Aqueous solutions

Solubility of different compounds in water

The dissolution of molecules into water (in any solvent actually) causes a **volume change** of the solution; the size of this volume change is often, but not always, larger than the van der Waals volume of the substance, and this is also found for the amino acids. This extra volume is thought to arise from the expanded structure of the water that is in the immediate vicinity of the surface of the "solute" molecule. There is a tendency for the nonpolar amino acids to show a larger volume difference, compared to the van der Waals volume, than the non-polar amino acids. The tendency to increase the effective molecular volume by a surrounding water sheath means that a protein will tend to remove these molecular groups from solution upon the application of pressure, if this is possible, and the volume of the amino acid groups is often smaller when they are removed from an aqueous environment, and just interact with each other.

***Table 4.3** Volume Properties of Individual Amino Acid Residues*

Residue	Van der Waals volume[a] (Å³)	Partial volume in solution[b] (Å³)	Partial specific volume[b] (cm³/g)
Ala (A)	67	86.4	0.732
Arg (R)	148	197.4	0.756
Asn (N)	96	115.6	0.610
Asp (D)	91	108.6	0.573
Cys (C)	86	107.9	0.630
Gln (Q)	114	142.0	0.667
Glu (E)	109	128.7	0.605
Gly (G)	48	57.8	0.610
His (H)	118	150.1	0.659
Ile (I)	124	164.6	0.876
Leu (L)	124	164.6	0.876
Lys (K)	135	166.2	0.775
Met (M)	124	160.9	0.739
Phe (F)	135	187.3	0.766
Pro (P)	90	120.6	0.748
Ser (S)	73	86.2	0.596
Thr (T)	93	113.6	0.676
Trp (W)	163	225.0	0.728
Tyr (Y)	141	190.5	0.703
Val (V)	105	136.8	0.831
Weighted average[c]			0.703

[a] Volume enclosed by van der Waals radius.

[b] Increase in volume of water after adding either one molecule or one gram of residue (A. A. Zamyatnin, *Ann. Rev. Biophys. Bioeng.* 13:145–165, 1984.)

[c] Weighted by frequency of occurrence in proteins, to give the value for an average residue in globular proteins.

One can approximately divide the properties of chemical groups according to the way water interacts with them, and the ability of water to dissolve the different substances. In this way we can speak of the **hydrophilic** and **hydrophobic** characteristics of the substances. The partition coefficient of a substance X is a measure of the tendency of a compound to be in water, compared to a vapor state, and it is defined as:

$$K_D = \frac{[X]_{H_2O}}{[X]vapor}$$

where [X] refers to the concentration of X in the corresponding environment. The free energy of transfer from the vapor to the water is: $\Delta G = -RT\ell nK_D$ and it is this **free energy** that is a **measure** of the **hydrophilicity** of a molecule.

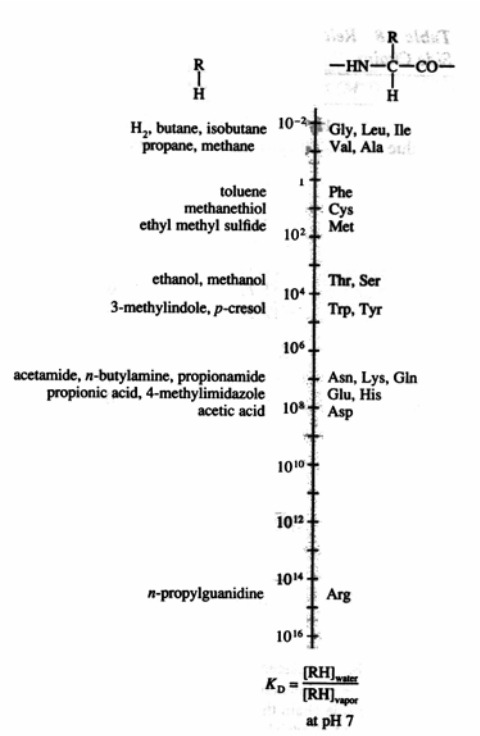


FIGURE 4.8
Relative hydrophilicities of amino acid side chains, measured by the partition coefficient between vapor of the appropriate small molecule and water at pH 7. The scale gives the equilibrium constant between the vapor and aqueous phase. The model compounds used for the side chains of the amino acid residues *(right)* are given on the *left*. (From R. Wolfenden et al., *Biochemistry* 20:849–855, 1981.)

In this figure the values for the ionizable molecules have been corrected for the fraction of molecules ionized at pH=7. Glycine is given the value of zero.

The tendency of substances to dissolve in water depends mainly on

1) the **polar character** of water,
2) its **H-bonding propensity**, and
3) the tendency for water molecules to be in certain **non-random orientations** relative to the other surrounding water molecules (often referred to as the "**structure**" of H_2O).

This **ordering propensity** for liquid water is a **statistical dynamic effect,** and does not refer to a static orientation or configuration (as in ice). It should be understood similarly to the inhomogeneous distribution of molecules that one sees in every condensed liquid phase, where the radial **distribution function** shows *tendencies of the liquid molecules to be in certain locations more than in other locations surrounding any particular solvent molecule*. For **water** the density distribution function is **not spherically symmetric** around a particular water molecule. However, on the average, for many molecules seen in a scattering experiment, they are spherically symmetric relative to the center of mass of the central water molecules, because of the rotational distribution of all the molecules. This reflects the tetrahedral structure of H_2O molecules, together with their H-bonding possibilities. The water molecule is approximately spherical – more correctly, a sphere that is slightly deformed into a "V" shape, so that there is little steric hindrance for the water molecules to change orientations, or move to new neighboring locations. Thus, the **configurational entropy** of **liquid water** is **high** (compared to what we might think when we hear of the "structure" of water.

We see exactly what we would expect from a solvent molecule with an asymmetric distribution of electronic charge. The **polar molecules** *interact* much **more favorably** with water, than the less polar - or the **non-polar** - amino acids The van der Waals interactions between water and the amino acids are not very different from the van der Waals interactions between the amino acids themselves. We cannot interpret these data until we discuss the hydrophobic effect (see below), but we can note here that the **tendency of molecules to dissolve in water** must be related to *the **total free energy** involved in making a "**cavity**" in the water the size of the guest molecule, and the **specific interactions (or lack of)** between the **water** and the "**surface**" of the **amino acid** molecules*.

- See table on next page -

Table 4.8 *Relative Hydrophilicities and Hydrophobicities of Amino Acid Side Chains*

Residue	Hydrophilicity[a] (kcal/mol)	Hydrophobicity (kcal/mol)			
		Side-chain analogues[a]	Amino acids[b]	*N*-acetyl amides[c]	Calculated[d]
Arg	−22.31	15.86	3.0	1.01	3.95
Asp	−13.34	9.66	2.5	0.77	3.81
Glu	−12.63	7.75	2.5	0.64	2.91
Asn	−12.07	7.58	0.2	0.60	1.91
Lys	−11.91	6.49	3.0	0.99	2.77
Gln	−11.77	6.48	0.2	0.22	1.30
His	−12.66	5.60	−0.5	−0.13	0.64
Ser	−7.45	4.34	0.3	0.04	1.24
Thr	−7.27	3.51	−0.4	−0.26	1.00
Tyr	−8.50	1.08	−2.3	−0.96	−1.47
Gly	0	0	0	0	0
Pro			−1.4	−0.72	−0.99
Cys	−3.63	−0.34	−1.0	−1.54	−0.25
Ala	−0.45	−0.87	−0.5	−0.31	−0.39
Trp	−8.27	−1.39	−3.4	−2.25	−2.13
Met	−3.87	−1.41	−1.3	−1.23	−0.96
Phe	−3.15	−2.04	−2.5	−1.79	−2.27
Val	−0.40	−3.10	−1.5	−1.22	−1.30
Ile	−0.24	−3.98	−1.8	−1.80	−1.82
Leu	−0.11	−3.98	−1.8	−1.70	−1.82

[a] Hydrophilicity was measured by the partition coefficient K_D of the model for each side chain (backbone replaced by hydrogen atom, Fig. 4.8) from vapor → water; hydrophobicity from water → cyclohexane. For ionizing side chains, the values were corrected for the fraction of each side chain that is ionized at pH 7. Both scales were normalized to zero for the value of Gly (A. Radzicka and R. Wolfenden, *Biochemistry* 27:1664–1670, 1988).

[b] Some values were measured from the relative solubilities of the amino acids in water and ethanol or dioxane (Y. Nozaki and C. Tanford, *J. Biol. Chem.* 246:2211–2217, 1971); others were extrapolated from these data (M. Levitt, *J. Mol. Biol.* 104:59–107, 1976).

[c] Measured from the partition coefficient between water and octanol of the N-acetyl amino acid amides (J. Fauchère and V. Pliska, *Eur. J. Med. Chem.* 18:369–375, 1983).

[d] Calculated from the hydrophobicities of the individual groups that make up each side chain, using data for the partition coefficient between water and octanol of many model compounds (M. A. Roseman, *J. Mol. Biol.* 200:513–522, 1988).

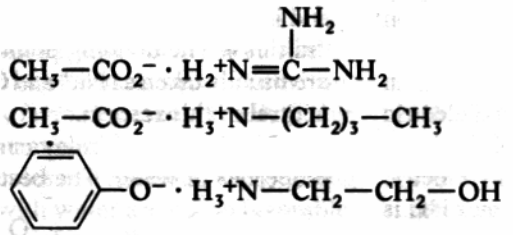
Molecules interacting in an aqueous environment

The interaction of molecules is described in terms of "*mass action*" as:

$$A \quad + \quad B \quad \leftrightarrow \quad (AB) \quad ; \quad K_{assoc} = \frac{[(AB)]}{[A][B]} \quad ; \quad \Delta G_{assoc} = -RT\ell nK_{assoc}$$

Substances that are very soluble in water, often do not associate very strongly with each other in water. This is because in order for the molecules A and B to interact, they must break their interactions at their interfaces with the surrounding solvent molecules. **Water plays a central role in these interactions of dissolved compounds** with each other, because water often **interacts** strongly in various **different ways with the "reactants" and "products"** of these non-covalent interactions. The interaction of water with some dissolved substances can also have a major effect on the properties of the "bulk" water. This is due to the "many body", or "all encompassing" statistical mechanical nature of the interactions which lead to the molecular distributions of water molecules. In addition, there is an entropic barrier to overcome, because the two molecules, A and B, become one entity (AB), but this often plays a minor role.

Table 4.9 Association in Water of Small Molecules Typical of Noncovalent Interactions in Proteins

Type of interaction	Example	Association constant (M^{-1})
Salt bridge		0.5[a] 0.37[b] 0.31[b] 0.20[c]
Hydrogen bond[i]	Formic acid dimers	0.04[d]
	Urea dimers	0.04[e]
	N-Methylacetamide dimers	0.005[f]
	δ-Valerolactam dimers	0.013[g]
Van der Waals	Benzene dimers	0.4[h]
	Cyclohexane · cyclohexanol	0.9[h]
	Benzene · phenol	0.6[h]

[a] C. Tanford, *J. Amer. Chem. Soc.* 76:945–946 (1954).

[b] B. Spriggs and P. Haake, *Bioorg. Chem.* 6:181–190 (1977).

[c] N. Stahl and W. P. Jencks, *J. Amer. Chem. Soc.* 108:4196–4205 (1986).

[d] A. Katchalsky et al., *J. Amer. Chem. Soc.* 73:5889–5890 (1951).

[e] J. A. Schellman, *Compt. Rendu Trav. Lab. Carlsberg Ser. Chim.* 29:223–229 (1955).

[f] I. M. Klotz and J. S. Franzen, *J. Amer. Chem. Soc.* 84:3461–3466 (1962).

[g] H. Susi et al., *J. Biol. Chem.* 239:3051–3054 (1964).

[h] S. D. Christian and E. E. Tucker, *J. Solution Chem.* 11:749–754 (1982).

[i] Interactions other than hydrogen bonding may contribute to the dimerization of these molecules, so the association constants are maximum values for hydrogen bonding.

However, it is also possible for the molecules to **interact through water molecules** placed **between** them.
It is even possible for two larger nonpolar surfaces to **interact over multiple layers of water**, and the interaction energy is not a smooth function of the distance between the two surfaces, but varies with a period of about 2.5 Å, the diameter of a water molecule. So it seems that the **most favorable interactions** happen when there are an **integral number of water molecules between the two surfaces**.
In addition, water can be shown to interact with certain areas of proteins, and **in the grooves** of DNA. In the DNA grooves, the water sometimes becomes an integral part of the structure, and it is thought that transitions between different conformational states involve also the dissociation of this "bound" (but labile) water.

The presence of ions in solution can have **large effects** on the "**structure**" and other properties of water. These effects are often well correlated with the

"***Hofmeister***" **series** (effectiveness of precipitating serum globulins):

***Cations*: $NH_4^+ > K^+ > Na^+ > Li^+ > Mg^+ > Ca^{2+} >$ guanidinium**

***Anions*: $SO_4^{2-} > HPO_4^{2-} >$ acetate > citrate > tartrate > $Cl^- > NO_3^- > ClO_3^- > I^- > ClO_4^- > SCN^-$**

Disrupt structure	←→	Increase structure of water
Increase surface tension	←→	No effect on surface tension
Decrease solubility of non-polar substances (salting out)	←→	Increase solubility of non-polar substances (salting in)

The ions at the **beginning** of each series, tend to *disrupt the structure* of water, *increase its surface tension*, and *decrease the solubility* of non-polar molecules (salting out).
The ions at the **end** of each series, increase the structure of water, do not affect the surface tension, and increase the solubility of non-polar molecules ("salting in").

Na^+ and Cl^- are at the dividing line. Part of the effect of these ions can be understood by realizing that they **remove water from the "bulk" phase** (by attracting a water sheath around themselves) and this leaves less water available for other compounds. This is an oversimplistic view of things, and glosses over the real statistical mechanical reason for the effects, but is a useful, and certainly partially true, paradigm.

Non-polar molecules, such as urea (H_2N-(C=O)-NH_2), also **interfere** with the **H-bonding** in the bulk water. Urea was the first biological molecule to be synthesized from inorganic compounds by Woehler. This was a very important happening, because it showed that "bio-organic" molecules were not the result of some "vital" characteristic of living organisms.

The **additives** are **usually excluded from the interface to the non-polar surfaces** of molecules, or from **air water interfaces**. This is the reason why the surface tension increases for those ions that disrupt the "structure" of water in the bulk phase. Remember what we discussed about the meaning of the "structure" of water.

Short **summary** of the different important **intermolecular forces** acting on **macro-biomolecules**.

Covalent bonds:

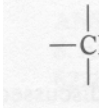
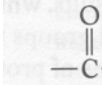
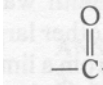
These are *Quantum Mechanical,* and act only at very **short range**. They are very **strong**, in general between **150 to 900 kJ/mol**, and they are **chemical bonds**, not physical bonds (as the rest which we discuss are). The form and stereochemistry of the smaller molecules and molecular groups are in general set by these covalent interactions, as well as the **van der Waals dimensions** (atomic radii) of the molecules.
The ***optimal van der Waals*** interactions occur at a distance of 1.2 Å *greater* than the covalent bond length. The ***atoms are in contact*** when they are 0.8 Å greater than when they are covalently attached to each other.

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

Atom	Observed range (Å)	Radius when singly bonded (Å)
Hydrogen	1.0–1.54	1.17
Oxygen	1.4–1.7	1.40
Nitrogen	1.55–1.60	1.55
Carbon	1.70–1.78	1.75
Sulfur	1.75–1.80	1.80

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

Table 4.2 *Van der Waals Surface Areas and Volumes of Chemical Groups When Bonded to Carbon Atoms*

Chemical group	Area (Å²)	Volume (Å³)
	1.0	5.5
	10.9	11.5
$-CH_2-$	20.9	16.8
$-CH_3$	33.4	22.3
Phenyl	94.9	76.1
—OH	19.3	12.6
	22.3	18.2
	43.4	
—SH		24.6
$-NH_2$	26.5	17.5
—NH—	16.4	13.4

Values from A. Bondi, *J. Phys. Chem.* 68:441–451 (1964) and A. Gavezzotti, *J. Amer. Chem. Soc.* 107:962–967 (1985).

Table 4.3 Volume Properties of Individual Amino Acid Residues

Residue	Van der Waals volume[a] (Å^3)	Partial volume in solution[b] (Å^3)	Partial specific volume[b] (cm^3/g)
Ala (A)	67	86.4	0.732
Arg (R)	148	197.4	0.756
Asn (N)	96	115.6	0.610
Asp (D)	91	108.6	0.573
Cys (C)	86	107.9	0.630
Gln (Q)	114	142.0	0.667
Glu (E)	109	128.7	0.605
Gly (G)	48	57.8	0.610
His (H)	118	150.1	0.659
Ile (I)	124	164.6	0.876
Leu (L)	124	164.6	0.876
Lys (K)	135	166.2	0.775
Met (M)	124	160.9	0.739
Phe (F)	135	187.3	0.766
Pro (P)	90	120.6	0.748
Ser (S)	73	86.2	0.596
Thr (T)	93	113.6	0.676
Trp (W)	163	225.0	0.728
Tyr (Y)	141	190.5	0.703
Val (V)	105	136.8	0.831
Weighted average[c]			0.703

[a] Volume enclosed by van der Waals radius.

[b] Increase in volume of water after adding either one molecule or one gram of residue (A. A. Zamyatnin, *Ann. Rev. Biophys. Bioeng.* 13:145–165, 1984.)

[c] Weighted by frequency of occurrence in proteins, to give the value for an average residue in globular proteins.

The **accessible surface area** of a molecule (small or large) is found by rolling a ball (1.4 Å in diameter – a water molecule) over the surface of the macromolecular structure (in the computer). This parameter is often given for structures presented in the literature, and many parameters are correlated with this surface area.

We saw this for instance when we calculated the solubility of cyclohexane in water (see earlier notes).

Table 4.4 *Accessible Surface Areas of Amino Acid Residues in a Gly-X-Gly Tripeptide in an Extended Conformation*

Residue	Total (Å²)	Main-chain atoms (Å²)	Side-Chain Atoms (Å³)		
			Total	Nonpolar	Polar
Ala	113	46	67	67	
Arg	241	45	196	89	107
Asn	158	45	113	44	69
Asp	151	45	106	48	58
Cys	140	36	104	35	69
Gln	189	45	144	53	91
Glu	183	45	138	61	77
Gly	85	85			
His	194	43	151	102	49
Ile	182	42	140	140	
Leu	180	43	137	137	
Lys	211	44	167	119	48
Met	204	44	160	117	43
Phe	218	43	175	175	
Pro	143	38	105	105	
Ser	122	42	80	44	36
Thr	146	44	102	74	28
Trp	259	42	217	190	27
Tyr	229	42	187	144	43
Val	160	43	117	117	

From S. Miller et al, *J. Mol. Biol.* 196:641 – 656 (1987).

Electrostatic forces:

a) **point charges**: energy -> $\Delta E = {Z_A Z_B e^2}/{Dr_{AB}}$

Calculate the energy of Na^+ and Cl^- ions that are separated by 2.76 Å in a crystal , D=1 (this is the optimum distance). The energy is 2.0×10^{-19} cal/molecular pair; in molar units this is 120 kcal/mol! This is a **very large number** (about 200 kT units!), *of the order of a covalent bond*. However this interaction energy for ions is **much less in water**, where the water can affect the ionic interaction; the strong electrostatic interaction is decreased drastically due to the high dielectric constant of the water (this is ~80). For most solvents, as already shown, the dielectric constants are between 2 and 110. The effective range of the electrostatic forces between ionic charges becomes very limited by the "***screening***" effects of the other free charges in solution. See the discussion below about the **Debye-Hueckel** theory.

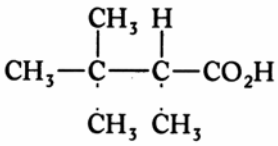
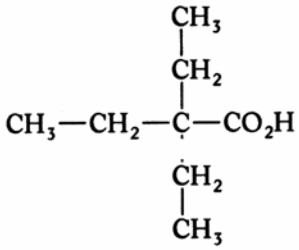
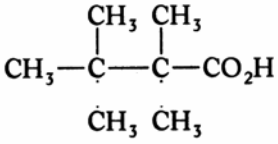
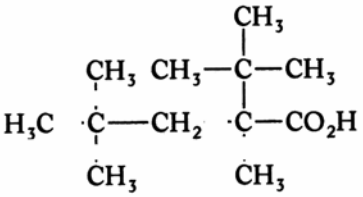
Very closely spaced oppositely charged charges in proteins are termed "**salt bridges**", and these ions can also participate in strong hydrogen bonds, and are often physically "linked" by intervening water molecules. The ionic interactions change as a function of ionic strength. The **acid dissociation constants, pK_a,** of the amino acids are in the pH range where the amino acids can easily dissociate, and this will create charges, which will produce electrostatic effects in proteins. The magnitude of these effects will depend on the pH of the solution environment (even the local environment). The pK_as are themselves affected by environmental effects such as ionic strength, by other dissociating groups in the region of interest on the protein, and by their environment in the region where they are located in the protein structure. The lower the polarity of the solution, the less the tendency to dissociate, forming an ion.

Table 4.5 *Effect of Nonaqueous Environment on the* pK_a *Values of Amino and Carboxyl Groups*

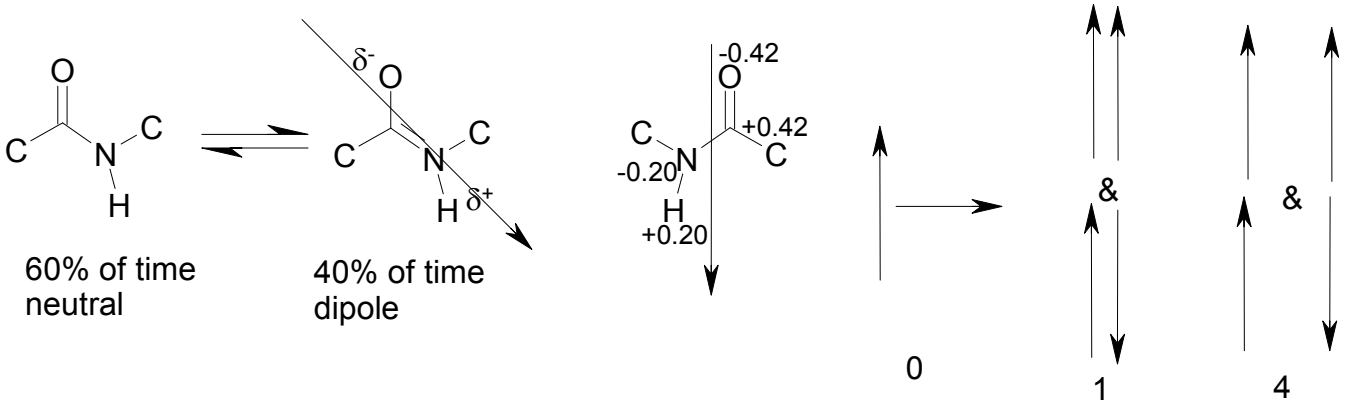
	pK_a Values for Various Wt % Dioxane in Water				
Acid or base	0	20	45	50	70
Acetic acid	4.76	5.29	6.31		8.34
$(HOCH_2)_3C\text{-}NH_2$	8.0	8.0	8.0	8.0	8.0
Benzoylarginine	3.34			4.59	4.60
Glycine: $\text{-}CO_2$	2.35	2.63	3.11		3.96
$\text{-}NH_2$	9.78	9.29	8.49		7.42

From A. Fersht, *Enzyme Structure and Mechanism*, W. H. Freeman, Reading, England, 1977.

Table 4.6 *Steric Effects on the Ionization of Carboxyl Groups*

Model compound	pK_a[a]
	5.55
	6.25
	6.44
	6.71
	6.97

[a] The pK_a values were measured in equal volumes of methanol and water at 40°C by G. S. Hammond and D. H. Hogle, *J. Amer. Chem. Soc.* 77:338–340 (1955).



average distribution of the electric charges for the peptide backbone & dipole moment

Relative Strength of interaction

b) **dipoles:** $\delta^+ - \delta^-$ **; orientation dependent**

1) remember the **peptide bond**; The dipole of the peptide backbone ($\left|\delta^{\pm}\right| = 0.4\, e^-$) is actually quite large. The dipole = Zd, is **3.5 D for a peptide bond**, and **1.85 D for a water molecule** (1 Å separating oppositely charged single charges has dipole moment of 4.8 D).

2) The **π electrons** of the faces of the aromatic rings of the amino acid side groups have a negative charge (~0.15 e^-). Neighboring rings tend to align so that the **edges** of the rings, with the positive hydrogens, are **perpendicular** to the phase of the other ring. That is, **the rings tend to align themselves perpendicular to each other**. O and S (electron rich) atoms tend to interact with the edges, and the NH groups interact with the faces.

3) In order to calculate the interaction energy due to all the charged groups in a protein, the Coulomb interactions are simply added (integrated).The energy of interaction goes as **$1/r^2$, $1/r^3$ or $1/r^6$ for ion-dipole, fixed dipole-dipole, and freely rapidly rotating dipole-dipole.** Note the dipole interactions fall off rapidly.

4) Polarizability: The charge distribution in molecules is easily perturbed by external charges. **Large atoms** are generally **more easily polarized**. **Induced dipoles** always lead to favorable interactions (**attraction**). The energy of interaction between a **permanent dipole-induced dipole** are **½** of what an equivalent **permanent dipole- permanent dipole** interaction is.

5) In **homogeneous solution** the electrostatic interactions can be calculated by using a simple dielectric constant. **But in proteins**, the **situation is very complex**; one must take into account interactions within the protein, with water, and with the effect of the other ions in the solvent. This is very difficult to calculate, even if the structure is known, especially when including the solvent structure at the surface of the protein, and often the water is in the protein. The effects of electrostatic interactions, and how to correctly describe them, is still an active, and contentious, area of research, where the models are vigorously defended by their proponents. The problem is the long range of the electrostatic forces.

van der Waals interactions:

These interactions actually consist of three parts: **1)** permanent dipoles, **2)** permanent-induced dipoles, and **3)** induced-induced dipoles.
The *induced-induced dipole interaction* – the **dispersion force** - is always present, and it is quantum mechanical in nature. The effects can be large, because this force is present between all every pair of atoms (and it is not pairwise additive). It involves the **correlation** of **transient dipoles** of two **interacting atoms** – it is a **dispersion force**. The transient dipoles originate from temporary asymmetric distributions and orientations of the electrons and nucleus of the atoms. These transient dipoles of neighboring atoms interact with each other. **The dispersion force varies as $1/r^6$, and it is basically electrostatic in nature**. The "**optimal**" van der Waals interaction is usually modeled as a balance between attraction and repulsion, as a **Lennard-Jones potential**.

$E_r = C_n / r^n - C_6 / r^6$, where n>6, and usually n=12.

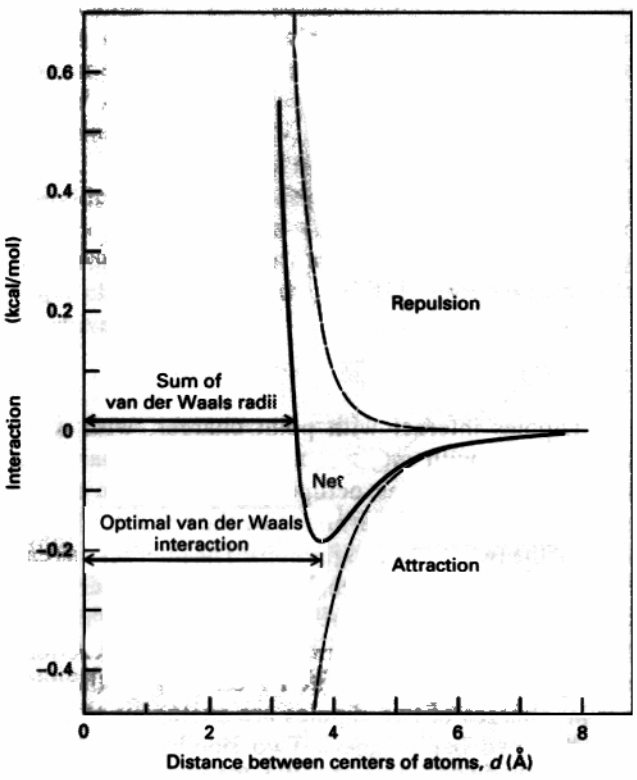


FIGURE 4.2
Representative profile of the energy of the van der Waals interaction as a function of the distance *d* between the centers of the two atoms. The individual attractive and repulsive components are indicated by the dashed lines, the net interaction by the solid line. The optimal interaction between the two atoms occurs where the energy is at a minimum. The sum of the van der Waals radii of the two atoms is given by the distance at which the energy increases sharply. The interaction energy was calculated using the Lennard–Jones 6,12 potential (Eq. 4.6) with $C_{12} = 2.75 \times 10^6$ Å^{12} kcal/mol and $C_6 = 1425$ Å^6 kcal/mol for the interaction between two carbons atoms (M. Levitt, *J. Mol. Biol.* 82:393–420, 1974).

The **optimum distance** is usually 0.3 to 0.5 Å larger than the sum of the van der Waals radii (see earlier). The interaction is **often considered to be independent of the orientation** of the interaction groups. But actually *it is dependent on the orientation of the atom groups* (e.g. a $-CH_3$ group interacting with a C-H bond is nearly 2x as strong when the groups are oriented along the bonds rather than perpendicular).

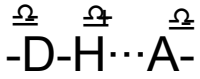
<u>Hydrogen-Bonds</u>:

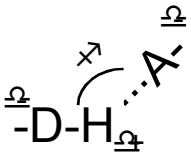
Two electronegative atoms competing for the same hydrogen:

$-D-H\cdots A-$; D=donor, and A=acceptor.

Earlier it was thought that the donor and acceptor shared the hydrogen more or less equally between them, but structure studies and calculations now show that this is not true – **the H remains associated with the donor,** and is **shared** only **partially** with the **acceptor**. The **D-H bond** distance is in general **shorter** than the **A-H distance**, but the **D-A distance** is **shorter** than the sum of the **van der Waals distances** of **all** the **atoms**. This indicates some covalent nature of the A-H bond.

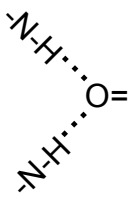
The **H-bond** is thought to be mainly **<u>electrostatic</u>** in nature, and this explains why the H-bond is **strongest** when the participating **bonds** are **linear**, but many H-bonds are at an angle.

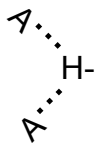




The **hydrogen atom** is quite special, because it is **highly charged** and has a very **small size**; in addition it can be **easily polarized**. As we said, linear H-bonds are thought to be strongest, but the *orientation dependence is controversial*.

Multiple donors and single acceptors are possible; for instance, there are two lone e^- pairs on the O-atom, and each can serve as an acceptor. And there are also single donors and multiple acceptors as with water in the grooves of DNA.





The **strengths of the H-bonds** depends on the **electronegativities** of the **donor** and **acceptor** atoms. The stronger the electronegativities, the stronger the H-bonds. Charged groups usually participate in stronger H-bonds. Of course, the stronger H-bonds are shorter.

The H-bonds are a ubiquitous. In proteins, the H···O distance is usually 1.9 – 2.0 Å, and the N-H distances 1.03 Å. So a typical N-H...O H-bond has a distance of about 3 Å (from the center of the two A and D atoms).

H-bond donors	**H-bond acceptors**
N-H, O-H	O=. –O-, -N=
sometimes:	sometimes:
S-H and C-H	-S-, and π e- of aromatics

The **strength** of the **H-bonds** varies from approximately **10 to 40 kJ/mol**; it is stronger than the **van der Waals bonds (about 1 kJ/mol)**. The covalent or ionic bonds are much stronger.

In water **every acceptor and donor group will be complexed with water molecules if the water can get to the groups**; this is true unless the acceptor/donor group either complexed with another H-bond complement, or sterically excluded from contact with water. Thus, the overall free energy contribution of the hydrogen bonds is often more important when they are **absent**. **That is, the free energy in a H-bonded complex compared to the complexes of the free compounds with water is not large** – there is just an interchange of the H-bonding parameters. Of course, at large concentrations this can have a major effect, but if a particular conformation of a protein macromolecule demands the absence of a H-bond (for steric reasons, for instance), then this loss of favorable free energy will raise the overall free energy of this conformation. This would be a **free energy penalty to pay for having this conformation with a overall loss of a H-bond**.