Supplementary Material

Ion conduction mechanism as a fingerprint of potassium channels

Carmen Domene[1,2], Riccardo Ocello[3], Matteo Masetti[3], Simone Furini[4]

[1]Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK
[2]Department of Chemistry, University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK
[3]Department of Pharmacy and Biotechnology, Alma Mater Studiorum – Università di Bologna, via Belmeloro 6, 40126 Bologna, Italy
[4]Department of Medical Biotechnologies, University of Siena, Siena, Italy

System	Membrane Potential [mV]	Initial SF Configuration	Replicas	Simulation Time [ns]	Conduction Events [#]
KcsA-WT	+100	with water	#1	1000	0
			#2	1000	0
			#3	1000	0
			#4	1000	0
	+100	without water	#1	1000	0
			#2	1000	1
			#3	1000	1
			#4	1000	2
	+200	with water	#1	1000	0
			#2	1000	0
			#3	1000	3*
			#4	1000	0
	+200	without water	#1	3900	43
			#2	4000	45
			#3	4000	38
			#4	3800	38
			#5	4000	35
			#6	4000	45
			#7	4000	32

Table S1. Details of the Molecular Dynamics simulations for channel model KcsA-WT. Conduction events marked with * are those sampled after the exit of water molecules from binding sites S3 and S2 of the selectivity filter.

System	Membrane Potential [mV]	Initial SF configuration	Replicas	Simulation Time [ns]	Conduction Events [#]
KcsA-E71A	+100	with water	#1	1000	3
			#2	1000	1
			#3	1000	6
			#4	1000	1
	+200	with water	#1	3900 (3700)	47
			#2	3900 (3800)	41
			#3	3900 (3800)	40
			#4	3900 (3600)	38
			#5	3900 (3700)	52
			#6	3700 (3600)	38
			#7	3700 (3600)	42
			#8	3900 (3800)	36

Table S2. Details of the Molecular Dynamics simulations for the channel model KcsA-E71A. All the conduction events were sampled only after the exit of water molecules from binding sites S3 and S2 of the selectivity filter. In all the simulated trajectories, water initially inside the selectivity filter left binding sites S3 and S2 in less than 300 ns. The sections of of trajectories after water left binding sites S3 and S2 were used for the analyses of conduction events. The length of the trajectory used for the analyses in each case is shown inside brackets.

System	Membrane Potential [mV]	Initial SF Configuration	Replicas	Simulation Time [ns]	Conduction Events [#]
TRAAK	+100	with water	#1	1000	1*
			#2	1000	0
			#2	1000	1*
	+100	without water	#1	1000	3
			#2	1000	5
			#3	1000	3
	+200	with water	#1	1000	0
			#2	1000	0
			#3	1000	0
	+200	without water	#1	1000	4
			#2	1000	8
			#3	1000	5
			#4	1000	4
			#5	1000	3
			#6	1300	10
			#7	1300	9
			#8	1300	5
			#9	1100	8
			#10	1300	9
			#11	1300	10
			#12	1300	4

Table S3. Details of the Molecular Dynamics simulations for the channel model TRAAK. Conduction events marked with * are those sampled after the exit of water molecules from binding sites S3 and S2 of the selectivity filter.

	KcsA-WT	KcsA-E71A	TRAAK
[C, S3, S2]	52±1 %	25±2 %	3.7±0.7 %
[S3, S2]	14±1 %	5.2±0.3 %	9±2 %
[C, S3, S2, S0]	10±1 %	32±1 %	5.9±0.8 %
[S3, S2, S0]	7.6±0.3 %	34±1 %	40±2 %
[S4, S2, S1]	8.0±1.0 %	1.2±0.1 %	27±4 %
[S4, S3, S2]	2.4±0.1 %	0.5±0.1 %	0.3±0.1 %
[S4, S3, S1]	2.3±0.1 %	1.0±0.1 %	5.9±0.7 %
[C, S3, S1]	2.3±0.1 %	0.8±0.1 %	1.0±0.2 %
[S4, S2, S0]	0 %	0 %	5.7±1.2 %

Table S4. Probabilities of the different ion occupancy states of the selectivity filter. For each channel model, 10 MSMs were estimated taking random subsets that included only half of the simulated trajectories. Average values and standard deviations are computed considering the occupancies estimated from these 10 MSMs.

	KcsA-WT	KcsA-E71A	TRAAK
[S3, S2, S0] → [C, S3, S2, S0]	0.90±0.10	0.94±0.06	0.85±0.07
[S3, S2, S0] → [S3, S2]	0.90±0.10	0.52±0.08	0.37±0.09
[S3, S2] → [C, S3, S2]	0.90±0.10	0.60±0.09	0.30±0.07
[C, S3, S2] → [S3, S2, S0]	0.86±0.07	0.98±0.03	0.07±0.05
[S4, S2, S1] → [S3, S2, S0]	0.71±0.08	0.31±0.04	0.99±0.01
[C, S3, S2, S0] → [C, S3, S2]	0.64±0.09	0.73±0.07	<0.01
[C, S3, S2] → [S4, S2, S1]	0.63±0.09	0.26±0.04	0.28+0.06
[C, S3, S2, S0] → [S3, S2]	0.28±0.05	0.17±0.04	<0.01
[S4, S3, S1] → [S3, S2, S0]	0.11±0.01	0.12±0.01	0.18+0.02
[S3, S2] → [C, S3, S1]	0.11±0.05	0.03±0.01	0.05±0.03
[S4, S3, S2] →[S3, S2, S0]	0.09±0.01	0.03±0.01	0.01±0.01
[C, S3, S2] →[S4, S3, S1]	0.09±0.03	0.09±0.02	0.07±0.03
[S3, S2] →[S4, S2, S1]	0.07±0.04	0.04±0.01	0.02±0.02
[C, S3, S1] →[C, S3, S2]	0.07±0.05	0.03±0.01	0.08±0.03
[S3, S2] →[S4, S3, S2]	0.05±0.01	0.01±0.01	0.01±0.01
[C, S3, S2, S0] →[S4, S3, S1]	0.01±0.01	0.02±0.01	0.15±0.2
[S4, S2, S0] →[S3, S2, S0]	<0.01	<0.01	0.06±0.03
[S4, S2, S1] →[S4, S2, S0]	<0.01	<0.01	0.12±0.02
[C, S3, S2, S0] →[S4, S2, S1]	<0.01	<0.01	0.68±0.05
[S4, S3, S1] → [S4, S2, S1]	0.02±0.02	<0.01	0.05±0.02

Table S5. Normalized net fluxes between pairs of ion occupancy states of the selectivity filter. For each channel model, 10 MSMs were estimated taking random subsets that included only half of the simulated trajectories. The new fluxes were normalized to the maximum net flux in the corresponding MSM. Average values and standard deviations are computed considering the normalized net fluxes estimated from these 10 MSMs.

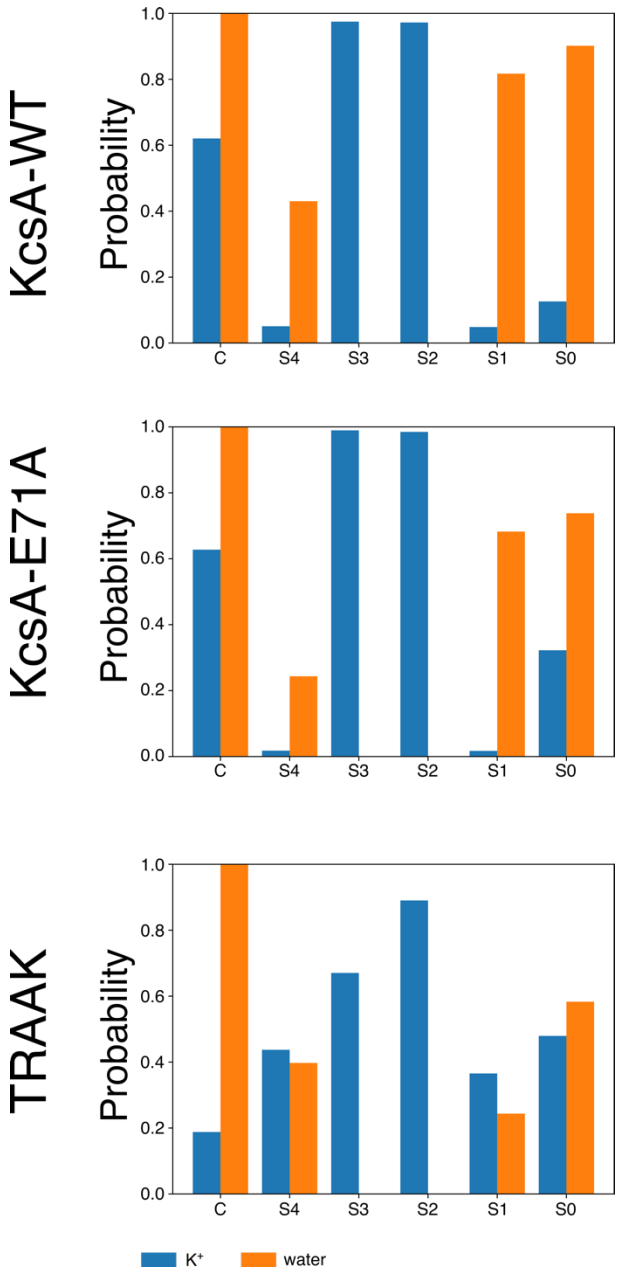

Figure S1. Potassium ions and water molecules inside the selectivity filter. Ions and water molecules were considered in a binding site when they occupy the space in-between the two layers of oxygens atoms delimiting the corresponding binding site. Binding sites S3 and S2 are vacant if they are not occupied by K^+; regardless of the channel model and membrane potential, water never occupied S2 or S3. The intracellular cavity, C, is always filled with several water molecules, and consequently the water probability is identical to 1. Data from simulations with membrane potentials equal to +100 mV and initialized without water molecules inside the SF (TRAAK and KcsA-WT), or after the exit of water molecules from the SF (KcsA-E71A) were used to calculate ion/water occupancy probabilities.

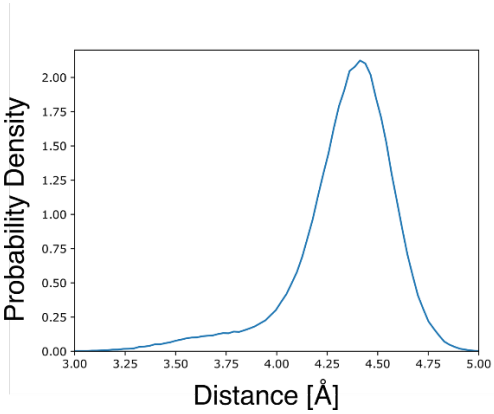

Figure S2. Distance between E71 and Y78 in MD simulations of KcsA-WT with +200 mV membrane potential. The distance was measured between the center of mass of the side chain oxygen atoms of E71 and the backbone nitrogen atom of Y78. Data from eight independent trajectories were considered.

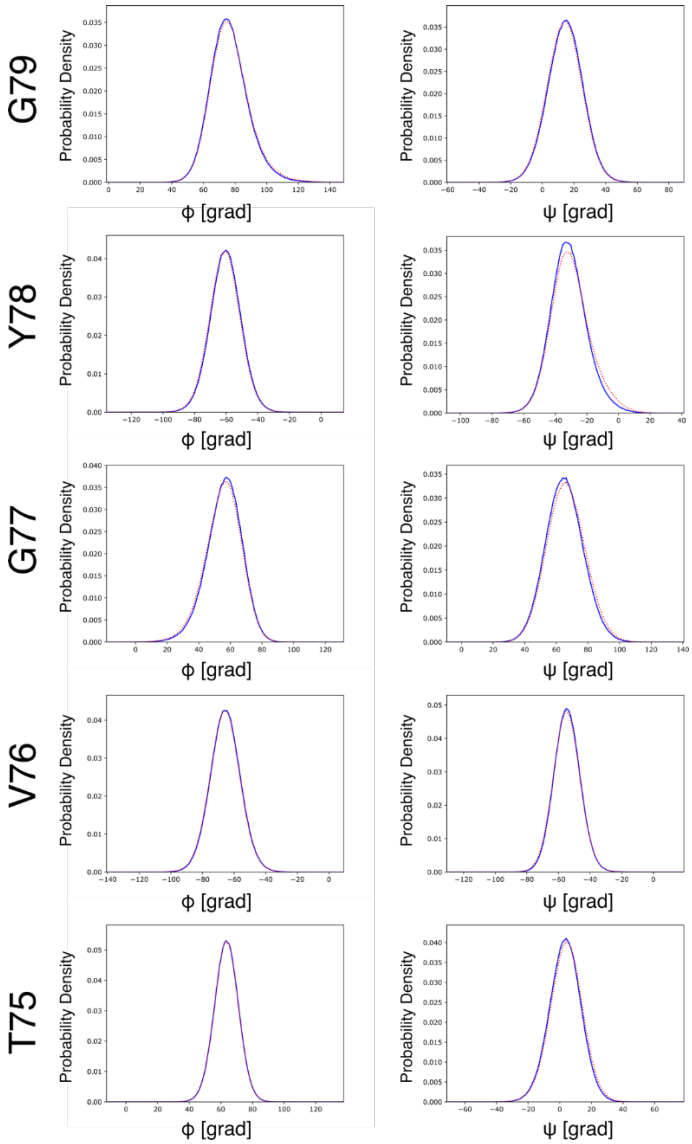

Figure S3. Selectivity filter backbone dihedral angles. Data from simulations with +200 mV applied membrane potential and initialized without water molecules inside the SF (KcsA-WT), or after the exit of water molecules from the SF (KcsA-E71A) were used.

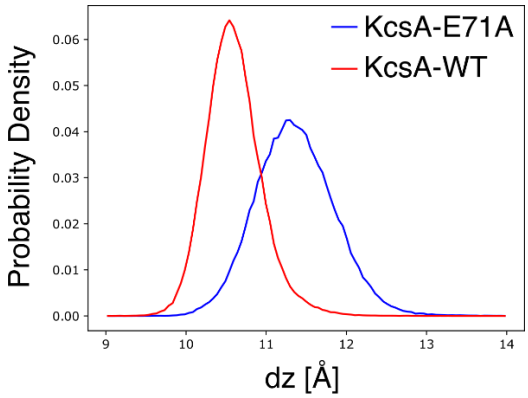

Figure S4. Position along the channel axis of the side chain oxygen atoms of D80 in simulations of KcsA-WT (red line) and KcsA-E71A (blu line) with a +200 mV membrane potential. The position was measure as the distance along the channel axis of the center of mass of the side chain oxygen atoms of each subunit from the center of mass of the Cα atoms of residues Thr75. The histograms were computed combining data from all the replicas.

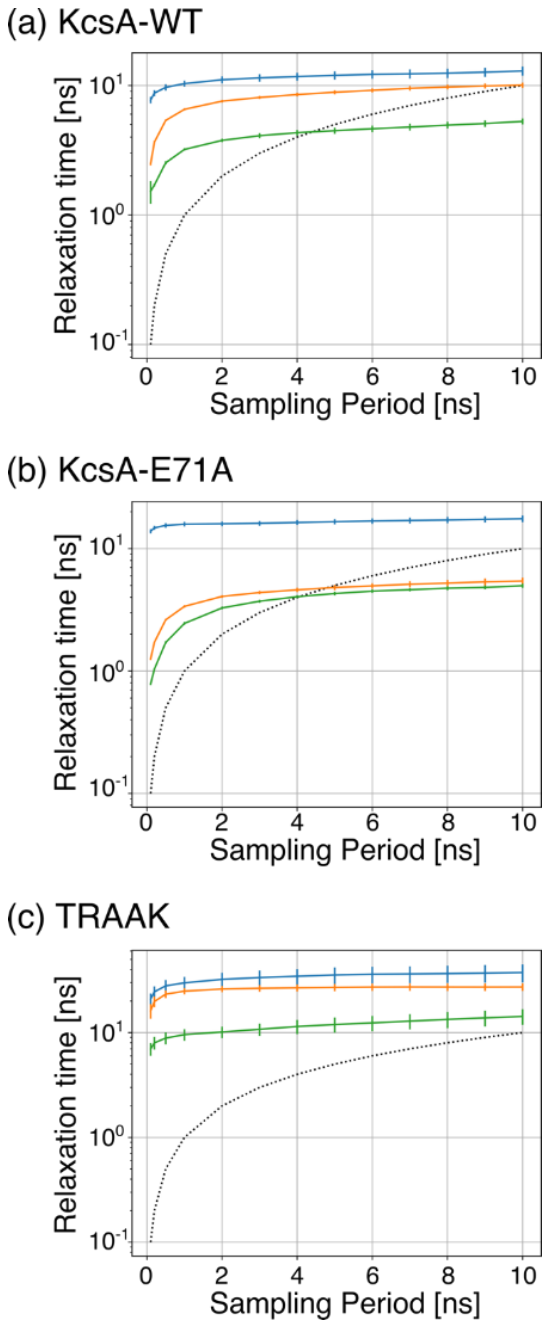

Figure S5. Relaxation times from MSMs estimated using different sampling periods. The first three relaxation times are shown. Average and standard deviations were estimated using MSMs obtained from different subsets of the available trajectories. The dashed black line corresponds to relaxation time equal to the sampling period.

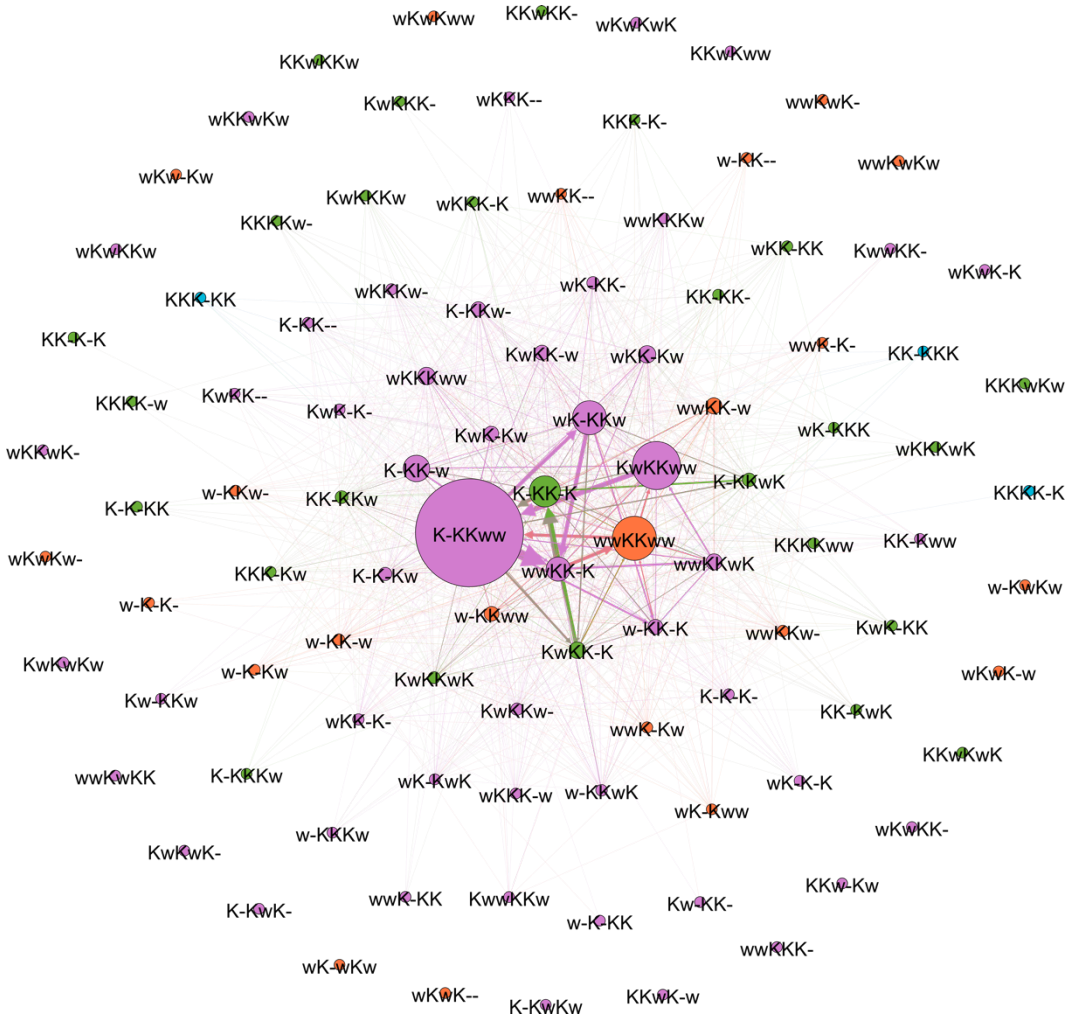

Figure S6. Complete network of states from the MSM of KcsA-WT. Nodes are labelled using a six-character string that describes the occupancy state of the channel starting from the cavity, and subsequently, binding sites S4-S0. K, w, and - corresponds respectively to a potassium ion, a water molecule, and an empty position. The colour of the node corresponds to the number of ions in the cavity and biding sites S4-S0. Node size is proportional to its probability. The colour of the edge is computed as the average between the color of the two connected nodes, and its width is proportional to the net flux along that direction.

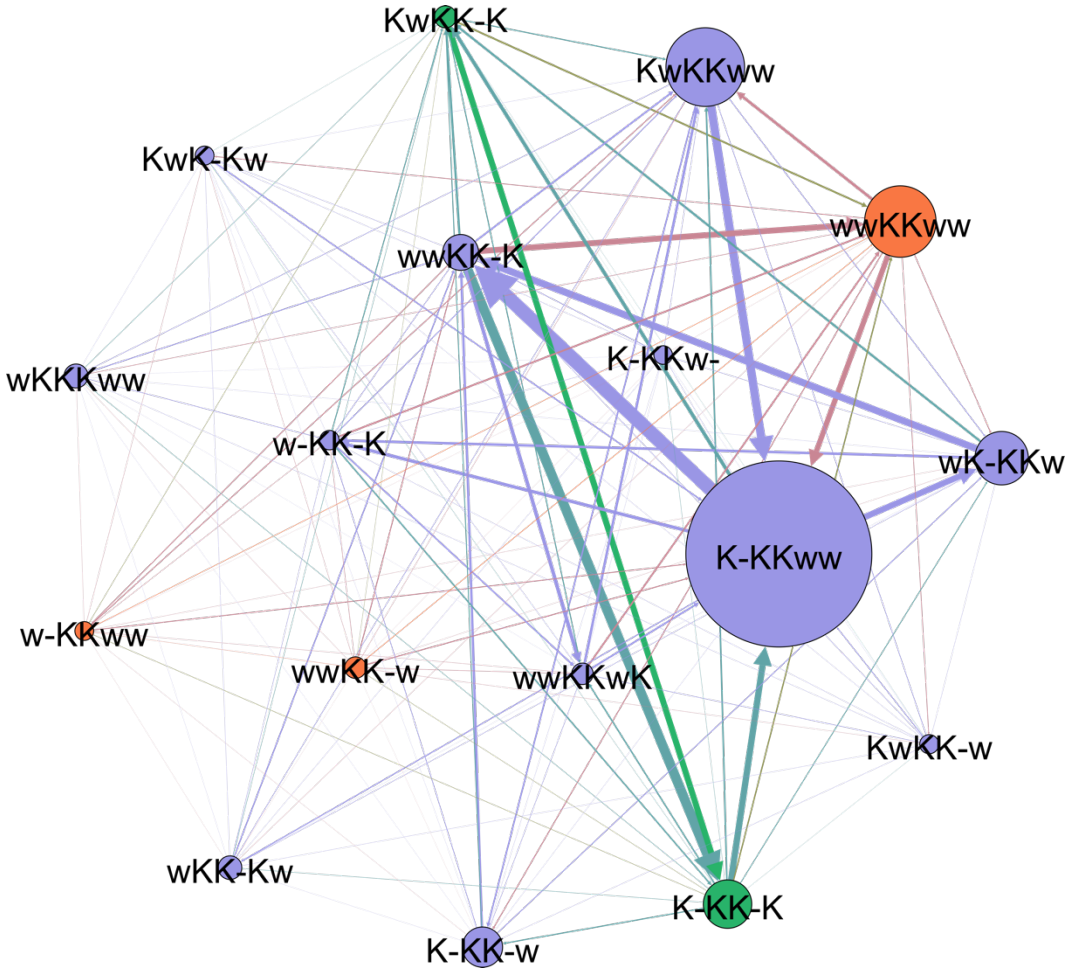

Figure S7. Network of state from the MSM of KcsA-WT filtered to show only the states with probability above 1%. Nodes are labelled using a six-character string that describes the occupancy state of the channel starting from the cavity, and subsequently, to binding sites S4-S0. K,w, and - correspond respectively to a potassium ion, a water molecule, and an empty position. The colour of the node corresponds to the number of ions in the cavity and biding sites S4-S0 (4 ions = green, 3 ions = purple, 2 ions = orange). The size of the node is proportional to its probability. The colour of the edge is computed as the average between the colour of the two connected nodes, and its width is proportional to the net flux along that direction.

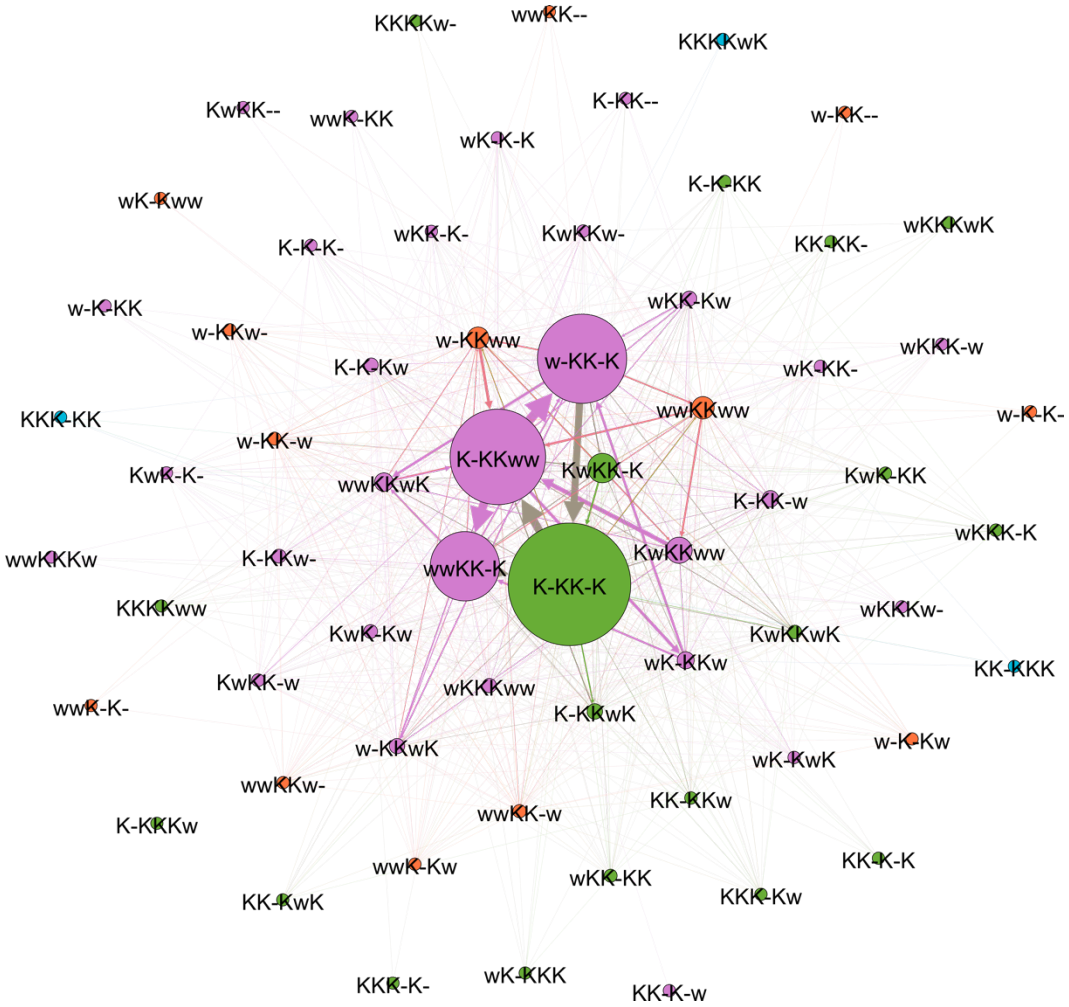

Figure S8. Complete network of states from the MSM of KcsA-E71A. Nodes are labelled using a six-character string that describes the occupancy state of the channel starting from the cavity, and subsequently, to binding sites S4-S0. K, w, and - corresponds respectively to a potassium ion, a water molecule, and an empty position. The colour of the node corresponds to the number of ions in the cavity and biding sites S4-S0. Node size is proportional to its probability. The colour of the edge is computed as the average between the color of the two connected nodes, and its width is proportional to the net flux along that direction.

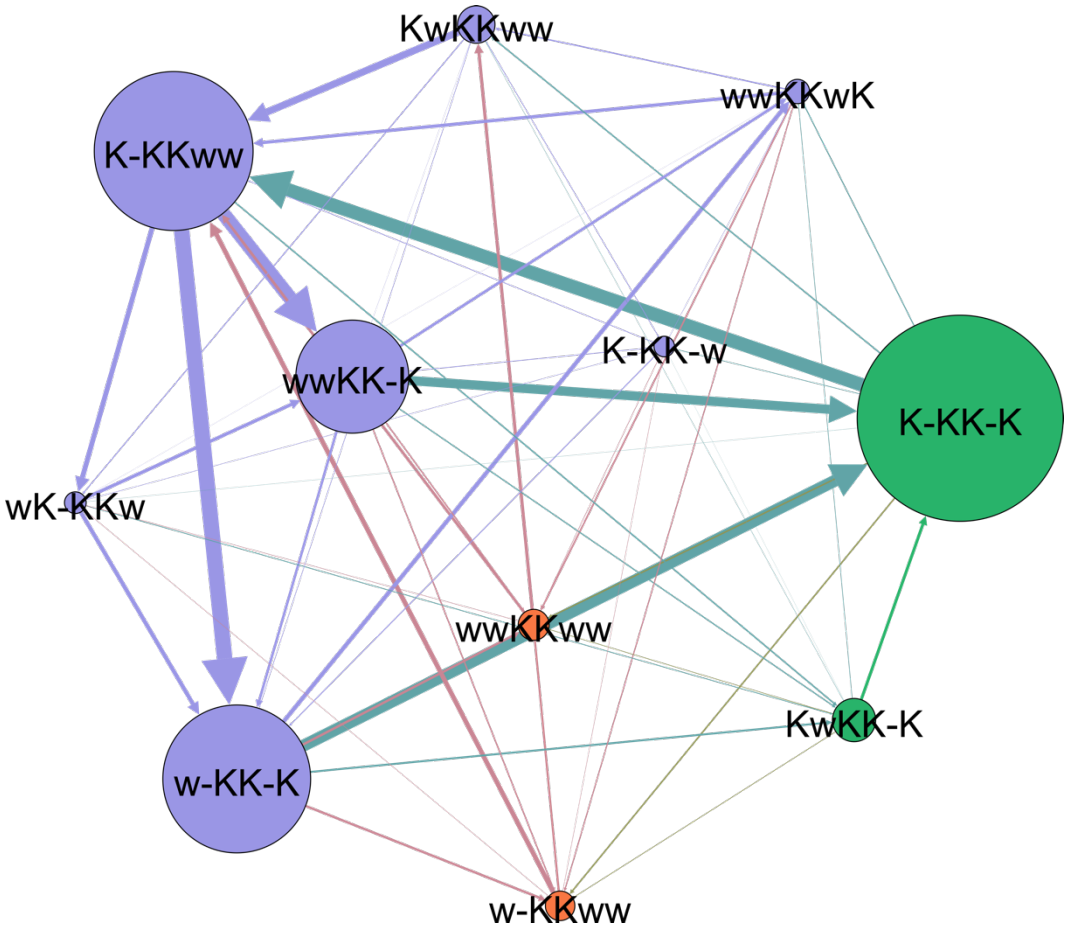

Figure S9. Network of state from the MSM of KcsA-E71A filtered to show only the states with probability above 1%. Nodes are labelled using a six-character string that describes the occupancy state of the channel starting from the cavity, and subsequently, to binding sites S4-S0. K,w, and - correspond respectively to a potassium ion, a water molecule, and an empty position. The colour of the node corresponds to the number of ions in the cavity and biding sites S4-S0 (4 ions = green, 3 ions = purple, 2 ions = orange). The size of the node is proportional to its probability. The colour of the edge is computed as the average between the colour of the two connected nodes, and its widht is proportional to the net flux along that direction.

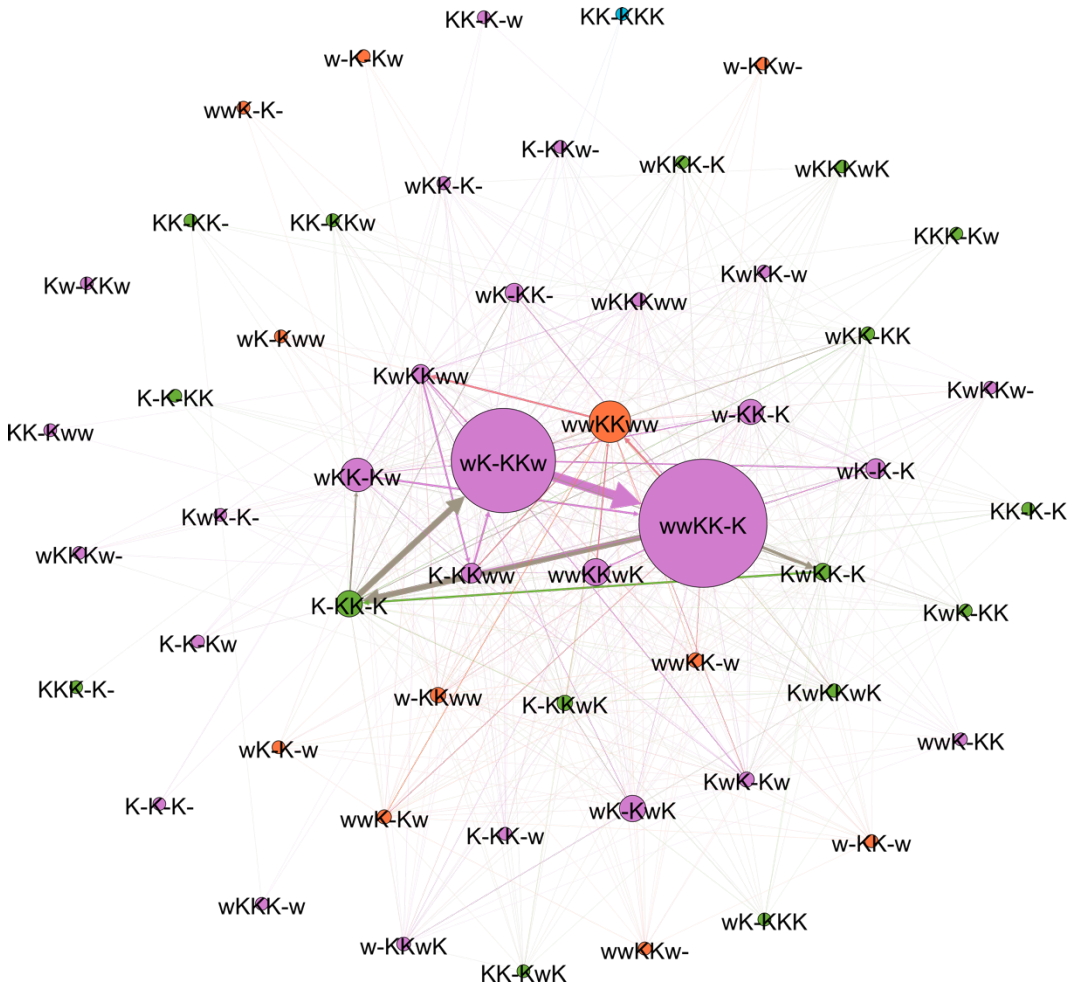

Figure S10. Complete network of states from the MSM of TRAAK. Nodes are labelled using a six-character string that describes the occupancy state of the channel starting from the cavity, and subsequently, to binding sites S4-S0. K, w, and - corresponds respectively to a potassium ion, a water molecule, and an empty position. The colour of the node corresponds to the number of ions in the cavity and biding sites S4-S0. Node size is proportional to its probability. The colour of the edge is computed as the average between the color of the two connected nodes, and its width is proportional to the net flux along that direction.

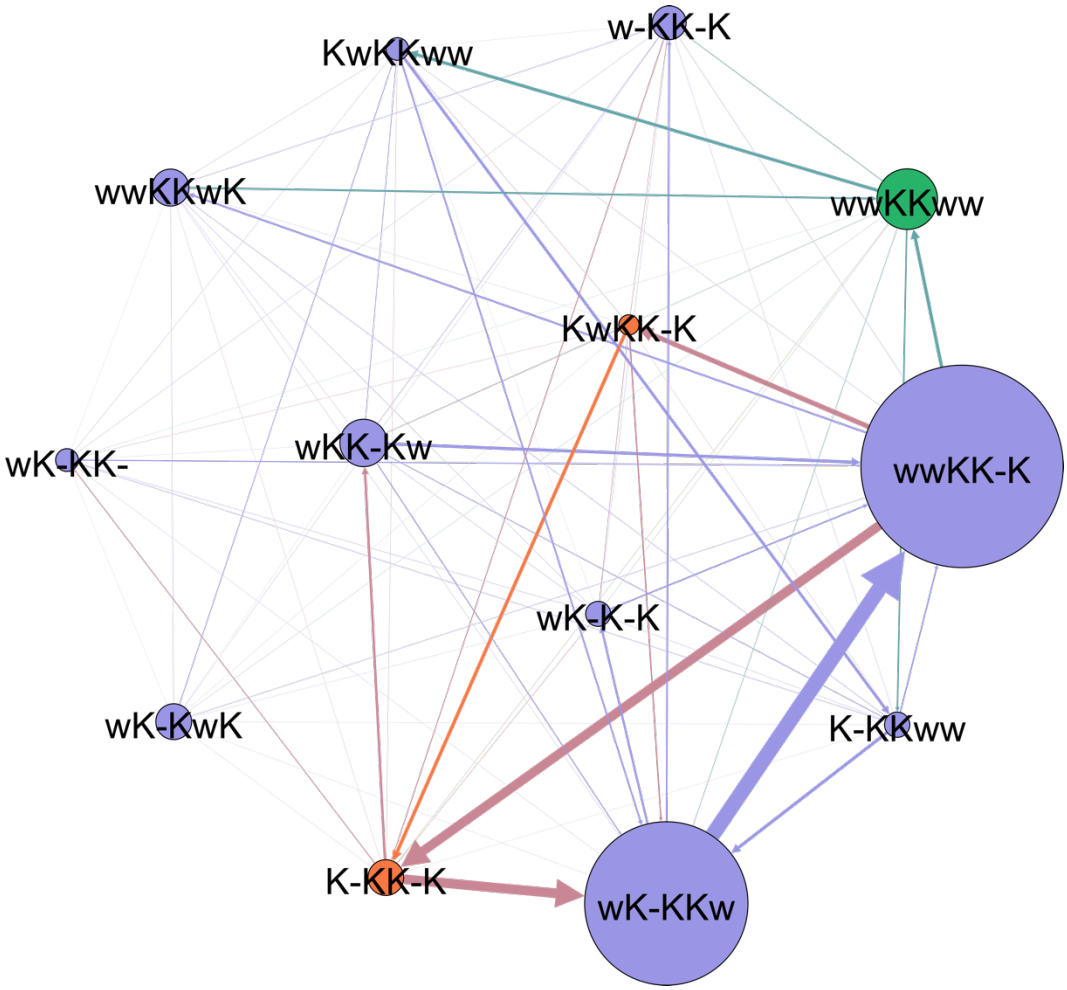

Figure S11. Network of state from the MSM of TRAAK filtered to show only the states with probability above 1%. Nodes are labelled using a six-character string that describes the occupancy state of the channel starting from the cavity, and subsequently, to binding sites S4-S0. K,w, and - correspond respectively to a potassium ion, a water molecule, and an empty position. The colour of the node corresponds to the number of ions in the cavity and biding sites S4-S0 (4 ions = green, 3 ions = purple, 2 ions = orange). The size of the node is proportional to its probability. The colour of the edge is computed as the average between the colour of the two connected nodes, and its widht is proportional to the net flux along that direction.

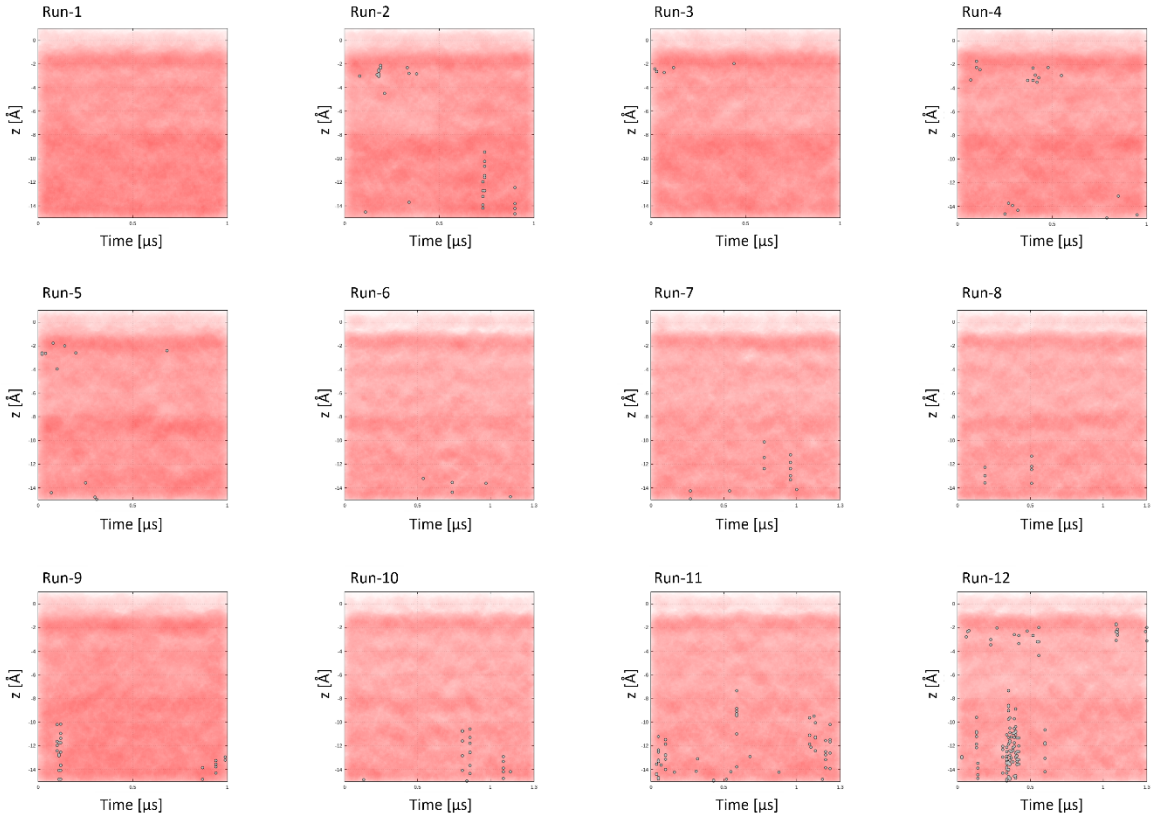

Figure S12. Water density and presence of lipids inside the cavity of the TRAAK channel. The density of water molecules along the channel axis is represented as shades of red against time for all the replicas of the TRAAK channel. Lipids' heavy atoms are shown as black-framed grey dots.. Only atoms found within a radius of 8 Å from the channel axis are shown. Data were saved every 1 ns.