Comment on "Anti-cooperativity in hydrophobic interactions: A simulation study of spatial dependence of three-body effects and beyond" [J. Chem. Phys. 115, 1414 (2001)]

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COMMENTS

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In their recent paper,[1] Shimizu and Chan presented results of their Monte Carlo study of the hydrophobic effect, which suggest that there is significant anti-cooperativity in hydrophobic interactions (i.e., that the deviation of the free energy of hydrophobic interactions from pairwise additivity is positive, thereby weakening the hydrophobic interactions). This result contradicts that of our molecular simulation study[2] of the hydrophobic effect, which revealed *strengthening* of hydrophobic interactions.

In our view, the results of Shimizu and Chan do not contain sufficient evidence for anti-cooperativity in hydrophobic interactions for the following reasons.

(1) According to the definition of the cooperative term in the potential of mean force of three hydrophobic particles [Eq. (4) in Ref. 1] (and from the definition of the potential of mean force of two hydrophobic particles [Eq. (8) in Ref. 3], the total potential of mean force of three particles [Eq. (1) in Ref. 1], as well as from the definition of the pairwise-additive potential of mean force [Eq. (3) in Ref. 1]), the cooperative free-energy term can be expressed as follows:

$$\text{cooperative term} = \mu_{abc}^* - 2\mu_{ab}^* + \mu_a^*, \qquad (1)$$

where μ_{abc}^* is the free energy of insertion of particle a into the aqueous environment of the fixed dimer bc, μ_{ab}^* is the free energy of insertion of particle a in the aqueous environment of particle b, and μ_a^* is the free energy of insertion of particle a into the aqueous environment. The baseline for the distance dependence of the cooperative term and of its components (μ_{abc}^* and μ_{ab}^*) is established by estimating the value of μ_a^*, which the authors obtained by the particle-insertion method while, in our earlier study,[2] we estimated the baseline of the cooperative term in the PMF by assuming

that the cooperative term vanishes with the distance faster than the PMF itself. Shimizu and Chan consider their method of baseline estimation superior to ours; this is the basis of their criticism. Their estimated errors in μ_a^* and μ_{abc}^* are 0.05 kcal/mol[1] and 0.005 kcal/mol,[1] respectively. There is *no* estimate of the error in μ_{ab}^*. We return to this question in point (2).

We now analyze the distance dependence of the cooperative term of Shimizu and Chan, which was *not* presented explicitly in their paper.[1] From their curves for the distance dependence of $2\mu_{ab}^* - 2\mu_a^*$ and $\mu_{abc}^* - \mu_a^*$ (Fig. 2 in Ref. 1), we constructed a graph for the distance dependence of the cooperative term [Eq. (1)]. The resulting curve is shown in Fig. 1. The cooperative term decreases sharply to zero at $\xi \approx 12$ Å, where ξ is the separation coordinate defined in Ref. 1. This decrease coincides with the end of the largest distance considered in their calculations,[1] and can therefore be an artifact of edge effects. To demonstrate that the anti-cooperative effect continues until 12 Å, a more extensive study beyond the distances considered by the authors[1] would be required. Until the distance of about 11.5 Å, the distance dependence of the PMF is similar to the curve found in our study[2] for the same system [Fig. 8(d) in Ref. 2] except that the coordinate in our study is not exactly the same as that of Shimizu and Chan[1] and that there is a downward shift [see point (2)] of the baseline of Shimizu and Chan[1] by about 0.2 kcal/mol.

The downward shift of the baseline of Shimizu and Chan[1] is due to errors in determining μ_{ab}^* [see point (2)]. According to the authors,[1] the number of configurations taken to estimate μ_{ab}^* was of the order of 10^5, while that to estimate μ_{abc}^* was of the order of 10^7. In fact, the fluctua-
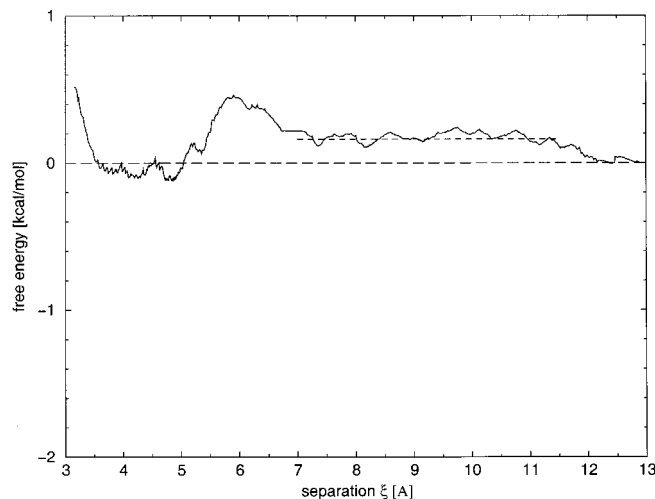
FIG. 1. Cooperativity curve calculated from the data of Fig. 2 in Ref. 1. The data from the original graph were digitized using the program UNSCANIT. The lower horizontal dashed line is the zero baseline in Ref. 1. The upper horizontal dashed line is our corrected baseline; see text for explanation. The abscissa is the coordinate ξ defined in Ref. 1.
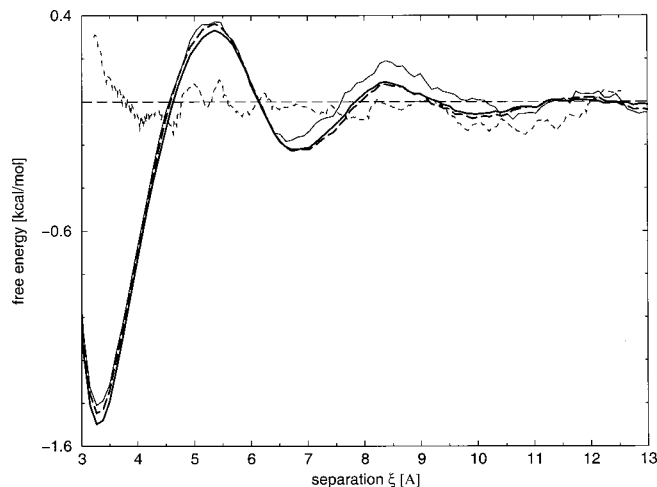

FIG. 2. Calculated PMF curves of a methane dimer in water. Heavy solid line: 21 windows from 3.5 Å to 13.5 Å; a 4 ns simulation in each window and 21 windows from 3.75 Å to 13.25 Å, a 2 ns simulation in each window. Heavy dashed line: 20 windows from 3.5 Å to 13.5 Å; a 4 ns simulation in each window. Light solid line: 21 windows from 3.5 Å to 13.5 Å; a 1 ns simulation in each window. Light dashed line: the difference between the PMF calculated by Shimizu and Chan[1] and our results using all data.

tions in the curve corresponding to μ_{ab}^* seem to be much larger compared to those corresponding to μ_{abc}^*. Unlike the case of μ_{abc}^*, the authors did not present any comparison of μ_{ab}^* curves corresponding to different numbers of configurations. This indicates that their μ_{ab}^* curve might contain significant errors [see point (2)].

(2) The value of ξ ranges until 13 Å (this means that the methane–methane distance ranges until $[\xi_{max}^2+(1/2d_0)^2]^{1/2}$ $=13.15$ Å (with $d_0=3.9$ Å being the distance between the particles constituting the dimer). The authors carried out their simulations in a periodic box with 396 water molecules, which corresponds to a cubic box dimension of 23 Å (with a water density of 1 g/cm^3). This means that the largest allowed solute–solute distance resulting from the minimum-image convention in computing the interaction energy was 11.5 Å (half of the box dimension). In view of this, the fact that the second solvent-separated minimum in the methane–methane PMF at about 10.5 Å (Fig. 2 in Ref. 1) has almost the same depth as the first one at about 7 Å seems to be an artifact of approaching the half-box distance.

To check, whether the depth of the second solvent-separated minimum can be comparable with that of the first one, we now determined the PMF of two methane molecules in a 28 Å periodic box using umbrella-sampling MD simulations and the WHAM method, as described in our earlier paper.[2] The total length of the simulation was 4 ns, and 21 windows were used, starting from a methane–methane distance of 3.5 Å with a 0.5 Å distance between windows. The resulting PMF curve is shown in Fig. 2 (to estimate the error, we performed an additional 2 ns of simulations and compared the resulting PMF's; the maximum error was found to be 0.04 kcal/mol). As shown, the 4 ns curve is much smoother than that of Shimizu and Chan,[1] and the second solvent-separated minimum (appearing at about 10 Å) is about three times shallower than the first one. This very strongly suggests that (i) the curve of Shimizu and Chan is shifted downward, and (ii) their deep "second solvent-

separated minimum" arises from the artifact in their calculations in which the methane–methane distance exceeded half of the box distance. Their curve, describing the distance dependence of the three-body PMF, $\mu_{abc}^*-\mu_a^*$, seems to be shifted upward by 0.05 kcal/mol and exhibits a sharp decrease to zero after half of the box-dimension distance; however, the $+0.05$ kcal/mol shift is contained within their error estimate of the reference μ_a^* free energy.

In order to establish the baseline of our PMF curve, we assumed that the PMF is negligible at distances beyond 12 Å (this is also the distance at which the PMF approaches zero in the calculations of Shimizu and Chan[1]). The PMF curve is almost flat beyond 10 Å and, moreover, the baseline must be contained between the second solvent-separated minimum at 10 Å and the subsequent very shallow maximum at 11.5 Å separating the second and the third solvent-separated minima; this gives the upper bound of its estimated error as 0.05 kcal/mol, which is within the accuracy of the estimation of the PMF given by the 4 ns simulation time. In Fig. 2, we have also plotted the difference between our PMF and that of Shimizu and Chan for two methanes in water. Apart from the region close to the contact minimum, their curve lies mostly below ours, and in the region between 10 and 12 Å the difference reaches -0.2 kcal/mol. Because this is the region in which, according to our simulations, the PMF gradually becomes almost completely flat, we can infer that their methane–methane PMF curve should be shifted upward by about 0.2 kcal/mol and, consequently, the cooperativity curve (Fig. 1) should by shifted downward by this value. When this is done, cooperativity and *not* anti-cooperativity is observed between the contact minimum and the cavity maximum; this is exactly the conclusion that we drew in our paper.[2]

(3) In their work, Shimizu and Chan considered only *one* system (composed of a methane dimer and an approaching methane molecule in water) and *one* water model. This $2m$ $+m$ system with a *rigid* methane dimer is largely artificial,

because the interacting hydrophobic particles are not constrained in reality. In this regard, the $m+m+m$ system (methane molecules on the vertices of an equilateral triangles, which is *restrained*, but not *constrained* to equilateral) seems to be much more realistic. Therefore, their generalization of the conclusions of their $2m+m$ study seems premature.

Recently,[4] we carried out an MD study of a larger hydrophobic solute than methane in water, for the system analogous to $m+m+m$. As in our study of methane trimers,[2] we found an *increasing* strength of cooperative interactions. The simulations were carried out in a 28 Å periodic box with the solute–solute distances until 14 Å (i.e.,

beyond the second solvent-separated minimum), where all PMF curves become flat within the error inherent in simulations.

In conclusion, contrary to the statements of Shimizu and Chan,[1] the evidence supports the cooperativity of the hydrophobic interaction.

[1] S. Shimizu and H. S. Chan, J. Chem. Phys. **115**, 1414 (2001).
[2] C. Czaplewski, S. Rodziewicz-Motowidło, A. Liwo, D. R. Ripoll, R. J. Wawak, and H. A. Scheraga, Protein Sci. **9**, 1235 (2000).
[3] S. Shimizu and H. S. Chan, J. Chem. Phys. **113**, 4683 (2000).
[4] C. Czaplewski, D. R. Ripoll, S. Rodziewicz-Motowidło, A. Liwo, R. J. Wawak, and H. A. Scheraga, Int. J. Quantum Chem. (in press).