

Supplementary Information

An engineered opsin monomer scrambles phospholipids

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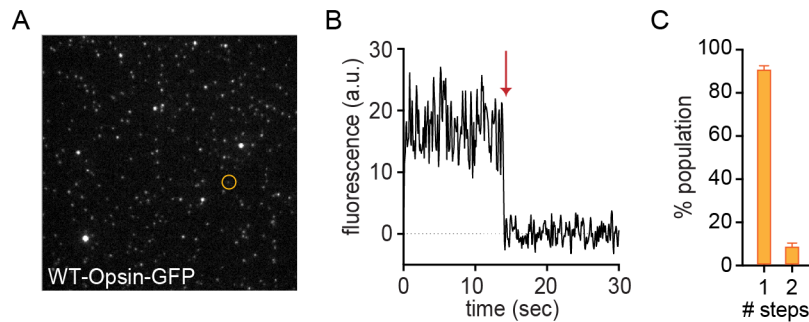
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Supplementary Figure S1

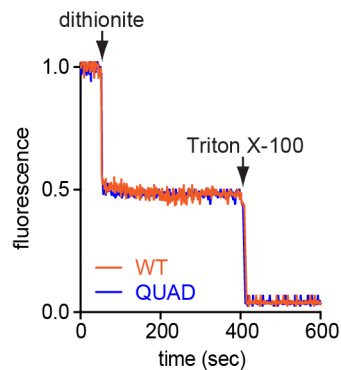


Supplementary Figure S1.

Single molecule photobleaching analysis of WT-Opsin-GFP.

WT-Opsin-GFP was expressed in HEK293T cells, extracted with 0.1% (w/v) DDM and tethered to a passivated glass slide using a biotinylated anti-GFP antibody. **(A)**. Representative TIRF image of the sample showing distribution of spots. Panel B shows photobleaching of the spot circled in orange. **(B)**. Representative trace showing single step photobleaching of a WT-Opsin-GFP molecule (corresponding to the circled spot in panel A). **(C)** Graph showing the fractional distribution of WT-Opsin-GFP molecules undergoing 1- and 2-step photobleaching in response to 488 nm illumination. 971 molecules were analyzed, 883 of which were bleached in a single step as shown in panel B. Error bars are standard errors calculated from 4 movies (>200 spots were analyzed per movie).

Supplementary Figure S2



Supplementary Figure S2.

Reconstituted vesicles protect encapsulated NBD-glucose from dithionite.

2-NBD-Glucose (10 μ M final concentration) and WT-opsin or QUAD opsin were added to DDM-destabilized vesicles and the samples were treated with BioBeads to reconstitute proteoliposomes containing encapsulated NBD-Glucose (PPR~1.1 mg/mmol (WT-opsin) and 0.45 mg/mmol (QUAD-opsin)). Fluorescence was recorded using the same settings as for NBD-PC (see Methods) and dithionite was added as indicated. For both WT-opsin and QUAD-opsin reconstituted samples, dithionite addition resulted in a sharp drop in fluorescence followed by a steady signal, indicating reduction of extravesicular NBD-Glucose and protection of the encapsulated pool. The extent of reduction was not as great as expected because of significant adsorption of NBD-Glucose to the BioBeads. Subsequent addition of Triton X-100 to disrupt the membrane barrier eliminated fluorescence. Thus, dithionite cannot cross the membrane of reconstituted vesicles to reduce trapped NBD-Glucose.