SUPPLEMENTARY MATERIALS

Supplementary text

Alternative modeling equations

Most biological processes are inherently non-linear. The Michaelis-Menten rate representation (3, 4) is one of several ways to describe such nonlinearities. We considered the following other representations:

First, reactions *V2* and *V3* can be rewritten in the form including the basal, receptor- and GAP-independent rates of trimeric complex dissociation and GTP hydrolysis, respectively:

$$V2 = k_2^0 n_1 + k_2 \frac{n_1}{K_2 + n_1}$$
$$V3 = k_3^0 n_3 + k_3 \frac{n_3}{K_3 + n_3} \qquad (1),$$

where k_2^0 and k_3^0 are the basal dissociation and hydrolysis rate constants, respectively. As these constants are very low (ca. 0.005 sec^{-1} and 0.05 sec^{-1}, respectively [1], 100-1000 fold lower than k_2 and k_3), exclusion of the basal components of *V2* and *V3* does not affect modeling presented in the Results.

Second, the Michaelis-Menten rate equation has been developed for situations where the substrate concentration strongly exceeds the enzyme concentration. When this condition is not maintained, the generalized rate equation can be used [2], and *V2* and *V3* will adopt the following form:

$$V2 = \frac{k_{diss}}{2}\left((K_2 + [Rc^*] + n_1) - \sqrt{(K_2 + [Rc^*] + n_1)^2 - 4[Rc^*]n_1}\right)$$
$$V3 = \frac{k_{hydr}}{2}\left((K_3 + [GAP] + n_3) - \sqrt{(K_3 + [GAP] + n_3)^2 - 4[GAP]n_3}\right) \quad (2)$$

Third, the three reactions (2-4) of the trimeric G protein cycle can also be expressed in terms of the power-law representation:

$$\begin{cases} V1 = \alpha 1 \cdot {n_4}^{g1} \cdot {n_2}^{g2}; \\ V2 = \beta 2 \cdot {n_1}^{h1} \cdot [Rc^*]^{h2}; \\ V3 = \beta 3 \cdot {n_3}^{h3} \cdot [GAP]^{h4} \end{cases} \quad (3),$$

where α1, β2, and β3 are rate constants, and g1, g2, h1, h2, h3, and h4 are kinetic orders [3]. Mass conservation (5a-5b) has to be applied to obtain the system of two independent differential equations:

$$\begin{cases} \frac{d[\beta\gamma]}{dt} = \frac{dn_2}{dt} = \beta 2 \cdot (M - n_2)^{h1} \cdot [Rc^*]^{h2} - \alpha 1 \cdot (n_2 - n_3)^{g1} \cdot {n_2}^{g2}; \\ \frac{d[G\alpha^{GTP}]}{dt} = \frac{dn_3}{dt} = \beta 2 \cdot (M - n_2)^{h1} \cdot [Rc^*]^{h2} - \beta 3 \cdot {n_3}^{h3} \cdot [GAP]^{h4} \end{cases} \quad (4).$$

The feedback loops (15-16) in the trimeric G protein cycle can also be successfully modeled using the power-law representation, such as

$$\begin{cases} V2 = \beta 2 \cdot {n_1}^{h1} \cdot [Rc^*]^{h2} \cdot {n_3}^{h6}; \\ \frac{d[Rc^*]}{dt} = V_{del} - \beta 4 \cdot [Rc^*]^{h5} \cdot {n_3}^{h7} \end{cases} \quad (5).$$

Such power-law representations have been widely used in metabolic modeling, and can be useful for non-ideal kinetics [4], as well as to incorporate additional regulations, as the Michaelis-Menten representation becomes cumbersome for complicated pathways [3].

It is important to note that all above-mentioned forms of modeling the trimeric G protein cycle have reproduced the diversity in behavior of the system reported in this work.

Supplementary Table 1: Cell volumes

Cell/organism	**volume**	**reference**
human platelet	10 fl	http://www.fpnotebook.com/HEM109.htm
S. cerevisiae(haploid)	30 fl	[5]
human erythrocyte	100 fl	http://web2.iadfw.net/uthman/blood_cells.html
human neutrophil	300 fl	[6]
human lymphocyte	400 fl	
Dictyostelium amoeba	500 fl	[7]
S49 lymphoma cell line/mouse	800 fl	[8]
human peritoneal fibroblast	2200 fl	http://www.pdiconnect.com/archive.php?op=read&mode=full&entryid=645
human ventricular myocyte	25 pl	[9]

Supplementary Table2. Amounts of GPCR/trimeric G protein/RGS molecules per cell

molecule	cell type	organism	molecules/cell	ref	cellular concentration
1. GPCR receptors					
prostaglandin E2 receptor -after stimulation	T cells	human	435±322 1035±357	[10]	2 nM 5 nM
β-adrenergic receptor	S49 lymphoma	mouse	1220±67	[11]	3 nM
bradykinin receptor	Rat-1 fibroblasts, ras-1 ransformed	rat	8350±160	[12]	6 nM
ß-adrenergic receptor	myocyte	rat	2.1×10^5	[13]	13 nM
PAF receptor	platelets	human pig rabbit	281±63 281±158 689±229	[14, 15]	50 nM 50 nM 115 nM
cAMP receptor	amoeba	*D.discoideum*	40,000	[7]	130 nM
fMLP receptor	HL60 cells	human	50,000	[16]	250 nM
Ste2 (pheromone receptor)	yeast	*S.cerevisiae*	8000	[17]	460 nM
Rhodopsin	rod outer segments	human		[18]	3 mM
2. G proteins					
Gpa1 -after stimulation	yeast	*S.cerevisiae*	8000 12000	[19]	460 nM 690 nM
pertussis toxin-sensitive Gα	HL60 cells	human	130000	[16]	650 nM
Gs	S49 lymphoma	mouse	130000	[20]	270 nM
Gs	myocyte	rat	4.7×10^7	[13]	3 μM
transducin	rod outer segments	human		[18]	300 μM
3. RGS					
RGS2L	NG108-15 neuroblastoma	rat		[21]	10-100 nM
Sst2 -after stimulation	yeast	*S.cerevisiae*	2000 5000	[19]	115 nM 290 nM

Supplementary Table 3. Data for $G\alpha^{GDP}$ + βγ association.

molecules	organism/source	k_{ass}	ref	method
α_i^{myr} + bovine brain βγ	rat (bacterial production), bovine	$0.7 * 10^6$ M^{-1} sec^{-1}	[22]	flow cytometry
α_i + bovine brain βγ	bovine brain	$4 * 10^4$ M^{-1} sec^{-1}	[23]	surface plasmon resonance spectroscopy
α_{i2} + $\beta_1\gamma_1$	human (baculovirus production)	$4.4 * 10^4$ M^{-1} sec^{-1}	[24]	optical biosensor
α_{i2} + $\beta_1\gamma_2$	human (baculovirus production)	$3.4 * 10^4$ M^{-1} sec^{-1}	[24]	optical biosensor
		K_d		
α_i^{myr} + bovine brain βγ	rat (bacterial production), bovine	3 nM	[22]	
α_{i1}^{myr}, bovine brain βγ	rat (bacterial production), bovine	0.2 nM	[25]	
α_o^{myr} + bovine brain βγ	rat (bacterial production), bovine	17 nM	[25]	
α_s + bovine brain βγ	rat (bacterial production), bovine	27 nM	[25]	
α_{41} / α_{39} + biotinyl βγ	bovine brain	20 nM / 350 nM	[26]	
FITC-α_o + rhodamine-βγ	bovine brain	10 nM	[27]	steady-state FRET
α_{39}	bovine brain	100 nM	[28]	pertussis toxin assay
α_s / α_o + $\beta_1\gamma_1$	rabbit liver, bovine brain, bovine (baculovirus production)	2 nM	[29]	
α_s / α_o + other βγ	rabbit liver, bovine brain, bovine (baculovirus production)	0.2 - 0.5 nM	[29]	
α_{i2}^{myr} + any βγ	bovine (bacterial and baculovirus production)	0.4 nM	[29]	
α_{i2}^{myr} + $\beta_1\gamma_1$	bovine (bacterial and baculovirus production)	85 nM	[24]	
α_{i2}^{myr} + $\beta_1\gamma_2$	bovine (bacterial and baculovirus production)	134 nM	[24]	

Supplementary Table 4. Data for GPCR-driven dissociation of the trimeric G proteins.

molecules	**organism, source**	k_{diss}	**ref**
Gs + β-adrenergic receptor	rabbit hepatocytes/ turkey erythrocytes	1-5 sec^{-1}	[1, 30]
Gq + muscarinic cholinergic (m1AChR) receptor	mouse/human (baculovirus production)	1.8 sec^{-1}	[31]
Gi + α_{2a}AR adrenoreceptor	hamster/porcine	5 sec^{-1}	[32]
Gi + muscarinic receptor (M2 mAChR)	baculovirus production	0.34 sec^{-1}	[33]
Gt + rhodopsin	bovine	286 sec^{-1}	[34]
Gt + rhodopsin	frog	120 sec^{-1}	[35, 36]
Golf + olfaction receptor	rat, olfactory cilia	at least 20 sec^{-1}	[35, 36]
Gq + rhodopsin	drosophila	at least 20 sec^{-1}	[36, 37]

Supplementary Table 5. Data for GAP-driven GTPase.

molecules	K_M	**reference**	**notes**
Gz + RGSZ1	2 nM	[38]	
Gz + RGSZ1	15 nM	[39]	5°C
	k_{hydr}		
Gq + PLC-β1	9-12 sec^{-1}	[31]	
Gq + RGS4	22-27 sec^{-1}	[31]	
Gz + RGSZ1/GAIP	40 min^{-1}	[38]	
Gt-RGS4	2.8 sec^{-1}	[40]	
Go-RGS4	2 sec^{-1}	[41]	8°C

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