Supporting Information

An Outer Membrane Protein undergoes Enthalpy- and Entropy-driven Transitions

Belete R. Cheneke[1], Mridhu Indic[2], Bert van den Berg[2] and Liviu Movileanu[1,3*]

[1]*Department of Physics, Syracuse University, 201 Physics Building, Syracuse, New York 13244-1130, USA*

[2]*Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts 01605, USA*

[3]*Syracuse Biomaterials Institute, Syracuse University, 121 Link Hall, Syracuse, New York 13244, USA*

[*]**Correspondence:**
Liviu Movileanu, PhD, Department of Physics, Syracuse University, 201 Physics Building, Syracuse, New York 13244-1130, USA; Phone: 315-443-8078; Fax: 315-443-9103;
E-mail: lmovilea@physics.syr.edu

1. Physical features of the extracellular loops of the WT-OccK1 protein

Table S1: Physical features of the extracellular loops of the WT-OccK1 protein (*1*).

Loops	Residues (position in sequence)	Total residues	Charges[#]	Range of the B-factor[*]	Average B-factor[*]
L1	RDHDAGKSL (25-33)	9	-2/+2	54.1 - 65.0	60.6 ± 5.7
L2	LNSGRGTSNSELLPLHDDGR AAD (68-90)	23	-4/+2	44.2 - 66.4	51.3 ± 6.4
L3	GEMLPDIPLLRYDDGRLLPQ (111-130)	20	-4/+2	19.6 - 28.5	23.4 ± 2.7
L4	LRNSADMQDLSAWSAPTQK SDG (157-178)	22	-3/+2	24.3 - 35.8	30.0 ± 2.8
L5	ED (203-204)	2	-2/0	25.5 - 27.2	26.4 ± 1.2
L6	DGAARAGEI (236-244)	9	-2/+1	26.7 - 36.9	30.7 ± 3.7
L7	GDSGWQSVYGSSGRSMGN DMFNGNFTNADE (271-300)	30	-4/+1	17.8 - 57.3	24.8 ± 7.2
L8	NATTKAGSGGK (330-340)	11	0/+2	26.6 - 47.5	36.2 ± 7.3
L9	SFNSD (372-376)	5	-1/0	28.8 - 34.9	32.1 ± 2.3

[#]The electric charges were determined at pH 7.4.
[*]The last two columns indicate the range of the temperature B-factor based upon the C_α atoms and the average B-factor of the fluctuating loop.

2. Titration experiments with potassium phosphate buffer

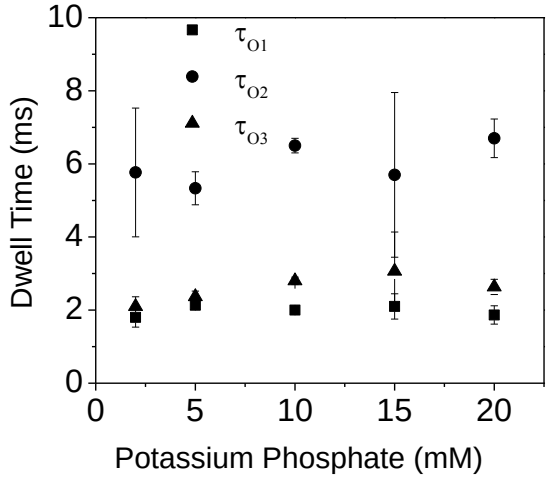
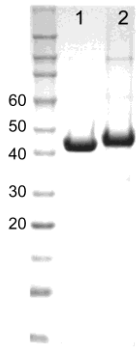


Figure S1: Titration experiments with potassium phosphate buffer in the concentration range 2-20 mM. The aqueous phase contained 1 M KCl at pH 7.4. The applied transmembrane potential was +40 mV.

3. Purification test of the OccK1 protein channel by SDS-PAGE assay.

Figure S2: Purification of *P. aeruginosa* OccK1 for single-channel electrical recordings. SDS-PAGE gel showing the final sample of OccK1 after cleavage of the N-terminal hepta-histidine tag by TEV protease (Lane 1). The protein before cleavage is shown in Lane 2. The molecular weights of marker proteins are shown on the left side (Novex Sharp Standard, Invitrogen). An amount of 3 µg OccK1 protein was loaded on each lane.



4. Temperature-dependent single-channel electrical recordings at a negative applied transmembrane potential

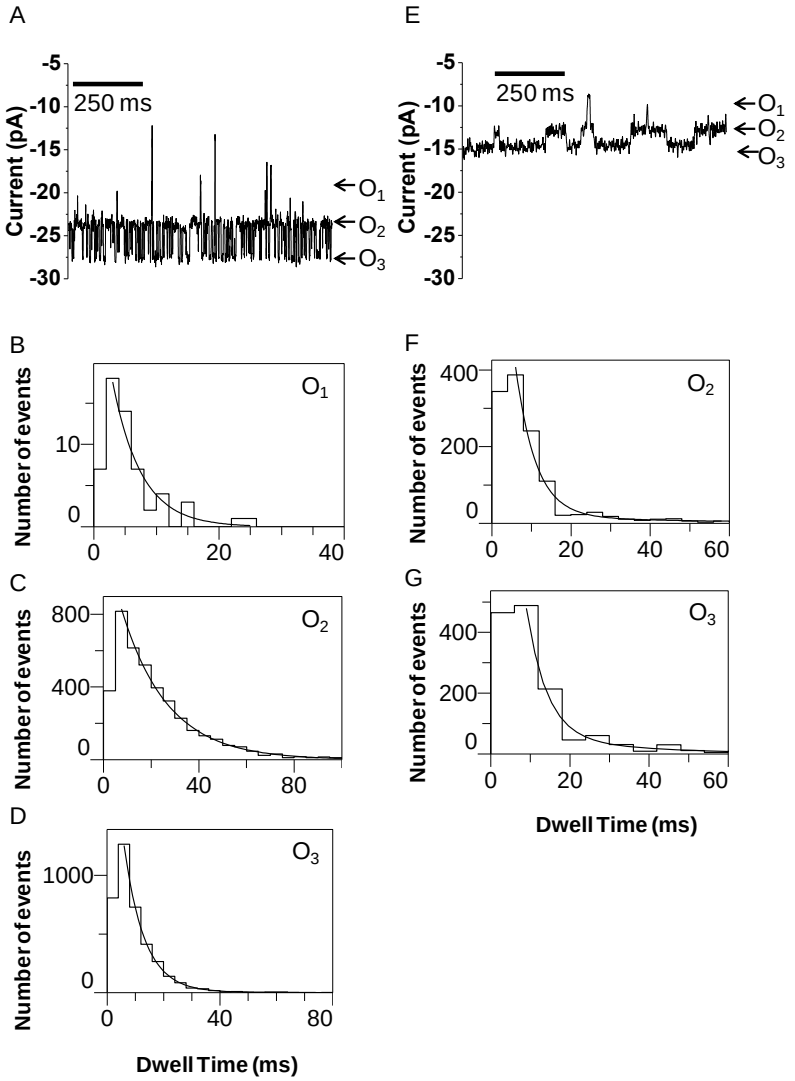


Figure S3: Representative single-channel electrical recordings acquired with the OccK1 protein at different temperatures. (A) Typical single-channel electrical trace recorded at 20 ^{O}C; (B) Dwell-time histogram of the O_1 events from (A); (C) Dwell-time histogram of the O_2 events from (A); (D) Dwell-time histogram of the O_3 events from (A); (E) Typical single-channel electrical trace recorded at 4 ^{O}C; (F)

Dwell-time histogram of the O_2 events from (E); (G) Dwell-time histogram of the O_3 events from (E). The fits were based upon a log likelihood ratio (LLR) test with a given confidence level of 0.95. The results of the fits were the following: (B) τ_{O1} = 4.6 ± 0.6 ms; (C) τ_{O2} = 19.35 ± 0.3 ms; (D) τ_{O3} = 7.6 ± 0.3 ms; (F) τ_{O2} = 23.5 ± 3.8 ms; (G) τ_{O3} = 38.9 ± 7.1 ms. The applied transmembrane potential was -40 mV. The other conditions are the same as in **Fig. 4**.

5. Standard histograms of fitted current amplitudes of the single-channel events

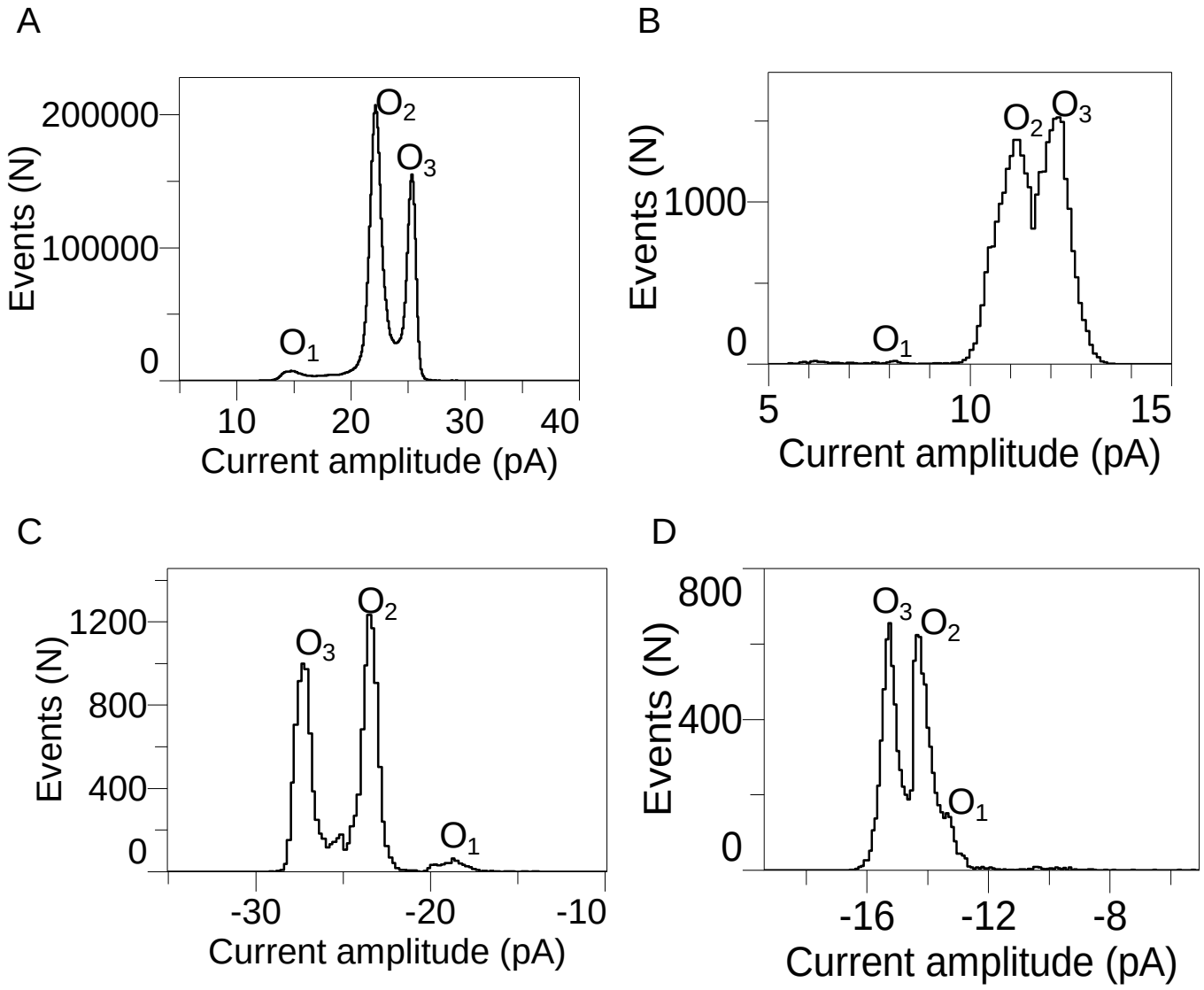


Figure S4: Typical standard histograms of fitted current amplitudes, which were taken from event-list protocol in ClampFit software (Axon). The vertical axis represents the number of events in the respective peak. **(A)** The transmembrane potential was +40 mV. The temperature in the chamber was 20°C; **(B)** The transmembrane potential was +40 mV. The temperature in the chamber was 4°C; **(C)** The transmembrane potential was -40 mV. The temperature in the chamber was 20°C; **(D)** The transmembrane potential was -40 mV. The temperature in the chamber was 4°C. The other conditions were the same as those in **Fig. 4**, the main text and in **Fig. S3** in **Supporting Information**.

6. Typical all-points current amplitude histograms of the WT-OccK1 protein channel

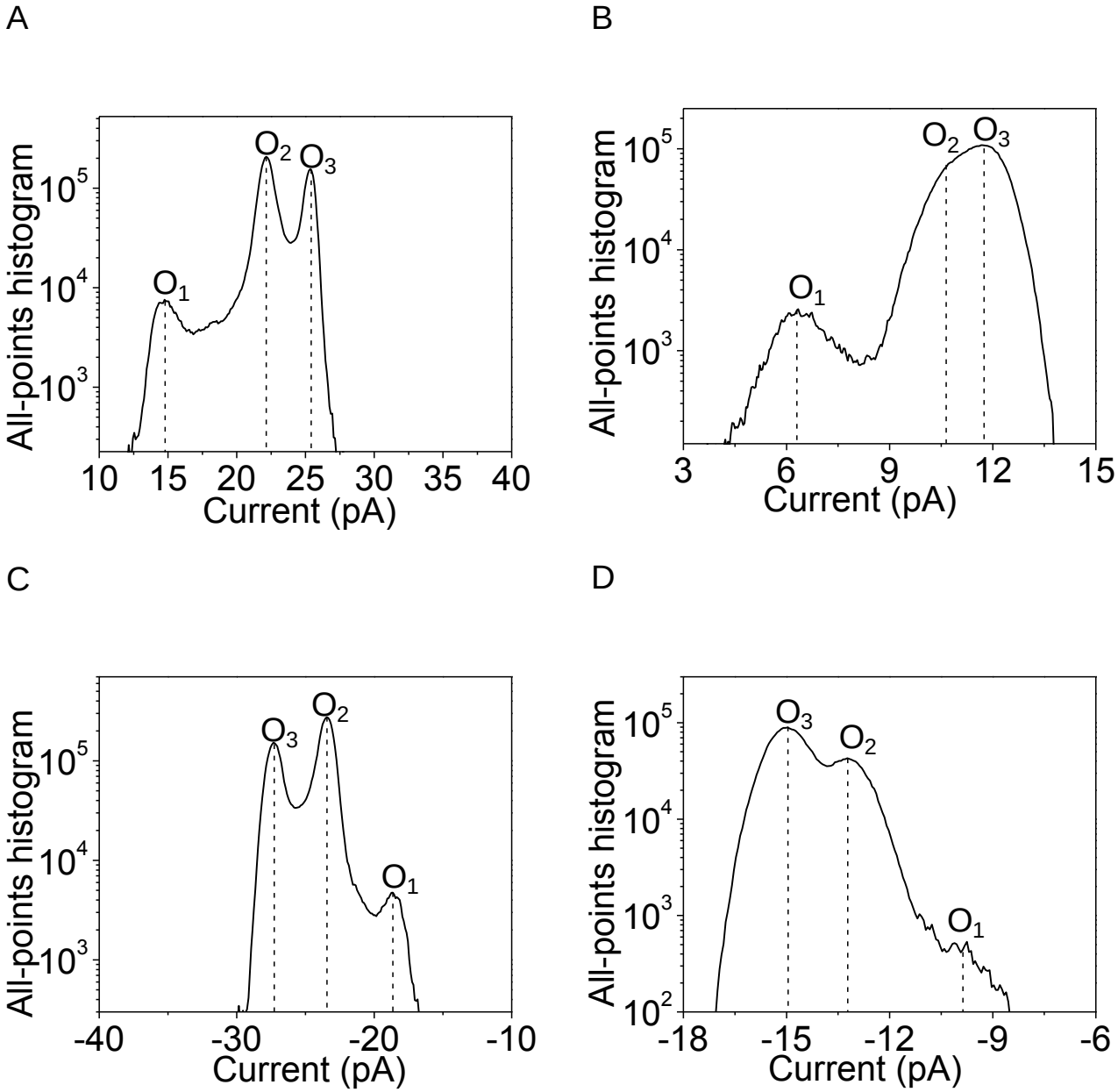


Figure S5: Typical all-points current amplitude histograms of the wild-type OccK1 protein channel. **(A)** The transmembrane potential was +40 mV. The temperature in the chamber was 20°C; **(B)** The transmembrane potential was +40 mV. The temperature in the chamber was 4°C; **(C)** The transmembrane potential was -40 mV. The temperature in the chamber was 20°C; **(D)** The transmembrane potential was -40 mV. The temperature in the chamber was 4°C. The other conditions were the same as those in **Fig. 4** in the main text and in **Fig. S3** in **Supporting Information**.

7. Semi-log Eyring plot of the four kinetic rate constants of the WT-OccK1 protein channel acquired at an applied transmembrane potential of -40 mV

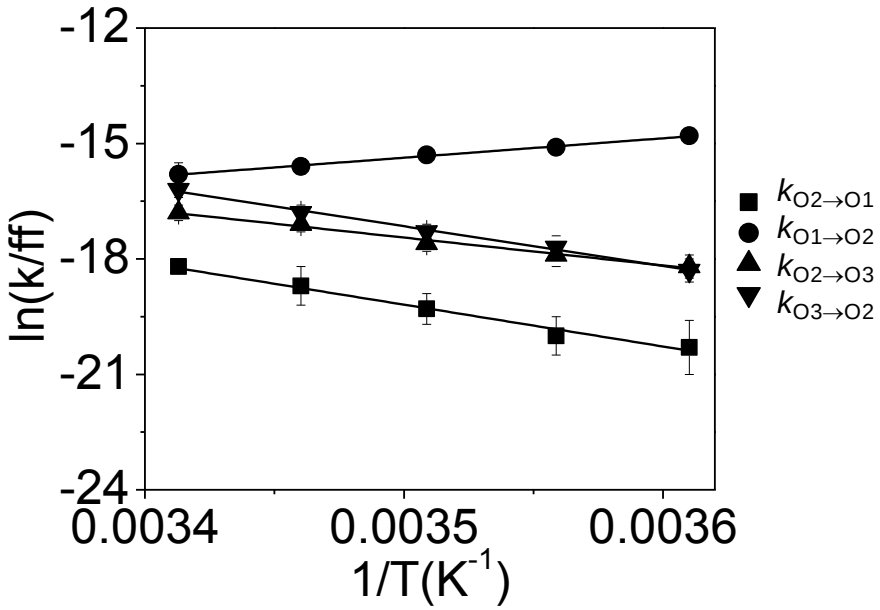


Figure S6: Semi-log Eyring plot of the four kinetic rate constants of the WT-OccK1 protein channel acquired at an applied transmembrane potential of -40 mV. All conditions are similar to those shown in **Fig. 6**.

8. Properties of the loop-deletion OccK1 mutants

Table S2. Properties of the loop-deletion OccK1 mutants.[a]

Loop	Deleted Residues	Charges	Number of residues	Salt Bridges	Hydrogen bonds	Van der Waals
L3	124-129 (DGRLLP)	1/-1	6	D124 (L3) – R16 (PW) [b]	G125 bb (L3) – Y18 sc (PW) R126 sc (L3) – R16 sc (PW) R126 sc (L3) – N76 sc (L2) R126 sc (L3) – S77 bb (L2) L127 bb (L3) – R126 sc (L3)	Several hydrophobic and Van der Waals interactions involving L127 (L3) – P129 (L3)
L4	166-175 (LSAWSAPTQK)	1/0	10	No salt bridge in the high-resolution crystal structure of OccK1	W169 sc (L4) – S281 bb (L7) S170 sc (L4) – Q276 sc (L7) Q174 sc (L4) – R240 bb (L6) K175 bb – R240 sc (L6)	P172 sc (L4) - Y279 sc (L7) There are also several hydrophobic and Van der Waals interactions with residues on PW

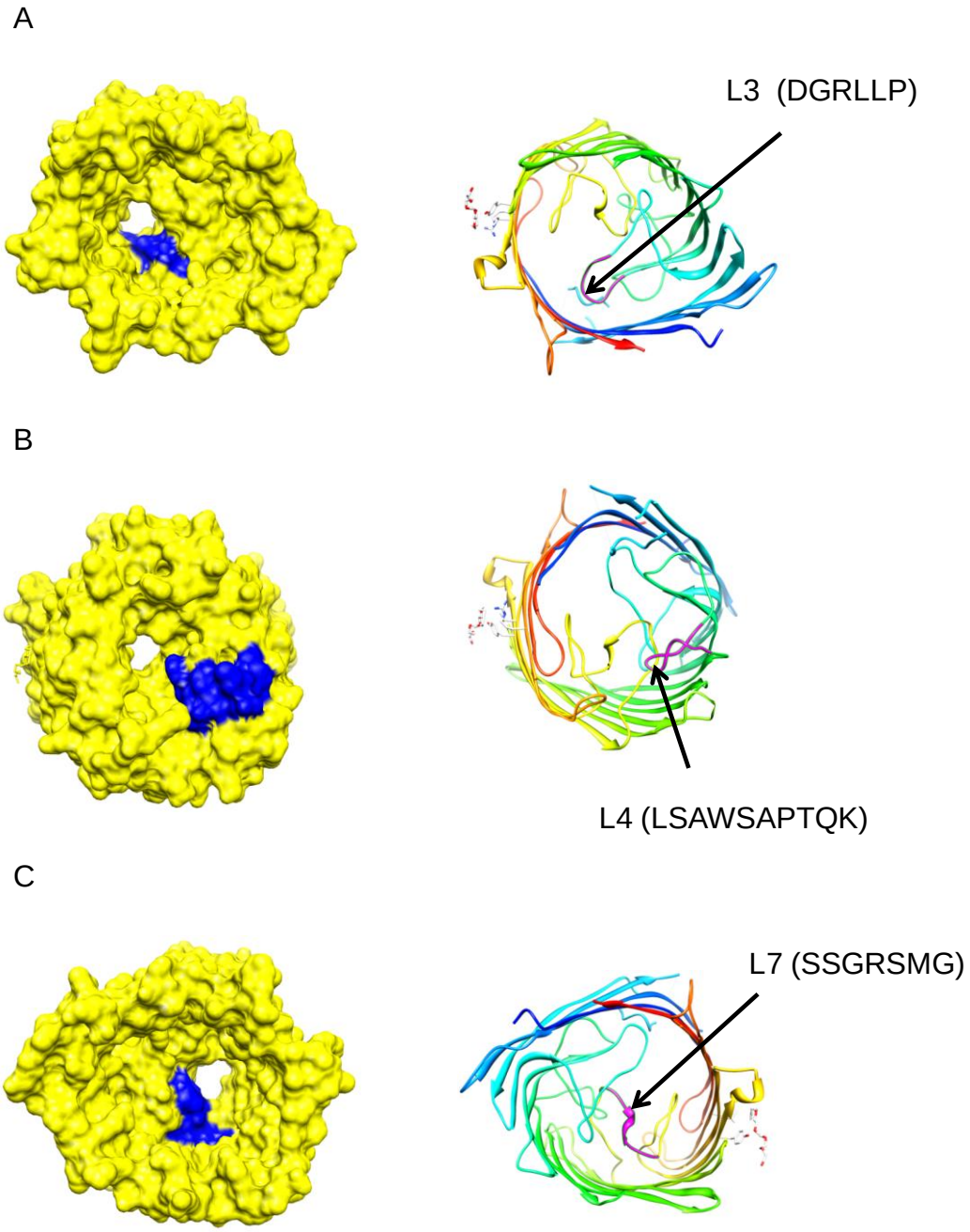
The table indicates the removed interactions between deleted fragments of the loops and other parts of the native protein (either extracellular loops or the pore walls). sc and bb indicates side chain and backbone, respectively. PW denotes the pore wall residues.

[a]Data were obtained using the recently published high-resolution X-ray crystal structure of the OccK1 protein (*2*).

[a]In a low-resolution X-ray crystal structure of the OccK1 protein (2qtk.pdb), we observed a second salt-bridge, R126 (L3) -E78 (L2) (*1*).

9. Graphic representations of the loop-deletion OccK1 mutants

Figure S7: Locations of the loop deletions examined in this work.



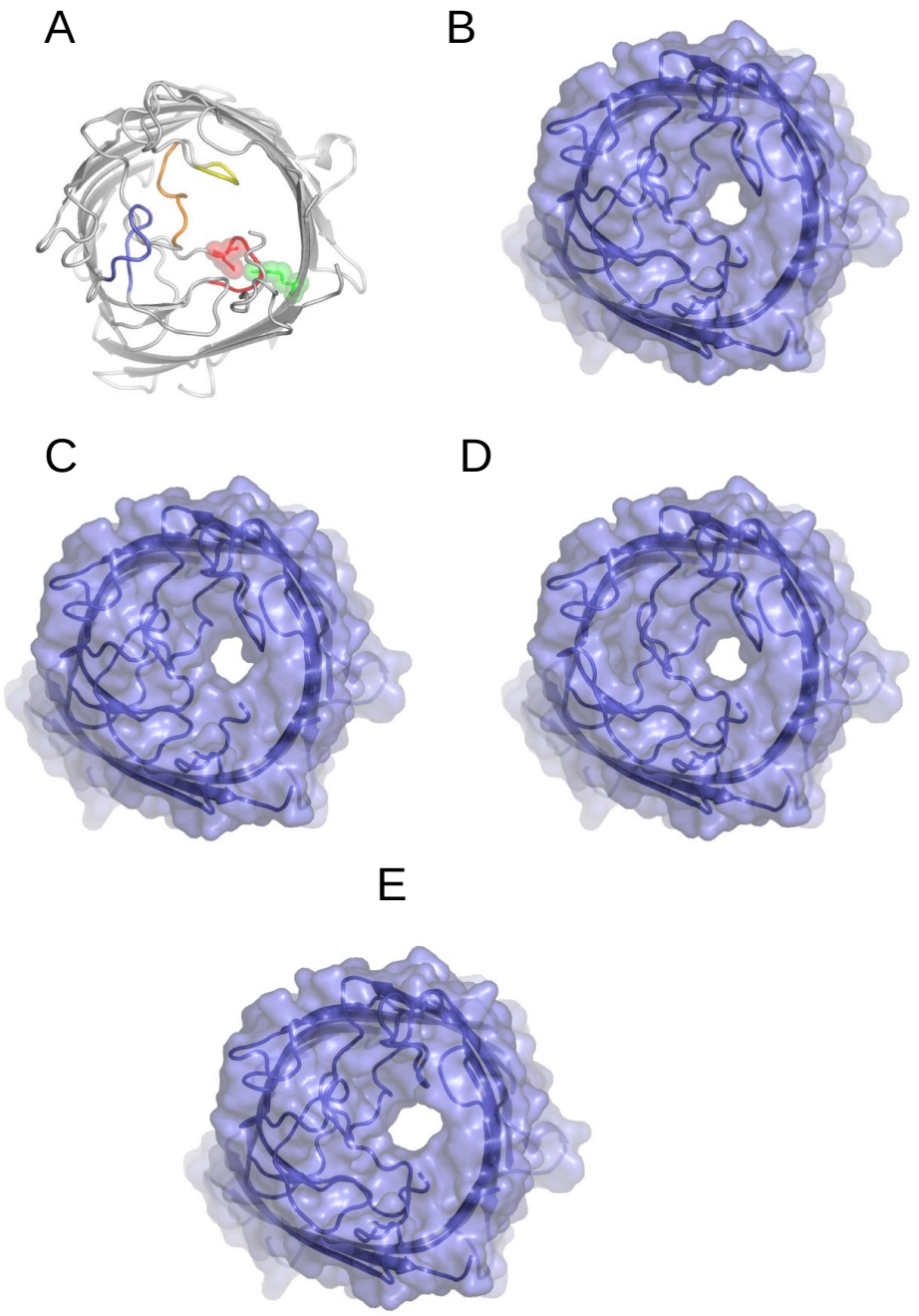


Figure S8: Location of the loop deletions in the high-resolution crystal structure of the OccK1 protein. (**A**) Backbone representations of the deleted loops. The panel also shows the removed salt bridge D124-R16 as sphere models, between loop L3 and β-barrel pore wall, respectively. Yellow, blue and red are the deleted fragments of loops L7, L4, and L3, respectively; (**B**) Transversal cross-sectional representation of the molecular surface of the WT-OccK1 protein; (**C**) Transversal cross-sectional representation of the predicted molecular surface of the OccK1 ΔL3 protein; (**D**) Transversal cross-sectional representation of the predicted molecular surface of the OccK1 ΔL4 protein; (**E**) Transversal cross-sectional representation of the predicted molecular surface of the OccK1 ΔL7 protein. The cross sectional area of the eyelet of the OccK1 ΔL7 protein is greater than those values corresponding to the WT-OccK1, WT-OccK1 ΔL3 and WT-OccK1 ΔL4 proteins, which is in accord with the single-channel electrical recordings carried out with these mutants at room temperature (*3*).

10. Single-channel electrical recordings with loop-deletion OccK1 mutants at room temperature

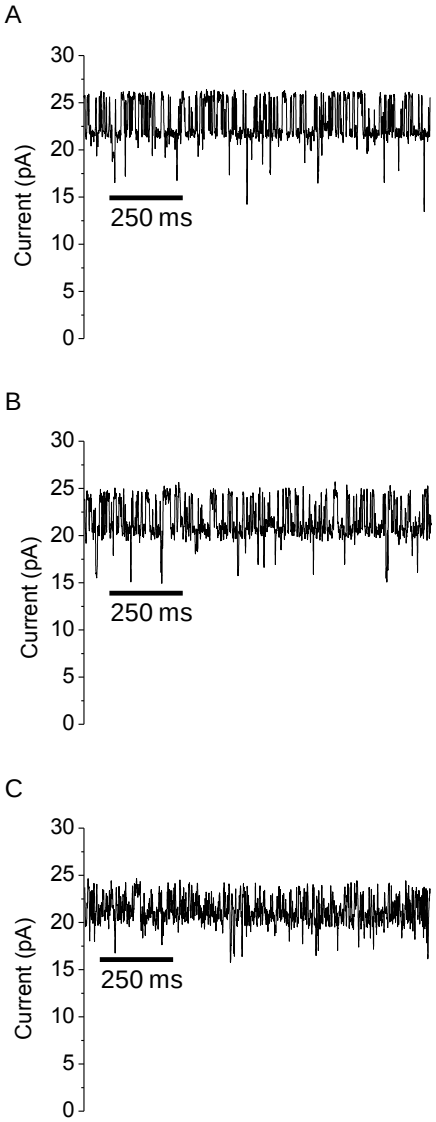


Figure S9: Single-channel electrical recordings with loop-deletion OccK1 mutants at room temperature. **(A)** WT-OccK1; **(B)** OccK1 ΔL3; **(C)** OccK1 ΔL4. The single-channel electrical recordings were acquired in 2 M KCl, 10 mM potassium phosphate, pH 7.4, and at 25°C. The applied transmembrane potential was +40 mV. For the sake of clarity, the electrical traces were low-pass Bessel filtered at 200 Hz.

11. Enthalpic contributions to single-channel current fluctuations of the WT-OccK1 protein and loop-deletion OccK1 mutants.

Table S3: Enthalpic contributions to single-channel current fluctuations of the WT-OccK1 protein and loop-deletion OccK1 mutants.

Parameter	Voltage (mV)	Construct	$O_2 \rightarrow O_1$	$O_1 \rightarrow O_2$	$O_2 \rightarrow O_3$	$O_3 \rightarrow O_2$
$\Delta H^{\#}$ (kJ/mol)	40	WT-OccK1	108 ± 16	-52 ± 5	56 ± 9	96 ± 11
		OccK1 ΔL3	126 ± 24	-37 ± 4	56 ± 10	115 ± 14
		OccK1 ΔL4	84 ± 9	-27 ± 2	66 ± 3	121 ± 17
	-40	WT-OccK1	91 ± 8	-42 ± 2	60 ± 3	86 ± 3
		OccK1 ΔL3	71 ± 10	-37 ± 4	57 ± 10	110 ± 12
		OccK1 ΔL4	151 ± 10	-34 ± 5	65 ± 8	98 ± 8

12. Determination of the minimum transition entropies and maximum transition energies for the WT-OccK1 protein

Table S4: Determination of the minimum transition entropies and maximum transition energies for the WT-OccK1 protein

Transition	Voltage (mV)	$\Delta H^{\neq}$ (kJ/mol)	$\Delta S^{\neq}_{min}$ (J/mol·K)	$T\Delta S^{\neq}_{min}$ (kJ/mol)	$\Delta G^{\neq}_{max}$ (kJ/mol)
$O_2 \rightarrow O_1$	+40	108 ± 16	145 ± 22	42 ± 6	66 ± 10
$O_2 \rightarrow O_1$	-40	91 ± 8	80 ± 12	26 ± 4	66 ± 5
$O_1 \rightarrow O_2$	+40	-52 ± 5	-405 ± 52	-119 ± 15	60 ± 10
$O_1 \rightarrow O_2$	-40	-42 ± 2	-346 ± 55	-101 ± 16	59 ± 14
$O_2 \rightarrow O_3$	+40	56 ± 9	-21 ± 6	-6.2 ± 1.5	62 ± 8
$O_2 \rightarrow O_3$	-40	60 ± 3	-6.8 ± 44	-2.0 ± 12	62 ± 16
$O_3 \rightarrow O_2$	+40	96 ± 11	122 ± 1	36 ± 1	60 ± 11
$O_3 \rightarrow O_2$	-40	86 ± 3	87 ± 31	25 ± 9	61 ± 12

[&]The temperature used to calculate $T\Delta S^{\neq}_{min}$ was 20°C.

References

1. Biswas, S., Mohammad, M. M., Movileanu, L., and van den Berg, B. (2008) Crystal structure of the outer membrane protein OpdK from Pseudomonas aeruginosa, *Structure 16*, 1027-1035.

2. Eren, E., Vijayaraghavan, J., Liu, J., Cheneke, B. R., Touw, D. S., Lepore, B. W., Indic, M., Movileanu, L., and van den Berg, B. (2012) Substrate specificity within a family of outer membrane carboxylate channels, *PLoS Biology 10*, e1001242.

3. Cheneke, B. R., van den Berg, B., and Movileanu, L. (2011) Analysis of gating transitions among the three major open states of the OpdK channel, *Biochemistry 50*, 4987-4997.