



Graphics Worksheet # 6 (Beese)

Protein-DNA Interactions

Kinemage file: 258protDNAinterWrksht.kin (Download from Website)

These questions are designed to approximate writing the first draft of a manuscript describing the 3-dimensional structure of Protein X, based on the structural information shown in the three kinemages in file 258protDNAinterWrksht.kin.

Background/ Introduction

Protein X controls the operon for the synthesis of ligand Y in *Bacillus loco*. In the absence of ligand Y, the protein is inactive, the operon is switched on and Y is produced. As the concentration of Y increases, it binds to protein X and converts it to an active form so that it can bind to the operator region and switch off the gene.

The structure of protein X was previously determined by X-ray crystallography in two different crystal forms, and you have now solved the structure of the DNA complex to 1.8 Å resolution. That complex, or “Structure 1” (Kinemage 1), has both the DNA operon and ligand Y bound to Protein X. The operon is represented by a canonical 18 base-pair two fold symmetrical duplex DNA. Protein X functions as a dimer. Structure 2 is unliganded Protein X (“apo”); without the bound ligand Protein X is inactive. Structure 3 has ligand Y bound to Protein X in the absence of DNA. The superposition of all three Protein X structures is shown in Kinemage 2. Kinemage 3 shows one subunit of Protein X, with sidechains grouped and color-coded.

Quality of the refined crystal structure

1) List all the information about the refined crystal structure of the Protein X / DNA complex that should be included in the paper (possibly in a tabular form) to enable the reviewer and reader to assess the quality and validity of the structure. Define your terms. Justify why the information is necessary. Take into account that the reviewers and readers of the target journal are very skeptical people.

2) In addition to the above list, suggest two figures to support the validity of the structure and justify why each should be included.

Description of the protein structure

3) Using the 3-D information in Kinemage 1, describe the structure of Protein X, including the following aspects:

- Number of chains, number of amino acids (are any disordered?);
- type, number, & location of secondary-structure elements.

What is ligand Y?

How is ligand Y bound? (That is, describe the major protein-ligand interactions, both polar and hydrophobic.

You may want to draw the H-bonds into the kinemage, remembering that the N...O distance should be <3.5Å.)

Protein-DNA binding interactions

5) Base your answers for this part on the 3-D information for Structure 1, in Kinemage 1. (Turn side chains on or off as needed.)

What common structural motif is present in Protein X?

What residues (numbers) form the motif?

What residues (numbers) form the DNA recognition helix?

6) What structural features contribute to sequence-specific DNA binding of Protein X? Illustrate each point with specific examples (using residue numbers, if appropriate) from Kinemage 1.

Consider the role of water. In developing your answer, consider some of the other members of this family of DNA-binding proteins that were discussed in class.

7) List five protein-DNA interactions observed in the Protein X complex with DNA (Kinemage 1) that are independent of the specific sequence of the operon.

Conformational changes

8) Use the dynamic 3-D information in Kinemage 2 to describe Protein X's conformational changes for apo vs liganded vs DNA-bound forms, including the following aspects:

- Describe the relative movements; how large is the motion? (measure some differences); is there a definite hinge point?
- how much of the change results from ligand binding and how much from DNA binding?
- can you propose a reason why the ligand might cause this type of movement?
- could the apo form bind to DNA? why, or why not?
- how much does the DNA conformation change on binding Protein X? (assuming it started as B form)

Regulation of DNA binding; future experiments

9) Building on your answer to question 8, hypothesize how ligand Y regulates the binding of Protein X to DNA.

10) Outline a set of experiments (genetic, biochemical, and/or structural) to test your hypothesis for the structural basis of sequence-specific binding of Protein X to its DNA operon.

11) Outline a set of experiments to test your hypothesis for the regulation by ligand Y of the DNA binding of protein X.