

BCH222 - Beta Proteins: Two Important Folds

Assignment, part A: α/β Proteins - Singly-wound (α/β)₈ Barrels

Reading (skim all, and refer to them for questions)

Richardson, "Anatomy & Taxonomy of Protein Structure", parts II.B. β Structure, and III.C. Parallel α/β Domains (on-line)

Lesk, Branden, and Chothia (1989) "Structural Principles of α/β Barrel Proteins: The Packing of the Interior of the Sheet", *Proteins* **5**, 139-148 -- geometry of (α/β)₈ barrels

Babbitt et al (1995) "A functionally diverse superfamily that abstracts the α -protons of carboxylic acids", *Science* **267**, 1159-61 -- function & evolution of a group of (α/β)₈ barrels

Graphics

1. Kinemage file  [ab8barrel.kin](#) (52KB) :

Run through the animation of the triose P isomerase (TIM) barrel in Kin.1, to get a feeling for why it is described as "singly-wound". TIM is the first such (α/β)₈ barrel that was found, but this is now clearly one of the commonest protein folds, especially for enzymes. The active site is almost always at the "top" end of the barrel, with specificity binding extending down toward the central core and catalysis at the rim (altho above different-numbered strands in convergently related families).

Compare that TIM barrel with the two α/β barrel domains of IGPS-PRAI in Kin.2.

How many β strands are in each of the barrels? _____, _____, _____

Are all the β strands connected by +1x crossovers? _____

Does every crossover connection contain a helix? _____

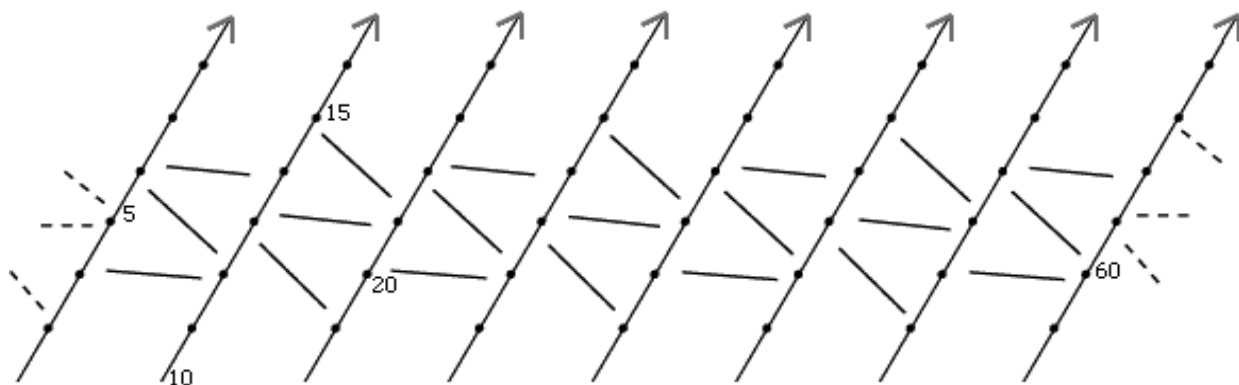
In file abBarrel.kin, kinemage 3 shows the type of interior packing typical of (α/β)₈ barrels. There are always at least three layers of hydrophobic sidechains, each layer perpendicular to the axis of the barrel, and sometimes there are additional hydrophilic layers on each end, as here.

What 4 residue types make up the central layer here? _____, _____, _____, _____

Why are there only 4 sidechains in each layer, rather than 8?

2. Kinemage file [babarel8.kin](#) (44KB)

Main chain, H-bonds, sequence labels, and a few numbers are shown. This is an idealized parallel $(\alpha/\beta)_8$ barrel modeled on TIM, pyruvate kinase, KDPG aldolase, taka-amylase, and glycolate oxidase. A very large number of parallel α/β -barrel proteins are known, all with 8 strands in the same singly-wound topology (with some minor exceptions). In this model structure all residues have the same ϕ , ψ (-114° , 124°). Strands were initially fit to a real barrel and then symmetrized as much as possible. (Departures from symmetry include some barrels being flattened and others round but conical.)



The above diagram shows the H-bonding pattern in babarel, as viewed from the outside and flattened into 2D. The H-bonds are, on average, perpendicular to the strands, so the strand twist gives the H-bond direction a twist, or effective offset from one strand to the next. Turn on & off the "row perp" button to see balls on the C β s for one row of 6 inward-pointing sidechains that are all adjacent in the direction perpendicular to the strands.

What is the total offset or shear (in residues) if one follows the perpendicular average H-bond direction all the way around the barrel back to the starting strand? _____

What is the offset per strand? _____

Turn on the side chains.

On the H-bond diagram, the dots represent α -carbons.

Circle the ones whose side chains extend toward the inside.

Using the Edit/draw/delete tool, draw lines (like the ones you saw in Kin.3 of abBarrel.kin) to connect the C α s for each of the two central layers of 4 internal sidechains.

Now find the two flanking layers of 4, and connect their C α s with dotted lines (change to 20 dots, since the lines are long). Afterward, turn on the "ref" button to compare and see how you did.

Each layer is made up of just one sidechain type in this simplified model; the 4 residue types are: _____, _____, _____, and _____.

Considering conformation, H-bonding, and sidechain direction, what is the rotational symmetry of this structure around the central axis of the barrel? (That is, is it 2-fold, 4-fold, 8-fold?) _____

3. Kinemage file [1tim_a.kin](#) (90KB) :

To start with, turn on just main chain and H-bonds, to compare this real barrel with the idealized babarel. Check that there are really 8 strands, that each connection is righthanded, and that they each move over by a single strand and all in the same direction.

Looking from the side of the barrel (view 2), estimate the angle (twist) between the strands in front and the ones in back. _____

Turn end-on to the barrel (view 1), so that in projection you see a central ellipse of beta strands surrounded by a ring of helices. The distance from one C α to the next is always a constant 3.8 Å, so that can be used as a yardstick to compare other distances.

Measure the flattening of the barrel cross-section by estimating its major and minor axes in C α -C α units.

_____ : _____

Turn on sidechains. The table below gives the composition of residue types on the inside surfaces of 3 singly-wound parallel beta barrels. What are the five commonest residue types here? (circle them)

G A P V I L M F Y W S T N D Q E K R H C
11 6 1 2 8 4 2 6 2 2 2 3 1 3 2 1 1 2 0 1

Large hydrophobics predominate, of course; however, one curious feature is the large number of glycines, which in general are found in turns and loops rather than regular secondary structures. Turn off "non-gly" to see the glycines.

Glycines have 3 unusual roles: 1) Flexibility; 2) Adoption of forbidden (usually positive Φ) conformations; and 3) Smallest side chain to fit in tight positions. In this case, flexibility is unlikely, the Gly Φ, Ψ values are more or less normal beta, and in many cases there are holes inside the barrel next to the Gly.

Turn "non-gly" back on, and see if you can find one of these holes, next to Gly ____.

From the babarel model building, we believe the function of these glycines is to relieve a bad contact between the internal C β and a CO on the neighboring strand.

4. Use a browser to visit the **SCOP** site, <http://scop.mrc-lmb.cam.ac.uk/scop/>

Enter the classification at the top of the heirarchy, go to "alpha and beta, a/b" and then to the TIM barrel fold. Do one level of outline expansion, to view down to the "family" level.

As of the October 2009 release 1.71, SCOP listed 33 superfamilies that have this fold. Most are enzymes and some entire metabolic pathways are populated with TIM barrels (such as the mandelate pathway of Babbitt et al).

Pick one of the specific TIM barrel PDB files in superfamilies 12-17 to download: what is its PDB code? _____
what is the protein's name? _____.

[If download from SCOP fails, get it from the PDB.]

Use KiNG to create and look at a simple kinemage of it (drag&drop the PDB onto the KiNG icon, ask for a ribbon kinemage. What aspects of this structure are classic and what is atypical for a TIM barrel? _____

[If it has more than one chain, just use the first one, or the one with fewer gaps. If you don't find your initial structure choice interesting or tractable, try another one.]

Assignment, part B: All- β Proteins - Greek Key β Barrels

Reading

Richardson "The Anatomy and Taxonomy of Protein Structure", part III.D. [Antiparallel \$\beta\$ Domains](#)" (on-line)

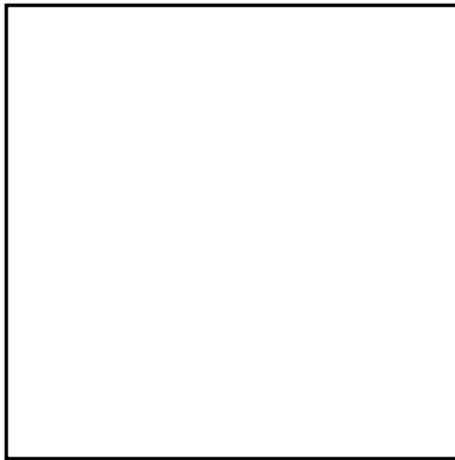
[Optional: Branden and Tooze (1999) [Introduction to Protein Structure](#), Second Edition, pp. 77-78 & 335-336 (look at the color figures, and Kin 6 of [c16Virus.kin](#) (513KB))]

Salemme (1983) "Structural Properties of Protein Beta Sheets", Prog. Biophys. Molec. Biol. **42**, several figures and pp. 127-130

Graphics

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.

1. The kinemage file [StSurvey_kin4.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 (otherwise), offset T shape: the common "helix-turn-helix" DNA-binding motif.
 - b. Topology
 This barrel is not very cylinder-shaped, but its arrangement helps illuminate the folding of such structures. In View2 with main chain on (turn off dimer helix and domain 2), 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. Turn on the barrel H-bonds.
 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 β sheets, rather than between the two terminal strands. Make a topology diagram of the barrel from the inside (as opened at the front in View 2 and laid flat). First draw 8 vertical lines for the β strands; then label the two termini (at lower left); then put direction arrows on the strands; then draw in their connections (using pencil is wise!).

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

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

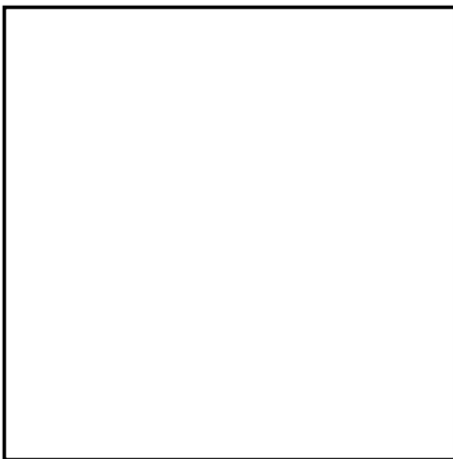
2. Exit and relaunch KiNG, and open kinemage file [1E43_GkKey.kin](#)(44KB), which contains one domain from the α -amylase enzyme of PDB file 1E43. In this structure, only 6 of the 8 strands form a Greek key topology, while the first 2 strands are a separate antiparallel hairpin ("end" button); this is also a commonly seen organization.

Remind yourself of the basic geometrical properties of antiparallel beta sheet. Pick a strand center and zoom in somewhat, or use View 2. 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? _____

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? _____

Turn off "end" and "sidechain", and use View 3 to look for the difference between twist and bend in this long, fairly isolated, 2-strand β -ribbon. A combination of both together makes an open, coiled structure (e.g. Salemme Figs. 22 & 23). To see the bend, drag to turn it around its long axis.

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. If those sidechains are hydrophobic, their interactions can help the long β -ribbon fold up. 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. Is that true here? _____



From View 4, draw a topology diagram of the amylase domain (just these 6 strands) with the barrel opened out and viewed from the inside. The N-terminus should be at lower left.

Like most Greek keys, the figure is potentially ambiguous between two possible neighboring choices for the central hairpin; for 1E43 the two choices are a hairpin near 454 between strands 3 and 4 or a hairpin near 464 between strands 4 and 5.

Use Views 5 and 6 to 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? _____