Supporting Information

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SI Estimation of Cytosolic Diffusion Rate of GFP-Tagged $\beta\gamma$ -Subunits (Upper Bound)

To examine when cytosolic diffusion, rather than membrane dissociation, becomes rate limiting for $\beta\gamma$ -subunit translocation, we estimated the diffusivity of a GFP-tagged $\beta\gamma$ -subunit on the basis of its molecular weight. By measuring cytosolic diffusion of engineered GFP multimers in *Escherichia coli*, cytosolic protein diffusivities have been calibrated within the 27- to 111-kDa range (1). On the basis of those measurements, we estimated that a GFP-tagged $\beta\gamma$ -dimer with a molecular weight of ~70 kDa (β , 35 kDa; γ , 7–9 kDa; GFP, 27 kDa) has a diffusion coefficient of about 7 μ m²/s. Cytosolic diffusion of the tagged $\beta\gamma$ -complex is unlikely to be any faster than this, but it could be slower due to

1. Nenninger A, Mastroianni G, Mullineaux CW (2010) Size dependence of protein diffusion in the cytoplasm of Escherichia coli. J Bacteriol 192(18):4535–4540.

hydrophobic interactions of cytosolic factors with the γ -subunit prenyl moiety. For 3D diffusion the mean square distance traveled $\langle r^2 \rangle$, is related to the diffusion time *t* by $\langle r^2 \rangle = 6Dt$, where *D* is the diffusion constant. Using $D = 7 \,\mu m^2/s$ gives the following relationship:

RMS displacement, µm	Time, s
1	0.02
5	0.6
10	2.3
20	9.5
30	21



Fig. S1. HeLa cells were transfected with α o, β 1, and GFP- γ 9 or GFP- γ 3. Endogenous CXCR4 receptors were activated with 100 ng/mL SDF-1 α and deactivated with 20 μ M AMD3100. (A) Confocal images before receptor activation, after receptor activation, and after receptor deactivation. (B) Forward and reverse translocation kinetics for γ 9 and γ 3. Data for three different cells are shown for each subunit. Yellow markers labeled "Ag." and "Antag." indicate the addition of SDF-1 α and AMD3100, respectively.



Fig. S2. (*A* and *C*) Translocation kinetics in HeLa cells expressing α o, β 1, and GFP-tagged γ -subunits: wild-type γ 9 or γ 3 or a γ 3 mutant in which the C-terminal sequence was changed from EKKFF(GerGer) to EAAFF(GerGer). Endogenous α 2 adrenergic receptors (*A*) or CXCR4 receptors (*C*) were activated at *t* = 0 s with 10 μ M norepinephrine or 100 ng/mL SDF-1 α , respectively. (*B* and *D*) Fluorescence recovery kinetics for intracellular membrane regions that were photobleached after translocation, in the continued presence of agonist, as in Fig. 2 of the main text. (*B*) Agonist: 10 μ M norepinephrine. (*D*) Agonist: 100 ng/mL SDF-1 α . The γ 3 and γ 9 curves in *C* and *D* are reproduced from Fig. 2 *D* and *E* of the main text, respectively.



Fig. S3. Concentration of activated receptors does not strongly influence translocation kinetics. Translocation curves were generated from the mathematical model at various concentrations of activated receptors ranging from 1 nM to 300 nM. Receptors were activated at t = 60 s in all plots. (A) Translocation curves generated using $k_{in} = k_{out} = 0.05$ s⁻¹, corresponding to values used to fit γ 9 data in the main text. Receptors deactivated at t = 600 s. (B) Translocation curves generated using $k_{in} = k_{out} = 0.006$ s⁻¹, corresponding to values used to fit γ 5 data in the main text. Receptors deactivated at t = 800 s. (C) Translocation curves generated using $k_{in} = k_{out} = 0.0025$ s⁻¹, corresponding to values used to fit γ 3 data in the main text. Receptors deactivated at t = 1,200 s. Changes in the concentration of activated receptors over this large range do not substantially influence the translocation kinetics.



Movie S1. Reversible $\beta\gamma$ translocation in HeLa cells coexpressing α o, β 1, and GFP- γ 9. Agonist: 100 ng/mL SDF-1 α . Antagonist: 20 μ M AMD3100. The movie plays at 30× real time and a running clock is displayed (h:min:s).

Movie S1

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Movie S2. Reversible $\beta\gamma$ translocation in HeLa cells coexpressing α o, β 1, and GFP- γ 3. Agonist: 100 ng/mL SDF-1 α . Antagonist: 20 μ M AMD3100. The movie plays at 120× real time and a running clock is displayed (h:min:s).

Movie S2