

Supplementary Information for

The first steps of the visual cycle in human rod and cone photoreceptors

Chunhe Chen, Leopold Adler IV, Cole Milliken, Bushra Rahman, Masahiro Kono, Lynn Poole Perry, Federico Gonzalez-Fernandez, Yiannis Koutalos

Includes: Figures S1 and S2 and SI References

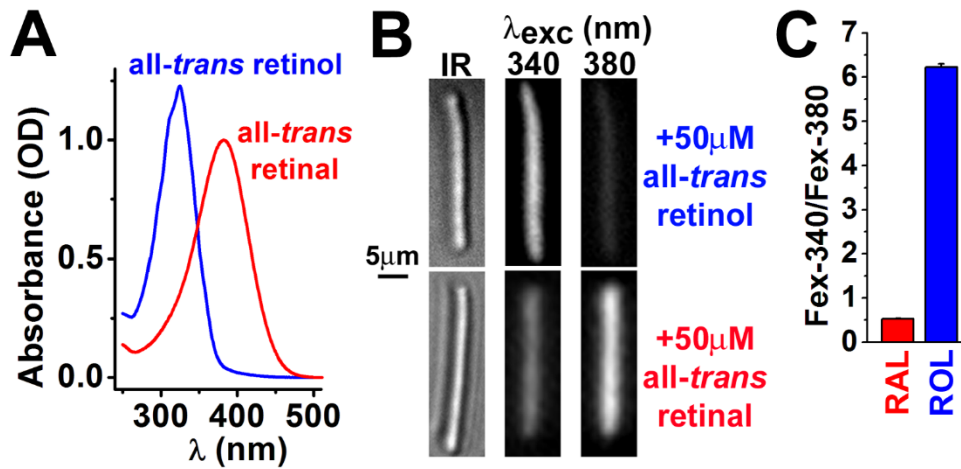


Fig. S1. Measurement of Fex-340 / Fex-380 fluorescence ratios for all-*trans* retinal and retinol. Fluorescence was excited with 340 and 380 nm light, and emission collected for >420 nm. (A) Absorption spectra of equimolar amounts of all-*trans* retinal and retinal in ethanol (replotted from ¹). (B) Fluorescence of human broken off rod outer segments (bROS) loaded with 50 μ M all-*trans* retinol or all-*trans* retinal (for 5 min, using 1% bovine serum albumin as carrier). IR, infrared images of the outer segments; fluorescence images of the outer segments after loading with retinoid are shown with the same intensity scaling to facilitate comparisons. (C) Fex-340 / Fex-380 fluorescence intensity ratios obtained by loading human bROS with 50 μ M of retinoid, giving values of 0.53 ± 0.01 for all-*trans* retinal (RAL, n = 9 cells) and 6.23 ± 0.07 for all-*trans* retinol (ROL, n = 8 cells), similar to those measured with mouse bROS ². Loading was carried out for 5 min, using 1% bovine serum albumin as lipophilic carrier. Error bars denote standard errors. All experiments at 37 $^{\circ}$ C.

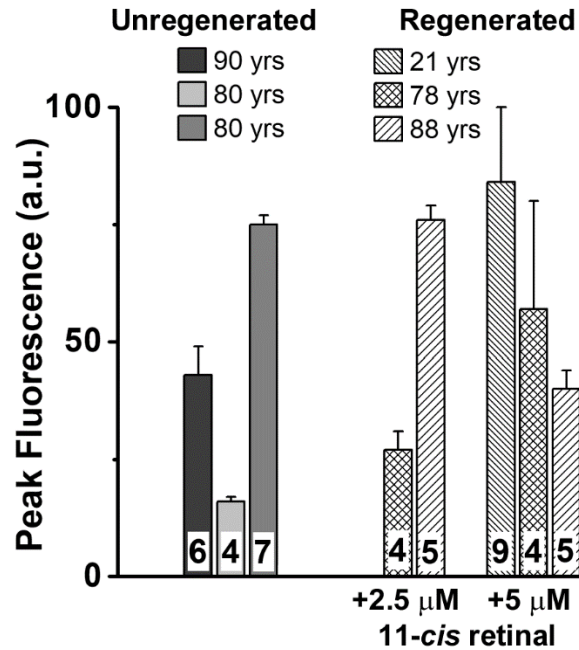


Fig. S2. Peak all-*trans* retinol fluorescence reached following bleaching in human rod photoreceptors isolated from different donors. Peak fluorescence reflects the amount of rhodopsin present before bleaching. For cells from three of the donors, isolated rod photoreceptors were not regenerated with 11-*cis* retinal prior to the measurement of all-*trans* retinol formation (Unregenerated). For cells from another three donors, isolated rods were regenerated by incubating for 10 min with 2.5 or 5 μM 11-*cis* retinal (Regenerated). All experiments at 37 °C. Number of cells are shown within each bar. Error bars represent standard errors.

SI References

1. Chen C, Thompson DA, Koutalos Y. Reduction of all-trans-retinal in vertebrate rod photoreceptors requires the combined action of RDH8 and RDH12. *J Biol Chem* 2012;287:24662-24670.
2. Adler L IV, Chen C, Koutalos Y. Mitochondria contribute to NADPH generation in mouse rod photoreceptors. *J Biol Chem* 2014;289:1519-1528.