

Reply to “Comment on ‘Molecular Origin of Anticooperativity in Hydrophobic Association’”

Cezary Czaplewski,^{†,‡} Sebastian Kalinowski,[‡]
Adam Liwo,^{†,‡} Daniel R. Ripoll,[§] and
Harold A. Scheraga^{*,†}

*Baker Laboratory of Chemistry and Chemical Biology,
Cornell University, Ithaca, New York 14853-1301, Faculty of
Chemistry, University of Gdańsk, ul. Sobieskiego 18,
80-952 Gdańsk, Poland, and Cornell Theory Center, Ithaca,
New York 14853-3801*

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In their comment¹ on our recent paper,² and also in their recent work,³ Shimizu et al. and Moghaddam et al., respectively, pointed out, among other issues, inconsistency in our results concerning the determination of the cooperative contribution to the potential of mean force (PMF) of two methane trimers: $m+m+m$, which consists of three methane molecules at equilateral-triangle geometry, and $2m+m$, which consists of two methane molecules at a contact distance characteristic of a methane dimer [m_1-m_2 in water and the third methane molecule (m_3) lying on the line passing through the middle of the dimer and perpendicular to the dimer axis (isosceles geometry)]. With a thermodynamic cycle, they have stated that, at the same geometries of both systems ($m+m+m$ and $2m+m$; see Figure 1 in ref 1), the PMF must be the same, which is not the case of the PMFs reported in our papers.^{2,4} Consequently, they argue that our plot of the cooperative contribution to the PMF of the $m+m+m$ system (Figure 4b in ref 4) should be upshifted to match that of the $2m+m$ system at the contact minimum. This would then result in demonstrating anticooperativity for both the $2m+m$ and $m+m+m$ systems.

First of all, we note that, although the comment refers to our recent paper² in which we used the TIP4P water model, most of ref 1, in fact, compares the PMF curves for the $2m+m$ and $m+m+m$ system reported in our earlier paper⁴ obtained with the TIP3P water model. Thus, our further discussion here refers mostly to our results reported in ref 4 and not to those reported in ref 2.

The thermodynamic argument could be applied literally (as Moghaddam et al. do in their comments) only if the errors in the PMF at the distance of 3.9 Å were negligible, which is not the case of the PMFs obtained in our simulations. From the convergence plots presented in Figures 2, 3a, and 3b in ref 4 for the $m+m$, $2m+m$, and $m+m+m$ systems, respectively, we note that the PMF curves are quite divergent in the region of the contact minimum (the discrepancy amounting to about 0.1 kcal/mol), and they are convergent only after the first desolvation maximum for 600,000 and more snapshots per window. It should be noted that the distance after which the curves are convergent (6 Å) is by far smaller than the distance at which the zero baseline of the cooperative PMF was established (11 Å). Poor convergence of the PMF curves near the contact minimum is even more apparent when the cooperative contributions to the PMF are compared; a good example is provided in

Figure 5 of ref 2, although this figure presents the results obtained with the TIP4P water. Moreover, we will show in this Reply that the discrepancy between the PMFs of the $2m+m$ and $m+m+m$ systems at 3.9 Å distance depends strongly on the details of simulations, suggesting that the errors are particularly large in this region. Thus, the thermodynamic argument can be used to point out the errors of the simulations but not the errors of logic, which downgrades this argument significantly. We also note that we were aware of possible increased errors in the contact-minimum region and, therefore, in the case of methane trimers, we *never* discussed the *value* of the excess (cooperative) PMF at the distance of 3.9 Å and even reiterated that our results showed that cooperativity was manifested only as a lowering of the desolvation barrier.

To demonstrate that details of a simulation procedure can influence the values of the PMF at the contact minimum, we note that our PMFs are computed as averages over a bin with 0.1 Å side rather than as values at single points. Thus, they contain contributions from regions of space outside the symmetric configurations corresponding to the $2m+m$ and $m+m+m$ systems, respectively. In this regard, averaging is different in the $2m+m$ system because a strong harmonic potential with a 100 kcal/(mol×Å²) force constant was imposed on the m_1-m_2 distance and weak harmonic potentials with a 2 kcal/(mol×Å²) force constant were imposed on the remaining distances.^{2,4,5}

To determine the effect of restraints on the PMF of the $2m+m$ system, we ran new umbrella-sampling MD simulations with imposed restraining potentials with a 2 kcal/(mol×Å²) force constant on all the methane–methane distances. In our previous studies,^{2,4,5} we either constrained the m_1-m_2 distance or applied a force constant of 100 kcal/(mol×Å²) to match the setup corresponding to that applied by other authors to the $2m+m$ system.^{3,6–8} We have applied the TIP3P water model and the van der Waals interaction parameters of Jorgensen et al.⁹ for the united methane atom. The other characteristics of the simulation were those used in our recent study.⁴ The $2m+m$ PMF from this new simulation and that from our previous study⁴ (i.e., with strong restraints imposed on the m_1-m_2 distance) are compared in Figure 1. It can be seen that the PMFs of Figures 1a and 1b are different in the contact-minimum region, the PMF determined in this new simulation being lower. However, there still is a discrepancy of 0.07 to 0.15 kcal/mol between the left and right sides of eq 3 of ref 1, with the higher discrepancy obtained with a larger number of data for the $2m+m$ PMF.

It should be noted that the convergence of the cooperative contribution of the $2m+m$ PMF to the zero asymptote is slower than that of the uniformly expanding $m+m+m$ cluster in Figure 4b of ref 4. Therefore, if the discrepancy between the PMF of the $2m+m$ and $m+m+m$ systems at equivalent configurations is in fact caused by problems with choosing the zero asymptote, the more reliable baseline of the cooperative contribution to the PMF of the $m+m+m$ system (seen in Figure 4b of ref 4) and not that of the $2m+m$ system (seen in Figure 4a of ref 4) should be used; hence, the plot in Figure 4a of ref 4 should be shifted downward rather than shifting the plot in Figure 4b of ref 4 upward. On the other hand, because the PMFs of the $2m+m$ and $m+m+m$ system converge at large distances,⁴ it is more probable that the discrepancy is caused by convergence problems in the contact-minimum region rather than by problems with selecting the asymptote. However, to prove this, we

* Corresponding author; phone: (607) 255 4034, fax: (607) 254 4700, e-mail: has5@cornell.edu.

[†] Cornell University.

[‡] University of Gdańsk.

[§] Cornell Theory Center.

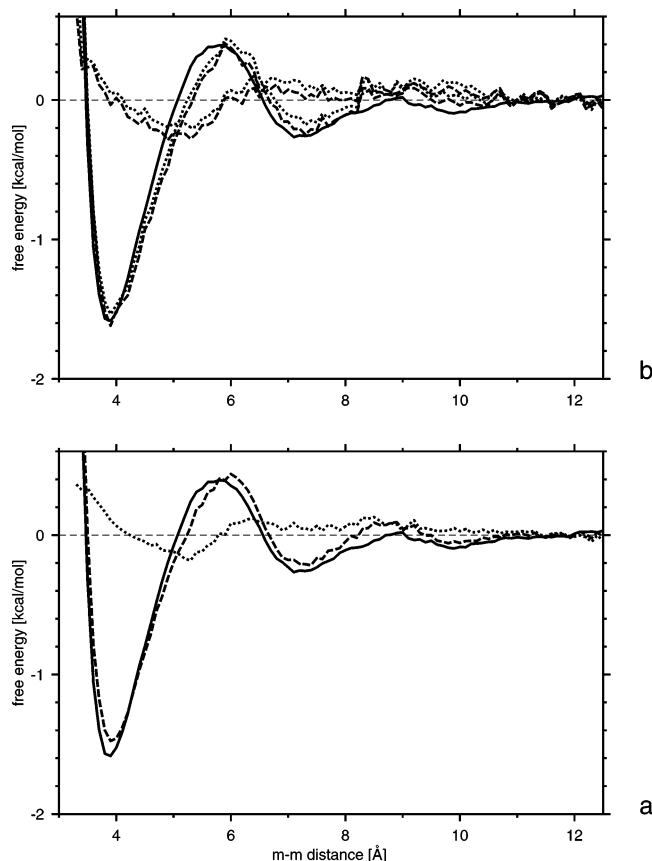


Figure 1. (a) Doubled PMF of the $m+m$ system (solid line) and the PMFs and the respective cooperative contribution to the PMF per methane pair (dashed line) of the $2m+m$ system obtained with the umbrella-sampling/WHAM method with imposed harmonic restraints with a $100 \text{ kcal}/(\text{mol} \times \text{\AA}^2)$ force constant on the m_1-m_2 distance. (b) As in (a), but restraints with a force constant of $2 \text{ kcal}/(\text{mol} \times \text{\AA}^2)$ were imposed, and the dashed and dotted lines mark the PMFs and the cooperative contributions to the PMF obtained for 2 and 4 ns/window, respectively. The TIP3P water model was used in the calculations.

have to check if the PMF of hydrophobic association does or does not have a long-distance dependence.

Recently,¹⁰ we carried out a particle-insertion (PI) study of the PMF of the hydrophobic association of two neon atoms and two methane molecules. We concluded that, for the smaller Ne atoms, the convergence of the PMF is very good while it is poorer in the case of the larger methane. In view of this, the Ne clusters appear to be a much better model with which to study hydrophobic association by the PI method, if the absolute value of the PMF is an issue, as it is here. Therefore, using the PI method for neon trimers, we now determined the cooperative contributions of the equilateral-triangle hydrophobic-cluster arrangement and that of the isosceles-triangle arrangement with one of the distances effectively constrained in the latter cluster. If multibody contributions to the PMF of hydrophobic association exhibit long-distance features as suggested by Chan and colleagues,^{7,8} they should also be manifested for neon trimers as the appearance of a significantly broad region where the cooperative contribution to the PMF is flat and yet remarkably greater than zero. As in our most recent study on applying the particle-insertion method to determine the PMFs,¹⁰ we used molecular dynamics to generate systems for particle insertion. We will designate the systems investigated as Ne+Ne+Ne and 2Ne+Ne, for the equilateral triangle and isosceles triangle geometry, respectively.

We carried out particle-insertion simulations with the TIP3P water model. The van der Waals parameters for the neon atom

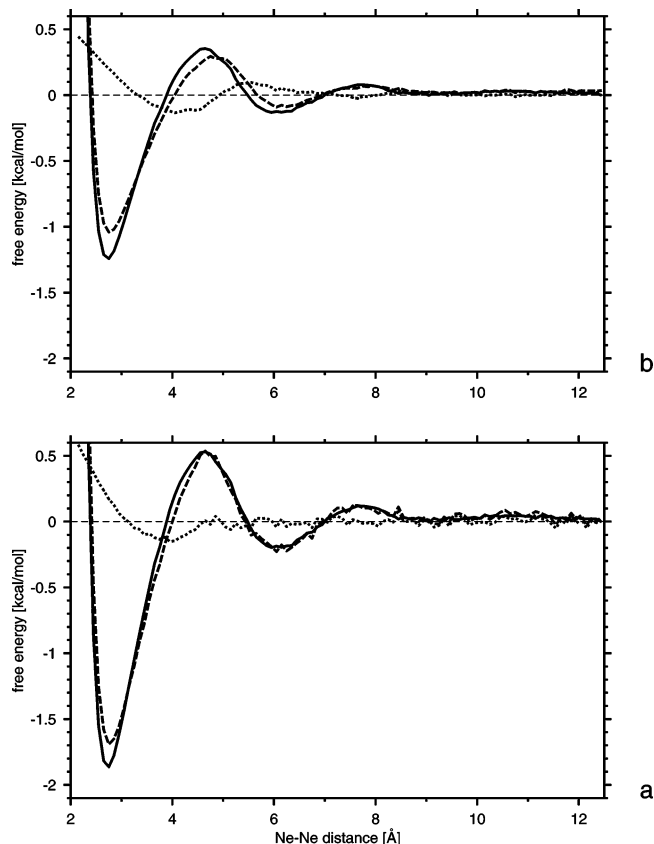


Figure 2. (a) Tripled PMF of the Ne+Ne system (solid line) and the PMF (dashed line) and the respective cooperative contribution to the PMF (dotted line) of the Ne+Ne+Ne system obtained by the particle-insertion method. (b) As in (a), for the 2Ne+Ne system; the doubled PMF of the Ne+Ne system is plotted. The TIP3P water model was used in the calculations.

were taken from the work of Forsman and Jönsson.¹¹ For the 2Ne+Ne system, we first carried out a 40 ns MD simulation of the neon dimer constrained to the contact-minimum distance (2.75 \AA)¹⁰ and then inserted the third neon atom. We collected data from two-dimensional bins in interatomic distances d_{13} and d_{23} with a bin side of 0.1 \AA and centered at $d_{13} = d_{23}$ from 2.0 to 12.5 \AA . For the Ne+Ne+Ne system, we performed a 20 ns MD simulation of one neon atom in water and then inserted the second and third neon atoms successively. We collected data from three-dimensional bins with a side of 0.1 \AA centered at $d_{12} = d_{13} = d_{23}$ from 2 \AA to 10 \AA .

For the Ne+Ne and Ne+Ne+Ne systems, we took 10,000 snapshots from the MD run of a single neon atom in water to perform insertions. For the Ne+Ne system, the second neon atom was inserted 10^5 times. For the Ne+Ne+Ne system, the second neon atom was inserted 2×10^5 times and, if the insertion was successful (i.e., the calculated statistical weight was significant¹⁰), the third neon atom was inserted 500 times, giving a total of 10^8 insertions per snapshot. The calculations on the Ne+Ne system have already been performed in our most recent study.¹⁰ For the 2Ne+Ne system, 20,000 snapshots from the Ne+Ne MD run were taken and the third neon atom was inserted 10^4 times. This gave accuracy comparable to that of the Ne+Ne+Ne and the 2Ne+Ne PMFs, because the bin is three-dimensional for the first and two-dimensional for the second system.

The resulting PMFs of the Ne+Ne+Ne and 2Ne+Ne systems as well as the respective cooperative contributions are shown in Figure 2. It can be seen that the cooperative contributions to the PMF are smaller in magnitude than in the methane system,

in agreement with the results of our earlier study of nonadditivity in hydrophobic interactions.¹² The cooperative contributions have been calculated using the free energy of solvation of a neon atom determined by the particle-insertion method in our recent study,¹⁰ as in the work of Chan and co-workers,^{3,7,8} and not by assuming that they tend to zero at large distances as in our work.^{2,4,5,12} It can be seen that, at the contact distance of the Ne+Ne system (2.65 Å), the cooperative contribution to the PMF of the 2Ne+Ne system is exactly equal to that of the Ne+Ne+Ne system, this being consistent with the thermodynamic argument of Shimizu et al.¹ because the configurations here are equivalent. The cooperative contributions to the PMF of the Ne+Ne+Ne and the 2Ne+Ne systems clearly tend to zero with increasing distance. For the Ne+Ne+Ne system, the zero asymptote is already reached after the first desolvation maximum, and for the 2Ne+Ne system one after the second desolvation maximum. No broad flat region with positive PMF is observed. This behavior is qualitatively the same as observed for the two methane clusters in Figure 4 of ref 4. Thus, there seem to be no long-range effects in the cooperativity of hydrophobic interactions, in contrast to the suggestions of Chan and co-workers.^{3,7,8,13} The flat region in their PMFs appears, therefore, to be a result of insufficient accuracy of the particle-insertion method to determine the PMF of hydrophobic association, as pointed out in our recent paper.¹⁰ Moreover, although the PMF is anticooperative at the contact distance for both the 2Ne+Ne and Ne+Ne+Ne systems, there is significant cooperativity in the desolvation-barrier region, as observed in our studies of methane clusters.^{4,5,12}

The quicker approach to the zero asymptote by the cooperative contribution to the PMF of the Ne+Ne+Ne system compared to that of the 2Ne+Ne system confirms our observation based on the methane-cluster studies⁴ that the asymptote is reached more quickly for uniformly expanding clusters compared to clusters with part of the molecules fixed at a certain distance.⁴ It appears, therefore, that the baseline of the m+m+m cluster (for methane) is reliable (in fact, the asymptote is clearly established after about 7 Å, the position of the solvent-separation minimum). The baseline of the 2m+m system (for methane) is less clear and, therefore, the thermodynamic argument of Moghaddam et al. should be used to set the value of the PMF of the 2m+m system to the PMF of the equivalent configuration of the m+m+m system; this implies a downward shift of the PMF of the 2m+m system presented in Figure 4a of ref 4. We also note that, in view of the results obtained here for neon trimers, using a different cutoff distance for establishing the baseline for different systems is, by no means, the same as setting “double standards” as suggested by the authors of the Comment, but results from different variation of the cooperative contribution to the PMF with distance for different systems.

The above observation partially addresses the remark of ref 1 concerning our setting a larger distance cutoff for the zero asymptote of the $L(2m+m)$ trimer where one of the distances is fixed compared to the $L(m+m+m)$ linear methane trimer. Given the short time allotted to us to reply to the comment of ref 1, we could not manage to perform a study on linear neon trimers to demonstrate this, but we are going to carry out such a study and publish the results in a separate paper. We also note that establishing the cutoff distance exactly is not necessary to judge that the three-body contribution to the PMFs of the linear trimers are anticooperative; they fall off with distance almost monotonically (see Figures 9 and 10 of ref 2) and, because the PMFs and their multibody components must all tend to zero with increasing distance, they can only be positive for the $L(m+m+m)$ and $L(2m+m)$ trimers.

We agree that the PMF of the 2m+m system at the contact minimum might be anticooperative (although, based on the results of our simulations, we cannot conclude that it is anticooperative, because of insufficient accuracy of the PMF in the contact-minimum region) and that the cooperativity of the m+m+m trimer at the contact minimum is small and is likely contained within the errors of the simulation. However, in reality, hydrophobic clusters are formed by assembling more than three separate hydrophobes larger than a methane molecule and not by adding a single methane molecule to a fixed dimer. As we have shown in our earlier study,⁴ already for a uniformly expanding methane tetrahedron, there is cooperativity even in the region to the right of the contact minimum (the accuracy of the PMF of the methane-tetrahedron system is sufficient to establish this fact as can be judged from the convergence plot in Figure 5b of ref 4). For the methane-tetrahedron system, the zero asymptote for the contribution of the cooperative term to the PMF is reached quickly, which excludes the possibility of making a significant error in baseline estimation (Figure 4b in ref 4). The cooperative contribution also increases with the size of the hydrophobic particles involved.¹² Thus, the multibody contribution to the hydrophobic effect seems to be mostly cooperative and not anticooperative.

In summary, the inconsistency between the left and right sides of eq 3 of ref 1 for our m+m+m and 2m+m data⁴ is explainable in terms of increased errors in the PMF in the contact-minimum region and, moreover, is largely reduced when harmonic potentials with the same force constants are applied to restrain all methane–methane distances of the 2m+m system in the umbrella-sampling simulations. Therefore, the problems with baseline estimation are not as severe as stated in ref 1. Moreover, our PI study of the cooperativity of the PMF of neon association demonstrated that the cooperative contribution to the PMF of the Ne+Ne+Ne trimer tends to zero rapidly with the distance and no nonzero baseline is established even in a short distance range. Thus, the zero asymptote of the cooperative contribution to the PMF of the m+m+m system can be established reliably. However, the asymptote is not so clear for the 2m+m system (from the PI study on the 2Ne+Ne system it appears that it is established only in the region of the second solvent-separated minimum, which is quite a large distance for the 2m+m methane system). Therefore, the PMF of the m+m+m system should be used to standardize the PMF of the 2m+m system and not the reverse.

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