Molecular details of the unique mechanism of chloride transport by a cyanobacterial rhodopsin

Andrew Harris,^a Mattia Saita,^b Tom Resler,^b Alexandra Hughes-Visentin,^a Raiza Maia,^b Franziska Sellnau,^b Ana-Nicoleta Bondar,^c Joachim Heberle^{b*} and Leonid S. Brown^{a*}

^a Department of Physics and Biophysics Interdepartmental Group, University of Guelph, 50 Stone Road East, Guelph, Ontario N1G 2W1, Canada ^b. Experimental Molecular Biophysics Group, Department of Physics, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany

" Experimental Molecular Biophysics Group, Department of Physics, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany ^C Theoretical Molecular Biophysics Group, Department of Physics, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany

Electronic Supplementary Information (ESI)

1. Supplementary figures

Figure S1. Sequence alignment for helices B-G for the cyanobacterial anion pumps and selected microbial rhodopsins. BR numbering is shown on top, MastR numbering shown in red. Asterisks show the mutation sites used in this work. Residues conserved in BR are shown in yellow on black, polar residues potentially involved in coordination of chloride in any group of chloride pumps are highlighted in grey. Abbreviations: BR – Halobacterium salinarum bacteriorhodopsin, sHR – Halobacterium salinarum halorhodopsin, pHR – Natronobacterium pharaonis halorhodopsin, NTQ FR – Fulvimarina pelagi NTQ rhodopsin, MastR – Mastigocladopsis repens rhodopsin, Tolypothrix_c – Tolypothrix campylonemoides rhodopsin, Scytonema_tol – Scytonema tolypothrichoides rhodopsin, Scytonema HK-05 – Scytonema sp. HK-05 rhodopsin, Tolypothrix b – Tolypothrix bouteillei rhodopsin, Nostoc T09 – Nostoc sp. T09 rhodopsin; Hassallia – Hassallia byssoidea rhodopsin, Synechocystis – Synechocystis sp. PCC 7509 rhodopsin, Chamaesiphon – Chamaesiphon sp. PCC 6605 rhodopsin, Aliterella – Aliterella atlantica rhodopsin, Calothrix HK-06 – Calothrix sp. HK-06 rhodopsin, Chroococcid. - Chroococcidiopsis thermalis rhodopsin, Scytonema mi - Scytonema millei rhodopsin, Cyanothece 7425 – Cyanothece sp. PCC 7425 rhodopsin, Leptol NIES2104 – Leptolyngbya sp. NIES-2104 rhodopsin, Leptol NIES3755 – Leptolyngbya sp. NIES-3755 rhodopsin, Myxosarcina – Myxosarcina sp. GI1 rhodopsin, Phormidesmis BC – Phormidesmis sp. BC1401 GrIS rhodopsin; Phormidesmis pr. – Phormidesmis priestleyi. The sequences are taken from public databases: NCBI Proteins and DOE JGI IMG.

Helices B and C

	46	5	57		82	85	89	96	
BR	AKK <mark>FY</mark> AI <mark>T</mark> TL <mark>N</mark>	7PA <mark>IA</mark> FTM	YL <mark>SM</mark> LL <mark>G</mark>	EQNP <mark>IY</mark> W	<mark>ary</mark> a	DWLF	TTPLLL	DL <mark>DL</mark> A <mark>LI</mark>	VD
sHR	PRLI <mark>W</mark> GA <mark>T</mark> LM	PL <mark>V</mark> SISS	YL <mark>GL</mark> LS <mark>G</mark>	AMVRSQW	G <mark>RY</mark> L	T <mark>w</mark> al	STPMIL	LA <mark>L</mark> G <mark>LI</mark>	AD
pHR	AKLIAVS <mark>T</mark> IL <mark>I</mark>	/PV <mark>V</mark> SIAS	YTG <mark>L</mark> AS <mark>G</mark>	DGVVTMW	G <mark>RY</mark> L	T <mark>W</mark> AL	STPMIL	LA <mark>L</mark> G <mark>LI</mark>	AG
NTQ FR	YRIVPIM <mark>S</mark> AI <mark>N</mark>	MVS <mark>A</mark> GLS	L <mark>L</mark> REFNA	NSTFTNA	Y <mark>RY</mark> G	N <mark>W</mark> TI	TV <mark>PILL</mark> I	ſQ <mark>l</mark> p <mark>l</mark> ⁄	ĀFG
	39					74	78	85	
MastR	WQIL <mark>Y</mark> TLNFF	CL <mark>IA</mark> AGL	YLA <mark>M</mark> AL <mark>G</mark>	NGRPT <mark>Y</mark> W	V <mark>RF</mark> V	T <mark>W</mark> FC	STPLLL	L <mark>DL</mark> TFI	GR
	*					*	*	*	_
Tolypothrix_c	WQIL <mark>Y</mark> TLNFF <mark>1</mark>	CL <mark>IA</mark> AGL	YLA <mark>M</mark> AF <mark>G</mark>	NGRPT <mark>Y</mark> W	V <mark>RF</mark> L	T <mark>W</mark> F <mark>F</mark>	STPLLL	L <mark>DL</mark> TF <mark>I</mark>	GK
Scytonema_tol	WKIL <mark>Y</mark> TLNFF <mark>l</mark>	CL <mark>IA</mark> AGL	YL <mark>AM</mark> AL <mark>G</mark>	NGRPT <mark>Y</mark> W	V <mark>RF</mark> V	T <mark>W</mark> FC	STPLLL	L <mark>DL</mark> TF <mark>I</mark>	GR
Scytonema HK-05	WQIL <mark>Y</mark> TLNFF <mark>1</mark>	CL <mark>IA</mark> AGL	YL <mark>AM</mark> AF <mark>G</mark>	NGRPT <mark>Y</mark> W	V <mark>RF</mark> L	T <mark>W</mark> F <mark>F</mark>	STPLLL	L <mark>DL</mark> TF <mark>I</mark>	GK
Tolypothrix_b	WKIL <mark>‡</mark> TLNFF <mark>1</mark>	CL <mark>IA</mark> AGL	YLA <mark>M</mark> AL <mark>G</mark>	NDRPT <mark>Y</mark> W	V <mark>RF</mark> L	T <mark>W</mark> F <mark>F</mark>	STPLLL	L <mark>DL</mark> TF <mark>I</mark>	GR
Nostoc_T09	WQIL <mark>Y</mark> TLNFF <mark>I</mark>	CL <mark>IA</mark> AGL	YLTMAL <mark>G</mark>	NGRPT <mark>Y</mark> W	V <mark>RF</mark> V	T <mark>W</mark> FC	STPLLL	L <mark>DL</mark> TY <mark>I</mark>	GK
Hassallia	WKIL <mark>Y</mark> TLNFF <mark>I</mark>	CL <mark>IA</mark> AGL	YL <mark>AM</mark> ALK	NDRPT <mark>Y</mark> W	V <mark>RY</mark> V	T <mark>W</mark> FL	STPLLL	L <mark>DL</mark> TF <mark>I</mark>	GR
Synechocystis	WKIL <mark>P</mark> TINFF	CA <mark>IA</mark> TGL	YL <mark>SM</mark> AL <mark>G</mark>	AGRPTVW	V <mark>RY</mark> I	T <mark>W</mark> FL	STPLLI	L <mark>DL</mark> TF <mark>I</mark>	GK
Chamaesiphon	WQIL <mark>Y</mark> TLNFF <mark>1</mark>	CL <mark>IA</mark> AGL	YLA <mark>M</mark> SL <mark>G</mark>	YDRPT <mark>f</mark> W	V <mark>RY</mark> I	T <mark>W</mark> FC	STPLLI I	L <mark>DL</mark> AF <mark>I</mark>	GR
Aliterella	WRIL <mark>P</mark> TLNFF <mark>I</mark>	CA <mark>IA</mark> AGL	YL <mark>SM</mark> AL <mark>G</mark>	EGRPTVW	V <mark>RY</mark> I	T <mark>W</mark> FL	STPLLL I	L <mark>DL</mark> TF <mark>I</mark>	GK
Calothrix HK-06	WKIL <mark>Y</mark> TLNFF <mark>I</mark>	CL <mark>IA</mark> AGL	YL <mark>GM</mark> AL <mark>G</mark>	NGRQT <mark>Y</mark> W	L <mark>RF</mark> F	T <mark>W</mark> FC	STPLLL	L <mark>DL</mark> TF <mark>I</mark>	GK
Chroococcid.	WQIL <mark>Y</mark> TLNFF <mark>\</mark>	CA <mark>IA</mark> AGL	YLI <mark>M</mark> AL <mark>G</mark>	NGRPT <mark>f</mark> W	V <mark>RY</mark> V	S <mark>W</mark> FI	STPLLL I	JI <mark>L</mark> TY <mark>I</mark>	GK
Scytonema_mi	WQIL <mark>Y</mark> TLNFF	CA <mark>IA</mark> AGL	YL <mark>IM</mark> AL <mark>G</mark>	NGRP <mark>IF</mark> W	I <mark>RY</mark> V	S <mark>W</mark> FI	STPLLL I	JI <mark>L</mark> TY <mark>I</mark>	GK
Cyanothece_7425	WQILLTLNFF	CL <mark>IA</mark> TTL	YL <mark>AM</mark> IL <mark>G</mark>	YDRP <mark>IY</mark> W	V <mark>RY</mark> L	T <mark>W</mark> GL	STPLIL I	JV <mark>I</mark> TR <mark>I</mark>	GG
Leptol_NIES2104	WQVV <mark>Y</mark> VLNFF <mark>1</mark>	CA <mark>IA</mark> SVL	YL <mark>AM</mark> TQR	FDRPS <mark>F</mark> W	V <mark>RY</mark> V	T <mark>W</mark> T <mark>F</mark>	STPLTI	/L <mark>L</mark> SY <mark>I</mark>	GR
Leptol_NIES3755	WQVV <mark>Y</mark> VLNFF <mark>1</mark>	CA <mark>IA</mark> AVL	YL <mark>AM</mark> TQK	FDRPT <mark>f</mark> W	V <mark>RY</mark> V	T <mark>W</mark> T <mark>F</mark>	STPLTI	/L <mark>L</mark> SY <mark>I</mark>	GK
Myxosarcina	LRIL <mark>F</mark> TLNFF	TA <mark>IA</mark> AGL	YL <mark>AM</mark> AFS	GERQ <mark>VY</mark> W	I <mark>RY</mark> F	T <mark>W</mark> FL	TTPLLL	JV <mark>L</mark> TY <mark>I</mark>	GR
Phormidesmis BC	WRIV <mark>Y</mark> TINFF <mark>I</mark>	AA <mark>IA</mark> ASL	YLAMVL <mark>G</mark>	YGRPT <mark>Y</mark> W	V <mark>RY</mark> V	T <mark>W</mark> AL	STPLTL	[L <mark>L</mark> SF <mark>]</mark>	GS
Phormidesmis pr	WRIVYTINFF	AATAAGT.	VT.AMX7T.C	VGRDTVW	(7 <mark>R V</mark> (7	TRMCT	STPT.TT.	7T	GG

Helices D and E

_	115	134
BR	DQG <mark>TI</mark> LA <mark>LV</mark> GA <mark>D</mark> GI <mark>MI</mark> G <mark>TG</mark> LVGALT	SY <mark>R</mark> FV <mark>WW</mark> AI <mark>ST</mark> AAMLY ILY VLFFGF
sHR	DLG <mark>SL</mark> FT <mark>VI</mark> AA <mark>D</mark> IG <mark>M</mark> CV <mark>TG</mark> LAAA <mark>MT</mark>	LF <mark>R</mark> WA <mark>FY</mark> AI <mark>S</mark> CAFF <mark>V</mark> VVLSALVTDW
pHR	NATK <mark>L</mark> FTA <mark>L</mark> TF <mark>D</mark> IA <mark>M</mark> CV <mark>TG</mark> LAAA LT	LM <mark>R</mark> WF <mark>WY</mark> AI <mark>S</mark> CACF <mark>LVVLY</mark> ILLVEW
NTQ FR	LHVRAAR <mark>M</mark> CIPALL <mark>MI</mark> W <mark>TG</mark> LVGQFG	LRLNV <mark>W</mark> GVI <mark>ST</mark> IFF <mark>V</mark> WLIIEVRGVI
	104	122
MastR	SLPLTGS <mark>LL</mark> GANAY <mark>ML</mark> V TG F V ATVT	PMSYI <mark>WY</mark> IV <mark>S</mark> CAAYLAIVYLLAQPY
Tolypothrix_c	SLPITGS <mark>LL</mark> GANAY <mark>ML</mark> A <mark>TG</mark> F V ATVT	PMSYI <mark>wy</mark> IV <mark>S</mark> CAAY <mark>L</mark> AIIYLLVQPY
Scytonema_tol	SLPITGS <mark>LL</mark> GANAY <mark>MI</mark> A <mark>TG</mark> F <mark>I</mark> AT <mark>VT</mark>	PTSYI <mark>WY</mark> VV <mark>S</mark> CGAY <mark>L</mark> AIF <mark>Y</mark> LLAQPY
Scytonema HK-05	SLPITGS <mark>LL</mark> GANAY <mark>ML</mark> A <mark>TG</mark> F <mark>V</mark> AT <mark>VT</mark>	PMSYI <mark>WY</mark> IV <mark>S</mark> CAAY <mark>L</mark> A <mark>IIY</mark> L <mark>L</mark> VQPY
Tolypothrix_b	SLPITGS <mark>LL</mark> GANAY <mark>ML</mark> A <mark>TG</mark> F <mark>V</mark> ATVT	PMSYI <mark>WY</mark> VT <mark>S</mark> CAAY <mark>L</mark> A <mark>IVY</mark> L <mark>L</mark> VKPY
Nostoc_T09	SLPITGS <mark>LL</mark> GANAY <mark>ML</mark> A <mark>TG</mark> F <mark>L</mark> AT IT	PTSYI <mark>WY</mark> II <mark>S</mark> CAAY <mark>L</mark> A <mark>I</mark> F <mark>Y</mark> L <mark>L</mark> VKPY
Hassallia	SLPITGS <mark>LL</mark> GANAY <mark>MI</mark> A <mark>TG</mark> FVAT <mark>IS</mark>	PISYI <mark>WY</mark> IV <mark>S</mark> CAAY <mark>L</mark> AVA <mark>Y</mark> M <mark>L</mark> LNQY
Synechocystis	SLPITAS <mark>LL</mark> GANAY <mark>MI</mark> A <mark>TG</mark> FVAT <mark>IS</mark>	AIGHI <mark>WY</mark> VV <mark>S</mark> CFAF <mark>L</mark> AT <mark>VY</mark> L <mark>L</mark> VNQY
Chamaesiphon	NLPITCS <mark>LI</mark> GANAY <mark>MI</mark> A <mark>TG</mark> FVGA <mark>IT</mark>	PMNQI <mark>WY</mark> LV <mark>S</mark> CGAF <mark>I</mark> AT <mark>LY</mark> LKPY
Aliterella	SLPITAS <mark>LI</mark> GANAY <mark>MI</mark> V <mark>TG</mark> FVAT <mark>IS</mark>	TIGHI <mark>WY</mark> VV <mark>S</mark> CFAF <mark>L</mark> AT <mark>VY</mark> L <mark>L</mark> VNQY
Calothrix HK-06	NIL <mark>T</mark> TSS <mark>LL</mark> GANAY <mark>MI</mark> I <mark>TG</mark> FAAT <mark>IS</mark>	PISYI <mark>WY</mark> IV <mark>S</mark> CGAY <mark>V</mark> A <mark>IMY</mark> L <mark>L</mark> IKPY
Chroococcid.	SVT <mark>T</mark> TAS <mark>LL</mark> GANAYT <mark>I</mark> AA <mark>G</mark> F V AT IS	PVNYI <mark>WY</mark> IV <mark>S</mark> CAAYCAT <mark>VY</mark> L <mark>L</mark> LNQY
Scytonema_mi	SIT <mark>T</mark> TAS <mark>LV</mark> GANAYT <mark>I</mark> AA <mark>G</mark> FVATIS	PVNYI <mark>WY</mark> IV <mark>S</mark> CAAYCAT <mark>VY</mark> L <mark>L</mark> LHQY
Cyanothece_7425	SLLLTAS <mark>LI</mark> GA <mark>D</mark> LF <mark>MI</mark> A <mark>TG</mark> YVAAVS	PINFI <mark>WY</mark> LV <mark>S</mark> CGAF <mark>V</mark> ALF <mark>Y</mark> LLRPY
Leptol_NIES2104	KPII <mark>L</mark> AS <mark>MV</mark> GA <mark>D</mark> VL <mark>MI</mark> A <mark>TG</mark> F <mark>V</mark> AA <mark>IS</mark>	PITNL <mark>WY</mark> IV <mark>S</mark> CGFY <mark>L</mark> G <mark>L</mark> A <mark>Y</mark> LLKHY
Leptol_NIES3755	KPAI <mark>L</mark> GS <mark>MI</mark> GA <mark>D</mark> VL <mark>MI</mark> A <mark>TG</mark> F <mark>V</mark> AA <mark>IS</mark>	PTTNL <mark>WY</mark> IV <mark>S</mark> CGFY <mark>L</mark> G <mark>L</mark> A <mark>Y</mark> LLKHY
Myxosarcina	RLS <mark>TI</mark> LG <mark>LI</mark> GANGY <mark>ML</mark> V <mark>TG</mark> FIATIS	PLNFV <mark>WY</mark> FV <mark>S</mark> CGAFAG <mark>ILY</mark> LLKPY
Phormidesmis BC	SLPIAAS <mark>MV</mark> GADIY <mark>MI</mark> A <mark>TG</mark> FVAA <mark>IS</mark>	PTSYI <mark>WY</mark> AV <mark>S</mark> CGAY <mark>L</mark> GLVYLLHHY
Phormidesmis pr	SLPIAAG <mark>MV</mark> GADIY <mark>MI</mark> ATGFVATIS	PTSYI <mark>WY</mark> FV <mark>S</mark> CGAY <mark>L</mark> GLVYLLHHY

Helices F and G

	178	182	189	194	204	212	216	
BR	FKVLRN <mark>V</mark> TV	VLW <mark>SA</mark> YP	VV <mark>WLI</mark> (SEC AC	IVPLNIETL:	L <mark>FMVLDV</mark> S	AKVGFG	ILLR <mark>SR</mark>
shr	FDTLRVLTV	VLWLG <mark>YP</mark>	IVWAV	VECLA	LVSVGVT <mark>S</mark> W	A <mark>Y</mark> S <mark>VLDV</mark> I	F <mark>AK</mark> YV <mark>F</mark> AI	T ILL RWV
PHR	FNTLKL <mark>L</mark> TV	VMWLG <mark>YP</mark>	IVWAL	VEGIA	VL P V G V T S W	G <mark>y</mark> SF <mark>LDI</mark> N	/ <mark>AK</mark> YI <mark>F</mark> AI	TILL NYL
NTQ FR	PKN <mark>I</mark> WWFFL	AF <mark>W</mark> GL <mark>YP</mark>	TAYALI	PQL <mark>G</mark> HT	GDI <mark>V</mark> V <mark>I</mark> RQL	L <mark>y</mark> siadv	S <mark>KL</mark> V YG	I <mark>IL</mark> SRYV
	166	170	177	182	192	197 200	204	211 214
MastR	ERTLVTVHL	VIW TL <mark>XP</mark>	TIMATT	SP <mark>DC</mark> FS	STFTQGS <mark>DH</mark> M.	FYTLLDI/	AS <mark>KVGFG</mark> I	SUNIL
	*	_			**	*		* *
Tolypothrix_c	FRKLVTVHL	VLWTLYP	TIMATT	SP <mark>DC</mark> FS	SFGQGS <mark>D1</mark> M	TYTLLDI/	ASKVGFG	
Scytonema_tol	FHKLVIVHL	VLWTL <mark>YP</mark>	VVWIL S	SP <mark>EC</mark> FS	SAFGQGS <mark>D1</mark> M	FYTLLDI/	AS <mark>KVGFG</mark> I	TISLNIL
Scytonema HK-05	FRKLVT V HL	VLWTL <mark>YP</mark>	IIVWIL S	SP <mark>DC</mark> FS	SFGQGS <mark>DU</mark> MI	F Y T <mark>LLDI</mark> /	AS <mark>KVGFG</mark> I	TLSLNTL
Tolypothrix_b	FRK <mark>L</mark> VT <mark>V</mark> HL	VLWTL <mark>YP</mark>	IIW NIT	SP <mark>EG</mark> FR	AFGQGS <mark>DT</mark> M	T <mark>Y</mark> T <mark>LLDI</mark>	AS <mark>KVGFG</mark> I	TISLNTL
Nostoc_T09	FRK <mark>L</mark> VT <mark>V</mark> HL	VLWTL <mark>YP</mark>	IIW VIL	SP <mark>EG</mark> FS	SAFDQG <mark>LE</mark> AM	S <mark>Y</mark> T <mark>ILDI</mark> A	AS <mark>KVGFG</mark> I	TISLNTL
Hassallia	FRK <mark>L</mark> VT <mark>V</mark> HL	VLWTL <mark>YP</mark>	V <mark>WIL(</mark>	TEC FN	I <mark>V</mark> FAQGT <mark>ET</mark> MI	F <mark>F</mark> T <mark>LLDL</mark> /	AS <mark>KVGFG</mark> I	TISINTL
Synechocystis	FRK <mark>L</mark> LS <mark>V</mark> HL	VLWTL <mark>YP</mark>	」 [™] [™] [™] 	NT <mark>G</mark> FN	IA <mark>V</mark> NQGT <mark>ET</mark> MI	F <mark>Y</mark> T <mark>ILDI</mark> T	S <mark>KVGFG</mark> I	T <mark>LSLNS</mark> M
Chamaesiphon	FRK <mark>L</mark> LTAHV	VLWTL <mark>YP</mark>	V <mark>WIL</mark> Z	AT <mark>G</mark> L <mark>G</mark>	VLSQGY <mark>ET</mark> M	G <mark>y</mark> T <mark>LLDL</mark> /	AS <mark>KVGFG</mark> I	F <mark>L</mark> S <mark>L</mark> N <mark>S</mark> L
Aliterella	FRK <mark>L</mark> LA <mark>V</mark> HL	VLWTL <mark>YP</mark>	ס <mark>עעדר(</mark>	INT <mark>G</mark> LN	IA <mark>I</mark> NQG <mark>VET</mark> MI	T <mark>YTLLDI</mark>	S <mark>KVGFG</mark> I	F <mark>LSLNS</mark> M
Calothrix HK-06	FTK <mark>L</mark> VT <mark>I</mark> HL	VLWTL <mark>YP</mark>	V <mark>₩IL</mark> S	SP <mark>p</mark> afn	I <mark>VL</mark> NQGG <mark>B</mark> AM(G <mark>y</mark> T <mark>LLDI</mark> /	AS <mark>KVGFG</mark> I	F <mark>LSLNT</mark> L
Chroococcid.	FHK <mark>L</mark> LK <mark>V</mark> HL'	T <mark>IW</mark> TL <mark>YP</mark>	ע <mark>עדד</mark> מ <mark>עדד</mark>	SPA <mark>G</mark> LN	IF <mark>LNI</mark> S <mark>VET</mark> MI	FVT <mark>LLDM</mark> /	AS <mark>KVGFG</mark> I	F <mark>LSLQT</mark> M
Scytonema_mi	FHK <mark>L</mark> LK <mark>V</mark> HL	IIWTF <mark>YP</mark>	ע <mark>עדד</mark> א	SPA <mark>G</mark> LN	IF <mark>LNI</mark> S <mark>VET</mark> MI	FVT <mark>LLDL</mark> (SS <mark>KVGFG</mark> I	F <mark>LSLQT</mark> M
Cyanothece_7425	IDQ <mark>L</mark> LS <mark>V</mark> QL	A <mark>lw</mark> tl <mark>yp</mark>	ע <mark>עדד</mark> מ <mark>עדר</mark>	SKT <mark>G</mark> FN	ILLNPT <mark>LET</mark> I:	S <mark>y</mark> t lldl a	AKVGFG	FFA <mark>L</mark> A <mark>T</mark> L
Leptol_NIES2104	FNRLLTVHL	VIW <mark>SL</mark> YP	VV <mark>WIL</mark> Z	GT <mark>G</mark> IN	I <mark>VI</mark> NSTT <mark>ET</mark> AI		AKVGFG	F <mark>L</mark> ALS <mark>S</mark> L
Leptol_NIES3755	FNR <mark>L</mark> LT <mark>V</mark> HL	VIWTL <mark>YP</mark>	VV <mark>WIL</mark> Z	ART <mark>G</mark> IN	I <mark>VI</mark> NSTT <mark>ET</mark> AI	F <mark>Y</mark> T <mark>ILDV</mark> /	AKVGFG	F <mark>L</mark> ALS <mark>S</mark> L
Myxosarcina	FKK <mark>L</mark> LT <mark>V</mark> HV	VLW <mark>SC</mark> YP	LVWIL (DT <mark>G</mark> FA	LIGDRW <mark>D</mark> AM	F <mark>Y</mark> T <mark>LLDI</mark> /	AKVGFG	F <mark>LSLNS</mark> F
Phormidesmis BC	FYR <mark>L</mark> LT <mark>V</mark> HL	LLWTA <mark>YP</mark>	IVWIL Z	AKT <mark>G</mark> YS	VIDSG <mark>LET</mark> M	S <mark>Y</mark> T LLDL A	AKVGFG	F <mark>LSL</mark> S <mark>S</mark> L
Phormidesmis pr	FGK <mark>L</mark> LT <mark>V</mark> HL	VLW <mark>TA<mark>YP</mark></mark>	TV <mark>WML</mark> Z	AKT <mark>G</mark> YS	S <mark>VI</mark> DSSA <mark>PT</mark> M;	S <mark>y</mark> T <mark>lldl</mark> a	AKVGFG	F <mark>L</mark> S <mark>L</mark> N <mark>S</mark> L



Figure S2. Chloride transport assays using whole *E. coli* cells expressing MastR suspended either in unbuffered 50 mM NaCl (left) or Na₂SO₄ (right), with and without 10 μ M CCCP (proton uncoupler). pH changes were measured by glass electrode, the arrows show the time of turning yellow (570-590 nm) illumination on and off. See supplementary methods below for the full description.



Figure S3. (Left) Absorption spectrum of the polyacrylamide-encased membranes of *E. coli* expressing MastR (black solid line) measured at pH 6, buffered by 0.05 M KH₂PO₄ and 0.05 M MES, at 22°C, with 2.5 M NaCl. Spectrum of the purified DDM-solubilized MastR (0.02 M NaCl, 0.05 M MES and 0.05 M KH₂PO₄, pH 6, 0.05% DDM) is given for comparison (red dashed line). (**Right**) Comparison of the photocycle kinetics of the polyacrylamide-encased membranes of *E. coli* expressing MastR and hydrated films of the same membranes, measured at pH 6, buffered by 0.05 M KH₂PO₄ and 0.05 M MES, at 22°C, with 0.02 M NaCl.



Figure S4. A) Comparison of chloride-transporting and chloride-free photocycles of MastR in *E. coli* membranes encased in polyacrylamide gels, measured at pH 6, buffered by 0.05 M KH₂PO₄ and 0.05 M MES, at 22°C, with either 0.1 M NaCl or 0.1 M Na₂SO₄. **B)** Verification of the chloride binding affinity of the dark state of MastR by chloride titration of the amplitude of the 460 nm signal (the L/N intermediates) of the MastR photocycle. The specified sodium chloride concentrations (from 4 M stock) were added on top of 0.1 M Na₂SO₄, other conditions are as in A).



Figure S5. The photocycle of lipid-reconstituted MastR in proteoliposome film (DMPC/ DMPA 9/1) measured at 22°C, pH 6 (buffered by MES) and ~3 M KCl, used for the time-resolved infrared spectroscopy experiments. The buffer and salt concentrations are approximate due to the film drying effects (see Experimental).



Figure S6. Rapid-scan (29 ms time resolution) light-minus-dark difference FTIR spectra of MastR proteoliposomes (sample conditions as in Fig. S5). Spectral resolution is 2 cm⁻¹, average of 6000 individual spectra. The black spectrum with the largest amplitude corresponds to 29 ms after the photoexcitation, and the other spectra represent relaxation of that state recorded every ~74 ms.



Figure S7. Kinetics of the amide I vibration at 1670 cm⁻¹ measured with step-scan FTIR. The evolution of the conformational changes of the sample in H_2O (data from Fig. 8, black) compared to that in D_2O (red). The better signal-to-noise ratio in the late ms time range of the D_2O data is due to the lower absorption of the D_2O sample in this range.

2. Supplementary methods

2.1. Ion transport assays

Ion transport assays for MastR in the whole *E. coli* cells were performed according to the published protocol.¹ The cells from 1 L culture grown as described in the main text were collected at 4680 ×g and 4 °C for 10 min. Half of the cells were washed three times with unbuffered solution (50 mM NaCl, 10 mM MgSO₄·7H₂O, 100 μ M CaCl₂), and then re-suspended for ion transport measurements. The other half were washed and resuspended similarly with chloride-free unbuffered solution (50 mM Na₂SO₄, 10 mM MgSO₄·7H₂O, 100 μ M CaCl₂). A glass electrode (Accumet Microprobe Extra Long Calomel Combo Electrode) was used to monitor pH changes of the cells suspended in unbuffered solution with gentle stirring. A digital oscilloscope (Agilent Technologies DSO 1052B Digital Storage Oscilloscope) was used for recording the pH. The sample was illuminated (Cole Parmer 9741-50 illuminator) with yellow light (570–590 nm) using a glass filter.

2.2. Structure modeling

A homology model of MastR was derived using Modeller 19.9^{2,3} and the crystal structure of halorhodopsin PDB ID:1E12.⁴ During homology modeling, the retinal molecule and the chloride ion bound to the active site of HR were treated as ligand molecules and modeled into MastR. A total of 20 homology models were generated, and the structure with the lowest DOPE score (Discrete Optimized Protein Energy)⁵ was used for further optimization using the CHARMM software,⁶ as follows.

Coordinates for hydrogen atoms were generated using CHARMM with the all-atom c36 parameters for protein groups^{7,8} and the retinal force field parameters described earlier.⁹⁻¹¹ Inspection of the homology model of MastR indicated that the C_{15} =N bond twist was ~64°, as compared to -160.3° in the template structure of HR; this discrepancy could be due to limitations of the homology modeling software in describing the geometry and interactions of the retinal polyene chain. To circumvent this issue, we subjected the homology model of MastR to constrained geometry optimizations with CHARMM whereby we first drove the C_{14} - C_{15} =N-C ϵ dihedral angle to ~174°, and then performed a new geometry optimization with the dihedral angle constraint switched off. During both CHARMM geometry optimizations, backbone heavy atoms were fixed to their starting coordinates; non-bonded interactions were smoothly switched off using an atom-based switch function between 10Å and 12Å. In the resulting geometry-optimized structure of MastR, retinal is all-*trans* with C_{13} =C₁₄ and C_{15} =N bond twists of ~19° and ~2°, respectively.

A caveat of the current structure analysis of MastR is that according to tests using the Phyre2 server¹² the sequence identity between the MastR sequence and the sHR structure used as a template⁴ is somewhat low, ~27%. As observed when modelling channelrhodopsins using bacteriorhodopsin as a template,¹³ the relatively low level of sequence identity means that details of the MastR structural model might be inaccurate. In spite of this caveat, the geometry-optimized homology model used here suffices to derive clues about potential intra-and inter-helical interactions of protein groups known to be important for function.

3. Supplementary references

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