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

Outline

Functional selectivity, biased signaling

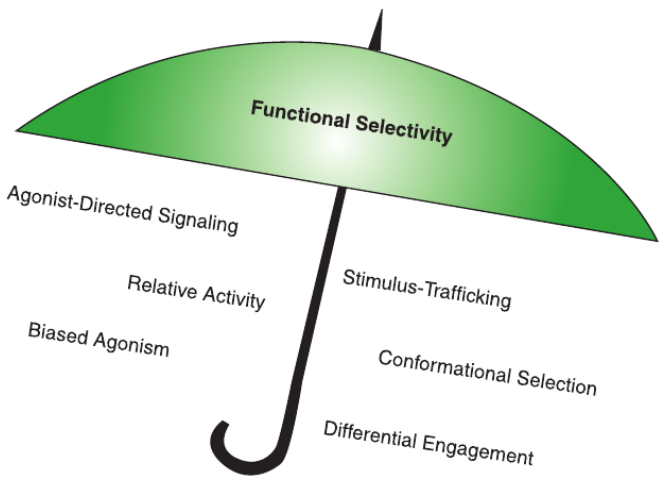
Functional selectivity

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

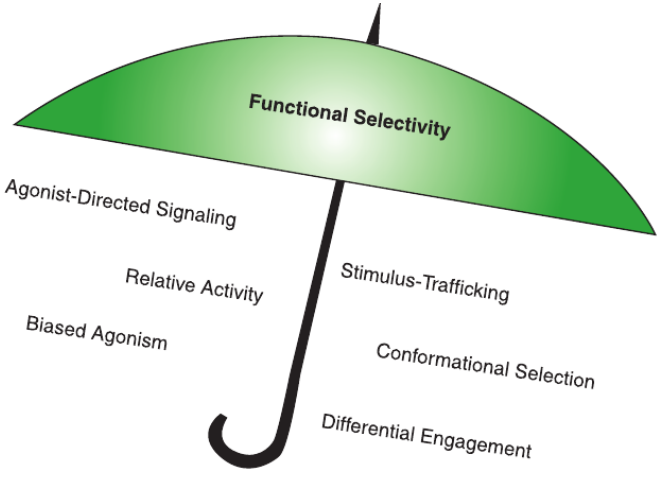
Functional selectivity

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]

- Ligand-specific activity in one receptor



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

Functional selectivity, biased signaling

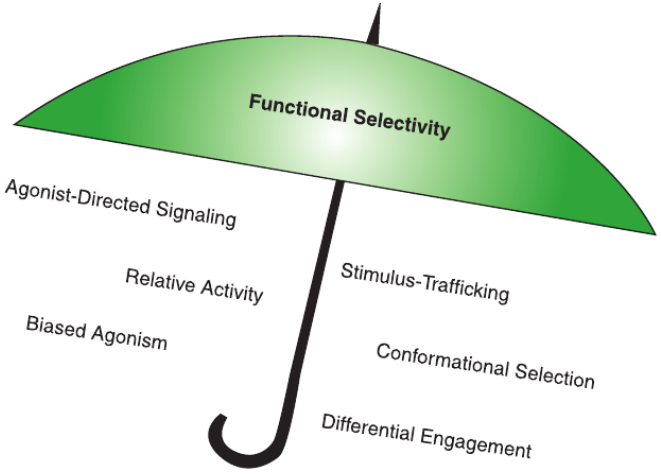
Functional selectivity

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]

- Several potential pathways associate to a given receptor



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

Functional selectivity, biased signaling

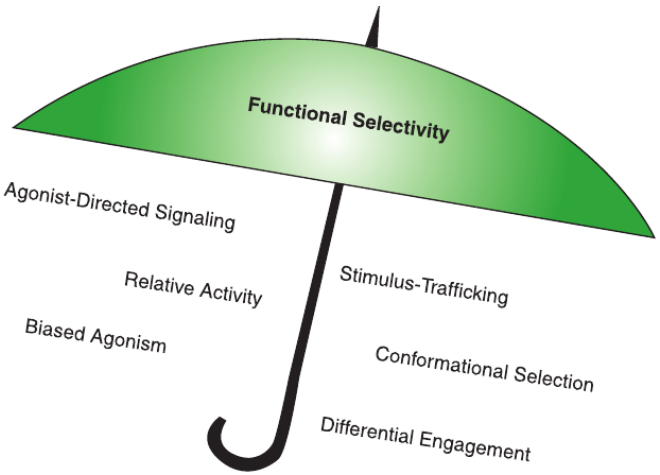
Functional selectivity

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]

- 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

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

Key concept in pharmacology

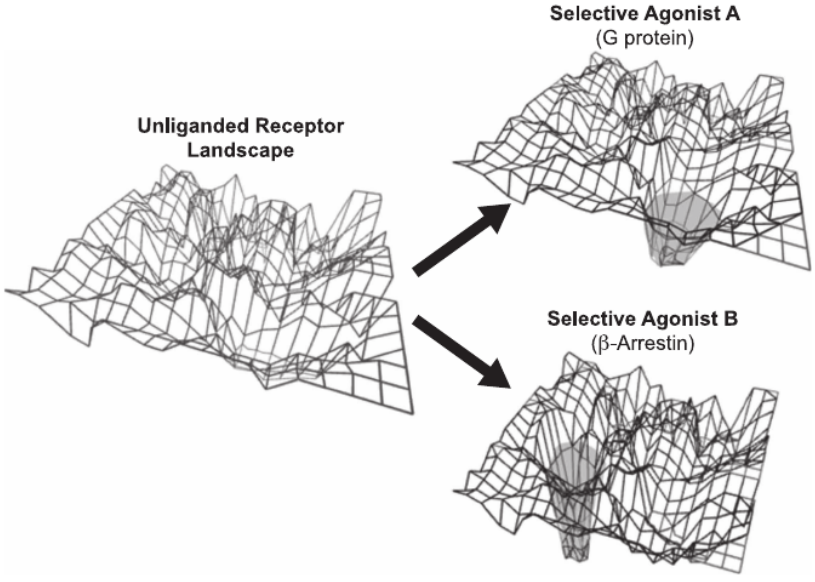
- ◇ Biased agonism is a key concept to be distinguish from
  - ▸ Partial or full agonist.
  - ▸ Antagonist, inverse agonist.
  - ▸ Affinity (K_d), potency (EC_{50}), efficacy (E_{max}).
- ◇ A bias might be **context-dependent** (cell type, physiological state, etc.)
- ◇ Biased agonism is becoming a major 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.

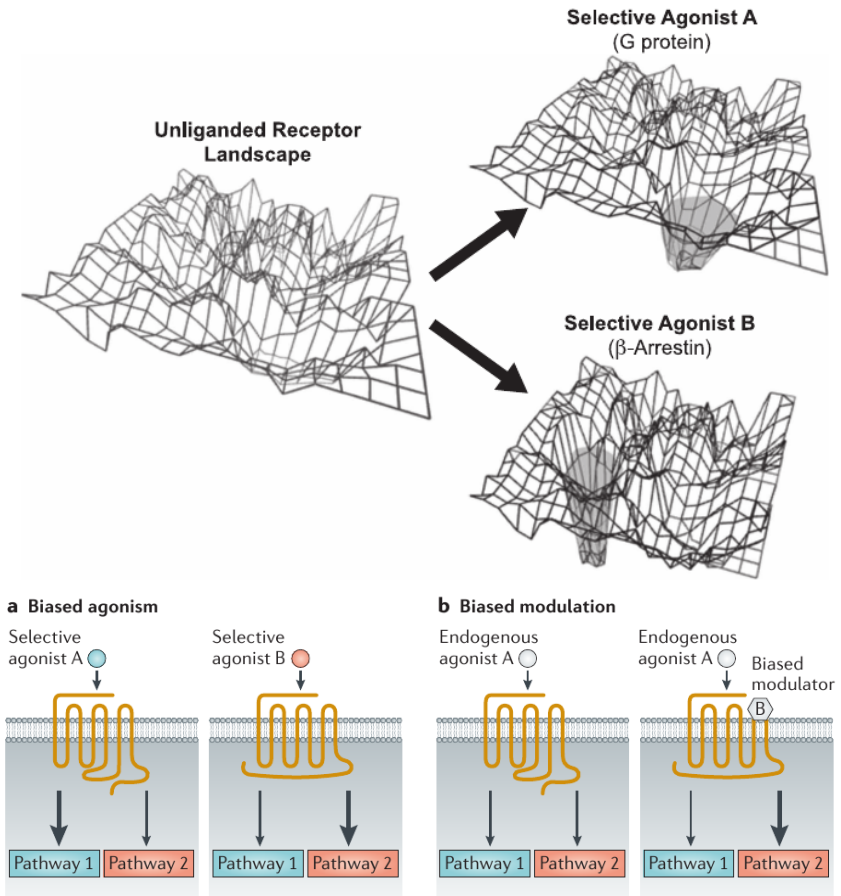


Kenakin, *J Pharmacol Exp Ther* (2011)

Theoretical foundation

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

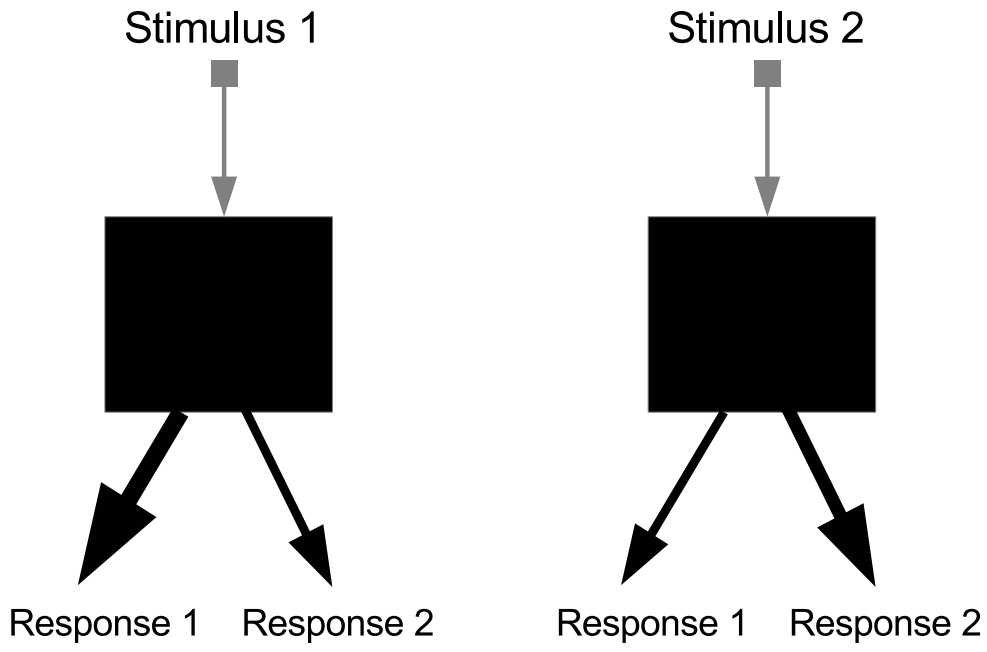
- Conformational selectivity = Ligand-specific modification of the energetic landscape, changing affinities and efficacies of signaling patways.
- Similar concept : modulating bias



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

Summary so far

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



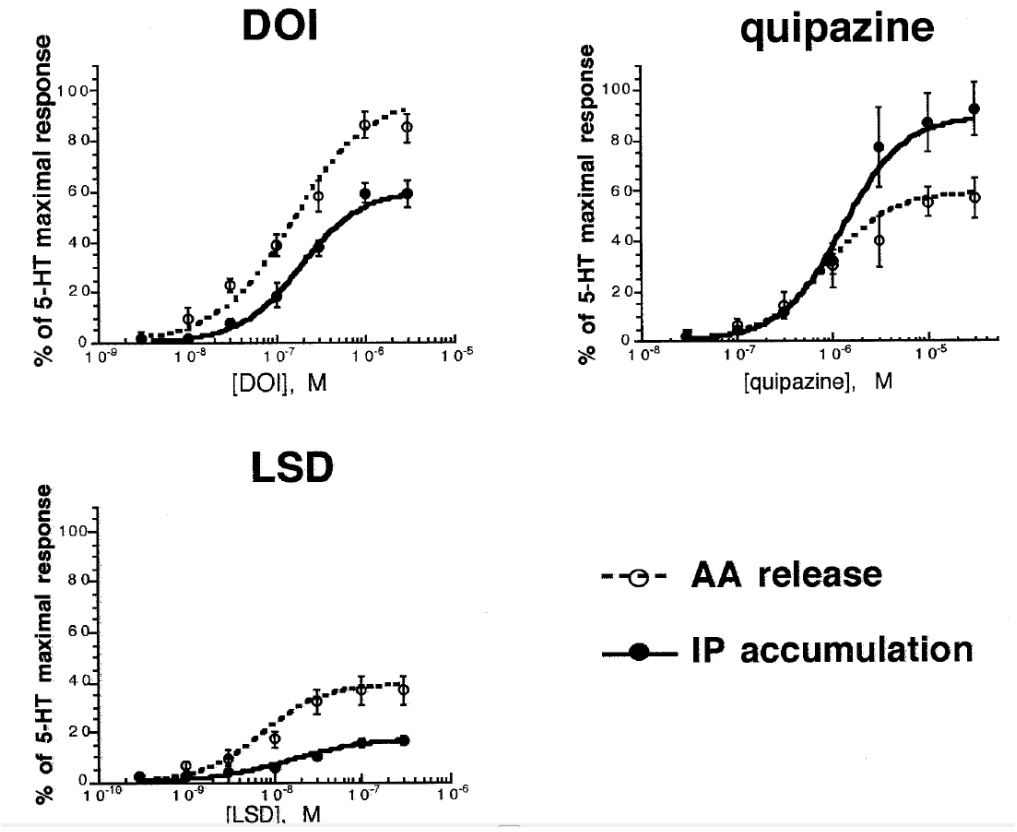
$\Rightarrow$ We always compare *a ligand with respect to a reference one.*

Outline

Serotonine receptor 5 – HT_{2C}

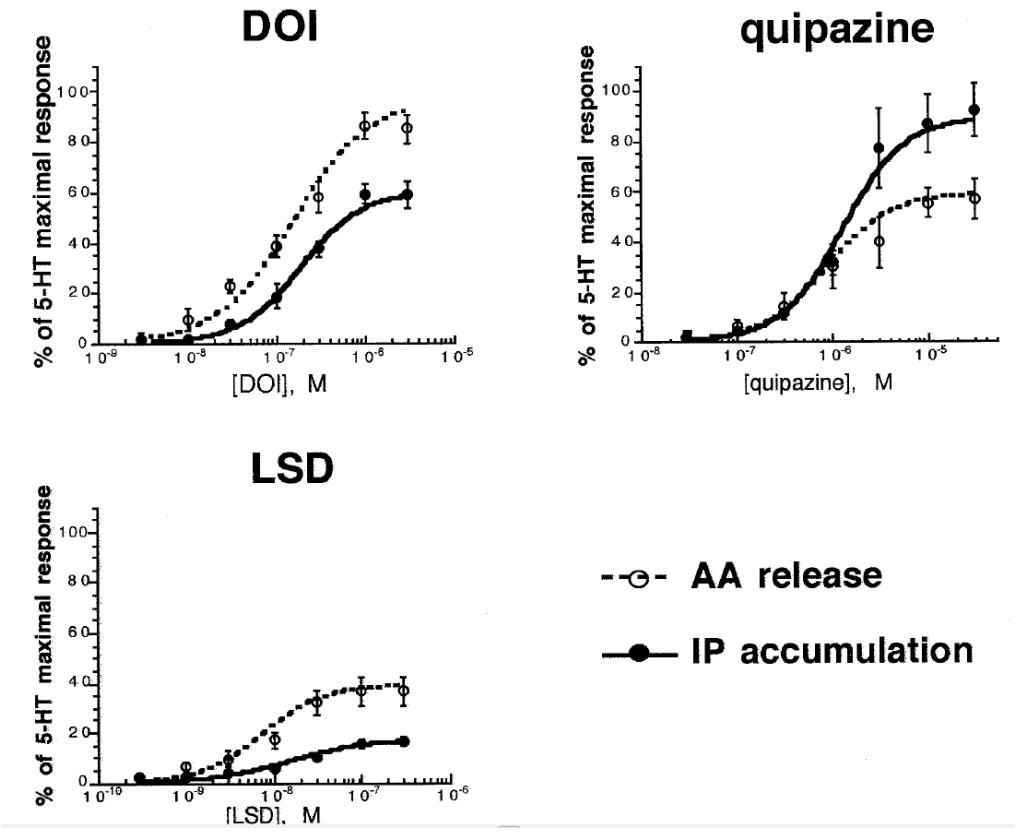
- Quipazine is biaised towards *PI* accumulation with respect to *AA* production, *compared to the reference agonist DOI*.
- *LSD* is not biased.

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



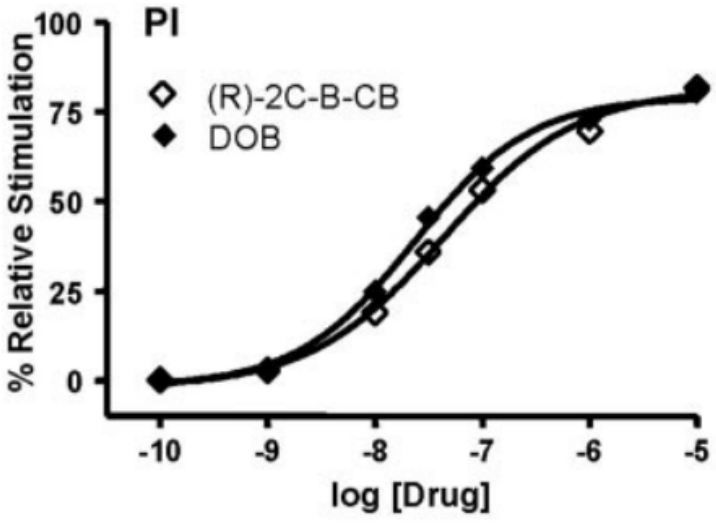
Serotonine receptor 5 – HT_{2C}

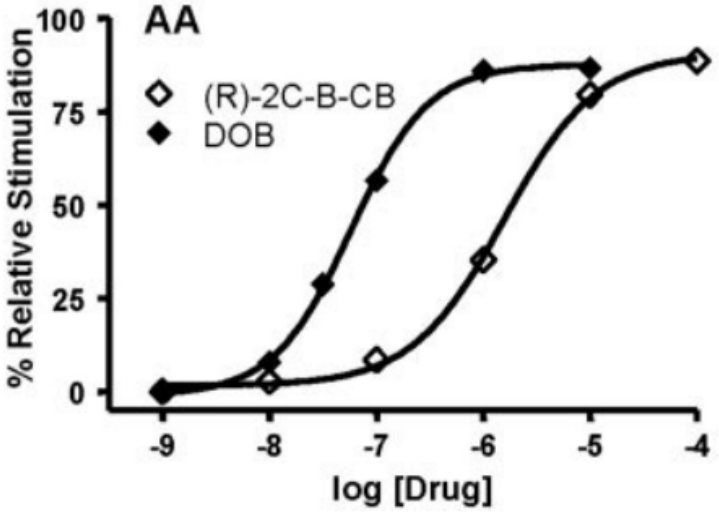
- Quipazine is biaised towards *PI* accumulation with respect to *AA* production, *compared to the reference agonist DOI*.
- *LSD* is not biased.
- Bias due to an E_{max} difference.



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

Serotonine receptor 5 – HT_{2A}

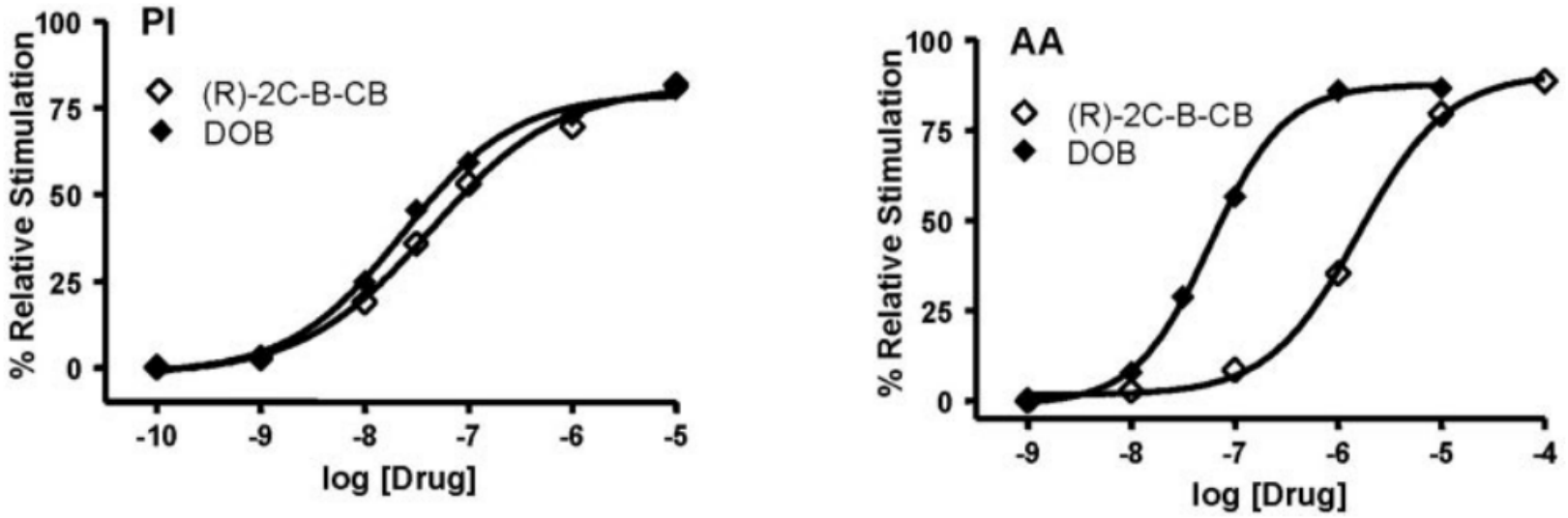




$(R) - 2C - B - CB$ is biaised towards *PI* accumulation with respect to *AA* production, *compared to the reference agonist DOB*.

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

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*.

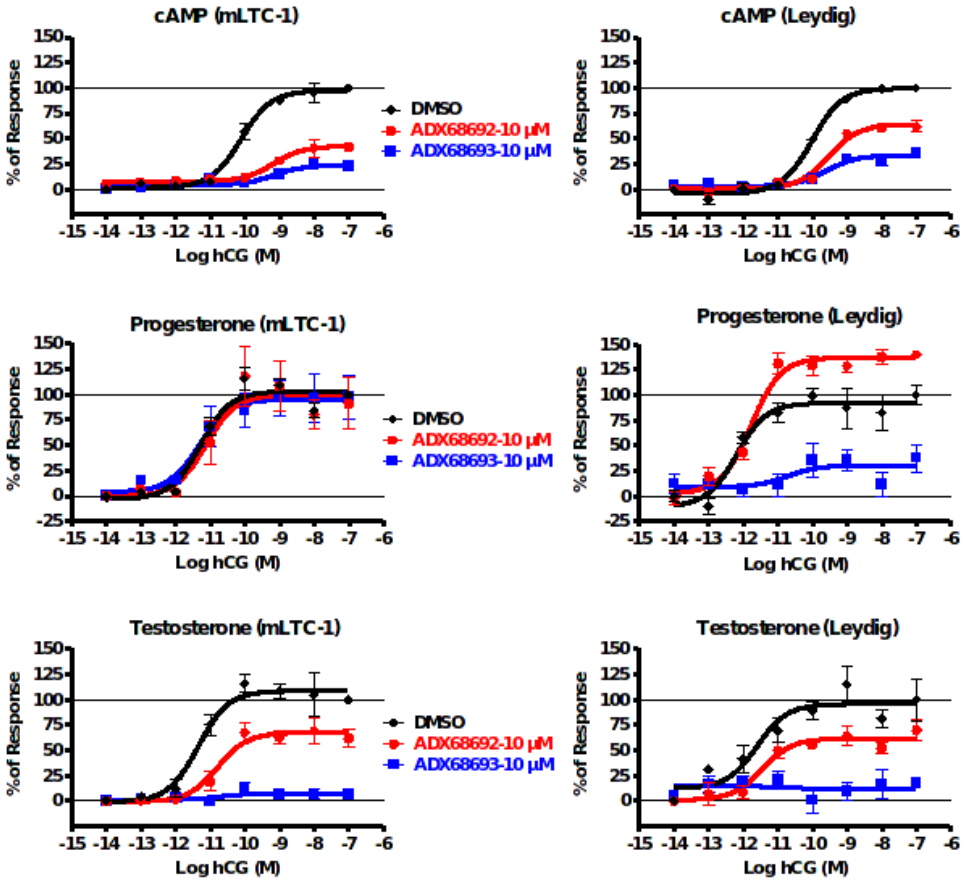
- Bias due to an EC_{50} difference.

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)

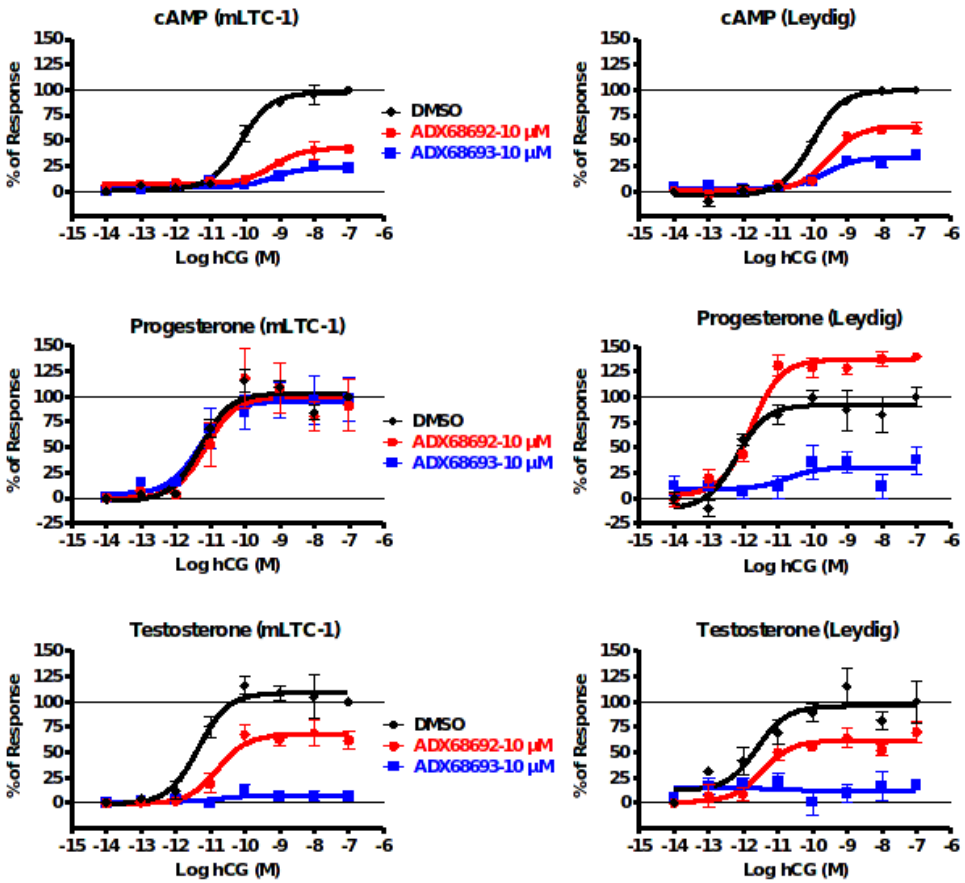


Steroidogenesis modeulated by NAM

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)

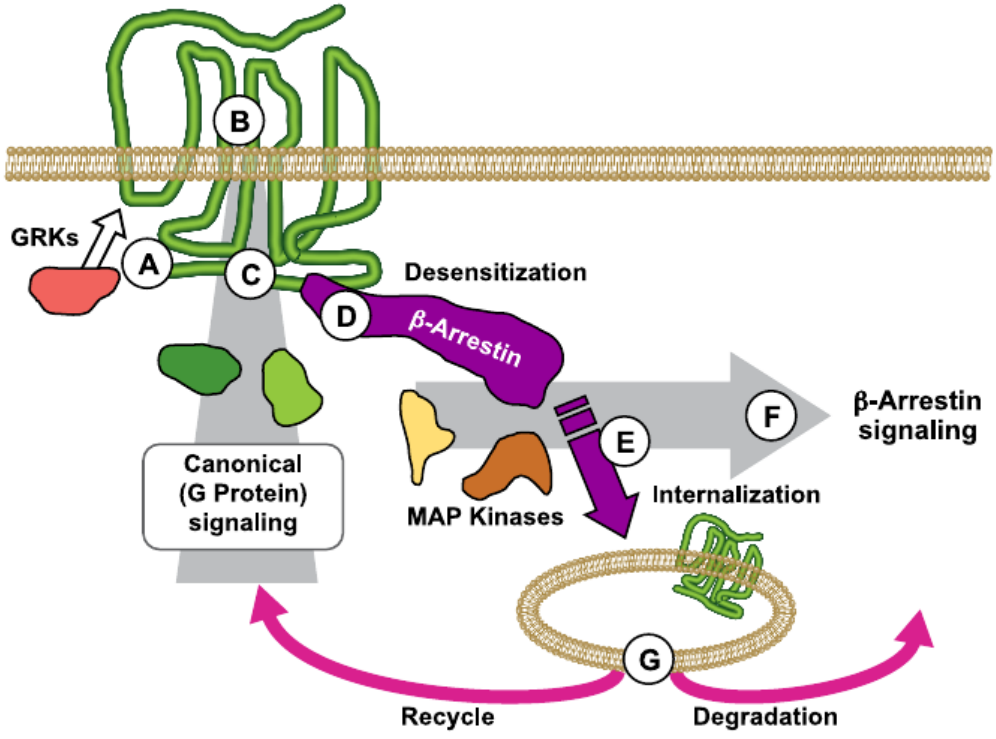


GPCR

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

Kenakin, *J Pharmacol Exp Ther* (2011)

Kenakin, *Chem Rev* (2017)



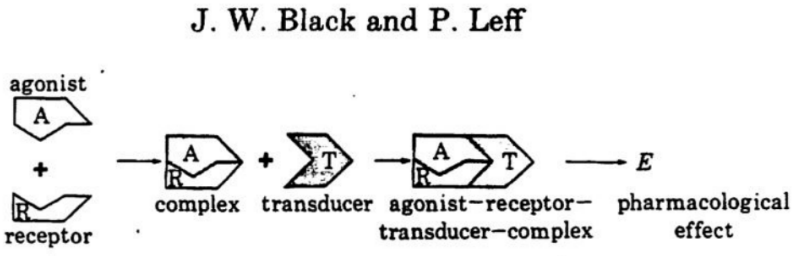
Outline

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)



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

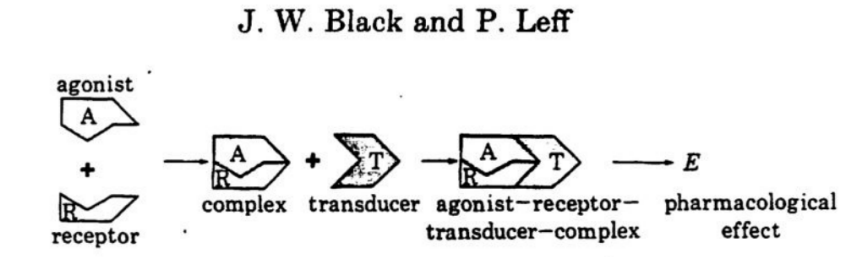
Operational model

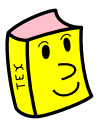
Dose-response data are fitted with the function

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

For $n = 1$,

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





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

Operational model

Dose-response data are fitted with the function

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

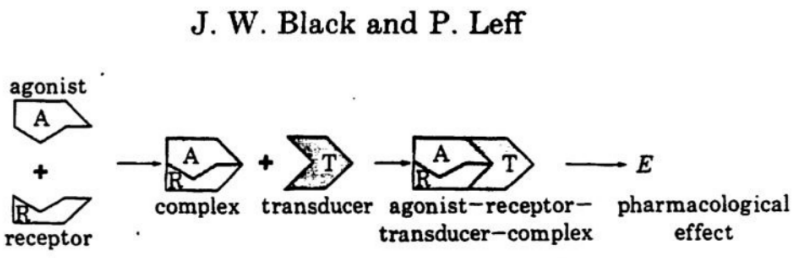
For $n = 1$,

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

Then, we define

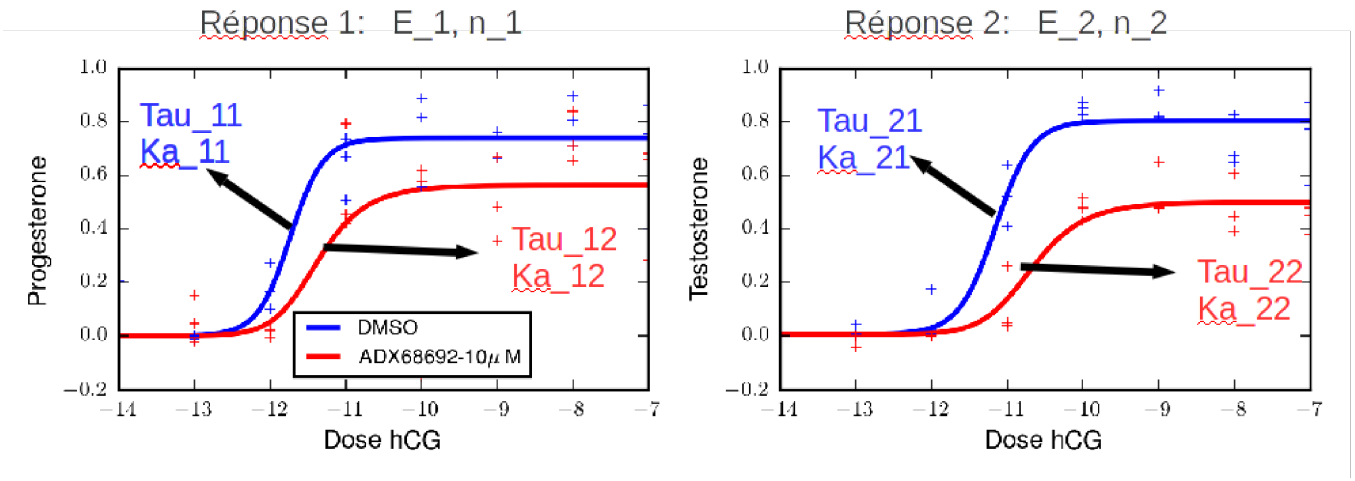
- **Transduction coefficient** :

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



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

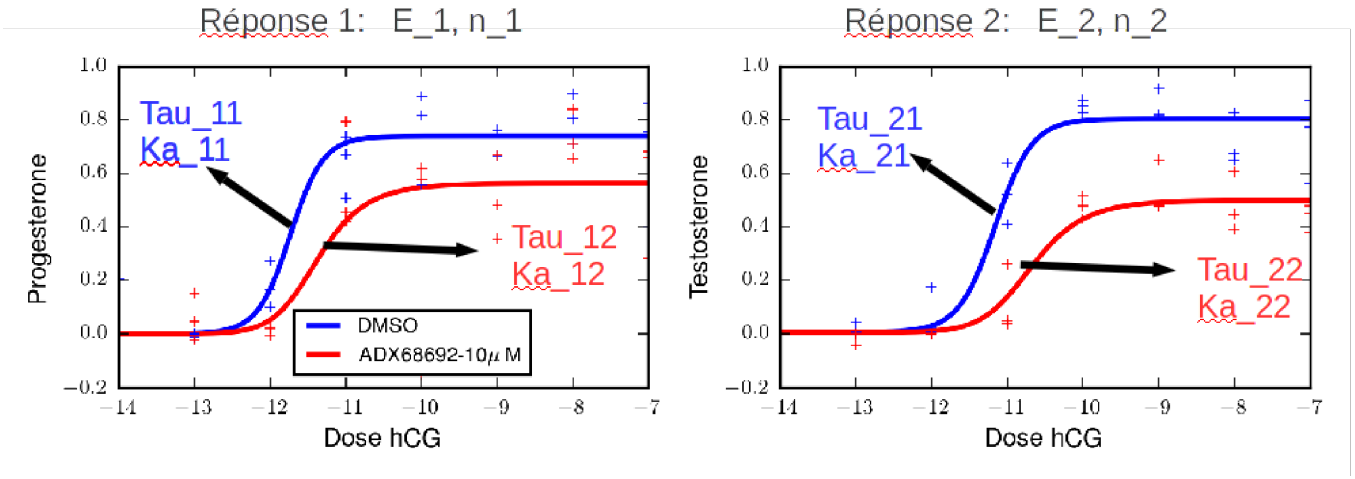
Bias definition With the operational model



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

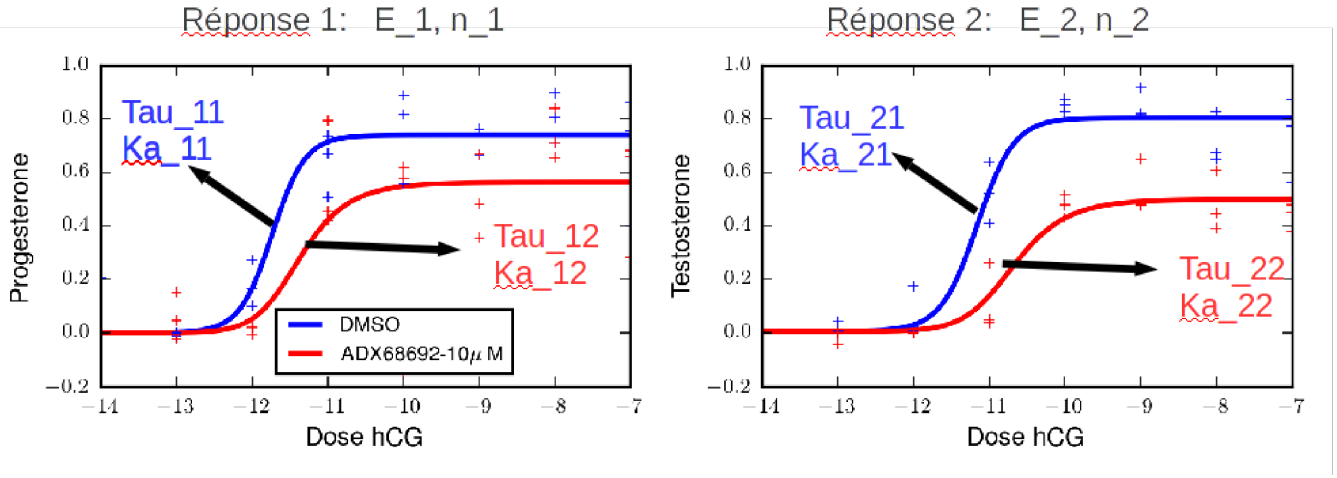


Two ligands ($j = 1, 2$) and **two** measured responses ($i = 1, 2$) :
Each dose-response data is fitted with the operational model :

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

For a given response i, we calculate
$\Delta_i \log(\tau/K_a) = \log(\tau_{i2}/Ka_{i2}) - \log(\tau_{i1}/Ka_{i1})$.

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 :

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

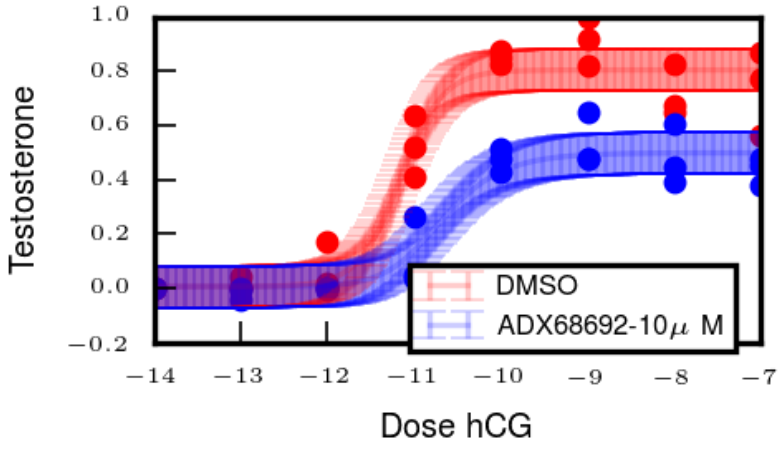
For a given response i, we calculate

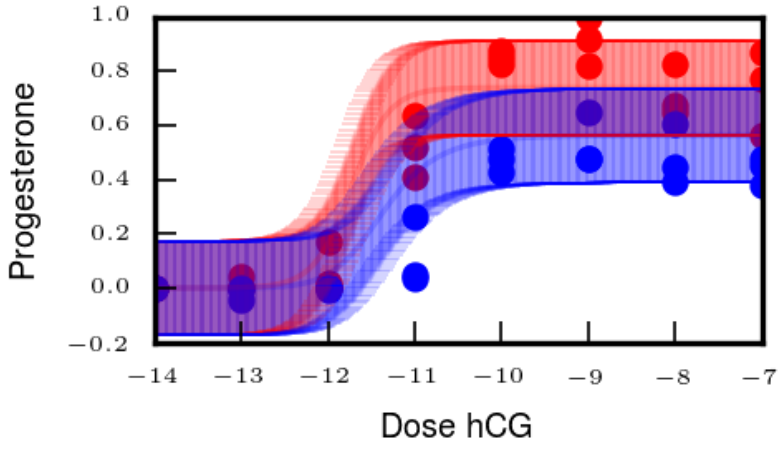
$$\Delta_i \log(\tau/K_a) = \log(\tau_{i2}/Ka_{i2}) - \log(\tau_{i1}/Ka_{i1}).$$

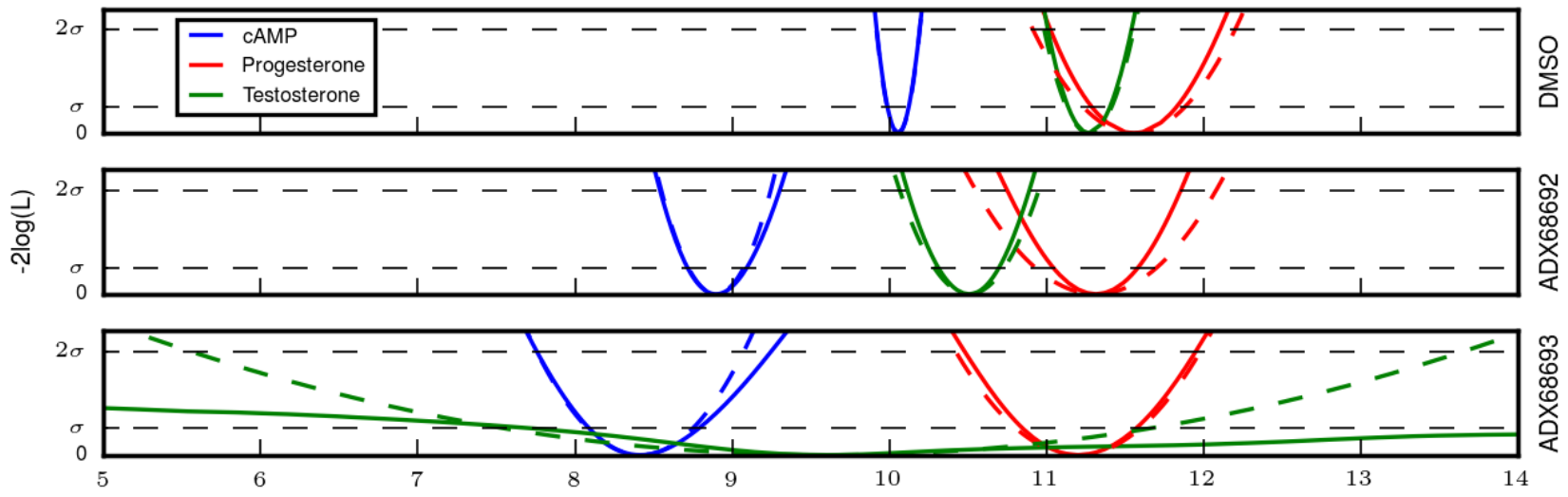
The **Bias** is then defined by

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

Statistical consideration

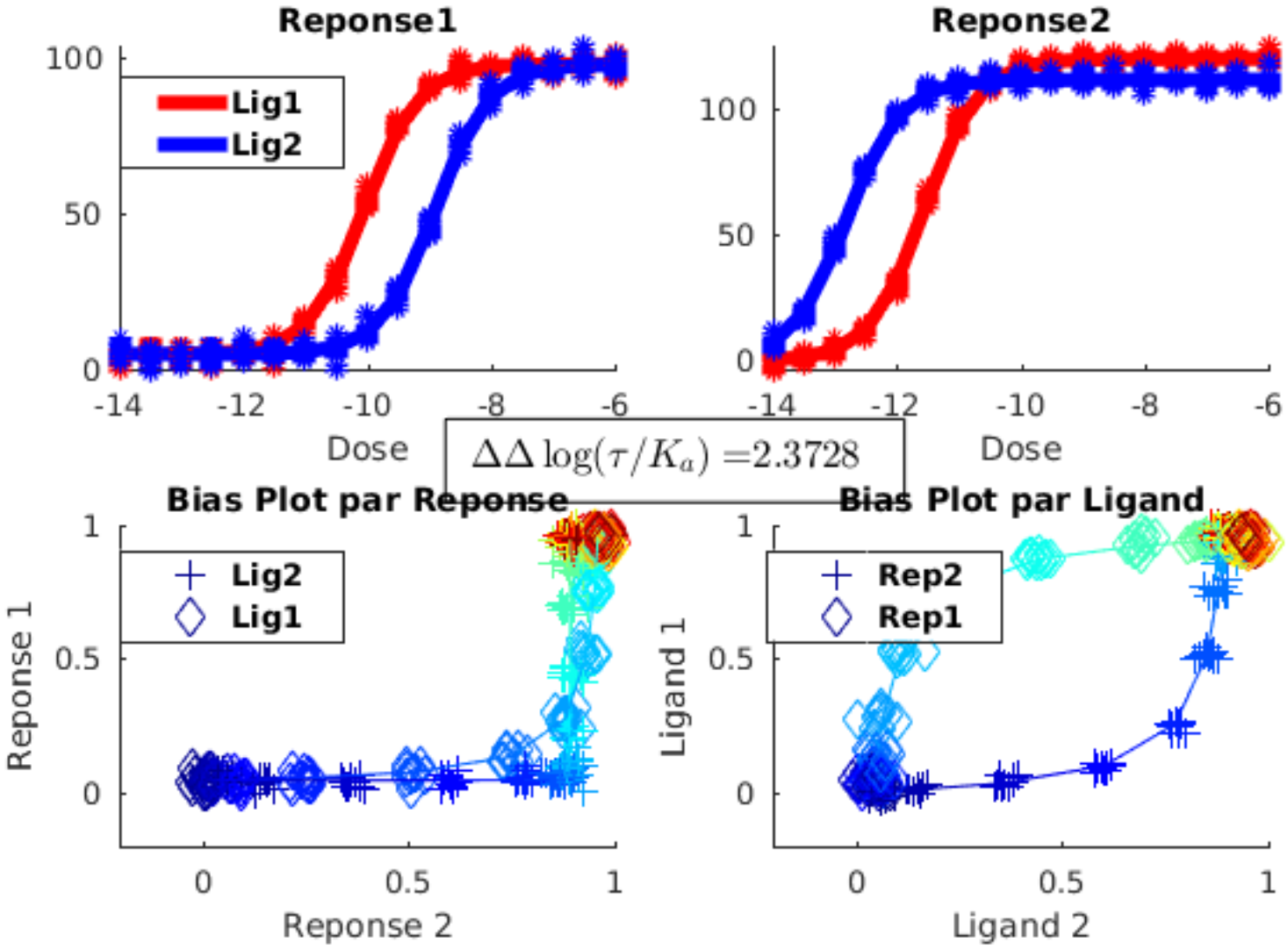






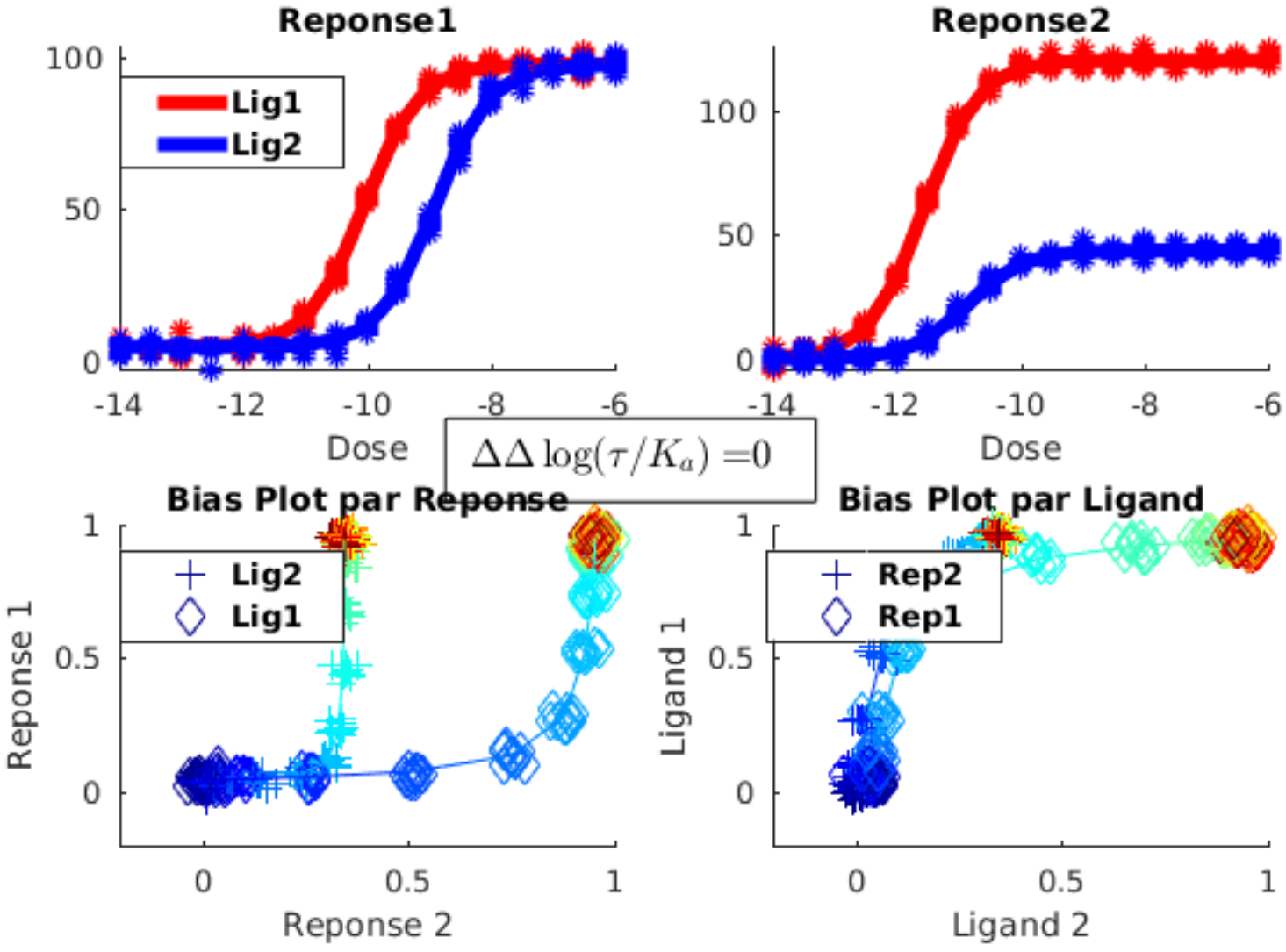
Raue A., et al. *Bioinformatics* (2015)

Is bias calculation intuitive ? (simulated data)



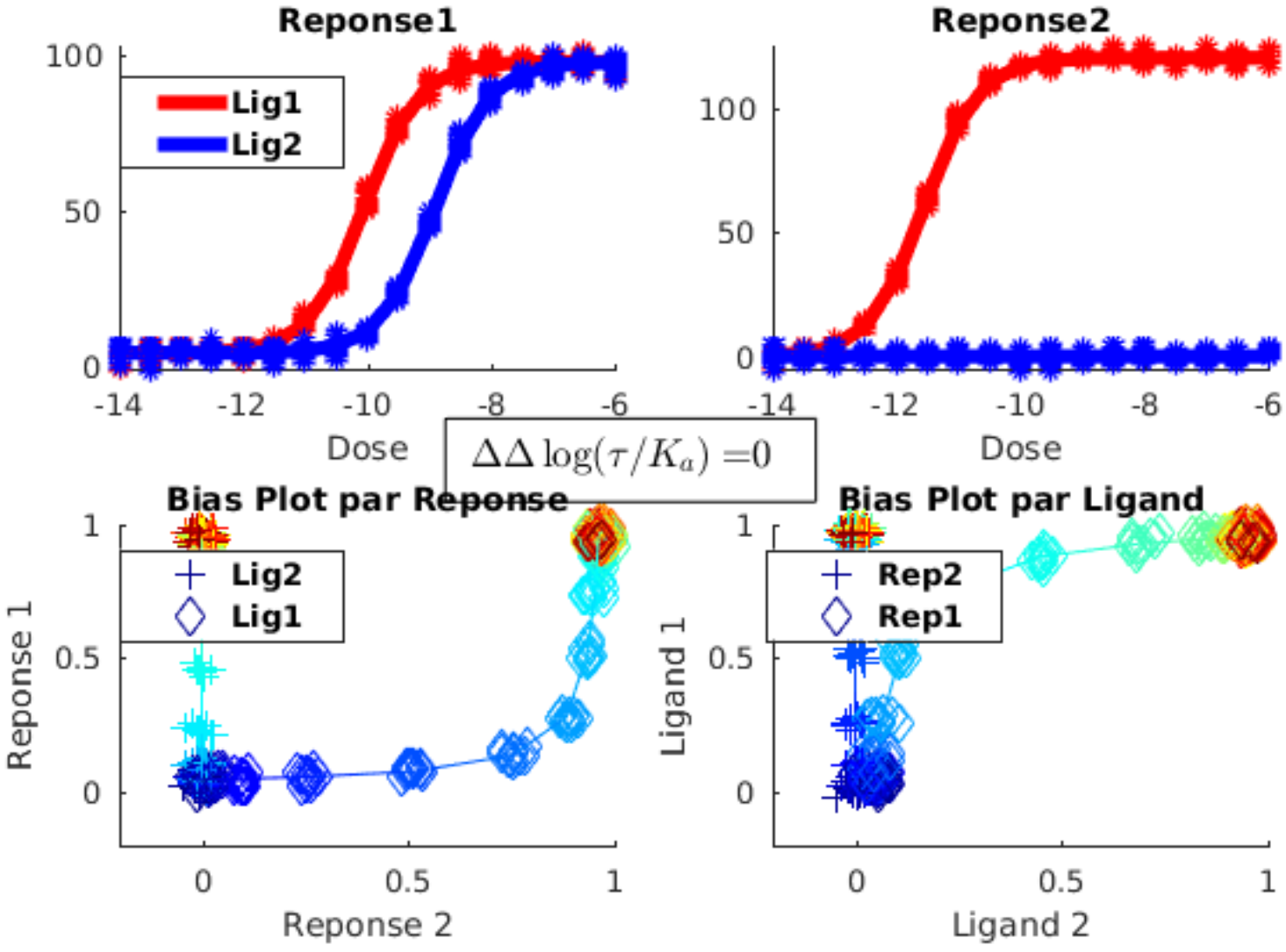
- A strong bias is usually 'apparent' on dose-response curves or bias plot

Is bias calculation intuitive ? (simulated data)



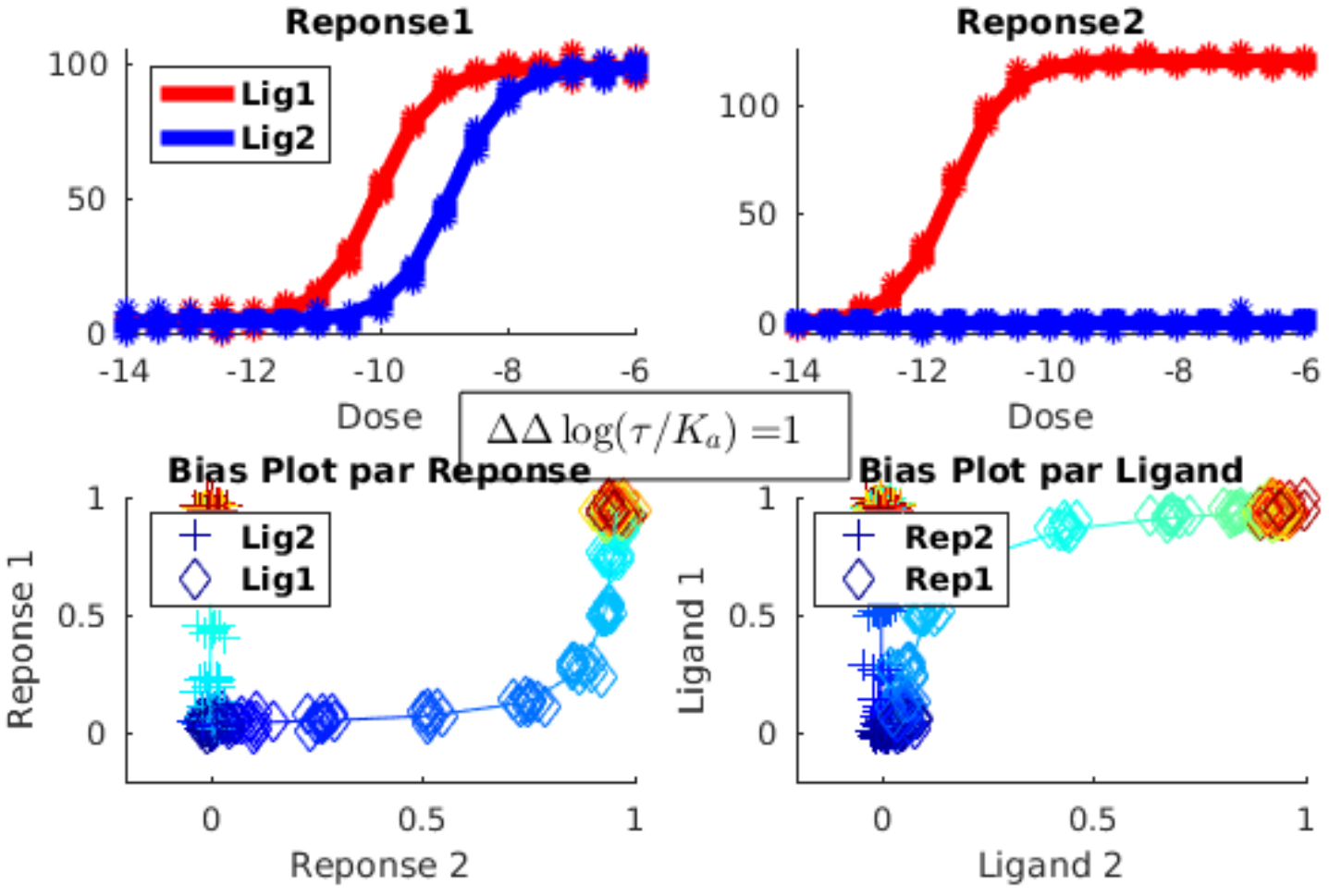
- But there may be counter-intuitive situation...

Is bias calculation intuitive ? (simulated data)



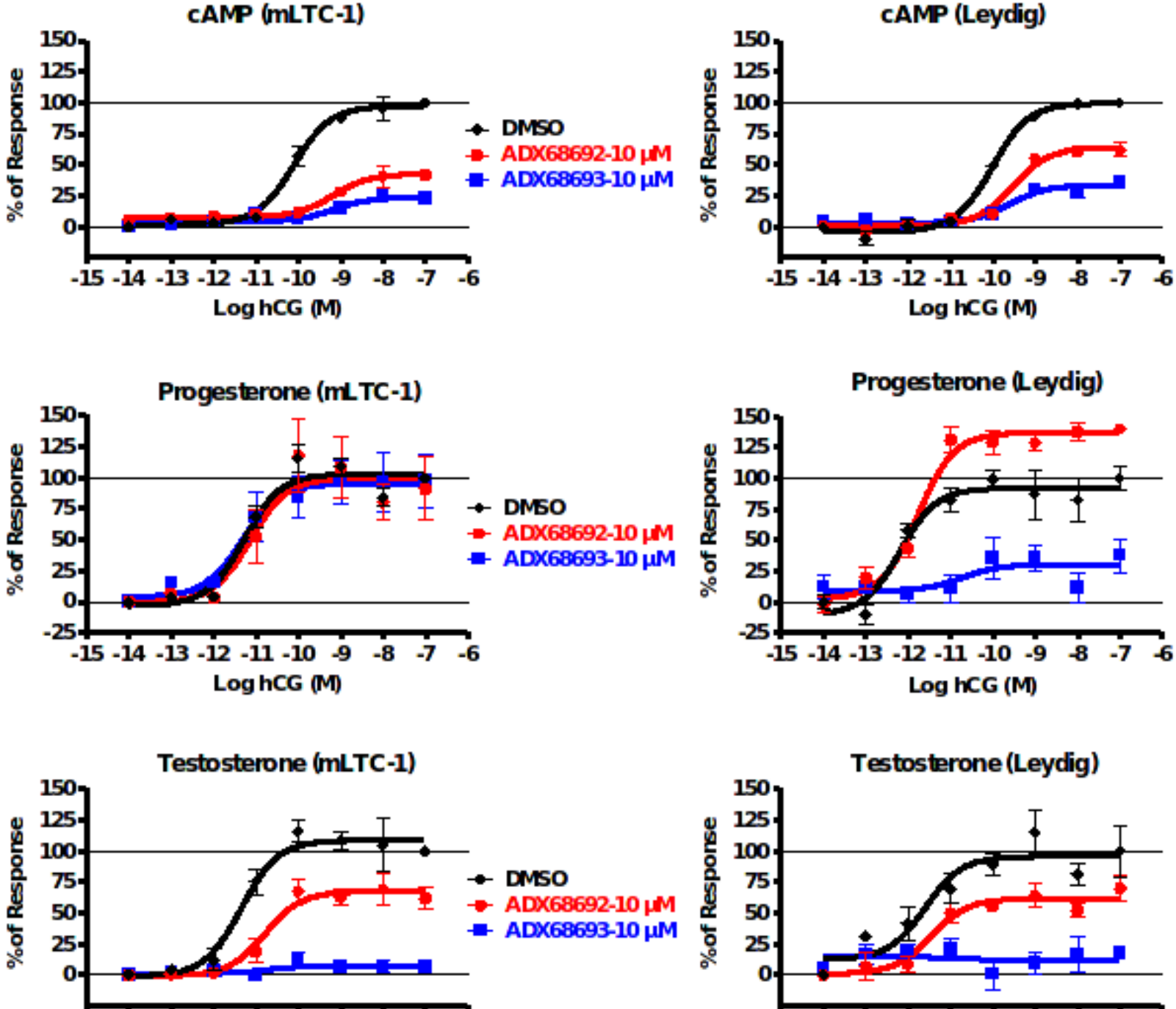
- But there may be counter-intuitive situation...

Is bias calculation intuitive ? (simulated data)



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

- … and those situations occur in real life !



Outline

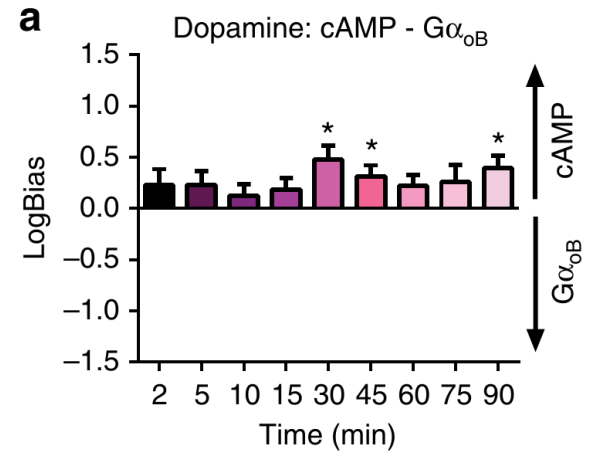
Time-dependent bias

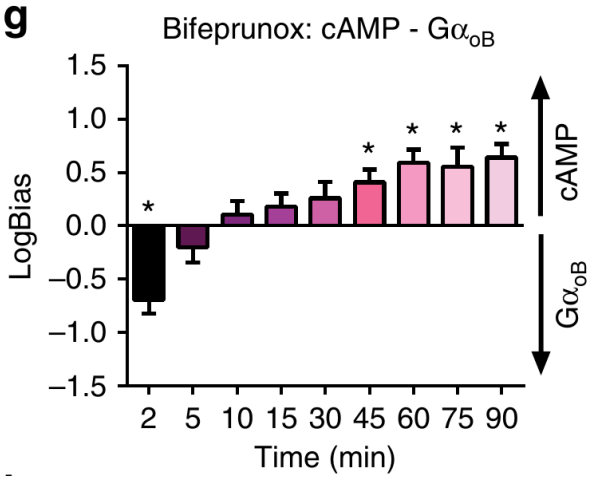
The role of kinetic context in apparent biased agonism at GPCRs

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)





Other extensions

- Dose-dependent bias

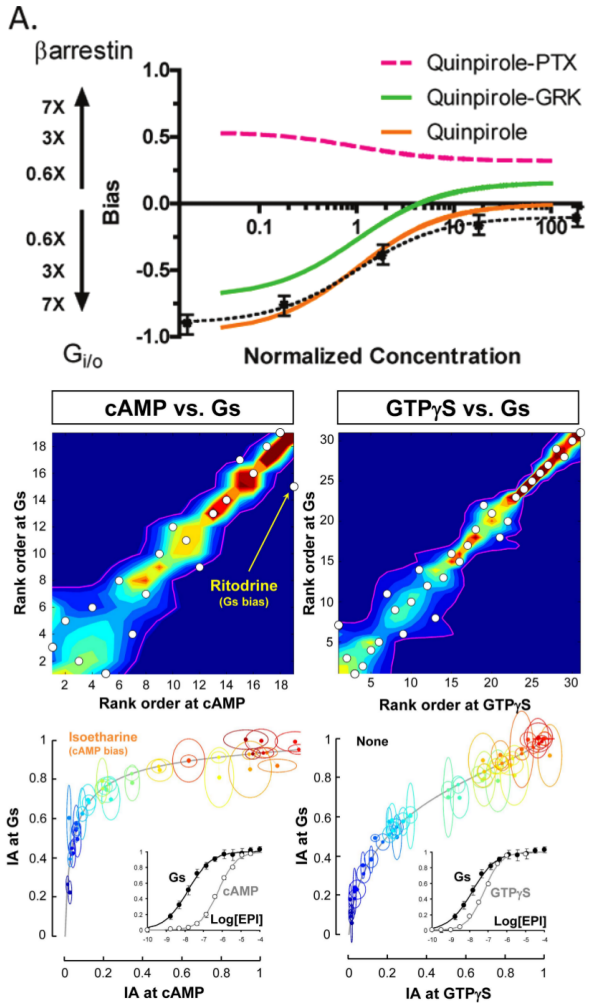
Barak and Peterson et al., *Biochem.* (2012)

- Extension of the operational model

Kenakin, *Chem. Rev.* (2017)

- Method based on Intrinsic activities and rank ordering

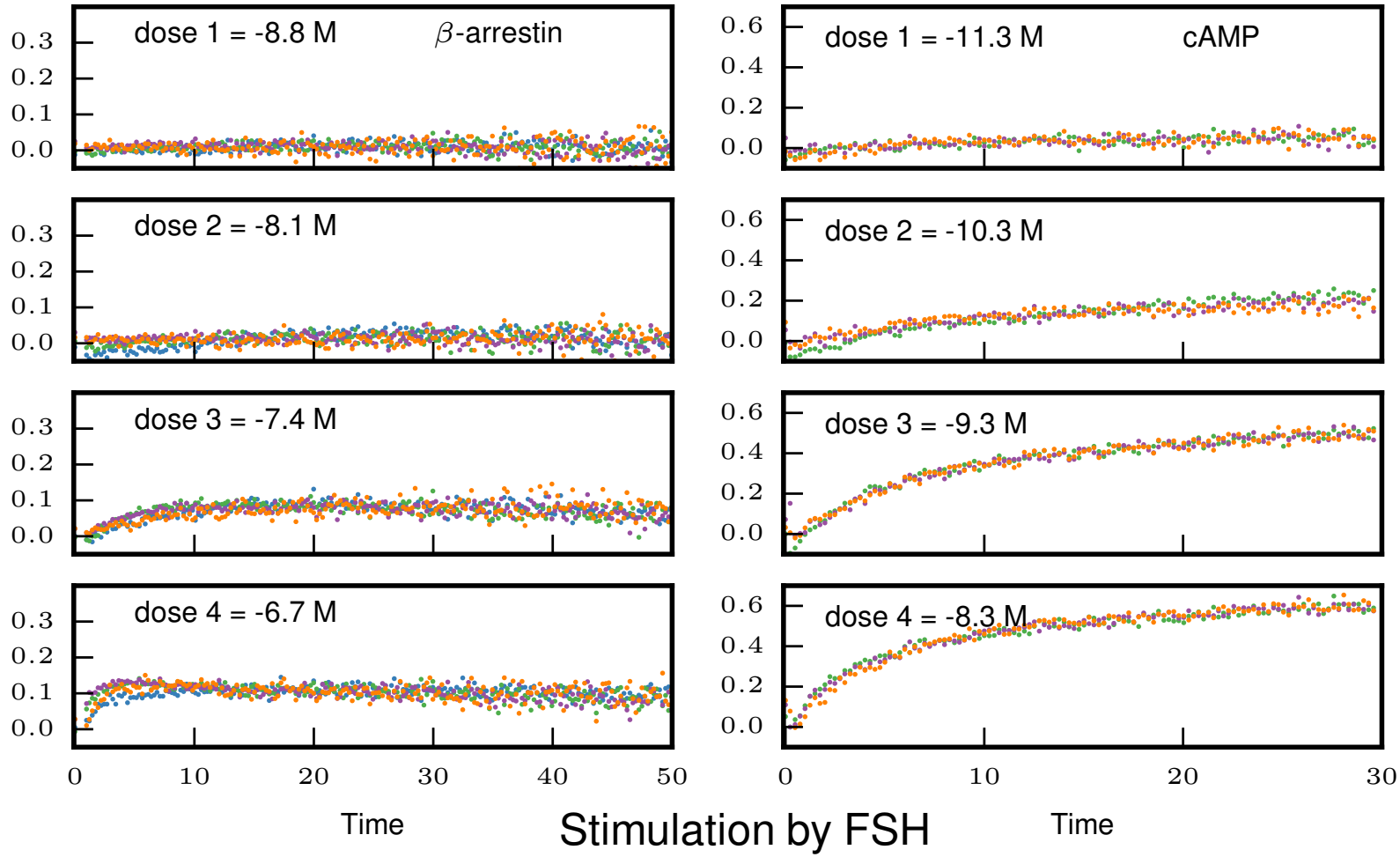
Onaran et al., *Sci. Rep.* (2017)



Outline

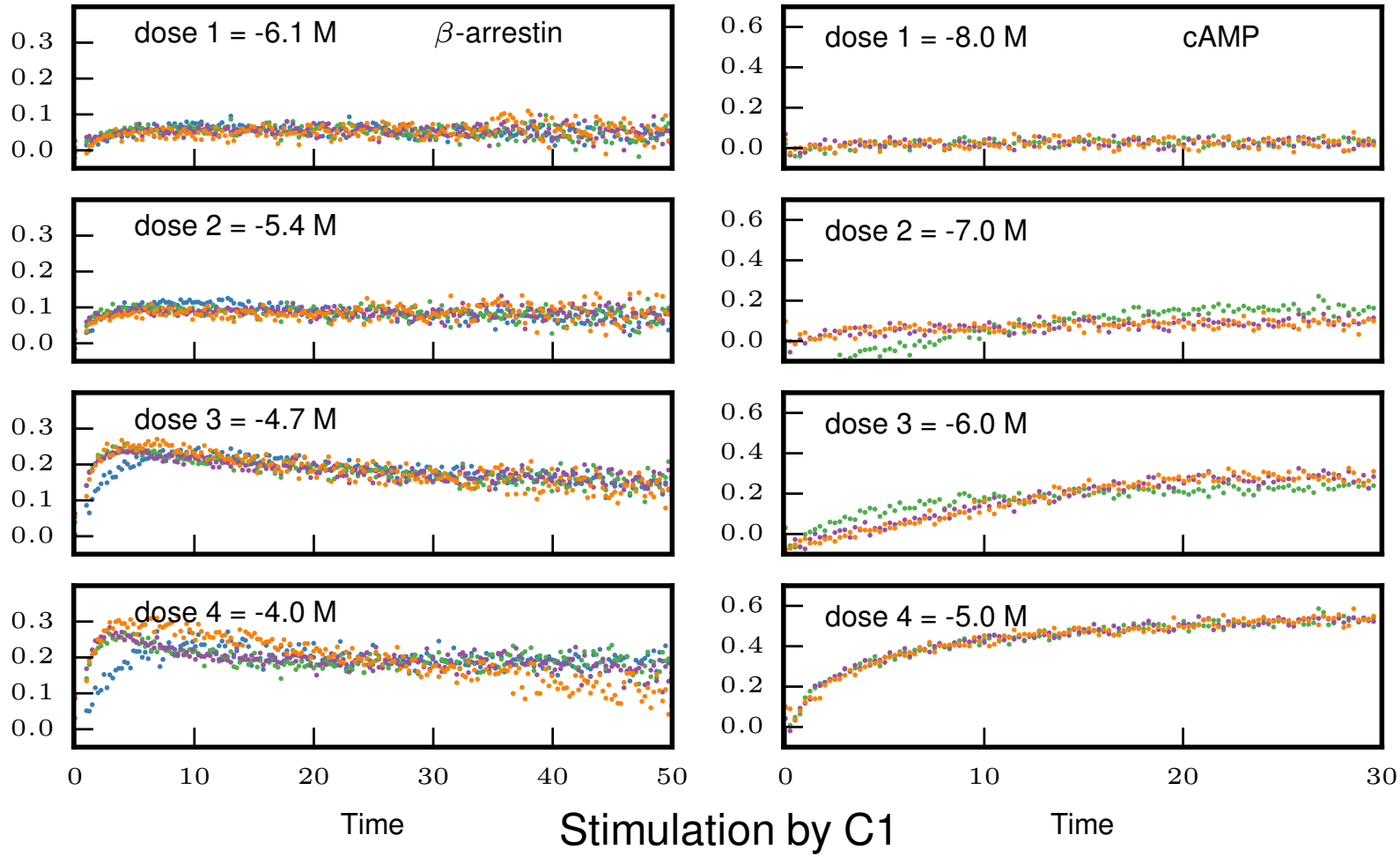
Dynamic data (on FHSR in HEK cells)

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



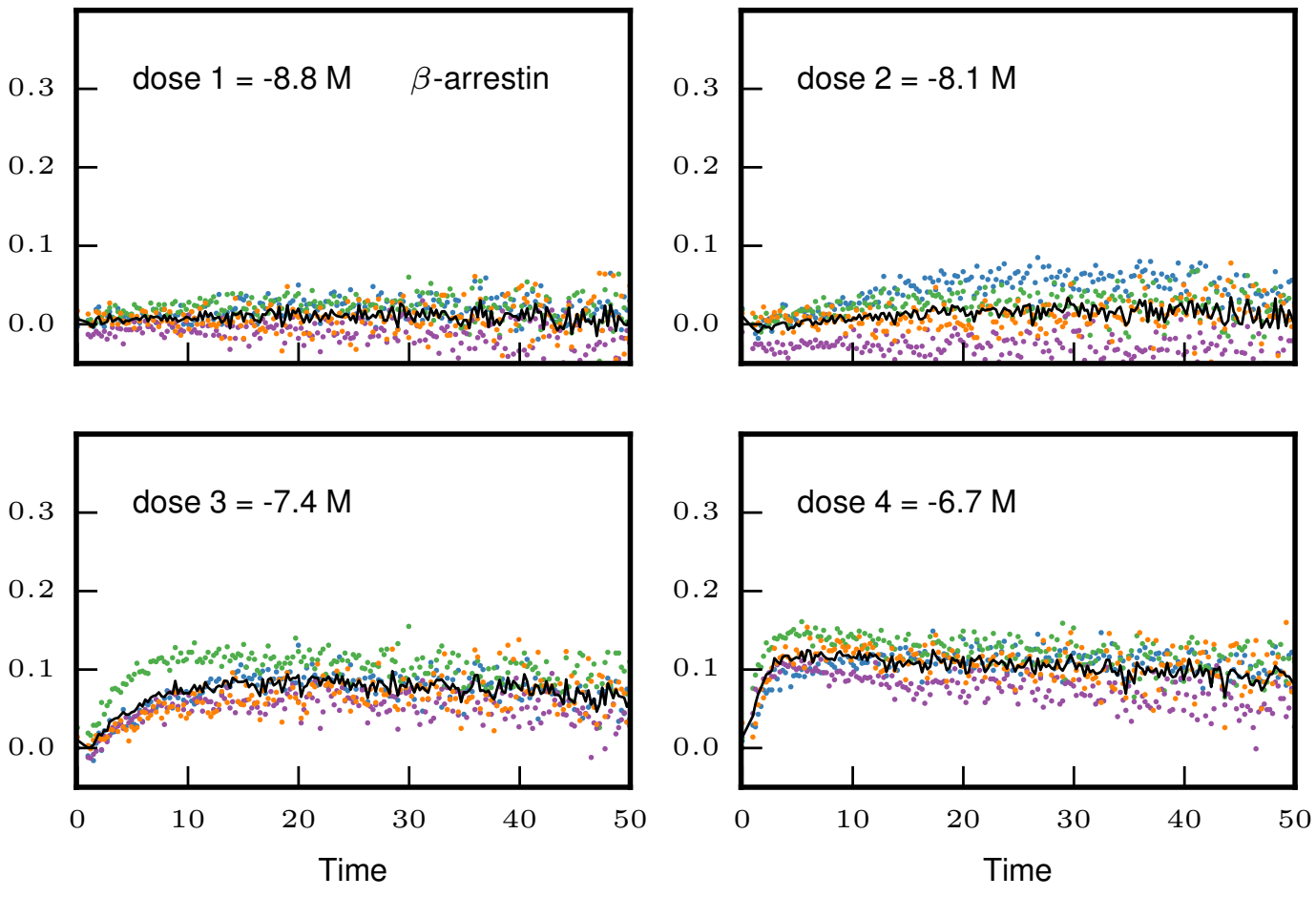
Dynamic data (on FHSR in HEK cells)

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



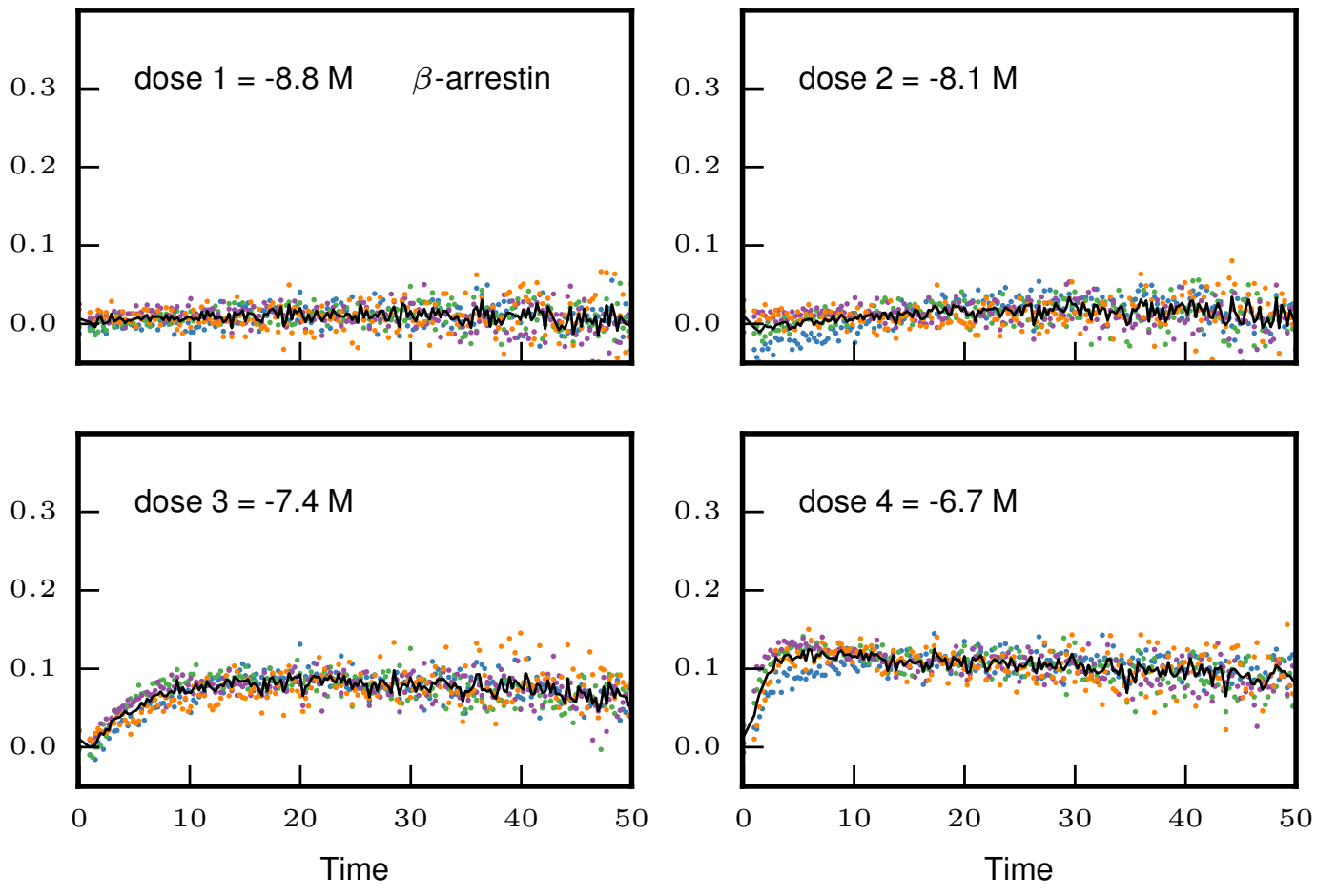
("trick" to minimize variance...)

Original "raw" data



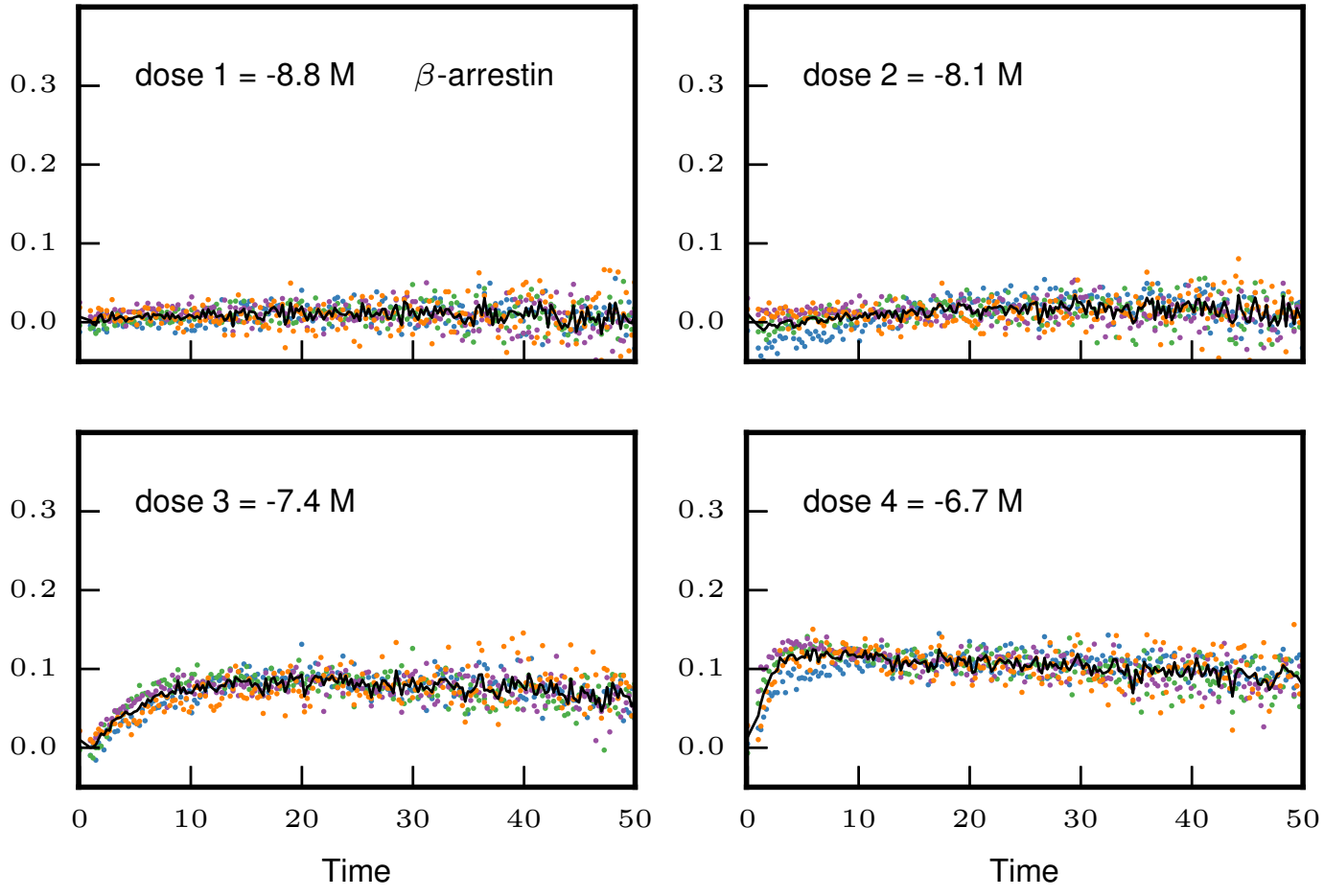
("trick" to minimize variance...)

"Adjusted" data



("trick" to minimize variance...)

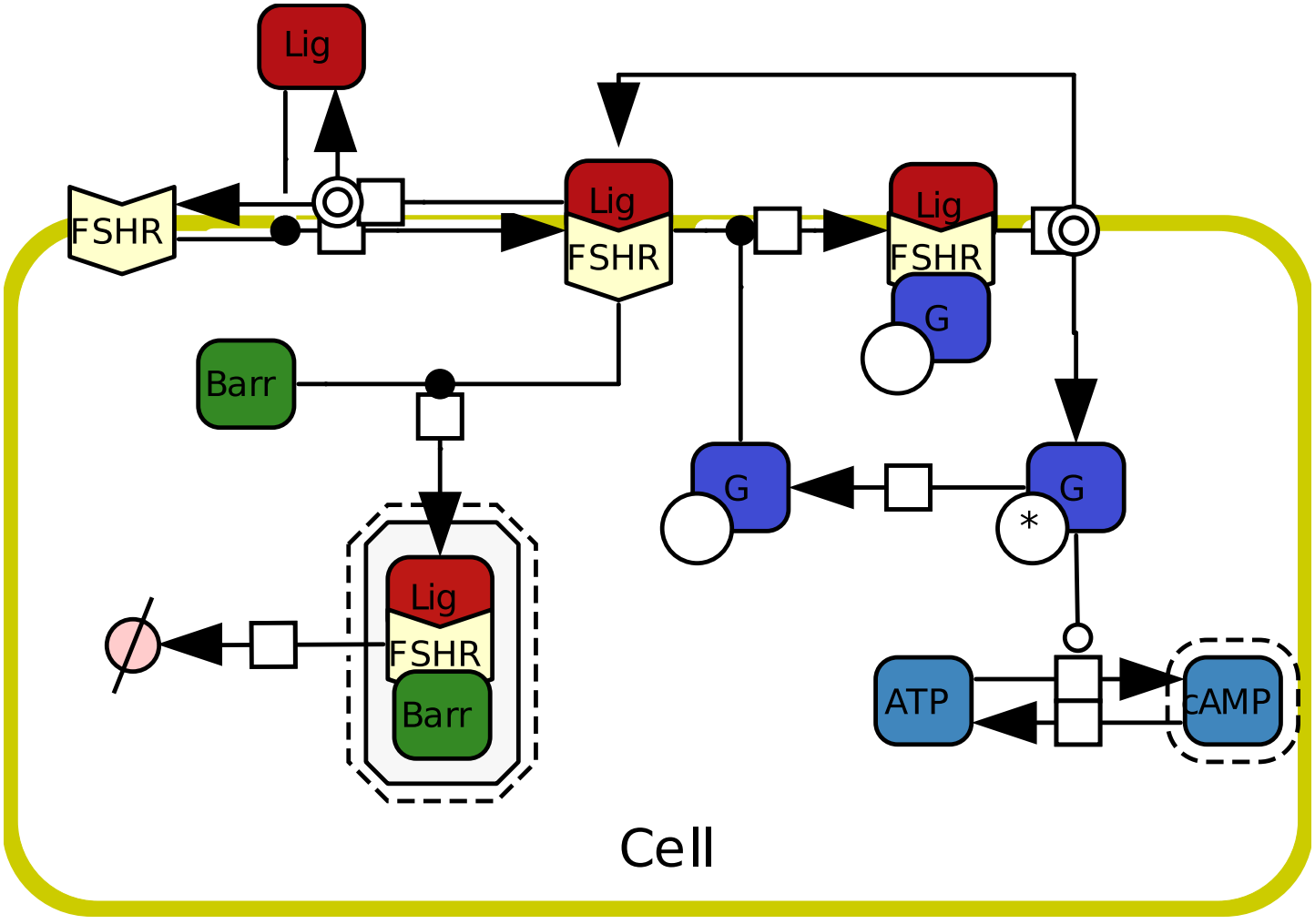
"Adjusted" data



+ adjusting the number of data points ...

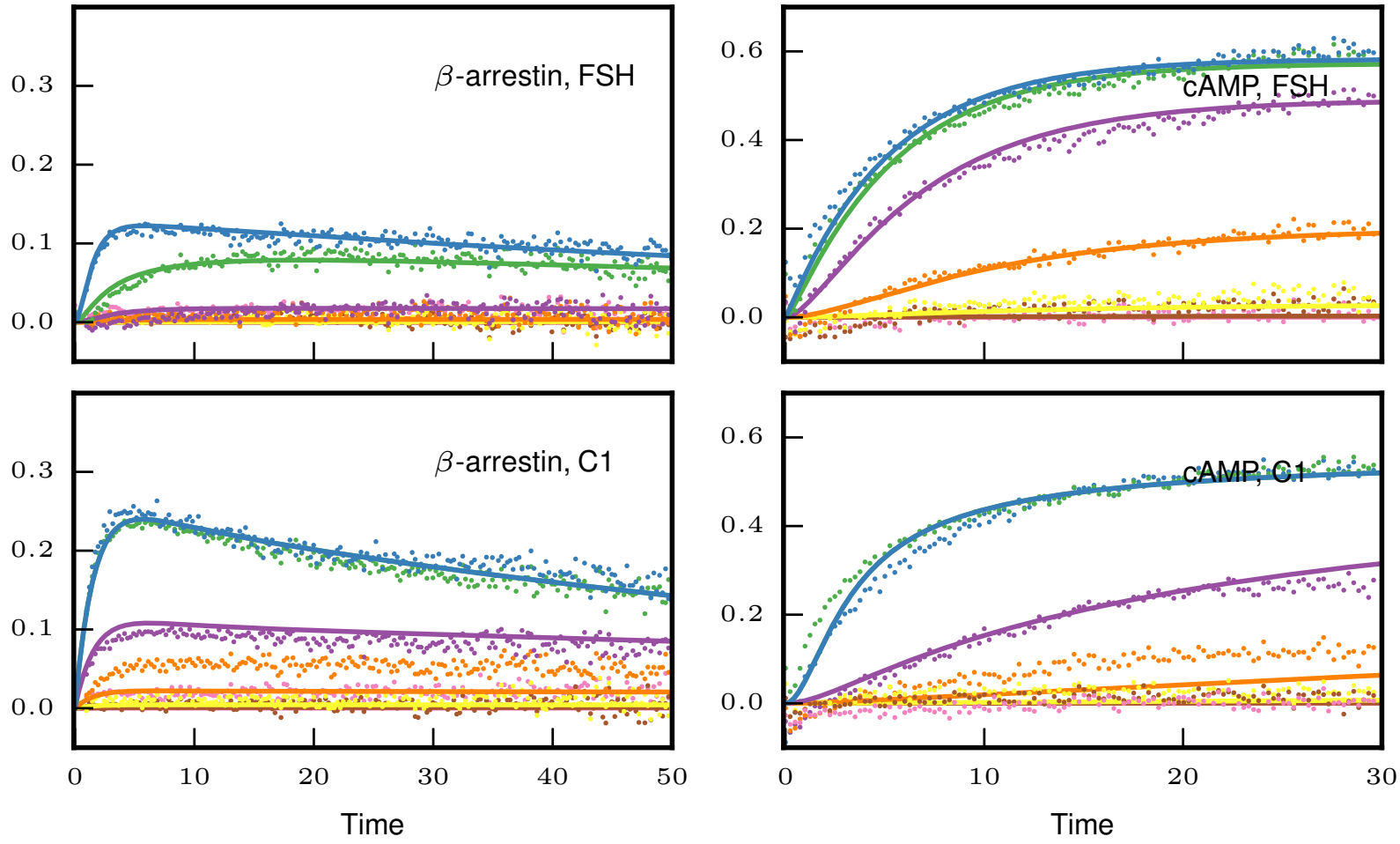
Principle

I)We start with a sufficiently detailed chemical reaction network



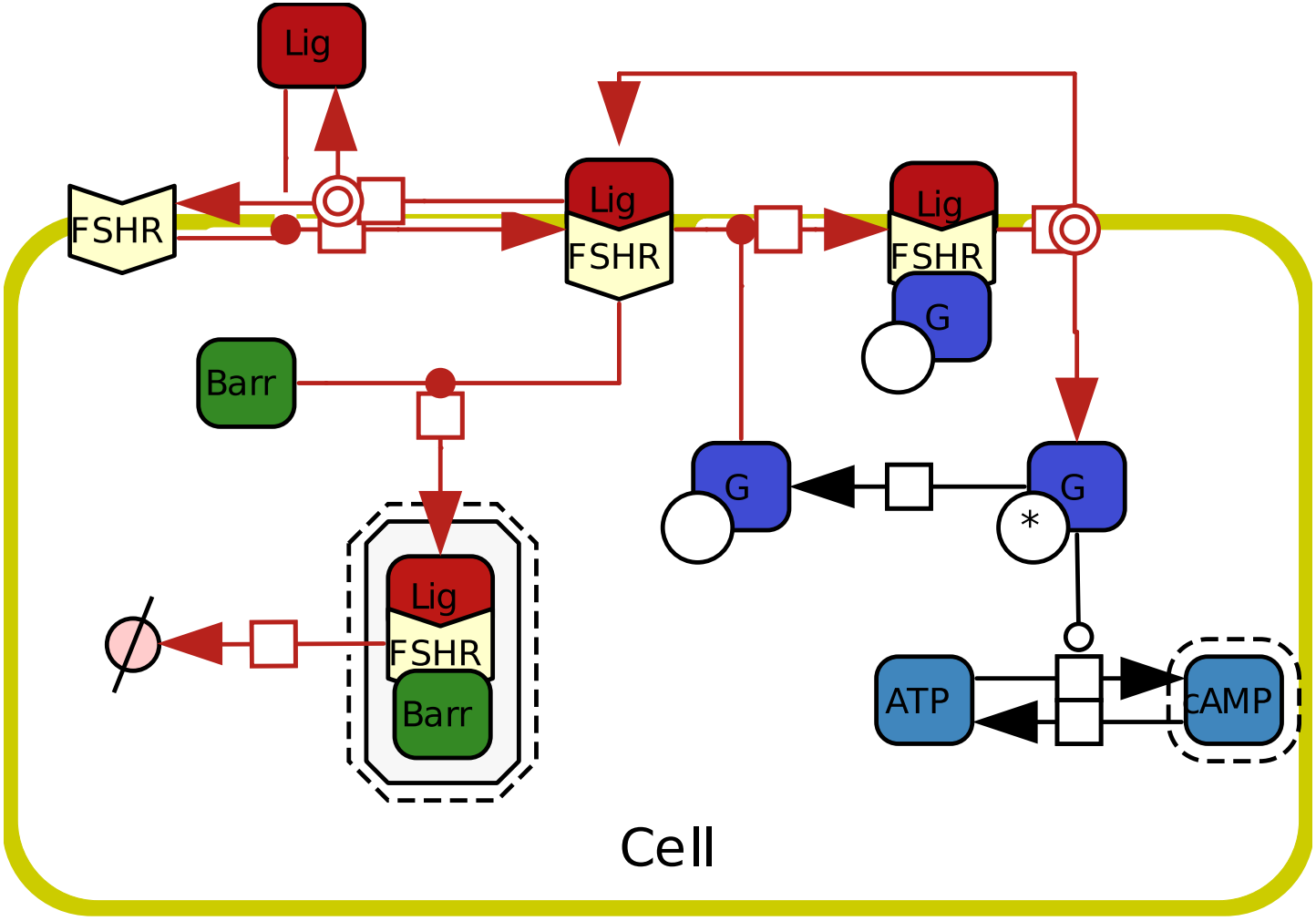
Principle

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



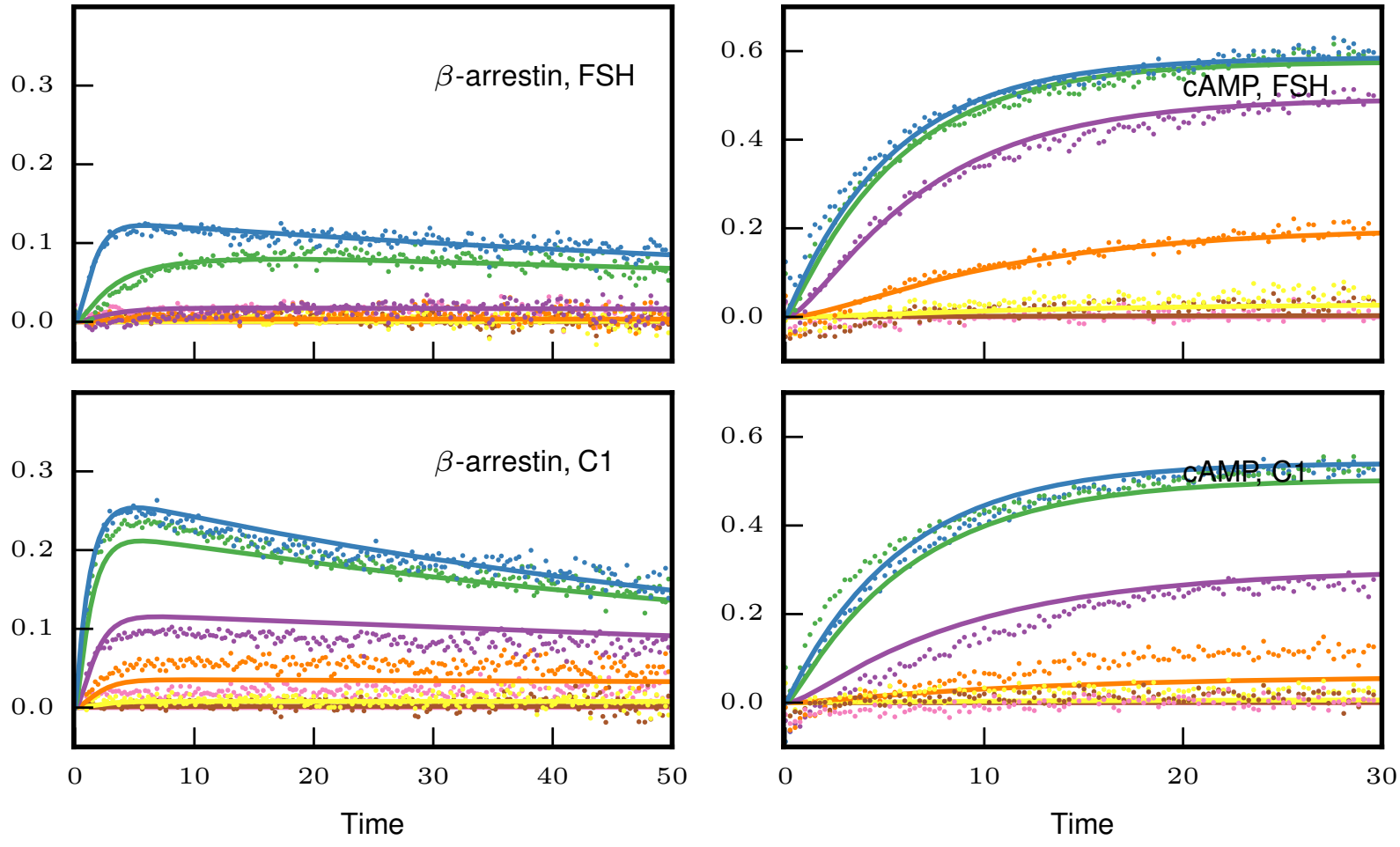
Principle

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



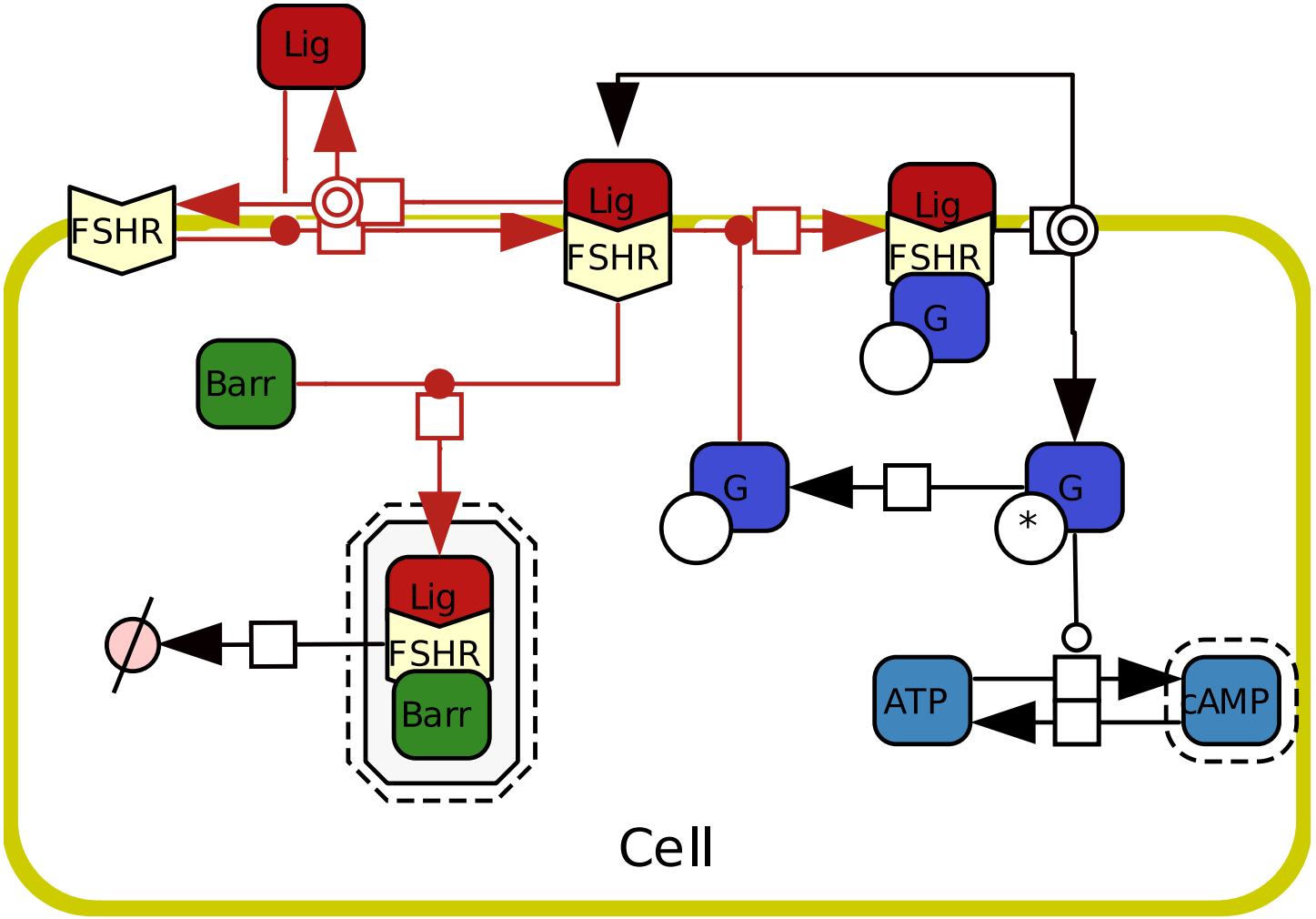
Principle

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



Principle

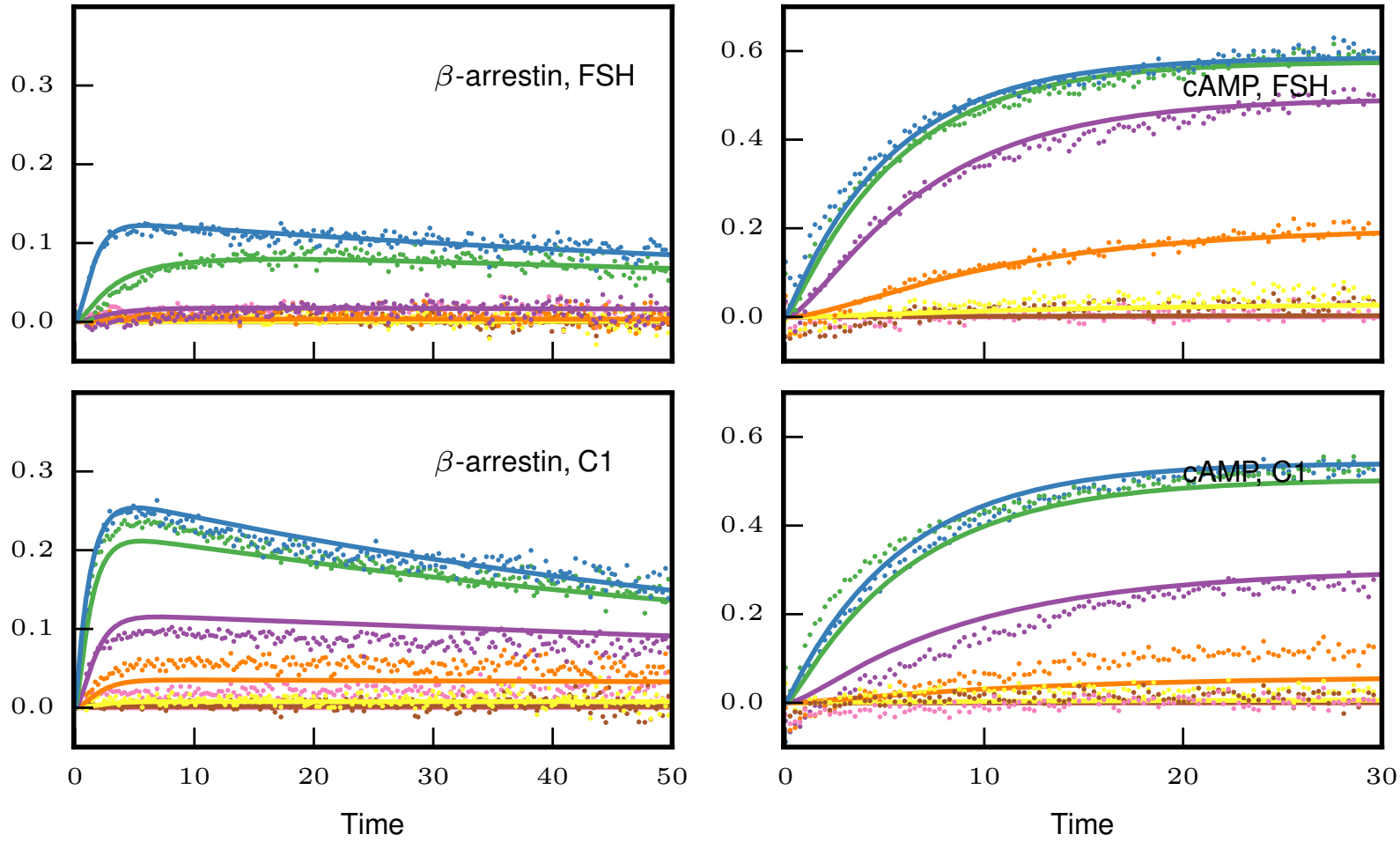
III) We use L^1-penalization to find the needed ligand specific parameters

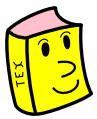


Steiert, Timmer and Kreutz, *Bioinformatics* (2016)

Principle

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

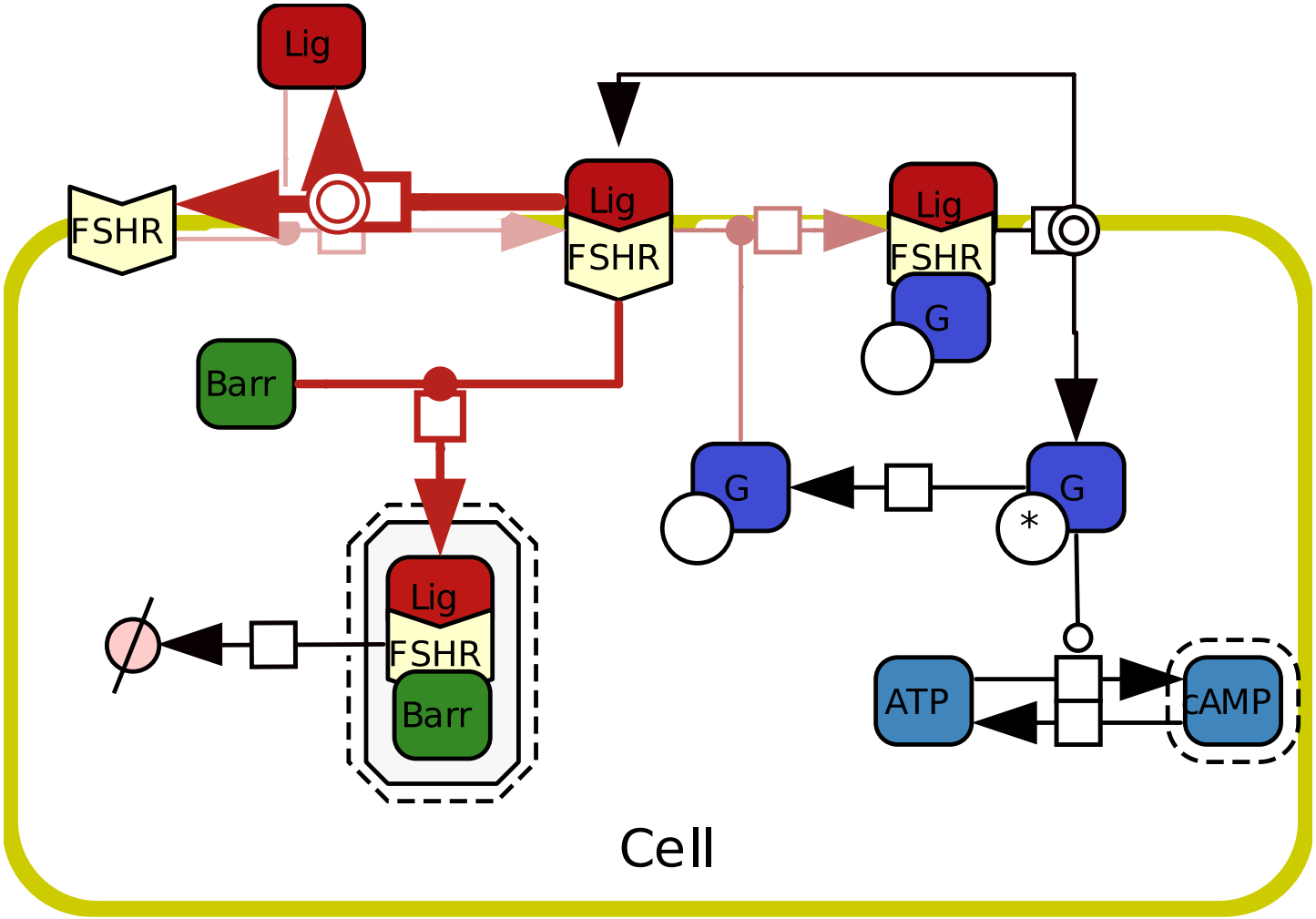




Steiert, Timmer and Kreutz, *Bioinformatics* (2016)

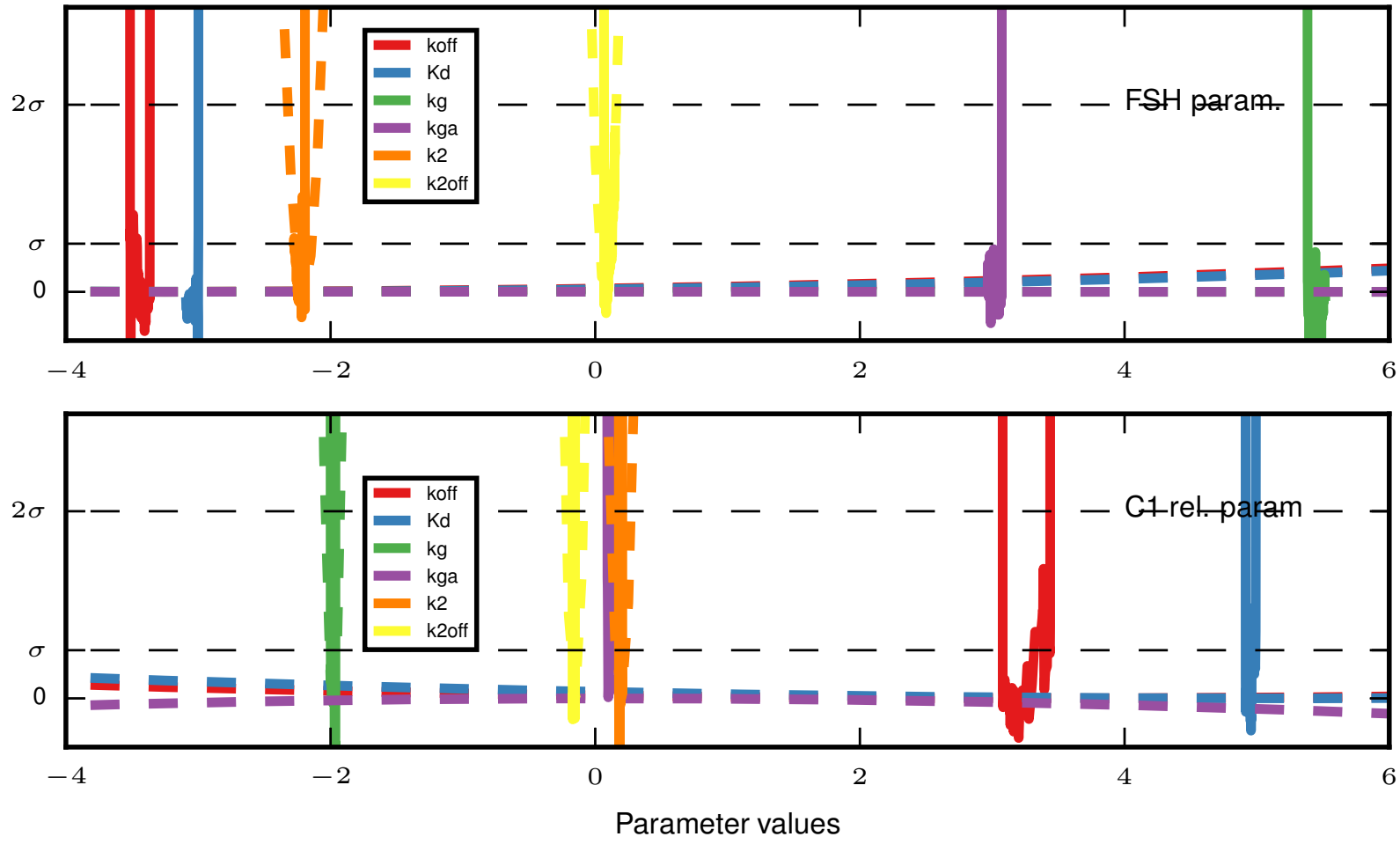
Principle

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



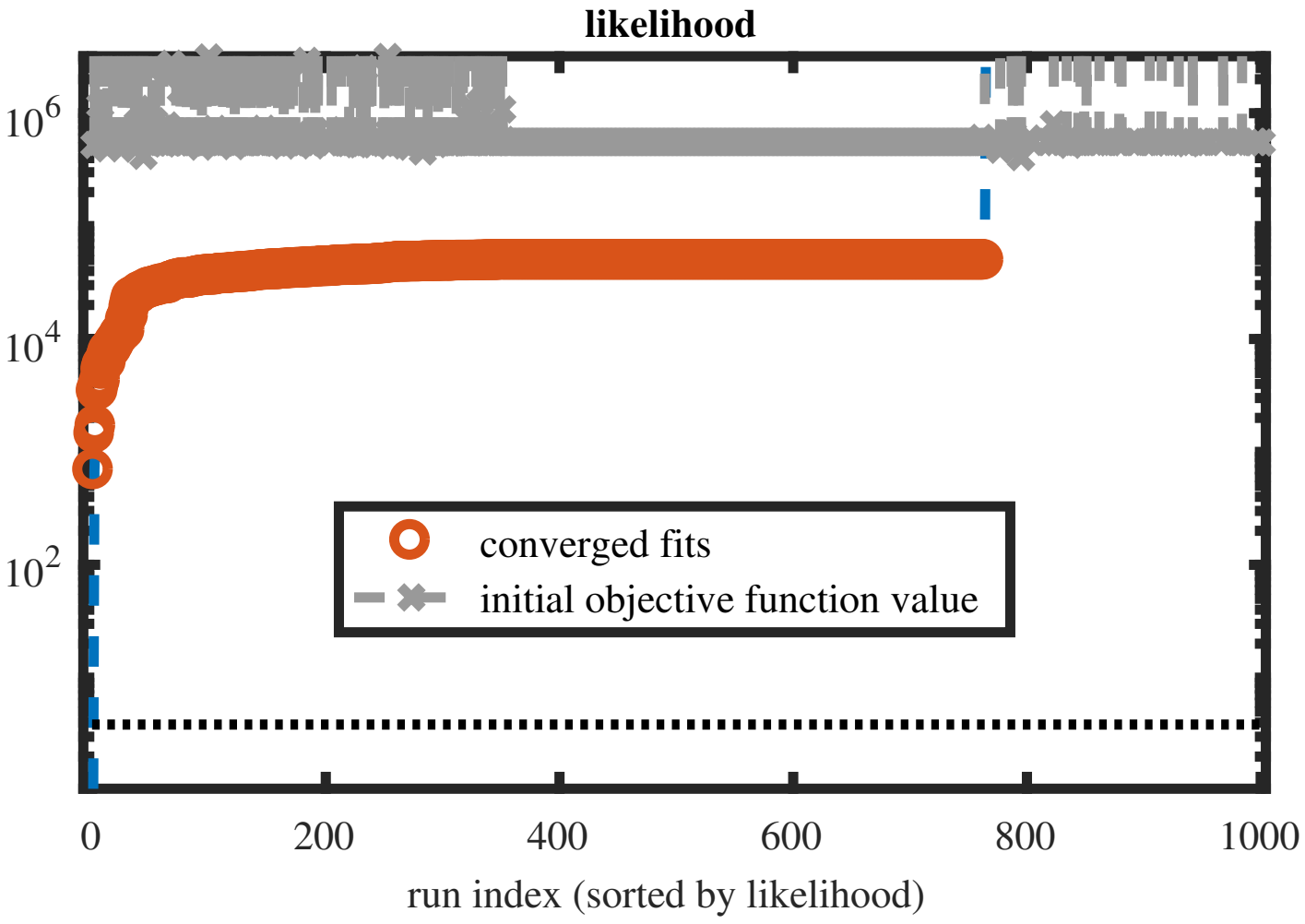
Principle

V) Significant differences between parameters is assessed by PLE

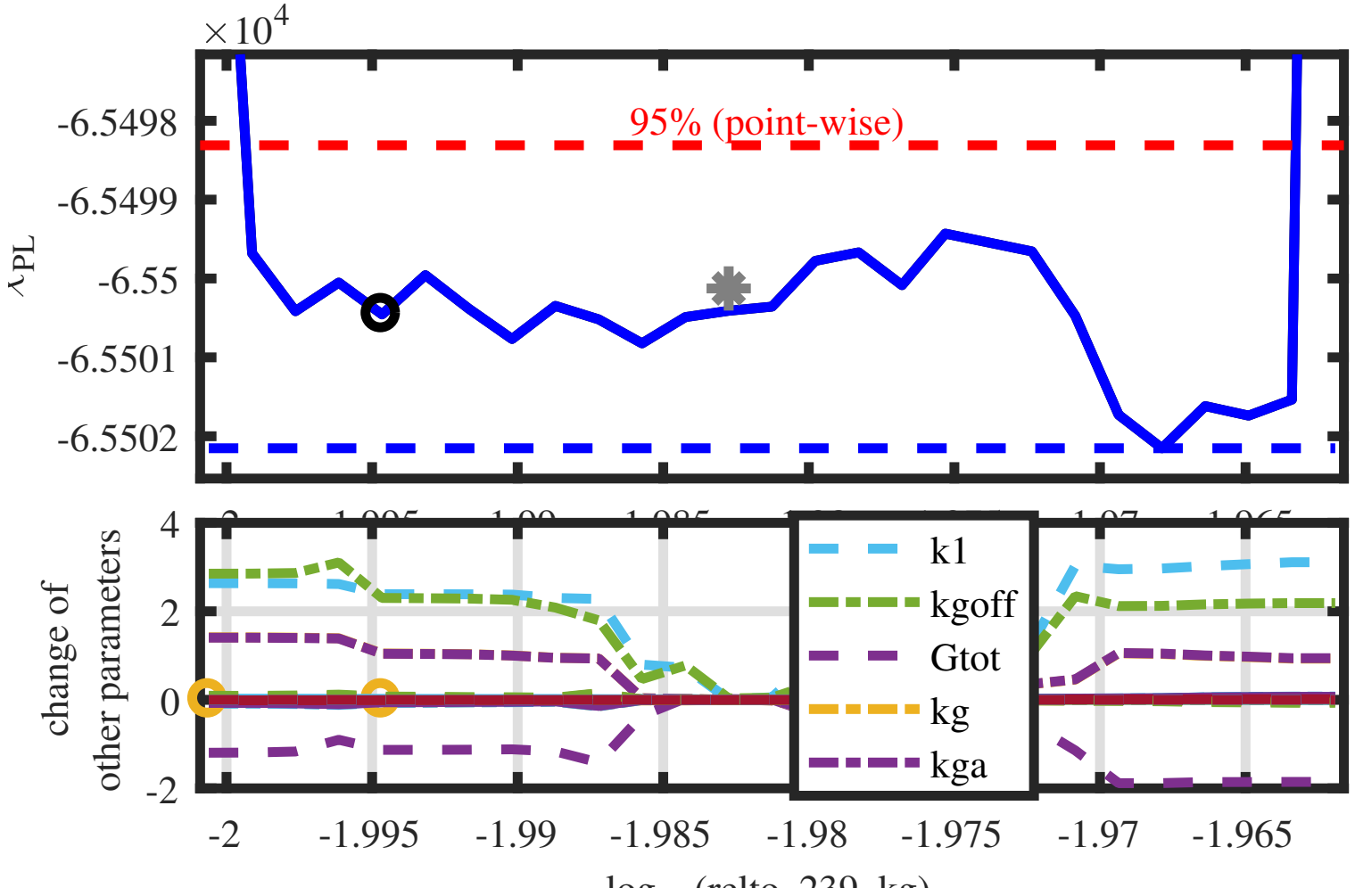


C1 is biased towards β-arr, compared to cAMP, in comparison to FSH.

Practical problems...

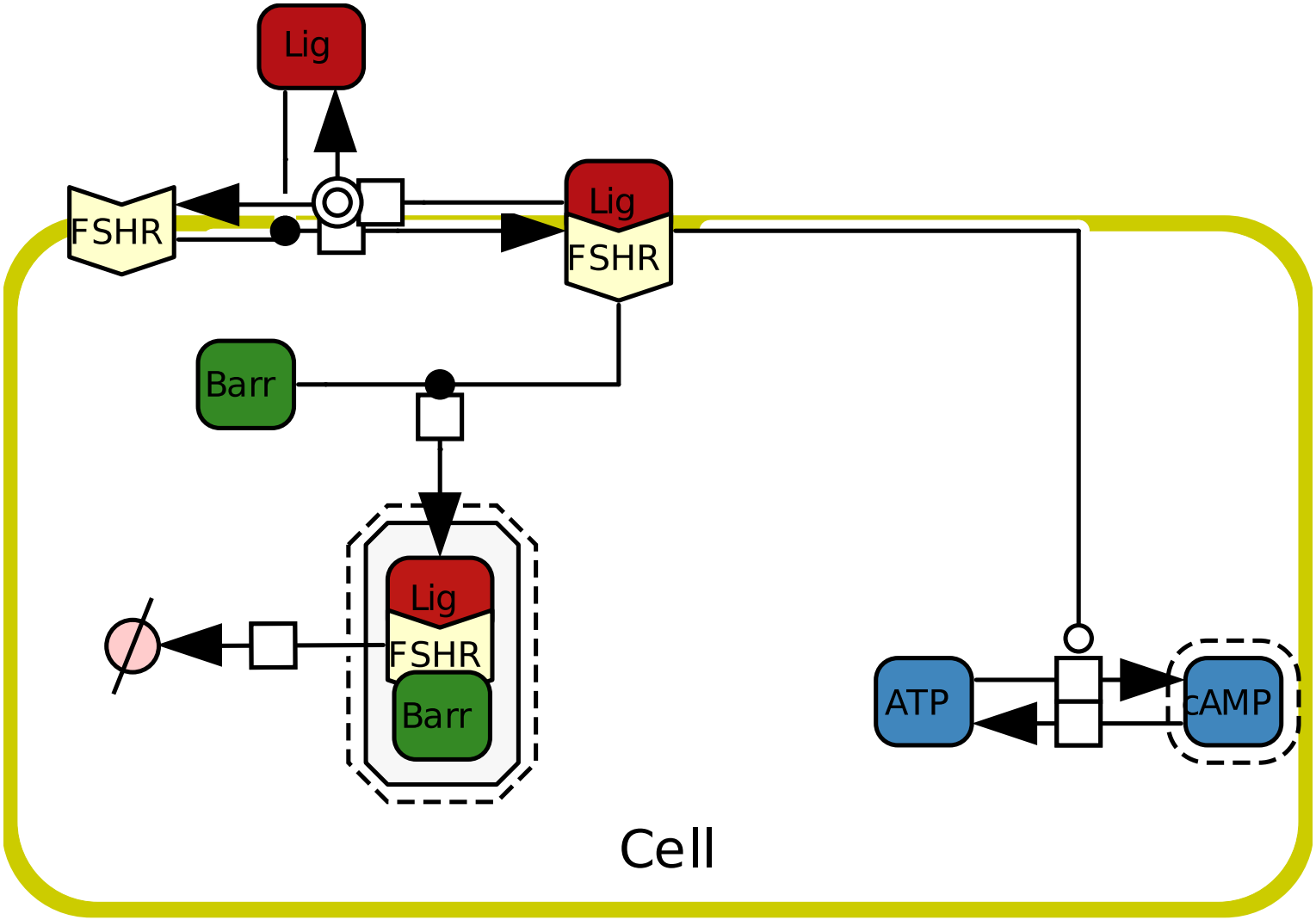


Practical problems...



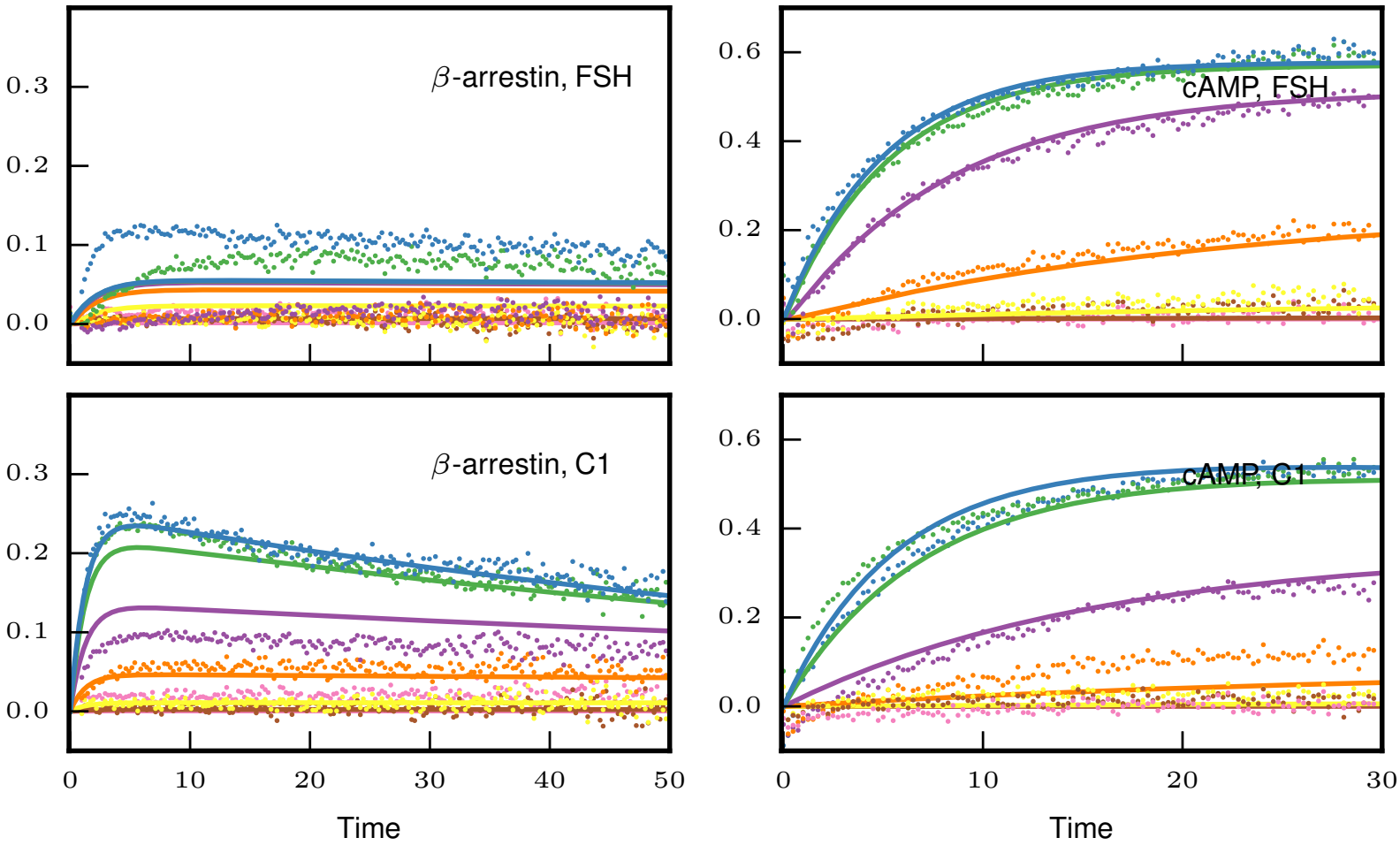
With a "simpler" model

Kinetic model without G-protein



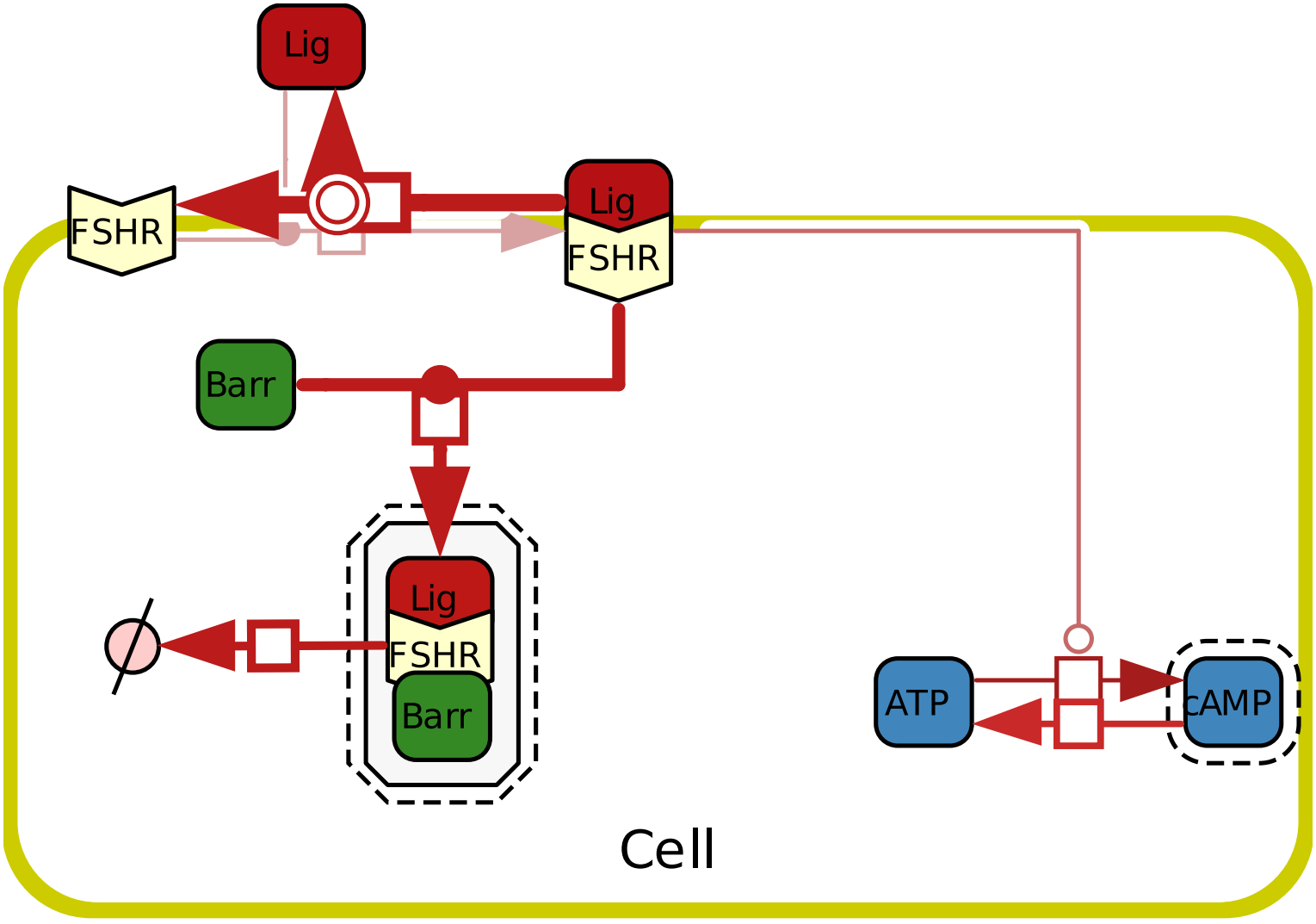
With a "simpler" model

We obtain a slightly worse fit



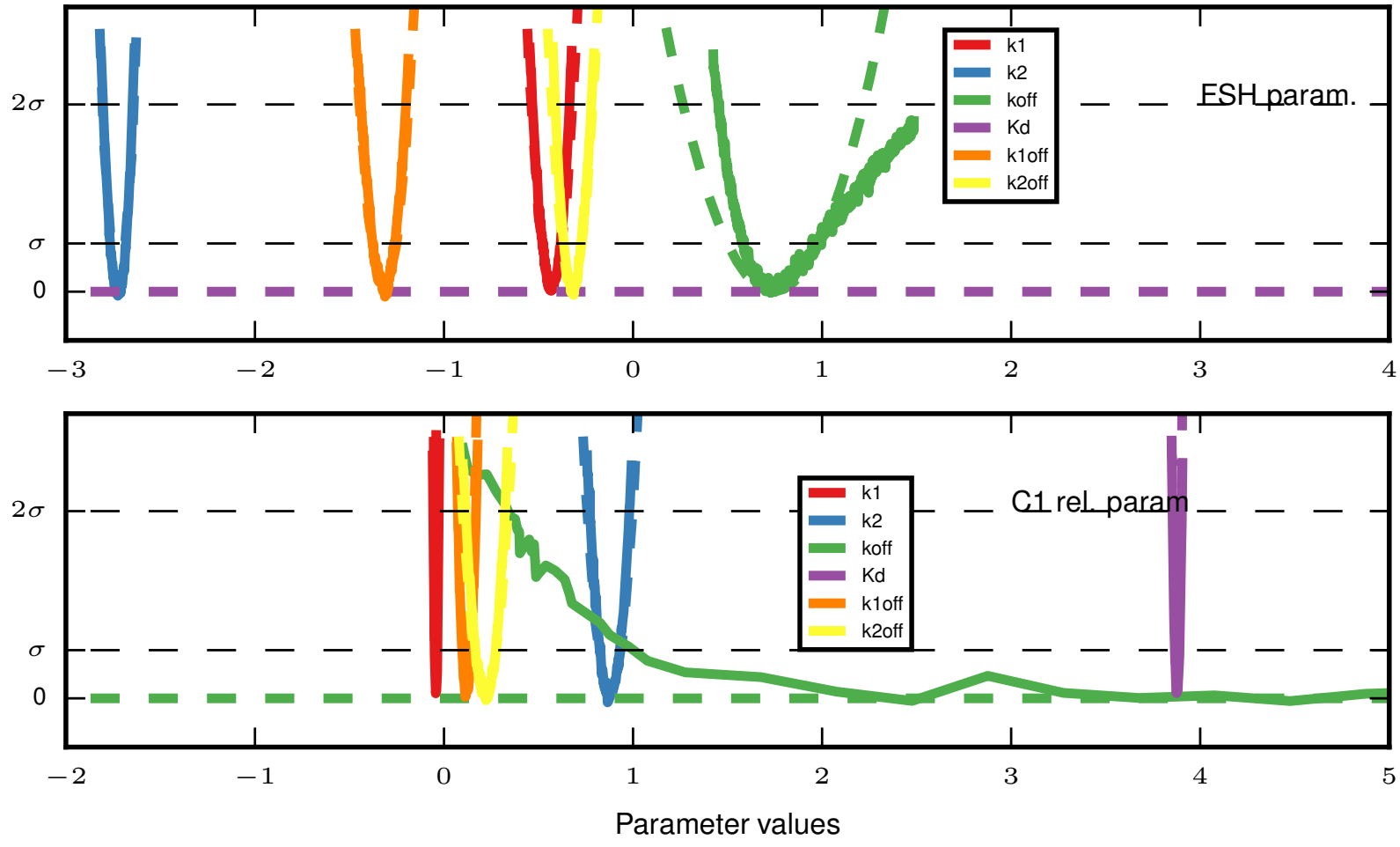
With a "simpler" model

But consistent results



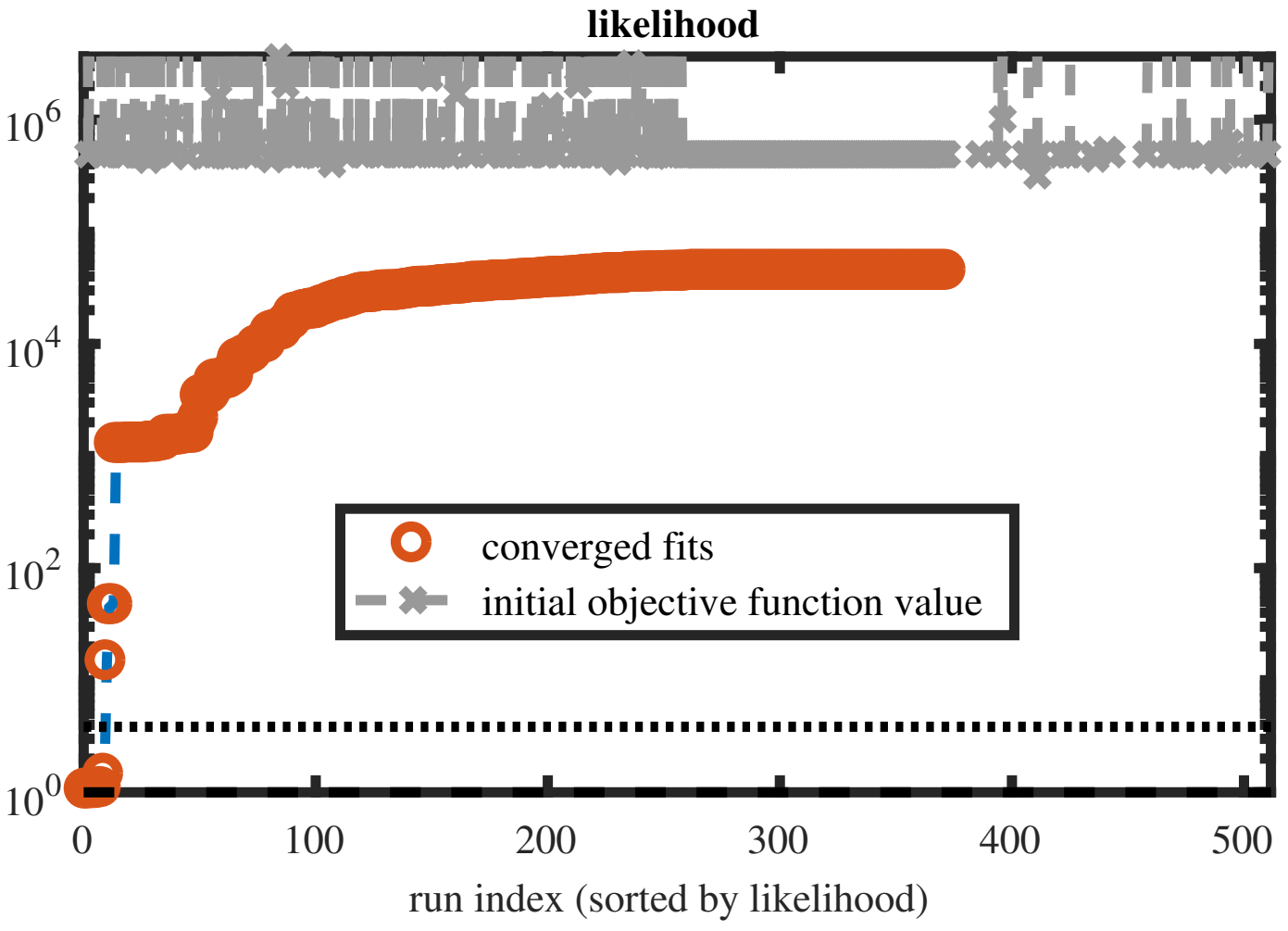
With a "simpler" model

And "better" parameter identifiability



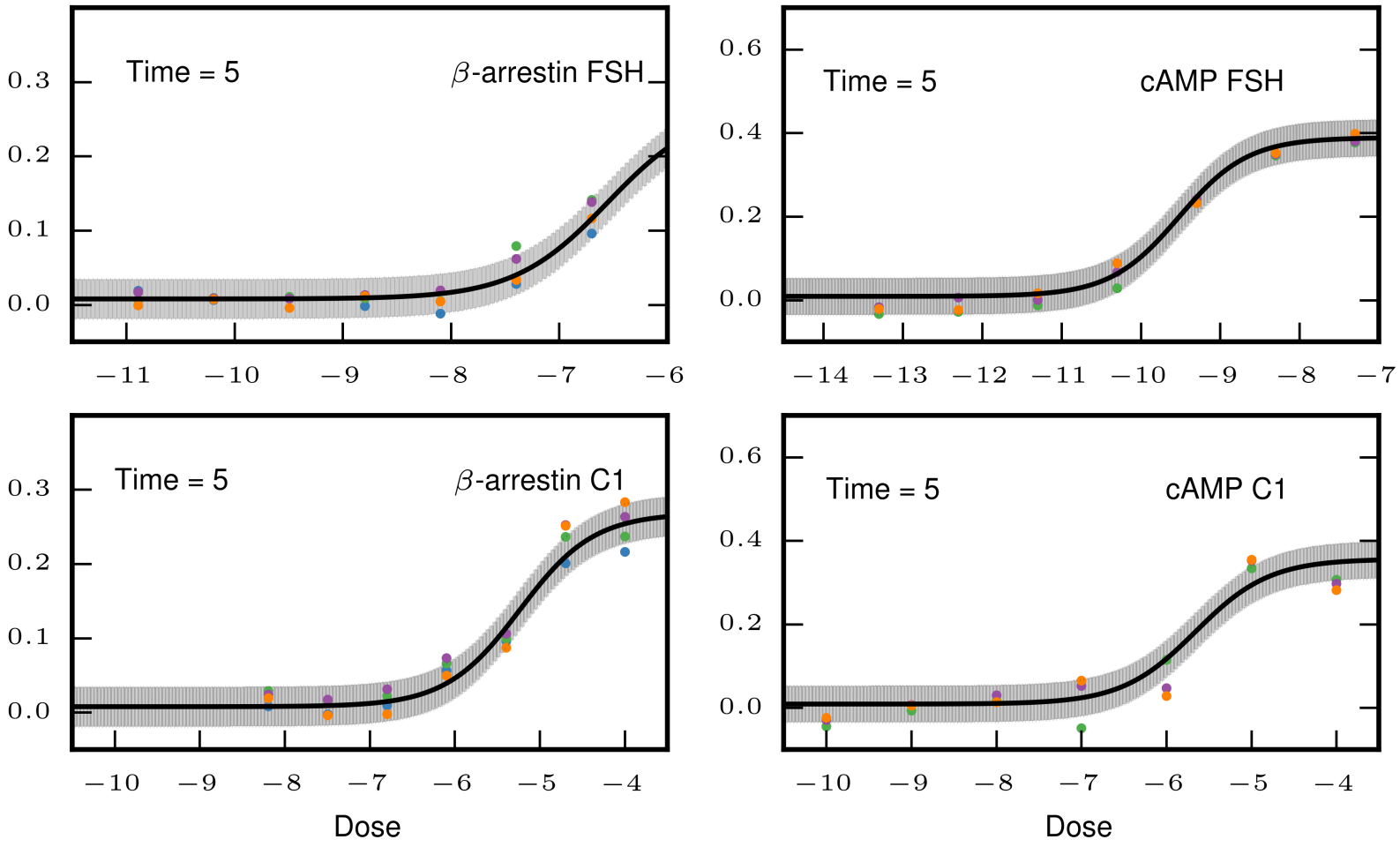
C1 is biased towards β-arr, compared to cAMP, in comparison to FSH.

With a "simpler" model



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

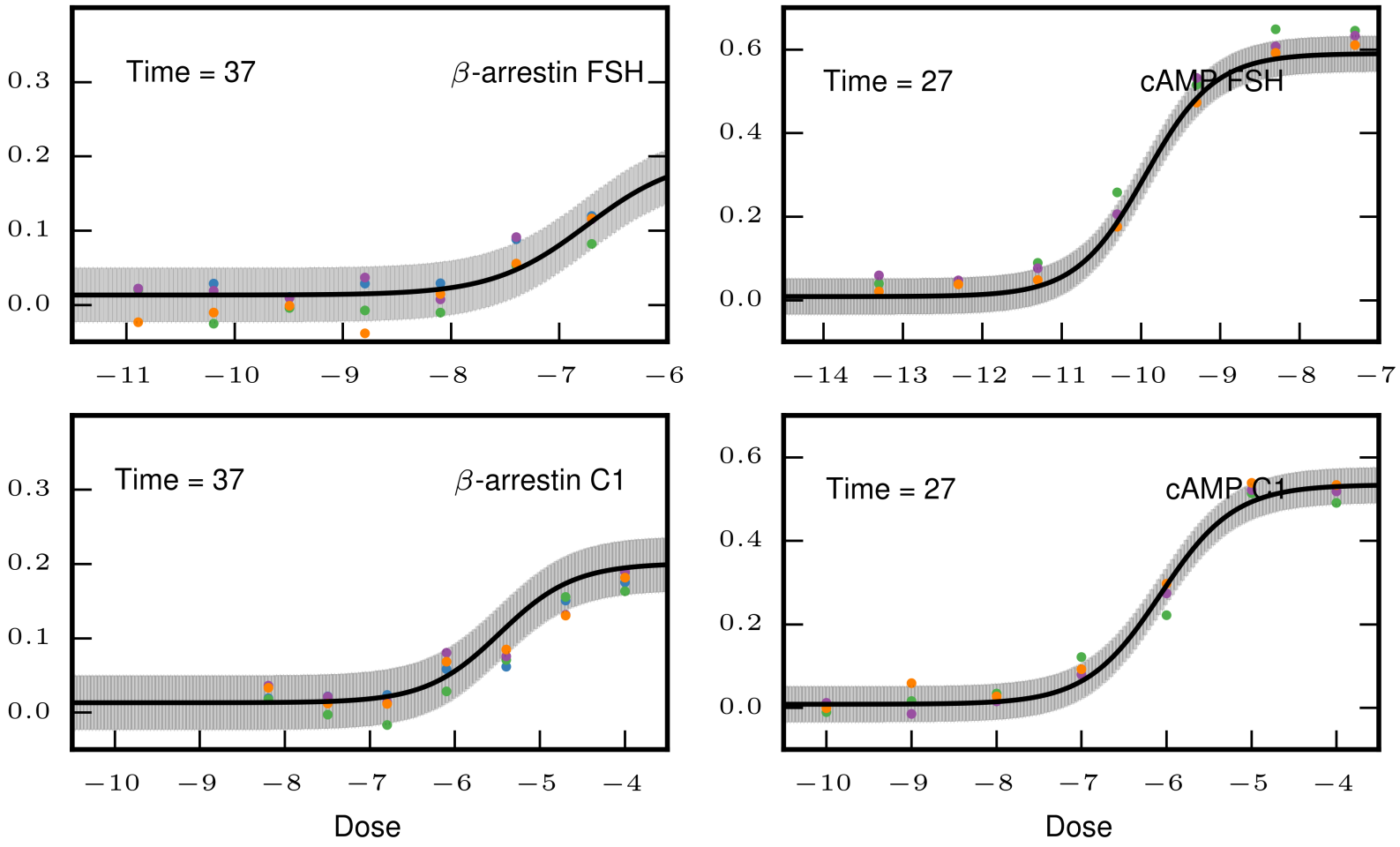
We systematically calculate bias value using standard method (operational model on dose-response curves :)



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 :)

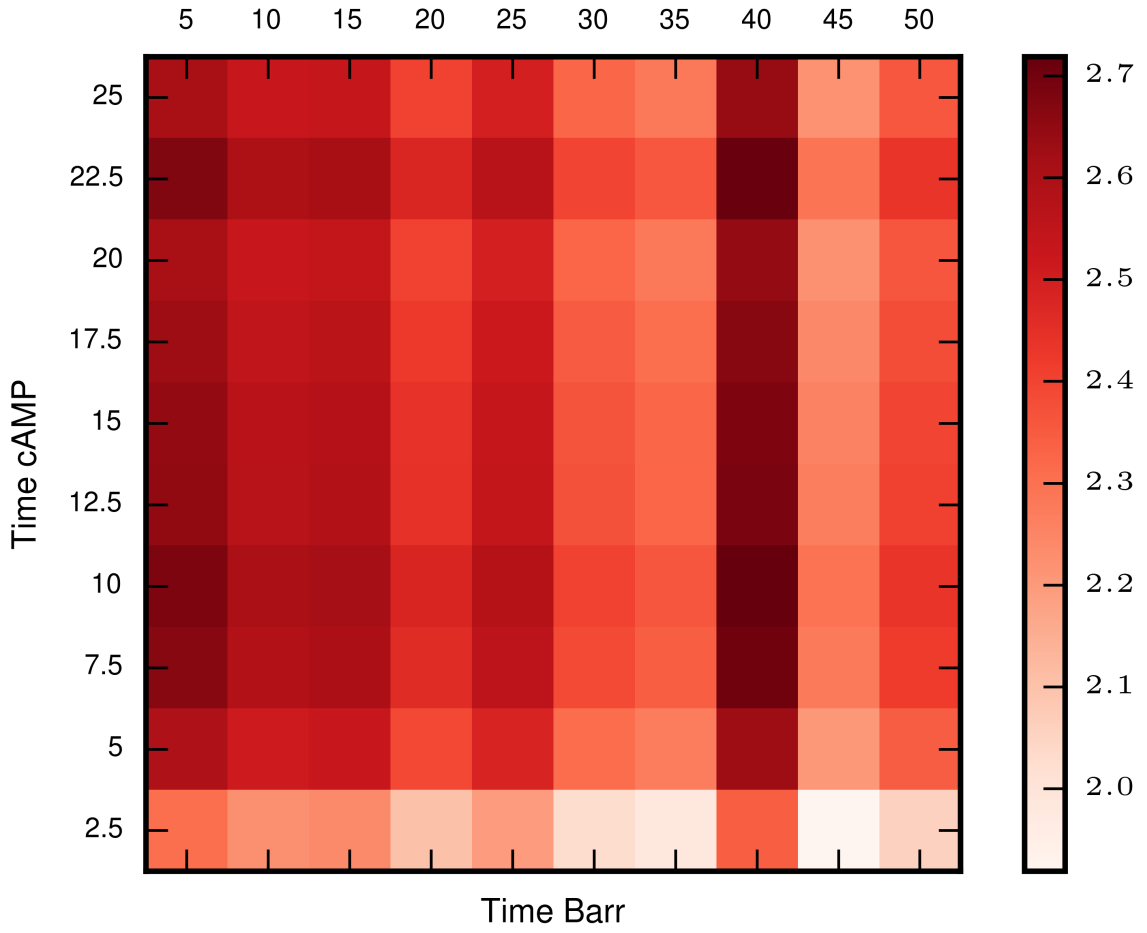


Bias=2.64 : C1 is biased towards β-arr, compared to cAMP, in comparison to FSH.

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

We systematically calculate bias value using standard method

Different times gives (slightly) different bias values

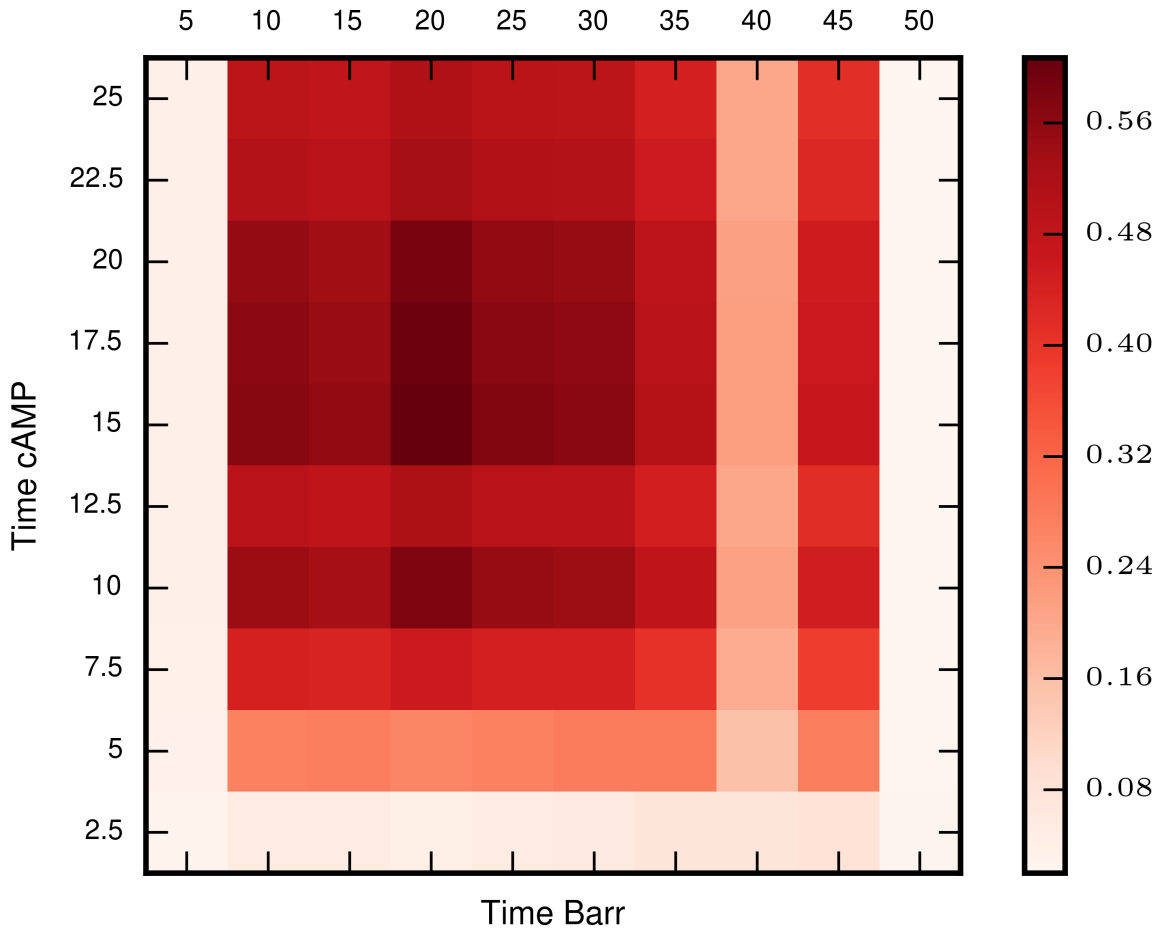


C1 is biased towards β-arr, compared to cAMP, in comparison to FSH.

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



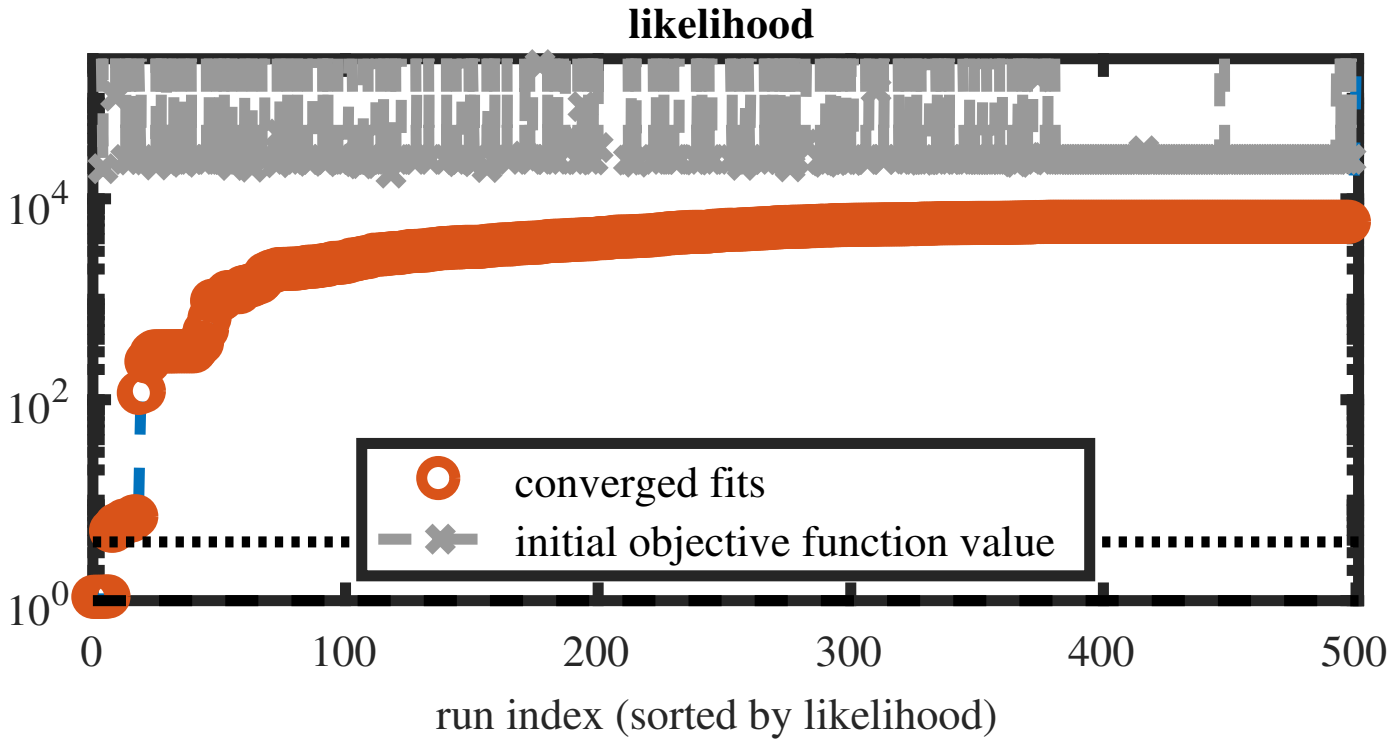
Thanks for your attention !

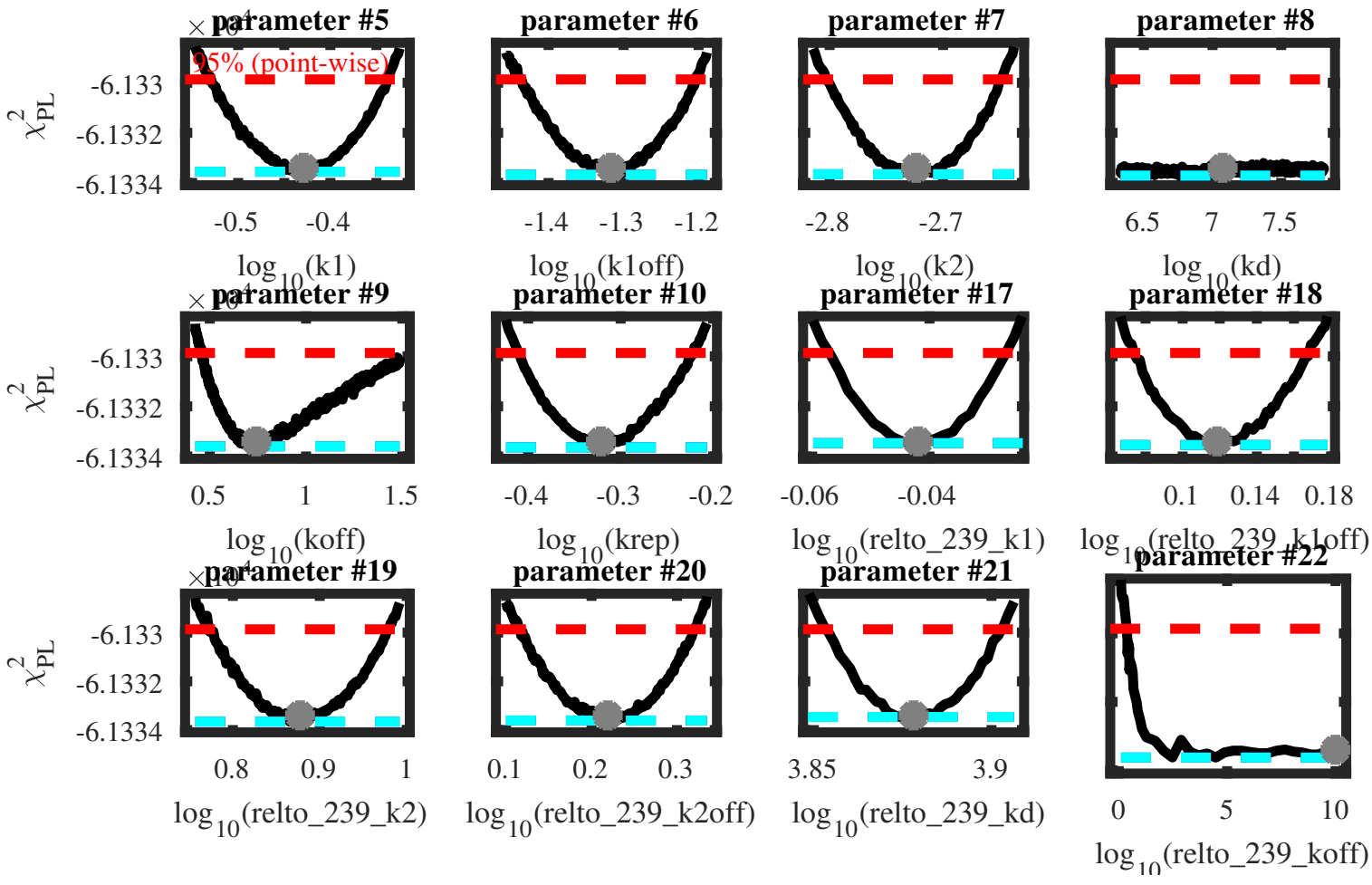
- Notion of signaling bias to quantify differential activation of several pathways by a Ligand at a given receptor.
- Standard quantification has several drawbacks (no time, limited to sigmoid scenario,..).
- We gave a kinetic interpretation of Ligand biased, which rely on dynamic (ODE) modeling and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


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)