Colloidal membranes are two dimensional (2D) surfaces composed of a one rod-length thick monolayer of aligned nanorods. Equilibrium formation of such structures requires assembly to readily propagate in two dimensions while self-limiting the third. Previous approaches towards assembly of colloidal membranes utilized chemically heterogeneous rods that mimic the dichotomic structures of amphiphilic molecules comprising biological membranes [1]. Here, we use computer simulations to demonstrate that structurally and chemically homogeneous hard rods can form equilibrium monolayers in the presence of depletant molecules, suggesting that geometry as well as chemical heterogeneity can be used to design assembly pathways of self-limited structures. Furthermore, we discover bounds on the molecular parameters that support formation of equilibrium membranes. These results have fundamental as well as practical significance. Extensive research has shown that hard particle fluids undergo entropy-driven assembly into a myriad of 3D structures [2–5]. Our work demonstrates that entropic forces can also drive formation of 2D structures. From a practical perspective, equilibrium colloidal membranes may enable manufacture of inexpensive and easily scalable optoelectronic devices [6].

Our study is motivated by recent experiments on suspensions of monodisperse rod-like colloidal viruses and the non-adsorbing polymer Dextran [7] (Fig. 1). *fd* viruses alone approximate the behavior of homogenous rods interacting with repulsive hard-core interactions [3]. The polymer induces an entropy-driven attractive (depletion) potential between the rods, the strength and range of which can be tuned by changing the polymer concentration and radius of gyration respectively (Fig. 1A) [3]. At high polymer concentrations viruses condense into smectic-like stacks of 2D membranes (Fig. 1B) [11]. With decreasing polymer concentration (attraction strength), individual 2D monolayers (membranes) within a smectic filament unbind, indicating that the membrane-membrane interaction switches from attractive to repulsive [7] (Fig. 1C).

Understanding the molecular origin of the repulsive membrane-membrane interactions is our primary goal. Experiments revealed significant protrusions of rods from isolated colloidal membranes, the magnitude of which could be tuned by changing the concentration of non-adsorbing polymer [7]. In contrast, these fluctuations were suppressed in stacked membranes. It was proposed that the entropy penalty associated with suppressing protrusion fluctuations of individual rods leads to repulsive interactions that stabilize isolated membranes under moderate osmotic pressure [11]. However, other plausible factors could also stabilize membranes, including attractive interactions between virus tips and depletant, repulsions due to bending (Helfrich) modes, or kinetic trapping of membrane intermediates. To elucidate these issues, we develop a computational model which demonstrates that protrusion interactions alone are sufficient to stabilize membranes in equilibrium. In contrast to the previous model which considered only protrusions of isolated rods [7], our work indicates that collective protrusion undulations dominate repulsive interactions between membranes. Surprisingly, the simulations predict that membranes are stable only for a certain range of rod aspect ratios and depletant sizes. We experimentally confirm the latter prediction.

We model the *fd* rods as hard spherocylinders with diameter \( \sigma \) and length \( L \). The non-adsorbing polymer is modeled as ghost spheres of diameter \( \delta \) which freely interpenetrate one another but behave as hard spheres when interacting with rods [12]. Compared with an effective pair potential approach, this model accounts for multi-rod interactions induced by polymers [13–15]. We perform Metropolis Monte Carlo (MC) with periodic boundary conditions [16]. The total number of rods \( N \) is fixed, the sphere osmotic pressure \( p_0 \) is set by insertion/deletion moves, and constant pressure is maintained in the \( xy \) plane by performing volume-change moves, while the box size is fixed in \( z \) direction [17]. Simulation results are reported with \( \sigma \) as the unit of length, \( k_B T \) as the unit of energy, and \( k_B T \sigma^{-3} \) as the unit of pressure.

Membrane-like structures have two generic repulsive interactions of distinct origin which dominate at different separation lengthscales. At large separations, slowly decaying low energy bending (Helfrich) modes dominate [18]. In contrast, at separations comparable to the monolayer thickness (the rod length), protrusions of molecules from the membrane surface generate the primary repulsive force [19]. We expect that long ranged bending modes provide a negligible contribution to the stabilization of colloidal membranes for the following reasons. First, the range of the depletion attraction...
that balances repulsive forces to drive membrane stacking is comparable to the depletant size. On this scale protrusion repulsions dominate. Second, the bending modes involve deviations of rods from the preferred direction and their interaction strength scales with bending modulus as

\[ f_{\text{bend}} \sim \kappa_b^{-1}. \]

The large bending modulus measured for fd membranes \( \kappa_b = 150 k_B T \) thus results in very weak Helfrich repulsions. Consistent with these arguments, a theoretical calculation (SI Fig. 5) shows that the protrusion interactions in simulated membranes exceed the strength of the Helfrich interactions by four orders of magnitude at relevant separations.

Based on the preeminence of protrusion modes, which do not involve rod tilting, in most simulations we restrict spherical orientations to be perfectly aligned along the \( z \) direction. This simplification greatly enhances computational efficiency, allowing us to extensively map the phase diagram as a function of all relevant molecular parameters. Our approximation is justified by Fig. 5 in the SI and the fact that simulations in which the fixed orientation constraint is relaxed predict similar phase behavior and membrane-membrane interactions (e.g. Fig. 3).

**Membrane-membrane interaction potential.** We first use umbrella sampling [16] to measure the free energy per rod

\[ f, \]

as a function of the separation between the centers of mass of two membranes, \( d \) (Fig. 2). At low osmotic pressures (e.g. \( \rho_s = 0.06 \)), \( f(d) - f(\infty) \) has no attractive region sufficient to overcome translational entropy; i.e., the stacking of disks is suppressed and the isolated colloidal membrane phase is stable. For larger osmotic pressures (\( \rho_s \gtrsim 0.08 \)), the free energy has a substantial minimum at finite membrane separation, signifying that membranes will stack to form the smectic-like columns. Consistent with these free energy results, unbiased simulations for these parameters resulted in two membranes which were respectively isolated and stacked at low and high osmotic pressures, as shown in Fig. 2 (right). The free energy and a representative snapshot are also shown for rods with orientational fluctuations at \( \rho_s = 0.06 \). Note that isolated membranes are stable and the interaction free energy is comparable to the case with parallel rods; the repulsion is slightly weaker with orientational fluctuations because they decrease the equilibrium areal rod density.

**Phase diagram.** We computed the equilibrium phase behavior as a function of osmotic pressure, rod aspect ratio, and sphere diameter as follows (Fig. 3). To identify the nematic-membrane phase boundary, we performed separate unbiased simulations starting from initial conditions in which (1) rods have random positions and (2) rods are aligned in a flat layer. For all results shown, the simulation outcomes were independent of initial conditions. To identify the transition from membranes to smectic filaments, a parameter set was considered to yield smectic layers if the total free energy of the attractive basin in the membrane-membrane interaction potential satisfies

\[ F \leq F_0 = k_B T \ln \rho_m v_0 \text{ with } \exp(-\beta F) = \int_{f(s)=0} ds \exp(-2\beta M f(s)), \]

\( M \) the number of rods in one membrane, \( v_0 \) a standard state volume, and \( \rho_m \) a membrane concentration. A finite value of \( F_0 \) accounts for membrane translational entropy. We roughly estimate \( M = 10^4 \) and \( \rho_m v_0 = 10^{-8} \) from the experimental conditions; the location of the phase boundary is not sensitive to the value of \( \rho_m v_0 \).

Fig. 3A illustrates the location of the equilibrium nematic
3. Phase diagrams from simulation and experiment. Triangles ▲ denote parameters that lead to nematic configurations, + symbols correspond to isolated membranes, and ■ symbols correspond to smectic layers. (A),(B) Phase diagrams determined from simulations for varying osmotic pressure \( p_s \) and (A) aspect ratio \( L \) with sphere diameter (polymer radius of gyration) \( \delta = 1.5 \), and (B) varying sphere diameter with \( L = 100 \). The solid lines identify the the isolated membrane/smectic and nematic/isolated membrane phase boundaries. They are fit by eye to simulation results except for the nematic/isolated membrane boundary in (A), which is a theoretical prediction [22]. (C) The experimental phase diagram corresponding to (B) using mixtures of \( d^7 \) viruses and PEG/PEO polymers. The final concentration of viruses was fixed at 5mg/mL and both polymer concentration and molecular weight were varied to change osmotic pressure and polymer radius of gyration, \( R_g \), respectively. As noted in the text, chiral structures such as helical ribbons which appear near the nematic and isolated membrane boundary are not shown.

In contrast, for \( \delta < 1.7 \) colloidal membranes are the equilibrium phase at intermediate depletant concentrations between a low osmotic pressure nematic phase and high osmotic pressure smectic filament phase. Decreasing the depletant size further below this critical value significantly expands the range of osmotic pressures for which colloidal membranes are stable. These results can be understood as follows. Increasing the depletant size expands the effective range of the attractive potential between two colloidal membranes, which in turn requires longer range repulsive interactions to stabilize colloidal membranes. For large enough depletant molecules, the repulsive protrusion interactions are not sufficiently long-ranged to overcome the attractive potential and colloidal membranes become unstable for all osmotic pressures.

**Origins of monolayer stability.** To understand the nature of the repulsive membrane-membrane interactions, we determine their functional form \( f_{pr} \) by subtracting the depletion interaction \( f_d \) from the measured membrane-membrane free energy, \( f_{pr}(d) = f(d) - f_d(d) \). The depletion term is given by \( f_d(d) = p_s\langle v_{ex}\rangle_d \), where \( v_{ex} \) is the volume excluded to spheres by rods, and \( \langle \cdot \rangle_d \) indicates an ensemble average over configurations at a particular separation \( d \). We then adapt a calculation in Ref. [21] to obtain the membrane-membrane interaction due to collective protrusions as \( 2\rho_{2d}f_{pr} = B \exp[-\pi\gamma(d - L)^2/3k_B T] \) with \( \gamma \) the surface tension, \( \rho_{2d} \) the area per rod, and \( B \) a constant. The calculations are presented in further detail in the SI. As shown in Fig. 3B the measured repulsive interaction \( f_{pr} \) is well described by this functional form, with fit values of \( \gamma \) that are close to the surface tension extracted from simulated height-height correlation spectra (SI Fig. 1). Thus, the membrane-membrane repulsion primarily arises from collective protrusion undulations.

**Experimental phase diagram.** Simulations predict a criti-
The protrusion interaction potential is well-fit by the theory in some parameter ranges. The dotted lines show the repulsive interaction potential $f_{\text{pr}}$ measured from simulations and the solid lines correspond to the best fit to the protrusion undulation potential given in the text with $B$ and $\gamma$ as fit parameters. Parameters are $L = 100$, $\delta = 1.5$ and (A) $p_s = 0.06$, (B) $p_s = 0.08$ and the best fit values are (A) $B = 0.8$, $\gamma^{-1} = 213$, (B) $B = 0.9$, $\gamma^{-1} = 156$.

The simulations also predict that the width of the isolated membrane phase depends strongly on aspect ratio and depletant size. While most previous simulations of hard rods considered small aspect ratios, our prediction of a critical aspect ratio below which the colloidal membrane phase disappears suggests that large aspect ratios are crucial for the phase behavior observed in Ref. [7]. The predicted critical aspect ratio is only qualitative, but can be tested by monitoring the phase behavior of depletant and rods with varying lengths, as the prediction of a critical depletant size was tested here.

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![Diagram](image-url)

**TABLE**

| Parameter | Value |
|-----------|-------|
| $L$       | 100   |
| $\delta$  | 1.5   |
| $p_s$     | 0.06  |
| $B$       | 0.8   |
| $\gamma^{-1}$ | 213 |

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1. S. Park, J. Lim, S. Chung, and C. Mirkin, Science **303**, 348 (Jan 16 2004).
2. P. Bolhuis and D. Frenkel, J. Chem. Phys. **106**, 666 (1997).
3. L. Onsager, Ann. N. Y. Acad. Sci. **51**, 627 (1949).
4. D. Chandler, J. D. Weeks, and H. C. Andersen, Science **220**, 787 (1983).
5. P. N. Pusey and W. Van Magen, Nature **320**, 340 (1986).
6. J. L. Baker, A. Widmer-Cooper, M. F. Toney, P. L. Geissler, and A. P. Alivisatos, Nano Lett. **10**, 195 (2010).
7. E. Barry and Z. Dogic, Proc. Nat. Acad. Sci. **107**, 10348 (2010).
8. K. R. Purdy, Z. Dogic, S. Fraden, A. Rühm, L. Lurio, and S. G. J. Mochrie, Phys. Rev. E **67** (2003).
9. S. Asakura and F. Oosawa, J. Chem. Phys. **22**, 1255 (1954).
10. D. Frenkel and T. Schilling, Phys. Rev. E **66** (2002).
11. J. N. Israelachvili and H. Wennerstrom, J. Phys. Chem. **96**, 520 (1992).
12. S. Asakura and F. Oosawa, J. Polym. Sci. **33**, 183 (1958).
13. S. V. Savenko and M. Dijkstra, J. Chem. Phys. **124**, 8 (2006).
14. A. Patti and M. Dijkstra, Phys. Rev. Lett. **102**, 128301 (2009).
15. A. Cuetos, E. Sanz, and M. Dijkstra, Faraday Discuss. **144**, 253 (2010).
16. D. Frenkel and B. Smit, *Understanding molecular simulation: from algorithms to applications*, 2nd ed. (Academic, San Diego, Calif.: London, 2002).
17. The number of rods is $N_r = 512$, except for simulations that examine finite size effects, in which $128 \leq N_r \leq 1152$, and with orientational fluctuations, in which $N_r = 1560$. For free energy calculations with orientational fluctuations and large aspect ratios, rods are allowed to interact with multiple periodic images of other rods (following Ref. [2]) and orientational fluctuations beyond a maximum angle are rejected to prevent any rod from interacting with itself. The maximum allowed angle is well beyond typical orientational fluctuations since rods in membranes are nearly aligned. Varying the maximal allowed angle showed that the constraint did not affect the free energy.
18. W. Helfrich and R. M. Servuss, Nuovo Cimento D **3**, 137 (1984).
19. R. Goetz, G. Gompper, and R. Lipowsky, Phys. Rev. Lett. **82**, 221 (1999).
20. W. Helfrich, Z. Naturforsch **33a**, 305 (1978).
21. S. Safran, *Statistical Thermodynamics of Surfaces, Interfaces, and Membranes* (Addison-Wesley Pub., 1994).
22. Y. Yang and M. F. Hagan, in preparation (2011).
23. Z. Dogic, K. R. Purdy, E. Grelet, M. Adams, and S. Fraden, Phys. Rev. E **69** (2004).

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*hagan@brandeis.edu*