

Biochemistry 530:
Introduction to Structural Biology

Autumn Quarter 2016

Course Information

BIOC 530

Graduate-level discussion of the structure, function, and chemistry of proteins and nucleic acids, control of enzymatic reactions. Please see the course syllabus and course website for more information concerning lecture topics.

If you are not a graduate student and have not completed Biochemistry 440,441,442; please speak with David Baker.

Course Website

URL: <http://courses.washington.edu/bioc530/>

Recommended Readings

Proteins: Structure & Molecular Properties, Thomas Creighton

Structure and Mechanism in Protein Science, Alan Fersht

Introduction to Protein Structure, Carl Branden, John Tooze

Protein Physics, Alexei Finkelstein, Oleg Ptitsyn

Course Information

Assignments & Exams

Problems sets will be given near the end of each block of lectures and usually will be due one week after the end of the block. There will be only one exam for this course at the end of the quarter. Your final grade will be based on homework assignments, participation in class and your performance on the final exam. Questions regarding grading can be addressed to David Baker.

Grading

Your final grade will be based on weekly homework assignments (60%), class participation (10%), and the final exam (30%).

TA Office Hours

TA office hours are held each week in Health Sciences J-475 on Fridays from 9 to 10 am.

Please email Jiayi at doujy@uw.edu to make an appointment.

Lecture Schedule(2016)

Week	Date	Monday	Wednesday	Friday
1	9/26 – 9/30	No Class	Forces I (DB)	Forces II (DB)
2	10/3 – 10/7	Forces III (DB)	X-Ray Crystallography I (EM)	X-Ray Crystallography II (EM)
3	10/10 – 10/14	X-Ray Crystallography III (EM)	X-Ray Crystallography IV (EM)	Protein Folding I (DB)
4	10/17 – 10/21	Protein Folding II (DB)	Protein Folding in vivo (DB)	Membrane Proteins (WH)
5	10/24 – 10/28	The RNA World I (AW) RNA folding movie	The RNA World II (AW)	The RNA World III (AW)
6	10/31 – 11/4	Sequence Comparison (DB)	Structure Prediction (DB)	Electron Microscopy (DV)
7	11/7 – 11/11	NMR I (RK)	NMR II (RK)	No Class (Veterans Day)
8	11/14 – 11/18	NMR III (RK)	Protein Dynamics I (VD)	Protein Dynamics II (VD)
9	11/21 – 11/25	Protein Design (DB) Design movie	Discussion Section	No Class (Thanksgiving)
10	11/28 – 12/2	Signaling and Protein Modification I (NZ)	Signaling and Protein Modification II (NZ)	Membrane Proteins (WH)
11	12/5 – 12/9	Structure-Based Drug Design (WH)	Chemical Biology I (DM)	Chemical Biology II (DM)
12	12/12 – 12/16	Exam		

Lectures 1-2: Review of forces and elementary statistical mechanics

Part I. Review of forces

- Covalent bonds
- Non-covalent Interactions:
 - Van der Waals Interactions
 - Electrostatics
 - Hydrogen Bonds
 - Hydrophobic Interactions

Part II. Review of key concepts from Stat. Mech.

Part III. Contributions to protein stability and binding

FORCES:

Covalent Bonds

Note on units:

Energy : kcal/mol (1kcal = 4.184 kJ).

Distance : Å (1Å = 10^{-10} m)

Thermal energy at room temperature = $RT = .59$ kcal/mol

Covalent bonds.

Some approximate bond energies:

C-C 82 kcal/mol

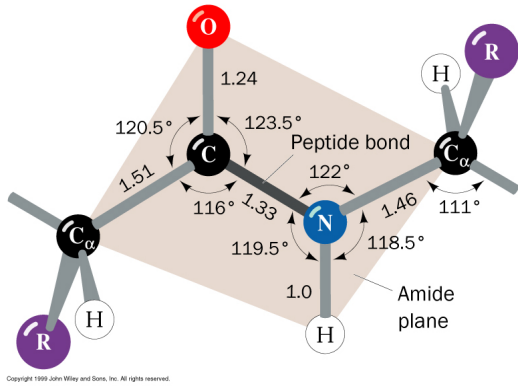
C-N 70 kcal/mol

Stronger than most non covalent interactions.

Important for chemistry, biosynthesis, metabolism but not for macromolecular conformation (the bonded structure of a macromolecule is independent of its conformation)

Recall the basic geometry of the peptide backbone (next page)

1) Covalent structure in the Peptide Bond plane



2) O and H atoms clashing

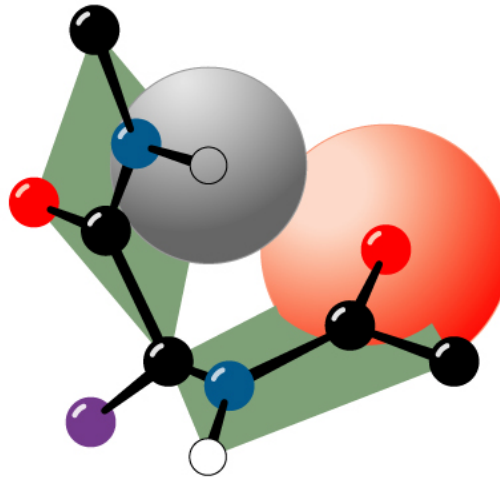


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3) Geometry of a di-peptide $\Phi=180, \Psi=180$

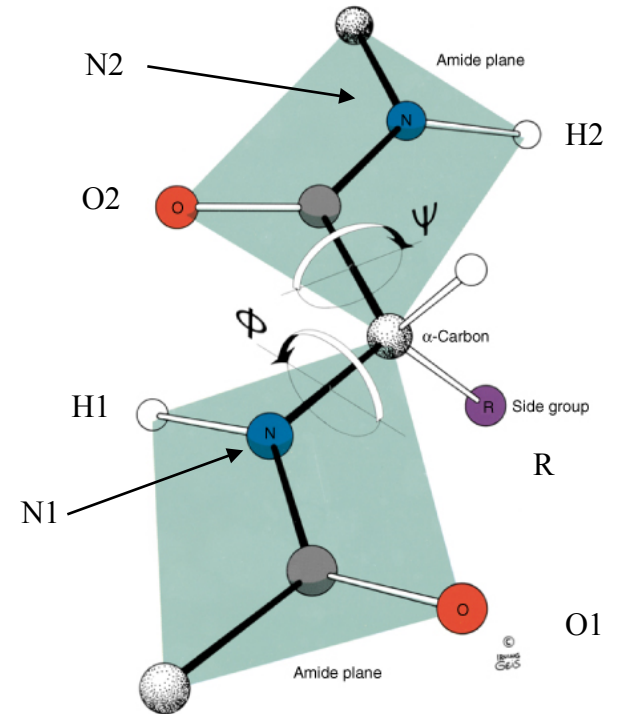


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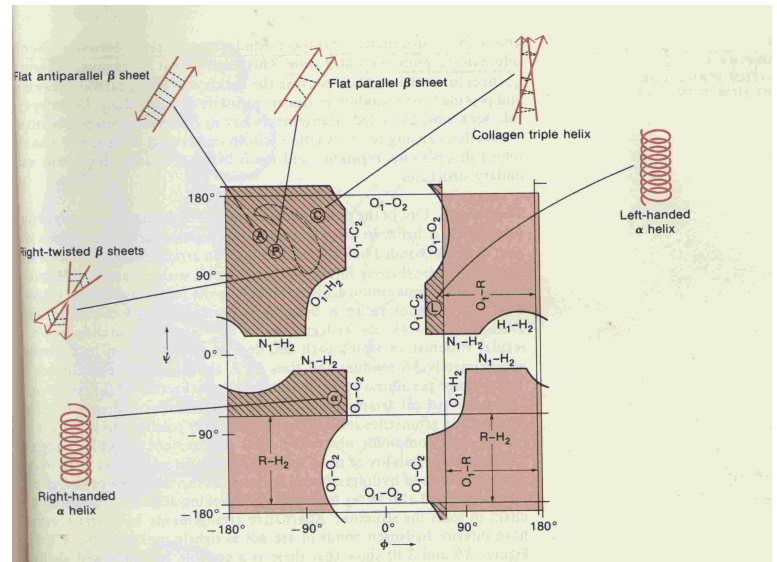
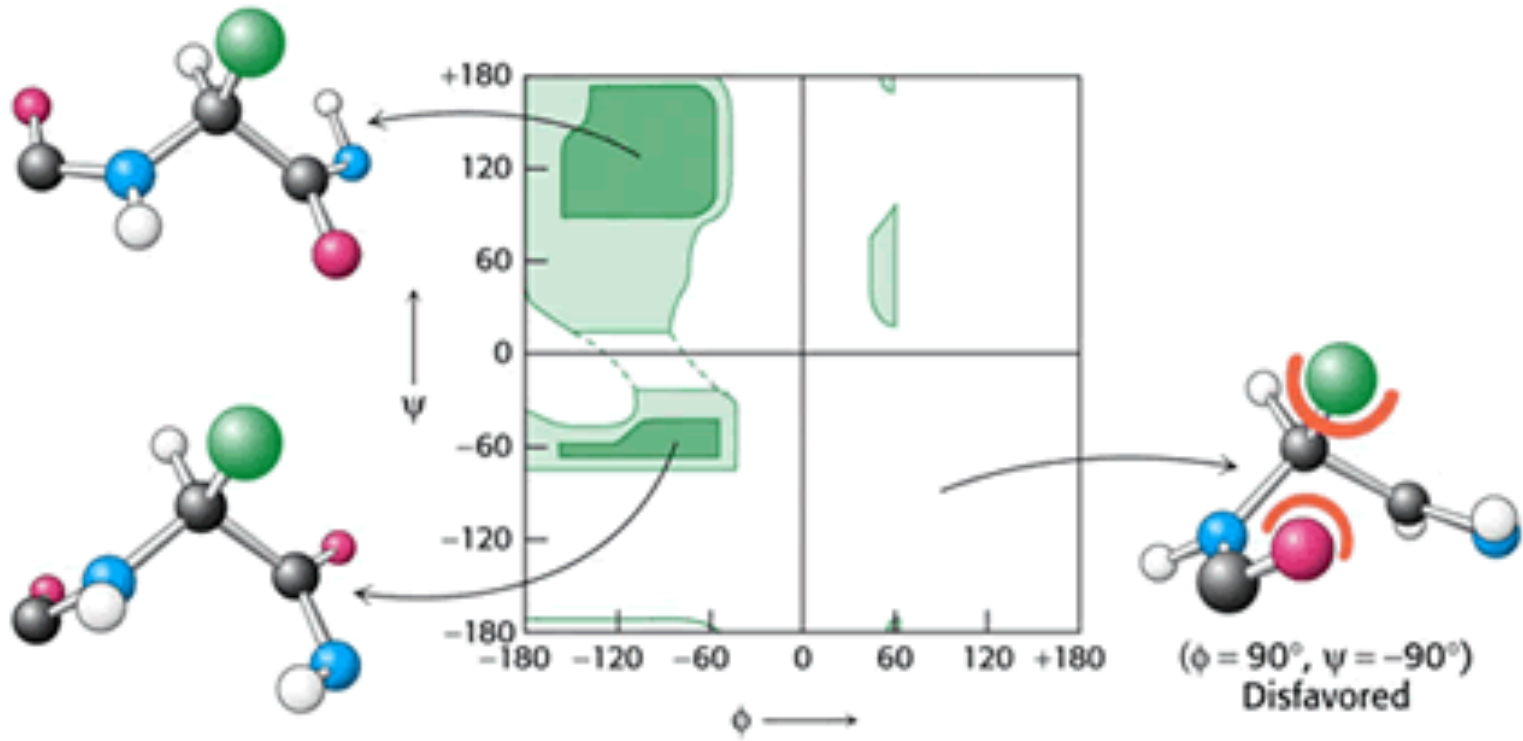
FORCES:

Non-covalent Interactions: Van der Waals Interactions

Two contributions to the interactions between nonpolar atoms:

A. strong repulsion at short distances (Pauli exclusion principle)

- usually modeled either as overlap between hard spheres (where each atom is assigned a radius and overlap of two atomic spheres is considered to have infinite energy), an exponential, or as a function of $1/r^n$
- important determinant of protein structure, because it leads to:
 - i) tight packing without atomic overlaps in protein cores
 - ii) restrictions on backbone torsional angles (next page)



FORCES:

Non-covalent Interactions: Van der Waals Interactions (cont'd)

B. Weak attraction at distances just greater than the sum of the atomic radii:

Induced dipole-dipole interaction:

- Non polar atoms have no net dipole moment, but at any given time, the dipole moment has a non zero value depending on the positions of the electrons around the nucleus. This transient dipole has an associated electric field that polarizes any nearby non polar atom, producing a transient dipole in it. The interaction between the two transient dipoles is attractive.
- The magnitude of the interaction depends on the polarizability of the atoms and the inverse sixth power of the distance between the atoms. You will also encounter this inverse sixth power dependence later in the course (NMR and fluorescence energy transfer).
- The functional form that is most often used to describe van der Waals interactions in biochemistry is the “6-12” potential where the numbers refer to the values of the exponents (next page).

FORCES:

Non-covalent Interactions: Van der Waals Interactions (cont'd)

$$E_{\text{VDW}} = \epsilon_{ij} [(\sigma_{ij}/r_{ij})^{12} - 2(\sigma_{ij}/r_{ij})^6]$$

$$\sigma_{ij} = r_i + r_j$$

$$\epsilon_{ij} = (\epsilon_i \epsilon_j)^{1/2}$$

Although individually weak (order of 0.2kcal/mol) a given atom may interact with many other atoms in a molecule: typical interaction energies of CH₂ groups in crystalline hydrocarbons are ~2kcal/mol (comparable in magnitude to other non-covalent interactions found in proteins).

The 6-12 potential above is often referred to as a Lennard Jones potential.

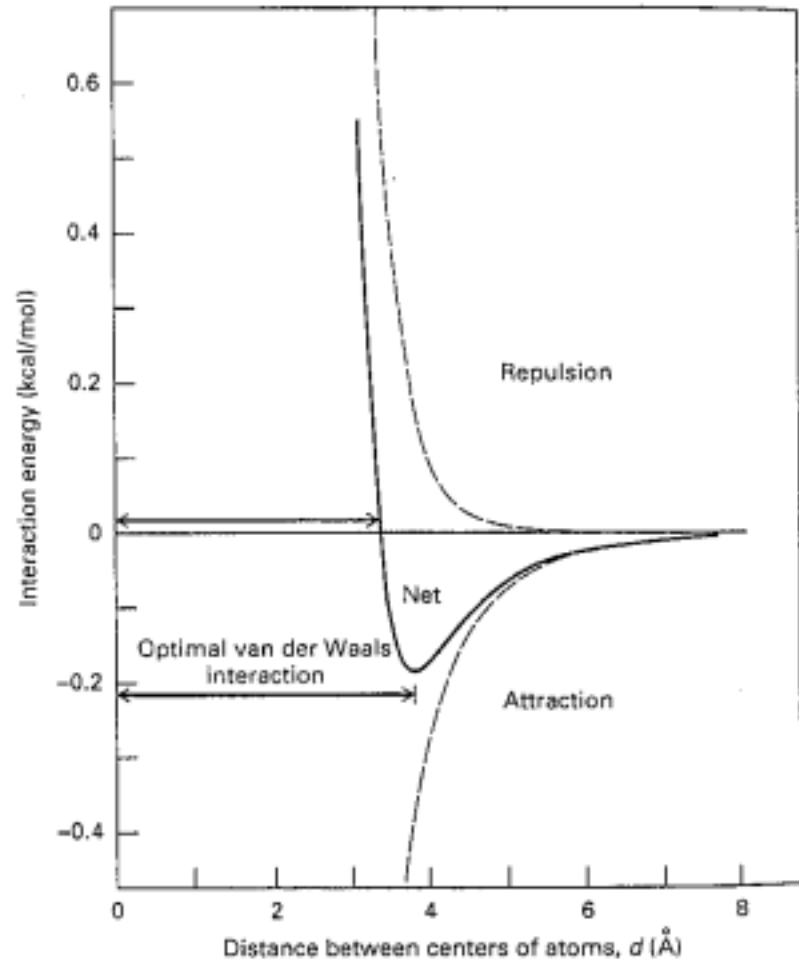


FIGURE 4.2

Representative profile of the energy of the van der Waals interaction as a function of the distance d between the centers of the two atoms. The individual attractive and repulsive components are indicated by the dashed lines, the net interaction by the solid line. The optimal interaction between the two atoms occurs where the energy is at a mini-

Example of Lennard Jones Parameters

Here is a typical set of parameters for the atoms in proteins; the values needed in the 6-12 potential are obtained from these parameters using the formulas on the previous page.

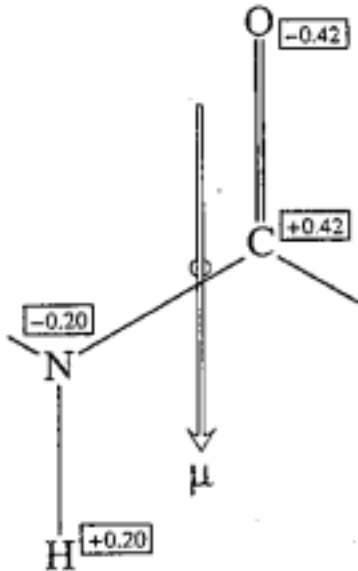
Atom symbol	ϵ_i in kcal/mol	r_i in A	Group represented
C	0.12	2.1	carbonyl carbon
H	0.05	0.8	polar hydrogen
N	0.24	1.6	nitrogen
O	0.16	1.6	carbonyl oxygen

FORCES:

Non-covalent Interactions: Electrostatics

Coulomb's law (1785): $E = k \frac{q_1 q_2}{r}$

The charges q_1 and q_2 are in units in which the electron has a charge of -1 , r is in Angstroms and E in kcal/mol. With these units, the conversion factor k is $332 \text{ (kcal/mol) } \text{\AA}/e^2$.



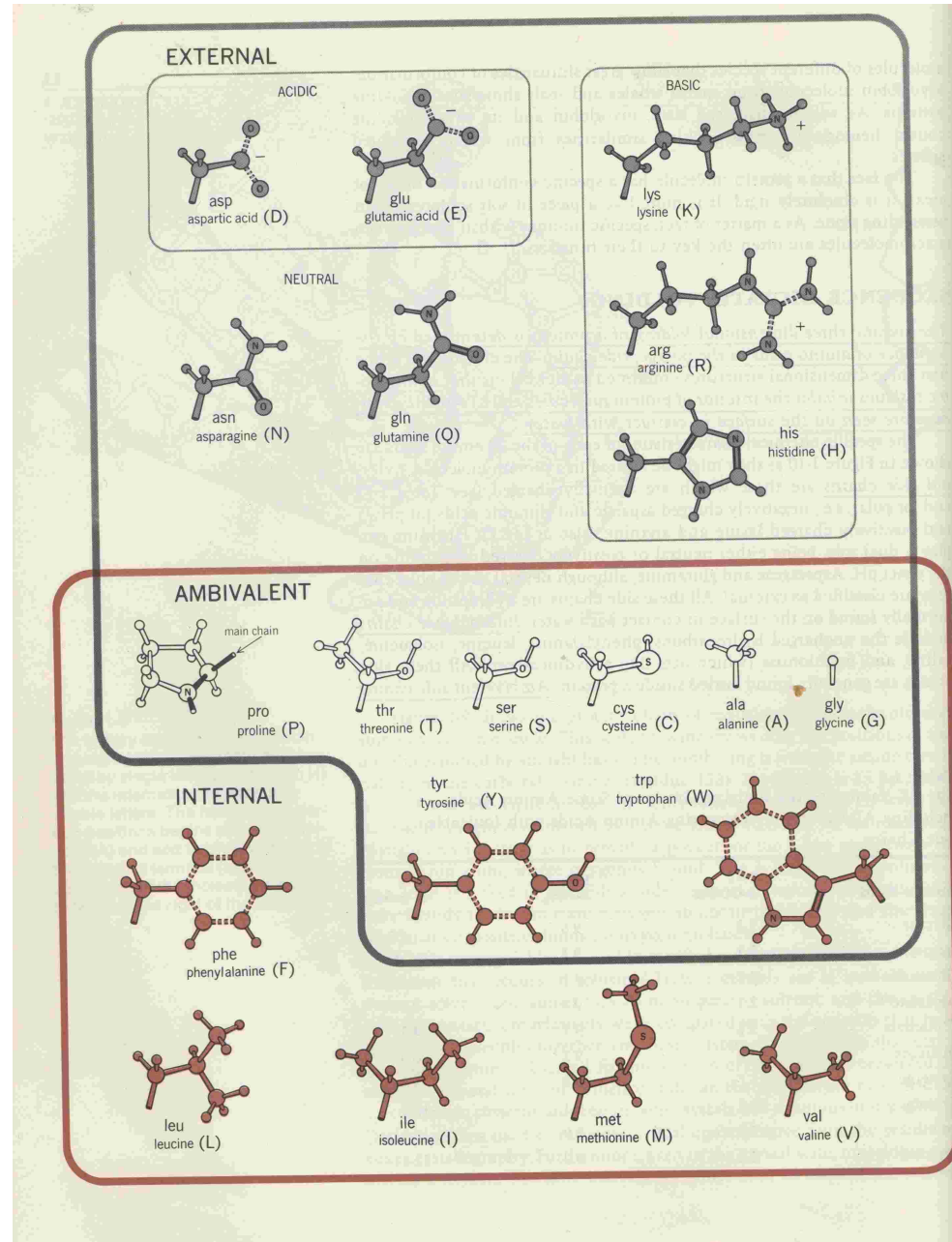
- For example, the interaction energy between an Na^+ and Cl^- separated by 3\AA is $\sim 110 \text{ kcal/mol}$.
- Note that the interaction energy falls off like $1/r$ (much less rapidly than vdW interactions) and hence there can be quite long range effects: in a vacuum, interaction energy between two ions separated by 50 nm is still greater than RT . May influence rate of protein-protein association (electrostatic steering) and catalysis.
- In proteins, have many polar groups which are overall neutral but have significant dipole moments due to differences in the electro-negativities of their constituent atoms (carboxyl groups, amide groups, aromatic rings). Charge-dipole interactions fall off like $1/r^2$, dipole-dipole interactions, like $1/r^3$.

The Different Groups of Amino-Acids

Charged (depending on pH) →

Polar →

Non-polar
(generally hydrophobic) →



FORCES:

Non-covalent Interactions: Electrostatics (cont'd)

Electrostatic interactions in proteins are usually modeled by assigning partial charges to each atom. In some models, all protons are represented explicitly, in others, only heavy atoms and hydrogen atoms bonded to polar atoms (polar hydrogens) are represented. The partial charges differ between models due to difficulties in accurately determining them.

Residue	Charge on Polar atoms
LYS	N ζ (-0.3) H ζ (0.35) C ϵ (0.25)
GLU	C γ (0.36) O ϵ 1 (-0.60) O ϵ 2 (-0.60)
ARG	N ϵ (-0.4) H ϵ (0.30) N η 2 (-0.45) H η 21 (0.35) C ζ (0.5)

FORCES:

Non-covalent Interactions: Electrostatics (cont'd)

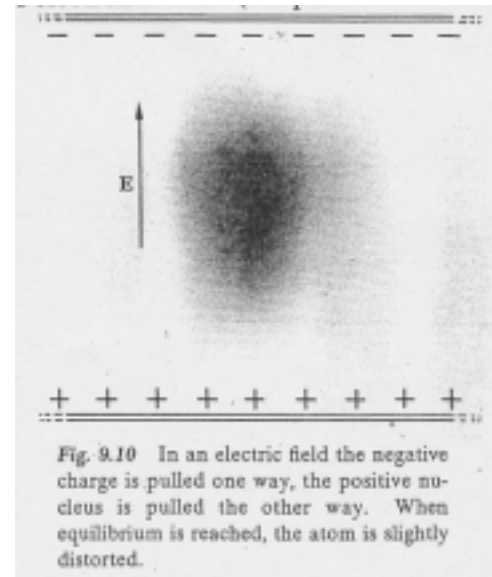
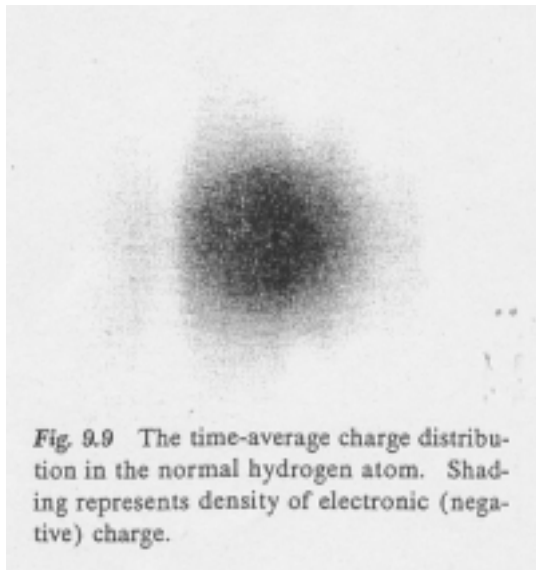
The electrostatic energy of a collection of charges in a vacuum can be computed using Coulomb's law and summing over all pairs of charged atoms

However, biological macromolecules are almost always immersed in a solvent medium (in cells, in aqueous buffer, etc). The presence of solvent has a profound effect on electrostatic interactions, which we now consider

Coulomb's Law in a Polarizable Medium

$$E = k q_1 q_2 / (\epsilon r)$$

where ϵ is the dielectric constant of the medium. The energy is reduced in a dielectric medium because redistribution of charge in the medium. This redistribution of charge may be due to shifting of electron clouds (nonpolar substances)...



Coulomb's Law in a Polarizable Medium (cont'd)

...or from partial orienting of dipole moments of polar molecules, e.g. water molecules

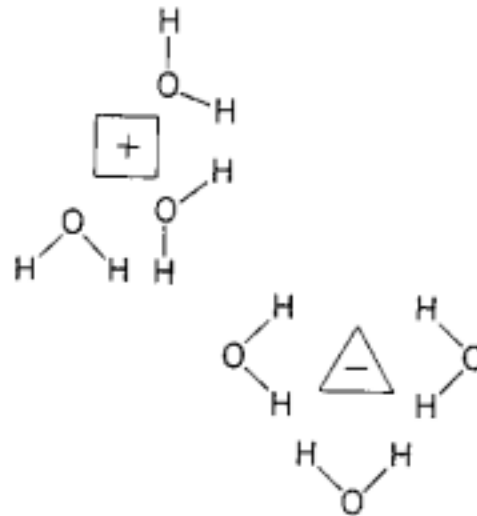
Polar molecules re-ordering around a positive charge



A particularly important example are water molecules, which have a significant dipole because the H-O-H bond angle is non linear. Partial reordering of the molecules partially compensates any applied electric field.



Electrostatic interaction in a nonpolar environment



Water surrounds the charged groups and attenuates their interaction

Water and Non-polar Solvent Dielectric Constants

- ✓ The dielectric constant of a solvent (“ ϵ ” in Coulomb’s law shown two slides ago) reflects how polar that solvent is
- ✓ The dielectric constant of water is **~80**, that of a non-polar liquid is close to **1**.
- ✓ The dielectric constant of the *protein interior* is usually taken to be between **1** and **4**. There is thus a very big difference between the interaction energy between two charged atoms at the surface of a protein and the same two atoms buried inside the protein.

The Poisson Equation

Coulomb's law holds in a dielectric medium if the dielectric constant is the same throughout all of the medium. However, the dielectric constant varies considerably in macromolecule-solvent systems--as we have seen, the dielectric constant on the surface of a protein is close to 80, while it is close to 1 in the interior.

Analysis of such systems is more complex because charge builds up at the interfaces between regions with different dielectric constants, and this induced charge in turn modifies the original field. To determine electrostatic interaction energies and the electric field throughout the system, need to solve the *Poisson equation* for the electrostatic potential $\Phi(\mathbf{r})$ ($\rho(\mathbf{r})$ is the charge density and ϵ the dielectric constant):

$$\nabla \cdot (\epsilon(\mathbf{r}) \nabla \Phi(\mathbf{r})) = -4 \pi \rho(\mathbf{r})$$

The program DELPHI solves the Poisson equation numerically and produces very pretty pictures of electrostatic fields around proteins. An excellent review of this area is *Science* **268**, 1144-1149.

Approximations for Computing Electrostatics

- Solving the Poisson equation numerically is too slow for many applications. There are some useful approximate solutions which start from the expression for the energy of a charged sphere immersed in a dielectric medium:

$$E = kq^2/2\epsilon R$$

- The energy associated with transferring a charge q from a vacuum (dielectric constant of 1) to a dielectric medium is thus:

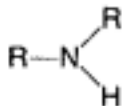
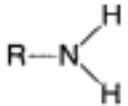
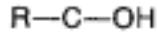
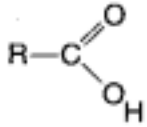
$$(1/\epsilon - 1) k q^2/2R$$

- R in the above expressions is the distance from the center of the ion to the solvent, which, for an isolated ion, is a bit more than the van der Waals radius of the ion. But for an ion buried in the middle of the protein, the appropriate value of R (the distance to the solvent) is much larger (roughly the radius of the protein) and the resultant reduction in energy of the ion upon transfer from the vacuum is much less. Efficient methods for computing this “average distance to solvent” have been developed (generalized Born approximation), and allow rapid approximate calculations of the free energies of charges in proteins that appear to be close to those obtained using the Poisson equation directly.
- Experts will note that the modeling the interior of a protein as a uniform dielectric is itself an approximation (different atoms have different polarizabilities, etc.)

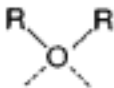
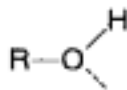
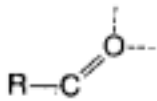
FORCES:

Non-covalent Interactions: Hydrogen Bonds

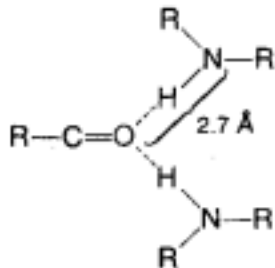
Donors



Acceptors



Complex



- Covalent bonds between hydrogen atoms and electronegative atoms can be quite polarized, with the hydrogen atom effectively having a significant positive partial charge. Because of their relatively small size, these positively polarized hydrogen atoms can interact strongly with electronegative atoms such as O and N.



- While thought to be primarily electrostatic in origin, this “Hydrogen bond” has some partial covalent properties, for example the distance between a hydrogen atom and an oxygen expected given the van der Waals radii of the atoms is $\sim 2.6\text{\AA}$, while in a hydrogen bond the distance is usually $\sim 1.8\text{\AA}$. The angular dependence of the interaction is also quite strong: the angle between the three atoms involved in the hydrogen bond is usually close to 180 degrees.

Hydrogen Bonds: water

Hydrogen bonding is a critical feature of the structure of liquid water. Water molecules are extensively hydrogen bonded to one another, and these strong interactions account for the unusually high boiling point of water compared to other simple liquids and many of the other anomalous features of water.

The strengths of most hydrogen bonds are $\sim 2\text{-}10\text{ kcal/mol}$. However, in most of the applications we will be interested, there is little net change in the number of hydrogen bonds since solvent exposed polar atoms in proteins generally make hydrogen bonds with water, and formation of hydrogen bonds within a protein molecule requires breaking these interactions with water. The free energies associated with these exchanges of hydrogen bonding partners are considerably smaller than the cost of burying a hydrogen bonding donor or acceptor such that it cannot make intra-molecular hydrogen bonds.

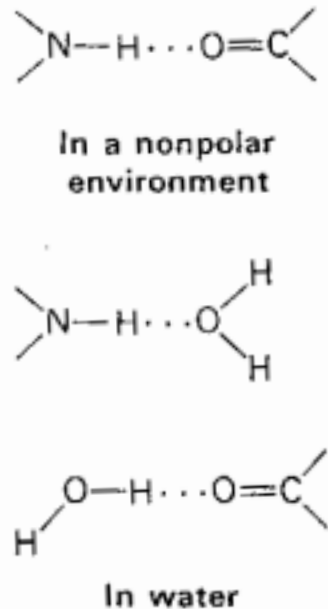


Figure 1-11
Water competes for hydrogen bonds.

Hydrogen Bonds in α -Helices ...

The regular secondary structures in proteins—alpha helices and beta sheets—allow the polypeptide chain to maintain hydrogen bonding while traversing the core of the protein.

Hydrogen bond

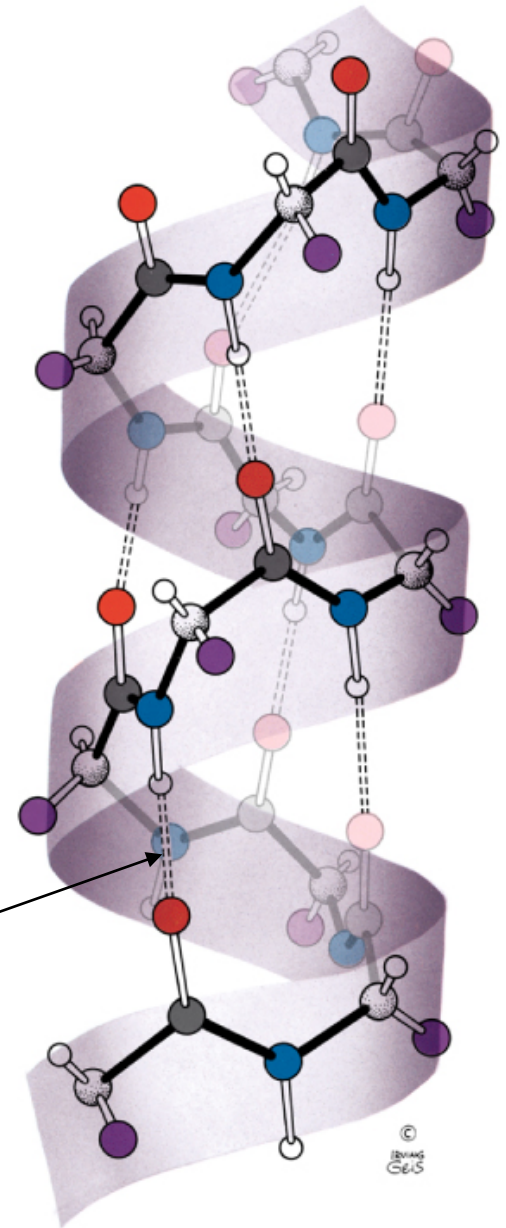
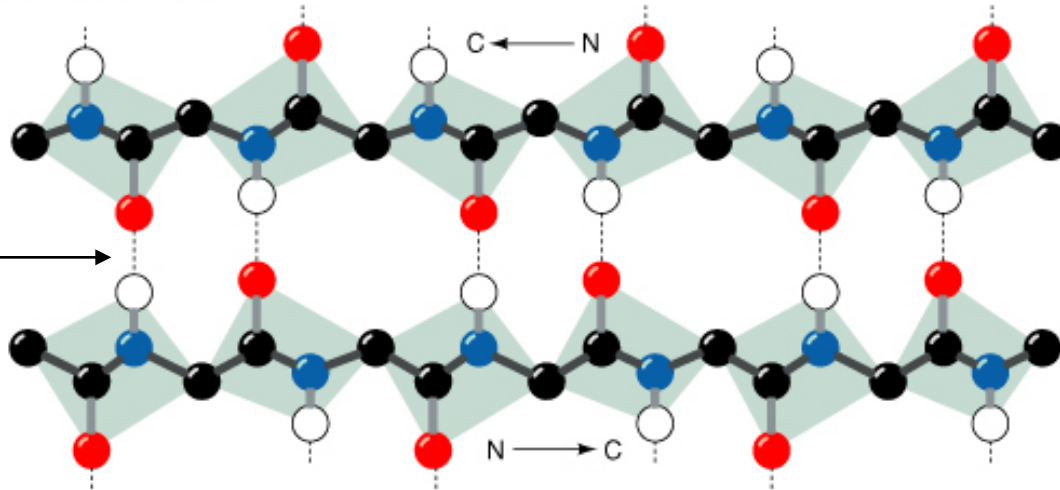


Figure 6-7. Key to Structure. The α helix.
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...and β -sheets

(a) Antiparallel



(b) Parallel

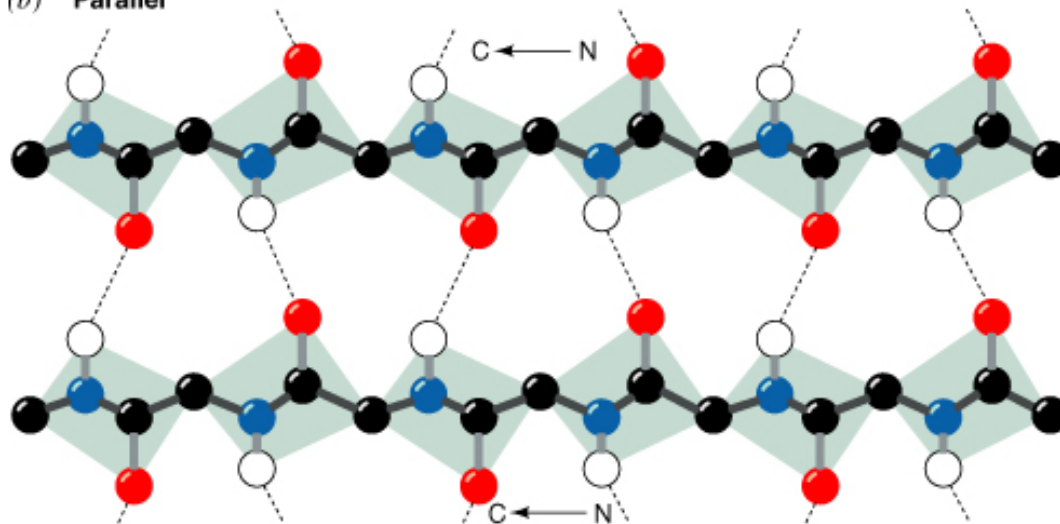


Figure 6-9. Key to Structure. β Sheets.
[Figure copyrighted © by Irving Geis.]

FORCES:

Non-Covalent Interactions: Hydrophobic Interactions

Solvation/ Hydrophobic interactions

The hydrophobic interaction is the pronounced attraction of non-polar solutes in water. Non-polar substances are poorly soluble in water (as is evident in mixing oil and water) and the free energies of transfer of non-polar substances to water are large and positive.

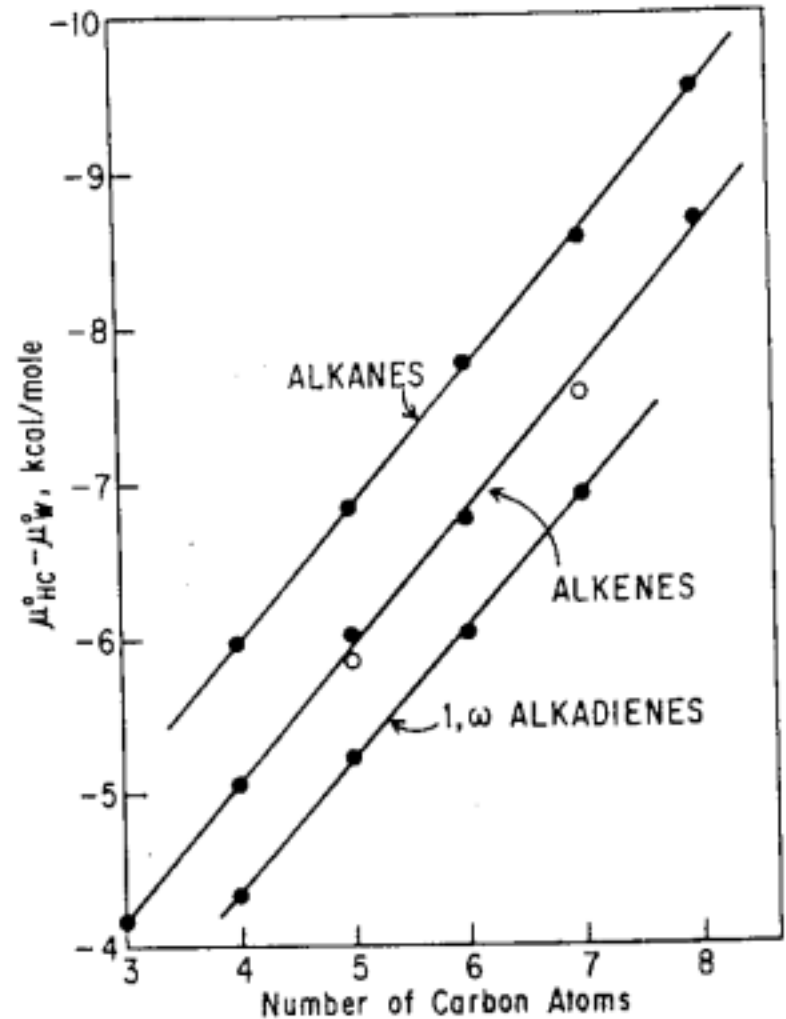


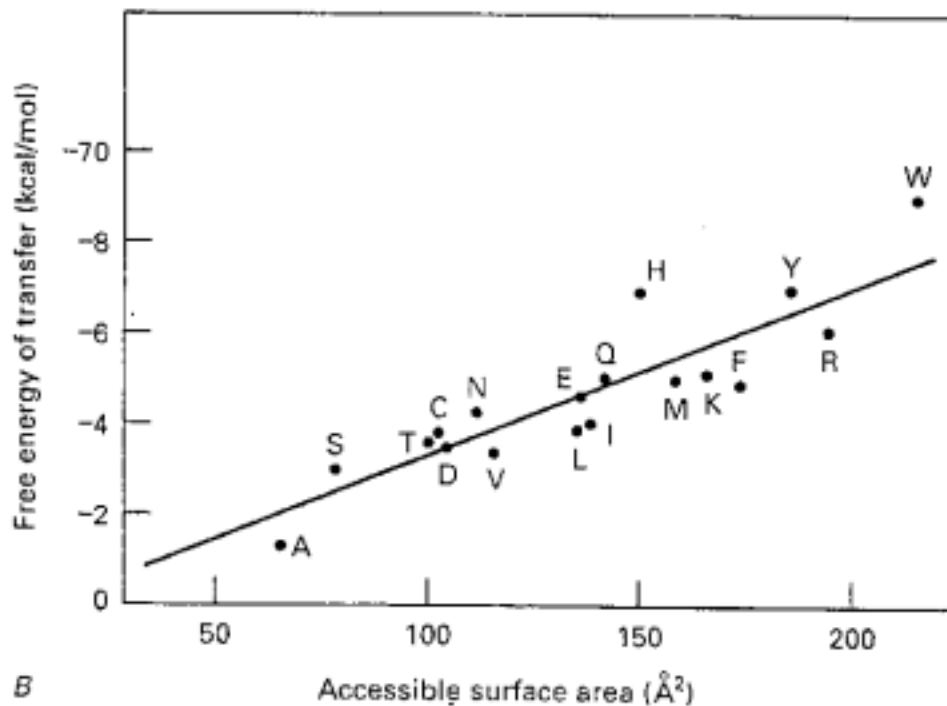
Table II.5 Free energies of transfer of amino acid side chains to water from cyclohexane or the gas phase

Side chain of	Cyclohexane \rightarrow H ₂ O		Vapor \rightarrow H ₂ O	
	kcal/mol	kJ/mol	kcal/mol	kJ/mol
Leu	4.92	20.59	2.28	9.54
Ile	4.92	20.59	2.15	9.00
Val	4.04	16.91	1.99	8.33
Pro	3.58	14.98	1.50	6.28
Phe	2.98	12.47	-0.76	-3.18
Met	2.35	9.83	-1.48	-6.19
Trp	2.33	9.75	-5.88	-24.61
Ala	1.81	7.57	1.94	8.12
Cys	1.28	5.36	-1.24	-5.19
Gly	0.94	3.93	2.39	10.00
Tyr	-0.14	-0.59	-6.11	-25.57
Thr	-2.57	-10.75	-4.88	-20.42
Ser	-3.40	-14.23	-5.06	-21.18
His	-4.66	-19.50	-10.27	-42.98
Gln	-5.54	-23.18	-9.38	-39.25
Lys	-5.55	-23.23	-9.52	-39.84
Asn	-6.64	-27.79	-9.68	-40.51
Glu	-6.81	-28.50	-10.24	-42.85
Asp	-8.72	-36.49	-10.95	-45.82
Arg	-14.92	-62.44	-19.92	-83.36

Free energies of transfer at 25°C and pH 7.0, adjusted for the effects of ionization, assuming that ionized forms move entirely into the aqueous phase. The difference between these two sets (i.e., the value for vapor \rightarrow cyclohexane transfer) is presumably a measure of susceptibility to van der Waals attractions and seems to be simply related to surface area. A. Radzicka and R. Wolfenden, *Biochemistry* **27**, 1664–1670 (1988); R. Wolfenden, L. Andersson, P. M. Callis, and C. C. B. Southgate, *Biochemistry* **20**, 849–855 (1981); P. R. Gibbs, A. Radzicka, and R. Wolfenden, *J. Am. Chem. Soc.* **118**, 6105 (1996); **113**, 4714–4715 (1991); and A. Radzicka, G. B. Young, and R. Wolfenden, *Biochemistry* **32**, 6807–6809 (1993).

Relationship between Solvation Free Energy and Surface Accessibility Area

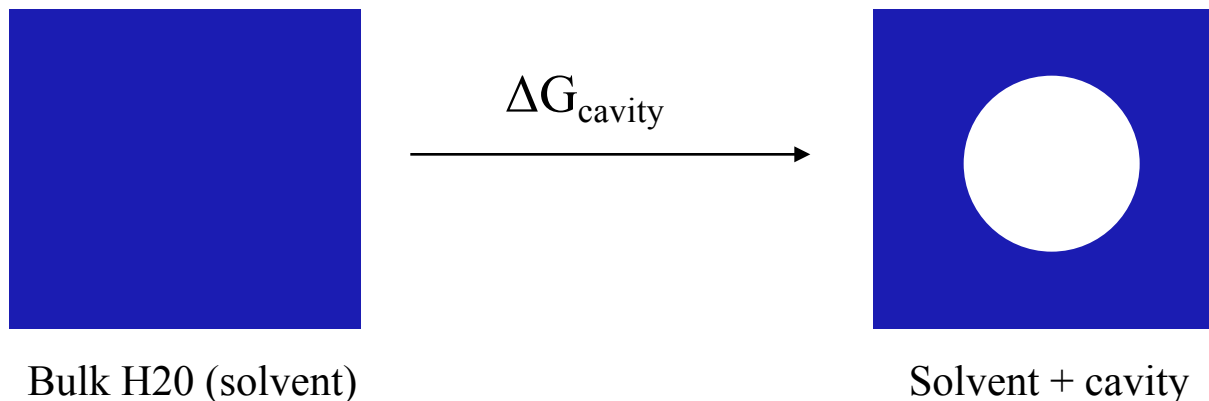
Empirically, the free energy of transfer of simple non-polar compounds to water is found to be roughly proportional to their surface area. Values reported in the literature are in the range of $10\text{cal/mole}\cdot\text{\AA}^2$.



Origin of the Hydrophobic Effect (1)

The origins of the hydrophobic effect are surprisingly still somewhat controversial. It is convenient to divide solvation processes into two steps:

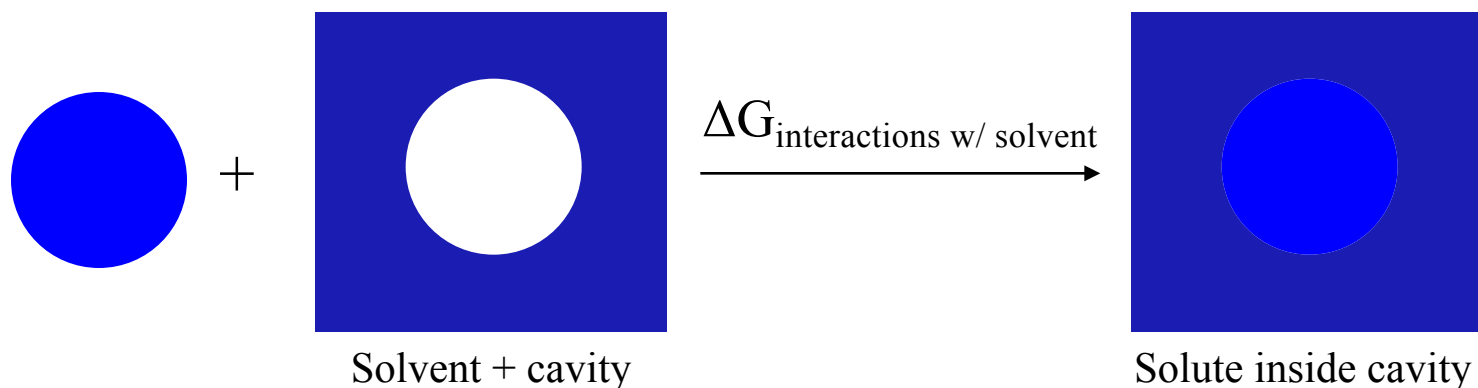
1) the creation of a cavity in the liquid large enough to accommodate the solute,



Because water is a strongly cohesive liquid, and because of its small size, the free energy of forming a cavity is higher than in other simple liquids (the probability of finding a reasonably large cavity is quite small). This is probably the main source of the anomalously low solubility of non-polar compounds in water (for polar and charged molecules, this cost is more than offset by the favorable electrostatic and hydrogen bonding interactions that can be formed; see the expression above for transferring an ion to a high dielectric solvent).

Origin of the Hydrophobic Effect (2)

2) the placement of the solute into the cavity.



The free energy changes associated with #2 are due to interactions between the solvent and the solute that we have already discussed: hydrogen bonding, van der Waals interactions, electrostatics (for non polar compounds, only van der Waals interactions are important).

The van der Waals interactions between non-polar solutes and water are of the same order of magnitude as those between water molecules, but to retain hydrogen bonding in the vicinity of the non-polar solutes requires some ordering of water molecules. There is thus also a decrease in entropy associated with exposing non-polar compounds to water and a change in heat capacity which lead to anomalous temperature dependencies that characterize “hydrophobic” interactions.

How Solvation Free Energy Compares to VdW

Because of the free energy cost associated with exposed non-polar surface in water, non-polar solutes are quite strongly driven together in water. The free energy gain for bringing two methane molecules together in water is significantly more than the free energy associated with their Van der Waals interaction. Hydrophobic interactions are the primary driving force for protein folding and association.

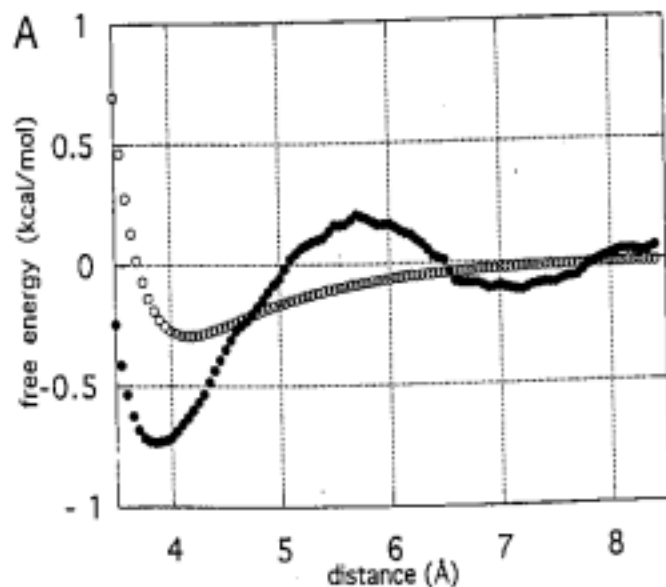


Fig. 2. Methane dimer PMF. A: Comparison of the PMF (closed symbols) to the vdW interaction between the solutes (open symbols). B: Comparison of the solvent contribution to the PMF (squares) to the molecular surface area (circles) and the solvent-accessible surface area (triangles).

References

The topics covered in this lecture are covered in more details in the following textbooks (available in Health Science Library and Biochemistry Library on *reserve*)

Non-covalent interactions relevant to protein structure: Creighton, ch. 4 p139

The same, with an emphasis on Protein-protein interactions: Fersht, ch.11, p324

The three dimensional structure of proteins (α -helix, β -sheet) : Fersht, ch1 p1, Branden & Tooze ch. 1

The Generalized-Born Approximation for Macromolecules:

Onufriev *et al.*, Modification of the Generalized Born Model Suitable for Macromolecules, *J. Phys. Chem. B.* **2000**, *104*, 3712-3720