

# COMMENTS

## Comment on “Molecular Origin of Anticooperativity in Hydrophobic Association”

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Recently, using the TIP4P water model, Czaplewski et al.<sup>1</sup> showed that the three-methane potential of mean force (PMF) in water at 298 K for their 2m+m system (i.e., the trimer case with isosceles-triangle geometry in their Figure 1b) is anticooperative at the contact minimum. Apparently, this conclusion is in qualitative agreement with our previous TIP4P simulation results, which have indicated anticooperativity at 25 °C for the same three-methane configuration,<sup>2–4</sup> but contradicts their previous conclusions from TIP4P and TIP3P simulations that it is cooperative<sup>5,6</sup> (cf. Figure 5 in ref 1 and the  $\phi = 0$  panel of Figure 9 in ref 3, as well as Figure 8 in ref 5).

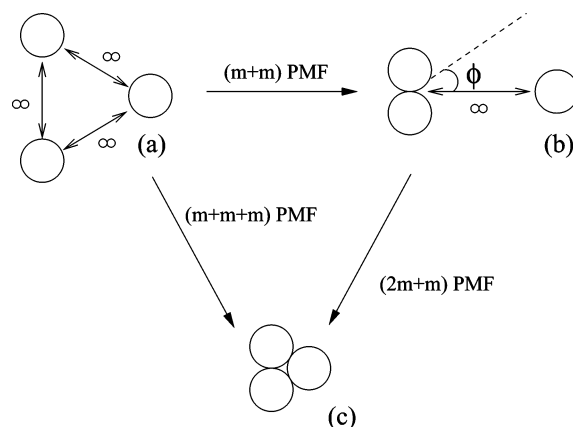
Despite their new 2m+m anticooperative result at the contact minimum, Czaplewski et al.<sup>1</sup> asserted, citing their previous TIP3P study,<sup>7</sup> that “there is only cooperativity” in the three-methane PMF simulated in an equilateral-triangle geometry (i.e., their m+m+m system, see also the corresponding TIP4P result in Figure 7B of ref 5). By construction, however, the contact-minimum three-methane configuration for the 2m+m and m+m+m systems are essentially identical. Can the cooperativity or anticooperativity of a given final three-methane configuration depend on the pathway by which it is assembled?

A straightforward analysis of the simple thermodynamic cycle in Figure 1 demonstrates that this is impossible. Let  $W_{m+m}^{(2)}(\xi_c)$  and  $W_{2m+m}^{(3)}(\xi_c)$  denote, respectively, the two-methane and the three-methane 2m+m PMFs at the position defined by the center-of-mass methane-methane spatial separation  $\xi_c = 3.9$  Å at contact minimum. The quantity  $W_{2m+m}^{(3)}(\xi_c)$  corresponds to the free energy change of the (b) → (c) process in Figure 1. Although the separation between each of the two methanes in the methane dimer and the third methane at the 2m+m contact minimum need not be exactly equal to  $\xi_c$ , in practice they are essentially identical. Therefore, in the present notation, the 2m+m cooperativity term at contact minimum is

$$\delta F_{2m+m}^{(3)}(\xi_c) = W_{2m+m}^{(3)}(\xi_c) - 2W_{m+m}^{(2)}(\xi_c) \quad (1)$$

Similarly, let  $W_{m+m+m}^{(3)}(\xi_c)$  denote the free energy change of the (a) → (c) process, the corresponding m+m+m cooperativity term at intermethane separation  $\xi_c$  is given by

$$\delta F_{m+m+m}^{(3)}(\xi_c) = W_{m+m+m}^{(3)}(\xi_c) - 3W_{m+m}^{(2)}(\xi_c) \quad (2)$$



**Figure 1.** A thermodynamic cycle for potentials of mean force of three methanes (denoted here by circles) in water.  $W_{m+m}^{(2)}(\xi_c)$ ,  $W_{2m+m}^{(3)}(\xi_c)$ , and  $W_{m+m+m}^{(3)}(\xi_c)$  express, respectively, the free energy changes upon (a) → (b), (b) → (c), and (a) → (c). The symbols “∞” in (a), (b) represent infinite methane-methane separation. The angle  $\phi$  in (b) indicates a general direction of the single methane relative to the methane dimer;<sup>2–4</sup> the geometry shown here is for  $\phi = 0$ . The 2m+m and L(2m+m) geometries of Czaplewski et al.<sup>1</sup> correspond, respectively, to  $\phi = 0$  and  $\phi = \pi/2$ .

Now, since the free energy change of the (a) → (b) process is simply  $W_{m+m}^{(2)}(\xi_c)$ ,

$$W_{m+m+m}^{(3)}(\xi_c) = W_{2m+m}^{(3)}(\xi_c) + W_{m+m}^{(2)}(\xi_c) \quad (3)$$

Hence,

$$\delta F_{m+m+m}^{(3)}(\xi_c) = \delta F_{2m+m}^{(3)}(\xi_c) \quad (4)$$

i.e., the cooperativity terms for the 2m+m and m+m+m cases are identical at intermethane distance  $\xi_c$ . We note that the intermethane separation at the m+m+m contact minimum can be slightly different from  $\xi_c$  by  $\sim 0.05$  Å. Nonetheless, the value of  $W_{m+m+m}^{(3)}$  at contact minimum is practically identical to  $W_{2m+m}^{(3)}$ . In this context, the exact relation in eq 4 may also be viewed as a requirement that the contact-minimum 2m+m and m+m+m cooperativity terms have to be essentially identical.

It follows that Czaplewski et al.’s new finding of 2m+m anticooperativity<sup>1</sup> implies anticooperativity for the essentially identical contact-minimum configuration in the m+m+m case as well. If the  $\delta F^{(3)}$  terms here were expressed as “per methane pair” quantities,<sup>1,5</sup>  $\delta F_{m+m+m}^{(3)}$  and  $\delta F_{2m+m}^{(3)}$  would be replaced by  $\delta F_{m+m+m}^{(3)}/3$  and  $\delta F_{2m+m}^{(3)}/2$ , respectively. But the fact that they have to be of the same sign remains unchanged. Therefore, the assertion by Czaplewski et al.<sup>1</sup> that their nonadditivity are of opposite signs is untenable. In other words, if the m+m+m contact minimum is cooperative, their new 2m+m results in ref 1 cannot be correct; conversely, if the 2m+m contact minimum is anticooperative, as stipulated in ref 1, the m+m+m contact minimum has to be anticooperative as well.

The study of anticooperativity and cooperativity of hydrophobic interactions entails comparing different PMFs. Thus, a

reliable and physically justifiable general procedure for setting zero-PMF baselines is of critical importance.<sup>2,3</sup> The Widom particle insertion approach has been applied to compute hydration free energies of single solutes of sizes as big as Xenon.<sup>8</sup> (It should be noted that in the study of Xenon in ref 8, the test particle approach has only been applied to single-solute hydration but not pairs of solutes.) For a given simulation box size, the test-particle insertion method of PMF determination, which sets zero-PMF baselines using the chemical potential of an isolated solute in pure water, is more reliable<sup>4,9</sup> than identifying the zero-PMF baseline with the largest intersolute distance in the simulation.<sup>1,6</sup> In this connection, the thermodynamic cycle in Figure 1 can be utilized to check the consistency of the zero-PMF baselines used in the  $m+m$ ,  $2m+m$ , and  $m+m+m$  simulations. Equation 3 should hold if they are consistent. However, it has been noted<sup>4</sup> that the zero-PMF baselines assumed by the TIP3P  $2m+m$  and  $m+m+m$  simulations of Czaplewski et al.<sup>7</sup> are not consistent. In that case, the reported  $W_{m+m}^{(2)}(\xi_c)$ ,  $W_{2m+m}^{(3)}(\xi_c)/2$ , and  $W_{m+m+m}^{(3)}(\xi_c)/3$  are, respectively, approximately equal to  $-0.79$ ,  $-0.74$ , and  $-0.83$  kcal/mol (Figures 2 and 3 of ref 7), resulting in a discrepancy  $\approx 0.22$  kcal/mol between the left and right sides of eq 3. We obtain the same discrepancy irrespective of whether the comparison is made at exactly  $\xi_c = 3.9$  Å for both the  $2m+m$  and  $m+m+m$  cases or at their respective contact-minimum configurations (see above). This significant inconsistency implies that the published simulation procedure and conclusions in ref 7 are problematic.

Two additional collinear three-methane configurations termed  $L(2m+m)$  (which is equivalent to our  $\phi = \pi/2$  case<sup>2-4</sup>) and  $L(m+m+m)$  have also been considered in the recent study of Czaplewski et al.<sup>1</sup> For these linear trimer geometries, a thermodynamic cycle very similar to the one in Figure 1 can be constructed by changing the  $2m+m$  and  $m+m+m$  specifications of the  $W^{(3)}$  terms in eqs 1–4 to  $L(2m+m)$  and  $L(m+m+m)$ , respectively, and replacing one factor of  $W_{m+m}^{(2)}(\xi_c)$  in each of eqs 1 and 2 by  $W_{m+m}^{(2)}(2\xi_c)$ . These lead to the same eqs 3 and 4, with the only modification being the added “L” specifications on the  $W^{(3)}$  and  $\delta F^{(3)}$  terms. Accordingly, we have used this linear trimer version of eq 3 to check the consistency of Czaplewski et al.’s<sup>1</sup> zero-PMF baselines in these cases. If one assumes that the quantities plotted in their Figures 7 and 8 in ref 1 are in fact  $W_{L(2m+m)}^{(3)}/2$  and  $W_{L(m+m+m)}^{(3)}/3$ , respectively, the zero-PMF baselines in the two plots are consistent inasmuch as they satisfy the linear trimer thermodynamic cycle just described. However, Czaplewski et al.<sup>1</sup> did not use a common criterion for the two baselines. Instead, they set the PMF = 0 levels for  $L(2m+m)$  and  $L(m+m+m)$  by matching the linear

trimer  $W_{L(m+m+m)}^{(3)}/3$  and  $W_{L(2m+m)}^{(3)}/2$  with the dimer  $W_{m+m}^{(2)}$  at two different spatial ranges: 11.5–13.0 Å and 8.5–10.0 Å, respectively.

In the absence of physical justifications, such double standards are questionable. First, any a priori assumption about the spatial range of nonadditivity is problematic.<sup>9</sup> Second, if matching at 8.5–10.0 Å is appropriate for  $L(m+m+m)$ , why should not it be appropriate for  $L(2m+m)$  as well? If one employs a consistent procedure of matching both trimer cases with dimer PMF at 8.5–10.0 Å, the zero-PMF baseline for  $W_{L(2m+m)}^{(3)}$  would be upshifted by  $\approx 0.09$  kcal/mol and differ significantly with the PMF values at larger distances. This consideration suggests strongly that the same problem would arise for their current  $L(m+m+m)$  baseline, though simulation data beyond 10.0 Å are not available for comparison.

Notwithstanding these uncertainties, it is noteworthy that the recent  $2m+m$  and  $L(2m+m)$  results of Czaplewski et al.<sup>1</sup> indicating essential anticooperative behavior are qualitatively consistent with the previous findings of anticooperativity for our corresponding  $\phi = 0$  (see above) and  $\phi = \pi/2$  cases (cf. Figure 9 in ref 1 and the  $\phi = \pi/2$  panel of Figure 9 in ref 3). Some issues such as the different predictions from the two sets of studies on the sign of nonadditivity for the  $\phi = 0$  desolvation barrier<sup>1-3</sup> remain to be resolved. Nonadditivity properties of hydrophobic interactions are temperature dependent.<sup>4</sup> As well, they are expected to depend on solute size, the model atomic interaction scheme, and may also be sensitive to the choice of water model.<sup>8,10</sup> Nonetheless, for the TIP4P model at 1 atm and  $\approx 25$  °C, with the publication of the latest results of Czaplewski et al.,<sup>1</sup> a consensus pointing to a prevalence of anticooperativity in three-methane hydrophobic interactions has apparently emerged.

## References and Notes

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