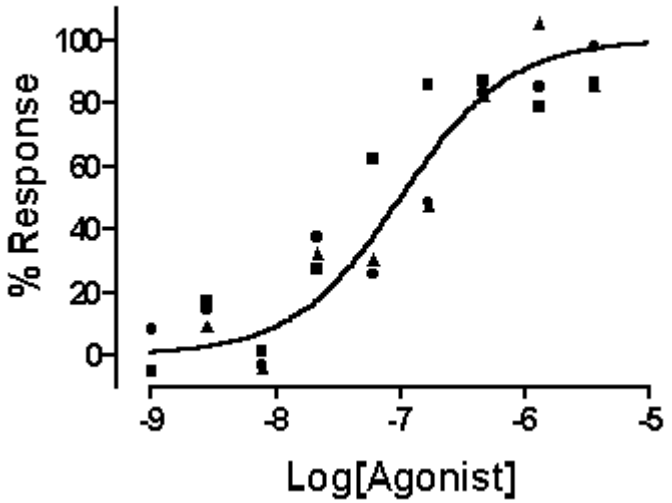
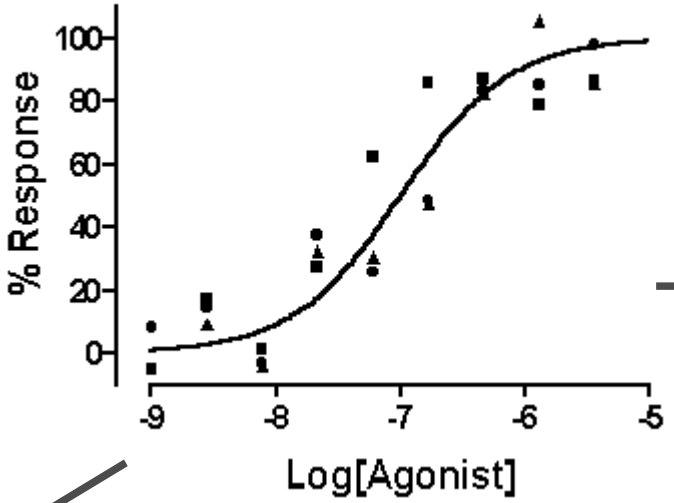
Ligand depletion *in vivo* modulates the dynamic range and cooperativity of signal transduction

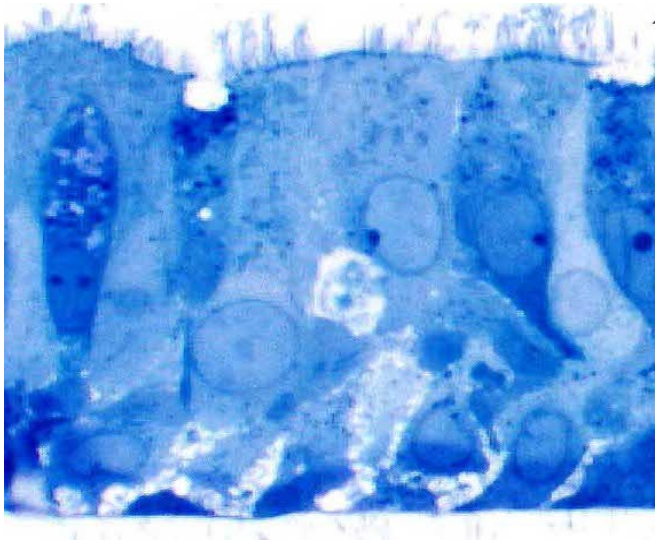
Nicolas Le Novère, EMBL-EBI

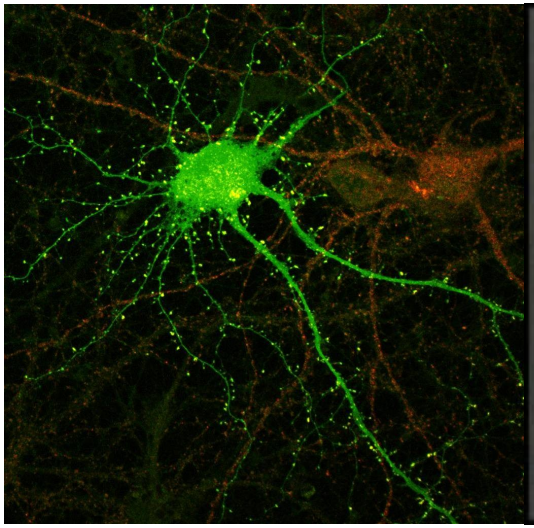
Dose-response is the most general measurement in biomedical sciences

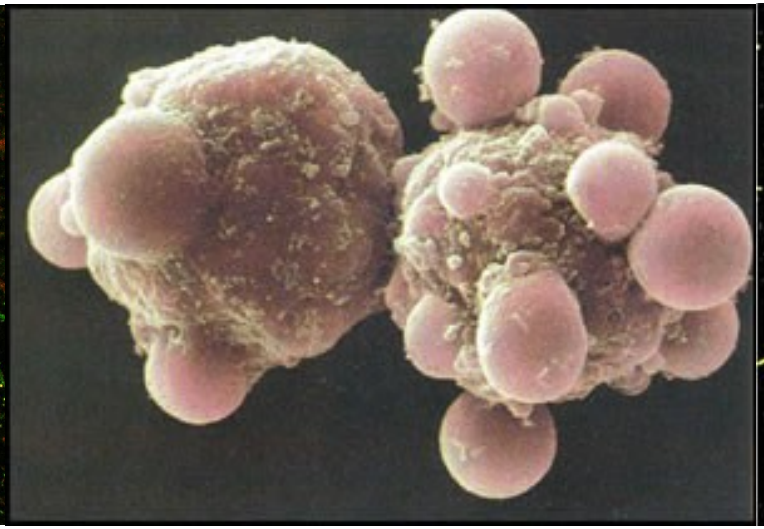


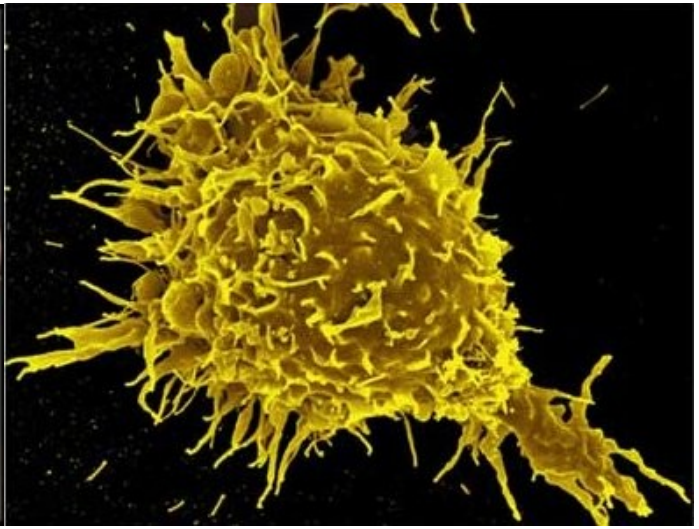
How general is a dose-response?



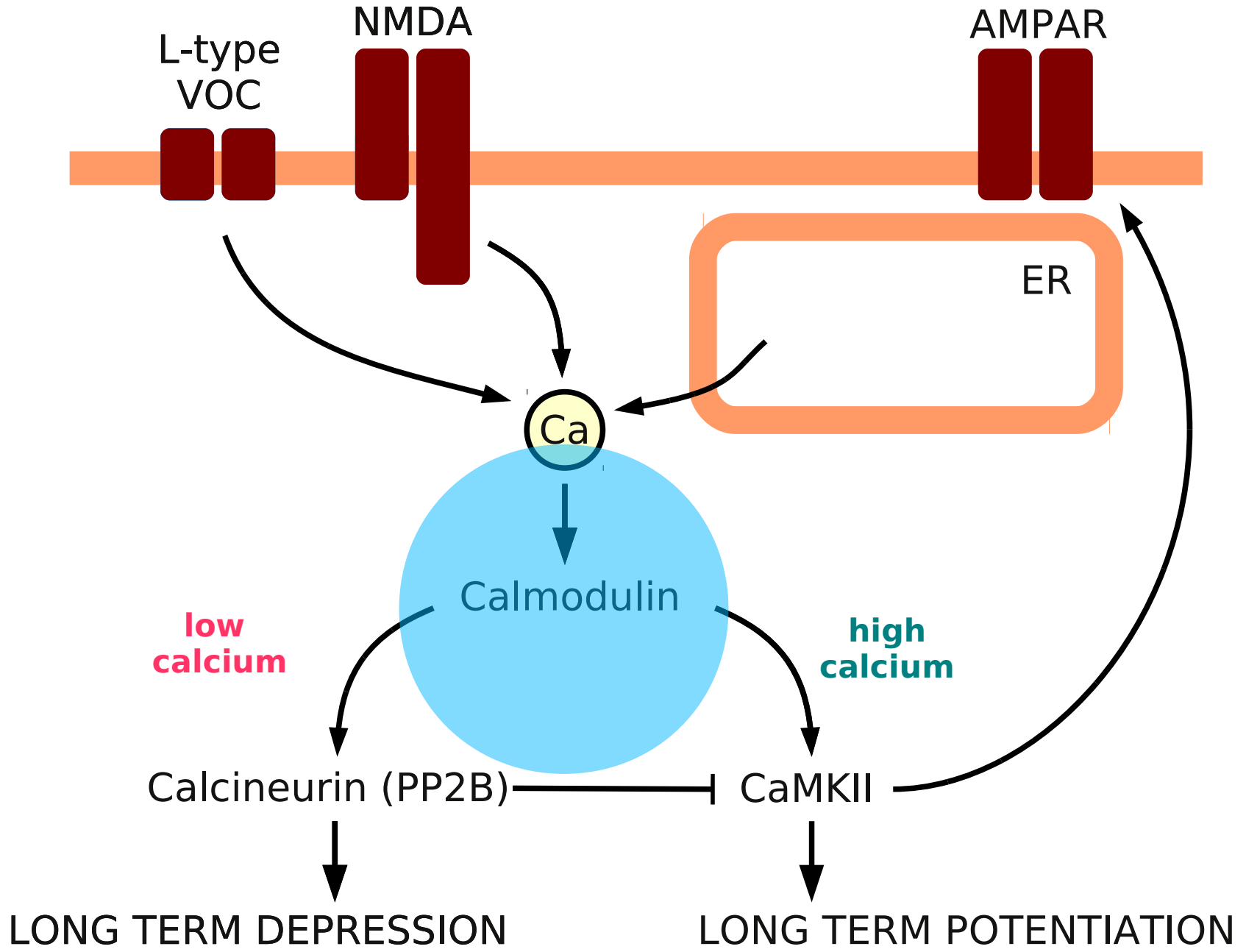






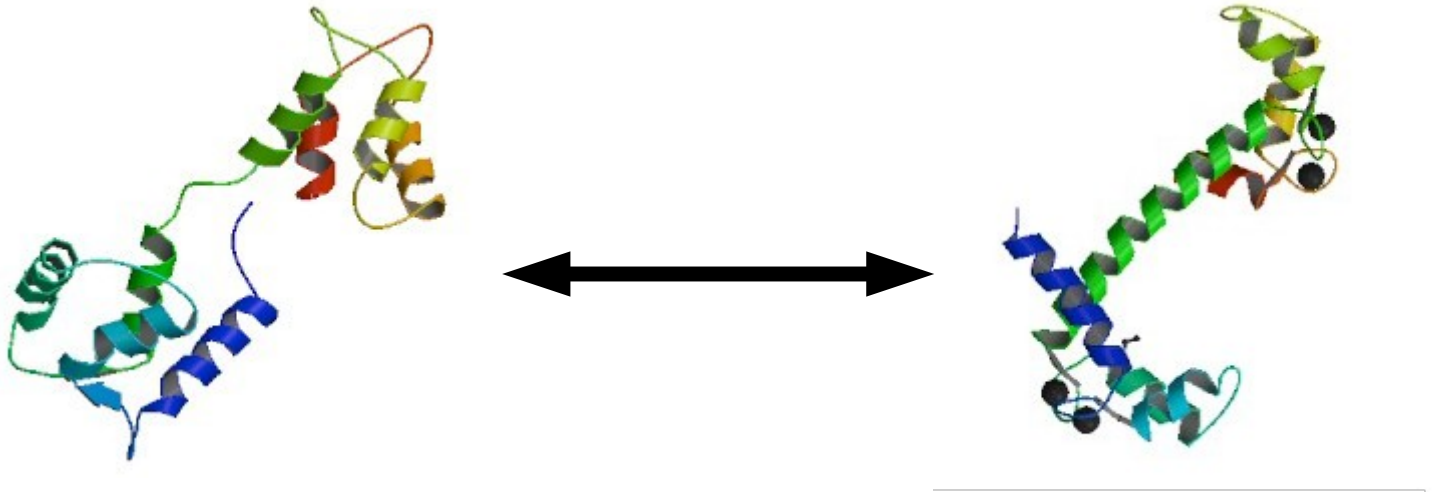


Calmodulin, the memory switch



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

State transitions of calmodulin



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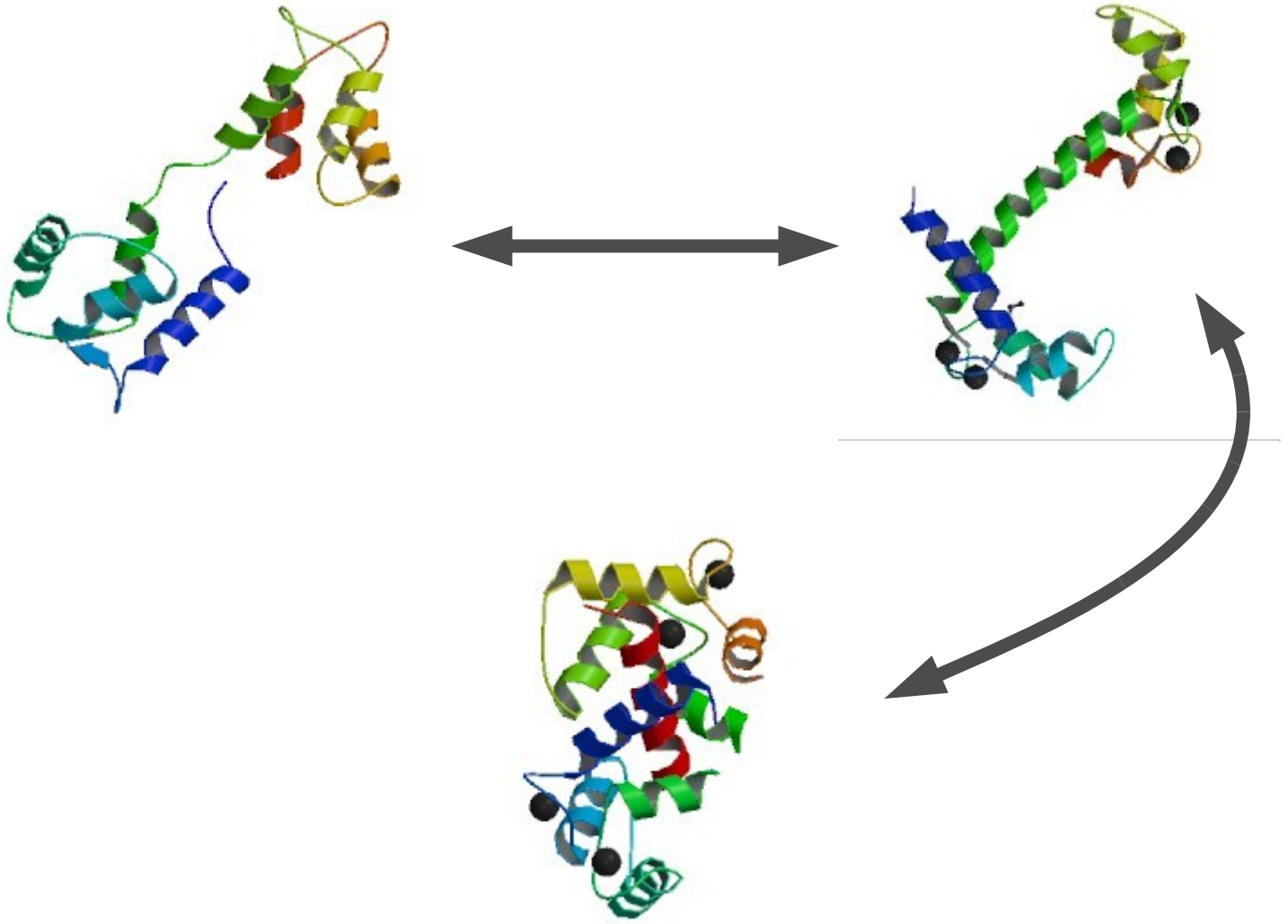
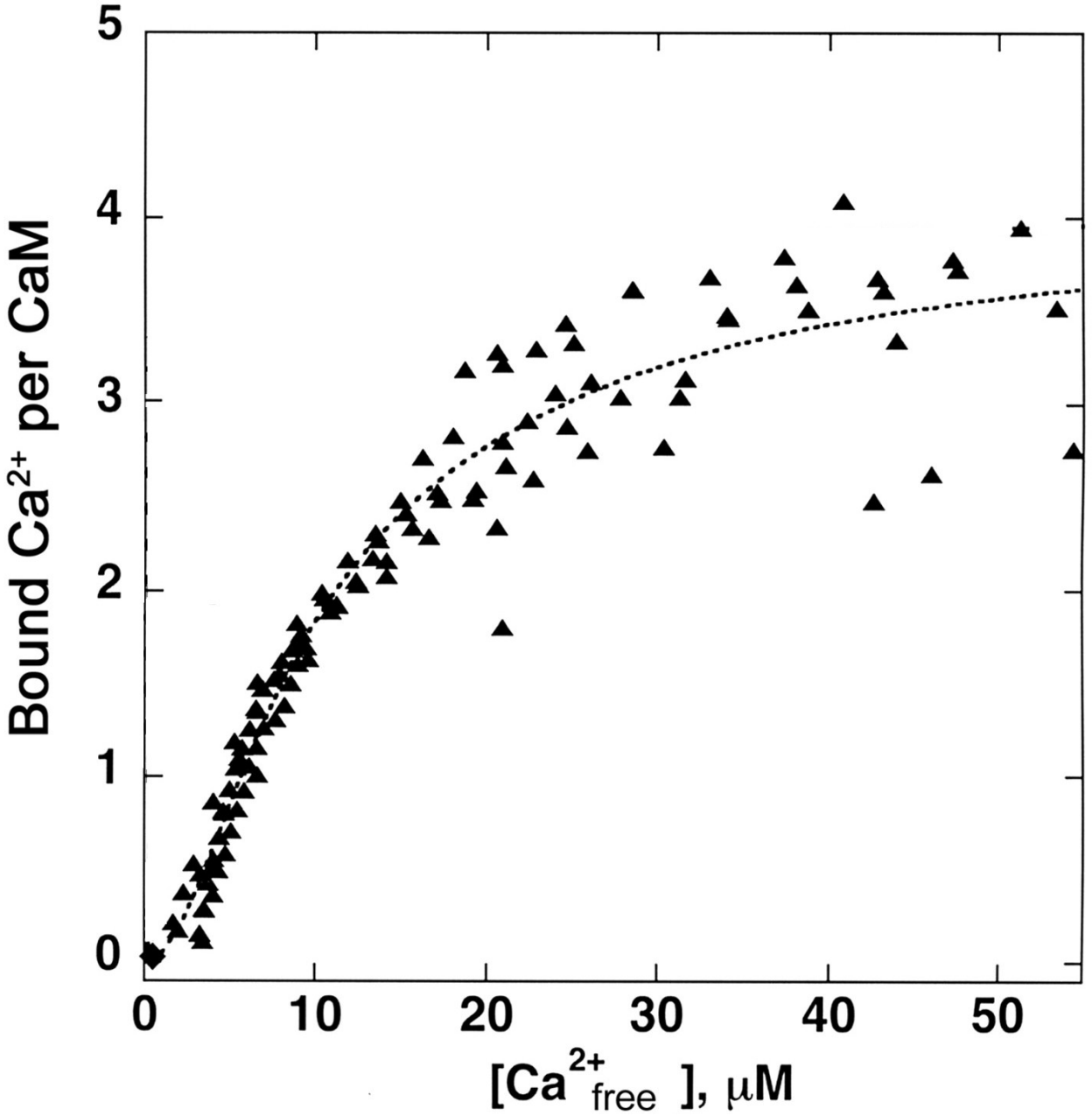
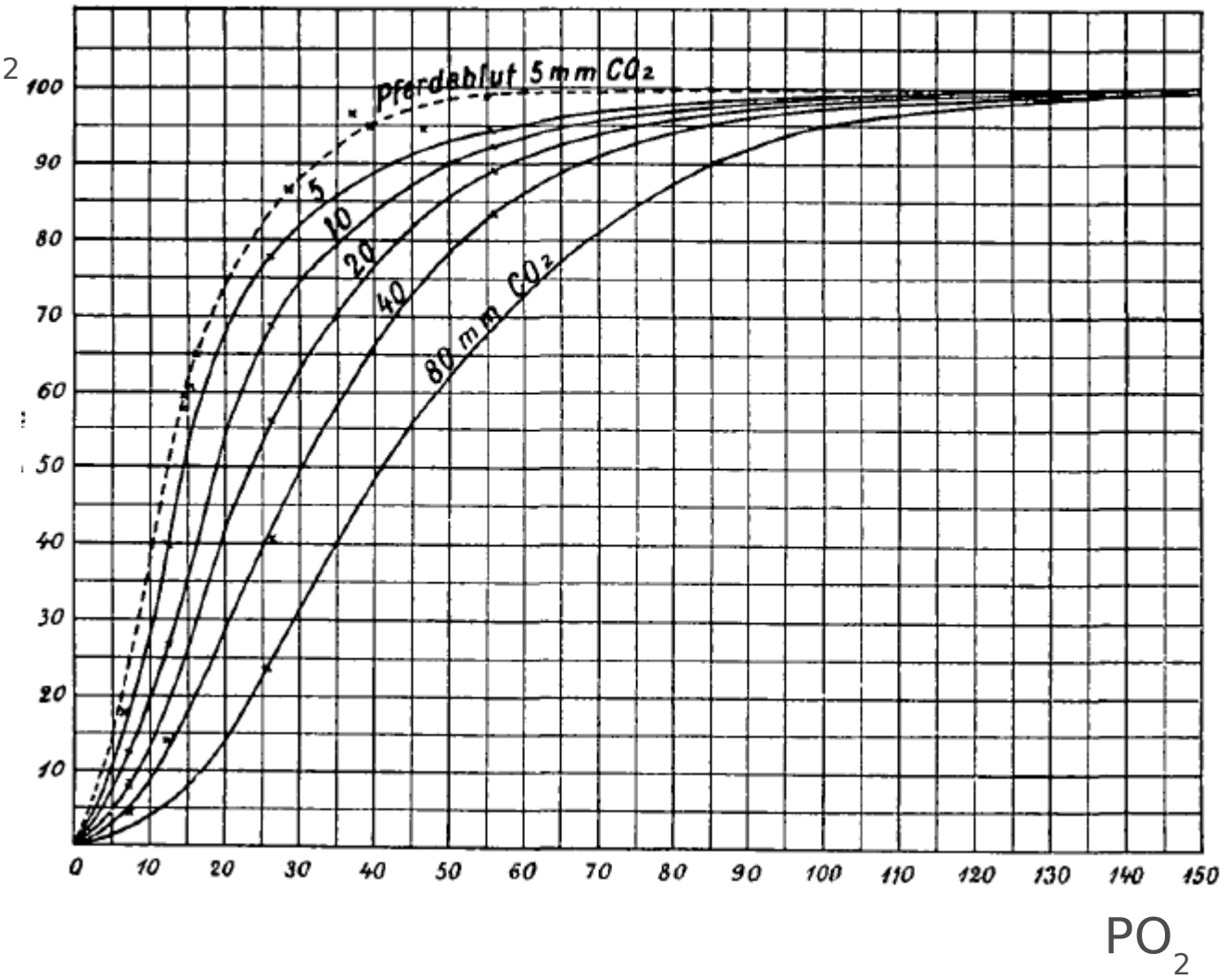


Calmodulin is ultra-sensitive



Shifman et al (2006)
PNAS, 103: 13968-13973

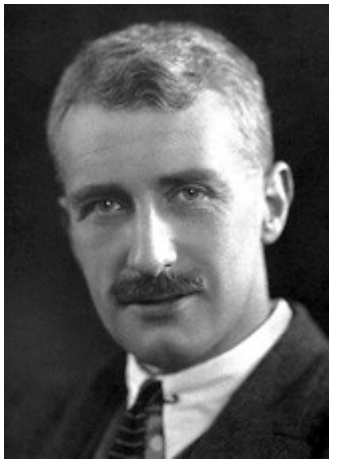
Origins of cooperativity: Bohr



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

Origins of cooperativity: Hill

Hill (1910) *J Physiol* 40: iv-vii.



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

$$\mathrm{Hb} + \mathrm{O_2} \rightleftarrows \mathrm{HbO_2},$$
$$\mathrm{Hb_n} + n\mathrm{O_2} \rightleftarrows \mathrm{Hb_nO_{2n}},$$

where $\mathrm{Hb_n}$ 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).

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Now it is unlikely that in either of these cases there is only Hb and $\mathrm{Hb_2}$: and as the calculation of the constants in these equations is very tedious I decided to try whether the equation

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

would satisfy the observations.

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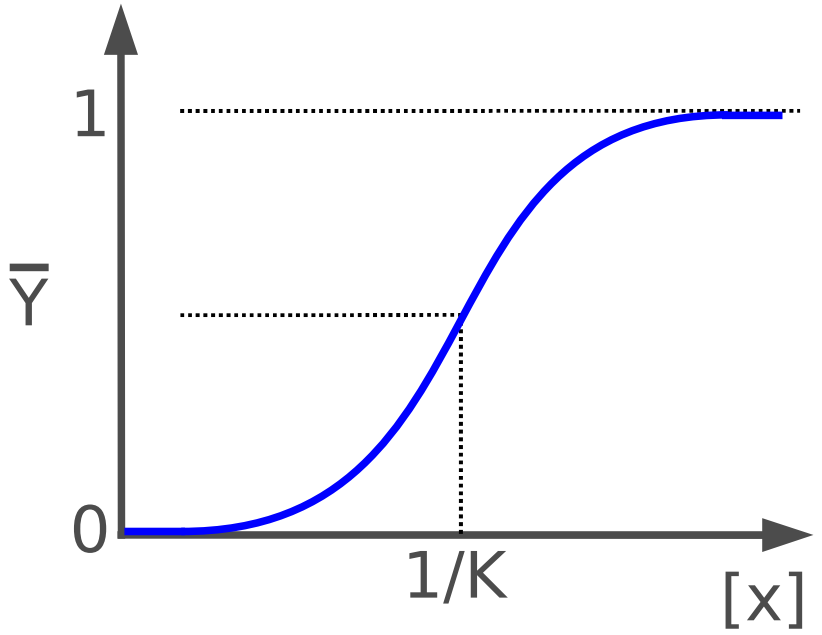


Hill Plot

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

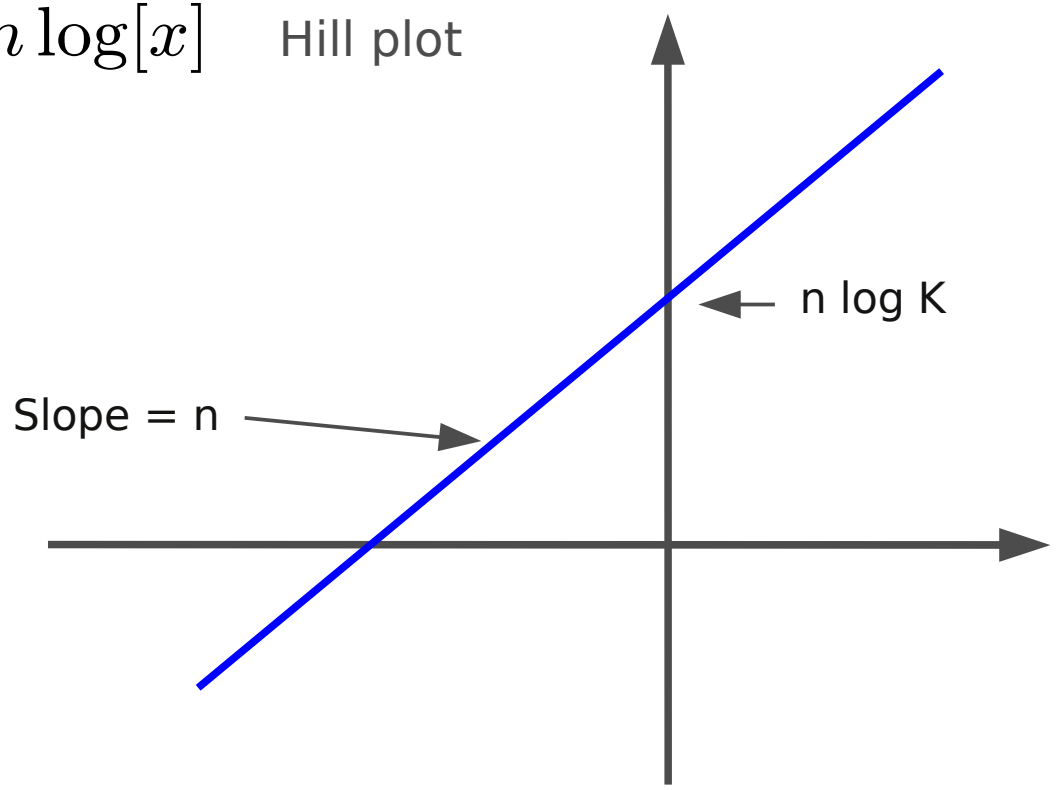
Hill equation

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

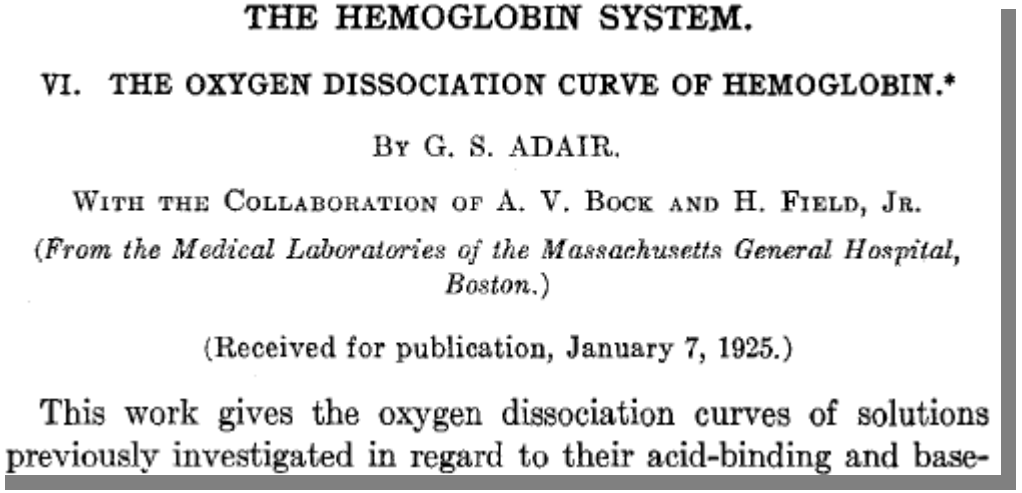
Hill plot

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-Klotz



THE HEMOGLOBIN SYSTEM.

VI. THE OXYGEN DISSOCIATION CURVE OF HEMOGLOBIN.*

By G. S. ADAIR.

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

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

(Received for publication, January 7, 1925.)

This work gives the oxygen dissociation curves of solutions previously investigated in regard to their acid-binding and base-

Adair (1925) *J Biol Chem* 63: 529

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$$\bar{Y} = \frac{1}{n}\frac{K_1[x] + 2K_2[x]^2 + 3K_3[x]^3 + 4K_4[x]^4}{1 + K_1[x] + K_2[x]^2 + K_3[x]^3 + K_4[x]^4}$$

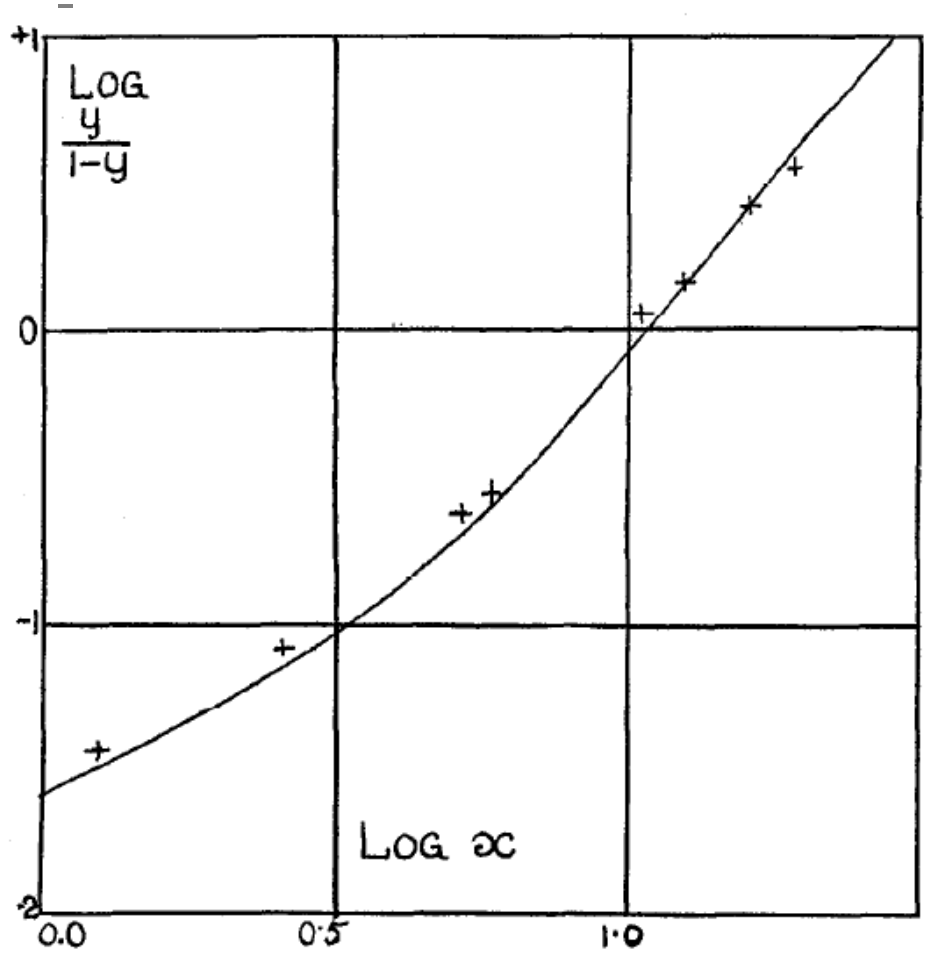

FIG. 2. Test of formula (6). Curve drawn from 6 experimental points from Table IV.

Origins of cooperativity: Adair-Klotz

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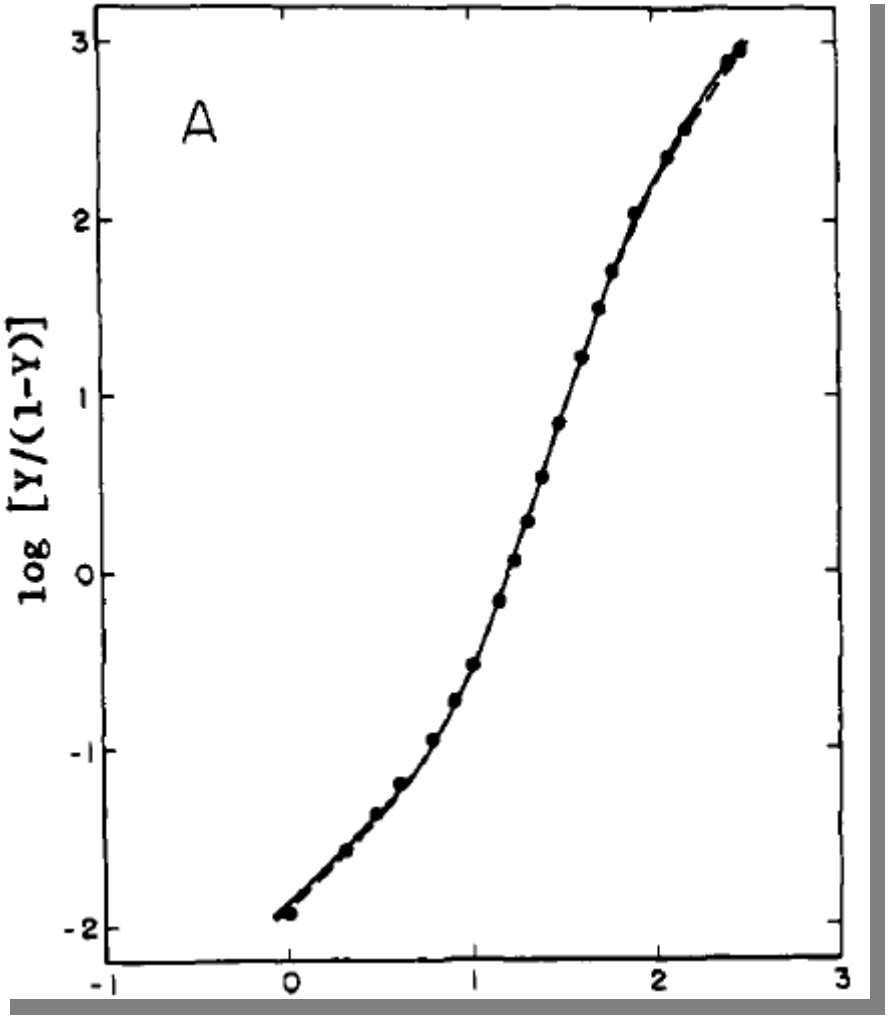
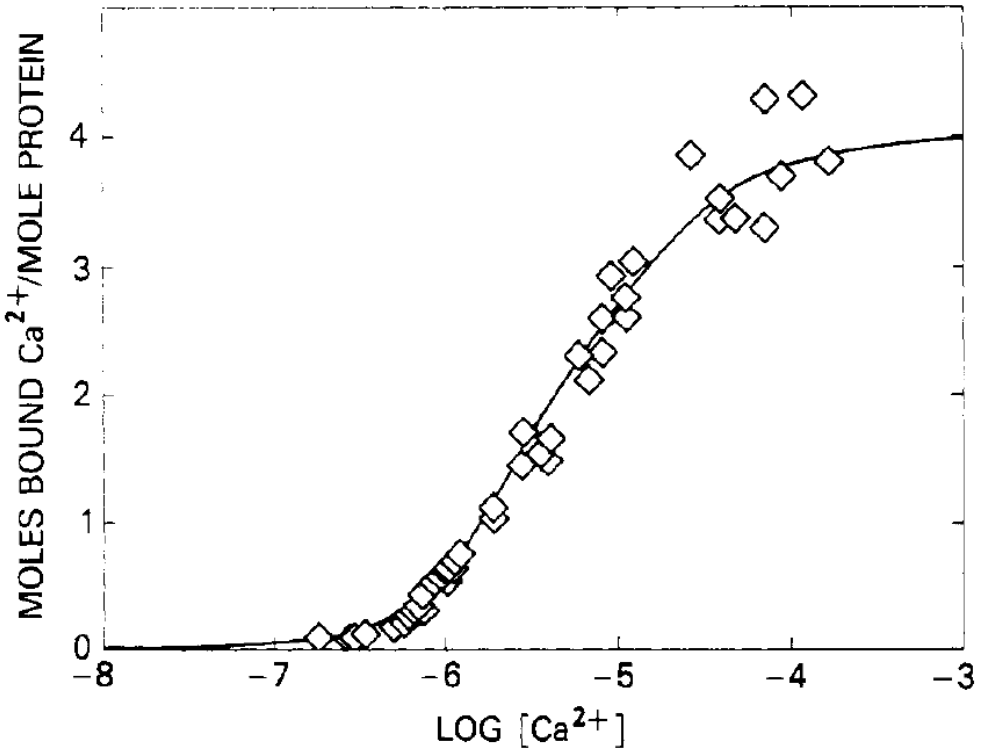


Imai (1973) *Biochemistry* 12: 798-808

Adair-Klotz model applied to Calmodulin

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

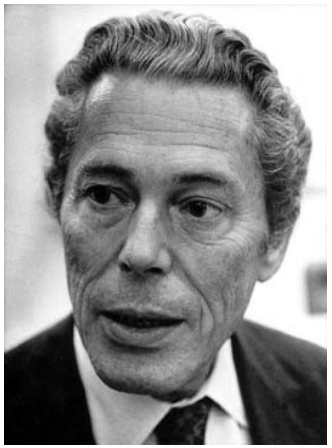
$$\bar{Y} = \frac{1}{n}\frac{K_1[Ca] + 2K_1K_2[Ca]^2 + 3K_1K_2K_3[Ca]^3 + 4K_1K_2K_3K_4[Ca]^4}{1 + K_1[Ca] + K_1K_2[Ca]^2 + K_1K_2K_3[Ca]^3 + K_1K_2K_3K_4[Ca]^4}$$



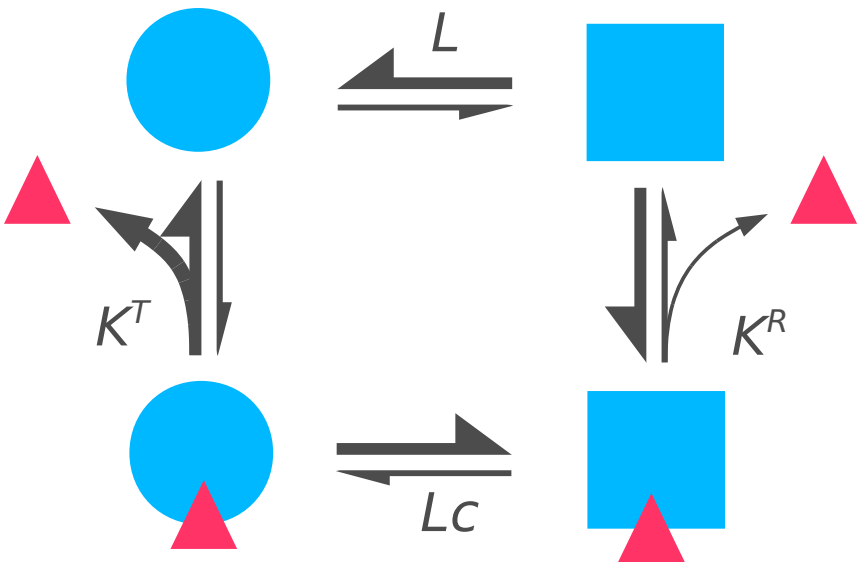
Crouch and Klee (1980) *Biochemistry*, 19: 3692-3698

Allostery and state selection

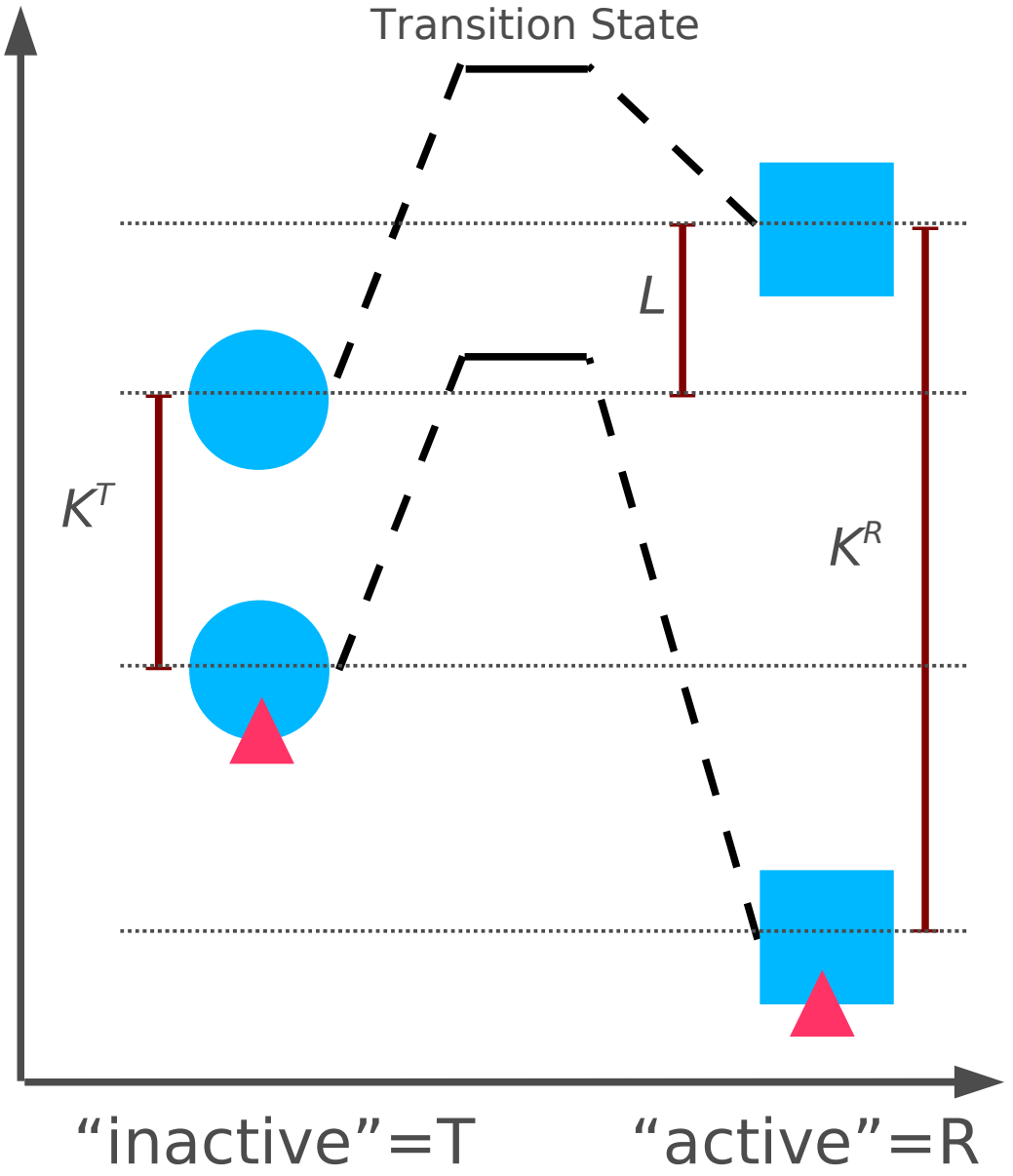
- Monod, Wyman, Changeux (1965). On the nature of allosteric transitions: a plausible model. *J Mol Biol*, 12: 88-118



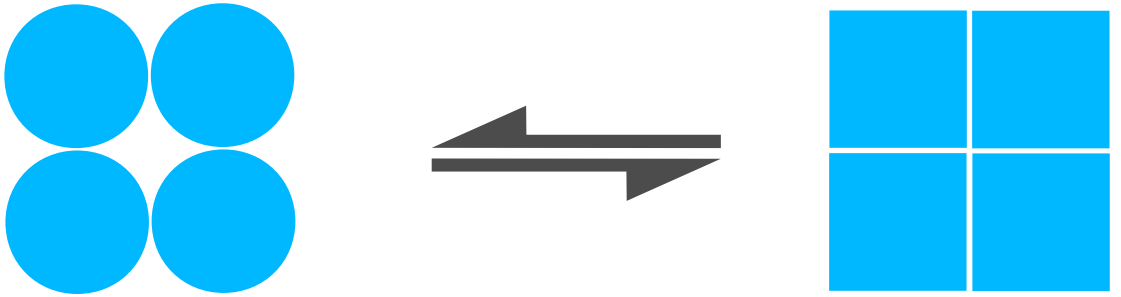
Modulation of thermal equilibria ≠ induced-fit



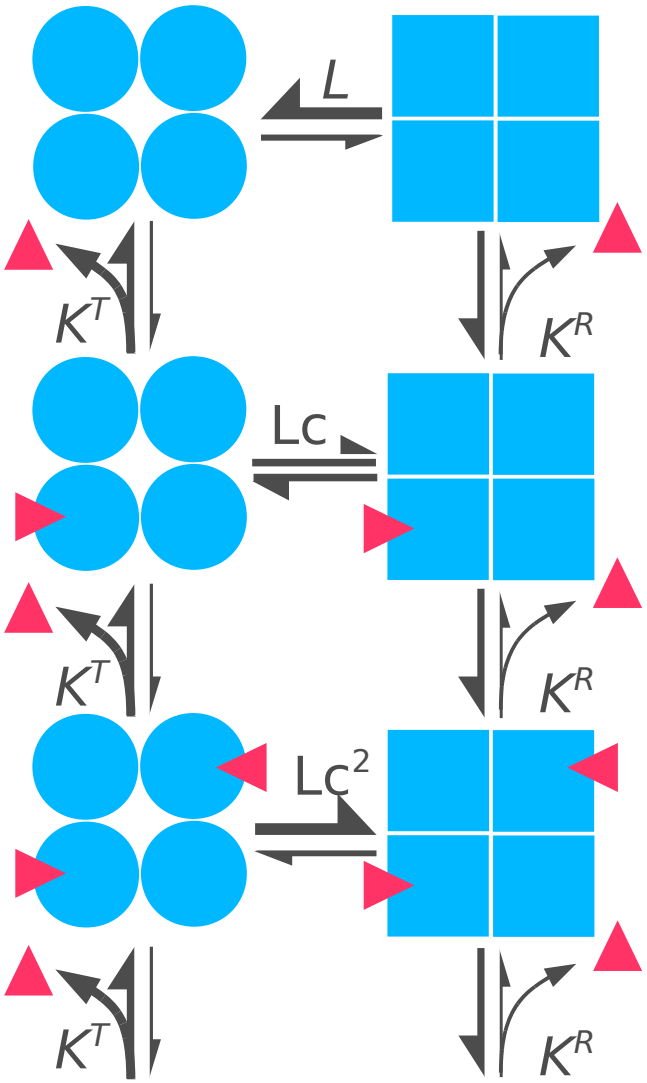
$$L = \frac{[T_0]}{[R_0]} \qquad c = \frac{K^R}{K^T}$$



Concerted transitions ≠ sequential model



Monod-Wyman-Changeux model



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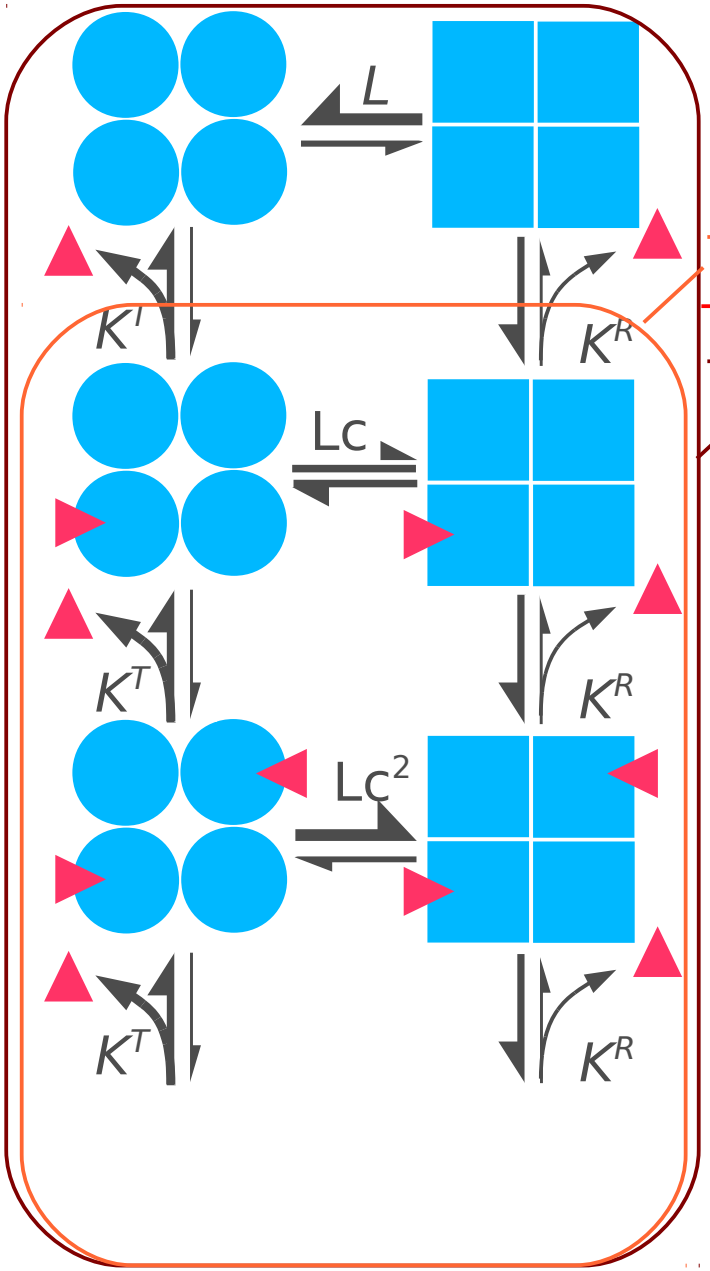
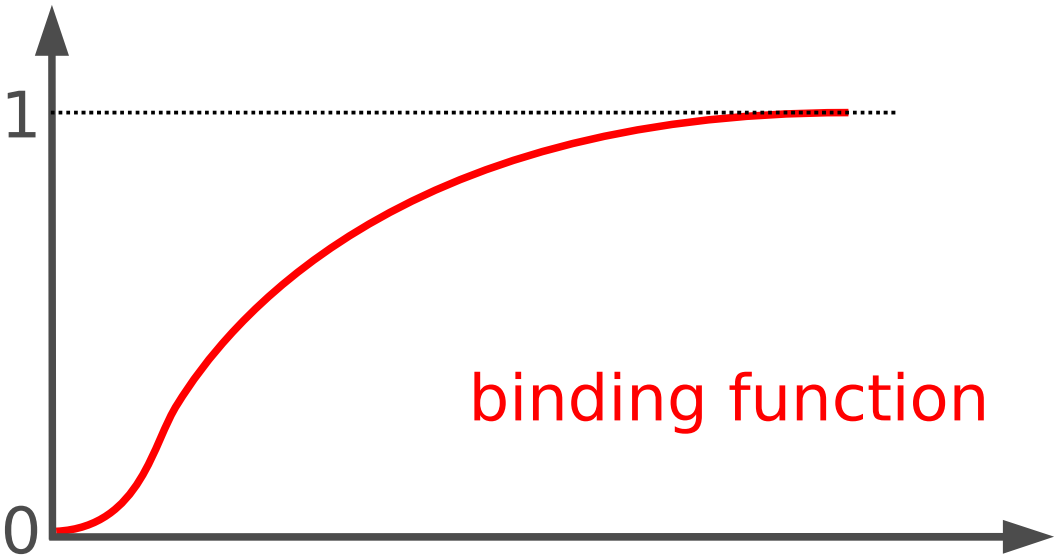


this = that

$$\alpha = \frac{[x]}{K^R}$$

$$\bar{Y} = \frac{\alpha(1+\alpha)^{n-1} + Lc\alpha(1+c\alpha)^{n-1}}{(1+\alpha)^n + L(1+c\alpha)^n}$$



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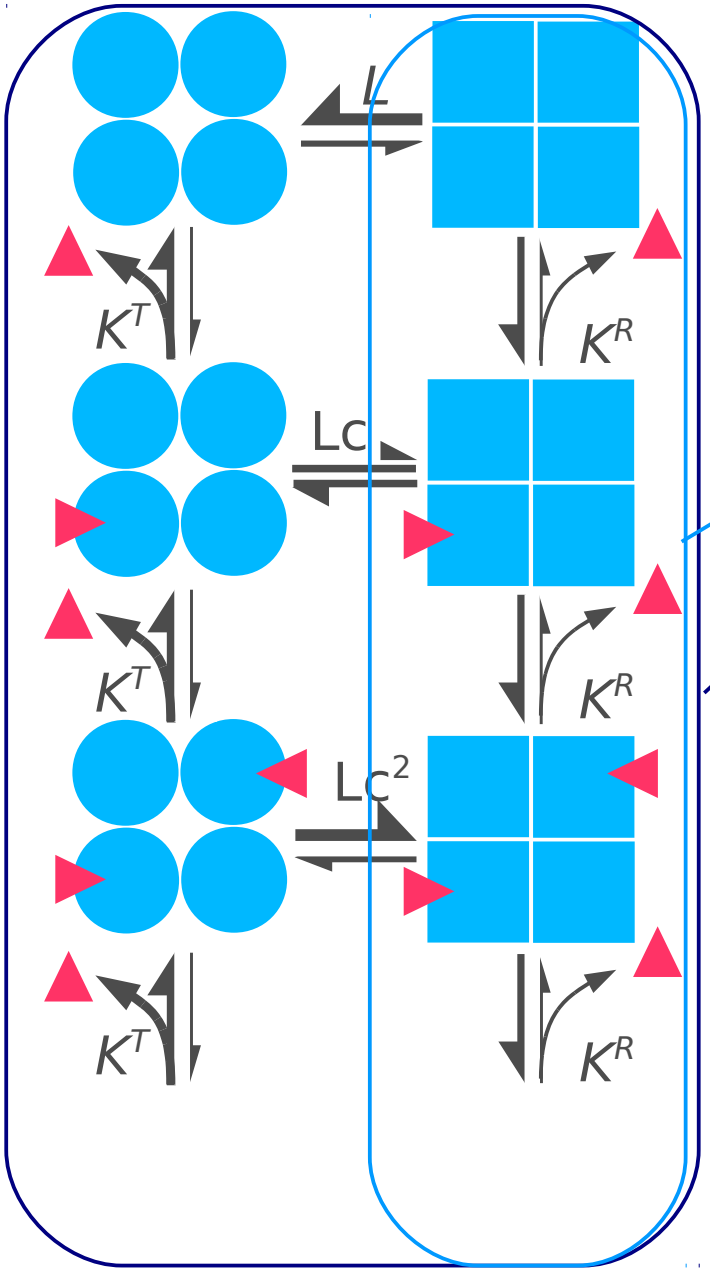

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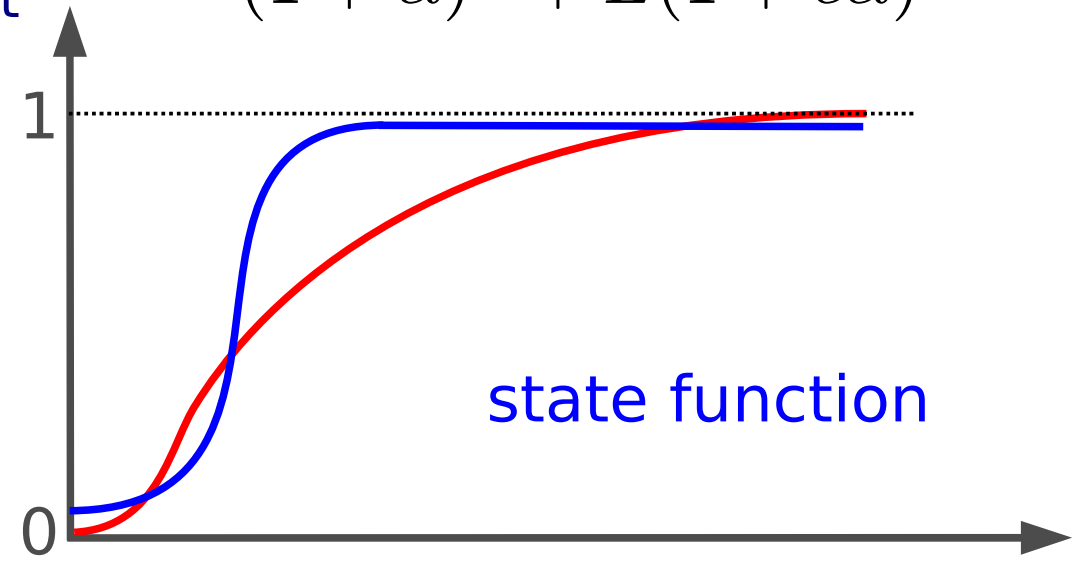
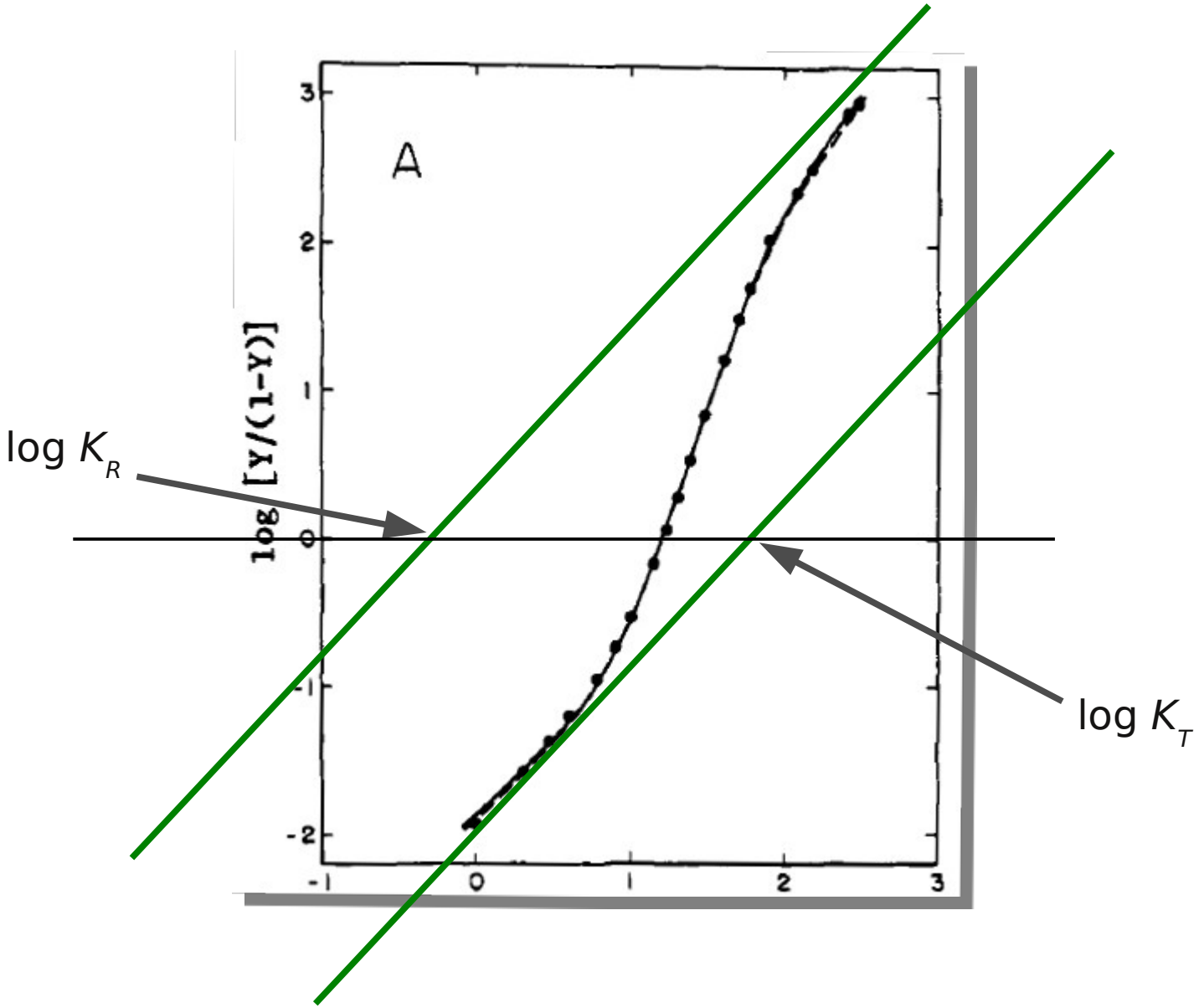
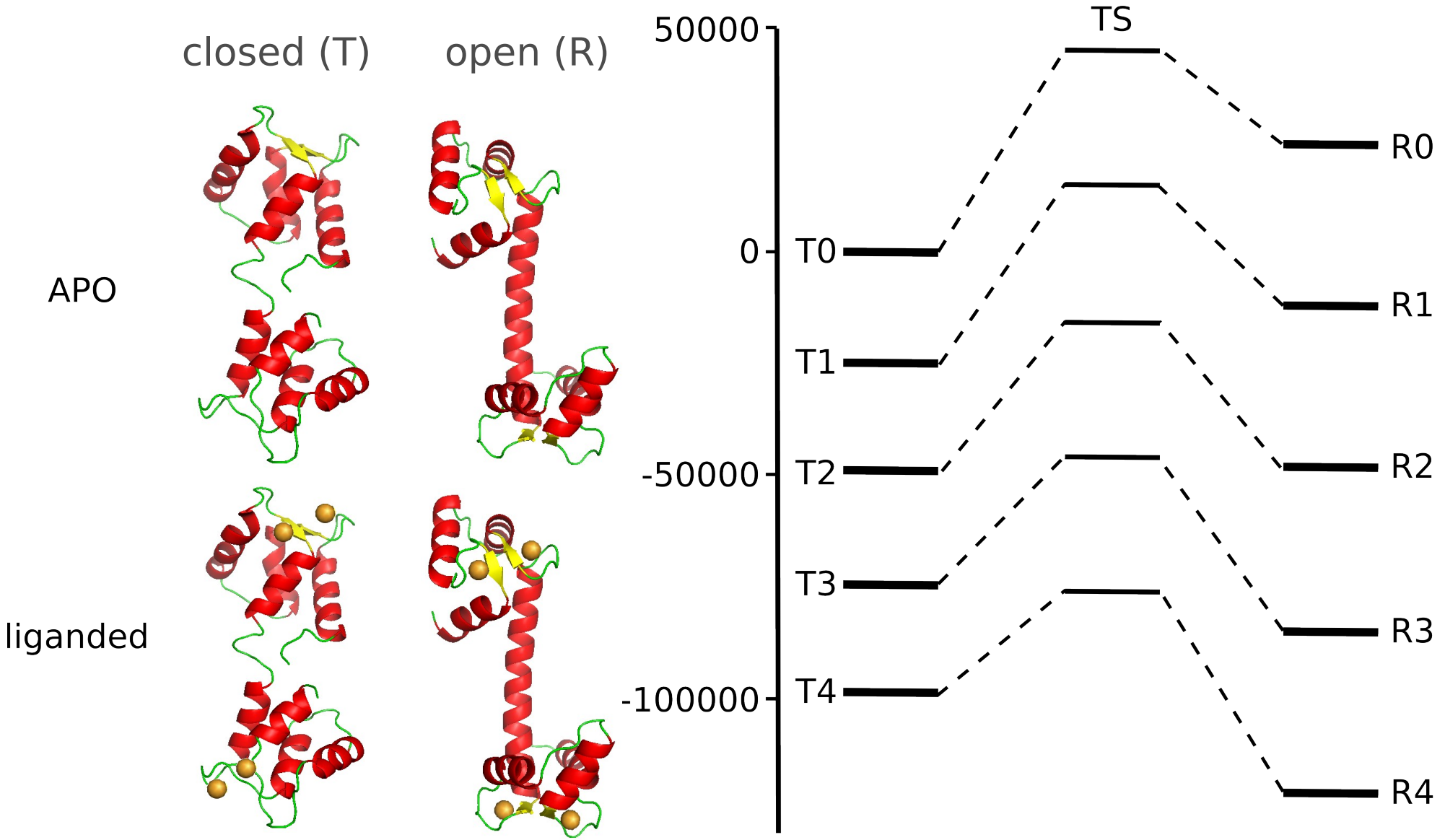
$$\bar{R} = \frac{(1+\alpha)^n}{(1+\alpha)^n + L(1+c\alpha)^n}$$



“Hill” Plot for MWC model

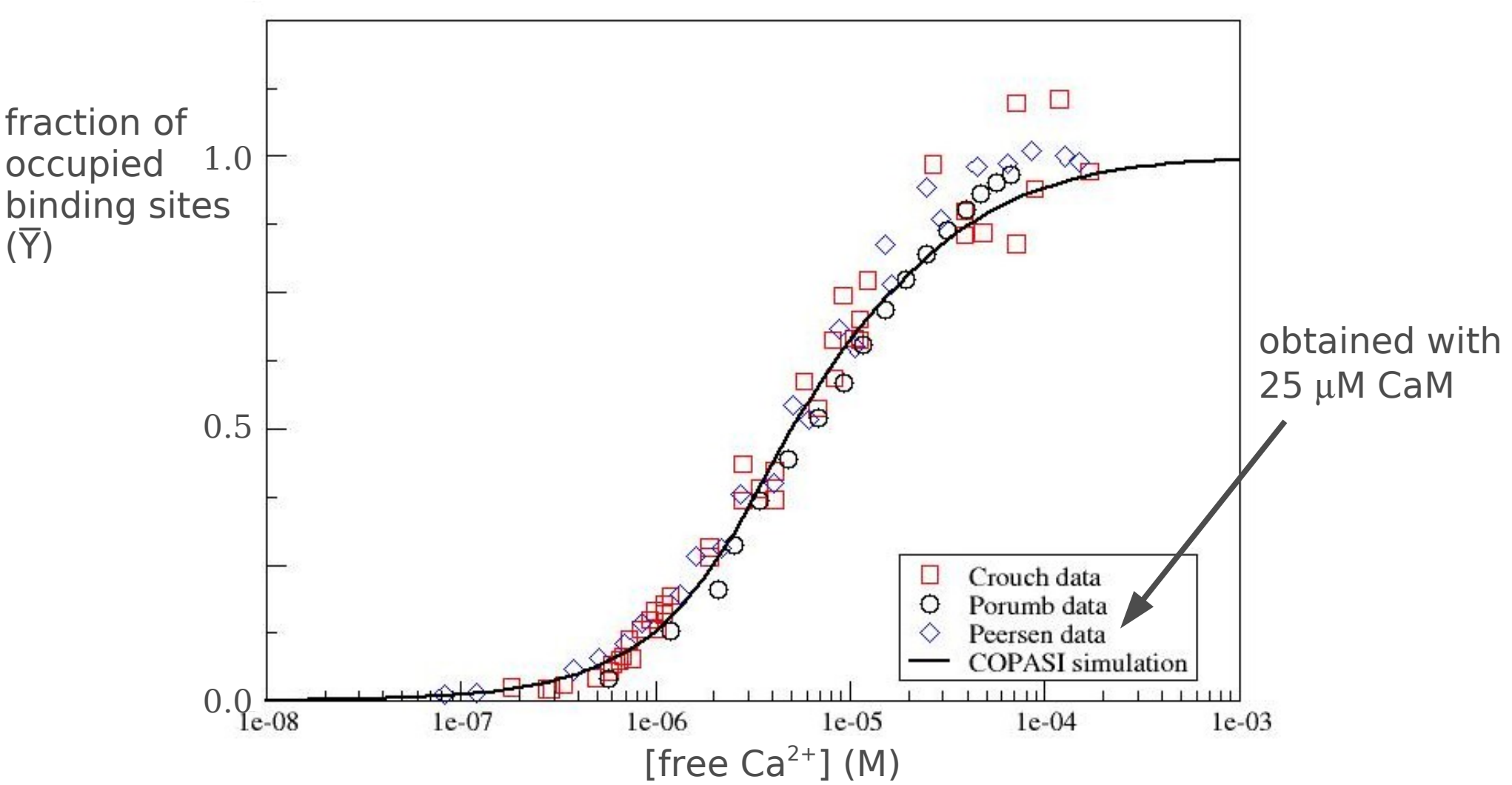


Concerted transition

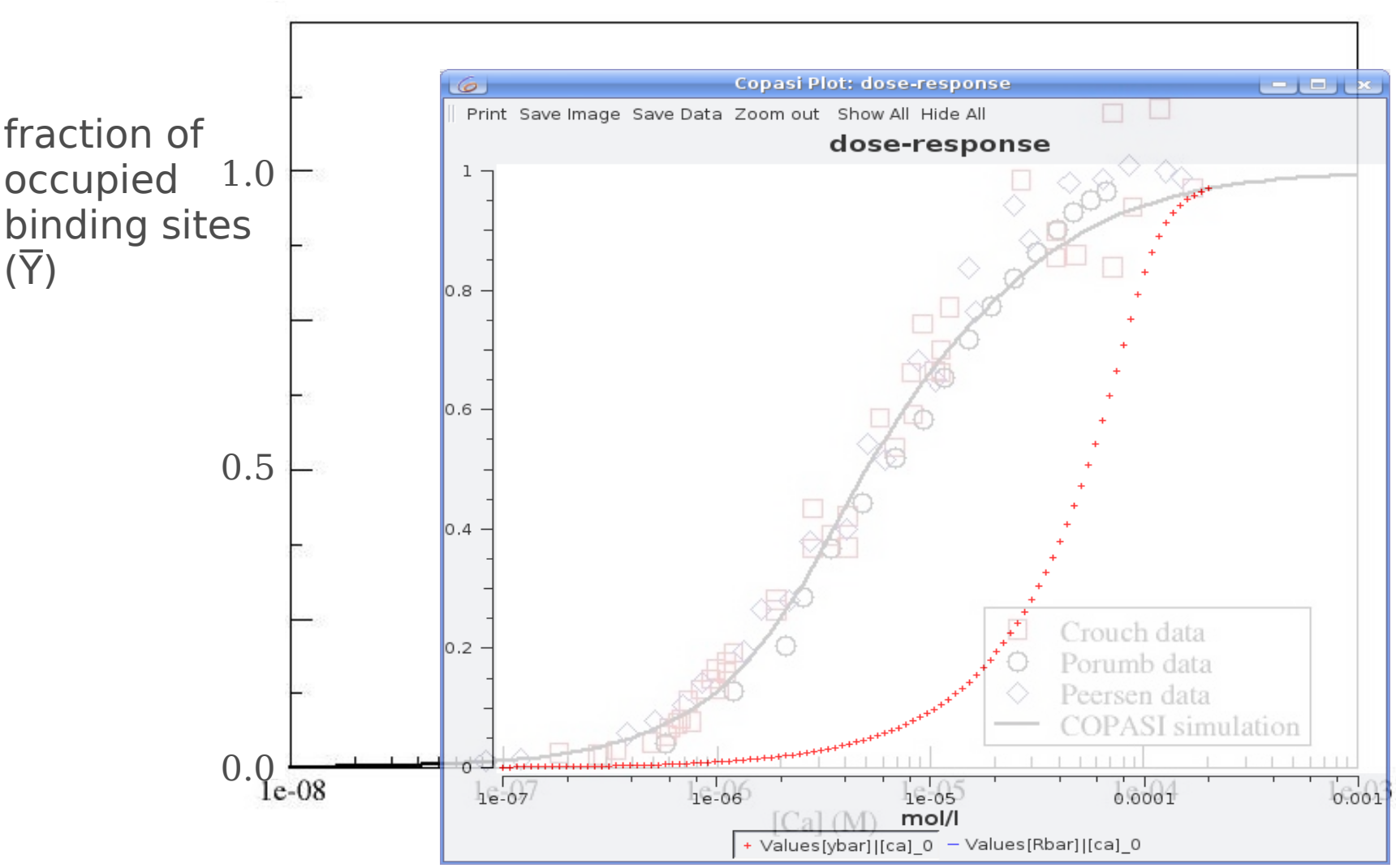


Stefan M.I., Edelstein S.J., Le Novère N (2009) Computing phenomenologic Adair-Klotz constants from microscopic MWC parameters. *BMC Syst Biol*, 3: 68

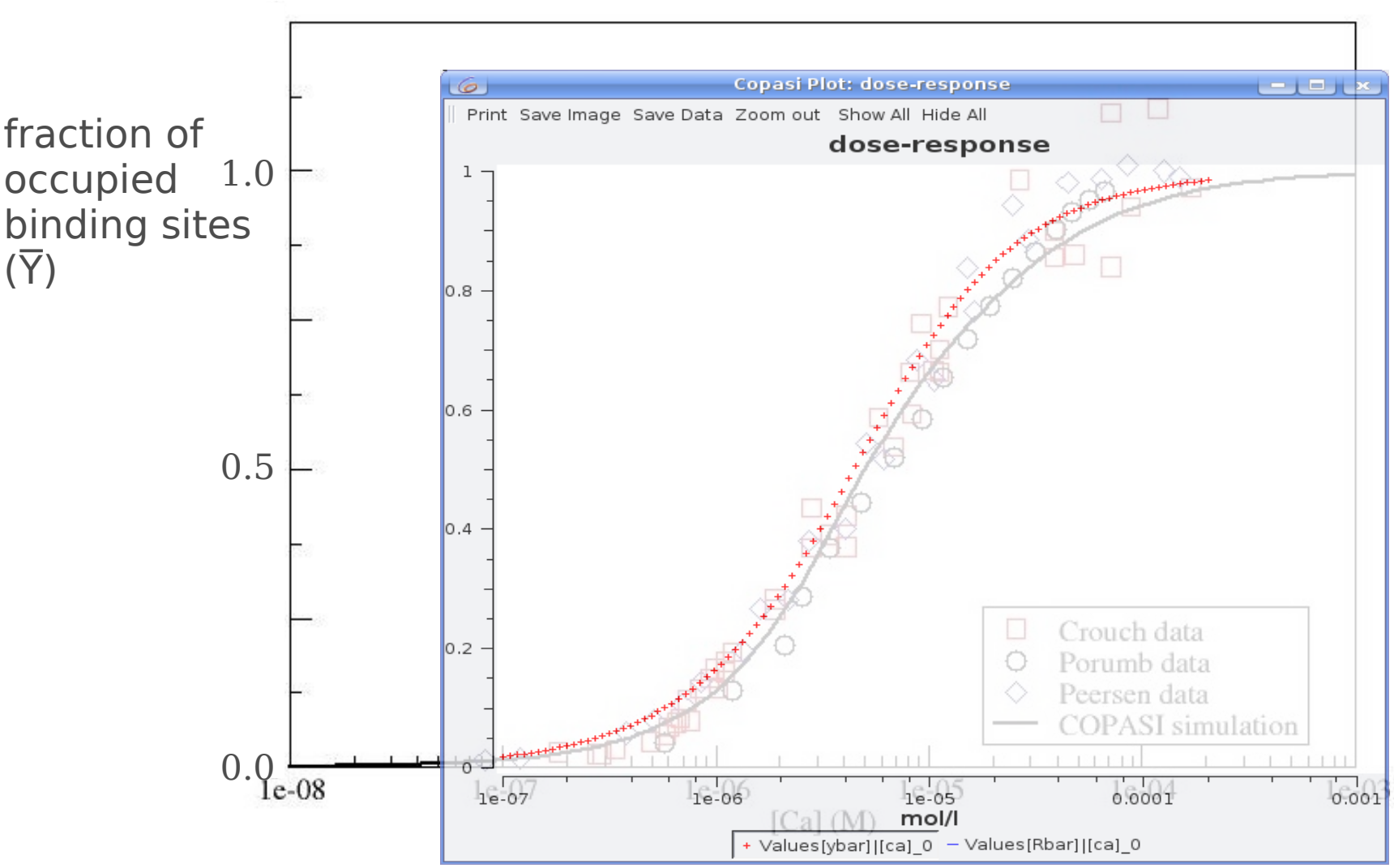
Allosteric model of Calmodulin function



Calcium dose-response on 25 μM Calmodulin



Calcium dose-response on 0.1 μM Calmodulin



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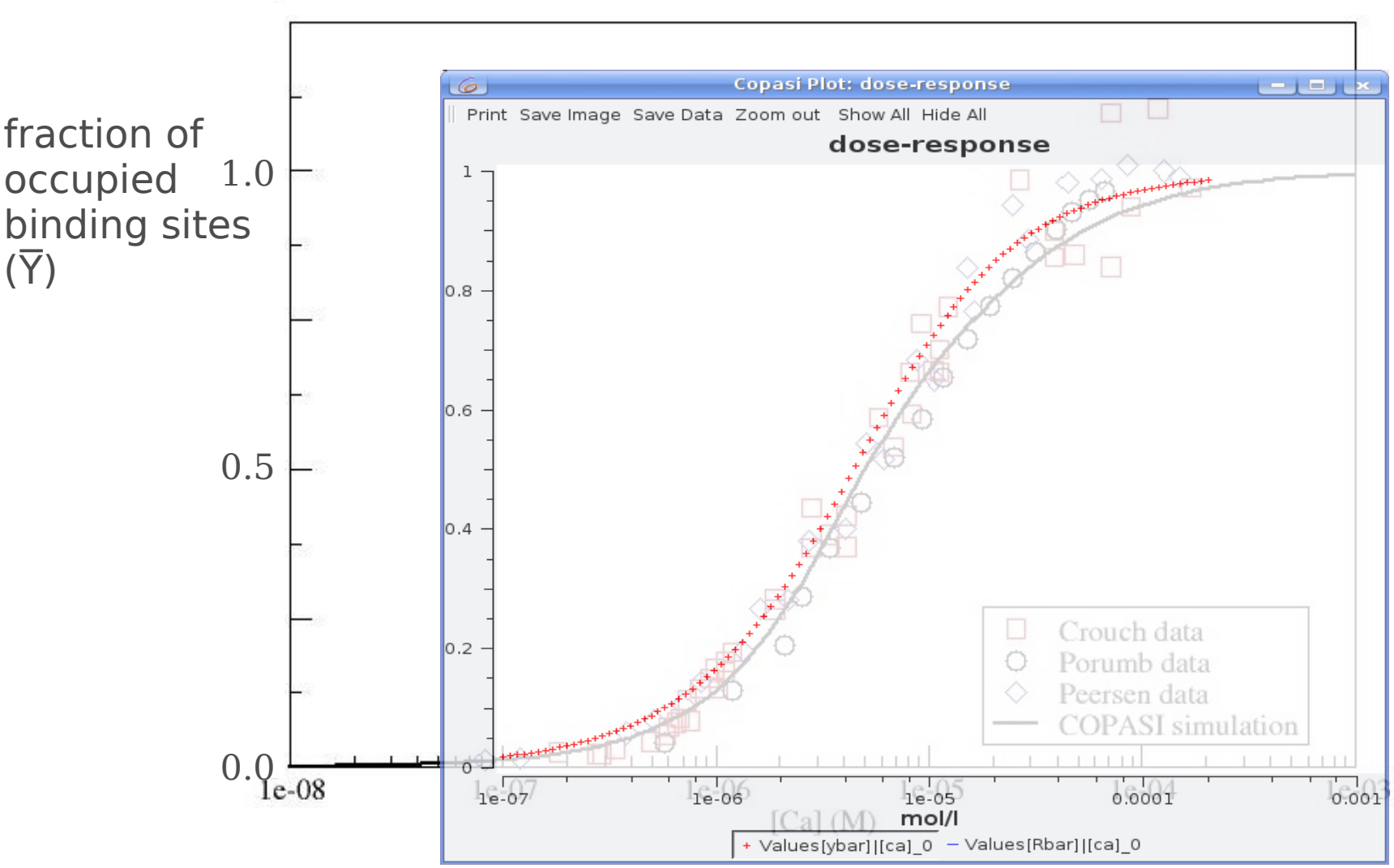
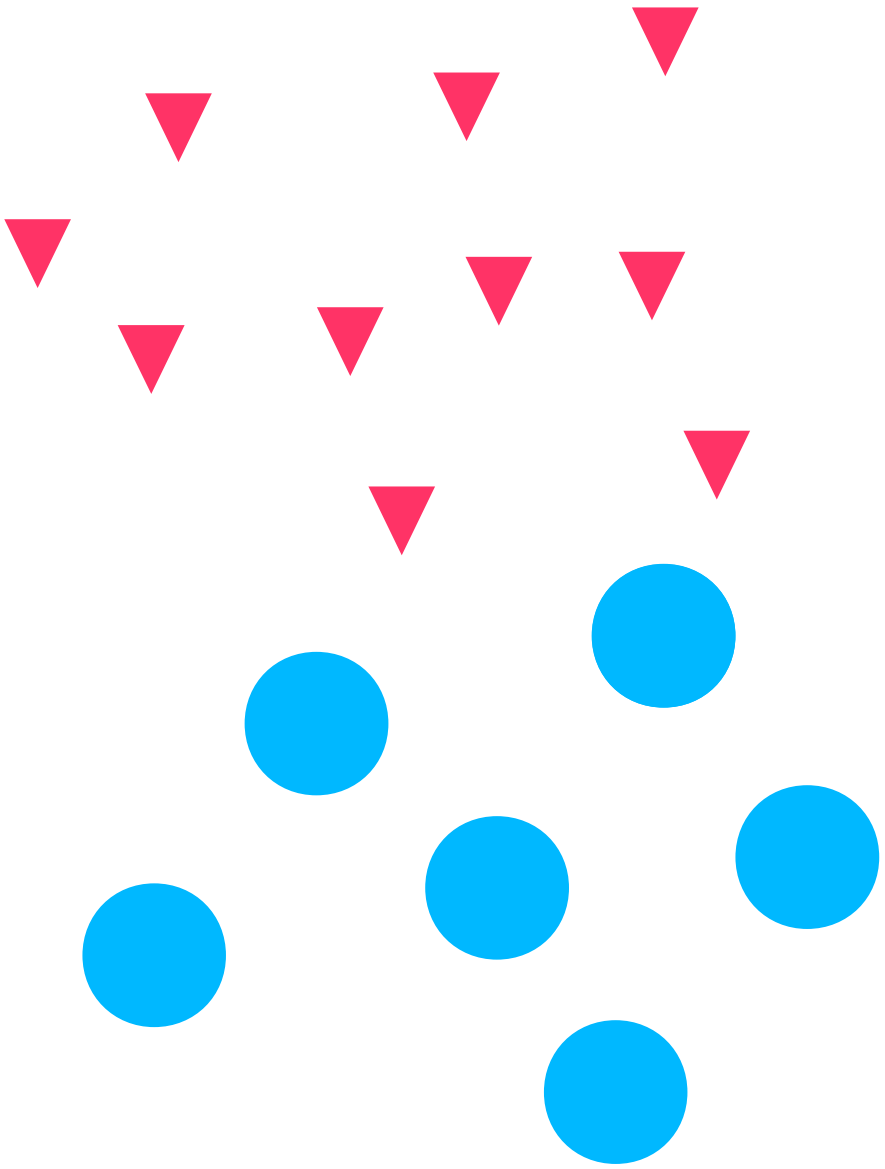


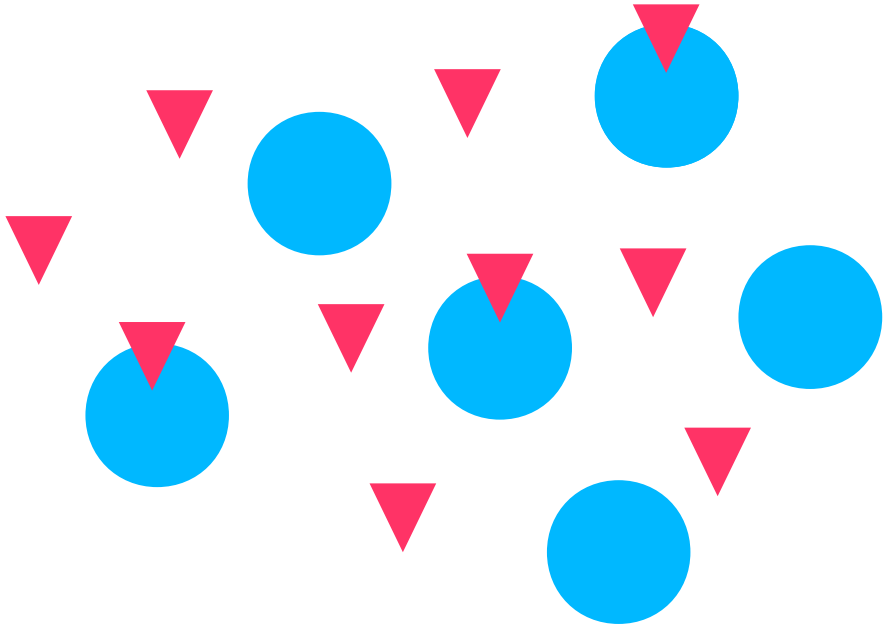
Edelstein S.J., Stefan M.I, Le Novère N. Ligand depletion in vivo modulates the dynamic range and cooperativity of signal transduction. PLoS One (2010), 5(1): e8449

What is ligand depletion?



What is ligand depletion?

Chemistry (mass-action law)



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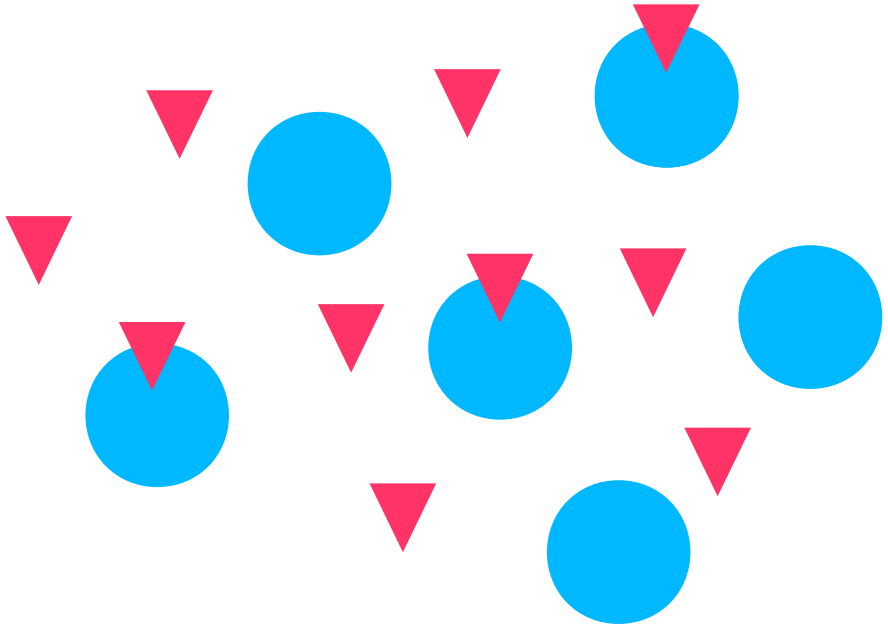
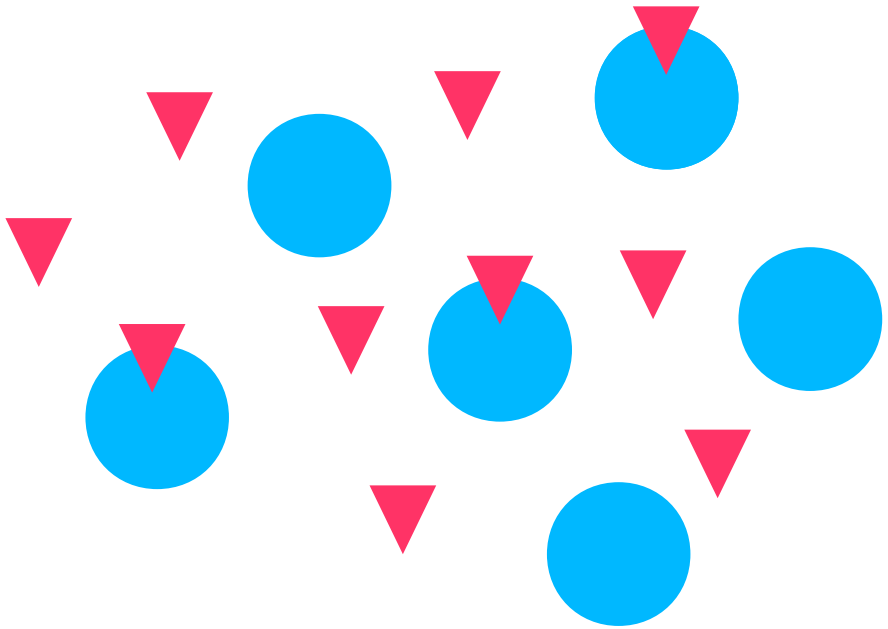


Chemistry (mass-action law)

Cellular signalling

What is ligand depletion?



Chemistry (mass-action law)

= f (free ligand)

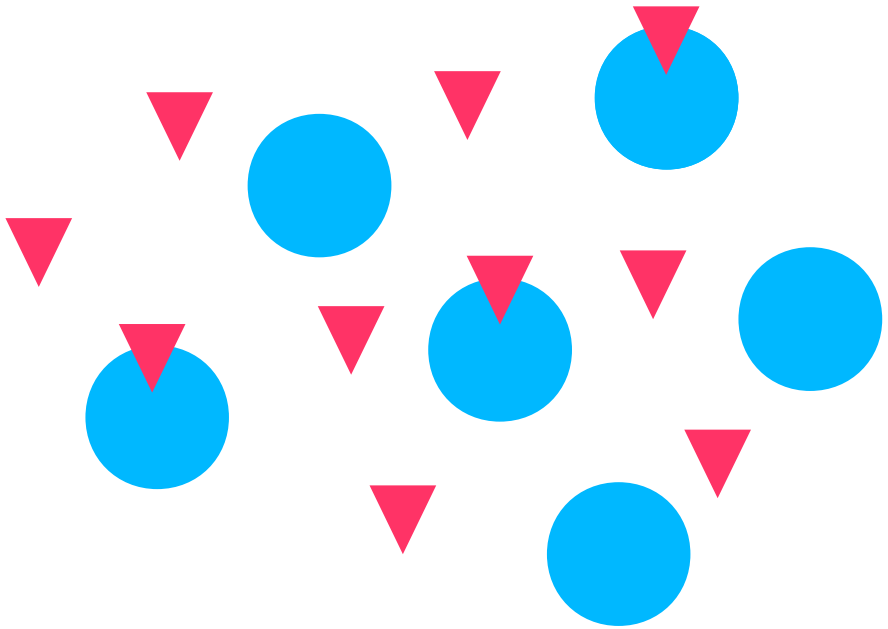
if + << Kd =

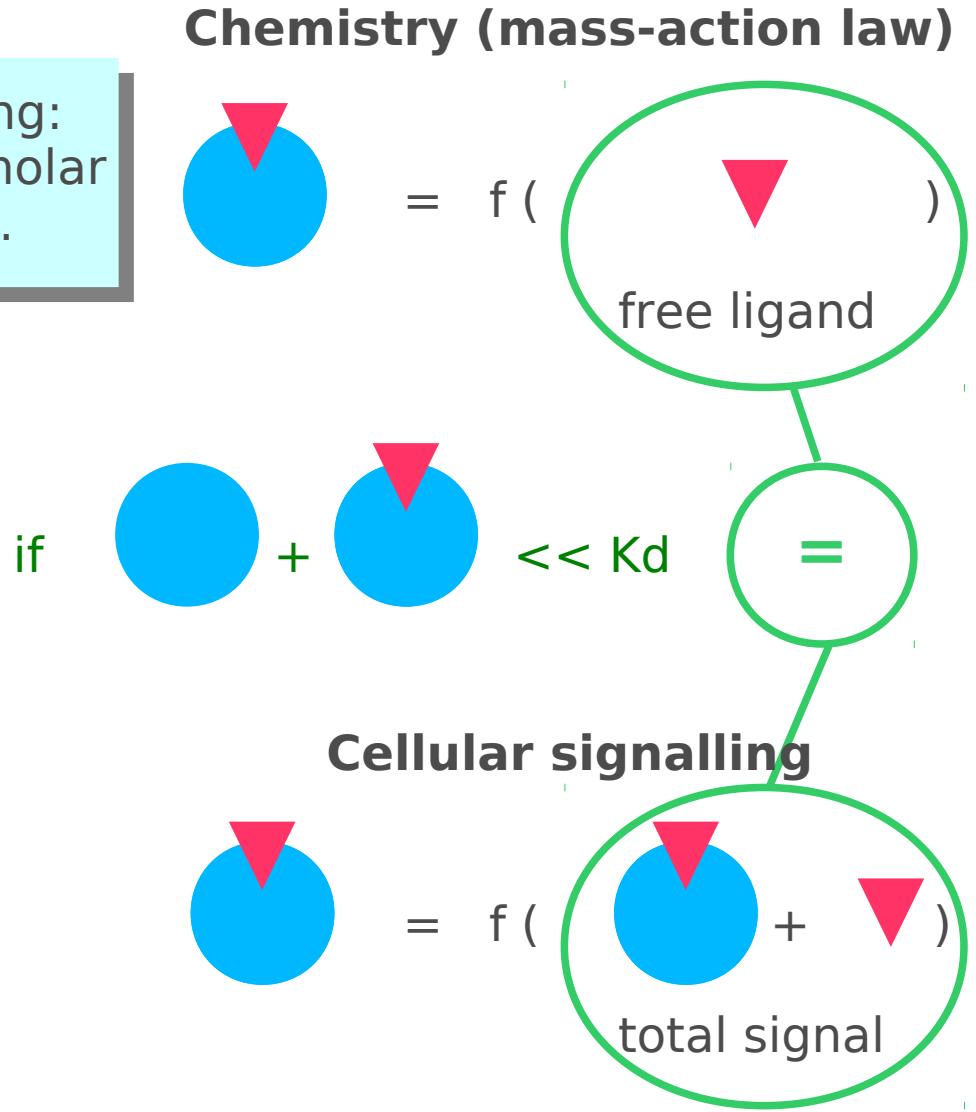
Cellular signalling

= f (+) total signal

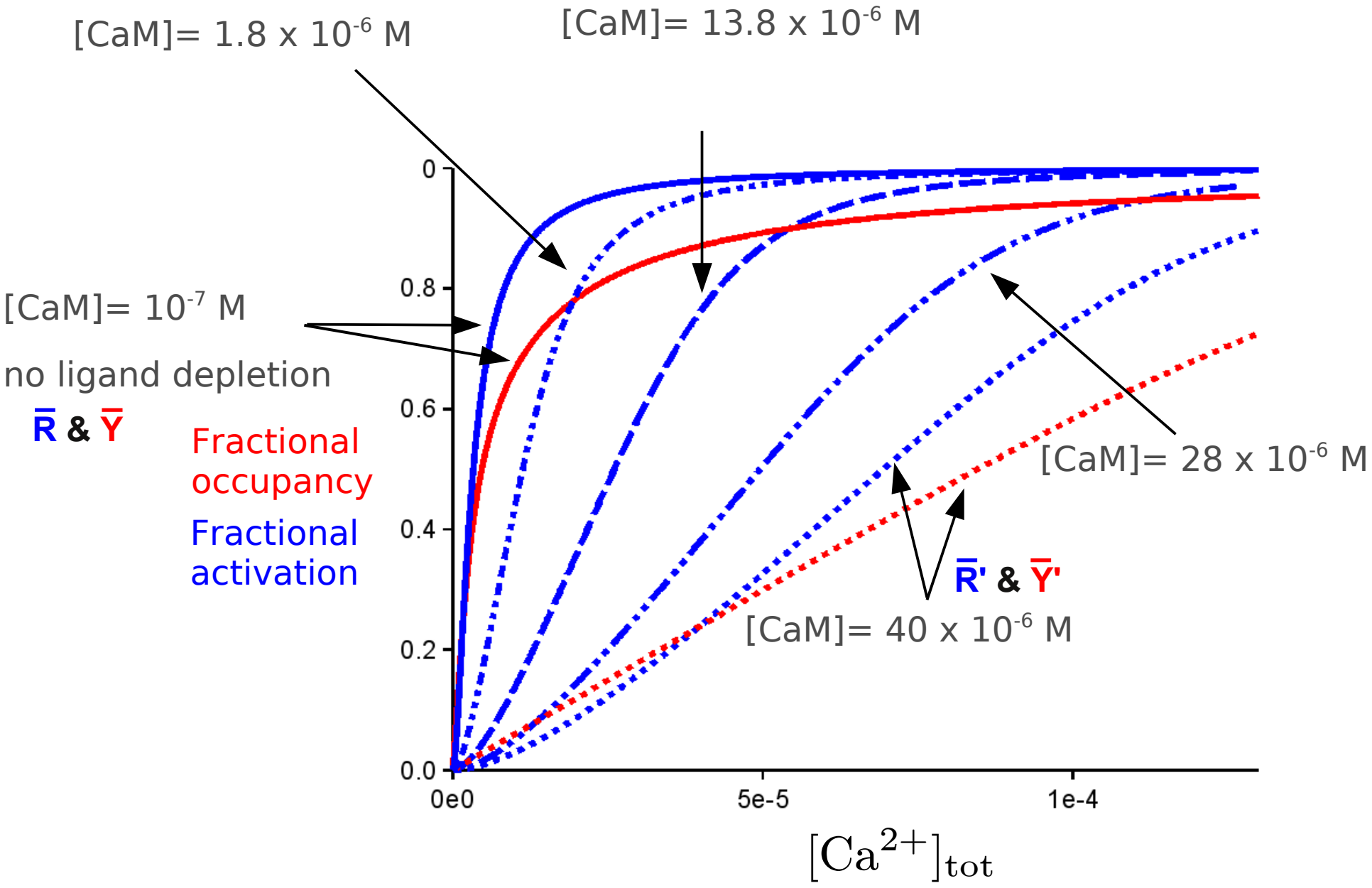
What is ligand depletion?

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

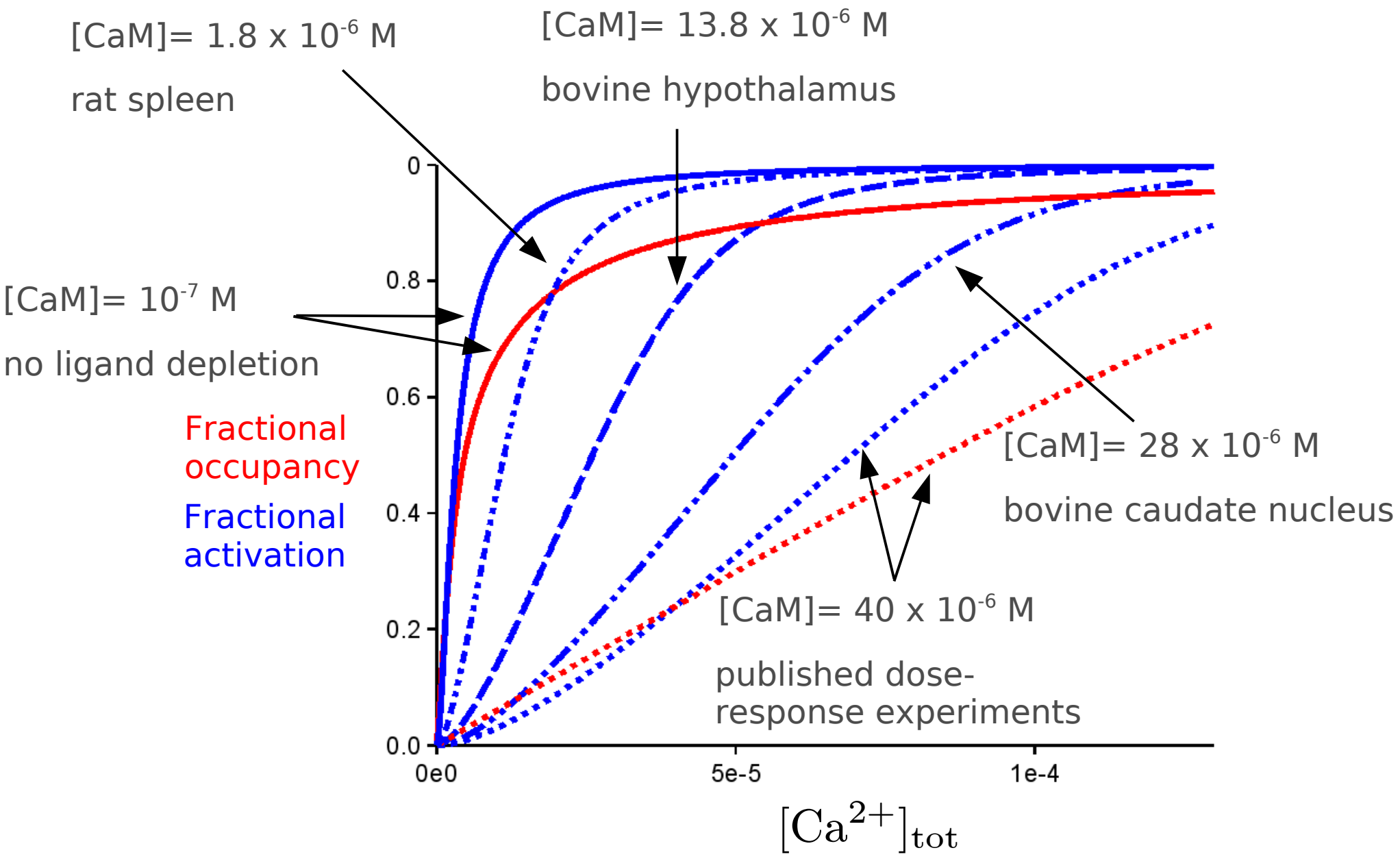




Dose-response depends on Calmodulin concentration



Dose-response depends on Calmodulin concentration



Ligand-depletion modifies sensitivity

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

bovine caudate nucleus

~5 μM to 120 μM

[CaM]= 1.8 x 10^{-6} M

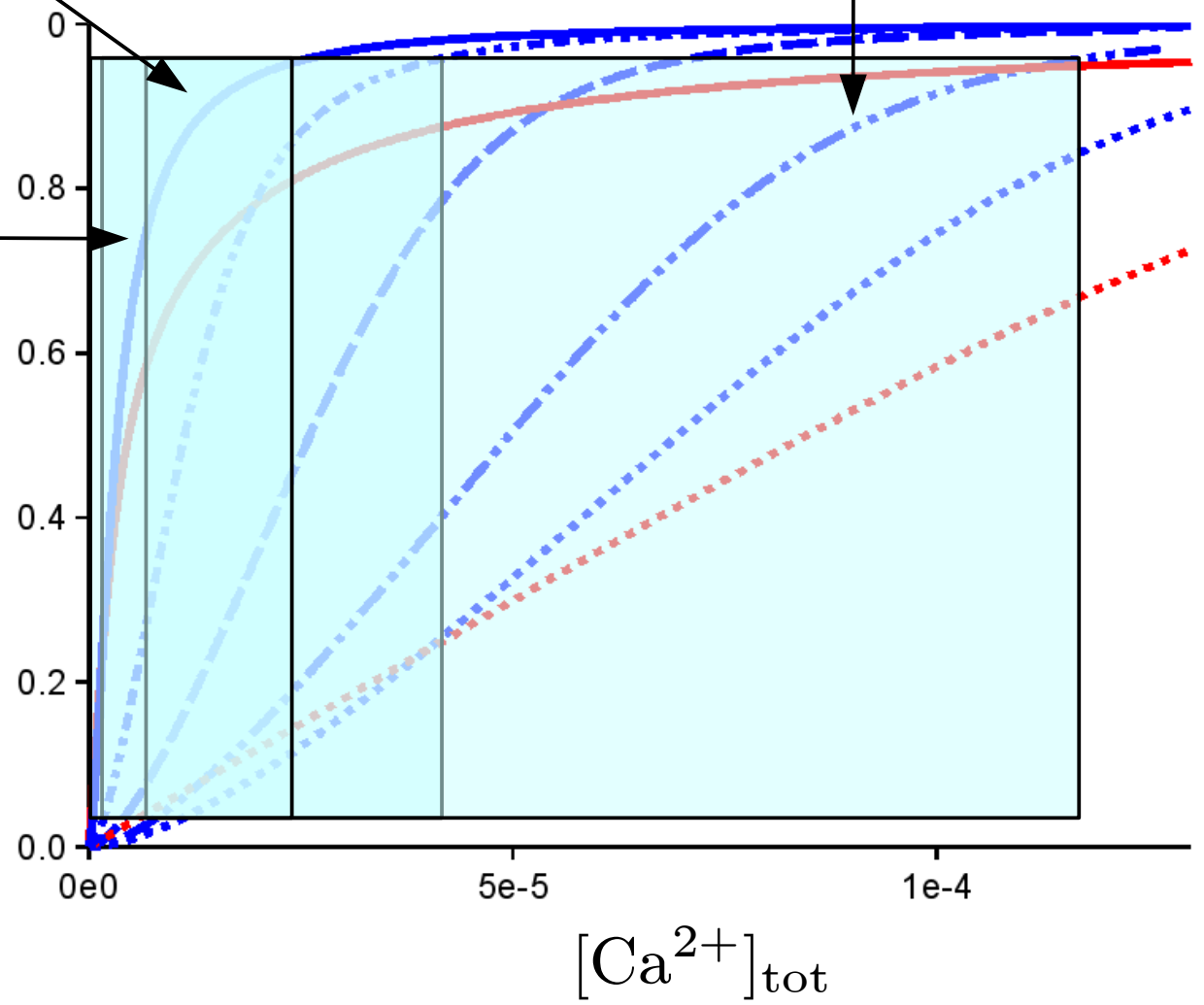
rat spleen

~1 μM to 45 μM

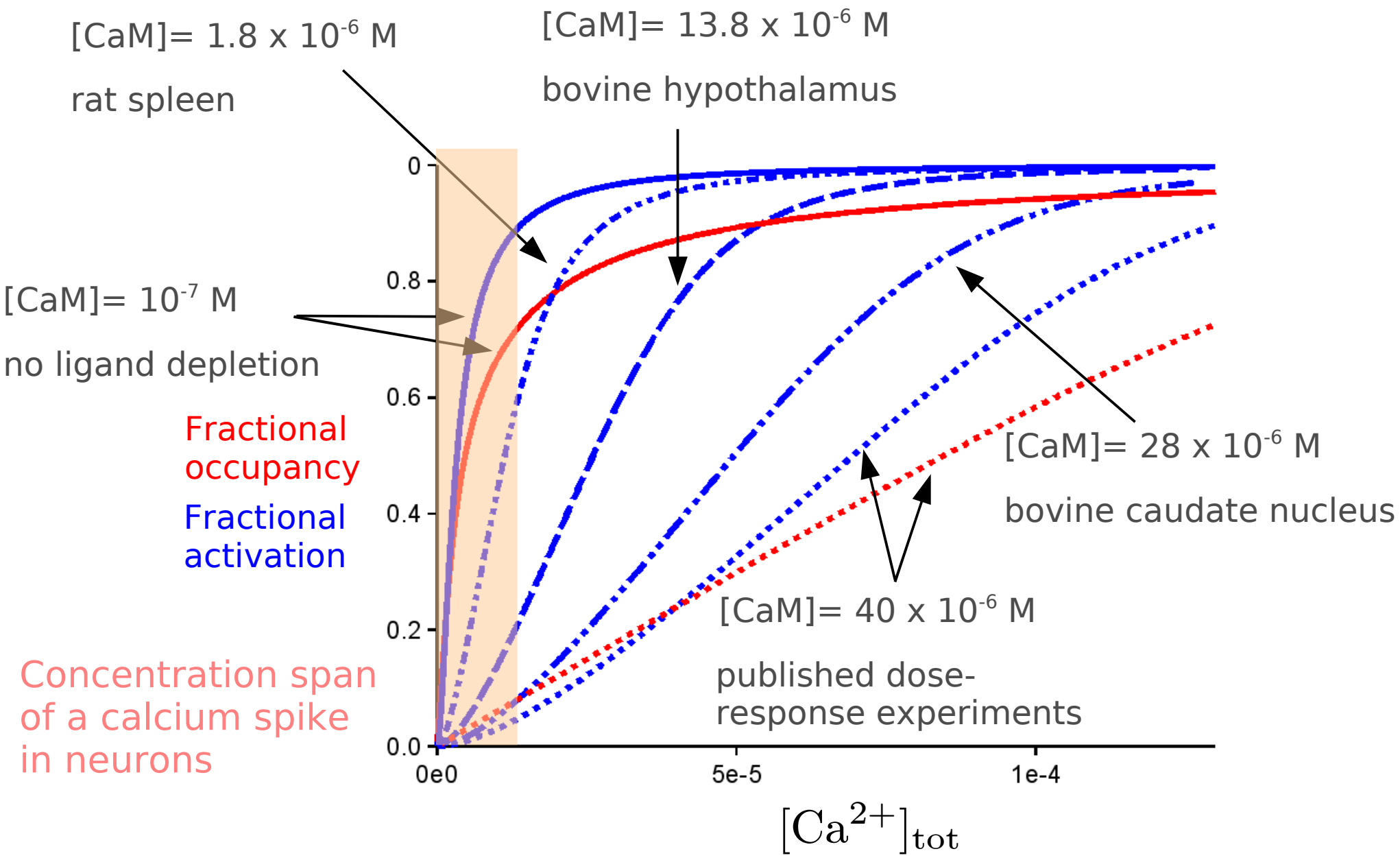
[CaM]= 10^{-7} M

no ligand depletion

~100 nM to 25 μM



But we cannot build a large $[Ca^{2+}]$ in neurons ...



Evaluating cooperativity: Hill Plot?

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

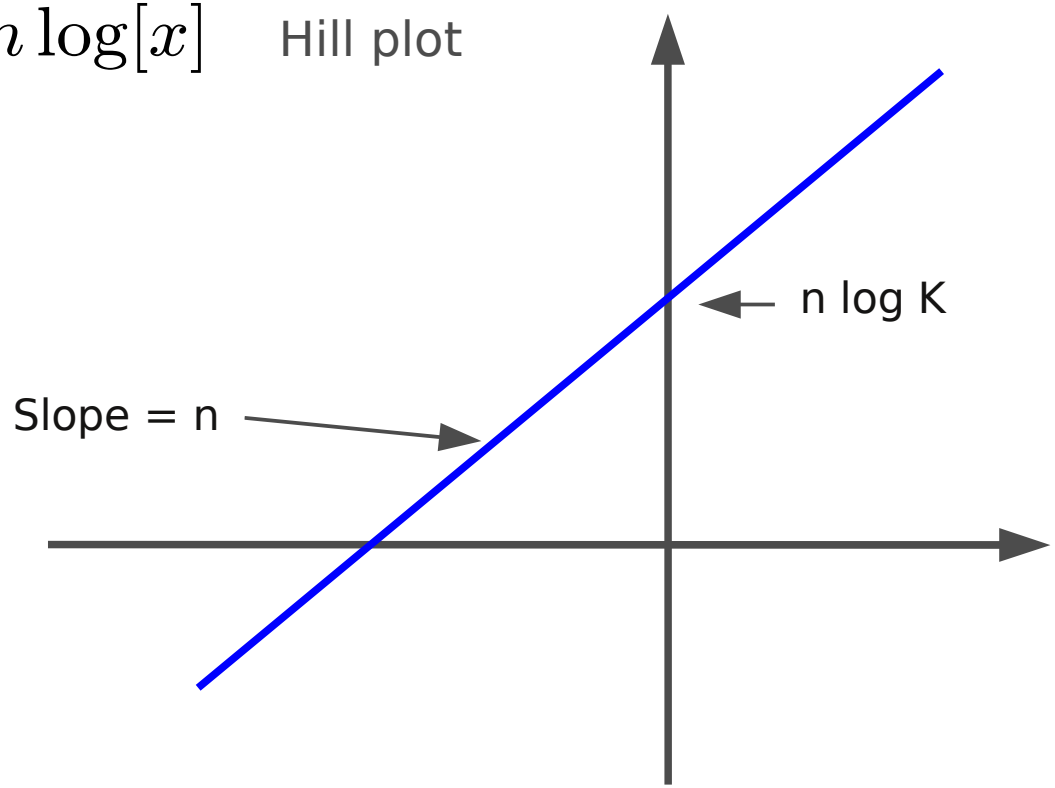
Hill equation

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

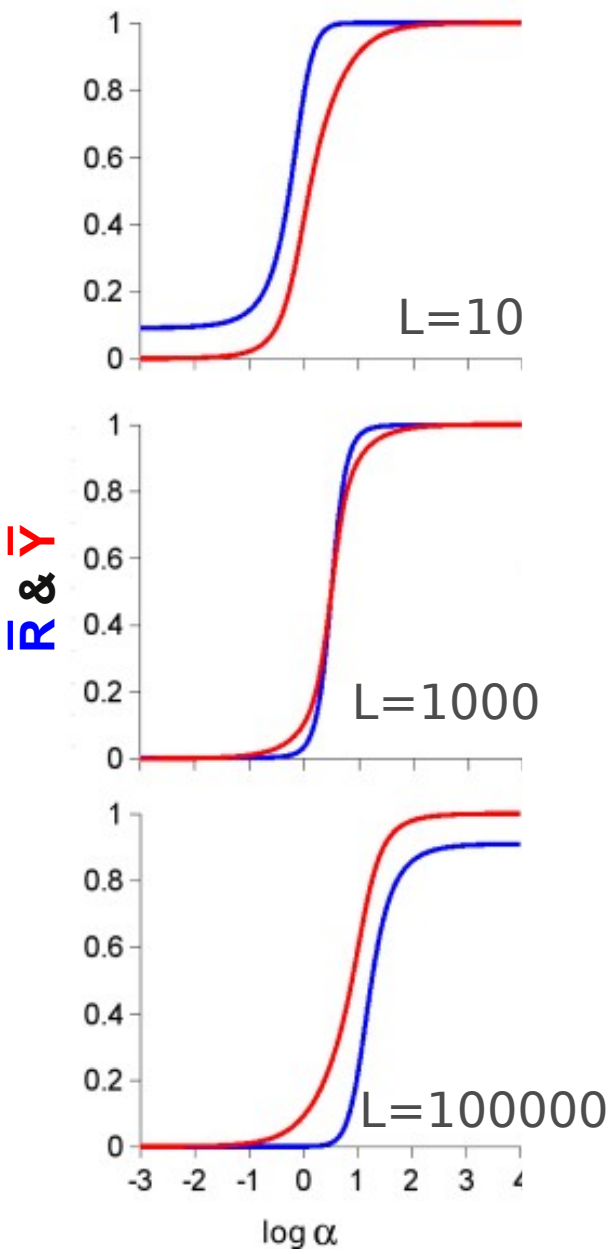
Hill plot

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



Hill number not suitable for state function



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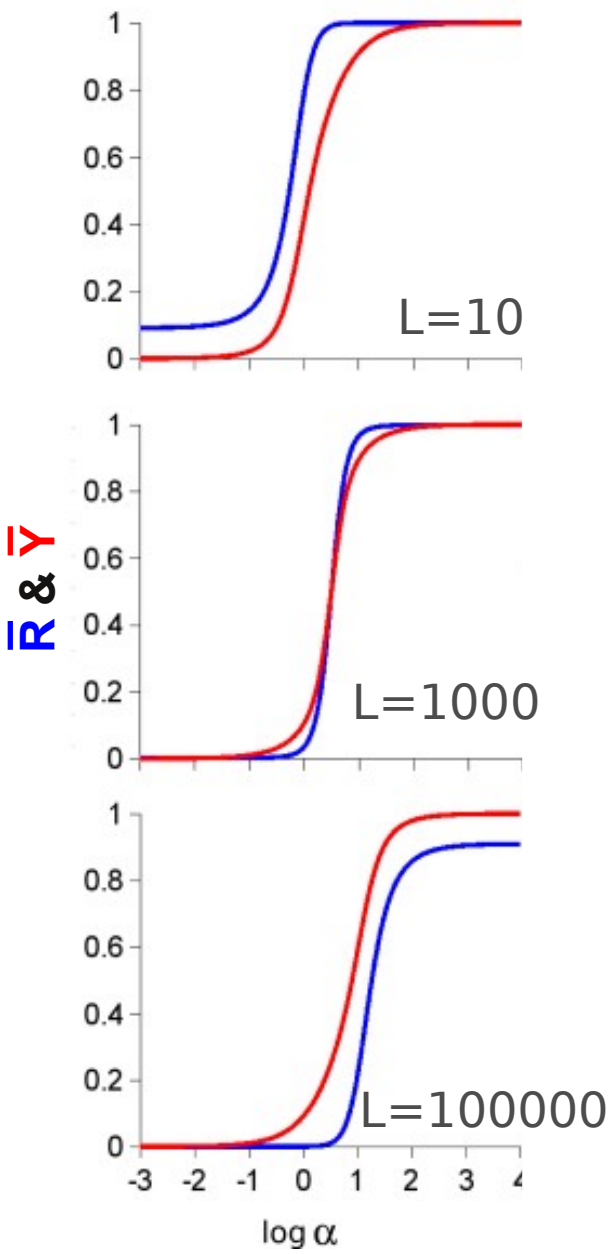

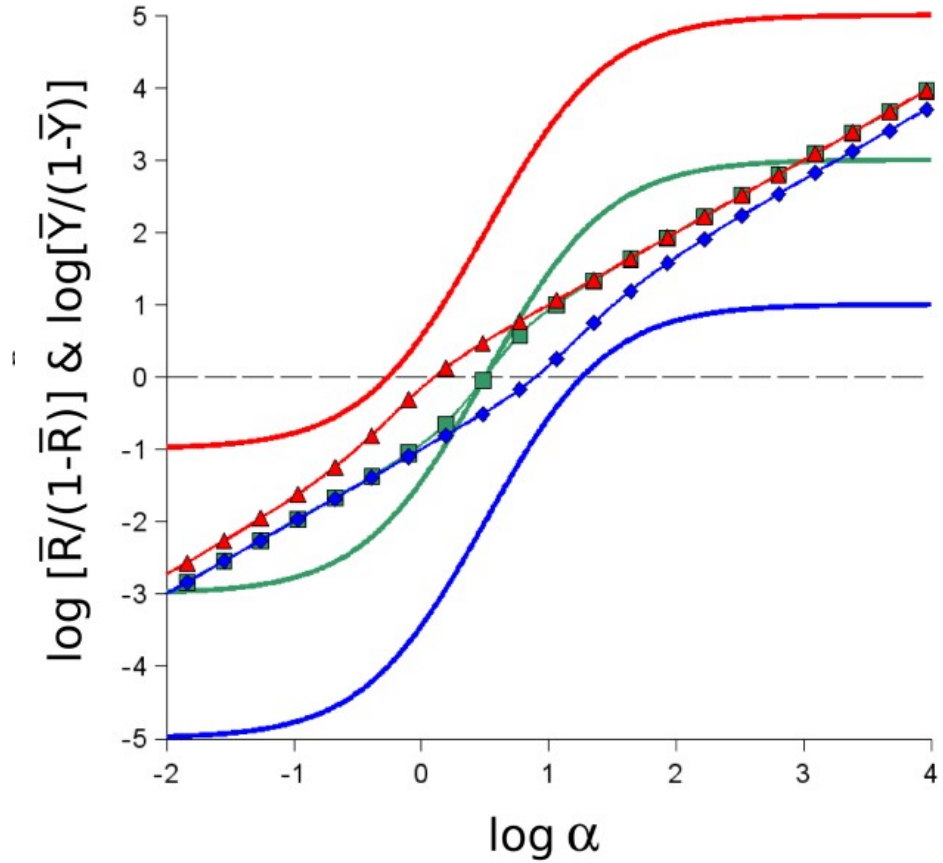

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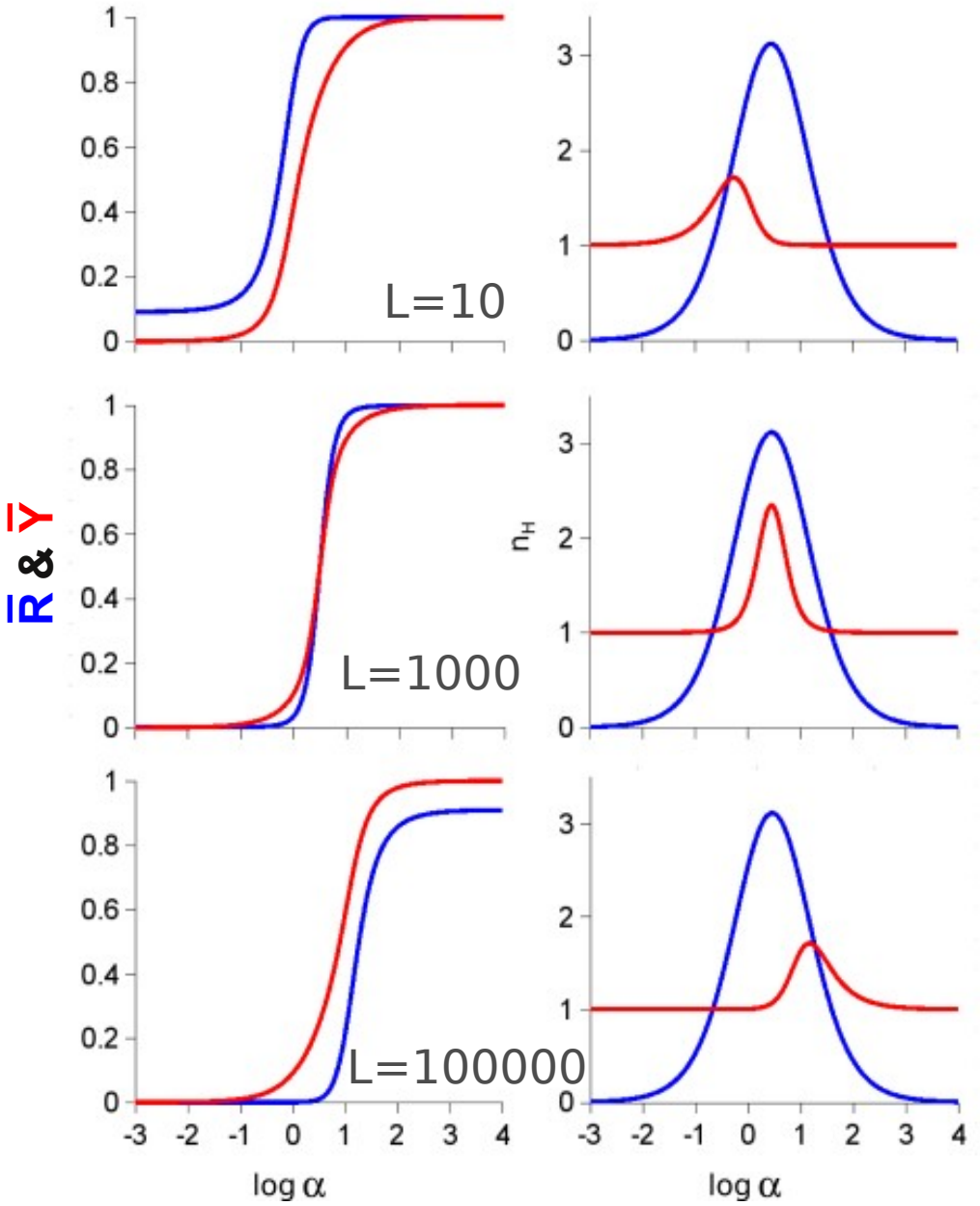
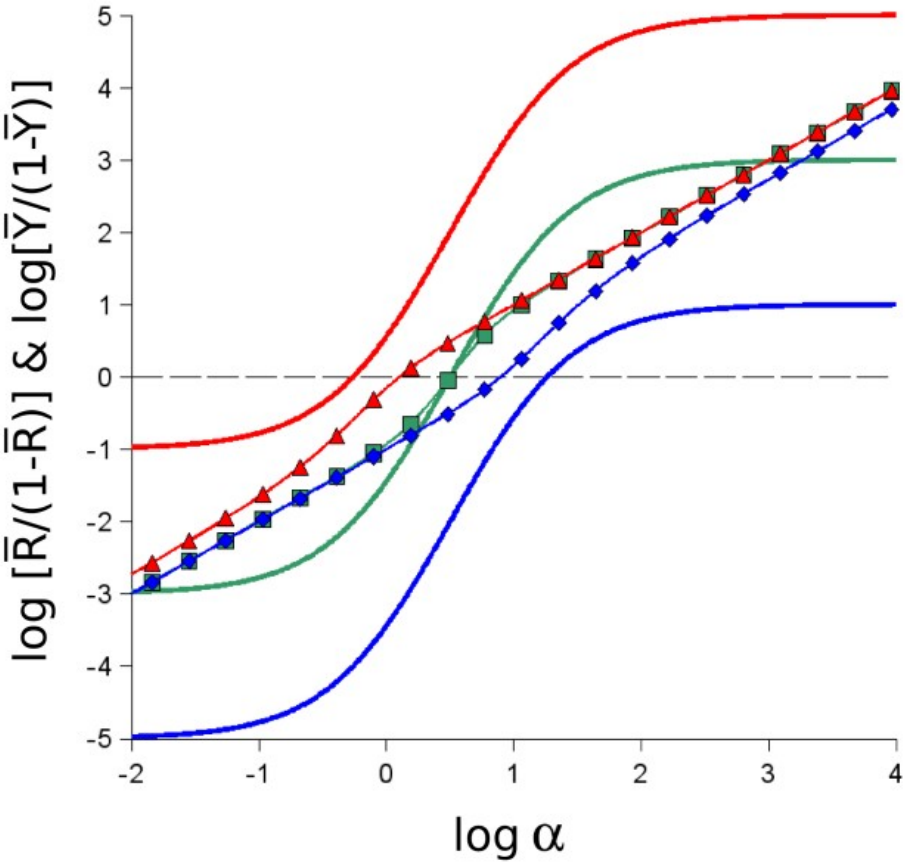
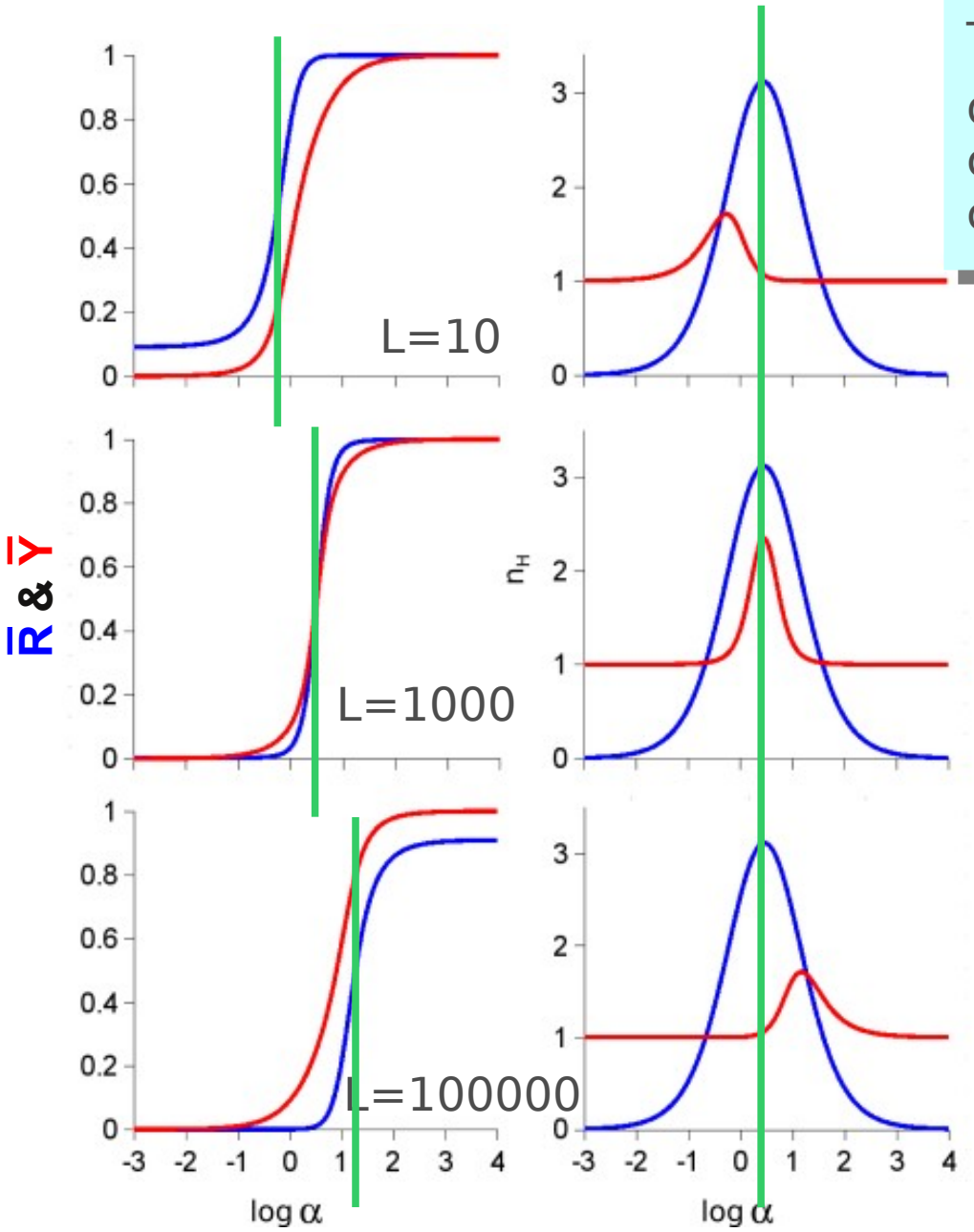
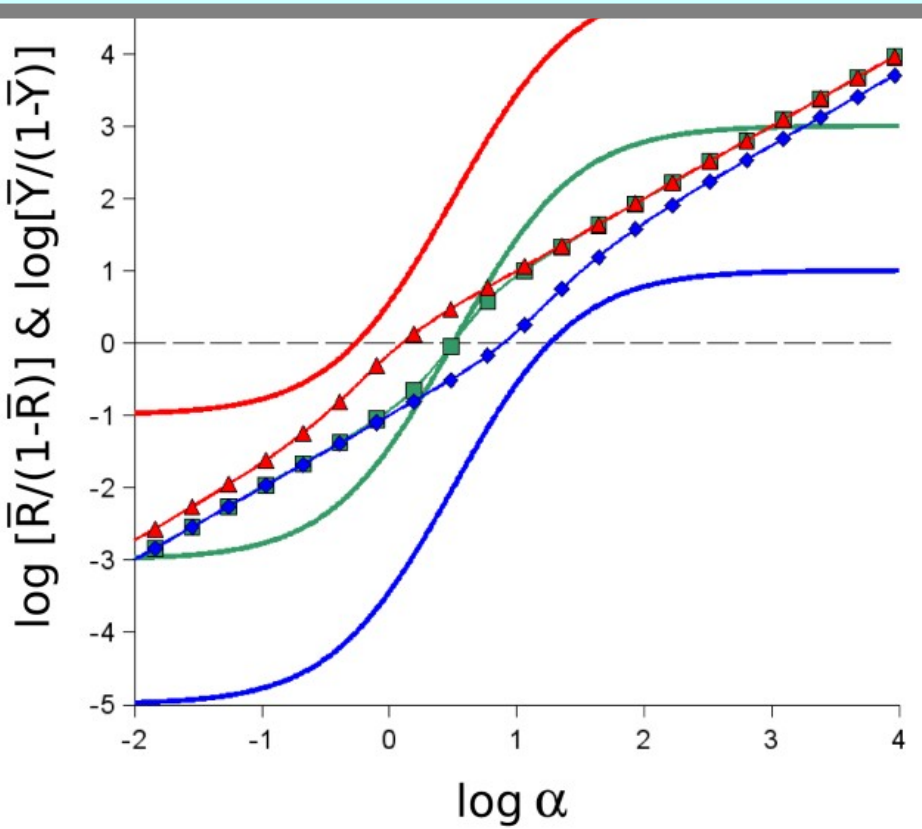




Hill number not suitable for state function

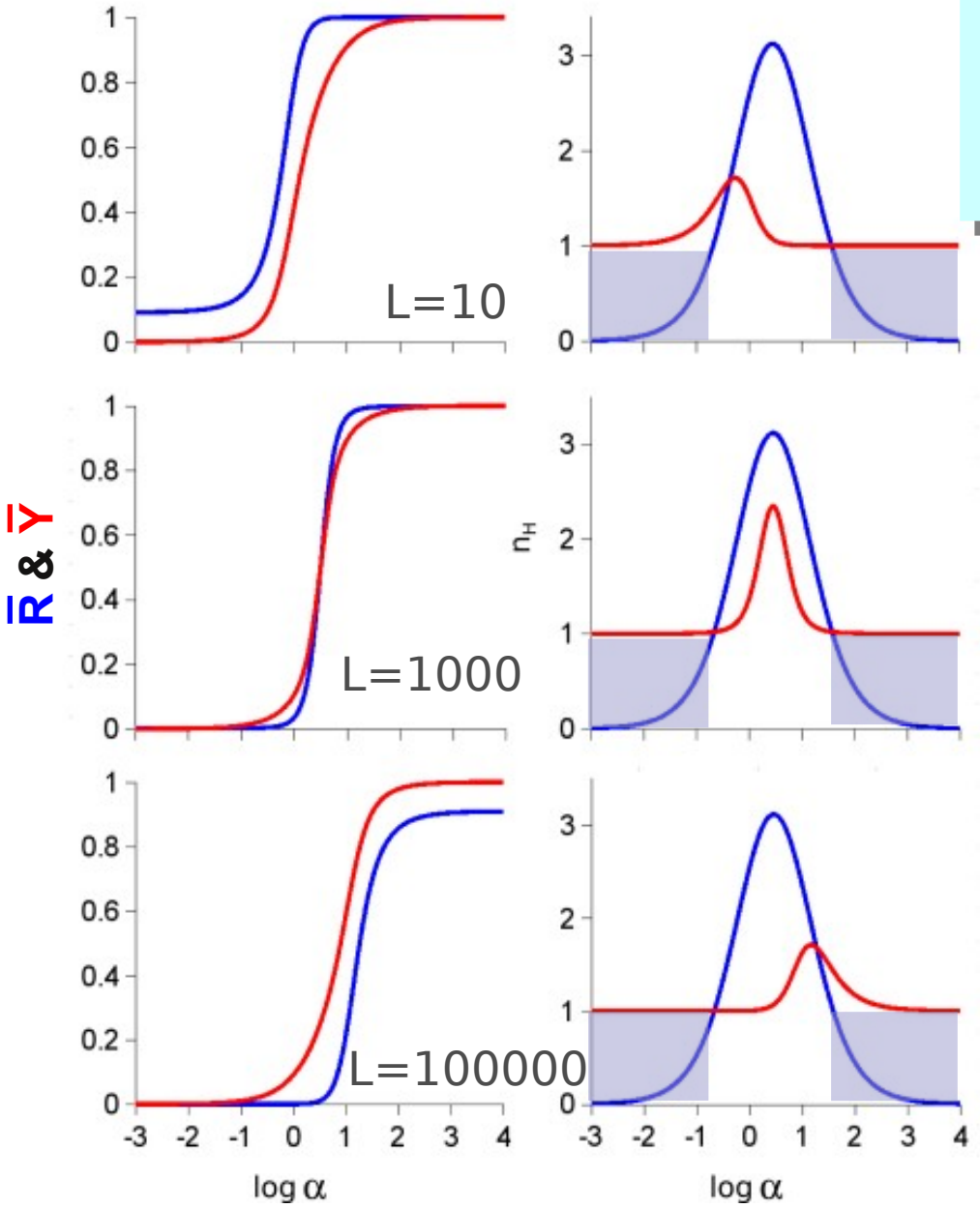
The maximum value of n_H does not depend on the free energy of conformational transition and does correspond to the maximal cooperativity

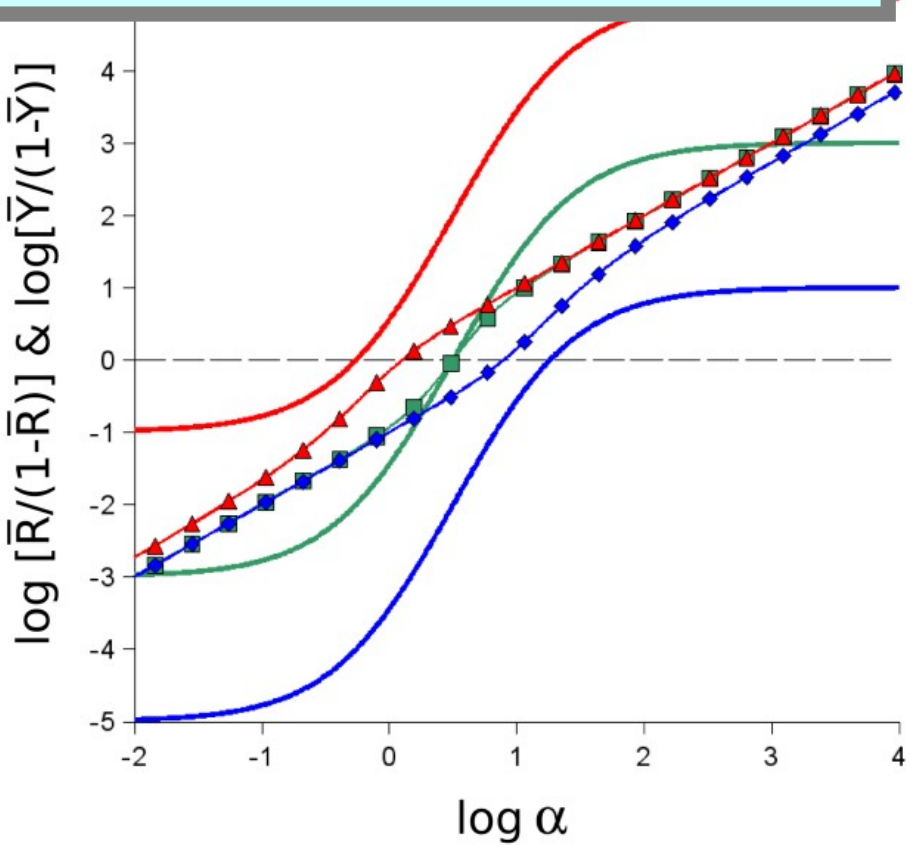




Hill number not suitable for state function

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





Equivalent monomer

$$\bar{R} = \frac{(1+\alpha)^N}{L(1+c\alpha)^N + (1+\alpha)^N}$$ can be rearranged $$\bar{R} = \frac{1}{1 + L\left(\frac{1+c\alpha}{1+\alpha}\right)^N}$$

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

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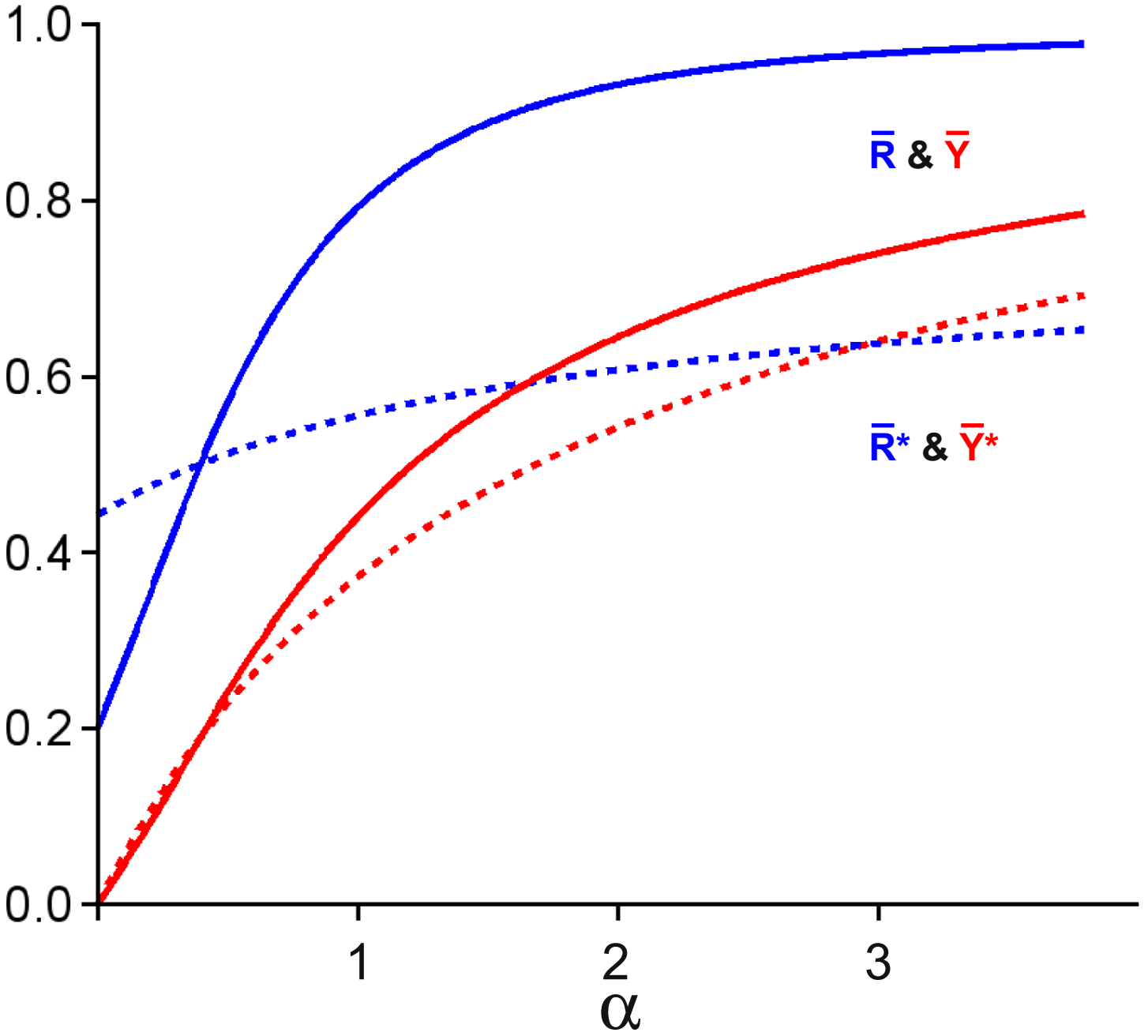
$$\lambda = \sqrt[N]{L}$$

State function for an equivalent monomer

$$\bar{R}^* = \frac{1}{1+\lambda\Omega}$$

Equivalent monomer for calmodulin



New index of cooperativity

$$\bar{R} = \frac{1}{1 + L\Omega^N}$$

$$\bar{R}^* = \frac{1}{1 + \lambda\Omega}$$

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$$\nu = \frac{d\bar{R}/d\alpha}{d\bar{R}^*/d\alpha} = \frac{N(1+\lambda\Omega)^2(\lambda\Omega)^{N-1}}{(1+(\lambda\Omega)^N)^2}$$

ν is insensitive too ligand depletion!

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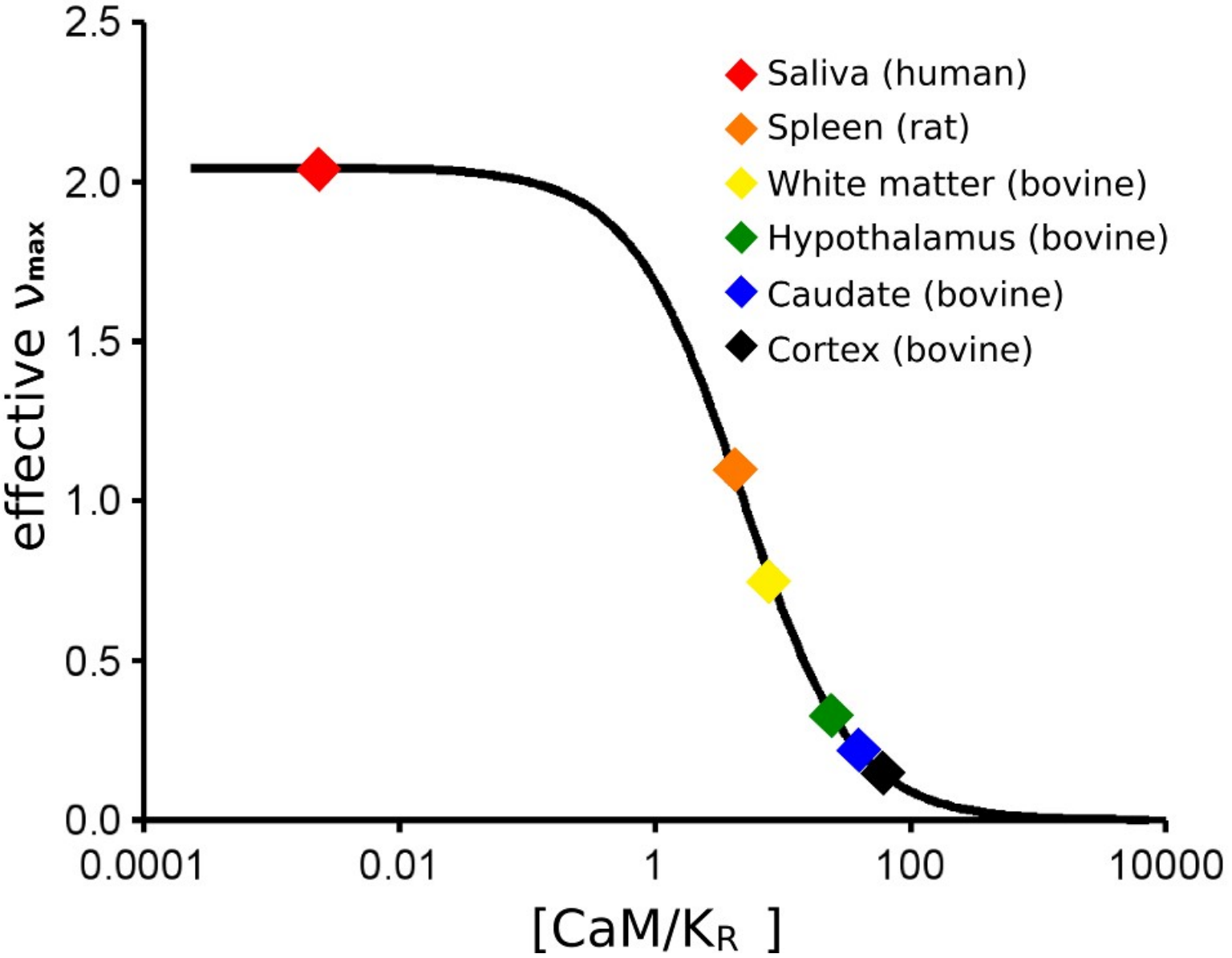
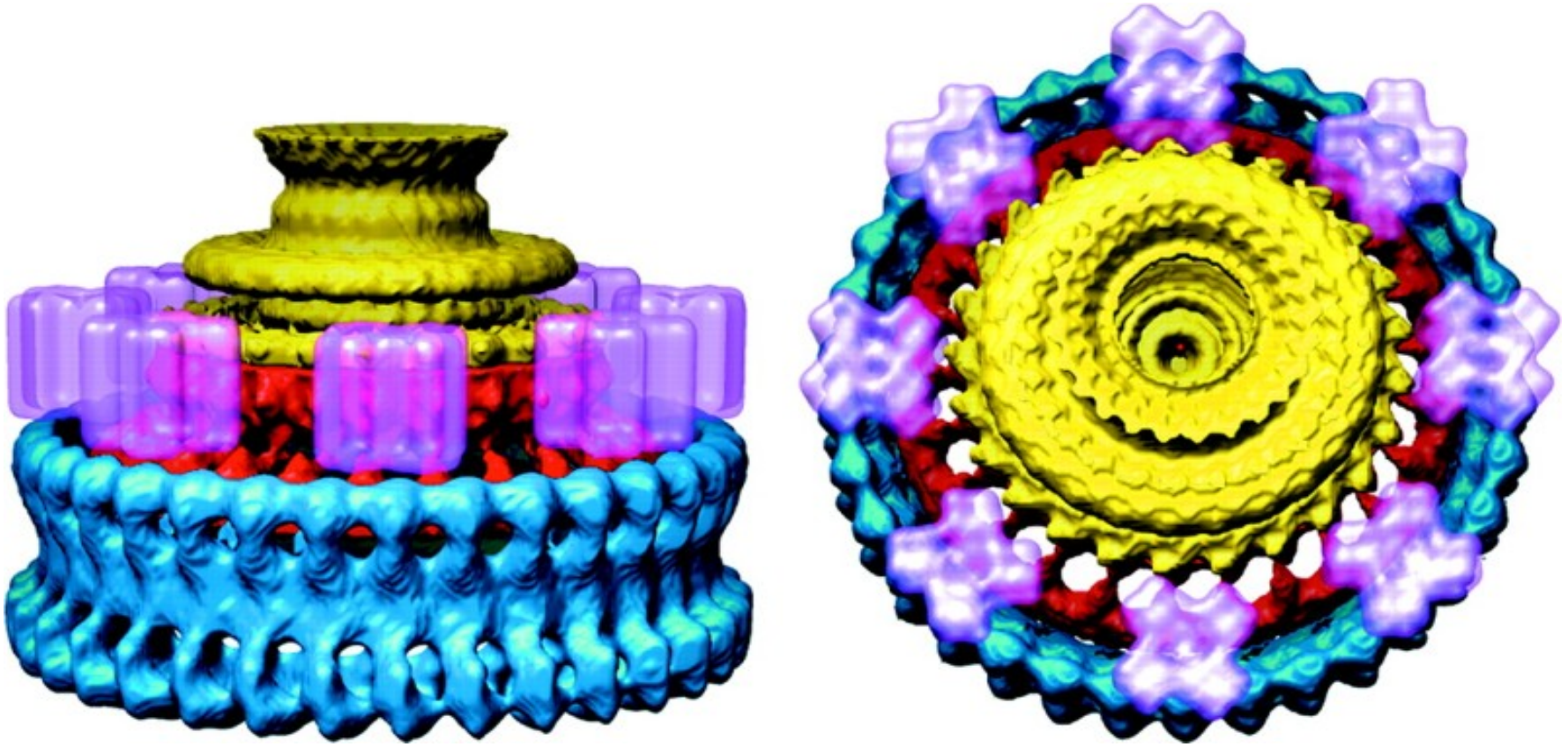
$$\text{effective } \nu = \frac{d\bar{R}'/d\alpha}{\nu}$$

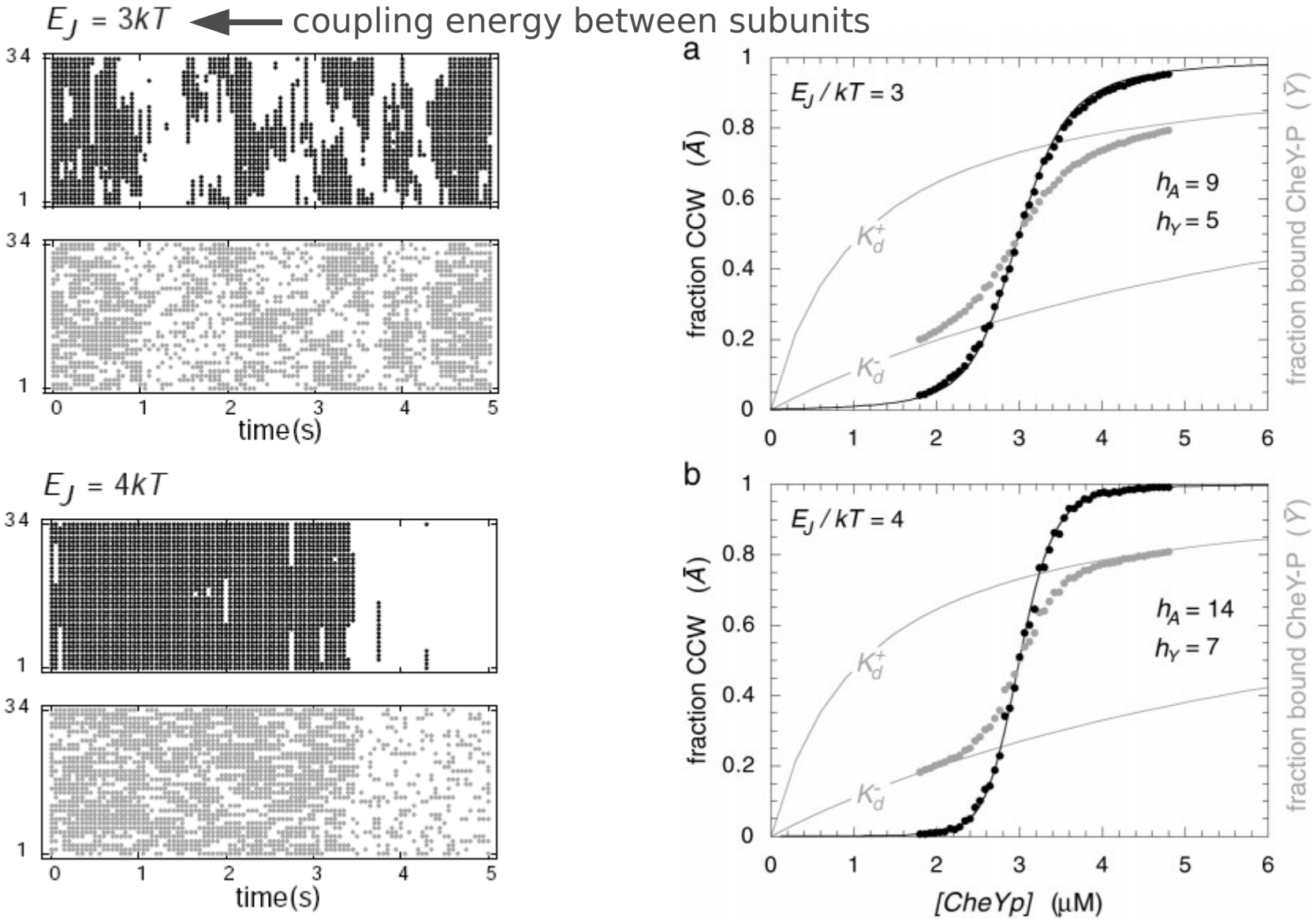
Ligand-depletion decreases effective cooperativity



Highly cooperative: bacterial flagellar motor

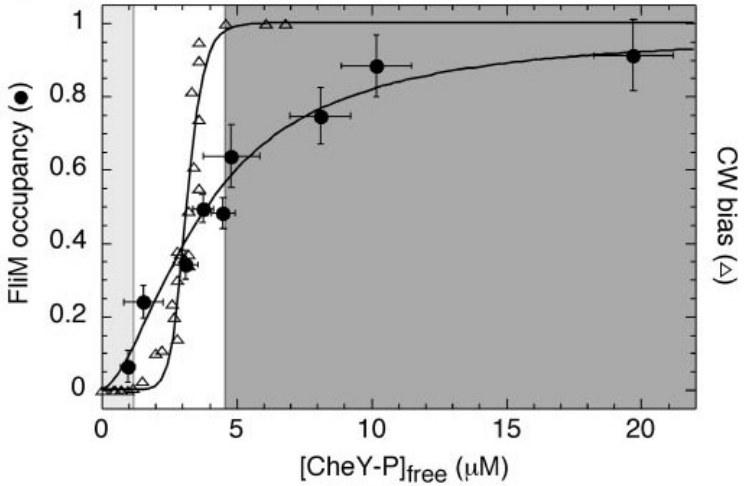


Concerted behaviour of bacterial flagellar



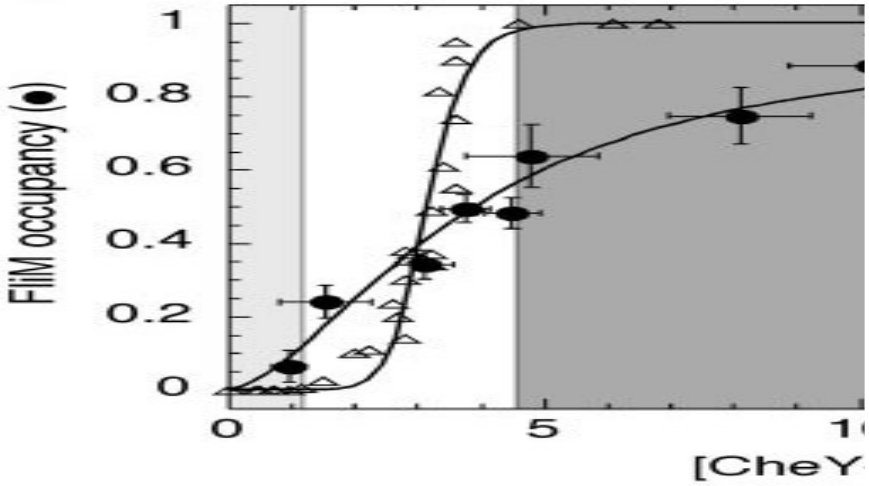
Duke, Le Novère and Bray (2001) Conformational spread in a ring of proteins: a stochastic approach to allostery. *J Mol Biol*, 308:541-553.

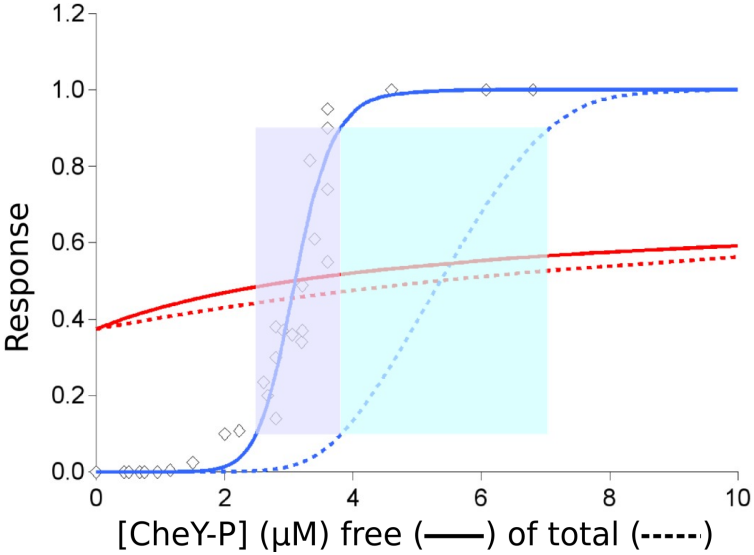
Effect of ligand depletion on dynamic range



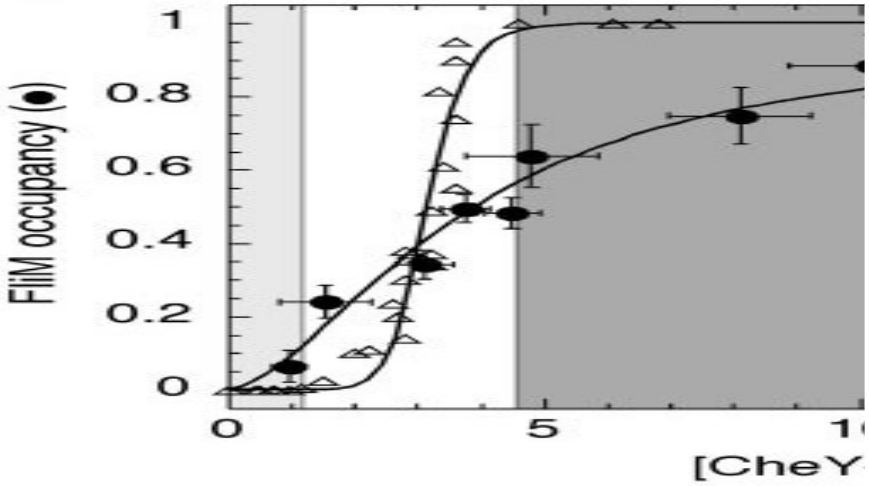
Sourjick and Berg (2002) Binding of the Escherichia coli response regulator CheY to its target measured in vivo by fluorescence resonance energy transfer. *PNAS* 99: 12669-12674

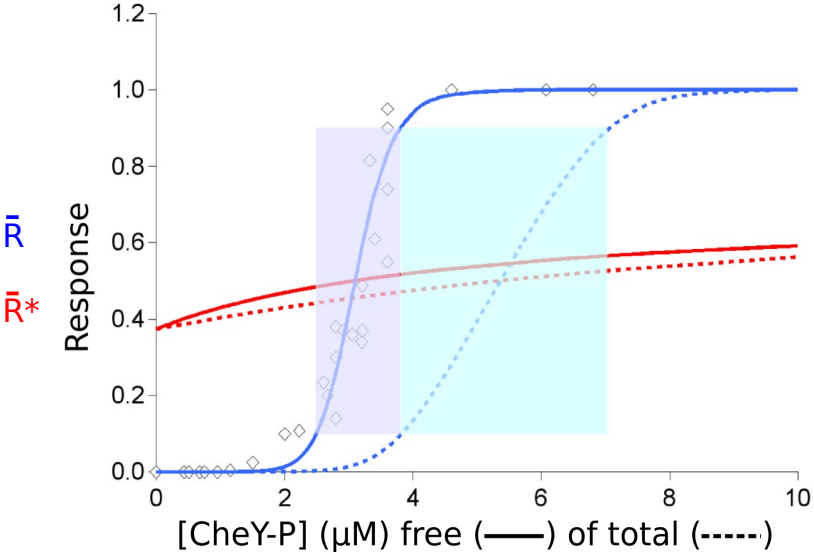
Ligand-depletion increases dynamic range

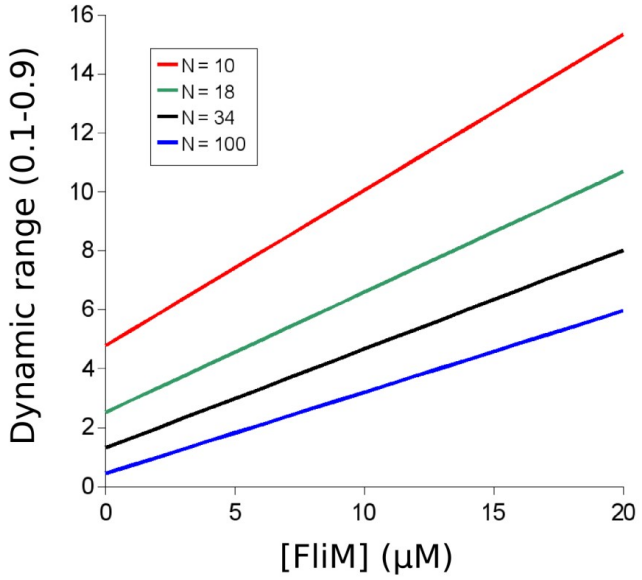


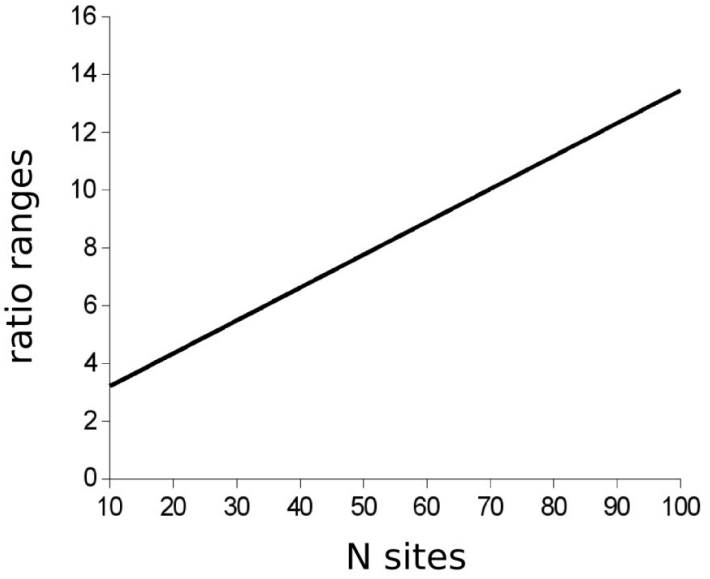


Ligand-depletion increases dynamic range

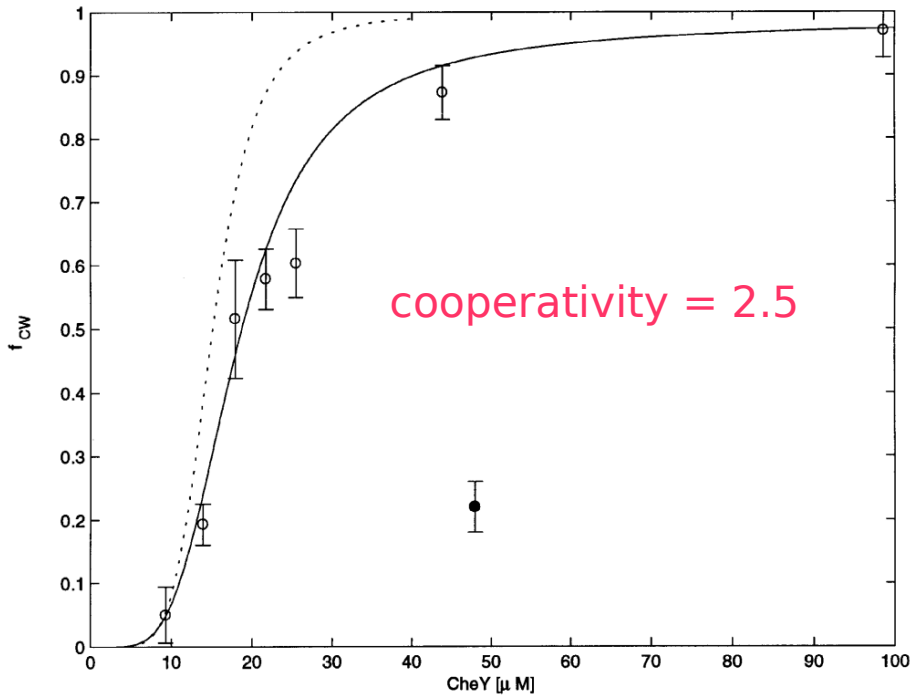




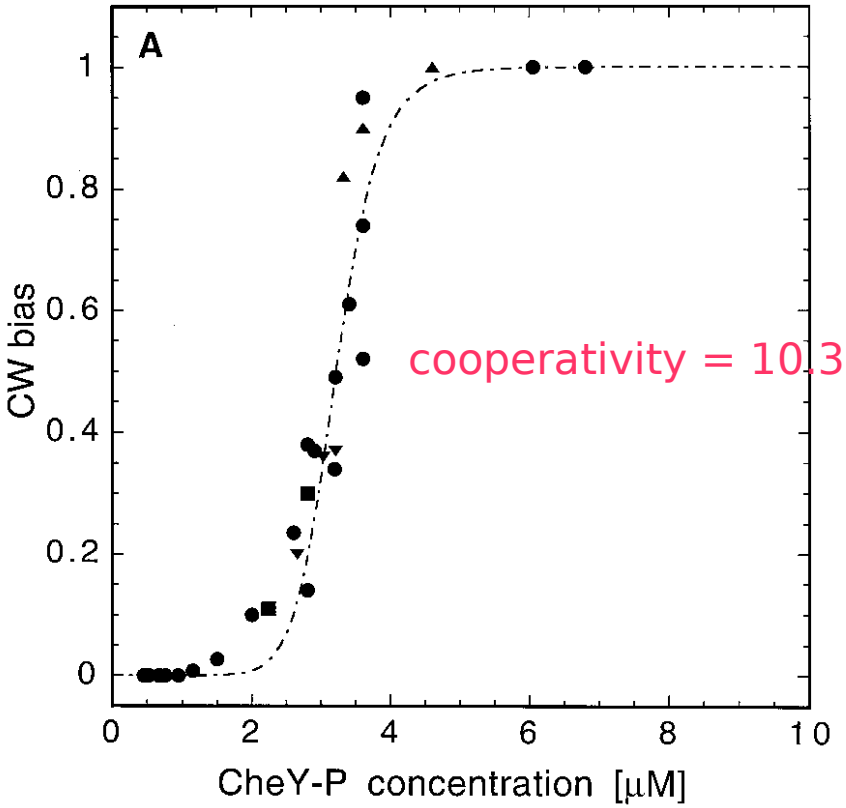




Ligand depletion explains different reports

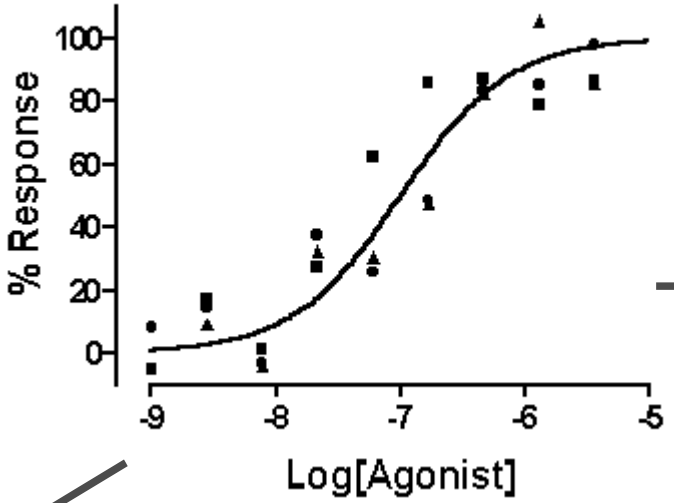


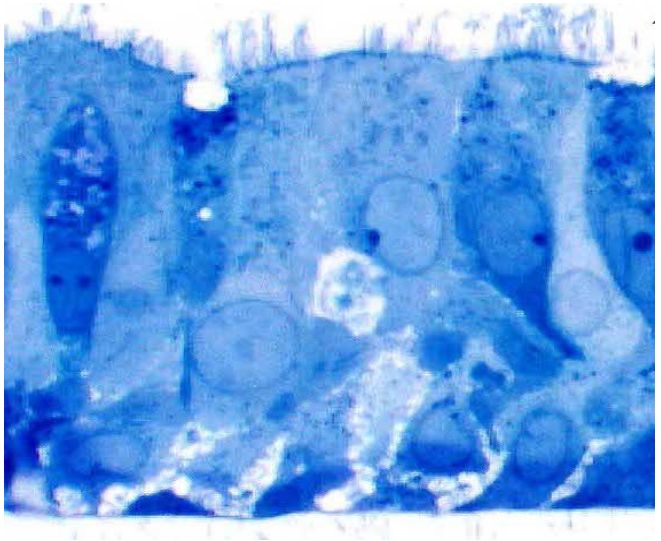
Alon et al (1998) Response regulator output in bacterial chemotaxis. *EMBO J* 17: 4238-4248

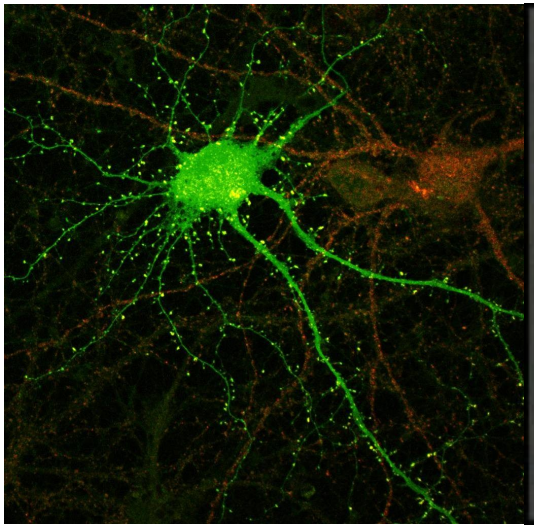


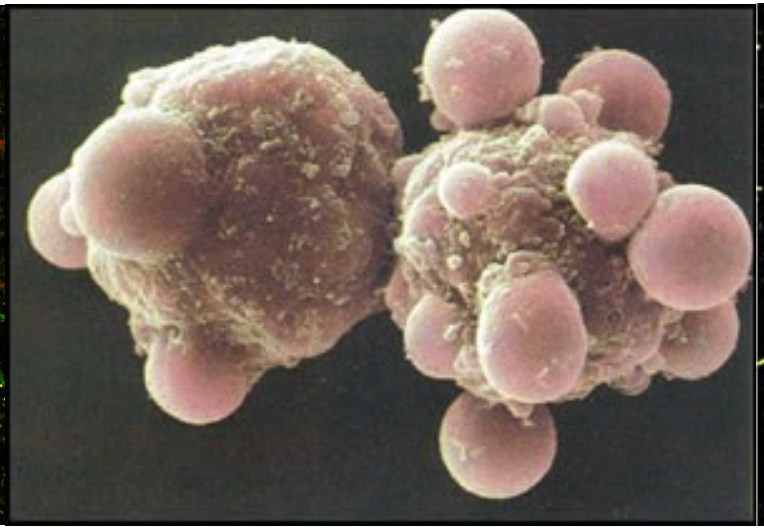
Cluzel et al (2000) An ultrasensitive bacterial motor revealed by monitoring signalling proteins in single cells. *Science* 287: 1652-1655

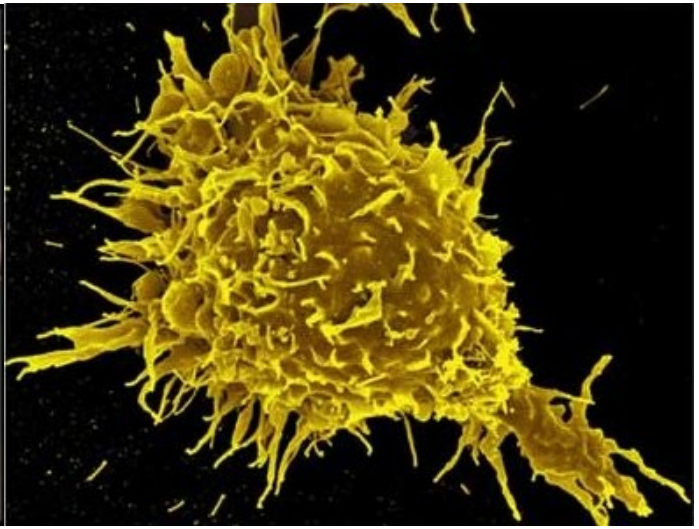
How general is a dose-response?



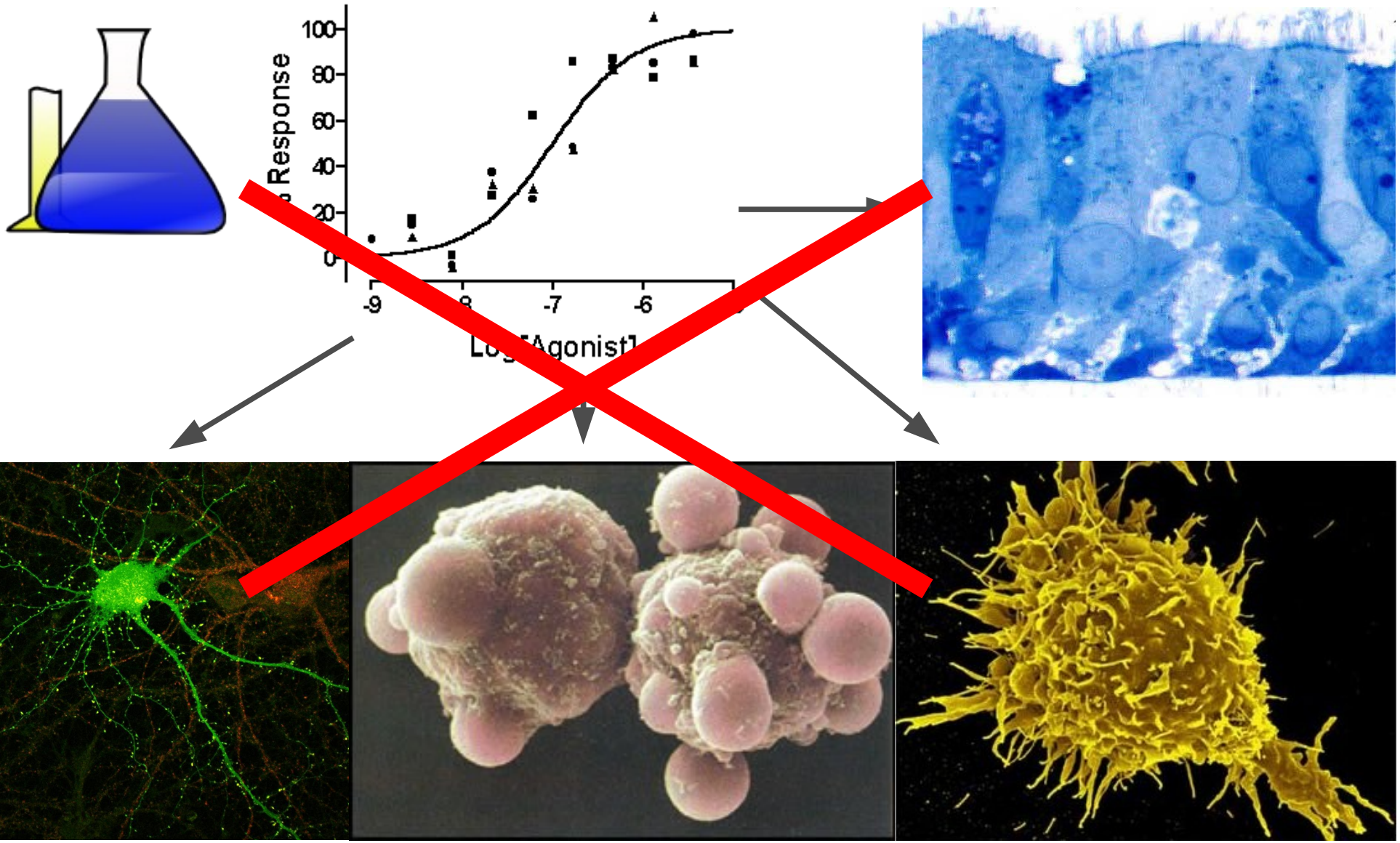








A “dose-response” cannot be reused directly!



Conclusions

- Dose-responses are the basic characterisations of “systems”, but also at the core of pharmacological treatments. Here we show that:
  - A “dose-response”cannot be reused directly in models of signalling systems. Instead one needs to build “mechanistic” models and run parameter-fitting approaches.
  - Ligand depletion decreases the effective cooperativity of transducers *in situ*
  - 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.

Acknowledgements

- Developers of COPASI
  - Sven Sahle
  - Stefan Hoops
  - Ursula Kummer
  - Pedro Mendes
- Developers of Scilab
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- Stephen Martins

Melanie Stefan

Stuart Edelstsein