BCH622 - Beta Proteins: Two Important Folds

Assignment, part A: α/β Proteins - Singly-wound (α/β)$_8$ Barrels

Reading and references

Richardson, "Anatomy & Taxonomyof Protein Structure",
Navigate to various parts by returning to "Table of Contents" (Links in online worksheet not connected)
parts II.B. β Structure, , III.A.1. Principles esp layers (see Fig 71) α/β, III.A.3 Gallery of Drawings (Fig 72-86), and III.C. Parallel α/β Domains (on-line)

Po-Ssu Huang, ..., &David Baker (2015) "De-novo_design-of-symm-TIM-..." Nature Chemical Biology online: 23 November 2015

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 sidechains, each layer perpendicular to the axis of the barrel, and sometimes there are additional layers on each end, as here.
List the residue types that make up the three middle layers:

layer _____   , _____   , _____   , _____

central layer _____   , _____   , _____   , _____

layer _____   , _____   , _____   , _____

Circle the "hydrophobic" residues in your list.
If not all "hydrophobic" what might allow those to be buried in the center of this protein?

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

2. Kinemage file 📥 babarel8.20160228.kin (66KB)

babarel8.20160228.kin FILE has two kinemages: babarel8 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.)

babarel8 Kinemage #1: Main chain, H-bonds, sequence labels, and a few numbers are shown.
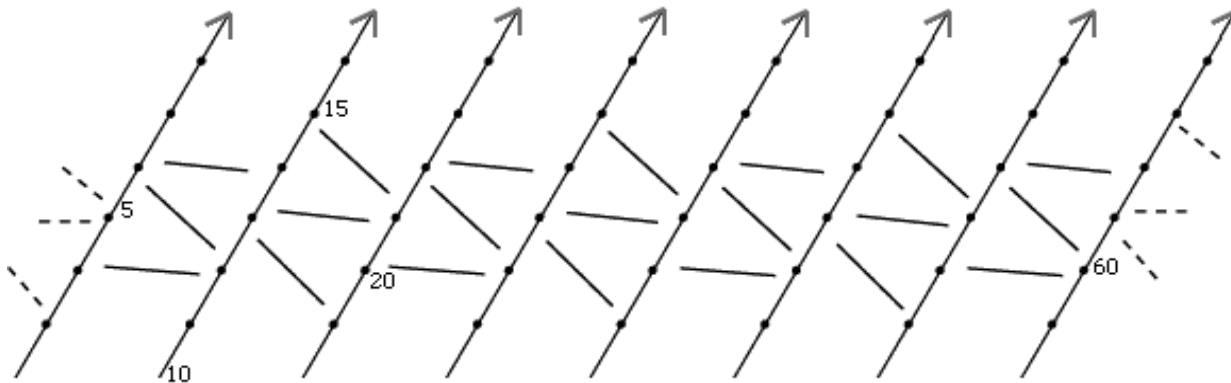
The above diagram shows the H-bonding pattern in babarel, as viewed from the outside and flattened into 2D with barrel axis vertical in the page. The H-bonds are, on average, perpendicular to the strands, so the strand twist gives the H-bond direction a twist, or effective offset from one strand to the next. Turn on & off the "row perp" button to see balls on the Cβs 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.

This design has a simple barrel interior, with 5 layers of 4 sidechains, each layer with 4 identical amino acids in identical conformations.
Using the Edit/draw/delete tool, draw lines (like the ones you saw in Kin.3 of abBarrel.kin) to connect the Cαs for each of the three most central layers of 4 internal sidechains.

Now find the two flanking layers, and connect their 4 Cαs 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 model; the 5 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?)   _____

babarel8 Kinemage #2 emphasizs the H-bonded pairs of strands that will guide us in getting the offset and twist for construction (rolling) the brass model of this structure.

Go through the views (like AB, BC, CD, ...) that place each pair in front and vertical. Note that alternatE sets (those with 5 H-bonds: AB, CD, EF, GH) are offset around the barrel axis. H-bonds between these pairs complete the cylinder barrel. Turn off and on "Other H-bonds" to see the completed pattern.
What is the offset in residues between the 5-H-bonded pairs? _____

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 Cα to the next is always a constant 3.8 Å, so that can be used as a yardstick to compare other distances.
Get a feeling for the flattening of the barrel cross-section by estimating its major and minor axes in Cα-Cα units. (Estimate by eye: this will help you develop your judgement about space and packing in proteins.)
approximate ratio: _____ : _____

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 find one of these holes: try Gly 42
(e.g. Edit/Find point... gly ca 42) What atom of what neighbor might clash if there were a side chain on this residue? _____   .

Estimate the size of the hole in the core next to this Gly in Cα-Cα units. _____
From the babarel model building, we believe one function of these glycines is to relieve a bad contact between the internal Cβ and a CO on the neighboring strand.

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4. Kinemage file 📥 5bvl1H-multi.kin.gz (747KB)
📥 Possu Huang design paper.pdf (2.3MB)

Now analyze the layers in the successful de-novo design-to-crystal structure of a "TIM" barrel protein. (i.e., layout the residue types in the inward pointing sidechains of this designed protein.)

What is the symmetry around the central axis of the design?_____

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

Reading

  Richardson "The Anatomy and Taxonomy of Protein Structure", part III.D. Antiparallel β Domains" (on-line)

Graphics: kinemages and their text windows

Read the text window for Kinemage 6 of Branden and Tooze supplement kins ⬇ c16Virus.kin (513KB)], and animate it in 3D from different View points.
[optional: Read and look at the other kins in c16Virus.kin.]

We will be looking at PDB entries 3GAP => 1G6N and 1E43 as a way of understanding what is said in the reading about Greek keys, plus "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 now superseded by 1G6N). 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.
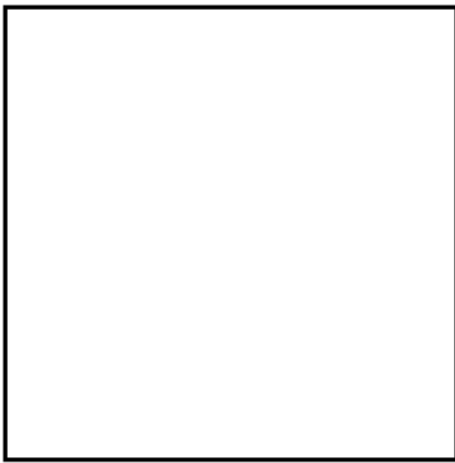   b. Topology
        This barrel is not very cylinder-shaped, but its arrangement helps illuminate the folding of such structures. In View2 with main chain on (turn off dimer helix and domain 2), identify the 8 strands which make up the barrel. Imagine starting at the barrel N- and C-termini (residues 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 <u>inside</u> (as opened at the front in View 2 and laid flat, with the first strand at left and pointing up). 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:

_____ _____ _____ _____ _____ _____ _____ _____

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_2016.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. Using 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 two pair of residues (on neighboring strands) that are within a narrow pair of H-bonds:
_____ and _____ or _____ and _____
How many of these 4 are hydrophobic? _____
Do they point inside or outside the β strands? _____
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. To see the bend, drag right 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. Using View 4, What are 2 hydrophobics on the inside of each strand (give residue type & number) _____ ; _____ ; _____ ; _____
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? _____