

# The hydrophobic effect and the excess free energy of solvation

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## Abstract

The hydrophobic effect has been explained by the peculiar properties of liquid water. However, recent works by Marmur proposed a novel explanation: the hydrophobic effect is caused by the self-assembly of hydrophobic solutes driven by the theoretical upper bound of excess free energy. Here I show that this argument is erroneous, based upon a rigorous statistical mechanical analysis.

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## 1. Introduction

Water plays an important and active role in biochemical processes [1–8]. In particular, the hydrophobic effect, i.e., the low solubility of non-polar solutes in water, is considered to be one of the major driving forces of protein folding [1–8].

What is the origin of the hydrophobic effect? In 1945, Frank and Evans [9] proposed that a hydrophobic solute in water promotes, in its surrounding, an enhanced hydrogen bond network of water. The accompanying decrease in entropy has been suggested to be the cause of the low solubility of hydrophobic solutes in water. Since then, water structure around the hydrophobic solutes has been studied extensively [3–8,10,11]. Another explanation is that the small molecular size of water is responsible for the hydrophobic effect [12,13]. This explanation is based upon the free energy of cavity formation via scaled particle theory [14,15]. The relationship between the two explanations, which emphasises different aspects of water, is not completely understood [7,16].

Recently, Marmur [17,18] proposed a novel explanation of the origin of the hydrophobic effect, which is completely different from above explanations. Marmur has shown from a thermodynamic analysis that there is

a theoretical upper bound  $kT = 0.58$  kcal/mol (at 293 K) of the excess free energy ( $\Delta G_a^{\text{ex}}$ ). However, the experimental  $\Delta G_a^{\text{ex}}$  for hydrophobic solutes is much larger than the upper bound (Marmur's paradox). For example,  $\Delta G_a^{\text{ex}} = 10.8$  kcal/mol for octane in water [19]. Marmur attributed this discrepancy to the self-assembly of hydrophobic solutes in water: the entropy loss or free energy increase upon self-assembly accounts for the large  $\Delta G_a^{\text{ex}}$  [17]. This approach is novel because it emphasises that the hydrophobic effect is not caused by the property of water. The origin of the hydrophobic effect is the self-assembly of solutes driven by a purely thermodynamic requirement [17,18].

The upper bound of  $\Delta G_a^{\text{ex}}$  was derived in the following manner: consider a system in which water phase is contacting the pure solute phase, in equilibrium.  $\Delta G_a^{\text{ex}}$  at ambient pressure ( $P = P_0$ ) is defined by,

$$\Delta G_a^{\text{ex}} = kT \ln \gamma, \quad (1)$$

where  $\gamma$  is Raoult's law activity coefficient [20]. By using the condition of equilibrium between the water phase and the pure solute phase, it follows that [20]:

$$\Delta G_a^{\text{ex}} = -kT \ln x_{a,w}, \quad (2)$$

where  $x_{a,w}$  is the mole fraction of the solute in the water phase. The second equation was derived by the equilibrium between two phases. This  $\Delta G_a^{\text{ex}}$ , according to Marmur [17,18,20], is calculated via the integration of partial molar volumes along the pressure axis as

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$$\Delta G_a^{\text{ex}} = \int_0^{P_0} dP (V_{a,w}(x_{a,w}) - V_{a,a}), \quad (3)$$

where  $V_{a,a}$  and  $V_{a,w}$ , respectively, represent the partial molar volumes of the solute a in its own phase and in water phase.  $V_{a,w}$  was calculated under the fixed solute mole fraction  $x_{a,w}$ . Applying an order-of-magnitude analysis, Marmur [17] showed that  $\Delta G_a^{\text{ex}}$  from Eq. (3) is much smaller than  $kT$ , by using the fact that  $V_{a,w}(x_{a,w}) - V_{a,a}$  is smaller than the partial molar volume of the ideal gas. Although the later analysis [18] relaxed this strict upper bound, the theoretical upper bound of  $\Delta G_a^{\text{ex}}$  was still shown to be much smaller than the experimental  $\Delta G_a^{\text{ex}}$  for hydrocarbons in water, thereby necessitating the consideration of self-assembly to account for the discrepancy.

In spite of the novelty of Marmur's approach, many theoretical and experimental difficulties have been pointed out with regards to this approach [21–24]. Abraham and Blandamer [22] have accumulated cases and examples which contradict Marmur's picture. For example, the equilibrium between the monomeric solutes and self-associated solutes is ignored entirely in Marmur's approach [22,23]. Moreover,  $\Delta G_a^{\text{ex}}$  has been calculated accurately by various theoretical and computational methods which involve no consideration of self-assembly [22].

However, Marmur's paradox remains unsolved. Is there really a theoretical upper bound in excess free energies? Or is Marmur's analysis flawed? What is the dominant contribution to the excess free energies? To answer these questions, a rigorous statistical mechanical analysis will be presented in this Letter.

## 2. A statistical thermodynamic foundation

Consider a system in which a liquid hydrocarbon phase is in contact with a water phase in equilibrium. At room temperature and ambient pressure, both phases are in the liquid state.

The transfer free energy  $\Delta G_a^{\text{tr}}$  of a hydrocarbon (solute) molecule a from its own phase  $i = a$  to water  $i = w$  is calculated as follows:

$$\Delta G_a^{\text{tr}} = \mu_{a,w}^* - \mu_{a,a}^* = -kT \ln \frac{\rho_{a,w}}{\rho_{a,a}}, \quad (4)$$

where  $\rho_{a,i}$  is the number density (molarity) of the solute molecule in phase  $i$  [19].  $\mu_{a,i}^*$  is Ben-Naim's 'pseudo-chemical potential' defined from chemical potential  $\mu_{a,i}$  as

$$\mu_{a,i}^* = \mu_{a,i} - kT \ln \rho_{a,i} \Lambda_a^3, \quad (5)$$

where  $\Lambda_a$  is the momentum distribution function [19].  $\mu_{a,i}^*$  has a statistical mechanical interpretation

$$\mu_{a,i}^* = kT \ln \left\langle \exp \left( - \frac{U_{a,i}}{kT} \right) \right\rangle_i, \quad (6)$$

where  $U_{a,i}$  is the interaction energy between a newly inserted solute a and the rest of the molecules in phase  $i$ . According to Eq. (6),  $\mu_{a,i}^*$  represents the 'binding free energy' of the solute molecule a to the phase  $i$  [19,25]. Transfer free energy  $\Delta G_a^{\text{tr}}$  thus can be interpreted as the difference in solute binding free energies between the two phases.

$\Delta G_a^{\text{tr}}$  can be related to the mole fraction  $x$  of the solute molecule in the water phase, by using

$$x_{a,w} = \frac{\rho_{a,w}}{\rho_{a,w} + \rho_{w,w}}, \quad (7)$$

where  $\rho_{w,w}$  is the molarity of water in the water phase. By Eq. (7), Eq. (4) can now be rewritten as

$$\begin{aligned} \Delta G_a^{\text{tr}} &= -kT \ln \frac{x_{a,w}}{1 - x_{a,w}} - kT \ln \frac{\rho_{w,w}}{\rho_{a,a}} \\ &\simeq -kT \ln x_{a,w} - kT \ln \frac{\rho_{w,w}}{\rho_{a,a}} \\ &= kT \ln \gamma - kT \ln \frac{\rho_{w,w}}{\rho_{a,a}} = \Delta G_a^{\text{ex}} - kT \ln \frac{\rho_{w,w}}{\rho_{a,a}}, \end{aligned} \quad (8)$$

where the approximation holds when  $x_{a,w} \ll 1$ .

Consider, as an example, the dissolution of octane in water. At 1 atm and 293.15 K, experimental values are  $\Delta G_a^{\text{tr}} = 8.193$  kcal/mol [19],  $\rho_{a,a} = 6.145 \times 10^{-3}$  mol/ml,  $\rho_{w,w} = 5.540 \times 10^{-1}$  mol/ml [26]. Therefore,  $\Delta G_a^{\text{ex}} = 10.814$  kcal/mol. Since  $-kT \ln \rho_{w,w}/\rho_{a,a} = -2.622$  kcal/mol is a negative value, a theoretical upper bound of  $\Delta G_a^{\text{ex}}$  is an upper bound of  $\Delta G_a^{\text{tr}}$ .

## 3. Transfer free energies

Here I examine the possibility of a theoretical upper bound to  $\Delta G_a^{\text{tr}}$ .

Differentiation of  $\Delta G_a^{\text{tr}}$  with respect to pressure  $P$  leads to

$$\begin{aligned} \left( \frac{\partial \Delta G_a^{\text{tr}}}{\partial P} \right)_{T, \rho_{a,w}} &= \left( \frac{\partial \mu_{a,w}^*}{\partial P} \right)_{T, \rho_{a,w}} - \left( \frac{\partial \mu_{a,a}^*}{\partial P} \right)_{T, \rho_{a,w}} \\ &= V_{a,w}^* - V_{a,a}^*, \end{aligned} \quad (9)$$

where  $V_{a,i}^* = V_{a,i} - kT \kappa_i$  is the volume change upon introducing a into phase  $i$ , calculated from  $V_{a,i}$ , the partial molar volume of a in phase  $i$ , and  $\kappa_i$ , the isothermal compressibility of the phase  $i$ .

Now I examine whether the differentiation performed in Eq. (9) is always defined. To do so, consider the process in which the pressure is lowered from ambient. Water and hydrocarbon phases, at given pressures, evaporate. Since  $\mu_{a,i}^*$  represents the binding free energy of solute a to phase  $i$ ,  $\mu_{a,i}^*$  is discontinuous at  $P = P_i^T$

when condensation (gas to liquid transition) takes place. The differentiation is not defined at this pressure.

Now I calculate  $\Delta G_a^{\text{tr}}$  at  $P = P_0$  by integrating the volumetric data along the pressure axis. Incorporating the discontinuities at  $P = P_a^T$  and  $P_w^T$ ,  $\Delta G_a^{\text{tr}}$  is expressed in the case of  $P_w^T < P_a^T$  as

$$\begin{aligned} \Delta G_a^{\text{tr}}(P = P_0) = & \Delta G_a^{\text{tr}}(P = 0) + \int_0^{P_w^T} dP (V_{a,w}^{*,g} - V_{a,a}^{*,g}) \\ & + \Delta \mu_{a,w}^*(P_w^T) + \int_{P_w^T}^{P_a^T} dP (V_{a,w}^{*,l} - V_{a,a}^{*,g}) \\ & - \Delta \mu_{a,a}^*(P_a^T) + \int_{P_a^T}^{P_0} dP (V_{a,w}^{*,l} - V_{a,a}^{*,l}), \end{aligned} \quad (10)$$

where the superscripts g and l emphasise the state, gas and liquid, respectively, of the phases.  $\Delta \mu_{a,i}^*(P_i^T)$  is the change in pseudochemical potential when  $P = P_i^T$ . In the case of  $P_w^T > P_a^T$ , the equation can be expressed in a similar manner.

Which are the leading terms of Eq. (10)? To answer this question, I analyse the order-of-magnitude of each terms in Eq. (10).

- (i) The first term:  $\Delta G_a^{\text{tr}}(P = 0) = 0$ . This can be shown by employing the definition  $\Delta G_a^{\text{tr}}(P = 0) = \mu_{a,w}^{*,g}(P = 0) - \mu_{a,a}^{*,g}(P = 0)$ . According to Eq. (6), the binding free energy of a solute to an ideal gas is zero. Hence both  $\mu_{a,w}^{*,g}(P = 0)$  and  $\mu_{a,a}^{*,g}(P = 0)$  are zero.
- (ii) The second term: as can be shown easily,  $V_{a,i}^{*,g} = 0$  for ideal gases. Therefore,  $\int_0^{P_w^T} dP (V_{a,w}^{*,g} - V_{a,a}^{*,g})$  is zero.
- (iii) The third term: at  $P = P_w^T$ , the water phase condenses from the gaseous to liquid state. This is followed by a large  $\Delta \mu_{a,w}^*$ , since the binding energy difference of a solute a between gaseous and liquid states is large, as is clear from Eq. (6). Since the binding energy of a solute in the gas phase is zero, i.e.,  $\mu_{a,w}^{*,g} \simeq 0$ , it follows that  $\Delta \mu_{a,w}^* \simeq \mu_{a,w}^{*,l}$ .
- (iv) The fourth term: as in (ii),  $V_{a,a}^{*,g} \simeq 0$ . Therefore,  $\int_{P_w^T}^{P_a^T} dP (V_{a,w}^{*,l} - V_{a,a}^{*,g}) \simeq \int_{P_w^T}^{P_a^T} dP V_{a,w}^{*,l} \ll V_{a,w}^{*,l}(P_0) \times P_0$ .  $V_{a,w}^{*,l}$  for octane in water is not reported in the literature, because of octane's low solubility in water. However, judging from the trend observed for lower hydrocarbons, it is not unrealistic to assume that  $V_{a,w}^*$  has the same order as  $V_{a,a}^*$ , and  $V_{a,a}^*$  is slightly smaller than  $V_{a,a} = 162.60$  ml/mol. Therefore,  $P_0 V_{a,w}^*$  is on the order of 3.9 cal/mol, which is negligible compared to  $\Delta G_a^{\text{ex}}$ .
- (v) The fifth term: as (iii), this term reflects the condensation of the solute phase, and  $\Delta \mu_{a,a}^* \simeq \mu_{a,a}^{*,l}$ .
- (vi) The sixth term:  $V_{a,w}^{*,l}(P_0)$  for octane in water is not given. However, by taking a gross overestimation  $|\int_{P_w^T}^{P_0} dP (V_{a,w}^{*,l} - V_{a,a}^{*,l})| \ll P_0 (V_{a,a}^{*,l}) = 3.9$  cal/mol, which is quite negligible.

Combining (i)–(vi), I obtain

$$\Delta G_a^{\text{tr}} \simeq \mu_{a,w}^{*,l}(P_w^T) - \mu_{a,a}^{*,l}(P_a^T), \quad (11)$$

which means that the changes in  $\mu_{a,i}^*$ s at the condensation pressures ( $P = P_i^T$ ) contribute dominantly to  $\Delta G_a^{\text{tr}}$ .

Eq. (11) can be verified from a different route: since  $V_{a,i}^* = (\partial \mu_{a,i}^* / \partial P)_{T,P,\rho_{a,i}}$ , as in Eq. (9), integration of this relationship yields,

$$\mu_{a,i}^{*,l}(P_i^T) \simeq \mu_{a,i}^{*,l}(P_0) - (P_0 - P_i^T) V_{a,i}^{*,l}(P_0), \quad (12)$$

by retaining up to the first order term in  $(P_0 - P_i^T)$ . Since  $(P_0 - P_i^T) V_{a,i}^{*,l}(P_0) < P_0 V_{a,i}^{*,l}(P_0)$ ,  $P_0 V_{a,i}^{*,l}(P_0)$  gives the upper limit of  $|\mu_{a,i}^{*,l}(P_i^T) - \mu_{a,i}^{*,l}(P_0)|$ . This term, as estimated in (iv), is less than 3.9 cal/mol. This is negligible compared to the transfer free energies of hydrophobic solutes. Therefore,  $\mu_{a,i}^{*,l}(P_i^T) \simeq \mu_{a,i}^{*,l}(P_0)$ . Combining this with Eq. (4) leads to:

$$\begin{aligned} \Delta G_a^{\text{tr}}(P_0) = & \mu_{a,w}^{*,l}(P_0) - \mu_{a,a}^{*,l}(P_0) \\ \simeq & \mu_{a,w}^{*,l}(P_w^T) - \mu_{a,a}^{*,l}(P_a^T), \end{aligned} \quad (13)$$

an equation identical to Eq. (11).

Unlike Marmur's analysis, the upper bound of each integrals in Eq. (10) did not lead to the upper bound of  $\Delta G_a^{\text{tr}}$ . The dominant contribution comes from the changes of binding free energies when the phases undergo condensation transition, which was ignored by Marmur. Therefore, there is no upper bound of  $\Delta G_a^{\text{tr}}$ .

#### 4. Excess free energies

The analysis in Section 3 confirmed that the major contribution to  $\Delta G_a^{\text{tr}}$  comes from the discontinuity in  $\mu_{a,i}^*$  at  $P_i^T$ s. Here I examine whether this discontinuity can account for the majority of  $\Delta G_a^{\text{ex}}$ .

Now  $\mu_{a,w}$ s at  $P = P_w^T$ , when  $x_{a,w}$  is fixed, can be expressed as:

$$\begin{aligned} \mu_{a,w}^g(P_w^T; x_{a,w}) = & \mu_{a,w}^{g,*}(P_w^T; x_{a,w}) + kT \ln \rho_{a,w}^g(P_w^T; x_{a,w}) A_a^3, \\ \mu_{a,w}^l(P_w^T; x_{a,w}) = & \mu_{a,w}^{l,*}(P_w^T; x_{a,w}) + kT \ln \rho_{a,w}^l(P_w^T; x_{a,w}) A_a^3. \end{aligned} \quad (14)$$

As before,  $\mu_{a,w}^{g,*}(P_w^T; x_{a,w}) = 0$  and  $\mu_{a,w}^{l,*}(P_w^T; x_{a,w})$  is a large positive number. And since gases are more dilute than liquids,  $\rho_{a,w}^l(P_w^T; x_{a,w}) \gg \rho_{a,w}^g(P_w^T; x_{a,w})$ . Taken together, I obtain  $\mu_{a,w}^g(P_w^T; x_{a,w}) \ll \mu_{a,w}^l(P_w^T; x_{a,w})$  (note that  $x_{a,w}$  is fixed). This clearly shows that there is a discontinuity in chemical potential at  $P_w^T$ , when the solute mole fraction in water is fixed. On the other hand,  $\mu_{a,a}$  is continuous at  $P = P_a^T$ , because of the phase equilibrium condition for the pure systems. Incorporating this discontinuity, Eq. (3) can be corrected as

$$\begin{aligned} \Delta G_a^{\text{ex}} = & \int_0^{P_0} dP (V_{a,w}(x_{a,w}) - V_{a,a}) + \mu_{a,w}^l(P_w^T; x_{a,w}) \\ & - \mu_{a,w}^g(P_w^T; x_{a,w}). \end{aligned} \quad (15)$$

Marmur [17,18] showed that the first term is small. Therefore

$$\Delta G_a^{\text{ex}} \simeq \mu_{a,w}^l(P_w^T; x_{a,w}) - \mu_{a,w}^g(P_w^T; x_{a,w}), \quad (16)$$

should hold.

Eq. (16) can be verified by another route: combining Eq. (16) with Eq. (14) gives

$$\begin{aligned} \Delta G_a^{\text{ex}} &\simeq \mu_{a,w}^{*,l}(P_w^T; x_{a,w}) - kT \ln \frac{\rho_{a,w}^g(P_w^T; x_{a,w})}{\rho_{a,w}^l(P_w^T; x_{a,w})} \\ &= \mu_{a,w}^{*,l}(P_w^T; x_{a,w}) - kT \ln \frac{\rho_{a,a}^l(P_w^T)}{\rho_{a,w}^l(P_w^T; x_{a,w})} \\ &\quad - kT \ln \frac{\rho_{a,w}^g(P_w^T; x_{a,w})}{\rho_{a,a}^g(P_w^T)} - kT \ln \frac{\rho_{a,a}^g(P_w^T)}{\rho_{a,a}^l(P_w^T)}. \end{aligned} \quad (17)$$

Since the density of an ideal gas does not depend upon the chemical constitution of the gas,  $\rho_{a,a}^g(P_w^T) = \rho_{w,w}^g(P_w^T)$  holds. Since  $x_{a,w}$  is small for hydrocarbons in water,

$$\begin{aligned} -kT \ln \frac{\rho_{a,w}^g(P_w^T; x_{a,w})}{\rho_{a,a}^g(P_w^T)} &= -kT \ln \frac{\rho_{a,w}^g(P_w^T; x_{a,w})}{\rho_{w,w}^g(P_w^T)} \\ &= -kT \ln x_{a,w}. \end{aligned} \quad (18)$$

By using Eq. (18), as well as Eqs. (4) and (7), Eq. (17) can be rewritten as

$$\begin{aligned} \Delta G_a^{\text{ex}} &\simeq \mu_{a,w}^{*,l}(P_w^T; x_{a,w}) + \left( \mu_{a,a}^{*,l}(P_w^T) - \mu_{a,w}^{*,l}(P_w^T; x_{a,w}) \right) \\ &\quad - kT \ln x_{a,w} + \left( -\mu_{a,a}^{*,l}(P_w^T) \right) \\ &= -kT \ln x_{a,w}, \end{aligned} \quad (19)$$

which is identical to Eq. (2). Since Eq. (16) is shown to be equivalent to Eq. (2), Eq. (16) represents a dominant contribution to  $\Delta G_a^{\text{ex}}$ . Therefore, the term on which Marmur focused when deriving the theoretical upper bound of  $\Delta G_a^{\text{ex}}$ , is a negligible term. The dominant contribution (e.g., Eq. (16)) was completely ignored in Marmur's analysis. Therefore, the analysis which derived the theoretical upper bound of excess free energies is flawed. It follows that there is no self-assembly caused by the upper bound of excess free energies.

## 5. Conclusion

A statistical mechanical analysis has been carried out to identify the dominant contribution to hydrophobic transfer free energies and excess free energies, calculated via the pressure axis. The binding free energy of a solute molecule to each phase (water or solute) undergoes a sharp discontinuous change when the water phase condenses from the gaseous to liquid state. This disconti-

nuity contributes dominantly to the hydrophobic effect. The contribution from the integration of partial molar volumes is negligible. The proposed upper bound of excess free energies [17,18] incorporated only a negligible contribution, and ignored the dominant contribution. The present analysis supports the previous investigations which accumulated the evidence against an explanation of the hydrophobic effect based upon self-assembly [21–23].

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