

Collective Properties of Hydration: Long Range and Specificity of Hydrophobic Interactions

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ABSTRACT We report results of molecular dynamics (MD) simulations of composite model solutes in explicit molecular water solvent, eliciting novel aspects of the recently demonstrated, strong many-body character of hydration. Our solutes consist of identical apolar (hydrophobic) elements in fixed configurations. Results show that the many-body character of PMF is sufficiently strong to cause 1) a remarkable extension of the range of hydrophobic interactions between pairs of solute elements, up to distances large enough to rule out pairwise interactions of any type, and 2) a SIF that drives one of the hydrophobic solute elements toward the solvent rather than away from it. These findings complement recent data concerning SIFs on a protein at single-residue resolution and on model systems. They illustrate new important consequences of the collective character of hydration and of PMF and reveal new aspects of hydrophobic interactions and, in general, of SIFs. Their relevance to protein recognition, conformation, function, and folding and to the observed slight yet significant nonadditivity of functional effects of distant point mutations in proteins is discussed. These results point out the functional role of the configurational and dynamical states (and related statistical weights) corresponding to the complex configurational energy landscape of the two interacting systems: biomolecule + water.

INTRODUCTION

Nonadditivity of hydration and of related free-energy G_{SW} of solutes and its relevance to solute-solute interactions has long been appreciated (see, e.g., Lifson and Oppenheim, 1960). It causes a solvent-induced free energy of interaction, the negative gradient of which with respect to coordinates of a given solute is the solvent-induced force (SIF) acting on that solute. SIFs therefore express the local response of the solvent to perturbations caused jointly by all solutes. Molecular dynamics (MD) studies of simple solutes in explicit molecular water solvent have recently shown that SIFs among solutes (or elements of composite solutes) are strongly non-pair-additive, that is, dependent upon the conformational context of all solutes (Brugé et al., 1996; Martorana et al., 1996, and manuscript to be published). This highlights the correspondingly strong relevance of higher order interactions contributing to G_{SW} that have often been neglected, so far, for practical reasons. Hydration free energy $G_{\text{SW}}(1, 2, \dots, j, \dots, n)$ for a system of n solutes or solute elements can be written as

$$G_{\text{SW}}(1 + 2 + \dots + n) = \sum_i^n G_{\text{SW}}(i) + \sum_{i<j}^n \delta G_{\text{SW}}(i + j) + \dots + \sum_{i<j<\dots}^n \delta G_{\text{SW}}(i + j + \dots) + \delta G_{\text{SW}}(1 + 2 + \dots + n) \quad (1)$$

where δG_{SW} terms of different order represent contributions of pair, triplet, . . . n -plet interactions in establishing (self-consistently and collectively) the total hydration free energy and related SIFs. In principle, changing the coordinates (or the character) of even one of the elements affects δG_{SW} terms to all orders and, consequently, the total G_{SW} and its derivatives with respect to coordinates of any other element. On the other hand, it is well known that the potential of mean force (PMF), the gradients of which equivalently express SIFs, has a many-body nature that makes it resistant to exact analytical treatment (Kirkwood, 1935; Hill, 1956). In Kirkwood's superposition approximation (KSA), factorizability of higher order terms is assumed, so as to retain the term responsible for the existence of SIFs, but not those responsible for their non-pair-additivity. KSA is equivalent to neglecting in Eq. 1 all δG_{SW} terms of three and more particles, responsible for the many-body character of hydration and of PMF, and for the non-pair-additivity of SIFs.

Consequences of the strong many-body character of G_{SW} and of PMF are evidenced both by MD simulations and by experimental data. In the simplest case of model solutes, SIFs themselves show a strong nonadditivity and context dependence (Brugé et al., 1996). In the case of a realistic system such as bovine pancreatic trypsin inhibitor (BPTI) in water, Fourier spectra and time cross-correlations of time-resolved SIFs obtained from MD simulations concur in suggesting that individual SIFs are the result of solvent perturbations caused jointly by all solute elements in the given conformational context (Martorana et al., 1996). In the same system, a sign reversal of some individual SIFs, relative to expectations based on the given side-chain type, has been observed (Martorana et al., manuscript to be published). All of this goes beyond the recognized breakdown of the surface area proportionality assumption (Lazaridis et

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al., 1995) and beyond the limited nonadditivities retained in KSA and in highly efficient computing approaches based on it, even if embodying specific extensions in special circumstances (Hirata et al., 1982; Pettitt et al., 1986; Klement et al., 1991; Pellegrini and Doniach, 1995; Garde et al., 1996). It also goes beyond triple and quadruple correlations of solute interactions, already sufficient to question the validity of hydrophobicity scales (Ben-Naim, 1990a,b), and, in general, beyond nonadditivity concerning hydration free energy only (Wallqvist and Covell, 1995). A variety of experimental data also concur in revealing the highly collective character of hydration. They include the nonadditive dependence of total hydration water upon solute concentration (Bulone et al., 1991); proximity effects in the solvation of small groups (Haberfield et al., 1984; Ben-Naim, 1990a); and nonlinear modulability of, e.g., hydrophobic interactions by small cosolutes (Cordone et al., 1989, and references therein; Somero, 1992; Palma et al., 1994).

The present work concerns MD simulations of composite model solutes (consisting of five- and six-member arrays of hydrophobic spheres) in water. Results show that the highly collective character of hydration extends the range of interactions between solute elements to distances too large for direct or solvent-induced (e.g., hydrophobic) pairwise interactions. They further show that in the appropriate configurational context, the many-body character of PMF can cause an amplification of SIFs driving all solute elements toward "hydrophobic collapse." In contrast, in a different configurational context, we observe the opposite effect, that is, a repulsive SIF on one of the elements. As we shall discuss, this effect is not simply amenable to the existence of solvent separated configurations. Implications of these findings cover areas as diverse as protein folding, biomolecular recognition, conformational changes, and nonadditivity of functional effects of distant point mutations in proteins. In the final section, we comment briefly on such implications. We also comment on respective advantages and disadvantages of the present approach, which takes water molecules explicitly into full account, and of approximated approaches based on KSA, which offer very high computer efficiencies.

METHODS

Molecular dynamics (MD) simulations were performed in the microcanonical (NVE) ensemble. The water-water interaction potential was TIP4P (Jorgensen et al., 1983). Different composite solutes were studied by means of independent MD trajectories and compared. One of the composite solutes had six identical and fixed Lennard-Jones (LJ) hydrophobic particles as "solute elements." A planar arrangement of equilateral triangles was adopted, as shown in the figures. The other two composite solutes used differed from the first one in the absence (at different sites) of one of the six LJ solute elements. In these two cases the absence of one LJ particle in the simulation box was compensated for by the introduction of one additional water molecule. Parameters of the LJ potential of the solute elements were the same as those of the LJ part of the TIP4P potential. Periodic boundary conditions were used. The simulation box contained 722 or 723 water molecules (as just made clear), allowing at least three layers of water molecules around solutes. Ewald sums were used to handle long-range electrostatic forces. Long (800 ps) independent MD runs were performed

at 300 K for each of the three composite solutes as well as for isolated pairs of LJ particles. To obtain more reliable statistical significance, each 800 ps run was obtained as a sequence of four 200-ps trajectories decoupled from each other by 20-ps annealings at 800 K followed by 40 ps of equilibration at 300 K. SIFs on each LJ element were computed as time averages of instantaneous force vectors exerted by all water molecules upon that element (Brugé et al., 1994). Related errors (≤ 4 pN) and statistical significance were evaluated, with identical results, as in Flyvbjerg and Petersen (1989) and in Brugé et al. (1996). Furthermore, we checked that the total torque and the total force on the composite solute were zero within the respective error bars. To obtain hydration patterns, the simulation box was divided into cells of 0.7 Å side length. The probability, p , that the center of the oxygen atom of one solvent molecule falls within one cell was computed over each composite 800-ps MD run, normalized against the value relative to bulk water and used for 3-D visualizations of hydration in Fig. 3. Using this space occupancy probability and the interaction potential, contributions from each space cell to SIFs, as also visualized in Fig. 3, were calculated.

RESULTS AND DISCUSSION

The first question addressed is whether the strong-many body character of hydration free energy and PMF can cause significant interactions between very distant elements of composite solutes (e.g., distant protein residues), which would not interact if alone in the solvent. The question is biologically very significant, e.g., in view of the interest raised by nonadditive effects of distant point mutations in proteins (Green and Shortle, 1993; LiCata and Ackers, 1995). Preliminarily, we have determined time-averaged SIFs versus distance for a single pair of our LJ solutes, from many independent 800-ps MD runs, each for a different distance. Fifteen such data points are shown in Fig. 1 (*top*). Runs at distances beyond the figure scale showed that SIFs are fading out above some 11 Å, in agreement with findings of other authors (see, e.g., Lee and Rossky, 1994). PMF was obtained from time-averaged SIFs by polynomial best-fitting of the many data points and subsequent integration. The figure shows the known oscillatory dependence of hydrophobic interactions upon distance (Lüdemann et al., 1996; Pratt and Chandler, 1980; Ravishanker et al., 1982; Zichi and Rossky, 1985). In Fig. 2 we show SIFs on each solute element in the six-element case. It is seen that, in this case, true SIFs are $\sim 60\%$ larger than those computed from Fig. 1 in the additivity hypothesis (corresponding to KSA). This amplifying action causes a much stronger drive toward hydrophobic collapse. As we shall see below, however, the opposite can also happen, because the effect depends very sensitively upon the specific configuration.

Next we performed MD simulations using five- and six-membered composite solute systems, as described in Methods and shown in Fig. 3 (*top*). Effects of particle 6 on particle 3 are evident from comparison of the left and center columns of Fig. 3. At the chosen 4.6-Å nearest-neighbor pair distance (marked by *arrows* in Fig. 1), the (hydrophobic) SIF acting within a single pair is 16 pN (Fig. 1, *top*). The distance between elements 3 and 6 in Fig. 2 and in Fig. 3 is 12.17 Å center to center and, as just mentioned, no hydrophobic interaction occurs at this distance within a

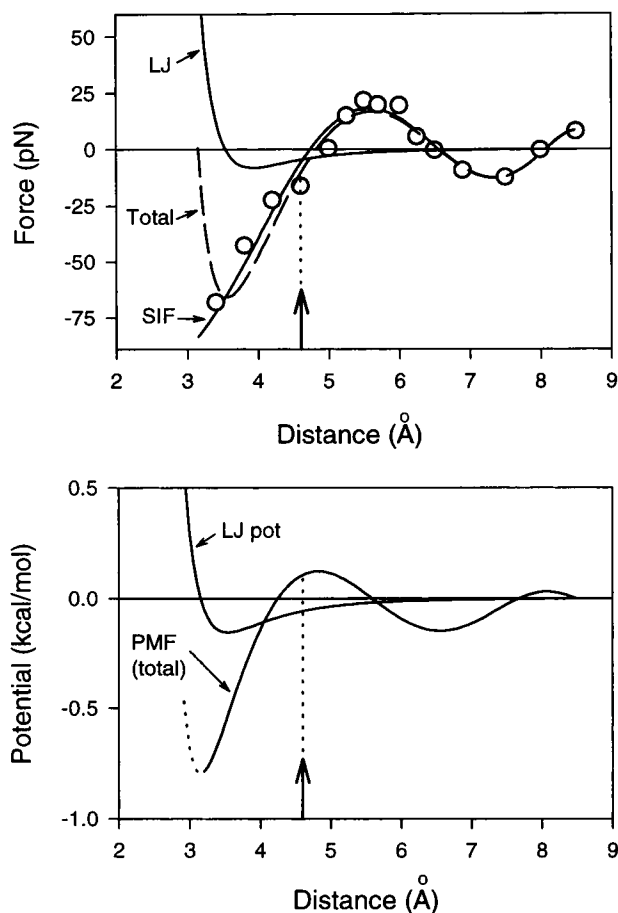


FIGURE 1 Direct and solvent-induced forces and potentials for two Lennard-Jones (LJ) particles in explicit water. (*Top*) Direct force (LJ) and solvent-induced force (SIF). (*Bottom*) Direct interaction potential (LJ) and potential of mean force (PMF). As confirmed by additional MD simulations, SIFs vanish to zero above ~ 11 Å.

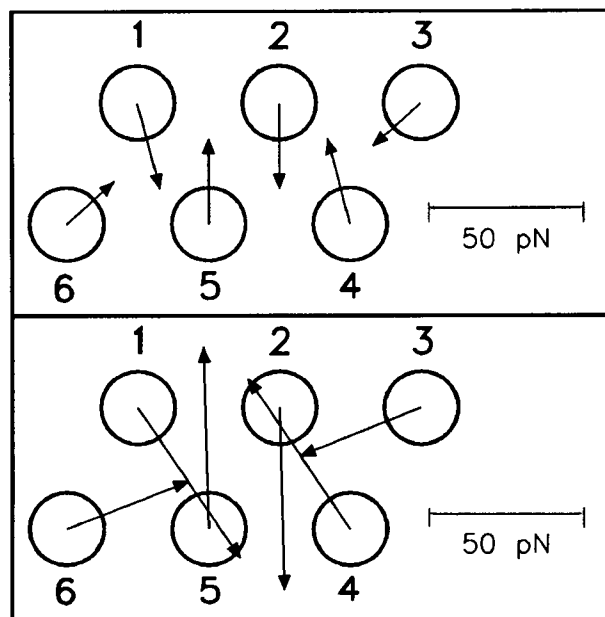


FIGURE 2 SIF vectors acting on each LJ element of the six-element solute. All nearest-neighbor center-to-center distances are 4.6 Å (fixed). It should be noted that SIFs on an isolated pair of the same LJ elements at the 4.6 Å distance are 16 pN (Fig. 1). (*Top*) SIFs computed under the pair additivity hypothesis (Kirkwood's superposition approximation). (*Bottom*) Actual SIFs obtained from MD simulations without approximations.

hydration. In the latter (more intuitive) view, we refer to Eq. 1, and, in accord with what we have just mentioned, we recall that the $\delta G_{\text{SW}}(3 + 6)$ contribution is zero. Consequently, the effect of particle 6 on the SIF vector on particle 3 can only come from δG_{SW} terms involving particles 3 and 6 to higher orders, up to $\delta G_{\text{SW}}(1 + 2 + 3 + 4 + 5 + 6)$. In other words, hydration and related free energy and SIFs are concurrently determined by all solute elements in the given configuration. The long-range interaction between elements 3 and 6 is the result of a relay action spreading self-consistently along overlapping hydration regions of all solute elements.

This extension of the range of solvent-induced interactions is already notable. It poses the further question of whether one can, with the present relatively simple solute system, observe effects like those observed in the far more complex case of BPTI mentioned in the Introduction (Martorana et al., manuscript to be published). More precisely, the question is whether specific configurations of our composite solute exist, such that some of its hydrophobic elements can be driven toward the solvent, against hydrophobic collapse. This question is of particular interest with the present type of composite solute, because all of its individual elements are hydrophobic and identical. The answer is provided by an additional 800-ps MD run using a configuration where the solute element 2 is missing. Results are shown in the right-hand column of Fig. 3, to be compared to those at their left. We see that when element 2 is absent,

single pair. On these grounds, in the case of pair-additive SIFs, we would expect that adding or removing particle 6 could in no way alter SIF on particle 3. Instead, Fig. 3 (*center top*) shows that element 6, when present, alters the SIF vector on element 3 by a sizable transversal 10-pN contribution (difference between red and blue vectors). Corresponding effects of the presence of element 6 on the hydration pattern of the composite solute as a whole and (more specifically) in the region around 3 are shown in Fig. 3 (*central row, left and center panels*). Here hydration is visualized by 3-D views of isoprobability surfaces at $p = 1.6$ (p defined as in Methods). The figure clearly shows that the presence of element 6 alters the hydration pattern around element 3. Panels in the bottom row (*left and center*) show space regions providing strong contributions to the average SIF acting on element 3 in the presence and absence of 6. These results prove the existence of interactions between particles 3 and 6, which are made possible by the presence of the intervening particles 1, 2, 4, and 5. Interactions can be equivalently described in terms of PMF or free energy of

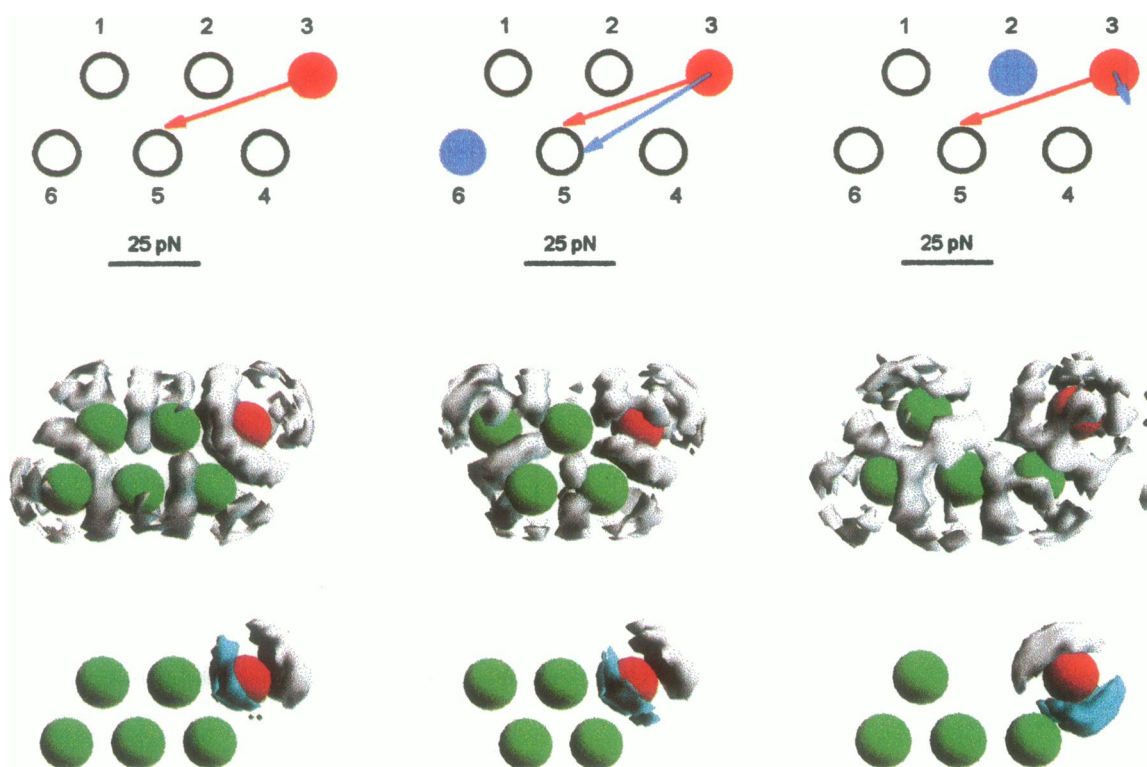


FIGURE 3 Effects of removing element 6 or 2 in the composite solute of Fig. 2. (Top) SIF acting on element 3 in three different cases: 1) all solute elements present (left); 2) element 6 missing (center); 3) element 2 missing (right). In all cases the red arrow represents SIF acting when all solute elements are present (as in Fig. 2, bottom, except for a change of scale). The blue arrow represents SIF acting when the blue-colored element is missing. (Center) 3-D representation of hydration isoprobability surface ($p = 1.6$) relative to cases shown in the top row. Element 3 is colored in red to facilitate comparisons. (Bottom) 3-D representation of space regions providing strong and opposite contributions (blue and gray) to the average SIF on element 3, relative to cases shown in the top row. Graphics by Pepke and Lyons (1993).

element 3 is driven toward the solvent (blue arrow in Fig. 3, top right), indeed opposing hydrophobic collapse, notwithstanding its intrinsic apolar character.

This behavior might be thought to be related to solvent-separated hydrophobic interactions, known to be caused by the particulate structure of the solvent (Lüdemann et al., 1996; Pratt and Chandler, 1980; Ravishanker et al., 1982; Zichi and Rossky, 1985). However, we see that the situation is more complex, because of the following strong nonadditivity effects: 1) When element 2 is present, SIF additively computed on element 3 should be ~ 28 pN, directed as shown in Fig. 2 (top). Actually, it is instead ~ 42 pN and turned slightly clockwise (bottom panel of Fig. 2 or, equivalently, red arrow in any panel of the top row in Fig. 3). 2) When element 2 is absent, SIF additively computed on 3 should be close to 16 pN, essentially directed toward element 4 (this is easily visualized, although it is not shown in the figures). Actually, it is instead ~ 11 pN, remarkably turned toward the solvent (blue arrow in Fig. 3). 3) The effect on particle 3 of adding or taking away element 2 is a change in SIF by some 50 pN (difference between red and blue vectors in Fig. 3, top right) along an unpredicted direction.

Finally, the sensitivity of SIFs and of their nonadditivity to relatively small hydration details is evident from a comparison of the left-hand column of Fig. 3 with the remaining two. Hydration differences in the presence and in the absence of element 2 or of element 6 are in fact not very impressive, although they are perfectly visible. In contrast, very large differences are seen in SIFs. This is because they usually are (as in Fig. 3) the result of opposite and large contributions. In fact, the size of each contribution usually exceeds that of SIFs by one or even two orders of magnitude (Brugé et al., 1994, 1996). Consequently, small changes of hydration and related contributions are apt to cause large changes in SIFs.

COMMENTS AND CONCLUSIONS

In this MD study we have taken into account explicitly and without approximations all solvent water molecules. By computing space-resolved SIFs at the scale of individual solute elements, we have revealed microscopic features of solvent-induced interactions that have not been scrutinized so far. These features have been traced to high-order δG_{SW}

terms in Eq. 1. A quantity we have used to compute SIFs is the inhomogeneous distribution of the normalized space-occupancy probability p (defined as in Brugé et al., 1996, and in Methods). In this view, it helps to refer to the complex energy landscape in the multidimensional configurational space of water (Stillinger, 1988). Steric and interaction potential constraints imposed by solutes alter this landscape, its statistically populated regions, related configurational and residence times, and consequently, the spatial distribution of p and related free energy.

Inhomogeneous p distributions cause, on each solute element, forces that do not balance out. The resulting SIF vector acts on that element as part of a general drive toward a configuration of all elements corresponding to a minimum of hydration free energy. We have already discussed the quantitative relation of SIFs to hydration and their great sensitivity to hydration details. The latter feature combines with the observed relay of hydrations and related interactions. Together, they endow SIFs with extraordinary specificity, which is very sensitive to the context of solute elements and biologically very significant. This context-specificity is sufficiently strong to blur, occasionally, the relevance of the character (hydrophobic/philic) of a given element (see Fig. 3, *top right*, and Martorana et al., 1996, and manuscript to be published).

The present MD approach is, in a way, complementary to highly efficient simulation approaches essentially based on KSA (see e.g. Hirata et al., 1982; Pettitt et al., 1986; Kitao et al., 1991; Klement et al., 1991; Pellegrini and Doniach, 1995; Garde et al., 1996). The convenience of using either type of approach depends on the type of problem addressed and particularly on the scale of details sought. Highly efficient “quasiadditive” approaches based on KSA have been tested in a number of cases and shown to reproduce with reasonable accuracy features of free energies and of hydration. This is in line with the foregoing remarks, because nonadditivities elicited here are less likely to show up in cases involving averages of large numbers of interactions (de Gennes, 1979) than in cases such as the present one, involving differences of large terms (Lazaridis et al., 1995). Interestingly, the adequacy of approximated methods to the understanding of mesoscopic (coarse-grained) situations and phenomena is endorsed by both experimental and theoretical work. For example, in several cases, even when a strong role of solvent-induced interactions is present, the description of important nonmicroscopic events in complex systems in fact obeys predictions based on mean-field theory (San Biagio and Palma, 1991; Sciortino et al., 1993; Bulone et al., 1993; San Biagio et al., 1996a,b). Furthermore, treatments in terms of additive hydrophobic interactions (Kauzmann, 1959; Dill et al., 1995) can be adequate to predict coarse-grained, yet not detailed, features of protein folding/unfolding. Of course, we do not expect mean-field approaches to be adequate at the detailed scale of, say, one or a few residues. Nonetheless, down to a scale of some 1000 \AA^2 , an approach in terms of average effect has proved adequate. This is the case of hemoglobin (HbA), whose

conformational T-R change involved in oxygen transport involves a change of some 1000 \AA^2 of its area exposed to solvent (Chotia et al., 1976). More precisely, using and extrapolating averaged (although nonadditive) perturbations has revealed that in the functional $G_F = \Delta G$ (T-R) free energy of HbA, the contribution of solvent-induced interactions overwhelms intramolecular ones and for this reason dictates the functional uptake/release of oxygen (Bulone et al., 1991, 1992, 1993; Palma et al., 1993, 1994).

At variance with the cases above, a complementary situation is met when the main concern is to understand in causal (physical) terms single, microscopic events (say, at the biologically significant single-residue resolution) and the physical drive governing them, involving (as noted above) differences of large terms. In these cases, the reasons discussed above make clear that accuracies of, say, 10% or even much better in computing such large terms are in general no longer adequate. A fully explicit and nonapproximated treatment of solvent molecules then becomes necessary, as shown by the present and other recent work (Brugé et al., 1996; Martorana et al., 1996). To appreciate this, it is sufficient to refer, e.g., to the functionally crucial details of how proteins fold and work at the single-residue level. Indeed, it is hardly conceivable that pathways and the net result of biomolecular folding and interactions at this level could be the same in the presence and the absence of strong and highly nonadditive effects, such as those elicited in the present work.

The reported large size of high-order δG_{SW} terms in Eq. 1 and the consequently extended range of solvent-induced interactions can also be responsible for biologically significant effects at the microscopic, single-residue scale. As an example, let us consider the frequently observed, small, and still biologically important nonadditivities of point mutation effects in proteins (Green and Shortle, 1993; LiCata and Ackers, 1995). We note that protein function involves encounters with other solutes and (or) conformational changes. Correspondingly, and in accord with Eq. 1, there will be changes ΔG_{SW} in the hydration free energy. Therefore, the free energy G_F associated with a given function of a protein will in general contain contributions from all δG_{SW} terms in Eq. 1. In light of the present results, it is very plain to see how mutations in two or more even distant sites can nonadditively alter ΔG_{SW} and thus G_F via δG_{SW} terms of all orders. This provides the “general molecular mechanism” acting through some “global elements of the protein” sought by LiCata and Ackers (1995). Here, the global feature is provided by the many-body character of PMF and G_{SW} . As for the associated redistribution of “statistical mechanical substates accessible to the system” that LiCata and Ackers propose to be involved in the mechanism, we recall that the origin of hydration free energy lies in the changes of energy, multiplicity, and related dynamic states of the statistically populated solvent configurations, caused globally and collectively by solutes (Brugé et al., 1996; Martorana et al., 1996). Consequently, the configurational and dynamic states (and related statistical weights) respon-

sible for function are those corresponding to the very complex configurational energy landscape of the two interacting systems: protein + $n\text{H}_2\text{O}$ (Bulone et al., 1993; Palma et al., 1993, 1994). Here n is on the order of 10^2 – 10^3 , and the functional role is played by the complex energy landscape of the entire system in the configurational space.

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REFERENCES

- Ben-Naim, A. 1990a. Solvent effects on protein association and protein folding. *Biopolymers*. 29:567–596.
- Ben-Naim, A. 1990b. Strong forces between hydrophobic macromolecules: implications in biological systems. *J. Chem. Phys.* 93:8196–8210.
- Brugé, F., S. L. Fornili, G. G. Malenkov, M. B. Palma-Vittorelli, and M. U. Palma. 1996. Solvent-induced forces on a molecular scale: non-additivity, modulation and causal relation to hydration. *Chem. Phys. Lett.* 254:283–291.
- Brugé, F., S. L. Fornili, and M. B. Palma-Vittorelli. 1994. Solvent-induced forces between solutes: a time- and space-resolved molecular dynamics study. *J. Chem. Phys.* 101:2407–2420.
- Bulone, D., I. D. Donato, M. B. Palma-Vittorelli, and M. U. Palma. 1991. Density, structural lifetime, and entropy of H-bond cages promoted by monohydric alcohols in normal and supercooled water. *J. Chem. Phys.* 94:6816–6826.
- Bulone, D., M. B. Palma-Vittorelli, and M. U. Palma. 1992. Enthalpic and entropic contributions of water molecules to the functional T-R transition of human hemoglobin in water. *Int. J. Quantum Chem.* 42:1427–1437.
- Bulone, D., P. L. San Biagio, M. B. Palma-Vittorelli, and M. U. Palma. 1993. On the role of water in hemoglobin function and stability. *Science*. 259:1335–1336.
- Chotia, C., S. Wodak, and J. Janin. 1976. Role of the subunit interfaces in the allosteric mechanism of hemoglobin. *Proc. Natl. Acad. Sci. USA*. 73:3793–3797.
- Cordone, L., A. Cupane, and E. Vitrano. 1989. Conformational and functional properties of hemoglobin in perturbed solvent: relevance of electrostatic and hydrophobic interactions. *J. Mol. Liq.* 42:213–229.
- de Gennes, P. G. 1979. *Scaling Concepts in Polymer Physics*. Cornell University Press, Ithaca, NY.
- Dill, K. A., S. Bromberg, K. Yue, K. M. Fiebig, D. P. Yee, P. D. Thomas, and H. S. Chan. 1995. Principles of protein folding—a perspective from single exact models. *Protein Sci.* 4:561–602.
- Flyvbjerg, H., and H. G. Petersen. 1989. Error estimates on averages of correlated data. *J. Chem. Phys.* 91:461–466.
- Garde, S., G. Hummer, A. F. Garcia, L. R. Pratt, and M. B. Paulaitis. 1996. Hydrophobic hydration: inhomogeneous water structure near nonpolar molecular solutes. *Phys. Rev. E*. 53:R4310–R4313.
- Green, S. M., and D. Shortle. 1993. Patterns of nonadditivity between pairs of stability mutations in staphylococcal nuclease. *Biochemistry*. 32:10131–10139.
- Haberfield, P., J. Kivuls, M. Haddad, and T. Rizzo. 1984. Enthalpies, free energies, and entropies of transfer of phenols from nonpolar solvents to water. *J. Phys. Chem.* 88:1913–1915.
- Hill, T. L. 1956. *Statistical Mechanics*. McGraw-Hill, New York, and Dover Publications, Mineola, NY.
- Hirata, F., B. M. Pettitt, and P. J. Rossky. 1982. Application of an extended RISM equation to dipolar and quadrupolar fluids. *J. Chem. Phys.* 77:509–520.
- Jorgensen, W. L., J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein. 1983. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 79:926–935.
- Kauzmann, W. 1959. Some factors in the interpretation of protein denaturation. *Adv. Protein Chem.* 14:1–63.
- Kirkwood, J. G. 1935. Statistical mechanics of fluid mixtures. *J. Chem. Phys.* 3:300–313.
- Kitao, A., F. Hirata, and N. Gō. 1991. The effects of solvent on the conformational and collective motions of proteins: normal mode analysis and molecular dynamics simulations of melittin in water and in vacuum. *Chem. Phys.* 158:447–472.
- Klement, R., D. M. Soumpasis, and T. M. Jovin. 1991. Computation of ionic distributions around charged biomolecular structures: results for right-handed and left-handed DNA. *Proc. Natl. Acad. Sci. USA*. 88:4631–4635.
- Lazaridis, T., G. Archontis, and M. Karplus. 1995. Enthalpic contribution to protein stability: insights from atom based calculations and statistical mechanics. *Adv. Protein Chem.* 47:231–306.
- Lee, S. H., and P. J. Rossky. 1994. A comparison of the structure and dynamics of liquid water at hydrophobic and hydrophilic surfaces—a molecular dynamics simulation study. *J. Chem. Phys.* 100:3334–3345.
- LiCata, V. J., and G. K. Ackers. 1995. Long-range, small magnitude nonadditivity of mutational effects in proteins. *Biochemistry*. 36:3133–3139.
- Lifson, S., and I. Oppenheim. 1960. Neighbour interactions and internal rotations in polymer molecules. IV. Solvent effect on internal rotations. *J. Chem. Phys.* 33:109–115.
- Lüdemann, S., H. Schreiber, R. Abseher, and O. Steinhauser. 1996. The influence of temperature on pairwise hydrophobic interactions of methane-like particles: a molecular dynamics study of free energy. *J. Chem. Phys.* 104:286–295.
- Martorana, V., G. Corongiu, and M. U. Palma. 1996. Correlated solvent-induced forces on a protein at single residue resolution: relation to conformation, stability, dynamics and function. *Chem. Phys. Lett.* 254:292–301.
- Palma, M. U., D. Bulone, P. L. San Biagio, and M. B. Palma-Vittorelli. 1994. Physical origin and biological significance of solvent-induced forces. In *Hydrogen Bond Networks*. M. C. Bellissent-Funel and J. C. Dore, editors. Kluwer Academic Publishers, Dordrecht, The Netherlands. 457–479.
- Palma, M. U., F. Parak, and M. B. Palma-Vittorelli. 1993. Foreword. In *Water-Biomolecule Interactions*. Conference Proceedings by the Italian Physical Society, Vol. 43. M. U. Palma, F. Parak, and M. B. Palma-Vittorelli, editors. Compositori, Bologna, XIX–XXII.
- Pellegrini, M., and S. Doniach. 1995. Modeling solvation contributions to conformational free energy changes of biomolecules using a potential of mean force expansion. *J. Chem. Phys.* 103:2696–2702.
- Pepke, E., and J. Lyons. 1993. *SciAn User's Manual*. Supercomputer Computations Research Institute, Florida State University, Tallahassee, FL.
- Pettitt, B. M., M. Karplus, and P. J. Rossky. 1986. Integral equation model for aqueous solvation of polyatomic solutes: application to the determination of the free energy surface for the internal motion of biomolecules. *J. Phys. Chem.* 90:6335–45.
- Pratt, L., and D. Chandler. 1980. Effects of solute-solvent attractive forces on hydrophobic correlations. *J. Chem. Phys.* 73:3434–3441.
- Ravishanker, G., M. Mezei, and D. L. Beveridge. 1982. Solvent-separated hydrophobic interaction. *Faraday Symp. Chem. Soc.* 83:477–483.
- San Biagio, P. L., D. Bulone, A. Emanuele, and M. U. Palma. 1996a. Self-assembly of biopolymeric structures below the threshold of random cross-link percolation. *Biophys. J.* 70:494–499.
- San Biagio, P. L., D. Bulone, A. Emanuele, M. B. Palma-Vittorelli, and M. U. Palma. 1996b. Spontaneous symmetry-breaking pathways: time-resolved study of agarose gelation. *Food Hydrocolloids*. 10:91–97.

- San Biagio, and M. U. Palma. 1991. Spinodal lines and Flory-Huggins free energy for solutions of human hemoglobins HbS and HbA. *Biophys. J.* 60:508–512.
- Sciortino, F., K. U. Prasad, D. W. Urry, and M. U. Palma. 1993. Self-assembly of bioelastomeric structures from solutions: mean-field critical behavior and Flory-Huggins free energy of interaction. *Biopolymers.* 33:743–752.
- Somero, G. N. 1992. Osmotic solutes: evolution, function, and regulation. *In Water and Life.* G. N. Somero, C. B. Osmond, and C. L. Bolis, editors. Springer Verlag, Berlin. 3–18.
- Stillinger, F. H. 1988. Supercooled liquid, glass transitions, and the Kauzmann paradox. *J. Chem. Phys.* 88:7818–7825.
- Wallqvist, A., and D. G. Covell. 1995. Free energy cost of bending *n*-dodecane in aqueous solutions. Influence of the hydrophobic effect and solvent exposed area. *J. Phys. Chem.* 99:13118–13125.
- Zichi, D. A., and P. J. Rossky. 1985. The equilibrium solvation structure for the solvent-separated hydrophobic bond. *J. Chem. Phys.* 83:797–808.