

KINEMAGE AUTHORSHIP TUTORIAL USING RICIN



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This tutorial will use the **PREKIN** kinemage construction program and the **KING** kinemage viewing/editing program. All software is available at kinemage.biochem.duke.edu

Obtain the ricin protein databank file at <http://www.rcsb.org/pdb/>
Search for 2AAI. Then Download/Display → Download the structure file
→ Right mouse click on PDB file format (no compression). Save target
as 2AAI on your desktop.

KINEMAGE 1- Overview of the molecule

To start - a simple alpha carbon backbone kinemage

For an initial look at the ricin molecule, we will run a simple default script on file [2AAI.pdb](#). Launch (i.e. start) PREKIN and open 2AAI.pdb from its menu. PREKIN will ask for an **output file name**, so call the output file **backbone.kin**. When the first dialog box of choices comes up, accept the default "Backbone browsing script", which will execute a simple script producing Calphas (a connected series of alpha carbons), disulfides, and non-water het groups for all subunits in the file. A 'het' group is anything not part of the polypeptide (or polynucleotide) chain. When PREKIN is done, launch KING and open the resulting kinemage.

Comment [BB1]: Tip: Pay attention to where your file is being saved. It is easy to accidentally lose a file by saving to the wrong directory.

You should see Calpha backbones for the two ricin chains in different colors, with yellow disulfides and several bound sugars (pink). Move the image around by dragging with the mouse. Such a simple, default kinemage shows many of the features of the structure, and is useful for many purposes. Note, however, that the viewpoint is arbitrary and the default colors, names, and arrangement of buttons are not ideal. If you want to show particular details and express your point clearly to someone unfamiliar with the structure, then there are many ways to make the kinemage more informative and persuasive.

Choosing and saving views in KING

In this section you will prepare a 3-D portrait of the ricin molecule and similar portraits of each of the two individual subunits (chains).

Creating an overview: First move the image around to find a view that spreads out the three domains in the plane of the screen, with the A chain (the white one) at the top. See

if you can enlarge the zoom factor by one or two arrow-clicks on the zoom slider without going off the screen edges. Type 's' on the keyboard to toggle into stereo, to make sure your zoom and orientation allow seeing most of the important parts in stereo (you can check for that, even if you can't see stereo yourself). Re-center if needed by right mouse clicking on the atom you want in the center of the screen. Alternately, you can hold down the control key and drag the structure around with the mouse. Once satisfied, choose "Save Current View" under the Views menu, and save the view as a descriptive name such as "overview". Move the image, then choose "overview" under the View menu, which should reproduce the view you just saved.

Creating views of subunit features: Right click on an atom near the middle of the A chain and zoom in somewhat. Check the "markers" box at the bottom of the window, then choose the "Find Point" function under the Edit menu. In the dialog box ask to search for " 177 ", which is the active-site Glu of ricin; KING will center on Glu 177 and mark it with a box. Choose a view for the A chain that shows both the central beta sheet and this active-site Glu, and save the view as "A chain". Now pickcenter between the two domains of the B chain, zoom in, and save a view that shows the domains fairly equivalently, in a vertical orientation to allow for stereo. It might help to unclick the A chain button so you can see the B chain better, although your view shouldn't absolutely require that since your readers may not think of doing it.

Renaming and coloring groups: Go Tools -> Kin editing -> edit properties, and click on any sugar atom. The resulting dialog box will show you the program's internal data structure for this atom. Click the "edit group" button and name the group "sugars". Accept the result, and see how the buttons have changed. In the same way, edit the group names for the protein, from 2AA1a and 2AA1b to "Ricin A chain" and "Ricin B chain" (remember that you only get about 11 characters for a group button name).

Go Tools -> Kin editing -> recolor kins and click "color all". Now you need to change the colors of the two subunits, A to differ from the red sugars and B to contrast better with the yellow SS bonds. The "tint" colors work best for Calpha backbones, because they can be distinguished without overwhelming small features you want to emphasize. Make the A chain yellowtint and the B chain greentint (try out some other possibilities, too) by choosing the color from the dropdown menu and then clicking on an atom in the chain. To get out, close the recolor box and then turn off the kin editor by clicking Tools-> Navigate.

Saving your modified kinemage: Choose "Save as", under the File menu; you will be given a dialog box to place and name the saved file. You will notice that the file ends in ".2.kin" rather than ".kin". This simply means that it is the second modification of the original kinemage. Quit out of KING.

Highlighting important features of the protein

In this section you will highlight one important feature of the active site of the ricin molecule, the active site Glutamate 177 sidechain in the A chain.

Adding the active site Glutamate: Use PREKIN to open the 2AAI.pdb file again, but this time name the output file "**sidechain.kin**". In the initial dialog box of PREKIN, choose "New Ranges". In the range dialog specify both start and end residue as 177, check the 'sc' (sidechain) and 'at' (balls for non-C atoms) boxes, the "OK accepts and ends ranges" button, and the OK button. In the following dialogs accept the defaults for no focus and 0.2A balls. In the last dialog window ask for "only first subunit" by making sure both entry windows have a 1 in them and the middle radio button is checked. When the program quits running, you have generated a side chain for Glu 177 of the A chain.

Merging files in KING : Now launch KING with your **backbone.kip** file, then choose "Append" on the File menu to add in the **sidechain.kin**. This merges the two kinemages into one. Change the color of the Glu 177 sidechain vectors to something bright, contrasting, and oxygenish, such as pink or hotpink. Rename the appropriate 2AAI button as Glu 177.

Look at the resulting kinemage to see if you did what you intended and whether you like the results. Modify views if needed, and note anything that needs to be changed during future editing. Before you save the completed kinemage, return to view 1 and make sure all of the buttons needed for this initial view are checked. You can edit the order and names of views by going Views->Edit saved views. Save the completed kinemage as **ricin1.kin**.

KINEMAGE 2- Secondary Structure in the A Chain

Highlighting secondary structure with ribbons

The alpha helices and beta sheets are easiest to visualize by portraying them as ribbons, which is an artificial but useful construct. PREKIN has a built-in script which reads the secondary structure information at the top of the original PDB file and uses it to construct the ribbons for the backbone.

Running the built-in ribbon script: Make a new kinemage by launching PREKIN and again selecting the 2AAI.pdb. Save output in the desired directory with the name "**ricin2.kin**". Select the "built-in scripts" menu, then the "ribbon: HELIX_SHEET" option. Accept all defaults in the "ribbon" box. In the "run conditions" box only select the first subunit (A chain) by clicking the middle radio button and then OK. When done and before exiting, select "new pass" under the file menu and again select "built-in scripts". This time select the "mChb" script. OK and again select the first subunit in the "run conditions"



box. Launch KING at the end to see the created kinemage. You will have two representations of the A chain overlaid. In the ribbon structure, [beta sheets](#) should be green arrows and alpha helices gold spirals. Irregular secondary structure, i.e. coils and turns, will be connecting ropes. In the mainchain structure you will see the peptide backbone and hydrogen bonding.

Using animations as alternate views of structure

Animating between two different representations of an object can be very informative, even more so if they have different conformations. You have made two different representations of the ricin A chain: a line tracing of the peptide backbone, and a ribbon diagram of the secondary structures. Next you will overlay and animate between these two representations. KING allows animations between groups using the hierarchy editor.

While viewing ricin2.kin in KING you can set up an animation between the two versions of the A chain. The top button will be the ribbon structure. Unclick the group buttons if you are unsure. Now open the hierarchy editor (under edit) and change the name of the ribbon structure from "2AA1a" to "ribbons A". Make sure the "dominant" and "animate" boxes are checked, then accept changes. Do the same with the other group except change the group name to "*mainchain". Accept changes and turn off the hierarchy editor. You have now created an animation between two representations of the A chain. Hit the 'a' key on the keyboard or click the ANIMATE button on the bottom left of the graphics window to see the two representations. Save some good views of the helices and the central beta sheet. Is the sheet parallel or antiparallel? Can you follow the polypeptide chain as it wanders in and out of the beta sheet? Can you pick out the secondary structure in the mainchain kinemage alone?

KINEMAGE 3 - Active site, with hydrogen bonds

The philosophy behind kinemages is that the most revealing way to illustrate what is important is to remove extraneous parts of the structure. In this kinemage, you will concentrate on the active site of ricin, which resides solely in the A chain. To see what is going on at the active site, you will eliminate everything that is not within 12 angstroms of the critical active site residue Glu 177, which you highlighted in the first kinemage. Before beginning, I suggest you review your amino acid sidechains so that you will recognize them easily.

Generating an active site with the Focus option

Run PREKIN on file 2AA1.pdb again, with output file [actsite.kin](#). Choose "New Ranges", then check the 'mc', 'sc', 'at' and the 'OK accepts and ends ranges' buttons. Hit "OK". In the next dialog box, choose to do the focus on a residue by number. In the "Focus Point Values" dialog, specify residue 177; for radii try 8A for sidechains, 12A for main chain, and 0 for everything else; ignore the special logic controls. Do only first subunit. When it finishes, launch the kinemage.

Cleaning up the kinemage

Drawing hydrogen bonds: Choose and keep a view that gives a good close-up of the active site. Find the sidechains Glu 177 and 208, as well as the Gln, the two Tyr, the Arg, and the Trp that surround the two glutamates. In Tools->kin editing->draw & delete, set "shorten lines to" 0.7. Draw in the Hbonds from the sidechains of Arg, Trp, and one Tyr to the closest mainchain carbonyl oxygens (the carbonyl of the peptide bond). It is helpful to zoom in on each side chain and measure the distances to the nearest oxygen. Remember that Hbonds are 3Å or less between atoms that share the hydrogen.

Pruning main chain: Now, in the same "draw & delete" menu, experiment using "punch", "prune" and "augar" to trim away all extraneous main chain that is either floating in space in bits or nowhere near the sidechains or Hbonds. If you want, prune away any uninteresting sidechains also. Use the "undo" button to recover from mistakes.

Save a view of each sidechain hydrogen bond, remembering to use descriptive titles for each view. Finally, save the modified kinemage to your hard drive as **ricin2.kin**.

KINEMAGE 4 – Superimposing the two domains of ricin chain B

Chain B has two domains that each have the "beta trefoil" fold; superimposing them can show how similar they really are. We will use the beta strands and the Trp sidechains as landmarks for doing the superposition in KING with its docking function.

Using the 2aai PDB file as input to PREKIN and naming the output file ricin4.kin, go to "New Ranges". Since the N-terminal tail is not equivalent and the changeover point between the domains is at about residue 138, specify numbers 7 to 138 for the range of domain 1, check "ca", and hit OK. Leave 7-138, replace the "..." with "css" (to get the disulfides), uncheck "ca", check "sc", and hit OK. Leave 7-138 and "sc", replace "..." with "trp", but this time check "OK accepts and ends ranges" before hitting OK. No focus. Check the "Do subunits in range above" radio button and specify subunits 2 to 2. When PREKIN is done, choose "New Pass" from the File menu. In "New Ranges", do exactly the same three ranges as above, but for residues 138 to 9999 (to get domain 2). This time, when PREKIN is done, launch the kinemage in KING.

You should see all of chain B, with Trp sidechains in cyan and SS in yellow. Turn off the second button on the button panel (domain 2) and use "Change color" to make the domain 1 Calphas white. Drag with the mouse down and then a little left to get a view down the 3-fold axis of domain 1 - you should see three Trp sidechains as symmetrical "T" shapes, with a triangle of Calpha strands evenly around a central opening. Save this view as View1. There are two other Trp in domain 1 that are not symmetrically related around the 3-fold; use "Prune" to delete them. Turn on domain 2 and make its Calphas some other color (maybe pinktint). Give the two groups names like "B dom1" and "B dom2". Find the 3 symmetrical Trp in domain 2, and prune away the extra, fourth one.

Find a view where the two domains are side by side and the three Trp sidechains in each are in roughly the same orientation. Turn on Tools->kin editing-> dock 3 on 3. Now

zoom in on the left domain so that you can clearly see the Trp sidechain connections to the alpha carbon backbone. Click on these alpha carbon connections in a clockwise fashion. You should see all three atom identifiers appear in the docking box. This is the reference structure. Repeat this procedure with the second domain, which will be the mobile structure. Now unclick the group button for the reference domain and click the “dock visible on invisible” button in the docking box. The mobile structure should jump into the space occupied by the reference structure. Turn on the reference structure again and you should see the overlay.

Use "Save as" to save the kinemage with your superimposed coordinates. Can you indeed see convincingly that these two domains have the same fold? They are almost certainly related by a gene duplication. Animate between the two structures, using the hierarchy editor to change the properties of the two group names. You have now made four [kinemages](#) that illustrate a number of interesting things about ricin. Congratulations!

Writing text in the text window and merging kinemages.

To add text to the text windows of any of your kinemages, simply click into the text window and type as if in a text editor. Right click with the mouse shows the cut/copy/paste mouse menu.

To place all four of your kinemages in one file, simply open them sequentially in KING. Once they are all open, save as a new kinemage.