Biophysical Journal, Volume *110*

Supplemental Information

Structurally Distinct Cation Channelrhodopsins from Cryptophyte Algae

Elena G. Govorunova, Oleg A. Sineshchekov, and John L. Spudich

Supporting Material

Structurally distinct cation channelrhodopsins from cryptophyte algae

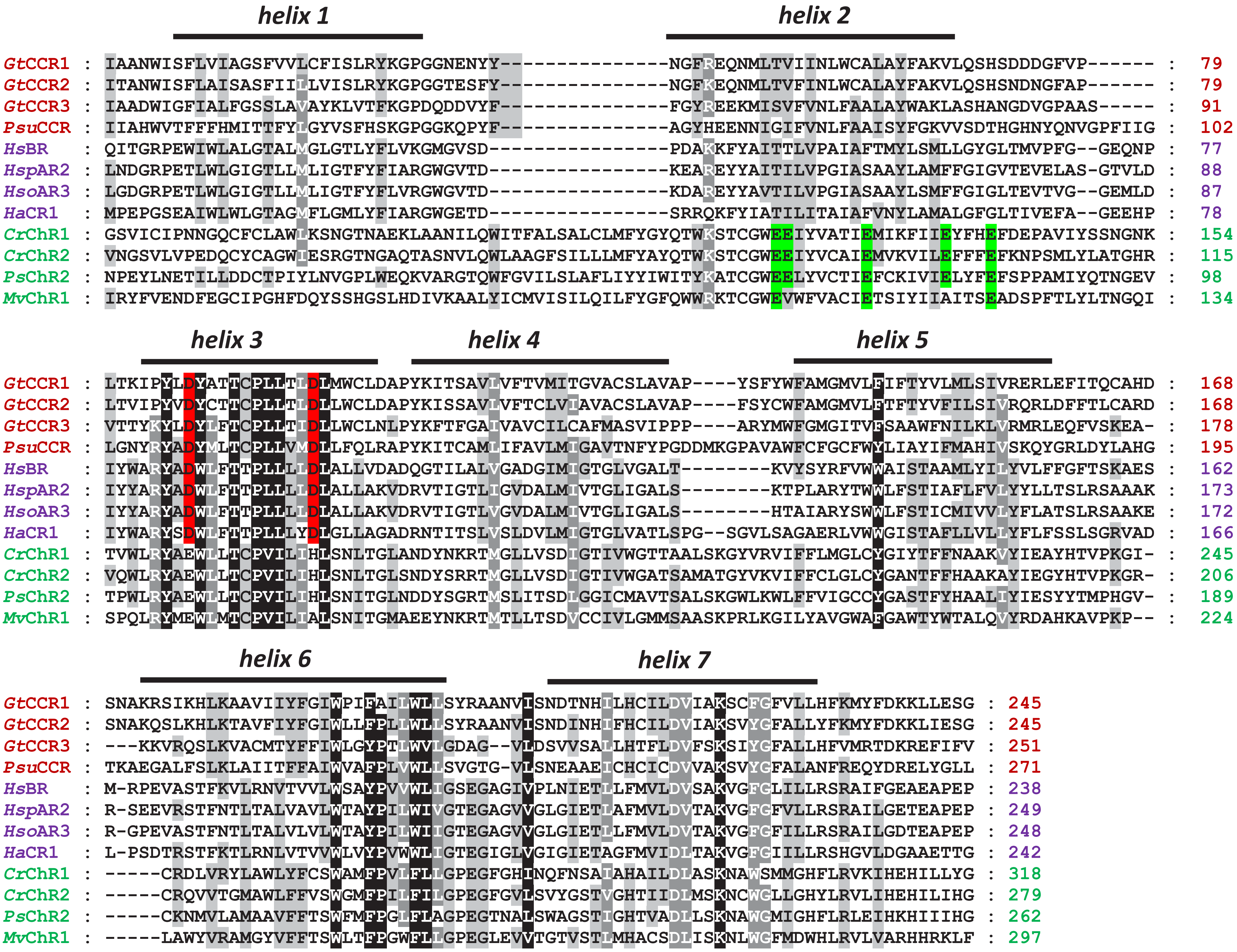
Elena G. Govorunova, Oleg A. Sineshchekov, and John L. Spudich

Center for Membrane Biology, Department of Biochemistry and Molecular Biology, University of Texas Health Science Center at Houston McGovern Medical School, Houston, Texas, USA

Address reprint requests and inquiries to John.L.Spudich@uth.tmc.edu

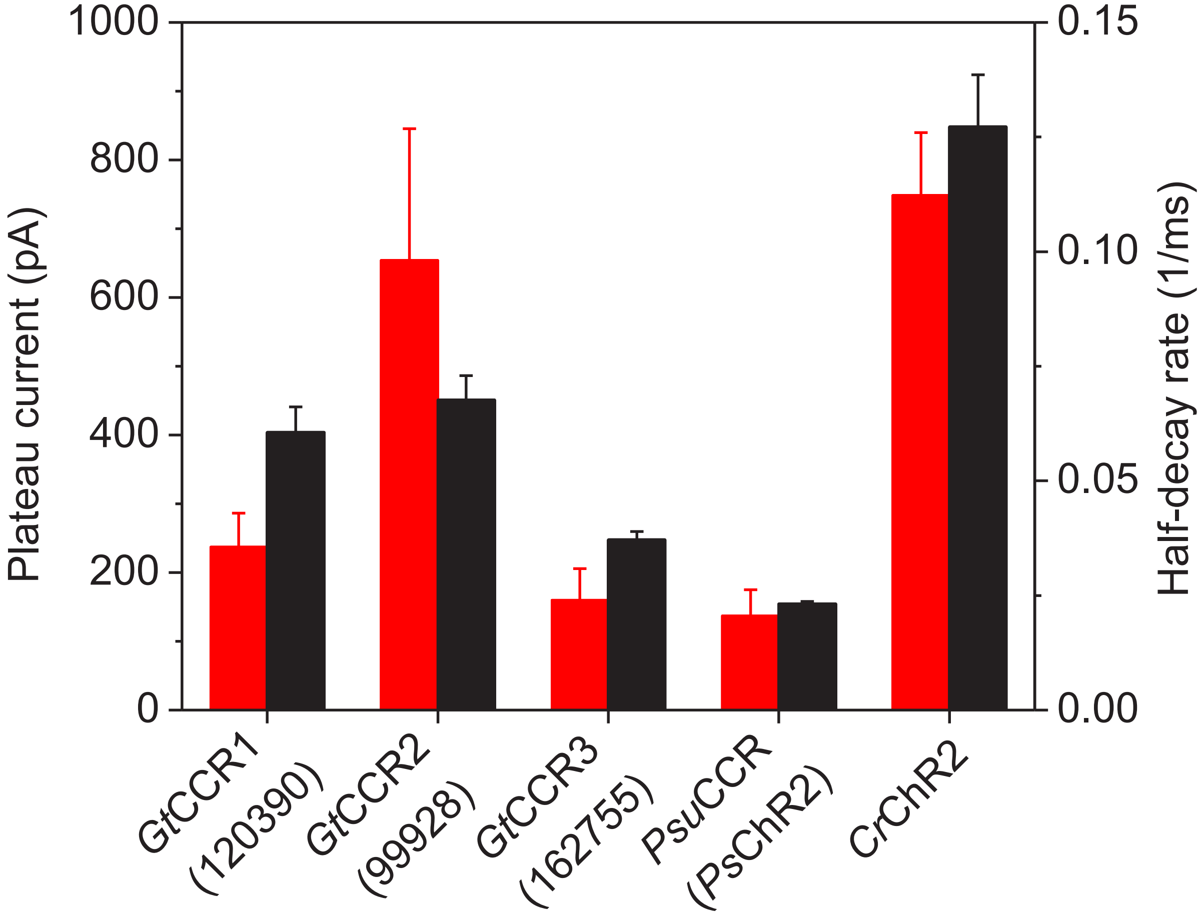

protein 110 [synthetic construct]
protein 99 [synthetic construct]
hypothetical protein GUITHDRAFT 159333 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 85745 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 62605 [Guillardia theta CCMP2712]
protein 72 [synthetic construct]
opsin 2 [Cyanophora paradoxa]
hypothetical protein GUITHDRAFT 86360 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 106800 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 99837 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 109252 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 152884 [Guillardia theta CCMP2712]
hypothetical protein [Nodosilinea nodulosa]
hypothetical protein [Kushneria aurantia]
hypothetical protein [Halomonas sp. PR-M31]
rhodopsin [Chroococcidiopsis thermalis]
rhodopsin [Gloeocapsa sp. PCC 7428]
hypothetical protein [Chlorogloeopsis fritschii]
hypothetical protein [Leptolyngbya boryana]
bacteriorhodopsin [Nostoc sp. NIES-3756]
bacteriorhodopsin [Chamaesiphon minutus PCC 6605]
hypothetical protein [Tolypothrix bouteillei]
rhodopsin [Natronomonas moolapensis]
bacteriorhodopsin [Natronomonas moolapensis 8.8.11]
bacteriorhodopsin [uncultured haloarchaeon]
rhodopsin [Halobacterium salinarum]
bacteriorhodopsin [Halorubrum coriense]
ArchT-GFP [synthetic construct]
rhodopsin [Halorubrum sp. 5]
bacteriorhodopsin [uncultured archaeon A07HB70]
bacteriorhodopsin [uncultured haloarchaeon]
bacteriorhodopsin [uncultured haloarchaeon]
bacteriorhodopsin [uncultured archaeon]
bacteriorhodopsin [uncultured haloarchaeon]
bacteriorhodopsin [Synechocystis sp. PCC 7509]
rhodopsin [Leptolyngbya sp. NIES-3755]
bacteriorhodopsin [Chamaesiphon minutus]
rhodopsin [Hassallia byssoidea]
rhodopsin [Scytonema tolypothrichoides]
rhodopsin [Tolypothrix bouteillei]
rhodopsin [Tolypothrix campylonemoides]
hypothetical protein [Mastigocladopsis repens]
rhodopsin [Aliterella atlantica]
hypothetical protein [Erythrobacter sp. KA37]
hypothetical protein [Sphingomonas sp. PAMC 26621]
hypothetical protein [Sphingomonas sp. Leaf38]
hypothetical protein [Sphingomonas sp. Leaf34]
hypothetical protein [Sphingomonas sp. STIS6.2]
rhodopsin [Methylobacterium sp. GXS13]
sensory rhodopsin I [Haloarcula vallismortis]
bacteriorhodopsin [uncultured archaeon A07HR67]
rhodopsin [Halorubrum lacusprofundi]
sensory rhodopsin-2 [Halapricum salinum]
sensory rhodopsin-2 [Halorhabdus tiamatea]
sensory rhodopsin-2 [Haloarcula sp. SL3]
sensory rhodopsin 1 [Haloarcula vallismortis]
sensory rhodopsin 1 [Haloarcula amylolytica]
sensory rhodopsin-2 [Haloarcula sp. CBA1127]
sensory rhodopsin 1 [Haloarcula argentinensis]
sensory rhodopsin 1 [Haloarcula californiae]
sensory rhodopsin-2 [Haloarcula hispanica]
sensory rhodopsin 1 [Haloarcula sinaiiensis]
sensory rhodopsin-2 [Haloarcula marismortui]
sensory rhodopsin-2 [Natronomonas pharaonis]
sensory rhodopsin-2 [Halobacterium hubeiense]
sensory rhodopsin II [Halobacterium hubeiense]
sensory rhodopsin-2 [Halobacterium salinarum]
family A G protein-coupled receptor-like protein [Phialocephala scopiformis]
hypothetical protein COCCADRAFT 26476 [Bipolaris zeicola 26-R-13]
hypothetical protein SBOR 9638 [Sclerotinia borealis F-4157]
hypothetical protein BC1G 13906 [Botrytis cinerea B05.10]
hypothetical protein SS1G 04339 [Sclerotinia sclerotiorum 1980]
hypothetical protein PV05 11623 [Exophiala xenobiotica]
hypothetical protein EPUS 05370 [Endocarpon pusillum Z07020]
hypothetical protein PV06 03044 [Exophiala oligosperma]
hypothetical protein PV08 05307 [Exophiala spinifera]
hypothetical protein G647 06224 [Cladophialophora carrionii CBS 160.54]
hypothetical protein A1O7 06308 [Cladophialophora yegresii CBS 114405]
hypothetical protein MYCFIDRAFT 211441 [Pseudocercospora fijiensis CIRAD86]
related to YRO2-putative plasma membrane protein
transcriptionally regulated by Haa1p [Melanopsichium pennsylvanicum 4]
hypothetical protein TREMEDRAFT 17826 [Tremella mesenterica DSM 1558]
hypothetical protein MELLADRAFT 39586 [Melampsora larici-populina 98AG31]
hypothetical protein SCLCIDRAFT 141768 [Scleroderma citrinum Foug A]
hypothetical protein PFL1 01891 [Anthracocystis flocculosa PF-1]
protein of bacterial rhodopsin family [Rhodotorula toruloides NP11]
Rhodopsin
retinal binding site [Xanthophyllomyces dendrorhous]
hypothetical protein G7K 1936-t1 [Saitoella complicata NRRL Y-17804]
hypothetical protein PDE 04448 [Penicillium oxalicum 114-2]
hypothetical protein SNOG 00341 [Parastagonospora nodorum SN15]
hypothetical protein LEMA P114110.1 [Leptosphaeria maculans JN3]
family A G protein-coupled receptor-like protein [Sphaerulina musiva SO2202]
hypothetical protein DOTSEDRAFT 75607 [Dothistroma septosporum NZE10]
FDD123 protein [Verticillium dahliae VdLs.17]
hypothetical protein BN1723 001539 [Verticillium longisporum]
hypothetical protein BN1708 017586 [Verticillium longisporum]
hypothetical protein BN1723 017257 [Verticillium longisporum]
putative bacteriorhodopsin [Colletotrichum sublineola]
bacteriorhodopsin [Colletotrichum simmondsii]
bacteriorhodopsin [Colletotrichum salicis]
heat shock protein 30 [Colletotrichum orbiculare MAFF 240422]
heat shock protein 30 [Colletotrichum gloeosporioides Nara gc5]
bacteriorhodopsin [Colletotrichum gloeosporioides Cg-14]
bacteriorhodopsin [Colletotrichum graminicola M1.001]
hypothetical protein S40285 08856 [Stachybotrys chlorohalonata IBT 40285]
hypothetical protein S7711 01519 [Stachybotrys chartarum IBT 7711]
hypothetical protein GGTG 05062 [Gaeumannomyces graminis var. tritici R3-111a-1]
Family A G protein-coupled receptor-like protein [Glarea lozoyensis ATCC 20868]
hypothetical protein M7I 6795 [Glarea lozoyensis 74030]
opsin-like protein [Marssonina brunnea f. sp. multigermtubi MB m1]
hypothetical protein AB675 5839 [Phialophora attae]
hypothetical protein HMPREF1541 00158 [Cyphellophora europaea CBS 101466]
hypothetical protein PV10 08466 [Exophiala mesophila]
hypothetical protein HMPREF1120 02861 [Exophiala dermatitidis NIH/UT8656]
Opsin-1 [Taphrina deformans PYCC 5710]
hypothetical protein Micbo1qcDRAFT 167924 [Microdochium bolleyi]
archaerhodopsin-2 precursor [Sphaerulina musiva SO2202]
bacteriorhodopsin [Aureobasidium pullulans EXF-150]
hypothetical protein DOTSEDRAFT 133285 [Dothistroma septosporum NZE10]
hypothetical protein PV11 03940 [Exophiala sideris]
hypothetical protein AC579 7431 [Pseudocercospora musae]
hypothetical protein AC578 1260 [Mycosphaerella eumusae]
hypothetical protein FAVG1 09553 [Fusarium avenaceum]
hypothetical protein FGSG 03064 [Fusarium graminearum PH-1]
hypothetical protein FG05 03064 [Fusarium graminearum]
hypothetical protein FPSE 02162 [Fusarium pseudograminearum CS3096]
opsin [Fusarium langsethiae]
opsin-like protein [Stemphylium lycopersici]
hypothetical protein PTT 09940 [Pyrenophora teres f. teres 0-1]
Rhodopsin bacterial [Macrophomina phaseolina MS6]
family A G protein-coupled receptor-like protein [Zymoseptoria brevis]
hypothetical protein MYCGRDRAFT 106573 [Zymoseptoria tritici IPO323]
hypothetical protein AC579 8497 [Pseudocercospora musae]
hypothetical protein AC578 3015 [Mycosphaerella eumusae]
hypothetical protein MYCFIDRAFT 56178 [Pseudocercospora fijiensis CIRAD86]
hypothetical protein FE78DRAFT 107577 [Acidomyces richmondensis]
hypothetical protein AUEXF2481DRAFT 3502 [Aureobasidium subglaciale EXF-2481]
family A G protein-coupled receptor-like protein [Aureobasidium melanogenum CBS 110
family A G protein-coupled receptor-like protein [Aureobasidium pullulans EXF-150]
opsin-like protein [Aureobasidium namibiae CBS 147.97]
putative opsin-like protein [Eutypa lata UCREL1]
putative opsin-like protein [Rosellinia necatrix]
hypothetical protein PFICI 15171 [Pestalotiopsis fici W106-1]
hypothetical protein M422DRAFT 215320 [Sphaerobolus stellatus SS14]
hypothetical protein MELLADRAFT 50312 [Melampsora larici-populina 98AG31]
protein of unknown function [Taphrina deformans PYCC 5710]
Opsin-1 [Taphrina deformans PYCC 5710]
putative opsin 1 [Phaeomoniella chlamydospora]
hypothetical protein EPUS 00641 [Endocarpon pusillum Z07020]
Opsin-like protein [Acremonium chrysogenum ATCC 11550]
hypothetical protein [Podospora anserina S mat+]
hypothetical protein S40285 06423 [Stachybotrys chlorohalonata IBT 40285]
hypothetical protein S40288 06367 [Stachybotrys chartarum IBT 40288]
opsin-1 [Fusarium graminearum PH-1]
hypothetical protein FG05 07554 [Fusarium graminearum]
hypothetical protein COCC4DRAFT 158477 [Bipolaris maydis ATCC 48331]
opsin-1 [Sclerotinia sclerotiorum 1980]
hypothetical protein BC1G 02456 [Botrytis cinerea B05.10]
hypothetical protein Z520 10478 [Fonsecaea multimorphosa CBS 102226]
hypothetical protein PV07 05899 [Cladophialophora immunda]
hypothetical protein Z517 07125 [Fonsecaea pedrosoi CBS 271.37]
hypothetical protein A1O3 08726 [Capronia epimyces CBS 606.96]
hypothetical protein A1O1 01146 [Capronia coronata CBS 617.96]
hypothetical protein A1O7 01290 [Cladophialophora yegresii CBS 114405]
hypothetical protein G647 01353 [Cladophialophora carrionii CBS 160.54]
hypothetical protein PV04 00680 [Capronia semiimmersa]
Opsin-1 [Phialophora attae]
hypothetical protein G7K 4380-t1 [Saitoella complicata NRRL Y-17804]
rhodopsin-like 3 [Oxyrrhis marina]
hypothetical protein L969DRAFT 95897 [Mixia osmundae IAM 14324]
hypothetical protein M422DRAFT 149760 [Sphaerobolus stellatus SS14]
rhodopsin
a chromoprotein [Ktedonobacter racemifer]
rhodopsin [Haloquadratum walsbyi]
bacteriorhodopsin II [Haloquadratum walsbyi C23]
rhodopsin [Haloquadratum walsbyi]
bacteriorhodopsin [Haloquadratum walsbyi J07HQW2]
rhodopsin [Pyrocystis lunula]
rhodopsin [Oxyrrhis marina]
rhodopsin [Oxyrrhis marina]
rhodopsin [Oxyrrhis marina]
hypothetical protein KNAG 0H01270 [Kazachstania naganishii CBS 8797]
hypothetical protein GUITHDRAFT 162755 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 148916 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 148915 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 135937 [Guillardia theta CCMP2712]
protein 100 [synthetic construct]
hypothetical protein GUITHDRAFT 164280 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 164579 [Guillardia theta CCMP2712]
PsChR2 [synthetic construct]
protein 107 [synthetic construct]
protein 109 [synthetic construct]
protein 84 [synthetic construct]
hypothetical protein GUITHDRAFT 107989 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 120390 [Guillardia theta CCMP2712]
hypothetical protein GUITHDRAFT 99928 [Guillardia theta CCMP2712]
proteorhodopsin [Candidatus Pelagibacter ubique]
bacteriorhodopsin [Candidatus Pelagibacter ubique]
proteorhodopsin [Candidatus Pelagibacter ubique HTCC1002]
proteorhodopsin [Candidatus Pelagibacter ubique]
bacteriorhodopsin [Candidatus Pelagibacter ubique]

Supporting figure 1. A phylogenetic tree of 500 closest homologs of the rhodopsin domain of *Guillardia theta* protein model 99928 found in the NCBI non-redundant translated nucleotide database by Position-Specific Iterated BLAST. The hits are color-coded according to the phylogenetic position of their source organisms: orange, cryptophytes; yellow, fungi; olive, other eukaryotes; magenta, haloarchaea; cyan, cyanobacteria; pink, other prokaryotes; not colored, microbial rhodopsins from undefined source organisms. Proteins tested in this study are marked with the black arrows on the right. Some redundant entries have been manually removed by the Dendroscope 3 software (1). The tree was visualized using the EvolView server (2).

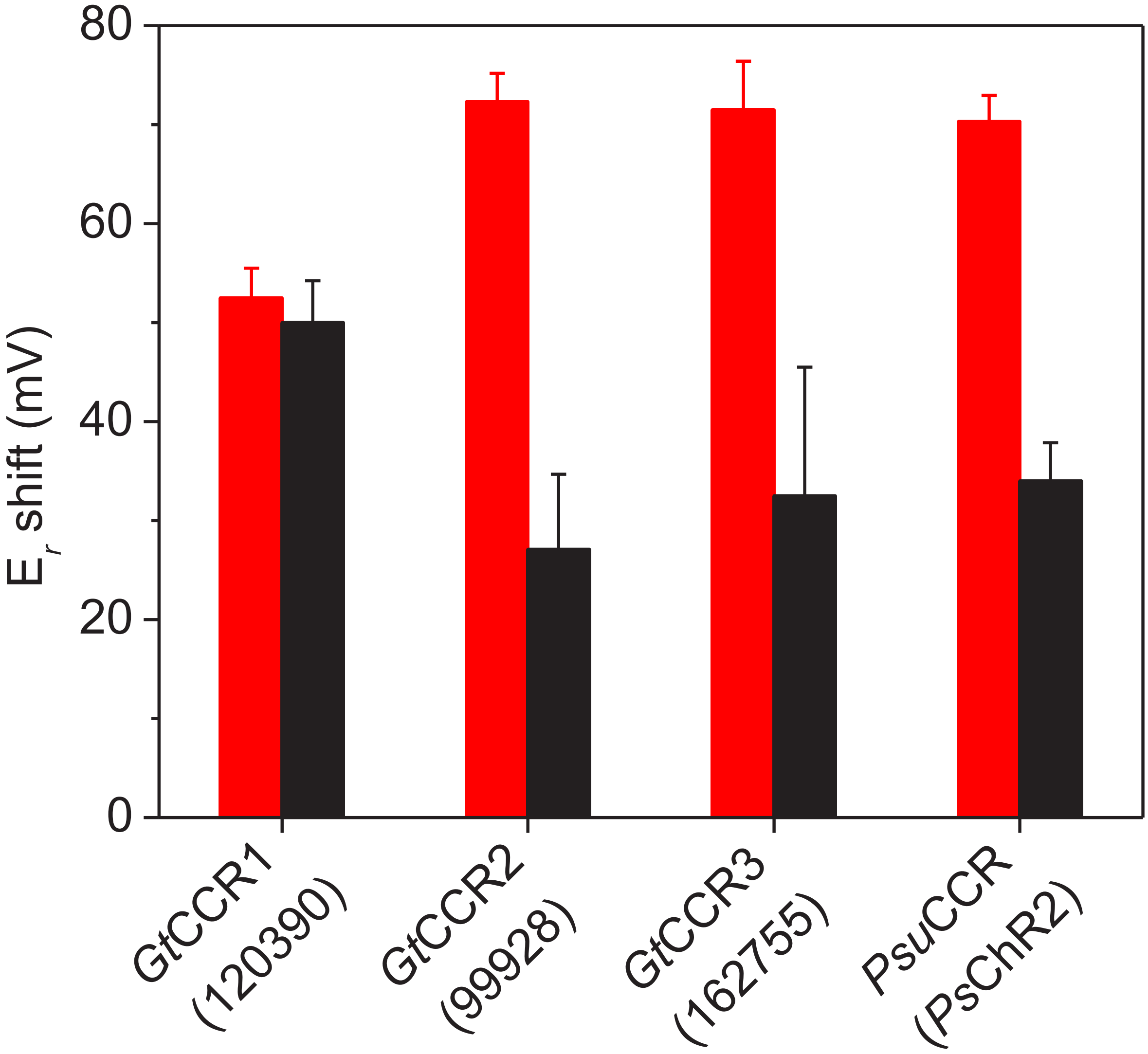


```
                 helix 1                                                   helix 2
GtCCR1  : IAANWISFLVIAGSFVVLCFISLRYKGPGGNENYY---------------NGFREQNMLTVIINLWCALAYFAKVLQSHSDDDGFVP------ : 79
GtCCR2  : ITANWISFLAISASFIILLVISLRYKGPGGTESFY---------------NGFKEQNMLTVFINLWCALAYFAKVLQSHSNDNGFAP------ : 79
GtCCR3  : IAADWIGFIALFGSSLAVAYKLVTFKGPDQDDVYF---------------FGYREEKMISVFVNLFAALAYWAKLASHANGDVGPAAS----- : 91
PsuCCR  : IIAHWVTFFFHMITTFYLGYVSFHSKGPGGKQPYF---------------AGYHEENNIGIFVNLFAAISYFGKVVSDTHGHNYQNVGPFIIG : 102
HsBR    : QITGRPEWIWLALGTALMGLGTLYFLVKGMGVSD----------------PDAKKFYAITTLVPAIAFTMYLSMLLGYGLTMVPFG--GEQNP : 77
HspAR2  : LNDGRPETLWLGIGTLLMLIGTFYFIARGWGVTD----------------KEAREYYAITILVPGIASAAYLAMFFGIGVTEVELAS-GTVLD : 88
HsoAR3  : LGDGRPETLWLGIGTLLMLIGTFYFLVRGWGVTD----------------KDAREYYAVTILVPGIASAAYLSMFFGIGLTEVTVG--GEMLD : 87
HaCR1   : MPEPGSEAIWLWLGTAGMFLGMLYFIARGWGETD----------------SRRQKFYIATILITAIAFVNYLAMALGFGLTIVEFA--GEEHP : 78
CrChR1  : GSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCGWEEIYVATIEMIKFIIEYFHEFDEPAVIYSSNGNK : 154
CrChR2  : VNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQWLAAGFSILLLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHR : 115
PsChR2  : NPEYLNETILLDDCTPIYLNVGPLWEQKVARGTQWFGVILSLAFLIYYIWITYKATCGWEELYVCTIEFCKIVIELYFEFSPPAMIYQTNGEV : 98
MvChR1  : IRYFVENDFEGCIPGHFDQYSSHGSLHDIVKAALYICMVISILQILFYGFQWWRKTCGWEVWFVACIETSIYIIAITSEADSPFTLYLTNGQI : 134

               helix 3                  helix 4                          helix 5
GtCCR1  : LTKIPYLDYATTCPLLTLDLMWCLDAPYKITSAVLVFTVMITGVACSLAVAP----YSFYWFAMGMVLFIFTYVLMLSIVRERLEFITQCAHD : 168
GtCCR2  : LTVIPYVDYCTTCPLLTLDLLWCLDAPYKISSAVLVFTCLVIAVACSLAVAP----FSYCWFAMGMVLFTFTYVFILSIVRQRLDFFTLCARD : 168
GtCCR3  : VTTYKYLDYLFTCPLLTIDLLWCLNLPYKFTFGAIVAVCILCAFMASVIPPP----ARYMWFGMGITVFSAAWFNILKLVRMRLEQFVSKEA- : 178
PsuCCR  : LGNYRYADYMLTCPLLVMDLLFQLRAPYKITCAMLIFAVLMIGAVTNFYPGDDMKGPAVAWFCFGCFWYLIAYIFMAHIVSKQYGRLDYLAHG : 195
HsBR    : IYWARYADWLFTTPLLLLDLALLVDADQGTILALVGADGIMIGTGLVGALT--------KVYSYRFVWWAISTAAMLYILYVLFFGFTSKAES : 162
HspAR2  : IYYARYADWLFTTPLLLLDLALLAKVDRVTIGTLIGVDALMIVTGLIGALS--------KTPLARYTWWLFSTIAFLFVLYYLLTSLRSAAAK : 173
HsoAR3  : IYYARYADWLFTTPLLLLDLALLAKVDRVTIGTLVGVDALMIVTGLIGALS--------HTAIARYSWWLFSTICMIVVLYFLATSLRSAAKE : 172
HaCR1   : IYWARYSDWLFTTPLLLYDLGLLAGADRNTITSLVSLDVLMIGTGLVATLSPG--SGVLSAGAERLVWWGISTAFLLVLLYFLFSSLSGRVAD : 166
CrChR1  : TVWLRYAEWLLTCPVILIHLSNLTGLANDYNKRTMGLLVSDIGTIVWGTTAALSKGYVRVIFFLMGLCYGIYTFFNAAKVYIEAYHTVPKGI- : 245
CrChR2  : VQWLRYAEWLLTCPVILIHLSNLTGLSNDYSRRTMGLLVSDIGTIVWGATSAMATGYVKVIFFCLGLCYGANTFFHAAKAYIEGYHTVPKGR- : 206
PsChR2  : TPWLRYAEWLLTCPVILIHLSNITGLNDDYSGRTMSLITSDLGGICMAVTSALSKGWLKWLFFVIGCCYGASTFYHAALIYIESYYTMPHGV- : 189
MvChR1  : SPQLRYMEWLMTCPVILIALSNITGMAEEYNKRTMTLLTSDVCCIVLGMMSAASKPRLKGILYAVGWAFGAWTYWTALQVYRDAHKAVPKP-- : 224

                 helix 6                            helix 7
GtCCR1  : SNAKRSIKHLKAAVIIYFGIWPIFAILWLLSYRAANVISNDTNHILHCILDVIAKSCFGFVLLHFKMYFDKKLLESG : 245
GtCCR2  : SNAKQSLKHLKTAVFIYFGIWLLFPLLWLLSYRAANVISNDINHIFHCILDVIAKSVYGFALLYFKMYFDKKLIESG : 245
GtCCR3  : ---KKVRQSLKVACMTYFFIWLGYPTLWVLGDAG--VLDSVVSALLHTFLDVFSKSIYGFALLHFVMRTDKREFIFV : 251
PsuCCR  : TKAEGALFSLKLAIITFFAIWVAFPLVWLLSVGTG-VLSNEAAEICHCICDVVAKSVYGFALANFREQYDRELYGLL : 271
HsBR    : M-RPEVASTFKVLRNVTVVLWSAYPVVWLIGSEGAGIVPLNIETLLFMVLDVSAKVGFGLILLRSRAIFGEAEAPEP : 238
HspAR2  : R-SEEVRSTFNTLTALVAVLWTAYPILWIVGTEGAGVVGLGIETLAFMVLDVTAKVGFGFVLLRSRAILGETEAPEP : 249
HsoAR3  : R-GPEVASTFNTLTALVLVLWTAYPILWIIGTEGAGVVGLGIETLLFMVLDVTAKVGFGFILLRSRAILGDTEAPEP : 248
HaCR1   : L-PSDTRSTFKTLRNLVTVVWLVYPVWWLIGTEGIGLVGIGIETAGFMVIDLTAKVGFGIILLRSHGVLDGAAETTG : 242
CrChR1  : -----CRDLVRYLAWLYFCSWAMFPVLFLLGPEGFGHINQFNSAIAHAILDLASKNAWSMMGHFLRVKIHEHILLYG : 318
CrChR2  : -----CRQVVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHG : 279
PsChR2  : -----CKNMVLAMAAVFFTSWFMFPGLFLAGPEGTNALSWAGSTIGHTVADLLSKNAWGMIGHFLRLEIHKHIIIHG : 262
MvChR1  : -----LAWYVRAMGYVFFTSWLTFPGWFLLGPEGLEVVTGTVSTLMHACSDLISKNLWGFMDWHLRVLVARHHRKLF : 297
```

Supporting figure 2. A ClustalW partial protein alignment of four cryptophyte CCRs used in this study: *Gt*CCR1 *aka* 120390 (KU761994), *Gt*CCR2 *aka* 99928 (KU761992), *Gt*CCR3 *aka* 162755 (KU761993) and *Psu*CCR *aka Ps*ChR2 (KF992056). For comparison are also included haloarchaeal proton pumps: *Hs*BR, *Halobacterium salinarum* bacteriorhodopsin (V00474), *Halobacterium* sp aus-2 archaeorhodopsin-2 (S56354), *Halorubrum sodomense* archaeorhodopsin-3 (GU045593), *Haloarcula argentinos* cruxrhodopsin-1 (D31880), and chlorophyte CCRs: *Chlamydomonas reinhardtii* ChR1 (AF508965), *Chlamydomonas reinhardtii* ChR2 (AF508966), *Platymonas (Tetraselmis) subcordiformis* ChR2 (JX983143), and *Mesostigma viride* ChR1 (JF922293). Numbers in parentheses are GenBank accession numbers. Amino acid residue numbering and helical regions are according to the *Hs*BR sequence. The Glu residues conserved in helix 2 of chlorophyte CCRs but not found in cryptophyte CCRs or haloarchaeal rhodopsin proton pumps are highlighted green, and the Asp residues conserved in helix 3 of haloarchaeal rhodopsin proton pumps and cryptophyte CCRs are highlighted red.



Supporting figure 3. The mean amplitudes of stationary photocurrents (red bars, left axis) generated by cryptophyte CCRs in HEK293 cells at the saturating light intensity and the wavelengths of the maximal sensitivity for each protein (see Fig. 2 in the main text), and half-decay rates of these currents upon switching off the light (black bars, right axis). The data are the mean values ± SEM (*n* = 5-14 cells). Although the current amplitudes also reflect expression levels, expression yield was not the reason for the observed differences, because the intensities of the tag fluorescence of all tested constructs were similar. The half-decay rate of the current decay (half-closing of the channel) is the reciprocal of the time when the stationary photocurrent amplitude decreases 50% after switching off the light.



Supporting figure 4. The shifts of E_r upon a 100-fold reduction of the Na^+ (from 150 mM to 1.5 mM; red bars) or H^+ (from pH 5.4 to pH 7.4; black bars) concentration in the bath determined for cryptophyte CCRs by measuring the IE curves as shown in Fig. 3 *c* in the main text. The data points are the mean values ± SEM (*n* = 3-5 cells).

Supporting References

1. Huson, D. H., C. Scornavacca. 2012. Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. *Syst. Biol.* 61:1061-1067.
2. Zhang, H., S. Gao, …, W. H. Chen. 2012. EvolView, an online tool for visualizing, annotating and managing phylogenetic trees. *Nucleic Acids Res.* 40:W569-572.