Regulation of Ion Channel Function by the Host Lipid Bilayer Examined by a Stopped-Flow Spectrofluorometric Assay

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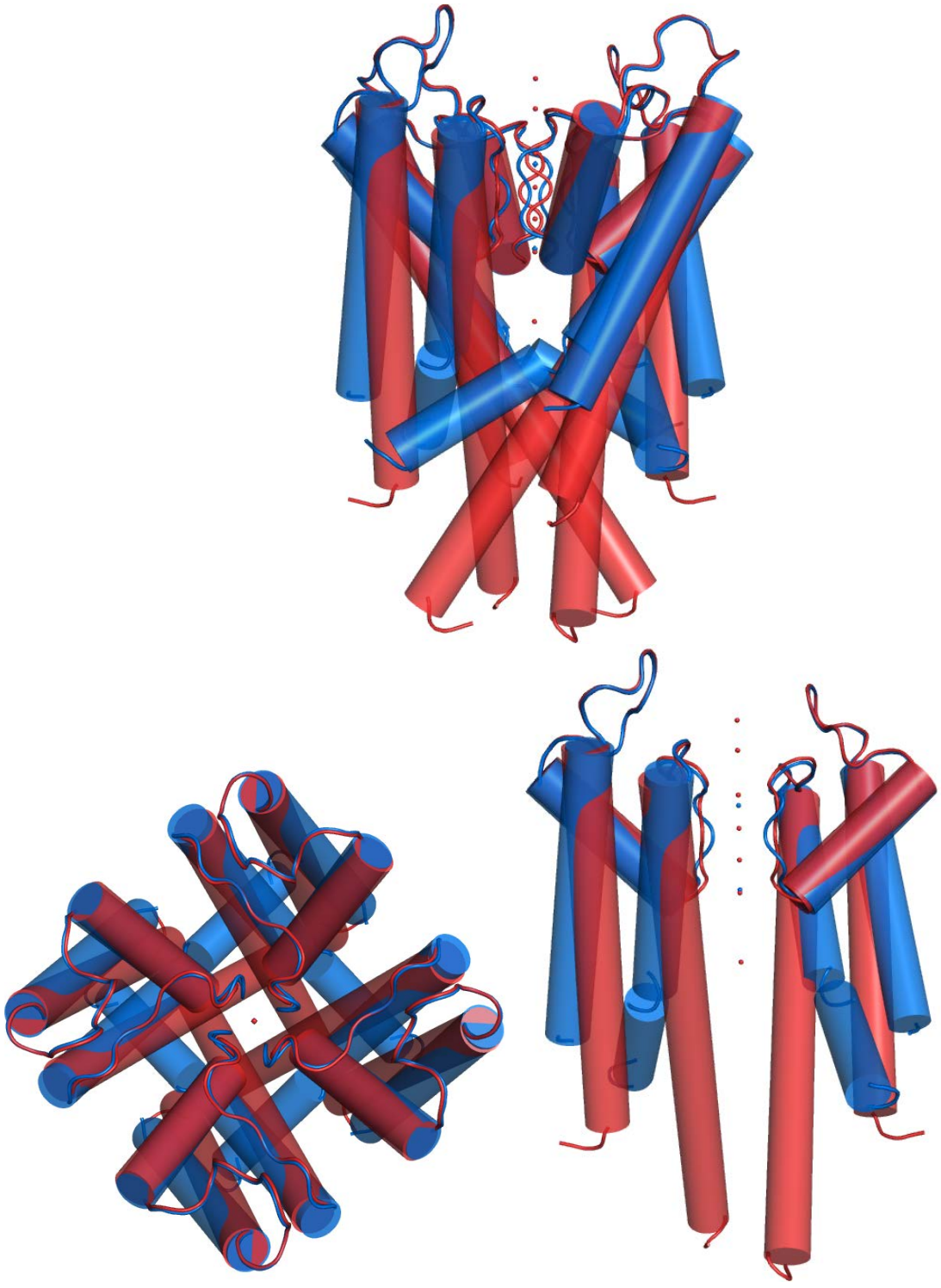
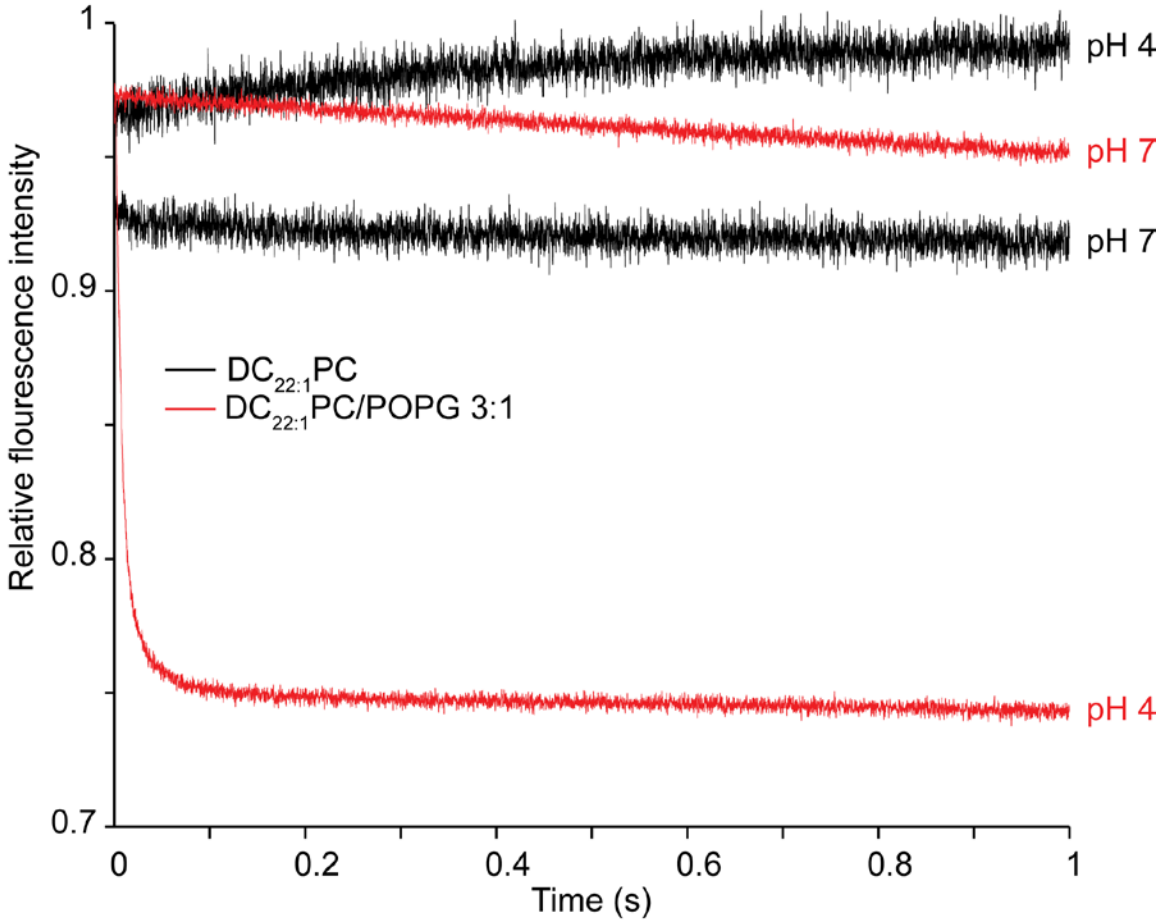
SUPPLEMENTAL INFORMATION

KcsA expression and purification. KcsA protein was expressed and purified according to the protocol previously reported in Thompson *et al*, 2008 (1) with minor modifications. Briefly, WT KcsA and the E71A variant were expressed from the pQE60 vector in BL21 (DE3) T1-R cells (Sigma). Cells were grown with aeration at 37 °C until an OD_{600} of ~ 1.0 and expression was induced by addition of 0.5 mM IPTG. After 3 h induction at 37° C, the cells were spun at 5,000 rpm for 10 min at 4 °C. Cell pellets were resuspended in buffer containing 50 mM Tris-HCl and 100 mM KCl, pH 7.6 and lysed by probe sonication. Membrane extraction was achieved by incubation with 25 mM *n*-Decyl β-D-maltopyranoside (DM, Affymetrix) for 2 h at room temperature with gentle agitation, followed by centrifugation at 17,500 rpm at 4° C for 45 min. The supernatant was applied to a Hi-Trap column (GE) charged with Co^{2+} and equilibrated with Buffer B (20 mM Tris-HCl, 100 mM KCl, pH 7.6, 5 mM DM). The column then was washed with Buffer B supplemented with 30 mM imidazole. The protein was eluted with Buffer B supplemented with 300 mM imidazole and concentrated prior to further purification by FPLC. The sample was applied to a Superdex-200 column (GE) equilibrated with Buffer B and fractions containing KcsA were pooled and assessed by SDS-PAGE. Protein concentration was calculated from absorption measurement at 280 nm using an extinction coefficient of 34950 M^{-1} cm^{-1}.



Supplemental Figure 1. Overlay of closed (red) (2) 1K4C and open-inactivated (blue) (3)3F5W KcsA structures. Note the differences in the bilayer-spanning domains.



Supplemental Figure 2. Fluorescence quench time-course observed when E71A KcsA was reconstituted into LUVs composed of either $DC_{22:1}PC$ (black traces) or $DC_{22:1}PC/POPG$ 3:1 (red traces), n=1.

SUPPORTING REFERENCES

1. Thompson, A.N., D.J. Posson, P.V. Parsa, and C.M. Nimigean. 2008. Molecular mechanism of pH sensing in KcsA potassium channels. *Proc. Natl. Acad. Sci. USA*

2. Zhou, Y., J.H. Morais-Cabral, A. Kaufman, and R. MacKinnon. 2001. Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 Å resolution. *Nature* 414:43-48.

3. Cuello, L.G., V. Jogini, D.M. Cortes, and E. Perozo. 2010. Structural mechanism of C-type inactivation in K(+) channels. *Nature* 466:203-8.