

Activation of G proteins by guanine nucleotide exchange factors relies on GTPase activity

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1 **Abstract**

2 G proteins are an important family of signalling molecules controlled by gua-
3 nine nucleotide exchange and GTPase activity in what is commonly called an
4 'activation/inactivation cycle'. The molecular mechanism by which guanine nu-
5 cleotide exchange factors (GEFs) catalyse the activation of monomeric G pro-
6 teins is well-established, however the complete reversibility of this mechanism
7 is often overlooked. Here, we use a theoretical approach to prove that GEFs are
8 unable to positively control G protein systems at steady-state in the absence of
9 GTPase activity. Instead, positive regulation of G proteins must be seen as a
10 product of the competition between guanine nucleotide exchange and GTPase
11 activity – emphasising a central role for GTPase activity beyond merely signal
12 termination. We conclude that a more accurate description of the regulation
13 of G proteins via these processes is as a 'balance/imbalance' mechanism. This
14 result has implications for the understanding of many intracellular signalling
15 processes, and for experimental strategies that rely on modulating G protein
16 systems.

17 **Introduction**

18 G proteins are an important and universal family of intracellular signalling mol-
19 ecules, incorporating both the alpha subunits of heterotrimeric G proteins and
20 the Ras small monomeric G proteins. Most G proteins bind guanine nucleotides
21 (GDP, GTP) in a strongly conserved nucleotide binding pocket – an ancient

22 mechanism preserved in both eukaryotes and prokaryotes (Simon et al. 1991;
23 Dong et al. 2007; Rojas et al. 2012). Typically, G proteins transition between two
24 discrete conformations with distinct signalling functions depending on which
25 nucleotide is bound, and so G proteins are often referred to as ‘molecular switches’.
26 G protein regulatory systems are crucial components of many intracellular pro-
27 cesses – incorrect regulation of G proteins has been implicated in disease: cancer
28 (Young et al. 2009; Vigil et al. 2010; O’Hayre et al. 2013), cardiovascular disease
29 (Loirand et al. 2013), genetic disorders (Seixas et al. 2013), among many others.
30 Regulation of G protein activation is largely controlled by two mechanisms (Fig-
31 ure 1A) and is commonly described as an ‘activation/inactivation cycle’ be-
32 tween the GTP-bound ‘on/active’ state and the GDP-bound ‘off/inactive’ state
33 (Vetter and Wittinghofer 2001; Oldham and Hamm 2008). Activation of G pro-
34 teins is controlled by accessory proteins which catalyse guanine nucleotide ex-
35 change – the sequential release of GDP and binding of GTP. For monomeric G
36 proteins these are known as guanine nucleotide exchange factors (GEFs). For
37 heterotrimeric G proteins, G protein coupled receptors (GPCRs) fulfil this role.
38 Inactivation of G proteins is controlled by GTPase activity which may either be
39 intrinsic, or be provided via accessory GTPase-activating proteins (GAPs). It
40 is generally thought that GTPase activity is required for the termination of G
41 protein signalling but that it is not essential for signal transmission (Takai et al.
42 2001).

43 An often overlooked property of GEFs is that their catalytic mechanism is com-
44 pletely reversible (Figure 1B) (Goody 2014). GEF-binding is not specific to GDP-
45 bound G protein – GEFs can also bind to GTP-bound G protein and catalyse the
46 reverse nucleotide exchange, GTP to GDP. In this way, GEFs are capable of inac-
47 tivating G proteins (Bos et al. 2007). The extent to which the reversibility of this
48 mechanism has been overlooked is demonstrated by the sheer number of publi-
49 cation which include diagrams where arrows corresponding to GEF-mediated
50 regulation are drawn as unidirectional – missing the reverse arrowhead high-
51 lighted in Figure 1A. This error is perhaps best illustrated by its occurrence in
52 core biology textbooks, for example:

- 53 • Figures 3–66 and 3–68 in Alberts et al. (2014)
- 54 • Figures 16–15 and 16–16 in Alberts et al. (2013)
- 55 • Figure 4, box 12–2 in Nelson and Cox (2013)
- 56 • Figure 13.40 in Berg et al. (2010)
- 57 • Figure 19–40 in Voet and Voet (2010)
- 58 • Figure 7.12A in Hancock (2010)

- 59 • Figure 10.3 and 10.4 in Bolsover et al. (2011)
- 60 • Figure 42.4 in Baynes and Dominiczak (2014)

61 There has been recent renewed interest in understanding the roles and functions
62 of GEFs based on a proper consideration of their enzyme kinetics (Northup et
63 al. 2012; Randazzo et al. 2013; Goody 2014). Here we develop the theoretical
64 understanding of G protein regulation by GEFs and GTPase activity through
65 exploring the consequences of the reversibility of the GEF mechanism. We use
66 mathematical methods to investigate G protein regulatory systems independent
67 of measured kinetic rates, in the context of the physiologically important steady-
68 state dynamics. This allows us to comment and draw conclusions on the qual-
69 itative behaviours of G protein:GEF:GTPase systems under a wide variety of
70 conditions.

71 **Results**

72 **Qualitative differences between reversible and irreversible mechanisms**

73 To demonstrate the qualitative difference between a reversible and an irreversible
74 mechanism we derived mass-action models of the GEF mechanism (Figure 1B,
75 Methods) and an artificial irreversible mechanism generated by disallowing re-
76 lease of GTP from the G protein·GEF complex.

77 The reversible and irreversible models were simulated: in the absence of GTPase
78 activity (Figures 2A, 2D); with intrinsic GTPase activity, modelled by exponen-
79 tial decay (Figures 2B, 2E); and with GAP-mediated GTPase activity, modelled
80 using the Michaelis-Menten equation (Figures 2C, 2F). To ensure that simula-
81 tions were physiologically plausible, kinetic rates measured for the the Ran:RCC1
82 system were used (Klebe et al. 1995). A GTP:GDP ratio of 10:1 was used to em-
83 ulate the relative levels in eukaryotic cells.

84 In the presence of either form of GTPase activity both reversible and irreversible
85 mechanisms display similar behaviour which is consistent with observations of
86 GEF-mediated activation of G proteins in a wide range of biological systems
87 (Janetopoulos et al. 2001; Peyker et al. 2005; Adjobo-Hermans et al. 2011; Chang
88 and Ross 2012; Oliveira and Yasuda 2013).

89 In the absence of GTPase activity we see a qualitative difference in the behaviour
90 of the two mechanisms; each distinct from their shared behaviour in the pres-
91 ence of GTPase activity. While both mechanisms show an inhibitory effect (which

92 will discussed below in more detail for the GEF mechanism), the steady-state
93 concentrations of active and inactive G protein differ substantially. Through
94 this example we demonstrate how the assumption of an irreversible model would
95 lead to incorrect conclusions when considering extremal (i.e. diseased) states.

96 **GEFs act to attain a constant ratio of inactive to active G protein**

97 We derived a simplified quasi-steady-state model of the GEF mechanism (Fig-
98 ure 1B) in an equivalent manner to the derivation of the Michaelis-Menten equa-
99 tion (Michaelis and Menten 1913; Briggs and Haldane 1925; Johnson and Goody
100 2011; Gunawardena 2012). This quasi-steady-state model captures the behaviour
101 of a generic G protein regulatory system in a single equation:

$$\frac{d[G_{GTP}]}{dt} = \frac{k_{cat}([G_{GDP}] - \kappa[G_{GTP}])e_0}{K_0 + K_1[G_{GDP}] + K_2[G_{GTP}]} - f_{GTPase}$$

102 Here $[G_{GXP}]$ is the concentration of GXP-bound G protein and κ is the ratio of the
103 backwards to the forwards kinetic rates. (For definitions of the other parameters
104 see the Methods section.)

105 At steady-state (setting the above equation equal to zero), in the absence of
106 GTPase activity, we find that the ratio of inactive to active G protein must al-
107 ways equal the value of the constant κ . An equivalent statement is: GEFs act
108 to produce a constant proportion of active G protein. While the ratio of inac-
109 tive to active G protein (κ) and proportion of active G protein ($1/\kappa + 1$) will vary
110 for different G protein:GEF systems, these values will remain constant within a
111 system, independent of the G protein or GEF concentrations.

112 **GEFs can be inhibitory**

113 The commonly used description of GEFs as ‘activators’ of G proteins is contra-
114 dicted by the inhibitory effect seen when the GEF mechanism is simulated in
115 the absence of GTPase activity (Figure 2D). This demonstrates the inadequacy
116 of this description.

117 The inhibitory effect can be explained by an equivalent increase in the concentra-
118 tions of intermediate G protein-GEF complexes. Values for the concentrations
119 of these intermediate complexes were derived as part of the construction of the
120 quasi-steady-state model. Using these values, we obtained an equation for the
121 proportion of (free) active G protein in terms of the total concentration of GEF.

122 This equation is plotted with the rates described for the Ran:RCC1 system in Fig-
123 ure 3A. Using this equation we are able to prove that in the absence of GTPase
124 activity the concentration of active G protein is inversely related to the total con-
125 centration of GEF. As the concentration of GEF increases, the concentration of
126 G protein will always decrease, and vice-versa.

127 Note that a high concentration of GEF will also lead to a faster total catalytic rate
128 (a larger V_{\max}). This suggests that there will be a tradeoff in terms of increasing
129 the concentration of GEF: a low concentration of GEF means that there will be
130 little inhibition, but a slow total rate; a high concentration of GEF will lead to
131 inhibition, but a fast total rate. We therefore hypothesise that for a healthy G
132 protein system, the concentration of GEF will lie in a physiologically relevant
133 region, where the inhibitory effect is not so pronounced, but where there is still
134 sufficient GEF to catalyse nucleotide exchange at an appropriate rate.

135 **GTPase activity has a functional role in the observed activation of G proteins**

136 The simulations of the GEF mechanism show that GTPase activity is sufficient
137 to restore an apparent GEF-mediated activation (Figures 2E, 2F). By comparing
138 these with the simulation of the system without GTPase activity (Figure 2D),
139 we can see how this activation arises. Initially, due to the GTPase activity, the
140 activation state reached by the system is suppressed – it is much reduced from
141 the activation state reached in the absence of GTPase activity. An increase in the
142 concentration of GEF is then able to positively regulate the system by moving
143 the activation state closer to the activation state reached in the absence of GTPase
144 activity (even though this state may itself be reduced).

145 For intrinsic GTPase activity we obtained an equation which describes the effect
146 of the relative rates of GEF-catalysed nucleotide exchange and GTPase activity
147 on the proportion of G protein which is active. This equation is plotted with
148 example parameters in Figure 3B, where we see a sigmoidal response such that
149 increasing the concentration of GEF (relative to the GTPase activity) increases
150 the concentration of active G protein. Again this allows us to hypothesise that,
151 for a healthy G protein system, the relative rates of nucleotide exchange and
152 GTPase activity must lie in this sigmoidal region, in order for the system to
153 properly respond to an activating or inhibitory signal.

154 Together, this clearly demonstrates a requirement for GTPase activity for the
155 observable activation of G proteins by GEFs. The proposed mechanism of reg-
156 ulation for a generic G protein:GEF:GTPase system can be summarised as fol-

157 lows: 1. GTPase activity inactivates the G protein system by altering the ratio
158 of inactive to active G protein away from a GEF-mediated equilibrium. 2. If the
159 rate of guanine nucleotide exchange increases or the GTPase activity decreases,
160 the proportion of active G protein will then move towards the GEF-mediated
161 equilibrium, generating an observed activation.

162 **Discussion**

163 We have shown that there are certain universal properties of GEF-mediated reg-
164 ulation of G proteins that arise from the reversibility of its mechanism and which
165 are independent of specific kinetic rates. The complete reversibility of the GEF
166 mechanism means that at steady-state any GEF acts to produce a constant ratio
167 of inactive to active G protein – giving a theoretical maximum proportion of ac-
168 tive G protein. Once this maximum is attained, then any subsequent increase
169 in the concentration of GEF—the ‘activator’ of the system—cannot increase the
170 concentration of active G protein. Instead this will lead to inhibition caused by
171 creation of excess intermediate G protein·GEF complexes.

172 We urge caution against naïve description of GEFs as ‘enzymes that activate G
173 proteins’ and against representations that show this mechanism as irreversible
174 as we have shown how these shorthands distort our understanding of the un-
175 derlying biology. We have demonstrated that GEFs should not be described as
176 enzymes that convert a substrate into product, but as enzymes that act to attain
177 an equilibrium—a balance—of active and inactive G protein. The two key roles
178 of GTPase activity are then: to drive the system away from this equilibrium—to
179 create an imbalance—and so permit positive regulation by GEFs; and to confer
180 a unique directionality on the G protein regulatory ‘cycle’. Therefore we sug-
181 gest that G protein signalling controlled by GEFs and GTPase activity should
182 not be described as an ‘activation/inactivation’ cycle but rather as a system that
183 is controlled through ‘regulated balance/imbalance’.

184 Both the complete reversibility of guanine nucleotide exchange and associated
185 requirement for GTPase activity as a functional component in the activation of G
186 proteins has previously been under-appreciated. This may be due to the almost
187 exclusive use of experimental systems where the GDP form of the G protein
188 is the unique starting condition and where uptake of GTP is monitored as the
189 GEF assay. We also note that our simulations show that an artificial irreversible
190 mechanism (Figures 2B, C) and reversible GEF mechanism (Figures 2E, F) have
191 similar profiles in the presence of GTPase activity and so under many conditions
192 it may be difficult to experimentally distinguish these mechanisms.

193 We predict that experimental protocols which attempt to regulate G proteins by
194 the over-expression of a GEF are likely to produce unexpected behaviour. We
195 expect that in many cases this may cause inhibition of the G protein rather than
196 activation (Figure 3A). Activation of G proteins should therefore be preferen-
197 tially targeted by reduction of the relevant GTPase activity (Figure 3B). Note
198 that these results remain consistent with the long-established use of dominant
199 negative mutants for the inhibition of G protein systems (Feig 1999; Barren and
200 Artemyev 2007). We accept that many previous studies that have ignored the
201 reversibility of GEFs will have made conclusions that are valid under many con-
202 ditions. But we stress that in extremal scenarios (such as in disease) those con-
203 clusions may not always hold.

204 Additionally, we hope that this new perspective in considering the control of
205 G proteins will lead to novel approaches for the control of G protein systems.
206 GEFs have previously been suggested as potential therapeutic targets (Bos et al.
207 2007). Our results extend this to a novel, and seemingly paradoxical, mecha-
208 nism by which over-expression of an activator could lead to the inhibition of
209 its substrate. This may have implications in G protein systems with diminished
210 GTPase activity, for example constitutively active transforming mutations in
211 Ras common in cancers (Stephen et al. 2014), where additional GAP activity
212 would have no effect but where sequestration of active G protein by a GEF may
213 be useful alternative.

214 The mathematical underpinning to our results mean that they should hold for
215 any G proteins:GEF system so long as the mechanism is consistent with that
216 studied here (Figure 1A), and under the reasonable assumption that the ma-
217 jority of its functional signalling is due to the steady-state behaviour. The pre-
218 cise tradeoffs for any system (equilibrium ratios, total rates, and scale of inhi-
219 bition) will depend on the specific kinetic rates for the GEF and the strength of
220 GTPase activity, but the overall qualitative characteristics should remain consis-
221 tent across all such systems. Conclusions based on alternative mechanisms, for
222 instance systems with an implicit G protein-GEF-GAP complex (Berstein et al.
223 1992), would require further analysis.

224 **Methods**

225 The following mathematical analysis uses the notation:

- 226 • G protein without nucleotide bound $\rightarrow G$

- 227 • G protein with GDP bound $\rightarrow G_{\text{GDP}}$
- 228 • G protein with GTP bound $\rightarrow G_{\text{GTP}}$
- 229 • GEF $\rightarrow E$

230 The volume concentration of a species S will be denoted by $[S]$.

231 Mass-action model

232 A deterministic ordinary differential equation (ODE) model of the GEF mecha-
233 nism (Figure 1B) was derived using the law of mass-action:

$$\begin{aligned} \frac{d[E]}{dt} &= - [E](k_1[G_{\text{GDP}}] + k_5[G_{\text{GTP}}]) + k_2[E \cdot G_{\text{GDP}}] + k_6[E \cdot G_{\text{GTP}}] \\ \frac{d[E \cdot G_{\text{GDP}}]}{dt} &= - (k_2 + k_3)[E \cdot G_{\text{GDP}}] + k_1[G_{\text{GDP}}][E] + k_4[E \cdot G][\text{GDP}] \\ \frac{d[E \cdot G_{\text{GTP}}]}{dt} &= - (k_6 + k_7)[E \cdot G_{\text{GTP}}] + k_5[G_{\text{GTP}}][E] + k_8[E \cdot G][\text{GTP}] \\ \frac{d[E \cdot G]}{dt} &= - (k_4[\text{GDP}] + k_8[\text{GTP}])[E \cdot G] + k_3[E \cdot G_{\text{GDP}}] + k_7[E \cdot G_{\text{GTP}}] \\ \frac{d[G_{\text{GDP}}]}{dt} &= - k_1[E][G_{\text{GDP}}] + k_2[E \cdot G_{\text{GDP}}] + f_{\text{GTPase}} \\ \frac{d[G_{\text{GTP}}]}{dt} &= - k_5[E][G_{\text{GTP}}] + k_6[E \cdot G_{\text{GTP}}] - f_{\text{GTPase}} \end{aligned}$$

234 We assume: for systems with no GTPase activity, $f_{\text{GTPase}} = 0$; for systems
235 with intrinsic GTPase activity, $f_{\text{GTPase}} = k_{\text{ase}}[G_{\text{GTP}}]$; and for systems with GAP-
236 mediated GTPase activity, $f_{\text{GTPase}} = \frac{k_{\text{ase}}[G_{\text{GTP}}]f_0}{K_m + [G_{\text{GTP}}]}$ where f_0 is the total concentra-
237 tion of GAP.

238 There is an equation for the conservation of mass of GEF:

$$e_0 = [E] + [E \cdot G_{\text{GDP}}] + [E \cdot G_{\text{GTP}}] + [E \cdot G] \quad (1)$$

239 And an equation for the conservation of mass of G protein:

$$g_0 = [G_{\text{GDP}}] + [G_{\text{GTP}}] + [E \cdot G_{\text{GDP}}] + [E \cdot G_{\text{GTP}}] + [E \cdot G] \quad (2)$$

240 Simulation of the mass-action model

241 The parameters used for the simulations in Figure 2 are summarised in Table
242 S1. Wherever possible, parameters measured for the Ran:RCC1 system were

243 used (Klebe et al. 1995). The irreversible model was generated by setting $k_7 = 0$.
244 (Alternative irreversible models could be generated by setting any one or more
245 of the reverse reaction rates to zero.)

246 All simulations were started from steady-state and generated by numerical in-
247 tegration of the mass-action equations, with the exception of free enzyme con-
248 centration $[E]$ which was calculated from the total mass of enzyme equation (1)
249 with:

- 250 • $e_0 = 0.05$ during $0 \leq t < 2$
- 251 • $e_0 = 0.2$ during $2 \leq t < 4$
- 252 • and free GEF (E) removed from the simulation until $e_0 = 0.05$ during $t \geq 4$

253 Quasi-steady-state model

254 Quasi-steady-state solutions for the intermediate enzyme complexes of the GEF
255 mechanism (Figure 1B) were derived using the framework of Gunawardena
256 (2012) (Figure S1):

$$\begin{aligned} [E] &= \left(\frac{K_0}{K_0 + K_1[G_{\text{GDP}}] + K_2[G_{\text{GTP}}]} \right) e_0 \\ [E \cdot G_{\text{GDP}}] &= \left(\frac{K_1^d[G_{\text{GDP}}] + K_2^d[G_{\text{GTP}}]}{K_0 + K_1[G_{\text{GDP}}] + K_2[G_{\text{GTP}}]} \right) e_0 \\ [E \cdot G_{\text{GTP}}] &= \left(\frac{K_1^t[G_{\text{GDP}}] + K_2^t[G_{\text{GTP}}]}{K_0 + K_1[G_{\text{GDP}}] + K_2[G_{\text{GTP}}]} \right) e_0 \\ [E \cdot G] &= \left(\frac{K_1^g[G_{\text{GDP}}] + K_2^g[G_{\text{GTP}}]}{K_0 + K_1[G_{\text{GDP}}] + K_2[G_{\text{GTP}}]} \right) e_0 \end{aligned}$$

257 where the K_i^x and the K_i are summary parameters (defined in Table S1).

258 These quasi-steady-state solutions were substituted into the equation for the
259 rate of change of $[G_{\text{GTP}}]$ given in the mass-action model, to obtain a quasi-steady-
260 state model for a generic GEF acting on a generic G protein:

$$\frac{d[G_{\text{GTP}}]}{dt} = \frac{k_{\text{cat}}([G_{\text{GDP}}] - \kappa[G_{\text{GTP}}])e_0}{K_0 + K_1[G_{\text{GDP}}] + K_2[G_{\text{GTP}}]} - f_{\text{GTPase}} \quad (3)$$

261 where k_{cat} is the forward catalytic rate; κ is the ratio of the backwards to the
262 forwards kinetic rates, multiplied by the ratio of GDP to GTP.

263 This equation does not consider mass held in G protein·GEF intermediate com-
264 plexes and so is only a good approximation when $e_0 \ll g_0$. Note that with

265 $f_{\text{GTPase}} = 0$ this model reduces to the Michaelis-Menten equation when $y = 0$,
266 and is equivalent to the equation used by Randazzo et al. (2013) when the con-
267 centration of GTP is absorbed into the summary paramters.

268 **Steady-state ratio of inactive to active G protein**

269 At steady-state with $f_{\text{GTPase}} = 0$, equation (3) implies:

$$[G_{\text{GDP}}] = \kappa [G_{\text{GTP}}] \quad (4)$$

270 Assuming that $e_0 \ll g_0$, equation (2) simplifies to $g_0 = [G_{\text{GDP}}] + [G_{\text{GTP}}]$, into
271 which equation (4) can be substituted to obtain:

$$\frac{[G_{\text{GTP}}]}{g_0} = \frac{1}{\kappa + 1}$$

272 This is the maximum steady-state proportion of active G protein.

273 **Active G protein as a function of GEF concentration (without GTPase activity)**

274 The effect of increasing the concentration of GEF on the steady-state concen-
275 tration of active G protein in the absence of GTPase activity ($f_{\text{GTPase}} = 0$) was
276 investigated.

277 The quasi-steady-state solutions for the intermediate enzyme complexes and
278 equation (4) were substituted into equation (2) to obtain:

$$0 = (\kappa + 1)[G_{\text{GTP}}]^2 + 2b[G_{\text{GTP}}] - K_s g_0$$

279 where $b = \frac{1}{2}(e_0 - g_0 + (\kappa + 1)K_s)$ and $K_s = \frac{K_0}{(K_1\kappa + K_2)}$.

280 This quadratic equation has one positive solution:

$$[G_{\text{GTP}}] = \frac{1}{\kappa + 1} \left(-b + \sqrt{b^2 + (\kappa + 1)K_s g_0} \right)$$

281 Alternatively, the proportion of active G protein is:

$$\frac{[G_{\text{GTP}}]}{g_0} = \frac{1}{g_0(\kappa + 1)} \left(-b + \sqrt{b^2 + (\kappa + 1)K_s g_0} \right) \quad (5)$$

282 We are interested in the rate of change of $[G_{\text{GTP}}]$ with respect to e_0 , the total
 283 concentration of GEF. As b (and only b) is a function of e_0 , we can examine:

$$\frac{d[G_{\text{GTP}}]}{db} = \frac{1}{\kappa + 1} \left(\frac{b}{\sqrt{b^2 + (\kappa + 1)K_s g_0}} - 1 \right) < 0$$

284 As this equation is always negative, the concentration of active G protein must
 285 decrease as the concentration of GEF is increased (and vice-versa).

286 Active G protein as a function of GEF concentration (with GTPase activity)

287 The effect of increasing the concentration of GEF on the steady-state concentra-
 288 tion of active G protein with GTPase activity ($f_{\text{GTPase}} = k_{\text{ase}}[G_{\text{GTP}}]$) was investi-
 289 gated.

290 At steady-state $\frac{d[G_{\text{GTP}}]}{dt} = 0$ implies:

$$[G_{\text{GDP}}] = \frac{K_2 y^2 + (K_0 + \kappa \hat{\kappa})[G_{\text{GTP}}]}{\hat{\kappa} - K_1 [G_{\text{GTP}}]} \quad (6)$$

291 where $\hat{\kappa} = \frac{k_{\text{cat}} e_0}{k_{\text{ase}}}$.

292 Again assuming that $e_0 \ll g_0$, equation (2) simplifies to $g_0 = [G_{\text{GDP}}] + [G_{\text{GTP}}]$,
 293 into which equation (6) can be substituted to obtain:

$$0 = (K_2 - K_1)[G_{\text{GTP}}]^2 + 2\hat{b}[G_{\text{GTP}}] - \hat{\kappa}g_0e_0$$

294 where $\hat{b} = \frac{1}{2}(K_0 + K_1 g_0 + (\kappa + 1)\hat{\kappa}e_0)$.

295 This quadratic equation has one solution that lies in the region $0 \leq [G_{\text{GTP}}] \leq g_0$:

$$[G_{\text{GTP}}] = \frac{1}{K_2 - K_1} \left(-\hat{b} + \sqrt{\hat{b}^2 + (K_2 - K_1)\hat{\kappa}g_0e_0} \right)$$

296 Alternatively, the proportion of active G protein is:

$$\frac{[G_{\text{GTP}}]}{g_0} = \frac{1}{g_0(K_2 - K_1)} \left(-\hat{b} + \sqrt{\hat{b}^2 + (K_2 - K_1)\hat{\kappa}g_0e_0} \right) \quad (7)$$

297 This equation describes the steady-state concentration of active G protein as a
 298 function of $\hat{\kappa}$, the ratio of the rate of forwards GEF-mediate nucleotide exchange
 299 to the rate of GTPase activity.

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306 **Author contributions**

307 This work formed part of the doctoral research of RS under the supervision of
308 GT. RS produced mathematical results, and GT and RS wrote the paper.

309 **Conflict of interest**

310 The authors declare that they have no conflict of interest.

311 **References**

- 312 Adjobo-Hermans, M. J. W., Goedhart, J., van Weeren, L., Nijmeijer, S., Manders,
313 E. M. M., Offermanns, S., and Gadella, T. W. J. (2011). "Real-time visualiza-
314 tion of heterotrimeric G protein Gq activation in living cells." *BMC Biol.* 32.
315 DOI: 10.1186/1741-7007-9-32.
- 316 Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., and
317 Walter, P. (2013). *Essential Cell Biology*. 4th ed. Taylor & Francis Group. ISBN:
318 9781317806271.
- 319 Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., and Walter,
320 P. (2014). *Molecular Biology of the Cell*. 6th ed. Taylor & Francis Group. ISBN:
321 9781317563754.
- 322 Barren, B. and Artemyev, N. O. (2007). "Mechanisms of dominant negative G-
323 protein alpha subunits." *J Neurosci Res* **85**, 3505–3514. DOI: 10.1002/jnr.
324 21414.
- 325 Baynes, J. and Dominiczak, M. (2014). *Medical Biochemistry*. 4th ed. Elsevier Health
326 Sciences UK. ISBN: 9781455745814.
- 327 Berg, J., Tymoczko, J., and Stryer, L. (2010). *Biochemistry*. 7th ed. W. H. Freeman.
328 ISBN: 9781429229364.

- 329 Berstein, G., Blank, J. L., Jhon, D.-Y., Exton, J. H., Rhee, S. G., and Ross, E. M.
330 (1992). "Phospholipase C- β 1 is a GTPase-activating protein for Gq/11, its
331 physiologic regulator." *Cell* **70**, 411–418. DOI: 10.1016/0092-8674(92)90165-
332 9.
- 333 Bolsover, S., Shephard, E., White, H., and Hyams, J. (2011). *Cell Biology: A Short*
334 *Course*. 3rd ed. Wiley. ISBN: 9781118008744.
- 335 Bos, J. L., Rehmann, H., and Wittinghofer, A. (2007). "GEFs and GAPs: critical
336 elements in the control of small G proteins." *Cell* **129**, 865–877. DOI: 10.1016/
337 j.cell.2007.05.018.
- 338 Briggs, G. E. and Haldane, J. B. (1925). "A Note on the Kinetics of Enzyme Ac-
339 tion." *Biochem J* **19**, 338–339.
- 340 Chang, S. and Ross, E. M. (2012). "Activation biosensor for G protein-coupled re-
341 ceptors: a FRET-based m1 muscarinic activation sensor that regulates G(q)." *PLoS One* **7**, e45651. DOI: 10.1371/journal.pone.0045651.
- 342
- 343 Dong, J.-H., Wen, J.-F., and Tian, H.-F. (2007). "Homologs of eukaryotic Ras su-
344 perfamily proteins in prokaryotes and their novel phylogenetic correlation
345 with their eukaryotic analogs". *Gene* **396**, 116–124. DOI: 10.1016/j.gene.2007.
346 03.001.
- 347 Feig, L. A. (1999). "Tools of the trade: use of dominant-inhibitory mutants of
348 Ras-family GTPases." *Nat Cell Biol* **1**, E25–E27. DOI: 10.1038/10018.
- 349 Goody, R. S. (2014). "How not to do kinetics: examples involving GTPases and
350 guanine nucleotide exchange factors." *FEBS J* **281**, 593–600. DOI: 10.1111/
351 febs.12551.
- 352 Gunawardena, J. (2012). "A linear framework for time-scale separation in non-
353 linear biochemical systems." *PLoS One* **7**, e36321. DOI: 10.1371/journal.pone.
354 0036321.
- 355 Hancock, J. (2010). *Cell Signalling*. 3rd ed. OUP Oxford. ISBN: 9780199232109.
- 356 Janetopoulos, C., Jin, T., and Devreotes, P. (2001). "Receptor-mediated activa-
357 tion of heterotrimeric G-proteins in living cells." *Science* **291**, 2408–2411. DOI:
358 10.1126/science.1055835.
- 359 Johnson, K. A. and Goody, R. S. (2011). "The original Michaelis constant: Trans-
360 lation of the 1913 Michaelis-Menten Paper." *Biochemistry* **50**, 8264–8269. DOI:
361 10.1021/bi201284u.
- 362 Klebe, C., Prinz, H., Wittinghofer, A., and Goody, R. S. (1995). "The kinetic
363 mechanism of Ran–nucleotide exchange catalyzed by RCC1." *Biochemistry*
364 **34**, 12543–12552. DOI: 10.1021/bi00039a008.

- 365 Loirand, G., Sauzeau, V., and Pacaud, P. (2013). "Small G proteins in the car-
366 diovascular system: physiological and pathological aspects." *Physiol Rev* **93**,
367 1659–1720. DOI: 10.1152/physrev.00021.2012.
- 368 Michaelis, L. and Menten, M. L. (1913). "Die kinetik der invertinwirkung." *Biochem*
369 *Z* **49**, 333–369.
- 370 Nelson, D. and Cox, M. (2013). *Lehninger Principles of Biochemistry*. 6th ed. W. H.
371 Freeman. W.H. Freeman. ISBN: 9781464109621.
- 372 Northup, J. K., Jian, X., and Randazzo, P. A. (2012). "Nucleotide exchange fac-
373 tors: Kinetic analyses and the rationale for studying kinetics of GEFs." *Cell*
374 *Logist* **2**, 140–146. DOI: 10.4161/cl.21627.
- 375 O'Hayre, M., Vázquez-Prado, J., Kufareva, I., Stawiski, E. W., Handel, T. M.,
376 Seshagiri, S., and Gutkind, J. S. (2013). "The emerging mutational landscape
377 of G proteins and G-protein-coupled receptors in cancer." *Nat Rev Cancer* **13**,
378 412–424. DOI: 10.1038/nrc3521.
- 379 Oldham, W. M. and Hamm, H. E. (2008). "Heterotrimeric G protein activation by
380 G-protein-coupled receptors." *Nat Rev Mol Cell Biol* **9**, 60–71. DOI: 10.1038/
381 nrm2299.
- 382 Oliveira, A. F. and Yasuda, R. (2013). "An improved Ras sensor for highly sen-
383 sitive and quantitative FRET-FLIM imaging." *PLoS One* **8**, e52874. DOI: 10.
384 1371/journal.pone.0052874.
- 385 Peyker, A., Rocks, O., and Bastiaens, P. I. H. (2005). "Imaging activation of two
386 Ras isoforms simultaneously in a single cell." *Chembiochem* **6**, 78–85. DOI:
387 10.1002/cbic.200400280.
- 388 Randazzo, P. A., Jian, X., Chen, P.-W., Zhai, P., Soubias, O., and Northup, J. K.
389 (2013). "Quantitative analysis of guanine nucleotide exchange factors (GEFs)
390 as enzymes." *Cell Logist* **3**, e27609. DOI: 10.4161/cl.27609.
- 391 Rojas, A. M., Fuentes, G., Rausell, A., and Valencia, A. (2012). "The Ras protein
392 superfamily: evolutionary tree and role of conserved amino acids." *J Cell Biol*
393 **196**, 189–201.
- 394 Seixas, E., Barros, M., Seabra, M. C., and Barral, D. C. (2013). "Rab and Arf pro-
395 teins in genetic diseases." *Traffic* **14**, 871–885. DOI: 10.1111/tra.12072.
- 396 Simon, M. I., Strathmann, M. P., and Gautam, N. (1991). "Diversity of G proteins
397 in signal transduction." *Science* **252**, 802–808.
- 398 Stephen, A. G., Esposito, D., Bagni, R. K., and McCormick, F. (2014). "Dragging
399 Ras back in the ring." *Cancer cell* **25**, 272–281. DOI: 10.1016/j.ccr.2014.02.017.
- 400 Takai, Y., Sasaki, T., and Matozaki, T. (2001). "Small GTP-binding proteins."
401 *Physiol Rev* **81**, 153–208.

- 402 Vetter, I. R. and Wittinghofer, A. (2001). "The guanine nucleotide-binding switch
403 in three dimensions." *Science* **294**, 1299–1304. DOI: 10.1126/science.1062023.
- 404 Vigil, D., Cherfils, J., Rossman, K. L., and Der, C. J. (2010). "Ras superfamily
405 GEFs and GAPs: validated and tractable targets for cancer therapy?." *Nat*
406 *Rev Cancer* **10**, 842–857. DOI: 10.1038/nrc2960.
- 407 Voet, D. and Voet, J. (2010). *Biochemistry*. 4th ed. John Wiley & Sons. ISBN: 9781118139936.
- 408 Young, A., Lyons, J., Miller, A. L., Phan, V. T., Alarcón, I. R., and McCormick,
409 F. (2009). "Ras signaling and therapies." *Adv Cancer Res* **102**, 1–17. DOI: 10.
410 1016/S0065-230X(09)02001-6.

411 **Figure captions**

412 **Figure 1. The activation of G proteins is regulated by GEFs and GTPase**
413 **activity.**

414 **A** G proteins are controlled by GEFs which catalyse the sequential release and
415 binding of guanine nucleotides, and by GTPase activity (both intrinsic and GAP-
416 mediated) which hydrolyses the bound GTP to form GDP. The red circle high-
417 lights that the GEF mechanism is completely reversible.

418 **B** The reversible mechanism by which a GEF catalyses guanine nucleotide ex-
419 change on a G protein proceeds through a series of GEF·G protein complexes
420 (Bos et al. 2007). Parameters k_i are kinetic rates which are unique to each G
421 protein:GEF system. Associated species (free GEF, GTP, GDP) have not been
422 drawn. The grey arrow identifies forwards nucleotide exchange, catalysing the
423 activation of the G protein. The red arrow identifies reverse nucleotide exchange,
424 catalysing the inactivation of the G protein.

425 **Figure 2. Apparent activation of G proteins via GEFs is only observed**
426 **when GTPase activity is present.**

427 Simulation of mass-action models, using parameters described in Table S1, and
428 where G_{GXP} denotes GXP-bound G protein. Where indicated as present, intrinsic
429 GTPase activity was modelled as exponential decay, GAP-mediated GTPase
430 activity by the Michaelis-Menten equation. The shaded region denotes stim-
431 ulation of the system through increasing the active GEF 4-fold from its basal
432 concentration. For all simulations, steady-state concentrations were used as the
433 initial conditions. Mass corresponding to GEF·G protein complexes has not been
434 drawn.

435 **A, B, C** An artificial irreversible model, constructed by assuming the rate of re-
436 lease of GTP from the active G protein·GEF complex is zero.

437 **D, E, F** The reversible GEF mechanism (Figure 1B).

438 **Figure 3. GTPase activity restores the ability of GEFs to positively reg-**
439 **ulate a G protein by moving the system away from equilibrium.**

440 The relationship between the concentration of GEF and the steady-state propor-
441 tion of active G protein (equation (5), equation (7)) illustrated using parameters
442 described for the Ran:RCC1 system (Klebe et al. 1995) and unit concentration

443 of G protein. The activation cannot be increased above a theoretical maximum
444 equilibrium value derived from the ratio of the total forwards and backwards
445 catalytic rates of the GEF (κ). The shaded region denotes the region which is
446 most likely to be physiologically relevant.

447 **A** In the absence of GTPase activity (equation (5)), increasing the GEF concentra-
448 tion can only decrease the steady-state concentration of active G protein, instead
449 producing irrelevant GEF·G protein complexes.

450 **B** In the presence of GTPase activity (equation (7)), the steady-state concentra-
451 tion of active G protein is suppressed. Increasing the (relative) concentration of
452 GEF acts to counter this suppression, driving the activation state back towards
453 the maximum equilibrium value.

454 **Figure S1. Application of the framework of Gunawardena (2012) to**
455 **the mechanism for the GEF mediated release and binding of guanine**
456 **nucleotides to G proteins.**

457 **A** The graph on the enzyme complexes with complexes as vertices and edges
458 representing reactions labelled by rates and partner species.

459 **B** All possible directed spanning trees of the graph on the enzyme complexes.
460 The red vertex denotes the root of each spanning tree.

461 **C** The basis element, ρ , generated from the each spanning trees: the sum over
462 each root vertex, of the products of the labels of each spanning tree. Every
463 steady-state of the original system $X = ([E], [E \cdot G_{GDP}], [E \cdot G_{GTP}], [E \cdot G])^T$ is
464 a solution to the equation $X = \lambda \rho$ where λ is a constant. We manipulate this
465 equation to obtain $X_i = \frac{\rho_i}{\sum_i \rho_i} \times \sum_i X_i$.

466 **Table S1.**

467 Concentrations, kinetic parameters, and summary parameters used for Figures
468 2 and 3. Where applicable, the definitions of the summary parameters in terms
469 of the individual kinetic parameters are stated.

470 Value obtained from (Klebe et al. 1995).

Figure 1

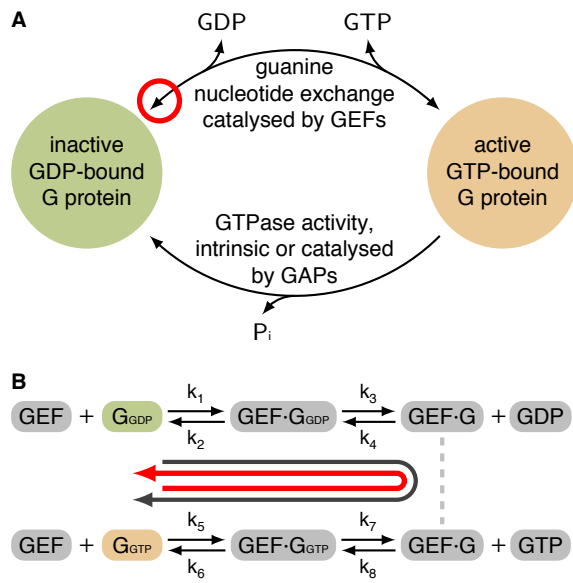


Figure 2

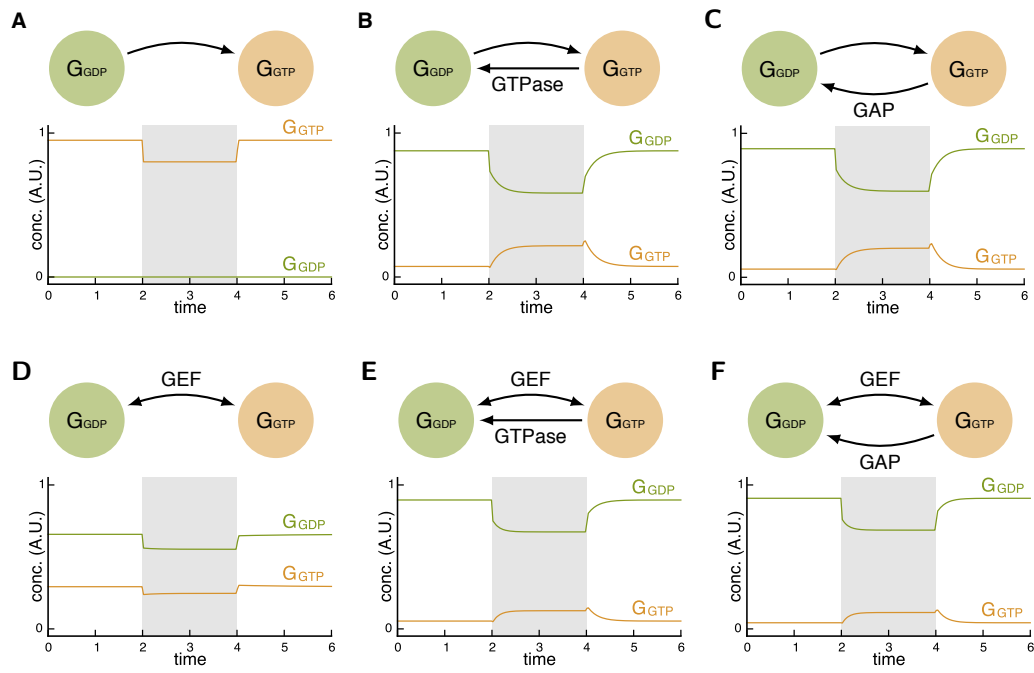


Figure 3

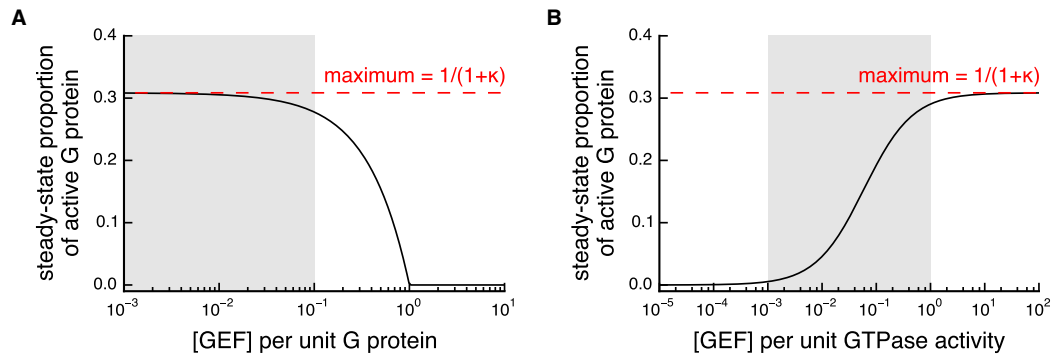


Figure S1

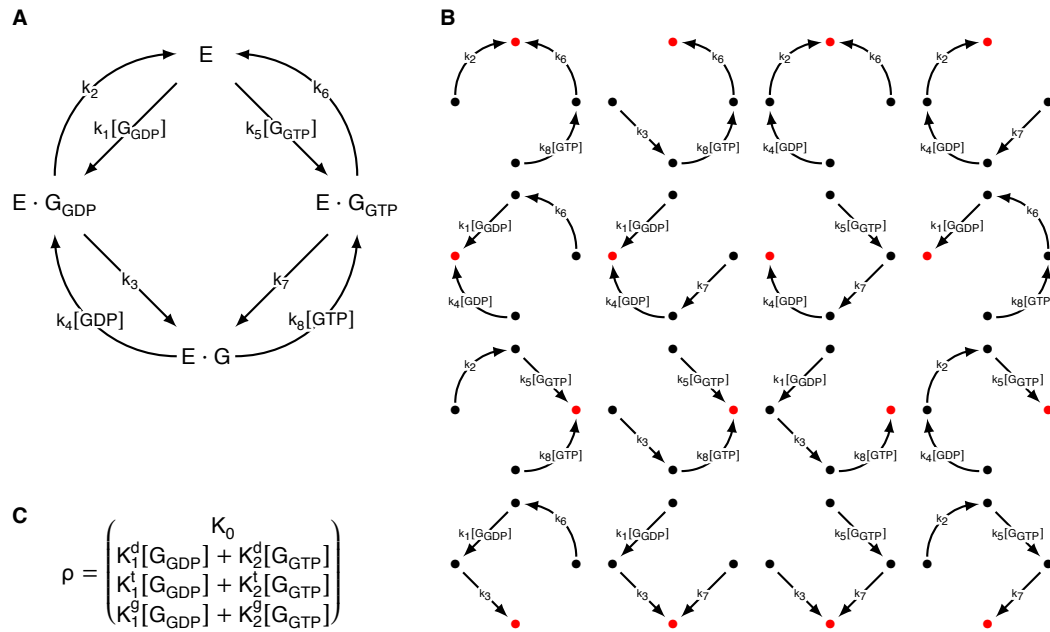


Table S1

Rate	Reversible model	Irreversible model	Definition
f_0	1	1	
[GTP]	10	10	
[GDP]	1	1	
k_1^*	7.4×10^7	7.4×10^7	
k_2^*	55	55	
k_3^*	21	21	
k_4^*	1.1×10^7	1.1×10^7	
k_5^*	1.0×10^8	1.0×10^8	
k_6^*	55	55	
k_7^*	19	0	
k_8^*	0.6×10^6	0.6×10^6	
k_ase	4	4	
K_m	0.7	0.7	
K_ic	100	100	
K_1^d	8.466×10^{16}	6.919×10^{16}	$k_1(k_6k_8[\text{GTP}] + k_4(k_6 + k_7)[\text{GDP}])$
K_2^d	2.090×10^{16}	0	$k_4k_5k_7[\text{GDP}]$
K_1^g	1.150×10^{11}	8.547×10^{10}	$k_1k_3(k_6 + k_7)$
K_2^g	1.444×10^{11}	0	$k_5k_7(k_2 + k_3)$
K_1^t	9.324×10^{15}	9.324×10^{15}	$k_1k_3k_8[\text{GTP}]$
K_2^t	1.061×10^{17}	1.061×10^{17}	$k_5(k_8(k_2 + k_3)[\text{GTP}] + k_2k_4[\text{GDP}])$
K_0	6.985×10^{10}	5.836×10^{10}	$k_6k_8(k_2 + k_3)[\text{GTP}] + k_2k_4(k_6 + k_7)[\text{GDP}]$
K_1	9.398×10^{16}	7.851×10^{16}	$K_1^d + K_1^g + K_1^t$
K_2	1.270×10^{17}	1.061×10^{17}	$K_2^d + K_2^g + K_2^t$
k_{cat}	5.128×10^{17}	5.128×10^{17}	$k_1k_3k_6k_8[\text{GTP}]$
κ	2.242	0	$\frac{k_2k_4k_5k_7[\text{GDP}]}{k_1k_3k_6k_8[\text{GTP}]}$
K_s	2.069×10^7	5.500×10^{-7}	$\frac{K_0}{(K_1\kappa + K_2)}$