Supplementary Information for

Controlling the shape and topology of two-component colloidal membranes

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This PDF file includes:
Supplementary text
Figs. S1 to S8 (not allowed for Brief Reports)
Legends for Movies S1 to S9
SI References

Other supplementary materials for this manuscript include the following:
Movies S1 to S9
Supporting Information Text

**Equilibrium equations.** The Euler-Lagrange equation for the energy (Eq. 4, main text) is given by

\[ \kappa (\Delta H + 2H^3 - 2HK) - \mu H = 0, \]  

where \( \Delta \) is the Laplace-Beltrami operator for surfaces (1).

Following the notation of Tu and Ou-Yang (1), we define an orthonormal frame \( \{ \hat{e}_1, \hat{e}_2, \hat{e}_3 \} \), where \( \hat{e}_3 \) is normal to the membrane, and \( \hat{e}_1 \) and \( \hat{e}_2 \) lie in the tangent plane to the membrane such that \( \hat{e}_1 \) is tangent to the edge of the membrane. The condition of vanishing shear force (normal to the membrane) at the edge is (1, 2)

\[
B \left[ \frac{d^2k_n}{ds^2} + k_n(k^2/2 - \tau_\kappa^2) + \tau_\kappa \frac{dk_n}{ds} + \frac{d(\tau_\kappa k_n)}{ds} \right] \\
+ c^* \left[ 2\tau_\kappa k_n - \frac{dk_n}{ds} \right] + B' \left[ 2\tau_\kappa^2 k_n - \frac{d}{ds}(\tau_\kappa k_n) \right] \\
+ \kappa \hat{e}_2 \cdot \nabla (2H) - \kappa \frac{d\tau_\kappa}{ds} - \gamma k_n = 0,
\]

where \( k_n \) is the normal curvature of the boundary, \( \tau_\kappa \) is the geodesic curvature of the boundary, \( \tau_\kappa \) is the geodesic torsion of the boundary and \( s \) is the arc-length parameter for the boundary. If \( \mathbf{T} \) is the tangent vector of the boundary (i.e., \( \hat{e}_1 = \mathbf{T} \) on the boundary) and \( \mathbf{n}_C \) is the membrane normal on the boundary, then \( k_n = \mathbf{n}_C \cdot d\mathbf{T}/ds \), \( k_\kappa = d\mathbf{T}/ds \cdot \mathbf{n}_C \times \mathbf{T} \), and \( \tau_\kappa = \mathbf{T} \cdot d\mathbf{n}_C / ds \). Note that \( k_n^2 + k_\kappa^2 = k^2 \). The condition of zero in-plane force (tangential to the membrane and normal to the edge) at the edge is (1, 2)

\[
B \left[ \frac{d^2k_\kappa}{ds^2} + k_\kappa(k^2/2 - \tau_\kappa^2) - \tau_\kappa \frac{dk_\kappa}{ds} - \frac{d(\tau_\kappa k_\kappa)}{ds} \right] \\
+ c^* \left[ \frac{dk_\kappa}{ds} + 2\tau_\kappa k_\kappa \right] + B' \left[ (k_n - 2H) \frac{d\tau_\kappa}{ds} + \frac{d}{ds}(k_n\tau_\kappa) + 2k_\kappa \tau_\kappa^2 \right] \\
- \left( \kappa/2 \right)(2H)^2 + kK + \mu + \gamma k_n = 0,
\]

and the condition of vanishing bending torque at the edge is (1, 2)

\[ 2\kappa H + \kappa k_n - B \frac{d\tau_\kappa}{ds} = 0. \]

**Numerical approach.** Here, we provide the details of the numerical solution of the ordinary differential equation (Eq. S3) to find the boundary \( r_{edge}(\phi) \) of the Enneper surface that satisfies the in-plane force balance. We express the equation as a first order system of ordinary differential equations and employ the MATLAB routine \texttt{bvp4c}. Since the equation is fourth order in \( r \) with two constraints, we can convert it into a six equation system by defining the auxiliary variables

\[
y_1 = r_{edge} \\
y_2 = \frac{dr_{edge}}{d\phi} \\
y_3 = k_\kappa \\
y_4 = \frac{dk_\kappa}{ds} \\
y_5 = A \\
y_6 = \mu,
\]

where \( A(\phi) \) is the area of the surface over the polar interval \([0, \phi]\) and \( \mu \) is the membrane tension. The equations that \( y_1, y_2, y_3, \) and \( y_4 \) satisfy are

\[
\frac{dy_1}{d\phi} = y_2 \\
\frac{dy_2}{d\phi} = F(y_1, y_2, y_3) \\
\frac{dy_3}{d\phi} = \frac{d}{d\phi} y_4 \\
\frac{dy_4}{d\phi} = \frac{ds}{d\phi} \frac{d^2k_\kappa}{ds^2}.
\]
Here, we have defined $F(y_1, y_2, y_3) = d^2 r_{\text{edge}}/d\phi^2$, which can be obtained by writing the definition of $k_\phi = y_3$ in terms of $r_{\text{edge}}$ and inverting; the exact expression for $F$ is cumbersome and is omitted here in the interest of brevity. For the Enneper surface geometry, $ds/d\phi = R[1 + y_i'^2](\sqrt{y_1^2 + y_2^2})$.

The differential equations for $y_6$ and $y_5$ are

$$\frac{dy_5}{d\phi} = \frac{R^2}{2} \left( y_1^2 + \frac{2y_1^{2(m+1)}}{m+1} + \frac{y_1^{4m+2}}{2m+1} \right)$$  \hspace{1cm} \text{[15]}$$

and

$$\frac{dy_6}{d\phi} = 0.$$  \hspace{1cm} \text{[16]}$$

The six corresponding boundary conditions to be enforced are: $A(0) = 0$ and $A(2\pi) = \pi R_0^3$, which come from the fixed area condition; and $y_i(0) = y_i(2\pi)$ for $i = 1, 2, 3, 4$, which amount to periodicity of $r_{\text{edge}}(\phi)$ and its derivatives. The disc of area $\pi R_0^2$ and zero tension is used as an initial guess. After the equations have been solved for a certain set of parameters, the solution is saved and used as an initial guess for a new problem with slightly perturbed parameters. Once the boundary coordinates are determined, energies are computed by numerically integrating the results with a trapezoidal quadrature rule.

The parameter $R$ is generally not known and must be selected through minimization of the energy. We use MATLAB’s constrained optimization routine `fmincon` to perform this task. There are potentially multiple solution branches of Eq. S3 that satisfy the boundary conditions. We often find, for example, a branch of relatively small amplitude solutions where a local minimum is typically located, in addition to a branch of higher energy, large amplitude solutions that may or may not have a local minimum. The two branches are separated by a steep energy barrier as $R$ decreases due to the large difference in the length of the boundary.

**Changing the Gaussian curvature modulus.** For completeness, we examine the theoretically predicted stable saddles at a hypothetical value of $\hat{\kappa} = 500$ $k_B T$ as well as a value of $\hat{\kappa} = 1500$ $k_B T$. These numbers were chosen to bracket the 1000 $k_B T$ value obtained from fitting the experimental edge profile. We keep the other parameters fixed at their aforementioned values. Figure S3 summarizes the simulations using these adjusted values of $\hat{\kappa}$. The general trend of increasing saddle order with increasing area is preserved. For $\hat{\kappa} = 500$ $k_B T$, we find a vein of lower order saddles that are possible, although they are not global minima. Increasing $m$ further causes such saddles to have higher energy than the disk, possibly due to the relative energies of different branches. After $m$ is increased even more, there is a “reentrance” phenomenon where the minimum can be found on a different branch and the saddles return to having lower energy than the disk. As with Fig. 4 (main text), these calculations do not account for non-Enneper surface shapes or other energetic barriers to formation. None of the minima exhibit self-intersection.

For $\hat{\kappa} = 1500$ $k_B T$, we find the overall trend to be very similar to Fig. 4 (main text). For a given $m$, the overall saddle orders are slightly lower. Again, none of the minima exhibit self-intersection.

**Deconvolution of widefield fluorescence z-stacks.** Z-stack images obtained by widefield microscopes are convolutions of ideal distortion-free images with point spread function (PSF) of the microscope. The significant axial width of the PSF in widefield microscopes hinders rendering and visualization of the structures in 3D due to significant contribution of light from out of focus planes. We perform an iterative deconvolution process on the experimentally obtained z-stacks to account for the effect of PSF.

We calculate the approximate 3D PSF from the Gibson & Lanni model (3), by inserting relevant microscopic parameters into the PSF generator plugin (4) in Fiji (a distribution of ImageJ software). The background fluorescence is deducted from the z-stack, and DeconvolutionLab2 plugin (5) is used to run Richardson Lucy deconvolution for 50 iterations.

In the Gibson & Lanni model, the PSF is dependent on the fluorophore height, $z_p$, measured from the coverslip. Although this value is not constant for the structures measured, we are limited by the plugin which accepts only a constant value. We optimize $z_p$ to minimize signal from defocused planes.

We further refine the images by adjusting the brightness and contrast levels. Line artifacts are observed in a few of the deconvolved structures (Fig. 9a, main text).

**Correction of focal plane shifts due to refractive index mismatch.** We observe our samples containing aqueous buffers with oil immersion objectives. The focal plane is changed by moving the objective in the z-direction. The refractive index mismatch between the immersion oil and the aqueous buffer causes the focal plane shift to be always smaller than the displacement of the objective. This results in an apparent elongation of the structures in the axial direction in both widefield and confocal microscopes.

We collect z-stack of small catenoid-like membranes (diameter $\approx 5 \mu m$). The symmetry axis of each catenoid-like shape is along $z$-axis and their diameters in $z$ and $y$ direction, $z_d$ and $y_d$ respectively, are measured. Catenoid-like membranes are axisymmetric objects, yet $z_d/y_d = 1.33$ for confocal z-stacks and 1.49 for widefield z-stacks. We assume that this scaling factor is constant in the range of observed structure sizes (6). The voxel depth is reduced by the factor 1.33 and 1.49 for confocal and widefield z-stacks, respectively, to correct for the axial scaling.

**Z-stack visualization.** All z-stacks are color coded by increasing the hue value of the stack slices with increasing $z$. We use 3D Viewer plugin (Fiji) for rendering (7) and record animations using Record 360 Degree Rotation tool.
Mesh creation from confocal z-stacks. The confocal images are 3D gauss smoothed on Fiji to reduce noise. A simple threshold operation is enough to binarize the z-stacks of smaller structures correctly. This method does not work well for larger structures, as the detected intensity decreases with increasing z. These structures are binarized using ilastik software (8). Z-stacks or raw images are resized by a factor of 5-10 before performing image processing operations such as rotation, registration, distance transformation, etc., to avoid pixelation.

We determine the shortest distance of each white voxel from the background voxels (black) in the binarized z-stack using the 3D Distance Transform tool. This distance is maximum at the mid-surface. The regions of maximum values in each slice of the distance-transformed z-stack are identified using Ridge Detector plugin (9). As this plugin works in 2D, it has trouble capturing the areas of mid-surfaces that are parallel to the z plane. We reslice the stack in the x and y directions as well, run the plugin, and combine all of the detected ridges to get a point cloud corresponding to the mid-surface.

This point cloud is then imported into Meshlab (10) and manually cleaned to remove outlier points. Poisson Disk Sampling and Compute Normal For Point Set tools are used to subsample the point cloud and calculate normal vectors. A mesh is constructed from the point cloud through the Screened Poisson Surface Reconstruction tool. Our mid-surface point cloud doesn’t represent a watertight surface, yet this tool attempts to create a watertight surface by extending the mesh far beyond the point clouds near the edges. The extended parts of the mesh are cleaned up with the Z-Painting tool. Mean and Gauss curvatures of the mid-surfaces are computed with Discrete Curvatures tool. The area of a mid-surface is measured with Compute Geometric Measures tool. The genus of the mid-surface mesh is determined with Compute Topological Measures tool.

Fitting Enneper surfaces to experimental saddle mid-surfaces. The order of a saddle, m, can be determined from observation, leaving R as the only unknown parameter in Eqs. 1-3 (main text). However, an experimentally obtained mid-surface will not, in general, be aligned with the Enneper surface. The mid-surface position and orientation naturally become fit parameters. Therefore, seven parameters, namely translation of the mid-surface along the x, y and z axes, rotation of the mid-surface about the x, y and z axes, and the R parameter of the Enneper surface are used to fit the experimental mid-surface to an Enneper surface described by Eqs. 1-3 (main text). We calculate the minimum distance of each point in the experimental point cloud from the theoretical Enneper surface. The average of the absolute distance values is minimized by a grid search method to optimize the seven parameters. Translation, rotation, and R parameters are refined up to 0.025 μm, 2º, and 0.07 μm during the optimization.

Fitting r-φ parameters to saddle mid-surface edge. We fit an Enneper surface to the experimental mid-surface, as described in the previous section, but this time keeping the x and y translation parameters fixed so that the center of the mid-surface coincides with the origin (0, 0). We calculate the valence of each vertex of the translated and rotated mid-surface mesh. Most of the boundary vertices can be extracted by selecting vertices with valence ≤ 3. The (r, φ) value for each boundary vertex (x_edge, y_edge, z_edge) is found by minimizing the quantity \[ |x(r, \phi) - x_{edge}|^2 + |y(r, \phi) - y_{edge}|^2 + |z(r, \phi) - z_{edge}|^2, \]
where \( x(r, \phi), y(r, \phi) \) and \( z(r, \phi) \) are given by Eqs. 1-3 (main text).

Determination of curvature of catenoid-like membranes from ρ-h data. We obtain widefield fluorescence z-stacks for catenoid-like membranes with their symmetry axis parallel to the focal plane. Light from out of focus planes is removed by deconvolution, and the stack is rotated about z axis, so that the catenoid symmetry axis is along y axis. The image in the middle of the stack is binarized, distance transformed, and the two mid-lines are calculated with ridge detection plugin (red curves in Fig. S6c inset). h is the coordinate along catenoid symmetry axis and \( \rho \) is the coordinate along an axis perpendicular to it. The origin of the ρ-h coordinate system is set to the centroid of the two mid-line data.

For surfaces of revolution, the mean curvature, H, and Gaussian curvature, K, are given by,

\[ K = \frac{-\rho''}{\rho (1 + \rho'^2)^2}, \]
\[ H = \frac{\rho \rho'' - (\rho^2 + 1)}{2|\rho| (\rho^2 + 1)^{3/2}}, \]
where \( \rho' = d\rho/dh \) and \( \rho'' = d^2\rho/dh^2 \) (11).

Measurement of rod orientation within catenoid-like membranes and saddles. Catenoid-like membranes and saddles are self-assembled using virus suspensions in which approximately 1 out of 30,000 long rods are fluorescently labeled. A series of composite phase-contrast/fluorescence images are captured for catenoid-like membranes with their symmetry axis parallel to the focal plane. The catenoid-like membranes move slowly within the sample chamber due to Brownian motion. Therefore, the image series needs to be aligned. This is established by setting the first phase-contrast image of the series as “fixed” and the subsequent images as “moving”. Multimode registration is performed using MATLAB’s imregtform (transform type: rigid) function. The transformation required for alignment is saved, and then applied to the fluorescence images with imwarp function.

The ρ-h data is extracted by binarization and distance transformation of the first phase-contrast image of the series. The binarization of fluorescence images yields rod position and angle between the rod and x axis of image through centroid and
orientation options within the regionprops function in MATLAB. The normal vector to the \( p-z \) curve at each rod position (equivalent to the surface normal vector, due to symmetry of the catenoid-like membrane) is calculated, and its absolute angular deviation from the rod vector, \( \theta \), is recorded.

Due to the helical nature of the experimentally observed saddles, there is no ideal imaging plane that symmetrically divides the saddle. We choose the imaging plane approximately in the middle of an edge-on saddle (Fig. S7a). The rod vector and normal vector to the mid-line of saddle wall are calculated using the method described above, though the calculated normal vector to the mid-line is only a projection of the surface normal vector, \( \hat{n} \).

The major axis length of each detected rod in the aligned and binarized fluorescence images is determined using regionprops function. This projected rod length increases with increasing distance from edge and then saturates. The saturation value (determined from \( y = a - be^{-x/c} \left( t \right) \) fit) is 1.3 \( \mu \)m, close to the known long rod length 1.2 \( \mu \)m. The twist angle, or the angle the rod vector makes with the imaging plane, is given as \( \cos^{-1} (\text{projected rod length}/1.3 \mu \text{m}) \). When the argument of inverse cosine function exceeds the value of one due to noise in the images, the twist angle is set to zero.

**Average position of short rods within the membranes.** We prepare samples using Dylight 488 labeled M13-wt rods and Dylight 550 labeled M13KO7 rods at \( n_{\text{short}} = 0.20 \), Dextran concentration 50 mg/ml. A catenoid-like membrane with its symmetry axis along the axial direction of the microscope is selected for imaging and 50 two-channel confocal images are captured at the catenoid midplane. Cross-talk between the two channels is negligible. The cross-sectional images (Fig. S8a-b) are binarized and the centroids are determined with the regionprops function. Dylight550 channel images are translated to align the centroids of images of both channels. Intensity profiles are calculated for lines passing through the centroid at varying angles and averaged over all angles. The plot of average intensity, \( I \), as a function of distance from centroid, \( x \), has two peaks. The peak positions are calculated by separating \( x > 0 \) and \( x < 0 \) data and taking a weighted average, \( \sum xI/\sum I \). The distance between the two peaks is the diameter of the cross-section image. If the curvature of the membrane is generated due to collective migration of short rods towards the inner(outer) wall of the catenoid-like membrane, the Dylight488 channel image should have smaller(larger) diameter than the Dylight550 channel image. Experimentally, the diameter difference between images of the two channels is 4 \pm 54 \text{nm}. The negligible diameter difference indicates that the short rods prefer to stay near the midplane of the catenoid wall.

Though, rod splay in the catenoid-like membrane affects the intensity profile as well as the measured diameter difference. We assume that all rod midpoints lie exactly on the mid-surface of the catenoid wall, and simulate the effect of rod splay on the intensity distribution along the \( x \) axis.

We first discuss the effect of rod splay in the \( x-y \) plane, ignoring splay in other planes. Assume \( r \) is the catenoid radius at \( z = 0 \) plane and \( L \) is the rod length. If there are \( N \) rods emitting \( m \) photons per unit length, radially oriented at \( z = 0 \) plane, the number of photons collected by each pixel of area \( l \times l \) along the \( x \) axis between \( r - L/2 < x < r + L/2 \) is proportional to

\[
\left( \frac{N}{2\pi x} \right) \times (lm).
\]

Thus the intensity profile along \( x \) axis in the interval \( r - L/2 < x < r + L/2 \) varies as \( I_{x,y}(x) \propto 1/x \) (Fig. S8d blue curve).

We now calculate the effect of rod splay in the \( z-x \) plane alone. Composite phase-contrast/fluorescence microscopy data is collected for a catenoid-like membrane of similar size to determine the rod splay in the \( z-x \) plane. A plot of the angle of rod vectors with the \( z = 0 \) plane, \( \theta_x \), as a function of the \( z \) coordinate of the rod midpoint, \( z_m \), is linear. The best fit equation is \( \theta_x = z_m \times 22^\circ/\mu \text{m} \). The pinhole aperture of the confocal microscope during data collection is 1.6 AU, and theoretically the optical section thickness at this aperture is 736 nm (12). For simplicity, we assume every fluorophore within a 736/2 nm \( = 368 \text{nm} \) axial distance from the focal plane (\( x-y \) plane) is visible at the focal plane.

For a rod with its midpoint at height \( z_m \), the total detected fluorescence emission within \( x \) to \( x + \Delta x \) can be expressed as,

\[
\Phi(x, \Delta x, z_m) \propto \Delta x / \cos \theta_x,
\]

when \(-368 \text{nm} < z_m + (x - r) \tan \theta_x < 368 \text{nm} \). If this condition is not satisfied, the rod element is outside of the optical section and \( \Phi(x, \Delta x, z_m) = 0 \).

Assuming a constant rod midpoint density in the \( z \) direction, the total intensity transmitted is the integral of \( \Phi(x, \Delta x, z_m) dz_m \),

\[
I_{x,z}(x) \propto \int_{z_m = -z_{\text{max}}}^{z_{\text{max}}} \Phi(x, z_m) dz_m,
\]

where \( z_{\text{max}} \) is the absolute value of \( z \)-coordinate at top/bottom edge of the catenoid.

The total intensity contribution, approximated as the product of \( I_{x,z} \) and \( I_{x,y} \), resembles a top hat (Fig. S8d black curve).

As the distribution is symmetric with respect to \( x = r \), it should not affect the diameter difference result.
Fig. S1. Helicoid-like shape of saddles. 3-D rendered deconvolved z-stack (widefield) image of a saddle self-assembled at \( n_{\text{short}} = 0.20 \) and 50 mg/ml Dextran concentration. Scale bar, 2 µm.
Fig. S2. Effect of short rod inclusion on line tension of membranes. Fluctuation spectra of edges of flat colloidal membranes composed of only M13KO7 rods (black circles) and uniform mixtures of 90% M13KO7 and 10% M13-wt rods (red circles) assembled at 47.5 mg/ml Dextran concentration. Fit to equation $\langle a^2_q \rangle = k_B T/(\gamma + Bq^2)$ (solid lines) yields line tension $\gamma$ and bending rigidity $B$. Uniformly mixed bi-disperse membranes have $\gamma = 620 \pm 20$ $k_B T/\mu$m and $B = 150 \pm 10$ $k_B T/\mu$m. Membranes composed of only M13KO7 rods have a comparatively lower $\gamma = 550 \pm 10$ $k_B T/\mu$m and $B = 130 \pm 10$ $k_B T/\mu$m.
Fig. S3. Stability of Enneper surfaces at various Gaussian moduli. Blue dots indicate the existence of an Enneper surface that is stable and lower energy than a disk of the radius $R_0 = \sqrt{A/\pi}$; red dots indicate such a stable saddle of the lowest energy; for most areas, this happens at the maximum allowable $m$. Energies were computed for a membrane with $\gamma = 620 \ k_B T/\mu m$, $B = B' = 150 \ k_B T \mu m$, $c^* = 100 \ k_B T$, and (a) $\bar{\kappa} = 500 \ k_B T$ or (b) $\bar{\kappa} = 1500 \ k_B T$. Saddles of order up to and including $m = 11$ were compared in this plot.
Fig. S4. Sponge-like structures observed at higher virus concentrations. Samples were made at $n_{\text{short}} = 0.20$, 3 mg/ml virus concentration and 50 mg/ml Dextran concentration, along with sample tilting to promote coalescence. The green bounding box dimension is 212 µm × 212 µm × 23 µm.
Fig. S5. Mid-surfaces of structures shown in figs. 2 and 6 of main text, colorized with mean and Gaussian curvatures. (a-c) 3rd order, 4th order, and 5th order saddle surface. (d-f) Catenoid, 3-noid, and 4-noid like surfaces. (g-i) Membranes with topology of saddle with a handle, catenoid with a handle, and 3-noid with a handle. (j-k) Two complex surfaces of genus 2, with 2 and 4 boundaries, respectively. (l) A catenoid-like membrane with wavy edge. Colormaps are shown on the left.
Fig. S6. Effect of catenoid-like membrane's size on its geometry and curvatures. (a) A bulge at the midsection develops in larger catenoid-like membranes even at \( n_{\text{short}} = 0.20 \) and Dextran concentration 50 mg/ml. Scale bars, 2 μm. Variation of (b) the angular deviation between the surface normal (\( \mathbf{n} \)) and the rod vector projection (\( \mathbf{r}_{yz} \)), (c) Gaussian curvature and (d) mean curvature, as functions of the arclength, for catenoid-like membranes of different heights assembled at same condition as (a). Inset of (c) shows a deconvolved fluorescence image of catenoid-like membrane, and the \( \rho-h \) axes. The arclength in (b-d) was measured from the midsection (i.e., from \((\rho_h = 0, 0)\) point) along the catenoid wall. Data for each curve in (b-c) was averaged over 5 catenoid-like membranes.
Fig. S7. Rod orientation in saddles of order \( m = 1 \). (a) An ideal saddle and its mid-plane. (b) A saddle-shaped membrane imaged at its mid-plane with phase-contrast microscopy. The randomly colored lines show the rods detected with fluorescence microscopy. Scale bar, 2 µm. (c) Plot of \( \theta \), the angle between the mid-plane projection of the surface normal, \( \mathbf{n}_{xy} \), and the mid-plane projection of rod vectors, \( \mathbf{r}_{xy} \). Distance was measured from the edge along the saddle wall. The membrane was self-assembled at \( n_{\text{short}} = 0.20 \) and 50 mg/ml Dextran concentration.
Fig. S8. Short rods do not preferentially reside near the outer or inner surface of the catenoid-like membranes. Self-assembled catenoid-like membranes composed of Dylight 488 labeled M13-wt rods and Dylight 550 labeled M13KO7 rods are imaged using different excitation wavelengths in a confocal microscope. Cross-section of a catenoid-like membrane at \( z = 0 \) plane is shown in (a) with only short rods visible, and in (b) with only long rods visible. Scale bar, 2 \( \mu \text{m} \). (c) Angular averaged intensity profiles measured along lines passing through the centroid (red line in (a)). Diameter of the cross-section images (a-b) is given by the distance between the two peaks in the intensity plots. Experimentally, no proof of short rod migration is found as the diameter difference is negligible (4 ± 54 nm). (d) Intensity distribution of a catenoid-like membrane due to rod splay in \( z-x \) plane (red line) and \( x-y \) plane (blue line). As the resultant intensity distribution (black line) resembles a top-hat profile, the effect of the rod-splay in (c) is negligible.
Movie S1. Coalescence of two saddle-shaped membranes into a larger saddle-shaped membrane as viewed in DIC imaging. The newly formed saddle then coalesces with another saddle to form a catenoid-like membrane. Scale bar, 2 µm.

Movie S2. 3D views of intermediate structures formed during coalescence of two saddles into a catenoid-like membrane. These images have been rendered by deconvolving fluorescence microscopy z-stacks. Scale bar, 2 µm.

Movie S3. Coalescence of two saddle-shaped membranes into a 3rd order saddle, as viewed in DIC imaging. Scale bar, 2 µm.

Movie S4. Coalescence of a saddle with a catenoid-like membrane to form a tri-noid, as viewed in DIC imaging. Scale bar, 2 µm.

Movie S5. 3D rendered views of topologically complex cubosomes of different genus values, g, and number of boundaries, b: (a) catenoid, 3-noid and 4-noid like surfaces; (b) membranes with topology of saddle with a handle, catenoid with a handle, and 3-noid with a handle; (c-d) two complex surfaces of genus 2, with 2 and 4 boundaries, respectively. The z-stacks have been captured using confocal microscopy. Scale bar, 2 µm.

Movie S6. Top (a) and side views (b) of a network-like structure, resembling a sponge phase, with genus 41. The layers are consecutively removed to reveal the periodic structure. The z-stacks have been captured using confocal microscopy. Scale bar, 5 µm.

Movie S7. Dynamics of long rods within a catenoid-like membrane imaged using composite phase-contrast/fluorescence imaging. Approximately 1 long rod out of 30,000 long rods is fluorescently labeled. Phase-contrast and fluorescence images are acquired simultaneously and are subsequently overlaid. Fluorescently labeled rods appear as green segments. Scale bar, 2 µm.

Movie S8. Reversible shape transformations induced by changes in temperature, as viewed in DIC imaging. A 3rd order saddle membrane transforms into a flat membrane on heating and vice versa on cooling of the sample. Scale bar, 2 µm.

Movie S9. 3D views of a membrane, assembled with PEG 35K as depletant, at different temperatures. Increasing osmotic pressure of the depletant with decreasing temperature increases the surface curvature of the membrane. These images have been rendered by deconvolving fluorescence microscopy z-stacks. Scale bar, 2 µm.

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