Supplementary Method

Generation of the animations. In order to generate the morph to visualize the conformational change between Na_vRh and Na_vAb, the homology-based model of Na_vRh was generated using online SWISS-MODEL workspace [1-3] with the structure of Na_vAb (PDB code: 3RVY, Chain A). The resulted structure was then superimposed to that of Na_vRh (PDB code: 4DXW) relative to the indicated structural elements. The shifted coordinates of the modeled structure and the original coordinates of Na_vRh were used as the initial and end states, respectively, for morph generation. The intermediate morphs were obtained with the multiple-chain morphing script [4,5] for Crystallography & NMR System (CNS) [6,7]. The animations were finally produced using PyMol [8].

Legends for Supplementary Movies

Supplementary Movie S1. An animation of the overall conformational changes between Na_vRh and Na_vAb. The morph was generated based on the structures of Na_vAb (starting frame, PDB accession code: 3RVY) and Na_vRh (ending frame, PDB accession code: 4DXW) with their pore domains superimposed. The generation of the animation is detailed in Method.

Supplementary Movie S2. The C-terminal half of S2 and the N-terminal half of S3 move almost as one module during the conformational change of the VSDs. The animation is similar to that shown in Movie S1 except that the distances (in the unit of Å) between a number of selected residues on the S2 and S3 segments are shown to highlight the synchronized motion of the intracellular fragments of S2 and S3.

Supplementary Movie S3. The conformational changes of S1, S3, and S4 segments relative to the charge transfer center (CTC) which is located on the S2 segment. The morph was generated based on the VSD structures of Na_vAb and Na_vRh with their CTCs superimposed.

Supplementary Movie S4. The conformational changes of S2-S4 segments relative to S1. The morph was generated based on the VSD structures of Na_vAb and Na_vRh with the S1 segments superimposed.

Supplementary Movie S5. The coupled movements between VSDs and the pore domain. Note the motion of the S5 segments from both the same and the adjacent protomers during the conformational changes of the VSD. The generation of the animation is identical to that of Movie S1 except for the distinct structural elements to be shown. The distances are shown in the unit of Å. Refer to S*upplementary Figure S5B* for the identity of the measured residues.

Supplementary Movie S6. The coupled movements between VSDs and the pore domain viewed from the cytoplasmic side. The animation shows a different view from *Supplementary Movie S1*.

Supplementary Movie S7. An animation of the R4 transfer in the context of the tetrameric protein. The morph was the same as in *Supplementary Movie S1*. R1-R4 as well as the CTC are highlighted in one protomer.

Supplementary References:

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