Noncovalent energy in protein structure
Conformational changes in proteins

Steve Long

Noncovalent Interactions that determine the properties of macromolecules.

Concepts to know:

van der Waals radius

electrostatic interactions:

- *Coulomb's law*
- *Dielectric constant*
- *Salt bridges*
- *Dipoles and dipole-dipole interactions*

van der Waals interactions:

- *induced dipoles*
- *London dispersion forces*
- *Lennard-Jones potential*

Hydrogen Bonds

Hydrophobic Effect

p*K*a

Textbook: Proteins by T.E. Creighton (Chapter 4)

The bond lengths and bond angles in biological macromolecules, such as protein or dna, are known with high accuracy from studies of model compounds (C-C bond length, . . .). What we do not know, without a structure determination, are the angles of rotation about single bonds (torsion or dihedral angles). For a 20 kDa protein there are a couple thousand of bonds with unkown torsion angles.

Charges in water

NaCl

Charges in vacuum

Na^+ Cl^-

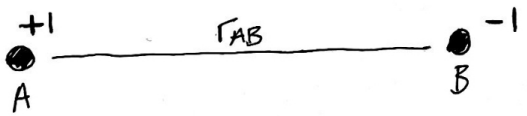
Charges in vacuum

$$\Delta E = \frac{z_A \cdot z_B \varepsilon^2}{r_{AB}}$$

z_A: charge of A; z_B: charge of B; ε is the elementary charge; r_{AB}: distance between A and B

side note:
F=E/r

Coulomb's law



Na^+ Cl^-

like charges repel: positive ΔE
opposite charges attract: negative ΔE

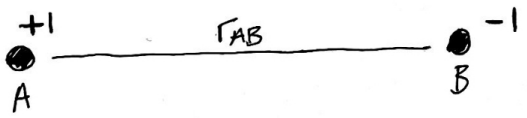
Charges in water

$$\Delta H^{\circ}_{A^+B^-} = \Delta E = \frac{z_A \cdot z_B \varepsilon^2}{r_{AB} D}$$

z_A: charge of A; z_B: charge of B; ε is the elementary charge; r_{AB}: distance between A and B; D: dielectric constant

enthalpy
(-) for NaCl

Coulomb's law



Na^+ Cl^-

D = 1 for vacuum
D ~ 80 for water

D ~ 2 for lipid membrane
D << 80 for interior of proteins, sometimes as low as 2.

All forces involved in non-covalent interactions have the electrostatic force as their basis.

Water plays a key role in strongly modulating all of these forces.

Ionic interactions are the simplest and obey Coulomb's law:

$$\Delta H^{\circ}_{A^+B^-} = \Delta E = \frac{Z_A Z_B \varepsilon^2}{r_{AB}}$$

ε = e⁻ charge
Z = # of charges
r_{AB} = distance b/ween A & B.

Note the inverse dependency to the inverse of the first power of the distance. They can have effects over large distances.

For the Na^+ Cl^- pair, ΔE = 120 kCal / mol. in vacuum.
But in non-vacuum, the strength is reduced by the **dielectric constant.**

$$\Delta H^{\circ}_{A^+B^-} = \Delta E = \frac{Z_A Z_B \varepsilon^2}{r_{AB} \cdot D}$$

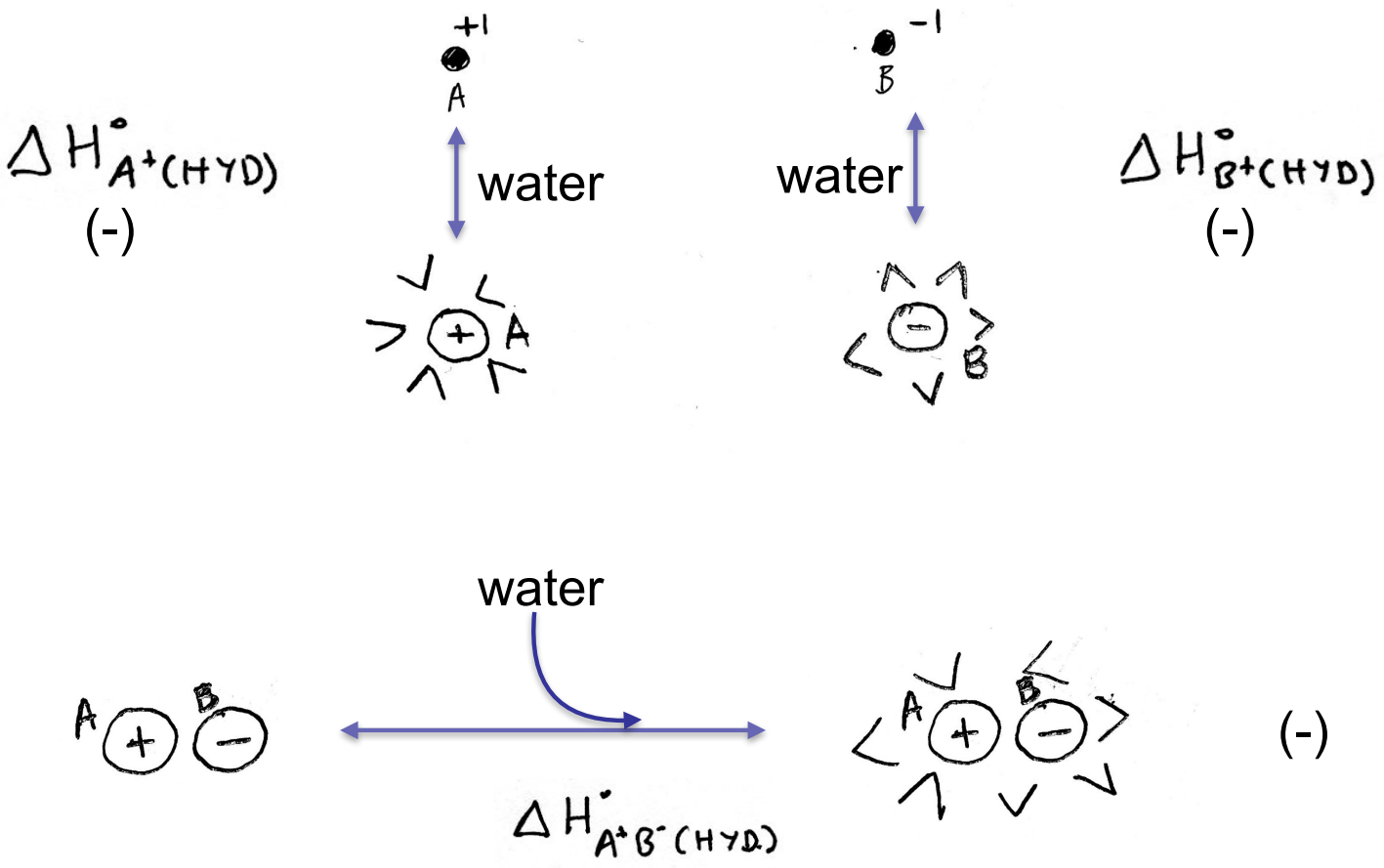
, D = dielectric constant.

Think of it as insulator whose strength varies depending on the charges, partial or full, in the environment. The more polar/charged the environment is the larger the dielectric constant.

In water, D = 80, => the interaction energy in the Na^+ Cl^- pair is only 1.5 kCal / mol !

Charges in water - another consideration, the hydration effect

NaCl



Charges in water - another consideration, the hydration effect

So, for an ion pair $A^+ B^-$

$$\Delta H^\circ = \underset{(-)}{\Delta H^\circ_{A^+B^-}} + \underset{(-)}{\Delta H^\circ_{A^+B^-(HYD)}} - \underset{(-)}{\Delta H^\circ_{A^+(HYD)}} - \underset{(-)}{\Delta H^\circ_{B^+(HYD)}}$$

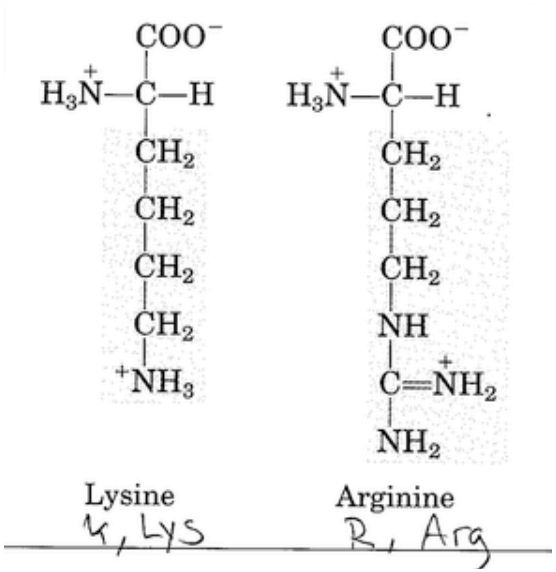
total enthalpy

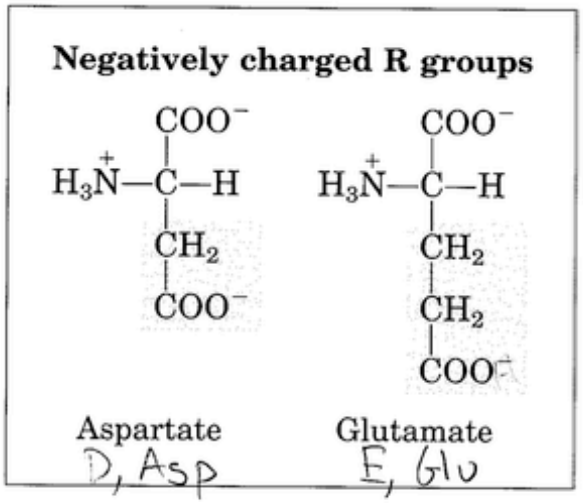
$$(\Delta G) \quad = \Delta H - T\Delta S$$

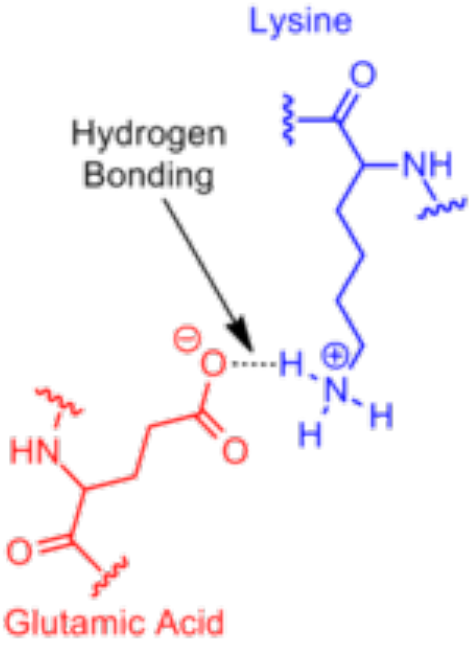
large -ΔH, indifferent ΔS

An ‘ion pair’ (sometimes referred to as a ‘salt-bridge’) can occur between positively and negatively charged amino acids. Forming charge-stabilized hydrogen bonds.

positively charged R groups





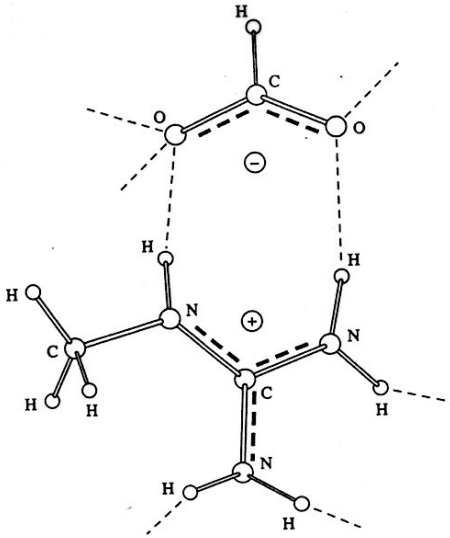


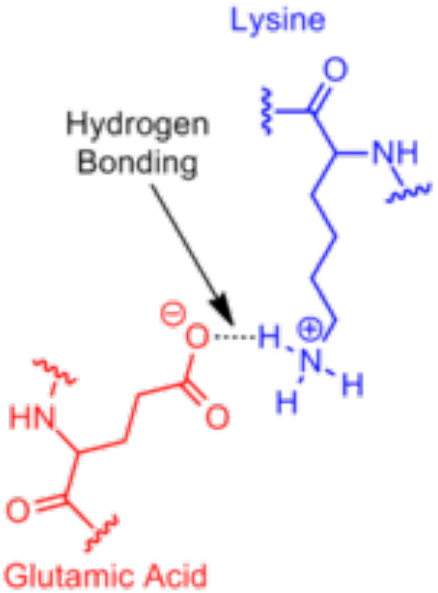
- In general, an ion pair is of little energetic consequence in aqueous solutions, except in some special cases:

-the dielectric constant away from the surface of a protein (buried inside) can be much << 80.

-may contribute to specificity of folding, intermolecular interactions (penalty of repulsion, of a buried charge etc.)

-some charged amino acids have hydrogens; these can then form charge-stabilized hydrogen bonds (**salt bridges**) which are energetically more important. Such electrostatic interactions can have big effects on the pKa values of the side chains involved; they would increase the tendency of the side chain to ionize.



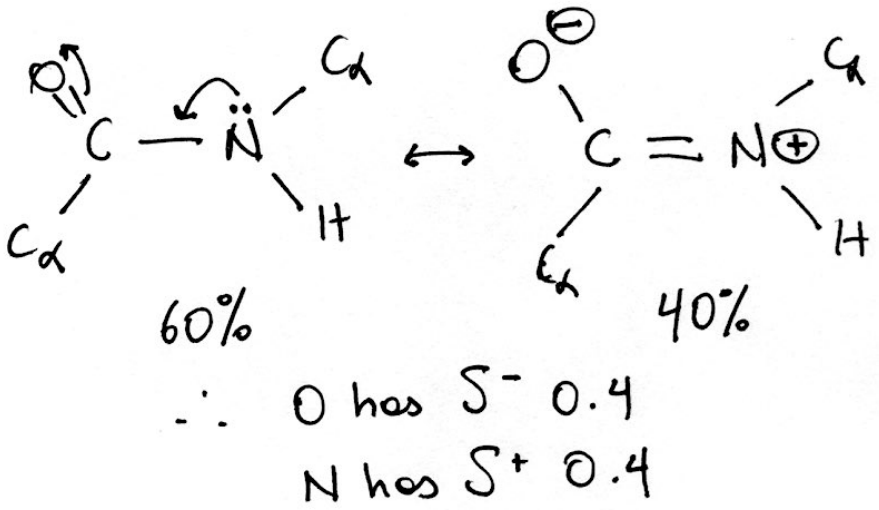


Dipoles

- partial charges in the absence of a net charge $\delta^{+/-}$
- depends on electronegativity of atom (excess e^- charge; attraction of electrons):

O 3.45
N 2.98
C 2.55
S 2.53
H 2.13

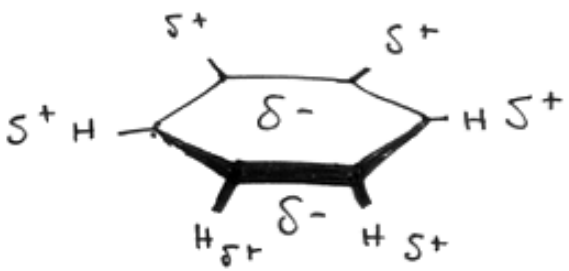
-resonance of peptide bond, δ 0.4 ε :



Dipoles

-in aliphatic bonds, $\delta \lesssim 0.1\,\varepsilon$;

-in aromatic systems, phenylalanine:

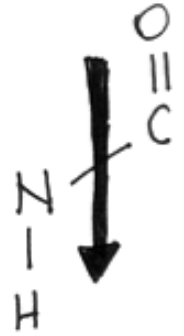


—— | or

staggered,

more v.d.W. contacts

-separation of charge -> **dipole moment** (vector quantity).

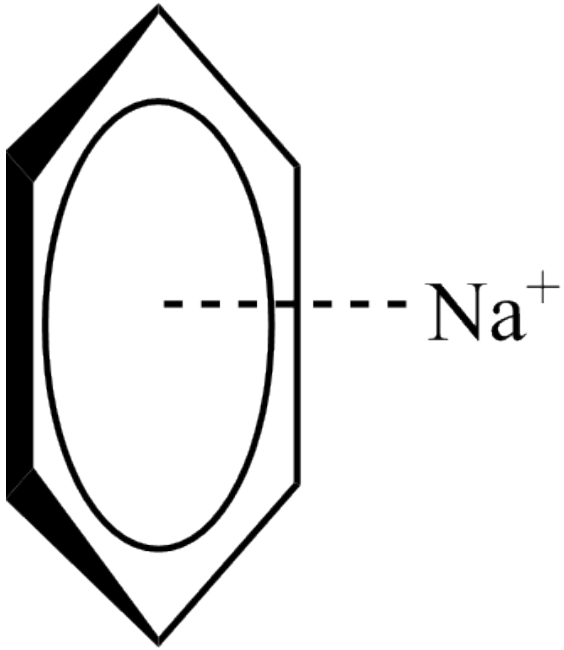


$\vec{\mu} = Z \cdot d$ $\quad 1\,e^- @ 1\,\text{Å} = 4.8$ Debye units.

3.5D for peptide bond

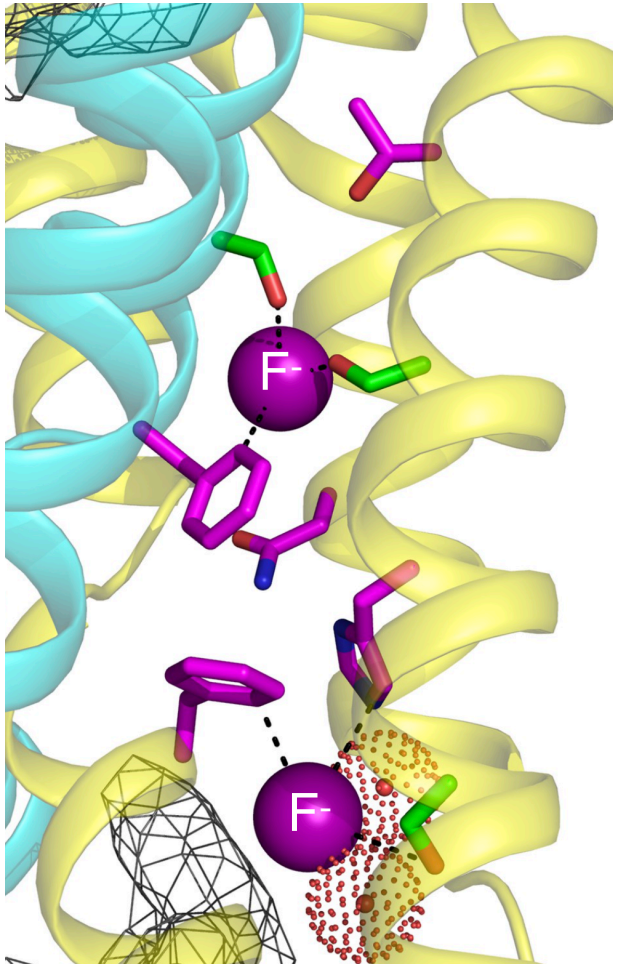
1.85D for H_2O

Cation – π interaction



Cation – π interaction (very common)

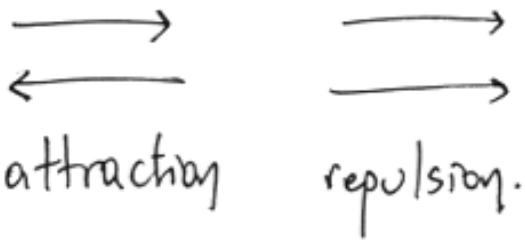
Anion – π interaction (less common)



Molecular determinants of permeation
in a fluoride-specific ion channel.
eLife (2017) N. Last et al. & C. Miller

Dipoles

- dipole - dipole interactions have directionality.



$\frac{1}{d^3}$ in fixed orientation

$\frac{1}{d^6}$ if dipoles free to rotate.

- a dipole is essentially an uneven distribution of electron density; therefore it is easily perturbed (in dipole-dipole interactions, for example), but it can also easily be induced by an electric field: **induced dipole**

- This depends on the electronic **polarizability** of the atom, which depends on how tightly the electrons are held by the nucleus => larger atoms (down the periodic table) have higher polarizability.

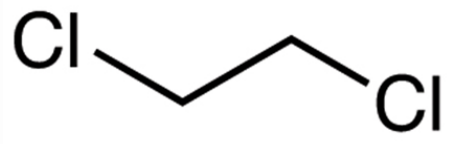
van der Waals radius

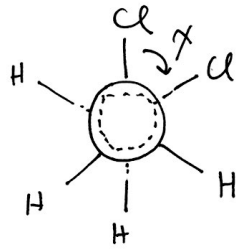
One obvious force in noncovalent interactions is due to space limitations (steric hindrance), as two atoms cannot occupy the same space. For the purposes of structural biology, the atom can be though of as having definite dimensions and can be modeled as a sphere having a **van der Waals radius**.

Table 4.1 *Van der Waals Radii of Atoms Found in Proteins*

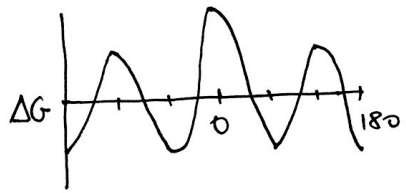
Atom	Radius when singly bonded (Å)
Hydrogen	1.17
Oxygen	1.40
Nitrogen	1.55
Carbon	1.75
Sulfur	1.80

Values from A. Bondi, *J. Phys. Chem.* 68:441–451 (1964) and A. Gavezzotti, *J. Amer. Chem. Soc.* 105:5220–5225 (1983).





di-chloro ethane



ΔG differences can be 'relatively' very small.

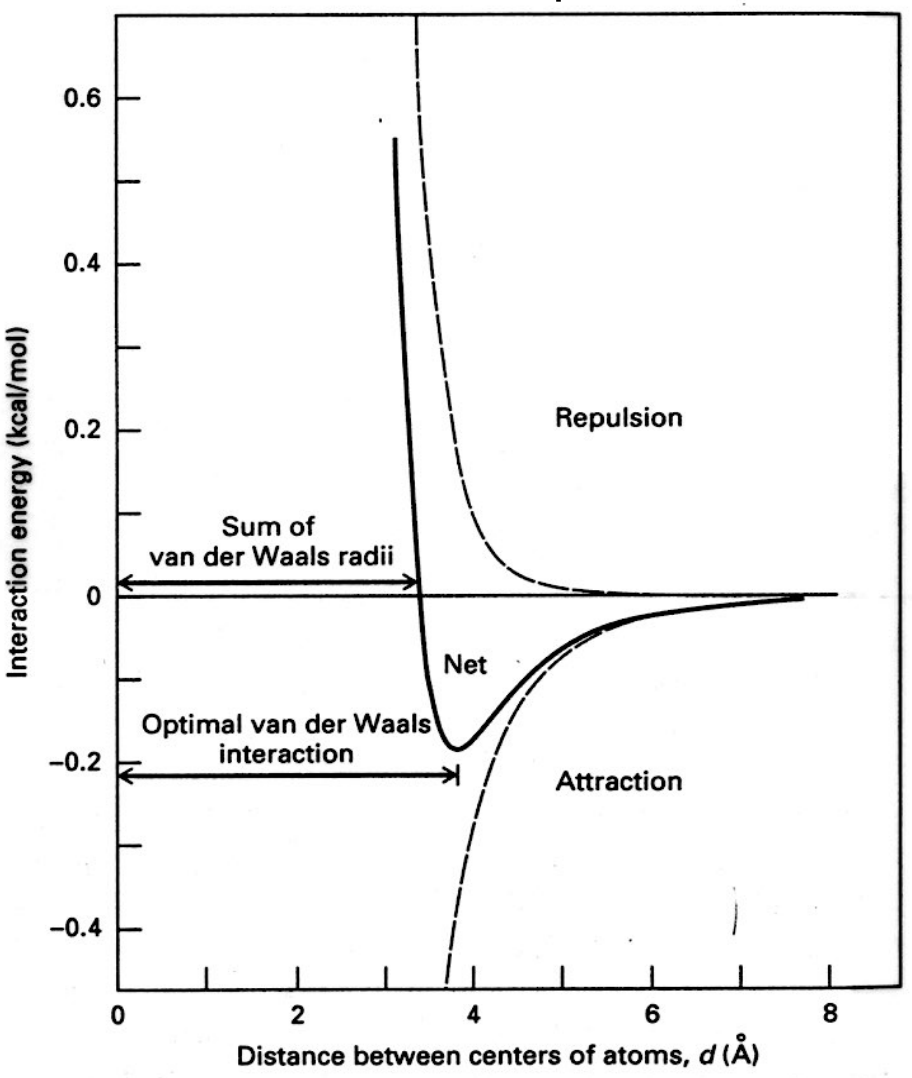
van der Walls interactions

van der Waals interactions Combination of induced dipoles and repulsive force

- van der Waals repulsion: cannot get closer than van der Waals radii unless bonded (two electrons cannot occupy same atomic orbital unless their spins are paired/covalently bonded - Pauli exclusion principle). Repulsive force rises w/ the 12th power of 1/distance; can safely consider a sphere model.

- van der Waals attraction (contact in short): formally **London** or **Dispersion** force; attraction rises with the 6th power of 1/distance. Based on quantum mechanical behavior of the electron, but classical analogy is **mutually induced dipoles**. Optimal v. d. Waals contact distance is 0.3 - 0.5 Å longer than the sum of the van der Waals radii.

Lennard-Jones potential



Lennard-Jones potential

$$E(d) = \frac{C_n}{d^{12}} - \frac{C_6}{d^6}$$

C_n, C_6 constants

d = distance

$\frac{C_n}{d^{12}}$ ↑ repulsions

$\frac{C_6}{d^6}$ ↑ attractions

hydrogen bonds

Hydrogen bonds

- two <u>electronegative</u> atoms 'competing' for the same hydrogen atom:

—**D**—H·····**A**—

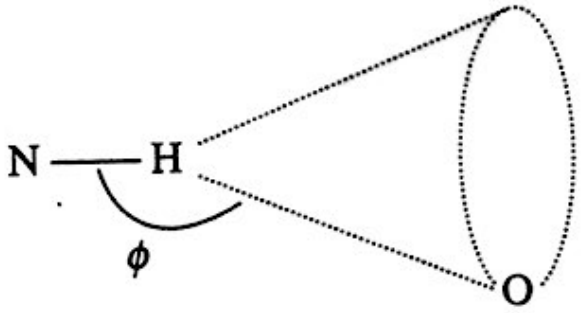
The hydrogen atom is bonded to donor, and is closer to it than to the acceptor; in some highly favorable cases, the hydrogen may appear to be equally shared by the two.

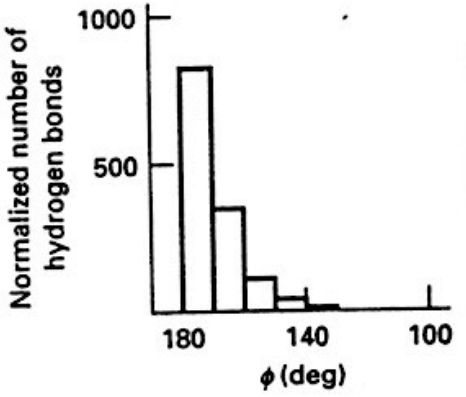
- primarily electrostatic interaction between the positive dipole of the D—H bond and the partial negative charge on the acceptor.

$-D^{\delta-}-H^{\delta+}\cdots A^{\delta-}-$

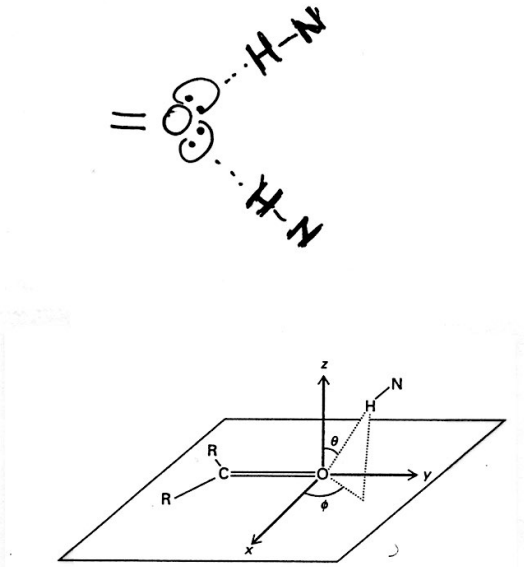
- only hydrogen atom can do this, owing to its high tendency to become positively polarized (only one proton is holding the hydrogen electron in place, and it is easy for the electron to wander away, relative to a nucleus with many protons).

- Strong preference for linearity:





- not as strong of a tendency to be pointed at lone electron pairs of the acceptor:



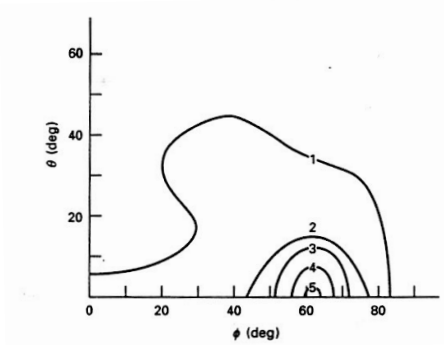


FIGURE 4.4
The geometries of C═O ··· H—N hydrogen bonds observed in crystal structures of small molecules. The definitions of the angles ϕ and θ are illustrated at the *top*, and the relative frequencies of their observed values in intermolecular hydrogen bonds (R. Taylor et al., *J. Amer. Chem. Soc.* 105:5761–5766, 1983) are given by the contours. The angle ϕ measures departures from linearity of the C═O bond and the H atom; the most frequently observed values are in the region of 50°–60°. The angle θ measures the extent to which the H atom lies out of the plane defined by the R, C, and O atoms; the most commonly observed values are in the region of 0°–7°. The lone-pair electrons of the oxygen atom are believed to project at angles of $\phi = 60°$, $\theta = 0°$. The spherical polar coordinate system used here gives a bias toward small values of θ that could be corrected by plotting sin θ.

- Dielectric constant, competition with water hydrogen bonds, and the nature of the donor/acceptor pair strongly influences the net energy of a hydrogen bond, can be upto 10 kcal / mol; 3 - 5 kcal / mol would be considered a good one.

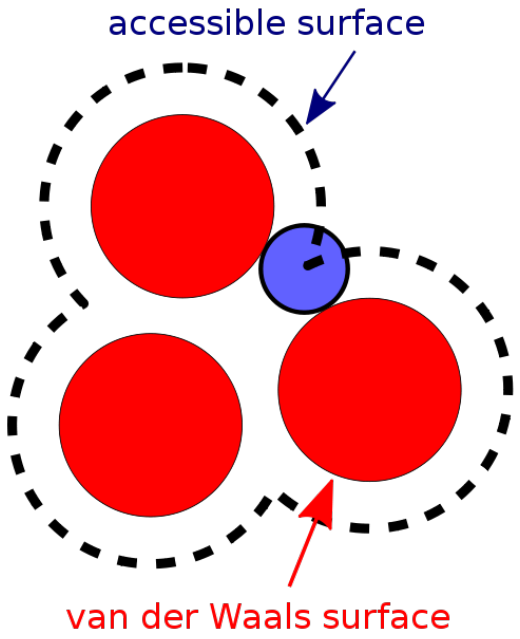
- In biological macromolecules,

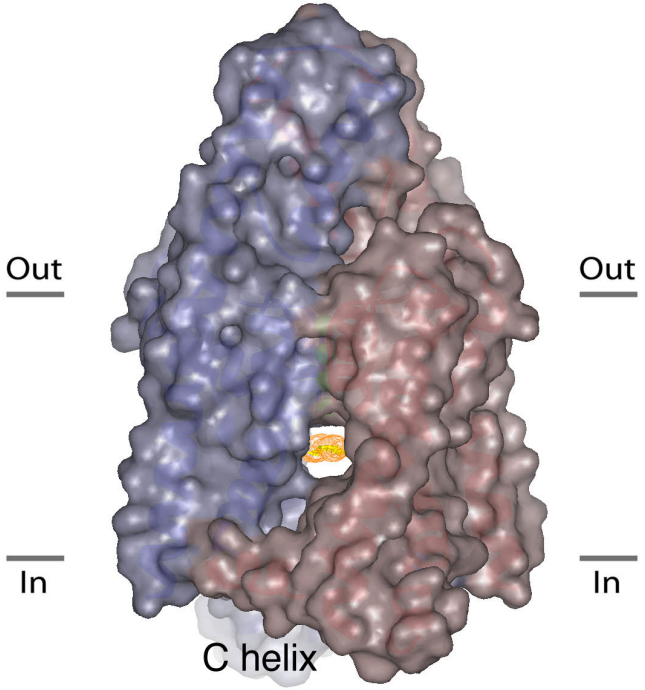
strong donors:	N—H, O—H
weak donors:	S—H, C—H (from edges aromatic systems)
strong acceptors:	=O, —O—H, =N— C
weak acceptors:	S^-, —S— , and faces of aromatic systems

- Typical H-bond distances are 2.8 Å for strong bonds to 3.2 Å for weaker ones.
  H-bonds are typically measured as the distance between donor and acceptor (not to the hydrogen)

The surface of a molecule modeled as a collection of spheres will have many small nooks and crannies that may not be accessible to other atoms. A useful model of the surface of a molecule is the **accessible surface area**.

The solvent accessible surface is sometimes referred to as the molecular surface.





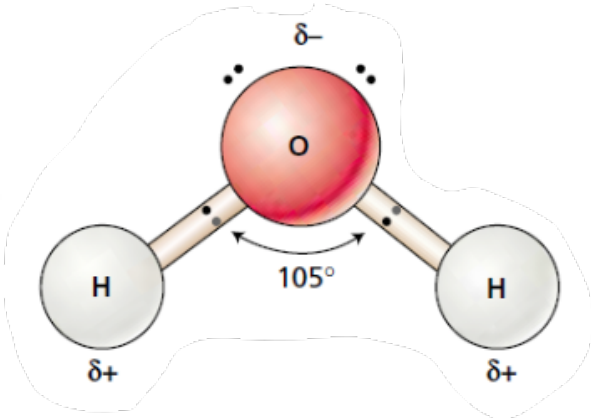
potassium channel K2P1
accessible surface

hydrophobic effect

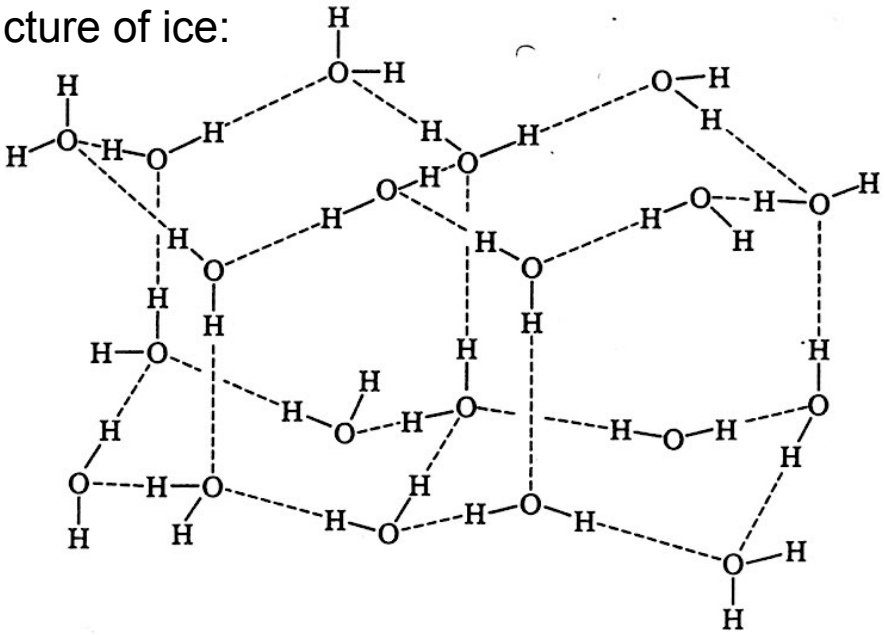
why do oil and water not mix?

Introduction to the **Hydrophobic** effect

- Key to the hydrophobic effect is water does not 'like' (+ΔG) having its hydrogen-bond network disrupted.



structure of ice:

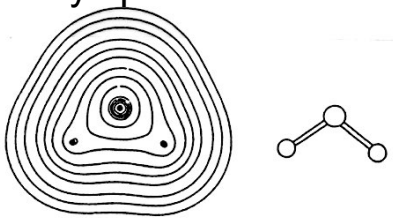


in liquid:

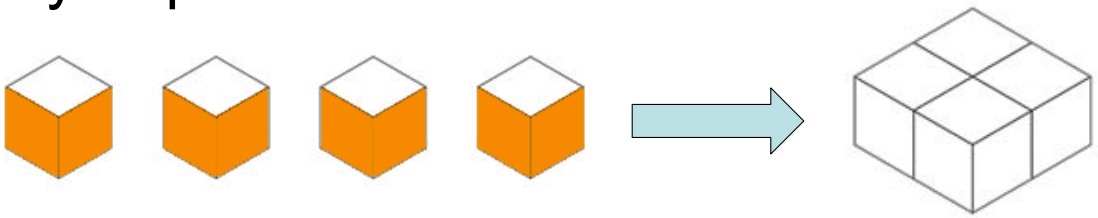
- 5th neighbor ?
- orientations not fixed

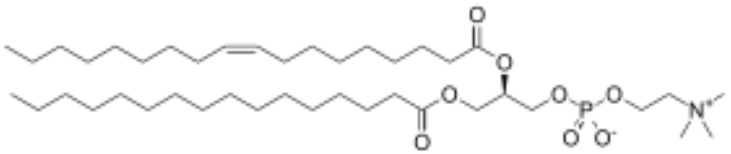
FIGURE 4.7
Structure of normal ice. Each H_2O molecule is involved in four hydrogen bonds (thin, dashed lines), each 2.76 Å between oxygen atoms. The water molecule is donor in two hydrogen bonds, acceptor in the other two. Substantial empty channels run between the molecules.

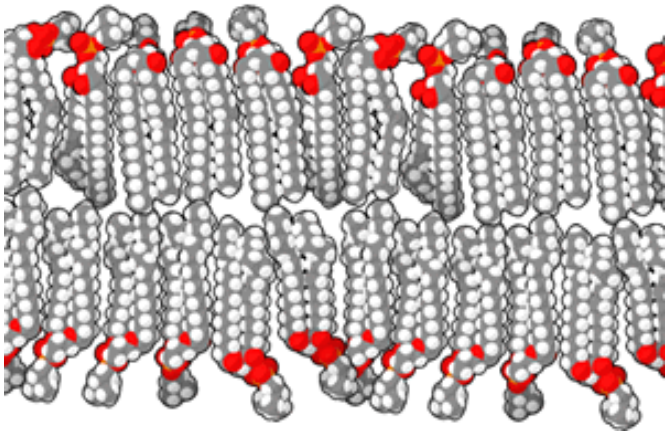
electron density of H_20 nearly spherical



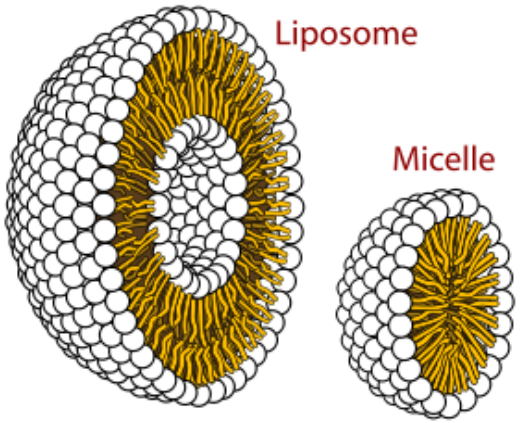
hydrophobic effect

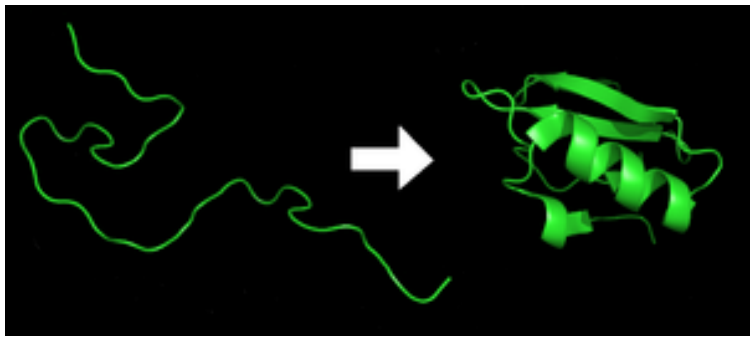




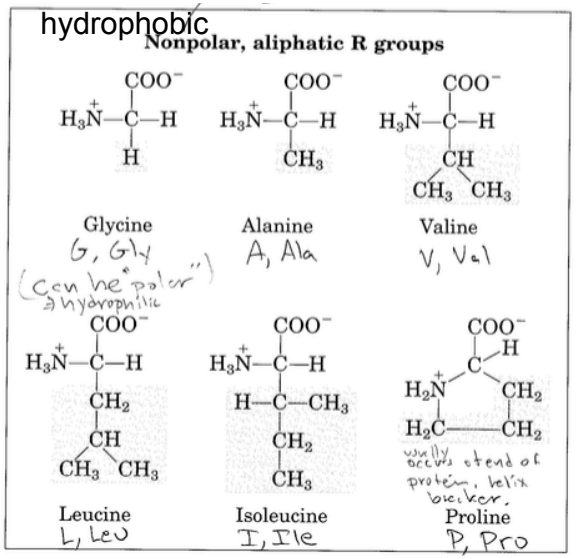


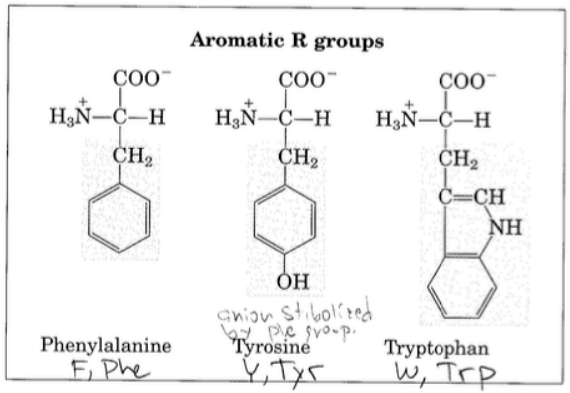
lipid bilayer

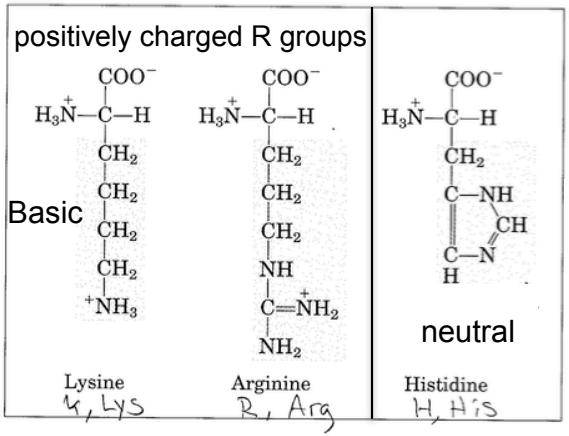


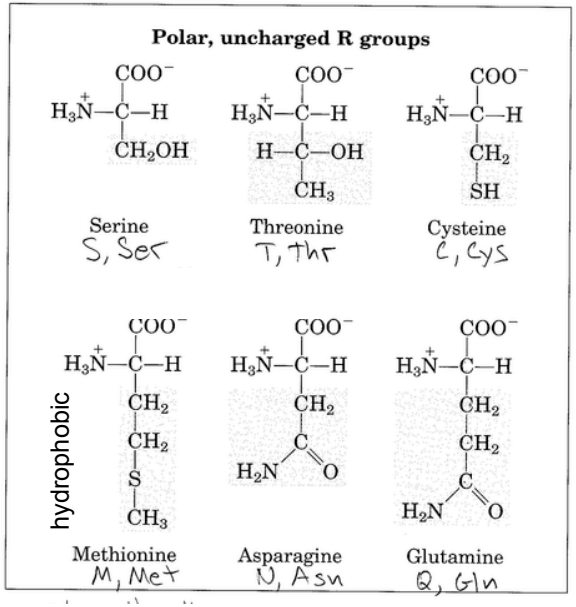


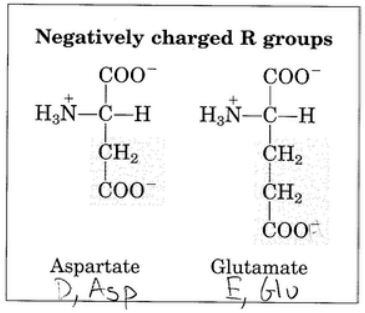
hydrophobic







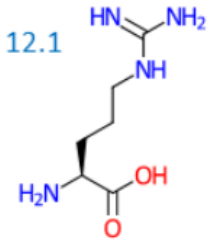




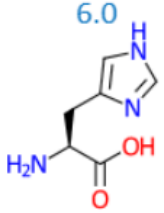
Acidic

standard amino acids,
as they would occur at pH 7.0

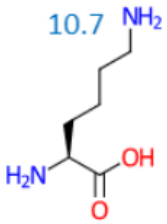
Positively charged



Arginine
Arg **R**



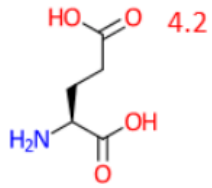
Histidine
His **H**



Lysine
Lys **K**

Negatively charged

Aspartic Acid
Asp **D**



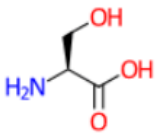
Glutamic Acid
Glu **E**

12.1 or 6.0: pKa of side chain

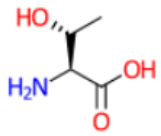
Sulfur or Selenium

The amino acids never exist in solution in these forms. Refer to previous slide for their form at pH 7. Textbooks can be confusing!

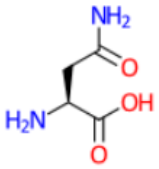
Polar uncharged



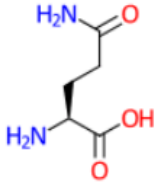
Serine
Ser **S**



Threonine
Thr **T**

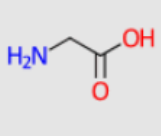


Asparagine
Asn **N**

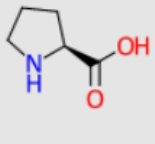


Glutamine
Gln **Q**

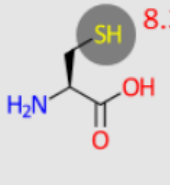
Special cases



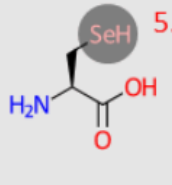
Glycine
Gly **G**



Proline
Pro **P**

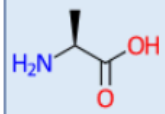


Cysteine
Cys **C**

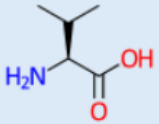


Selenocysteine
Sec **U**

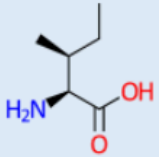
Hydrophobic



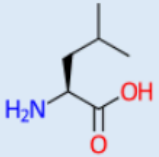
Alanine
Ala **A**



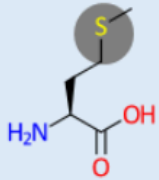
Valine
Val **V**



Isoleucine
Ile **I**

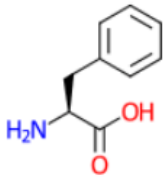


Leucine
Leu **L**

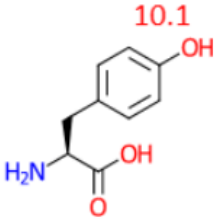


Methionine
Met **M**

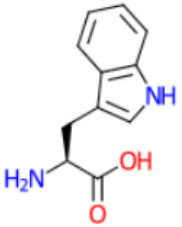
Hydrophobic



Phenylalanine
Phe **F**



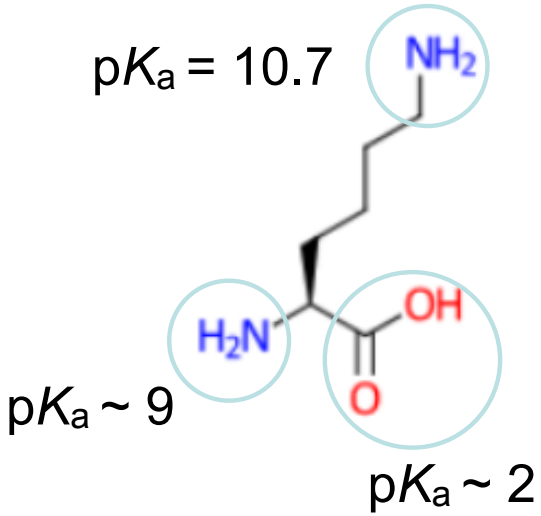
Tyrosine
Tyr **Y**



Tryptophan
Trp **W**

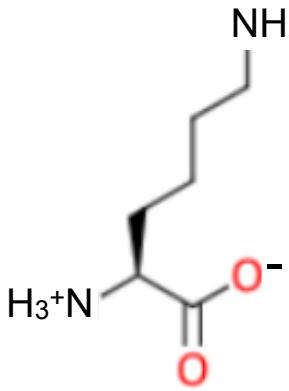
Thomas Ryckmans 2021

Lysine (Lys, K)

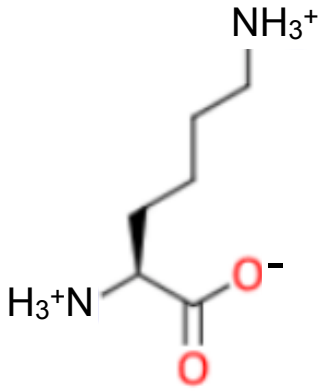


It never exists in solution in this form.
Textbooks can be confusing!

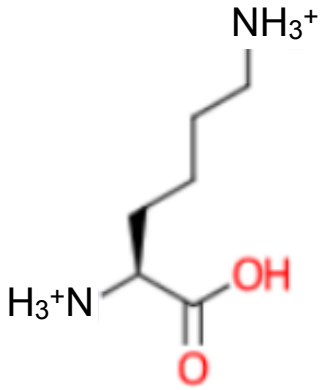
at neutral pH (pH 7)



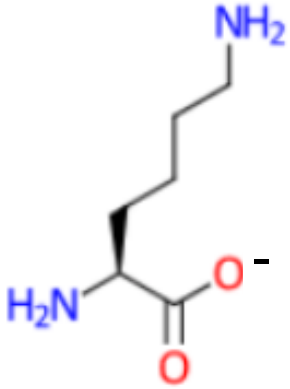
at pH 4



at pH 1.5



at pH 12

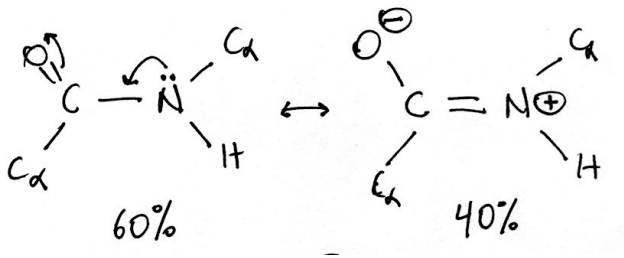


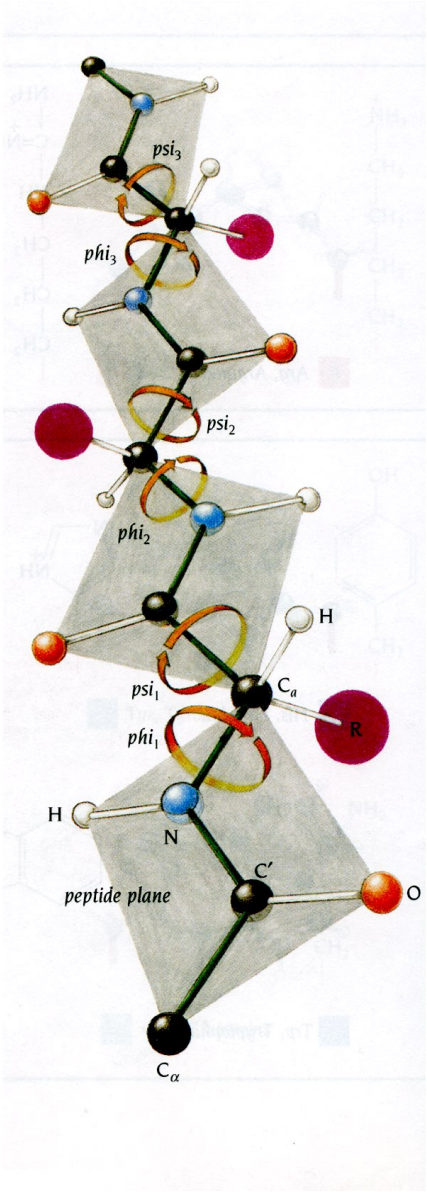
When the pH is below the pK_a, the protonatable group is protonated.

When the pH is above the pK_a, the protonatable group is deprotonated.

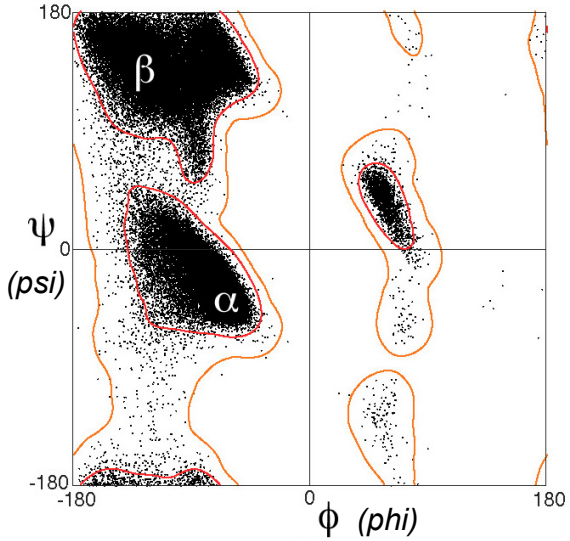
Introduction to protein structure

polypeptide bond: phi/psi angles

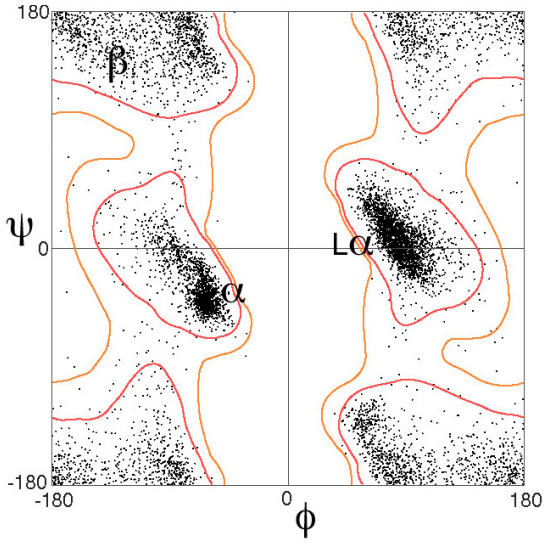




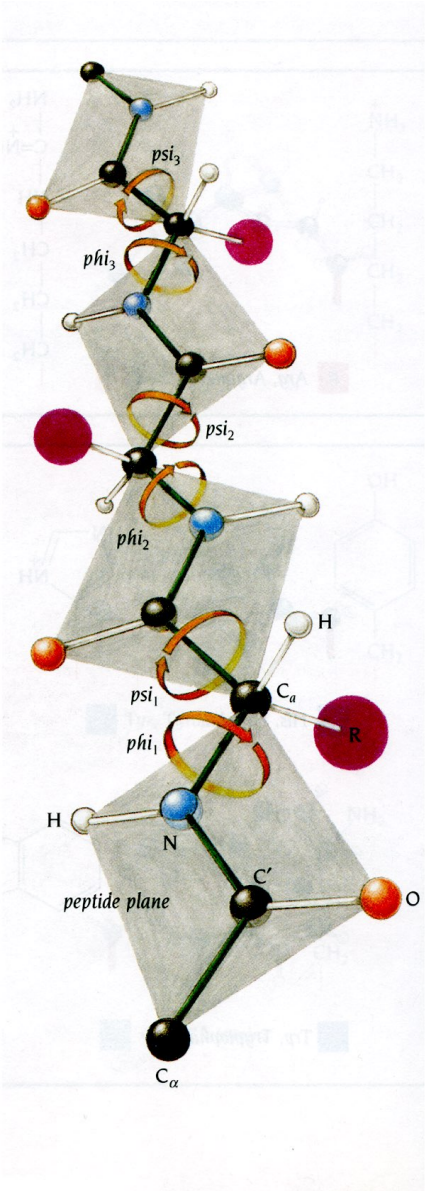
Ramachandran plot



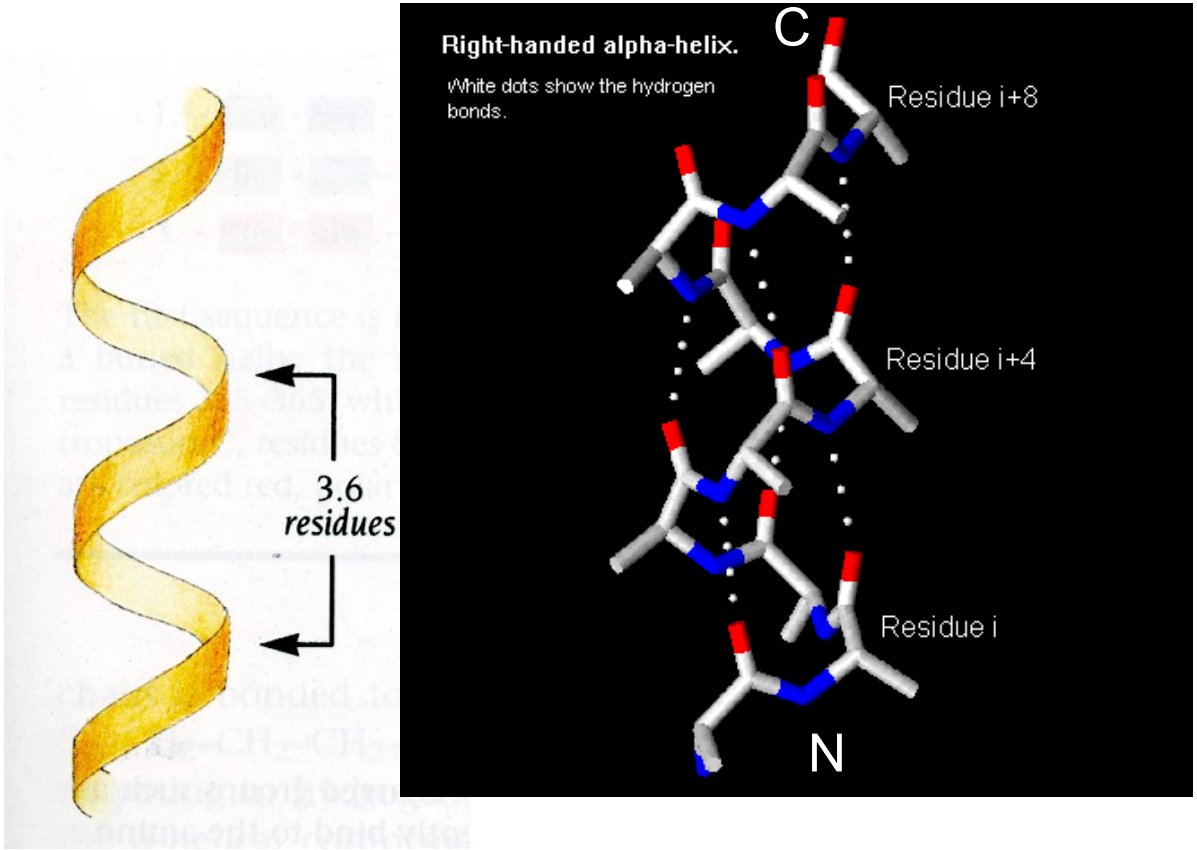
all residues
except Gly/Pro



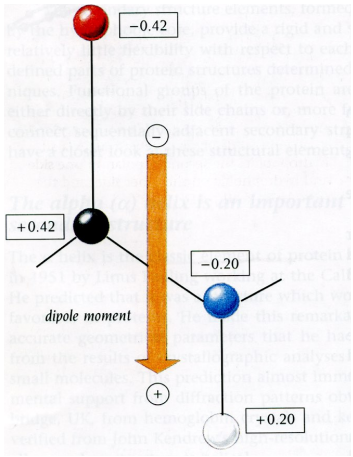
Gly residues

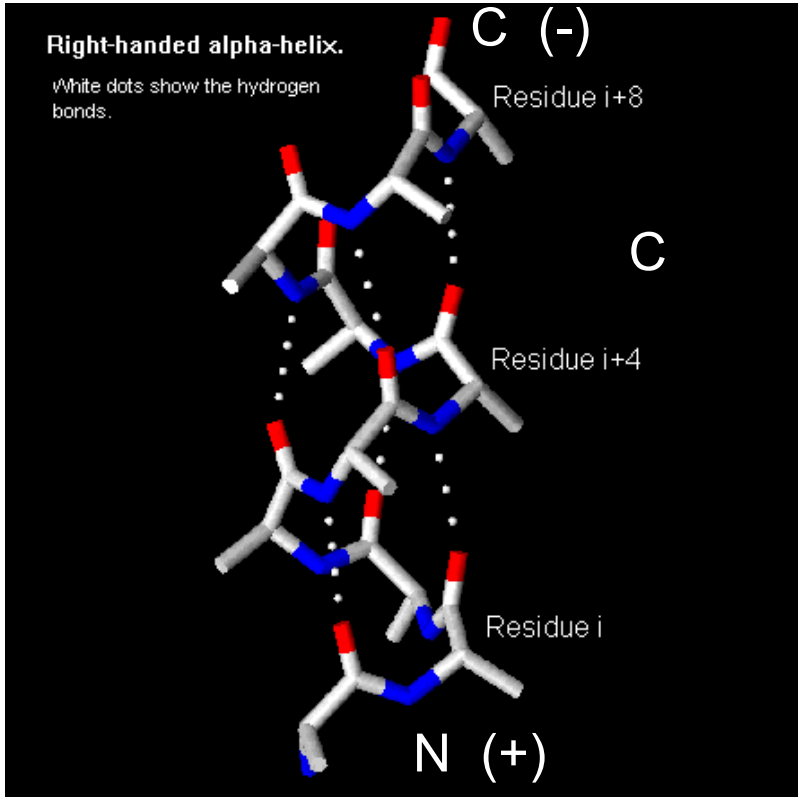


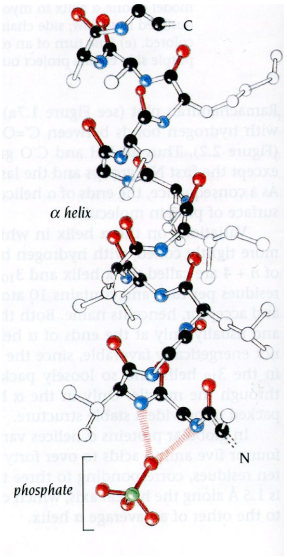
secondary structure: the alpha helix



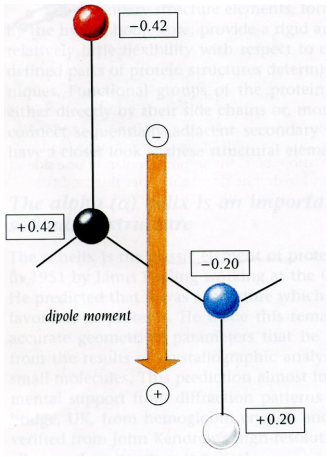
alpha helix dipole moment

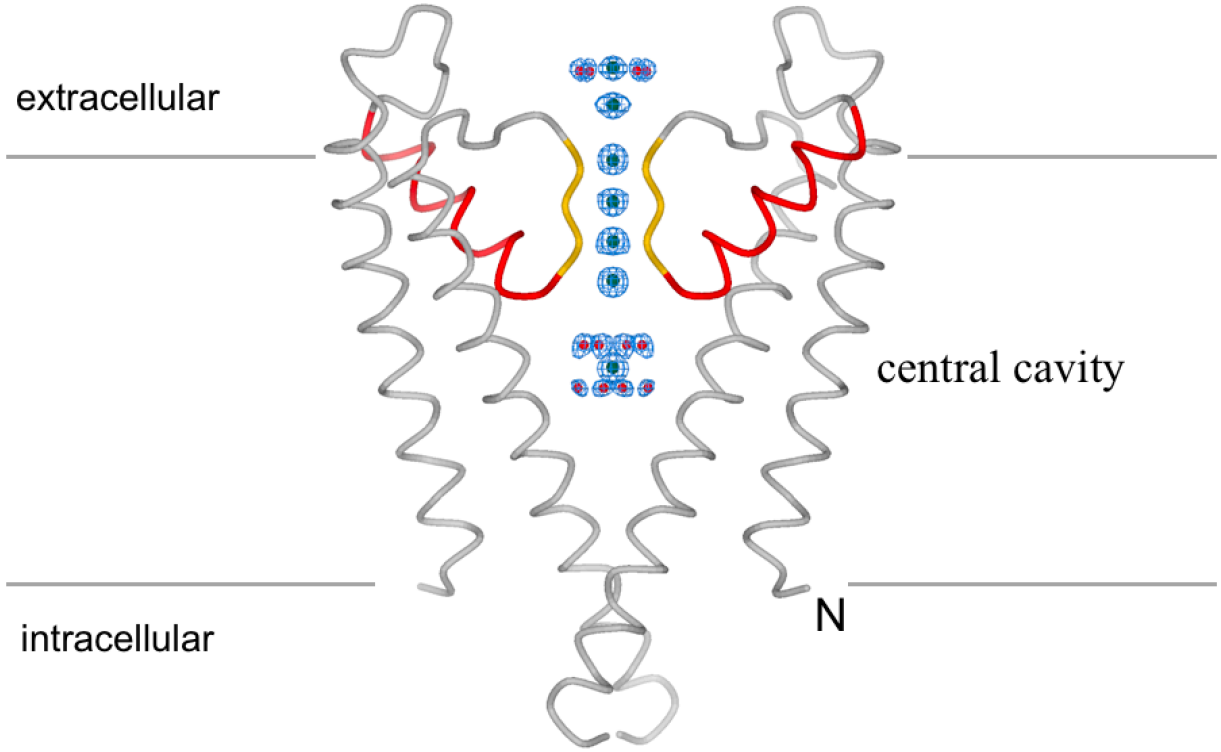






alpha helix dipole moment

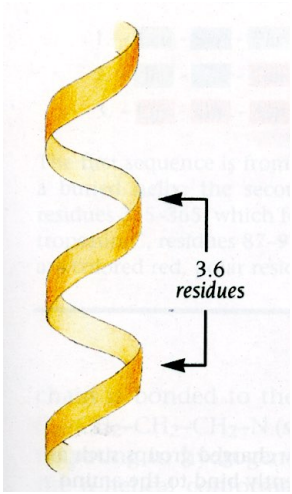


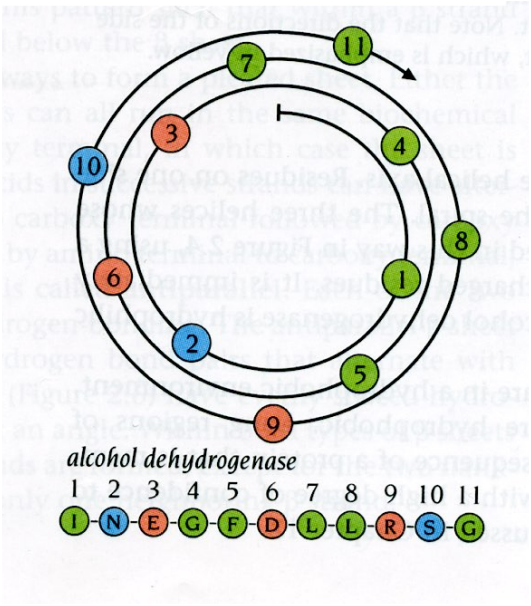


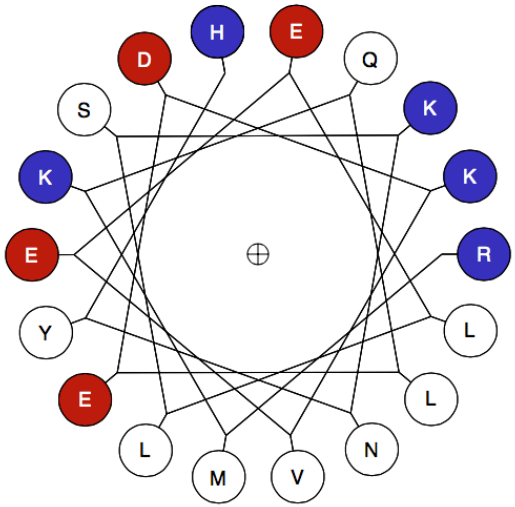
potassium channel KcsA: D.A. Doyle et al. 1998

helical wheel projections

amphipathic helices:

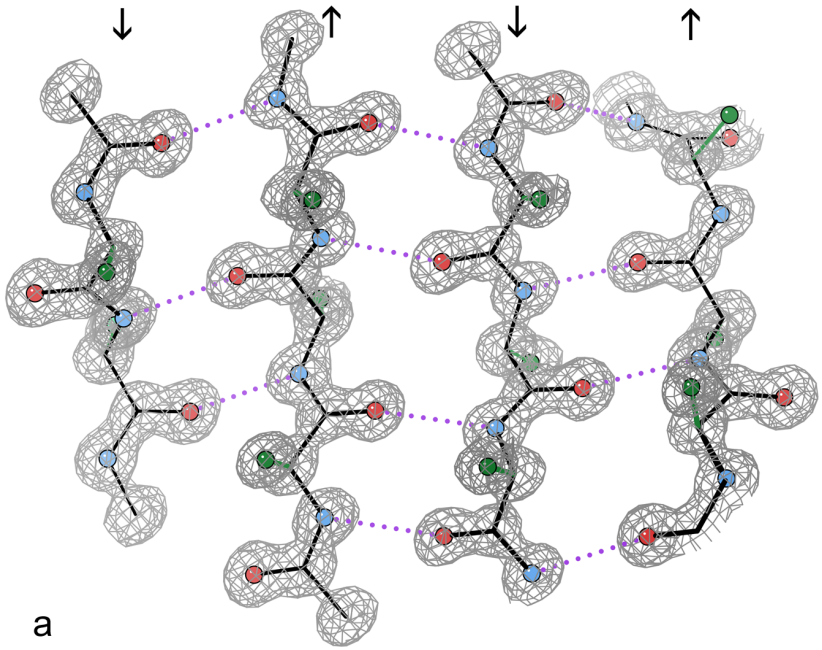


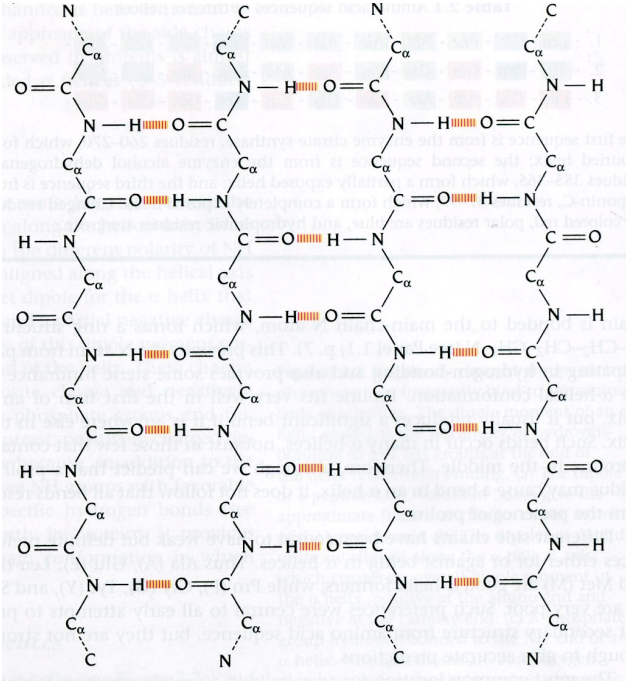


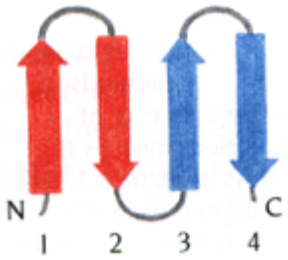


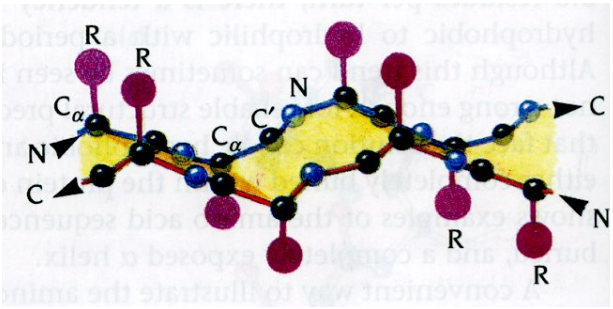
most common place for alpha helix: partially buried

secondary structure: the antiparallel beta sheet

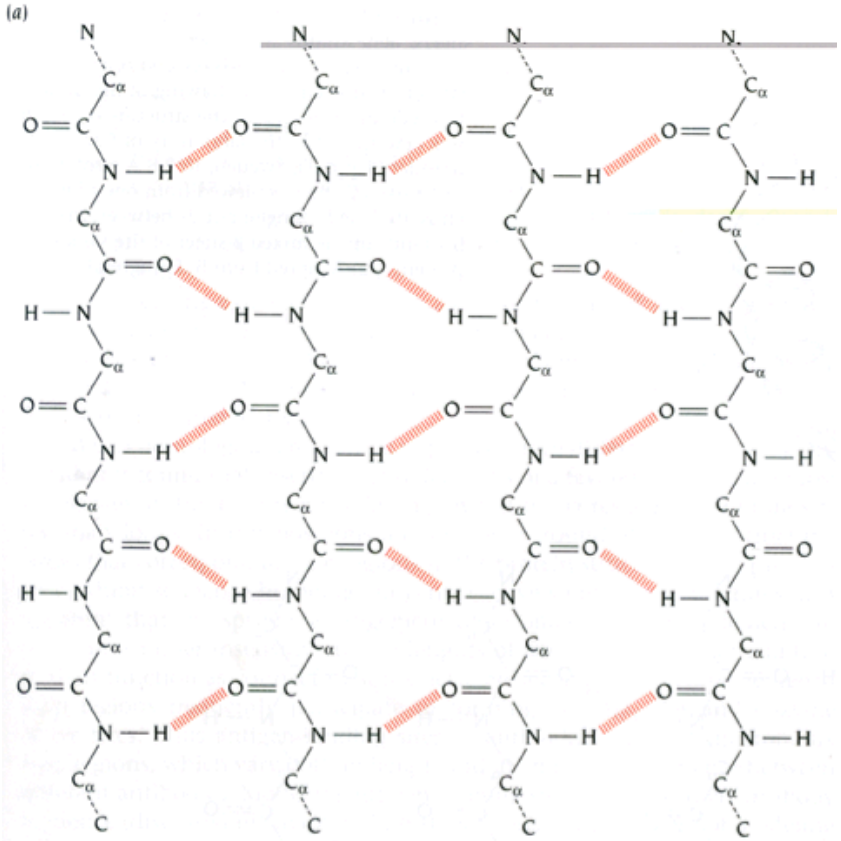


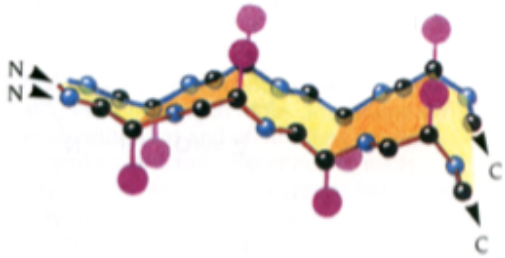


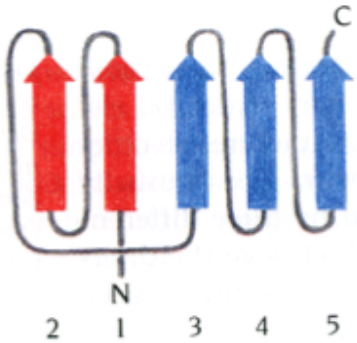




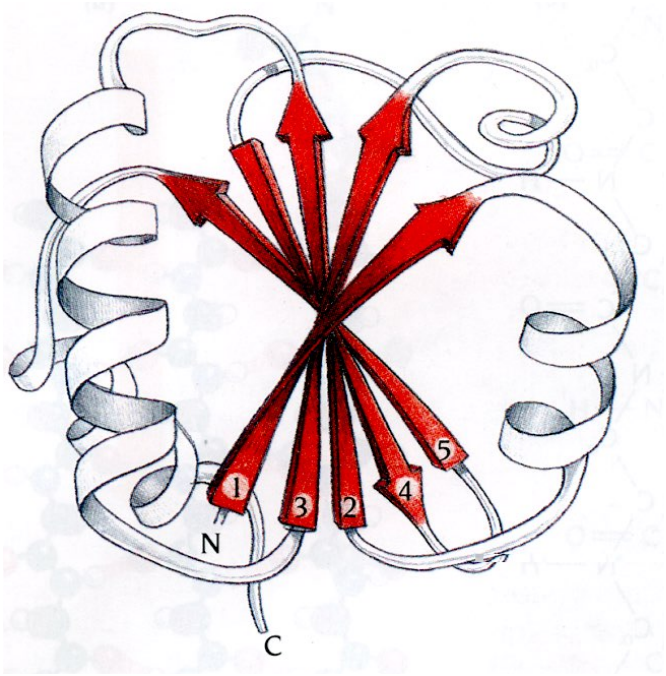
parallel beta sheet





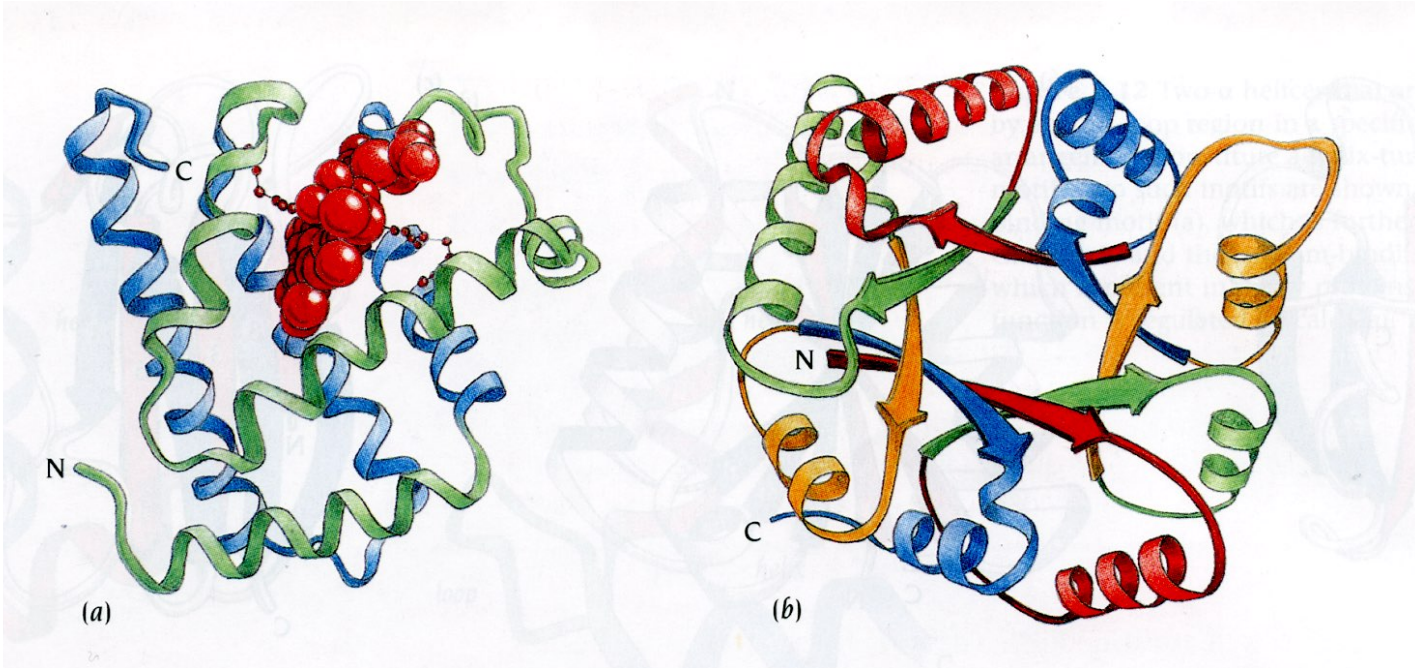


sheets have a ‘right-handed’ twist



thioredoxin

schematic diagrams: secondary structure dominates

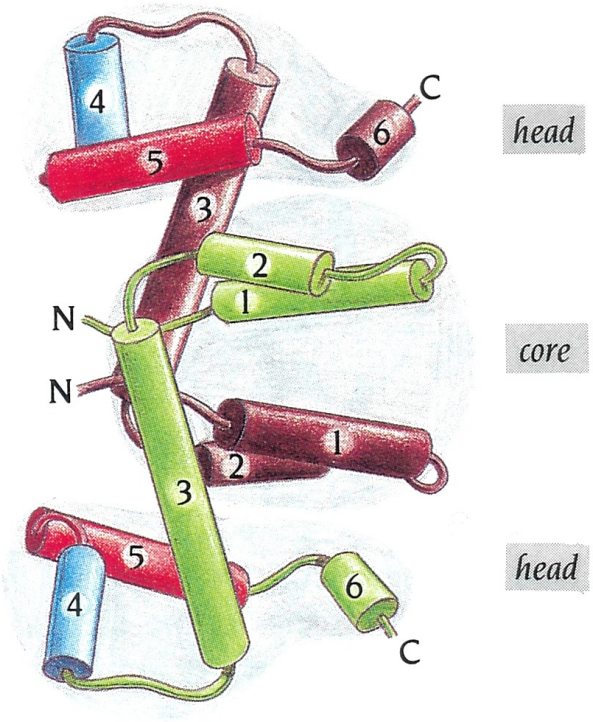


suggested texts:

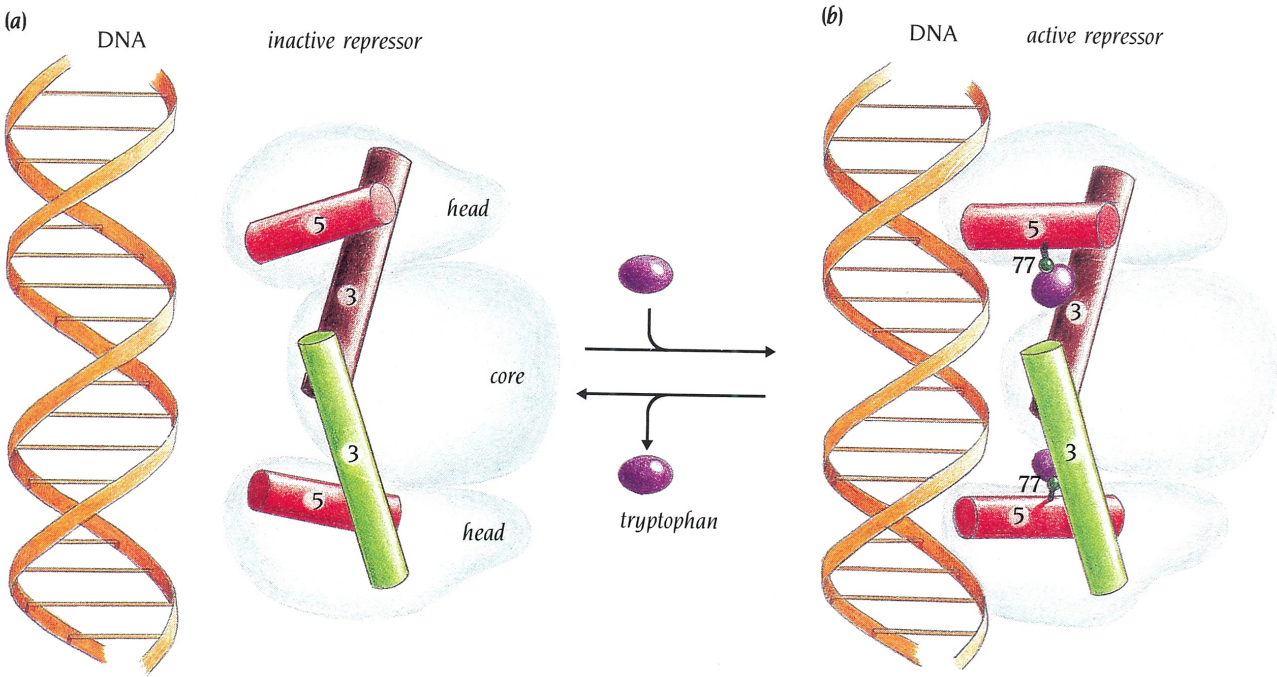
Proteins, by TE Creighton

Introduction to Protein Structure
by Carl Branden and John Toonze

Protein Conformational Changes



Trp repressor



concept of <u>induced fit</u>

Proteins are flexible

Small scale changes:
Individual amino acids
local regions of peptide backbone

large scale changes:
Domain movements
Sliding movements
Metamorphosis

Occur over several time scales

Proteins are flexible. Changes in conformation may be subtle, reflecting molecular vibrations and small movements of amino acid residues throughout the protein. A protein flexing in this way is sometimes said to "breathe." Changes in conformation may also be more dramatic, with major segments of the protein structure moving as much as several nanometers. Specific conformational changes are frequently essential to a protein's function.

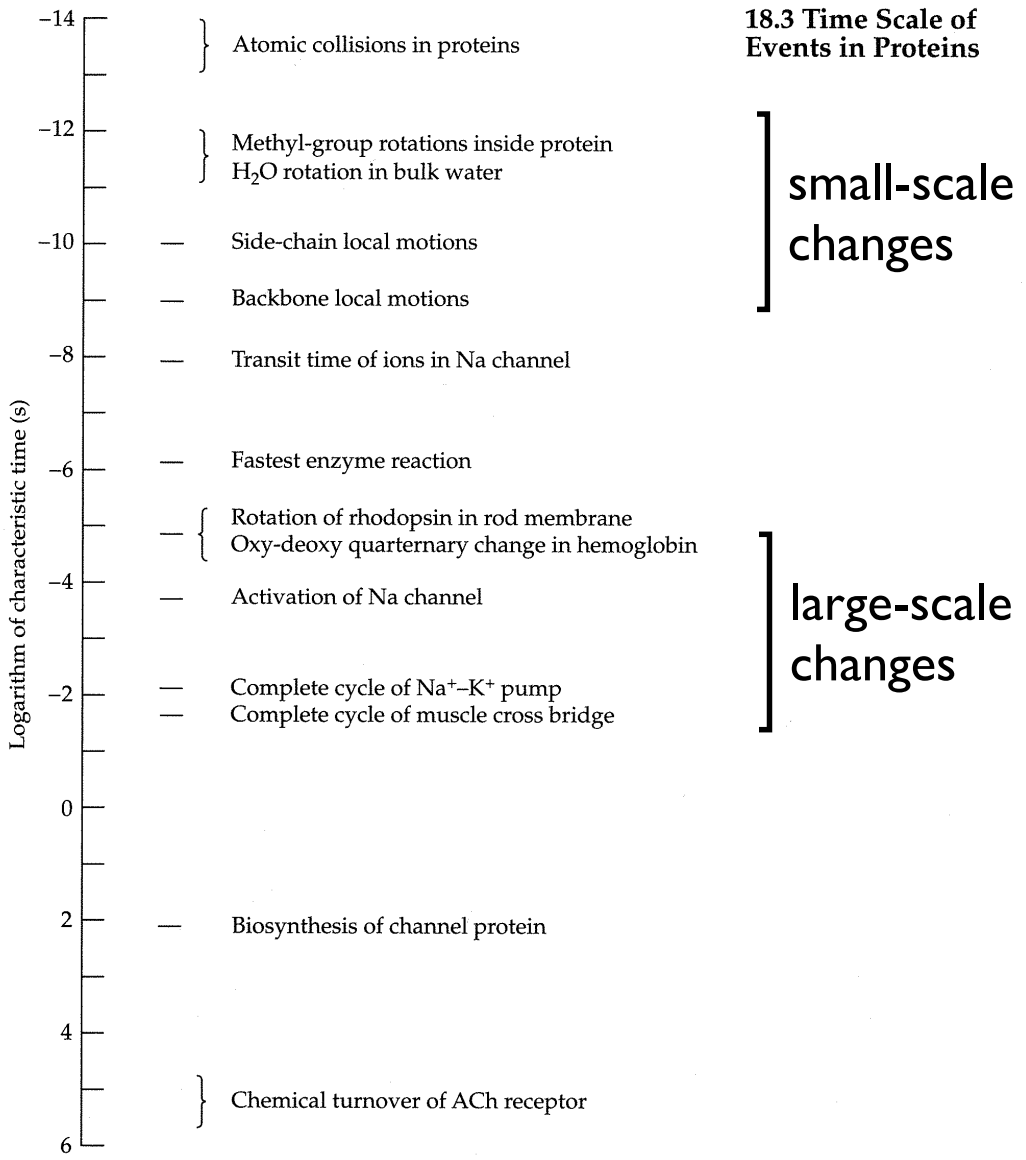
The binding of a protein and ligand is often coupled to a conformational change in the protein that makes the binding site more complementary to the ligand, permitting tighter binding. The structural adaptation that occurs between protein and ligand is called **induced fit**.

Proteins are flexible

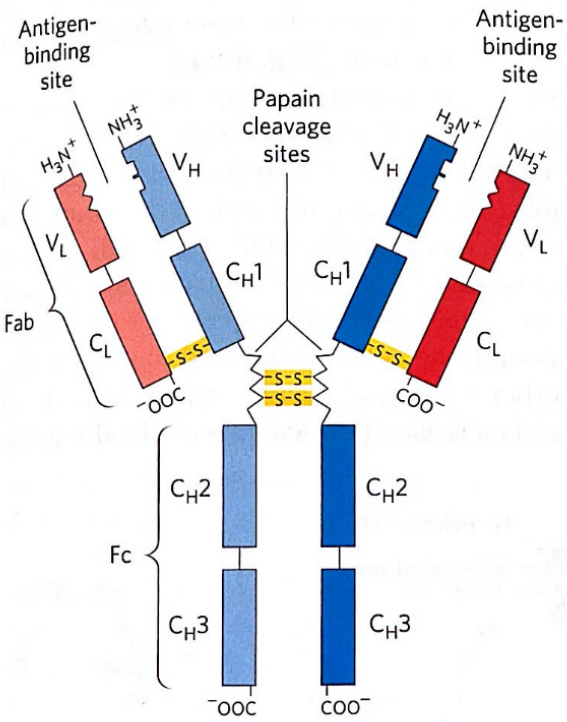
Small scale changes:
Individual amino acids
local regions of peptide backbone

large scale changes:
Domain movements
Sliding movements
Metamorphosis

Occur over several time scales



regions of flexibility can sometimes be deduced by looking at the structure or amino acid sequence alignment.



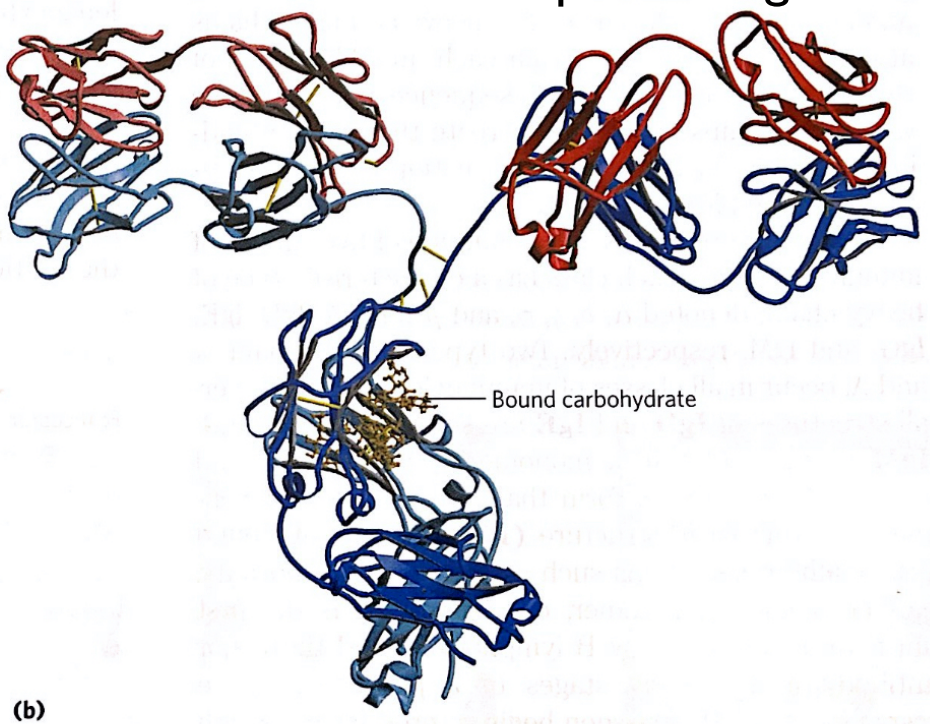
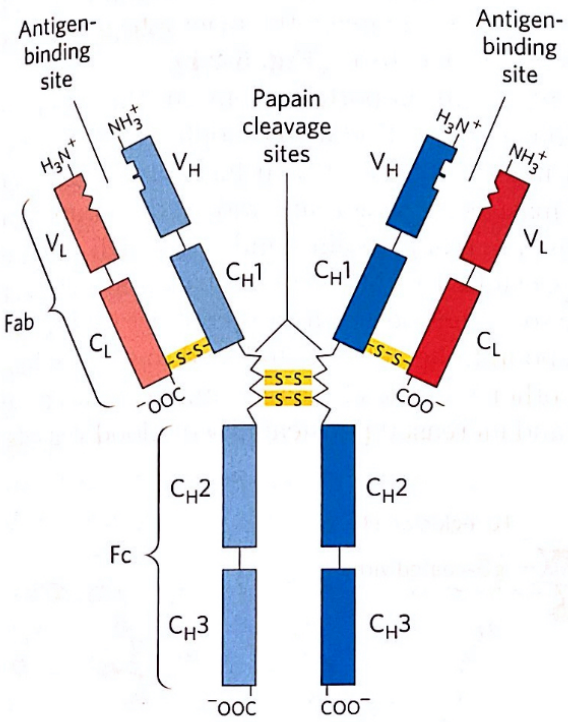
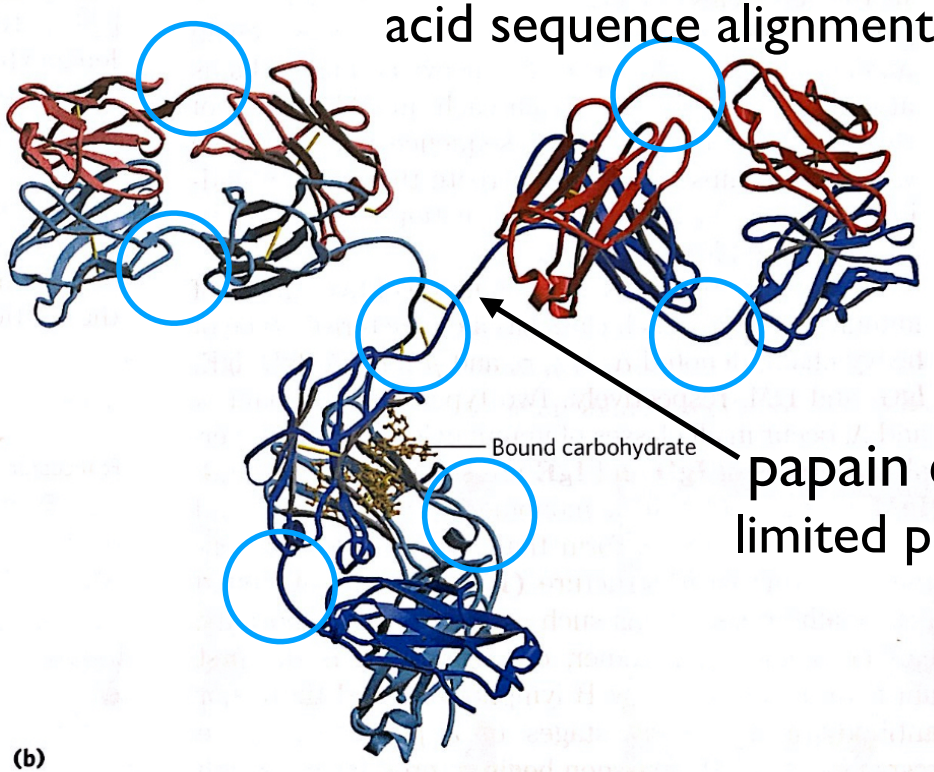


FIGURE 5-21 Immunoglobulin G. (a) Pairs of heavy and light chains combine to form a Y-shaped molecule. Two antigen-binding sites are formed by the combination of variable domains from one light (V_L) and one heavy (V_H) chain. Cleavage with papain separates the Fab and Fc portions of the protein in the hinge region. The Fc portion also contains bound carbohydrate (shown in (b)). **(b)** A ribbon model of the first complete IgG molecule to be crystallized and structurally analyzed. Although the molecule has two identical heavy chains (two shades of blue) and two identical light chains (two shades of red), it crystallized in the asymmetric conformation shown here. Conformational flexibility may be important to the function of immunoglobulins. [Source: (b) PDB ID 1IGT, L. J. Harris et al., *Biochemistry* 36:1581, 1997.]

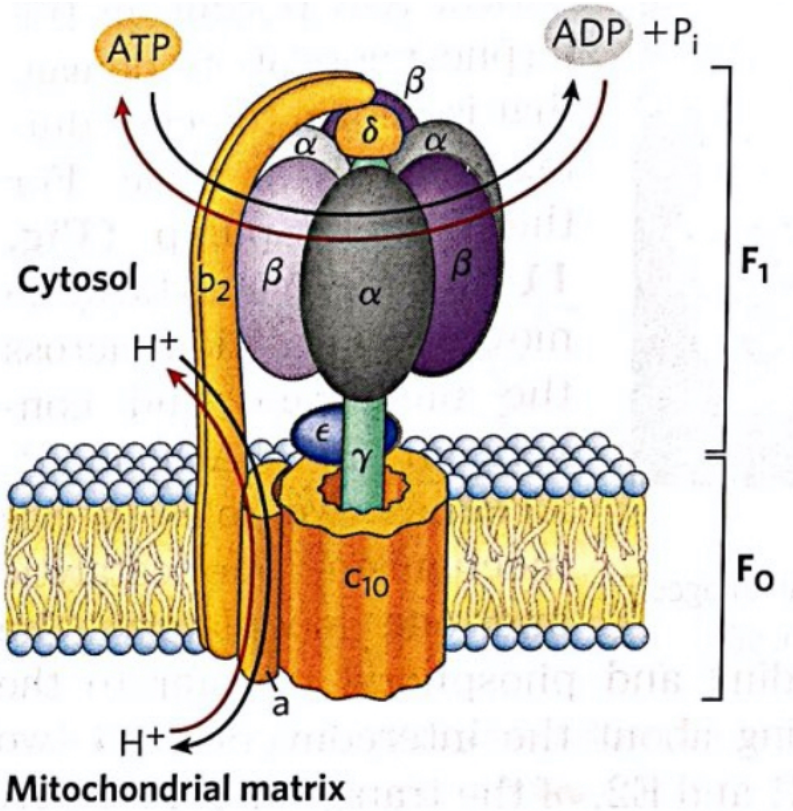
regions of flexibility can sometimes be deduced by looking at the structure or amino acid sequence alignment.





papain cleavage
limited proteolysis

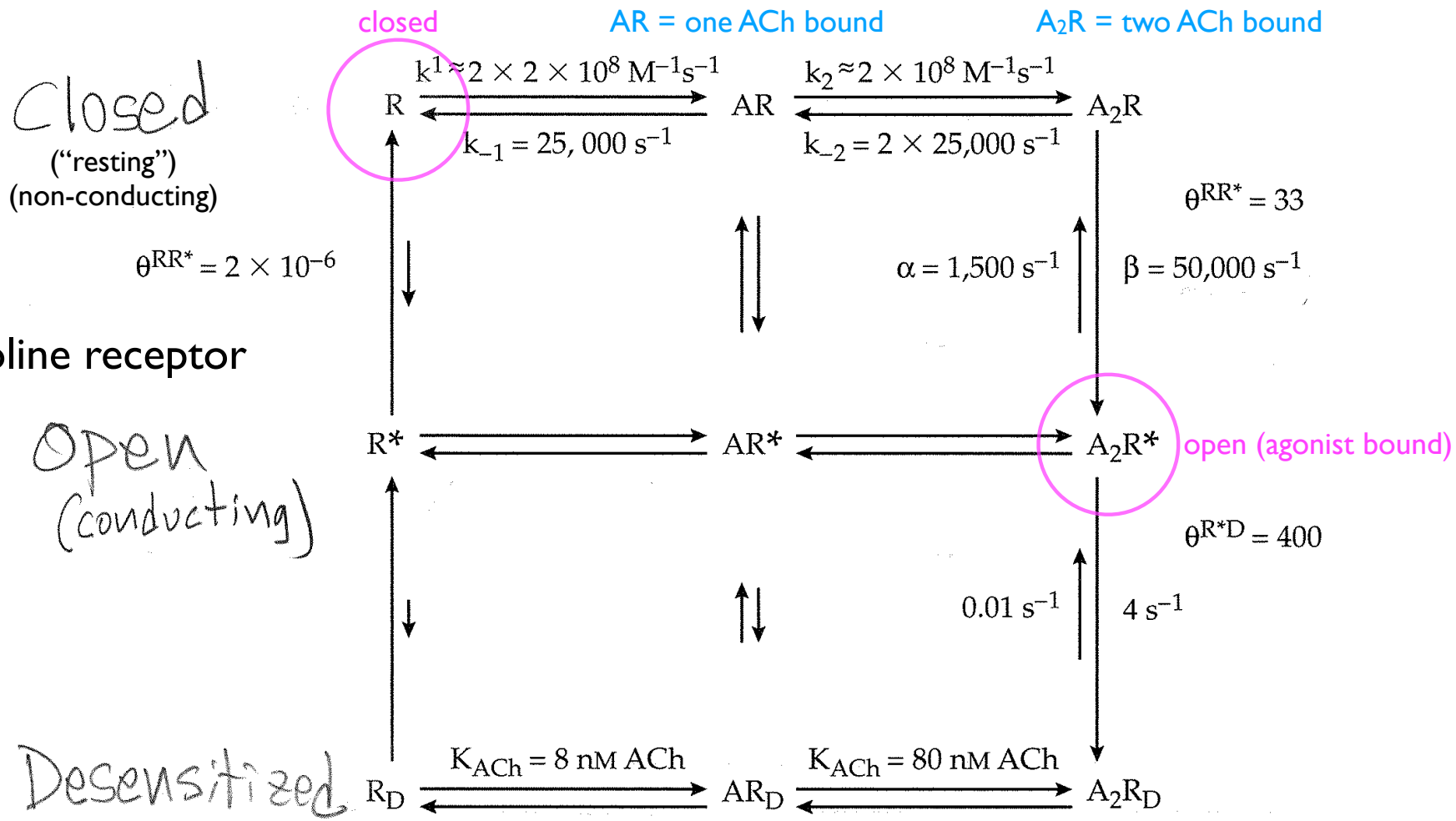
FIGURE 5-21 Immunoglobulin G. (a) Pairs of heavy and light chains combine to form a Y-shaped molecule. Two antigen-binding sites are formed by the combination of variable domains from one light (V_L) and one heavy (V_H) chain. Cleavage with papain separates the Fab and Fc portions of the protein in the hinge region. The Fc portion also contains bound carbohydrate (shown in (b)). **(b)** A ribbon model of the first complete IgG molecule to be crystallized and structurally analyzed. Although the molecule has two identical heavy chains (two shades of blue) and two identical light chains (two shades of red), it crystallized in the asymmetric conformation shown here. Conformational flexibility may be important to the function of immunoglobulins. [Source: (b) PDB ID 1IGT, L. J. Harris et al., *Biochemistry* 36:1581, 1997.]



ATP synthase

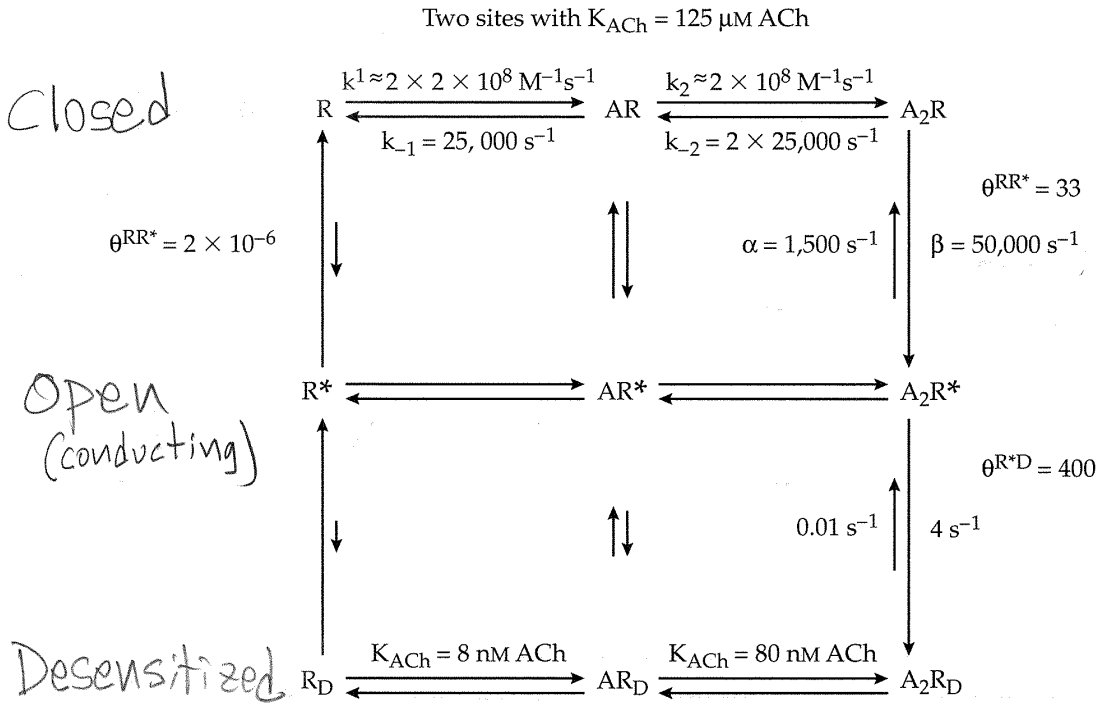
https://www.youtube.com/watch?v=k_DQ1FjFuYM&t=48s

https://www.youtube.com/watch?v=kXpzp4RDGJI&t=3s



6.10 A Kinetic Model for nAChR Gating

Ion Channels of Excitable Membranes. Bertil Hille



6.10 A Kinetic Model for nAChR Gating An allosteric state diagram for kinetic analysis of transitions of nACh receptors. Proceeding from left to right, the agonist binding sites go from unliganded, to singly liganded, to doubly liganded. Proceeding from top to bottom, the overall channel conformation goes from closed (R), to open (R^*), to desensitized (R_D). Some of the forward and backward rate constants of individual steps are labeled with first-order (s^{-1}) or second-order ($M^{-1}s^{-1}$) rate constants. Some of the ACh binding steps are labeled with their apparent ACh equilibrium dissociation constants (K_{ACh}). Some of the conformational transitions are labeled with their equilibrium constants (θ). Numerical values are for mouse $\alpha_2\beta\gamma\delta$ AChR at –100 mV and T = 22°C. [Values from Auerbach and Akk 1998 and Salamone et al. 1999.]

paper discussion