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Advanced Biochemistry 8010 Dr. Kelley Moremen
Homework problem set Fall 2008

This problem set will be due October 28, 2008
Print this PDF file and answer the questions in the space provided.

1. In the structure described in the coordinate file 1e0o (that is: number "1", letter "e", number "0", letter "o") contains multiple chains and an extended sugar polymer chain in the coordinate file. Answer the following questions about the structure:
   What is the name of the two proteins in the coordinate file and how many chains are shown for each protein? Protein 1 _____, # of chains _____.
   Protein 2: _____, # of chains _____.
   How many sugar residues are shown in the coordinate file _____.
   How many different kinds of sugar residues are there in the coordinate file _____.
   What is the species source of the two proteins? _____.
   What other hetero atoms are present in the coordinate file?_____.
   What was the genus and species for the expression host for the two proteins? _____.
   What was the resolution of the protein structure? _____.
   Which protein chains interact with the sugar polymer _____.
   The protein chain designated chain C interacts with the sugar polymer. **The following questions pertain *ONLY* to the interactions between chain C and the sugar polymer**.
   Which atoms in protein chain C are within 3.5Å of the sugar polymer? List the protein atom and the corresponding sugar atom that it is closest to. Also list the potential mode of interaction between the two atoms (i.e. ionic, H-bond, hydrophobic, or no real interaction) An example of one amino acid is shown in the table. *Remember the correct command syntax for Rasmol or Pymol: Rasmol: select within (3.5, *:e) and not *:e and not water Pymol: select protein, c//; select sugar, e//; select interact, (protein w. 3.5 of sugar)*

Amino acids in Chain C		Sugar polymer atoms		
Amino acid number	Atom designation	Residue no.	Atom designation	Interaction (H-bond, ionic, hydrophobic, or no real interaction)
Lys112	nz	5	O2S	ionic

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2. Look at the structure of the protein in the coordinate file 1H4G. The protein is associated with a substrate analog in the coordinate file. Answer the following questions about this structure.
   If you look at chain A in the coordinate file, describe the structure of the protein fold.

   What is the structure of the substrate analog?

   How is the substrate analog associated with the protein?

   What is unusual about the structure and conformation of the substrate analog?

   What is the reaction catalyzed by the enzyme?

   Describe the mechanism of bond cleavage by 1H4G being certain to discuss the proposed structure of the transition state of the substrate during the catalytic cycle.

   Create a display of the substrate analog bound to chain A of 1H4G in Pymol or Rasmol using the following characteristics:
   a) display only chain A and its bound substrate analog
   b) color the background white
   c) display the substrate analog in stick display
   d) color the substrate analog with CPK colors
   e) display the protein as a cartoon display
   f) color helices as magenta and beta strands as yellow
   g) Save one image of the protein zoomed-out so that the image shows the entire protein structure.
   h) Save a second image that is zoomed-in and only show the side chains of chain A for amino acids that are within 4Å of the substrate analog (show the full amino acid structure if at least one atom in the amino acid is within 4Å of the substrate analog).
   i) Import the two images saved from Pymol or Rasmol into Powerpoint as two side-by side images.

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j) Label the protein and substrate analog in each image. In the zoomed-in image, label each of the amino acids in the image and note which amino acid is the nucleophile in the reaction mechanism and which amino acid is the general acid in the reaction mechanism.
k) Save the Powerpoint file as a PNG-fomatted graphics file and import it into an MS Word document.
l) Create a legend in MS Word that effectively describes the graphic in each panel being certain to include (1) the PDB ID code, (2) programs used in the creation of the graphic, (3) the citation for the original source of the structure, and (4) a description of all aspects of the molecule that are being displayed in the figure.
m) Print out the graphic from the Word document and include it with this homework problem set.

3. The protein in the coordinate file 2RH1 is the structure of an unusual fusion protein expression product. Answer the following questions about the structure.
What is the identity of the protein that comprises residues 29-341 in the coordinate file _____.
What is the identity of the protein that comprises residues 1001-1161 in the coordinate file _____.
What is the species source of the protein sequence of residues 29-341_____.
What is the species source of the protein sequence of residues 1001-1161 _____.
The protein structure that comprises residues 1001-1161 is actually inserted into the middle of the domain that comprises residues 29-341. Where in the 29-341 sequence is the residue 1001-1161 insertion found? _____.
Explain why the authors chose to crystallize the sequence comprised of residues 29-341 as a fusion protein with residues 1001-1161 inserted into the middle of the protein rather than just residues 29-341 alone.

There are 8 different non-protein “hetero” molecules in the coordinate file. List each, draw their corresponding structures, and explain, if possible, why they are found in the protein structure.
Molecule 1: Structure:
Can you explain why it is there?

Molecule 2: Structure:
Can you explain why it is there?

Molecule 3: Structure:
Can you explain why it is there?

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Molecule 4: Structure:
Can you explain why it is there?

Molecule 5: Structure:
Can you explain why it is there?

Molecule 6: Structure:
Can you explain why it is there?

Molecule 7: Structure:
Can you explain why it is there?

Molecule 8: Structure:
Can you explain why it is there?

4. The structure in the coordinate file 2c58 is a co-complex of an enzyme with an uncleavable substrate analog. Answer the following questions about the structure.
What is the reaction catalyzed by the enzyme?

Draw the structure of the substrate analog in the active site:
Why is the enzyme unable to cleave the substrate?

What are the critical three residues that are a part of the catalytic site for this enzyme?

A review describing the mechanism of the enzyme can be found on a link from the class website (Siolman and Sussman (2008) Chemico-Biological Interactions 175: 3-10). How do the three residues described above accomplish bond cleavage by the enzyme? Draw the proposed catalytic mechanism.

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Figure 1 in the review shows the protein structure of 2c58 in a ribbon form with a ball-and-stick representation of the substrate analog. Create a display of 2c58 in Rasmol or Pymol that is identical to Fig. 1 in the review except also show the three key catalytic residues in a stick form. Save the graphic and load the saved graphic into a Powerpoint file. Label the three catalytic residues as well as the substrate analog in Powerpoint. Export the graphic from Powerpoint as a PNG file and import the graphic into a MS Word document. Create a legend for the figure in the MS Word document that effectively describes the protein structure image being certain to include (1) the PDB ID code, (2) programs used in the creation of the graphic, (3) the citation for the original source of the structure, and (4) a description of all aspects of the molecule that is being displayed in the figure. Print out the graphic from the Word document and include it with this homework problem set.

5. A similar structure to the one above can be found in the coordinate file 1eve. The main difference is that an inhibitor is bound in the active site (a PDF of the primary paper can be found on a link from the class website).
What is the inhibitor? _____.

Draw the structure of the inhibitor:

Why is the inhibitor being used as a therapeutic drug?

Does the inhibitor interact directly with the catalytic residues that are displayed in the answer to question 4?

How does the inhibitor block enzyme activity?

Create a display of the enzyme-inhibitor complex that looks similar to the figure generated in question 4 except show the inhibitor bound to the active site as a ball-and-stick display. Export the graphic from Rasmol or Pymol, label it in Powerpoint, and import the labeled graphic into a MS Word document as in question 4. Write a legend for the figure similar to the legend for the figure in question 4 and print out the graphic from the MS Word document and include it with this homework problem set.