**Description of Additional Supplementary Files**

File Name: Supplementary Movie 1

Description: **Outward K+ ion permeation within a 500 ns trajectory from the computational electrophysiology MD simulations.** The simulations were performed with the crystal conformation in all subunits (simulation I, Supplementary Table 1). For clarity, only two subunits of NaK are shown. K+ ions are shown as red spheres. Most of the time, two K+ ions simultaneously occupied the S3 and S4 ion binding sites together with one ion in the vestibule.

File Name: Supplementary Movie 2

Description: **Inward K+ ion permeation within a 400 ns trajectory from the computational electrophysiology MD simulations**. The simulations were performed with the crystal conformation in all subunits (simulation I, Supplementary Table 1). For clarity, only two subunits of NaK are shown. K+ ions are shown as red spheres. Most of the time, two K+ ions simultaneously occupied the S3 and S4 ion binding sites together with one ion in the vestibule.

File Name: Supplementary Movie 3

Description: **Outward Na+ ion permeation within a 200 ns trajectory from the computational electrophysiology MD simulations.** The simulations were performed with a mixture of crystal and flipped conformations in different subunits (simulation V, Supplementary Table 1). For clarity, only two subunits of NaK are shown. Na+ ions are shown as blue spheres. Most of the time, only one Na+ ion resided in the SF.

File Name: Supplementary Movie 4

Description: **Inward Na+ ion permeation within a 500 ns trajectory from the computational electrophysiology MD simulations.** The simulations were performed with a mixture of crystal and flipped conformations in different subunits (simulation V, Supplementary Table 1). For clarity, only two subunits of NaK are shown. Na+ ions are shown as blue spheres. Most of the time, only one Na+ ion resided in the SF. Na+ ions entered the SF through the side entry instead of passing through the S1 and S2 ion binding sites.