Supporting Information

Real-time monitoring of membrane-protein reconstitution by isothermal titration calorimetry

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Additional electrophysiological experiments.

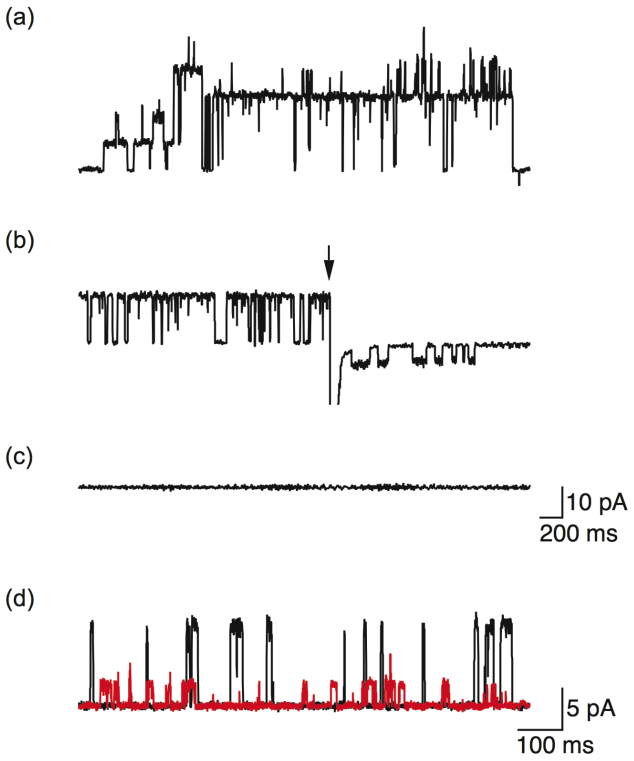

Figure S1. Modulation of KcsA channel activity by pH and small-molecule inhibition. After reconstitution and detergent removal, KcsA was transferred from proteoliposomes into planar membranes consisting of 3:2 (*w*/*w*) *E. coli* polar lipid extract/1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG). (a) pH 4.0 on both sides, +150 mV. (b) pH 7.0 on *cis* side, pH 4.0 on *trans* side, change from +75 mV to –75 mV (arrow). (c) pH 7.0 on both sides, +150 mV. (d) pH 4.0 on both sides, +150 mV, either in the absence (black trace) or in the presence (red trace) of 10 mM tetraethylammonium.

Quantitative comparison with simple lipid/detergent systems. Under equilibrium conditions, as they have been demonstrated for protein-free *E. coli* polar lipid extract/OG mixtures,[39] the slopes of the phase boundaries in Figure 3 can be converted to standard molar Gibbs free energies of micelle-to-bilayer transfer. These amount to $\Delta G_L^{b/m,0} = -RT \ln((1+R_D^{m,SOL})/(1+R_D^{b,SAT})) = -1.7$ kJ mol^{-1} for the lipid and $\Delta G_D^{b/m,0} = -RT \ln((R_D^{b,SAT}/R_D^{m,SOL}(1+R_D^{m,SOL})/(1+R_D^{b,SAT})) = 1.0$ kJ mol^{-1} for OG, where the negative and positive signs reflect the intrinsic preferences of the lipid for bilayer structures and of the detergent for micellar assemblies, respectively.[39] In a naïve approach neglecting competing, unproductive reactions during IMP reconstitution, it is tempting to quantify the influence of the protein by treating the KcsA/*E. coli* lipid/OG system as a pseudobinary lipid/detergent mixture. In other words, one may account for the protein as if its presence merely reflected a change in experimental conditions such as a shift in temperature or pressure rather than explicitly treating it as an additional component and without considering irreversible processes that remove material from the reconstitution mixture. Then, the slopes of the critical lipid/detergent concentration pairs in Figure 3 would result in standard molar Gibbs free energies of micelle-to-bilayer transfer of $\Delta G_L^{b/m,0} = -2.8$ kJ mol^{-1} for *E. coli* polar lipid extract and $\Delta G_D^{b/m,0} = 0.7$ kJ mol^{-1} for OG, implying that KcsA renders reconstitution even more favorable for the lipid and less unfavorable for the detergent. As pointed out above, however, these considerations are overly simplistic, as they are based on equilibrium assumptions, and hence must not be used to derive conclusions on the thermodynamics of bilayer formation in the presence of KcsA. At best, they may provide a simple, descriptive means of parameterizing the supramolecular state of the reconstitution mixture as a function of lipid concentration under a given set of experimental conditions.