This is an electronic reprint of the original article.
This reprint may differ from the original in pagination and typographic detail.

Vierros, Sampsa; Sammalkorpi, Maria

Phosphatidylcholine reverse micelles on the wrong track in molecular dynamics simulations of phospholipids in an organic solvent

Published in:
Journal of Chemical Physics

DOI:
[10.1063/1.4914022](https://doi.org/10.1063/1.4914022)

Published: 01/01/2015

Document Version
Publisher's PDF, also known as Version of record

Published under the following license:
Unspecified

Please cite the original version:
Vierros, S., & Sammalkorpi, M. (2015). Phosphatidylcholine reverse micelles on the wrong track in molecular dynamics simulations of phospholipids in an organic solvent. *Journal of Chemical Physics*, 142(9).
<https://doi.org/10.1063/1.4914022>

Phosphatidylcholine reverse micelles on the wrong track in molecular dynamics simulations of phospholipids in an organic solvent

S. Vierros and M. Sammakorpi

Department of Chemistry, Aalto University, P.O. Box 16100, 00076 Aalto, Finland

(Received 5 December 2014; accepted 23 February 2015; published online 6 March 2015)

Here, we examine a well-characterized model system of phospholipids in cyclohexane via molecular dynamics simulations using a force field known for reproducing both phospholipid behavior in water and cyclohexane bulk properties to a high accuracy, CHARMM36, with the aim of evaluating the transferability of a force field parametrization from an aqueous environment to an organic solvent. We compare the resulting reverse micelles with their expected experimental shape and size, and find the model struggles with reproducing basic, experimentally known reverse micellar structural characteristics for common phosphatidylcholine lipids such as 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC), 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), and 1,2-dilinoleoyl-sn-glycero-3-phosphatidylcholine (DLPC) in cyclohexane solvent. We find evidence that the deviation from the experimental behavior originates from an underestimation of the lipid tail-cyclohexane interaction in the model. We compensate for this, obtain reverse micellar structures within the experimentally expected range, and characterize these structurally in molecular detail. Our findings indicate extra caution and verification of model applicability is warranted in simulational studies employing standard biomolecular models outside the usual aqueous environment. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4914022>]

I. INTRODUCTION

Leading biomolecular force fields are dominantly designed for studying molecular systems in aqueous environment and their parametrizations fine-tuned to reproduce the aqueous assembly characteristics. For lipid force fields, the focus in force field parametrization is on accurate reproduction of bilayer properties, see, e.g., Refs. 1–4 for recent reviews. Employing the same models also for describing different molecular environments, for example, organic solutions and non-aqueous mixtures, is attractive as the force fields typically contain parameters for all the components. However, the transferability of the parameters and evaluation of the overall model performance in these different environments has received very little attention both in parameter design and in simulational studies. We examine here this important topic on an experimentally well-characterized phosphatidylcholine (PC) lipid/cyclohexane organogel system.

The ternary mixture of PC lipids, water, and oil (including cyclohexane) is one of the most studied organogel forming systems. In most cases, lecithin which is a mixture of PC lipids (exact composition depends on the source of origin) has been studied^{5–10} but also organogels of single PC lipids such as 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC)¹⁰ have been examined. Regardless of the exact PC lipid composition, PC organogels are thermodynamically stable, optically clear, and isotropic solutions¹¹ of entangled wormlike reverse micelles (RMs) with similar viscosity characteristics. In them, water acts as the gelation agent and gelation typically occurs at a certain water-to-lipid ratio, w_0 , range that is dependent on the chemical nature of the organic media.¹¹ Increase in temperature can move the gelation range to a higher

water-to-lipid ratio or prevent it altogether.^{10–12} Furthermore, the stability region of the organogels is influenced also by modifications in the structure of the phospholipid tails.¹⁰ In particular in cyclohexane, the addition of water triggers uniaxial growth of the initially spherical RMs^{13,14} into disconnected wormlike RMs.^{5,7} Maximum viscosity is attained around $w_0 = 11$ followed by a second shape transition to water-in-oil (w/o) microemulsion droplets at $w_0 = 14$.⁵ Maximum water uptake in cyclohexane is around $w_0 = 24$; at higher water-to-lipid ratios the system phase separates into a microemulsion phase and an excess water phase.⁵

The organogel transition and the viscous characteristics of the solution, as well as, the size change in the amphiphilic aggregates can be characterized experimentally very well with, e.g., rheological measurements,¹⁰ NMR self-diffusion,⁵ and scattering techniques including light,⁸ neutron,^{7,9} and X-ray⁶ scattering. This provides experimental comparison data for simulations at the level of basic structural characteristics. However, at the level of molecular interactions, experimental characterization of these non-crystalline, highly dynamic soft materials systems is challenging and important open questions remain regarding the role of solvent, gelating agent, and additives in controlling the gelation transition. This makes these fascinating systems also for simulational characterization which enables the microstructure and dynamics of the RMs to be probed in detail not achievable through present experimental methods. Although gaining popularity, so far the computational investigation of RM systems has been restricted to only a handful of systems. In particular, ternary systems composed of water, supercritical carbon dioxide, and acid functionalized perfluoroalkane or perfluoropolyether surfactants,^{15–20} as well as, systems composed of water,

apolar solvent (oil) and Aerosol OT (AOT)^{21–31} have been characterized. Additionally, alkyl-PEG³² and malonamide derived surfactant³³ systems have been studied by computer simulations. Most studies so far utilize united-atom or coarse grained description for either the hydrophobic tails, the solvent, or both to reduce the computational load but also some all-atom simulations exist.^{22–24,32}

A well-founded, but often omitted, question in studying RM systems is, whether the simulation models parametrized for aqueous environments typically employed for studying also these systems are applicable for describing molecular systems in which an organic solvent replaces water as the solvating species. The aspect of force field accuracy in description has been given very little attention in the previous computational RMs studies. To our knowledge only Martinez *et al.* have discussed the issue in more detail.²² For the AOT RM system they studied, however, detailed structural data are not available and comparison to experiments was made on a qualitative level. In the present work, we evaluate the performance of one of the most common biomolecular force fields, the CHARMM force field, in describing an experimentally well-characterized PC lipid/cyclohexane system and evaluate under what conditions the model can be employed to describe such systems. After this, we characterize the structure of PC RMs in cyclohexane. The PC lipid/cyclohexane system, and the organogel transition there, is an ideal model system for assessing model accuracy as PC lipids, and especially DPPC, are thoroughly parametrized in biomolecular force fields, and the force field behavior on them detailly evaluated in aqueous environment, see, e.g., Refs. 1–4. Cyclohexane is a common organic solvent with no specific interactions such as hydrogen bonding capability which would complicate the interactions. Furthermore, the gelating agent in these systems is water which is an integral part of all biomolecular force fields. The system is chosen because the exact structure of PC RMs in cyclohexane, as well as, the phase behavior, is known experimentally to a high degree of accuracy; the force field is chosen because it reproduces PC lipid characteristics excellently in aqueous environment, the lipids are parametrized also in low hydration, and a consistent cyclohexane model exists. To our knowledge, the study is the first atomistic detail characterization of a reverse micellar system in which the employed simulation model is explicitly evaluated and validated against experimental structural data of the system. The study also serves to benchmark the CHARMM36 lipid parametrization under conditions of low lipid hydration and non-planar assembly geometry.

II. METHODS

The GROMACS 4.6 simulation package^{34,35} was used for the simulations. The intermolecular interactions were modelled using the empirical CHARMM force field.³⁶ Parameters for the lipids are those of the CHARMM36 force field,³⁷ which is the most recent version of the CHARMM lipid force field. Cyclohexane parameters were taken from the compatible CHARMM carbohydrate force field of Ref. 38. Water was modelled using CHARMM's modified version

of TIP3P water model,³⁶ which has Lennard-Jones (LJ) interactions also for the hydrogen atoms. The original TIP3P water model³⁹ restricts LJ interactions to the oxygen. We chose these particular models because the lipid model has been validated also at low hydration with the water model,³⁷ verified parameters are available for both saturated and unsaturated lipids,³⁷ and force field consistent parameters for cyclohexane exist.³⁸ Furthermore, the accuracy of the lipid model compares favourably to other commonly used lipid force fields in a recent review,¹ or is among the best performing in several others^{2,4} with specifically the deuterium order parameters most accurately reproduced.⁴ In total, the force field is a very good candidate for good performance in describing the PC/cyclohexane system.

Periodic boundary conditions were applied in all three dimensions and long-range electrostatic interactions were calculated using the PME method,⁴⁰ with a fourth-order smoothing spline. A real space cut-off of 1.2 nm and reciprocal space grid of 0.12 nm were used. Lennard-Jones potentials were smoothly shifted to zero between 0.8 nm and 1.2 nm. Equations of motion were integrated with the leap-frog algorithm using a time step of 2 fs and bonds involving hydrogen were constrained to their equilibrium distance using LINCS⁴¹ and SETTLE⁴² algorithms. A stochastic velocity rescaling thermostat⁴³ was used with reference temperature of 325 K and a relaxation time constant of 0.5 ps. Pressure was kept constant using Parinello-Rahman barostat⁴⁴ with a time constant of 4.0 ps. Pressure control was applied isotropically with compressibility set to $8.2 \times 10^{-5} \text{ bar}^{-1}$.

In describing the PC lipid RMs in cyclohexane, the basic starting point is that the lipid aggregate has the accurate structural characteristics. For organogel forming PCs, this corresponds to the formation of wormlike RMs which have cylindrical form. In general, phospholipids solvated by oil have an effectively inverted cone shape which packs as RMs. Increased hydration of the PC headgroup increases its size making the apparent shape of the lipid more cylindrical. This promotes an increase of the aggregation number of the RM which first results in elongation of the micelle into a cylinder, and at more cylindrical lipid shapes, bicellar or lamellar assembly. On the other hand, an increase in the effective size of the tails typically caused by increasing temperature (introduction of disorder by gauche defects in the acyl chains) has an opposite effect. Gelation occurs at a range where long, wormlike micelles which entangle in the solution form; the exact size distribution of the aggregates in the system is naturally determined by the interplay of entropy and the packing considerations.

First, to map the simulation model performance in terms of packing parameter for lipids in cyclohexane, reverse micelles composed of 5 different PC lipids with symmetric fatty acid tails of 16–18 carbon atoms in length and 0–4 *cis* double bonds per tail were modelled. The selected lipids were DPPC, 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), 1,2-dilinoleoyl-sn-glycero-3-phosphatidylcholine (DLPC), 1,2-digammalinolenoyl-sn-glycero-3-phosphatidylcholine (DGPC) and 1,2-distearidonoyl-sn-glycero-3-phosphatidylcholine (DSPC). Figure 1 shows the respective structures and their expected aggregation behavior in cyclohexane. Of these

FIG. 1. Structures of the simulated PC lipids and their expected aggregate radius and length in cyclohexane. The chain unsaturation increases systematically from top to bottom.

DPPC, DOPC and DLPC form stable organogels in progressively lower temperatures¹⁰ while, to our knowledge, the gelation of DGPC and DSPC organosols has not been studied experimentally. Our simulation temperature $T = 325$ K is chosen from the middle of the stability region of DPPC organogels (313–333 K)¹⁰ because DPPC is a common model surfactant and its parametrization in the simulational model extensively tested in aqueous environment. DOPC and DLPC form organogels in cyclohexane at lower temperatures in experiments.¹⁰ In our simulations, however, we maintain the temperature constant but increase the *cis* double bonds count. This is to enable us to observe systematically how the size and form of the reverse micelle and the solvent penetration depend on lipid chain disorder.

We chose $N_{agg} = 78$ as the primary studied aggregation number as this corresponds to a clearly elongated micelle (as opposed to spherical) and is computationally a feasible system size in all-atom detail. Water-to-lipid ratio was selected to be $w_0 = 7$ which is located well in the range where DPPC (and lecithin) form wormlike RMs in cyclohexane.^{5,7,8,10} The sensitivity of the results to the choice of aggregation number and water-to-lipid ratio was examined throughout by simulations of different size aggregates and also both smaller ($w_0 = 5$) and larger ($w_0 = 11$) water-to-lipid ratios. The studied water-to-lipid ratios fall all in the DPPC (and lecithin) gelling regime corresponding to wormlike RMs and experimental comparison data in this data range also exist, see, e.g., Refs. 6, 9, and 45. The RMs formed of the different PC lipids were simulated between 60 ns and 100 ns depending on the system, see Table I.

To speed up the equilibration, the RMs were preassembled rather than allowed to self-assemble from random initial configuration. Initial configurations for the RMs were constructed by first placing N_w water molecules inside a spherical domain with radius R_w . The radius R_w was calculated assuming that each water molecule occupies a volume of $30 \text{ \AA}^3$. Next, the lipids were placed with their heads on the

TABLE I. Studied systems and their abbreviations. The table presents the lipids, water-to-lipid ratio w_0 , aggregation number N_{agg} , number of water N_w and cyclohexane N_{CHX} molecules, simulation duration t , and the simulation box edge length L in each simulation. The simulation box is cubic, $V = L^3$.

System	Lipid	w_0	N_{agg}	N_w	N_{CHX}	t (ns)	L (nm)
	DPPC	7	78	546	6616	64	11.4
	DOPC	7	78	546	6522	63	11.2
	DLPC	7	78	546	6925	63	11.5
	DGPC	7	78	546	6557	64	11.3
RM78	DSPC	7	78	546	6561	100	11.3
RM57	DSPC	7	57	399	6765	87	11.2
RM70	DSPC	5	70	350	6572	100	11.0
RM94	DSPC	11	94	1034	8118	100	12.1
RM129	DSPC	11	129	1419	9297	60	12.7
	DPPC	11	129	1419	9297	93	12.7
	DPPC	7	78	546	6616	80	11.4
	DPPC	7	94	658	8173	58	12.1

surface of the water droplet and tails pointing outward: the lipids were positioned so that the choline nitrogen atom was less than $R_w + 0.4$ nm and the last carbon atom in both fatty acid tails more than $R_w + 2.9$ nm from the geometric center of the water droplet. To satisfy the specified constraints and to generate molecular packings without overlap, the Packmol software package⁴⁶ was used. These configurations were then put under initial relaxation by a 200 ps NVT ensemble simulation in vacuum. To preserve the initial spherical shape of the RM while still allowing the structure to relax, the water molecules restrained to their initial coordinates with loose springs (force constant $100 \text{ kJ nm}^{-2} \text{ mol}^{-1}$). Next, the system was solvated with bulk cyclohexane. Steepest descent method was utilized to energy minimize the initial configurations. After this, the actual production simulations were performed in NPT ensemble.

To root the origins of the observed simulation model behavior, also bulk solvent simulations on cyclohexane and hexadecane mixtures of varying composition were conducted. Hexadecane here shares the parameters of the PC lipid hydrocarbon tails in CHARMM36 lipid description³⁷ while two cyclohexane models were compared. One is by Guvench *et al.*³⁸ (based on alkane parameters by Vorobyov *et al.*⁴⁷) and the other has the aliphatic non-bonded parameters replaced by those of Yin and MacKerell⁴⁸ corresponding to reverting the non-bonded aliphatic parameters back to those of CHARMM27. Additionally, the cyclohexane by Vorobyov *et al.*⁴⁷ was tested but as the models differ by only angle and dihedral parameters, no improvement over Guvench *et al.*³⁸ model was found. Initial configurations for these systems were prepared by first randomly placing the hexadecane and cyclohexane molecules inside a cubic box ($10 \times 10 \times 10 \text{ nm}^3$). Then, the box vectors and the coordinates were repeatedly scaled by the factor of 0.98 and the resulting configuration energy minimized. This was repeated until the size of the box was significantly compressed to size $(\sim 3.7 \text{ nm})^3$. This ensured no substantial voids were left in the system. After this, the system was allowed to expand in the NPT ensemble (barostat time constant $\tau_p = 40$ ps) until the volume reached $\sim 125 \text{ nm}^3$ corresponding to a density close to the experimental value.

Finally, actual production runs were performed in the NPT ensemble with identical parameters to those used in the RM simulations. Simulations were run for 20–40 ns.

System coordinates and energies were recorded every 10 ps. VMD was used to visualize the trajectories.⁴⁹ In the data analysis, the first 25 ns of the RM simulations (50 ns for the $w_0 = 5$ system in which the relaxation is slower) and 2 ns of the cyclohexane-hexadecane mixture simulations were counted as initial relaxation period and were therefore disregarded.

In analysing the micelle forms resulting from the different PCs quantitatively, we follow the example set by previous computational studies of RMs^{22,23,29} and calculate the semi-axes lengths a , b , and c from the principal moments of inertia by assuming that the RMs were ellipsoids of uniform density. For ellipsoids, the relations between semi-axes and principal moments of inertia are given by equations

$$\begin{aligned} I_1 &= \frac{M}{5} (b^2 + c^2), \\ I_2 &= \frac{M}{5} (c^2 + a^2), \\ I_3 &= \frac{M}{5} (a^2 + b^2), \end{aligned} \quad (1)$$

where I_1 , I_2 , and I_3 are the principal moments of inertia and M the mass of the RM (lipids and water). Eccentricity, e , is given by

$$e = \sqrt{1 - \frac{c^2}{a^2}}, \quad (2)$$

where $e = 0$ for spherical shapes and $e \rightarrow 1$ for disc-like and rod-like shapes. To distinguish between disc-like and rod-like RMs, we also calculated a so called rod-to-disc parameter. For disc-like particles $a \approx b > c$ and for rod-like particles $a > b \approx c$. Rod-to-disc parameter s is given by

$$s = \frac{b - c}{a - c}, \quad (3)$$

where $s = 0$ for a rod-like shape and $s = 1$ for disc-like shape. We point out that these equations require $a > b > c$.

In analysing whether the dominantly cylindrical DSPC aggregates in cyclohexane match overall PC lipid aggregates in cyclohexane in experiments, instead of ellipsoids, we treat the RMs as cylinders of constant density. This is to obtain a better match with the actual structural dimensions. Then the relation between principal moments of inertia and the cross-sectional radius R and length H of the cylinder is given by

$$\begin{aligned} I_1 &= \frac{1}{12}MH^2 + \frac{1}{4}MR^2, \\ I_2 &= \frac{1}{12}MH^2 + \frac{1}{4}MR^2, \\ I_3 &= \frac{1}{2}MR^2. \end{aligned} \quad (4)$$

Bulk solvent parameterization performance was measured by comparing densities and heats of vaporization calculated from our simulations to experimental values reported in the literature. The heat of vaporization H_m was calculated from

the simulations according to the formula⁵⁰

$$H_m = (E_{pot}(g) + k_bT) - E_{pot}(l), \quad (5)$$

where $E_{pot}(l)$ and $E_{pot}(g)$ are the average potential energies per molecule in liquid phase and gas phase, respectively. For the gas phase potential energy, a single solvent molecule was simulated in vacuum for 50 ns. In these calculations, all interactions were included. To characterize the mixing behaviour of cyclohexane and hexadecane, excess molar volumes were calculated and compared to experimental values. Excess molar volume V^E of a cyclohexane-hexadecane mixture is given by

$$V^E = x_1M_1(\rho_{mix}^{-1} - \rho_1^{-1}) + x_2M_2(\rho_{mix}^{-1} - \rho_2^{-1}), \quad (6)$$

where x_i is the molar fraction, M_i the molar mass, ρ_i the density of pure solvent i , and ρ_{mix} is the density of a binary mixture composed of the two solvents (cyclohexane and hexadecane).

III. RESULTS

In the simulations, the shape of the RMs composed of the different PC lipids evolved from the initially spherical aggregate into distinctly disc- or rod-like, or ended up into fluctuating intermediary forms of these. Such fluctuation reveals the disc- and rod-like structures have only a small free energy difference. Time-series of the shape parameters and snapshots are presented in the supplementary material.⁵¹ Initially, the RMs often relaxed towards a different structure than what stabilized as its final shape indicating relatively long relaxation periods are needed to equilibrate the form; in our simulations at water-to-lipid ratios of $w_0 = 7$ or above, 25 ns appeared by all measures sufficient to stabilize the form, or the fluctuations. To characterize the shape and dimensions of each RM, we calculated time-averaged semi-axes lengths, eccentricities (Eq. (2)), and rod-to-disc parameters (Eq. (3)) from the remainder of the simulation trajectories. Figure 2 shows the average values of eccentricities and rod-to-disc parameters as a function of fatty acid tail unsaturation. The figure shows that all RMs deviate from spherical form (eccentricity $e = 0$) and that the eccentricity increases as a function of tail unsaturation. Furthermore, the rod-to-disc parameter shows a transition from disc-like RMs (formed by DPPC and DOPC) to a rod-like RM (formed by DSPC) with DLPC and DGPC forming intermediary shapes. Snapshots of corresponding to the average values of the rod-to-disc parameters of DPPC, DLPC, and DSPC are presented in Figure 3. The intermediate lipids behave analogously as indicated by Figure 2. Also the snapshot of Figure 3 show a clear disc-to-rod transition as the lipid tail unsaturation increases. Further examination of the trajectories reveals all RMs, except perhaps DSPC, exhibit significant fluctuations in their shape: DPPC and DOPC fluctuate around a disk-like shape, DSPC, and DGPC around a rod-like shape, and DLPC between these two extremes, see supplementary material for time-dependent data.⁵¹

Experimentally, DPPC/cyclohexane solution is in the organogel state in conditions corresponding to the simulations.¹⁰ This means that the microstructure of the RMs DPPC

FIG. 2. Average eccentricities (black squares) and rod-to-disc parameters (blue circles) for the different RMs. The unsaturation of the fatty acid tails increases from left to right. Standard deviation of the data set is used as an error estimate.

forms should be rod-like – contrary to the disc-like shape predicted by our DPPC simulation. Of course, the fixed aggregation number of the simulated RM could bias the results, and indeed, both theoretical⁵² and computational studies⁵³ indicate that for small micelles with a fixed aggregation number disc-like shape may be preferred even though for larger aggregates cylindrical shape is the most stable form. To test this, we simulated larger DPPC RMs with aggregation numbers 94 ($w_0 = 7$) and 129 ($w_0 = 11$). The former adopted disk-like structure while the latter relaxed into a shape that can be characterized as tri-axial ellipsoid (see supplementary material for time-series of the shape parameters and snapshots⁵¹). The absence of cylindrical structure with none of the studied water-to-lipid ratios even with the larger micelles provides confidence that the observed flat structures result from an imbalance in the force field rather from size effects or an insufficient water-to-lipid ratio. However, even though these simulations are extensive in size for atomistic molecular simulations, the micelles are still relatively small. Therefore,

FIG. 3. Simulation snapshots corresponding to DPPC, DLPC, and DSPC reverse micelles with the average value of rod-to-disc parameter of each simulation. The same micelles are viewed perpendicular (top row) and parallel to their longest axis (bottom row). Water is shown as light blue and phosphorylcholine moieties as red space-fill. The rest of the lipid is black. Cyclohexane solvent is omitted in the visualization for clarity. Figures are in scale.

we can not completely rule out the possibility of finite-size effects influencing the structure.

For the lipids composed of unsaturated fatty acids, the situation is more complicated. Experimentally, DOPC and DLPC should be in the organosol state, since the simulation temperature (325 K) is above the threshold temperature where their organogels collapse back to organosol state. This means the RMs in the solutions are either shorter wormlike RMs or small spherical RMs. Due to the fixed number of lipids, initially single aggregate, and insufficient simulation time for micellar fission, we cannot differentiate between the two types. In principle, the RMs could be elongating to initiate a fission event⁵⁴ (disc-to-sphere transition) but we consider much more likely that the elongation results from a disc-to-rod transition because the former is in accordance with the theoretical predictions of packing parameter. Assuming, we see a disc-to-rod transition with increasing unsaturation of the lipid tails, we conclude the simulation model seems to behave systematically. However, the effective shape of the lipid in cyclohexane is predicted off by the model as DPPC, which is supposed to be in the organogel regime, forms clearly disc-like micelles, and only the most unsaturated lipid in this study, DSPC, forms clearly rod-like aggregates.

Since the shape of a RM and the effective sizes of fatty acid tails and headgroups in the solution are all coupled, pin pointing the exact cause of the incorrect microstructure of DPPC RM is difficult. For the same reason, the evolution of the RM shape as a function of fatty acid tail unsaturation serves only to establish that the simulation model behaves systematically. However, the parametrization history of the CHARMM lipid force field gives valuable clues to what could be the origins of the lipid shape being off. While the lipid model has undergone numerous refinements since CHARMM27,⁵⁵ the current version CHARMM36³⁷ retains non-bonded parameters by Yin and MacKerell⁴⁸ for the hydrocarbon tails. These parameters were fitted against short-chain alkanes and consequently slightly overestimate heat of vaporization of bulk hexadecane while underestimating density.⁵⁵ Priorly, in other all-atom force fields besides CHARMM, hydrocarbon chain parameters have been a common source of simulation artefacts in bilayer and bulk alkane simulations which are sensitive to exact balance between individual parameter values. For instance, alkane parameters of all-atom Optimized Potentials for Liquid Simulations (OPLS-aa) force field significantly overestimated densities of long-chain alkanes and yielded too high transition temperatures for lipid bilayers.⁵⁶ Additionally, initial versions of General Amber Force Field (GAFF) and Amber lipid force fields were also unable to reproduce the experimental area per lipid in a tensionless ensemble.^{57–59} In these two closely related parametrizations, the problem was later remedied by refitting torsional and LJ parameters of the hydrocarbon tails^{60,61} against bulk pentadecane.

In light of the problems experienced by Amber and OPLS lipid models and taking into account the fatty acid tail LJ interactions were not reparametrized for CHARMM36, we concentrated our attention to the hydrocarbon LJ parameters in examining the cause of the RMs adopting a disc-like shape in the simulations. To narrow down the exact interaction responsible for the incorrect microstructure, we first performed

TABLE II. Pure cyclohexane and hexadecane solvent density ρ and heat of vaporization H_m calculated based on simulations at 325 K. The error percentages are relative errors to experimental values reported in literature. N is the simulation system size.

Solvent	N molecules	ρ (kg m ⁻³)	Error (%)	H_m (kJ mol ⁻¹)	Error (%)
Hexadecane	256	729.96 ^a	-2.4	71.66 ^b	-6.6
Cyclohexane ^c	920	721.91 ^d	-3.5	32.19 ^e	-0.2
Cyclohexane ^f	920	685.96	-8.3	27.37	-15.1

^aExperimental value 751.29 kg m⁻³ interpolated from Ref. 62.

^bExperimental value 76.74 kJ mol⁻¹ calculated from vapor pressures from Ref. 63.

^cNon-bonded parameters of Vorobyov *et al.*⁴⁷

^dExperimental value 748.29 kg m⁻³ extrapolated from Ref. 64.

^eExperimental value 32.24 kJ mol⁻¹ calculated from vapor pressures from Ref. 63.

^fNon-bonded parameters of Yin and MacKerell.⁴⁸

simulations of pure cyclohexane and hexadecane, see Table II. Here, hexadecane shares the hydrocarbon parameters of the lipid hydrocarbon tails. With the set of simulation parameters used in this paper, the heat of vaporization and density of pure cyclohexane were within 0.2% and 3.5%, respectively, of experimental values. The density of bulk hexadecane, on the other hand, was underestimated by 2.4% and heat of vaporization by 6.6%. For cyclohexane with non-bonded parameters originating from the work of Yin and MacKerell,⁴⁸ the deviations are larger. For heat of vaporization, the error percentages are guidelines of magnitude at best as there is considerable scatter in the reported experimental values.

Since neither the cyclohexane nor the hexadecane model with parameters used for the RM simulations showed significantly more deviation from the experimental values than the other, we next examined the balance of the tail-solvent interactions. We examined binary mixtures of hexadecane and cyclohexane by simulations and compared the calculated excess molar volumes (Eq. (6)) to experimental values, see Figure 4 and Table III. The data show the shape of the curve is well reproduced for the parameters used in the RM simulations but the value may be overestimated significantly (by up to 110%). Overestimation of the magnitude of positive excess molar volumes indicates the tail-solvent interaction is too weak in the model. Consequently, cyclohexane prefers cyclohexane interaction to interaction with the lipid tails

which leads to lack of solvent penetration between the tails. As a result, the lipid molecule tails become too compact in structure and pack into a bilayer-like morphology. On the other hand, reverting the non-bonded aliphatic parameters back to those of CHARMM27 (originating from Yin and MacKerell⁴⁸) for cyclohexane, leads to underestimation of the excess molar volume. Indeed, in the updated linear and cyclic ethers non-bonded parameters by Vorobyov *et al.*⁴⁷ used for the cyclohexane, the LJ well-depth of aliphatic hydrogens was increased in comparison to hydrogen parameters by Yin and MacKerell.⁴⁸ This makes cyclohexane-cyclohexane interaction favourable in comparison to cyclohexane-tail interactions in CHARMM36.

As the excess molar volume curves show opposite behavior for the two cyclohexane models, we tested the DPPC RM behavior also with cyclohexane with the Yin and MacKerell non-bonded parameters.⁴⁸ This shifted the form and fluctuations of the DPPC RM to be comparable to DGPC RM, that is, to a rod-like RM with occasional fluctuations to a disk-like shape. This demonstrates that the small change in solvent parameters has (1) a measurable effect on the RM shape and (2) moves the shape and fluctuations to the right direction, i.e., towards rod-like structure. However, the rod-like shape does not stabilize in the simulation which could result from the small size of the aggregate; a larger RM studied by this model might adopt a stable cylindrical form.

TABLE III. Densities (ρ_{mix}) and excess molar volumes (V^E) at 325 K calculated from different cyclohexane-hexadecane mixture simulations using non-bonded parameters of Vorobyov *et al.*⁴⁷ or Yin and MacKerell.⁴⁸ Experimental comparison data is from Ref. 65. x_1 is the cyclohexane molar fraction and N_{CHX} and N_{HD} the numbers of cyclohexane and hexadecane molecules in the system, respectively.

x_1	N_{CHX}	N_{HD}	Vorobyov <i>et al.</i> ^{47,a}		Yin and MacKerell ^{48,b}		Expt. Ref. 65
			ρ_{mix} (kg m ⁻³)	V^E (cm ³ mol ⁻¹)	ρ_{mix} (kg m ⁻³)	V^E (cm ³ mol ⁻¹)	V^E (cm ³ mol ⁻¹)
0.10	26	234	...	...	727.69 ± 0.12	0.17	0.13
0.25	72	217	727.27 ± 0.71	0.65	724.54 ± 0.12	0.11	0.30
0.35	109	203	...	...	721.71 ± 0.13	0.18	0.39
0.50	178	178	724.40 ± 0.47	0.99	717.04 ± 0.17	0.14	0.49
0.63	253	149	722.53 ± 0.67	1.12	711.67 ± 0.14	0.17	0.53
0.75	344	115	721.04 ± 0.18	1.07	705.77 ± 0.17	0.08	0.50
0.90	502	56	720.42 ± 0.32	0.63	695.13 ± 0.17	0.10	0.30

^aSimulations run for 20 ns.

^bSimulations run for 40 ns.

FIG. 4. Excess molar volume (V^E) of cyclohexane/hexadecane mixture as a function of cyclohexane molar fraction. Solid lines represent the second (this work) and fourth (experimental) order Redlich-Kister equations ($V^E = x_1 x_2 \sum c_i (x_1 - x_2)^i$) fit to the data points. Experimental data is from Ref. 65.

We must consider also the possibility that headgroup parameters could add to inducing the incorrect bilayer-like structure in the RM simulations here. After all, the disk-like structure can be equally well due to a too large effective size of the headgroup as it can be due to too small effective size of the tails. The PC lipid headgroup parametrization is, however, extremely complex, and to test it properly we would need NMR data of the lipid heads under low hydration—we are not aware of such data existing. However, a recent study shows that CHARMM36 lipid parameters perform well in the case of high-curvature inverse hexagonal phases.⁶⁶ Additionally, Piggot *et al.*⁴ report CHARMM36 to be the only one of the force fields they examined to reproduce correctly also the C2 carbon atom deuterium order parameters for both DPPC and POPC (palmitoyllecithin) lipids. This means the C2 carbon atom which is facing the head group could be assumed to sample the same conformations as the lipids in the NMR experiments. These findings in particular, and the overall decent behavior of this model in describing hydrated bilayers in recent reviews, see, e.g., Refs. 1–4, lead us to believe the lipid-lipid and lipid-water interactions are relatively well balanced in the model. Therefore, as the lipid tail-cyclohexane interactions show a clear imbalance, we conclude the most likely cause for the reverse micelles adopting the wrong shape in the simulations is due to the lipid tail-cyclohexane interactions. We stress, however, that the influence of other force-field terms cannot be completely ruled out.

Having thus concluded the simulational model biases the lipid tails in cyclohexane into a too ordered state likely due to an underestimation of cyclohexane-lipid tail interaction

strength, we next characterize the DSPC reverse micelles which adopt the correct elongated form and compare the structure to what is known experimentally about PC lipid reverse micelles in cyclohexane. As the viscosity behaviour, and hence the structure of the RMs, is very similar in all experimentally studied cyclohexane/PC organogels provided that the temperature is not outside the organogel stability region,¹⁰ very similar internal structure and dynamics (which dominate the viscosity behavior) are attained with a range of PC lipids and temperatures. Hence, if the overall structure of the DSPC RMs in our simulations matches with the experiments, the findings on them should be representative of PC organogels even though the simulational model needs to be corrected for the underestimated lipid tail-cyclohexane mixing.

First, the sensitivity of the DSPC RM shape to the aggregation number and hydration was probed. Figure 5 presents snapshots corresponding to the final configuration for the RMs with different aggregation numbers and water-to-lipid ratios. The DSPC RMs with aggregation numbers 70 ($w_0 = 5$), 78 ($w_0 = 7$), and 129 ($w_0 = 11$) settle into a rod-like form with little fluctuations. However, the smaller reverse micelles RM57 ($w_0 = 7$) and RM94 ($w_0 = 11$) end fluctuating between spherical and rod-like forms in our simulations. Furthermore, the figure shows RMs with equal water-to-lipid ratios have similar cross-sectional radii and the increase in aggregation number elongates the RM. Additionally, increasing w_0 increases the cross-sectional radii of the RMs—consistent with experimental observations.⁹

To further quantify whether the RM structures match PC lipid aggregates in cyclohexane, we calculated the cross-sectional radii of the aggregates, the water channel (only water), and the polar core (water and phosphorocholine). Next, in Figure 6, we compare these dimensions with those of lecithin reverse micelles measured by Schurtenberger *et al.* using SANS⁹ and with those of lecithin inverse hexagonal phase measured by Angelico *et al.* using SAXS.⁶ We argue the comparison of the structure of simulated DSPC RMs to the structural trends of lecithin RMs at a lower temperature is justified because the viscosity behavior of all PC/cyclohexane organogels is very similar despite significant differences in temperature and fatty acid tail composition. Figure 6 shows the cross-sectional radius of lecithin RMs increases between water ratios 6–14 experimentally and this trend is visible also in the radii calculated from the simulated RMs. However, the simulations result in a clear underestimation of the absolute value (14%–26% relative error). This difference is not surprising and likely originates from the difference in

FIG. 5. Final simulation configuration from each DSPC simulation. Dashed lines indicate the average diameter of the micelle polar core (water and phosphorylcholine) and the black bars at the right the core size summary at water-to-lipid ratios of 5, 7, and 11, respectively. Water is shown in light blue and phosphorylcholine in red space fill. Glycerol and tails are shown in black. Cyclohexane solvent is omitted in the visualization for clarity. Figures are in scale.

FIG. 6. Comparison of the calculated cross-sectional radius of the whole RM (water and lipids), water core, and polar interior (water and phosphorylcholine moiety) to experimental values. The black solid symbols correspond to experimental values from Refs. 9 and 6 and the colored open symbols to the simulations in this work.

the fatty acid tail composition – the artificially high number of unsaturated bonds which compensates for the lack of natural solvent penetration in the simulation model also results in radially shorter hydrocarbon tails and hence a smaller micelle radius. Unlike the overall cross-sectional radii, the experimental values of water and polar core do not directly correspond to the radius of gyration (R_g) but are derived from the scattering data following a more complex procedure. Nevertheless, the water region size in the simulations compares closely to that of the experimentally reported. However, in the simulations, the polar region is almost identical in size as the water core size but experimentally the size is estimated lower. This discrepancy may be influenced by the differences in methodology: experimental water core radii relies on the assumption that PC occupies $70 \text{ \AA}^2$ at the polar-apolar interface while the definition of polar core includes water and phosphorylcholine moiety only. Regardless, the trends and rate of radius increase with water-to-lipid ratio increase match with those reported experimentally for both the water channel and polar core.

Figure 7 presents the density profiles of water, lipid, and cyclohexane, as well as, profiles detailing the distribution of different lipid moieties on the water-hydrocarbon interface. To obtain profiles unbiased by the hemispherical endcaps, the simulation box was divided along the RM principal axis

direction into 5 slices and the data presented in Figure 7 corresponds to the center slab of these, i.e., to the most cylindrical section of the RM. The data show the interface between the polar interior of the RMs and the apolar solvent phase resides near the lipid glycerol moiety, and that the interface is sharp with little overlap between the two regions. As already said, the penetration of solvent into the fatty acid tail region is likely underestimated in the model and thus very little conclusions in regards to real RM systems can be drawn from the tail region behavior; in the following, we concentrate in the core structure.

For water-to-lipid ratios of 7 and 11, the density profiles of water indicate the presence of a central water channel—in line with experimental measurements.^{5,9} At water-to-lipid ratio $w_0 = 11$, the density of water in the core region approaches bulk water density and there is only a slight overlap with the distribution of lipid molecules. At $w_0 = 7$, however, choline groups penetrate occasionally even to the center of the water channel, indicating the channel is very narrow and its width fluctuates. Finally, at water-to-lipid ratio of 5, no bulk-like water core exists but rather a mixture of lipid head groups and water molecules. This behaviour differs from AOT RMs²³ where a much more well-defined water core is present even at a water-to-surfactant ratio of 3. This is consistent with the conclusion of Willard *et al.*, that PC headgroup sequesters more water than the sulphate headgroup of AOT.⁴⁵ Furthermore, the emergence of bulk-like water core between water ratios of 5 and 7 coincides with the observation of Willard *et al.*, that only at $w_0 > 5.8$, water molecules whose dynamics can be characterized as being bulk-like or free exist in lecithin RMs. The behaviour of the water core can be thus viewed as further validation that the headgroups in the hydrophilic interior behave accurately.

IV. DISCUSSION

Here, we examined the performance of the CHARMM36 force field for describing PC lipids in cyclohexane. The motivation rises from questioning the validity of the increasingly common usage of biomolecular force fields in describing systems outside their parametrization environment. CHARMM36 force field performs very well in describing all the studied components individually or in aqueous environment,^{37,38} and is currently one of the highest regarded lipid force fields, with good accuracy in water environment.¹⁻⁴ However, the

FIG. 7. Density profiles of the cylindrical mid-section of the PC RMs with water-to-lipid ratios $w_0 = 5$ (at left), $w_0 = 7$ (middle), and $w_0 = 11$ (at right). Radial distance corresponds to the 2D distance from the RM center of mass.

work here shows the non-bonded hydrocarbon parameters, specifically the CHARMM36 lipid force field linear alkane parameters originating from Ref. 48 and the newer alkane and cycloalkane non-bonded parameters from Ref. 47, contain an interaction imbalance in describing mixtures of lipids and cyclohexane. Here, the imbalance results in an aphysical disc-like structure for the PC lipid RMs in our simulations as the lipid tails pack too tightly in the RM structures due to lack of cyclohexane solvent penetration between the tails.

Actually, the same incompatibility with the CHARMM36 lipids is likely to affect all solvent models utilizing parameters from the updated alkane force field⁴⁷ as the non-bonded characteristics are the same. Furthermore, the finding also raises a concern on the accuracy of the description of organic solutes and their partitioning in lipid bilayers and regular micelles even in aqueous environments as also here an imbalance in interaction strengths would influence the outcome. However, we expect the effect not to be so dramatic as apolar interactions are but one among many types of interactions governing solute partitioning. Nevertheless, our results show in transferring aqueous environment parameters of, e.g., lipids to organic media, checking the bulk solvent characteristics is insufficient. Additionally, the compatibility of the different parametrizations and balances of the interactions needs to be verified.

We found that the compensation for the too tight tail packing in the simulation model by introduction of disorder by excess *cis* double bonds widens the lipid tails sufficiently to result in micelle structures matching with experimental data in their structure and water response. In our simulations, the DSPC lipid forms cylindrical, elongated RMs which were considered to represent wormlike RMs at the fixed aggregation number employed in the simulations. We analysed these micelles and their response to hydration change, as well as, sensitivity to aggregation number. We found the structural changes of the DSPC RMs as a function of water-to-lipid ratio are consistent with experimental data on PC/cyclohexane RM systems, see, e.g., Refs. 6, 9, and 45. In particular, the core water forms well-defined, sharp interfaced region at water-to-lipid ratios above 5, but at lower water-to-lipid ratio, no well-defined water core exists. This influences both the dynamics and the structure of the RM. Furthermore, the cross-sectional radii of the water channel and polar core match closely those found in lecithin RMs at varying water-to-lipid ratios while the total radius seems slightly underestimated most likely due to the introduction of excess kinks in the PC lipid tails to compensate for the underestimated solvent penetration in the simulation model.

The findings indicate molecular dynamics simulations could reveal control factors about RM structure and dynamics. However, taking into account the interaction imbalance in the model, we restrained ourselves to conservative observations about the RM micelle core behavior as drawing any conclusions on the tail region or solvent behavior is clearly not warranted. In total, until this imbalance in intermolecular interactions for apolar species is addressed, we would advice against any extensive conclusions on the RM behavior based on the employed simulation model. Furthermore, we recommend extra caution when drawing conclusions from a

simulated systems where accurate experimental comparison data are not available.

V. CONCLUSIONS

In conclusion, our results show that in transferring a parametrization of a surfactant molecule valid in aqueous environment into an organic medium, ensuring the tail-solvent interaction is correctly reproduced is crucial. Actually, this conclusion extends to describing general partitioning of apolar moieties in surfactant aggregates (also in aqueous environment). For example, characterization of drug partitioning in micelles and lipid bilayers also in aqueous environment may suffer from similar imbalance between the interactions, and this will influence the predictive capability of the simulations. Hence, our findings call for verification of the performance of the employed model whenever priorly uncharacterized mixtures are studied even though the individual simulation components would have been thoroughly validated as is the case here for all the employed parameters.

ACKNOWLEDGMENTS

This research was supported by Fortum Foundation, Academy of Finland, and a Marie Curie Career Integration Grant within the 7th European Community Framework Programme (Grant Agreement No. 293861). Computational resources by CSC IT Centre for Science, Finland, are gratefully acknowledged.

- ¹M. Paloncv, G. Fabre, R. H. DeVane, P. Trouillas, K. Berka, and M. Otyepka, *J. Chem. Theory Comput.* **10**, 4143 (2014).
- ²A. L. Rabinovich and A. P. Lyubartsev, *Polym. Sci., Ser. C* **55**, 162 (2013).
- ³A. P. Lyubartsev and A. L. Rabinovich, *Soft Matter* **7**, 25 (2011).
- ⁴T. J. Piggot, A. Pineiro, and S. Khalid, *J. Chem. Theory Comput.* **8**, 4593 (2012).
- ⁵R. Angelico, G. Palazzo, G. Colafemmina, P. A. Cirkel, M. Giustini, and A. Ceglie, *J. Phys. Chem. B* **102**, 2883 (1998).
- ⁶R. Angelico, A. Ceglie, U. Olsson, and G. Palazzo, *Langmuir* **16**, 2124 (2000).
- ⁷P. Schurtenberger, L. J. Magid, S. M. King, and P. Lindner, *J. Phys. Chem.* **95**, 4173 (1991).
- ⁸P. Schurtenberger and C. Cavaco, *Langmuir* **10**, 100 (1994).
- ⁹P. Schurtenberger, G. Jerke, C. Cavaco, and J. S. Pedersen, *Langmuir* **12**, 2433 (1996).
- ¹⁰D. Capitani, A. L. Segre, F. Dreher, P. Walde, and P. L. Luisi, *J. Phys. Chem.* **100**, 15211 (1996).
- ¹¹R. Scartazzini and P. L. Luisi, *J. Phys. Chem.* **92**, 829 (1988).
- ¹²Z. Shervani, T. Jain, and A. Maitra, *Colloid Polym. Sci.* **269**, 720 (1991).
- ¹³H.-Y. Lee, K. K. Diehn, S. W. Ko, S.-H. Tung, and S. R. Raghavan, *Langmuir* **26**, 13831 (2010).
- ¹⁴G. Palazzo, *Soft Matter* **9**, 10668 (2013).
- ¹⁵S. Salaniwal, S. T. Cui, P. T. Cummings, and H. D. Cochran, *Langmuir* **15**, 5188 (1999).
- ¹⁶S. Salaniwal, S. Cui, H. D. Cochran, and P. T. Cummings, *Ind. Eng. Chem. Res.* **39**, 4543 (2000).
- ¹⁷S. Senapati and M. L. Berkowitz, *J. Chem. Phys.* **118**, 1937 (2003).
- ¹⁸S. Senapati and M. L. Berkowitz, *J. Phys. Chem. B* **107**, 12906 (2003).
- ¹⁹S. Senapati and M. L. Berkowitz, *J. Phys. Chem. A* **108**, 9768 (2004).
- ²⁰W. Lu, J. Kim, W. Qiu, and D. Zhong, *Chem. Phys. Lett.* **388**, 120 (2004).
- ²¹A. V. Nevidimov and V. F. Razumov, *Mol. Phys.* **107**, 2169 (2009).
- ²²A. V. Martinez, L. Dominguez, E. Malolepsza, A. Moser, Z. Ziegler, and J. E. Straub, *J. Phys. Chem. B* **117**, 7345 (2013).
- ²³S. Abel, F. Sterpone, S. Bandyopadhyay, and M. Marchi, *J. Phys. Chem. B* **108**, 19458 (2004).

- ²⁴S. Abel, M. Waks, M. Marchi, and W. Urbach, *Langmuir* **22**, 9112 (2006).
- ²⁵J. Tian and A. E. García, *J. Chem. Phys.* **134**, 225101 (2011).
- ²⁶M. H. H. Pomata, D. Laria, M. S. Skaf, and M. D. Elola, *J. Chem. Phys.* **129**, 244503 (2008).
- ²⁷J. Chowdhary and B. M. Ladanyi, *J. Phys. Chem. B* **113**, 15029 (2009).
- ²⁸G. Mudzhikova and E. Brodskaya, *Colloid J.* **68**, 738 (2006).
- ²⁹E. N. Brodskaya and G. V. Mudzhikova, *Mol. Phys.* **104**, 3635 (2006).
- ³⁰G. Mudzhikova and E. Brodskaya, *Colloid J.* **68**, 729 (2006).
- ³¹V. R. Vasquez, B. C. Williams, and O. A. Graeve, *J. Phys. Chem. B* **115**, 2979 (2011).
- ³²S. Abel, M. Waks, W. Urbach, and M. Marchi, *J. Am. Chem. Soc.* **128**, 382 (2006).
- ³³B. Qiao, T. Demars, M. Olvera de la Cruz, and R. J. Ellis, *J. Phys. Chem. Lett.* **5**, 1440 (2014).
- ³⁴B. Hess, C. Kutzner, D. van der Spoel, and E. Lindahl, *J. Chem. Theory Comput.* **4**, 435 (2008).
- ³⁵S. Pronk, S. Pall, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M. R. Shirts, J. C. Smith, P. M. Kasson, D. van der Spoel, B. Hess, and E. Lindahl, *Bioinformatics* **29**, 845 (2013).
- ³⁶A. D. MacKerell, D. Bashford, M. Bellott, R. L. Dunbrack, J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, W. E. Reiher, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin, and M. Karplus, *J. Phys. Chem. B* **102**, 3586 (1998).
- ³⁷J. B. Klauda, R. M. Venable, J. A. Freites, J. W. O'Connor, D. J. Tobias, C. Mondragon-Ramirez, I. Vorobyov, A. D. MacKerell, and R. W. Pastor, *J. Phys. Chem. B* **114**, 7830 (2010).
- ³⁸O. Guvench, S. N. Greene, G. Kamath, J. W. Brady, R. M. Venable, R. W. Pastor, and A. D. Mackerell, *J. Comput. Chem.* **29**, 2543 (2008).
- ³⁹W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein, *J. Chem. Phys.* **79**, 926 (1983).
- ⁴⁰U. Essmann, L. Perera, M. L. Berkowitz, T. Darden, H. Lee, and L. G. Pedersen, *J. Chem. Phys.* **103**, 8577 (1995).
- ⁴¹B. Hess, H. Bekker, H. J. C. Berendsen, and J. G. E. M. Fraaije, *J. Comput. Chem.* **18**, 1463 (1997).
- ⁴²S. Miyamoto and P. A. Kollman, *J. Comput. Chem.* **13**, 952 (1992).
- ⁴³G. Bussi, D. Donadio, and M. Parrinello, *J. Chem. Phys.* **126**, 7 (2007).
- ⁴⁴M. Parrinello and A. Rahman, *J. Appl. Phys.* **52**, 7182 (1981).
- ⁴⁵D. M. Willard, R. E. Riter, and N. E. Levinger, *J. Am. Chem. Soc.* **120**, 4151 (1998).
- ⁴⁶L. Martinez, R. Andrade, E. G. Birgin, and J. M. Martinez, *J. Comput. Chem.* **30**, 2157 (2009).
- ⁴⁷I. Vorobyov, V. M. Anisimov, S. Greene, R. M. Venable, A. Moser, R. W. Pastor, and A. D. MacKerell, *J. Chem. Theory Comput.* **3**, 1120 (2007).
- ⁴⁸D. Yin and A. D. MacKerell, *J. Comput. Chem.* **19**, 334 (1998).
- ⁴⁹W. Humphrey, A. Dalke, and K. Schulten, *J. Mol. Graphics* **14**, 33 (1996).
- ⁵⁰W. L. Jorgensen, D. S. Maxwell, and J. Tirado-Rives, *J. Am. Chem. Soc.* **118**, 11225 (1996).
- ⁵¹See supplementary material at <http://dx.doi.org/10.1063/1.4914022> for time-series of shape parameters and additional snapshots from the simulations.
- ⁵²B. Halle, M. Landgren, and B. Jönsson, *J. Phys.* **49**, 1235 (1988).
- ⁵³A. G. Dafil, J. B. Avalos, and A. D. Mackie, *Langmuir* **28**, 3730 (2012).
- ⁵⁴M. Sammalkorpi, M. Karttunen, and M. Haataja, *J. Am. Chem. Soc.* **130**, 17977 (2008).
- ⁵⁵S. E. Feller and A. D. MacKerell, *J. Phys. Chem. B* **104**, 7510 (2000).
- ⁵⁶S. W. I. Siu, K. Pluhackova, and R. A. Böckmann, *J. Chem. Theory Comput.* **8**, 1459 (2012).
- ⁵⁷B. Jójárt and T. A. Martinek, *J. Comput. Chem.* **28**, 2051 (2007).
- ⁵⁸S. W. I. Siu, R. Vácha, P. Jungwirth, and R. A. Böckmann, *J. Chem. Phys.* **128**, 125103 (2008).
- ⁵⁹Å. A. Skjervik, B. D. Madej, R. C. Walker, and K. Teigen, *J. Phys. Chem. B* **116**, 11124 (2012).
- ⁶⁰C. J. Dickson, L. Rosso, R. M. Betz, R. C. Walker, and I. R. Gould, *Soft Matter* **8**, 9617 (2012).
- ⁶¹C. J. Dickson, B. D. Madej, Å. A. Skjervik, R. M. Betz, K. Teigen, I. R. Gould, and R. C. Walker, *J. Chem. Theory Comput.* **10**, 865 (2014).
- ⁶²A. Queimada, S. Quinones-Cisneros, I. Marrucho, J. Coutinho, and E. Stenby, *Int. J. Thermophys.* **24**, 1221 (2003).
- ⁶³R. M. Stephenson and S. Malanowski, *Handbook of the Thermodynamics of Organic Compounds* (Elsevier, 1987).
- ⁶⁴A. A. Silva, R. A. Reis, and M. L. L. Paredes, *J. Chem. Eng. Data* **54**, 2067 (2009).
- ⁶⁵S. N. Bhattacharyya and D. Patterson, *J. Chem. Soc., Faraday Trans. 1* **81**, 375 (1985).
- ⁶⁶A. Sodt and R. Pastor, *Biophys. J.* **104**, 2202 (2013).