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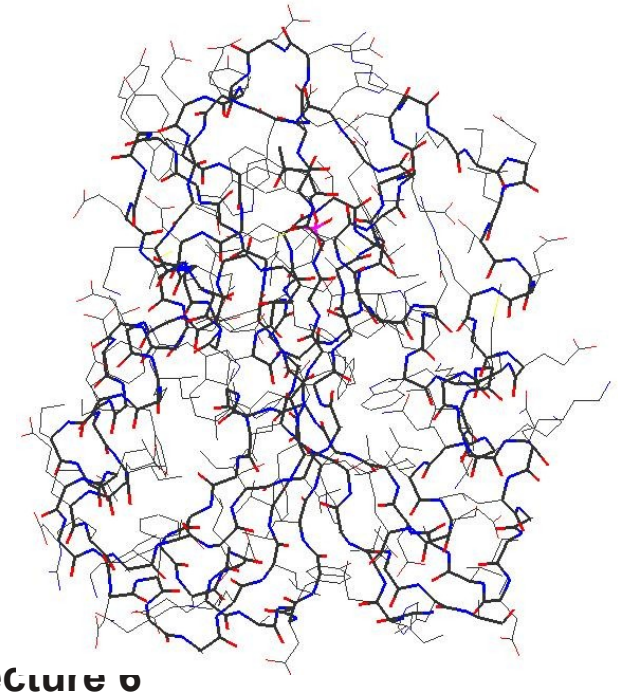
Tertiary Structure

Tertiary Structure - Spatial arrangement of secondary structures and side chains in a polypeptide

- **Polypeptide backbone adopts one (or very few) conformation or overall fold**

  ~50% of the residues are in secondary structures (α-helix or β-sheet)

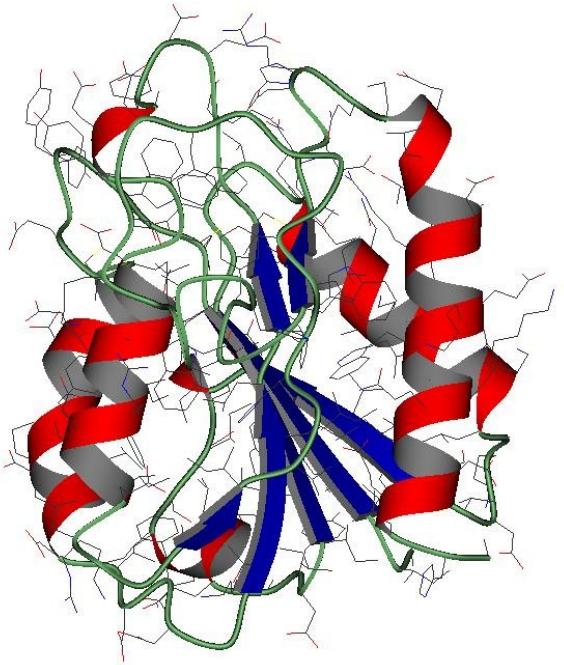
  ~50% adopt irregular connecting structures



Flavodoxin tertiary structure:

Left – Backbone in heavy lines, side chains in light

Right – Backbone as "cartoon"; helix = ribbon, sheet = arrow, connections as coils



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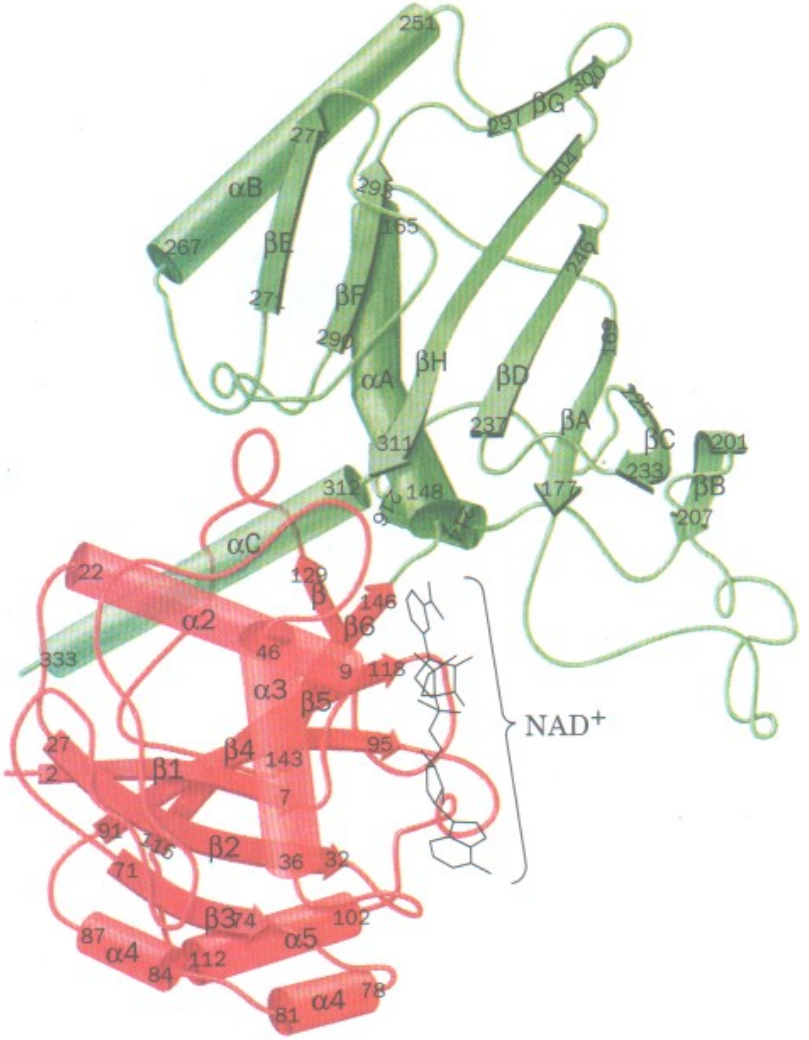
Domains in Tertiary Structures

Tertiary structure of polypeptides larger than 200 residues are generally composed of 2 or more domains

Domains (in protein architecture) are structurally independent units with the characteristics of small globular proteins

- **Domains are typically between 100-200 residues in size and give large proteins a bi- or multilobal appearance**
- **Domains are normally connected by a single (or less commonly two) peptide segments**

(Domain) Fold is the spatial arrangement of secondary structures and side chains in a polypeptide domain



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Folds (of domains)

The structures of over 18000 (2006) domains are known

- while the number of folds would seem unlimited, comparisons of the known structures indicates that relatively few folds are unique
- many proteins with unrelated primary sequences share similar folds
  - current estimates suggest there may be as few as 1000 unique folds
  - apparently folds are "scaffolds" on which a variety of active sites or binding sites may evolve

One of the simplest means of categorizing folds is to classify them according to secondary structure content

(1) α-domains

(2) β-domains

(3) α/β domains (parallel β-sheets)

(4) α+β domains (antiparallel β-sheets)

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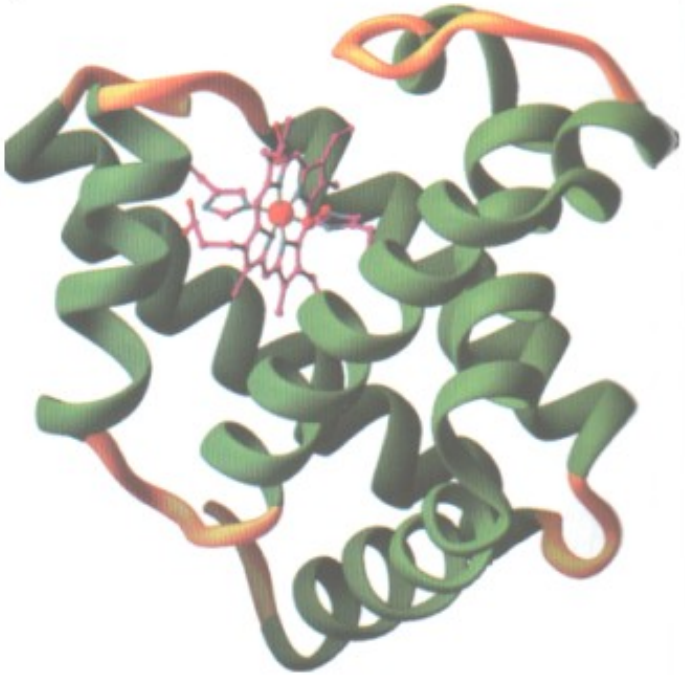
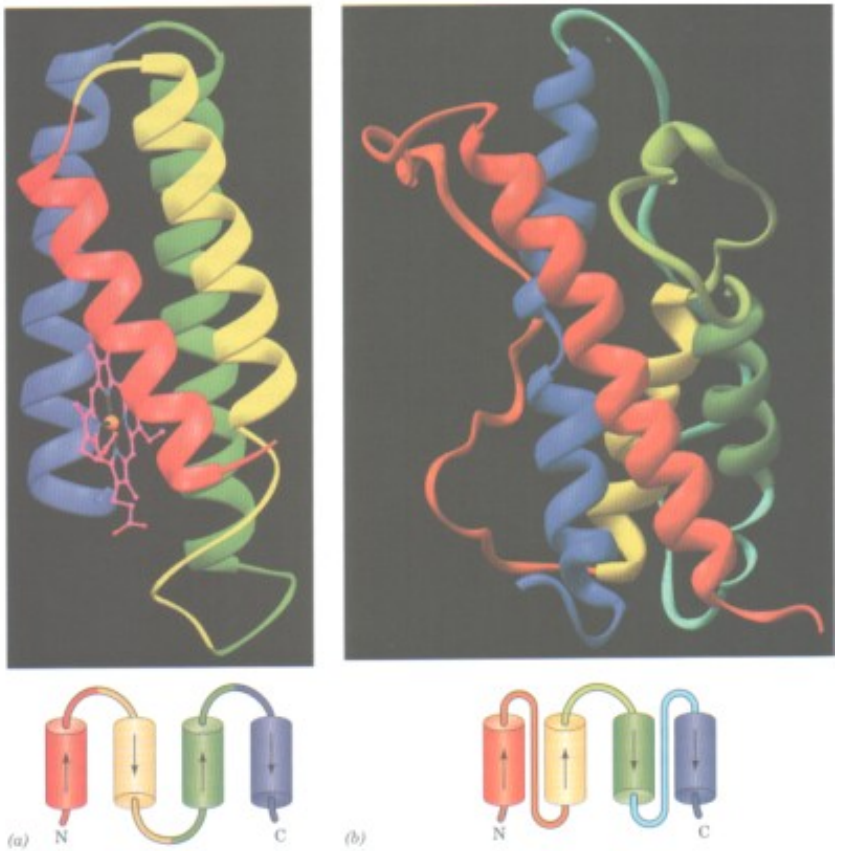
α-domains

- α-domains are composed of orthogonal helices or parallel helices
  - domains composed of parallel helices are referred to as bundles



Orthogonal helices



Center: antiparallel or 'up-and-down' bundle

Right: mixed bundle with both parallel and antiparallel helices

Not shown: parallel bundle example

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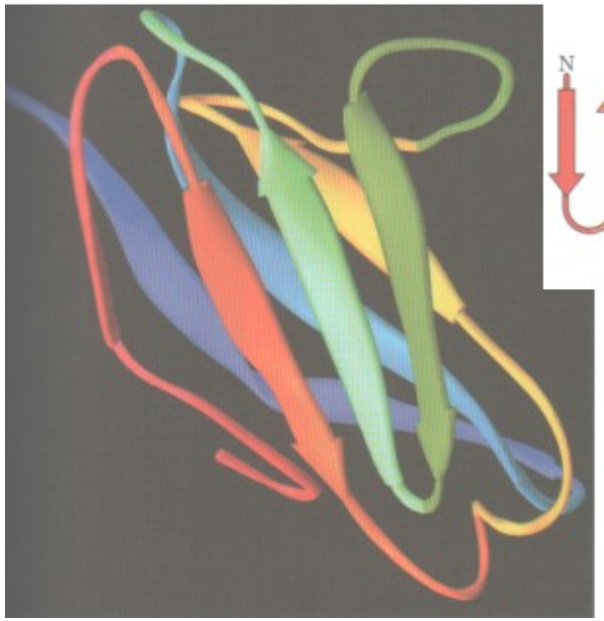
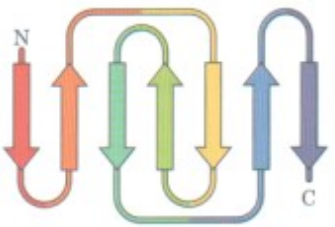
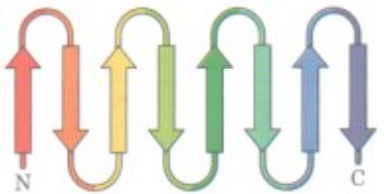
β-domains

- β-domains are composed of β-sheets that may be parallel or orthogonal
  - Shorter β–sheets are "open" or form a plane
  - β–sheets containing more than 6 strands often fold into β-barrels







Two antiparallel open β-sheets
(2-layer antiparallel β-sandwich)

Antiparallel β-barrel

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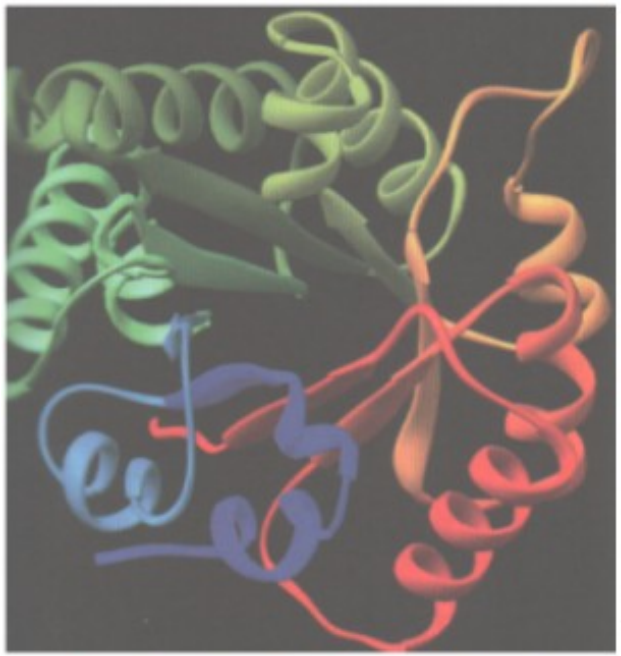
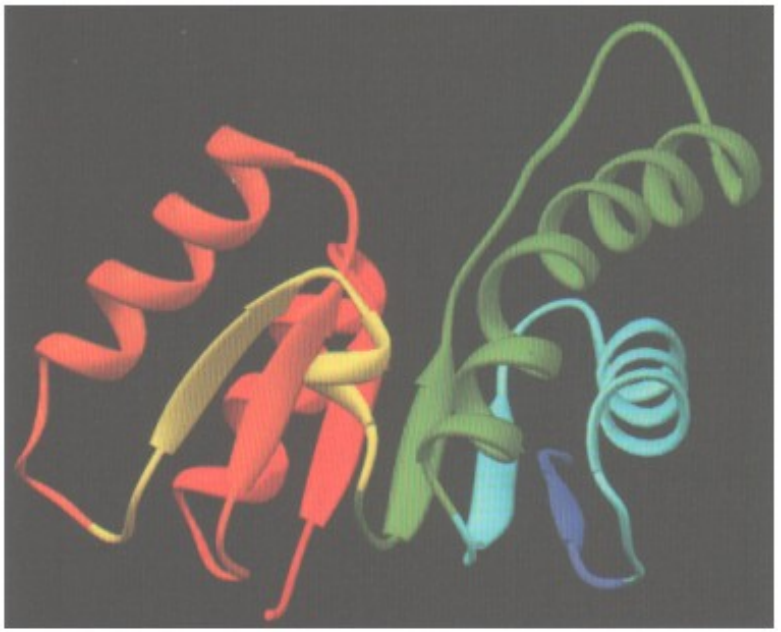
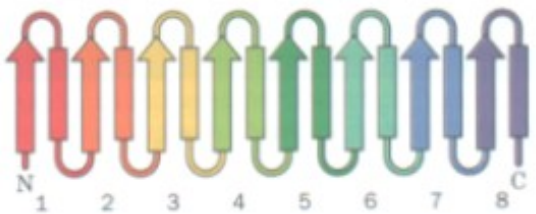
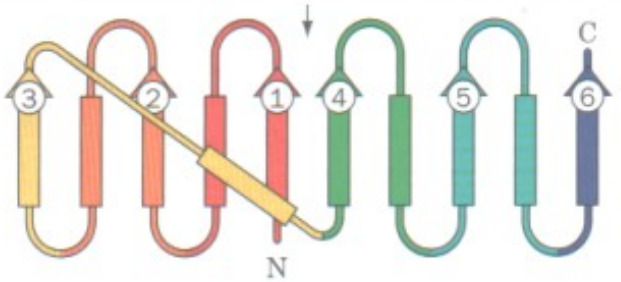
Domains with α-helices and β-sheets

- α/β domains have parallel β-sheets
- α+β domains have antiparallel β-sheets









Two α/β domains:

Left: Parallel β-barrel
(Parallel α/β barrel)

Right: Parallel open
(αβα open sandwich)

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Protein Stability

Thermodynamics studies indicate:

Proteins are only marginally stable under physiological conditions !!!

- **ΔG to denature is generally 0.4 kJ/mol of residues**
  - **~40 kJ/mol to denature a 100 residue protein ... in contrast, the energy required to break a single H-bond is ~20 kJ/mol**
- **Various noncovalent forces that are part of a protein (electrostatic, H-bonding, hydrophobic interaction) include both attractive and repulsive components**

Protein structure arises from a delicate balance among powerful countervailing forces

- **Presumably, this delicate balance facilitates the flexibility that many protein require to function**

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Protein Stability: Forces

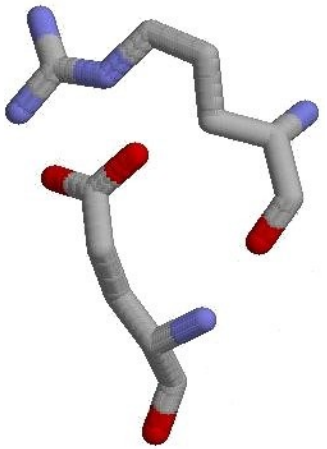
(1) Electrostatics

Salt bridges: Interactions between polar charged residues

Ion-dipole: Interactions between polar charge and polar uncharged residues

Dipole-dipole: Interactions between polar uncharged residues

Van der Waal's: Interactions between nonpolar residues



Salt bridge

(2) Hydrogen Bonds

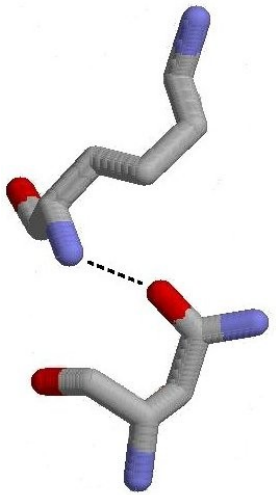
Interaction between a potential acid and potential base

(3) Hydrophobic Effect

Interaction between nonpolar residues and water

(4) Disulfide bridges

Covalent bond between the side chains of Cys residues



Hydrogen bond

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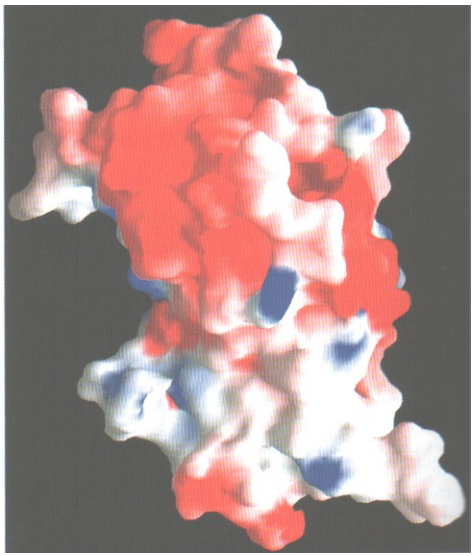
Electrostatics

- Interactions of charged particles is governed by classical electrostatics (Coulomb's Law(vacuum): $F=kq_1q_2/r$)
- Require modified Coulomb's Law for interactions in solution

$$F = kq_1q_2/Dr$$

F	is the energy of interaction
k	is a constant
q_1, q_2	are the electric charges
r	is separation distance
D	is the dielectric constant



Dielectric constant increases with solvent polarity – water has an exceptionally high dielectric constant (78.5). The protein interior (~4) more closely resembles a liquid hydrocarbon.

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Hydrogen Bonds

Interaction between weakly acidic donor and an acceptor with lone-pair electrons

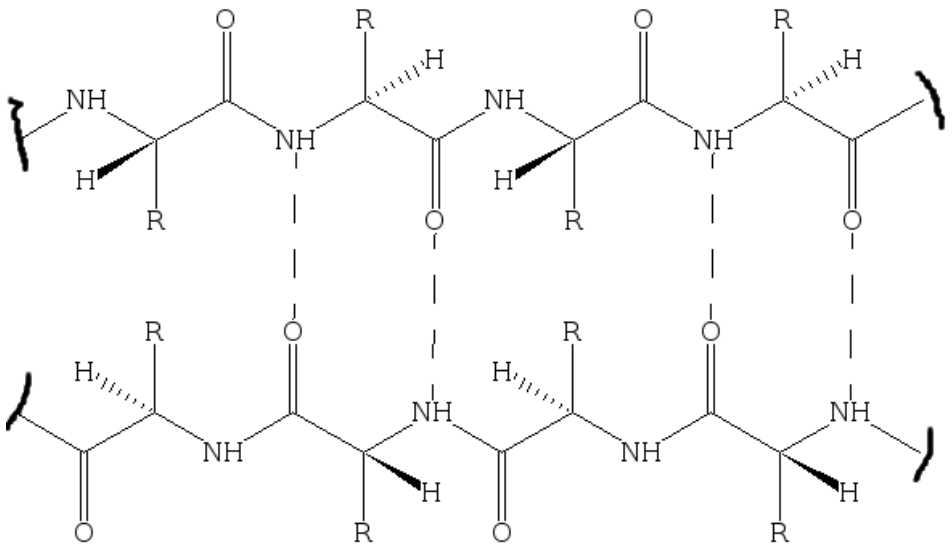
- Generally involves the electronegative O and N atoms
- In principle can involve S, C-H and π electrons (~1/10 the strength)

Energies (~ -30 kJ/mol) are intermediate between covalent bonds and van der Waal's forces suggesting partial covalent character

Interaction distance between D-H ---- :A is less than the sum of the van der Waal's radii of atoms (optimal D to A distance 2.7 Å)

Linear hydrogen bonds are strongest (D – H ---- :)

- **significant deviation from linearity is observed**



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H Bonds weakly stabilize Proteins

H bond energetics are similar whether they involve two protein atoms or protein and solvent atoms

- Likely have weak stabilizing role given the low dielectric of the protein interior

Despite lack of stabilizing role, H bonds provide structural basis for native folded state

- unsatisfied H bonding potential in the hydrophobic core have significant destabilizing role
- native folded state minimize unsatisfied H bonding potential in hydrophobic core
  - Likely why H bonding is important in secondary structures --- secondary structures occupy the hydrophobic core and require satisfied H bonding potential

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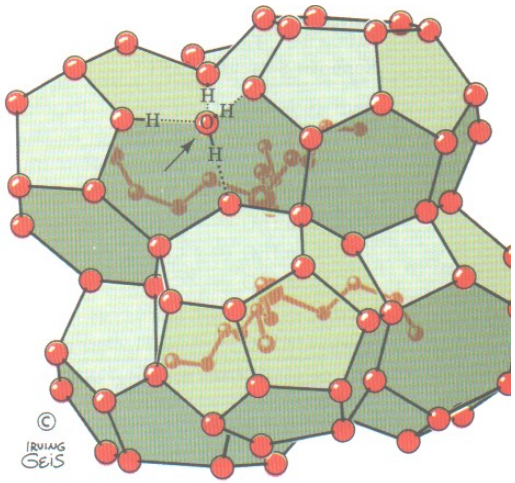
Hydrophobic Effect

Hydrophobic effect is name given to *factors that cause nonpolar substances to* ***minimize their contacts with water***

- Results from the special properties of water (not just its high dielectric constant)
- Maximizes Van der Waal's interactions between nonpolar residues

Water 'pushes' nonpolar residues together to minimize contact between water and nonpolar residues



Example of interaction between water and solute:

Aggregation of nonpolar substances minimizes contact with water molecules

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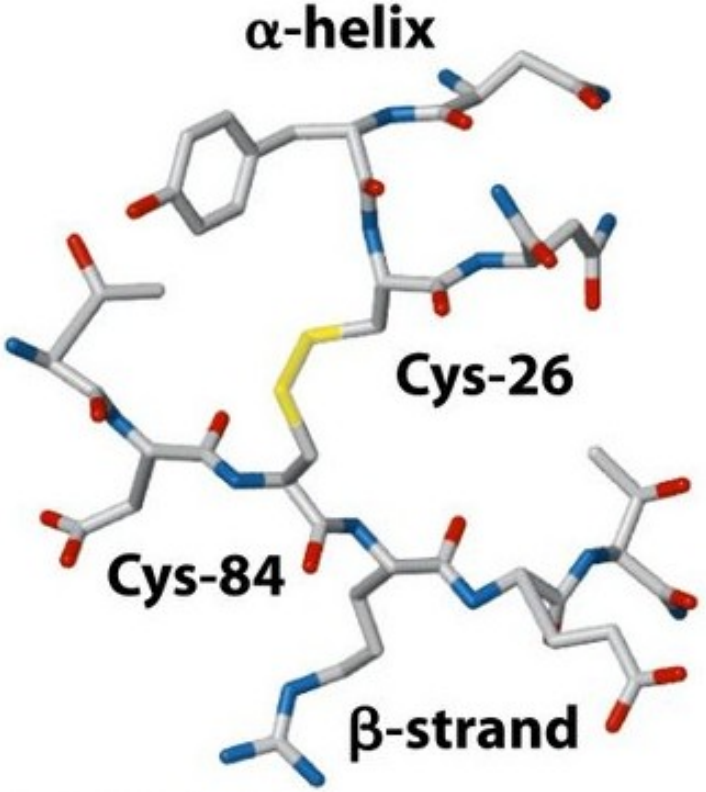
Disulfide Bonds

Disulfide bonds form as a protein folds and function to stabilize its native 3D structure

- **Cytoplasmic proteins (reducing environment) – disulfide bond stability is low (eg. disulfides break)**
- **Secreted proteins (oxidizing environment) - Most proteins with disulfide bonds are secreted to oxidizing extracellular environments.**
  - **Disulfide are stable in 'hostile' extracellular environments and provide additional structural stability**



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Protein denaturation

Factors or conditions that promote unfolding include:

(1) pH extremes – alter ionization states and H bonding

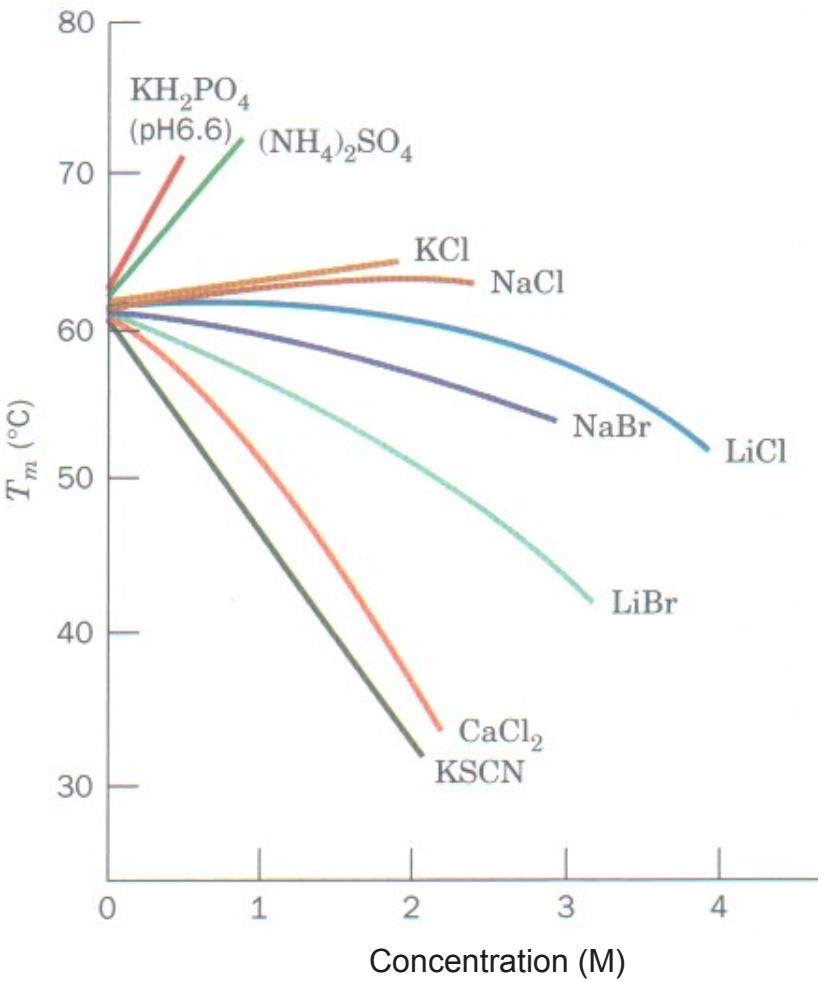
(2) detergents – associate with nonpolar residues and disrupt hydrophobic core

(3) (some) water soluble organics – interfere with hydrophobic forces

(4) chaotropic salts – increase solubility of nonpolar substances and disrupt hydrophobic core (eg. Guanidine HCl)

(5) heat – kinetic energy overcomes energy of folded state

Nonionic urea is another commonly used denaturant



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Quaternary Structure

- Nearly all proteins with molecular mass >100 kDa are composed of multiple polypeptide chains or subunits
  - subunits associate in a geometrically specific manner
  - spatial arrangement of subunits is known as quaternary structure
- Quaternary structures have several functional roles

(1) facilitate large macromolecular assemblies

assembly of small subunits into larger quaternary structures conserves genetic material and decreases chances of error

(2) protein polymorphism

expression of multiple quaternary structures from several related gene products

(3) regulation

Allostery and cooperativity are regulatory mechanism specific to multisubunit proteins