

Allosteric GPCR pharmacology equations for GraphPad Prism

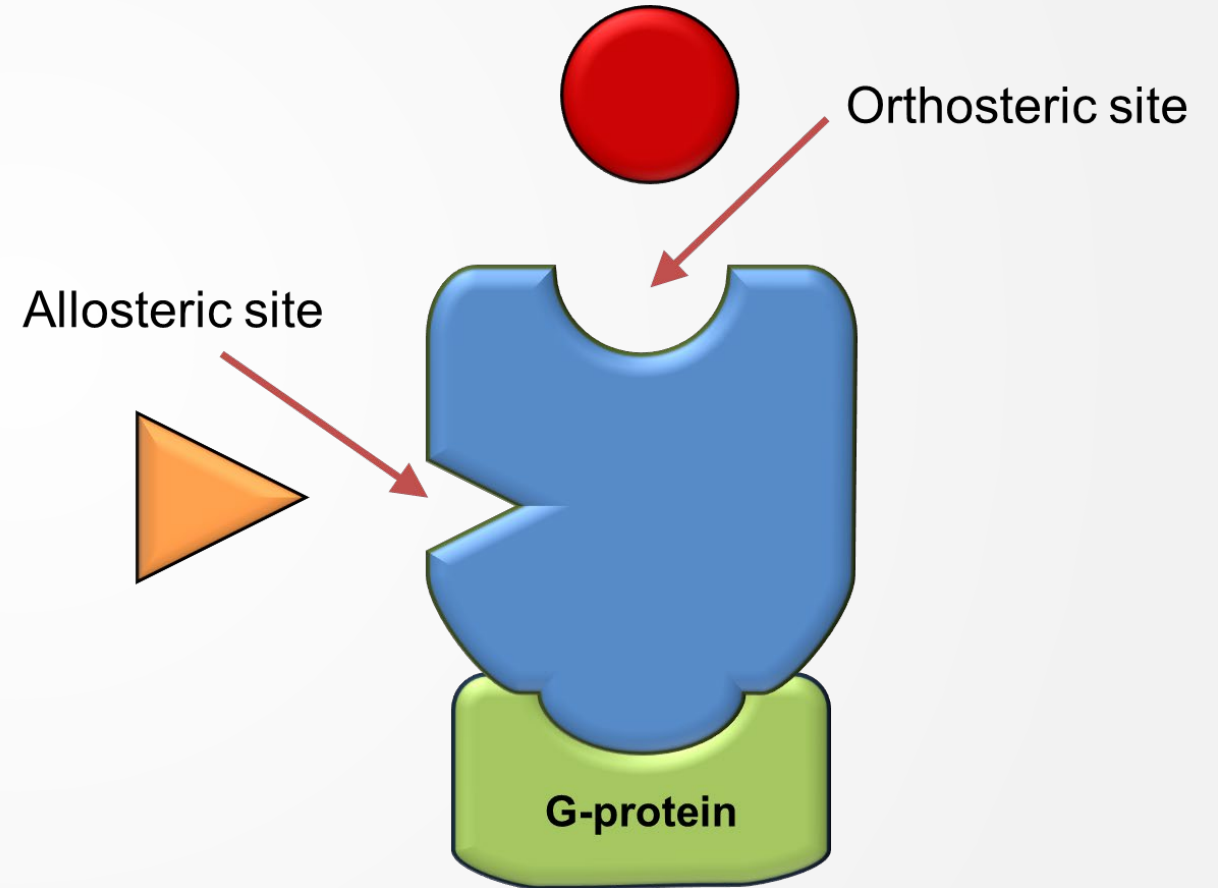
Samuel Hoare, Ph.D.

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June 17 2024

Summary

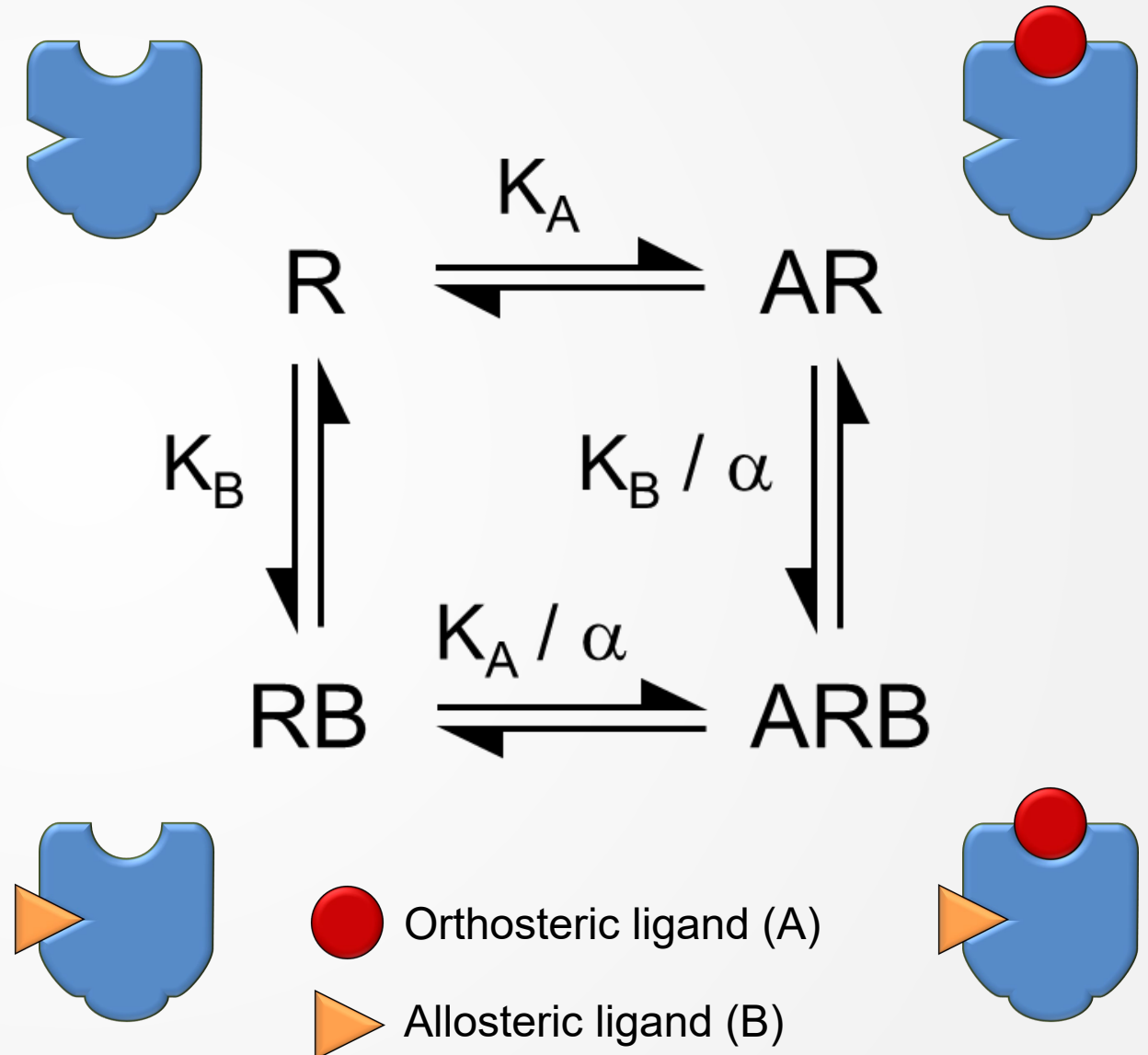
- Equations for quantifying allosteric modulator pharmacology for GPCRs have been derived by numerous investigators, for both binding and functional assays.
- These equations have been loaded into [GraphPad Prism](#) in custom templates designed by Pharmechnics.
- We are grateful to Harvey Motulsky, Trajen Head and the Support desk at GraphPad Software for guidance.



Allosteric pharmacology analysis for GPCRs

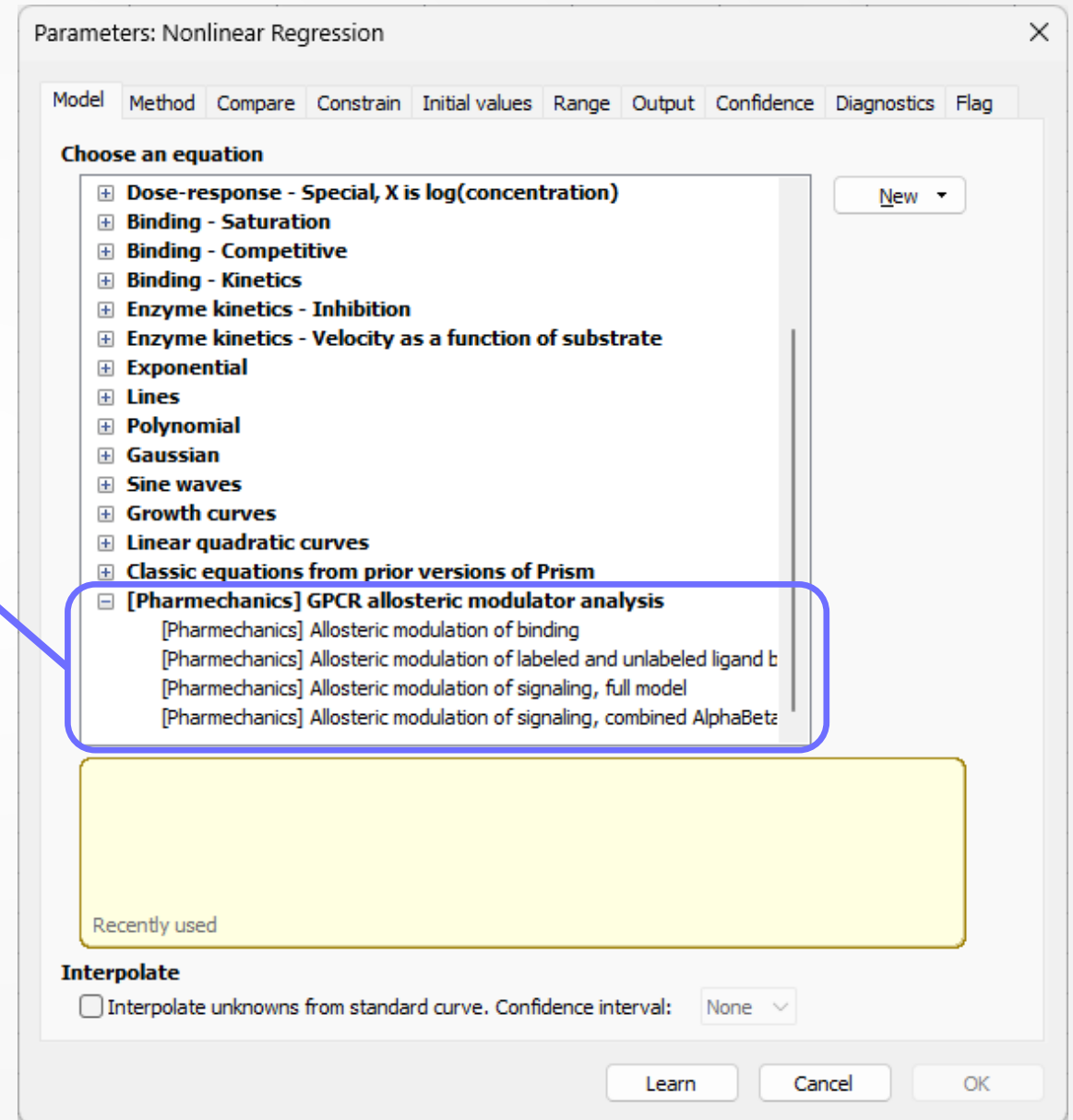
- Equations for analyzing binding and functional data for GPCR allosteric modulators are built on classical models of ligand-receptor interaction (e.g. see diagram on right).
- These equations quantify allosteric effects in terms of cooperativity (the effect of modulator binding on affinity and/or efficacy of the orthosteric ligand) and modulator affinity.

- [Stockton et al. Mol Pharm 1983, 23: 551-7](#)
- [Ehlert Mol Pharm 1988, 33: 187-94](#)
- [Lazareno & Birdsall Mol Pharm 1996, 48: 362-78](#)
- [Hall Mol Pharmacol 2000, 58: 1412-23](#)
- [Leach, Sexton & Christopoulos 2007, TIPS 28:382-9](#)
- [Kenakin Nat Rev Drug Discov 2005, 4: 919-27](#)
- [Ehlert JPET 2005, 315: 740-54](#)



Loading equations overview

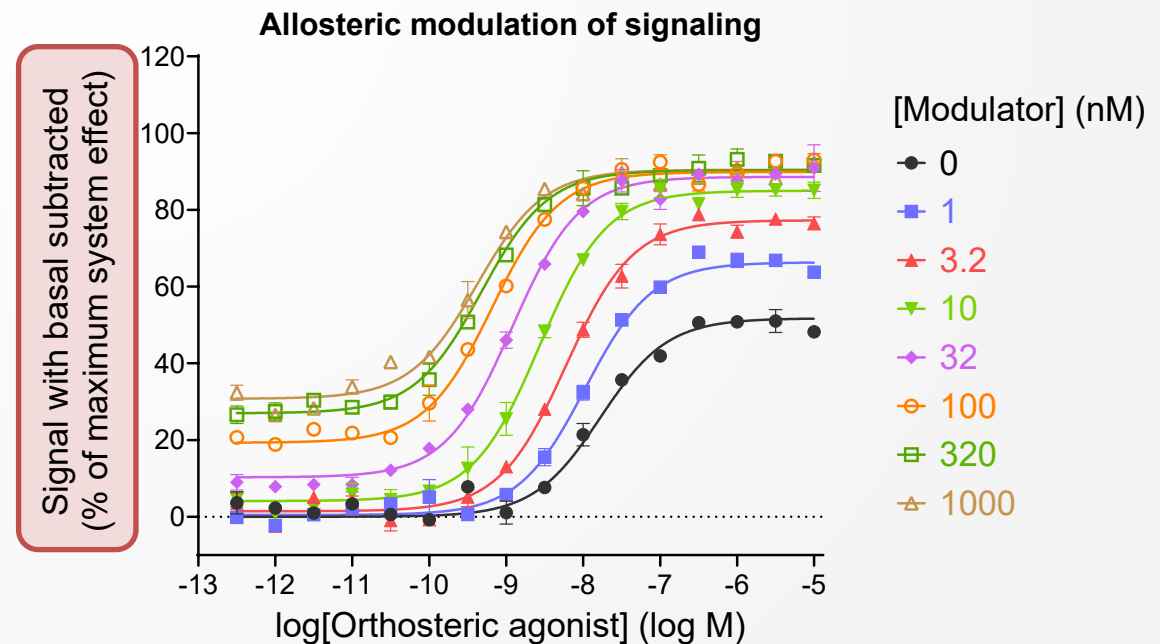
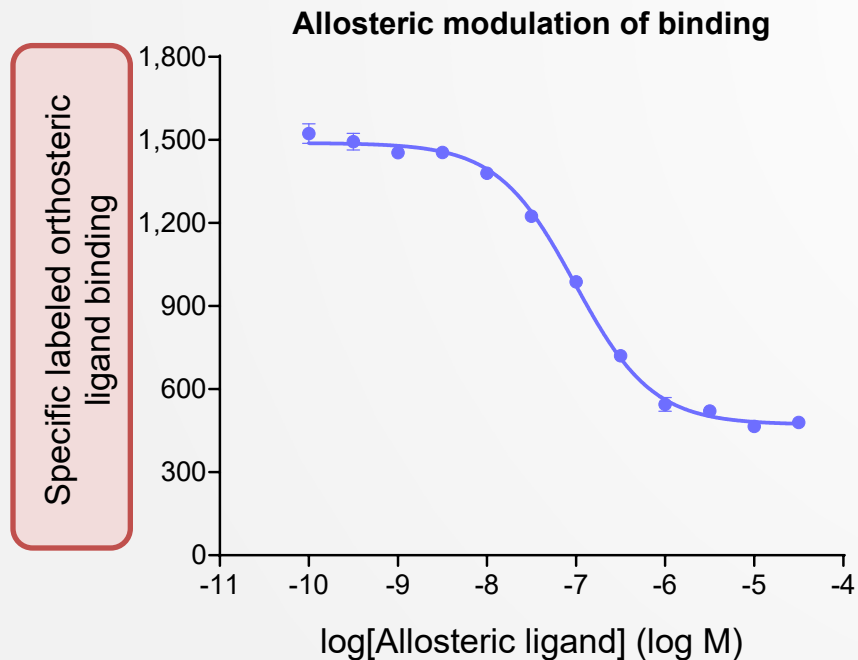
- Download the relevant Prism template from [here](#).
- The equations can be loaded in batch or individually into your equation library.
- See “Loading allosteric equations from a Prism file” document [here](#) for instructions.
- Questions? Email sam.hoare@pharmechanics.com
- The equations are supported by [Pharmechanics](#), not by GraphPad.



Equation descriptions and instructions

Baseline considerations

- For all the equations, baseline or basal signals are assumed to be zero.
 - Binding assays – baseline is nonspecific tracer ligand binding.
 - Functional assays – baseline is signaling response in the absence of orthosteric and allosteric ligand.
- In all cases the baseline will need to be subtracted from the data before the data are entered into Prism.

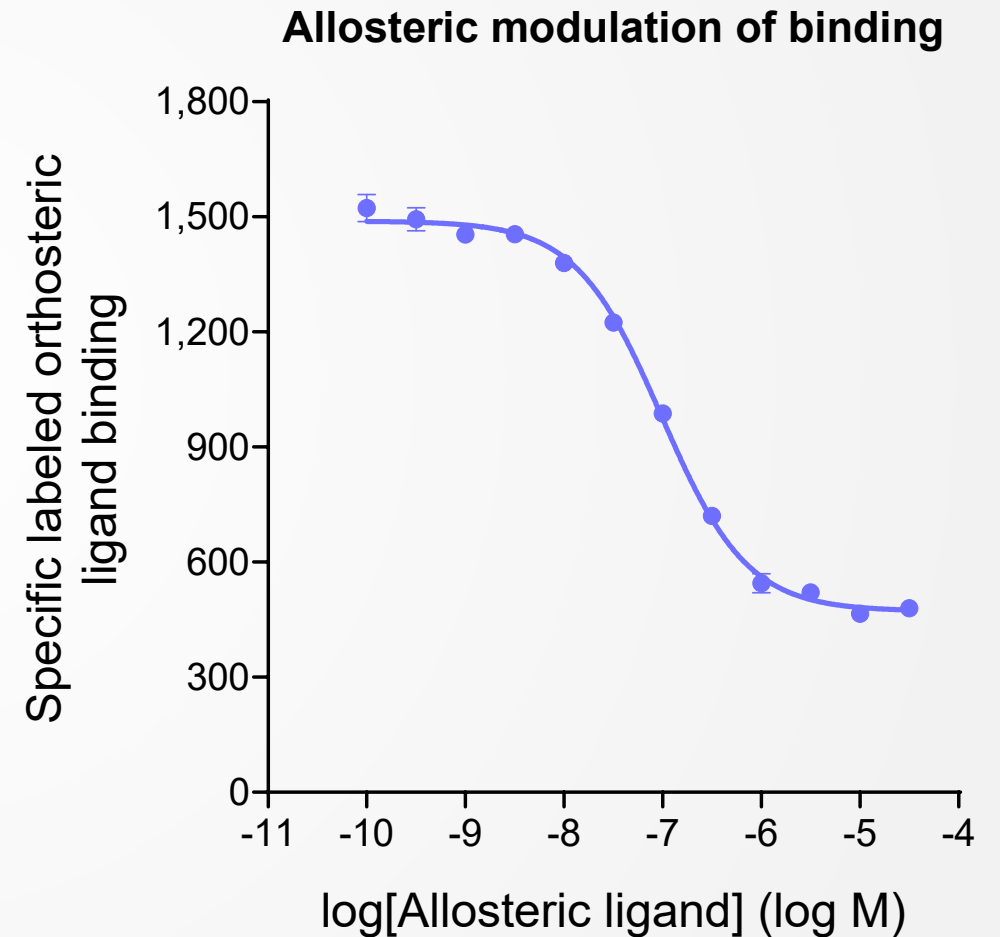
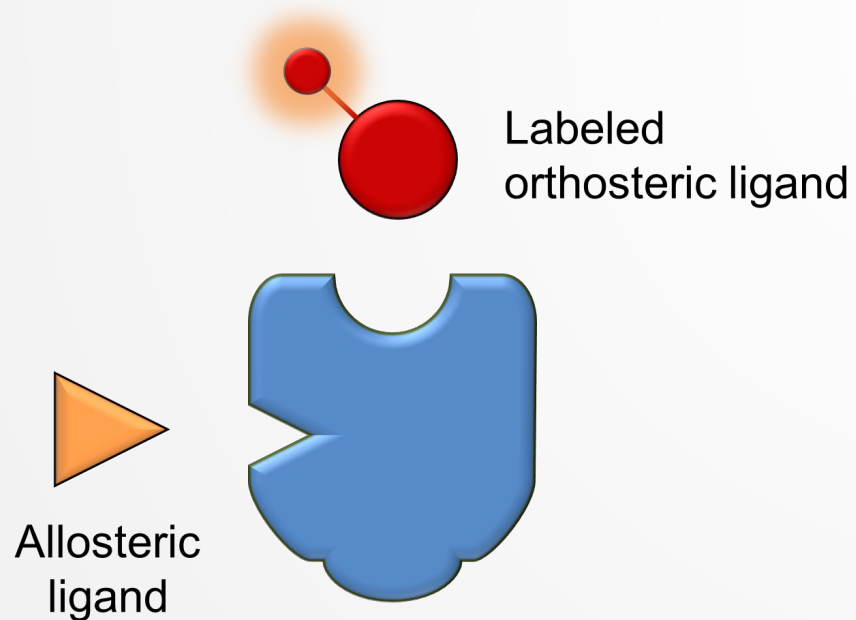


Modulation of labeled orthosteric ligand binding

Modulation of labeled orthosteric ligand binding

Used for a binding assay in which an allosteric ligand is titrated versus a single concentration of a labeled orthosteric ligand.

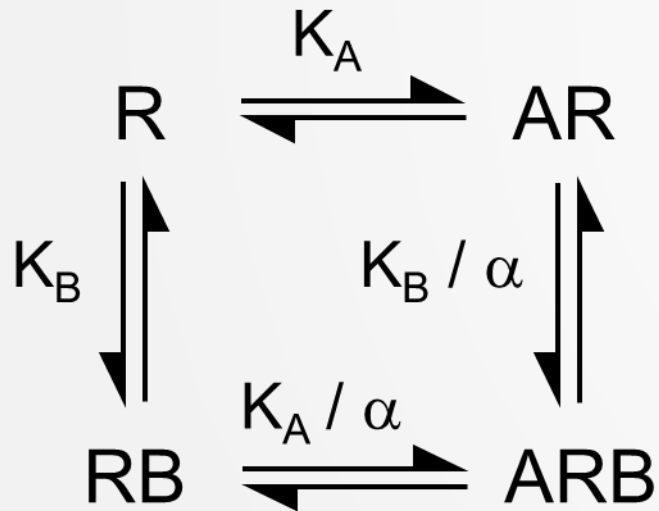
For equation derivation see [here](#) and [here](#).



Modulation of labeled orthosteric ligand binding

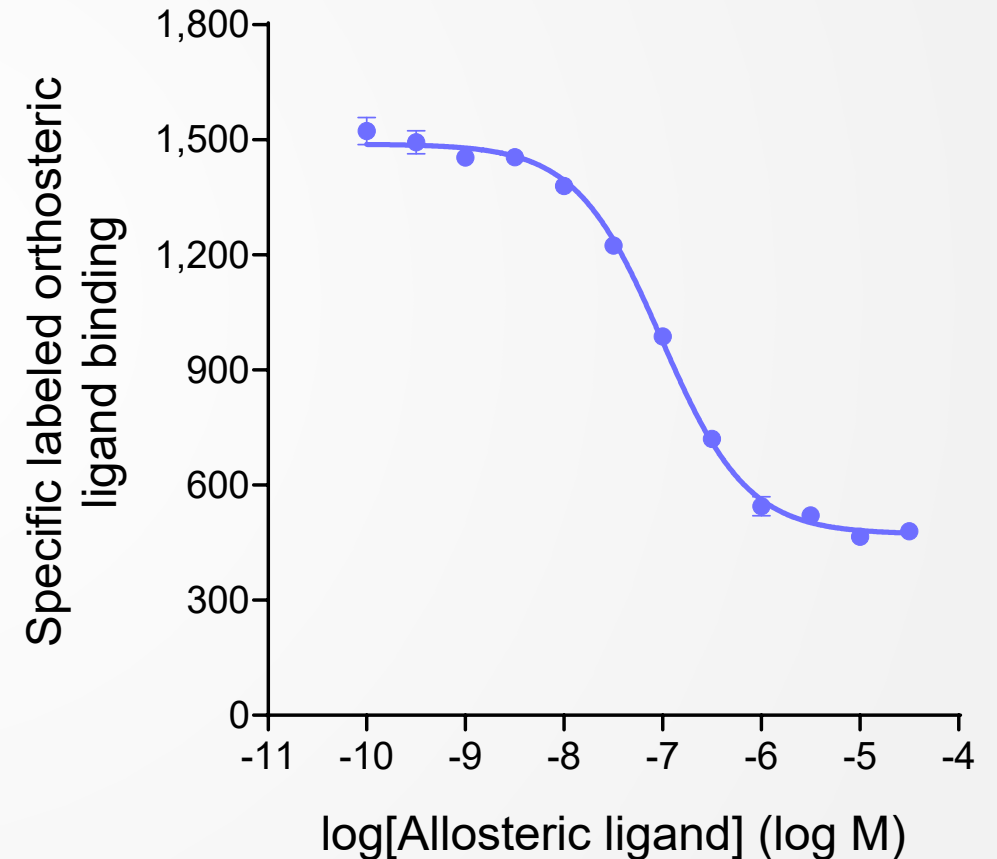
Fitted parameters are:

- α , binding cooperativity between allosteric modulator and orthosteric ligand
- K_B , affinity of allosteric modulator



A is orthosteric ligand
B is allosteric ligand

Allosteric modulation of binding



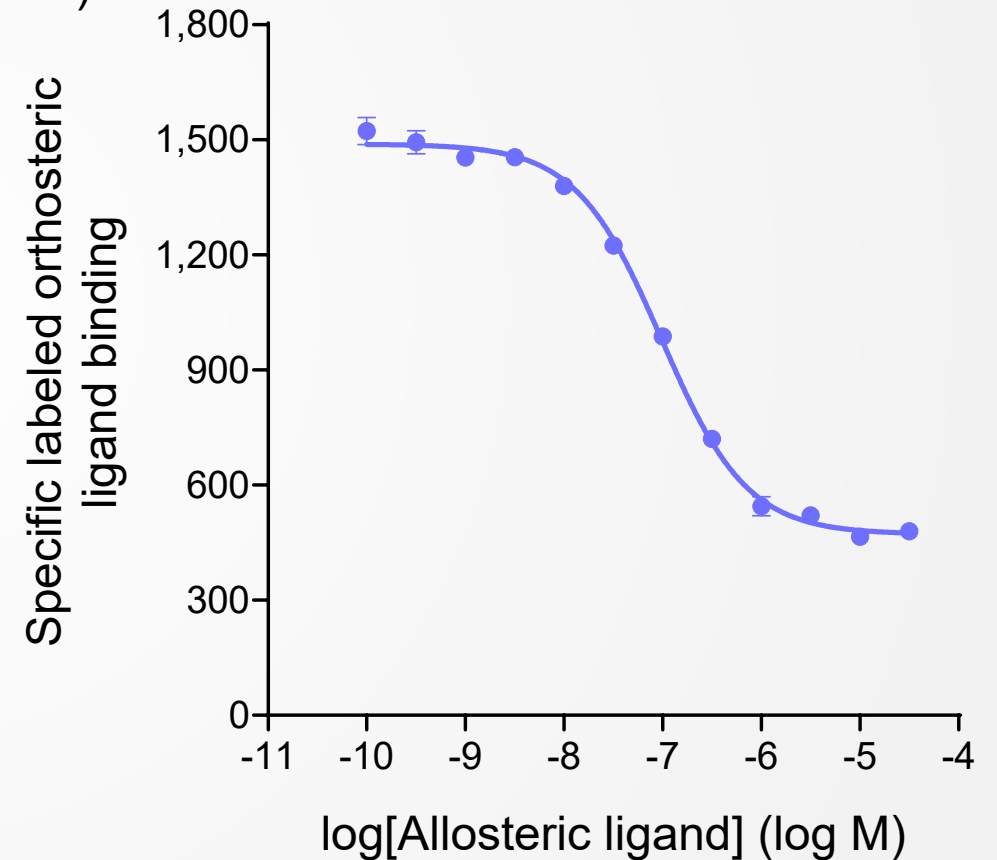
Data entry

X values: Log of allosteric modulator concentration (units of log M)

Y values: Specific binding of labeled orthosteric ligand (nonspecific binding subtracted)

		X	Group A	
		log[Allosteric ligand] (log M)	Data Set A	
	×	X	A:Y1	A:Y2
1	Titl	-10.000	1558	1488
2	Titl	-9.500	1464	1524
3	Titl	-9.000	1453	1454
4	Titl	-8.500	1467	1442
5	Titl	-8.000	1373	1387
6	Titl	-7.500	1242	1207
7	Titl	-7.000	990	984
8	Titl	-6.500	736	705
9	Titl	-6.000	569	520
10	Titl	-5.500	501	542
11	Titl	-5.000	450	481
12	Titl	-4.500	489	471

Allosteric modulation of binding



Model

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Choose an equation

- Dose-response - Special, X is log(concentration)
- Binding - Saturation
- Binding - Competitive
- Binding - Kinetics
- Enzyme kinetics - Inhibition
- Enzyme kinetics - Velocity as a function of substrate
- Exponential
- Lines
- Polynomial
- Gaussian
- Sine waves
- Growth curves
- Linear quadratic curves
- Classic equations from prior versions of Prism
- [Pharmechanics] GPCR allosteric modulator analysis
 - [Pharmechanics] Allosteric modulation of binding
 - [Pharmechanics] Allosteric modulation of labeled and unlabeled ligand b
 - [Pharmechanics] Allosteric modulation of signaling, full model
 - [Pharmechanics] Allosteric modulation of signaling, combined AlphaBeta

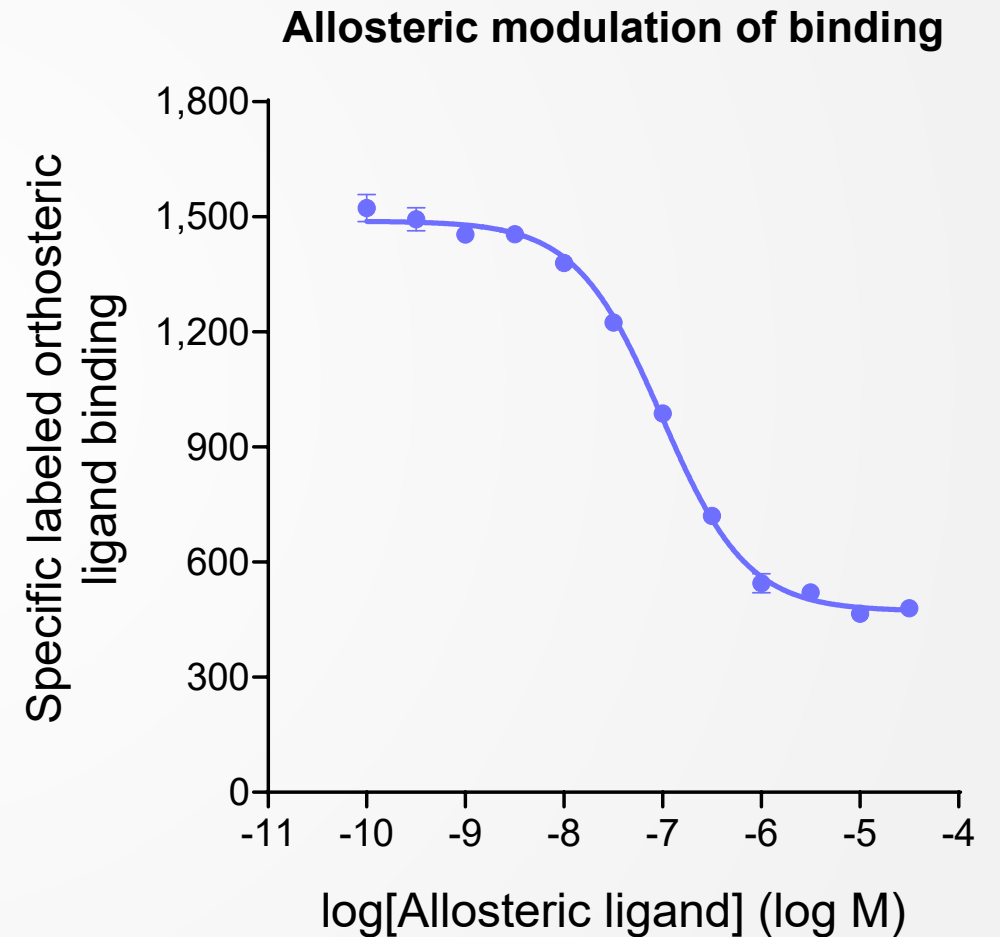
For binding assays titrating an allosteric ligand (B) versus a single concentration of a labeled orthosteric ligand (A). Allosteric ligand conc is the X value, specific binding of the orthosteric ligand is the Y value.

[Pharmechanics] Allosteric modulation of binding
Numerical derivatives

Interpolate
 Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK

“Allosteric modulation of binding”



Results table

1	[Pharmechanics] Allosteric	
2	Best-fit values	
3	Bmax	1985
4	A_nM	= 3.000
5	KA_nM	= 1.000
6	Alp	0.1039
7	logKB	-7.496
8	B0	1489
9	KB	3.188e-008

Parameters for allosteric modulator

Alp: α , binding cooperativity ^A

logKB: Logarithm of modulator affinity (log M)

KB: Modulator affinity (M)

Parameters for labeled orthosteric ligand

Bmax (y axis units)

A_nM: Concentration in nM (entered as constant)

KA_nM: Affinity in nM (entered as constant)

B0: Binding in absence of allosteric modulator (y axis units)

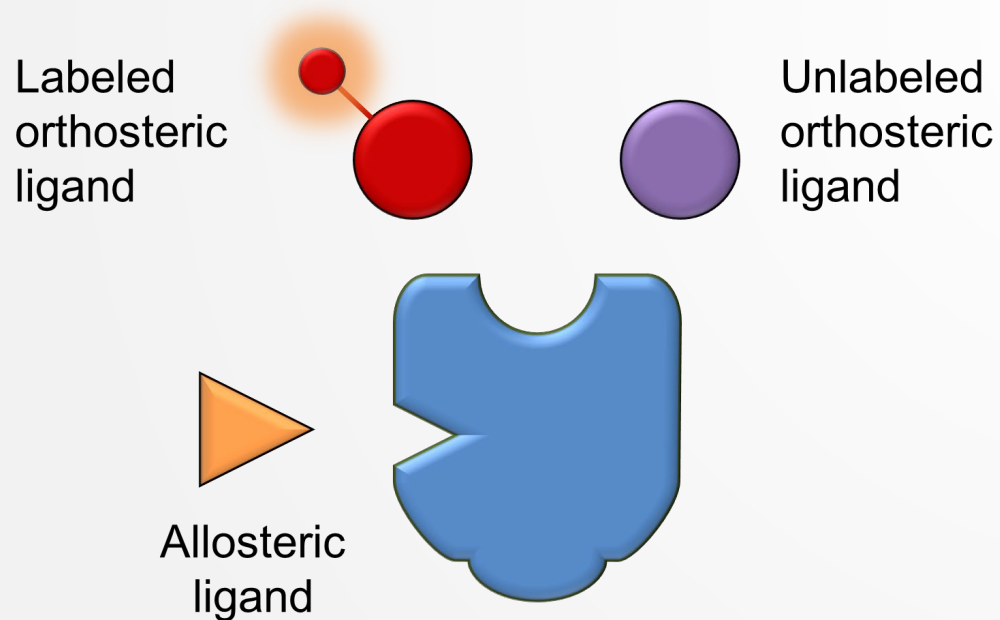
A) α value > 1 is positive cooperativity, < 1 is negative cooperativity

Modulation of labeled and unlabeled orthosteric ligand binding

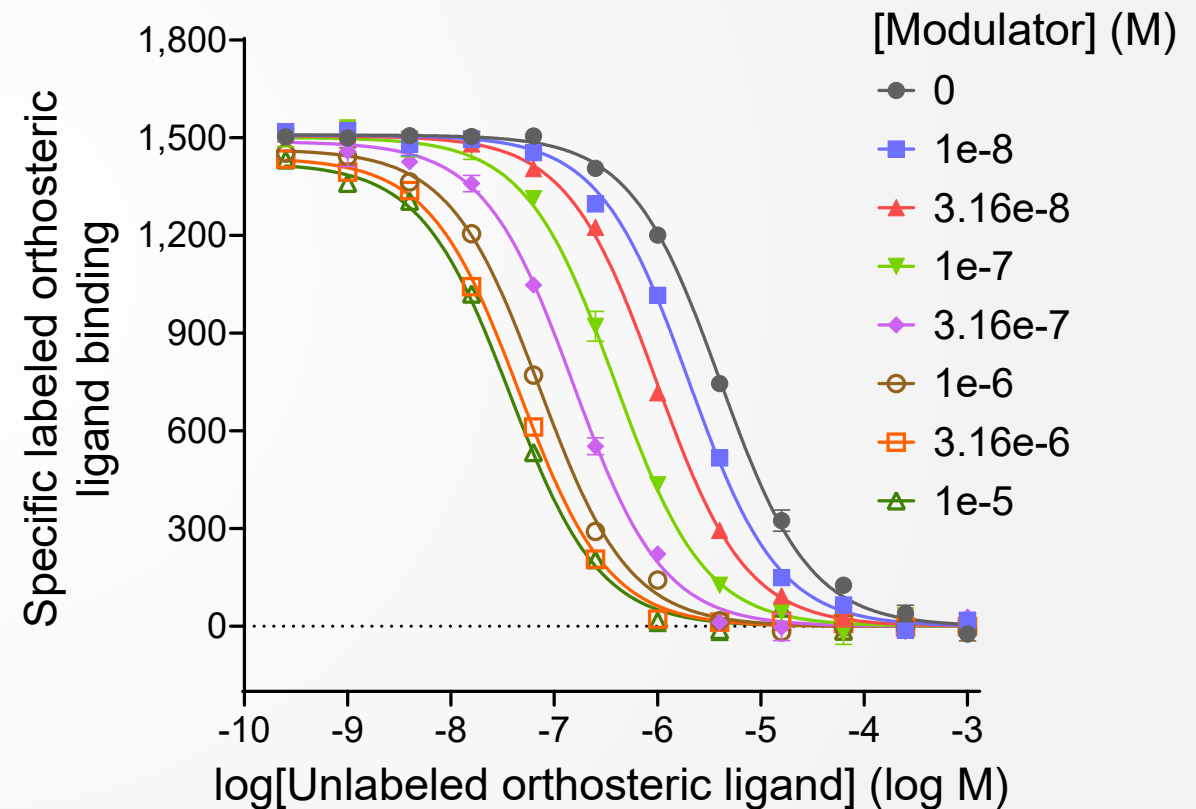
Modulation of labeled and unlabeled orthosteric ligand binding

Used for a “Triple ligand” binding assay in which an unlabeled orthosteric ligand and an allosteric modulator are titrated versus a labeled orthosteric ligand.

Equation derived by Lazareno and Birsdall – see [here](#).



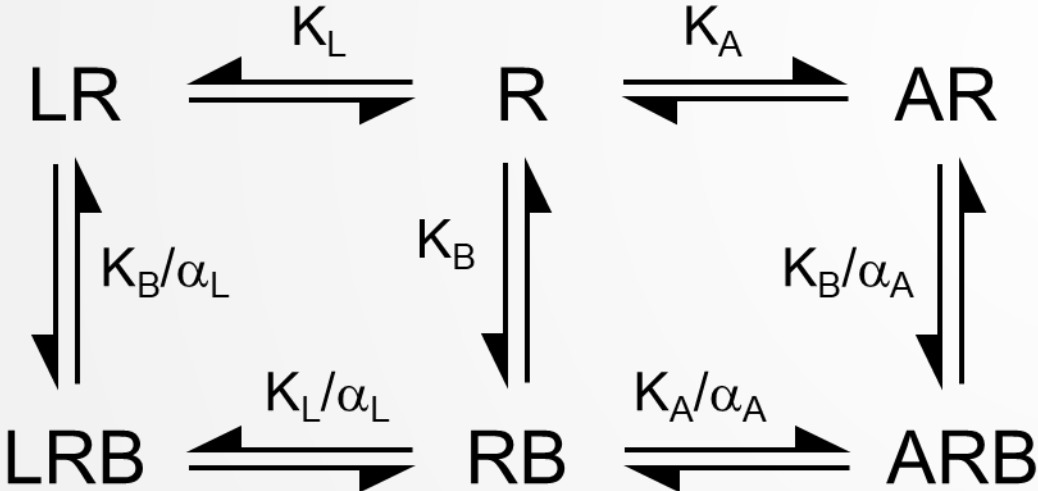
Allosteric modulation of labeled & unlabeled ligand binding



Modulation of labeled and unlabeled orthosteric ligand binding

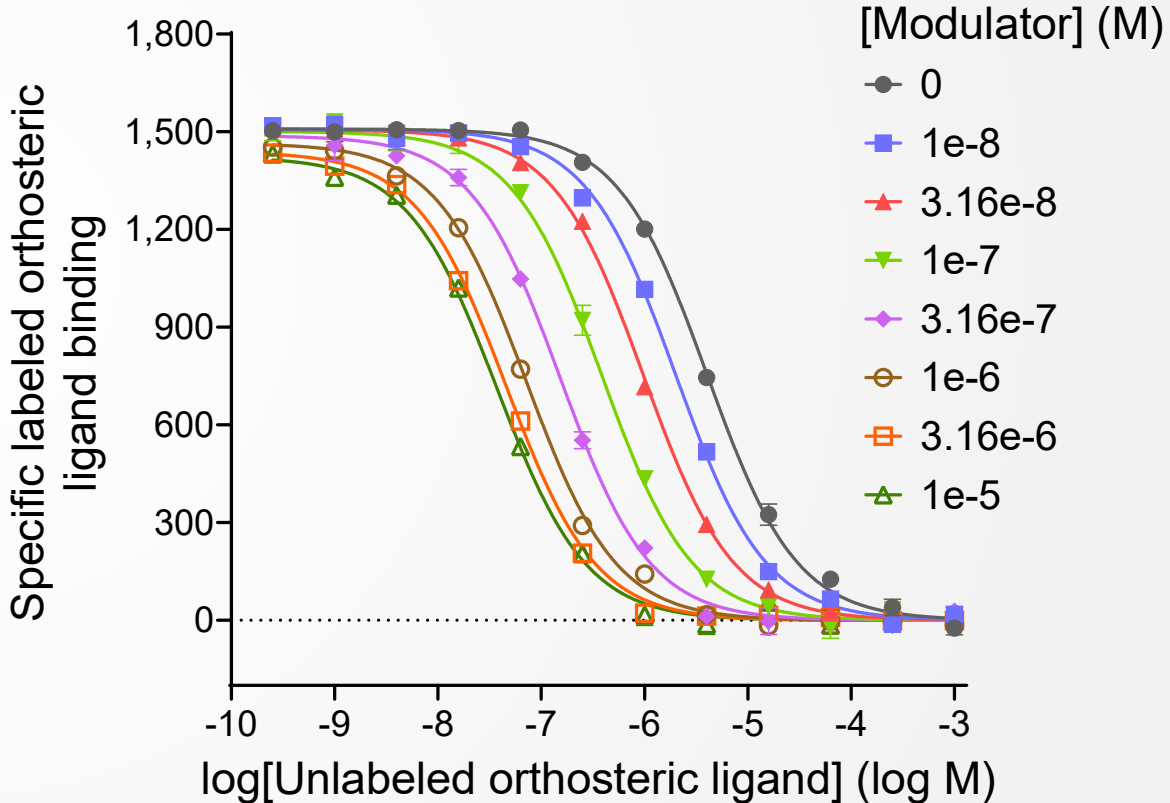
Fitted parameters are:

- Cooperativity between allosteric modulator & labeled or unlabeled orthosteric ligand (α_A & α_L respectively)
- Affinity of allosteric ligand (K_B) and unlabeled orthosteric ligand (K_L)



A is labeled orthosteric ligand
 B is allosteric ligand
 L is unlabeled orthosteric ligand

Allosteric modulation of labeled & unlabeled ligand binding



Data entry

		X	Group A		Group B		Group C		Group D		Group E		Group F		Group G		Group H	
		log[Unlabeled orthosteric ligand] (log M)	0		1e-8		3.16e-8		1e-7		3.16e-7		1e-6		3.16e-6		1e-5	
	X		A:Y1	A:Y2	B:Y1	B:Y2	C:Y1	C:Y2	D:Y1	D:Y2	E:Y1	E:Y2	F:Y1	F:Y2	G:Y1	G:Y2	H:Y1	H:Y2
1	Title	-3.00	-7	-40	10	27	-16	31	15	-25	36	20	17	-44	-7	6	40	-17
2	Title	-3.60	66	14	-24	2	1	-1	38	26	-36	5	-20	15	-16	12	23	-22
3	Title	-4.20	108	144	51	81	59	-1	-55	14	-14	19	-38	31	8	10	-16	-14
4	Title	-4.80	291	357	142	158	79	108	39	46	38	-43	-18	-16	-12	44	74	39
5	Title	-5.40	741	749	522	513	307	282	106	146	1	23	-5	40	4	23	19	-43
6	Title	-6.00	1194	1209	1016	1016	732	702	431	438	214	229	140	144	3e-001	44	48	-16
7	Title	-6.60	1410	1403	1293	1301	1232	1219	967	876	579	527	296	287	224	188	206	203
8	Title	-7.20	1511	1501	1470	1438	1396	1416	1314	1312	1048	1048	779	764	607	617	548	519
9	Title	-7.80	1490	1518	1509	1483	1471	1492	1521	1434	1385	1335	1211	1199	1032	1054	1034	1007
10	Title	-8.40	1523	1490	1515	1446	1487	1485	1521	1442	1406	1446	1351	1378	1337	1339	1318	1293
11	Title	-9.00	1512	1487	1527	1513	1533	1485	1512	1549	1412	1506	1414	1470	1377	1412	1364	1357
12	Title	-9.60	1521	1486	1514	1523	1506	1518	1472	1528	1509	1504	1436	1470	1430	1439	1431	1434

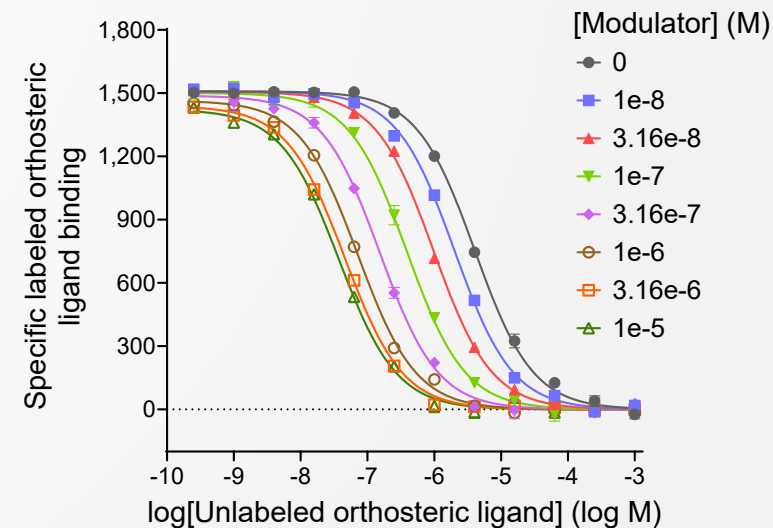
X values: Log of unlabeled orthosteric ligand concentration (units of log M)

Y values: Specific binding of labeled orthosteric ligand (nonspecific binding subtracted)

Different column groups: Different concentrations of allosteric modulator.

Column headers: Allosteric modulator concentration in M

Allosteric modulation of labeled & unlabeled ligand binding



Model

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Choose an equation

- Dose-response - Special, X is log(concentration)
- Binding - Saturation
- Binding - Competitive
- Binding - Kinetics
- Enzyme kinetics - Inhibition
- Enzyme kinetics - Velocity as a function of substrate
- Exponential
- Lines
- Polynomial
- Gaussian
- Sine waves
- Growth curves
- Linear quadratic curves
- Classic equations from prior versions of Prism
- [Pharmechanics] GPCR allosteric modulator analysis
 - [Pharmechanics] Allosteric modulation of binding
 - [Pharmechanics] Allosteric modulation of labeled and unlabeled ligand binding
 - [Pharmechanics] Allosteric modulation of signaling, full model
 - [Pharmechanics] Allosteric modulation of signaling, combined AlphaBeta

Titrate allos. ligand (B) and unlabeled orthos. ligand (L) versus a single conc. of ortho. ligand (A). Unlabeled ortho. ligand is X. Specific binding of labeled ortho. ligand is Y. Allos. ligand cons is column title

Suggest manual initial values of Alp

[Pharmechanics] Allosteric modulation of labeled and unlabeled ligand binding

Numerical derivatives

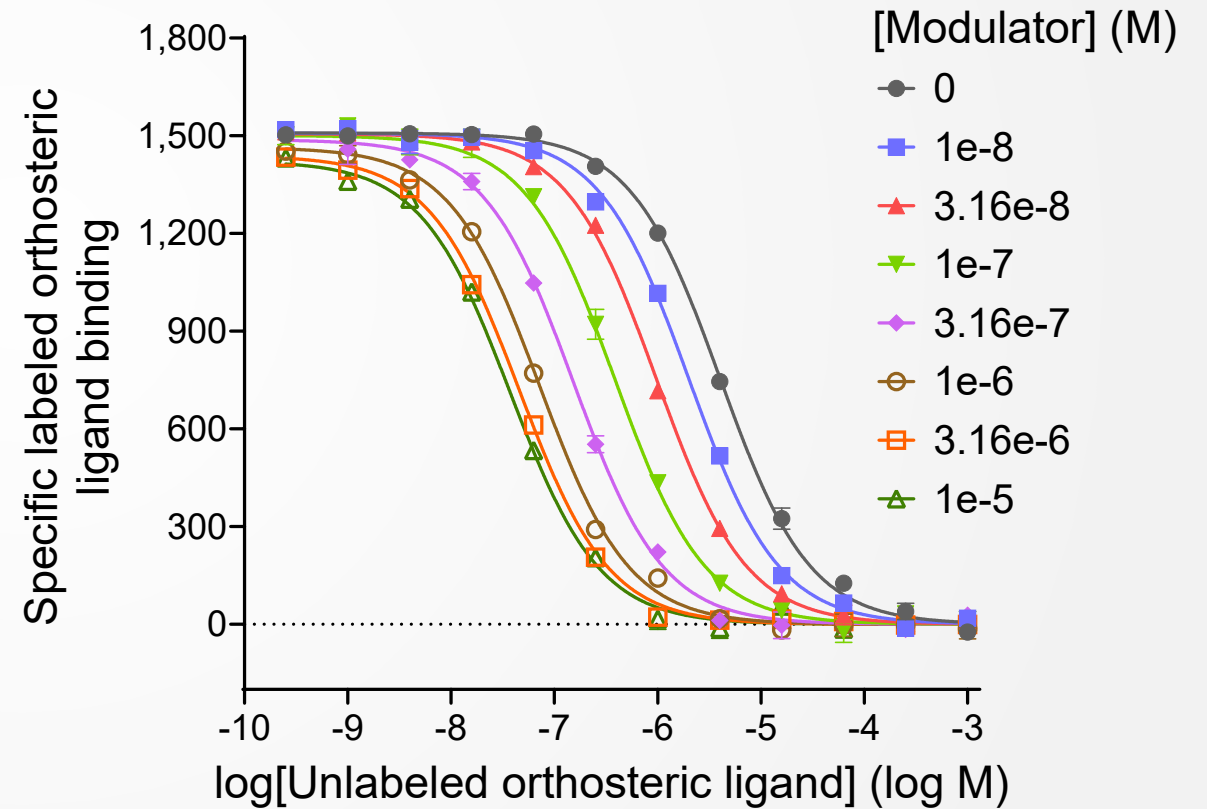
Interpolate

Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK

“Allosteric modulation of labeled and unlabeled ligand binding”

Allosteric modulation of labeled & unlabeled ligand binding



Analysis details

Parameters: Nonlinear Regression

Model Method Compare **Constrain** Initial values Range Output Confidence Diagnostics Flag

Parameter Name	Constraint Type	Value	Hook
Bmax	Shared, and must be greater than	0	
A_nM	Constant equal to	3	
KA_nM	Constant equal to	1	
B	Data set constant (from column title)		
AlpA	Shared, and must be greater than	0	
logKB	Shared value for all data sets		
logKL	Shared value for all data sets		
AlpL	Shared, and must be greater than	0	

Global analysis – all curves fit simultaneously with shared fit parameters.

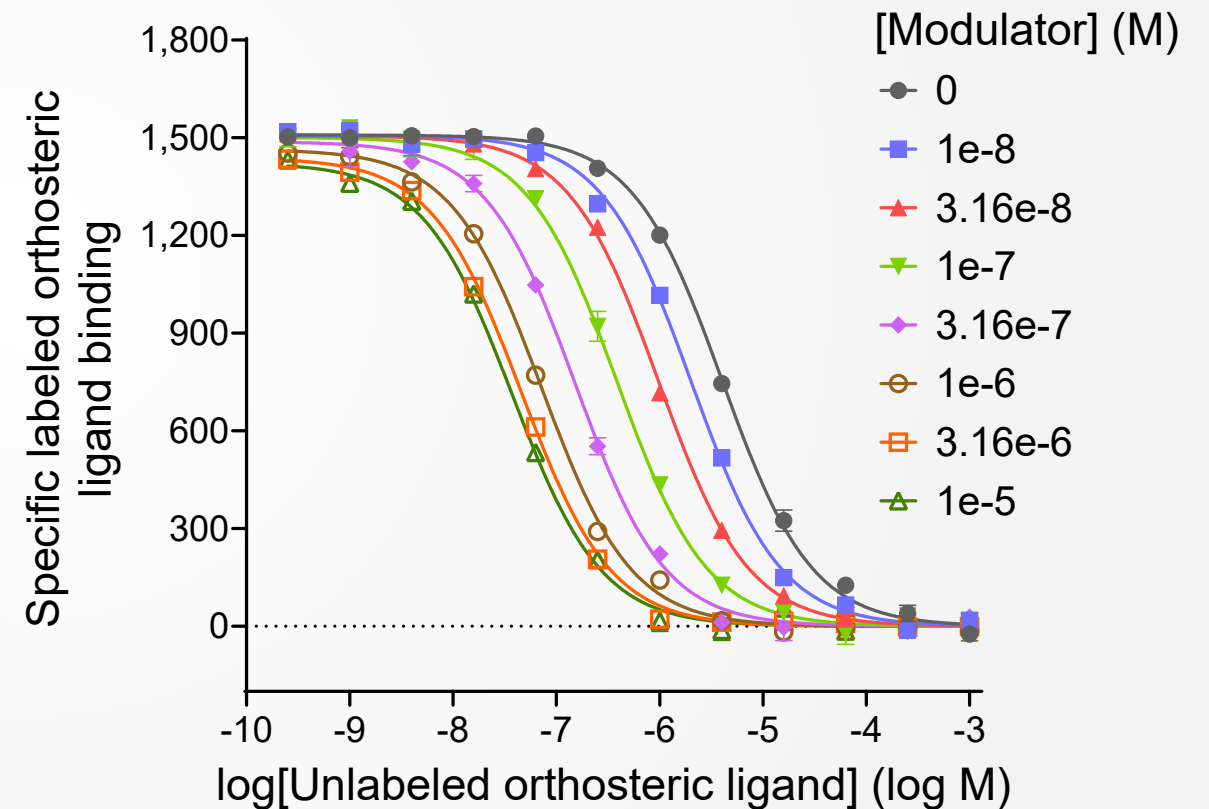
Constrain one parameter relative to another

must be greater than 1 times

must be greater than 1 times

Learn Cancel OK

Allosteric modulation of labeled & unlabeled ligand binding



Results table

Nonlin fit Table of results		I
		Global (shared)
1	[Pharmechanics] Al	
2	Best-fit values	
3	Bmax	2012
4	A_nM	= 3.000
5	KA_nM	= 1.000
6	B	
7	AlpA	0.7869
8	logKB	-6.008
9	logKL	-5.997
10	AlpL	101.0
11	B0	1509
12	KB	9.809e-007
13	KL	1.006e-006

Parameters for allosteric modulator

AlpA: α_A , binding cooperativity on labeled orthosteric ligand ^A

AlpL: α_L , binding cooperativity on unlabeled orthosteric ligand ^A

logKB: Logarithm of modulator affinity (log M)

KB: Modulator affinity (M)

Parameters for labeled orthosteric ligand

Bmax (y axis units)

A_nM: Concentration in nM (entered as constant)

KA_nM: Affinity in nM (entered as constant)

B0: Binding in absence of allosteric modulator (y axis units)

Parameters for unlabeled orthosteric ligand

logKL: Logarithm of unlabeled orthosteric ligand affinity (log M)

KL: Unlabeled orthosteric ligand affinity (M)

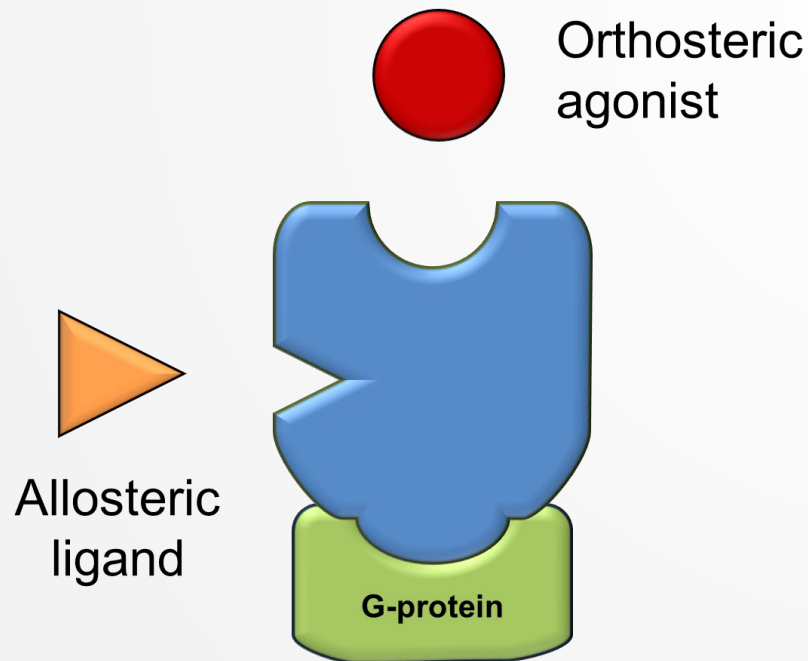
A) α value > 1 is positive cooperativity, < 1 is negative cooperativity

Modulation of orthosteric agonist signaling, full model

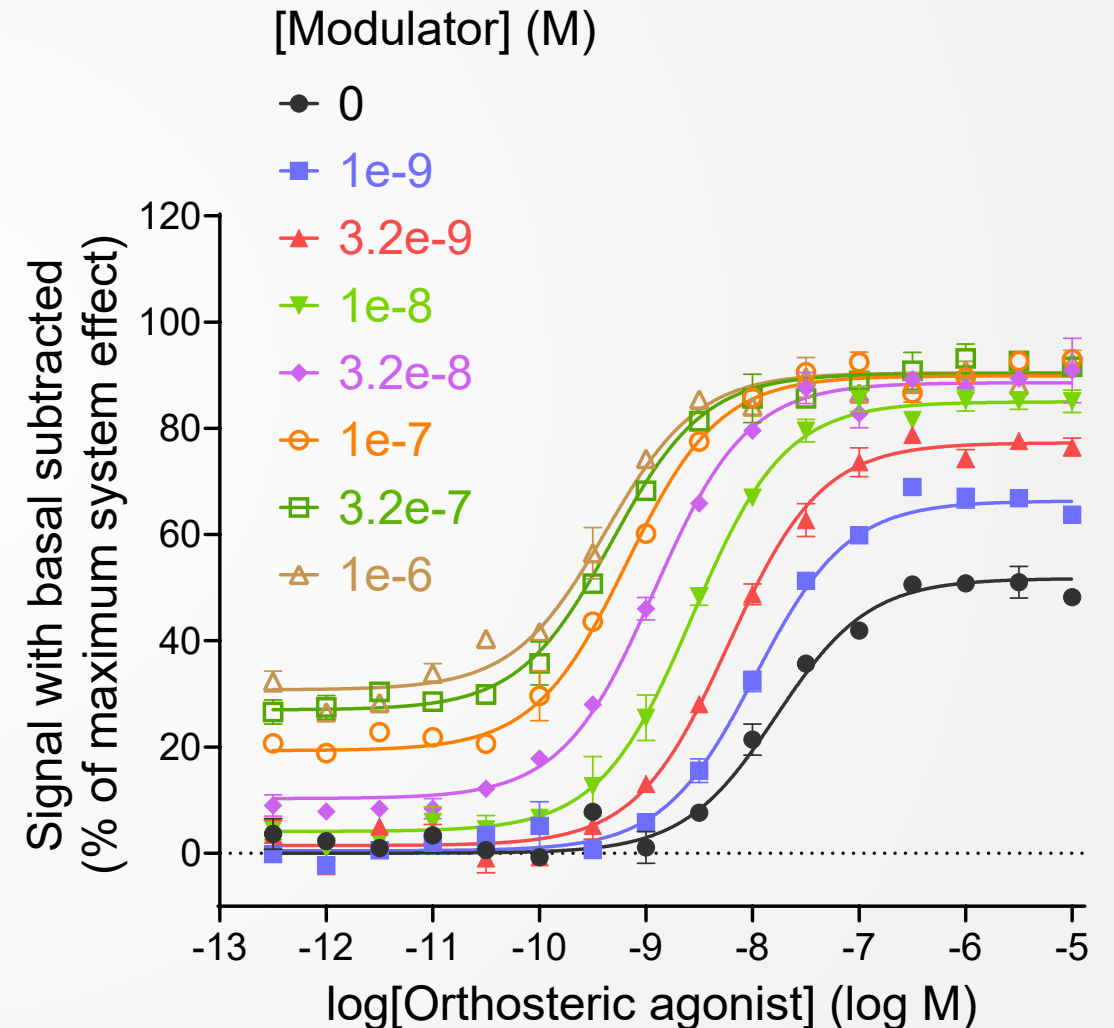
Modulation of orthosteric agonist signaling, full model

Used for a signaling assay in which an allosteric ligand is titrated versus a concentration-response of an orthosteric agonist.

Equation derived by Christopoulos – see [here](#). Similar equation derived by [Kenakin](#) and [Ehlert](#).



Allosteric modulation of signaling, full model



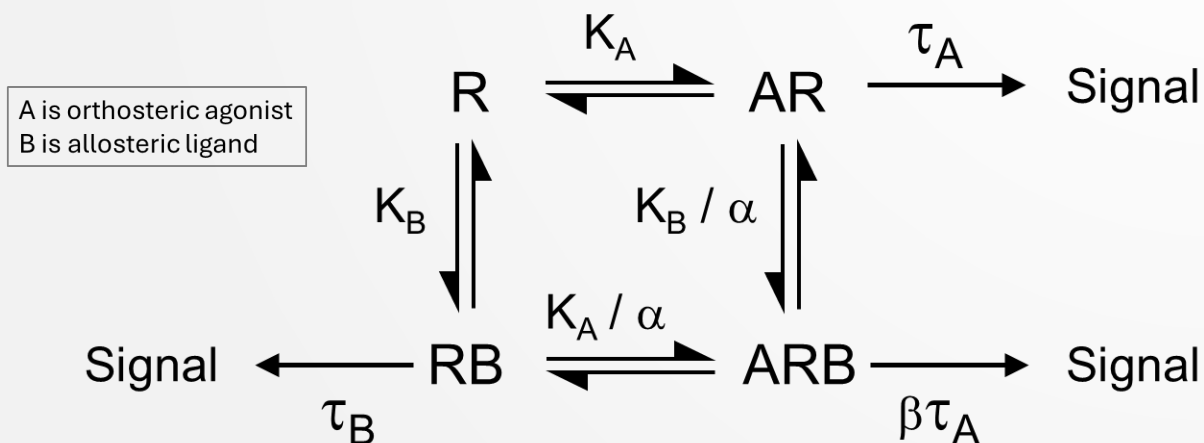
Modulation of orthosteric agonist signaling, full model

An understanding of the [operational model of agonism](#) is required to apply this equation and interpret the results.

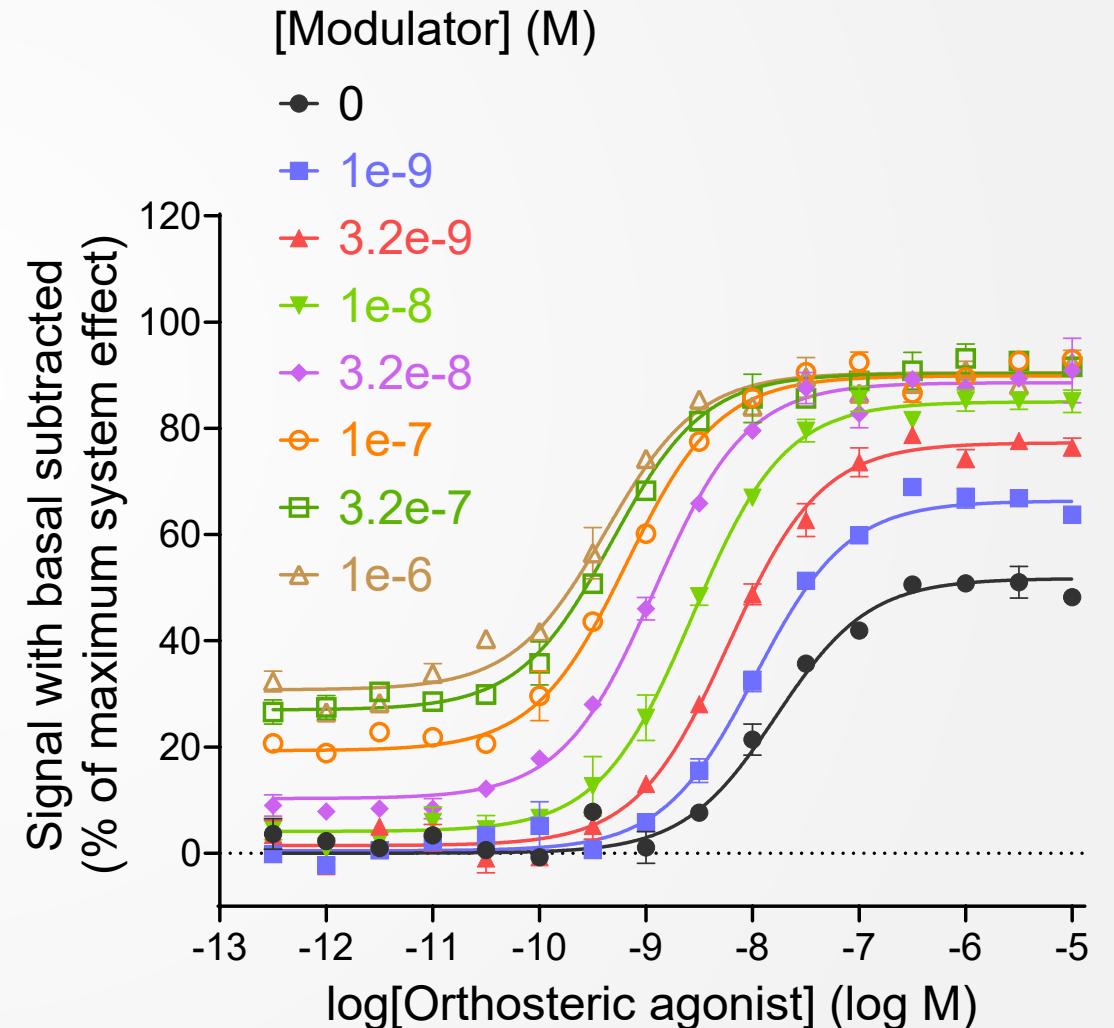
The model allows for agonism of allosteric modulator (but not inverse agonism).

There are two cooperativity factors:

- α , binding cooperativity
- β , signaling cooperativity (effect of allosteric modulator on efficacy of orthosteric agonist)



Allosteric modulation of signaling, full model



Data entry

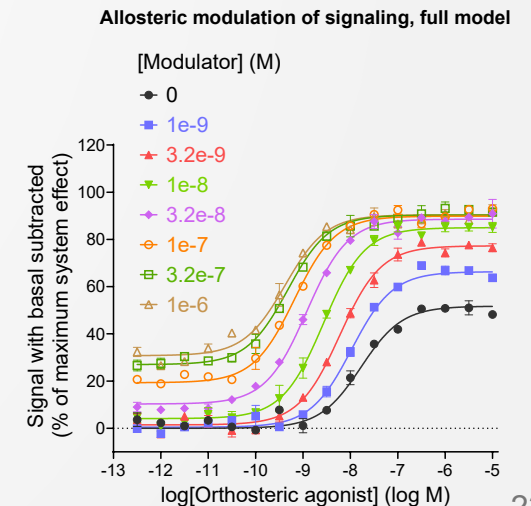
		X	Group A		Group B		Group C		Group D		Group E		Group F		Group G		Group H	
		log[Orthosteric agonist] (log M)	0		1e-9		3.2e-9		1e-8		3.2e-8		1e-7		3.2e-7		1e-6	
	X	A:Y1	A:Y2	B:Y1	B:Y2	C:Y1	C:Y2	D:Y1	D:Y2	E:Y1	E:Y2	F:Y1	F:Y2	G:Y1	G:Y2	H:Y1	H:Y2	
1	Titl	-12.500	0.7	6.5	-1.2	0.9	6.2	0.7	6.5	2.5	11.0	7.0	20.2	21.2	24.3	28.9	34.3	30.4
2	Titl	-12.000	2.5	2.1	-3.0	-1.5	-1.8	-3.1	-1.2	2.8	7.0	8.8	19.0	18.7	25.2	29.7	25.9	27.1
3	Titl	-11.500	0.9	1.0	0.4	0.7	5.5	4.6	4.0	0.7	8.2	8.5	23.6	21.9	30.5	30.2	27.1	29.4
4	Titl	-11.000	3.4	3.2	3.2	1.2	0.4	5.4	3.1	8.7	10.3	6.5	21.1	22.6	27.0	30.1	32.0	35.7
5	Titl	-10.500	1.3	-0.1	5.0	1.2	1.8	-3.7	7.1	1.9	12.3	11.9	19.8	21.5	28.1	31.7	39.0	41.6
6	Titl	-10.000	-0.9	-0.6	9.7	0.6	-1.5	-3.1e-002	8.1	5.3	17.0	18.6	25.0	34.3	39.8	31.7	42.6	40.7
7	Titl	-9.500	9.0	6.6	1.2	4.4e-002	7.6	2.6	18.2	7.0	29.5	26.5	45.2	41.9	50.3	51.2	61.3	51.7
8	Titl	-9.000	4.1	-1.9	6.5	5.1	12.0	14.1	21.2	29.8	43.9	48.1	61.8	58.6	69.5	67.0	75.6	72.9
9	Titl	-8.500	8.1	7.1	17.8	13.3	26.8	29.3	46.7	49.9	66.6	65.1	77.4	77.6	79.5	83.2	85.5	85.3
10	Titl	-8.000	24.3	18.4	34.2	30.4	46.8	50.7	66.1	67.8	79.4	79.7	85.0	86.5	90.2	81.1	84.0	84.2
11	Titl	-7.500	35.8	35.6	50.7	51.8	65.8	59.6	77.5	81.7	90.5	84.6	91.1	90.0	84.7	86.6	85.8	93.3
12	Titl	-7.000	41.8	42.0	59.1	60.5	70.8	76.3	87.9	83.2	80.0	85.4	90.6	94.4	87.7	90.2	85.5	87.6
13	Titl	-6.500	49.9	51.3	70.1	67.8	79.7	77.9	80.8	82.2	88.8	89.6	88.3	84.9	87.3	94.3	86.6	90.4
14	Titl	-6.000	50.9	50.7	68.7	65.0	72.6	76.0	83.2	87.2	88.5	86.8	90.1	89.0	95.9	90.4	89.1	92.6
15	Titl	-5.500	54.0	48.0	68.0	65.7	78.8	76.4	86.8	83.5	88.1	90.7	93.7	91.7	93.5	91.9	87.9	88.3
16	Titl	-5.000	48.7	47.7	63.0	64.5	78.2	77.8	87.2	82.9	84.8	96.9	93.0	93.0	90.6	92.6	94.7	89.8

X values: Log of orthosteric agonist concentration (units of log M)

Different column groups: Different concentrations of allosteric modulator.

Y values: Signal response with basal signal subtracted (basal is signal in the absence of orthosteric and allosteric ligand)

Column headers: Allosteric modulator concentration in M



Model

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Choose an equation

- + Dose-response - Special, X is log(concentration)
- + Binding - Saturation
- + Binding - Competitive
- + Binding - Kinetics
- + Enzyme kinetics - Inhibition
- + Enzyme kinetics - Velocity as a function of substrate
- + Exponential
- + Lines
- + Polynomial
- + Gaussian
- + Sine waves
- + Growth curves
- + Linear quadratic curves
- + Classic equations from prior versions of Prism
- [Pharmacokinetics] GPCR allosteric modulator analysis
 - [Pharmacokinetics] Allosteric modulation of binding
 - [Pharmacokinetics] Allosteric modulation of labeled and unlabeled ligand b
 - [Pharmacokinetics] Allosteric modulation of signaling, full model
 - [Pharmacokinetics] Allosteric modulation of signaling, combined AlphaBeta

For functional assays combining orthosteric agonist (A) and allosteric ligand (B). Orthosteric agonist conc is the X value, the signal response is the Y value, and the signal response for different allosteric ligand concs go in separate columns.

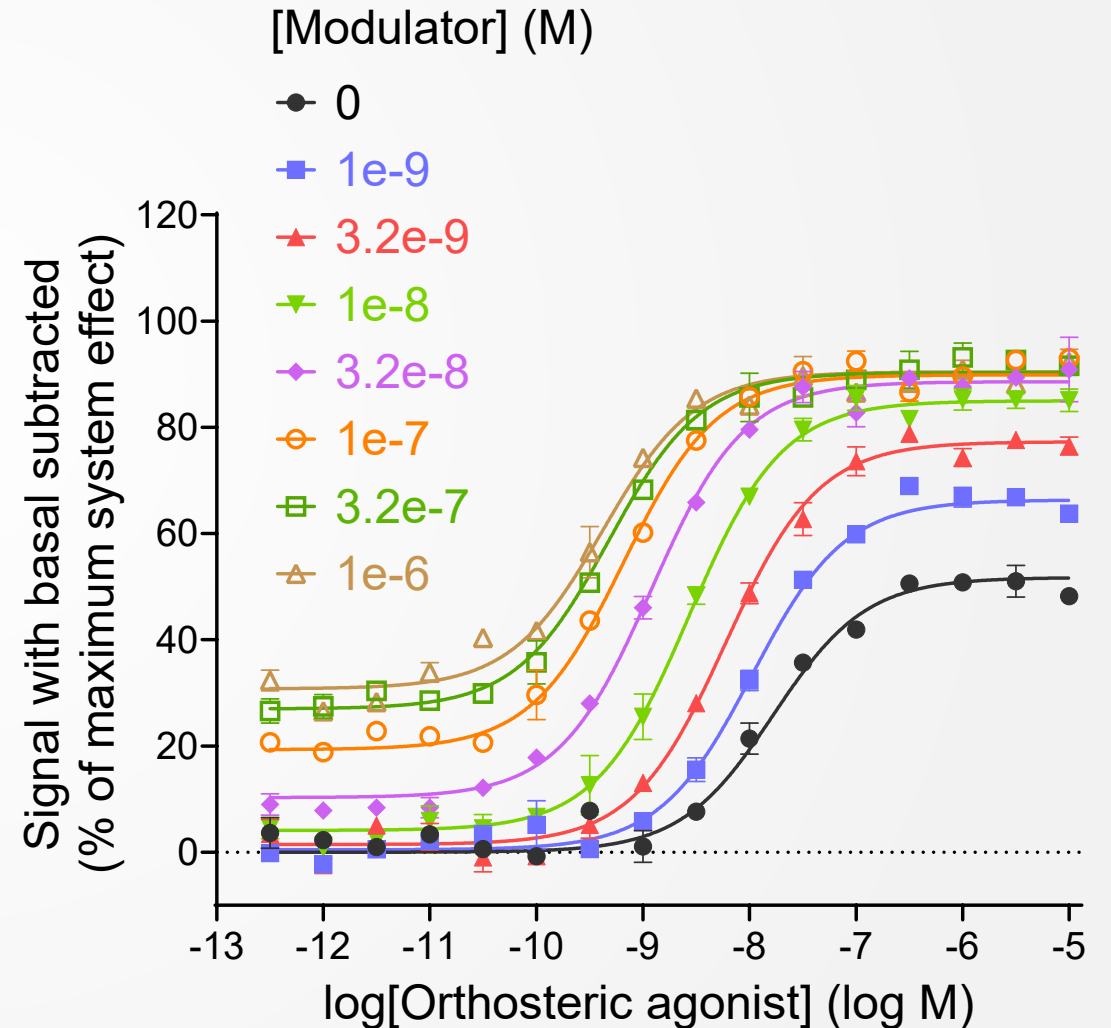
[Pharmacokinetics] Allosteric modulation of signaling, full model
Numerical derivatives

Interpolate
 Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK

“Allosteric modulation of signaling, full model”

Allosteric modulation of signaling, full model



Analysis details

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Parameter Name	Constraint Type	Value	Hook
Em	Shared value for all data sets		
TauA	Shared, and must be greater than	0	
logKB	Shared value for all data sets		
Alp	Shared, and must be greater than	0	
Bet	Shared, and must be greater than	0	
B	Data set constant (from column title)		
TauB	Shared, and must be greater than	0	
logKA	Shared value for all data sets		
n	Shared, and must be greater than	0	

Global analysis – all curves fit simultaneously with shared fit parameters.

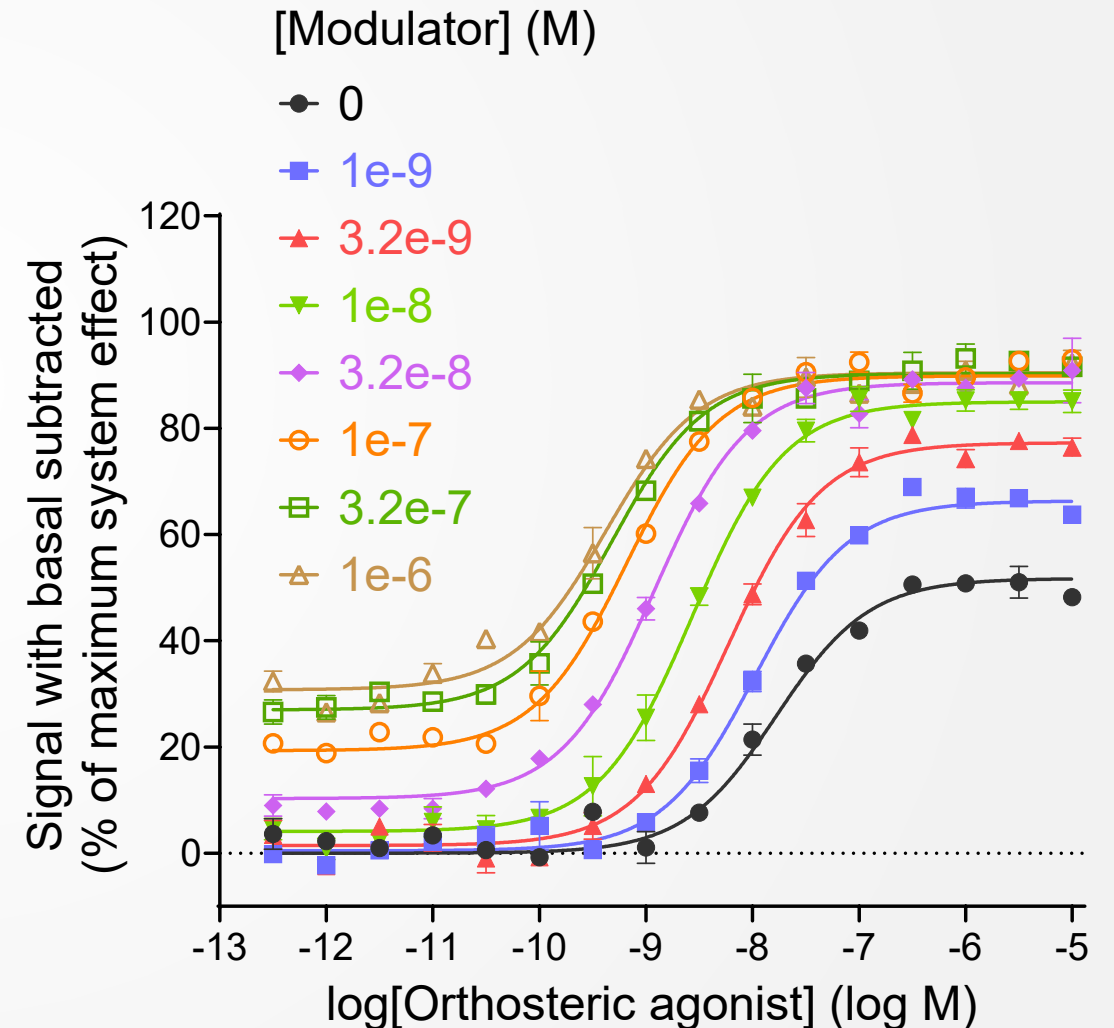
Constrain one parameter relative to another

must be greater than 1 times

must be greater than 1 times

Learn Cancel OK

Allosteric modulation of signaling, full model



Analysis tip

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Parameter Name	Constraint Type	Value	Hook
Em	Constant equal to	100	
TauA	Shared, and must be greater than	0	
logKB	Shared value for all data sets		
Alp	Shared, and must be greater than	0	
Bet	Shared, and must be greater than	0	
B	Data set constant (from column title)		
TauB	Shared, and must be greater than	0	
logKA	Shared value for all data sets		
n	Shared, and must be greater than	0	

Recommend constraining Em (system maximum effect) if this value can be independently determined, e.g. as the Emax of a full agonist.

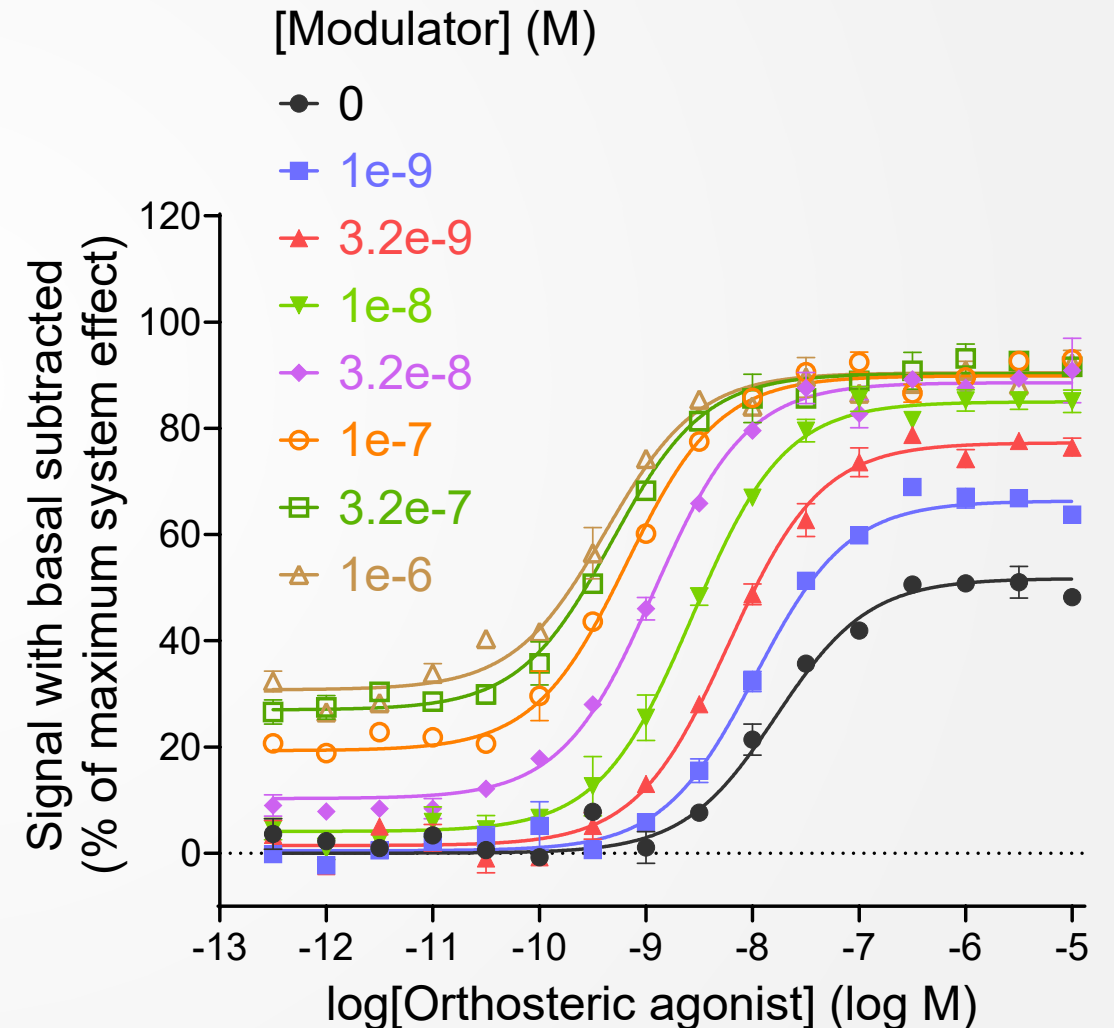
Constrain one parameter relative to another

must be greater than 1 times

must be greater than 1 times

Learn Cancel OK

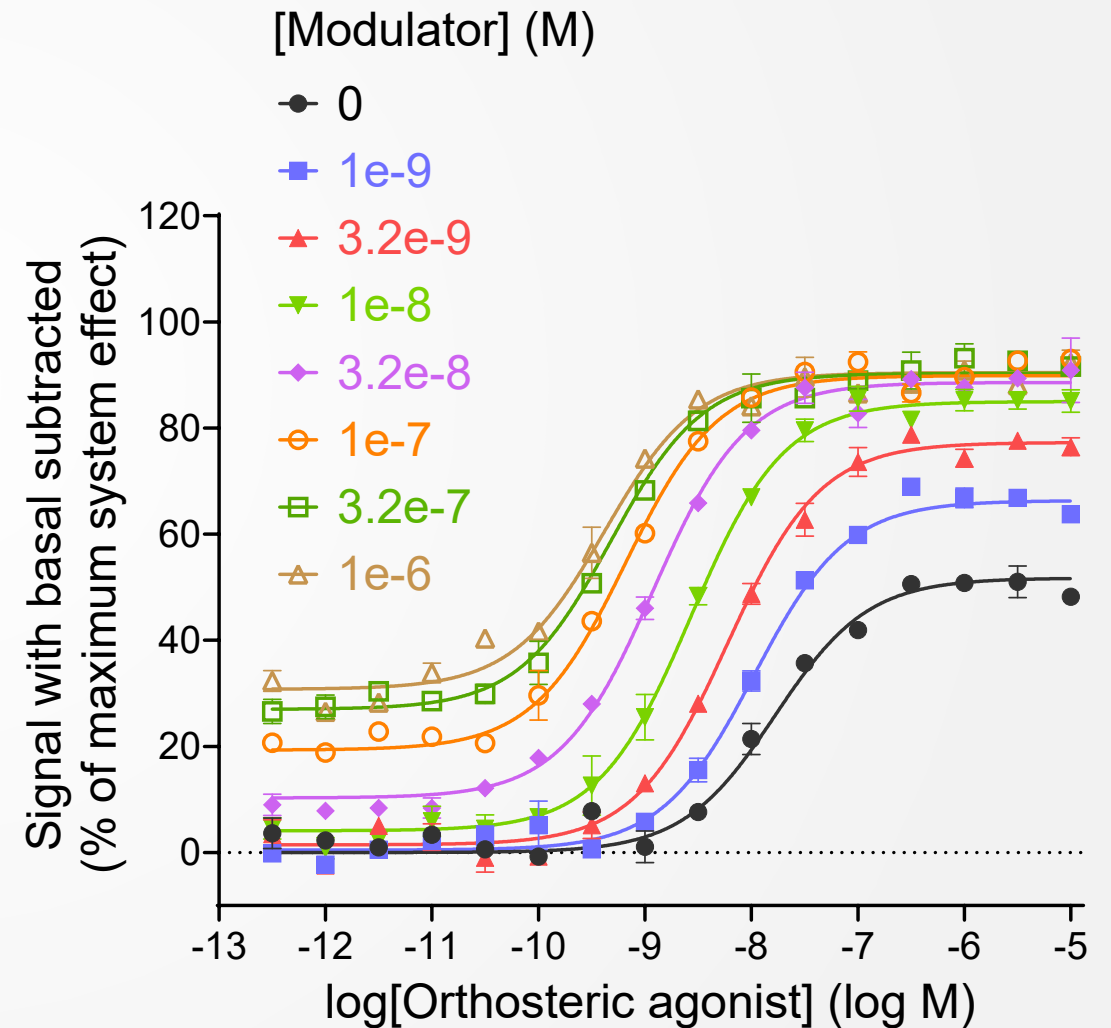
Allosteric modulation of signaling, full model



Results table

1	[Pharmechanics] Allosteric	
2	Best-fit values	
3	Em	= 100.0
4	TauA	1.072
5	logKB	-6.977
6	Alp	12.50
7	Bet	8.922
8	B	
9	TauB	0.4903
10	logKA	-7.459
11	n	0.9983
12	KA	3.472e-008
13	KB	1.054e-007
14	EC50 A	1.672e-008
15	EC50 B	7.075e-008
16	Emax A	51.72
17	Emax B	32.90
18	Std. Error	
19	TauA	0.03466
20	logKB	0.05005
21	Alp	1.535
22	Bet	1.173
23	TauB	0.02371
24	logKA	0.05261
25	n	0.05099
26	Emax A	0.7945
27	Emax B	1.068

Allosteric modulation of signaling, full model



Results table

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26	Emax A	0.7945
27	Emax B	1.068

Parameters for allosteric modulator

Alp: α , binding cooperativity on orthosteric agonist ^A

Bet: β , signaling cooperativity on orthosteric agonist ^A

logKB: Logarithm of modulator affinity (log M)

KB: Modulator affinity (M)

TauB, signaling efficacy of allosteric modulator

EC50 B, EC_{50} of allosteric modulator (M)

Emax B, E_{max} of allosteric modulator (Y axis units)

Parameters for orthosteric agonist

logKA: Logarithm of orthosteric agonist affinity (log M)

KA: Orthosteric agonist affinity (M)

TauA, signaling efficacy of orthosteric agonist

EC50 A, EC_{50} of orthosteric agonist (M)

Emax A, E_{max} of orthosteric agonist (Y axis units)

n, transducer Hill slope of orthosteric agonist

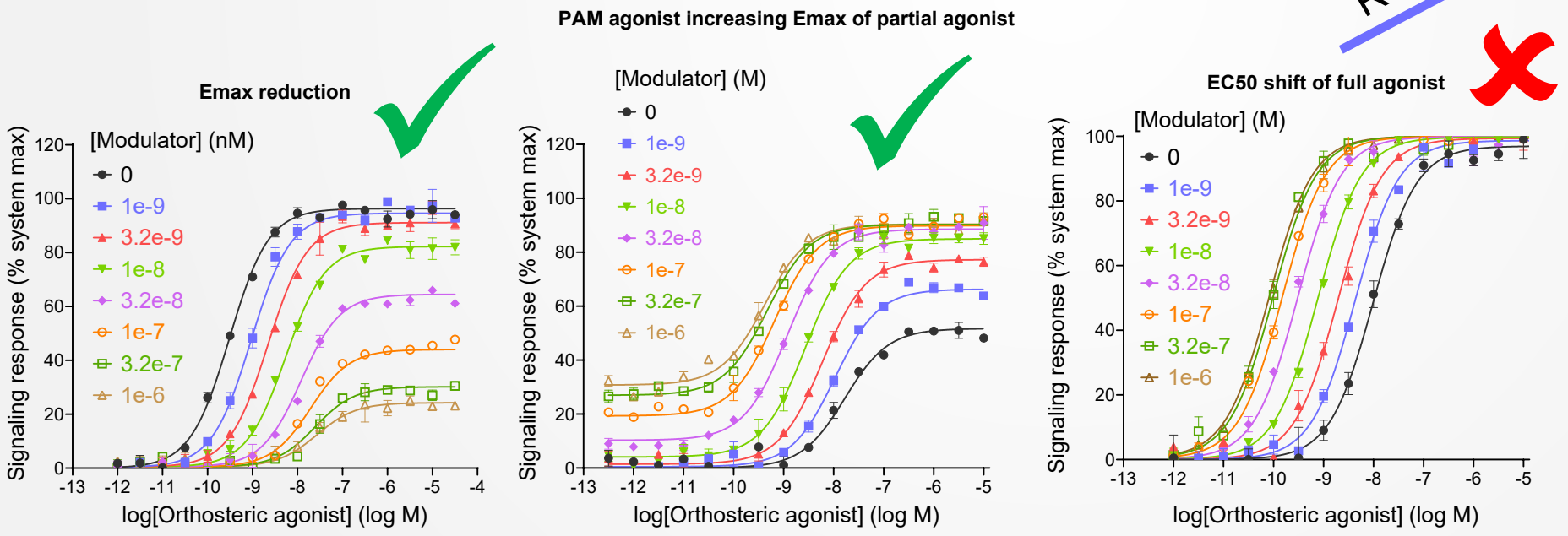
System parameter

Em, system maximum effect (Y axis units)

A) Cooperativity value > 1 is positive cooperativity, < 1 is negative cooperativity

Issues with full model

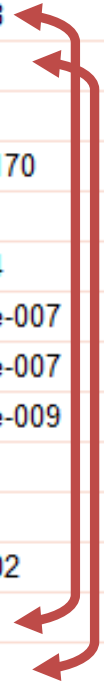
- The full model (fitting α and β independently) can work well when the allosteric modulator changes the orthosteric agonist E_{max} , or when the allosteric modulator or orthosteric agonist is a partial agonist.
- The full model does not work well when the only effect of the modulator is to shift the EC_{50} of a full orthosteric agonist. Under these and some other conditions the variability of the fitted values can be very large.



Results

1	[Pharmechanics]	
2	Best-fit values	
3	Em	
4	TauA	32.27
5	logKB	-6.988
6	alp	0.1878
7	bet	726.9
8	B	
9	TauB	0.005170
10	logKA	-6.489
11	n	0.9964
12	KA	3.245e-007
13	KB	1.028e-007
14	EC50 A	9.752e-009
15	Std. Error	
16	TauA	12.61
17	logKB	0.04202
18	alp	18.16
19	bet	70289
20	TauB	0.01524
21	logKA	0.1818
22	n	0.02749

Very large SE values!

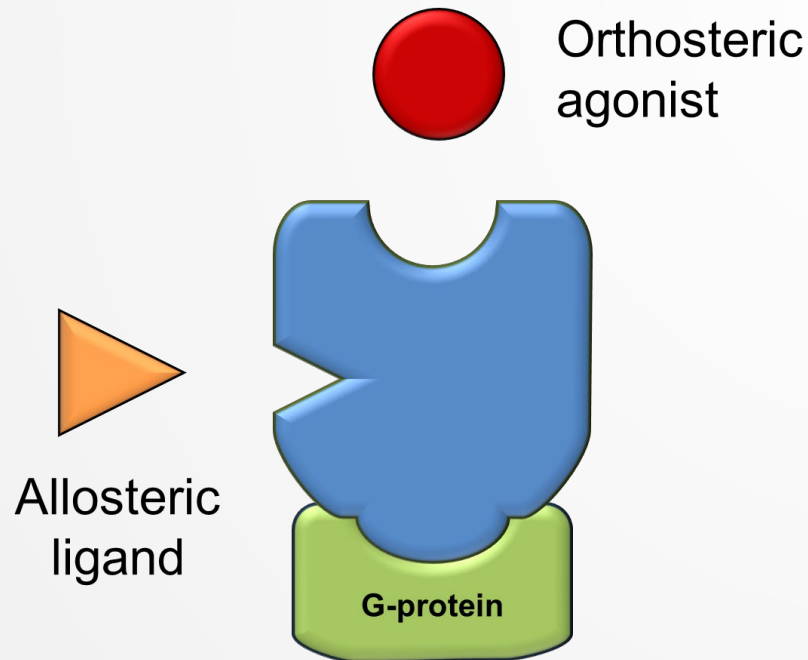


Modulation of orthosteric agonist signaling, combined $\alpha\beta$ model

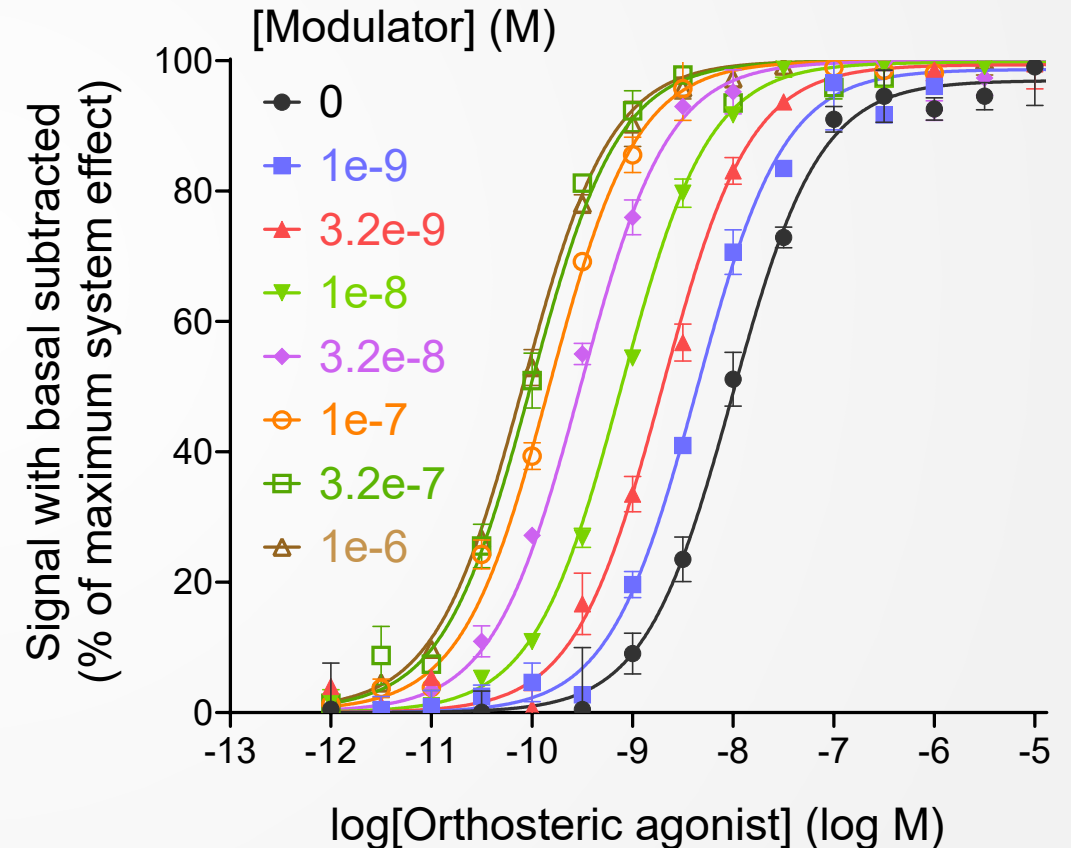
Modulation of orthosteric agonist signaling, combined $\alpha\beta$ model

Used for a signaling assay in which α and β cannot be independently determined, e.g. when the only effect of the modulator is to shift the EC_{50} of a full orthosteric agonist.

A reduced equation was derived by Christopoulos that allows a combined allosteric parameter ($\alpha\beta$) to be estimated when the orthosteric agonist is a full agonist – see [here](#).



Allosteric modulation of signaling, combined AlphaBeta



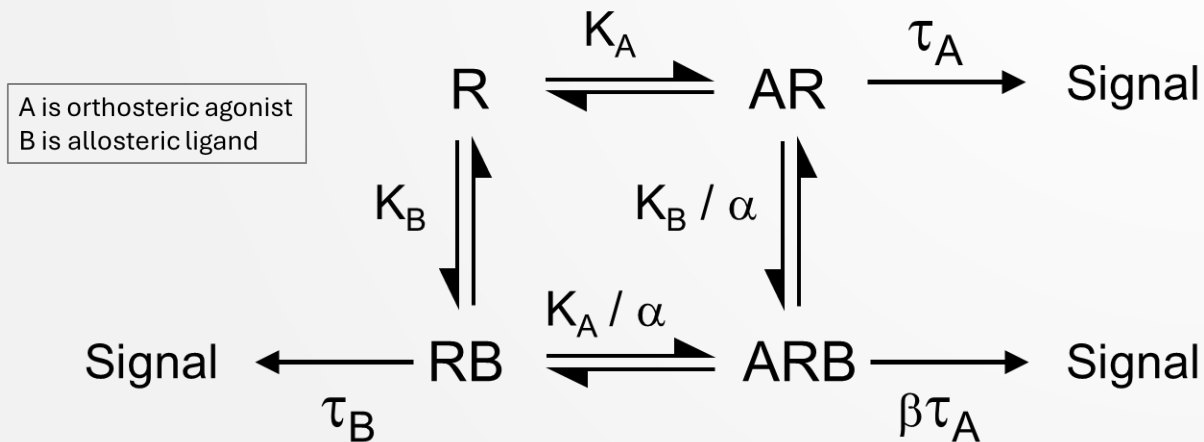
Modulation of orthosteric agonist signaling, combined $\alpha\beta$ model

An understanding of the [operational model of agonism](#) is required to apply this equation and interpret the results.

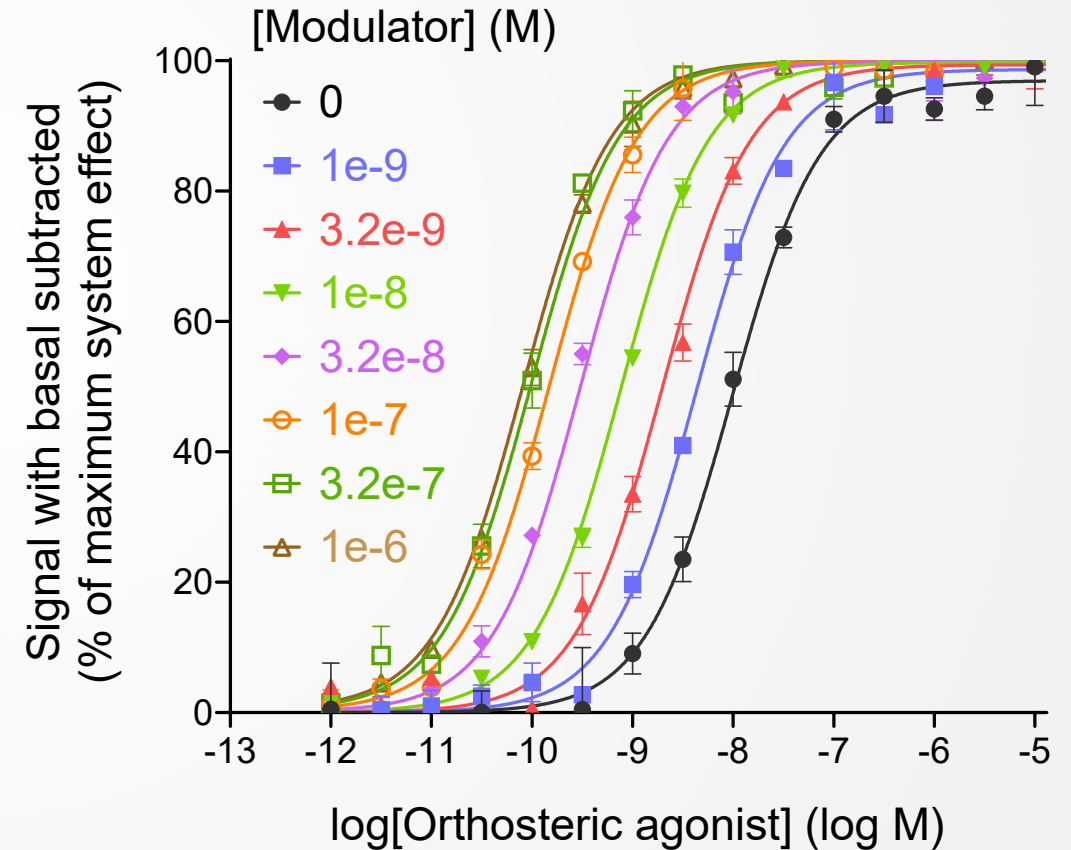
Model allows for agonism of allosteric modulator (but not inverse agonism).

There is a combined cooperativity factor:

- $\alpha\beta$, binding cooperativity multiplied by signaling cooperativity.



Allosteric modulation of signaling, combined AlphaBeta



Data entry

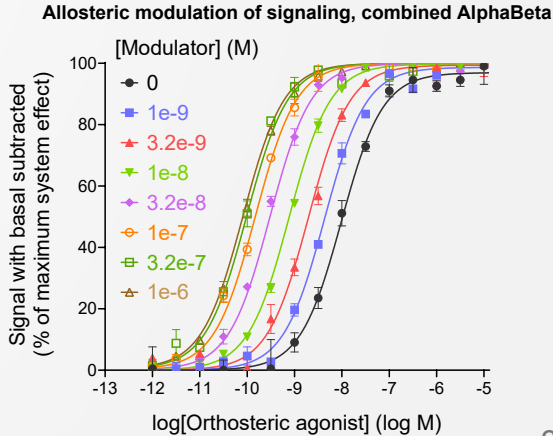
Table format: XY		X	Group A		Group B		Group C		Group D		Group E		Group F		Group G		Group H	
		log[Orthosteric agonist] (log M)	0		1e-9		3.2e-9		1e-8		3.2e-8		1e-7		3.2e-7		1e-6	
	X	X	A:Y1	A:Y2	B:Y1	B:Y2	C:Y1	C:Y2	D:Y1	D:Y2	E:Y1	E:Y2	F:Y1	F:Y2	G:Y1	G:Y2	H:Y1	H:Y2
1	Title	-12.000	-6.5	7.6	1.0	-1.2	4.3	3.7	3.5	-0.2	-4.2	-3.5	0.8	1.8	2.0	0.9	-2.2	2.6
2	Title	-11.500	-6.7	-0.4	2.5	-1.6	2.3	0.1	1.6	-1.5	-4.9	-1.1	2.3	5.2	13.2	4.3	4.6	4.5
3	Title	-11.000	-0.7	0.3	-1.5	3.4	5.0	5.9	3.4	-1.2	3.1	3.8	4.0	3.8	7.4	7.5	10.5	9.0
4	Title	-10.500	3.3	-3.2	0.8	4.2	2.4	3.2	5.8	4.8	13.4	8.5	26.5	22.0	22.2	28.9	26.4	26.9
5	Title	-10.000	-3.1	3.0	7.6	1.7	3.4	-2.1	10.3	11.5	26.0	28.4	37.3	41.4	46.7	55.2	50.2	55.7
6	Title	-9.500	10.0	-9.0	1.5	4.0	12.0	21.4	25.4	28.3	53.4	56.6	68.3	70.1	81.3	81.2	76.6	79.5
7	Title	-9.000	12.2	5.9	17.6	21.6	30.8	36.2	55.2	53.6	78.6	73.3	82.8	88.3	95.5	89.2	93.8	86.8
8	Title	-8.500	26.9	20.1	40.0	42.0	59.6	53.9	81.8	77.5	92.3	93.7	100.6	90.9	96.6	99.1	97.5	94.1
9	Title	-8.000	55.3	47.0	67.2	74.1	81.1	85.2	91.5	91.9	98.6	91.8	100.3	106.4	93.5	93.5	96.5	98.0
10	Title	-7.500	74.5	71.3	84.6	82.3	94.7	92.7	97.5	100.4	105.5	100.0	100.4	102.8	100.5	103.0	100.2	98.2
11	Title	-7.000	93.0	89.1	103.9	89.4	100.6	103.8	98.9	102.1	99.3	107.0	100.2	97.6	94.1	97.7	103.7	103.1
12	Title	-6.500	98.6	90.6	91.4	91.9	101.3	99.7	95.9	102.7	101.0	98.5	97.7	99.3	97.8	96.9	104.1	100.8
13	Title	-6.000	94.3	90.9	95.4	96.7	96.9	100.2	105.5	95.6	93.8	90.8	98.7	97.5	99.7	101.3	103.3	98.8
14	Title	-5.500	92.5	96.7	104.0	102.0	99.5	101.1	100.3	97.9	100.1	94.7	99.6	100.8	104.8	109.5	97.8	102.1
15	Title	-5.000	93.1	105.0	106.4	99.2	103.3	95.7	104.3	109.9	102.9	108.1	98.6	103.3	104.4	100.2	98.8	103.9
16	Title	-4.500	102.0	89.1	92.7	101.1	102.6	104.3	97.2	91.4	99.1	104.0	107.7	98.1	103.0	98.1	103.2	96.8

X values: Log of orthosteric agonist concentration (units of log M)

Different column groups: Different concentrations of allosteric modulator.

Column headers: Allosteric modulator concentration in M

Y values: Signal response with basal signal subtracted (basal is signal in the absence of orthosteric and allosteric ligand)



Model

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Choose an equation

- + Dose-response - Special, X is log(concentration)
- + Binding - Saturation
- + Binding - Competitive
- + Binding - Kinetics
- + Enzyme kinetics - Inhibition
- + Enzyme kinetics - Velocity as a function of substrate
- + Exponential
- + Lines
- + Polynomial
- + Gaussian
- + Sine waves
- + Growth curves
- + Linear quadratic curves
- + Classic equations from prior versions of Prism
- [Pharmacokinetics] GPCR allosteric modulator analysis
 - [Pharmacokinetics] Allosteric modulation of binding
 - [Pharmacokinetics] Allosteric modulation of labeled and unlabeled ligand b
 - [Pharmacokinetics] Allosteric modulation of signaling, full model
 - [Pharmacokinetics] Allosteric modulation of signaling, combined AlphaBeta

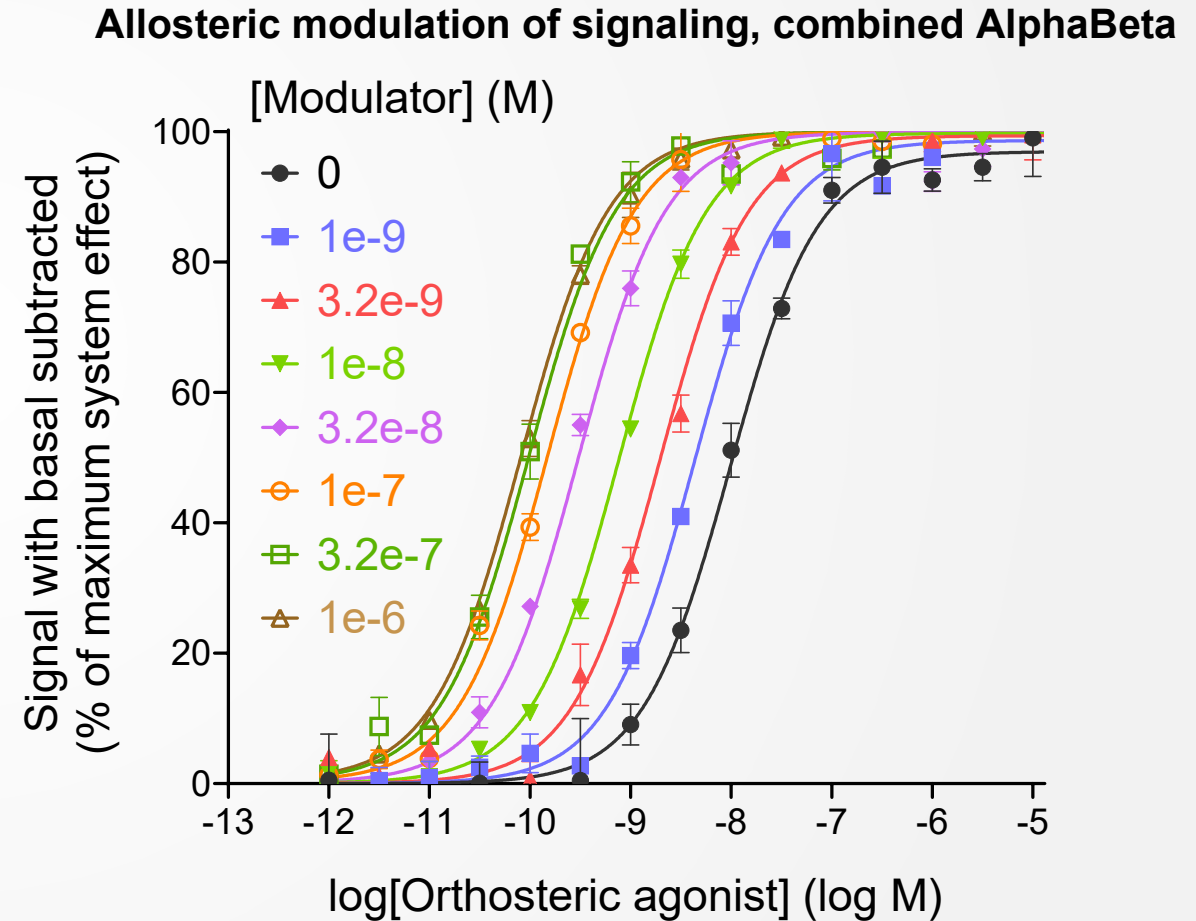
“Allosteric modulation of signaling, combined AlphaBeta”

For functional assays combining orthosteric agonist (A) and allosteric ligand (B) for fitting combined AlphaBeta. Ortho. agonist conc is X value, signal response is Y value, and signal response for different allosteric ligand concs go in seperate columns.

[Pharmacokinetics] Allosteric modulation of signaling, combined AlphaBeta
Numerical derivatives

Interpolate
 Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK



Analysis details

Parameters: Nonlinear Regression

Model Method Compare **Constrain** Initial values Range Output Confidence Diagnostics Flag

Parameter Name	Constraint Type	Value	Hook
Em	Must be greater than	0	
logKB	Shared value for all data sets		
AlpBet	Shared, and must be greater than	0	
B	Data set constant (from column title)		
TauB	Shared, and must be greater than	0	
logEC50A	Shared value for all data sets		
n	Shared, and must be greater than	0	

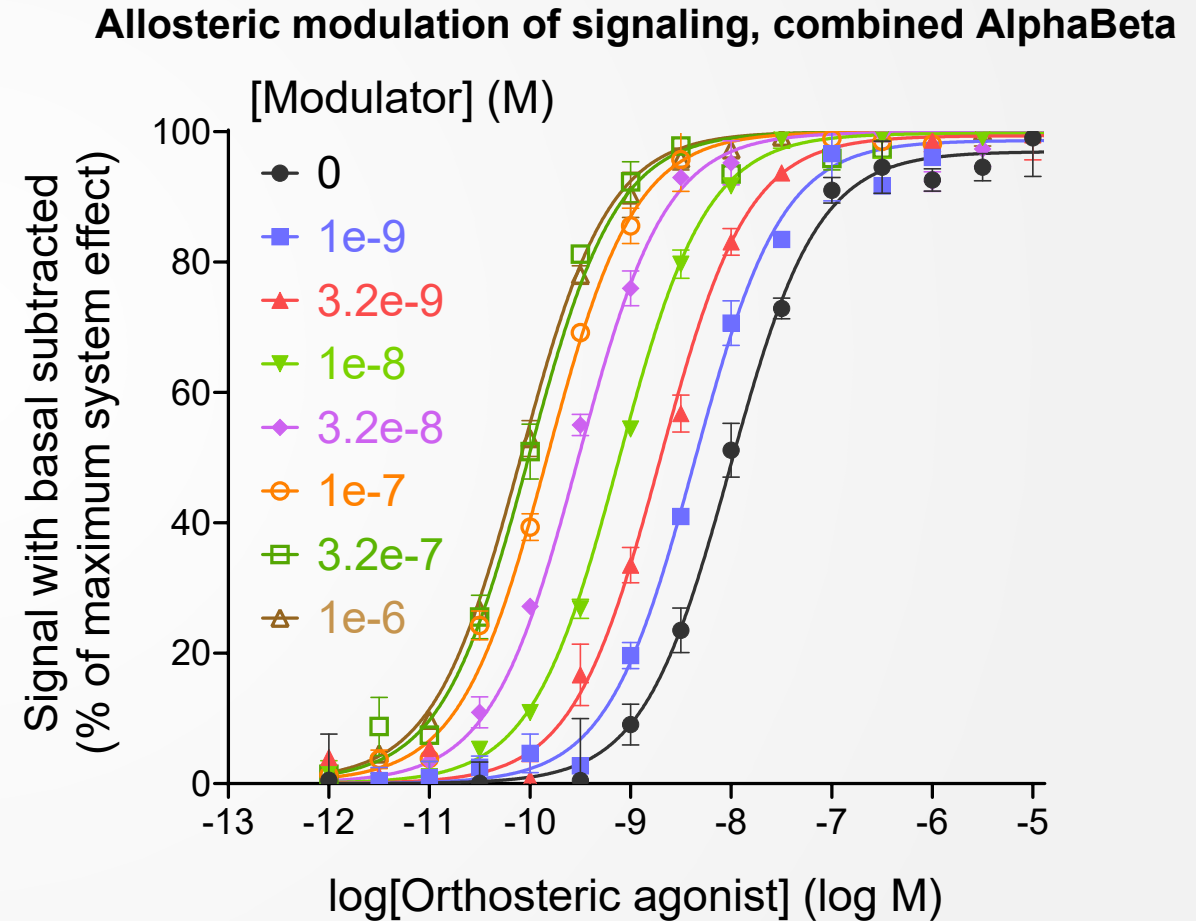
Global analysis – all curves fit simultaneously with shared fit parameters.

Constrain one parameter relative to another

must be greater than 1 times

must be greater than 1 times

Learn Cancel OK



Analysis details

Parameters: Nonlinear Regression

Model Method Compare **Constrain** Initial values Range Output Confidence Diagnostics Flag

Parameter Name	Constraint Type	Value	Hook
Em	Constant equal to	100	
logKB	Shared value for all data sets		
AlpBet	Shared, and must be greater than	0	
B	Data set constant (from column title)		
TauB	Shared, and must be greater than	0	
logEC50A	Shared value for all data sets		
n	Shared, and must be greater than	0	

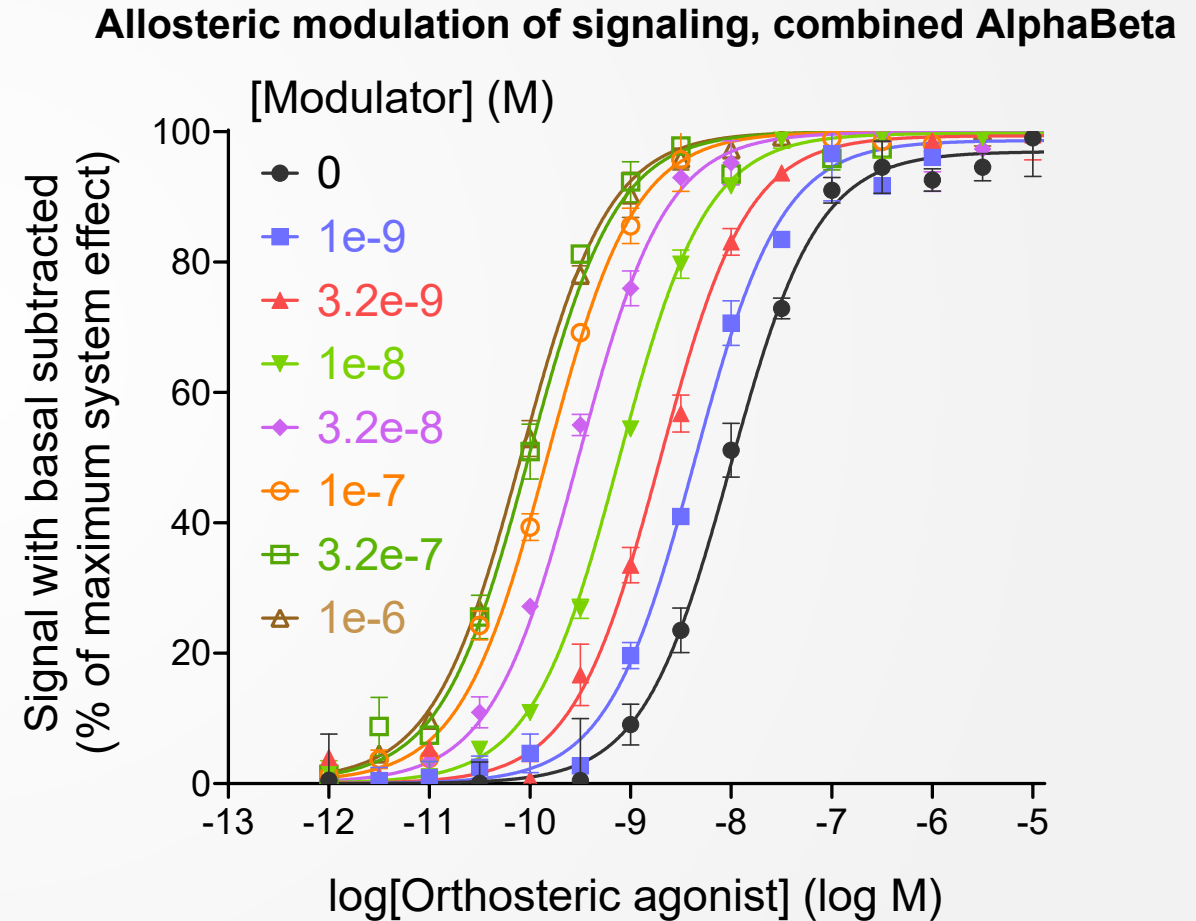
Recommend constraining Em (system maximum effect) if this value can be independently determined, e.g. as the Emax of a full agonist.

Constrain one parameter relative to another

must be greater than 1 times

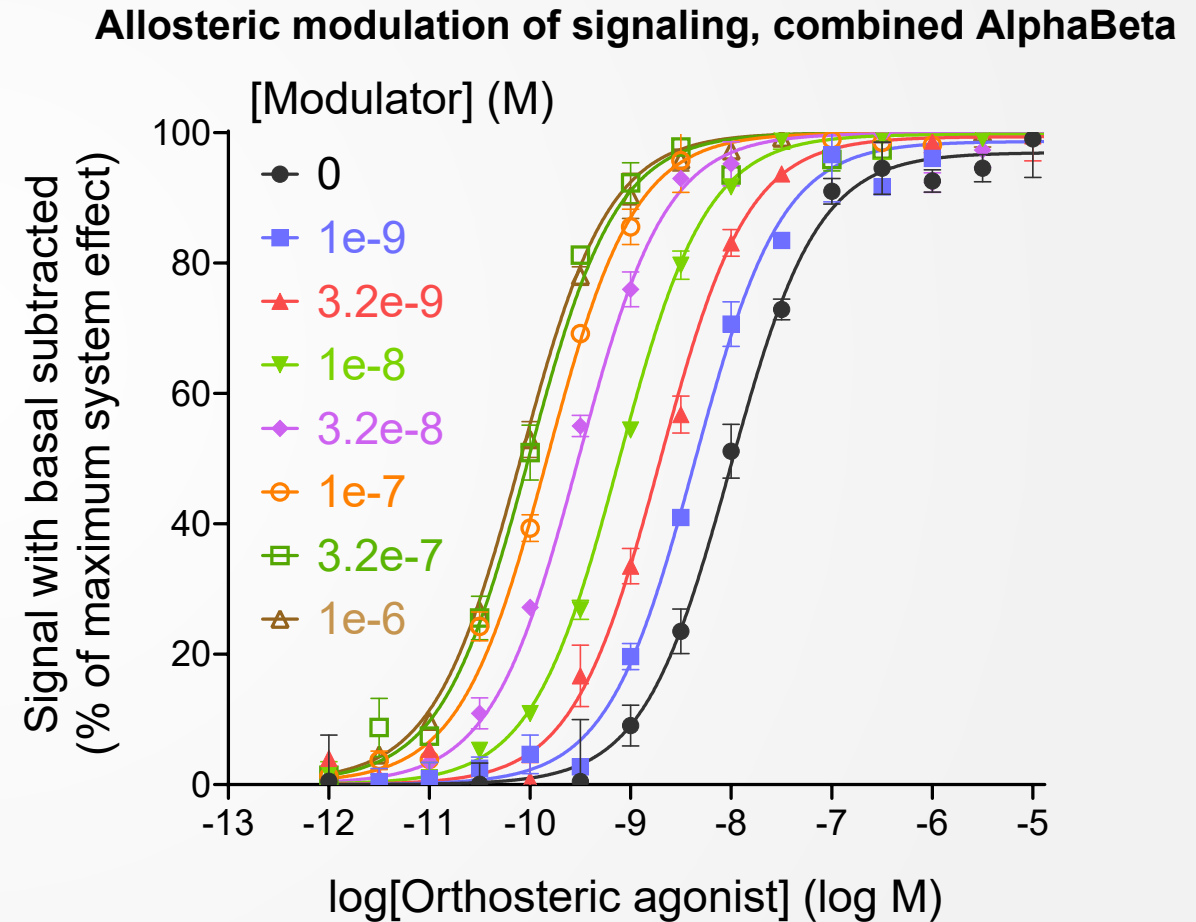
must be greater than 1 times

Learn Cancel OK



Results table

Nonlin fit Table of results		Global (shared)
1	[Pharmechanics] Allosteric	
2	Best-fit values	
3	Em	= 100.0
4	logKB	-7.512
5	AlpBet	26.05
6	B	
7	TauB	2.980e-008
8	logEC50A	-7.962
9	n	0.9850
10	KB	3.078e-008
11	EC50 A	1.090e-008
12	EC50 B	-7.512
13	Emax A (=Em)	= 100.0
14	Emax B	2.980e-008
15	Std. Error	
16	logKB	0.03715
17	AlpBet	1.668
18	TauB	0.007104
19	logEC50A	0.02321
20	n	0.01894
21	Emax B	0.007104



Results table

Nonlin fit Table of results		Global (shared)
1	[Pharmechanics] Allosteric	
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11	EC50 A	1.090e-008
12	EC50 B	-7.512 ^A
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17	AlpBet	1.668
18	TauB	0.007104
19	logEC50A	0.02321
20	n	0.01894
21	Emax B	0.007104

Parameters for allosteric modulator

AlpBet: $\alpha\beta$, binding cooperativity on orthosteric agonist (α) multiplied by signaling cooperativity on orthosteric agonist (β)

logKB: Logarithm of modulator affinity (log M)

KB: Modulator affinity (M)

TauB, signaling efficacy of allosteric modulator

EC50 B, EC_{50} of allosteric modulator (M)^A

Emax B, E_{max} of allosteric modulator (Y axis units)

Parameters for orthosteric agonist

logEC50A : Logarithm of orthosteric agonist EC_{50} (log M)

EC50 A, EC_{50} of orthosteric agonist (M)

Emax A, E_{max} of orthosteric agonist (Y axis units)

n, transducer Hill slope of orthosteric agonist

System parameter

Em, system maximum effect (Y axis units)

A) In this example the EC50 B value is meaningless because the allosteric modulator did not stimulate signaling.