

BCH222 :: Greek Key β Barrels

Reading

J.S. Richardson (1981) "The Anatomy and Taxonomy of Protein Structure, III.D. Antiparallel β Domains", [optional: II.C. TightTurns, II.D. Bulges], Adv. Prot. Chem. **34**, pp. 299-303.

[C.I. Branden and J. Tooze (1999) Introduction to Protein Structure, Second Edition, pp. 77-78 & 335-336 (look at the color figures, and if desired Kin 6 of [c16Virus.kin](#) (513KB))]

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. Ray's Rule says that for the privileged pair of β -hairpin strands that wind around together to form the Greek key or jellyroll, the sidechains between narrow H-bond pairs should point toward the inside core of the structure.

At the end, you will make your own kinemage of a chosen structure and analyze its Greek key. The control buttons, which appear on the right-hand side of the KiNG or Mage graphics window, appear in the text below [LIKE THIS](#).

1. Kinemage 4 of the kinemage file [StSurvey.kin](#) (412KB) 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

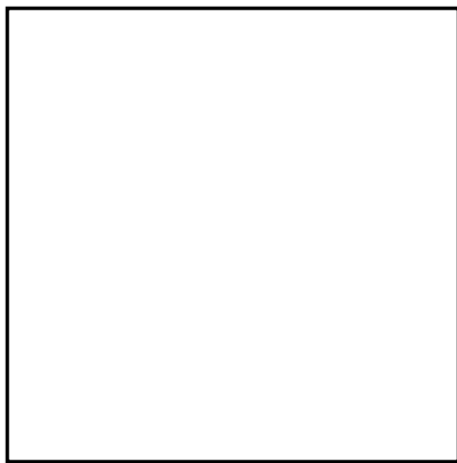
This barrel is not very cylinder-shaped, but its arrangement helps illuminate the folding of such structures. In View2, with [MAIN CH](#) on (turn off & on again) and [H-BONDS](#) on, just for domain 1, identify the 8 strands which make up the barrel. Imagine starting at the barrel N- and C-termini (residues 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 central 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?) _____



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. Exit and relaunch KiNG, and open kinemage file [1E43gk.kin](#), which 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 center, and zoom in somewhat. 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 (described in Anatax II.D.). 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? _____

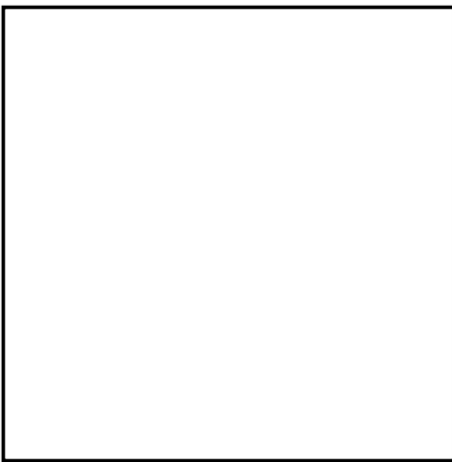
Identify a pair of residues (on neighboring strands) that are within a narrow pair of H-bonds: _____ and _____.

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 (includes residues in 430's and 460's). About how many degrees per residue does it twist? _____ To see that it also bends, look down the long axis of the two strands.

Turn off "end" and "sidechain". 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? _____ (turn sidechains back on to check this)

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

Enter at top of heirarchy and go to "All-beta proteins". Each person should pick one of the folds of at least 8 strands and notation of jelly-roll topology. Expand the outline a step, or click to enter the fold, 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. _____ Note the chain and range of residue numbers, if given (meaning the Greek key jellyroll is only part of the whole structure).

Navigate to the [PDB database site](http://www.rcsb.org/pdb/) (<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. Exit the browser.

Launch KiNG, and import your PDB file. In the dialog, pick the default C α Balls & stick, plus Ribbons for the relevant chain and residue range. View the kinemage, to see your jellyroll Greek key in context of the structure. Save a view of the jellyroll. Return to the Molikin dialog and add Ball & stick of backbone, sidechain, and N,O balls for your jellyroll chain and residue range. Identify the pair of β strands that forms the most convincing Greek key, judging by the number, length, and H-bond quality of the strands that can be included. How many β s strands take part in the jellyroll pair? _____

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

Center on a regular portion of a priveleged strand pair, zoom in, and z-clip to view ribbon, mc with N&C balls, and sc. Turn your strand pair to view it from the inside of the barrell, and locate a pair of sidechains pointed toward you; they are residues _____ and _____ Using your skills from the H-bond exercise, are they surrounded by a wide or a narrow pair of H-

bonds? _____ Choose Tools -> Edit/draw/delete -> Draw line segments, shorten by 0.5, and draw in the H-bond pairs around your sidechains and at least 2 more neighborin H-bonds along the ribbon. Save and name your closeup view of this sidechain and H-bond pair.

So, have you shown that this best strand pairing matches Ray's Rule or contradicts it? _____

Save your kinemage and email it to the TA.