Functional Characterization of Procaryotic NCX by Solid Supported Membrane Technology

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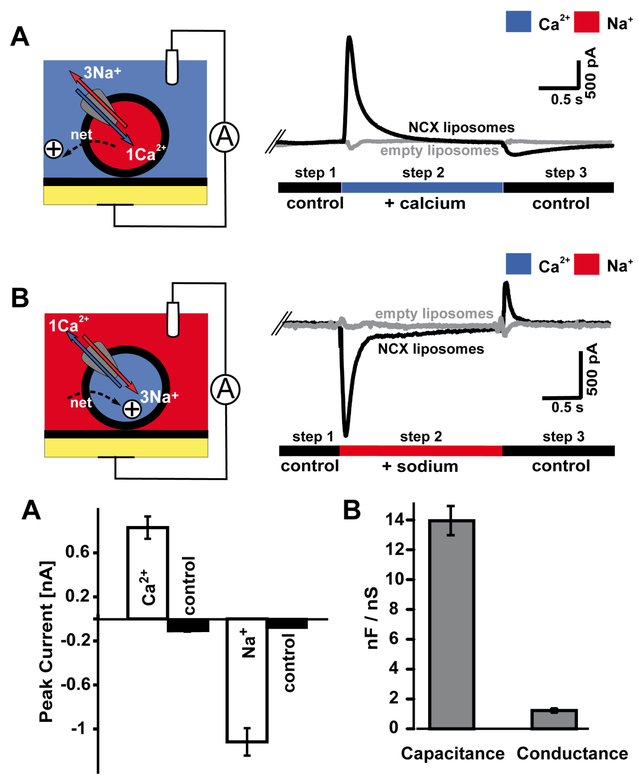
Abstract

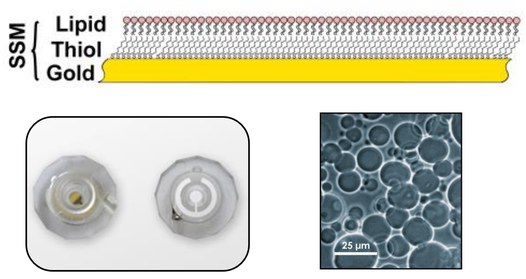
Sodium/calcium exchangers (NCXs) are membrane transporters which play an important role in Ca^{2+} homeostasis and Ca^{2+} signaling. These proteins have been implicated in various Ca^{2+} dependent physiological processes such as neurotransmission, skeletal and smooth muscle contraction, cardiac contractility and apoptosis. Here, we use solid supported membrane (SSM) technology to performed functional analysis of NCX_Mj, an archaeal NCX isoform which has recently been crystalized. Using this approach we characterized the substrate affinity, ion specificity and inhibition by divalent cations of this archaeal exchanger protein. In conclusion we found a high functional similarity of NCX_Mj and eukaryotic NCX isoforms, although the prokaryotic protein lacks a large regulatory domain. Furthermore our results demonstrate that SSM based technology is well suited for state-of-the-art functional characterization of transporter proteins.

Method

The NCX_Mj protein was reconstituted in liposomes, which were immobilized on the SSM sensor. The NCX current is generated by the exchange of Na^+ versus Ca^{2+}, usually with an electrogenic stoichiometry of 3 Na^+ to 1 Ca^{2+}, leading to a net cation flux. To induce an outward flowing $I_{Na/Ca}$, lipid vesicles were loaded with 140 mM Na^+ and $I_{Na/Ca}$ was activated by application of calcium-containing buffer (100 µM free Ca^{2+}). Alternatively liposomes were loaded with 20 mM Ca^{2+} and transport activated with 10 mM Na^+.

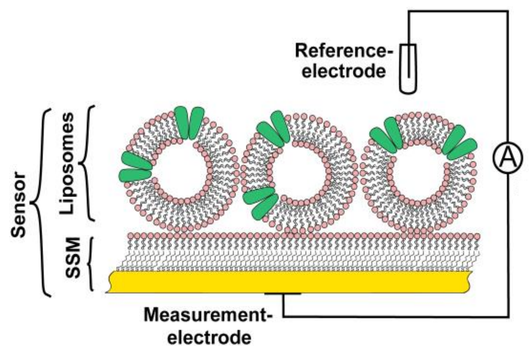


SSM Technique



SSM based biosensors
A gold electrode of 3 mm diameter is functionalized by a thiol- and a lipid layer. This allows to immobilize membrane fragments or liposomes containing the target transporter on the sensor.

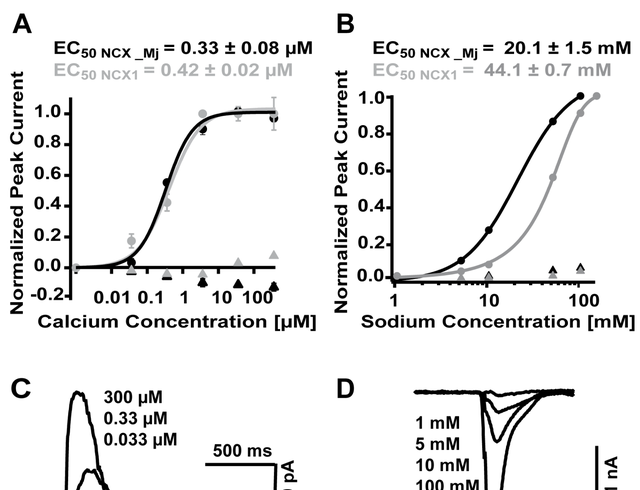
Accumulated transport current
The protein on the sensor is synchronously activated by a fast perfusion system. The transport of charged substrates over the membrane generates an electrical current. It's measured as a sum signal of all the protein on one sensor, thereby enabling a high amplification.



Platform
The experiments were performed on the SURFE2R N1, a system for SSM based transporter recordings. It's including a specialized perfusion system and performs amplification and data recording.

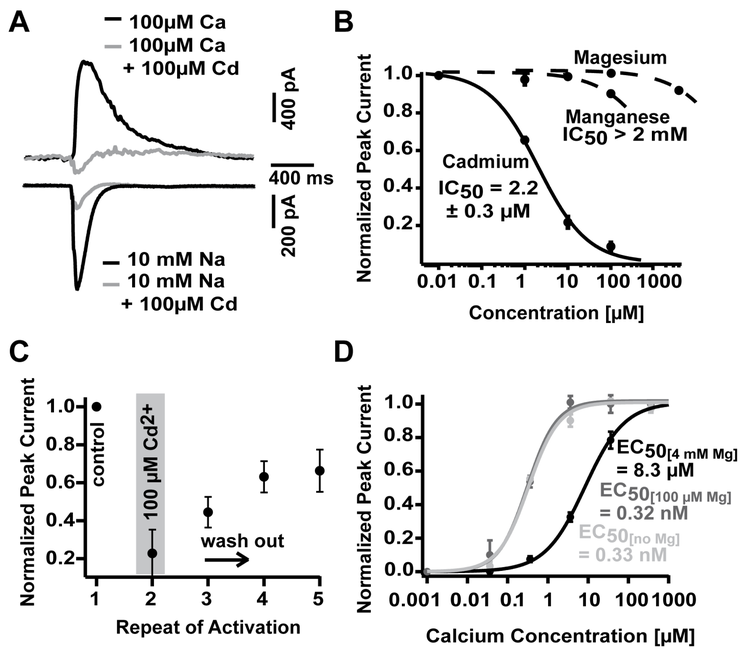
Apparent Affinity

Apparent Ca^{2+} and Na^+ affinity of NCX_Mj (black circles) and human NCX1 (grey circles). Control experiments (triangles) were performed with empty liposomes/parental HEK cell membranes (A) Apparent Ca^{2+} affinity was measured using vesicles loaded with 140 mM Na^+. Outward $I_{Na/Ca}$ was activated by application of solution containing differing free Ca^{2+} concentration as indicated (n=5). (B) Apparent Na^+ affinity was determined using vesicles loaded with 20 mM Ca^{2+}. Inward $I_{Na/Ca}$ was activated by application of varying Na^+ concentrations as indicated. (C+D) Representative current traces of outward/inward NCX_Mj $I_{Na/Ca}$, evoked by different Ca^{2+}/ Na^+ concentrations, respectively.



Inhibition

Inhibition of NCX_Mj by divalent cations.
(A) Na^+ and Ca^{2+}-activated NCX_Mj currents are inhibited by 100 µM Cd^{2+}.
(B) Concentration response curves of Cd^{2+}, Mg^{2+} and Mn^{2+}.
(C) Reversibility of Cd^{2+} inhibition. After activation of $I_{Na/Ca}$ by Ca^{2+} and subsequent block by Cd^{2+} only partial recovery of $I_{Na/Ca}$ upon washout of Cd^{2+} was observed.
(D) Ca^{2+} affinity of NCX in the absence and presence of 100 µM or 4 mM Mg^{2+}. In the presence of 4 mM Mg^{2+}, Ca^{2+} induced NCX_Mj activity is shifted towards higher Ca^{2+} concentrations indicating competition between Mg^{2+} and Ca^{2+}.



Selectivity