Supplemental Information Physical Properties of *Escherichia coli* Spheroplast Membranes

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Fig. S1 Geometries of an aspirated GUV and an aspirated spheroplast are the same except in size. Two different situations are shown. A. Aspiration produced a cylindrical protrusion (shown with a GUV image; scale bar = 10 µm): definitions for R_v , R_p , L_p . B. Aspiration produced a spherical protrusion (shown with a spheroplast image; scale bar = 2.5 µm): definitions for r, h, and H. C. Formulas used for the situation B (1): the volume on the right side of the red plane = $\pi h^2 \left(r - \frac{h}{3}\right)$; the area of the partial sphere on the right side of the red plane = $2\pi rh$.



Fig. S2 (Left image) A spheroplast was held by a micropipette with a small suction pressure initially in 30% STOP solution. (Middle image) Then the solution was changed to ~85% STOP solution. (Right image) Finally the solution was changed back to ~30% STOP solution. Both the swelling and the phase contrast changes were reversible. In sequence, the diameter of the spheroplast was 5.8, 4.7, 5.8 μ m; the phase contrast ($I_0 - I$)/ I_0 was 0.15, 0.25, 0.17 (see Fig. 1). Scale bar = 2 μ m.



Fig. S3 The images from left to right show the response of a spheroplast in 100% STOP solution to a sucking pressure by micropipette aspiration. Note that the applied suction pressure was very small ~50 Pa, but the spheroplast was already entirely sucked into the pipette. Scale bar = 5 μ m. The images were taken, from left to right, at t = 0, 79, 110, 169 s. This could be due to the size of the micropipette being too close to the size of the spheroplast, or it could be what was described as a liquid drop model in the micropipette-aspiration experiments of human blood cells (2-4). Since our interests are solely on the membrane property of spheroplasts, we will not discuss spheroplasts from 100% STOP solution.



Fig. S4 The tension vs. area change measurement of a spheroplast is reversible and repeatable. In this example, the spheroplast membrane was stretched and released twice (four different colors). The error bars are explained in Fig. 3.

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