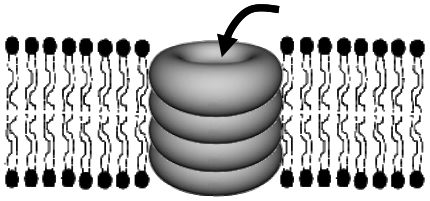
Molecular Biology Through Discovery

Problem Set 2: Protein + observations/assertions

Submit reasoning and process, not merely answers!



2.1. Some antibiotics form rings that stack and create a pore through the membrane. Consider a cyclic polypeptide antibiotic in which each of four rings (shown as doughnuts) is composed of one instance of each of the four amino acids: serine, glycine, threonine, and alanine. If the atoms of the backbone are approximated by touching spheres of about 0.2 nanometers in diameter,* estimate the circumference of the pore (presume it to be a circle) and the diameter of the largest molecule that could fit through it. Approximate the circumference (π·diameter) to be 3·diameter. (Show work: First read, then draw a picture of the atoms of a ring, then think, then draw some more, then calculate, then think some more)

2.2. Before you cook an egg, the egg "white" is not at all white: it's clear. After you cook the egg, the "white" is white, because the large amount of globular protein has denatured (i.e., unfolded), and as a consequence, the protein has precipitated. Why should unfolding globular protein that are normally soluble in water cause them to stick to each other (which is what "precipitate" means)? Create and describe a picture that illustrates your reasoning.

2.3. Lactate dehydrogenase (the last enzyme in human anaerobic glycolysis) is a soluble, multimeric protein. If you were to try to fold a single linear polypeptide chain of lactate dehydrogenase, you would find it impossible to do so without leaving a large number of hydrophobic amino acids exposed to water. Create and describe a picture that illustrates why this should be.

2.4. Use only the results of Sanger and Tuppy (1951) [Biochem J 49:463-481] to deduce as much of the structure of insulin you can. Do this as a group effort (as described below), something like a geometric proof, appealing to observations – specific lines within the tables (axioms) -- and assertions you derive from them (theorems). A justification may consist of one observation or two prior assertions (assertions from Table 14 don't count towards this total). Make sure that your reasoning is transparent. For example:

	Assertion	Justification
A.	Thr-Pro*	Table 6, Line 8
B.	Thr-(Ala,Lys,Pro)¶	Table 9, Line 6
C.	Only one Pro	Table 14
D.	Thr-Pro-(Ala,Lys)	Assertions **A**+**B**+**C**†

*Meaning "The dipeptide N-Thr-Pro-C lies somewhere in the insulin polypeptide chain". The form N-Xxx-Yyy-C means that the amino acids are read from amino end to carboxyl end.

¶ Meaning "A tetrapeptide somewhere in insulin begins N-Thr and is immediately followed by Ala, Lys, and Pro in some unknown order"

† Meaning "The assertion on this line follows from the assertions on lines A, B, and C"

It would take a fair bit of time to reconstruct the structure of insulin by yourself. Big scientific problems, however, are generally solved not by individuals but by communities. Even though this question isn't too big of a scientific problem, we can still approach it in that spirit.

Accordingly, I've divided you into working groups, giving each person an area of expertise. Each person is associated with an amino acid. That person should consider all experiments from Sanger &

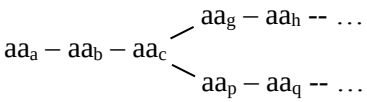
* How big are nanometers? Try visiting Scale of the Universe and/or Proton to Protein.

Tuppy (1951) that bear on the specific amino acid, deducing all that's possible from the results, in the form shown above. When you are satisfied by a non-trivial deduction (the last line of your chain of reasoning), publish it, by posting it to the community bulletin board.

You'll be able to reach increasingly sophisticated deductions (perhaps even the entire sequence of the insulin B-chain!) as you combine your results with those of others in your group. You might also make some use of results from other groups, but beware! There might be all sorts of garbage posted to the community bulletin board – not intentionally, of course, but people do make mistakes. If you accept the results of others uncritically, you may accumulate mistakes and reach erroneous conclusions yourself.

How can you avoid contaminating your growing chain of deductions?

2.5. Suppose that Sanger and Tuppy tried used their methods to deduce the structure of a protein that was not a linear array of amino acid but rather had branch points:



Make up specific experimental results they might have obtained that would have allowed them to detect this structure.

2.6 Choose what you believe to be the two most contradictory observations in the research articles on which the four newspaper articles on Vitamin D are based. If you believe the two observations to be truly irreconcilable, then describe the contradiction. If you believe that they can be reconciled, then describe how. You can save yourself the task of reading all four articles by consulting with colleagues who have read articles different from the one you read. To that end, here's a list of who was assigned which newspaper article.

Extra – in case you have the time and inclination

2.7. Make a set of 20 different graphical symbols representing the 20 amino acids. You may use colors, shapes, fill style, etc, but no letters or numbers. The symbols should be organized so that they are ***easy to write*** and ***easy to remember*** and that if two amino acids share some important characteristics then their symbols also are similar in some respect (but not identical).