Technical Comments

The Meaning of Hydrophobicity

K. P. Murphy *et al.* (*1, 2, 3*) recently proposed what would appear to be a profound change in our way of thinking about hydrophobic interactions. On the basis of their elegant and careful experiments on small molecules (*4*) and on proteins (*5*), they propose that "for globular proteins . . . hydrophobic interactions lead to destabilization" (*1*) and that "the hydration effect of nonpolar solutes stabilizes the dissolved state and thus in itself cannot be regarded as a cause of their hydrophobicity" (*2*). These statements would appear to conflict with the current dogma that hydration of nonpolar solutes is opposed by the entropy of water ordering at 25°C and that protein folding is driven by hydrophobicity. It appears, however, that the statements of Murphy *et al.* illuminate a *specific aspect* of nonpolar solvation and do not contradict the traditional dogma. The apparent contradiction can be traced to the use by Murphy *et al.* of a novel meaning for the terms "hydrophobic interactions" and "hydration effect," defined by a specific hypothetical process they describe.

Several times in the past there have been disagreements about the meaning of the terms "hydrophobicity," "hydrophobic effect," and "hydrophobic hydration" (*6*). There appear to be at least three distinct meanings of these terms: (i) "Hydrophobic" has been used to refer to any transfer of a nonpolar solute to any aqueous solution. (ii) Alternatively, it has been used more specifically to refer to transfers of nonpolar solutes into aqueous solution when a particular characteristic temperature dependence is observed, as noted below. These two meanings describe experimental observations and make no reference to a particular molecular interpretation. (iii) "Hydrophobicity" has also been used to refer to particular molecular models, generally involving the ordering of water molecules around the nonpolar solute. Whereas the common usage of the term now appears to be definition (ii) (*7*), Murphy *et al.* (*1*) appear to have used a particular variant of definition (iii).

Hydrophobicity is as good a term as any with which to describe the unusual temperature dependence of the solvation of nonpolar solutes in water (ii). The unusual feature is that nonpolar solvation in water is strongly opposed by entropy at around 25°C and has a large positive heat capacity change. Simpler solvation processes are instead opposed by enthalpy over a wide range of temperatures and have small heat capacity changes. This is the feature of nonpolar solvation first identified by Butler (*8*) and by Frank and Evans (*9*) that merits special terminology. Definition (i) needs no special term because it describes otherwise ordinary solution processes. There are also two problems with using meaning (iii) to define hydrophobicity: the molecular mechanism is not yet fully understood, and "water ordering" is an appropriate description of the entropic explosion of nonpolar solutes at 25°C, but not over a broader temperature range (*2*). Hence I believe the most sensible use of "hydrophobicity" is in situation (ii); it would therefore be simply an operational definition of an experimental observation.

The apparent contradiction can be traced to a particular, novel meaning that Murphy *et al.* attribute to "hydrophobic interactions" and "hydration effect." According to meaning (ii), "hydrophobicity" describes a complete transfer process as measured in partitioning experiments: (a) removal of the solute from the pure medium, with the breaking of solute-solute bonds; (b) closing the cavity therein; (c) creating a cavity in water; and (d) making the solute-water bonds. This is an experimentally measurable process. Murphy *et al.*, however, define "hydrophobic interactions" and "hydration effect" in terms of the free energy of creating a cavity in water minus the corresponding free energy at a special temperature, $T = T_s$, where the transfer is least favored. They obtain this hydration free energy by subtracting the transfer enthalpy at T_s from the total transfer free energy. [Murphy *et al.* state that the transfer enthalpy at T_s equals the vaporization enthalpy, but that is not completely correct; rather it equals the oil-water transfer enthalpy at T_s and includes the enthalpy of solute-water interactions, (d), in addition to (a) and (b).] Using this hypothetical "compact gas" reference state (*2*), Murphy *et al.* make the point that, relative to T_s, the opening of the water cavity at other temperatures is more energetically favored than it is entropically disfavored.

In short, Murphy *et al.* express the free energy of transfer as (*2*):

$$\Delta G(T) = \Delta H(T_s) + \Delta C_p[(T - T_s) - T \ln(T/T_s)]$$

where ΔH is the enthalpy change and ΔC_p is the heat capacity change. They refer to the second term on the right-hand side of this equation as the "hydration effect," to which I have no objection. But Murphy *et al.* (*1*) now also interchangeably refer to this second term as the "hydrophobic interaction." Instead, in my opinion, "hydrophobic interaction" should not refer to the second term alone, but to the full free energy, the sum of both terms on the right. Otherwise, to define hydrophobicity at temperature T in terms of a hypothetical reference state with the enthalpy "turned off" at a different temperature, T_s, constitutes a definition, which, to my knowledge, has not been used before. On the other hand, if we adhere instead to meaning (ii) to define "hydrophobicity" and "hydrophobic hydration," then data of these authors (*2*) provides clear evidence, and much of the underpinning, for the widely held view that water ordering opposes nonpolar solvation at 25°C and that at least one large driving force for protein folding is the burial of nonpolar residues in a nonpolar core. Such processes undoubtedly involve van der Waals interactions, as Murphy *et al.* point out.

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Response: The question has been raised as to whether our recent report (*1*) was in agreement with the viewpoint formulated in a previous paper concerning the nature of hydrophobic interactions (*2*). The problem arose from an attempt to use Baldwin's general expression (*3*) for the free energy change of hydrophobic interaction, which uses two reference temperature values, T_h

and T_s. These are the temperatures where the enthalpy and entropy of transfer of liquid hydrocarbons into water are zero (*3*). The equation is given as

$$\Delta G^0_{\mathrm{hyd}} = \Delta C_{p,\mathrm{hyd}}(T - T_h) + \Delta C_{p,\mathrm{hyd}} T \ln(T_s/T)$$

In our 1988 analysis (*2*) of the hydrophobic effect, we used the single reference temperature T_s, which leads to the expression

$$\Delta G^0_{\mathrm{hyd}} = \Delta H^* - \Delta C_p[(T_s - T) + T \ln(T/T_s)]$$

Here ΔH^* is the enthalpy change at T_s, which is given by $\Delta C_p(T_s - T_h)$. This last expression separates the temperature-independent term, ΔH^* from the temperature-dependent terms and provides the basis for the interpretation of hydrophobic interaction in terms of hydration and nonhydration components. The temperature-dependent term is regarded as describing the hydration effect. This term is always negative below the reference temperature T_s and increases in magnitude the greater the departure from T_s. We thus concluded that the hydration effect results in a decrease of hydrophobic interactions. From this point of view the positive free energy change is of enthalpic origin and arises from van der Waals contributions.

In our 1990 report, we wanted to call special attention to the existence of a common temperature, denoted by T_s^*, where different classes of apolar compounds (gases, liquids, solids, and proteins) have a unique value that depends only on the class of compound for the entropy change of dissolution into water or for denaturation. For liquid hydrocarbons, T_s is the same as T_s^*. As the hydration of apolar groups is the only common element in all of these processes, T_s^* appears to be a key parameter for describing the hydration effect. Regrettably, in our report (*1*) the two presentations of hydrophobic interactions given above were not clearly delineated. In particular, the term "hydrophobic effect" was used rather than "hydration" to describe the heat capacity temperature-dependent component of the free energy expression based on a single reference temperature. What we actually meant was that the hydration effect, not the hydrophobic effect, leads to destabilization of protein structures.

We agree with Dill that the most distinguishing feature of hydrophobicity is the unusually large heat capacity change. The generally accepted view is that its molecular origin is based on significant structural changes of the water surrounding apolar groups. Our definition of the hydration effect, which is phenomenologically based on the heat capacity–sensitive term of the free energy of dissolution, must reflect the effects of restructuring of solvated water as well as the properties of water and the pure apolar substance. At the reference temperature T_s^*, we observed that the enthalpy of dissolution of liquid hydrocarbons is approximately equal to the enthalpy of vaporization, since the enthalpy of dissolution of hydrocarbon gases is zero near T_s^* (*4*). Consequently, with regard to Dill's parenthetical statement "(Murphy *et al.* state that the transfer enthalpy at T_s equals the vaporization enthalpy. . . .)", at T_s^* the enthalpy change attributed to water restructuring must cancel the enthalpy change due to van der Waal's solute-solvent contacts. Overall, the hydration effect mirrors the decrease in hydrophobicity as one moves in temperature away from T_s^*.

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"You Nobel laureates are all alike!"