The purpose of this exercise is:

1). to examine the case of a tandem head-to-tail homodimer binding to DNA
2). to view a Zn finger motif
3). to consider the case of a beta sheet binding to the major groove of DNA
4). to consider both direct protein-DNA H-bonding patterns and also water-mediated H-bonds
5). to examine the electrostatic interactions between positive histone proteins and negative DNA in the nucleosome

LEUCINE ZIPPERS

File - Open - "01 zipper positions.rsm" in folder "Exercise 08 DNA binding proteins - II"
Load and run the script “Protein-DNA-II.01.doc”

We considered leucine zippers in Modeling Exercise 3 (Protein folding). These are an important class of DNA-binding proteins. For review we will display one model and consider one question.

Q1. The zipped leucines (red) are spaced ______ residues apart, which approximately equals ____ turns of alpha helix.

<table>
<thead>
<tr>
<th># of residues</th>
<th># of turns</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 4</td>
<td>1</td>
</tr>
<tr>
<td>B. 7</td>
<td>2</td>
</tr>
<tr>
<td>C. 10</td>
<td>3</td>
</tr>
</tbody>
</table>

TANDEM HEAD-to-TAIL DIMERS

File - Open - "02 vdr tandem subunits.rsm". Do not rotate until after a first read thru this section.
Load and run the script “Protein-DNA-II.02.doc”

There are numerous examples of head-to-head dimers such as lambda repressor which bind to palindromic DNA sequences. A tandem head-to-tail arrangement of subunits is less common but also is possible. One example is the vitamin-D receptor (vdr) illustrated here. Segments of each monomer are colored according to their position in the polymer sequence, using the visible spectrum (blue to red).

Q2. The protein secondary structural element binding in the major groove of the DNA is a ____ .

A. blue alpha helix  C. red alpha helix
B. blue beta sheet  D. red beta sheet
Q3. The grey spheres bound to the whole dimer are ____.

A. two Fe (one per subunit)
B. two Zn (one per subunit)
C. four Fe (two per subunit)
D. four Zn (two per subunit)

Note how the yellow end of the monomer at the left is near the green end of the monomer at the right, in the head-to-tail arrangement. Both red helices are in front and run identically (N to C) from lower left to upper right.

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**BETA SHEET IN THE MAJOR GROOVE**

*File - Open - "03 met repressor homodimer.rsm"*

Do not reorient until the following introduction is read.

*Load and run the script “Protein-DNA-II.03.A.doc”*

Although we have seen several cases of helices bound in the major groove, such as lambda repressor and vitamin-D receptor, there is at least one case of a protein that binds to DNA via a small beta sheet lying in the major groove. This example is the methionine (met) repressor. The DNA strands are colored white and grey. The protein is a homo-dimer. Each protein chain is colored sequentially by the visible spectrum from blue to red. The small violet wireframe structures at the top of the model are S-adenosyl methionine molecules, which bind to the protein dimer and in turn affect protein dimer binding to DNA. Look at the blue amino-terminal ends of the protein subunits to get a sense of head-to-head or head-to-tail orientation. One N-terminal end is at the left rear, the other is at the right front.

Rotate the molecule to get a top view of the violet S-adenosyl methionine molecules. Also, rotate the molecule to get an end-on view of the major groove, with the strands of the sheet in the groove oriented perpendicular to the computer screen (namely running in and out of the "page").

Q4. The protein dimer is arranged ____.

A. head-to-head  B. head-to-tail

Q5. The entire dimer has ____ total S-adenosyl methionine molecules bound to it.

A. 2  B. 4

Q6. The number of strands in the beta sheet in the major groove is ____.

A. two, namely one from each monomer  B. four, namely two from each monomer
Q7. The beta sheet is ____ .

A. parallel  B. antiparallel

*Continue using the same .rsm structure.*
*Load and run the script Protein-DNA-II.03.B.doc”*

Hint: Consider the blue segment attached at the end of each strand in the beta sheet.

The beta sheet considered above now is represented by two yellow ribbons. Protein sidechains are represented by single spheres. In the opening view, sidechains extending below the sheet are colored blue and those extending above the sheet are colored green. The red spheres are water molecules. As we learned previously in this course, there is an alternating up-down every-other-residue periodicity to the orientations of the sidechains in the sheet.

Q8. All of the green sidechains are _______ residues.

A. odd-numbered nonpolar  
B. odd-numbered polar  
C. even-numbered nonpolar  
D. even-numbered polar

Q9. All of the blue sidechains are _______ residues.

A. odd-numbered nonpolar  
B. odd-numbered polar  
C. even-numbered nonpolar  
D. even-numbered polar

View the sheet end-on. Also view it from the top (the green side).

*Continue using the same .rsm structure*  
*Load and run the script “Protein-DNA-II.03.C.doc”*

The protein is colored orange, except for the green and blue spheres representing sidechains of the beta sheet bound to DNA. The DNA is colored white.

Q10. The nonpolar (green) sidechains in the beta sheet face ____ .

A. the DNA  
B. the rest of the protein

The blue polar sidechains in the beta sheet face the DNA, and would be completely exposed to solvent if the protein was not bound to DNA. Although we often consider cases where H-bond donor and acceptor atoms of protein sidechains directly form H-bonds with acceptor and donor atoms of DNA groups, this is not always the case. Sometimes water molecules lie between the protein and the DNA, mediating the interactions between them. For example you could have a DNA O: acceptor atom indirectly interact with a protein O: acceptor atom in the following way.

Normally you would not expect a DNA-to-protein acceptor-to-acceptor interaction because you would be thinking of a single direct acceptor-donor pairing. Here in this hypothetical example, the water molecule provides two donors (the H's) that indirectly bridge the DNA and protein acceptor groups together.

Q11. In this model, the buried waters lying in the major groove between the protein and DNA are colored ____.
   
   A. grey  B. violet  C. red

Before leaving this model, rotate it to get a side view with the beta sheet strands running left-right or vice versa.

**ZINC FINGERS**

File - Open - "06 three Zn finger domains.rsm"
Load and run the script “Protein-DNA-II.06.doc”

In this view, the DNA strands are colored white and grey. Three green Zn atoms are present and are bound to a single protein chain. The protein is colored violet, except for non-carbon sidechain atoms within 4.0 Angstroms of the Zn. Those sidechain atoms are colored CPK. Use your cursor to click on the yellow and blue sidechain atoms. They come from two types of sidechains which frequently bind Zn atoms in proteins.

Q12. Considering the yellow sulfur atoms, each Zn is bound to two ____.
   
   A. met  B. cys

Q13. Considering the blue nitrogen atoms, each Zn is bound to two ____.
   
   A. lys  B. arg  C. his  D. asn  E. gln

Q14. The four atoms bound to the Zn form a ____.
   
   A. square  B. tetrahedron

Each of the Zn-binding domains is called a zinc finger. The function of the Zn is to help the protein fold. Does the Zn also bind to the DNA?

Q15. The Zn of zinc fingers ____ directly bind to the DNA.
   
   A. does  B. does not

Type in the following script by hand:

```
select protein
ribbon on
color structure
```
Helices are red, sheets are yellow, and other portions are white

Q16. This protein ____ appear to be a case of internal homology.

   A. does       B. does not

Each zinger finger domain in this protein contains a Zn atom, an alpha helix, and a two-strand beta sheet.

Q17. The portion of the protein domain which binds to the DNA is the ____.

   A. alpha helix       B. beta sheet       C. metal atom

DIRECT PROTEIN-DNA H-BONDING INTERACTIONS

The figure below depicts two of the specific protein-DNA contacts in a particular protein. Panel (a) shows the two donor hydrogens at the end of one arginine pairing up with the N:\ and O:\ acceptor groups of one guanine ring. In this protein, the positive arginine also is stabilized by the presence of an adjacent negative aspartate sidechain. Panel (b) shows a donor NH and an acceptor C=O:\ of one asparagine sidechain pairing up with an N:\ acceptor and NH donor of one adenine ring. These are especially nice examples of protein-DNA interactions, since each of them includes two direct protein-DNA hydrogen bonds at one site.

(a) ![Image of protein-DNA interaction](image1.png)

(b) ![Image of protein-DNA interaction](image2.png)

File - Open - "07 direct H-bond interactions.rsm" Load and run the script “Protein-DNA-II.07.A.doc”
This model uses spacefilled atoms to highlight some of the interactions between a particular transcription regulation protein and the segment of DNA to which it binds.

\[
\begin{align*}
\text{protein} & : \quad \text{asn155} \quad \text{arg152}(+) \quad \text{asn125} \\
\text{DNA} & : \quad \text{A7} \ldots \text{G8} \ldots \text{G9} \ldots \text{A10}
\end{align*}
\]

The DNA chains are colored yellow and orange. The AGGA nucleic acid segment noted above is from the yellow strand. The protein is colored green, except for the sidechains noted above which are colored CPK.

**Q18.** The protein-DNA interactions occur ____.

A. along the phosphate-sugar ridge  
B. in the major groove  
C. in the minor groove

*Load and run the script “Protein-DNA-II.07.B.doc”*

Nucleotides 7, 8, and 10 on the yellow strand are spacefilled and colored yellow. Nucleotide 9 is not spacefilled. You should have a structure showing the three protein/DNA interactions given at the top of this page at A7, G8, and A10. Hydrogens are not included in the computer model. You will have an edge-on view of the nucleotides.

**Q19.** In each interaction, the plane of the amino acid sidechain ____.

A. lies in the same plane as the nucleotide sidechain  
B. is perpendicular to the nucleotide sidechain

This is similar to the drawings on the previous page.

*Load and run the script “Protein-DNA-II.07.C.doc”*

This is a close-up view of the G-arg-asp unit illustrated in panel “a” of the figure on the previous page.

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**IONIC INTERACTIONS BETWEEN HISTONES AND DNA IN THE NUCLEOSOME**

In eukaryotes, the highly negative DNA polymer is packaged into a more stable form by wrapping the DNA around an octamer of histone protein subunits. The histone molecules have a high % of positively charged lysine and arginine residues. The complex is called the nucleosome. It contains 2 copies each of histones H2A, H2B, H3, and H4. This is a very large structure (8 protein subunits and one long DNA double helix).
File - Open “09 nucleosome.txt”. This may take awhile, given the large size of the file.
Load and run the script “Protein-DNA-II.09.A.doc”. An animation will run.

You will see the DNA as white and grey strands forming a double helix, which then is wrapped around the histone core. The eight histone subunits are rendered in various color shades. The opening view is a "side" view. Rotate the model around the vertical axis to get a sense of the number of DNA loops. Also view it from the top.

Q20. The DNA makes approximately ______ loops around the histone core in this model.
   A. 1.25   B. 1.75   C. 2.25   D. 2.75

Load and run the script “Protein-DNA-II.09.B.doc”

The eight protein chains are named A thru H (but labels are not displayed). The two DNA chains are named I and J (but labels are not displayed). Use your cursor and read the status bar or the console to get a sense of chain locations. The chain ID name appears right after the colon (:) in the atom’s identity.

Q21. In the top view (where the DNA looks like a circle), the protein subunit that sticks way out into solution is ____ .
   A. chain E  B. chain F  C. chain G

Rotate the molecule (to help unfreeze the large structure) and click on a residue. In the status bar the identity is given as [amino acid type] residue-number.chain.atom .

Load and run the script “Protein-DNA-II.09.C.doc”

This colors lysine and arginine sidechain nitrogens blue, colors one histidine sidechain N blue, and colors asp and glu sidechain oxygens red, thereby using CPK colors at the site of atoms which are ionized at pH 7.

Q22. In the opening view, the negative DNA phosphate groups (spacefilled orange and yellow spheres) are mostly nearby ____ .
   A. positive (blue) protein groups
   B. negative (red) protein groups

Rotate the model around the vertical axis to view other portions of the DNA, maintaining the DNA approximately in a horizontal plane. Occasionally you will see both red and blue spheres at the portion of the protein surface in contact with the DNA, but the predominant pattern will be the same choice you answered above.

Load and run the script “Protein-DNA-II.09.D.doc”

This script rotates the model to a top view. Also rotate the model to get a bottom view in which you can see the black background through a "channel" of empty space thru the center of the protein octamer.
Q23. Look at the subunit which extends far out into solution. In that extension, the charges on the sidechains are entirely ____ .

   A. positive (blue) protein groups
   B. negative (red) protein groups

The "cylindrical" side surface of the histone core is predominantly one type of charge as noted above.

Q24. The "flat" top and bottom surfaces of the histone core ____ .

   A. are predominantly positive so they attract DNA
   B. have a mixture of approximately equal amounts of positive (blue) and negative (red) charges, so these surfaces are approximately net neutral and do not attract negative DNA

Close Jmol (File - Exit)