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Supporting material *for*

The impact of lipid composition on the tear fluid lipid layer

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Materials and Methods
Simulations

Simulation models
Multi-component lipid systems comprised of DPPC, cholesteryl oleate (CO), trioleate (TO), and water were modeled. In some systems we also included DPPE, but its effect was observed to be minor and did not change the conclusions (data not shown). All systems were described using the coarse-grained (CG) representation in terms of the Martini model (1, 2). The initial structure of the model is a previously published (3) periodic system of two lipid monolayers separated by vacuum on one side and water on the other side. This system of lipid composition POPC:FFA:TO:CO 4:2:1:1 (FFA standing for free fatty acid) was then modified to gain new starting structures where FFA was no longer included. 8 lipids were added to both sides to yield 800 lipids in both monolayers. The lipid types were interchanged so that new randomly distributed lipid compositions were reached. The new compositions were DPPC/TO 8:2, DPPC/DPPE/TO 4:4:2, DPPE/TO 8:2, DPPC/CO 9:1, DPPC/TO 9:1, DPPC/DPPE/TO 4.5:4.5:1, DPPE/TO 9:1, DPPC/TO/CE 4:3:3, pure DPPC, DPPC/DPPE 1:1, and pure DPPE. The number of water molecules per lipid and the thickness of the vacuum layer were checked to be large enough, preventing the constructed lipid system from seeing its own periodic image during the simulation.

The force field for all the systems was based on the Martini model (version 2.0) (1, 3). The simulations were carried out by using the Gromacs software package (version 4.0.4) (4-6). The simulation temperature was maintained at 305 K with the Berendsen temperature coupling (7) using the time constant of 0.3 ps. All simulations used a time step of 20 fs, and the data was stored every 200 ps.

We first carried out simulations at constant pressure, each conducted with only a modest increase/decrease in pressure to reach a wide variety of systems with different areas per lipid. The area per lipid is defined here as the total area of the layer divided by the total number of lipid moleculesVelocities were regenerated at the beginning of each run. Pressure was held constant with the semi-isotropic Berendsen pressure coupling in the plane of the membrane by using a time constant of 3 ps and a compressibility of 0.00005 bar. After each area change of 5Å^2/lipid, each system was simulated for 10 ns in constant volume to reassure the equilibration of the system. This was continued until we had reached all the simulation-accessible areas per lipid (from 40-50Å^2/lipid to 50-70Å^2/lipid) for all simulated systems.

Next the selected systems were simulated for a time scale of at least 1μs in constant volume simulations (600 ns for the one-component DPPC standard), of which at least 400 ns were under equilibrium conditions. It is worth pointing out that the time scales mentioned above do not include the speed-up factor of four arising from the fact that the dynamics in a CG system using the MARTINI description are faster than atomistic dynamics by an approximate factor of four (3). The effective simulation time considered in the final production simulations was thus 4 microseconds, of which at least 1.6 microseconds corresponded to equilibrium conditions used in

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analysis. The selected systems were 50, 55, 60, 65 and 70 Å^2/lipid for all systems having composition PL/TG 9:1 (where PL stands for phospholipid) and PL/TG 8:2; 50, 55, 60 and 65 Å^2/lipid for DPPC/CO 9:1; 40, 45 and 50 Å^2/lipid for DPPC/TO/CE 4:3:3; and 40, 45, 50, 55, 60 and 65 Å^2/lipid for systems containing only PL lipids.

Analysis of simulation data

The number densities of the systems were calculated from the simulation data with the Gromacs analysis g_density tool, version 4.0.7. For the density profile analysis, the simulation box was divided in slices that were 0.5 Å thick. The values were calculated for each simulated system in two ways: i) based on the whole equilibrium trajectory and ii) by dividing the equilibrium trajectory to 4-6 non-overlapping parts in order to calculate the 95 % confidence limit. The error bars determined in this manner are shown in the figures. The simulation snapshots were visualized with VMD software, and number densities with xmgrace software.

The surface pressure (π)-area (A) data for the equilibrium runs was fitted to experimental data presented in the present article using the approach described by Baoukina et al. (8). The fit confirmed that the simulated systems can be compared to experimental results, i.e. the simulations and Langmuir film balance experiments both show the identical π-A relationship in the average A/acyl chain range considered.

References

1. S.J. Marrink, H.J. Risselada *et al.* and A.H. de Vries, The MARTINI force field: coarse grained model for biomolecular simulations, *J. Phys. Chem. B.* **111** (2007), pp. 7812–7824.

2. S.J. Marrink, A.H. de Vries and A.E. Mark, Coarse grained model for semi-quantitative lipid simulations, *J. Phys. Chem. B.* **108** (2004), pp. 750–760.

3. P. Kulovesi, J. Telenius et al. and J. M. Holopainen, Molecular Organization of the Tear Fluid Lipid Layer, Biophysical Journal, 99(2010), pp. 2559-2567.

4. H.J.C. Berendsen, D. van der Spoel, R. and R. van Drunen, GROMACS: a message-passing parallel molecular dynamics implementation, *Comput. Phys. Commun.* **91** (1995), pp. 43–56.

5. D. Van Der Spoel, E. Lindahl *et al.* and H.J. Berendsen, GROMACS: fast, flexible, and free, *J. Comput. Chem.* **26** (2005), pp. 1701–1718.

6. B. Hess, C. Kutzner *et al.* and E. Lindahl, GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation, *J. Chem. Theory Comput.* **4** (2008), pp. 435–447.

7. H.J.C. Berendsen, J.P.M. Postma *et al.* and J.R. Haak, Molecular dynamics with coupling to an external bath, *J. Chem. Phys.* **81** (1984), pp. 3684–3690.

8. S. Baoukina, L. Monticelli *et al.* and D.P. Tieleman, Pressure-area isotherm of a lipid mono-

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layer from molecular dynamics simulations, *Langmuir* **23** (2007), pp. 12617–12623.