Measuring Agonist Bias

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Terminology

Biased Signalling

(Functional Selectivity)

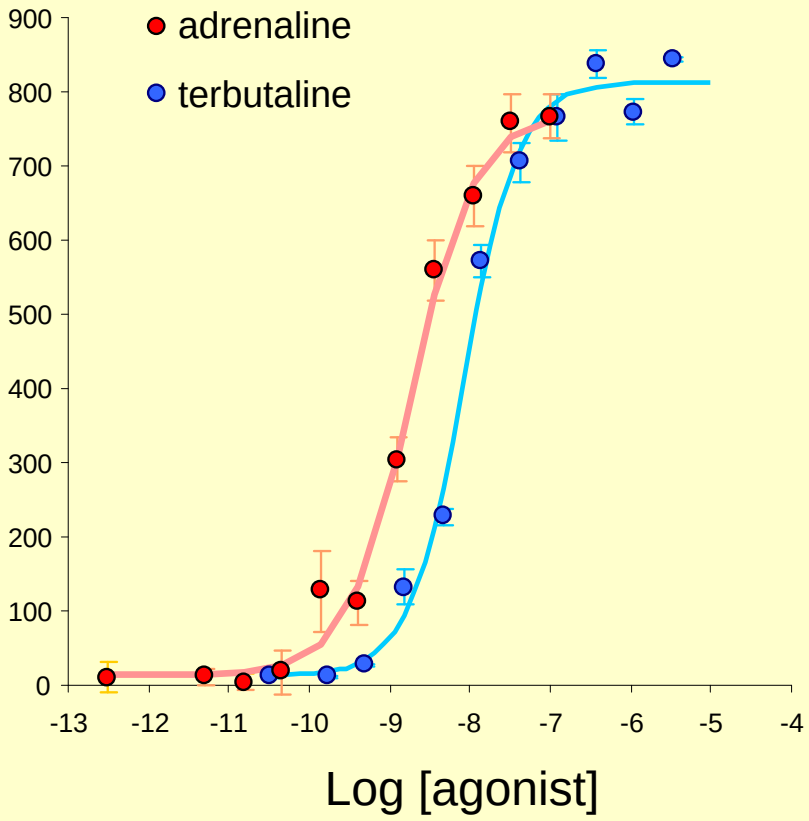
(may or may not be due to bias efficacy)

vs.

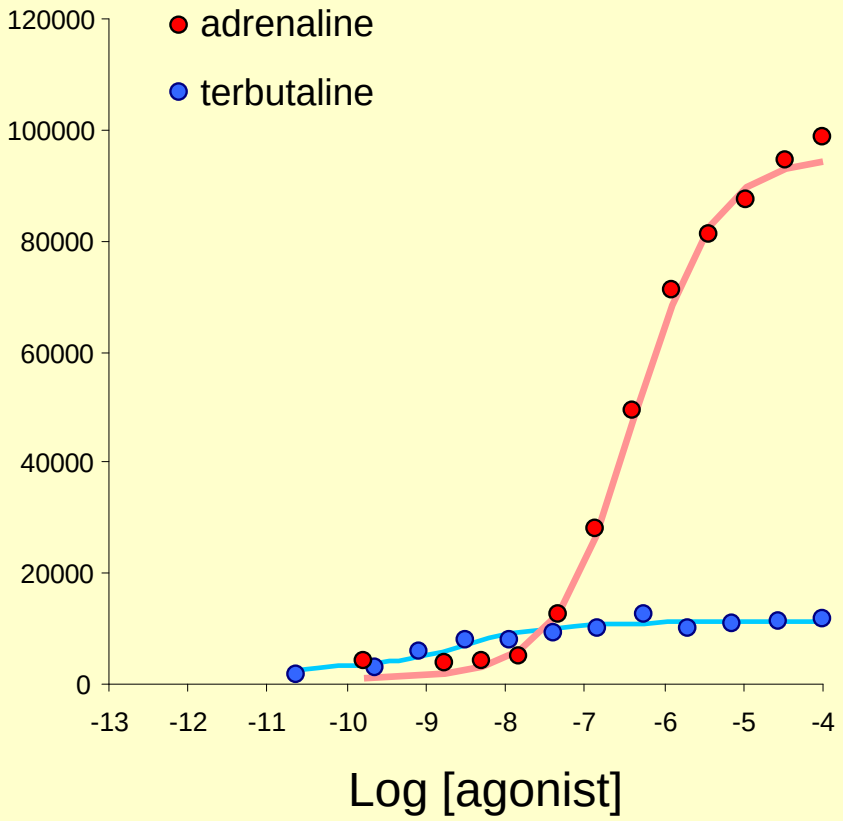
Bias efficacy or Bias Agonism

Functional Selectivity (β_2-AR)

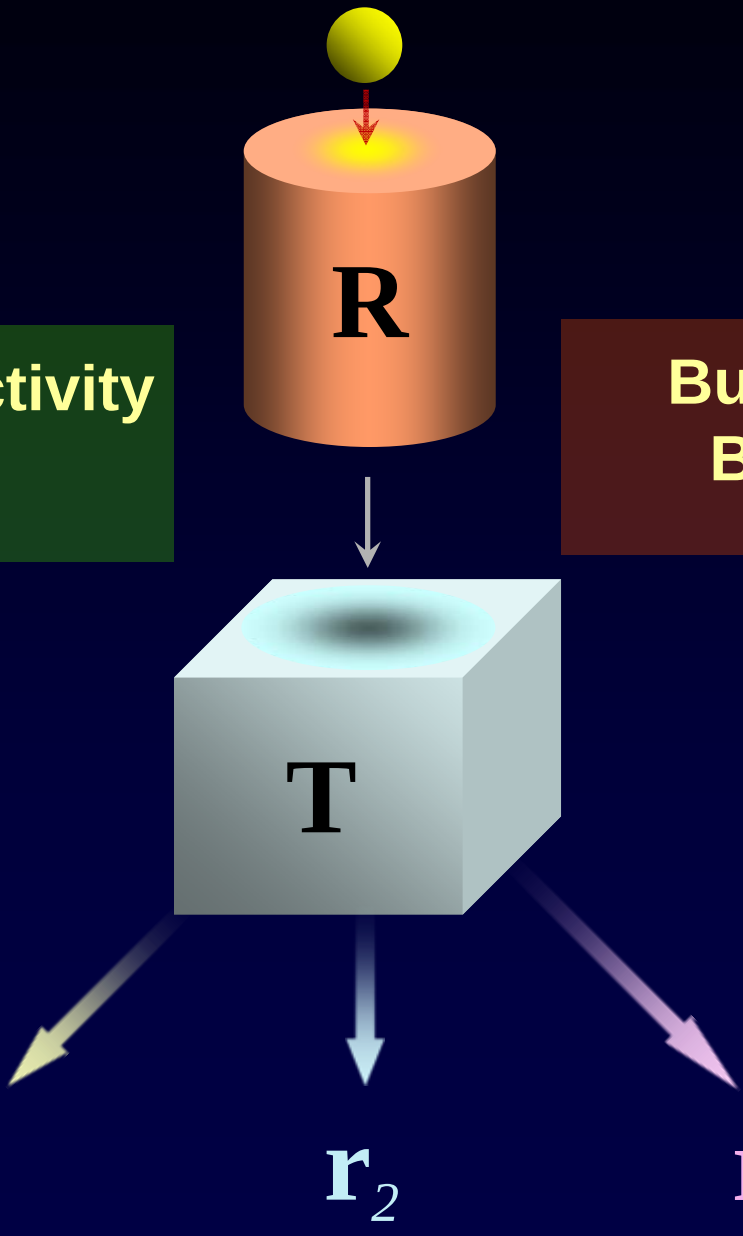
cAMP accumulation



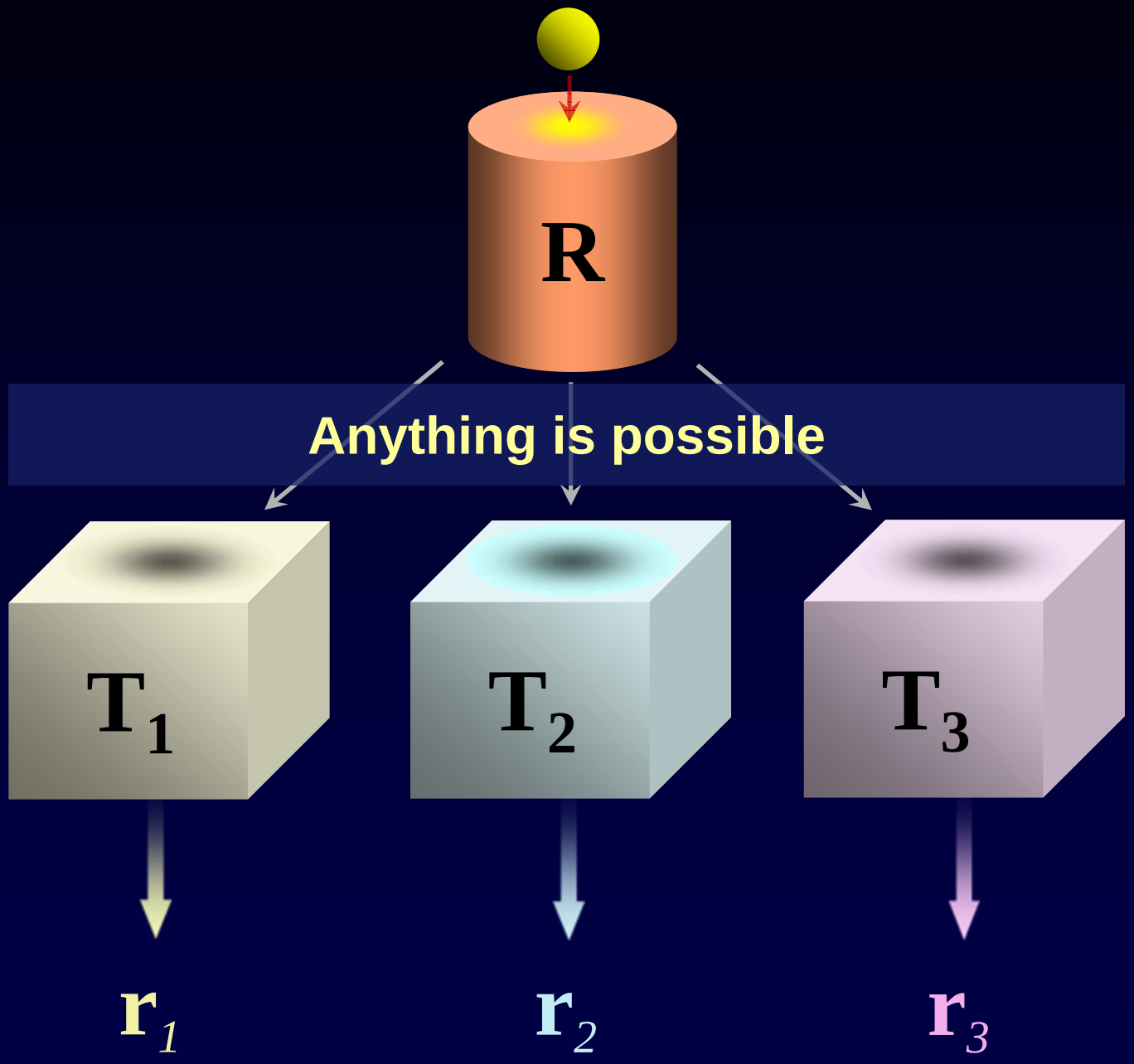
ERK Phosphorylation

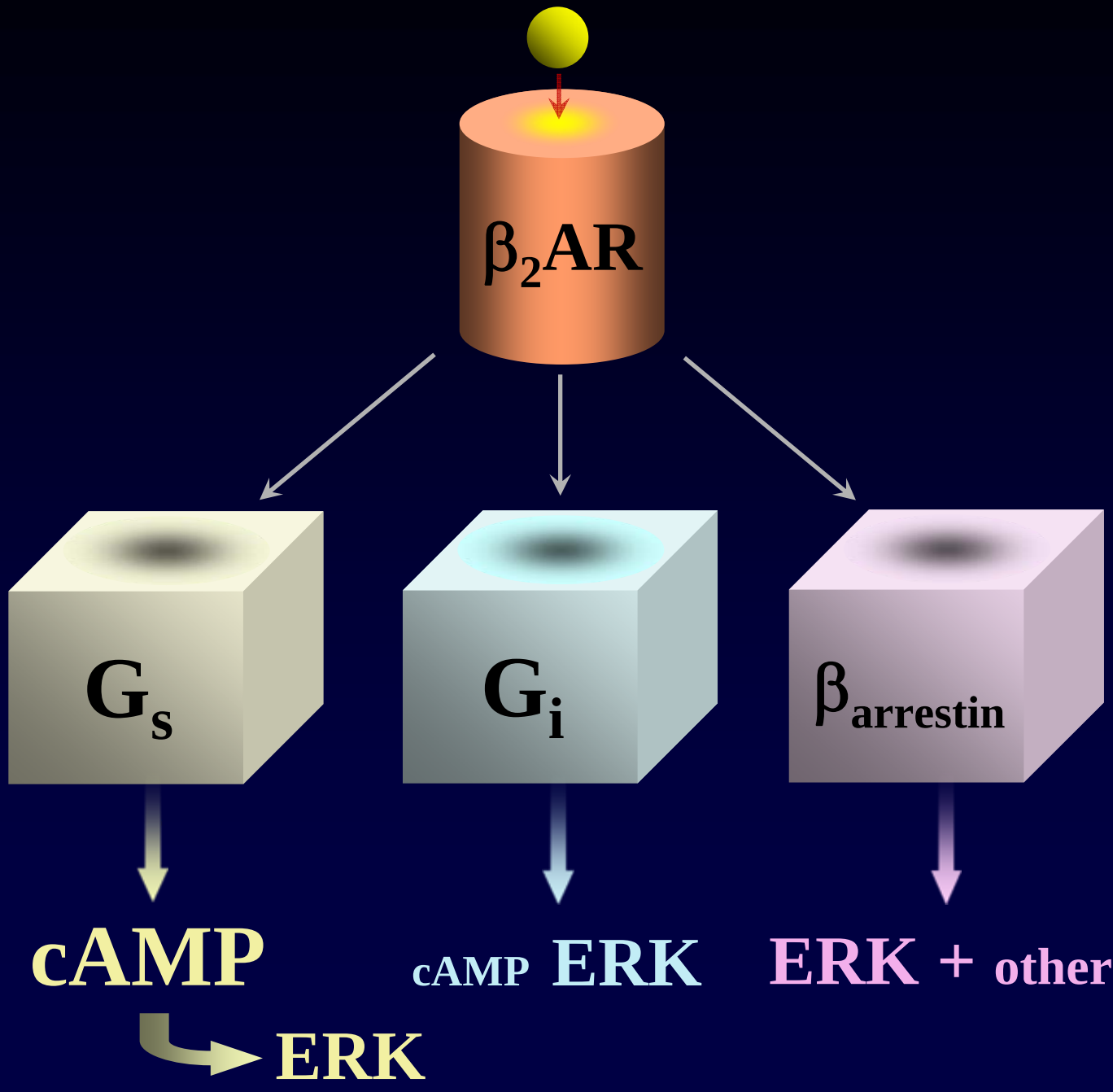


Functional Selectivity is possible



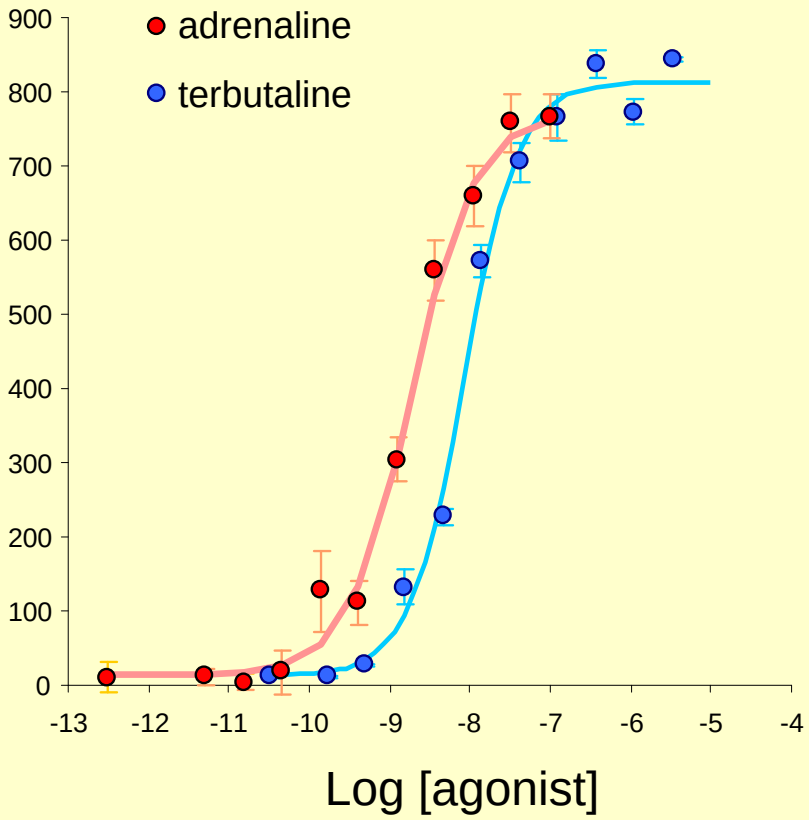
But no room for Bias Efficacy


R
Anything is possible
T1
T2
T3
r1
r2
r3

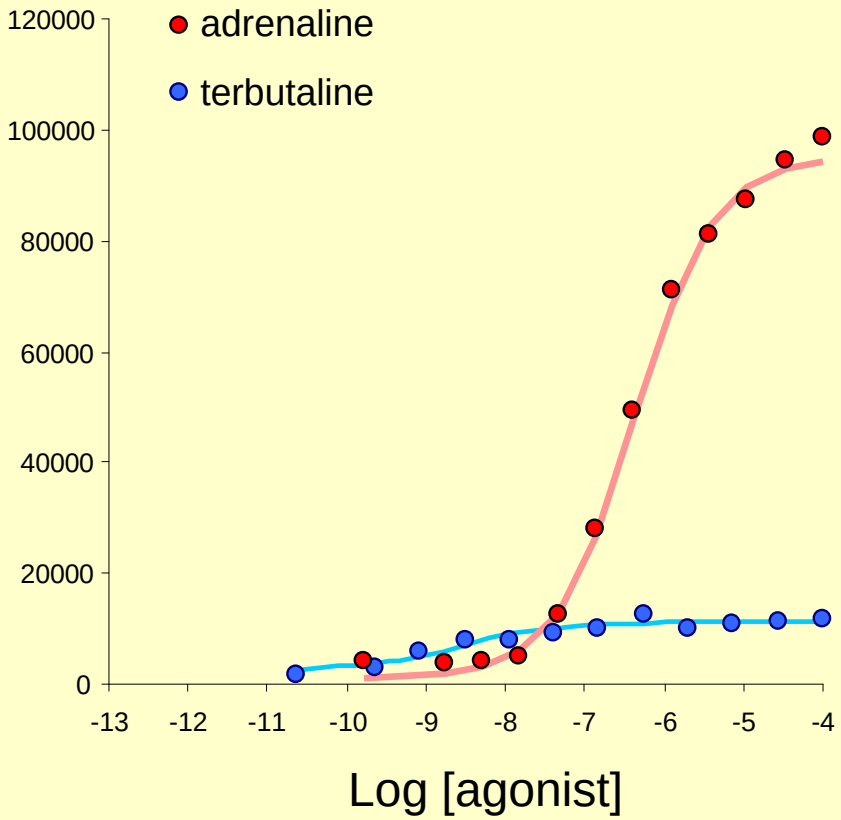

β2AR
Gs
Gi
βarrestin
cAMP
ERK
cAMP ERK
ERK + other

Functional Selectivity but no bias efficy (β_2-AR)

cAMP accumulation



ERK Phosphorylation



1

Make sure that the observed responses are mediated by different transducers.

Otherwise, agonist bias cannot be an explanation for the observed functional selectivity.

2

Understanding & Measuring Bias
Agonism requires a theory of efficacy.

ASF Theory

1935 – 1966 – 2014

binding $b = \dfrac{R_t[\mathrm{L}]}{[\mathrm{L}] + K_L}$

stimulus $s = \varepsilon\, b$

response $r = f(s)$

Operational Model

1981 – 1983 – 2014

binding $b = \dfrac{R_t[\mathrm{L}]}{[\mathrm{L}] + K_L}$

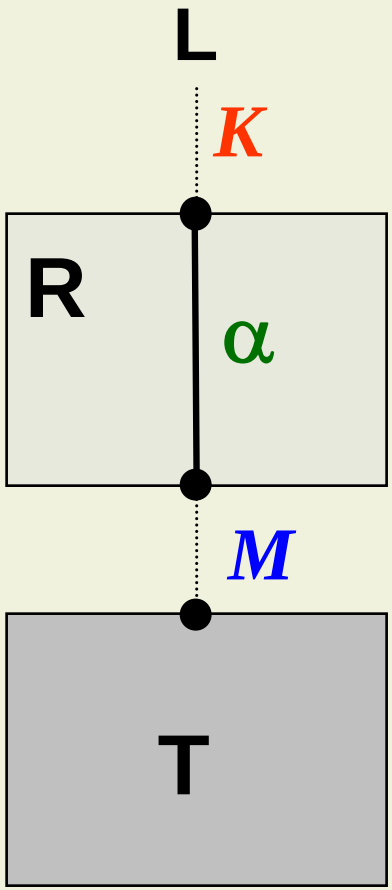
stimulus $s = \varepsilon\, b$

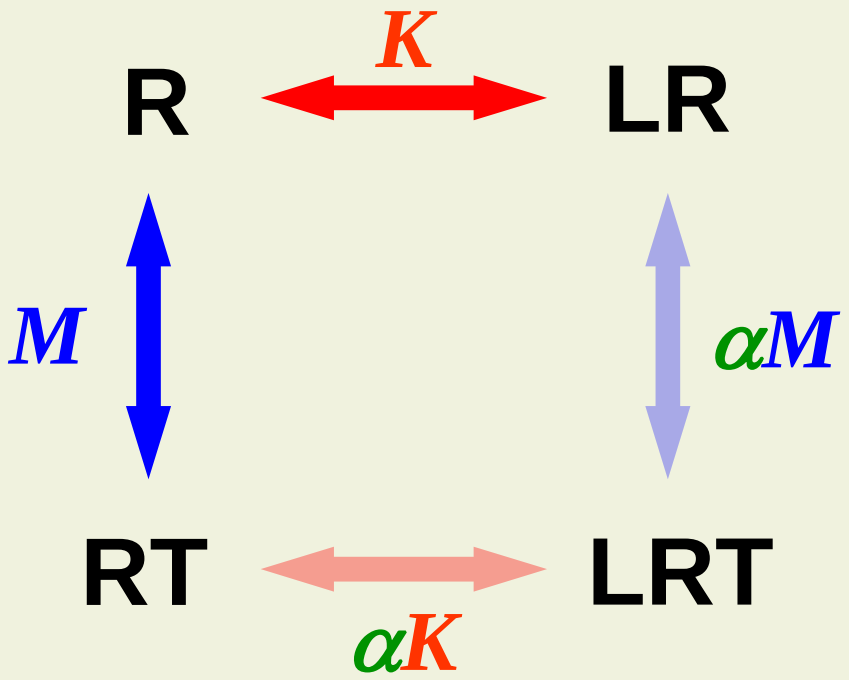
response $r = f(s) = \dfrac{r_{\max} s}{s + K_E}$

$$r = \frac{r_{\max}\,\tau/(1+\tau)\,[\mathrm{L}]}{[\mathrm{L}] + K_L/(1+\tau)} \qquad \tau = \varepsilon\, R_t / K_E$$

Molecular Description of efficacy

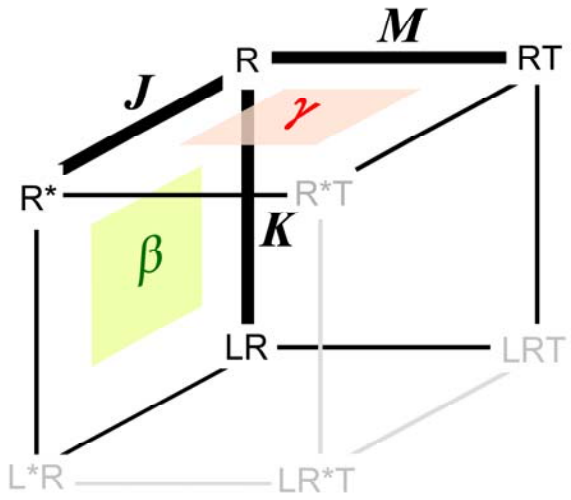
The TCM

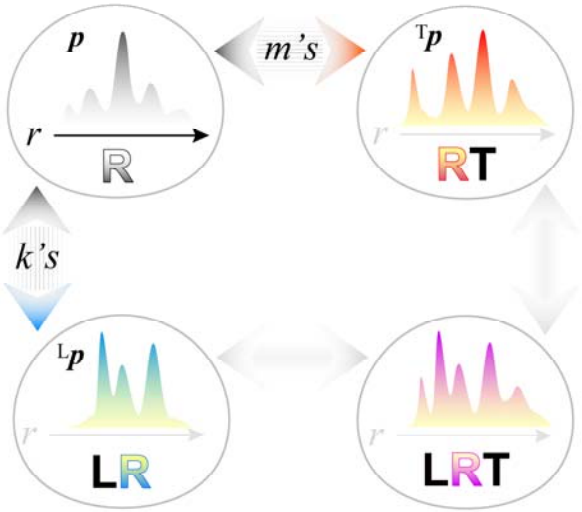




TCM

Two-state TCM





Multi-state Probabilistic model

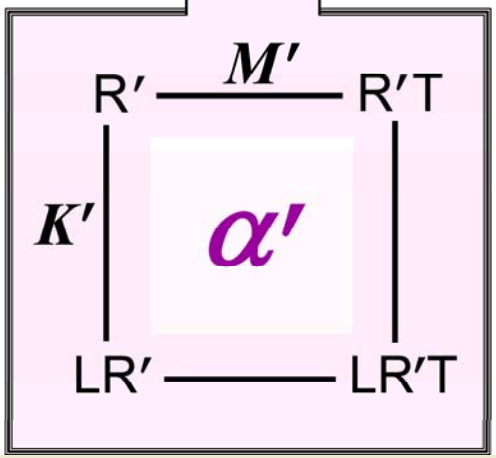
$R \cup R^* \equiv R'$

$R' \equiv \bigcup_i r_i$

$$K' = K\frac{1+\beta J}{1+J} = \langle k \rangle$$

$$M' = M\frac{1+\gamma J}{1+J} = \langle m \rangle$$

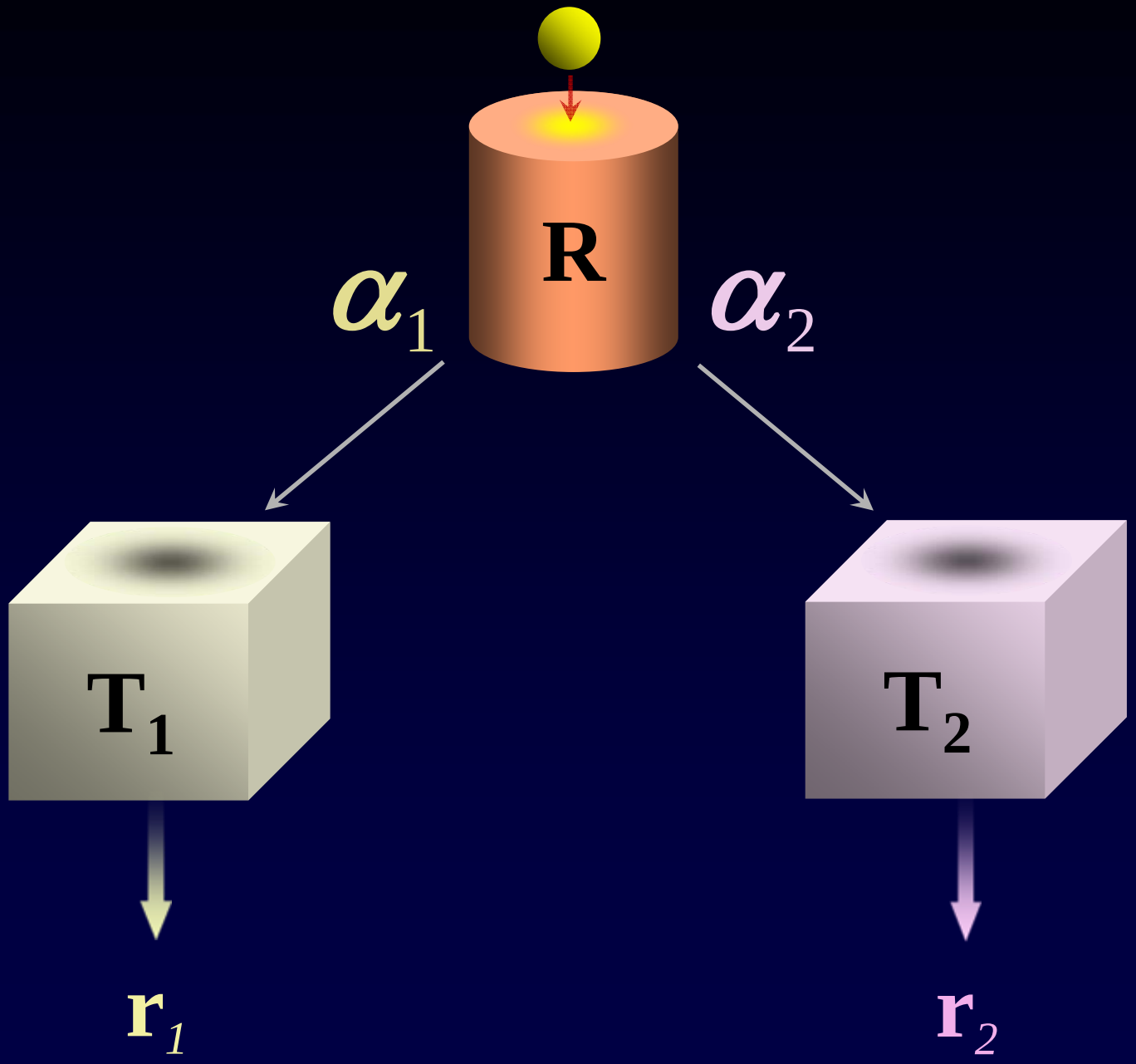
$$\alpha' = \frac{(1+\beta\gamma J)(1+J)}{(1+\beta J)(1+\gamma J)}$$



$$K' = \sum_i p_i k_i = \langle k \rangle$$

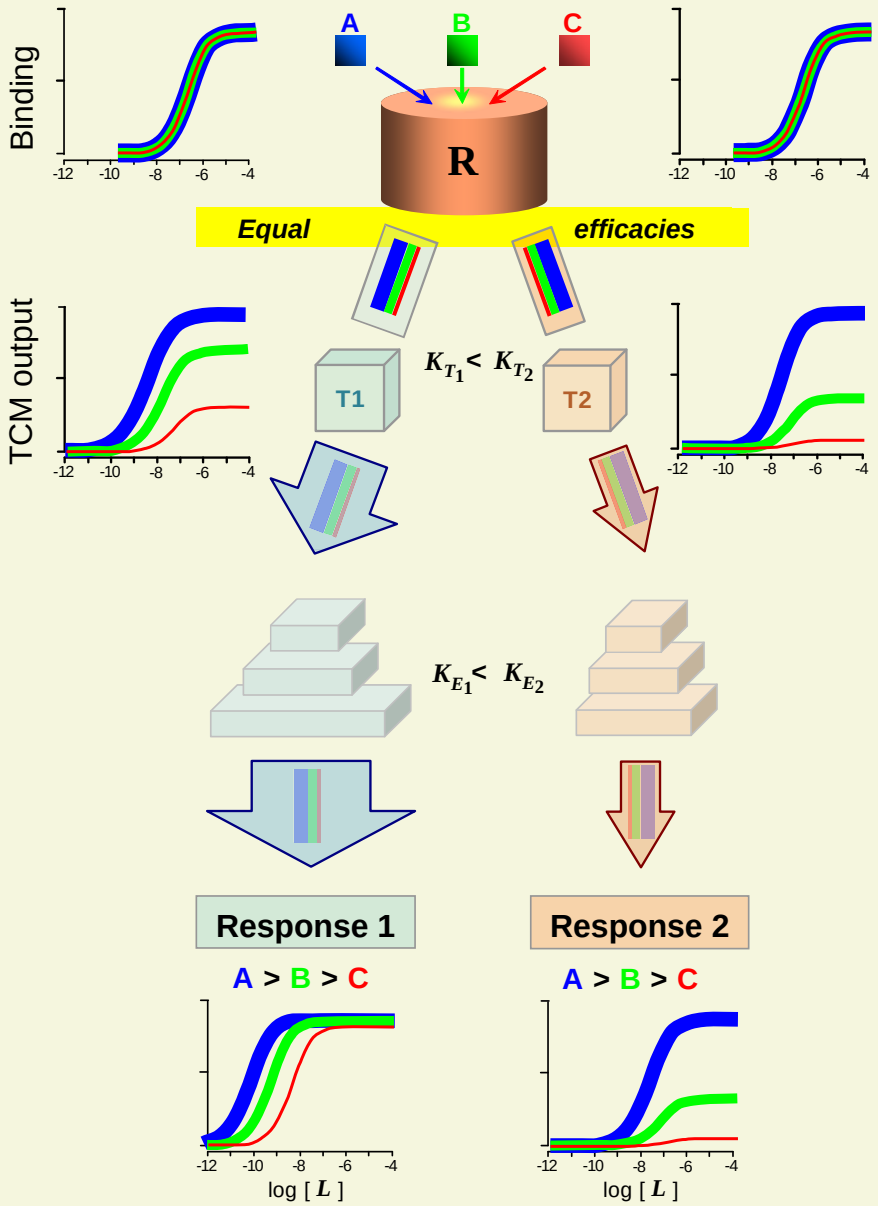
$$M' = \sum_i p_i m_i = \langle m \rangle$$

$$\alpha' = \sum_i \frac{{}^{T}p_i \, {}^{L}p_i}{p_i}$$


R
α_1
α_2
T_1
T_2
r_1
r_2

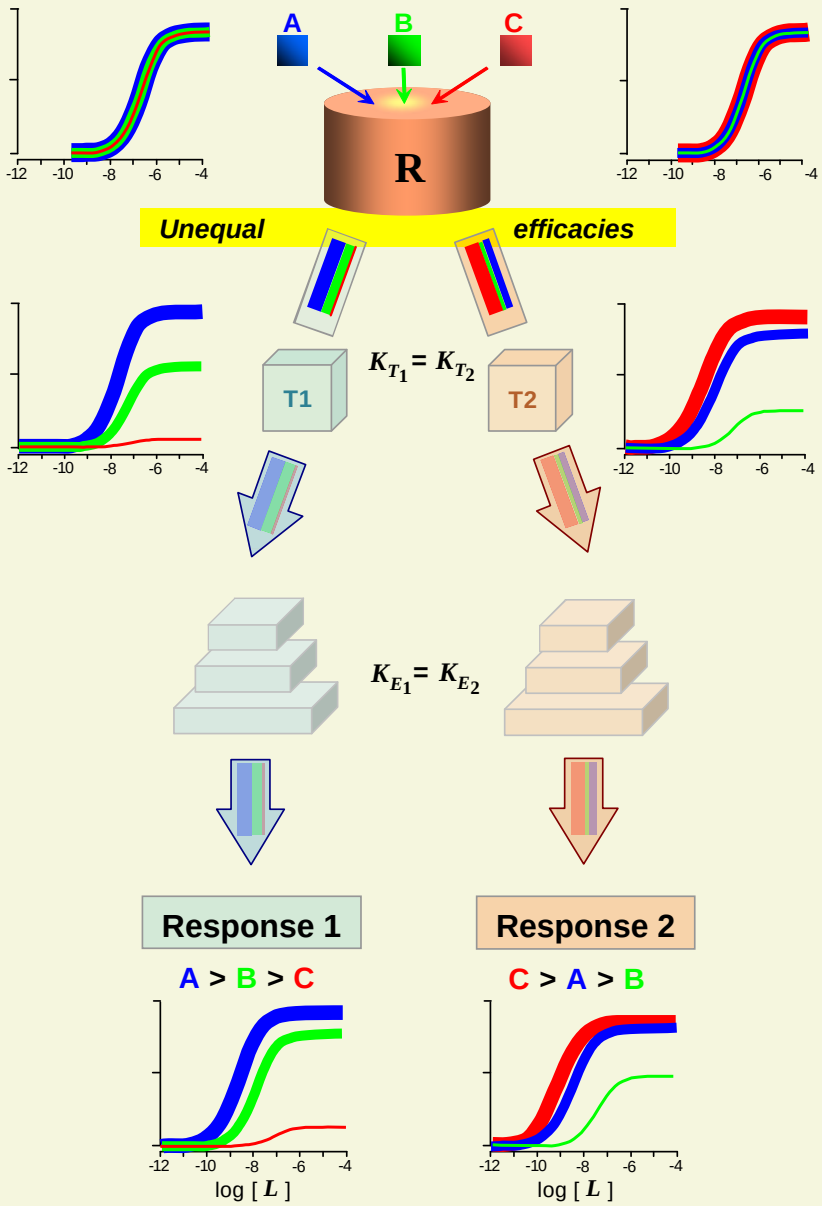
Functional Selectivity (+)

Bias efficacy (-)



Functional Selectivity (+)

Bias efficacy (+)



3

Measures of relative efficacy and agonist bias

In principle, all available strategies based on the ASF theory (including Operational Model approach) are equivalent.

What actually matters is the kind of information fed to the estimation procedures.

A simulation scheme

to show what actually these methods measure

GPCR activation is represented by ligand-induced Receptor-Transducer coupling
(TCM)

Simulate different ligands with different α values

↓

Take **[RT]+[LRT]** *as activity and correct for basal activity*

↓

Feed the previous output to a saturable hyperbolic function

↓

Fit the resulting curves with the operational model and see how relative τ's compare with simulated relative α's

Binding $\xrightarrow{\varepsilon}$ s $\xrightarrow{K_E}$ $f(s) \rightarrow r$

$\mathrm{L} + \mathrm{R} + \mathrm{T} \xrightarrow{\alpha, M}$ [RT] + [LRT] $\rightarrow r$

$$r_{op} = \frac{E_{max}[\mathrm{L}]}{[\mathrm{L}] + EC_{50}}$$

$$\frac{K_L}{1+\tau}$$

$$K_L \frac{1+\hat{T}/K_T}{1+\alpha\hat{T}/K_T}$$

$$r_{tcm} = \frac{E_{max}[\mathrm{L}]}{EC_{50} + [\mathrm{L}]}$$

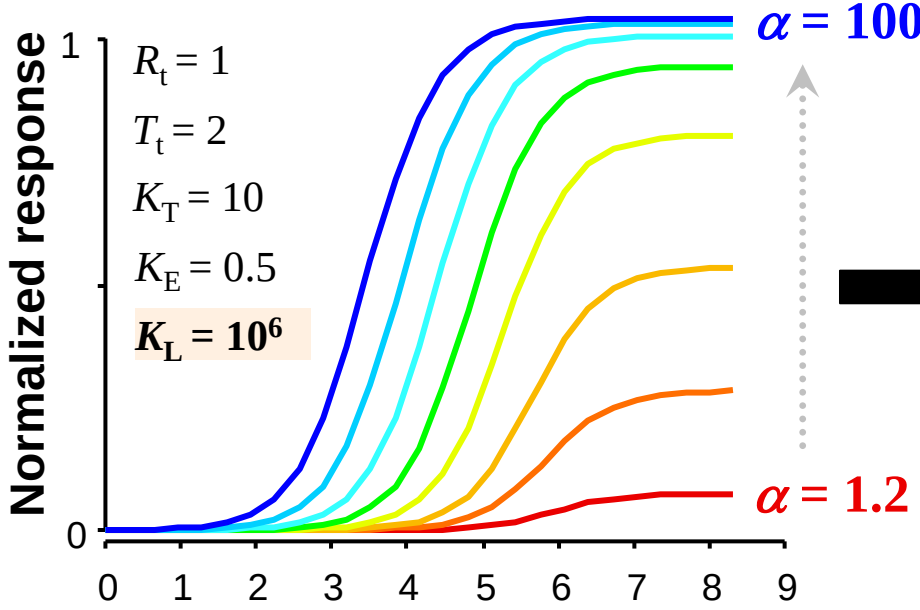
ASF

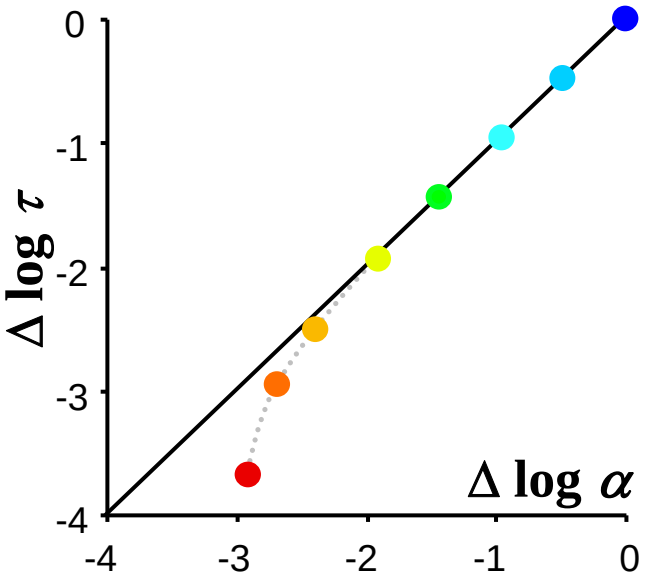
$$\tau = \varepsilon \frac{R_t}{K_E}$$

$$\varepsilon \frac{R_t}{K_E} = (\alpha - 1)\frac{\hat{T}/K_T}{1+\hat{T}/K_T}$$

$$\varepsilon \equiv \alpha - 1$$

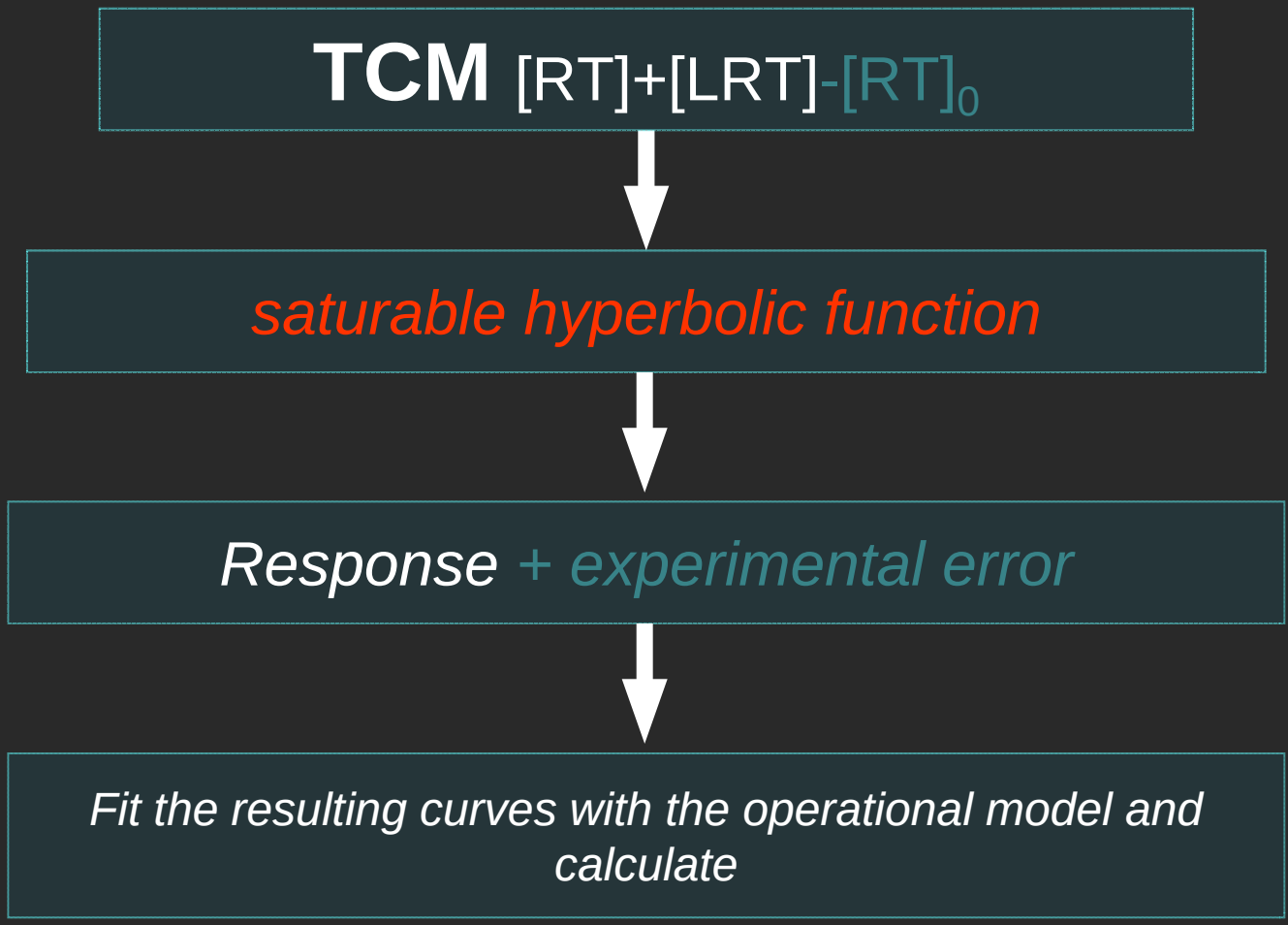
TCM



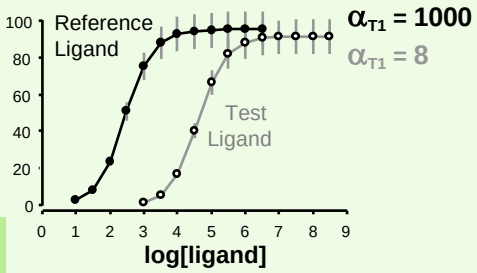
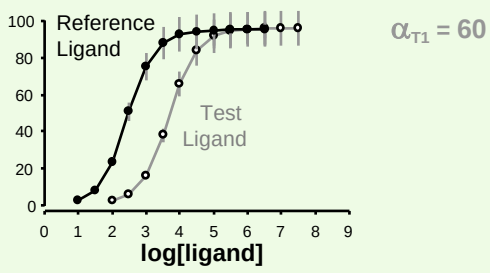
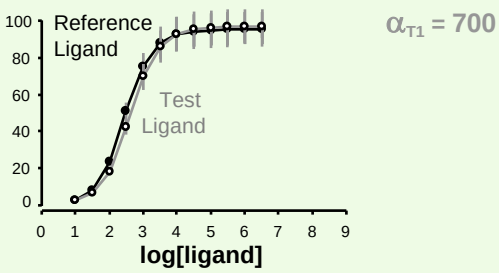
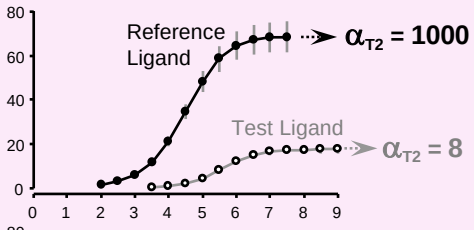
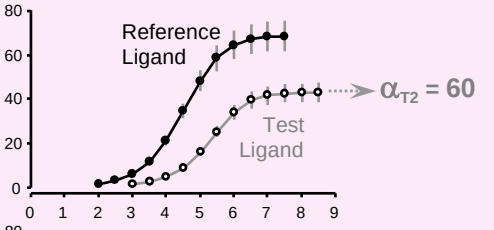
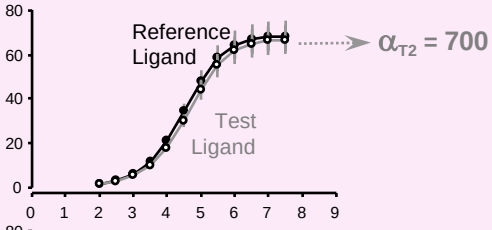
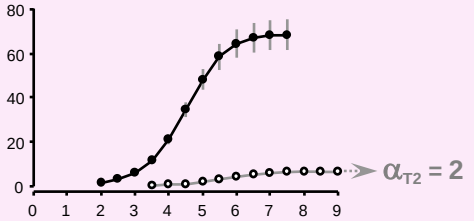
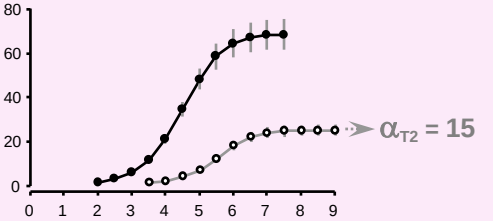
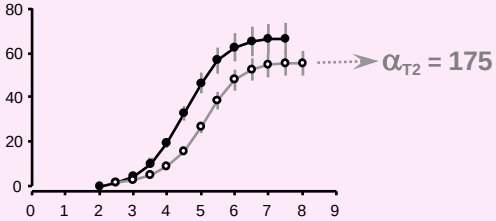
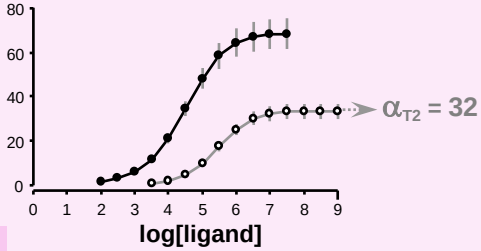
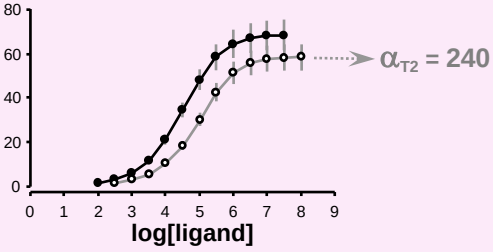
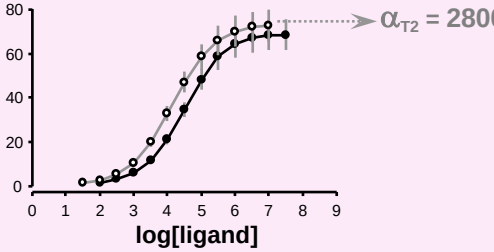


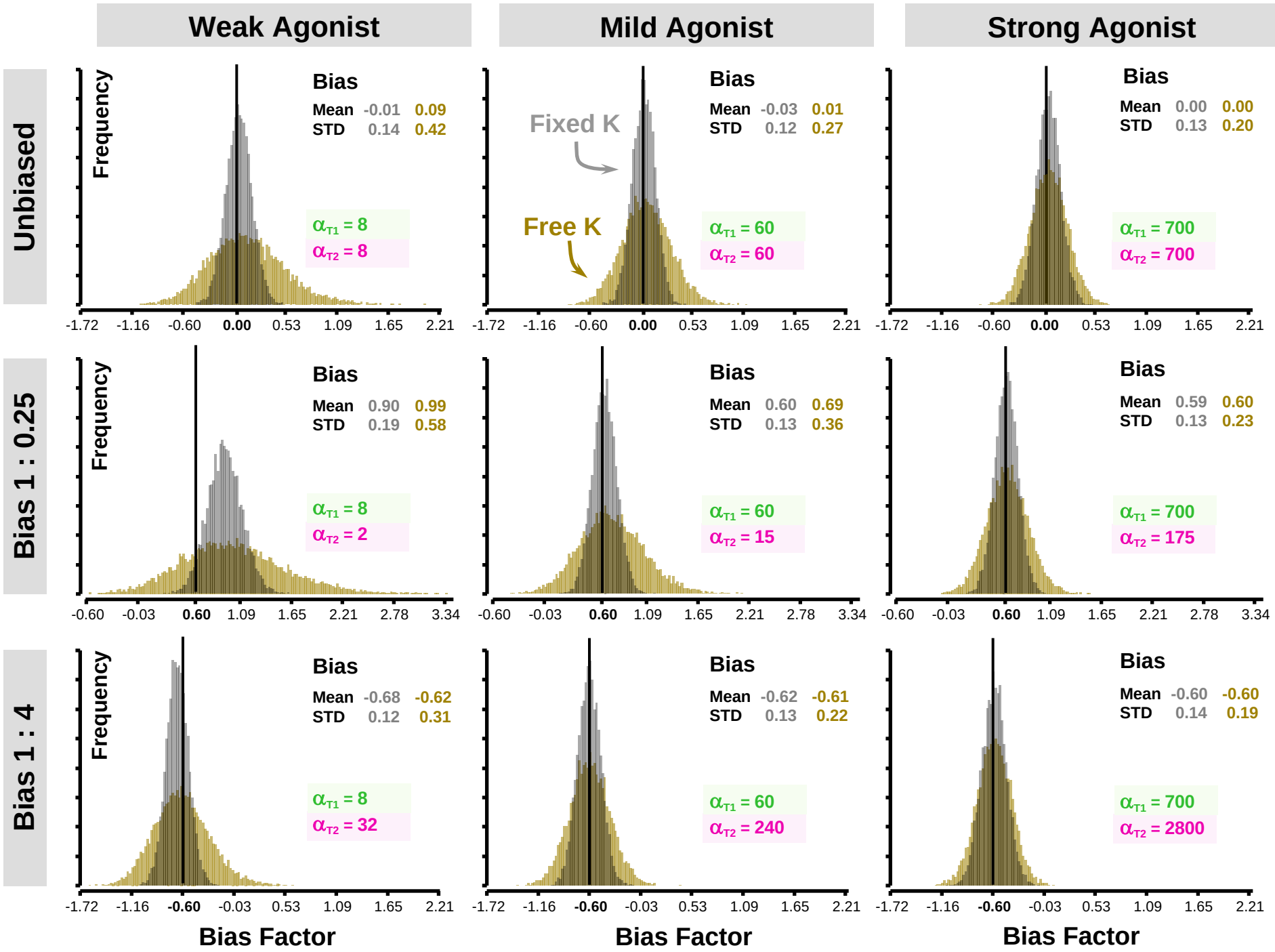
Simulation of an experimental case:

Two transducers / two pathway / biased and unbiased ligands



Test ligands are :

	Weak Agonists	Mild Agonists	Strong Agonists
Response 1			
Unbiased			
Bias 1 : 0.25			
Bias 1 : 4			
Response 2			


Weak Agonist
Mild Agonist
Strong Agonist
Unbiased
Bias 1 : 0.25
Bias 1 : 4
Frequency
Bias
Mean -0.01 0.09
STD 0.14 0.42
α_{T1} = 8
α_{T2} = 8
Fixed K
Free K
Mean -0.03 0.01
STD 0.12 0.27
α_{T1} = 60
α_{T2} = 60
Mean 0.00 0.00
STD 0.13 0.20
α_{T1} = 700
α_{T2} = 700
Mean 0.90 0.99
STD 0.19 0.58
α_{T1} = 8
α_{T2} = 2
Mean 0.60 0.69
STD 0.13 0.36
α_{T1} = 60
α_{T2} = 15
Mean 0.59 0.60
STD 0.13 0.23
α_{T1} = 700
α_{T2} = 175
Mean -0.68 -0.62
STD 0.12 0.31
α_{T1} = 8
α_{T2} = 32
Mean -0.62 -0.61
STD 0.13 0.22
α_{T1} = 60
α_{T2} = 240
Mean -0.60 -0.60
STD 0.14 0.19
α_{T1} = 700
α_{T2} = 2800
-1.72 -1.16 -0.60 0.00 0.53 1.09 1.65 2.21
-0.60 -0.03 0.60 1.09 1.65 2.21 2.78 3.34
-1.72 -1.16 -0.60 -0.03 0.53 1.09 1.65 2.21
Bias Factor

Conclusion

1. Agonist Bias is measureable from CR curves.
2. All available methods are equivalent in that respect, if appropriate information is used in the estimation procedures.
3. If possible, use independently measured ***unconditional-K*** values to improve statistical efficiency of estimation.
4. Unconditional-K values can be best obtained from binding experiments performed in the presence guanine nucleotides/PTX treatement/KO/purified receptors systems etc.