Supplementary Table 1

Gaussians widths (σ) and temperature bias (ΔT) used in each of the simulation replicas in the Bias-Exchange Well-Tempered Metadynamics calculations[a].

Replica	Collective variables biased	σ (V_1) [nm]	σ (V_2)[b] [nm]	σ (V_3) [nm]	σ (V_4)	σ (V_5)	298 K + ΔT (K)
1	V_1, V_2	0.035	0.035	-	-	-	4470
2	V_1, V_2	0.035	0.035	-	-	-	4470
3	V_1, V_2, V_3	0.116	0.116	0.116	-		2214
4	V_1, V_2, V_3	0.100	0.100	0.100	-		1831
5	V_1, V_2, V_3	0.083	0.083	0.083	-		1447
6	V_1, V_2, V_3	0.066	0.066	0.066	-		1064
7	V_1, V_2, V_3	0.050	0.050	0.050	-		681
8	unbiased	-	-	-	-	-	-
9	V_1, V_2, V_4, V_5	0.150	0.150	-	0.1050	0.002250	2214
10	V_1, V_2, V_4, V_5	0.125	0.125	-	0.0875	0.001875	1831
11	V_1, V_2, V_4, V_5	0.100	0.100	-	0.0700	0.001500	1447
12	V_1, V_2, V_4, V_5	0.075	0.075	-	0.0525	0.001125	1064
13	V_1, V_2, V_4, V_5	0.050	0.050	-	0.0350	0.000750	681
14	V_4, V_5	-	-	-	0.0350	0.000750	4470
15	V_4, V_5	-	-	-	0.0350	0.000750	4470
16	V_4, V_5	-	-	-	0.0350	0.000750	4470

(a) Gaussian functions were added to the biasing potential acting on each variable at 5-ps intervals, and were initially 2 kJ/mol in height. The height of these Gaussians functions was progressively scaled down as prescribed by the Well-Tempered Metadynamics method (Branduardi, D. et al. *J. Chem. Theor. Comp.* **8**, 2247-2254, 2012). To avoid systematic errors, the inversion condition was applied to the upper and lower limits of each collective variable biased (Crespo, Y. et al. *Phys. Rev. E* **81**, 055701, 2010). Note the specified 'temperature bias' is not the simulation temperature, which was in all cases 298 K. This scheme allows for an efficient exploration of the multidimensional collective-variable space: replicas with larger values of the temperature-bias and/or Gaussians widths are able to access high free-energy regions, albeit at low spatial resolution. Replicas with smaller values primarily sample low free-energy regions but at high spatial resolution.

(b) In the simulations of the Ca^{2+}-bound state, all σ (V_2) values were scaled down by a factor 1.4, so as to provide greater spatial resolution.

Supplementary Table 2

Deviation in the geometry of the (truncated) ion-binding region of NCX_Mj upon optimization via quantum-mechanical calculations, with respect to the input structure obtained via classical MD simulations, for different ions and occupancies.

	$3\times Na^+$	$2\times Na^+$	$1\times Ca^{2+}$	Ca^{2+} vs Sr^{2+}
RMSD, all atoms (Å)[a]	0.45	0.40	0.32 (0.40)[c]	0.10[d]
RMSD, cation site (Å)[b]	0.26	0.42	0.17 (0.23)[c]	0.14[d]

[(a)] The truncated ion-binding regions are depicted in Supplementary Fig. 6; the all- atom RMSD value does not include hydrogen atoms.

[(b)] This RMSD value is that of the bound ions and the coordinating oxygen atoms.

[(c)] Values in parenthesis refer to the geometry optimization using the SDD basis set (rather than with cc-pv-dz).

[(d)] These RMSD values compare the optimized geometries of the Ca^{2+}- and Sr^{2+}-bound states (both with the SDD basis set).