

A physiologically-relevant intermediate state structure of a voltage-gated potassium channel.

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Supplementary information;

Supplementary Tables 1-4;

Supplementary Figures 1-14.

Further exploration of residue interrelationships in the S1, S2 and VSD

The S1 and S2 helices in the xE1R/R2E structure adopt a different conformation than in the xWT (Figs. 3, 5), changing the intra VSD interactions and contacts with the PD. To further explore the conformation of the VSD in the xE1R/R2E structure, we measured the average shortest distance between the C α atoms of the VSD helices and compared these values with the xWT structure (Supplementary Fig. 7). We observed that the lower half of S1 in the xE1R/R2E structure moves very little with respect to the FH' (< 0.4 Å) but moves ~1.3 Å away from the S5'. For the upper half of S1, the distances to S5' and FH' increase by 4.3 Å and 4.8 Å, respectively, indicating that this region of the VSD has not yet approached the filter helix in this intermediate structure. This distance changes minimally (<0.3Å) for the S3-S4 pair or for S4 in relation to the lower parts of S1 and S2, (Supplementary Fig. 7), while the average shortest distance to S4 increases by 1.6 Å and 3.4 Å for the upper halves of S1 and S2, respectively. The average shortest distance between helices S1 and S2 is similar between the two structures (<0.6 Å, Supplementary Fig. 7), showing that the upper parts of the S1 and S2 helices tilt together, setting them away from the S4 and increasing the solvent cavity. This dissociation from the FH' and the S4 increases conformational heterogeneity, especially for the upper halves of S1 and S2, as reflected by the lower local resolution for the xE1R/R2E VSD in comparison to the PD (Supplementary Fig. 4d) and xWT (Supplementary Fig. 2b).

To further explore the conformational landscape, we performed 3D variability analysis, which revealed clusters with similar yet non-equivalent conformations for the upper part of S1 (Supplementary Fig. 8). Combination of clusters with similar S1 conformations (Supplementary Fig. 8e; clusters 1-2 and clusters 4-5) highlighted the movement of the upper S1-S2 which is still distinct from the S1 conformation in xWT, where the S1 is attached to the filter helix from the adjacent subunit (Supplementary Fig. 8f-h). This further emphasizes that the E1R/R2E mutations do not lock the VSD in a single distinct conformation but rather allow flexibility within the upper halves of helices S1 and S2.

It is likely that the movement of the VSD from rest to the intermediate state has resulted in the breaking of contacts that stabilized the closed resting state and that the next step is for stabilizing interactions seen in xWT to form as the VSD moves to the fully activated state. Although we do

not observe large differences in the selectivity filter conformation between xWT and xE1R/R2E, the lack of interactions between the VSD and the FH' likely underlie electrophysiological differences (enhanced Rb⁺ conductance and XE991 sensitivity of the intermediate compared to activated state, Fig. 1e-1h)¹, possibly by affecting its flexibility at a level that cannot be observed at this resolution.

Supplementary Table 1. Summary characteristics of the intermediate and fully activated channel states of KCNQ1.

	WT hKCNQ1	Intermediate state (hE1R/R2E)	Activated state (hE1R/R4E)	References
Ion conduction and selectivity				
Permeability I_{Rb}/I_K	2.51 ± 0.22	1.95 ± 0.10	1.54 ± 0.06	Fig. 1E-F; Fig. 9F; Zaydman et al., 2014 ¹
Pharmacology				
XE991 IC ₅₀	0.07 μ M	0.24 μ M	>30 μ M	Fig. 1G-H
ML277 fold increase	7.26 $\pm$ 1.78	3.75 $\pm$ 0.59 Lower	5.86 $\pm$ 1.30 Higher	Fig. 9G; Eldstrom et al., 2021 ²
VSD-pore coupling				
Coupling efficiency		High	Low	Hou et al., 2017 ³
Modulation by β-subunits				
Action of KCNE1		Suppressed	Enhanced	Fig. 9; Zaydman et al., 2014 ¹ ; Hou et al., 2017 ³
Action of KCNE3		Enhanced	Suppressed	Fig. 10; Taylor et al., 2020 ⁴

Supplementary Table 2. Cryo-EM data collection, refinement and validation statistics.

Cryo-EM data collection, refinement and validation statistics		
Data collection and processing	xE1R/R2E PDB ID: 9MY4 EMDB ID: EMD-48726	xWT PDB ID: 9MY3 EMDB ID: EMD-48725
Magnification	130,000	130,000
Voltage (kV)	300	300
Electron exposure (e⁻/Å²)	50	50
Defocus range (μm)	-0.5 to -2.0	-0.5 to -2.0
Pixel size (Å)	0.96	0.96
Symmetry imposed	C4	C4
Initial particle images (no.)	10,095,344	1,348,161
Final particle images (no.)	95,597	47,918
Map resolution (Å)	3.53	3.45
FSC threshold	0.143	0.143
Map resolution range (Å)	2.86 – 7.00	2.91 – 7.00
Refinement		
Initial model used (PDB code)	xWT	7TCP
Model resolution (Å)	3.4	3.4
FSC threshold		
Model resolution range (Å)	3.4-3.7	3.3-3.6
Map sharpening B factor (Å²)	-	40
Model composition		
Non-hydrogen atoms	15000	15004
Protein residues	1904	1904
Ligands	-	-
B factors (Å²)		
Protein	118.68	148.16
Ligand	-	-

R.m.s. deviations		
Bond lengths (Å)	0.004	0.004
Bond angles (°)	0.971	0.939
Validation		
MolProbity score	1.56	1.16
Clashscore	5.58	2.62
Poor rotamers (%)	1.31	0.52
Ramachandran plot		
Favored (%)	97.01	94.44
Allowed (%)	2.99	2.56
Disallowed (%)	0.00	0.00

Supplementary Table 3. xE1R/R2E Cryo-EM structure system

Box dimensions	140 Å (x axis) * 140Å (y axis) * 107Å (z axis)
Total number of atoms	192059
Total number of water molecules	39389
Salt concentration	[K ⁺ Cl ⁻]= 0.15M
Lipid composition	100% POPC (427 molecules)

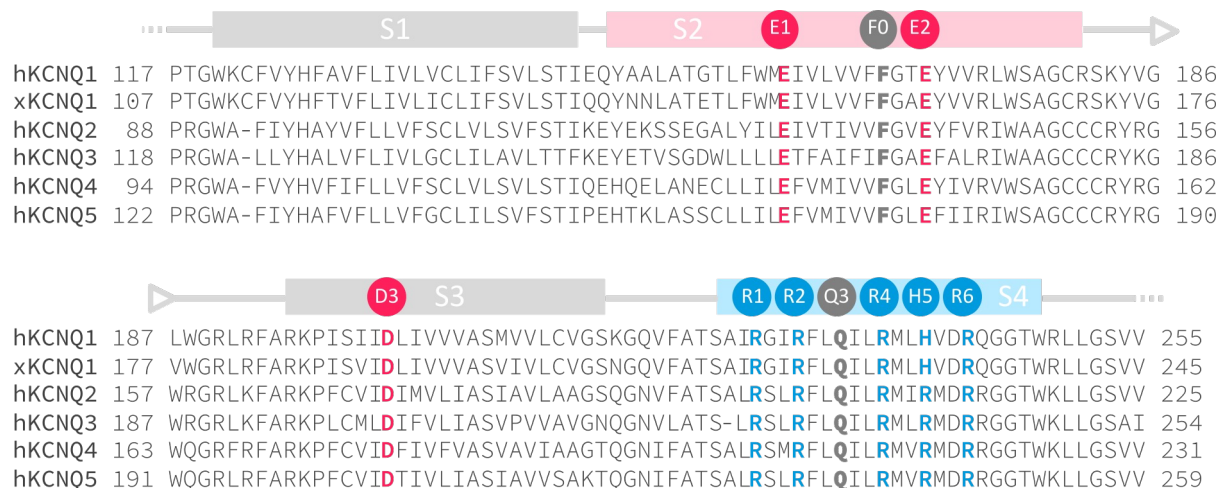
xWT-R system

Box dimensions	140 Å (x axis) * 140Å (y axis) * 107Å (z axis)
Total number of atoms	192019
Total number of water molecules	39331
Salt concentration	[K ⁺ Cl ⁻]= 0.15M
Lipid composition	100% POPC (428 molecules)

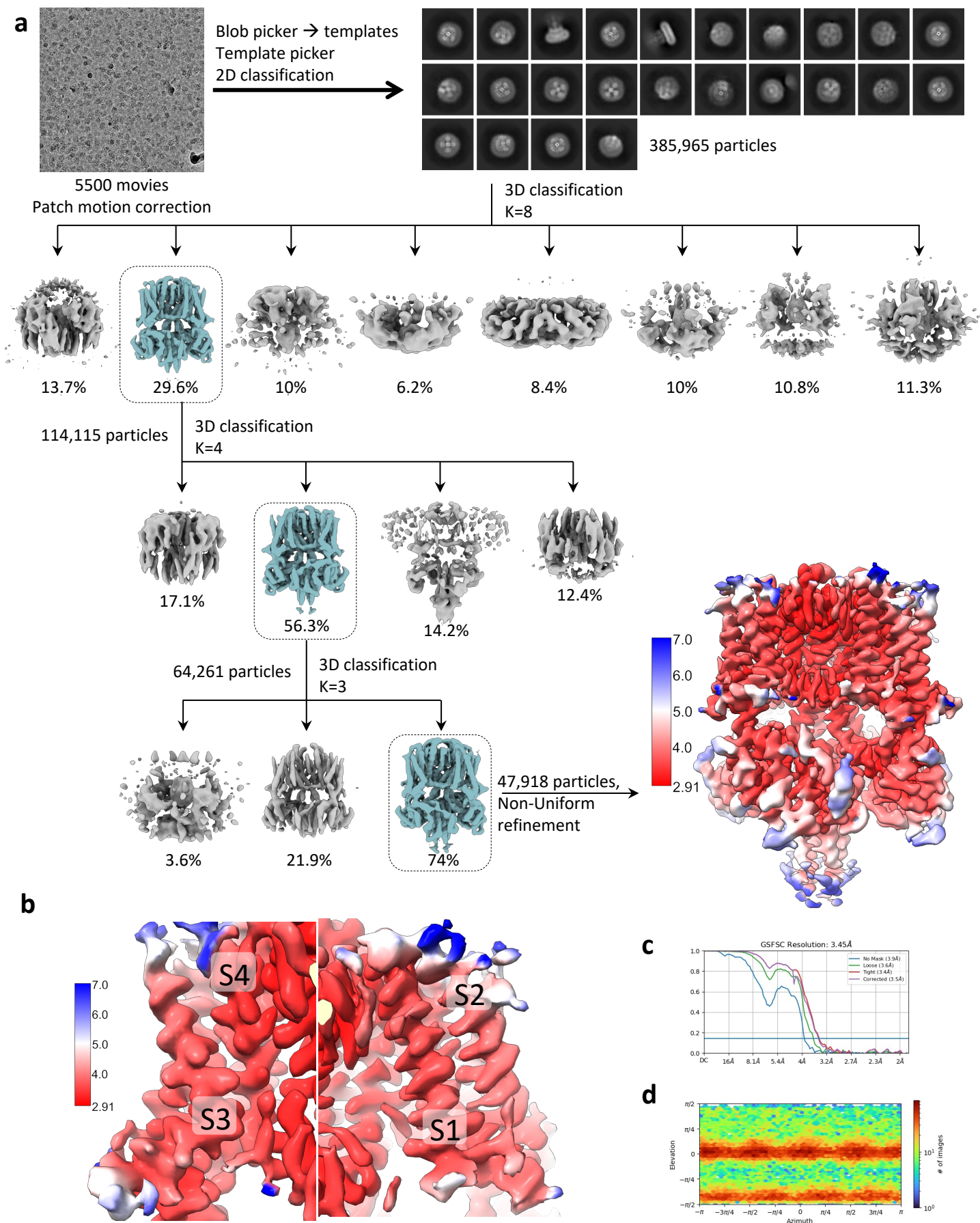
Supplementary Table 4. MD Simulation Checklist.

Reliability and reproducibility checklist for molecular dynamics simulations *All boxes must be marked YES by acceptance unless “Response not needed if No”.	Yes	No	Response (Please state where this information can be found in the text)
1. Convergence of simulations and analysis			
1a. Is an evaluation presented in the text to show that the property being measured has equilibrated in the simulations (e.g. time-course analysis)?	☒	☐	Measured properties include side-chain positions, inter-atomic distances, whose stability is shown in Fig.6 and discussed in Results section and Discussion section.
1b. Then, is it described in the text how simulations are split into equilibration and production runs and how much data were analyzed from production runs?	☒	☐	Methods section
1c. Are there at least 3 simulations per simulation condition with statistical analysis?	☒	☐	We performed three replicated trajectories per system; Methods section and Results section.
1d. Is evidence provided in the text that the simulation results presented are independent of initial configuration?	☒	☐	Methods sect. The three replicated trajectories of each system were run independently since energy minimization/ equilibration stage. By analyzing the production runs, we observed consistent behavior across the replicas in terms of key observables as RMSD over time and other structural features (backbone conformations and side-chain orientations), discussed e.g. in Results sect., Fig. 6. Discussion.
2. Connection to experiments			
2a. Are calculations provided that can connect to experiments (e.g. loss or gain in function from mutagenesis, binding assays, NMR chemical shifts, J-couplings, SAXS curves, interaction distances or FRET distances, structure factors, diffusion coefficients, bulk modulus and other mechanical properties, etc.)?	☒	☐	Structural observables (backbone and side-chain conformations, inter-atomic distances), discussed e.g. in Results sect., Fig. 6. Discussion.
3. Method choice			

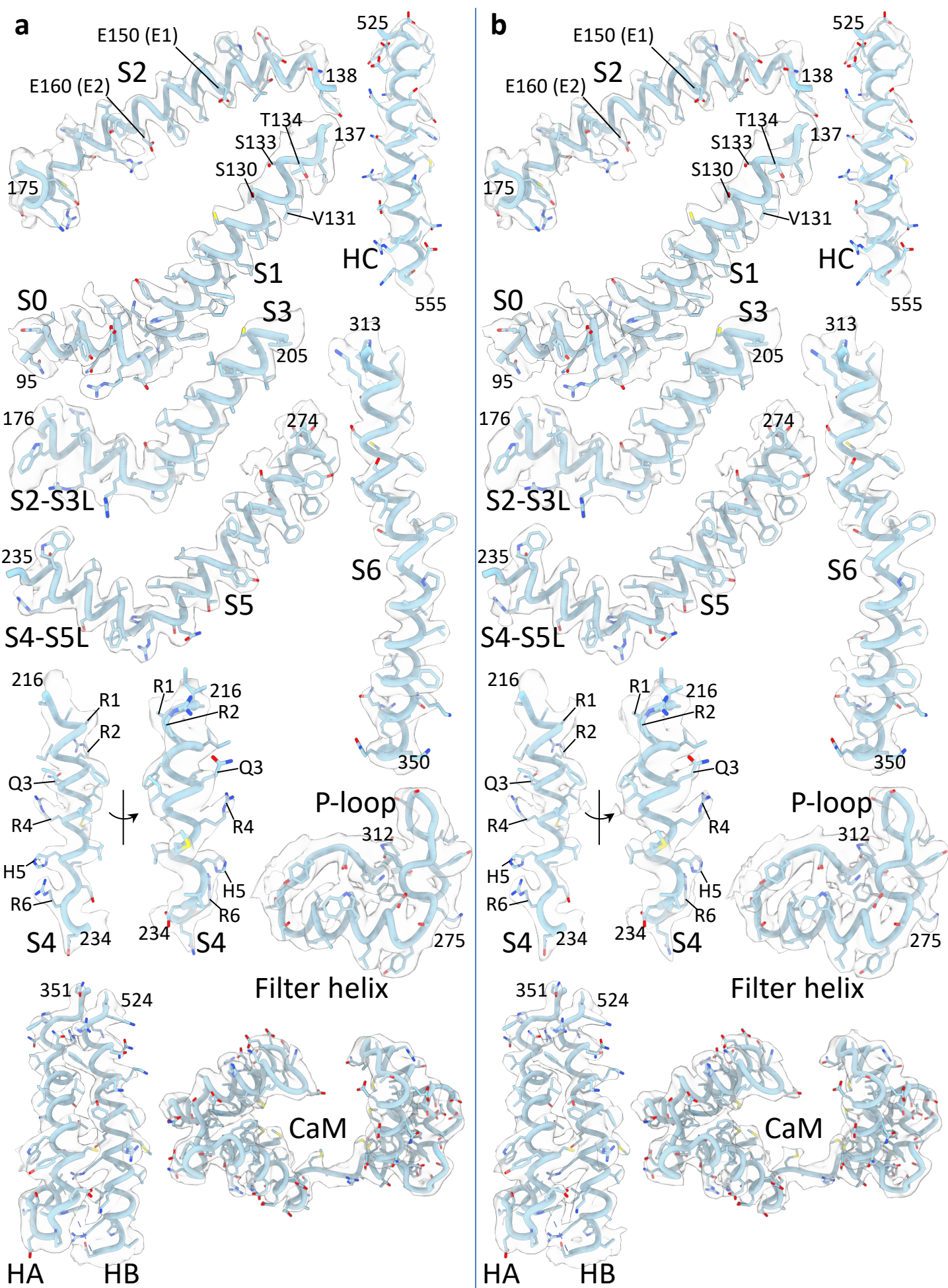
3a. Do simulations contain membranes, membrane proteins, intrinsically disordered proteins, glycans, nucleic acids, polymers, or cryptic ligand binding?	☒	☐	Simulations contain membrane and membrane proteins, Methods sect.
3b. Is it described in the text whether the accuracy of the chosen model(s) is sufficient to address the question(s) under investigation (e.g. all-atom vs. coarse-grained models, fixed charge vs. polarizable force fields, implicit vs. explicit solvent or membrane, force field and water model, etc.)?	☒	☐	We used the all-atom CHARMM force field (Methods section), with fixed charges and explicit solvent, a standard choice for validating experimentally determined structures at atomic resolution when analyzing structural observables such as backbone conformations or side-chain orientations.
3c. Is the timescale of the event(s) under investigation beyond the brute-force MD simulation timescale in this study that enhanced sampling methods are needed?	☐	☒	
If YES , are the parameters and convergence criteria for the enhanced sampling method clearly stated?	☐	☐	
If NO , is the evidence provided in the text?	☒	☐	Converged and stationary structural observables; Results sect.
4. Code and reproducibility			
4a. Is a table provided describing the system setup that includes simulation box dimensions, total number of atoms, total number of water molecules, salt concentration, lipid composition (number of molecules and type)?	☒	☐	Written in text in Methods sect.; table attached
4b. Is it described in the text what simulation and analysis software and which versions are used?	☒	☐	Methods sect.
4c. Are other parameters for the system setup described in the text, such as protonation state, type of structural restraints if applied, nonbonded cutoff, thermostat and barostat, etc.?	☒	☐	Methods sect.
4d. Are initial coordinate and simulation input files and a coordinate file of the final output provided as supplementary files or in a public repository?	☒	☐	10.5281/zenodo.16615177
4e. Is there custom code or custom force field parameters?	☐	☒	Response not needed if No
If YES , are they provided as supplementary files or in a public repository?	☐	☐	



Supplementary Fig. 1 Sequence alignment of the VSDs of hKCNQ1-5 and xKCNQ1 with highlighted gating residues.



Supplementary Fig. 2 Processing pipeline for xWT+CaM dataset. **a** Processing workflow for xKCNQ1+CaM. 3D classification refers to ab-initio reconstruction and heterogeneous refinement performed using Cryosparc. A local resolution estimation map is shown illustrating the variation in resolution across the map **b** Local resolution estimation map focused on the VSD. **c** Fourier shell correlations (FSC) curves. **d** Distribution of the particles used for non-uniform refinement.



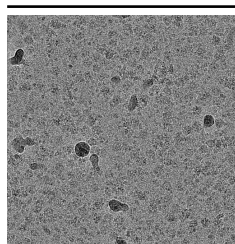
Supplementary Fig. 3 Sample maps for xWT and CaM. a CryoEM map. **b** Sharp map. The start and end point of each segment as well as residues discussed in Results are labeled.

Data set #1

Data set #2

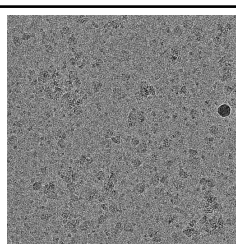
a

- Template picker
- 2D classification (2 rounds)



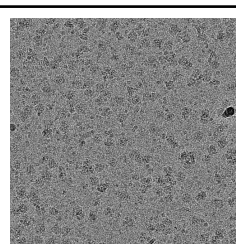
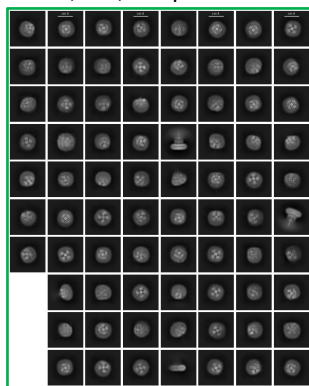
13,160 movies

3,291,943 particles



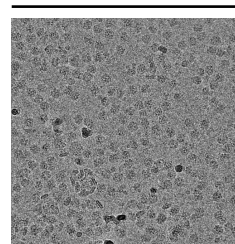
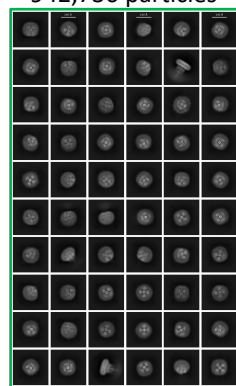
15,253 movies

3,260,457 particles



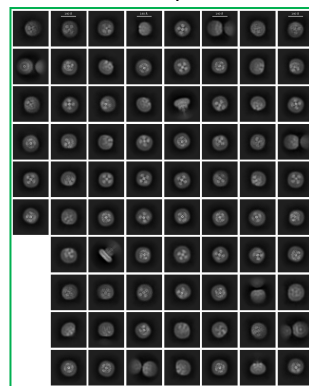
3,927 movies

942,736 particles

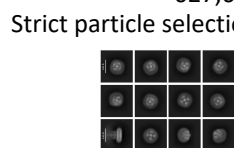


11,044 movies

2,600,208 particles



627,636 particles

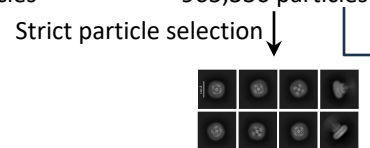
343,741 particles
ab-initio, K = 3

Maps

Heterogeneous refinement, K=3

668,710 particles

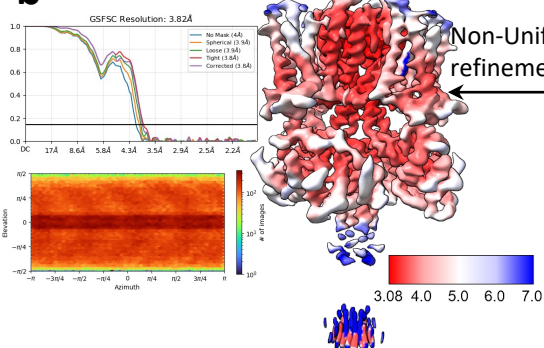
253,723 particles

144,822 particles
ab-initio, K = 3

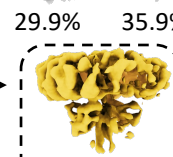
Maps

Heterogeneous refinement, K=3

b Initial map

Non-Uniform
refinement, C4

46.6%



29.9% 35.9%

34.2%

833,545 particles

Heterogeneous refinement, K=3

29.8%

23.6%

388,672 particles

3D classification, K=3

54%

26.7%

19.3%

210,150 particles

3D classification, K=3

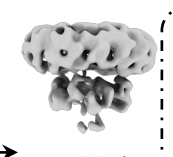
141,822 particles

3D classification, K=3

67.5%

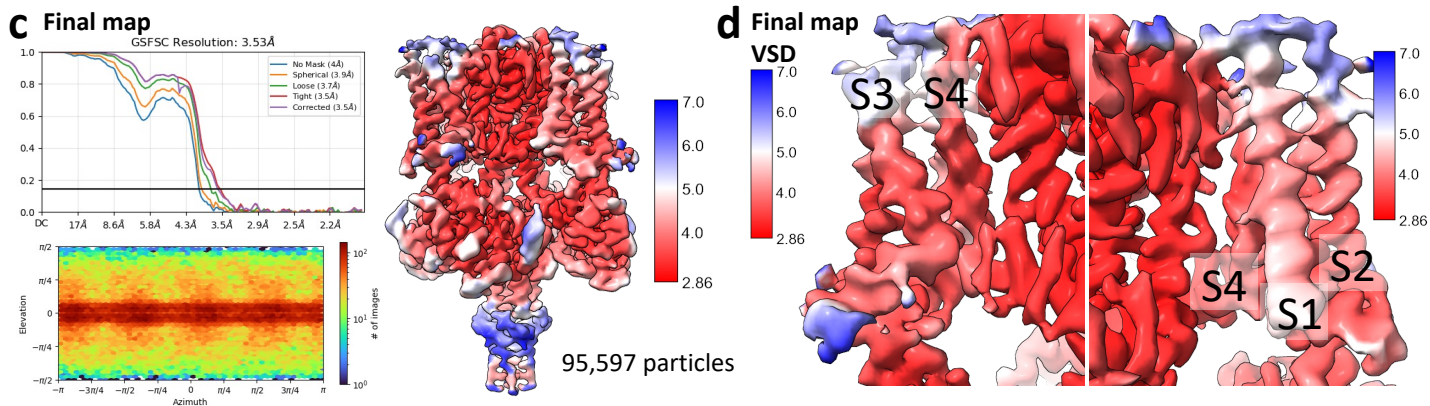
95,597 particles

Non-uniform refinement, C4

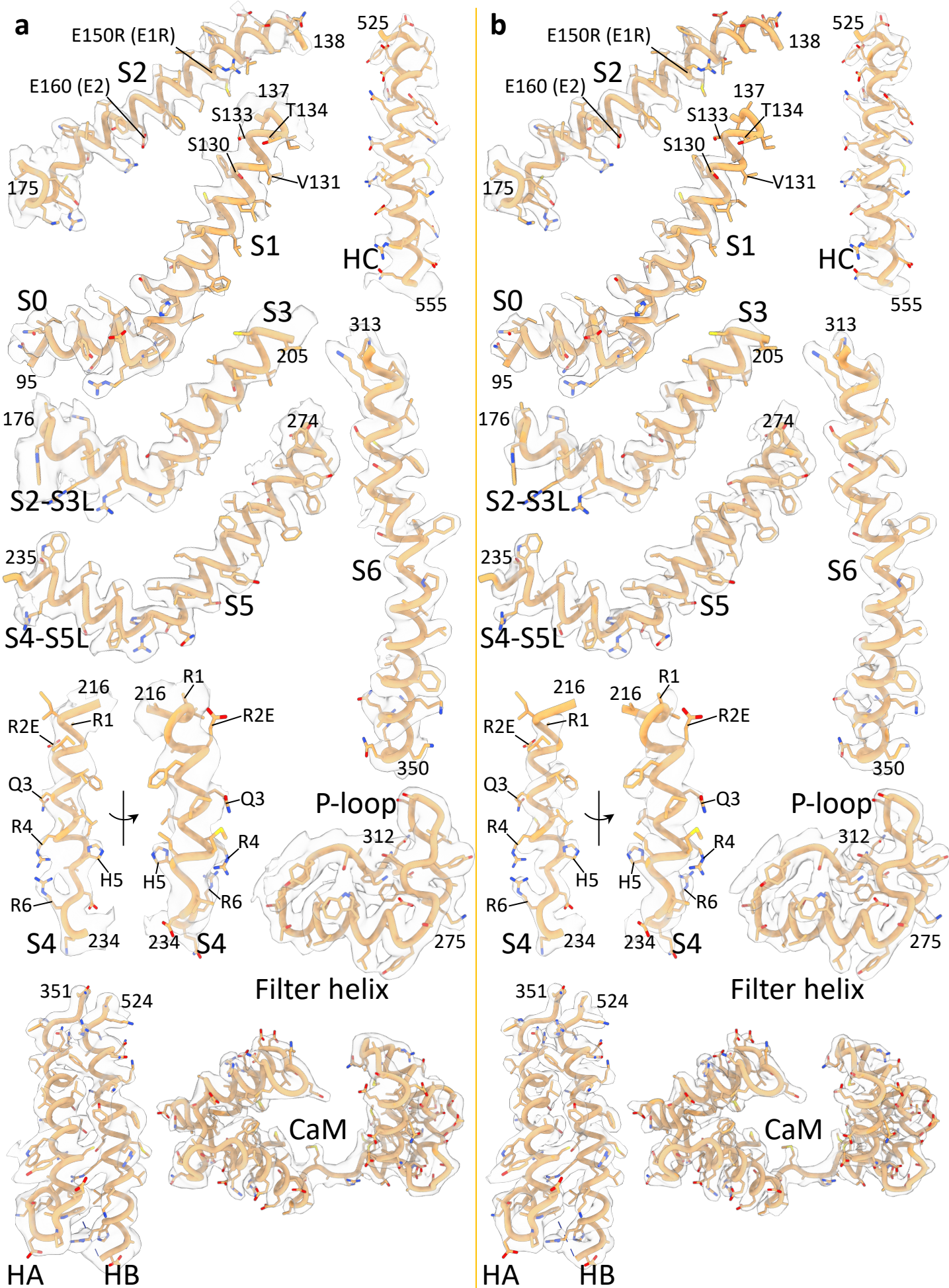


10.9% 67.4% 21.7%

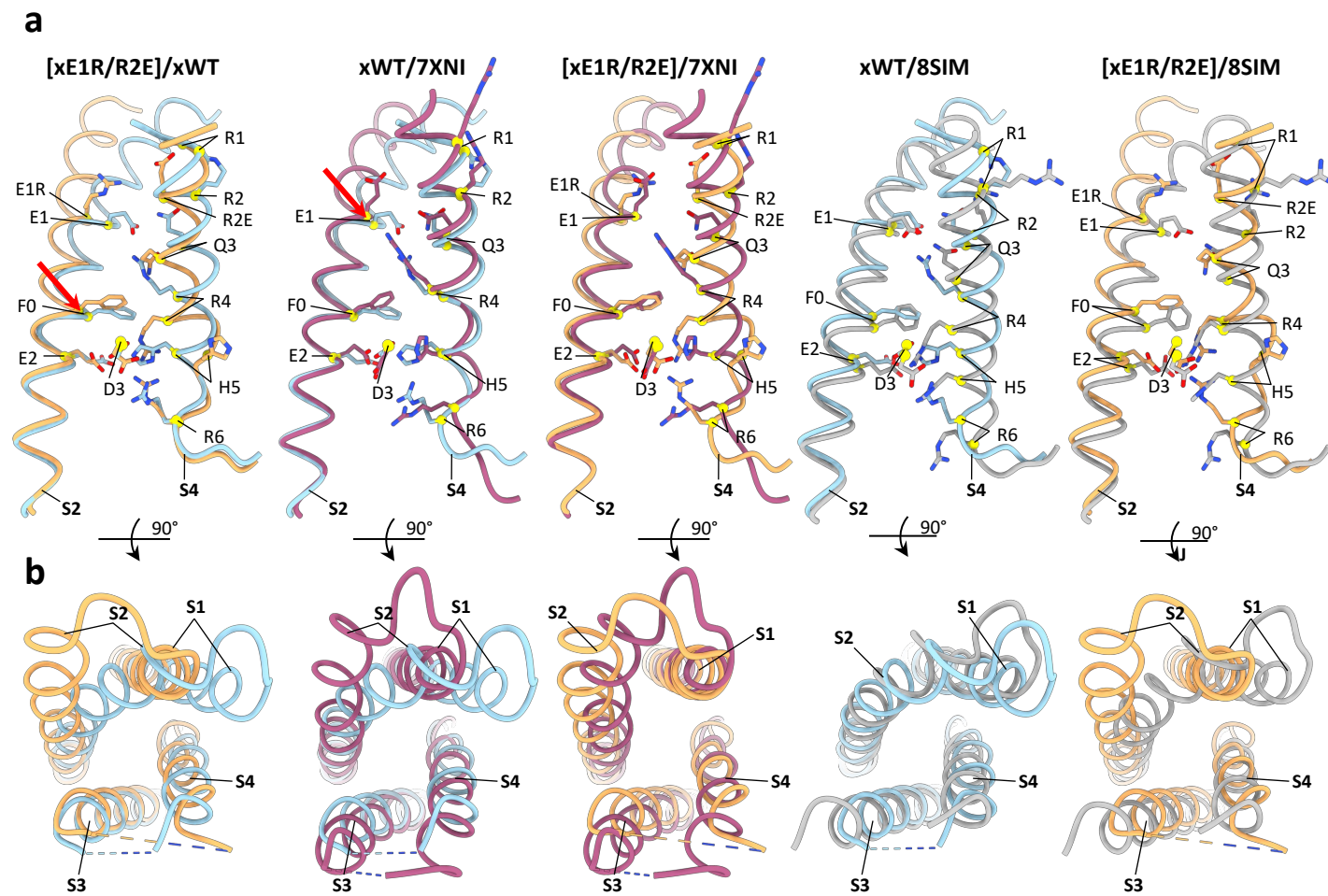
Final map (see next page)



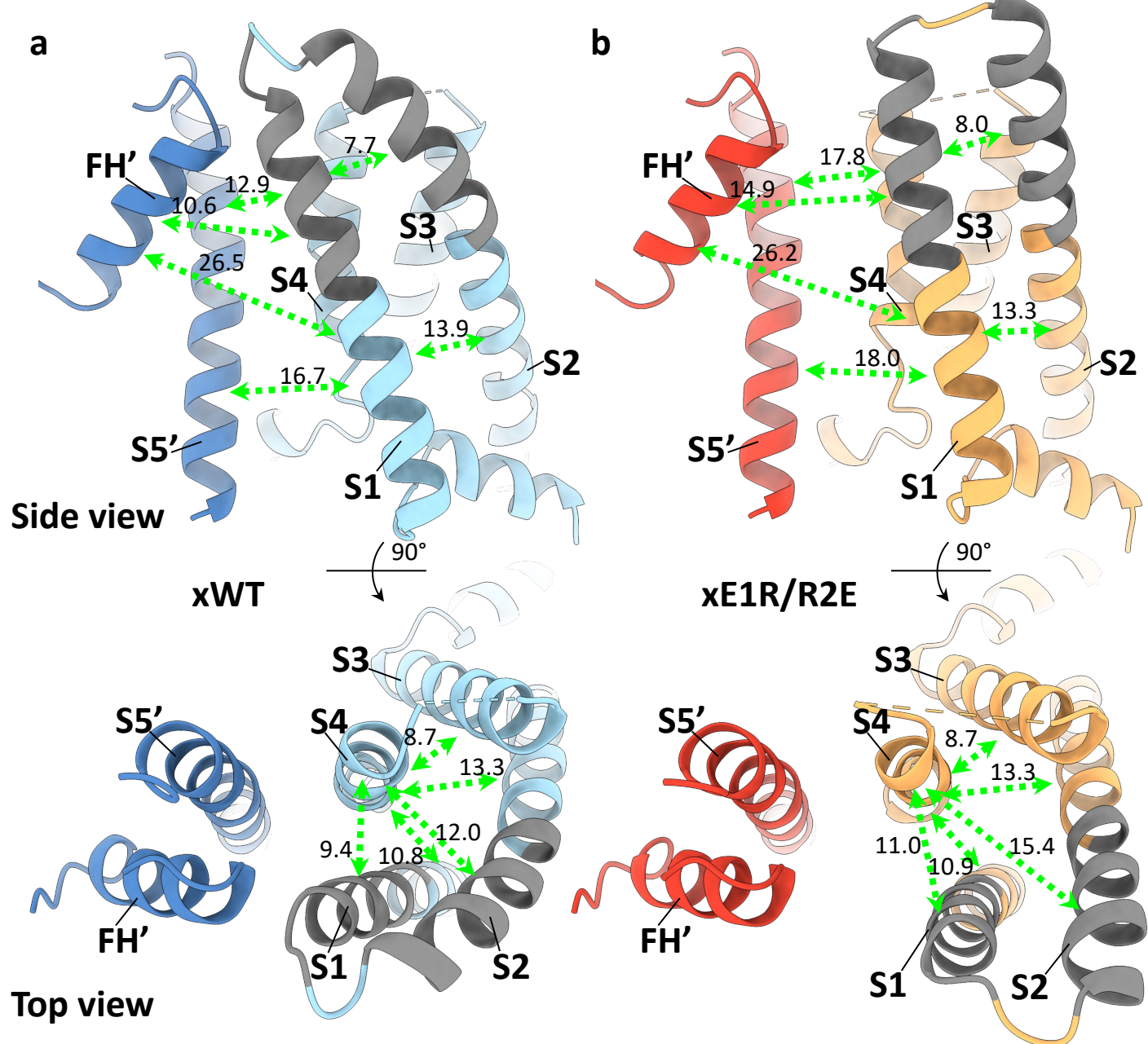
Supplementary Fig. 4 Processing pipeline for xE1R/R2E+CaM dataset. **a-c** Workflow for xE1R/R2E+CaM particles. Two rounds of 2D classification were performed for selection of high-quality particles. 3D classification refers to ab-initio reconstruction and heterogeneous refinement and was used to further sort KCNQ1 particles. **b** Local resolution estimation (LRE) map, FSC curves and particle distribution plot for an initial map that was used later for 3D variability analysis. Particles from the “purple class”, that were used to obtain the initial map, were subjected to further rounds of classification to sort KCNQ1 particles. Those steps improved the overall and local VSD resolution and led to the final map (**c**). An additional classification step beyond this point was tested but not pursued, as it resulted in only KCNQ1 classes with decreased overall and VSD resolution. **c** FSC curves, particle distribution plot, and LRE map for the final map. **d** VSD LRE final map. The final map was used for the generation of the density-modified map.



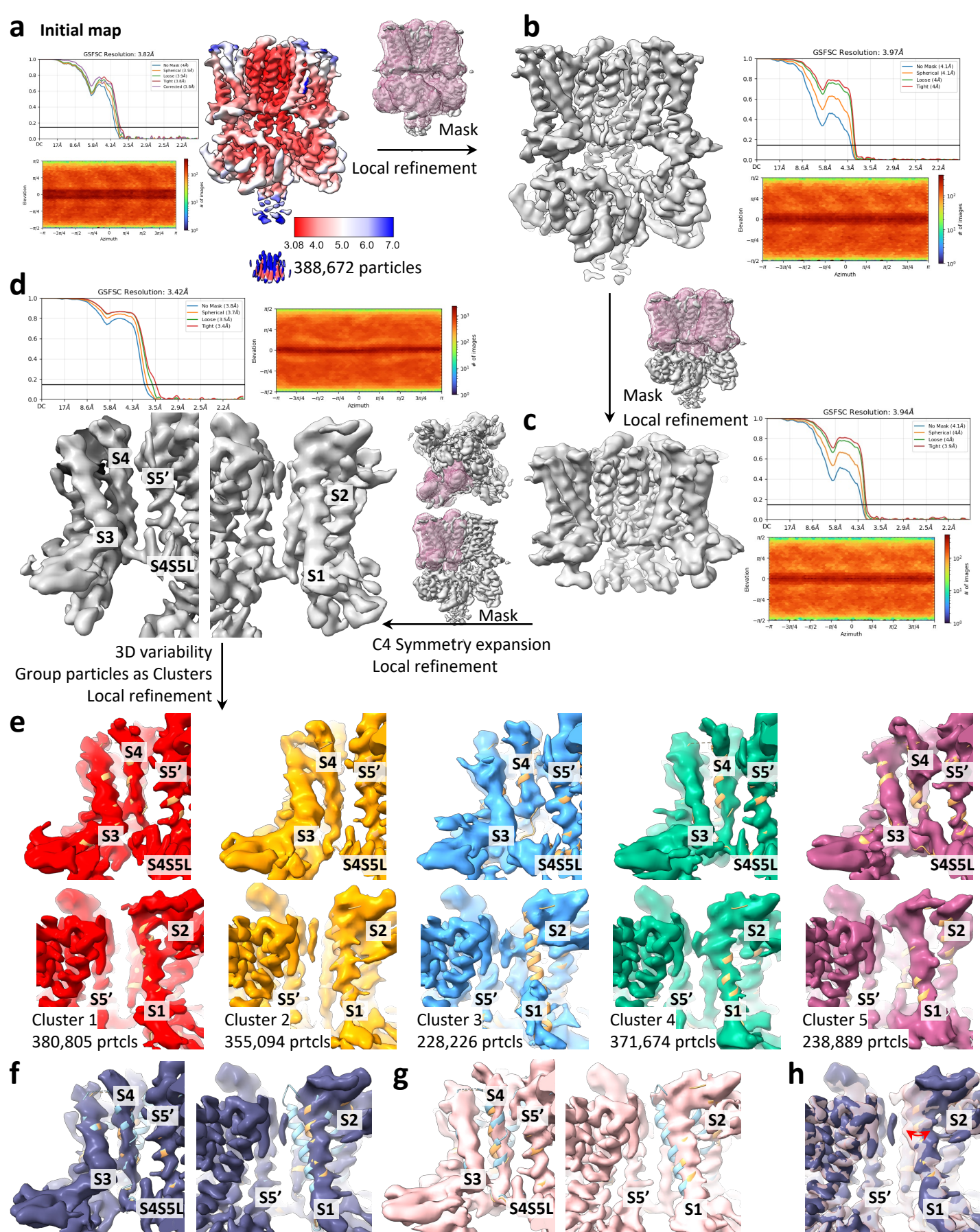
Supplementary Fig. 5 Sample maps for xE1R/R2E and CaM. a CryoEM map. **b** Density modified map. The start and end point of each segment as well as residues discussed in Results are labeled.



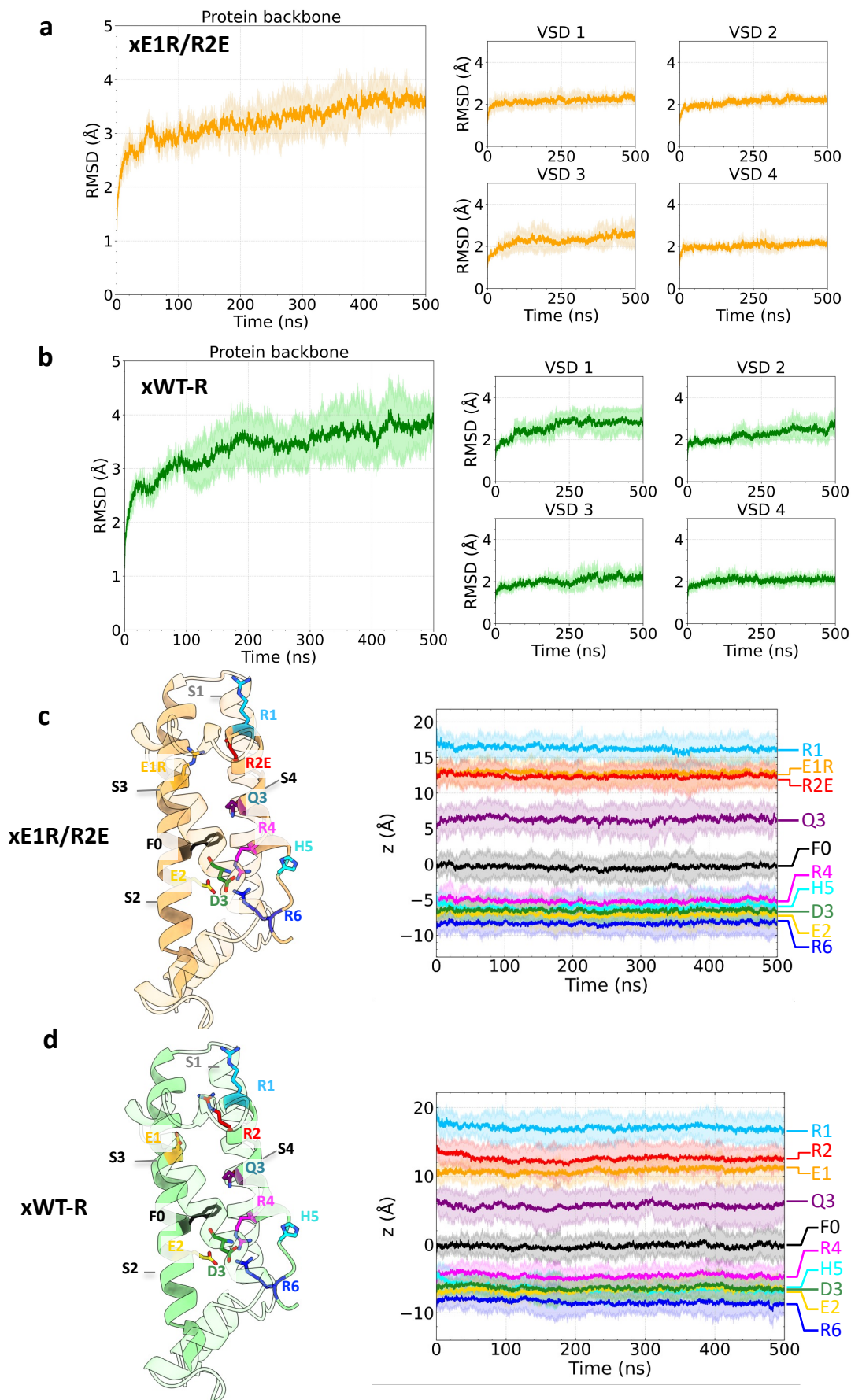
Supplementary Fig. 6 Superposition of the VSD of [xE1R/R2E]/xWT, xWT/7XNI, [xE1R/R2E]/7XNI, xWT/8SIM, and [xE1R/R2E]/8SIM. **a** Comparison of CTC architecture of various reported KCNQ1 structures. xE1R/R2E is shown in orange, xWT in blue (both from this paper), 7XNI⁵ in purple, and 8SIM⁶ in gray. The important CTC residues in (*Xenopus*; xE150 (E1), xE160 (E2), xF157 (F0), and xD192 (D3), and Human; hE160, hE170, hF167, hD202) and the relative position of the S4 residues xR218 (R1), xR221E (R2), xQ227 (Q3), xH230 (H5), and xR233 (R6) (human equivalent; R228, R231, Q237, H240, and R243) are shown as sticks. α atoms are highlighted as yellow spheres. Helices S1 and S3 were removed for clarity. D3 from the S3 helices is shown in sticks. 7XNI tilts somewhat toward the S1-S2 conformation seen in our xE1R/R2E models. The red arrows in panel (**a**) highlight the point of tilt in xE1R/R2E and 7XNI. **b** Representations viewed from above reveal similarities in VSD positioning between xE1R/R2E and 7XNI. As in panel (**a**), xE1R/R2E is shown in orange, xWT in blue, 7XNI in purple and 8SIM in gray.



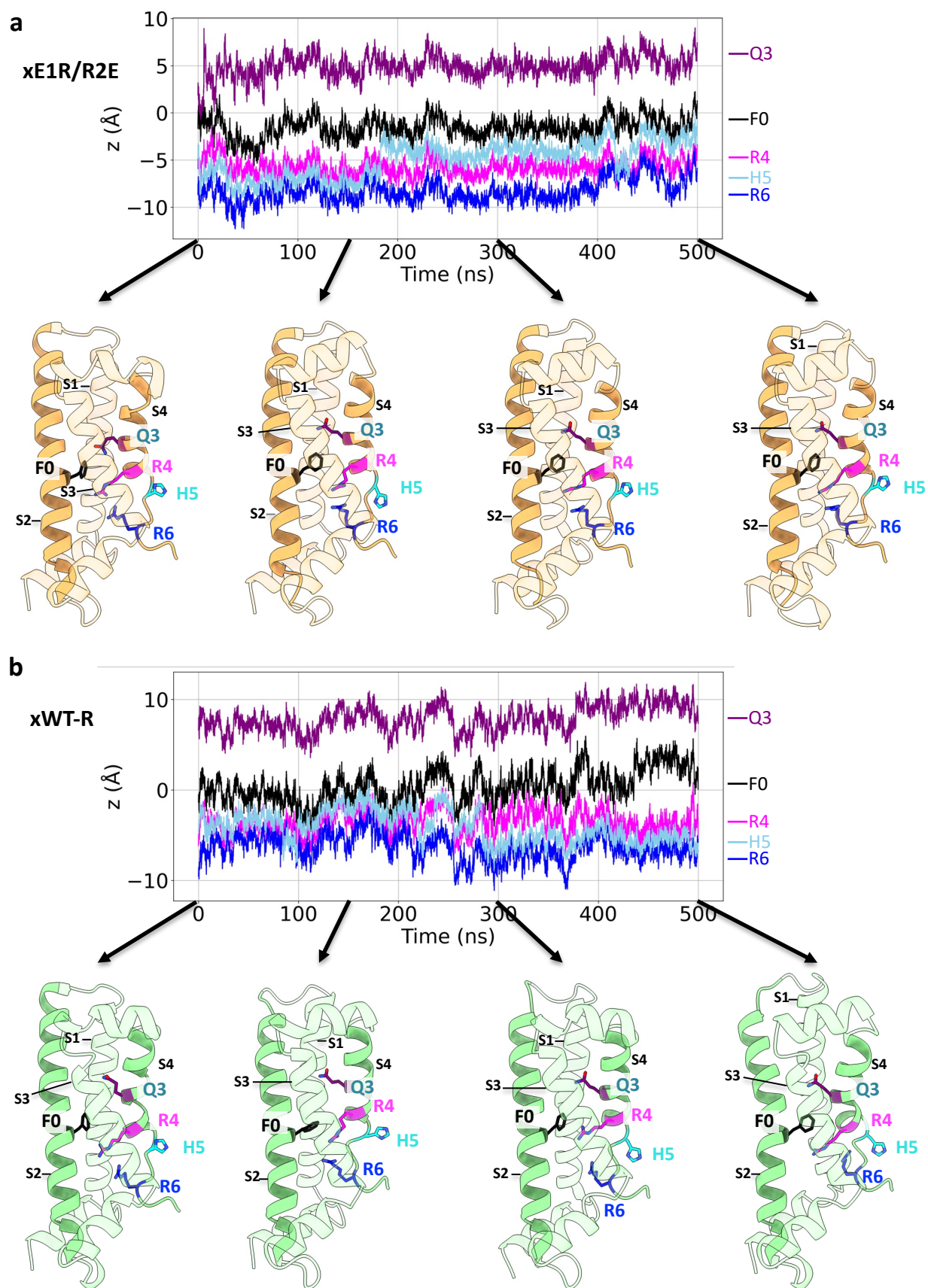
Supplementary Fig. 7 Shortest average distances between the S1-S4 helices and the PD. xWT **a** is colored blue, and xE1R/R2E **b** is colored orange with the upper parts of the S1 and S2 colored gray. The adjacent subunits are shown in a darker shade. Side and top views are shown. Dashed arrows show where measurements were made of the average shortest C α distances between the helices. The distances are in Angstroms. The upper S1 and S2 helices are further from the S4 and the pore domain in the xE1R/R2E structure.



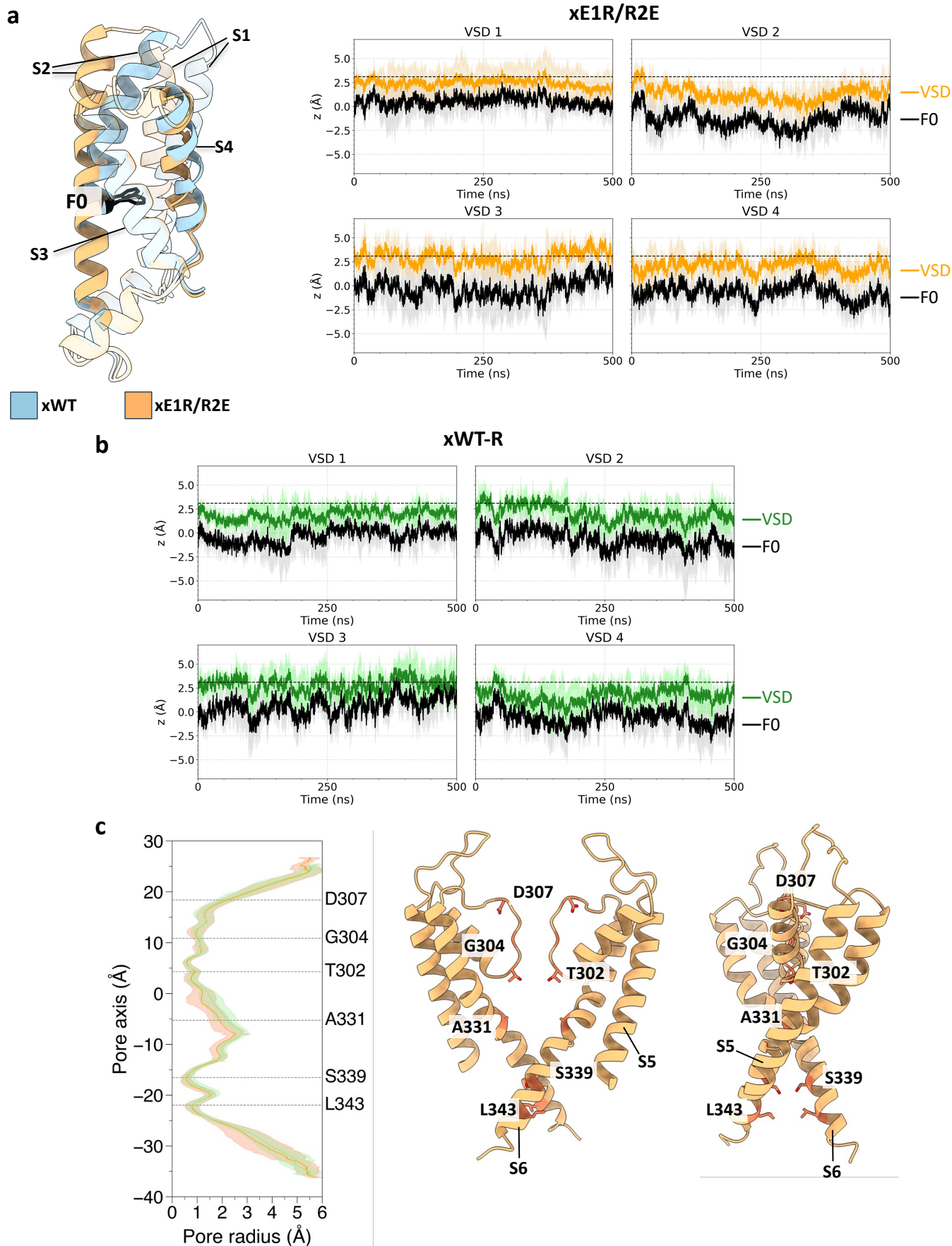
Supplementary Fig. 8 3D variability analysis for xE1R/R2E. **a** Map and particles used. **b** Local refinement focused on the tetramer. **c** Local refinement focused on the tetramer TM domain. **d** Local refinement focused on the protomer TM domain after C4 symmetry expansion. **e** 3D variability analysis results. Particles were grouped in 5 clusters followed by local refinement. The xE1R/R2E structure (orange) is docked in the maps for reference. **f** Local refinement after combining clusters 1 and 2. xWT is shown in blue for comparison. **g** Local refinement after combining clusters 4 and 5. xWT is shown in blue for comparison. **h** Alignment of (f) and (g) maps showing variability in upper S1, which accounts for lower resolution.



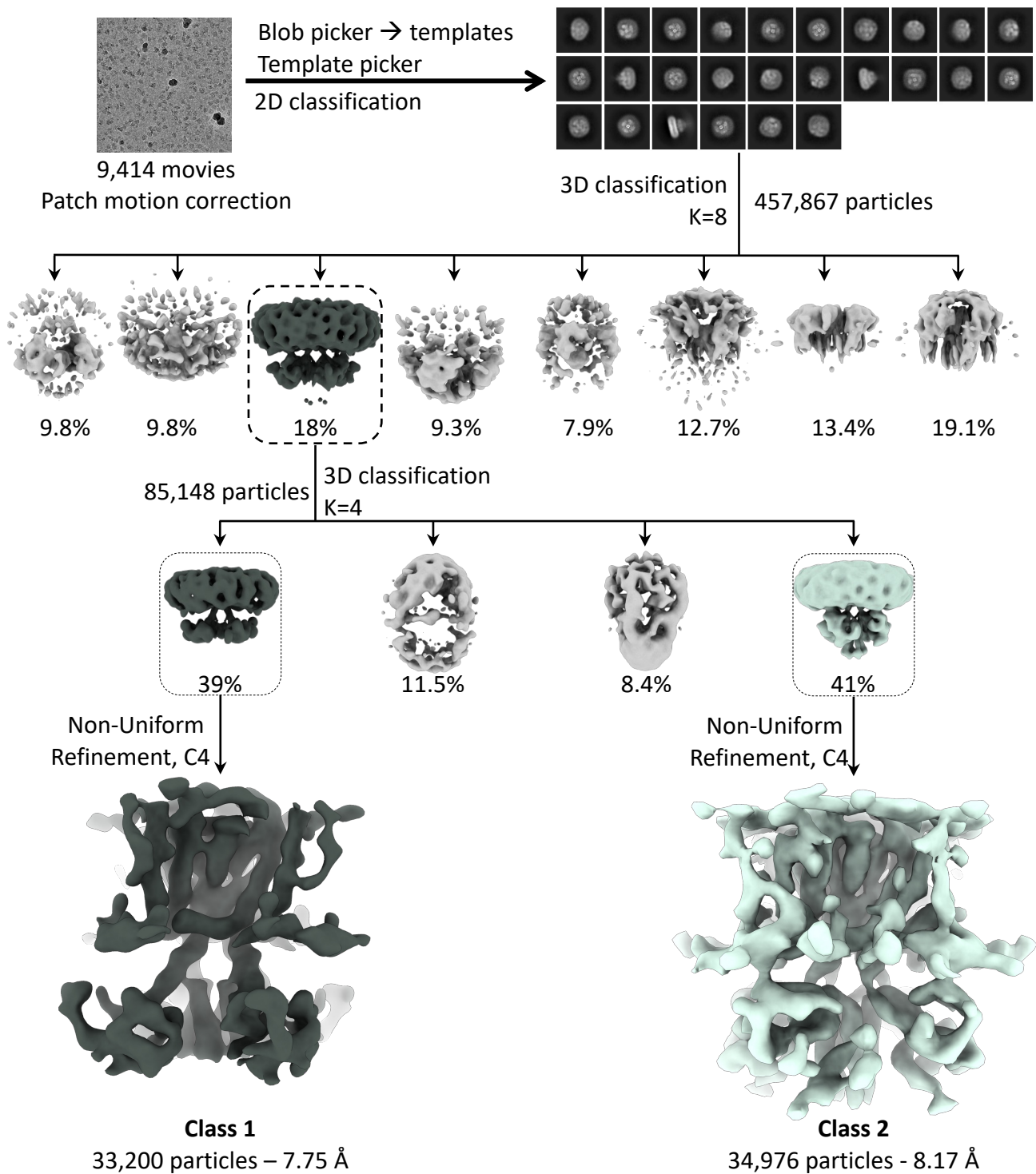
Supplementary Fig. 9 MD simulations of xE1R/R2E and xWT-R conformations. Time evolution of backbone atom RMSDs for the entire TM domain and each of the four VSDs separately, averaged over the three simulated replicates for **a** xE1R/R2E and **b** xWT-R. Time evolution of the z-coordinates of selected S4 side chains for **c** xE1R/R2E and **d** xWT-R. F0 (F157) is set as zero.



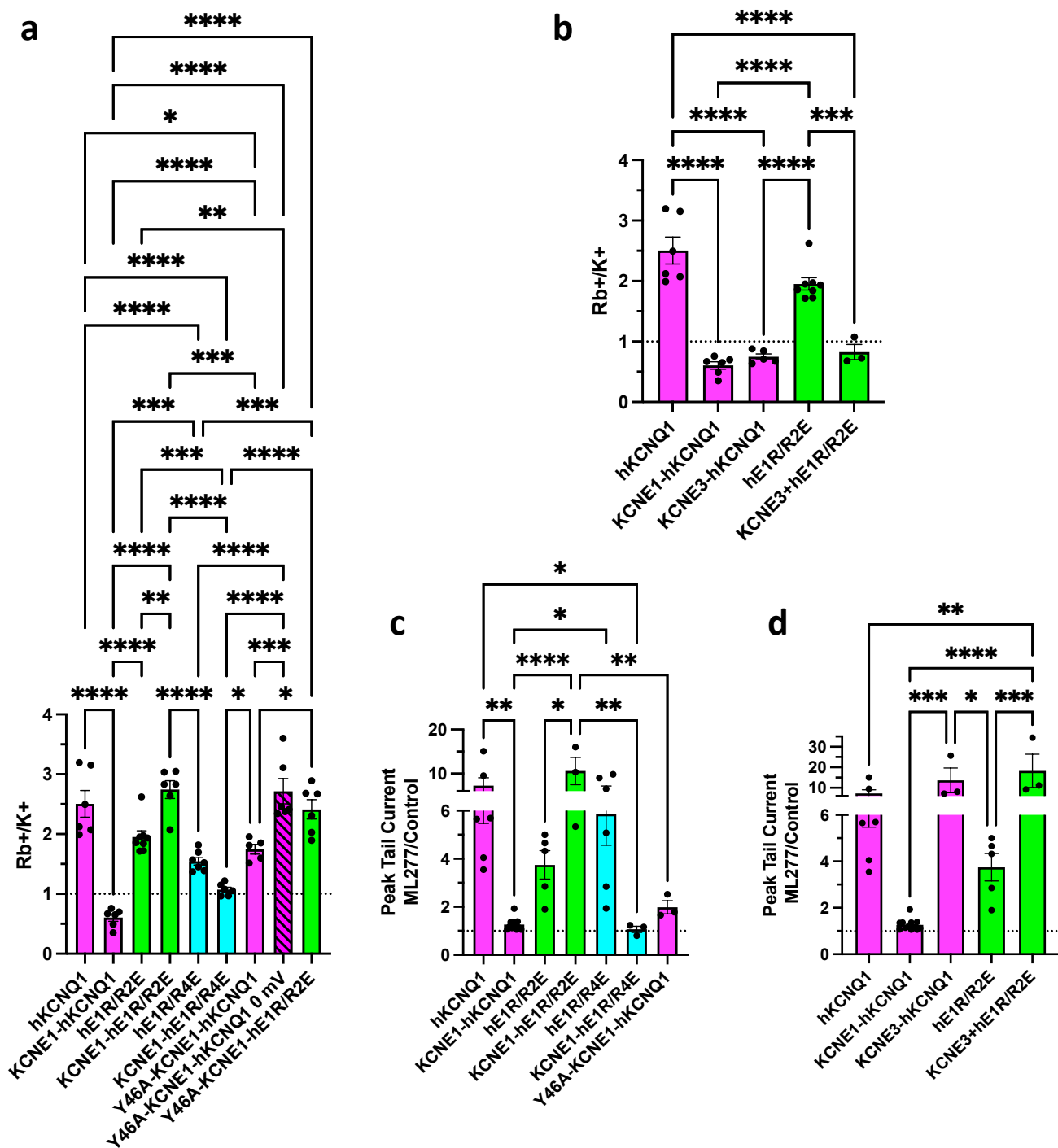
Supplementary Fig. 10 Selected side-chain conformations. Time evolution of the z-coordinates of VSD side chains with F0 (F157) set as zero, in a single simulated replicate for xE1R/R2E **a** and xWT-R **b**. Snapshots of VSD structures at selected time-frames are shown below each time evolution.



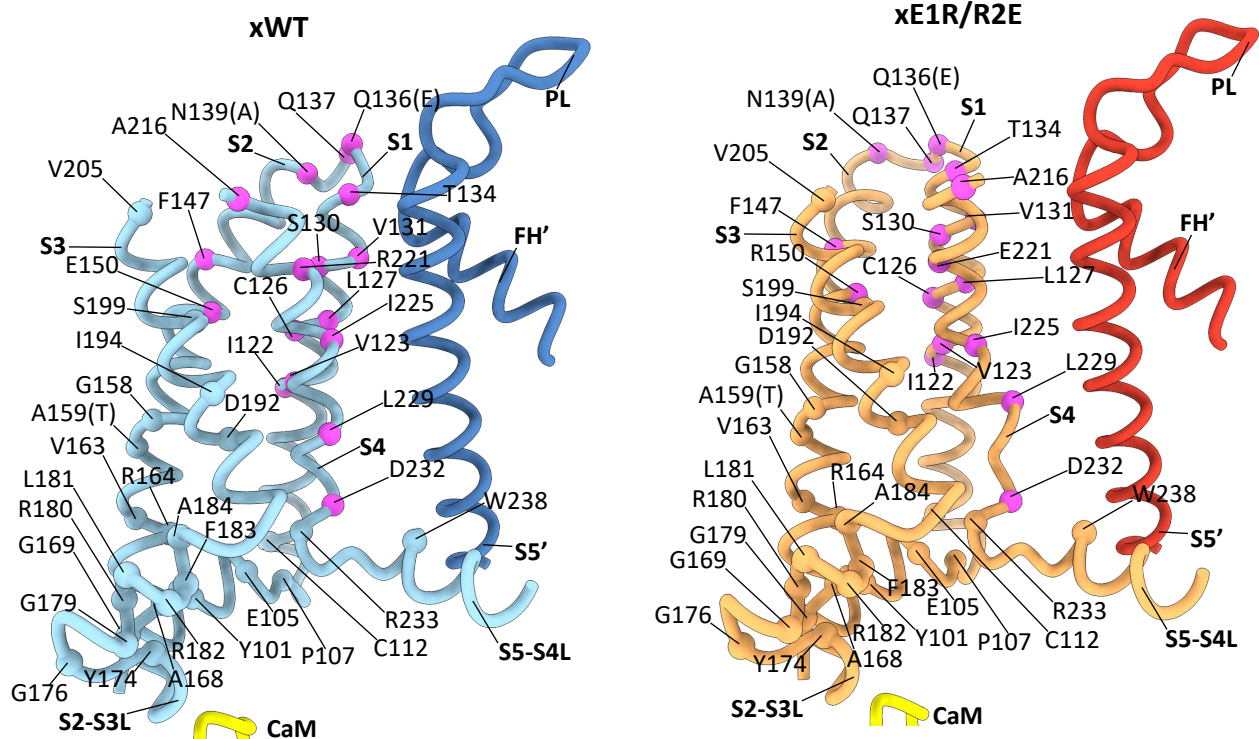
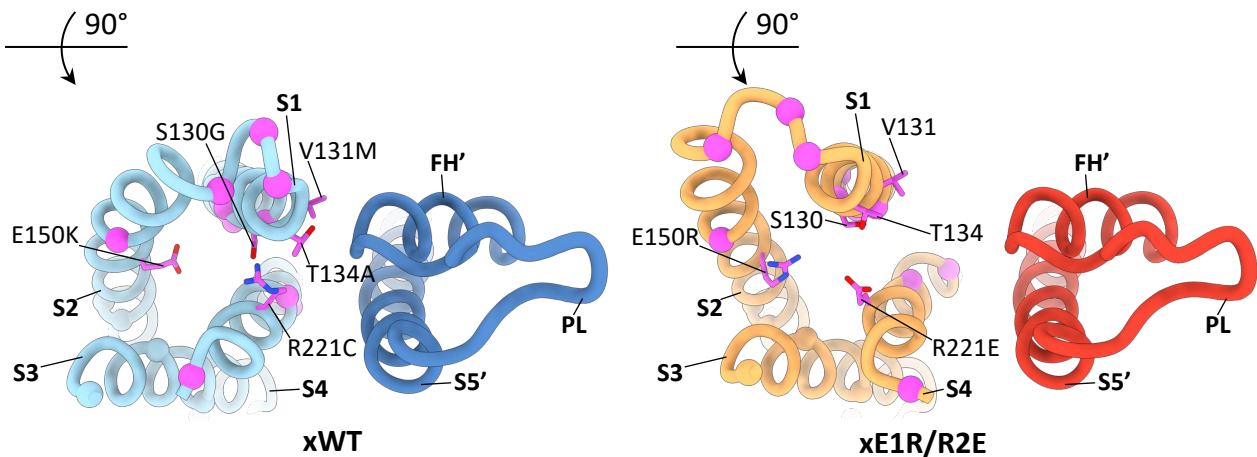
Supplementary Fig. 11 S4 position. Time evolution of S4 center of mass z-coordinate (orange) and F0 sidechain (black) in each of the four VSDs, averaged over the three simulated replicas for **a** E1R/R2E and **b** xWT-R. The black dashed line is the coordinate value in the xE1R/R2E cryoEM structure. **c** Pore profiles of xE1R/R2E (orange) and xWT-R (green), averaged over simulated replicas with dashed lines indicating the positions of pore-lining residues, which are shown as red sticks in the structure.



Supplementary Fig. 12 xE1R/R2E-PIP2 dataset processing workflow. 3D classification refers to ab-initio and heterogeneous refinement performed using Cryosparc. Two different KCNQ1 classes were observed. Class 1 can fit an open PIP2-bound channel and Class 2 can fit an open-gate channel with CaM attached to the VSD (see Fig. 7).



Supplementary Fig. 13 Bar charts from Fig. 9 and Fig. 10 with statistical comparison included. **a-b** Rb^+/K^+ permeability ratio measured from -60 mV tails after a 5 s pulse to +60 mV (or 0 mV, crosshatch bar). **c-d** Tail current response to 1 μ M ML277. Cells were pulsed to +60 mV for 4 s and then to -40 mV for 0.9 s. The holding potential was -90 mV. Data were compared using a one-way Anova with Tukey's multiple comparisons test in GraphPad Prism 10 for Mac. * <0.05, ** <0.01, *** <0.001, **** <0.0001.

a**b**

Supplementary Fig. 14 Altered interactions for missense KCNQ1 mutations in the xE1R/R2E VSD compared to xWT. **a** Side view of the VSD with C α atoms of residues with reported missense mutations associated with LQTS, SQTS and AF highlighted by spheres. The numbering is based on the xKCNQ1 sequence and were identified from the sequence alignment in Sun and MacKinnon, 2017⁷. When different, human residues are shown after in parenthesis. The xE1R/R2E mutant S1-S2 VSD helices are displaced from the FH'; mutations whose position changes in the xE1R/R2E structure in comparison to the xWT are indicated in magenta. The S5, FH and the P-loop from the adjacent protomer are shown in a darker shade and the part of CaM that interacts with the S2-S3L is colored yellow. **b** Top view representation of the interactions of the residues that seem to be important in stabilizing the S1-FH' attached conformation in the xWT structure. Those residues, shown as magenta sticks, when mutated can lead to arrhythmia. Helices are shown in tube form to allow clear identification of mutation locations.

References

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- 6 Mandala, V. S. & MacKinnon, R. The membrane electric field regulates the PIP(2)-binding site to gate the KCNQ1 channel. *Proc Natl Acad Sci U S A* **120**, e2301985120 (2023). <https://doi.org:10.1073/pnas.2301985120>
- 7 Sun, J. & MacKinnon, R. Cryo-EM Structure of a KCNQ1/CaM Complex Reveals Insights into Congenital Long QT Syndrome. *Cell* **169**, 1042-1050 e1049 (2017). <https://doi.org:10.1016/j.cell.2017.05.019>