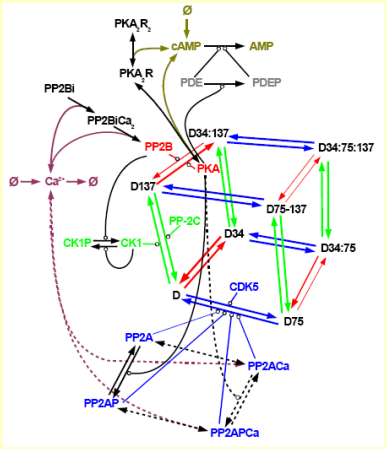
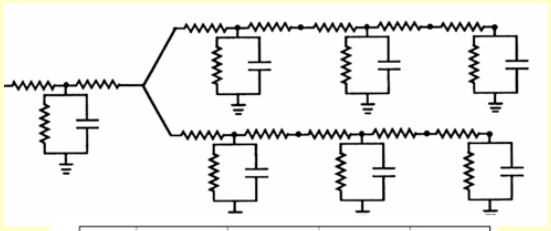
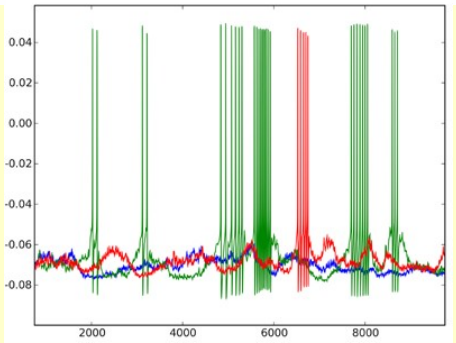
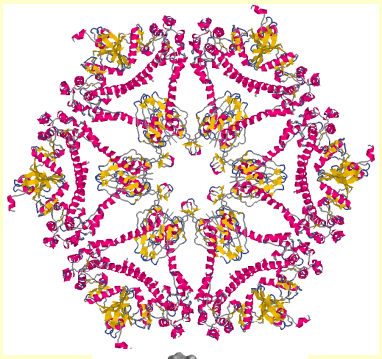
"Compneur" themes and projects

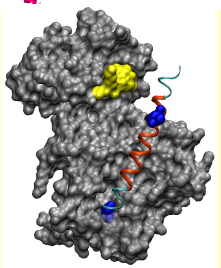
Computational Neurobiology

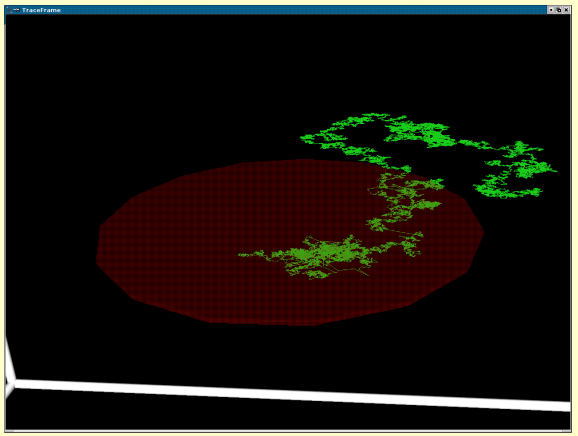












Systems Biology

SBML

SBGN

SBO

TEDDY

KISAO

e-MeP

Ligand-Gated Ion Channel database

MIRIAM

MIASE

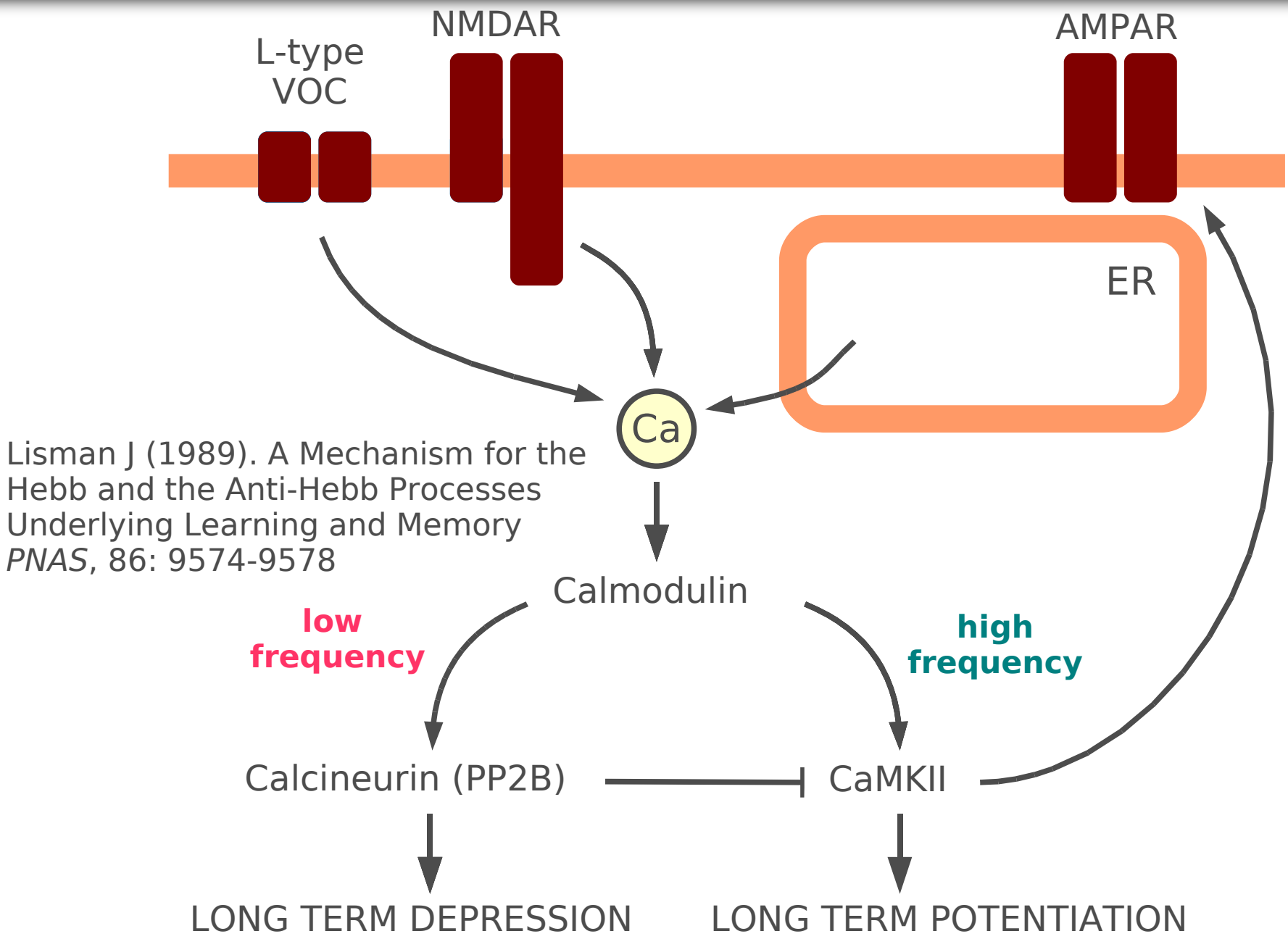
BioModels

EMBL-EBI

Cooperativity in signal transduction

Nicolas Le Novère

Calcium theory of Synaptic Plasticity



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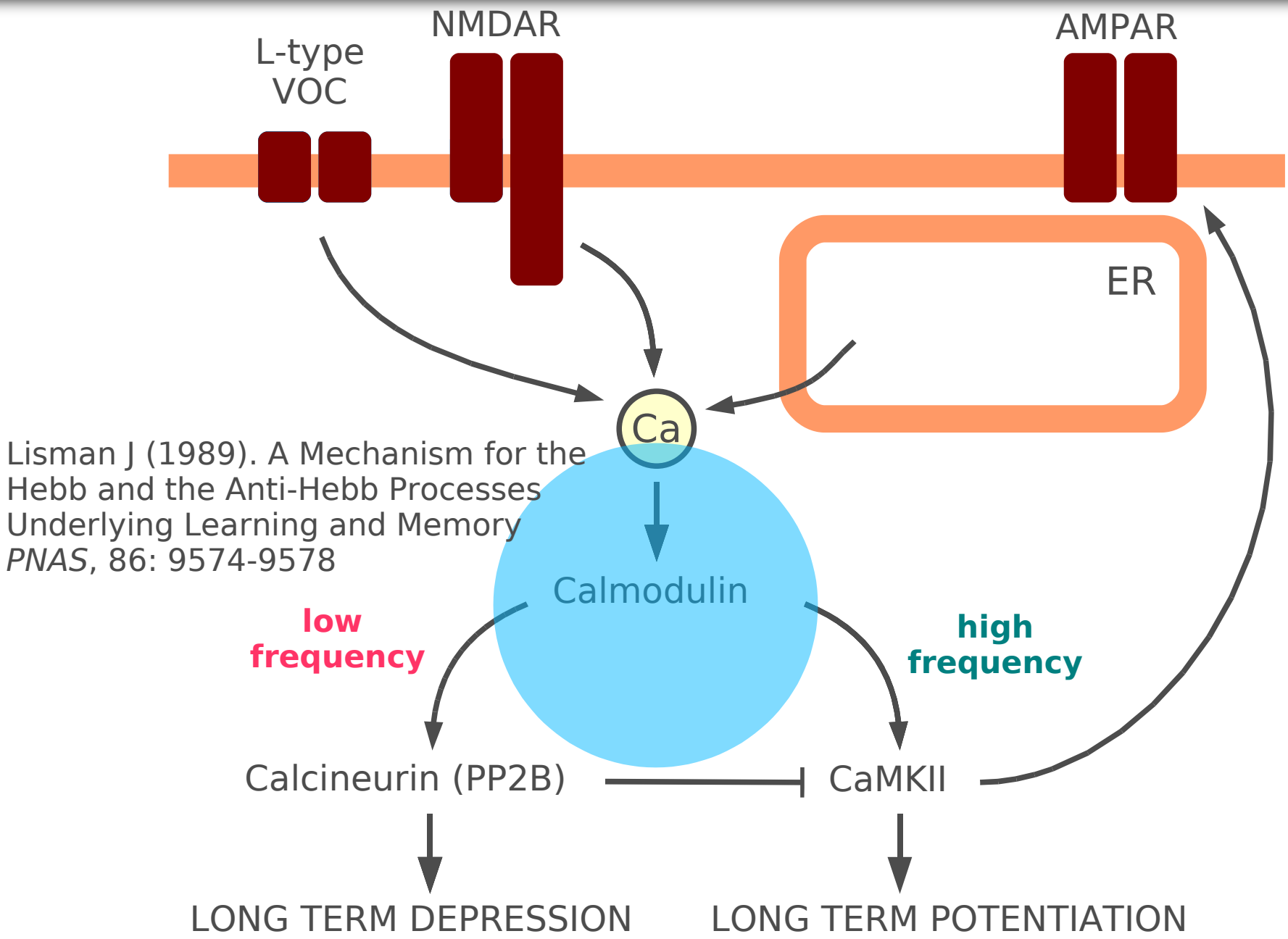
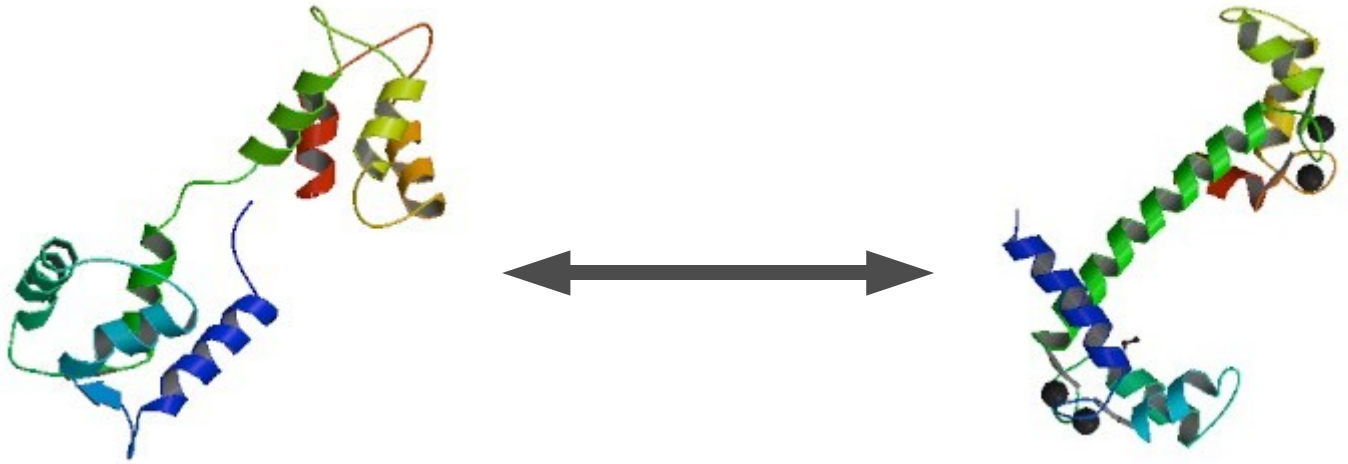


Lisman J (1989). A Mechanism for the Hebb and the Anti-Hebb Processes Underlying Learning and Memory *PNAS*, 86: 9574-9578

State transitions of Calmodulin



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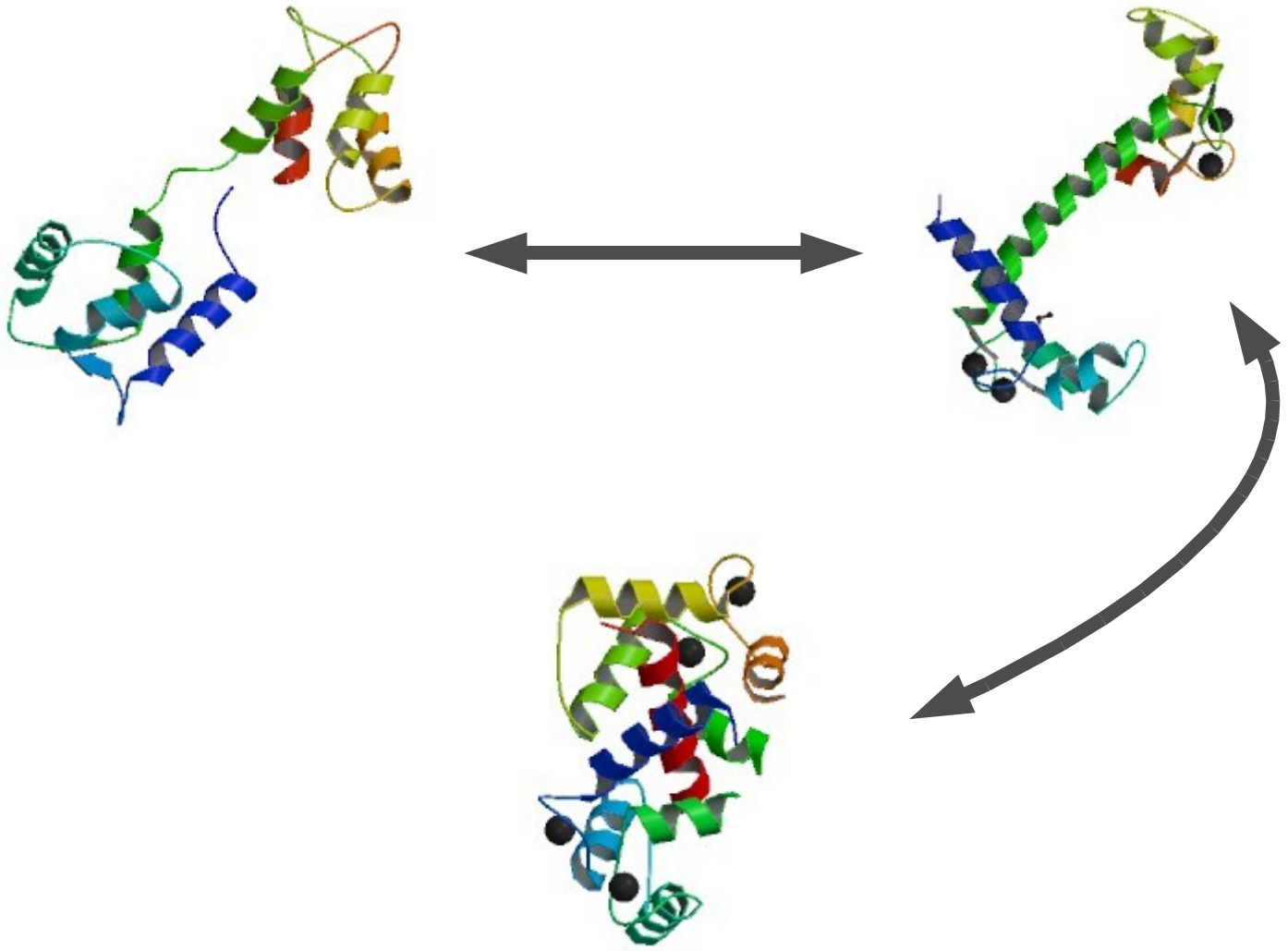
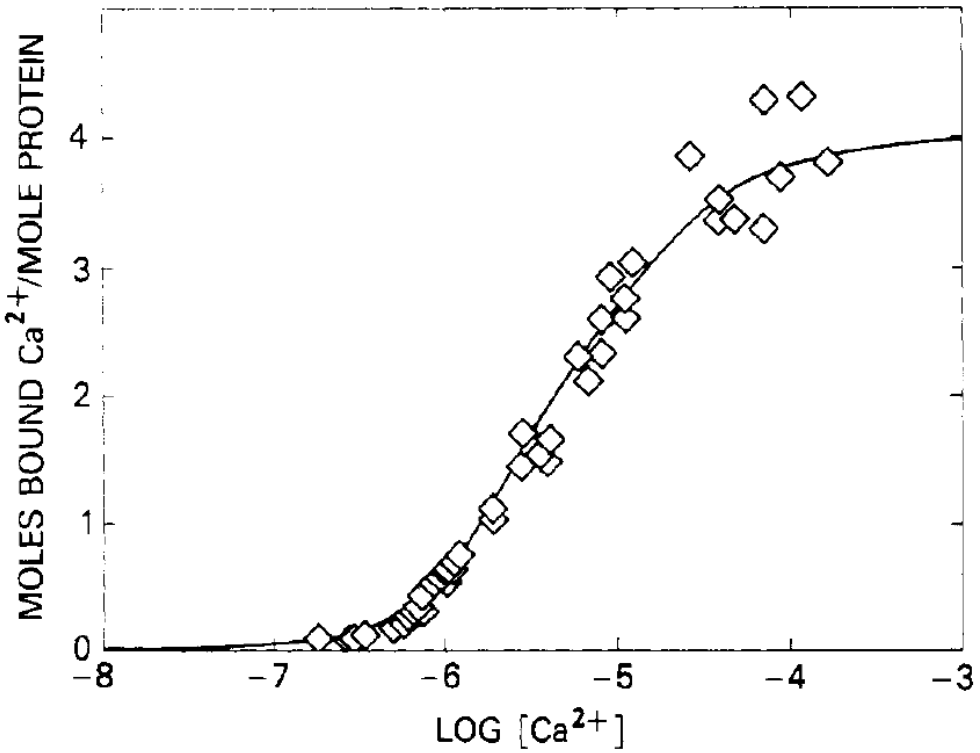
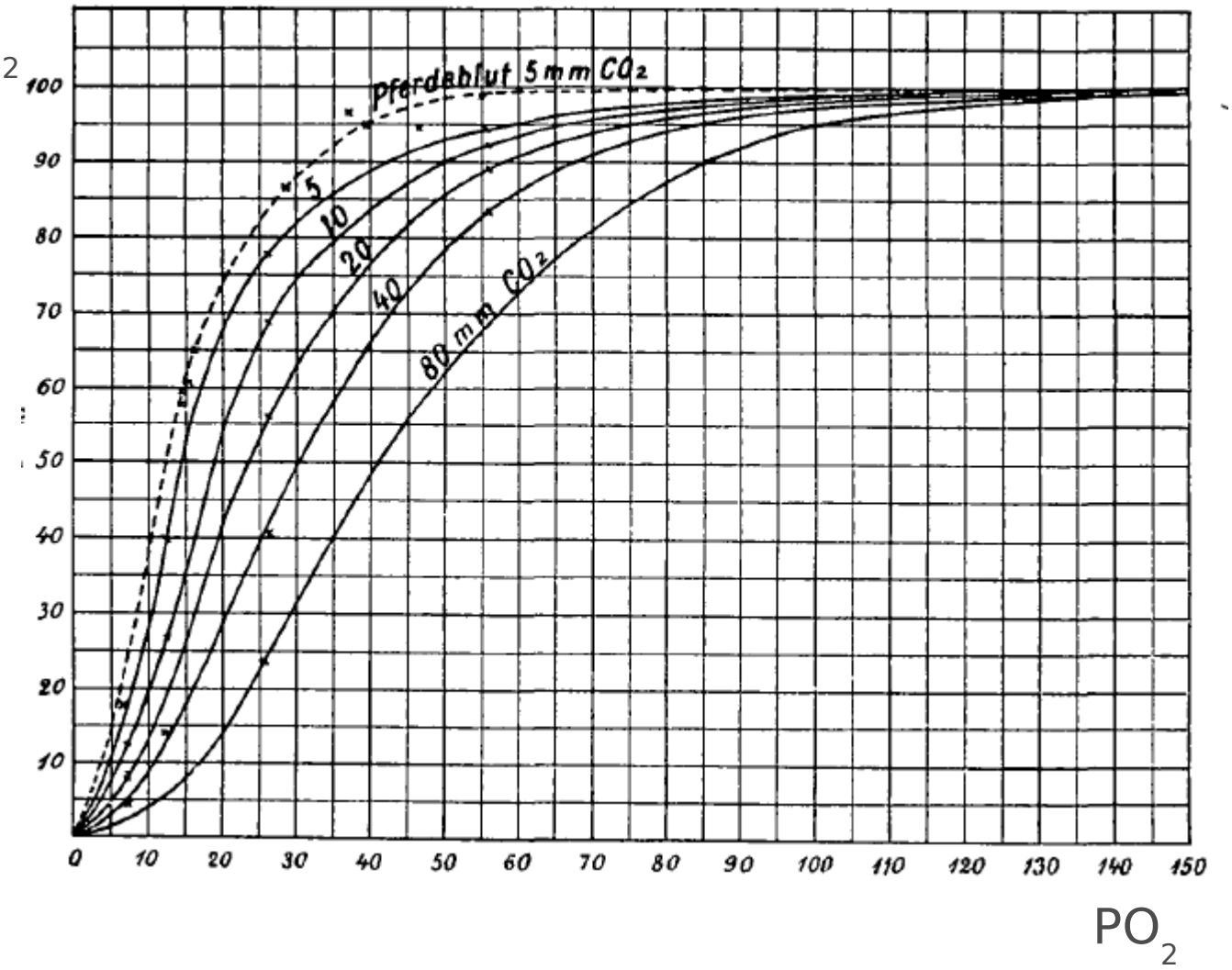


Calmodulin is ultra-sensitive



Crouch TH and Klee CB (1980)
Positive cooperative binding of calcium to bovine brain calmodulin.
Biochemistry, 19: 3692-3698

Origins of cooperativity: Bohr



Bohr C (1903) Theoretische behandlung der quantitativen verhältnisse bei der sauerstoff aufnahme des hämoglobins *Zentralbl Physiol* 17: 682

Origins of cooperativity: Hill

iv PROCEEDINGS OF THE PHYSIOLOGICAL

The possible effects of the aggregation of the molecules of hæmoglobin on its dissociation curves. By A. V. HILL.

In a previous communication Barcroft and I gave evidence which seemed to us to prove conclusively that dialysed hæmoglobin consists simply of molecules containing each one atom of iron. The molecular weight is therefore Hb = 16,660. These experiments have not been published yet, but I shall assume the results.

Other observers (Reid, Roaf, Hüfner and Gansser) working on different solutions have obtained divergent results. The method used by all of them was the direct estimation of the osmotic pressure, by means of a membrane permeable to salts, but not to hæmoglobin. The method involves a relatively large error, because the quantity measured is small. It is doubtful however whether this can explain the discordant results.

Our work led me to believe that the divergence between the results of different observers was due to an aggregation of the hæmoglobin molecules by the salts present in the solution, a consequent lowering of the number of molecules, and an increase in the average molecular weight as observed by the osmotic pressure method. To test this hypothesis I have applied it to several of the dissociation curves obtained by Barcroft and Camis with hæmoglobin in solutions of various salts, and with hæmoglobin prepared by Bohr's method.

The equation for the reaction would be

$$\mathrm{Hb} + \mathrm{O_2} \rightleftarrows \mathrm{HbO_2},$$
$$\mathrm{Hb}_n + n\mathrm{O_2} \rightleftarrows \mathrm{Hb}_n\mathrm{O}_{2n},$$

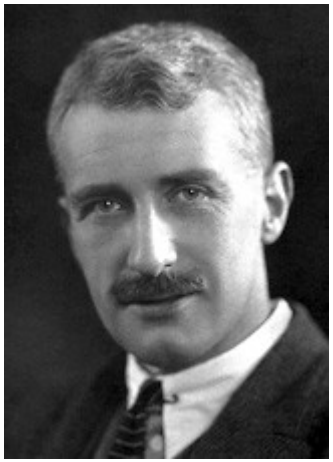
where Hb_n represents the aggregate of n molecules of Hb. I have supposed that in every solution there are many different sized aggregates, corresponding to many values of n.

If there were in the solution only Hb and $\mathrm{Hb_2}$ the dissociation curve would be

$$y = \lambda \frac{K'x^2}{1+K'x^2} + (100-\lambda)\frac{Kx}{1+Kx} \quad \ldots\ldots\ldots\ldots\ldots\ldots (\mathrm{A}),$$

where λ % is as $\mathrm{Hb_2}$, $(100-\lambda)$ % as Hb, K' is the equilibrium constant of the reaction $\mathrm{Hb_2} + 2\mathrm{O_2} \rightleftarrows \mathrm{Hb_2O_4}$ and K that of $\mathrm{Hb} + \mathrm{O_2} \rightleftarrows \mathrm{HbO_2}$: K has the value ·125 (Barcroft and Roberts).

Hill AV (1910) The possible effects of the aggregation of the molecules of hæmoglobin on its dissociation curves. *J Physiol* 40: iv-vii.



Origins of cooperativity: Hill

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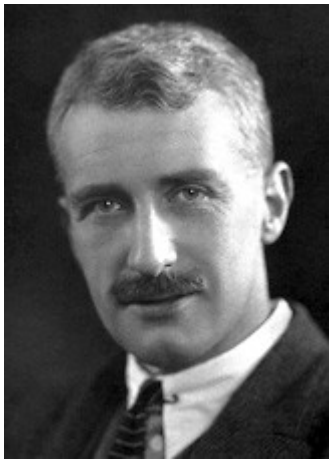
$$y = 100\frac{Kx^n}{1+Kx^n} \quad \ldots\ldots\ldots\ldots\ldots\ldots (B)$$

would satisfy the observations.



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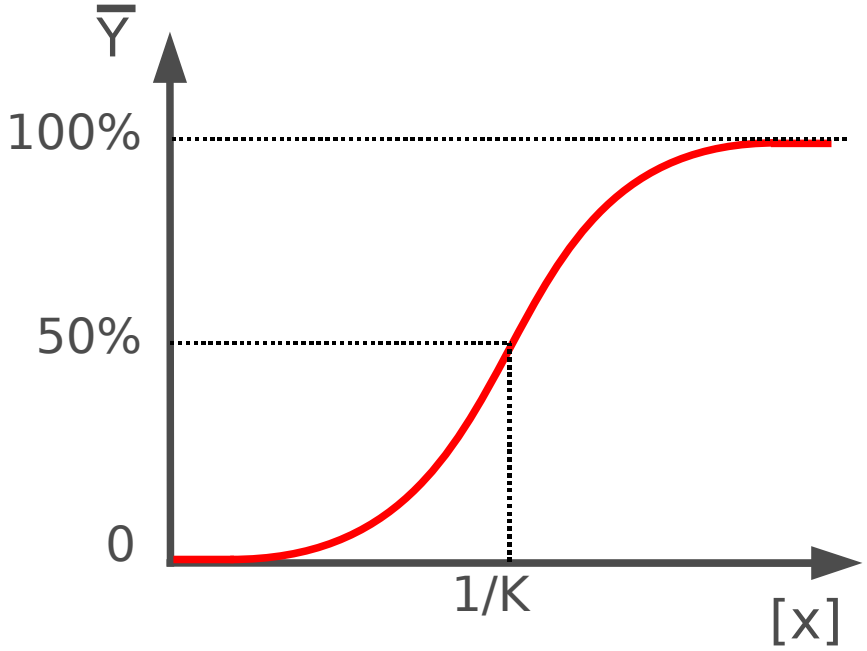


Origins of cooperativity: Hill Plot

$$\bar{Y} = \frac{K^n[x]^n}{1 + K^n[x]^n}$$

Hill equation

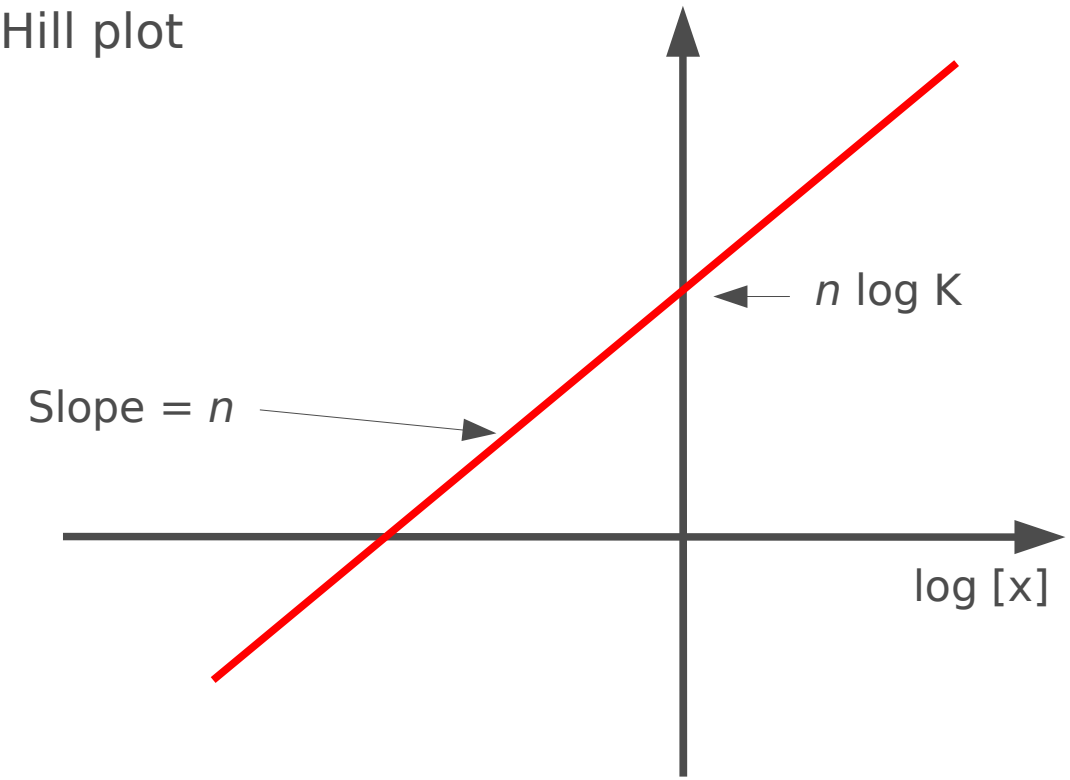
Origins of cooperativity: Hill Plot

$$\bar{Y} = \frac{K^n[x]^n}{1 + K^n[x]^n}$$

Hill equation

$$\log \frac{\bar{Y}}{1 - \bar{Y}} = n \log[x] + n \log K$$

Hill plot



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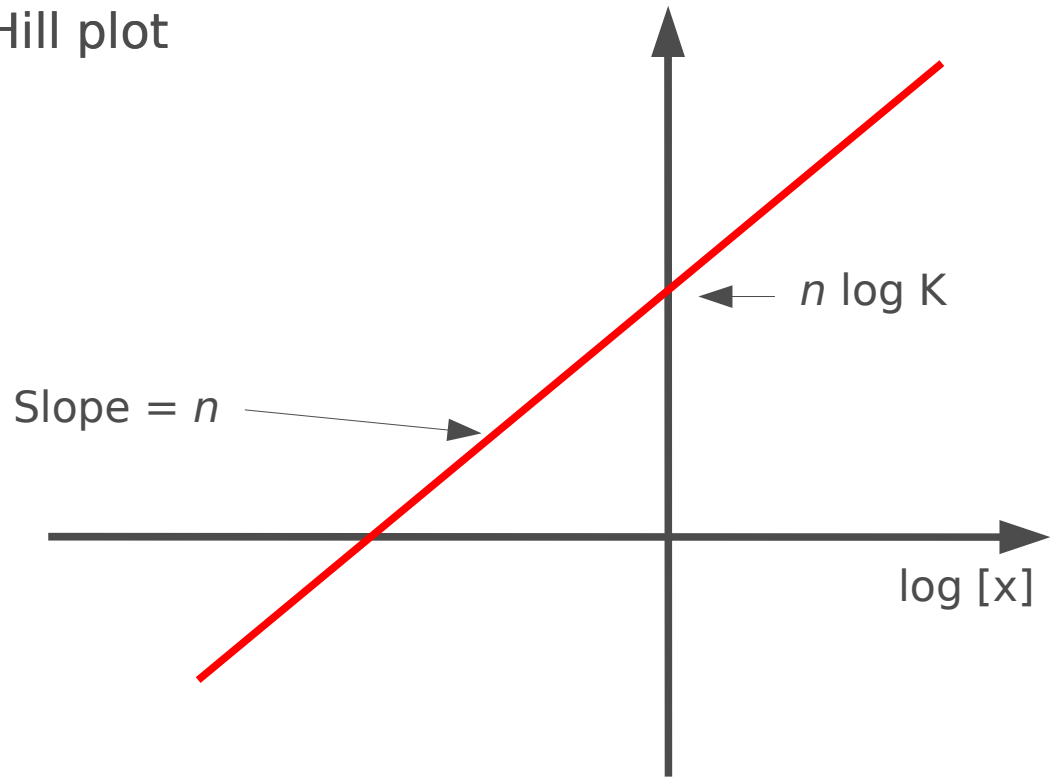

Effect increases in function of the signal to the power of *n*:
n>1, ultra-sensitive
n<1, infra-sensitive

BUT cooperativity of ligand, not of binding sites: unique affinity

Origins of cooperativity: Adair

THE HEMOGLOBIN SYSTEM.

VI. THE OXYGEN DISSOCIATION CURVE OF HEMOGLOI

By G. S. ADAIR.

With the Collaboration of A. V. Bock and H. Field, J

(*From the Medical Laboratories of the Massachusetts General Hos Boston.*)

(Received for publication, January 7, 1925.)

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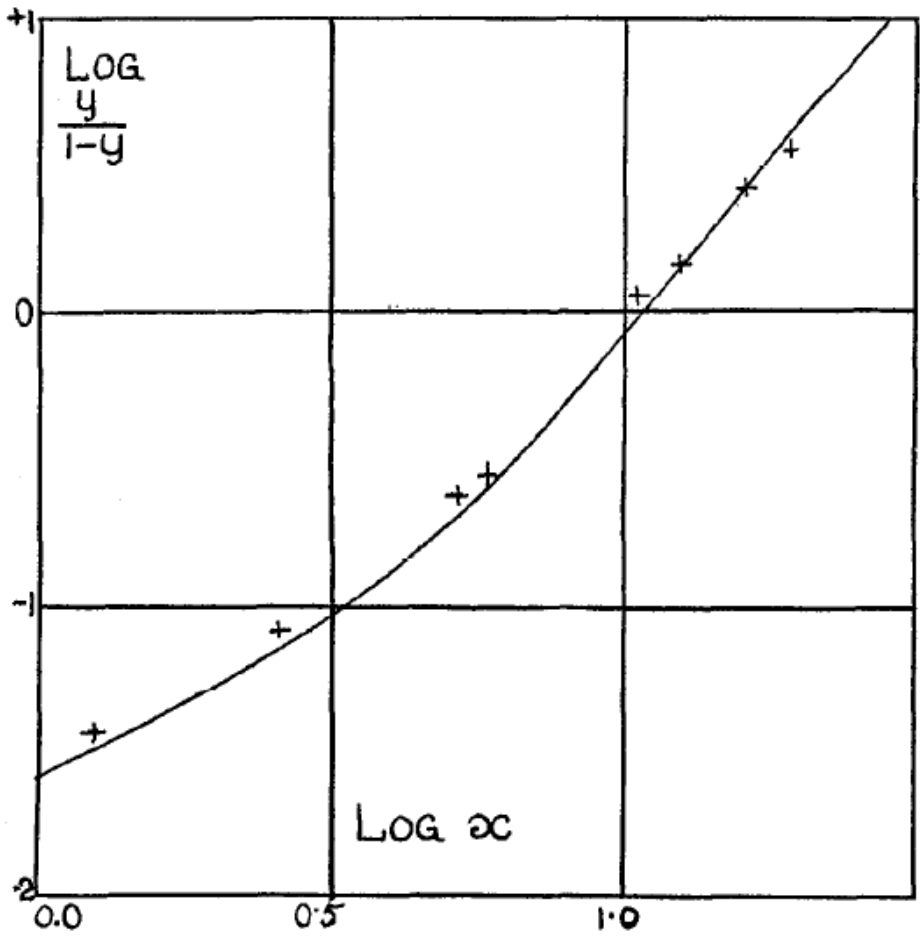

FIG. 2. Test of formula (6). Curve drawn from 6 experimental points from Table IV.

Adair GS (1925) The hemoglobin system VI. the oxygen dissociation curve of hemoglobin. *J Biol Chem* 63: 529

Origins of cooperativity: Adair

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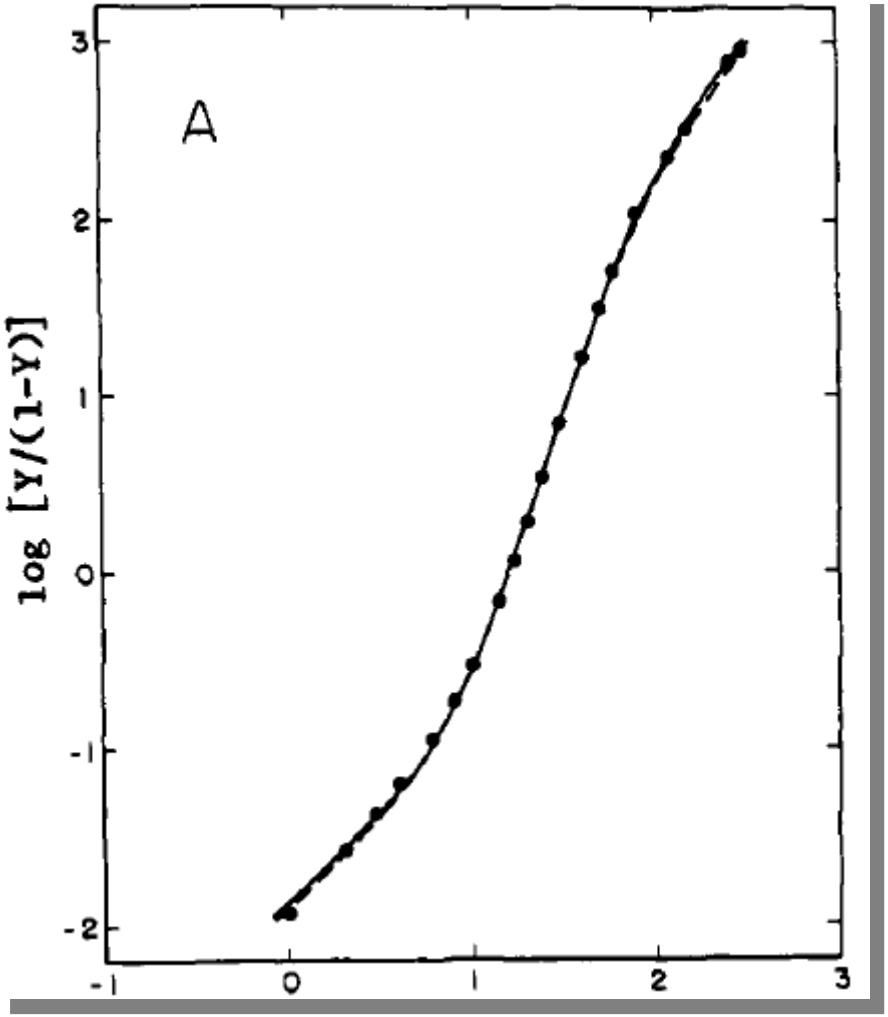
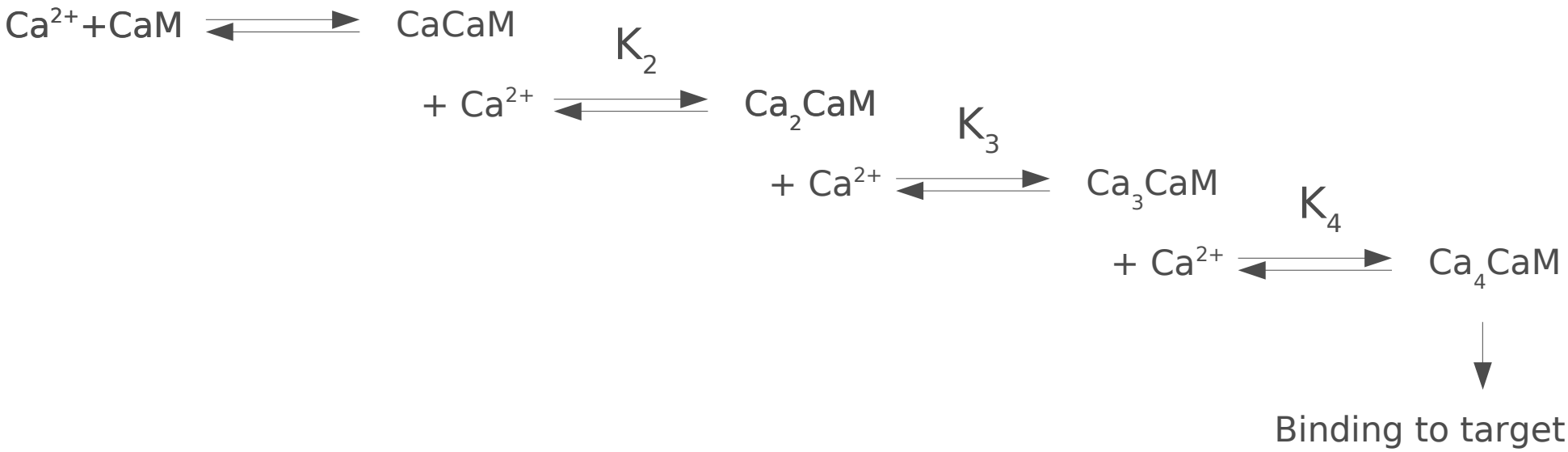


Imai (1973) Analyses of Oxygen Equilibria of Native and Chemically Modified Human Adult Hemoglobins on the Basis of Adair's Stepwise Oxygenation Theory and the Allosteric Model of Monod, Wyman, and Changeux. *Biochemistry* 12: 798-808

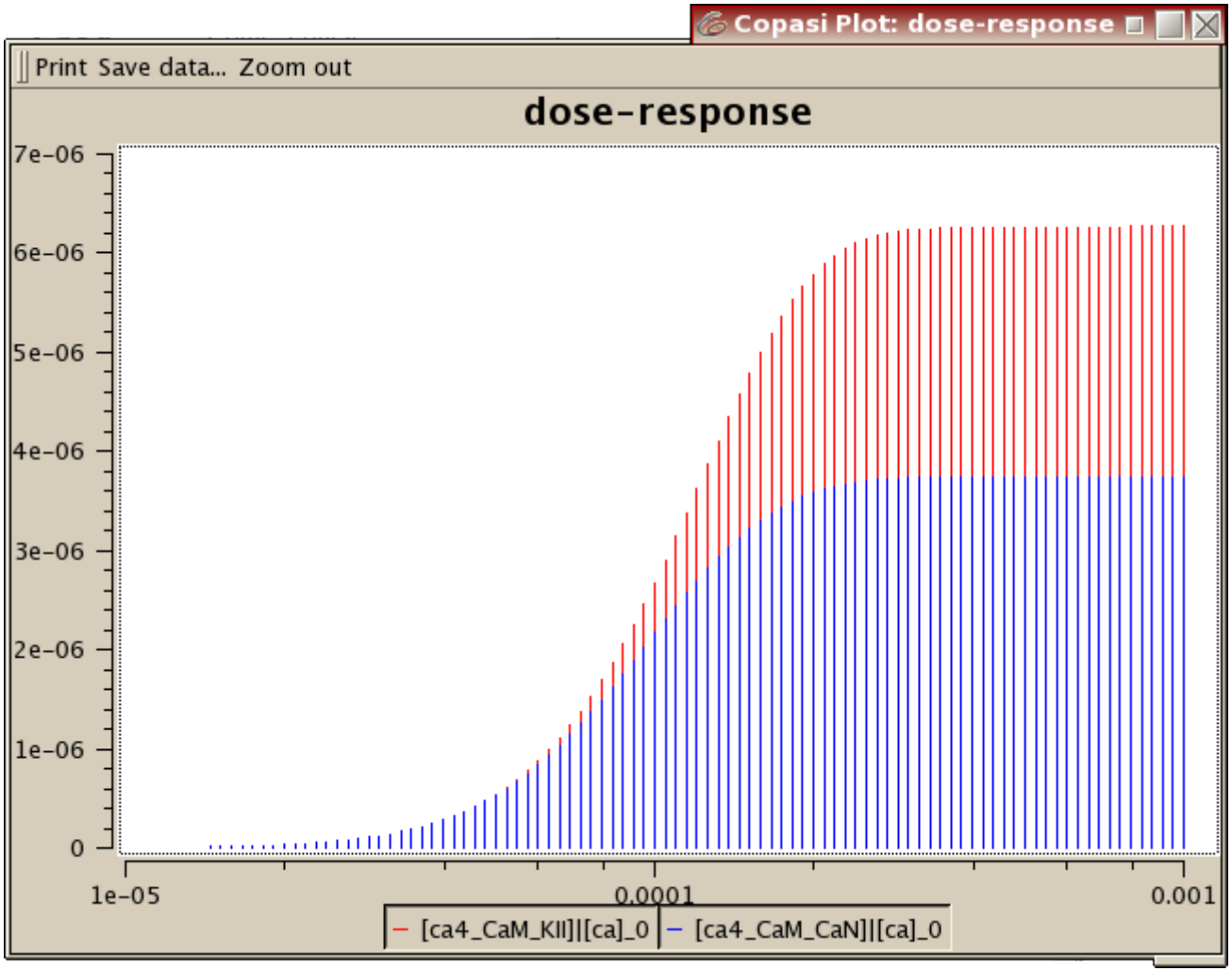
Corresponding induced-fit model

Klotz I (1946) The Application of the Law of Mass Action to Binding by Proteins. Interactions with Calcium. *Arch Biochem*, 9:109–117.

$$\bar{Y} = \frac{1}{n}\frac{K_1[\mathrm{Ca}^{2+}] + 2K_1K_2[\mathrm{Ca}^{2+}]^2 + 3K_1K_2K_3[\mathrm{Ca}^{2+}]^3 + 4K_1K_2K_3K_4[\mathrm{Ca}^{2+}]^4}{1 + K_1[\mathrm{Ca}^{2+}] + 2K_1K_2[\mathrm{Ca}^{2+}]^2 + 3K_1K_2K_3[\mathrm{Ca}^{2+}]^3 + 4K_1K_2K_3K_4[\mathrm{Ca}^{2+}]^4}$$



That does not work ...

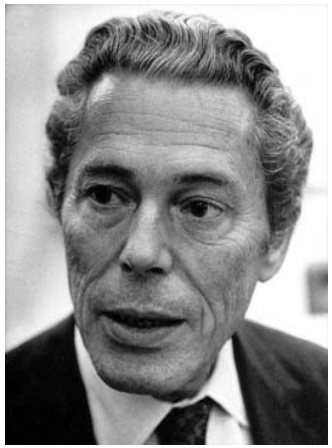


That does not work ...

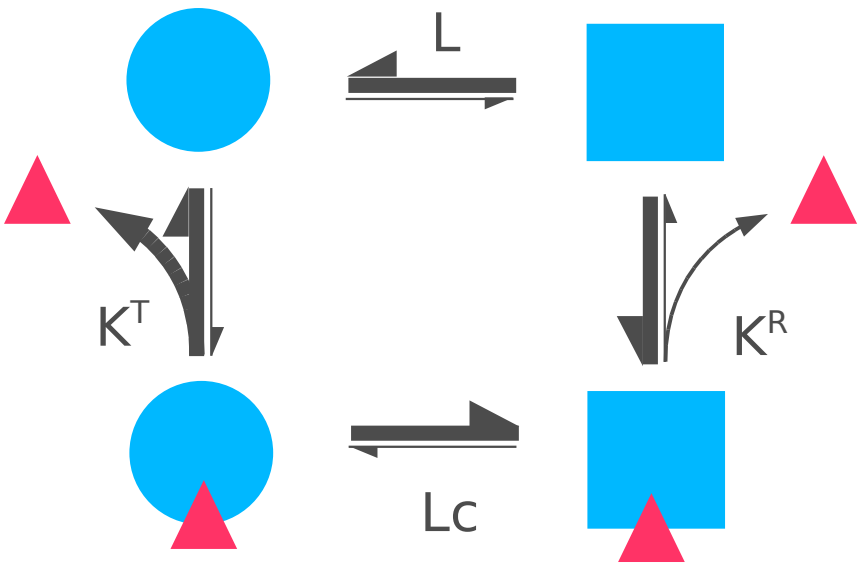
- Calmodulin bound to three calcium ions activates calcineurin
  - Kincaid and Vaughan (1986). *PNAS*, 83: 1193-1197
- Calmodulin bound to two calcium ions can bind CaMKII
  - Shifman et al (2006). *PNAS*, 103: 13968-13973
- Calmodulin affinity for calcium increases once bound to CaMKII
  - Shifman et al (2006) [but many previous reports on other targets: e.g. Burger et al (1983). *JBC*, 258: 14733-14739 ; Olwin et (1984). *JBC* 259: 10949-10955]
- Calcium activates both LTP and LTD through calmodulin
  - Lisman (1989) *PNAS*, 86: 9574-9578
  - High [Ca^{2+}] (high freq) ≅ CaMKII ; Low [Ca^{2+}] (low freq) ≅ Calcineurin
- What is the cause of the increasing affinity? How do binding sites communicate?
- What is the relationship between the known structures and the quantitative model? What causes the structural transitions?

Allostery and state selection

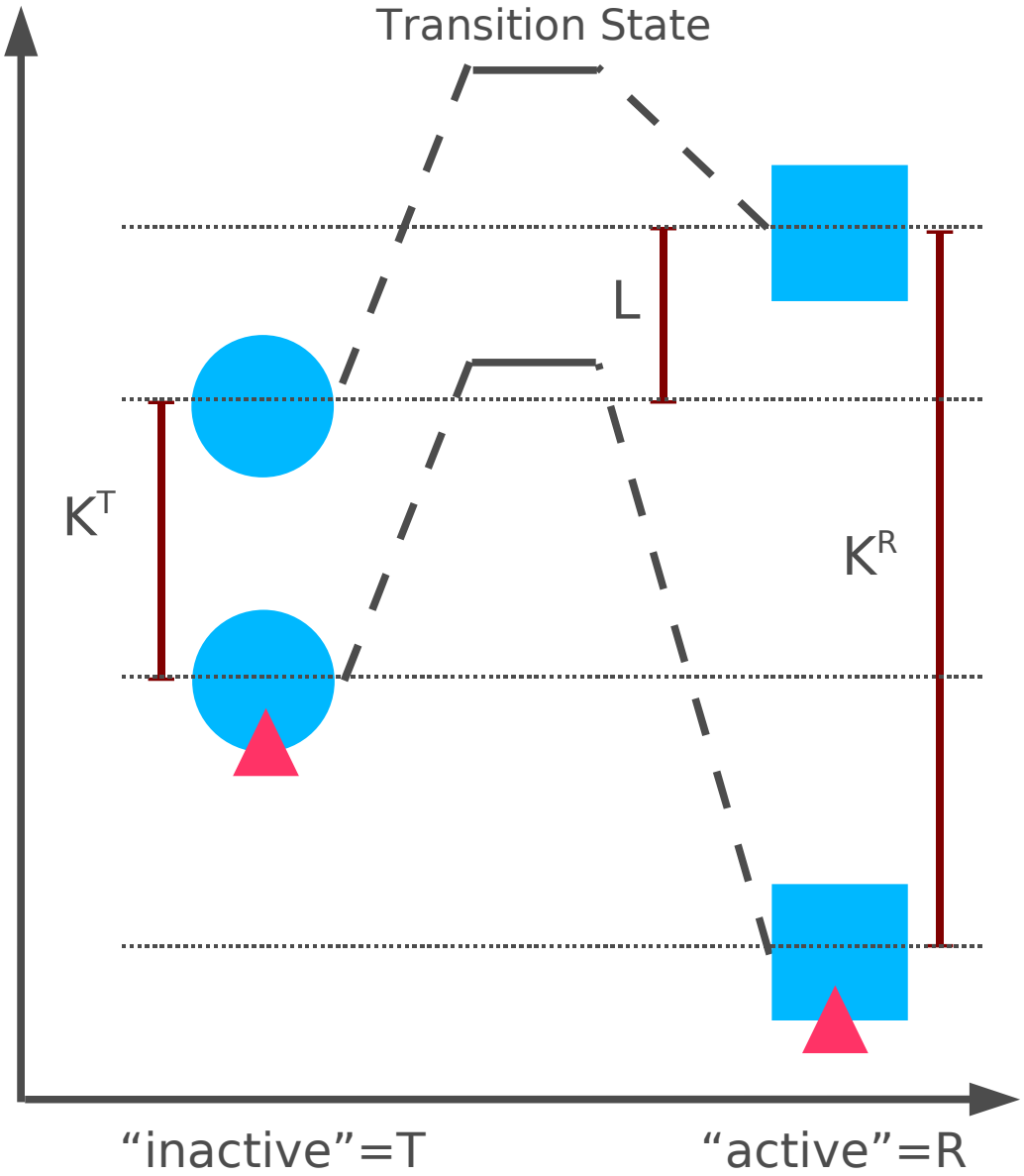
- Monod, Wyman, Changeux (1965). On the nature of allosteric transitions: a plausible model. *J Mol Biol*, 12: 88-118



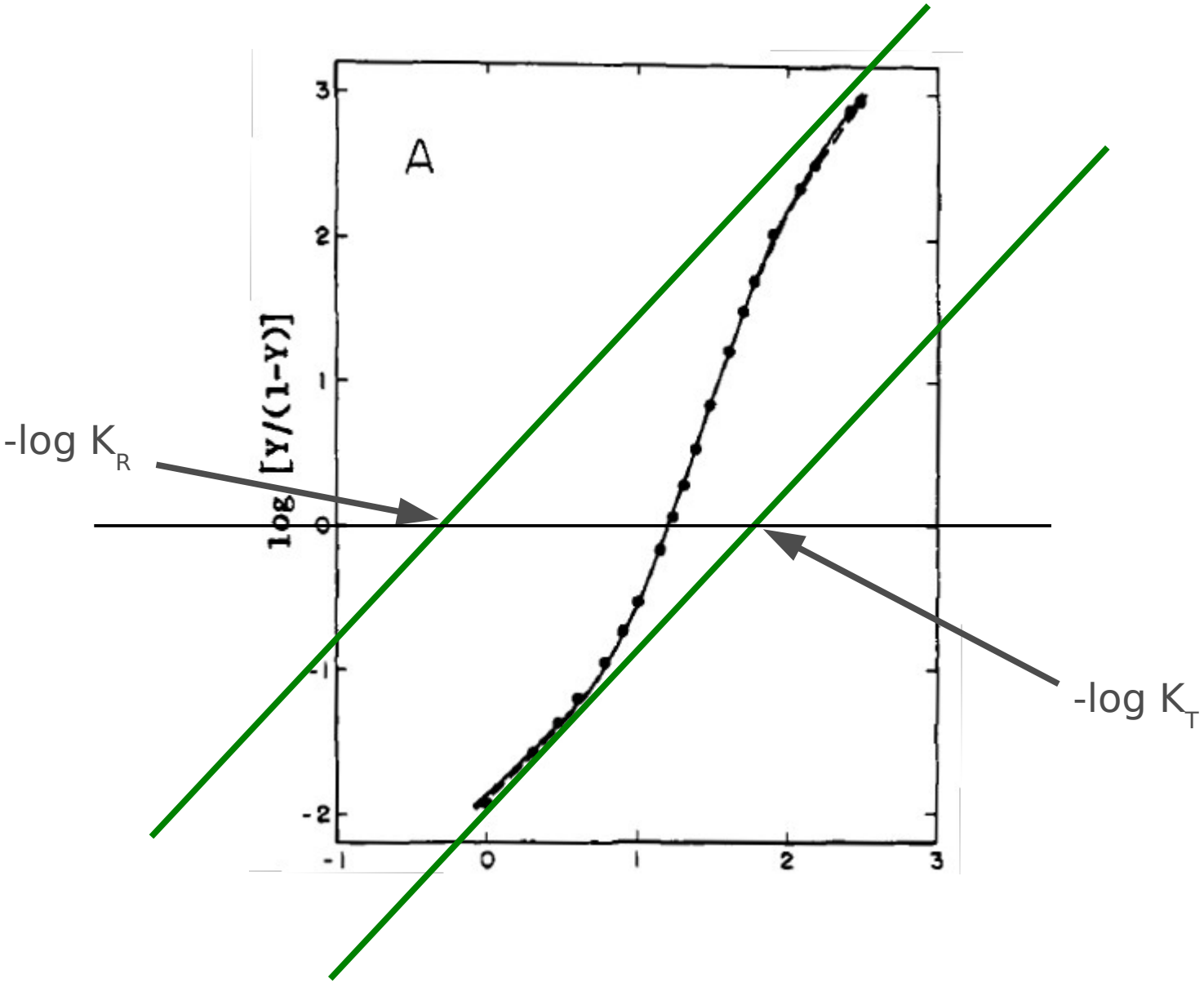
Modulation of thermal equilibria ≠ induced-fit



$$L = \frac{[T]_0}{[R]_0} \qquad c = \frac{K_R}{K_T}$$

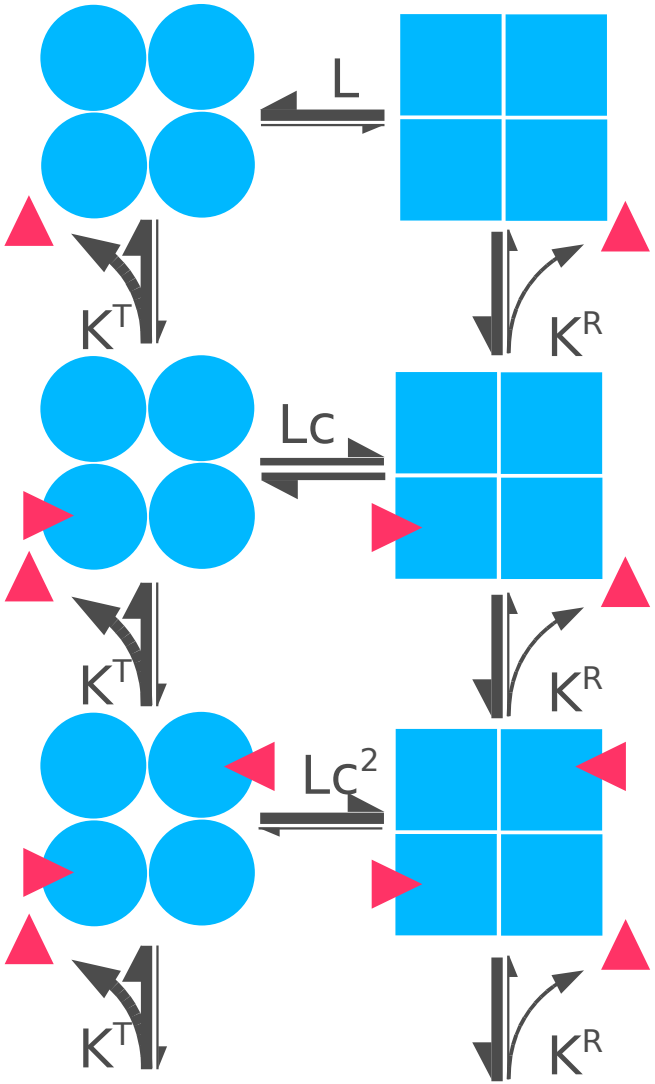


“Hill” Plot for MWC model

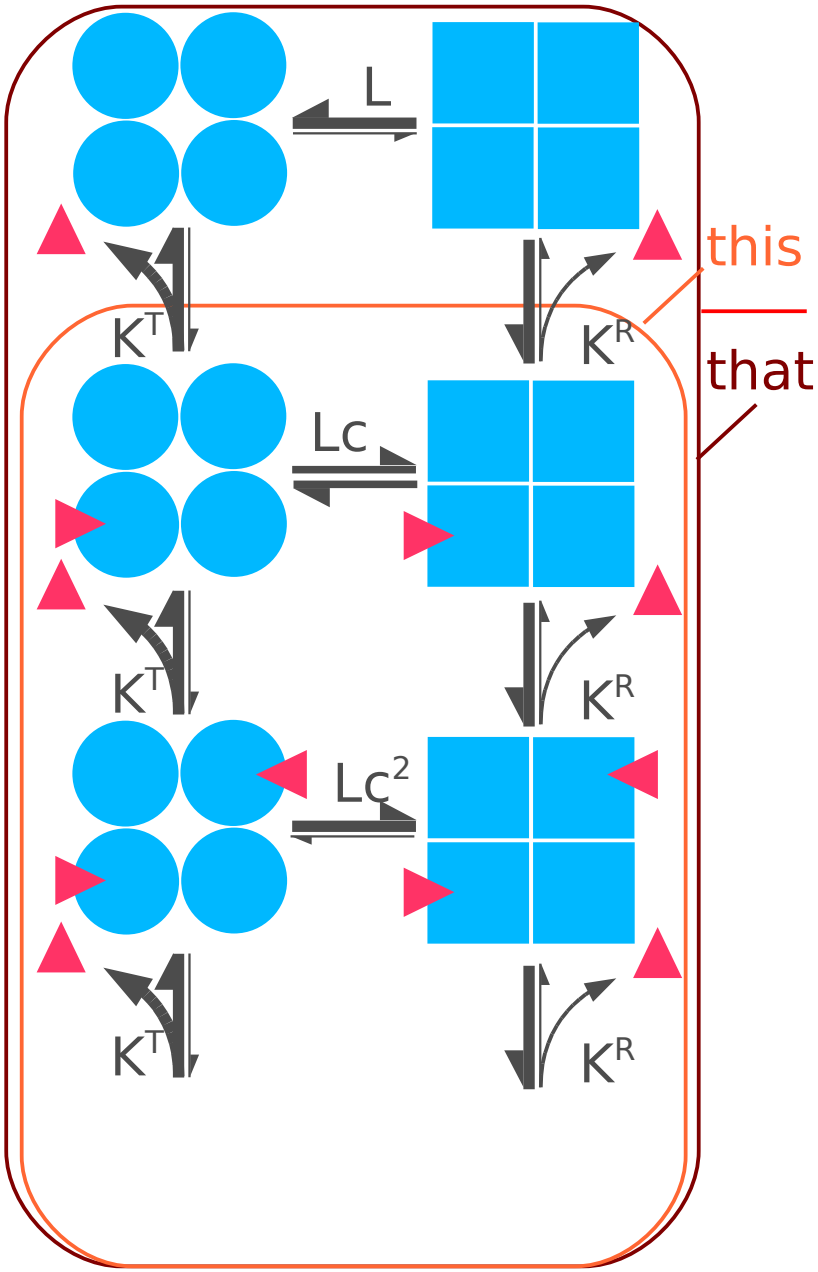


Concerted transitions ≠ sequential model

Monod-Wyman-Changeux model



Binding function



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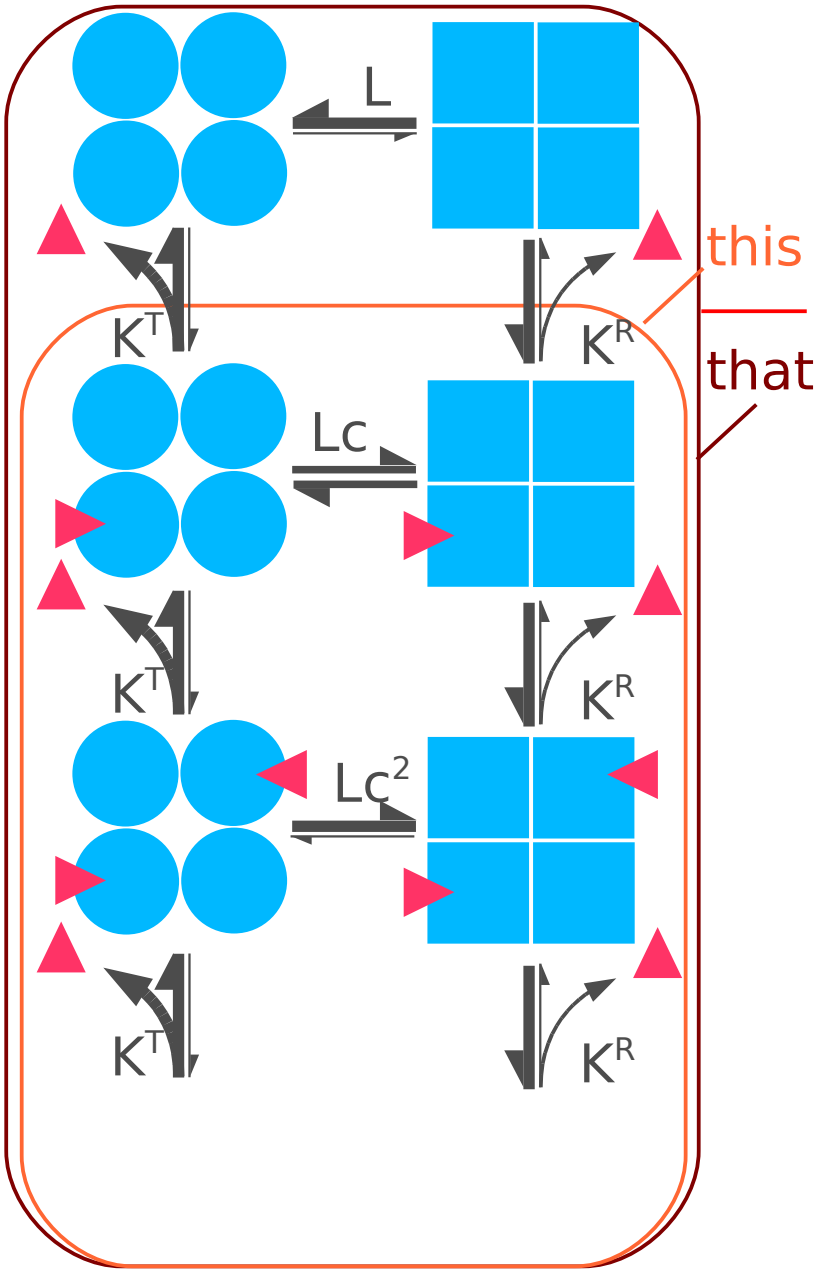
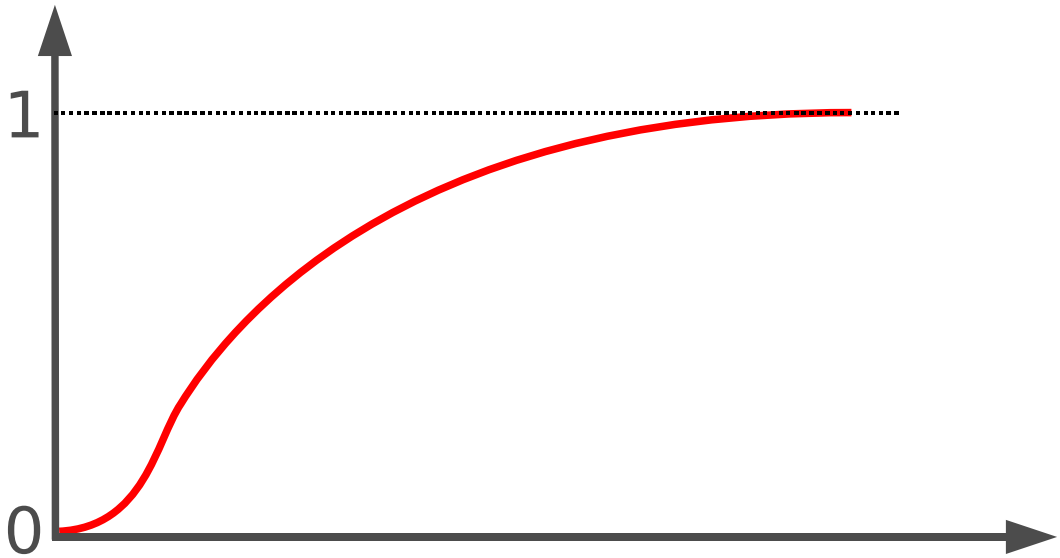
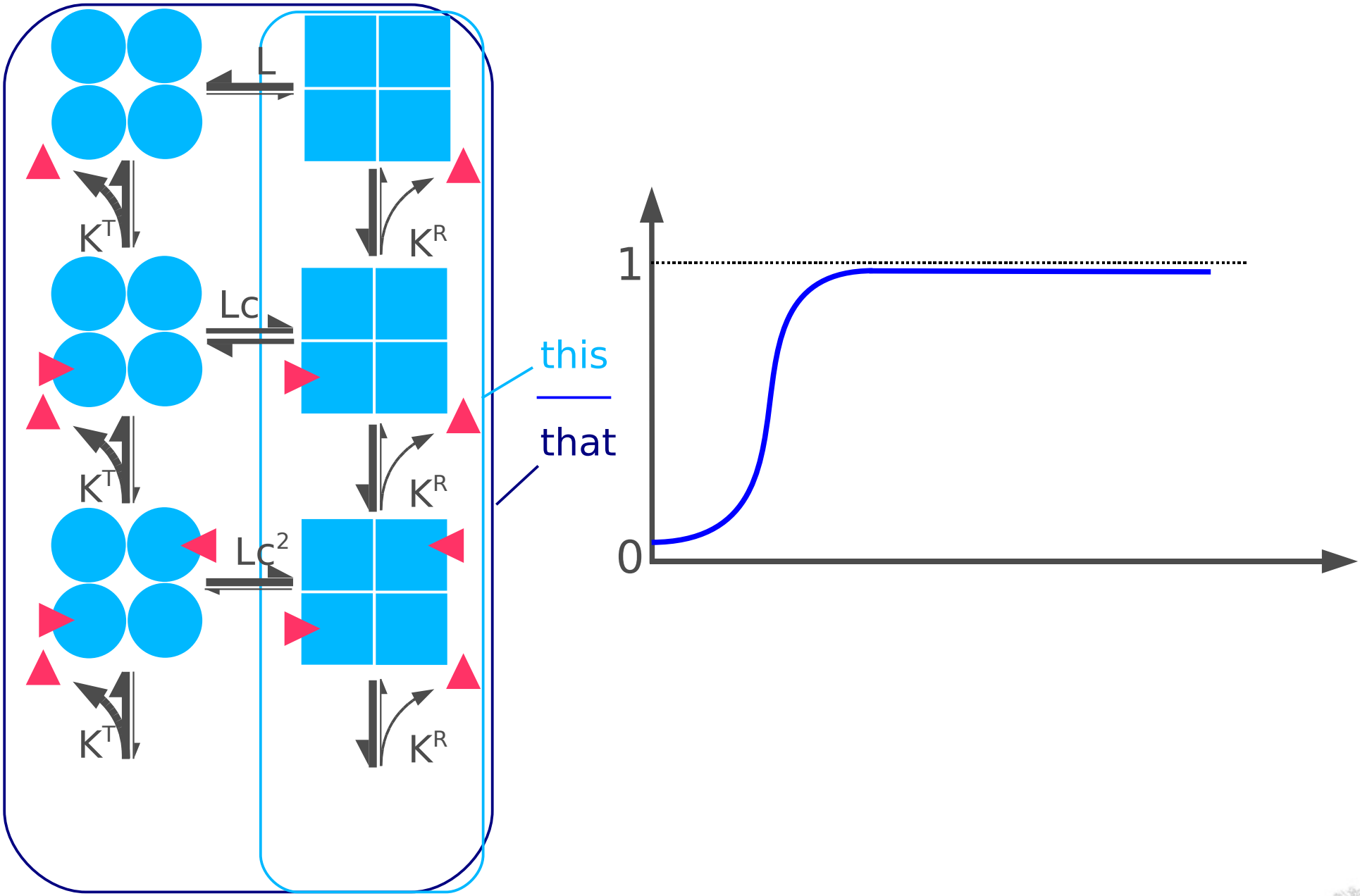
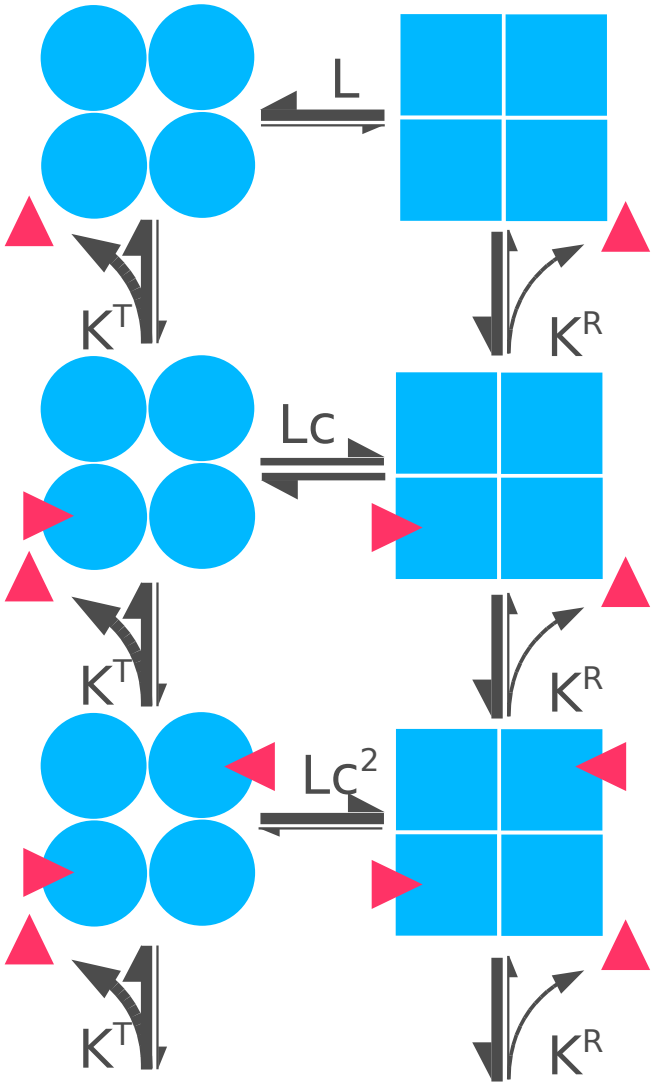


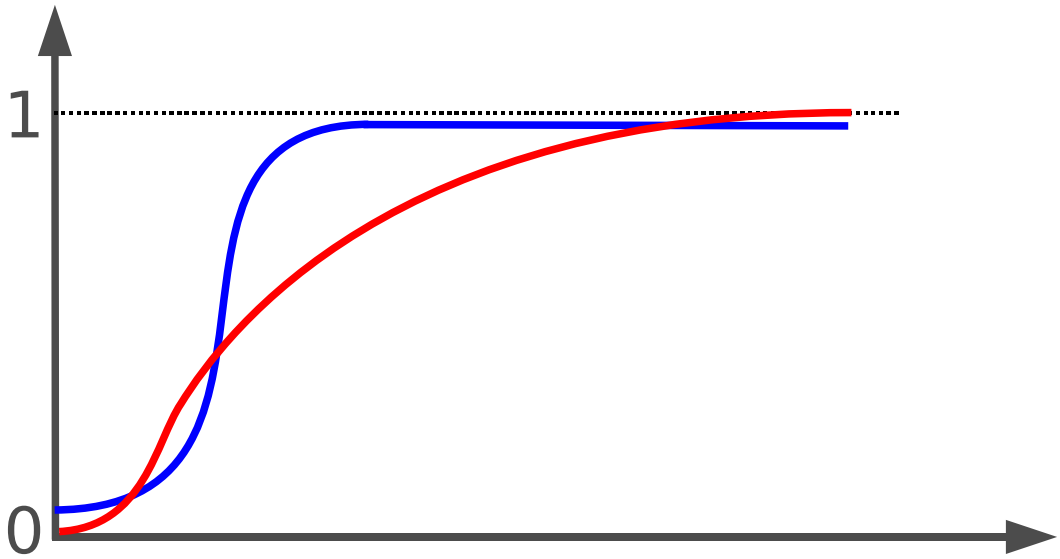


State function

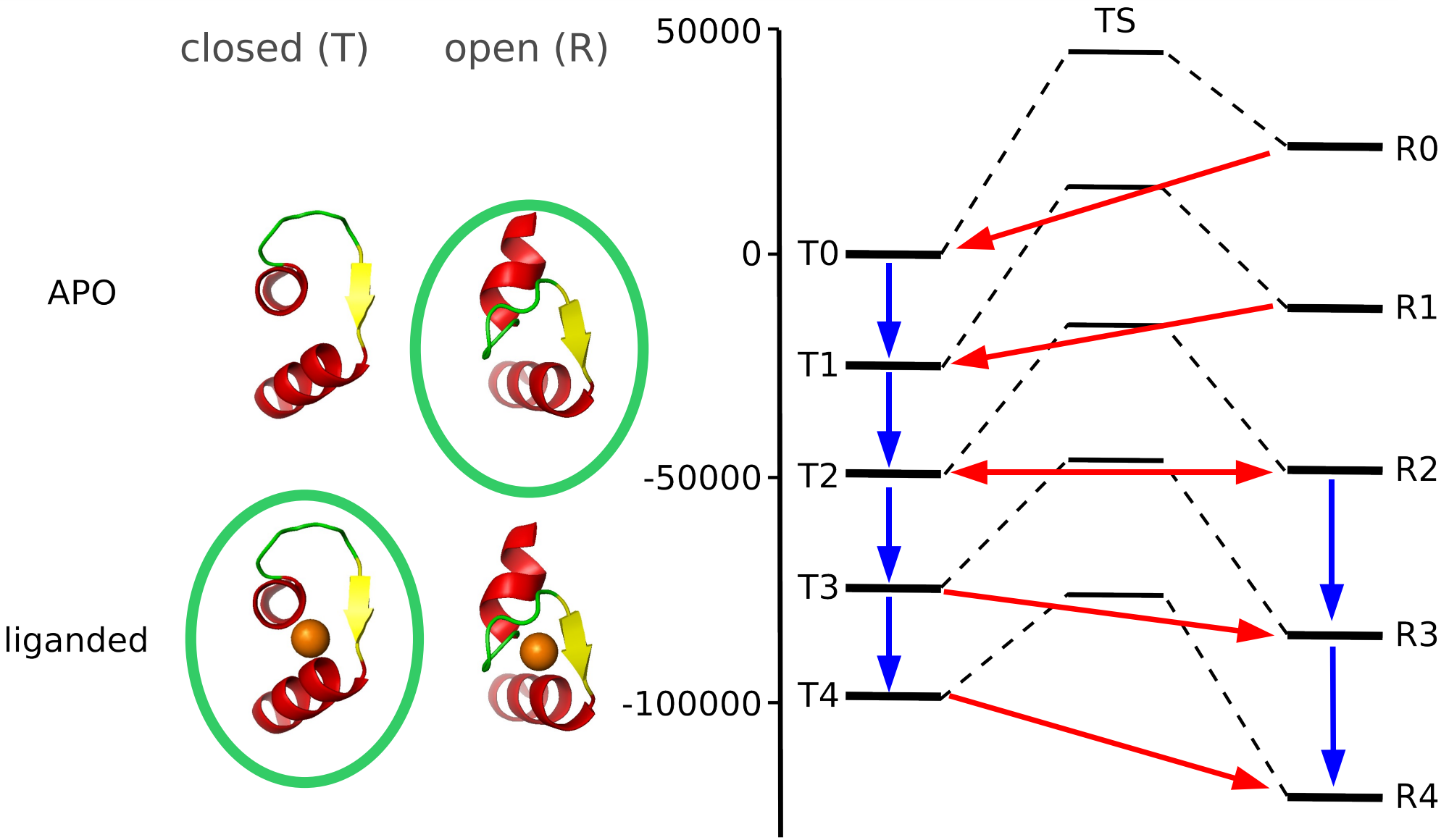


Binding and State functions do not overlap



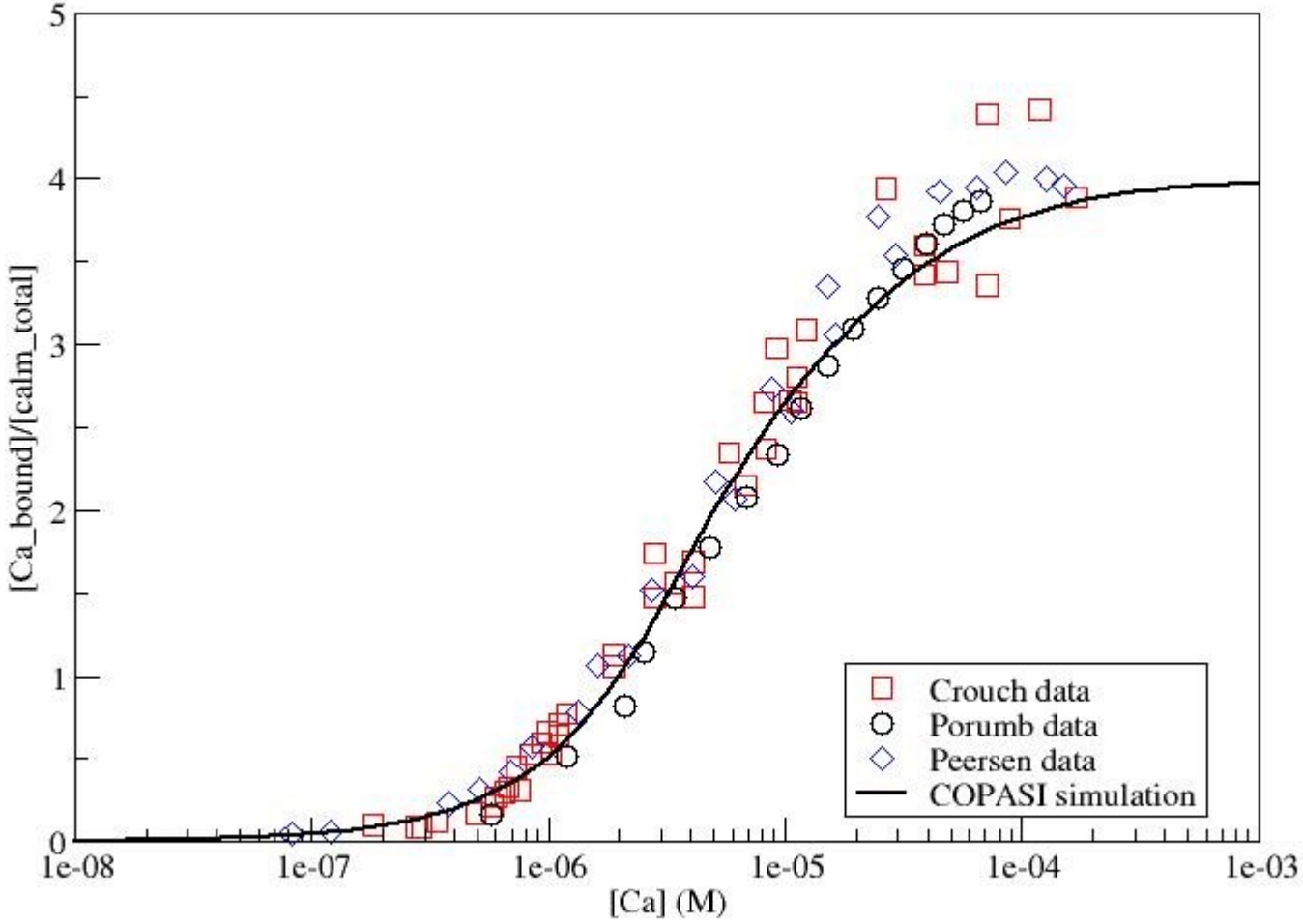


Allosteric model of Calmodulin

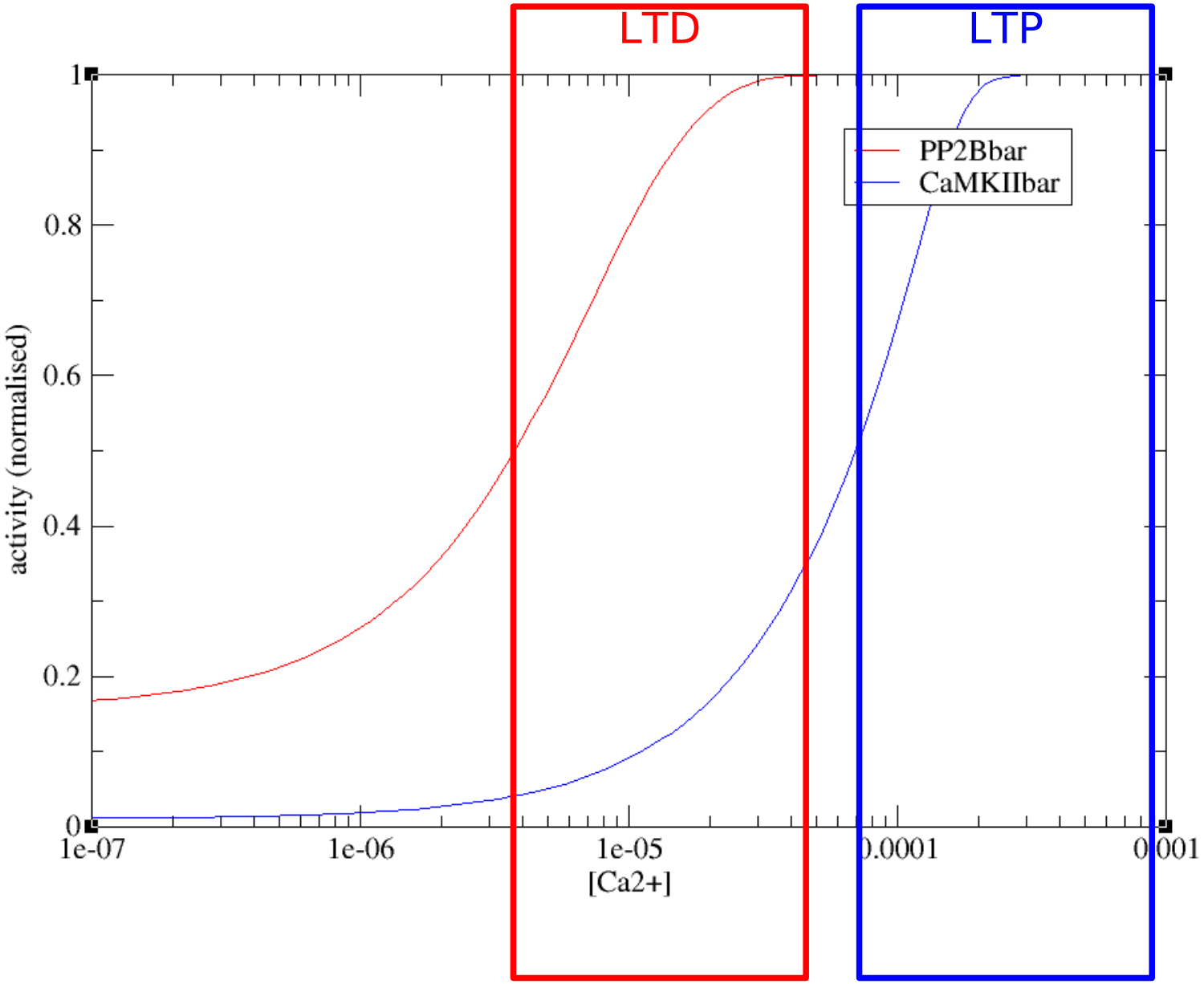


Stefan MI, Edelstein SJ, Le Novère N (2008) An allosteric model of calmodulin explains differential activation of PP2B and CaMKII. *Proc Natl Acad Sci USA*, 105:10768-10773

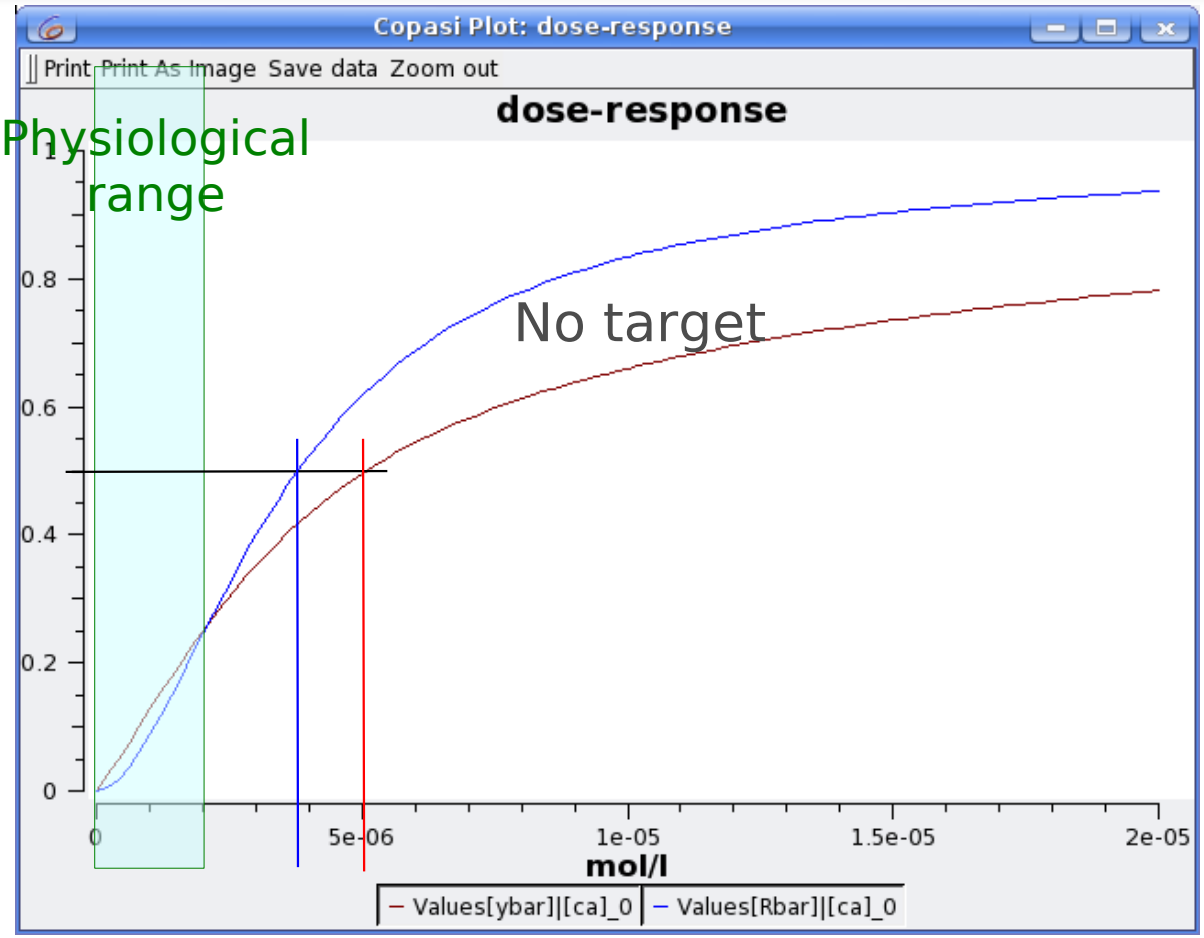
Comparison with experiments



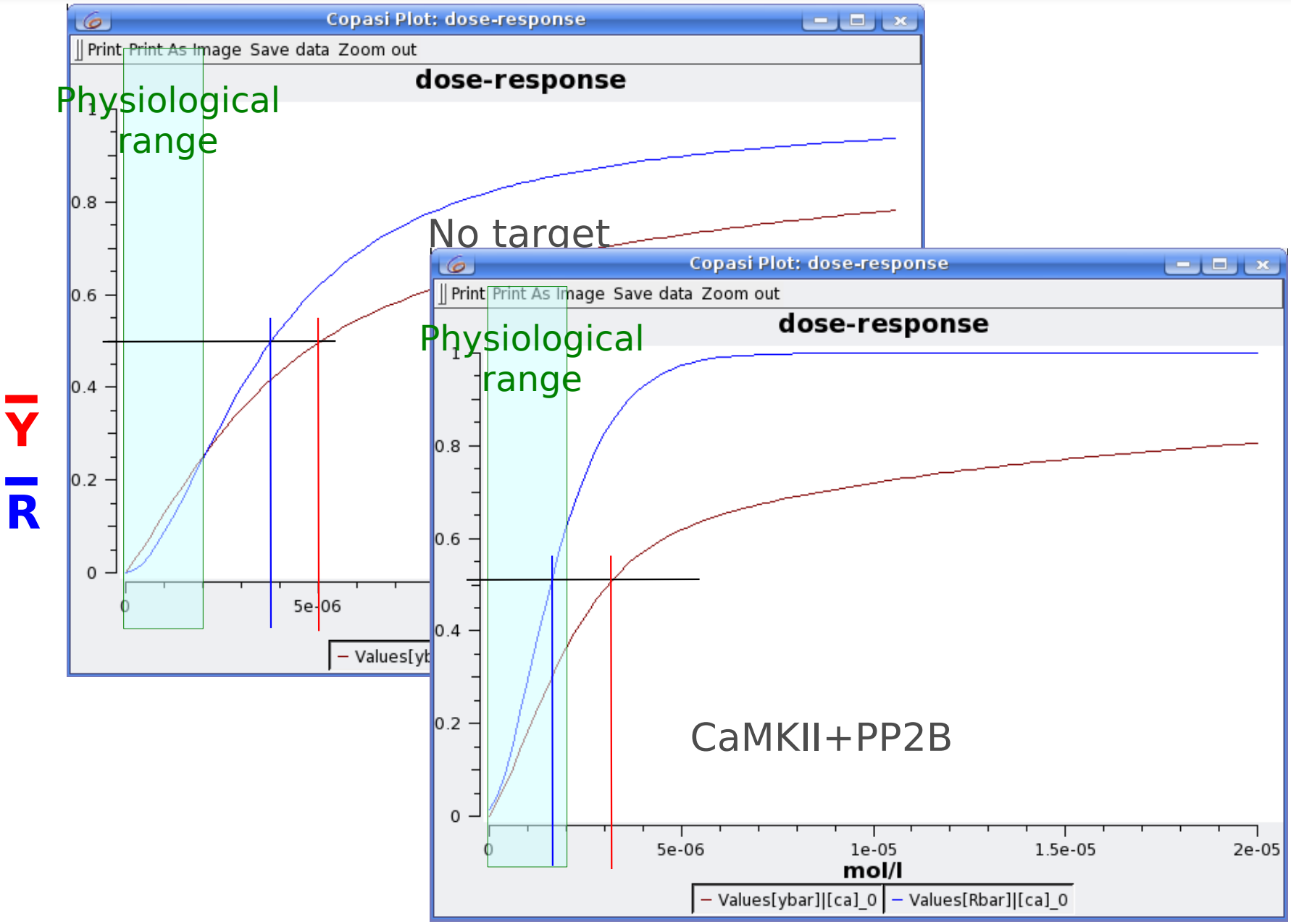
Bidirectional synaptic plasticity



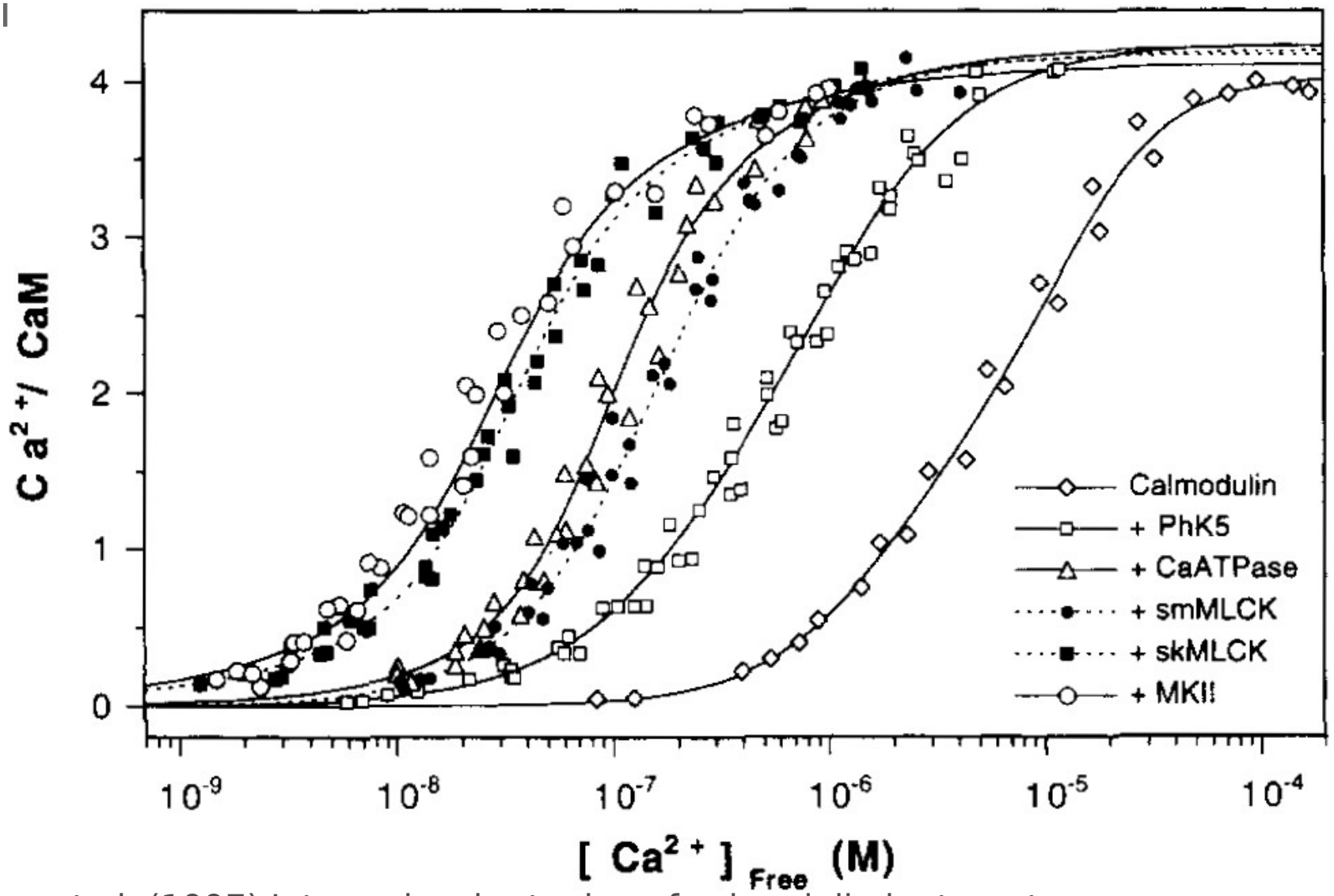
But ... we're out of the physiological range?



This is Systems Biology!

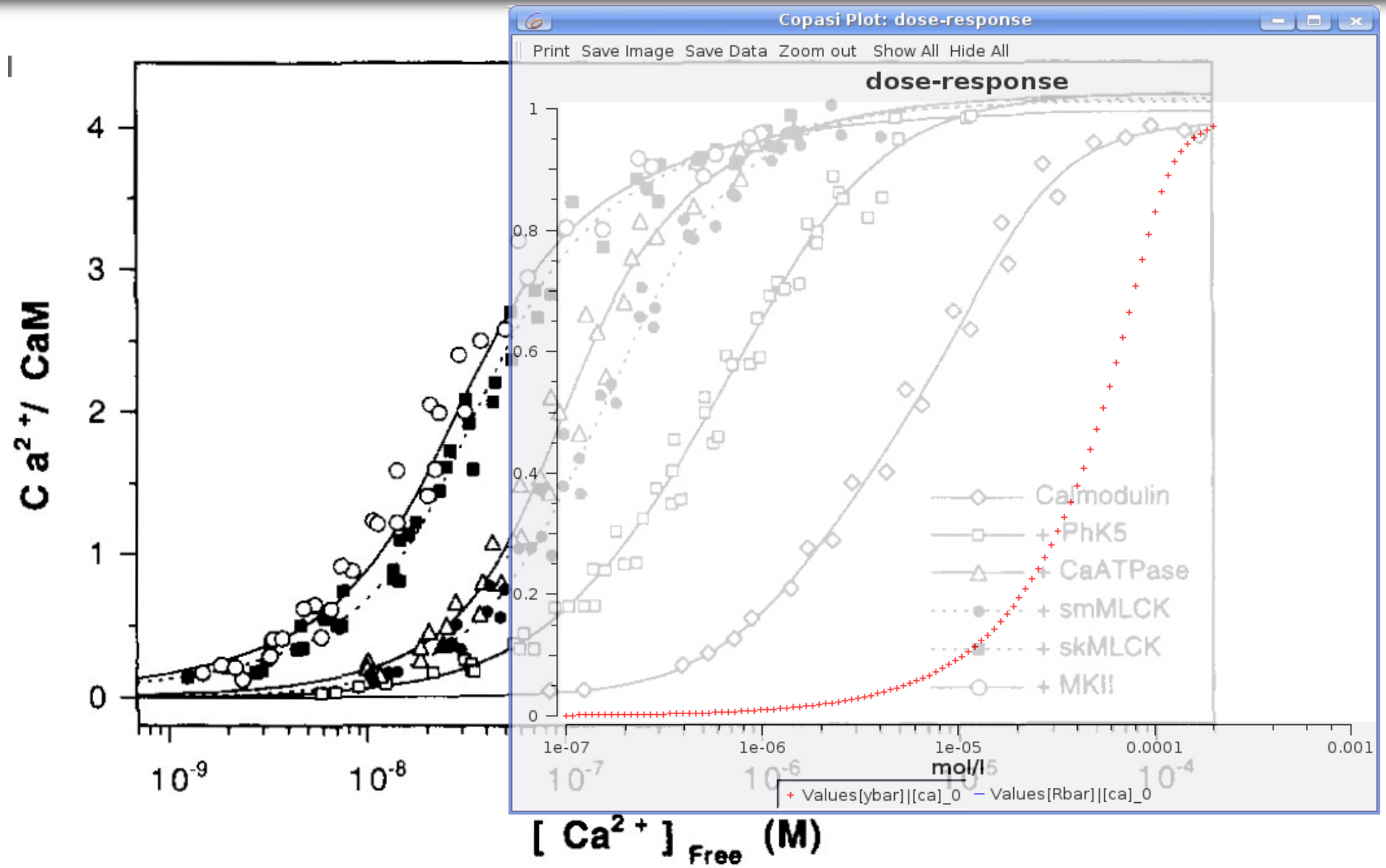


Binding function on 25 μM CaM

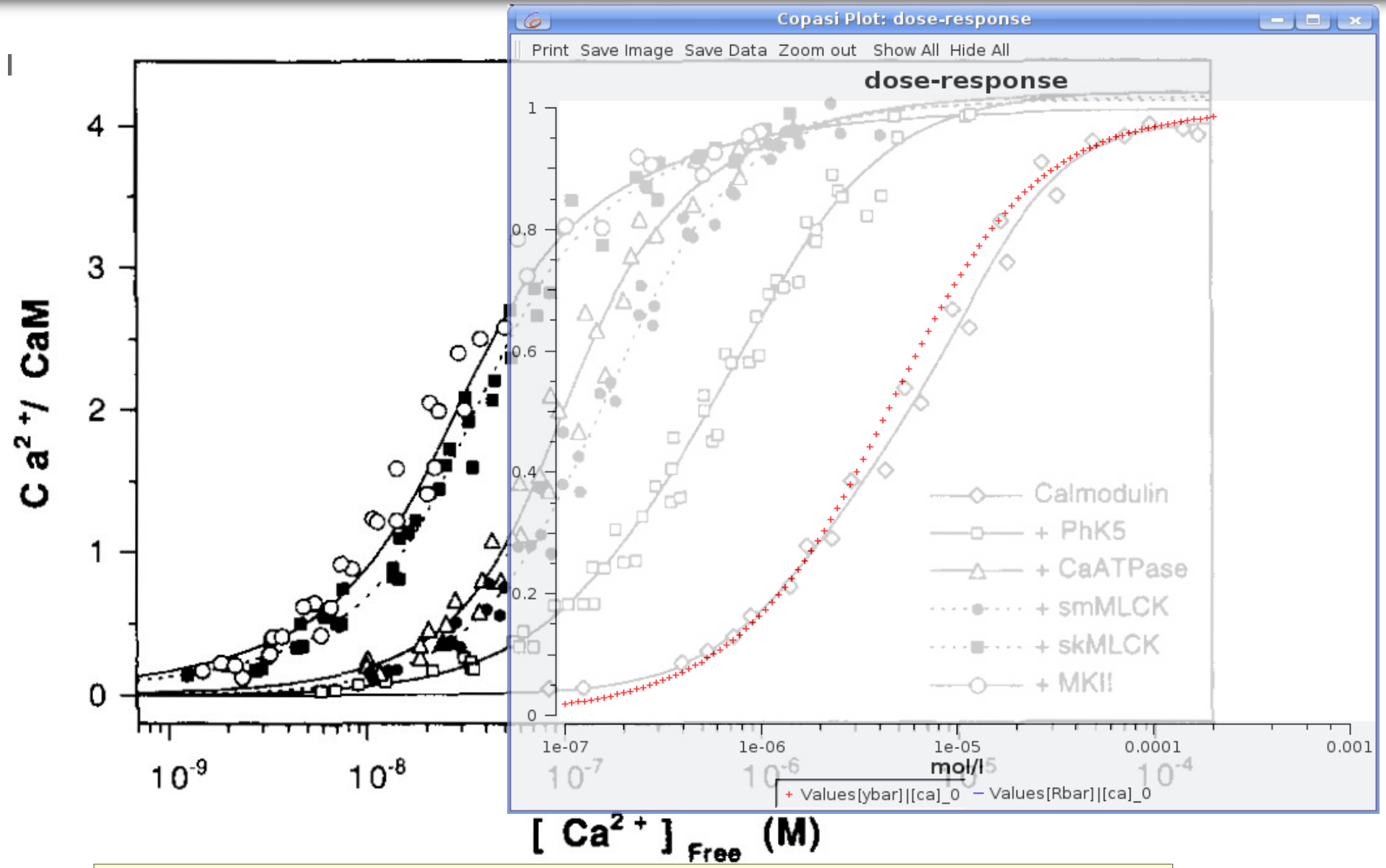


Peersen et al. (1997) Intermolecular tuning of calmodulin by target peptides and proteins: Differential effects on Ca2+ binding and implications for kinase activation *Prot Sci*, 6: 794-807

Parameter-scan on 25 μM CaM

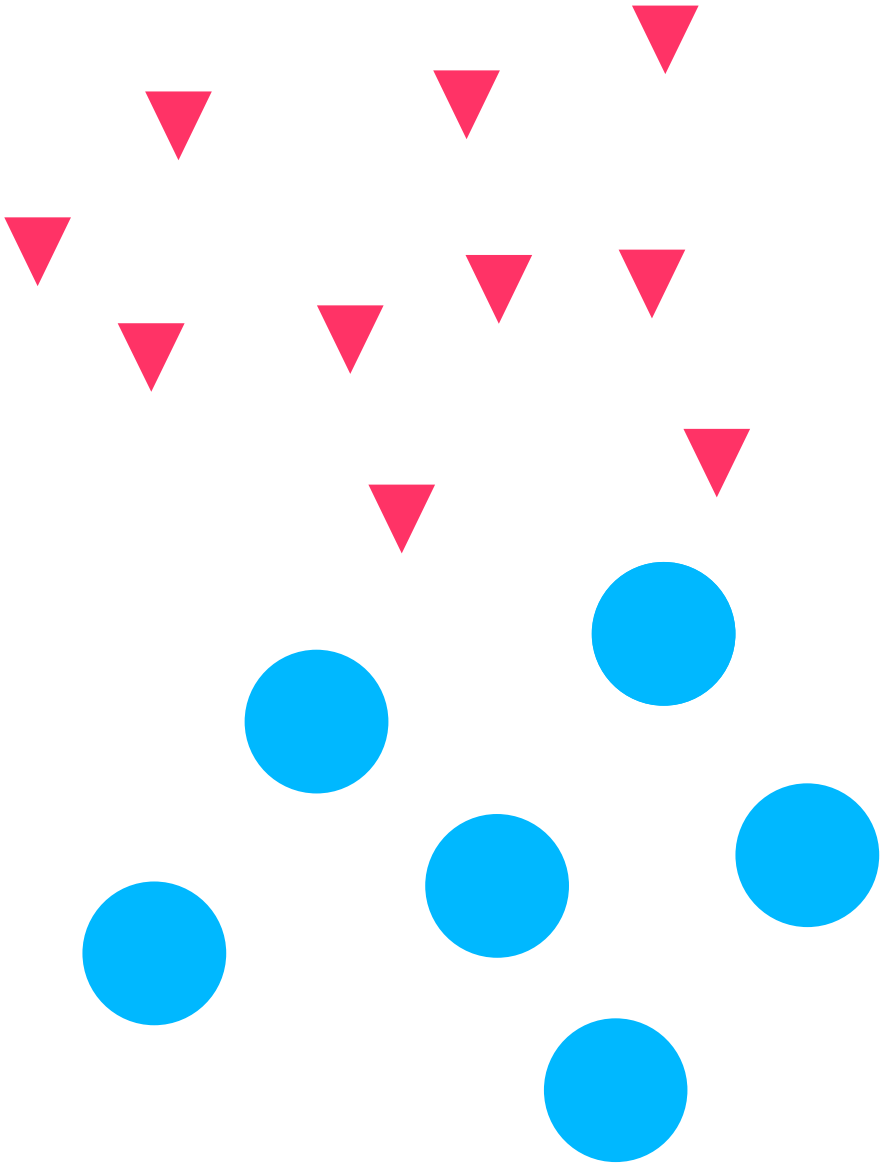


Parameter-scan on 0.1 μM CaM



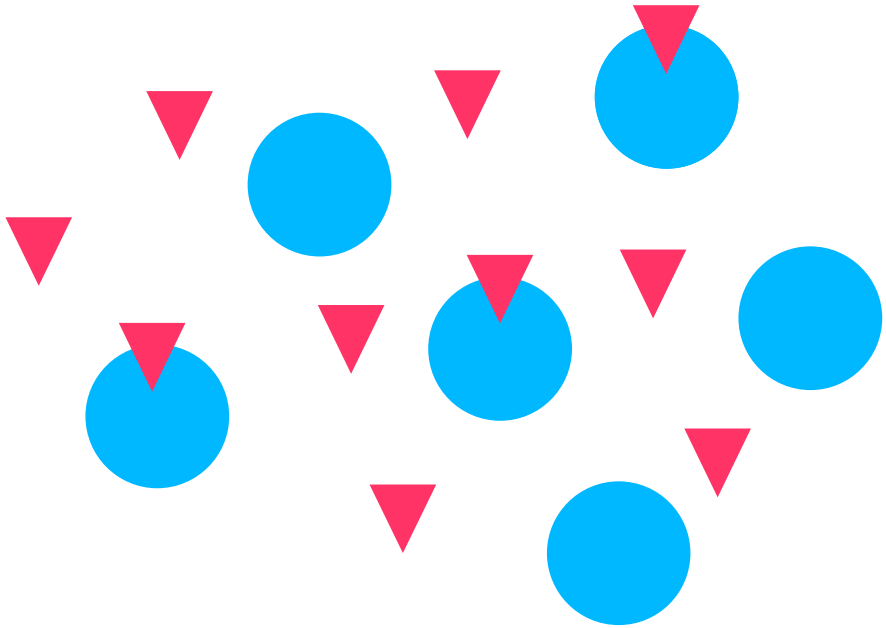
Edelstein SJ, Stefan MI, Le Novère N. Ligand depletion *in vivo* modulates the dynamic range of cooperative signal transduction. *submitted*

What is ligand depletion?

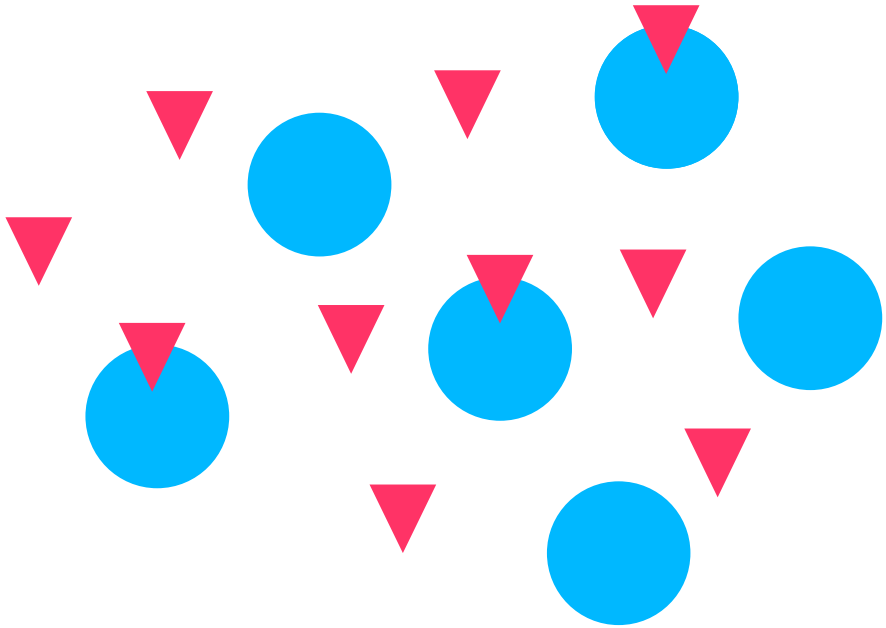


What is ligand depletion?

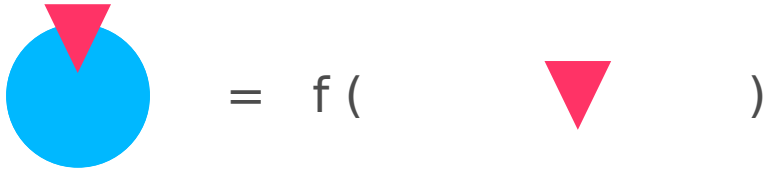
Chemistry (mass-action law)



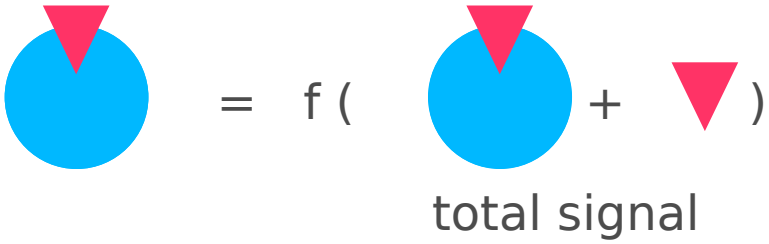
What is ligand depletion?



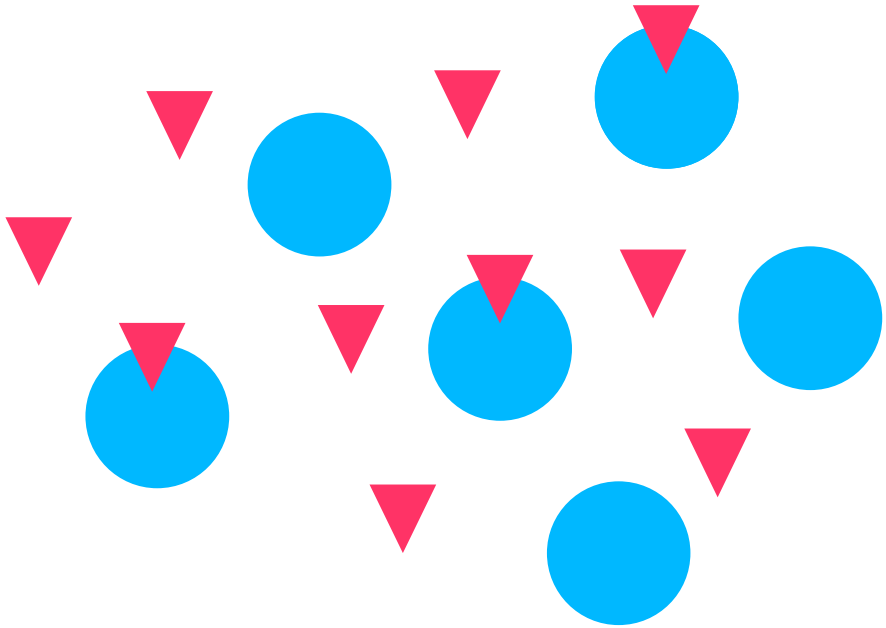
Chemistry (mass-action law)



Cellular signalling



Ligand depletion



Chemistry (mass-action law)

= f (free ligand)

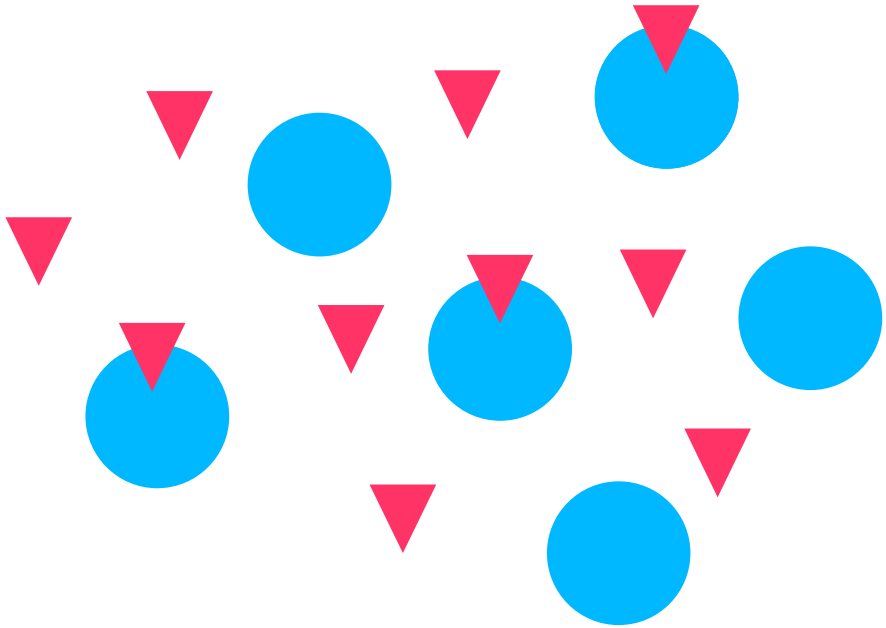
if + << Kd =

Cellular signalling

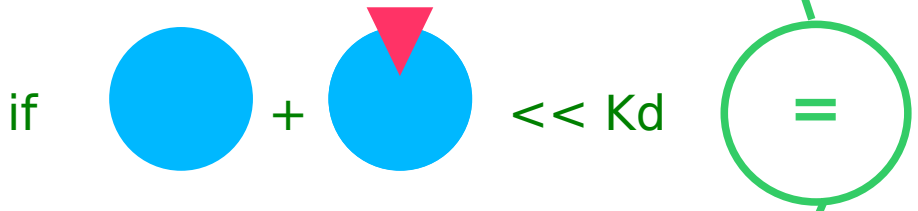
= f (+ total signal)

Ligand depletion

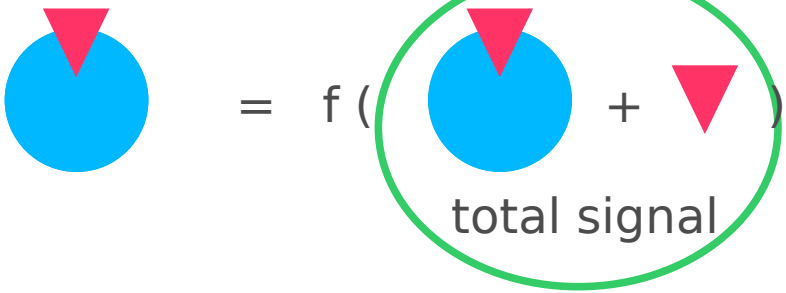
This is generally not the case in signalling: Concentrations of sensor are in micromolar range, as are the dissociation constants



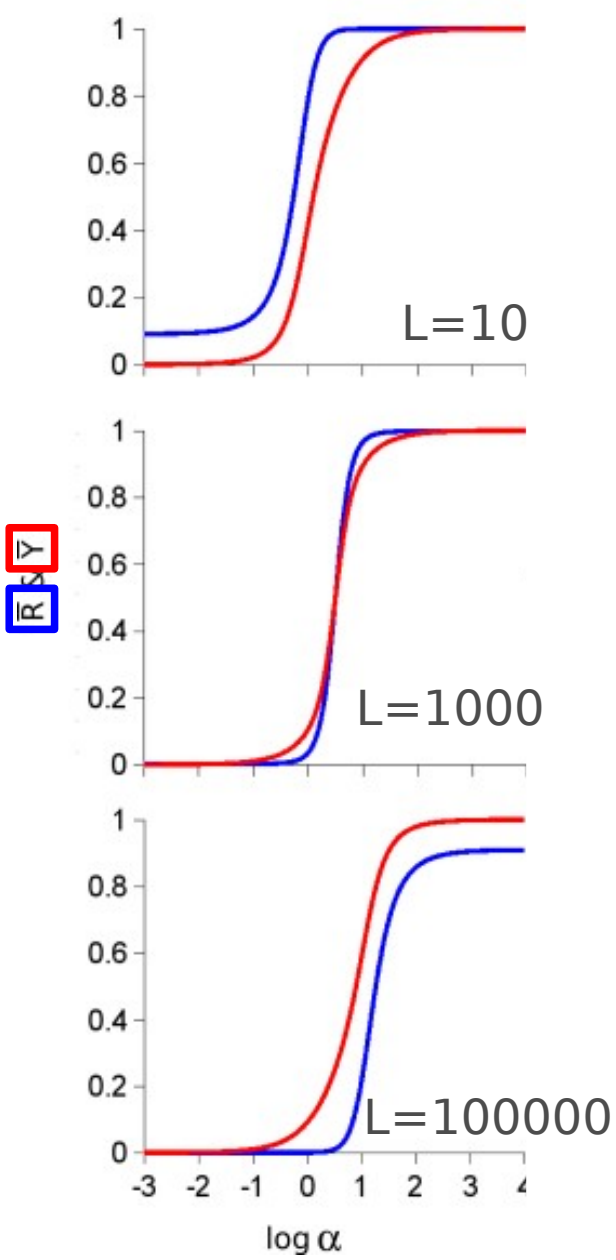
Chemistry (mass-action law)



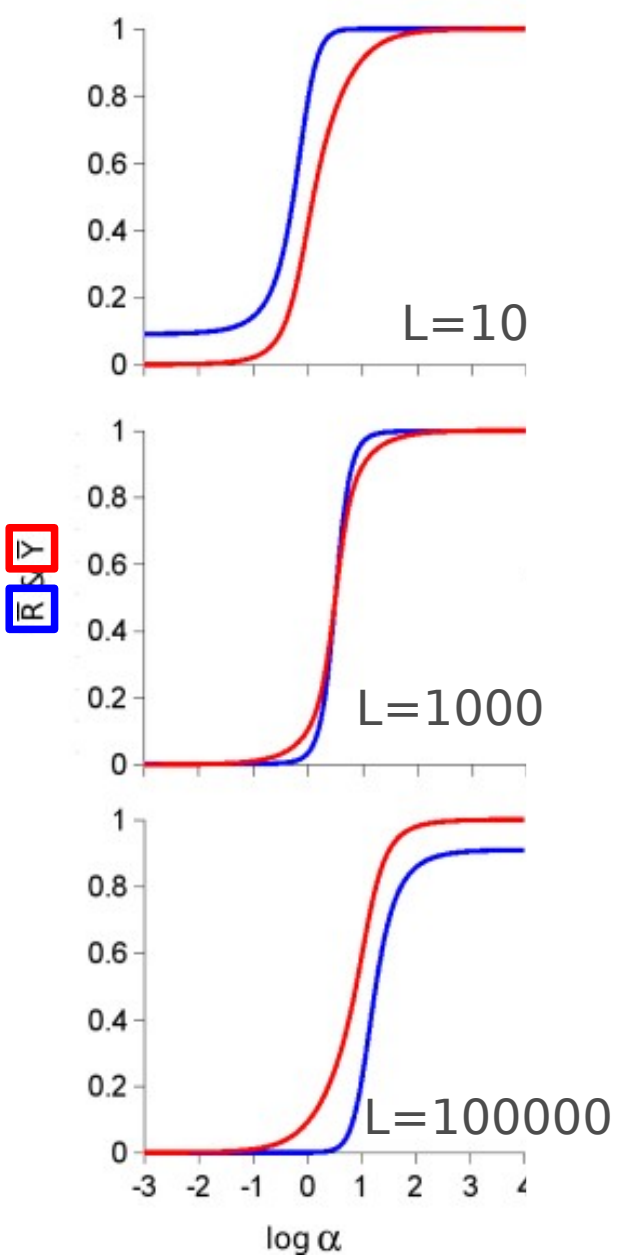
Cellular signalling

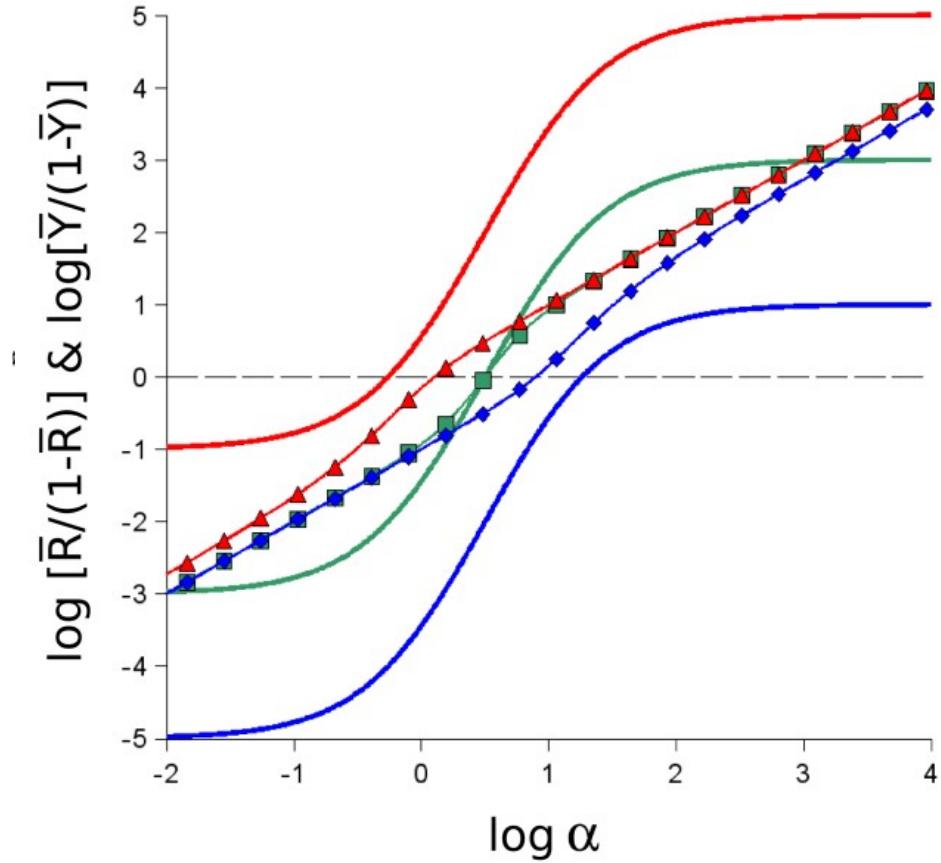


Hill number not suitable for state function



Hill number not suitable for state function





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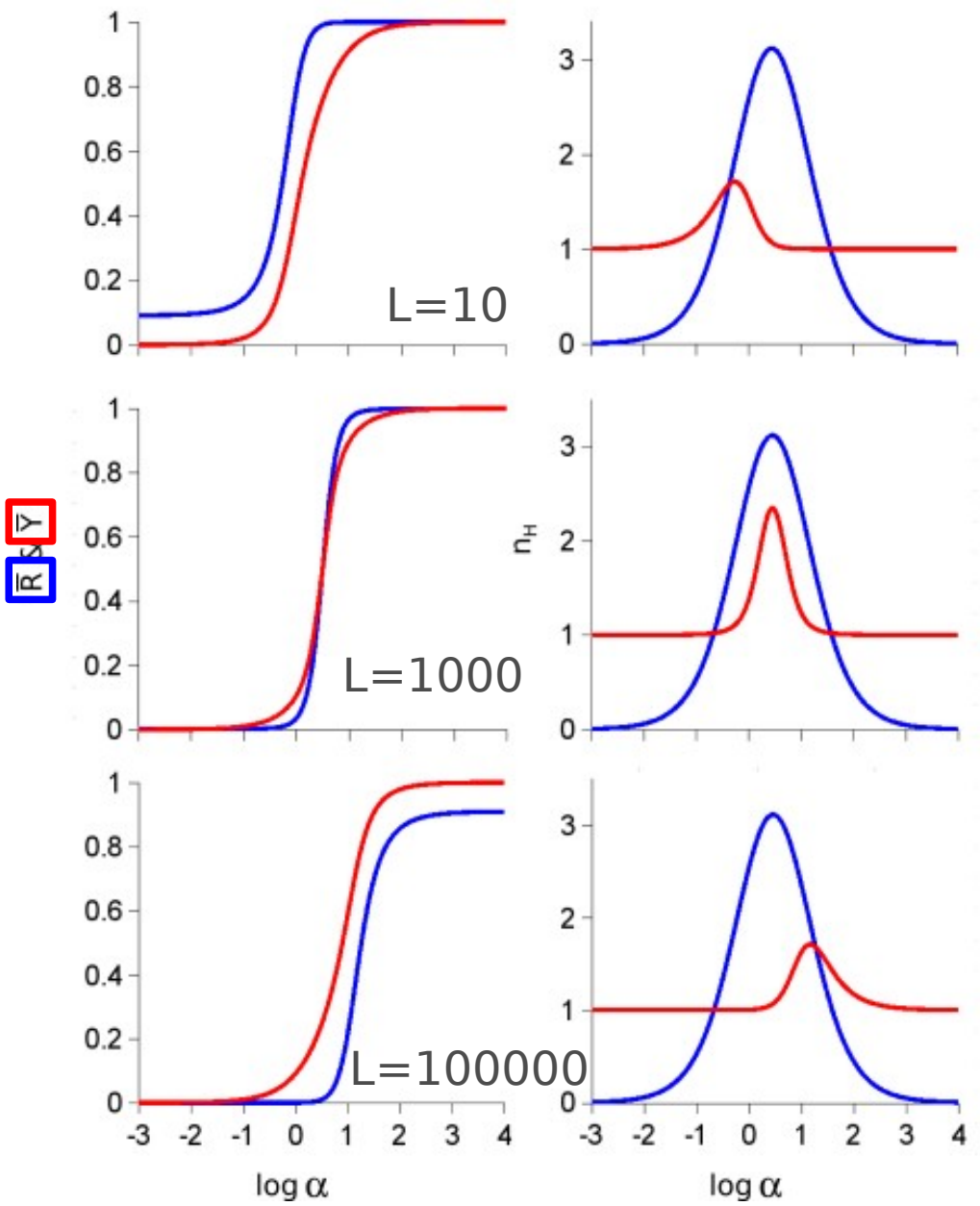
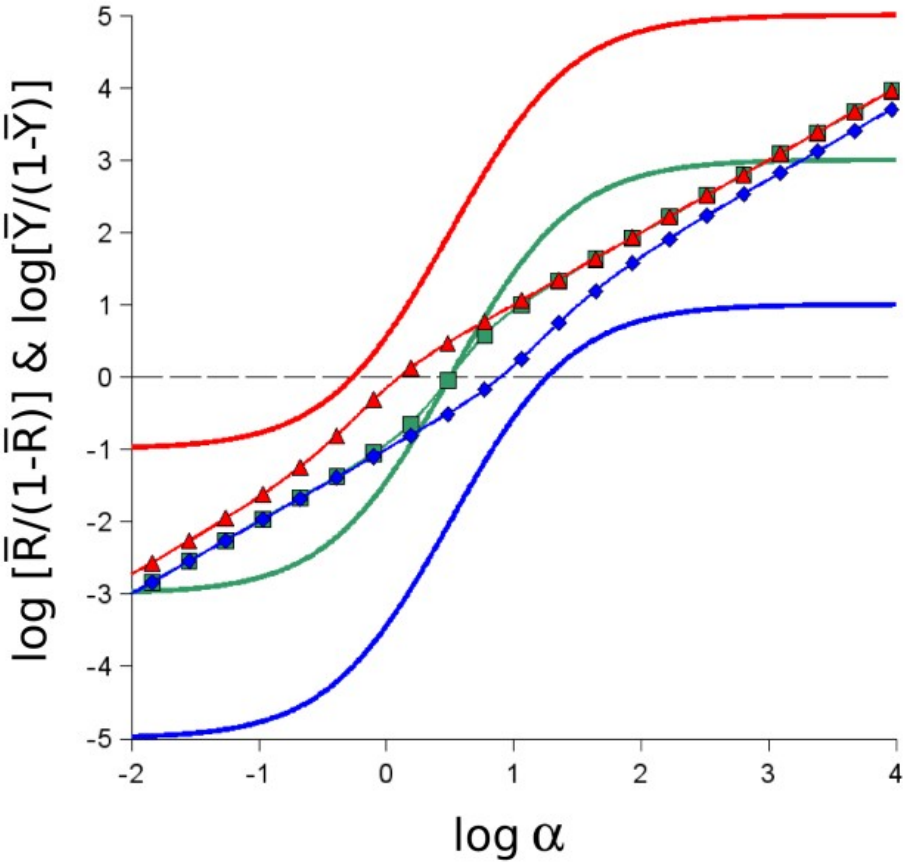
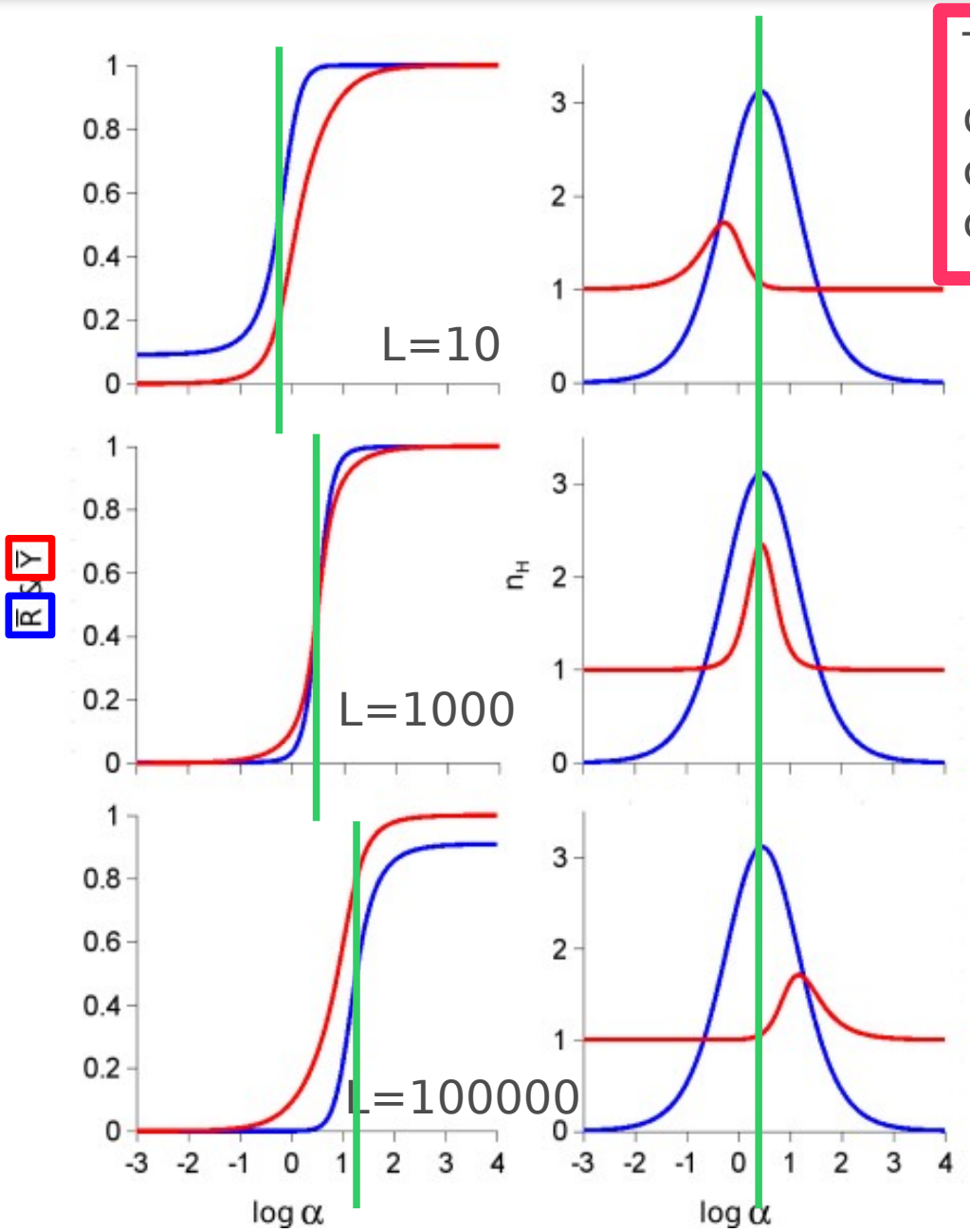
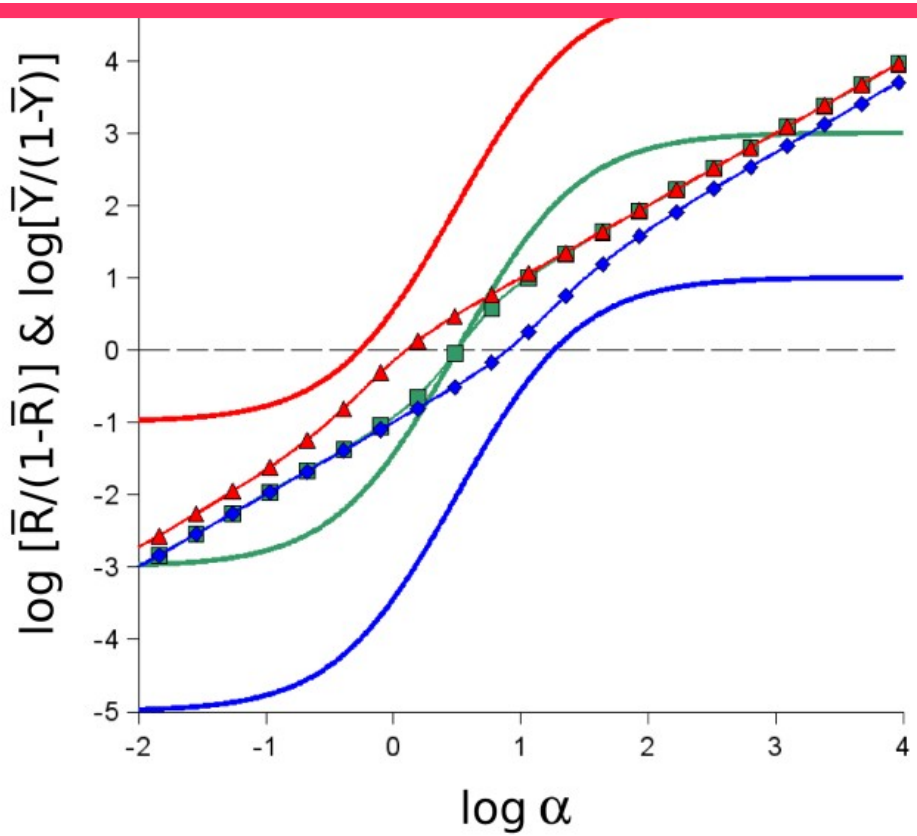




Hill number not suitable for state function

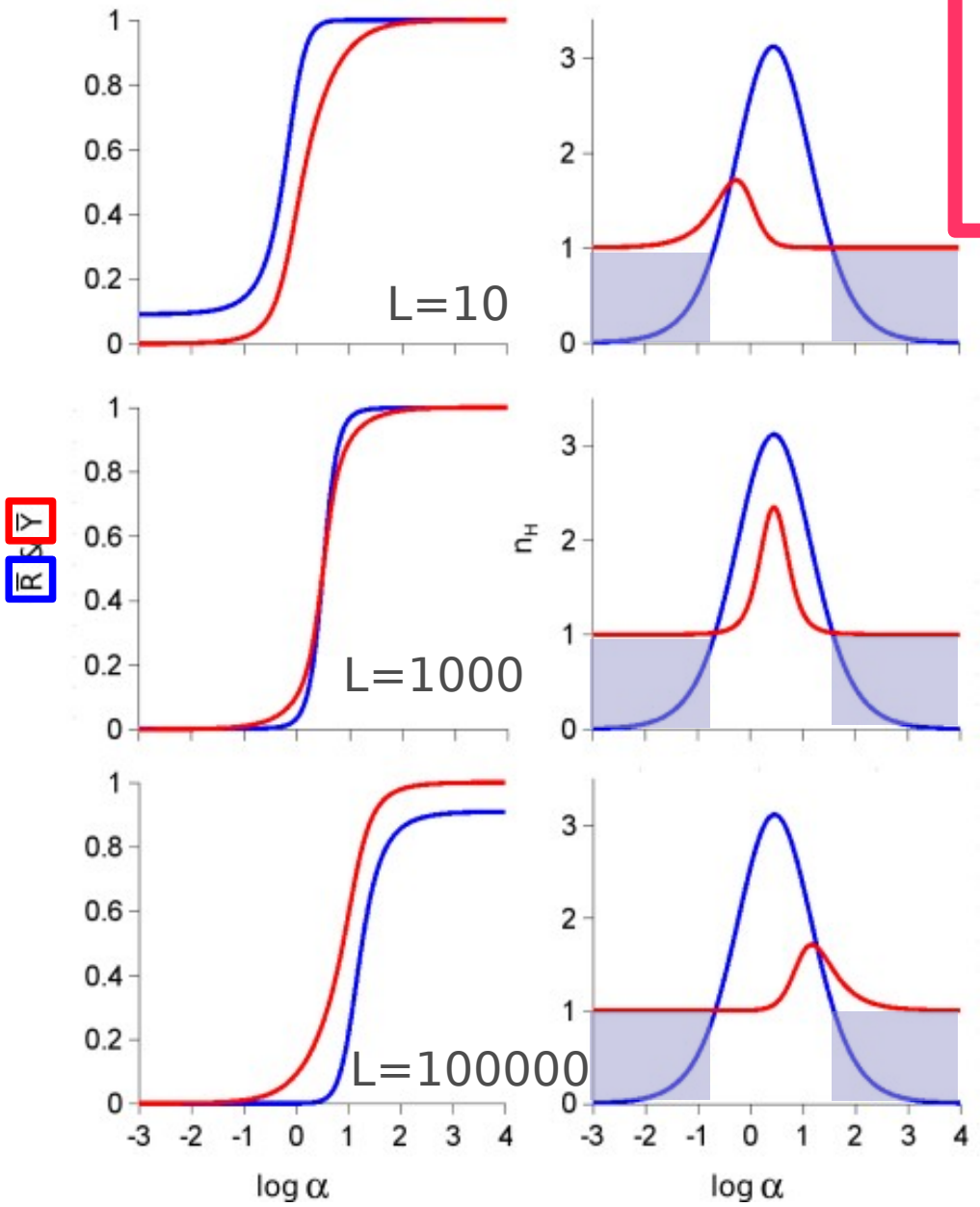
The maximum value of n_H does not depend on the free energy of conformational transition and does correspond to the maximal cooperativity

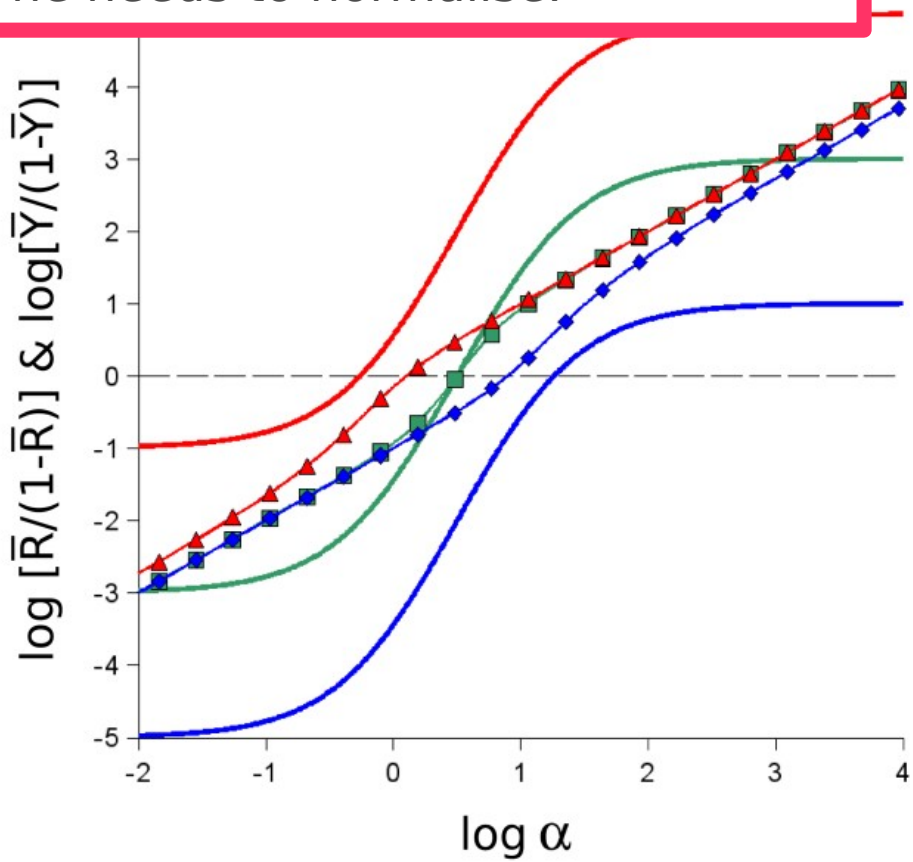




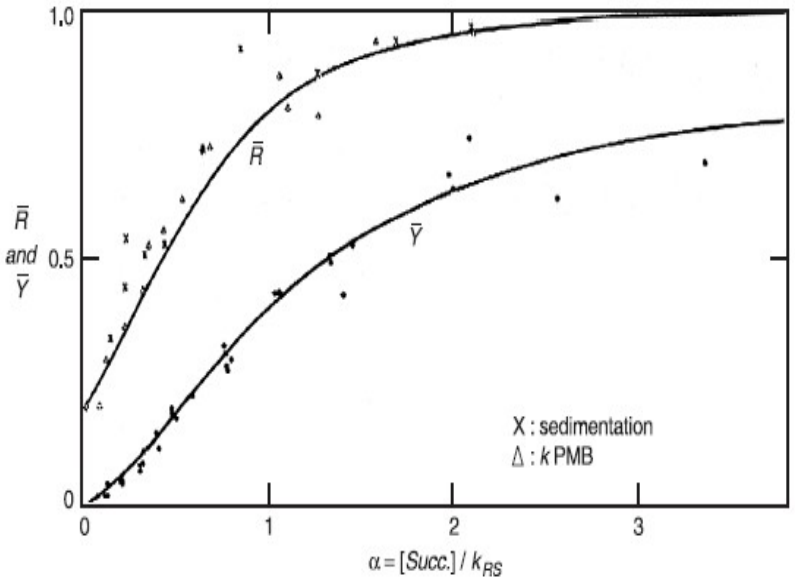
Hill number not suitable for state function

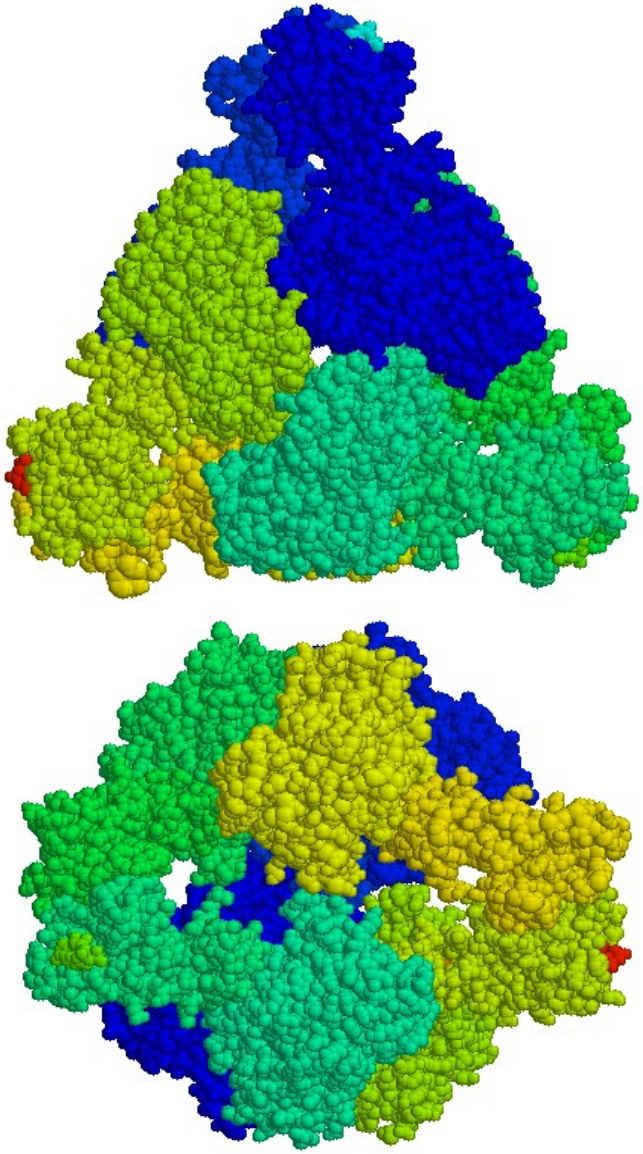
n_H is significantly lower than 1 for many values of α, which is usually associated to negative cooperativity. One needs to normalise.





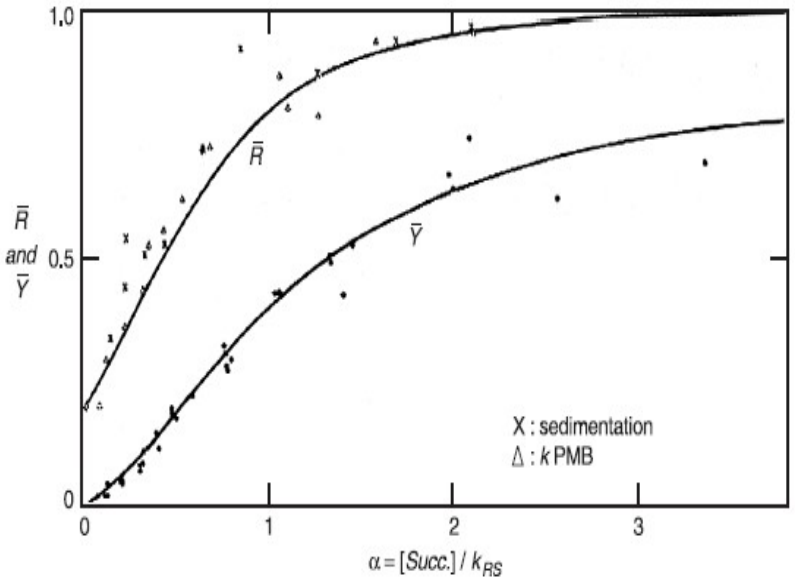
Aspartate transcarbamylase

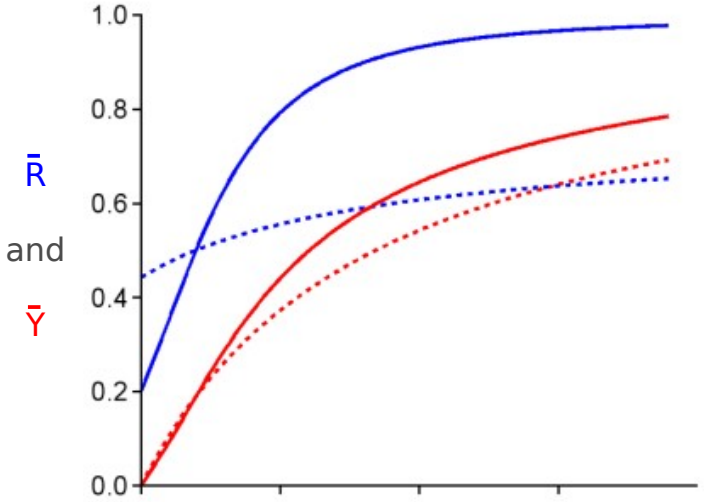




Changeux and Rubin (1968) Allosteric Interactions in Aspartate Transcarbamylase. III. Interpretation of Experimental Data in Terms of the Model of Monod, Wyman, and Changeux. *J Biol Chem* 63: 529

Equivalent non-cooperative monomer

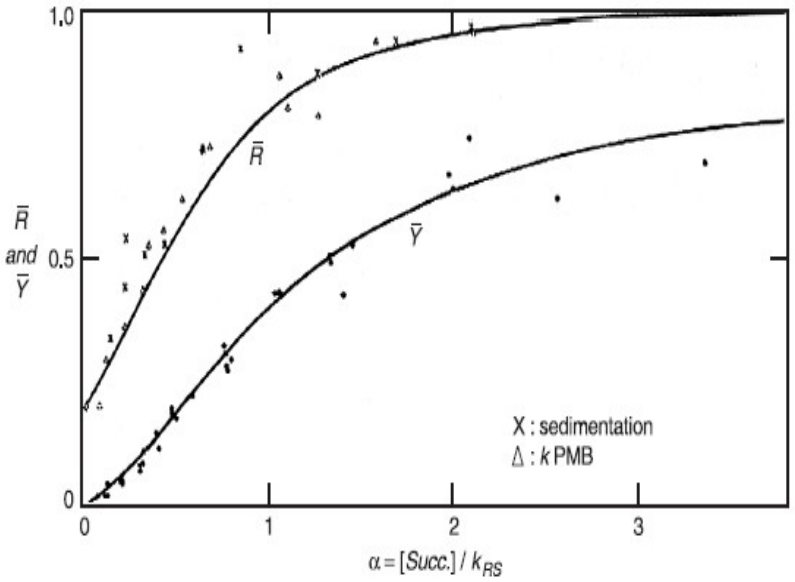


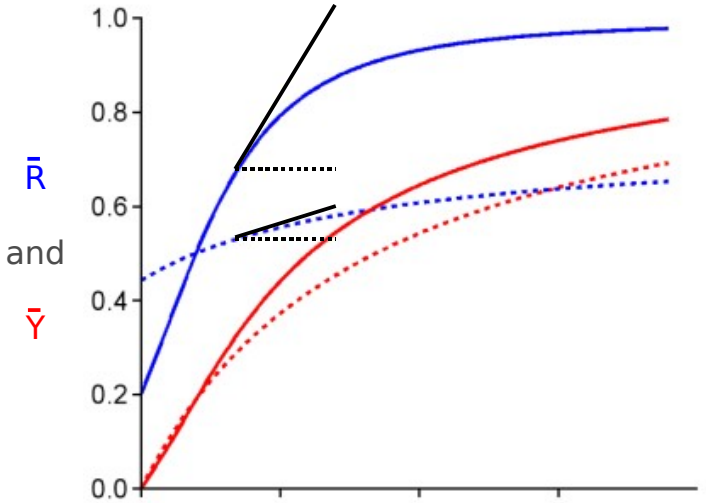


We assume that the free energy of conformational change spread over all the subunits (symmetrical protein)

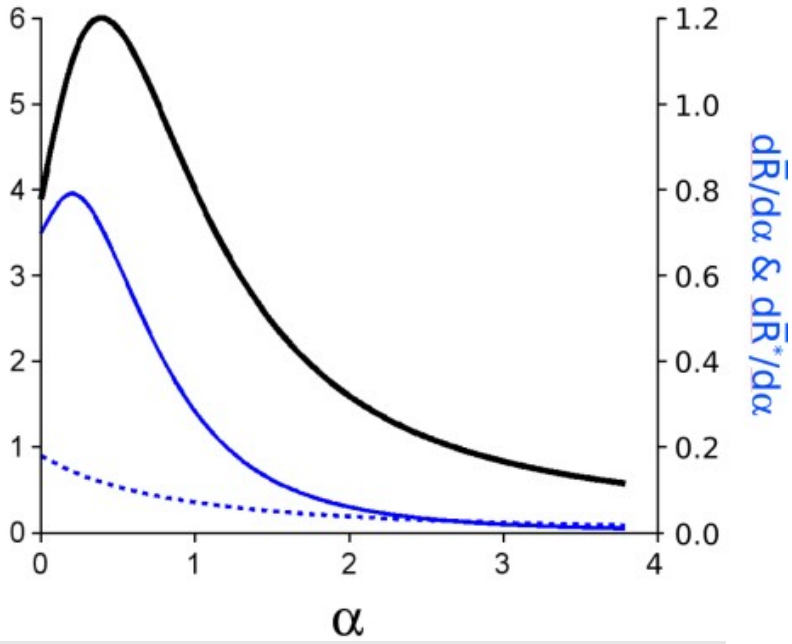
$$\lambda = \sqrt[N]{L}$$

New index of cooperativity

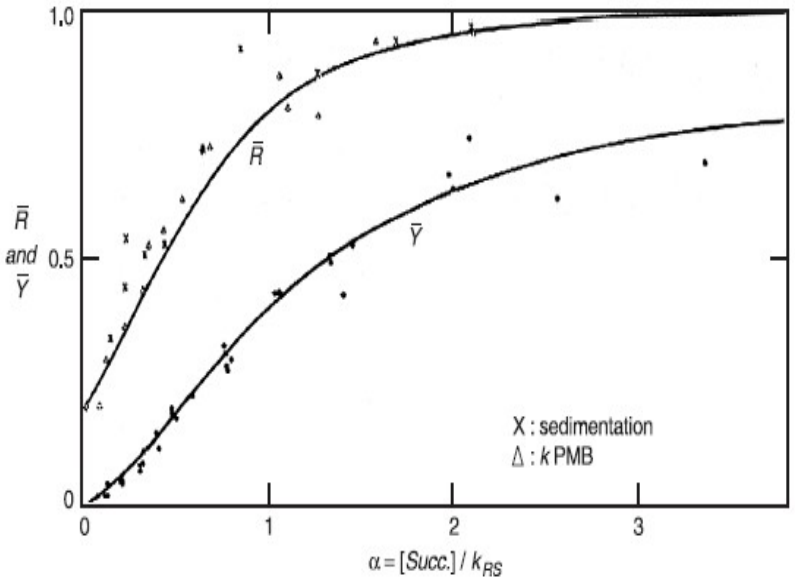


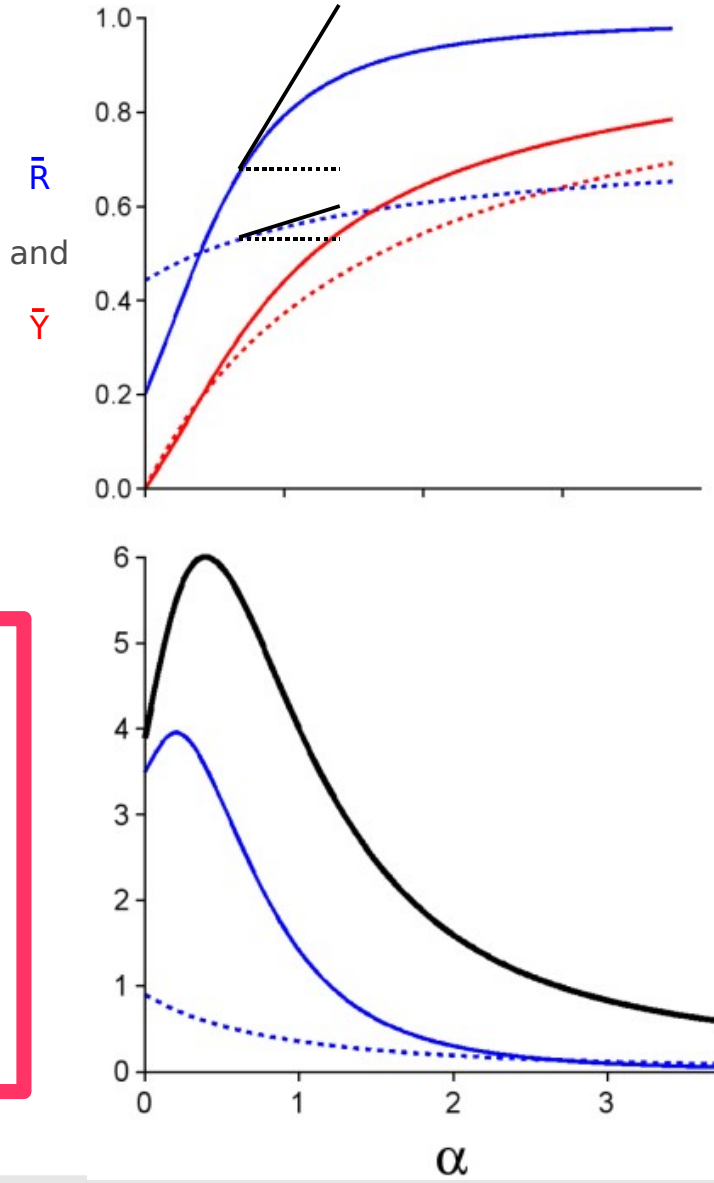


$$\nu = \frac{d\bar{R}/d\alpha}{d\bar{R}^*/d\alpha}$$



Aspartate transcarbamylase





If all sites identical, the cooperativity is maximum when $\bar{R} = \bar{R}^* = 1/2$ with a value of $\nu = N$ that is the **number of binding sites**.

Effect of ligand depletion on calmodulin response

[CaM]= 13.8 10^{-6} M

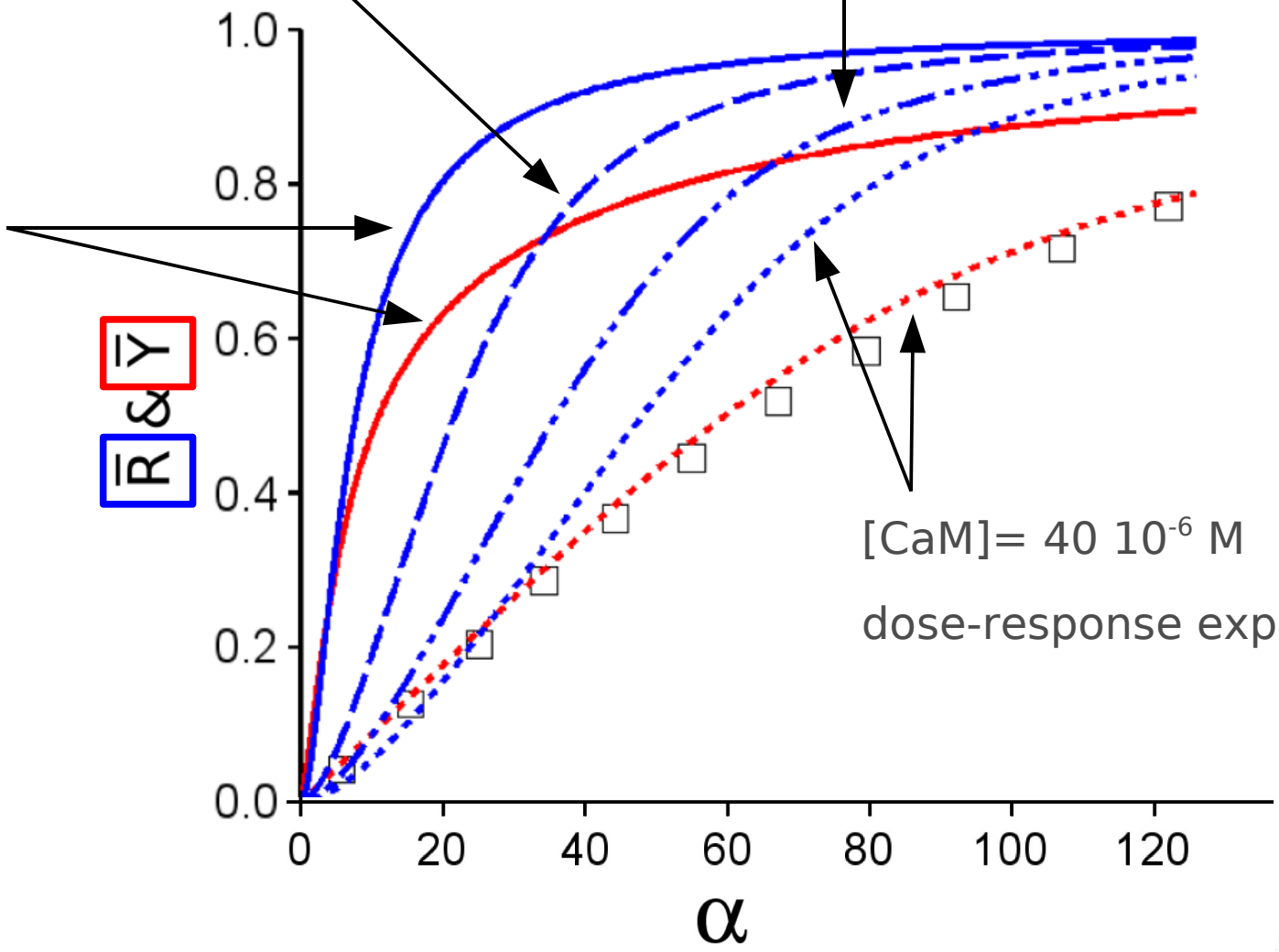
bovine hypothalamus

[CaM]= 28 10^{-6} M

bovine caudate

[CaM]= 10^{-7} M

no ligand depletion



[CaM]= 40 10^{-6} M

dose-response exp

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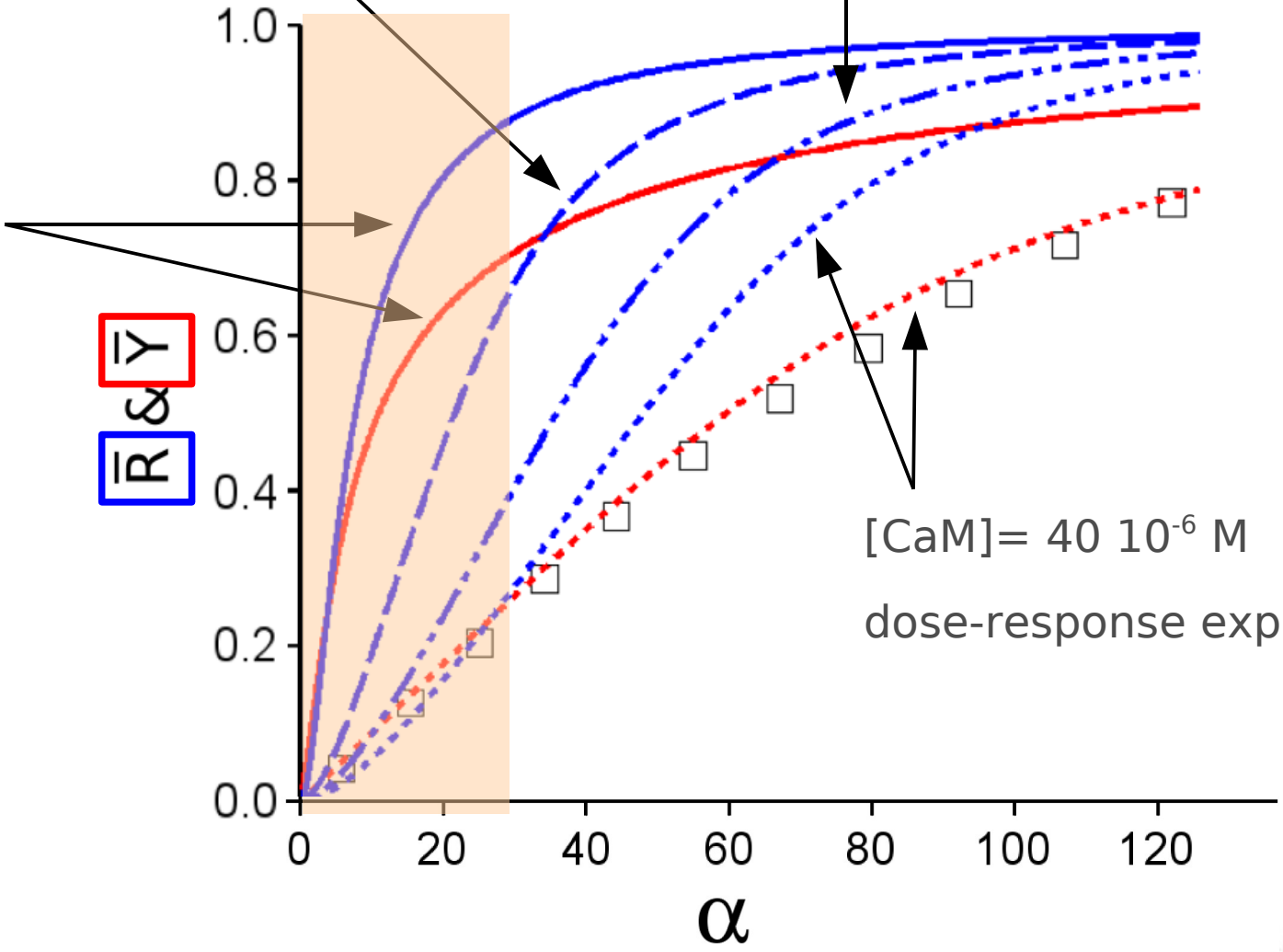
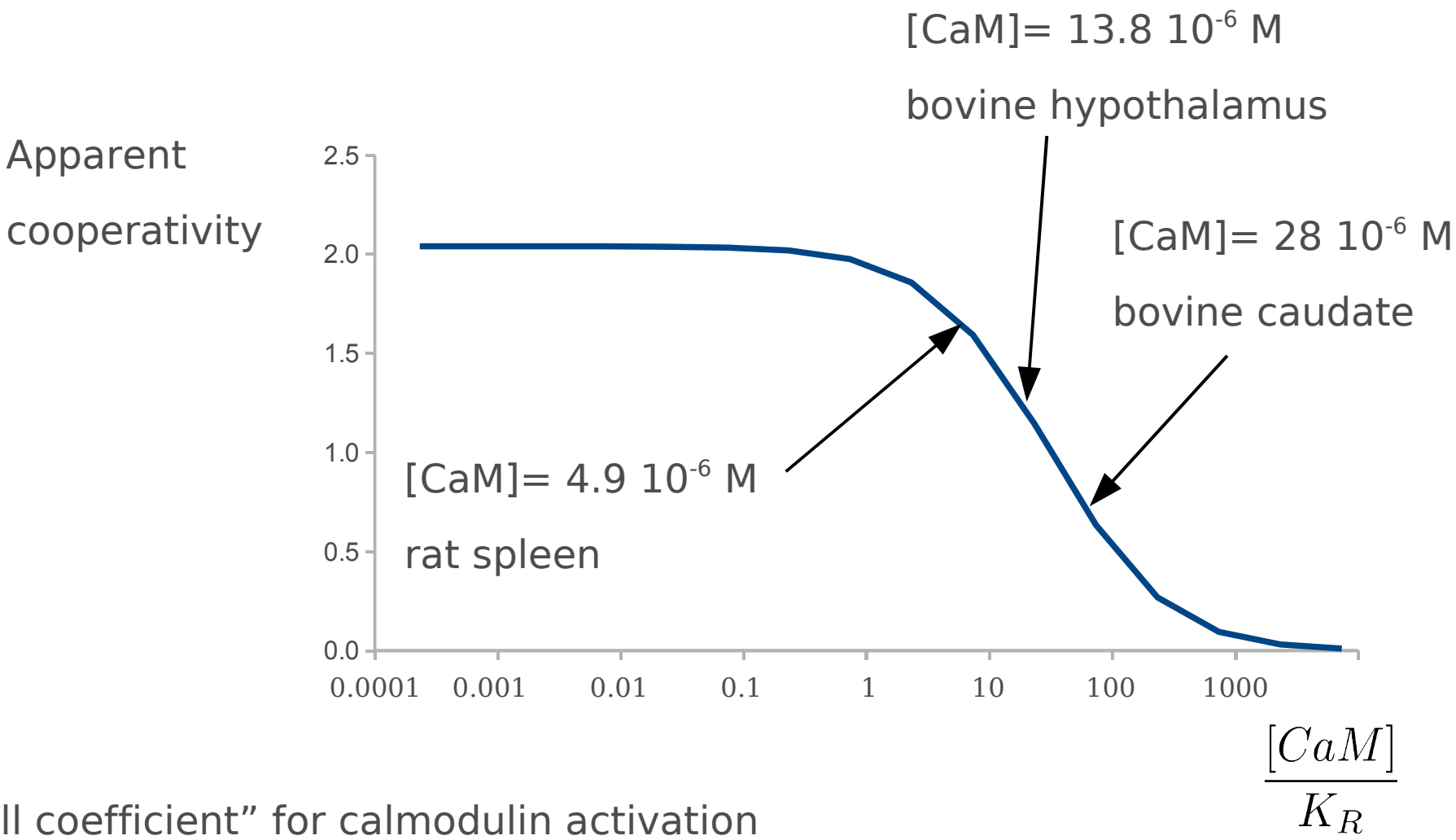


[CaM]= 40 10^{-6} M

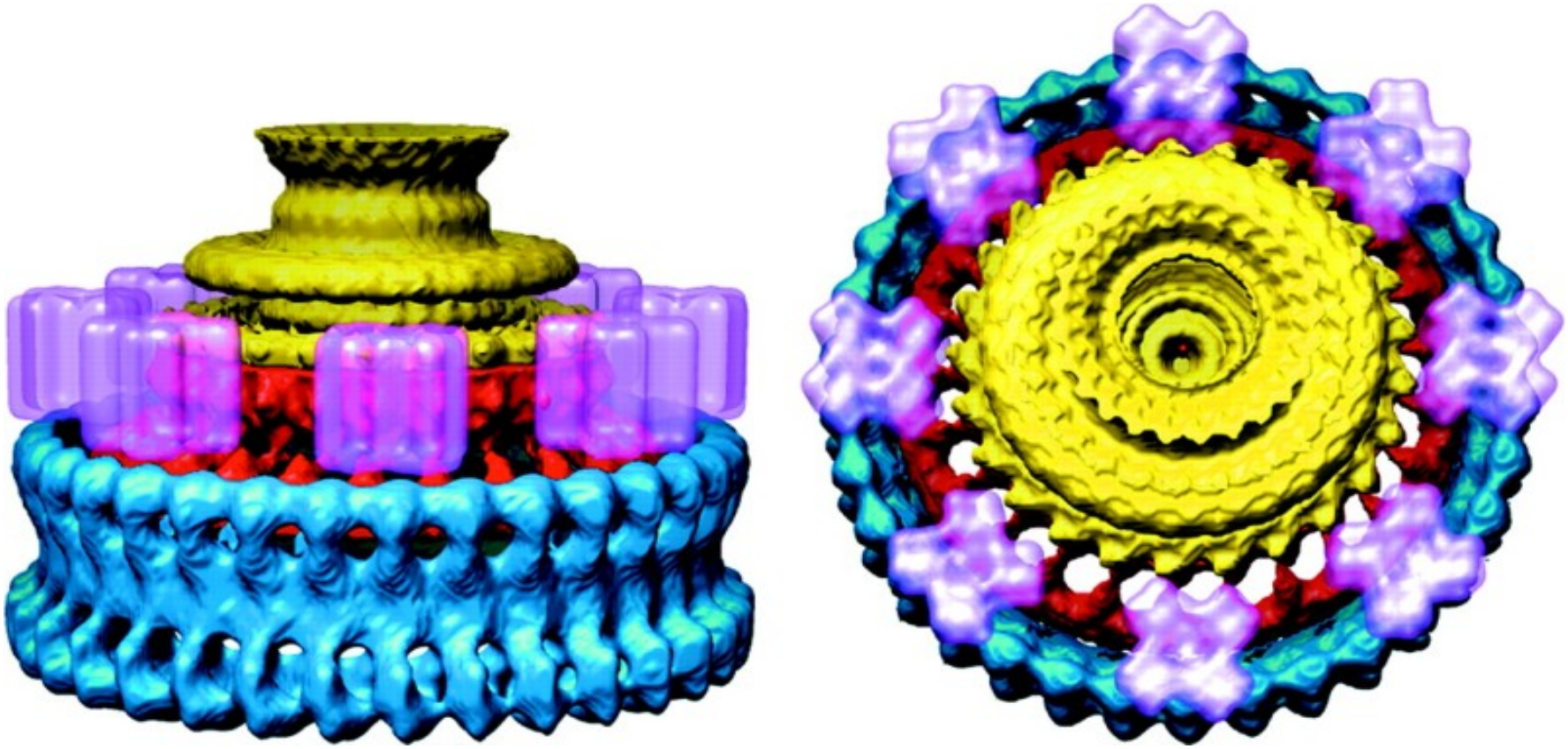
dose-response exp

Effect of ligand-depletion on cooperativity

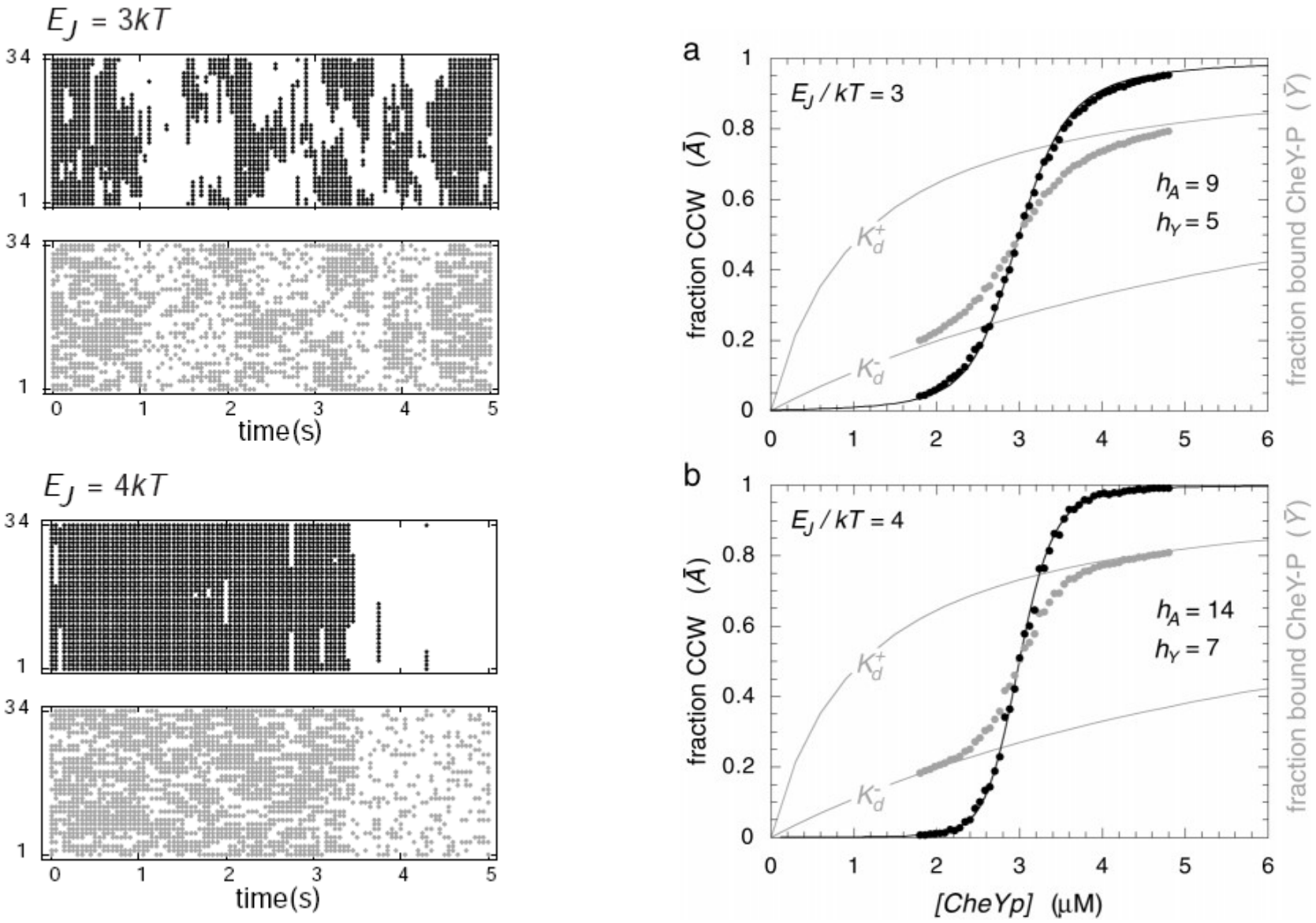


"Hill coefficient" for calmodulin activation ranges between 1 and 1.8 ...

Bacterial flagellar motor

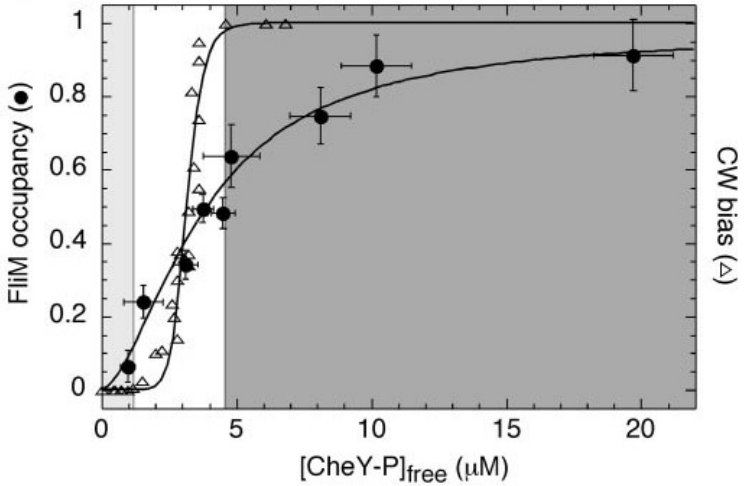


Concerted behaviour of bacterial flagellar



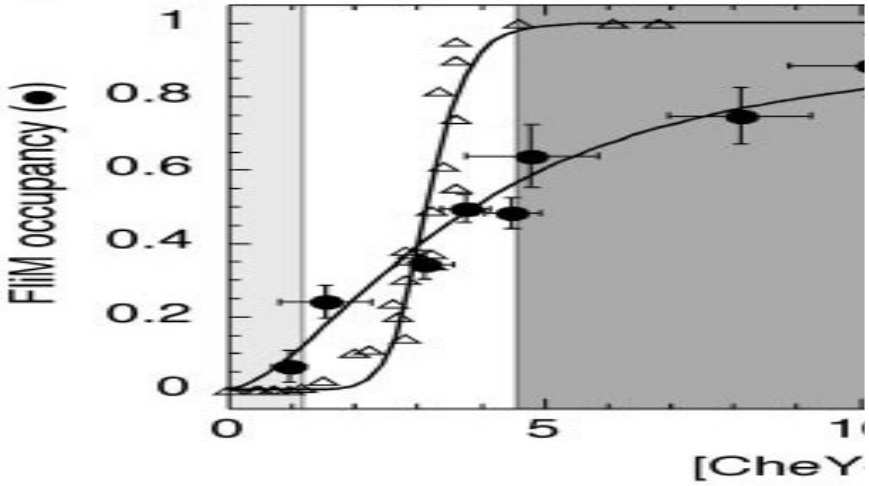
Duke, Le Novère and Bray (2001) Conformational spread in a ring of proteins: a stochastic approach to allostery. *J Mol Biol*, 308:541-553.

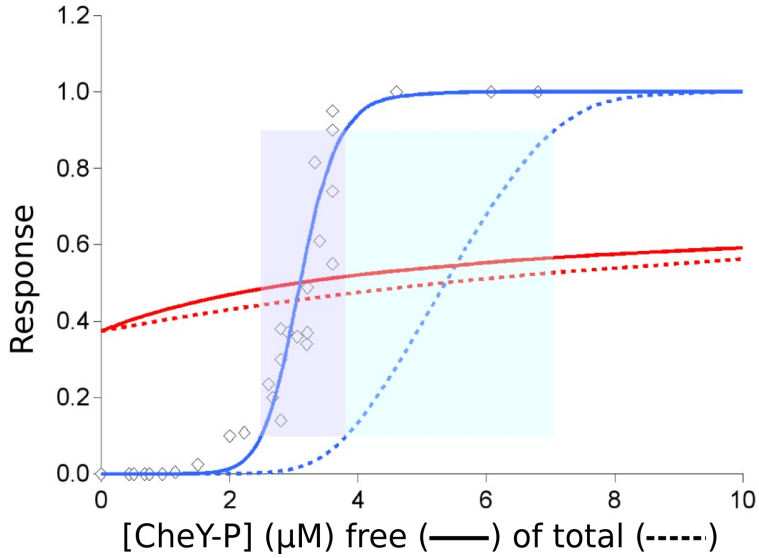
Effect of ligand depletion on motor response



Sourjick and Berg (2002) Binding of the Escherichia coli response regulator CheY to its target measured in vivo by fluorescence resonance energy transfer. *PNAS* 99: 12669-12674

Effect of ligand depletion on motor response





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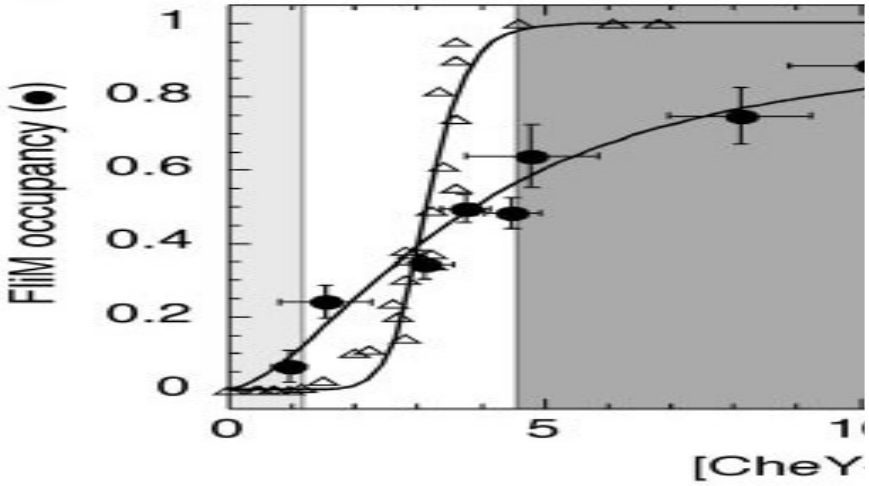
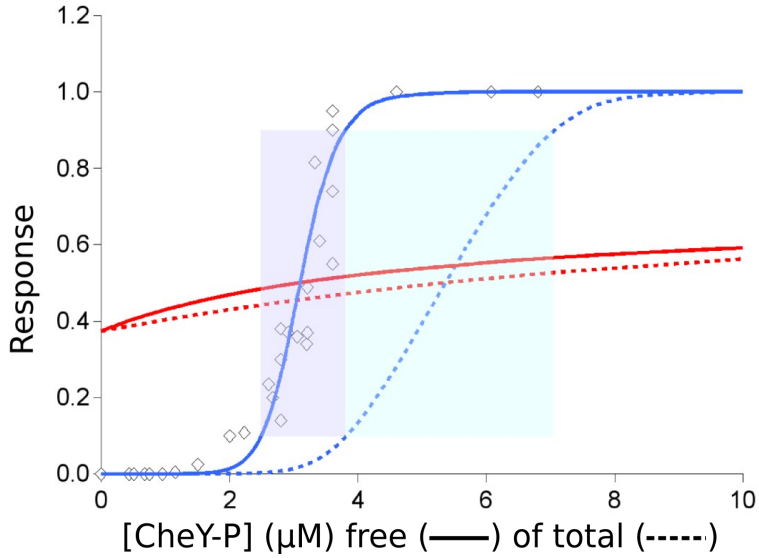
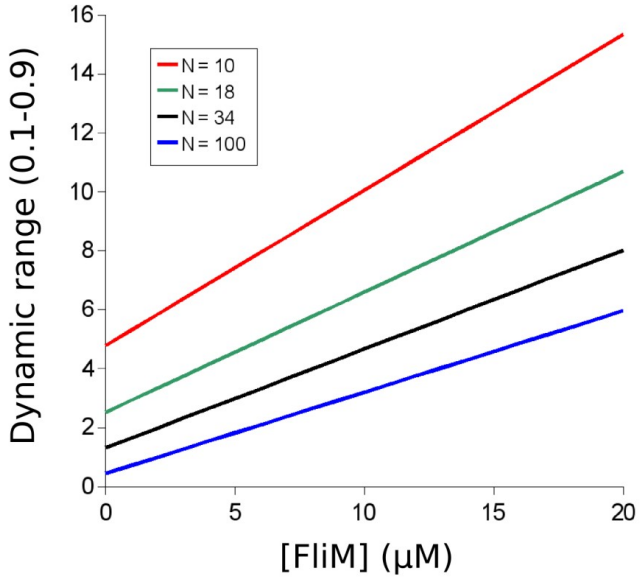
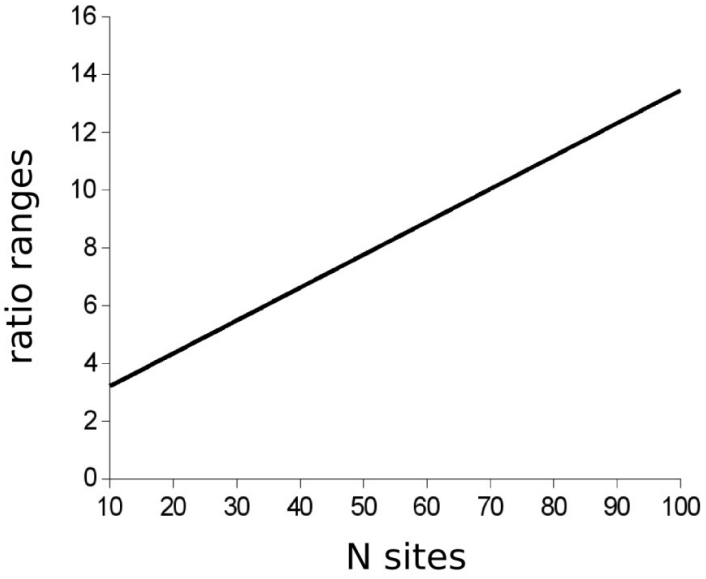
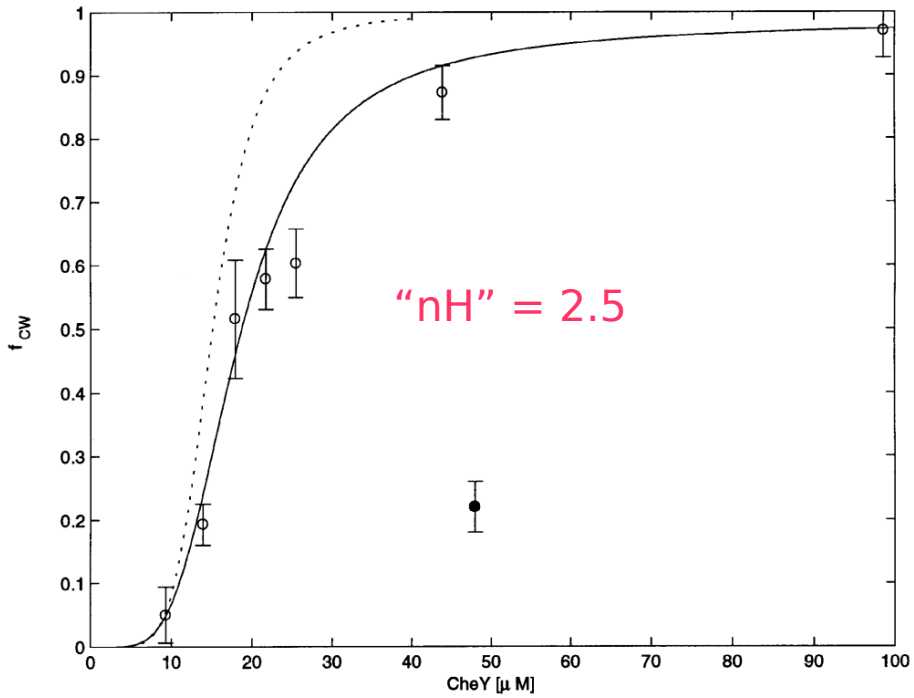




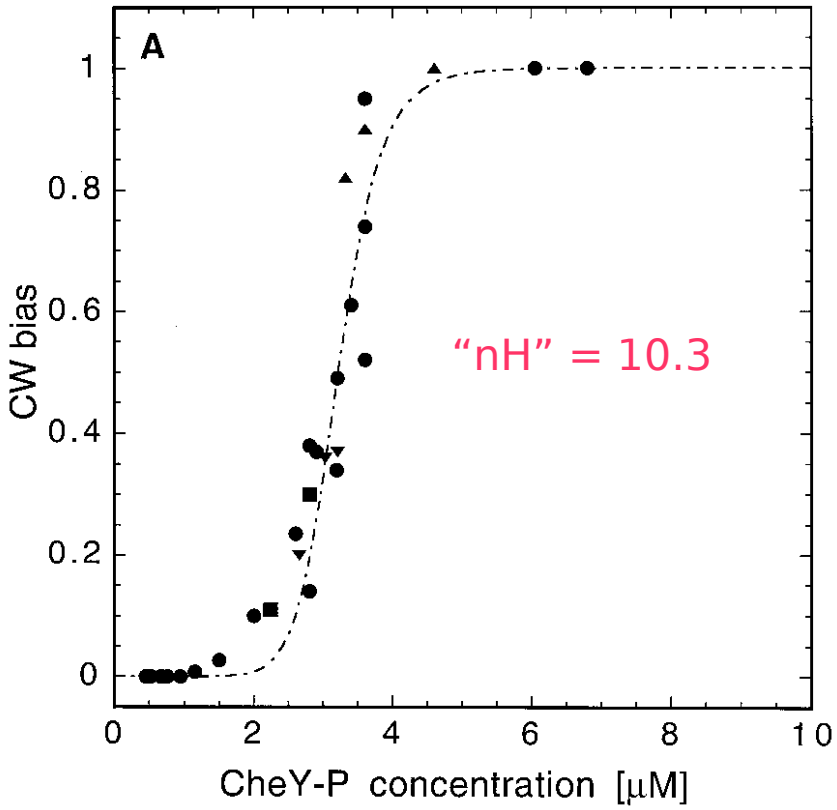




Ligand depletion explains different measures



Alon et al (1998) Response regulator output in bacterial chemotaxis. *EMBO J* 17: 4238-4248



Cluzel et al (2000) An ultrasensitive bacterial motor revealed by monitoring signalling proteins in single cells. *Science* 287: 1652-1655

Conclusions

- Ligand depletion decreases the effective cooperativity

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- Ligand depletion increases the dynamic range

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- Modifying the concentration of the sensor may be a powerful way to quickly adapt to a new environment, and switch from a measurement mode to a detection mode.

Conclusions

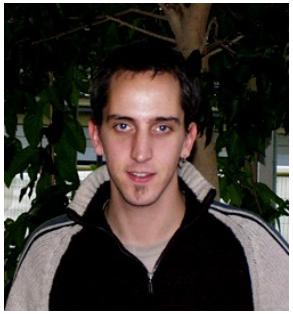
- Ligand depletion decreases the effective cooperativity
- Ligand depletion increases the dynamic range
- Modifying the concentration of the sensor may be a powerful way to quickly adapt to a new environment, and switch from a measurement mode to a detection mode.
- Generic advice for the quantitative study of cellular signalling:
  - Measure the parameters *in vitro* in controlled conditions
  - Measure quantities of interactants and subcellular location
  - Develop accurate quantitative models

The team

Melanie Stefan, AU

Stuart Edelstein, CH, US

Cooperativity crew

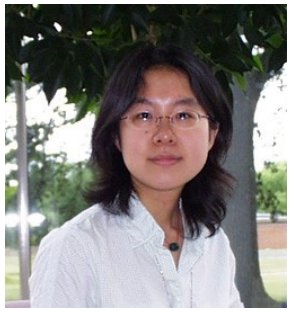


Dominic Tolle, EI, DE

Michele Mattioni, IT

Spatial simulation crew

BioModels crew



Lu Li, CN

Noriko Hiroi, JP

Signalling pathways crew

Viji Chelliah

Lukas Endler

Chen Li

Nicolas Rodrigues, FR

Nick Juty, UK

Camille Laibe, FR