

BCH222 - Greek Key β Barrels

Reading

C.I. Branden and J. Tooze (1999) Introduction to Protein Structure, Second Edition, pp. 77-78 & 335-336 (look at the color figures)

J.S. Richardson (1981) "The Anatomy and Taxonomy of Protein Structure, III.D. Antiparallel β Domains", Adv. Prot. Chem. **34**, pp. 299-303.

F.R. Salemme (1983) "Structural Properties of Protein Beta Sheets", Prog. Biophys. Molec. Biol. **42**, Fig.28 and pp. 127-129

Graphics assignment

We will be looking at PDB entries **3GAP** and **1E43** as a way of understanding what is said in the reading about Greek keys, especially "Ray's Rule" for the sidedness of 2-stranded beta ribbons and their preferred direction of bend. At the end, you will make your own kinemage of a chosen structure and analyze its Greek key. The control buttons of Mage, which appear on the right-hand side of the Mage graphics window, appear in the text below **LIKE THIS**.

1. Kinemage 4 of the kinemage file **StSurvey.kin** shows subunit A of c-AMP Receptor Protein (CAP protein) (PDB file **3GAP**). CAP protein includes a nice example of a Greek key beta barrel from the all-beta category (for domain 1).

- a. Parts of CAP protein

Residues 1-109 (domain 1) are predominantly an antiparallel beta barrel, explicitly residues 18-99 (closeup in View2). Cyclic AMP (in pink), which acts as a regulator, can be seen bound at the back of the beta barrel.

Residues 110-136 (View3) are a long alpha helix (how many turns? _____) which forms the primary contact with the other subunit in the dimer. Residues 137-208 are the DNA-binding domain, a small open-face sandwich antiparallel beta sheet (View4). The pair of helices at residues 170-190 and their corner form the specific DNA-binding site (View5).

Approximately what is the angle between those two helices? _____ They form a rather unusual, offset T shape: the common "helix-turn-helix" motif.

- b. Topology

This barrel is not very cylinder-shaped, but its arrangement helps illuminate the folding of such structures. With **MAIN CH** on (turn off & on again) and **H-BONDS** on, just for domain 1 (in View2), identify the 8 strands which make up the barrel. Imagine starting at the barrel N- and C-termini (residues 18 and 99) and following along that pair of chains as they coil next to one another around and around the barrel, to a tight foldover point in the middle of that sequence.

That hairpin foldover is at what residues? _____

Click repeatedly on the **ANIMATE** button to see the buildup of these paired strands. We believe that this sort of Greek key beta structure folds up by first forming a long 2-stranded ribbon, which then curls up into the barrel. How many beta H-bonds are there between this "privileged" pair of strands? _____

How many beta H-bonds are there that join one portion of the privileged pair to another portion of it? (i.e. how many H-bonds to complete the rest of the barrel?) _____



- c. Because of the strand pairing, it is better to visualize the barrel opened out between the two beta sheets, rather than between the two terminal strands. Make a topology diagram of the barrel from the outside (as opened at the back in View6 and laid flat). First draw 8 vertical lines for the beta strands; then label the two termini (at lower right); then put direction arrows on the strands; then draw in their connections.

If the strands are lettered A through H along the sequence, list the order in which they occur around the barrel:

List the connections by type (e.g., +1x, -3):

You can see why this type of barrel topology is called a "jellyroll" Greek key.

2. Kinemage file [1E43gk.kin](#) contains one domain from the α -amylase enzyme of PDB file 1E43.

Remind yourself of the basic geometrical properties of antiparallel beta sheet. Pick a strand and identify the 3 perpendicular directions that represent 1) N to C direction along the chain; 2) H-bond direction, or peptide dipole direction, perpendicular to the chain; and 3) side chain direction, perpendicular to both the first two. Does the side chain direction really alternate one in and one out? _____

The commonest breakdown of sidechain alternation is a bulge, where one strand has an extra residue. Find a β bulge on the less regular sheet: at residues _____ and _____, opposite residue _____.

A less common irregularity is a convex shape formed by the reverse pleat at a Gly in L β conformation. On the more regular sheet, there is such a convexity at Gly _____; its ϕ = _____, ψ = _____. Is the surrounding H-bonding normal? _____

Identify a pair of residues (on neighboring strands) that are within a narrow pair of H-bonds: _____ and _____.

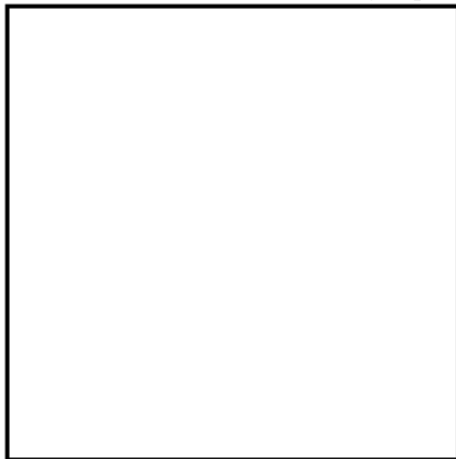
Wide and narrow pairs alternate along the chain direction; are they the same or alternate along the H-bond direction perpendicular to the strands? _____

For the central part of the more regular sheet, about how many degrees per residue does the strand twist? _____

Lefthanded or righthanded twist (of the peptide or H-bonding plane, along the strand direction)? _____

Look for the difference between twist and bend (e.g. Salemme Fig. 26b). A combination of both together makes a coiled structure (e.g. Salemme Figs. 22 & 23), especially evident for isolated 2-strand ribbons. For an example, look at the long β -ribbon that makes half of the less regular sheet here. About how many degrees per residue does it twist? _____ To see that it also bends, look down the long axis of the two strands.

Consider the properties of coiled 2-strand ribbons as formulated in "Ray's Rule": the preferred bend is such that side chains between narrow H-bond pairs are on the inner, concave side. Equivalently, if you are looking at the ribbon from that inner side, the chain should go up on the left and down on the right.



Draw a topology diagram of the amylase domain with the barrel opened out and viewed from the inside:

Like all Greek keys, the figure is ambiguous between two possible neighboring choices for the central hairpin; for 1E43 the two choices are a hairpin between strands _____ and _____ or a hairpin between strands _____ and _____.

Look at each of these possible strand pairs by Ray's Rule: which pair has side chains between narrow H-bonds projecting toward the interior? _____

Which pair goes up on the left and down on the right viewed from inside? _____

Turn on the RR Hbonds to emphasize the interactions between the Ray's Rule strand pairs. Does the pair here which is correct by Ray's Rule also have the most strands and H-bonds? _____


3. Go to the **SCOP site** (<http://scop.mrc-lmb.cam.ac.uk/scop/>)
 What is the current version number? _____ release date? _____

Enter at top of hierarchy and go to "All-beta proteins". Each person should pick one of the folds of at least 8 strands and a Greek key or jelly-roll topology (marked on the SCOP display page). Expand the outline a step and pick either the first superfamily or one that interests you. Go down to an individual PDB file, make sure it's a crystal structure at 2.5 Å or better, and note its PDB code. _____

Navigate to the **PDB database site** (<http://www.rcsb.org/pdb/>) and ask for your file. Does it list structure factors under the summary information? _____

Download your file as PDB-format, uncompressed, onto your local area (your PC's hardisk or the L_GreekKey directory on a Richardson-cluster machine). Exit the browser.

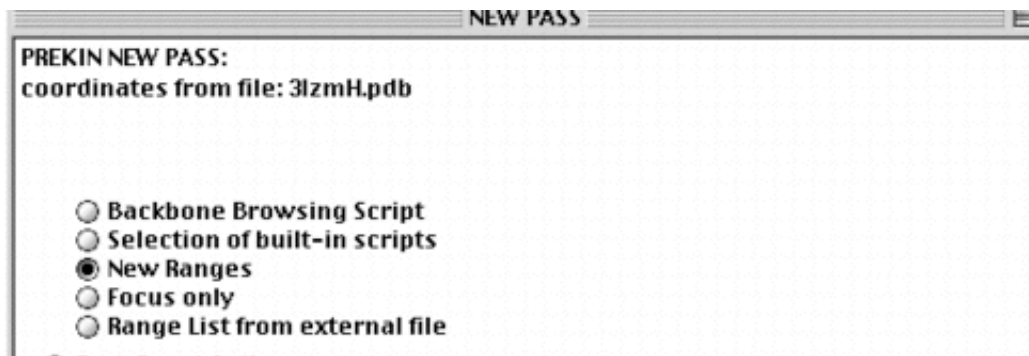
Now use Prekin to create a kinemage file from your downloaded PDB file. The commands to invoke Prekin and read in the PDB file vary depending upon the computer type you are using. Follow the appropriate set of computer inputs:

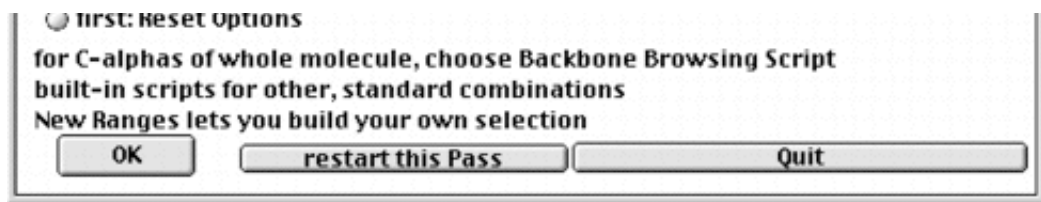
if your computer is then do these commands ...
a unix or linux box (command line)	<ol style="list-style-type: none"> Within an X-windows environment and in the directory in which you downloaded the PDB file: at the command line prompt, type <code>prekin #xyz.pdb</code>, substitute for "#xyz" the PDB ID code. Name the output file "<code>#xyzCAabc.kin</code>" where "CA" is the usual mnemonic for a Cα backbone model and then substitute for "abc" your initials. Choose the backbone browser option. This should generate the kinemage file you requested AND launch Mage to display that file. (If not, then launch Mage and the new "<code>#xyzCAabc.kin</code>" file by typing <code>mage "#xyzCAabc.kin</code>" on the command line.
a Mac OS9 or Windows computer (graphics interface)	<ol style="list-style-type: none"> For recent versions of Mage for MacOSX, Prekin is bundled in the Mage.app application. To invoke Prekin, drag-n'-drop a *.pdb file to the MAGE icon. (Note that the filetypeing script decides whether to invoke Prekin or Mage by looking at the file extension. For a dropped file with an extension of .pdb or .ent, Prekin is invoked; for an extension of .kin, Mage is invoked. For MacOS9 or Windows, try dragging the icon for your newly downloaded PDB file onto the icon/alias/shortcut for the Prekin program:  Sometimes this may not work because of inappropriate file-typing. If dragging-and-dropping doesn't work, then start-up the prekin program (typically) via double-clicking the icon/alias/shortcut and read in the PDB file as prompted. After Prekin is started, follow the instructions for steps b, c & d, that are given above.

When the display launches in Mage, decide whether there are extra subunits or domains and, if so, which part you want. Locate the β barrel or sandwich, and make a note of its start and end residue numbers. _____ Exit Mage.

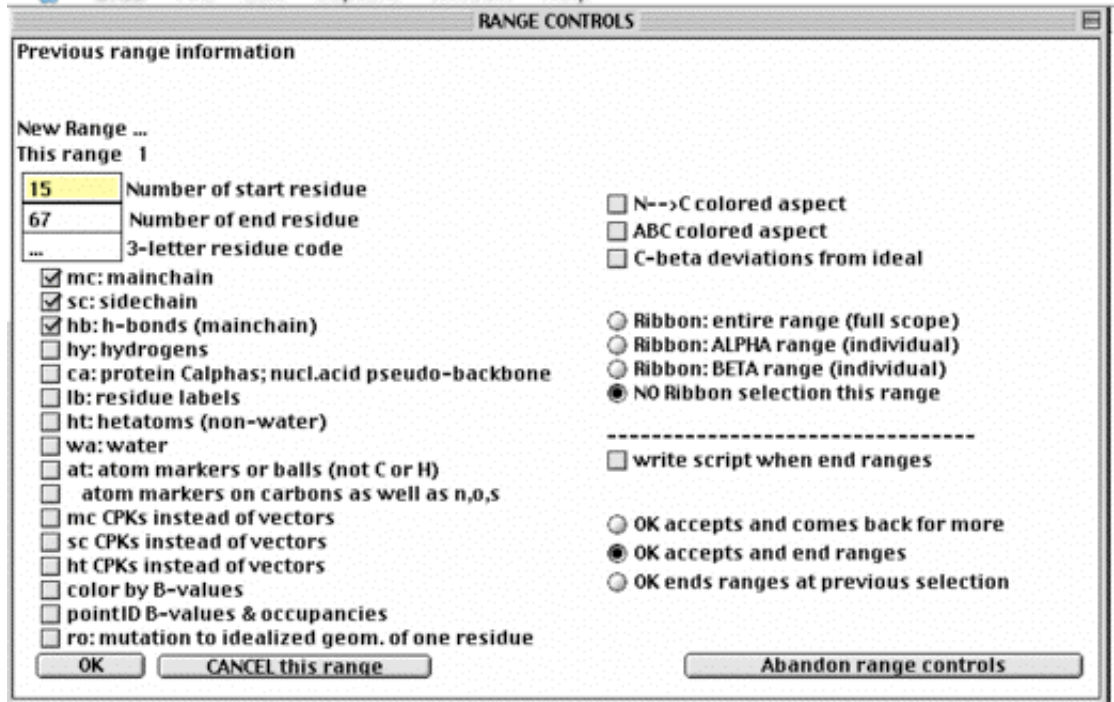
Start up Prekin again and with the same PDB file. This time name the kinemage to be generated as "`#xyzabc.kin`" (no CA).

On the Prekin dialog, choose range controls...

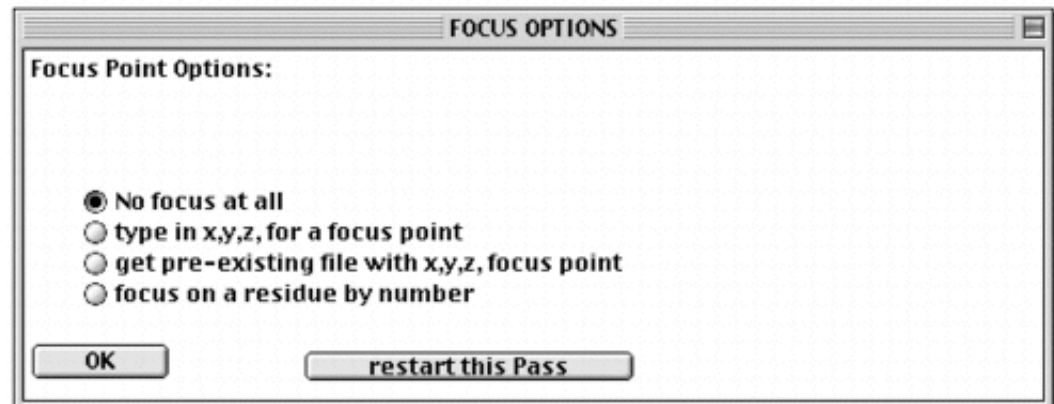




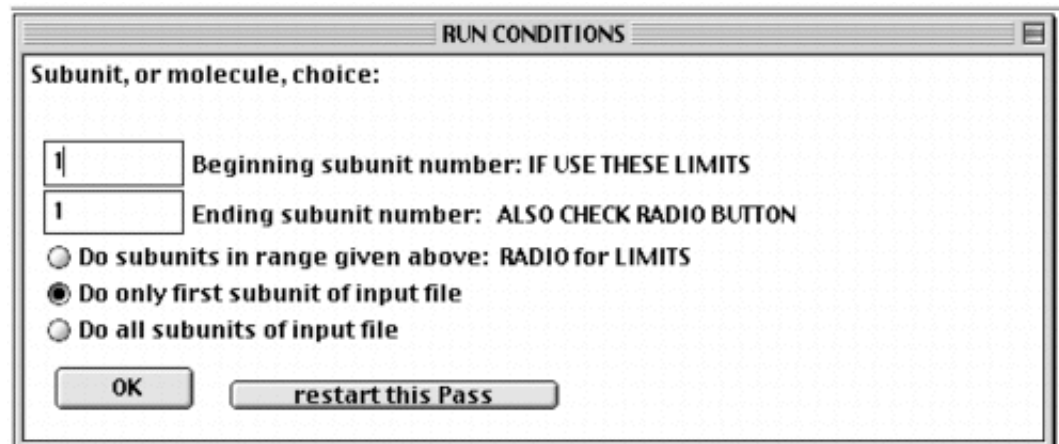
... specify the start and end numbers, check the "mc", "sc", and "hb" boxes; check the "OK accepts and end ranges" radio button; and **then** accept/OK ...



... In the next dialogs, ask for "no focus" ...



... and first subunit (unless you know you want a different subunit).



In Mage, identify the pair of β strands that forms the most convincing Greek key, either by length and H-bonding, or by Ray's Rule. How many β strands take part in the pair? _____

Is this an example where there is a clear and unambiguous choice of preferred strand pairing? _____

If so, does the best structural pairing match Ray's Rule or contradict it? _____

Find a good view; save and name it with "keep current view" on the Edit menu. On the Edit menu, turn on "Superpick" and "Draw New". Draw a green emphasis line on top of each H-bond that forms part of the special Greek key ribbon. Save your kinemage (with "save as" on the file menu) as "#xyzabc1.kin". Exit Mage. Check that your newly created files do exist. Remove "#xyzCAabc.kin" and "#xyzabc.kin"; keep the PDB and "#xyzabc1.kin"