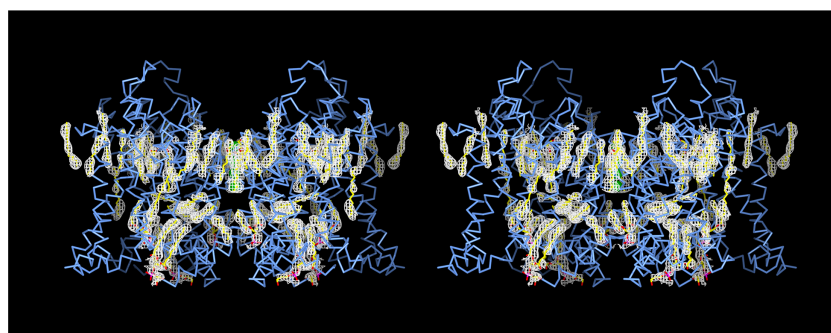


**Supplementary Figure 1. | Overall structure of the channel- $\beta$  subunit complex.** A ribbon representation from the side with the extracellular solution 'above' and the intracellular solution 'below', shown in stereo. Each subunit of the channel is colored uniquely. The  $\beta$  subunits are colored according to the channel subunit they contact. The NAD<sup>+</sup> cofactor bound to each  $\beta$  subunit is drawn as gray sticks. TM indicates the integral membrane component of the complex.



**Supplementary Figure 2. | Electron density for lipid molecules.** The view is of the transmembrane region of the channel from the side (extracellular solution 'above' and the intracellular solution 'below'), in stereo. The channel is drawn as blue  $\alpha$ -carbon trace. Electron density for lipid molecules is drawn as white mesh. The lipid molecules are shown as sticks and colored according to atom type (carbon, yellow; oxygen, red; phosphorous, magenta). Phases for the map were calculated by removing the lipid molecules from the coordinates and refining these modified coordinates using a simulated annealing protocol in the program CNS (38). This procedure, which is used to generate a 'simulated annealing omit map', minimizes bias in the map. The map has Fourier coefficients  $3F(\text{observed}) - 2F(\text{calculated})$ , and was calculated from 50-2.4 Å, contoured at  $1.0\sigma$ , and drawn around the lipid molecules.

## Supplementary Table | Data collection and refinement statistics

**Data collection**

Spacegroup	P4 <sub>2</sub> 1 <sub>2</sub>
Cell parameters (Å)	144.0, 144.0, 284.4
Source	NLSL X-29
Resolution (Å)	50.0 – 2.4
Wavelength (Å)	1.08 Å
No. of reflections (total)	278,768
No. of reflections (unique)	112,726
Completeness (%)	96.0 (86.3) ‡
R <sub>sym</sub> (%) *	6.4 (43.6)
I/σ	13.8 (1.4)

**Refinement** (50-2.4 Å)

No. of reflections	104,891	No. of atoms	11,816
No. of molecules/ASU	2	R <sub>work</sub> /R <sub>free</sub> (%) §	21.2/24.4
Bond length / angle †	0.01 Å / 1.4 °		
Ramachandran	99.8 % (allowed region) / 0.02% (disallowed region)		
Average protein B-factors			
T1/β	molecule 1: 37.2 Å <sup>2</sup> ; molecule 2: 41.2 Å <sup>2</sup>		
pore	molecule 1: 45.8 Å <sup>2</sup> ; molecule 2: 71.0 Å <sup>2</sup>		
voltage sensor	molecule 1: 64.0 Å <sup>2</sup> ; molecule 2: 148.0 Å <sup>2</sup>		
No. and average B-factors			
waters	molecule 1: 193 waters, 41.9 Å <sup>2</sup> molecule 2: 126 waters, 39.0 Å <sup>2</sup>		
lipid molecules	molecule 1: 16 lipids (210 atoms), 84.1 Å <sup>2</sup> molecule 2: 1 lipid (22 atoms), 88.4 Å <sup>2</sup>		

\* R<sub>sym</sub> =  $\sum |I_i - \langle I_i \rangle| / \sum I_i$ , where  $\langle I_i \rangle$  is the average intensity of symmetry-equivalent reflections

§ R factor =  $\sum |F(\text{obs}) - F(\text{calc})| / \sum F(\text{obs})$ ; 5% of the data that were excluded from refinement were used in the R<sub>free</sub> calculation.

† r.m.s.d of bond is the root-mean-square-deviation of bond angle and length

‡ parentheses indicate outer resolution shell (2.5 - 2.4Å).