

Supplementary Information for

Dragonfly red opsins share a common tuning mechanism with mammalian red opsins and further enhancement of near-infrared sensitivity

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		Third intracellular loop		
Sf_LWF1	217	PLATIIYSYWFIVQAVAAHEKQMREQAKKMNVASLRSADASNTSAECKLAKVALMTISLW	276	
Sf_LWF2	223	PLATIIYSYWFIVQAVAAHEKQMREQAKKMNVASLRSADASNTSAECKLAKVALMTISLW	282	
Sf_LWF3	228	PLATIIYSYWFIVQAVAAHEKQMREQAKKMNVASLRSADASNTSAECKLAKVALMTISLW	287	
Sf_LWF4	226	PLATIIYSYWFIVQAVAAHEKQMREQAKKMNVASLRSADASNTSAECKLAKVALMTISLW	285	
Sf_LWA1	230	PLGVIIYAYWFIVKAVSAHERQMREQAKKMNVASLRSAEAQNTSAEIKLAKIAMMTISLW	289	
Sf_LWA2	229	PLGIIIIYAYWFIVQAVAAHEKQMREQAKKMNVASLRSSEAQNTSAECKLAKIAMMTISLW	288	
Oa_LWA2	229	PLGVIIYAYWFIVQAVAAHEKQMREQAKKMNVASLRSSEAQNTSAECKLAKIAMMTISLW	288	
Su_LWA2	229	PLGIIIIYAYWFIVQAVAAHEKQMREQAKKMNVASLRSSEAQNTSAECKLAKIAMMTISLW	288	
Ma_LWA2	229	PLGIIIIYAYWFIVQAVAAHEKQMREQAKKMNVASLRSSEAQNTSAECKLAKIAMMTISLW	288	
As_LWA2	229	PLGIIIIYAYWFIVQAVAAHERQMREQAKKMNVASLRSSEAQNTSAECKLAKIAMMTISLW	288	
Tp_LWA2	229	PLGIIIIYAYWFIVQAVAAHEKQMREQAKKMNVASLRSSEAQNTSAECKLAKIALMTISLW	288	
Am_LWA2	228	PLGIIIIYAYWFIVQAVAAHERQMREQAKKMNVASLRSSEAQNTSAECKLAKIAMMTISLW	287	
Ap_LWA2	230	PLGVIIYAYWFIVQAVAAHEKQMREQAKKMNVASLRSSEAQNTSAECKLAKIALMTISLW	289	
Es_LWA2	229	PLGVIIYAYWFIVQAVAAHERQMREQAKKMNVASLRSSEAQNTSAECKLAKIALMTISLW	288	
Ia_LWA2	227	PLGIIIIYAYWFIVQAVAAHERQMREQAKKMNVASLRSSEAQNTSAECKLAKVALMTISLW	286	
Mc_LWA2	227	PLGIIIIYAYWFIVQAVAAHERQMREQAKKMNVASLRSSEAQNTSAECKLAKVALMTISLW	286	
Ip_LWA2	229	PLAMIIYAYWFIVQAVATHERNMREQAKKMNVASLRSSEAQNTSAECKLAKVAMVTISLW	288	
Bovine Rh	215	PLIVIFFCYGQLVFTVKEAAAQQQE-----SATTQKAEKEVTRMVIIMVIAF	261	
Dragonfly Gs-RhLW		Jellyfish opsin		
Jellyfish opsin	184	PVLLMVATYVLVQG---EMQNMRGAAQLFGSESEAALKN-IKAEKRHTRLVFMILSF	238	

Figure S1

Fig. S1. Alignment of amino acid sequences of the third intracellular loops of opsin. Third intracellular loops of opsins (black plane letters) were replaced with that of Gs-coupled jellyfish opsin (cyan bold letter). The schematic drawing of chimeric mutants (Dragonfly Gs-RhLW) is also shown.

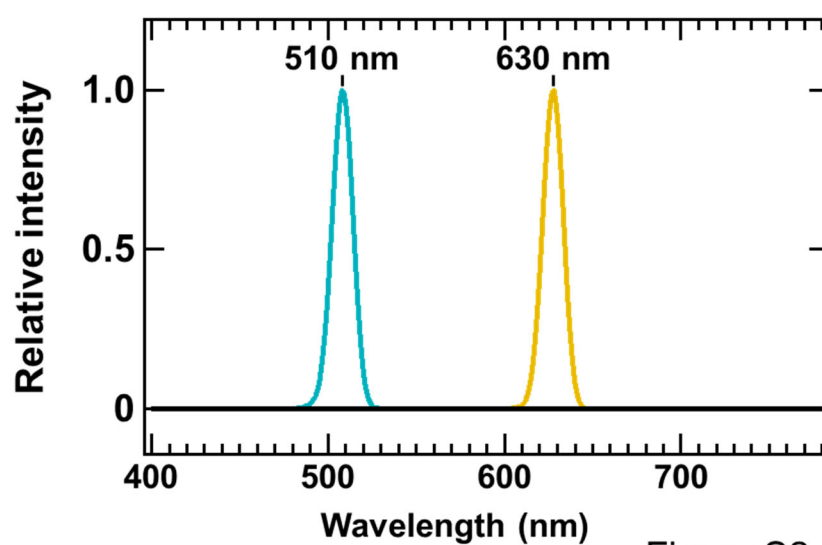


Figure S2

Fig. S2. Spectra of lights for HAS stimulation. Spectra of narrow-band 510 nm and 630 nm lights are shown.

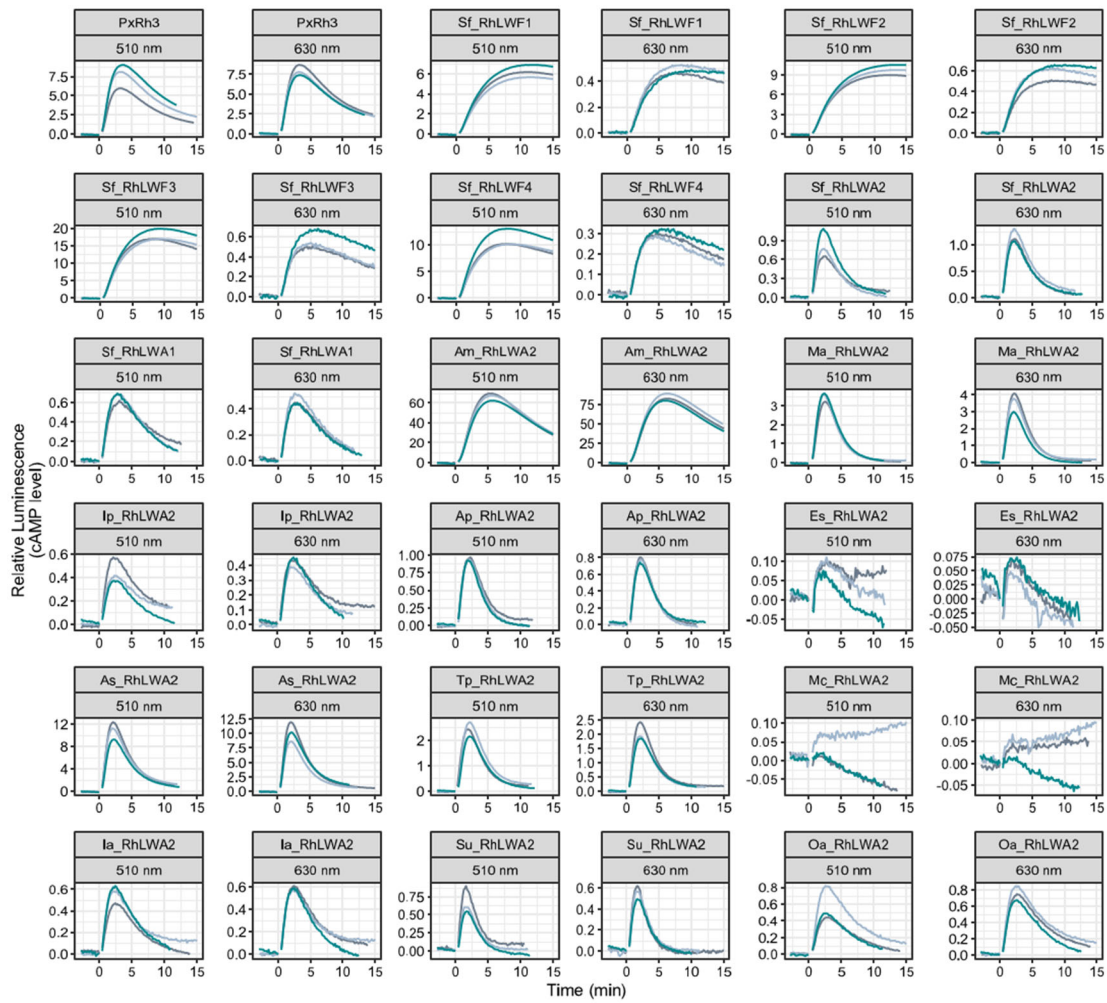


Figure S3

Fig. S3. The datasets of time-dependent luminescence changes for Fig. 1. Light-induced changes in intracellular cAMP in HEK293 cells expressing Dragonfly Gs-RhLW in response to 630-nm light and 510-nm light measured by GloSensor cAMP assay. At time 0 indicates the timing of light stimulation. The three colored curves correspond to the three experimental replicates. Am, *Asiagomphus melaenops*; Ma, *Macromia amphigena*; Sf, *Sympetrum frequens*; Ip, *Indolestes peregrinus*; Ap, *Anax parthenope*; Es, *Epiophlebia superstes*; As, *Anotogaster sieboldii*; Tp, *Tanypteryx pryeri*; Mc, *Mnais costalis*; Ia, *Ischnura asiatica*; Su, *Somatochlora uchidai*; Oa, *Orthetrum albistylum*.

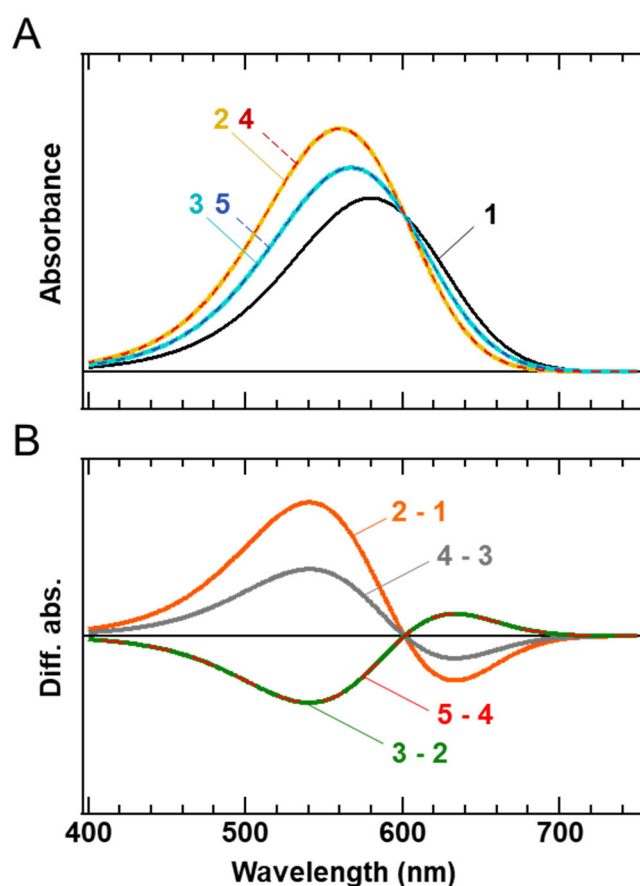


Figure S4

Fig. S4. Schematic representations of the relationship between absolute and difference spectra of bistable pigments.

(A) Diagrams of absolute spectra of opsin-based pigments in the dark (1: black curve), after red-light irradiation (2: yellow curve), after subsequent green-light irradiation (3: cyan curve), after a second red-light irradiation (4: red curve), and after a second green-light irradiation (5: blue curve). (B) The difference spectra are calculated by subtracting two spectra (after minus before irradiation) according to the notations.

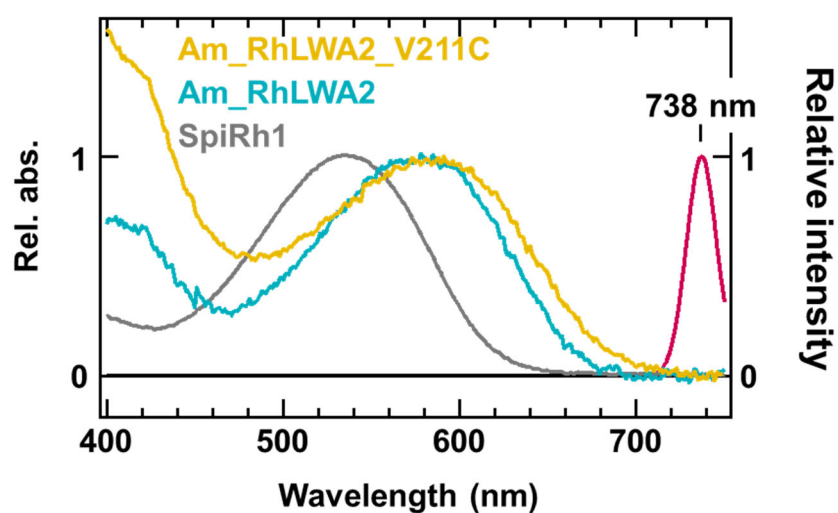


Figure S5

Fig. S5. Absorption spectra of SpiRh1, Am_RhLWA2, and the V211C mutant, along with the NIR light spectrum.

Absorption spectra of SpiRh1 (gray), Am_RhLWA2 (cyan) and the V211C mutant (yellow) are shown for comparison. Each absorption spectrum was normalized to the maximum absorbance in the visible light region. The spectrum of tightly filtered 738 nm light is also shown.