



Supporting Information for

Dynamic bidirectional coupling of membrane morphology and rod organization in flexible vesicles

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Movies S1 to S5

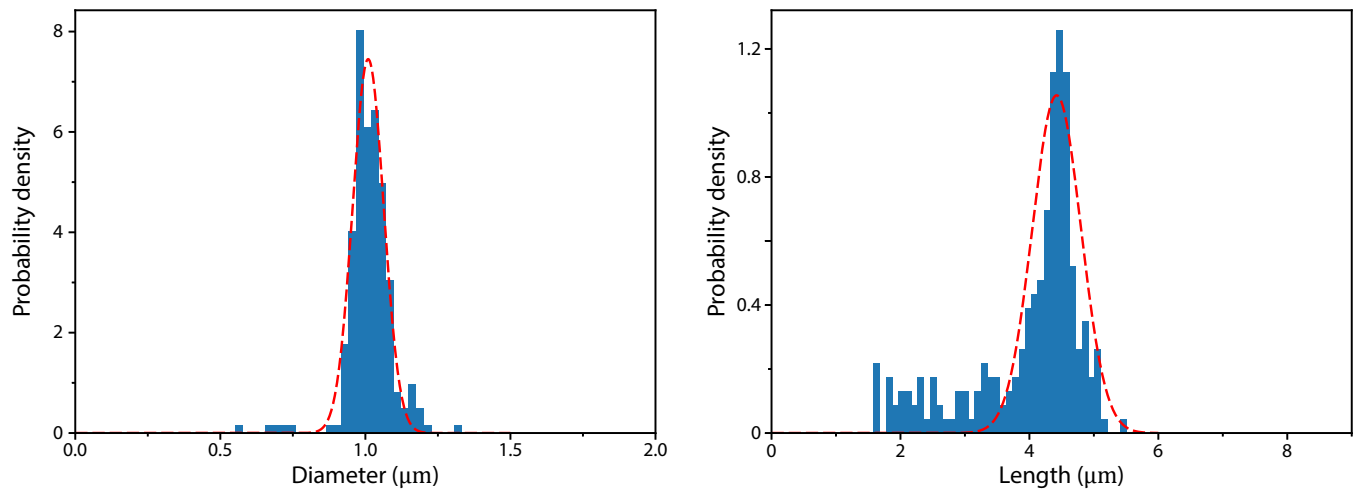


Fig. S1. Probability density distribution function of rod diameter (left panel) and rod length (right panel) as measured from SEM images. A total of ~ 200 rods were measured. Both distributions were fitted with a normal distribution, such that we find for the diameter $D = 1.01 \pm 0.05 \mu\text{m}$ and for the length $L = 4.42 \pm 0.38 \mu\text{m}$, where the error denotes the standard deviation. The average rod length of the full population is slightly shorter $\langle L \rangle = 3.99 \mu\text{m}$, due to an extended left-side tail, causing some deviation from the normal distribution, while the average diameter $\langle D \rangle = 1.01 \mu\text{m}$ is the same.

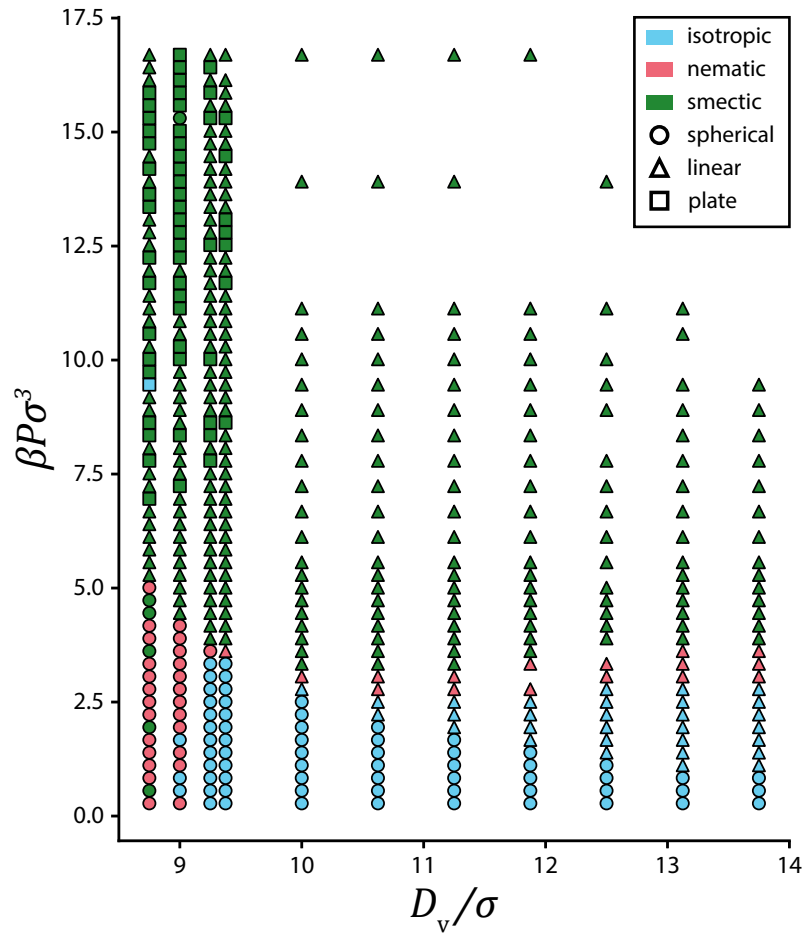


Fig. S2. Simulated phase diagram of rod order and vesicle shape as a function of the dimensionless vesicle diameter (D_v/σ) versus the dimensionless pressure ($\beta P \sigma^3$) for $N = 30$ and $L/D = 5$. The colors indicate the internal rod ordering: blue for isotropic, red for nematic, and green for smectic. The symbols denote the vesicle shape: circles for spherical, triangles for linear, and squares for plate-like vesicles.

Table S1. Measurement parameters corresponding to the experimental vesicle configurations shown in the snapshots of Fig. 2 along with their corresponding shape and rod order classification.

Vesicle	V_{ves}	A_{ves}	ν	N	η	b/R_g^2	c/R_g^2	Shape	$\langle S_i \rangle$	$\langle \tau_i \rangle$	Order
1	1352	610	0.95	55	0.13	0.081	0.702	Sphere	0.19	0.13	Isotropic
2	539	353	0.86	20	0.12	0.376	0.106	Linear	0.29	0.22	Isotropic
3	648	525	0.57	32	0.15	0.883	0.002	Linear	0.81	0.56	Smectic
4	310	238	0.90	25	0.25	0.150	0.169	Sphere	0.63	0.49	Smectic
5	371	348	0.61	33	0.28	0.801	0.026	Linear	0.73	0.54	Smectic
6	184	176	0.84	18	0.30	0.359	0.113	Plate	0.66	0.50	Smectic

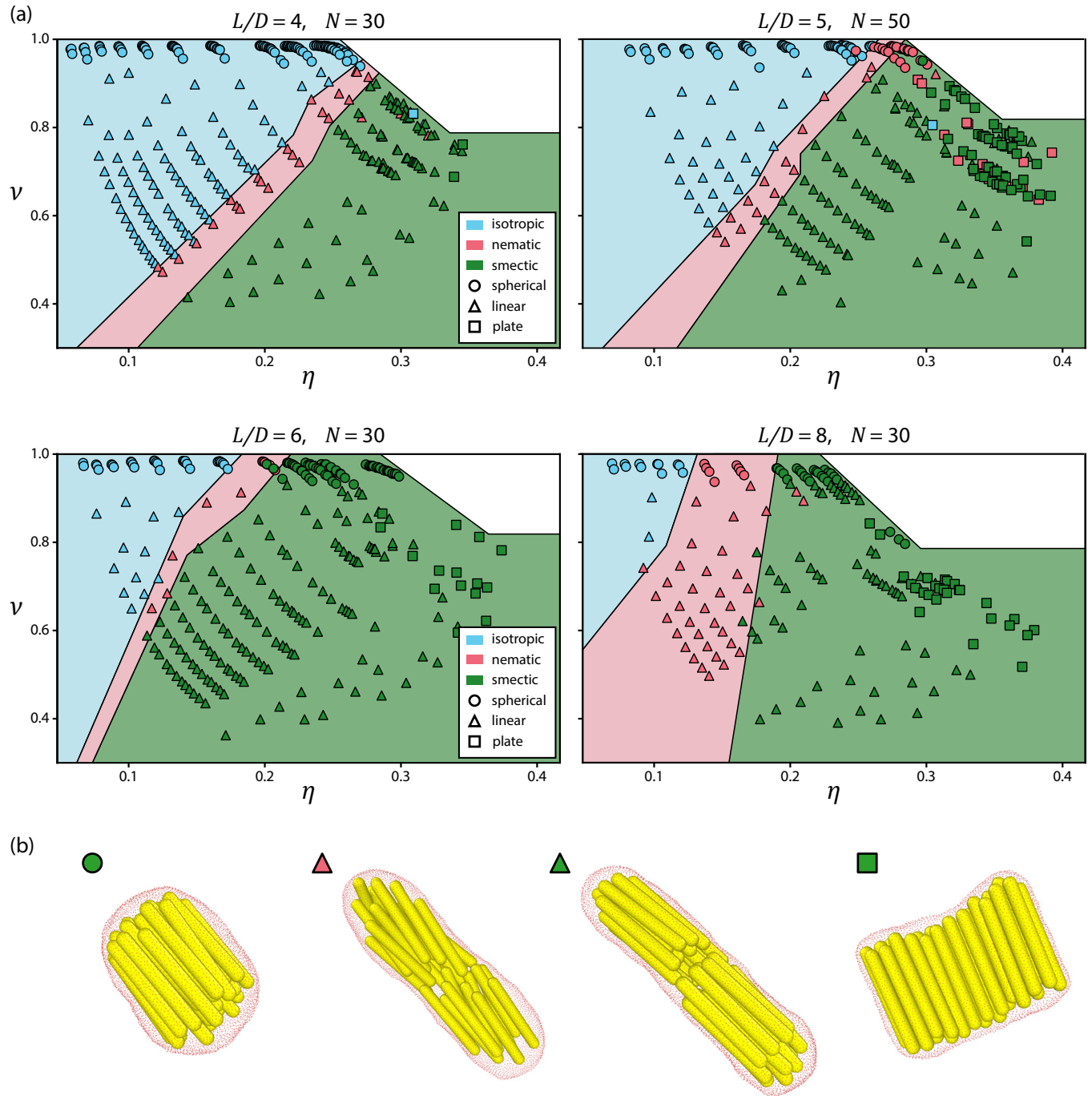


Fig. S3. (a) Phase diagrams of rod order and vesicle shape as a function of packing fraction η and the vesicle's reduced volume ν , obtained from simulations, for various rod numbers and aspect ratios, as indicated above each respective panel. The top left panel, bottom left panel, and bottom right panel, show the phase diagram for vesicles encapsulating $N = 30$ rods with $L/D = 4$, $L/D = 6$, and $L/D = 8$, respectively. The top right panel shows the corresponding plot for vesicles encapsulating $N = 50$ rods with $L/D = 5$. The colors indicate the internal rod ordering: blue for isotropic, red for nematic, and green for smectic. The symbols denote the vesicle shape: circles for spherical, triangles for linear, and squares for plate-like vesicles. The background colors denote the approximate boundaries of the different ordering regimes. (b) Simulation snapshots of a smectic sphere, nematic linear, smectic linear, and smectic plate-like morphology for rods with aspect ratio $L/D = 8$, corresponding to the bottom-right panel in (a).

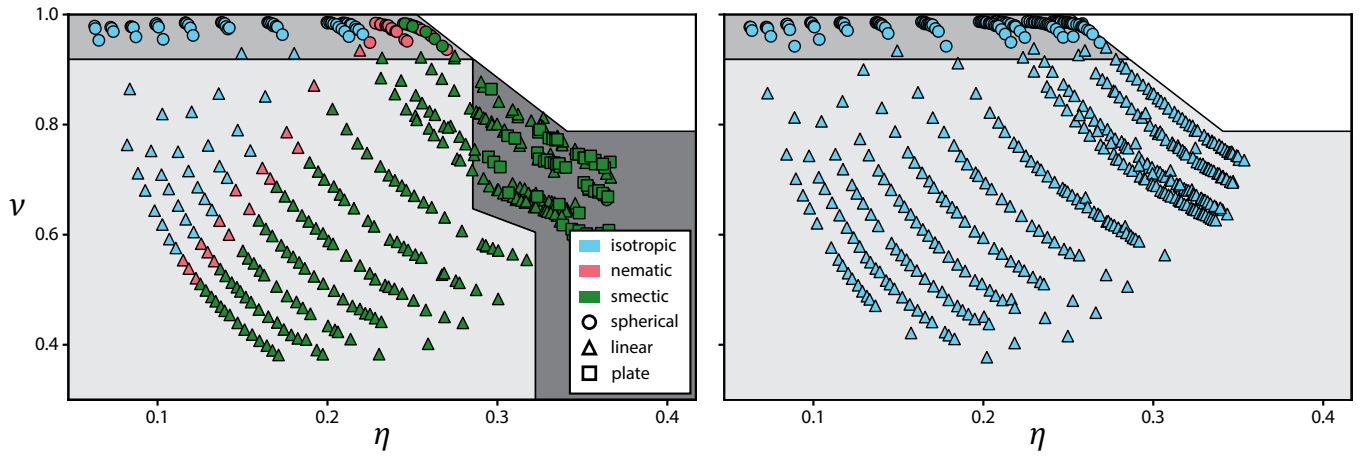


Fig. S4. Phase diagram of rod order and vesicle shape as a function of packing fraction η and the vesicle's reduced volume ν , obtained from simulations. The left panel shows the phase diagram for vesicles encapsulating rods ($N = 30$, $L/D = 5$), while the right panel shows the corresponding plot for vesicles encapsulating an equivalent volume of spheres ($N_{\text{sph}} = 150$, $L/D = 1$). The colors indicate the internal particle ordering: blue for isotropic, red for nematic, and green for smectic. The symbols denote the vesicle shape: circles for spherical, triangles for linear, and squares for plate-like vesicles. The background colors denote the approximate boundaries of the different vesicle shape regimes, distinguishing spherical (grey), linear (light grey), and linear/plate (dark grey) regions.

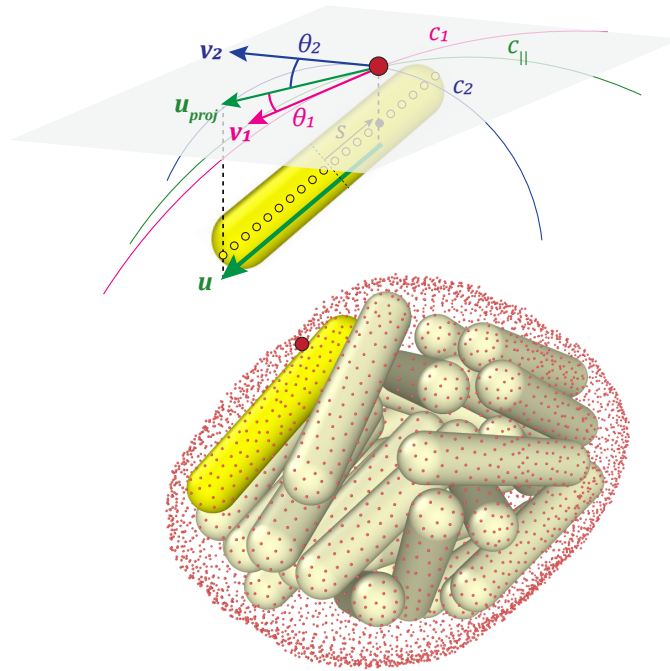


Fig. S5. Schematic illustration of the measurement of $c_{\parallel}(s)$ for a single rod segment. The rod (yellow) is divided into 20 segments (yellow circles). For a given segment (black circle), located at a distance s from the rod center, the nearest membrane vertex is identified (red circle). If this vertex lies within a cutoff distance of 1.5σ , the local curvature $c_{\parallel}(s)$ is recorded. Each membrane vertex is characterized by two orthogonal principal curvatures, c_1 and c_2 , which define the local tangent plane of the membrane. The rod orientation vector (green arrow) is projected onto this tangent plane, and the membrane curvature along the projected direction is computed as $c_{\parallel} = (\theta_1 c_2 + \theta_2 c_1) / (\theta_1 + \theta_2)$, where θ_1 and θ_2 are the angles between the projected rod orientation and the principal curvature directions. This procedure is applied to all segments of each rod and subsequently repeated for all rods in the vesicle.

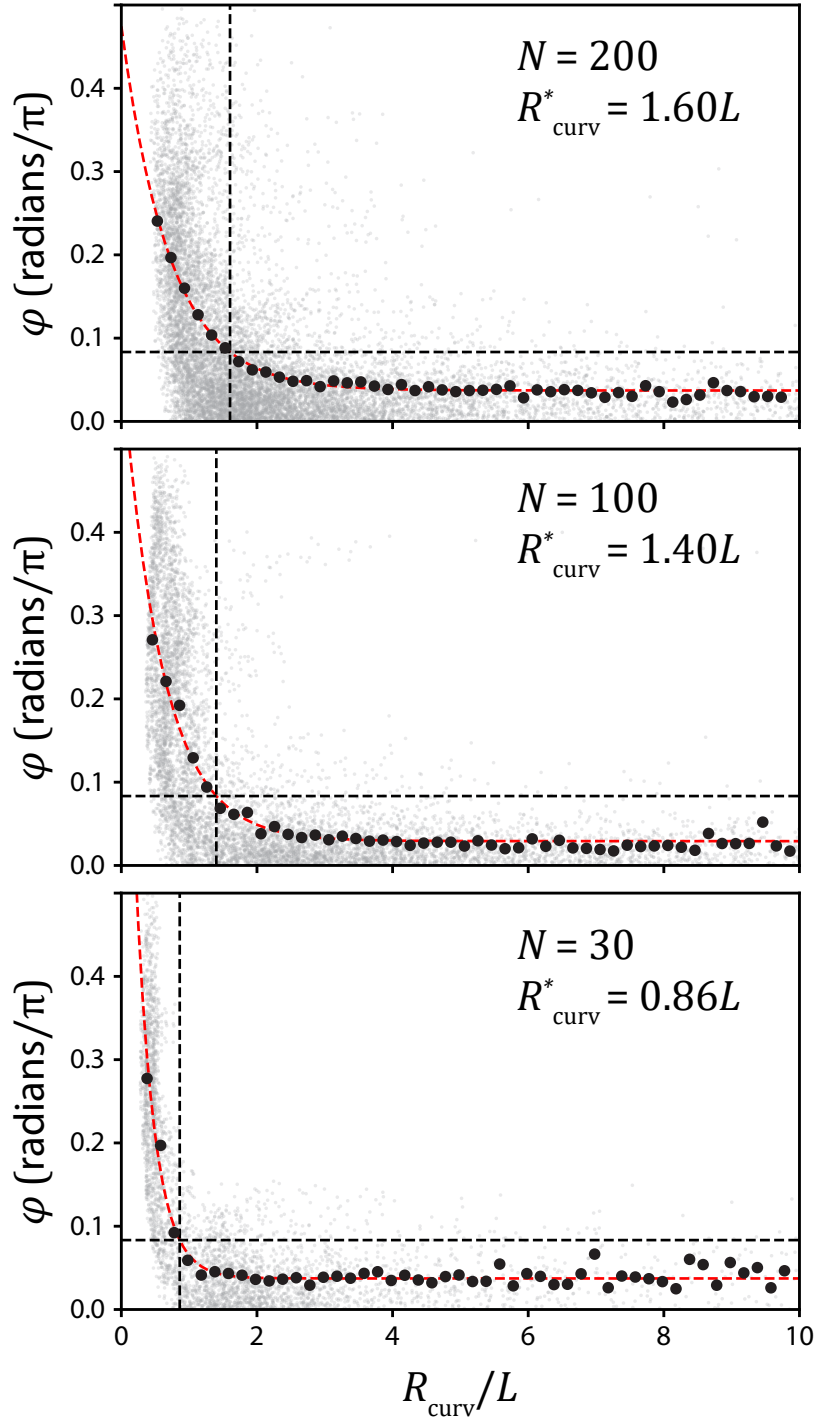


Fig. S6. The angle φ , defined as the angle between the orientation vector of a rod and its projection onto the tangent plane of a nearby membrane vertex, plotted as a function of the vertex radius of curvature measured along the direction of the projected rod vector, $R_{\text{curv}} = c_{\parallel}^{-1}$ (see Supporting Fig. S5). Grey points denote membrane vertices located within 1.5σ of at least one rod. The data are binned with width σ and fitted to an exponential decay (red dashed line). Results are shown for three system sizes: $N = 200$, 100, and 30, from top to bottom. The critical radius of curvature, R_{curv}^* , indicated by black dashed lines in each panel, is defined as the value of R_{curv} at which φ reaches 15° (i.e., $\varphi = 0.08\pi$).

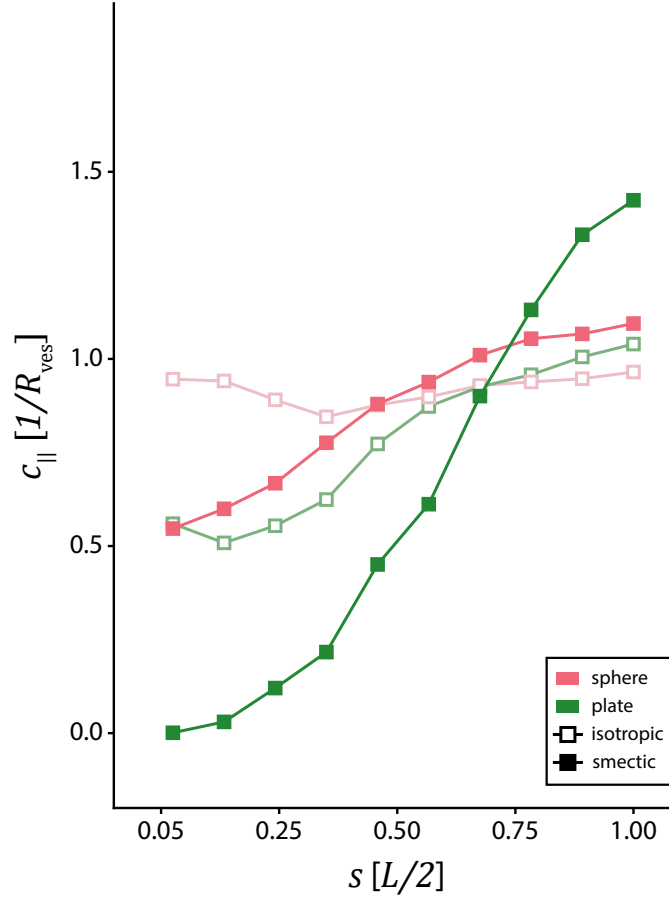


Fig. S7. Correlation between position along the rod, s , and the membrane curvature along the rod axis, $c_{||}$, for simulated spherical (red) and plate-like (green) vesicle morphologies. Data are averaged over all rod segments located within 1.5σ of the membrane. Smectic packings are indicated by filled squares, while isotropic packings at comparable reduced volumes ν are shown as open squares. Because the plate-like morphology occurs only at high packing fraction, the isotropic comparison is made using a linear vesicle with a similar ν . For the spherical vesicle, the smectic and isotropic cases correspond to $\nu \approx 0.97$ with packing fractions $\eta = 0.25$ and $\eta = 0.07$, respectively. For the plate-like vesicle, the corresponding smectic and isotropic cases have $\nu \approx 0.77$ with $\eta = 0.36$ and $\eta = 0.08$, respectively.

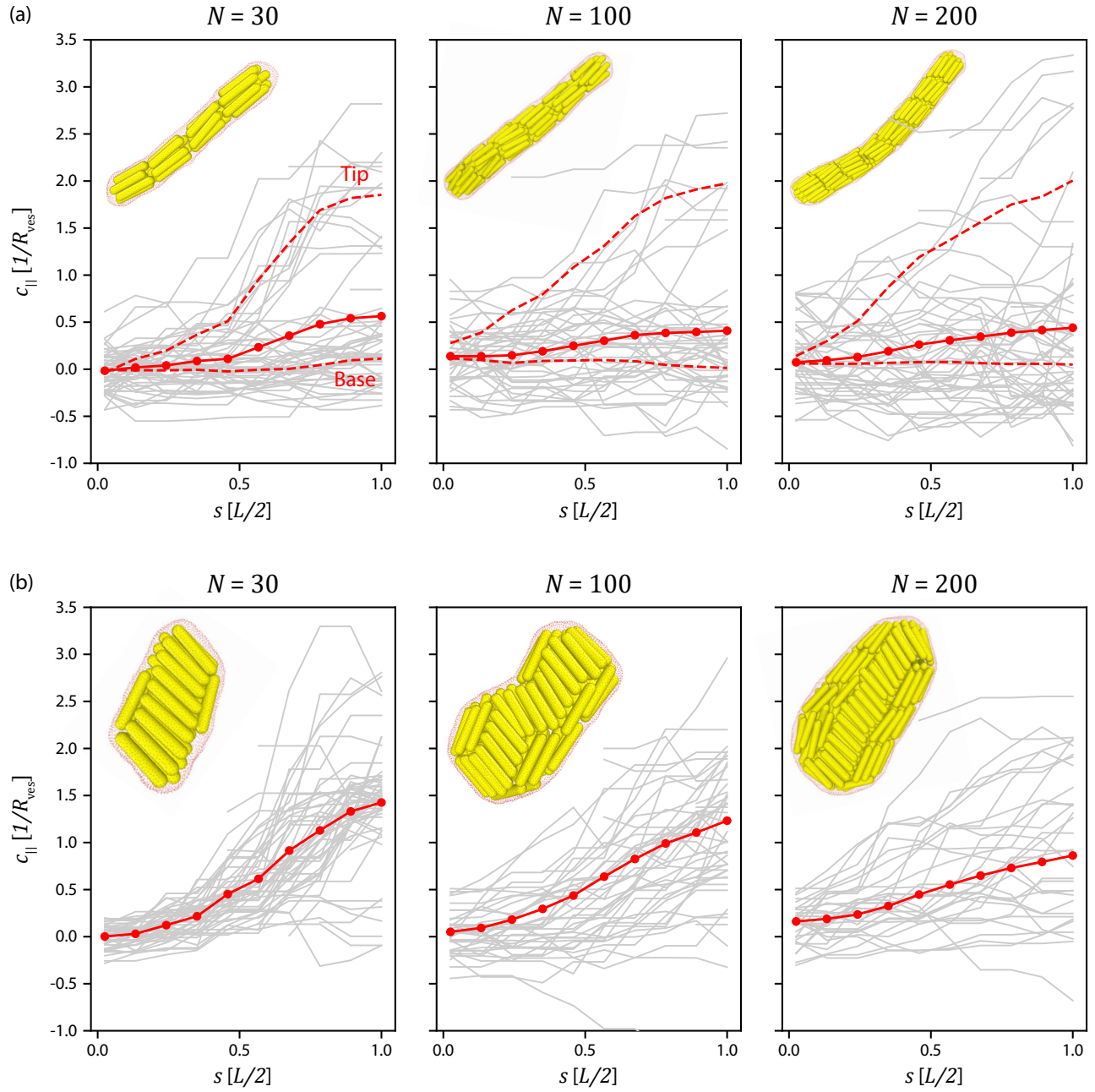


Fig. S8. Correlation between the position along the rod, s , expressed in units of $L/2$, and the membrane curvature along the rod axis, $c_{||}$ (see Methods), for linear (a) and plate-like (b) vesicles encapsulating $N = 30$, 100, and 200 rods. Red lines with circular markers show averages over all rod segments located within $1.5 \mu\text{m}$ of the membrane, while gray lines represent $c_{||}(s)$ for individual rods. For the $N = 100$ and 200 cases, a randomized subset of approximately 30 rods is shown to avoid visual clutter. For linear vesicles, rods located near the vesicle tips and along the base exhibit distinct curvature responses, highlighted by dashed red lines. Insets show representative vesicle morphologies and corresponding rod organization.

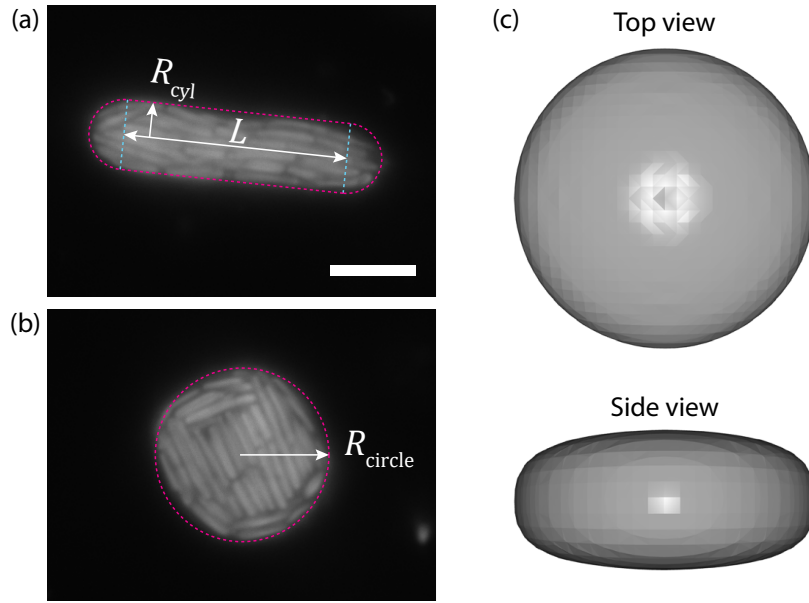


Fig. S9. Volume and membrane area estimation of the vesicle before and after area expansion. a) After area expansion, the vesicle is approximated as a spherocylinder with length $L \approx 17.25 \mu\text{m}$ and radius $R_{cyl} \approx 2.25 \mu\text{m}$. The final volume and surface area are then estimated as $V_f \approx \pi R_{cyl}^2 L + 4/3 \pi R_{cyl}^3 = 251 \mu\text{m}^3$ and $A_f \approx 2\pi R_{cyl} L + 4\pi R_{cyl}^2 = 244 \mu\text{m}^2$, respectively. b) Prior to area expansion, the vesicle is assumed to be a flattened circular plate with radius $R_{sph} \approx 5 \mu\text{m}$, which is modeled as a superellipsoid $\left(|x/R_{sph}|^2 + |y/R_{sph}|^2\right)^{3/2} + |z/a_3|^3 = 1$, with $a_3 = 2 \mu\text{m}$. This geometry yields an initial volume $V_i \approx V_f \approx 255 \mu\text{m}^3$ and surface area $A_i \approx 220 \mu\text{m}^2$. The corresponding reduced volumes are $\nu_i = 0.82$ initially and $\nu_f = 0.70$ after expansion, corresponding to an approximate 11% increase in membrane area. c) Top and side views of the superellipsoid. The scale bar is $5 \mu\text{m}$.

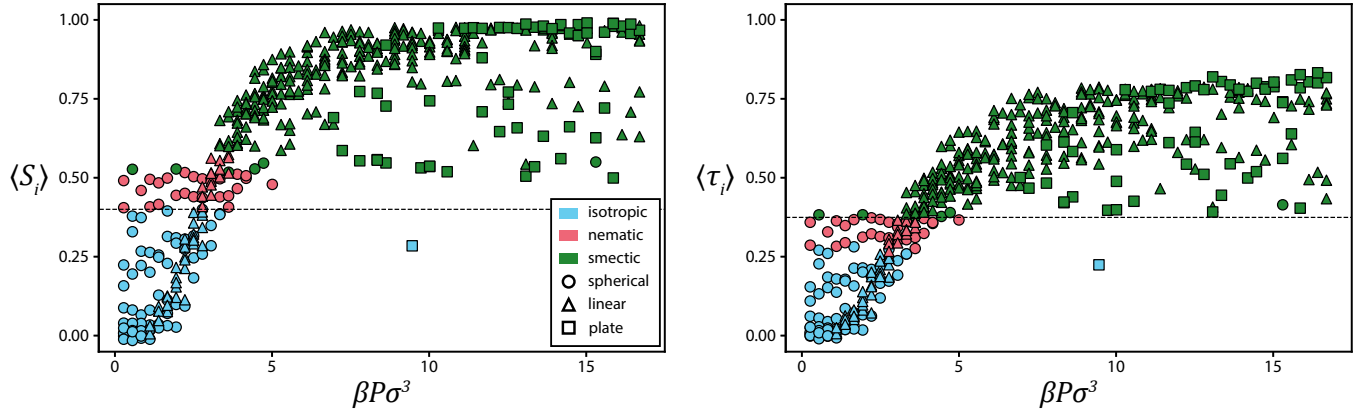


Fig. S10. Average local nematic order parameter $\langle S_i \rangle$ (left panel) and smectic order parameter $\langle \tau_i \rangle$ (right panel) as a function of the dimensionless pressure ($\beta P \sigma^3$) for $N = 30$ and $L/D = 5$. The dashed lines represent the threshold values $S_{\text{crit}} = 0.4$ (left panel) and $\tau_{\text{crit}} = 0.375$ (right panel) that were used to determine the rod order. The colors indicate the internal rod ordering: blue for isotropic, red for nematic, and green for smectic. The symbols denote the vesicle shape: circles for spherical, triangles for linear, and squares for plate-like vesicles.

Movie S1. Two channel composite confocal fluorescence movie illustrating encapsulated FITC-coated silica rods (green) inside a RITC-labelled GUV (red). The movie shows isotropic rod packing in a spherical vesicle (top left), smectic rod packing in a spherical vesicle (top right), and smectic rod packing in a linear vesicle (bottom). The movies were recorded at a rate of 7 fps and are played back at 10 fps.

Movie S2. Simulation protocol for six different combinations of vesicle size and pressure with $N = 30$ rods. The simulation starts with the rods ordered and the pressure increase up to the desired one, visible by the changes in the box dimensions. After equilibrium is reached we take time averages of the relevant quantities. From top left to bottom right we have an isotropic sphere, a smectic sphere, a smectic plate-like, an isotropic linear, a nematic linear, and a smectic linear vesicle.

Movie S3. Numerical simulation of a vesicle with $N = 30$ rods illustrating the morphology change when the pressure is increased. Representative snapshots are in Fig. 5.

Movie S4. Fluorescence microscopy movie illustrating the morphology change of a GUV encapsulating SU-8 rods as the membrane area increases. The movie was recorded at a rate of 5 fps and is played back at 10 fps. Representative snapshots are in Fig. 5.

Movie S5. Numerical simulation of a vesicle with $N = 200$ rods illustrating the morphology change when the vesicle surface area increases 10%. Representative snapshots are in Fig. 5.