Biological space aqueous phase



Figure 1.9 Physical Biology of the Cell (© Garland Science 2009)

Water as a reactant $C_6H_{12}O_6 + 6O_2 \Rightarrow 6H_2O + 6CO_2 + energy$

$ATP+H_2O \Rightarrow ADP + H_3PO_4 + energy$



The water molecule

- **↓** OH distance = 0.958 Å
- 4 HOH angle = 104.5°





Oxygen is electronegative it draws the electrons in the bonds it shares with the hydrogen atoms towards it.

This results in the water molecule having a large dipole moment.

Dipole moment = 1.85 Debye = $6x10^{-30}$ Cm

Two water molecules can therefore form a strong electrostatic interaction.

The source of hydogen bonds is that uncharged species can still have a large inherent polarization.

A hydrogen bond consists of a hydrogen atom lying between two small, strongly electronegative atoms with lone pairs of electrons (N, O, F).





Strength of an H-bond is related to

- the **D-H**---A distance
- the D-H-A angle.

DistanceVan der Waals radius of
H: 1.1Å, O 1.5 Å.The closest approach should be 2.6 Å.Separation is about 1 Å less! It is 1.76 Å.

Hydrogen bond =1.77 Å Covalent bond =0.965 Å

Intermediate between VdW distance and typical O-H covalent bond of 0.96Å.

The strength

The hydrogen bonds is in the range of 0.1 - 40 kcal mol⁻¹.

The covalent bond between H and O in water (about 492 kJ mol⁻¹).

The van der Waals interaction (about 5.5 kJ mol⁻¹).

The hydrogen bond is
part electrostatic (90%)
and part covalent (10%)

The hydrogen bond is stronger than typical electrostatic interactions between partial charges, but *it is easily disassociated by heat or by interaction with other atoms.*



Hydrogen bond is directional

can hold two H-bonded molecules or groups in a specific geometric arrangement



Hydrogen bond potential energy





H-bonding network



Iower density than liquid water



Solid Ice vs. Solid Benzene



Only 42% of the volume is filled by the van der Waals volumes of the atoms, compared to 74% for spherical close packing.



Solid phase - ice

Liquid phase - water

↓ The number of nearest neighbors in water is 4.4 (4 in ice). ↓ Hydrogen bonds half life = $10^{-11} - 10^{-15}$ sec.

4 Cooperativity in hydrogen bond formation.

Upon breakage of one hydrogen bond, another hydrogen bond forms, with the same partner or a new one, within 0.1 ps

The H-bonding propensity of the water together with the tetrahedral geometry, leads to *a higher entropy in the bulk phase*.

Network of hydrogen bonds in liquid water





Selected properties of water

4 High boiling point

4 High viscosity (0.89 cP, at 25°C)



4 High surface tension (72.75 mJ/m², at 20°C)

4 A low thermal expansivity (0.00021/°C at 20°C)

4 High specific heat capacity ($C_V = 4.18 J g^{-1} K^{-1}$ at 25°C)

4 The dielectric constant is high (78.4 at 25°C)

4 Conductivity of protons is anomalously high

Dissociation - the separation of a water molecule into a hydrogen ion (H⁺) and a hydroxide ion (OH⁻).

$$H_{2}O + H_{2}O \iff H_{3}O^{+} + OH^{-}$$

$$K_{eq} = \frac{[H_{3}O^{+}][OH^{-}]}{H_{2}O}$$

$$H \longrightarrow H^{+} \bigoplus H^{+}$$

 $[H_2O] \sim 55M$ and ionization is very weak, then $[H_2O] \sim constant$.

For pure water $K_W = [H_3O^+][OH^-] = 10^{-14}$

 $[H^+] = [H_3O^+] = [OH^-] = 10^{-7}M$ In a neutral solution $[H_3O^+] = 10^{-7}M; \quad [OH^-] = 10^{-7}M$ That is only 1 H⁺ for every 560,000,000 water molecules!



pН

Definition of pK_a

The p K_a of a titrating site is defined as the pH for which the site is 50% occupied, HA + H₂O \Leftrightarrow A⁻ + H₃O⁺

 $K_{a} = \frac{a_{A^{-}}a_{H_{3}O^{+}}}{a_{HA}} = \frac{a_{A^{-}}}{a_{HA}}a_{H_{3}O^{+}} = \frac{1-\theta}{\theta}a_{H_{3}O^{+}}$ Deprotonation reaction



θ is degree of protonation or occupancy:
number of bound protons as a function of pH

Titration curve:

$$\theta(\mathbf{p}H) = \frac{1}{1 + e^{-\ln 10(\mathbf{p}K_a - \mathbf{p}H)}}$$

$$pH = pK_a + 1 \quad f_{A^-} = \frac{10}{11} \approx 90\%$$

$$pH = pK_a - 1 \quad f_{A^-} \approx 9\%$$

$$pH = pK_a - 2 \quad f_{A^-} \approx 0.9\%$$

Proton transport in bulk water

Hydronium ion gives up a proton





Figure 1.9 Physical Biology of the Cell (© Garland Science 2009)

'Bound water' in biological systems

- Intracellular water very close to any membrane or organelle (sometimes called vicinal water)
- Organized very differently from **bulk water**
- This structured water plays a significant role in governing the shape (and thus biological activity) of large folded biopolymers.

The density of bound water is 10% higher and it has a 15% greater heat capacity suggesting much reduced molecular motion.



Lysozyme molecule in a aqueous environment.

The whole cell water (70–80% of the total mass) is distributed into only two to three hydration layers around macromolecules.





Representation of the first layer of interfacial water at the surface of a protein.

Its highly heterogeneous structure reflects the heterogeneity of the macromolecule surface.



Solubility in water

Water is a polar solvent: dissolves charged or polar compounds by replacing solute-solute H-bonds with solute-water H-bonds.

Water structure is different around the solute molecules:



Compounds that dissolve easily in water are <mark>hydrophilic</mark>. *Non-polar* molecules such as lipids and side chains of some amino acids are *hydrophobic*

Amphipathic molecules have polar or charged regions, as well as non-polar regions.

Water interacts electrostatically with charged solutes



Entropy increases as crystalline substances dissolve

Small ions perturb water over only a short distance, e.g., ~5 Å.



The radial distribution functions $g_{10}(r)$ for Li⁺, Na⁺, water, and K⁺ in liquid water.

- These curves measure the distance from the monovalent cation to the nearest solvent oxygen.

- The curve labeled " H_2O " measures the oxygen–oxygen distance in liquid water.



Types of ions

- Structure-breaking ion 'chaotrope' (disorder-maker) (Na⁺)
- structure-forming ion '**kosmotrope**' (order-maker) (K⁺)



more dense water ↔ less dense water

- Kosmotropes shift the local equilibrium to the right.
- Chaotropes shift it to the left.

Ion	Surface charge density	Intra-cellular	Extra-cellular	Water preference	
<i>Ca</i> ²⁺	2.11	10 nM	2.5 mM	High density	
Na ⁺	1.00	10 mM	150 mM	High density	
K +	0.56	159 mM	4 mM	Low density	

Water in biological systems

- outer hydration shell (cybotactic region, semi-ordered)
- inner hydration shell (chemisorbed and ordered water)
- bulk water (random arrangement)

- Intracellular water very close to any membrane or organelle (sometimes called *vicinal water*).
- The density of bound water is 10% higher and it has a 15% greater heat capacity suggesting much reduced molecular motion.



Moisture Sorption Isotherm



 a_{w}

- Activity "effective concentration"
- Ion-ion and ion-H₂O interactions (hydration shell) cause number of ions available to react chemically ("free" ions) to be less than the number present
- Concentration can be related to activity using the activity coefficient γ , where $[a] = \gamma(c)$



- Concentration of ions
- Charge of the ion
- Diameter of the ion



Hydrophobic effect is crytical for biological systems

This is not an intermolecular force, but rather the effect due to the peculiar solvent – water.



Hydrophilic molecules

Polar groups



Polar molecule dissolved in water



4 Introduction of a hydrocarbon molecule creates a unfavourable cavities in water.

4 By clumping together in water hydrophobic molecules can reduce the total surface area of the cavity ($\Delta S > 0$).



Unaggregated state: Water population highly ordered Lower entropy; energetically unfavorable Aggregated state: Water population less ordered Higher entropy; energetically more favorable

There is no "hydrophobic force"!!!

Lipid supension

- ✤ Water concentration 55 M
- Lipid concentration $< 1 \ \mu M$

 $\frac{water \quad molecules}{lipid \quad molecules} \approx 5.5 \times 10^7$

□ The water molecules easily change orientations, or move to new neighboring locations.



System explores different conformations through diffusion and random thermal motion.

System will stay with the lowest energy conformation it encounters.







Getting old !!





Figure 1.9 Physical Biology of the Cell (© Garland Science 2009)



Diffusion Thermal fluctuations Low Reynold's number

The radius of a water molecule is about 0.1 nm.



Protein radius is in the range 2 - 10 nm.

Fluid can be considered as a continuum

Transport Phenomena

A system is not in equilibrium when the macroscopic parameters (T, P, etc.) are not constant throughout the system.

To approach equilibrium, these non-uniformities have to be dissipated through **the transport of energy, momentum, and mass**.

The mechanism of transport is molecular movement.

$$\frac{3}{2}kT = \frac{1}{2}M\langle v \rangle^{2}$$
$$\langle v \rangle = \left(\frac{3kT}{M}\right)^{\frac{1}{2}}$$

Molecular speed

For T = 300 K

500 Da (ATP) - v = 70 m/s

50 000 Da (protein) -v = 7 m/s

6.25 GDa (200 nm diameter vesicle) – $v = 600 \mu m/s$



Actual velocity ⇒ Maxwell's distribution

$$f(v) = 4\pi \left(\frac{M}{2\pi RT}\right)^{3/2} v^2 e^{-\frac{Mv^2}{2RT}}$$



Macroscopic theory of diffusion:

Assumptions:

- 1. conservation of mater
- 2. the relation between gradient and flux is linear



In thermodynamic terms, we're watching the increase in entropy within a small, isolated system without an input of energy.

The random walk of a large number of particles results with deterministic flow of particles.

In three dimensions:

$$= -D\nabla C$$
 and $\frac{\partial}{\partial t}$

$$\frac{\partial C}{\partial t} = D\nabla^2 C$$



$$\frac{dc(x,y,z)}{dt}\bigg|_{x,y,z} = D\left(\frac{d^2C}{dx^2} + \frac{d^2C}{dy^2} + \frac{d^2C}{dz^2}\right)\bigg|_t$$

The solution which corresponds to an initial condition that all particles are at x = 0 at t = 0:

$$n(x,t) = \frac{k}{\sqrt{Dt}} \exp\left(-\frac{x^2}{4Dt}\right)$$

The *rms* displacement of particles:

$$\sqrt{\left\langle x^2\right\rangle} \approx \sqrt{Dt}$$

k is a normalization factor

$$\langle x \rangle = 0$$

Diffusive transport in biology

 $J_{r} = -D$

A concentration penalty
No directional specificity
The time penalty - <X²> = 4Dt

D ~ 10⁻⁵ for most small molecules in water





Distance		Ave. Diffusion Time	Significance	
	100 angstroms	0.0000001 sec	Cell membrane thickness	
	1 micron	0.001 sec	Size of most bacteria or mitochondria	
	10 microns	0.1 sec	Diameter of small eukaryotic cells	
	100 microns	10 sec	Diameter of large eukaryotic cells	
	250 microns	1 min	Radius of giant squid axon	
	2 millimeters	1 hr	Thickness of frog sartorius muscle, half thickness of lens of eye	
	5 millimeters	7 hr	Radius of mature ovarian follicle	
	2 centimeters	5 days	Thickness of ventricular myocardium	
10 centimeters		120 days	Diameter of sea urchins & other small animals	
	1 meter	32 yrs	Half height of human	

The Langevin approach – dissipative force

Averageing over a large number of particles

Forces acting on a particle due to the solvent:

- (i) Stochastic thermal (Langevin) force:
 - **4** changes direction and magnitude

4 averages to zero over time

$$\langle \boldsymbol{\xi}(t) \rangle = 0$$

(ii) a viscous drag force that always slows the motions.

$$f = -\xi v$$

$$f = -\xi v$$

$$f = -\xi v$$

$$f = 6\pi \eta R$$

$$f \approx 4.5nN$$

$$F_g \approx 10^{-14} nN \ll f$$

Newton's law for the protein motion in a one-dimensional domain of length L, x(t):

$$\frac{dx}{dt} = v, \quad m\frac{dv}{dt} = -\xi v + f_B(t) \quad 0 \le x(t) \le L$$
$$\frac{m}{2}\frac{d^2(x^2)}{dt^2} - mv^2 = -\frac{\xi}{2}\frac{d(x^2)}{dt} + xf_B(t)$$

The average over a large number of proteins

$$\frac{m}{2}\frac{d^2\langle x^2\rangle}{dt^2} - \langle mv^2\rangle = -\frac{\xi}{2}\frac{d\langle x^2\rangle}{dt} + \langle xf_B(t)\rangle$$

Integrating twice between t = (0, t) with x(0) = 0:

$$\frac{d\langle x^2 \rangle}{dt} = \frac{2k_BT}{\xi} (1 - e^{-t/\tau}), \quad \langle x^2 \rangle = \frac{2k_BT}{\xi} \left[t - \tau (1 - e^{-t/\tau}) \right]$$

where $\tau = m/\zeta$.

$$\left\langle x^{2} \right\rangle = \frac{2k_{B}T}{\xi} \left[t - \tau (1 - e^{-t/\tau}) \right]$$
$$\left\langle x^{2} \right\rangle = \frac{k_{B}T}{m} t^{2} \quad (t \ll \tau)$$

The protein behaves as a ballistic particle moving with a velocity $v = (k_B T/m)^{1/2}$. For a protein with $m = 10^{-21}$ kg, v = 2 m/s.

For $\mathbf{t} \ll \boldsymbol{\tau}$, the exponential

can be expand to second

order:

In a fluid the protein moves at this velocity only for a time $\tau \sim m/\zeta = 10^{-13}$ sec – shorter than any motion of interest in a molecular motor.

During this time the protein travels a distance $\mathbf{v} \cdot \boldsymbol{\tau} \sim 0.01$ nm before it collides with another molecule.

$$\langle x^2 \rangle = \frac{2k_B T}{\zeta} \left[t - \tau (1 - e^{-t/\tau}) \right]$$

When $t >> \tau$, the exponential term disappears and:

$$\langle x^2 \rangle = \frac{2k_BT}{\zeta}t \quad (t >> \tau)$$

Because $\langle x^2 \rangle = 2Dt$ (Einstein relation – 1905):

$$D = \frac{k_B T}{\zeta}$$

Friction is quantitatively related to diffusion

For protein typically $D \sim 10^{-11} \text{ m}^2/\text{sec.}$



Brownian motion

Brown (1827): observed irregular movement of pollens in water under microscope.

Major contribution of Brown: made sure non-organic particles also have Brownian motion, confirmed that Brownian motion is not a manifestation of life.

 $\langle x(t) \cdot f_B(t) \rangle = 0$

The random impulses from the water molecules are uncorrelated with position.



Einstein, Brownian motion, and atomic hypothesis



Albert Einstein, 1905

Albert Einstein published 4 papers in the *Annalen der Physik* in 1905.

- Photoelectric effect
- Brownian motion
- Special theory of relativity

- Drag force: $f = \gamma v$
- Diffusion due to random walk: $d^2 = 6Dt$
- To reach equilibrium: $D\gamma = kT$
- Random collisions (random walk) are related to the dissipation of kinetic energy to solvent molecules.

External forces acting on macromolecules

$$\zeta \cdot \frac{dx}{dt} = F(x,t) + f_B(t)$$

The inertial term is neglected.

Forces acting on proteins can be characterized by a potential

$$F(x,t) = -\frac{\partial \varphi(x,t)}{\partial x}$$

$$\xi \frac{dx}{dt} = -\frac{\partial \phi(x,t)}{\partial t} + f_B(t)$$

Langevin equation



Figure 1.9 Physical Biology of the Cell (© Garland Science 2009)

Characteristics of Fluids

(1)Fluids have density (ρ), and thus moving fluids have momentum (requires a force to start or stop them).

(2)Fluids have viscosity

- (1) Viscosity changes with temperature and salinity
- (2) When fluids contact a solid, there is a thin layer that sticks very tightly to the solid surface. = "Noslip condition".

Momentum Transfer, Viscosity

Drag – transfer of the momentum in the direction perpendicular to velocity.



 $\frac{\Delta p_x}{\Delta t} \equiv F_x \propto \frac{A \cdot \left(v_{x, \text{top}} - v_{x, \text{bottom}}\right)}{\Delta z}$

Laminar flow between two surfaces moving with respect to each other.

F_x	$-n\frac{d}{d}$	u_x
\overline{A}	- // <u>/</u>	ΔZ

 F_x – the viscous drag force, η - the coefficient of viscosity F_x/A – shear stress

Shear viscosity η is the proportionality between the velocity gradient and the force required, per area, to keep the plates moving at constant velocity.

	η (kg/m•sec at 20° C)
Water	10-3
Olive oil	0.084
Glycerine	1.34
Glucose	1013

The Reynolds Number Dimensionless constant

 $\frac{inertial \ term}{friction \ term} = \frac{va\rho_m}{\eta}$

- a radius of a particle
- v particle velocity
- ρ_m medium density

When the Reynolds number 'R' is small the viscous forces dominate.



		Mass [g]	Diffusion time	Swimming speed [cm/ s]	Reynolds number
Bacterium	1 µm	10- 12	1 msec	10 ⁻³	10-5
Whale	10 m	<i>10</i> 9	10 ³ years	1000	<i>10</i> ⁸

Low Reynolds Number = Laminar Flow





Shark skin delays transition to turbulence

