

# Alpha-Beta Structures

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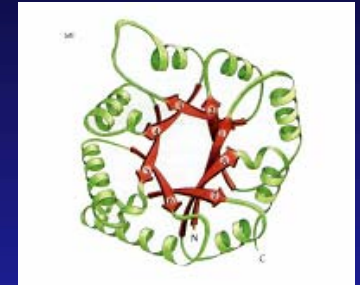
- are the most frequent
- consist of a central parallel or mixed  $\beta$  sheet surrounded by  $\alpha$  helices
- are found in
  - all glycolytic enzymes
  - many other enzymes as well as proteins that bind and transport metabolites
- binding crevices are formed by loop regions; these regions do not contribute to the structural stability of the fold but participate in binding and catalytic action

## Alpha-Beta Structures

### Parallel $\beta$ strands are arranged in barrels or sheets

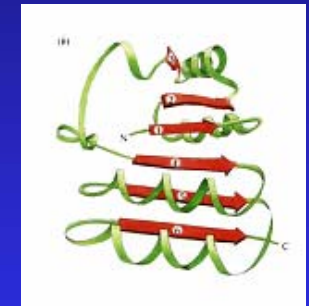
#### TIM barrel

In TIM barrels there is a core of twisted  $\beta$  strands arranged close together, like the staves of a barrel. The  $\alpha$  helices that connect the parallel  $\beta$  strands are on the outside of this barrel.



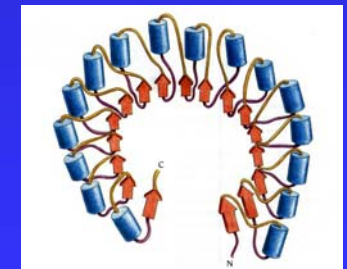
#### Rossmann fold

The Rossmann fold contains an open twisted  $\beta$  sheet surrounded by  $\alpha$  helices on both sides.

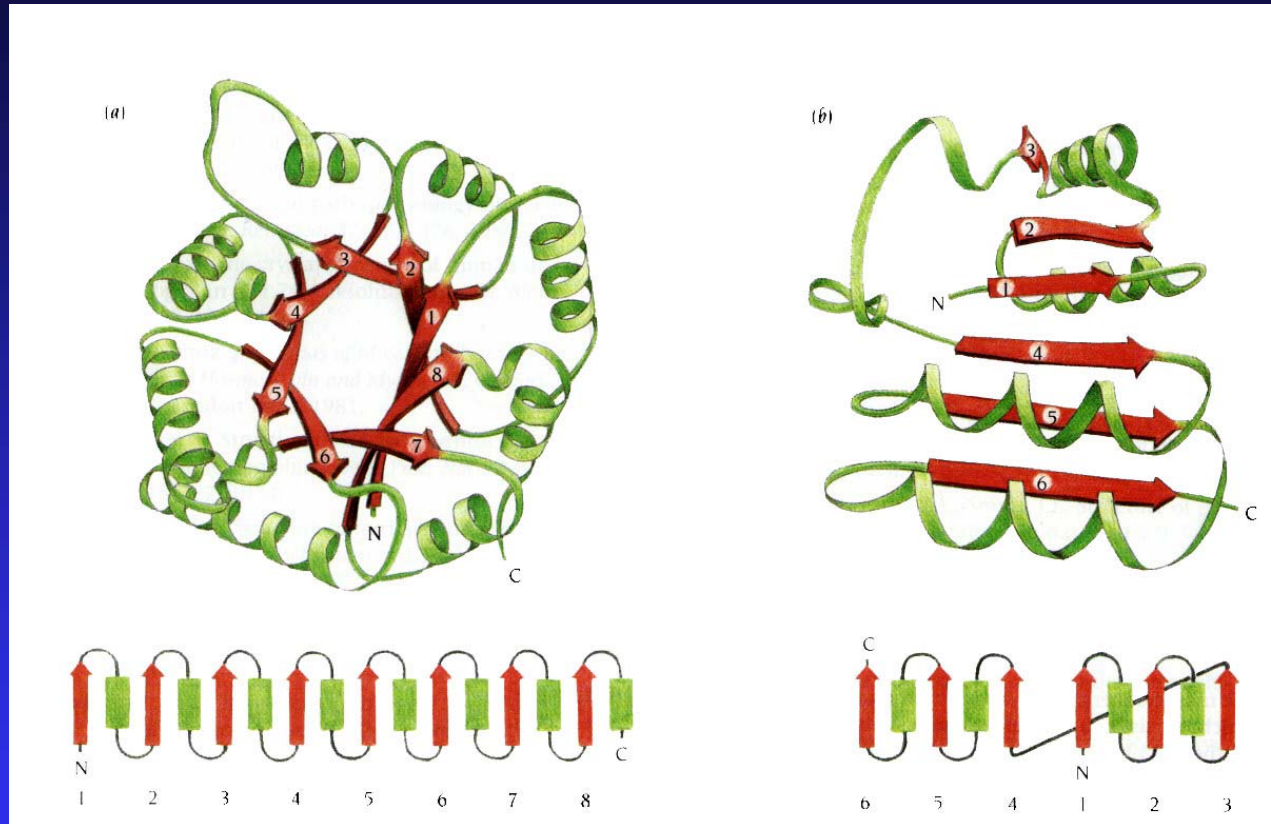


#### Horseshoe fold

The horseshoe fold is formed by amino acid sequences that contain repetitive regions of a specific pattern of leucine residues, so-called **leucine-rich motifs**, which form  $\alpha$  helices and  $\beta$  strands. The  $\beta$  strands form a curved parallel  $\beta$  sheet with all the  $\alpha$  helices on the outside.



## Parallel $\beta$ strands are arranged in barrels or sheets

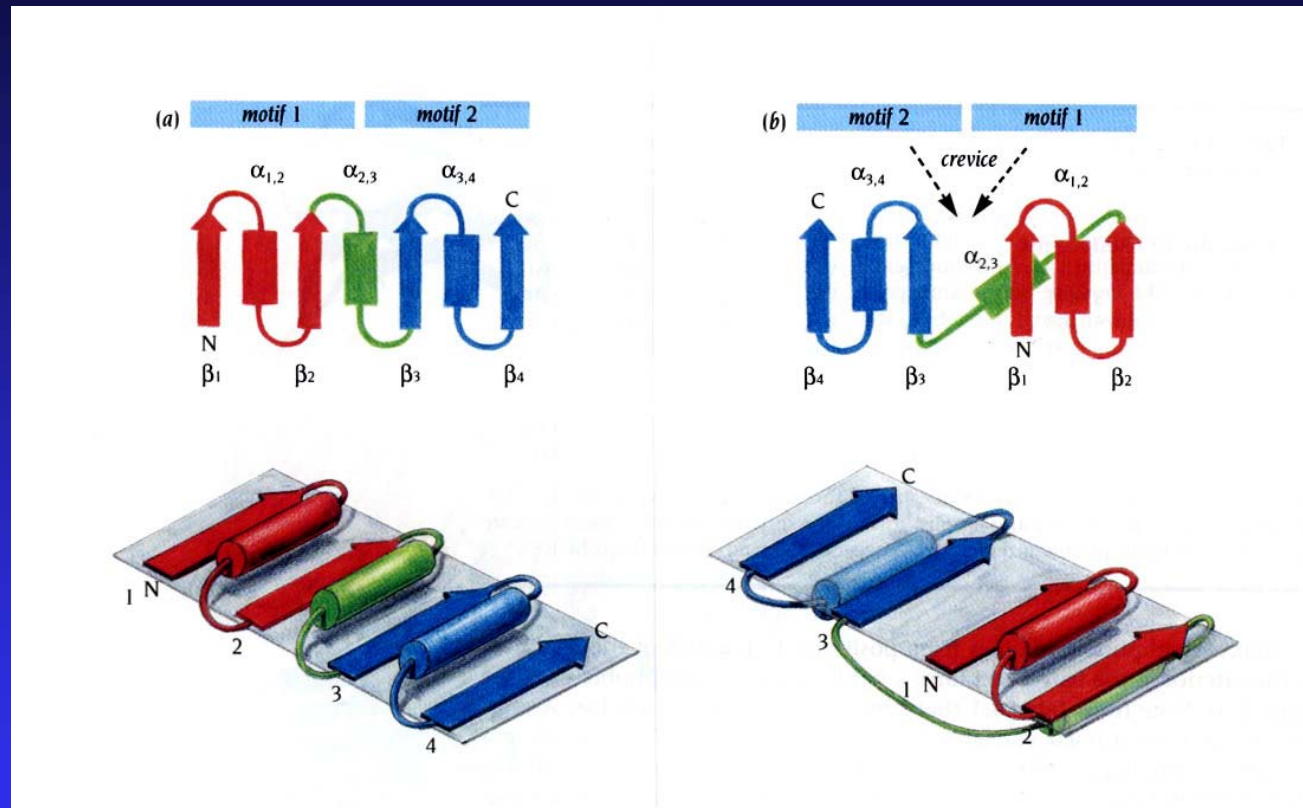


a) closed barrel exemplified by schematic and topological diagrams of the enzyme triosephosphate isomerase

b) an open twisted sheet with helices on both sides, as in coenzyme-binding domain of some dehydrogenases

Both classes are built up from  $\beta$ - $\alpha$ - $\beta$  motifs that are linked such that  $\beta$  strands are parallel.

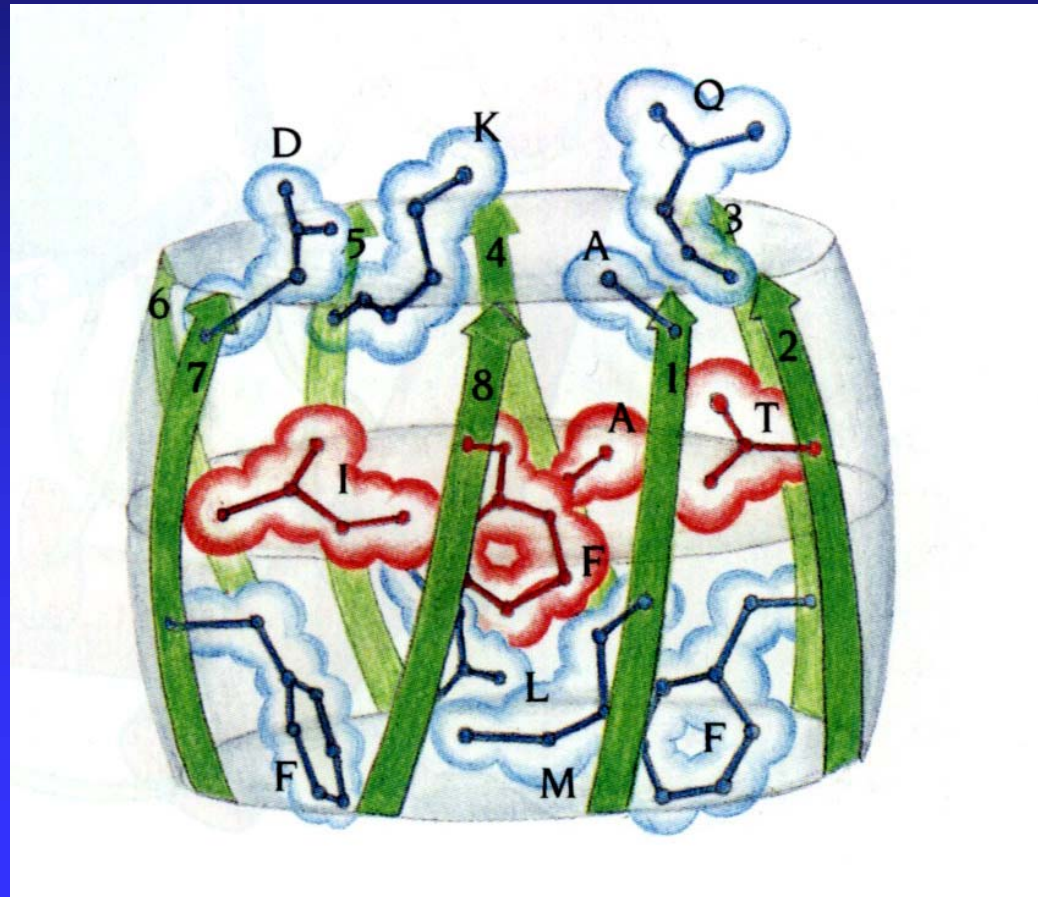
## Two $\beta$ - $\alpha$ - $\beta$ motifs can be joined into a four-stranded parallel $\beta$ sheet in two different ways



- a) The last  $\beta$  strand of motif 1 is adjacent to the first  $\beta$  strand motif 2, giving the strand order 1 2 3 4. The motifs are aligned in this way in barrel structures and in the horseshoe fold.
- b) The first  $\beta$  strands of both motifs are adjacent, giving the strand order 4 3 1 2. Open twisted sheets contain at least one motif alignment of this kind. In both cases the motifs are joined by an  $\alpha$  helix (green).

## Branched hydrophilic side chains dominate the core of $\alpha/\beta$ barrels

The core is arranged in three layers, with each layer containing four side chains from alternate  $\beta$  strands as shown for the enzyme glycolate oxidase.





## The packing interactions between $\alpha$ helices and $\beta$ strands are dominated by Val, Ile, and Leu

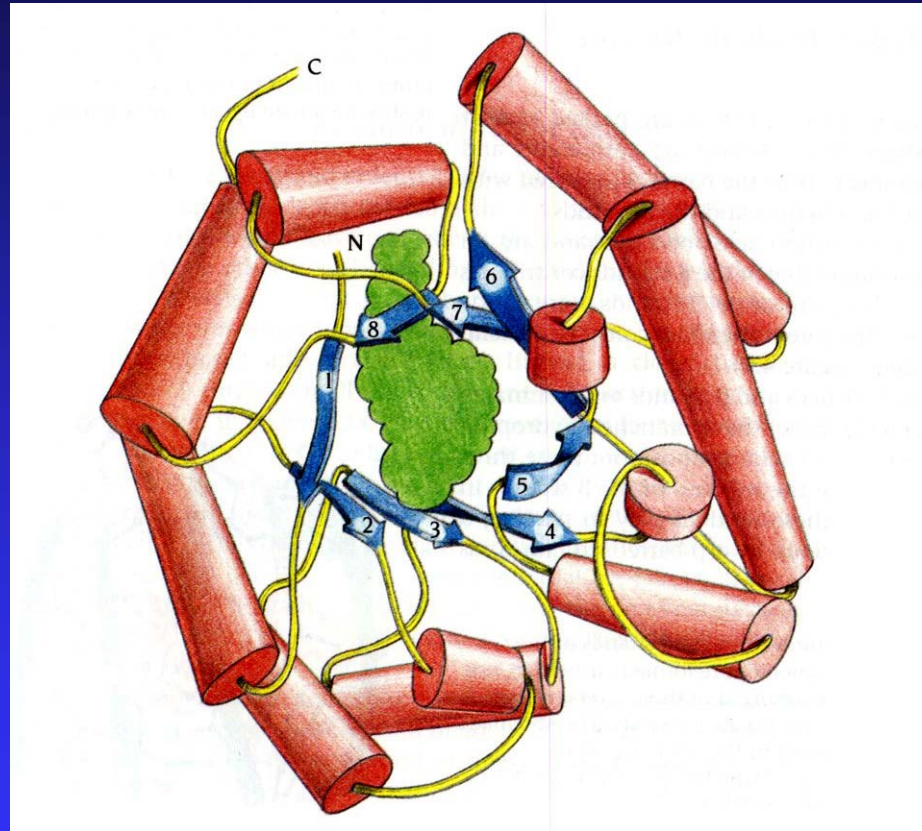
**Table 4.1** The amino acid residues of the eight parallel  $\beta$  strands in the barrel structure of the enzyme triosephosphate isomerase from chicken muscle

Strand no.	Residue no.	Positions				
		1	2	3	4	5
1	6–10	Phe	Val	Gly	Gly	Asn
2	37–41	Glu	Val	Val	Cys	Gly
3	59–63	Gly	Val	Ala	Ala	Gln
4	89–93	Trp	Val	Ile	Leu	Gly
5	121–125	Gly	Val	Ile	Ala	Cys
6	158–162	Lys	Val	Val	Leu	Ala
7	204–208	Arg	Ile	Ile	Tyr	Gly
8	227–231	Gly	Phe	Leu	Val	Gly

The sequences are aligned so that residues in positions 1, 3, and 5 point into the barrel and residues in positions 2 and 4 point toward the  $\alpha$  helices on the outside and are involved in the hydrophobic interactions between the  $\beta$  strands and the  $\alpha$  helices.

This is reflected in the amino acid composition: these three amino acids comprise approximately 40% of the residues of the  $\beta$  strands in parallel  $\beta$  sheets.

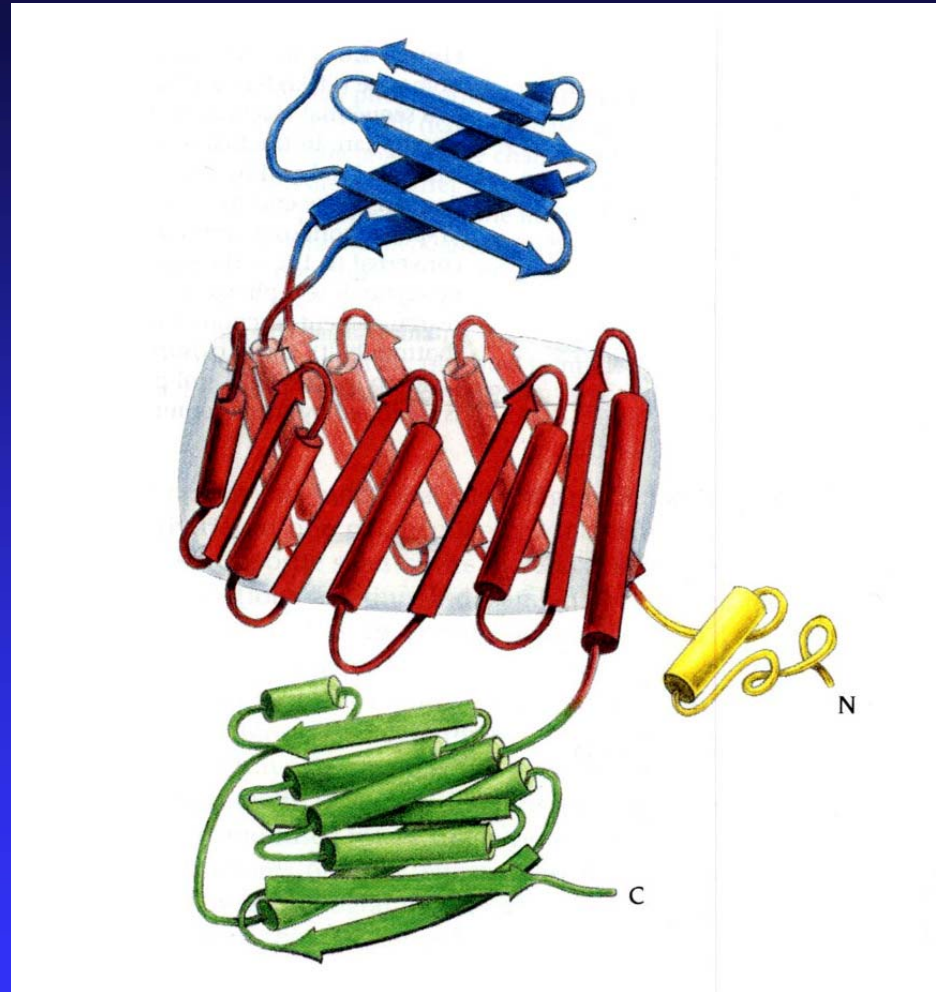
Methylmalonyl-coenzyme A mutase has a whole  
in the middle of its  $\beta/\alpha$ -barrel domain



The inside of the barrel is lined by small hydrophilic side chains (Ser, Thr) from the  $\beta$  strands, which creates a hole in the middle where one of the substrate molecules, coenzyme A (green), binds along the axis of the barrel from one end to the other.

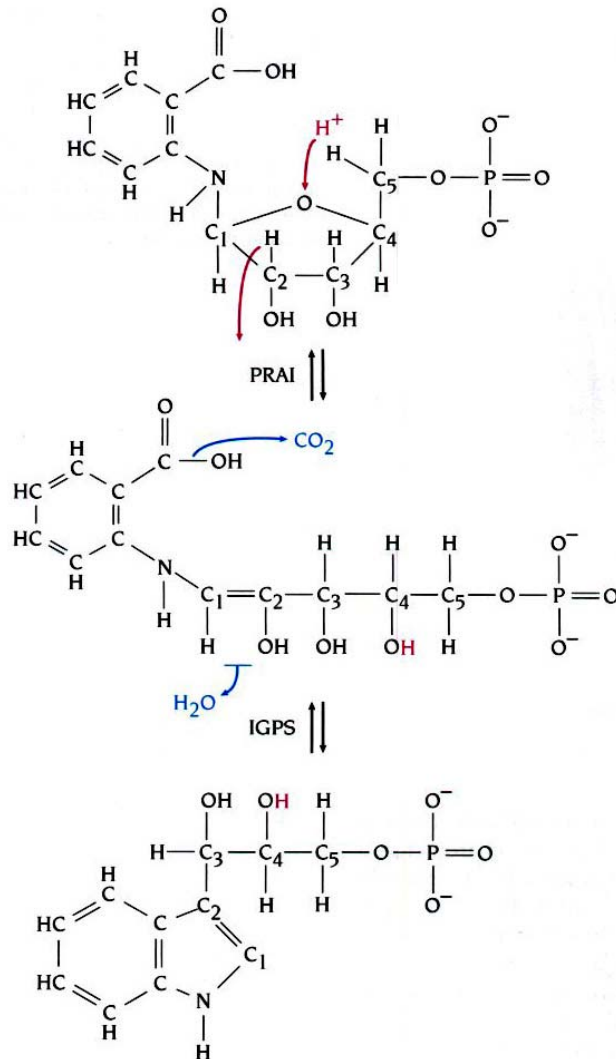


Pyruvate kinase contains several domains,  
one of which is an  $\alpha/\beta$  barrel



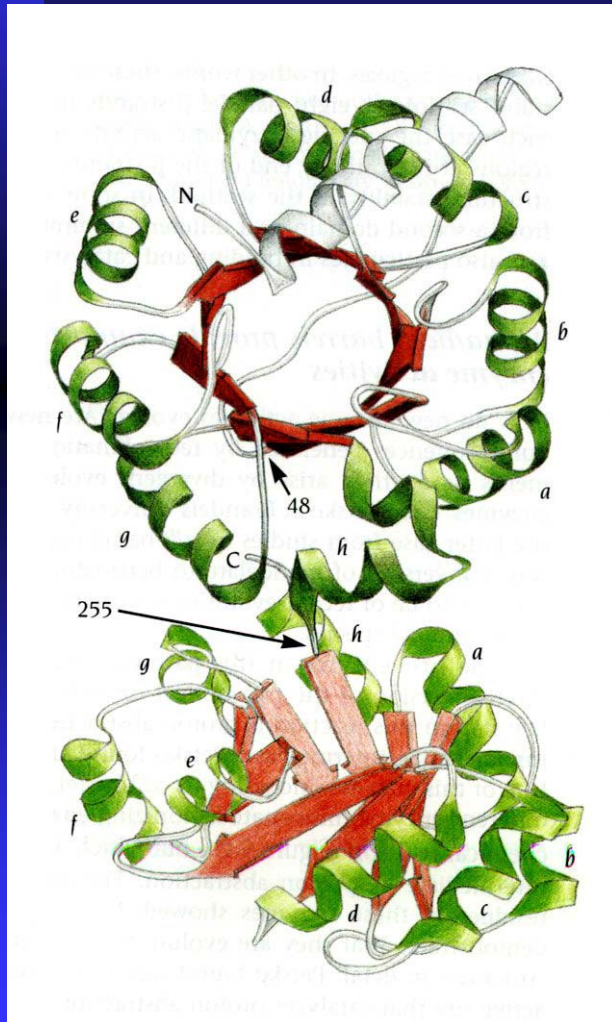
This structure illustrates perfectly how a long polypeptide chain can be arranged in domains of different structural types.

## Two sequential reactions in tryptophan biosynthesis are catalyzed by a bifunctional enzyme in *E. coli*



In the first reaction which is catalyzed by the **C-terminal PRA-isomerase domain** of the enzyme, the substrate **N-(5'-phosphoribosyl) anthranilate (PRA)** is converted to **1-(o-carboxyphenylamino)-1-deoxyribulose 5-phosphate (CdRP)** by a rearrangement reaction. The succeeding step, a ring closure reaction from **CdRP** to **indole-3-glycerol phosphate (IGP)**, is catalyzed by the **N-terminal IGP-synthase domain**.

## PRA-isomerase and IGP-synthase activities are performed by two separate domains in the polypeptide chain of a bifunctional enzyme PRA:IGPS in *E. coli*

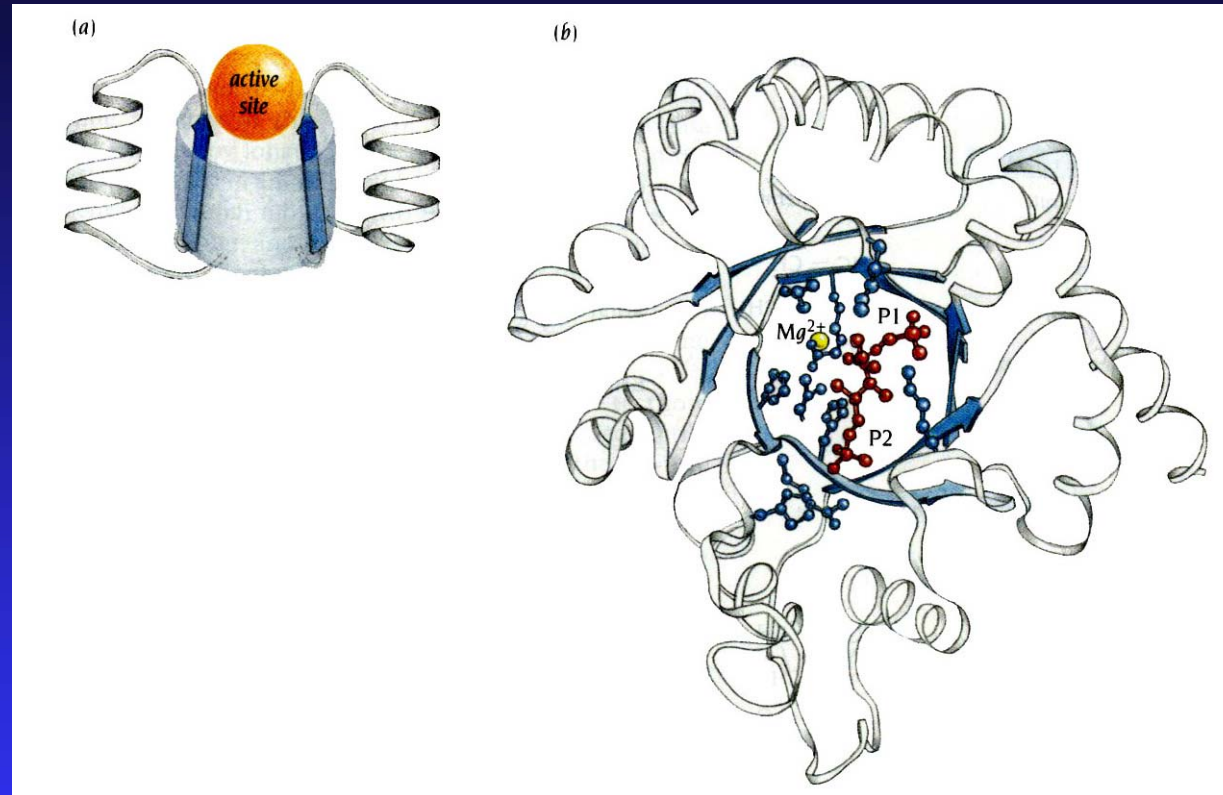


Both these domains are  $\alpha/\beta$ -barrel structures, oriented such that their active sites are on opposite sides of the molecule. The two reactions are therefore independent of each other.

In *Bacillus subtilis* these two reactions are catalyzed by **two separate enzymes** that have amino acid sequences homologous to the corresponding regions of the enzyme from *E. coli*. *Neurospora crassa* has an enzyme with **three catalytic activities within the same polypeptide chain**; here two domains similar to those of *E. coli* enzyme are linked to a third domain that has yet another enzymatic function in the same biosynthetic pathway.

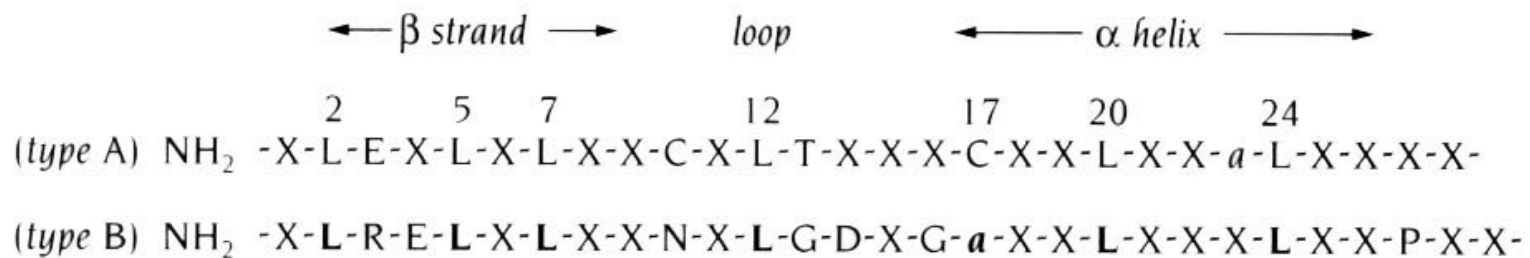
**These differences between the species reflect different ways to organize the genome.** DNA sequences that code for protein domains with different functions are organized into separate genes in one organism and fused into a single gene in another.

## The active site is formed by loops at one end of the $\alpha/\beta$ barrel



- a) The active site in all  $\alpha/\beta$  barrels is in a pocket formed by the loop regions that connect the carboxy ends of the  $\beta$  strands with the adjacent  $\alpha$  helices.
- b) A view from the top of the barrel of the active site of the enzyme RuBisCo (ribulose biphosphate carboxylase), which is involved in  $CO_2$  fixation in plants. A substrate analog binds across the barrel with the two phosphate groups, P1 and P2, on opposite sides of the pocket. A number of charged side chains (blue) from different loops as well as a  $Mg^{2+}$  ion form the substrate-binding site and provide catalytic groups.

## Leucine-rich motifs form an $\alpha/\beta$ -horseshoe fold

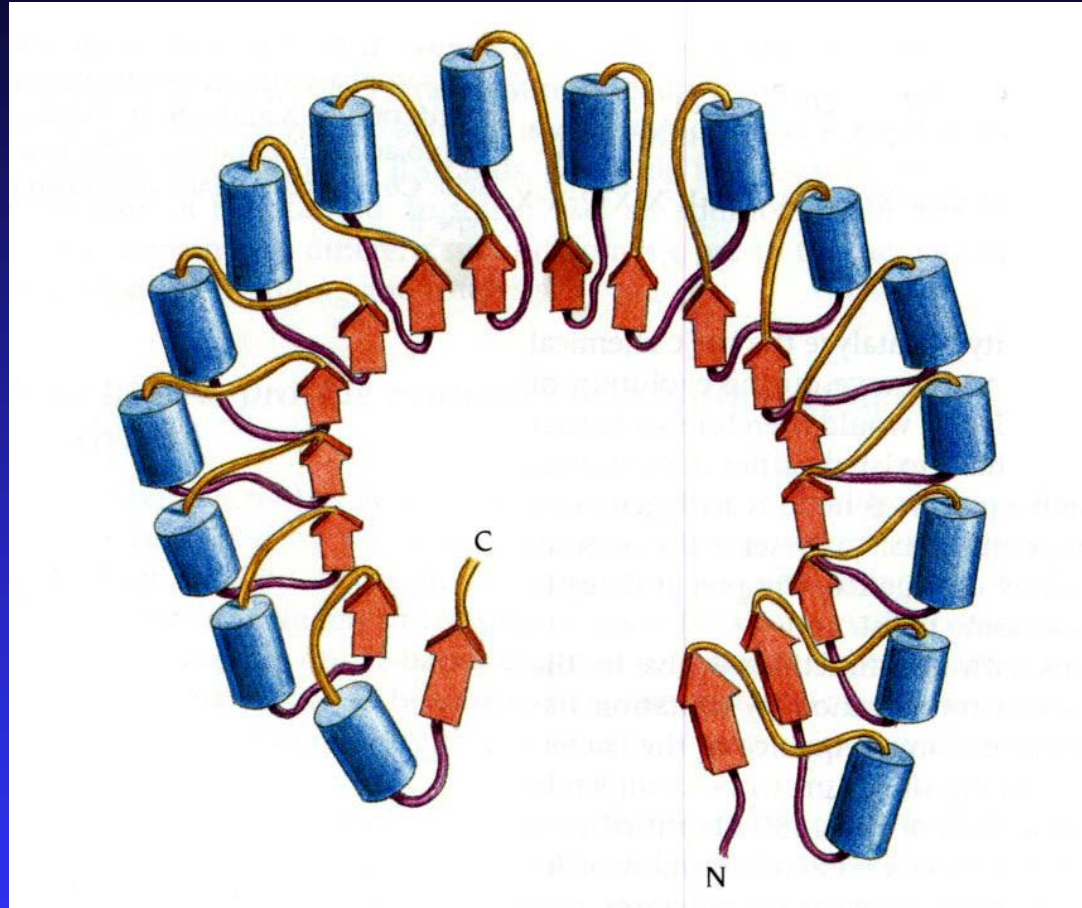


Consensus amino acid sequence and secondary structure of the leucine-rich motifs of type A and B. "X" denotes any amino acid; "a" denotes an aliphatic amino acid. Conserved residues are shown in type B.

The leucine-rich motifs have been identified from sequence studies in over 60 different proteins, including receptors, cell adhesion molecules, bacterial virulence factors, and molecules involved in RNA splicing and DNA repair.



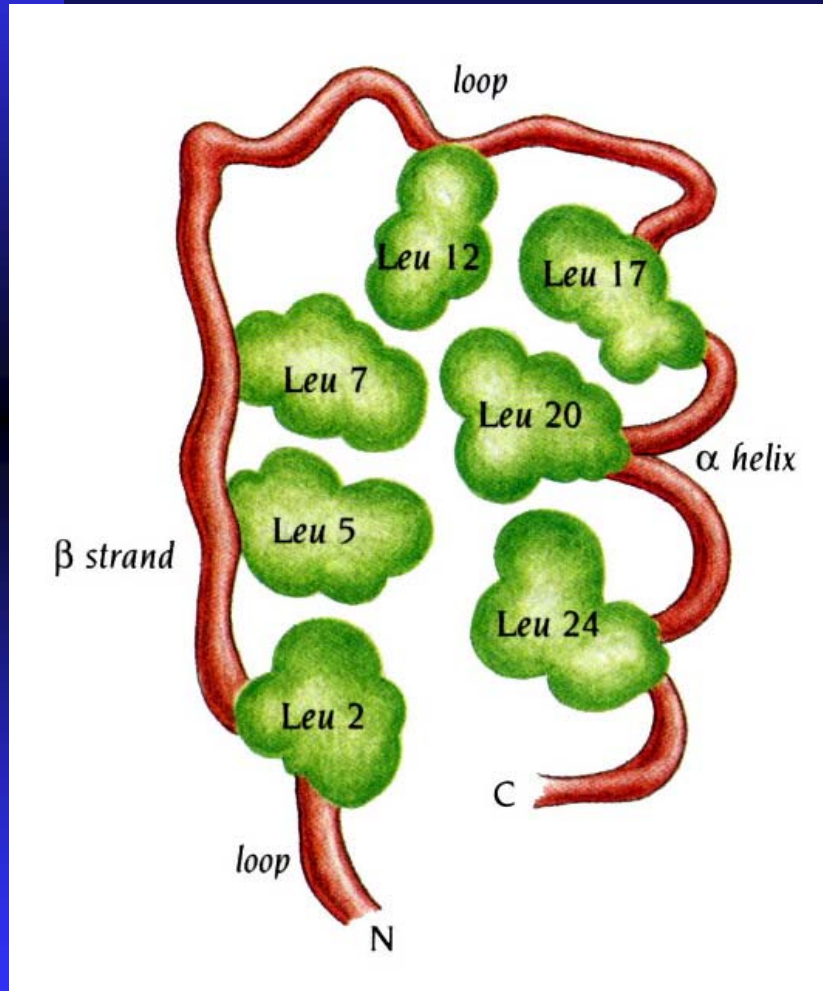
## Leucine-rich motifs form an $\alpha/\beta$ -horseshoe fold



Schematic diagram of the structure of the **ribonuclease inhibitor**. The molecule, which is built up by **repetitive  $\beta$ -loop- $\alpha$  motifs** (**leucine-rich motifs of A and B types that alternate along the chain**), resembles a horseshoe with a **17-stranded parallel  $\beta$ -sheet** on the inside and **16  $\alpha$  helices** on the outside

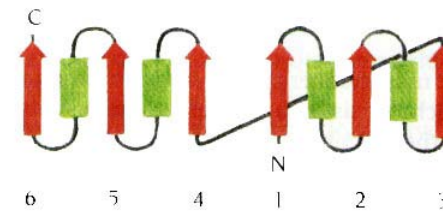
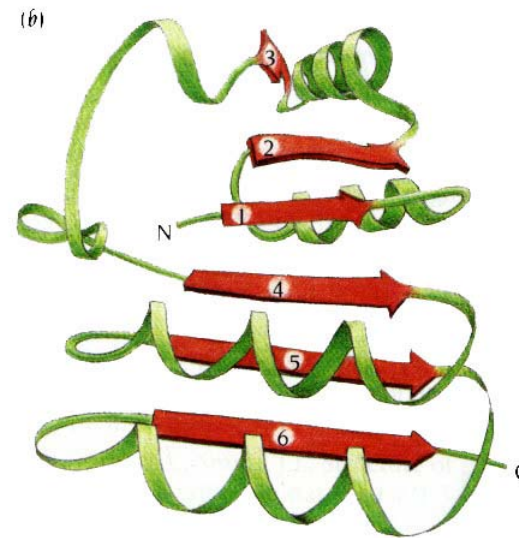
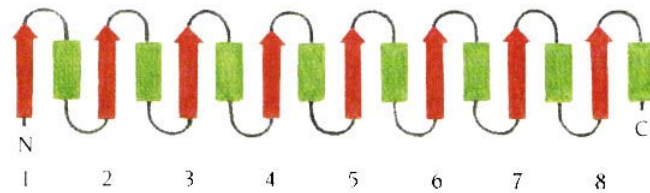
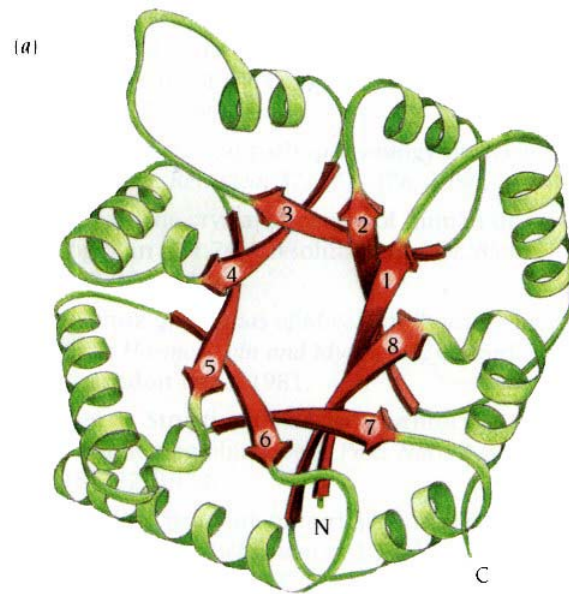


## Leucine-rich motifs form an $\alpha/\beta$ -horseshoe fold

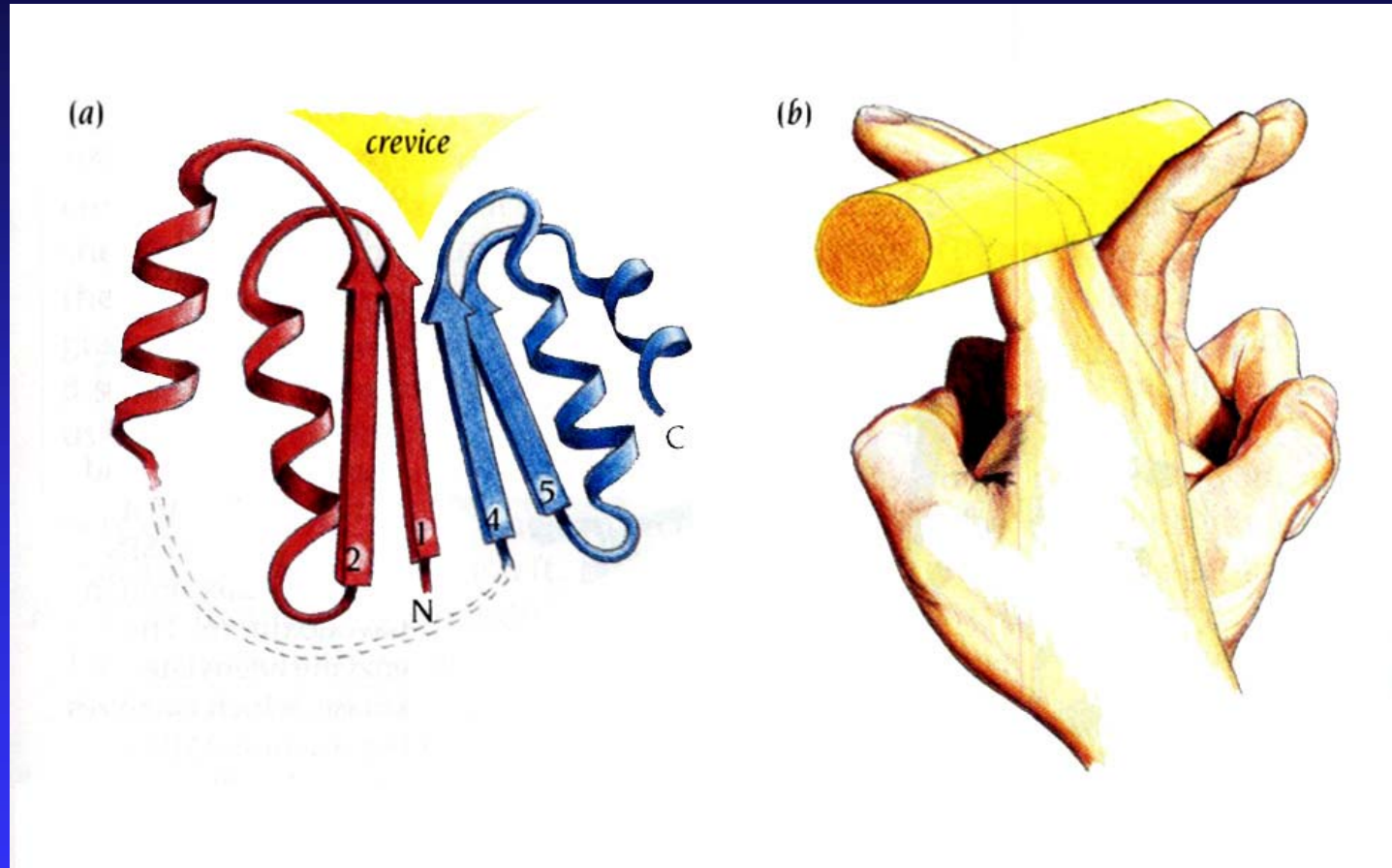


Schematic diagram illustrating the role of the conserved leucine residues in the leucine rich motif in stabilizing the  $\beta$ -loop- $\alpha$  structural module. In the ribonuclease inhibitor, leucine residues 2, 5, and 7 from the  $\beta$  strand pack against leucine residues 17, 20, and 24 from the  $\alpha$  helix as well as leucine residue 12 from the loop to form a hydrophobic core between the  $\beta$  strand and the  $\alpha$  helix.

Alpha/beta twisted open-sheet structures contain  $\alpha$  helices on both sides of the  $\beta$  sheet



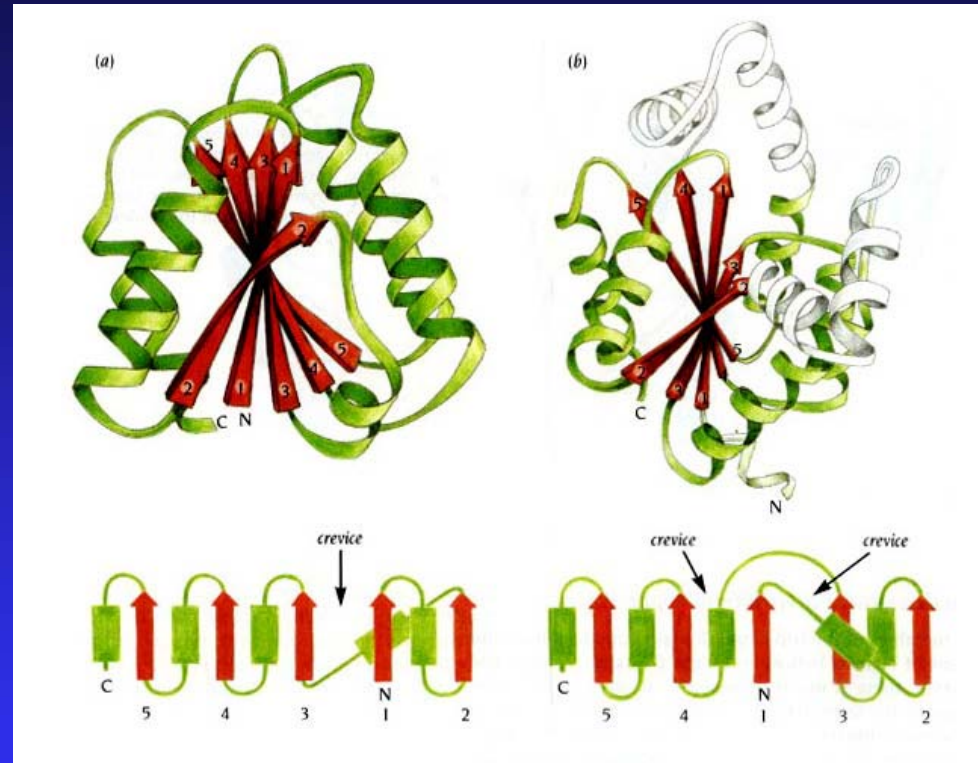
The active site in open twisted  $\alpha/\beta$  domains is in a crevice outside the carboxy ends of the  $\beta$  strands



The crevice is formed by two adjacent loop regions that connect the two strands with  $\alpha$  helices on opposite sites of the  $\beta$  sheet.

Open  $\beta$ -sheet structures have a variety of topologies, however, the positions of active sites can be predicted in the structures

a) The FMN-binding protein flavodoxin. The loops from  $\beta$  strands 1 and 3 to their respective  $\alpha$  helices form the major part of the binding cleft for the coenzyme FMN (flavin mononucleotide).

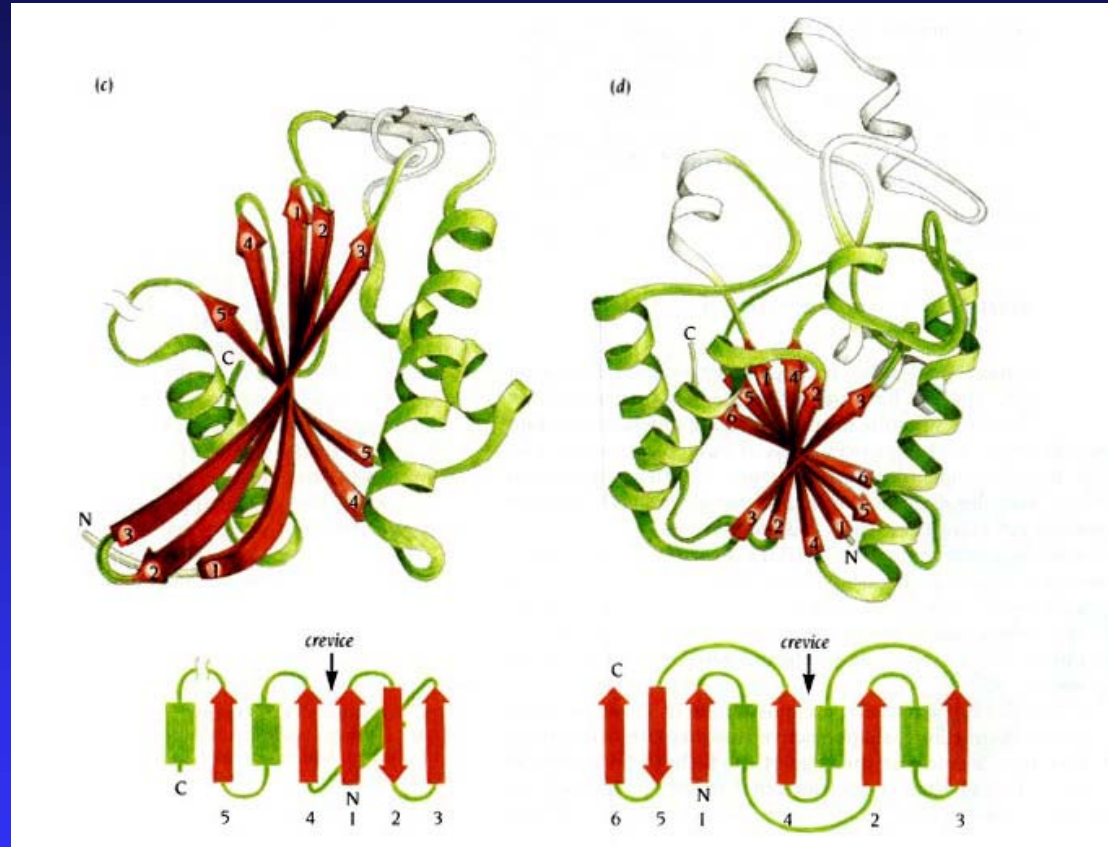


b) The enzyme adenylate kinase, which catalyzes the reaction  $\text{AMP} + \text{ATP} = 2 \text{ ADP}$ . Crevices are formed between  $\beta$  strands 1 and 3 and between strands 1 and 4. One of these crevices forms part of an AMP-binding site, and the other crevice forms part of an ATP-binding site that catalyzes the formation of ADP from AMP and ATP.



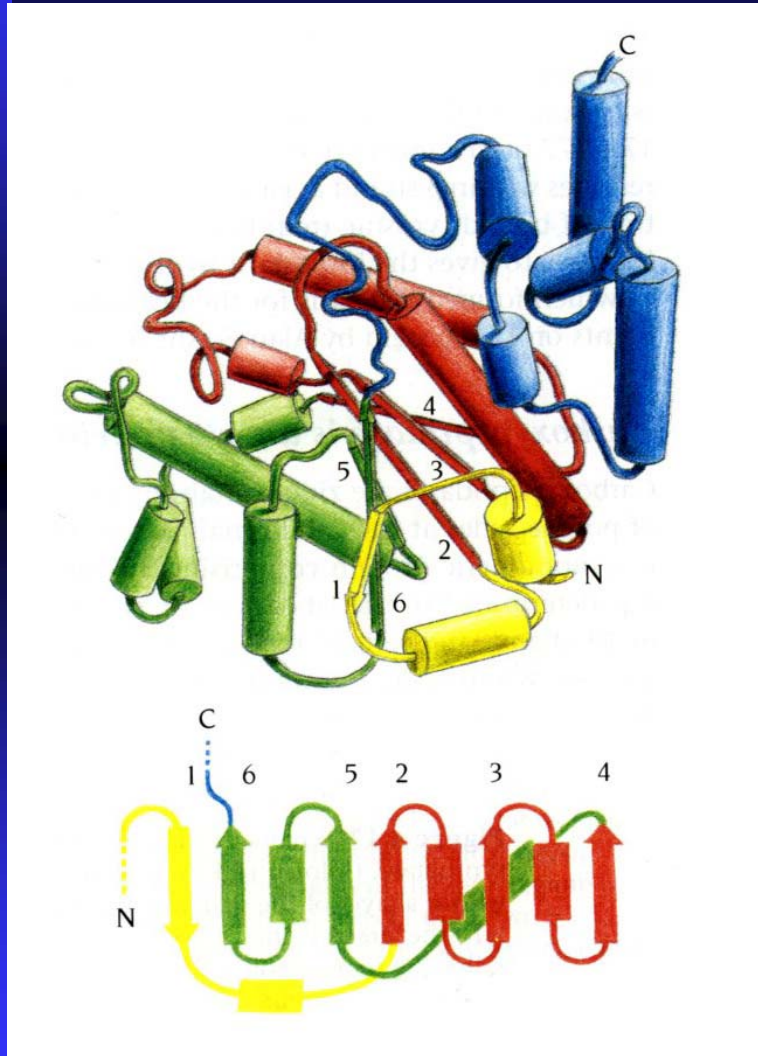
Open  $\beta$ -sheet structures have a variety of topologies, however, the positions of active sites can be predicted in the structures

c) The ATP-binding domain of the glycolytic enzyme hexokinase, which catalyzes the phosphorylation of glucose. The loops from  $\beta$  strands 1 and 4 to their respective  $\alpha$  helices form a part of the enzyme active center.



d) The glycolytic enzyme phosphoglycerate mutase, which catalyzes transfer of a phosphoryl group from carbon 3 to carbon 2 in phosphoglycerate. The loops from  $\beta$  strands 2 and 4 to their respective  $\alpha$  helices form a part of the enzyme active center

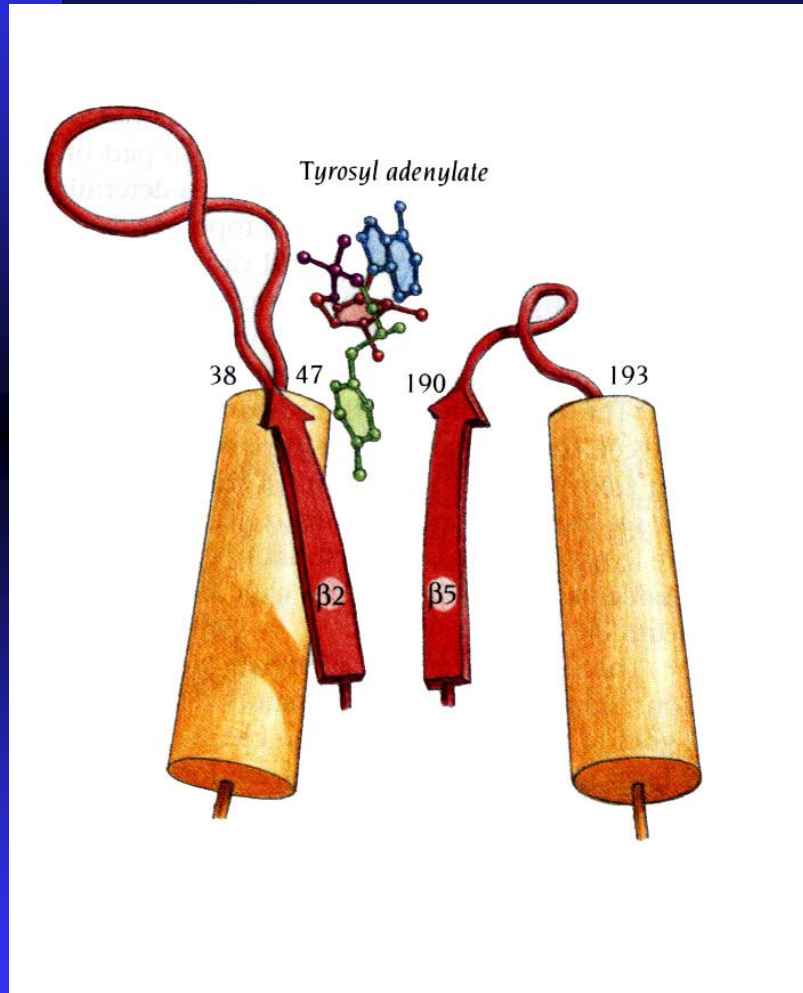
## Tyrosyl-tRNA synthetase has two different domains ( $\alpha/\beta + \alpha$ )



The enzyme couples tyrosine to its cognate transfer RNA. The central region of the catalytic domain (red and green) is an open twisted  $\alpha/\beta$  structure with five parallel  $\beta$  strands. The active site is formed by the loops from the carboxy ends of  $\beta$  strands 2 and 5. These two adjacent strands are connected to  $\alpha$  helices on opposite sides of the  $\beta$  sheet.

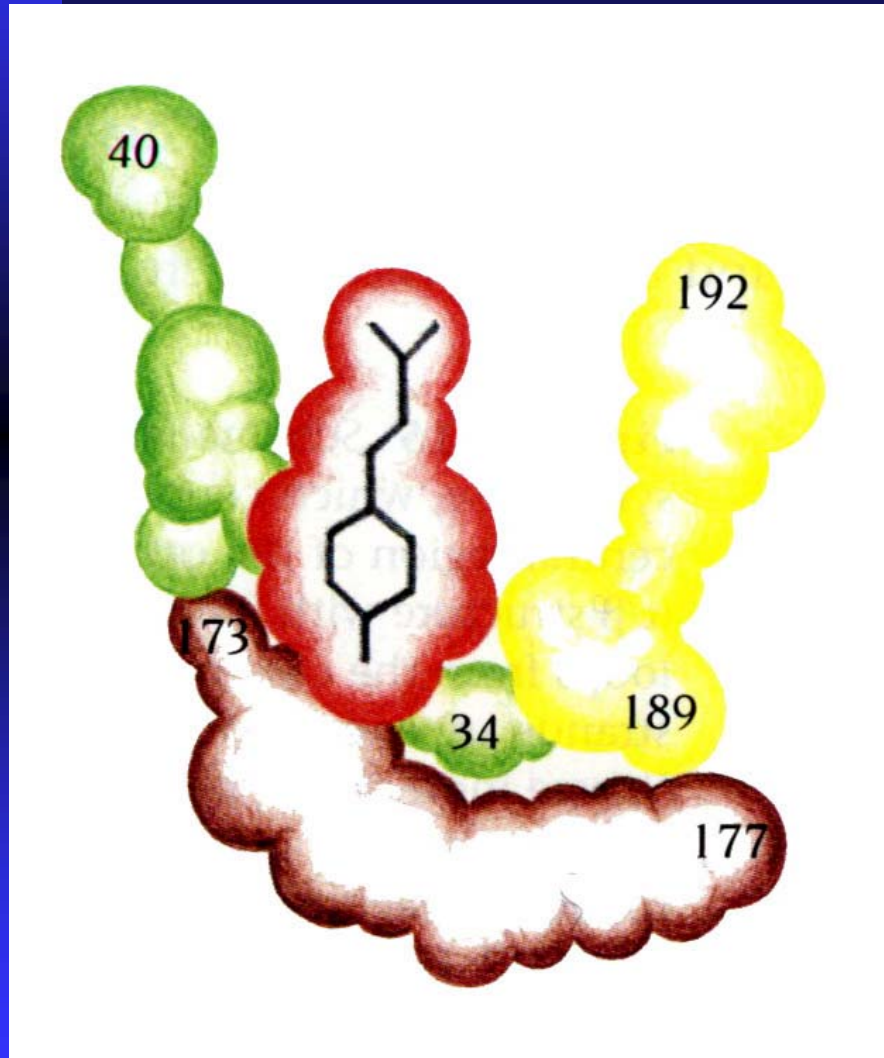


## The active site of tyrosyl-tRNA synthetase



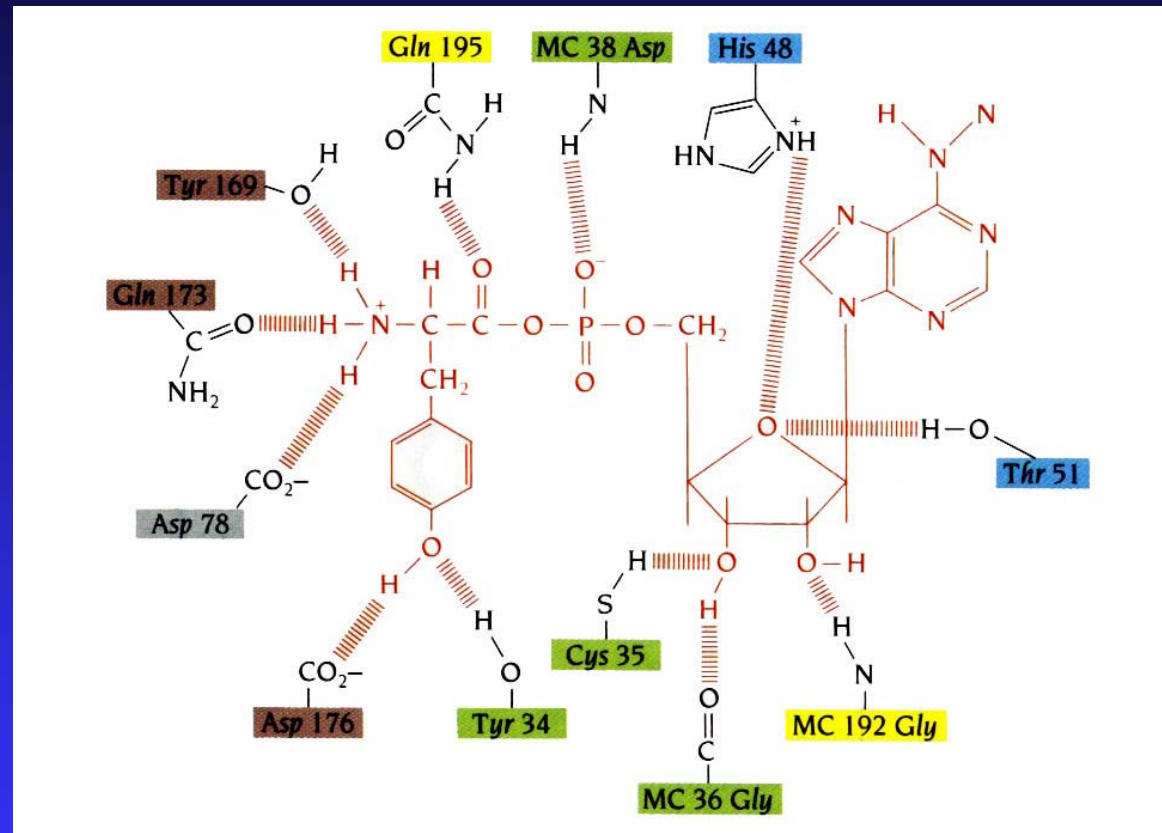
Tyrosyl adenylate, the product of the first reaction catalyzed by the enzyme, is bound to two loop regions: residues 38-47, which form the loop after  $\beta$  strand 2, and residues 190-193, which form the loop after  $\beta$  strand 5. The tyrosine and adenylate moieties are bound on opposite sides of the  $\beta$  sheet outside the carboxy ends of  $\beta$  strands 2 and 5. The phosphate and the sugar moieties are hydrogen-bonded to the main chain nitrogen atoms of residues 38 and 192, respectively.

## Schematic diagram of bound tyrosine to tyrosyl-tRNA synthetase



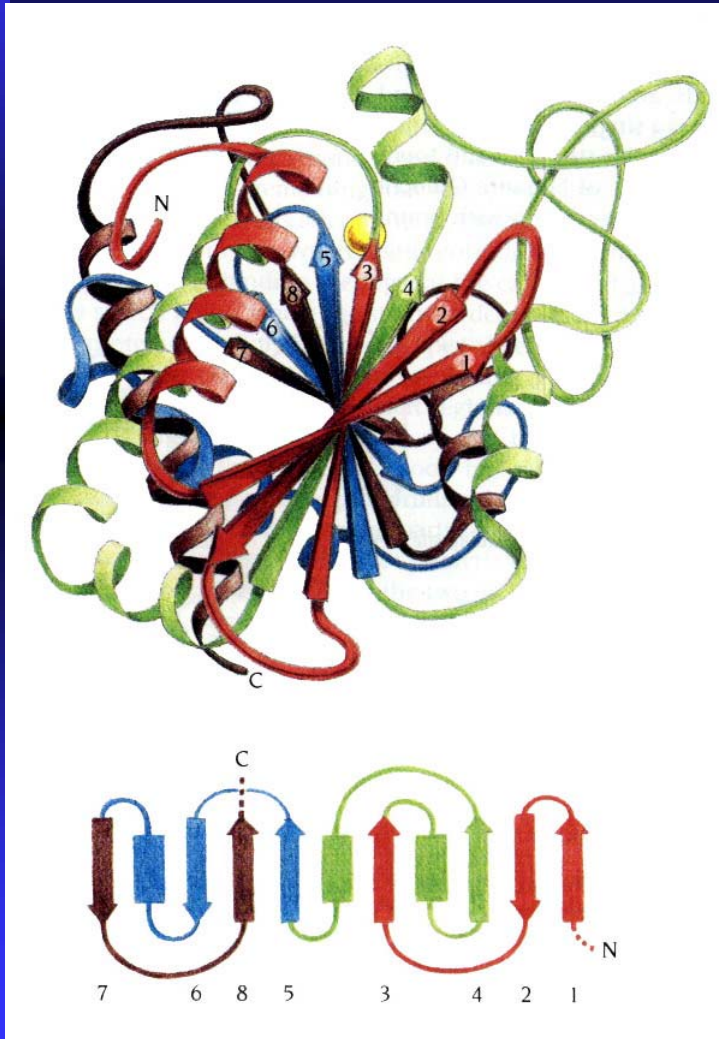
Colored regions correspond to van der Waals radii of atoms within a layer of the structure through the tyrosine ring. Red is bound tyrosine; green is the end of  $\beta$  strand 2 and the beginning of the following loop region; yellow is the loop region 189-192; and brown is part of the  $\alpha$  helix in loop region 173-177.

## Side chains of the tyrosyl-tRNA synthetase that form hydrogen bonds to tyrosyl adenylate



Green residues are from  $\beta$  strand 2 and the following loop regions, yellow residues are from the loop after  $\beta$  strand 5, and brown residues are from  $\alpha$  helix before  $\beta$  strand 5.

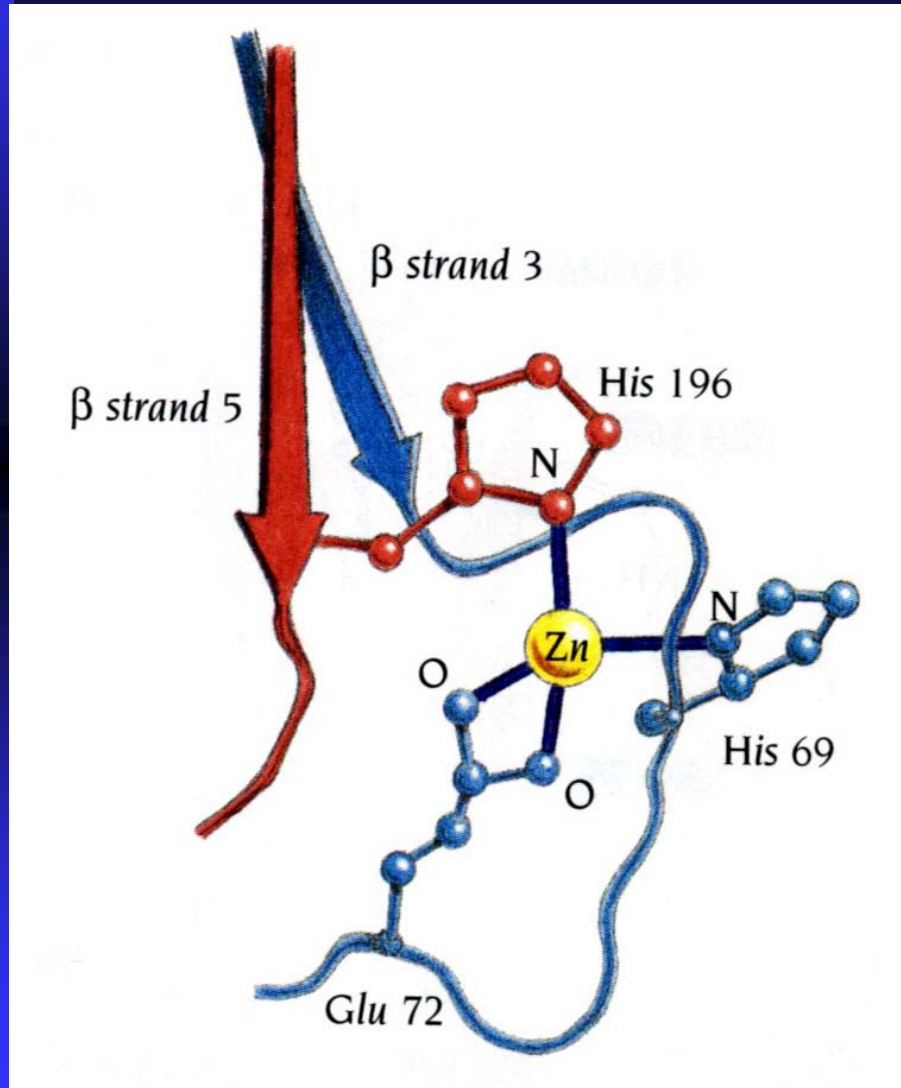
## Carboxypeptidase is an $\alpha/\beta$ protein with a mixed $\beta$ sheet



Carboxypeptidases are zinc-containing enzymes that catalyze the hydrolysis of polypeptides at the C-terminal peptide bond. The zinc atom is essential for catalysis by binding to the carbonyl oxygen of the substrate that weakens the C=O bond by abstracting electrons from the carbon atom and thus facilitates cleavage of the adjacent peptide bond.

The central region of the mixed  $\beta$  sheet contains four adjacent parallel  $\beta$  strands (8, 5, 3, and 4) where the strand order is reversed between strands 5 and 3. The active-site zinc atom (yellow circle) is bound to side chains in the loop regions outside the carboxy ends of these two  $\beta$  strands.

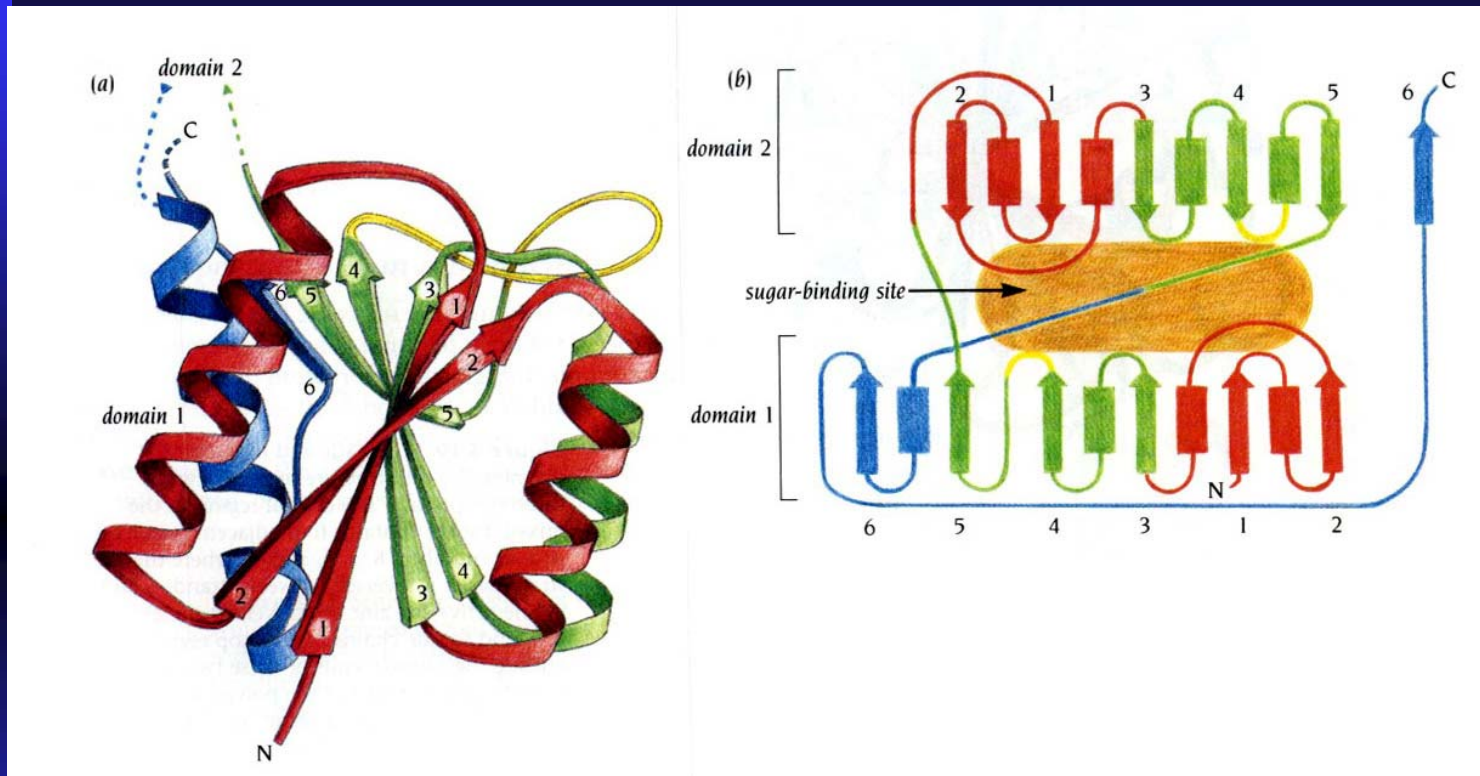
## Detailed view of the zinc environment in carboxypeptidase



The active-site zinc atom is bound to His 69 and Glu 72, which are part of the loop region outside  $\beta$  strand 3. In addition, His 196, which is the last residue of  $\beta$  strand 5, also binds zinc.



## Arabinose-binding protein has two similar $\alpha/\beta$ domains



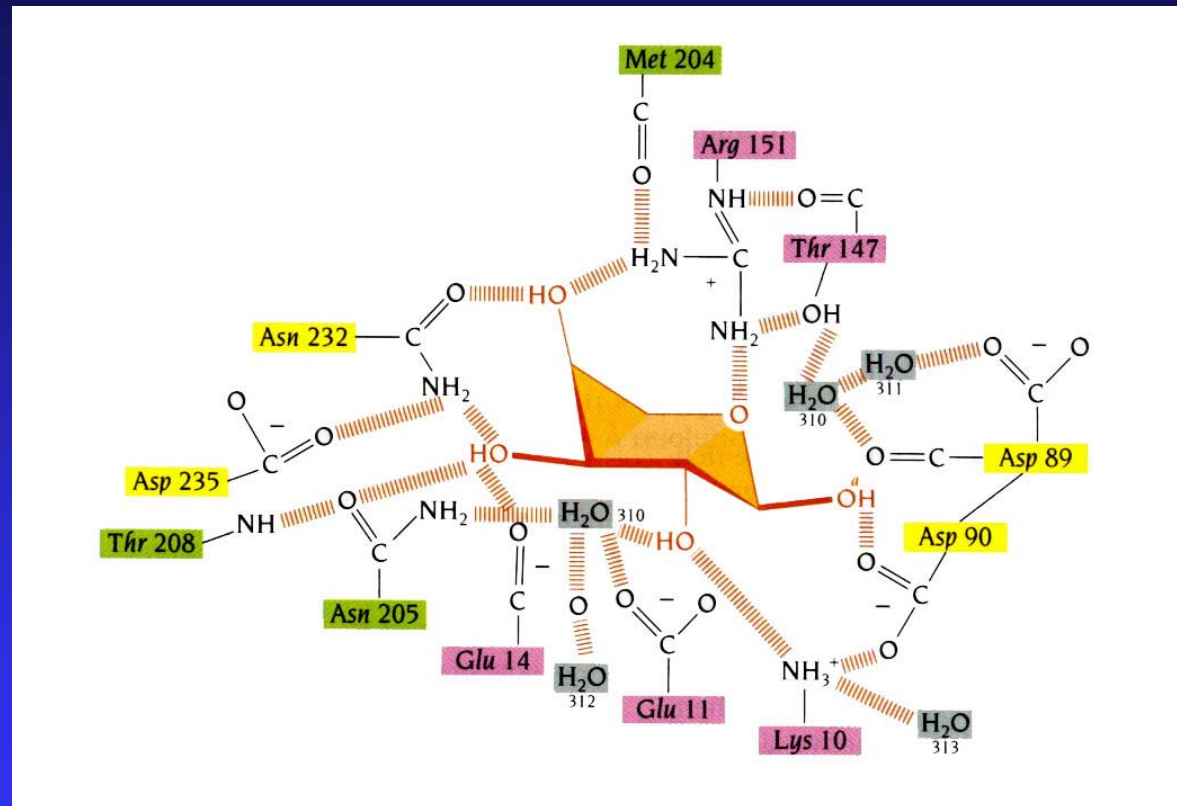
Arabinose-binding protein is a periplasmic space protein that is involved in arabinose transport in *E. coli*. It is a single polypeptide chain of 306 amino acids folded into two open twisted domains of similar structure and topology.

a) A schematic diagram of one of these domains.

b) The two domains are oriented such that the carboxy ends of the parallel  $\beta$  strands face each other on opposite sides of a crevice in which the sugar molecule binds.



## Schematic diagram of the complex networks of hydrogen bonds formed by polar side chains from the arabinose-binding protein and L-arabinose



The residues that interact with the sugar are in turn hydrogen-bonded to each other or to other residues or isolated water molecules. The pink and green residues are in loop regions that, from the topology diagram, are predicted to form the binding site. The yellow residues are from adjacent loop regions.

# Alpha/Beta Structures - Conclusion

-Alpha/beta structures are the most frequent and most regular of the protein structures. They fall into three classes:

- $(\alpha/\beta)_8$  barrel (TIM barrel)

- open twisted parallel or mixed  $\beta$  sheet with  $\alpha$  helices on both sides

- $\alpha/\beta$  horseshoe fold – formed by leucine rich motif, a curved  $\beta$  sheet with all  $\alpha$  helices on the outside of the sheet.

-The  $(\alpha/\beta)_8$  barrel structure is one of the largest and most regular of all domain structures, comprising about 250 amino acids.

- It has so far been found in more than 20 different proteins, with completely different amino acid sequences and different functions.

- They all have their active sites in very similar positions, at the bottom of a funnel-shaped pocket created by the loops that connect the carboxy end of the  $\beta$  strands with the amino end of the  $\alpha$  helices.

- The specific enzyme activity is, in each case, determined by the lengths and amino acid sequences of these loop regions, which do not contribute to the stability of the fold.

# Alpha/Beta Structures - Conclusion

-The horseshoe structure is formed by homologous repeats of leucine-rich motifs, each of which forms a  $\beta$ -loop- $\alpha$  unit. The units are linked together such that the  $\beta$  strands form an open curved  $\beta$  sheet, like a horseshoe, with  $\alpha$  helices on the outside of the  $\beta$  sheet and the inside exposed to the solvent. The invariant leucine residues of these motifs form the major part of the hydrophobic region between the  $\alpha$  helices and the  $\beta$  sheet.

-The open  $\alpha/\beta$ -sheet structures vary considerably in size, number of  $\beta$  strands, and strand order. However, they all have their active site at the carboxy edge of the  $\beta$  strands, and these active sites are lined by the loop regions that connect the  $\beta$  strands with the  $\alpha$  helices. In this respect, they are similar to  $(\alpha/\beta)_8$  barrel structures. However, the active-site regions are created differently in open structures. They are formed in those regions outside the carboxy edge of  $\beta$  sheet, where two adjacent loops are on opposite sides of the  $\beta$  sheet. The positions of these regions can be predicted from topology diagrams.

-The rules that relate the general position of functional binding sites to the overall structure of the protein are thus known for  $\alpha/\beta$  barrels and open-sheet  $\alpha/\beta$  proteins.