USING DYNAMICAL REACTION NETWORK TO INFER DRUGS SELECTIVITY IN PHARMACOLOGY

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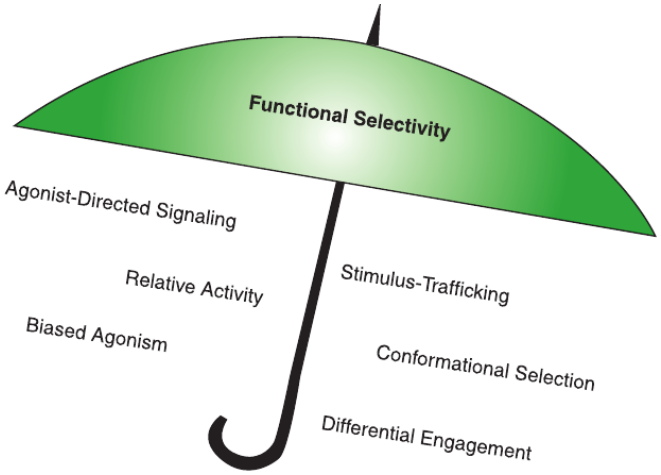
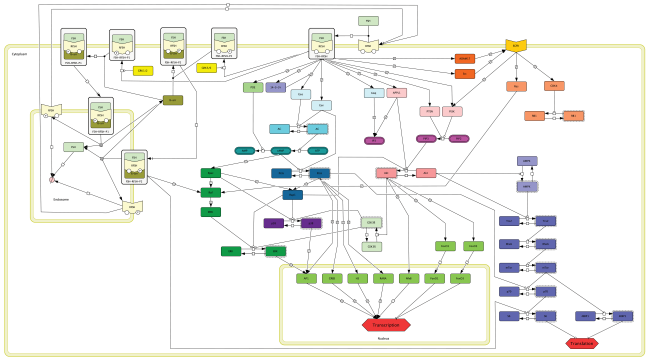
Outline

Functional selectivity, biased signaling

What is Drugs Selectivity ?

- Several reaction pathways are generally associated to a given receptor, and lead to various cell response.





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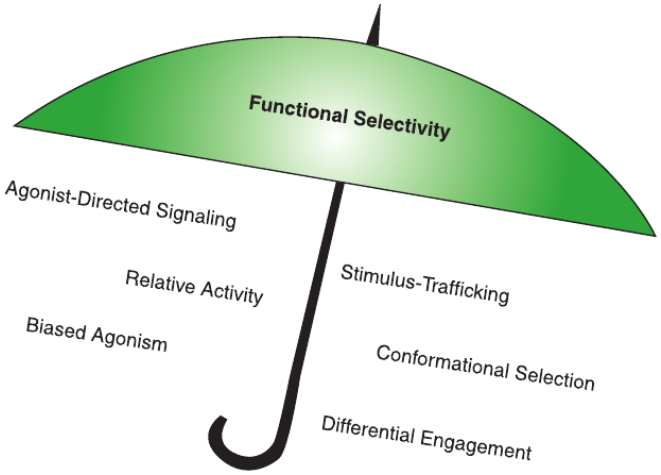

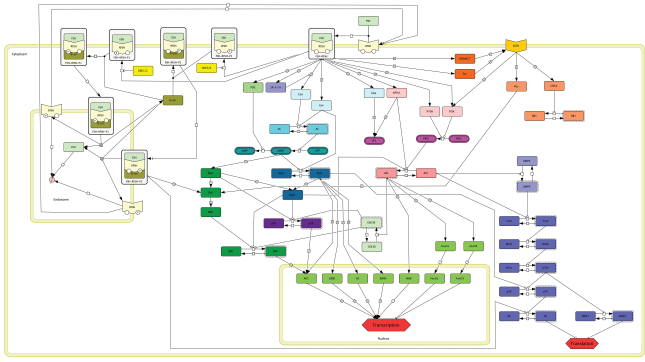

Functional selectivity, biased signaling

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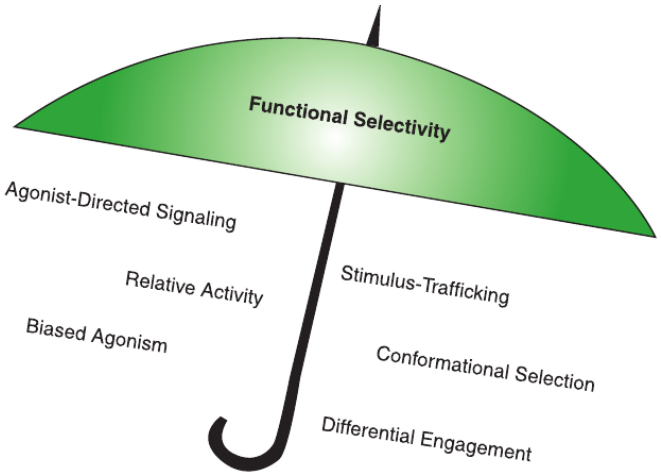
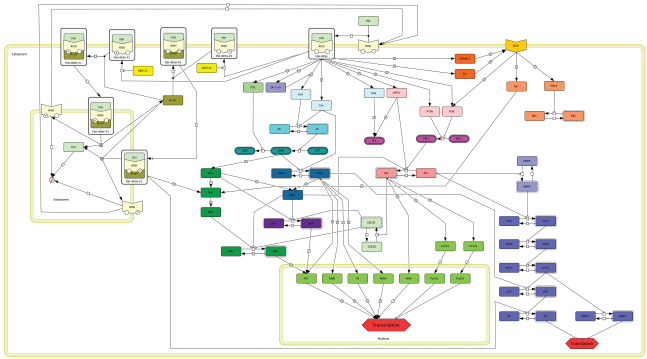
- Several reaction pathways are generally associated to a given receptor, and lead to various cell response.
- Differential activation of those reaction pathways, that differs between (natural or synthetic) ligand
- **Drugs Selectivity** = Ligand-dependent selectivity for certain signal transduction pathways at one given receptor





Key concept in pharmacology

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

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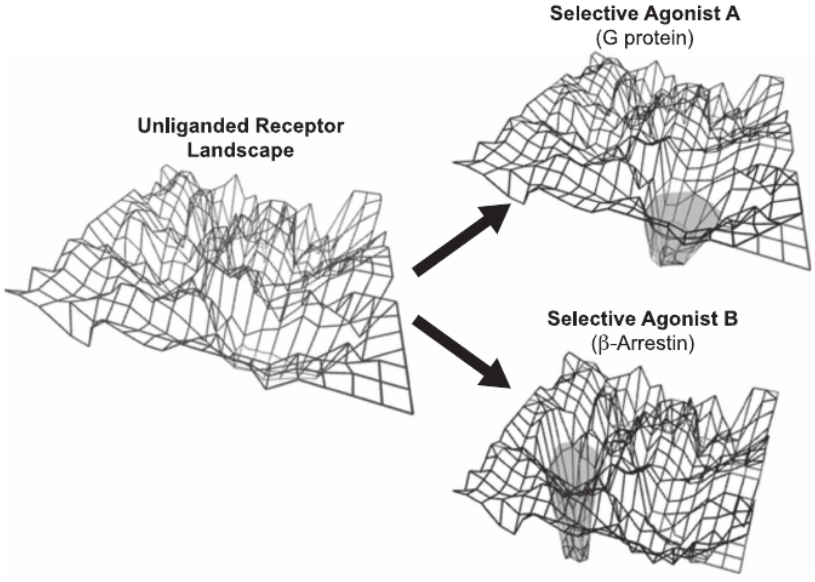
  - Affinity (K_d), potency (EC_{50}), efficacy (E_{max}).
- ◇ A bias might be **context-dependent** (cell type, physiological state, etc.)
- ◇ Biased agonism is becoming a major tool in drug discovery.

$\Rightarrow$ 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 patways.



Kenakin, *J Pharmacol Exp Ther* (2011)

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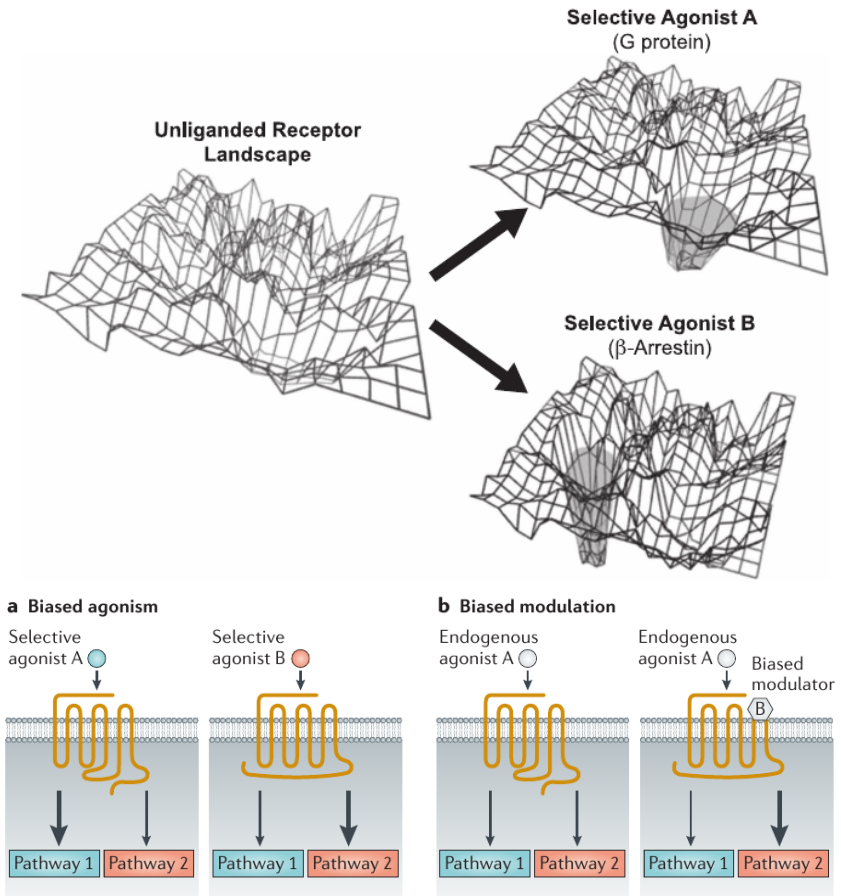
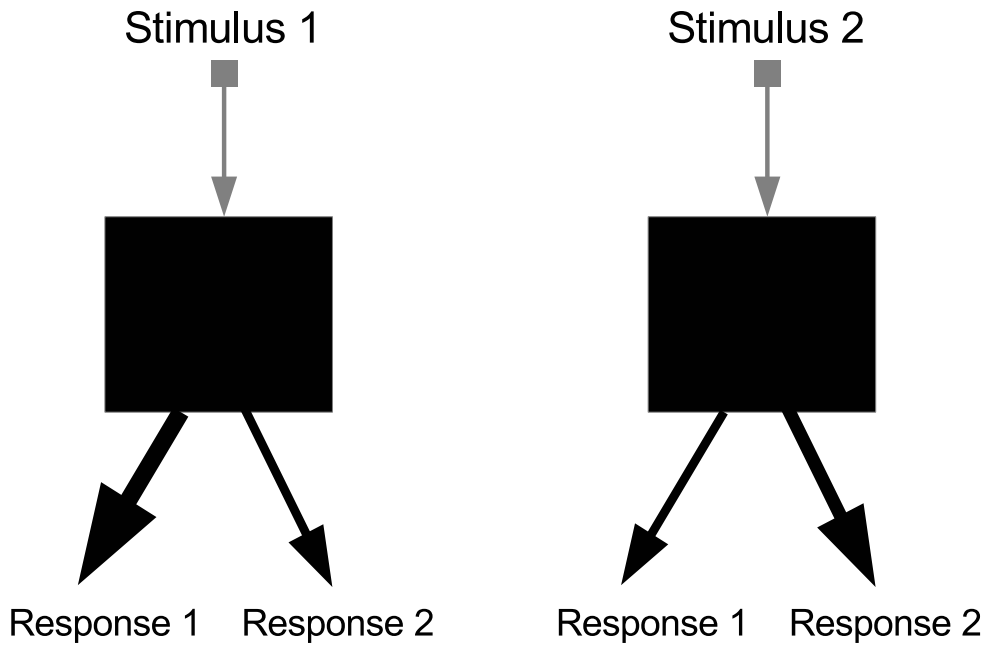
Similar concept : modulating bias



Kenakin and Christopoulos, *Nat. Rev. Drug Discov.* (2013)

Minimal setting

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



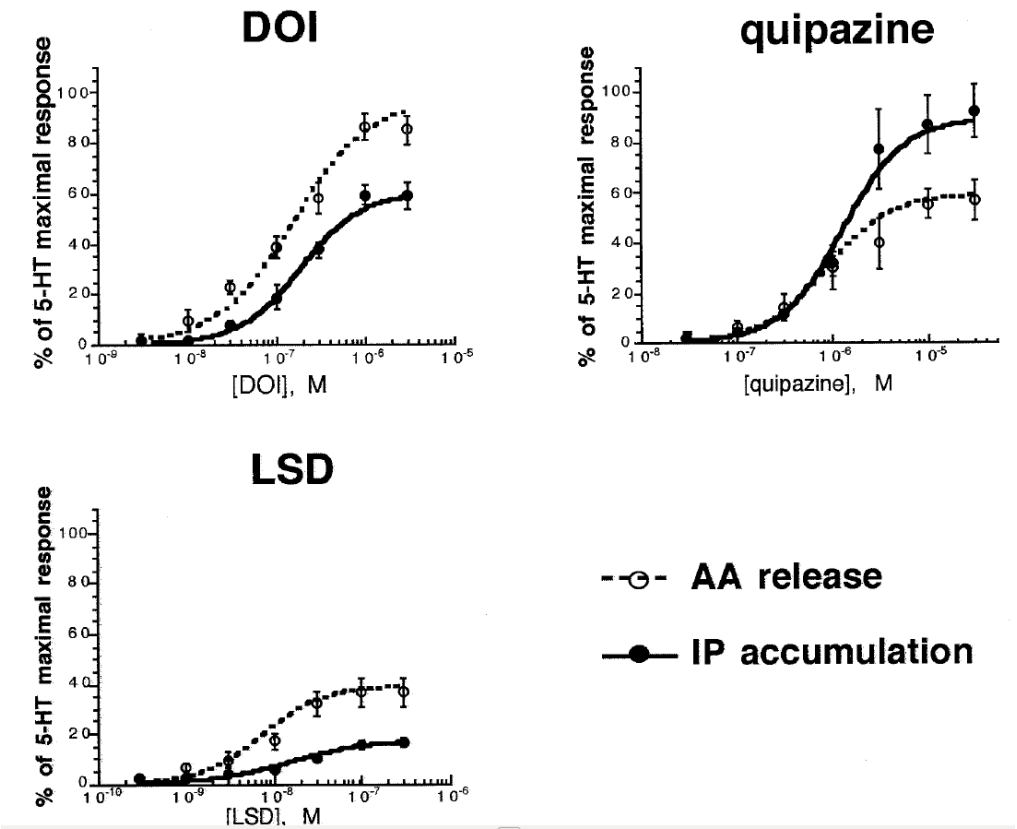
$\Rightarrow$ We always compare *a ligand with respect to a reference one.*

Outline

Serotonine receptor 5 – HT_{2C}

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

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



Serotonine receptor 5 – HT_{2C}

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$\Rightarrow$ Bias due to an E_{max} difference.

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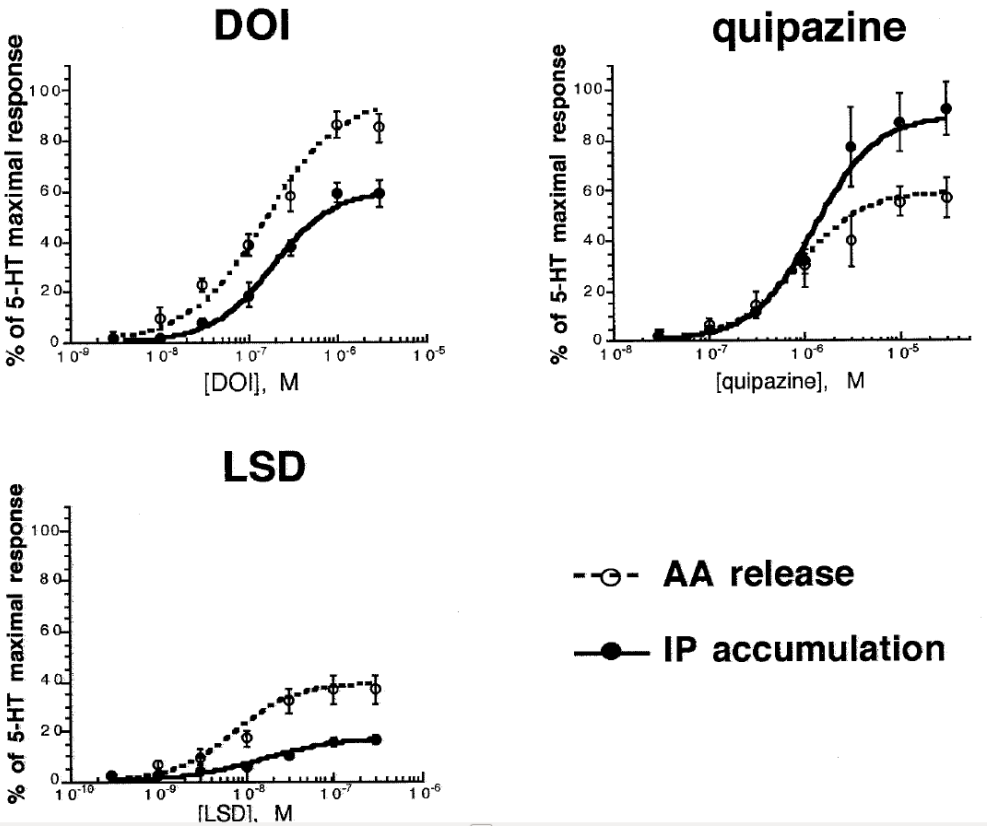
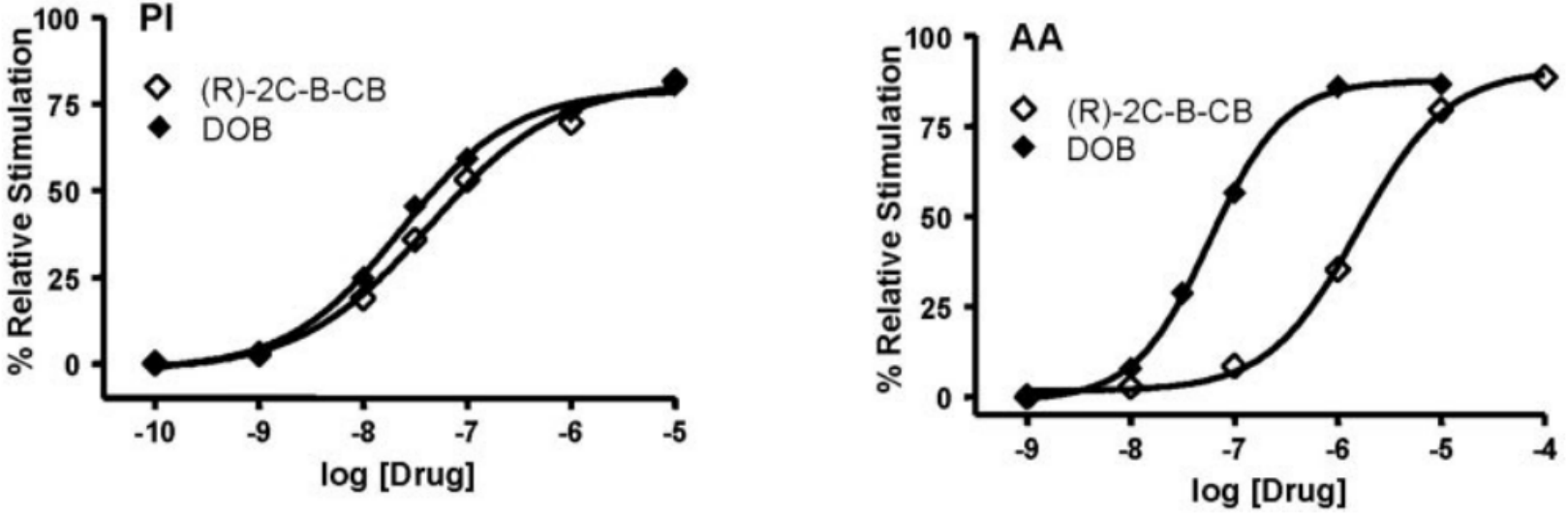


Serotonine receptor 5 – HT_{2A}



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

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

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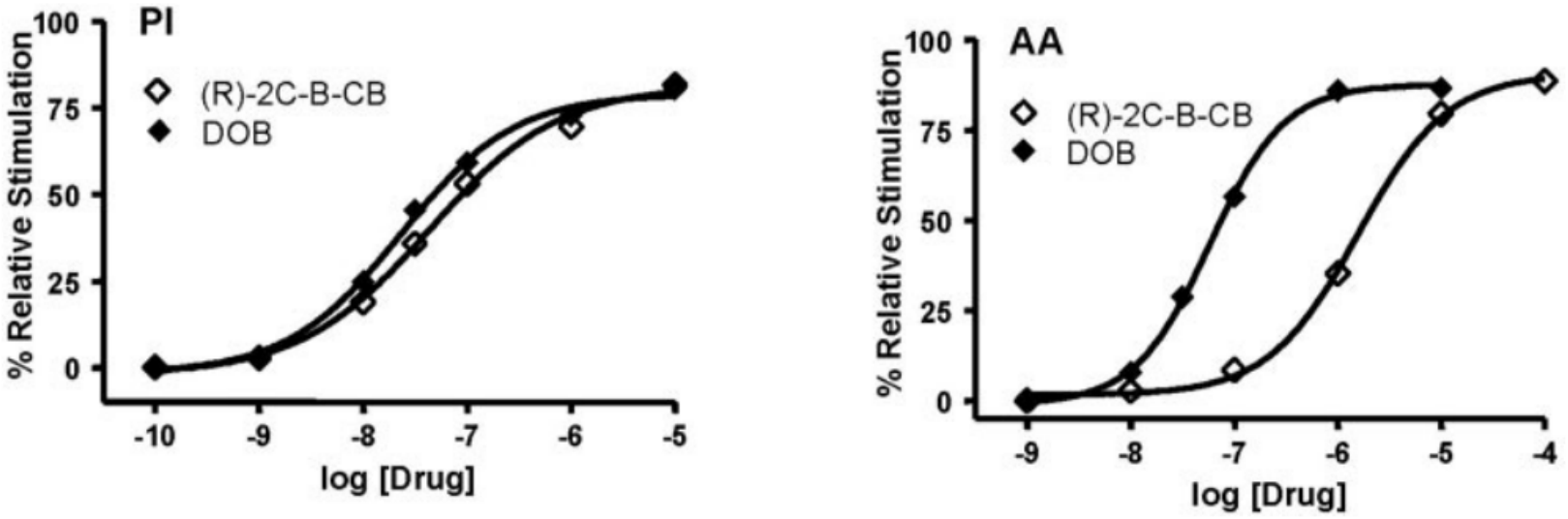

- $(R) - 2C - B - CB$ is biaised towards PI accumulation with respect to AA production, *compared to the reference agonist* DOB.

$\Rightarrow$ Bias due to an EC_{50} difference.

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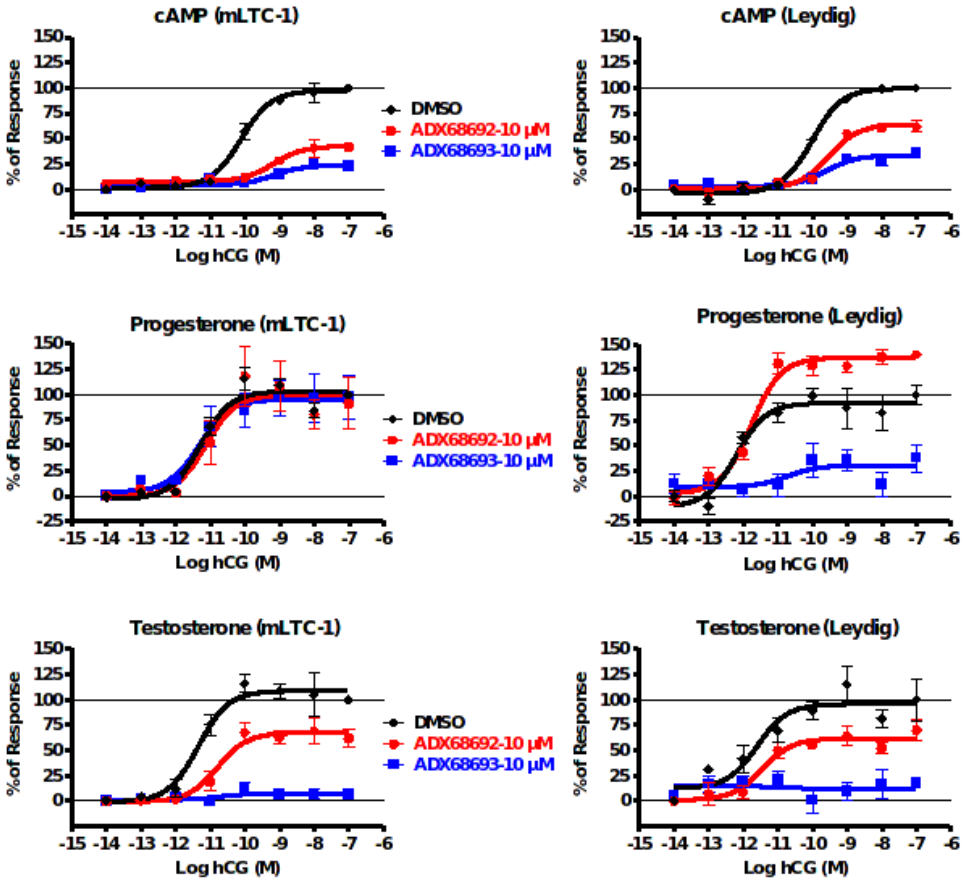
Steroidogenesis modulated by NAM

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)



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$\Rightarrow$ Selective (biased) allosteric modulation

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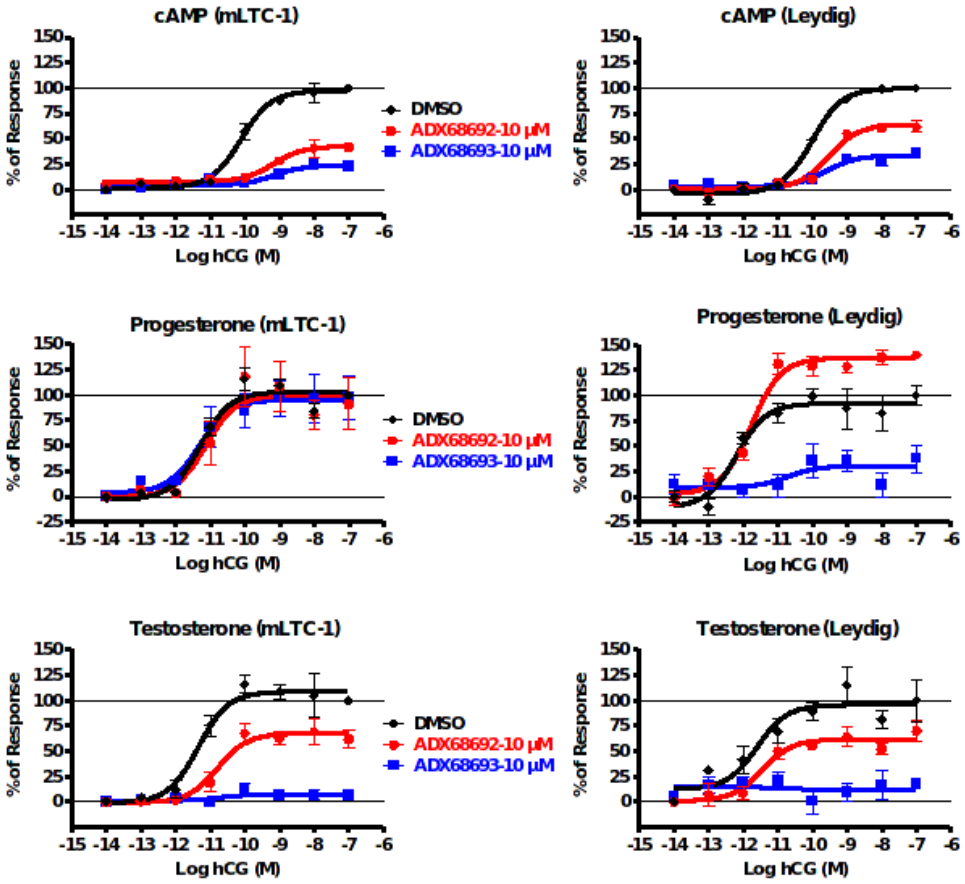
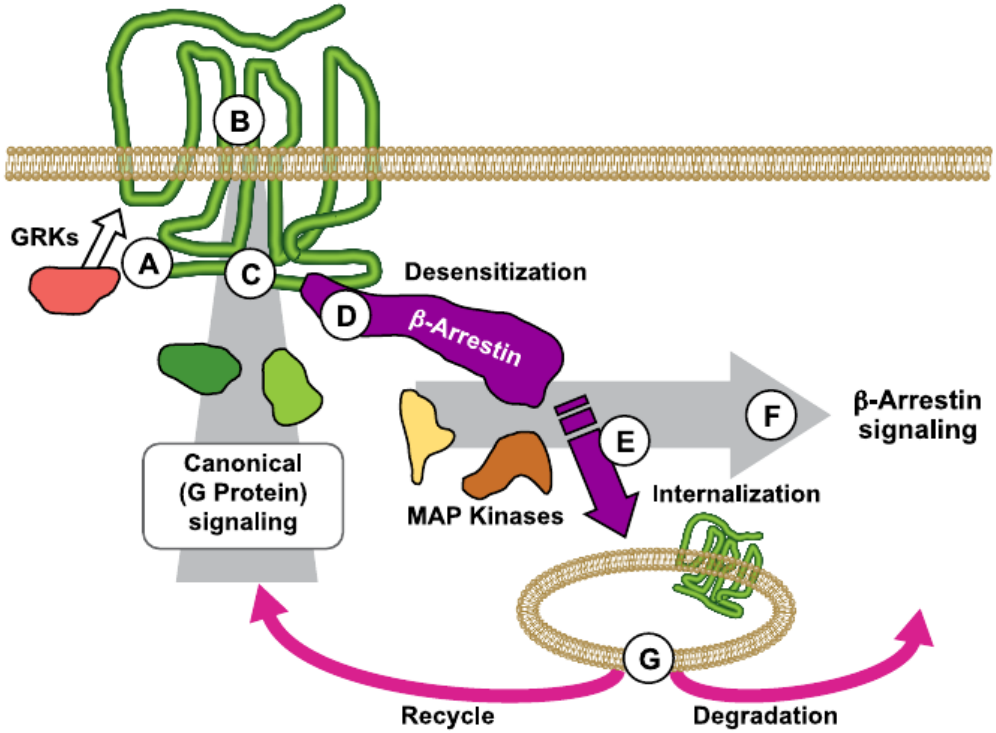


Many more examples on GPCR (principle drug target)

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

Kenakin, *Chem Rev* (2017)



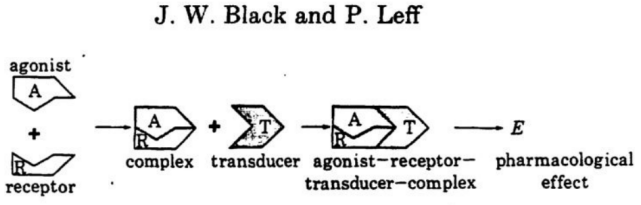
Outline

Operational model

Dose-response data are fitted with the function

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

- Response at equilibrium of a Michaelis-Menten type model.
- $Ka =$ **Dissociation constant** of the couple Ligand/Receptor
- $\tau =$ **Efficacy coefficient** of the transduction pathway



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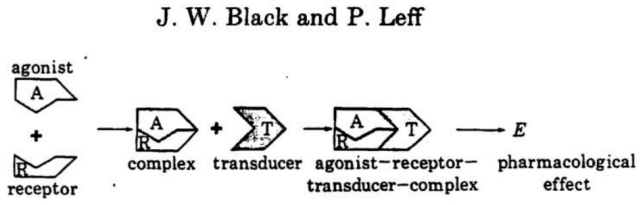
For $n = 1$,

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



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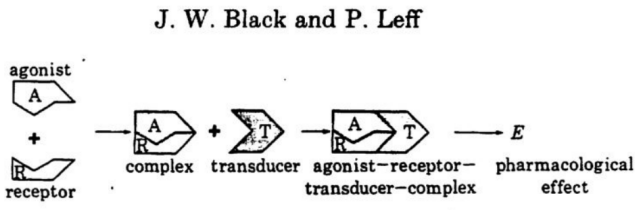
Then, we define

$\Rightarrow$ **Transduction coefficient** :

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



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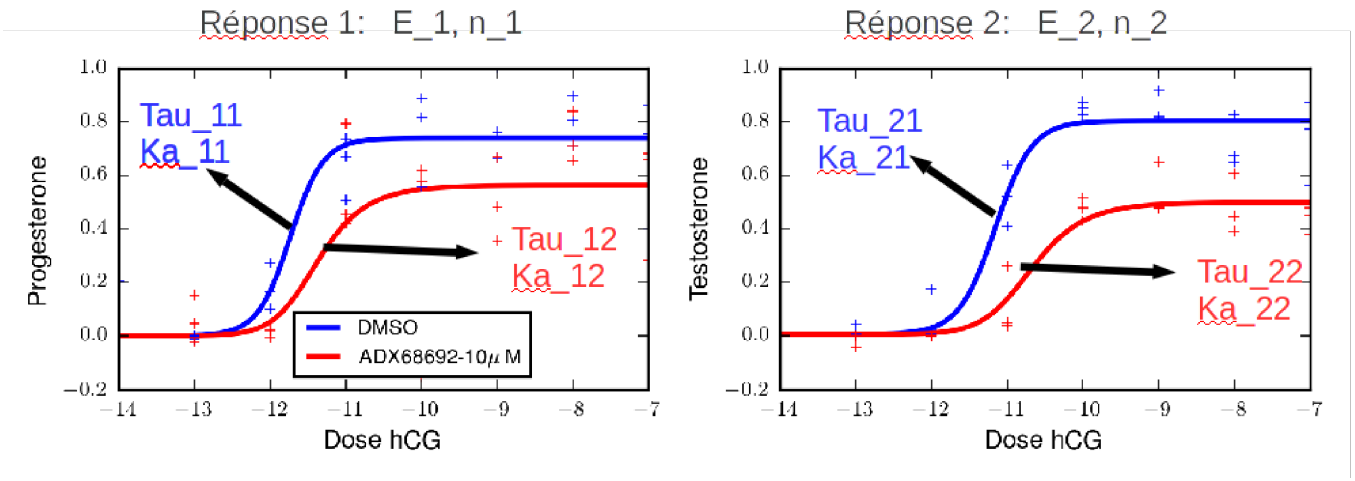
Bias quantification : with the operational model



Two ligands ($j = 1, 2$) and **two** measured responses ($i = 1, 2$) :
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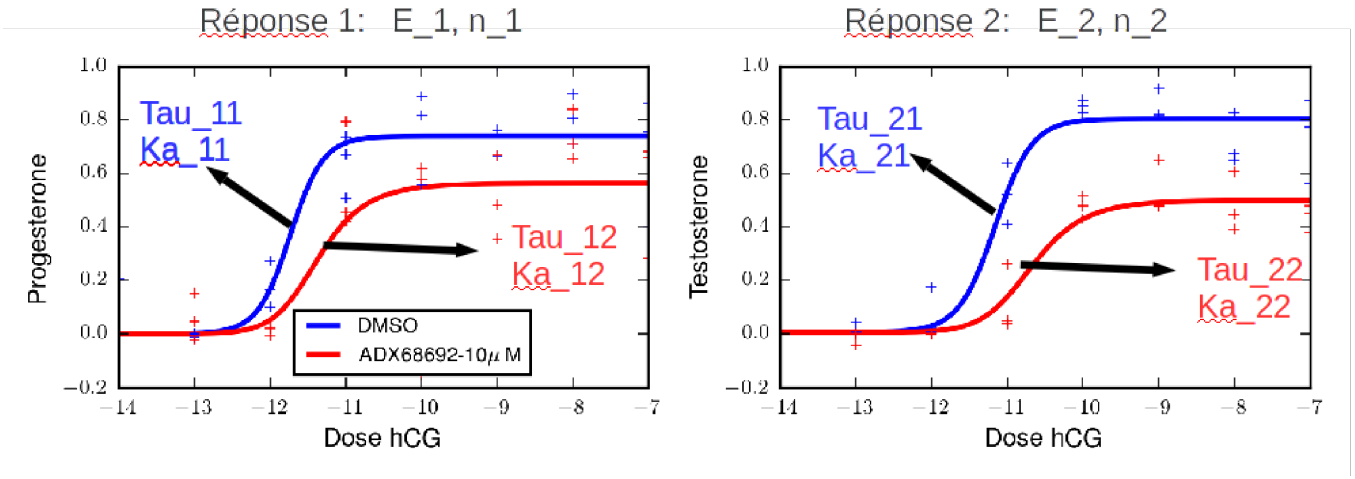

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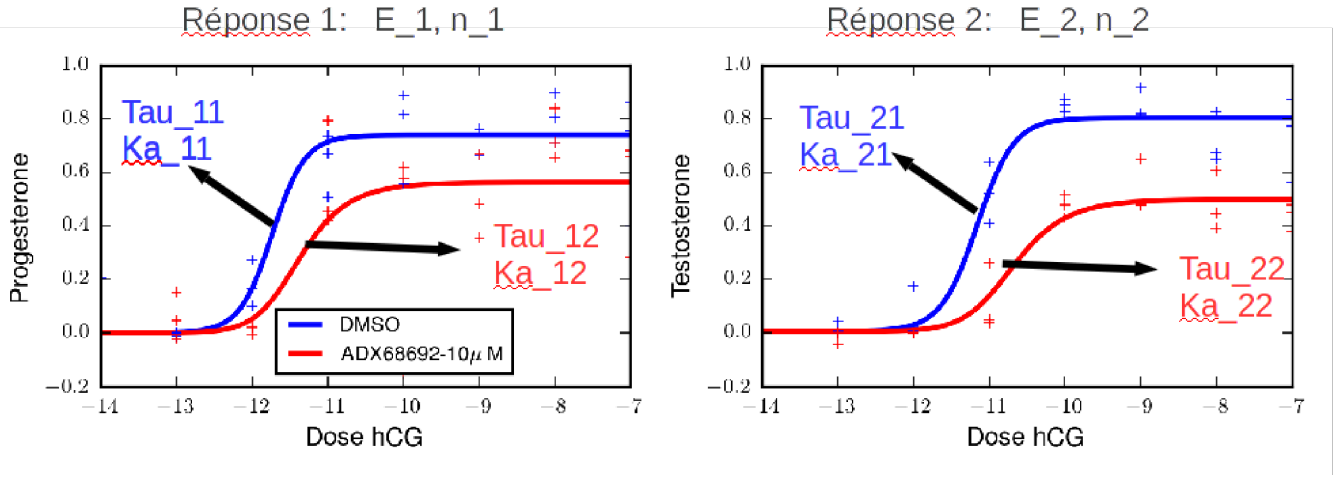

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The **Bias** is then defined by

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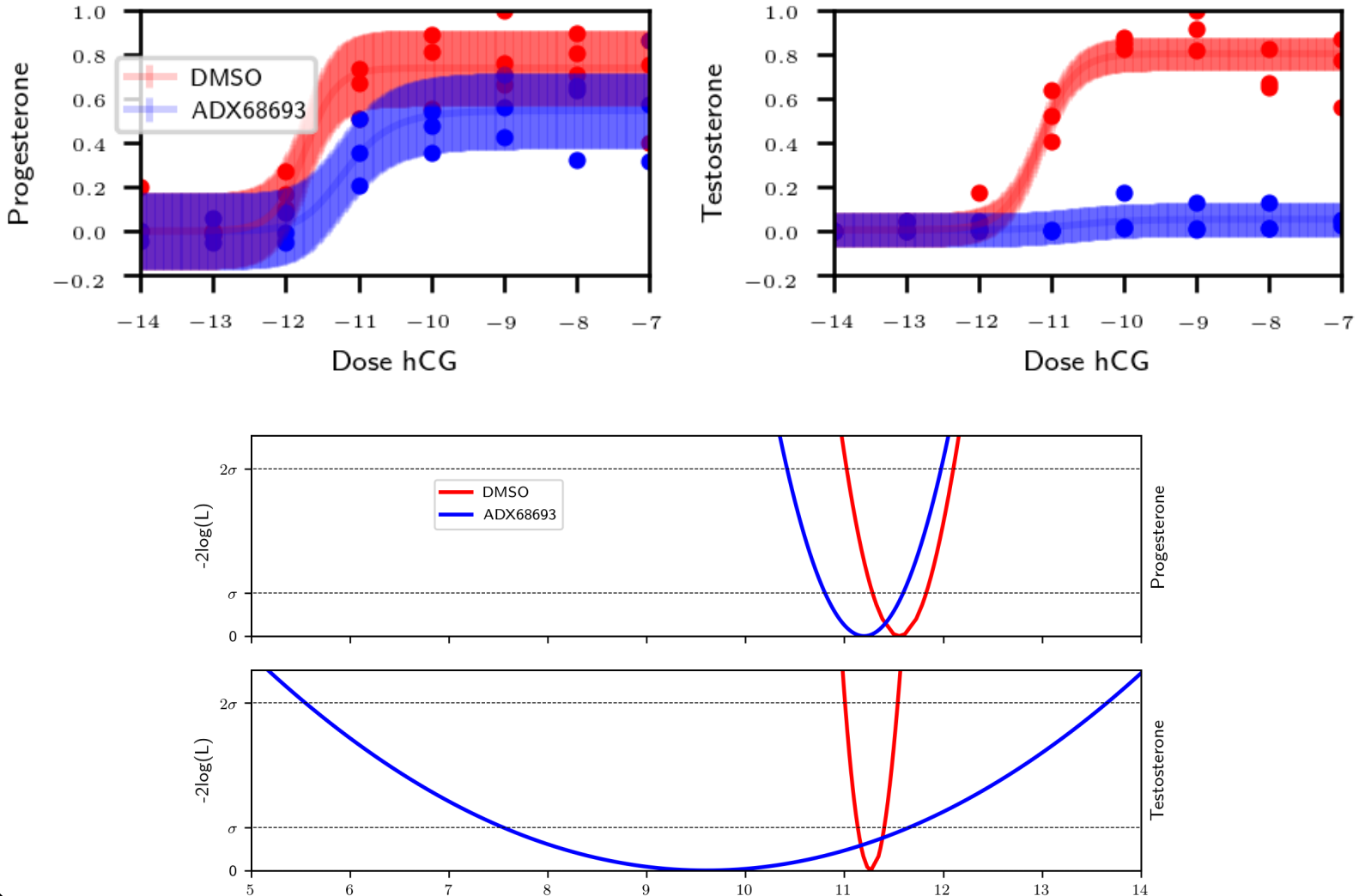
Statistical consideration : parameter confidence interval and (un-)identifiability



Data2Dynamics : Raue A., et al. *Bioinformatics* (2015)

Outline

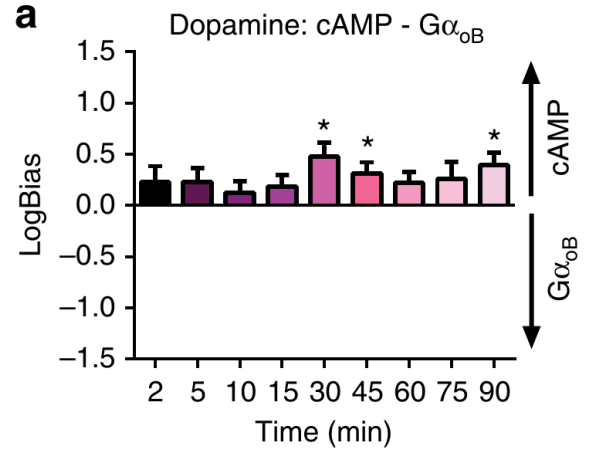
Time-dependent bias ?

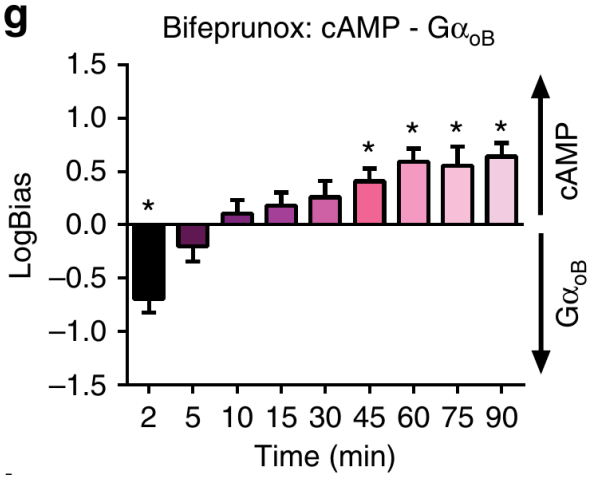
The role of kinetic context in apparent biased agonism at GPCRs

Carmen Klein Herenbrink[1], David A. Sykes[2], Prashant Donthamsetti[3,4], Meritxell Canals[1], Thomas Coudrat[1], Jeremy Shonberg[5], Peter J. Scammells[5], Ben Capuano[5], Patrick M. Sexton[1], Steven J. Charlton[2], Jonathan A. Javitch[3,4,6], Arthur Christopoulos[1] & J Robert Lane[1]

- 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)





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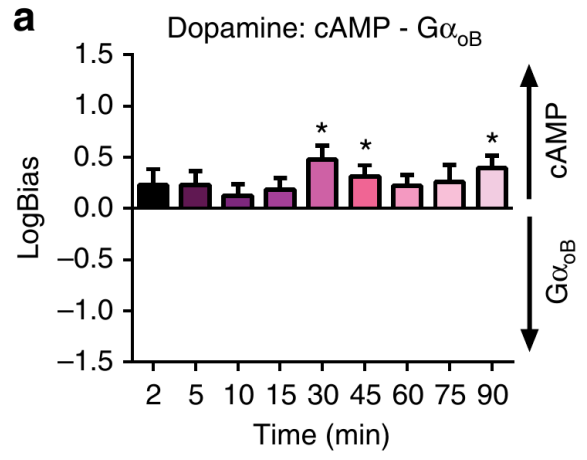
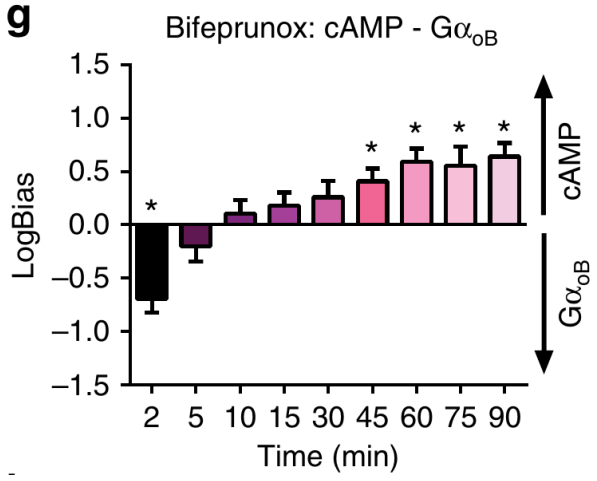
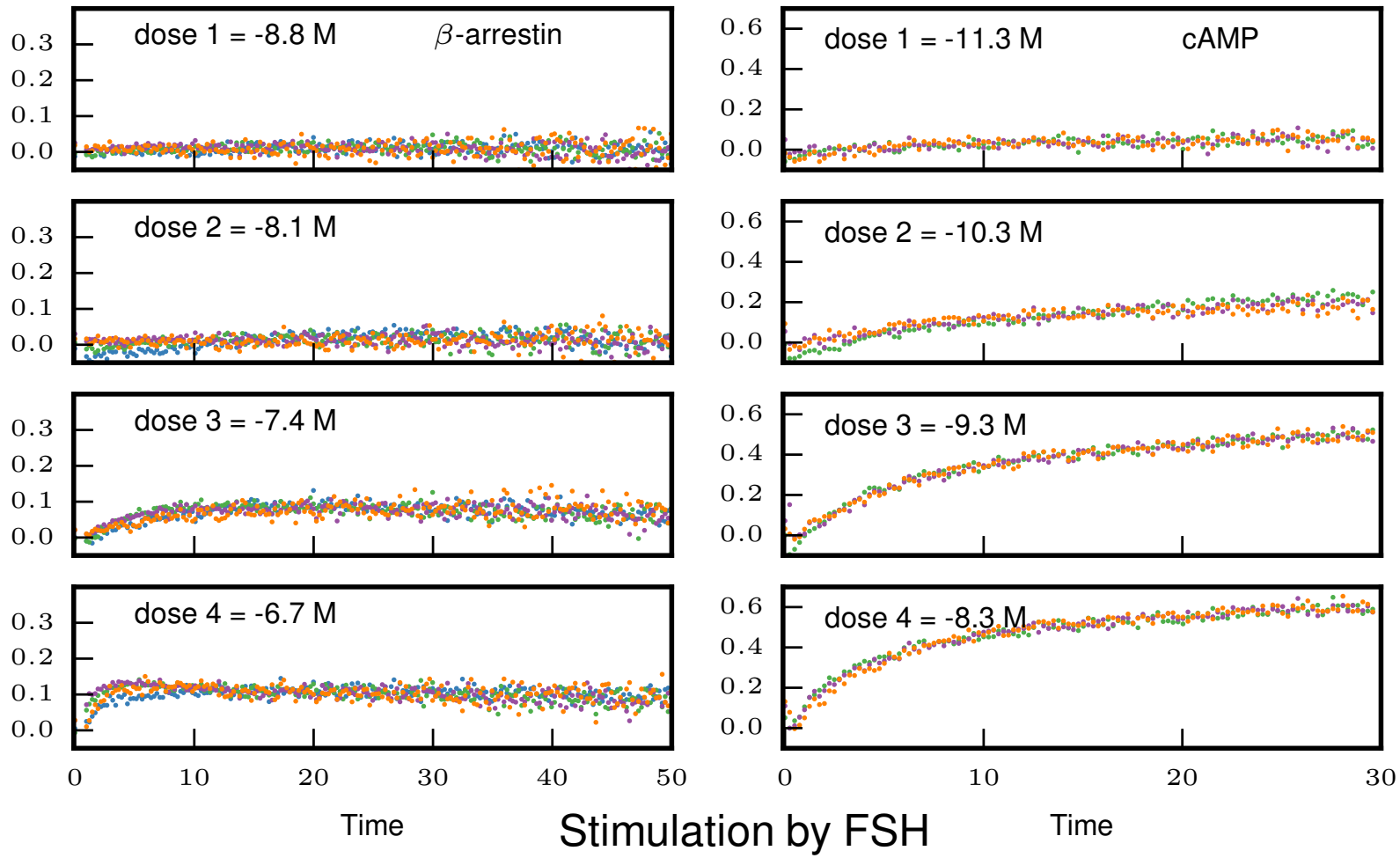
⇒ **We need to take into account dynamic patterns in bias quantification**





Dynamic data (on FHSR in HEK cells)

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



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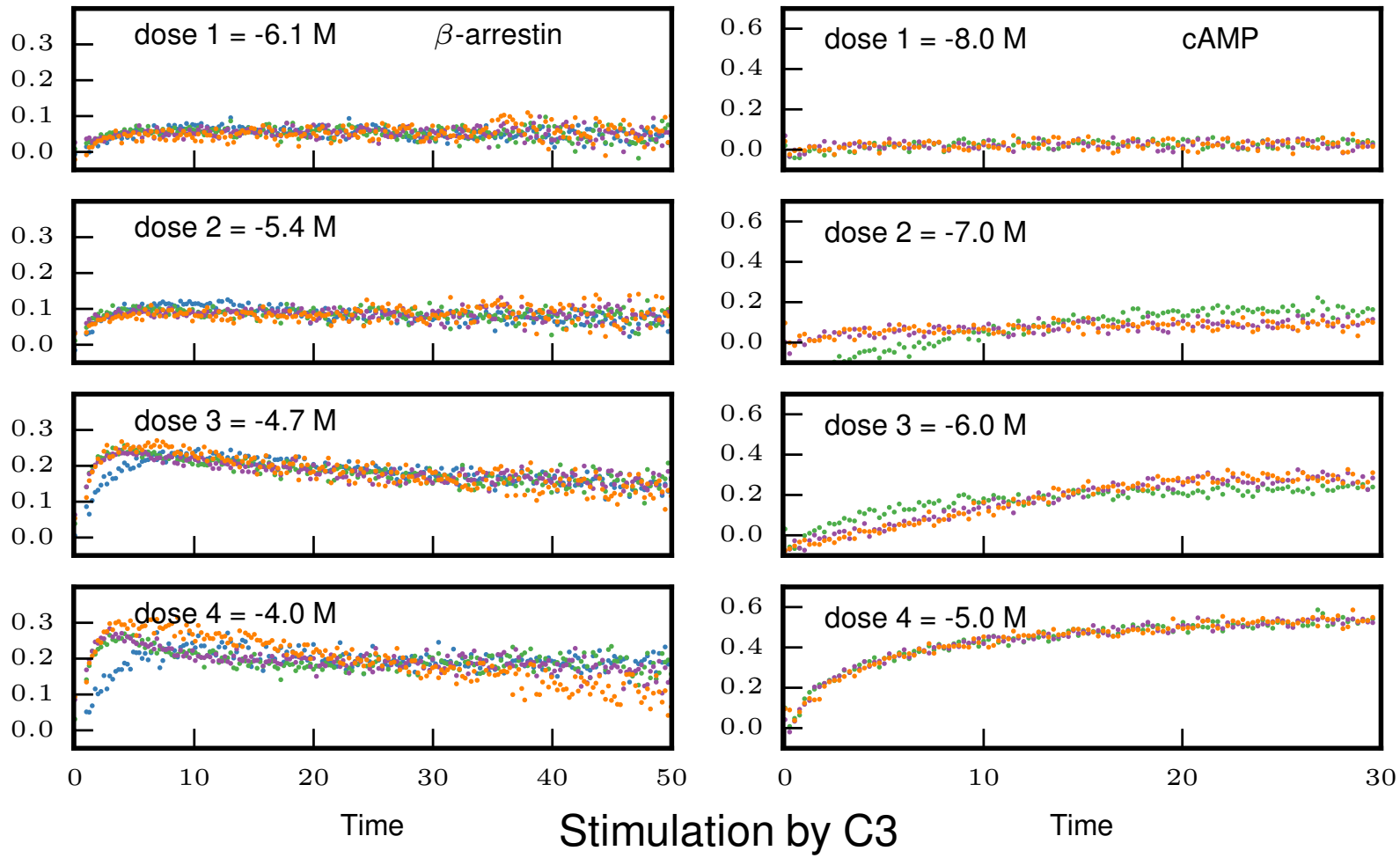
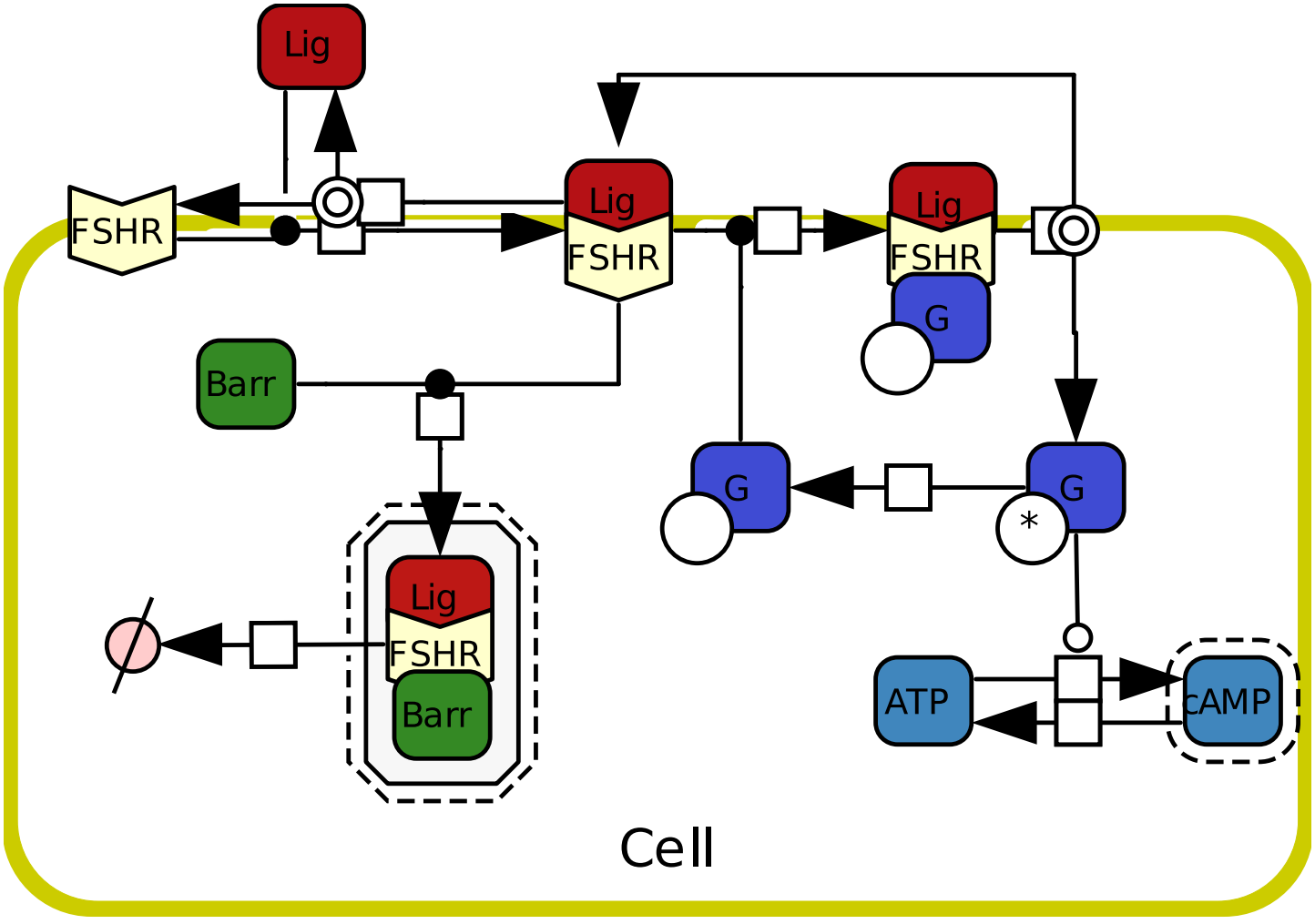


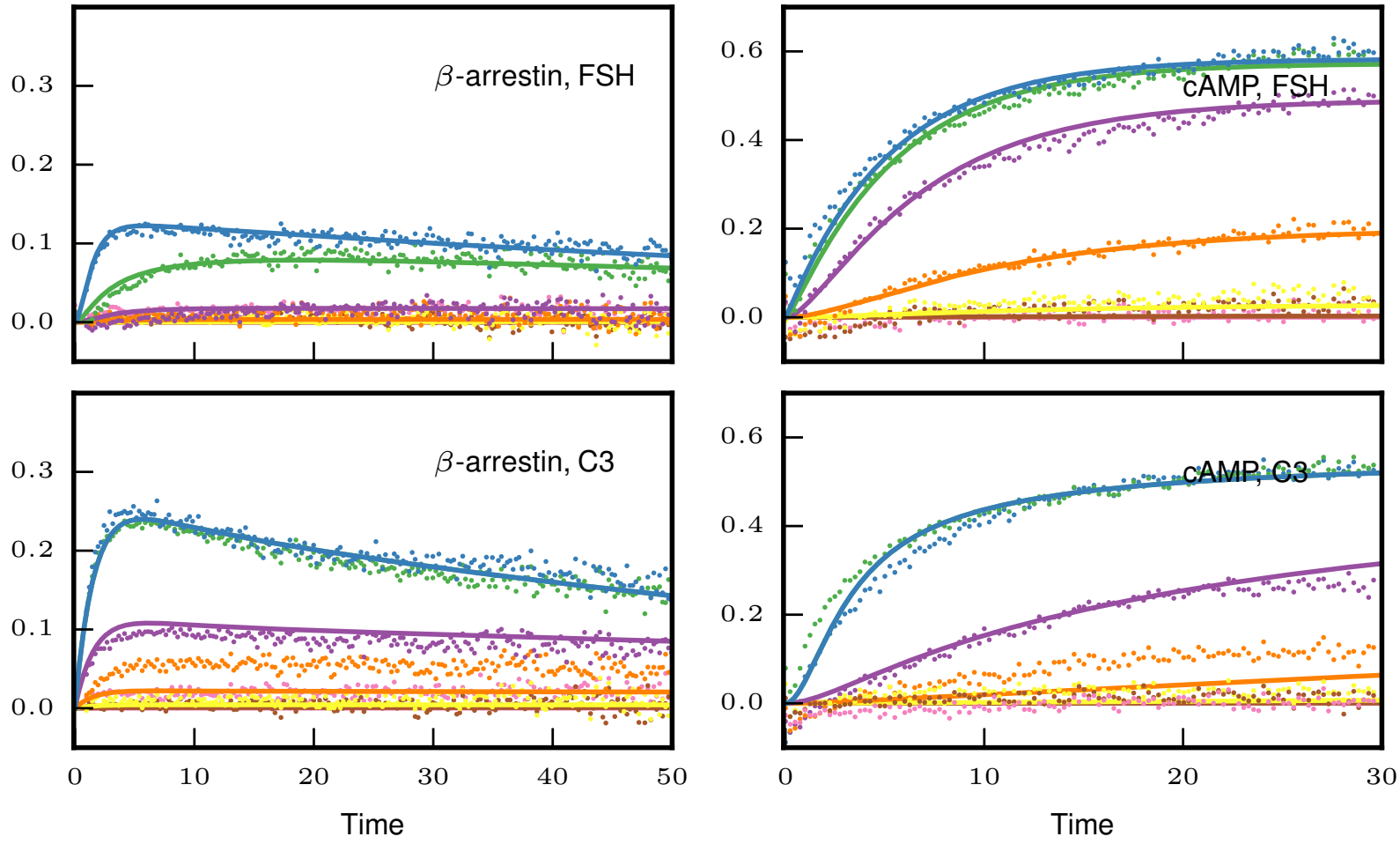
Principle of the methodology

I) We start with a sufficiently detailed chemical reaction network



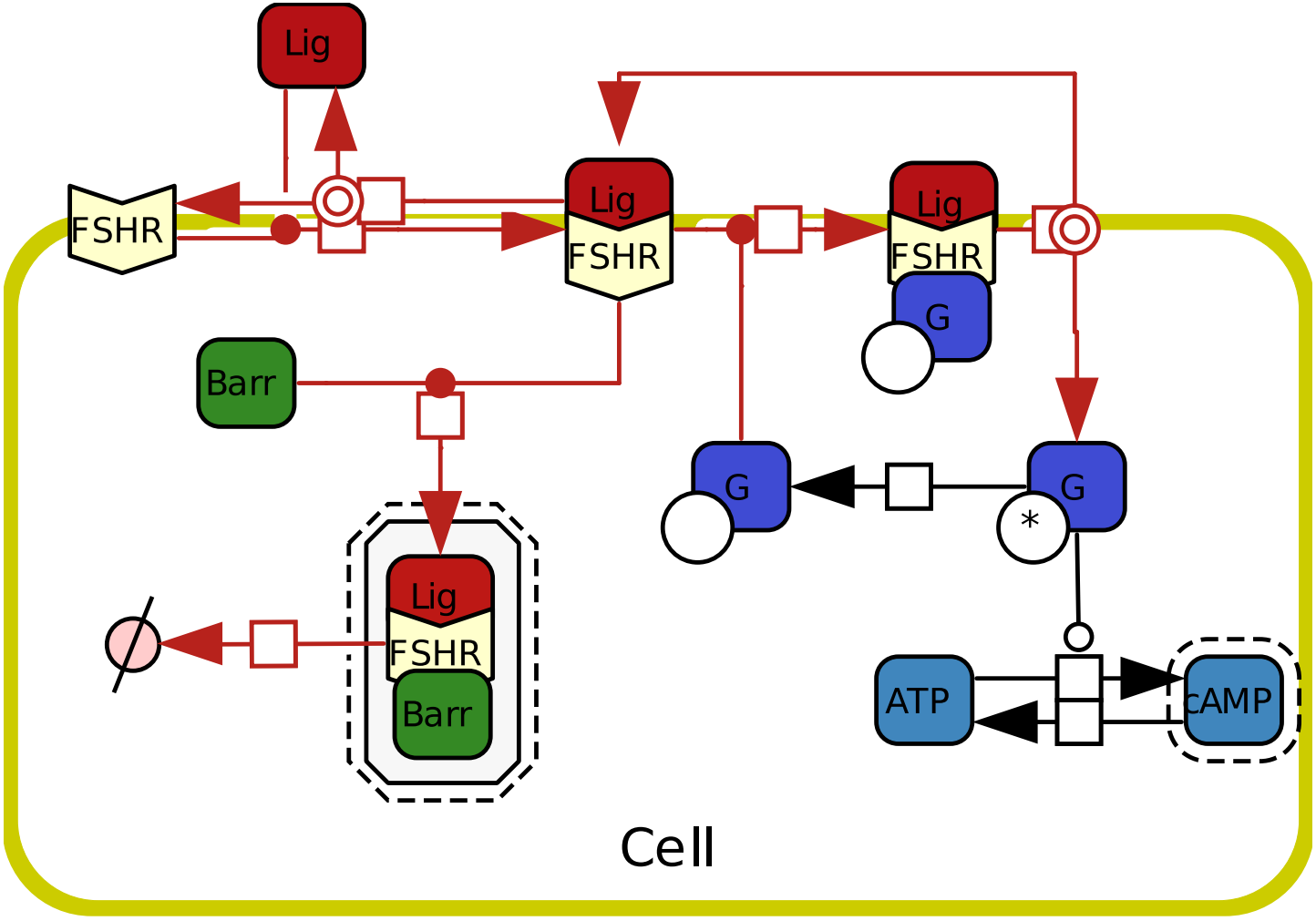
Principle of the methodology

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



Principle of the methodology

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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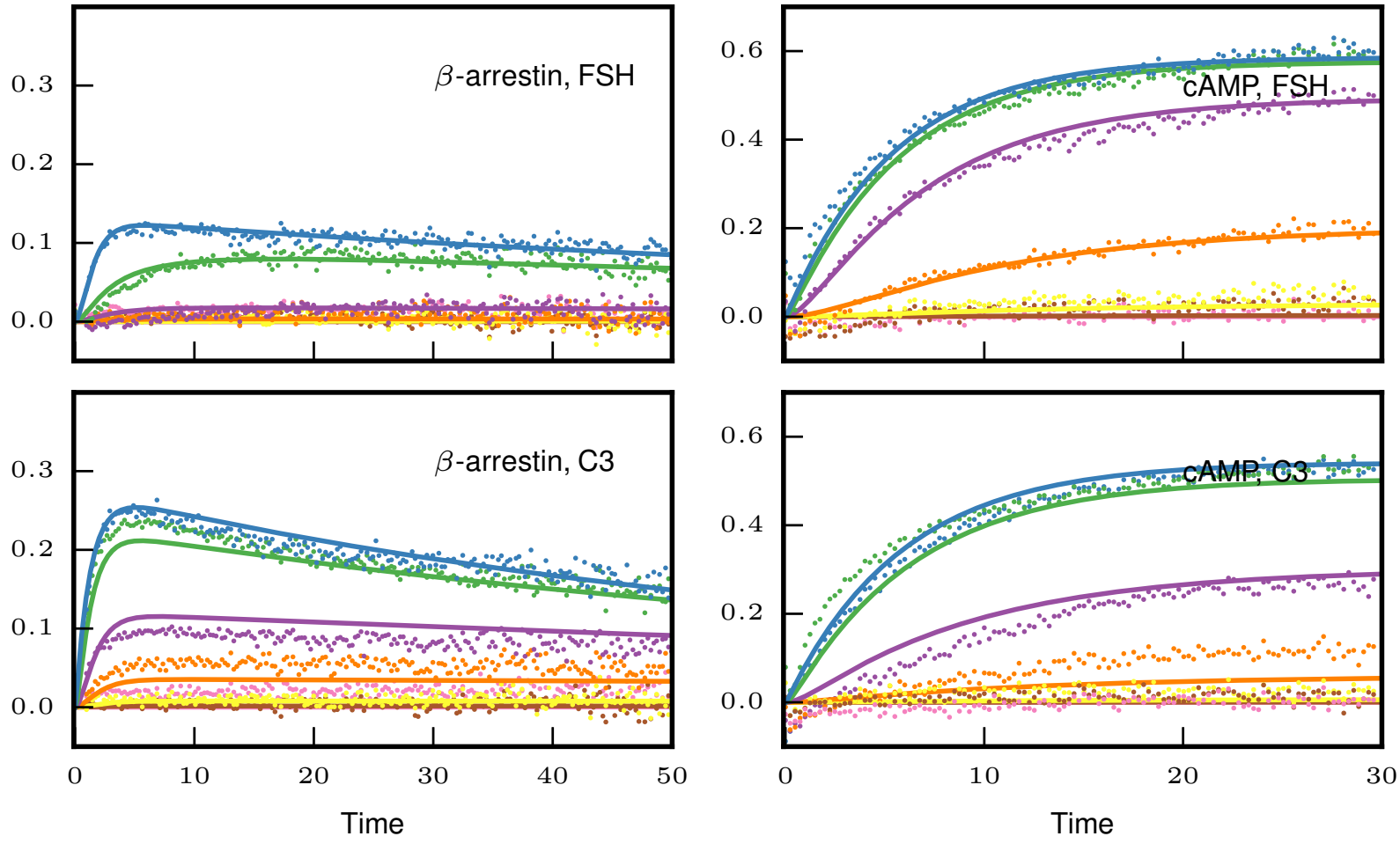

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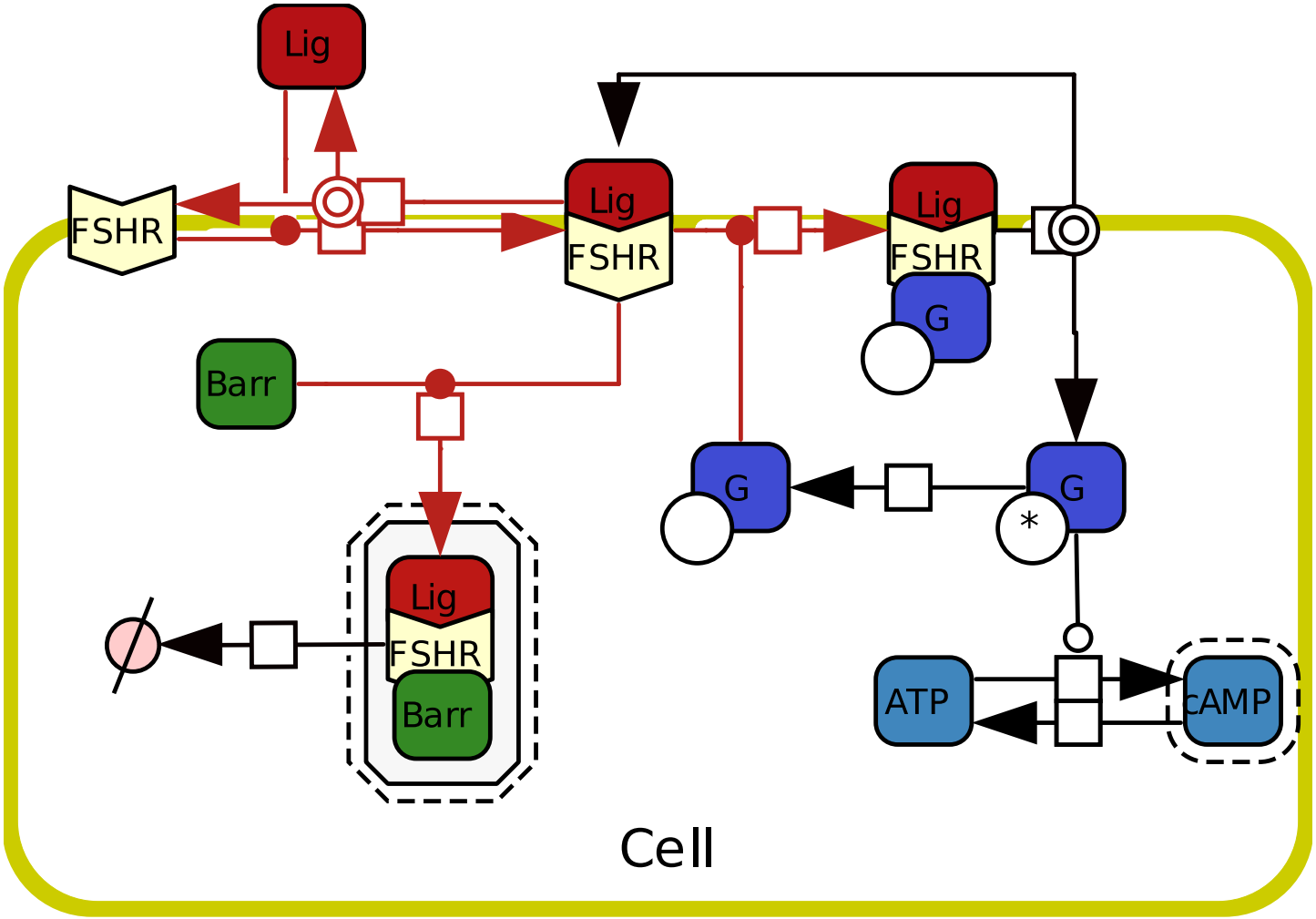
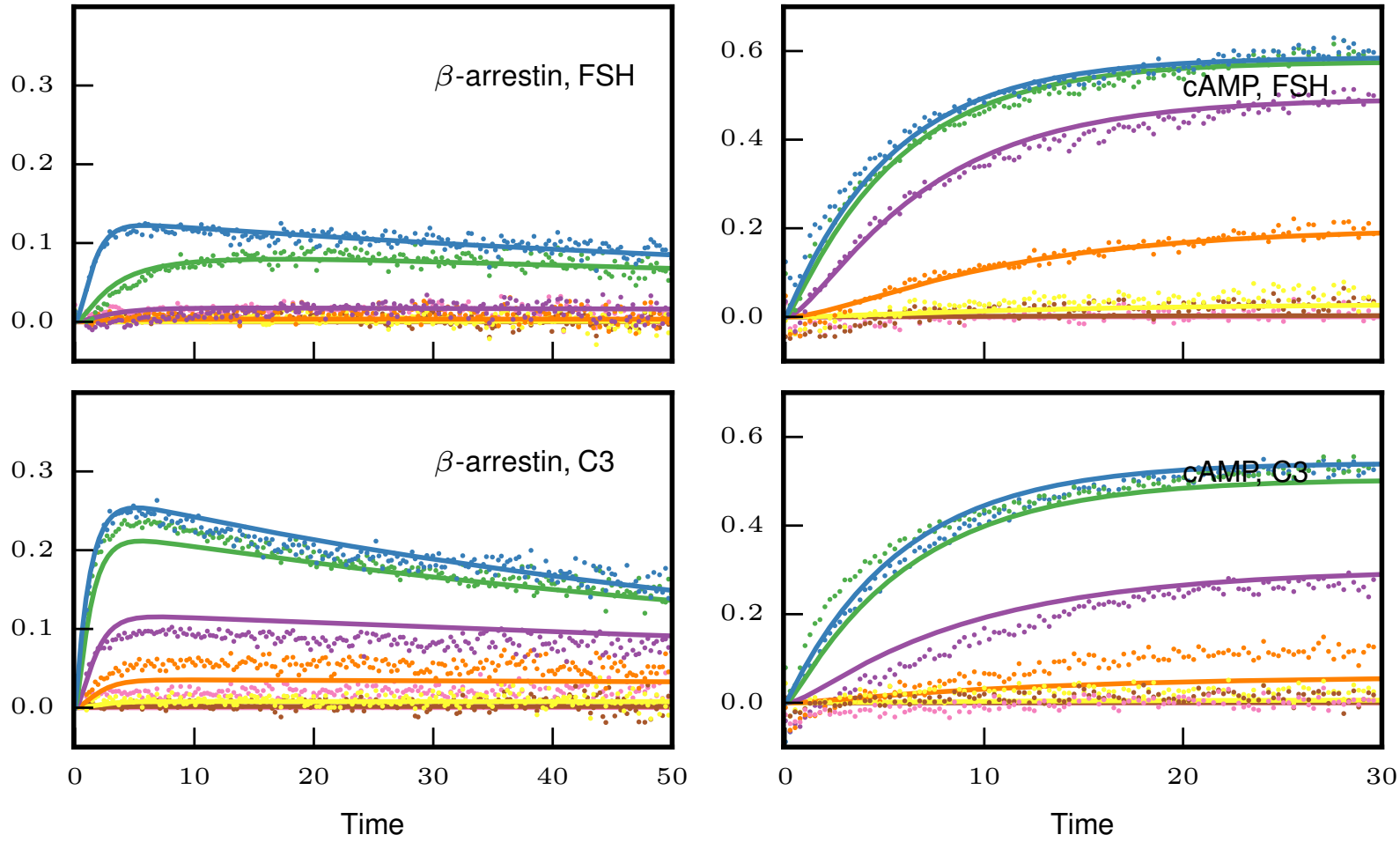
III) We use L^1-penalization to find **ligand specific parameters**

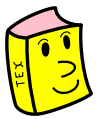


Data2Dyanmics : Steiert, Timmer and Kreutz, *Bioinformatics* (2016)

Principle of the methodology

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

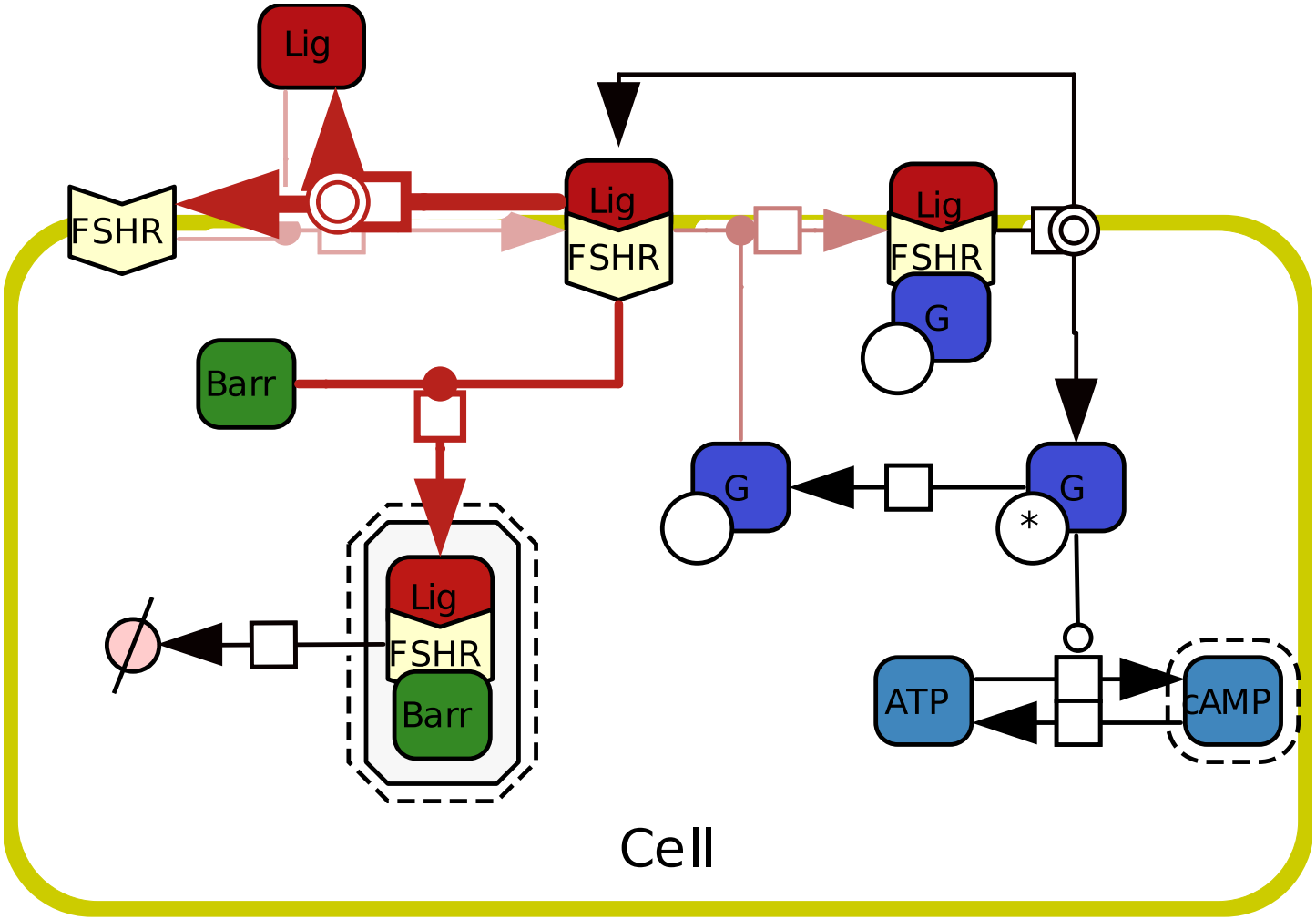




Steiert, Timmer and Kreutz, *Bioinformatics* (2016)

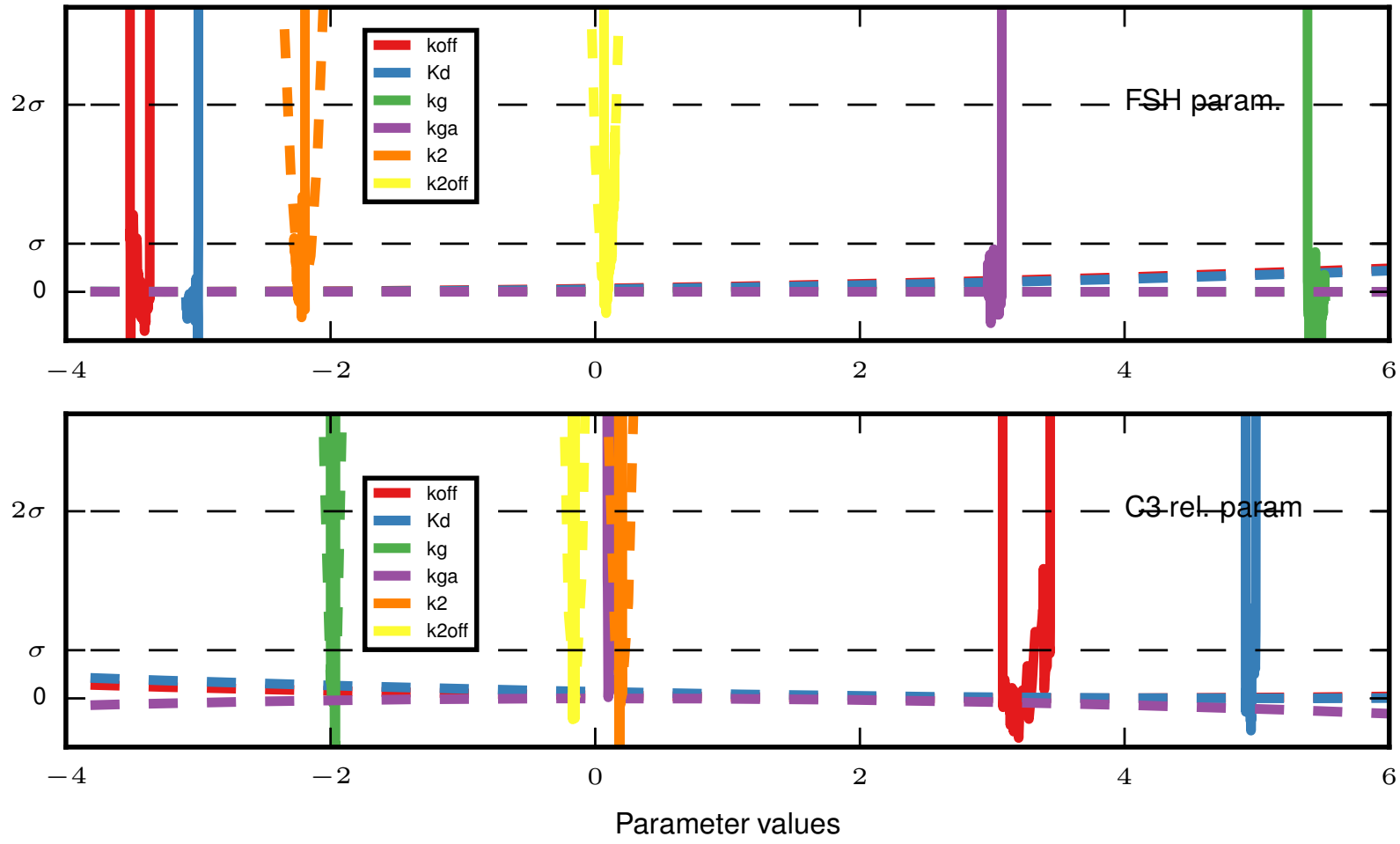
Principle of the methodology

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



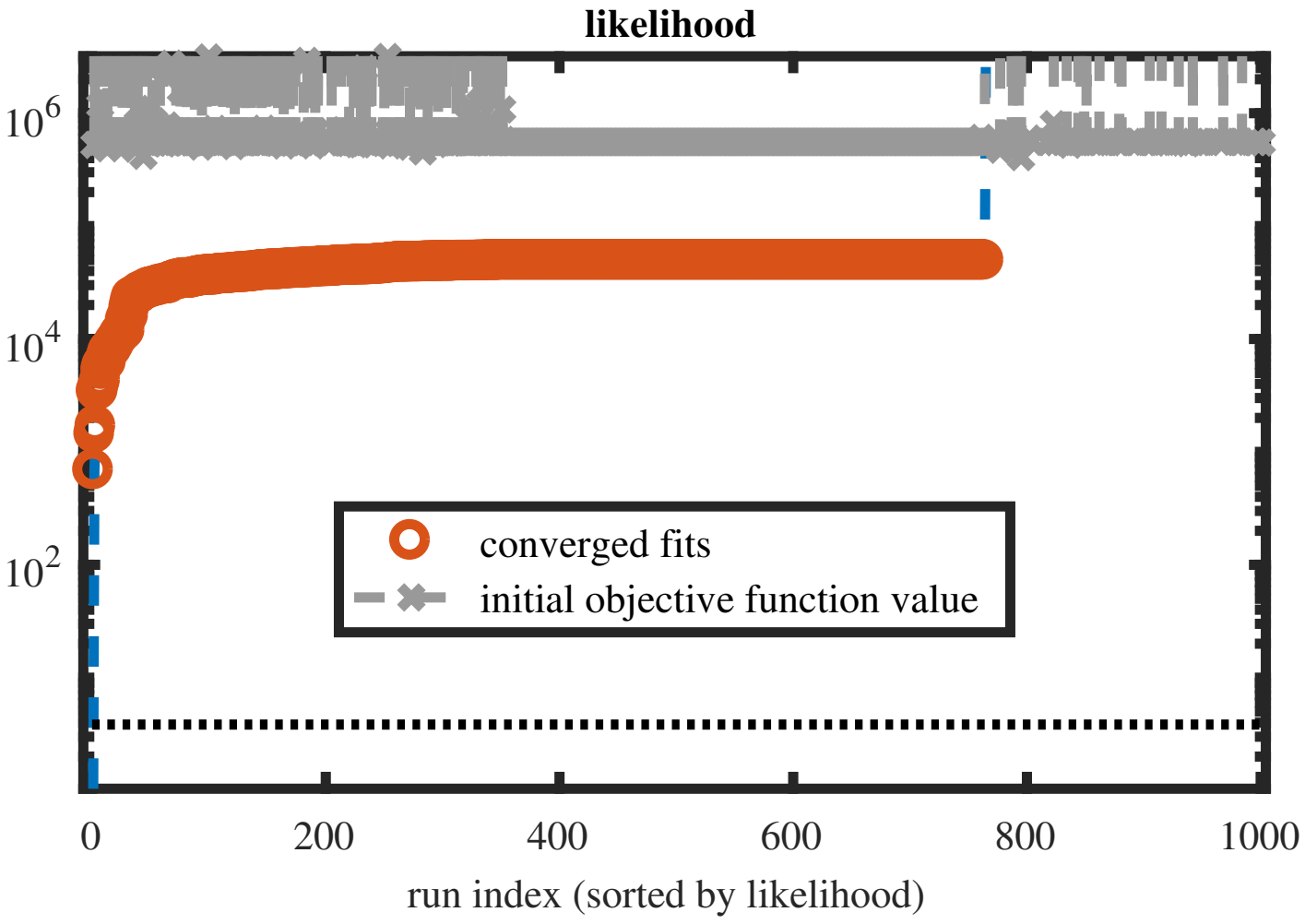
Principle of the methodology

V) Significant differences between parameters is assessed by PLE

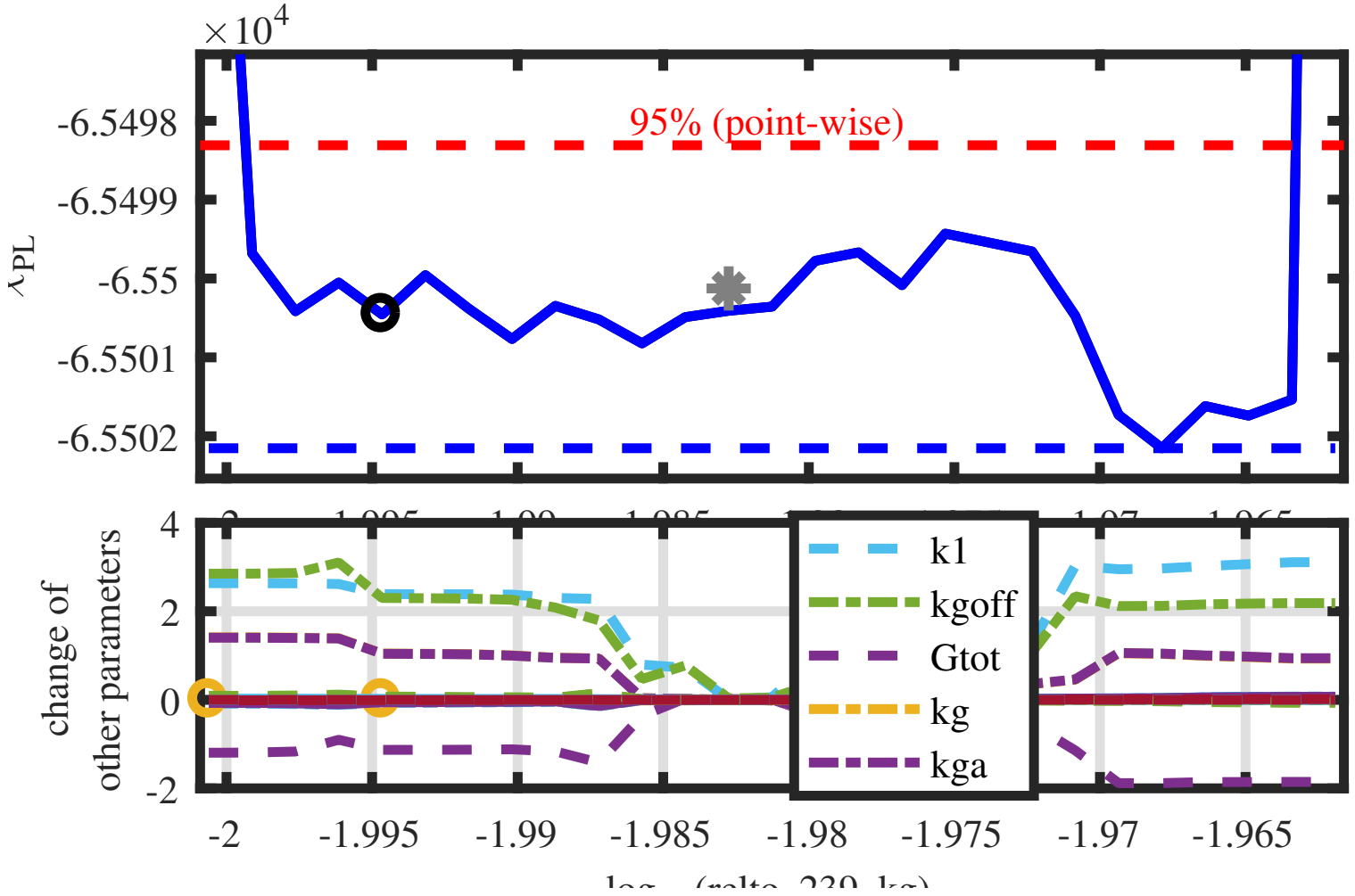


→**here :** C3 is biased towards β-arr, compared to cAMP, in comparison to FSH.

Practical problems...

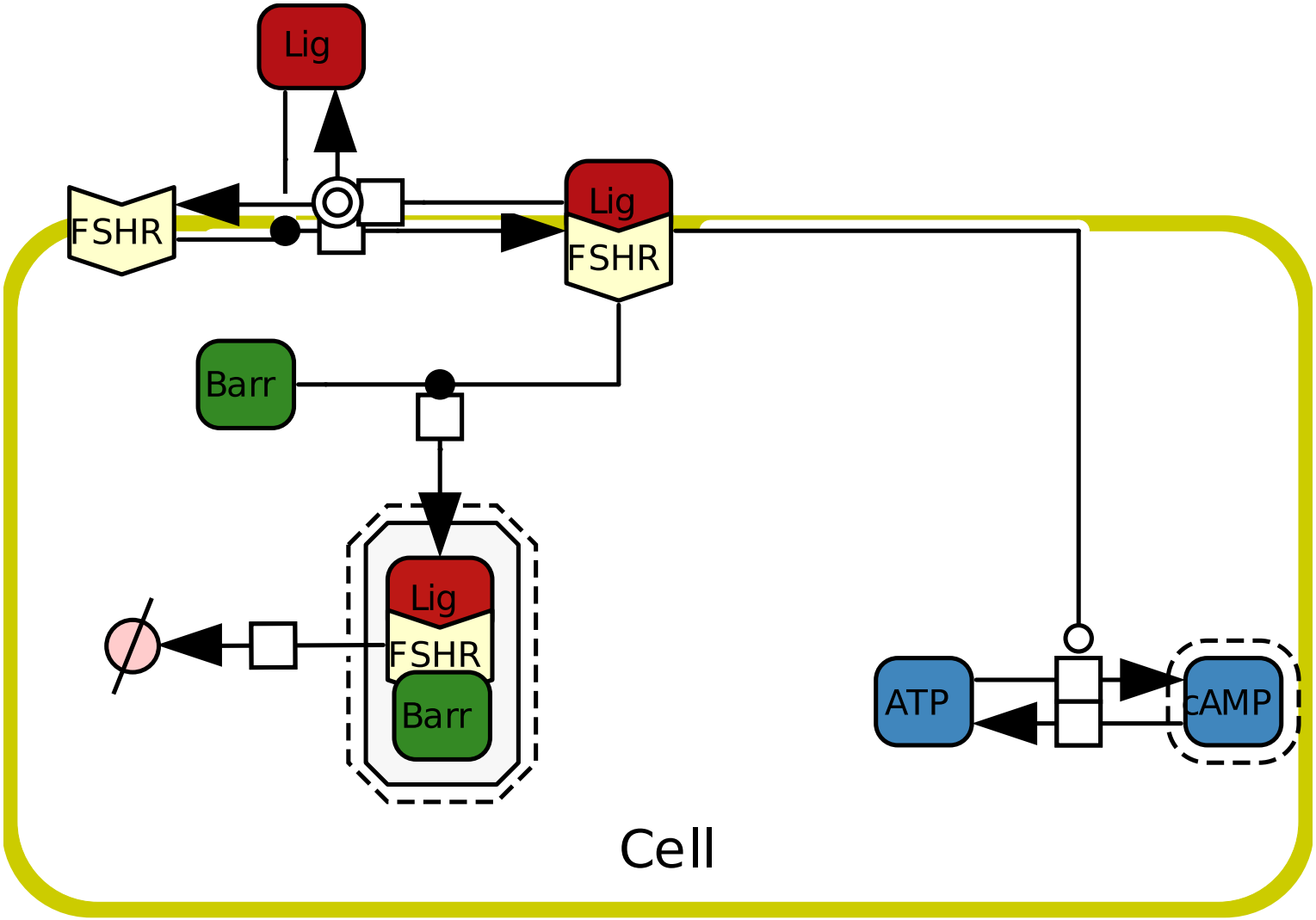


Practical problems...



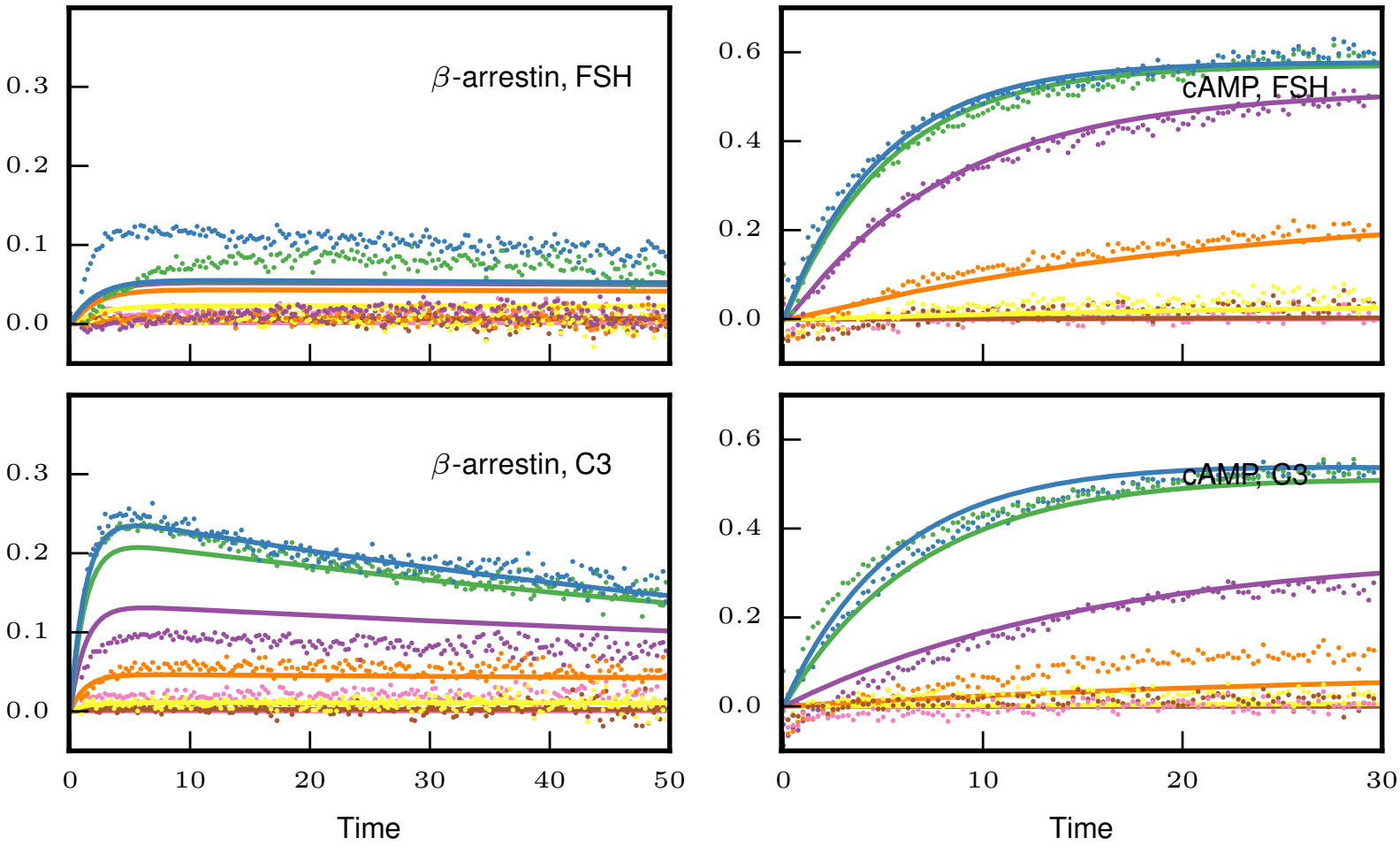
With a "simpler" model

Kinetic model without G-protein



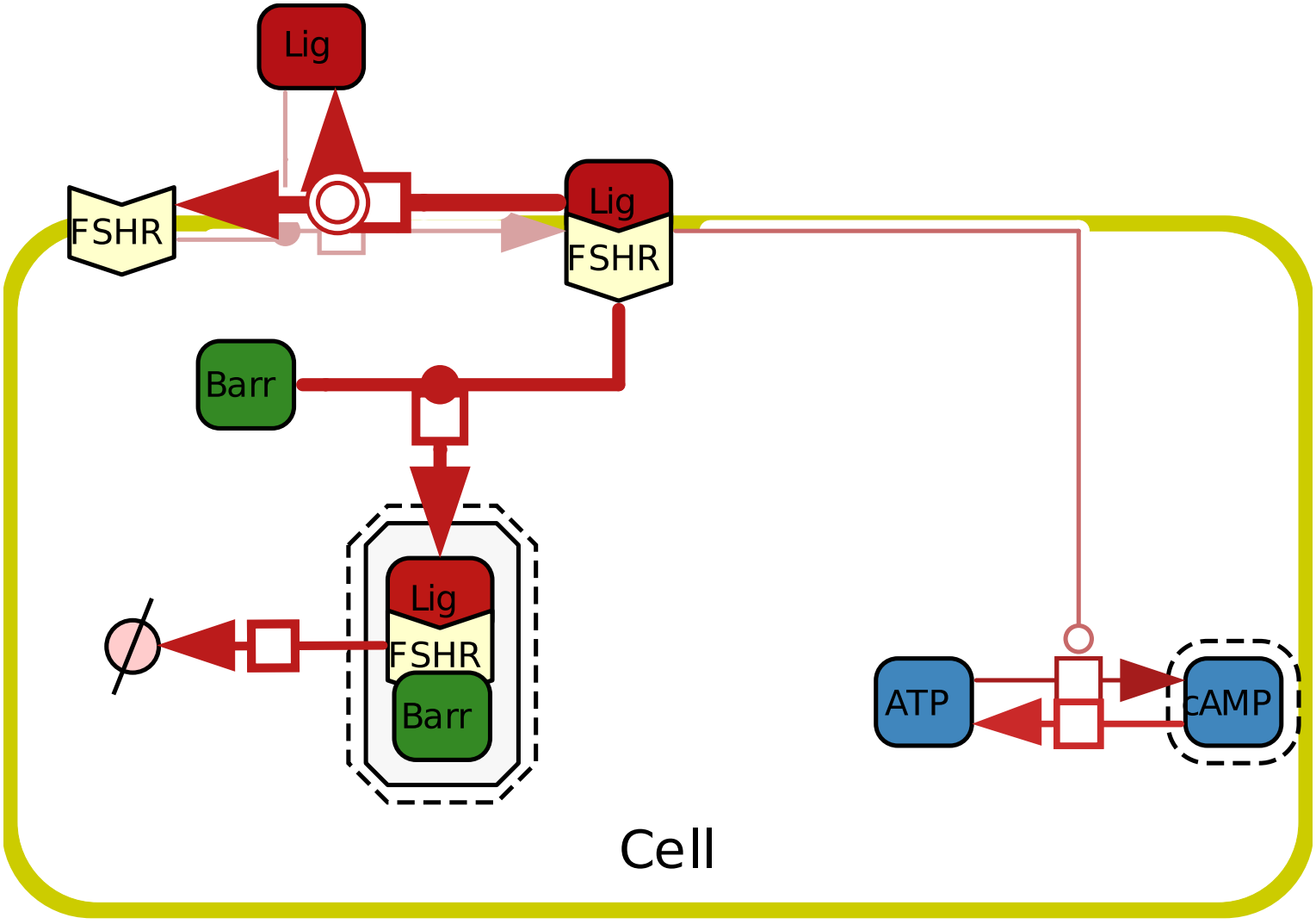
With a "simpler" model

We obtain a slightly worse fit



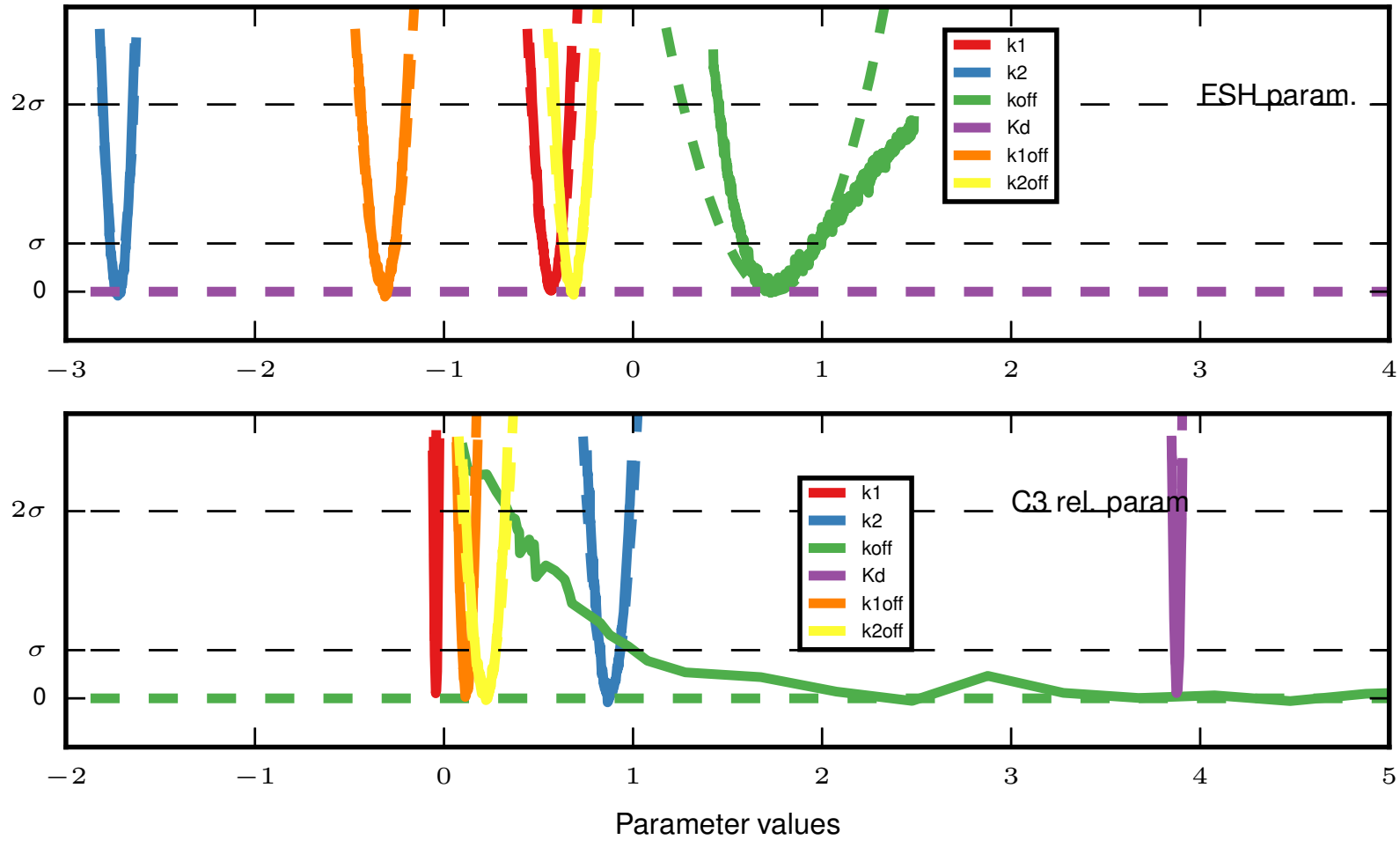
With a "simpler" model

But consistent results



With a "simpler" model

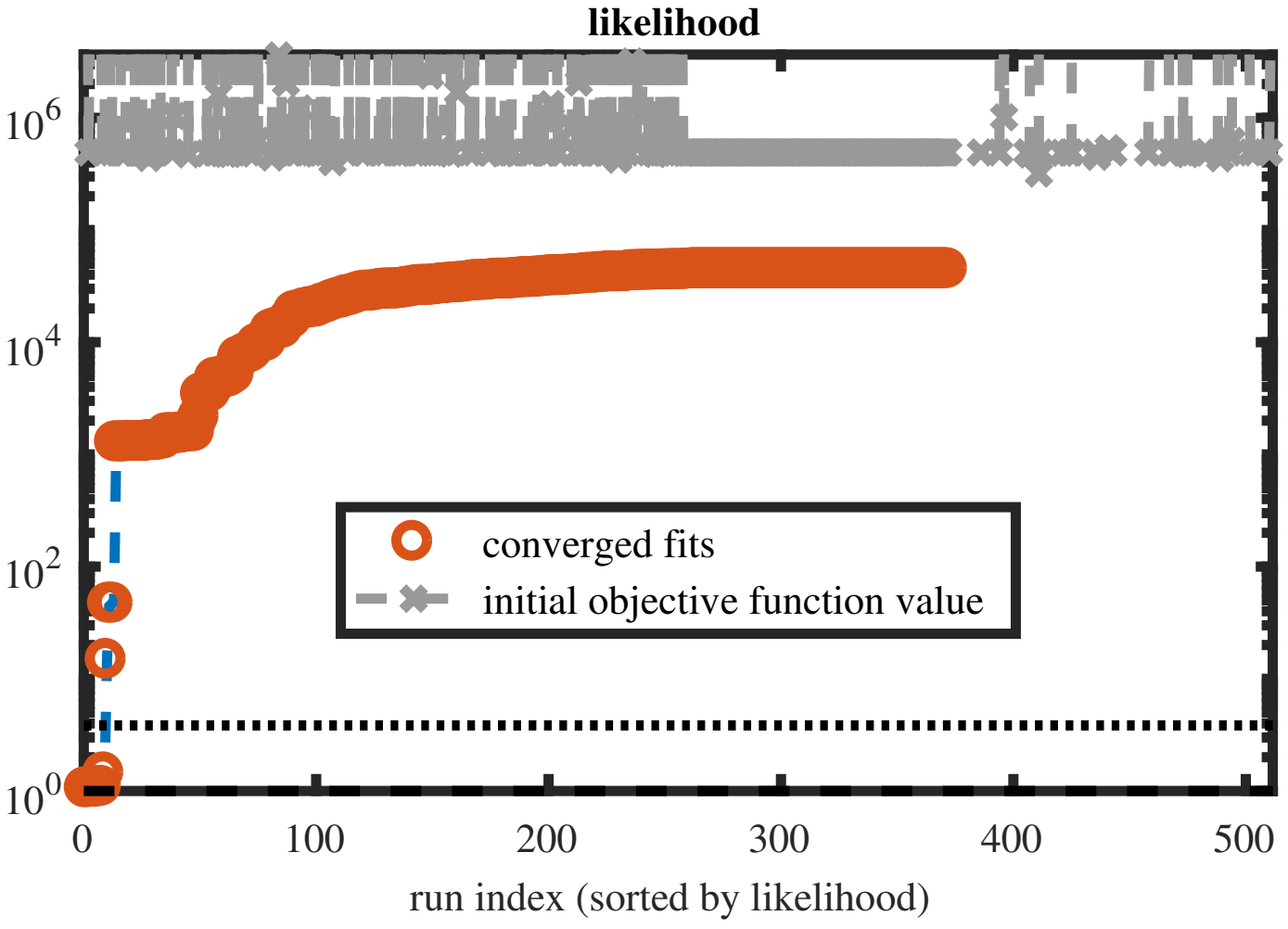
And "better" parameter identifiability



C3 is biased towards β-arr, compared to cAMP, in comparison to FSH.

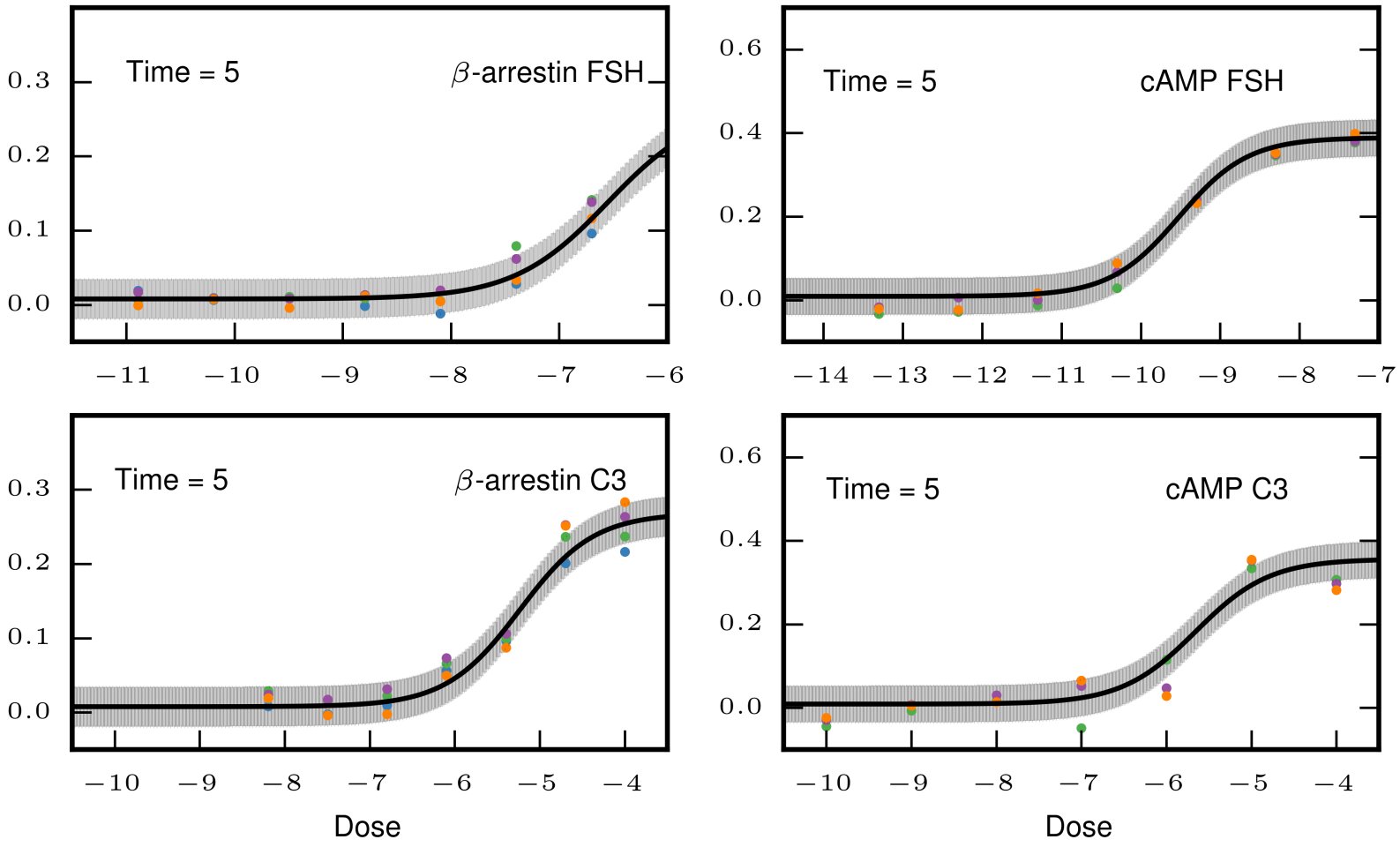
With a "simpler" model

And "better" convergence curves



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

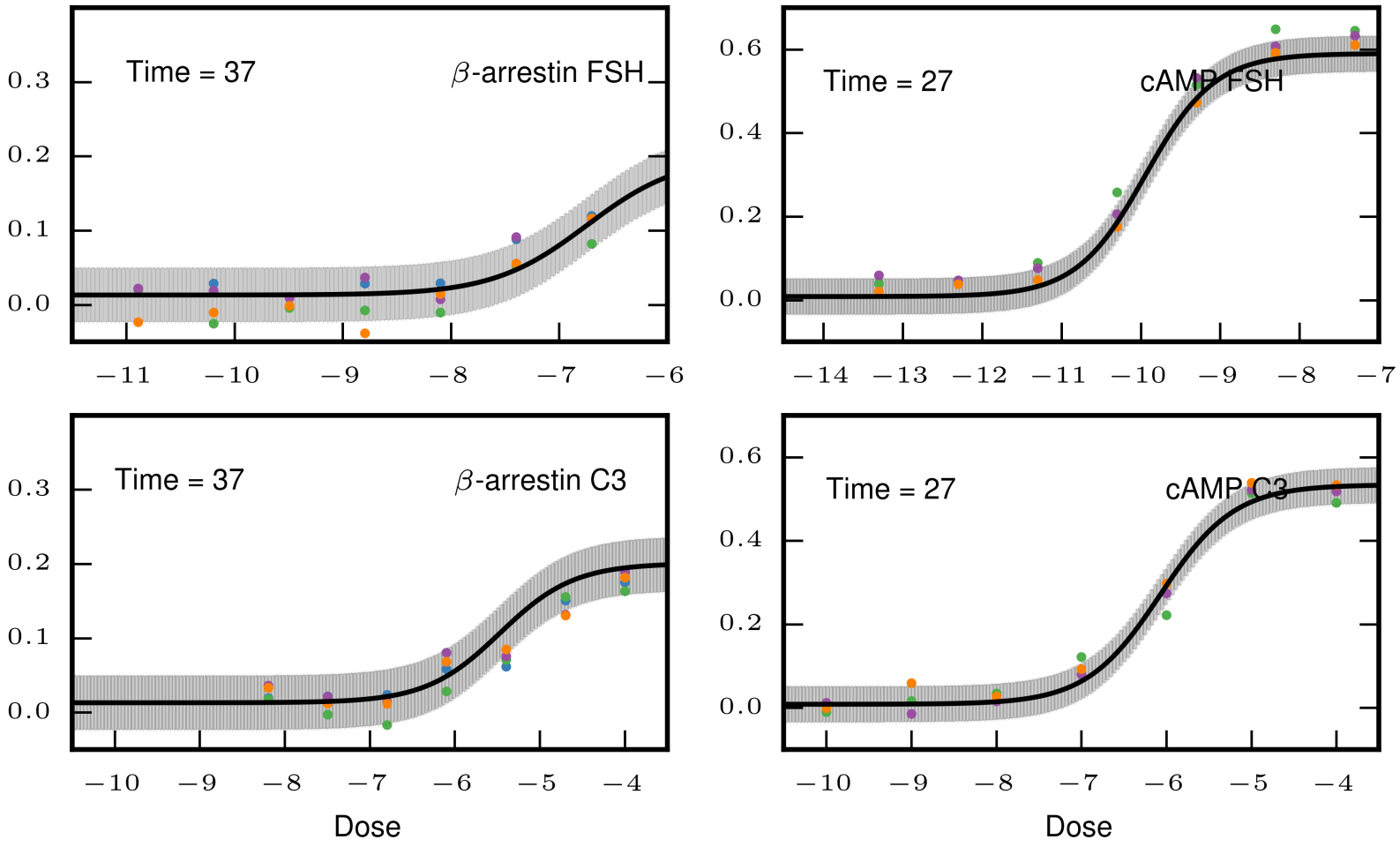
We systematically calculate bias value using standard method (operational model on dose-response curves :)



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 :)

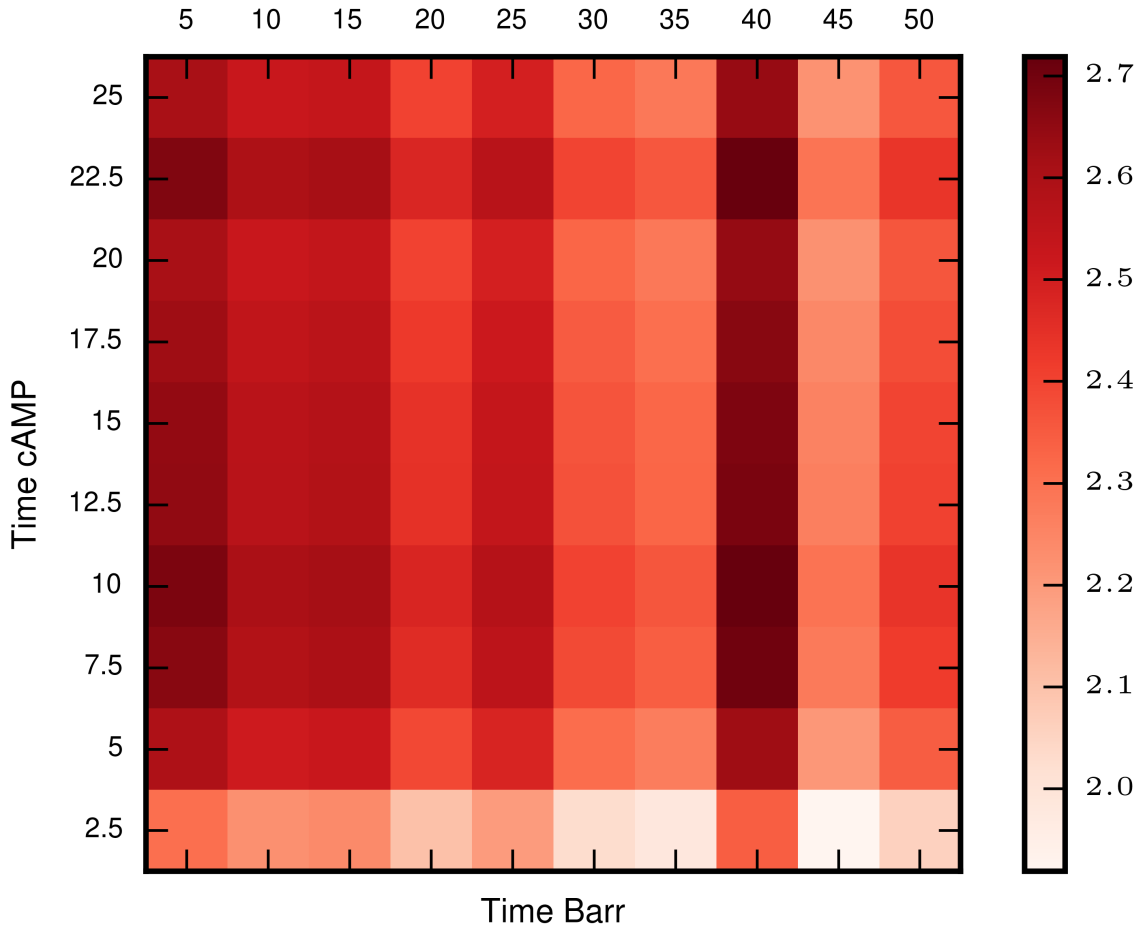


Bias=2.64 : C1 is biased towards β-arr, compared to cAMP, in comparison to FSH.

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

We systematically calculate bias value using standard method

Different times gives (slightly) different bias values

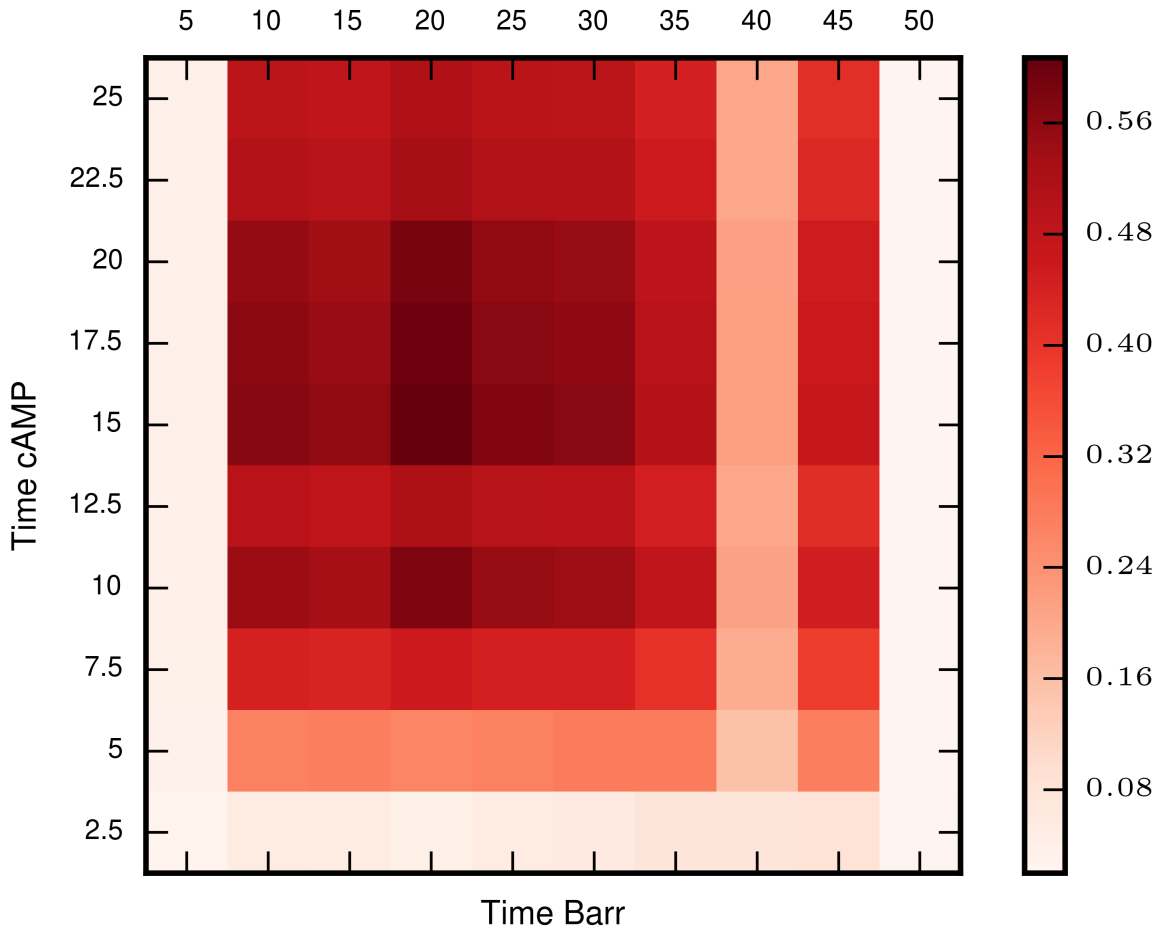


C1 is biased towards β-arr, compared to cAMP, in comparison to FSH.

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



Summary

- 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, et).
- We gave a kinetic interpretation of Ligand biased, which rely on dynamic (ODE) modeling and parameter estimation with L^1 penalization.

Summary

- 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, et).
- We gave a kinetic interpretation of Ligand biased, which rely on dynamic (ODE) modeling and parameter estimation with L^1 penalization.

$\Rightarrow$ How to deal with "fuzzy/noisy" PLE / Densely sampled time data ?

$\Rightarrow$ How to deal with non uniqueness of the penalized solution ?

$\Rightarrow$ How to perform a model reduction that would lead to both a satisfactory fit and identifiable parameters ?

Thanks for your attention !

Bios Team, PRC, INRA (Tours, Fr)

- ⋆ Eric Reiter
- ⋆ Pascale Crépieux
- ⋆ Anne Poupon
- ⋆ Francesco De Pascali

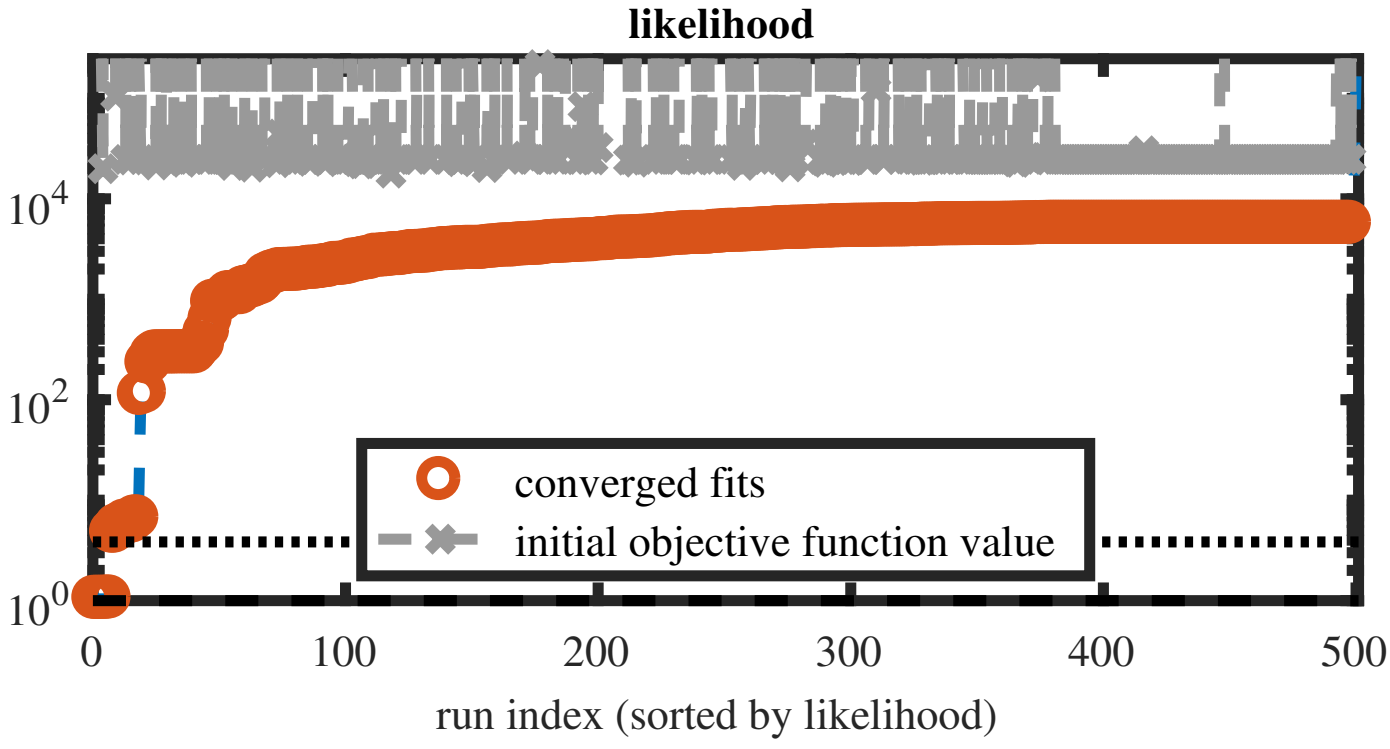
United Arab Emirates University

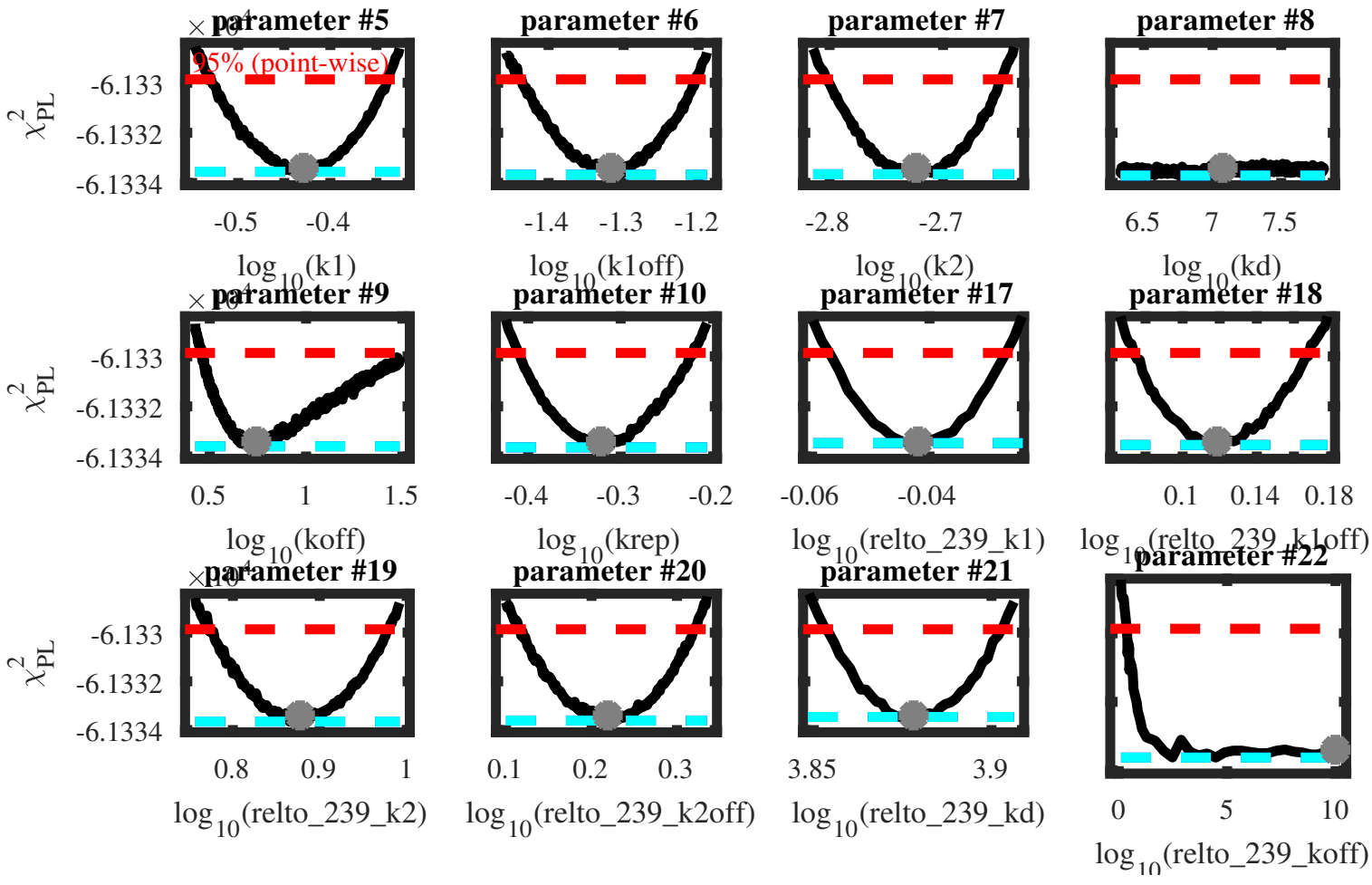
- ⋆ Mohammed Ayoub

M. Ayoub et al., Molecular and Cellular Endocrinology 436 (2016)

L. Riccetti et al., Scientific Reports 7 :940 (2017)

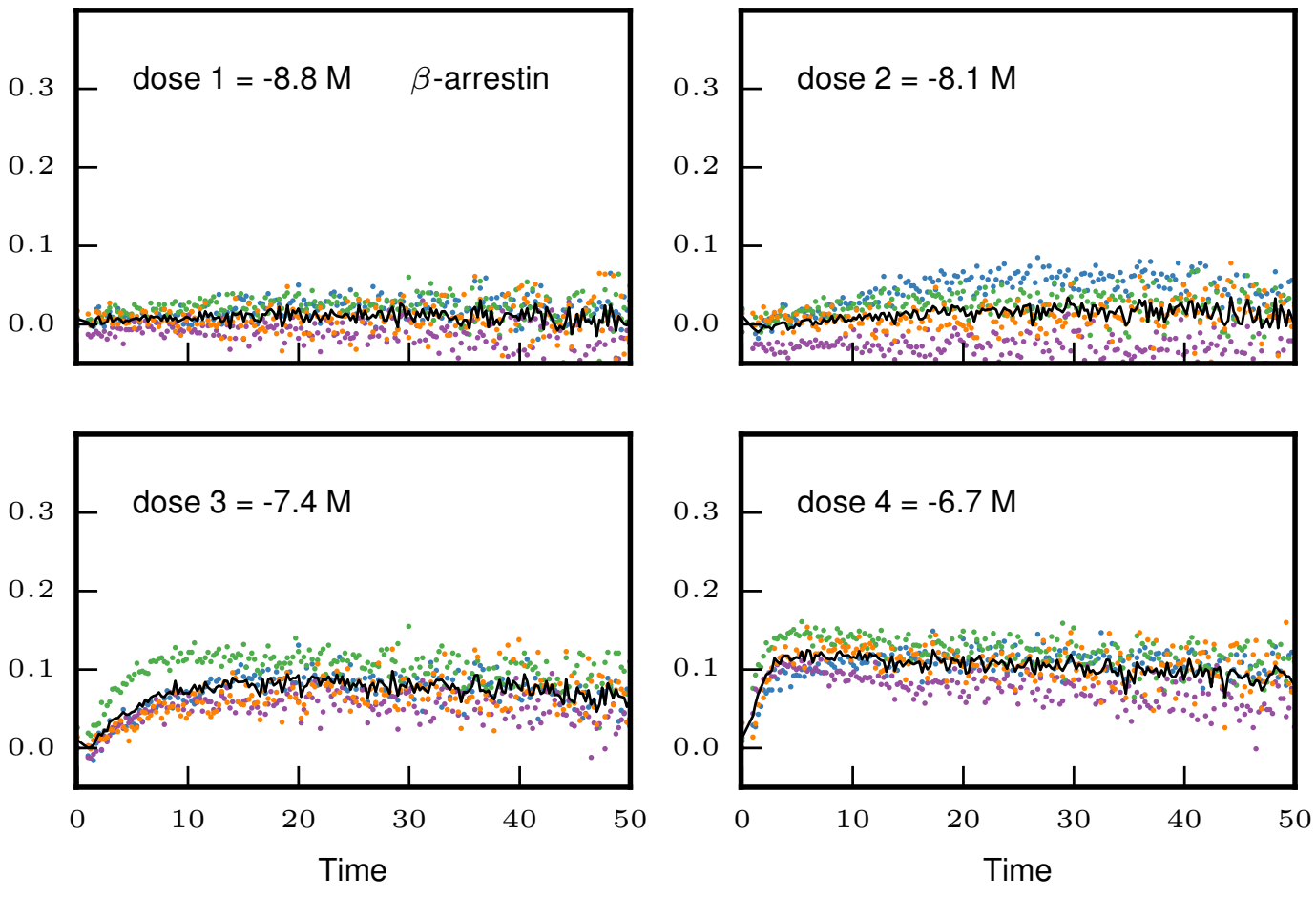
R.Y. et al., Methods in Molecular Biology, in press (2018)


likelihood
10^4
10^2
10^0
converged fits
initial objective function value
0
100
200
300
400
500
run index (sorted by likelihood)


parameter #5
parameter #6
parameter #7
parameter #8
parameter #9
parameter #10
parameter #17
parameter #18
parameter #19
parameter #20
parameter #21
parameter #22
95% (point-wise)
χ^2_{PL}
-6.133
-6.1332
-6.1334
$\log_{10}$(k1)
$\log_{10}$(k1off)
$\log_{10}$(k2)
$\log_{10}$(kd)
$\log_{10}$(koff)
$\log_{10}$(krep)
$\log_{10}$(relto_239_k1)
$\log_{10}$(relto_239_k1off)
$\log_{10}$(relto_239_k2)
$\log_{10}$(relto_239_k2off)
$\log_{10}$(relto_239_kd)
$\log_{10}$(relto_239_koff)

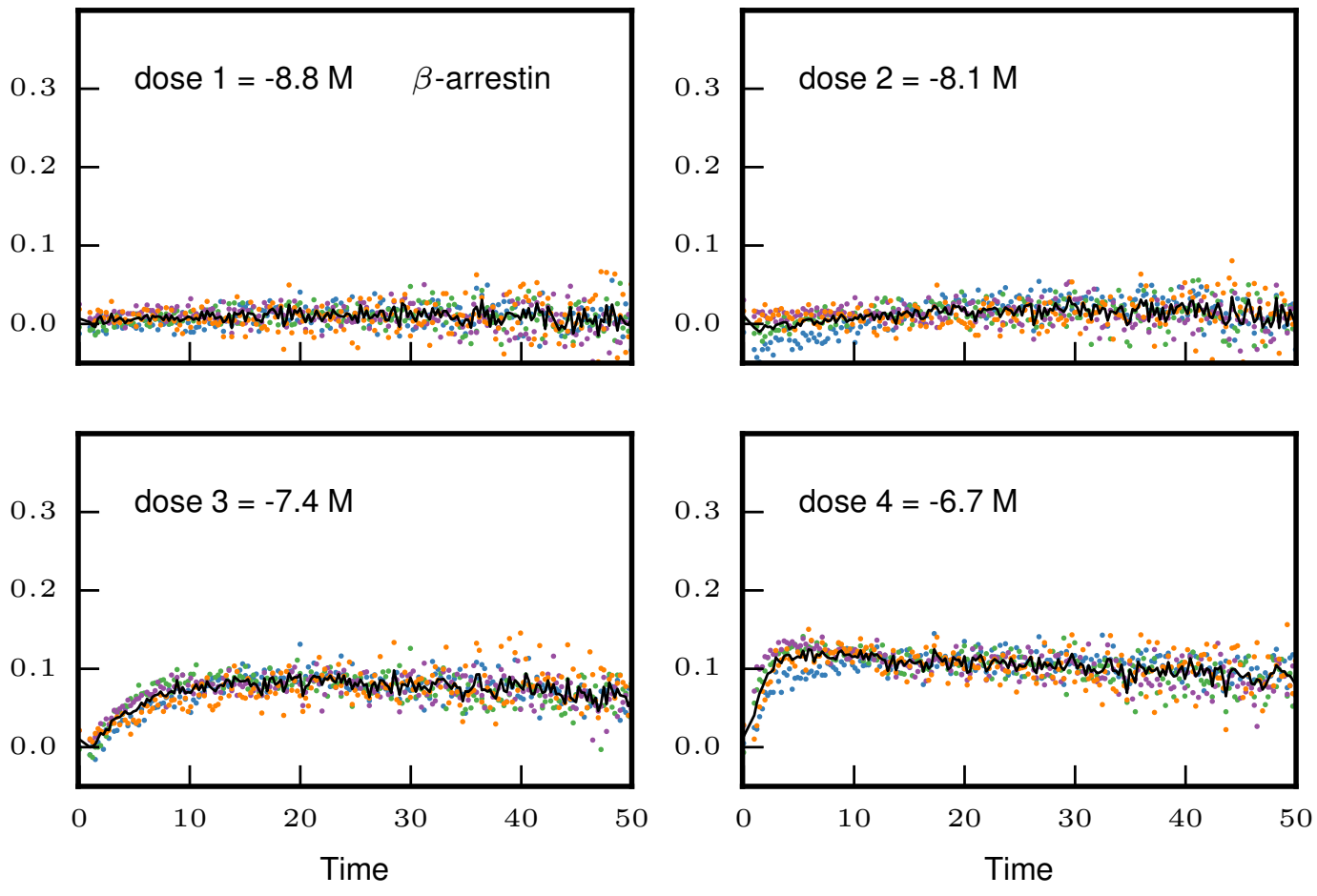
("trick" to minimize variance...)

Original "raw" data



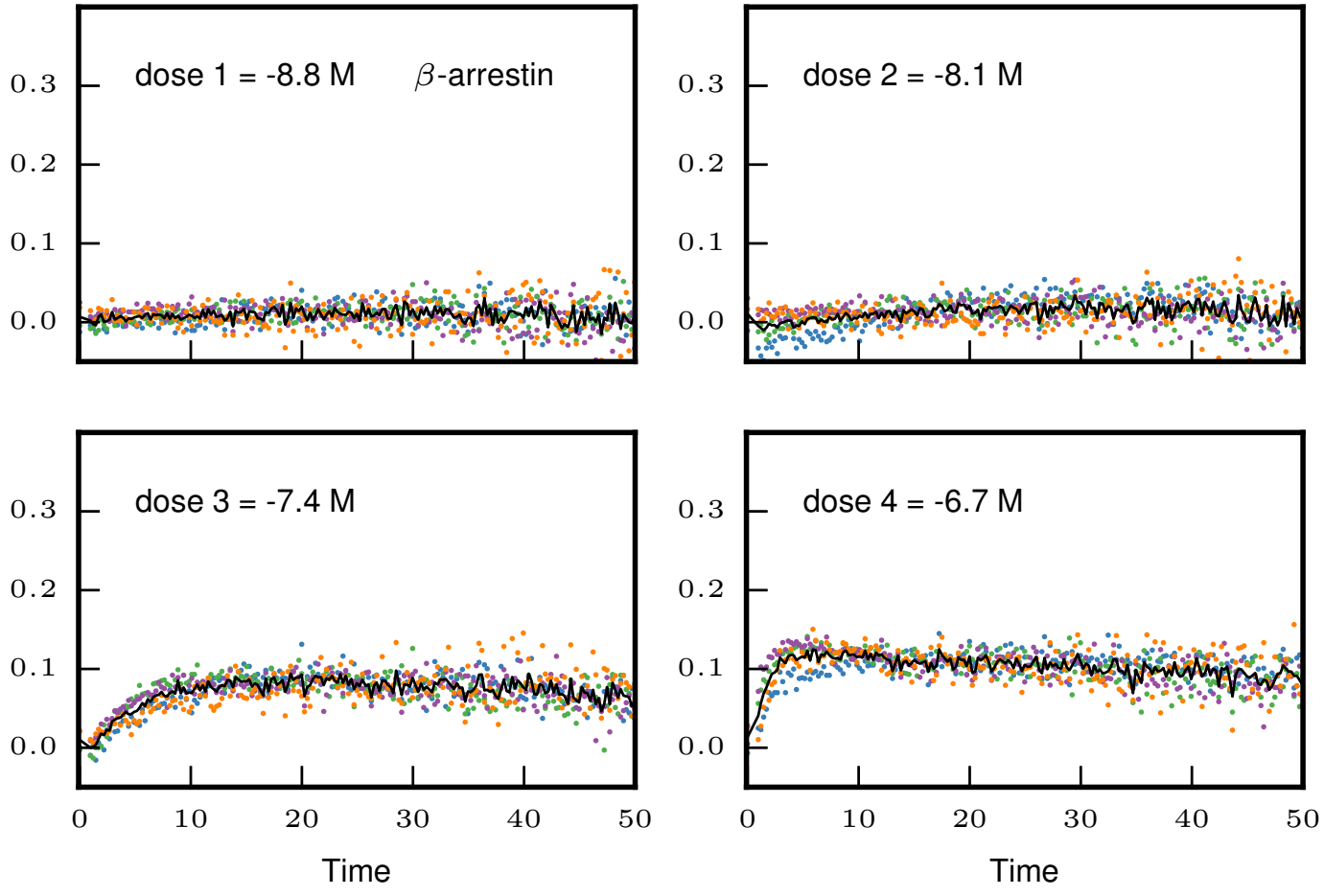
("trick" to minimize variance...)

"Adjusted" data



("trick" to minimize variance...)

"Adjusted" data



+ adjusting the number of data points ...

Other extensions

Dose-dependent bias

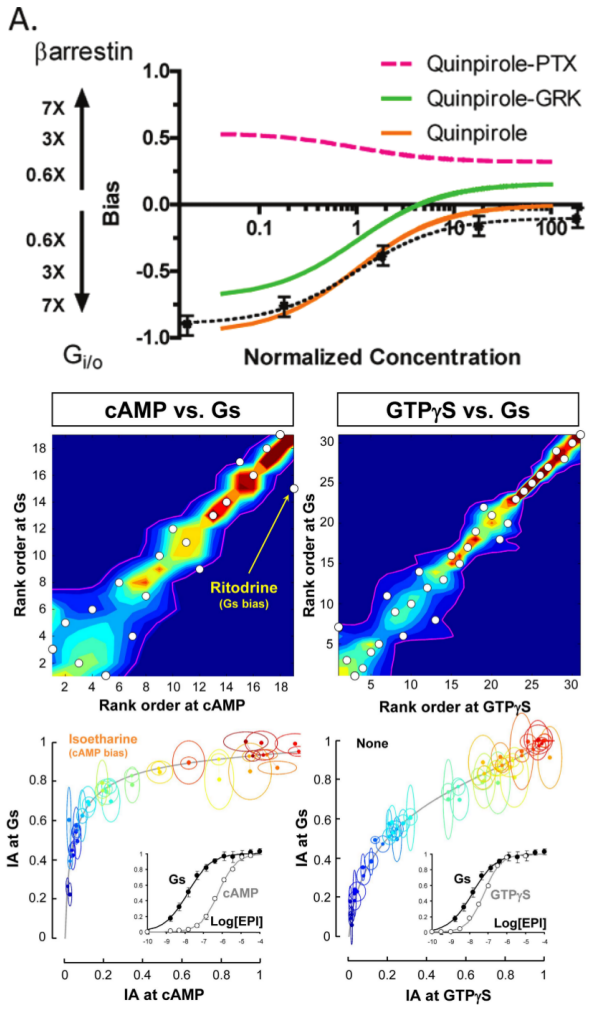
Barak and Peterson et al., *Biochem.* (2012)

Extension of the operational model

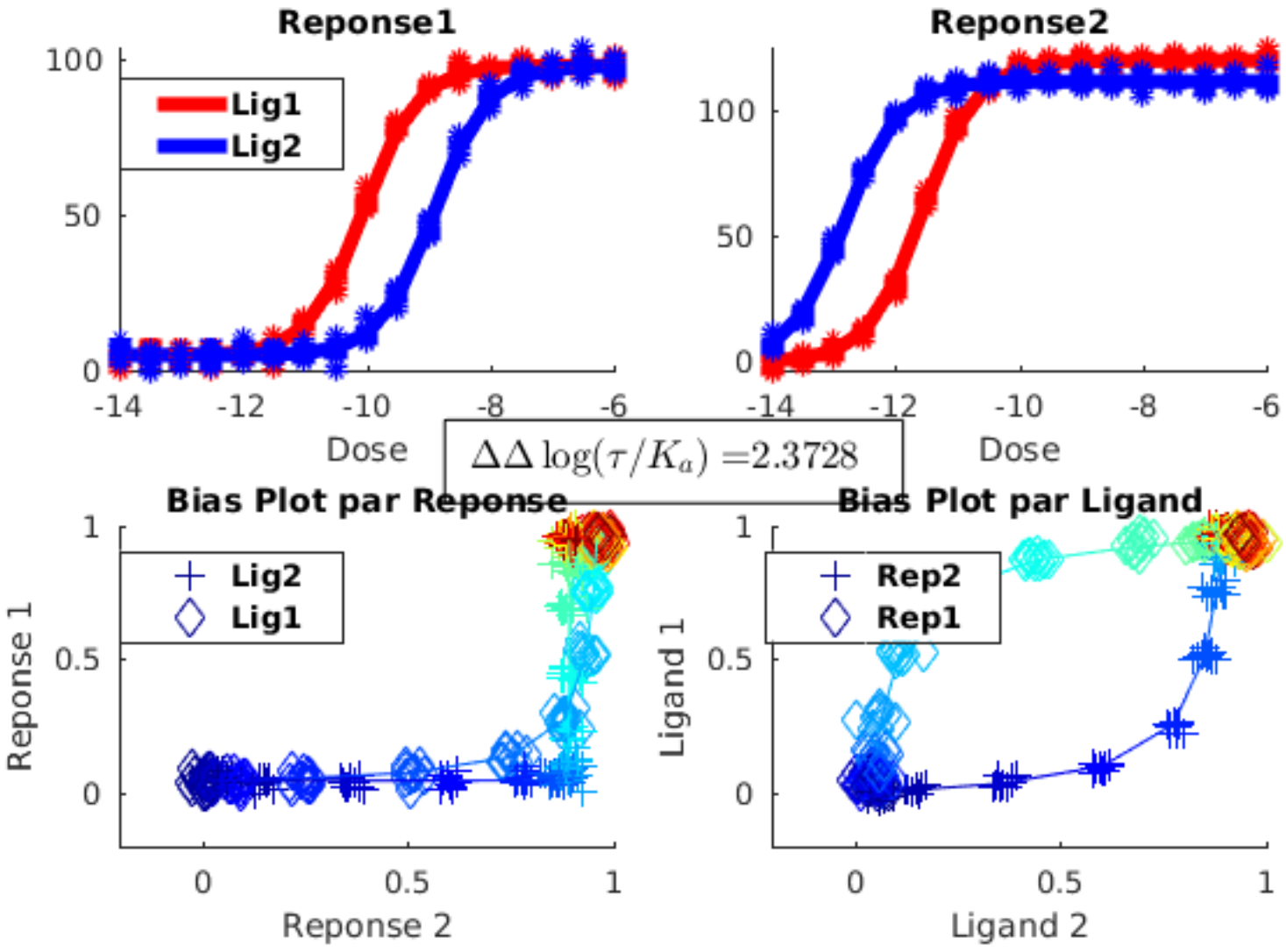
Kenakin, *Chem. Rev.* (2017)

Method based on Intrinsic activities and rank ordering

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

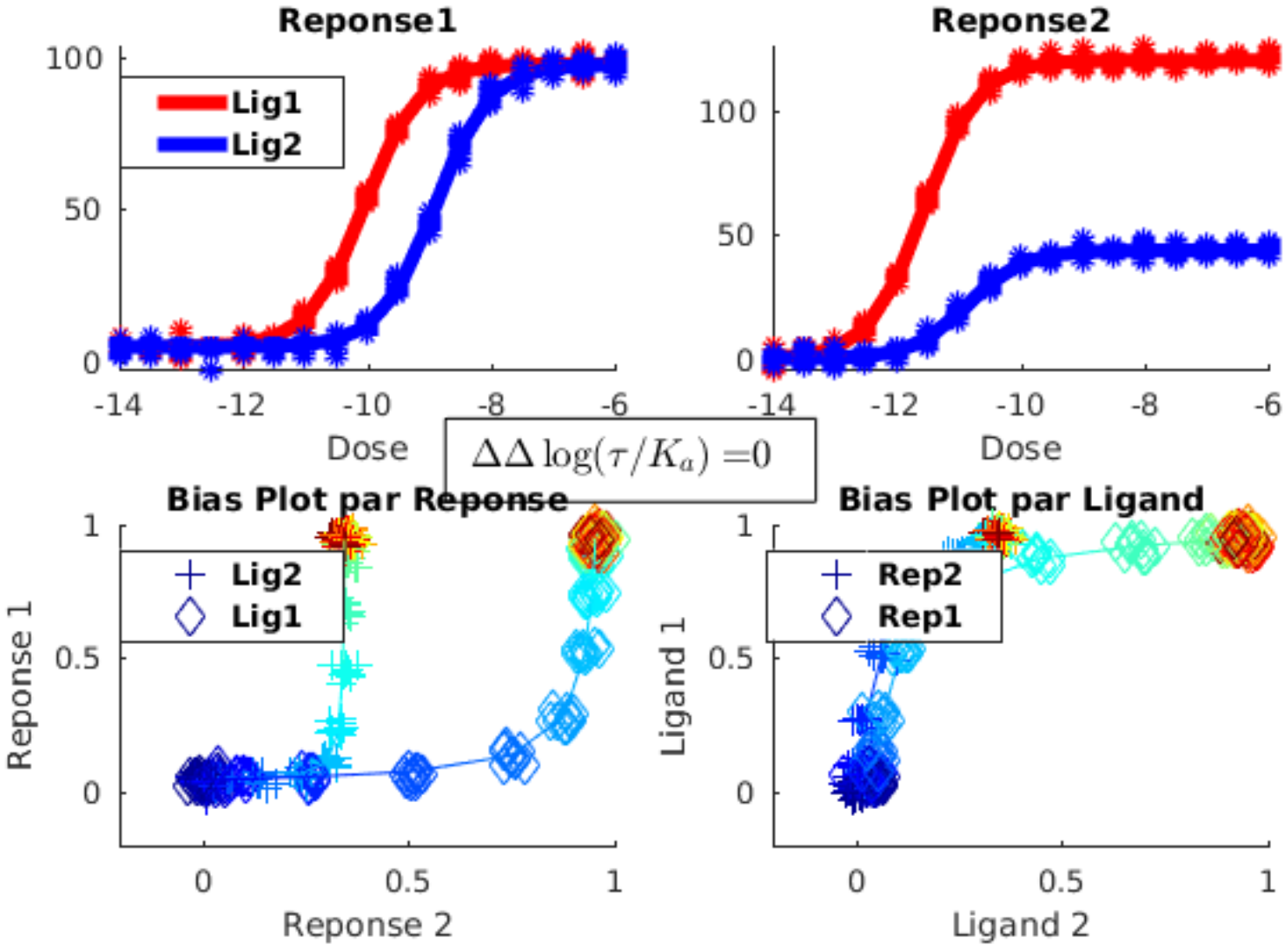


Is bias calculation intuitive ? (simulated data)



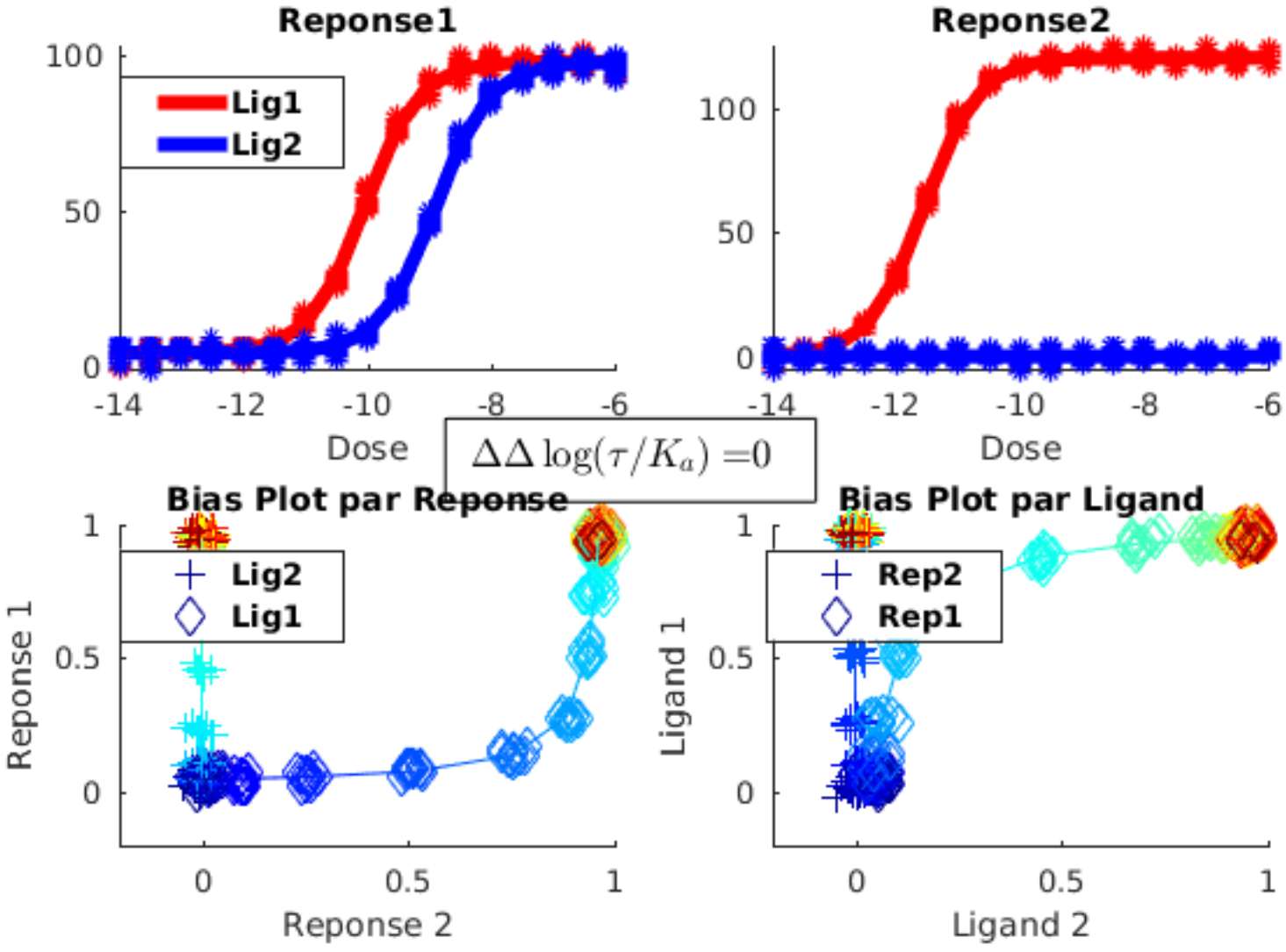
A strong bias is usually 'apparent' on dose-response curves or bias plot

Is bias calculation intuitive ? (simulated data)



But there may be counter-intuitive situation...

Is bias calculation intuitive ? (simulated data)



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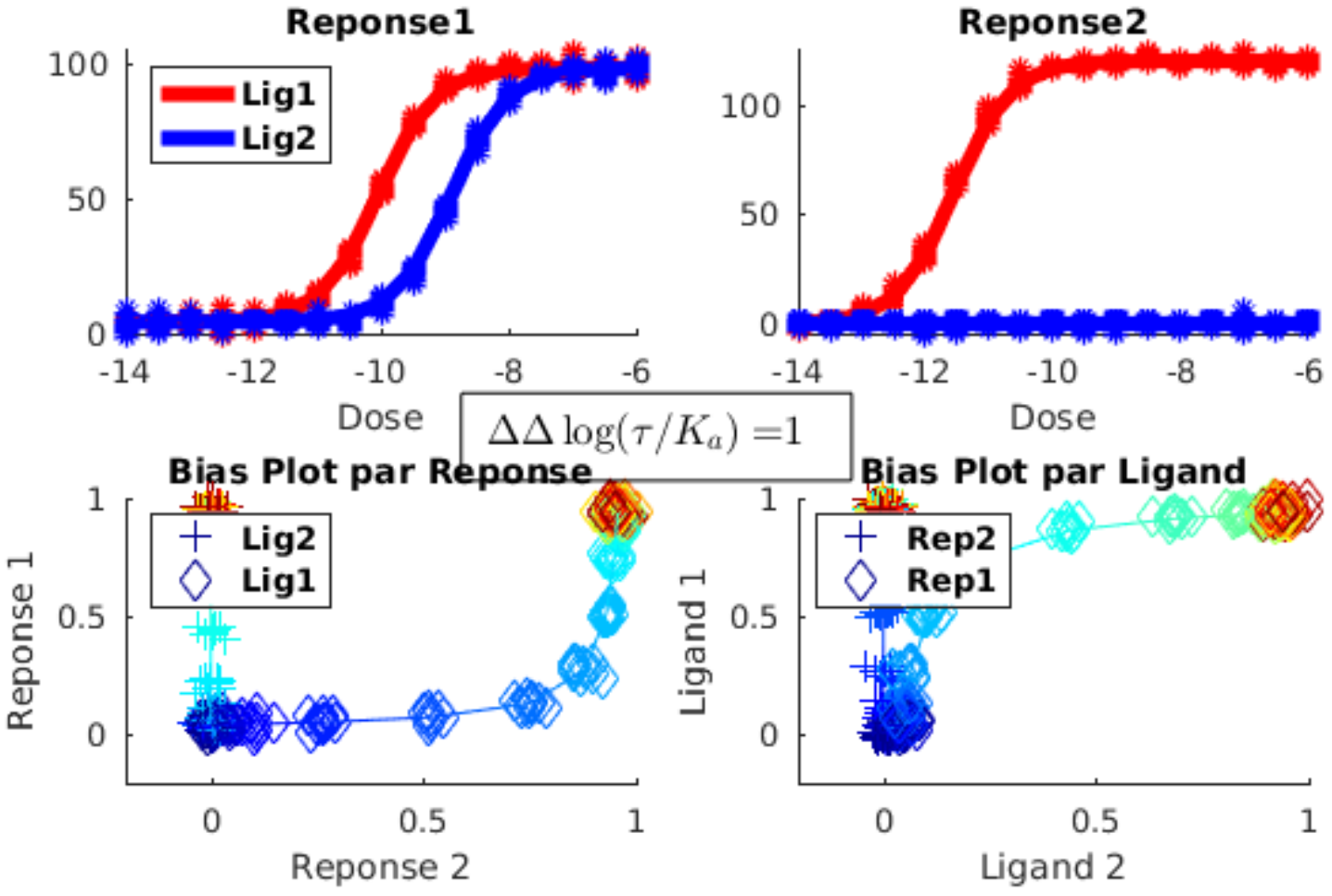

But there may be counter-intuitive situation...

... and those situations occur in real life !