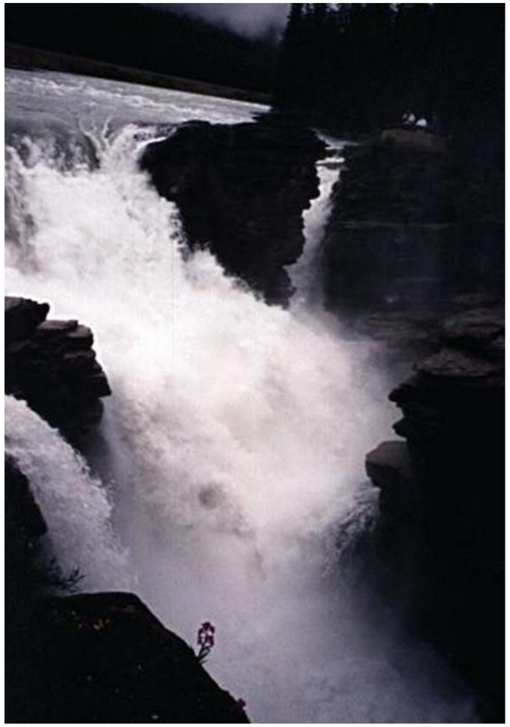


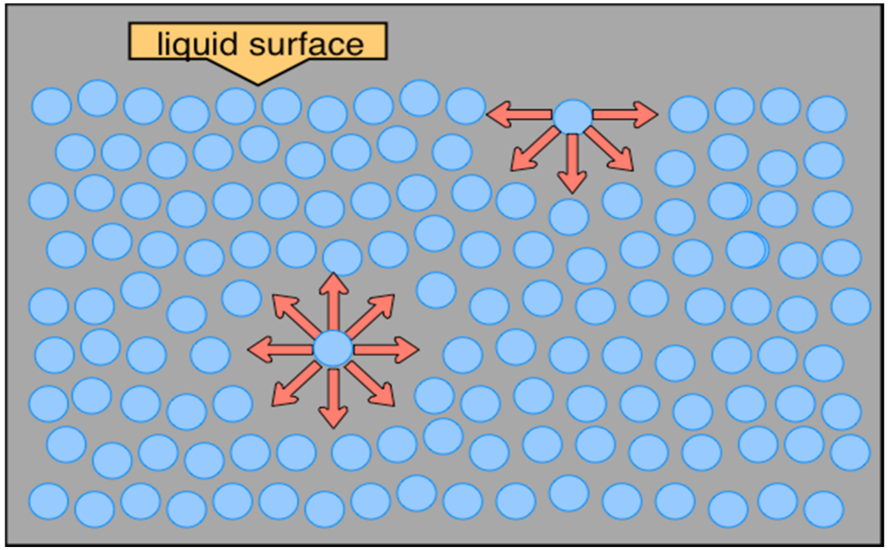
WATER AND HYDRATION

”We all owe our existence to the weirdness of water”

Henderson 1913

http://www.chem1.com/acad/sci/aboutwater.html
http://www1.lsbu.ac.uk/water/

Hydration/Solvation



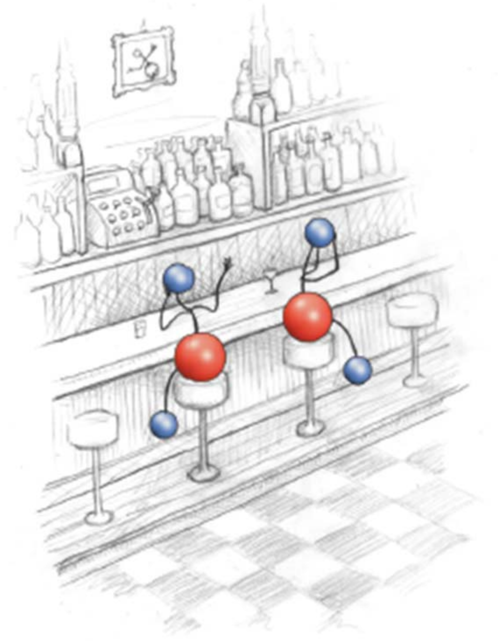
Molecules at the interface of a liquid has different properties that molecules in the bulk

Today’s plan

- **Pure water**
  - Hydrogen bonding
  - Water structure
  - Eccentricities of water and how they are modeled
  - Water pseudo-science
- **Water and biomolecules**
  - Hydration and activity
  - Hydration and dynamics
  - Hydration and stability
  - The hydrophobic effect
  - Preferential hydration

Water: the forgoten molecule of life?

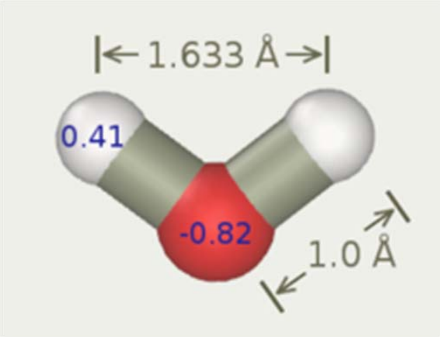
Compared to macromolecules like proteins and macromolecular assemblies like membranes the water molecule apprears simple and intuitavely unable to sustain complex life processes



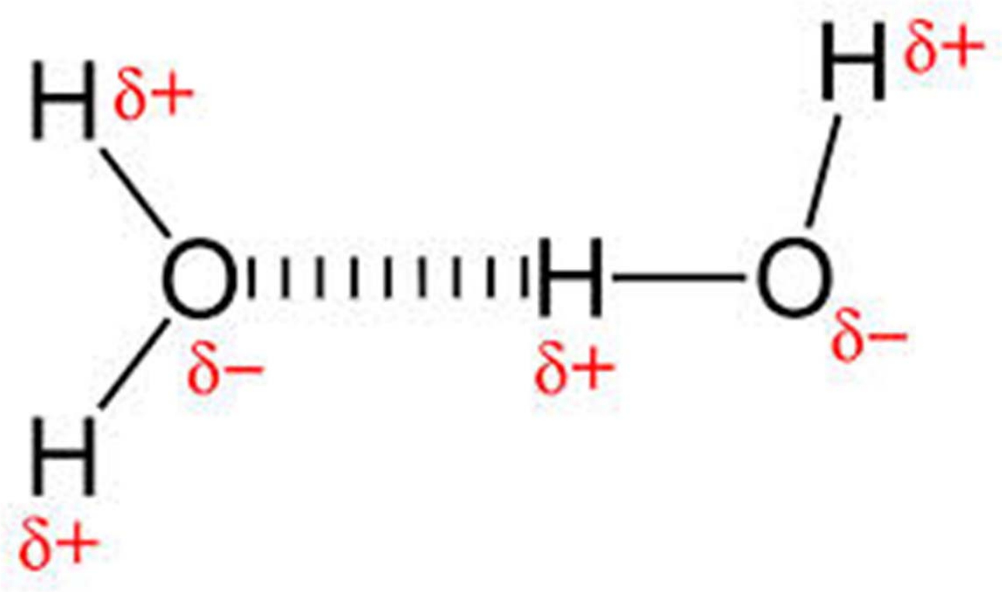
Gerald Pollack (2013) The fourth phase of water

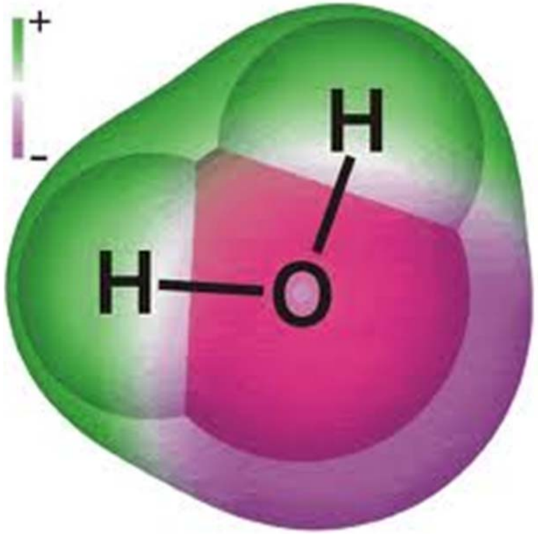
But water has a rich social life with strong, long-ranged interactions and distinct structure formation. This collectivity of water behavior may reperesent a level of ”macromolecular” sophistication which is crucial for biology .

The water molecule



The Name is Bond !
Hydrogen Bond





The hydrogen bond

Latimer & Rodenbush (1920)

A particularly strong attraction between molecules occurs when a hydrogen atom is located between two electronegative atoms (e.g. N, O, F eller Cl) and the arrangement is linear. Particularly when:

-The H atom is bound to another electronegative atom.

-When the three atoms (e.g. O$\cdots$H-O) are in line.

* *

Bond energy (ΔH) 20-30 kJ/mol

(10x Van der Waals interaction

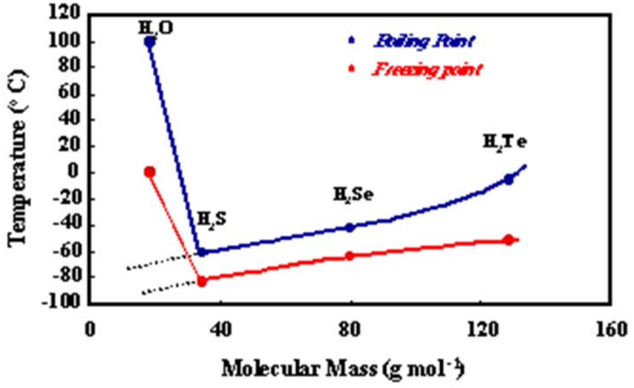
0.1 x covalent bond)

Directional

(H-bonds can be both ”stretched” and ”bent”)

Strength proportional to r $^{-3}$

(ion-ion r^{-1} Van der Waals r^{-6})



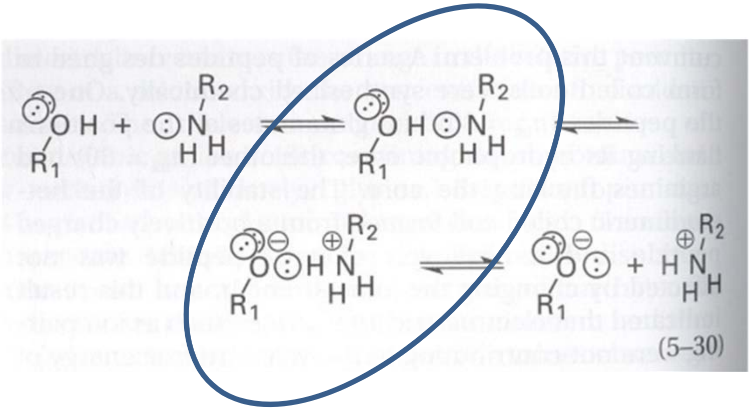
Boiling and Freezing Points of Group 16 Hydrides

Exercise

- Explain the interpretation:

”A hydrogen bond is an intermediate on the trajectory of an acid-base reaction”

Reaction of acid R_1-OH and base R_2NH_2

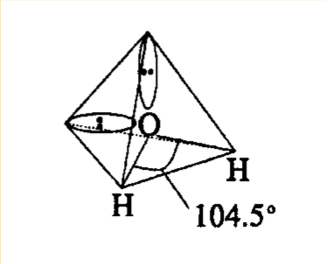


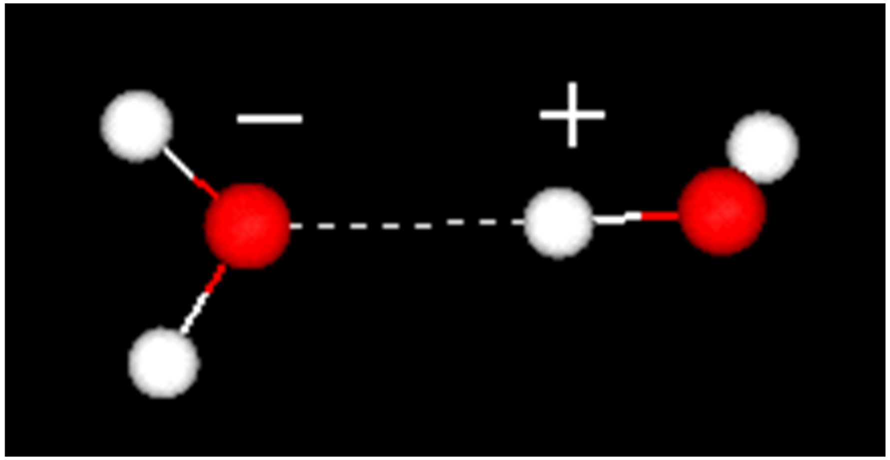
Hydrogen bond

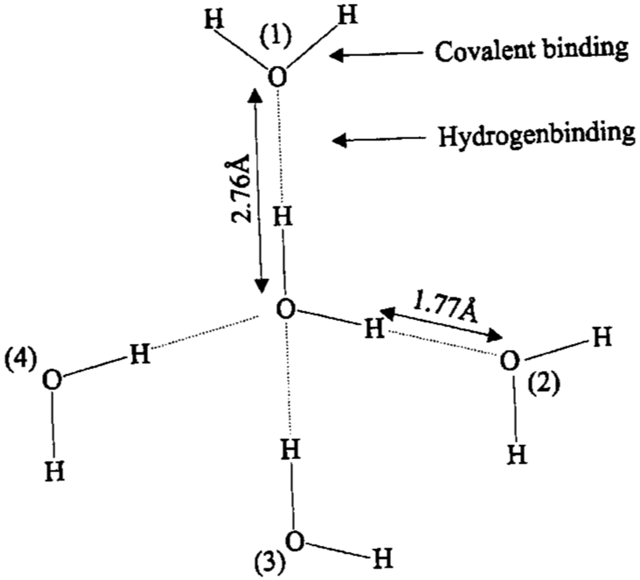
In water hydrogen bonding is an intermediate between hydroxide (OH^-) and hydronium (H_3O^+)

Water and hydrogen bonding

Tetrahedral angle 109.5^{o}



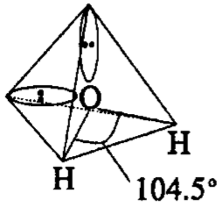




Because of its small bond energy and the small activation energy involved in its formation and rupture, the hydrogen bond is especially suited to play a part in reactions occurring at normal temperatures. It has been recognized that hydrogen bonds restrain protein molecules to their native configurations, and I believe that as the methods of structural chemistry are further applied to physiological problems it will be found that the significance of the hydrogen bond for physiology is greater than that of any other structural feature.

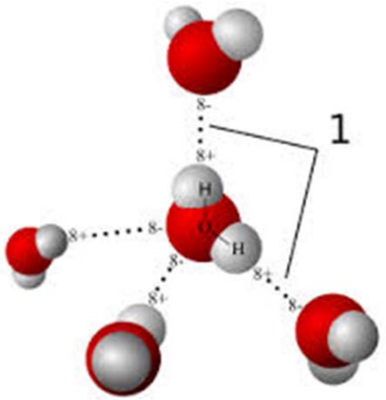
Pauling (1940) The Nature of the Chemical Bond.

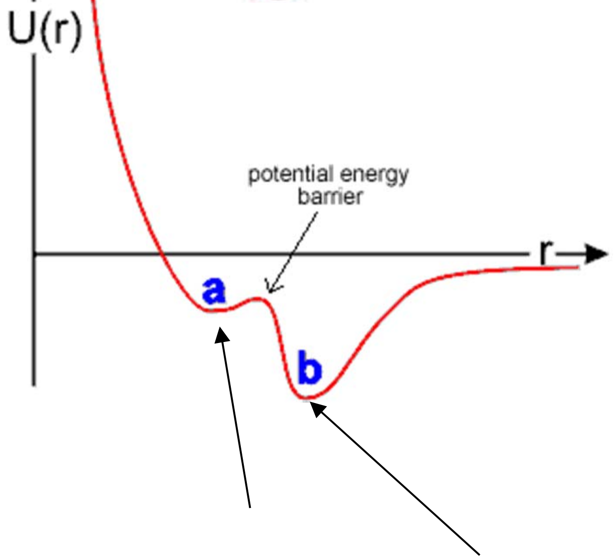
WATER STRUCTURE



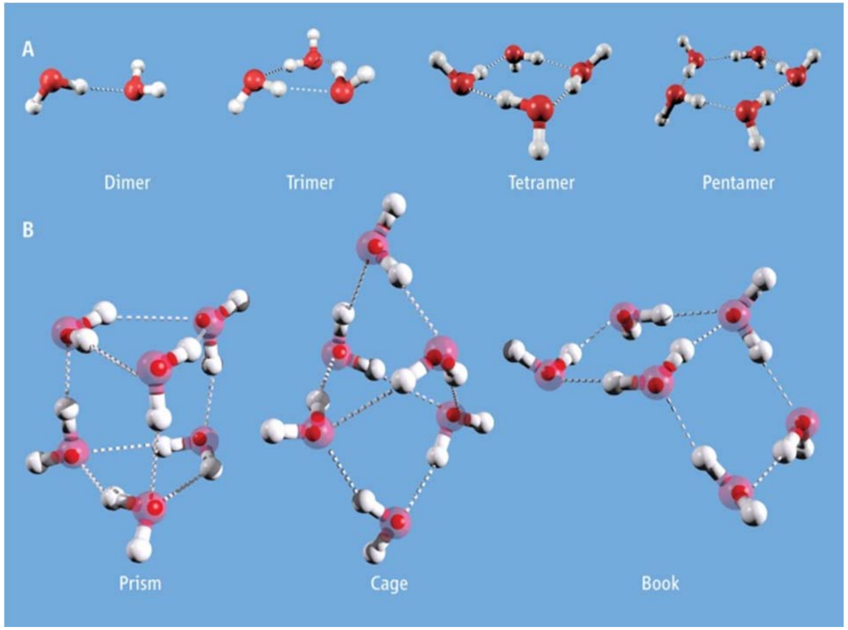
Water H-bond structure

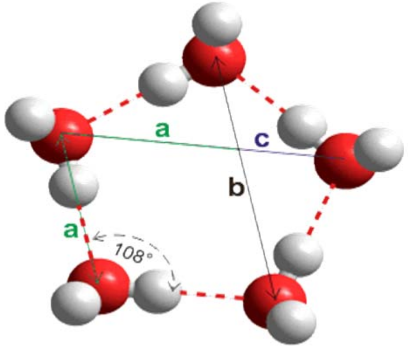
Tetraedic water pentamer

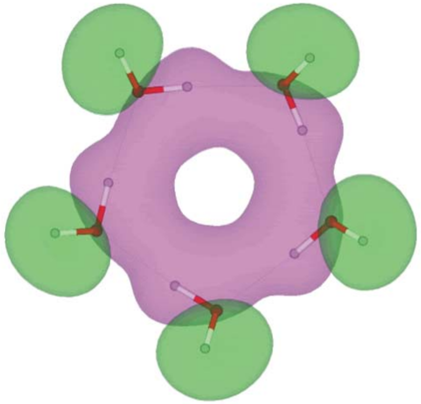




Dominates at HIGH T / LOW T

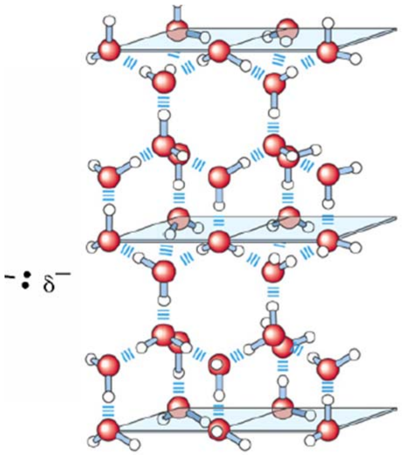




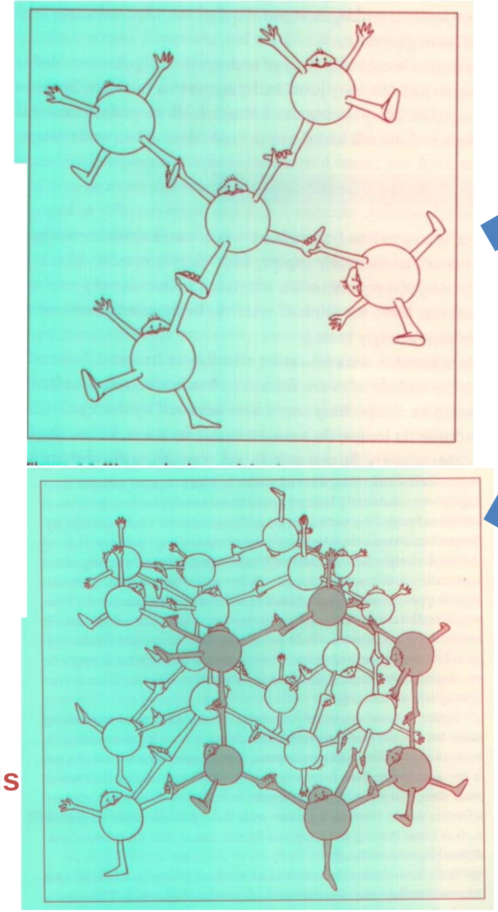


Networks of hydrogen bonded water molecules: Ice

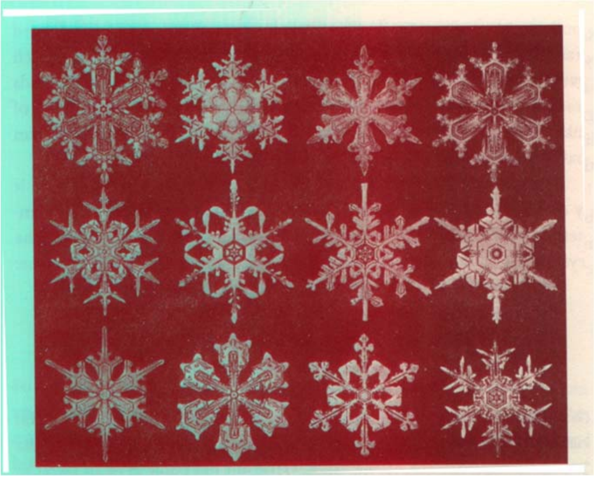
**Two Donors and two acceptors
”two hands and two feet”**



Two H-bonds per molecule or ~100 M H-bonds

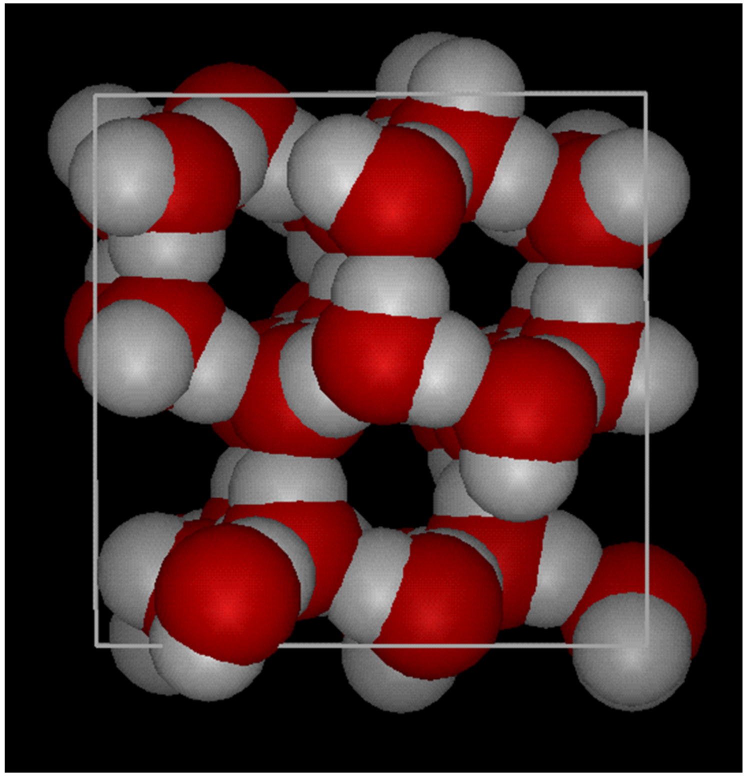


Life’s Matrix, P. Ball

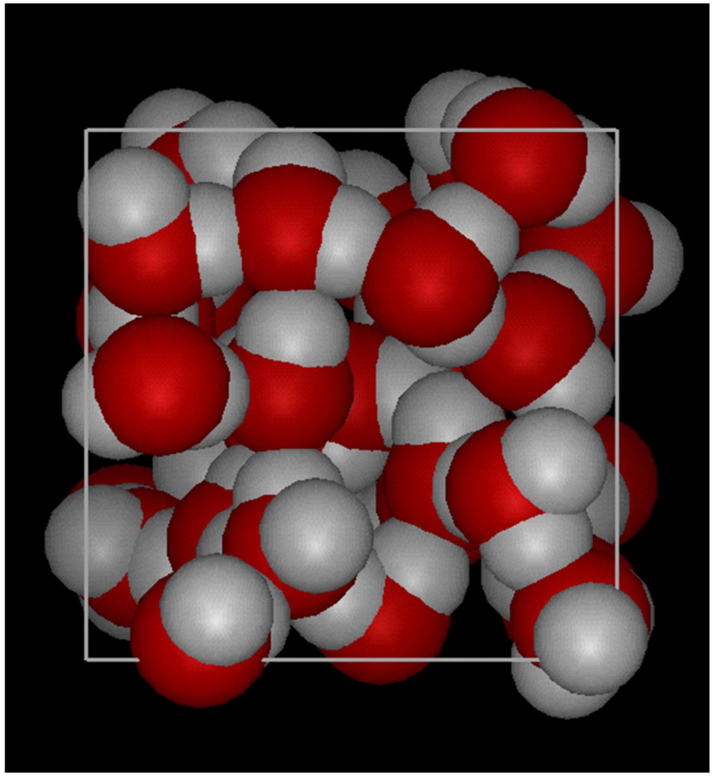


The structure of Ice and liquid water

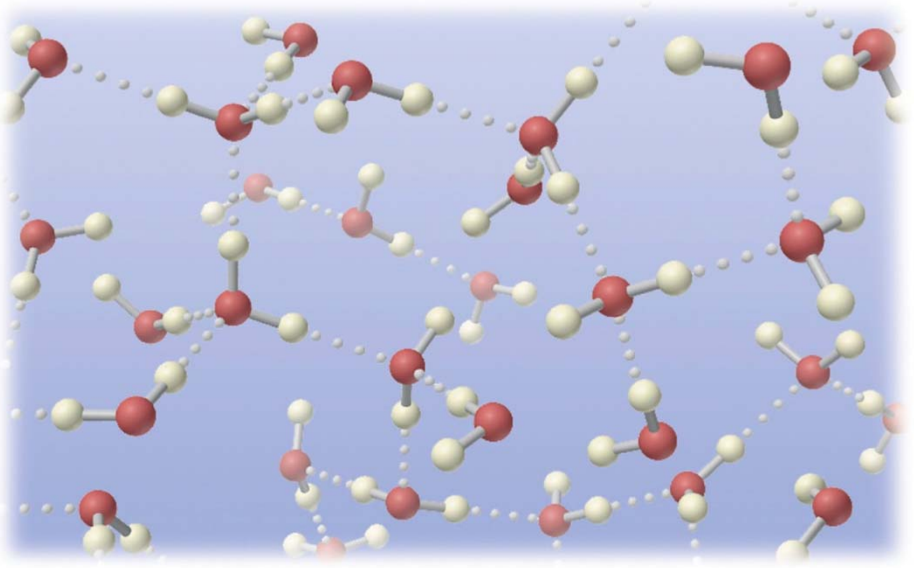
Ice



Water (liq.)



Water is a ”structured” liquid



- **Gas theories: Randomness**
- **Solid theories: long-range and long-time order**
- **Liquid theories: Both ??**

- **WHAT IS WATER STRUCTURE?**

”Extent” of H-bonding

ΔH_{evap} = 44 kJ/mol (=2.4 kJ/g; the highest known value)

ΔH_{melt} = 6 kJ/mol

Broken hydrogen bonds upon melting

α = 6/(44+6) 100% = 12%

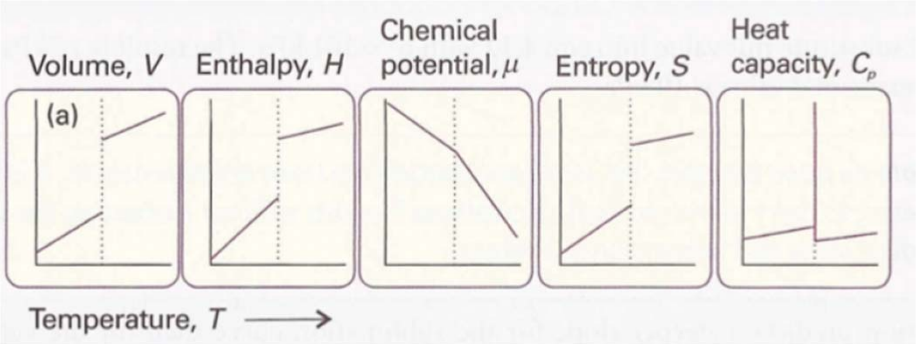
Liquid water has 88% intact H-bonds i.e. 1.8 per molecule.

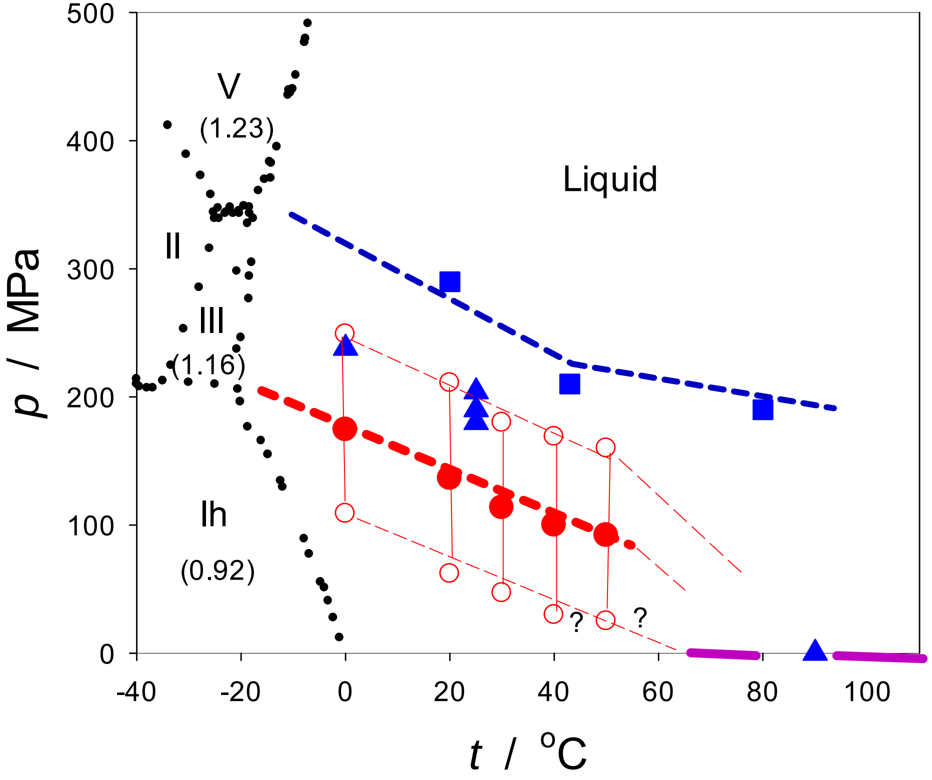
Some investigations even suggest >95% non-broken bonds!

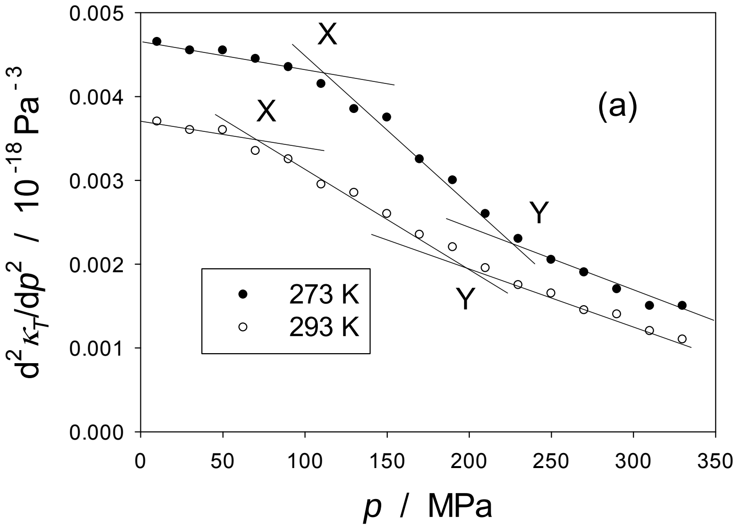
Cho et al 1997, Suresh et al 2000

”Ice-structure” in water

Phase transitions in liquid water







Koga et al, submitted

The molecular organization of liquid H_2O has some reminiscence of the solid that share the solid-liquid boundary

WATER MODELS

Water has highly unusual properties can we model them?

41 eccentricities: www.lsbu.ac.uk/water/

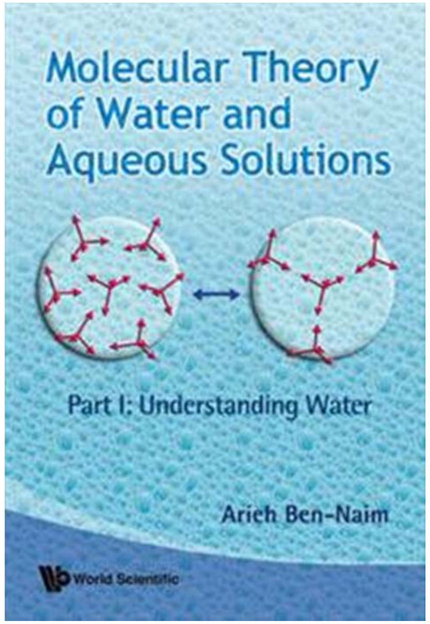
1. Water has unusually high melting point.
2. Water has unusually high boiling point.
3. Water has unusually high critical point.
4. Water has unusually high surface tension and can bounce.
5. Water has unusually high viscosity.
6. Water has unusually high heat of vaporization.
7. Water shrinks on melting.
8. Water has a high density that increases on heating (up to 3.984°C).
9. The number of nearest neighbors increases on melting.
10. The number of nearest neighbors increases with temperature.
11. Pressure reduces its melting point (13.35 MPa gives a melting point of -1°C)
12. Pressure reduces the temperature of maximum density.
13. D2O and T2O differ from H2O in their physical properties much more than might be expected from their increased mass; *e.g.* they have increasing temperatures of maximum density (11.185°C and 13.4°C respectively).
14. Water shows an unusually large viscosity increase but diffusion decrease as the temperature is lowered.
15. Water's viscosity decreases with pressure (at temperatures below 33°C).
16. Water has unusually low compressibility.
17. The compressibility drops as temperature increases down to a minimum at about 46.5°C. Below this temperature, water is easier to compress as the temperature is lowered.
18. Water has a low coefficient of expansion (thermal expansivity).
19. Water's thermal expansivity reduces increasingly (becoming negative) at low temperatures.
20. The speed of sound increases with temperature (up to a maximum at 73°C).
21. Water has over twice the specific heat capacity of ice or steam.

Water models

Equilibrium cluster models (mixture models)

RÖNTGEN CA. 1900

Water is considered a mixture of
- a low density, high interaction energy species
And
- a high density, low interaction energy species



Molecular Theory of Water and Aqueous Solutions

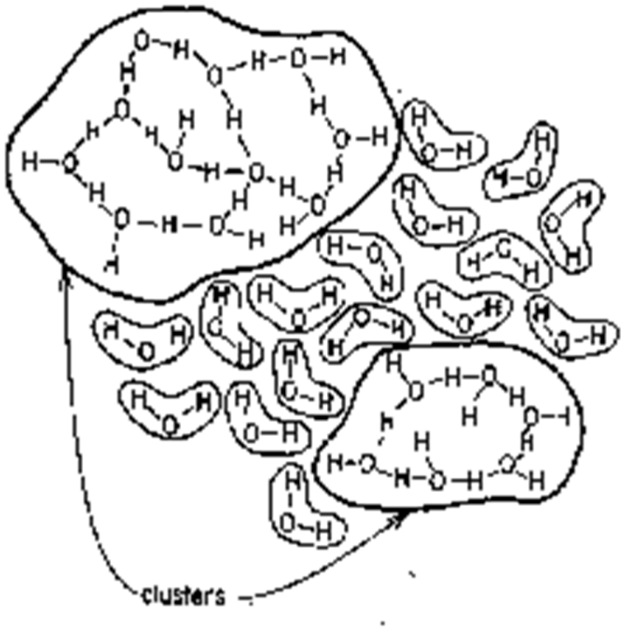
Part I: Understanding Water

Arieh Ben-Naim

World Scientific

The ”Flickering Cluster” model

Frank and Wen 1957



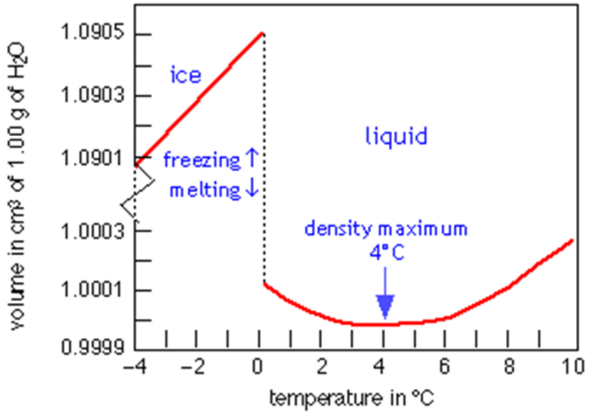
Water is a MIXTURE of a normal (disordered) liquid and clusters of hydrogen bonded molecules.

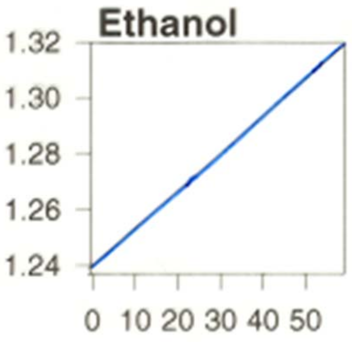
Many other (including earlier) ”MIXTURE MODELS” have been proposed

Basic macroscopic properties of cluster:

$$nH_2O \leftrightarrows (H_2O)_n$$

$$\Delta H^\circ<0, \Delta S^\circ<0, \Delta V^\circ>0$$





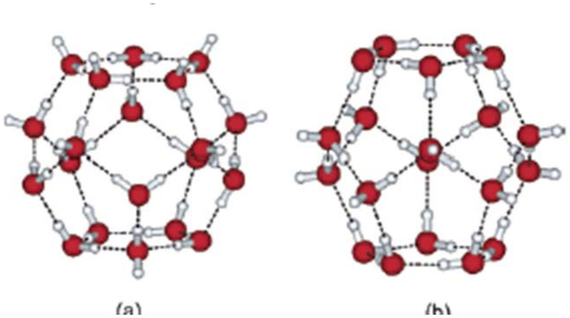
Formation of water structures has:

$$\Delta H \Delta V < 0$$

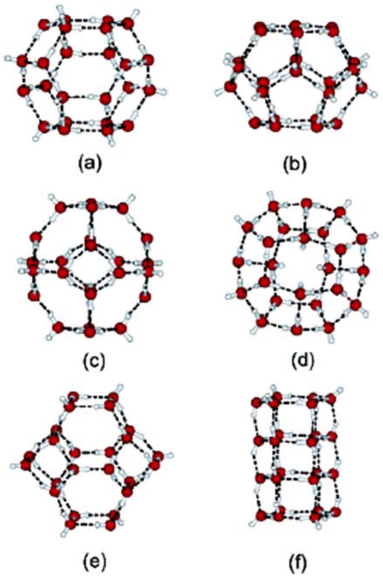
Modern Simulation approaches to water clustering

Water cluster anions

Herbert et al 2005



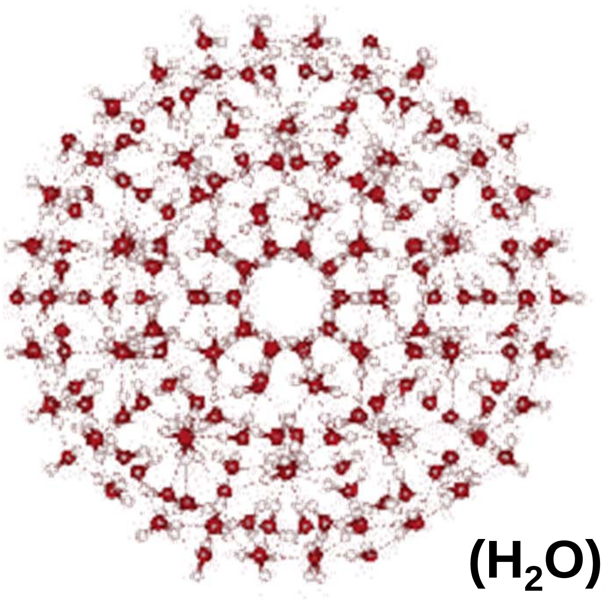
$(H_2O)_{20}^-$



$(H_2O)_{24}^-$

icosohedral clustering model

Martin Chaplin 2000



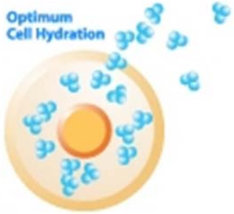
$(H_2O)_{280}$

Water cluster anions of 20-200 water molecules

Turi et al. Science 2005

Water Cluster Quackery !

Clusterwater$^{\text{TM}}$: Shelf-life ~ 5 psec ?



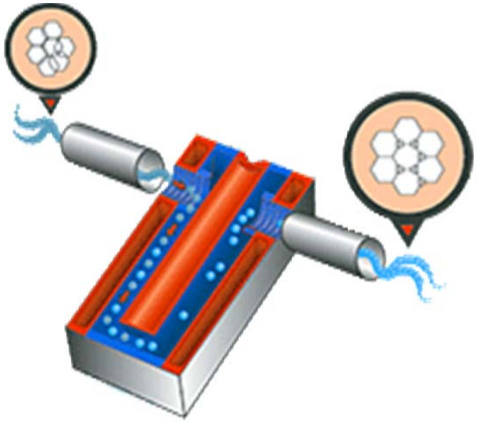
Preparation of clustered water:

U.S. patent 6,033,678

U.S. Patent 5,247,179

Grander Water

The mystery of the millennium or pseudoscience?



Revitalized water ”remembers” the changes and

Improves human health
Improves water’s washing power
Improves agricultural yields
Prolongs lifetime of water pipes
Etc etc....

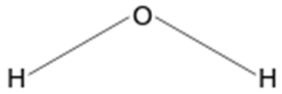
Can long-lived changes in water structure be envisioned
- or is water quite forgetful with a memory in the ps-range

http://www.chem1.com/CQ/

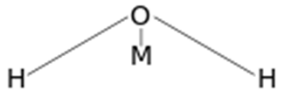
Back to modeling:

Other approaches to modeling water

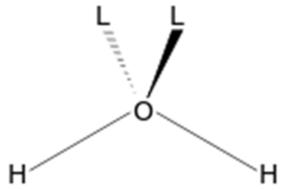
Parametrization for Molecular Dynamics simulation



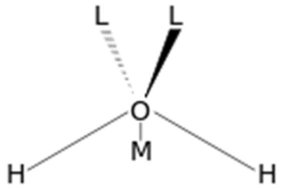
3-site



4-site



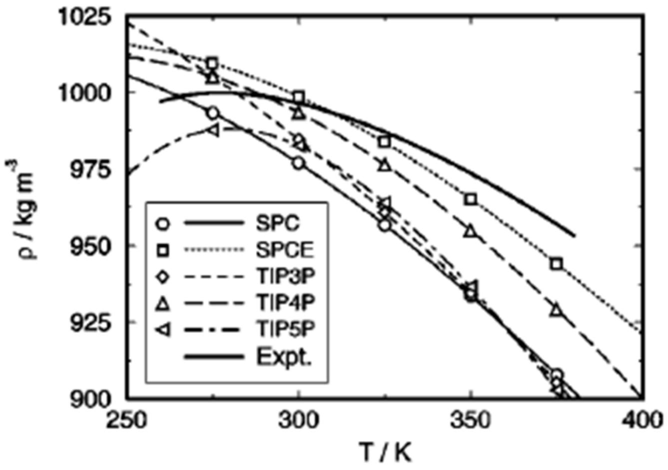
5-site



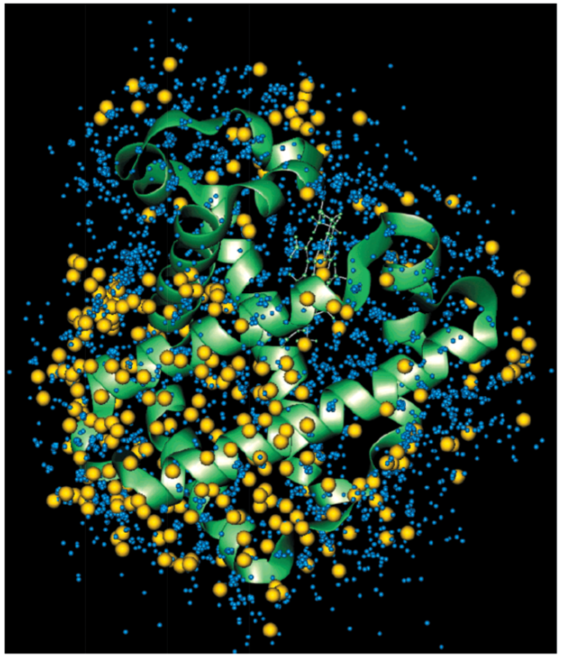
6-site

	BNS[17]	ST2[17]	TIP5P[18]	TIP5P-E[19]
r(OH), Å	1.0	1.0	0.9572	0.9572
HOH, deg	109.47	109.47	104.52	104.52
r(OL), Å	1.0	0.8	0.70	0.70
LOL, deg	109.47	109.47	109.47	109.47
$A \times 10^{-3}$, kcal $Å^{12}$/mol	77.4	238.7	544.5	554.3
B, kcal $Å^6$/mol	153.8	268.9	590.3	628.2
q(L)	−0.19562	−0.2357	−0.241	−0.241
q(H)	+0.19562	+0.2357	+0.241	+0.241
R_L, Å	2.0379	2.0160		
R_U, Å	3.1877	3.1287		

Reproducing experimental data



Calculating density with different force fields
Pascheck 2004



Hydration sphere structure of myoglobin elucidated by crystallography (blue) and MD simulation (yellow)
Makarov et al 2002

A lot of improvements have been gained, but subtleties of water properties still cannot be accounted for. Simulation can strongly guide the interpretation of experiments

”Toy models”

Mercedes-Benz Model

Performance vs. Exp. And MD simulation

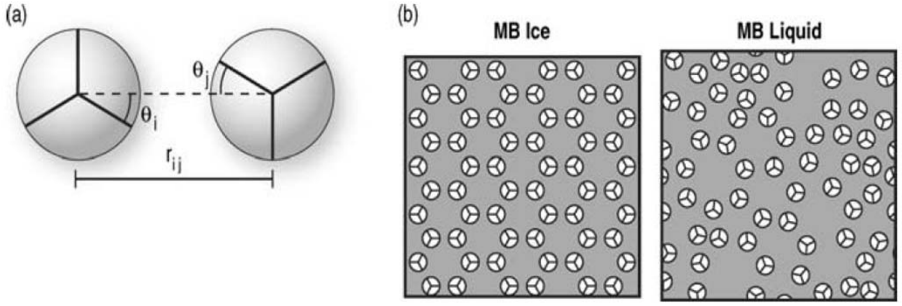
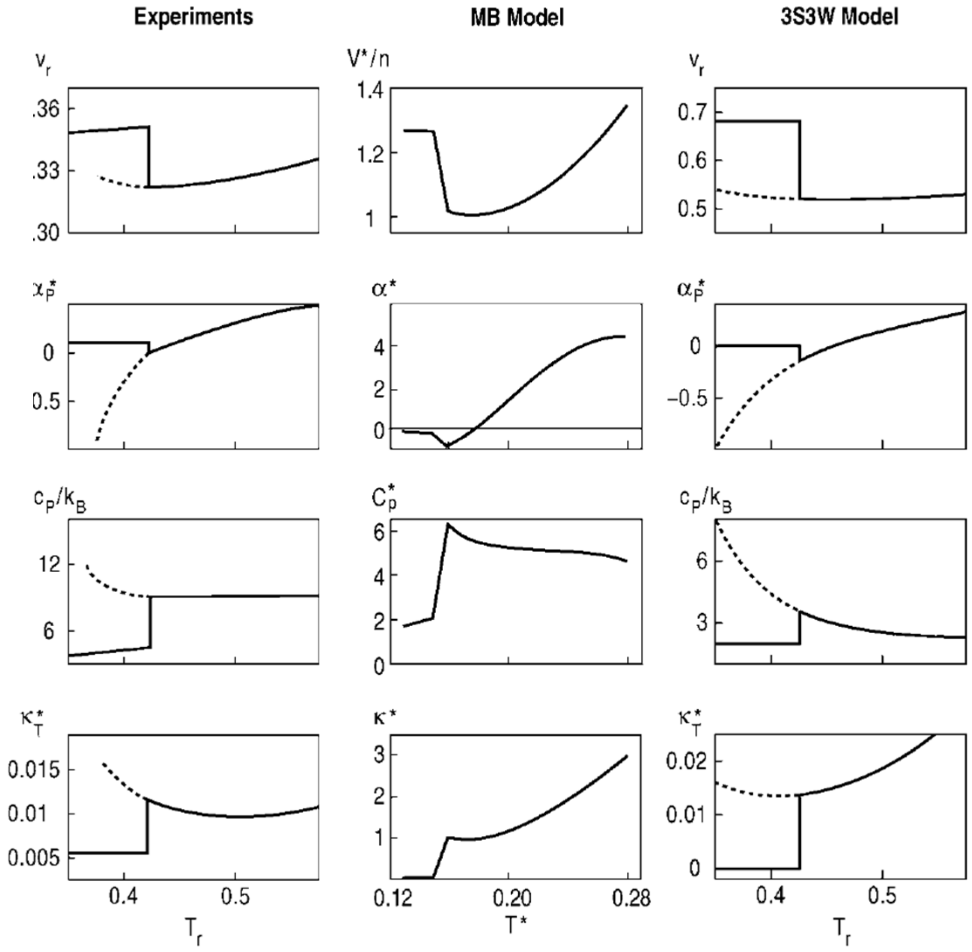


Figure 1 (*a*) MB water model. Each water is a LJ disk, with three hydrogen bonding arms. (*b*) (*Left*) MB ice, the stable state at low temperature. (*Right*) A typical liquid configuration at T* = 0.20, density $\rho^* = 0.9$ (72).

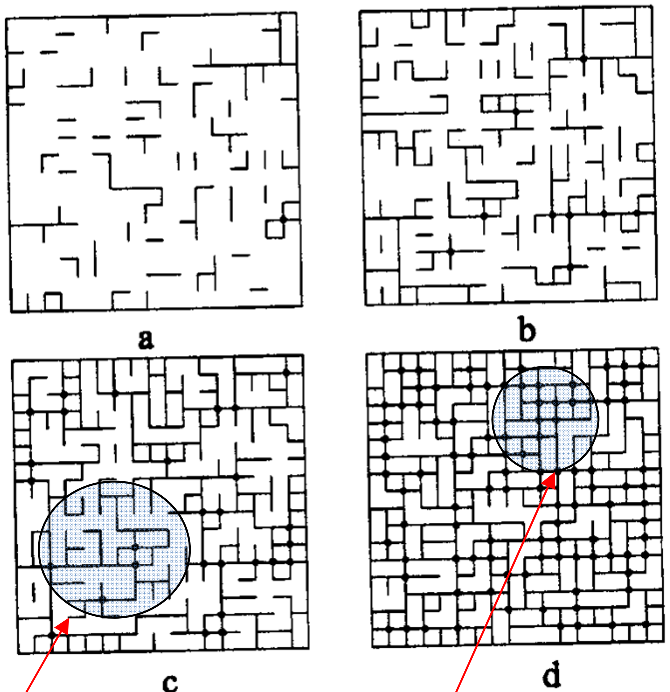
Dill et al 2005



Percolation model

and long-range (mediated) interactions

HOT WATER

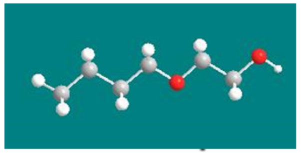


COLD WATER

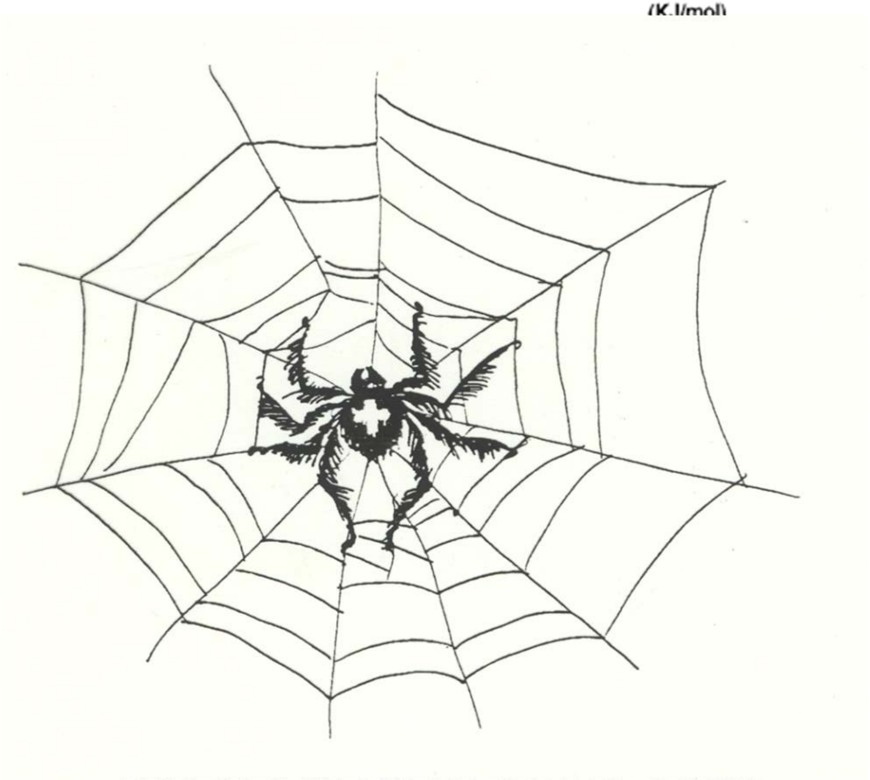
Bond percolation

Site percolation ”cluster”

Stanley & Teixeira 1980



(KJ/mol)



ethanol interact measurably even when hydrated by 10^5 -10^6 water molecules.

In summary

- Two key features underlie the unusual behavior of water
  - High density of hydrogen bonding (50 - 100 mol/l at room T)
  - Tetrahedral arrangement gives rise to the formation of clusters with icelike structure and properties.

- Clustering is perturbed by changes in T and P – and the presecence of solutes and surfaces.

- While modeling of water is still incomplete, both sophisticated MD based on QM-parameters and simple toy-models reproduce many central water properties

Water and surfaces

The hydrogen bonded network in water responds strongly to interfaces

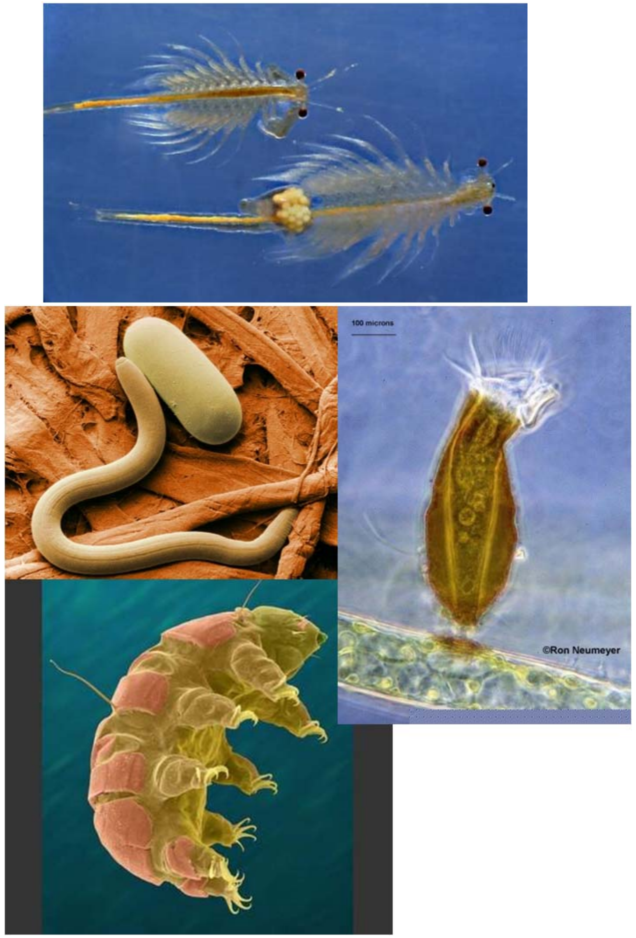
Hydration and metabolism

Hydration dependence of cellular metabolism in Artemia cysts

Hydration level **g H_2O / g dry weight**	**Metabolic events initiated**
0 – 0.1	None observed
0.1	Decrease in ATP level
0.1 – 0.3	No additional events observed
0.3 ± 0.05 (24 % H2O)	Metabolism involving several amino acids, Krebs-cycle intermediates, short chain aliphatic acids and nucleotides. Slow decrease in glycogen level
0.3 – 0.6	No additional events observed
0.6 ± 0.07 (38% H2O)	Respiration, carbohydrate synthesis, net increase in ATP, RNA and protein synthesis, embryonic development
0.6 – 1.4 (full hydration)	No additional metabolic events observed

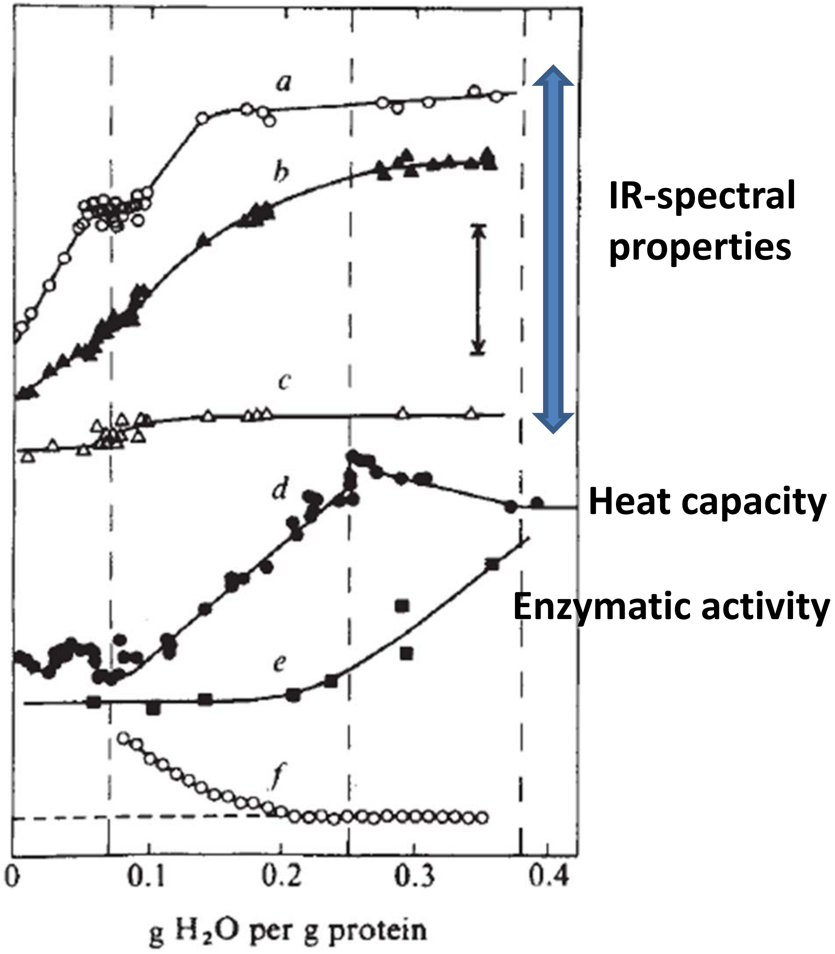
J.S. Clegg 1978

Anhydrobiotic animals (and plants) survive complete dehydration



Hydration and activity on a molecular level

Gradual hydration of lysozyme



IR-spectral properties

Heat capacity

Enzymatic activity

Hydration level g H_2O / g Lyso	Event
0-0.07	Interaction with ionizable groups (charge re-distribution). No structural change i enzyme
0.07-0.25	Hydration of hydrophilic groups. Water-water interactions. Formation of native 2nd structure
Ca. 0.25	Enzymayic activity commences. Strong increase in enzyme flexibility
0.38	Monolayer coverage (ca 300 water per enzyme)

Careri et al, 1980

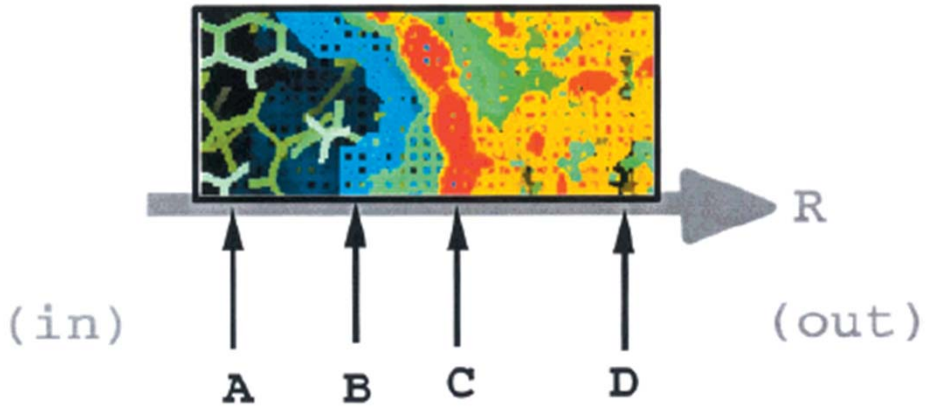
Water at the interface of a (fully hydrated) protein

Protein
(essentially water free)

Low water density
(interpenetration
Protein/water). Very high
residence times

High water density –
”Hydration site”
(2.5-4 Å from surface)
$D_{H2O} \approx 0.5 \times D_{H2O,bulk}$
Anisotropic. High
residence times. High H_2O
density (5-15% higher)

Remote hydration zone –
D_{H2O} slightly lower than
$D_{H2O,bulk.}$ Anisotropic.

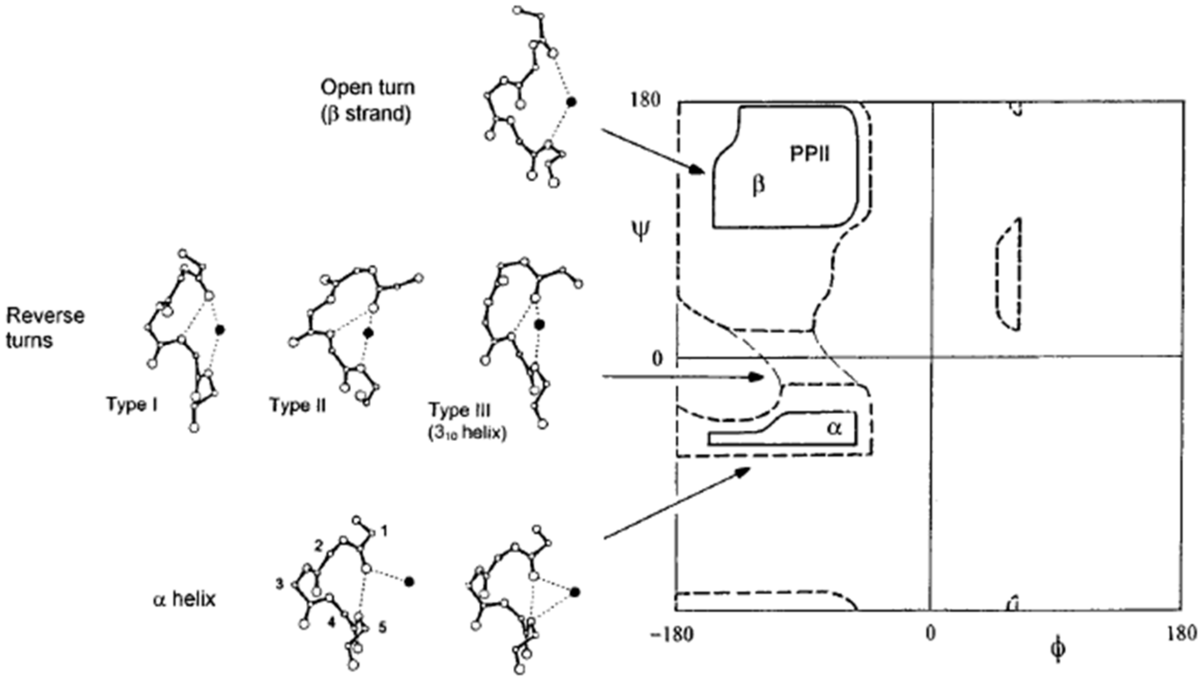


Makarov et al. 2002

Hydration and macromolecular dynamics

Lubrication by water

Raman spectroscopy of unfolded proteins and in disordered loop regions of molten globule-like states

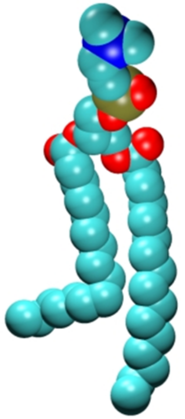


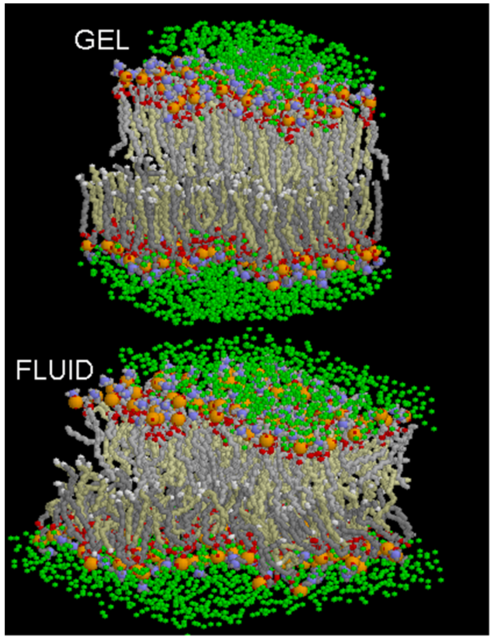
Water hydrogen bonding promotes low energy barriers for *e.g.* α→β "flickering" on ps time scales at room temperature.

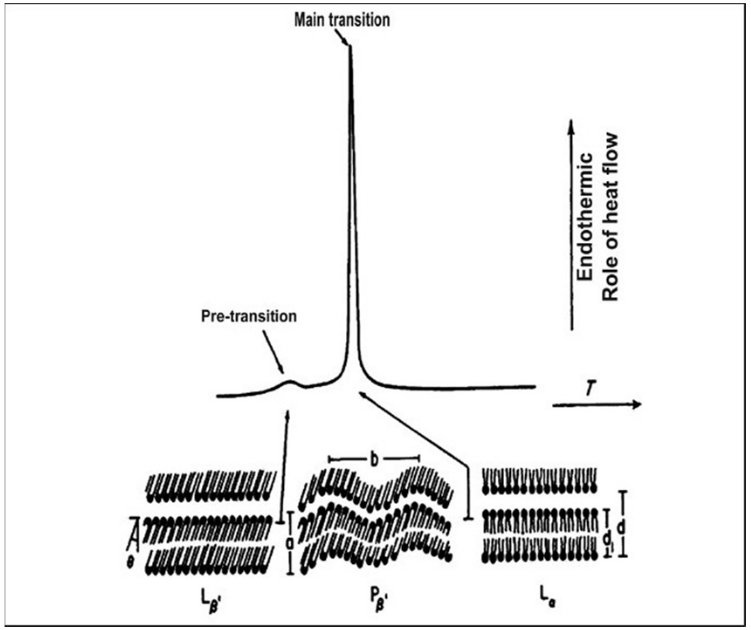
Same time scale as flickering clusters in pure water

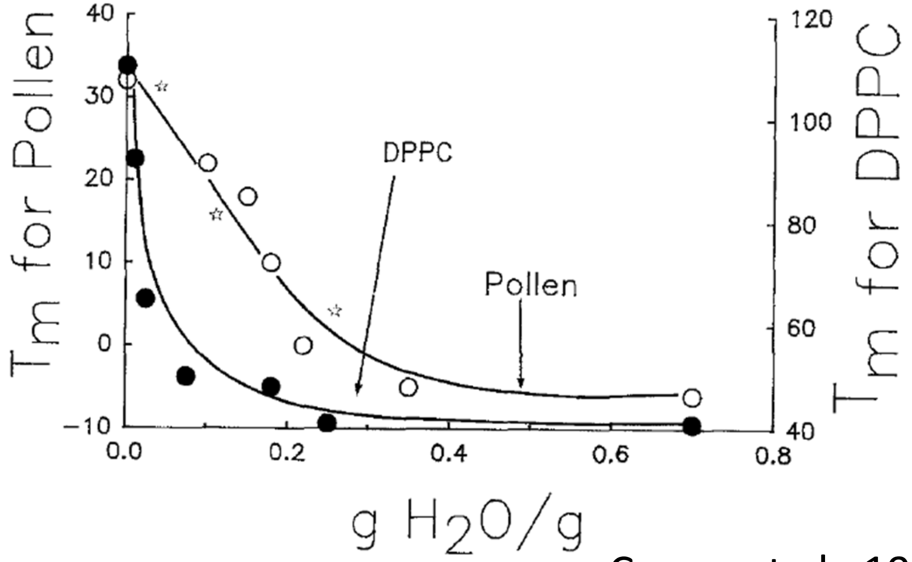
Barron et al. 1997

Membrane hydration (Lubrication)









Crowe et al. 1989

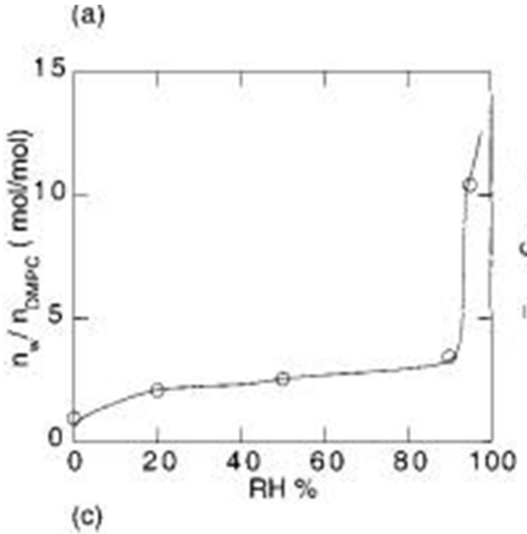
Water interchelates between DPPC molecules and hence

- **Increases lateral area**
- **Promotes molecular movement**

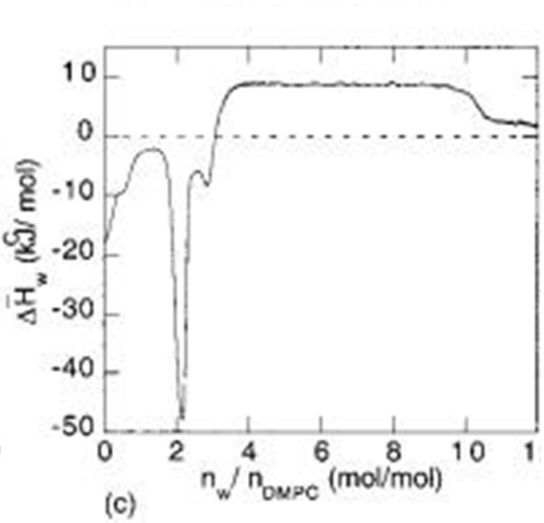
Entropically driven hydration lipid membranes

Hydration of DMPC (25^oC)

Water binding isotherm



Water binding enthalpy



Water lubrication

“Swelling of the lipids is due to increased thermal excitations in the lipid head group as water is added”

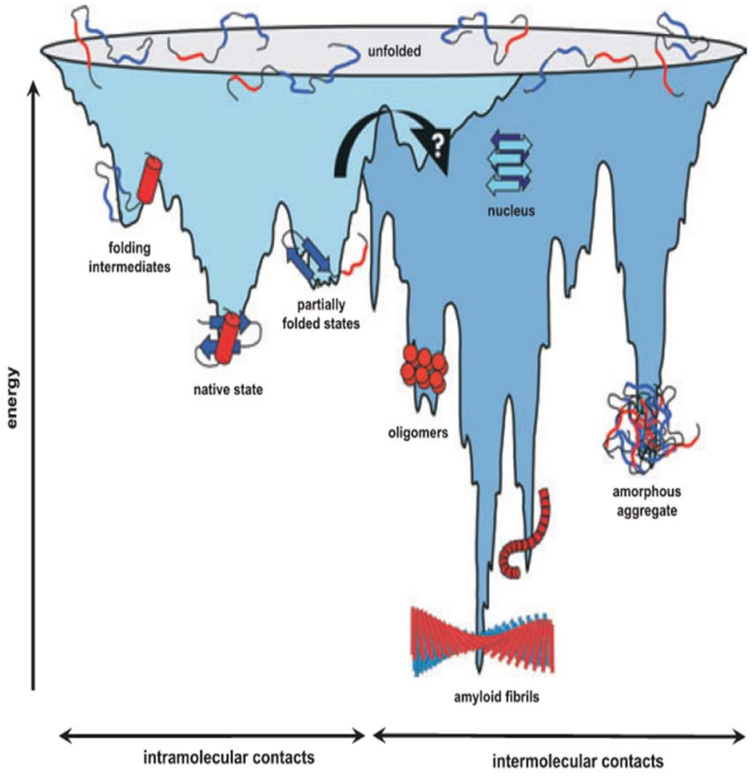
The ”binding” of water is driven by increased thermal fluctuations in the system

Sparr et al 2000

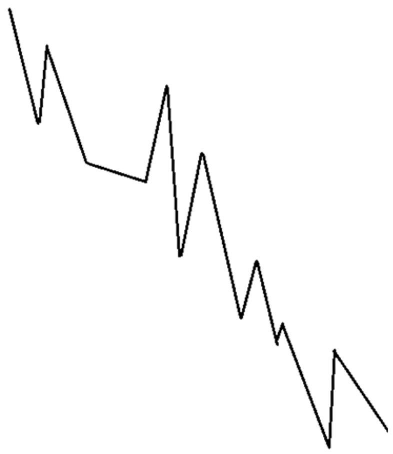
Why is a freeze dried protein conformation stable?

Folding dynamics:

Water smoothening of the energy landscape



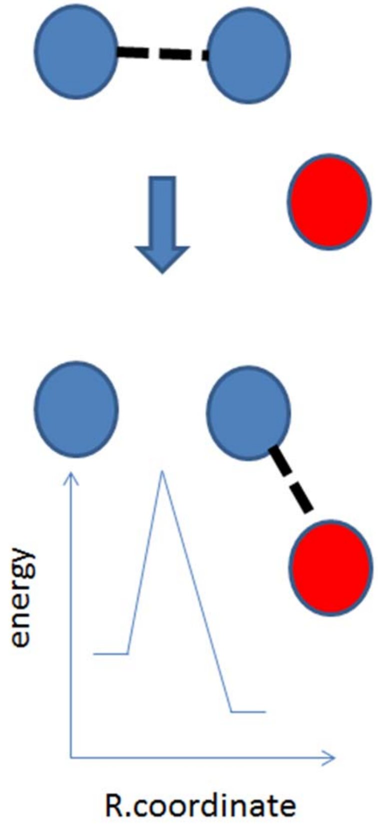
Jahn & Radford 2004

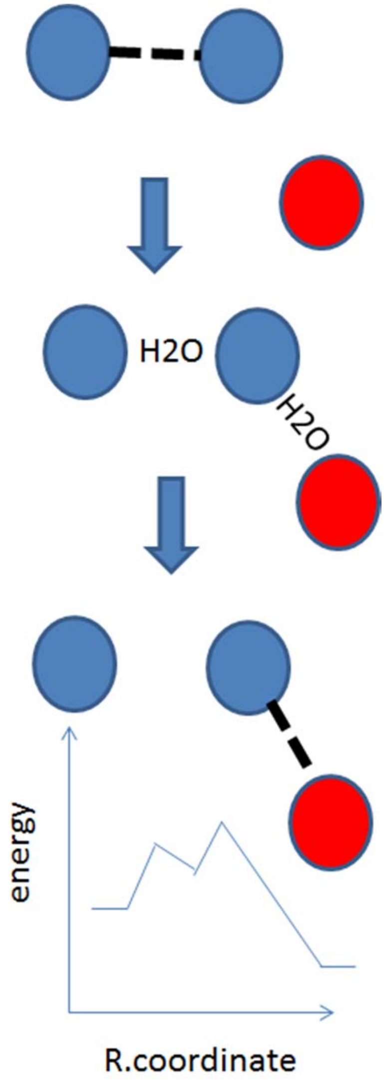


Energy landscape no H2O

with H2O

Water mediated folding
Polishing the rugged landscape



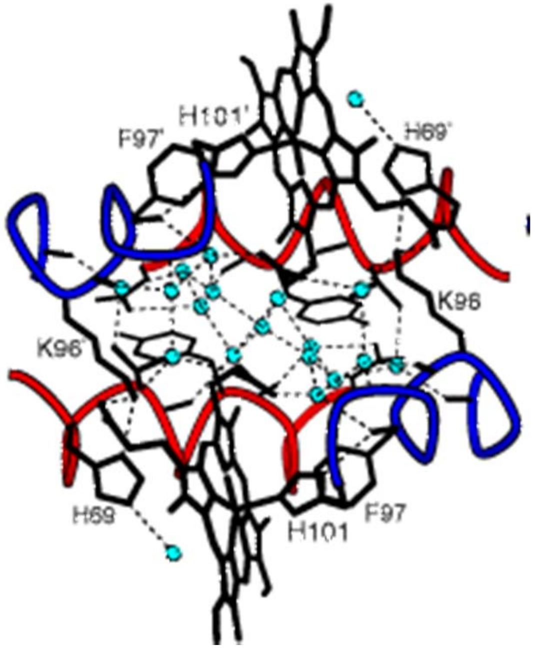


Water and protein stability

Water and protein-protein interactions

Many protein-protein interfaces are ”wet”. Statistically there is about 1 water molecule for every 100Å^2 (9%)
(Rodier et al 2005)

Water is most often hydrogen bound at the interface, but ”entropically driven capture” has also been suggested for water at hydrophobic interfaces.
(Petrone & Garcia 2004)



Royer *et al.*, 1996

The water cluster between two Hemoglobin molecules modulates stability and enables communication between monomers.

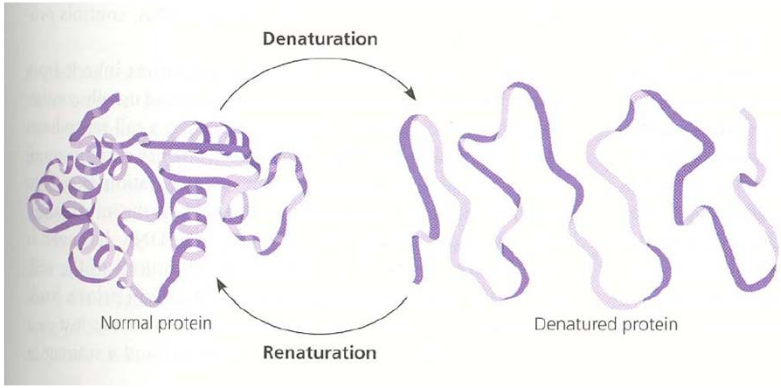
Ligation of O_2 (or CO) disrupts the water cluster

Stabilizing forces – book keeping

For a standard 100 amino acid globular protein:

$$\mathbf{N \leftrightarrows D} \qquad K=[D]/[N]$$

$$(\Delta G^\circ=-RT\ln K)$$

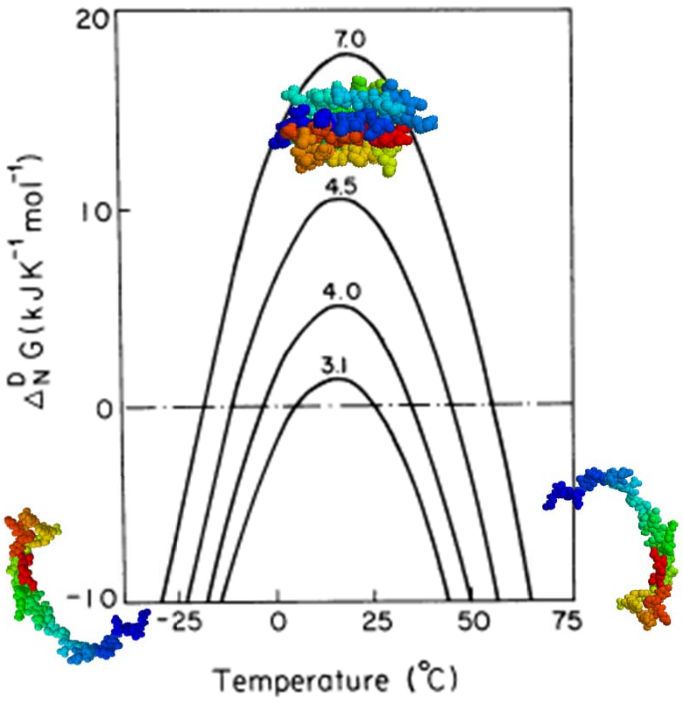


Conformational entropy	- 1300 to – 4500 kJ/mol
Steric hindrance (native)	-900 kJ/mol
Hydrophobic interactions	+ 1100 kJ/mol
Change in dispersion forces	+ 950 kJ/mol
Hydrogen bonding	+200 to 3000 kJ/mol
Net stability (ΔG°)	+20 to 40 kJ/mol

Creighton 1984

Water and protein stability

Recall: $$\frac{d\Delta G}{dT} = -\Delta S$$



Proteins denature upon both heating and cooling

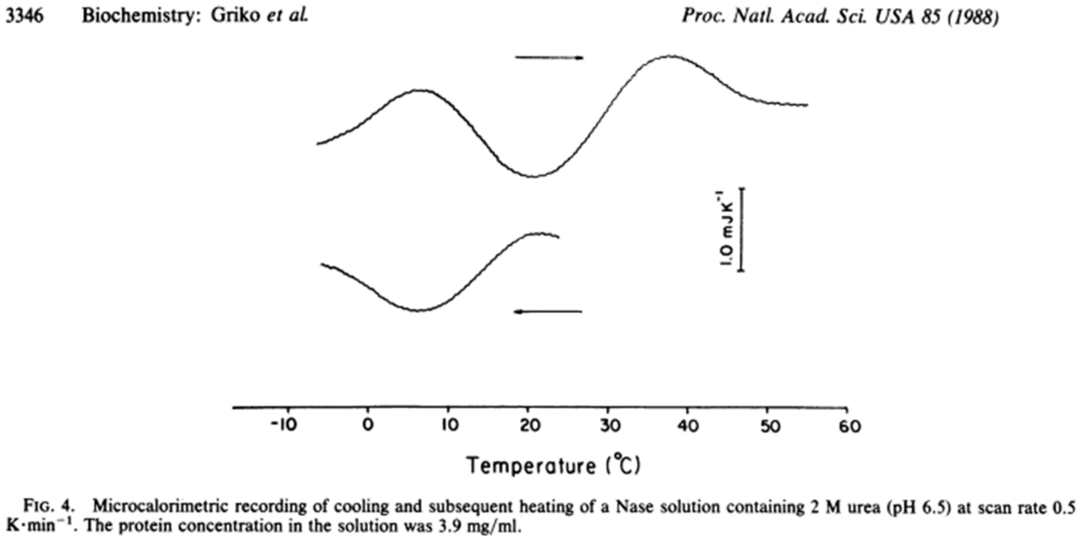

3346 Biochemistry: Griko *et al.* *Proc. Natl. Acad. Sci. USA 85 (1988)*

FIG. 4. Microcalorimetric recording of cooling and subsequent heating of a Nase solution containing 2 M urea (pH 6.5) at scan rate 0.5 K·min^{-1}. The protein concentration in the solution was 3.9 mg/ml.

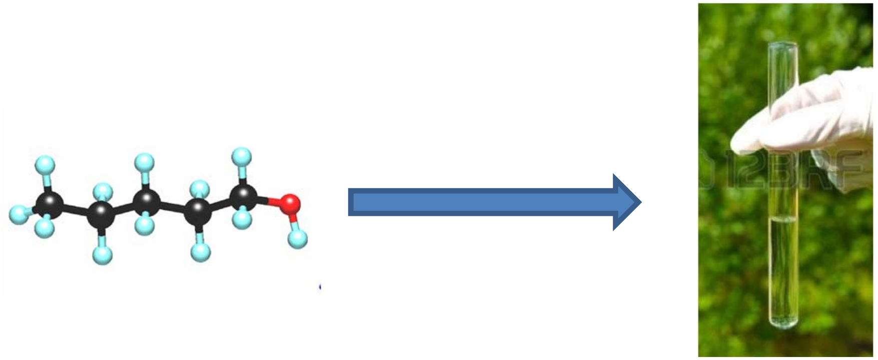
Water ”dissolves” the protein conformation at low temparature while thermal motion rips it apart at high temperature

The former reflects **HYDRPHOBIC HYDRATION**

Oil and water do not mix !

The Hydrophobic Effect: ”The entropic bond”

Kauzmann 1959



$$PenOH(l) \rightarrow PenOH(aq)$$

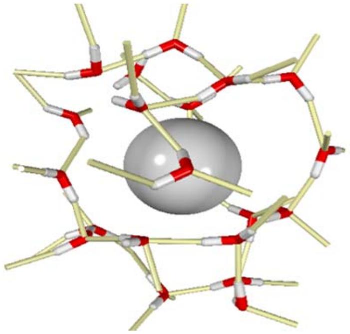
1-pentanol in water	
ΔG° (kJ/mol)	13.5
ΔH° (kJ/mol)	-7.8
$T\Delta S^\circ$ (kJ/mol)	-21.3
ΔC_p°(kJ/mol/K)	0.35

$$\Delta G^o = \Delta H^o - T\Delta S^o$$

Hydrophobic solutes INCREASE the number of H-bonds in water.

The entropic penalty drives the hydrophobic effect

”Icebergs” and ”Flickering Clusters”

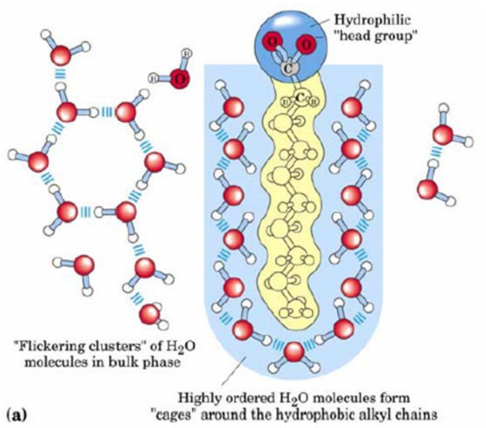


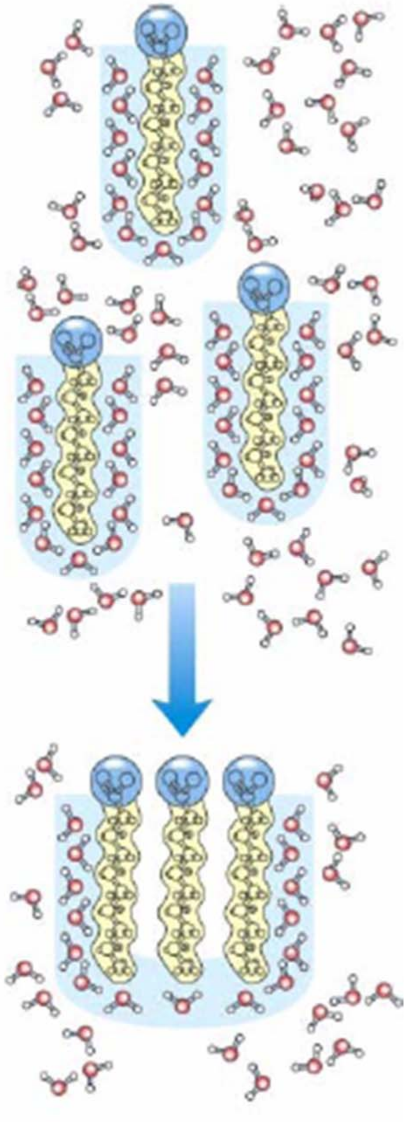
For both types of water structures:

$\Delta H° < 0, \Delta S° < 0, \Delta V° > 0$

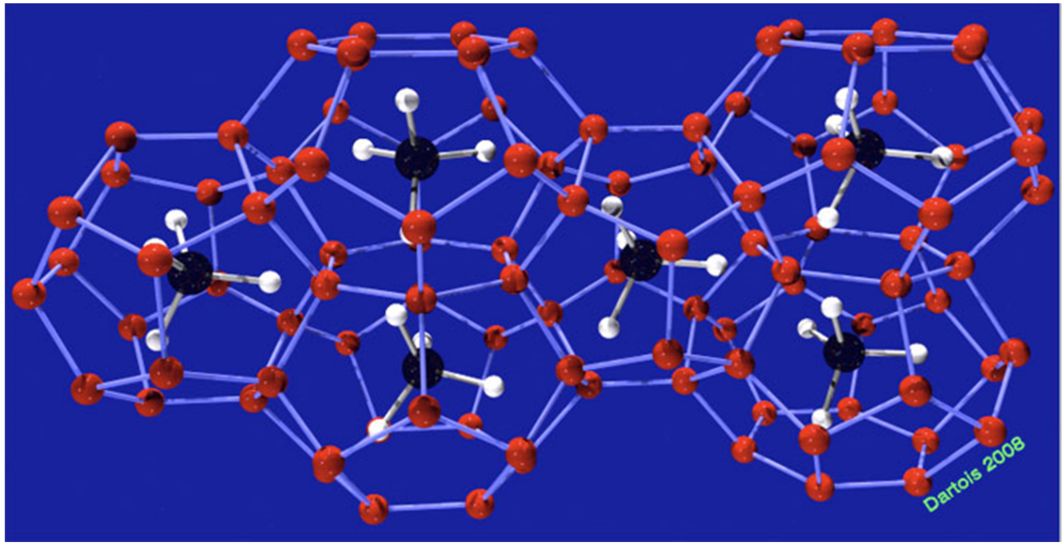
Energy-entropy compensation.

Distinctively different conformations have nearly equal probability – strong density flucuations

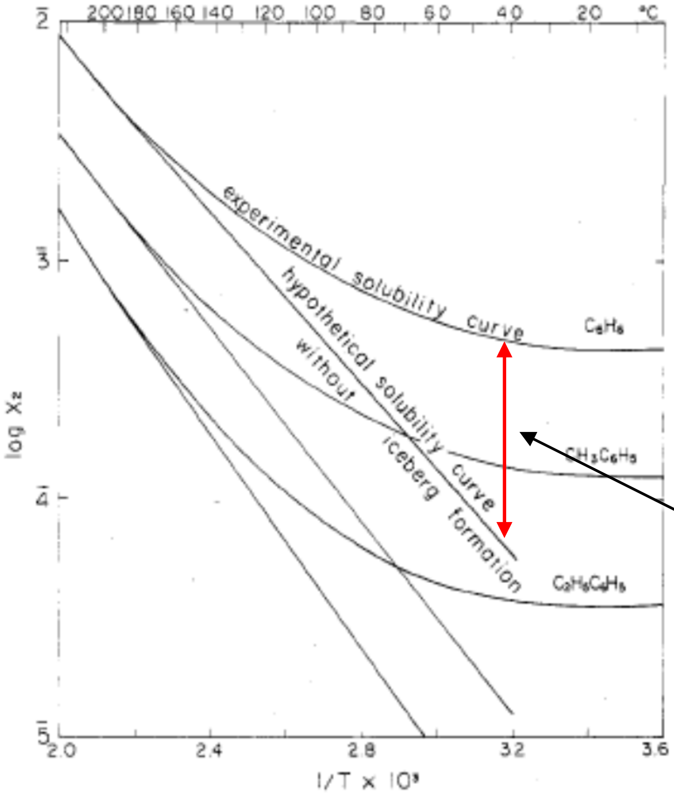




Methane clathrate
Ultimate cluster formation



Is hydrophobicity really entropically driven?



Shinoda 1977

Icebergs around non-polar solutes form spontaneously – hence they promote the solubiliy.

Increase in solubility of benzene in water due to the formation of ”Icebergs”

The increased solubility at low temperature has the same origin as cold denaturation of proteins: Hydrophobic interactions are weakened at low temperature.

Hydrophobic effects are ENERGY driven

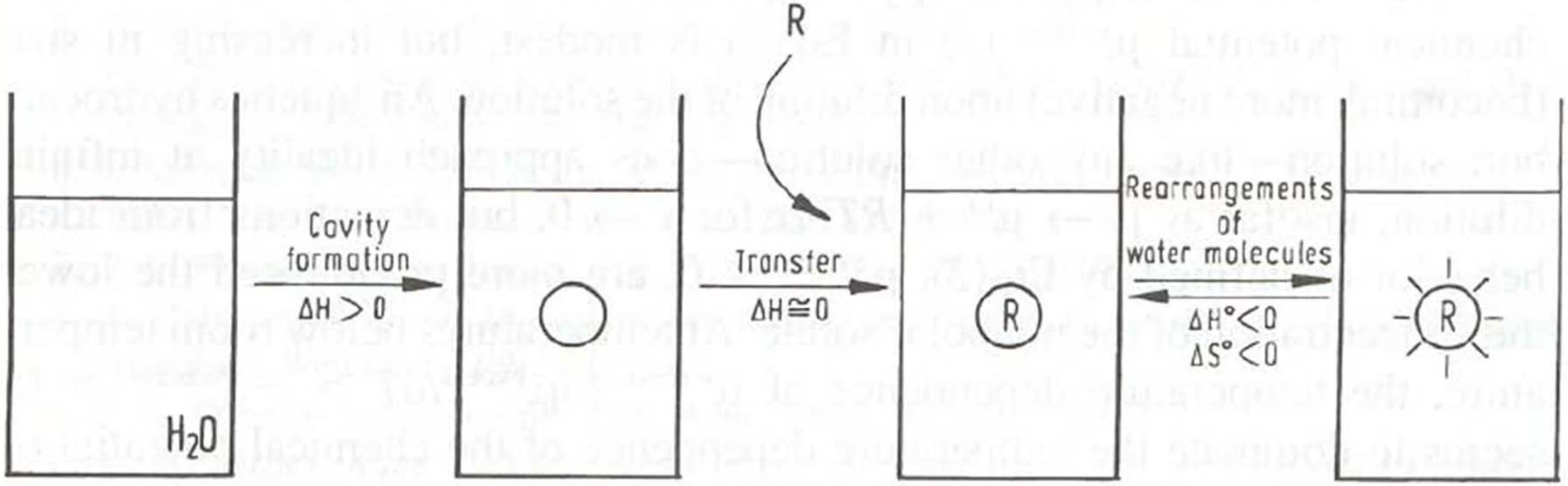


Fig. 1. A process, discussed in the text, for dissolution of a nonpolar molecule R in liquid water.

Hvidt 1980

The cost of creating a cavity which can accomondate the hydrophobic solute strongly limits solubility – relaxation of interfacial water molecules into ”icebergs” slightly promotes mixing.

Hydrophobic solutes are equally (sparsely) soluble in water and ohter polar solvents such as hydrazine or ethylene glycol.

Preferential hydration:
Proteins (and membranes) in mixed solvents

Proteins hydration in mixed solvents

The theory of preferential interactions

What happens when a protein is hydrated in a mixed aqueous solvent rather than in pure water?

Small molecules modulate the stability (and function) of proteins

Urea, for example, is a well-known denaturant, while sucrose acts as a stabilizer

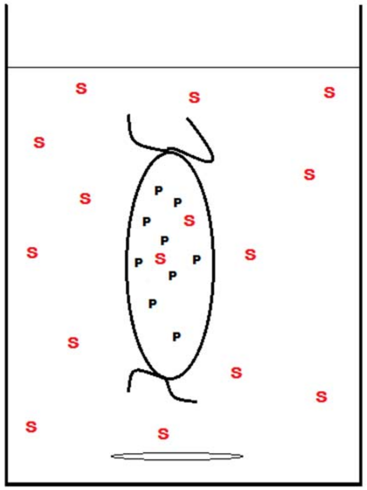
These ”osmolytes” are extensively used both in Nature and biotechnology

How do these solutes bring about these effects?

The preferential binding parameter, Γ

A thermodynamic measure of stoichiometry

A dialysis bag with protein (P) is bathed in an aqueous solution of a solute (S)



Measure $[S]_{inside}$ and $[S]_{outside}$

$$\Gamma \approx \frac{[S]_{inside} - [S]_{outside}}{[P]}$$

$$\Gamma = \left(\frac{\partial [S]}{\partial [P]} \right)_{\mu_S, P, T}$$

Preferential binding

Preferential hydration

Γ>0: Attractive P-S interactions – S accumulates at the surface of P

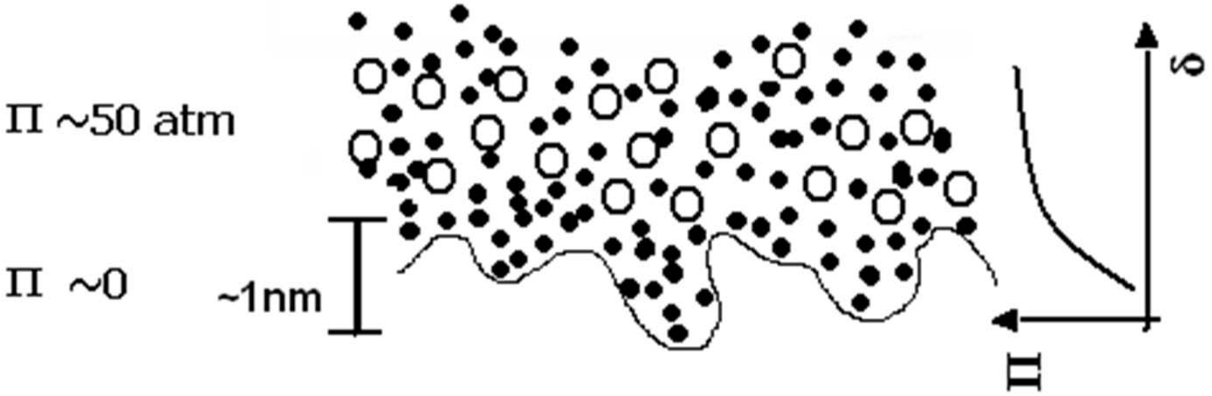
Γ=0: No interactions (ideal mixture, Random distribution of S)

Γ<0: Repulsive P-S interactions – S is repelled from the surface of S

What is Γ<0 ?

Preferential hydration or preferential exclusion

Osmotic stress



Biomolecule

Osmotic gradient

- Smoother interface
- Tighter molecular packing

or

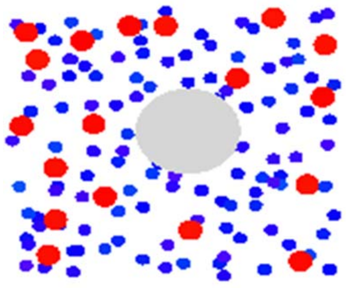
Increased interfacial tension

Gibbs adsorption equation (The surface excess)

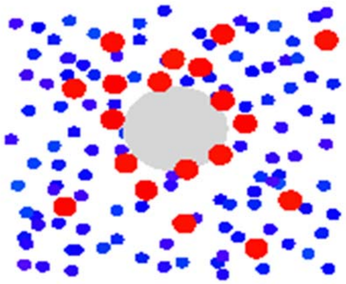
$$\Gamma = -\frac{1}{RT}\left(\frac{\partial \gamma}{\partial \ln c}\right)_T$$

(ideal solution)

Linkage and preferential interactions



Kosmotropes
Pref. Exclusion
$\Gamma<0$



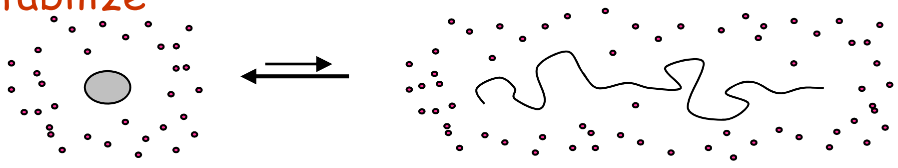
Chaotropes
Pref. Binding
$\Gamma>0$

- **Stability, solubility, oligomerization** etc are governed by preferential interactions

---

Preferential exclusion : promotes low-interface conformations

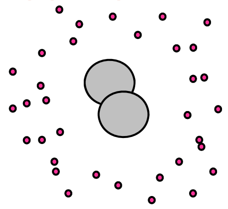
Stabilize



Native protein

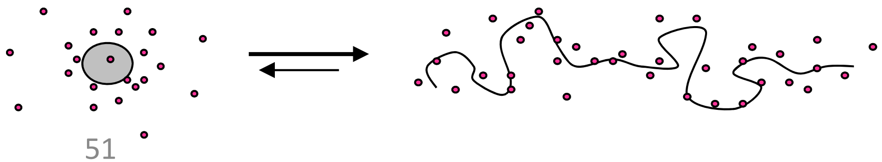
Denatured protein

Aggregate/Precipitate

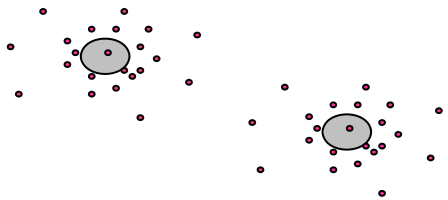


Preferential binding : promotes larger interfaces

Destabilize

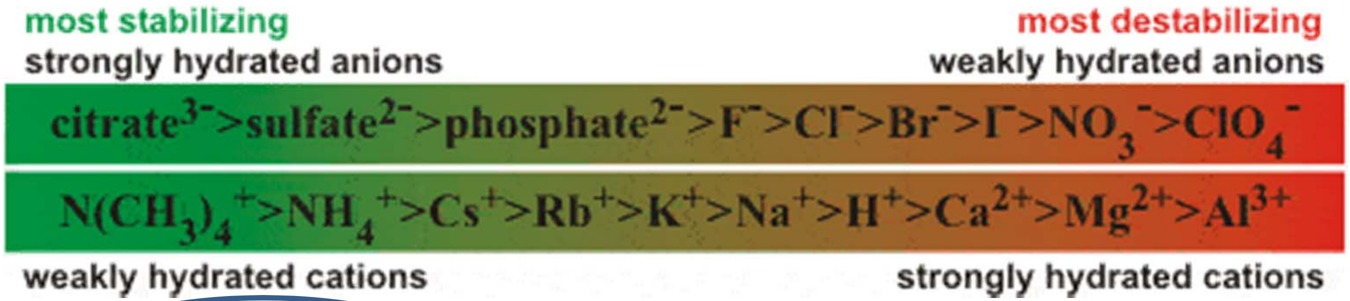


Solvate



Hofmeister effects and the Lyotropic Series

Kosmotropes **Chaotropes**



most stabilizing
strongly hydrated anions

most destabilizing
weakly hydrated anions

$\text{citrate}^{3-} > \text{sulfate}^{2-} > \text{phosphate}^{2-} > F^- > Cl^- > Br^- > I^- > NO_3^- > ClO_4^-$

$N(CH_3)_4^+ > NH_4^+ > Cs^+ > Rb^+ > K^+ > Na^+ > H^+ > Ca^{2+} > Mg^{2+} > Al^{3+}$

weakly hydrated cations

strongly hydrated cations

Natural
protectants

sucrose
sorbitol
betaine

Glycine

Urea

Michael Chaplin

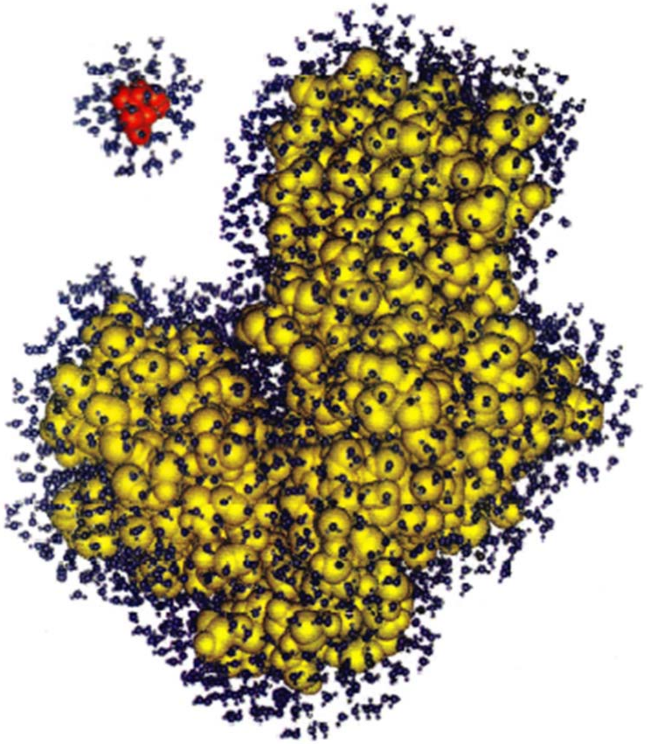
Relies on surface charge density and polarizability

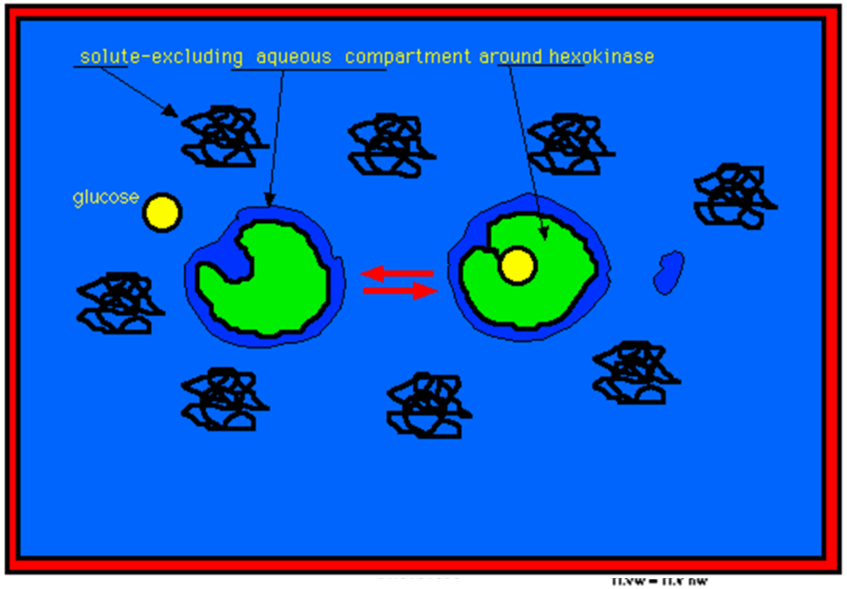
Anionic kosmotropes (e.g. F^- and SO_4^{2-}) bind 10-20 water molecules strongly but leaves the bulk rather unperturbed.

Anionic chaotropes affect bulk properties

Koga et al. 2004,
Westh et al. 2006

Osmotic stress analysis





$$n_w = \frac{RT}{V} \frac{\Lambda \ln (G.w)}{\Delta \Pi}$$

Osmotic stress promotes the affinity of hexokinase and glucose

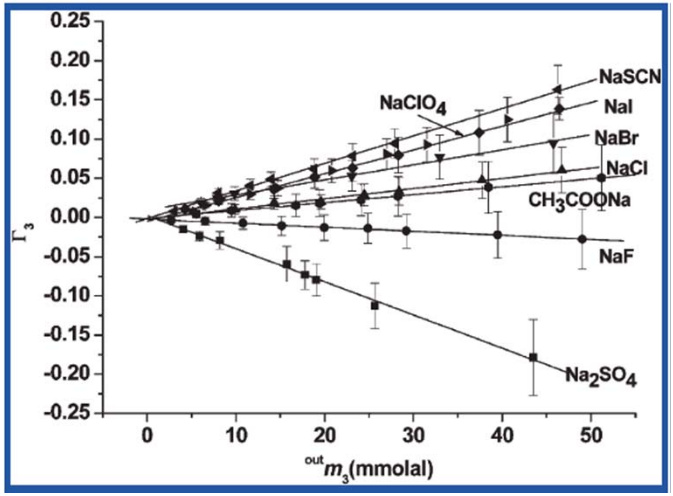
Or

Binding of glucose releases som 300 water molecules !

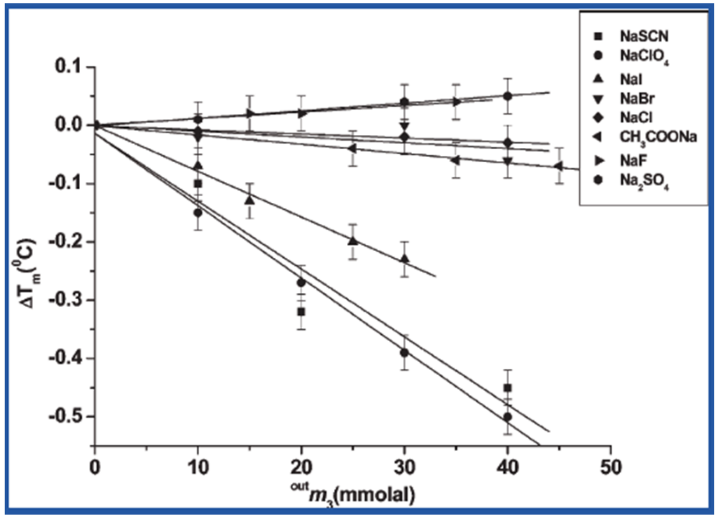
Parsegian and Rand 1997

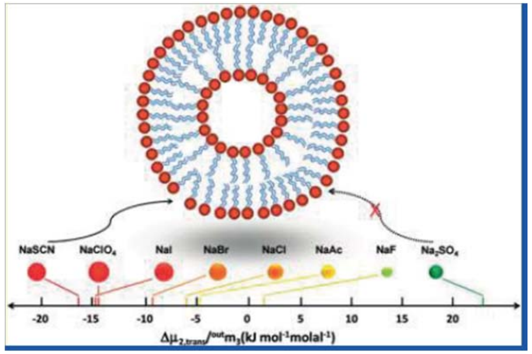
Preferential interactions of membranes

Preferential interaction

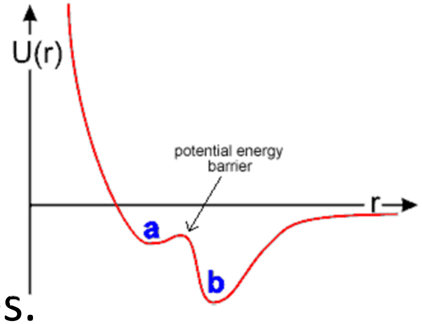


Membrane property: melting temp





Closing remarks



- Water forms large, short-lived clusters with ice-like structure
- Similar structures are formed at hydrophobic interfaces
- High molecular cohesion of water expels hydrophobic moieties.
- Water ”lubricates” folding and fluctuation through bridging
- Water binds strongly to charged groups
- Water in crevices have long residence times (ns - μs); the hydration shell has intermediate residence time (100’th of ps)
- The hydration shell has moderately reduced diffusion coefficient and moderately increased density.

Macromolecules and their aqueous solvent have co-evolved

Micromolecular Evolution – the aqueous environtment is regulated to meet the functional requirements.
Brought about by regulating solvent composition.
The evolutionary benefit of this strategy is genetic simplicity

Yancey et al. 1982