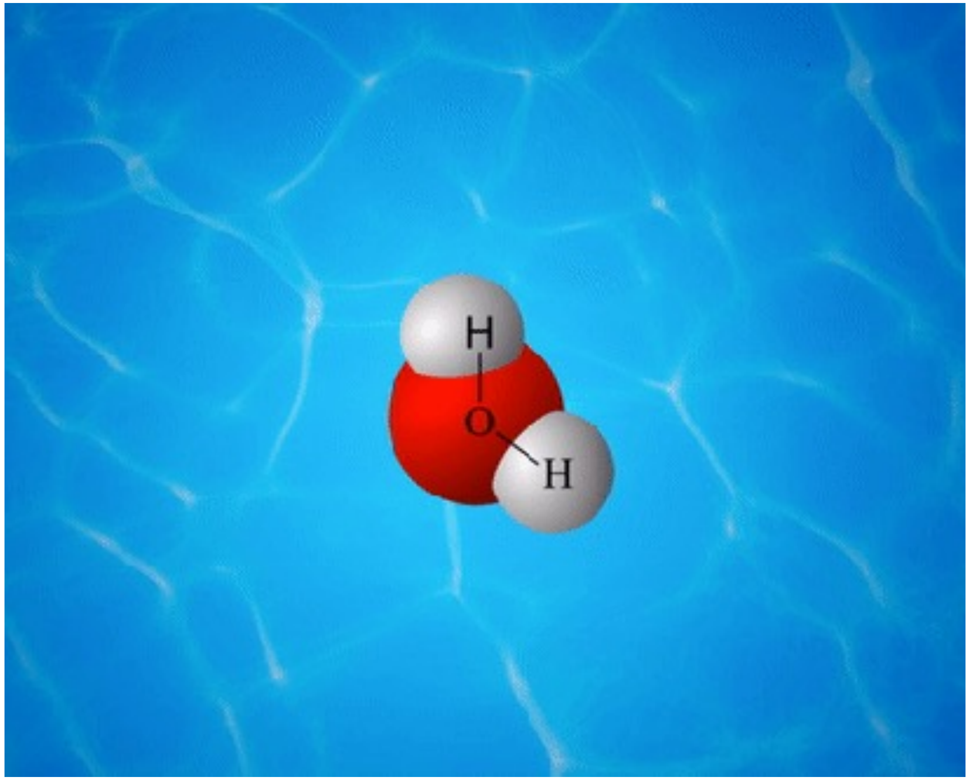
Macromolecular Structures

Sc. in Nanotechnology Engineering
Sapienza University of Rome



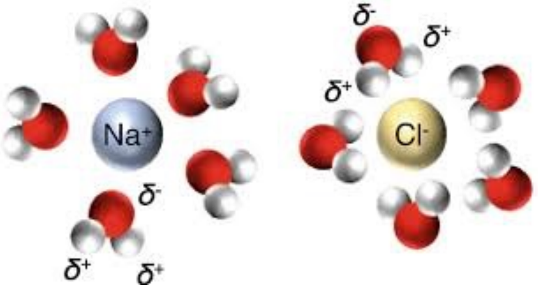
Beatrice Vallone
beatrice.vallone@uniroma1.it
www.vallonelab.com

Water & Life: from molecular properties to biological macromolecules

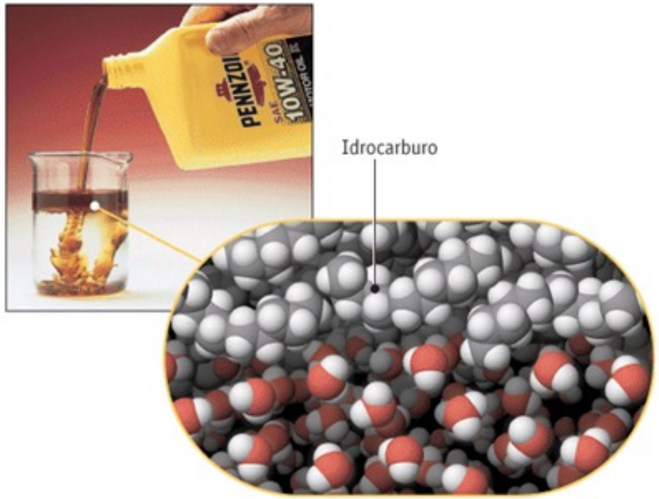
Puzzling phenomena

- Why does ice float?

- Why are electric fields weakened in water?

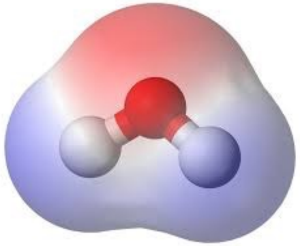


- Why do oil and water not mix?

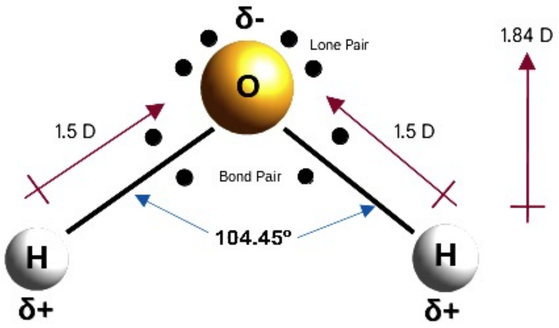


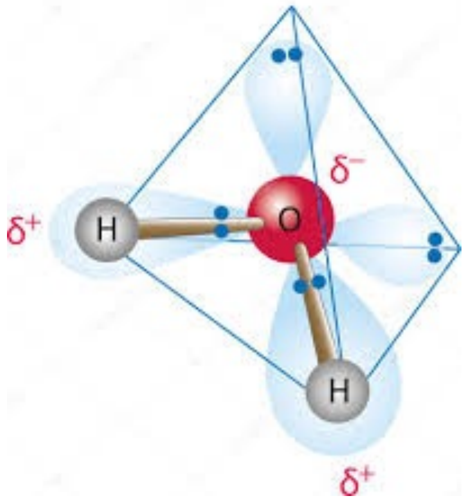
Hydrophobic effect: ΔH or ΔS?

The water molecule



- Tetrahedral geometry, 104.5° angle
- Permanent dipole → high polarity
- Small molecule, but extraordinarily strong

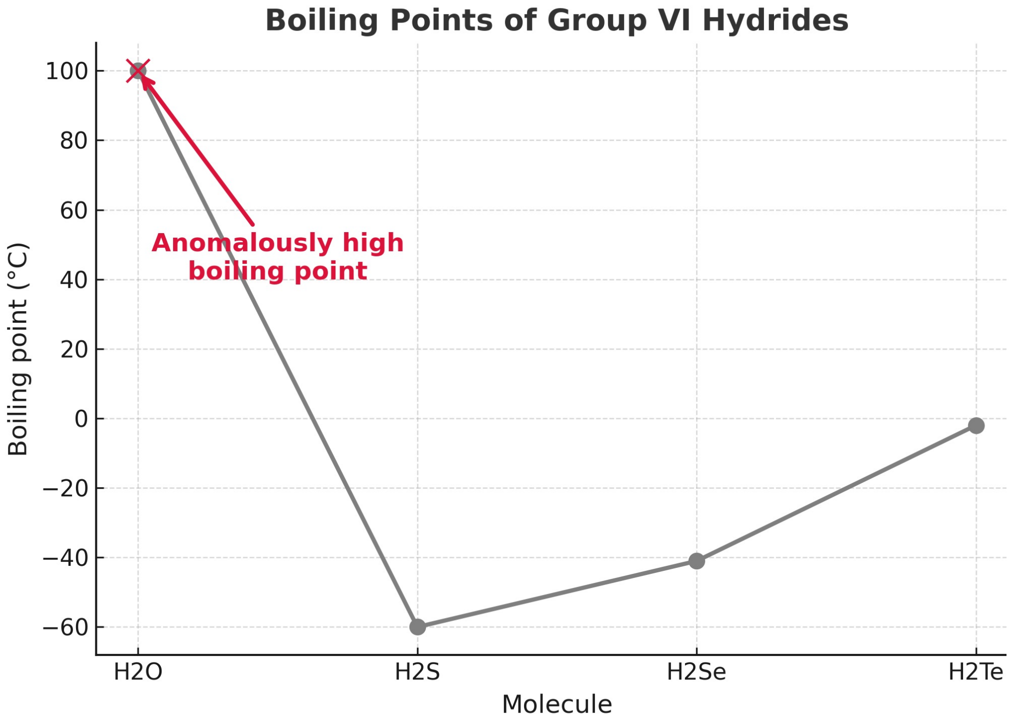


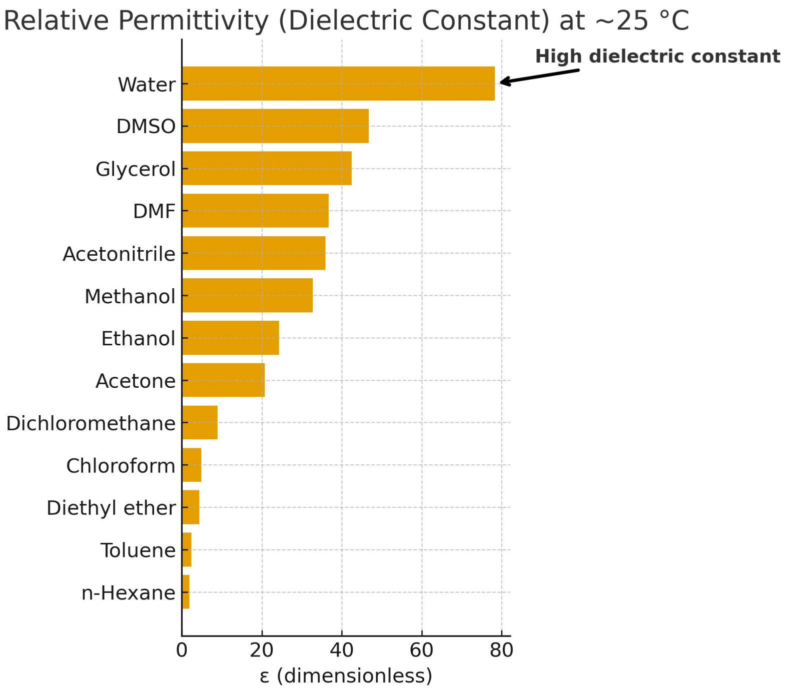


Oxygen has sp^3 hybridization

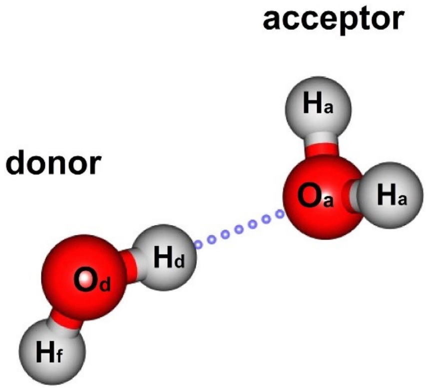
Extremely strong – what do we mean?

- Unusually high boiling and melting points
- High surface tension
- High heat capacity
- Ice less dense than liquid water
- Powerful dielectric constant

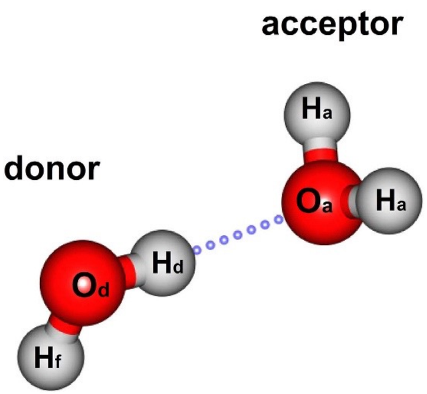




Is H-bonding only dipolar?



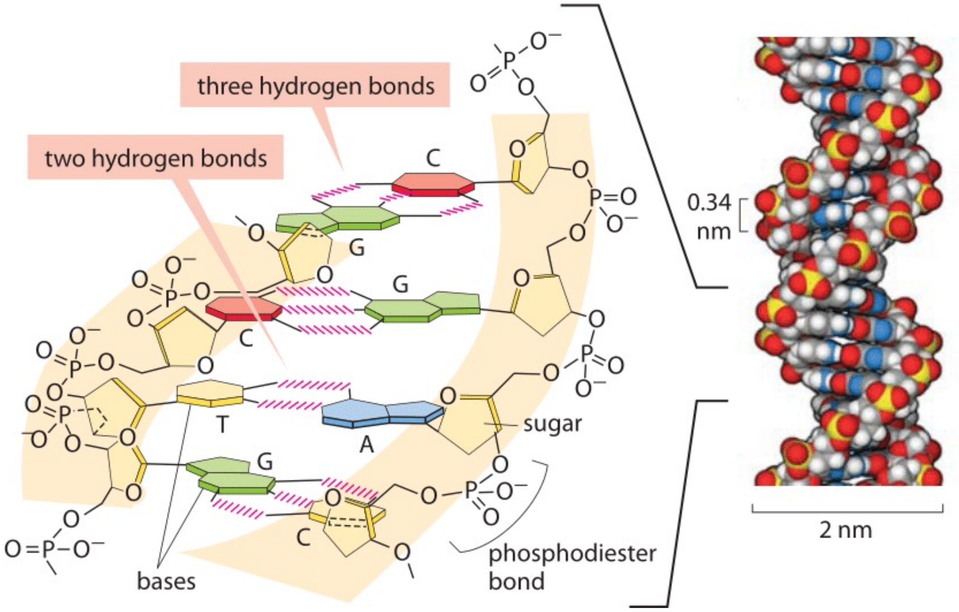
Is H-bonding only dipolar?



Hydrogen bonds are often described as simple dipole–dipole interactions.

In reality, hydrogen bonds combine electrostatic attraction with other components: directionality, partial covalent character, and cooperative stabilization.

This explains why they are stronger and more specific than van der Waals interactions, yet weaker and more flexible than covalent bonds.



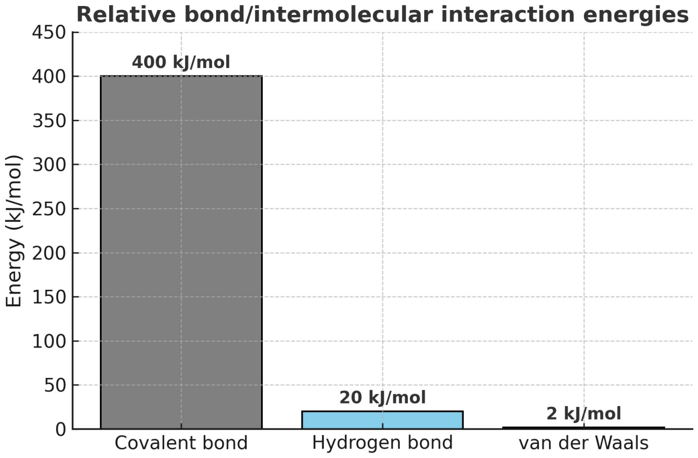
In biological macromolecules, hydrogen bonds are fundamental determinants of structural organization and molecular recognition.

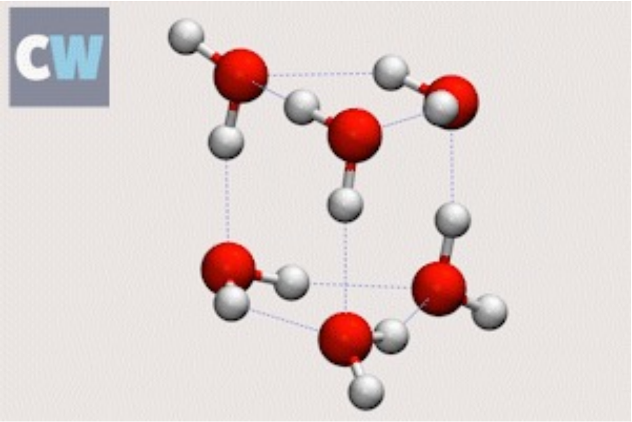
They have a dual role, structural stability and selective recognition.

Hydrogen bonding is indispensable for the chemistry of life.

Hydrogen bonding

Typical strength (5–20 kJ/mol) makes them significantly stronger than most dispersion forces, but weaker than covalent bonds. In liquid water, the hydrogen-bond network is highly dynamic: bonds are constantly breaking and reforming on the picosecond timescale.





J O Richardson *et al*, *Science*, 2016, **351**, 1310 10.1126/science.aae0012

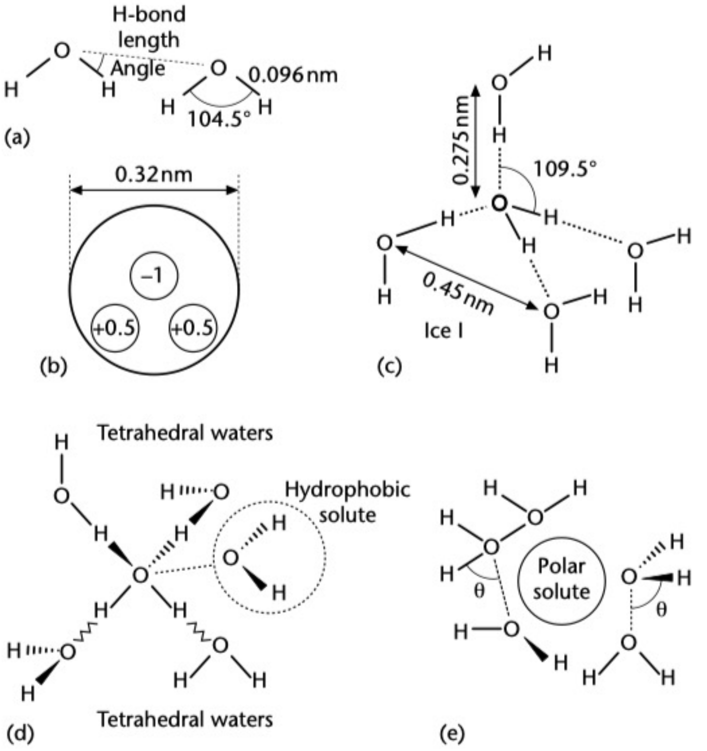


Figure 1 Structure of water. (a) Definition of key lengths and angles. (b) Model of water. (c) Structure of Ice I. (d) Schematic of H bonding structure in liquid water, and in presence of an apolar solute. (e) Schematic of H bonding structure around a positively charged ion of polar atom.

Hydrogen bond geometry

- The oxygen atom in water is **sp^3 hybridized**, with two covalent O–H bonds and two lone pairs arranged in a tetrahedral geometry.
- As a result, hydrogen bonds are **directional**: their stability depends not only on the donor–acceptor distance, but also on the **bond angle**.
- Maximum strength is achieved when the donor–H···acceptor atoms are aligned in a nearly straight line (≈180°).
- This angular dependence explains the ordered network of hydrogen bonds in ice and the structural specificity in proteins and nucleic acids.

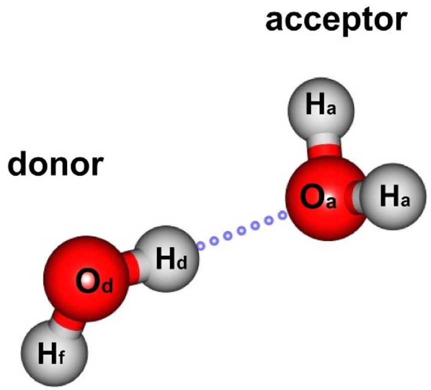
From: Sharp, Kim. (2001). Water: Structure and Properties. 10.1038/npg.els.0003116.

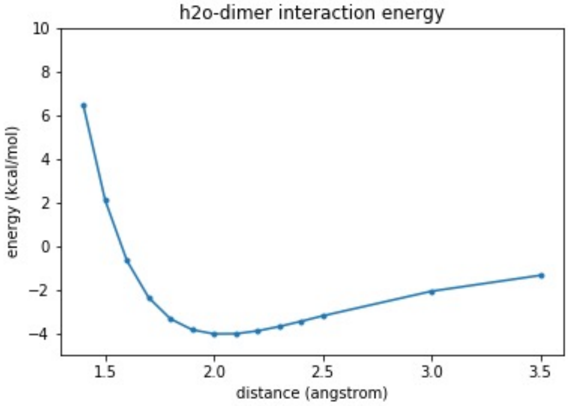
Evidence for partial covalent character

- The actual O···O distance in the water dimer (2.98 Å) is shorter than the sum of the van der Waals radii of two oxygens (3.04 Å), indicating that the oxygen atoms approach closer than the non-bonded limit, consistent with electron density overlap.

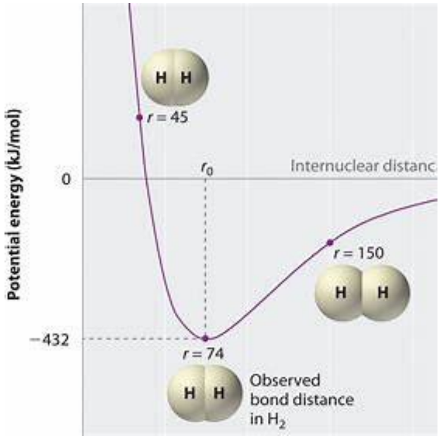
- The H···O distance of ~1.95 Å, significantly shorter than the sum of the van der Waals radii of H and O, further supports the idea that the interaction is not purely electrostatic but involves partial electron sharing, i.e. covalent character.

Interaction	Typical distance (Å)
O–H covalent bond	~1.0 Å
H···O (hydrogen bond)	~1.95 Å
O···O distance in water dimer	~2.98 Å
Sum of van der Waals radii (O + O)	3.04 Å
Sum of van der Waals radii (O + H)	~2.72 Å



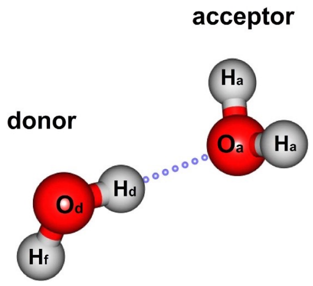


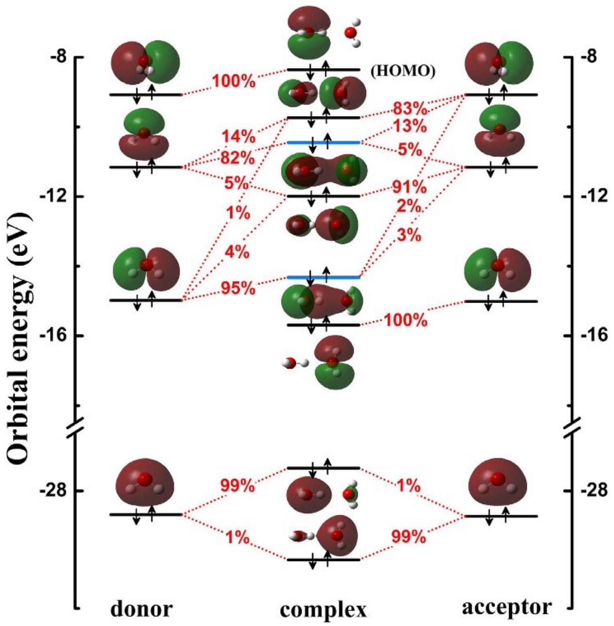
For the O…H hydrogen bond
E=5-25 kJ/mol



For the O-H bond
E=460 kcal/mol

Quantum chemical analyses reveal overlap between molecular orbitals of donor and acceptor atoms, which contributes to a partial covalent character.

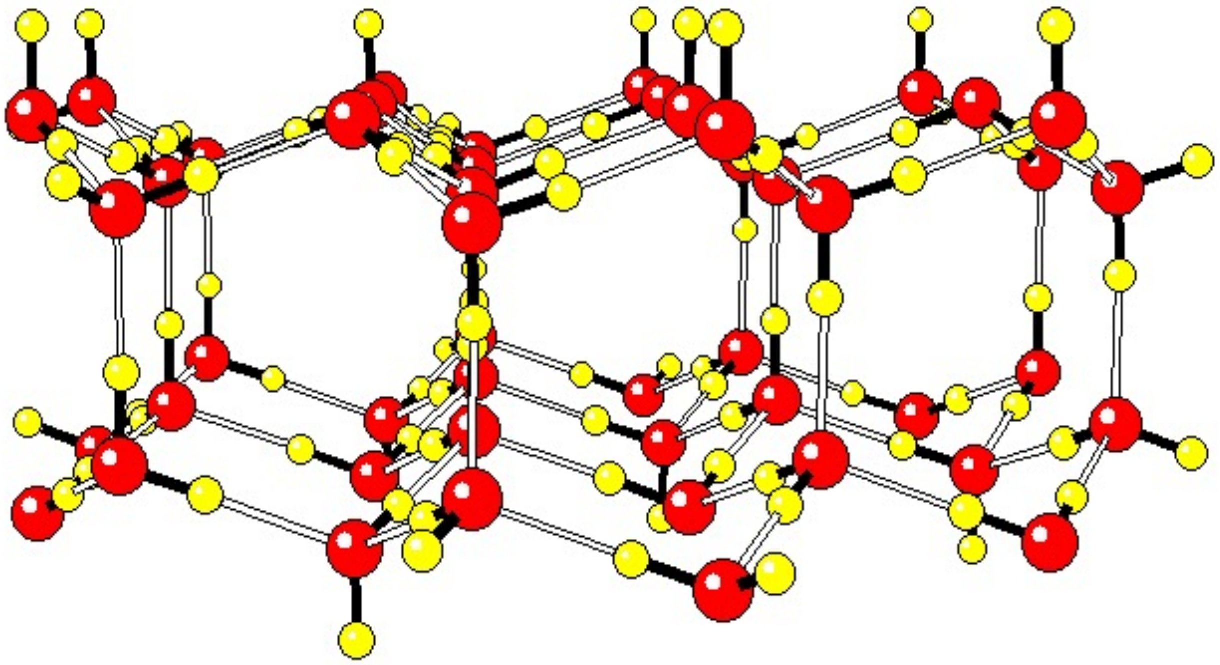




Wang, B., Jiang, W., Dai, X. *et al.* Molecular orbital analysis of the hydrogen bonded water dimer. *Sci Rep* **6**, 22099 (2016). https://doi.org/10.1038/srep22099

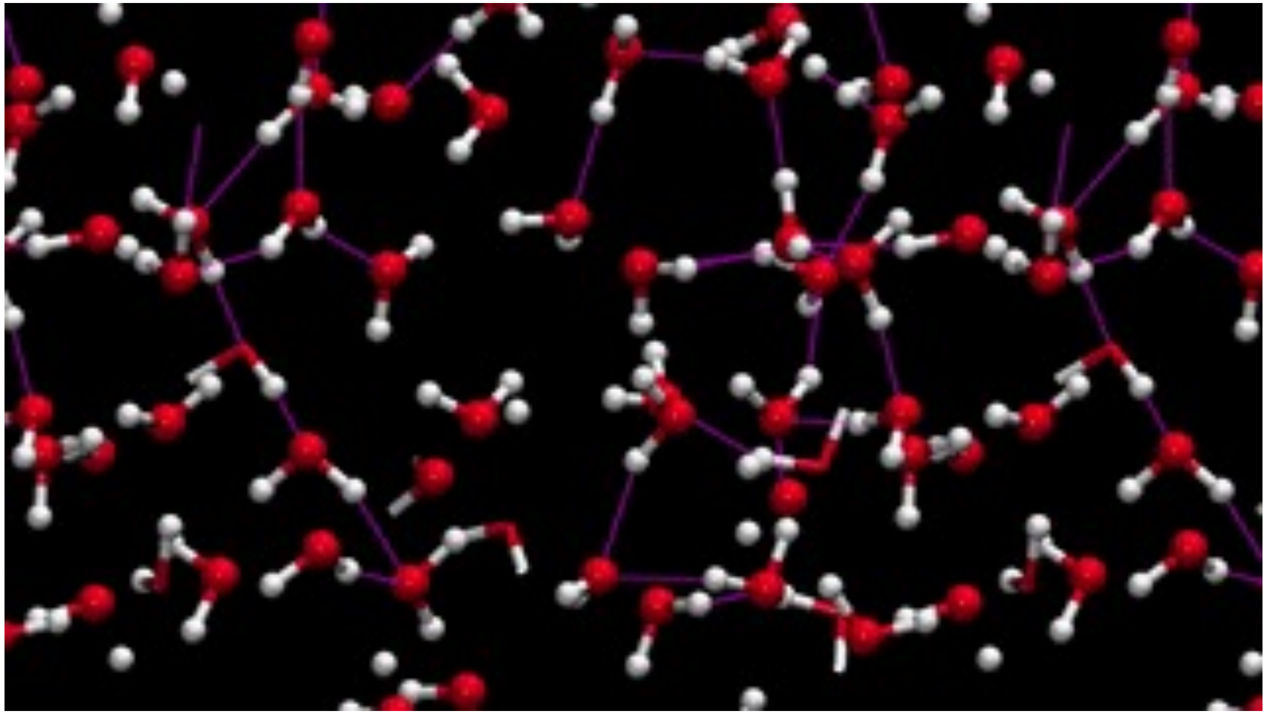
Hydrogen bond network in ice

- Each water molecule forms **4 hydrogen bonds** (2 donors, 2 acceptors)
- Nearly perfect **tetrahedral arrangement**
- Open, crystalline structure with voids

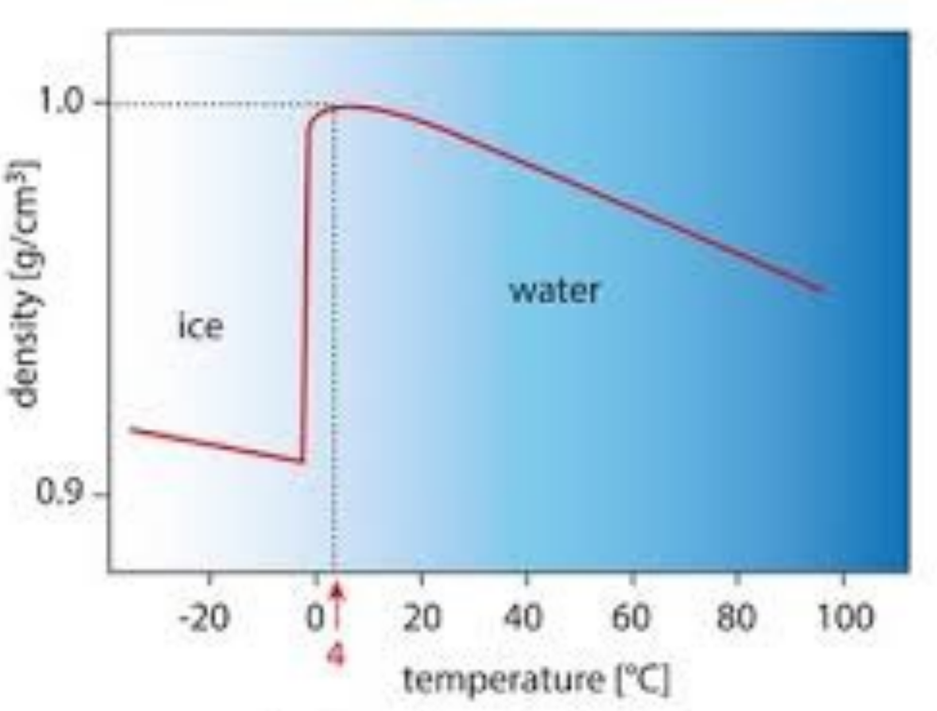


Hydrogen bond network in liquid water

- On average **3.4 hydrogen bonds per molecule**
- Highly **dynamic network**: bonds continuously break and reform on the **picosecond timescale**
- More compact structure compared to ice



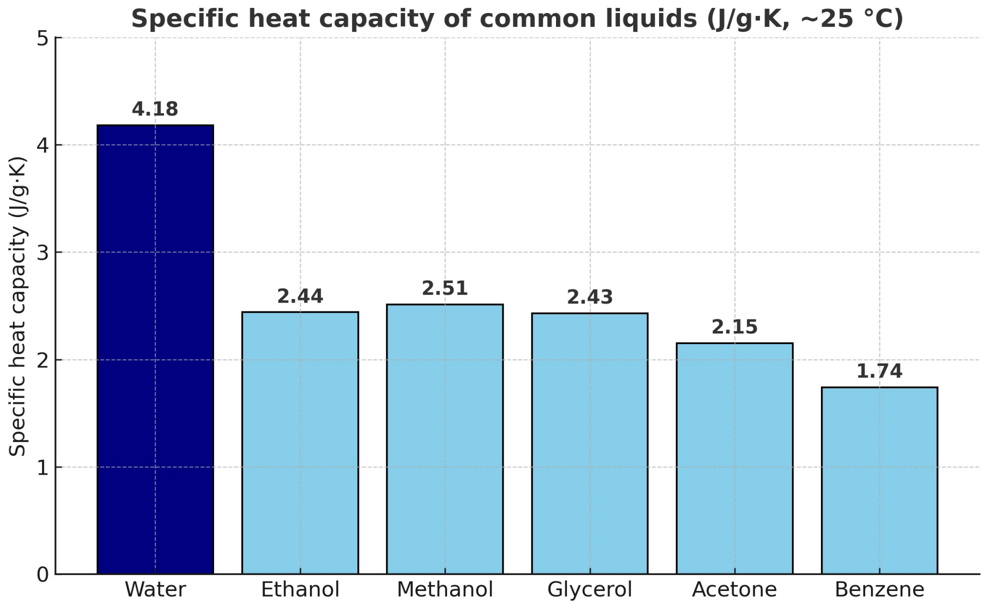
Macroscopic consequences of H-bonding



Why does water have maximum density at 4 °C?

- At 0 °C, water still retains an open, tetrahedral H-bond network (like ice) → lower density.
- Between 0 °C and 4 °C, parts of this network collapse, molecules pack closer → density increases.
- Above 4 °C, thermal motion dominates, molecules move apart → density decreases.
- At 4 °C the two effects balance → **maximum density**.

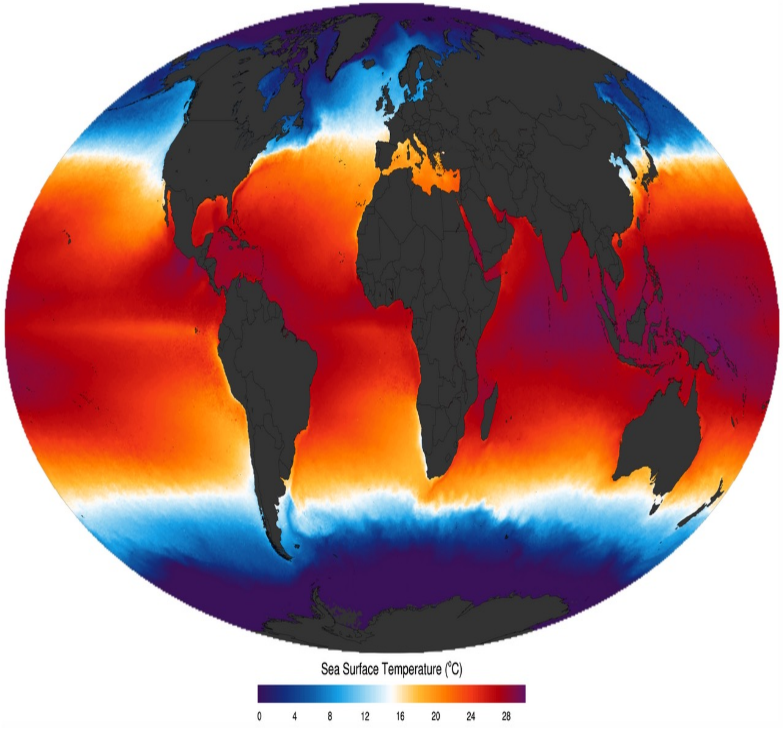
High heat capacity

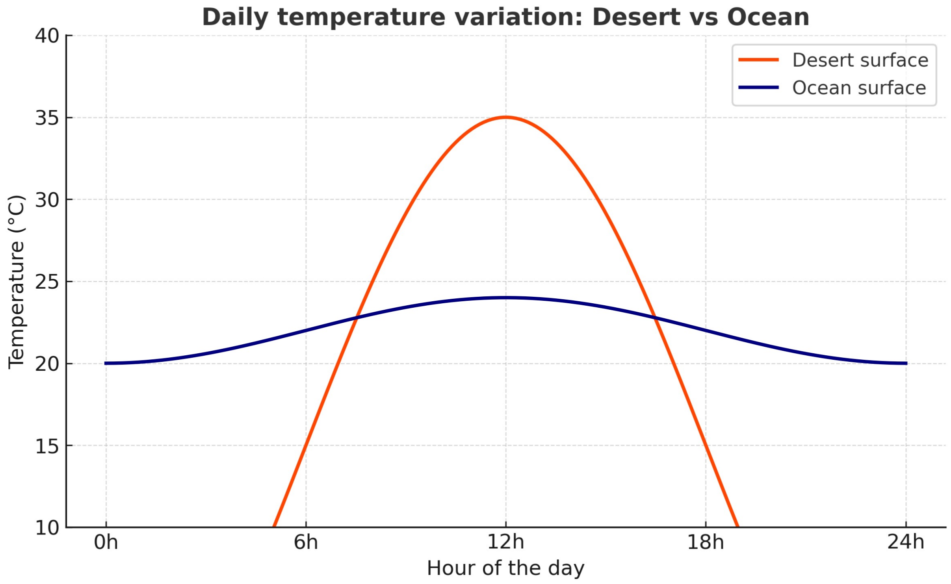


Water has an exceptionally high specific heat capacity (4.18 J/g·K), almost twice that of most common liquids.

This anomaly reflects the energy needed to break and reform the hydrogen-bond network, making water much more resistant to temperature changes.

High heat capacity → “ocean thermostat”





Sea surface temperatures show remarkable stability thanks to water’s high heat capacity: oceans absorb and release heat slowly, moderating global climate.

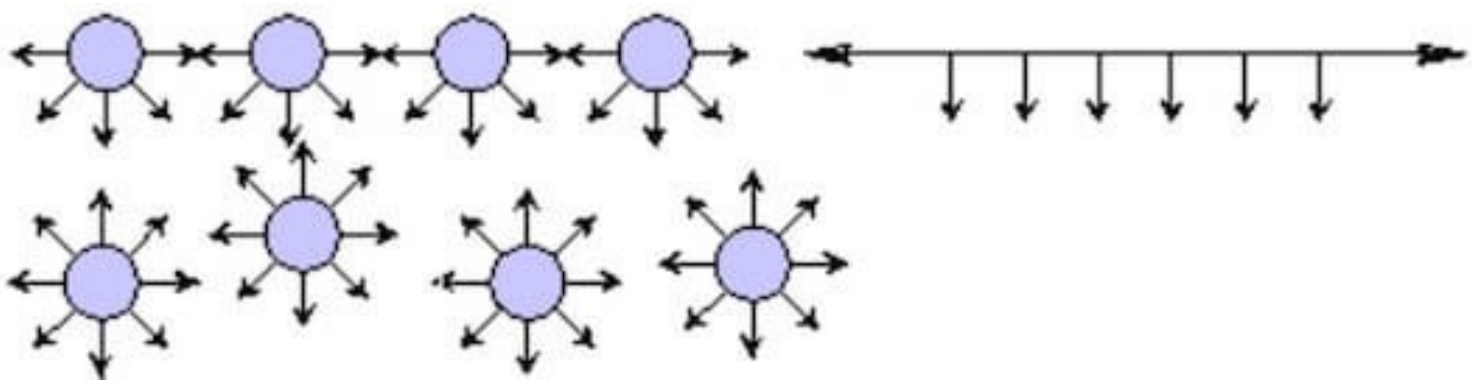
Daily temperature variation: deserts heat up and cool down rapidly, while ocean surfaces remain stable — evidence of water’s role as a planetary thermostat.

Surface Tension – Physical and Biomedical Aspects

Why is water’s surface tension so high?

Physical definition

- Property of liquids due to molecular cohesion
- Molecules at the surface experience an inward force



- The surface behaves like an elastic membrane
- Unit: J/m² = N/m
- Energy required to increase the liquid surface area

Why is water’s surface tension so high?

- Extensive **hydrogen-bond network** between molecules
- Surface molecules lack neighbors above → stronger inward pull
- Requires more energy to create new surface → exceptionally high surface tension (~72 mN/m at 25 °C)

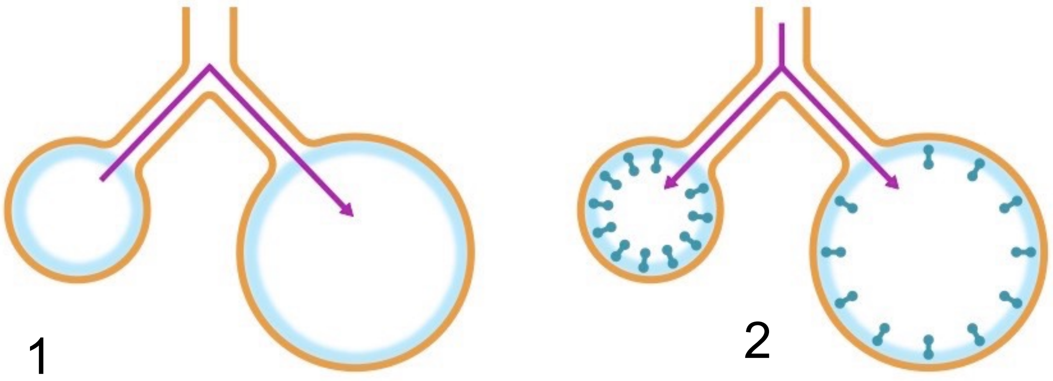
Biomedical aspects – Respiratory system

- In the alveoli, surface tension would tend to cause collapse
- Pulmonary surfactant reduces surface tension
- In premature infants surfactant may be absent → neonatal respiratory distress
- Therapy: exogenous surfactant, assisted ventilation

Engineering & nanotechnology relevance

- **Interface physics**
  - The alveolus is essentially a **liquid–air interface** problem.
  - Surface tension is controlled by molecules that self-assemble at the interface
    → exactly what nanotechnologists study in coatings, detergents and emulsions.

Alveoli without (1) and with (2) surfactant



1.Without surfactant

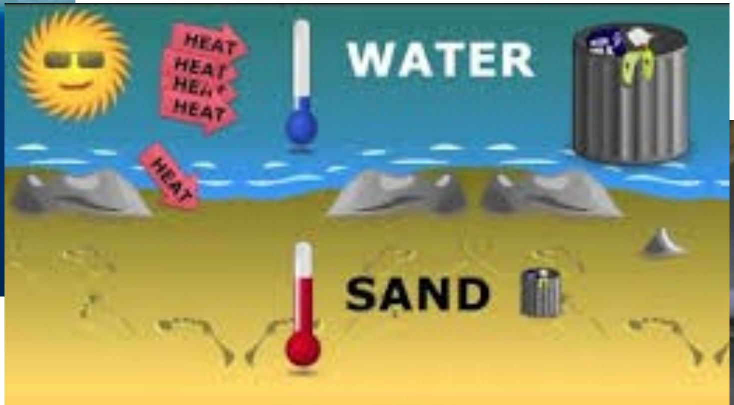
- The surface tension of the liquid film lining the alveoli is high
- Smaller alveoli tend to collapse, larger ones over-distend
- This leads to severe respiratory difficulties

2.With surfactant

- A mixture of phospholipids and proteins spreads at the air–liquid interface
- It drastically reduces surface tension
- Alveoli remain stable regardless of their size
- Gas exchange can proceed efficiently

"Anomalous" properties of water

- Density maximum at 4 °C
- Very high heat capacity
- High surface tension and cohesion

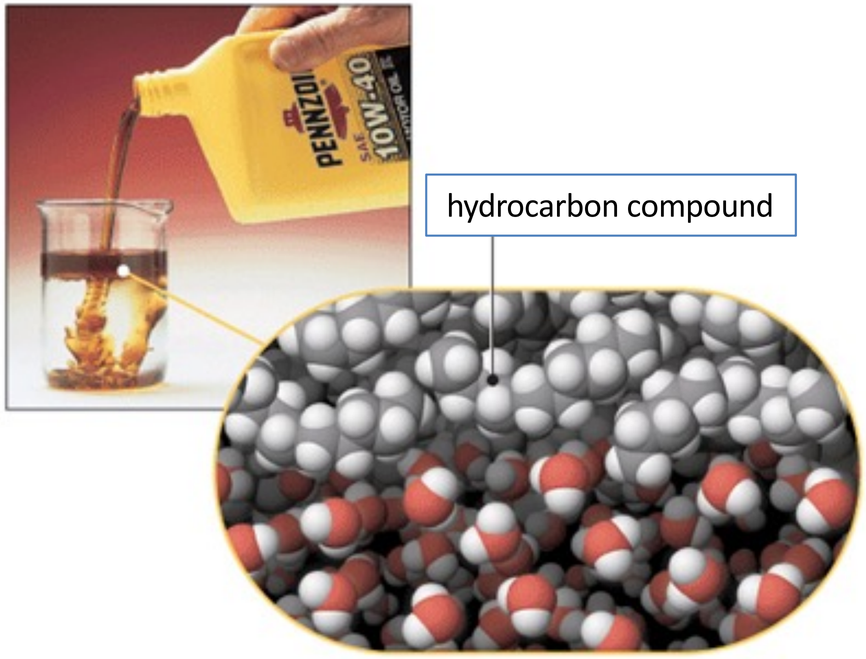


Water as a biomolecule

- Not just a solvent, but an active player
- Controls conformations and dynamics
- Essential for life and complexity

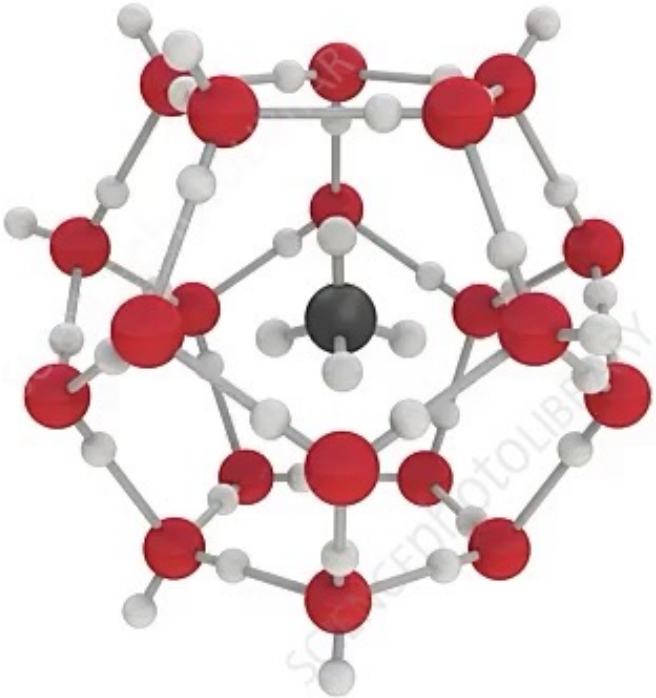
The “entropic paradox”

- Normally, mixing increases entropy ($\Delta S > 0$)
- With hydrophobic solutes, the opposite happens: there is a phase separation → $\Delta S < 0$.
- Paradox: why doesn’t mixing of water and nonpolar molecules increase entropy as expected?



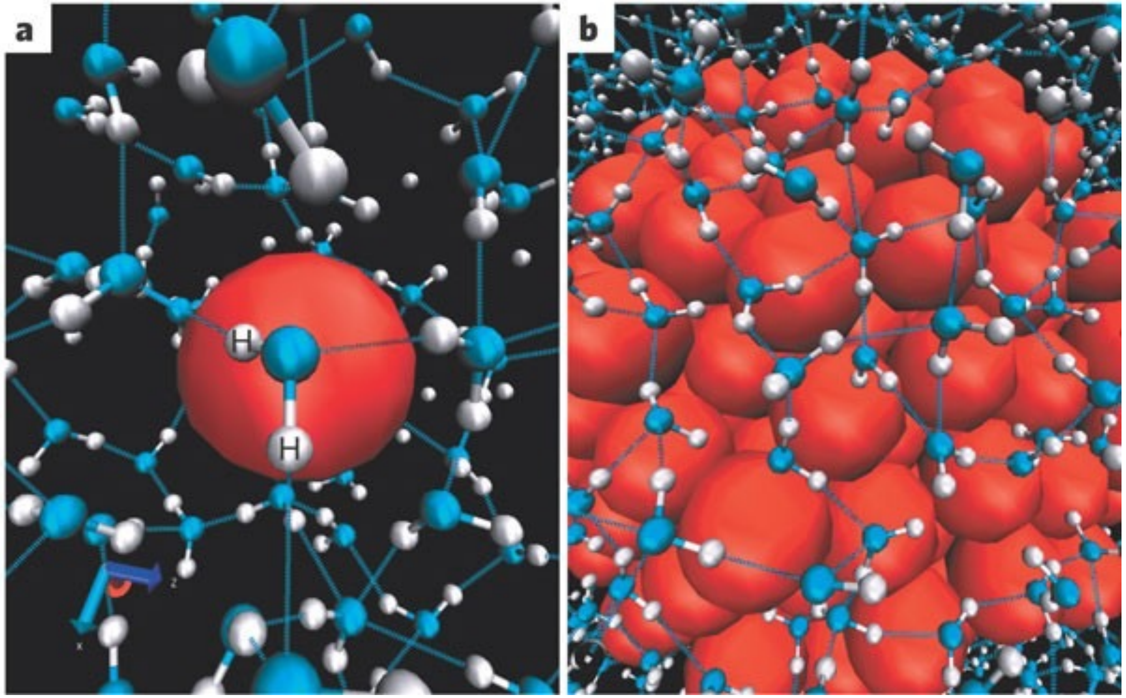
Hydrophobicity: the entropic paradox

- Liquid water forms a **dynamic hydrogen-bond network**
- Nonpolar solutes cannot form H-bonds
- Surrounding water arranges into **structured cages** (clathrate-like)
- This local ordering reduces entropy ($\Delta S < 0$)



Resolution: the hydrophobic effect

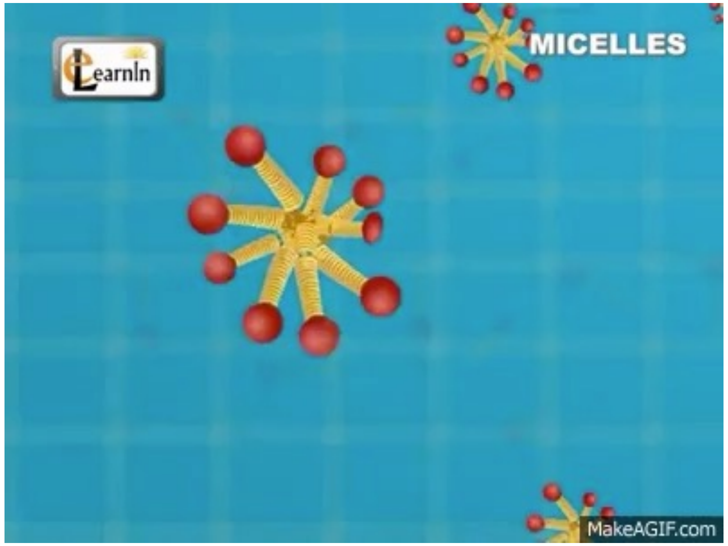
- Hydrophobic solutes aggregate.
- Ordered water released → entropy increases.
- $\Delta G = \Delta H - T\Delta S$ → aggregation becomes spontaneous.

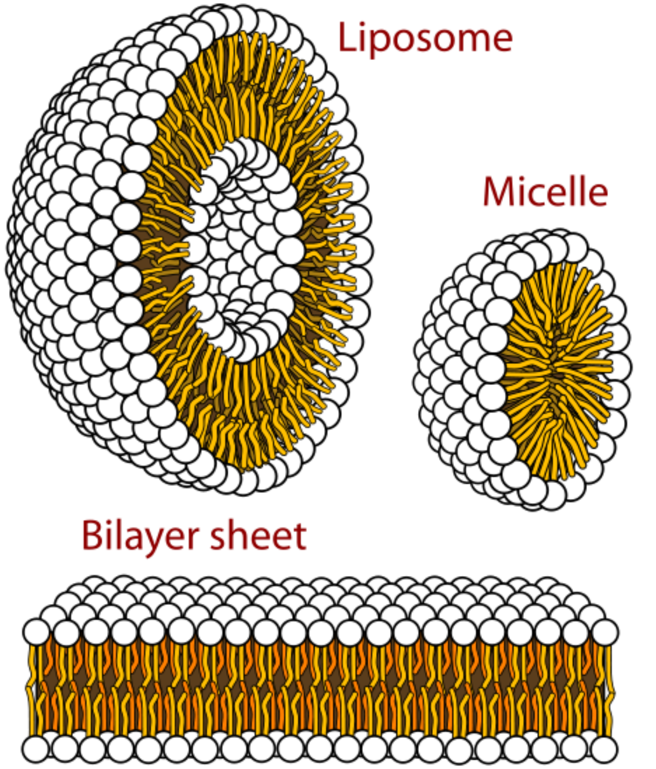


From: Chandler, D. Interfaces and the driving force of hydrophobic assembly. *Nature* **437**, 640–647 (2005). https://doi.org/10.1038/nature04162

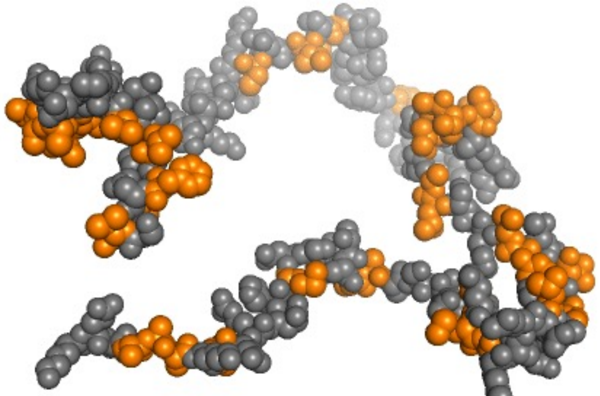
Biological & technological relevance

- **Protein folding**: hydrophobic residues collapse into the core
- **Membranes**: lipids self-assemble into bilayers
- **Molecular recognition**: hydrophobic patches stabilize complexes
- **Nanotechnology**: hydrophobic effect drives **micelle and vesicle formation**, drug delivery systems, self-assembled nanostructures

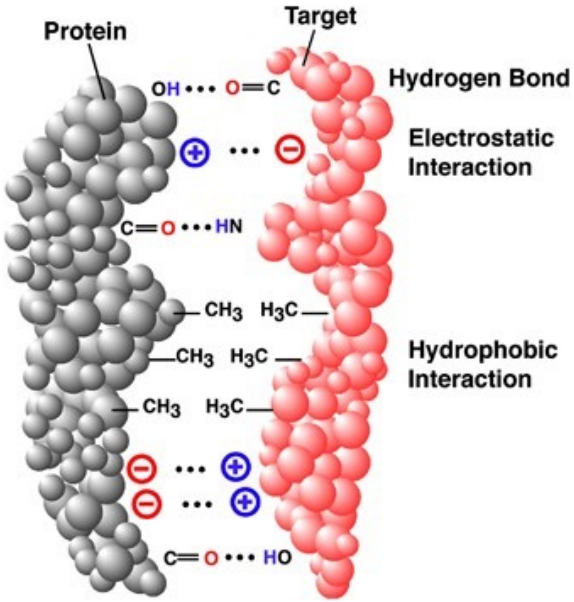




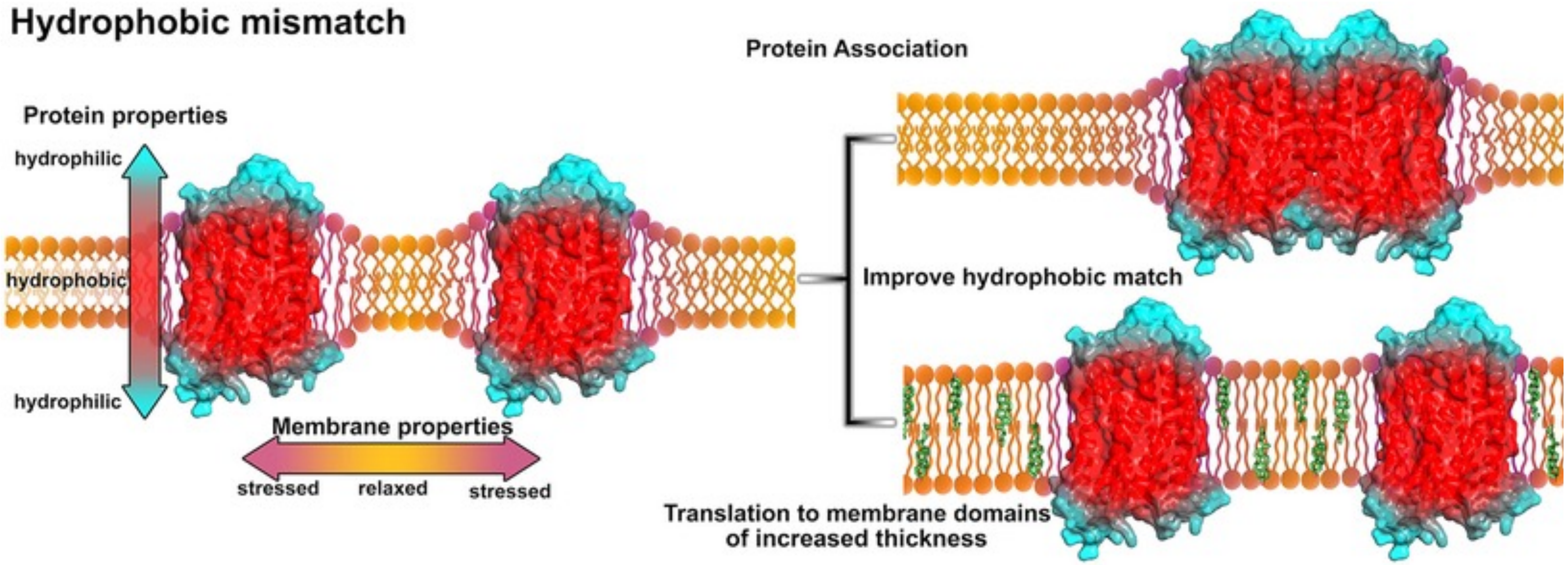
Role in protein folding and supramolecular structures



Hydrophobic residues = orange
Hydrophilic residues = grey



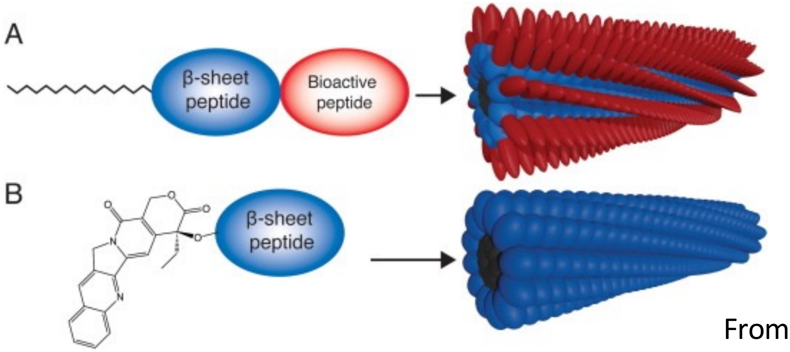
From: Hoshino, Y., Lee, H. & Miura, Y. Interaction between synthetic particles and biomacromolecules: fundamental study of nonspecific interaction and design of nanoparticles that recognize target molecules. *Polym J* **46**, 537–545 (2014). https://doi.org/10.1038/pj.2014.33



From: Gahbauer, Stefan & Böckmann, Rainer. (2016). Membrane-Mediated Oligomerization of G Protein Coupled Receptors and Its Implications for GPCR Function. Frontiers in Physiology. 7. 10.3389/fphys.2016.00494.

Nanotechnology applications of the hydrophobic effect

- **Micelles and liposomes**: self-assembled carriers for drug delivery
- **Nanoparticles**: hydrophobic ligands control stability and aggregation in solution
- **Polymeric nanostructures**: hydrophobic blocks drive self-assembly into nanoparticles or hydrogels
- **Surface engineering**: hydrophobic patterning for biosensors, lab-on-a-chip, and antifouling coatings
- **Nanoporous materials**: hydrophobic channels for selective molecular recognition and separation
- **Protein-based nanomaterials**: engineered hydrophobic patches to stabilize assemblies or scaffolds



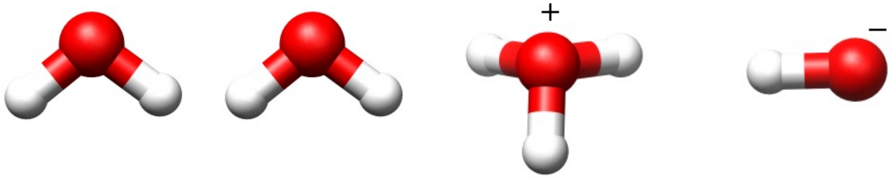
Bioactivity can be covalently incorporated into self-assembled hydrogels by (A) the addition of cell-signaling peptides on the exterior of nanostructures or (B) the inclusion of hydrophobic drugs on the interior.

From: Webber, Matthew & Pashuck, E.. (2021). (Macro)molecular self-assembly for hydrogel drug delivery. Advanced Drug Delivery Reviews. 172. 10.1016/j.addr.2021.01.006.

Autoprotolysis of water

- $2\ H_2O \rightleftharpoons H_3O^+ + OH^-$

$H_2O\ (l) + H_2O(l) \rightleftarrows H_3O^+(aq) + OH^-(aq)$



- $K_w = 10^{-14}$ at 25 °C

$$K = \frac{[H_3O^+]\cdot[OH^-]}{[H_2O]^2}$$

- Definition of pH and pOH

pH scale

pH is defined as the decimal logarithm of the reciprocal of the hydronium concentration:

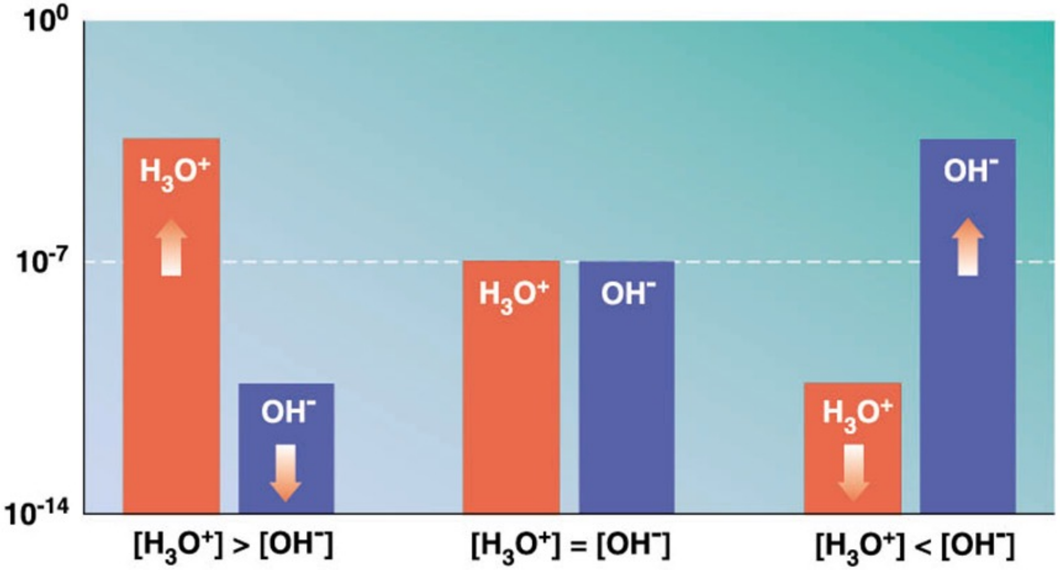
$$pH = -\log_{10}[H_3O^+]$$

pOH is defined as the decimal logarithm of the reciprocal of the oxydril concentration:

$$pOH = -\log_{10}[OH^-]$$

In water $[H_3O^+] = [OH^-] = 10^{-7}$ M and pH = pOH = 7

$pH = -\log_{10}[H_3O^+] = -\log_{10}10^{-7} = 7$



Buffer systems

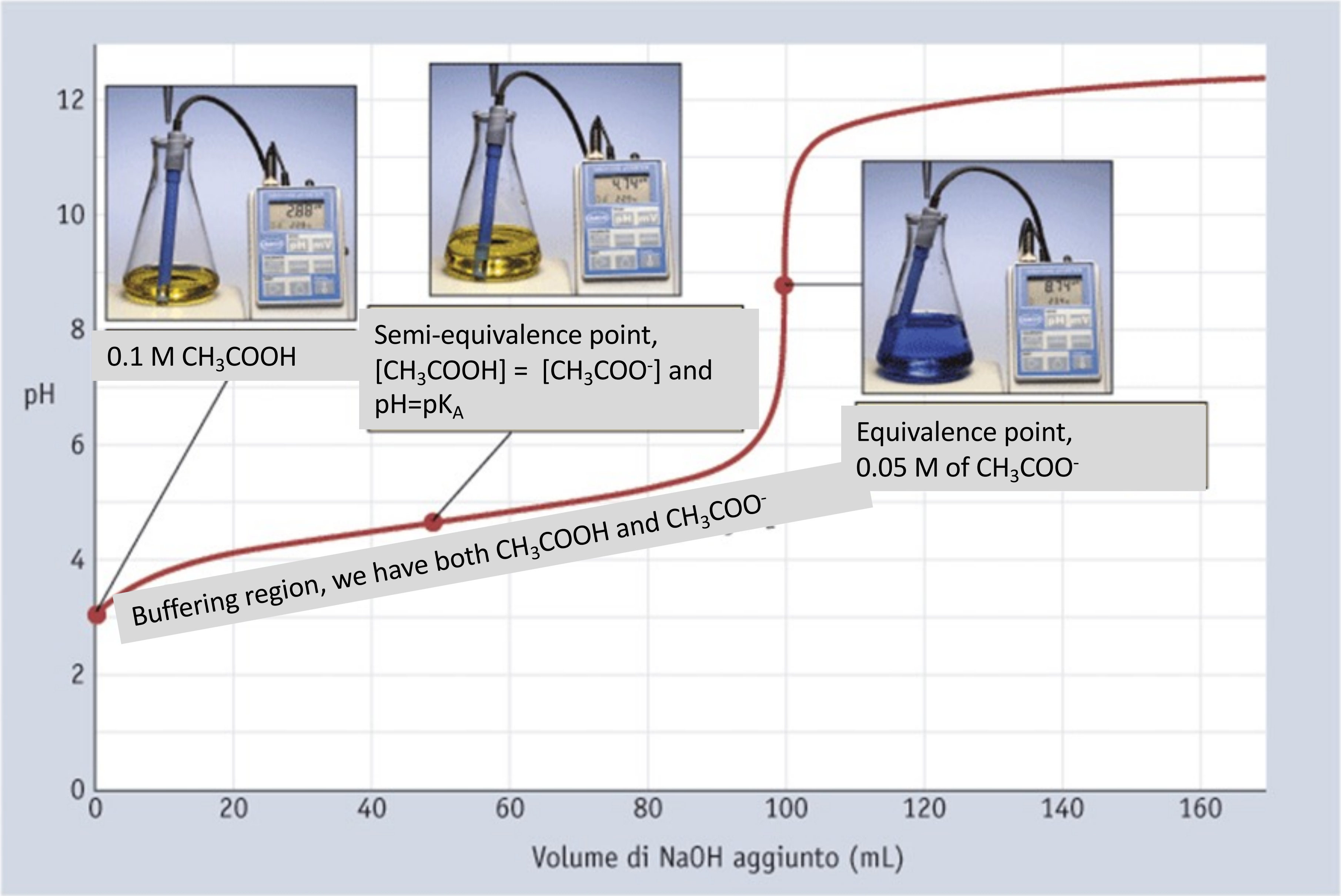
- Buffers are solutions that **resist changes in pH** when small amounts of acid or base are added.
- Composed of a **weak acid (HA) and its conjugate base (A^-)**.
- Governed by the **Henderson–Hasselbalch equation**:

$$pH = pK_A + \log_{10} \frac{[\text{coniugate base}]}{[\text{acid}]}$$

- Function: maintain **biological pH homeostasis**.

Titration of a weak acid with a strong base

100 ml of acetic acid 0.1 M with NaOH 0.1 M.



0.1 M CH_3COOH

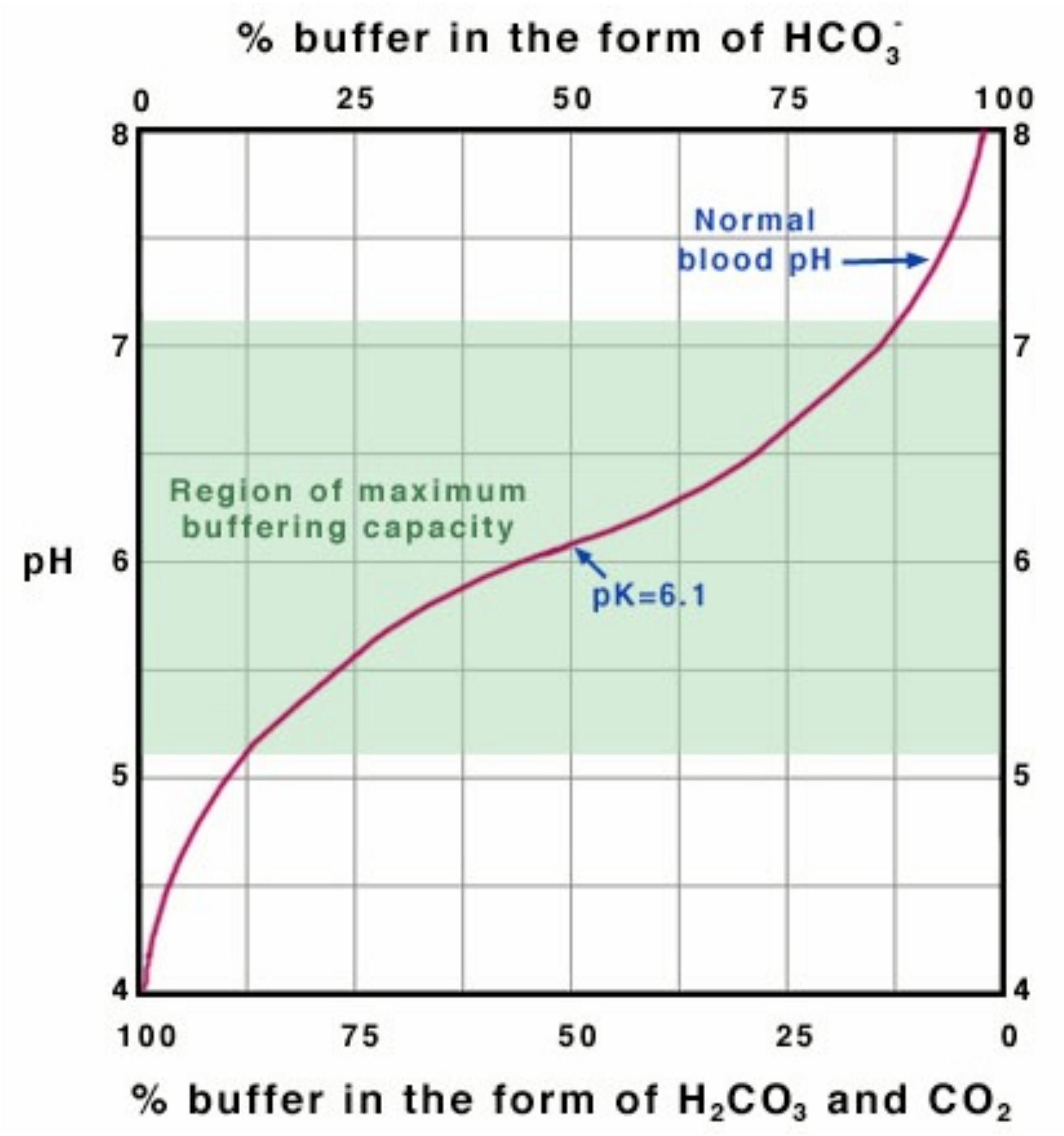
Semi-equivalence point, $[CH_3COOH] = [CH_3COO^-]$ and $pH=pK_A$

Equivalence point, 0.05 M of CH_3COO^-

Buffering region, we have both CH_3COOH and CH_3COO^-

TITRATION CURVE OF BICARBONATE BUFFER

CO_2 (gas) ⇆ CO_2 (aq) + H_2O ⇆ H_2CO_3 ⇆ HCO_3^- + H^+



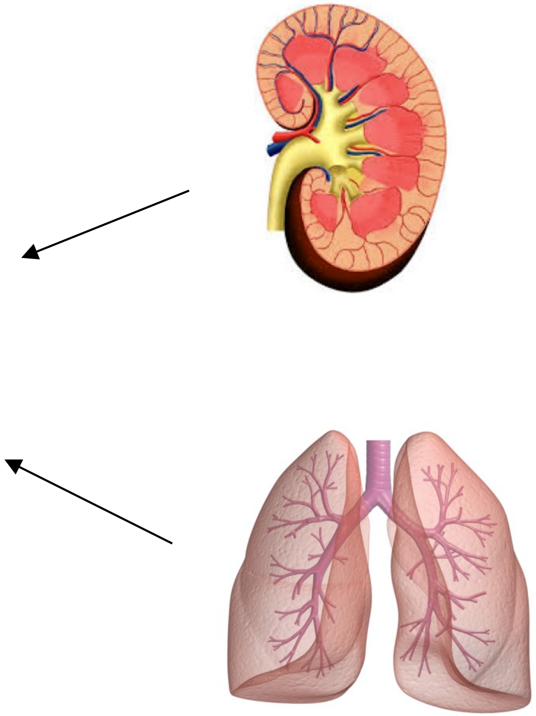
A pH = 7.4 other mechanisms are also acting to control the concentrations of HCO_3^- e CO_2 necessary to keep pH constant

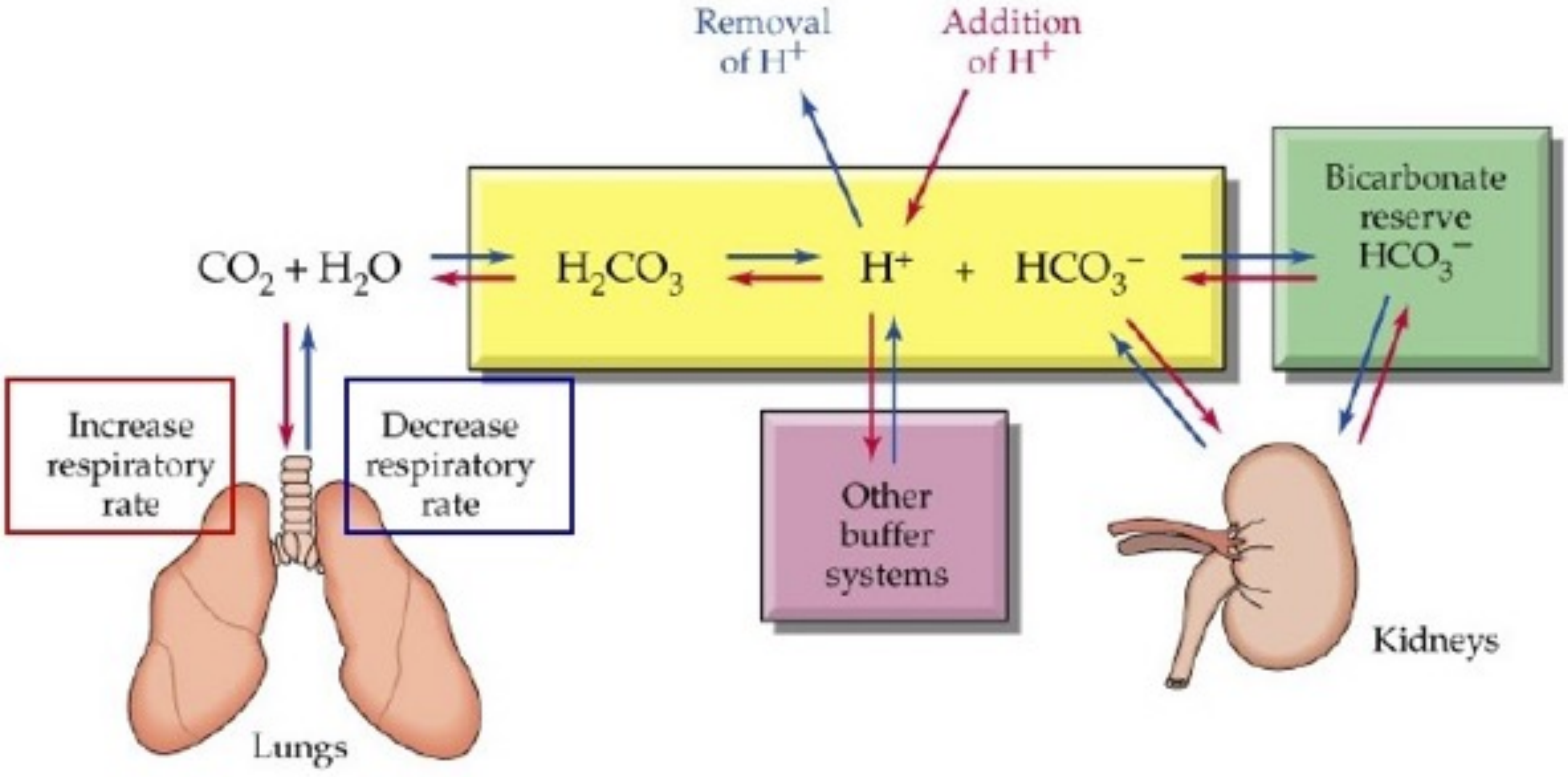
Biological relevance

- Blood buffer system: **bicarbonate / carbonic acid**
- Other biological buffers: phosphate buffer, protein side chains (e.g., histidine).
- Engineering relevance: design of **nanomaterials, biosensors, drug delivery systems** requires buffer environments to control stability and activity.

$$CO_2\,(\text{gas}) \leftrightarrows CO_2\,(\text{aq}) + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+$$

$$pH = pK'_{a1} + \log \frac{\left[HCO_3^-\right]}{\left[CO_2\right]_{AQ}}$$

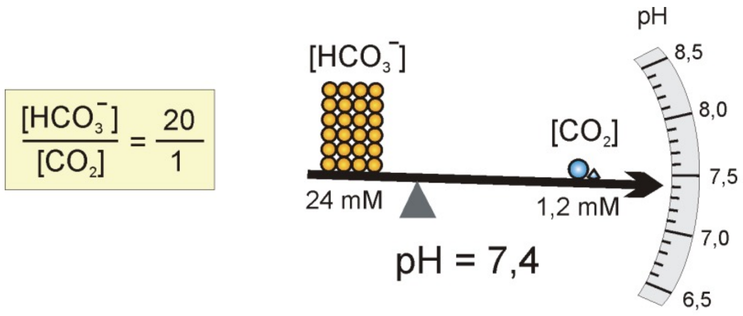



Removal of H^+
Addition of H^+
$CO_2 + H_2O$
H_2CO_3
H^+ + HCO_3^-
Bicarbonate reserve HCO_3^-
Increase respiratory rate
Decrease respiratory rate
Other buffer systems
Lungs
Kidneys

The buffer system CO2 / bicarbonate is efficient only in a open system because it is far from the maximum buffering power

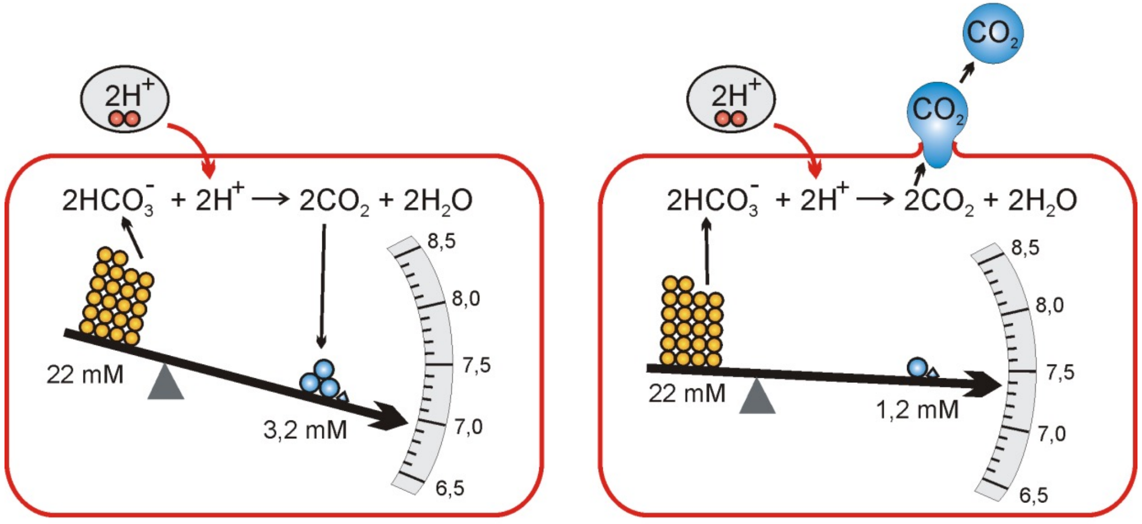
In normal conditions

$$\frac{HCO_3^-}{CO_2} = \frac{20}{1}$$



Increased acidity

$2H^+$



sistema “chiuso” pH = 6,93

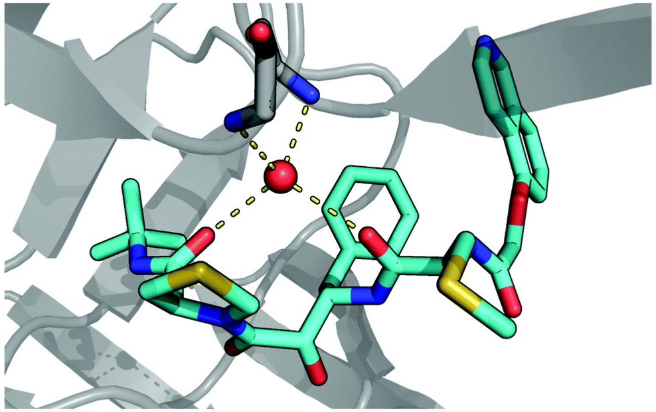
sistema “aperto” pH = 7,36

An increase in the velocity of the CO_2 exchange between capillary and alveolus is sufficient in an open system to partially compensate for the increase of H^+ (acidosis) or OH^- (alkalosis)

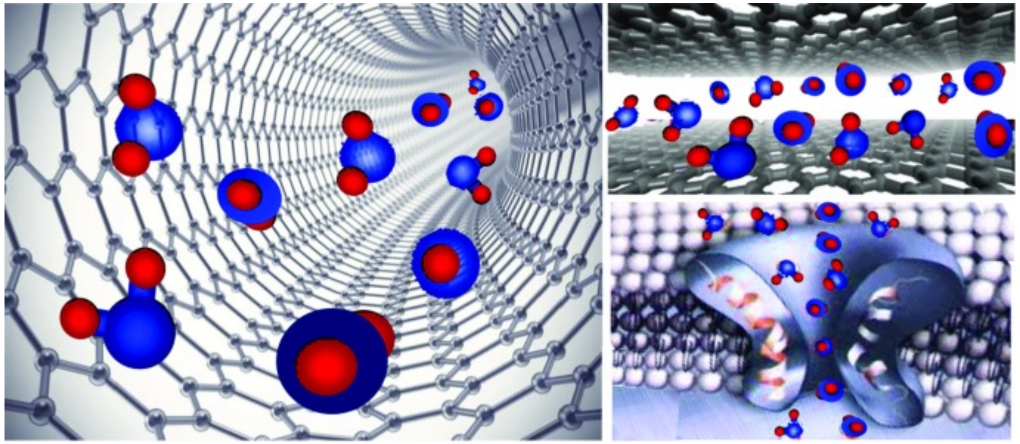
Confined water and crowding

In cells, water ≠ bulk water.

- Water is confined in narrow spaces
  (protein cavities, membranes, cytoskeleton mesh).
- Reduced mobility and altered hydrogen-bonding network.
- Leads to different physical properties than bulk water.



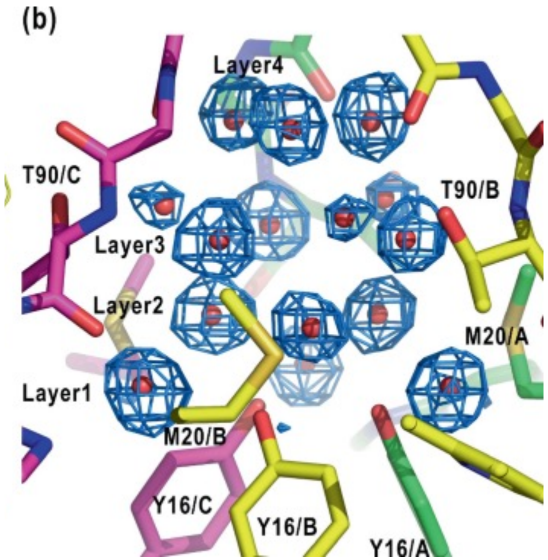
Samways ML , Taylor RD , Bruce Macdonald HE , Essex JW . Water molecules at protein-drug interfaces: computational prediction and analysis methods. Chem Soc Rev. 2021 Aug 21;50(16):9104-9120. doi: 10.1039/d0cs00151a.



Corti, H.R. *et al.* Structure and dynamics of nanoconfined water and aqueous solutions. *Eur. Phys. J. E* **44**, 136 (2021). https://doi.org/10.1140/epje/s10189-021-00136-4

Confinement alters pH, kinetics, solubility

- pH shifts: proton mobility is altered in nanoconfinement (different buffering).
- Kinetics: enzymatic reactions speed up or slow down depending on hydration layer.
- Solubility: proteins, salts, and metabolites may behave differently in crowded environments.



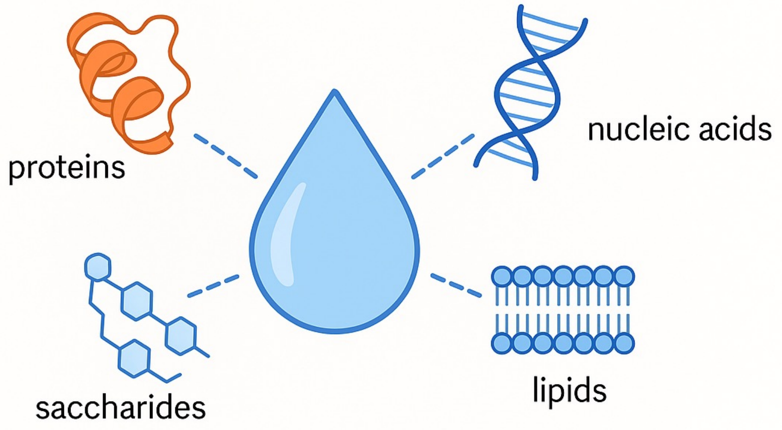
Water as a unifying factor across all macromolecules

- All macromolecules (proteins, nucleic acids, lipids, saccharides) depend on structured water layers.
- Water mediates stability, folding, recognition, and interactions.
- Without water, macromolecules lose their biological meaning (and properties).

Take-home message

- Water = the hidden architect of biochemistry
- Every macromolecule is in constant interaction with water
- Designing nano-biosystems means also designing water

Water as a unifying factor across macromolecules



Proteins, nucleic acids, lipids, saccharides –
all depend on water.

References in the presentation.

- Wang, B., Jiang, W., Dai, X. *et al.* Molecular orbital analysis of the hydrogen bonded water dimer. *Sci Rep* **6**, 22099 (2016). https://doi.org/10.1038/srep22099
- J O Richardson *et al*, *Science*, 2016, **351**, 1310 10.1126/science.aae0012
- Chandler, D. Interfaces and the driving force of hydrophobic assembly. *Nature* **437**, 640–647 (2005). https://doi.org/10.1038/nature04162
- Hoshino, Y., Lee, H. & Miura, Y. Interaction between synthetic particles and biomacromolecules: fundamental study of nonspecific interaction and design of nanoparticles that recognize target molecules. *Polym J* **46**, 537–545 (2014). https://doi.org/10.1038/pj.2014.33
- Gahbauer, Stefan & Böckmann, Rainer. (2016). Membrane-Mediated Oligomerization of G Protein Coupled Receptors and Its Implications for GPCR Function. Frontiers in Physiology. 7. 10.3389/fphys.2016.00494.
- Webber, Matthew & Pashuck, E.. (2021). (Macro)molecular self-assembly for hydrogel drug delivery. Advanced Drug Delivery Reviews. 172. 10.1016/j.addr.2021.01.006.
- Corti, H.R. *et al.* Structure and dynamics of nanoconfined water and aqueous solutions. *Eur. Phys. J. E* **44**, 136 (2021). https://doi.org/10.1140/epje/s10189-021-00136-4
- Sharp, Kim. (2001). Water: Structure and Properties. 10.1038/npg.els.0003116.

References for further resding.

- Chandler D. Interfaces and the driving force of hydrophobic assembly. Nature 437, 640–647 (2005). doi:10.1038/nature04162
- Ball P. Water as an active constituent in cell biology. Chem. Rev. 108, 74–108 (2008). doi:10.1021/cr068037a
- Bagchi B. Water in Biological and Chemical Processes: From Structure and Dynamics to Function. Cambridge Univ. Press (2013).
- Franks F. Water: A Matrix of Life. 2nd edition. Royal Society of Chemistry (2000).
- Phillips R., Kondev J., Theriot J., Garcia H. Physical Biology of the Cell. 2nd edition. Garland Science (2012).
- Petsko G.A., Ringe D. Protein Structure and Function. Oxford Univ. Press (2003).