

Supporting information for:
**Structural Properties of Nonionic Monorhamnolipid Aggregates in
Water Studied by Classical Molecular Dynamics Simulations**

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Free Energy Perturbation Simulations: Perturbation theory is a method of choice for calculating binding free energies.¹ The binding free energy may be estimated computationally using the thermodynamic cycle of **Figure S1**, which involves double annihilation, or double creation of the guest in its free and bound states. More details could be found elsewhere.² Before starting the FEP simulations, the system was first energy minimized for 10000 steps without any constraints. Starting from 0 K, the temperature of the system was raised to 300 K in increments of 1.5 K using MD, simulating the system at each temperature for 2000 fs. At 300 K, the system was equilibrated for 1 ns before starting the simulations. All FEP simulations were carried out in the NPT ensemble. The temperature was maintained at 1 bar using the Langevin piston method. Periodic boundary conditions were applied. All other conditions were same as the classical MD simulations described in the main article.

During the FEP simulations, each perturbation converting (N20+N40) to N60 was divided

into 400 windows. For each window, a total of 2000 MD steps were performed, the first 1000 being considered equilibration and the remaining 1000 used for data collection. FEP simulations were performed for both forward and backward directions. Lennard-Jones interactions were adjusted linearly over the entire range of λ , the coupling parameter that smoothly connects the initial ($\lambda=0$) and the final state ($\lambda=1$) of the perturbed system. Electrostatic interactions were decoupled for disappearing atoms at ($\lambda=0.5$). FEP calculations were analyzed using the VMD plugin,³ and the binding free energy differences and statistical errors were calculated using the Bennett acceptance ration method. Probability distribution and free energy convergence plots for few windows are shown in **Figure S2**. The result of the FEP simulation is presented in **Figure S3**.

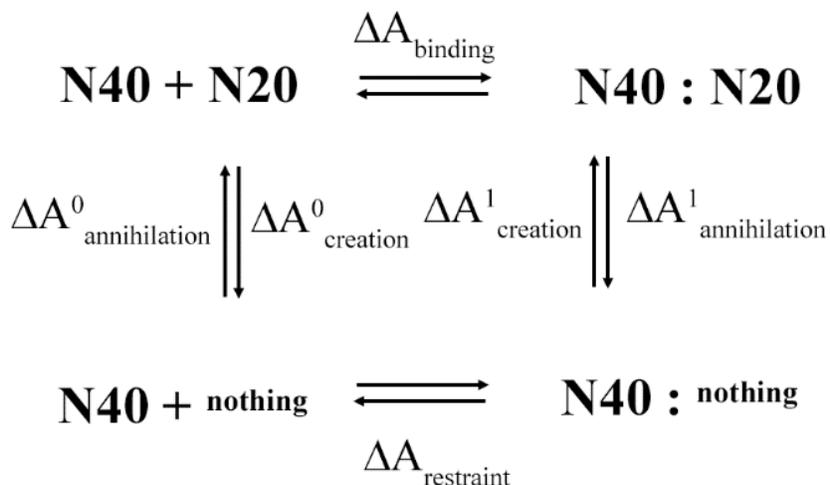
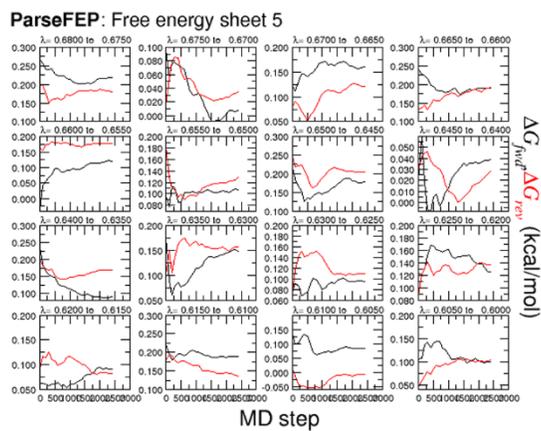


Figure S1. Thermodynamic cycle used to estimate binding free energy, $\Delta A_{\text{binding}}$.

(a)



(b)

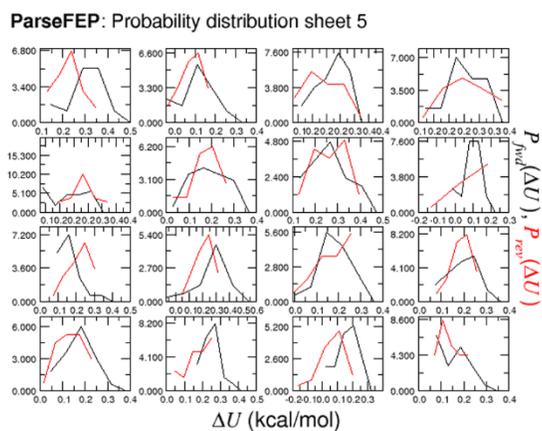


Figure S2. (a) Free energy profile of forward and reverse simulations. (b) Probability distribution of forward and reverse simulations.

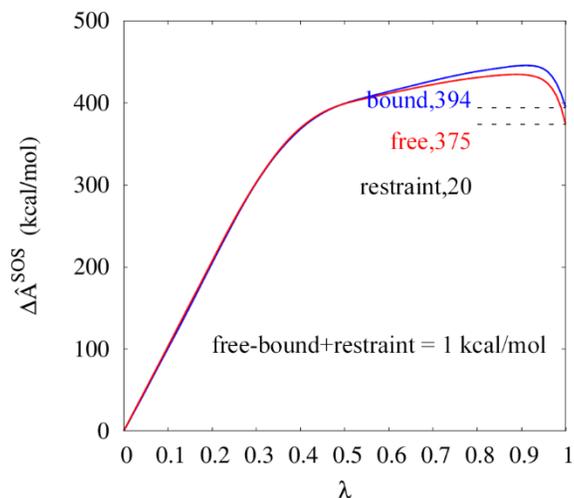


Figure S3. Binding energy for the fusion of aggregate N20 and N40 to form a larger aggregate N60. Free-energy change for the annihilation of the aggregate N20 in free (red) and bound state (blue). The difference at $\lambda = 1$, between the net free energy changes, $\Delta A^0 - \Delta A^1 = 375 - 394 = -19$ kcal/mol, corresponds to the binding free energy, to which the contribution to the confinement of the (N20:N40) *viz.* 20 kcal/mol ought to be added. The resulting binding free energy of 1 kcal/mol is not in favor of the formation of N60 from N40 and N20.

Force field parameters:

The molecule was divided into several groups as given in Figure to generate force field parameters. The force field parameters are given in a separate file.

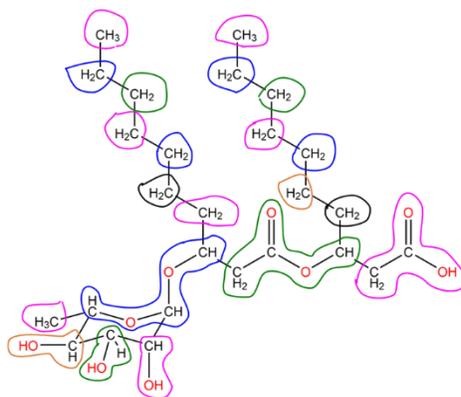


Figure S4. Grouping of Rha-C10-C10 for the purpose of force field parameters generation. Different colors are used to show different groups. Similar colors donot have any relationship.

REFERENCES

1. Jorgensen, W. L.; Thomas, L. L. Perspective on Free-Energy Perturbation Calculations for Chemical Equilibria. *J Chem Theory Comput* **2008**, *4*, 869-876.
2. Pohorille, A.; Jarzynski, C.; Chipot, C. Good Practices in Free-Energy Calculations. *The Journal of Physical Chemistry B* **2010**, *114*, 10235-10253.
3. Liu, P.; Dehez, F.; Cai, W.; Chipot, C. A Toolkit for the Analysis of Free-Energy Perturbation Calculations. *J Chem Theory Comput* **2012**, *8*, 2606-2616.