Coupling Efficiency of Rhodopsin and Transducin in Bicelles

Supplementary Information

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TITLE RUNNING HEAD. Rhodopsin and transducin coupling in negatively-charged bicelles.

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Supplementary Table 1

Bicelle Composition	D_T ($10^{-9}cm^2/s$)	R_h (nm)	MW (kDa)	PolyD (nm)	% PolyD	PolyD Index
Conalbumin	659.9	3.8	74.4	0.8	21.4	0.1
DDM	737.2	3.5	65.2	1.0	27.6	0.1
2% DMPC:DHPC	198.6±14.9	10.3±1.1	816.8±207.2	2.6±0.8	25.5±8.0	0.1±0.1
4% DMPC:DHPC	384.9±104.6	5.8±1.2	232.0±87.4	1.3±0.6	20.6±6.5	0.1±0.1
8% DMPC:DHPC	641.4±28.3	3.3±0.1	55.6±2.2	0.3±0.1	8.1±4.1	0.0
2% PS (70:30)	201.5±7.1	11.2±1.3	996.7±265.8	3.4±0.6	29.6±2.1	0.1±0.0
4% PS (70:30)	307.8±13.9	6.8±0.1	292.3±12.7	1.1±0.1	16.5±2.0	0.0
8% PS (70:30)	370.6±5.7	5.4±0.1	1721±3.5	0.7±0.2	13.2±2.7	0.0
2% PA (70:30)	222.2±10.9	9.9±1.6	769.4±292.2	2.7±0.9	26.1±4.6	0.1±0.1
4% PA (70:30)	327.2±18.2	6.7±0.4	290.6±42.1	1.7±0.1	26.5±3.7	0.1±0.1
8% PA (70:30)	373.8±4.1	5.7±0.1	194.4±7.9	1.4±0.1	24±1.6	0.1 ± 0.1
2% PS (50:50)	252.2±33.8	9.8±1.9	794±375.1	2.7±0.5	27.5±1.8	0.1±0.1
4% PS (50:50)	279.6±21.6	7.4±0.3	369±31.2	1.1±0.3	15±3.4	0.0
8% PS (50:50)	380±21.5	5.4±0.3	177.8±22.4	1.2±0.1	22.8±2.7	0.1±0.1
2% PS+PA (70:30)	230.4±21.1	9.7±1.4	742.4±244.	2.7±0.6	26.5±2.6	0.1±0.1
4% PS+PA (70:30)	297.0±15.9	7.3±0.3	350.2±31.0	1.6±0.1	21.6±0.7	0.1±0.1
8% PS+PA(70:30)	387.5±6.1	5.4±0.1	171.7±5.9	1.4± 0.1	26.4±1.5	0.1±0.1
2% PS+PA (60:40)	197.5±24.2	11.6±2.4	1188±574.7	3.0±1.0	24.2±3.5	0.1±0.1
4% PS+PA (60:40)	309.9±17.6	6.8±0.3	300.5±33.9	1.6±0.2	23.6±3.9	0.1±0.1
8% PS+PA(60:40)	386±3.4	5.4±0.1	171.4±1.7	1.5±0.1	28.7±1.3	0.1±0.1

Supplementary Table 1. Dynamic Light Scattering (DLS) measurements of negatively charged bicelles. All bicelle samples were prepared in extra MII assay buffer (50 mM HEPES pH 8.0, 100 mM NaCl, 1 mM $MgCl_2$). 2 mg/mL Conalbumin (75 kDa) and 0.5 mM DDM (70 kDa) were prepared as positive controls. Hydrodynamic radii were determined by Dynamics V5 software

with light scattering data collected at 18-22 °C on a DynaPro detector. The final concentration of phospholipids was 2%, bicelles was 4 or 8%. The ratio of neutral to negatively charged phospholipids is indicated within the parentheses in the table. Results are means ± S.E.M. of at least 25 scans with three independent experiments.

Supplementary Table 2

	EC_{50} ± S.E.M. (μM)	n
ROS	0.64 ± 0.09	6
DDM	3.14 ± 0.05	4
PA(70:30)	1.08 ± 0.12	3
PS(70:30)	1.08 ± 0.09	3
PS(50:50)	1.07 ± 0.07	4
PA+PS(70:30)	0.94 ± 0.06	3
PA+PS(60:40)	0.79 ± 0.05	3

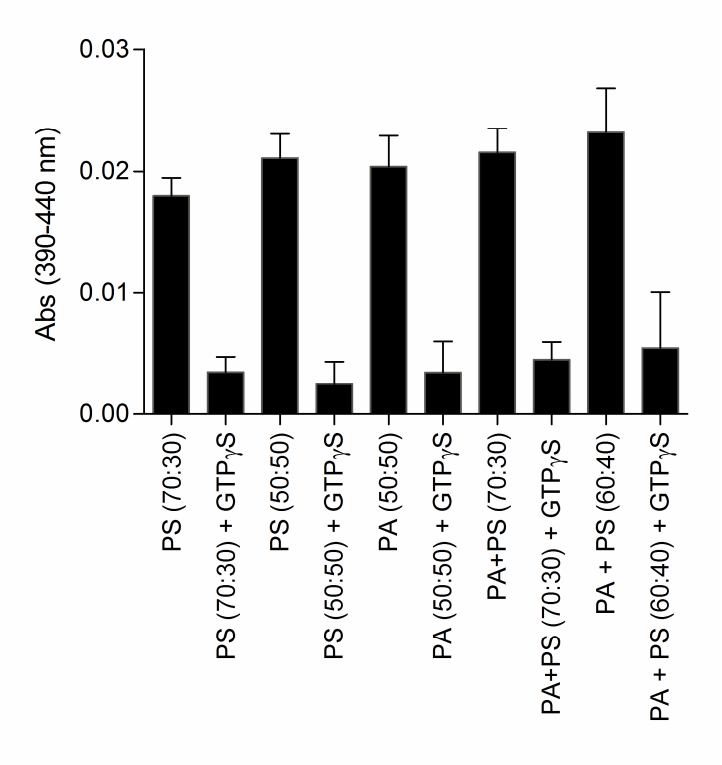
Supplementary Table 2. The affinity of G_t for Rhodopsin in the presence or absence of bicelles. The concentration response curves were measured at 4 °C in the presence of different amount of G_t. Final concentration of bicelle was 8% (lipid:rhodopsin ratio of approximately 12800:1). The ratio of neutral to negatively charged phospholipids is indicated with parenthesis in the table. The concentration-response curves were analyzed using a four parameter logistic equation. Results are mean ± S.E.M. values from at least three independent experiments.

Supplementary Table 3

	4 ^{0}C, pH 8.2	15 ^{0}C, pH 8.2
	$t_{1/2}$ ± S.E.M. (day)	$t_{1/2}$ ± S.E.M. (day)
ROS	0.6 ± 0.01	0.5 ± 0.01
DDM	0.1 ± 0.01	0.1 ± 0.5
PS(70:30)	5.4 ± 0.2	3.3 ± 0.3
PS(50:50)	5.8 ± 0.3	3.9 ± 0.1
PA(70:30)	5.9 ± 0.2	3.1 ± 0.1
PA+PS(70:30)	6.8 ± 0.3	4.6 ± 0.3
PA+PS(60:40)	7.0 ± 0.3	4.9 ± 0.4

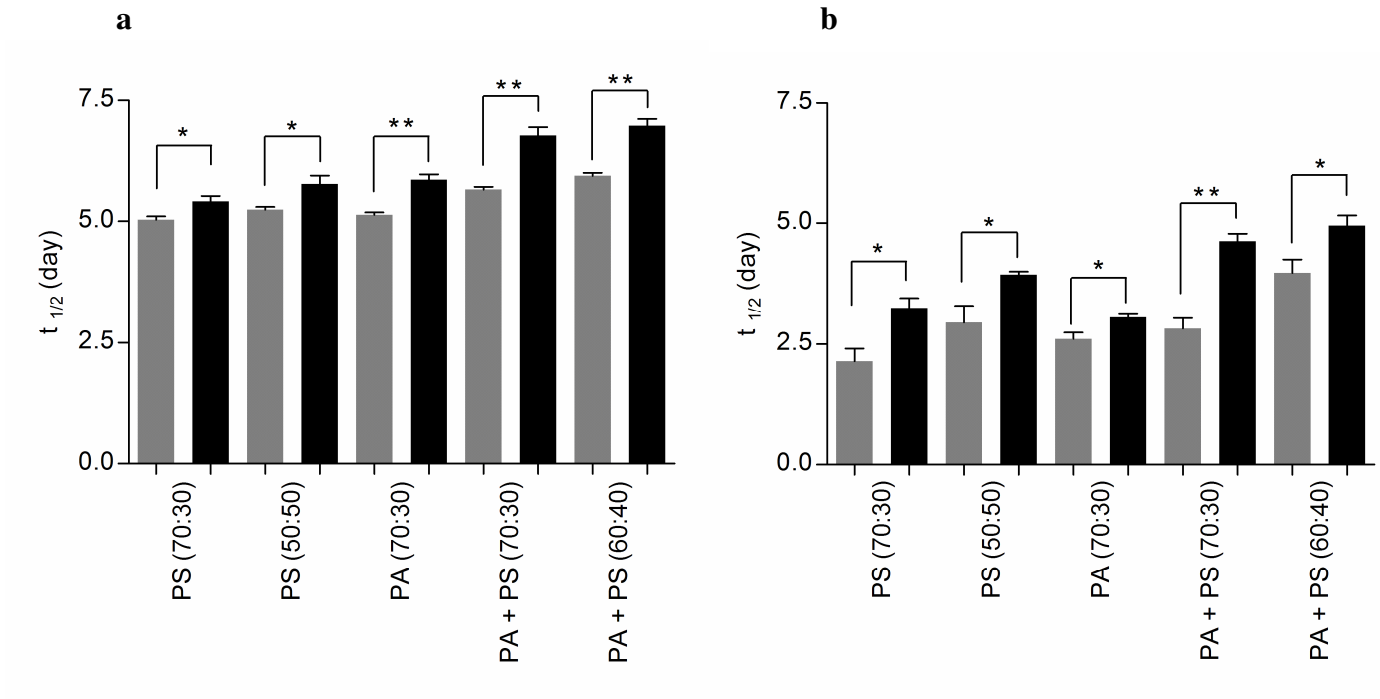
Supplementary Table 3 Extra-metarhodopsin II decays of rhodopsin in the presence of negatively charged bicelles. The extra-metarhodopsin II decay was measured at 4 °C and 15 °C. The final concentration of each bicelle mixture is 8% (lipid:rhodopsin ratio of approximately 12800:1). The ratio of neutral to negatively charged phospholipids is indicated within parentheses. The half life of extra MII signal was calculated using an exponential decay equation. Results are mean ± S.E.M. values from at least three independent experiments.

Supplementary Figure 1



Supplementary Figure 1 The extra MII decay was measured at 4 °C and pH 8.2. To test Receptor-G protein coupling, we added excess GTPγS which terminates receptor-G protein interaction, and we measured this loss of coupling as the loss of the extra-MII signal. We added 150 μM GTPγS 48 hours after starting from the decay experiment. Then, after 5 min. incubation with GTPγS, we measured extra-metarhodopsin signal again. The final bicelle concentration is 8%. Results are mean ± S.E.M. values of at least three independent experiments.

Supplementary Figure 2



Supplementary Figure 2 Stability of rhodopsin-G_t(empty) complex in the presence of negatively charged bicelles. The effect of negatively charged bicelles on complex stability was evaluated at (**a**) 4 °C and (**b**) 15 °C. The final concentration of phospholipids was 2% (grey bars), bicelles was 8% (black bars). The lipid:rhodopsin ratio was approximately 12800:1. The ratio of neutral to negatively charged phospholipids is indicated within the parentheses in the graphs. The half life of the extra MII signal was calculated by using an exponential decay equation. Results are mean ± S.E.M. values from at least three independent experiments (* $p <$ 0.05; ** $p < 0.005$).

Supplementary Table 4

	Initial rates ± S.E.M. ($1 / sec \times 10^{-2}$)
G_t	0.15 ± 0.06
ROS	2.21 ± 0.12
DDM	1.08 ± 0.07
PS (70:30)	2.59 ± 0.26
PS (50:50)	2.88 ± 0.13
PA (70:30)	2.82 ± 0.34
PA+PS (70:30)	4.62 ± 0.30
PA+PS (60:40)	4.48 ± 0.51

Supplementary Table 4. Basal and receptor mediated nucleotide exchange rates of G_t in the presence of different bicelles. Final concentration of bicelles was 8% (lipid:rhodopsin ratio of approximately 12800:1). The ratio of neutral to negatively charged phospholipids is indicated within parentheses. The initial nucleotide exchange rates are shown in $sec^{-1} \times 10^{-2}$ for G_t. The exchange rate was determined by fitting the data to an exponential association equation $F\lambda = F\lambda_{max}(1-e^{-kt})$. Results are mean ± S.E.M. values from at least three independent experiments.