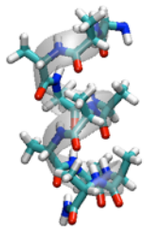


# Non-covalent interactions and how macromolecules fold

**Lecture 4:** The hydrophobic effect

Dr Philip Fowler

First-year Biophysics course

**Objective:**

What is hydrophobicity?

What is the experimental evidence for the formation of hydrogen bond networks by water?

What is the temperature dependence of the hydrophobic effect for neopentane and what is the physical (i.e. molecular) description that accounts for this temperature dependence?

Can we correlate the hydrophobic effect with any molecular properties?

What are the consequences for the folding of biological macromolecules, especially proteins?

**Summary:**

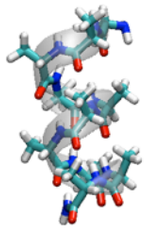
Oil and water don't mix; this is because oil is 'hydrophobic'.

Water has many anomalous properties that can be explained by its propensity to form hydrogen bonding networks that 'melt out' with increasing temperature.

The temperature dependence of the hydrophobic effect is complex; at physiological temperatures the hydrophobic effect is entropically driven.

The hydrophobic effect correlated with the accessible surface area of small molecules.

The hydrophobic effect is the primary driver of protein folding.



# Oil and water don't mix: oil is hydrophobic

water

fearing

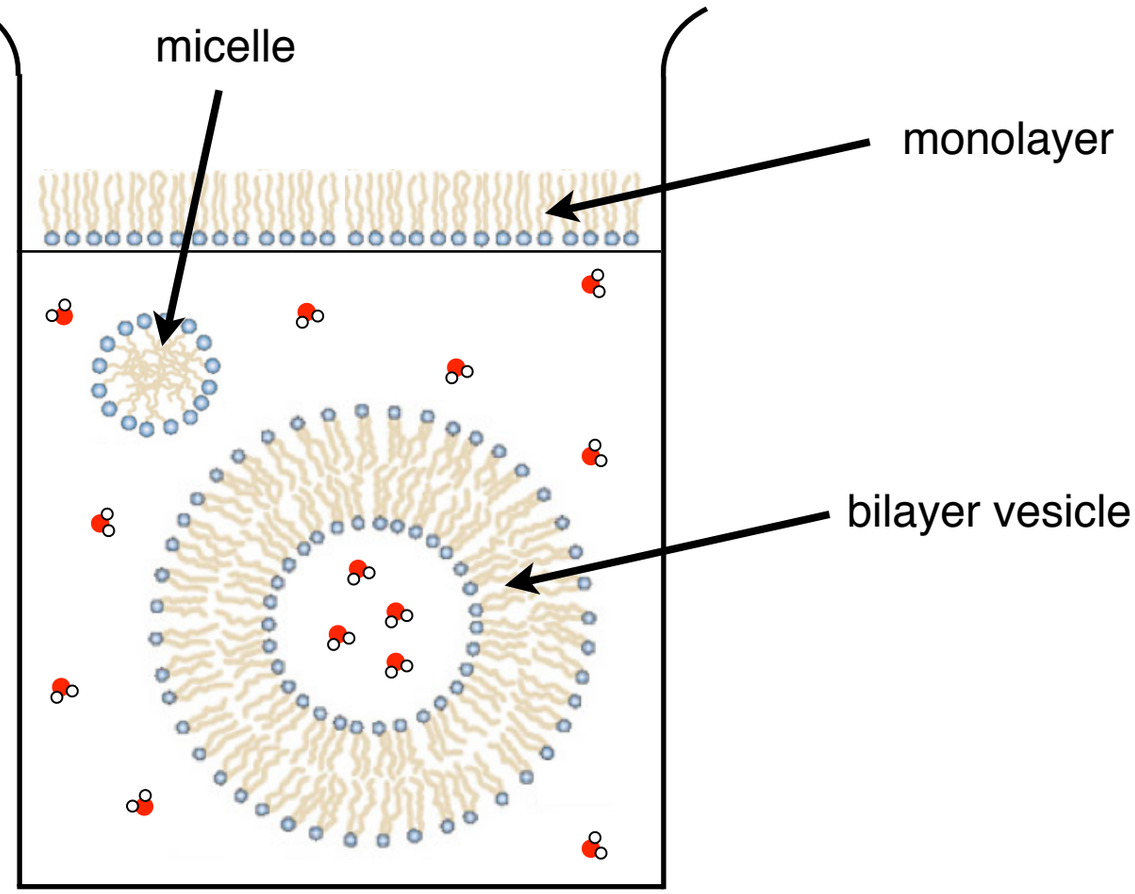
We intuitively know this and can explain simple effects without knowing the physics

polar headgroup

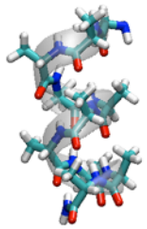
non-polar hydrocarbon tails

e.g. when added to water lipids form regular structures to bury their non-polar tails and put their polar headgroups at the interface between the lipids and water

oil and water

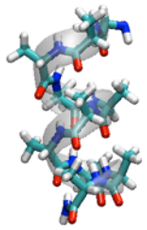



http://www.youtube.com/watch?v=I_DelF5XJYM

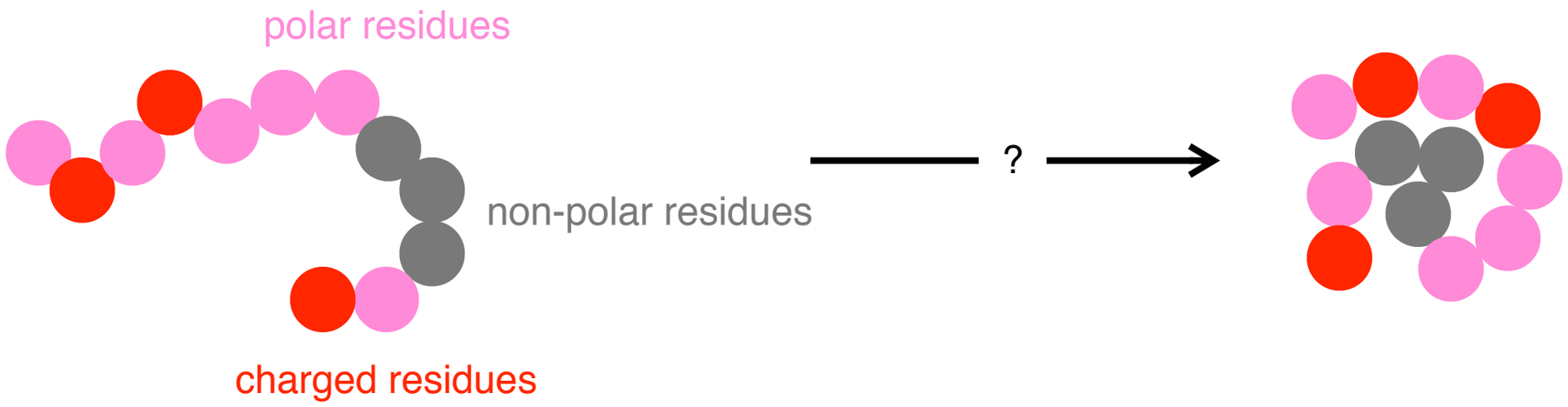


# The Louisiana oil slick was a very large monolayer of oil


Picture: NASA/CSTARS-University of Miami/GETTY IMAGES



# ..but proteins are polymers with irregular sequences of amino acids




Proteins do not form simple structures when added to water

The **hydrophobic effect** is the most important energetic driving force in the folding of proteins and other biological macromolecules

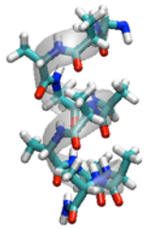
It is difficult to understand

It results from the unusual properties of water e.g. proteins will denature if placed in non-aqueous solvent

Another reason why statements like below are incorrect:

> "..the **information** needed to fold a macromolecule into its native 3D structure is **contained** within its sequence."
>
> -van Holde, 2nd edition, pg 107

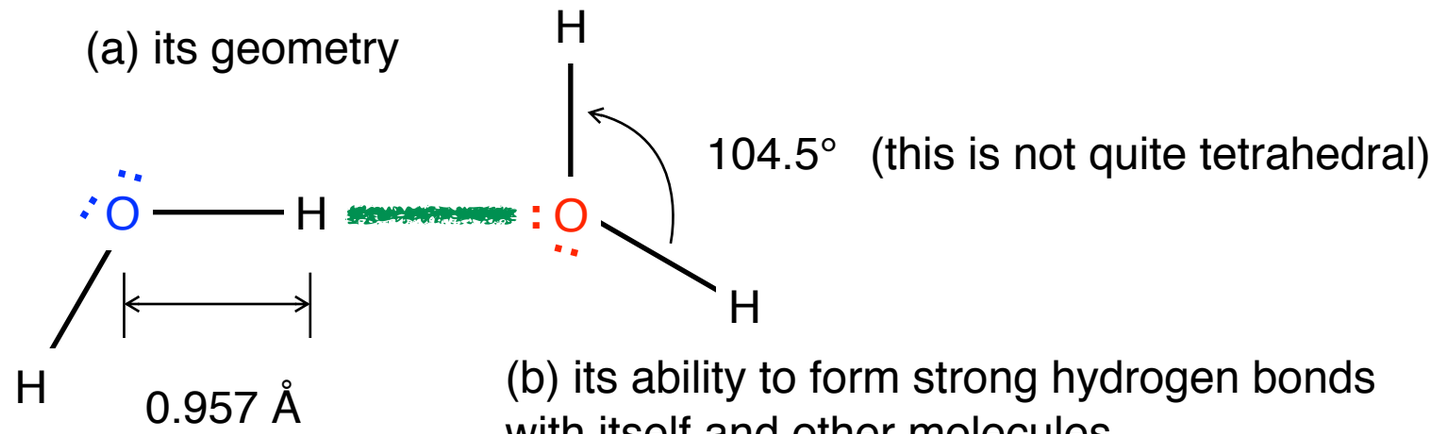
Voet (3rd ed) pg 26 van Holde (2nd ed) pg 16, 153 Creighton (2nd ed) pg 156



# Properties of Water

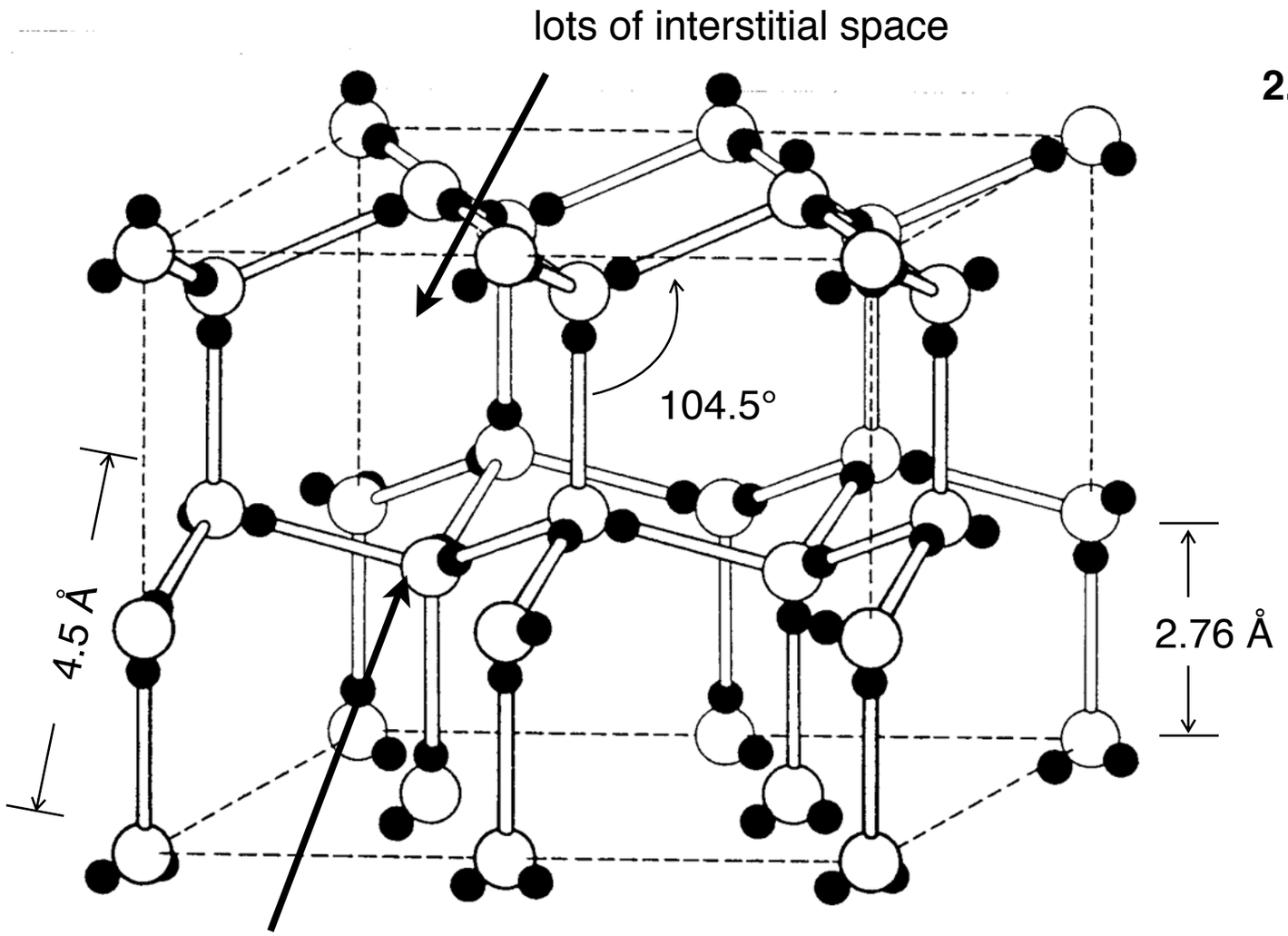
The unusual properties of water stem from:

(a) its geometry




(b) its ability to form strong hydrogen bonds with itself and other molecules

The structure of ice

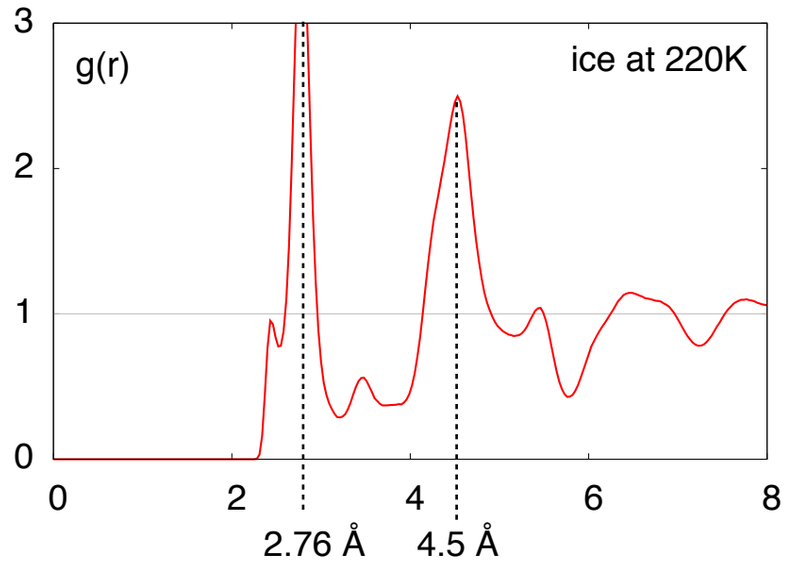


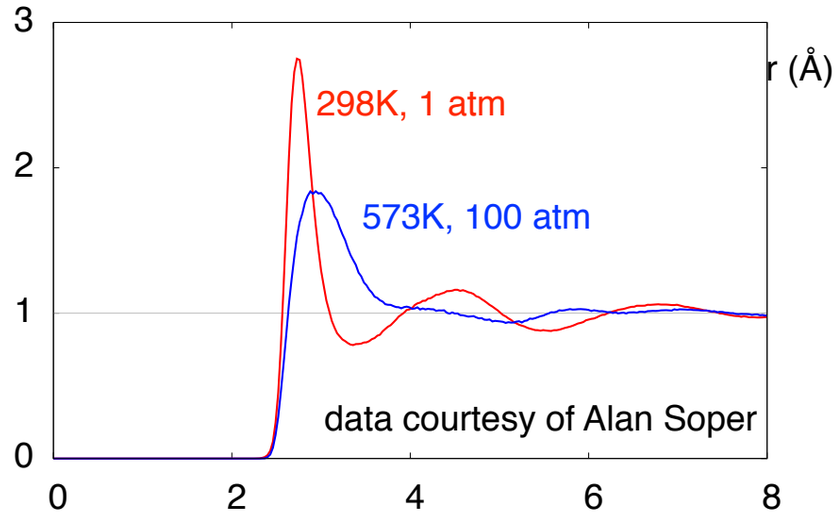

each water accepts a hydrogen from 2 neighbours and donates a hydrogen to 2 other neighbours:

4 nearest neighbours
12 next-nearest neighbours

**1. high molar volume when liquid**

**2. radial distribution functions**




Science (2002) **297** 1288, Chem. Phys. (2000) **258** 121

# Properties of Water

**3. High $C_p$ (specific heat capacity)** additional energy required to break hydrogen bonds

**4. High surface tension**

**5. Infrared absorption spectra**

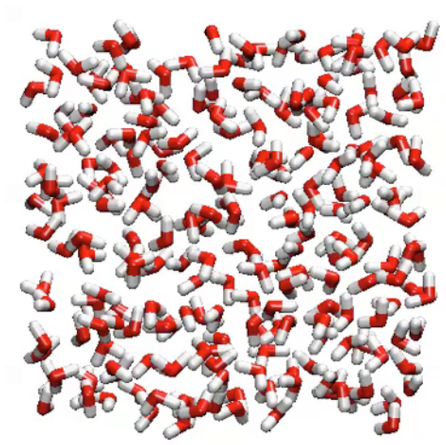
Frequency $\propto$ Strength (energy) of covalent bond

| Ice | Water | Steam |
|---|---|---|
| 3220 cm | 3490 cm | 3700 cm |

← Strength of OH bond increases as hydrogen bonding decreases →

**6. High dielectric constant**

positively charged

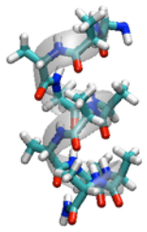


negatively charged

| | $\mu$ (D) | $\varepsilon$ |
|---|---|---|
| water | 3.37 | 78.5 |
| methanol | 3.96 | 32.6 |
| chloroform | 1.15 | 4.8 |
| CCl | 0 | 2.2 |
| hexane | 0 | 1.9 |

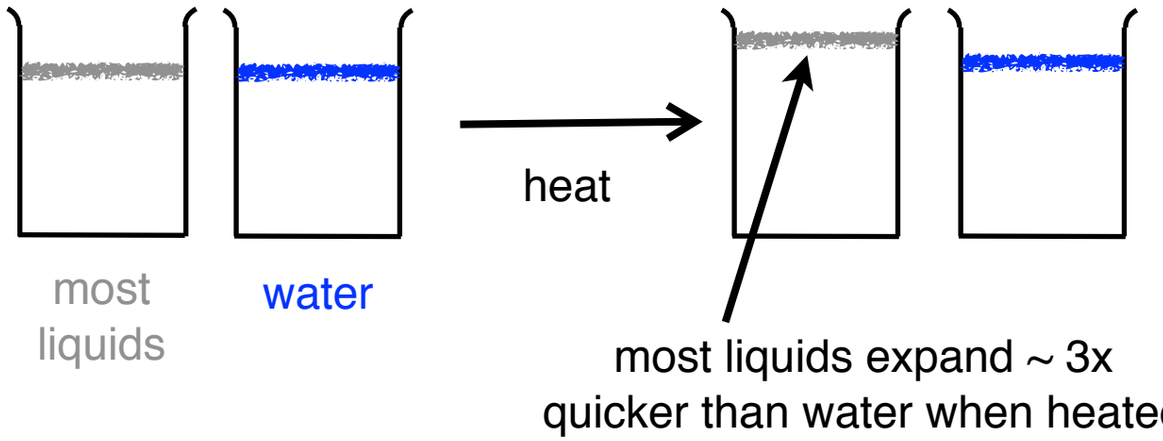
← for the magnitude of its dipole moment water has an anomalously high dielectric constant

when the electric field starts to align the water molecules, hydrogen bonding allows water to stabilise the new conformation

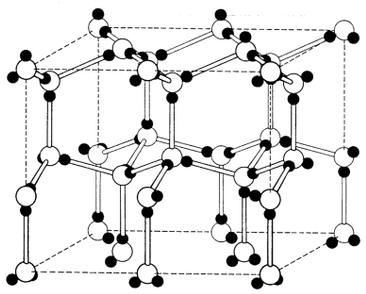


# Properties of Water

## 7. Small thermal expansion coefficient



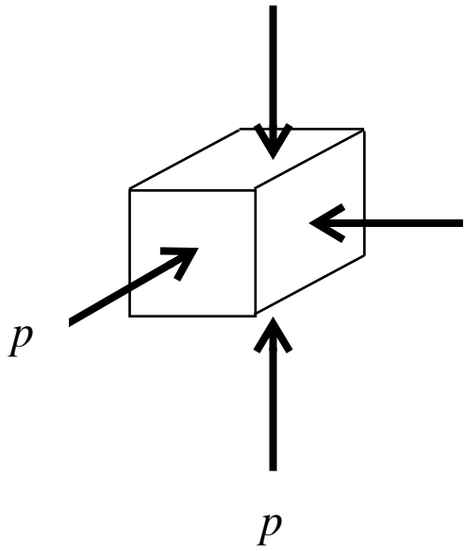

as the temperature is increased the structured volume of water decreases as hydrogen bonds are broken



interstitial space begins to fill as hydrogen bonds broken

interstitial space filled

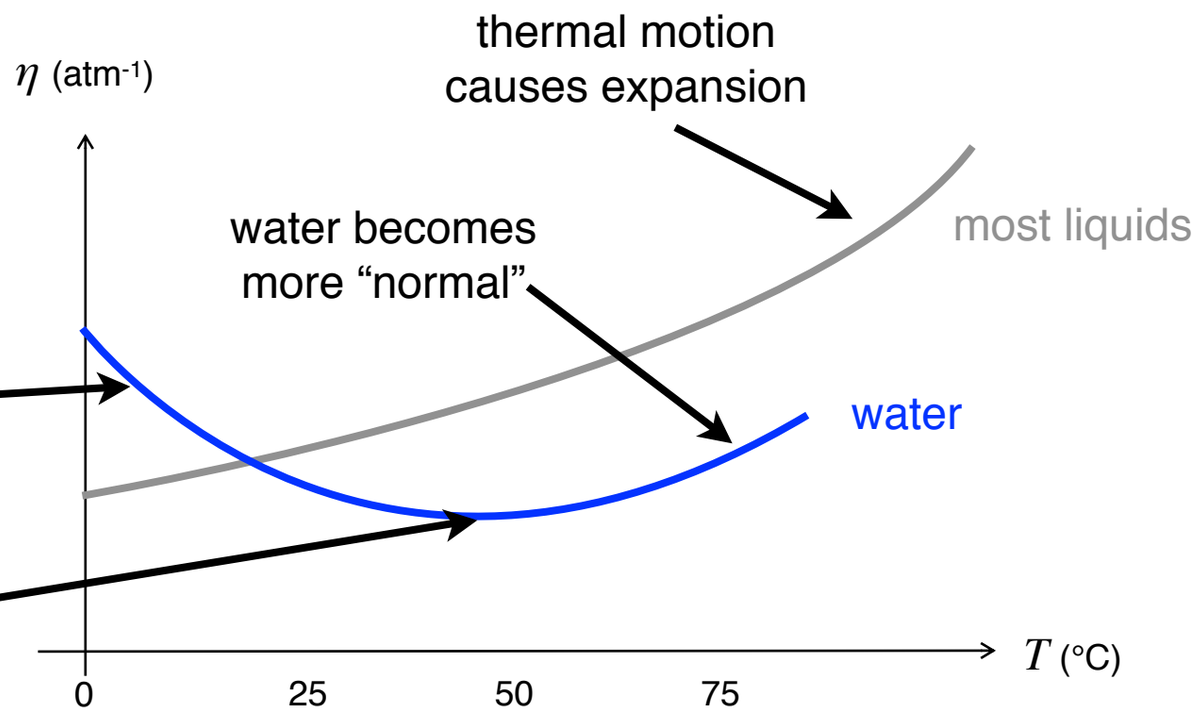
## 8. anomalous isothermal compressibility coefficient

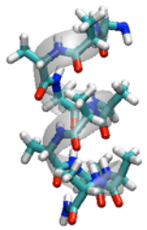



the isothermal compressibility coefficient, $\eta$, measures how much the volume, $V$, changes when put under pressure, $p$

$$\eta = -\frac{1}{V}\left(\frac{\partial V}{\partial p}\right)$$

most liquids: $\eta \uparrow \quad T \uparrow$

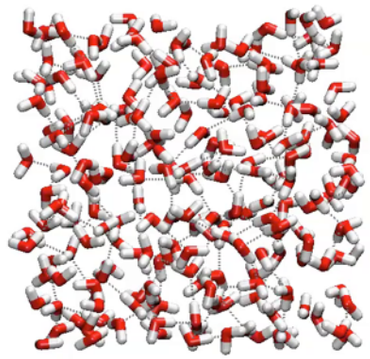


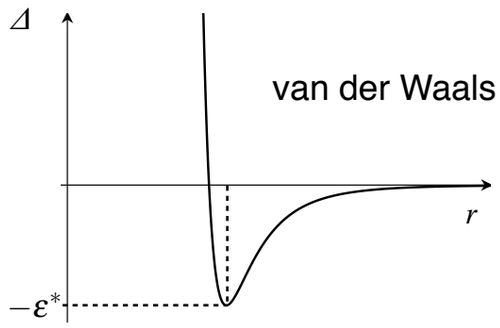


# The hydrophobic effect results from...

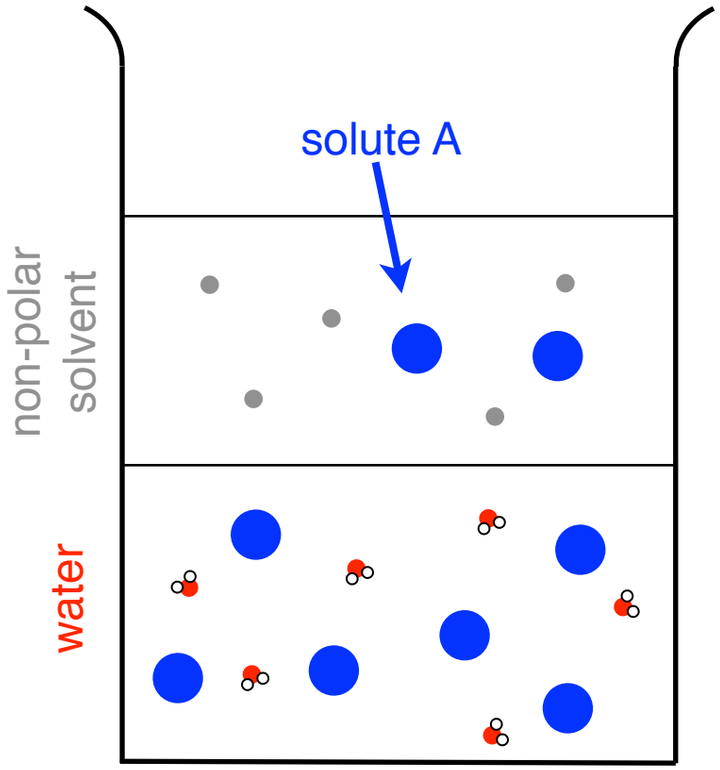
**1**. the extensive and cohesive hydrogen bonding networks in water

**2**. non-polar molecules having stronger interactions with themselves than with water






We can characterise it by measuring the **free energy** of transferring a solute from a non-polar solvent to water:




partition coefficient

$$K_p = \frac{X\ (H_20)}{X\ (\text{non-polar solvent})}$$

mole fraction (i.e. [A]) in water

but $K_p$ will vary depending on the [solute], $X_A$

transfer free energy

$$\Delta G = \lim_{X_A \to 0} \left(-RT \ln K_p\right)$$

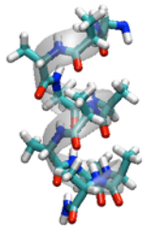
gas constant = 8.314 J $K^{-1}$ $mol^{-1}$

| $K_p$ | $\Delta G$ |
|---|---|
| > 1 | < 0 |

usually just quoted as

$$\Delta G = -RT \ln K_p$$

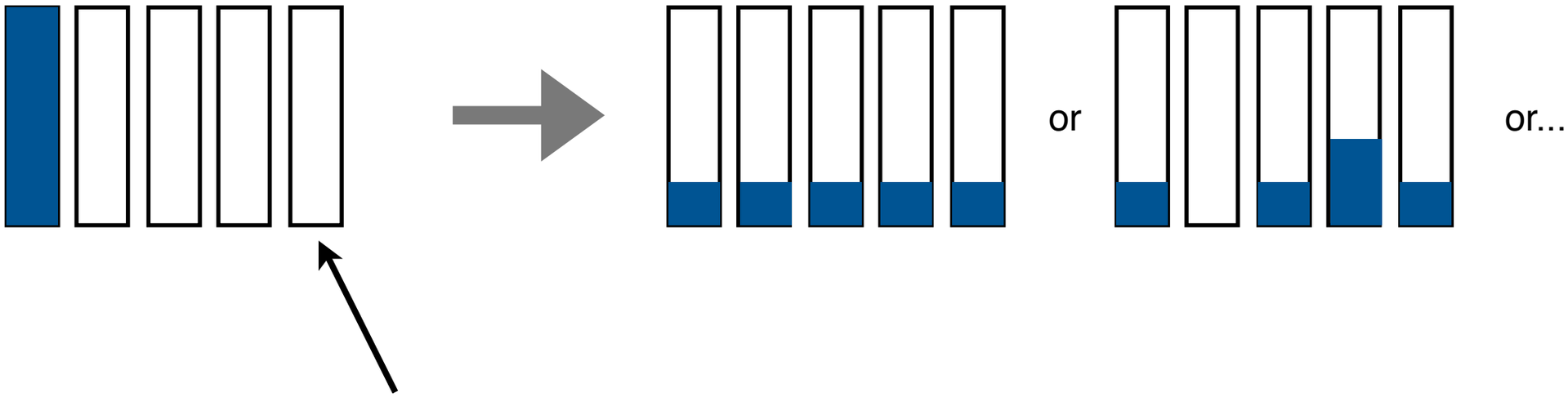
solute A partitions into water



# Entropy as energy dispersal: reminder

Macroscopic level: entropy measures how dispersed the energy is (usually between molecules)

Molecular level: entropy measures how dispersed the energy is (including within a molecule)




each bar could be

individual molecules

energy states within individual molecules
(e.g. translational, rotational, vibrational, electron states)

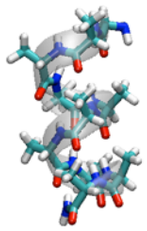
Boltzmann's constant
$k = 1.38 \times 10^{23}$ J/K

to determine how dispersed the energy is imagine counting the number of ways the energy could be arranged

Entropy (J/K)

$$S = k \ln W$$

fewer ways = more ordered
(but not like a deck of cards!)



# A large specific heat capacity is characteristic of the hydrophobic effect

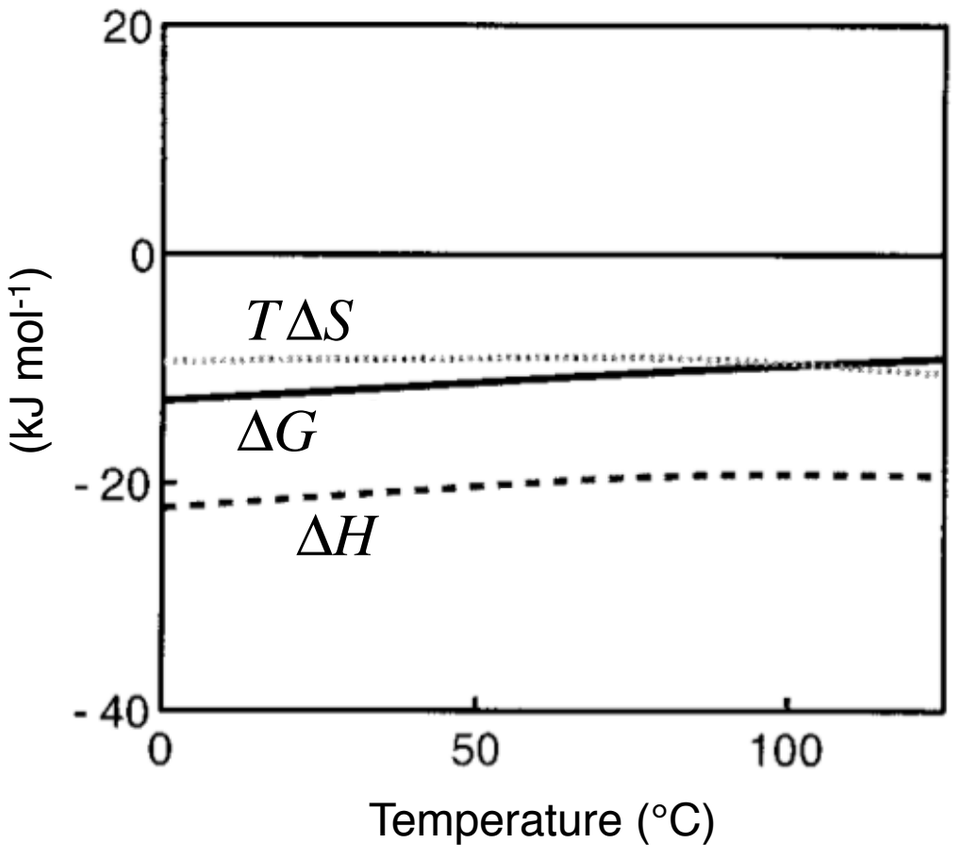
$$\Delta C_p = \frac{\partial \Delta H}{\partial T} = T\frac{\partial \Delta S}{\partial T}$$

the change in heat capacity tells us how quickly the transfer enthalpy and entropy are changing with temperature

→ a large $\Delta C_p$ tells us that $\Delta H$ and $T\Delta S$ are changing very rapidly with temperature

**low $C_p$ : 'normal'**

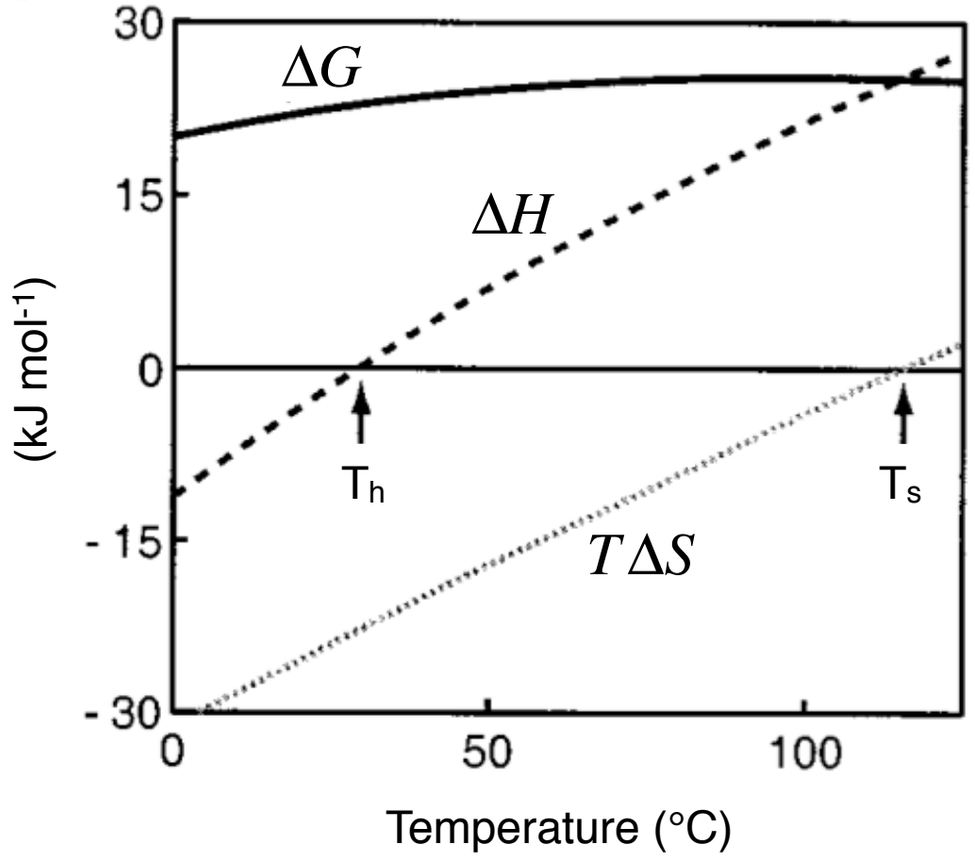
pentane$_{(gas)}$ → pentane$_{(liquid)}$




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

**anomalously high $C_p$ : hydrophobic effect**

pentane$_{(liquid)}$ → pentane$_{(aq)}$




→ $\Delta G > 0$ for $0 < T < 100$ °C and therefore pentane does not dissolve in water

→ can we develop a physical (i.e. molecular) explanation that could also be applied to proteins?

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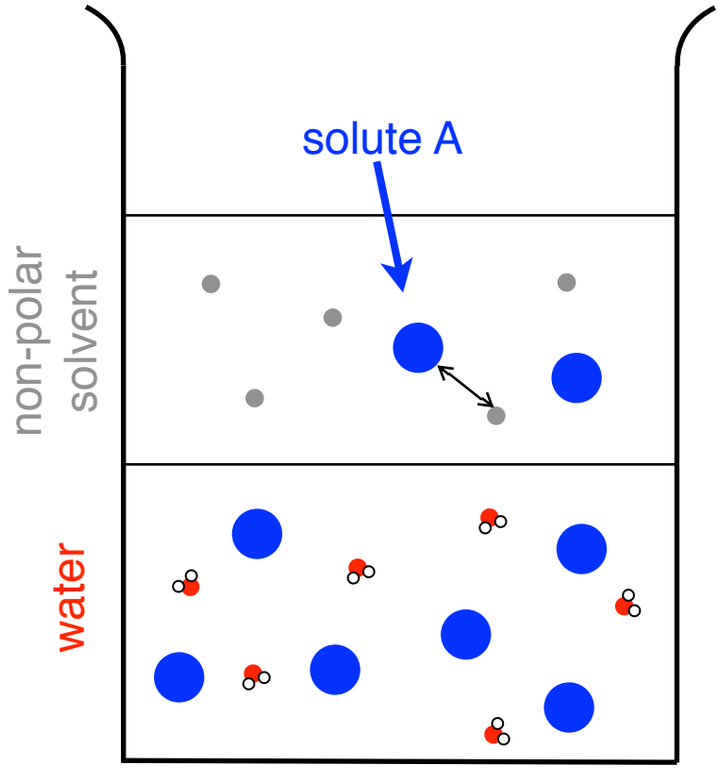
# Possible molecular contributing factors to the free energy of transfer



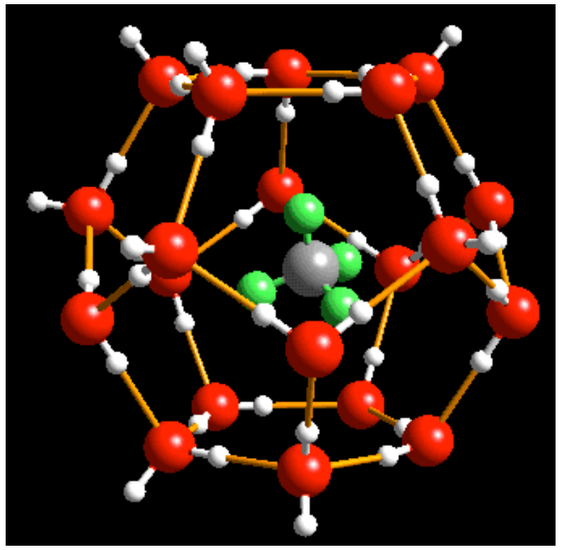

$\Delta H$: interactions between **solute** and **non-polar solvent**

$\Delta H$: interactions between **solute** and **water**

$T\Delta S$: changes in the local order* of the **water** due to the **solute**

$\Delta H$: resulting changes in the number of **water** hydrogen bonds

For example, when water and some non-polar molecules are crystallised they are observed to form **clathrates.**



a clathrate

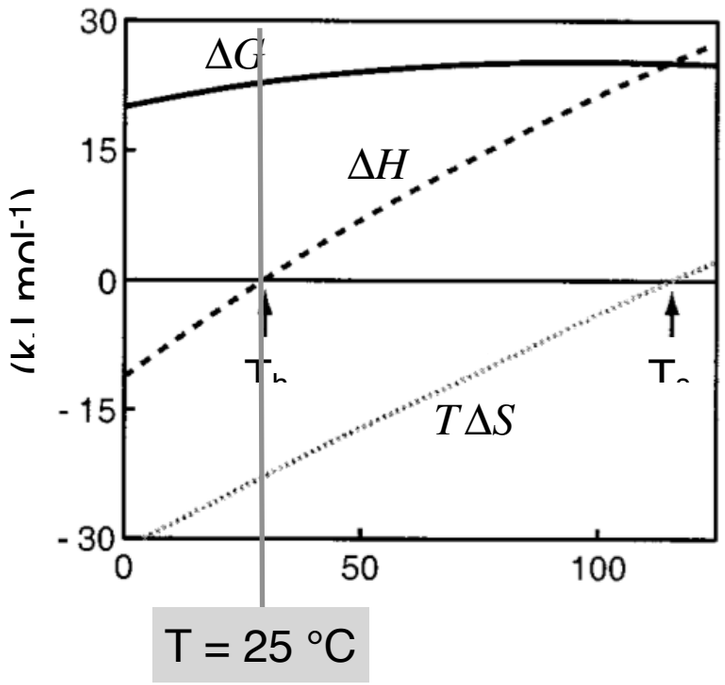
The water forms a highly-ordered ‘cage’ around the non-polar molecule

This is a extreme illustration of the local structure that water can adopt

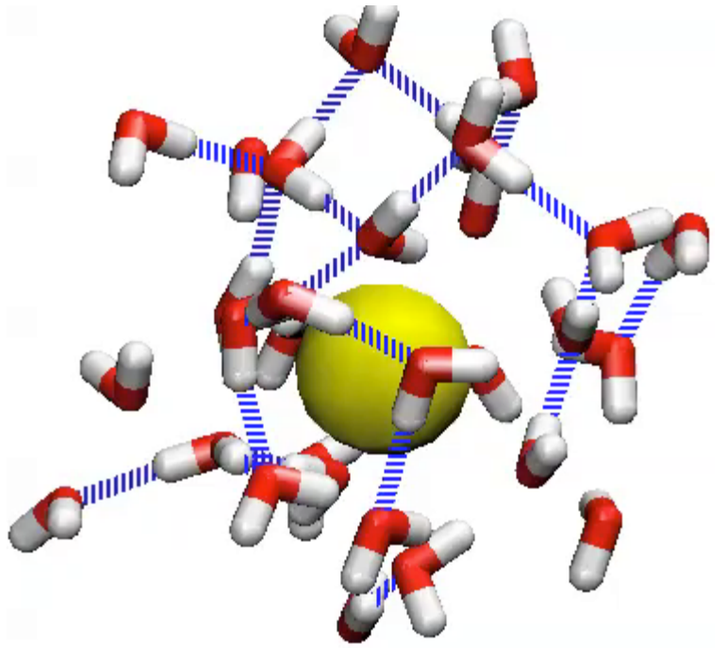
Water does **not** form such highly-ordered structures around proteins at physiological temperature.

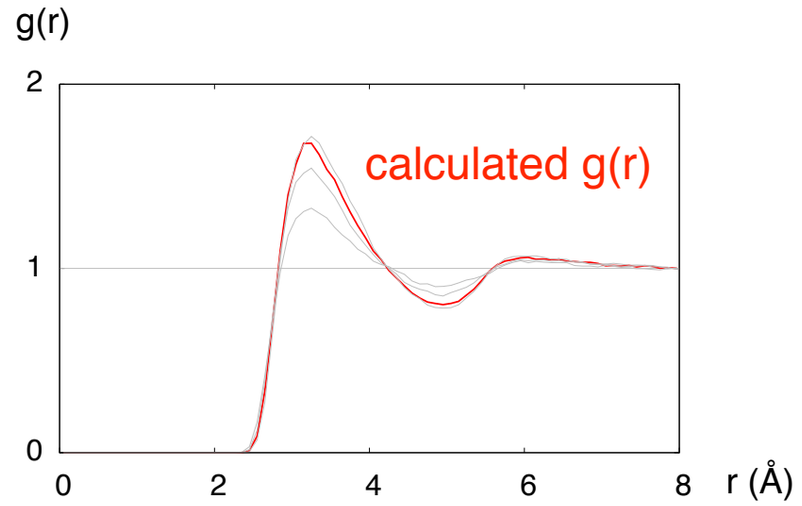
* hence changing the number of energy states of the water

# At physiological temperature the hydrophobic effect is entropically driven




Argon in water (25 °C)






**Caution**: the thermodynamic data is for neopentane and the simulations are of Argon

$\Delta H$

| | | |
|---|---|---|
| 1. no interactions between **solute** and **water** | 0 | |
| 2. interactions between **solute** and **non-polar solvent** lost | > 0 | ☹☹ |
| 3. increase in the number of **water** hydrogen bonds | < 0 | ☺☺ |
| 2 and 3 have about the same magnitude therefore: | $\Delta H = 0$ | |

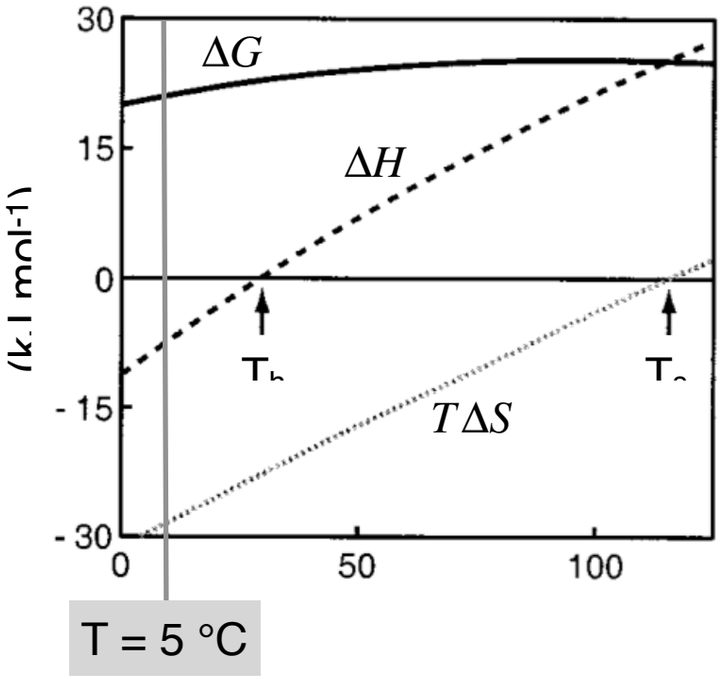
$T\Delta S$

1. the **water** orders itself around the **solute** as it cannot hydrogen bond to it $< 0$ ☹☹

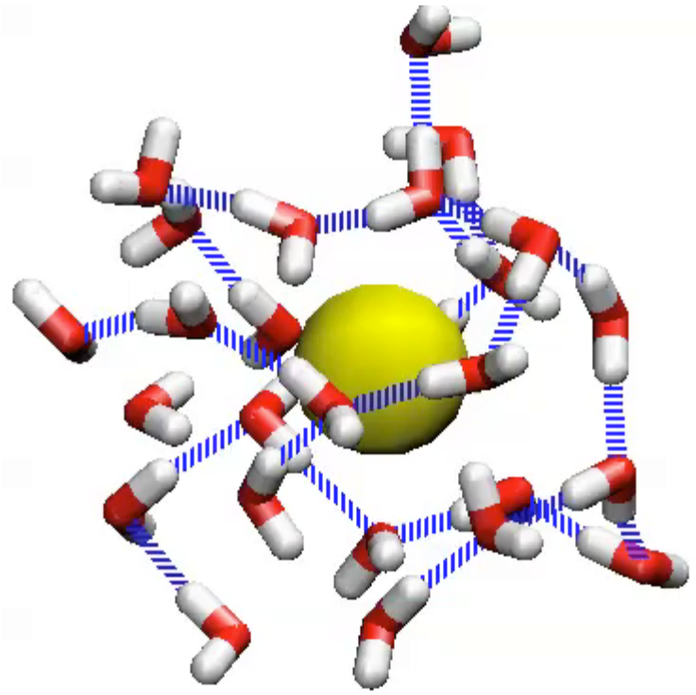
$T\Delta S < 0$

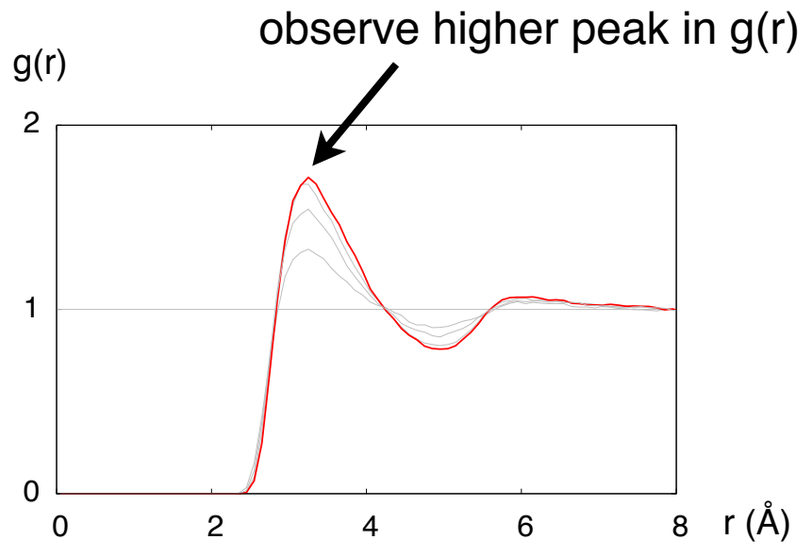
$\Delta G > 0$ driven by the large decrease in entropy as water orders itself around the non-polar solute

# At lower temperatures the water has more local structure




Argon in water (5 °C)



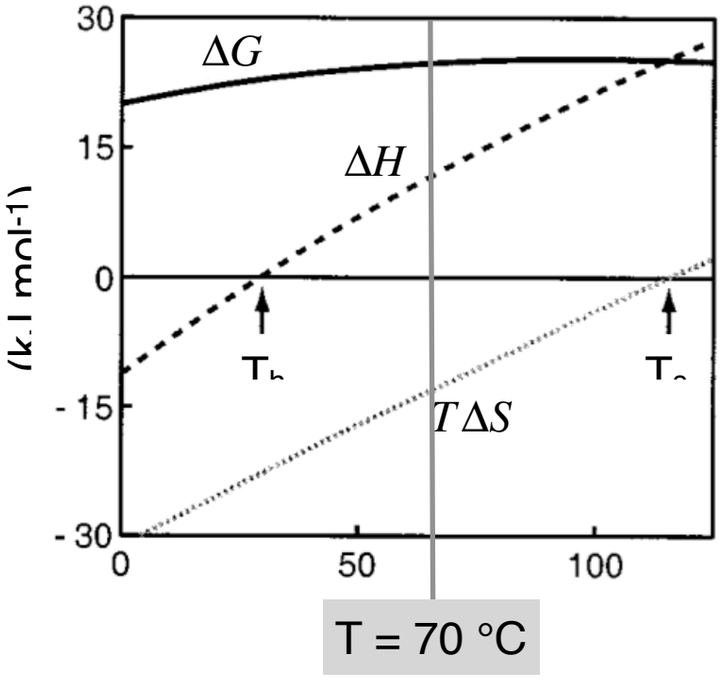



| $\Delta H$ | 25 °C | 5 °C |
|---|---|---|
| 1. no interactions between **solute** and **water** | - | - |
| 2. interactions between **solute** and **non-polar solvent** lost | ☹☹ | ☹☹ |
| 3. increase in the number of **water** hydrogen bonds | ☺☺ | ☺☺☺ |
| now 3 dominates 2: | $\Delta H = 0$ | $\Delta H < 0$ |

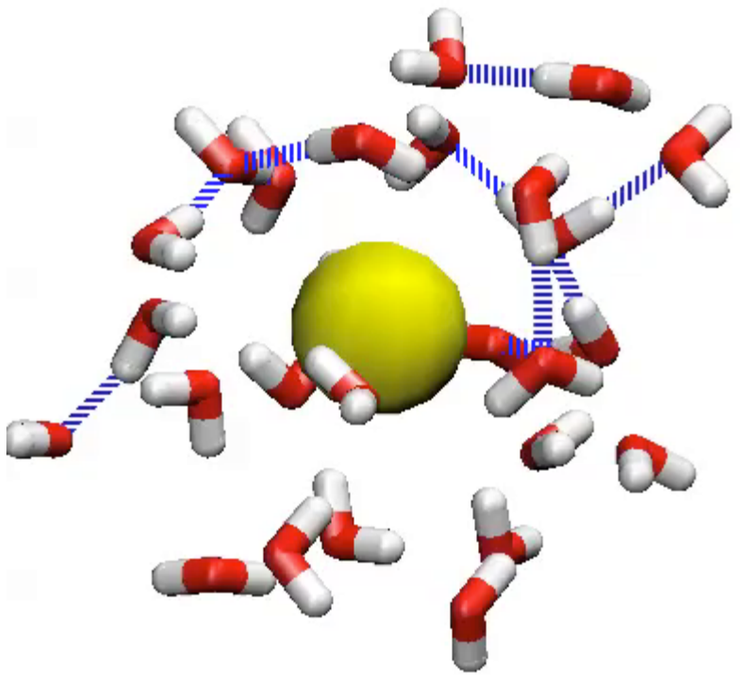
| $T\Delta S$ | 25 °C | 5 °C |
|---|---|---|
| 1. the **water** is more ordered around the **solute** | ☹☹ | ☹☹☹ |
| | $T\Delta S < 0$ | $<< 0$ |

$\Delta G > 0$ and is a balance between an even larger decrease in entropy as the water becomes more structured around the non-polar solute and a corresponding increase in the number of hydrogen bonds between water molecules

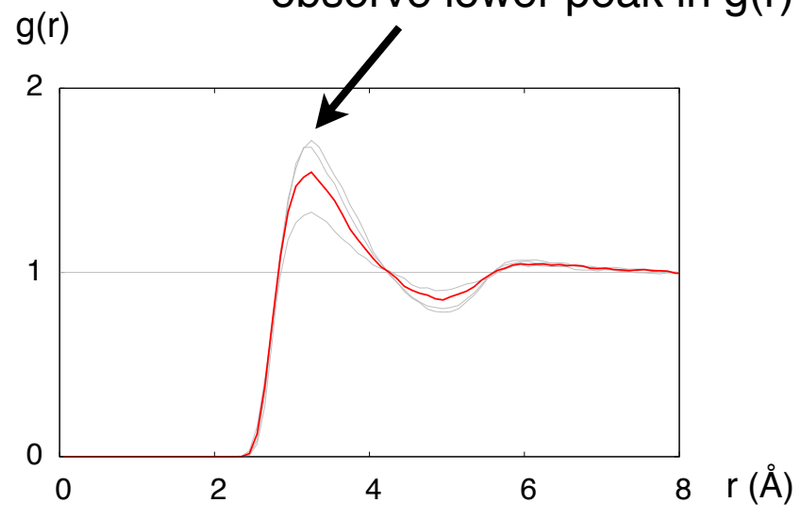
# At higher temperatures the water has less local structure




Argon in water (70 °C)



observe lower peak in g(r)



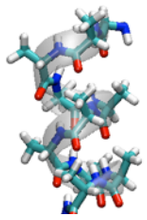

| $\Delta H$ | 25 °C | 70 °C |
|---|---|---|
| 1. no interactions between **solute** and **water** | - | - |
| 2. interactions between **solute** and **non-polar solvent** lost | ☹☹ | ☹☹ |
| 3. increase in the number of **water** hydrogen bonds | ☺☺ | ☺ |
| now 2 dominates 3: | $\Delta H = 0$ | $\Delta H > 0$ |

| $T\Delta S$ | 25 °C | 70 °C |
|---|---|---|
| 1. the **water** is ordered around the **solute** but less so than at physiological temperatures | ☹☹ | ☹ |

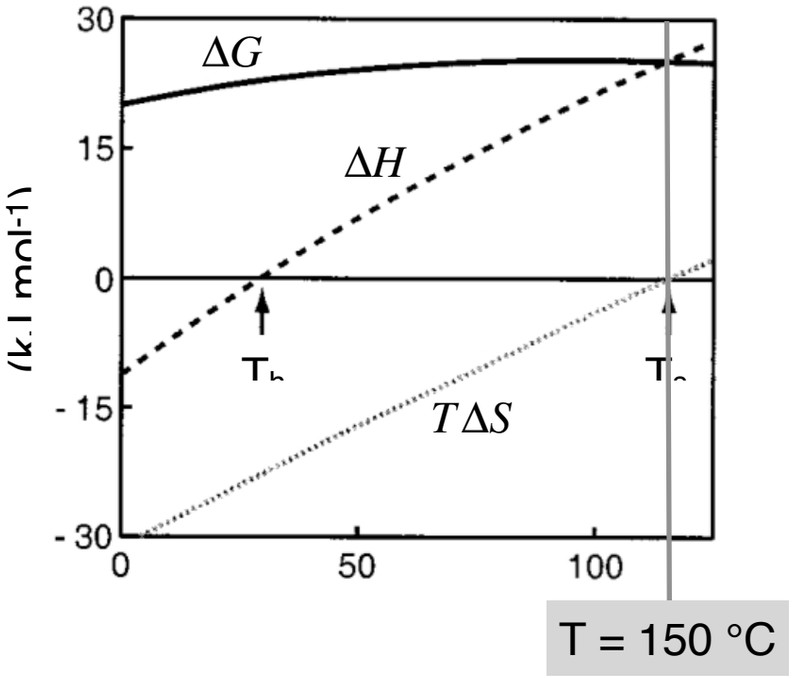
$T\Delta S < 0$

less negative

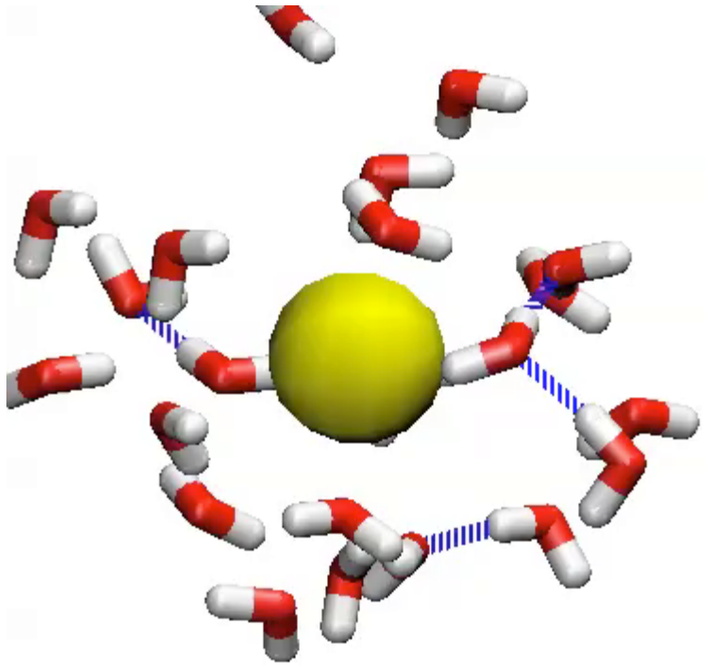
$\Delta G > 0$ and roughly split between the (smaller) decrease in entropy as the water becomes more less structured and the loss of interactions between the solute and the non-polar solvent

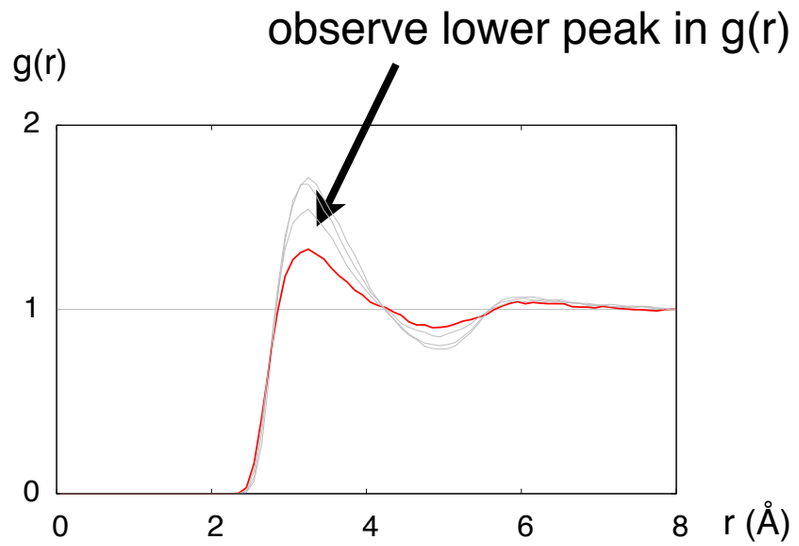


# At even higher temperatures water begins to behave like a normal liquid




Argon in water (150 °C, 100 atm)





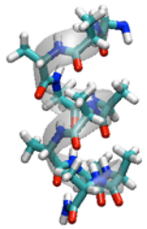

| $\Delta H$ | 25 °C | 150 °C |
|---|---|---|
| 1. no interactions between **solute** and **water** | - | - |
| 2. interactions between **solute** and **non-polar solvent** lost | ☹☹ | ☹☹ |
| 3. increase in the number of **water** hydrogen bonds | ☺☺ | - |
| only 2 contributes: | $\Delta H = 0$ | $\Delta H > 0$ |

| $T\Delta S$ | 25 °C | 150 °C |
|---|---|---|
| 1. the **water** adopts little structure around the **solute** | ☹☹ | - |

$T\Delta S < 0$

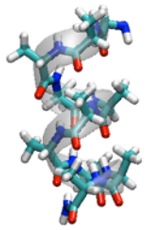
$T\Delta S = 0$

$\Delta G > 0$ and is now dominated by the loss of enthalpic interactions between the solute and the non-polar solvent



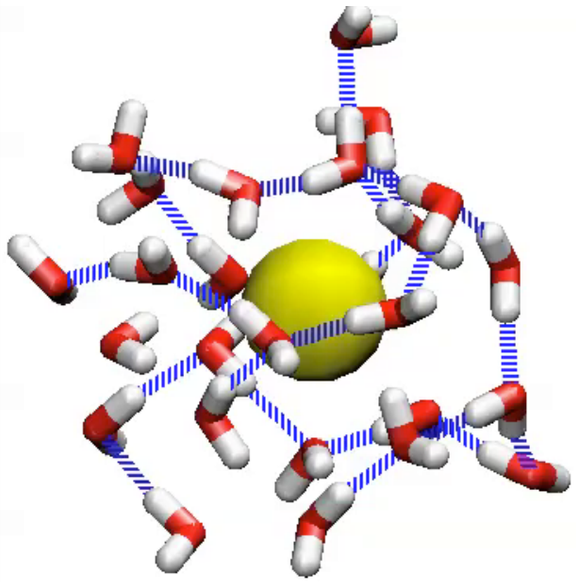
# FoldIt!


http://fold.it/

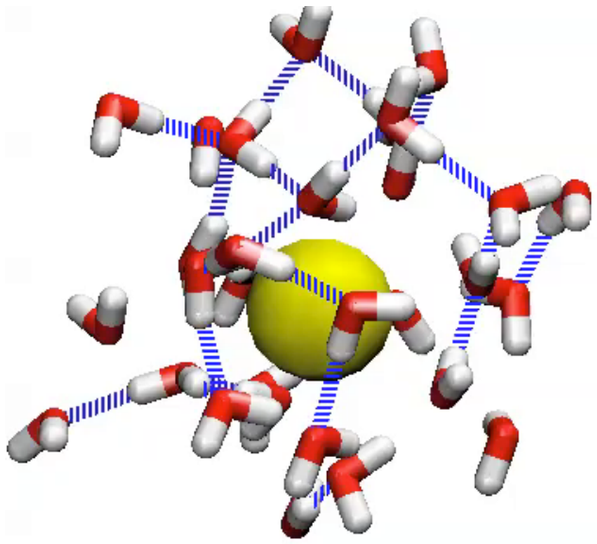


# The hydrophobic effect

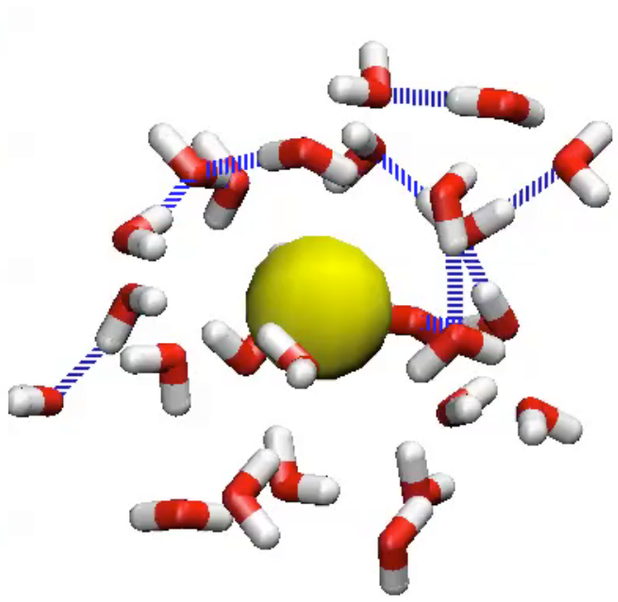
5 °C



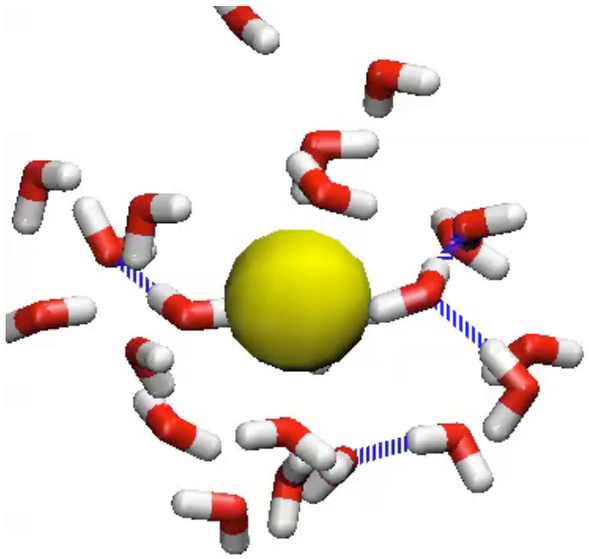
25 °C

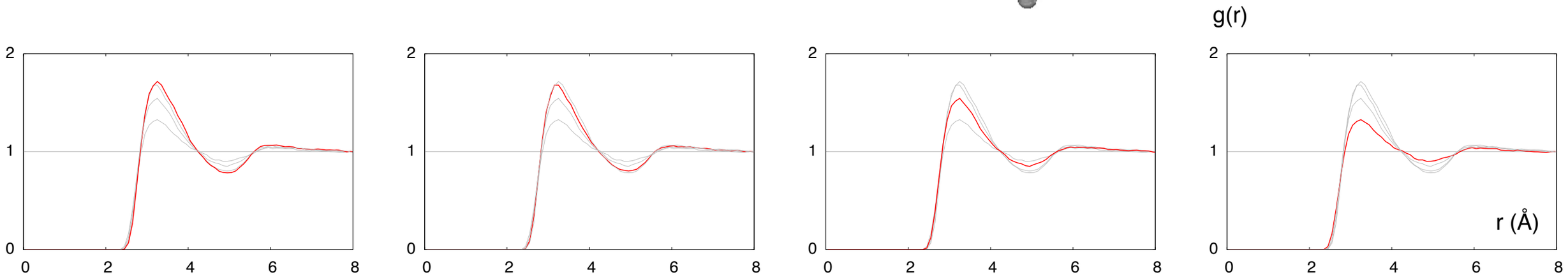


70 °C



150 °C

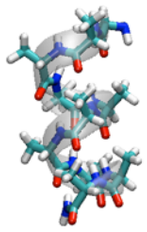





The large heat capacity is because energy is required to break the hydrogen bonds of the water as it is warmed

This physical picture can also be applied to proteins (e.g. cold denaturation) but we must be careful not to infer too much from such simple studies

Nature (2005) **437** 640



# A common misconception: the hydrophobic ‘force’

the hydrophobic effect is often called:

the hydrophobic interaction

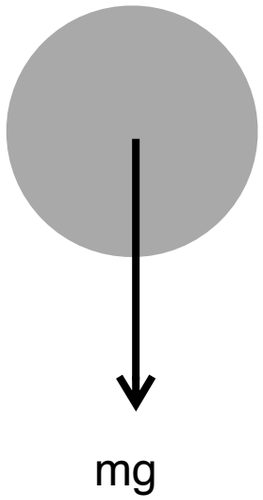
this is ok but ‘interaction’ implies either force (see right) or that the effect is enthalpic in origin (which is often not true)

the hydrophobic force

it is not a force in the sense that gravity is a force

because we can draw a diagram showing how a force due to gravity acts on a body

ball in free fall

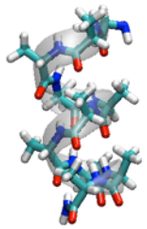



...and we can write a mathematical expression for how large the force is

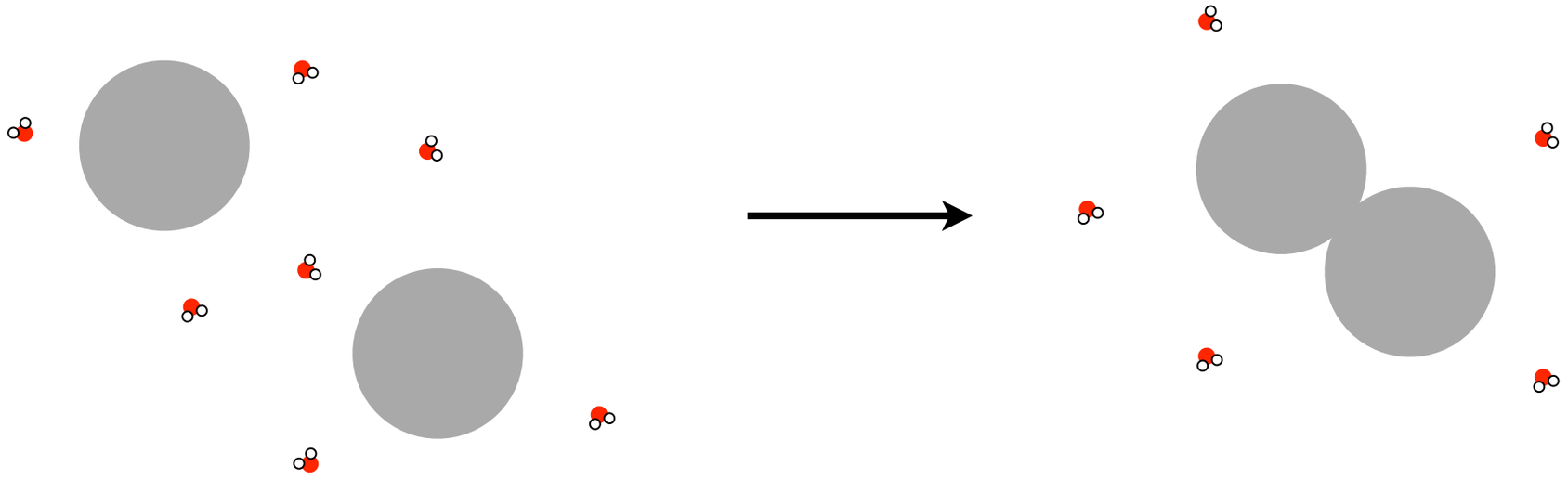
the hydrophobic effect causes molecules to move and therefore forces are applied to individual molecules

but the net motion depends on many factors including the environment (e.g. the solvent)

also calling it a ‘force’ disguises the fact that at physiological temperature it is **entropic**



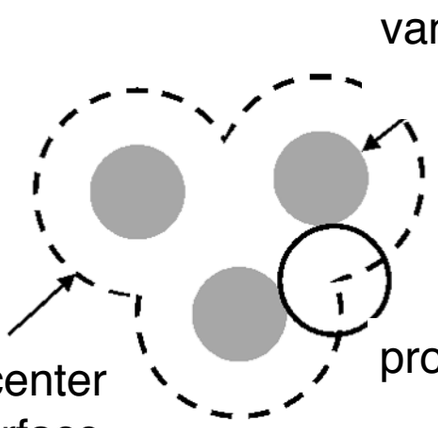
# The partitioning of hydrophobic molecules is a consequence of the hydrophobic effect

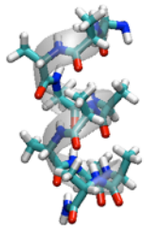


at physiological temperatures the hydrophobic molecules associate with one another (increase in entropy) so that the water can form the maximum number of hydrogen bonds

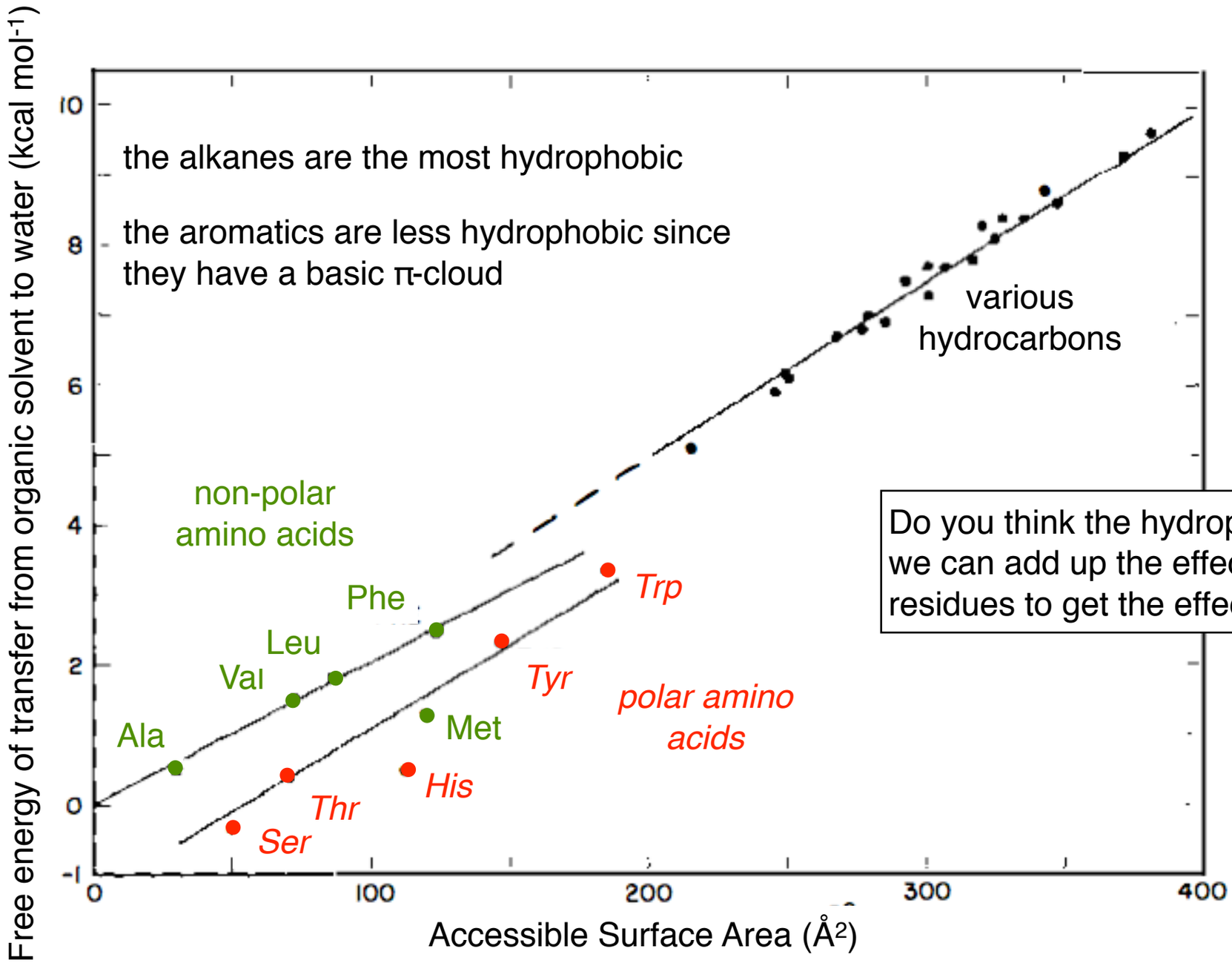
this can restated as the hydrophobic molecules are reorganising to minimise the surface area they present to water

Definition of accessible surface area (ASA):





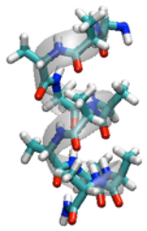
# The transfer free energy of small molecules correlates with their accessible surface area




Do you think the hydrophobic effect is linear (i.e. we can add up the effects of all the individual residues to get the effect for the whole protein)?

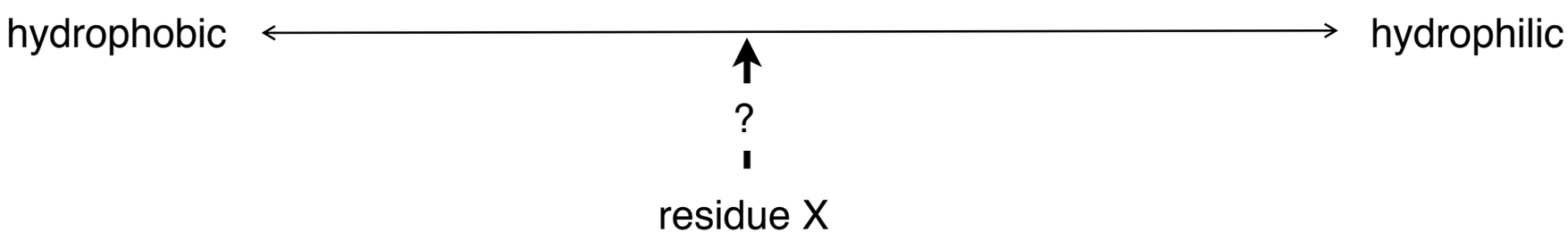
suggests that the hydrophobic effect is proportional to the accessible surface area

Annu. Rev. Biophys. Bioeng. (1977) **6** 151



# The hydropathy of amino acid residues

The hydropathy of an amino acid residue refers to the degree that it is hydrophilic or hydrophobic.



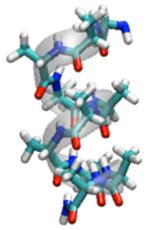

Data like the transfer free energy from a non-polar solvent to water can be used to construct a **hydropathy scale**

(and hence not accessible by water)

How often an amino acid is found buried in the interior of a protein compared to how often it is found on the surface of a protein correlates well with its hydropathy.

More recent studies have attempted to use the translocon to produce a **biological hydropathy scale**

Nature (2005) 433 377



# Consequences for the folding of proteins

the hydrophobic effect is the primary driver of protein folding

non-polar residues are much more likely to be found in the interior of proteins

polar residues and charged residues are much more likely to be found on the surface of proteins*

polar residues

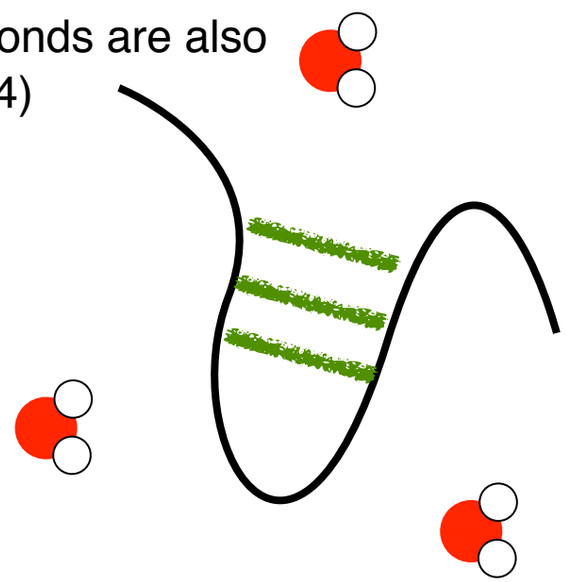
non-polar residues

charged residues

hydrogen bonds form in the interior of the proteins in clusters (i.e. in a cooperative way - see next Lecture)

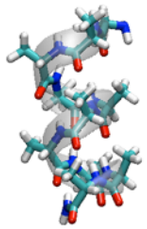
the interior of proteins is a densely packed environment (the sidechains are interdigitated)

these hydrogen bonds are also strong since ($\varepsilon \approx 4$)



| | volume occupied |
|---|---|
| proteins | 75% |
| liquids | 45% |
| water | 36% |
| spheres | 75% |
| crystals of small organic molecules | 70-80% |

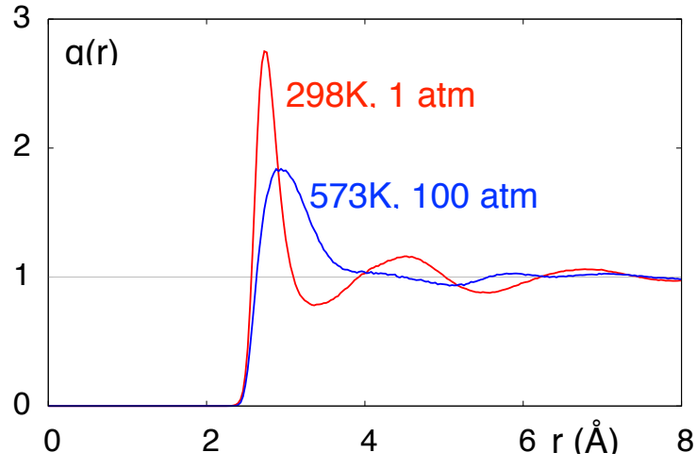
*membrane proteins an exception!



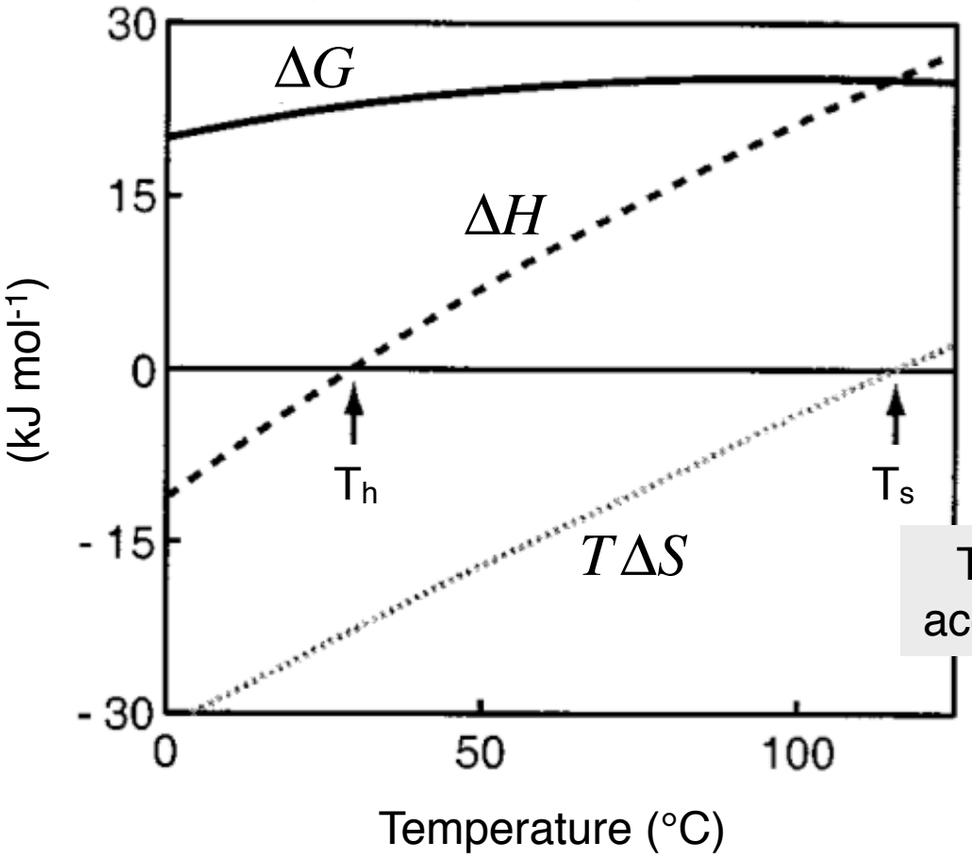
# Lecture 4: Summary

Oil and water don't mix; this is because oil is 'hydrophobic'.

Water has many anomalous properties that can be explained by its propensity to form hydrogen bonding networks that 'melt out' with increasing temperature.

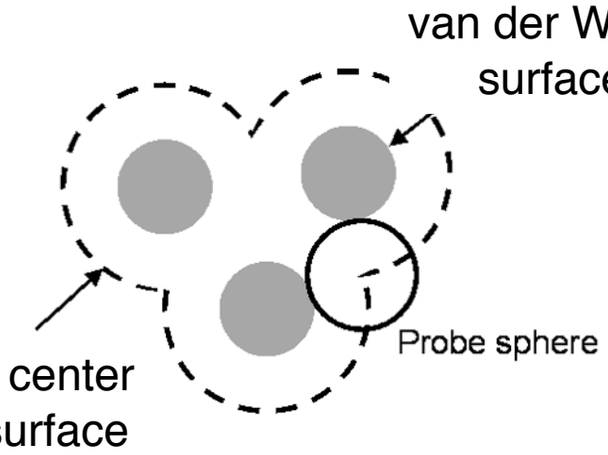


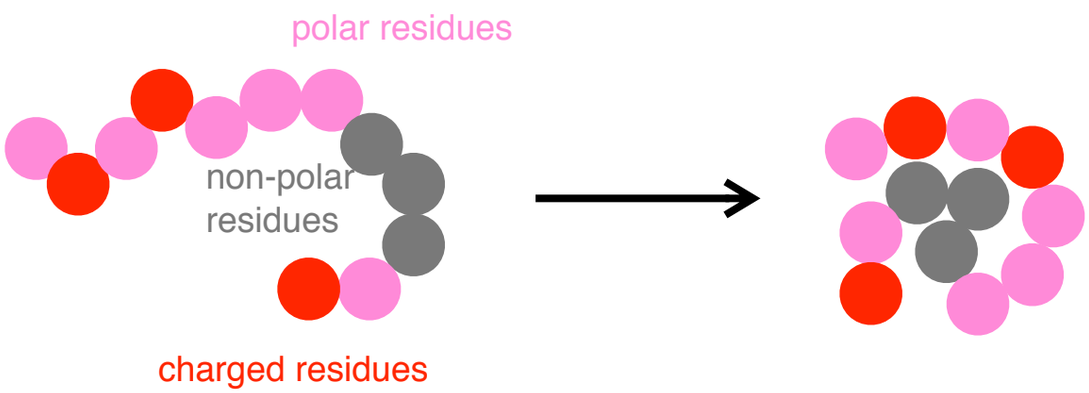

pentane$_{(liquid)}$ → pentane$_{(aq)}$




The temperature dependence of the hydrophobic effect is complex; at physiological temperatures the hydrophobic effect is entropically driven.

The hydrophobic effect correlates with the accessible surface area of small molecules.




The hydrophobic effect is the primary driver of protein folding.