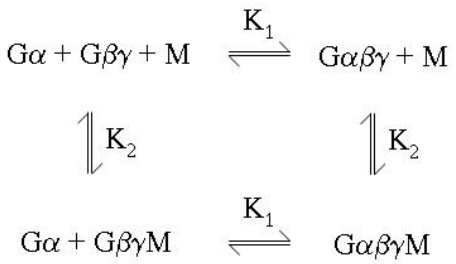
SI Methods

Under the conditions of the experiments, the effective G_t concentration ([Gαβγ], i.e., the concentration of G_t that is immediately available for interaction with R*) is lower than the total G_t concentration $[G_t]_{tot}$. This is due to dissociation of G_t into its subunits (Gα and Gβγ) and G_t binding to detergent micelles (M), which do not contain rhodopsin. In other words, dissociated G_t and G_t bound to micelles without rhodopsin is not competent to interact with R*. Because the isolated Gα subunit is highly soluble, we assume that only Gαβγ and Gβγ bind to detergent micelles. The resulting equilibria are given below:



According to the reaction scheme, the dissociation constant of G_t subunit dissociation (K_1) is given by

$$K_1 = \frac{[G\alpha]\cdot[G\beta\gamma]}{[G\alpha\beta\gamma]} = \frac{[G\alpha]\cdot[G\beta\gamma M]}{[G\alpha\beta\gamma M]} \quad , \qquad [1]$$

and the dissociation constant of micelle interaction with Gαβγ or Gβγ (K_2) is given by

$$K_2 = \frac{[G\beta\gamma]\cdot[M]}{[G\beta\gamma M]} = \frac{[G\alpha\beta\gamma]\cdot[M]}{[G\alpha\beta\gamma M]} \quad . \qquad [2]$$

The mass balance equations for G_t, Gα, Gβγ, and M are:

$$[G_t]_{tot} = [G\alpha]_{tot} = [G\beta\gamma]_{tot} \qquad [3]$$

$$[G\beta\gamma]_{tot} = [G\beta\gamma] + [G\beta\gamma M] + [G\alpha\beta\gamma] + [G\alpha\beta\gamma M] \qquad [4]$$

$$[G\alpha]_{tot} = [G\alpha] + [G\alpha\beta\gamma] + [G\alpha\beta\gamma M] \qquad [5]$$

$$[M]_{tot} = [M] + [G\beta\gamma M] + [G\alpha\beta\gamma M] \qquad [6]$$

The mass balance equations assume that the concentration of G_t complexed with R* (< 3 nM) or bound to detergent micelles containing R* is negligible.

Because of the mutual dependence of the variables, it is not possible to find an analytic solution for [Gαβγ] as a function of $[G_t]_{tot}$. For numerical computing, two additional variables are defined:

$$x_1 = \frac{G\alpha}{K_1} = \frac{G\alpha\beta\gamma}{G\beta\gamma} \quad [7]$$

$$x_2 = \frac{M}{K_2} = \frac{G\beta\gamma M}{G\beta\gamma} \quad [8]$$

Using Eqs. 2, 7, and 8, the variables [Gαβγ], [GβγM], and [GαβγM] can now be expressed as functions of [Gβγ]:

$$[G\alpha\beta\gamma] = x_1 \cdot [G\beta\gamma] \quad [9]$$

$$[G\beta\gamma M] = x_2 \cdot [G\beta\gamma] \quad [10]$$

$$[G\alpha\beta\gamma M] = \frac{[G\alpha\beta\gamma]\cdot[G\beta\gamma M]}{[G\beta\gamma]} = x_1 \cdot x_2 \cdot [G\beta\gamma] \quad [11]$$

Substituting for [Gαβγ], [GβγM], and [GαβγM] in the balance equations (Eqs. 4–6) yields

$$[G\beta\gamma] = \frac{[G\beta\gamma]_{tot}}{1 + x_1 + x_2 + x_1 \cdot x_2} \quad [12]$$

$$[G\alpha] = [G\alpha]_{tot} - x_1 \cdot [G\beta\gamma] - x_1 \cdot x_2 [G\beta\gamma] \quad [13]$$

$$[M] = [M]_{tot} - x_2 \cdot [G\beta\gamma] - x_1 \cdot x_2 \cdot [G\beta\gamma] \quad [14]$$

Eqs. 7–9 and 12–14 were used for numerical calculation of the effective G_t concentration [Gαβγ] as a function of total added G_t ($[G_t]_{tot}$) in the fitting procedure (see below). Together with the numerical calculation of [Gαβγ], the dependence of the initial G_t activation rate (v) on $[G_t]_{tot}$ was fitted to a Michaelis–Menten type hyperbolic function

$$v = \frac{V_{max} \cdot [G\alpha\beta\gamma]}{K_m + [G\alpha\beta\gamma]} \quad , \quad [15]$$

where V_{max} and K_m denote the maximum value of v and the Michaelis constant, respectively. The data points of the titration experiments performed at three different DDM concentrations were

simultaneously fitted using the same set of parameters V_{max}, K_1, and K_2 but with individual Michaelis constants (K_m^1, 0.006% DDM; K_m^2, 0.008% DDM, and K_m^3, 0.01% DDM).

The total concentration of DDM micelles $[M]_{tot}$ was estimated as follows. The data shown in the inset of Fig. 5*B* indicate that under the experimental conditions, the critical micelle concentration of DDM is 0.005%. Together with the molecular mass of DDM (511 g/mol) and the aggregation number of DDM [98 monomers per micelle (1)], the concentrations of DDM micelles are 1.2 µM (0.006% DDM), 1.6 µM (0.008% DDM), and 2.0 µM (0.01% DDM), respectively.

Fitting Procedure

The data points of the titration experiments performed at three different DDM concentrations (Fig. 5*B*) were numerically fitted with the following fitting procedure using Scientist software (MicroMath). The superscript of the variables identifies the set of titration experiment (1: 0.006% DDM; 2: 0.008% DDM, and 3: 0.01% DDM). All concentrations are given in µM.

Independent variable: $[G_t]_{tot}$
Dependent variables: v^1, v^2, v^3
Parameters: $K_1, K_2, V_{max}, K_m^1, K_m^2, K_m^3$

$$[G\alpha]_{tot} = [G_t]_{tot}$$
$$[G\beta\gamma]_{tot} = [G_t]_{tot}$$

$$[M]_{tot}^1 = 1.2$$
$$[M]_{tot}^2 = 1.6$$
$$[M]_{tot}^3 = 2.0$$

$$x_1^1 = [G\alpha]^1 / K_1$$
$$x_1^2 = [G\alpha]^2 / K_1$$
$$x_1^3 = [G\alpha]^3 / K_1$$

$$x_2^1 = [M]^1 / K_2$$
$$x_2^2 = [M]^2 / K_2$$
$$x_2^3 = [M]^3 / K_2$$

$$[G\beta\gamma]^1 = [G\beta\gamma]_{tot} / (1 + x_1^1 + x_2^1 + x_1^1 \cdot x_2^1)$$
$$[G\beta\gamma]^2 = [G\beta\gamma]_{tot} / (1 + x_1^2 + x_2^2 + x_1^2 \cdot x_2^2)$$
$$[G\beta\gamma]^3 = [G\beta\gamma]_{tot} / (1 + x_1^3 + x_2^3 + x_1^3 \cdot x_2^3)$$

$$[G\alpha]^1 = [G\alpha]_{tot} - x_1^1 \cdot [G\beta\gamma]^1 - x_1^1 \cdot x_2^1 \cdot [G\beta\gamma]^1$$
$$[G\alpha]^2 = [G\alpha]_{tot} - x_1^2 \cdot [G\beta\gamma]^2 - x_1^2 \cdot x_2^2 \cdot [G\beta\gamma]^2$$
$$[G\alpha]^3 = [G\alpha]_{tot} - x_1^3 \cdot [G\beta\gamma]^3 - x_1^3 \cdot x_2^3 \cdot [G\beta\gamma]^3$$

$$[M]^1 = [M]_{tot}^1 - x_2^1 \cdot [G\beta\gamma]^1 - x_1^1 \cdot x_2^1 \cdot [G\beta\gamma]^1$$
$$[M]^2 = [M]_{tot}^2 - x_2^2 \cdot [G\beta\gamma]^2 - x_1^2 \cdot x_2^2 \cdot [G\beta\gamma]^2$$
$$[M]^3 = [M]_{tot}^3 - x_2^3 \cdot [G\beta\gamma]^3 - x_1^3 \cdot x_2^3 \cdot [G\beta\gamma]^3$$

$$[G\alpha\beta\gamma]^1 = x_1^1 \cdot [G\beta\gamma]^1$$
$$[G\alpha\beta\gamma]^2 = x_1^2 \cdot [G\beta\gamma]^2$$
$$[G\alpha\beta\gamma]^3 = x_1^3 \cdot [G\beta\gamma]^3$$

$$v^1 = V_{\max} \cdot [G\alpha\beta\gamma]^1 / (K_m^1 + [G\alpha\beta\gamma]^1)$$
$$v^2 = V_{\max} \cdot [G\alpha\beta\gamma]^2 / (K_m^2 + [G\alpha\beta\gamma]^2)$$
$$v^3 = V_{\max} \cdot [G\alpha\beta\gamma]^3 / (K_m^3 + [G\alpha\beta\gamma]^3)$$

$$0 < [G\alpha]^1 < [G\alpha]_{tot}$$
$$0 < [G\alpha]^2 < [G\alpha]_{tot}$$
$$0 < [G\alpha]^3 < [G\alpha]_{tot}$$

$$0 < [M]^1 < [M]_{tot}^1$$
$$0 < [M]^2 < [M]_{tot}^2$$
$$0 < [M]^3 < [M]_{tot}^3$$

References

1. Rosevear P, VanAken T, Baxter J, Ferguson-Miller S (1980) *Biochemistry* 19:4108-4115.