

Posson et al., <http://www.jgp.org/cgi/content/full/jgp.201311057/DC1>

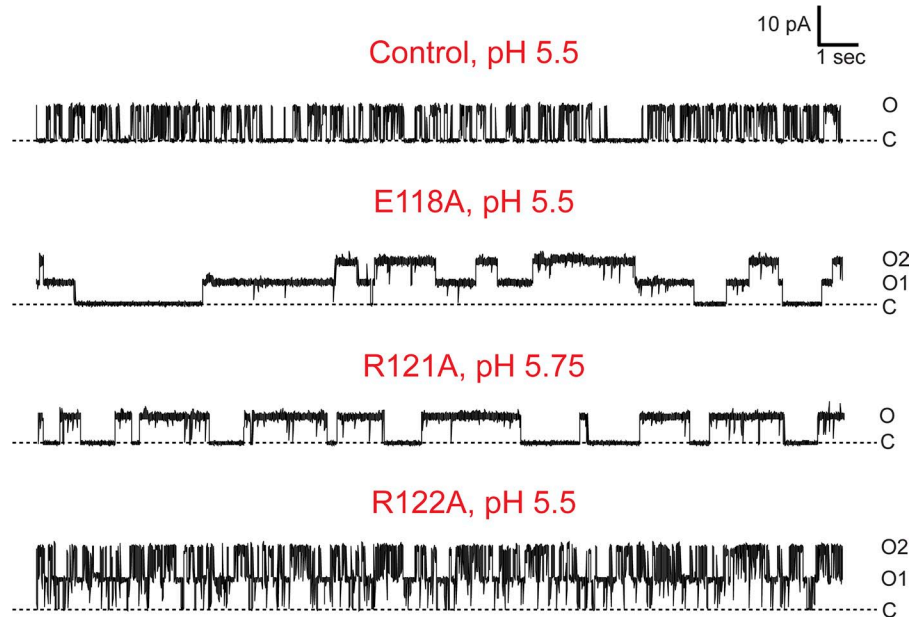


Figure S1. Single-channel gating kinetics at intermediate activation for individual mutations compared with the E71A control channel. Both E118A and R121A slowed the transitions between open and closed states, whereas R122A had a much smaller effect on kinetics. Fig. 2 showed that the combination of E118A and R121A as well as E118A and R122A had profound effects on the channel kinetics, indicating energetic coupling of these residues during intermediate states. All traces were recorded at 100 mV and filtered offline to 500 Hz for display.

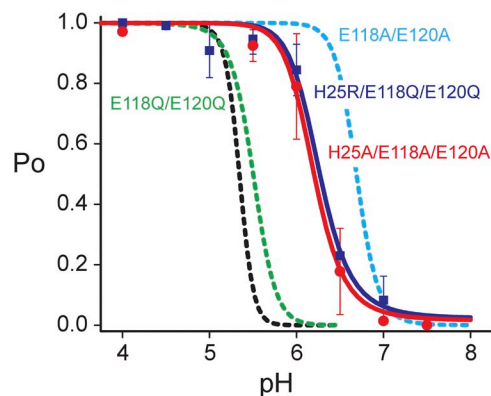


Figure S2. Two H25/E118/E120 mutants that remained pH dependent indicate the potential presence of unidentified pH sensors. The control channel (black dashed line) and E118A/E120A (cyan dashed line) are the same as in Fig. 1. E118Q/E120Q was modeled as the H25 sensor (green dashed line). H25R/E118Q/E120Q (squares, blue line) and H25A/E118A/E120A (circles, red line) have an unknown pH sensor modeled as a single proton-binding site. All data are from Thompson et al. (2008, *Proc. Natl. Acad. Sci. USA*, 105:6900–6905). Model fit parameters are given in Table 1.

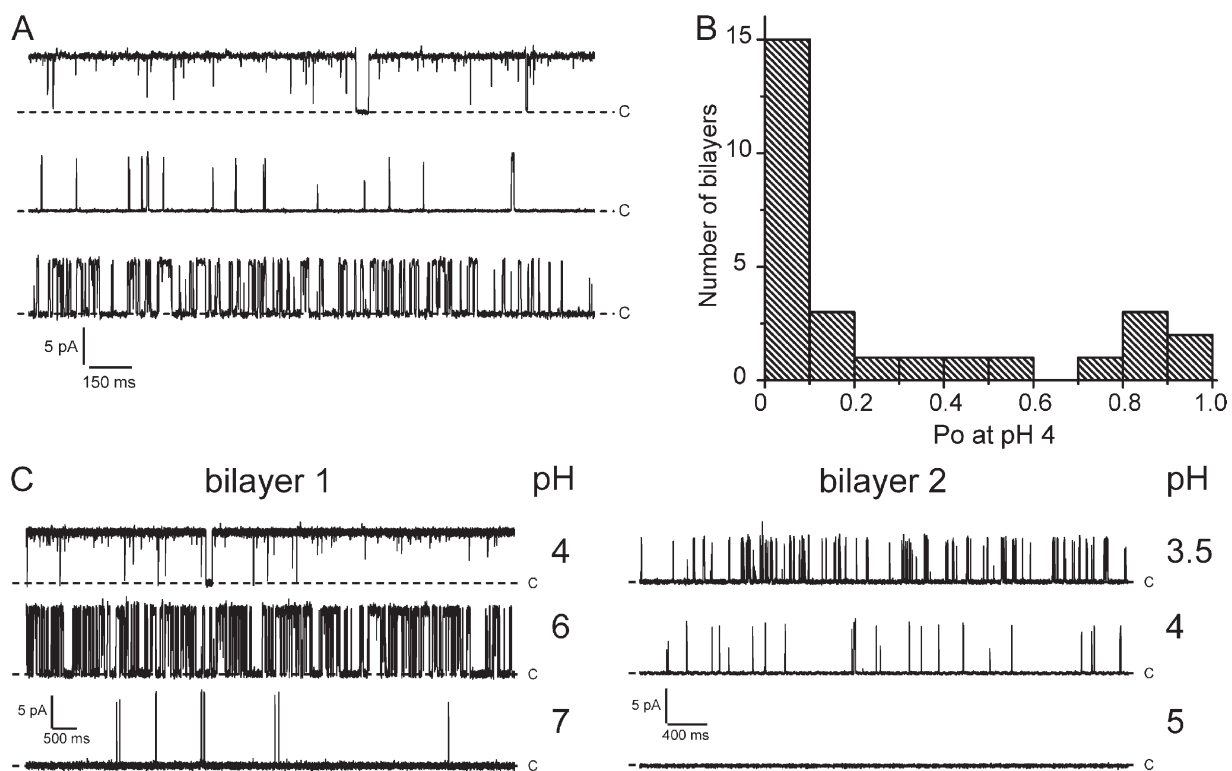


Figure S3. The H25A mutant is pH dependent. (A) Three separate bilayer recordings from H25A single channels at pH 4.0 exemplifying the high degree of channel to channel variability in P_o . (B) Histogram of P_o values for H25A channels spanning values from a few percent to 100% open. The majority of bilayers contained very low P_o channels (15 out of 28). (C) pH dependence for one high- P_o bilayer and one low- P_o bilayer. The mutant channel retains a pH sensor. Single-channel recordings in A and C were at 100 mV and filtered offline to 500 Hz for display.