

Name: _____



Worksheet 4: Making Kinemages

rev 090831

text like this is a menu or selection item in KiNG

Making kinemages of your own

1. (prep.) Download the KiNG program from kinemage.biochem.duke.edu, if you don't already have it.

-> On MicroSoftWindows, so you can tell pdb files from kine files: from any folder, choose "Tools/Folder Options/View (tab)". Uncheck the "Hide extensions for known file types", choose "Apply".

2. With a web browser, go to the PDB (Protein Data Bank) site at <http://www.rcsb.org/pdb>. Enter "alpha-hemolysin" in the search field at the top of the page and hit the "Search" button (or return). In the resulting list, skip the monomer files (with just an A chain) and the tetramers, and click on the code or name text of the alpha hemolysin heptamer (7AHL) for info about it. [Copy the name and location of the protein; here: _____ since KiNG does not write PDB header information into the kinemage text window.] Click on "Download file" at upper right, choose PDB file, either text or gz (KiNG can read gzipped files) and save it in your working folder. Back in the browser window, enter "1AD1" (not case sensitive) in the space next to the "Search" button, and download that file as well; its name is _____. Do the same for 1IG5: _____. [Getting the name and any other descriptive information is valuable for constructing the text part of a kinemage -- of course, you can always open the PDB coordinate file itself in a text-editor or wordprocessor to read this information, but reading the paper abstract on the rcsb PDB page is good practice.]

3. On your computer open KiNG and select **File/Import/to quick kin/Pseudo-backbone**, then navigate to where you placed 7AHL.pdb and open it. (Alternatively, on the Mac, drag-n-drop the PDB file 7AHL onto the KiNG icon.) A Calpha-backbone kinemage will be displayed in the KiNG graphics window. Move it around and identify the chains by turning on&off their buttons or by clicking on an atom. Choose at least 2 different views of this 7-chain mushroom whose stalk is a _____ strand transmembrane β barrel, and use **Save Current View** on the Views menu to save each of them with an appropriate View ID; they will then show up on the **VIEWS** menu.

About how far is it between backbone on opposite sides of the barrel? _____ Å (enough to make a pore, even allowing for side chains). Does the pore continue through the larger, nonmembrane domains? _____ .

4. Changing colors at the list level: Choose **Edit hierarchy** on the Edit menu; this brings up a "Hierarchy window" (move it off to the side). It shows a collapsed view of the group/subgroup/list kinemage organization (as also seen in the upper portion of the right-hand side-panel). For 7AHL A chain, expand the outline down to the 3rd (list) level (pick on the small open circle symbols), pick the list, then select **Properties** and click on various color patches until you get one that you like (as seen interactively in the graphics window). **OK** button sets your choice. .

5. Choose **Edit hierarchy** again. Without expanding the hierarchy, select each group in turn (click on either the name or the blue ball next to it), then select **Properties** to get the "Edit group properties" window, and select (turn on, check) the "**animate**" check-box. An asterisk will appear as a prefix to the group name in the graphics window button panel. When all 7 groups are so marked as animatable, click in the graphics window to get the keyboard focus there. The "a" key or the Animate buttons in the lower part of the right-hand side-panel select each animate group in turn (most interesting viewed down the 7-fold symmetry axis). Pushing the + key (without a shift) accumulates turning the groups on.

What happens when you hold down the "a" key on the keyboard? _____

[Don't save yet.]

This time, either use just **File/Import/Molecules**, or drag-n-drop the 1AD1 PDB file onto the open graphics window and choose "**Open file in Molikin**", which will give you more control. .

6. Now build a kinemage with file 1AD1. (Unselect default Ball&Stick) then pick **+ Ribbons** for the ribbon script using the HELIX & SHEET records in the pdb header, select just **Chains A** to get just the first subunit, and keep the other default ribbon parameters. Choose **As new kinemage** and a ribbon representation will appear in the KiNG graphics window. It should show red helices and green β strands. Find KiNG's text window, check **Allow text to be edited**, and type in the protein name, write it here also:

_____.

This enzyme is the target of an antibiotic drug. Save a nice view. Only 7 green arrows show, as assigned by the automatic pdb program; can you find the eighth possible β strand? near residue _____.

[Don't save yet.]

7. Now, for file 1IG5, in the Molikin window keep the default **Ball & stick** and additionally check ON "**water**", "**balls on N,O,P,etc.**", "**backbone**", "**sidechain**". Accept with the **As new kinemage** button. Main chain is shown in _____ color, side chains are cyan, and the peach balls are waters.

Pickcenter on the larger gray sphere and zoom in; the usual Ca^{++} is replaced here with _____.

Identify the metal ligands: side chains _____, _____, _____;

backbone _____; waters _____, _____.

Turn off the sidechains and waters, zoom back out, and rotate to see the backbone structure.

Use "**Save As**" on the File menu to save your set of 3 modified kinemages; edit the name to "worksheet4-yourname.kin".

8. Quit KiNG, then relaunch with your constructed kinemages, and admire your own work! Note that if you added text to the text window, it now is there to help explain your kinemage.

9. Open a kinemage in a text-editor (like NotePad on MSWindows, TextEdit.app on Macs, (nedit, vi, or emacs on linux). Note the obvious @group/@subgroup/@list hierarchical organization. Try editing some things e.g. change color name on a vectorlist. Save the modified kinemage as PLAIN TEXT -- text-editors do this naturally, word-processor programs like MS-Word can do this as a special request, otherwise, all the fancy word-processing controls will confuse the KiNG parser! Now you can view your edited kinemage in KiNG and appreciate the iterative process of building a kinemage.

10. Attach kin to e-mail: nicholas.warischalk@duke.edu (also could attach this answered worksheet).