

Cut it out: Out-of-plane stresses in cell sheet folding of *Volvox* embryosPierre A. Haas^{1,2,3,*} and Steph S. M. H. Höhn^{4,*}¹*Max Planck Institute for the Physics of Complex Systems, Nöthnitzer Straße 38, 01187 Dresden, Germany*²*Center for Systems Biology Dresden, Pfotenhauerstraße 108, 01307 Dresden, Germany*³*Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, 01307 Dresden, Germany*⁴*Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Wilberforce Road, Cambridge CB3 0WA, England, United Kingdom*

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The folding of cellular monolayers pervades embryonic development and disease, and is often caused by cell shape changes such as cell wedging. However, the function and mechanical role of different active cellular changes in different regions of folding tissues remain unclear in many cases, at least partially because the quantification of out-of-plane mechanical stresses in complex three-dimensional tissues has proved challenging. The gastrulationlike inversion process of the green alga *Volvox* provides a unique opportunity to overcome this difficulty: Combining laser ablation experiments and a mechanical model, we infer the mechanical properties of the curved tissue from its unfurling on ablation. We go on to reproduce the tissue shapes at different developmental timepoints quantitatively using our mechanical model. Strikingly, this reveals out-of-plane stresses associated with additional cell shape changes away from those regions where cell wedging bends the tissue. Moreover, the fits indicate an adaptive response of the tissue to these stresses. In this way, our paper provides not only the experimental and theoretical framework to quantify out-of-plane stresses in tissue folding, but it also shows how additional cell shape changes can provide another source of out-of-plane stresses in development complementing cell wedging.

DOI: [10.1103/PhysRevE.111.014420](https://doi.org/10.1103/PhysRevE.111.014420)**I. INTRODUCTION**

The folding of tissues into three-dimensional shapes is a crucial part of morphogenetic processes such as neurulation and gastrulation in natural embryonic development [1–4], associated birth defects such as spina bifida [5], and synthetic morphogenesis in organoids [6–9]. These dramatic shape changes are regulated by a complicated interplay of mechanical forces and molecular signaling [10–17]. The latter can be visualized by fluorescent tagging [18–20], but the mechanical forces must be inferred indirectly and have therefore remained one of the biggest mysteries in the life sciences.

The best-known mechanism underlying the bending of cell sheets is cell wedging through apical or basal constriction [3,10,21,22]. Whether such cell wedging is active or the passive mechanical result of active processes elsewhere is a fundamental question of morphogenesis, but remains debated in many cases, such as the formation of the hinge in the

optic cup [7,23–25]. Active cell wedging locally imparts a preferred, intrinsic curvature to the tissue which drives tissue bending by generating out-of-plane forces. This intrinsic curvature need not however be compatible with the global geometry of the tissue, leading to persisting tissue stresses termed residual stresses [26,27]. Now the stresses accompanying morphogenetic movements impact both their mechanics and biochemical responses [28]. In particular, tissues are known to have the ability to alleviate tensile stresses through cell movements or changes in cell adhesion or shape [29] and this adaptation can be crucial for maintaining tissue integrity [30]. The role of adaptation to stresses in tissue folding and how the mechanical state of the tissue is involved in this adaptation in general remain, however, open questions. Addressing these outstanding problems fundamentally relies on quantifying the spatiotemporal distribution of mechanical forces in tissues.

Over the past decade, different techniques to infer mechanical forces and stresses in tissues have indeed emerged, ranging from atomic force microscopy, traction force microscopy, and fluorescence resonance energy transfer-based molecular force sensors to optical tweezers, micropipette aspiration, magnetic beads, and liquid droplets [31,32]. However, methods for quantifying out-of-plane stresses in folding epithelia are still lacking. In fact, we are only aware of a single quantitative study of out-of-plane stresses in cell sheets: Fouchard *et al.* [33] measured the force needed to unfurl curled synthetic cell sheets and went on to estimate the active torques and the bending modulus of epithelial monolayers

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[34]. In particular, many studies have thus used laser ablation to infer in-plane forces from quantifications of the ensuing recoil of the tissue: see, e.g., Refs. [35–38]. However, extending these methods to quantifying out-of-plane forces in curved tissues *in vivo* has proved challenging because of (i) the difficulty of imaging out-of-plane recoils on ablation and (ii) the need for a mechanical model of these complex deformations to link the measured recoil to the mechanical forces causing it. Here, we present our framework combining parallel laser ablation [PLA; Fig. 1(a)] and a mechanical model to quantify out-of-plane stresses and infer mechanical properties of curved cell sheets. We apply our framework to the gastrulationlike inversion process of the microalga *Volvox*.

Inversion in *Volvox* and the related volvocine algae (Chlorophyta) is an emerging model developmental event, during which the spherical embryonic cell sheet turns itself inside out through a program of cell shape changes [40–48]. *Volvox* embryos are free to deform within a fluid-filled compartment within their transparent parent, without confinement from extracellular matrix (ECM) structures. Their chlorophyll autofluorescence serves as a label-free cell marker. These features make *Volvox* inversion uniquely accessible to experimental visualization and perturbations via laser ablations. Additionally, the axisymmetry of the morphogenetic movements of inversion makes the process ideally suited to theoretical modeling.

Here, we focus on what is called type-B inversion in *Volvox globator* [Refs. [39,40,43,49] and Figs. 1(b)–1(g) and 1(b′)–1(g′)]. We have previously described this morphogenetic process with a mechanical model [39,50,51] in which the program of cell shape changes driving tissue movements appears as variations of the intrinsic curvatures and intrinsic stretches of an elastic shell [39,50] and which reproduces the inversion process quantitatively [51]. These *in silico* approaches have made a number of mechanical predictions [39,51], but direct experimental tests of the underlying framework are still missing.

We therefore start by using our ablation framework to reveal persistent residual out-of-plane stresses in the posterior hemisphere throughout inversion by showing how the cell sheet unfurls at the boundary of a circular ablation at the posterior pole. We use our mechanical model to show that the geometric incompatibilities causing this recoil result from mismatched intrinsic and observed curvatures of the cell sheet. From quantifications of the recoil, we infer mechanical properties and the intrinsic curvature of the posterior hemisphere during its invagination, the latter being consistent with the observed cell shape changes. Thus these results not only provide proof of principle of our ablation framework, but also test the elastic framework underlying our previous *in silico* approaches. We go on to extend our elastic model [51,52] to reproduce the tissue shapes quantitatively at different stages of inversion. Strikingly, the resulting fitted mechanical sequence and the ablation data show a clear signature of out-of-plane stresses in the posterior hemisphere and suggest an adaptive response to these stresses. This shows that the additional cell shape changes in the posterior hemisphere, which generate these out-of-plane stresses, and cell wedging are complementary mechanical mechanisms for tissue folding in *Volvox*.

Type-B inversion in *Volvox globator*

We close this introduction with a more detailed description of type-B inversion in *Volvox globator*, summarizing results that this paper relies on. After completion of an initial cell cleavage phase [53,54], the embryos of *Volvox globator* are spherical monolayers of ≈ 3000 cells [43]. Each embryo is located within a fluid-filled embryonic vesicle that is embedded in the ECM of the parent [55–58]. The embryonic cells are connected to each other by a network of cytoplasmic bridges (CBs) resulting from incomplete cytokinesis [59]. A ring of cells at the anterior pole lacks CBs, leaving an opening, the phialopore. During the morphogenetic process of inversion, the embryos turn themselves inside out through this phialopore within ≈ 60 – 80 min in order to expose the two cilia that grow at each apical cell pole [40].

During inversion the embryonic cells, which have a microtubule-based cortical cytoskeleton, but neither cell walls nor ECM [60], remain connected to each other by CBs, so the positions of the cells relative to their neighbors are maintained [40]. This suggests the elastic description of the cell sheet that we have adopted previously [39,50–52,61]. The global deformations of the embryonic cell sheet during inversion are driven by cell shape changes [Figs. 1(b)–1(g)] that have previously been described based on electron and confocal microscopy [40].

Prior to inversion, all embryonic cells are teardrop shaped and connected at their broadest point by CBs [Figs. 1(b) and 1(b′)]. Type-B inversion begins (“early invagination”) with the appearance of a circular bend region (BR) just below the equator of the embryo, caused by cell wedging and relocation of the CBs to the thinner basal cell poles [40]. Simultaneously, the cells in the posterior hemisphere undergo likely actin-dependent thinning [62,63] and become spindle shaped [Figs. 1(c) and 1(c′)]. Our previous *in silico* analyses [39,51] indicate that active contraction of the posterior cell sheet, associated with this cell thinning, is necessary to explain the resulting “mushroom shape” of the embryos. The questions of whether this contraction is associated with residual stresses and, if so, whether the cells alleviate such stresses during inversion have remained open, however.

As inversion proceeds (“midinvagination”), a wave of cell wedging travels from the equator towards the posterior pole and the posterior hemisphere moves into the anterior one [40]. The cells at the posterior pole retain their spindle shapes [Figs. 1(d) and 1(d′)]. At late invagination, a “dimple” remains where the cell sheet around the posterior pole has not yet inverted. The cells at the posterior pole remain spindle shaped while the basal cell poles in the BR are less wedge shaped than before, resulting in a relaxation of the curvature in the BR and near the posterior pole [Figs. 1(e) and 1(e′)]. By the time the posterior hemisphere has inverted fully, the phialopore has widened and the anterior hemisphere is peeling over the inverted posterior. The cells in the inverted posterior have adopted a pencil shape [Figs. 1(f) and 1(f′)] at this stage. Closure of the phialopore marks the completion of inversion and the end of embryogenesis in *Volvox*. At this stage, all cells are pencil shaped with pointy apical cell poles [40]. Within 30–60 min after inversion, all cells shorten, widen, and adopt a columnar shape while the radius of the juvenile spheroid increases [Figs. 1(g) and 1(g′)].

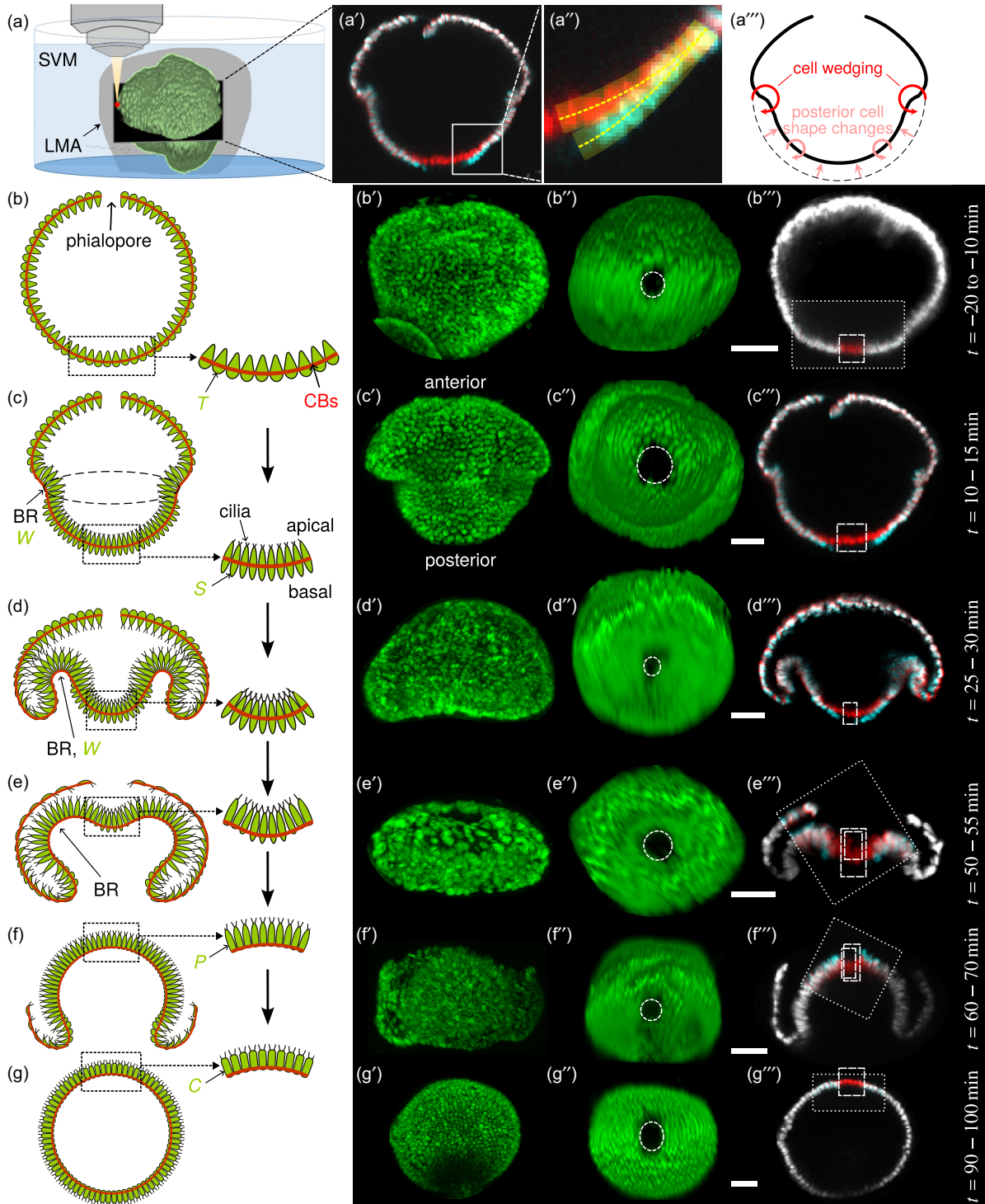


FIG. 1. Experiments: Parallel laser ablation (PLA) of inverting *Volvox globator* embryos. (a) Experimental setup for two-photon microscopy and PLA, illustrated for a surface rendering of a *V. globator* embryo at an early inversion stage. Samples were embedded in low-melting point agarose (LMA), submerged in standard *Volvox* medium (SVM), and imaged using two-photon microscopy of chlorophyll autofluorescence. Holes were created using a separate two-photon laser (Appendix A) at the posterior pole of embryos before, during, and after inversion. (a') Overlay (white) of micrographs of the midsagittal cross section of the embryo in panel (a), just before ablation (red) and after relaxing into its postablation equilibrium shape (cyan), showing the unfurled edge of the cell sheet. (a'') Tracing of the cell sheet (transparent area) and its midline (dashed line) in the square magnified from panel (a'). (a''') Mechanical hypothesis: Posterior cell shape changes not only contract the posterior hemisphere [39], but also induce a curvature mismatch in it that causes out-of-plane stresses distant and distinct from those generated by the wedge-shaped cells in the bend region. (b)–(g) Schematic cross sections of *V. globator* embryos in consecutive developmental stages, modified from Ref. [40]. The cross-section schematics show the cell shapes associated with the different stages of inversion, the network of cytoplasmic bridges (CBs, red line), and the growing cilia. BR, bend region; W, wedge-shaped cells. Insets show

II. EXPERIMENTS

A. Parallel laser ablation in type-B *Volvox* inversion

To quantify out-of-plane stresses in *Volvox* inversion, we performed laser ablation experiments on *Volvox globator* embryos in consecutive stages of inversion (Appendix A). To capture out-of-plane elastic responses to these ablations, they were performed on midsagittal cross sections, i.e., cross sections in a plane containing the axis defined by the anterior and posterior poles of the axisymmetric *Volvox globator* embryos. Cross sections were imaged using two-photon microscopy [Fig. 1(a) and Appendix A], and approximately circular holes were created at the posterior poles of the embryos by laser ablation using a separate two-photon laser (Appendix A). The axisymmetry of the embryos and the ablations ensures that any subsequent deformation of the cell sheet is approximately axisymmetric, too. The dimensions of the resulting hole in the cell sheet were determined in three-dimensional datasets recorded subsequently [Figs. 1(b'')–1(g'') and Appendix A].

B. Observations

Laser ablations were performed at the posterior pole of embryos in consecutive developmental stages before, during, and after inversion [Figs. 1(b'')–1(g'') and 1(b''')–1(g''') and Movies S1–S7 in the Supplemental Material [64]], i.e., no deformation within at least 45 s after ablation ($N = 4$ embryos), indicating that the cell sheet is not residually stressed before inversion starts. However, at early invagination [Figs. 1(c'') and 1(c''') and Fig. S1 and Movies S2 and S3 in the Supplemental Material [64]] and midinvagination [Figs. 1(d'') and 1(d''') and Movie S4 in the Supplemental Material [64]], ablation led to outward unfurling of the edges of the cell sheet and relaxation into a new equilibrium shape ($N = 14$), showing that out-of-plane residual stresses have appeared in the cell sheet. When ablations were performed at the “dimple stage” of late invagination [Figs. 1(e) and 1(e')], the unfurling cell sheet adopted a curvature closer to that in the BR [Figs. 1(e'') and 1(e''') and Fig. S2 and Movie S5 in the Supplemental Material [64]; $N = 4$], indicating a mechanical effect of the BR on the postablation equilibrium shape. Interestingly, for ablations at the stage where the posterior hemisphere has fully inverted [Figs. 1(f) and 1(f')], the direction of unfurling changed compared to earlier timepoints [Figs. 1(f'') and 1(f'''), and Fig. S3 and Movie S6 in the Supplemental Material [64]; $N = 7$]. Finally, for posterior ablations after completion of inversion, when the cells have become columnar [Figs. 1(g) and 1(g')], the cell sheet no longer showed any recoil within at least 90 s after laser ablation

[Figs. 1(g'') and 1(g''') and Movie S7 in the Supplemental Material [64]; $N = 3$], indicating that the residual stresses have relaxed.

In all experiments, unfurling of the edges of the cut upon laser ablation and relaxation into the postablation equilibrium shape took 4–10 s (Fig. 1; see also Figs. S1–S3 in the Supplemental Material [64]). Within this time, the embryos otherwise maintained their shape: Morphogenetic inversion movements, i.e., global embryonic shape changes distinct from the local ablation response, were only distinguishable after at least 30 s, but did continue after ablation (Movie S8 in the Supplemental Material [64]).

The observed outward unfurling of the cell sheet suggests that the preferred, intrinsic curvature of the cell sheet differs from its observed curvature before ablation. We therefore hypothesize that such a curvature mismatch gives rise to out-of-plane stresses across the posterior hemisphere [Fig. 1(a''')]. The thinning of cells from teardrop to spindle shapes driving contraction of the posterior hemisphere also changes its curvature [Ref. [50] and Figs. 1(a'''), 1(b), and 1(c)], and so could contribute to this mismatch of curvatures that would therefore result not only from cell wedging near the equator [Figs. 1(a''') and 1(c)]. The change of the direction of unfurling with the change of the sign of the curvature of the posterior hemisphere [Figs. 1(b''') and 1(f'')] is consistent with this hypothesis if the intrinsic curvature of the tissue is smaller in magnitude than its actual curvature. However, alternative hypotheses for the source of these out-of-plane stresses might be anisotropic stretching or an active cell response to the ablation.

III. ORIGIN OF OUT-OF-PLANE STRESSES IN VOLVOX INVERSION

A. Mechanical toy problems

To test these hypotheses, we need to understand the mechanics of ablations in elastic spheres. For this purpose, we introduce three mechanical toy problems.

1. Toy problem 1: Deformations of a spherical elastic shell with mismatched intrinsic curvatures

Our first toy problem studies the effect of curvature mismatch on cell sheet shapes before ablation: We consider a complete spherical shell of unit radius and (relative) thickness $h \ll 1$. The intrinsic curvatures of the shell are $\kappa^0 = k \neq 1$, different from the undeformed curvature of the shell. This curvature mismatch causes the shell to deform; we assume that the shell remains spherical, and denote by f its deformed radius [Fig. 2(a)].

This radius is determined by the competition of the strains $E = f - 1$ due to the stretching of the shell and the bending

cell shapes [40] near the posterior pole of the embryo (T, teardrop-shaped cells; S, spindle-shaped cells; P, pencil-shaped cells; C, columnar cells). (b')–(g') Maximum intensity projections of z stacks showing lateral views of *V. globator* embryos in the developmental stages in panels (b)–(g). The anterior and posterior hemispheres are labeled in panel (c'). (b'')–(g'') Posterior view of the embryos in panels (b')–(g') after laser ablation. Dashed white line: outline of the hole created by laser ablation after relaxation into its postablation equilibrium shape. (b''')–(g''') Overlays (white) of micrographs of the midsagittal cross section of the embryos in panels (b')–(g') just before ablation (red) and after relaxing into their postablation equilibrium shapes (cyan). Dashed lines: areas of laser ablation. Thin dotted lines in panels (b''') and (e''')–(g''') denote fields of view in which the laser ablation was performed. Times t are given relative to the start time $t = 0$ of inversion. Scale bars: 20 μm .

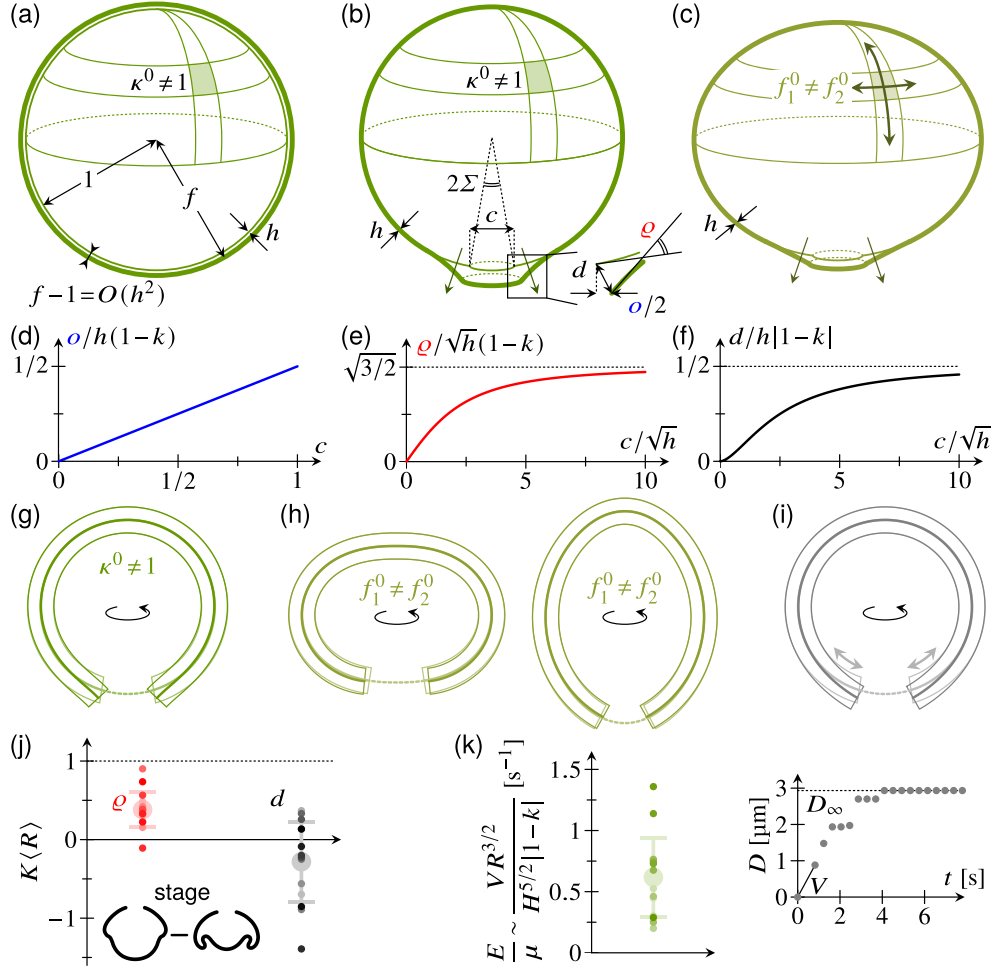


FIG. 2. Origin of out-of-plane stresses in *Volvox* inversion: Mechanical toy problems and analysis of experiments. (a) Toy problem 1: A spherical shell of unit radius and thickness $h \ll 1$ deforms to a radius $f = 1 + O(h^2)$ due to mismatched intrinsic curvatures $\kappa^0 = k \neq 1$. (b) Toy problem 2: This mismatch of intrinsic curvatures causes a circular cut of angle 2Σ and radius $c = 2 \sin \Sigma$ to open. Inset: Definition of the opening o , rotation ϱ , and displacement d of the cut edge. (c) Toy problem 3: Anisotropic contraction also causes recoil on ablation. (d) Plot of $o \sim h(1-k)$ against c . (e) Plot of asymptotic approximation for $\varrho/\sqrt{h}(1-k)$ against $c/\sqrt{h}$. (f) Plot of asymptotic approximation for $d/h|1-k|$ against $c/\sqrt{h}$. (g) Numerical solution of toy problem 2: Plot of cross-section preablation (light) and postablation (dark), showing a recoil qualitatively consistent with experiments. (h) Numerical solutions of toy problem 3: The recoil is much smaller than in experiments despite the excessive anisotropy. (i) Fast localized relaxation can also reproduce the experimental recoil qualitatively. (j) Estimated dimensional intrinsic curvatures K , nondimensionalized with the average posterior radius $\langle R \rangle$ during invagination. Estimates are computed from numerical solutions of toy problem 2 using measurements of ϱ, d for $N = 14$ *Volvox* embryos at early invagination and midinvagination (inset). Large markers and error bars: mean and standard deviation of estimates. (k) Estimates of the ratio E/μ from Eq. (4) and measurements of the initial recoil velocity V for the $N = 14$ ablations of panel (j). Variables: elastic modulus E , effective viscosity μ , (dimensional) cell sheet thickness H , posterior radius R during invagination, and (dimensionless) estimated intrinsic curvature k from panel (j). Inset: Example measurement (Fig. S1 in the Supplemental Material [64]) of the (dimensional) cut displacement D against time, showing the initial velocity V and equilibrium displacement D_∞ used for the estimates in panels (j) and (k).

strains $K = 1/f - k$ resulting from the difference of the actual and intrinsic curvatures of the shell. The elastic energy of the shell is the sum of its stretching and bending energies [65], and so is proportional to

$$E^2 + \frac{h^2}{12} K^2 = (f-1)^2 + \frac{h^2}{12} (1/f - k)^2. \quad (1)$$

Assuming $k = O(1)$, this is minimized for

$$f \sim 1 - \frac{h^2}{12} (k-1). \quad (2)$$

As expected, the shell grows ($f > 1$) or shrinks ($f < 1$) if its preferred curvature is flatter ($k < 1$) or larger ($k > 1$) than its undeformed curvature, but the deformations are asymptotically small compared to the shell thickness.

There is an interesting mechanical subtlety of this calculation: Though this argument gives the correct $O(h^2)$ scaling for the deformations of the shell, the smallness of the deformations is beyond the realm of validity of the shell theory assumed in writing down Eq. (1). For this reason, the correct prefactor has to be calculated within bulk nonlinear solid mechanics; the calculation is given in Appendix B. There we

also discuss some of the surprising behavior revealed by that prefactor which shows how seemingly innocuous problems in nonlinear mechanics of this ilk can break our intuition.

2. Toy problem 2: Circular ablation of a spherical elastic shell with mismatched intrinsic curvatures

Next, we study the mechanics of a circular ablation in a shell with mismatched intrinsic curvatures. The mismatched intrinsic curvatures and the no-torque boundary condition at the rim of the cut cause the shell to deform near the cut. For an ablation of angle 2Σ , i.e., radius $c = 2 \sin \Sigma$ [Fig. 2(b)], asymptotic solution of the equations of shell theory for $h \ll 1$ (Appendix B) shows that the cut opens by an amount [Fig. 2(d)]

$$o \sim h(1 - k) \sin \Sigma = \frac{h}{2}c(1 - k). \quad (3)$$

Importantly, this is asymptotically larger than the deformation without ablation described by Eq. (2). Asymptotic approximations can also be found (Appendix B) for the rotation ϱ of the rim of the cut and its displacement d due to the ablation [Figs. 2(e) and 2(f)].

3. Toy problem 3: Circular ablation of a spherical elastic shell with anisotropic contraction

Opening of an ablation does not require intrinsic curvature mismatches, however: As explained in more detail in Appendix B, geometry requires the stretches of an axisymmetric spherical shell to be equal at its poles, so anisotropic contraction will cause stresses there and hence result in a recoil on ablation [Fig. 2(c)].

B. Discussion

These results allow us to test our hypothesis, that there is a curvature mismatch during *Volvox* inversion. First, toy problems 1 and 2 emphasize that ablation experiments are needed: The unablated deformations are too small to be visualized in experiments; only the deformations from ablations are comparable to the thickness of the cell sheet, and so allow quantification of curvature mismatches that cannot be quantified from the unablated cell sheet only.

1. The observed recoil during *Volvox* invagination results from a curvature mismatch

Next, solving for postablation shapes in toy problems 2 and 3 numerically (Appendix A), we observe that the recoil for an intrinsically flat shell ($k = 0$) agrees with experiments qualitatively [Figs. 1(c''') and 2(g)]. By contrast, the recoil resulting from anisotropic contraction is much smaller than the experimentally observed one, even for blatantly excessive anisotropies [Figs. 1(c''') and 2(h)]. This does not yet allow the conclusion that the observed recoil results from a curvature mismatch: Indeed, fast biological processes could *a priori* be triggered close to the ablation site to lead to a fast active relaxation of the shell there. The resulting geometric incompatibility could, it turns out, lead to a recoil qualitatively consistent with experiments [Fig. 2(i) and Appendix A]. If however this mechanism underlied the experimental observations, we would expect to observe a recoil for pre- and postinversion ablations, too, which we do not [Figs. 1(b''') and 1(g''')].

We therefore discard this possibility, and conclude that the observed recoil is only consistent with a curvature mismatch that existed before ablation.

2. Quantitative estimates of the curvature mismatch during *Volvox* invagination are consistent with the observed cell shape changes

To make this analysis more quantitative, we measured the recoil on ablation in $N = 14$ *Volvox* embryos at early invagination and midinvagination [Appendix A and Figs. 1(c)–1(c'''), 1(d)–1(d'''), and Fig. S1 in the Supplemental Material [64]], and extracted estimates of intrinsic curvatures [Fig. 2(j)] from measurements of the cut rotation ϱ and displacement d using numerical solutions of toy problem 2. The hemispherical shape of the posterior at these early inversion stages [Fig. 2(j), inset] justifies using toy problem 2 for this inference. The consistent outward curling of the cell sheet on ablation (Fig. S1 in the Supplemental Material [64]) indicates $k < 1$, and, indeed, averaging these estimates, we find $\langle k \rangle \approx 0.05 \ll 1$, i.e., very much smaller than the observed posterior curvature, stressing the mismatch of these curvatures.

Importantly, this estimate is consistent with the observed cell shape changes: The spindle-shaped cells in the posterior during early *Volvox* invagination are symmetric, and connected at their midplane by cytoplasmic bridges [Fig. 1(c), inset], suggesting $k = 0$. By contrast, the teardrop-shaped cells in preinversion embryos [Fig. 1(b), inset] suggest $k > 0$, and indeed the absence of recoil observed preinversion (i.e., the absence of residual stresses) requires $k \approx 1$.

In this way, the observed cell shapes, which our mechanical model is agnostic to, provide an independent way of testing a mechanical prediction of our ablation approach and hence of validating it. This relies on the structure of the cell sheet of *Volvox* (cells connected by CBs as opposed to a confluent tissue, say), and is one of the reasons for using the emerging model organism that is *Volvox* for this validation.

We could also have tried to estimate the intrinsic curvature from measurements of the cut opening o or based on the asymptotic solutions of toy problem 2. These estimates cannot be relied upon however; the reasons for this are discussed in Appendix C.

3. Recoil velocity measurements yield a bound on the elastic modulus of the cell sheet

The (dimensional) recoil velocity V is set by the interplay between the elastic force driving it and viscous dissipation in the tissue and surrounding fluid. A scaling argument (Appendix B) yields

$$V \sim \frac{E}{\mu} \frac{H^{5/2}}{R^{3/2}} |1 - k|, \quad (4)$$

in which E is the elastic modulus of the cell sheet, μ is an effective viscosity, and H and R are the dimensional thickness and posterior radius of the cell sheet. Using this relation, we estimate in Fig. 2(k) the ratio E/μ based on the initial recoil velocity of the $N = 14$ ablations also analyzed in the previous paragraph. We stress our use of this initial velocity for these dynamic measurements, by contrast with the above matching of (quasi)equilibrium quantifications of the recoil to a static toy problem [Fig. 2(k), inset].

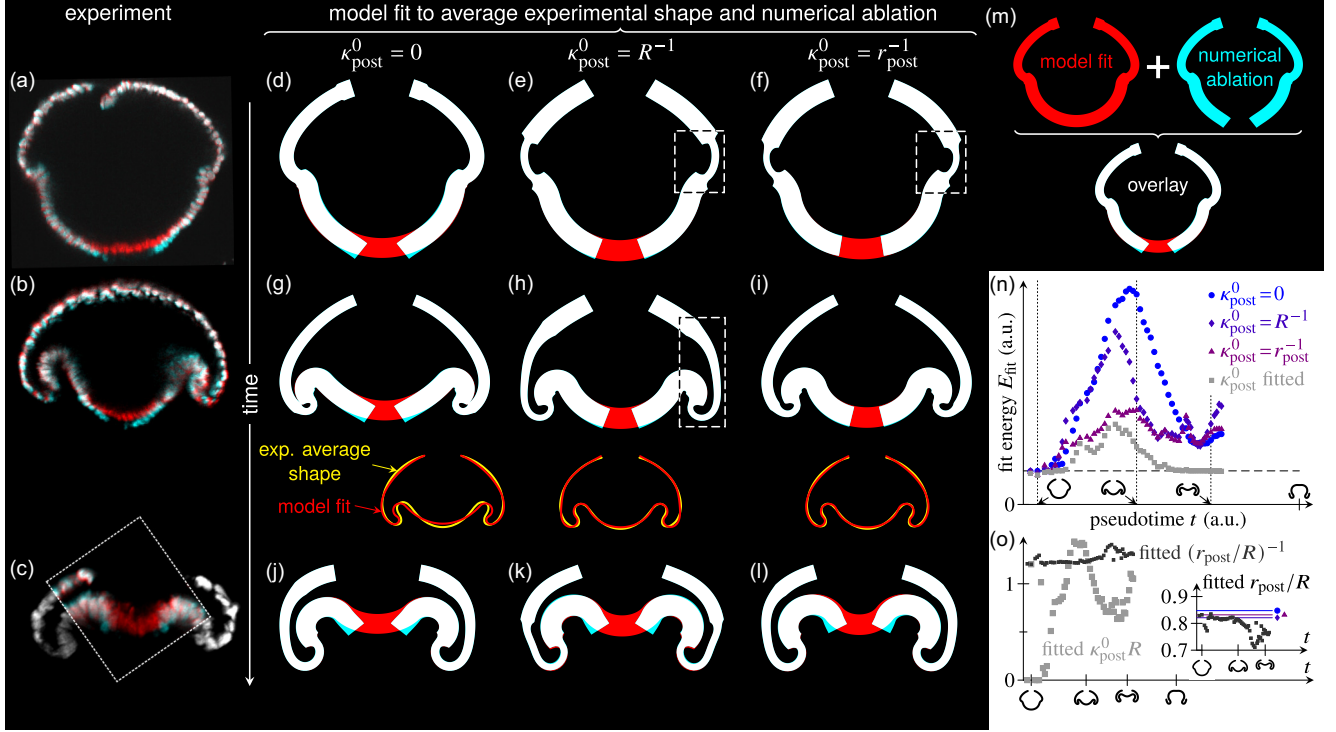


FIG. 3. Effect of out-of-plane stresses in *Volvox* inversion: Fits of the elastic model to average experimental cross sections [51] and numerical ablations. (a) Overlay of experimental cross sections (white) before (red) and after (cyan) a posterior ablation at an early invagination stage [Fig. 1(c'')]. (b) Analogous plot for an ablation at a midinvagination stage [Fig. 1(d'')]. (c) Analogous plot for an ablation at the “dimple stage” [Fig. 1(e'')]. Outside the dotted rectangle, only preablation shapes are plotted (white). (d) Fit, for zero posterior intrinsic curvatures $\kappa_{\text{post}}^0 = 0$, of the elastic model to the average experimental cross section at an inversion timepoint corresponding to panel (a) (red), overlaid with the equilibrium shape after numerical ablation (cyan), showing a recoil on ablation. (e) Analogous plot, for posterior intrinsic curvatures $\kappa_{\text{post}}^0 = R^{-1}$, equal to the undeformed curvature of the embryo. The recoil is reduced. The dashed box highlights thinning of the cell sheet in the fitted shape. (f) Analogous plot, for posterior intrinsic curvatures $\kappa_{\text{post}}^0 = r_{\text{post}}^{-1}$, equal to the inverse contracted intrinsic radius of the posterior. There is almost no recoil. (g) Fit, for $\kappa_{\text{post}}^0 = 0$, of the elastic model to the average experimental cross section at an inversion timepoint corresponding to panel (b), and numerical ablation. Inset: Comparison of fitted shape and average experimental cross section. (h) Analogous plot for $\kappa_{\text{post}}^0 = R^{-1}$. The fitted shape reproduces the experimental shape better than that in panel (g). (i) Analogous plot for $\kappa_{\text{post}}^0 = r_{\text{post}}^{-1}$. The fit is again better than that in panel (g). (j) Fit, for $\kappa_{\text{post}}^0 = 0$, of the elastic model to the average experimental cross section at an inversion timepoint corresponding to panel (c), and numerical ablation. (k) Analogous plot with $\kappa_{\text{post}}^0 = R^{-1}$. The recoil on ablation is qualitatively similar. (l) Analogous plot with $\kappa_{\text{post}}^0 = r_{\text{post}}^{-1}$. (m) Illustration of the numerical ablation plots using the plots in panel (d): The superposition of the fitted shape before numerical ablation (red) and that after numerical ablation (cyan) defines the overlaid shape (white). (n) Plot of the fit energy E_{fit} (arbitrary units) against inversion (pseudo)time for fits with $\kappa_{\text{post}}^0 \in \{0, R^{-1}, r_{\text{post}}^{-1}\}$ and for a fit in which $\kappa_{\text{post}}^0, r_{\text{post}}$ are among additional fitting parameters. $E_{\text{fit}} = 0$ would be a perfect fit; the horizontal dashed line indicates the value of E at which fitting was cut off. Pseudotimepoints used for the fits in panels (d)–(l) are highlighted. (o) Plots of fitted $\kappa_{\text{post}}^0 R$ and $(r_{\text{post}}/R)^{-1}$ against inversion (pseudo)time. Inset: Plot of fitted r_{post}/R against inversion pseudotime. The constant values used for the fits with $\kappa_{\text{post}}^0 \in \{0, R^{-1}, r_{\text{post}}^{-1}\}$ are highlighted.

We thus obtain a mean value $\langle E/\mu \rangle \approx 0.6 \text{ s}^{-1}$ [Fig. 2(k)]. With the lower bound $\mu \gtrsim 0.8 \text{ mPa s}$ corresponding to the viscosity of an algal growth medium [66], so neglecting dissipation within the tissue, this yields $E \gtrsim 0.5 \text{ mPa}$. This bound is much lower than the values reported for confluent tissues [67–69], yet perhaps appropriate for these cells connected only by CBs. With $H \approx 11 \mu\text{m}$ [40], the estimated bending modulus $EH^3 \gtrsim 7 \times 10^{-19} \text{ J}$, well above the energy of thermal fluctuations, provides a sanity check of this estimate.

IV. EFFECT OF OUT-OF-PLANE STRESSES IN VOLVOX INVERSION

Having thus shown that out-of-plane stresses resulting from a curvature mismatch persist during *Volvox* invagination,

we ask the following: What is the effect of these out-of-plane stresses on the tissue shape sequence during inversion?

A. Quantitative model of *Volvox* inversion

The toy models in the previous section can describe the shapes of *Volvox* embryos during early invagination locally, close to the posterior ablation, but cannot capture the global embryo shapes and mechanics. We therefore extended our previous detailed morphoelastic continuum model of the cell shape changes of inversion [51] and fitted parameters encoding the cell shape changes driving inversion [40] to the average cell sheet midlines at various timepoints of inversion [51], as described in detail in Appendices A and D. We then performed numerical ablations on these fitted shapes and compared *in vivo* and *in silico* results [Figs. 3(a)–3(m)].

1. Results for early invagination stages are consistent with the observed cell shape changes and estimates from ablation recoils

We first considered an early invagination timepoint [Fig. 3(a)], for which we fitted the average embryo shape (cell sheet midline) for three different values of the posterior intrinsic curvature κ_{post}^0 [Figs. 3(d)–3(f)]: (1) $\kappa_{\text{post}}^0 = 0$, corresponding to the flat posterior intrinsic curvature predicted by the quantitative estimates of the previous section and the observed cell shape changes in the posterior; (2) $\kappa_{\text{post}}^0 = R^{-1}$, equal to the inverse radius of the undeformed embryo, i.e., the value suggested at preinversion stages by the lack of recoil on ablation; and (3) $\kappa_{\text{post}}^0 = r_{\text{post}}^{-1}$, equal to the inverse intrinsic contracted radius of the posterior resulting from the cell shape changes to spindle-shaped cells [Fig. 1(c)]. With this last value, the contracted shape of the uninverted posterior is unstressed. The model can reproduce the average embryo shape well for all three values of κ_{post}^0 [Figs. 3(d)–3(f)], although the fit parameters inferred from the cell sheet midlines for the latter two values imply a thinning of the anterior hemisphere of the cell sheet [highlighted in Figs. 3(e) and 3(f)]. This thinning is only observed experimentally at later stages of invagination, when it is associated with the formation of pancake-shaped cells there [Ref. [40] and Fig. 1(d)]. However, numerical ablations reveal that these three fitted shapes are mechanically different [Figs. 3(d)–3(f)]: Only the first leads to a sizable recoil on ablation, qualitatively consistent with experiments. The fitting thus suggests $\kappa_{\text{post}}^0 = 0$, in agreement with the estimates of the previous section and the observed cell shape changes.

2. Results for late invagination stages are consistent with the observed expansion of the region of cell wedging

Next, we considered a late invagination timepoint [Fig. 3(c)], around the time of phialopore opening. Again, all three values of posterior intrinsic curvature could reproduce the observed cell sheet midlines [Figs. 3(j)–3(l)] and the fitted shapes even reproduce the observed thinning of the anterior hemisphere [40] although the fits are based on the cell sheet midlines only. Moreover, numerical ablations lead to similar recoils in all three cases [Figs. 3(j)–3(l)]. This is consistent with the observed expansion of the wave of cell wedging towards the posterior pole [40]: The recoil magnitude is set no longer by the geometric incompatibilities of the few remaining spindle- or pencil-shaped cells [Fig. 1(e), inset], but by that of the wedge-shaped cells in the bend region [Fig. 1(c)].

B. Discussion

These results thus provide a check of the fitting and suggest that the mechanics of out-of-plane stresses in *Volvox* inversion can be captured correctly by this fitting. In turn, this indicates that we can use the fit results to understand out-of-plane stresses at other stages of inversion.

1. Fitted mechanics at midinvagination stages predict changing posterior out-of-plane stresses

Hence we next turned to a midinvagination stage [Fig. 3(b)], again fitting the model for the three different values of κ_{post}^0 to the average shape [Figs. 3(g)–3(i)]. Inter-

estingly, the quality of the fit to the average experimental inversion shape is considerably worse for $\kappa_{\text{post}}^0 = 0$ [Fig. 3(g), inset] than for the other two values [Figs. 3(h)–3(i), insets]. Strikingly, the fit parameters inferred from the cell sheet midlines in the case $\kappa_{\text{post}}^0 = R^{-1}$ imply a thickness gradient in the anterior hemisphere [Fig. 3(h)]. This gradient is consistent with the formation of pancake-shaped cells starting in the anterior fold and propagating towards the phialopore, as described previously [Ref. [40] and Fig. 1(d)]. All of this suggests that the intrinsic curvature of the spindle-shaped cells and hence the out-of-plane stresses in the uninverted posterior change at this stage. At the cell scale, such a change might result from small changes of the relative position of the CBs, as described for later stages of inversion [40]. The fact that a better fit [Figs. 3(h)–3(i)] is obtained for positive values of κ_{post}^0 that are associated with reduced geometric incompatibilities in the uninverted posterior begs the following question: Could this change constitute an adaptive response of the tissue to the stresses resulting from the geometric incompatibility generated by the spindle-shaped cells in the posterior?

2. The fitted mechanical sequence hints at an adaptive response to out-of-plane stresses

To provide a hint of an answer to this question, we considered fits of our model, for the three values of κ_{post}^0 , to more average shapes [51] corresponding to other timepoints before phialopore opening.¹ While the fit energies [Fig. 3(n)] are comparable at early and late stages of invagination, those at midinvagination stages are lowest, by some margin, for the fit with $\kappa_{\text{post}}^0 = r_{\text{post}}^{-1}$. We have already noted above that the contracted shape of the uninverted posterior is unstressed in this case. This therefore hints that κ_{post}^0 changes in such a way as to relieve the geometric incompatibilities in the uninverted posterior, and hence at an adaptive response to the stresses. To substantiate this hint, we performed a further fit in which κ_{post}^0 and r_{post} are among additional fitting parameters (Appendix D). The resulting fit energy [Fig. 3(n)] is not substantially lower than that of the more constrained fit with $\kappa_{\text{post}}^0 = r_{\text{post}}^{-1}$ at midinvagination stages. Moreover, the fitted values of κ_{post}^0 [Fig. 3(o)] increase from zero at the start of invagination to values comparable to r_{post}^{-1} at midinvagination stages. All of this lends further support to the hypothesis of an adaptive response to stresses in the tissue. Interestingly, the fitted values of κ_{post}^0 [Fig. 3(o)] decrease and those of r_{post} [Fig. 3(o), inset] decrease before increasing again at mid-to-late invagination stages, when the fit energy of this fit becomes rather smaller than that of the other fits [Fig. 3(n)]. All of this could be a signature of the pencil-shaped cells [Ref. [40] and Fig. 1(e), inset] that begin to form at these stages of inversion (and another adaptive response). Indeed, the position of cytoplasmic bridges in these cells may suggest $\kappa_{\text{post}}^0 R < 1$, and they are narrower than spindle-shaped cells [40], in-

¹We did not fit our model beyond the onset of phialopore opening because that is associated with poorly understood cell rearrangements near the phialopore [51], the mechanics of which cannot be described by our elastic model.

dicating reduced r_{post} . We discuss these results further in Appendix D.

V. CONCLUSION

We have combined ablation experiments and a detailed elastic model to establish the origin and analyze the mechanical effects of out-of-plane stresses during *Volvox* inversion. Strikingly, our results show how changes of posterior intrinsic curvature—albeit small compared to the intrinsic curvature imposed by the wedge-shaped cells in the bend region—contribute to the observed tissue shape sequence of *Volvox* inversion. In particular, the theory predicts an intriguing adaptive response of the geometric incompatibilities in the cell sheet to its out-of-plane stresses.

Tissue bending is not therefore all about cell wedging. Rather, out-of-plane stresses from other cell shape changes also contribute mechanically to *Volvox* inversion. It is curious that these cell shape changes from teardrop-shaped cells to spindle-shaped cells [Figs. 1(b) and 1(c)] are actually the opposite of apical constriction from the point of view of shape. This is possible because biological tissues are not asymptotically thin: If they were, the asymptotic separation of stretching and bending energies [65] would imply that only the large bending deformations of cell wedging [34,52] that break this asymptotic separation would have an effect comparable to that of in-plane stretching deformations. The question now becomes the following: Do these out-of-plane stresses that impact the shapes of inverting *Volvox* embryos and their adaptive response predicted by theory have a mechanical function? Our results [Figs. 3(d)–3(l)] show that it is possible to invaginate an elastic sheet in their absence, suggesting that they are not required mechanically for inversion, unlike the formation of wedge-shaped cells and contraction, which are known to be required mechanically for the closely related type-A inversion in *Volvox carteri* [61–63]. Still, one might speculate that these stresses or equivalently the stored elastic energy contribute to the mechanical robustness of *Volvox* inversion. Moreover, the predicted adaptation of these stresses during inversion suggests that they have a function related to the unknown mechanochemical signaling processes that orchestrate inversion. Testing such hypotheses will require again close integration of experiment and theory and, in particular, dynamic quantification of cell shapes to link “microscopic” cell-level changes to geometric incompatibilities and hence stresses at the “macroscopic” tissue level.

More generally, our experimental and theoretical approach sets out a framework for inferring the mechanical state and properties of curved tissues based on their unfurling on ablation. We expect our framework to be applicable to a broad range of problems in cell sheet folding if ablation experiments are complemented by an appropriate mechanical model of the local geometry of these tissues. For our ablations in *Volvox*, this mechanical model is provided by toy problem 2.

The inverse problem of inferring intrinsic stretches and curvatures of a cell sheet based on the deformations of its midline is clearly ill posed in general: For example, contraction of a spherical sheet of cells can be driven by contraction of the cells or by a mismatch of intrinsic curvatures, as discussed in toy problem 1. Interestingly, very recent work [70] has

shown how to solve this inverse problem in a membrane theory neglecting bending effects. In this context, the additional information provided by our ablation framework relieves this illposedness.

The use of the emerging model organism that is *Volvox* has been a key element of the proof-of-principle analysis of our framework, not only because its axisymmetric deformations and the absence of cell rearrangements at the stages of inversion analyzed simplify the mechanical model. More importantly, the structure of the cell sheet of *Volvox* allowed our model to make a special mechanical prediction: The intrinsic curvature of the spindle-shaped cells [Fig. 1(c)] is approximately zero, $k \approx 0$. This prediction is special because we could confirm it independently of our model, just based on the symmetry of the observed cell shapes. This test would have been very challenging in, e.g., a confluent curved epithelium because its curvature would distort the cell shapes and their symmetry. The test would therefore have required other invasive measurements such as microsurgery experiments to isolate clusters of cells and quantify their flattening on being released from the global confinement in the tissue. Still, this proof-of-principle validation of our ablation method needed such a special prediction. This is because tests of general mechanical predictions are intrinsically linked to mechanical models, in very much the same way as measuring the stiffness of metal springs in a school physics practical relies on Hooke’s law.

Finally, from a more physical point of view, it is striking that the “simple” tissue shapes of early *Volvox* invagination did not constrain the mechanical state of the cell sheet to the same extent as the more complex shapes at late invagination stages. Indeed, at early invagination stages, knowledge of the unfurling of the tissue on ablation was required to distinguish between the three mechanical scenarios discussed in Figs. 3(d)–3(f). By contrast, at later stages, we could distinguish between these possibilities based on the shapes of cell sheets alone [Figs. 3(g)–3(i)]. It is tempting to ask whether this observation generalizes: Do more complex tissue shapes intrinsically contain more information about the mechanical state of the tissue? This begs a more basic question: What is the right way of quantifying tissue shape complexity? Addressing these questions both in simple physical model problems and simple biological systems like *Volvox* or organoids will allow us to take the first steps towards addressing, too, the much more fundamental biological problem that is the relation between tissue shape (complexity) and biological function.

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DATA AVAILABILITY

Experimental data and MATLAB code are available in Ref. [73].

APPENDIX A: MATERIALS AND METHODS

In this appendix, we describe the experimental methods and summarize the elastic model, its numerical solution, and its fitting to the experimental data.

1. Model organism cultivation

Wild-type strain *Volvox globator* Linné (SAG 199.80) was obtained from the Culture Collection of Algae at the University of Göttingen, Germany [71], and cultured as described previously [39,72] in liquid standard *Volvox* medium (SVM) with a cycle of 16 h light at 24 °C and 8 h dark at 22 °C. *Volvox globator* cultures in the asexual life cycle of these monoecious microalgae were used.

2. Two-photon microscopy and laser ablation experiments

Volvox spheroids containing embryos undergoing inversion were embedded in 2 μ l of 1% low-melting-point agarose, covered with SVM, and imaged using a Trim Scope two-photon microscope (LaVision). A laser line at $\lambda = 1040$ nm was used for imaging and a separate laser line at $\lambda = 900$ nm was used for performing laser ablations. As a single chloroplast mostly fills each *Volvox* cell, chlorophyll autofluorescence was detected at $\lambda > 647$ nm, and used for visualizing the embryonic cell sheet. Z stacks were recorded before and after each laser ablation experiment, with a z step of 2 μ m. To maximize acquisition speed and capture the elastic response to the laser cut, a single plane was imaged during ablation experiments, which removed cells within a radius of 3–6 μ m of the posterior pole of the cell sheet. Videos of the midsagittal plane were recorded at 1–4.5 fps for at least 5 s before and 30 s after laser ablation. Videos used for determining velocities of elastic recoils were recorded at the maximal speed possible for the field-of-view size used, corresponding to 2.5–4.5 fps.

3. Analysis of experimental data

Outlines of the posterior hemisphere were traced manually on recordings of midsagittal embryo cross sections using FIJI [74]. To trace the midline of the cell sheet, the linewidth was set to fit the thickness of the cell sheet. Recoil quantification (Figs. S1–S3 in the Supplemental Material [64]) was then performed using custom MATLAB (The MathWorks, Inc.) scripts; in particular, the fit in Fig. 2(k) uses the `polyfitZero` function by M. Mikofski, obtained from the MATLAB file exchange (file 35401) [75]. Posterior cell sheet radii were estimated by fitting a circle to the cross sections in FIJI. Cell sheet thicknesses were estimated by scaling the chloroplast thicknesses measured in FIJI so that the resulting mean value matched that reported previously [40].

4. Morphoelastic shell theory

To describe *Volvox* morphogenesis in terms of the axisymmetric deformations of an elastic shell with varying intrinsic stretches and curvatures, we use the shell theory derived in the biologically relevant limit of “large bending deformations” in Ref. [52]: For an axisymmetric elastic shell of relative thickness h , we denote by s and S the respective arclengths of the undeformed and deformed cross sections of the axisymmetric shell. These cross sections are described by their distances ρ and r from the axis of symmetry and the tangent angle ψ of the deformed cross section. The meridional and circumferential stretches and curvatures of the deformed shell are thus

$$f_s(s) = \frac{dS}{ds}, \quad f_\phi(s) = \frac{r(s)}{\rho(s)}, \quad (\text{A1a})$$

$$\kappa_s(s) = \frac{1}{f_s} \frac{d\psi}{ds}, \quad \kappa_\phi(s) = \frac{\sin \psi(s)}{r(s)}, \quad (\text{A1b})$$

and we denote by $f_s^0(s)$, $f_\phi^0(s)$, $\kappa_s^0(s)$, $\kappa_\phi^0(s)$ their intrinsic, preferred values. The differences between the actual stretches and curvatures and their intrinsic values define the shell strains

$$E_s = \frac{f_s - f_s^0}{f_s^0}, \quad E_\phi = \frac{f_\phi - f_\phi^0}{f_\phi^0} \quad (\text{A2a})$$

and curvature strains

$$K_s = \frac{\tilde{f}_s \kappa_s - f_s^0 \kappa_s^0}{(f_s^0)^2 f_\phi^0}, \quad K_\phi = \frac{f_\phi \kappa_\phi - f_\phi^0 \kappa_\phi^0}{f_s^0 (f_\phi^0)^2} \quad (\text{A2b})$$

that appear in the elastic energy

$$\mathcal{E} = 2\pi \int_{\mathcal{C}} e(s) \rho(s) ds, \quad (\text{A3a})$$

where the integration is along the cross section $\mathcal{C}$ of the shell, and the energy density is

$$e = \frac{C}{2} \left\{ h [\alpha_{ss} E_s^2 + (\alpha_{s\phi} + \alpha_{\phi s}) E_s E_\phi + \alpha_{\phi\phi} E_\phi^2] + h^2 [\beta_{ss} E_s K_s + \beta_{s\phi} E_s K_\phi + \beta_{\phi s} E_\phi K_s + \beta_{\phi\phi} E_\phi K_\phi] + h^3 [\gamma_{ss} K_s^2 + (\gamma_{s\phi} + \gamma_{\phi s}) K_s K_\phi + \gamma_{\phi\phi} K_\phi^2] \right\}, \quad (\text{A3b})$$

in which C is a material parameter and α_{ss} , $\alpha_{s\phi}$, $\alpha_{\phi s}$, $\alpha_{\phi\phi}$, β_{ss} , $\beta_{s\phi}$, $\beta_{\phi s}$, $\beta_{\phi\phi}$, γ_{ss} , $\gamma_{s\phi}$, $\gamma_{\phi s}$, $\gamma_{\phi\phi}$ are functions of $\eta = \kappa_s^0 h / 2 f_s^0 f_\phi^0$ only, the (complicated) explicit expressions for which are given in Eqs. (58) and (64) of Ref. [52].

To find the deformed shape of the shell resulting from the imposed intrinsic stretches and curvatures numerically, we solve the boundary value problem associated with Eqs. (A3) derived in Ref. [52] using the `bvp4c` solver of MATLAB.

5. Numerical solution of the toy problems

We solve these equations for a circular cross section $\rho(s) = \sin s$ for $\Sigma \leq s \leq \pi$. At $s = \pi$, we impose the boundary conditions $r = \psi = 0$; if $\Sigma > 0$, we impose no-force and no-torque conditions at $s = \Sigma$ which, from Ref. [52], take the form $\alpha_{ss} E_s + \alpha_{s\phi} E_\phi + h(\beta_{ss} K_s + \beta_{s\phi} K_\phi) = 0$, $\beta_{ss} E_s + \beta_{\phi s} E_\phi + h(\gamma_{ss} K_s + \gamma_{s\phi} K_\phi) = 0$; if $\Sigma = 0$, the boundary conditions at $s = 0$ are instead $r = \psi = 0$.

For toy problem 2, we take $f_s^0 = f_\phi^0 = 1$, $\kappa_s^0 = \kappa_\phi^0 = k$. For toy problem 3, we take $f_s^0 = 1 + f_0 = 1/f_\phi^0$, where f_0 is a constant that expresses the anisotropy of contraction, and $\kappa_s^0 = \kappa_\phi^0 = 1$. To describe hypothetical fast active relaxation of the tissue on ablation, we take $f_s^0 = f_\phi^0 = 1 + F_0(\Sigma + s_0 - s)/s_0$ for $\Sigma \leq s \leq \Sigma + s_0$ for some length scale s_0 , and $f_s^0 = f_\phi^0 = 1$ otherwise, as well as $\kappa_s^0 = \kappa_\phi^0 = 1$; results do not seem to depend strongly on the form of the decay of f_s^0, f_ϕ^0 away from $s = \Sigma$. The parameter values for [Figs. 2(g)–2(i)] are $h = 0.3$, $\Sigma = 0.3$, $k = 0$, $f_0 = \pm 0.1$, $F_0 = 0.35$, $s_0 = 0.2$.

6. Fitting the morphoelastic model to the experimental data

For a quantitative description of *Volvox* inversion, similarly to Ref. [51], we consider again a circular cross section $\rho(s) = \sin s$, but now for $\Sigma \leq s \leq \pi - P$, where P is the phialopore size. We set $P = 0.3$ and $h = 0.15$, as in Ref. [51]. The boundary conditions are those of the toy problems discussed above, except for $s = \pi - P$, where we impose no-force and no-torque conditions.

We define functional forms for the intrinsic stretches and curvature functions $f_s^0(s), f_\phi^0(s), \kappa_s^0(s), \kappa_\phi^0(s)$ that allow a minimal representation of the cell shape changes observed during *Volvox* inversion [40], described in detail in Appendix D. These functional forms define a number of fitting parameters, which, with $\Sigma = 0$, were fitted to the average inversion shapes [51], by minimizing a fit energy E_{fit} that measures the difference between the average and fitted shapes using the MATLAB function `fminsearch`, similarly to Ref. [51]. This fitting is described in detail in Appendix D.

For numerical ablations, the value of Σ was increased from $\Sigma = 0$ in these fitted shapes, with boundary conditions as in the toy problems above.

APPENDIX B: SOLUTION OF THE MECHANICAL TOY PROBLEMS

In this appendix, we derive the analytical solutions of the mechanical toy problems discussed in the main text.

1. Toy problem 1: Deformations of a spherical elastic shell with mismatched intrinsic curvatures

As in the main text [Fig. 2(a)], we consider a thin incompressible elastic shell of undeformed (nondimensional) unit radius, and thickness $h \ll 1$, and hence undeformed curvature $\kappa = 1$. The shell has uniform intrinsic curvatures $\kappa^0 = k$. If $k \neq 1$, there is a curvature mismatch, due to which the shell deforms. We assume the shell to remain spherical and that $k = O(1)$, and denote by f its deformed radius.

a. Geometry of the deformed shell

The (spherical) polar radius of a point in the undeformed configuration of the shell is $r = a + \zeta$, where a is the midsurface radius, to be determined in the calculation, and ζ is a transverse coordinate, with $-h^- \leq \zeta \leq h^+$. By definition, $h^+ + h^- = h$, and $[(a + h^+) + (a - h^-)]/2 = 1$, i.e., $a = 1 - (h^+ - h^-)/2$. Analogously, a point in the deformed configuration of the shell has polar

radius $\tilde{r} = \tilde{a} + \tilde{\zeta}$, where $\tilde{a}$ and $\tilde{\zeta}$ are the deformed midsurface radius and transverse coordinate, now with $-\tilde{h}^- \leq \tilde{\zeta} \leq \tilde{h}^+$ and hence $\tilde{a} = f - (\tilde{h}^+ - \tilde{h}^-)/2$. With respect to spherical polars (r, θ, ϕ) , the deformation gradient is diagonal, with principal stretches

$$\lambda_r = \frac{\partial \tilde{\zeta}}{\partial \zeta}, \quad \lambda_\theta = \lambda_\phi = \frac{\tilde{a}}{a} \frac{1 + \tilde{\zeta}/\tilde{a}}{1 + \zeta/a}. \quad (\text{B1})$$

Incompressibility requires $\lambda_r \lambda_\theta \lambda_\phi = 1$, which is a differential equation for $\tilde{\zeta}$ as a function of ζ . Imposing $\tilde{\zeta} = 0$ at $\zeta = 0$, required by the definition of the midsurface, we find its implicit solution

$$\left(\frac{\tilde{a}}{a}\right)^2 \left(\tilde{\zeta} + \frac{\tilde{\zeta}^2}{\tilde{a}} + \frac{\tilde{\zeta}^3}{3\tilde{a}^2}\right) = \zeta + \frac{\zeta^2}{a} + \frac{\zeta^3}{3a^2}. \quad (\text{B2})$$

We now make use of the framework of morphoelasticity [76]: By analogy with Eqs. (B1), we define the intrinsic configuration of the cell sheet (which need not be embeddable into three-dimensional space) by its intrinsic stretches:

$$\lambda_r^0 = \frac{\partial Z}{\partial \zeta}, \quad \lambda_\theta^0 = \lambda_\phi^0 = \frac{1 + kZ}{1 + \zeta/a}. \quad (\text{B3})$$

To write down these expressions, we have identified $\tilde{a}/a$ and $1/\tilde{a}$ in Eqs. (B1) as the stretch and curvature of the deformed configuration, respectively, and have replaced them with their respective intrinsic values 1 and k . We will refer to Z as the intrinsic transverse coordinate. We are left to define the midsurfaces (a different choice of these would change the interpretation of k , so the existence of this freedom of choice is not surprising). We do so by imposing $-h^0/2 \leq Z \leq h^0/2$; the intrinsic thickness h^0 is determined by the incompressibility condition $\lambda_r^0 \lambda_\theta^0 \lambda_\phi^0 = 1$, which integrates to

$$\zeta + \frac{\zeta^2}{a} + \frac{\zeta^3}{3a^2} = Z + kZ^2 + \frac{k^2 Z^3}{3} \quad (\text{B4})$$

on requiring $Z = 0$ at $\zeta = 0$. Requiring that $Z = \pm h^0/2$ and $\tilde{\zeta} = \pm \tilde{h}^\pm$ correspond to $\zeta = \pm h^\pm$ in Eqs. (B2) and (B4) yields

$$\begin{aligned} & \left(\frac{f - \frac{\tilde{h}^+ - \tilde{h}^-}{2}}{1 - \frac{h^+ - h^-}{2}}\right)^2 \left[\pm \tilde{h}^\pm + \frac{(\tilde{h}^\pm)^2}{f - \frac{\tilde{h}^+ - \tilde{h}^-}{2}} \pm \frac{(\tilde{h}^\pm)^3}{3\left(f - \frac{\tilde{h}^+ - \tilde{h}^-}{2}\right)^2} \right] \\ &= \pm h^\pm + \frac{(h^\pm)^2}{1 - \frac{h^+ - h^-}{2}} \pm \frac{(h^\pm)^3}{3\left(1 - \frac{h^+ - h^-}{2}\right)^2} \end{aligned} \quad (\text{B5a})$$

$$= \pm \frac{h^0}{2} + \frac{k(h^0)^2}{4} \pm \frac{k^2(h^0)^3}{24}. \quad (\text{B5b})$$

Together with the condition $h^+ + h^- = h$, these form a system of equations for $h^\pm, \tilde{h}^\pm$, and h^0 , the solution of which defines the deformed geometry of the shell.

b. Elasticity of the deformed shell

Let $\mathbf{F}$ and $\mathbf{F}^0$ be the diagonal tensors with principal stretches $\lambda_r, \lambda_\theta, \lambda_\phi$ and $\lambda_r^0, \lambda_\theta^0, \lambda_\phi^0$, respectively, relative to the standard basis of spherical polars. By the fundamental relation of morphoelasticity [76], the elastic deformation gradient is

$\mathbf{F}(\mathbf{F}^0)^{-1}$. The elastic deformation gradient therefore has principal stretches

$$\Lambda_\theta = \Lambda_\phi = \frac{\lambda_\theta}{\lambda_\theta^0} = \frac{\lambda_\phi}{\lambda_\phi^0} = \frac{\tilde{a}}{a} \frac{1 + \tilde{\zeta}/\tilde{a}}{1 + kZ} \equiv \Lambda, \quad (\text{B6a})$$

$$\Lambda_r = \frac{\lambda_r}{\lambda_r^0} = \frac{1/\lambda_\theta \lambda_\phi}{1/\lambda_\theta^0 \lambda_\phi^0} = \frac{1}{\Lambda^2}, \quad (\text{B6b})$$

where we have used the incompressibility conditions. The first two principal invariants of the associated Cauchy–Green tensor are thus $\mathcal{I}_1 = 2\Lambda^2 + \Lambda^{-4}$ and $\mathcal{I}_2 = \Lambda^4 + 2\Lambda^{-2}$.

Since the problem that we are solving is *not* a leading-order problem (in an expansion in the small thickness of the shell), the constitutive assumptions matter. We therefore consider general elastic energy densities with a regular power series expansion [77], viz.,

$$e = \frac{1}{2} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} C_{mn} (\mathcal{I}_1 - 3)^m (\mathcal{I}_2 - 3)^n, \quad (\text{B7})$$

where $C_{00} = 0$ without loss of generality, and where thermodynamic stability requires $C_{10}, C_{01} \geq 0$ [78]. By incompressibility, we have equality $dV^0 = dV$ of the volume elements of the intrinsic and undeformed configurations. Explicitly,

$$\begin{aligned} dV^0 = dV &= (a + \zeta)^2 \sin \theta \, d\zeta \, d\theta \, d\phi \\ &= ha^2(1 + khz)^2 \sin \theta \, dz \, d\theta \, d\phi, \end{aligned} \quad (\text{B8})$$

using incompressibility again, and where $z = Z/h$. The elastic energy of the shell is the integral of the energy density with respect to the intrinsic configuration, viz.,

$$\mathcal{E} = \int_{V^0} e \, dV^0 = 4\pi h \int_{-h^0/2h}^{h^0/2h} ea^2(1 + khz)^2 \, dz. \quad (\text{B9})$$

c. Asymptotic solution

We now determine the deformed radius f by minimizing $\mathcal{E}$ by asymptotic expansion for $h \ll 1$. To keep the algebraic expressions that arise in the calculation (somewhat) simple, it will be useful to divide the asymptotic solution into two steps.

(i) *Leading-order calculation.* We begin by showing that $f = 1 + O(h^2)$, recovering the scaling behavior obtained in the main text using a shell theory. To this end, we introduce expansions

$$h^\pm = \frac{h}{2} + h^2 h_2^\pm + O(h^3), \quad (\text{B10a})$$

$$\tilde{h}^\pm = \frac{h}{2} + h^2 \tilde{h}_2^\pm + O(h^3), \quad (\text{B10b})$$

$$h^0 = h + h^2 h_2^0 + O(h^3), \quad (\text{B10c})$$

and

$$f = 1 + hf_1 + h^2 f_2 + O(h^3). \quad (\text{B10d})$$

Using MATHEMATICA (Wolfram, Inc.), Eqs. (B5) yield

$$h_2^\pm = \pm \frac{k-1}{4}, \quad \tilde{h}_2^\pm = \pm \frac{k-1}{4} - f_1, \quad h_2^0 = 0. \quad (\text{B11})$$

We then set $Z = hz$, $\tilde{\zeta} = h\tilde{\zeta}_1 + h^2\tilde{\zeta}_2 + O(h^3)$ and solve Eqs. (B2) and (B4) for $\tilde{\zeta}_1, \tilde{\zeta}_2$ as functions of z . We find

$$\tilde{\zeta}_1 = z, \quad \tilde{\zeta}_2 = -2f_1 z + (k-1)z^2, \quad (\text{B12})$$

and hence compute

$$\mathcal{E} = 2\pi(C_{10} + C_{01})[12f_1^2 + (k-1)^2]h^2 + O(h^3), \quad (\text{B13})$$

which is minimized for all k at $f_1 = 0$. This proves that $f = 1 + O(h^2)$ as claimed.

(ii) *Next-order calculation.* To determine the leading-order deformation of the shell, we extend expansions (B10) using the leading-order results (B11), writing

$$h^\pm = \frac{h}{2} \pm \frac{h^2(k-1)}{4} + h^3 h_3^\pm + h^4 h_4^\pm + O(h^5), \quad (\text{B14a})$$

$$\tilde{h}^\pm = \frac{h}{2} \pm \frac{h^2(k-1)}{4} + h^3 \tilde{h}_3^\pm + h^4 \tilde{h}_4^\pm + O(h^5), \quad (\text{B14b})$$

$$h^0 = h + h^3 h_3^0 + h^4 h_4^0 + O(h^5) \quad (\text{B14c})$$

and

$$f = 1 + h^2 f_2 + h^3 f_3 + h^4 f_4 + O(h^5). \quad (\text{B14d})$$

Solving Eqs. (B5) order by order for $h_3^\pm, h_4^\pm, \tilde{h}_3^\pm, \tilde{h}_4^\pm, h_3^0, h_4^0$, leaving only f_2, f_3, f_4 as unknowns, we find

$$h_3^\pm = 0, \quad \tilde{h}_3^\pm = -f_2, \quad h_3^0 = -\frac{5-6k+k^2}{12}, \quad (\text{B15a})$$

and

$$h_4^\pm = \pm \frac{3-10k+9k^2-2k^3}{48}, \quad h_4^0 = 0, \quad (\text{B15b})$$

$$\tilde{h}_4^\pm = \pm \frac{3-10k+9k^2-2k^3}{48} - f_3 \pm \frac{f_2}{4}(5-2k). \quad (\text{B15c})$$

We set $Z = hz$ again and write

$$\tilde{\zeta} = hz + (k-1)h^2 z^2 + \tilde{\zeta}_3 h^3 + \tilde{\zeta}_4 h^4 + O(h^5), \quad (\text{B16})$$

using the leading-order results (B12). From Eqs. (B2) and (B4), we obtain

$$\tilde{\zeta}_3 = -2f_2 z + \frac{5-6k+k^2}{3} z^3, \quad (\text{B17a})$$

$$\tilde{\zeta}_4 = -2f_3 z - \frac{z^2}{4}[k-1-4(5-2k)f_2] - \frac{10-15k+9k^2}{3} z^4. \quad (\text{B17b})$$

Using MATHEMATICA, we compute

$$\begin{aligned} \frac{\mathcal{E}}{2\pi} &= (C_{10} + C_{01})(k-1)^2 h^2 \\ &+ h^4 \{12(C_{10} + C_{01})f_2^2 - (k-1)f_2[C_{10}(5k-11) \\ &+ C_{01}(k-7)] + g(k; C_{10}, C_{01}, C_{20}, C_{11}, C_{02})\} \\ &+ O(h^5), \end{aligned} \quad (\text{B18})$$

where g is a quartic polynomial in k with coefficients depending on the material parameters $C_{10}, C_{01}, C_{20}, C_{11}, C_{02}$, the explicit expression of which is of no relevance to our calculation. Minimizing $\mathcal{E}$ to determine f_2 finally yields

$$\begin{aligned} f &= 1 - \frac{h^2}{12}(k-1) \frac{(11C_{10} + 7C_{01}) - (5C_{10} + C_{01})k}{2(C_{10} + C_{01})} \\ &+ O(h^3). \end{aligned} \quad (\text{B19})$$

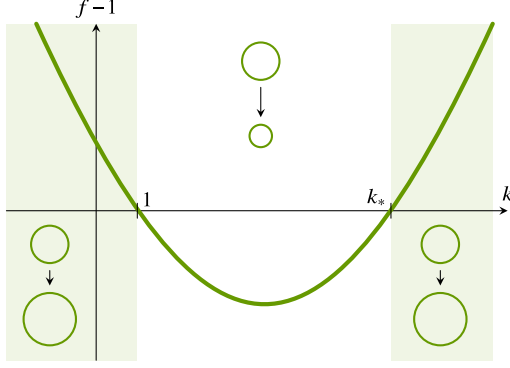


FIG. 4. Surprising mechanical behavior in toy problem 1. Plot of $f - 1$ against k from Eq. (B19). As expected, the sphere grows if $k < 1$ and shrinks if $1 < k < k_*$, but, surprisingly, the sphere also grows if $k > k_*$, where k_* is given by Eq. (B20).

d. Surprising mechanical behavior

While this result has the same scaling behavior as the result derived in the main text from a shell theory not formally valid for these small deformations, it features a complicated prefactor depending on the material parameters C_{10} , C_{01} defined by Eq. (B7) and also on k that is not predicted by the shell theory, but that gives rise to surprising and somewhat counterintuitive behavior: Indeed, the $O(h^2)$ correction in Eq. (B19) vanishes and changes sign not only at $k = 1$, but also at $k = k_*$, where

$$k_* = \frac{11C_{10} + 7C_{01}}{5C_{10} + C_{01}} = 1 + \frac{6(C_{10} + C_{01})}{5C_{10} + C_{01}}. \quad (\text{B20})$$

Since thermodynamic stability requires $C_{10}, C_{01} \geq 0$ [78], it follows that $k_* > 1$, and hence that $f > 1$ if $k < 1$ and $f < 1$ if $1 < k < k_*$ (Fig. 4). This shows that, as expected, the sphere grows (shrinks) if k is just below (above) unit value, but it is surprising that the sphere grows ($f > 1$) if $k > k_*$ (> 1) (Fig. 4). It is likely that the reason for which the intuition that the sphere should simply grow and shrink if $k < 1$ and $k > 1$ respectively fails is because these are very small deformations beyond leading order, asymptotically smaller than the thickness of the shell.

This surprising mechanical behavior is reminiscent of a classical result of elasticity, the inflation jump instability of an elastic balloon [76,79,80], which also arises in the mean-field limit of a vertex model of a cell sheet [81].

In this context, we also note that (again because the problem is not a leading-order problem) the definition of the midsurfaces (and hence of the intrinsic curvatures) matters: Had we chosen to define the midsurface in the undeformed configuration rather than in the intrinsic configuration, we would have obtained a different answer, simply because the middle surface of the undeformed configuration does not map to the middle surface of the intrinsic configuration, and so the intrinsic curvatures do not directly correspond to each other. This ambivalence does not however change the fact that there is “surprising” behavior as discussed in the previous paragraph.

2. Toy problem 2: Circular ablation of a spherical elastic shell with mismatched intrinsic curvatures

As in the main text [Fig. 2(b)], we consider a circular cut of angular extent 2Σ in a thin incompressible elastic spherical shell of unit radius, of thickness $h \ll 1$, and with intrinsic curvatures $\kappa^0 = k$. If $k \neq 1$, these are mismatched; to determine the resulting deformation of the shell, we take cylindrical polar coordinates defined by the axis of the cut.

a. Governing equations and boundary conditions

With respect to these coordinates, the undeformed spherical shell has polar radius $\rho(s) = \sin s$, where s is arclength, measured from the center of the cut, so its rim corresponds to $s = \Sigma$. The deformed shell has arclength $S(s)$, corresponding polar radius $r(s)$, and tangent angle $\psi(s)$. The stretches and curvatures of the deformed shell are thus

$$f_s(s) = \frac{dS}{ds}, \quad f_\phi(s) = \frac{r(s)}{\rho(s)}, \quad (\text{B21a})$$

$$\kappa_s(s) = \frac{1}{f_s} \frac{d\psi}{ds}, \quad \kappa_\phi(s) = \frac{\sin \psi(s)}{r(s)}. \quad (\text{B21b})$$

These define the shell strains and curvature strains

$$E_s = f_s - 1, \quad E_\phi = f_\phi - 1, \quad (\text{B22a})$$

$$K_s = f_s \kappa_s - k, \quad K_\phi = f_\phi \kappa_\phi - k. \quad (\text{B22b})$$

For this toy problem, the curvatures remain small compared to the shell thickness. We do not therefore need to use the “large bending theory” [52] used in the numerical calculations in the main text for our asymptotic calculations. On defining

$$N_s = 2E_s + E_\phi, \quad N_\phi = E_s + 2E_\phi, \quad (\text{B23a})$$

$$M_s = 2K_s + K_\phi, \quad M_\phi = K_s + 2K_\phi, \quad (\text{B23b})$$

and letting $\varepsilon = h/\sqrt{12}$, the deformed configuration of the shell satisfies the (nondimensionalized) force-balance equations [50]

$$\varepsilon^2 \frac{d}{ds} (rM_s) = f_s (rN_s \tan \psi + \varepsilon^2 M_\phi \cos \psi), \quad (\text{B24a})$$

$$\frac{d}{ds} (rN_s \sec \psi) = f_s N_\phi, \quad (\text{B24b})$$

complemented by the geometric equations

$$\frac{dr}{ds} = f_s \cos \psi, \quad \frac{d\psi}{ds} = f_s \kappa_s. \quad (\text{B24c})$$

It will however turn out to be convenient to express these equations in terms of ψ , E_s , E_ϕ , N_s for the asymptotic solution. To this end, rearranging the first of Eqs. (B24c) using definitions (B22), we notice that

$$\frac{d}{ds} [E_\phi \rho(s)] = f_s \cos \psi - \rho'(s). \quad (\text{B25})$$

Moreover, we obtain, from Eqs. (B22) and (B23), the algebraic relations

$$2N_\phi = 3E_\phi + N_s, \quad 2f_s = 2 + N_s - E_\phi. \quad (\text{B26})$$

Inserting these into Eqs. (B24) and (B25) and expanding, we conclude that deformed configuration of the shell is described

by the equations

$$4 \frac{d}{ds} [(1 + E_\phi) N_s \sec \psi \sin s] = (2 + N_s - E_\phi)(3E_\phi + N_s), \quad (\text{B27a})$$

$$2\varepsilon^2 \frac{d}{ds} \left[(1 + E_\phi) \left(2 \frac{d\psi}{ds} + \frac{\sin \psi}{\sin s} - 3k \right) \sin s \right] = (2 + N_s - E_\phi) \left[(1 + E_\phi) N_s \tan \psi \sin s + \varepsilon^2 \cos \psi \left(\frac{d\psi}{ds} + 2 \frac{\sin \psi}{\sin s} - 3k \right) \right], \quad (\text{B27b})$$

$$2 \frac{d}{ds} (E_\phi \sin s) = 2(\cos \psi - \cos s) + (N_s - E_\phi) \cos \psi. \quad (\text{B27c})$$

The boundary conditions to be applied at the rim of the cut, $s = \Sigma$, are

$$N_s(\Sigma) = 0, \quad M_s(\Sigma) = 0 \iff 2\psi'(\Sigma) + \frac{\sin \psi(\Sigma)}{\sin \Sigma} = 3k. \quad (\text{B28})$$

b. Asymptotic solution

In the asymptotic limit $\varepsilon \ll 1$, the shell deforms in a region of characteristic extent $\delta \ll 1$ near $s = \Sigma$. In this inner region, we let $s = \Sigma + \delta\xi$, defining an inner coordinate ξ . Let $\beta(\xi) = \psi(s) - s$ be the deviation of the tangent angle due to the cut; this deviation is small, $\beta \ll 1$. Expanding the no-torque boundary condition,

$$\frac{2}{\delta} \dot{\beta}(0) + \beta(0) \cot \Sigma = 3(k - 1), \quad (\text{B29})$$

where the dot denotes a derivative with respect to ξ . This condition therefore requires $\beta = O(\delta)$. Scaling from Eqs. (B27), we also find

$$\frac{N_s}{\delta} \sim E_\phi, \quad \frac{\varepsilon^2 \beta}{\delta^2} \sim N_s, \quad \frac{E_\phi}{\delta} \sim \max\{\beta, N_s, E_\phi\}. \quad (\text{B30})$$

The first scaling yields $N_s \ll E_\phi$, and hence the third scaling becomes $E_\phi/\delta \sim \beta$. The scalings then reduce to $\delta \sim \sqrt{\varepsilon}$, and hence $E_\phi \sim \delta^2$, $N_s \sim \delta^3$. [The asymptotic expansion of elasticity [52] that leads to the shell equations (B24) includes the scalings $E_s, E_\phi = O(\varepsilon)$ and $K_s, K_\phi = O(1)$, consistent with these scalings.] We therefore posit regular expansions

$$\psi = s + \delta[b + O(\delta)], \quad (\text{B31a})$$

$$E_\phi = \delta^2[e + O(\delta)], \quad (\text{B31b})$$

$$N_s = \delta^3 \cot \Sigma [n + O(\delta)], \quad (\text{B31c})$$

so that, on Taylor expanding and at leading order, Eqs. (B27) become

$$2\dot{n} = 3e, \quad 2\ddot{b} = n, \quad \dot{e} = -b. \quad (\text{B32})$$

The leading-order problem is therefore, on recalling Eqs. (B28) and (B29),

$$4\ddot{\ddot{b}} + 3\dot{b} = 0 \quad \text{subject to} \quad \begin{cases} b \rightarrow 0 \text{ as } \xi \rightarrow \infty, \\ 2\dot{b}(0) = 3(k - 1), \\ \ddot{b}(0) = 0. \end{cases} \quad (\text{B33})$$

Its solution is

$$b(\xi) = -3^{3/4}(k - 1)e^{-3^{1/4}\xi/2} \cos\left(\frac{3^{1/4}\xi}{2}\right). \quad (\text{B34})$$

c. Opening of the cut edges

One experimentally accessible geometric measure that characterizes the deformation resulting from the curvature mismatch is the cut opening o , i.e., the amount by which the radius of the hole created by the ablation increases during the recoil of the edge of the hole [Fig. 2(b), inset]. To compute o , we note that the radial displacement $u(s) = r(s) - \rho(s)$ of the shell satisfies the differential equation

$$\frac{du}{ds} = f_s \cos \psi - \cos s \implies \frac{du}{d\xi} = -\delta^2 b \sin \Sigma + O(\delta^3), \quad (\text{B35})$$

where we have made use of $f_s = 1 + O(\delta^2)$. By definition, $o = 2[u(\xi = 0) - u(\xi \rightarrow \infty)]$, twice the radial displacement of the cut edge across the boundary layer. We thus obtain

$$\begin{aligned} o &= 2\delta^2 \sin \Sigma \int_0^\infty b(\xi) d\xi + O(\delta^3) \\ &= -h(k - 1) \sin \Sigma + O(\delta^3). \end{aligned} \quad (\text{B36})$$

As expected, this shows that the rim of the cut curls outwards if $k < 1$, and inwards if $k > 1$. We also note that o is proportional to the radius $\sin \Sigma$ of the ablation.

d. Rotation and displacement of the cut edges: Breakdown of the leading-order asymptotics

There are (at least) two other experimentally accessible measures of the deformation [Fig. 2(b), inset]: the rotation ϱ of the edge of the cut, and its displacement d during the recoil. By definition,

$$\varrho = \delta b(0) + O(\delta^2) = -\sqrt{\frac{3h}{2}}(k - 1) + O(\delta^2). \quad (\text{B37})$$

Moreover, the calculation of the cut opening above shows that the radial displacement of the edge of the cut is $-(h/2)(k - 1) \sin \Sigma + O(\delta^3)$. Similarly we find that its axial displacement is $-(h/2)(k - 1) \cos \Sigma + O(\delta^3)$, from which

$$d = \frac{h}{2}|k - 1| + O(\delta^3). \quad (\text{B38})$$

Both leading-order results are independent of the cut size Σ . This is clearly absurd, because we must have $\varrho, d \rightarrow 0$ as $\Sigma \rightarrow 0$. This signals a breakdown of the leading-order asymptotics that we will analyze in the next subsection.

e. Asymptotic solution for small ablations

We now address the breakdown of the leading-order asymptotic solution for small cut sizes that we highlighted above when deriving the leading-order expressions for the cut rotation and cut displacement.

(i) *Breakdown of asymptoticity.* To understand this breakdown of the asymptotic validity of the leading-order solution, we expand further, writing

$$\psi = s + \delta[b_0 + \delta b_1 + O(\delta^2)], \quad (\text{B39a})$$

$$E_\phi = \delta^2[e_0 + \delta e_1 + O(\delta^2)], \quad (\text{B39b})$$

$$N_s = \delta^3 \cot \Sigma [n_0 + \delta n_1 + O(\delta^2)], \quad (\text{B39c})$$

where b_0 is given by Eq. (B34), and $n_0 = 2\ddot{b}_0$, $e_0 = 4\ddot{b}_0/3$ from Eqs. (B32). On expanding Eqs. (B27) and the boundary conditions pertaining thereto using MATHEMATICA, we obtain

$$4\ddot{b}_1 + 3b_1 = \cot \Sigma (4\ddot{b}_0^2 + 8\dot{b}_0\ddot{b}_0 - 8\ddot{b}_0 - \frac{9}{2}b_0^2), \quad (\text{B40a})$$

subject to

$$\dot{b}_1(0) = -\frac{1}{2}b_0(0) \cot \Sigma, \quad \ddot{b}_1(0) = -\dot{b}_0(0) \cot \Sigma. \quad (\text{B40b})$$

This shows that $b_1(\xi)$ is proportional to $\cot \Sigma$, so the expansion $b = b_0 + \delta b_1 + O(\delta^2)$ loses asymptoticity when $\delta \cot \Sigma = O(1)$, which is when $\Sigma = O(\delta)$.

(ii) *Asymptotic scalings for small ablations.* We must therefore derive the asymptotic scalings for small cut sizes $\Sigma = O(\delta)$. We thus write $\Sigma = \delta\sigma$, where $\sigma = O(1)$. The scaling $\beta = O(\delta)$ still holds, but establishing the other scalings takes more effort.

First, expanding Eq. (B27a) shows that $E_\phi \lesssim N_s$. If $E_\phi \ll N_s$, then we may posit $N_s = \nu n + o(\nu)$, $E_\phi = o(\nu)$ for some $\nu \ll 1$, and Eq. (B27a) becomes, at leading order,

$$2\frac{d}{d\xi}[(\sigma + \xi)n] = n \implies n(\xi) = \frac{C_1}{\sqrt{\xi + \sigma}}, \quad (\text{B41})$$

where C_1 is a constant of integration. The no-force condition at the rim of the cut is $n(0) = 0$, yielding $C_1 = 0$ and hence $n \equiv 0$, which is a contradiction. This shows that $N_s \sim E_\phi$.

Next, on expanding Eq. (B27c), we find that $\delta^2 \lesssim E_\phi \sim N_s$. If $\delta^2 \ll E_\phi \sim N_s$, then $N_s = \nu n + o(\nu)$, $E_\phi = \nu e + o(\nu)$, for some $\nu \ll 1$, and hence, from Eqs. (B27a) and (B27c),

$$2\frac{d}{d\xi}[(\sigma + \xi)n] = 3e + n, \quad 2\frac{d}{d\xi}[(\sigma + \xi)e] = n - e, \quad (\text{B42a})$$

which imply

$$2(\sigma + \xi)\dot{n} = 3e - n, \quad 2(\sigma + \xi)\dot{e} = n - 3e. \quad (\text{B42b})$$

Summing these equations yields $n + e = \text{const.}$, and then integrating the first gives, in particular,

$$n(\xi) = C_2 + \frac{C_3}{(\sigma + \xi)^2}, \quad (\text{B42c})$$

wherein C_2, C_3 are more constants of integration. The no-force condition at the rim of the cut is $n(0) = 0$, while matching

to the undeformed configuration $f_s = f_\phi = 1$ as $\xi \rightarrow \infty$ requires $n \rightarrow 0$ in this limit. Hence $C_2 = C_3 = 0$, so $n \equiv 0$. This is another contradiction, so $E_\phi \sim N_s \sim \delta^2$.

Finally, expanding Eq. (B27b) gives $N_s \delta^2 \lesssim \varepsilon^2$. If $N_s \delta^2 \ll \varepsilon^2$, then, on positing $\psi = s + \delta b + O(\delta^2)$ again, Eq. (B27b) becomes, at leading order,

$$\begin{aligned} \frac{d}{ds}[2(\sigma + \xi)\dot{b} + b] &= \dot{b} + \frac{2b}{\sigma + \xi} \\ \implies (\xi + \sigma)^2 \ddot{b} + (\xi + \sigma)\dot{b} - b &= 0, \end{aligned} \quad (\text{B43a})$$

which is a homogeneous equation with general solution

$$b(\xi) = C_4(\xi + \sigma) + \frac{C_5}{\xi + \sigma}, \quad (\text{B43b})$$

where C_4, C_5 are yet more constants of integrations. Matching to the undeformed shell as $\xi \rightarrow \infty$ requires $\beta \rightarrow 0$ in this limit, and hence $C_4 = 0$. Let $v(s)$ be the vertical displacement of the shell, which satisfies $dv/ds = f_s \sin \psi - \sin s$. Expanding yields $\dot{v} = \delta^2 C_5 (\xi + \sigma)^{-1} + O(\delta^3)$, which does not integrate to a finite displacement unless $C_5 = 0$, i.e., unless $b \equiv 0$.

This is a final contradiction, so $N_s \delta^2 \sim \varepsilon^2$. Since we have shown above that $E_\phi \sim N_s \sim \delta^2$, this yields $\delta \sim \sqrt{\varepsilon}$, which finally determines the asymptotic balance. In particular, $E_s, E_\phi = O(\varepsilon)$, as expected from the scalings of the asymptotic expansion underlying the derivation of the shell theory [52].

(iii) *Asymptotic governing equations for small ablations.* Having determined the asymptotic balance for $\Sigma = O(\delta)$, we can now obtain the governing equations in this limit. According to the scalings derived above, we replace Eqs. (B31) with

$$\psi = s + \delta[b + O(\delta)], \quad (\text{B44a})$$

$$E_\phi = \delta^2[e + O(\delta)], \quad (\text{B44b})$$

$$N_s = \delta^2[n + O(\delta)]. \quad (\text{B44c})$$

Equations (B27) yield

$$2(\sigma + \xi)\dot{n} = 3e - n, \quad (\text{B45a})$$

$$2(\sigma + \xi)^2 \ddot{b} = -2(\sigma + \xi)\dot{b} + 2b + (\sigma + \xi)^3 n + (\sigma + \xi)^2 bn, \quad (\text{B45b})$$

$$2(\sigma + \xi)\dot{e} = n - 3e - 2(\sigma + \xi)b - b^2. \quad (\text{B45c})$$

On eliminating e , these equations reduce to the pair of nonlinear second-order equations

$$4(\sigma + \xi)^2 \ddot{n} = -12(\sigma + \xi)\dot{n} - 6(\sigma + \xi)b - 3b^2, \quad (\text{B46a})$$

$$2(\sigma + \xi)^2 \ddot{b} = -2(\sigma + \xi)\dot{b} + 2b + (\sigma + \xi)^3 n + (\sigma + \xi)^2 bn, \quad (\text{B46b})$$

which are subject to the boundary conditions

$$\begin{aligned} n(0) &= 0, \quad 2\dot{b}(0) + \frac{b(0)}{\sigma} = 3(k - 1), \\ b, n &\rightarrow 0 \text{ as } \xi \rightarrow \infty. \end{aligned} \quad (\text{B47})$$

Since Eqs. (B46) are nonlinear, their solution is not proportional to $k - 1$, unlike the solution of the leading-order

problem for $\Sigma = O(1)$. They must be solved numerically in general.

(iv) *Approximate solution for small ablations.* It is therefore all the more remarkable that it is possible to find an analytical approximate solution of Eqs. (B46) that satisfies the boundary conditions (B47) and for which the cut rotation and cut displacement match onto Eqs. (B37) and (B38) in the limit $\sigma \rightarrow \infty$.

Our starting point is the observation that the boundary conditions for $\xi \rightarrow \infty$ imply that $b \ll \sigma + \xi$ there, and hence the nonlinear terms in Eqs. (B46) can be neglected there. On setting $x = \sigma + \xi$, Eqs. (B46) reduce to

$$2x^2\ddot{n} = -6x\dot{n} - 3xb, \quad 2x^2\ddot{b} = -2x\dot{b} + 2b + x^3n, \quad (\text{B48})$$

in the limit $x \rightarrow \infty$, where dots now denote derivatives with respect to x . The solution of these equations subject to boundary conditions (B47) will constitute our approximate solution.

To obtain this solution, we introduce the operator $\mathcal{D} = x^2 d^2/dx^2 + x d/dx - 1$. By definition, $\mathcal{D}b = x^2(xn)/2$. We observe that $\mathcal{D}(xn) = x^3\ddot{n} + 3x^2\dot{n} = -3x^2b/2$ using Eqs. (B48), so

$$\begin{aligned} \mathcal{D}\left(b \pm \frac{i}{\sqrt{3}}xn\right) &= \mp \frac{\sqrt{3}}{2}ix^2\left(b \pm \frac{i}{\sqrt{3}}xn\right) \\ &= \left(\frac{3^{1/4}}{\sqrt{2}}e^{\mp i\pi/4}x\right)^2\left(b \pm \frac{i}{\sqrt{3}}xn\right), \end{aligned} \quad (\text{B49})$$

which are complex Bessel equations [82], the solutions of which can be written as

$$b + \frac{i}{\sqrt{3}}xn = c_1\mathcal{K}_1\left(\frac{3^{1/4}}{\sqrt{2}}e^{-i\pi/4}x\right) + c_2\mathcal{I}_1\left(\frac{3^{1/4}}{\sqrt{2}}e^{-i\pi/4}x\right), \quad (\text{B50a})$$

$$b - \frac{i}{\sqrt{3}}xn = c_3\mathcal{K}_1\left(\frac{3^{1/4}}{\sqrt{2}}e^{i\pi/4}x\right) + c_4\mathcal{I}_1\left(\frac{3^{1/4}}{\sqrt{2}}e^{i\pi/4}x\right), \quad (\text{B50b})$$

where c_1, c_2, c_3, c_4 are constants of integration, and where $\mathcal{I}_1, \mathcal{K}_1$ are the modified Bessel functions of the first order [82] which satisfy $\mathcal{I}_1(z) \sim (2\pi z)^{-1/2}e^z$ and $\mathcal{K}_1(z) \sim (\pi/2z)^{1/2}e^{-z}$ as $z \rightarrow \infty$ for $\text{Re}(z) > 0$. Accordingly, $b, n \rightarrow 0$ as $x \rightarrow \infty$ requires $c_2 = c_4 = 0$. Hence

$$\begin{aligned} b(x) &= \frac{c_1}{2}\mathcal{K}_1\left(\frac{3^{1/4}}{\sqrt{2}}e^{-i\pi/4}x\right) + \frac{c_3}{2}\mathcal{K}_1\left(\frac{3^{1/4}}{\sqrt{2}}e^{i\pi/4}x\right) \\ &= c'_1\text{ker}_1\left(\frac{3^{1/4}x}{\sqrt{2}}\right) + c'_2\text{kei}_1\left(\frac{3^{1/4}x}{\sqrt{2}}\right), \end{aligned} \quad (\text{B51a})$$

$$\begin{aligned} n(x) &= \frac{\sqrt{3}}{x}\left[-\frac{ic_1}{2}\mathcal{K}_1\left(\frac{3^{1/4}}{\sqrt{2}}e^{-i\pi/4}x\right) + \frac{ic_3}{2}\mathcal{K}_1\left(\frac{3^{1/4}}{\sqrt{2}}e^{i\pi/4}x\right)\right] \\ &= \frac{\sqrt{3}}{x}\left[-c'_1\text{kei}_1\left(\frac{3^{1/4}x}{\sqrt{2}}\right) + c'_2\text{ker}_1\left(\frac{3^{1/4}x}{\sqrt{2}}\right)\right], \end{aligned} \quad (\text{B51b})$$

where $c'_1 = i(c_3 - c_1)/2$, $c'_2 = -(c_1 + c_3)/2$, and where we use $\text{ker}_0, \text{ker}_1, \dots$ and $\text{kei}_0, \text{kei}_1, \dots$ to denote the Kelvin functions [82]. Applying the remaining first two boundary conditions in Eqs. (B47) using MATHEMATICA and setting $\varsigma = 3^{1/4}\sigma/\sqrt{2}$, we find

$$c'_1 = \frac{3^{3/4}\sqrt{2}(1-k)\varsigma\text{ker}_1\varsigma}{(\text{ker}_1\varsigma)^2 + (\text{kei}_1\varsigma)^2 + \sqrt{2}\varsigma[\text{ker}_1\varsigma(\text{ker}_0\varsigma + \text{kei}_0\varsigma) + \text{kei}_1\varsigma(\text{kei}_0\varsigma - \text{ker}_0\varsigma)]}, \quad (\text{B52a})$$

$$c'_2 = \frac{3^{3/4}\sqrt{2}(1-k)\varsigma\text{kei}_1\varsigma}{(\text{ker}_1\varsigma)^2 + (\text{kei}_1\varsigma)^2 + \sqrt{2}\varsigma[\text{ker}_1\varsigma(\text{ker}_0\varsigma + \text{kei}_0\varsigma) + \text{kei}_1\varsigma(\text{kei}_0\varsigma - \text{ker}_0\varsigma)]}. \quad (\text{B52b})$$

From this solution, we obtain the leading-order rotation of the edge of the cut,

$$\varrho \sim \delta b(\sigma) = \frac{\sqrt{3}\varsigma[(\text{ker}_1\varsigma)^2 + (\text{kei}_1\varsigma)^2]}{(\text{ker}_1\varsigma)^2 + (\text{kei}_1\varsigma)^2 + \sqrt{2}\varsigma[\text{ker}_1\varsigma(\text{ker}_0\varsigma + \text{kei}_0\varsigma) + \text{kei}_1\varsigma(\text{kei}_0\varsigma - \text{ker}_0\varsigma)]}\sqrt{h}(1-k). \quad (\text{B53})$$

In particular, $\varrho \rightarrow 0$ as $\sigma \rightarrow 0$: The cut rotation vanishes with the cut size as expected, so this solution resolves the unphysical behavior of the expression in Eq. (B37) computed from the leading-order solution. Remarkably, we find using MATHEMATICA that $\varrho \rightarrow \sqrt{3h/2}(1-k)$ as $\sigma \rightarrow \infty$, recovering the result for $\Sigma = O(1)$ obtained earlier.

This solution resolves the unphysical behavior in Eq. (B38) for the cut displacement in a similar way: The leading-order equations for the radial and vertical displacements of the shell are now

$$\frac{du}{d\xi} = -\delta^3\left[\frac{b^2}{2} + (\sigma + \xi)b - f\right], \quad \frac{dv}{d\xi} = \delta^2b, \quad (\text{B54})$$

where $f(\xi) = [n(\xi) - e(\xi)]/2 = [n(\xi) - (\sigma + \xi)\dot{n}(\xi)]/3$ using the first of Eqs. (B45). The first equation cannot be integrated in closed form, so the approximate solution does not yield a correction to the expression for the cut opening in a straightforward manner. However, Eqs. (B54) show that $v \gg u$ for $\Sigma = O(\delta)$, whence the cut edge displacement is $d \sim |v(\xi=0) - v(\xi \rightarrow \infty)|$. This leads to

$$d = \frac{\varsigma[\text{ker}_1\varsigma(\text{kei}_0\varsigma - \text{ker}_0\varsigma) - \text{kei}_1\varsigma(\text{kei}_0\varsigma + \text{ker}_0\varsigma)]}{\sqrt{2}\{(\text{ker}_1\varsigma)^2 + (\text{kei}_1\varsigma)^2 + \sqrt{2}\varsigma[\text{ker}_1\varsigma(\text{ker}_0\varsigma + \text{kei}_0\varsigma) + \text{kei}_1\varsigma(\text{kei}_0\varsigma - \text{ker}_0\varsigma)]\}}h|1-k|. \quad (\text{B55})$$

We find $d \rightarrow h|1 - k|/2$ as $\sigma \rightarrow \infty$, which recovers the result for $\Sigma = O(1)$. This is quite remarkable, because the radial displacement, which is negligible for $\Sigma = O(\delta)$, contributes to the expression for d obtained for $\Sigma = O(1)$. Moreover, $d \rightarrow 0$ as $\sigma \rightarrow 0$, resolving the limitation of the solution for $\Sigma = O(1)$ as announced.

f. Recoil velocity: Scaling argument

With the leading-order asymptotic solution, we can understand the scaling of the velocity of the recoil from the undeformed to the deformed solution. If the shell were to remain in its undeformed configuration,

$$E_s = E_\phi = 0, \quad K_s = K_\phi = 1 - k. \quad (\text{B56})$$

The deformed solution has $N_s \ll E_\phi$ from Eqs. (B31), whence, from the first of Eqs. (B23), $E_s = -E_\phi/2 + O(\delta)$. It follows that, in the asymptotic inner region,

$$E_s \sim -\frac{1}{2}\delta^2 e, \quad E_\phi \sim \delta^2 e, \quad (\text{B57a})$$

so definitions (B22) imply that $f_s, f_\phi \sim 1$. Hence, from Eqs. (B22) and (B31),

$$K_s \sim 1 - k + \dot{b}, \quad K_\phi \sim 1 - k. \quad (\text{B57b})$$

The elastic energy density [50] is thus

$$\begin{aligned} & E_s^2 + E_s E_\phi + E_\phi^2 + \varepsilon^2 (K_s^2 + K_s K_\phi + K_\phi^2) \\ & \sim \begin{cases} 3\varepsilon^2(1 - k)^2 & \text{in the undeformed configuration,} \\ \frac{3\delta^4 e^2}{4} + \varepsilon^2[3(1 - k)(1 - k + \dot{b}) + \dot{b}^2] & \text{in the deformed configuration.} \end{cases} \end{aligned} \quad (\text{B58})$$

On noting that $e = \frac{4}{3}\ddot{b}$ from Eqs. (B32), it follows that the elastic energy released during the recoil from the undeformed to the deformed configuration is

$$\begin{aligned} \Delta\mathcal{E} &= 2\pi \int_0^\infty \delta^4 \left[\frac{4}{3}\ddot{b}^2 + \dot{b}^2 + 3\dot{b}(1 - k) \right] \sin \Sigma (\delta d\xi) \\ &= -3^{7/4}\pi\delta^5(k - 1)^2 \sin \Sigma \leq 0, \end{aligned} \quad (\text{B59})$$

using the leading-order solution (B34) and in which the factor $\sin \Sigma$ is the leading-order Jacobian of polar coordinates. Of course, the fact that elastic energy is released is hardly surprising, but we needed to compute this number to find the leading-order scaling of this energy release.

We now make this result dimensional: In our derivation of the leading-order solution, we have implicitly nondimensionalized distances with the dimensional radius R of the shell, and energies with YR^2 , where $Y = EH$ is Young's modulus of the shell, with E its elastic modulus and H its dimensional thickness, so that $h = H/R$. On unraveling this nondimensionalization, the dimensional energy released during the recoil thus scales as $\Delta\mathcal{E} \sim EHR^2\delta^5(k - 1)^2 \sin \Sigma$. During the recoil, the sheet moves a (dimensional) distance $D \sim R\delta^2|1 - k| \sin \Sigma \sim H|1 - k| \sin \Sigma$ from Eq. (B38). The effective elastic force driving the recoil is thus $F \sim \Delta\mathcal{E}/D \sim EHR\delta^3|1 - k| \sim EH^{5/2}R^{-1/2}|1 - k|$. The recoil is resisted by viscous dissipation in the tissue and the surrounding fluid. The hydrodynamic strain rate

scales as $\mu V/(\delta R)$, where μ is an effective viscosity and V is the initial recoil velocity, i.e., the fluid velocity that decays over the extent δR of the asymptotic inner region. The latter has surface area $R^2\delta$, and so $F \sim (\mu V/(\delta R))(R^2\delta) \sim \mu VR$. The initial recoil velocity and hence the hydrodynamic force are independent, at leading order, of k because the initial shape of the cell sheet is, by the results of toy problem 1. The balance of elastic and hydrodynamic forces then yields the scaling of the recoil velocity,

$$V \sim \frac{E}{\mu} \frac{H^{5/2}}{R^{3/2}} |1 - k|. \quad (\text{B60})$$

This scaling breaks down for small cut sizes, because it does not satisfy $V \rightarrow 0$ as $\Sigma \rightarrow 0$, but resolving this would require a more detailed understanding of the dependence of the fluid velocity on cut size and its decay away from the rim of the cut, which is beyond the scope of our analysis.

3. Toy problem 3: Circular ablation of a spherical elastic shell with anisotropic contraction

Finally, we show how (spatially uniform) anisotropic contraction of a spherical shell of unit radius [Fig. 2(c)] leads to stresses (and hence to recoil on ablation) irrespective of the intrinsic curvatures of the shell. Let $f_1 \neq f_2$ be the principal contractions, and take cylindrical coordinates such that the (orthogonal) principal directions of contraction align with the azimuthal and circumferential directions of the shell. As in the previous section, we denote by s and $S(s)$ the undeformed and deformed arclengths of the shell along its meridian (measured from a pole), and by $\rho(s) = \sin s$ and $r(s)$ the undeformed and deformed polar radii of the shell. The strains of the midsurface of the shell [52] are thus

$$E_s(s) = \frac{f_s(s)}{f_1} - 1, \quad E_\phi(s) = \frac{f_\phi(s)}{f_2} - 1, \quad (\text{B61})$$

where $f_s(s) = S'(s)$, $f_\phi(s) = r(s)/\rho(s)$ and where dashes denote differentiation with respect to s . Let $\psi(s)$ denote the deformed tangent angle to the shell, so that $r'(s) = f_s \sin \psi$. At the pole of the shell, we have $r(0) = \rho(0) = \psi(0) = 0$ by geometric continuity, so l'Hôpital's rule implies $f_\phi(0) = r'(0)/\rho'(0) = f_s(0) \cos \psi(0)/\cos 0 = f_s(0)$. Since $f_1 \neq f_2$, it follows that $E_s(0)$ and $E_\phi(0)$ cannot both vanish, so the meridional and circumferential stresses $N_s = 2E_s + E_\phi$ and $N_\phi = E_s + 2E_\phi$, the expressions of which are derived in Ref. [50], cannot both vanish near the pole by continuity, and hence the shell will recoil on ablation.

While these stresses are caused by anisotropic contraction, we note that they also generate a curvature mismatch. Indeed, let K_s, K_ϕ denote the mismatch of the actual and intrinsic curvatures of the shell in the meridional and circumferential directions. Then $M_s = 2K_s + K_\phi$, $M_\phi = K_s + 2K_\phi$ satisfy the differential equation $\varepsilon^2(rM_s)' = f_s(rN_s \tan \psi + \varepsilon^2 M_\phi \cos \psi)$ as derived in Ref. [50], where ε is again the (scaled) relative thickness of the shell. Hence M_s, M_ϕ cannot both vanish identically since N_s does not, and hence neither can K_s, K_ϕ , as claimed.

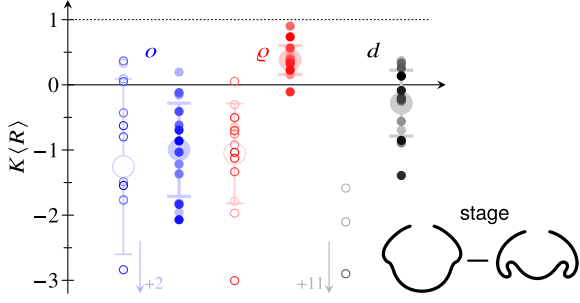


FIG. 5. Estimated dimensional intrinsic curvatures K , nondimensionalized with the average posterior radius $\langle R \rangle$ during invagination. Estimates are computed from asymptotic results (open markers) and numerical solutions of toy problem 2 (filled markers) using measurements of o , ρ , d for $N = 14$ *Volvox* embryos at early invagination and midinvagination (inset and Fig. S1 in the Supplemental Material [64]). Arrows, off-scale values; large markers and error bars, mean and standard deviation of estimates. Measurements and estimates based on ρ , d and numerical solutions are copied from Fig. 2(j).

APPENDIX C: EXTENDED DISCUSSION OF EXPERIMENTAL RESULTS

In the main text [Fig. 2(j)], we estimate the intrinsic curvature during invagination based on measurements of the cut rotation ρ and cut displacement d and numerical solution of toy problem 2. The resulting separate estimates are within a standard deviation of each other, and yield a mean estimate of intrinsic curvature of $\langle k \rangle \approx 0.05 \ll 1$.

Here, we extend the discussion of these estimates: The estimate of the intrinsic curvature based on measurements of the cut opening o and numerical solution of toy problem 2 is not within a standard deviation of the other estimates (Fig. 5). Since measuring o requires quantifying smaller deformations, this is perhaps not unexpected. The cut opening o might be the traditional way of quantifying ablation recoils in two dimensions, but we note that its three-dimensional analog for quantifying out-of-plane recoils is the cut displacement d that we use in the main text. The cut rotation ρ is an additional measure of the ablation recoil that only becomes available in three dimensions.

We can also estimate the intrinsic curvature based on the asymptotic expressions or approximations for o , ρ , d derived above. The resulting estimates (Fig. 5) have a much larger variance than those based on numerical solutions. This suggests that, while the asymptotic estimates are useful to understand the mechanical basis of the recoil upon ablation, the cell sheet of *Volvox* is too thick for them to yield good quantitative estimates.

APPENDIX D: FITTING OF THE MODEL TO THE CELL SHEET SHAPES OF *VOLVOX* INVERSION

We fit our morphoelastic model (Appendix A), based on the elastic shell theory for large bending deformations of Ref. [52], to the consensus shapes of *Volvox* inversion, i.e., the averaged cell sheet midlines from previous experiments [51], in a similar way to the fitting described in Ref. [51]. The use of the upgraded shell theory of Ref. [52] is the main mechanical difference between the quantitative inversion model of this

paper and that of Ref. [51]. In what follows, we describe this quantitative model and the fitting in detail.

1. Fitting parameters encoding cell shape changes in intrinsic stretches and curvatures

Similarly to Ref. [51], we define piecewise constant or linear functional forms for the intrinsic stretch and intrinsic curvature functions f_s^0 , f_ϕ^0 , κ_s^0 , κ_ϕ^0 (Appendix A) that encode the cell shape changes observed during *Volvox* inversion [Ref. [40] and Fig. 6(a)]. These functional forms are shown in Figs. 6(b)–6(d). They define fitting parameters $f_1, f_2, f_3, f_4, f_5, \kappa_1, \kappa_2, \kappa_3, \kappa_4, s_1 < s_2 < s_3 < s_4$ that encode the intrinsic stretches and intrinsic curvatures of these different cell shape changes, and the positions of the boundaries between regions of different cell shapes [Ref. [51] and Fig. 6(a)]. There are thus 13 fitting parameters. Since the intrinsic stretches and curvatures change during development, as the program of cell shape changes driving inversion is run through, each of these parameters is *a priori* a function of time, so it has to be fitted for at each developmental timepoint. This number of fitting parameters may therefore appear to be large, but we emphasize, as discussed in detail in Ref. [51], that the functional forms of intrinsic stretches and curvatures are the minimal ones that can encode the cell shape changes observed during *Volvox* inversion [40] and are appropriately continuous.

a. Fitting methods and details

For fitting this model to the average shape of *Volvox* at a given developmental timepoint of inversion, similarly to Ref. [51], we place fitting points (R_i, Z_i) for $i = 1, 2, \dots, N_{\text{fit}} = 100$ at equal spacing along the arclength of the meridian of this average shape. For a given set of parameter values, we solve the boundary value problem associated with Eqs. (A3) using the `bvp4c` solver of MATLAB (The MathWorks, Inc.) or a custom implementation of arclength continuation based on the same solver. The solution of this boundary value problem defines a cross section $(r(s), z(s))$, where $dz/ds = f_s \sin \psi$ (Appendix A). This yields points (r_i, z_i) equally spaced along this cross section, for $i = 1, 2, \dots, N_{\text{fit}} = 100$. The fitting minimizes the fit energy

$$E_{\text{fit}} = \left\{ \sum_{i=1}^{N_{\text{fit}}} [(r_i - R_i)^2 + (z_i - Z_i)^2] \right\}^{1/2} \quad (\text{D1})$$

over the fitting parameters. We perform this minimization using a modification of the `fminsearch` function of MATLAB that uses the modified parameters for its underlying Nelder-Mead algorithm suggested by Ref. [83] and that restricts the initial simplex used by the algorithm. The second modification avoids parameter values that are “too far way” from the initial guess and that might lead to the algorithm failing due to bifurcations that lie between the initial guess and these parameter values in parameter space. Further, we stopped the minimization once E_{fit} dropped below a certain value [Fig. 3(n)]; empirically, we found that this avoids excessive variations of fit parameters. We fit the average shapes for the developmental timepoints $t_1 < t_2 < \dots$ one after the other,

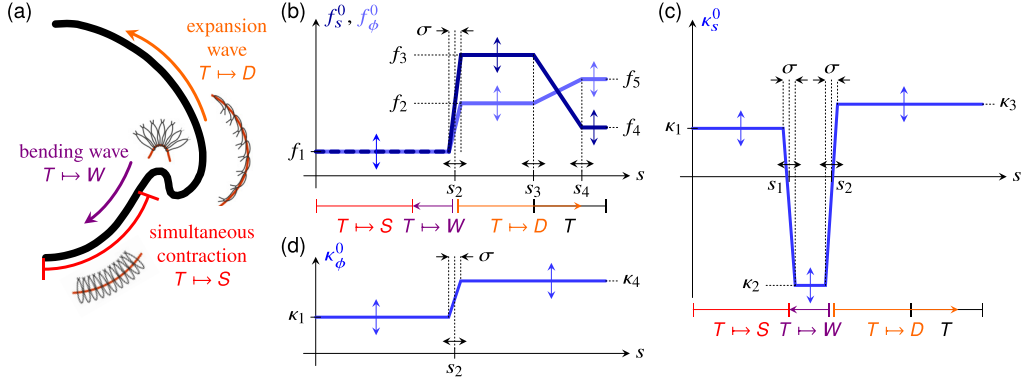


FIG. 6. Cell shape changes during *Volvox* invagination encoded into functional forms of intrinsic stretches and curvatures for fitting of the elastic model. (a) Cell shape changes during *Volvox* invagination, before the appearance of pencil-shaped cells during late invagination [40]; T, teardrop-shaped cells; W, wedge-shaped cells; S, spindle-shaped cells; D, disk-shaped cells. Insets show W, S, and D cell shapes. (b) Plots of the piecewise linear or constant functional forms of the intrinsic stretches f_s^0, f_ϕ^0 against arclength s (Appendix A), defining fitting parameters $f_1, f_2, f_3, f_4, f_5, s_2 < s_3 < s_4$ encoding the intrinsic stretches of the different cell shapes observed during *Volvox* inversion [40,51] and the positions of the boundaries between regions of different cell shapes. Regions corresponding to the cell shapes and their changes in panel (a) are shown below the axis; overlaps indicate the possibility of a gradient of cell shapes. (c) Plot of the meridional intrinsic curvature κ_s^0 against s , defining additional fitting parameters $\kappa_1, \kappa_2, \kappa_3$ encoding the intrinsic curvatures of the different cell shapes and the additional positional fitting parameter $s_1 < s_2$. Cell shape changes are shown below the axis as in panel (b). (d) Plot of the circumferential intrinsic curvature κ_ϕ^0 against s , defining the additional fitting parameter κ_4 that defines yet another intrinsic curvature value for a cell shape. As in Ref. [51], the parameter σ regularizing what would otherwise be discontinuities in $f_s^0, f_\phi^0, \kappa_s^0, \kappa_\phi^0$ is not fitted for. The figure has been redrawn from Ref. [51].

using the fitted parameter values from the previous timepoints to obtain initial guesses.

b. Fitting details for the different fits

For the fits discussed in the main text, the number of fitting parameters was reduced as follows: For the fits of constant $\kappa_1 = \kappa_{\text{post}}^0 \in \{0, R^{-1}, r_{\text{post}}^{-1}\}$, the parameters that were fitted for at each timepoint were $f_2, f_3, f_4, f_5, \kappa_2, s_1, s_2, s_3, s_4$. Additionally, $f_1 = r_{\text{post}}/R, \kappa_3, \kappa_4$ were fitted only at the first timepoint and held constant for later timepoints. This assumption reduces the number of fitting parameters. For the final “free” fit, $f_1 = r_{\text{post}}/R, \kappa_1 = \kappa_{\text{post}}^0, \kappa_3, \kappa_4$ were additionally fitted for at each timepoint.

2. Further discussion of the fitting results

In this final subsection, we discuss the fitting results of the main text further. In particular, we must discuss the fact that we did not fit the parameters f_1, κ_3, κ_4 at each timepoint. Based on the observed cell shape changes [40], no variation of f_1 is expected while the cells in the posterior are spindle shaped. This is borne out by the “free” fit, which suggests that f_1 remains (approximately) constant until around the time of phialopore opening, when the cells in the uninverted posterior become pencil shaped [40], as discussed in the main text. Not fitting the parameters κ_3, κ_4 is *a priori* a simplifying assumption, because the preferred curvatures of the teardrop-shaped cells and disk-shaped cell shapes that are observed in the anterior hemisphere during early and late invagination [40] could be different. Could the worse fit energies for the fits with $\kappa_{\text{post}}^0 \in \{0, R^{-1}\}$ [Fig. 3(n)] result from the different

values of r_{post} [Fig. 3(o), inset] or κ_3, κ_4 fitted at the first timepoint? To exclude this possibility, we have run additional fits with $\kappa_{\text{post}}^0 = 0$ using the values of $f_1 = r_{\text{post}}$ and/or κ_3, κ_4 used for $\kappa_{\text{post}}^0 = r_{\text{post}}^{-1}$. These fits did not lead to better fit energies (Fig. 7). The fact that the variations of the fitted values of κ_{post}^0 and r_{post} in the final “free” fit [Fig. 3(o)] are comparable to the differences between these values in the earlier fits is also consistent with this. All of this shows that these simplifying assumptions do not cause the worse fits, and hence that these result indeed from the “wrong” values of κ_{post}^0 .

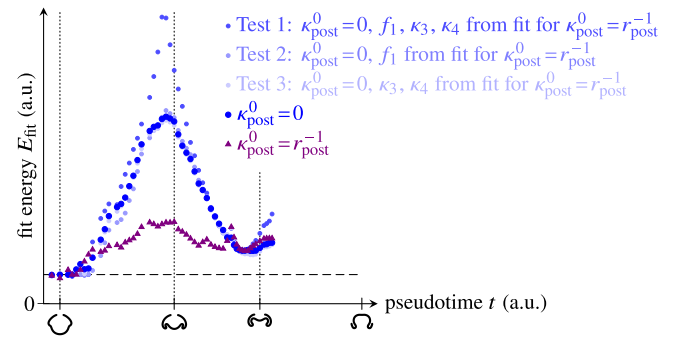


FIG. 7. Test of the fit results in the main text. Plot of the fit energy E_{fit} against developmental pseudotime t : Repeating the fits for $\kappa_{\text{post}}^0 = 0$ using the values of the parameters $f_1 = r_{\text{post}}$ and/or κ_3, κ_4 from the (better) fit with $\kappa_{\text{post}}^0 = r_{\text{post}}^{-1}$ does not lead to better fits. The energies of the fits for $\kappa_{\text{post}}^0 \in \{0, r_{\text{post}}^{-1}\}$ are repeated from Fig. 3(n) for comparison. Pseudotimepoints used for the discussion in Figs. 3(a)–3(l) are highlighted by the shape insets on the horizontal axis.

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