Supplementary Information for

Membrane Environment Modulates the pK_a Values of Transmembrane Helices

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System preparation and equilibration

The helical peptides were generated using the sequences listed in Table 1 of the main text and were acetylated and amidated at the N and C termini, respectively. Each peptide was then inserted in the membrane bilayer using the CHARMM-GUI[1] server and the system was solvated with TIP3P water molecules[2]. Na^+ and Cl^- ions were added to adjust the concentration to 150 mM. The CHARMM36[3] force-field was used to represent the lipid molecules and the CMAP-corrected CHARMM27 all-atom force-field[4,5] was applied to describe the proteins. The lipid bilayers were chosen to resemble the membranes used in the SSNMR experiments. In addition, the carboxyl groups in the lipid bilayers that are used in the simulations of H12, H14, E12, E14, D12 and D14 are substituted with ether to mimic the experimental conditions more closely

(Koeppe *et al*. personal communications). Both charged and neutral states of the side chains in the TM helices were equilibrated at 300K using a CHARMM-GUI[1] protocol in the NVT ensemble for 80 ns, from which the first 40 ns were discarded. For all simulations the SHAKE algorithm[6] was applied to constrain bonds involving hydrogen atoms and the PME algorithm[7], with a non-bonded cutoff of 12 Å and a van der Waals switching function was used to calculate the long range electrostatic interactions. The Leapfrog Verlet integrator was used with an integration time step of 2 fs. A Langevin thermostat with frictional coefficient of $10ps^{-1}$ was employed to maintain the temperature at 300K. All equilibration simulations were performed on a GPU platform utilizing the CHARMM/OpenMM interface.

The structures obtained from the last 40 ns of the simulated charged and neutral states of the peptides were clustered using the kclust technique implemented in the MMTSB tool set.[8] The least-square fitting procedure was modified to conserve the peptide tilt angles and insertion depth in the membrane.[9] The centers of the most populated clusters were extracted from the trajectories and saved as initial structures for the pH-replica exchange simulations.

All simulations in this work were done using CHARMM[10] simulation package.

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