Supplementary Information

The Cation Selectivity is a Conserved Feature in the OccD Subfamily of *Pseudomonas aeruginosa*

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Fig. S1

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OccD1      -------------------------DAFVSDQAEAKGFIEDSSLDLLLRNYYFNRDGKSG 35
OccD2      -----------------------------DEGEAKEGFIEGSSLQLLTRNYYFNHDRRHA 31
OccD3      ADEQENPPAPDNPSYAAEVQSIPSVAKPIKGQAGATGLVEGQSLTLTTRNFYSRENMKDS 60
OccD4      ---------------------------EENAERSKEGFIEGSEVNLLLRNFYFNRDFRKG 33
OccD5      --------------------------------GDG--GFVEDSELQFLARTYYFNRDYRDS 27
OccD6      ----------------------------NDQEAAKGFVEDSHLDLFFRNGYISRDYKHG 31
                                          *::*.. : :  *. *  .: : .

OccD1      SGDRVD---------------WTQGFLTTYESGFTQGTVGFGVDAFGYLGLKLDGTSDKT 80
OccD2      SGHDSK--------------EWAQGFIATFQSGYTPGVVGFGVDAYGMLGLKLDGGGGTG 77
OccD3      FTFRIPKAGG-GSQRIHQRNAWVQGTVLKYSSGYTQGTVGFGFDVAAFNEIALERGKGRI 119
OccD4      QSSPAGG---------GYTEEWVQGFMANFSSGFTQGTLGVGIDAFAQLGVRLDSGGGRS 84
OccD5      PNNAGRNRFKPRSERNGYREEATQGLRLQFASGYTPGSLGFGLDAHAMLGLQLDSGGGRT 87
OccD6      RQDKAE---------------WGQAATATFTSGFTQGTVGVGVDAFGLYAVRLDGGKGRS 76
                                  *.    : **:* * :*.*.*. .   : *:   .

OccD1      GTG----NLPVMNDGKP---RDDYSRA-------GGAVKVRISKTMLKWGEMQPTAPVFA 126
OccD2      GTS----ILPITSPSKE---GYESGKAPDEFSSGGAALKIRAFDTELKLGDQFLSNPVVA 130
OccD3      GGGG---NRTLANSDGE--ALGEWSKLG------VANIRLRASNTEFKAGRFLVNTPVFS 168
OccD4      GAGGSVDLLPYDDQGRP---QDDYSRA-------GGAVKLRWYGTVLRVGDVFPETPVIQ 134
OccD5      GTG----NLPVGADGHP---DHRYGKV-------GGALRLRHGETRLKYGQTTTSAPVFA 133
OccD6      GAA----GIDFFKQGDSGSAADDLSKG-------GAAVKFRISNTVLKYGDQMPSLPVLS 125
           * .           .         .:         . ::.*   * :: *      **.

OccD1      AGGSRLFPQTATGFQLQSSEFEG-LDLEAGHFTEGKEPTTVKSRGELYATY-----AGET 180
OccD2      GGESRMLPQTFRGVSLTNNSFED-LTLTAGQVSFTKYYNQSGHR-RLGSYYGELPGD-RD 187
OccD3      YIDNRALPSSFTGFAVTSEELDN-LSLQAGSFRKVSPRTGSGDEDMTTEYG----TRQVK 223
OccD4      YGNSRLFPSSFRGFTLVNDSLAKGLTLQAGKLNSMTQPNSTSGSDDFYSFY---TGRRID 191
OccD5      ASSNRTLAGMAYGLLLEDRSFDG-LLLEGGRFTAASGPGESKVRGDISTVYGRLGAYPVR 192
OccD6      YDNSRLLPESYSGTLITSKEIEG-LELNAGRFT-------AESRKSAEGRD-----SGG- 171
              .* :.    *  : . .:   * * .* .

OccD1      AKSADFIGGRYAITDNLSASLYGAELEDIYRQYYLNSNYTI-PLASDQSLGFDFNIYRTN 239
OccD2      SHHLSWLGGTWGGIEGFTSSLYAAELQNVWKQYYADVDYTY-EIDDNWSLNPGAHYYKTV 246
OccD3      GDRLNYLGGNYKPLDGLEISLYGSHFQDVWNQYYLGVTHDIGDLENGIALRTAFNGYHTG 283
OccD4      SPWVAYAGGDYQATEHWSVSLYGSRQKDAWDQYYAGTSFNY-PLDDKLSLLGGANYYKVK 250
OccD5      LDAVGFLGGQWQATERLQLSLYASRFDDIWQQAYFGASHRQ-PLGGERALRVDLDAYRTR 251
OccD6      LKSINVFGGKYAFTDHFNASLYASDVEDVLKKQYINLNYTI-PLQADQSLNFDFNGYRTK 230
                 ** :   :    ***.:  .:   : * .  .    :    :*    . *:.

OccD1      DE----GKAKAGDISNTTWSLAAAYTLDAHTFTLAYQKVHGDQPFDYIGFGRNG-SGAGG 294
OccD2      DS----GDSLLGRIDNNTYSLHFAVGYRQHTVTAVLQKVNGNTPFDYINQGD-------- 294
OccD3      DT----GAREAGYIDNDTWSLAFTLGHRAHALTLAYQQVDGNEYFDYVHETS-------- 331
OccD4      DQ----GRQVMGELDNDIWSVRGGFAYGPHQVLLSYQRNNGDDDFDYLRQTD-------- 298
OccD5      DS----GQSRFGRIDTLTSSLALGYEHGPQRITLAYQRVHGEQPFDYMAFGDGR----SS 303
OccD6      LDSDFADQNFNGNRDNKIWSLAASYTIDAHTFMIAHQRNTGDTGYNYGWYQNAGGIGDGG 290
                .    *  ..   *:        : .    *:  *:  ::*

OccD1      DSIFLANSVQYSDFNGPGEKSWQARYDLNLASYGVPGLTFMVRYING-KDIDGTKMSDNN 353
OccD2      -SIFLDNSQQYSDFNGPNEKSWKLQYDYDFVALGLPGLSASASYSRGKLDLTRVDPDSPG 353
OccD3      -AIFLANS-MLADYNSPNEKSAQIRYETDWSYYGVPGLSTGVWYVKGWDIDGTHYDGDRN 389
OccD4      -SIYLDNSIQYSDFNSPKERSLMLRYDLDMAAFGVPGLSFMTRYGKGWDADYSNANSVYM 357
OccD5      ASMVLANSVGYSDFNGPGERSWQLRYDLDLGALGLPGLSLHALHARGRAGASASSAAESI 363
OccD6      TTIWLANS-YWSDFNAEDERSWQVSYALDFAKYGVPGLTYRVAYVRG-DNIKTAETSN-- 346
            :: * **   :*:*.  *:*    *  :    *:***:  . : .*
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OccD1        VGYKNYGYGED---GKHHETNLEAKYVVQSGPAKDLSFRIRQAWHRANADQG--EGDQNE 408
OccD2        YGGWYSADGKN---AKHWERDLDLQYVVQGGPAKDLSLRLRWATHRGTGGYSAVDNDIDE 410
OccD3        GAYGNYAEVRAQDGEKHHELGLMAAYKVQNGPIKDSTFKLTYMMHKASQNQ--IDGSVNE 447
OccD4        RTDANGNPLTN---QGRWERDVEVKYVVQGGAAKDLAFRVRQATVR-SDSF---ESDLDE 410
OccD5        YAGLYGRDG------RHRENDLGFAYRVKAGPLAGLALRASQAWHRGNASY--LDGDIDE 415
OccD6        --------------GKEREIFNQVQYVVQSGPAKDLTLRLRSSFLRVSNDARSYNDDGNE 392
                                . *      * *: *.  . :::      : . .    :.. :*

OccD1        FRLIVDYPLSIL 420
OccD2        YRVIVDYPIDVF 422
OccD3        LRLVSTFPFNLL 459
OccD4        VRLIVEYPLQVL 422
OccD5        TRLVVDYSRSIW 427
OccD6        IRAFVEYPFSVF 404
              * .  :. .:
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<u>Figure S1</u> Amino acid sequence analysis of the OccD proteins. Sequence homology analysis generated by Clustal Omega (Version 1.0.3, The European Bioinformatics Institute,UK), where conserved sites are marked with '*', ':', or '.' in the alignment . 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 by comparing sequences to the PBD generated from the crystal structures of OccD1, OccD2, and OccD3. Amino acid residues that are part of the basic ladder are highlighted in red. Amino acid residues that are part of the basic ladder are highlighted in red.

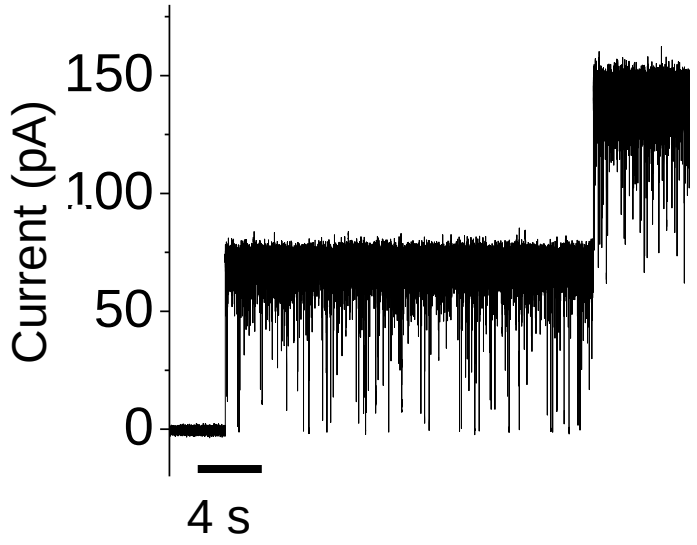

Fig. S2

<u>Figure S2</u> A two-channel insertion of the OccD3 channel into a planar lipid bilayer. The applied transmembrane potential was of +80 mV. The single-channel electrical trace was low-pass Bessel filtered at a frequency of 2 kHz.