

An information theory model of hydrophobic interactions

(solvation/hydrophobic effects/biomolecule solution structure)

GERHARD HUMMER*, SHEKHAR GARDE*†, ANGEL E. GARCÍA*, ANDREW POHORILLE‡§, AND LAWRENCE R. PRATT*¶

*Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545; †Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143; and ‡§National Aeronautics and Space Administration, Ames Research Center, MS-239-4, Moffett Field, CA 94035-1000

Communicated by David Chandler, University of California, Berkeley, CA, February 27, 1996 (received for review December 13, 1995)

ABSTRACT A molecular model of poorly understood hydrophobic effects is heuristically developed using the methods of information theory. Because primitive hydrophobic effects can be tied to the probability of observing a molecular-sized cavity in the solvent, the probability distribution of the number of solvent centers in a cavity volume is modeled on the basis of the two moments available from the density and radial distribution of oxygen atoms in liquid water. The modeled distribution then yields the probability that no solvent centers are found in the cavity volume. This model is shown to account quantitatively for the central hydrophobic phenomena of cavity formation and association of inert gas solutes. The connection of information theory to statistical thermodynamics provides a basis for clarification of hydrophobic effects. The simplicity and flexibility of the approach suggest that it should permit applications to conformational equilibria of nonpolar solutes and hydrophobic residues in biopolymers.

Hydrophobic interactions are widely believed to be of dominating importance for protein structure, aggregation, and function. However, the molecular theories of hydrophobic interactions (1–10) have not been used so far in molecular studies of protein structure. This is partly because these theories have limitations that are still being clarified (5, 11–21) and partly because of their complexity. This paper suggests a new approach to molecular theories of hydrophobic effects and then tests the simplest model to which this suggestion leads. It is argued that the simplicity and flexibility of this approach should eventually permit its application to issues of protein structure in solution.

Alternative descriptions of hydrophobic effects that are used are based upon parameterizations of solubility data (22–26). Those hydrophobicity models have not changed essentially from the concepts of Kauzmann (27) but the solubility data have been parameterized in a variety of ways (28–31). Although solubility models of hydrophobic effects have been useful, molecular-level theories are expected to have wider applicability and to improve our understanding of hydrophobic effects on biomolecular structure. This could be particularly important to recent work that probes protein solution structure in new ways.

One example of such work is reversible denaturation experiments. The observed destabilization of folded proteins with decreasing temperature is an evidence of hydrophobic interactions. Cold/heat denaturation of globular proteins (32–34), pressure denaturation (35–39), and the effects of osmotic stress (40–49) demonstrate that the solvent activity affects the structure. However, parameterizations of hydrophobicity models that reflect the activity of the aqueous medium have not been pursued extensively (50).

The adequacy of solubility models is also not obvious in studies of the structures of folding intermediates on renatur-

ation pathways. These studies are expected to teach how proteins fold and perhaps then lead to better methods of predicting the folded structures (51, 52). The solubility models are parameterized for well-defined structures of native proteins. For folding intermediates, in contrast, several structures are available, the exposures of different residues to water might be quite different from those known in fully folded proteins, and, furthermore, the characteristics of the protein-water interface will be different from those known. It is relevant, for example, that the hydrophobicity models do not describe solvent-separated hydrophobic interactions or desolvation barriers to contact that might be significant for folding intermediates (53).

As a final example that motivates renewed theoretical work on hydrophobic effects, we mention the current research on predicting the folded structures of polypeptides using “knowledge-based” contact potentials obtained by analyzing a training set of protein structures (31, 54). Such approaches have produced interesting results; however, in the absence of a clear connection between the contact potentials and molecular principles, the incompletely resolved question of adequacy of the training set is crucial. For example, the solution conditions of crystal structures are a potentially important but generally ignored feature of the training set. In addition, it seems likely that economy of description could be obtained “from a deeper understanding of how the various physical contributions can be represented with minimum redundancy” (31). A molecular-level theory of hydrophobic interactions might provide such a representation.

Here we develop an information theory model of hydrophobic effects. It has some conceptual overlap with knowledge-based contact potentials but it is sharply distinguished from the latter by providing a clear connection to molecular principles of statistical thermodynamics. We present the simplest such model, based upon information readily accessible from experiment or computer simulations, and test it for prediction of the most primitive hydrophobic phenomena: the thermodynamics of *hydration* and *association* of model hard core solutes. As we will show, this model proves sufficient to describe these primitive hydrophobic phenomena *quantitatively*. We will note also and comment upon the significant overlap that this simplest model has with the most detailed of the available molecular theories (3, 15).

THEORETICAL DEVELOPMENT

Chemical potentials of nonpolar solutes at infinite dilution in water are the quantities of ultimate interest here. We shall be concerned with the excess chemical potential $\Delta\mu^{ex}$ of model solutes that perfectly repel centers of water molecules from a solute excluded volume. We will identify the center of a water molecule as the position of its oxygen atom. This definition of

Abbreviation: pmf, potential of mean force.

†Present address: Department of Chemical Engineering, University of Delaware, Newark, DE 19716.

¶To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

a solute molecular volume is consistent with molecular models that are commonly used in detailed calculations and for the simulation data considered below. It is known that the effects of more general solute-solvent interactions can be treated at a subsequent step (4). Statistical mechanics relates $\Delta\mu^{ex}$ to the probability p_0 of finding an empty volume Δv , or cavity, of given size and shape in water, $\Delta\mu^{ex} = -k_B T \ln p_0$. For atomic and small molecule-sized cavities, p_0 can be calculated directly. However, similar calculations for larger cavities become difficult. We seek models that are applicable to cavity shapes and sizes inaccessible to direct calculations.

We address the problem of modeling p_0 by considering the set of probabilities p_n of finding exactly n solvent centers in the cavity volume, $\sum_{n=0}^{\infty} p_n = 1$, and modeling the distribution p_n . Observation of no solvent molecules in the cavity region is then just one of the elementary events and p_0 is just one of the desired probabilities.

Information theory (55, 56) provides an approach to the modeling of p_n . We adopt a relative or cross entropy (55)

$$\eta = - \sum_{n=0}^{\infty} p_n \ln \left[\frac{p_n}{\hat{p}_n} \right], \quad [1]$$

with \hat{p}_n representing a chosen "default model." The \hat{p}_n of the default model serve as a reference estimate of the probabilities: they will equal the default model if no further information is supplied. The probabilities p_n are then obtained by maximizing η subject to the constraints of the available information. For our problems that information includes the moments

$$\langle n \rangle = \rho \Delta v, \quad [2]$$

$$\langle n(n-1) \rangle = \rho^2 \int_{\Delta v} d\mathbf{r} \int_{\Delta v} d\mathbf{r}' g(|\mathbf{r} - \mathbf{r}'|), \quad [3]$$

where $g(\mathbf{r})$ is the water oxygen radial distribution function and ρ is the water density.

In principle, this model can be incrementally improved by including higher moments. However, we only use the moments Eqs. 2 and 3 because the density ρ and radial distribution information $g(\mathbf{r})$ are available and the integrations over the solute volume to calculate the two moments are simple enough.

Another way to improve such models is to learn a good default model \hat{p}_n . A sophisticated default model would be the results for a simple liquid (13, 17–19) solvent, presumed separately known. Another default model is $\hat{p}_n \propto 1/n!$. This produces the Poisson distribution if only the mean value $\langle n \rangle$ is supplied as information. This form is associated with Gibbs's development of classical statistical thermodynamics and we call this the Gibbs default model. Another alternative is the flat default model, where \hat{p}_n is a positive constant for $n \leq n_{max}$ and zero otherwise. If n_{max} is chosen sufficiently large, its value has no appreciable effect on the p_n .

We explicitly view this approach as a heuristic method for discovering simple models for p_n . We don't attempt to justify more basically the \hat{p}_n used or the p_n obtained except to note what works well.

We have found that the p_n observed by simulation of liquid water are simple. A sophisticated consideration of \hat{p}_n has not been necessary so far. Fig. 1 shows the p_n obtained from Monte Carlo simulation of the simple point charge (SPC) model of water (58) at the thermodynamic state 298 K and 1.0 g/cm³ for a spherical exclusion volume Δv with radius d ; this parameter d would be the solute-solvent exclusion diameter, or distance of closest solvent approach, for a hard sphere solute. The $\ln p_n$ are seen to be closely parabolic. This would be the behavior predicted using the flat default model with $n_{max} \rightarrow \infty$ and the

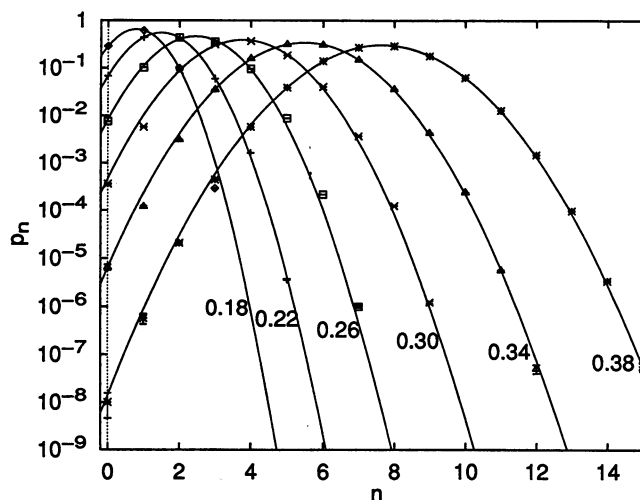


FIG. 1. Probabilities p_n of observing n solvent centers in spherical cavity volumes. Results from Monte Carlo simulation (57) of 512 SPC water molecules (58) are shown as symbols. The parabolas are the predictions of information theory using the flat default model and the moments of Eqs. 2 and 3. The center-to-center exclusion distance, d (in nanometers), is noted next to each curve.

moments of Eqs. 2 and 3. Thus, we here dispense with other considerations and use this extremely simple model only.

We emphasize the simplicity of this quadratic model. What is required is the calculation of the moments Eqs. 2 and 3 and, since $n_{max} \rightarrow \infty$, the fitting of the form $p_n = \exp(\lambda_0 + \lambda_1 n + \lambda_2 n^2)$ with Lagrange multipliers $\lambda_0, \lambda_1, \lambda_2$ determined by the conditions $\sum_{n=0}^{\infty} p_n = 1$, $\sum_{n=0}^{\infty} n p_n = \langle n \rangle$, and $\sum_{n=0}^{\infty} n^2 p_n = \langle n^2 \rangle$. From this, we extract the p_0 that provides the desired thermodynamic result. This procedure can be readily applied to solutes of arbitrary shapes in bulk water or in anisotropic environments near the surface of a protein.

RESULTS

Fig. 2 shows the chemical potential of a hard sphere solute in water calculated from the model and directly from cavity statistics. We find excellent agreement in the range considered. This simple model accurately reproduces the thermodynamics of cavity formation in the region that is accessible to direct computer simulations (13, 17–19).

We next proceed from *hydrophobic hydration* of a hard sphere solute to *hydrophobic interactions* between solutes. The free energy changes associated with bringing together two inert gas atoms correspond to the potential of mean force (pmf). The model provides us with the means of calculating this pmf. With the known density and radial distribution function, we calculate the two moments Eqs. 2 and 3 for the volume enclosed by the two solute spheres with given distance of closest solvent approach d and solute center-to-center separation r . Fitting the distribution to this information and extracting p_0 produces an approximate chemical potential $\Delta\mu^{ex}(r)$ for the two-sphere solute. The pmf is then defined as $W(r) = \Delta\mu^{ex}(r) - \lim_{s \rightarrow \infty} \Delta\mu^{ex}(s)$.

Here we study the association of two cavities of methane size in water. For the distance of closest solvent approach to the spheres, we have chosen a value of $d = 0.33$ nm. This is the smallest distance at which methane-water pair correlations reach 1.0 in commonly used models (60). The cavity pmf is shown in Fig. 3. As a reference, the cavity potential produced by the molecular dynamics simulation of Smith and Haymet (16) is included. This was obtained by subtracting the solute-solute potential from the pmf of methane association. Again, we find quantitative agreement between the simple model and the results of direct computer simulations. The model cavity

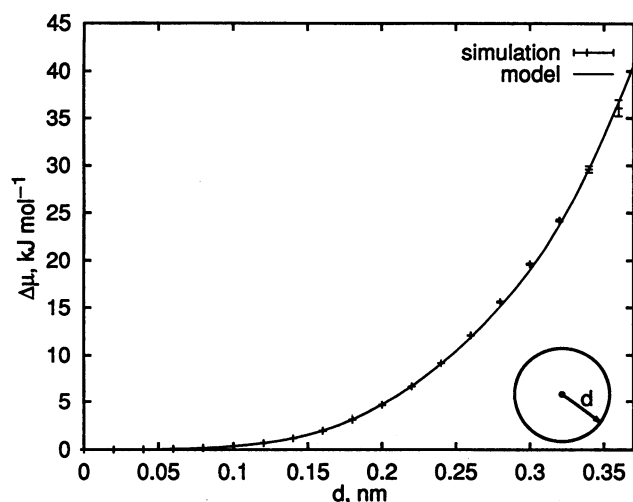


FIG. 2. Hydrophobic hydration: comparison of the chemical potential calculated from simulation (symbols) and the information theory model (line) for hard sphere solutes with d the distance of closest approach of a water oxygen to the solute. The integral Eq. 3 required by the model was reduced to one-dimensional integration (59). The simulation results were gathered from test-particle insertion, where 8000 configurations (separated by 50 passes each) of a Monte Carlo simulation (57) of 512 SPC water molecules (58) were used. The same simulation was used to calculate the water-oxygen radial distribution function $g(r)$.

pmf shows a strongly favored region with overlapping cavities, separated by a substantial barrier from a solvent-separated, stable minimum at about 0.7 nm distance. We observe also a shallow third minimum at 1.1 nm separation.

We note that structural data on the solvation of one sphere, if they were available (61), could be used as information to predict the opening of a cavity for a second sphere in the neighborhood of the first sphere. Similarly, structural data on the solvation of two spheres could be used with information theory to predict the potential of mean force for three spheres, and so on.

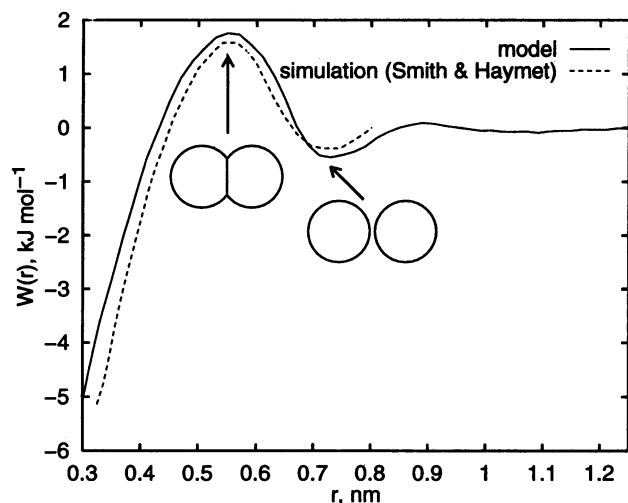


FIG. 3. Hydrophobic interaction: pmf of cavity association. r is the separation between the centers of two spherical cavities. The distance of closest solvent approach is $d = 0.33$ nm. The result of the information theory model (solid line) is compared with the cavity pmf of Smith and Haymet (16) (dotted line; from figure 4 of ref. 16). The simulation result was based upon continuous solute-solvent interactions (16) rather than the hard core interactions treated by the model. The insets illustrate the size of the excluded volume. At the solvent-separated pmf minimum (0.7 nm), a water molecule barely fits between the two hard sphere solutes.

As a last example, we study the torsional equilibrium of n -butane. Fig. 4 shows the cavity pmf as a function of the torsional angle ϕ , which is compared to explicit computer simulation results of Beglov and Roux (62). In complete agreement with the computer simulations, we find that the more compact cis ($\phi = 0$) and gauche ($\phi = \pi/3$) structures are favored over the extended trans conformation ($\phi = \pi$) by about 1.8 kJ mol $^{-1}$ and 0.7 kJ mol $^{-1}$, respectively.

DISCUSSION

The successes of the present applications are tied to the remarkable simplicity of the data in Fig. 1. Those results show that the $\ln p_n$ are closely parabolic in the range considered. However, they are not *precisely* parabolic. For example, a close examination of Fig. 1 for the larger radii shows that p_1 is somewhat depressed relative to the quadratic model. We conjecture that this is associated with the fact that the solvent water is expected to pull away from the walls of macroscopically large hard spheres since it does not wet those nearly flat surfaces (2). Physical effects such as these can be built into information theory models. For example, the information underlying the moments Eqs. 2 and 3 would be used more fully by stratifying the observation volume to examine and constrain the probability of solvent occupancy in thin surface shells. It is an important virtue of this approach that physical insights can often be directly incorporated into the model and tested. The discovery and codification of effective default models \hat{p}_n (13, 17-19) will surely be the path to advance this method of describing hydrophobic effects.

Chandler (15) has given a helpful interpretation of the Pratt-Chandler theory (3) of hydrophobic effects using an assumption of Gaussian fluctuations of the solvent density field. Such a model may be referred to as a Gaussian field model (15). Chandler concludes that if the solvent density fluctuations were distributed precisely as a Gaussian functional then the solvent density distortion due to the imposition of a hard blocking object—the solute—would be described by the Pratt-Chandler theory. The present model also relies on the nearly parabolic behavior shown in Fig. 1. Thus, these two approximate theories have an important qualitative similarity. Both models use information on a central portion of a distri-

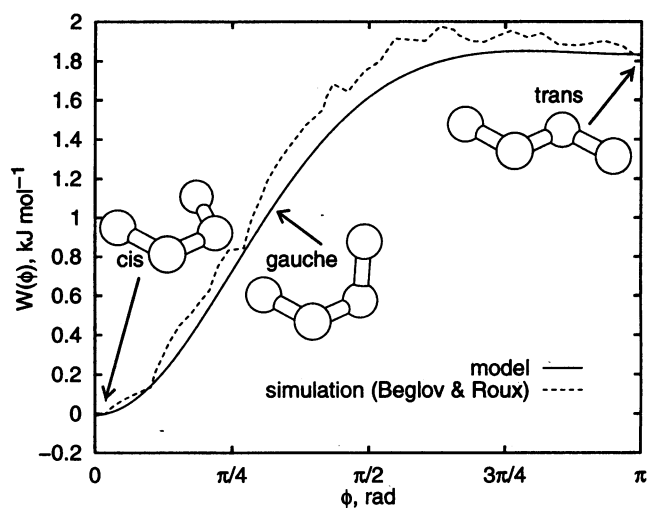


FIG. 4. Torsional pmf of n -butane. Butane was modeled as four spheres with distance of closest solvent approach $d = 0.33$ nm, bond length 0.153 nm, and tetrahedral bond angles. The result of the information theory model (solid line) is compared with the cavity pmf of Beglov and Roux (62) (dotted line; from figure 8 of Ref. 62). The simulation result was based upon continuous solute-solvent interactions (62) rather than the hard core interactions treated by the model. The cis, gauche, and trans rotational states are shown as insets.

bution and then extract behavior in a far wing of that distribution. However, these two theories are not identical. The most important distinction is that the Gaussian field model is most appropriate for long wavelength density fluctuations and does not require that spatial integrals of density fields be natural numbers, i.e., nonnegative integers. Chandler (15) emphasizes this limitation by stating that the Gaussian field model does not well describe the particulate nature of matter. For example, the Gaussian field model can predict positive probabilities of occupancy by negative numbers, $n < 0$, of molecules.

The present model has been limited to using only the known density ρ and the oxygen–oxygen radial distribution function $g(r)$. The exploitation of empirical information of that sort has frequently been a feature of molecular theories of hydrophobic effects (1–3, 13, 15, 17–19). Since the present development continues that tradition but strives for a minimum of extraneous assumptions, we regard it as a synthesis of those previous theories for the solvation properties discussed here.

The development here has made a clear separation of the effects of short ranged, repulsive solute–solvent interactions and longer ranged, attractive interactions. Such a separation is a basic idea of the theory of liquids (63) and was a feature of the earliest molecular theories of hydrophobic effects (1–3). A full treatment of realistic solutes must also include longer ranged attractive interactions (4). A clear separation of the effects of interactions that have different physical character is important for the microscopic interpretation of surface tension models of hydrophobicity (64–76).

CONCLUSIONS

The two-moment information theory model provides an accurate description of the primitive hydrophobic effects, including solvent-separated hydrophobic interactions, for which we have molecularly detailed computer experimental data. When the cavities of interest are much larger than the size of the solvent molecules, other physical effects need to be considered (2). In addition, more general solute–solvent interactions need to be treated (4). However, problems of biophysical interest, such as interactions of ligands with binding sites (77), effects of point mutations on protein–solvent interactions (78), and conformational equilibria of side chains, are within the range of applicability of this model. These applications are more direct than the problems mentioned above as motivation for further theoretical development of molecular theories of hydrophobic effects. The present model is simple and has a clear connection to statistical thermodynamics. The information theory approach is sufficiently flexible that a variety of additional effects can be incorporated into the model. These qualities open the opportunity for a constructive, quantitative, and molecular description of hydrophobic effects on biopolymer structure in solution.

Note Added in Proof: Notable results on these problems have appeared very recently. Computer simulations have showed a third, shallow minimum in the methane pair cavity pmf at about 1 nm distance (79), as predicted by the information theory. Using field-theoretic concepts with evident relations to the work of ref. 15, Callaway (80) has studied the relation of a geometric entropy to hydrophobic effects and calculated accurate results for the surface tensions of several liquids.

A.P. acknowledges support by the National Aeronautics and Space Administration Grant NCC 2-772.

1. Pierotti, R. A. (1963) *J. Phys. Chem.* **67**, 1840–1845.
2. Stillinger, F. H. (1973) *J. Solut. Chem.* **2**, 141–158.
3. Pratt, L. R. & Chandler, D. (1977) *J. Chem. Phys.* **67**, 3683–3704.
4. Pratt, L. R. & Chandler, D. (1980) *J. Chem. Phys.* **73**, 3434–3441.
5. Pratt, L. R. (1985) *Annu. Rev. Phys. Chem.* **36**, 433–449.
6. Lazaridis, T. & Paulaitis, M. E. (1992) *J. Phys. Chem.* **96**, 3847–3855.
7. Lazaridis, T. & Paulaitis, M. E. (1993) *Fluid Phase Equilibria* **83**, 43–49.
8. Lazaridis, T. & Paulaitis, M. E. (1993) *J. Phys. Chem.* **97**, 5789–5790.
9. Lazaridis, T. & Paulaitis, M. E. (1994) *J. Phys. Chem.* **98**, 635–642.
10. Paulaitis, M. E., Ashbaugh, H. S. & Garde, S. (1994) *Biophys. Chem.* **51**, 349–357.
11. Watanabe, K. & Andersen, H. C. (1986) *J. Phys. Chem.* **90**, 795–802.
12. Pohorille, A. & Pratt, L. R. (1990) *J. Am. Chem. Soc.* **112**, 5066–5074.
13. Pratt, L. R. & Pohorille, A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2995–2999.
14. Madan, B. & Lee, B. (1994) *Biophys. Chem.* **51**, 279–289.
15. Chandler, D. (1993) *Phys. Rev. E* **48**, 2898–2905.
16. Smith, D. E. & Haymet, A. D. J. (1993) *J. Chem. Phys.* **98**, 6445–6454.
17. Head-Gordon, T. & Stillinger, F. H. (1993) *J. Chem. Phys.* **98**, 3313–3327.
18. Head-Gordon, T. (1994) *Chem. Phys. Lett.* **227**, 215–220.
19. Head-Gordon, T. (1995) *J. Am. Chem. Soc.* **117**, 501–507.
20. Wallqvist, A. & Berne, B. J. (1995) *J. Phys. Chem.* **99**, 2885–2892.
21. Wallqvist, A. & Berne, B. J. (1995) *J. Phys. Chem.* **99**, 2893–2899.
22. Franks, F. (1975) in *Water. A Comprehensive Treatise*, ed. Franks, F. (Plenum, New York), Vol. 4, pp. 1–94.
23. Richards, F. M. (1977) *Annu. Rev. Biophys. Bioeng.* **6**, 151–176.
24. Edsall, J. T. & McKenzie, H. A. (1983) *Adv. Biophys.* **16**, 53–183.
25. Dill, K. A. (1990) *Biochemistry* **29**, 7133–7155.
26. Blokzijl, W. & Engberts, J. B. F. N. (1993) *Angew. Chem. Int. Ed. Engl.* **32**, 1545–1579.
27. Kauzmann, W. (1959) *Adv. Prot. Chem.* **14**, 1–63.
28. Hermann, R. B. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4144–4145.
29. Eisenberg, D., Weiss, R. M., Terwilliger, T. C. & Wilcox, W. (1982) *Faraday Symp. Chem. Soc.* **17**, 109–120.
30. Eisenberg, D. & McLachlan, A. D. (1986) *Nature (London)* **319**, 199–203.
31. Wodak, S. J. & Rooman, M. J. (1993) *Curr. Opin. Struct. Biol.* **3**, 247–259.
32. Privalov, P. L. (1990) *Crit. Rev. Biochem. Mol. Biol.* **25**, 281–305.
33. Makhatadze, G. I. & Privalov, P. L. (1993) *J. Mol. Biol.* **232**, 639–659.
34. Privalov, P. L. & Makhatadze, G. I. (1993) *J. Mol. Biol.* **232**, 660–679.
35. Peng, X., Jonas, J. & Silva, J. L. (1994) *Biochemistry* **33**, 8323–8329.
36. Zhang, J., Peng, X., Jonas, A. & Jonas, J. (1995) *Biochemistry* **34**, 8631–8641.
37. Jonas, J. & Jonas, A. (1994) *Annu. Rev. Biophys. Biomol. Struct.* **23**, 287–318.
38. Cléry, C., Renault, F. & Masson, P. (1995) *FEBS Lett.* **370**, 212–214.
39. Vidugiris, G. J. A., Markley, J. L. & Royer, C. A. (1995) *Biochemistry* **34**, 4909–4912.
40. Colombo, M. F., Rau, D. C. & Parsegian, V. A. (1992) *Science* **256**, 655–659.
41. Colombo, M. F., Rau, D. C. & Parsegian, V. A. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 10517–10520.
42. Rand, R. P., Fuller, N. L., Butko, P., Francis, G. & Nicholls, P. (1993) *Biochemistry* **32**, 5925–5929.
43. Bellelli, A., Brancaccio, A. & Brunori, M. (1993) *J. Biol. Chem.* **268**, 4742–4744.
44. Robinson, C. R. & Sligar, S. G. (1993) *J. Mol. Biol.* **234**, 302–306.
45. Robinson, C. R. & Sligar, S. G. (1994) *Biochemistry* **33**, 3787–3793.
46. Robinson, C. R. & Sligar, S. G. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 3444–3448.
47. Di Primo, C., Sligar, S. G., Hoa, G. H. B. & Douzou, P. (1992) *FEBS Lett.* **312**, 252–254.
48. Garner, M. M. & Rau, D. C. (1995) *EMBO J.* **14**, 1257–1263.
49. Sidorova, N. Y. & Rau, D. C. (1995) *Biopolymers* **35**, 377–384.
50. Kauzmann, W. (1987) *Nature (London)* **325**, 763–764.
51. Shortle, D. (1993) *Curr. Opin. Struct. Biol.* **3**, 66–74.
52. Baldwin, R. L. (1993) *Curr. Opin. Struct. Biol.* **3**, 84–91.
53. Pratt, L. R. & Pohorille, A. (1993) in *Association of European Biophysical Societies Conference Proceedings on Water-Biomol-*

- ecule Interactions*, eds. Palma, M. U., Palma-Vittorelli, M. B. & Parak, F. (Società Italiana DiFisica, Bologna, Italy), Vol. 43, pp. 261–268.
54. Maiorov, V. N. & Crippen, G. M. (1992) *J. Mol. Biol.* **227**, 876–888.
 55. Shore, J. E. & Johnson, R. W. (1980) *IEEE Trans. Information Theory* **IT-26**, 26–37.
 56. Jaynes, E. T. (1983) in *E. T. Jaynes: Papers on Probability, Statistics and Statistical Physics*, ed. Rosenkrantz, R. D. (Reidel, Dordrecht, The Netherlands), pp. 315–336.
 57. Hummer, G., Pratt, L. R. & García, A. E. (1995) *J. Phys. Chem.* **99**, 14188–14194.
 58. Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F. & Hermans, J. (1981) in *Intermolecular Forces: Proceedings of the 14th Jerusalem Symposium on Quantum Chemistry and Biochemistry*, ed. Pullman, B. (Reidel, Dordrecht, The Netherlands), pp. 331–342.
 59. Hill, T. L. (1958) *J. Chem. Phys.* **28**, 1179–1182.
 60. Hummer, G., Pratt, L. R. & García, A. E. (1996) *J. Phys. Chem.* **100**, 1206–1215.
 61. Garde, S., Hummer, G., García, A. E., Pratt, L. R. & Paulaitis, M. E. (1996) *Phys. Rev. E* **53**, R4310–R4313.
 62. Beglov, D. & Roux, B. (1994) *J. Chem. Phys.* **100**, 9050–9063.
 63. Chandler, D., Weeks, J. D. & Andersen, H. C. (1983) *Science* **220**, 787–794.
 64. Sharp, K. A., Nicholls, A., Fine, R. F. & Honig, B. (1991) *Science* **252**, 106–109.
 65. Nicholls, A., Sharp, K. A. & Honig, B. (1991) *Proteins Struct. Funct. Genet.* **11**, 281–296.
 66. Sharp, K. A., Nicholls, A., Friedman, R. & Honig, B. (1991) *Biophys. Chem.* **30**, 9686–9697. Sitkoff, D., Sharp, K. A. & Honig, B. (1994) *Biophys. Chem.* **51**, 397–409.
 67. Kumar, S. K., Szeifei, I., Sharp, K., Rossky, P. J., Friedman, R. & Honig, B. (1995) *J. Phys. Chem.* **99**, 8382–8391.
 68. Holtzer, A. (1992) *Biopolymers* **32**, 711–715.
 69. Holtzer, A. (1994) *Biopolymers* **34**, 315–320.
 70. Irida, M., Nagayama, K. & Hirata, F. (1993) *Chem. Phys. Lett.* **207**, 430–435.
 71. Ben Naim, A. & Mazo, R. M. (1993) *J. Phys. Chem.* **97**, 10829–10834.
 72. Lee, B. (1993) *Prot. Sci.* **2**, 733–738.
 73. Tunon, I., Silla, E. & Pascualahir, J. L. (1994) *J. Phys. Chem.* **98**, 377–379.
 74. Giesen, D. J., Cramer, C. J. & Truhlar, D. G. (1994) *J. Phys. Chem.* **98**, 4141–4147.
 75. Simonson, T. & Brünger, A. T. (1994) *J. Phys. Chem.* **98**, 4683–4694.
 76. Lee, B. (1994) *Biophys. Chem.* **51**, 263–269.
 77. Ringe, D. (1995) *Curr. Opin. Struct. Biol.* **5**, 825–829.
 78. Eriksson, A. E., Baase, W. A., Zhang, X. J., Heinze, D. W., Blaber, M., Baldwin, E. P. & Matthews, B. W. (1992) *Science* **255**, 178–183.
 79. Ludemann, S., Schreiber, H., Abseher, R. & Steinhauser, O. (1996) *J. Chem. Phys.* **104**, 286–295.
 80. Callaway, D. J. E. (1996) *Phys. Rev. E* **53**, 3738–3744.