# Allosteric Modulation

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Topics

- What is an allosteric modulator?

- How do allosteric modulators behave?
  - Build up theory from known properties
  - Use theory to predict & qualify behaviours (illustrated with real world examples)

- How can we characterise allosteric modulators?
  - To drive SAR
  - To understand mechanism
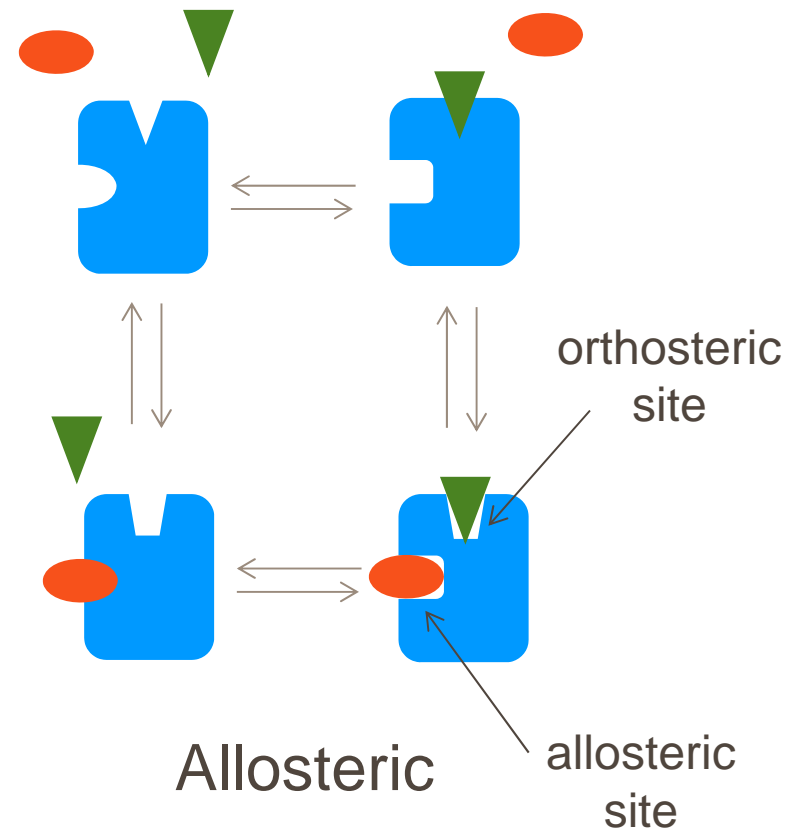 - For PK/PD modelling or 'dose prediction'

What is an allosteric modulator?

- A ligand which binds to a receptor at a site distinct from that of the endogenous agonist.
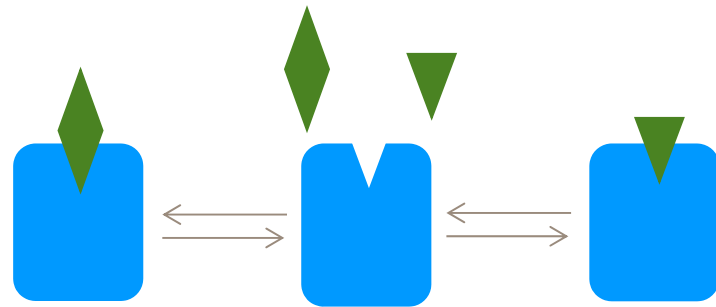
Competitive
≡ Orthosteric

Orthosteric binding is mutually exclusive.

An allosteric ligand can bind to the receptor at the same time as an orthosteric ligand.

orthosteric site

Allosteric

allosteric site

Immediate consequences of this mechanism

- The effects of an allosteric modulator are saturable – they have an upper limit.

- The effects of an allosteric modulator must be due to an effect on receptor conformation (to which the orthosteric ligand is sensitive).

- Presumably then the orthosteric ligand induces a conformational change in the receptor to which the allosteric ligand is sensitive.

- Allosterism can be formally described in terms of ligand effects on receptor conformation:
  - Positive cooperativity $\Rightarrow$ the ligands have highest affinity for a common (set of) conformation(s) of the receptor
  - Negative cooperativity $\Rightarrow$ the ligands have highest affinity for distinct a (set of) conformation(s) of the receptor

Dihydrofolate Reductase
Hydrogen-deuterium exchange during allosteric ligand binding

Trimethoprim (TMP) & NADPH positively cooperative

Folinic acid & NADPH negatively cooperative

Access of backbone amide protons to solvent

Decreased

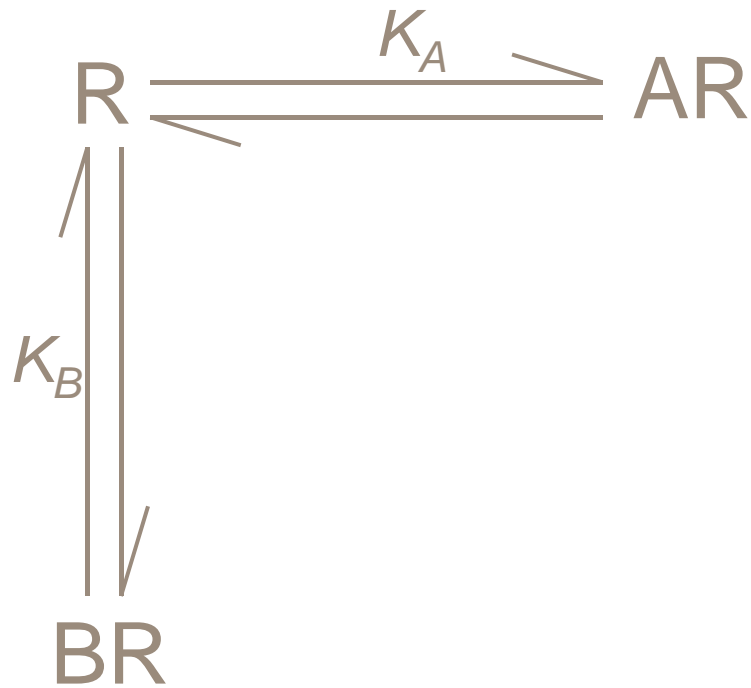Fig 4: Polshakov et al. (2006) J. Mol. Biol. 356, 886-903

Increased

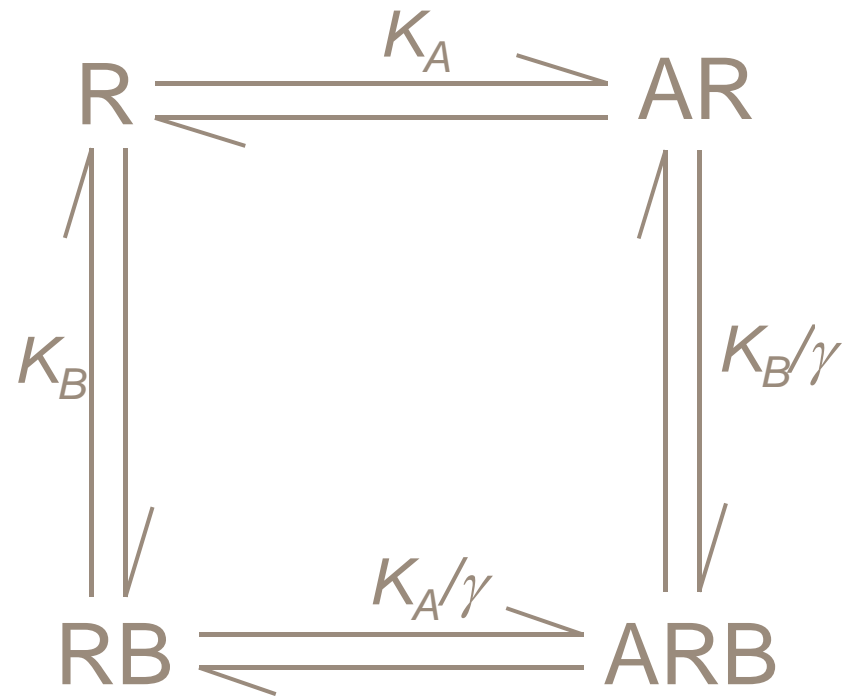^{15}N-^{1}H heteronuclear single quantum coherence spectroscopy

More formally, in terms of binding

Competitive

$$R \xrightleftharpoons{K_A} AR$$

K_B (R — BR)

BR

Allosteric

$$R \xrightleftharpoons{K_A} AR$$

K_B (R — RB)

K_B/γ (AR — ARB)

$$RB \xrightleftharpoons{K_A/\gamma} ARB$$

γ is the 'allosteric constant'.

K_A & K_B are dissociation constants, $\gamma > 1$ indicates positive cooperativity

# A further property of allosteric modulation

- Reciprocity – the orthosteric ligand has the same effect on the allosteric ligands affinity as the allosteric ligand has on the orthosteric ligand's affinity.

- This is quantified by the allosteric constant.

- Reciprocity is a thermodynamic requirement of the system at equilibrium (otherwise allosteric binding would provide a route to a perpetual motion machine).
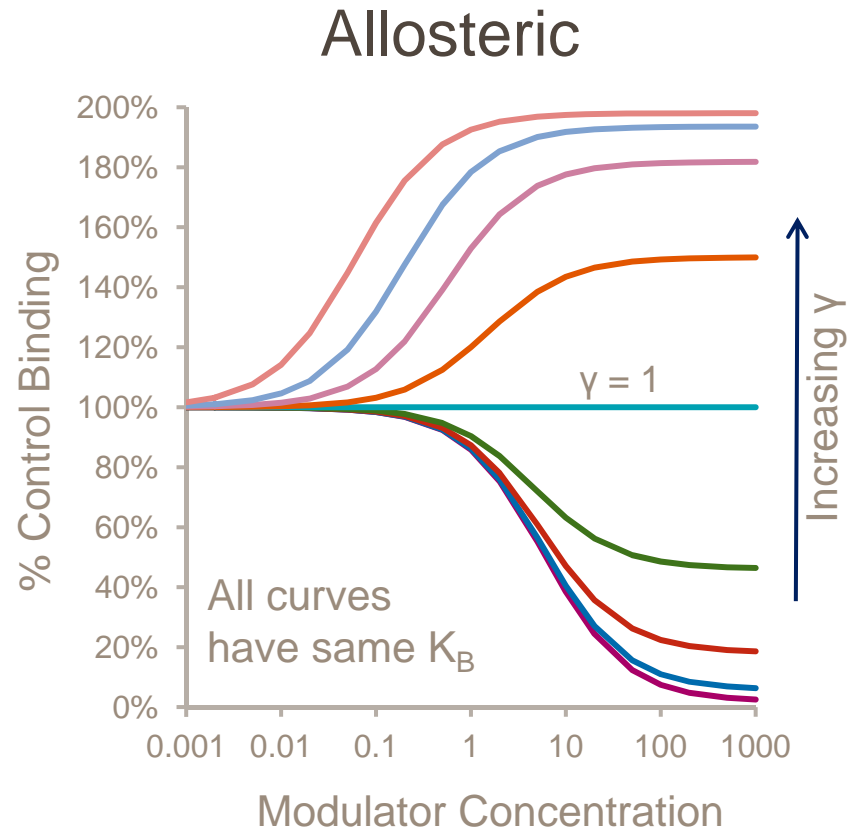
So what does allosterism look like: effect on binding

In both cases, [radioligand] = K_A

Competitive
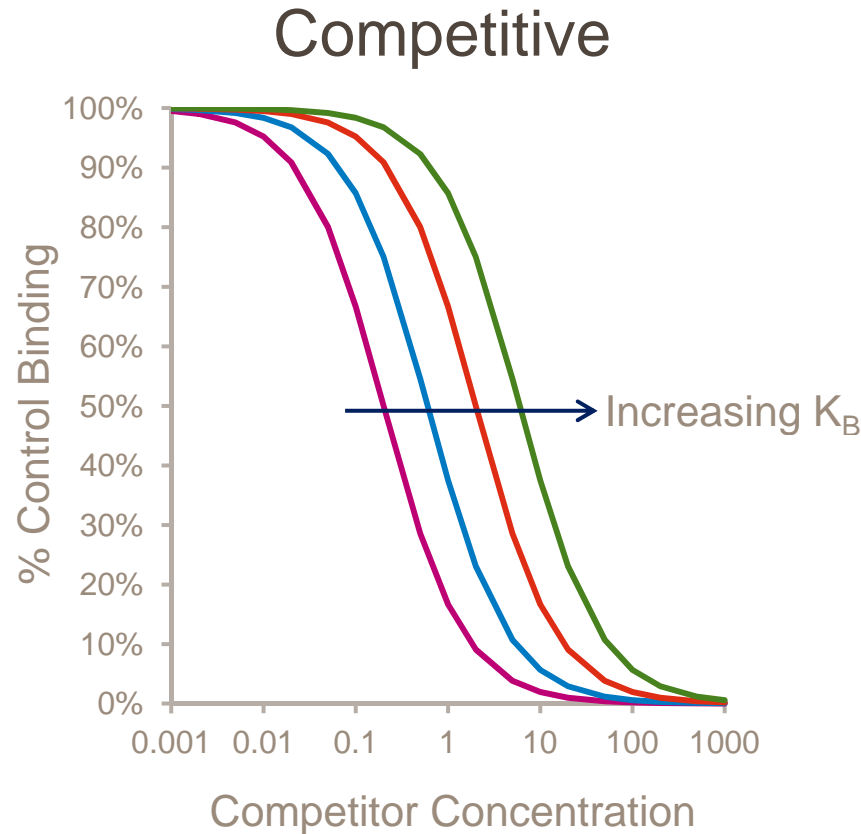
Analysed by Cheng-Prusoff equation

Allosteric

DO NOT use Cheng-Prusoff equation!

The effects of an allosteric modulator on binding are described by 2 parameters

Fig 3: Proška & Tuček (1995) Mol. Pharm. 48, 696-702

Probe Dependence

- The allosteric constant characterises the interaction of a pair of ligands – the same allosteric ligand can modulate different orthosteric ligands to different extents:

Ligand	Cooperativity with:	
	$[^3H]NMS$	ACh
Strychnine (M_2)	2.2 ± 0.3	0.15 ± 0.02
Brucine (M_2)	1.6 ± 0.06	0.28 ± 0.05
Brucine (M_1)	0.9 ± 0.04	1.6 ± 0.1

Lazareno et al (1998) Mol. Pharm. 53, 573 – 589.
Lazareno & Birdall (1995) Mol. Pharm. 48, 362 - 378

The properties of allosteric modulation

- Saturability – the effect of an allosteric modulator is inherently limited.

- Reciprocity – the orthosteric ligand affects the modulator's properties to the same extent as the modulator affects those of the orthosteric ligand.

- Probe dependence – the cooperativity constants describe the interaction between *pairs* of ligands – screen with the endogenous agonist, where ever possible!

An advantage of positive allosteric modulation

Maintains the temporal characteristics of signalling

- An agonist activates receptors continually when present and may well induce desensitisation.

- A positive allosteric modulator only activates receptors when the endogenous agonist is present.

- Particularly advantageous for neurotransmitter receptors

Normal

Agonist

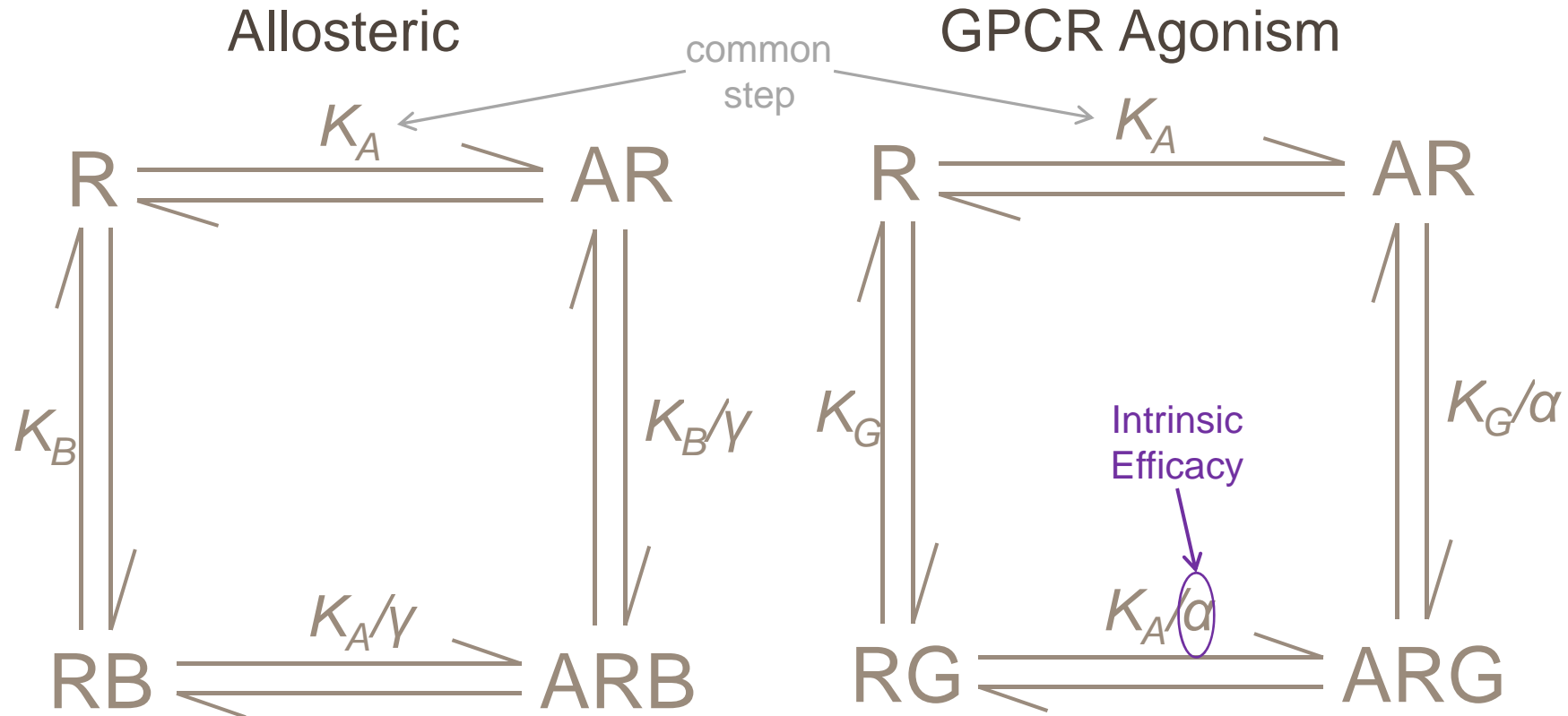+ Positive modulator
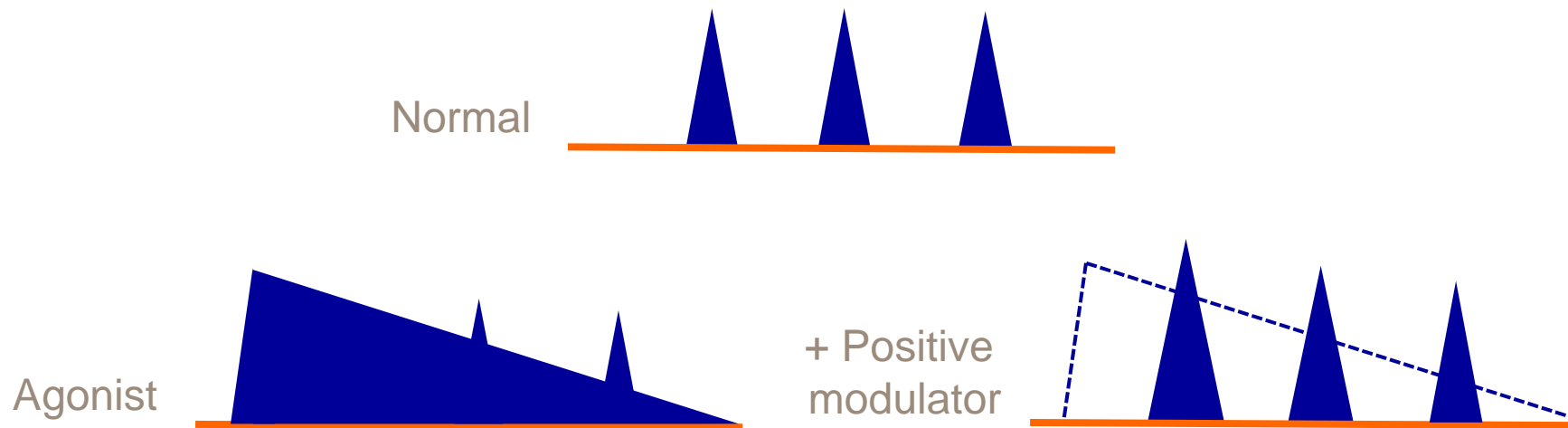
Further advantages & disadvantages

- Allosteric sites may be less well conserved between receptor subtypes than the orthosteric site (which has evolved to bind to the same ligand) giving the potential for greater selectivity.

- The potential range of effects of allosteric modulators is more varied than that of orthosteric ligands.

- Demonstrating that a non-competitive ligand is actually binding to your target receptor requires more effort than for competing ligands.

  - By definition a competing ligand binds to the same binding site on the receptor as the endogenous agonist.

  - An allosteric ligand binds anywhere but the orthosteric site and may not displace an orthosteric radioligand
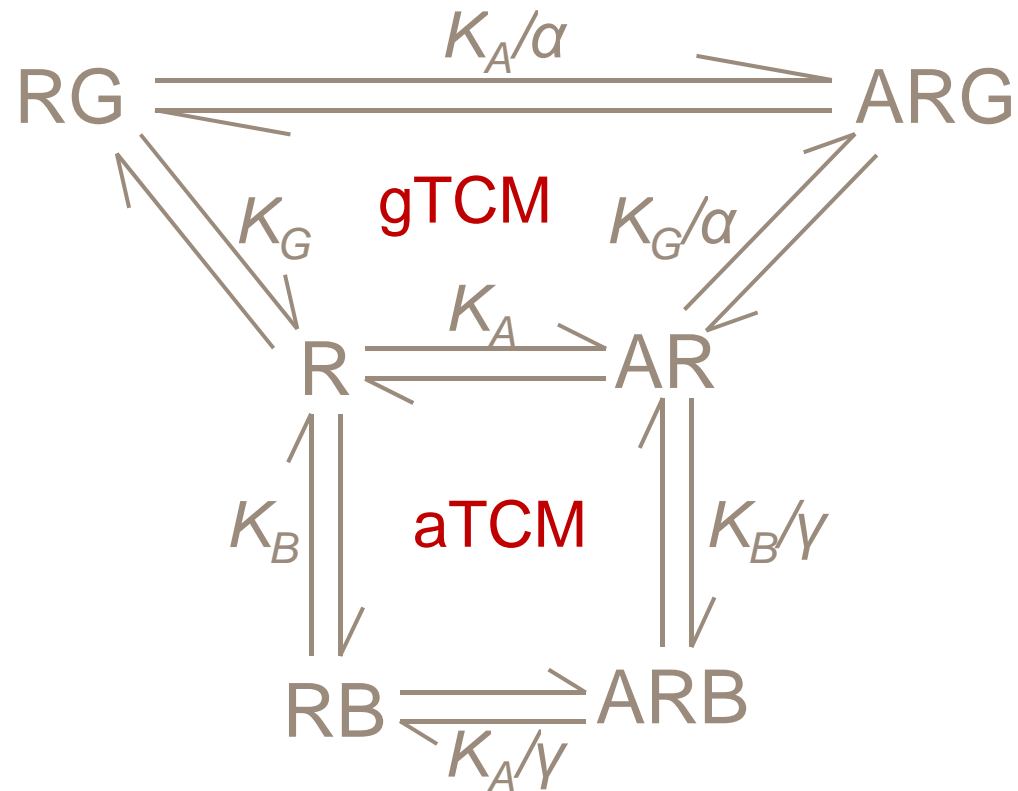
Analogy with Agonism

The two ternary complex models...

Allosteric

$$R \xrightleftharpoons[]{K_A} AR$$

common step

GPCR Agonism

$$R \xrightleftharpoons[]{K_A} AR$$

K_B K_B/γ

$$RB \xrightleftharpoons[]{K_A/\gamma} ARB$$

K_G Intrinsic Efficacy K_G/α

$$RG \xrightleftharpoons[]{K_A/\alpha} ARG$$

- Ligand intrinsic efficacy in the GPCR TCM is an allosteric constant
- Biased agonism is essentially a manifestation of the probe dependence of allosteric modulation

RG $\xrightarrow{K_A/\alpha}$ ARG

gTCM

K_G $\quad$ K_G/α

R $\xrightarrow{K_A}$ AR

K_B $\quad$ aTCM $\quad$ K_B/γ

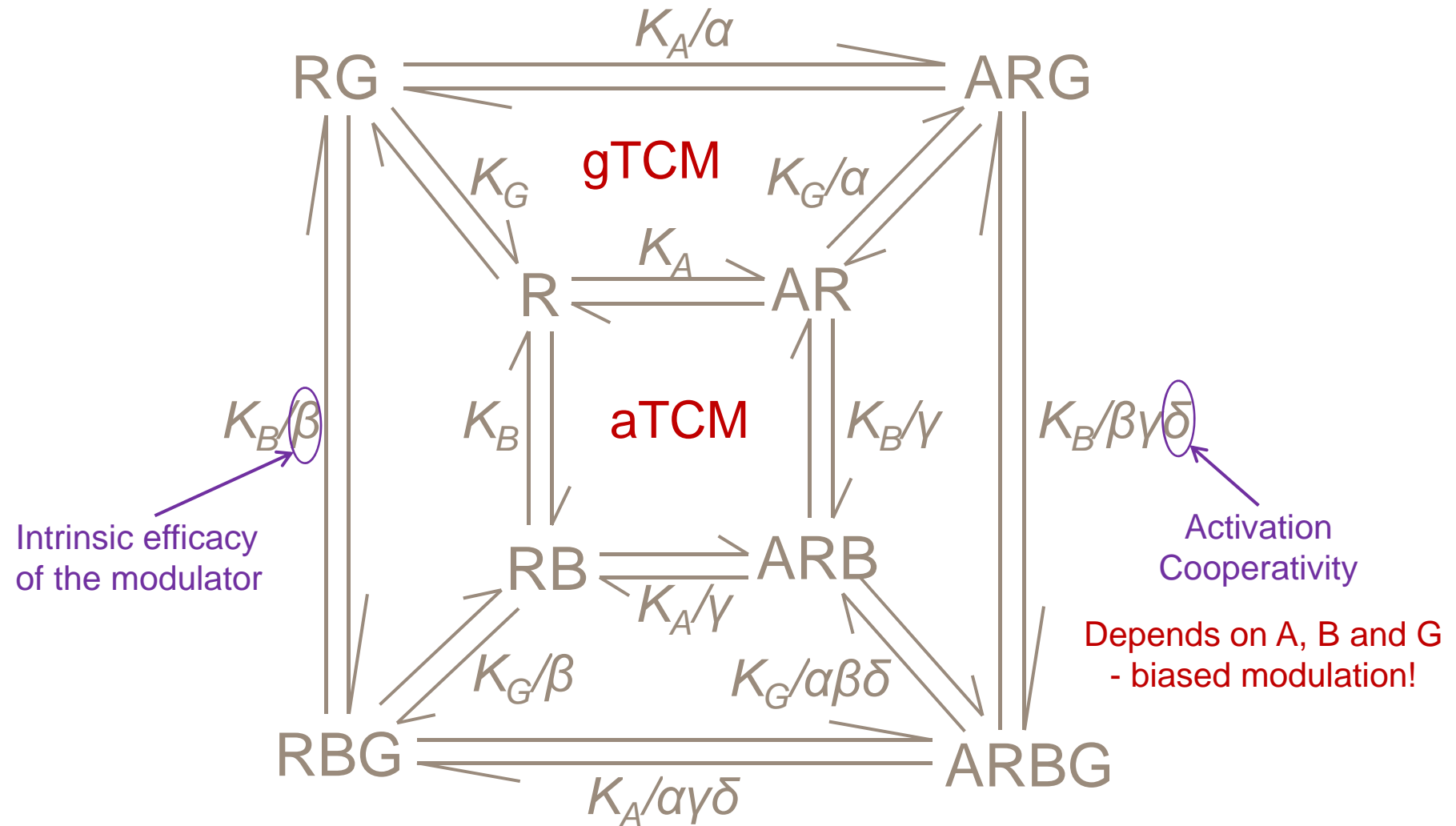RB $\xrightarrow{K_A/\gamma}$ ARB

Completing the reaction scheme results in a model of allosterism in functional assays ...

Functional effects of allosteric modulators

Exemplifies the complexity of the system

Intrinsic efficacy of the modulator

Activation Cooperativity

Depends on A, B and G - biased modulation!

Hall, 2006, In: Bowery NG (ed). Allosteric Receptor Modulation in Drug Targeting. Taylor & Francis: New York, pp 39–78.

Characterising a modulator requires 4 parameters!

- The affinity (K_B) and (intrinsic) efficacy (β) of the allosteric modulator

    - i.e., characterise the modulator as a ligand in its own right

- The binding (γ) and activation (δ) cooperativity

    - The characteristics of the allosteric interaction

- Each signalling pathway needs to be characterised separately!

- If there is more than one endogenous agonist, the cooperativity constants need to be measured for each one!

- However, this means it is (theoretically) possible to design an allosteric ligand that selectively affects only the biological process that you want to and has no effect on any other response via that receptor.

    - This is impossible for an orthosteric ligand

Example of biased positive allosteric modulator

GLP-1 receptor biased allosteric agonists

cAMP
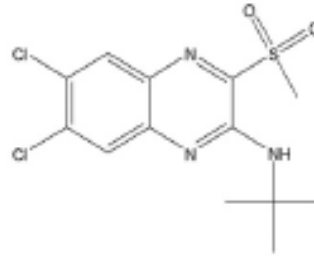
ERK Phosph

Fig 3D: Koole et al (2010) Mol. Pharm. 78, 456-465.

Fig 5E: Koole et al (2010) Mol. Pharm. 78, 456-465.

Potentiates cAMP production but has no effect of on ERK phosphorylation

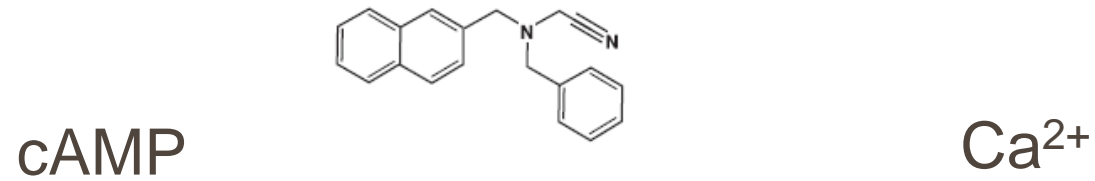# Example of a biased negative allosteric modulator.

LPI805 at NK2 receptors

cAMP   Ca^{2+}

Fig4C: Maillet et al (2007) FASEB J. 21, 2124-2134.   Fig4A: Maillet et al (2007) FASEB J. 21, 2124-2134.

Negatively modulates cAMP production but (very weakly) positively modulates Ca^{2+}

The properties of allosteric modulation

- Saturability – the effect of an allosteric modulator is inherently limited.

- Reciprocity – the orthosteric ligand affects the modulator's properties to the same extent as the modulator affects those of the orthosteric ligand.

- Probe dependence – the cooperativity constants describe the interaction between *pairs* of ligands – screen with the endogenous agonist, where ever possible!

- Transducer dependence – allosteric effects may depend on the signaling pathway that you measure.

How can we quantify allosteric effects?

What can we actually measure or calculate?

First we need a theoretical model
The previous model is a little too mechanistic and specific

Pragmatic solution for experimental systems which lack constitutive activity – doesn't permit inverse agonism

Leach et al. (2007) Trends Pharmacol. Sci. 28, 382 – 389.
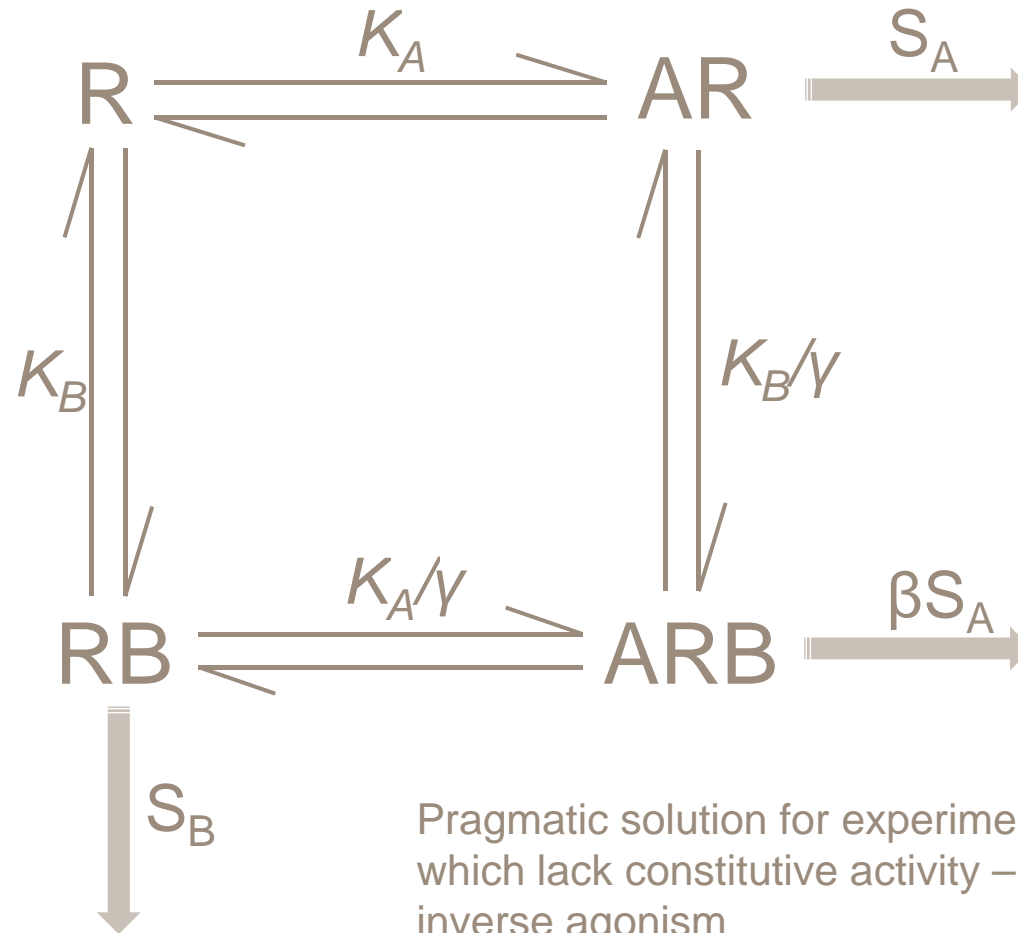
A more complete model

Includes constitutive activity and the possibility of inverse agonism

Hall (2013) Prog. Mol. Biol. Translational Sci. (Kenakin T, ed) 115, 217 – 290
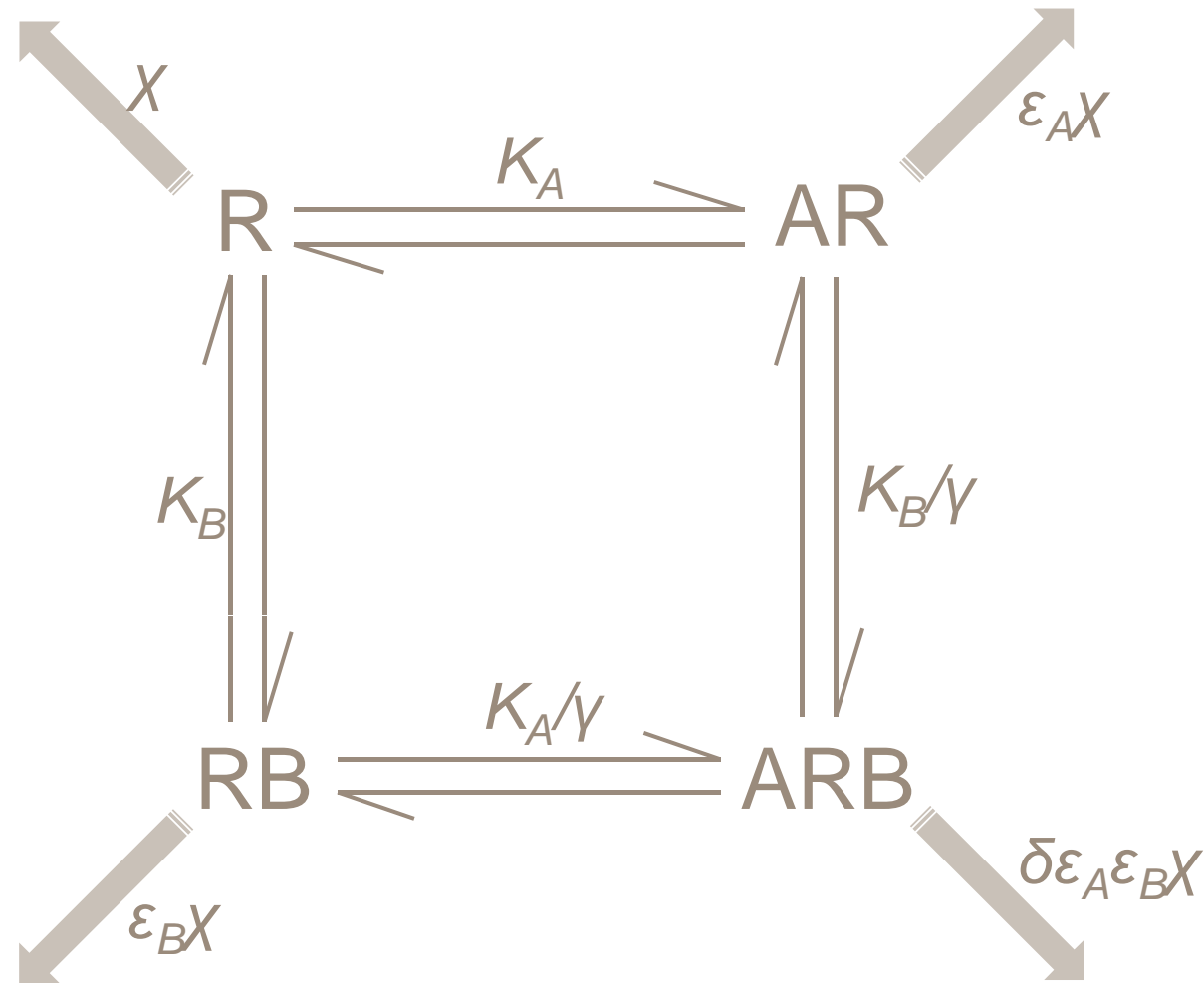
Behaviour of the model

Interaction of intrinsic efficacy: $\gamma = \delta = 1$, vary ε_B
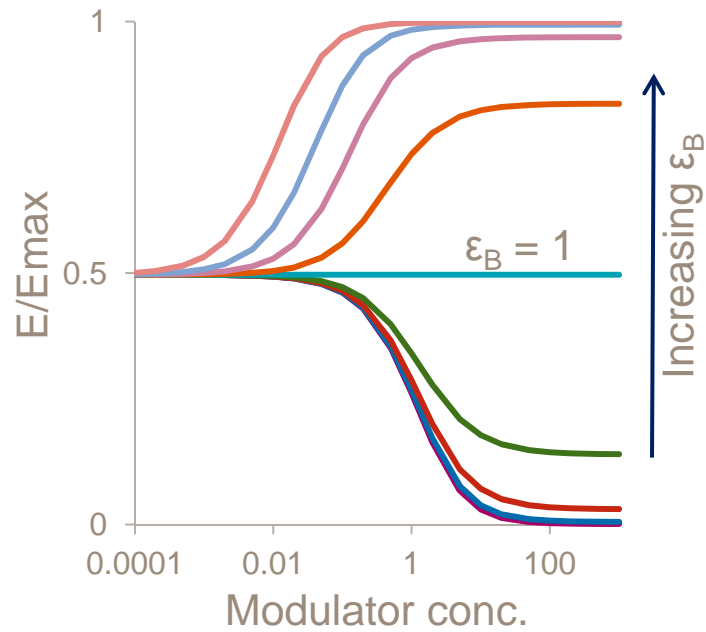
The Leach et al model doesn't account for this aspect of an allosteric interaction
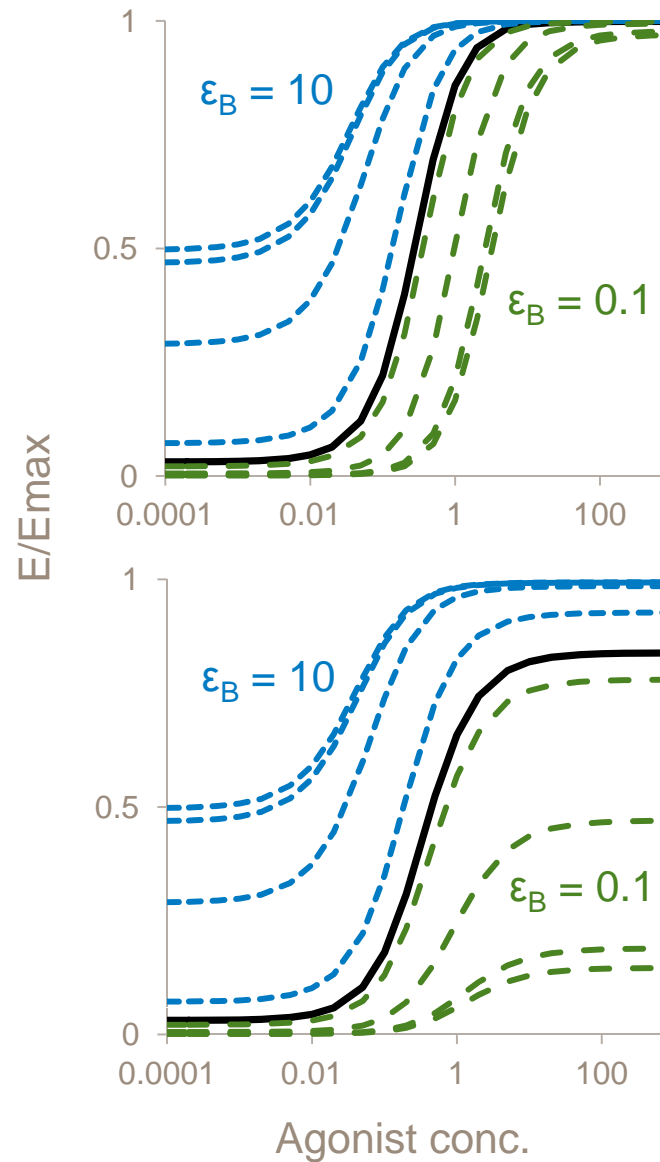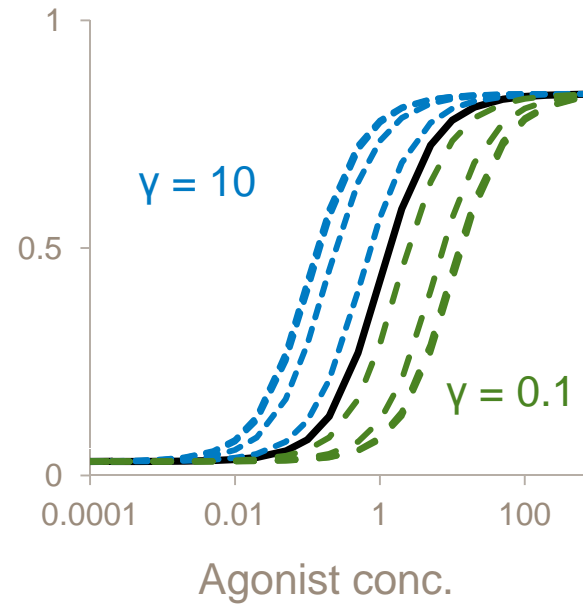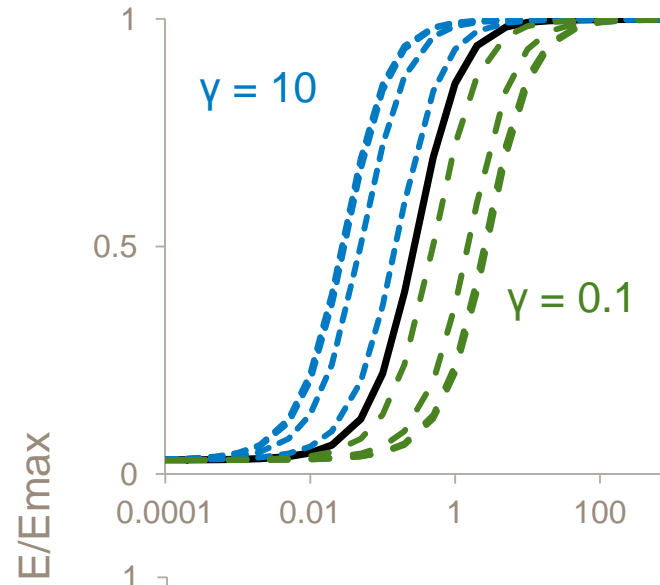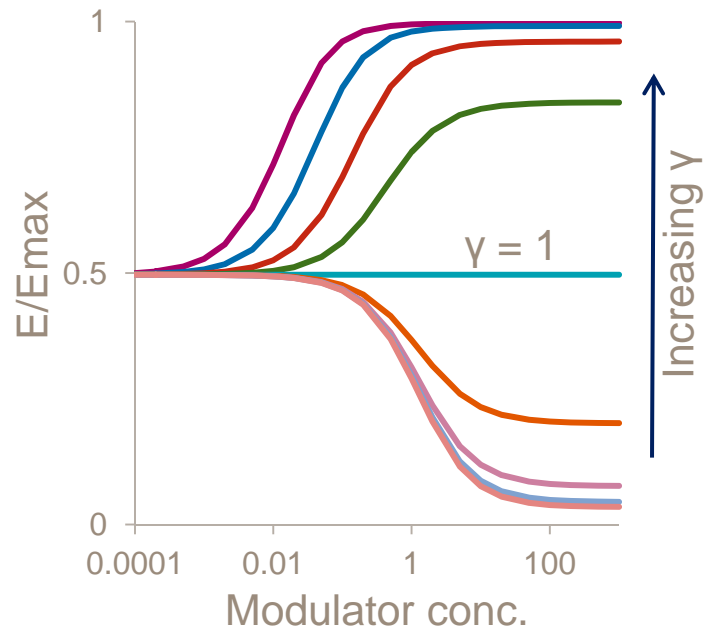
Real World Example?

Allosteric GLP1 agonist
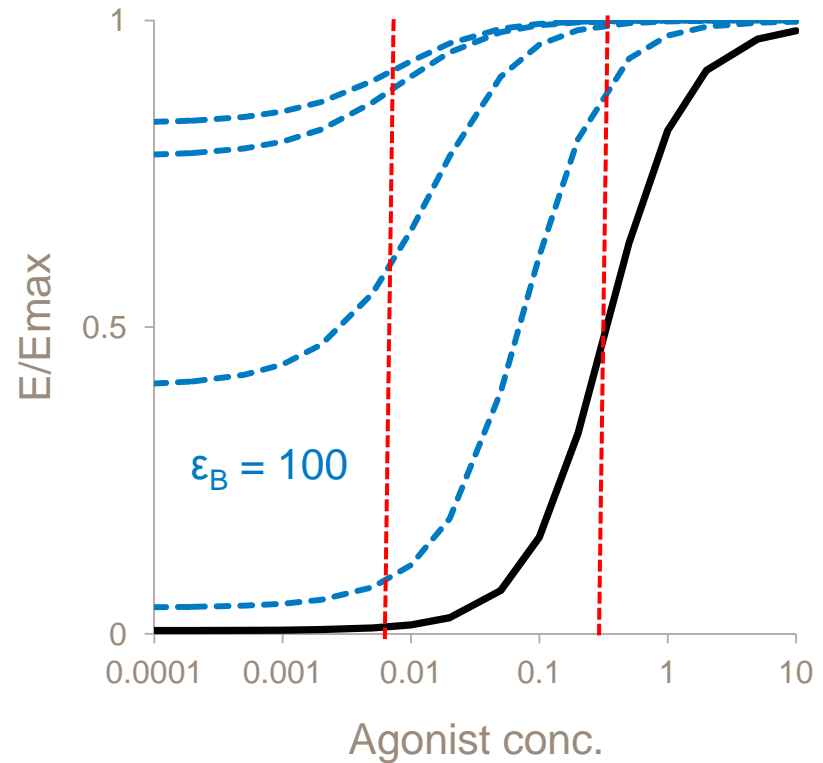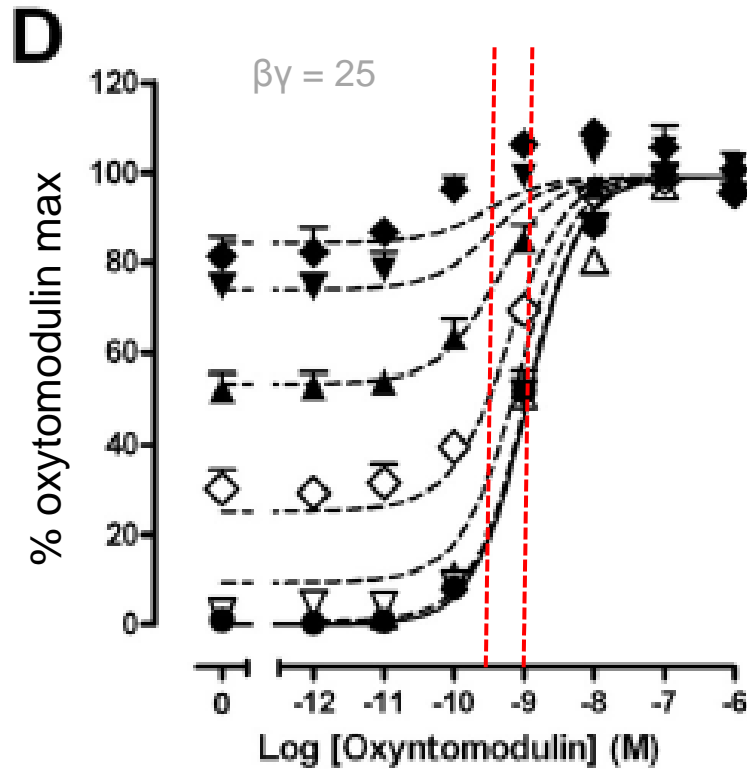
Note the GLP1 receptor data requires some negative cooperativity to cause the relatively small level of leftward shift seen in this case.

Koole et al (2010) Mol. Pharm. 78, 456-465.

Behaviour of the model

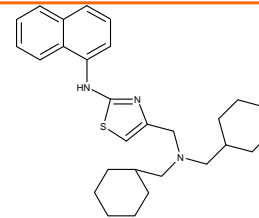Binding cooperativity: $\varepsilon_B = \delta = 1$, vary γ

Real World Examples?

DFB at mGluR5; CCR4 antagonist
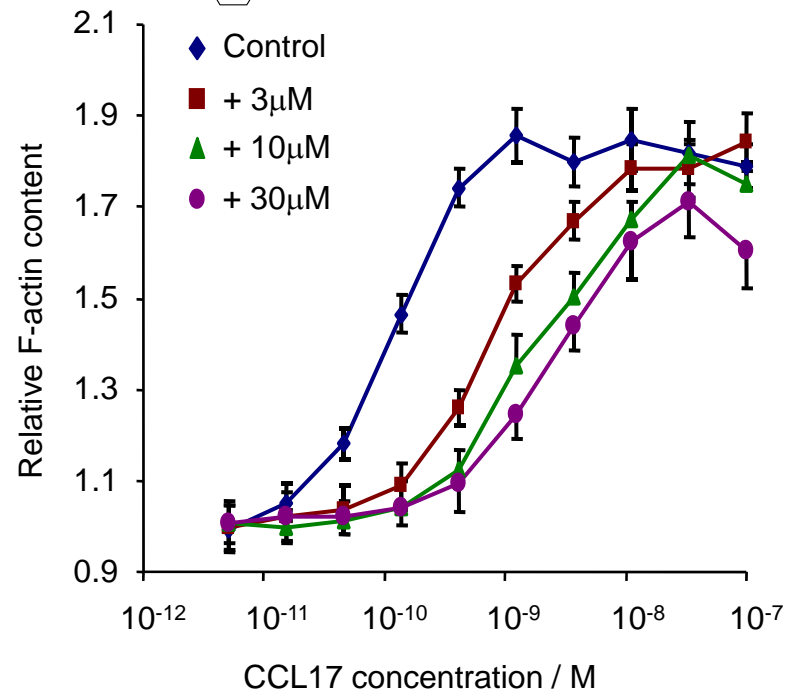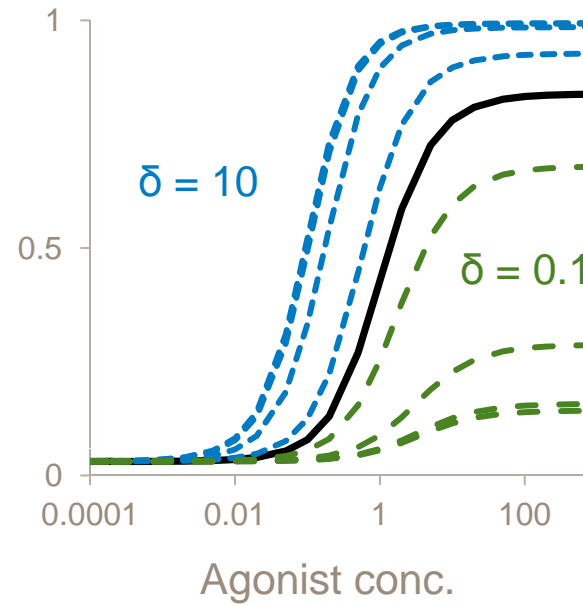
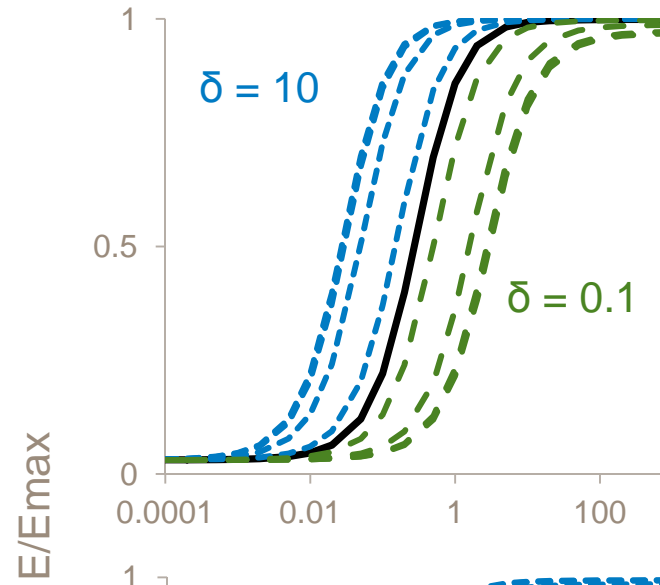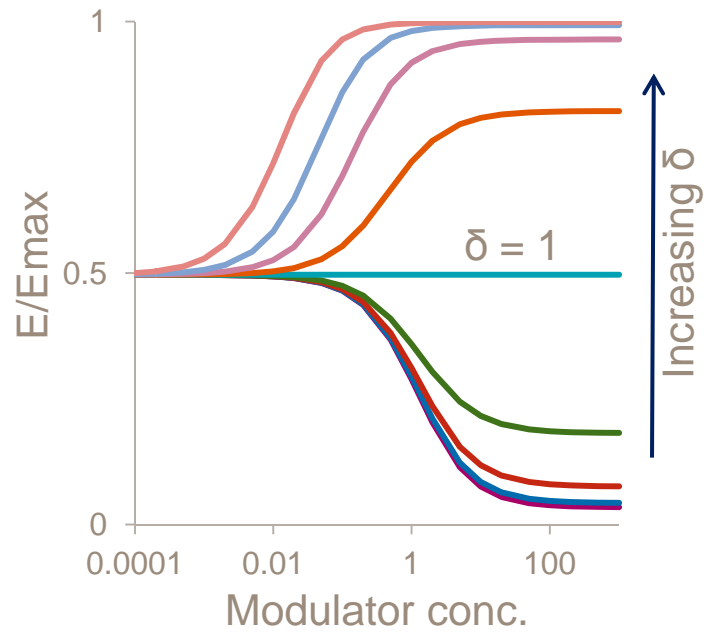Difluorobenzaldazine: PAM at mGluR5

NAM at CCR4

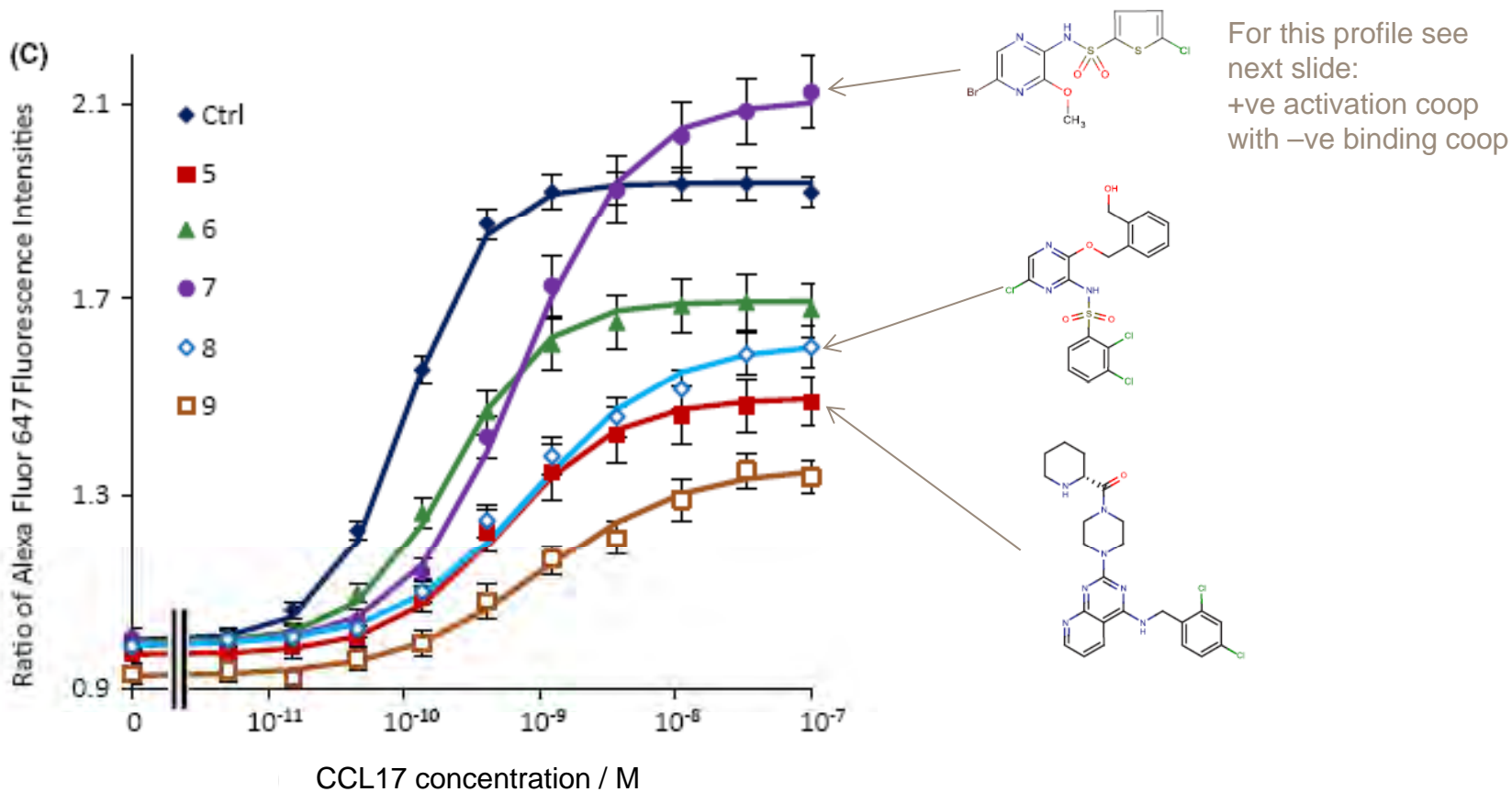Fig 4: O'Brien et al (2003) Mol. Pharm. 64, 731-740

Behaviour of the model

Activation cooperativity: $\varepsilon_B = \gamma = 1$, vary δ

Real World Examples?
CCR4 antagonists

We have seen little evidence of inverse agonism with these compounds in any system

Slack et al. (2013) Pharm. Res. Persp. 1, e00019 29

The product γδε_B

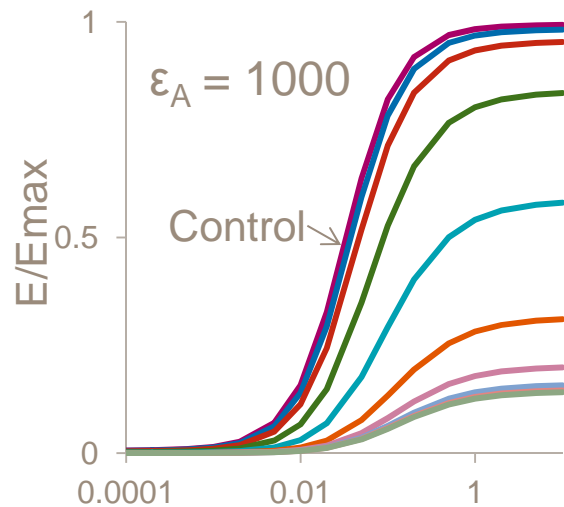(≈βγ in the Leach et al. model)

- Can be used to characterise the overall effect of an allosteric modulator
- But DOES NOT represent a unique profile of effect

$$\gamma\delta\varepsilon_B = 0.1$$

$\gamma = 10, \delta = 0.1, \varepsilon_B = 0.1$ $\gamma = 10, \delta = 0.0003, \varepsilon_B = 30$ $\gamma = 0.01, \delta = 10, \varepsilon_B = 1$

$\varepsilon_A = 1000$

Control

E/Emax

Control

$\varepsilon_A = 1000$

Agonist conc.

$\varepsilon_A = 30$

Control
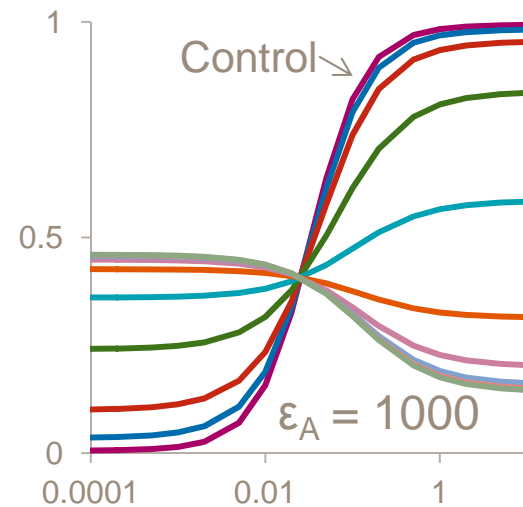
cf. cpd 7 on previous slide

The overall effect of a compound is the summation of its properties

30

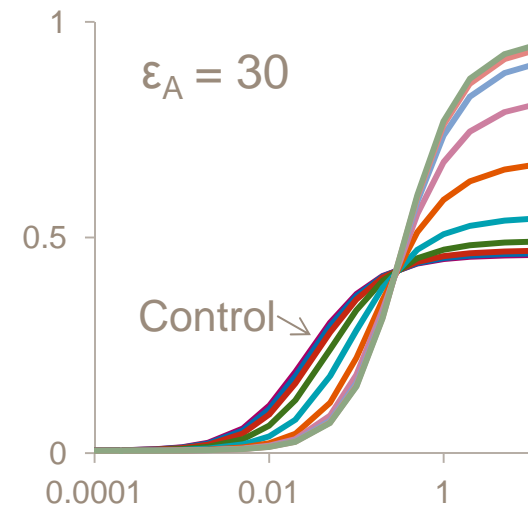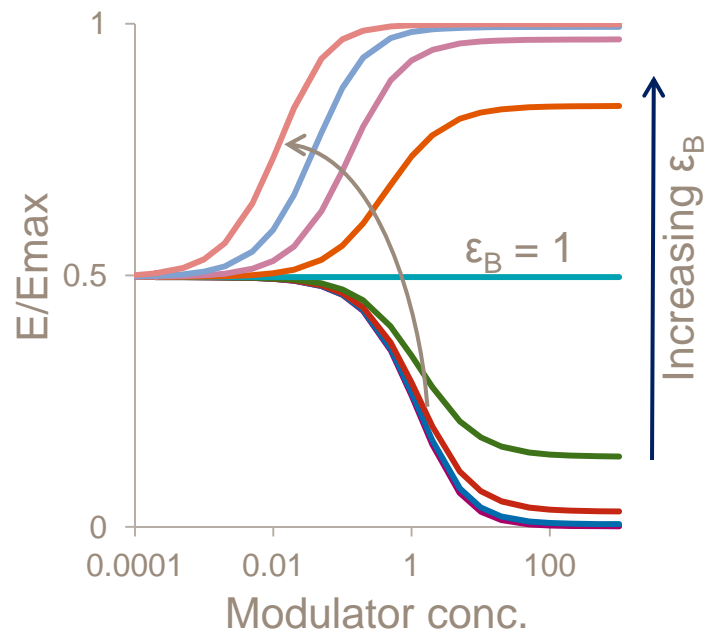$$XC_{50} = \dfrac{K_B\left(1 + \chi + \dfrac{[A]}{K_A}\left(1 + \varepsilon_A \chi\right)\right)}{1 + \varepsilon_B \chi + \dfrac{\gamma[A]}{K_A}\left(1 + \delta\varepsilon_A\varepsilon_B \chi\right)}$$

This is a complicated function of the affinity, intrinsic efficacy and the cooperativity constants.

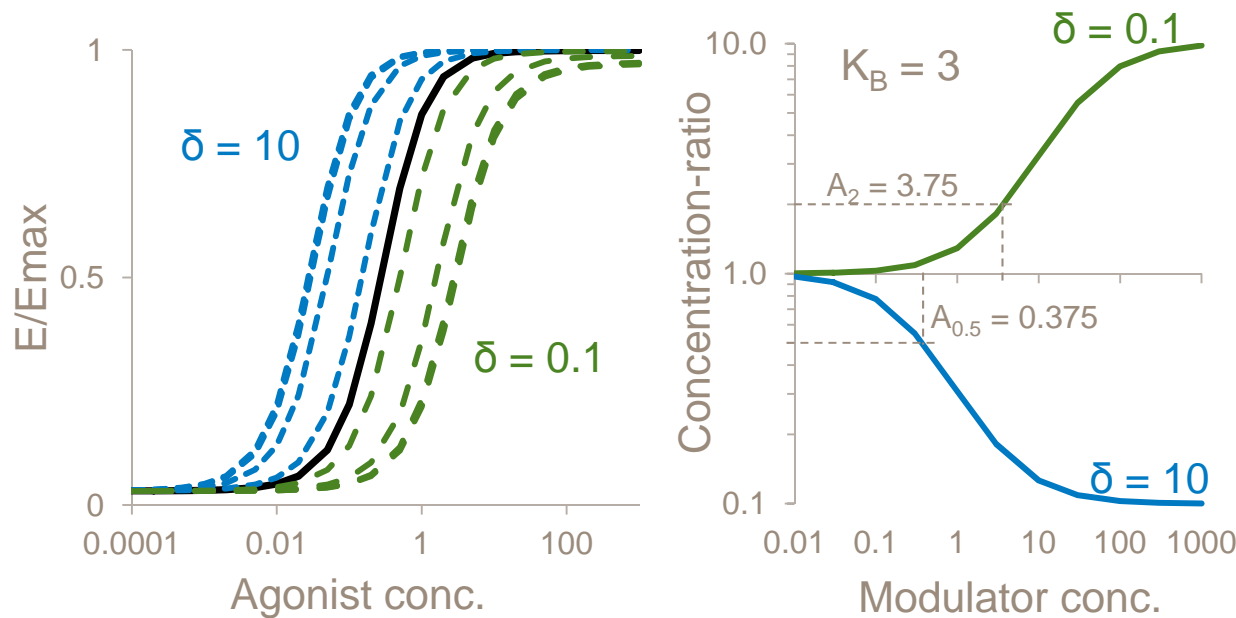Optimising potency DOES NOT optimise any specific property.

The maximal effect of a modulator is a similarly composite parameter.

NB: XC$_{50}$ does NOT translate between experimental systems – it CAN'T be used to predict effects in one system based on another

What can we measure? Use of concentration-ratios

When the curves are 'sufficiently parallel'

Assuming $\varepsilon_A \gg (1, \varepsilon_B)$,

$$DR_{max} \approx (\gamma \delta \varepsilon_B)^{-1}$$

$$K_B \approx A_2 \left(1 - 2DR_{max}^{-1}\right)$$

or, $K_B \approx A_{0.5}\left(DR_{max}^{-1} - 2\right)$

$$pA_2 \neq pK_B$$

Thus, under some generally reasonable (and testable) assumptions, a classical null analysis of the curve shifts can provide an estimate of affinity and the overall allosteric effect of a modulator.

This does rely on us being able to define a meaningful concentration-ratio, so the behaviour can't be too exotic (e.g. $\gamma = 10$, $\delta = 0.0003$, $\varepsilon_B = 30$).

Hall (2013) Prog. Mol. Biol. Translational Sci. (Kenakin T, ed) 115, 217 – 290

What can we measure? Model Fitting
Can we actually fit the Leach et al or Hall Models?

- Yes, but the experiments are very labour intensive.

- To fit the Leach et al model requires a 'complete' family of concentration-response curves at two different receptor densities.
  - There must be no evidence of constitutive activity in the system
  - The orthosteric agonist must become partial at one of the receptor densities

- To fit the Hall model requires a complete family of concentration-response curves at two different receptor densities in a system with constitutive activity.
  - Again, the orthosteric agonist must become partial at one of the receptor densities and the basal activity must change

An illustration – allosteric inverse agonist

Simulated data from Hall (2013)
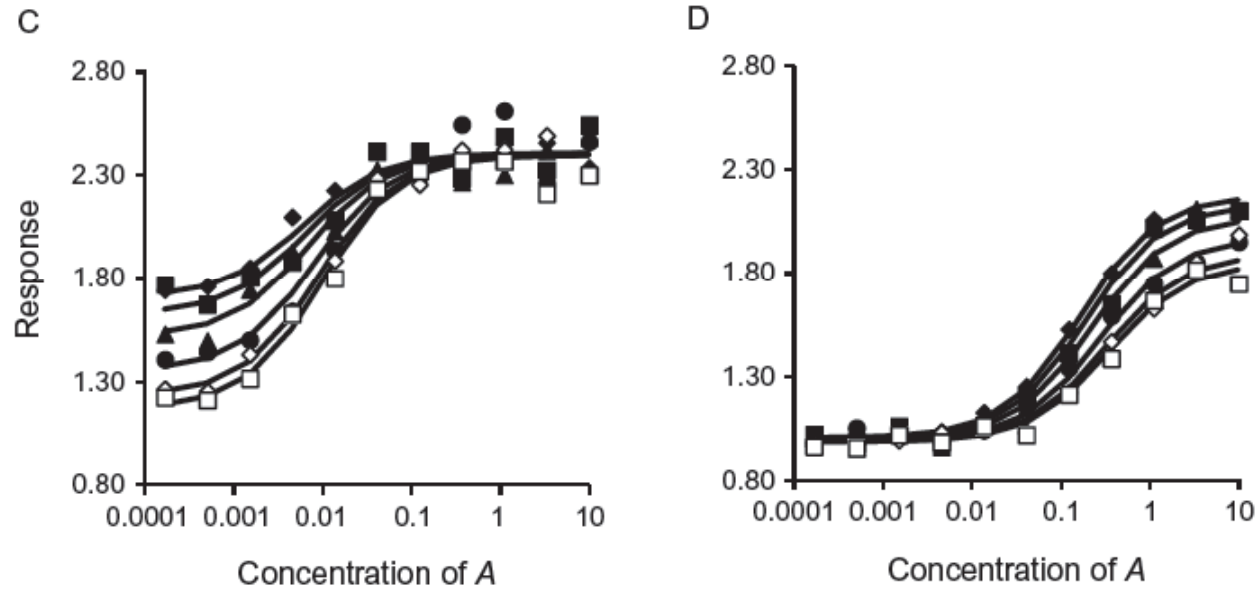
Allosteric inverse agonist, high coupling efficiency

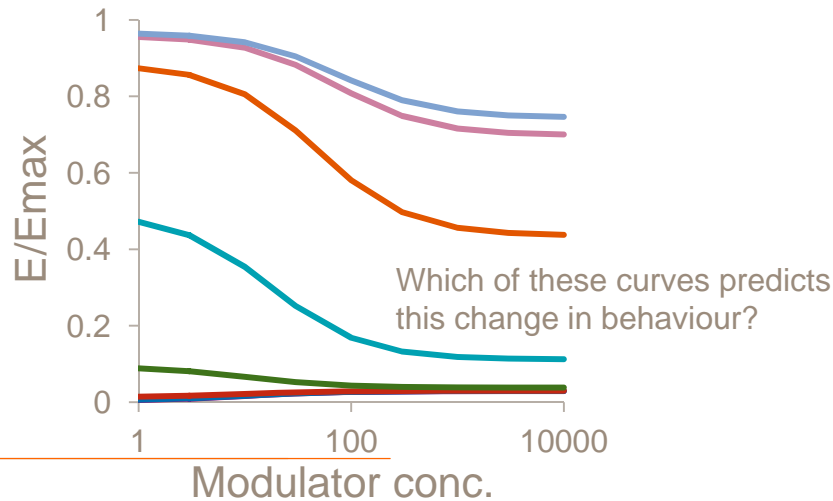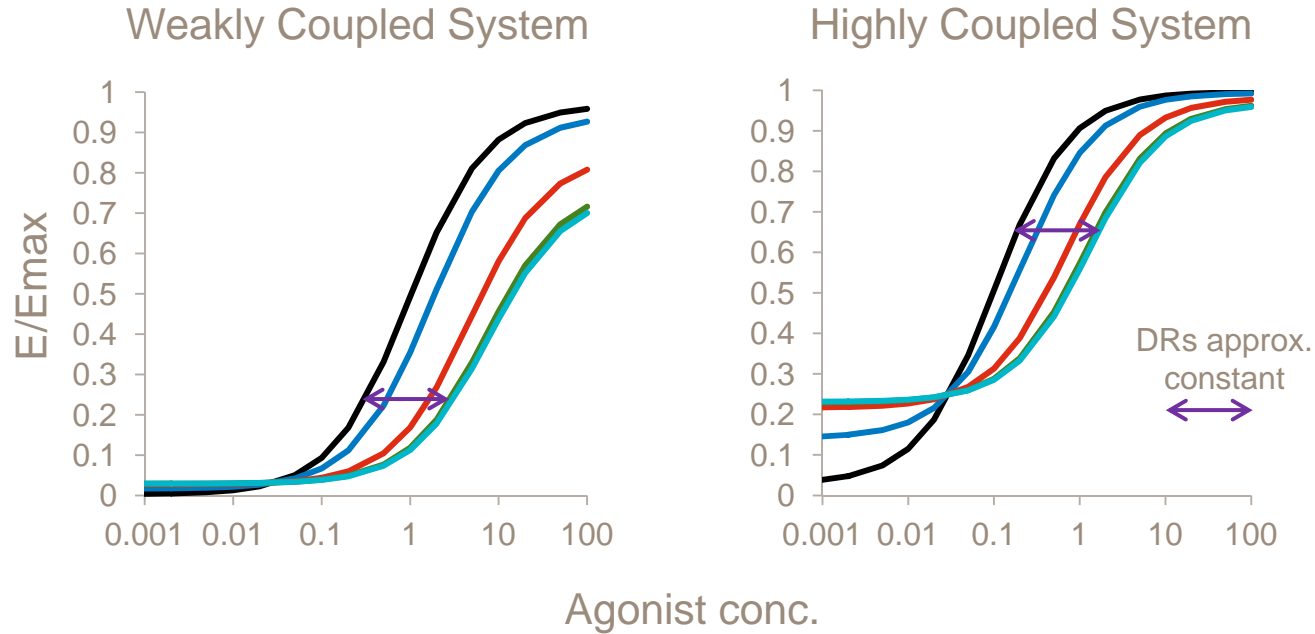| Parameter	Input	Estimate	
E_{max}	1.50	1.52 ± 0.13	
$\log K_a$	0.00	0.01 ± 0.09	
$\log K_b$	0.48	0.46 ± 0.06	
$\log \varepsilon_A$	2.48	2.50 ± 0.08	
$\log \varepsilon_B$	−1.00	-1.00 ± 0.06	
$\log \varepsilon_{AB}$	2.00	2.00 ± 0.06	$(\varepsilon_{AB} = \delta\varepsilon_A\varepsilon_B)$
$\log \alpha$	0.00	-0.02 ± 0.10	$(\alpha = 1/\gamma)$

Is this level of analysis really necessary?

- For screening work NO

  - Tracking XC_{50} and maximal effect is probably enough to drive routine SAR decisions

  - In many cases curve shifts can provide quantitative information on affinity and overall cooperativity and qualitative information on underlying mechanisms

  - Very strong negative cooperativity can be treated as competitive antagonism

- For dose prediction and PK/PD modelling work concentration-ratios or model fitting approaches are the only ways to provide system independent parameters which can be translated into complex physiological systems.

  - The more complex your therapeutic hypothesis is, the more likely you are to need to use the fitting approaches.

Translation between systems: negatively cooperative agonist

Weakly Coupled System

Highly Coupled System

DRs approx. constant

Agonist conc.

Which of these curves predicts this change in behaviour?

Modulator conc.

Kb	10
alpha	1
eA	10000
eB	10
delta	0.01

These allow me to specify what will happen

Summary

- Characteristics of allosteric modulation

  - Saturability – the effect of an allosteric modulator is inherently limited.

  - Reciprocity – the orthosteric ligand affects the modulator's properties to the same extent as the modulator affects those of the orthosteric ligand.

  - Probe dependence – the cooperativity constants describe the interaction between *pairs* of ligands – screen with the endogenous agonist, where ever possible!

  - Transducer dependence – allosteric effects may depend on the signaling pathway that you measure.

- The effects of allosteric modulators on binding do not necessarily translate directly into functional systems

- XC_{50} and maximal effect are of limited value in the characterisation of allosteric modulators

- At a minimum curve shift analysis (if not model fitting) is required to predict behaviour across experimental or physiological systems