Supporting Information

Tuning the Photocycle Kinetics of Bacteriorhodopsin in Lipid Nanodiscs

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1. SDS-PAGE 5-13% Tris-glycine gel and MALDI-TOF mass spectrum of both bacteriorhodopsin and MSP1E3D1

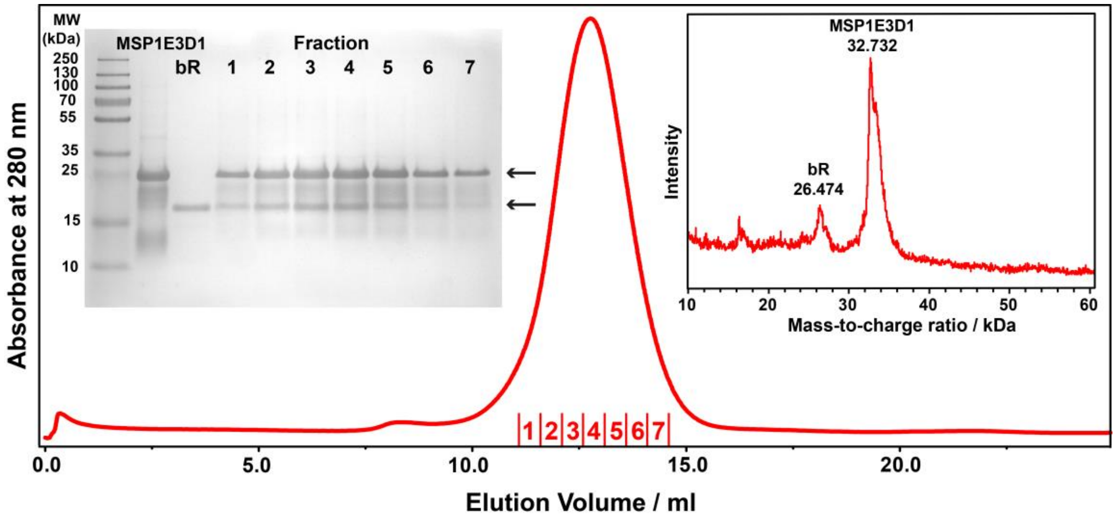

Figure S1. Size exclusion chromatography with Superdex 200 10/300 GL of bR embedded DOPG only nanodiscs, SDS-PAGE gel of fractions collected during size exclusion chromatography and MALDI-TOF mass spectrum of combined fraction of 3 and 4, recorded using Bruker Microflex LRF MALDI mass spectrometer (Bruker Daltonics). Each lane on the SDS-PAGE gel corresponds to the fraction taken at specific elution volume stated in the size exclusion chromatograph, with arrows emphasizing the molecular weight of MSP1E3D1 and bacteriorhodopsin.

2. The circular dichroism spectra

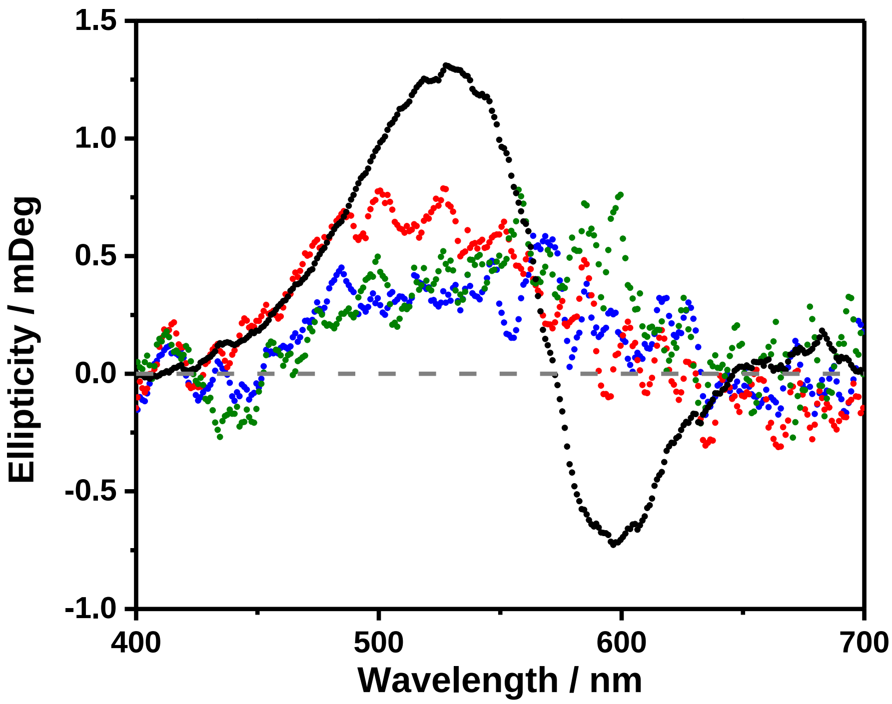

Figure S2. The representative circular dichroism spectra of bR in PM (black) and in nanodiscs of PC/PG=50/50 for DMPC/DMPG (red), DOPC/DOPG (blue) and DMPC/DOPG (green), respectively. The ellipticity of bR in PM was divided by 3.

3. Excitation repetition rates

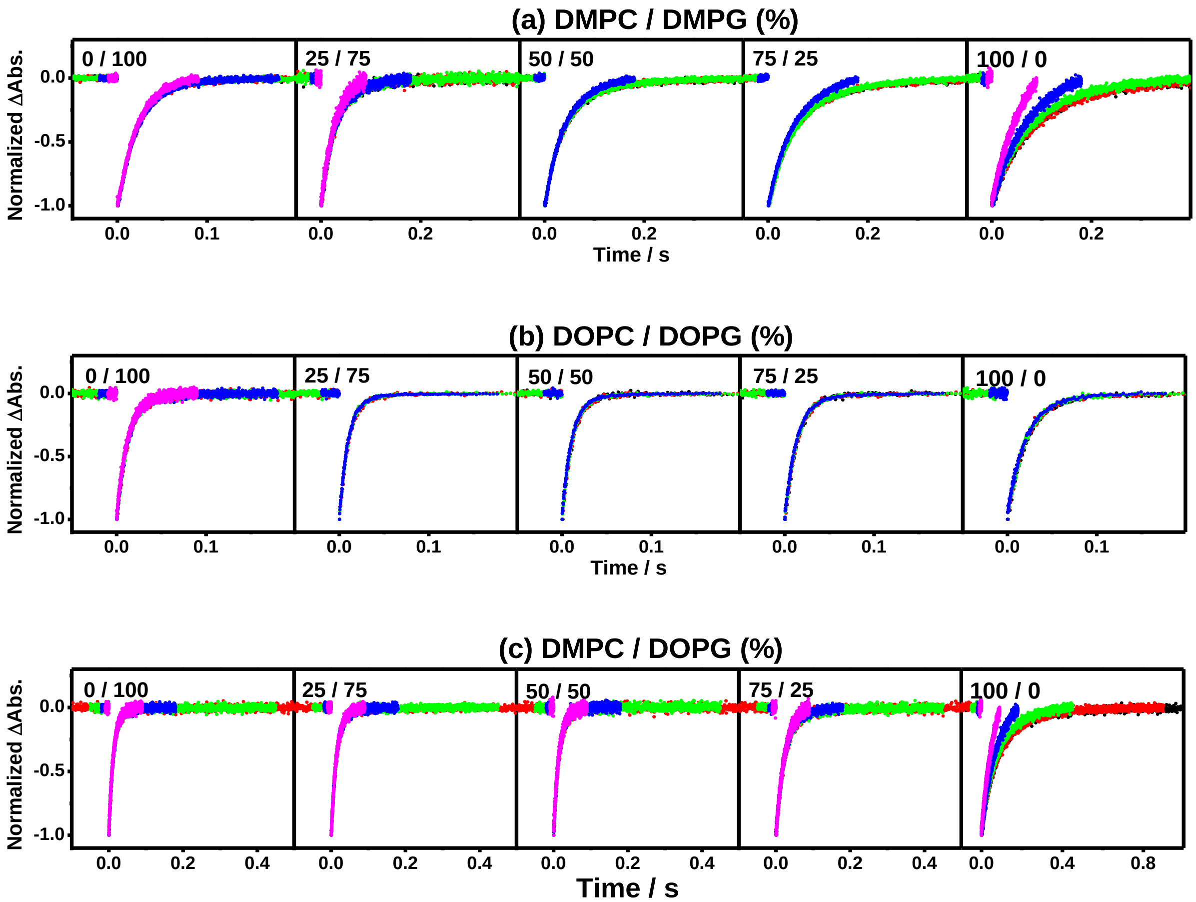

Figure S3. The normalized temporal profiles of the recoveries of bR at 560 nm upon 532-nm pulsed excitation with repetition rates of 0.5 Hz (black), 1 Hz (red), 2 Hz (green), 5 Hz (blue) and 10 Hz (pink) for monomeric bR in nanodiscs consisted of different PC/PG molar ratios (a) DMPC/DMPG, (b) DOPC/DOPG, and (c) DMPC/DOPG. The flux of the 532 nm pulsed laser was controlled at 0.4 mJ/cm^2.

4. Zeta potential results were consistent with the analysis using anionic exchange chromatography

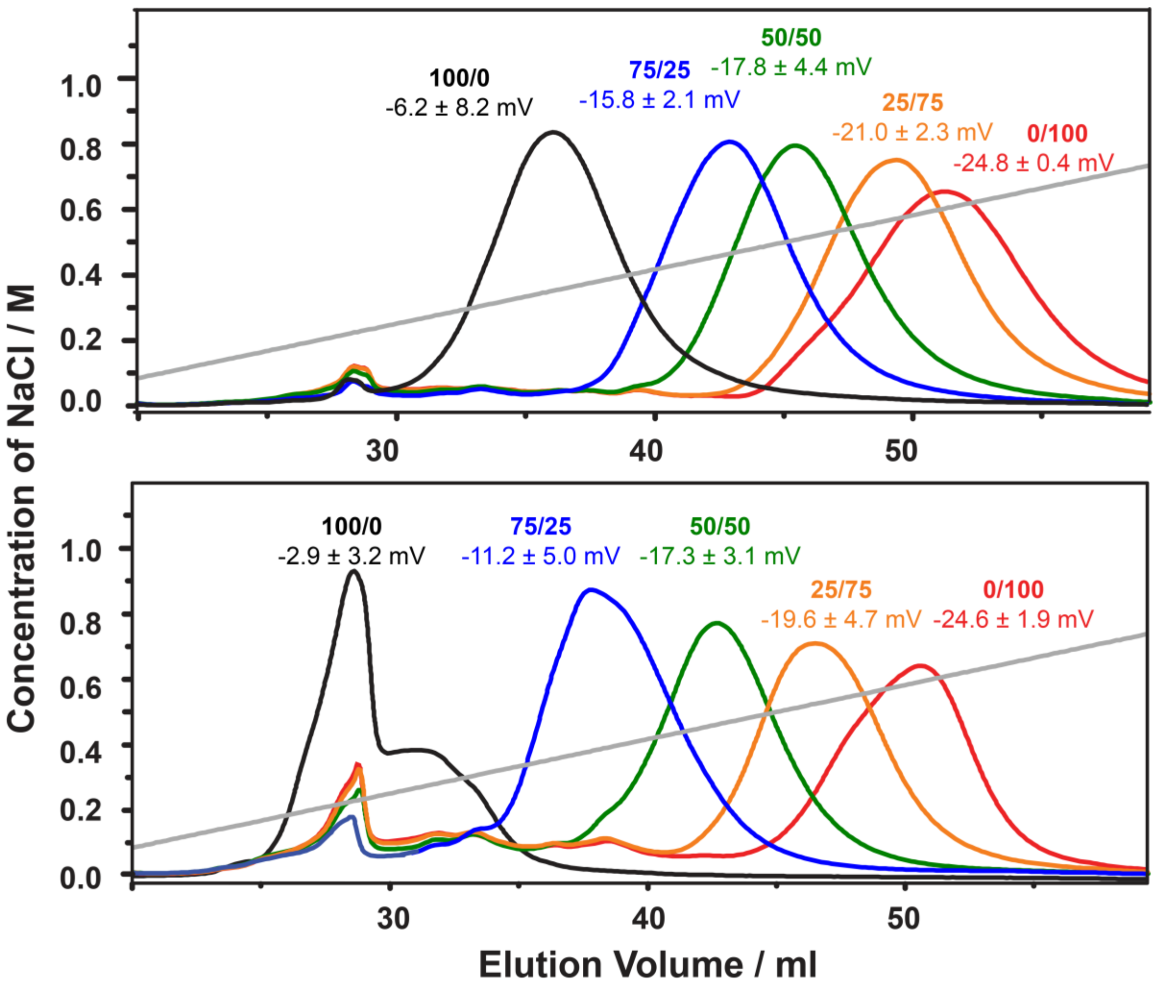

Figure S4. Surface potentials of (above) bR embedded into nanodiscs and (below) empty nanodiscs composed of DMPC/DOPG molar ratios of 100/0 (black), 75/25 (blue), 50/50 (green), 25/75 (orange) and 0/100 (red), characterized by elution profiles of anionic exchange chromatography and zeta potential. The electrokinetic potentials of the nanodisc samples were determined using a zeta potential analyzer (ZetaPlus, Brookhaven Instruments). The cuvette was equipped with carbon electrodes. The concentrations of bR embedded nanodisc sample and empty nanodisc sample were diluted to 1.1 μM and the temperature was controlled at 25 °C. The zeta potential of the empty nanodisc increases the negativity as the content of the negatively-charged lipid DOPG is increased. As the monomeric bR was incorporated into the nanodiscs, the similar increment of negativity of the surface charge slightly increased in each nanodisc sample, indicating that the monomeric bR played a minor role in changing the surface charge of the nanodiscs.

5. Untruncated time-resolved difference absorption spectra

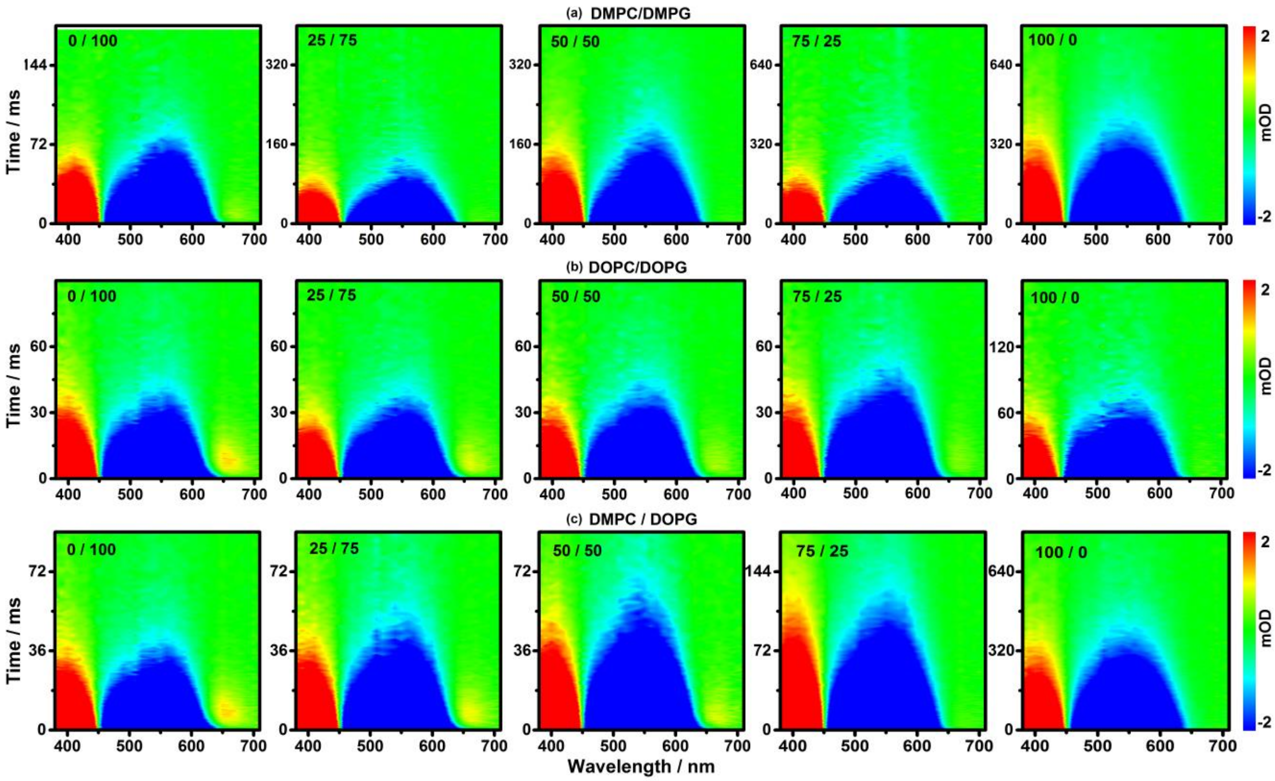

Figure S5. Untruncated time-resolved difference absorption spectra in two-dimensional contours of monomeric bR in nanodiscs consisted of different PC/PG molar ratios for (a) DMPC/DMPG, (b) DOPC/DOPG, and (c) DMPC/DOPG. The flux of the 532 nm pulsed laser was controlled at 0.4 mJ/cm^2.

Table S1. The repetition rates of the excitation 532-nm pulsed for various bR samples for acquiring time-resolved difference spectra.

	DMPC/DMPG				
	100/0	75/25	50/50	25/75	0/100
Number of Averages	550	600	1200	1200	1200
Repetition Rate / Hz	0.5	1	2	2	5
	DOPC/DOPG				
	100/0	75/25	50/50	25/75	0/100
Number of Averages	1200	1200	1200	1200	1100
Repetition Rate / Hz	2	5	5	5	5
	DMPC/DOPG				
	100/0	75/25	50/50	25/75	0/100
Number of Averages	550	1100	1100	1100	1100
Repetition Rate / Hz	0.5	2.5	5	5	5