

2. Solvation and Hydrophobicity

Solvation

Solvation describes the intermolecular interactions of a molecule or ion in solution with the surrounding solvent, which for our purposes will usually refer to water. Aqueous solvation influences an enormous range of problems in molecular biophysics, including (1) charge transfer and stabilization; (2) chemical reactivity; (3) hydrophobicity; (4) solubility, phase separation, and precipitation; (5) binding affinity; (6) self-assembly; and (7) transport processes in water.

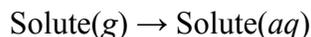
Solute–solvent interactions are mostly dominated by electrostatics (interactions of charges, dipoles, and induced dipoles), and includes hydrogen bonding and repulsion (which have some electrostatic components). The terms solute and solvent commonly apply to dilute mixtures in the liquid phase in which the solute (minor component) is dispersed into the solvent (major component). For this reason, the concept of solvation is also at times extended to refer to the influence of any surrounding environment in which a biomolecule is embedded, for instance, a protein or membrane.

Unlike most solvents, the presence of water as a solvent for biological molecules fundamentally changes their properties and behavior from the isolated molecule. Hydrogen bonding interactions with water can be strong enough that it is hard to discern where the boundary of solute and solvent ends. Furthermore, the fluctuating hydrogen bond network of water introduces a significant entropy to the system which can be competitive or even the dominant contributor in free energy calculations.

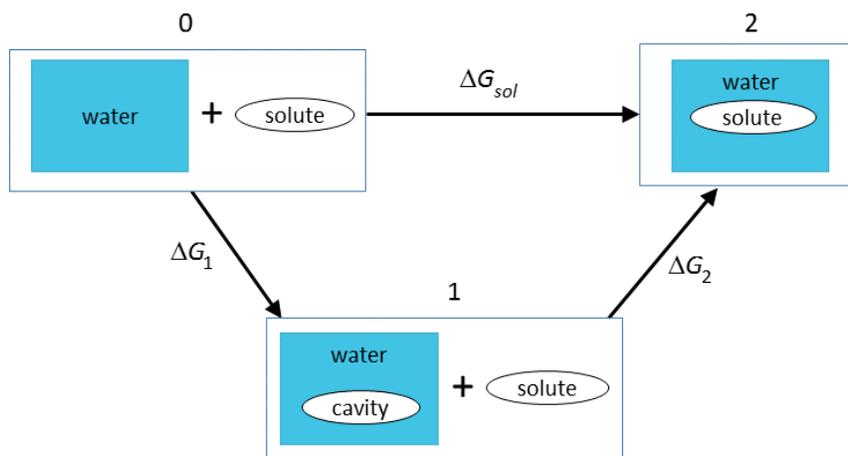
Although we will deal primarily with chemical equilibria in water, solvation is a highly dynamical process. Solvation dynamics refers to the time-dependent correlated motions of solute and solvent. How does a solvent reorganize in response to changes in solute charge distribution or structure? Conversely, how do conformational changes to the intermolecular configuration of the solvent (flow) influence changes in structure or charge distribution in the solute?

Solvation Thermodynamics

Let's consider the thermodynamics of a general aqueous solvation problem. This will help us identify various physical processes that occur in solvation, and also identify certain limitations. The reference state in solvation problems is commonly taken to be the isolated solute in vacuum:



Conceptually, it is helpful to break the solvation process into two steps: (1) the energy required to open a cavity in the liquid and (2) the energy to put the solute into the cavity and turn on the interactions between solute and solvent.



Each of these terms has enthalpic and entropic contributions:

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

$$\Delta G_{sol} = \Delta G_1 + \Delta G_2$$

$$= \Delta H_1 - T \Delta S_1 + \Delta H_2 - T \Delta S_2$$

ΔG_1 : Free energy to open a cavity in water. We are breaking the strong cohesive intermolecular interactions in water (ΔH_1), and reducing the configurational entropy of the water hydrogen-bond network (ΔS_1). Therefore ΔG_1 is large and positive. Most of the hydrophobic effect is dominated by this term.

ΔG_2 : Free energy to insert the solute into the cavity, turn on the interactions between solute and solvent. Ion and polar solvation is usually dominated by this term. This includes the favorable electrostatic and H-bond interactions (ΔH_2). It also can include a restructuring of the solute and/or solvent at their interface due to the new charges.

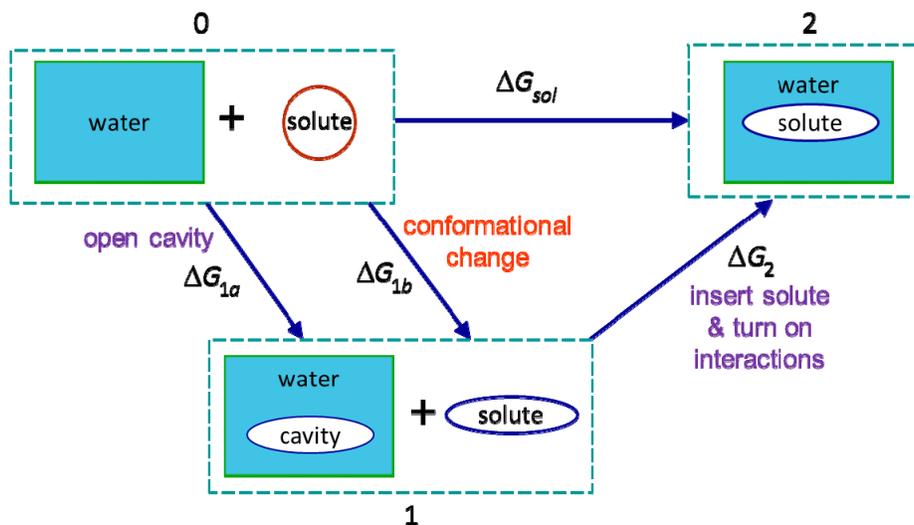
The simplest treatment describes the solvent purely as a homogeneous dielectric medium and the solute as a simple sphere or cavity embedded with point charges or dipoles. It originated from

the Born–Haber cycle first used to describe ΔH_{rxn} of gas-phase ions, and formed the basis for numerous continuum and cavity-in-continuum approaches to solvation. With a knowledge of this cycle, it becomes possible to construct thermodynamic cycles for a variety of related solvation processes:

Solvation of solute X	$X(g) + H_2O \rightarrow X(aq)$	ΔG_{sol}
Solubility	$X(s) + H_2O \rightarrow X(aq)$	$K_{sp} = [X(aq)] = \exp[-\Delta G_{sp}/k_B T]$
Transfer free energy	$X(oil) \rightarrow X(water)$	$P = [X(oil)]/[X(aq)]$
Association/Aggregation	$2X(aq) \rightarrow X_2(aq)$	$K_a = [X_2]/[X]^2$
Binding	$X(aq) + Y(aq) \rightarrow XY(aq)$	$K_a = [XY]/[X][Y]$

Looking at the cycle above also illustrates many of the complications from this approach relevant to molecular biophysics, even without worrying about atomistic details. From a practical point of view, the two steps in this cycle can often have large magnitude but opposite sign, resulting in a high level of uncertainty about ΔG_{sol} —even its sign! More importantly, this simplified cycle assumes that a clean boundary can be drawn between solute and solvent—the solvent accessible surface area. It also assumes that the influence of the solvent is perturbative, in the sense that the solvent does not influence the structure of the solute or that there is conformational disorder or flexibility in the solute and/or solvent.

To overcome some of these limitations, a more detailed approach can be used:



ΔG_{1a} : Free energy to create a cavity in water for the final solvated molecule.

ΔG_{1b} : Free energy to induce the conformational change to the solute for the final solvated state.

ΔG_2 : Free energy to insert the solute into the cavity, turn on the interactions between solute and solvent. This includes turning on electrostatic interactions and hydrogen bonding, as well as allowing the solvent to reorganize around the solute:

$$\Delta G_2 = \Delta G_{\text{solute-solvent}} + \Delta G_{\text{solvent reorg}}$$

Configurational entropy may matter for each step in this cycle, and can be calculated using¹

$$S = -k_B \sum_i P_i \ln P_i$$

Here sum is over microstate probabilities, which can be expressed in terms of the joint probability of the solute with a given conformation and the probability of a given solvent configuration around that solute structure. In step 1, one can average over the conformational entropy of the solvent for the shape of the cavity (1a) and the conformation of the solute (1b). Step 2 includes solvent configurational variation and the accompanying variation in interaction strength.

1. See C. N. Nguyen, T. K. Young and M. K. Gilson, Grid inhomogeneous solvation theory: Hydration structure and thermodynamics of the miniature receptor cucurbit[7]uril, *J. Chem. Phys.* **137** (4), 044101 (2012).

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 ΔG is what determines the affinity of one substance for another rather than just ΔH . 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, ΔG_{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:

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

- 1) $\Delta S_{\text{sol}} < 0$. 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 ΔS_1 .
- 2) $\Delta H_{\text{sol}} > 0$. The energy penalty of breaking up the hydrogen bond network (ΔH_1) is the dominant contributor to the enthalpy. This can be estimated from a count of the net number of H-bonds that needed to be broken to accommodate the solute: ΔH_{sol} increases by 1–3 kcal mol⁻¹ of hydrogen bonds. The interaction energy between a hydrocarbon and water (ΔH_2) is weakly favorable as a result of dispersion interactions, but this is a smaller effect. So, $\Delta H_{\text{sol}} \approx \Delta H_1$.

The net result is that ΔG_{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 ΔS_{sol} . Qualitatively, we are talking about limiting the configurational space that water molecules can adopt within the constraints of a tetrahedral potential.

Approximation

Bulk water: 4 HBs/tetrahedron

Within a tetrahedral lattice the orientation of an H₂O has:

6 configurations: 1,2 1,3 1,4
 2,3 2,4 3,4

$$\Omega_{\text{bulk}} = 6$$

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

3 configurations 1,2 1,3 1,4

$$\Omega_{\text{surface}} = 3$$

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

$$-T\Delta S_{\text{sol}} = k_B T \ln 2$$

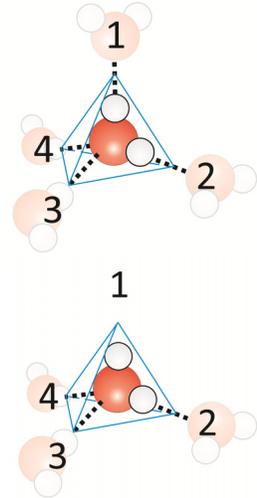
Evaluating at 300 K,

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

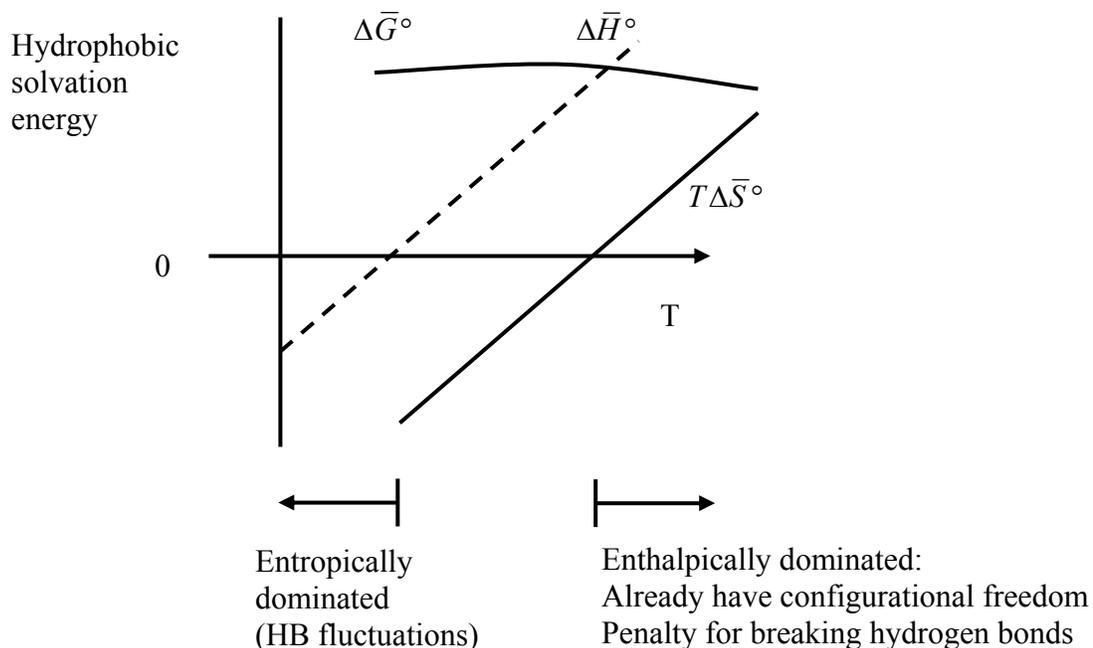
This value is less than the typical enthalpy for hydrogen bond formation, which is another way of saying that the hydrogen bonds like to stay mostly intact, but have large amplitude fluctuations.

Temperature Dependence of Hydrophobic Solvation

From ΔS_{sol} we expect ΔG_{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 ΔG_{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 ΔH is independent of temperature. The temperature dependence of all of these variables can be described in terms of a large positive heat capacity.

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

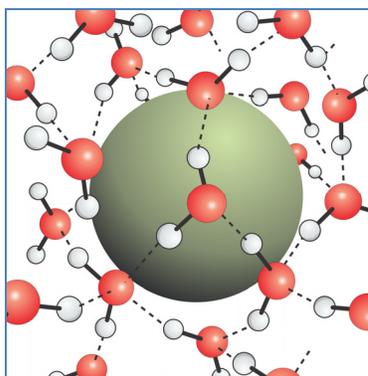
At low temperatures, with a stronger, more rigid hydrogen-bond network, the ΔS_2 term dominates. But at high temperature, approaching boiling, the entropic penalty is far less.

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, ℓ , $\sim 0.5\text{--}1.0$ nm).

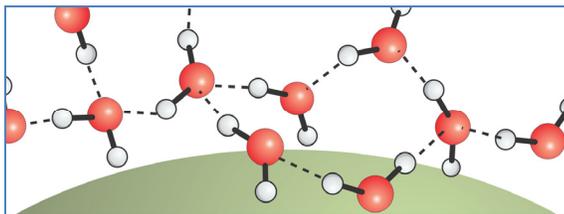
For small solutes ($R < \ell$): Network deformation

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 $\Delta G_{\text{sol}}(R < \ell) \propto R^3$.



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 ΔG_{sol} to scale as the surface area $\Delta G_{\text{sol}}(R > \ell) \propto R^2$. 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 ΔG 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 γ is the energy required to deform a liquid–vapor interface: $\gamma = \left(\partial U / \partial a\right)_{N,V,T}$, 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 γ should include entropic and enthalpic contributions to altering the hydrogen bond network at a surface, so we associate this with ΔG_{sol} . For water at 300 K, $\gamma = 72$ pN/nm. γ 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. $\gamma = 72$ mJ/m². 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 3.0×10^{-26} L/molecule, and the corresponding surface area per molecule deduced from geometric arguments is ~ 10 Å². 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

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

where E is an energy density.²

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

Remembering that $-\partial G / \partial V|_{N,T} = p$, the energy density E corresponds to units of pressure and corresponds to $E = 2.4 \times 10^3$ atm. If we divide E by the molarity of water (55M), then we find E 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

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

2. D. Chandler, Interfaces and the driving force of hydrophobic assembly, Nature **437**, 640-647 (2005).

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

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

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

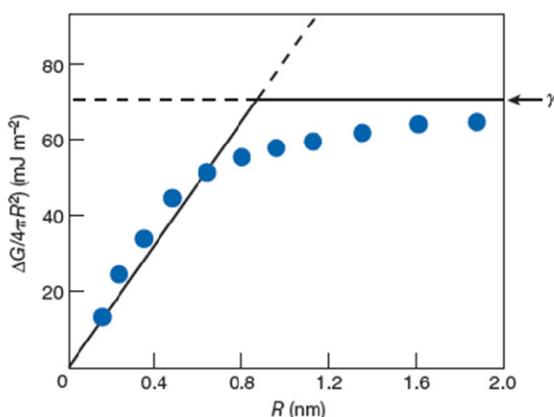


Figure 2 | Solvation free energy, ΔG , for a spherical cavity in water as a function of the cavity size. The results are for ambient conditions (room temperature and 1 atm pressure). The circles show the results of detailed microscopic calculations²⁵. The liquid–vapour surface tension is shown by γ . 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 ΔG 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 :

$$\begin{aligned} \Delta \bar{G} &= -k_B T \ln P(r) \\ P(r) &= \frac{e^{-U(r)/k_B T}}{\int_0^\infty e^{-U(r)/k_B T} dr} = \frac{\exp\left[\frac{-4\pi\gamma r^2}{k_B T}\right]}{\frac{1}{2} \sqrt{\frac{k_B T}{4\gamma}}} \\ &= \frac{2}{\sqrt{\pi} R_{\text{surf}}} \exp\left[-r^2/R_{\text{surf}}^2\right] \end{aligned}$$

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 isn't 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 $\delta r = 0.028 \text{ nm}$. 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

$$\Delta\mu^0 = -k_B T \ln(P)$$

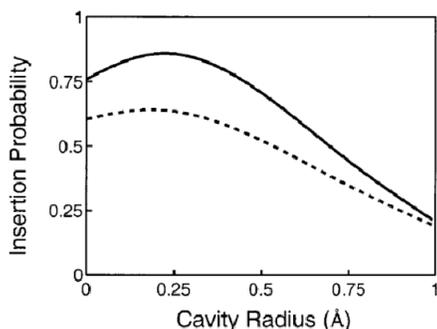


Figure 9. Free volume distributions from molecular dynamics simulations of a simple liquid, *n*-hexane (dashed line), and water (solid line). The probability of finding a cavity of a given radius is plotted. Water has more small cavities (<1 Å) than *n*-hexane. Data adapted from G. Hummer et al., *J.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

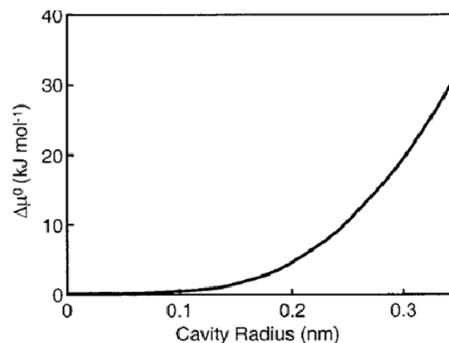


Figure 10. Free energy cost of creating a cavity, from molecular simulations. Data adapted from Hummer, G., et al. *J. Phys. Chem. B* **1998**, *102*, 10 475.

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Hydrophobic Collapse³

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.

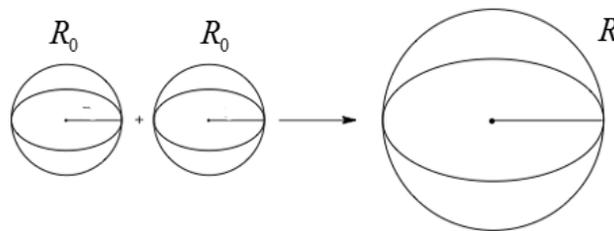
Let's calculate the free energy change for two oil droplets coalescing into one. The smaller droplets both have a radius R_0 and the final droplet a radius of R .

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

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 γ is positive.

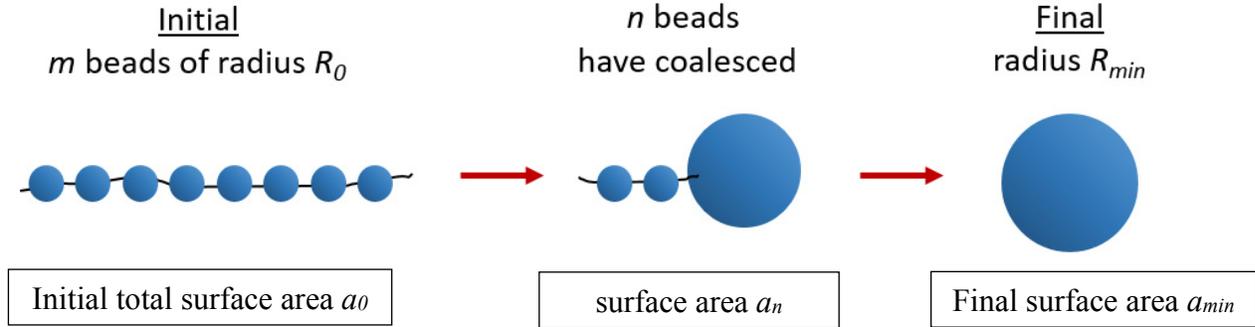


<u>Initial State</u>	<u>Final State</u>
Total Volume $V = 2\left(\frac{4}{3}\pi R_0^3\right)$	$V = \frac{4}{3}\pi R^3 \rightarrow R = 2^{1/3} R_0$
Initial surface area $a_0 = 2(4\pi R_0^2)$	$a = 4\pi R^2 = 4\pi(2^{1/3})^2 R_0^2 = 4\pi(1.59)R_0^2$
$\frac{a_0}{4\pi R_0^2} = 2$	$\frac{a}{4\pi R_0^2} = 1.59$
$\Delta G_{\text{collapse}} = (a - a_0)\gamma = (-0.41)4\pi R_0^2\gamma$	

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 $\Delta S_{\text{collapse}} \approx k_B \ln(3/6)$. In other words, a small number compared to the surface term.

3. 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, $V = n\left(\frac{4}{3}\pi R_0^3\right)$, 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

$a_n = (\text{surface area of drop formed by } n \text{ coalesced beads}) + (\text{total area of remaining } m-n \text{ beads})$

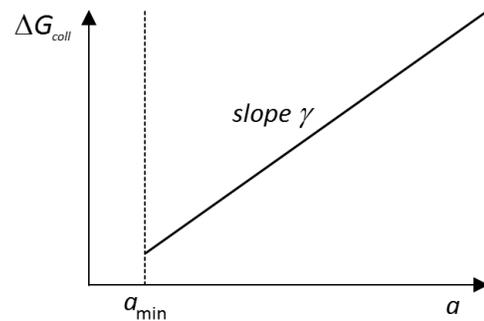
$$\begin{aligned} a_n &= \left(n^{2/3}4\pi R_0^2\right) + (m-n)4\pi R_0^2 \\ &= \left(m + n^{2/3} - n\right)4\pi R_0^2 \\ &= a_0 + \left(n^{2/3} - n\right)4\pi R_0^2 \end{aligned}$$

The free energy change for coalescing n beads is

$$\begin{aligned} \Delta G_{\text{coll}} &= (a_n - a_0)\gamma \\ &= \left(n^{2/3} - n\right)4\pi R_0^2\gamma \end{aligned}$$

The minimization of surface area drives the collapse of the chain, and is proportional to the surface tension γ .

$$f_{\text{coll}} = -\frac{\partial \Delta G_{\text{coll}}}{\partial a_n} = \gamma$$



This is not a real force expressed in Newtons, but it does indicate the direction of spontaneous change. We can think of it as a pseudo-force, with the area 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)

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

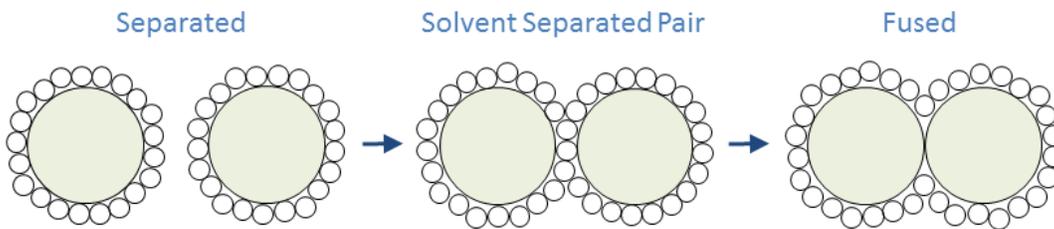
Here we still have to figure out the relationship between extension and surface area, $\partial a_n / \partial x$. Alternatively, we can think of the collapse coordinate as the number of coalesced beads, n .

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

	$n^{2/3} - n$	$1 - \frac{2}{3} n^{-1/3}$
$n = 2$	-0.41	0.47
$n = 3$	-0.92	0.54
$n = 5$	-2.08	0.61
$n = 10$	-5.35	0.69

Molecular Scale

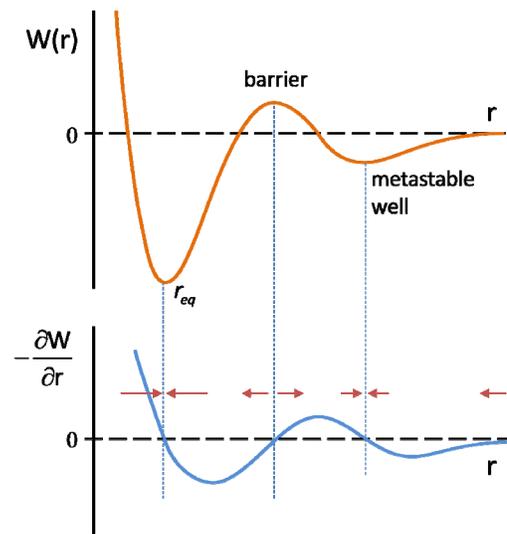
When looking at a molecular scale, in reality there are barriers to collapse. Consider the hydrophobic solvation structure about two hydrophobic spheres.



The free energy for bringing the spheres together as a function of inter-sphere separation is

$$W(r) = -k_B T \ln g(r)$$

Here $g(r)$ is the radial pair distribution function, and $W(r)$ is known as the potential mean force. We are taking a free energy that is a function of many internal variables and projecting it onto a single coordinate. $W(r)$ is a potential function that can be used to obtain the mean effective forces that a particle will experience at a given separation $f = -\partial W / \partial r$.



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).
2. 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** (51), 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).