your lab instructor will assign these groups during discussion the week prior to the lab experiment. You have a fixed amount of time at each station and should not move on to the next one until your lab instructor tells you to do so.

The timing in lab will be as shown in the table below. The time refers to the number of minutes into the lab period at which you will start the particular station.

	Prelab talk	**Station A**	**Station B**	**Station C**	**Station D**
Groups 1 and 2	0 mins	10 mins	45 mins	80 mins	~110 mins
Groups 3 and 4	35 mins	45 mins	80 mins	115 mins	~145 mins
Time spent at station	10 mins	35 mins	35 mins	30 mins	~30 mins

Data Collection

The following sections tell you what to do at each station and give you a space to write in answers to some questions. Note that there is a table (similar to Table 12.8 from Prof. Moore's *new* textbook) attached to the back of this packet. You will need this table to answer some of the questions.

Station A – Fundamental Structures

Divide your group into two. One sub-group should work with the models 1a and 1b and answer the questions on those models, the other sub-group should work with models 2a and 2b and answer the questions on those models. Once you have completed these tasks, each sub-group should explain to the members of the other sub-group what the models they worked on are and how they arrived at the answers to the questions.

Models 1a and 2a

Take a close look at models 1a and 2a and answer the following questions:

Model 1a **Model 2a**

i. How many amino acids are depicted in this structure?

_____ _____

ii. Identify the four atoms that make up the repeating structural unit of this model

iii. Describe the path in 3D space this repeating unit takes.

iv. What kind of secondary structural feature does this model represent?

v. 1a If the *pitch* of this structure is defined as the number of repeating units per turn, what is the pitch of the structure modeled?

2a Look at the three strands in the model. Are they parallel or anti-parallel? Explain briefly.

vi. What one feature contributes most to the stabilization of both structures?

Models 1b and 2b

Take a close look at models 1b and 2b, which include the amino acid side chains, and answer the following questions:

Model 1b **Model 2b**

vii. How many amino acids are present in the protein fragment modeled?

viii. Beginning at the N-terminal end, sequence the first 6 amino acids in the protein.

ix. Work with the other subgroup to answer this question. Which structure is *denser*? i.e. which structure packs the most amino acids into the least space?

x. One "side" of the structure modeled faces/contacts the interior of the protein and one is in contact the environment around the protein it is a part of. How can you tell which "side" faces which way?

xi. Use the remainder of your time at this station to look at all four models as a single group. Fill in your copy of this handout with *all* the answers and think about the similarities and differences between the two structures modeled.

Station B – The Zinc Finger System

Model A

i. What level of structure (primary, secondary, tertiary or quaternary) is depicted *within* this model?

_____

ii. Taken as a unit, what level of structure does the entire model represent?

_____

iii. In figure 1 (below), circle or highlight the α-helix in this structure.

Model B

i. What is the model intended to represent?

ii. Use the model to identify the hydrogen bonding pairs of N and O atoms throughout the molecule. Draw these bonds on figure 2 below.

Figure 1

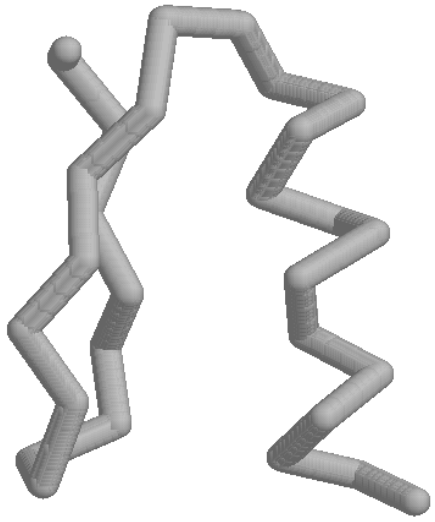
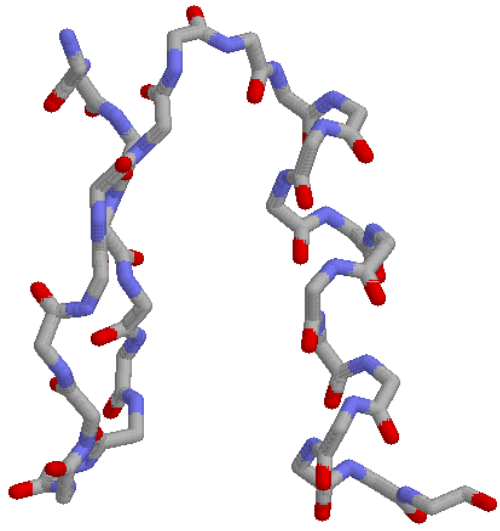


Figure 2



Model C

i. Identify the amino acids that bind the zinc atom. (Zinc is green, sulfur is yellow.)

ii. What is the geometry around the zinc atom? (Hint: think back to VSEPR – e.g. octahedral, square pyramid, tetrahedral etc.)

_____

Model D

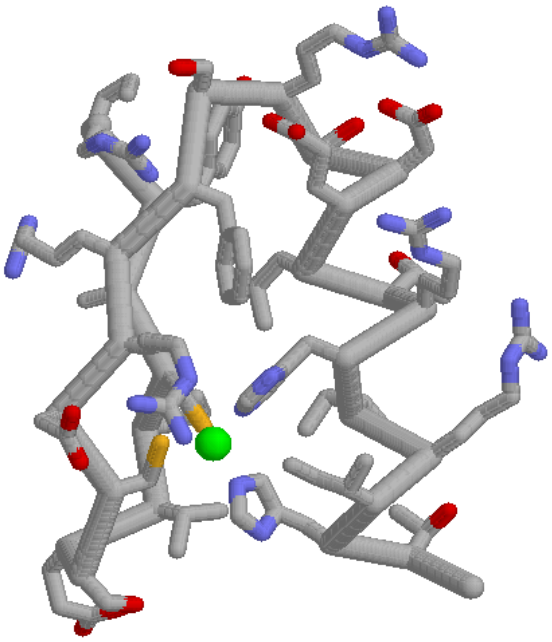
i. What does this model show that the other models did not?

ii. On the diagram of this model (figure 3), circle the amino acid side chains on the interior of the protein motif. (Exclude those attached to the zinc atom.) Identify each side chain you circled with the three-letter code representing the amino acid of which it is a part. (see table)

iii. What do most of these amino acids have in common?

iv. What do most of the side chains/amino acids on the *exterior* of the protein fragment have in common?

Figure 3



Model E

i. What does this model show? (Hint: what doesn't it show?)

ii. (No written answer required) Try to pick out all the features you identified in the previous four models.

iii. In which model is it easier to see the α-helix and β-sheet structures, model A or model E?

Model F

i. What does this model show?

ii. How might this model be useful in a way that the previous models are not? (Hint: Think about how this biomolecular structure "looks" to another molecule.)

Station C - β-Globin

i. What kind of secondary structural type/motif studied in station A is found within the β-globin model? How many of them are there in this model?

ii. The larger of the colored areas in this model represents a "heme" group. Heme consists of a planar porphyrin ring around an iron cation. Identify the two amino acids in the protein backbone that bind to the iron to hold the heme group in place?

iii. This β-globin model represents a tertiary structure. Four of these bound together by weak, non-covalent forces form the oxygen-carrying biomolecule "hemoglobin" (a quaternary structure). The small colored area on the exterior of the of the model represents the side chain that, if replaced with a different amino acid, leads to sickle cell anemia. What is the name of the amino acid represented in this model?

iv. When the amino acid in the small colored area is replaced by valine, the modified (sickle-cell)hemoglobin molecules spontaneously clump together forming large protein aggregates that distort the shapes of red blood cells and cause them to plug up capillary beds. Does the substitution of valine into this position involve replacing a hydrophilic amino acid with a hydrophobic one, or *vice versa*?

Green Fluorescent Protein (GFP)

i. What kind of <u>secondary</u> structural motif does the *outside* of the model represent?

_____

ii. What kind of <u>tertiary</u> structure does the *entire* model represent? (Hint: You saw it in "proteins 2".)

iii. Three consecutive amino acids in GFP (Ser65, Tyr66 and Gly67) undergo a cyclization reaction and oxidation to form the "fluorophore" -- the chemical group that gives off a green fluorescence. Many other proteins contain a Ser-Tyr-Gly sequence, and yet they do not form a fluorophore. Why?

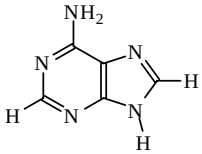
iv. All the strands in this structure are antiparallel except one pair. Try to find the parallel pair. No written answer required.

Station C –DNA

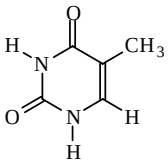
Large scale models of a section of a DNA strand are provided for you to examine as you consider some of the questions below.

Task 1

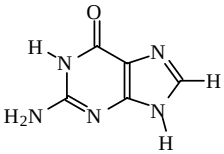
The nitrogenous bases diagramed below have been built for you. Label each as a purine or a pyrimidine.



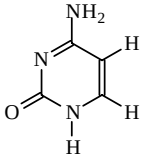
adenine



thymine



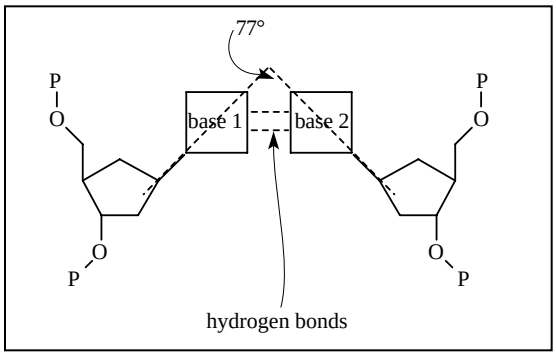
guanine



cytosine

i. On the molecular diagrams above, circle the hydrogen atom which is replaced when each of the molecules attaches to the deoxyribose sugar in a DNA strand.

Each of these molecules is planar. Lay the molecules next to one another to show how they would hydrogen bond in DNA. The angle between the bond of one base to deoxyribose and the bond of the other base to deoxyribose should be 77°.

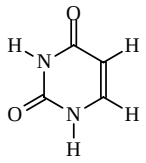


ii. In how many ways can the above bases hydrogen bond with each other?

iii. What pairing arrangement is found in the Watson-Crick structure of DNA; draw this, using the structures above, in the space below.

iv. Why do you suppose that only this pairing is found?

v. In RNA, uracil replaces thymine. What is the difference in structure between uracil and thymine?



uracil

vi. What kind of effect do you think this will have on the hydrogen-bonding properties of this base?

vii. RNA can be both double stranded (in some viruses) or, more usually, a single strand. These single strands can loop back on themselves, allowing Watson-Crick type base-pairing between bases from different parts of the same strand to stabilize secondary structure. Draw a hydrogen bonding structure diagram like the two in part (iii) that would fit this structure. Make uracil one of the bases in the base pair.

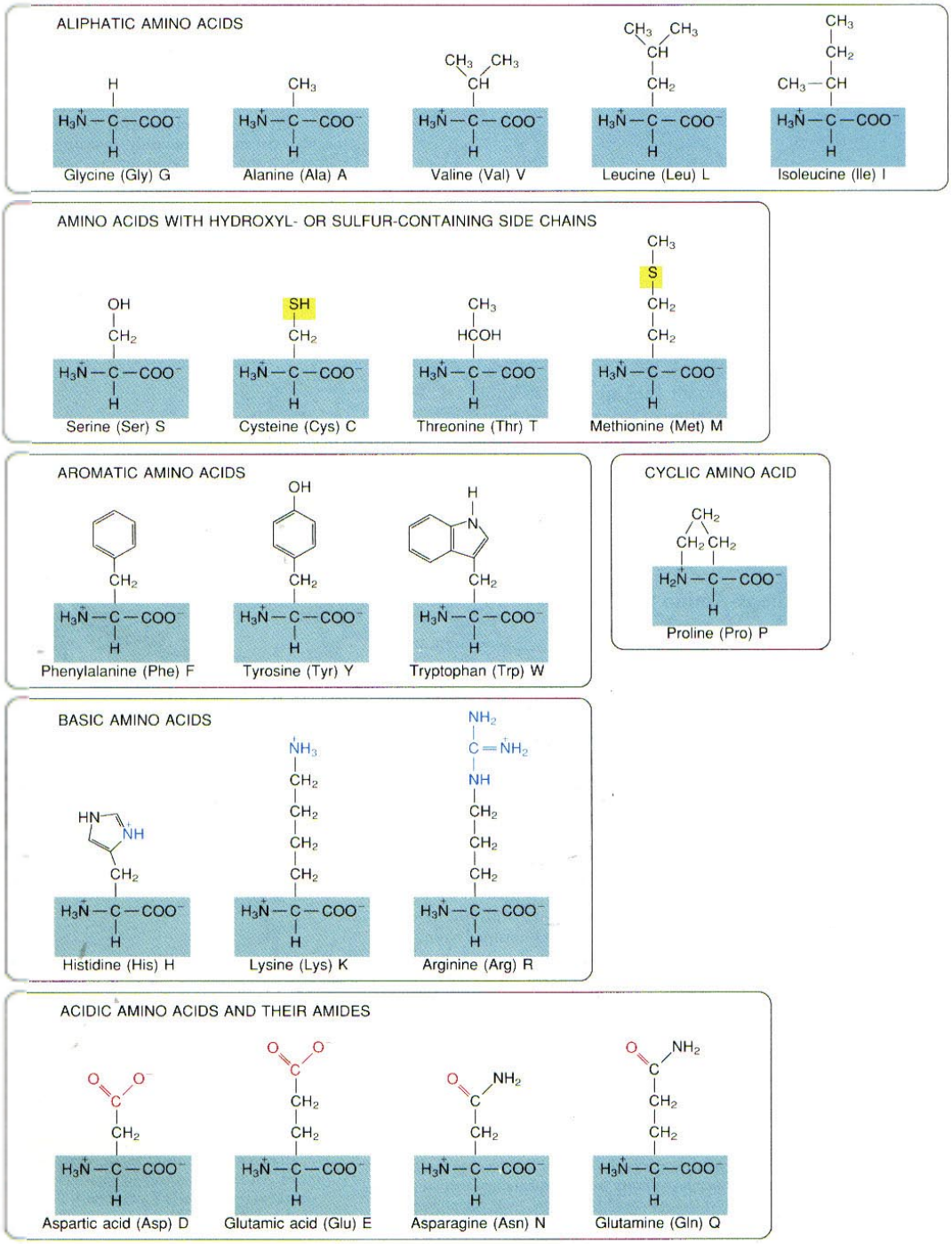


Table similar to Moore et. al. Table 12.8
Amino acids classified according to their general properties