Supporting information for

Structure-based engineering of lithium transport capacity in an archaeal sodium-calcium exchanger (NCX_Mj)

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Experimental Procedures

Cloning and Mutagenesis

DNA encoding the wild-type NCX_Mj was cloned to a pET-28a plasmid, as previously described. The NCX_Mj/NCLX construct was generated by subsequent introduction of nine point mutations (E54D, T50N, S51G, S77A, N81V, T209N, E213D, S236G, D240N) into the WT-NCX_Mj construct by QuickChange mutagenesis (Stratagene). All mutations were confirmed by sequencing.

Preparation of *E. coli* membrane vesicles containing overexpressed NCX_Mj or NCX_Mj/NCLX

The DNA constructs of NCX_Mj and NCX_Mj/NCLX were transformed into *E. coli* BL21 (DE3)pLysS competent cells. Cells were grown in 2xYT media until OD_{600}= 0.5–0.6 was reached and expression was induced at 16 °C by adding 400 μM IPTG. Cells were harvested 12–16 h after induction and immediately resuspended in 50 mM Mops–Tris pH 7.4, 250 mM sucrose, 1 mM EDTA, 1 mM DTT, 100 Units/ml DNase, and 1 mM PMSF. Homogenized cells were then lysed 3 times using a microfluidizer and membrane vesicles containing a given overexpressed protein were obtained using a three-step sucrose gradient (2.0,1.4, and 0.7 M) as previously described.[1]

$^{45}Ca^{2+}$-uptake assay in *E. coli* membranes vesicles

Initial rates (t = 5 s) of Na^+/Ca^{2+} and Li^+/Ca^{2+} exchange reactions were assayed by measuring $^{45}Ca^{2+}$-uptake in isolated membrane vesicles. To measure the Ca^{2+} dependency of these reactions, Na^+ or Li^+ (160 mM) loaded vesicles were diluted 25–50-fold at 35°C in an assay medium containing 30 mM Mops/Tris, pH 6.5, 160 mM choline chloride, and 5–2000 mM $^{45}CaCl_2$. To measure the Na^+ or Li^+ dependency of these reactions, vesicles were loaded with 1-160 mM Na^+ or Li^+ (+3 mM EGTA) and diluted in an assay medium to give a concentration of 250 μM $^{45}CaCl_2$. $^{45}Ca^{2+}$-uptake was quenched at different times (1–60 s) by rapid injection of cold EGTA-buffer into the assay medium. The quenched solutions were immediately filtrated on GF/C filters (Tamar Ltd) and uptake was assessed as previously described.[2-4] GraFit 7.0 software (Erithacus Software, Ltd.) was used for data fitting. The kinetic parameters (K_m and V_{max}) were evaluated under conditions in which the concentration-dependent curves reach more than 85% saturation. The K_m and V_{max} values were measured in three independent experiments and the obtained data are presented as the mean±SE.

References

1. Almagor, L., Giladi, M, van Dijk L., Buki, T, Hiller, R., and Khananshvili, D. (2014) Functional asymmetry of bidirectional Ca^{2+}-movements in an archaeal sodium-calcium exchanger (NCX_Mj). *Cell Calcium* 56, 276-284.
2. Khananshvili, D. (1991) Voltage-dependent modulation of ion binding and translocation in the cardiac Na^{+}–Ca^{2+}exchange system. *J. Biol. Chem*. 266, 13764–13769.
3. Khananshvili, D. (1991) Mechanism of partial reactions in the cardiac Na^{+}–Ca^{2+}exchanger. *Ann. N.Y. Acad. Sci.* 639, 85–98.
4. Khananshvili, D., Shaulov, G., and Weil-Maslansky, E. (1995) Rate-limiting mechanisms of exchange reactions in the cardiac sarcolemma Na^{+}–Ca^{2+}exchanger. *Biochemistry* 34, 10290–10297.

Table S1. Kinetic parameters of Na^{+}/Ca^{2+} or Li^{+}/Ca^{2+} exchange reactions mediated by NCX_Mj or NCX_Mj/NCLX

Na^{+}- or Li^{+}-dependent $^{45}Ca^{2+}$-uptake	K_m values	V_{max} pmol Ca^{2+}·mg protein^{-1}·s^{-1}
NCX_Mj		
K_m $(Na)_i$ at fixed $[Ca]_o$	23 ± 3 mM	560 ± 34
K_m $(Ca)_o$ at fixed $[Na]_i$	73 ± 9 µM	526 ± 15
NCX_Mj/NCLX		
K_m $(Na)_i$ at fixed $[Ca]_o$	1.4 ± 0.4 mM	218 ± 14
K_m $(Ca)_o$ at fixed $[Na]_i$	113 ± 11 µM	272 ± 7.0
K_m $(Li)_i$ at fixed $[Ca]_o$	1.4 ± 0.4 mM	234 ± 17
K_m $(Ca)_o$ at fixed $[Li]_i$	102 ± 12 µM	234 ± 8.0

The initial rates (t = 5 s) of $Na^{+}{}_i$- or $Li^{+}{}_i$-dependent $^{45}Ca^{2+}$-uptake were measured in *E. coli* derived cell-membrane vesicles containing over-expressed NCX_Mj or NCX_Mj/NCLX, as described in Figure 2. For measuring the $K_m(Ca)_o$ values, the concentrations of extravesicular $^{45}Ca^{2+}$ was varied (25-1600 µM) at fixed (saturating) concentrations of intravesicular Na^{+} or Li^{+} (160 mM). For measuring the $K_m(Na)_i$ or $K_m(Li)_i$ values, the intravesicular concentrations of Na^{+} or Li^{+} were varied at fixed concentration of extravesicular $^{45}Ca^{2+}$ (250 µM). The kinetic parameters represent mean ± SE values of at least three independent experiments.

Figure S1

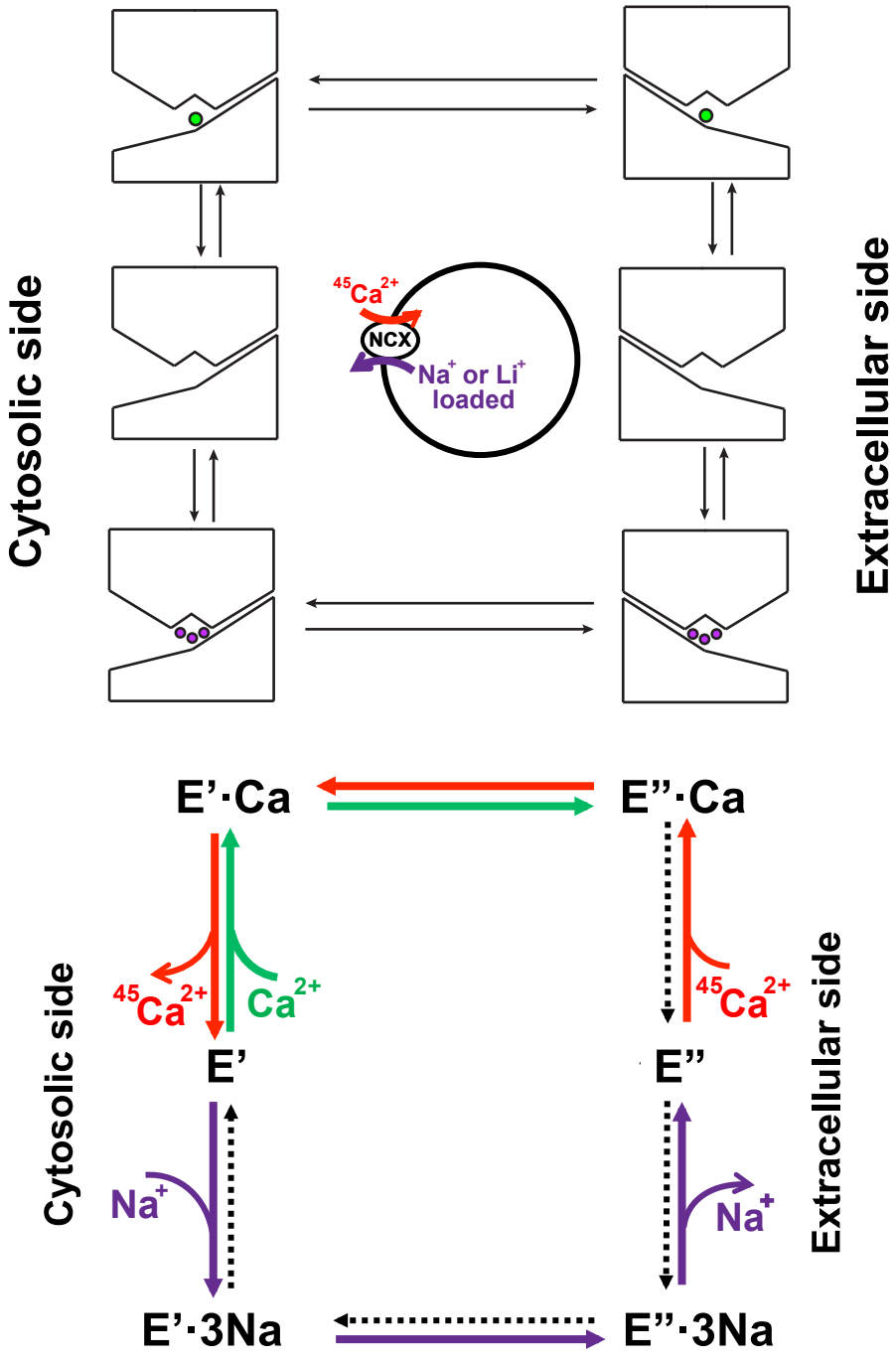

Figure S1. NCX_Mj and its ion-exchange reactions. Schematic representation of the ping-pong mechanism describing the exchange reactions for $Na^+/^{45}Ca^{2+}$ exchange or $Ca^{2+}/^{45}Ca^{2+}$ exchange. The red, green, and purple arrows represent Ca^{2+}-entry, Ca^{2+}-exit, and Na^+-exit steps of the transport cycle, respectively. The doted arrows represent the reactions of the transport cycle, which negligibly contribute to the observed ion exchange reactions under the given experimental conditions.