Supplemental Information

Energetics of Ion Permeation in an Open-Activated TRPV1 Channel

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Supporting Material

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Figure S1. Potential of mean force (PMF) as a function of the position along the permeation pathway from BE-meta simulations with two K⁺ ions (BE_2K). PMF evolution along the collective variable ξ of two different K⁺ ions (a and b), revealing convergence at t=160 ns. Colour scheme: 80 ns (red), 140 ns (blue), 160 ns (black).

Figure S2. Potential of mean force (PMF) as a function of the position along the permeation pathway from BE-meta simulations with two Na⁺ ions (BE_2NA). PMF evolution along the collective variable ξ of two different Na⁺ ions (a and b), revealing convergence at t=110 ns. Colour scheme: 60 ns (red), 100 ns (blue), 110 ns (black).
**Figure S3.** Potential of mean force (PMF) as a function of the position along the permeation pathway from BE-meta simulations with four Na\(^+\) ions (BE_4NA). (a-d) PMF evolution along the collective variable \(\xi\) of four different Na\(^+\) ions. Colour scheme: 120 ns (red), 130 ns (blue), 150 ns (black).

**Figure S4.** Potential of mean force (PMF) as a function of the position along the permeation pathway from BE-meta simulations with two Na\(^+\) ions and one K\(^+\) ion (BE_2NA_1K). PMF evolution along the collective variable \(\xi\) of (a-b) two Na\(^+\) ions and (c) one K\(^+\) ion. Colour scheme: 100 ns (red), 160 ns (blue), 180 ns (black).
**Figure S5.** Potential of mean force (PMF) as a function of the position along the permeation pathway from BE-meta simulations BE-meta simulation with two $K^+$ ions using a model of the entire pore domain (BE_2K_pore). Colour scheme: 160 ns (red), 180 ns (blue), 200 ns (black).

**Figure S6.** Potential of mean force (PMF) as a function of the position along the permeation pathway from BE-meta simulations with two $Na^+$ ions using a model of the entire pore domain (BE_2NA_pore). Colour scheme: 120 ns (red), 160 ns (blue), 250 ns (black).

**Figure S7.** (a) $K^+$ (red) and $Na^+$ (black) coordination number as a function of the position along the permeation pathway. The coordination number was measured as the number of oxygen atoms from water molecules closer than a certain cut-off distance to an ion (3.0 Å for $K^+$ and 2.8 Å for $Na^+$). Data were collected from the neutral replica trajectory of simulations BE_2K_pore and BE_2NA_pore respectively. (b) TRPV1 pore with reference axis. (c-d) Model systems in BE_2K and BE_2NA simulations showing partially-hydrated ion diffusion.
Figure S8. RMSD of TRPV1 pore backbone (residues E570-V868; black) and selectivity filter atoms (residues G643-D646; red) in the neutral replica of simulations (a) BE_2K_pore and (b) BE_2NA. Evolution of G643 Cα-Cα distance for residues in opposite subunits in the neutral replica (c) BE_2K_pore and (d) BE_2NA_pore. Formation of the asymmetric clipped conformation is observed after 120 ns in simulations with Na⁺ ions.

Figure S9. (a) TRPV1 pore model (E570–V686), with ions and POPC lipids. (b) TRPV1 model selectivity filter (F587 to Y671) with cations. Potassium ions are shown in blue, and sodium ions in yellow.
**Figure S10.** (a) TRPV1 S5-S6 and selectivity filter structure (residues E570–V686) used as starting point for BE-meta simulations. Key residues in the calculation of the PMF of permeation (E684, Y671, G643, M644, G654 and D646) are shown in licorice representation. The black arrow at the right side of the structure indicates the position of key residues along the permeation pathways with respect to the carbonyl oxygen atoms of G643. (b) NavAb S5-S6 and selectivity filter structure, showing S178, E177, L176 and T175 in licorice, and five ion binding sites $S_{\text{EXT}}$, $S_{\text{HFS}}$, $S_{\text{CEN}}$, $S_{\text{IN}}$ and $S_{\text{CAV}}$. 