

Biophysics 204

Graphics problem set - nucleic acid-protein interactions

DNA-binding proteins often recognize specific binding sites by making sets of hydrogen bonds and van der Waals contacts to functional groups in the DNA major groove. Zinc finger proteins are especially useful for examining sequence-specific interactions because they illustrate how the same basic structural unit can be used to bind DNA in different ways. In this exercise, we will examine the crystal structures of two zinc finger-DNA complexes, Zif268 and GLI.

Throughout the exercise, you should keep a few general principles in mind: 1) many proteins use α -helices, sometimes called "recognition helices" to make contacts in the DNA major groove, 2) the orientation of the recognition helix is determined largely by the docking of the surrounding protein scaffold, 3) the same type of amino acid can be used to recognize bases in different ways, and 4) the structure of the DNA (the helix type, the presence of irregularities, or the location of water molecules) determines the relative positioning of functional groups on the bases and thus determines the nature of the binding surface.

There are 2 key papers that should be used as guides:

(1) Pavletich, N. P. and Pabo, C. O. (1991) Zinc finger-DNA recognition: Crystal structure of a Zif268-DNA complex at 2.1Å, *Science* **252**, 809-817.

(2) Pavletich, N. P. and Pabo, C. O. (1993) Crystal structure of a five-finger GLI-DNA complex: New perspectives on zinc fingers, *Science* **261**, 1701-1707.

Two Pymol sessions have been set up to display the Zif and GLI complexes.

The Pymol session displays the 3-finger Zif268 protein as a yellow ribbon. The structure of each finger consists of an α -helix, a sharp turn, an antiparallel β -sheet, and a zinc ion (shown in CPK). Note how the 3 fingers wrap around the DNA major groove. Side chains that coordinate the zinc ion and side chains that make contacts to specific bases and to the DNA backbone are shown in finger 2. **[Q1]** Which side chains coordinate the zinc ion and what secondary structures are they located on?

Figure 5 from reference (1) provides a helpful map of sequence-specific DNA contacts. Identify the arginine-guanine H-bonds in finger 2. **[Q2]** Which groups on the base are contacted and what are the lengths of the H-bonds? This arginine-guanine contact is a very common interaction in protein-DNA complexes, but this one has a twist. **[Q3]** What determines the orientation of the arginine guanidinium group against the base? **[Q4]** Identify the second base-specific contact (amino acid and base) in finger 2. **[Q5]** Could other side chains be used at this position?

Backbone contacts help orient the fingers against the DNA. Follow the contact from the zinc ion in finger 2. This is an interesting interaction because the metal ion polarizes the histidine and enhances H-bonding to the phosphate oxygen. **[Q6]** Identify the two other backbone contacts made by finger 2 (amino acid and base).

GLI complex

(Note that the amino acid numbering is 100 greater than the actual numbering described in the paper.)

Notice the different arrangement of the 5 fingers compared to the Zif complex. [Q7] Describe two features of the GLI complex (such as orientation of fingers relative to DNA, spacing of fingers, structure of fingers, etc.) that are obviously similar to the Zif complex and two that are different. [Q8] What is finger 1 doing? The DNA to which fingers 2 and 3 are bound probably does not represent a specific binding site. [Q9] Is there anything about the way these fingers bind (such as orientation or number or types of amino acid contacts) that might suggest how fingers bind to nonspecific DNA?

Specific amino acid-base contacts are shown in finger 4 (green bases) and finger 5 (orange bases). Figure 5 from reference (2) provides an especially helpful map. Identify the arginine-guanine H-bonds in finger 5. [Q10] How does this H-bonding configuration compare to the one in Zif finger 2? [Q11] What is unusual about the Asp144 interaction and how might the DNA structure influence this contact? Examine the base-specific contact in finger 4 shown in CPK. This is an example of a hydrophobic contact to a thymine methyl group. [Q12] Do you expect to see this interaction in RNA-protein complexes?

A couple of basic questions to consider: Do you think that general rules (i.e. a recognition code) exist for zinc finger-DNA recognition? What about a recognition code for other classes of complexes, or for DNA-protein interactions in general?