PNAS COMMENTARY

A protein curvature for sensing touch

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With the discovery of the proteins Piezo 1 and 2 in 2010 (1), the long-sought force-sensing molecules that underly the perception of touch, proprioception, and the control of blood pressure in all vertebrates were found. Piezo 1 and 2 are large membrane proteins that open a small ion channel when force is exerted on the membrane. In 2017, the first high-resolution cryoelectron microscopy (cryo-EM) structure of Piezo 1 revealed three identical arms that spiral out from a central ion channel (Fig. 1) (2). The three Piezo arms do not lie in a plane, which led to the suggestions that the Piezo protein curves the cell membrane into a nanodome and that this protein–membrane nanodome flattens when forces induce tension in the membrane, leading to channel opening (2, 3). In the first Piezo structure and subsequent high-resolution structures of Piezo 1 (4, 5) and Piezo 2 (6), the Piezo proteins were embedded in detergent molecules, not in lipid membranes. These detergent molecules form droplet-like micelles that shield the numerous transmembrane helices of the Piezo proteins from water, similar to the lipids of cell membranes. However, unlike detergent micelles, the sheet-like lipid membranes prefer a planar conformation, and curving this planar conformation into a nanodome costs bending energy. Central questions for understanding the function of Piezo proteins, therefore, are: Which conformation do the three Piezo protein arms adopt in a lipid membrane, and how does this membrane-embedded Piezo conformation respond to force (7)? In two articles in PNAS, Haselwandter et al. (8, 9) give answers to both questions by analyzing and modeling the shapes of lipid vesicles with embedded Piezo proteins obtained from cryoelectron tomography.

In the cryoelectron tomography images, vesicles that contain a single Piezo 1 protein adopt a teardrop-like shape with the protein located in the region of highest curvature. Haselwandter et al. model both the more highly curved protein–membrane nanodome visible in the images and the more gently curved "free" vesicle membrane around the nanodome based on the local bending energy (10):

$$E_{\text{be}} = 2\kappa\,(M - M_o)^2\,. \qquad [1]$$

This bending energy depends on the local curvature M, includes the bending rigidity κ and spontaneous curvature M_o as parameters, and needs to be integrated ("summed up") over the whole vesicle to obtain the overall elastic energy of the vesicle shape. The free membrane around the nanodome has no intrinsic tendency to curve and, therefore, a spontaneous curvature M_o of zero. For the protein–membrane nanodome, Haselwandter et al. use the spontaneous curvature M_o as a fit parameter to quantify the curvature imprint of the Piezo protein on the membrane.

The bending energy of Eq. **1** has been used for decades to model the shapes of "giant" vesicles with diameters of tens of micrometers, which can be observed by optical light microscopy (11, 12). Haselwandter et al. apply this bending energy to model vesicle shapes with diameters of tens of nanometers and carefully validate this "nanoscopic" modeling approach by comparing cryoelectron tomography shapes and calculated shapes of the free vesicle membrane around the Piezo nanodome in their first article (8). In their second article (9), Haselwandter et al. use the calculated elastic energies of the free vesicle membrane to determine the forces on the Piezo nanodome that are exerted by the surrounding curved vesicle membrane. From the modeled forces, and from the observed shapes of the Piezo nanodome that result from these forces in differently sized vesicles with diameters from about 25 to 70 nm, Haselwandter et al. obtain the spontaneous curvature $M_o \simeq 1/40\ \text{nm}^{-1}$ for the Piezo protein–membrane nanodome (9). This spontaneous curvature corresponds to a radius $R_o = 1/M_o \simeq 40$ nm for the Piezo nanodome in large vesicles with curvatures comparable to those of cell membranes. The curvature radius of 40 nm for the membrane-embedded Piezo protein determined by Haselwandter et al. is clearly smaller than the curvature radius of about 10 nm of the Piezo protein in the detergent micelles of the high-resolution cryo-EM structures (2).

Recent high-resolution cryo-EM structures of Piezo 1 embedded in membrane vesicles show Piezo protein conformations for two opposite orientations (13). In one orientation, the extracellular side of Piezo 1 points toward the vesicle inside, as in the cryoelectron tomography shapes of Haselwandter et al. (8, 9). In this orientation, the Piezo protein structure is highly curved because of the small mean diameter of 20 nm of the thousands of imaged vesicles that have been averaged to obtain high resolution. In the other orientation, the extracellular side of Piezo 1 points toward the vesicle outside, and the Piezo protein structure is nearly completely flattened because the vesicle curvature opposes the intrinsic Piezo curvature in this orientation. The flattened high-resolution Piezo 1 structure reveals a widening of the central ion channel compared with the curved structure. However, these two vesicle-embedded Piezo 1 structures depict two rather extreme conformations that Piezo 1 is unlikely to adopt in cell membranes.

In contrast, the lower-resolution cryoelectron tomography shapes of individual vesicles with varying diameters ranging from about 25 to 70 nm allow Haselwandter et al.

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Author contributions: T.R.W. wrote the paper.

The author declares no competing interest.

See companion articles, "Quantitative prediction and measurement of Piezo's membrane footprint," 10.1073/pnas.2208027119, and "Elastic properties and shape of the Piezo dome underlying its mechanosensory function," 10.1073/pnas.2208034119.

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Published October 5, 2022.

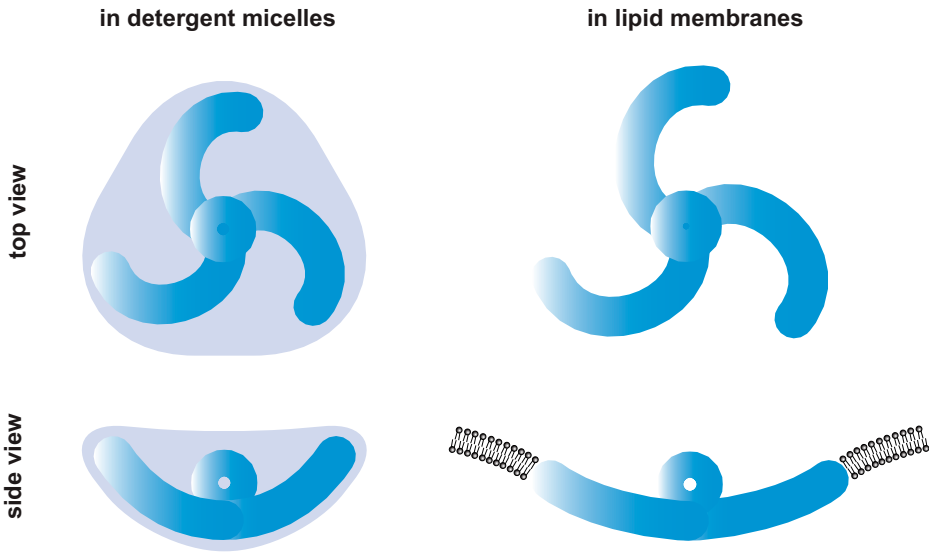

Fig. 1. Schematic illustrations of Piezo protein shapes. The energetic cost of membrane bending leads to a smaller curvature of Piezo in lipid membranes compared with Piezo structures in detergent micelles.

to infer the intrinsic, "unstressed" curvature M_o of membrane-embedded Piezo 1 from modeling and extrapolation (9). In addition to this curvature, Haselwandter et al. obtain the bending rigidity κ of the protein–membrane nanodome from model fitting and find that the nanodome rigidity is identical to the bending rigidity $\kappa \simeq 20\, k_BT$ of the surrounding lipid membrane within the numerical modeling accuracy. Equipped with these two elastic parameters M_o and κ of the nanodome, the shape changes and flattening of membrane-embedded Piezo 1 under external forces that induce membrane tension can be obtained from standard elasticity calculations (9).

The Piezo protein–membrane nanodome is a prime example of how membrane curvature can be coupled to protein function. Curvature-generating proteins attained a prominent role in cell biology with the discovery of the proteins that induce and maintain the intricately formed membrane shapes of cellular organelles (14). These proteins generate membrane curvature by imposing their curved shape when binding to membranes, or by inserting helices as curvature-inducing wedges into membranes (15, 16). The curvatures generated by the proteins lead to membrane-mediated protein interactions, because the overall membrane bending energy depends on the distance and orientation of the proteins, and to protein cooperativity in membrane shaping (17). The Piezo proteins stand out among these curvature-inducing proteins by their sheer size and number of transmembrane helices (38 in each of the three arms) and by using the generated membrane curvature to achieve high sensitivity for membrane tension. In cell membranes, the curved membrane shape in and around the Piezo nanodome leads to an area increase ΔA compared to the projected area of this curved shape in the plane of the surrounding membrane. This area increase is associated with an energy term $\gamma \Delta A$ that leads to the flattening of the nanodome with increasing membrane tension γ. The area increase ΔA of the curved membrane shape at small tensions is much larger than the area difference between the open and closed states of the Piezo ion channel and, thus, decouples the tension sensitivity of Piezo proteins from the rather small area increase of channel opening (2). For the mechanosensitive ion channel protein MscL of bacterial membranes, in contrast, the area increase ΔA that drives the channel opening in tense membranes is the area difference between the open and closed states of the ion channel (2).

ACKNOWLEDGMENTS. My research is supported by Deutsche Forschungsgemeinschaft Grant CRC 1114/A4.

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