

## FAQ: Questions not covered in the tutorial

### How do I

- Know what is important about my structure? Read the literature, particularly the article(s) published by the authors who deposited the structure in the Protein Data Bank (see the JNRL entry in the PDB file header, or use the PDB web “explore” window). Use PubMed (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>) to find other pertinent articles. Run the default browser script in Prekin and look at the overall organization. Run the “lots” built-in script on individual subunits to see everything in the PDB file, and zoom in on interesting features. Look around and see what you can find!
- Tell what amino acid sidechains interact with the bound ligand? In addition to the written literature, use the “lots” built-in Prekin script, or do a “focus” around the ligand (find its residue number or its XYZ first in your backbone kinemage. You can then center the ligand on the screen using pickcenter, zoom in, and see which sidechains are close to the ligand. Click on the sidechains to learn their identity and distance to the ligand. This will let you decide which sidechains to include in your more selectively authored kinemage.
- Locate secondary structure? The PDB file contains secondary-structure information in the header. PREKIN uses this with the built-in script called “ribbon: HELIX\_SHEET”. Running this script, as done in the tutorial, will generate a kinemage clearly indicating the pieces of secondary structure of the protein. If you want to see the hydrogen-bonding pattern of the main-chain peptide bonds, you can run the built-in script mcHb. Of course, you can also measure the dihedral angles of the amino acid residues and compare them to the Ramachandran plot found in any biochemistry text. If the PDB header doesn’t list helix and sheet, or if you want to change its assignments, you can specify ribbons by hand in the Ranges dialog.
- Identify het groups? Het groups are “nonpeptide” groups such as metals, cofactors, ligands, water, etc. Prekin outputs non-water het groups in pink; whether they are a separate group or under the subunits depends on the organization of chainIDs in the PDB file. You can turn the het button on and off to locate the het groups. Clicking directly on the het group will give you an abbreviated name. Looking in the PDB header (“HET” records) or even the PubMed abstract corresponding to that particular structure are probably the best ways to figure out exactly what the het group is.
- Find an axis of symmetry? Rotate the image slowly and look at every side. Imagine an axis coming from your nose into the image. If you rotated the image about that axis by a set number of degrees, e.g. 90, 120, 180, would you get back the same image?
- Save a static 2-D image for a written report or PowerPoint presentation? Do a bitmap screen capture: on the PC, use the “print screen” button at upper right of keyboard, which puts a screen image in the system clipboard (you may want to temporarily fill the entire screen by tugging out all corners of the image window); on the Mac, type “shift apple 4” and drag the new cursor around the part you want, to save a Pict file to your hard drive, or “shift control apple 4” saves to the clipboard. An image in the clipboard can be pasted directly into PowerPoint, or can be pasted into a bitmap

editor like Adobe Photoshop or Microsoft Photo Editor to make modifications. *For written reports, you should first change the kinemage to a white background (Display menu) so you don't use up an entire black ink cartridge.*

MAGE will write out images as postscript (.eps) files either in color or black&white (set on Display menu), which can be read by Adobe Illustrator or printed with the Downloader utility (Mac). Sometimes they will print directly if you just drag the file to the printer icon. MAGE can also write files for input to the freeware rendering programs Raster3D or POV.Ray, but then you would need to get those programs and learn to use them. They can make ribbons look especially nice.

- Use kinemages interactively in a presentation? One way is to give your presentation in html using a web browser. If you have configured your browser to use MAGE as an application, you can set links in your presentation to kinemages on your computer. Alternatively, you can simply use MAGE directly.
- Configure my browser to use MAGE? In Netscape, under Edit go to Preferences, then Applications. The file type is kinemage, the file extension is .kin, the MIME type is chemical/x-kinemage, and use the Browse button to point the application to your MAGE program. Internet Explorer has a similar setup protocol.
- Best illustrate the protein backbone? It depends on what you want to see. The “Calpha” backbone used in the first part of tutorial is the simplest method, but doesn't show the peptide bonds. To identify mainchain hydrogen bonds and to measure dihedral angles, you must show “mainchain” (either in scripts or ranges). Ribbons make an artistically pleasing introductory overview, as described below.
- Make ribbons? There are three ways in PREKIN, two of which are built-in scripts which will either make a simple ribbon of the whole protein backbone, or will highlight the secondary structures as colored arrows and spirals (easiest and best choice, unless your PDB file is missing the records Prekin needs). Alternatively, you can select particular regions and their ribbon style using the “range control” boxes.
- Color domains within a single protein chain? Make the domains separately with PREKIN using New Pass. They will then be separate groups that you can color or turn off separately. For continuity in a Calpha trace, make the domains overlap by one amino acid (if 150 is the changeover residue, first do 1 to 150, then 150 to 9999).
- Access Help while using MAGE? Most individual items on the Help menu bring up terse explanations in a dialog box: e.g., “Mage Key Shortcuts” lists the effects of all the keyboard shortcuts, and “Input Output” explains the items on the File menu. The “Mage->text” options are probably appropriate only for advanced users.
- Choose good colors? Demo5\_4b.kin has a palette display and advice about colors.
- Get the image to move sideways in the screen without rotating? Hit the ‘f’ key to enter flatland, then use your mouse to drag the image wherever you want. Hit ‘f’ again to leave flatland.
- Put labels on particular features? Labels in kinemages are copied from the PointID (what is shown at the bottom of the screen when you pick an atom or point). Choose Draw New on the Edit menu, check the Labels button, and pick a point to label it. If you click and drag, you can move the new label away from the point. To edit the label, turn off the molecule (unless the label was moved away from it), turn on Show

Object Properties, pick the label's lower left corner, and edit what it says in the rightmost field of the dialog box.

- Eliminate unneeded buttons? In the MAGE Edit menu choose "Show Object Properties", then in the graphics window pick a point in the feature whose button you want to hide. The resulting dialog box shows the hierarchy of Group, Subgroup, List, and PointID for what you picked; that hierarchy controls the buttons in Mage, with indenting. (You can edit the names in those fields to change the button labels.) Checking the "no button" box hides just that button, while checking "dominant" hides the buttons of all members below that button.
- Control display objects in different groups with one button? Use what are called "master buttons", which show up beneath the regular hierarchy on the button panel. (For example, if you have multiple subunits or conformations, you can set up a master button to turn on or off the side chains in all of them at once.) Open the kinemage in a word-processor, find the line that starts each subgroup or list you want controlled (scroll, or search for "sc" in this case), and add "master= {side ch}" to that line. Then you should eliminate display of the individual "sc" buttons (see above).
- Optimize the depth perception of my kinemage? Although this is usually not necessary, you can use the "slab" slider to change the depth of the image included in the direction in and out of the screen (sometimes a thinner slab is good for crowded closeups). This also modifies the degree of "depth cueing" or dimming toward the back, since its range is the slab depth. Typing x or X moves the molecule toward you, and z or Z moves it away.
- Change color on (or delete) a feature that won't let me select it? Some features, such as shortened new bonds, are "unpickable". To pick them, go to Edit and turn on Superpick. Then click the end of the bond. Turn off Superpick when finished. [In general, remember you can pick only endpoints, not in the middle of lines.]
- Troubleshoot problems? If things do not work correctly as described in the help and documentation, a good strategy is to look on the kinemage web site for newer versions of Mage or Prekin and check to see if your faulty feature has been fixed. If not, please report the problem by Email to [mage@kinemage.biochem.duke.edu](mailto:mage@kinemage.biochem.duke.edu), giving your program version, hardware platform, and operating system.

### **Advanced features:**

- Superimpose structures from two different PDB files? You probably want to animate between two (or more) conformations, such as an enzyme with and without a bound inhibitor, or to compare two related proteins. To superimpose the coordinate set of one structure onto another, you can use the "magic fit" feature of the Swiss-Model PDBviewer (<http://www.expasy.ch/spdv>), an excellent freeware program complimentary to Mage. Run the moved PDB file through PREKIN, append it to the kinemage of the reference structure, and use "Show Object Properties" to put an asterisk at the beginning of both group names to set up the animation (see tutorial for details).  
Alternatively, you can dock or superimpose groups in MAGE. Turn on only the group you wish to be mobile, select "Docking Scope" on the Tools menu, then turn the reference group back on. Use the new set of sliders to move the mobile group

relative to the reference group until the desired parts overlap as well as possible. Pickcenter is useful to make the rotations move around an understandable center point. The usual global rotation by mouse drag, the flatland scrolling, and the “n”, “N” 90-degree image rotations still operate as well; use them often to see what you are doing. An alternative to the sliders is to hold down the shift key and use mouse drag to move just the mobile group. “Save As” will write out the modified kinemage, but there is currently no easy way to get modified PDB coordinates.

- See the hydrogens in a protein structure? If the PDB file does not contain hydrogens, and most don't, you can add the hydrogens using a program called REDUCE. This program, available from the Richardson web site at [kinemage.biochem.duke.edu](http://kinemage.biochem.duke.edu), has SGI Unix, Linux, Mac OSX, and PC versions. See the Readme.txt file for details. Use the “reduced” PDB file to make your kinemage. An even easier way is to have the MolProbity web service do it for you: go to MolProbity on the navigation bar of the kinemage web site at Duke, and specify the PDB code or upload your file.
- See how the protein interior is packed together, or how ligand and protein fit? Take a PDB file which contains hydrogens and calculate the interactions between sidechains (or between ligand and protein, or DNA and protein) using the program PROBE, available from the Richardson web site quoted above. Again, MolProbity can do this for you and show the result on-line in JavaMAGE, or you can download the kinemage.

Tip: Students wanting to know about advanced features such as master buttons, the color palette, and kinemage file format should look at [demo5\\_4b.kin](#).