Supplementary Information for:

Powdered G-Protein-Coupled Receptors

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1. Preparation of rhodopsin disk membranes

For extraction of rhodopsin from retinal disk membranes (RDM), bovine retinas were obtained from W. L. Lawson, Co. (Lincoln, NE) in 30 % sucrose solution containing aprotinin, a competitive serine protease inhibitor, and DTT (dithiothreitol). The bovine retinas were kept in a −80 °C freezer before use. Note that the yield of RDM is much higher in cases where retinas are dissected from bovine eyes and stored in a 30% sucrose solution. Rhodopsin was extracted from bovine retinal disk membranes as described.[1] Briefly, the retinas were thawed followed by homogenization and sucrose density gradient centrifugation at 4 °C. The complete protocol[1] was performed in the cold room at 4 °C unless stated otherwise. The RDM have a characteristic band in the sucrose density gradient (26–30% w/w). The band containing RDM was collected and the excess sucrose was removed by dilution with deionized water, followed by centrifugation at 48,000 × g (Sorvall SS-34 rotor) for 30 min. After centrifugation the pellet was collected and re-suspended in deionized water. The process was repeated three times. Finally, the RDM membranes were suspended in 15 mM sodium phosphate buffer pH 6.9, and the protein was characterized using UV-visible absorption spectroscopy (Fig. S1).

2. Purification of rhodopsin in CHAPS and determination of yield

Next, we purified the rhodopsin in CHAPS detergent using a zinc acetate extraction method.[2] It should be pointed out that this is the first instance of successfully using a zinc acetate extraction procedure with a zwitterionic detergent. For determining the yield of the purified rhodopsin, we compared the amount of rhodopsin before and after purification. First, the amount

of RDM before purification was estimated using UV-visible spectroscopy.[3] Next, the characterization of the purified protein was determined using UV-visible spectroscopy. The percentage ratio for the amount of the purified protein to the amount of rhodopsin in the RDM before purification gives the yield of purified rhodopsin.

3. Lyophilization of RDM and rhodopsin-detergent solutions

The extracted RDM membranes (0.3 mg/mL rhodopsin) were flash-frozen with liquid nitrogen followed by lyophilization or cryodessication. The freeze-drying was done for 12 hrs at 100-mTorr vacuum pressure. Lyophilization was performed until constant mass was obtained. The lyophilized RDM were dissolved in 3% Ammonyx LO detergent in 15 mM sodiumphosphate buffer containing 30 mM hydroxylamine, and UV-visible spectra were obtained[1] (Fig S1). The UV-visible spectra of the rehydrated lyophilized RDM in the dark state and photobleached states were collected to obtain the amount and purity of the protein[3] (Fig. S1). The UV-visible spectra of the solubilized RDM before lyophilization and after lyophilization were compared and no significant difference was obtained, implying high stability of the lyophilized RDM. Notably rhodopsin in native disk membranes is more stable than the purified protein in detergents. Lastly, to address whether the process of freeze-drying can be accomplished with rhodopsin-detergent solutions, we performed lyophilization with purified rhodopsin (1.85 mM) in CHAPS detergent (50 mM). The concentrations are prior to lyophilization. Successful freeze-drying was obtained with purified rhodopsin in CHAPS detergent (see text). The lyophilization conditions for the rhodopsin-detergent solutions were the same as for lyophilization of rhodopsin in disk membranes.

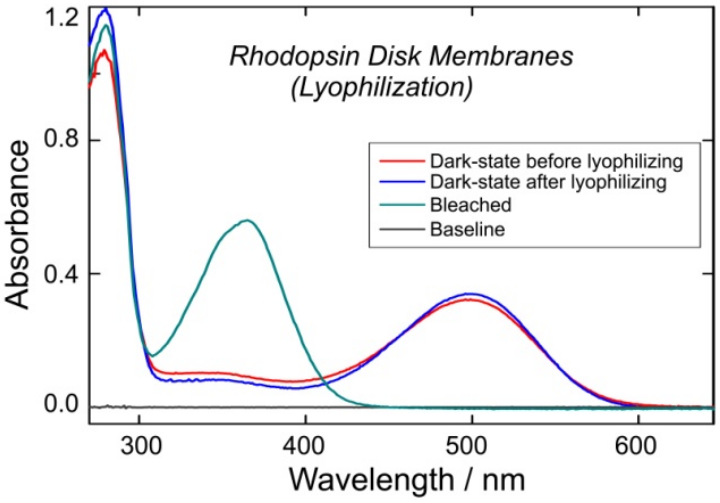

Figure S1. Characterization of the powdered disk membranes containing rhodopsin prepared from bovine retinas upon rehydration with detergent buffer. Dark-state rhodopsin absorbs strongly at 500 nm. After complete photobleaching in the presence of hydroxylamine, the spectral purity (A_{280}/A_{500}) was determined. The A_{280} is due to aromatic amino acids whereas A_{500} is specific to the retinylidene protonated Schiff base. The rhodopsin in disk membranes was solubilized in 3% Ammonyx LO detergent containing 15 mM sodium phosphate buffer (pH 6.9) containing 30 mM hydroxylamine at 15 ˚C and A_{280}/A_{500} was determined to be 2.4.

4. References

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3. Brown, M. F. UV–Visible and Infrared Methods for Investigating Lipid–Rhodopsin Membrane Interactions. *Methods Mol. Biol.* **2012,** *914*, 127-153.