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Simulation of the Spontaneous Aggregation of Phospholipids into Bilayers

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The self-aggregation of lipid molecules to form bilayer membranes is a process fundamental to the organization of life. Although qualitatively explained by the hydrophobic effect,[1] the molecular aggregation itself is a complex phenomenon that has not been possible to study in detail experimentally. Here, we report a series of molecular dynamics computer simulations that for the first time demonstrate the possibility to observe the entire process at atomic detail with realistic lipids. Starting from random solutions, bilayers are formed on time scales of 10−100 ns, with properties matching experimental data. Several key steps and approximate time scales of the aggregation can be identified. The final rate-limiting process is the reduction and disappearance of large hydrophilic transmembrane water pores, of biological relevance for, for example, ion permeation.

Singer and Nicholson were the first to recognize the implications of the extreme flexibility of membranes for the structure of cellular walls, leading to the famous fluid-mosaic model[2] with diffusing lipids and proteins. The bilayer formation process is, however, extremely fast and involves subtle rearrangements at the molecular level, making it elusive to current experimental methods. Simplified computer models have been used to mimic aggregation of surfactant-like molecules into monolayers and micelles,[3] bilayerlike structures,[4,5] and even vesicles.[6] These models are theoretically important to extend length and time scales, but they do not include atomic detail like hydrogen bonds and represent the collective entropic effects driving aggregation[1] as pairwise interactions. Detailed molecular dynamics simulations have on the other hand, provided accurate models of up to nanometer and nanosecond scales, but previously only for preassembled bilayers.[7−11] This work demonstrates the first simulations of aggregation of lipids into bilayers with atomic detail of the structure and interactions. Compared to micelle aggregation studies,[12,13] bilayer formation is considerably more challenging due to the balance between hydrophobicity and solvation, and the aggregation involves collective mesoscopic dynamics.

The phospholipid dipalmitoylphosphatidylcholine (DPPC) was initially chosen for the study, since it is present in biological membranes and well studied both experimentally and computationally. Six simulations were performed on systems containing 64 DPPC lipids and 3000 water molecules, using the GROMACS software.[14] To test the dependence on lipid type and system size, additional simulations were performed using palmitoyloleoylphosphatidylcholine (POPC), dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylethanolamine (DOPE), and on larger systems consisting of 128 and 256 DPPC lipids. The total simulation time exceeded 0.5 μs. All systems were subject to periodic boundary conditions, and the temperature was coupled to 323 K, well above the phase transition at 315 K. Pressure was controlled by separate coupling to 1 atm in normal and lateral directions, corresponding to a stress-free bilayer. Recent improvements in bond constraining[15] and methods to remove fast oscillations[16] made it possible to extend the time step to 5 fs. The absence of lipid net charge enabled the use of a group-based 1.5 nm cutoff for electrostatics and 1.0 nm for Lennard-Jones instead of computationally expensive lattice sums (particle mesh Ewald summation used in a test run showed no significant effect of the long-range electrostatics on the aggregation mechanism). Similar setups have been reported for several other simulations that accurately reproduce available experimental data.[17,18]

A typical aggregation process is illustrated in Figure 1, with an initially random solution of DPPC lipids gradually forming a perfect bilayer. All starting configurations were cubic, and no bias introduced to any direction. The anisotropic coupling allowed the simulation box to deform according to the natural forces acting within each system. There is a rapid initial separation into water and lipid phases, driven by strong thermodynamic forces that separate the hydrophobic tails from the aqueous environment. The subsequent rearrangement into a bilayer is slower, requiring about 3 ns. This intermediate configuration contains a large transmembrane water pore stabilized by a few misplaced lipids. The breakdown of the pore is the rate-limiting step in the overall process, requiring more than 20 ns in the illustrated case. Once the disruption starts, it disappears rapidly (less than 1 ns), and the system relaxes to an equilibrium bilayer within about 5 ns. This final structure is indistinguishable from those of preassembled bilayers simulated with the same interaction parameters.[18] The surface area per lipid is 0.62 ± 0.01 nm^2, very close to the experimental result[19] 0.629 ± 0.013 nm^2.

Another five independent simulations with 64 DPPC lipids were performed with different random initial configurations. All of these evolved in a similar manner and with the same characteristic time scales, apart from the pore lifetime t_{pore} that varied substantially. Lifetimes of 5−80 ns were observed, with typical values around 15 ns. Similar formation pathways and characteristic times were also identified for the systems containing 64 POPC, DOPC, and DOPE lipids. Enlargement of the system to 128 or 256 DPPC lipids did not change the aggregation mechanism with the bilayer containing a single water pore as an intermediate phase. This suggests a typical pathway for bilayer formation, as illustrated in Figure 2. Note that some caution is advisable when extrapolating this observed mechanism toward truly macroscopic scales since even the large simulated systems are relatively small.

Remarkably, the bilayers that form almost always contain equal numbers of lipids in the two monolayers. In fact, only in one 64

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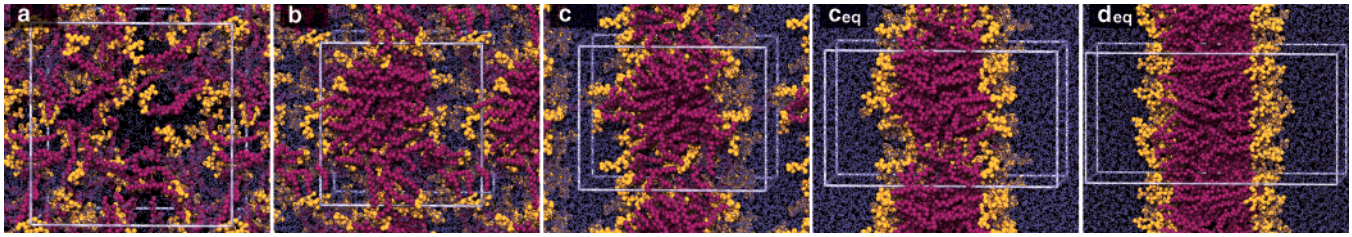

Figure 1. Snapshots of the phospholipids assembling into a bilayer. Headgroup atoms are depicted with orange spheres, while tail atoms are purple and water molecules are small in blue. For clarity, the system is repeated in space and the actual simulation box shown as a light-blue frame. Starting from the random solution (**a**), large thermodynamic gradients drive an aggregation into irregular clusters at 200 ps (**b**). This is followed by the formation of a primordial bilayer shown at 3 ns (**c**) and subsequent relaxation toward a metastable structure with a transmembrane pore reached after 10 ns ($\mathbf{c_{eq}}$). This water pore is relatively stable and involves a significant free energy barrier, but once the disruption starts at about 20 ns it quickly disappears and the system reaches an equilibrated bilayer configuration after a total 25 ns ($\mathbf{d_{eq}}$). Figure S1 in the Supporting Information shows the process in more detail, and supporting Figure S2 contains the corresponding densities along the eventual membrane normal.

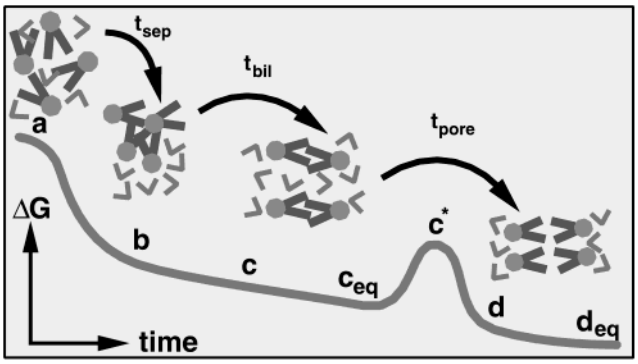

Figure 2. Schematic model of the free energy variation ΔG during the assembly process. There is a rapid separation of the random mixture (**a)** into lipid and aqueous domains (**b)** on time scale $t_{sep} \cong 200$ ps. This is followed by the formation of a bilayerlike phase with defects (**c**) on time scale $t_{bil} \cong 5$ ns, which eventually surmounts the free energy barrier to form a defect free bilayer (**d**). The last step depends on the defect lifetime $t_{pore} \cong 15$ ns, which is determined by the energy of the transition state (**c***). Both the metastable phase (**c**) and the final bilayer (**d**) slowly relax toward their equilibrium structures $\mathbf{c_{eq}}$ and $\mathbf{d_{eq}}$.

lipid simulation did we observe aggregation into an asymmetric bilayer with 30/32 DPPC lipids and two more remaining in solution for the subsequent 20 ns simulation. In the largest simulation to date, involving 256 lipids, 12 molecules remained in the water phase organized into a small micelle. Fusion with the bilayer was not observed in the simulation, indicating that this process requires longer time scales.

The transient formation and destabilization of the water pore illustrated in Figure 3 appears to be a basic feature of the formation mechanism. Initially the pore in the bilayer is very large, containing about 100 dissolved waters and 8−10 buried headgroups. During the relaxation, the pore gradually narrows with the final metastable structure measuring 1−1.5 nm in diameter, consisting of 4−6 headgroups and roughly 50 ± 10 water molecules. The ratio of dissolved waters to lipid headgroups is about 10:1, similar to the 11 waters reported for primary DPPC hydration,[20] which indicates that the buried lipids essentially maintain their hydration shells. It is not until the headgroups reorient toward the membrane surface that the pore is destabilized. Given that we observe lifetimes up to 80 ns in our simulations, a substantial free energy barrier must be involved in this headgroup reorientation. It is likely explained by the breaking of hydrogen bonds with pore waters and other headgroups, and steric hindrance imposed by the membrane environment. The stabilization is, however, a cooperative effect and cannot easily be separated into various components. With less than four buried headgroups, the pore disrupts very quickly, occasionally leaving

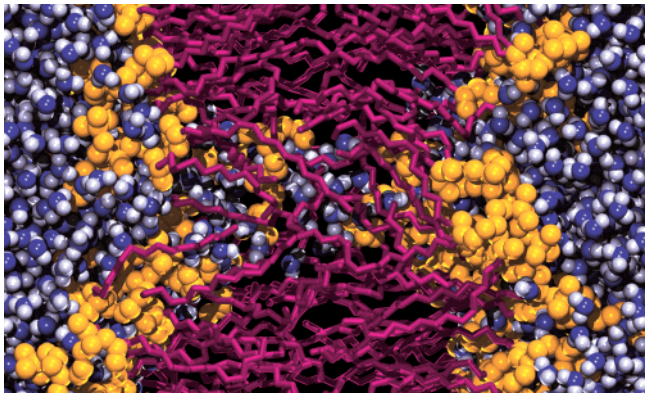

Figure 3. The metastable transmembrane water pore after 15 ns. Headgroup atoms are orange, tails shown as purple bonds. Water is drawn with deep-blue oxygen and light-blue hydrogen atoms. Figure S3 in the Supporting Information shows a full sequence of the pore equilibration, destabilization, and final breakdown.

a small number of waters in the membrane that are rapidly expelled.

The existence of hydrophilic pores in equilibrium membranes is biologically important. Even a small population can explain experimentally observed permeation rates for ions in the absence of channel or translocation proteins. Instead of permeating the membrane by a solvation−diffusion mechanism, the occasional presence of pores offers charged molecules an energetically very favorable permeation route.[21] Under mechanical, osmotic, or electrocompressive stress, such defects can become stable, leading to electrical breakdown or even rupture of the entire membrane. The ability of membranes to form hydrophilic pores is also important as intermediate stages in phase transitions and in fusion and budding events. In fact, the simulations reported here are an example of this, with the pore as an intermediate stage in the transition from isotropic fluid to a bilayer phase. The present report clearly demonstrates that it has become possible to simulate collective mesoscopic phenomena with atomic detail. No prior knowledge of the aggregation state is required, as the lipids spontaneously form the most favorable phase on time scales accessible to simulations. It is very reassuring that the defect-free bilayer is indeed the most stable phase with current force fields. Further, the simulations provide realistic time scales of several key bilayer formation and reorganization processes and make it possible to examine the detailed structure and dynamics of the biologically interesting intermediate phases, such as the hydrophilic transmembrane water pores.

Supporting Information Available: Additional figures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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