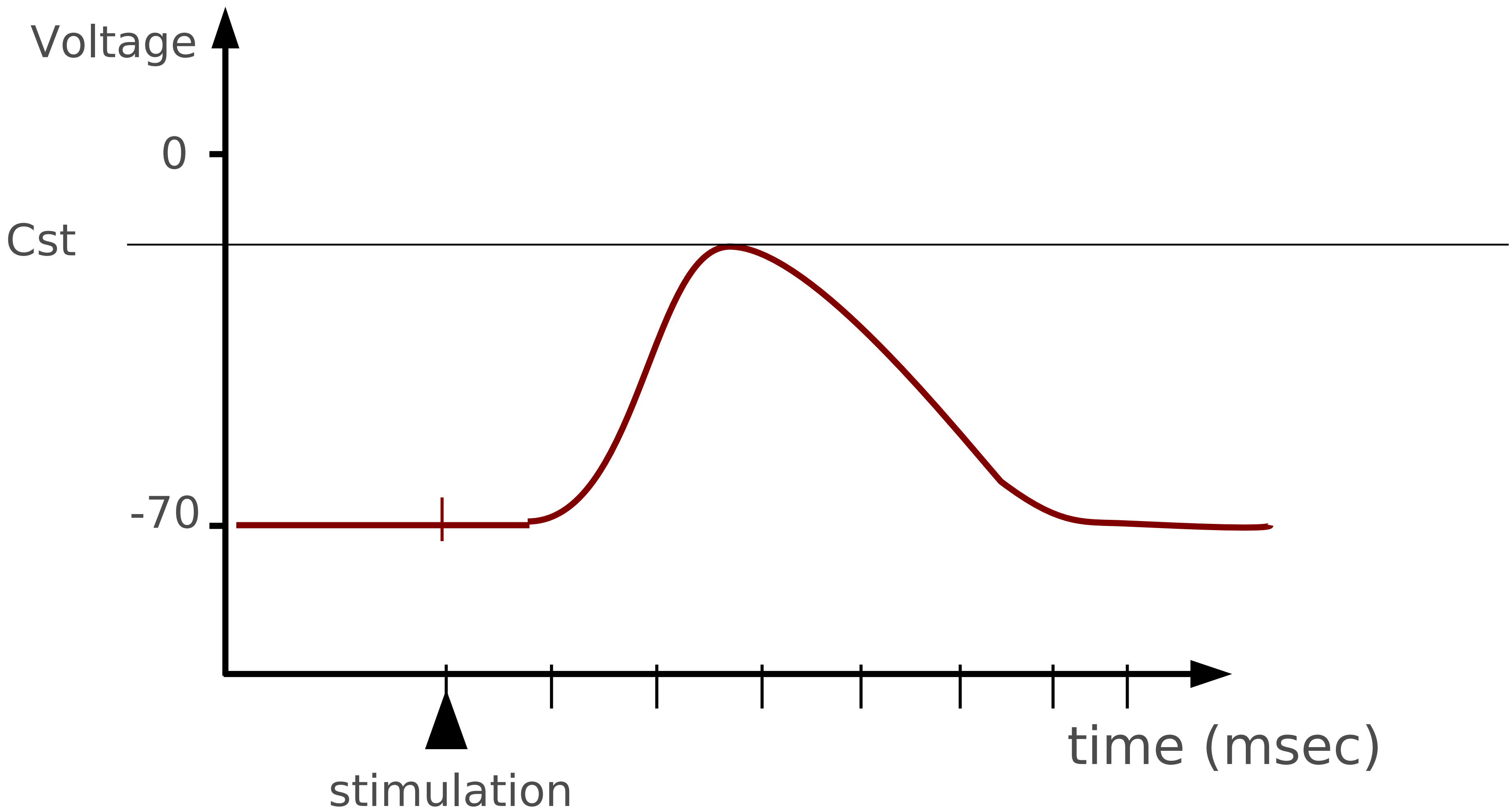
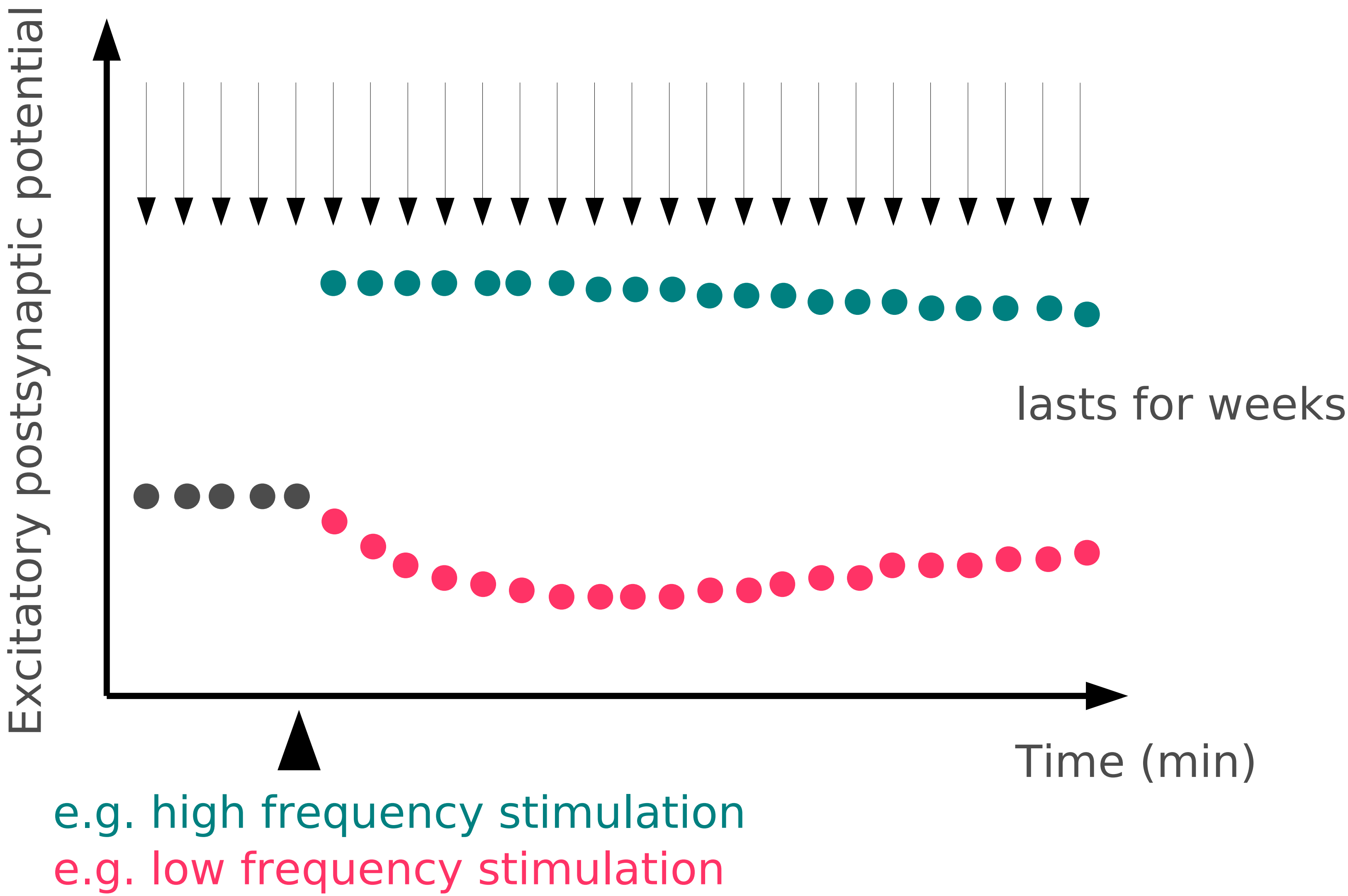
Modelling the Response of Allosteric Calcium Sensors Involved in Synaptic Plasticity

Nicolas Le Novère, EMBL-EBI

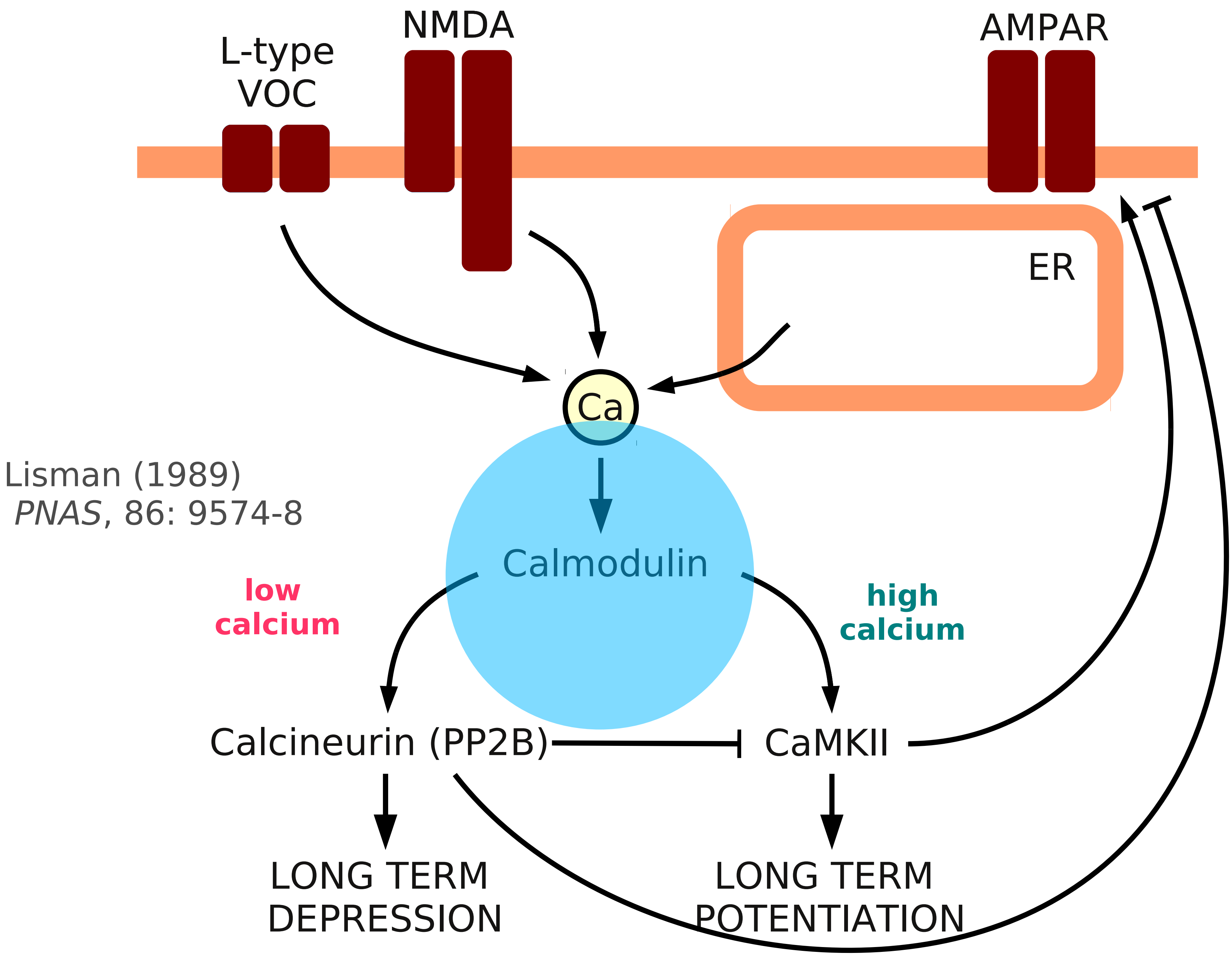
Excitatory post-synaptic potential



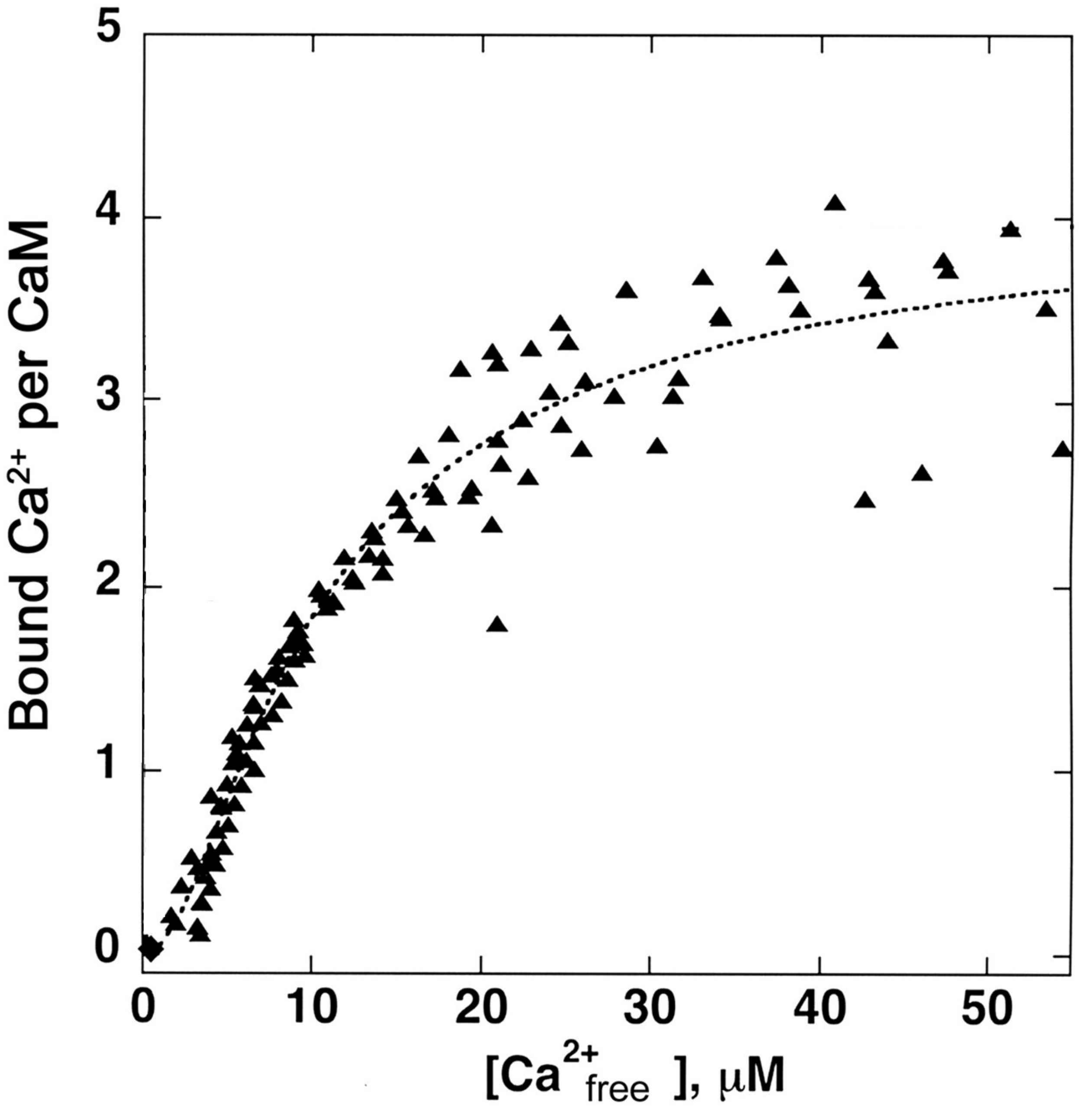
Bidirectional synaptic plasticity



Calmodulin, the memory switch

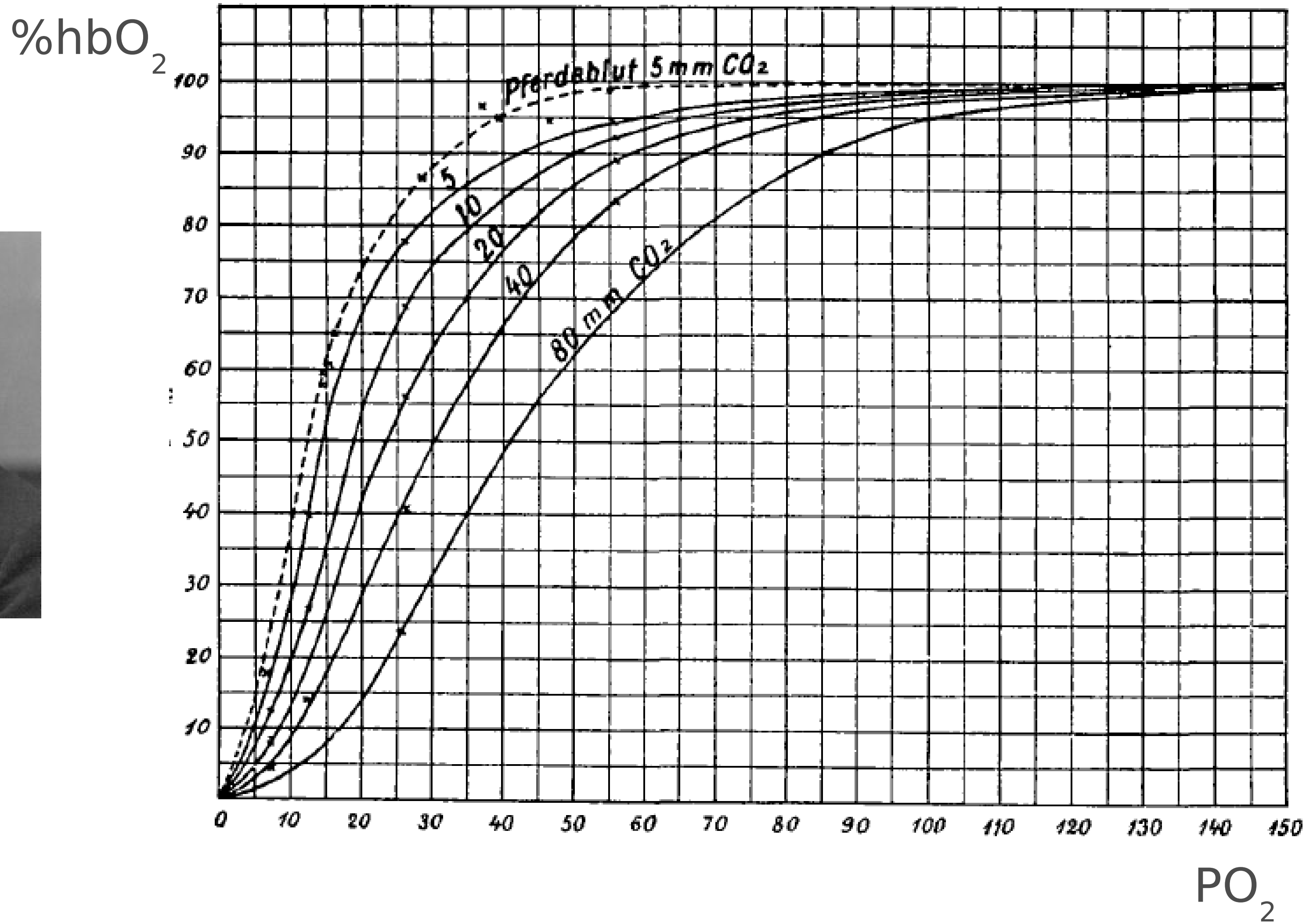


Calmodulin is ultra-sensitive



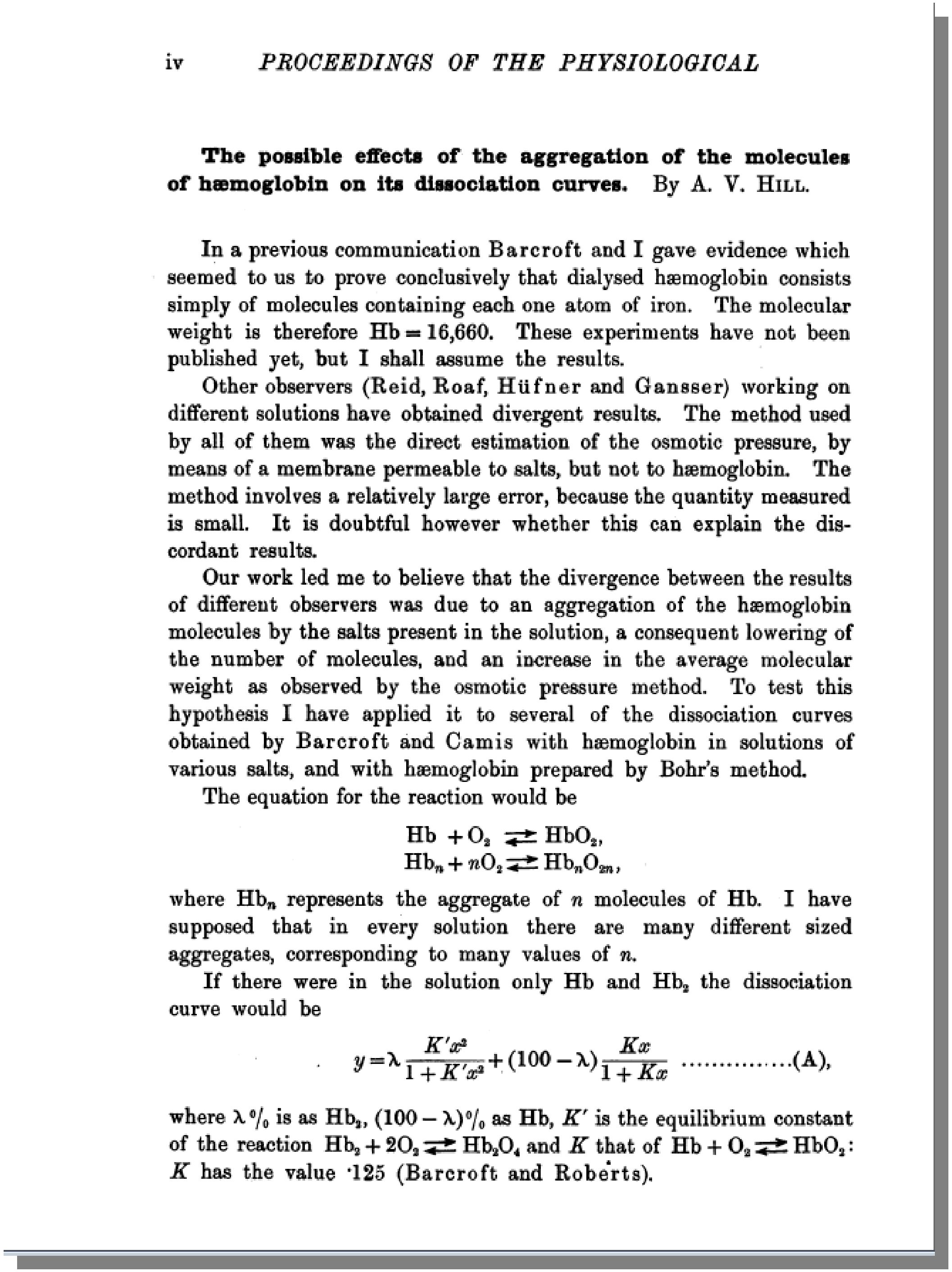
Shifman et al (2006)
PNAS, 103: 13968-13973

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



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

$$Hb + O_2 \rightleftarrows HbO_2,$$
$$Hb_n + nO_2 \rightleftarrows Hb_nO_{2n},$$

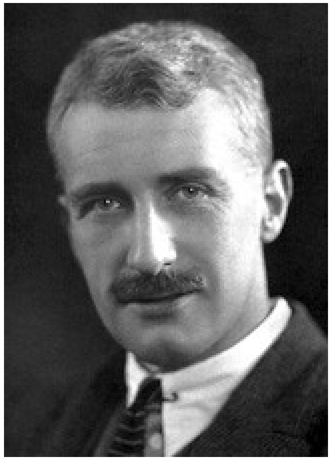
where Hb_n represents the aggregate of n molecules of Hb. I have supposed that in every solution there are many different sized aggregates, corresponding to many values of n.

If there were in the solution only Hb and Hb_2 the dissociation curve would be

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

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

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



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

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

would satisfy the observations.

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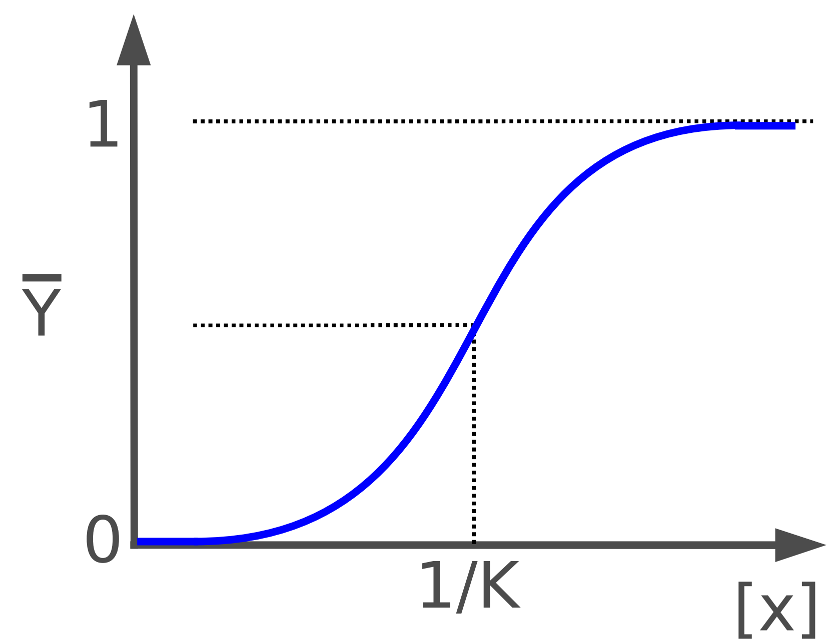


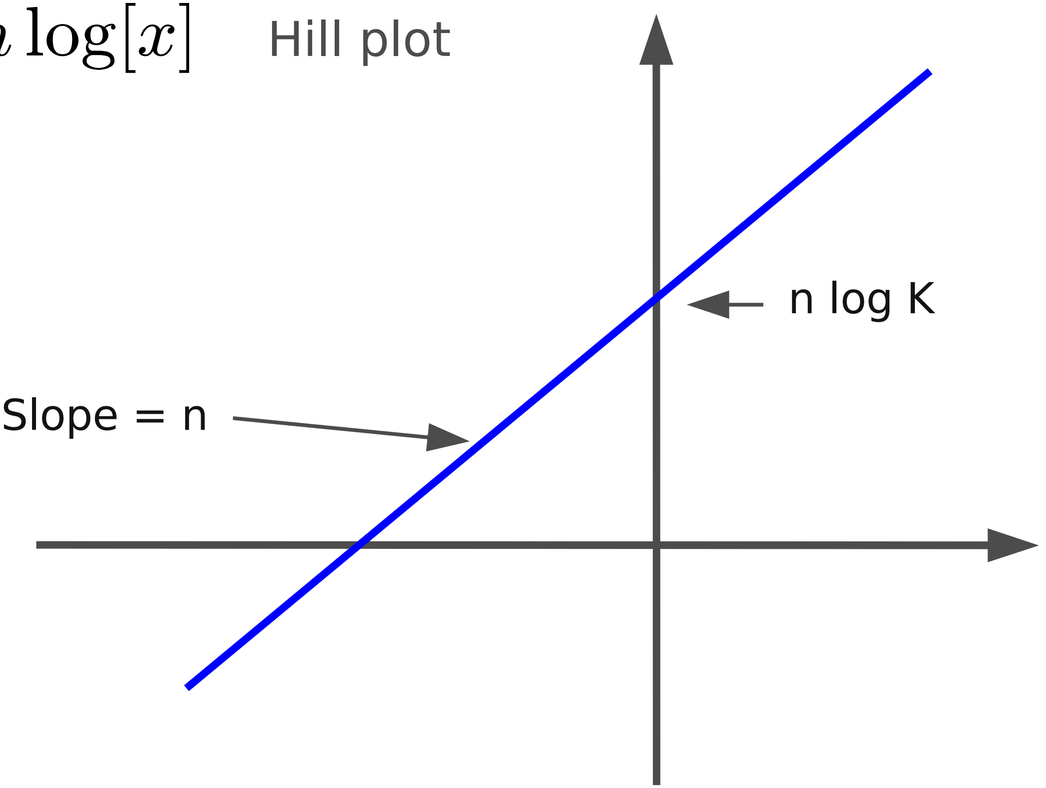
Hill Plot

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

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 HEMOGLOI

BY G. S. ADAIR.

WITH THE COLLABORATION OF A. V. BOCK AND H. FIELD, .

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

(Received for publication, January 7, 1925.)

This work gives the oxygen dissociation curves of so previously investigated in regard to their acid-binding anc

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

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

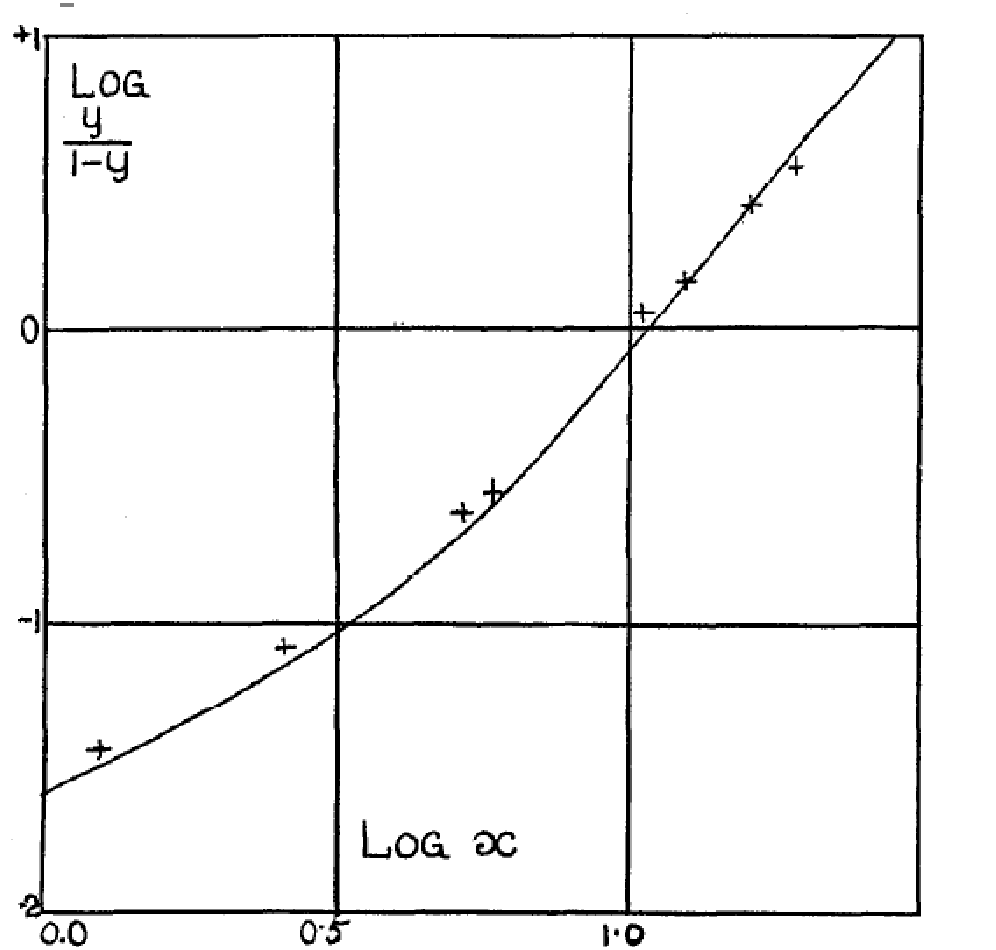
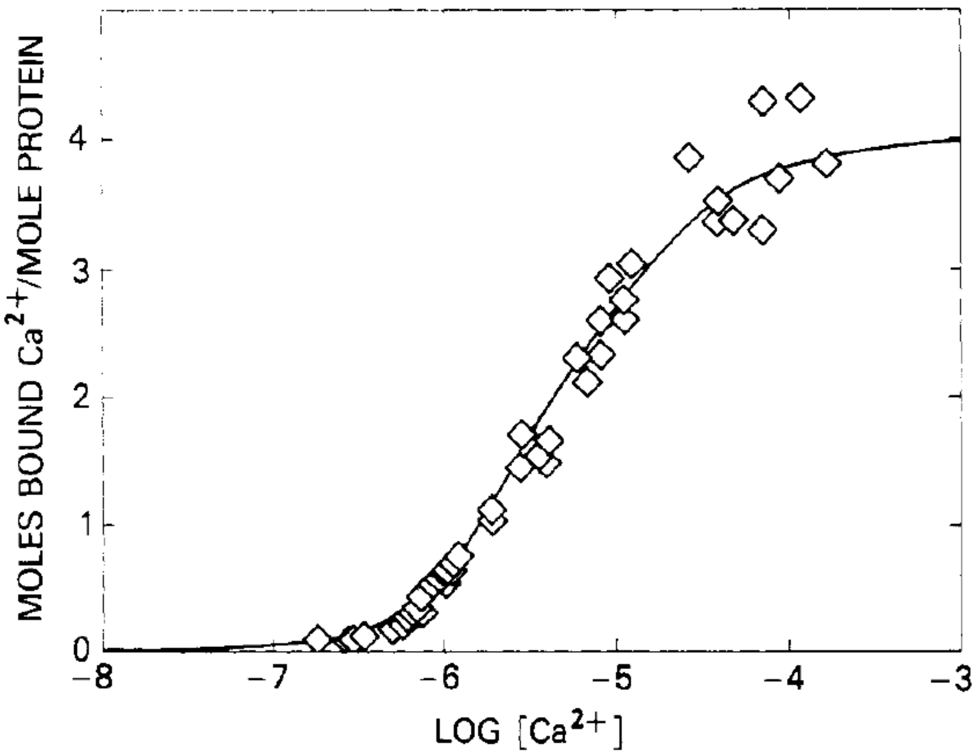


FIG. 2. Test of formula (6). Curve drawn from 6 experimental points from Table IV.

Adair-Klotz model applied to Calmodulin

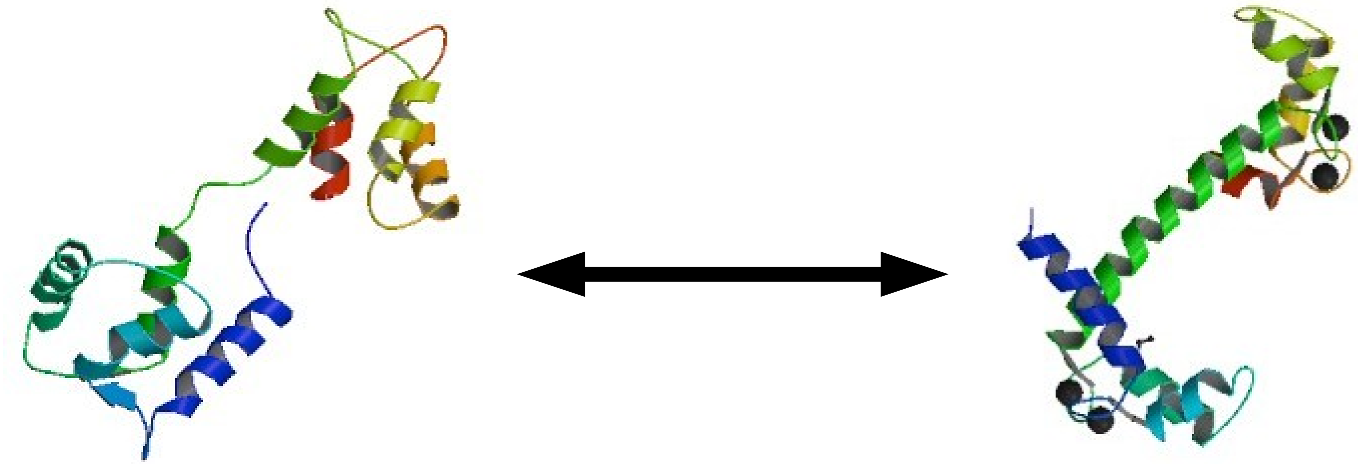
Klotz (1946) The Application of the Law of Mass Action to Binding by Proteins. Interactions with Calcium. *Arch Biochem*, 9:109–117.

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

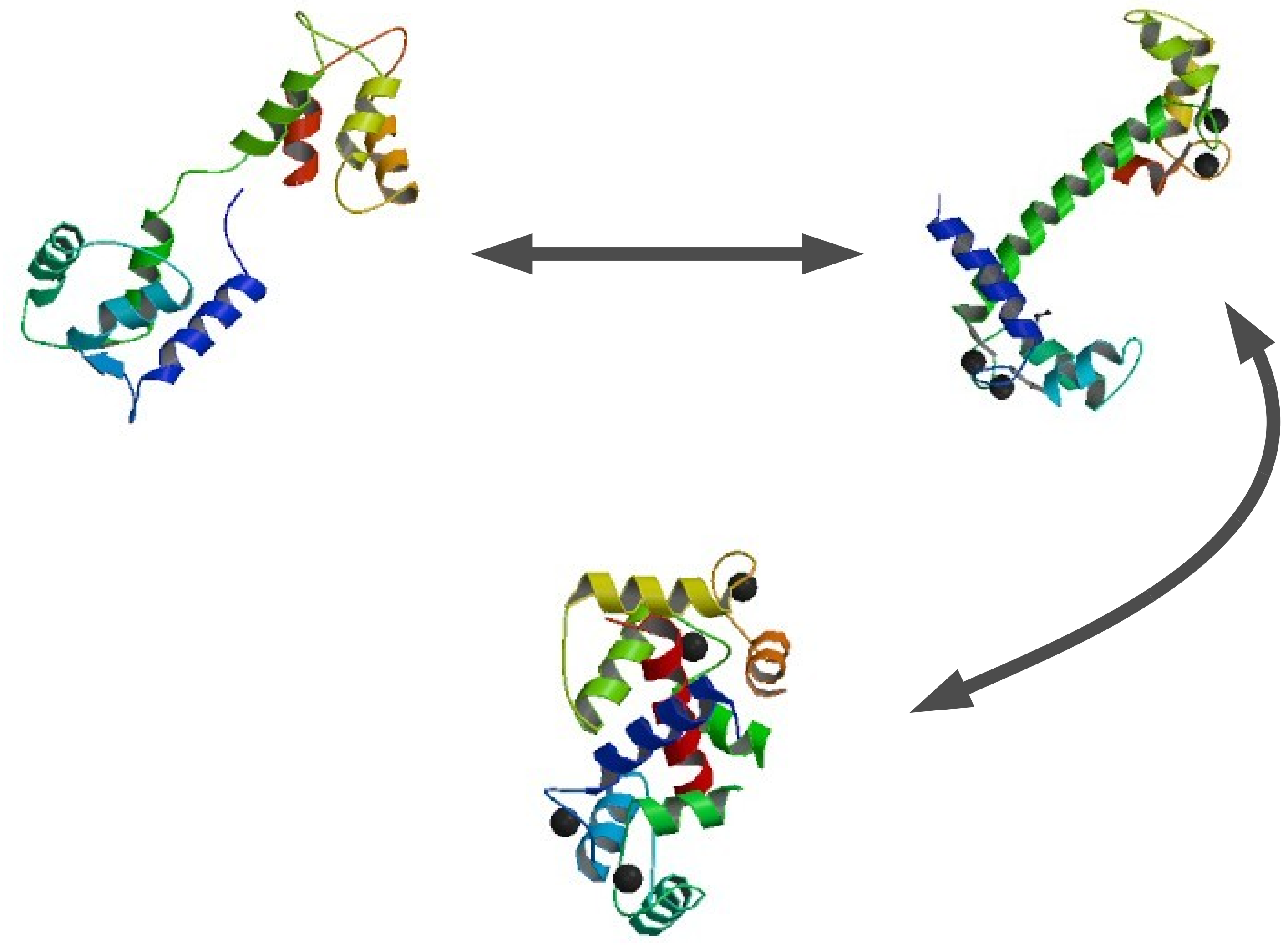


Crouch and Klee (1980) *Biochemistry*, 19: 3692-3698

State transitions of calmodulin



State transitions of calmodulin

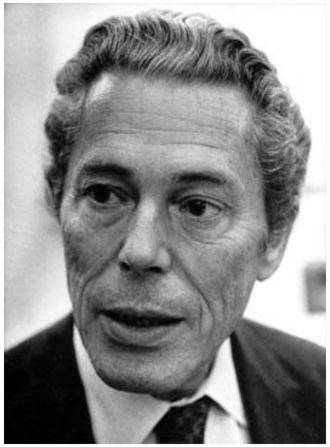


Adair-Klotz (induced-fit sequential) model not adequate

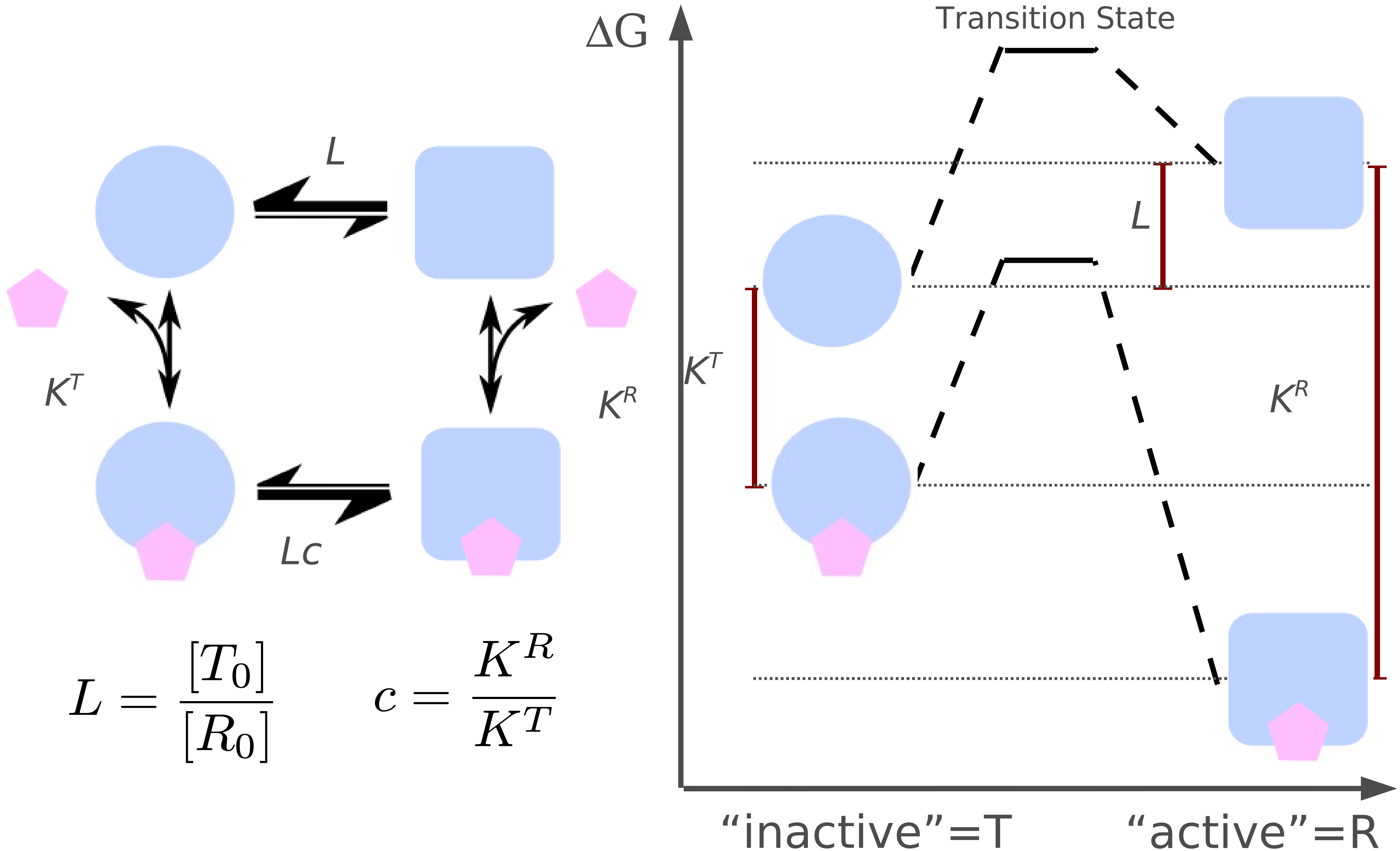
- Calmodulin bound to three calcium activates calcineurin
  - Kincaid and Vaughan (1986). *PNAS*, 83: 1193-1197
- Calmodulin bound to two calcium can bind CaMKII
  - Shifman et al (2006). *PNAS*, 103: 13968-13973
- Calmodulin affinity for calcium increases once bound to CaMKII
  - Shifman et al (2006) [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) $\cong$ CaMKII ;
    Low [Ca^{2+}] (low freq) $\cong$ Calcineurin

Allostery and state selection

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

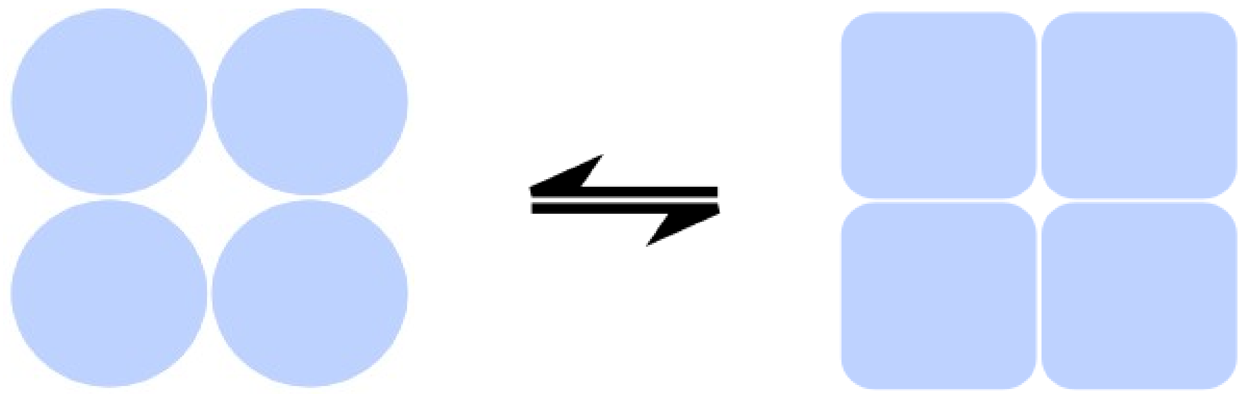


Modulation of thermal equilibria ≠ induced-fit

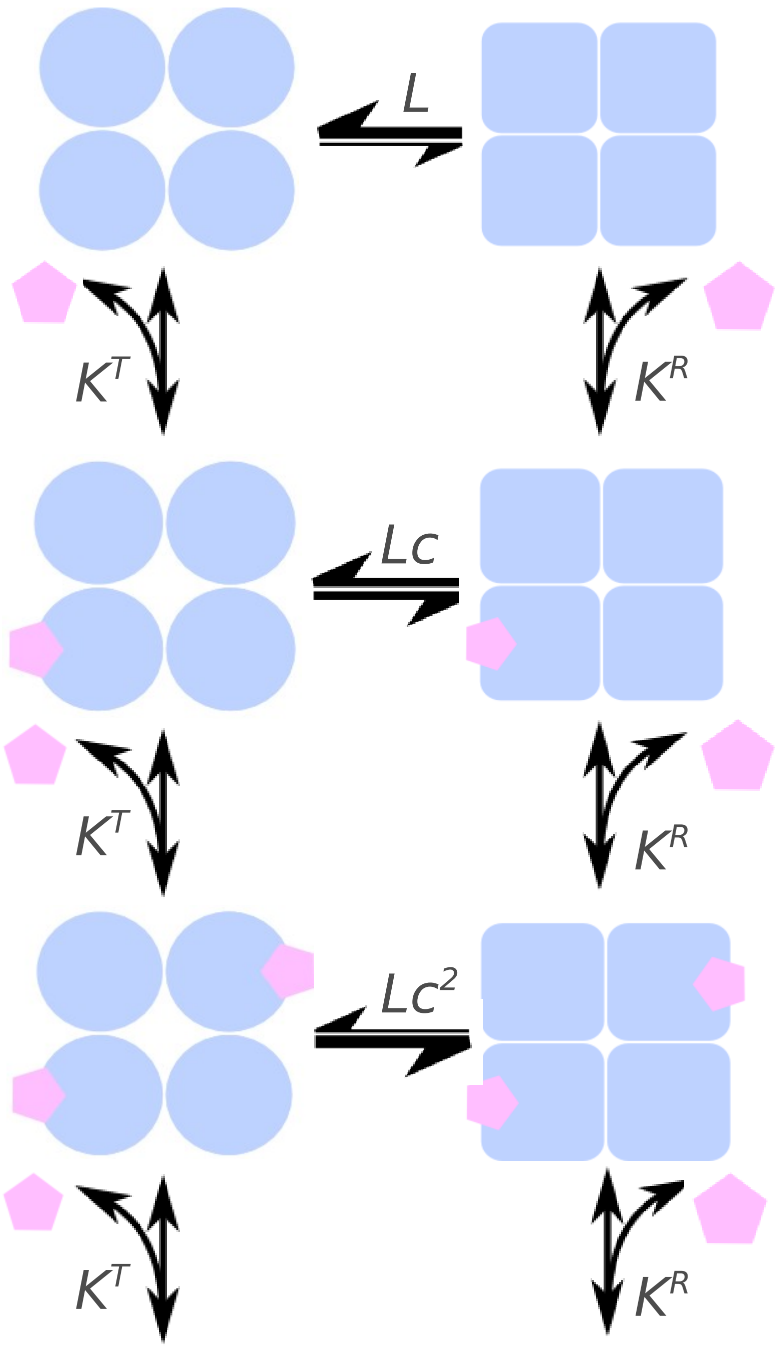


$$L = \frac{[T_0]}{[R_0]} \qquad c = \frac{K^R}{K^T}$$

Concerted transitions ≠ sequential model



Monod-Wyman-Changeux model



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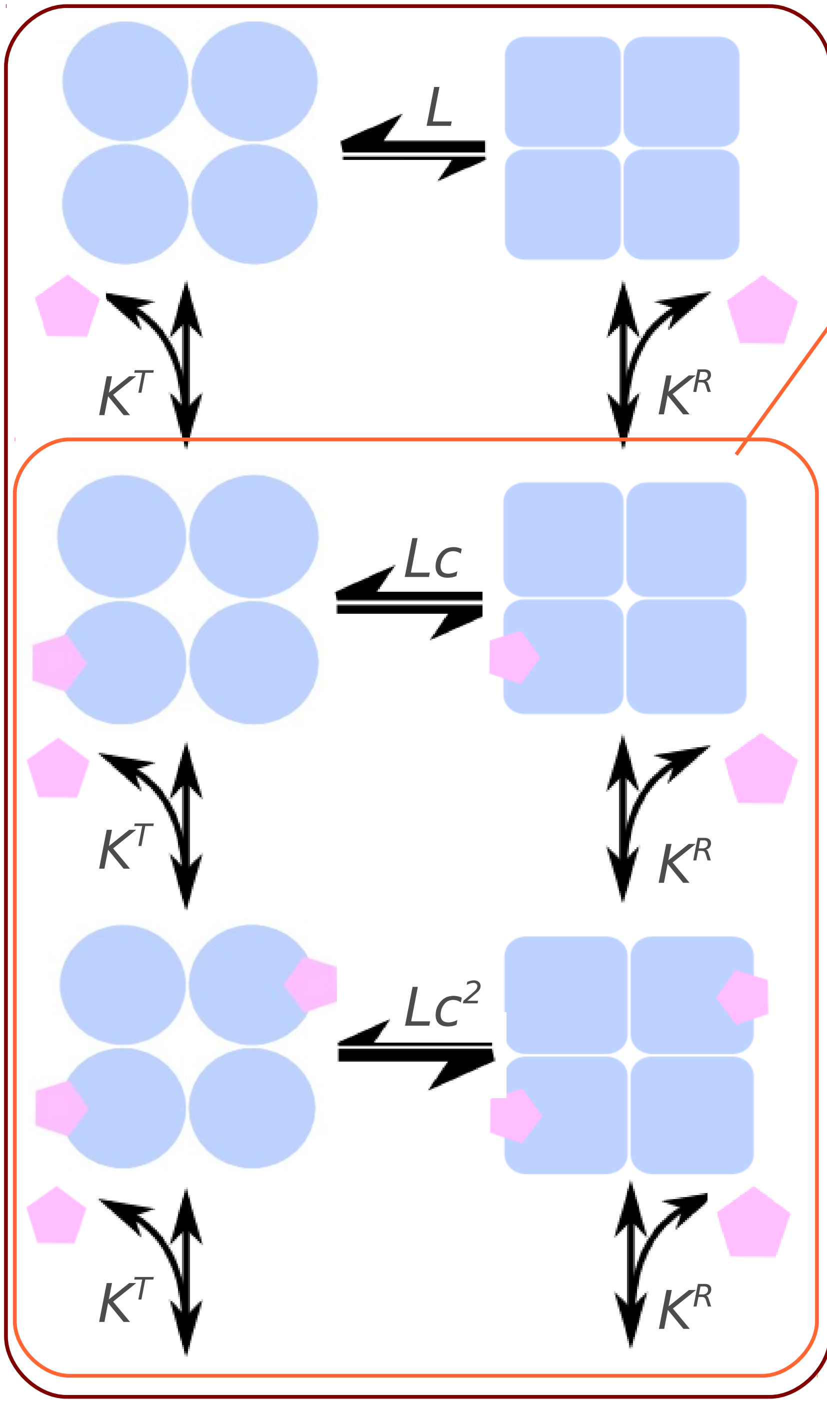
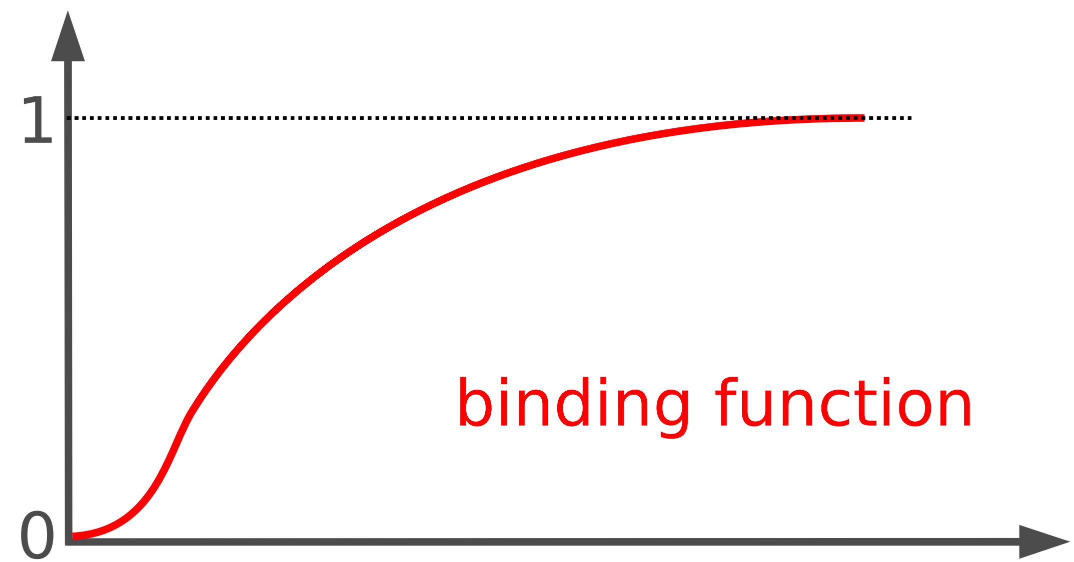


$$\alpha = \frac{[x]}{K^R}$$

this

that

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



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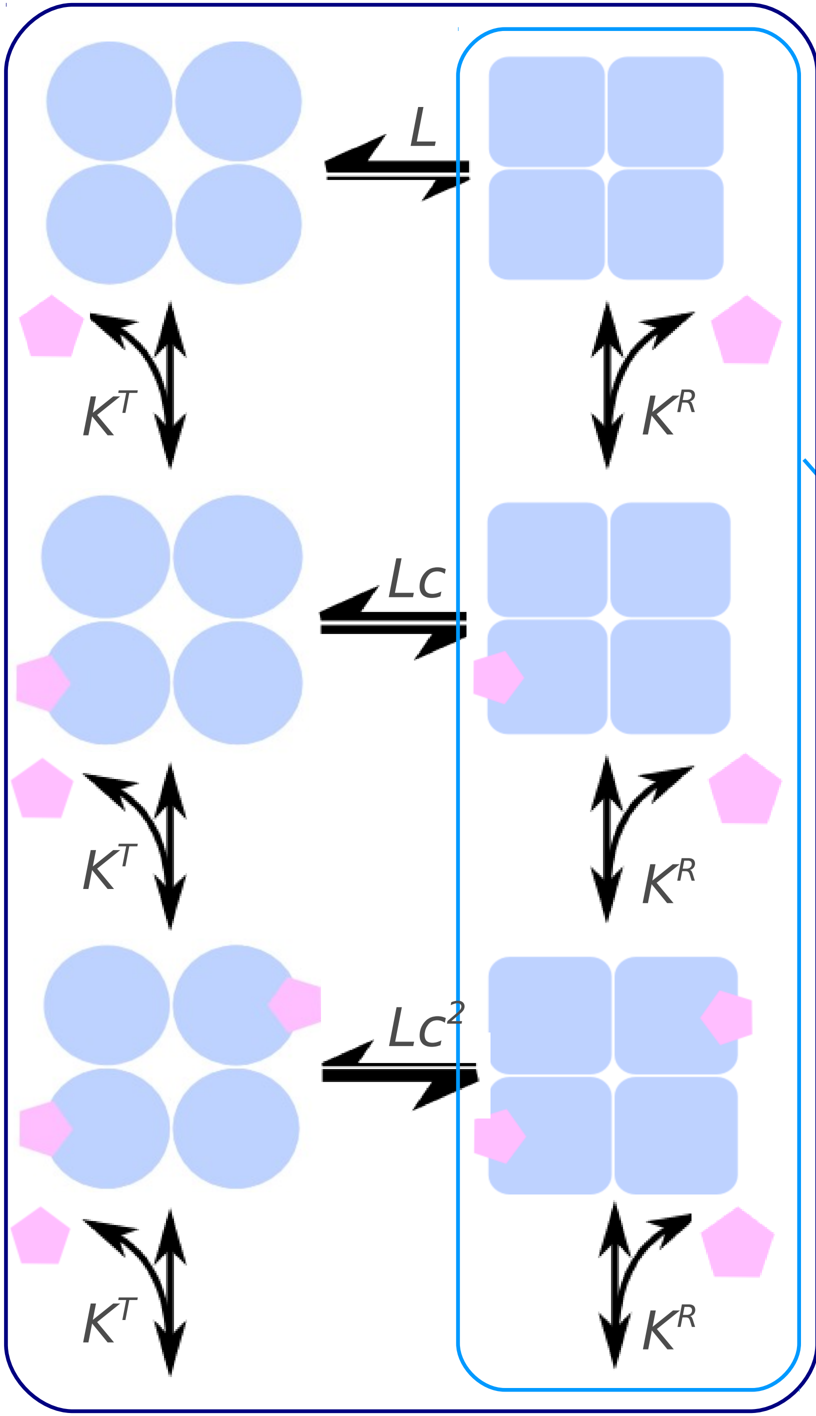

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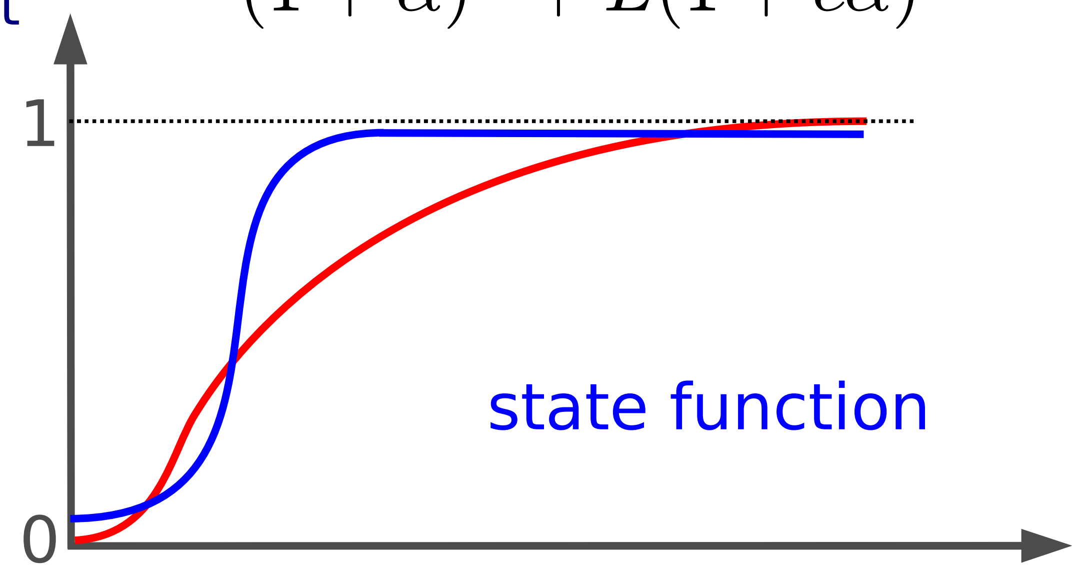
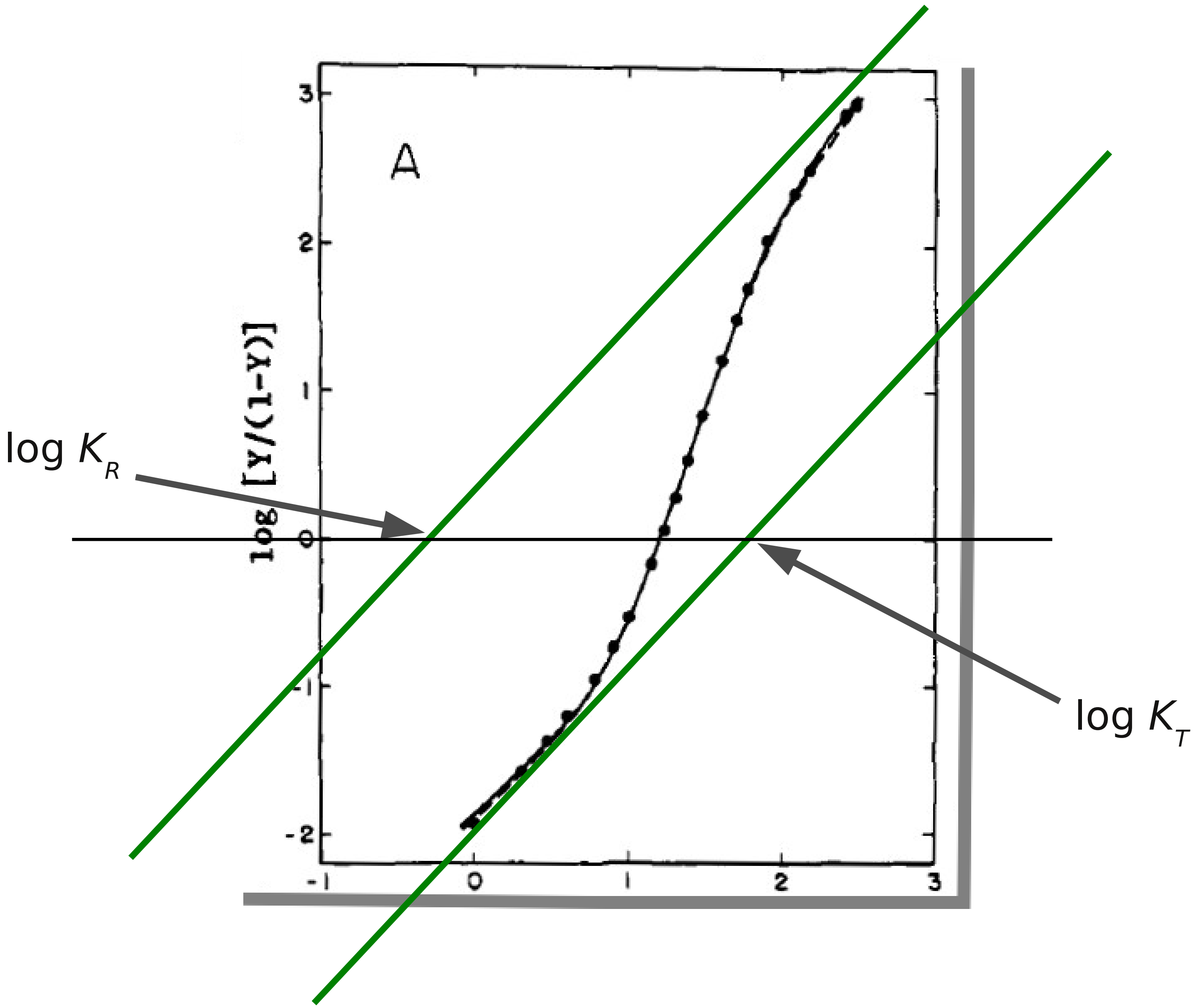
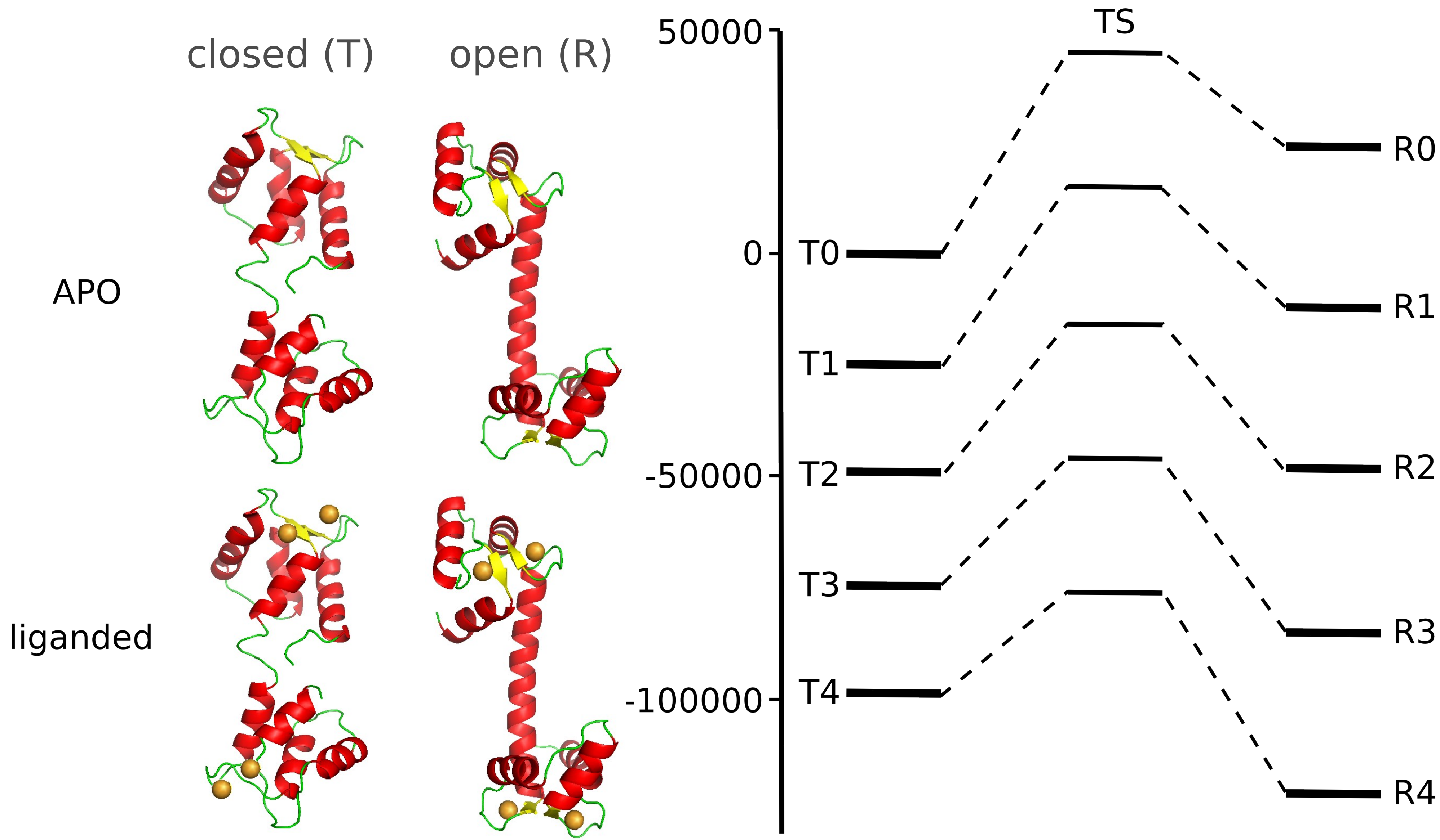


“Hill” Plot for MWC model



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

Concerted transition



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

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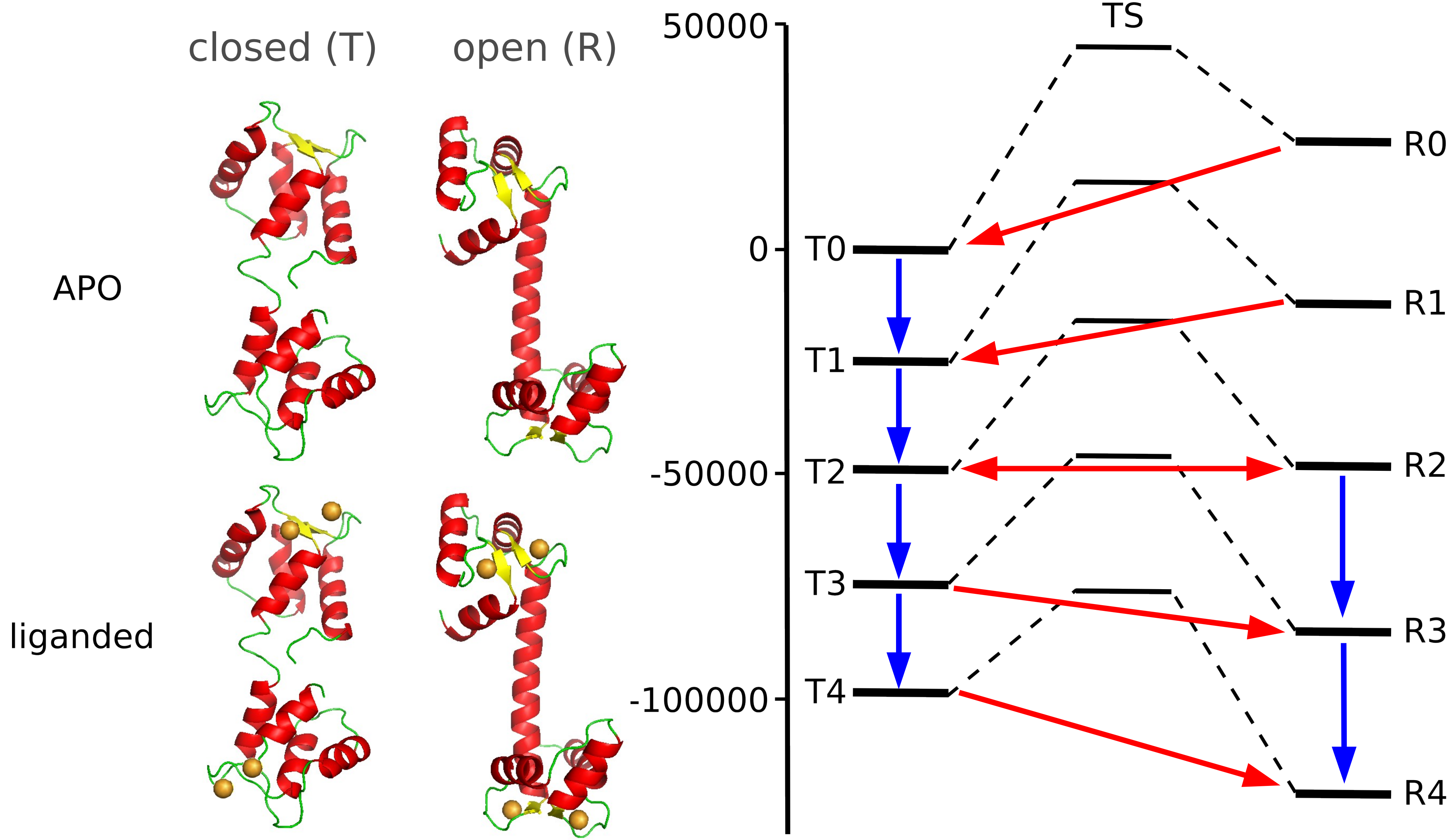


Extended MWC model necessary for Calmodulin

$$\bar{Y} = \frac{1}{n}\frac{\sum_i\left(\alpha_i\prod_{j\neq i}(1+\alpha_j)\right)+L\prod_k\left(\frac{1+e_k\gamma_k}{1+\gamma_k}\right)\sum_i\left(c_i\alpha_i\prod_{j\neq i}(1+c_j\alpha_j)\right)}{\prod_i(1+\alpha_i)+L\prod_k\left(\frac{1+e_k\gamma_k}{1+\gamma_k}\right)\prod_i(1+c_i\alpha_i)}$$

Any number of different sites per protomer. Several protomers can be carried by one subunit

- α_i = [ligand]/$K^{R}_{i,\mathrm{lig}}$
- γ_k = [modulator]/$K^{R}_{k,\mathrm{mod}}$
- $c_i = K^{R}_{i,\mathrm{lig}} / K^{T}_{i,\mathrm{lig}}$
- $e_k = K^{R}_{k,\mathrm{mod}} / K^{T}_{k,\mathrm{mod}}$

Based on Rubin and Changeux (1966) *J Mol Biol*, 21: 265-274

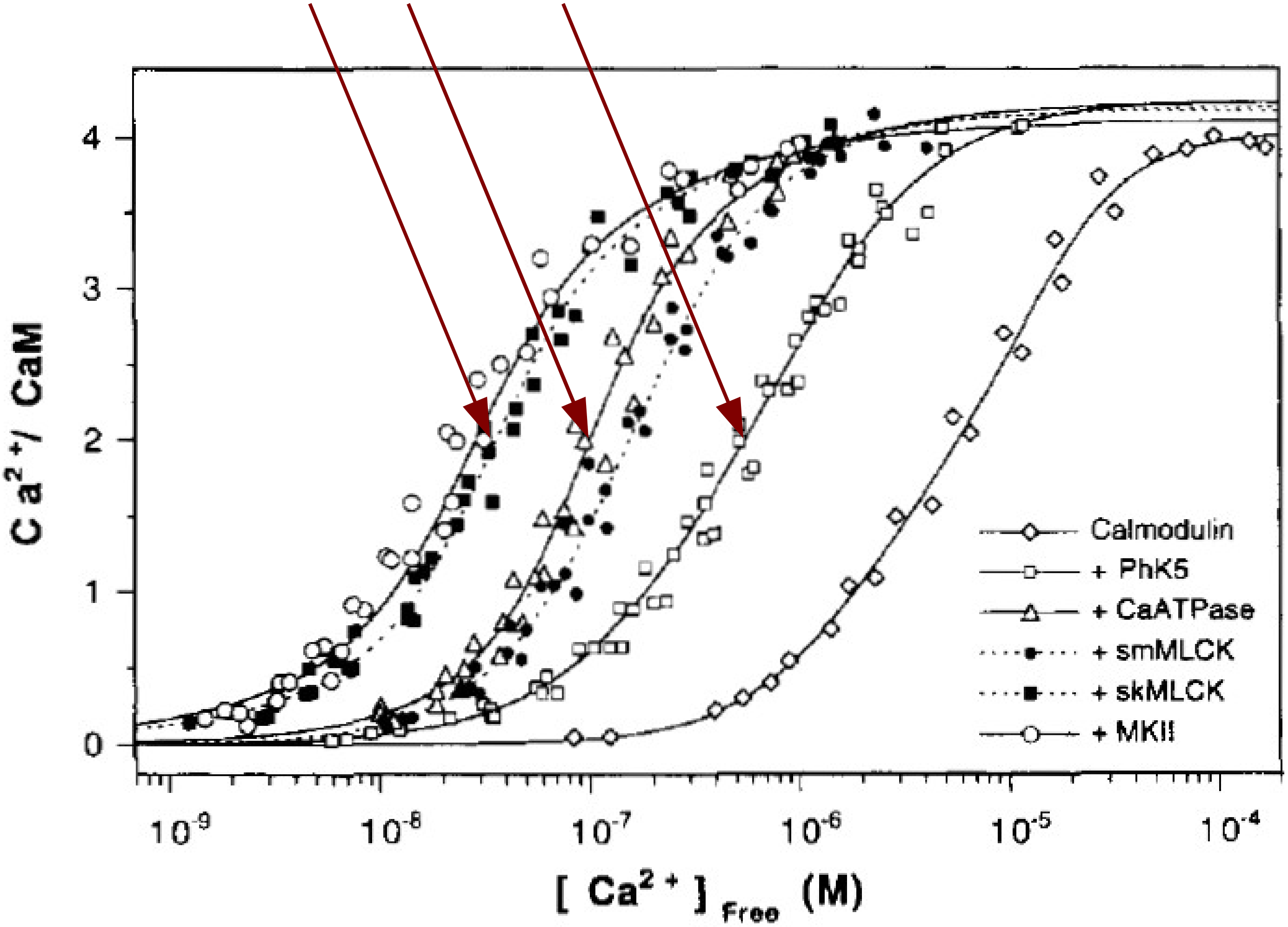
Stefan M.I., Edelstein S.J., Le Novère N (2009) Computing phenomenologic Adair-Klotz constants from microscopic MWC parameters. *BMC Syst Biol*, 3: 68

Simplification of the model for finding *L* and *c*

- Hypothesis for the whole model: free energy of conformational transition is evenly distributed: c is unique
- Additional simplification to determine L: affinities are identical

$$\bar{Y} = \frac{\alpha(1+\alpha)^3 + L\left(\dfrac{1+\gamma e}{1+\gamma}\right)c\alpha(1+c\alpha)^3}{(1+\alpha)^4 + L\left(\dfrac{1+\gamma e}{1+\gamma}\right)(1+c\alpha)^4}$$

Targets as allosteric effectors



Peersen et al. (1997) *Prot Sci*, 6: 794-807

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- Model constraints for the determination of c and L
  - Ca binding in presence of target: none, skMLCK, PhK5, CaATPase (Peersen et al (1997) Prot Sci 6: 794-807). Concentration at 50% saturation.
  - 100 000 parameter sets plus least-square
  - 13 identical minima. e for skMLCK is 10^{-15}, which can be taken as skMLCK binding only to R state.

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L=20670

c=3.96.10^{-3}

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Relaxation of the model for finding *Ki*

- Determination of individual affinities:

$$\bar{Y} = 0.25\,\frac{\sum_i \left(\alpha_i \prod_j (1+\alpha_j)\right) + L \sum_i \left(c\,\alpha_i \prod_j (1+c\,\alpha_j)\right)}{\prod_i (1+\alpha_i) + L \prod_i (1+c\,\alpha_i)}$$

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- Model constraints for calcium dissociation constants
  - Complete CaM (Bayley et al (1996) *Prot Sci* 5: 1215-1228)
  - N and C term Mutants (Shifman et al (2006) *PNAS*, 103: 13968-13973)
  - R-only – skMLCK (Peersen et al (1997) Prot Sci 6: 794-807)
  - Concentration at 25% and 50% saturation.
  - Systematic logarithmic sampling of the affinity space (coarse-grained, 50 values per affinity, then refined 66 values per affinity) = 25 millions parameter sets

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$K^R_A = 8.32\ 10^{-6}$

$K^R_B = 1.66\ 10^{-8}$

$K^R_C = 1.74\ 10^{-5}$

$K^R_D = 1.45\ 10^{-8}$

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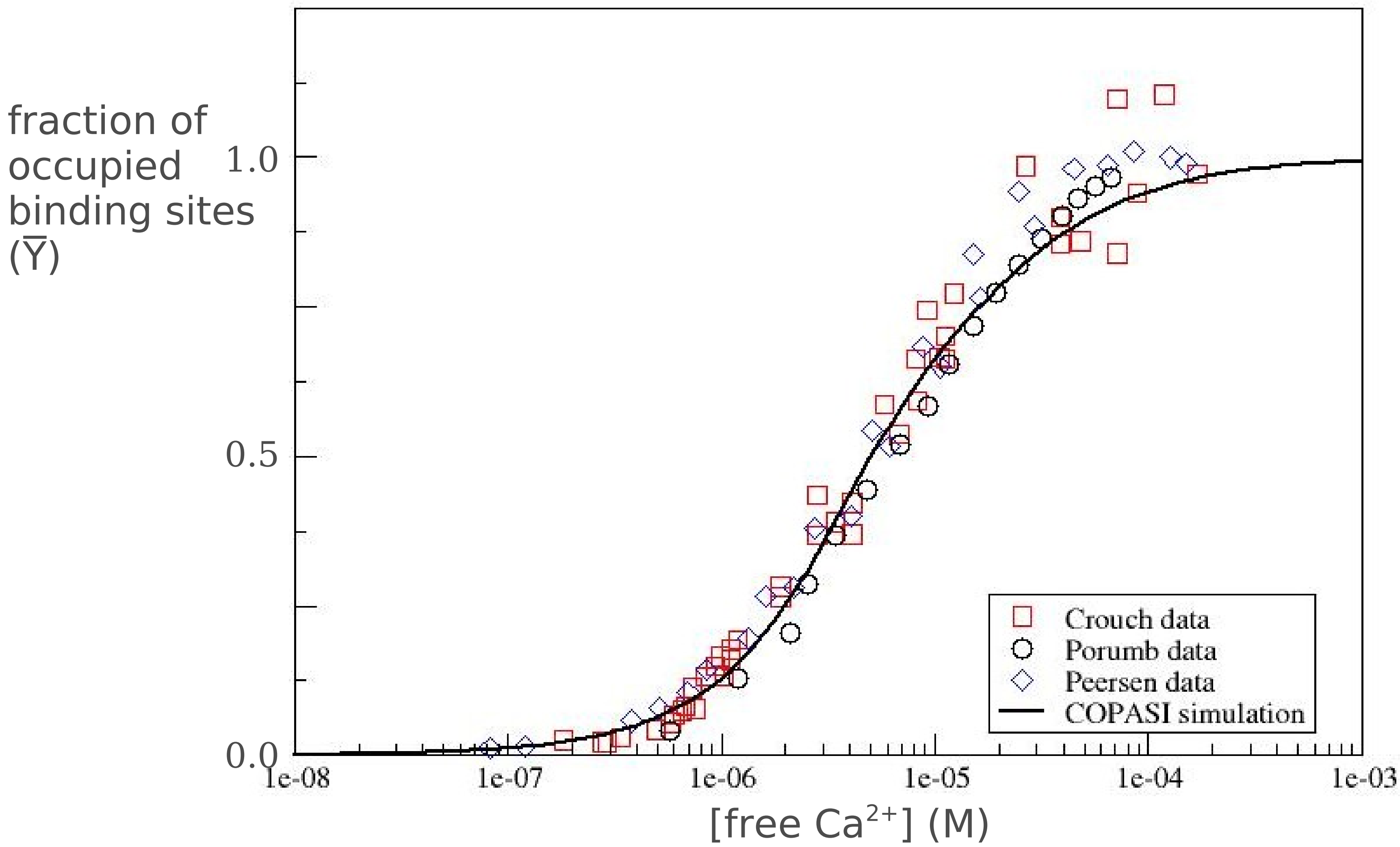
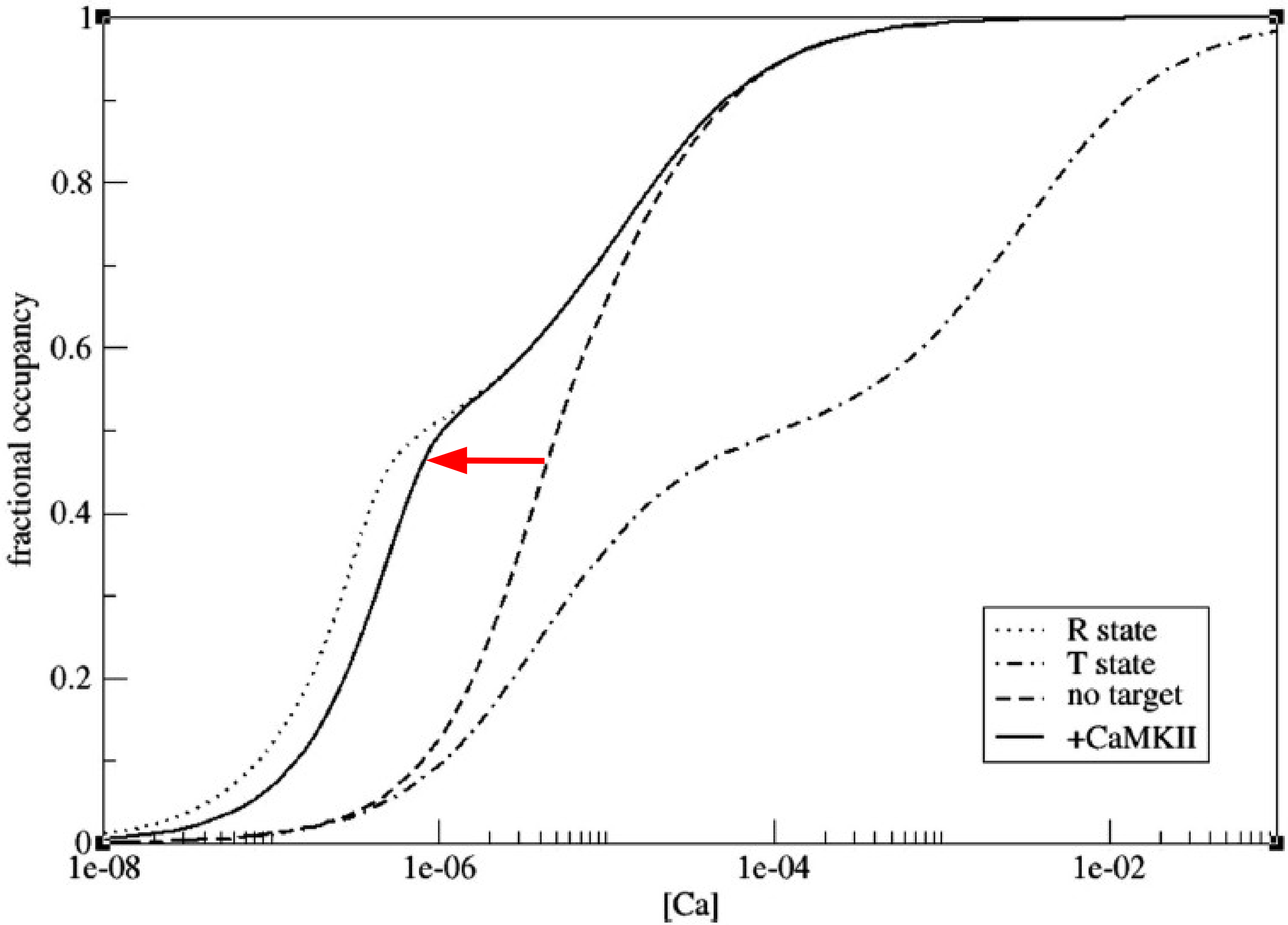
Allosteric model of Calmodulin function



Binding to target increases the affinity for Ca^{2+}



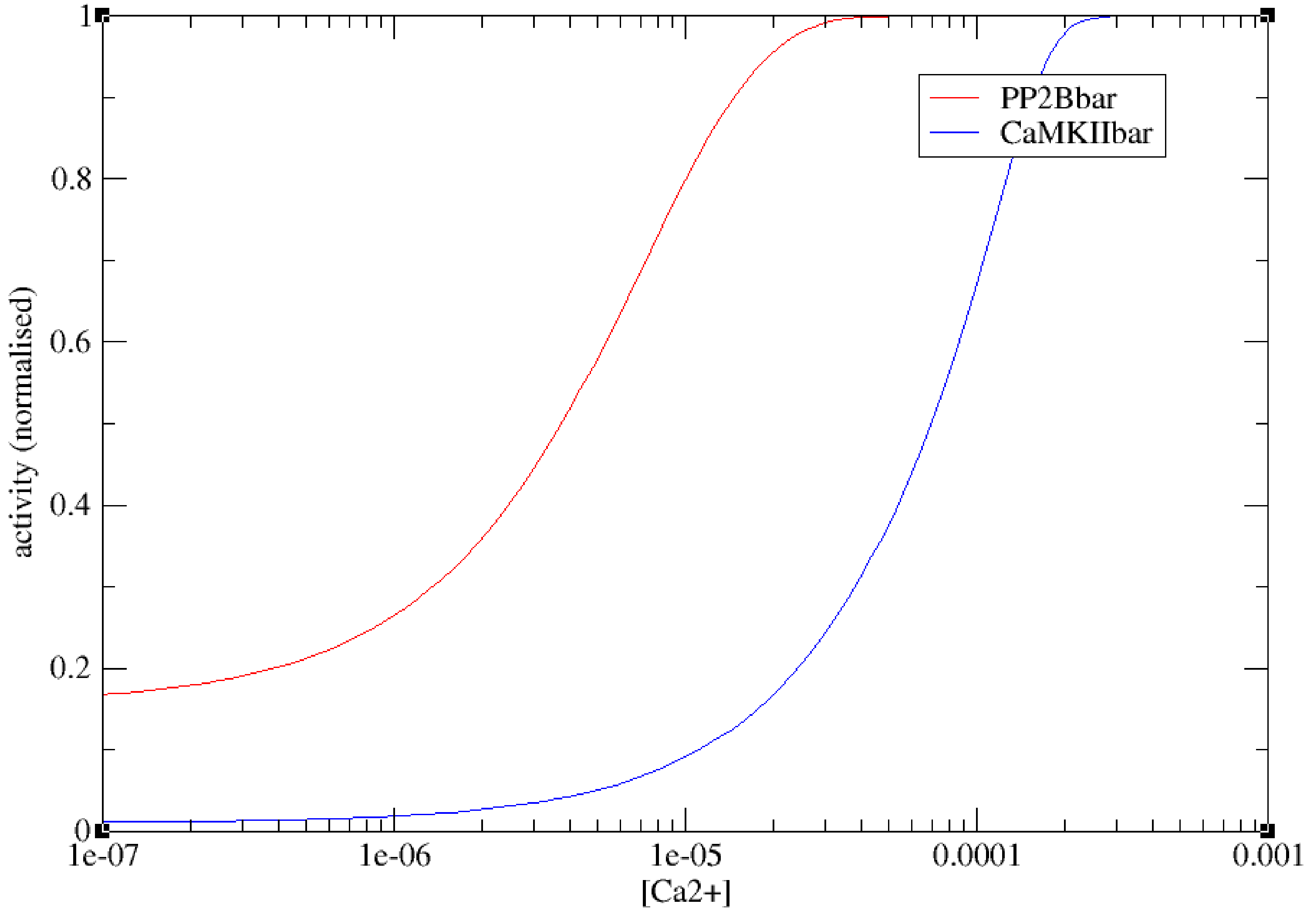
Activity of unsaturated calmodulin

- Fractional activity depends on the number of calcium ions bound. E.g.:

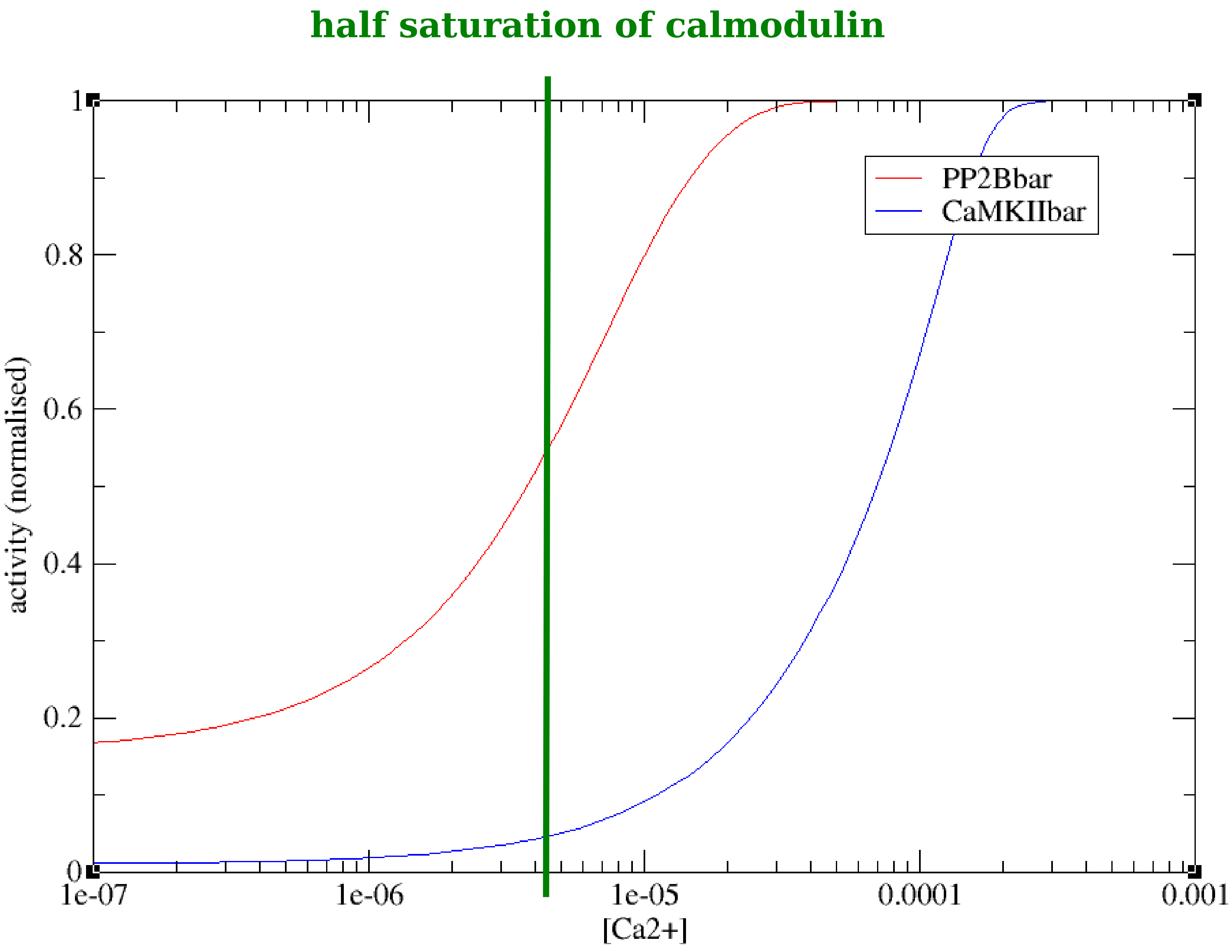
$$\frac{R_2}{T_2} = \frac{1}{L \cdot c^2}$$

- R_0/T_0 = 1/20000 (1/L)
- R_1/T_1 = 1/170
- R_2/T_2 = 0.69 → half-saturation = equi-probability
- R_3/T_3 = 80
- R_4/T_4 = 10000

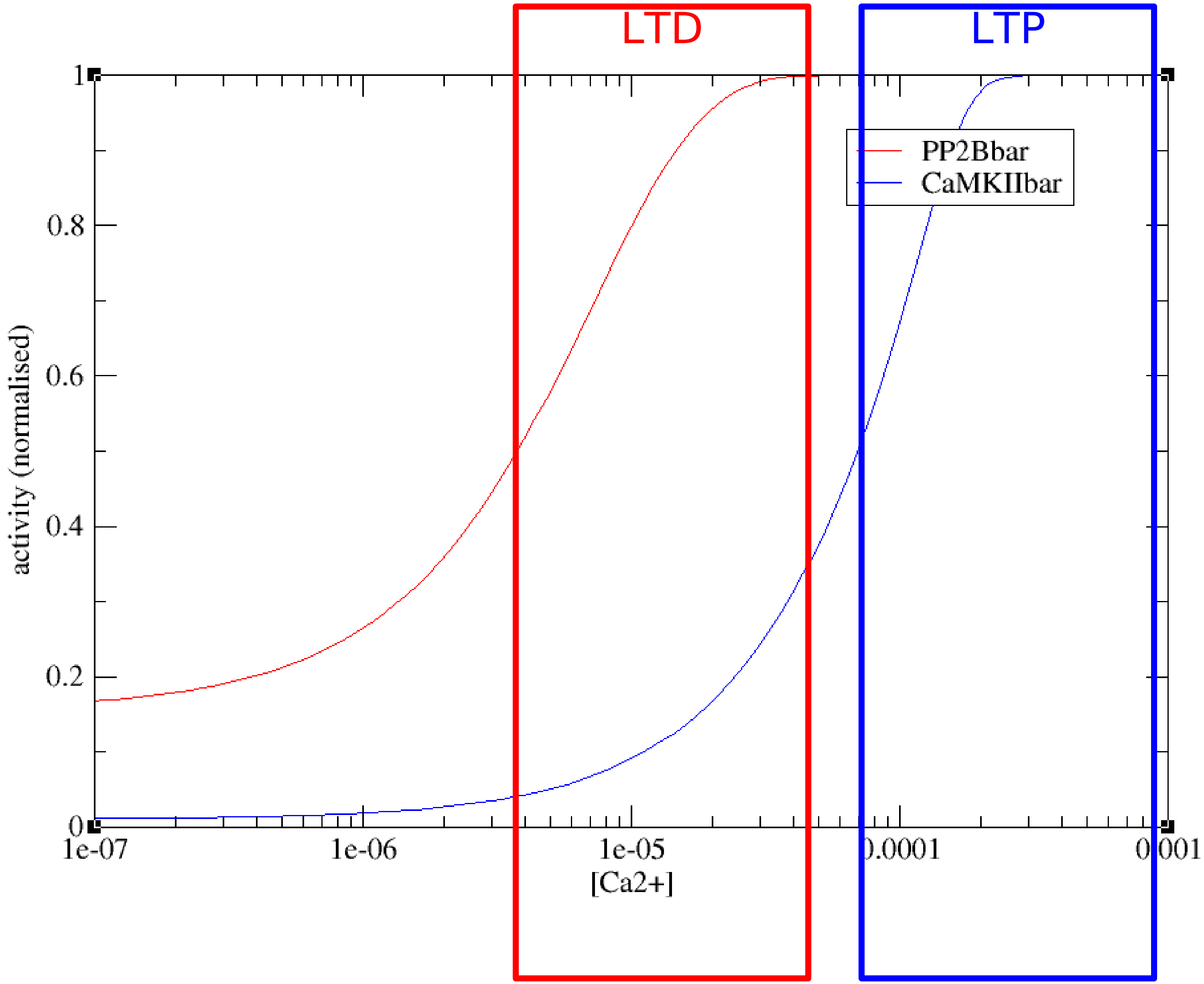
Bidirectional synaptic plasticity



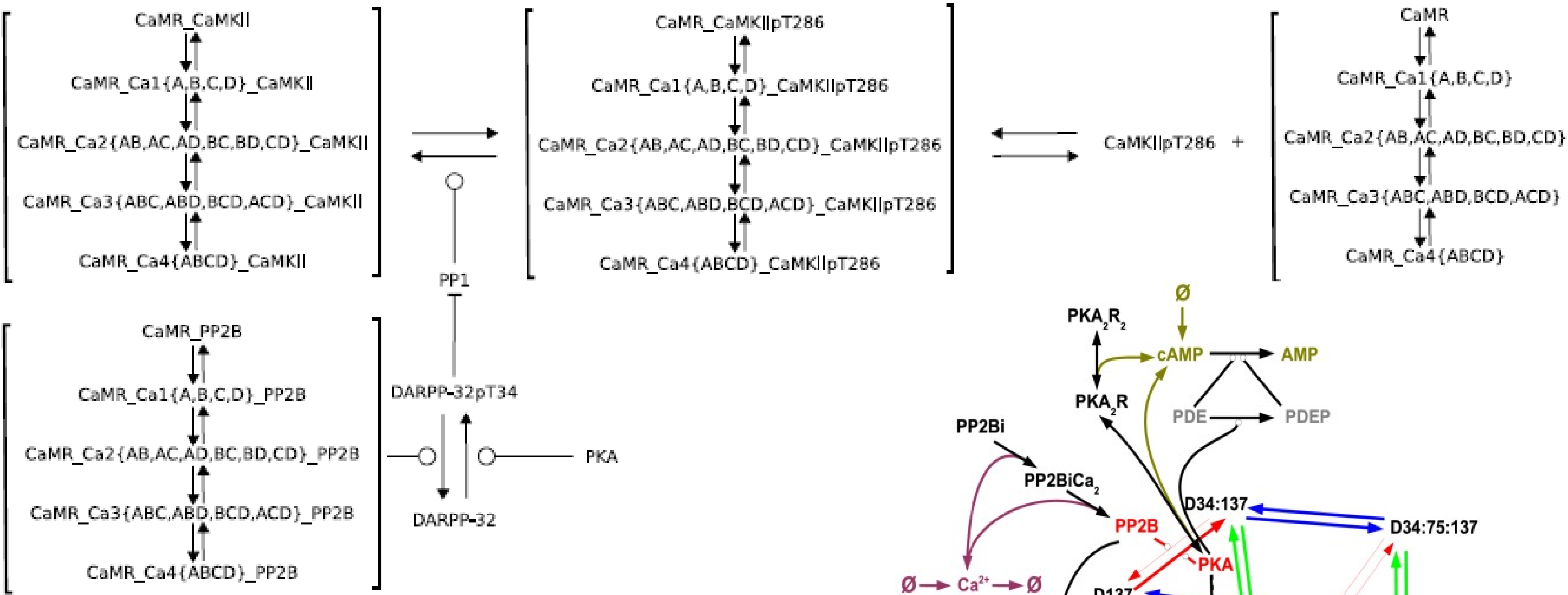
Bidirectional synaptic plasticity

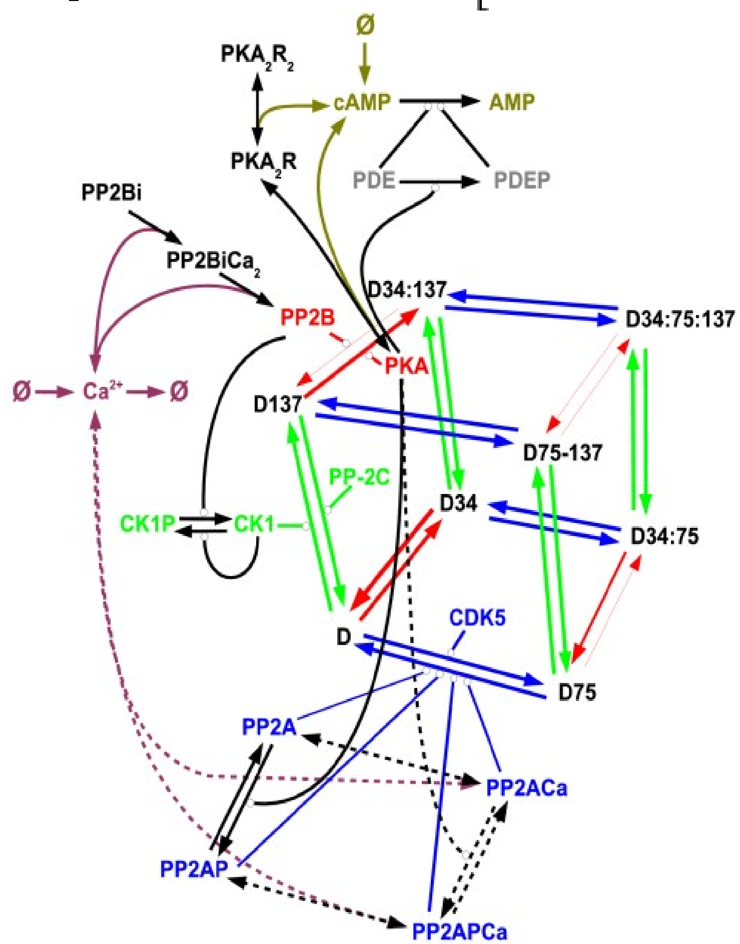


Bidirectional synaptic plasticity



Including kinetics



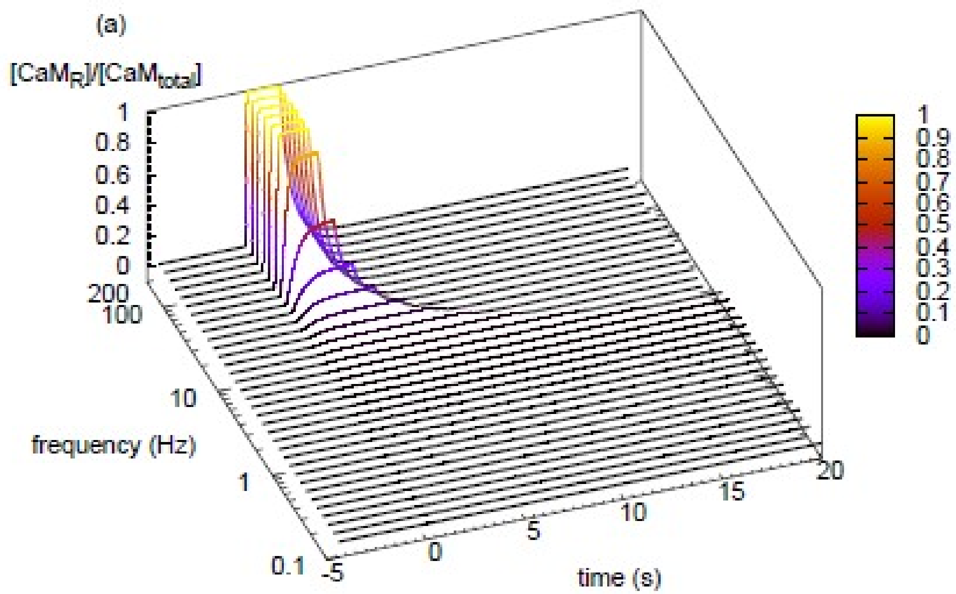


Li L., Stefan M.I., Le Novère N. Calcium input frequency, duration and amplitude differentially modulate the relative activation of calcineurin and CaMKII. Submitted

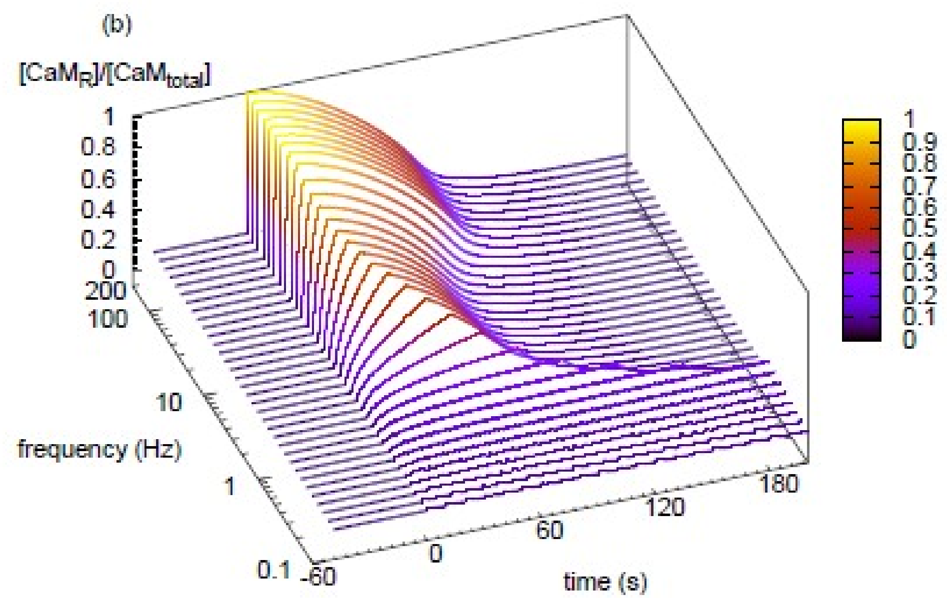
Fernandez E., Schiappa R., Girault J.A., Le Novère N. DARPP-32 is a robust integrator of dopamine and glutamate signals. PLoS Computational Biology (2006), 2(12): e176.

Binding to allosteric targets increase the effect of signals

No targets



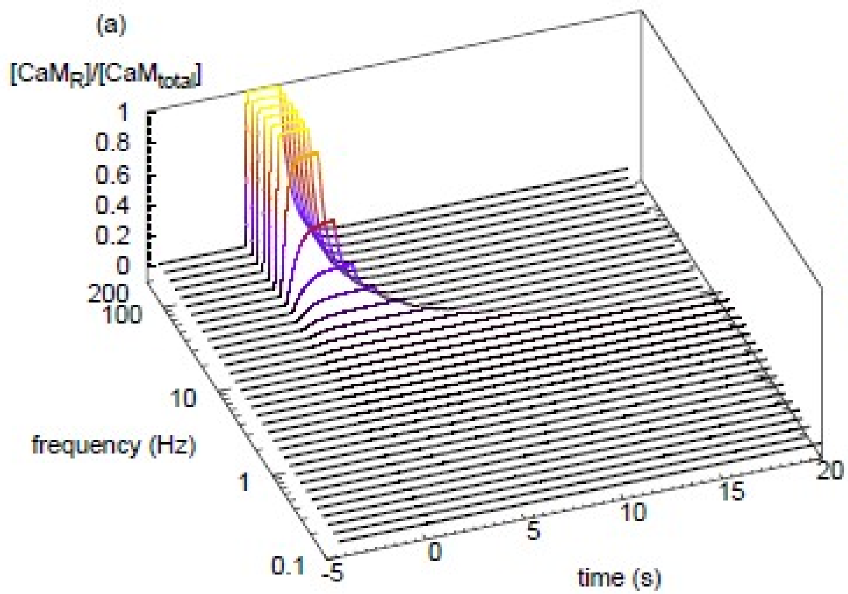
CaMKII and calcineurin



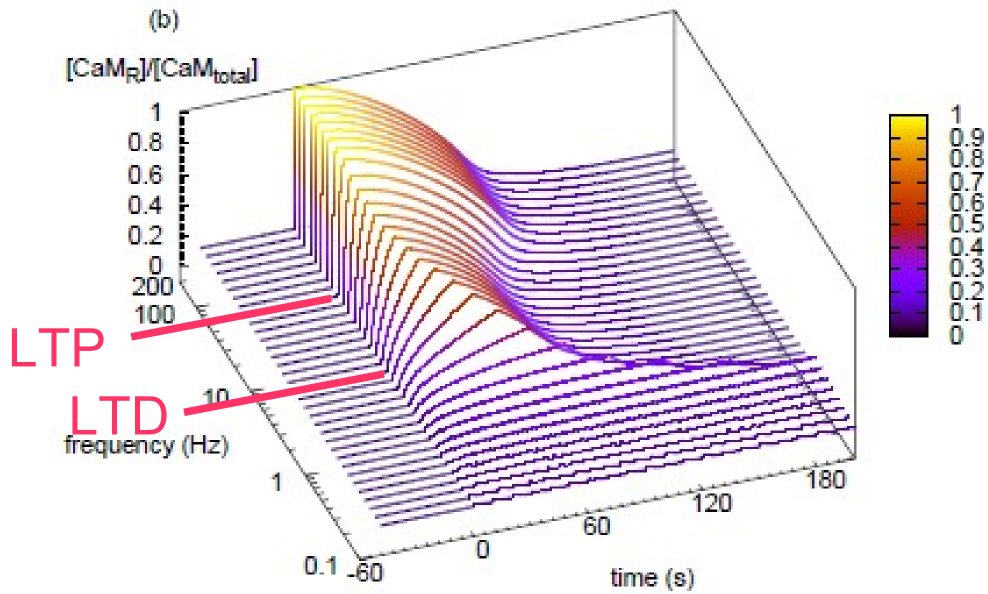
Total amount of calcium put in the system is kept constant:
When the frequency increases, the duration of the signal decreases.

Binding to allosteric targets increase the effect of signals

No targets

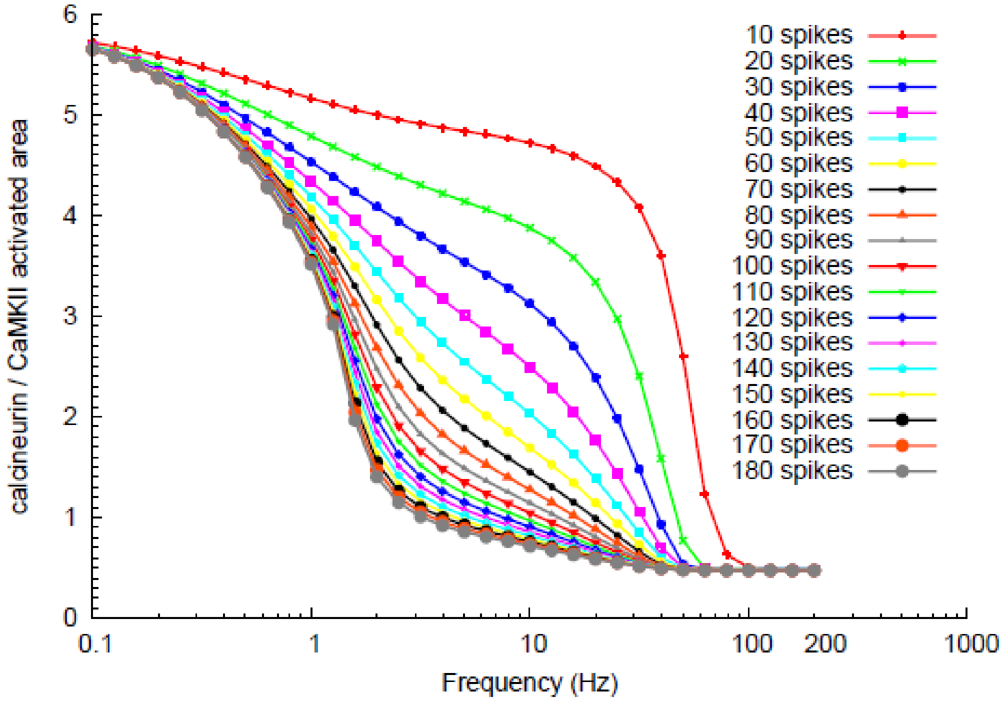


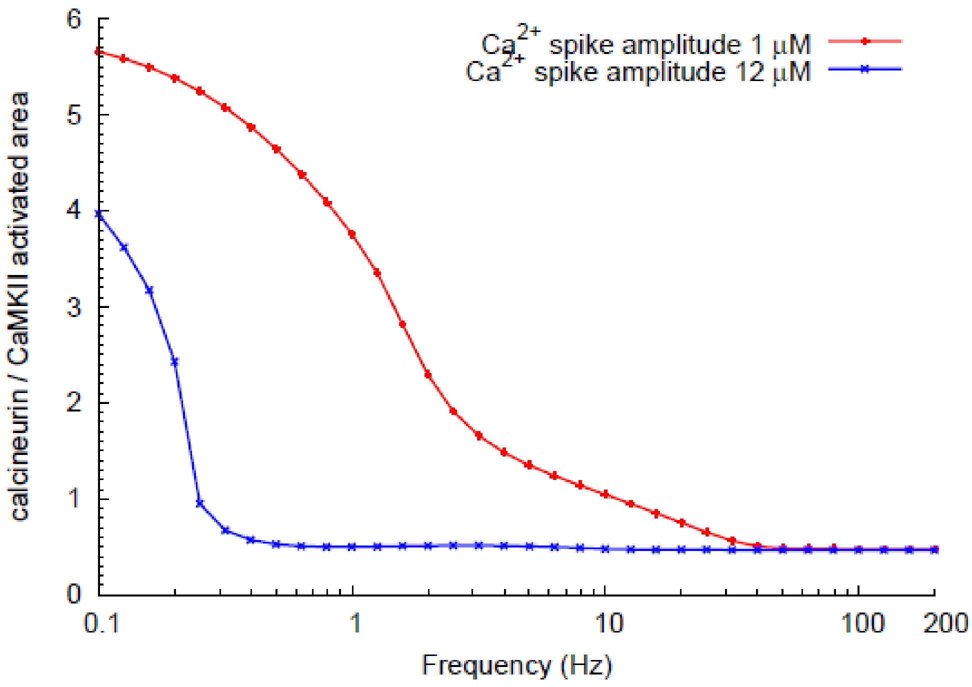
CaMKII and calcineurin



Total amount of calcium put in the system is kept constant:
When the frequency increases, the duration of the signal decreases.

Amplitude and duration change response to frequency



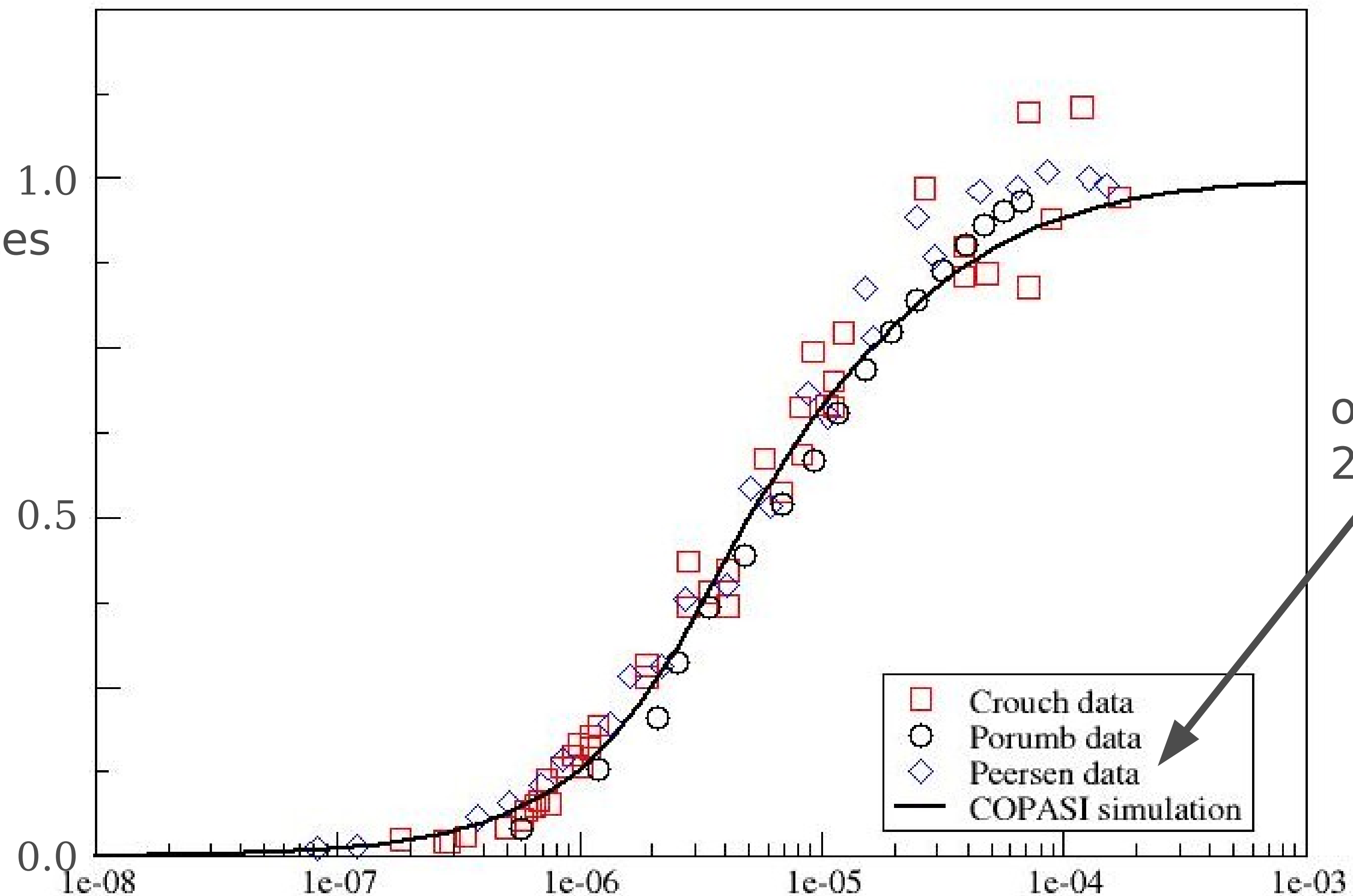


Conclusions on plasticity

- We designed an allosteric model of Calmodulin, based on only two states for the EF hands, both binding calcium with different affinities, and a concerted transition for all 4 EF hands.
  - The model fits independent experimental datasets.
  - Affinitity of CaM for calcium increases upon binding of the target.
  - CaM can exist in the open state, bind its targets, even with less than 4 calcium bounds.
- The model displays an activation of the sole PP2B at low concentration of calcium, while high concentrations activate both PP2B and CaMKII.
- Kinetic simulations show that:
  - Both CaMKII and calcineurin increases for all signals, but differentially
  - Allosteric stabilisation of CaM by targets enhance the signal
  - Frequency, amplitude and duration of the signal are important for regulating synaptic plasticity

Allosteric model of Calmodulin function

fraction of occupied binding sites ($\bar{Y}$)

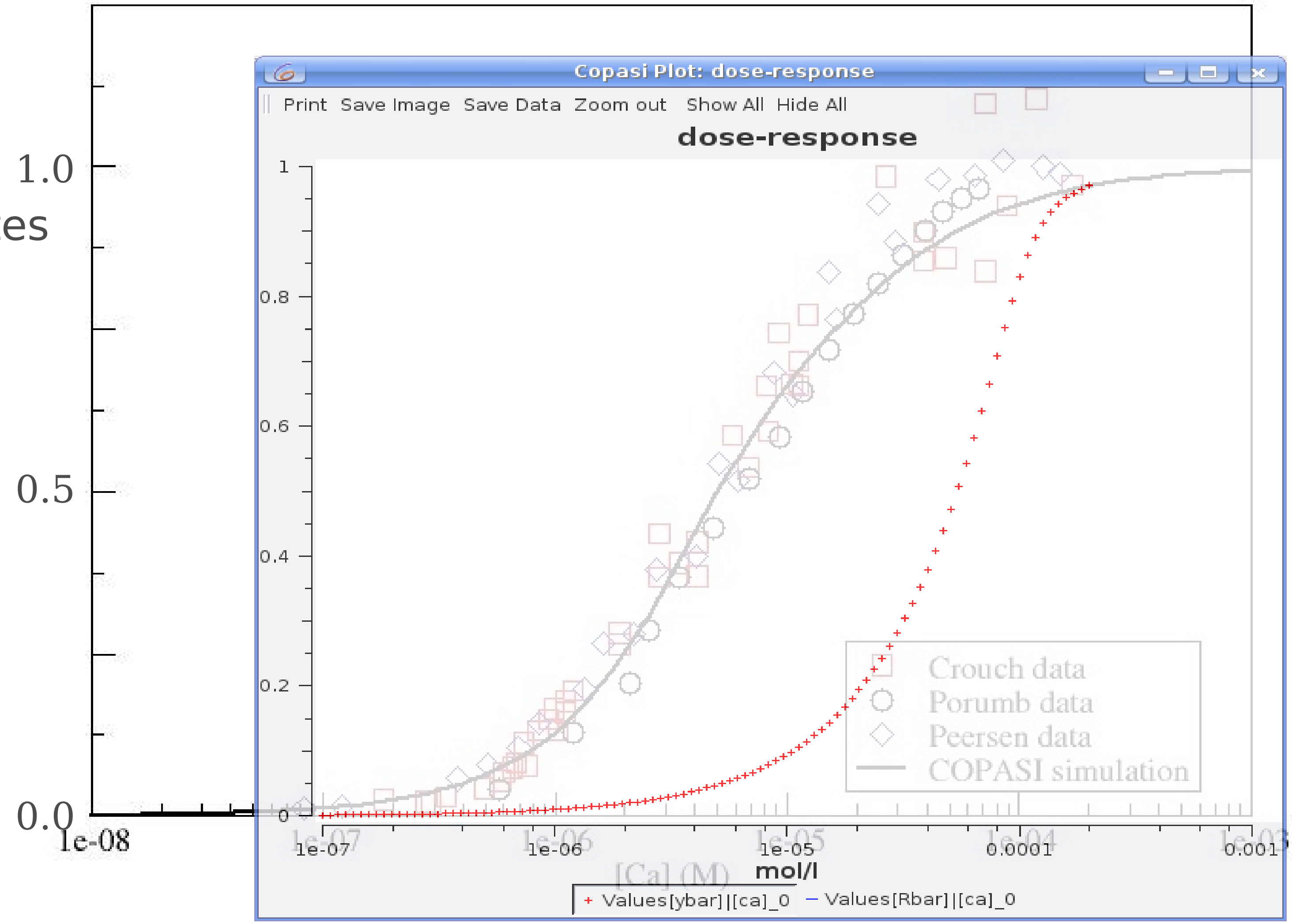


[free Ca^{2+}] (M)

obtained with 25 μM CaM

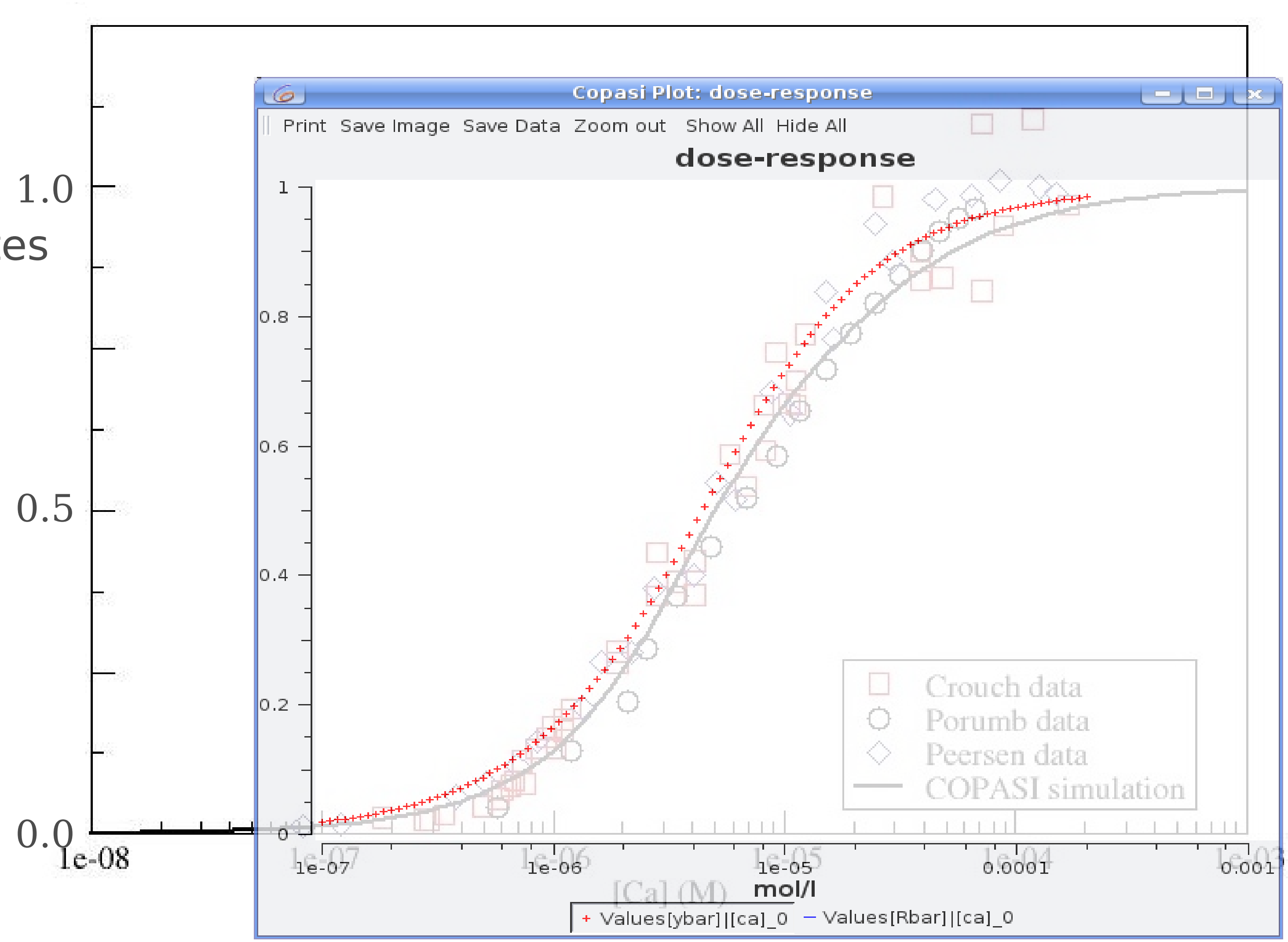
Calcium dose-response on 25 μM Calmodulin

fraction of occupied binding sites ($\bar{Y}$)



Calcium dose-response on 0.1 μM Calmodulin

fraction of occupied binding sites ($\bar{Y}$)



Calcium dose-response on 0.1 μM Calmodulin

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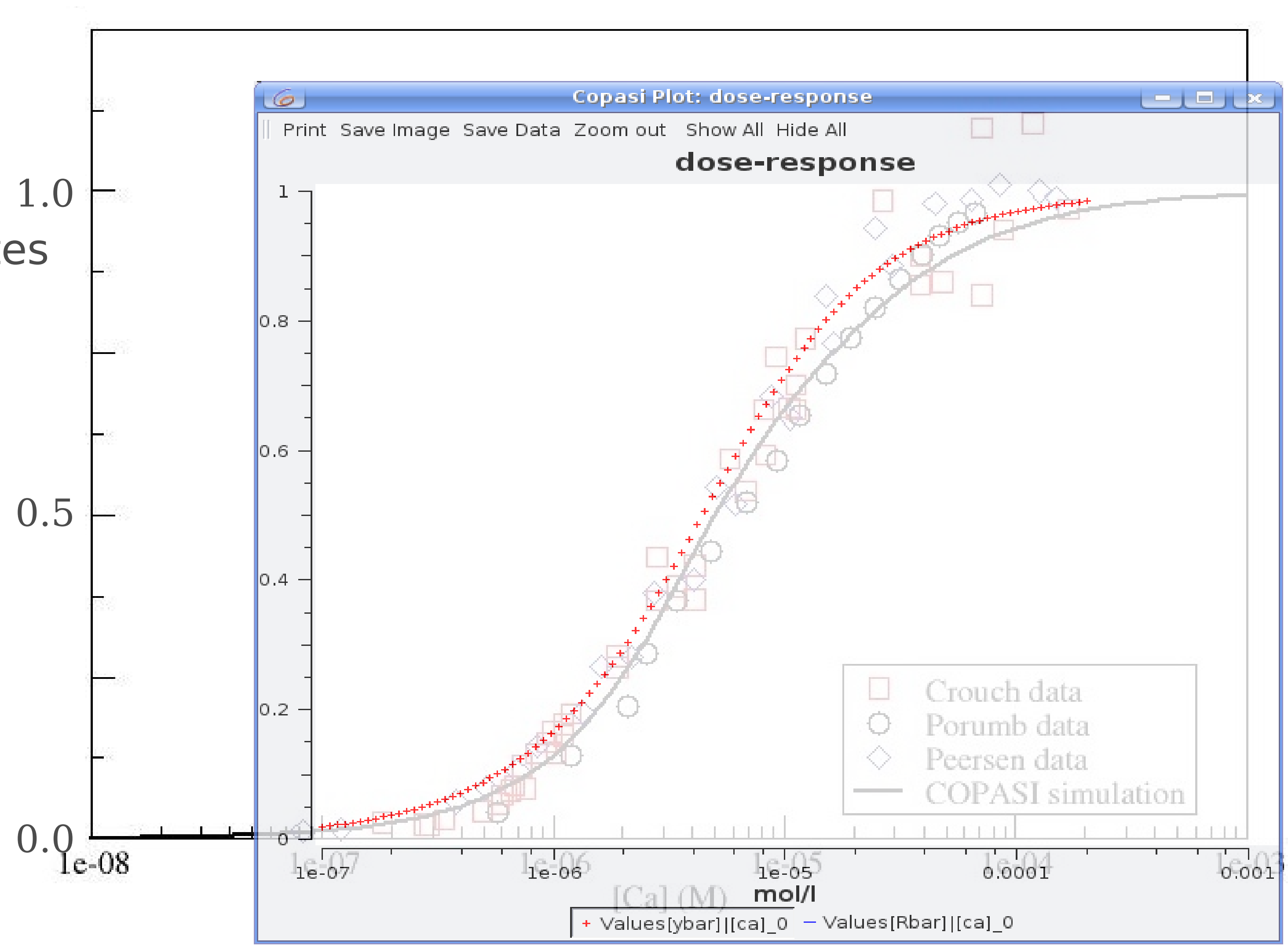
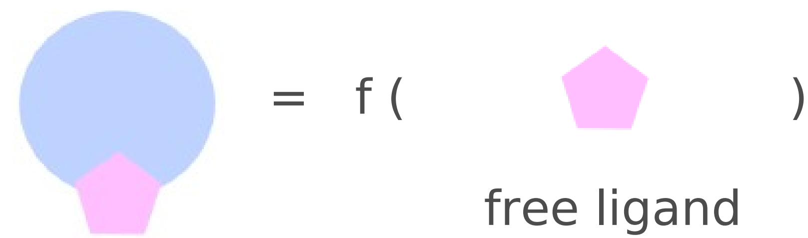


Edelstein S.J., Stefan M.I, Le Novère N. Ligand depletion in vivo modulates the dynamic range and cooperativity of signal transduction. PLoS One (2010), 5(1): e8449

What is ligand depletion?

What is ligand depletion?

Chemistry (mass-action law)



What is ligand depletion?

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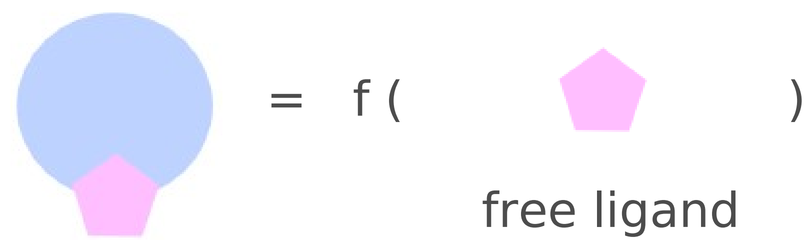
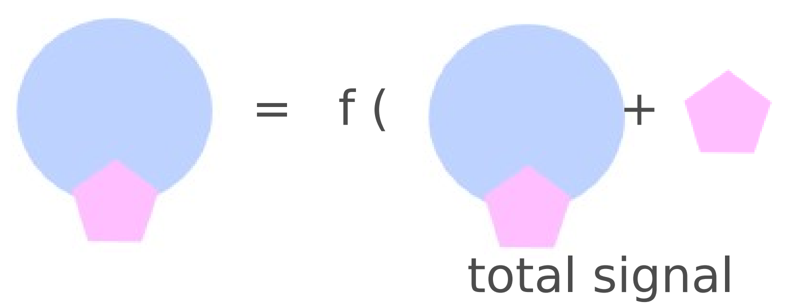


Cellular signalling



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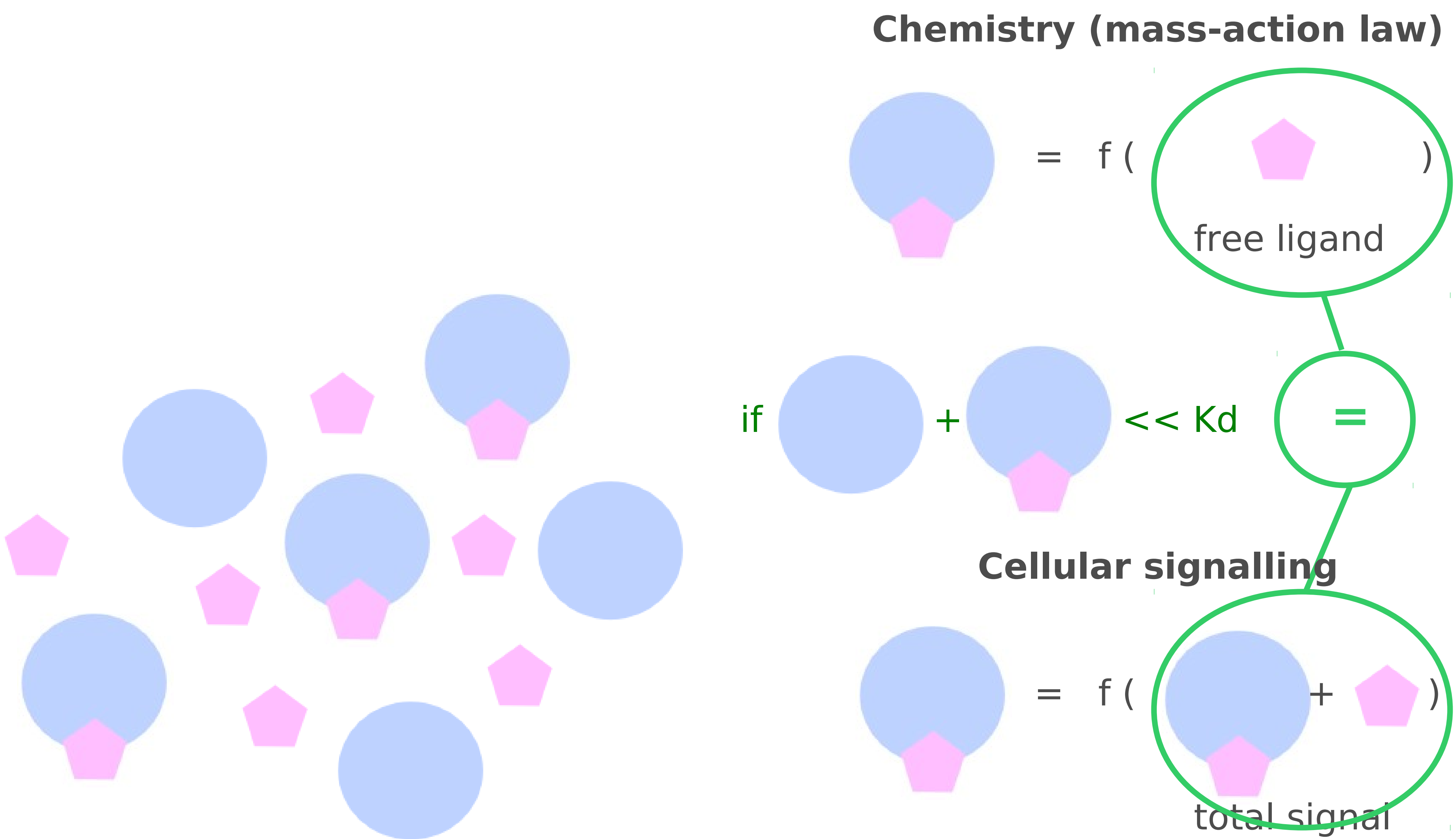
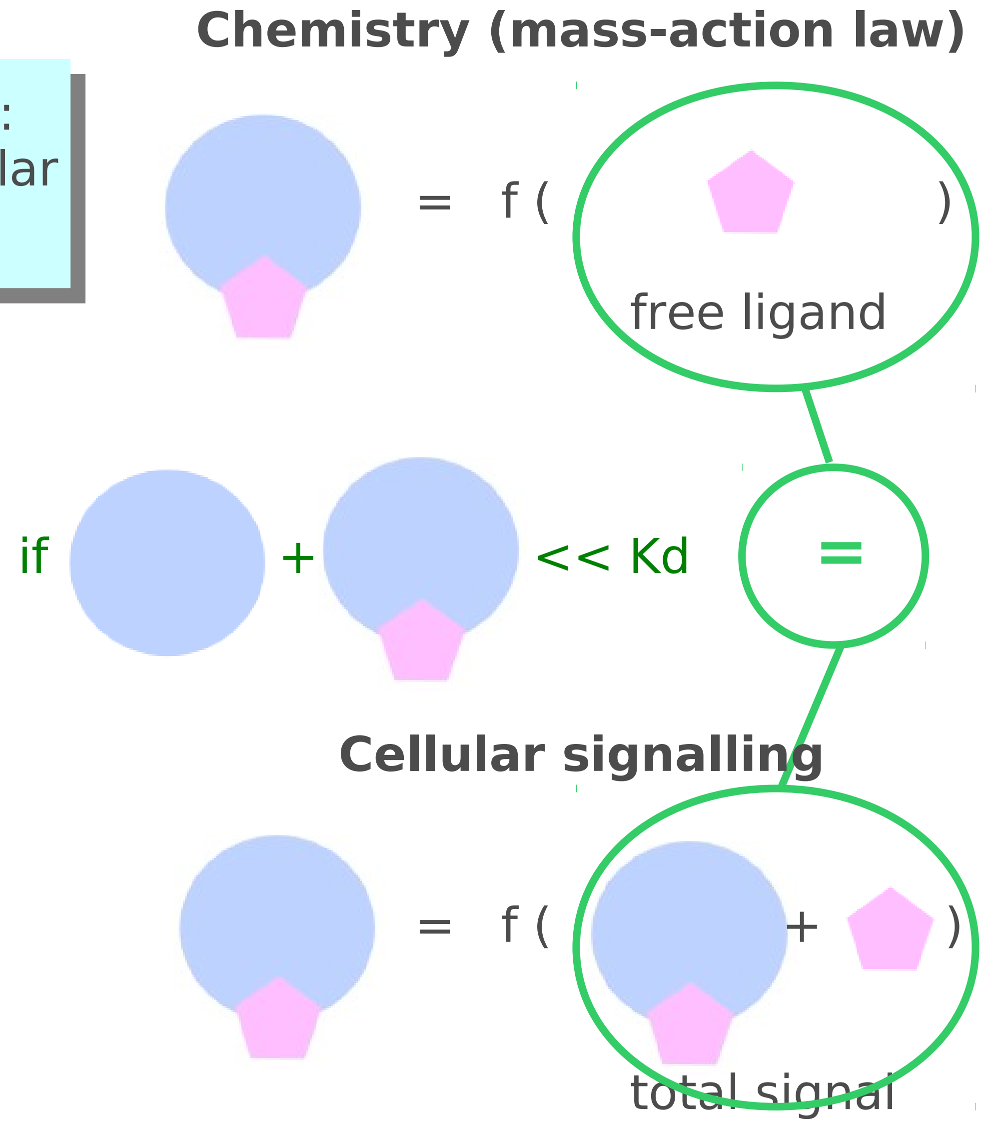
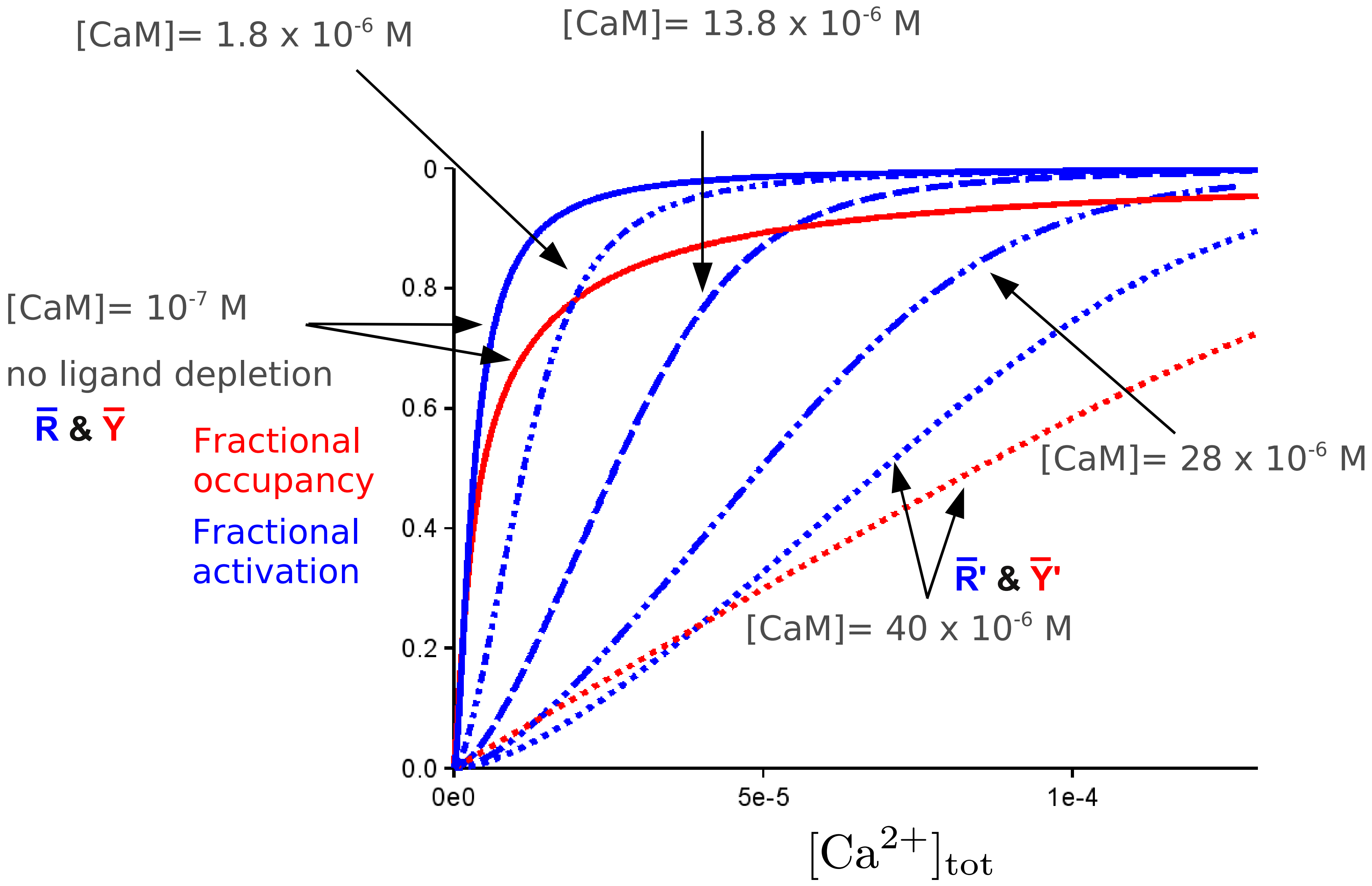


What is ligand depletion?

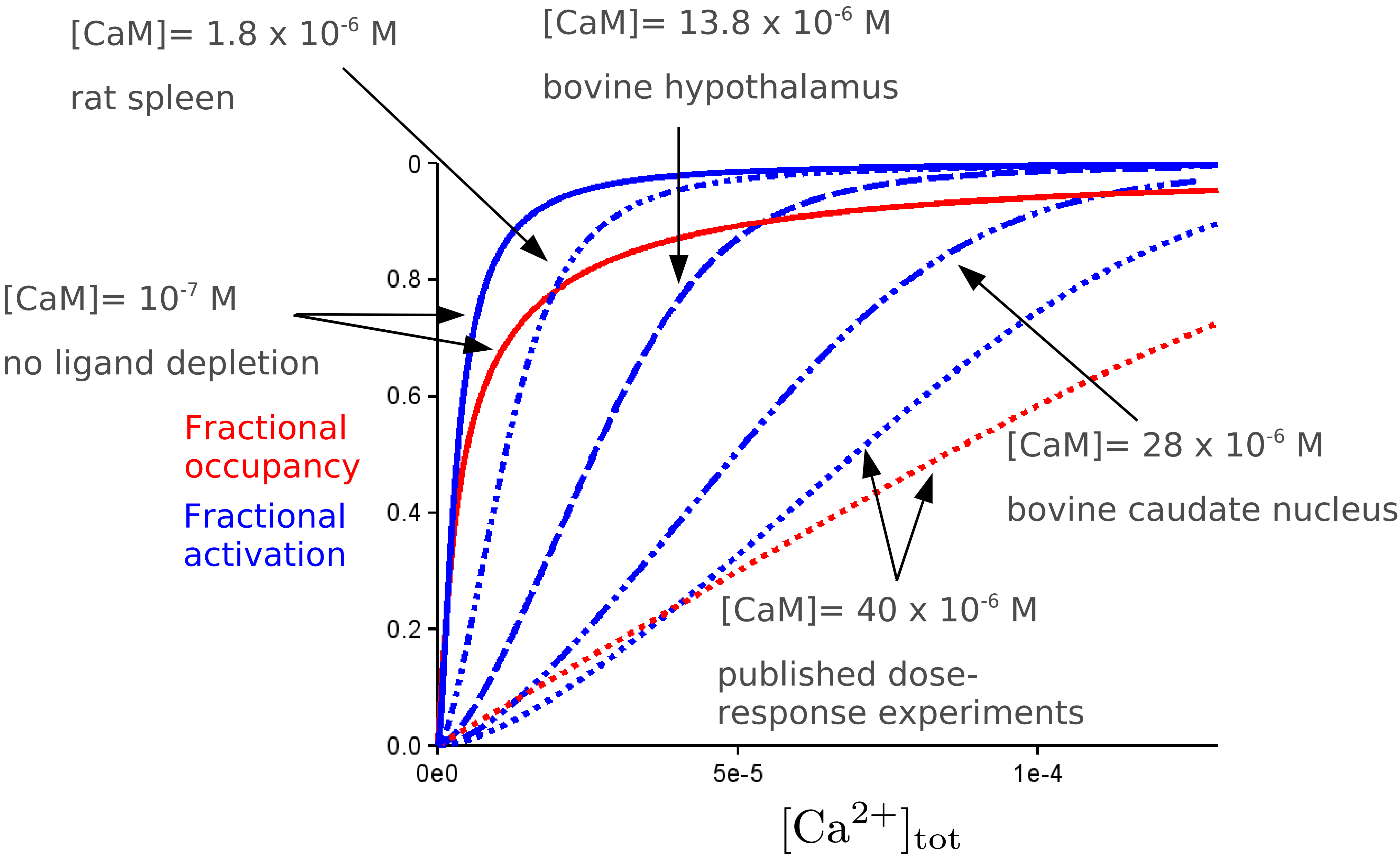
This is generally not the case in signalling: Concentrations of sensors are in micromolar range, as are the dissociation constants.



Dose-response depends on Calmodulin concentration



Dose-response depends on Calmodulin concentration



Ligand-depletion modifies sensitivity

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

bovine caudate nucleus

~5 μM to 120 μM

$[CaM] = 1.8 \times 10^{-6}$ M

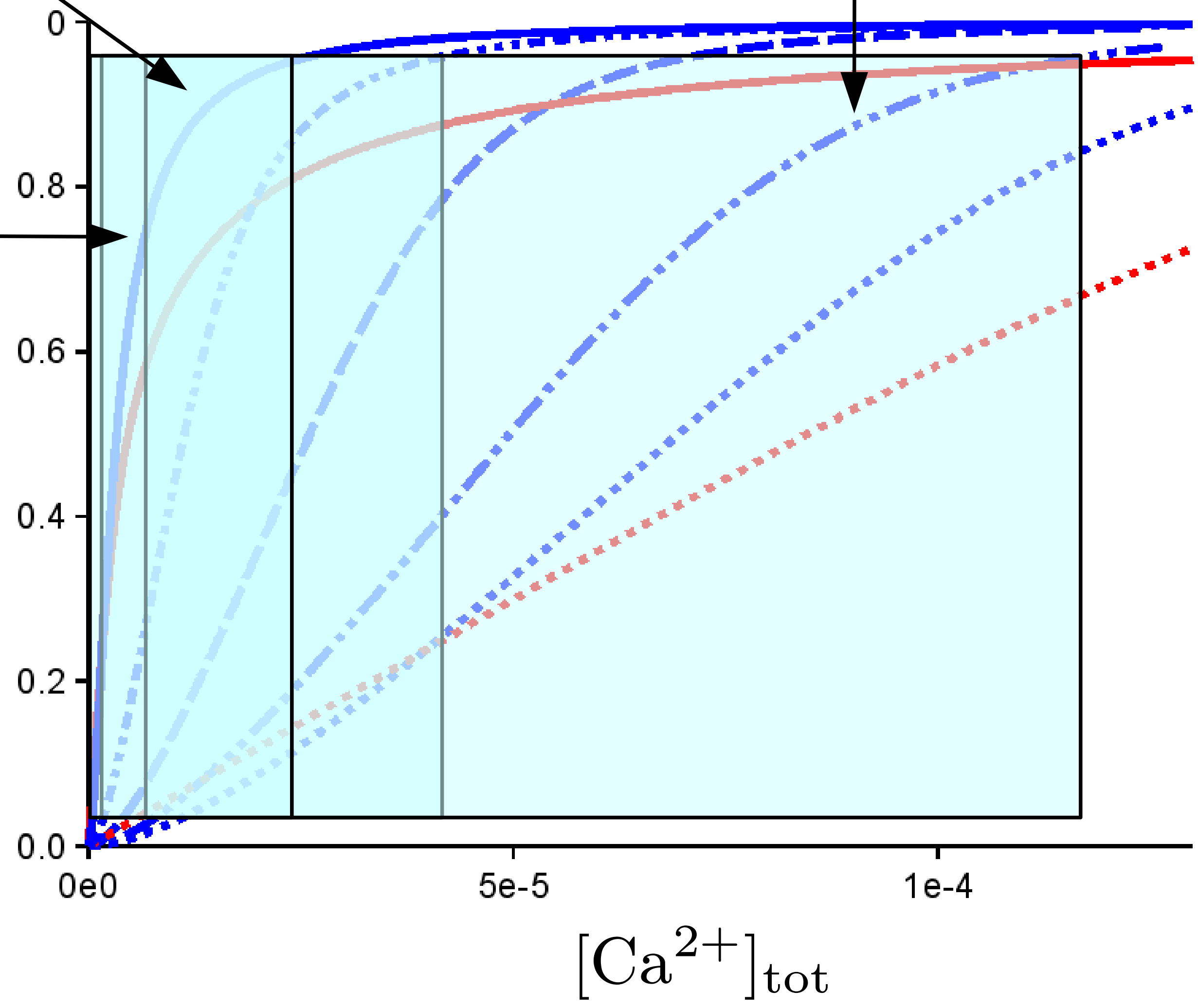
rat spleen

~1 μM to 45 μM

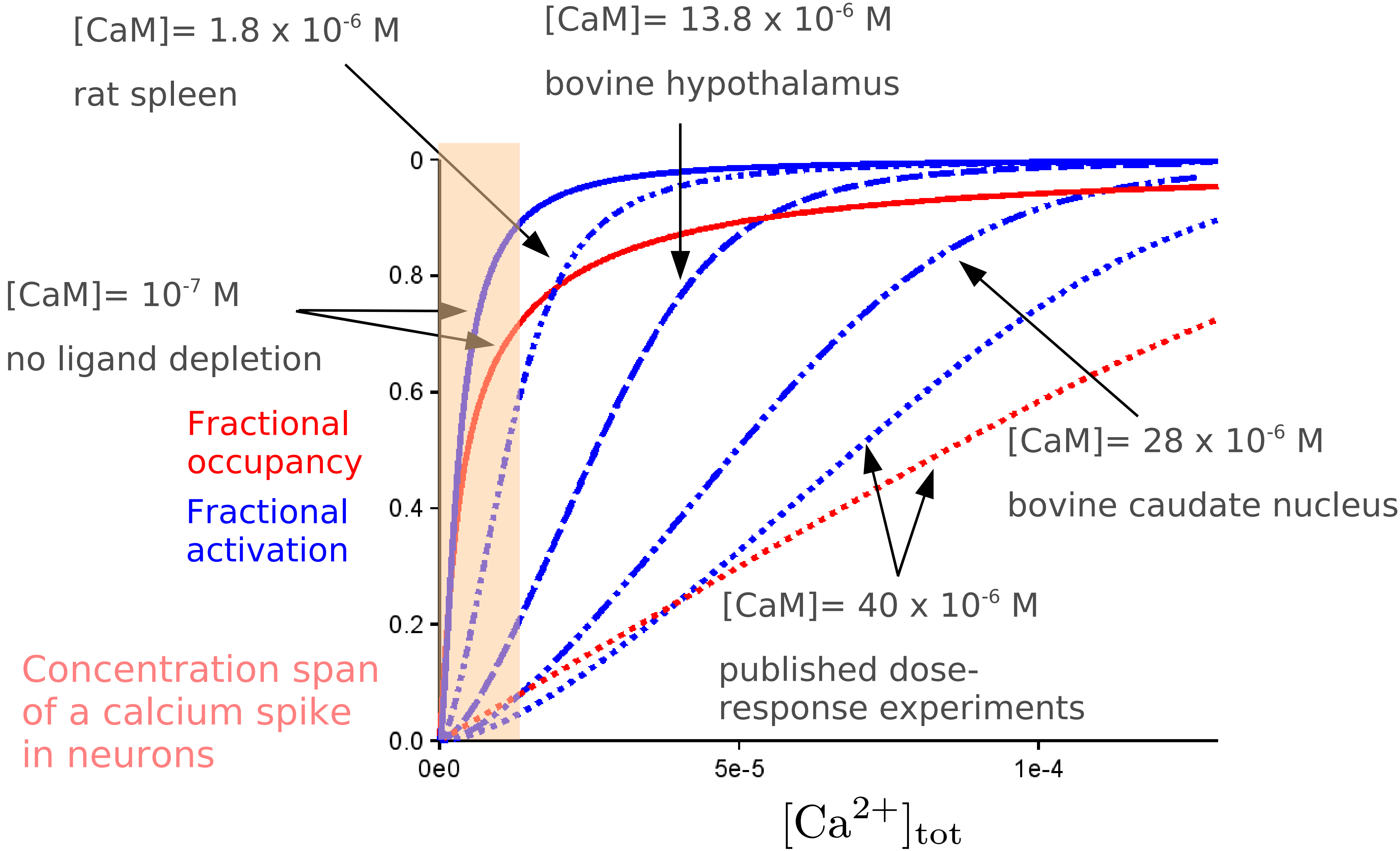
$[CaM] = 10^{-7}$ M

no ligand depletion

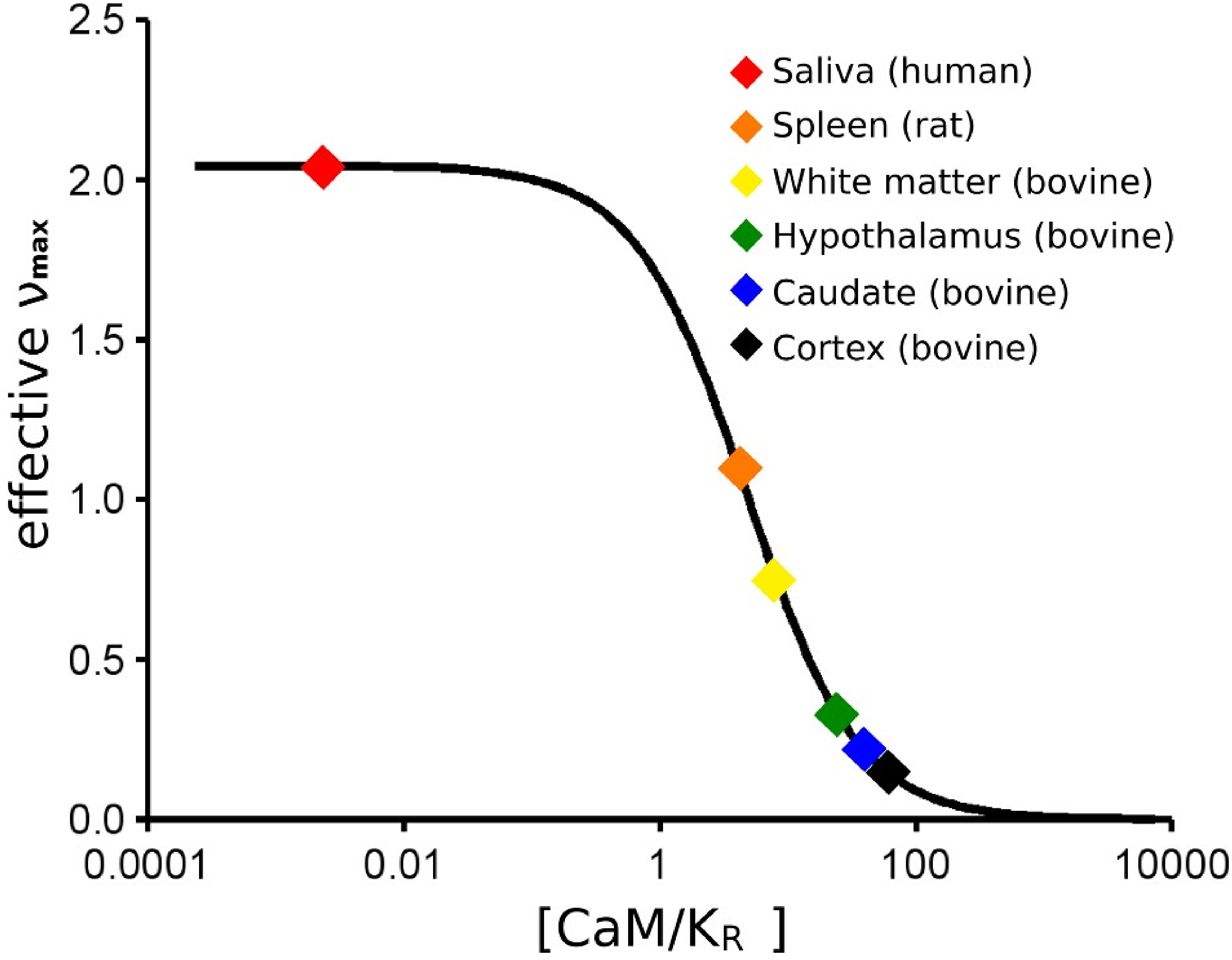
~100 nM to 25 μM



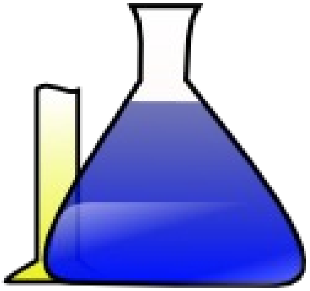
But we cannot build a large $[Ca^{2+}]$ in neurons ...

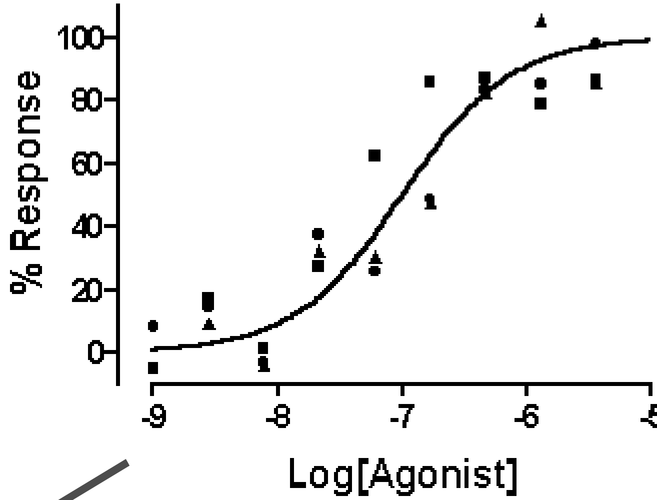


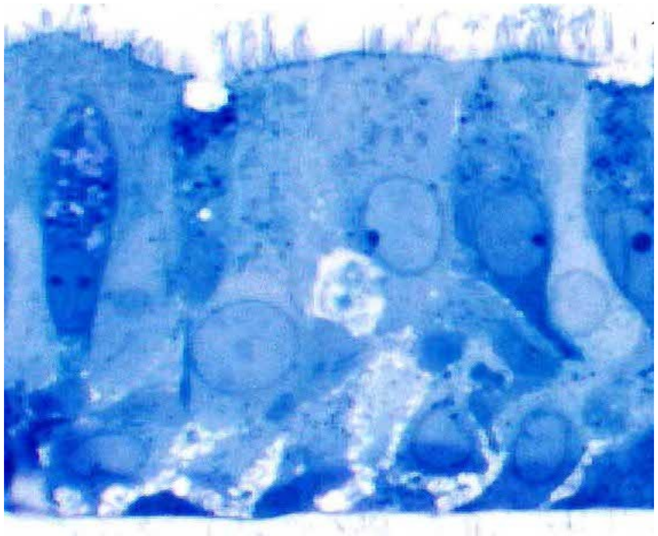
Ligand-depletion decreases effective cooperativity

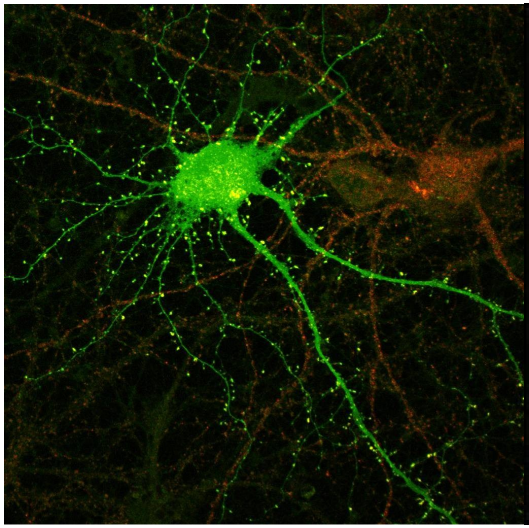


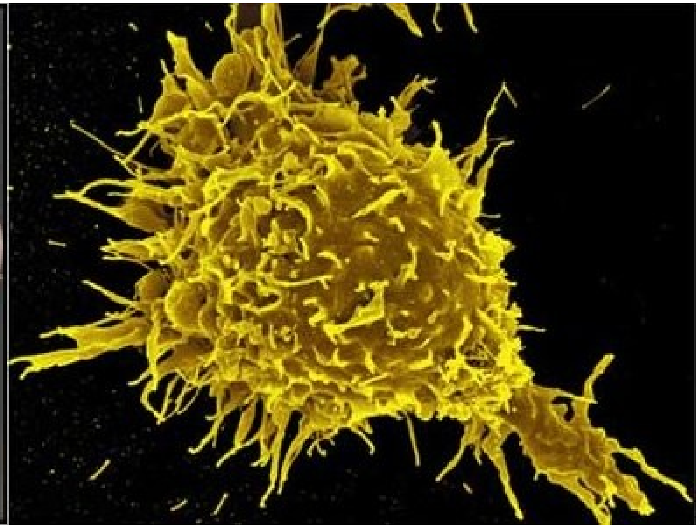
How general is a dose-response?



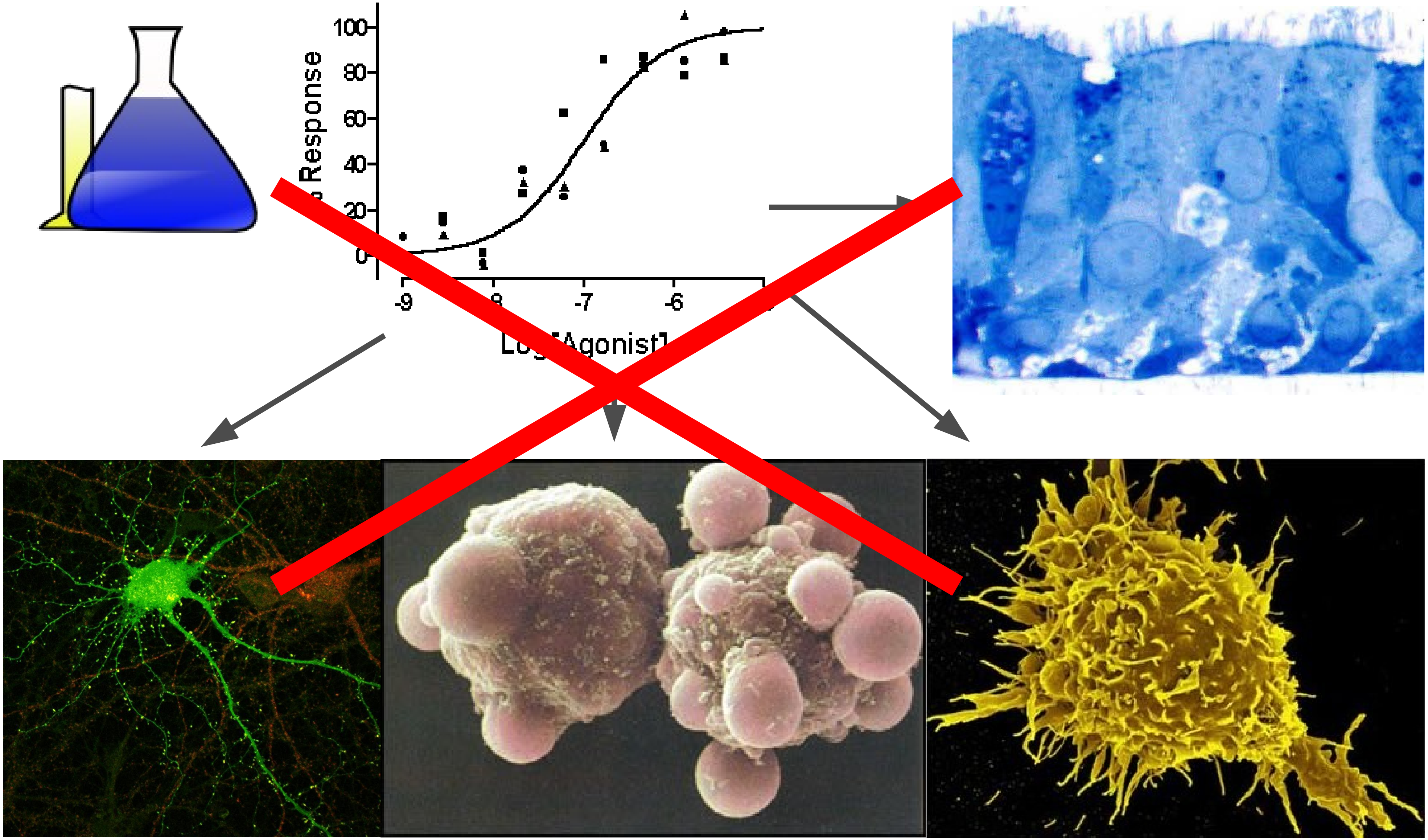








A “dose-response” cannot be reused directly!



Conclusions on depletion

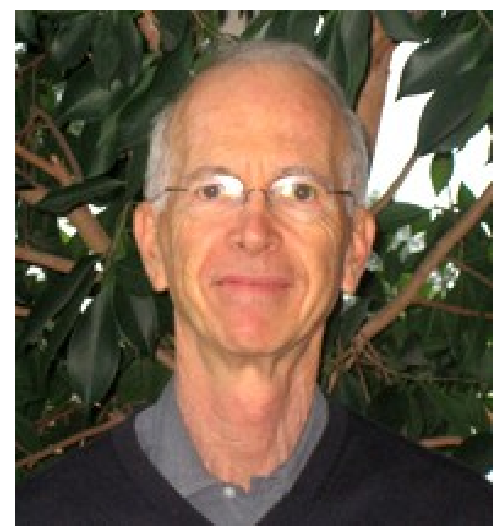
- Dose-responses are the basic characterisations of “systems”, but also at the core of pharmacological treatments. Here we show that:
  - A “dose-response”cannot be reused directly in models of signalling systems. Instead one needs to build “mechanistic” models and run parameter-fitting approaches.
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

Melanie Stefan

Lu Li



Stuart Edelstein