1. INTERMOLECULAR ATTRACTION

The interactions that occur between molecules, or between non-bonded portions of the same molecule, are the fundamental forces of life. It is the ability of matter to organize itself that leads to the growth, reproduction and even death of an organism. This first chapter is going to focus on the origins of affinity between and within molecules. Although there is nothing particularly compelling about the weak attractive interaction felt between helium atoms, the same forces involved in that contact are critical in determining the affinity and specificity of antibodies for their antigens, regulatory proteins for their operator DNA sequences and for the assembly of biological membranes from phospholipids. By starting small, and adding increasing layers of complexity to the interactions between biological molecules, we can work towards a complete understanding of all the functions of a cell.

Elementary Thermodynamics

Equilibrium and Free Energy

Underlying all molecular interactions, and in fact all aspects of organic and inorganic behavior, are the principles of thermodynamics. Consider the association of two molecules, **A** and **B**, which combine to form a non-covalent complex **A•B**. The complex will exist in equilibrium with the individual molecules (Scheme 1.1):

$$A + B \Leftrightarrow A\bullet B \qquad \text{(Scheme 1.1)}$$

Their affinity for one another can be measured by the equilibrium constant for the association, K_a, also called the **association constant**, which will have units of M^{-1} (Eq. 1.1):

$$K_a = \frac{[A \bullet B]}{[A][B]} \qquad \text{(Eq. 1.1)}$$

The size of that equilibrium constant is a measure of the affinity of two molecules for one another. Practically speaking, in biochemistry the value of K_a ranges from 10^{15} M^{-1} for extremely strongly interacting molecules to 10^3 M^{-1} for interactions between molecules with low affinities for one another.[1]

At a given temperature, the size of the association constant is solely dependent upon the difference in standard state free energy (ΔG°) between the complex and the separated molecules (Eq. 1.2):

$$K_a = e^{-\Delta G^\circ / RT} \qquad \text{(Eq. 1.2)}$$

[1] As is now obvious, equilibrium constants will be cited with units this semester. Formally, that is inappropriate. Equilibrium constants are, by definition, unitless. We'll return to that point later, but beware that physical chemists sneer at biochemists for this transgression. We won't even talk about kcal vs. kJ.

where R is the ideal gas constant (1.987 $^{cal}/_{mol \cdot K}$ or 8.3145 $^{J}/_{mol \cdot K}$), T is the temperature in units of Kelvins and ΔG° is defined as $G^\circ_{products} - G^\circ_{reactants}$ where all compounds are at unit concentration (1 M). Thus, for two molecules to combine with a high association constant, the free energy of the complex must be low in comparison to the free energy of the individual molecules. What determines the magnitude of ΔG°? The Gibb's free energy equation shows that the change in free energy can be related to the difference in two more fundamental thermodynamic parameters (Eq. 1.3):

$$\Delta G = \Delta H - T\Delta S \qquad \text{(Eq. 1.3)}$$

where ΔH is the change in the enthalpy associated with a process and ΔS is the change in entropy. The values for these parameters are essential for understanding the origins for all molecular interactions and biological catalysis, but they reflect very different phenomena.

Energy and Enthalpy

The change in enthalpy (ΔH) is defined as the heat released or consumed in a chemical process (at constant pressure, which happens to be appropriate to biological conditions). Endothermic processes, with a positive value of ΔH, absorb heat from the surroundings and exothermic processes release heat. But how is that related to the affinity of molecules for one another? There is an important relationship between energy (E) and enthalpy. Energy is defined as the ability to do work by applying a **force** over a distance. In any chemical reaction, energy must be conserved according to the first law of thermodynamics. A change in energy associated with converting reactants to products will lead to a transfer of kinetic energy with the surroundings, with the change in kinetic energy measured as a change in temperature. Change in temperature is the result of heat flow, and is therefore related to the change in enthalpy (we'll get to heat capacity later).

In addition, a change in energy of products vs. reactants can result in work done upon the surroundings, usually in the form of a volume change against a constant pressure, so that the change in energy (at constant pressure) can be defined as (Eq. 1.4).

$$\Delta E = \Delta H - P\Delta V \qquad \text{(Eq. 1.4)}$$

In biochemical reactions we can usually assume that the change in volume is zero, which equates the change in enthalpy with the change in energy of the system.

Since energy and enthalpy are essentially equal under biological conditions, the ΔH accompanying the association of two molecules is a measure of difference in forces acting upon them between the free and bound states. When the change in energy is negative for a molecular association, the complex is lower in energy than the individual molecules, which results from the stronger forces acting within the complex than acting upon the isolated molecules. Other things being equal, the association constant will be larger when intermolecular forces acting within the complex are stronger than those acting on the isolated molecules. Although that may seem obvious, the important thing is that it relates to a more negative value for ΔH, which in turn gives a more negative value for ΔG.

Entropy and Disorder

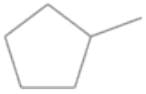
The other component determining the size of the change in free energy is the change in entropy. Entropy is commonly thought of as a measure of the disorder in a system. For a chemical system (say a sample of a pure compound), the entropy is defined as (Eq. 1.5):

$$S = k\ln\Omega \qquad \text{(Eq. 1.5)}$$

where Ω is the number of "microstates" available to the system and k is Boltzman's constant (the ideal gas constant divided by Avogadro's number). The concept of microstates is tricky. Formally, it is determined by the number of ways in which the energy of the molecule can be distributed into translational, rotational, vibrational and electronic modes. More colloquially, it relates to the positional and conformational options available to the molecule. For example, a molecule of glucose dissolved in one liter of water has more entropy than a molecule of glucose in 10 mL of water. The more space, the more entropy.

Also, certain molecules have greater inherent entropy than others due to conformational flexibility. Cyclohexane has less inherent entropy than 1-hexene (both have the formula C_6H_{12}) because it has less conformational flexibility (Table 1.1). It has been estimated that a typical carbon-carbon single bond possesses about 2-3 eu ("entropy units" or cal/mol•K) of entropy.[2]

Table 1.1. Standard state molar entropy (S°) of some isomers of C_6H_{12} in the liquid state. Note that the more "freely" rotating carbon-carbon single bonds, the greater the molar entropy for an isomer.

C_6H_{12} Isomer	**S° (cal/mol•K)**
	49.2
	59.2
	70.6

Returning to molecular association, note that a high association constant, which is favored by a negative value for ΔG, is going to be promoted by a more positive change in entropy (Eq. 1.3). Of course, the very process of combining two molecules removes entropy. It has been estimated that the loss of independent motion of two molecules forming a single complex causes a loss of about 20

[2] M. Mammen, E. I. Shakhnovich and G. M. Whitesides (1998) Using a Convenient, Quantitative Model for Torsional Entropy to Establish Qualitative Trends for Molecular Processes That Restrict Conformational Freedom" *J. Org. Chem.* **63**, 3168-3175.

eu or about 6.0 $^{kcal}/_{mol}$ at 300 K.[3] Other entropic factors may, however, contribute to the change in entropy of association. Individual portions of the molecules may have more or less conformational freedom in the complex, and the entropy of the surroundings may be affected by the formation of a complex. All of these factors can contribute to the relative affinity of two molecules for one another.

Summary

- If two molecules interact through stronger **forces** than are provided by their interactions with the solvent, there is an **enthalpic** motivation for their association.

- If the change in the **positional or conformational freedom** of the associating molecules (and their surroundings) is not too negative, or even positive, then the complex will be relatively favored from an **entropic** perspective.

Of course, the nature of the Gibb's free energy equation (Eq 1.3) says that a favorable change in enthalpy can overcome an unfavorable change in entropy, and visa versa, under the right circumstances (low temperature tilts to enthalpic favorability, high temperature tilts to entropic favorability). Some of the subtleties of how molecules interact will be found in weighing the relative contributions of each of these factors in stabilizing or destabilizing a given interaction. In order to evaluate their contributions, however, it will be important to identify the specific nature of the intermolecular forces and forms of molecular disorder that are involved in the interactions between and within molecules.

Bonding and IMFs - The Enthalpic Contribution

The Covalent Bond

We tend to complicate the basis of the covalent bond as this weird thing that arises from orbital overlap or linear combination of atomic orbitals through various theories of bonding that tip their hat to quantum mechanics. I'm not going there. Instead, let's just get to the most important issue. Bonds form because the attractive interaction of shared valence electrons to the nuclei of two covalently bonded atoms is more favorable than the repulsion of those valence electrons from each other or the repulsion of the nuclei from each other. Chemical bonding relies on a favorable **enthalpic** interaction between atoms, mediated by the electrostatic attraction of electrons and protons in molecules.

Rather than dwell on the details of the chemical bond, I'd rather focus on a structural consequence. Atoms in molecules are very close to each other when covalent bonds form. Among my goals for this course is that everyone know how close they are and how strongly attracted they are. I expect to be able to drop in on your thesis oral and ask "how long is a C-C bond" and then "what is the

[3]G. Patrick Brady and Kim A. Sharp (1997) "Entropy in protein folding and in protein-protein interactions", *Curr. Opin. Struct. Biol.*, **7**, 215-221. Note however, that this number varies a great deal depending upon the size of the molecules involved and the nature of their interaction with one another.

bond enthalpy for a C-C bond" and to get an intelligent answer. So, I present the necessary information in Table 1.2. By the way, the answers are 1.5 Å and about 80 kcal/mol.

Table 1.2. The lengths of covalent bonds and their bond enthalpies (how much energy is needed to break them). Note that bond enthalpies are larger for shorter bonds and for higher bond orders.

Bond	Distance (Å)	Enthalpy ($^{kcal}/_{mol}$)
C-C	**1.54**	**83**
C-H	0.96	99
O-H	1.10	111
C=C	1.34	146
C-O	1.43	86
C=O	1.20	127
N ≡ N	1.10	227
S-S	2.05	63

Electrostatics and Bond Dipoles

All **forces** operating between bonded and unbonded atoms are **electrostatic** in nature. The properties of the negatively charged electron clouds that surround the dense positively charged nucleus define the distribution of charge in a molecule. By extension, it also defines the enthalpy of interaction *between* atoms and molecules. In some instances, these electrostatic interactions are handled through the equations of quantum mechanics, principally in discussing bonding, but most intermolecular forces are described in classical terms, with molecules and atoms behaving as bodies with a somewhat complex charge distribution over their surfaces. The electrostatic potential between two charged bodies can be calculated from **Coulomb's law**, which relates electrostatic force to the distance of separation. Equation 1.6 defines the energy of interaction between two charged bodies.

$$E = \frac{q_1 q_2 e^2}{D r_{12}} \qquad \text{(Eq. 1.6)}$$

Where q_1 and q_2 are the unit charges on two bodies, **e** is the charge on an electron, r_{12} is the distance between the two bodies and D is the dielectric constant of the medium (D=1 in a vacuum).[4] If the charges are opposite, then the energy of interaction is negative and favorable. The electrostatic energy is also moderated by distance (the closer two charged objects are, the larger the absolute value of the energy of interaction) and the dielectric constant. In polar solvents like water (D = 80, the electrostatic energy will be reduced, but in non-polar media, like hexane (D = 1.7), the electrostatic attraction or repulsion between two objects will be accentuated.

[4]Dielectric constants are generally given relative to the unit value for a vacuum. In SI units, the value of D for a vacuum is 1.1×10^{-10} $C^2/J \cdot m$.

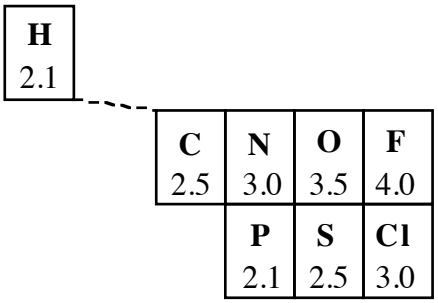


Figure 1.1 The electronegativities of several elements of interest.[5]

In ions, charge is the result of an imbalance of electrons and protons in the atomic or molecular species, which leads to an excess of positive or negative charge of multiples of **e** (the charge on an electron = 1.6 x 10^{-19} C). In neutral molecules, the most important contribution to charge distribution on the molecule results from differences in **electronegativity** between atoms. Figure 1.1 shows the electronegativity of some important biochemical elements. When two atoms of differing electronegativity are bonded to one another, the electron density of the bonded electron pair is not distributed evenly between the two atoms. The more electronegative element picks up the greater share, and hence a partial negative charge. The less electronegative element will receive a partial positive charge. The asymmetry in charge distribution leads to a **bond dipole**, whose strength is proportional to distance and the magnitude of the charge that is separated (Eq. 1.7).

$$\mu = Z \cdot r \qquad \text{(Eq. 1.7)}$$

This product will give a value of the dipole in units of C·m and can be converted to the standard unit, the **debye** (D), by use of the conversion factor relating 1 D to 3.3 x 10^{-30} C•m. Some sample molecular structures with their molecular dipoles are shown in Figure 1.2. Typically, the most polar bonds to be found in biological molecules are between oxygen and hydrogen, which makes water an unusually polar molecule for its small size.

At short range, interactions between dipoles can be quite important in determining the energy of interaction, but the energy drops off more quickly with distance, as $^{1}/_{r^3}$, because at greater distance the separate, but paired charges that create the dipole appear to blur together, as the negative and positive poles of the dipole are relatively closer together and a greater distance of separation from a second species. In essence, at long distances, the ends of the dipole merge to create the singular neutral species that they comprise.

[5]Linus Pauling, "The Nature of the Chemical Bond", Third Ed., Cornell University Press, Ithaca, 1960 pp. 93-96.

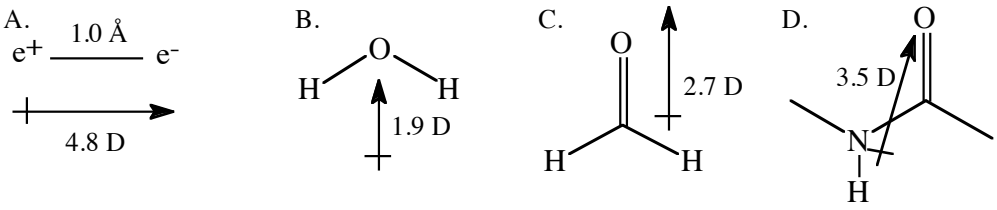


Figure 1.2 Some sample molecular dipoles. (A.) A separation of unit charges by 1.0 Å leads to a dipole of 4.8 D. (B.) Note that, though the O-H bond of water is among the most polar in biochemistry, the bent geometry and short bond distance moderate the molecular dipole. (C.) The single polar bond in formaldehyde creates a strong molecular dipole. (D.) In N-methyl acetamide, the larger dipole is the result of two polar bonds (N-H and C=O) aligned with one another.

van der Waals Forces

The first class of electrostatic forces acting between molecules are the weakest but, at the same time, the most abundant. Named for Johannes van der Waals, who quantified the non-ideality of gas behavior in terms of molecular size and intermolecular attraction. van der Waals (vdW) forces describe the net attraction felt between any two atoms. In actuality, however, the appellation "vdW forces" is somewhat misleading, because in fact this single term encompasses the contributions of two counter-acting forces: exclusion forces and dispersion forces.

Exclusion forces (sometimes called Pauli exclusion forces) refer to a repulsive force acting between any two non-bonded, closed-shell atoms (a closed-shell atom has a filled valence shell of electrons). At a classical level, these forces can be understood as the repulsion that takes place between the electron clouds surrounding the two atoms. As they approach each other from a distance, the two neutral objects interact exceedingly weakly, but as the atoms are moved closer to each other, there will come a point at which the two electron clouds start to overlap with one another, and the negative charge on the electrons repels the atoms from one another. At a quantum level, this interaction can more accurately be described in terms of the wavefunctions that result when two atoms approach.

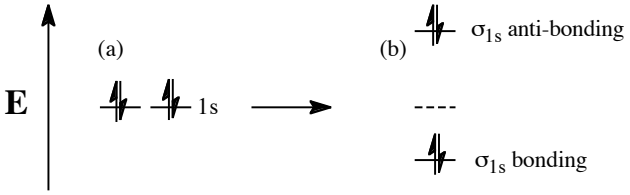


Figure 1.3 When two helium atoms are far apart from one another (a) their 1s atomic orbitals are isolated and possess the same energy. However, when the atoms are close together (b) the atomic orbitals combine to form σ_{1s} bonding and anti-bonding molecular orbitals. Because σ_{1s}* is sufficiently high in energy to offset the stabilization afforded by the σ_{1s} bonding orbital (compare their energies to the 1s orbital energy, represented by the dashed line), the He_2 molecule is unstable with respect to two He atoms.

For example, if two helium atoms are brought into close proximity with one another, the wavefunctions that describe their independent, and filled, 1s atomic orbitals will overlap and can be combined to create a resulting set of molecular orbitals, one bonding and one anti-bonding (Figure 1.3). Because of the Pauli exclusion principle, only two electrons can share a given orbital (by possessing opposite electron spins), so of the four electrons on the two helium atoms, only two are used to fill the stable (in comparison to the 1s atomic orbitals) bonding molecular orbital. The other two are advanced in energy to an anti-bonding molecular orbital which is more destabilizing relative to the atomic orbitals than the bonding orbital is stabilizing relative to the atomic orbitals. As a result, the net electronic energy of the He_2 molecule is higher than that of the independent He atoms. The closer together the two atoms come, the more pronounced this effect. Similarly, any two closed-shell atoms will be repelled from each other if placed in too close proximity.

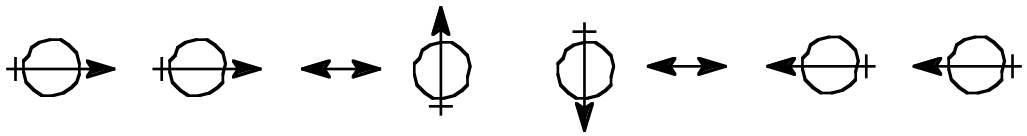


Figure 1.4 Dispersion forces arise through of momentary dipoles that are generated by the asymmetry of distribution of electron density on a given atom. When two atoms are in close proximity to each other, electrostatic stabilization is available by coupling the fluctuations in the dipoles of the adjoining atoms.

With exclusion forces dictating that atoms remain apart, vdW forces must therefore include an attractive component to atomic interactions. This comes in the form of **DISPERSION FORCES** (also known as London forces), which describe an interaction between **two induced dipoles** on two molecules in close proximity. A molecule's electron density can fluctuate so as to create momentary electric dipoles as a result of the asymmetry in electronic distribution about the nuclei. If a second molecule is close enough to sense this small fluctuation in molecular dipole, its electrons can fluctuate synchronously with those on the first molecule to generate a small electrostatic stabilization between the two molecules (Figure 1.4). The magnitude of this interaction is directly dependent upon the polarizability of the two species. For small atoms like helium, this interaction is quite weak, but it becomes more substantial for larger atoms, such as xenon, whose relatively high boiling point (-108°C vs. -270°C for He) is due to enhanced dispersion forces acting in the condensed phase of Xe.

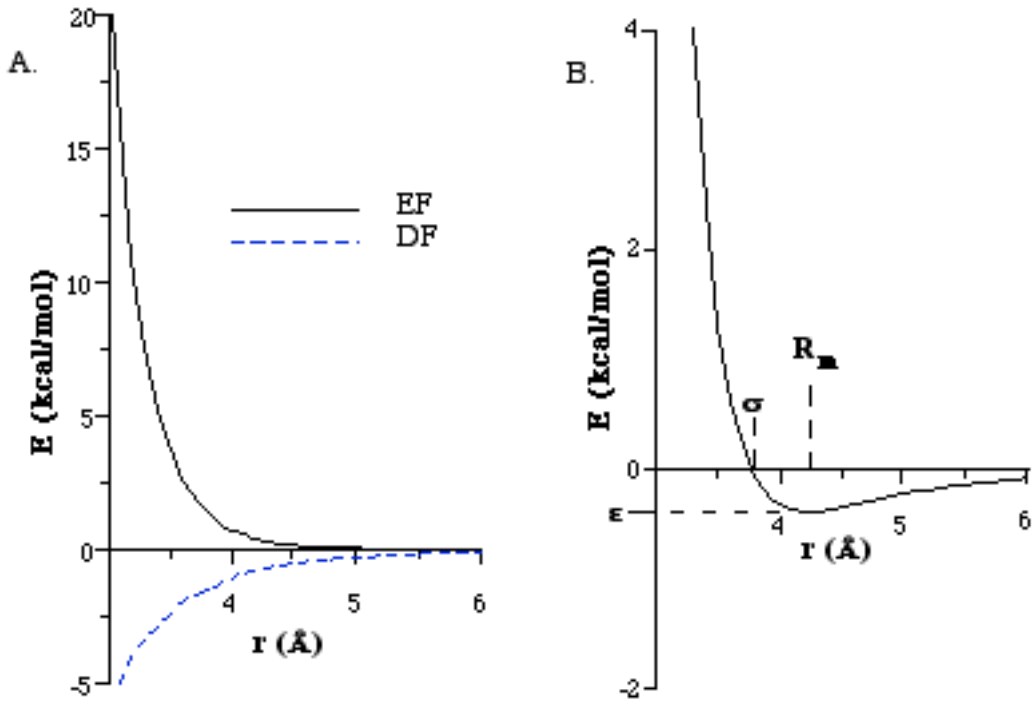


Figure 1.5 (A.) A plot of the two contributing terms to the Lennard-Jones 6-12 potential for methane. The solid line shows the contribution assigned to exclusion forces and the dashed line shows the contribution from dispersion forces. (B.) The sum of these two functions is plotted vs. intermolecular distance. The function crosses zero energy at 3.76 Å (2 x vdW radius) and the minimum energy is -0.39 $^{kcal}/_{mol}$, at the vdW contact distance (R_m) of $\sqrt[6]{2}\cdot\sigma$, or 4.2 Å.

With these two counteracting influences, the vdW forces acting between two atoms have the potential to be either attractive or repulsive. As it turns out, the primary determinant of whether the interaction is favorable is interatomic distance (r). The attractive interaction arising from dispersion forces acts over a longer range than the repulsion arising from exclusion forces. The **Lennard-Jones 6-12 potential** (eq. 1.8) models this phenomenon by attaching two variables (σ and ε) to an equation that relates the potential energy of interaction between two atoms to a repulsive term that varies with $^{1}/_{r12}$ and an attractive term that varies as $^{1}/_{r6}$.

$$E = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] \qquad \text{(Eq. 1.8)}$$

While the equation is empirical, the attractive term can be rationalized on the basis of the fact that the strength of an electric field generated by a dipole varies as $^{1}/_{r3}$. The sixth power in the 6-12 potential reflects the coupled attraction between two dipoles. The twelfth power used to describe

exclusion forces has no theoretical basis, but is the most common representation used in biochemical calculations. The importance of this term is that it rises quite sharply at short distances.

When equation 1.8 is plotted (Figure 1.5) it can be seen that ε is the absolute value of the minimum energy of interaction between the two atoms and σ is the distance at which the energy of interaction is zero (more on that soon). As an example, methane has a σ of 3.76 Å and a ε of -0.39 $^{kcal}/_{mol}$, which reflects the energy of stabilization at the optimal distance of interaction (referred to as R_m). When the energy value is compared to the average energy of a carbon-carbon bond (about 83 $^{kcal}/_{mol}$) it can be seen that, in absolute terms, that the net vdW attraction between two methyl groups will be very small (see also Table 1.3). However, because of the large number of atomic contacts that exist in most biochemical complexes, the sum of all vdW forces can prove to be a significant source of enthalpic stabilization for the complex.

The Size of Atoms - An Interlude

Based on the preceding discussion, it should be clear that vdW forces indicate a distance of closest approach that is defined by the sharp increase in energy at values of r smaller than the σ value for a given atom or atom group. Because σ represents the closest distance at which two species can be interacting without having their energy of interaction skyrocket, this value is typically used to define the **van der Waals radius** of an atom. The vdW radius is used to define the spatial boundaries of an atom, or atom group. If two atoms are placed much closer than their combined vdW radii, they will strongly repel one another. But if they are placed at that distance or somewhat further apart, the energy of interaction between the atoms will be negative and favorable. Practically speaking, vdW radii are determined from crystal structures of small molecules, using the contact distances between non-bonded atoms to determine the appropriate values. Needless to say, there is some variation in the data just as there is even in the radius of an atom of a given element that depends on its specific bonding environment in a molecule, but Table 1.2 provides a list of values that can be used as a general guide.

Table 1.3 Sizes of atoms and contact energies.[6] The vdW radius results in zero energy of interaction, and 0.5 R_m reflects the surface that gives the optimal energy of interaction, listed as the minimum energy of interaction.

Group	**Polarizability ($Å^3$)**	**vdW Radius (Å)**	**0.5 R_m (Å)**	**Min. Contact Energy ($^{kcal}/_{mol}$)**
Hydrogen	0.7	1.1		0.02
Carbon	0.8	1.7	2.0	0.21 (OH)
Nitrogen	1.1	1.5-1.6	1.8	0.17 (amide)
Oxygen	1.8	1.4-1.5	1.9	0.11 (sp^3)
Sulfur	2.9	1.85		0.25 (thioether)

[6] *Data are taken from Schulz and Schirmer, Principles of Protein Structure, Springer-Verlag, 1979 and Lesk, Protein Architecture, IRL Press, 1991.

The distances given in Table 1.3 are only intended to be representative. In fact, two atoms can approach closer than their vdW radius, but at some energetic cost, and, likewise, they can be separated by greater than the sum of their R_m values, but again with an energetic cost (although the cost of deviating from this value is less than that for too close an approach). Also note that the vdW radius of methane (~1.9 Å) is not the sum of the hydrogen atom's vdW radius (1.1 Å) and the C-H bond distance (1.1 Å, as well). Instead, it is somewhat closer to the vdW radius of carbon (1.7 Å) because two interacting methane molecules can be oriented in such a way as to place the hydrogens away from the zone of contact.

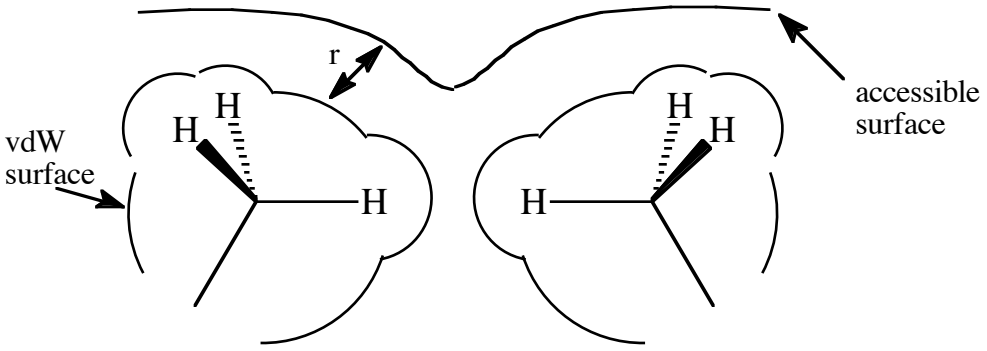


Figure 1.6 Comparison of the vdW surface and the solvent accessible surface. The vdW surface is the aggregate of the surfaces of each atom, like bubbles upon bubbles. The accessible surface is drawn at a distance r (the radius of the spherical probe) away from the vdW surface, and it smoothes over gaps in the vdW surface.

This raises an additional point that the vdW surface of a molecule, defined by the aggregate vdW surfaces of its atoms, is not the best description of the molecule's shape. Instead, the **solvent accessible surface area**, is frequently used to describe the shape of a molecule. This surface is generated by a sphere of 1.4 Å radius (approximating a water molecule) rolled across the vdW surface of the molecule. Where deep, narrow valleys appear between atom groups, the sphere cannot penetrate, and so the accessible surface is smooth at this cleft, not unlike a snow bridge over a glacial crevasse (Figure 1.6). The accessible surface represents the shape of the molecule as it would appear to other molecules and provides a measure of the surface area that is able to interact with other molecular surfaces.

Dipole- Dipole and Dipole- Induced Dipole Interactions

As a final comment on vdW interactions, it should be noted that this catch-all term is also used to describe the interactions of all atom groups, including those that might have **fixed dipoles** (see Figure 1.2), but *excluding* those that have hydrogens bonded to oxygen and nitrogen, for reasons to be seen later. If polar atom groups interact with themselves, **dipole-dipole interactions** arise. Since the bond dipoles are fixed, there is no need to induce the second dipole. In calculating the energy of interaction between two molecules, the 6-12 potential is used, but an extra electrostatic term is used to account for the permanent electrostatic contribution to the interatomic interaction. In real terms, the contribution of these electrostatic contributions can be significant. For example, formaldehyde (Figure 1.2C) has the same number of electrons as O_2, but its boiling point is 160°C

higher, largely due to the stabilizing contributions of interactions between the static dipoles on neighboring formaldehyde molecules in the liquid phase.

Polar atom groups can also interact favorably with non-polar groups through **dipole-induced dipole interactions**. After all if a transient dipole can induce another transient dipole (Figure 1.4), why shouldn't a fixed dipole be able to. Thus there are attractive interactions between groups that we typically don't think should interact all that favorably. Water and methane interact three times more favorably than two methane molecules, though not nearly as favorably as two water molecules (Table 1.4). But that is an unfair comparison as will be seen below.

Table 1.4. van der Waals interactions between pairs (dimers) of polar and non-polar molecules. Note the role of atomic size and polaritity on the enthalpy of interaction. Note also the special case of water, which forms a strong dimer at very short intermolecular distances.

Molecular dimer	$\Delta H_{interaction}$ ($^{kcal}/_{mol}$)	**distance (Å)**
$CH_4 \bullet CH_4$	0.3	3.5
$SiH_4 \bullet SiH_4$	0.6	4.2
$H_2O \bullet CH_4$	0.9	3.5
HCl•HCl	1.2	3.8
$H_2O \bullet H_2O$	3.2	2.8

The Hydrogen Bond

It was noted above that polar bonds including hydrogen atoms are generally excluded from the dipole-dipole interactions that fall into the vdW category. Why? Because these interactions are both geometrically and energetically distinct from all other dipole-dipole type interactions. Moreover, they play an unusually important role in the enthalpy of interaction between biomolecules and are frequently identified as the determining factor in the affinity and specificity of these contacts. So, dipole interactions involving hydrogen are placed in a separate category. When one electronegative atom (usually oxygen or nitrogen in biochemical systems) makes contact to a hydrogen bonded to another heteroatom, a **hydrogen bond** is formed (Figure 1.7). The birth of structural biochemistry was brought about by Linus Pauling's recognition of the importance of hydrogen bonding in determining protein structure, and though Watson and Crick didn't claim much chemical knowledge, they fully appreciated the importance of hydrogen bonding in determining the structure of DNA.

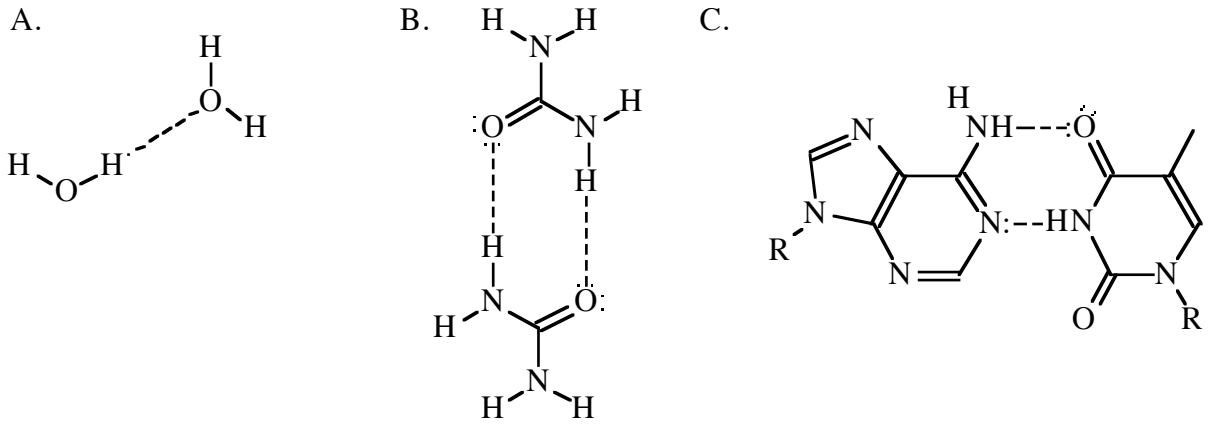


Figure 1.7 Some examples of hydrogen bonding. (A) Note that the oxygens in water are both hydrogen bond acceptors and hydrogen bond donors. (B) In urea, the nitrogens are the donors and the oxygens are the acceptors. (C) The A:T base pair shows ring nitrogens acting as H-bond acceptor and donor.

The heteroatom with a covalent bond to the hydrogen is said to be the hydrogen bond **donor** and the heteroatom in close contact with the hydrogen (but not bonded to it) is the **acceptor**. Identifying possible H-bond donors is easy; one need only look for a hydrogen covalently attached to an electronegative element (though *rarely*, S-H and C-H bonds can also act as donors - see below). A general trait of the accepting atom is the presence of a lone pair of electrons that would be capable of forming a covalent bond to the hydrogen if the proton were to be transferred from one atom to another. So, for example, oxygen generally has two lone pairs of electrons and can therefore accept two H-bonds. Nitrogen, however, is not as straightforward. Amines have free lone pairs to accept H-bonds, but at neutral pH, most amines are protonated to ammonium groups and therefore are only able to act as donors. Amide nitrogens, which are poor bases, remain neutral down to very low pH's and possess a lone pair, but because they are such poor bases they are also quite poor H-bond acceptors. As it turns out, the most common form of nitrogen acting as an H-bond acceptor is when it is part of an aromatic ring, as is the case in DNA bases (Figure 1.7C). **Only lone pairs in hybrid orbitals (sp^3, sp^2 and sp) are capable of acting as H-bond acceptors**.

As mentioned earlier, the geometry associated with hydrogen bonds sets them apart from vdW type contacts. While it isn't always the case, hydrogen bonds frequently place the donor and acceptor atoms closer than the sum of their vdW radii would appear to allow (Table 1.5). This is particularly surprising, since there is the additional presence of a hydrogen atom getting swallowed up in the contact. The proton frequently penetrates the vdW radius of the acceptor atom. Note, however, that the H-bond does not usually have any substantial covalent character with respect to the accepting atom. There are some additional geometrical restrictions that are placed on H-bonds. For starters, the proton typically lies close to the line defined by the donor and acceptor atoms. Most H-bonds have a D···H···A angle of 160° ± 20°, though the majority of H-bonds cluster at 180° (Figure 1.8A). In general, the acceptor atom's lone pair is directed towards the proton, as though to accept the proton in the position where it could expect to form a bond (Figure 1.8 B). However, there is a fair amount of structural flexibility in this parameter as well. One interesting side-light of the flexible geometries allowed in H-bonds is that you'll occasionally see one H-bond donor interacting

with two H-bond acceptors in what is known as a "bifurcated" or "three-centered" H-bond (Figure 1.8 C).

Table 1.5 List of acceptor-donor atom distances in H-bonds.

Donor-Acceptor	Ave. Distance (Å)	Range (Å)
N-H···N	3.1	2.9-3.4
N-H···O		
Amide	2.9	2.6-3.0
Amine	3.0	2.6-3.2
O-H···N	2.8	2.6-2.9
O-H···O		
Carboxylate	2.6	2.5-2.8
Alcohol	2.7	2.6-3.0

The close approach, coupled to the relatively high polarity of N-H and O-H bonds, makes hydrogen bond more enthalpically important than other dipole-dipole interactions. In the gas phase, H-bond energies can range between 3-6 $^{kcal}/_{mol}$, approximately 10 fold larger than the vdW interaction between methane molecules, though still considerably less than a covalent bond. Because of the electrostatic nature of the H-bond, the energy of interaction is closely related to the environment, so that the H-bonds between water molecules in the gas phase (5.4 $^{kcal}/_{mol}$) are considerably stronger than H-bonds in liquid water (3.4 $^{kcal}/_{mol}$). This is another qualifier for the H-bond - sort of. It's been noted that C-H and S-H groups can act as H-bond donors to nitrogen and oxygen acceptors, judging by contact distances, but because of the low polarity associated with those bonds, and the larger donor-acceptor distances, the interactions aren't much more significant in energy than a vdW interaction, and so they are usually neglected in discussions of H-bonding interactions.

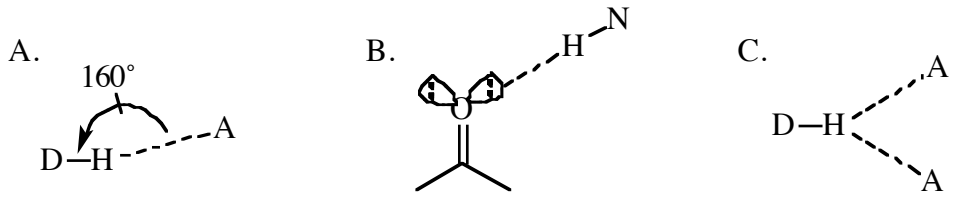


Figure 1.8 (A) The angle of this H-bond (160°) is not unusual, thought most H-bonds cluster at about 180°. (B) H-bonds form in line with the lone pairs of the acceptor. (C) It is possible to have three-center H-bonds, such as this one with one donor, but two acceptors.

Ion-Ion Interactions

From an electrostatic perspective, ion-ion interactions are perhaps the easiest to understand, since they result from the influence of fixed unit charges. It is not difficult to see that the phosphate groups in polynucleotides, each carrying a negative charge, will repel each other, and conversely will attract cations dissolved in solution (Figure 1.9A). However, like other intermolecular contacts, the energy of interaction will vary with distance and with the polarity of the medium. Among the

strongest ion-ion contacts are those that involve hydrogen bonding as well. For example, the interaction of an alkyl ammonium group and a carboxylate (Figure 1.9B) involves cationic and anionic groups that are an H-bond donor and acceptor respectively. Because of the short distance of approach permitted in H-bonding and the high charges on the participating groups, these contacts are among the most energetically important between biomolecules. Of course, the biochemical macromolecules may also interact with inorganic species, such as hydrogen phosphate (HPO_4^{2-}) and metal ions (such as Mn^{2+}, Fe^{3+}, etc.) which possess greater charge and are therefore capable of even stronger binding interactions.

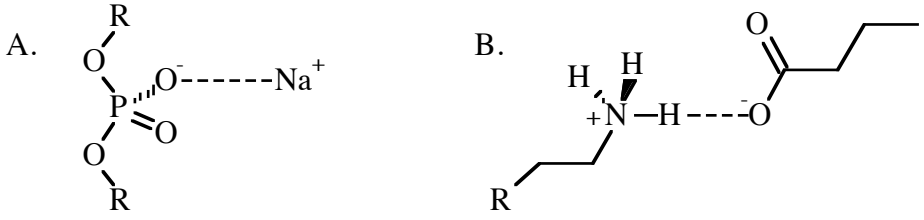


Figure 1.9 (A) An ion-ion interaction between a phosphodiester anion and a sodium cation. (B) An ion-ion interaction mediated via a hydrogen bond.

Summary

The enthalpic contributions to the contacts between and within biological macromolecules all arise from electrostatics. From the weakest vdW contact to the strongest ion-ion attraction, the affinity between opposite charges is the dominating theme. In general, one can rate the energy of interactions as vdW < H-bond < ion-ion, but that doesn't really capture the full picture. For one, because of the numerical superiority enjoyed by vdW contacts relative to the other two interactions, they can contribute significantly to the enthalpy of interaction between two biological molecules. Likewise, remember that the high enthalphic values of H-bonds and ion-ion interactions are often mediated in biochemistry because of the composition of the solvent. Sure, an H-bond may form between two biomolecules, but only at the expense of a loss of H-bonding between the individual species and the solvent water molecules. Also, the polar nature of water reduces the electrostatic attraction that anions and cations feel for one another. As a result, the energetic impact of a given type of interaction may not necessarily correlate with its individual importance in the gas phase. While the nature of these forces are relatively straightforward to understand, their application in biochemical function is often subtle and surprising, as will be seen in the coming weeks.

The Hydrophobic Effect - The Entropic Contribution

Oil and Water

The goal of this chapter is to explain how macromolecules attract one another to form stable complexes. As a model then, one couldn't do much better than looking at the assembly of non-polar molecules, such as alkanes, from aqueous solution to form a separate immiscible phase. Just like mixing salad oil and vinegar, the separation of the **hydrophobic** ("water-fearing"), non-polar species from solution is a spontaneous process, indicating a negative change in free energy. However, "hydrophobic" is a misleading term, since non-polar molecules actually prefer interactions with water relative to self interactions. The dipole/induced-dipole interaction is stronger than the induced-dipole/induced-dipole interaction.

Instead, it might be said that the water molecules "fear" non-polar molecules, preferring to interact with themselves. This phenomenon is driven by entropy. Consider the transfer of methane from the gas phase to two different solvents, water and carbon tetrachloride (Table 1.6). Dissolving methane in carbon tetrachloride has a positive change in free energy, due to the loss of entropy that results from leaving the gas phase, though there is some enthalpic stabilization that results from vdW contacts with CCl_4 molecules. Likewise, dissolving methane in water is unfavorable, but even more so than with carbon tetrachloride. Although methane has more favorable intermolecular interactions with water than it does with CCl_4 (ΔH is more negative), the change in entropy is considerably more negative and, causing a positive change in free energy.

Table 1.6 Thermodynamics of methane dissolving in water and carbon tetrachloride at 37°C.

Transfer	**ΔG**	**ΔH**	**ΔS**
gas to CCl_4	+3.5 $^{kcal}/_{mol}$	-0.5 $^{kcal}/_{mol}$	-14 $^{cal}/_{mol \cdot K}$
gas to water	+6.3 $^{kcal}/_{mol}$	-3.2 $^{kcal}/_{mol}$	-32 $^{cal}/_{mol \cdot K}$
CCl_4 to water	+2.8 $^{kcal}/_{mol}$	-2.7 $^{kcal}/_{mol}$	-18 $^{cal}/_{mol \cdot K}$

The take-home lesson from Table 1.6 is that transferring methane from CCl_4 to water costs 18 cal/mol•K, or 5.5 $^{kcal}/_{mol}$ at 37°C ($T\Delta S$), more than enough to offset the greater enthalpic stabilization that water offers. Since it seems unlikely that methane loses more entropy by being dissolved in water vs. an organic solvent, we're left looking for an alternative. What we've overlooked to this point is the role of the solvent in solvation, and the impact of placing a molecule of methane into an aqueous environment. As it turns out, the loss of 18 e.u. associated with transferring methane from CCl_4 to water *is the result of a loss of solvent entropy*, not solute entropy. The **hydrophobic effect**, which is the observation that non-polar molecules aggregate in aqueous solution, **is driven by solvent entropy**.

Solvent entropy *does not* play a role in the solvation of polar atom groups. In Table 1.7, the thermodynamic parameters associated with the phase separation of several organic compounds from water are shown. In a comparison of the entropies associated with the transfer of solute into a pure solvent phase, ethanol stands out with a significantly lower $\Delta S_{transfer}$, implying that water interacts with the hydroxyl group of ethanol without the significant loss of entropy that accompanies the interaction of water with the methyl group of propane, which is isosteric with ethanol. This is an important distinction. Certainly, water forms close associations with all solutes it encounters, but the loss of entropy that accompanies solvation of hydrophobic molecules is special. Why does that loss occur?

Table 1.7 Thermodynamic parameters for the transfer of an organic solute into a pure solvent phase.

Transfer	**ΔG**	**ΔH**	**ΔS**
$C_2H_{6(aq)}$ to $C_2H_{6(l)}$	-3.8 $^{kcal}/_{mol}$	+2.4 $^{kcal}/_{mol}$	+21$^{cal}/_{mol\cdot K}$
$C_3H_{8(aq)}$ to $C_8H_{8(l)}$	-5.0 $^{kcal}/_{mol}$	+1.9 $^{kcal}/_{mol}$	+22 $^{cal}/_{mol\cdot K}$
$C_2H_5OH_{(aq)}$ to $C_2H_5OH_{(l)}$	-0.8 $^{kcal}/_{mol}$	+2.4 $^{kcal}/_{mol}$	+10 $^{cal}/_{mol\cdot K}$

Clathrates

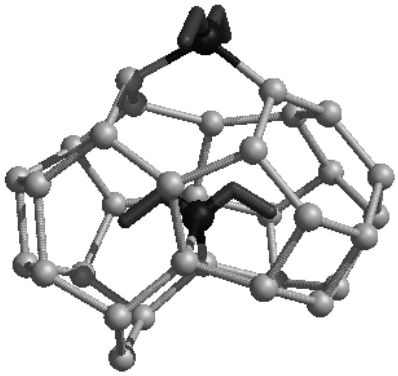


Figure 1.10. The crystal structure of diethylamine hydrate. It can be seen that the amino groups (dark spheres) participate in hydrogen bonds (depicted as thin rods) with the surrounding waters, but not the ethyl groups. Instead a cage of waters (light spheres) forms around those non-polar groups, maximizing H-bonding between water molecules, while still providing vdW contacts to the ethyl groups.

The rationale accepted by most biochemists is that water forms ordered structures when interacting with non-polar solutes.[7] That order results in the loss of solvent entropy. An analogy is often drawn

[7] An alternate view is that water loses entropy because it has to create unusually large cavities to accommodate a non-polar solute. By giving up positional freedom (water can't occupy those cavities) the solvent loses entropy. Different rationale, same outcome.

to the interaction of methane with water. When high pressure methane mixes with water at low temperatures (such as near gas vents on the sea floor or at leaks in gas pipelines) an unusual solid forms, known as a gas **clathrate**. This mixture of water and methane has many of the characteristics of ice, but in fact melts at a higher temperature. A similar phenomenon occurs when a single molecule of methane is dissolved in water; an ice-like cage of water molecules surrounds the CH_4 molecule. In allowing the methane molecule to become solvated, the water molecules must create a hole into which the methane molecule fits. This hole is costly in enthalpy, since hydrogen bonds between adjacent water molecules have to be broken. Although the CH_4 molecule occupies that space, the weaker vdW forces don't fully restore the lost enthalpy of interaction between water molecules. However, it is possible for the water molecules lining the methane-containing cavity to reorient themselves in such a way that almost every molecule retains full H-bonding, though exposed to a non-polar molecule. To create this "cage" (see Figure 1.10), the water molecules must freeze in position and, in effect, form part of a complex of a single methane molecule surrounded by a number of associated water molecules.

In these iceberg-like structures, each water molecule gives up about 2 cal/mol•K in forming the cage (~0.6 $^{kcal}/_{mol}$ at 25°C). But each molecule maintains a relatively constant enthalpy of interaction with its fellow water molecules while the solvated methane picks up modestly favorable enthalpic interactions with water (dipole/induced-dipole; see Table 1.4). So despite a mildly favorable enthalpy of interaction, the dominating negative entropy of interaction leads to a positive free energy of solvation.

This explains the separation of non-polar molecules from water. By aggregating, hydrophobic molecules decrease the amount of non-polar surface area exposed to solvent. The number of waters locked into cage structures decreases, so the overall change in entropy for phase separation is positive, even though there is a cost associated with restricting water molecules to one phase and greasy, hydrophobic molecules to a separate phase. The association of hydrophobic molecules is therefore the result of an increase in solvent entropy, not in any inherent molecular forces that hold them together. In biochemistry, exposed non-polar atom groups on the surface of a macromolecule can thus contribute to the affinity of an interaction without providing any significant enthalpic contribution to binding. In general, the hydrophobic effect contributes 0.0045 $^{kcal}/_{mol}$ to the free energy of binding per $Å^2$ of non-polar surface area.

The Hofmeister Series

Other species dissolved in an aqueous medium can play a role in the ordered structure of solvent water, and therefore play a role in the hydrophobic effect. Two general trends have been identified in the ability of dissolved cations and ions to accentuate the hydrophobic effect. The so-called **Hofmeister series** is shown here:

$$NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+} > \text{guanidinium} \quad \text{(Eq. 1.9a)}$$

$$SO_4^{2-} > HPO_4^{2-} > \text{acetate} > \text{citrate} > Cl^- > I^- > SCN^- \quad \text{(Eq. 1.9b)}$$

Ammonium and sulfate are the cation and anion that cause the greatest enhancement of the hydrophobic effect, though in general anions play a more significant role that cations. These ions apparently disrupt the structure of free solvent water and thereby decrease the solubility of nonpolar

species, since the formation of ordered water cages will be even worse from an entropic standpoint. On the other hand, guanidinium (see Figure 1.11A) and thiocyanate ions tend to increase the order of water in solution and therefore reduce the loss of entropy associated with dissolved nonpolar molecules. These species tend to decrease the magnitude of the hydrophobic effect and thereby promote the solubility of normally hydrophobic molecules. One other molecular species worth mentioning in this context is urea (Figure 1.11B). Similar in structure to the guanidinium ion, it is uncharged, but also causes a significant decrease in the hydrophobic effect.

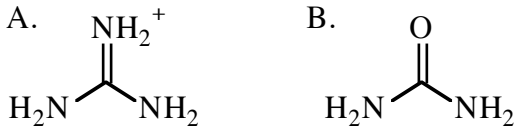


Figure 1.11. (A) The guanidinium cation. (B) Urea. Both species act to increase the order of water and therefore decrease the hydrophobic effect, making nonpolar molecules more soluble.

Summary

- The affinity of two molecules for one another is defined by an association constant, K_a, whose magnitude is dependent on the difference in free energy (ΔG) between the free and bound species.
- Enthalpy (H), which results from electrostatic forces operating between chemical species, and entropy (S), which is related to the disorder of those species, both contribute to free energy.
- The **forces** operating between nonbonded atoms and molecules can be generally categorized as van der Waals, hydrogen bonds and ion-ion interactions, in order of increasing contribution to enthalpy. However, vdW forces (which contribute to all interactions) are the most prevalent and essentially define the size and shape of molecules.
- **The hydrophobic effect is an important driver of molecular association in aqueous solutions.** Ice-like structures of water (**clathrates**) develop dissolved nonpolar molecules, which substantially reduces solvent entropy. When nonpolar molecules aggregate, these structures break up, increasing entropy and thereby contributing to a more negative value of ΔG - rendering the overall process spontaneous.

Further Reading

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