Supplementary material: On the role of membrane anisotropy and I-BAR proteins in the stability of tubular membrane structures

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February 28, 2012

In the first part, we describe isotropic and anisotropic models of tubular membrane structures. The aim of this comparison is to show the contribution of anisotropic and isotropic membrane components to the formation of tubular and spherical membrane protrusions, respectively. In the second part, we give more details of the lattice statistics and linear stability analysis employed to describe the effects of prominin nanodomain rafts and I-BAR proteins on the stability of the cell membrane protrusion.

Part I

Parametrization of the vesicle profile

The vesicle profile is parametrized by the angle, θ, of the tangent to the profile with the plane perpendicular to the axis of rotation, as a function of the arclength, s. The parametric equations of the vesicle profile are given by the following equations:

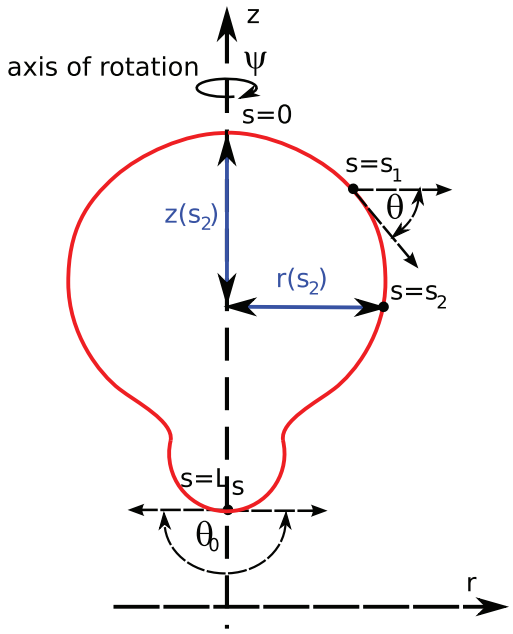

Figure 1: Schematic representation of a profile in the arclength parametrization $\theta(s)$, where s is the arclength and L_s is the length of the profile.

$$z(s) = \int_0^s ds' \sin(\theta(s')), \tag{1}$$

$$r(s) = \int_0^s ds' \cos(\theta(s')). \tag{2}$$

The principal curvatures can be derived from the equations describing the surface of the vesicle. In three-dimensional Euclidean space, the vector describing the points on a surface of revolution, parametrized with the function $\theta(s)$, is $\mathbf{R} = \{\cos(\psi)\, r(s),\, \sin(\psi)\, r(s),\, z(s)\}$. The angle of rotation ψ and the arclength s are coordinates on the surface. In order to calculate the principal curvatures C_1 and C_2 on the surface, the metric tensor g_{ij} is calculated in the following way:

$$g_{ij} = \begin{pmatrix} \frac{\partial \mathbf{R}}{\partial s} \cdot \frac{\partial \mathbf{R}}{\partial s} & \frac{\partial \mathbf{R}}{\partial \psi} \cdot \frac{\partial \mathbf{R}}{\partial s} \\ \frac{\partial \mathbf{R}}{\partial s} \cdot \frac{\partial \mathbf{R}}{\partial \psi} & \frac{\partial \mathbf{R}}{\partial \psi} \cdot \frac{\partial \mathbf{R}}{\partial \psi} \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 0 & (r(s))^2 \end{pmatrix}, \tag{3}$$

where $\partial\mathbf{R}/\partial s = \{\cos(\psi)\, \cos(\theta(s)), \cos(\theta(s))\, \sin(\psi), \sin(\theta(s))\}$, $\partial\mathbf{R}/\partial\psi = \{-\sin(\psi)\, r(s),\, \cos(\psi)\, r(s),\, 0\}$. The unit normal $\mathbf{n}$ can be calculated from $\mathbf{n} = (\partial\mathbf{R}/\partial s \times \partial\mathbf{R}/\partial\psi)/\sqrt{\det(g_{ij})} = \{-\cos(\psi)\, \sin(\theta(s)), -\sin(\psi)\, \sin(\theta(s)), \cos(\theta(s))\}$
Next we defined a supporting matrix $\mathbf{Y}$ as

$$\mathbf{Y} = \begin{pmatrix} \frac{\partial^2 \mathbf{R}}{\partial s^2} & \frac{\partial^2 \mathbf{R}}{\partial s \partial \psi} \\ \frac{\partial^2 \mathbf{R}}{\partial \psi \partial s} & \frac{\partial^2 \mathbf{R}}{\partial \psi^2} \end{pmatrix}. \tag{4}$$

The coefficients of the second fundamental form, L_{ij}, are defined as follows

$$L_{ij} = \mathbf{Y} \cdot \mathbf{n}. \tag{5}$$

The curvature tensor H_{ij} is then

$$H_{ij} = {g_{ij}}^{-1} L_{ij} = \begin{pmatrix} \frac{d\theta(s)}{ds} & 0 \\ 0 & \frac{\sin(\theta(s))}{r(s)} \end{pmatrix}. \tag{6}$$

The mean curvature of the surface, H, can be written as

$$H = \frac{1}{2}\mathrm{Tr}(H_{ij}) = \frac{1}{2}(C_1 + C_2). \tag{7}$$

Thus, $C_1 = \frac{d\theta(s)}{ds}$, $C_2 = \frac{\sin(\theta(s))}{r(s)}$.

The bending energy $\mathcal{F}$ for the profile parametrized with the function $\theta(s)$ is given by the formula

$$\mathcal{F}[\theta(s)] = \frac{\kappa}{2}\int_0^{2\pi} d\psi \int_0^{L_s} dsr(s)\left(\frac{d\theta(s)}{ds} + \frac{\sin(\theta(s))}{r(s)} - \mathcal{C}_0\right)^2, \tag{8}$$

where ψ is the angle of rotation as shown in Fig. 1, $\mathcal{C}_0$ is the spontaneous curvature. The surface area and the volume are calculated as $S = 2\pi\int_0^{L_s} dsr(s)$, $V = \pi\int_0^{L_s} dsr^2(s)\sin\theta(s)$.

The volume, V_0, and the radius, R_0, of the sphere having the same surface area, S, as the investigated vesicle are chosen as the volume and the length units, respectively: $R_0 = \sqrt{S/4\pi}$, $V_0 = \frac{4}{3}\pi R_0^3$. The dimensionless, reduced volume v and the dimensionless spontaneous curvature c_0 are defined as $v = V/V_0$, $c_0 = \mathcal{C}_0 R_0$.

In numerical minimization the shape profile is expanded into an appropriate Fourier series and the resulting functional is minimized with respect to the coefficients in the Fourier expansion. The proper series is the one which by construction fulfills boundary conditions for the shape profile, i.e. $\theta(0) = 0$ and $\theta(L_s) = \pi$. The sine series almost perfectly fulfills the requirements for the set of functions which we are looking for.

The following Fourier series is used to approximate the function $\theta(s)$:

$$\theta(s) = \theta_0\frac{s}{L_s} + \sum_{i=1}^{N} a_i \sin(i\frac{\pi}{L_s}s), \tag{9}$$

where $\theta_0 = \pi$ when the shape of the vesicle without up-down symmetry is parametrized, N is the number of Fourier modes, and a_i are the Fourier amplitudes. When Eq. (9) is inserted into equations (1), (2) and (8), the functional minimization can be replaced by the minimization of the function of many variables. The functional (8) becomes the function of the amplitudes a_i and the length of the shape profile L_s. The minimization is performed under the constraints of constant surface area S and volume V. The constraints in the numerical calculations are implemented by means of Lagrange multipliers.

Part II

Lattice statistics

In order to avoid high local lateral densities of I-BAR proteins, we consider the excluded volume principle, i.e. the finite volume of I-BARs by the application of lattice statistics. The inner membrane monolayer area is divided into small patches that contain a large enough number of attached proteins, allowing us to use statistical physics. The membrane curvature is taken to be constant over the patch. A lattice is imagined with all its M sites in one patch, which can be occupied by I-BARs. In the chosen patch, there are N I-BARs each with energy E_p.

The elastic curvature energy of an I-BAR protein attached to the inner membrane surface is (Perutkova et al., 2010; Baumgart et al., 2011):

$$E_p = \frac{\kappa_p L_0}{2}(C - C_{\mathrm{p}})^2 \ , \tag{10}$$

where κ_p and L_0 are the flexural rigidity and length of the protein, respectively. $C_{\rm p}$ is the intrinsic (spontaneous) curvature of an I-BAR protein, and $C = H + D\cos(2\omega)$ is the total membrane curvature seen by the I-BAR at the rotational angle ω between the normal plane in which the protein is lying and the plane of the first principal curvature C_1 (along the radial direction), where H is the mean local membrane curvature and D is membrane curvature deviator. Due to the assumption $C_{\rm p} \geqslant C$ for all ω, the I-BAR protein is oriented in the direction perpendicular (ω=0) to the major axis of the protrusion, and as a result, $C = C_1$. In Eq. (10) the sign of the curvatures C and $C_{\rm p}$ is determined with respect to the curvature of the outer membrane surface.

The added contribution of I-BARs to the membrane free energy density is:

$$F_p = \frac{\eta n_s}{2}(C_1 - C_{\rm p})^2 n \ , \tag{11}$$

where n is the uniform relative density of I-BARs, $\eta = \kappa_p L_0$, and n_s is the saturation area density of I-BARs (Kabaso et al., 2011c). In order to avoid high lateral densities of I-BAR proteins, we consider the excluded volume principle, i.e. the finite volume of I-BARs by the application of lattice statistics.

Direct interactions between I-BAR proteins are taken into account by using the Bragg-Williams approximation:

$$W_{\rm ii} = \bar{N}_{\rm ii} w \ , \tag{12}$$

where w is the interaction energy of a I-BAR-I-BAR pair (for $w < 0$ the interaction between the molecules is attractive) and $\bar{N}_{\rm ii}$ is the average number

of nearest neighbour I-BARs ,

$$\bar{N}_{ii} = \frac{1}{2}\ N\ c\ \frac{N}{M}\ , \tag{13}$$

where c is the number of nearest neighbours ($c = 4$ for a two-dimensional square net). The factor $1/2$ was inserted in order to avoid counting each I-BAR-I-BAR pair twice. The canonical partition function of the membrane patch is

$$Q^{\mathrm{P}} = \frac{M!}{N!\ (M-N)!} \exp\left(-N\ E_p/kT\right) \exp\left(-cN^2 w/2MkT\right)\ , \tag{14}$$

where kT is the Boltzmann energy, and w is the interaction energy of an I-BAR-I-BAR pair (for $w < 0$ the interaction between the molecules is attractive). The Helmholz free energy of the patch is $F^{\mathrm{P}} = -kT \ln Q^{\mathrm{P}}$,

$$\begin{aligned} F^{\mathrm{P}} = \quad & N\frac{\eta}{2}(C - C_{\mathrm{p}})^2 + \frac{cwN^2}{2M} \\ & + kTN\ln(N/M) + kT(M-N)\ln((M-N)/M)\ , \end{aligned} \tag{15}$$

where we applied the Stirling approximation $\ln x! = x\ln x - x$. The free energy of all the I-BARs is calculated by summing the contributions of all the patches in the membrane:

$$\begin{aligned} F_i = \quad & \int_A \frac{\eta n_s}{2}(C - C_{\mathrm{p}})^2 n \mathrm{d}A + \int_A \frac{cwn_s}{2} n^2 \mathrm{d}A + \\ & + kTn_s \int_A \left(n\ln n + (1-n)\ln(1-n)\right)\ \mathrm{d}A\ . \end{aligned} \tag{16}$$

Here the first term describes the energy contribution due to the anisotropic I-BAR proteins (see also Perutkova et al., 2010; Kabaso et al., 2011c).

The tubular aggregate of anisotropic prominin nandomains

Based on the results presented, we consider the case of the whole membrane as the tubular aggregate of homogeneously distributed anisotropic prominin nanodomains:

$$\epsilon_{b,aniso} = \int \left(\kappa \left(H - H_m \right)^2 + \kappa \left(D - D_m \right)^2 \right) dA \ , \tag{17}$$

where it is assumed that anisotropic nanodomains are completely oriented, i.e. the membrane curvature tensor and the intrinsic membrane curvature tensor coincide everywhere (Iglič et al., 2005; Iglič et al., 2007b)

It is further assumed that the anisotropic component has intrinsic spontaneous curvature $C_{2m} = 0$, and consequently, $H_m = \frac{1}{2} C_{1m}$, and $D_m = H_m$. Under these assumptions, the membrane free energy (Eq. (17)) becomes:

$$\begin{aligned} \epsilon_{b,aniso} = & \int \left(\kappa \left(H - H_m \right)^2 + \kappa \left(D^2 + H_m^2 \right) - 2\kappa D H_m \right) dA \\ = & \int \left(2\kappa H^2 - 2\kappa \left(H + D \right) H_m + 2\kappa H_m^2 \right) dA \ , \end{aligned} \tag{18}$$

where the identity $D^2 = H^2 - C_1 C_2$ was incorporated, and the term $-\kappa C_1 C_2$ was neglected, since for small undulations of the membrane tube $C_1 C_2 \approx 0$. The radius of the undulating tubular membrane along the axisymmetric z axis is written as:

$$r(z) = R + h(z) \tag{19}$$

where $h(z)$ is a small deviation from the initial radius R of a tubular membrane. The surface of revolution of the cylinder is defined by the two principal curvatures,

$$C_1 = \frac{1}{r(1 + r'^2)^{1/2}}, \ C_2 = -\frac{r''}{(1 + r'^2)^{3/2}} \ . \tag{20}$$

The mean membrane curvature is:

$$H = \frac{C_1 + C_2}{2} = \frac{-r''r + r'^2 + 1}{2r(1+r'^2)^{3/2}} = \frac{1}{2}\left(-h''(z) + \frac{1}{R} - \frac{h(z)}{R^2}\right) , \qquad (21)$$

where the curvature was expanded for small deviations from the radius of the membrane protrusion, $r(z) = R + h(z)$, $r'(z) = h'(z)$, and $r''(z) = h''(z)$. The membrane curvature deviator is:

$$D = \frac{C_1 - C_2}{2} = \frac{(1+r'^2) + r''r}{2r(1+r'^2)^{3/2}} = \frac{1}{2}\left(h''(z) + \frac{1}{R} - \frac{h(z)}{R^2}\right) , \qquad (22)$$

where the curvature was expanded for small deviations from the radius. The following is the derivation of the free energy for a membrane with isotropic elastic properties from the more general expression (Eq. (18)) . For a membrane of isotropic elastic properties D_m=0 and $H_m = C_{1m} = C_{2m}$. As a result, Eq. (18) transforms (up to the constant terms independent of H and D) into the following Helfrich expression for the isotropic free energy,

$$\begin{aligned} \epsilon_{b,iso} &= \int \left(\kappa \left(H - H_m\right)^2 + \kappa D^2\right) dA \\ &= \int \left(2\kappa H^2 - 2\kappa H_m H + \kappa H_m^2\right) dA , \end{aligned} \qquad (23)$$

where the identity $D^2 = H^2 - C_1 C_2$ was used, and the constant term $\kappa C_1 C_2$ was again neglected.

Derivation of the system free energy

We next consider the contribution of I-BAR proteins to a membrane of anisotropic (Eq. (18)) and isotropic (Eq. (23)) elastic properties. Addition of Eq. (16) to Eq. (23) gives the total free energy of a membrane with

isotropic elastic properties:

$$F_{iso} = \int [2\kappa H^2 - 2\kappa H_{\rm P} H + \kappa H_{\rm P}^2 + \frac{\eta n_s}{2}(C_1 - C_{\rm p})^2 n + kTn_s(n\ln n + (1-n)\ln(1-n)) + \sigma]\,{\rm d}A \ , \quad (24)$$

where $C_1 = C$ for $\omega = 0$, and $C_1 = 1/R - h(z)/R^2 - h'(z)^2/(2R)$. The infinitesimal area dA of the axisymmetric model is (up to quadratic order):

$$dA = r(z)\left(1 + \frac{1}{2}h'(z)^2\right) 2\pi\ dz \ . \quad (25)$$

The spontaneous curvature of the membrane H_m is taken as the spontaneous mean curvature of the tubular aggregate of prominin nanodomains ($H_{\rm P}$), and σ is the surface tension coefficient (Lagrange multiplier for a constraint of constant membrane area). Note that for the sake of simplicity the direct interaction term $\frac{cwn_s}{2}n^2$ is neglected. Similarly, the summing up of Eq. (16) and Eq. (18) gives the total free energy of a membrane with anisotropic elastic properties as:

$$F_{aniso} = \int [2\kappa H^2 - 2\kappa\,(H + D)\,H_{\rm P} + 2\kappa H_{\rm P}^2 + \frac{\eta n_s}{2}(C_1 - C_{\rm p})^2 n + kTn_s(n\ln n + (1-n)\ln(1-n)) + \sigma]\,{\rm d}A \ . \quad (26)$$

Linear stability analysis

The equation of motion of the membrane height deflection along the cylindrical main axis is given by:

$$\varphi\frac{\partial h(z)}{\partial t} = -\frac{\partial F_i}{\partial h(z)} \ , \ i = iso, aniso \ , \quad (27)$$

where φ is the friction coefficient describing the drag of the fluid surrounding the membrane, t is time, and F_i is redefined to the energy per unit length

of the isotropic or anisotropic case.

The equation of motion of the I-BAR proteins is:

$$\frac{\partial n(z)}{\partial t} = \frac{\Lambda}{n_s}\frac{\partial}{\partial z}\left(n(z)\frac{\partial}{\partial z}\frac{\partial F_i}{\partial n(z)}\right) \ , \tag{28}$$

where Λ is the mobility of I-BARs in the membrane. Note that the normal diffusion term $D_f\frac{\partial^2 n(z)}{\partial z^2}$ is included in the first term on the right hand side of Eq. (28), and that $\Lambda = \frac{D_f}{kT}$, where D_f is the diffusion coefficient of I-BARs. The aim of the following linear stability analysis was to investigate the interplay between anisotropic prominin nanodomain rafts and I-BAR proteins. The basic assumption of the linear stability analysis is that the system is investigated in the limit of a small perturbation from the uniform initial state to give:

$$\begin{aligned} r(z) &= R + \delta h(z,t) \\ n(z) &= \frac{N_t}{n_s\ R} + \delta n(z,t) \ , \end{aligned} \tag{29}$$

where $\delta h(z,t)$ and $\delta n(z,t)$ are small deviations from the corresponding uniform values.

By linearization of Eqs.(5),(6) in the manuscript, we obtain the following equations:

$$\varphi\frac{\partial h(\vec{z})}{\partial t} = \int \left(U + \delta L(h,n) + O(\delta^2)\right) dA \tag{30}$$

$$\frac{\partial n(z)}{\partial t} = \delta N(h,n) + O(\delta^2) \ , \tag{31}$$

where the functions $L(h,n)$ and $N(h,n)$ describe the forces and fluxes acting on the membrane in the limit of small undulations. The forces acting on the membrane in the equilibrium state are described by U. For the isotropic

case, the function $L(h,n)$ is:

$$L(h,n) = \frac{1}{8R^5}\varphi((N_t\eta + 4R(\kappa - 2C_{\rm p}N_t\eta) + 4R^2(-4H_P\kappa + C_{\rm p}^2N_t\eta)$$
$$+8R^3(H_P^2\kappa + \sigma))h(z) + R^2(n_s(1 - 4C_{\rm p}^2R^2)\eta n(z)$$
$$+(N_t\eta + 4R(\kappa - 3C_{\rm p}N_t\eta) + 4R^2(-6H_P\kappa + C_{\rm p}^2N_t\eta) + 8R^3(H_P^2\kappa + \sigma))h''(z)$$
$$-2R^2(n_s(-1 + 2C_{\rm p}R)\eta n''(z) + (4R\kappa + N_t\eta)h''''(z)))) \ . \qquad (32)$$

For the anisotropic case, the function $L(h,n)$ is:

$$L(h,n) = \frac{1}{2R^5}\varphi((N_t\eta + R(\kappa - 4C_{\rm p}N_t\eta) + R^2(-8H_P\kappa + C_{\rm p}^2N_t\eta)$$
$$+2R^3(2H_P^2\kappa + \sigma))h(z) + R^2(n_s(1 - C_{\rm p}^2R^2)\eta n(z)$$
$$+(R\kappa - N_t\eta + R^2(-4H_P\kappa + C_{\rm p}^2N_t\eta) + 2R^3(2H_P^2\kappa + \sigma))h''(z)$$
$$-2R^3\kappa h''''(z)))) \ . \qquad (33)$$

The function U is:

$$U = \frac{\varphi(-8R^3(H_P^2\kappa + \sigma) - 4R^2C_{\rm p}^2N_t\eta + 4R\kappa + N_t\eta)}{8R^4} \ . \qquad (34)$$

where for the anisotropic case, the term $H_P^2\kappa$ becomes $2H_P^2\kappa$, and the term $N_t\eta$ becomes $4N_t\eta$. For the isotropic case, the function $N(h,n)$ is:

$$N(h,n) = Dn''(z) + \frac{N_t(-1 + 2C_{\rm p}R)\Lambda\eta(h''(z) + R^2h''''(z))}{4n_sR^4} \ . \qquad (35)$$

For the anisotropic case, the function $N(h,n)$ is:

$$N(h,n) = Dn''(z) + \frac{N_t(-1 + C_{\rm p}R)\Lambda\eta h''(z)}{n_sR^4} \ . \qquad (36)$$

The equilibrium radius R_{eq} is obtained by assuming that the undulation U equals zero. There are three possible solutions for R_{eq}. By inserting the

parameter values into each solution of R_{eq}, it was shown that only one solution does not have an imaginary part. For the isotropic case, this function of R_{eq} is:

$$
\begin{aligned}
&R_{eq,iso} = -\frac{C_{\mathrm{p}}^2 N_t \eta}{6(H_P^2\kappa+\sigma)} + (6H_P^2\kappa^2 + C_{\mathrm{p}}^4 N_t^2\eta^2 + 6\kappa\sigma) \\
&/(3\ 2^{2/3}(H_P^2\kappa+\sigma))(-2C_{\mathrm{p}}^6 N_t^3\eta^3 - 18C_{\mathrm{p}}^2 N_t\kappa\eta(H_P^2\kappa+\sigma) \\
&+3(9H_P^4 N_t\kappa^2\eta + 18H_P^2 N_t\kappa\eta\sigma + 9N_t\eta\sigma^2 + \sqrt{3} \\
&(-(H_P^2\kappa+\sigma)^2(4C_{\mathrm{p}}^4 N_t^2\kappa\eta^2 - 27H_P^4 N_t^2\kappa^2\eta^2 + 4C_{\mathrm{p}}^6 N_t^4\eta^4 + 32\kappa^3\sigma + 36C_{\mathrm{p}}^2 N_t^2\kappa\eta^2\sigma \\
&-27N_t^2\eta^2\sigma^2 + 2H_P^2\kappa(16\kappa^3 + 18C_{\mathrm{p}}^2 N_t^2\kappa\eta^2 - 27N_t^2\eta^2\sigma))))^{1/3}) \\
&+\frac{1}{6\ 2^{1/3}(H_P^2\kappa+\sigma)}(-2C_{\mathrm{p}}^6 N_t^3\eta^3 - 18C_{\mathrm{p}}^2 N_t\kappa\eta(H_P^2\kappa+\sigma) \\
&+3(9H_P^4 N_t\kappa^2\eta + 18H_P^2 N_t\kappa\eta\sigma + 9N_t\eta\sigma^2 + \sqrt{3}(-(H_P^2\kappa+\sigma)^2 \\
&(4C_{\mathrm{p}}^4 N_t^2\kappa^2\eta^2 - 27H_P^4 N_t^2\kappa^2\eta^2 + 4C_{\mathrm{p}}^6 N_t^4\eta^4 + 32\kappa^3\sigma + 36C_{\mathrm{p}}^2 N_t^2 \\
&\kappa\eta^2\sigma - 27N_t^2\eta^2\sigma^2 + 2H_P^2\kappa(16\kappa^3 + 18C_{\mathrm{p}}^2 N_t^2\kappa\eta^2 - 27N_t^2\eta^2\sigma)))))^{1/3}\ . \qquad (37)
\end{aligned}
$$

For the anisotropic case, this function of R_{eq} is:

$$
\begin{aligned}
&R_{eq,aniso} = \frac{1}{6(2H_P^2\kappa+\sigma)}(-C_p^2 N_t\eta + (12H_P^2\kappa^2 + C_p^4 N_t^2\eta^2 + 6\kappa\sigma)/ \\
&(-18C_p^2 H_P^2 N_t\kappa^2\eta + 216H_P^4 N_t\kappa^2\eta - C_p^6 N_t^3\eta^3 - 9C_p^2 N_t\kappa\eta\sigma + 216H_P^2 N_t\kappa\eta\sigma + 54N_t\eta\sigma^2 + \\
&\frac{1}{2}(-4(12H_P^2\kappa^2 + C_p^4 N_t^2\eta^2 + 6\kappa\sigma)^3 + 4N_t^4\eta^2(C_p^6 N_t^4\eta^2 + 9C_p^2\kappa(2H_P^2\kappa+\sigma) \\
&-54(2H_P^2\kappa+\sigma)^2)^2)^{1/3} + (-18C_p^2 H_P^2 N_t\kappa^2\eta + 216H_P^4 N_t\kappa^2\eta - C_p^6 N_t^3\eta^3 - \\
&9C_p^2 N_t\kappa\eta\sigma + 216H_P^2 N_t\kappa\eta\sigma + 54N_t\eta\sigma^2 + \frac{1}{2}(-4(12H_P^2\kappa^2 + C_p^4 N_t^4\eta^2 + 6\kappa\sigma)^3 \\
&+4N_t^4\eta^2(C_p^6 N_t^4\eta^2 + 9C_p^2\kappa(2H_P^2\kappa+\sigma) - 54(2H_P^2\kappa+\sigma)^2)^2)^{1/3})\ . \qquad (38)
\end{aligned}
$$

The equilibrium shape analysis was performed using $R_{eq,iso}$ and $R_{eq,aniso}$. For the sake of simplicity, the phase diagram analysis was performed using

the list of parameter values mentioned in the manuscript (see Results section).

The Fourier transform of the linearized equations of motion (Eqs. (30),(31)) allowed us to rewrite the equations in a matrix form as follows:

$$\begin{bmatrix} \dot{h} \\ \dot{n} \end{bmatrix} = M \times \begin{bmatrix} h \\ n \end{bmatrix} , \tag{39}$$

where the derivatives $\dot{h}$ and $\dot{n}$ are with respect to the frequency mode q. The stability matrix obtained from the Fourier transform for a membrane with isotropic or anisotropic elastic properties is:

$$M = \begin{bmatrix} M_{hh} & M_{hn} \\ M_{nh} & M_{nn} \end{bmatrix} . \tag{40}$$

The solution of the determinant of the stability matrix gives two eigen vectors ($\omega(q)$). The first eigen vector is always negative, while the second eigen vector can be positive depending on the value of the biophysical parameters used in our system. In a dispersion relation, the positive regions ($\omega(q) >0$) describe regions of unstable growth of tubular dilations, while negative regions ($\omega(q) <0$) describe stable regions. A stable region is identified by exponential decay of an undulating distribution of membrane shapes ($h(z)$) or I-BAR proteins ($n(z)$) at a particular frequency (q), whereas an unstable region would exhibit exponential growth.

To have a better understanding of the interplay between prominin nanodomains and I-BAR proteins, a linear stability analysis was performed, revealing the transition between stable and unstable regimes. The solution of the stability matrix M was obtained from its determinant. The terms of

the determinant are collected according to their order in q as follows:

$$[\lambda_1, \lambda_2] = q^2(q^4 A + q^2 B + C) \ , \tag{41}$$

where the coefficients A, B, and C, are functions of the different biophysical parameters, λ_1 and λ_2 are the two possible solutions of the quadratic equation $r^2 A + rB + C$, where $r = q^2$. For the isotropic case, the coefficients of the quadratic equation are as follows:

$$\begin{aligned}
A = \quad & D\kappa + \frac{DN_t\eta}{4R} - \frac{N_t\Lambda\eta^2}{16R^3} + \frac{C_{\rm p}N_t\Lambda\eta^2}{4R^2} - \frac{C_{\rm p}^2N_t\Lambda\eta^2}{4R} \\
B = \quad & DH_P^2\kappa + \frac{D\kappa}{2R^2} - \frac{3DH_P\kappa}{R} + \frac{DN_t\eta}{8R^3} - \frac{3DC_{\rm p}N_t\eta}{2R^2} \\
& + \frac{DC_{\rm p}^2N_t\eta}{2R} + \frac{3N_t\Lambda\eta^2}{32R^5} \\
& - \frac{5C_{\rm p}N_t\Lambda\eta^2}{16R^4} + \frac{C_{\rm p}^2N_t\Lambda\eta^2}{8R^3} + \frac{C_{\rm p}^3N_t\Lambda\eta}{4R^2} + D\sigma \\
C = \quad & \frac{-D\kappa}{2R^4} + \frac{2DH_P\kappa}{R^3} - \frac{DH_P^2\kappa}{R^2} - \frac{DN_t\eta}{8R^5} \\
& + \frac{DC_{\rm p}N_t\eta}{R^4} - \frac{DC_{\rm p}^2N_t\eta}{2R^3} \\
& - \frac{N_t\Lambda\eta^2}{32R^7} + \frac{C_{\rm p}N_t\Lambda\eta^2}{16R^6} + \frac{C_{\rm p}^2N_t\Lambda\eta^2}{8R^5} \\
& - \frac{C_{\rm p}^3N_t\Lambda\eta^2}{4R^4} - \frac{D\sigma}{R^2} \ .
\end{aligned} \tag{42}$$

For the anisotropic case, the coefficients of the quadratic equation are as

follows:

$$
\begin{aligned}
A = \quad & D\kappa \\
B = \quad & 2DH_P^2\kappa + \frac{D\kappa}{2R^2} - \frac{2DH_P\kappa}{R} - \frac{DN_t\eta}{2R^3} + \frac{DC_{\mathrm{p}}^2N_t\eta}{2R} + D\sigma \\
C = \quad & \frac{-D\kappa}{2R^4} + \frac{4DH_P\kappa}{R^3} - \frac{2DH_P^2\kappa}{R^2} - \frac{DN_t\eta}{2R^5} \\
& + \frac{2DC_{\mathrm{p}}N_t\eta}{R^4} - \frac{DC_{\mathrm{p}}^2N_t\eta}{2R^3} \\
& - \frac{N_t\Lambda\eta^2}{2R^7} + \frac{C_{\mathrm{p}}N_t\Lambda\eta^2}{2R^6} + \frac{C_{\mathrm{p}}^2N_t\Lambda\eta^2}{2R^5} \\
& - \frac{C_{\mathrm{p}}^3N_t\Lambda\eta^2}{2R^4} - \frac{D\sigma}{R^2} \ . \qquad (43)
\end{aligned}
$$