Name:_____

BCH622 - H-bond Worksheet

File: HbondPractice-KiNG.kin

[For a quick review of protein backbone, read the first part of section 1B in the "Anatomy & Taxonomy of Protein Structure" (AnaTax), linked on the BCH622 website.
If you need a reminder about planar vs tetrahedral geometry of covalent bonds, or a quick overview of H-bonds and donors vs acceptors, refer to file "Chemistry1001.pdf".
For sidechain structures and their polar atoms (and 3-letter abreviation), refer to file "CornCrib.pdf".]

H-bond Practice

Open the file HbondPractice-KiNG.kin and do the exercises, followinging the instructions for each kinemage (also given in the text-window of the kinemage).

These instructions are printed out on the next few pages of this Worksheet, as well as in the text window of the kinemage file.

Answer the following questions as you go along: (Only this first page needs to be handed in.)

On the first try in kinemage 1, I got _____ right out of _____ and got _____ wrong.

Did you perservere and eventually get them all correct? _____

In kin. 3, The "N-cap at the sequence start of a helix is the residue half-out and half-in the helix: its CO group makes the first i to i+4 backbone H-bond in the alpha-helix.
What residue is the helix N-cap? _____ _____

In kin 4, how many H-bonds include a sidechain atom? _____

In kin. 5, the one direct backbone interaction is between the_____ atom of residue _____ on the protein backbone and the _____ atom of residue _____ on the DNA backbone.

These examples add various clues to the basic stick figure:
which of the following did you find especially helpful in identifying or understanding the H-bonds?
_____ the red O and blue N balls
_____ the grey hydrogens
_____ the pale green H-bond contact dots

Hydrogen Bond Practice

KINEMAGE 1 - A beta hairpin, with polar H's, pointID's, and balls:

This kinemage shows the backbone (in white), sidechains (cyan), and polar hydrogens (gray) for a small piece out of a protein structure, with red balls on the O atoms and skyblue balls on the N atoms. The ends are truncated at Calphas for clarity. Move the image around to see the arrangement in 3D. Occasionally return to View1, or find a view you like and choose "Save current view" on the Views pulldown menu so you can get back to it.

The object of the exercise is to find the hydrogen bonds and draw them in, starting on a simple example with clear, unambiguous H-bonds and lots of clues included.

Turn on the drawline function by selecting menu item "Tools:Edit/draw/delete". A separate small window appears that has "radio" buttons: choice of one turns off all others. So clicking "Draw line segments" or "Draw dotted linw" sets that function, and clicking "Do nothing (navigate)" turns off drawline so you can click on a graphics point to just reveal its pointID. [Move the edit/draw/delete dialog box off to one side so it can't get hidden behind the graphics window.]

To avoid confusion between covalent bonds and your drawn H-bonds, use "Draw dotted lines". Click on two atoms to draw a dotted line between them. In this exercise, you should work with the H atoms turned on, and draw the H-bond between the H and the O (either direction is always fine); when hydrogens are shown use them rather than draw between N and O, in later exercises you will practice finding H-bonds without the help of seeing the H's.

Turn off the sidechains and start looking for possible donor-acceptor pairs on the backbone, with suitable geometry. The H-bond donor should be a peptide NH and the acceptor a backbone CO. They should approximately point at each other, with an H-O distance less than 2.5A (1.7-2.0A is best). The N-H-O angle is best at 180 and should be above about 135 degrees. [For the NH, both the electrostatic dipole interaction and the bonding contribution in an H-bond are optimal at 180.] The C-O-H angle is less critical, but should be above 100 degrees. [For the CO, the electrostatic effect is optimal at 180, but the bonding contribution is best at the lone-pair positions, at an angle near 120.] H-bond energy decreases with each factor that is non-ideal, but of course setting specific limits is arbitrary. In this kinemage, however, all the possible H-bonds are close to ideal.

When you find a pair with suitable geometry, draw in the H-bond. (If you don't like it, once you move it around and look from all angles, then remove it with the "Undo drawing" button.)

Once you have drawn lines for all the backbone H-bonds, turn the sidechains back on and see if you can find any sidechain-to-backbone or sidechain-sidechain H-bonds and draw them in also, again checking for suitable geometry. There are many more potential H-bond donors (e.g., any OH, Lys NH3, Asn/Gln NH2) and acceptors (e.g., the exposed side of an OH O, Asp/Glu carboxyl O, Asn/Gln CO) on the sidechains.

When you are done, turn on the "answer key" group, and score yourself. The answer key shows the correct H-bonds as shortened orange lines. Correct ones will overlap your dotted lines. Study the ones you got wrong, to figure out what makes them unacceptable.

If you want to try again, turn off the "answer key" group. You can then either just add to your previous H-bonds (Ddrawn obj 1" subgroup in the "Drawn objs" group), or you can add H-bonds in a different color by using the "New subgroub" button to make a "Drawn objs 2" subgroup. Of course,

by turning off "Drawn objs 1" you can ignore the earlier ones and in effect start over. When done, score yourself again. When satisfied with this part of the exercise, choose the next Kinemage in the upper-right Kinemage panel to move on to the next exercise.

KINEMAGE 2 - Retrial on beta hairpin, without H's and balls

This is the same beta hairpin you last worked on, but this time the task is to draw all the H-bonds without the help of seeing the N and O balls and the polar H atoms in the display. Again, first draw in the backbone H-bonds, this time clicking on an O and an N atom. You may want to keep on the sidechains, to mark where the Calphas are. Then find and draw the sidechain H-bonds.

When done, turn on the "answer key" group, score yourself, and study any you got wrong. Cycle through the exercise again if you want, or choose the next Kinemage to move on.

KINEMAGE 3 - A small helix, with polar H's, pointID's, and balls:

This exercise is set up the same way as the first one, but for an alpha-helix plus a bit extra on each end. First, turn off the sidechains and identify and draw in the alpha-helical H-bonds in the regular central part of the helix (keep on the H atoms and the N and O balls, and draw between H and O, checking for suitable geometry as described above).

Most of the helical H-bonds have nearly ideal geometry and the classic alpha-helix pattern of CO(i)-NH(i+4). At the C-terminus, however, that pattern is distorted, with some of the H-bonds weak or marginal (with bad angles and/or longer distances), and sometimes bifurcated. Although the cutoff is fairly arbitrary, O(36)-NH(40) and O(38)-NH(41) are acceptable, O(37)-NH(40) is very marginal (both angles are bad), and O(37)-NH(41) is clearly unacceptable (the N-H-O angle is 110). You may need to use measures to check out marginal cases, but for good H-bonds you should learn to spot them by eye. Make your analysis, and draw in the H-bonds for the C-terminal end of the helix.

Once you have drawn lines for all the backbone H-bonds, turn the sidechains back on and see if you can find any sidechain-to-backbone H-bonds and draw them in also, again checking for suitable geometry. There are many more potential H-bond donors (e.g., any OH, Lys NH3) and acceptors (e.g., Asp Od, Glu Oe, His N) on the sidechains. In this case, there are no sidechain-sidechain H-bonds.

When you are done, turn on the "answer key" group, score yourself, and study any you got wrong. Turn off previous "Drawn objs #" subgroups and the "answer key" group, create a new "Drawn objs # subgroup using the "New subgroup" button", and try the task again with the N/O atom balls turned off (leave on the H atoms, and draw from them). Score again, and choose the next Kinemage to move on.

KINEMAGE 4 - A small loop and beta piece, without H's and balls

In this exercise, you see the structure with just the heavier atoms for backbone and sidechains, the way it would usually be shown in a published figure or in most graphics programs.

First identify the backbone H-bonds, and draw them in by clicking on the N and the O atoms. (In this kinemage, the answer key lines will be off-set if you draw from the H.) [The balls and H atoms can be turned on temporarily, if you get confused or to check your assignments, but try to do without them .]

This example includes good H-bonds with quite non-linear C-O-H angles.

Turn sidechains back on, look for sidechain-to-mainchain H-bonds, and draw them in. [Turn on H's temporarily to remind you of where they should be, but don't draw from them.]

When you are done, turn on the "answer key" group, score yourself, and study any you got wrong.

KINEMAGE 5 - Protein/DNA complex (partial)

This time you will identify H-bonds that make up one part of the functional binding site for the complex of lambda repressor with its DNA operator site. The protein is in shades of yellow, and the DNA has white backbone and lilac bases. Polar H atoms (gray) and atom balls on N, O, & P are included. The second, more distant, DNA strand has its backbone simplified.

First look for and draw H-bonds between protein sidechains and DNA bases, which provide sequence-specific interactions (mostly on the top half of the interface, in View1). Draw between the hydrogen and the acceptor atom.

Then draw lines to show the H-bonds between protein and the DNA backbone; at least one should involve an atom on the protein backbone.

Turn off the DNA but leave on the protein and your "Drawn objs" group of drawn H-bonds. Look for two places where sidechains that contact DNA are also held in place by H-bonds between two of the sidechains; draw in those H-bonds.

Score yourself, study any wrong or missing H-bonds, and cycle through the exercise again if you want.

Several features not used in the scoring are visible for study. As in many protein/DNA interactions, here there are indirect H-bonds that join lambda to the DNA through well-ordered water molecules; two such interfacial waters can be turned on for examination.

Turn on the "Hb dots" button to visualize directly the favorable atomic overlaps that constitute these protein/DNA H-bonds, shown as lens shapes of pale green dots (including the ones thru the waters).

Also, you may want to practice drawing the base-pair H-bonds in the DNA (3 for GC, 2 for AT). Remember that they are not included in the answer key, so you must evaluate their appearance for yourself.

This is the last H-bond practice example. When done, quit out of KiNG.