Lecture 10 (9/30/20)

- Reading: Ch4; 125, 138-141, 141-142
- Problems: Ch4 (text); 7, 9, 11
  Ch4 (study guide); **1, 2**

NEXT

- Reading: Ch4; 125 (Fig 4-10), 134-136 (struct. determin.)
- Problems: Ch4 (text); 10, 15

OUTLINE

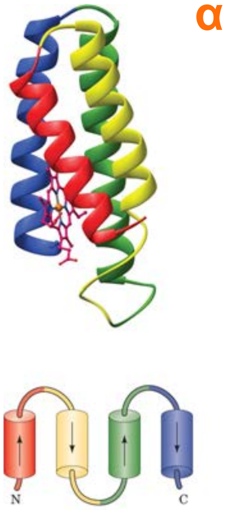
Lecture 10 (9/30/20)

I. Protein Structure
  A. Primary
    1. Determination
      a. Sequence determination; CHEMICAL
      b. Sequence determination; PHYSICAL
      c. Sequence determination; BIOLOGICAL
  B. Secondary
    1. Conformational structure; Levinthal paradox
    2. Pauling & Corey's predictions
      a. α-Helix
      b. β-sheets/strands
      c. Connections between β-strands
      d. Connections between α-helices; angle not important
    3. Super secondary structure
  C. Tertiary
    1. Picturing and classifications
    2. Topology
    3. Domains
    4. Intrinsically disordered
    5. Stability
  D. Quaternary
II. Protein Characterization; Structure determination

Tertiary Structure

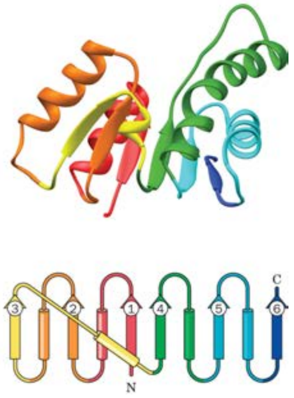
Protein Structure-Tertiary

Protein Classification:
α, β, α/β, or barrels



Cytochrome *b*562
PDBid 256B

Human immunoglobulin fragment
PDBid 7FAB



Dogfish lactate dehydrogenase
PDBid 6LDH

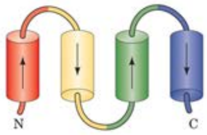
Protein Structure-Tertiary

Protein Classification:

α

Cytochrome *b*562
PDBid 256B

Growth hormone

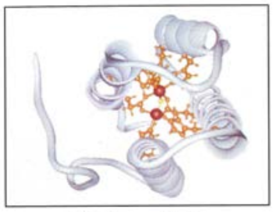


Protein Structure-Tertiary

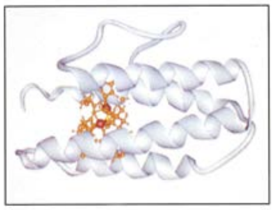
Protein Classification:

α

Four-helix bundle



Myohemerythrin
(top view)



Myohemerythrin
(side view)

Protein Structure-Tertiary

Protein Classification:

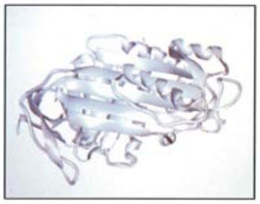
β

β–β Sandwich

Human immunoglobulin fragment
PDBid 7FAB

Immunoglobulin-fold

Insecticyanin



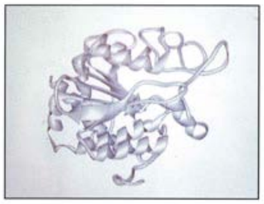
α_1-Antitrypsin

Protein Structure-Tertiary

Protein Classification:

α/β

αβ with saddle at core



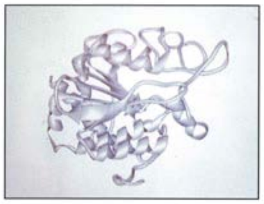
Carboxypeptidase

Carboxypeptidase
(b)

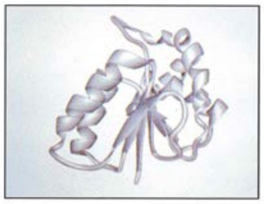
Protein Structure-Tertiary

Protein Classification: α/β

αβ with saddle at core



Carboxypeptidase



Lactate dehydrogenase domain 1

Flavodoxin

SADDLE SHAPE

Flavodoxin

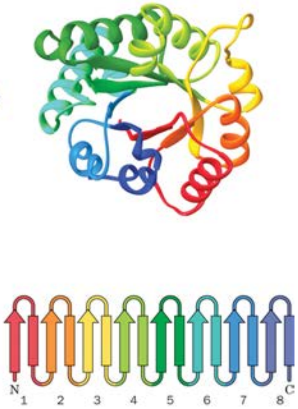
Carboxypeptidase (b)

Protein Structure-Tertiary

Protein Topology: 8-Stranded β Barrels

Human retinol binding protein
PDBid 1RBP

Peptide-N⁴-(N-acetyl-β-D-glucosaminyl) asparagine amidase
PDBid 1PNG



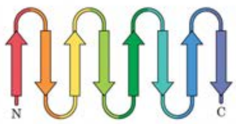
Triose phosphate isomerase
PDBid 1TIM

Protein Structure-Tertiary

Protein Topology: 8-Stranded β Barrels

Human retinol binding protein
PDBid 1RBP

β-meander



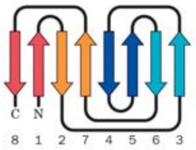
Protein Structure-Tertiary

Protein Topology: 8-Stranded β Barrels

Peptide-N^4-(N-acetyl-β-D-glucosaminyl) asparagine amidase
PDBid 1PNG

Greek key

(4-5-6-7)



Protein Structure-Tertiary

Protein Topology: 8-Stranded β Barrels

Triose phosphate isomerase
PDBid 1TIM

α/β-barrel

Protein Structure-Tertiary

Protein Topology: 8-Stranded β Barrels

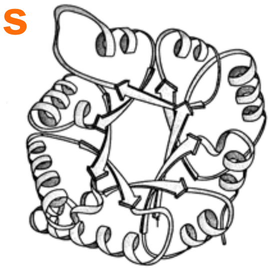
α/β Barrel

Triose phosphate
isomerase
(top view)

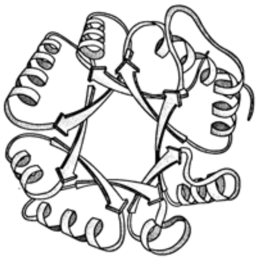
Triose phosphate
isomerase
(side view)

α/β-barrels

ß-BARREL SHAPE



Triose phosphate isomerase

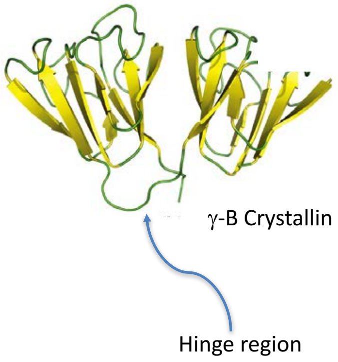


Pyruvate kinase domain 1
(a)

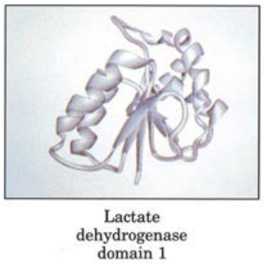
Protein Structure-Tertiary

Protein Domains

Domains:
Separate folded structures with hinge all with one polypeptide chain

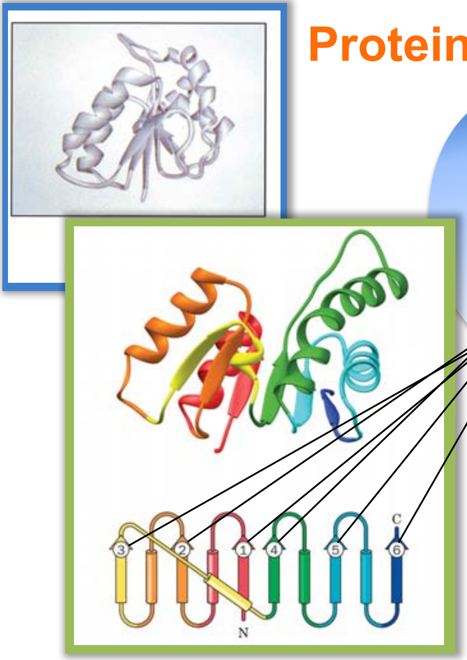


Recall:



Protein Structure-Tertiary

Protein Domains



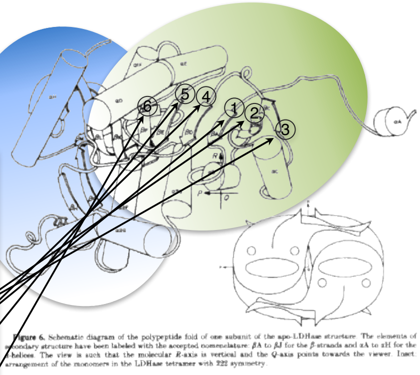


Figure 6. Schematic diagram of the polypeptide fold of one subunit of the apo-LDHase structure. The elements of secondary structure have been labeled with the accepted nomenclature: βA to βJ for the β-strands and αA to αH for the α-helices. The view is such that the molecular R-axis is vertical and the Q-axis points towards the viewer. Inset: arrangement of the monomers in the LDHase tetramer with 222 symmetry.

Dogfish lactate dehydrogenase
PDBid 6LDH

α/β-saddle
&
Rossmann Fold (β–α–β–α–β)

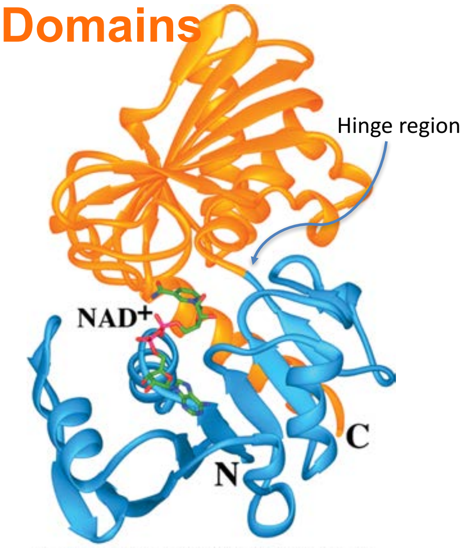
Protein Structure-Tertiary

Protein Domains

2-Domain Protein : GAPDH

Glyceraldehyde-3-phosphate dehydrogenase
PDBid 1GD1

α/β-saddle
&
Rossmann Fold (β–α–β–α–β)

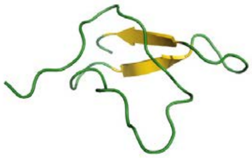


Based on an X-ray structure by Alan Wonacott, Imperial College, London, U.K. PDBid 1GD1.

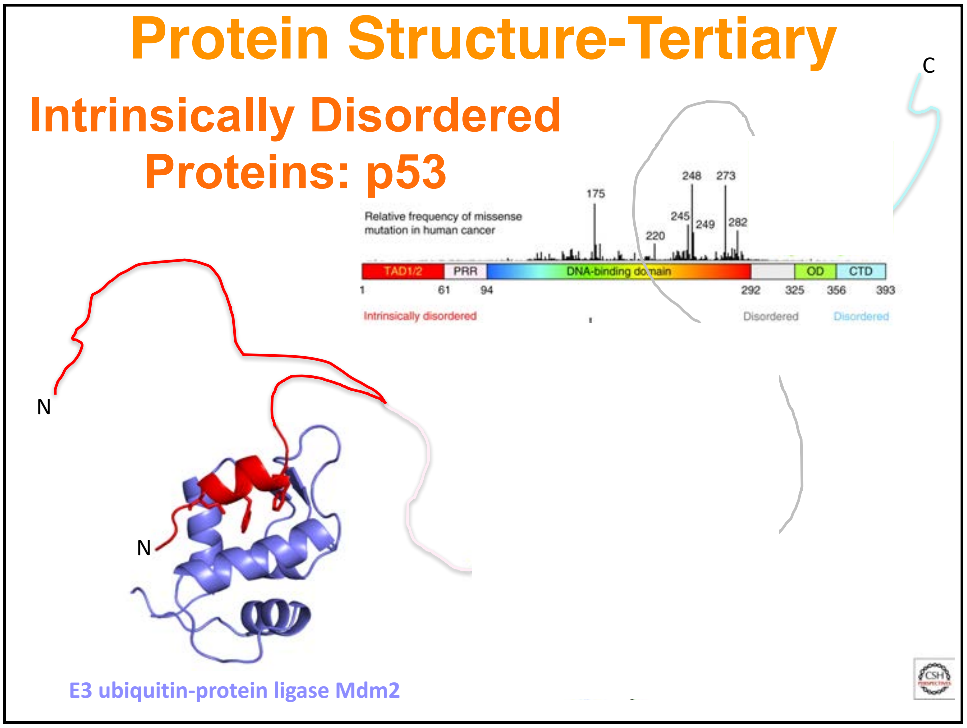
Protein Structure-Tertiary

Intrinsically Disordered Proteins: Tachystatin

Unstructured domains:
Part of primary structure without any defined conformation, changeable, dynamic, several possible structures depending on binding partners in cells



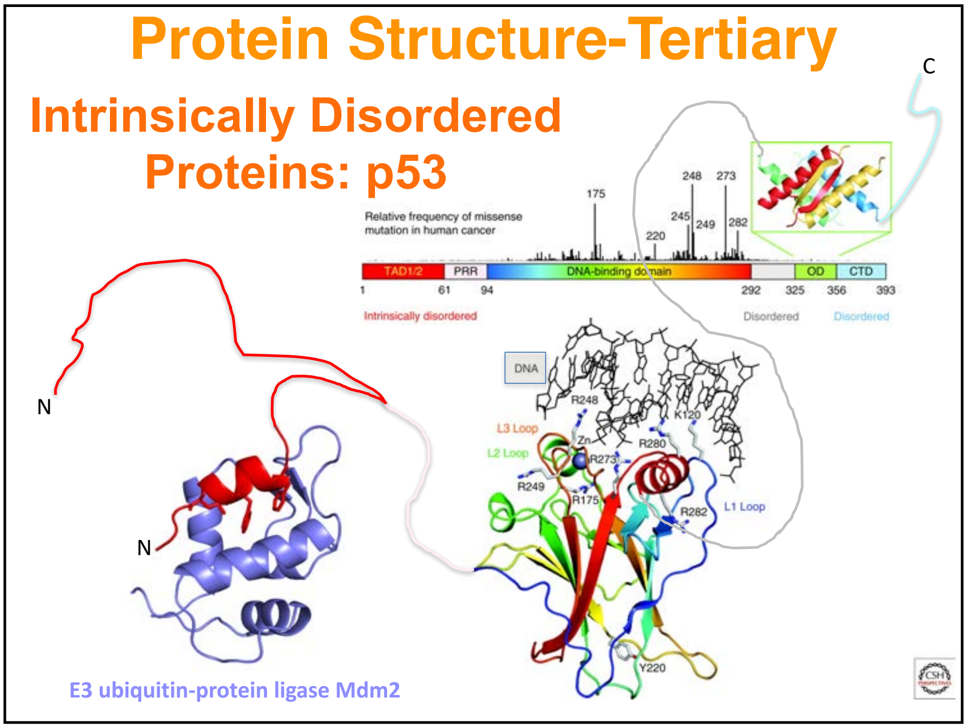
Tachystatin



Protein Structure-Tertiary

Intrinsically Disordered Proteins: p53

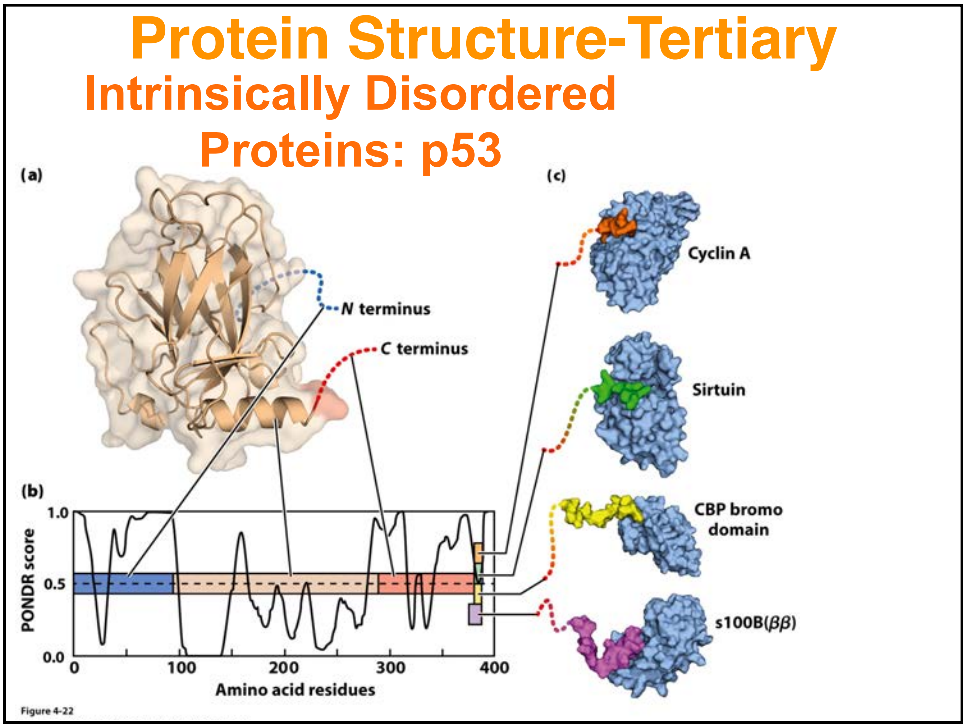
E3 ubiquitin-protein ligase Mdm2



Protein Structure-Tertiary

Intrinsically Disordered Proteins: p53

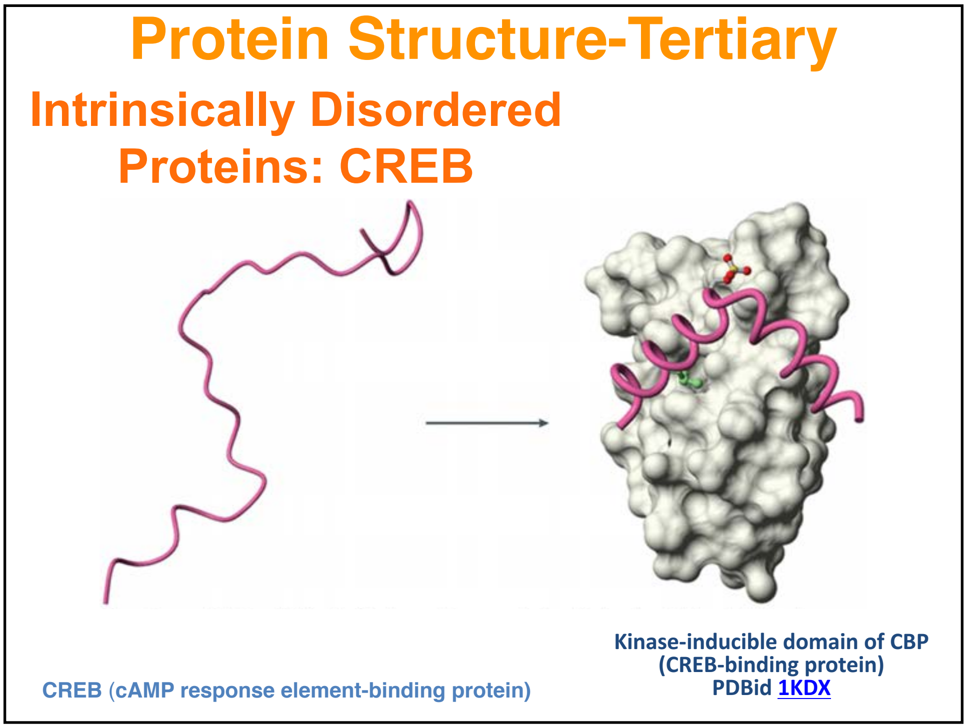
E3 ubiquitin-protein ligase Mdm2



Protein Structure-Tertiary

Intrinsically Disordered Proteins: p53

Figure 4-22



Protein Structure-Tertiary

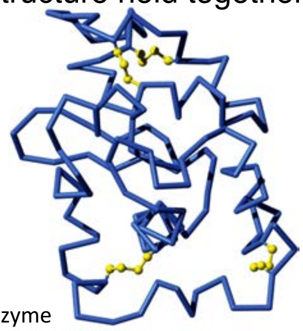
Intrinsically Disordered Proteins: CREB

CREB (cAMP response element-binding protein)

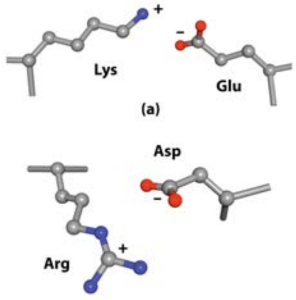
Kinase-inducible domain of CBP
(CREB-binding protein)
PDBid 1KDX

Protein Structure-Summary Tertiary

- 3° structure is a collection of 2° structures in motifs and/or domains
- Sum of weak interactions
- 3° structure held together with side-chain interactions



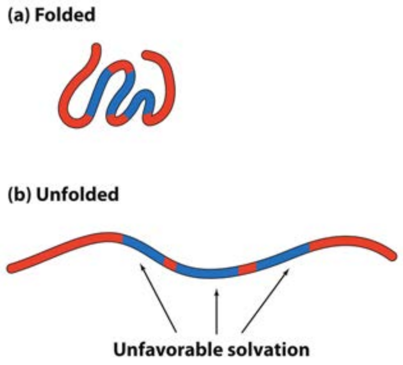
lysozyme

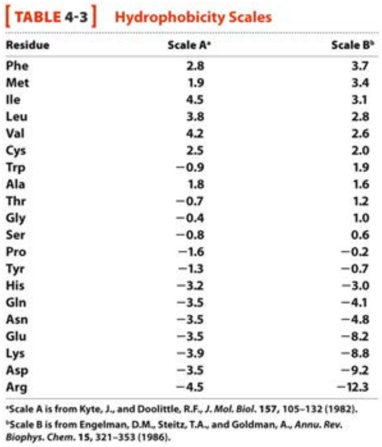


- Hydrophobic effect
- Hydrogen bonds
- Disulfide bonds
- Salt bridges
- van der Waals (Dispersion forces)

Protein Structure-Tertiary

Hydrophobic effect drives protein folding



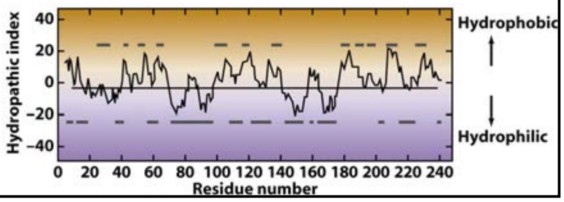


[TABLE 4-3] Hydrophobicity Scales

Residue	Scale A[a]	Scale B[b]
Phe	2.8	3.7
Met	1.9	3.4
Ile	4.5	3.1
Leu	3.8	2.8
Val	4.2	2.6
Cys	2.5	2.0
Trp	−0.9	1.9
Ala	1.8	1.6
Thr	−0.7	1.2
Gly	−0.4	1.0
Ser	−0.8	0.6
Pro	−1.6	−0.2
Tyr	−1.3	−0.7
His	−3.2	−3.0
Gln	−3.5	−4.1
Asn	−3.5	−4.8
Glu	−3.5	−8.2
Lys	−3.9	−8.8
Asp	−3.5	−9.2
Arg	−4.5	−12.3

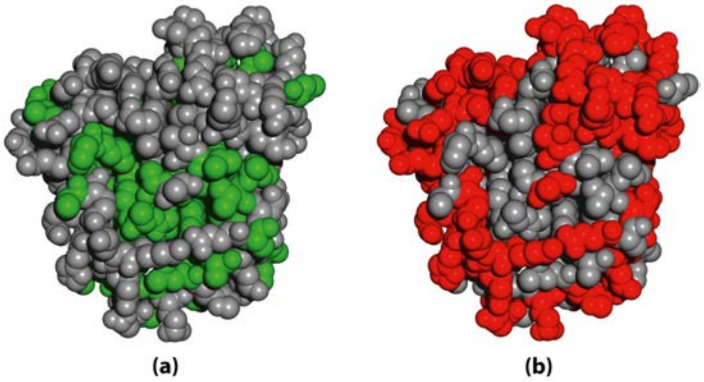
[a] Scale A is from Kyte, J., and Doolittle, R.F., *J. Mol. Biol.* 157, 105–132 (1982).

[b] Scale B is from Engelman, D.M., Steitz, T.A., and Goldman, A., *Annu. Rev. Biophys. Chem.* 15, 321–353 (1986).



Protein Structure-Tertiary

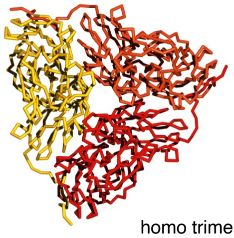
Globular proteins



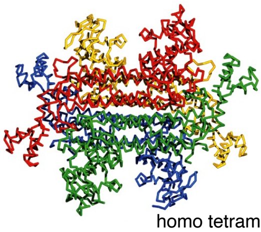
- a hydrophobic core and a hydrophilic surface

Quaternary Structure

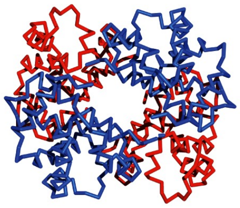
Protein Structure-Quaternary



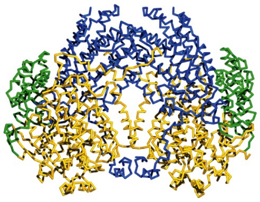
α_3 homo trimer



α_4 homo tetramer



$\alpha_2\beta_2$ hetero tetramer

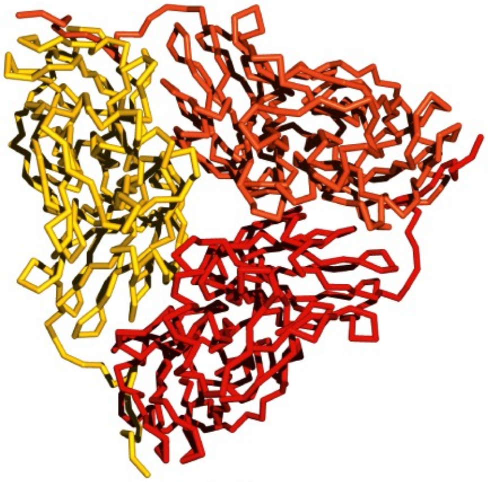


$\alpha_2\ \beta_2\ \gamma_2$ hetero hexamer

- Multiple subunits held together by primarily non-covalent interactions
- Nomenclature:
  - homo *vs.* hetero
  - subunits indicated with Greek letters (α (1st), β (2nd), γ (3rd), etc.

Protein Structure-Quaternary

Same weak interactions determine 4° structure

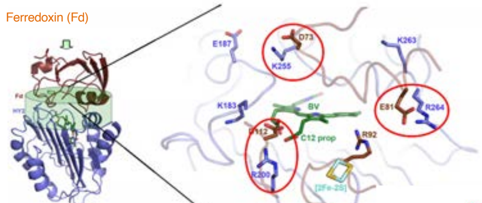


- Hydrophobic effect
- Hydrogen bonds
- Salt bridges
- van der Waals (Dispersion forces)

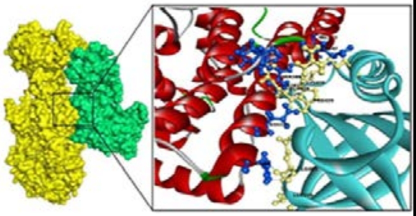
Protein Structure-Quaternary

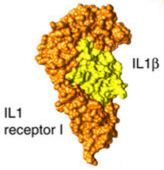
4° structure held together with side-chain interactions

Sum of weak interactions



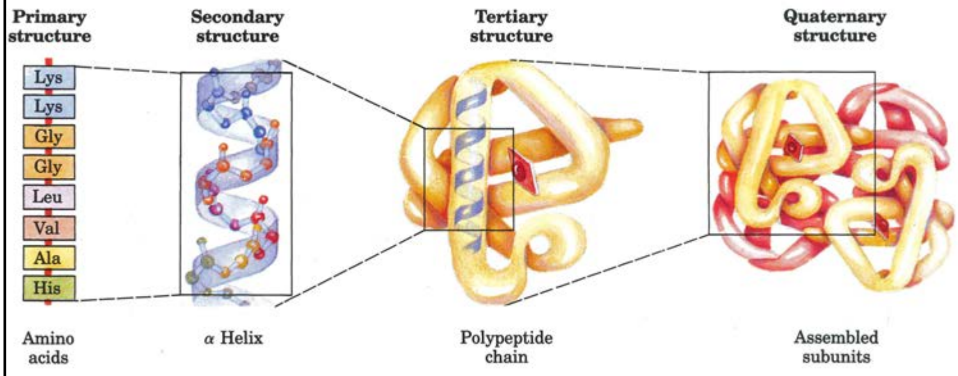
Intermolecular salt bridges formed by conserved, charged residues stabilize HY2-Fd interaction. HY2-bound substrate and the iron-sulfu cluster on AtFd2 are shown in stick representation. Important charged residues in AtFd2-HY2 binding interface are viewed from the top of Fc side of the docking complex. Residues identified in this study are show in stick representation with blue (HY2) and brown (AtFd2) carbon atoms. Red circles indicate the salt bridges in-between two proteins.





- Hydrophobic effect
- Hydrogen bonds
- Salt bridges
- van der Waals forces

Protein Structure-Summary



Protein Structure-Tertiary

Problem

Which residue would be more likely to be ____?

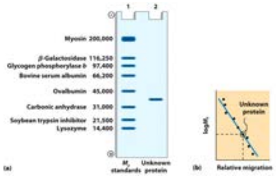
1. On a globular protein's surface: Trp or Gln
2. In the globular protein interior: Ser or Val
3. In the middle of an alpha-helix: Gly or Leu
4. In a beta-sheet: His or Pro

Protein Characterization

Quaternary Structure

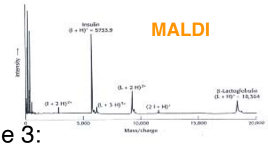
Protein Characterization: Structure Determination

Determination of Quaternary Structure



Need the “native” molecular weight
- Gel filtration
- Ultracentrifugation

Need the **subunit** stoichiometry and molecular weight
- SDS-PAGE
- MALDI-MS



MALDI

Example 1:
Native MW = 200 kDa
Subunit MW = 50 kDa

α_4

Example 2:
Native MW = 300 kDa
Subunit MW = 75 kDa, 50 kDa
Subunit stoichiometry =
(75:50) is 2:3

$\alpha_2\beta_3$

Example 3:
Native MW = 360 kDa
Subunit MW = 80 kDa, 40 kDa
Subunit stoichiometry = equal
(means with half the size, the smaller one would have to have half intensity on gel)

$\alpha_3\beta_3$