Electronic Supplementary Information

Minimum-energy Vesicle and Cell Shapes Calculated using Spherical Harmonics Parameterization

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S-1 The spherical harmonics functions and series expansion

The usual (non-parametric or direct) spherical harmonics expansion of a radial function $r(\theta, \phi)$ in spherical polar coordinates (θ, ϕ) can be given by,

$$r(\theta,\phi) = \sum_{L}^{\infty} \sum_{K=-L}^{L} C_{LK} Y_{LK}(\theta,\phi)$$

where,

$$Y_{LK}(\theta,\phi) = N_{LK} \cdot P_{LK}(\cos\theta)e^{iK\phi}$$

with $P_{LK}(\cos\theta)$ the associated Legendre functions and N_{LK} normalization constants. *L* and *K* are integers, $0 \le \theta \le \pi$ and $0 \le \phi \le 2\pi$. Since it is more straightforward to work with real functions, we define the real symmetric and anti-symmetric combinations of the above functions, $y(\theta, \phi)$.

$$y_{LK}(\theta,\phi) = \overline{P}_{LK}(\cos\theta)\cos(K\phi)$$

for $K \ge 0$, and

$$y_{LK}(\theta, \phi) = \overline{P}_{LK}(\cos \theta) \sin(|K|\phi)$$

for K < 0, where $\overline{P}_{LK}(x)$ is given by

$$\overline{P}_{LK}(x) = \sqrt{\left(2 - \delta_{0K}\right) \cdot \left(2L + 1\right) \cdot \frac{(L - K)!}{(L + K)!}} \cdot P_{LK}(x)$$

where we used the normalization given in Heiskanen and Moritz¹. Also

$$P_{LK}(x) = (1 - x^2)^{\frac{K}{2}} \frac{d^K}{dx^K} \cdot P_L(x)$$

where

$$P_{L}(x) = \frac{1}{2^{L} \cdot L!} \cdot \frac{d^{L}}{dx^{L}} (x^{2} - 1)^{L}$$

Note that:

1- The factor $(-1)^{\kappa}$, called the "Condon-Shortly phase factor", is not included in our definition.

2-
$$P_{L0}(x) = P_L(x)$$

The expressions for $P_{LK}(\cos\theta)$ for up to L=2 are given below ($x = \cos(\theta)$):

 $P_{00}(x) = 1$ $P_{10}(x) = x$ $P_{11}(x) = \sin \theta$ $P_{20}(x) = \frac{1}{2}(3x^2 - 1)$ $P_{21}(x) = 3\cos\theta\sin\theta$ $P_{22}(x) = 3\sin^2\theta$

S-2 Calculation of associated Legendre functions and derivatives

For constructing the basis it is necessary to calculate the associated Legendre functions efficiently. This is done by recursion. For the calculation of geometric properties, calculation of their derivatives is also necessary. We provide efficient and numerically stable expressions below².

The $P_{L,K}$ are calculated using backward recursion relations³. For each integer $L \ge 0$ the value of $P_{L,K}(\cos \theta)$ is evaluated using the relation

$$P_{L,L}(\cos(\theta)) = \frac{(2L)!}{L!} (\frac{1}{2}\sin\theta)^L$$

Other values are calculated from:

$$P_{L,K+2}(\cos\theta) = (2K+1) \cdot \cot(\theta) P_{L,K+1}(\cos\theta) - (L-K)(L+K+1) P_{L,K}(\cos\theta)$$

where $P_{L,K}(\cos\theta) = 0$ when K > L.

The order *k* derivatives of the associated Legendre polynomials with respect to θ are obtained by the following relations ^{2,4} (shown here without the normalization):

$$2\frac{d^{k}}{d\theta^{k}}P_{LK}(\cos\theta) = (L+K)\cdot(L-K+1)\cdot\frac{d^{k-1}}{d\theta^{k-1}}P_{L,K-1}(\cos\theta) - \frac{d^{k-1}}{d\theta^{k-1}}P_{L,K+1}(\cos\theta)$$

where

$$\frac{d^k}{d\theta^k} P_{L0}(\cos\theta) = -\frac{d^{k-1}}{d\theta^{k-1}} P_{L,1}(\cos\theta) \text{ and } \frac{d^k}{d\theta^k} P_{LL}(\cos\theta) = L\frac{d^{k-1}}{d\theta^{k-1}} P_{L,L-1}(\cos\theta)$$

These relations are stable for low order derivatives and efficient for computation, because the derivative expressions do not introduce additional trigonometric function evaluations. For concise FORTRAN code to compute normalized derivatives see Bosh 2000².

S-3 Calculation of surface properties

As stated in the main text, the surface is represented parametrically as:

$$\vec{S} = \begin{bmatrix} x(\theta, \phi) \\ y(\theta, \phi) \\ z(\theta, \phi) \end{bmatrix} = \begin{bmatrix} \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{X} Y_{LK}(\theta, \phi) \\ \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{Y} Y_{LK}(\theta, \phi) \\ \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{Z} Y_{LK}(\theta, \phi) \end{bmatrix}$$

Generally, surface properties are computed from partial derivatives of the surface functions using equations of classical differential geometry⁵. Some of this background will be repeated here for clarity as applied to spherical harmonics surface parameterization. Tests of our implementation are also provided.

The surface normal is given by

$$\hat{n} = \frac{\vec{S}_{\theta} \times \vec{S}_{\phi}}{\left| \vec{S}_{\theta} \times \vec{S}_{\phi} \right|}$$

where \vec{S}_{θ} and \vec{S}_{ϕ} are now the 3-vectors,

$$\vec{S}_{\theta} = \begin{bmatrix} \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{X} \frac{\partial Y_{LK}(\theta, \phi)}{\partial \theta} \\ \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{Y} \frac{\partial Y_{LK}(\theta, \phi)}{\partial \theta} \\ \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{Z} \frac{\partial Y_{LK}(\theta, \phi)}{\partial \theta} \end{bmatrix}$$

similarly for \vec{S}_{ϕ} , and the second derivatives. Derivatives of the associated Legendre functions are calculated using the recursion relations given above.

The total surface area A is given by,

$$A = \int dA = \int_{0}^{\pi} \int_{0}^{2\pi} \left| \vec{S}_{\theta} \times \vec{S}_{\phi} \right| d\theta d\phi$$

The total volume V is given by,

$$V = \frac{1}{3} \int_{0}^{\pi} \int_{0}^{2\pi} (\vec{S} \cdot \hat{n}) \left| \vec{S}_{\theta} \times \vec{S}_{\phi} \right| d\theta d\phi$$

The above, and other, quantities can be calculated from the coefficients of the first and second fundamental forms. The first fundamental form is given by,

$$I = aS \cdot aS$$
$$I = Ed\theta^2 + 2Fd\theta d\phi + Gd\phi^2$$

The second fundamental form is,

$$II = -d\vec{S} \cdot d\hat{n}$$
$$II = Ld\theta^2 + 2Md\theta d\phi + Nd\phi^2$$

The coefficients of the first (E, F, G) and second (L, M, N) fundamental forms are given in terms of the surface differentials and normals by,

$$E = \vec{S}_{\theta} \cdot \vec{S}_{\theta}$$

$$F = \vec{S}_{\theta} \cdot \vec{S}_{\phi}$$

$$G = \vec{S}_{\phi} \cdot \vec{S}_{\phi}$$

$$L = \vec{S}_{\theta\theta} \cdot \hat{n}$$

$$M = \vec{S}_{\theta\phi} \cdot \hat{n}$$

$$N = \vec{S}_{\phi\phi} \cdot \hat{n}$$

We calculate the local mean curvature *H* as,

$$H = \frac{EN + GL - 2FM}{2(EG - F^2)}$$

which is needed for calculating the shape energy.

As a self-check for the accuracy of shape property calculations and their implementation we also calculate the Gaussian curvature (K)

$$K = \frac{LN - M^2}{EG - F^2}$$

which when integrated over a closed surface of spherical topology must satisfy,

$$k = \frac{1}{4\pi} \int_{\bar{S}} K dA = 1$$

Other properties such as shape index and curvedness may also be obtained.

Quadrature:

The above integrals cannot be evaluated analytically. *A*, *V* and *k* are calculated using numerical integration by Gaussian quadrature⁶. If a function *f* is to be integrated from -1 to 1, and a Gaussian quadrature of order *N* is chosen, then

$$\int_{-1}^{1} f(x) dx \approx \sum_{n=1}^{N} f_n w_n$$

where the function is evaluated at the points x_n , $-1 < x_n < 1$, and w_n are the Gaussian quadrature weights. If the integration limits are instead *a* and *b*, then one uses the linear transformation:

$$x'_{n} = \frac{1}{2}(b-a)x_{n} + \frac{1}{2}(b+a)$$

f is evaluated at these new points.

In the following we will test our Gaussian quadrature implementation using surface area calculations for ellipsoids.

S-4 Testing convergence of SHP area calculation with Gaussian quadrature

We tested our implementation convergence with increasing Gaussian quadrature base points of an oblate ellipsoid. We compared the results with total surface area obtained from the ellipsoid surface triangulation that results from the iterative subdivision of the icosahedron mapped to the sphere.

A surface triangulation is an approximation of the continuous surface using discrete triangular tiles. The geometrical properties area, volume and total mean curvature are additive and continuous measures of a surface and are thus guaranteed to converge to the true value of the continuous surface in the limit of infinitesimal triangle areas of the mesh. This is an important property that we exploit when writing down the formulas for triangulated surfaces⁷. Surface area is given by the sum of the surface areas of the individual triangles:

$$A = \sum_{i=1}^{N_{\text{mesh}}} A_i$$

We found that the surface area calculations converge faster with the Gaussian quadrature method (Figure S-1).

S-5 Testing the accuracy of SHP area calculations with Gaussian quadrature against analytical methods

Even in the relatively simple case of a general ellipsoid, i.e. $L_{max} = 1$, there is no analytical formula for the total surface area. In this parameterization the ellipsoid, with half axis lengths *a*, *b* and *c*, is given by

$$\vec{S}(\theta, \phi) = \begin{bmatrix} a \cdot \cos(\theta) \sin(\phi) \\ b \cdot \sin(\theta) \sin(\phi) \\ c \cdot \cos(\phi) \end{bmatrix}$$

and the surface differentials with respect to θ and ϕ ,

$$\vec{S}_{\theta}(\theta,\phi) = \begin{bmatrix} a \cdot -\sin(\theta)\sin(\phi) \\ b \cdot \cos(\theta)\sin(\phi) \\ 0 \end{bmatrix} \text{ and } \vec{S}_{\phi}(\theta,\phi) = \begin{bmatrix} a \cdot \cos(\theta)\cos(\phi) \\ b \cdot \sin(\theta)\cos(\phi) \\ -c \cdot \sin(\phi) \end{bmatrix}$$

Accordingly, the coefficients of the first fundamental form are given by,

$$E = \vec{S}_{\theta} \cdot \vec{S}_{\theta} = \sin^2 \phi \cdot (a^2 \sin^2 \theta + b^2 \cos^2 \theta)$$

$$F = \vec{S}_{\theta} \cdot \vec{S}_{\phi} = (b^2 - a^2) \cos \theta \sin \theta \cos \phi \sin \phi$$

$$G = \vec{S}_{\phi} \cdot \vec{S}_{\phi} = \cos^2 \phi \cdot (a^2 \cos^2 \theta + b^2 \sin^2 \theta) + c^2 \sin^2 \phi$$

So the surface area, after some algebra, is given by,

$$A = \int_{0}^{\pi} \int_{0}^{2\pi} \sqrt{EG - F^2} d\theta d\phi = \int_{0}^{\pi} \sin \phi \int_{0}^{2\pi} \sqrt{a^2 b^2 \cos^2 \phi + c^2 \sin^2 \phi (b^2 \cos^2 \theta + a^2 \sin^2 \theta)} d\theta d\phi$$

=
$$\int_{0}^{\pi} \sin \phi \int_{0}^{2\pi} ab \cos \phi \sqrt{1 + \frac{c^2 \sin^2 \phi (b^2 \cos^2 \theta + a^2 \sin^2 \theta)}{a^2 b^2 \cos^2 \phi}} d\theta d\phi$$

=
$$2\sqrt{2}b \int_{0}^{\pi} \sqrt{a^2 + c^2 + (a^2 - c^2) \cos(2\phi)} \sin \phi \times E\left(\frac{c}{b} \sqrt{\frac{2(b^2 - a^2)}{a^2 + c^2 + (a^2 - c^2) \cos(2\phi)}}\right) d\phi$$

where E(k) is a complete elliptic integral of the second kind. This integral can be approximated by a series expansion as,

$$E(k) = \frac{\pi}{2} \left(1 - \sum_{n=1}^{\infty} \left[\frac{(2n-1)!!}{(2n)!!} \right]^2 \frac{k^{2n}}{2n-1} \right)$$

yielding expressions that can be integrated in a straightforward manner and evaluated analytically. The expansion was performed for several orders and compared to Gaussian quadrature and the approximate Knud Thomsen formula (Figure S-2).

We conclude that Gaussian quadrature is the most accurate and efficient strategy for evaluating geometric properties for general SHP shapes. For all our calculations we used 3600 base points and as a self check, the total Gaussian curvature never deviated from 1 up to the 5th decimal.

S-6 A note on the preferred curvature and area difference

 C_o is a local preference of the membrane bilayer to attain a particular curvature. This preference can be related to the microscopic conformational and steric interactions specific to the chemical species that make up the bilayer's outer and inner leaflets, i.e. it is an effect of the local microscopic geometry (and takes the area per molecule into account). The pure bending energy is then calculated as the deviation of the instantaneous local curvature (*H*) from that preferred local curvature.

At the time of formation of the membrane bilayer, there may be a non-zero difference between the areas of the inner and outer leaflets. This area difference results from either a difference in the number of lipid molecules in the outer and inner leaflets, or from an asymmetrical distribution of lipid species, which possess different areas per molecule, between the two leaflets. When the vesicle changes shape, the difference in areas between the two leaflets may change at an energy cost due to the deviation of the instantaneous area difference (ΔA) from the preferred one (ΔA_o). There is no shear resistance within a pure lipid membrane bilayer, and the lipid molecules are assumed to redistribute instantaneously, within their corresponding leaflet, to accommodate the change. The effect is non-local in this case.

In the shape minimization calculation, they do not enter in an independent fashion, as will be demonstrated below (neglecting the membrane skeleton energy for the moment).

$$E = \frac{k_b}{2} \left[\oint (c_1 + c_2)^2 dA + 2C_o \oint (c_1 + c_2) dA + \frac{\alpha \pi}{AD^2} \left(\Delta A^2 - 2\Delta A \Delta A_o \right) \right] + const.$$

$$E = \frac{k_b}{2} \left[\oint (c_1 + c_2)^2 dA + \frac{\alpha \pi}{A} \left(\oint (c_1 + c_2) dA \right)^2 - 2 \left(C_o + \frac{\alpha \pi \Delta A_o}{AD} \right) \oint (c_1 + c_2) dA \right] + const.$$

since,

$$\Delta A = D \oint (c_1 + c_2) dA$$

we can now rewrite the bending energy equation as

$$E = \frac{k_b}{2} \left[\oint (c_1 + c_2)^2 dA + \frac{\alpha \pi}{A} \left(\oint (c_1 + c_2) dA \right)^2 - 2\bar{C}_o \oint (c_1 + c_2) dA \right] + const$$

where,

$$\overline{C}_o = C_o + \frac{\alpha \pi \Delta A_o}{AD}$$

is an "effective" spontaneous curvature. This result is identical to Equations 6 and 7 in Mukhopadhyay et al. 2002^{8} .

S-7 Numerical implementation of the membrane skeleton energy

To calculate the local principal stretches, the deformation of a triangular mesh, associated with SHP, was constructed efficiently at every iteration in the optimization. A particular configuration of shape coefficients corresponds to a mesh configuration that generally represents a deformation Φ from the relaxed membrane skeleton shape. The shear and stretch energies discretized on this mesh are given by

$$E_{MS} = \frac{K_{\alpha}}{2} \sum_{i=1}^{N_{\text{Triangles}}} (\alpha^2 + a_3 \alpha^3 + a_4 \alpha^4) A_i + \mu \sum_{i=1}^{N_{\text{Triangles}}} (\beta + b_1 \alpha \beta + b_2 \beta^2) A_i$$

where A_i represents the area of the i^{th} undeformed triangle.

Shear and stretch calculations depend critically on the ability to calculate the local principal stretches (which can then be integrated in a material-model dependent manner over the whole shape). $\alpha = \lambda_1 \lambda_2 - 1$ and $\beta = (\lambda_1 - \lambda_2)^2 / 2\lambda_1 \lambda_2$ are the local area and shear strain invariants, and $\lambda_{1,2}$ the local principal stretches. The local principal stretches are calculated for each triangle from the eigenvalues of the strain tensor G (Figure S-3) as follows:

$$\Phi(V) = F(V) + b, \qquad G = \frac{1}{2}(F^{T}F - I)$$

$$D_{s} = FD_{m}$$
where
$$D_{m} = \left[\begin{bmatrix} d_{m_{1}} \end{bmatrix} \begin{bmatrix} d_{m_{2}} \end{bmatrix} \right], \qquad D_{s} = \left[\begin{bmatrix} d_{s_{1}} \end{bmatrix} \begin{bmatrix} d_{s_{2}} \end{bmatrix} \right]$$

$$F = D_{s}D_{m}^{-1}$$

$$\therefore G = \frac{1}{2}(D_{m}^{-T}D_{s}^{T}D_{s}D_{m}^{-1} - I)$$

The eigenvalues of G are related to the principal stretches by,

$$\varepsilon_{1} = \frac{1}{2} (\lambda_{1}^{2} - 1)$$

$$\varepsilon_{2} = \frac{1}{2} (\lambda_{2}^{2} - 1)$$

All calculations were implemented in efficient vectorized fashion in Matlab.

S-8 Numerical optimization under constraints using sequential quadratic programming

In order to give the energy model predictive power, it is assumed that the shape obtained corresponds to the minimum of the shape energy under constraints of surface area, volume and specific values of \overline{C}_o (or $\Delta \overline{a}_o$).

To obtain minimum energy shapes for a given model we used sequential quadratic programming^{9, 10}. The Lagrangian of our optimization problem is given by,

$$L(s,\lambda) = f(s) - \lambda^T C(s)$$

with f(s) the objective function that depends on the optimization parameter set *s* (in our case f(s) is the energy and *s* corresponds to the spherical harmonics coefficients), λ are the Lagrange multipliers and the C(s) the set of constraints. At the solution (s^*, λ^*)

the Lagrangian is stationary $\overline{\nabla}L(s,\lambda) = 0$.

The zeros of these equations are found using Newton's method by iteratively incrementing *s* and λ by *ds* and $d\lambda$, and solving

 $\overline{\nabla}\overline{\nabla}^T L(ds, d\lambda) = -\overline{\nabla}L$



Figure S-1|Convergence of total surface area: triangulation vs. Gaussian quadrature. Log-log plot of number of surface points vs. percent error in total surface area calculation. The surface used is an oblate spheroid and hence with known analytical value for surface area. The surface triangulation was generated with subdivisions of the icosahedron. The area is given as the sum of areas of individual triangular tiles. Gaussian quadrature base points and weights were generated according to^{6} .



Figure S-2 Comparison of accuracy of surface area calculations. General and prolate ellipsoid surface area calculations using Gaussian quadrature with only 360 base points, elliptic integral approximations over several orders and Thomsen's formula.



Figure S-3| Sketch of deformation Φ operating on a triangle V producing deformed coordinates.



Figure S-4| Examples of echinocyte type 1 shapes showing the top, bottom and side views of 6 cells. Images were recorded with confocal microscopy using DiI labelling (membrane label) in 250 mM NaCl solutions. Surfaces were triangulated for visualization using a marching cubes algorithm. Out-of-plane spicules can clearly be seen in cells 1, 5 and 6. Bar: 5µm

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