Biophysical Journal, Volume *110*

Supplemental Information

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Supporting Material

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MATERIALS AND METHODS

Fabrication of microapertures.

Microapertures were fabricated in silicon (Si) chips, according to the procedures described in ref. 1 (Fig. S1). In brief, a FZ Si (100) wafer, one side of which was coated with a Si_3N_4 layer, was thermally oxidized in dry oxygen, and then the Si_3N_4 side was coated with SiO_2 by RF sputtering method. The thermal oxide layer was photolithographically patterned, and anisotropically etched in tetramethylammonium hydroxide. Then SiO_2 was sputtered onto the Si side to cover bare Si_3N_4 and Si surfaces formed during the anisotropic etching. After photolithographic patterning, circular holes were formed in the Si_3N_4 layer by isotropic etching in 85% phosphoric acid at 150 ˚C. The SiO_2 layer beneath the holes was then removed by 5% hydrofluoric acid to form apertures. Next a SiO_2 layer was grown on the surface of the Si chips containing apertures by wet thermal oxidation for 1 h. A layer of SU8–3010 photoresist (MicroChem, Newton, MA) was spun onto a dummy wafer, and the chip was placed on the SU8-3010 layer from the Si_3N_4 side. A Teflon-AF1601 solution (DuPont Fluoroproducts, Wilmington, DE) was then spun onto the chip and the solvent (FC-40) was allowed to evaporate at 95 ˚C. Teflon-AF around the apertures was lifted off by soaking in hot Remover 1165. Finally, the chips were washed thoroughly with isopropanol, ethanol, acetonitrile, acetone and chloroform.

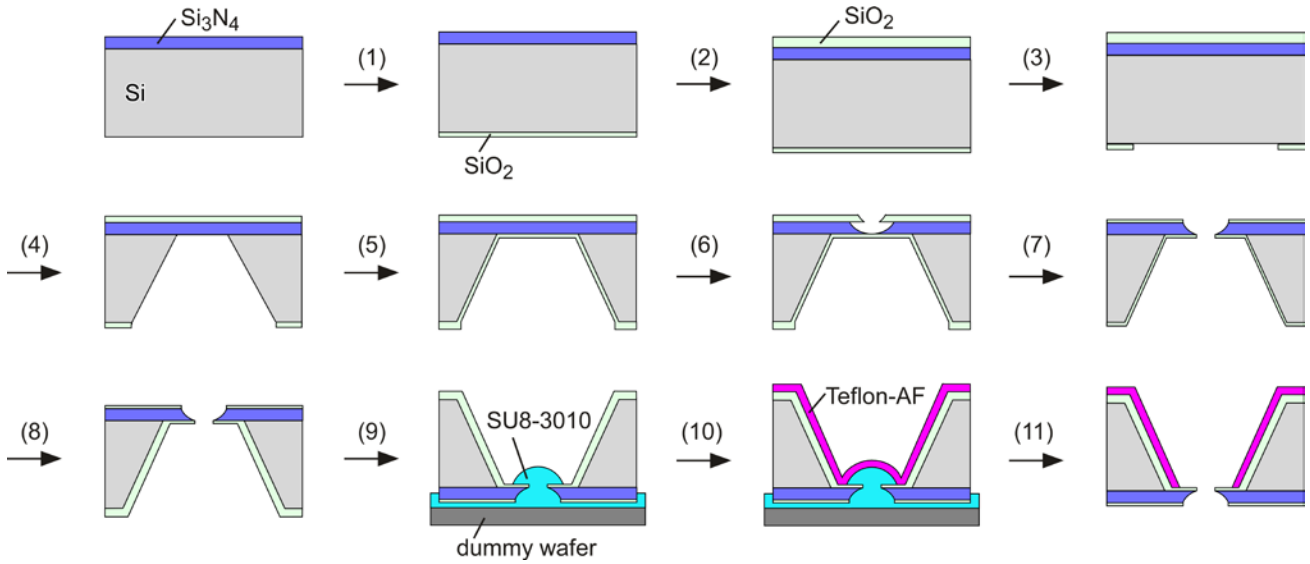

Fig. S1. Procedures for fabricating microapertures: (1) Thermal oxidation; (2) sputtering of SiO_2; (3) patterning of the thermal oxide layer; (4) anisotropic etching of Si; (5) RF sputtering of SiO_2; (6) patterning and isotropic etching of Si_3N_4; (7) SiO_2 removal; (8) wet thermal oxidation; (9) placing the Si chip on an SU8-3010 layer; (10) spin coating of Teflon-AF; (11) lifting off the Teflon-AF layer around the aperture.

Analysis of size distribution and density of membrane vesicles.

Size distribution of membrane vesicles was obtained using qNano (Izon Science, Oxford, United Kingdom) according to standard operating procedures (2, 3). Briefly, a nanopore membrane (NP800) was mounted onto the stretching pins of the qNano and stretched to a pin-to-pin width of 45 mm - 47 mm. Recording solution (120 mM KCl, 10 mM HEPES, pH 7.2 with KOH) was added to the both cell chambers which contained an Ag/AgCl electrode each, below and above the nanopore membrane. After removing air bubbles trapped in the nanopore, a voltage is applied across the membrane to establish a baseline electrical current through the nanopore. The solution in the upper cell chamber was then replaced with a solution containing standard particles of known size or membrane vesicles. Vesicles (particles) translocating the nanopore was detected as transient current blockade. The size and concentration of vesicles (particles) was obtained based on their relationship to the magnitude of

individual blockade currents and the rate of the blockade events, respectively. The density of membrane vesicle (ρ) was estimated based on the following equation,

$$\rho \sum_{d}^{d_{\max}} V_d C_d + \sigma \left(1 - \sum_{d}^{d_{\max}} V_d C_d \right) = \omega$$

where ρ and ω are density (g/mL) of the recording solution and sample solution containing membrane vesicles, respectively, and V_d and C_d are volume and concentration (particles/mL) of vesicles with a diameter of *d*, respectively.

Analysis of lipid compositions in cell membranes by using thin-layer chromatography (TLC) .

Lipid compositions in cell membranes of Chinese hamster ovary (CHO) cell lines expressing the hERG channels, Human Embryonic Kidney (HEK) 293 cell lines expressing the human $Na_v1.5$ and human $\alpha1\beta2\gamma2$ $GABA_A$ receptor channels were analyzed with TLC. In brief, the cell lines stored in liquid nitrogen were thawed and sonicated in phosphate buffered saline. Then the cell samples were mixed well with chloroform and methanol. After addition of water and chloroform, chloroform layer was collected and concentrated with nitrogen stream. TLC analysis was made using HPTLC plates of silica gel 60 (Merck Millipore, Darmstadt, Germany) and a software JustTLC (Sweday, Sweden). The weight % of each lipid component, i.e., phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cholesterol (Chol), thus analyzed was as follows: PC 43%, PE 25% and Chol 32% for CHO cells expressing the hERG channels, PC 40%, PE 35% and Chol 25% for HEK 293 cells expressing the $GABA_A$ receptor channels, and PC 49%, PE 26% and Chol 25% for HEK 293 cells expressing the $Na_v1.5$ receptor channels. Interestingly, cells containing higher amounts of PE and Chol compared with PC appeared to be robust against centrifugal forces and showed wider optimum centrifugal ranges for the channel integration into bilayer lipid membranes (BLMs).

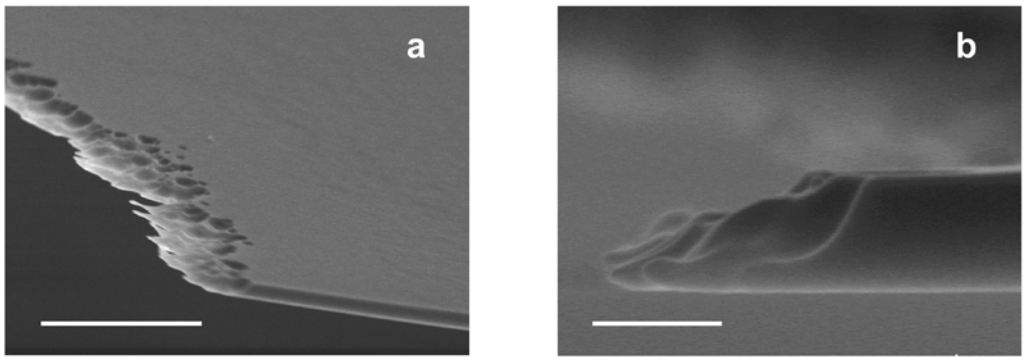

Fig. S2. Scanning electron microscopy (SEM) images of an aperture edge. (a) Overhead view. Scale bar 2 μm, tilt 66.8°. (b) Cross-sectional view. Scale bar 300 nm.

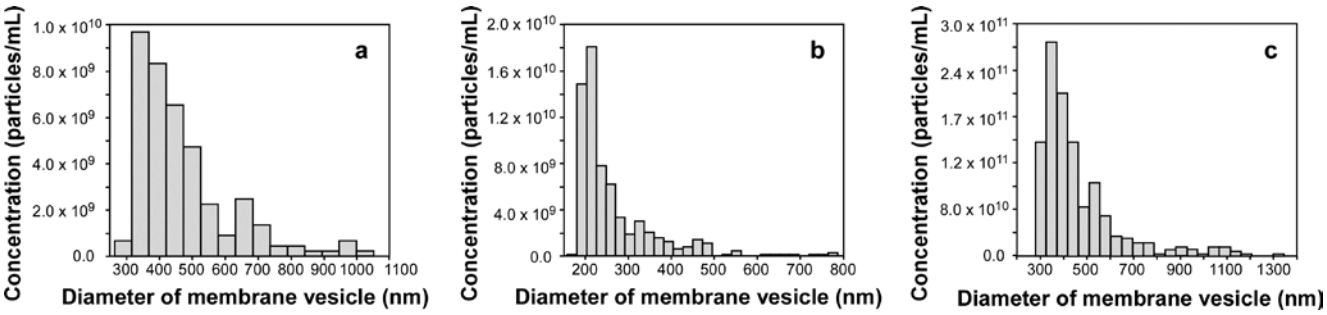

Fig. S3. Size distribution of membrane vesicles. Samples a-c were vesicles extracted from different cultures of CHO cell lines expressing hERG channels. Each sample was diluted 10-100 times with recording solution (120 mM KCl, 10 mM HEPES, pH 7.2 with KOH) and size distribution was determined using qNANO.

Supporting References

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