α/β structures
  • Closed barrels
  • Open twisted sheets
  • Horseshoe folds
The α/β domains

• Most frequent domain structures are α/β domains:
  ▫ A central parallel or mixed β sheet
  ▫ Surrounded by α helices
  ▫ All glycolytic enzymes, and proteins that bind and transport metabolites, have such structures.
  ▫ Binding crevice are formed by loop regions. These regions do not contribute to structural stability but in binding and catalytic action.

https://pdb101.rcsb.org/motm/50
Three main classes of \( \alpha/\beta \) proteins

1) TIM barrel (alpha/beta barrel)
   - A core of twisted parallel \( \beta \) strands arranged like staves of a barrel
   - The \( \alpha \) helices connect the parallel \( \beta \) strands are on the outside of this barrel.

2) Rossman fold (open sheet):
   - Open twisted \( \beta \) sheet
   - Surrounded by \( \alpha \) helices on both sides.

3) Horseshoe fold:
   - Repetitive regions of a specific pattern of “leucine rich motifs” --- forming \( \alpha \) helices & \( \beta \) strands
   - The \( \beta \) strands form a curving parallel \( \beta \) sheet (sometimes like a horseshoe).
   - All \( \alpha \) helices are on the outside.
All three are β-α-β motifs

Closed barrel

Open sheet

Horseshoe fold

248 residues, first beta strand starts at residue 6, last alpha ends at 246
How they differ: 2 βαβ can join in 2 ways

βαβ-Motif 1  βαβ-Motif 2

These experimental rules always apply, since βαβ motif is right handed

In both 2 motifs joined by green alpha helix

Barrels and horseshoes have this

Open sheets have at least one such alignment
Interesting stories

- In α/β structures where the strand order is 1 2 3 4, all connections are on the same side of the β sheet. An open β sheet of this sort with four or more parallel β strands would leave one side of the parallel β sheet exposed to the solvent and the other side shielded by the α helices.

Instead, a closed barrel of typically 8 twisted β strands is formed.

It has the α helices on the outside of the barrel, with β strand 8 hydrogen-bonded to strand 1.

Usually there is an alpha helix after the last beta strand
• The 8 beta strand barrel is one of the largest and most regular of all domain structures.
• Needs >200 aa.
• Many proteins have this, esp. enzymes, with completely different sequences/functions!
More interesting stories

- Superimposing the 8-stranded alpha/beta structures shows that around 160 residues are structurally equivalent – (they form beta strands and alpha helices.)

  The remaining residues form loops.

  The loops have very different lengths and conformations, thus for these enzymes:

  **Beta strands and alpha helices form structural framework**
  **Loops are for functions (catalytic chemistry)**

  Sometimes loops are very long and form independent domains.
1) The alpha/beta barrel

- the hydrophobic side chains of the alpha helices are backed against hydrophobic side chains of the beta sheet.

- The alpha helices are antiparallel and adjacent to the beta strands they connect.

- the side chains of the consecutive residues on beta strands are on the opposite sides of the beta sheet.

- The other side-chains point inside the barrel to form a hydrophobic core.

Glycolate oxidase: a typical alpha/beta barrel.
The packing interactions between alpha helices and beta strands are dominated by the residue Val, Ile, Leu, which have branched hydrophobic side chains.

These 3 amino acids comprise 40% of the residues of the beta strands in parallel beta sheets. (Arrows in the core or barrel, stars interact with alpha helices)

Some hydrophilic residue Lys, Arg, Gln having polar end group – these chains are in hydrophobic interior (chain) and goes to top of barrel with the hydrophilic end sticking out of the barrel.

Contribute to hydrophobic core
Exception

• No bulky hydrophobic residues to fill the core,
  ▫ has small hydrophilic side chains inside the barrel (serine and threonine) and has a hole in the middle.
• The coenzyme A (green) binds in the middle.

Methylmalonyl-coenzyme A mutase
Just as an example: Pyruvate kinase

- All known 8-stranded \(\alpha/\beta\)-barrel domains have enzymatic functions.
- Sometimes the barrel domain makes the whole domain.
- Some other times, the barrel is just a subunit – but it is always associated with some enzymatic function.
- 530 residue
A tale two functions: A double barrels have occurred by gene fusion!

- in *E. coli* performs two enzymatic activities in the biosynthesis of tryptophan:
  - PRA-isomerase (255-450 bottom barrel) / IGP-synthase (48-254, top barrel). (one polypeptide)

- *Bacillus subtilis* uses two separate sequences to do these two functions, each sequence is homologuous to the corresponding part, and forming two separate barrels. (2 function 2 polypeptide)

- *N. crassa* (fungi) **has an enzyme with 3 catalytic activities on the same sequence**, with two domains similar to that of *E. coli*, and linked to a 3rd domain

An e. coli protein involved in tryptophan Biosynthesis.
**Active site**

- In all alpha/beta barrels, the active site is at the bottom of a funnel shaped pocket created by **8 loops** that connect the C terminals of the beta strands and N terminals of the alphas.

- Difference in loop region determines the function!

- Sometimes, loop branch out a separate domain/subunit.

**Enzyme RuBisCo: CO$_2$ fixation in plants**

Blue side chains are charged residues from different loops.

In binding and catalysis in loop regions

Substrate (red), charged side chains (blue), Mg (yellow)
How do enzyme activities evolve?

2 enzymes similar in function but different in substrate specificity.

- Genome fragment duplication.
- Duplicated genes – one of them is free to mutate.

Alpha-beta barrel

- Only the loop region need to mutation to change the catalytic function and substrate specificity.
- Alpha helices and beta strands for structural stability.
- They are also excellent targets for in vitro protein engineering.

HOW?

By changing length and specific residue active site loop region, it might be possible to produce novel enzymes.
2) Horseshoe and leucine-rich motifs

- Leucine-rich motifs have been identified in over 60 different proteins including receptors, cell adhesion molecules, bacterial virulence factors, and molecules involved in RNA splicing and DNA repair.
A horseshoe structure (ribonuclease inhibitor) has 456 residues.

15 tandem (homologous) leucine-rich motifs

- Type A: 29 residues
- Type B: 28 residues
- There are 2 other nonhomologous part at two ends

Beta strands form parallel beta sheet

All alpha helices are on the outside forming hydrophobic core with beta strands as before, and with leucine: **leucine 2,5,7 from beta** pack against **leucine 20,24 from alpha**. Leucine 12 on loop is also part of hydrophobic core, and 17 on helix.

The other side of beta sheet faces solution, different from barrel.
Leucine-rich motif form an α/β-horseshoe fold

Figure 4.11 Schematic diagram of the structure of the ribonuclease inhibitor. The molecule, which is built up by repetitive β-loop-α motifs, resembles a horseshoe with a 17-stranded parallel β sheet on the inside and 16 α helices on the outside. The β sheet is light red, α helices are blue, and loops that are part of the β-loop-α motifs are orange. (Adapted from B. Kobe et al., Nature 366: 751–756, 1993)
3) Twisted open sheet with alpha helices on both sides

When the alpha helices are on **both sides** of the beta sheets, there are 3 consequences:

- A closed barrel cannot be formed – never found and unlikely to occur as a large number of beta strands are needed to enclose an alpha helix.

- There is always a binding crevice created by two beta strands from opposite sides.

- Each beta strand contributes hydrophobic side chains to pack against alpha helices in two similar hydrophobic core regions, one on each side of the beta sheet.
Open beta sheet has many variations

- Comparing to $\alpha/\beta$ barrel, which has the basic 8 alpha and 8 beta arrangement, open beta sheet has much more variations.

- Number of beta strands varies from 4 to 10.

- Two beta strands joined by crossover does not need to be neighbors, although that is preferred.

- There can also be mixed beta sheets, with hairpin connections giving antiparallel beta strands – next 4 figures.
Open twisted α/β structures: The FMN-binding redox protein flavodoxin
Open twisted α/β structures: The enzyme adenylate kinase

Catalyzes the reaction
AMP + ATP ⇔ 2ADP
Open twisted $\alpha/\beta$ structures: The ATP-binding domain of glycolytic enzyme hexokinase

Catalyzes the phosphorylation of glucose.
Open twisted α/β structures: The glycolytic enzyme phosphoglycerate mutase

Catalyzes the transfer of a phosphoryl group from carbon 3 to carbon 2 in phosphoglycerate

Although also a reverse here, but there is no crevice -- antiparallel.
Active sites for open α/β-sheet proteins

- The **barrel structure** active site can be predicted, with **funnel shaped** active site.

- For the **open α/β-sheet** structures, this can also be predicted – they form crevices at the edge of β-sheet.
  - The crevice occurs when there are 2 adjacent connections that are on opposite sides of the β-sheet.
  - Two strands: one goes from above, one below – observe the previous pictures.
  - This is called “**topological switch point**”, postulated by Carl Branden in 1980. Since then, almost all new α/β structures have been found to have active sites at such crevices.

This is in contrast to the other two main classes: α helical proteins and antiparallel β proteins: no such predictive rules have been found for them.
Story of Tyrosyl-tRNA

- A crucial step of protein synthesis: a group of enzymes (aminoacyl-tRNA synthetase) connect each amino acid with its specific tRNA.

- Applying the rules we learned: strands 2 and 5 form crevice (switch point), active site.
Open Twisted or Rossman Fold

- [http://blogs.oregonstate.edu/psquared/files/2012/04/Rossmann1.gif](http://blogs.oregonstate.edu/psquared/files/2012/04/Rossmann1.gif)
Conclusion

- Alpha/beta structures are the most frequent and most regular structures.
- We discussed three classes.
- The barrels are one of the largest and most regular domain structures, 250 residues.
- Found in many proteins with completely different amino acid sequences and functions. All enzymes. 8 betas surrounded by 8 helices. Active sites all same place – carboxy end. Functions depend on lengths of loops; loops do not affect structure stability.
- Horseshoe is from homologous repeats of leucine-rich motifs, each is a beta-loop-alpha unit. Alpha helices on the outside and curved beta sheet inside – exposed to solvent.
- The open alpha/beta structures varies more. But all their active sites are at the carboxy edge of the beta strand, with a special crevice between two parallel beta strands (their loops to opposite alpha, because of a turn)
- Unlikely to barrel structure active site, they are formed in those regions outside the carboxy edge of the beta sheet.