Supplementary Information File

OccK Channels from *Pseudomonas aeruginosa* Exhibit Diverse Single-channel Electrical Signatures, but Conserved Anion Selectivity

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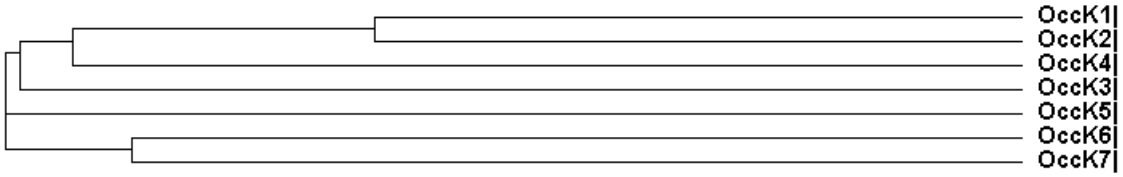
Figure S1

A



B

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OccK1|          ---------AEGGFLEDAKTDLVLRNYYFNRDFRDHDAGKSLVDEWAQGFILKFSSGYTP  51
OccK2|          -----GHVHAGQGFLEDAKASLTARNFHLHRNFVG-DASQGKAEEWTQSFILDARSGFTQ  54
OccK3|          ------------DLVEDSHASLELRNFYFNRDFRQSGARDN-ADEWAQGFLLRLESGFSE  47
OccK4|          ------------EFLADSSAHLDLRNFYQLRDYRQHDAPQSQAGNWSQGFVLRLQSGFTG  48
OccK5|          VLAGIAPLGNAAGFLEDSKASLETRNFYMNRDFRD-GPGQSKREEWAQGFILNLQSGYTQ  59
OccK6|          ------------EFIKDSKASIELRNFYFNRDFRQEGASQSKAEEWAQGFLLRYESGYTE  48
OccK7|          ------------EFFADGKAGLELRNFYFNRDYRQPGASQSYSEEWAQGFLLRYESGYTE  48
                             :. *. : :  **::  *::   .. ..   :*:*.*:*   **::

OccK1|          GTVGVGLDAIGLFGVKLNSGRGTSNSELLPLH-DDGRAADNYGRVGVAAKLRVSASELKI  110
OccK2|          GSVGFGLDVLGLYSLKLDGGKGTAGTQLLPIH-DDGRPADDFGRLAVAGKLRVSNSELKI  113
OccK3|          GTVGFGVDAIGLLGFKLDSGSGSGGTGLLPADGSAGGSQDDYAKLGLTAKARVSNSLLKV  107
OccK4|          GPLGFGLDATGLLGVKLDSGRGRSNDGTLPFGANSKEPVDDYSHLGLTAKLRYSQTQLQV  108
OccK5|          GTVGFGLDAMGMLGVKLDSGRGRSGTGLLPKD-SDGRAPDTYSKLGLTAKVKVSQSELKV  118
OccK6|          GTIGFGVDAIGLLGVKLDSSPDRSGTGLLKRDRETGRAQDDYGEAGITAKLRASKSTLKI  108
OccK7|          GLFGLGVDALGLLGVRLDSSPERSGSGLLPYSTSDRRAAHDYSSLGLTAKLRVSHSTLKI  108
                 * .*.*:*. *: ..:*:..   ..   *    .   . . :.  .::.* : * : *::

                      L3                                          L4
OccK1|          GEMLPDIPLLRYDDGRLLPQTFRGFAVVSRELPGLALQAGRFDAVSLRNSADMQDLSAWS  170
OccK2|          GEWMPVLPILRSDDGRSLPQTFRGGQLSANEIAGLTLYAGQFRGNSPRNDASMQDMSLFG  173
OccK3|          GALHFKSPLVSANDTRLLPELFRGALLDVQEIDGLTLRGAHLDRNKLNSSSDYQVFSANR  167
OccK4|          GILMPQLPVAFRDDVRLLPQTFDGALLTSSEIEGLTLTAGQLWKSRTRESAGSDDMYIMG  168
OccK5|          GTLIPKLPSVQPNNGRIFPQIFEGALLTSKEIKDLGFTAGRLEKTKIRDSSDSEDLALND  178
OccK6|          GTLTPKLPVIMPNDSRLLPQTFQGGALNSMEIDGLTLDAGRLKKVNQRDSSDNEDMTITG  168
OccK7|          GTLMPRLPVVQFNDTRLHPQTFQGGLLEVNEIDGLALQFGQLRQVKQRDSTNAEDLGITR  168
                 *      *    :: *  *: * *  :   *: .* :  .::     ...:. : :

                 ------------------------------------------
OccK1|          APTQK--------SDGFNYAGAEYRFNRERTQLGLWHGQLEDVYRQSYANLLHKQRVGDW  222
OccK2|          RPAAT--------SDRFDFAGGEYRFNGERSLLGLWNAELKDIYRQQYLQLQHSQPLGDW  225
OccK3|          IGGR---------SDAFDFAGGDYRLT-PALTASLHQGRLKDIYRQTFAGLVHTLDLGGQ  217
OccK4|          RDKAH-------ASDEFNLAGATYAFT-PRLSASYYYGQLKDIYRQHYLGLLHTLPLGEG  220
OccK5|          KNGR----FAGVSADHFDLGGLDYKLT-DQLTASYHYSNLQDVYRQHFVGLLHSWPIGPG  233
OccK6|          GGKRNIVVRSGLTSDKFDFAGGSYKWT-DNLSTSYHYGKLDNFYKQHYLGLVHTLPIADK  227
OccK7|          GNKRNVLAGRHPGSDRFDFAGGTYRWS-ERLSSSYHYANLEDFYRQHHLGVQHLLPLADD  227
                             :* *: .*  *  .      .   ..*.:.*:* .  : *   :.

                                                                  L7
OccK1|          T-LGANLGLFVDRDDGAARAGEIDSHTVYGLFSAGIGLHTFYLGLQKVGGDSGWQSVYGS  281
OccK2|          L-LGANLGGFRGRDAGSARAGKLDNRTVSALFSARYGLHTLYLGLQKVSGDDGWMRVNGT  284
OccK3|          RSLKSDLRFARASEDGGFR--ELDNRAFGALFSLRLGAHAVAAGYQRISGDDPYPYIAG-  274
OccK4|          LSLRSDLRYFDSGEDGAAISGPVDNRNLNAMLTLRAGAHAFGIGVQKMIGNDAFPVLNGY  280
OccK5|          E-LTSDLRFARSTDSGSAKAGGIDNKSLNGMFTYSLGNHAFGAAWQRMNGDDAFPYLEG-  291
OccK6|          QSLKSDIRWARSTDDGSS---NVDNKALNAMFTYSLGYHAFGVGYQKMSGDTGFAYING-  283
OccK7|          QSLKSDIRWARSTDEGGS---RVNNRALNALFTYRLGGHAFGLGYQRMSGDSGFAYLAG-  283
                   * :::      : *.     ::.: . .:::   * *:.  . *:: *:  :  : *

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OccK1|          SGRSMGNDMFNGNFTNADERSWQVRYDYDFVGLGWPGLIGMVRYGHGSNATTKAGSG-GK  340
OccK2|          SGGTLANDSYNASYDNPGERSWQLRYDFDFVGLGLPGLTFMTRYLHGDHVRLAGVTDDGS  344
OccK3|          SDPYLVNFIQIGDFGNVDERSWQLRYDYDFGALGLPGLSFMSRYVSGDNVARG-AANDGK  333
OccK4|          TTPYVANLMAYQTFTRPQEKSWQLRYDYDFAGLGLPGLNLMTRYVQGRDIDRGAGRADDS  340
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OccK5|          SNPYLVNFVQVNDFAGPKERSWQLRYDYDFVGLGIPGLTFMTRYVKGDNVELAGQSGEGR 351
OccK6|          ADPYLVNFIQIGDFANKDEKSWQARYDYNFAGVGIPGLTFMTRYVKGDNIDLLTTSGEGK 343
OccK7|          TDPYLVNFVQIGDFANKDERSWQLRYDYDFAAIGLPGLTFMSRYLRGEHIDLLDGGGRGK 343
                 :   : *      :    *:*** ***::* .:* ***  * **  * .         .

OccK1|          EWERDVELGYTVQSGPLARLNVRLNHASNRRSFNS--DFDQTRLVVSYPLSW- 390
OccK2|          EWGRESELGYTLQSGAFKRLNVRWRNSSQRRDWGSNTRFDENRLIVSYPLSLL 397
OccK3|          EWERNTDLGYVVQSGPLKNLGVKWRNATVRSNFAN--DLDENRLILSYSLALW 384
OccK4|          EWERNTDLSYVIQSGPLKSVALKWRNITYRSRYGA--DLDENRFIVNYTLKLW 391
OccK5|          EWERNTELQYVFQSGALKNLGIRWRNATFRSNFTR--DIDENRLIVSYTLPIW 402
OccK6|          EWERDMDIAYVFQSGPLKNLGVKWRNATMRTNYTN--DYDENRLIVSYTLPLW 394
OccK7|          EWERDTDIAYLVQSGPLKNLGIKLRNGTFRSDFGN--DIDETRLIVSYALPLW 394
                 ** *: :: * .***.:  : :: .: : *  :      *:.*:::.*.*
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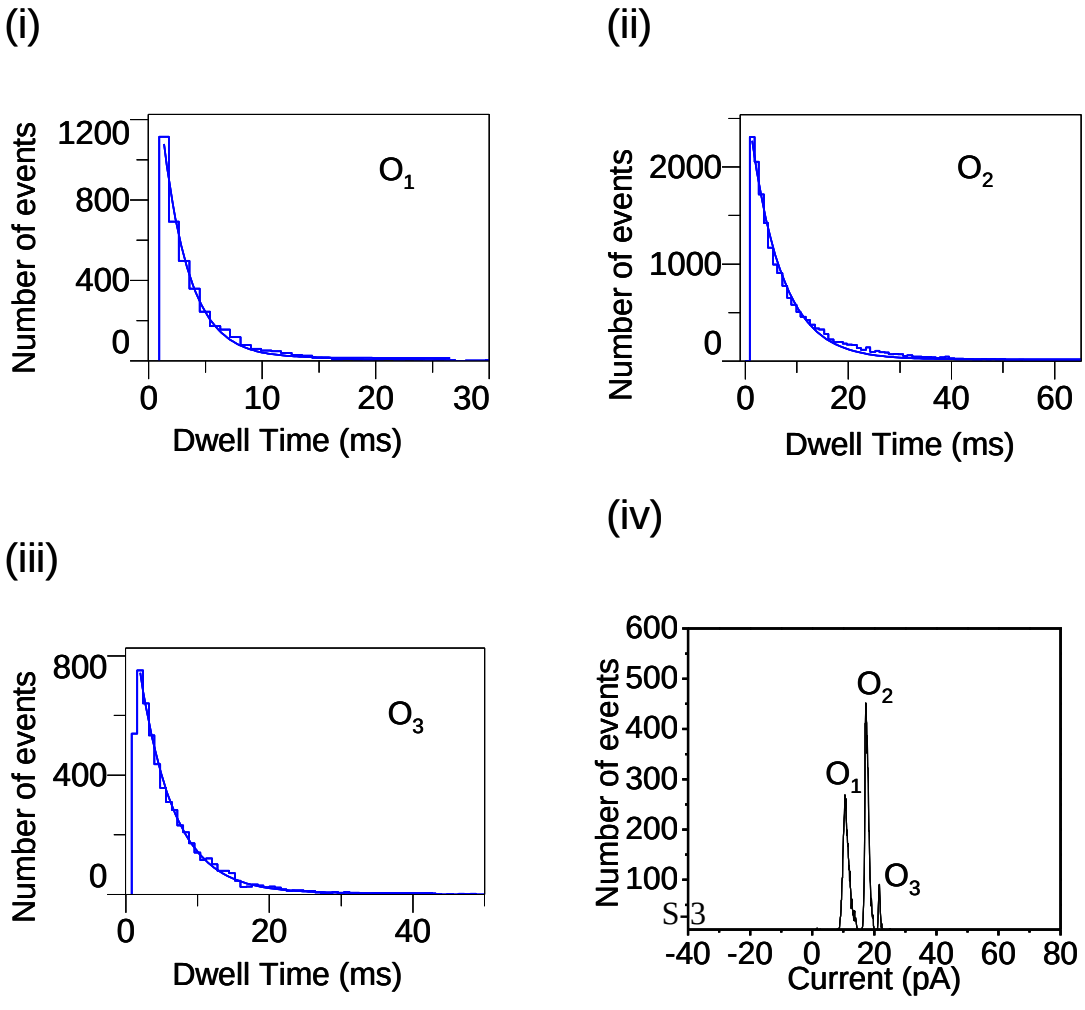
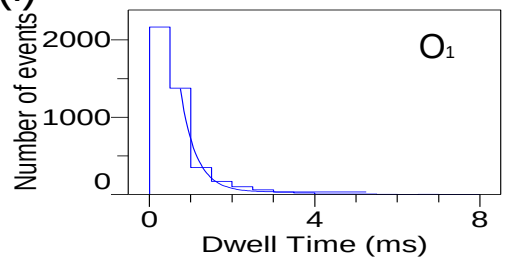
Figure S1 Amino acid sequence analysis of seven OccK subfamily members. (**A**) Phylogenetic tree generated by ClustalW2 (Version 2.1, The European Bioinformatics Institute, UK) and (**B**) Sequence homology analysis generated by Clustal Omega (Version 1.0.3, The European Bioinformatics Institute, UK), where conserved sites are marked with '*', ':', or '.' at the bottom of the sequences. An asterisk (*) indicates positions which have a single, invariant residue. A colon (:) indicates conservation between groups of strongly similar properties (scoring > 0.5 in the Gonnet PAM 250 matrix). A period (.) indicates conservation between groups of weakly similar properties (scoring =< 0.5 in the Gonnet PAM 250 matrix). The β-barrel regions are highlighted in yellow. The charged amino acids located near the central constriction are marked in red. Large extracellular loops L3, L4, and L7, which are folded back into the channel lumen, are indicated by horizontal arrows.

Figure S2
A OccK1



B OccK2

(i)



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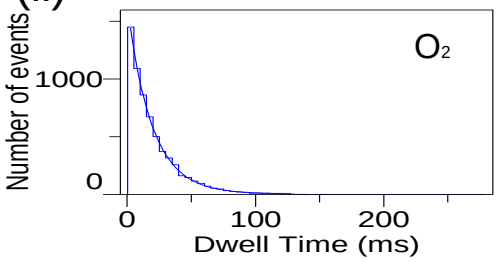

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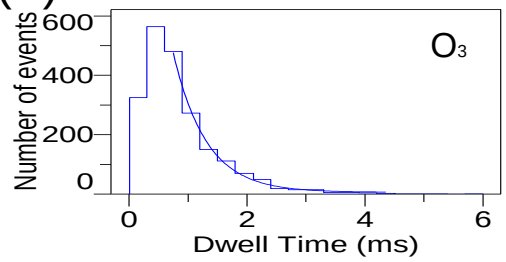

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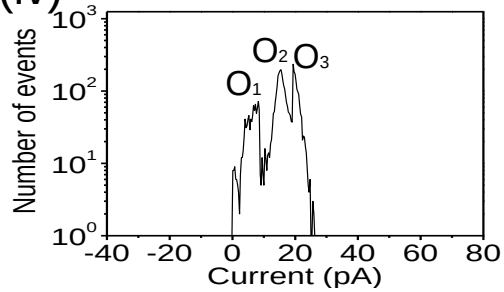
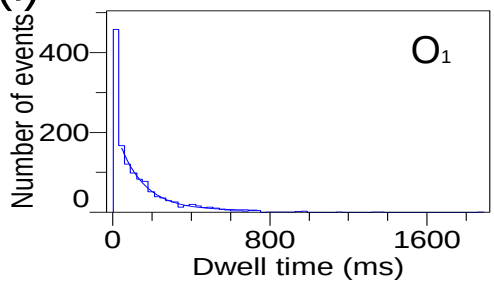


C OccK4

(i)



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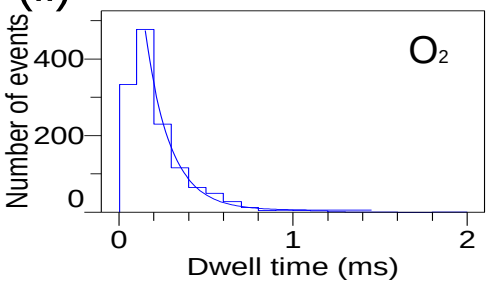

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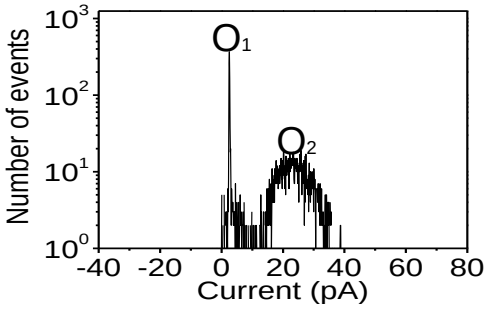
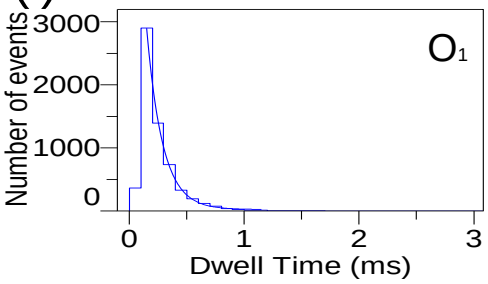
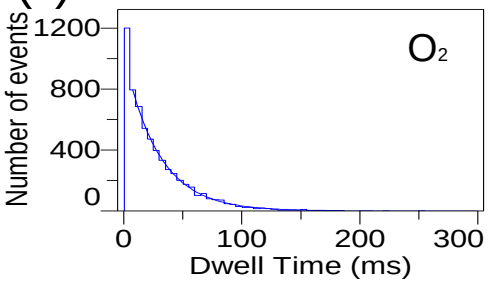


D OccK5

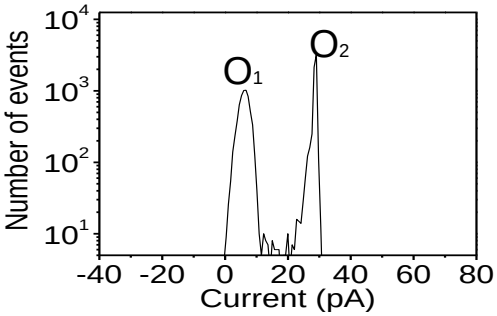
(i)



(ii)

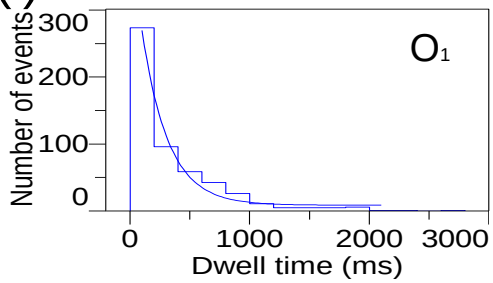


(iii)



E OccK6

(i)



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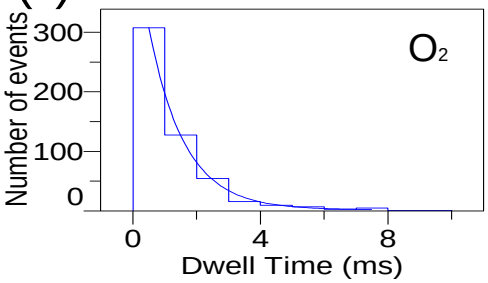
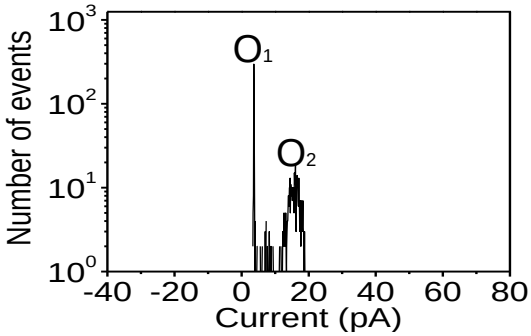


(iii)



F OccK7

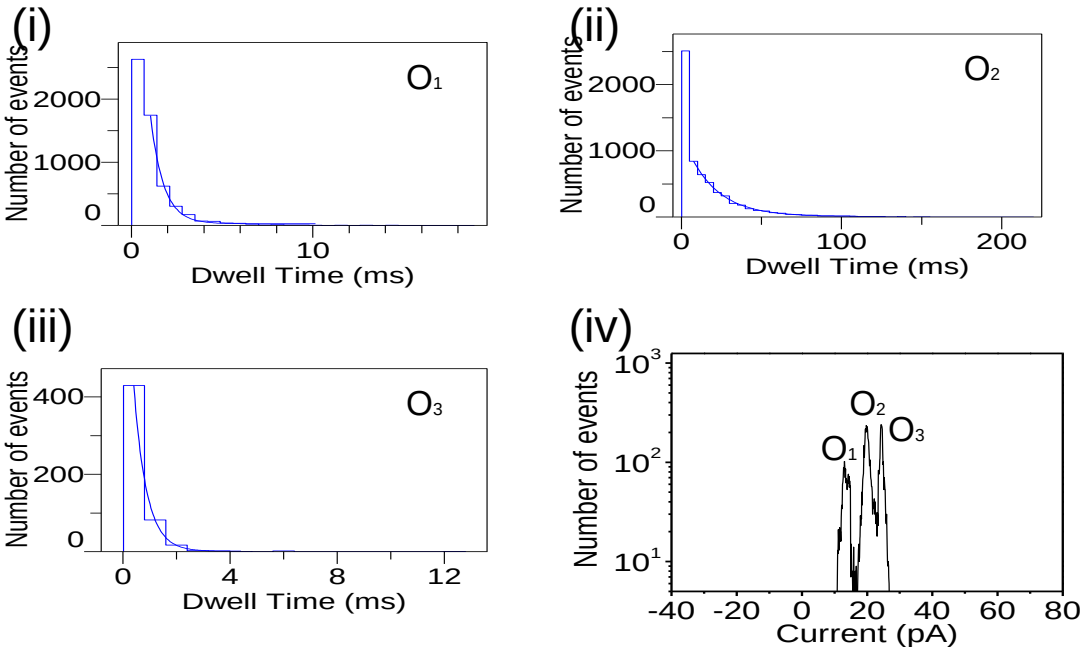


Figure S2 The single-channel sub-state transition analyses of the OccK subfamily members. (**A**) OccK1, (**B**) OccK2, (**C**) OccK4, (**D**) OccK5, (**E**) OccK6 and (**F**) OccK7. For (**A**), (**E**) and (**F**), (i),(ii), and (iii) are the dwell time histograms of the O_1, O_2, and O_3 open sub-states, respectively. In these cases, (iv) represent the fitted current amplitude histograms, indicating the number of events of each open sub-state. For (**B**), (**C**), and (**D**), (i) and (ii) are the dwell time histograms of the O_1 and O_2 open sub-states, whereas (iii) are the fitted current amplitude histograms. Data were collected in 1M KCl, 10 mM potassium phosphate, pH=7.4, and at a transmembrane potential of +60 mV. For OccK4 and OccK6, the traces were low-pass Bessel filtered at 5 kHz. In the case of the other protein channels, we used a filtering frequency of 2 kHz. All the dwell time histograms are fitted to a single-exponential distribution, as judged by log likelihood ratio (LLR) tests with a confidence level of 95% (*1;2*). The fitting results of the dwell time histograms were the following: τ_{O1} (OccK1) = 2.37 ± 0.06 ms; τ_{O2} (OccK1) = 6.09 ± 0.08 ms; τ_{O3} (OccK1) = 4.79 ± 0.06 ms ;τ_{O1} (OccK2) = 0.38 ± 0.02 ms; τ_{O2} (OccK2) = 19.16 ± 0.23 ms; τ_{O3} (OccK2) = 0.56 ± 0.02 ms; τ_{O1} (OccK4) = 129 ± 6 ms; τ_{O2} (OccK4) = 0.14 ± 0.01 ms; τ_{O1} (OccK5) = 29.1 ± 0.4 pS; τ_{O2} (OccK5) = 0.12 ± 0.01 ms; τ_{O1} (OccK6) = 219 ± 20 ms; τ_{O2} (OccK6) = 1.11 ± 0.03 ms; τ_{O1} (OccK7) = 0.72 ± 0.03 ms; τ_{O2} (OccK7) = 19.1 ± 0.3 ms; τ_{O3} (OccK7) = 0.48 ± 0.01 ms.

A three-open sub-state kinetic model for the current fluctuations of the OccK proteins

Using standard formalisms of chemical kinetics for single-molecule fluctuations of the OccK protein channel (*3*), we have the following system of partial differential equations (*4-6*):

$$\frac{dP_{O1}}{dt} = -k_{O1\to O2}P_{O1} + k_{O2\to O1}P_{O2}$$
$$\frac{dP_{O2}}{dt} = +k_{O1\to O2}P_{O1} - k_{O2\to O1}P_{O2} + k_{O3\to O2}P_{O3} - k_{O2\to O3}P_{O2} \quad \text{(S1)}$$
$$\frac{dP_{O3}}{dt} = -k_{O3\to O2}P_{O3} + k_{O2\to O3}P_{O2}$$

where P_{O1}, P_{O2} and P_{O3} are the probabilities to occupy the O_1, O_2 and O_3 sub-states, respectively. These probabilities are defined by the following expressions (*4-6*):

$$P_{O1} = \frac{T_{O1}}{T} = \frac{N_{O1}\tau_{O1}}{T} = f_{O1}\tau_{O1}$$
$$P_{O2} = \frac{T_{O2}}{T} = \frac{N_{O2}\tau_{O2}}{T} = (f_{O1} + f_{O3})\tau_{O2} \quad \text{(S2)}$$
$$P_{O3} = \frac{T_{O3}}{T} = \frac{N_{O3}\tau_{O3}}{T} = f_{O3}\tau_{O3}$$

Here, T_{O1}, T_{O2} and T_{O3} are the total times occupied by the O_1, O_2 and O_3 sub-states, respectively. N_{O1}, N_{O2} and N_{O3} are the total recorded events that correspond to the O_1, O_2 and O_3 sub-states, respectively. T indicates the total recording time. f and τ denote the event frequency and the average dwell time for a well-defined sub-state, respectively. The equations (S2) show two components for the O_2 state, corresponding to transitions toward the O_1 and O_3 sub-states. In other words, the well made by the O_2 sub-state is flanked by two barriers for reaching the O_1 and O_3 sub-states.

The rates for reaching the O_1 and O_3 sub-states are just the corresponding event frequencies, which are normalized to the P_{O2} probability:

$$k_{O2\to O1} = \frac{f_{O1}}{P_{O2}} = \frac{f_{O1}}{1 - f_{O1}\tau_{O1} - f_{O3}\tau_{O3}}$$
$$k_{O2\to O3} = \frac{f_{O3}}{P_{O2}} = \frac{f_{O3}}{1 - f_{O1}\tau_{O1} - f_{O3}\tau_{O3}} \quad \text{(S3)}$$

At equilibrium, the partial derivatives of equations (S1) are zero, since the event probabilities are constant. Therefore,

$$k_{O1\to O2} = \frac{1}{\tau_{O1}}$$
$$k_{O3\to O2} = \frac{1}{\tau_{O3}} \quad \text{(S4)}$$

The equations (S3) and (S4) indicate that the four rates, which describe the kinetic scheme with three open sub-states, can be calculated using the event frequencies and the average dwell times of the flanked O_1 and O_3 sub-states. In addition, the equations (S3) and (S4) confirm the general rule that the average dwell time in a particular sub-state is given by the reciprocal of the sum of the kinetic rate constants for the transitions occurring away from that respective sub-state (*3;7-9*):

$$\frac{1}{\tau_{O2}} = k_{O2\to O1} + k_{O2\to O3} \quad \text{(S5)}$$

<u>Table S1</u>: The standard free energies corresponding to various gating transitions of the OccK1 protein. (A) 1 M KCl; (B) 2 M KCl; (C) 3 M KCl; (D) 4 M KCl. The buffer solution contained 10 mM potassium phosphate, pH 8.0. All standard free energy (ΔG) values are given in $k_B T$. Data represent averages ± SDs over a number of at least three distinct single-channel electrical recordings.

A.

U (mV)	$\Delta G_{O1\rightarrow O2}$	$\Delta G_{O3\rightarrow O2}$
-80	-2.8 ± 0.2	-1.6 ± 0.1
-60	-3.4 ± 0.2	-1.6 ± 0.1
-40	-4.4 ± 0.5	-1.5 ± 0.1
-20	-4.9 ± 0.6	-1.8 ± 0.1
+20	-3.7 ± 0.1	-2.0 ± 0.1
+40	-3.4 ± 0.2	-1.6 ± 0.3
+60	-3.3 ± 0.5	-1.8 ± 0.2
+80	-2.6 ± 0.1	-1.6 ± 0.2

B.

U (mV)	$\Delta G_{O1\rightarrow O2}$	$\Delta G_{O3\rightarrow O2}$
-80	-3.0 ± 0.2	-1.2 ± 0.3
-60	-3.3 ± 0.4	-1.1 ± 0.3
-40	-3.6 ± 0.3	-0.8 ± 0.5
-20	-3.7 ± 0.1	-1.1 ± 0.4
+20	-3.5 ± 0.4	-1.1 ± 0.3
+40	-3.5 ± 0.4	-0.9 ± 0.3
+60	-3.3 ± 0.5	-1.4 ± 0.2
+80	-3.3 ± 0.5	-1.4 ± 0.2

C.

U (mV)	$\Delta G_{O1\rightarrow O2}$	$\Delta G_{O3\rightarrow O2}$
-80	-2.7 ± 0.3	-0.9 ± 0.5
-60	-3.3 ± 0.1	-0.9 ± 0.3
-40	-3.9 ± 0.2	-1.5 ± 0.1
-20	-4.0 ± 0.3	-1.3 ± 0.1
+20	-4.2 ± 0.3	-1.6 ± 0.3
+40	-4.0 ± 0.6	-1.4 ± 0.1
+60	-4.0 ± 0.9	-1.3 ± 0.1
+80	-3.6 ± 1.1	-1.1 ± 0.2

D.

U (mV)	$\Delta G_{O1\rightarrow O2}$	$\Delta G_{O3\rightarrow O2}$
-80	-3.2 ± 0.1	-0.4 ± 0.4
-60	-3.0 ± 0.1	-0.1 ± 0.4
-40	-3.6 ± 0.4	-0.2 ± 0.3
-20	-3.9 ± 0.2	-0.3 ± 0.2
+20	-4.8 ± 0.3	-0.4 ± 0.2
+40	-4.2 ± 0.6	-0.4 ± 0.3
+60	-4.1 ± 0.6	-0.4 ± 0.2
+80	-4.2 ± 0.6	-0.3 ± 0.2

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