

Supplementary material for:

A unique choanoflagellate enzyme rhodopsin with cyclic nucleotide phosphodiesterase activity

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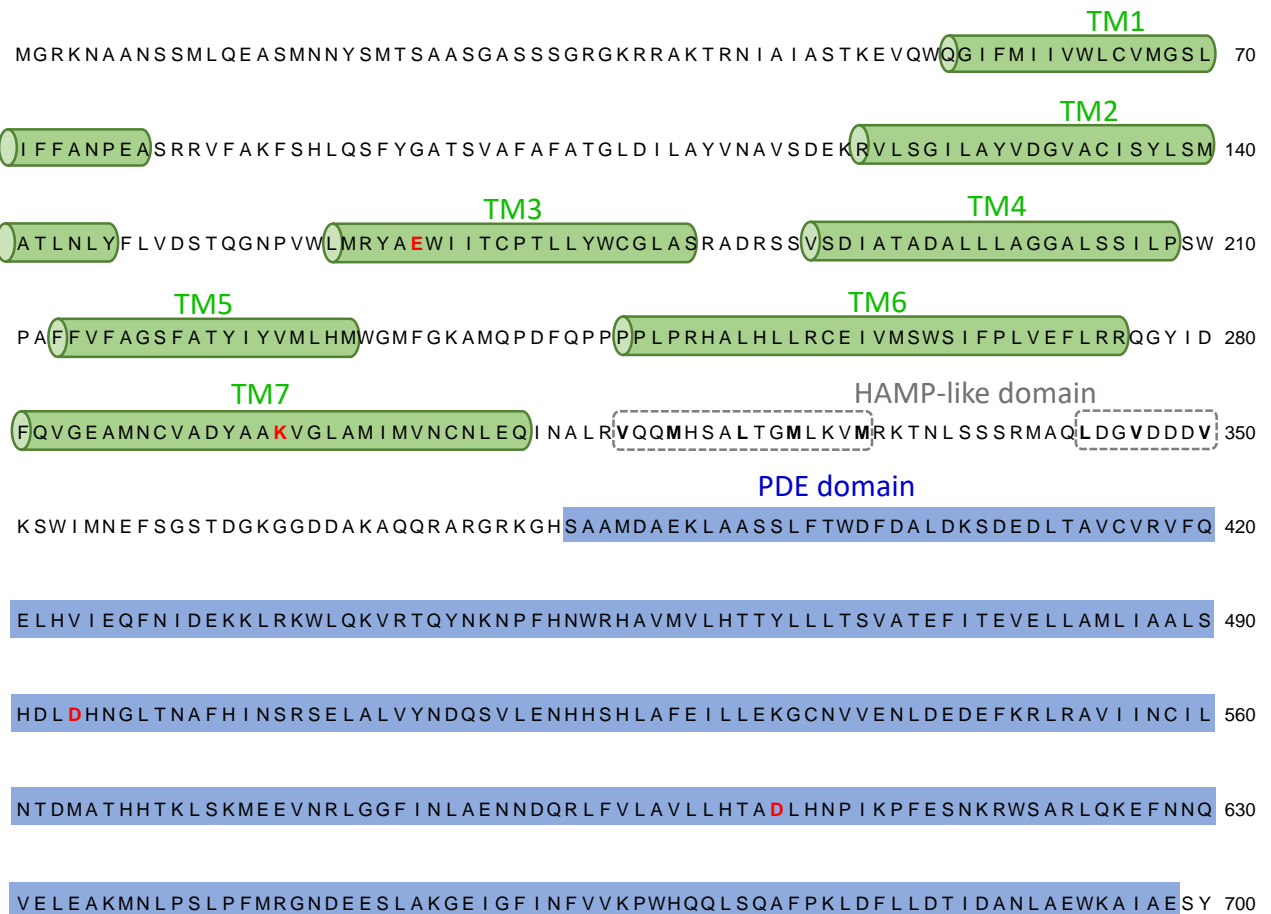
**Running title:** *Rhodopsin-PDE*

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RQMH 704

Figure S1 , Amino acid sequence of Rhodopsin-PDE  
 7 transmembrane helices (TM1-7) in the rhodopsin domain are shown as green rods. Membrane topology was predicted based on the X-ray structure of BR after the sequence alignment. The HAMP-like domain is marked by gray dotted boxes. The PDE domain is shown in blue. Key functional residues in the rhodopsin and PDE domains are indicated in red. The repeats of hydrophobic residues, characteristic for HAMP domains, are shown in bold letters.



TM6

No in Rh_PDE	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268				
No in BR	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186								
Rh-PDE	W	G	M	F	G	K	A	M	Q	-	-	-	-	P	D	F	Q	P	P	P	P	L	P	R	H	A	L	H	L	L	R	C	E	I	V	M	S	W	S	I	F	P
BR	F	F	G	F	T	S	K	-	-	-	-	-	-	A	E	S	M	R	P	E	-	-	V	A	S	T	F	K	V	L	R	N	V	T	V	V	L	W	S	A	Y	P
HR	L	V	Q	W	P	-	-	-	-	-	-	-	-	A	D	A	E	A	A	G	-	-	T	S	E	I	F	G	T	L	K	I	L	T	V	V	L	W	L	G	Y	P
KR2	K	K	V	I	N	E	G	-	-	-	-	-	-	K	E	G	I	S	P	A	-	-	G	Q	K	I	L	S	N	I	W	I	L	F	L	I	S	W	T	L	Y	P
ChR2	A	K	A	Y	I	E	-	-	-	-	-	-	-	G	Y	H	T	V	P	K	G	R	Q	V	V	T	G	M	A	W	L	F	F	V	S	W	G	M	F	P		
ACR1	F	A	I	F	A	I	T	I	H	D	F	Q	T	I	G	S	P	M	S	L	K	-	-	V	V	E	R	L	K	L	M	R	I	V	F	V	S	W	M	A	Y	P
SR11 NP	V	G	P	M	T	E	S	-	-	-	-	-	-	A	S	Q	R	S	S	G	-	-	I	K	S	L	Y	V	R	L	R	N	L	T	V	V	L	W	A	I	Y	P
ASR	W	N	P	L	R	A	K	-	-	-	-	-	-	T	R	T	Q	S	S	E	-	-	L	A	N	L	Y	D	K	L	V	T	Y	F	T	V	L	W	I	G	Y	P
Rh-GC	W	S	C	F	T	G	A	I	D	-	-	-	-	G	E	T	Q	C	N	V	-	-	E	K	S	G	L	R	W	I	R	F	S	T	I	T	W	S	L	F	P	
HKR1 CR	W	S	M	F	H	A	S	I	-	-	-	-	-	A	E	A	R	H	D	S	-	-	S	R	V	S	L	E	V	L	R	L	F	A	V	G	L	W	F	T	F	P

TM7

No in Rh_PDE	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302											
No in BR	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222									
Rh-PDE	-	L	V	E	F	L	R	R	Q	G	-	Y	I	D	F	Q	V	-	-	G	E	A	M	N	C	V	A	D	Y	A	A	K	V	G	L	A	M	I	-	-	-	-	-		
BR	-	V	W	L	I	G	S	E	G	A	G	I	V	P	L	N	I	-	-	E	T	L	L	F	M	V	L	D	V	S	A	K	V	G	F	G	L	I	-	-	-	-	-		
HR	-	I	L	W	A	L	G	S	E	G	V	A	L	L	S	V	G	V	-	-	T	S	W	G	Y	S	G	L	D	I	L	A	K	Y	V	F	A	F	L	-	-	-	-	-	
KR2	L	M	P	Y	L	T	G	V	D	G	F	L	S	E	D	G	V	M	A	R	Q	L	V	Y	T	I	A	D	V	S	K	V	I	Y	G	V	L	-	-	-	-	-			
ChR2	-	I	L	F	I	L	G	P	E	G	F	G	V	L	S	V	Y	G	-	-	S	T	V	G	H	T	I	D	L	M	S	K	N	C	W	G	L	L	G	H	Y	L	R	V	L
ACR1	-	I	L	W	S	F	S	T	G	A	C	I	M	S	E	N	T	-	-	S	S	V	L	Y	L	L	G	D	A	L	C	K	N	T	Y	G	I	L	-	-	-	-	-		
SR11 NP	-	F	I	W	L	L	G	P	T	G	V	A	L	L	T	P	T	V	-	-	D	V	A	L	I	V	Y	L	D	L	V	T	K	V	G	F	G	I	-	-	-	-	-		
ASR	-	I	V	W	I	I	G	P	S	G	F	G	W	I	N	Q	T	I	-	-	D	T	F	L	F	C	L	L	P	F	F	S	K	V	G	F	S	F	L	-	-	-	-	-	
Rh-GC	-	I	T	W	F	S	Y	T	S	G	-	-	L	I	S	F	T	V	-	-	A	E	A	G	F	S	M	I	D	I	G	A	K	V	F	L	T	L	V	-	-	-	-	-	
HKR1 CR	-	V	I	W	I	V	V	K	M	G	-	-	L	V	D	I	R	T	-	-	E	E	W	T	W	C	A	C	D	F	L	G	K	V	M	F	S	S	-	-	-	-	-		

No in Rh_PDE	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325																						
No in BR	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243																								
Rh-PDE	-	-	-	-	M	V	N	C	N	L	E	Q	I	N	A	-	-	-	-	-	-	-	-	-																					
BR	-	-	-	-	L	L	R	S	-	-	R	A	I	F	G	-	-	-	-	-	-	-	-	-																					
HR	-	-	-	-	L	L	R	W	-	V	A	A	N	E	G	-	-	-	-	-	-	-	-	-																					
KR2	-	-	-	-	L	G	N	L	A	I	T	L	S	K	N	-	-	-	-	-	-	-	-	-																					
ChR2	I	H	E	H	I	L	I	H	G	D	I	R	K	T	T	K	-	-	-	-	-	-	-																						
ACR1	-	-	-	-	L	W	A	T	T	W	G	L	L	N	G	K	W	D	R	D	Y	V	K	G	R	N	V	D	G	T	L	M	P	E	Y	E	Q	D	L	E	K	G	N	T	E
SR11 NP	-	-	-	-	A	L	D	A	-	A	A	T	L	R	A	-	-	-	-	-	-	-	-	-	-																				
ASR	-	-	-	-	D	L	H	G	-	L	R	N	L	N	D	-	-	-	-	-	-	-	-	-	-																				
Rh-GC	-	-	-	-	L	V	N	S	T	V	E	Q	A	Q	N	-	-	-	-	-	-	-	-	-																					
HKR1 CR	-	-	-	-	L	L	H	G	N	F	L	T	I	E	Q	-	-	-	-	-	-	-	-	-																					

No in Rh_PDE	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540																										
No in BR	244	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265																		
Rh-PDE	M	L	K	V	M	R	K	T	N	L	S	S	R	M	-	-	-	-	-	-	-	-	-																		
BR	A	A	A	T	S	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																		
HR	G	A	A	P	A	D	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																		
KR2	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																		
ChR2	V	E	D	E	A	E	A	G	A	V	P	-	-	-	-	-	-	-	-	-	-	-																			
ACR1	R	Y	E	D	A	R	A	G	E	T	F	E	V	K	M	F	G	R	T	L	T	S	V	R	R	S	R	-	-	-	-	-	-	-	-	-	-	-	-		
SR11 NP	T	P	A	V	A	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																		
ASR	T	L	Q	F	V	E	N	I	T	L	F	A	N	S	R	R	Q	Q	S	R	R	V	-	-																	
Rh-GC	L	E	N	Q	I	N	C	D	A	I	L	Q	K	M	M	P	E	G	V	L	E	Q	L	K	N	G	Q	A													
HKR1 CR	L	K	D	L	V	E	Q	K	E	R	F	M	S	S	M	S	H	E	L	R	T	P	L	N	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure S2 Amino acid sequence alignment of the rhodopsin domains of selected microbial rhodopsins

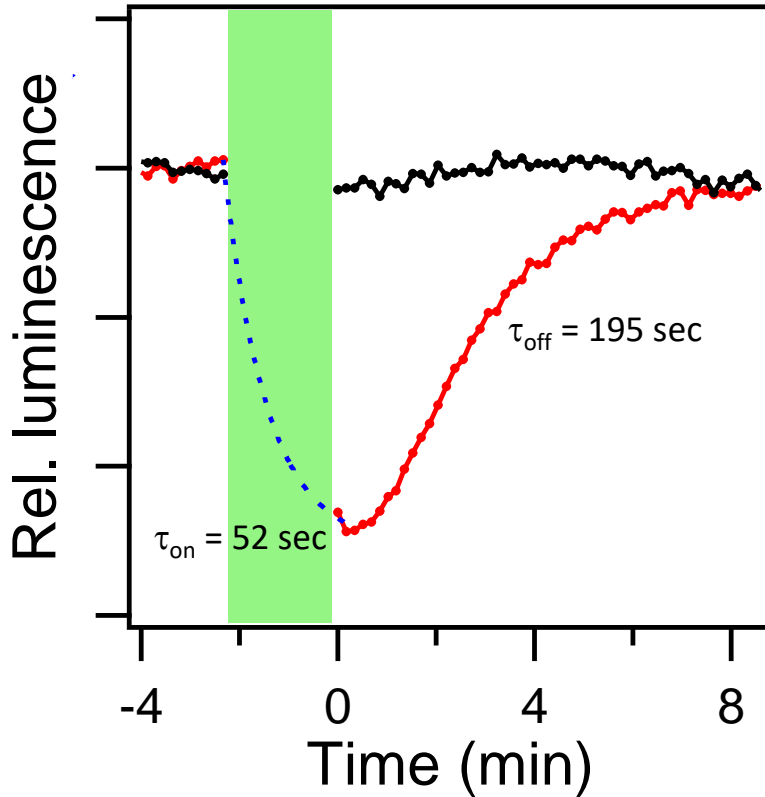
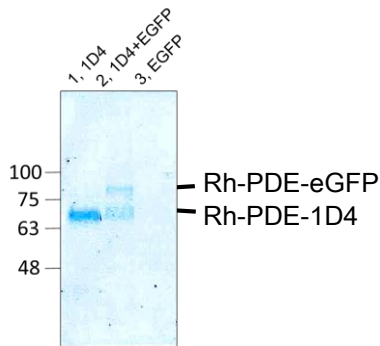


Figure S3 Estimation of the activated Rh-PDE lifetime

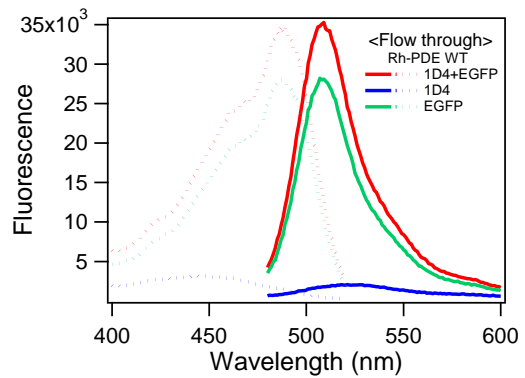
Signal decay during illumination (shown in light-green) represents degradation of cAMP by Rh-PDE, whereas the increase of the signal after shutting-off the light involves deactivation of Rh-PDE and production of cAMP by endogenous adenylyl cyclases. The decay and the recovery were fitted by a single exponential function to obtain  $\tau_{\text{on}}$  as 52 sec and  $\tau_{\text{off}}$  as 195 sec. Lifetime of Rh-PDE  $\tau_{\text{R}}$  was estimated as 71 sec by using the following equation.

$$1/\tau_{\text{R}} = 1/\tau_{\text{on}} - 1/\tau_{\text{off}}$$

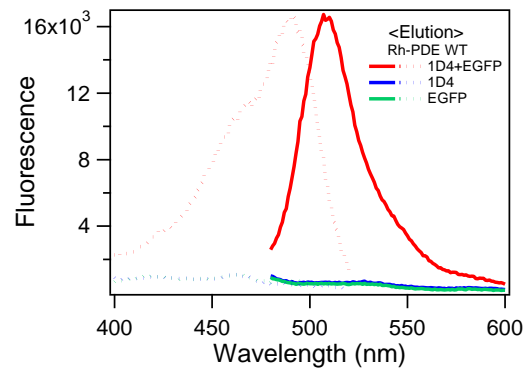
## A, SDS-PAGE



## B, unbound fraction



## C, elution



### Figure S4 Rh-PDE forms oligomers

1D4-tagged and eGFP-tagged Rh-PDE were cotransfected in HEK293 cells followed by purification by 1D4-resin. The eluted fraction contained both versions of Rh-PDE (A, lane 2). In contrast, only a single band is seen when only 1D4 tagged Rh-PDE is expressed (A, lane 1), and no protein was eluted from the cells transfected only the eGFP-tagged version (A, lane 3). B, Fluorescent spectra of the flow through solution after loading the solubilized protein on 1D4 resin. When the eGFP-tagged version is transfected, the flow through shows eGFP fluorescence, indicating the eGFP tagged Rh-PDE is not bound to the 1D4 resin. The data presented here indicate oligomeric assembly of Rh-PDE, at least in the solubilized form. Dotted curve; excitation spectra. Solid curve; emission spectra. C, Fluorescent spectra of the eluted proteins. eGFP fluorescence was observed only in the co-transfected cells, meaning eGFP-tagged Rh-PDE associates with 1D4-tagged Rh-PDE.