



Biased signaling and L1 penalization

Romain Yvinec

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Biased signaling and L1 penalization

Towards a system biology definition of drugs selectivity

Romain Yvinec

BIOS, INRA Centre Val-de-Loire

What is biased signaling?

Some examples

Bias calculus - standard method : operational model

Some extension to bias calculus

Biased signaling using dynamical model

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Biased signaling using dynamical model

Functional selectivity, biased signaling

Functional selectivity

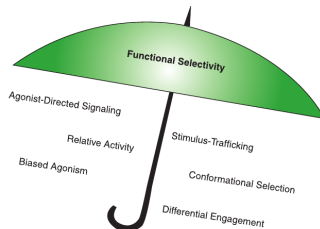
From Wikipedia, the free encyclopedia

Not to be confused with [binding selectivity](#).

Functional selectivity (or “agonist trafficking”, “biased agonism”, “biased signalling”, “ligand bias”, and “differential engagement”) is the [ligand](#)-dependent selectivity for certain [signal transduction](#) pathways in one and the same [receptor](#). This can be present when a receptor has several possible signal transduction pathways. To which degree each pathway is activated thus depends on which ligand binds to the receptor.^[1]



Exp. Biology 2005 (ASPET, San Diego)
Simmons, *Mol. Interventions* (2005)
Urban et al., *J Pharmacol Exp Ther*
(2007)



Functional selectivity, biased signaling

Functional selectivity

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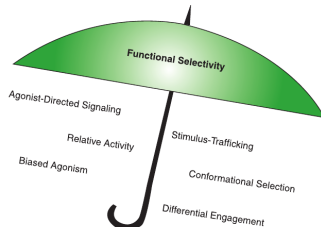
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- ▶ Ligand-specific activity in one receptor



Exp. Biology 2005 (ASPET, San Diego)
Simmons, *Mol. Interventions* (2005)
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Functional selectivity, biased signaling

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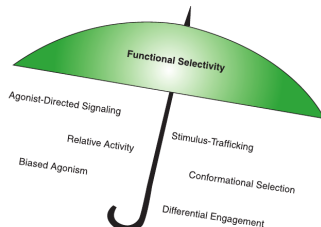
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- ▶ Several potential pathways associate to a given receptor



Exp. Biology 2005 (ASPET, San Diego)
Simmons, *Mol. Interventions* (2005)
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Functional selectivity, biased signaling

Functional selectivity

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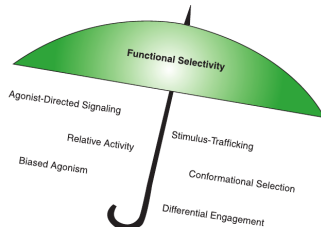
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- ▶ Differential activation of certain pathways



Exp. Biology 2005 (ASPET, San Diego)
Simmons, *Mol. Interventions* (2005)
Urban et al., *J Pharmacol Exp Ther*
(2007)



Key concept in pharmacology

- ◇ Biased agonism is a key concept to be distinguish from
 - Partial or full agonist.
 - Antagonist, inverse agonist.
 - Affinity (K_d), potency (EC_{50}), efficacy (E_{max}).

Key concept in pharmacology

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- ◇ A bias might be **context-dependent** (cell type, physiological state, etc.)

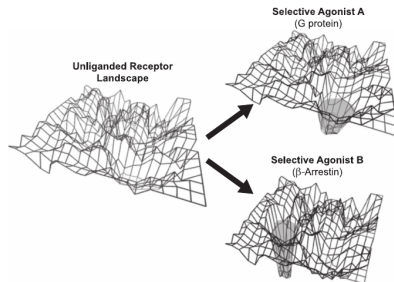
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 - Partial or full agonist.
 - Antagonist, inverse agonist.
 - Affinity (K_d), potency (EC_{50}), efficacy (E_{max}).
 - ◇ A bias might be **context-dependent** (cell type, physiological state, etc.)
 - ◇ Biased agonism is becoming a major tool in drug discovery.
- ⇒ Candidate screening requires to accurately quantify bias.

Theoretical foundation

A receptor may adopt several spatial conformations, each of which has different activation pathway profiles.

- ▶ Conformational selectivity = Ligand-specific modification of the energetic landscape, changing affinities and efficacies of signaling pathways.

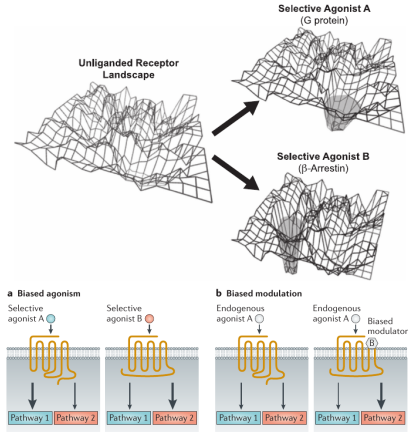


Kenakin, *J Pharmacol Exp Ther* (2011)

Theoretical foundation

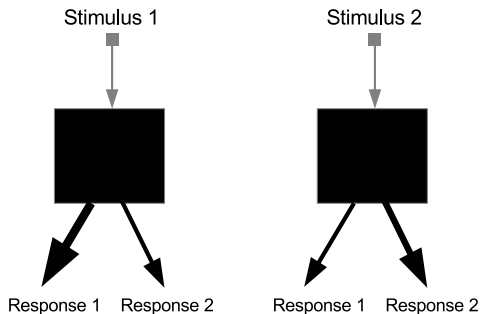
A receptor may adopt several spatial conformations, each of which has different activation pathway profiles.

- ▶ Conformational selectivity = Ligand-specific modification of the energetic landscape, changing affinities and efficacies of signaling pathways.
- ▶ Similar concept : modulating bias



Summary so far

To speak about signaling bias, one necessarily needs **two** ligands and **two** responses, in a **same** cellular context.



⇒ We always compare *a ligand with respect to a reference one*.

What is biased signaling ?

Some examples

Bias calculus - standard method : operational model

Some extension to bias calculus

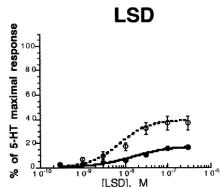
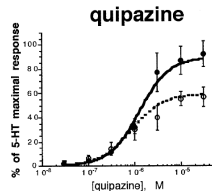
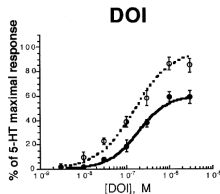
Biased signaling using dynamical model

Serotonin receptor 5 – HT_{2C}

- ▶ Quipazine is biased towards *PI* accumulation with respect to AA production, *compared to the reference agonist DOI*.
- ▶ *LSD* is not biased.



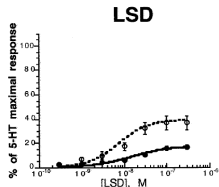
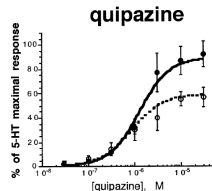
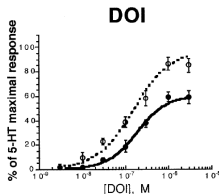
Berg et al., *Mol. Pharmacol.* (1998)



--○-- AA release
—●— IP accumulation

Serotonin receptor 5 – HT_{2C}

- ▶ Quipazine is biased towards PI accumulation with respect to AA production, compared to the reference agonist DOI .
- ▶ LSD is not biased.
- ▶ Bias due to an E_{max} difference.

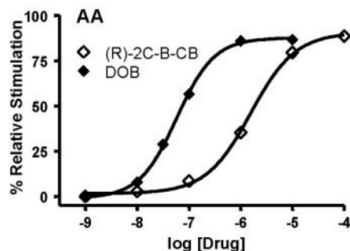
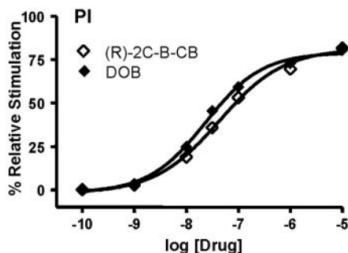


--○-- AA release
—●— IP accumulation



Berg et al., *Mol. Pharmacol.* (1998)

Serotonin receptor 5 – HT_{2A}

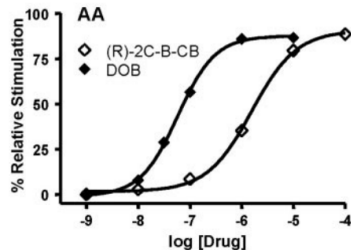
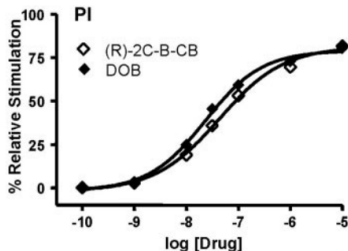


(R) – 2C – B – CB is biased towards PI accumulation with respect to AA production, compared to the reference agonist DOB.



Urban et al., *J Pharmacol Exp Ther* (2007)

Serotonin receptor 5 – HT_{2A}



(R) – 2C – B – CB is biased towards *PI* accumulation with respect to *AA* production, *compared to the reference agonist DOB*.

- Bias due to an EC_{50} difference.



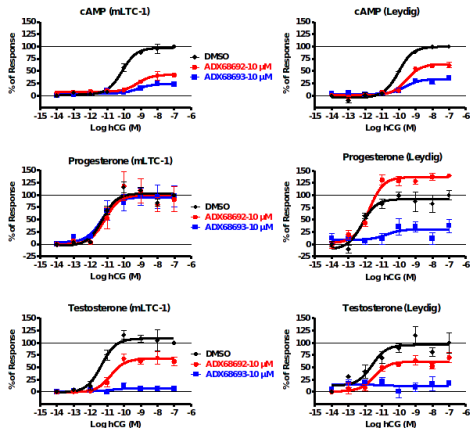
Urban et al., *J Pharmacol Exp Ther* (2007)

Steroidogenesis modeulated by NAM

Some negative allosteric modulators (NAM) can biased Progesterone production with respect to Testosterone production, under stimulation of LH/CG receptor by hCG.



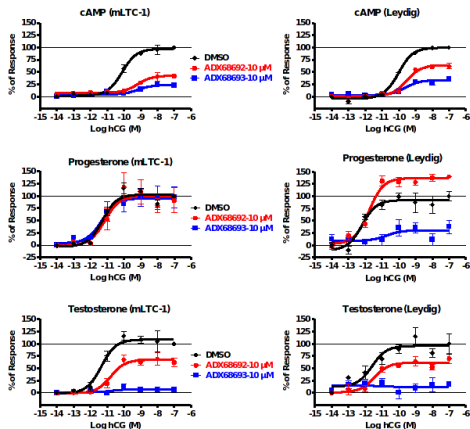
Ayoub et al., *Mol. Cell. Endocrinol* (2016)



Steroidogenesis modeulated by NAM

Some negative allosteric modulators (NAM) can biased Progesterone production with respect to Testosterone production, under stimulation of LH/CG receptor by hCG.

- ▶ Selective (biased) allosteric modulation



Ayoub et al., *Mol. Cell. Endocrinol* (2016)

GPCR

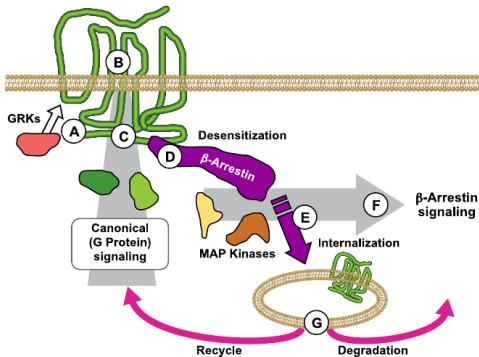
Many GPCR's are known
to have biased ligands
(G / β -arrestin)



Kenakin, *J Pharmacol
Exp Ther* (2011)



Kenakin, *Chem Rev*
(2017)



Outline

What is biased signaling ?

Some examples

Bias calculus - standard method : operational model

Some extension to bias calculus

Biased signaling using dynamical model

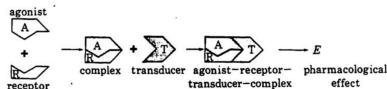
Operational model

Dose-response data are fitted with the function

$$y = E_{tot} \frac{\tau^n [L]^n}{([L] + K_A)^n + \tau^n [L]^n}.$$

- ▶ Response at equilibrium of a Michaelis-Menten type model.
- ▶ K_A = **Dissociation constant** of the couple Ligand/Receptor
- ▶ $\tau = R_{tot}/K_E$, **Efficacy coefficient** (K_E is dissociation constant of the ternary complex)

J. W. Black and P. Leff



Black and Leff, *Proc. R. Soc. Lond. B* (1983)

Operational model

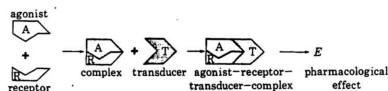
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$$y = E_{tot} \frac{\tau^n [L]^n}{([L] + K_A)^n + \tau^n [L]^n}.$$

For $n = 1$,

- ▶ $EC_{50} = \frac{K_A}{\tau + 1}$
- ▶ Efficacy $y_{\infty}/E_{tot} = \frac{\tau}{\tau + 1}$

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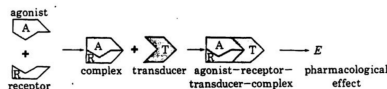
- ▶ $EC_{50} = \frac{K_A}{\tau + 1}$
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Then, we define

- ▶ **Transduction coefficient :**

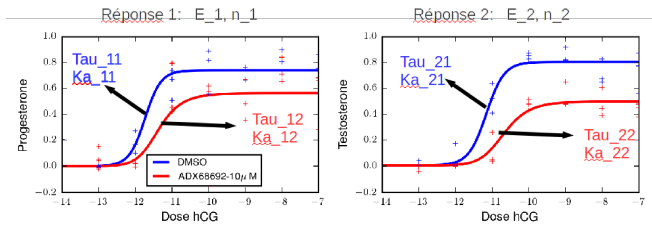
$$R := \log \left(\frac{\tau}{K_A} \right)$$

J. W. Black and P. Leff



Black and Leff, *Proc. R. Soc. Lond. B* (1983)

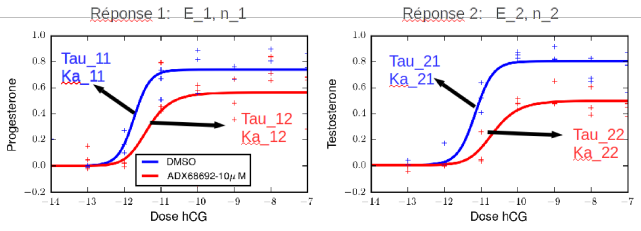
Bias definition With the operational model



Two ligands ($j = 1, 2$) and **two** measured responses ($i = 1, 2$) :
Each dose-response data is fitted with the operational model :

$$y_{ij} = E_i \frac{\tau_{ij}^{n_i} [L]^{n_i}}{([L] + Ka_{ij})^{n_i} + \tau_{ij}^{n_i} [L]^{n_i}} .$$

Bias definition With the operational model

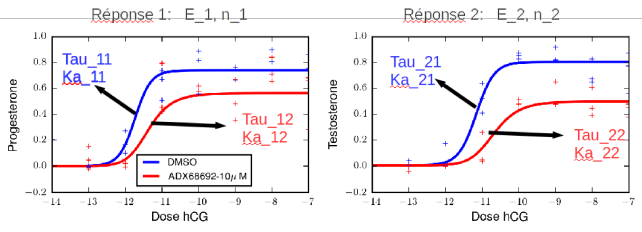


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 $\Delta_i \log(\tau/K_a) = \log(\tau_{i2}/Ka_{i2}) - \log(\tau_{i1}/Ka_{i1}) .$

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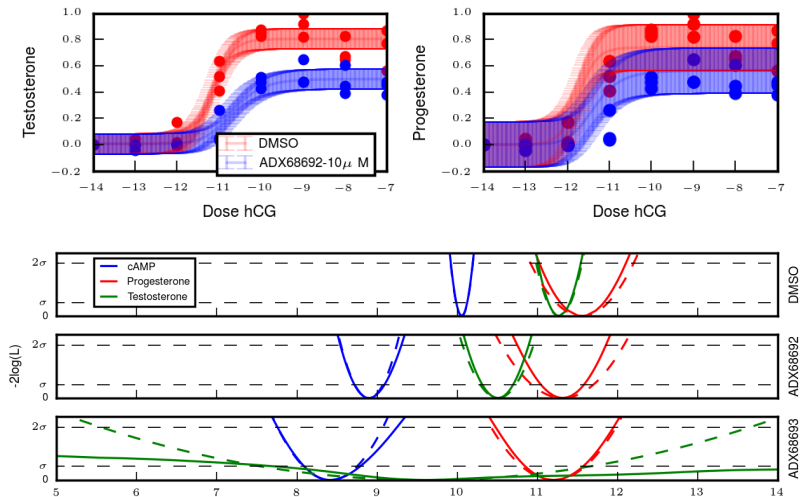
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The **Bias** is then defined by

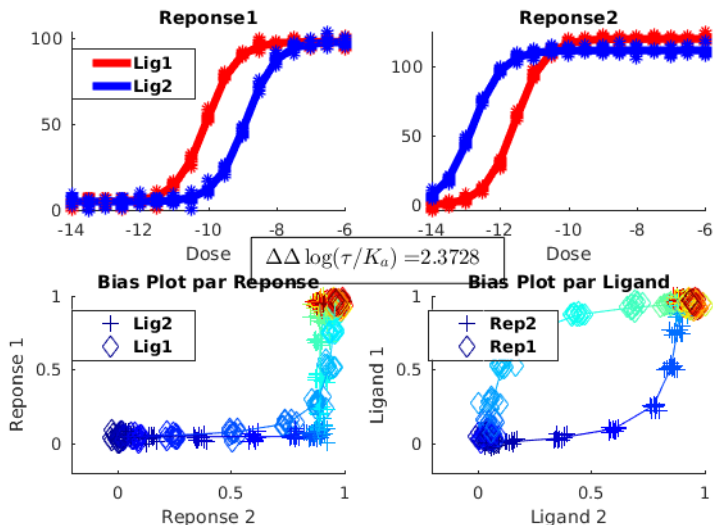
$$\Delta\Delta \log(\tau/K_a) = \Delta_2 \log(\tau/K_a) - \Delta_1 \log(\tau/K_a)$$

Statistical consideration



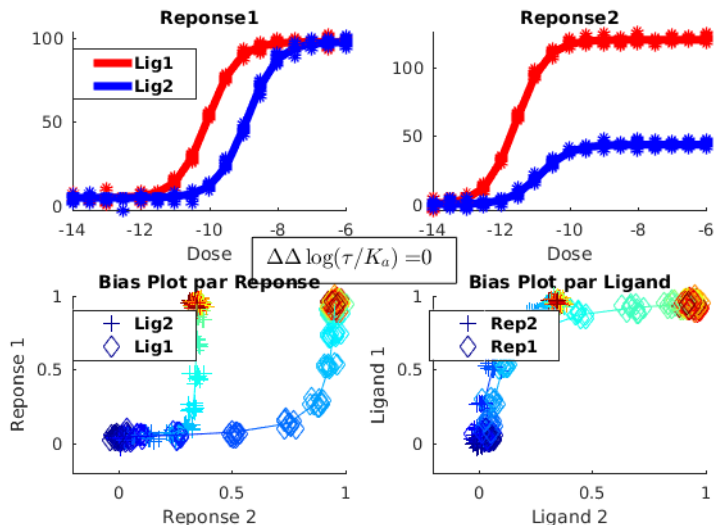
Raue A., et al. *Bioinformatics* (2015)

Is bias calculation intuitive? (simulated data)



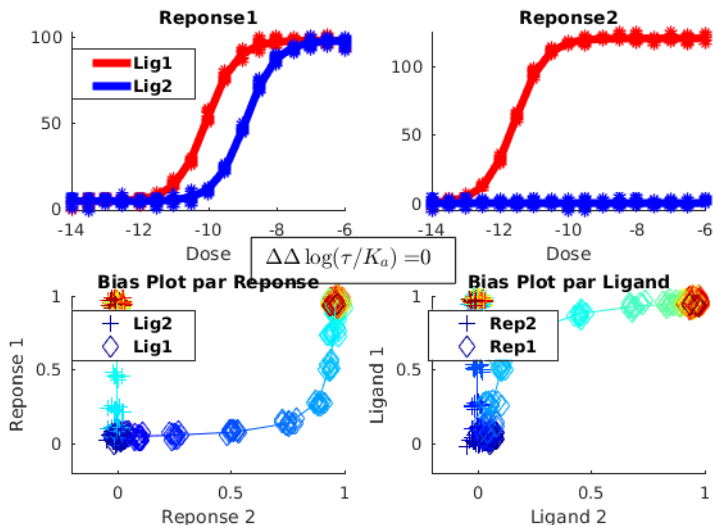
- ▶ A strong bias is usually 'apparent' on dose-response curves or bias plot

Is bias calculation intuitive? (simulated data)



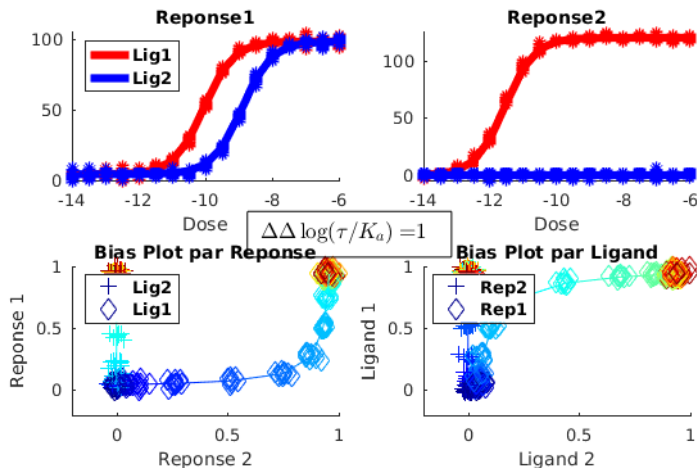
- But there may be counter-intuitive situation...

Is bias calculation intuitive? (simulated data)



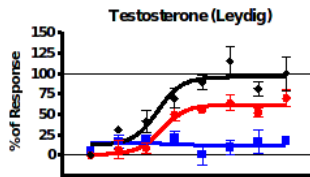
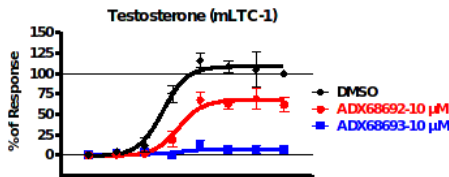
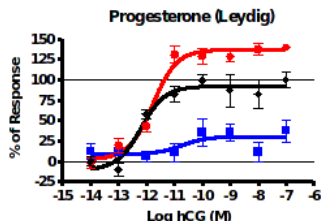
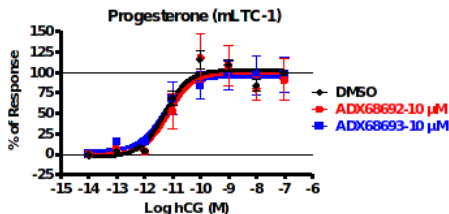
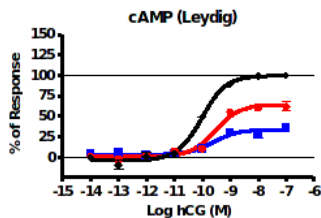
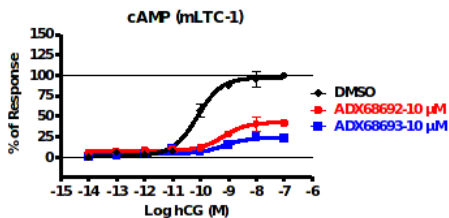
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Is bias calculation intuitive? (simulated data)



- But there may be counter-intuitive situation...

- ... and those situations occur in real life!



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Biased signaling using dynamical model

Time-dependent bias

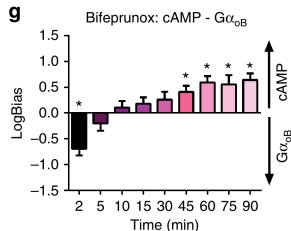
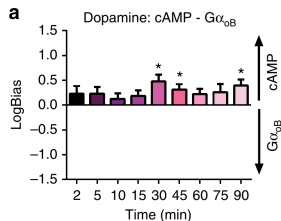
The role of kinetic context in apparent biased agonism at GPCRs

Carmen Klein Herenbrink¹, David A. Sykes², Prashant Donthamsetti^{3,4}, Meritxell Canals¹, Thomas Coudrat¹, Jeremy Shonberg⁵, Peter J. Scammells⁵, Ben Capuano⁵, Patrick M. Sexton¹, Steven J. Charlton², Jonathan A. Javitch^{3,4,6}, Arthur Christopoulos¹ & J Robert Lane¹

- ▶ Bias value may change according to the response time after stimulation.
- ▶ Kinetic explanation : Ligands with a slow binding kinetics may have changing bias value according to time.



Klein Herenbrink et al., *Nat. Commun* (2016)



Other extensions

- ▶ Dose-dependent bias



Barak and Peterson et al.,
Biochem. (2012)

- ▶ Extension of the operational model

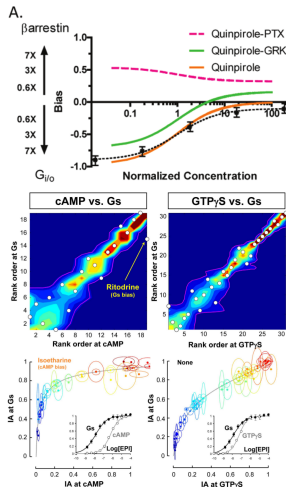


Kenakin, *Chem. Rev.* (2017)

- ▶ Method based on Intrinsic activities and rank ordering



Onaran et al., *Sci. Rep.*
(2017)



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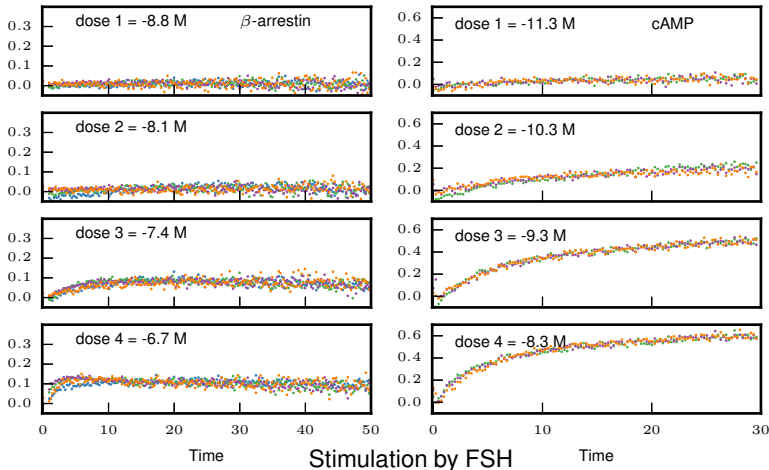
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Biased signaling using dynamical model

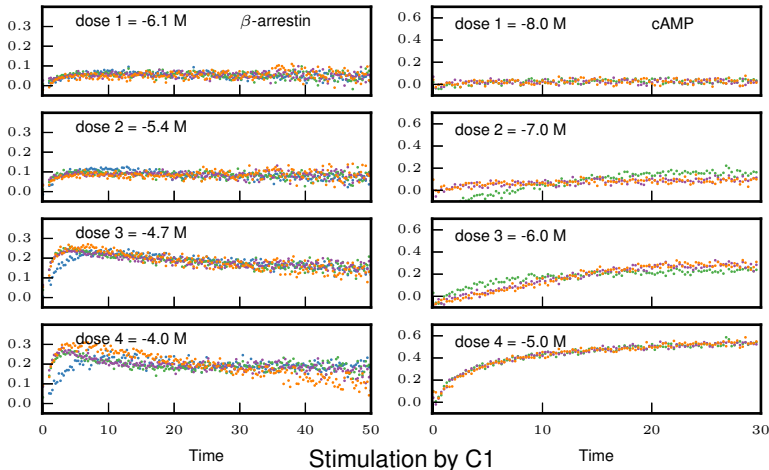
Dynamic data (on FHSR in HEK cells)

Instead of focusing on dose-response curves, we deal with several doses kinetic experiments (here : induced BRET data)



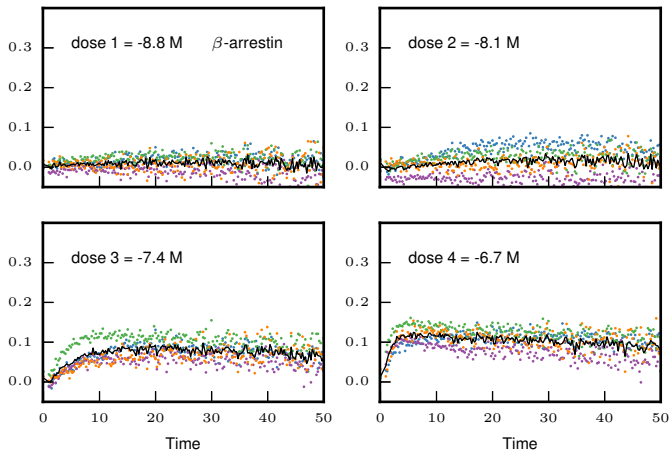
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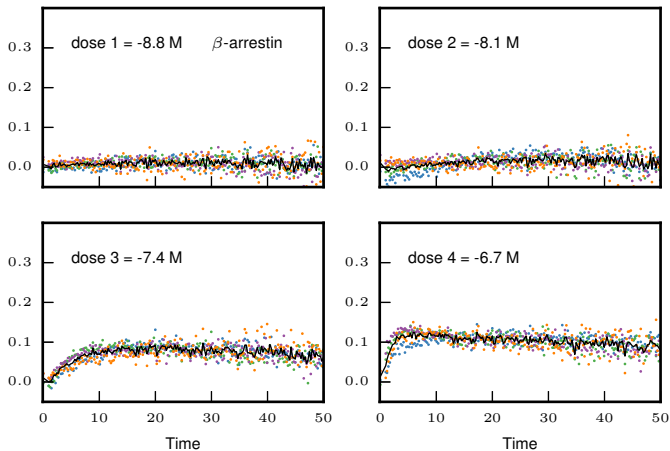
("trick" to minimize variance...)

Original "raw" data



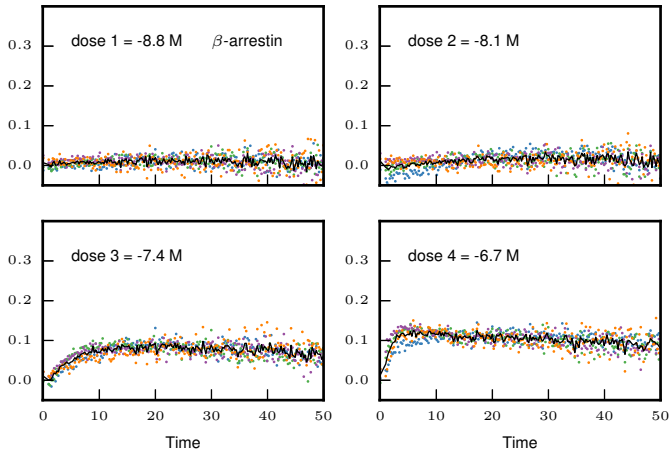
("trick" to minimize variance...)

"Adjusted" data



("trick" to minimize variance...)

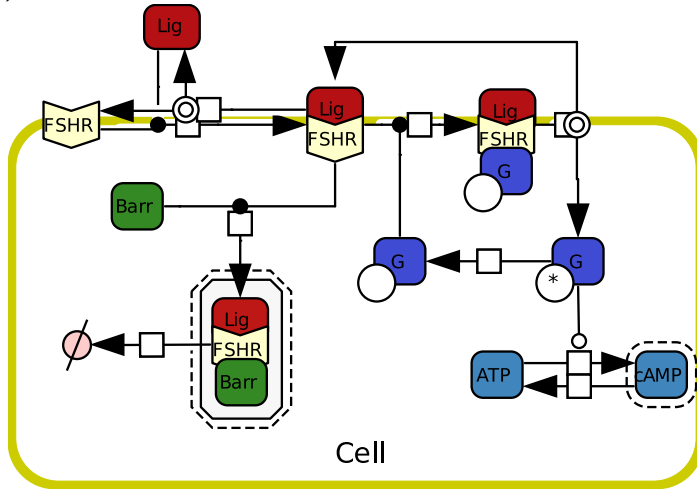
"Adjusted" data



+ adjusting the number of data points ...

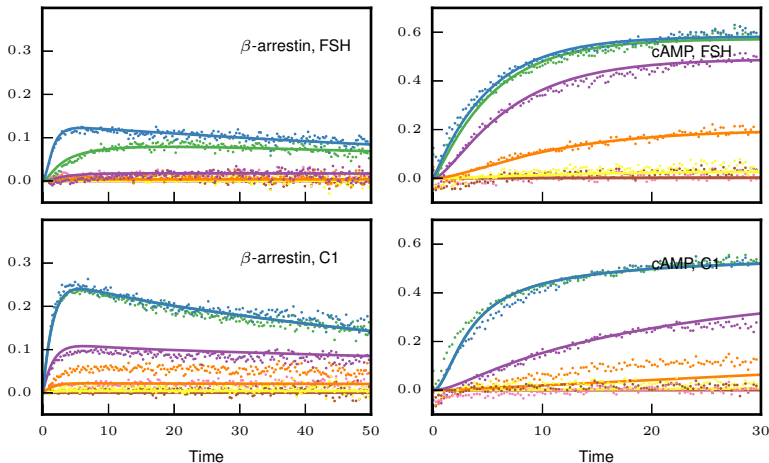
Principle

1) We start with a sufficiently detailed chemical reaction network



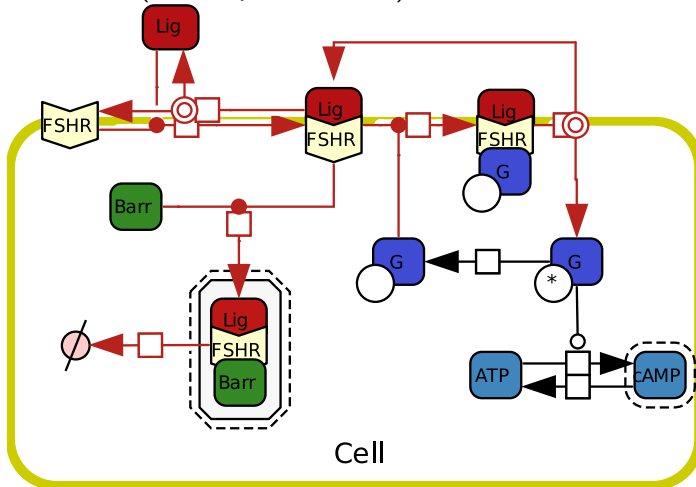
Principle

I) We start with a sufficiently detailed chemical reaction network to accurately fit the data (one separate model for each Ligand)



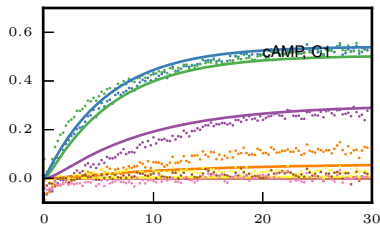
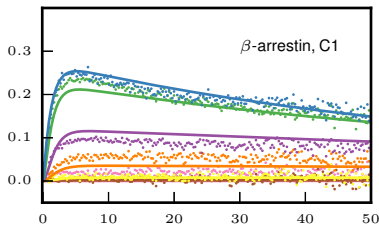
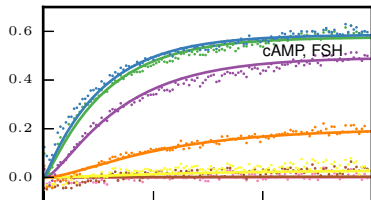
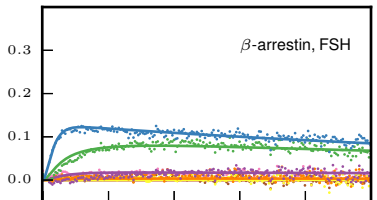
Principle

II) We fit all data at once, using some common parameters (initial concentration of molecules, measurement parameters...) and some different ones (kinetic parameters...)



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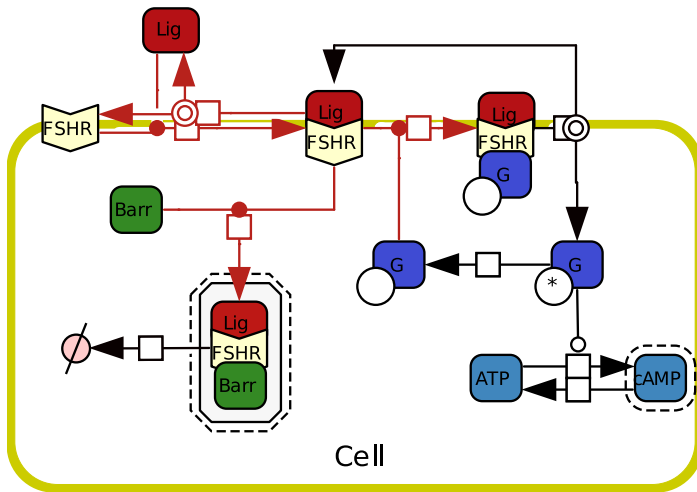


Time

Time

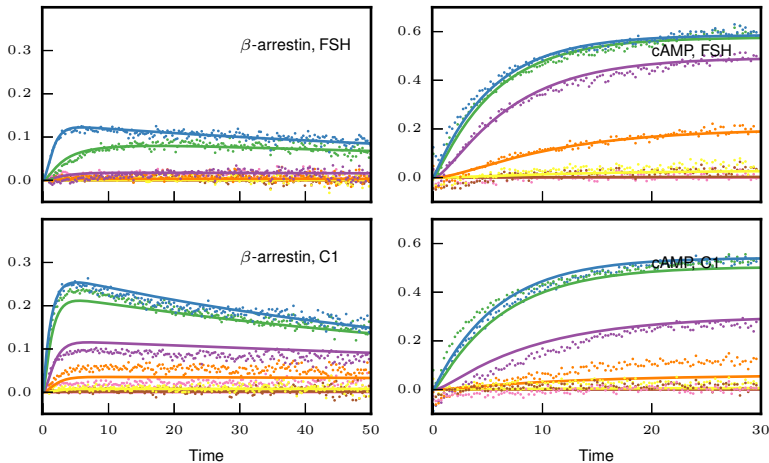
Principle

III) We use L^1 -penalization to find the needed ligand specific parameters



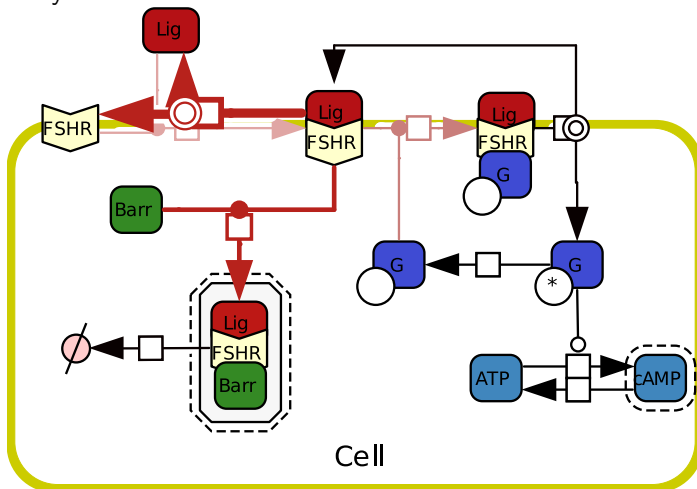
Principle

III) We use L^1 -penalization to find the needed ligand specific parameters, keeping the fit 'as good as before'



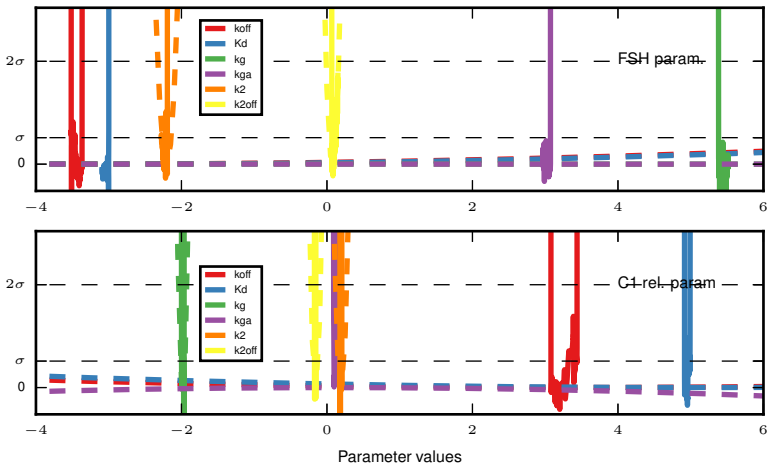
Principle

IV) After re-optimization, the set of distinct (ligand-specific) kinetic parameters gives us an accurate description of ligand specificity.



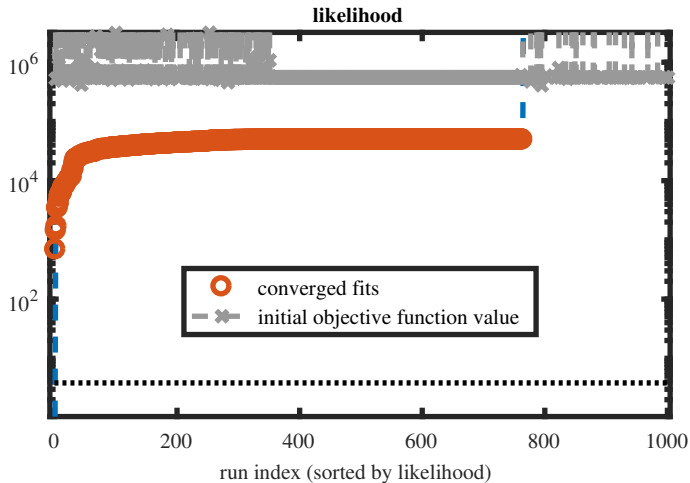
Principle

V) Significant differences between parameters is assessed by PLE

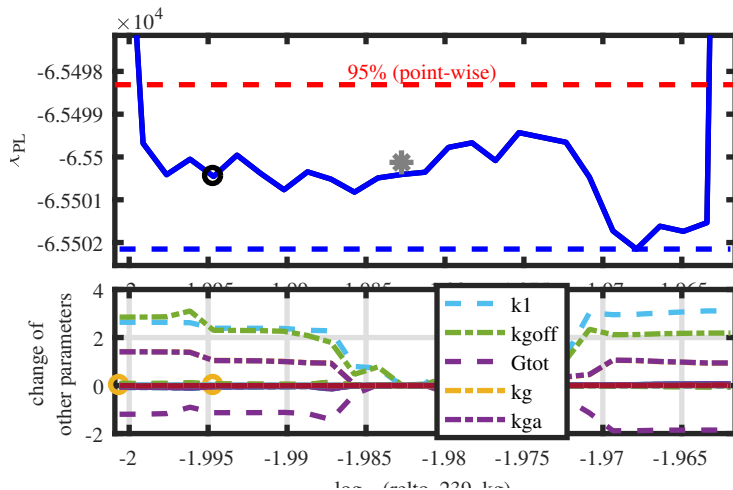


C1 is biased towards β -arr, compared to cAMP, in comparison to FSH.

Practical problems...

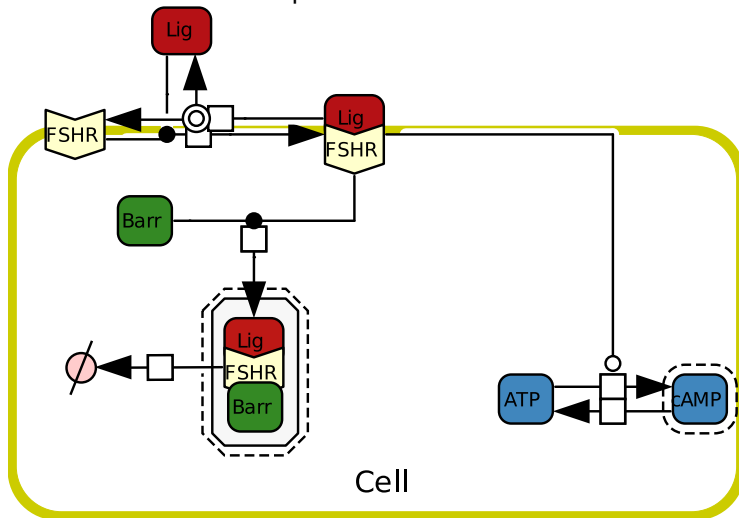


Practical problems...



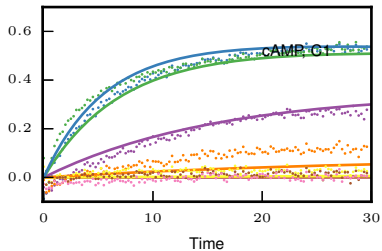
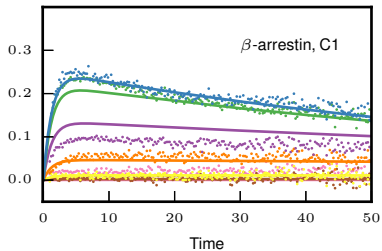
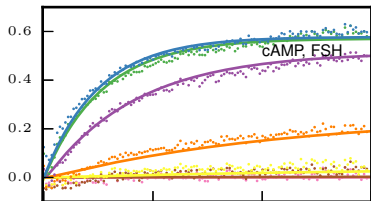
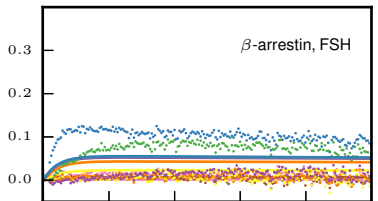
With a "simpler" model

Kinetic model without G-protein



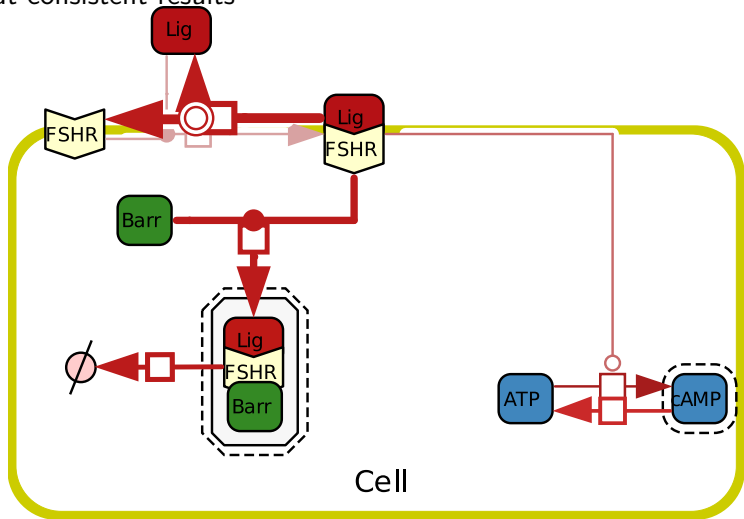
With a "simpler" model

We obtain a slightly worse fit



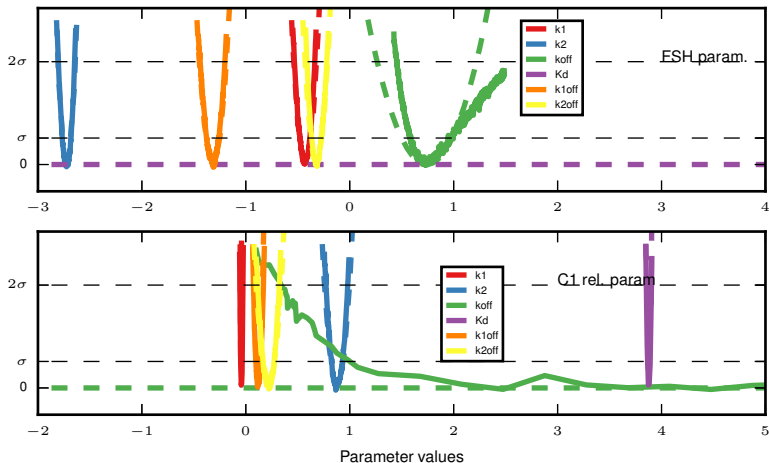
With a "simpler" model

But consistent results



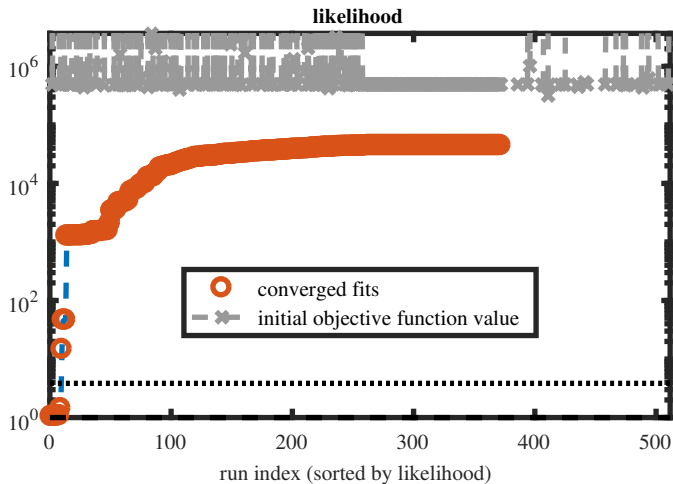
With a "simpler" model

And "better" parameter identifiability



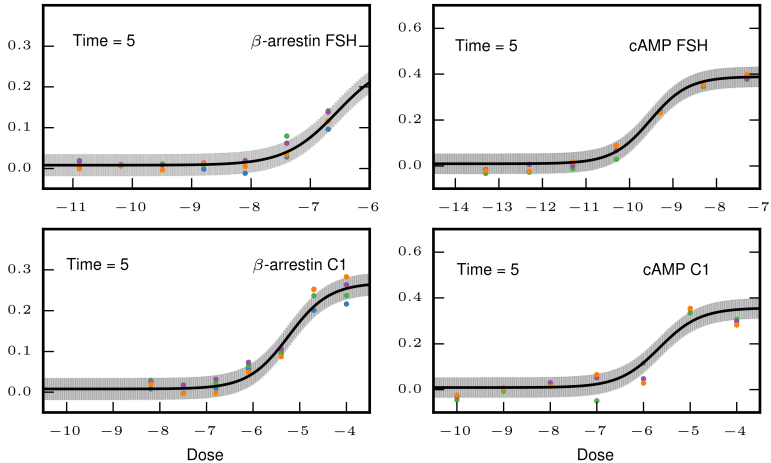
C1 is biased towards β -arr, compared to cAMP, in comparison to FSH.

With a "simpler" model



Comparison with dose-response (on FHSR in HEK cells)

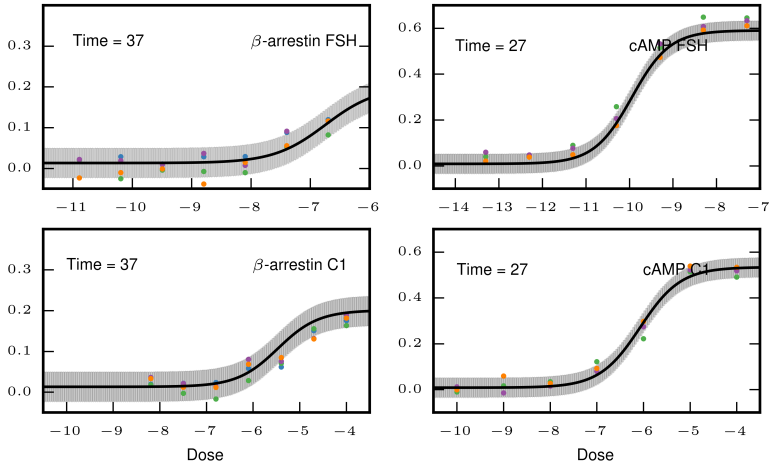
We systematically calculate bias value using standard method
(operational model on dose-response curves :)



Bias=2.3 : C1 is biased towards β -arr, compared to cAMP, in comparison to FSH.

Comparison with dose-response (on FHSR in HEK cells)

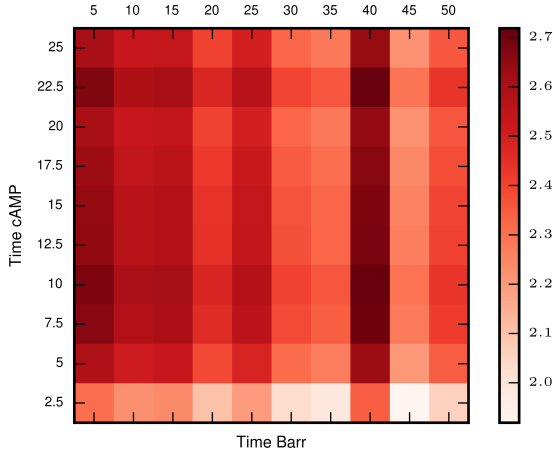
We systematically calculate bias value using standard method
(operational model on dose-response curves :)



Bias=2.64 : C1 is biased towards β -arr, compared to cAMP, in comparison to FSH.

Comparison with dose-response (on FHSR in HEK cells)

We systematically calculate bias value using standard method
Different times gives (slightly) different bias values

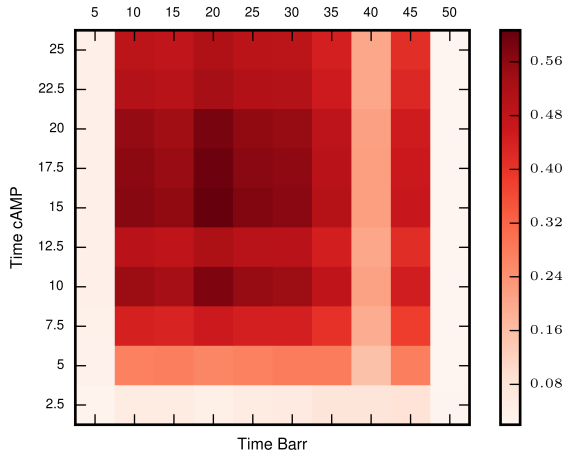


C1 is biased towards β -arr, compared to cAMP, in comparison to FSH.

Comparison with dose-response (on FHSR in HEK cells)

We systematically calculate bias value using standard method

Uncertainty can be large according to the time of measurement



Thanks for your attention !

- ▶ Notion of signaling bias to quantify differential activation of several pathways by a Ligand at a given receptor.
- ▶ Standard quantification has several drawbacks (no time, limited to sigmoid scenario,..).
- ▶ We gave a kinetic interpretation of Ligand biased, which rely on dynamic (ODE) modeling and parameter estimation with L^1 penalization.

Thanks for your attention !

- ▶ Notion of signaling bias to quantify differential activation of several pathways by a Ligand at a given receptor.
- ▶ Standard quantification has several drawbacks (no time, limited to sigmoid scenario,..).
- ▶ We gave a kinetic interpretation of Ligand biased, which rely on dynamic (ODE) modeling and parameter estimation with L^1 penalization.
- ▶ Any other ideas how to define *bias* ?
- ▶ How to deal with "fuzzy/noisy" PLE ?
- ▶ How to deal with non uniqueness of the penalized solution ?
- ▶ How to perform a model reduction that would lead to both a satisfactory fit and identifiable parameters ?

