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Supporting Material

Water Replacement Hypothesis in Atomic Detail – Factors Determining the Structure of Dehydrated Bilayer Stacks

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Water Replacement Hypothesis in Atomic Detail – Factors Determining the Structure of Dehydrated Bilayer Stacks

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Details of the Simulation

All MD simulations were carried out using the GROMACS molecular dynamics package version 4.0 (1). Orthorhombic periodic boundary conditions were applied in all three dimensions. The LINCS method (2) to constrain bonds allows the use of the leapfrog integration algorithm with a time step of 5 fs (3). For Lennard-Jones interactions we used a plain cutoff (without shift function) of 1.2 nm (4). Electrostatic interactions within 1.0 nm were calculated each time step, while interactions beyond this range every ten time steps. Long-range electrostatics was handled by means of the Particle-Mesh Ewald technique (5, 6). Neighbor searching used a twin-range approach with the cutoff (rlist) of 1 nm (manual Gromacs, version 4.0). POPC, trehalose and water molecules were separately coupled to a heat bath at T = 310 K, using the Berendsen algorithm (7) with a coupling constant of 0.1 ps. The pressure was maintained at 1 atm by separate coupling in all three directions with coupling constant of 2 ps (7).

For self-assembly one POPC molecule was randomly chosen from the POPC bilayer (website http://moose.bio.ucalgary.ca/files/popc.itp). 64 copies of this molecule were placed randomly into a cubic box, using the Gromacs genbox tool. A short simulation in vacuum (2 ns) with anisotropic pressure coupling at 1 atm fits the box size to its content. 3200 water molecules were then randomly added to obtain a 1:50 lipid/water ratio and a 2 ns simulation at 1 atm followed to again adjust the box (Fig. S1A). The conditions of self-assembly were the same as in (3). After 30 ns NPT simulation all lipids aggregated into the bilayer. As the self assembly not always led to symmetric bilayers, we selected a symmetric conformation, i.e. 32 lipids in either leaflet (Fig. S1B).

The starting point for dry bilayers was the self-assembled POPC bilayer with 32 lipids per leaflet and excess water. Because 28-30 waters/lipid are usually used to simulate fully hydrated bilayer, we reduced the number of waters per lipid to 28.5. For the systems with 28.5, 11.7 and 5.4 waters per lipid, water was removed from the midplane of the interlamellar region of self-assembled bilayer and the MD simulation continued for 20 ns under NPT conditions. The height

of the box (along the bilayer normal) decreased and effectively a multilamellar “stack” was formed through periodic boundary conditions. This approach to obtain bilayers with reduced water content was applied earlier (8,9). To avoid artifacts due to the very small number of lipids in the box, we multiplied the system 4 times in the plane. After multiplication we run the simulations with 256 POPC lipids and a corresponding number of water molecules (28.5; 11.7 and 5.4 waters per lipid) for 200 ns each.

To create a bilayer with trehalose, different numbers of trehalose molecules were placed randomly in the empty space after water removal and the procedure above followed. The initial constraints on the lipids resulted in preferential location of trehalose and water along the lipid interface before any changes in area per lipid take place. Again the box was multiplied 4 times and now the 256 lipids per box were simulated for 400 ns under NPT conditions. It is clear that after adding the trehalose the water will redistribute because trehalose and lipids compete for the water. The exact numbers of different molecules in all models together with our nomenclature is presented in Table 1.

Detailed Description of Analysis Techniques

The area per lipid (APL) was calculated by dividing the xy plane of the simulation box by the 128 lipids per leaflet. APL values were averaged over the time interval at (near) equilibrium conditions, i.e. between 100 and 200 ns for the models without trehalose and between 320 and 400 ns for the models with trehalose (Fig. 1). We also fitted the evolution of APL in time by the exponential function $y = A1*\exp(-x/t1) + y0$ (Fig. 1). The values of APL, obtained by fitting and by averaging do not deviate significantly (Table 2).

To obtain mass density profiles, the positions of the atom groups were averaged over the last 10 ns of the simulations within 100 slices along the bilayer normal. The density profiles were plotted separately for lipids, water, trehalose, P and N atoms. The profiles are symmetrized between leaflets and smoothed without changing the profile shape.

RDF was averaged over the last 10 ns of simulations without trehalose and 20 ns with trehalose.

Headgroup orientation is characterized by the cosine of the angle between the P-N vector and the bilayer normal which we compute over 5 ns of near-equilibrated trajectory. Cosines are used to avoid spurious peaks resulting from Jacobians. For characterizations we fit the distributions with several Gaussians.

Electrostatic potentials for lipids, water and trehalose are calculated separately. In order to do so charge distributions for all the components were calculated as a function of position along the bilayer normal and integrated twice. All potentials were taken as zero in the center of the bilayer and symmetrized over leaflets. Plots are centered in the middle of the interbilayer space.

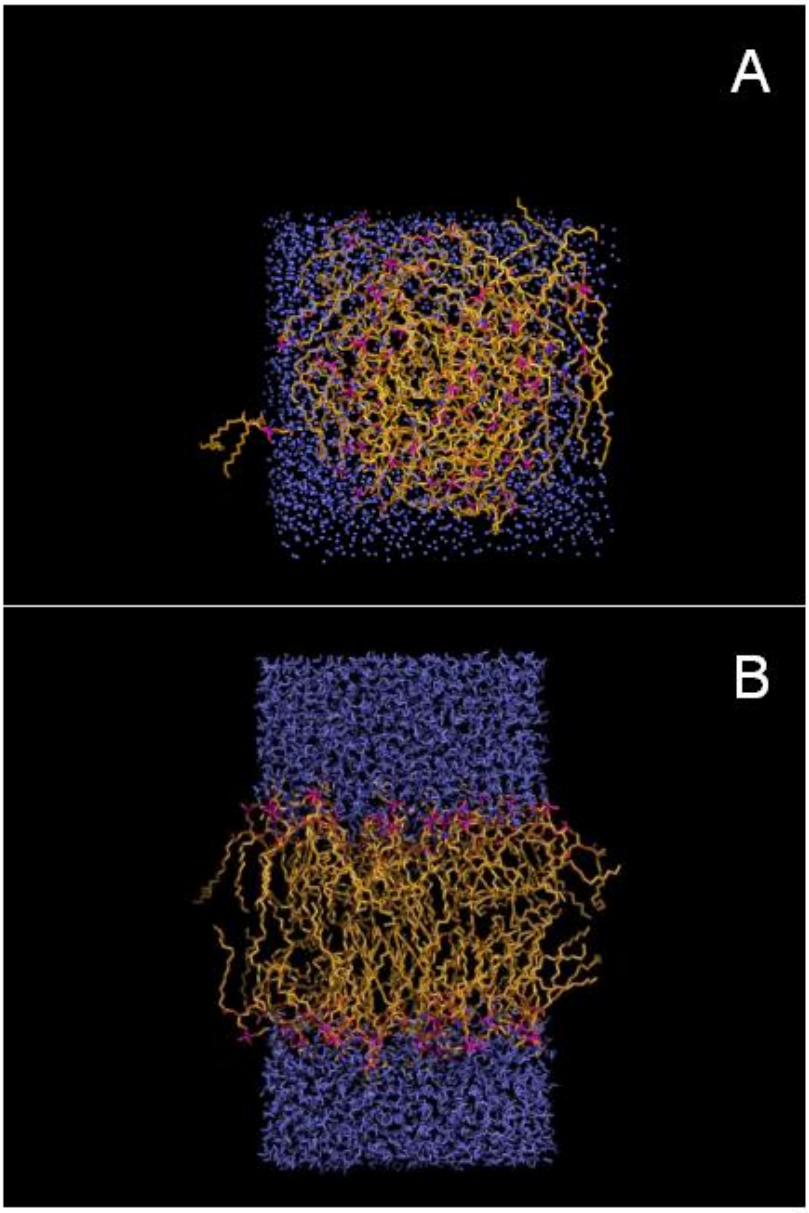

Fig. S1. Snapshots of simulation boxes (64 lipids and 3200 waters) at the beginning (A) and at the end (30 ns) (B) of the self-assembly.

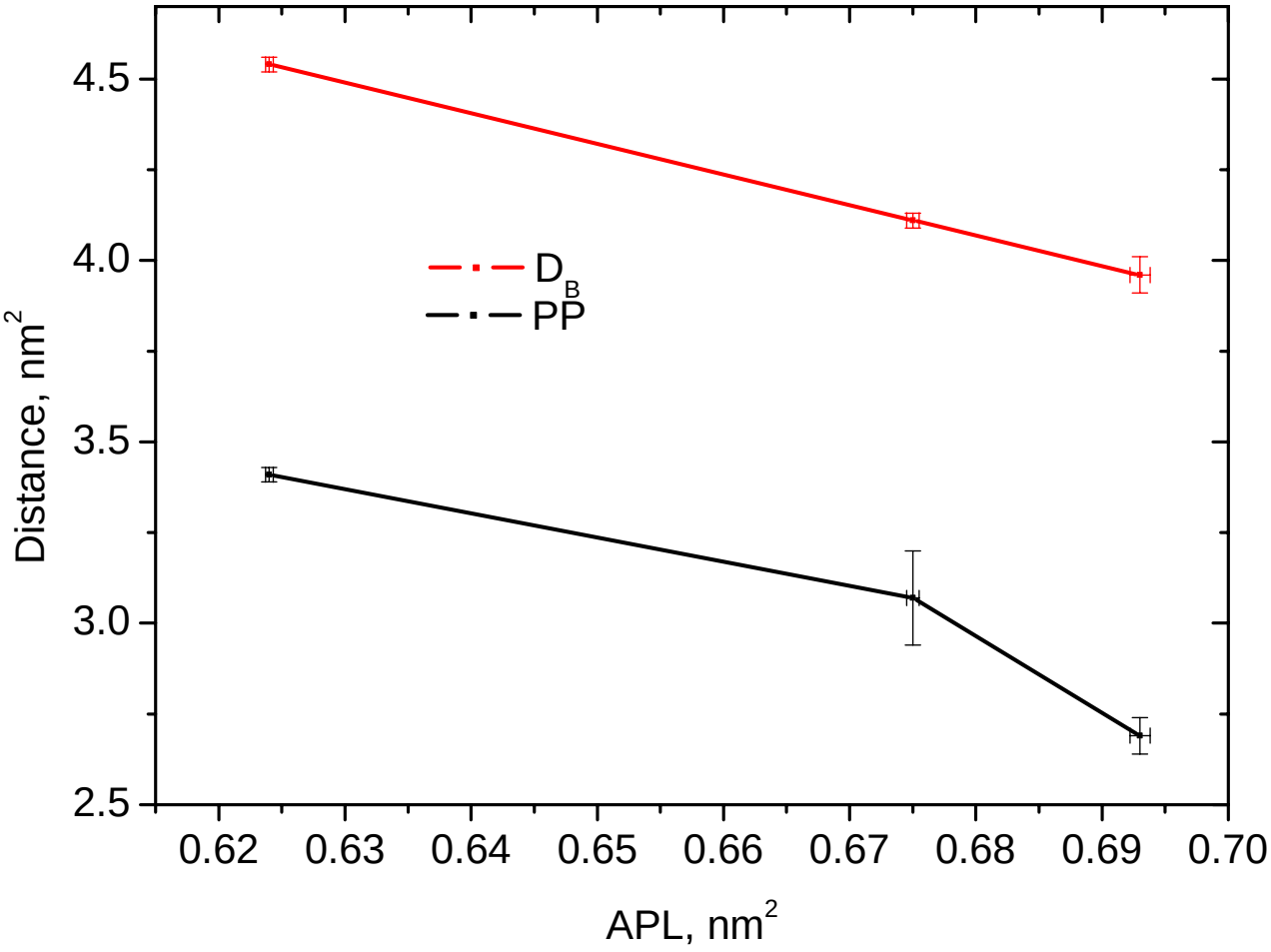

Fig S2 The correlation between APL and the thickness of the bilayer D_B or peak-to-peak distance PP in the models with different water contents without trehalose. SE is indicated.

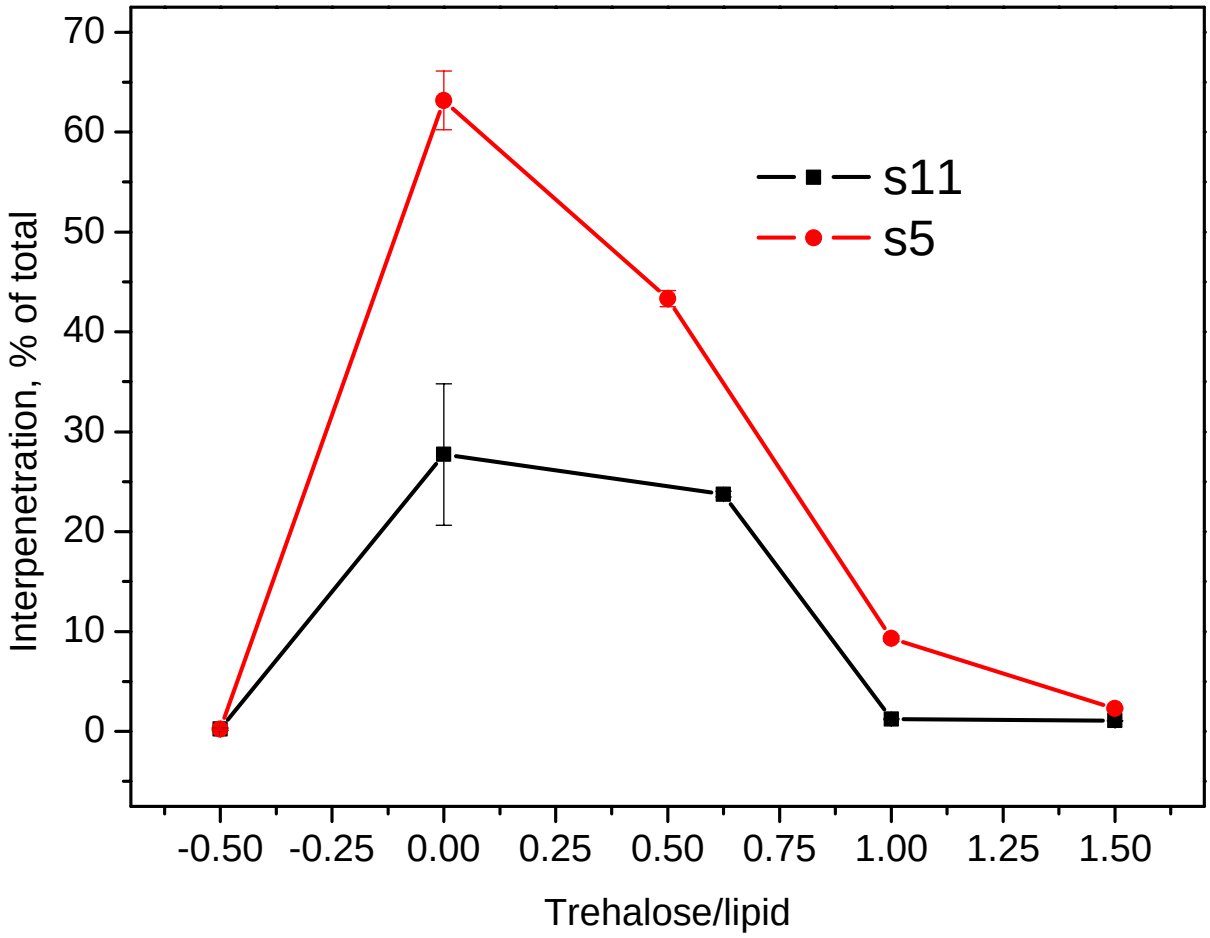

Fig S3 Interpenetration of the adjacent bilayers in s5 and s11 models at different trehalose:lipid molar ratio. Interpenetration is calculated as the % of lipid density in the mid of the interbilayer space from the peak value. SE is indicated.
In some models the size of SE bars are less than the size of the symbols.
Conditionally the position of the hydrated model h28-00 in x-axis is indicated as -0.5.

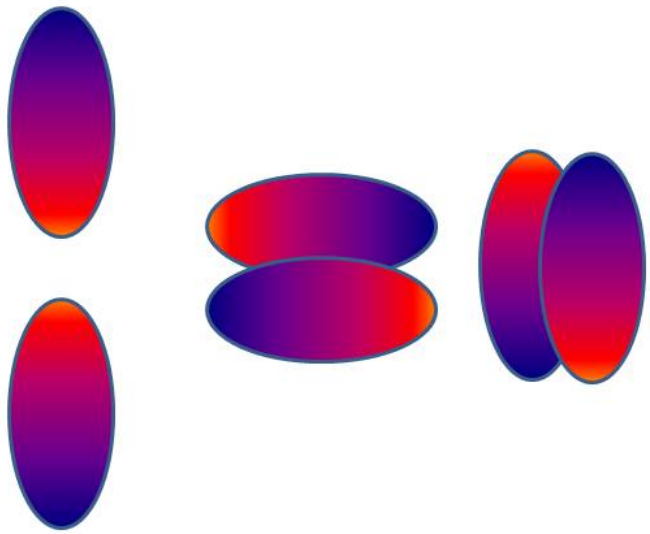

Fig. S4. At large separation the dipoles are opposite and repulsive, at intermediate separation the reorientation of dipoles switches off the repulsion. At very close range the dipoles reorient again, now a slight overlap will push towards strong interpenetration.

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