Download and open the file $\mathbf{4 m} \mathbf{4 j}$ _SS_CuHis using PyMOL. This is an insulin unit, which is a hormone which helps to regulate the amount of glucose in the blood stream. In the right-hand pane on PyMol, you will see a series of structures which you can select to show them on the screen. By working through them, and selecting each in turn, answer the following questions.

1. How many different polypeptide chains make up the quaternary structure of this insulin protein?
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2. Which secondary structures are visible?
$\alpha$-helices, with two $\beta$-strands forming an interchain $\beta$-sheet.
3. Which type of interactions maintain these secondary structures?

Hydrogen bonds.
4. The overall 3D shape which includes the secondary structures is called the tertiary structure.

Which type of bonding gives this protein its tertiary structure?
Hydrogen bonding.
5. Which types of bonds give the protein its overall quaternary structure?

Disulfide bridges between the cysteine residues of each chain, and some hydrogen bonds too.
6. The insulin protein has two $\mathrm{Cu}^{2+}$ copper ions attached to it. How are they attached? Through interactions with the histidine residues on the $\alpha$-helices.
7. What effect would changing one amino acid in the primary structure have on the overall shape and function of the protein?
This depends on which amino acid is changed, and what it is changed into. Changing one amino acid could affect and disrupt the secondary structures which form, and it could also affect the tertiary structure - the overall 3D shape of the polypeptide chains. This may be a positive, or a negative change, depending on the specific amino acids involved. The important point to remember is that changing one small amino acid, which is in effect just changing one of the side chains of an amino acid residue, can affect the overall structure of the protein. The overall structure of a protein, in this case not only the tertiary structure of each polypeptide chain, but also the quaternary structure of the whole protein, is directly related to its function. Therefore changing a few atoms in an amino acid residue could have great consequences to the function and effectiveness of a protein.

