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Supplemental Information

Complex Photochemistry within the Green-Absorbing Channelrhodop-

sin ReaChR

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FIGURE S1 Normalized Evolutionary Associated Difference Spectra (EADS) of ReaChR wild-type at pH 5 (*top*), 7.4 (*middle*) and 9 (*bottom*). EADS and their half-life times ($t_{1/2}$) are derived from a global fit routine. For illustration purposes, solid lines were added to the EADS. Photocycle intermediates are indicated by capital letters.



FIGURE S2 Slow-cycling mutants. Representative photocurrents of the C168A and C168S mutant after illumination with 530 nm light (25 s) and wild-type (wt) ReaChR (5 s, 530 nm). Bar graph summarizes monoexponentially fitted current decays (top right, mean \pm SE.; n = 6-7).



FIGURE S3 High-pressure liquid chromatography (HPLC) of retinal isomers isolated from ReaChR wild-type at pH 7.4 (room temperature). Before retinal extraction the sample was illuminated for 10 min (\sim 530 nm LED) followed by 10 min dark adaptation (apparent dark state, DA_{app}). The retinal isomers eluted after 23.3 min (13-*cis*) and 39.5 min (all-*trans*). The ratio of 78:22 all-*trans*:13-*cis* retinal was determined via peak integration.