# 5. Hydrophobicity

### Hydrophobic Solvation: Thermodynamics

Why do oil and water not mix? What is hydrophobicity? First, the term is a misnomer. Greasy molecules that do not mix with water typically do have favorable interaction energies, i.e.,  $\Delta H_{\text{int}} < 0$ . Walter Kauzmann first used the term "hydrophobic bonding" in 1954. This naming has been controversial from the beginning, but it has stuck presumably, because in this case  $\Delta G$  is what determines the affinity of one substance for another rather than just  $\Delta H$ . Generally speaking, the entropy of mixing governs the observation that two weakly interacting liquids will spontaneously mix. However, liquid water's intermolecular interactions are strong enough that it would prefer to hydrogen bond with itself than solvate nonpolar molecules. It will try to avoid disrupting its hydrogen bond network if possible.

The hydrophobic effect refers to the free energy penalty that one pays to solvate a weakly interacting solute. Referring to the thermodynamic cycle above,  $\Delta G_{sol}$ , the reversible work needed to solvate a hydrophobic molecule, is dominated by step 1, the process of forming a cavity in water. The free energy of solvating a hydrophobic solute is large and positive, resulting from two factors:

- 1)  $\Delta S_{sol} < 0$ . The entropy penalty of creating a cavity in water. We restrict the configurational space available to the water within the cavity. This effect and the entropy of mixing (that applies to any solvation problem) contribute to  $\Delta S_1$ .
- 2)  $\Delta H_{sol} > 0$ . The energy penalty of breaking up the hydrogen bond network ( $\Delta H_1$ ) is the dominant contributor to the enthalpy. This can be estimated from a count of the net number of H-bonds that needs to be broken to accommodate the solute:  $\Delta H_{sol}$  increases by 1–3 kcal mol<sup>-1</sup> of hydrogen bonds. The interaction energy between a hydrocarbon and water ( $\Delta H_2$ ) is weakly favorable as a result of dispersion interactions, but this is a smaller effect. (At close contact, van der Waals forces lower the energy by ~0.1-1.0 kcal mol<sup>-1</sup>). Therefore  $\Delta H_{sol} \approx \Delta H_1$ .

The net result is that  $\Delta G_{sol}$  is large and positive, which is expected since water and oil do not mix.

These ideas were originally deduced from classical thermodynamics, and put forth by Frank and Evans (1945) in the "iceberg model", which suggested that water would always seek to fulfill as many hydrogen bonds as it could—wrapping the network around the solute. This is another misnomer, because the hydrophobic effect is a boundary problem about reducing configurational space, not actual freezing of fluctuations. Hydrogen bonds continue to break and reform in the liquid, but there is considerable excluded configurational space for this to occur. Let's think of this as solute-induced *hydrogen-bond network reorganization*.



#### Water Configurational Entropy

Let's make an estimate of  $\Delta S_{sol}$ . Qualitatively, we are talking about limiting the configurational space that water molecules can adopt within the constraints of a tetrahedral potential.

2,4

3.4

Approximation

Bulk water: 4 HBs/tetrahedron Within a tetrahedral lattice the orientation of an H<sub>2</sub>O has: 6 configurations: 1,2 1,3 1,4

2,3

<b>O</b> 1,11,	=	6
<b>SZ</b> bulk	_	υ

At a planar interface, you satisfy the most hydrogen bonds by making one dangling hydrogen bond pointing toward the surface

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3 configurations 1,2 1,3 1,4
\Omega_{\text{surface}} = 3
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So an estimate for the entropy of hydrophobic solvation if these configurations are equally probable is  $\Delta S_{sol} = k_B \ln(\Omega_{surf} / \Omega_{bulk}) = -k \ln 2$  per hydrogen bond of lost configurational space:

$$-T\Delta S_{\rm sol} = k_{\rm B}T\ln 2$$

Evaluating at 300 K,

 $-T\Delta S_{sol} = 1.7 \text{ kJ/mol water molecules} @ 300 \text{ K}$ = 0.4 kcal/mol water molecules

This value is less than the typical enthalpy for hydrogen bond formation, which is another way of saying that the hydrogen bonds like to stay mostly intact, but have large amplitude fluctuations.

#### Temperature Dependence of Hydrophobic Solvation

From  $\Delta S_{sol}$  we expect  $\Delta G_{sol}$  to rise with temperature as a result of the entropic term. This is a classic signature of the hydrophobic effect: The force driving condensation or phase-separation increases with temperature. Since the hydrogen-bond strength connectivity and fluctuations in water's hydrogen-bond network change with temperature, the weighting of enthalpic and entropic factors in hydrophobic solvation also varies with *T*. Consider a typical temperature dependence of  $\Delta G_{sol}$  for small hydrophobic molecules:



The enthalpic and entropic contributions are two strongly temperature-dependent effects, which compete to result in a much more weakly temperature-dependent free energy. Note, this is quite different from the temperature dependence of chemical equilibria described by the van't Hoff equation, which assumes that  $\Delta H$  is independent of temperature. The temperature dependence of all of these variables can be described in terms of a large positive heat capacity.

$$\Delta C_{p,\text{sol}} = \frac{\partial \Delta H_{\text{sol}}^0}{\partial T} = T \frac{\partial \Delta S_{\text{sol}}^0}{\partial T}$$
$$= -T \frac{\partial^2 G_{\text{sol}}^0}{\partial T^2} \quad (\text{Curvature of } \Delta G^0)$$

At low temperatures, with a stronger, more rigid hydrogen-bond network, the  $\Delta S$  term dominates. But at high temperature, approaching boiling, the entropic penalty is far less.

### Hydrophobic Solvation: Solute Size Effect

To create a new interface there are enthalpic and entropic penalties. The influence of each of these factors depends on the size of the solute (*R*) relative to the scale of hydrogen bonding structure in the liquid (correlation length,  $\ell$ , ~0.5–1.0 nm).

#### For small solutes (R < $\ell$ ): Network deformation

The solute can insert itself into the hydrogen bond network without breaking hydrogen bonds. It may strain the HBs ( $\Delta H > 0$ ) and reduce the configurational entropy ( $\Delta S < 0$ ), but the liquid mostly maintains hydrogen bonds intact. We expect the free energy of this process to scale as volume of the solute  $\Delta G_{sol}(R < \ell) \propto R^3$ .



#### For large solutes, $R > \ell$ : Creating an interface

The hydrogen bond network can no longer maintain all of its HBs between water molecules. The low energy state involves dangling hydrogen bonds at the surface. One in three surface water molecules has a dangling hydrogen bond, i.e., on average five of six hydrogen bonds of the bulk are maintained at the interface.



We expect  $\Delta G_{sol}$  to scale as the surface area  $\Delta G_{sol}(R > \ell) \propto R^2$ . Of course, large solutes also have a large volume displacement term. Since the system will always seek to minimize the free energy, there will be a point at which the  $R^3$  term grows faster with solute radius than the  $R^2$  term, so large solutes are dominated by the surface term.

#### Calculating $\Delta G$ for Forming a Cavity in Water

Let's investigate the energy required to form cavities in water using a purely thermodynamic approach. To put a large cavity  $(R > \ell)$  into water, we are creating a new liquid-vapor interface for the cavity. So we can calculate the energy to create a cavity using the surface tension of water. Thermodynamically, the surface tension  $\gamma$  is the energy required to deform a liquid-vapor interface:  $\gamma = (\partial U/\partial a)_{N,V,T}$ , where *a* is the surface area. So we can write the change in energy as a result of inserting a spherical cavity into water as the product of the surface tension of water times the surface area of the cavity,

$$U(R) = 4\pi R^2 \gamma$$

In principle, the experimentally determined  $\gamma$  should include entropic and enthalpic contributions to altering the hydrogen bond network at a surface, so we associate this with  $\Delta G_{sol}$ . For water at 300 K,  $\gamma = 72$  pN/nm.  $\gamma$  varies from 75 pN/nm at 0 °C to 60 pN/nm at 100 °C.

The surface tension can also be considered a surface energy per unit area: which can also be considered a surface energy, i.e.,  $\gamma = 72 \text{ mJ/m}^2$ . To relate this to a molecular scale quantity, we can estimate the surface area per water molecule in a spherical cavity. The molecular volume of bulk water deduced from its density is  $3.0 \times 10^{-26}$  L/molecule, and the corresponding surface area per molecule deduced from geometric arguments is ~10 Å<sup>2</sup>. This area allows us to express  $\gamma \approx 4.3$  kJ/mol, which is on the order of the strength of hydrogen bonds in water.

For small cavities ( $R < \ell$ ), the considerations are different since we are not breaking hydrogen bonds. Here we are just constraining the configurational space of the cavity and interface, which should scale as volume. We define

$$\Delta G_{\rm sol}(R < \ell) = \frac{4\pi R^3}{3} \rho_E$$

where  $\rho_{E}$  is an energy density.<sup>1</sup>

$$\rho_E \approx 240 \times 10^{-9} \text{ pJ/nm}^3 = 240 \text{ pN nm}^{-2}$$

Remembering that  $-\partial G / \partial V|_{N,T} = p$ , the energy density corresponds to units of pressure with a value  $\rho_E = 2.4 \times 10^3$  atm. If we divide  $\rho_E$  by the molarity of water (55M), then we find it can be expressed as 4.4 kJ/mol, similar to the surface free energy value deduced.

So combining the surface and volume terms we write

<sup>1.</sup> D. Chandler, Interfaces and the driving force of hydrophobic assembly, Nature 437, 640–647 (2005).

$$\Delta G_{\rm sol}(R) = 4\pi\gamma R^2 + \frac{4}{3}\pi R^3 \rho_E$$

Alternatively, we can define an effective length scale (radius) for the scaling of this interaction

$$\frac{\Delta G_{\rm sol}}{k_B T} = \left(\frac{R}{R_{\rm surf}}\right)^2 + \left(\frac{R}{R_V}\right)^3 \qquad \qquad R_{\rm surf} = \sqrt{\frac{k_B T}{4\pi\gamma}} \quad R_V = \left(\frac{3k_B T}{4\pi\rho_E}\right)^{1/3}$$

where  $R_{\text{surf}} = 0.067$  nm and  $R_V = 1.6$  nm at 300 K. We can assess the crossover from volumedominated to area-dominated hydrophobic solvation effects by setting these terms equal and finding that this occurs when  $R = 3\gamma / \rho_E = 0.9$  nm. The figure below illustrates this behavior and compares it with results of MD simulations of a sphere in water.



Figure 2 | Solvation free energy,  $\Delta G$ , for a spherical cavity in water as a function of the cavity size. The results are for ambient conditions (room temperature and 1 atm pressure). The circles show the results of detailed microscopic calculations<sup>25</sup>. The liquid–vapour surface tension is shown by  $\gamma$ . The solid lines show the approximate scaling behaviour of  $\Delta G/4\pi R^2$  for small *R*, and the asymptotic behaviour for large *R*. This approach can be used to infer the typical length characterizing the crossover behaviour, but not the quantitative behaviour of  $\Delta G$  in the crossover regime.

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An alternate approach to describing the molar free energy of solvation for a hydrophobic sphere of radius *r* equates it with the probability of finding a cavity of radius *r*:

$$\Delta G = -k_B T \ln P(r)$$

$$P(r) = \frac{e^{-U(r)/k_B T}}{\int_0^\infty e^{-U(r)/k_B T} dr} = \frac{\exp\left[\frac{-4\pi\gamma r^2}{k_B T}\right]}{\frac{1}{2}\sqrt{\frac{k_B T}{4\gamma}}}$$

$$= \frac{2}{\sqrt{\pi}R_{\text{surf}}} \exp\left[-r^2/R_{\text{surf}}^2\right]$$

This leads to an expression much like we previously described for large cavities. It is instructive to determine for water @ 300 K:

$$\langle r \rangle = \int_0^\infty dr \, r \, P(r) = \pi^{-1/2} R_{\text{surf}} = \frac{1}{2\pi} \left(\frac{k_B T}{\gamma}\right)^{1/2} = 0.038 \text{ nm}$$

This is very small, but agrees well with simulations. (There is not much free volume in water!) However, when you repeat this to find the variance in the size of the cavities  $\delta r = (\langle r^2 \rangle - \langle r \rangle^2)^{1/2}$ , we find  $\delta r = 0.028$  nm. So the fluctuations in size are of the same scale as the average and therefore quite large in a relative sense, but still less than the size of a water molecule.

Simulations give the equilibrium distribution of cavities in water



Figure 9. Free volume distributions from molecular dynamics simulations of a simple liquid, *n*-hexane (dashed line), and water (solid line). The probability of finding a cavity of a given radius is plotted. Water has more small cavities ( $\leq 1$  Å) than *n*-hexane. Data adapted from G. Hummer et al., *J. Phys. Chem. B* **1998**, *102*, 10 475.

Figure 10. Free energy cost of creating a cavity, from molecular simulations. Data adapted from Hummer, G., et al. J. Phys. Chem. B 1998, 102, 10 475.

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## Hydrophobic Collapse<sup>2</sup>

We see that hydrophobic particles in water will attempt to minimize their surface area with water by aggregating or phase separating. This process, known as hydrophobic collapse, is considered to be the dominant effect driving the folding of globular proteins.

Let's calculate the free energy change for two oil droplets coalescing into one. The smaller droplets both have a radius  $R_0$  and the final droplet a radius of R.

$$\Delta G_{\rm collapse} = \Delta G_{\rm sol}(R) - 2\Delta G_{\rm sol}(R_0)$$

The total volume of oil is constant—only the surface area changes. If the total initial surface area is  $A_0$ , and the final total surface area is A, then

$$\Delta G_{\text{collapse}} = (A - A_0)\gamma$$

which is always negative since  $A < A_0$  and  $\gamma$  is positive.



$$\frac{\text{Initial State}}{\text{Total Volume } V = 2\left(\frac{4}{3}\pi R_0^3\right)} \qquad \frac{\text{Final State}}{V = \frac{4}{3}\pi R^3 \rightarrow R = 2^{1/3}R_0}$$

$$\text{Initial surface area } A_0 = 2\left(4\pi R_0^2\right) \qquad A = 4\pi R^2 = 4\pi (2^{1/3})^2 R_0^2 = 4\pi (1.59) R_0^2$$

$$\frac{A_0}{4\pi R_0^2} = 2 \qquad \frac{A}{4\pi R_0^2} = 1.59$$

$$\Delta G_{\text{collapse}} = (A - A_0)\gamma = (-0.41) 4\pi R_0^2\gamma$$

This neglects the change in translational entropy due to two drops coalescing into one. Considering only the translational degrees of freedom of the drops, this should be approximately 
$$\Delta S_{collapse} \approx k_B \ln(3/6)$$
. In other words, a small number compared to the surface term.

<sup>2.</sup> See K. Dill and S. Bromberg, Molecular Driving Forces: Statistical Thermodynamics in Biology, Chemistry, Physics, and Nanoscience. (Taylor & Francis Group, New York, 2010), p. 675.

We can readily generalize this to a chain of n beads, each of radius  $R_0$ , which collapse toward a single sphere with the same total volume. In this case, let's consider how the free energy of the system varies with the number of beads that have coalesced.



Again the total volume is constant,  $V = n(\frac{4}{3}\pi R_0^3)$ , and the surface area changes. The initial surface area is  $A_0 = m4\pi R_0^2$  and the final surface area is  $A_{min} = 4\pi (R_{min})^2 = m^{2/3} 4\pi R_0^2$ . Along the path, there is a drop of total surface area for each bead that coalesces. Let's consider one path, in which an individual bead coalesces with one growing drop. The total surface area once *n* of *m* particles have coalesced is

 $A_n = ($ surface area of drop formed by *n* coalesced beads) + (total area of remaining *m*–*n* beads)

$$A_{n} = (n^{2/3} 4\pi R_{0}^{2}) + (m-n) 4\pi R_{0}^{2}$$
$$= (m+n^{2/3}-n) 4\pi R_{0}^{2}$$
$$= A_{0} + (n^{2/3}-n) 4\pi R_{0}^{2}$$

The free energy change for coalescing n beads is



This free energy is plotted as a function of the bead number at fixed volume. This is an energy landscape that illustrates that the downhill direction of spontaneous change leads to a smaller

number of beads. The driving force for the collapse of this chain can be considered to be the decrease in free energy as a function of the number of beads in the chain:

$$f_{\text{coll}} = -\frac{\partial \Delta G_{\text{coll}}}{\partial r} \propto -\frac{\partial \Delta G_{\text{coll}}}{\partial n}$$
$$-\frac{\partial \Delta G_{\text{coll}}}{\partial n} = 4\pi R_0^2 \gamma \left(1 - \frac{2}{3}n^{-1/3}\right)$$

This is not a real force expressed in Newtons, but we can think of it as a pseudo-force, with the bead number acting as a proxy for the chain extension. If you want to extend a hydrophobic chain, you must do work against this. Written in terms of the extension of the chain x (not the drop area A)

$$w = -\int_{x_0}^{x} f_{ext} \, dx = \int_{x_0}^{x} \left( \frac{\partial \Delta G_{coll}}{\partial A_n} \right) \left( \frac{\partial A_n}{\partial x} \right) dx$$

Here we still have to figure out the relationship between extension and surface area,  $\partial A_n / \partial x$ . Alternatively, we can think of the collapse coordinate as the number of coalesced beads, *n*.

#### Hydrophobic Collapse and Shape Fluctuations

An alternate approach to thinking about this problem is in terms of the collapse of a prolate ellipsoid to a sphere as is seeks to minimize its surface area. We take the ellipsoid to have a long radius  $\ell/2$  and a short radius *r*. The area and volume are then:



$$A = 2\pi \left( r^2 + \frac{\ell^2}{4} \frac{\alpha}{\tan \alpha} \right) \qquad \alpha = \cos^{-1} \left( \frac{2r}{\ell} \right)$$
$$V = \frac{2}{3} \pi r^2 \ell \qquad \text{(constant)}$$
$$\therefore \quad r^2 = 3V / 2\pi \ell$$
$$A = \left( \frac{3V}{\ell} + \pi \frac{\ell^2}{2} \frac{\alpha}{\tan \alpha} \right)$$

Let's plot the free energy of this ellipsoid as a function of  $\ell$ . For  $V = 4 \text{ nm}^3$ , k<sub>B</sub>T=4.1 pN nm we find  $\ell_{\text{min}}=1.96 \text{ nm}$ . Note that at k<sub>B</sub>T the dimensions of the ellipsoid can fluctuate over many ~5 Å.



10

## Readings

- 1. N. T. Southall, K. A. Dill and A. D. J. Haymet, A view of the hydrophobic effect, J. Phys. Chem. B **106**, 521–533 (2002).
- D. Chandler, Interfaces and the driving force of hydrophobic assembly, Nature 437, 640–647 (2005).
- 3. G. Hummer, S. Garde, A. E. García, M. E. Paulaitis and L. R. Pratt, Hydrophobic effects on a molecular scale, J. Phys. Chem. B **102**, 10469–10482 (1998).
- 4. B. J. Berne, J. D. Weeks and R. Zhou, Dewetting and hydrophobic interaction in physical and biological systems, Annu. Rev. Phys. Chem. **60**, 85–103 (2009).