

## Reply to "Comment on 'Anti-cooperativity in hydrophobic interactions: A simulation study of spatial dependence of three-body effects and beyond'" [J. Chem. Phys. 116, 2665 (2002)]

Seishi Shimizu and Hue Sun Chan

Department of Biochemistry and Department of Medical Genetics and Microbiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, M5S 1A8, Canada

(Received 8 November 2001; accepted 21 November 2001)

[DOI: 10.1063/1.1434995]

Detailed simulation studies of anti-cooperativity or cooperativity in hydrophobic interactions are relatively new. The Comment by Czaplewski *et al.*<sup>1</sup> on our recent paper<sup>2</sup> and in defense of their previously published work<sup>3</sup> is useful in highlighting potential methodological limitations in the field. We recognize that much remains to be learned about the important question of hydrophobic interactions nonadditivity, and that sustained efforts are needed to overcome numerical and other sources of uncertainties in the simulation results reported thus far.

Figure 1 estimates the numerical uncertainties in the two-methane PMF we used in Ref. 3. By comparing full-simulation and half-simulation results, statistical errors are at most 0.06 kcal/mol except around  $\xi=4.8$  Å in the barrier region where the error can be  $\approx 0.08$  kcal/mol. Incorporating these errors leads to the possibility that the three-methane anti-cooperativity we reported for the contact minimum and an extended regime with  $\xi$  larger than that of the main barrier<sup>2</sup> can be reduced, in some cases coming close to being additive. We agree with Czaplewski *et al.* that the accuracy of the two-methane PMF should be improved in order to provide a more definitive resolution of the anti-cooperativity issue. However, the error estimates in Fig. 1 do not support the contention of Czaplewski *et al.* that the interactions at these positions are cooperative.<sup>1,3</sup>

Czaplewski *et al.* are correct in stating in their points 1 and 2 that simulated two-methane PMF at large spatial separations may contain artifacts (edge effects) as a result of the finite size of the simulation box and periodic boundary conditions. However, we take issue with their assertion that our two-methane PMF *as a whole* "should be shifted upward by about 0.2 kcal/mol." This suggestion clearly fails to take into account the basic principles of the test-particle insertion approach we have used, in which the absolute PMF at each position (spatial separation) is evaluated *independently* by comparing two-methane and single-methane chemical potentials.<sup>2,4</sup> Therefore, the accuracy of PMF values at small separations is independent of that at large separations. Consequently, even if PMF accuracy at large separations may be compromised because of edge effects, the accuracy of our PMF at small separations (including the regime of significant anti-cooperativity between 5 to 9 Å) should not be affected. It follows that the Czaplewski *et al.* assertion that our PMF

should be shifted as a whole because of uncertainties at large separations is not justified.

In fact, for periodic simulation boxes of similar sizes, edge-effect considerations argue in favor of employing test-particle insertion<sup>2,4,5</sup> as a more reliable means to approximate the true zero-PMF baseline at  $\xi \rightarrow \infty$  (effectively in pure water) than the practice of setting PMF to zero at a certain large separation.<sup>1,3</sup> For example, in the Czaplewski *et al.* new simulation of two-methane PMF using a 28 Å periodic box,<sup>1</sup> the methanes can be as close as 15 Å from an image methane in a neighboring periodic cell when the two methanes are separated by 13 Å in the simulation box. Although at this separation direct interactions between methanes and image methanes are cut off, effects of the image methanes can still be felt by the methanes through the intervening water molecules. Therefore, as a model configuration for estimating the zero-PMF baseline, two methanes 13 Å apart in a 28 Å periodic box would appear to be less adequate than test-particle inserting a single methane into a 23 Å periodic box. The single inserted methane is more isolated, and therefore serves

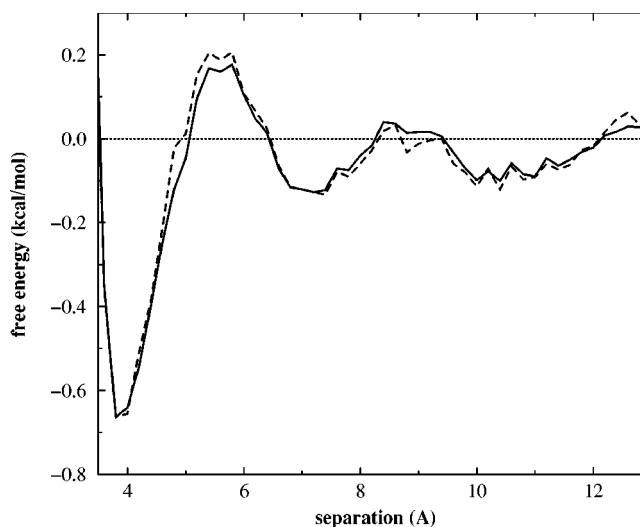


FIG. 1. Estimation of statistical uncertainties in the simulated two-methane PMF at 298 K in the studies of Shimizu and Chan (Ref. 2) PMFs are obtained by a test-particle insertion technique; here  $\xi$  is the distance between the two methanes' centers of mass. Accuracy of the calculation is assessed by comparing the full-simulation result (averaged over  $8.8 \times 10^6$  passes, solid curve) with the half-simulation result (averaged over  $4.4 \times 10^6$  passes, dashed curve).

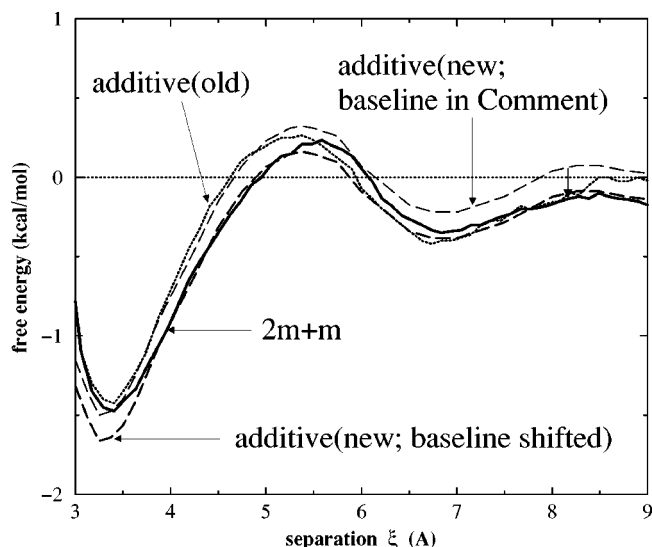


FIG. 2. Addressing the question of hydrophobic cooperativity by comparing the new Czaplewski *et al.* two-methane PMF (Ref. 1) with three-methane PMF results they reported previously (Ref. 3). The abscissa is the distance variable  $\xi$  defined in Refs. 1 and 2 (which is different from that in Ref. 4 and the present Fig. 1). The solid and dotted curves represent, respectively, the 12-window “ $2m+m$ ” three-methane PMF and the 12-window “ $m+m$ ” two-methane PMF in Fig. 8(D) of Czaplewski *et al.* (2000) (Ref. 3). Here two times the free energy given in Fig. 8(D) of Ref. 3 is plotted as a function of the present variable  $\xi$ ; the dotted curve is the additivity-assumed hypothetical three-methane PMF according to the previous (old) Czaplewski *et al.* simulation. The thin dashed curve labeled “additive (new; baseline in the Comment)” is a reproduction of the heavy solid curve in Fig. 2 of the Comment (Ref. 1) whereas the thick dashed curve labeled “additive (new; baseline shifted)” is obtained by shifting this additivity-assumed PMF newly obtained by Czaplewski *et al.* such that it approximately coincides with their previous  $2m+m$  three-methane PMF in the region  $\xi = 7.26\text{--}8.28 \text{ \AA}$ . An anti-cooperative effect between their previous  $2m+m$  result and the “additive (new; baseline shifted)” is seen around the contact minimum.

as a better model for the  $\xi \rightarrow \infty$  situation, because it is separated by a distance of  $23 \text{ \AA}$  from the nearest image methane.

The two-methane PMF reported by Czaplewski *et al.* in the Comment<sup>1</sup> represents a valuable advance. However, this new result casts further doubt on their previous conclusion that the three-methane hydrophobic interactions in question are cooperative.<sup>3</sup> As we have pointed out,<sup>2</sup> a major source of

uncertainty in the method of Czaplewski *et al.*<sup>1,3</sup> is their assumption that “the cooperative term vanishes with distance faster than the PMF itself”<sup>1</sup> as well as the somewhat arbitrary way of implementing this assumption. We conducted a test by following their procedure, using the region of methane–methane separation of  $7.5\text{--}8.5 \text{ \AA}$  (which translates into  $\xi = 7.26\text{--}8.28 \text{ \AA}$  for the  $\phi = 0$  case<sup>2</sup> in Fig. 2) to superimpose their new two-methane PMF (Ref. 1) with their previously published three-methane PMF (see pages 1239 and 1244 of Ref. 3). Figure 2 shows that this exercise leads to an anti-cooperative effect of  $\approx +0.19 \text{ kcal/mol}$  at the contact minimum, contrary to their previous prediction of cooperativity.<sup>3</sup> In our view, this implies one or more of the following: (i) The previous three-methane data of Czaplewski *et al.*<sup>3</sup> is not sufficiently reliable, (ii) their procedure for matching two- and three-methane PMFs is flawed, and (iii) hydrophobic anti-cooperativity is more prevalent than they have previously posited.

Finally, in response to point (3) in the Comment,<sup>1</sup> we emphasize that although prevalent hydrophobic anti-cooperativity is indicated for the three-methane configurations in our previous study,<sup>2</sup> we recognize that cooperativity or anti-cooperativity naturally depends on the nonpolar solutes involved and their spatial configuration. Indeed, we have been careful in specifying that our previous results apply only to methane-size nonpolar solutes at  $25^\circ \text{C}$  and 1 atm. Extensive efforts will be needed to ascertain the sign of hydrophobic nonadditivity in a wide range of solvent conditions for nonpolar solutes of different shapes and sizes. Nevertheless, based on the consideration above with the admittedly limited simulation data available to date, there is stronger support in favor of anti-cooperativity than cooperativity for a significant fraction of the three-methane configurations we have investigated.<sup>2</sup>

<sup>1</sup>C. Czaplewski, S. Rodziewicz-Motowidlo, A. Liwo, D. R. Ripoll, R. J. Wawak, and H. A. Scheraga, *J. Chem. Phys.* **116**, 2665 (2002), preceding paper.

<sup>2</sup>S. Shimizu and H. S. Chan, *J. Chem. Phys.* **115**, 1414 (2001).

<sup>3</sup>C. Czaplewski, S. Rodziewicz-Motowidlo, A. Liwo, D. R. Ripoll, R. J. Wawak, and H. A. Scheraga, *Protein Sci.* **9**, 1235 (2000).

<sup>4</sup>S. Shimizu and H. S. Chan, *J. Chem. Phys.* **113**, 4683 (2000).

<sup>5</sup>S. Shimizu and H. S. Chan, *J. Am. Chem. Soc.* **123**, 2083 (2001).