A ciliary opsin in the brain of a marine annelid zooplankton is UV-sensitive and the sensitivity is tuned by a single amino acid residue

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Supplemental Data

Supplemental Table S1 and supplemental Figs. S1 and S2

Supplemental Table S1

The list of PCR primers to make Lys-94 mutants of *Platynereis* c-opsin

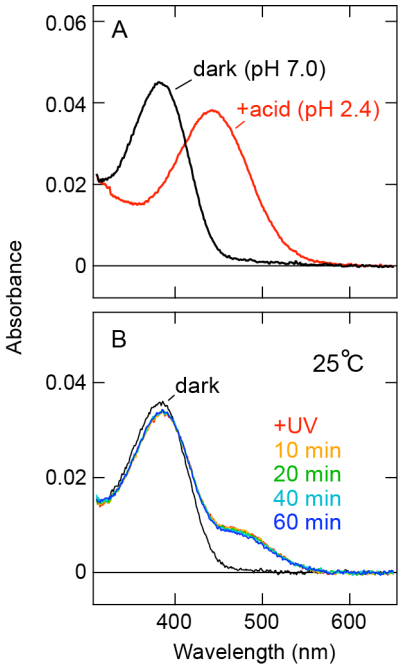
primer name	primer sequence
c-opsin_forward	5'-ccaactgcaagaattccaccatggatggggaaaat-3'
c-opsin_1D4_reverse	5'-ccggaatttgcggccgcttaagcaggagccacctgactagtctcggtagcagccacctg-3'
K94T_forward	5'-tttggaactgctttcaCgtttatcatcacagcc-3'
K94T_reverse	5'-ggctgtgatgataaacGtgaaagcagttccaaa-3'
K94A_forward	5'-tttggaactgctttcGCgtttatcatcacagcc-3'
K94A_reverse	5'-ggctgtgatgataaacGCgaaagcagttccaaa-3'
K94S_forward	5'-tttggaactgctttcaGCtttatcatcacagcc-3'
K94S_reverse	5'-ggctgtgatgataaaGCtgaaagcagttccaaa-3'
K94V_forward	5'-tttggaactgctttcGTgtttatcatcacagcc-3'
K94V_reverse	5'-ggctgtgatgataaacACgaaagcagttccaaa-3'
K94D_forward	5'-tttggaactgctttcGaCtttatcatcacagcc-3'
K94D_reverse	5'-ggctgtgatgataaaGtCgaaagcagttccaaa-3'
K94E_forward	5'-tttggaactgctttcGagtttatcatcacagcc-3'
K94E_reverse	5'-ggctgtgatgataaactCgaaagcagttccaaa-3'
K94H_forward	5'-tttggaactgctttcCaCtttatcatcacagcc-3'
K94H_reverse	5'-ggctgtgatgataaaGtGgaaagcagttccaaa-3'
K94R_forward	5'-tttggaactgctttcaGgtttatcatcacagcc-3'
K94R_reverse	5'-ggctgtgatgataaacCtgaaagcagttccaaa-3'

In the sequences of "K94X_forward" and "K94X_reverse" primers, mutated sequences are shown in capitals.

Supplemental Fig. S1 Tsukamoto et al.

<u>Supplemental Fig. S1.</u> Amino acid sequence alignment of *Platynereis* c-opsin, human Opn3, pufferfish TMT opsin, mosquito Opn3, lamprey parapinopsin, and bovine rhodopsin

Putative regions corresponding to seven transmembrane helices are indicated. Positions 94, 113, 181, and 296 are also indicated (*red arrowheads*), and amino acid residues at position 94 are highlighted in boldface. Positions 113/181 and 296 are the counterion sites and the retinal-binding site, respectively. The *red box* marks the conserved C-terminal motif of ciliary opsins, but the motif is not conserved in human Opn3. Note that residue numbering in this study is based on the sequence of bovine rhodopsin.



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<u>Supplemental Fig. S2.</u> Spectral data supporting arguments in this study.

A, Acid denaturing of *Platynereis* c-opsin to confirm formation of the covalent bond (the Schiff base linkage) with the retinal. 140 μL of purified c-opsin (*black*) at pH 7.0 was mixed with 6 μL of 1 N HCl, and the final pH value was converted to 2.4 (*red*). The product with λ_{max} of ~440 nm is due to protonation of the Schiff base linkage and clearly indicate the existence of the covalent bond with the retinal (7). *B*, Thermal stability of the photoproduct of *Platynereis* c-opsin. Purified c-opsin (*black*) was illuminated by UV light (*red*), and kept at 25 °C for 10 (*orange*), 20 (*green*), 40 (*pale blue*) and 60 (*blue*) minutes, respectively.