

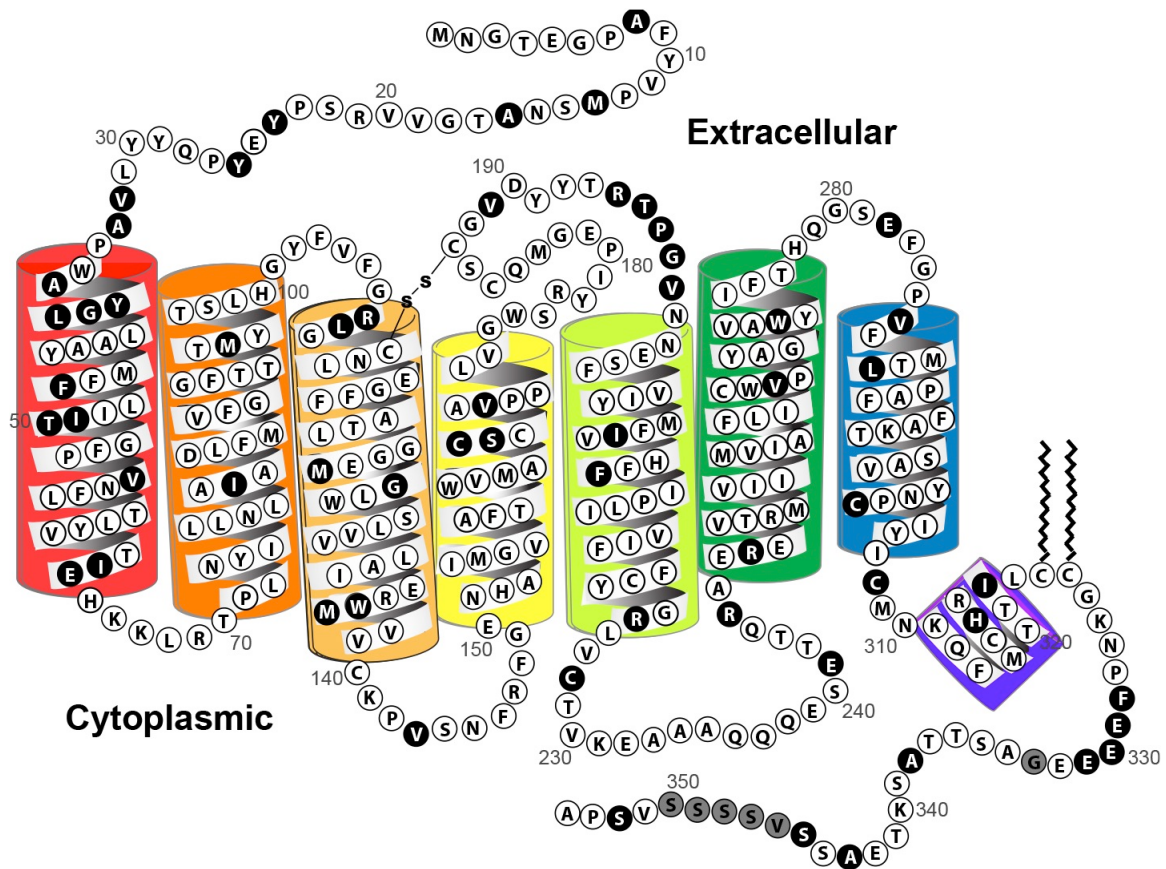


Figure S1. Two-dimensional model of zebrafish rhodopsin, based on secondary structural features highlighted in the crystal structure of bovine rhodopsin (3). The seven transmembrane domains and the cytoplasmic helix, are highlighted by coloured cylinders. Residues with a white background are identical to bovine rhodopsin identities, while residues with a black background differ. Residues with a grey background are additional amino acids in zebrafish rhodopsin, not found in bovine rhodopsin.

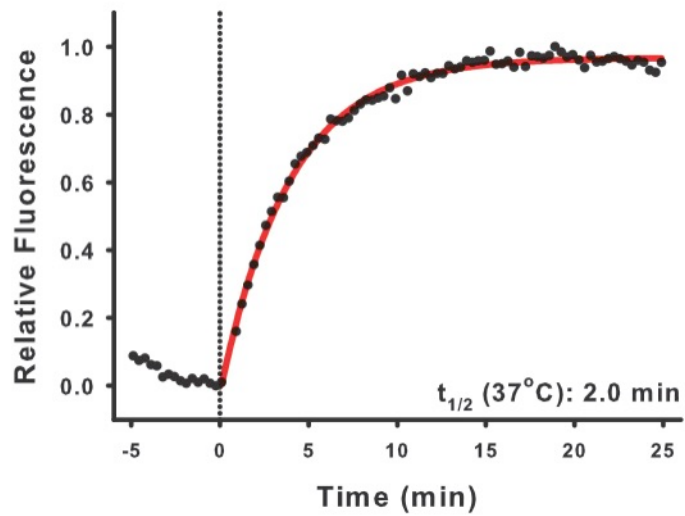


Figure S2. Retinal release of bovine rhodopsin measured at physiological temperature.

Measured as the increase in fluorescence intensity following photoactivation at $t = 0$

(dotted line) at 37°C, pH 7, in 0.1% dodecyl maltoside.



Figure S3. Sequence logo representing variation among vertebrate rhodopsins of eight residues shown to mediate retinal release. Generated using WebLogo (81) with standard settings. Sequences used were obtained from GenBank (www.ncbi.nlm.nih.gov) and listed in Table S1.

Table S1. List of rhodopsin sequences used to generate the sequence logo in Figure S3, along with respective GenBank accession numbers.

Scientific Name	Accession Number
<i>Plecoglossus altivelis</i>	AB074484
<i>Chlamydera maculata</i>	JQ034385
<i>Emberiza bruniceps</i>	JQ695942
<i>Ailuroedus crassirostris</i>	JQ034381
<i>Pseudobagrus tokiensis</i>	FJ197075
<i>Gallus gallus</i>	NM_001030606
<i>Bos Taurus</i>	NM_001014890
<i>Corvus macrorhynchos</i>	AB555651
<i>Tursiops truncatus</i>	AF055456
<i>Sminthopsis crassicaudata</i>	AY159786
<i>Tachyglossus aculeatus</i>	JX103830
<i>Anguilla Anguilla</i>	L78008
<i>Anguilla Anguilla</i>	L78007
<i>Xenopus tropicalis</i>	BC135234
<i>Lepisosteus osseus</i>	AF137207
<i>Carassius auratus</i>	L11863
<i>Poecilia reticulata</i>	Y11147
<i>Homo sapiens</i>	U49742
<i>Lethenteron camtschaticum</i>	AB116382
<i>Uta stansburiana</i>	DQ100323
<i>Lefua costata</i>	EU409634
<i>Macaca fascicularis</i>	S76579
<i>Anas platyrhynchos</i>	AF021240
<i>Trichechus manatus</i>	AF055319
<i>Oryzias latipes</i>	AB180742
<i>Chanos chanos</i>	FJ197072
<i>Mus musculus</i>	BC031766
<i>Cynops pyrrhogaster</i>	AB043890
<i>Polyodon spathula</i>	AF369050
<i>Scopelarchus analis</i>	EF517404
<i>Esox Lucius</i>	AY158044
<i>Takifugu rubripes</i>	AF201471
<i>Python regius</i>	FJ497236
<i>Oryctolagus cuniculus</i>	NM_001082349
<i>Ambystoma tigrinum</i>	U36574
<i>Salmo salar</i>	NM_001123537
<i>Mirounga angustirostris</i>	AY228452
<i>Xenopeltis unicolor</i>	FJ497233
<i>Acipenser sp.</i>	AF137206
<i>Astyanax mexicanus</i>	U12328
<i>Bufo bufo</i>	U59921
<i>Eubalaena glacialis</i>	JQ730751
<i>Taeniopygia guttata</i>	AF222329
<i>Danio rerio</i>	AB087811

Table S2: Retinal release half-life values for wild type and reverse triple mutant bovine rhodopsin, measured at 20°C, confirming the effects of several mutations in the bovine rhodopsin background.

Opsin	Retinal release $t_{1/2}$ (min)^a	Difference from WT zebrafish rhodopsin (min)
Bovine Rhodopsin	13.9 ± 1.6 (19)	----
I213F, L266V, F273W	9.6 ± 0.7 (3)	-4.3**

^a For retinal release rate half-life values, the number of replicates are indicated in brackets.

^b Significant differences from wild type bovine rhodopsin are indicated, based on a two-tailed t-test with unequal variance, where $p < 0.01$ (**).