Supporting Information

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SI Methods

Osmotic Pressure Calculations. Osmotic pressures of solutions of Na^+–acetate, Na^+–propionate, and K^+–acetate at various concentrations were calculated following the method of Luo and Roux (1). To prepare each calculation, a solution with the target molal concentration was prepared in a cubic box of ∼50-Å side length, and equilibrated at constant pressure (1 atm) and temperature (298 K) for 1 ns. Subsequently two water layers of thickness ∼20 Å were added at opposite sides of the solution, along the z axis. The resulting systems (∼23,500 atoms) were equilibrated for 1 ns, at constant temperature and pressure, with the cross-sectional area along the XY plane and the position of all ions also constant. Two virtual semipermeable membranes (flat-bottom harmonic potentials) were then introduced at the boundaries between the ionic solution and the water layers, so as to confine the ions within the central region, while water diffuses freely across the simulation box. Each system was then simulated for 20 ns at constant temperature and volume (determined from a time-average over the second equilibration stage). The osmotic pressure for each molal concentration was calculated as the average force per unit area exerted by the boundary potentials on the ions. To ascertain that 20 ns are sufficient to achieve convergence, the K^+–acetate solutions were simulated three times (with CHARMM27). Experimental values of the osmotic pressure, Π, were derived from measurements of osmotic coefficients, ϕ, using the following equation (2):

$$\Pi = \frac{\nu R T W_s}{1,000 V_s} \phi m, \qquad \textbf{[S1]}$$

where ν is the number of dissociated species in solution ($\nu = 2$ in this case), m the molal concentration of the solution, and W_S and V_S are the molecular weight and molal volume of water.

Dissociation Constant Calculations. To calculate the dissociation constants of the Na^+–carboxylate and K^+–carboxylate ion pairs, a series of simulation systems were prepared containing 1,470 water molecules, an acetate anion and either Na^+ or K^+. For each cation, 86 configurations were prepared in which the distance between the cation and the acetate molecule (defined by the central carbon atom) was set at values ranging from 2 to 5 Å in intervals of 0.05 Å, and from 5 to 10 Å in intervals of 0.2 Å. The potential of mean force (PMF) as a function of the cation–acetate distance, r, was then computed at constant temperature (298 K) and pressure (1 atm), using the Thermodynamic Integration method. Specifically, for each configuration, we carried out a 4-ns simulation in which the cation–acetate distance is restrained to the initial reference (using a harmonic potential of $k = 4,000$ kcal/mol·Å²). From each of these simulations, we computed the mean value of the projection of all interatomic forces (i.e., excluding those from the restraint) on the distance vector, using the implementation of the adaptive biasing force method in NAMD (3). The PMF for each cation was then obtained by integrating the mean-forces forces calculated at each value of r. The dissociation constant of each ion pair was derived from the PMF using the following equation (4):

$$K_d = \left[4\pi \int_0^{R_{\text{off}}} \exp\left\{ -\frac{\text{PMF}(r)}{k_B T} \right\} r^2 dr \right]^{-1}, \qquad \textbf{[S2]}$$

where R_{off} is the distance that defines the binding region. Note that if r is in angstroms, Eq. **S2** implies the K_d is in units of one molecule per cubic angstrom (to convert to moles per liter, multiply by 1,660.54).

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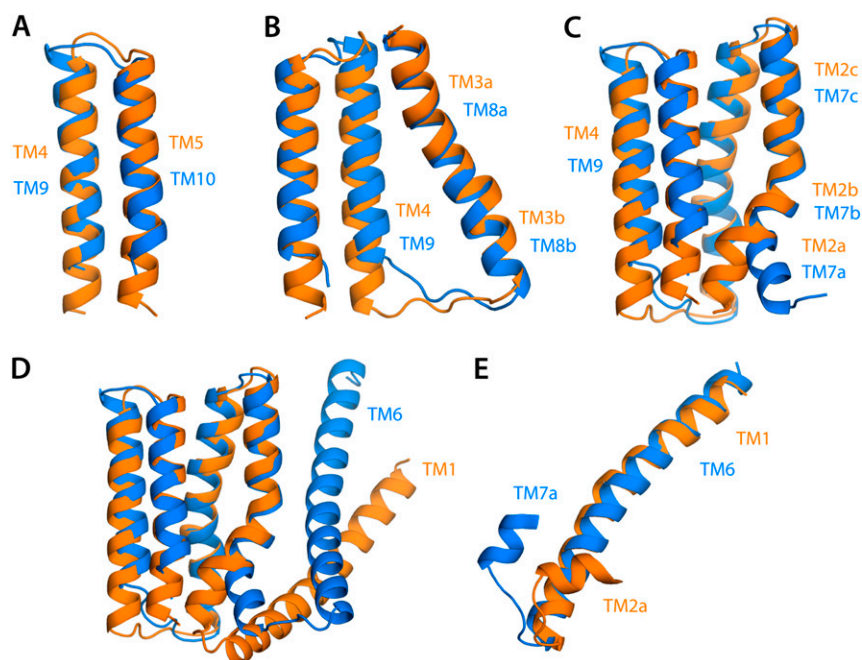
Fig. S1. Comparative analysis of the inverted-topology repeats in the outward-facing structure of NCX_Mj. (*A*) Superposition of TM4–TM5 on TM9–TM10; the root-mean-square difference (RMSD) in the Cα trace is 1.1 Å (for residues 101–118/125–142 and 257–274/ 282–299, respectively). (*B*) Superposition of TM3–TM4–TM5 on TM8–TM9–TM10; the RMSD is 1.2 Å (for the same residues as *A*, plus 67–92 and 226–251, respectively). (*C*) Superposition of TM2–TM3–TM4–TM5 on TM7–TM8–TM9–TM10; the RMSD excluding TM2a/TM7a is 1.1 Å (for the same residues as *B*, plus 46–64 and 205–223, respectively). (*D*) Superposition of TM1–TM2–TM3–TM4–TM5 on TM6–TM7–TM8–TM9–TM10. The fit is the same as that in *C*. (*E*) Superposition of TM1–TM2a on TM6–TM7a; the RMSD excluding TM2a/TM7a is 1.2 Å (residues 2–34 and 161–193, respectively).
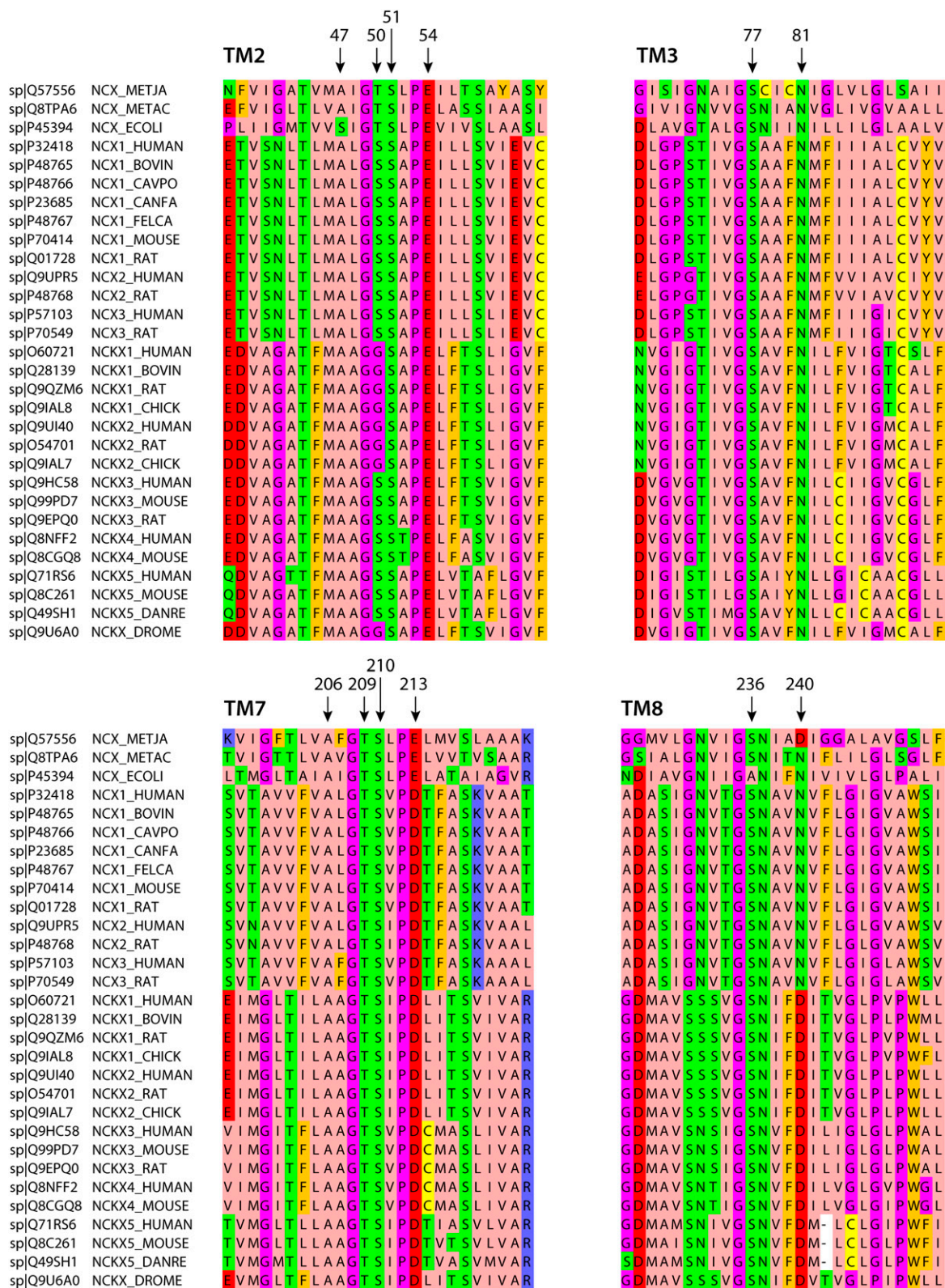
Fig. S2. Alignment of representative amino acid sequences of NCX and NCKX exchangers, including NCX_Mj. Only the four transmembrane helices flanking the ion-binding sites are shown. Residues directly involved in the coordination of ions/water in the structure of NCX_Mj are indicated. Note that D240 is substituted by asparagine in all NCX sequences, including those in *E. coli* (YrbG) and *M. acetivorans* (MaX1).
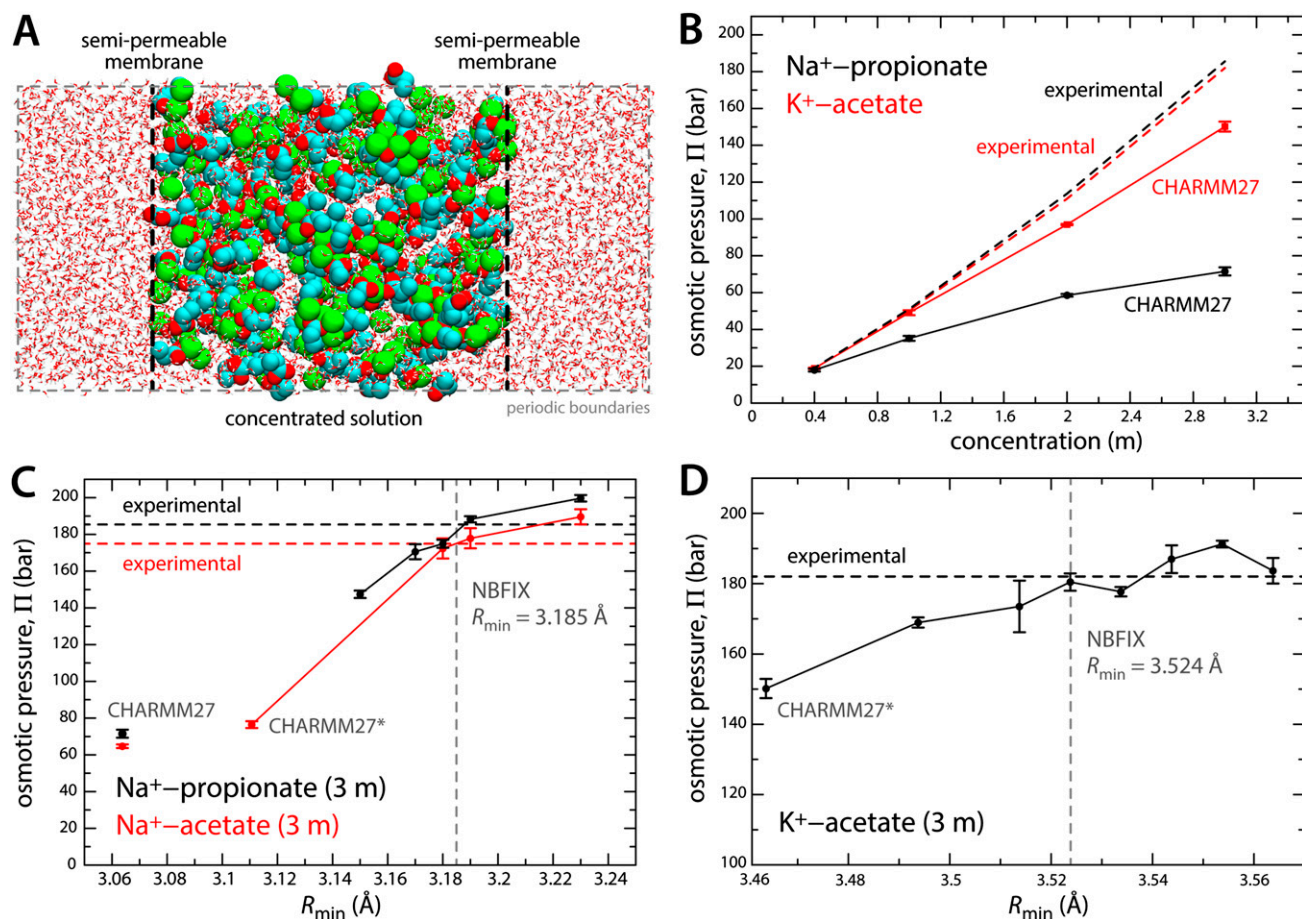
Fig. S3. Optimization of an NBFIX correction of the CHARMM force field for Na⁺–carboxylate and K⁺–carboxylate interactions, through osmotic-pressure calculations. (*A*) One of the simulation systems used for the calculation of osmotic pressures. (*B*) Comparison of experimental osmotic pressures of concentrated solutions of Na⁺–propionate and K⁺–acetate (2) with those calculated via MD simulations, using the standard CHARMM27 force field. The values for K⁺–acetate are averages from three independent simulations of 20 ns each; the error bars are the corresponding SDs. The values for Na⁺–propionate were obtained from single simulations of 20 ns; the error bars reflect differences in the calculated pressure between the two halves of each simulation. (*C*) Comparison of experimental osmotic pressures of 3 M solutions of Na⁺–propionate and Na⁺–acetate with those calculated via MD simulations, for different values of the Lennard–Jones (LJ) R_{min} parameter that describes the van der Waals interaction between Na⁺ and the carboxyl–oxygen atoms in propionate/acetate. CHARMM27* refers to the updated LJ parameters for Na⁺ and K⁺ developed by Noskov and Roux (5). The optimal value of R_{min} is indicated. All data derive from single simulations of 20 ns each; the error bars reflect differences in the calculated pressure between the two halves of each simulation. (*D*) Same as C, for a 3 M solution of K⁺–acetate.
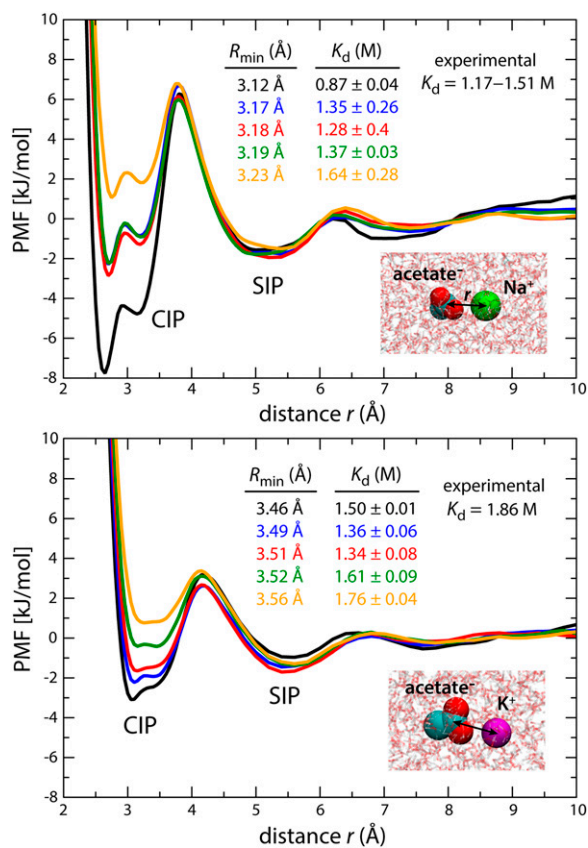
Fig. S4. Comparison of calculated and experimental dissociation constants of Na$^+$–acetate and K$^+$–acetate ion pairs. For each ion pair, the plots show the calculated potential of mean force (PMF) as a function of the distance r between the cation and the central carbon atom in the acetate anion. Alternative PMF profiles were computed for different values of the Lennard–Jones R_{min} parameter that describes the van der Waals interaction between the cations and the carboxyl–oxygen atoms in acetate. The corresponding values of the dissociation constant K_d are indicated, along with the experimental values (1). To derive the K_d values, the PMF profiles are integrated over the range in r that encompasses both the contact ion pair (CIP) and the solvent-shared ion pair (SIP) complexes (i.e., up to R_{off} = 6.2 Å).

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