The hydrophobic effect:

**Water loves water,
apolar loves apolar,
~~apolar and water hate each other~~**

Apolar ≠ Hydrophobic

Entropic effect due to the fact that water loves water a lot!

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Apolar ≠ Hydrophobic

Entropic effect due to the fact that water loves water a lot!

**So, for water, you don't necessarily have to be a great lover;
yet, you should still meet certain standard!**

HYDROPHOBIC INTERACTION

Unlike other interactions, it involves a collective process.
Its importance includes conceptual aspects in physical chemistry and biology with practical consequences. Therefore, the so-called hydrophobic effect deserves a careful study.

THERMODYNAMICS OF THE HYDROPHOBIC EFFECT

The solubility of non-polar substances in water is not only low (compared to polar substances) but also, unlike other interactions, **decreases with temperature.**

In thermodynamic terms it means that **the free energy of solubilization, ΔGsol**, defined as the free energy of transferring a non-polar substance to water from its vapor phase, **increases when we increase temperature.**

$$\Delta G_{sol} = \Delta H_{sol} - T\Delta S_{sol} > 0$$

where ΔH_{sol} y ΔS_{sol} are the enthalpy and entropy of solubilization, respectively.

Considering a small temperature change in which ΔH_{sol} y ΔS_{sol} remain constant, an increase of ΔG_{sol} with temperature leads us to conclude that $T\Delta S_{sol}$ should be negative ($-T\Delta S_{sol} > 0$). **That is, the solubilization of a non-polar substance in water produces a decrease in entropy.**

- ΔS_{sol} includes a term called **mixing entropy**, this being a **positive** contribution, due to the fact that the mixture of species generates a loss of information.
- Given that the experimental value of ΔS_{sol} is negative there should be a term of ΔS_{sol} that exceeds in absolute value the entropy of the mixture (positive) to yield a negative value.
- **Experiments (such as in monatomic gases, where the restriction on internal mobility is zero) determine that responsibility for entropy decrease should be attributed to the solvent.**
- The molar free energy is related to the variation of the molar enthalpy and entropy between the hydrocarbon medium (hc, or non-polar) and the aqueous medium (w) by:

$$\Delta g_{sol} = (h_{hc} - h_w) - T(s_{hc}\text{-}s_w) \rightarrow \Delta g_{sol} / T = (h_{hc} - h_w) \, 1/T - (s_{hc}\text{-}s_w)$$

- By detemining the free energy of solubility at different temperatures it is possible to separate the entropic and enthalpic components.

	ΔG / cal(mol)-1	ΔH / cal(mol)-1	ΔS / cal(mol K)-1
C_2H_6	3900	-2500	-21
C_3H_8	4900	-1700	-21
C_4H_{10}	5900	-800	-23
C_6H_6	4600	600	-13
$C_6H_5CH_3$	5300	600	-16
$C_6H_5C_2H_5$	6100	400	-19

Thermodynamic parameters for the transfer of hydrocarbons from an organic solvent to water. ΔH_{solv} **small or negative**!

Unfavorable contribution due to the fact that the entropic term ($-T\Delta S_{sol}$) becomes positive, since the entropy change is negative, which is attributed to water.

PARTITIONING AND THERMODYNAMICS
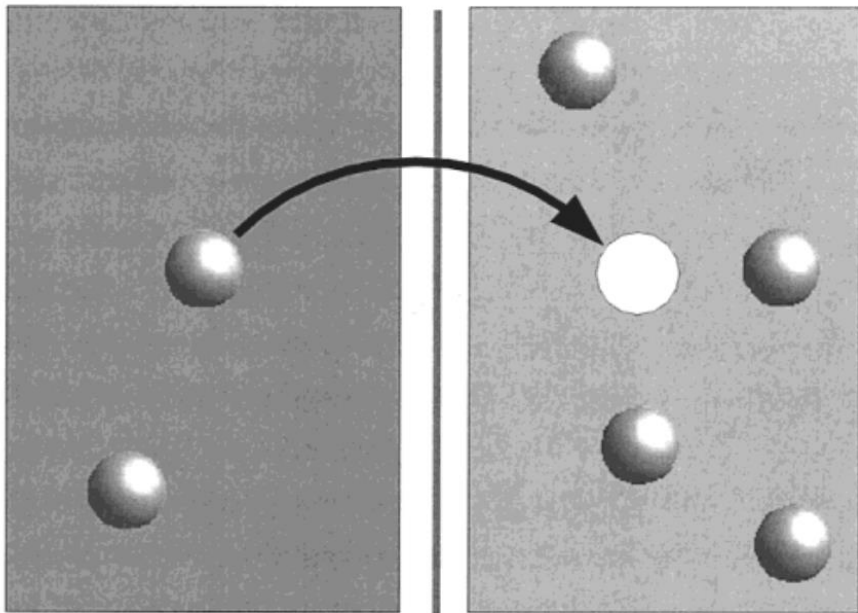(From K. Dill)

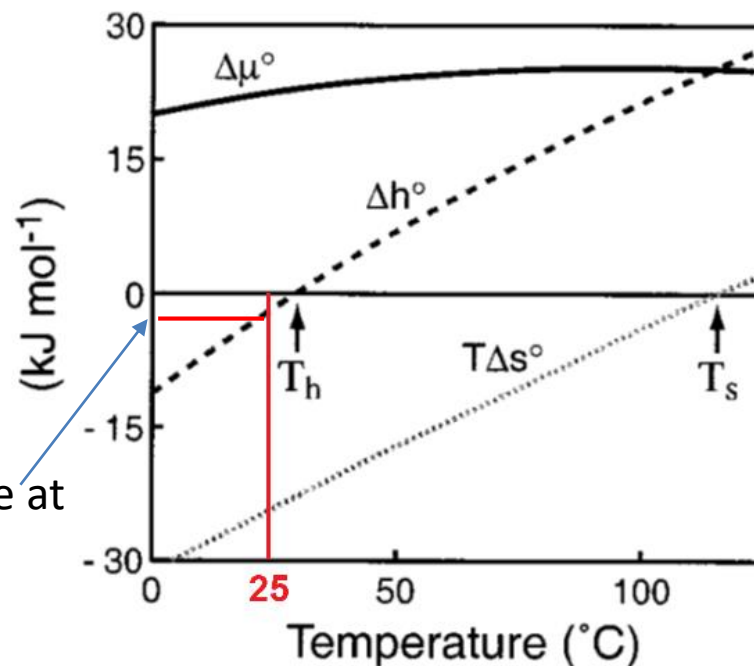The entropic term dominates

Figure 1. Partitioning is a balance. Hydrophobicity is measured by oil/water partitioning of solutes. At equilibrium, the chemical potentials in the two phases, 1 and 2, are equal $\mu_1^\circ + RT \ln C_1 = \mu_2^\circ + RT \ln C_2$. Measuring the solute concentrations $c1$ and $c2$ in the two phases give the difference in chemical affinities between phases, $\Delta\mu^\circ = -RT \ln C_1/C_2$.


Small negative value at room temperaturre

Figure 2. Hydrophobic Effect is the anomalous mixing thermodynamics of nonpolar solutes with water.   The transfer of neopentane from its own neat phase into water

Relaxation experiments

The molecular effects of hydration depend on local interactions.
The Figure shows the shift in the dielectric relaxation time of water per mole of solute as a function of the variation in the entropy of solubility for alcohols and carboxylic acids of different chain lengths. It can be observed that a larger chain length is correlated with a shift towards longer times and with a greater decrease in the solubility entropy.

Thus, the restriction in water movement is produced by the presence of the hydrophobic surface and this is responsible for the decrease in entropy.
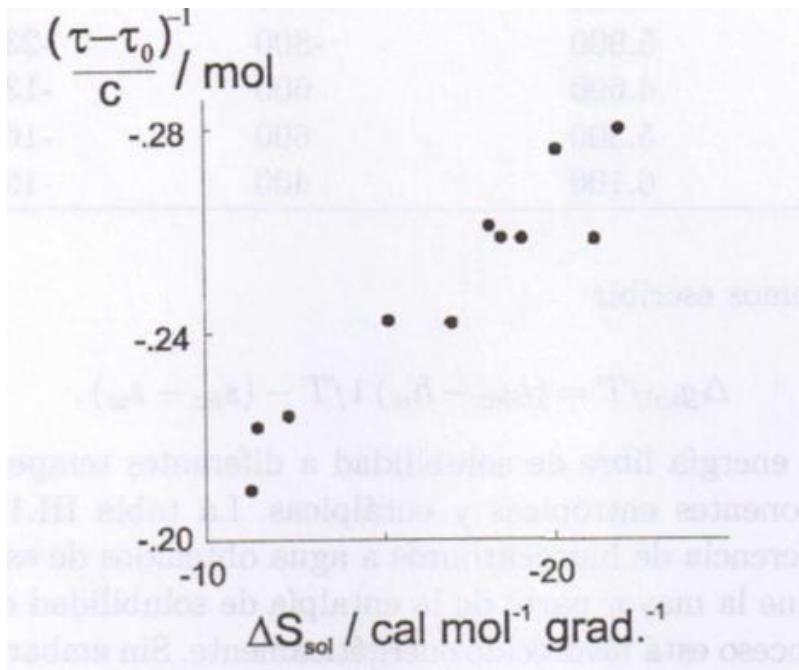
Relative shift of the dielectric relaxation time of water per mole of solute in solutions of alcohols and carboxylic acids as a function of the entropy of solubility. τ_0: pure water.

Dynamic properties can also be studied by NMR using isotopic substitution to characterize nuclei belonging to different solutes as well as the solvent. The Figure shows the relaxation of acetone/water solutions at different concentrations.
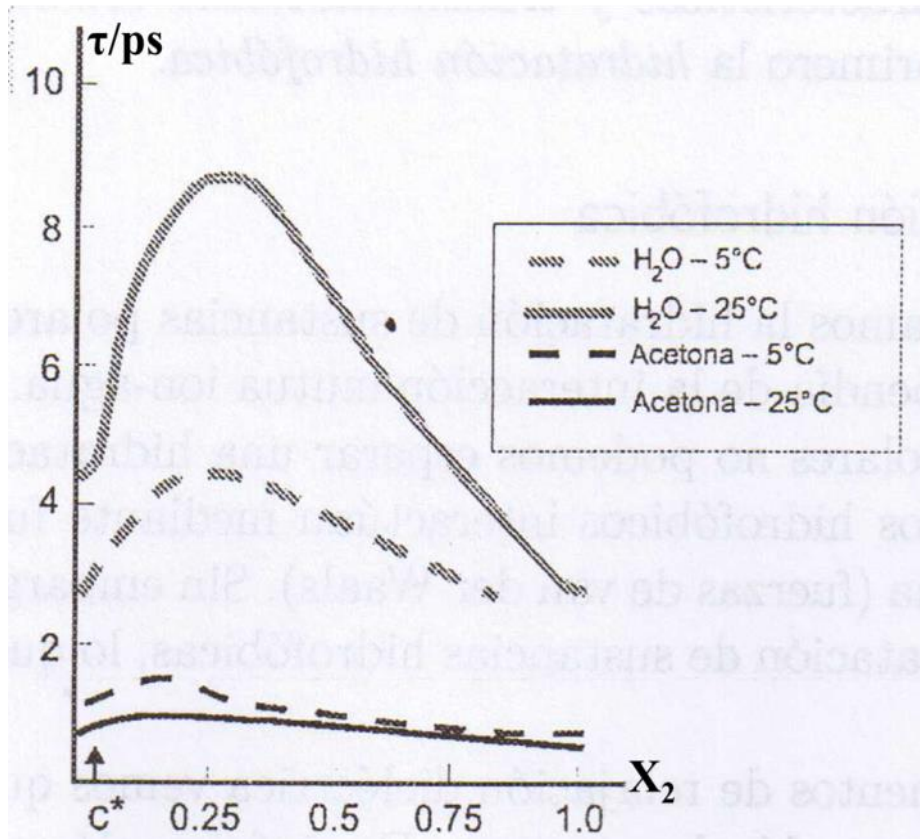

Figure: Rotational correlation times of water inacetone as a function of acetone concentration. Black lines show the relaxation of acetone's ^{17}O while the grey ones are the rotational correlation times of water's proton ^{1}H.

-The presence of acetone slows down the rotation of water (solution of acetone in water), an effect that disappears when the concentration of acetone is very high (solution of water in acetone).

-Unlike the case of water, the correlation times of acetone do not suffer considerable variations for different concentrations.

-The mobility of water is lower than acetone, although its molecular size is smaller.

-The mobility of water is more dependent on temperature, indicating a greater activation energy for rotation.

DYNAMICS, STRUCTURE AND THERMODYNAMICS

HYDROPHOBIC HYDRATION

In aqueous solutions of nonpolar substances, molecules and hydrophobic groups interact both with each other and with water through weak van der Waals forces. However, there is evidence of hydration in hydrophobic substances, which is called **hydrophobic hydration**.

-Estimates of the relaxation time of hydration water based on total relaxation times, would give very large values (extending to more than one hydration sphere).
-Solvents (e.g. acetone) rotate faster than water, so there is no bond between solute and solvent as in the case of ions.

Results from **computational simulations** bring relevant information:

- In small solutes, water has low residence times (<65ps) but a clathrate-type cage.
- If we consider flat walls formed by hydrophobic atoms that have thermal vibration, it can be seen that water modifies its properties at relatively high distances from the wall (10nm), which is consistent with dielectric relaxation and NMR results.
- Likewise, the hydrogen-bond network shows an improvement in the structuring of hydrogen bonds with respect to "free" water. This means that the hydrophobic wall can induce a structure of greater order and stability than that of the liquid core.

The existence of hydrophobic hydration produces a decrease in the entropy of solubility of hydrophobic substances since, as shown by dynamic and structural studies, non-polar substances induce restrictions in the movement of water molecules, which produces a decrease in the entropy.

Hydrophobic interaction:

It can be defined as the interaction that occurs not only through van der Waals forces between non-polar substances in water.

These interactions have a significant effect since they reduce the total area exposed to water, causing a certain number of water molecules from the environment to be transferred to regions of greater mobility. Thus, **while the solubility of non-polar substances is unfavorable since the immobilization of water *decreases* the entropy, the association of these molecules in water increases the entropy, a favorable process since they release water to the bulk.**

This can be reflected in the experimental results (dielectric relaxation and NMR) which show that for low concentrations the mobility of water decreases while as the concentration increases the mobility begins to grow.

The contributions of the interaction of non-polar molecules can then be separated into two parts, one corresponding to the van der Waals interaction (solvent independent) and another coming from changes in the solvent. The latter is called the **hydrophobic interaction**. (in other solvents where it also occurs, it is called solvophobic).
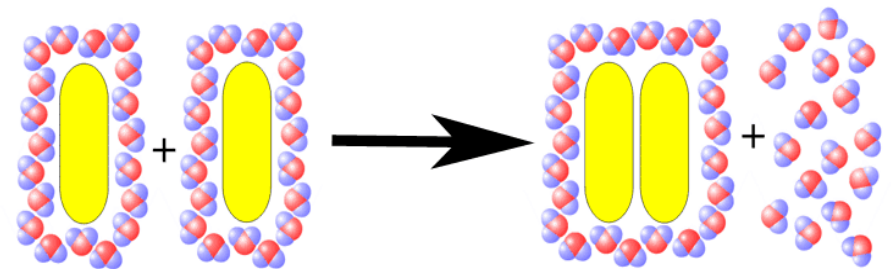
Estado 1: cada molécula hidrofóbica tiene su capa de solvatación

Estado 2: las dos moléculas hidrofóbicas se agregan y comparten capa de solvatación

Nonpolar solutes attract each other in water, as if pulled by certain "force". Hydrophobic effect. Water looses hydrogen bonds → reduce interface by removing water towards bulk (entropic effect). Barrier: cost of dehydration.
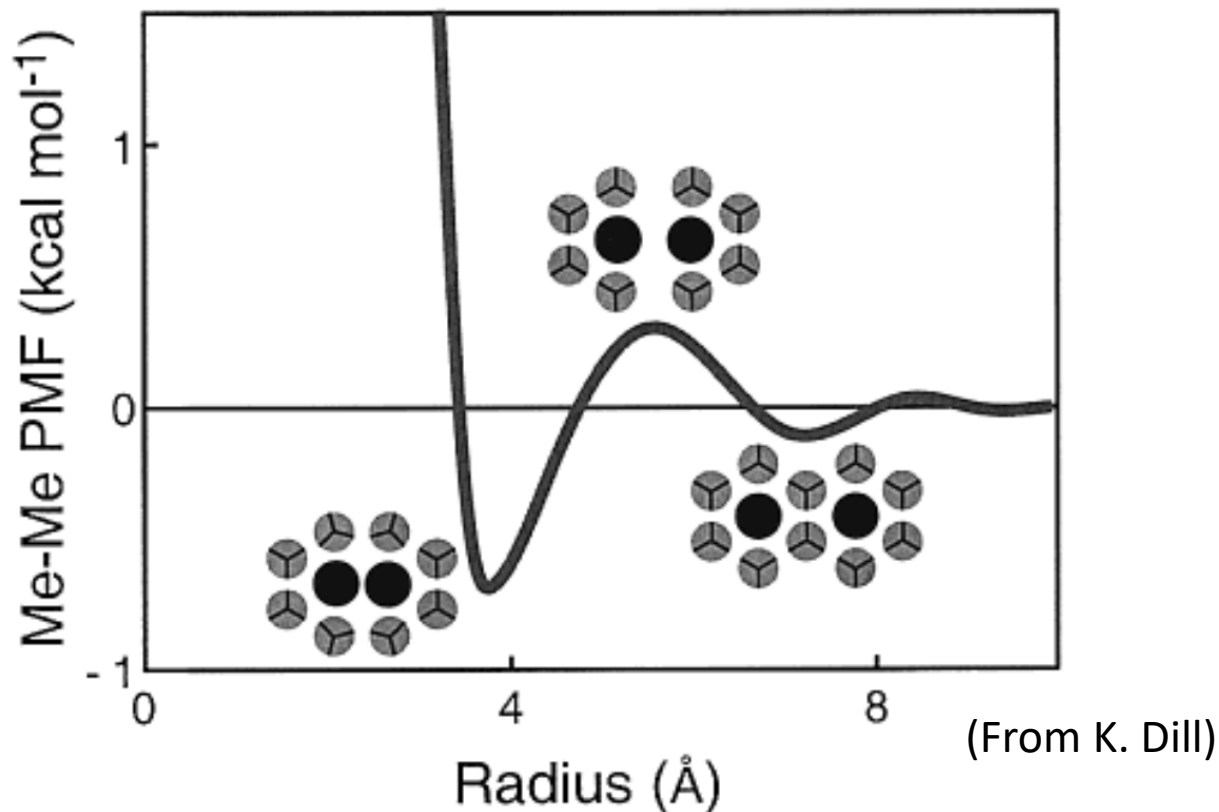

(From K. Dill)

Figure 15. Methane−methane potential of mean force. Idealized potential of mean force between two methanes as a function of distance in angstroms. Left: methanes (in black) at contact, middle: at the barrier distance (which has an unfavorable free energy), and right: in a solvent-separated configuration. Adapted from D. E. Smith, and A. D. J. Haymet, J. Chem. Phys. **1993**, 98, 6445.

The total interaction contains both entropic and enthalpic contributions, all linked to the rearrangement of the solvent. Therefore, the free energy of the hydrophobic interaction is considered as:

$$\Delta G_{ih} = \Delta H_{ih} - T\Delta S_{ih}$$

which includes all contributions to the binding process of non-polar substances, whether direct or indirect. Working in this way we lose specificity (we do not only consider the solvent) but we resort to parameters that can be determined experimentally.

What is the length extent of the hydrophobic interaction?
Experimental results conclude that the range reaches between 10 and 200 nm, while simulation results show that the range could be of the order of 20nm.

Temperature effects

Assuming a small temperature range, in which ΔH_{ih} y ΔS_{ih} are constant, the fact that $\Delta S_{ih} > 0$ would imply that an *increase in temperature leads to a decrease in the free energy*, that is, to an increase in the hydrophobic interaction. But it must be taken into account that entropy is linked to the *structure of water* and experimental results show that it *loses definition as the temperature increases*. **Two opposing effects are at play: at certain temperatures there is a positive entropic contribution (T. $\Delta S \uparrow$), so we will expect an increase in the interaction with temperature, and at the same time an increase in *temperature decreases water's order* and, therefore, there is a entropic contribution weakening the interaction ($\Delta S \downarrow$). It is then expected that there should be a temperature above which the process is reversed.**

Effect of pressure

At pressures of around 1 to 2 kbar water loses its regular tetrahedral structure. Considering that the hydrophobic effect is based on the existence of the hydrogen bond network, the loss of these structures turns water into a liquid without a solvophobic effect, weakening the hydrophobic interaction.
This is important for proteins, whose native structure depends on the hydrophobic effect. Proteins denature at high pressures.

Other factors that modify the hydrophobic interaction

Addition of **"structure destroying" substances**: Substances whose hydration structure is incompatible with the structure of free water. They form hydrogen bonds (HB) with water but are incompatible with water's network, influencing their ability to form HB with other water molecules (effect induced by hydrophobic substances) and to arrange themselves regularly. They are thus inhibitors of hydrophobic interaction. As there is less "order to destroy", the hydrophobic interaction is weakened. For example: urea (denatures proteins).
Addition of **"structure-promoting" substances**: Substances whose hydration structure is compatible with the structure of water. A molecule that stabilizes the structure of water will favor the formation of ordered structures induced by hydrophobic substances, therefore promoting hydrophobic interaction. For example: aldose sugars.

HIDROPHBIC MOMENTS AND AMPHIPHILICITY

Atomic solvation parameters, ASP
Biological molecules are neither totally hydrophobic nor totally hydrophilic. This property of sharing characteristics of each kind is called **amphiphilicity**.
To quantify the hydrophobic interaction, Eisemberg and collaborators proposed that **the water-solute interaction can be considered by the sum of a parameter, the *atomic solvation parameter* (ASP), which describes such interaction for each atom of the compound.** The basic idea is that the interaction free energy can be expressed as the sum of the free energy of each atomic group. The concept of *area exposed to the solvent, A_i, is also used (defined by rolling a 0.14 nm sphere around the molecular surface)*, which is not equal to the sum of the individual areas of each atom (and depends on the conformation of the molecule). The atomic contribution to the free energy is then equal to the sum of the exposed surface of each atom multiplied by the solvation parameter $\Delta\sigma_i$:

$$\Delta G_i = \Delta\sigma_i\, A_i$$

Regarding the total free energy of transfer of a molecule from the aqueous medium to the interior of a protein, it is expressed as:

$$\Delta G_i = \Sigma\, \Delta\sigma_i\, A_i$$

$$\Delta G = \sum_i \Delta \sigma_i A_i. \qquad \text{(III.11)}$$

a)

$$\Delta G = \sum_i \Delta \sigma_i A_i$$

b)

Agua

Superficie accesible
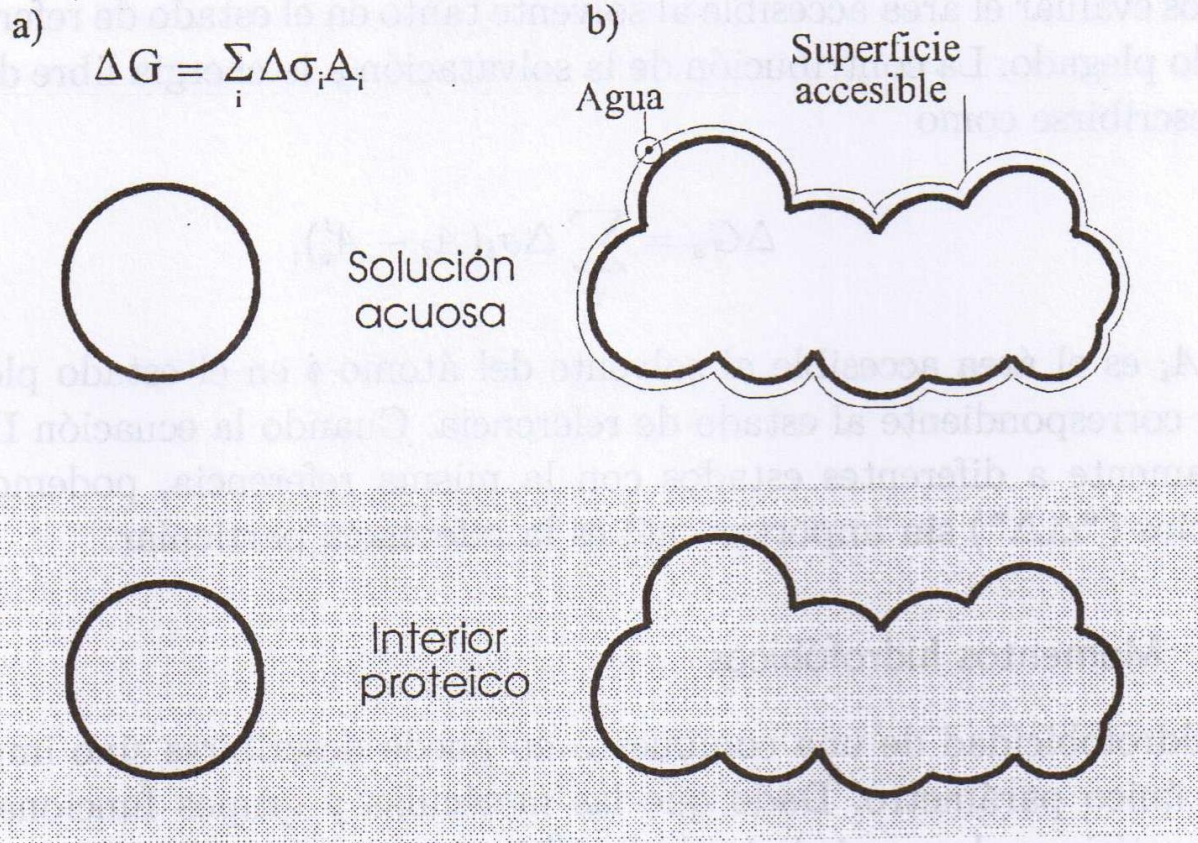
Solución acuosa

Interior proteico

Figura III.4: Esquema que muestra la base para calcular la energía libre de transferencia de un soluto desde el agua al interior de una proteína. a) un átomo aislado; b) una molécula más compleja. Se observa que para el último caso el área expuesta total no es igual a la suma de las áreas individuales debido al apantallamiento de ciertas regiones por los átomos vecinos.

Based on this magnitude, a scale of amino acid hydrophobicity could be established on the basis of calculations of σ_i :

RESIDUO	ESCALA
Ile	0.73
Phe	0.61
Val	0.54
Leu	0.53
Trp	0.37
Met	0.26
Ala	0.25
Gly	0.16
Cys	0.04
Tyr	0.02
Pro	-0.07
Thr	-0.18
Ser	-0.26
His	-0.40
Glu	-0.62
Asn	-0.64
Gln	-0.69
Asp	-0.72
Lys	-1.1
Arg	-1.8

Cuadro III.2: Escala de hidrofobicidad de los aminoácidos. Los valores corresponden a la escala de consenso descripta por Eisenberg, Weis, Terwillinger y Wilcox [*Faraday Symp. Chem. Soc.* 17: 109 (1982)]. Los residuos están dispuestos en orden decreciente de hidrofobicidad. La magnitudes son aproximadamente al valor necesario para transferir el aminoácido de una fase hidrofóbica a una hidrofílica expresada en kcal mol^{-1}

Table: Amino acid hydrophobicity scale. The magnitudes are approximately the value needed to transfer it from a hydrophobic phase to a hydrophilic one. (kcal mol^{-1}).

Compare side chains (Ile, Ala, Gly, His, Arg, etc.)

Solvation energy and protein folding

The ideas mentioned have been used to try to obtain information about protein folding. One could evaluate at least the solvation component of the folding free energy using $\Delta\sigma_i$ by **evaluating the solvent accessible area in both the reference state (A_i') and the folded state(A_i).** The contribution of solvation to the folding free energy can be written as:

$$\Delta G_i = \Sigma \, \Delta\sigma_i \, (A_i - A_i')$$

Hydrophobic moments

In order to correctly define hydrophobicity and the distribution of components involved in hydrophobic and hydrophilic properties, Eisenberg et al. defined the concept of **hydrophobic moments, μ_h, similarly to the case of interactions of electric charges**.
It was considered that **a molecule** perfectly balanced in its polar and non-polar components **can have a distribution such that a "momentum" can be defined**.
The zeroth order μ_h is the net hydrophobicity value. Regarding an amphiphilic molecule, the first order μ_h will depend on the distance between the center of the polar and non-polar regions and its magnitude.

A **hydrophobic dipole** is represented by a **vector whose direction goes in the direction of the hydrophobic portion**. For a complete macromolecule the hydrophobic dipole will be equal to the **vectorial sum of the dipole moments of each component**. Like any vector, for its identification it is necessary to indicate magnitude, direction and sense.
The following Table shows the established consensus scale for amino acids along with their hydrophobic moments.

AMINOÁCIDO	$\Delta G^{\ddagger}_{obs}$	ΔG_R	$\|\mu_a\|$	$\cos\theta$
Gly	(0)	(0)	(0)	–
Ala	0.42	0.67	0	–
Val	1.66	1.5	0.48	0.84
Leu	2.32	1.9	1.0	0.89
Ile	2.46	1.9	1.2	0.99
Pro	0.98	1.2	0.18	0.22
Cys (half)	1.34	0.38	0.17	0.76
Met	1.68	2.4	1.9	0.94
Thr	0.35	0.52	1.5	0.09
Ser	-0.05	0.01	0.73	-0.67
Phe	2.44	2.3	1.1	0.92
Trp	3.07	2.6	1.6	0.67
Tyr	1.31	1.6	1.8	-0.93
Asn	-0.82	-0.60	1.3	-0.86
Gln	-0.30	0.22	1.9	-1.0
Asp	-1.05	-1.2	1.9	-0.98
Glu	-0.87	-0.76	3.0	-0.89
His	0.18	0.64	0.99	-0.75
Lys	-1.35	-0.57	5.7	-0.99
Arg	-1.37	-2.1	10.0	-0.96

GRUPO	$\Delta G^{\ddagger}_{obs}$	ΔG_R
–CH$_2$–	0.67	0.56
–C$_6$H$_5$–	1.91	1.6
–OH–	-0.76	-0.66
–CH$_2$–CONH–	-1.14	0.66

El momento hidrofóbico se define como

$$\mu_h = \sum H_i r_i - \langle H_i \rangle r_i, \qquad \text{(III.13)}$$

donde r_i es el vector desde cualquier origen al centro del $i^{ésimo}$ grupo y H_i la hidrofobicidad del mismo grupo. La figura III.5 muestra una representación gráfica del momento dipolar hidrofóbico de la arginina.
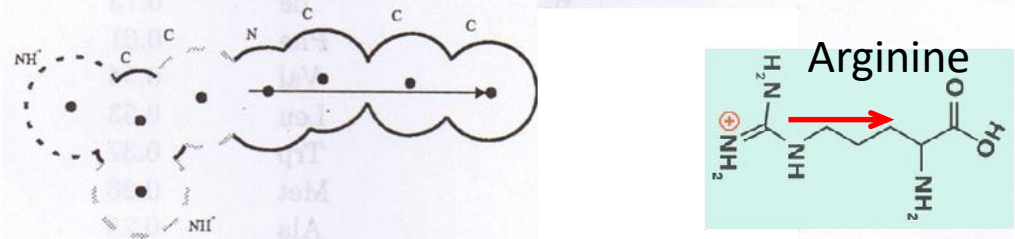

Arginine

Figura III.5: El momento hidrofóbico de la arginina puede representarse como se muestra en la figura. La flecha apunta desde a la región más hidrofílica a la más hidrofóbica de la molécula.

Table: Solvation free energies (kcal mol^{-1}) for **aminoacids** together with their hydrophobic moments (kcal mol^{-1} Å). The **direction of the hydrophobic momentum is defined from the α carbon to the geometric centre of the lateral chain (indicated in the table with cos θ).**

MEASUREMENT OF THE HYDROPHOBIC INTERACTION
Partition coeficients:

The **free energy of transfer from a non-polar phase to water** allows us to establish a **hydrophobicity scale** and it can be related to the **partition coefficient, P**.

The partition coefficient of a molecular species is **the ratio of its concentrations between the two phases in equilibrium**. Molar volumes not always available. **"Non-universal" scale** but a relative one between two given solvents. There is chemical affinity, but also conformational changes in the transfer between media.

$$\mu^\circ_0 + R\,T\,\ln x_0 = \mu^\circ_w + R\,T\,\ln x_w$$

$$\Delta\mu = R\,T\,\ln (x_0/x_w)$$

$$P \equiv x_0/x_w$$

$$\Delta\mu = R\,T\,\ln P$$

Thus, P is related to the free energy of transfer.

Figure 1. Partitioning is a balance. Hydrophobicity is measured by oil/water partitioning of solutes. At equilibrium, the chemical potentials in the two phases, 1 and 2, are equal $\mu^\circ_1 + RT \ln C_1 = \mu^\circ_2 + RT \ln C_2$. Measuring the solute concentrations $c1$ and $c2$ in the two phases give the difference in chemical affinities between phases, $\Delta\mu^\circ = -RT \ln C_1/C_2$.

We learn on the hydrophobicity of a substance by expressing the partition coefficient between water and a given reference organic solvent. **Using the same reference solvent it is possible to construct a hydrophobicity table.** Some additional corrections have been derived to this equation.
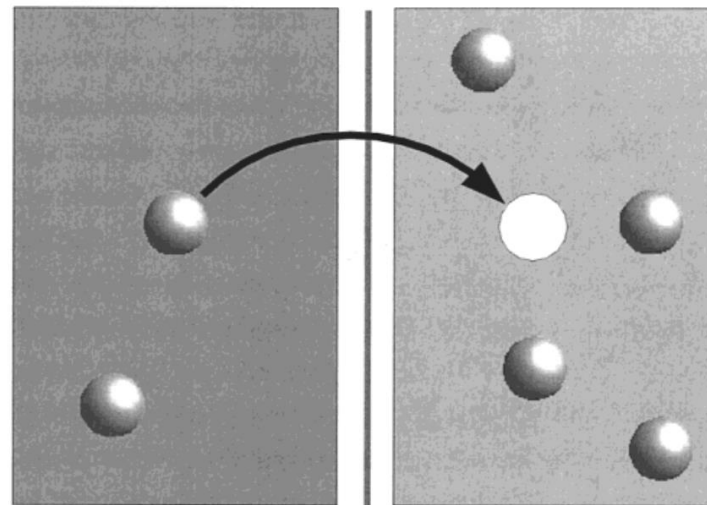
Leu, Ile	butano, isobutano
Val	propano
Pro	ciclopropano
Phe	tolueno
Met, Trp	etil metil sulfato, skatole
Ala	metano
Cys, Gly	metanotiol, hidrógeno
— nivel cero — Tyr	fenol
Thr	etanol
Ser	metanol
His	4-metilimidazol
Lys, Gln	N-butilamina, propionamida
Asn	acetamida
Glu	acido propiónico
Asp	acido acético
Arg	N-propilguanidina

Comparison of the hydrophobicity scale of amino acids with some compounds obtained from the cyclohexane-water distribution coefficients.

Since P refers to the solubility equilibrium between two phases (polar and non-polar), it is **related to both hydrophobicity and hydrophilicity**, that is, it **reflects the amphiphilicity of the substance**.

If the **hydrophobicity scales of the Tables shown are compared**, it can be seen that there are some **changes in the order** of certain substances making us *pay attention to hydrophobicity scales constructed with different methods.*

Hydrophilic-lipophilic balance

Like the partition coefficient, the hydrophilic-lipophilic balance (HLB) is a **measure of amphiphilicity and has been developed empirically**. It is interesting to measure the tendency to bind to a hydrophobic region and solubilize (important in drug design). A **relationship between HBL and the partition coefficient** was proposed, which can be expressed as
$HLB - 7 = 0.36 \ln (1/P)$

GENERAL AND SPECIFIC HYDROPHOBICITY

Since the **hydrophobic interaction does not include "bonds" it is nonspecific**. In principle, any pair of particles with hydrophobic regions can join together. However, there are cases in which a certain **specificity** is observed, for example in cases in which there is an agreement between the **geometries** involved.
Specific hydrophobic interaction plays an important role in, for example, drug binding to plasma proteins and muscle contraction.

FUTURE DEVELOPMENT

Three different hydrophobicity scale tables have been shown! which speaks of the hard work that remains to be done in order to improve the predictive capacity and, in this way, solve problems in the biological field.
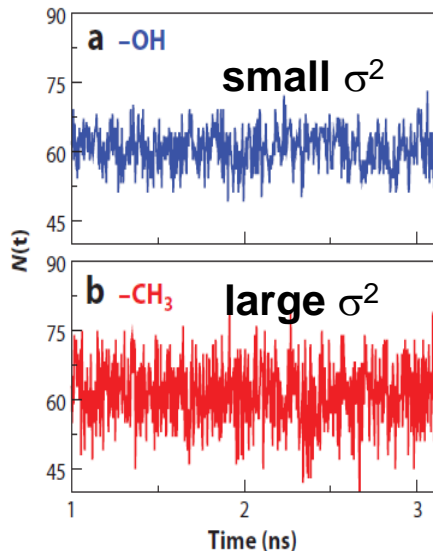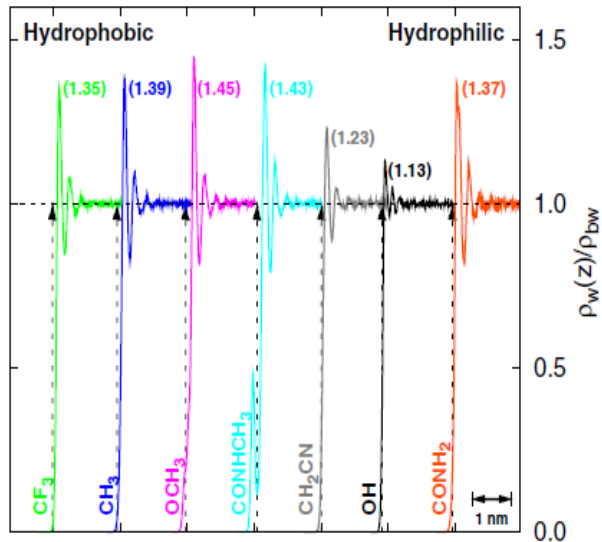
QUANTIFYING LOCAL HYDROPHOBICITY ON SOLID SURFACES:

Water density fluctuations
Water "abhors" vacuum ~ hydration, δ profiles/layering.
Density fluctuations enhanced at hydrophobic surfaces.
Generic contexts with controlled chemistry and geometry in order to determine how chemical topology and topography define the local hydrophobicity. Functionalized SAMs.
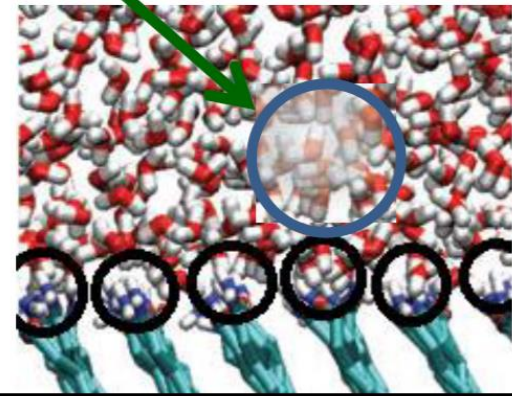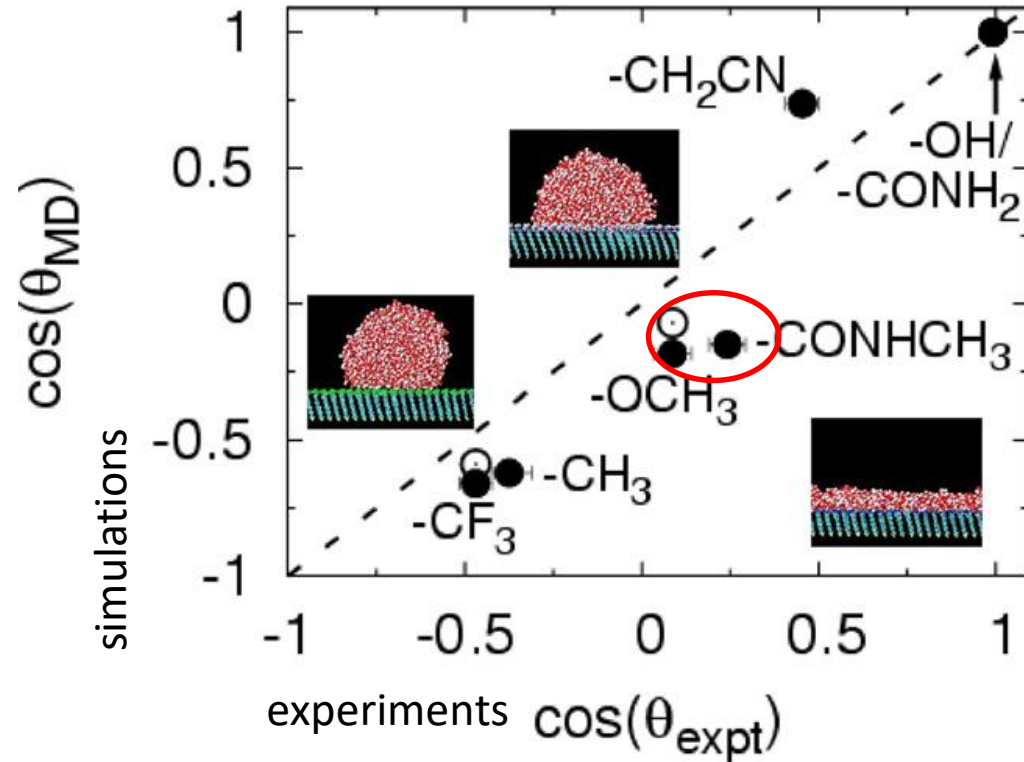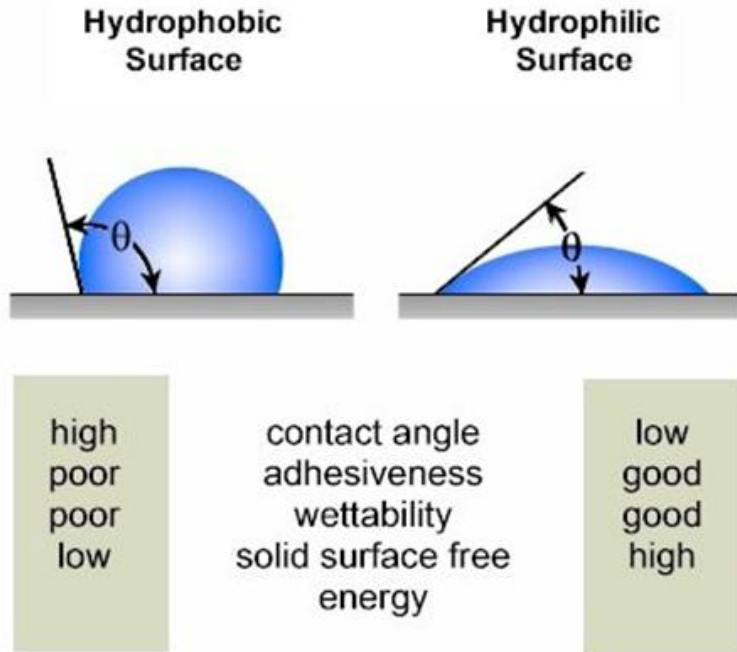
r=3.3Å

Self-assembled monolayer
(SAM: functionalized alkyl chains)

Local measure but can be extended to larger regions and other shapes

(free-energy of cavity creation at small volume)

$$\mu \approx k_b T \langle N \rangle^2 / 2\sigma^2$$

$$\mu = -k_b T \ln[p(N = 0)]$$

↑$\sigma^2/\langle N \rangle^2$, cavity creation, vacating probability

a –OH **small σ^2**

b –CH$_3$ **large σ^2**

d
- ■ –CH$_3$
- ■ –CONHCH$_3$
- ■ Bulk water
- ● –OH

Hydrophobicity $\ln p(N)$

$N/\langle N \rangle$

Hydrophobic   Hydrophilic

$\rho_w(z)/\rho_{bw}$

CF$_3$ CH$_3$ OCH$_3$ CONHCH$_3$ CH$_2$CN OH CONH$_2$

Form S. Garde's group, Proc. Natl Acad Sci U S A. (2009); Annu. Rev. Chem. Biomol. Eng. (2011).
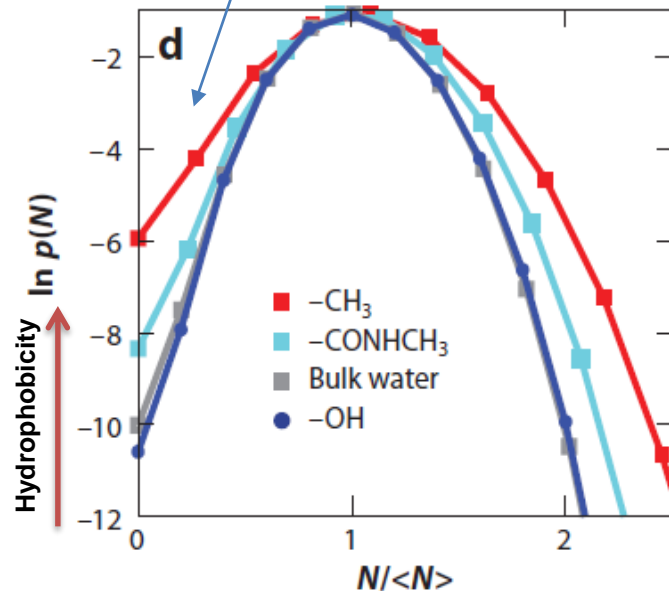
EXPERIMENTAL STANDARD MEASURE ON SOLID SURFACES: CONTACT ANGLE (θ)

Functionalized SAMs: R. Godawat, S. N. Jamadagni, and S. Garde Proc. Natl Acad Sci U S A. (2009)

The contact angle is the standard measure in materials science.

But, "*hydrophobicity depends on the eye of the beholder… One common definition is that water droplets on a planar hydrophobic surface possess a contact angle larger than 90°; but given that **nothing dramatic changes when the contact angle falls below this or any other point**, it is just a convenient but arbitrary definition*" (Garnick&Bae, Science (2008)).

We shall revisit this!