Quantitative prediction and measurement of Piezo's membrane footprint

Supplementary Information

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S1 Supplementary figures

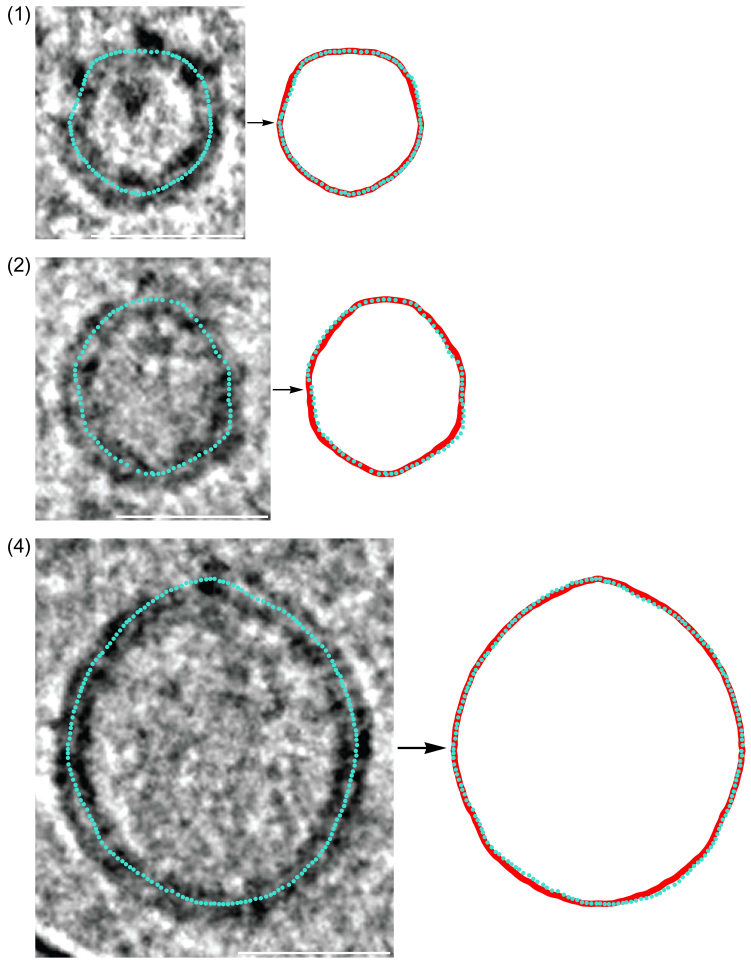

Figure S1: Oriented images of Piezo vesicles 1, 2, and 4 in Fig. 4 of the main text obtained by cryo-EM tomography (left panel) with traced mid-membrane vesicle profiles (cyan dots). Interpolation of the traced vesicle profiles and left-right averaging of the interpolated vesicle profiles about $r = 0$ yields the symmetrized Piezo vesicle profiles (red curves), with the lines of maximal length from Piezo's CED to the interpolated vesicle profiles corresponding to the vesicle symmetry axes (right panels). Scale bars, 26 nm. We use the same scale bars in the left and right panels. The corresponding results for Piezo vesicle 3 in Fig. 4 of the main text are shown in Fig. 2 of the main text.

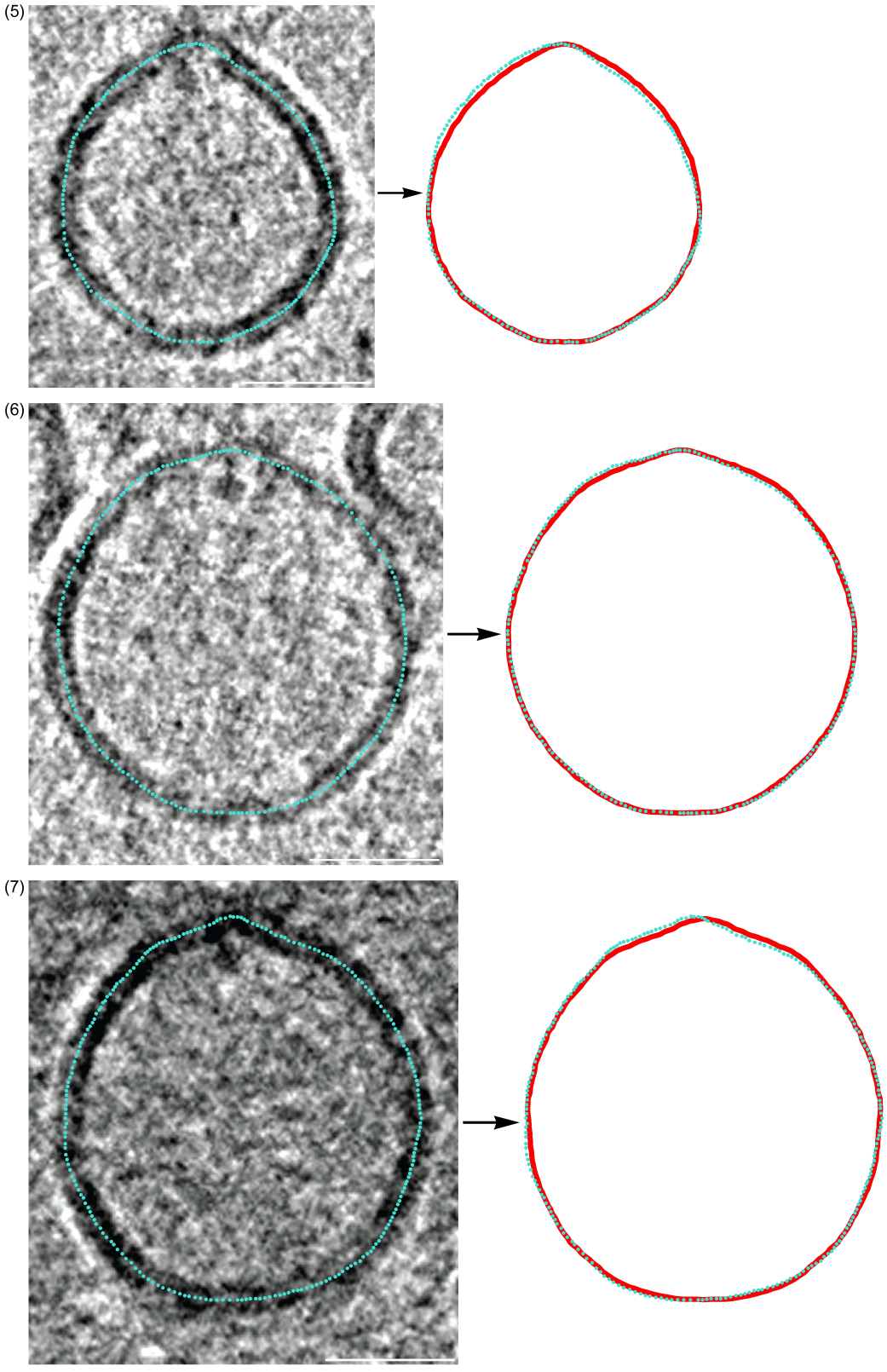

Figure S2: Oriented Piezo vesicle images obtained by cryo-EM tomography, traced vesicle profiles, and symmetrized vesicle profiles as in Fig. S1, but for Piezo vesicles 5–7 in Fig. 4 of the main text. Scale bars, 26 nm. We use the same scale bars in the left and right panels.

S2 Derivation of the Hamilton equations

As explained in the main text, the free membrane bending energy of Piezo vesicles can be rewritten as

$$\widetilde{G}_M = \int_0^{s_b} ds\, L\left(\psi, \dot{\psi}, r, \dot{r}, \dot{h}\right) , \tag{S1}$$

where the Lagrangian

$$L\left(\psi, \dot{\psi}, r, \dot{r}, \dot{h}\right) = \pi K_b\, r \left(\dot{\psi} + \frac{\sin\psi}{r}\right)^2 + 2\pi\lambda_a r + \lambda_r(s)\left(\dot{r} - \cos\psi\right) + \lambda_h(s)\left(\dot{h} - \sin\psi\right) , \tag{S2}$$

in which we use the same notation as in Eq. (4) of the main text. Subject to suitable boundary conditions, the Hamilton equations define the extremal functions $\psi(s)$, $r(s)$, and $h(s)$ that make Eq. (S1) with Eq. (S2) stationary. The purpose of this section is to derive the Hamilton equations for Eq. (S1) with Eq. (S2).

As described in the main text, the generalized momenta associated with the generalized displacements (shape variables) ψ, r, and h in Eq. (S1) with Eq. (S2) are defined by [1]

$$p_\psi \equiv \frac{\partial L}{\partial \dot{\psi}} = 2\pi K_b r \left(\dot{\psi} + \frac{\sin\psi}{r}\right) , \tag{S3}$$

$$p_r \equiv \frac{\partial L}{\partial \dot{r}} = \lambda_r \, , \tag{S4}$$

$$p_h \equiv \frac{\partial L}{\partial \dot{h}} = \lambda_h \, . \tag{S5}$$

As pointed out in the main text, the Hamiltonian associated with the Lagrangian in Eq. (S2), obtained through a Legendre transformation, is given by [1]

$$H \equiv p_\psi \dot{\psi} + p_r \dot{r} + p_h \dot{h} - L \, . \tag{S6}$$

S2.1 Variation of the energy functional

The energy functional in Eq. (S1) takes the general form

$$J\left[\psi, r, h\right] = \int_{s_1}^{s_2} ds F\left(s, \psi, \psi', r, r', h, h'\right) , \tag{S7}$$

where, throughout this section, we use the notation $\psi' \equiv d\psi/ds$, etc. It is instructive to allow, in principle, both boundaries of the integration domain, s_1 and s_2, to be variable. In this section, we denote the values of $\psi(s)$, $r(s)$, and $h(s)$ at $s = s_1$ and $s = s_2$ by

$$\psi(s_i) = \psi_i \, , \tag{S8}$$

$$r(s_i) = r_i \, , \tag{S9}$$

$$h(s_i) = h_i \, , \tag{S10}$$

where $i = 1, 2$. The extremal functions of J in Eq. (S7) can be determined through the calculus of variations using standard methods [2–4]. For completeness, we summarize here this calculation.

The perturbed functional in Eq. (S7) takes the form

$$J\left[\hat{\psi}, \hat{r}, \hat{h}\right] = \int_{\hat{s}_1}^{\hat{s}_2} ds F\left(s, \hat{\psi}, \hat{\psi}', \hat{r}, \hat{r}', \hat{h}, \hat{h}'\right) , \tag{S11}$$

where we define the perturbed functions as

$$\hat{\psi}(s) \equiv \psi(s) + \epsilon\eta_\psi(s) , \tag{S12}$$

$$\hat{r}(s) \equiv r(s) + \epsilon\eta_r(s) , \tag{S13}$$

$$\hat{h}(s) \equiv h(s) + \epsilon\eta_h(s) , \tag{S14}$$

in which ϵ is small and the functions $\eta_{\psi,r,h}(s)$ are arbitrary. At the boundaries, we have

$$\hat{s}_i = s_i + \epsilon S_i , \tag{S15}$$

$$\hat{\psi}(\hat{s}_i) = \psi_i + \epsilon\Psi_i , \tag{S16}$$

$$\hat{r}(\hat{s}_i) = r_i + \epsilon R_i , \tag{S17}$$

$$\hat{h}(\hat{s}_i) = h_i + \epsilon H_i , \tag{S18}$$

where $i = 1, 2$ and, depending on the boundary conditions under consideration (see Sec. S3), S_i, Ψ_i, R_i, and H_i may be arbitrary or zero.

Introducing, for convenience, the notation

$$F \equiv F\left(s, \psi, \psi', r, r', h, h'\right) , \tag{S19}$$

$$\hat{F} \equiv F\left(s, \hat{\psi}, \hat{\psi}', \hat{r}, \hat{r}', \hat{h}, \hat{h}'\right) , \tag{S20}$$

we have that

$$J\left[\hat{\psi}, \hat{r}, \hat{h},\right] - J\left[\psi, r, h,\right] = \int_{s_1}^{s_2} ds\left(\hat{F} - F\right) + \int_{s_2}^{\hat{s}_2} ds\hat{F} - \int_{s_1}^{\hat{s}_1} ds\hat{F} . \tag{S21}$$

Expanding $\hat{F}$ to $O(\epsilon)$ about $(s, \psi, \psi', r, r', h, h')$, Eq. (S21) yields the following leading-order change in J:

$$\begin{aligned}\delta J = &\int_{s_1}^{s_2} ds\left(\epsilon\eta_\psi\frac{\partial F}{\partial\psi} + \epsilon\eta'_\psi\frac{\partial F}{\partial\psi'} + \epsilon\eta_r\frac{\partial F}{\partial r} + \epsilon\eta'_r\frac{\partial F}{\partial r'} + \epsilon\eta_h\frac{\partial F}{\partial h} + \epsilon\eta'_h\frac{\partial F}{\partial h'}\right) \\ &+ \int_{s_2}^{s_2+\epsilon S_2} dsF - \int_{s_1}^{s_1+\epsilon S_1} dsF \qquad \text{(S22)} \\ = &\int_{s_1}^{s_2} ds\left(\epsilon\eta_\psi\frac{\partial F}{\partial\psi} + \epsilon\eta'_\psi\frac{\partial F}{\partial\psi'}\right) + \int_{s_1}^{s_2} ds\left(\epsilon\eta_r\frac{\partial F}{\partial r} + \epsilon\eta'_r\frac{\partial F}{\partial r'}\right) + \int_{s_1}^{s_2} ds\left(\epsilon\eta_h\frac{\partial F}{\partial h} + \epsilon\eta'_h\frac{\partial F}{\partial h'}\right) \\ &+ F\big|_{s=s_2}\epsilon S_2 - F\big|_{s=s_1}\epsilon S_1 \qquad \text{(S23)}\end{aligned}$$

$$= \int_{s_1}^{s_2} ds\, \epsilon\eta_\psi \left(\frac{\partial F}{\partial \psi} - \frac{d}{ds}\frac{\partial F}{\partial \psi'}\right) + \int_{s_1}^{s_2} ds\, \epsilon\eta_r \left(\frac{\partial F}{\partial r} - \frac{d}{ds}\frac{\partial F}{\partial r'}\right) + \int_{s_1}^{s_2} ds\, \epsilon\eta_h \left(\frac{\partial F}{\partial h} - \frac{d}{ds}\frac{\partial F}{\partial h'}\right)$$
$$+ \left[\epsilon\eta_\psi \frac{\partial F}{\partial \psi'}\right]_{s=s_1}^{s=s_2} + \left[\epsilon\eta_r \frac{\partial F}{\partial r'}\right]_{s=s_1}^{s=s_2} + \left[\epsilon\eta_h \frac{\partial F}{\partial h'}\right]_{s=s_1}^{s=s_2} + F\big|_{s=s_2}\epsilon S_2 - F\big|_{s=s_1}\epsilon S_1\,, \tag{S24}$$

where we obtained Eq. (S24) via integration by parts.

Note that Eqs. (S12) and (S16) with Eq. (S15) imply that

$$\hat{\psi}(\hat{s}_i) = \psi_i + \epsilon\Psi_i \tag{S25}$$
$$= \psi(s_i + \epsilon S_i) + \epsilon\eta_\psi(s_i + \epsilon S_i) = \psi_i + \epsilon S_i \psi'(s_i) + \epsilon\eta_\psi(s_i) + \ldots \tag{S26}$$

To $O(\epsilon)$, we therefore have that

$$\Psi_i = \eta_\psi(s_i) + S_i\psi'(s_i)\,. \tag{S27}$$

Upon rearranging Eq. (S27), and carrying out an analogous analysis for $r(s)$ and $h(s)$, we find

$$\eta_\psi(s_i) = \Psi_i - S_i\psi'(s_i)\,, \tag{S28}$$
$$\eta_r(s_i) = R_i - S_i r'(s_i)\,, \tag{S29}$$
$$\eta_h(s_i) = H_i - S_i h'(s_i) \tag{S30}$$

to $O(\epsilon)$. Substitution of Eqs. (S28)–(S30) into Eq. (S24) yields

$$\delta J = \epsilon\Bigg\{ \int_{s_1}^{s_2} ds\, \eta_\psi \left(\frac{\partial F}{\partial \psi} - \frac{d}{ds}\frac{\partial F}{\partial \psi'}\right) + \int_{s_1}^{s_2} ds\, \eta_r \left(\frac{\partial F}{\partial r} - \frac{d}{ds}\frac{\partial F}{\partial r'}\right) + \int_{s_1}^{s_2} ds\, \eta_h \left(\frac{\partial F}{\partial h} - \frac{d}{ds}\frac{\partial F}{\partial h'}\right)$$
$$+ \frac{\partial F}{\partial \psi'}\bigg|_{s=s_2}\Psi_2 - \frac{\partial F}{\partial \psi'}\bigg|_{s=s_1}\Psi_1 + \frac{\partial F}{\partial r'}\bigg|_{s=s_2}R_2 - \frac{\partial F}{\partial r'}\bigg|_{s=s_1}R_1 + \frac{\partial F}{\partial h'}\bigg|_{s=s_2}H_2 - \frac{\partial F}{\partial h'}\bigg|_{s=s_1}H_1$$
$$+ \left(F - \psi'\frac{\partial F}{\partial \psi'} - r'\frac{\partial F}{\partial r'} - h'\frac{\partial F}{\partial h'}\right)\bigg|_{s=s_2} S_2 - \left(F - \psi'\frac{\partial F}{\partial \psi'} - r'\frac{\partial F}{\partial r'} - h'\frac{\partial F}{\partial h'}\right)\bigg|_{s=s_1} S_1 \Bigg\}. \tag{S31}$$

Identifying F with the Lagrangian L, $F \equiv L$, the Hamiltonian of the system takes the form in Eq. (S6). Equation (S31) can then be rewritten as

$$\delta J = \epsilon\Bigg\{ \int_{s_1}^{s_2} ds\, \eta_\psi \left(\frac{\partial L}{\partial \psi} - \frac{d}{ds}\frac{\partial L}{\partial \psi'}\right) + \int_{s_1}^{s_2} ds\, \eta_r \left(\frac{\partial L}{\partial r} - \frac{d}{ds}\frac{\partial L}{\partial r'}\right) + \int_{s_1}^{s_2} ds\, \eta_h \left(\frac{\partial L}{\partial h} - \frac{d}{ds}\frac{\partial L}{\partial h'}\right)$$
$$+ p_\psi\big|_{s=s_2}\Psi_2 - p_\psi\big|_{s=s_1}\Psi_1 + p_r\big|_{s=s_2}R_2 - p_r\big|_{s=s_1}R_1 + p_h\big|_{s=s_2}H_2 - p_h\big|_{s=s_1}H_1$$
$$- H\big|_{s=s_2}S_2 + H\big|_{s=s_1}S_1 \Bigg\}, \tag{S32}$$

where we have used the definitions of the generalized momenta in Eqs. (S3)–(S5).

The extremal functions of the functional in Eq. (S7) satisfy $\delta J = 0$. Noting that the functions $\eta_{\psi,r,h}(s)$ are arbitrary, the first line of Eq. (S32) thus yields the Euler-Lagrange equations satisfied by the extremal $\psi(s)$, $r(s)$, and $h(s)$ (see also Sec. S2.2). The terms in the second line of Eq. (S32) mandate that the extremal $\psi(s)$, $r(s)$, and $h(s)$ satisfy fixed-value ($\Psi_i = 0$, $R_i = 0$, or $H_i = 0$) or natural (zero-force; $p_\psi = 0$, $p_r = 0$, or $p_h = 0$) boundary conditions. Natural boundary conditions thereby mean

that the extremal $\psi(s)$, $r(s)$, and $h(s)$ are such that the functional in Eq. (S7) is stationary under arbitrary perturbations $\epsilon\Psi_i$, ϵR_i, or ϵH_i at the respective domain boundaries. Finally, the terms in the third line of Eq. (S32) show that, if one or both of the limits of integration s_1 and s_2 in Eq. (S7) can be freely adjusted when minimizing Eq. (S7), then the extremal functions of Eq. (S7) must satisfy $H = 0$ at the corresponding $s = s_i$ to ensure that $\delta J = 0$ for arbitrary perturbations ϵS_i. Note that, if H does not explicitly depend on s, then H is conserved along s and $H = 0$ for some s implies $H = 0$ for all s (see Sec. S2.2).

S2.2 From the Euler-Lagrange to the Hamilton equations

The (three) Euler-Lagrange equations implied by the first line of Eq. (S32) are formulated in terms of the Lagrangian of the system, the generalized displacements $\psi(s)$, $r(s)$, and $h(s)$, and the generalized velocities $\dot{\psi}(s)$, $\dot{r}(s)$, and $\dot{h}(s)$, where, as in the main text, we use in this section the notation $\dot{\psi} \equiv d\psi/ds$, etc. Following the Hamiltonian formulation of classical mechanics one may, alternatively, use the generalized momenta in Eqs. (S3)–(S5) instead of the generalized velocities $\dot{\psi}(s)$, $\dot{r}(s)$, and $\dot{h}(s)$ to specify the state of the system, with the Hamilton equations taking the place of the Euler-Lagrange equations [1, 4]. Note, in particular, that the Hamiltonian in Eq. (S6) is a function of the generalized displacements and the generalized momenta, rather than the generalized displacements and the generalized velocities. We follow here Ref. [1] to provide, based on Sec. S2.1, a derivation of the Hamilton equations for Piezo vesicles.

To derive the Hamilton equations for Eq. (S1) with Eq. (S2) we first note that, in terms of the generalized momenta in Eqs. (S3)–(S5), the Euler-Lagrange equations implied by the first line of Eq. (S32) can be rewritten as

$$\dot{p}_\psi = \frac{\partial L}{\partial \psi}\,, \tag{S33}$$

$$\dot{p}_r = \frac{\partial L}{\partial r}\,, \tag{S34}$$

$$\dot{p}_h = \frac{\partial L}{\partial h}\,. \tag{S35}$$

Next, from the Legendre transformation in Eq. (S6) one finds that

$$\frac{\partial H}{\partial p_\psi} = \dot{\psi}\,, \tag{S36}$$

$$\frac{\partial H}{\partial p_r} = = \dot{r}\,, \tag{S37}$$

$$\frac{\partial H}{\partial p_h} = \dot{h}\,. \tag{S38}$$

Similarly, we have from Eq. (S6) that

$$\frac{\partial H}{\partial \psi} = -\frac{\partial L}{\partial \psi} = -\dot{p}_\psi \,, \tag{S39}$$

$$\frac{\partial H}{\partial r} = -\frac{\partial L}{\partial r} = -\dot{p}_r \,, \tag{S40}$$

$$\frac{\partial H}{\partial h} = -\frac{\partial L}{\partial h} = -\dot{p}_h \,, \tag{S41}$$

where, for the second equalities, we used the Euler-Lagrange equations in Eqs. (S33)–(S35). Combining Eqs. (S36)–(S41) we obtain the Hamilton equations for Eq. (S1) with Eq. (S2),

$$\dot{\psi} = \frac{\partial H}{\partial p_\psi} = \frac{1}{r}\left(\frac{p_\psi}{2\pi K_b} - \sin\psi\right) \,, \tag{S42}$$

$$\dot{r} = \frac{\partial H}{\partial p_r} = \cos\psi \,, \tag{S43}$$

$$\dot{h} = \frac{\partial H}{\partial p_h} = \sin\psi \,, \tag{S44}$$

$$\dot{p}_\psi = -\frac{\partial H}{\partial \psi} = \left(\frac{p_\psi}{r} - p_h\right)\cos\psi + p_r \sin\psi \,, \tag{S45}$$

$$\dot{p}_r = -\frac{\partial H}{\partial r} = \frac{p_\psi}{r^2}\left(\frac{p_\psi}{4\pi K_b} - \sin\psi\right) + 2\pi\lambda_a \,, \tag{S46}$$

$$\dot{p}_h = -\frac{\partial H}{\partial h} = 0 \,, \tag{S47}$$

as in Eqs. (5)–(10) of the main text. While the Euler-Lagrange equations in Eqs. (S33)–(S35) are a set of three differential equations and generally involve derivatives up to second order, the Hamilton equations in Eqs. (S42)–(S47) constitute a set of six first-order differential equations [1]. The solutions of Eqs. (S42)–(S47), subject to the boundary conditions mandated by the second and third lines of Eq. (S32), specify the extremal functions $\psi(s)$, $r(s)$, and $h(s)$ of Eq. (S1) with Eq. (S2), from which the membrane bending energy can be calculated by substituting these extremal functions back into Eqs. (1) or (2) of the main text and evaluating the integral.

Finally, note that the Hamiltonian in Eq. (S6) associated with Piezo vesicles only depends on s through the generalized displacements $\psi(s)$, $r(s)$, and $h(s)$ and the generalized momenta $p_\psi(s)$, $p_r(s)$, and $p_h(s)$. In particular, H in Eq. (S6) does not explicitly depend on s. As a result, the value of H is constant with s [1],

$$\frac{dH}{ds} = \frac{\partial H}{\partial \psi}\dot{\psi} + \frac{\partial H}{\partial r}\dot{r} + \frac{\partial H}{\partial h}\dot{h} + \frac{\partial H}{\partial p_\psi}\dot{p}_\psi + \frac{\partial H}{\partial p_r}\dot{p}_r + \frac{\partial H}{\partial p_h}\dot{p}_h = 0 \,, \tag{S48}$$

where the last equality follows from the Hamilton equations in Eqs. (S42)–(S47), which means that H is conserved along s.

S3 Boundary conditions

In this section we expand on the discussion of boundary conditions in the main text [5–8]. Note, in particular, that the variational calculation in Sec. S2.1 shows that two distinct kinds of boundary conditions may determine the membrane shape and elastic energy of Piezo vesicles: fixed-value or natural boundary conditions on the generalized displacements (shape variables) $\psi(s)$, $r(s)$, and $h(s)$ [see the second line of Eq. (S32)], and boundary conditions arising from the variable length of the integration domain in Eq. (S1) [see the third line of Eq. (S32)]. For the membrane bending energy in Eq. (S1) with Eq. (S2) we have the integration limits $s_1 = 0$ and $s_2 = s_b$ in the third line of Eq. (S32), with s_b being variable.

As discussed in the main text, the basic geometric properties of Piezo vesicles immediately yield the boundary conditions

$$\psi(0) = 0\,, \tag{S49}$$

$$r(0) = 0\,, \tag{S50}$$

$$h(0) = 0\,, \tag{S51}$$

$$\psi(s_b) = \pi - \alpha\,, \tag{S52}$$

$$r(s_b) = r_b \tag{S53}$$

on the shape variables $\psi(s)$, $r(s)$, and $h(s)$. However, these boundary conditions alone are not sufficient to solve the Hamilton equations in Eqs. (5)–(10) of the main text, or to ensure that the energy functional is stationary in Eq. (S32).

To proceed, first note that, from the definition of $p_\psi(s)$ in Eq. (S3), we have

$$p_\psi(0) = 2\pi K_b \lim_{s\to 0}\left[r\left(\dot{\psi} + \frac{\sin\psi}{r}\right)\right] = 2\pi K_b \lim_{s\to 0}\left(r\dot{\psi}\right) + 2\pi K_b \lim_{s\to 0}\left(\sin\psi\right) = 0\,, \tag{S54}$$

where we assumed that $\dot{\psi}(0)$ is finite and used Eqs. (S49) and (S50), yielding the boundary condition $p_\psi(0) = 0$.

Second, we note that, since the membrane bending energy in Eq. (S1) with Eq. (S2) does not depend on the absolute value of h, we can arbitrarily set $h(s) = 0$ at $s = 0$ without loss of generality, resulting in the boundary condition in Eq. (S51). Having fixed $h(0) = 0$, we assume that $h(s_b)$ and, hence, $h(s_b) - h(0)$ can be varied when minimizing the membrane bending energy in Eq. (S1) with Eq. (S2). From the second line of Eq. (S32) we see that we then must have $p_h(s_b) = 0$ for the first variation of Eq. (S1) with Eq. (S2) to be equal to zero. From the Hamilton equation for $\dot{p}_h(s)$, Eq. (S47), it thus follows that $p_h(s) = 0$ for $0 \le s \le s_b$, yielding

$$p_h(0) = 0\,. \tag{S55}$$

Equation (S55) implies that the system satisfies natural (zero-force) boundary conditions on $h(s)$ not only at $s = s_b$ but also at $s = 0$.

Third, we consider constraints arising from the variable length of the integration domain in Eq. (S1) with Eq. (S2). Note that the Lagrangian in Eq. (S2) does not depend on the absolute value of s. Without loss of generality, we therefore use $s = 0$ as the lower limit of integration in Eq. (S1). However, because the length of the integration domain in Eq. (S1) with Eq. (S2) is not fixed, we allow the value of the upper limit of integration, $s = s_b$, to be varied when minimizing the membrane bending energy. As shown in Sec. S2.1, stationarity of Eq. (S1) with Eq. (S2) then requires that the Hamiltonian of the system vanishes at $s = s_b$ [see the third line of Eq. (S32)]:

$$H\big|_{s=s_b} = 0\,. \tag{S56}$$

The Hamiltonian associated with Piezo vesicles in Eq. (S6) does not explicitly depend on s. As a result, H is conserved along s [see Eq. (S48)] [1]. Equation (S56) thus yields $H = 0$ for $0 \leq s \leq s_b$ [5, 6]. We therefore have $H = 0$ at $s = 0$, which implies that Eq. (S1) with Eq. (S2) is also stationary with respect to variations in the lower limit of the integration domain. Furthermore, note from Eq. (S6) that

$$H = \pi K_b r \left(\dot{\psi}^2 - \frac{\sin^2\psi}{r^2}\right) - 2\pi\lambda_a r + \lambda_r \cos\psi + \lambda_h \sin\psi \tag{S57}$$

and that, by L'Hôpital's rule together with the geometric relation $\dot{r} = \cos\psi$, the two principal curvatures $\dot{\psi}$ and $\sin\psi/r$ must be equal to each other at $s = 0$,

$$\lim_{s\to 0} \frac{\sin\psi}{r} = \dot{\psi}(0)\,, \tag{S58}$$

where we used the boundary conditions in Eqs. (S49) and (S50). Assuming that λ_a is finite, Eqs. (S57) and (S4) thus yield $H = p_r(s)$ at $s = 0$, where we have again used the boundary conditions in Eqs. (S49) and (S50) together with Eqs. (S5) and (S55). Since $H = 0$ at $s = 0$, one therefore finds [5]

$$p_r(0) = 0\,, \tag{S59}$$

as stated in the main text. Equations (S49)–(S51), (S54), (S55), and (S59) provide the initial conditions for the generalized displacements and generalized momenta in Eq. (12) of the main text, while Eqs. (S52) and (S53) specify the values of the shape variables $\psi(s)$ and $r(s)$ at the Piezo dome boundary. Note that solutions of the Euler-Lagrange or Hamilton equations satisfying these boundary conditions indeed yield a vanishing first variation of the membrane bending energy in Eq. (S1) with Eq. (S2) [see Eq. (S32)].

S4 Solving the Hamilton equations

We used *Mathematica* [9] to numerically solve the six Hamilton equations and area equation in Eqs. (5)–(11) of the main text subject to the initial conditions in Eq. (12) of the main text and the experimental constraints in Eq. (13) of the main text to obtain the shape of the free membrane in Piezo vesicles (see Fig. 4 of the main text as well as Fig. 1 of our companion paper). We used an analogous numerical solution approach to solve, instead, Eqs. (5)–(11) with Eq. (12) in the main text subject to the constraints $A(s_b) = A_P$, $\psi(s_b) = -\alpha$, and $r(s_b) = r_b$ associated with the minimization of the Piezo dome bending energy (see Fig. 5*A* of the main text), or the constraints $A(s_b) = A_F$, $\psi(s_b) = \pi - \alpha$, and $r(s_b) = r_b$ associated with Figs. 2 and 3 in our companion paper. For all of these calculations it is useful to rewrite Eqs. (5)–(11) of the main text in dimensionless form. To this end we use, for all calculations pertaining to the minimization of the free membrane bending energy in Piezo vesicles, as the unit of length the spatial scale $L_c = \sqrt{A_F}$, which characterizes the spatial extent of the free membrane in Piezo vesicles. For all calculations pertaining to the minimization of the Piezo dome bending energy we use, instead, as the unit of length the spatial scale $L_c = \sqrt{A_P}$, which characterizes the spatial extent of the Piezo dome. In either case we use as the unit of energy the characteristic energy scale in Eqs. (1) and (2) of the main text, K_b. We thus make the change of variables $s \to \bar{s} = \frac{s}{L_c}$, $r \to \bar{r} = \frac{r}{L_c}$, $\lambda_a \to \bar{\lambda}_a = \frac{L_c^2}{K_b}\lambda_a$, $u_0 \to \bar{u}_0 = L_c u_0$, etc.

The Hamilton equations in Eqs. (5)–(10) of the main text involve the factors $\frac{1}{r}$ and $\frac{1}{r^2}$ while, from the initial conditions in Eq. (12) of the main text, we have $r(s) \to 0$ as $s \to 0$. Furthermore, it is, in general, non-trivial to impose the limit in Eq. (S58) in a numerical solution scheme. To address these difficulties, we start the numerical solution of Eqs. (5)–(11) of the main text not at $\bar{s} = 0$ but at some small $\bar{s} = \bar{s}_0 > 0$. The (approximate) initial conditions at $\bar{s} = \bar{s}_0$ can be obtained by expanding the functions entering Eqs. (5)–(11) of the main text about $\bar{s} = 0$, substituting these expansions into Eqs. (5)–(11) of the main text, setting $\bar{s} = \bar{s}_0$, directly imposing the initial conditions in Eq. (12) of the main text together with Eq. (S58) and $\dot{\psi}(0) \equiv u_0$, and solving the resulting equations for the values of the shape variables, generalized momenta, and membrane area at $\bar{s} = \bar{s}_0$, to leading order in $\bar{s}_0$ in each equation. We set $\bar{s}_0 = 10^{-8}$.

For given values of λ_a, s_b, and u_0 the above procedure allows numerical solution of Eqs. (5)–(11) of the main text subject to the initial conditions in Eq. (12) of the main text. We employ a shooting method [10,11] to determine values of λ_a, s_b, and u_0 that satisfy the constraints in Eq. (13) of the main text (Fig. 4 of the main text as well as Fig. 1 of our companion paper), the constraints $A(s_b) = A_P$, $\psi(s_b) = -\alpha$, and $r(s_b) = r_b$ associated with the minimization of the Piezo dome bending energy (Fig. 5*A* of the main text), or the constraints $A(s_b) = A_F$, $\psi(s_b) = \pi - \alpha$, and $r(s_b) = r_b$

associated with Figs. 2 and 3 in our companion paper. In this shooting method, the values of λ_a, s_b, and u_0 are iteratively adjusted so that the respective constraints are satisfied. This is achieved conveniently and efficiently in *Mathematica* [9] by nesting the *NDSolve*-command, used to numerically solve Eqs. (5)–(11) of the main text, inside the *FindRoot*-command, used to adjust the values of λ_a, s_b, and u_0 so as to satisfy the respective constraints. To initialize the numerical optimization carried out by the *FindRoot*-command, it is thereby necessary to specify starting values for λ_a, s_b, and u_0. In dimensionless form, useful sets of starting values for the scenarios considered here are given by $\left(\bar{\lambda}_a, \bar{s}_b, \bar{u}_0\right) = (-1, 1, \pi)$ for the minimization of the free membrane bending energy in the present paper (Fig. 4 of the main text) and in our companion paper (Figs. 1–3 of our companion paper), and by $\left(\bar{\lambda}_a, \bar{s}_b, \bar{u}_0\right) = (-10, 1, -\pi)$ for the minimization of the Piezo dome bending energy (Fig. 5*A* of the main text). When carrying out a series of related shooting calculations with only minor changes to the constraints imposed on the Piezo vesicle shape, it is generally convenient to initialize $\left(\bar{\lambda}_a, \bar{s}_b, \bar{u}_0\right)$ using the optimized values of $\left(\bar{\lambda}_a, \bar{s}_b, \bar{u}_0\right)$ obtained for a 'nearby' (the preceding) solution. A general caveat is that the shooting method does not necessarily yield a unique solution to boundary value problems such as studied here.

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