# A G-Protein Subunit Translocation Embedded Network Motif Underlies GPCR Regulation of Calcium Oscillations

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## 1. Model description for Gi mediated Calcium oscillation - single γ subunit model

A mathematical model was developed to investigate the role of Gβγ translocation on Gi coupled GPCR induced $Ca^{2+}$ oscillation. We used the basic framework of a $G_q$ receptor-operated $Ca^{2+}$oscillation as proposed by Kummer *et. al.,*(1) and modified it to consider interactions specific for the Gi pathway and the spatial redistribution of the Gβγ subunit. The main components for this model are, (i) input (ii) activated Gβγ at the plasma membrane ($\beta\gamma_{PM}$), (iii) Gβγ at the internal membrane ($\beta\gamma_{IM}$), (iv) active phospholipase-C, (PLC-β), (v) cytosolic calcium ($Ca_{cyt}$) and (vi) calcium in ER ($Ca_{ER}$). The current model accounts for the formation and disappearance of Gβγ, translocation of Gβγ between plasma membrane and internal membranes, formation and degradation of PLC-β and calcium fluxes across the ER and plasma membrane. The model is presented by equations S1-S5.

The compartmental model for a single γ subunit is based on the following assumptions,

1. The external agonist interacts with the GPCR and leads to the dissociation of the heterotrimer to form active Gβγ . The rate of active Gβγ formation is given by the first term in equation S1., which is proportional to R (activated receptor concentration) and $R_0$ (basal receptor).
2. As the GTP hydrolysis of activated Gα is facilitated by active PLC-β (2), formation of the G protein heterotrimer by association of Gα and Gβγ is proportional to PLC-β and follows Michaelis-Menten kinetics with respect to Gβγ (Second term in equation S1).
3. There is a negative feedback on receptor activation through $Ca^{2+}$ mediated kinase that leads to Gβγ inactivation proportional to $Ca_{cyt}$ (3, 4) (Third term in equation S1)
4. Active Gβγ undergoes a spatial redistribution (last two terms in equation S1) through a membrane affinity based reversible translocation between plasma membrane and internal membranes (5) (fourth and fifth terms in equation S1). Here $k_{t1}$ and $k_{t2}$ represent rate constants for forward (from plasma to internal membrane) and backward (internal to plasma membrane) translocation, respectively.
5. Released Gβγ mediates PLC-β activation. Thus its formation rate is modeled as being proportional to active Gβγ (first term in equation S3). Further, its removal is quantified using a Michaelis-Menten kinetics which is consistent with Kummer et al. (1).
6. Consistent with Kummer et al, we assumed that the $Ca^{2+}$ influx to the cytosol is regulated by PLC-β mediated IP3 and is modeled as proportional to PLC-β (first term in equation

S4). As IP3 is generated by catalysis through active PLC-β, the calcium influx from the ER is directly taken to be dependent on PLC-β.

7. There is an immediate positive feedback on the calcium influx from ER by the cytosolic calcium itself, CICR (6) (second term in equation S4).
8. The cytosolic $Ca^{2+}$ fluxes across the plasma membrane are controlled by (i) PLC-β mediated IP3 (first term in equation S4) and (ii) ion-pumps at plasma membrane and ER (third and fourth term in equation S4) (7).

$$\frac{d[\beta\gamma_{PM}]}{dt} = K_a([R_0] + [R]) - k_1 \frac{[\beta\gamma_{PM}]}{Km_1+[\beta\gamma_{PM}]}[PLC-\beta] - k_2 \frac{[Ca_{cyt}][\beta\gamma_{PM}]}{Km_2+[\beta\gamma_{PM}]} - k_{t1}[\beta\gamma_{PM}] + k_{t2}[\beta\gamma f_{IM}] \quad \text{(S1)}$$

$$\frac{d[\beta\gamma_{IM}]}{dt} = k_{t1}[\beta\gamma_{PM}] - k_{t2}[\beta\gamma_{IM}] \quad \text{(S2)}$$

$$\frac{d[PLC-\beta]}{dt} = k_3[\beta\gamma_{PM}] - k_4 \frac{[PLC-\beta]}{Km_3+[PLC-\beta]} \quad \text{(S3)}$$

$$\frac{d[Ca_{cyt}]}{dt} = k_5[PLC-\beta] + k_6 \frac{[Ca_{ER}]}{Km_4+[Ca_{ER}]}[PLC-\beta][Ca_{cyt}] - k_7 \frac{[Ca_{cyt}]}{Km_5+[Ca_{cyt}]} - k_8 \frac{[Ca_{cyt}]}{Km_6+[Ca_{cyt}]} \quad \text{(S4)}$$

$$\frac{d[Ca_{ER}]}{dt} = -k_6 \frac{[Ca_{ER}]}{Km_4+[Ca_{ER}]}[PLC-\beta][Ca_{cyt}] + k_8 \frac{[Ca_{cyt}]}{Km_6+[Ca_{cyt}]} \quad \text{(S5)}$$

Parameters and initial conditions chosen for simulation of single γ–subunit model are described in the next section.

### 2.1 Description of Gi mediated calcium oscillation: two γ subunit model

To capture the presence of multiple γ subunits with distinct rates of translocation in a particular cell type(8), we incorporated a fast γ subunit ($\gamma_{fast}$) and a slow γ subunit ($\gamma_{slow}$) in the model, both of which are capable of translocation but with distinct translocation rates. The translocation rates, for $\gamma_{fast}$ and $\gamma_{slow}$ are $k_{tf}$ and $k_{ts}$, where $k_{tf}>k_{ts}$. It is assumed that both the γ subunits act additively on downstream signaling (activation of PLC-β). The two γ subunit model is presented by the following equations,

$$\frac{d\left[\beta\gamma_{fast_{PM}}\right]}{dt} = K_a m_1\left([R_0] + [R]\right) - k_1 \frac{\left[\beta\gamma_{fast_{PM}}\right]}{Km_1 + \left[\beta\gamma_{fast_{PM}}\right]}[PLC - \beta] - k_2 \frac{[Ca_{cyt}]\left[\beta\gamma_{fast_{PM}}\right]}{Km_2 + \left[\beta\gamma_{fast_{PM}}\right]}$$
$$-k_{tf}\left(\left[\beta\gamma_{fast_{PM}}\right] - \left[\beta\gamma_{fast_{IM}}\right]\right) \qquad \text{S6)}$$

$$\frac{d\left[\beta\gamma_{slow_{PM}}\right]}{dt} = K_a m_2\left([R_0] + [R]\right) - k_1 \frac{\left[\beta\gamma_{slow_{PM}}\right]}{Km_1 + \left[\beta\gamma_{slow_{PM}}\right]}[PLC - \beta] - k_2 \frac{[Ca_{cyt}]\left[\beta\gamma_{slow_{PM}}\right]}{Km_2 + \left[\beta\gamma_{slow_{PM}}\right]}$$
$$-k_{ts}\left(\left[\beta\gamma_{slow_{PM}}\right] - \left[\beta\gamma_{slow_{IM}}\right]\right) \qquad \text{(S7)}$$

$$\frac{d[\beta\gamma_{fast_{IM}}]}{dt} = k_{tf}\left(\left[\beta\gamma_{fast_{PM}}\right] - \left[\beta\gamma_{fast_{IM}}\right]\right) \qquad \text{(S8)}$$

$$\frac{d[\beta\gamma_{slow_{IM}}]}{dt} = k_{ts}\left(\left[\beta\gamma_{slow_{PM}}\right] - \left[\beta\gamma_{slow_{IM}}\right]\right) \qquad \text{(S9)}$$

$$\beta\gamma_{PM} = [\beta\gamma_{fast_{PM}}] + [\beta\gamma_{slow_{PM}}] \qquad \text{(S10)}$$

The dynamics of βγ is presented as the sum of five terms in the equation (S6). The first term indicates the activation rate of the βγ complex. $K_a$ represents the rate of receptor activation with $R_0$ and R being the basal and stimulated activated receptor concentration. To account for the effect of varying the ratio, $\beta\gamma_{fast}:\beta\gamma_{slow}$, we incorporated a factor $m_i$ into the activation rate term. If a particular γ subunit concentration is relatively high in a cell then $m_i$ will be higher for that particular γ subunit because probability of the presence of that γ subunit in the G protein complex is higher.

Here, we assume that the total Gprotein complex at the plasma membrane is in excess and constant irrespective of the amount of $\gamma$-subunit. Since experimentally only $\gamma$-subunit is over expressed, the assumption states that the $G_{\alpha\beta}$ complex is limiting and therefore due to the respective type of $\gamma$-subunit only the composition of Gprotein complex changes. Thus over expression of a specific type of $\gamma$-subunit only changes the composition of the complex of the fast and slow subunits of $\beta\gamma$ in the model. This formulation assumes a fixed $G_{\alpha\beta\gamma}$ concentration and depending on the type of $\gamma$-subunit (fast or slow), composition will vary. For example for the two $\gamma$-subunit model, total G protein, i.e., $G_T$ is as follows

$$G_T = \left( G_{\alpha\beta\gamma_{fast}} + G_{\alpha\beta\gamma_{slow}} \right) \qquad \text{(S11)}$$

The activation rate for the $\beta\gamma_{fast}$ and $\beta\gamma_{slow}$ are as following

$$\dot{\beta\gamma_{fast}} = K_{a0} * ([R] + [R_0]) * G_{\alpha\beta\gamma_{fast}} \quad \text{(S12)}$$

$$\dot{\beta\gamma_{slow}} = K_{a0} * ([R] + [R_0]) * G_{\alpha\beta\gamma_{slow}} \quad \text{(S13)}$$

Let $m1 = G_{\alpha\beta\gamma_{fast}}/G_T \; and \; m2 = G_{\alpha\beta\gamma_{slow}}/G_T$, corresponds to composition of specific type of Gprotein complex. Hence, following activation rate terms can be written as,

$$\dot{\beta\gamma_{fast}} = K_{a0} * ([R] + [R_0]) * m1 * G_T \quad \text{(S14)}$$

$$\dot{\beta\gamma_{slow}} = K_{a0} * ([R] + [R_0]) * m2 * G_T \quad \text{(S16)}$$

$Let\ K_a = K_{a0} * G_T$ and $hence$,

$$\dot{\beta\gamma_{fast}} = K_a * m1 * ([R] + [R_0]) \quad and \quad \dot{\beta\gamma_{slow}} = K_a * m2 * ([R] + [R_0]) \quad \text{(S17)}$$

This activation terms are used in the dynamic equations S6 and S7,

It should be noted that,

1. $m_1$ = 0.5, $m_2$ = 0.5 represents the case in which both fast and slow subunit have equal levels of expression.
2. $m_1$ = 1 represents the case of expression of only the fast γ subunit, (similar to overexpression of fast γ–subunit).
3. $m_1$ = 0 represents the case of expression of only the slow γ subunit, (similar to knockdown of fast γ–subunit).
4. We assumed $m_1$=0.25, and $m_2$=0.75 to represent the wild type HeLa cells.

The second and third terms (equation S6) are for deactivation of βγ complex regulated by PLC-β and $Ca^{2+}$. The last term in equation S6 captures the effect of reversible translocation. For the two-subunit model, we assumed that both forward and reverse translocation rates follow first order kinetics (5). For the rest of the variables (i.e. PLC-β, $Ca_{ER}$ and $Ca_{cyt}$) the dynamic equations are the same as those used for the single subunit model.

### 2.2 Initial conditions

To obtain the initial conditions for each of the components in the signaling pathway, we chose a vector of zeros as initial condition and obtained the steady state values for all the

variables. We then chose the vector of steady state values as the initial condition vector for the various simulation cases under consideration.

For demonstration here we present the case of $m_1$= 0.25, $m_2$= 0.75. In this case, first we chose the initial condition vector for the two $\gamma$ subunit ODE model as

$H_0$ = [$\beta\gamma_{fast\ PM\_0}$ $\beta\gamma_{fast\ IM\_0}$ $\beta\gamma_{slow\ PM\_0}$ $\beta\gamma_{slow\ IM\_0}$ PLC-$\beta_0$ $Ca_{cyt\_0}$ $Ca_{ER\_0}$] = [0 0 0 0 0 0 0];

Then we supplied this initial condition to model and set [R] =0 to obtain the steady state

$H_{ss}$ = [0.0870 0.0870 2.7427 2.7427 30.3761 0.0258 1.9425].

This steady state solution was then used as the initial working condition for $m_1$=0.25, $m_2$=0.75.

### 2.3. Simulation of the response in a cell population

The $Ca^{2+}$oscillations in cell populations with different proportions of $\gamma_{fast}$ and $\gamma_{slow}$ were simulated using our ODE model for two $\gamma$ subunits as discussed above. We assumed a Gamma distribution of the activation term for $\beta\gamma$ (for $K_a$ in Equation S6), that is

( $\mathrm{F}(K_a, k, \theta) = \frac{1}{\theta^k}\frac{1}{\Gamma(k)}K_a^{(k-1)}e^{-(K_a/\theta)}$ ) with a scale factor θ=4 and shape factors k=0.25 (resulting in a mean, μ=1.0 and standard deviation, σ =0.5). Such a simulation method for obtaining a cell population response based on variations in protein concentration has been used in several computational studies. In order to obtain the death time during apoptosis (response) in a HeLa cell population, Spencer et al., 2009 (9) used a lognormal distribution$\left(F(x,\mu,\sigma) = \frac{1}{x\sigma\sqrt{2\pi}}e^{-\frac{(lnx-\mu)^2}{2\sigma^2}},\ x>0\right)$ in the variation of protein concentration (a parameter in their ODE model). In other studies, Wang et al., 2012(10) used an uniform distribution ( $F(x,a,b) = \frac{1}{b-a},\ or\ a<x<b, F(x,a,b) = 0,\ for\ x<a\ and\ x>b$) of the parameter in their ODE model to obtain the heterogeneous PIP3 response in a *Dictyostelium* population. Ferrell et al 1998 (11) assumed a cumulative distribution function ($F(K,m,a) = \frac{K^m}{a^m+K^m}$) in the half saturation constant (K) of the hormone concentration (a parameter in their ODE model) to study the MAPK response and on-off maturation state in a *Xenopus* oocyte population.

**References**


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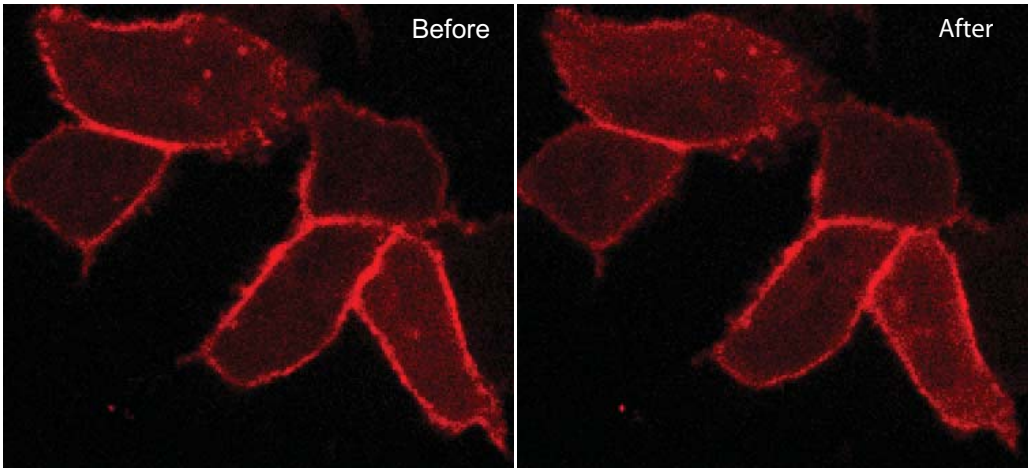
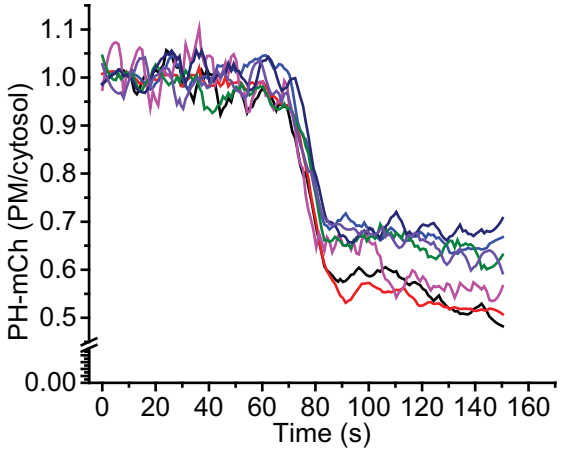
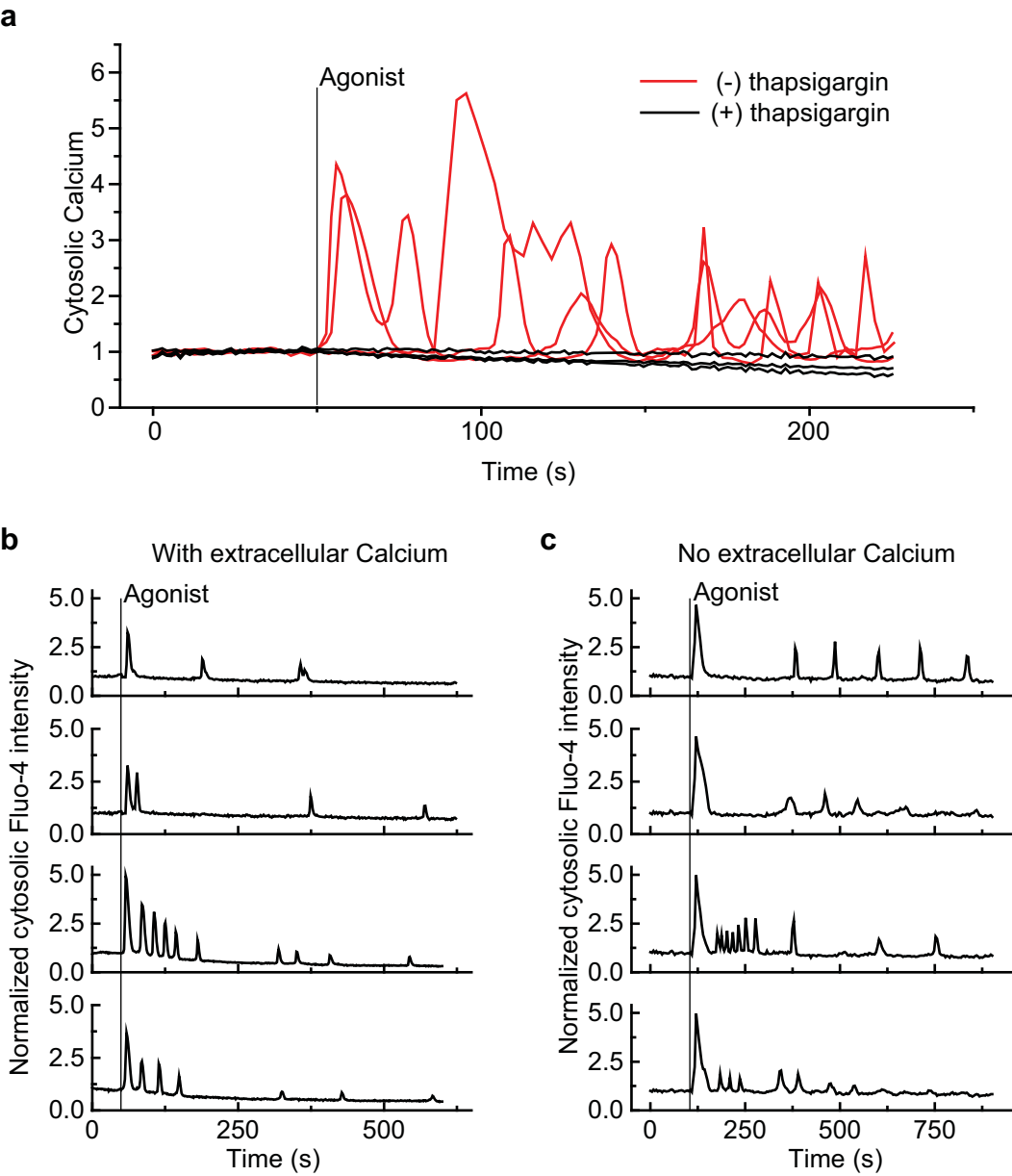
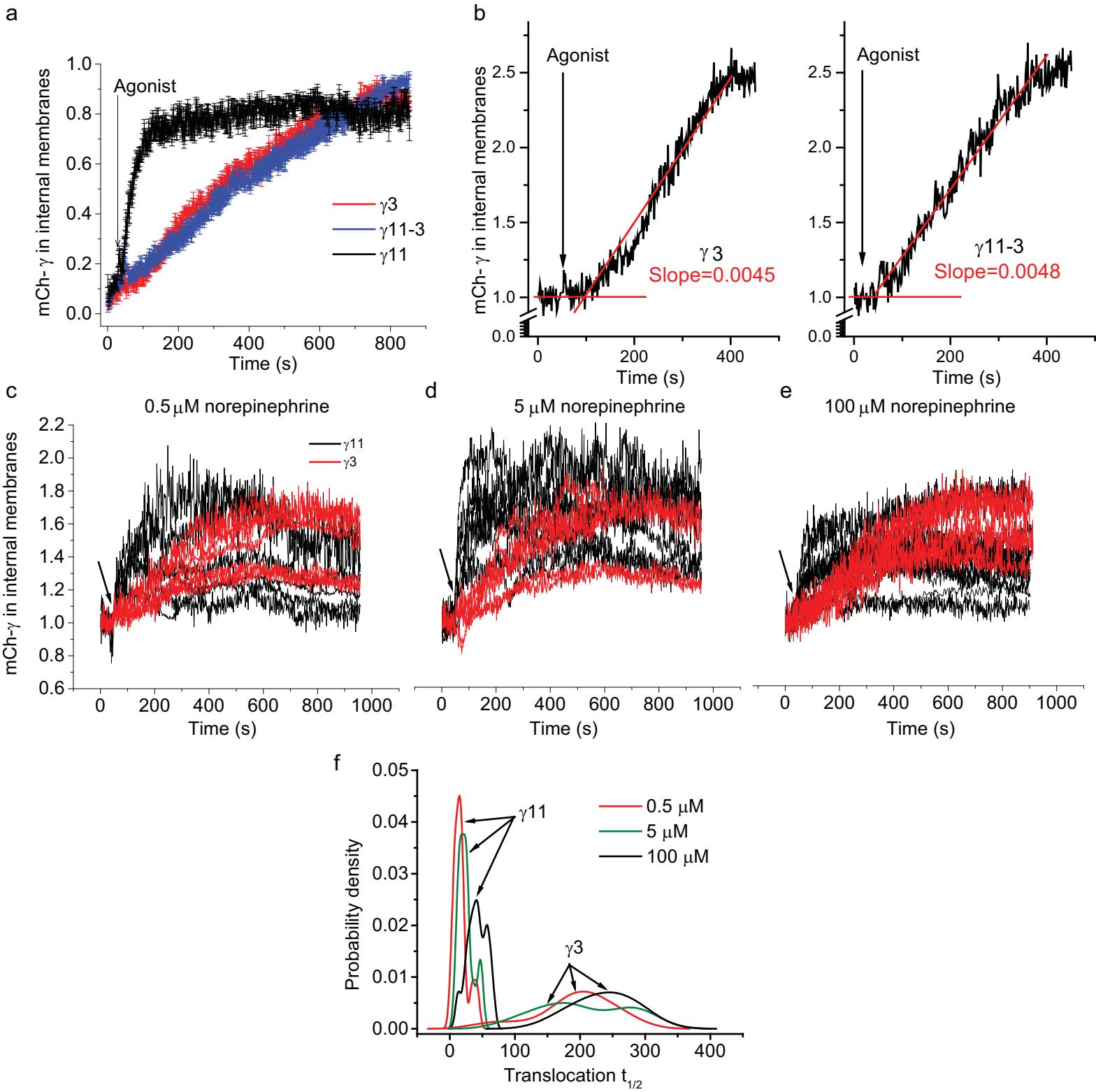
a

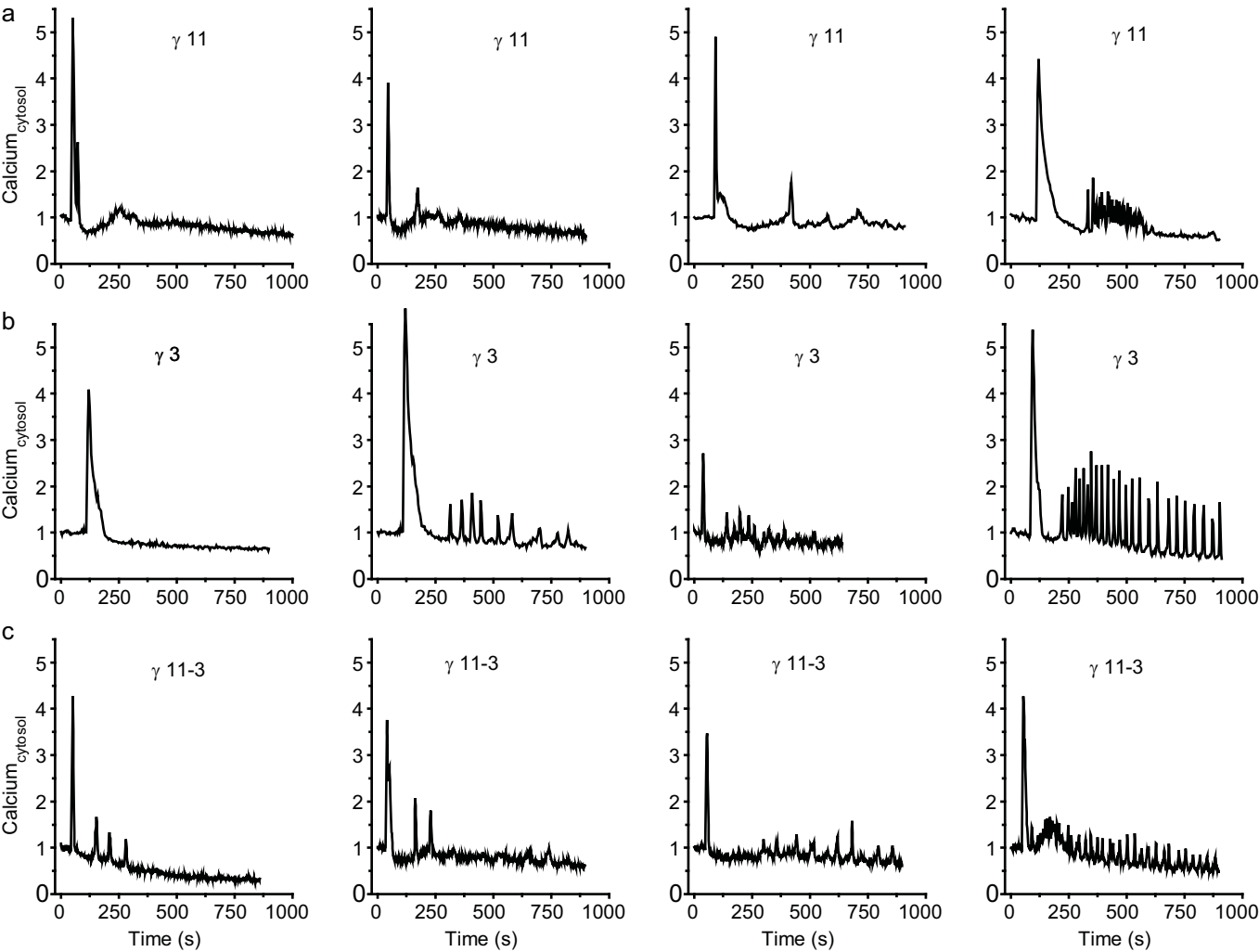

Before
After

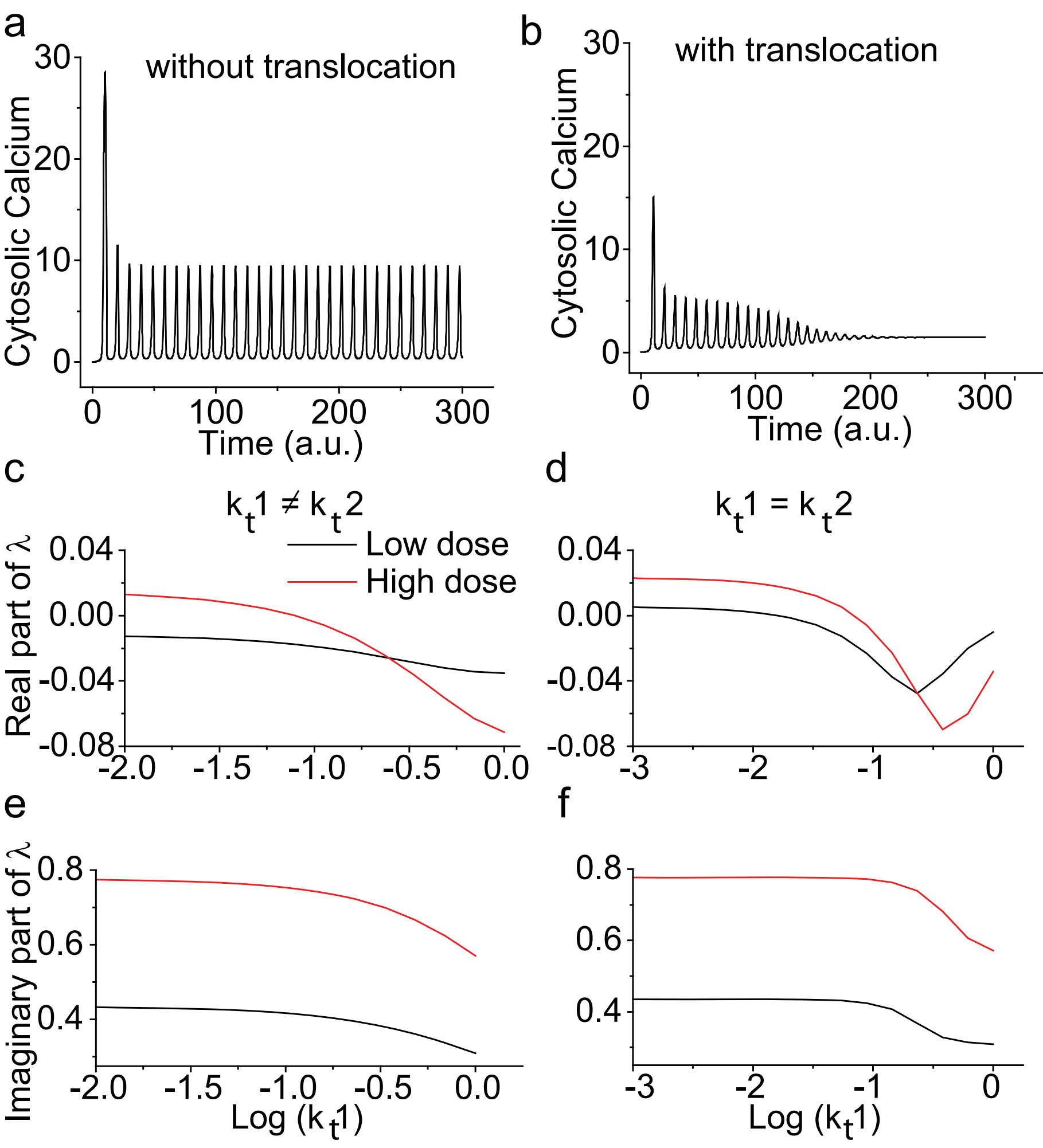
b

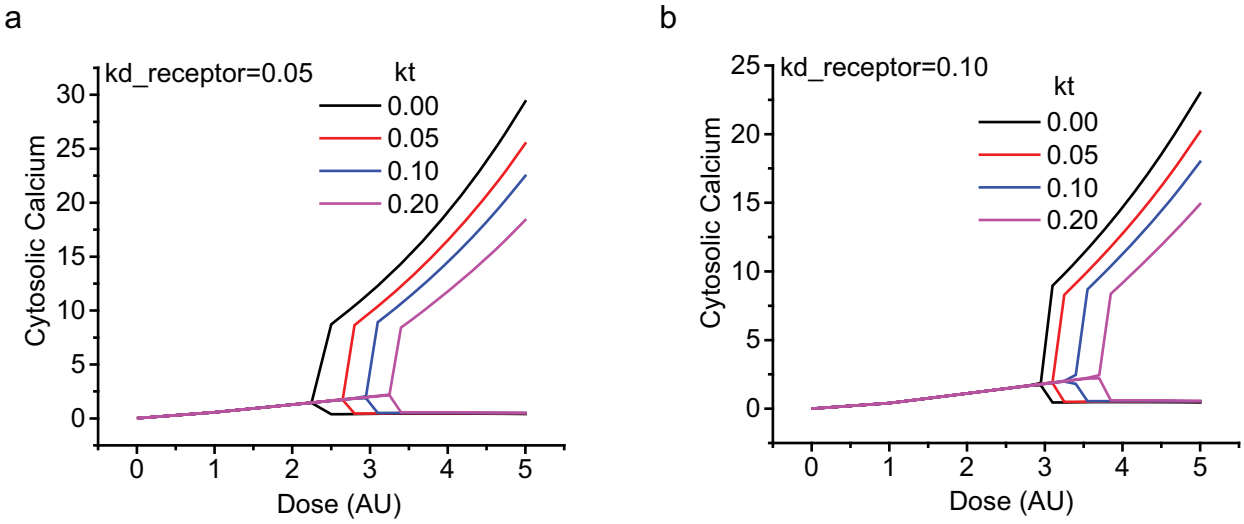

PH-mCh (PM/cytosol)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.00
0
20
40
60
80
100
120
140
160
Time (s)

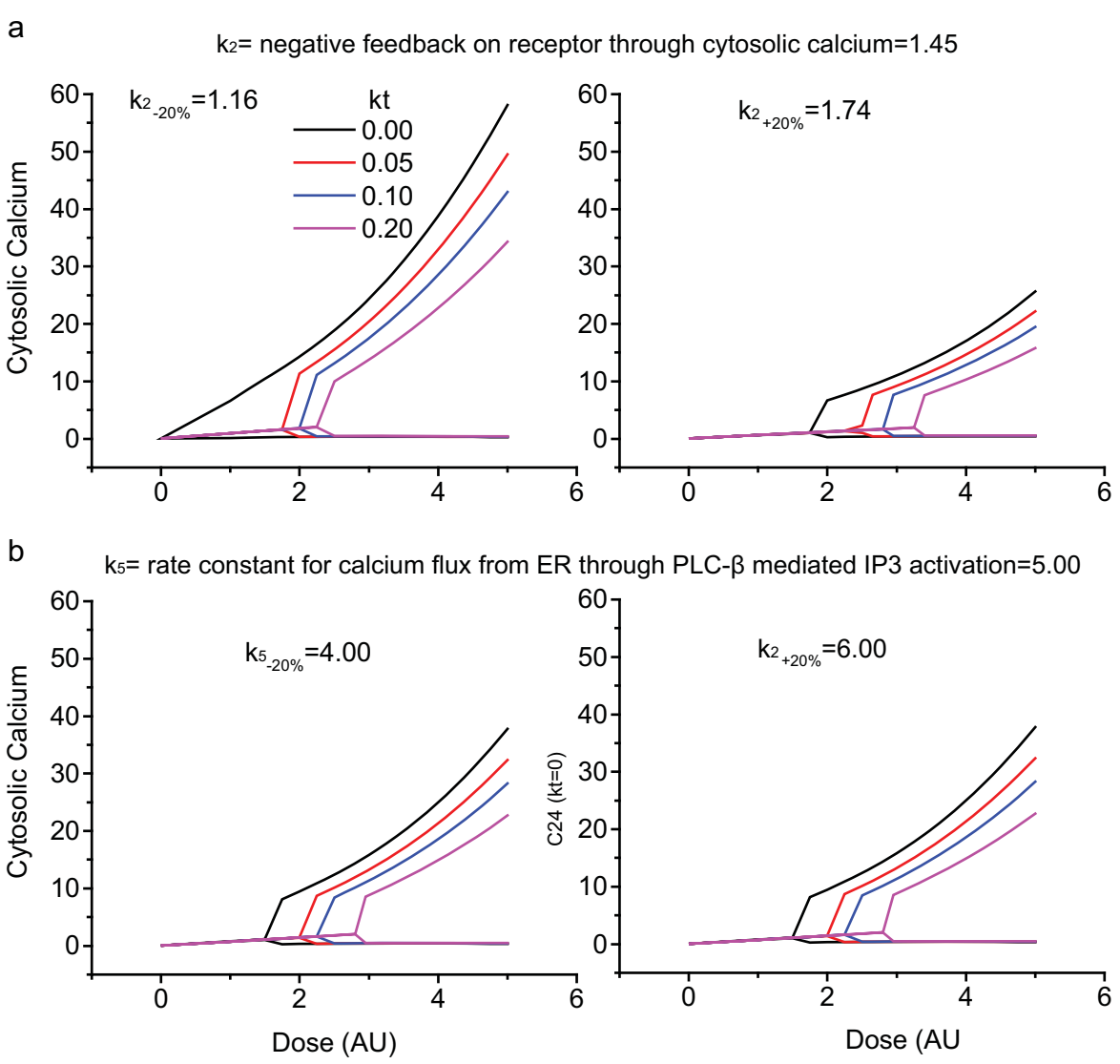

a
Agonist
(-) thapsigargin
(+) thapsigargin
Cytosolic Calcium
Time (s)
b
With extracellular Calcium
Agonist
Normalized cytosolic Fluo-4 intensity
Time (s)
c
No extracellular Calcium
Agonist
Normalized cytosolic Fluo-4 intensity
Time (s)

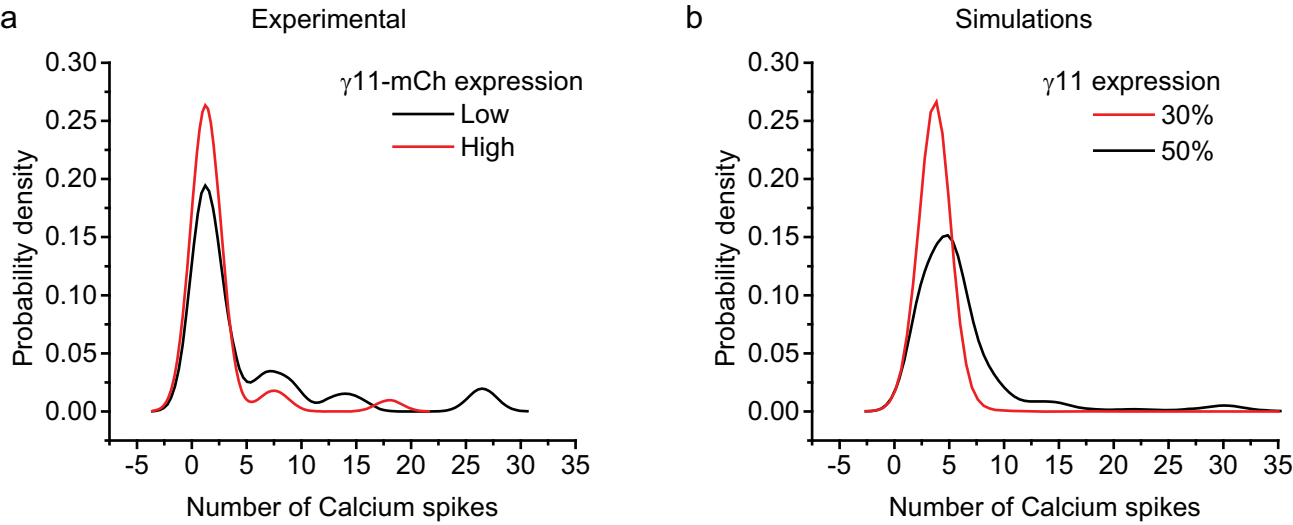

a
Agonist
mCh- γ in internal membranes
Time (s)
γ3
γ11-3
γ11
b
Agonist
γ 3
Slope=0.0045
γ11-3
Slope=0.0048
c
0.5 μM norepinephrine
d
5 μM norepinephrine
e
100 μM norepinephrine
f
Probability density
Translocation t1/2
0.5 μM
5 μM
100 μM

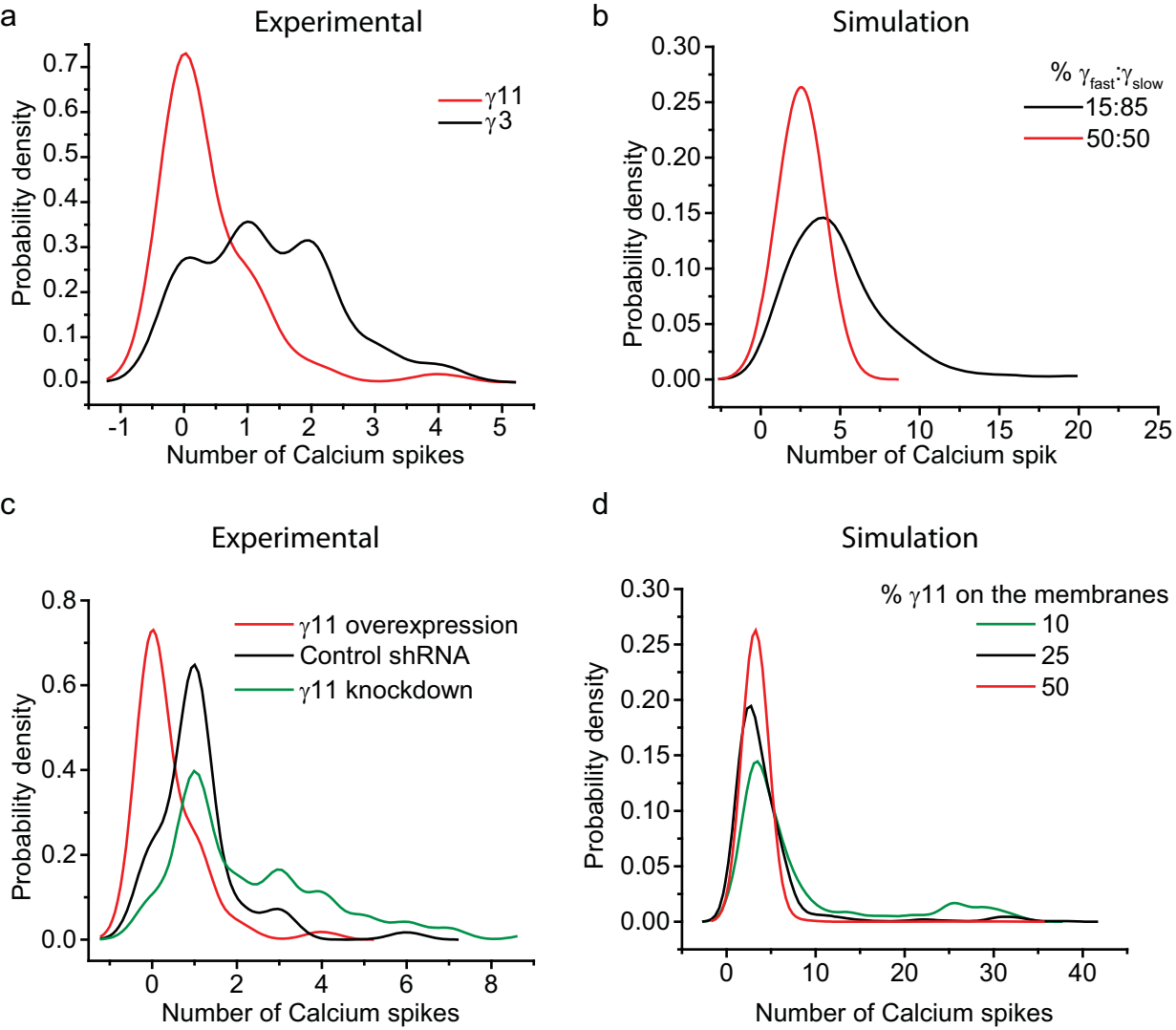

a
b
c
γ 11
γ 3
γ 11-3
Calcium cytosol
Time (s)
0
250
500
750
1000
1
2
3
4
5


a
without translocation
Cytosolic Calcium
Time (a.u.)
b
with translocation
Cytosolic Calcium
Time (a.u.)
c
$k_t1 \neq k_t2$
Low dose
High dose
Real part of λ
d
$k_t1 = k_t2$
e
Imaginary part of λ
Log ($k_t1$)
f
Log ($k_t1$)


a
kd_receptor=0.05
kt
0.00
0.05
0.10
0.20
Cytosolic Calcium
Dose (AU)
b
kd_receptor=0.10
kt
0.00
0.05
0.10
0.20
Cytosolic Calcium
Dose (AU)


a
k2= negative feedback on receptor through cytosolic calcium=1.45
k2 -20%=1.16
kt
0.00
0.05
0.10
0.20
k2 +20%=1.74
Cytosolic Calcium
b
k5= rate constant for calcium flux from ER through PLC-β mediated IP3 activation=5.00
k5 -20%=4.00
k2 +20%=6.00
C24 (kt=0)
Dose (AU)
Dose (AU


a
Experimental
γ11-mCh expression
Low
High
Probability density
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-5
0
5
10
15
20
25
30
35
Number of Calcium spikes
b
Simulations
γ11 expression
30%
50%
Probability density
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-5
0
5
10
15
20
25
30
35
Number of Calcium spikes


a
Experimental
Probability density
Number of Calcium spikes
γ11
γ3
b
Simulation
% γfast:γslow
15:85
50:50
Probability density
Number of Calcium spik
c
Experimental
Probability density
Number of Calcium spikes
γ11 overexpression
Control shRNA
γ11 knockdown
d
Simulation
% γ11 on the membranes
10
25
50
Probability density
Number of Calcium spikes

**Supplementary figure legends**

**Fig. S1.** Activation of α2-adrenergic receptor using norepinephrine induces IP3 production. (a) HeLa cells transfected with PLC-δ PH domain tagged with mCherry showed translocation from plasma membrane to cytosol. (b) Plot shows the ratio PH-mCh *PM*/PH-mCh *cytoplasm* ($N_{exp}$=4).

Figure S2 : Stimulation through norepinephrine induces intracellular calcium release in HeLa cells (a) Calcium dynamics in HeLa cells in presence of a blocker of sarcoplasmic reticulum $Ca^{2+}$-ATPase pump (thapsigargin = 120 nM). Black- three representative cells treated with thapsigargin; Red – Three representative cells not treated with thapsigargin (b) Calcium oscillation in 4 representative HeLa cells in presence of calcium in medium (HBSS) (c) Calcium oscillation in 4 representative HeLa cells in absence of calcium in medium (HBSS). We found HeLa cells show calcium oscillation even without calcium in medium. ($N_{exp}$= 6)

**Fig. S3.** Characterization of the translocation rate of γ11, γ3 and γ11-3. (a) Comparison of the translocation dynamics for γ11, γ3 and γ11-3. γ3 (red), 5 cells γ11-3 (blue), 5 cells; γ11 (black), 6 cells. Error bars: +/- SEM. Note that the γ11-3 chimera shows similar slower translocation kinetics as γ3. (b) Activation of alpha-2 adrenergic receptor using norepinephrine is associated with a change in slope in γ3 and γ11-3 signal in internal membranes. ($N_{exp}$= 30). (c) Translocation profile for γ11 and γ3 at 0.5 μm norepinephrine (d) Translocation profile for γ11 and γ3 at 5 μm norepinephrine (e) Translocation profile for γ11 and γ3 at 100 μm norepinephrine. (f) Comparison of the translocation speed distribution in cell populations at 3 different agonist concentrations (0.5, 5 and 100 μM) for γ11 and γ3. Here the translocation rate is defined as the time to reach half of the maximum Gγ signal intensity in internal membranes. Plots show the translocation profiles of γ11 and γ3 at different concentrations. (n = number of cells in a population = 30). ($N_{exp}$=6).

Figure S4. $Ca^{2+}$oscillations in four cells, each from a population expressing a specific γ subunit type. Note that there is cell to cell variability in the spiking pattern within a population expressing a specific γ subunit type. ($N_{exp}$=3)

Figure S5. Computational analysis of γ-translocation rate in the regulation of Gi mediated $Ca^{2+}$ oscillation at different drug doses (*in silico* analysis from single γ-subunit model). (a) Simulated

time course of $Ca^{2+}$ oscillation in the absence of $\beta\gamma$ translocation (i.e.,$k_t1 = k_t2 = 0$ in single subunit model) demonstrating sustained oscillations. $k_t1$ is the rate constant for forward translocation of $\beta\gamma$ from plasma membrane to internal membranes. $k_t2$ is the backward rate constant. Time (x axis) is in arbitrary units.(b) Simulated time-course of $Ca^{2+}$ oscillations in the presence of $\beta\gamma$ translocation yielding damped response. (c), (d) Damping of $Ca^{2+}$ oscillations (real part of Eigenvalue) as a function of $\beta\gamma$ translocation rate at low and high drug-doses. (c) $k_t1 \neq k_t2$ and (d) $k_t1 = k_t2$. (e), (f) Frequency of $Ca^{2+}$ oscillation (imaginary part of Eigenvalue) as a function of $\beta\gamma$ translocation rate ($k_t1$) at lower and higher drug-doses. (e) $k_t1 \neq k_t2$, (f) $k_t1 = k_t2$. Note that for conditions $k_t1 \neq k_t2$ and $k_t1 = k_t2$, the qualitative behavior of eigenvalues is similar.

**Fig. S6.** The effect of translocation (kt) on calcium concentration remains similar when receptor endocytosis is included in the model. (a) Steady state cytosolic calcium concentration (arbitrary units) as a function of agonist concentration for different translocation rates ($k_t$ =0, $k_t$ =0.05, $k_t$ =0.1, $k_t$ =5) at kd_receptor=0.05. (b) Steady state cytosolic calcium concentration (arbitrary units) as a function of agonist concentration for different translocation rates ($k_t$) at kd_receptor=0.1. Here, $k_t$=translocation rate; kd_receptor= receptor endocytosis rate. Note that the trend obtained for bifurcation plot from the simulation of the model with receptor endocytosis is comparable to the results from the model without receptor endocytosis (see Fig. 7a).

**Fig. S7.** Sensitivity analysis of the rate constants for the calcium model. (a) Sensitivity analysis of $k_2$ ($k_2$= negative feedback on receptor through cytosolic calcium), $k2_{-20\%}$=1.16, $k2_{+20\%}$ =1.74 (b) Sensitivity analysis of $k_5$ ($k_5$=rate constant for calcium flux from ER through PLC-β mediated IP3 activation), $k5_{-20\%}$=4, $k5_{+20\%}$ = 6 Left panel = 20% decrease in parameter value; Right panel = 20% increase in parameter value. The result shows that the effect of translocation on calcium oscillation is not valid for only one set of parameters but the trend remains comparable for different choices of parameters.

**Fig. S8**. Comparison of the experiment and simulation results for the dependence of Calcium oscillations on the ratio $\gamma_{fast}$: $\gamma_{slow}$ in a cell population. (a) Probability density distribution of number of calcium spikes at different proportions of $\gamma_{fast}$ from experiments (b) Probability density distribution of number of calcium spikes at different proportions (30% and 50%) of $\gamma_{fast}$ from simulations. Since the transiently transfected cells show intrinsic variation in $\gamma 11$ expression levels (reflected by the mcherry intensity level), we grouped the cells into low *(mch- $\gamma 11$ intensity*

*level =1.5-5),* and high *(mch- γ11 intensity level > 10)* expression levels to evaluate the dependence of the ratio $\gamma_{fast}$: $\gamma_{slow}$ on calcium oscillation. The trend obtained from experimental data matches simulation results from the model. (n= number of cells in population =30, $N_{exp}$=3).

**Fig. S9.** Characteristics of calcium oscillation distribution in a cell population depends on $\gamma_{fast}$: $\gamma_{slow}$ (obtained through γ11 expression, γ3 expression and γ11 knockdown) at lower agonist concentrations (norepinephrine concentration = 0.5 μM). We also compared these with simulation results to show that the experimental result matches the simulations from the model. (a) Comparison of the distribution of number of calcium spikes in mCh-γ11, and mCh-γ3 transfected cell populations at dose = 0.5 μM from experiment (b) Comparison of the distribution of number of calcium spikes in cell population with 50% $\gamma_{fast}$ (corresponding to γ11 expression) and 15% $\gamma_{fast}$ (corresponding to γ3) at lower dose (k1=0.75) from simulation, (c) Comparison of the distribution of number of calcium spikes in mCh-γ11 transfected, control shRNA and γ11 knockdown cell population at dose = 0.5 μM from experiment, (d) Comparison of the distribution of number of calcium spikes in cell population with 50% $\gamma_{fast}$ (corresponding to γ11 expression), 25% $\gamma_{fast}$ (corresponding to control shRNA HeLa cells) and 10% $\gamma_{fast}$ (corresponding to γ11 knockdown) at lower dose (k1=0.75) from simulation. (n = number of cells in a population = 30, $N_{exp}$=3).

**Supplementary movie legends**

Movie S1: $Ca^{2+}$ oscillation in HeLa cells with higher $\gamma_{fast}$:$\gamma_{slow}$ (overexpression of $\gamma$11, fast translocating subunit). Cells here and below were transfected with $\gamma$11-mCh (red) and incubated with Fluo-4 (green) to monitor $Ca^{2+}$ response.

Movie S2: $\alpha$2AR mediated $Ca^{2+}$oscillation in HeLa cells with lower $\gamma_{fast}$:$\gamma_{slow}$ (overexpression of $\gamma$3, slow translocating subunit).

Movie S3: $\alpha$2AR mediated$Ca^{2+}$oscillation in HeLa cells with lower $\gamma_{fast}$:$\gamma_{slow}$ (Introduction of chimeric $\gamma$11-3, slow translocating subunit).

Movie S4: $\alpha$2AR mediated $Ca^{2+}$ oscillation in HeLa cells transfected with a control shRNA.

Movie S5: $\alpha$2AR mediated $Ca^{2+}$ oscillation in $\gamma$11 knockdown HeLa cells.

**Table S1: Comparison of statistical parameters for the distribution of calcium oscillation characteristics between mch γ11, mch γ3 and mch γ11–3 transfected HeLa cell populations.**

| **Number of $Ca^+$ spikes (N)** | γ11 | γ3 | γ11–3 | **Duration $Ca^+$ oscillation (T) (Sec)** | γ11 | γ3 | γ11–3 |
|---|---|---|---|---|---|---|---|
| **Mean** | 3.6 | 5.3 | 5.1 | **Mean** | 230.81 | 369.51 | 416.0 |
| **Median** | 1 | 3 | 3 | **Median** | 160 | 280 | 360 |
| **SD** | 5.4 | 6.26 | 7.67 | **SD** | 252.75 | 286.46 | 264.68 |
| **Variance** | 29.36 | 39.84 | 57.72 | **Variance** | 63995 | 84079 | 70559 |
| **% of population showing N <3** | 70% | 49% | 55% | **% of population showing 0<T<100** | 47 | 22 | 13 |

**Table S2: List of parameter values used for simulation from the single and two-subunit models**

The unit of the concentration/activity is arbitrary (AU).

| Parameter | Value | Unit |
|---|---|---|
| $K_a$ | 1.0 | $[Second]^{-1}$ |
| $k_{t1}$ | 0.001 to 0.500 | $[Second]^{-1}$ |
| $k_{t2}$ | 0.001 to 0.500 | $[Second]^{-1}$ |
| $k_{tf}$ | 0.50 | $[Second]^{-1}$ |
| $k_{ts}$ | 0.02 | $[Second]^{-1}$ |
| $m_1$ and $m_2$ | 0.00 to 1.00 | Dimension less |
| $[R_0]$ | 0.05 | AU |
| $k_1$ | 0.0001 | AU/Second |
| $k_2$ | 1.45 | $[Second]^{-1}$ |
| $k_3$ | 5.82 | $[Second]^{-1}$ |
| $k_4$ | 32.24 | AU/Second |
| $k_5$ | 0.70 | $[Second]^{-1}$ |
| $k_6$ | 5.00 | 1/(AU*Second) |
| $k_7$ | 153.00 | AU/Second |
| $k_8$ | 4.85 | AU/Second |
| $Km_1$ | 0.788 | AU |
| $Km_2$ | 0.18 | AU |
| $Km_3$ | 29.09 | AU |
| $Km_4$ | 2.67 | AU |
| $Km_5$ | 0.16 | AU |
| $Km_6$ | 0.05 | AU |

Note that for generating responses as shown in various figures the activated receptor concentration [R] was assumed to be 2.0, 1.5 and 1 for high, medium and low doses, respectively.

**Table S3: Comparison of statistical parameters for the distribution of calcium spiking number between γ11 knockdown, control shRNA and mch-γ11 transfected HeLa cell population**

| **Number of $Ca^{+}$ spikes** | **γ11 knockdown** | **Control shRNA** | **γ11 transfected** |
|---|---|---|---|
| **Mean** | 8.2 | 4.67 | 3.6 |
| **Median** | 8 | 3.5 | 1 |
| **Mode** | 8.8 | 7 | 1 |
| **Variance of dominant mode** | 13.8 | 11.0 | 5.8 |
| **% of population showing N<3** | 17% | 40.9% | 70% |