Protein Molecule Modeling

Creating a Protein Model

Proteins are chains of amino acids (**aa**) that are covalently linked together through peptide bonds. In this model, you will generate a "ribbon" — the peptide bonded backbone of the protein chain. You will not visualize the amino acid residues that give the protein its particular structure and chemistry. However, you will get a feel for the different types of structural elements that make up a protein because of the amino acid residues.

- The **primary** structure is the amino acid sequence.
- Two main **secondary** structures are α-helix and β-sheet.
- **Tertiary** structure is how the random coils, helices, and sheets interact. Tertiary structure is often stabilized by the disulfide (S-S) bonds that form between cysteine amino acids. Notably, insulin has 3 disulfide bridges for only 51 **aa**.
- Some proteins have **quaternary** structures also, because they are made up of subunits, e.g., hemoglobin.

Based on analysis of the crystal structures of thousands of proteins, scientists have found more than 200 motifs or small patterns that they can now relate to the chemistry and structural characteristics of these short stretches of **aa** sequence. With this large amount of sequence/structure data, scientists are getting close to being able to PREDICT the structure of a protein from its **aa** sequence. This information is also enabling scientists to rationally design proteins with predictable activities. To do this, scientists will use a DNA synthesizer to prepare DNA segments of defined coding sequence and then clone this sequence into bacteria or eukaryote cells where the sequence will be expressed through the normal processes of gene expression, and a novel protein will be generated. Researchers then will assay the protein for the specific activity they seek.

This model does NOT resemble protein synthesis. In protein synthesis, individual amino acids (**aa**) connect to the growing (nascent) protein chain on the ribosome. We are "renaturing" or "re-folding" a "denatured" protein.

We can model the end-product (a folded protein) by simply coiling a fine wire around a pencil, pen or applicator stick. You will usually need only 1 length of wire per protein. Hemoglobin would have 4 wires, one representing each subunit.

Materials:	**Wire (10-30 cm)**	**Pencil, pen or applicator stick (hereafter called "stick")**

1. Coil the wire around the stick. You can make some coils and then leave some wire hanging, then coil some more to make α-helices. The coils can have different numbers of turns, BUT, should have the same diameter. Proteins themselves are NOT symmetrical in general.
2. Gently pull off the wire from the stick.
3. Stretch out the non-coiled areas and fold some of them back to form β-sheets.
4. Gently smush the whole thing in your cupped hands, as if you are forming a meatball or dumpling.
5. When you open your hands you will have a somewhat spherical entity, a model of the backbone of a globular protein.
    - If you press OR stretch the model gently, you will notice that it springs back. Many proteins have some flexibility in their active (native) state (conformation).
    - If you stretch more you'll model what happens at high temperature or chemical (e.g., soap) denaturation. The protein can no longer go back to its native conformation (you may not remember how you folded it initially) and it stays denatured and inactive**.**

Amazingly, thousands of substrates and their enzymes find each other all the time as we breathe, think, and laugh. Multiple identical copies of different proteins are made on ribosomes through the process of translation every second in our cells. Ask students to consider the role of mutations again after they have built their models. How could a single amino acid change influence a protein's overall structure or activity?

Sources for modeling wire: (Give out lengths that vary between 10 and 30 cm)

Craft store beading wire (shown).	Phone service cabling. Use a single-edge razor blade to expose the thin, colorful inner wires. Get this cabling from your local telephone office! Ask them to donate!	Rolls of twist-tie, available at "dollar" stores or hardware stores.

After you have built a model, visit the NIH web site **http://www.ncbi.nlm.nih.gov** and click on "**structures**" to go to information about the 3-D structures of hundreds of proteins and to download ITS free software, CN3D, which works better with IE and on PC's. Enter "insulin" or "1XGL." TRY QuickPDB, a NEW molecule viewer! You may need to download some other free software (Chime is available for FREE at http://www.umass.edu/molvis/martz/ which will have links to the protein structure pages. When you open the protein structure files in these software applications, choose "**ribbon**" or "**cartoon**" and then choose "**structure**." Ribbon will show the backbone more clearly and structure will show the α-helix as pink (or red) and the β-sheets as yellow. Find the Chime or CN3D structures of insulin and catalase and model their 3-D structures.

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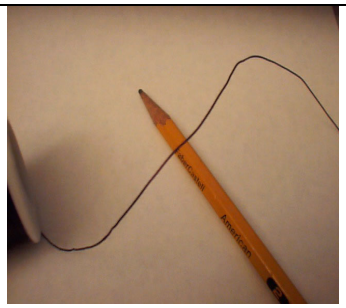
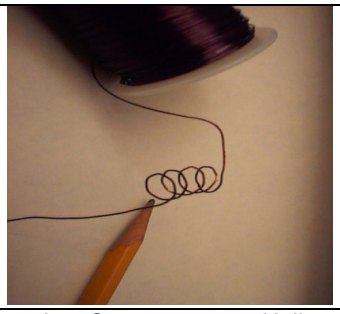
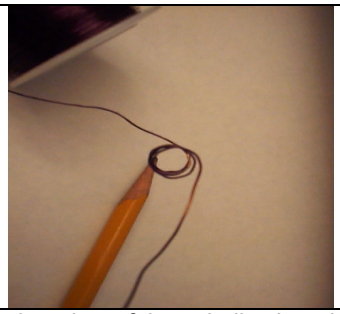
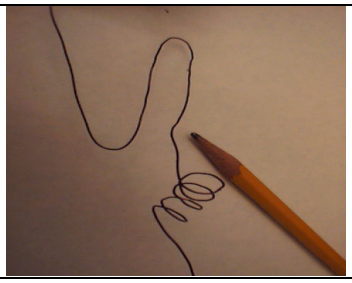
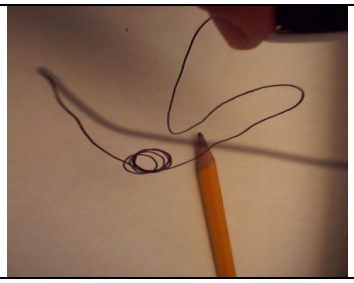
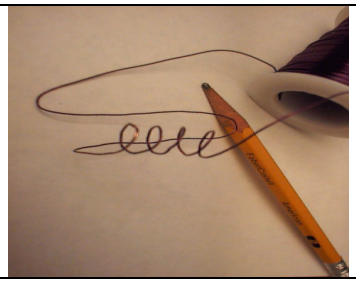
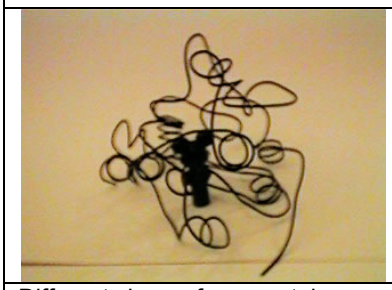
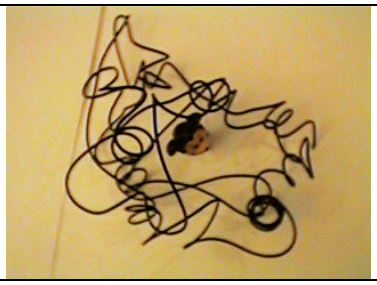
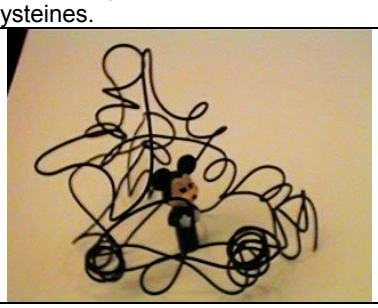
Making a Protein Backbone Model with Wire and a Pencil

		
Primary Sequence/Structure: The chain of amino acids. Random coil regions look like this.	Secondary Structures --- □-Helix: Just curl the wire around a pencil, a pen, or an applicator stick.	Another view of the □-helix, thought of as a "barrel." Helices may be long or short but have about the same diameter.
		
Secondary Structures: □-Sheet (left) is folded like a mountainous switchback path, and is relatively flat (planar).	Helices and sheets may be next to each other in the final structure, separated only by a small segment of random coil.	Tertiary Structures: □-helices are stabilized by H-bonds between the coils. Distant segments are often stabilized by disulfide bonds between cysteines.
		
Different views of one protein. The N and C terminal **aa**'s may be nearby or far away. Mickey substrate is a pencil top!	This substrate helps orient us. Most substrates are asymmetric. Substrates often fit into the clefts of protein folds.	Most active sites have **aa's** from different parts of the chain that become neighbors because of structural folds.

Molecular model-making is NOT a "Mickey Mouse" **exercise. Wire models help scientists to visualize protein structure in 3 dimensions just like they can help us to teach our students.** Jane Richardson, from Duke University, earned a MacArthur Genius Award for developing ways to visualize proteins. Look for MAGE/kinemage http://kinemage.biochem.duke.edu/

Many biotechnologies rely on the universality of the genetic code and the common functions of proteins from different organisms. An important example is the change from using the DNA polymerase from E.coli bacteria to polymerases isolated and then genetically cloned from organisms that can live at the high temperatures of geysers and deep oceanic vents. In realizing that "a polymerase is a polymerase is a polymerase." AND, polymerase from a geyser organism is still active at 95C. Biotechnologists realized a PCR machine could be built to automate the heating stages. Kary Mullis earned his Nobel Prize by establishing the concept of PCR. Companies are earning profits now by producing automated, programmable PCR machines and by providing proteins (DNA polymerases) from unusual organisms whose genes are now cloned and propagated in E.coli.

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Ways to Incorporate Models for Molecule Biology into VA SOL-based Lessons

Biochemistry, molecular biology and biotechnology methods and study subjects deal with cells, the basic units of life and health. As macromolecules, proteins provide study material about amino acids and the basic chemical elements of life (CHONPS) as well as interesting examples of metallochemistry regarding iron (hemoglobin), zinc (transcription factors), and sodium and potassium for nerve and other forms of signal transduction, and salt and water balance (e.g., cystic fibrosis).

Thousands of proteins are at work in us as I write this page and as you read the page! Here are some ideas for enhancing student learning through model building and analysis.

For biological scientists, molecular model-building has been an important discovery tool, along with deep thought about basic concepts of chemistry and biology, and extensive literature research. Linus Pauling, Watson and Crick, Max Perutz, Jane Richardson, and Gertrude Elion all built models of their biochemicals for study, interpretation and idea generation.

Now we have access right from our schools to the identical data that a professional researcher uses for "computational biology," the study of biological information using computers to digest and process the data. Students can thus have the opportunity to apply what they have learned through the hands-on model-building to gain a deeper and more extensive understanding of proteins, learning like a scientist through internet research!

Adopting a protein can be a theme for organizing many aspects of biology through your curriculum. Students can find out about evolutionary comparisons among diverse organisms, develop an appreciation of the universality of the genetic code, and begin to understand the relationships between what we ingest or expose ourselves to and what happens to our bodies through actions (or inactivation) of proteins.

Bio.3 Biochemical principles for life (macromolecules/enzymes)

Enzymes are biological catalysts that speed up chemical reactions by getting the interacting molecules together and in a higher energy state so the reaction can occur under the "gentle" conditions of life. No one knows WHY protein enzymes are so big, when only 3 to 5 of 150-400 amino acids actually DO the chemistry at the enzyme's active site. I like to think of the example of holding a baby. All the rest of our body works together so we can use our hands and arms to hug the child yet hold it securely. And, our hands are on far sides of our bodies if we stretch out, while in a hug, our arms are very close to each other.

1. Go through the protein building process as described.
2. Guide students through the heat/chemical denaturation steps. During a fever, many of our proteins become inactive. Since thousands of different proteins have to work together (even though indirectly), upsetting even a few proteins by heat or alcohol or drug ingestion can cause major global molecular effects that lead to disease.
3. Ask students to relate protein enzyme structure to tools that accomplish what humans cannot do unaided. They should come up with examples like cars, cranes, steamrollers, scissors, staplers, glue, sewing machines and ovens. The parts that actually DO the work may be located in a small part of the machine!

Scientists found about the structures of enzymes long after they accumulated a lot of evidence about the actions of enzymes. These days, biochemical studies intermix investigating enzyme activity WITH studies of protein structure.

Bio.4: Structure and function at the MOLECULAR level

See a wonderful animated description at **http://www.johnkyrk.com/aminoacid.html** (linked to Virginia's SOL website!).

- Introduce the amino acids and proteins: subunits (building blocks) and polymers, including the peptide bond. Explain that amino acid chemistry and structure influences protein chemistry and structure.
- Introduce the primary and secondary structures and have the students model these structures individually. Have students find out properties of helices and sheets by gently stretching and crushing the forms.
- Introduce tertiary and quaternary structures and have students build a "protein," then tweak that product.
- Download some protein examples such as insulin, hemoglobin, keratin, avidin, which have vastly different structures and clearly different a and b secondary structures.
- Students, in teams, can search other proteins.
- Ask students to relate their modeling to what might be happening to food when it is cooked.
- Ask students to consider the chemistry of the various amino acids in relation to pH, relating this to enzyme activation and inactivation as foods transit through our digestive system.

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Bio.6 Common mechanisms of inheritance and protein synthesis

Many diseases, including cancers, are now known to be due to single DNA base changes or deletions that result in altered protein sequences that frequently generate proteins that are folded incorrectly.

- Students read about the classic cases in their text, namely sickle cell anemia, HIV and cancer transforming factors
- Show students how to build the protein models
- Show students how to find protein structures they can rotate in XYZ planes (using the PDB viewer, RasMol, Chime or Kinemage)
- Student teams adopt a genetic disease with a known protein effect, then conduct a literature and structure search
- Each student on the team (or pairs) can build a normal and mutant protein model to discuss and compare
- Teams then present their models and describe through their models, orally, and in writing, the normal vs mutant structures for the adopted genetic disease
- Examples include: sickle cell anemia, cystic fibrosis, p21, HIV protease, insulin, ras, urease.

References

PLEASE NOTE: For the molecular modeling web sites, you may have to download software. Plan on **two hours** to download, install and troubleshoot. Work with your technology specialist. Rest assured that all the software is professional AND free. Some work better on PC's *vs.* Mac and *vice versa*; some work better with Netscape Browser *vs.* Internet Explorer. Read the installation notes carefully.

1. Eric Martz's web site with CHIME professional (free) software browser plug-in --- http://www.umass.edu/molvis/martz/
2. Protein databanks
3. NIH http://www.ncbi.nlm.nih.gov pick "**struc**teins http://chemistry.gsu.edu/glactone/PDB/pdb.html

Interesting Protein Stories

Dorothy Crowfoot Hodgkin http://nobelprizes.com/nobel/chemistry/dch.html

Prions http://www.hhmi.org/news/lindquist3.html

Green Fluorescent Protein http://pantheon.cis.yale.edu/~wfm5/gfp_gateway.html

Examining the Molecular Structure of Urease

Urease is a popular enzyme to study in school because it is easy to isolate from Jack Beans (1926).
http://www.sciencenews.org/sn_arch/11_16_96/timeline.htm
James Sumner earned a Nobel Prize in 1946 for this work.
http://www.nobel.se/chemistry/laureates/1946/press.html and
http://www.wmich.edu/acs/Puzzlers/jan00puzzler.htm

A simple pH assay with an indicator dye is also available.

Recently, urease was found to be an indicator enzyme for the bacteria that causes 30% of all ulcers, *Helicobacter pylori.* Urease is a large protein that complexes with a smaller protein (coded by a separate gene) that is needed for activity regulation. We will examine it in a variety of ways to show how to use the molecule visualizing software.
Urease has been flown on the Space Shuttle for the microgravity research project
http://pcg.tecmasters.com/flown_proteinsorg.html#U

Production of urease from Helicobacter in transgenic tobacco plants
http://www.ibmb.uni.wroc.pl/cmbl/c53_05.pdf

For orientation, visit an amino acid, and then visit insulin, a small protein of only 51 aa.
Urease has
http://www.siu.edu/departments/biochem/chime_rasmol/nickel_proteins/urease_structures.htm

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1. A great website for enzyme studies http://www.glue.umd.edu/~nsw/ench485/ench485.htm of Dr. Nam Sun Wang.**ture**"
2. Protein DataBank http://www.rcsb.org/pdb pick: "**get educated**"
4. Branden, C., and Tooze, J. 2nd ed. 1999. Introduction to Protein Structure. Garland Publishing, Inc. NY.
5. Atkins, P.W. 1996. Molecules. W.H. Freeman and Company. NY.
6. MolyMod space-filling atom kits http://www.indigo.com (also marketed as Prentice-Hall molecular model set for organic chemistry).
7. Toothpickase, a truly EXCELLENT model for enzyme action by Peggy Skinner. http://www.accessexcellence.org/AE/ATG/data/released/0166-PeggySkinner/description.html
8. http://www.biotech.vt.edu/~tmhorn and scroll to "proteins"
9. The Duke University Kinemage Home Page http://kinemage.biochem.duke.edu/

You may want to start your students off here, with structures of SMALL molecules, especially the amino acid building blocks of pro

Notes for looking at a protein.
The DEFAULT setting is wireframe and CPK. CPK has the color coding for atoms as gray=carbon, white=hydrogen, red=oxygen and blue=nitrogen

For a small molecule like an amino acid, the wireframe format is fine
http://chemistry.gsu.edu/glactone/PDB/Amino_Acids/aa.html

Toby's first choice is

1. Set **display to** "**ribbons** or **cartoons**" and then set **colors to** "**structure**"
This will show the backbone and point out areas of alpha helix (pink)and beta sheet (yellow).

> In insulin, which is shown as a dimer, you can easily make out the helices, and there are only 2 in one of the subunits.
>
> In urease it's harder, since the protein is so large with 767 amino acids and 3 separate chains, contributed by 3 different genes.

2. To see the 3 chains, set **color** to **"chains"** and the 3 are much easier to make out.

3. Next, since the protein is really made up from linked amino acids, set **color** to **"amino acid"**. Each of the 20 aa has a separate color.

4. Next set **display** to "**spacefill"** and each aa will show all its atoms as one color. If you knew the color code it would be easier to see which are positively charged+, negatively charged - , etc. Find the look-up table using the help link. I find that it's easier by using the CPK coloring, so you can see the Os and H and S and N more easily, but then, I am OLD and have been making model molecules since 1962.

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