Modeling Self-Assembly in a Variety of Molecular Systems

Sandia National Laboratories

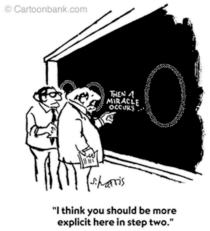
Mark J. Stevens

msteve@sandia.gov

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under Contract DE-AC04-94AL8500.

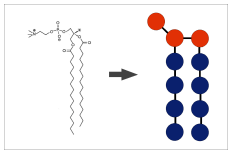
Introduction

The process of membrane fusion is central to biology, and plays a role in events such as vesicular trafficking, fertilization, viral entry and mitosis. While fusion between two biological membranes is normally a process mediated by proteins, many pure lipid bilayer systems will spontaneously fuse and it is likely that the underlying physical chemistry in both cases has important similarities. In addition, mechanisms of fusion between pure lipid systems have a number of technological applications such as liposome based drug delivery and the formation of supported lipid bilayer biosensors and other devices. As a result, fusion between pure lipid membranes has been the subject of a wide range of experimental, computational and theoretical studies. These efforts have produced a number of models, typically involving the local deformation of a membrane, the formation of an inverted hexagonal phase like structure as an intermediate, and the subsequent complete merger of the two sides. In the hemifused intermediate the outer leaflets are fused while the inner leaflets remain separate, to form a connection between membranes referred to as a stalk. However, the molecular aspects of membrane fusion are not treated by the continuum models and have yet to be determined experimentally. We have performed MD simulations that show new processes in the fusion mechanisms and reveal novel molecular details of lipid movement.



Model

Our coarse-grained model for the lipid molecules consists of a double tail bead-spring molecule composed of two types of beads (tail and head). The simulations presented here have 11 beads per lipid with 3 in the head group and 4 each in the tails.



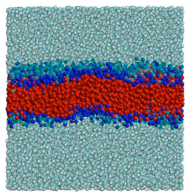
Lennard-Jones (LJ) potential $4\varepsilon\left[\left(\frac{\sigma}{r_{ij}}\right)^{12}-\left(\frac{\sigma}{r_{ij}}\right)^{6}\right]$

The purely repulsive LJ potential u_{RLJ} is cutoff and shifted at $r_c = 2^{1/6}\sigma$.

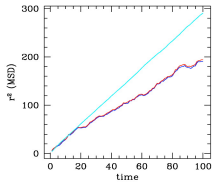
FENE bond potential $-\frac{1}{2}kR_0^2\ln\left(1-r_{i,i+1}^2/R_0^2\right)+u_{RLJ}\left(r_{i,i+1}\right)$

Angle bend potential $k_\theta(\theta-\theta_0)^2$

Self-Assembly

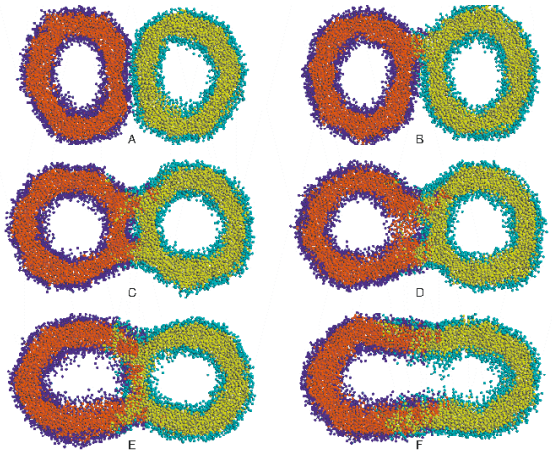


We first demonstrated that our model system self-assembles into a bilayer. The image above shows a bilayer formed starting from a random initial configuration of lipids and solvent. In this simulation there are $N_{lip} = 488$ lipid molecules and an initial bead density of $0.67\,\sigma^{-3}$. After about 1 million time steps at $T = 1.35\,\varepsilon$ the bilayer formed. In order to allow the system to choose the appropriate cell dimensions that fit a bilayer, the simulation was continued in the NPT ensemble at $P = 1$ where the **area per lipid,** $A = 3.7\,\sigma^2$.



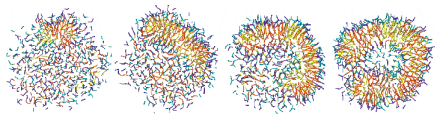
A key aspect of biomembranes is their fluidity. Unfortunately, lipid diffusion is too slow to treat with atomistic simulations. However, using the coarse-grained models we are able to see lipids diffuse across the system. The plot at the right shows the mean square displacement of the head groups (blue), tail groups (red) and solvent (cyan).

Membrane Fusion



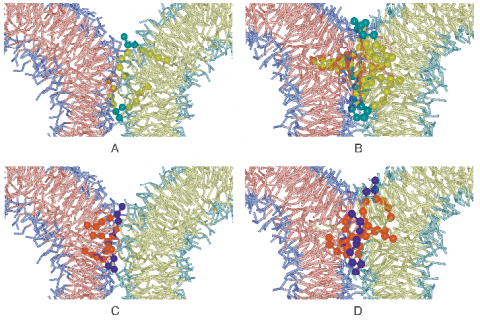
Cross sections (5 nm thick) of fusing liposomes showing sequence of events to complete fusion. (A) flat interface at t = 55 τs (B) initial stalk at t = 94 τs (C) growth of stalk to other side with solvent cavity at t = 140 τs (D) dissolution of one connector and solvent cavity t = 149 τs (E) intermediate fusion state at t = 204 τs (F) complete fusion at t = 231 τs. The nonspherical shape of the fused liposome is a consequence of the geometric constraints on the surface area and volume of the fused liposome. This system is under osmotic pressure with internal solvent density 20% larger than external density.

In a typical fusion simulation, the process begins with the formation of a flattened contact between the liposomes, and fusion between the outer membrane leaflets is initiated at the edge of the contact surface. There are several factors that may promote fusion at the contact edge, including strain that is relieved by fusing with the neighboring liposome and an increase in area per lipid giving more mobility to molecules in this region. While this finding of edge originated fusion is intuitive, it offers a contrasting view to that described in existing models. For example, Kuzmin and coworkers have suggested that formation of a nipple shaped protrusion in opposing bilayers, serves as a point of initiation for fusion, and leads to the formation of a stable intermediate. The present work suggests a second although not mutually exclusive mechanism in which first a membrane-membrane contact forms (here through an initially applied force). This causes membrane bending at the contact edge bringing two strained points on the membranes into close proximity where fusion initiates. Hence the point of fusion can be significantly distal to the initial point of closest approach.



Cross sectional images of fusion in plane of fusion (yz): A) Initial stalk at time t = 69 τs, growing stalks at B) 86 τs, C) 108 τs, and D) almost complete fusion of outer leaflets at t = 140 τs. Only lipids in outer leaflets are shown.

A somewhat surprising finding is that following the initial formation of the stalk, this structure expands highly asymmetrically along the strained membrane at the contact edge. In current fusion models the stalk forms in a central location and growth occurs radially outward from the center with cylindrical symmetry. A consequence of these dynamics is the formation of a partially confined solvent cavity between the two liposomes (see. Fusion Dynamics part C). Some of the confined solvent joins the external solvent, and some empties into the left liposome as its inner bilayer dissolves and an extended hemifused structure forms (cf. Fusion Dynamics part D). The simulations emphasize the three-dimensional nature of the process.



Stalk structure upon initiation of fusion. The left side is at t = 58 ms and right side at t = 66 ms. A) - D) close ups of lipid conformation in the stalk. These lipids have typically rotated or one of the tails has rotated so that the orientation better matches the stalk structure. The line in D) is a guide to the eye to indicate the ordering of the upper bilayer to become part of the stalk.

Splayed Tails Promote Initial Fusion

The simulations also provide direct insight into the dynamics of individual lipids during the early stages of fusion. To begin with there is a tilting of the lipids at the presumptive point of fusion, similar to that proposed for the modified stalk model (Kuzmin et al.), which appears to be facilitated by the local increase in the area per lipid. The first exchange between the membranes occurs when an aliphatic tail rotates out of the parent membrane and inserts into the opposing membrane, resulting in a tilted lipid with the aliphatic tails in a splayed (trans) conformation that is shared between the two fusing bilayers. This immediately suggests a mechanism for accommodating curvature strain on a molecular level. The possibility of splayed lipid conformations has been considered in some models for membrane fusion, however the picture that emerges here is distinctly different. In our simulations the splayed lipids tend to orient such that their aliphatic tails contact each other, thus creating the beginnings of a new hydrophobic core. As additional (tilted) lipids associate with the splayed bridging lipids, the aliphatic tails of these molecules extend into a more cis-like conformation, establishing a hydrophobic core (cf. Fusion Dynamics part C) and eventually forming a classical stalk (part F).

Conclusions

While many of the general features of our simulations agree well with models based on a continuum mechanics framework, the present work offers significant insights into the molecular details of the early stages of fusion. In particular the simulations suggest a model for specific molecular rearrangements that result in the formation of a stalk. This model builds on the notion of tilted lipids in a strained prestalk structure, which here facilitates the splaying of lipids to connect the two fusing membranes. A critical event is then the association of the aliphatic tails of splayed lipids, which nucleates a new hydrophobic core that spans the gap between the original membranes. This core grows as the chains extend and resolves completely the fusion between the outer leaflets. In addition, the importance of mechanisms that accommodate changes in membrane curvature is highlighted. Continuum models of the stalk do not provide a clear molecular view of how the stresses are resolved during the fusion process.

References

P. I. Kuzmin, J. Zimmerberg, Y. A. Chizmadzhev, F. S. Cohen, Proc. Natl. Acad. Sci. USA 98, 7235 (2001).

This work on membrane fusion is in collaboration with Tom Woolf and Jan Hoh at the Johns Hopkins Medical School.