Lecture 2-3: Review of forces (ctd.) and elementary statistical mechanics. Contributions to protein stability

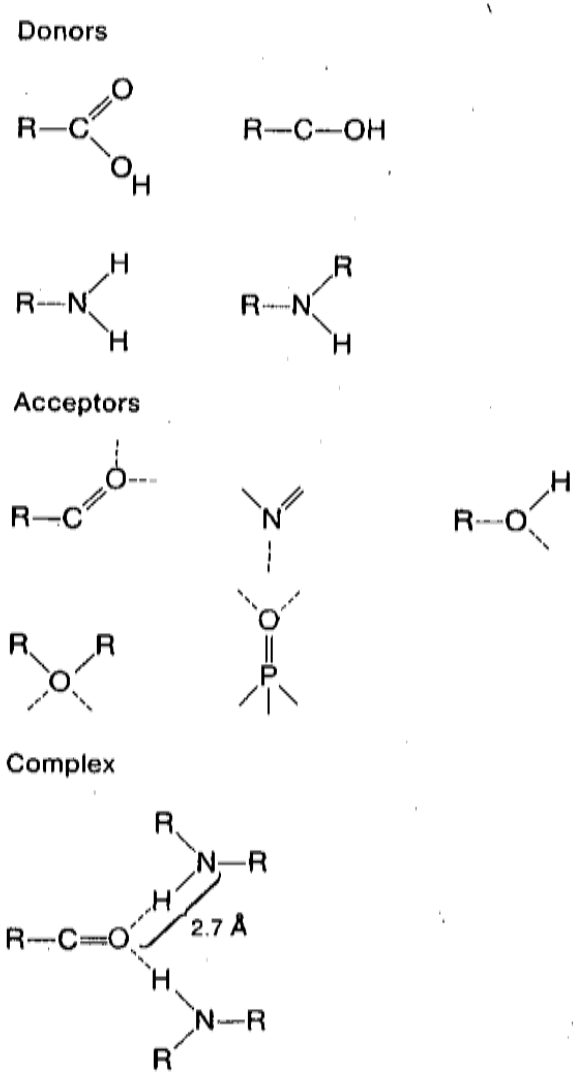
Part I. Review of forces

- 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:
Non-covalent Interactions: Hydrogen Bonds



- 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.

-D-H … A-

- 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 ~2.6Å, while in a hydrogen bond the distance is usually ~1.8Å. 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 ~2-10kcal/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.

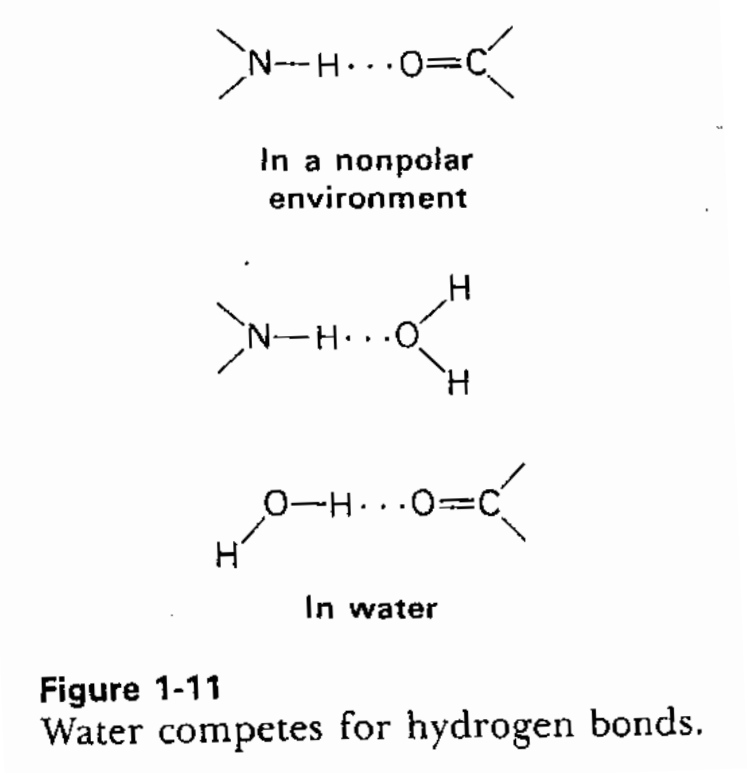


Figure 1-11
Water competes for hydrogen bonds.

Hydrogen Bonds in α-Helixes …

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.

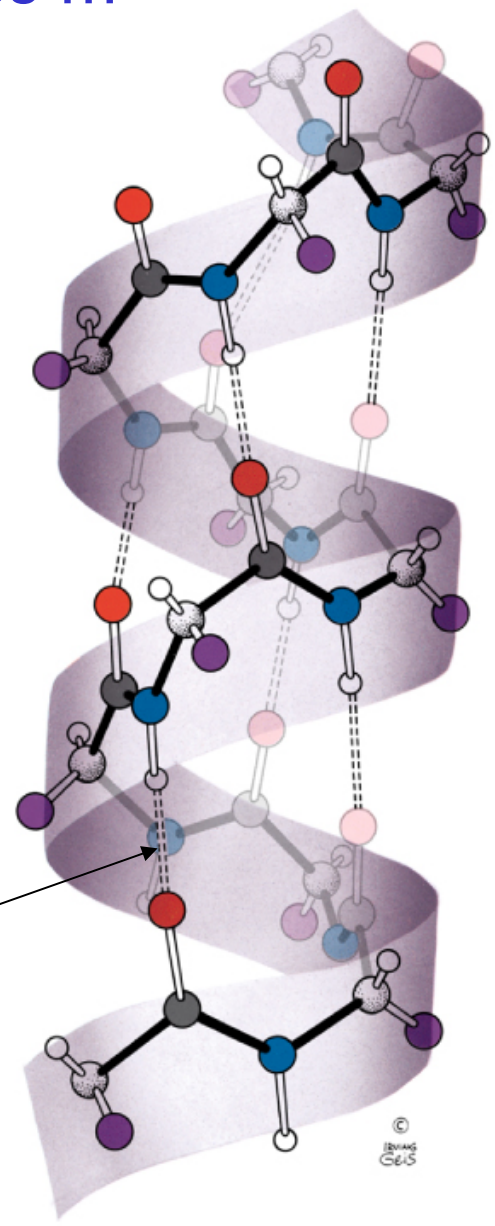


Figure 6-7. Key to Structure. The α helix.
[Figure copyrighted by © Irving Geis.]

…and β-sheets

Hydrogen bond

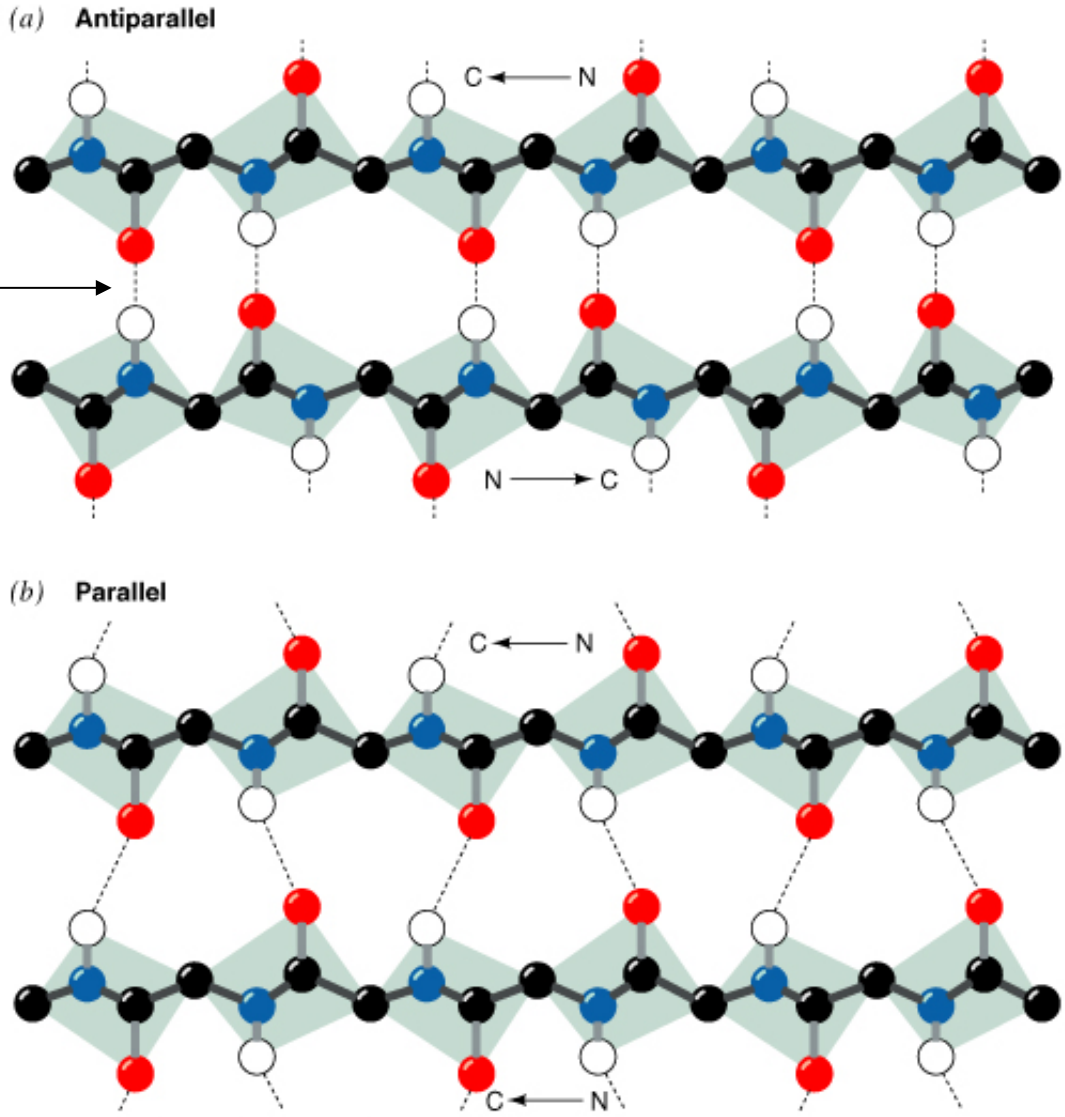


Figure 6-9. Key to Structure. ß Sheets.
[Figure copyrighted © by Irving Geis.]

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.

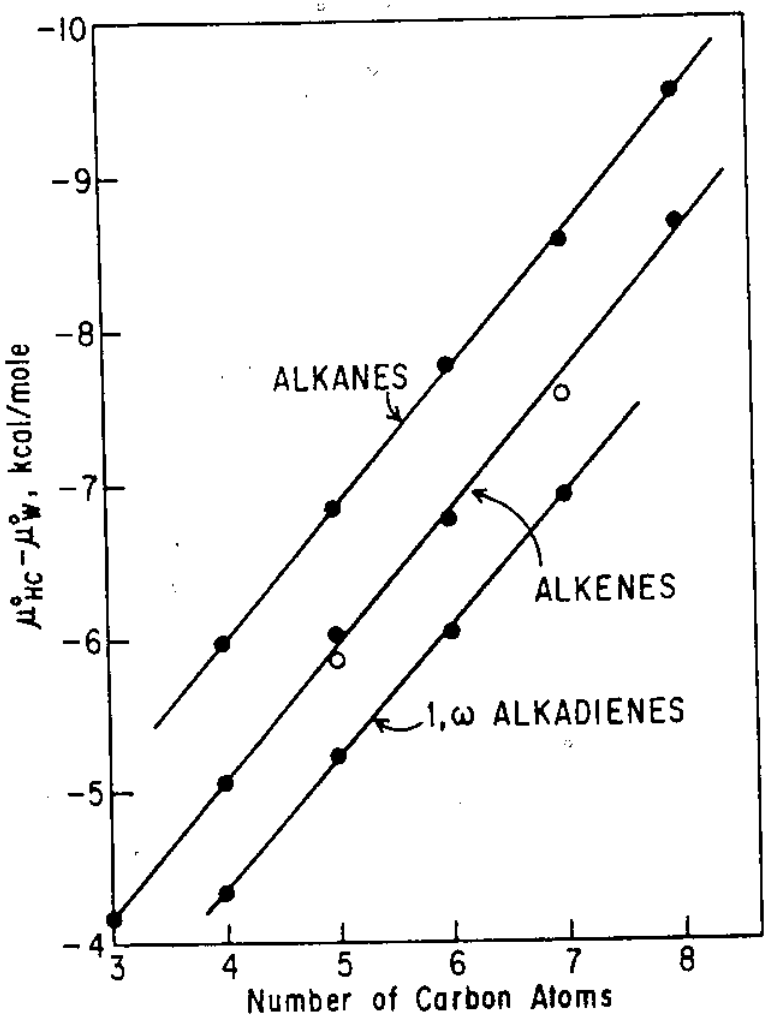


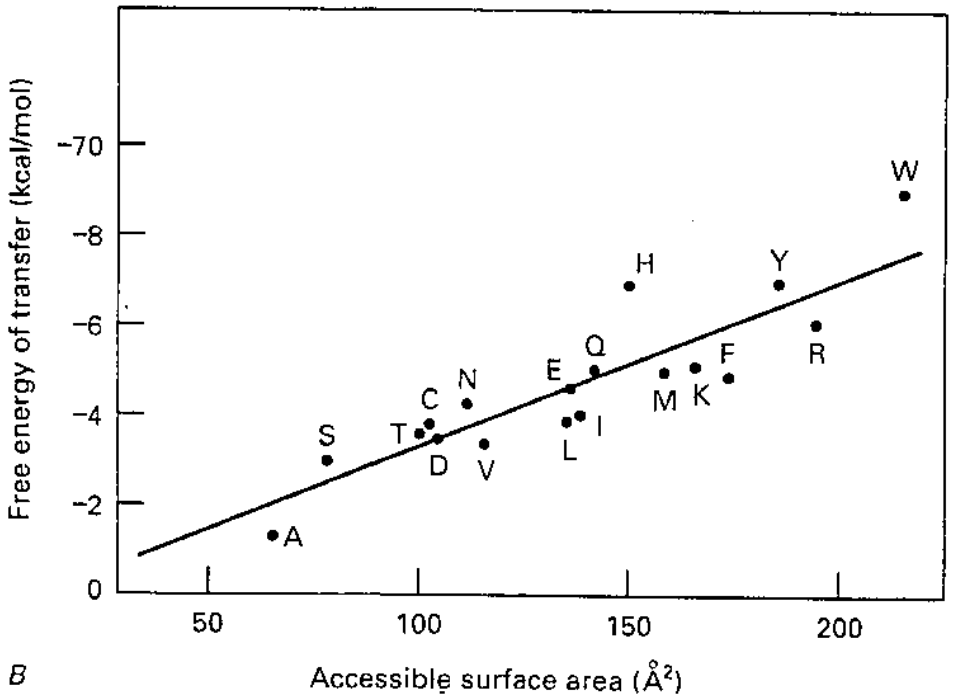
Table 11.5 *Free energies of transfer of amino acid side chains to water from cyclohexane or the gas phase*

	Cyclohexane → H_2O		Vapor → H_2O	
Side chain of	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→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. Cullis, 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

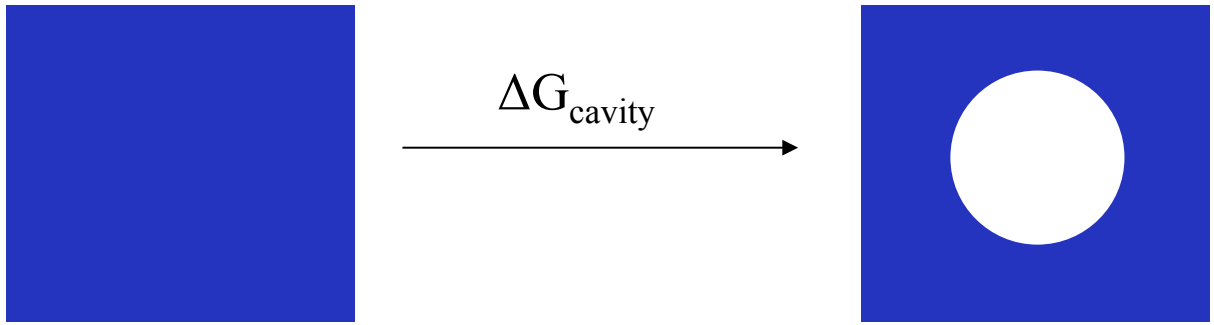
Empirically, the free energy of transfer of simple non-polar compounds to water is found to be roughly proportional to their surface area. Values reported in the literature are in the range of 10cal/mole*Å2.



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,

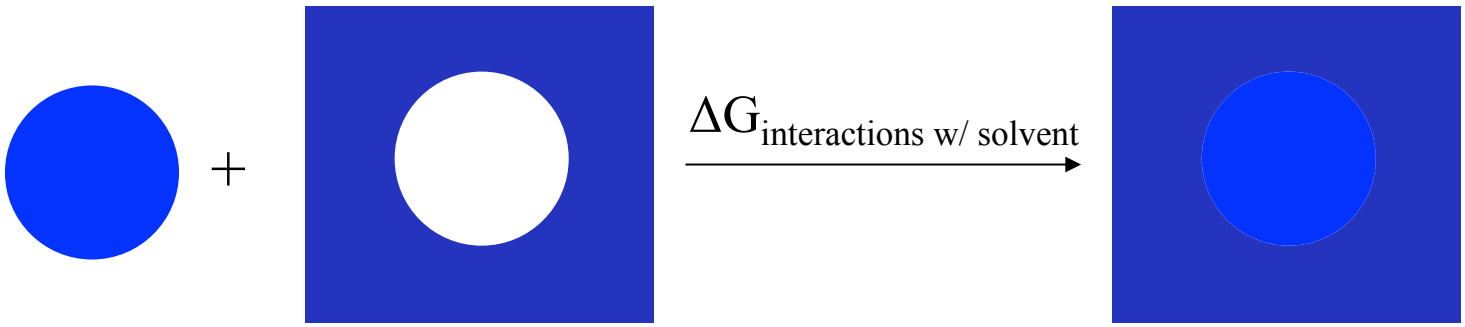


Bulk H20 (solvent) Solvent + cavity

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.



Solvent + cavity Solute inside 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.

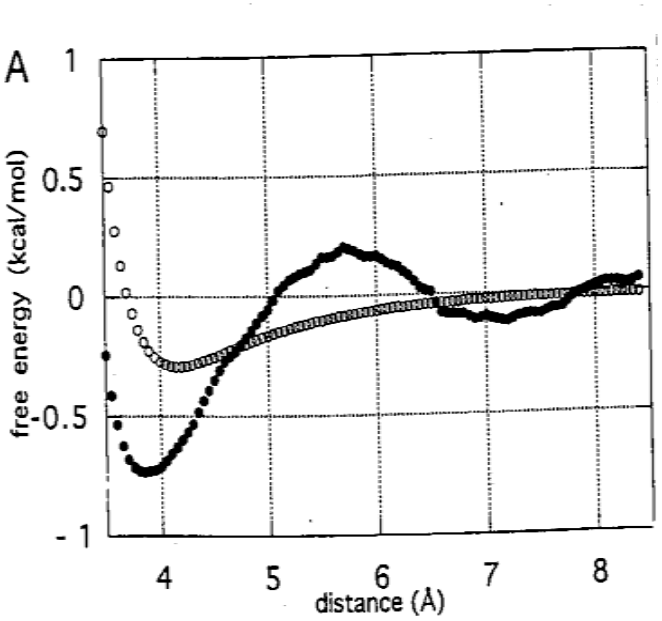


Fig. 2. Methane dimer PMF. **A:** Comparison of the PMF (closed symbols) to the vdW interaction between the solutes (open symbols). **B:** Comparison of the solvent contribution to the PMF (squares) to the molecular surface area (circles) and the solvent-accessible surface area (triangles).

Non-Covalent Interactions: Hydrophobic Interactions

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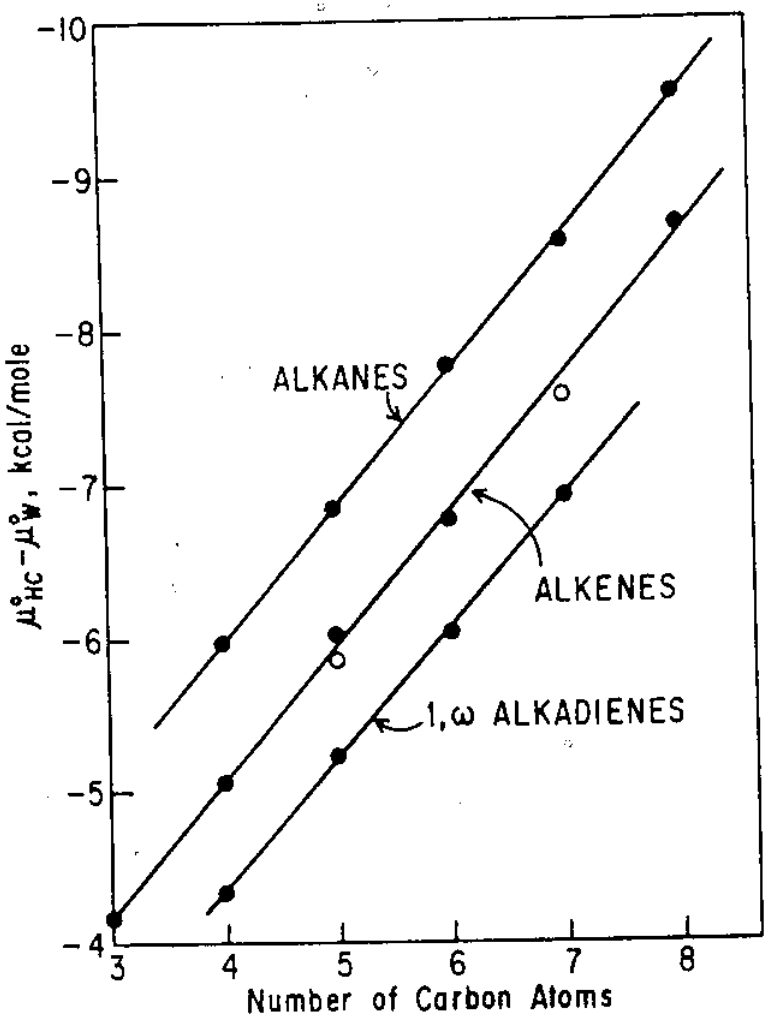

Some Simple but Key Results from Stat Mech

(1) The energy of an isolated system is constant.

(2) The entropy is proportional to the logarithm of the number of states of a system: $\mathbf{S = k \ln \Omega}$

(3) The entropy of an isolated system increases in any spontaneous process.

(4) For a sub system in thermal equilibrium with a larger system (the outside world), the condition that the entropy of the combined system increases is equivalent to the condition that the free energy of the smaller system, $\mathbf{G = E - TS + PV}$ decreases.

The Boltzman distribution

In most of biochemistry, the PV term is very small, and thus, to determine whether a reaction occurs spontaneously, we must consider the balance between the energy change and the entropy change:

$$\Delta G = \Delta E - T\Delta S$$

The probability of observing a particular state of a system with free energy G is:

$$\text{Prob} \propto \exp [S_{tot}/k] \propto \exp [-G/kT]$$

This is a very important result as it relates free energy differences to differences in populations.

The Boltzman distribution (ctd.)

Consider a protein with two different conformations, conf1 and conf2, that differ in free energy by some amount ΔG.

From previous page,

$$\text{Prob(conf1)} \propto \exp[-G(\text{conf1})/kT]$$

$$\text{Prob(conf2)} \propto \exp[-G(\text{conf2})/kT] = \exp[-(G(\text{conf1}) - \Delta G)/kT]$$

The ratio between the populations (concentrations) of the two conformations at equilibrium is called the equilibrium constant (Keq).

$$\mathbf{Keq = Prob(conf1)/Prob(conf2) = \exp[-\Delta G/kT]}$$

Taking the logarithm of both sides gives the familiar expression

$$\mathbf{\Delta G = -kT \ln K_{eq}}$$

which relates the free energy difference in a reaction to the log of the equilibrium constant.

The Boltzman distribution (ctd.)

For example, if the free energy difference between the two conformations is 1kcal/mol, what is the ratio of the two populations (the equilibrium constant) at 300K?

$$P(\text{conf1})/P(\text{conf2}) = \exp -(\Delta G/kT) = \exp -(1/0.6) = .19$$

It is useful to remember the free energy difference that corresponds to a ten fold difference in the populations of two states in equilibrium:

$$\Delta G = -kT \ln K_{eq} = -(0.6 \times \ln 10) = -1.38 \text{ kcal/mol}$$

Link with Protein Folding/Protein-protein Association

Thus, to determine whether a protein will fold or whether two macromolecules will associate, one needs to determine the change in free energy in the process.

$$\Delta G = \Delta E - T\,\Delta S$$

At low temperature, the ΔE term dominates, but as the temperature is increased, the $T\,\Delta S$ term becomes increasingly important.

We learned how to compute ΔE for processes involving changes in van der Waals interactions, hydrogen bonds, etc; but how to compute ΔS ?

Entropic Change in Protein Folding

From above, S = k ln (number of states), so we need to determine the change in the number of states during the process.

This counting is simplest for amino acid side chains, which adopt a small number of discrete states called rotamers (each torsion angle has three possible values).

Example: valine side chains have three possible rotamers (one torsion angle). How much entropy is lost in a change from a conformation in which the valine can adopt all three rotamers to a conformation in which only one rotamer is tolerated?

$$\Delta S = k \ln 3 - k \ln 1 = 0.00198 \bullet \ln 3 - 0 = 0.0022 \text{ kcal/mol} \bullet K$$

How much free energy does this correspond to?

$$\Delta \text{free energy} = - T \Delta S = - 300 \ (0.0022) = -0.66 \text{ kcal/mol}$$

Entropic Change in Protein Folding (cont'd)

The entropy changes in protein folding are estimated to be **~0.007 kcal/mol·K** per residue for the main chain, and **~0.003 kcal/mol·K** per residue for the side chains. For a 100 residue protein the total entropy change in folding is thus ~ 1kcal/mol K; at 300 degrees K (room temperature) this corresponds to **~300 kcal/mol. For a protein to fold, this large unfavorable contribution to the folding free energy must be compensated by the non-covalent interactions discussed last time, which are individually much weaker.**

(*Current Opinion in Structural Biology,* **7**, 215-221)

Entropic Change in Macromolecular Association

What is the entropic change caused by the association of two macromolecules?

There are three components:

1) large decrease in translational entropy
2) decrease in rotational entropy
3) gain in entropy associated with intermolecular motions

The net contribution of these three effects is estimated to be ~5-15kcal/mol at 300K.