

## Supplementary Information

# Oriented Reconstitution of the Full-Length KcsA Potassium Channel in a Lipid Bilayer for AFM Imaging

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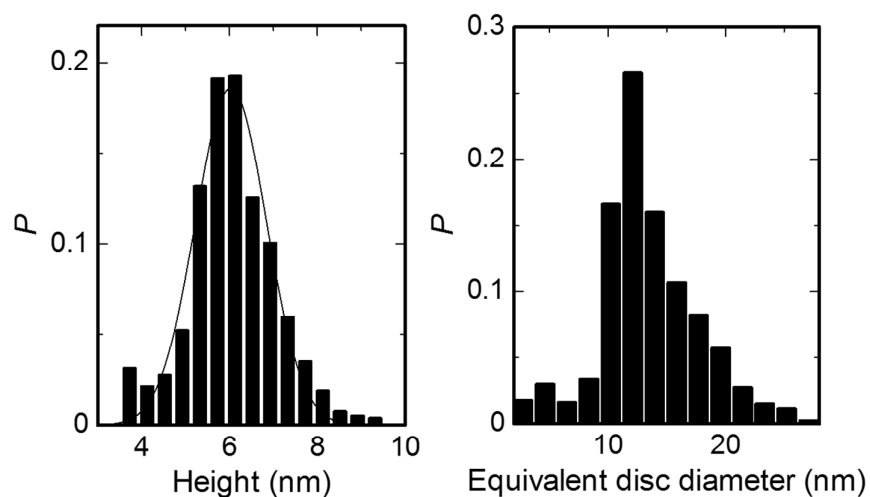


Figure S1. Histograms of the maximum height and the equivalent disc diameter of the KcsA channels adsorbed onto mica imaged in Figure 2A.

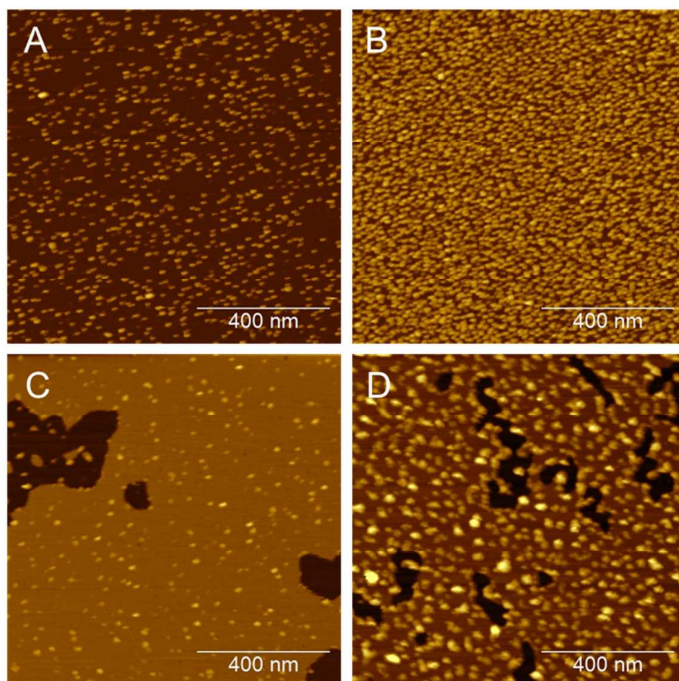
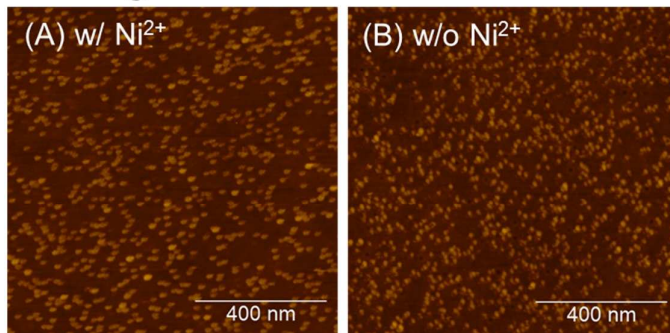
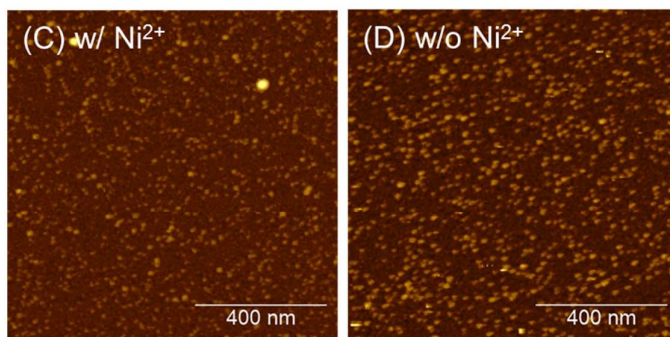


Figure S2. Formation of the supported bilayer around the high-density KcsA channels attached on the mica surface. AFM images of the channels are shown before (A, B) and after (C, D) bilayer formation. The concentration of the channels was 1 (A, C) and 10 (B, D)  $\mu\text{g/mL}$ , reflecting the density of the channel on the mica. Dispersed channels attached to the mica, despite the high density, became membrane-embedded after treatment of the destabilized liposomes, and the channels were clustered in the membrane. Images were taken in 10 mM HEPES (pH 7.5), 300 mM KCl. Scale bars: 400 nm.

### Coverglass



### NTA-mica



**Figure S3.** Non-specific adsorption of the KcsA channel on bare coverglass and NTA-modified mica. AFM images of adsorbed KcsA channels are shown on  $\text{Ni}^{2+}$  coated coverglass (A), bare coverglass (B),  $\text{Ni}^{2+}$ -NTA-modified mica (C) and NTA-modified mica (D). Images were taken in 10 mM HEPES (pH 7.5), 300 mM KCl. Scale bars: 400 nm.

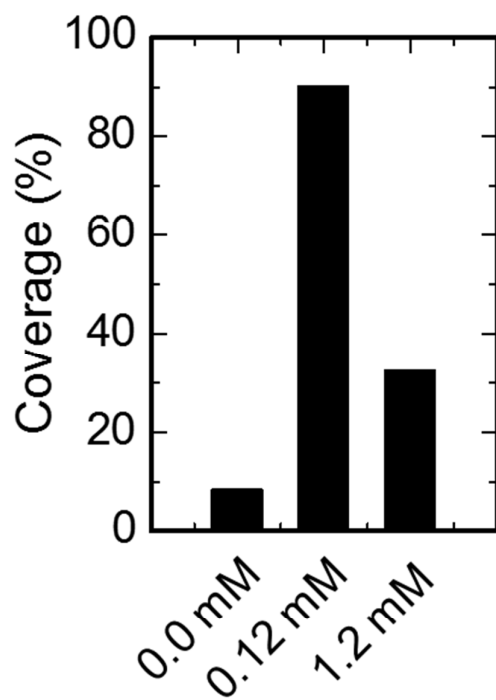


Figure S4. Effect of DDM concentration on the coverage of supported bilayers on the mica surface. The coverage levels were analyzed using the AFM images shown in Figure 3.

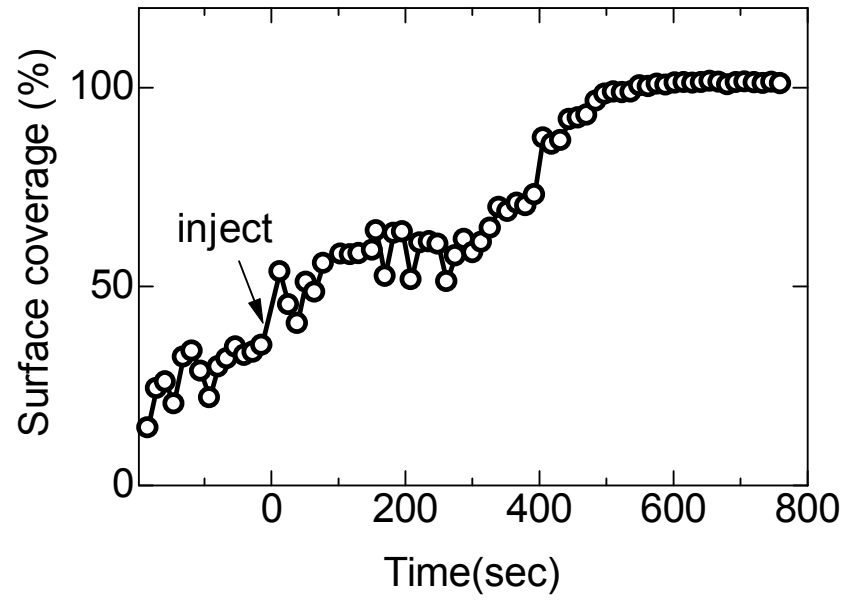


Figure S5. Time-course of bilayer filling. The surface coverage of the channels and bilayers in figure 4A are analyzed. Destabilized liposome solution was injected at 0 sec.

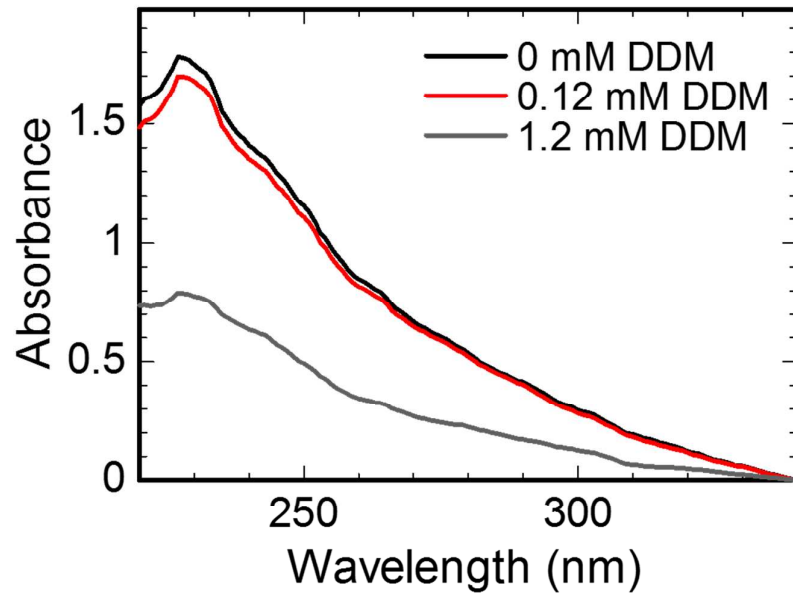


Figure S6. Light scattering of destabilized liposome. The DDM molecules were added to a liposome suspension of asolectin (0.5 mg/mL).

Movie S1. Time-lapse image of bilayer filling. This movie is corresponding to the images in figure 4A. Imaging buffer was replaced by destabilized liposome solution at 3 min 17 sec. Scan rate was 13 sec/frame.