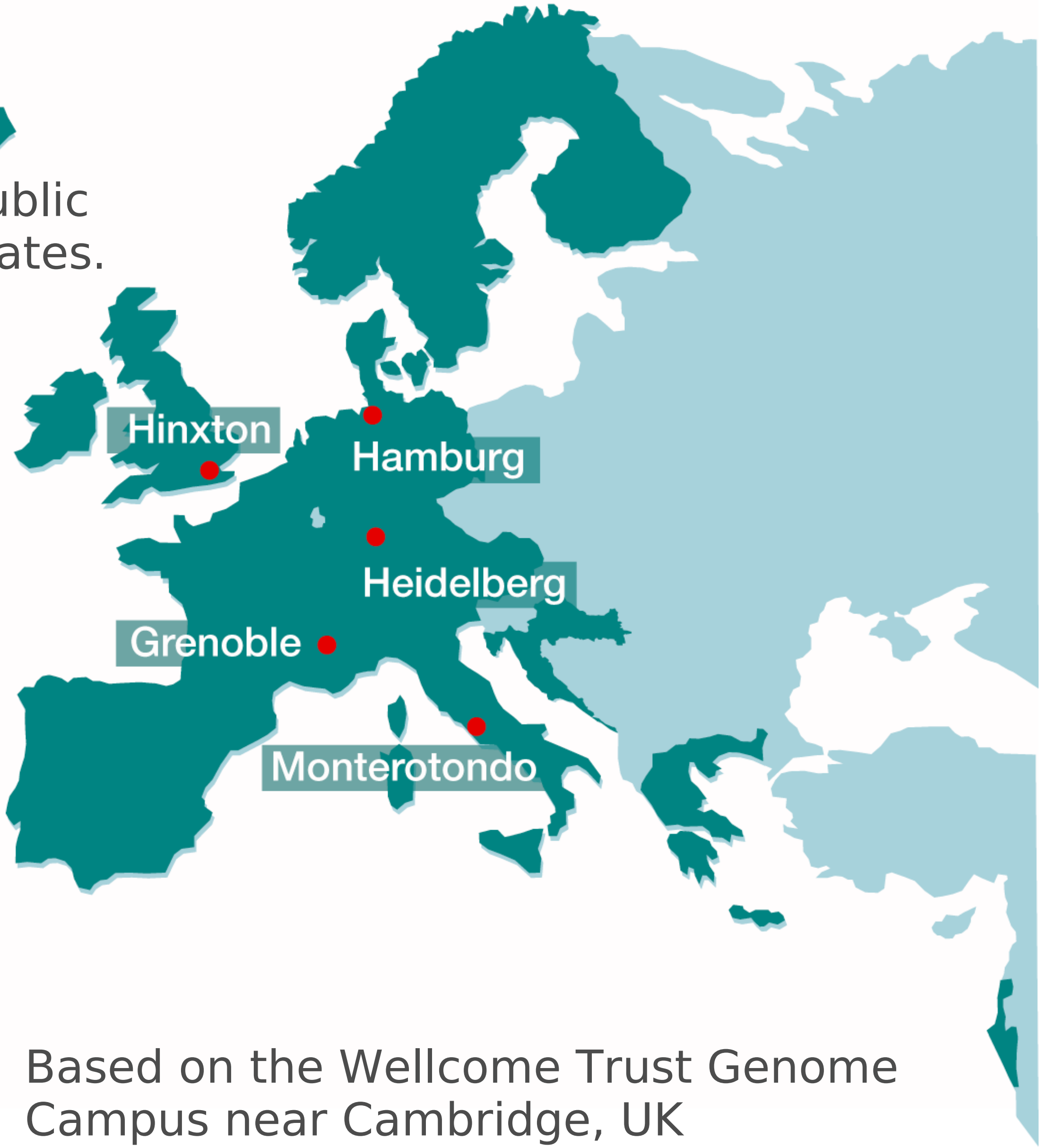
Modélisation du comportement des senseurs allosteriques du calcium impliqués dans la plasticité synaptique

Cooperativité, sensibilité et tout ça

Nicolas Le Novère, EMBL-EBI

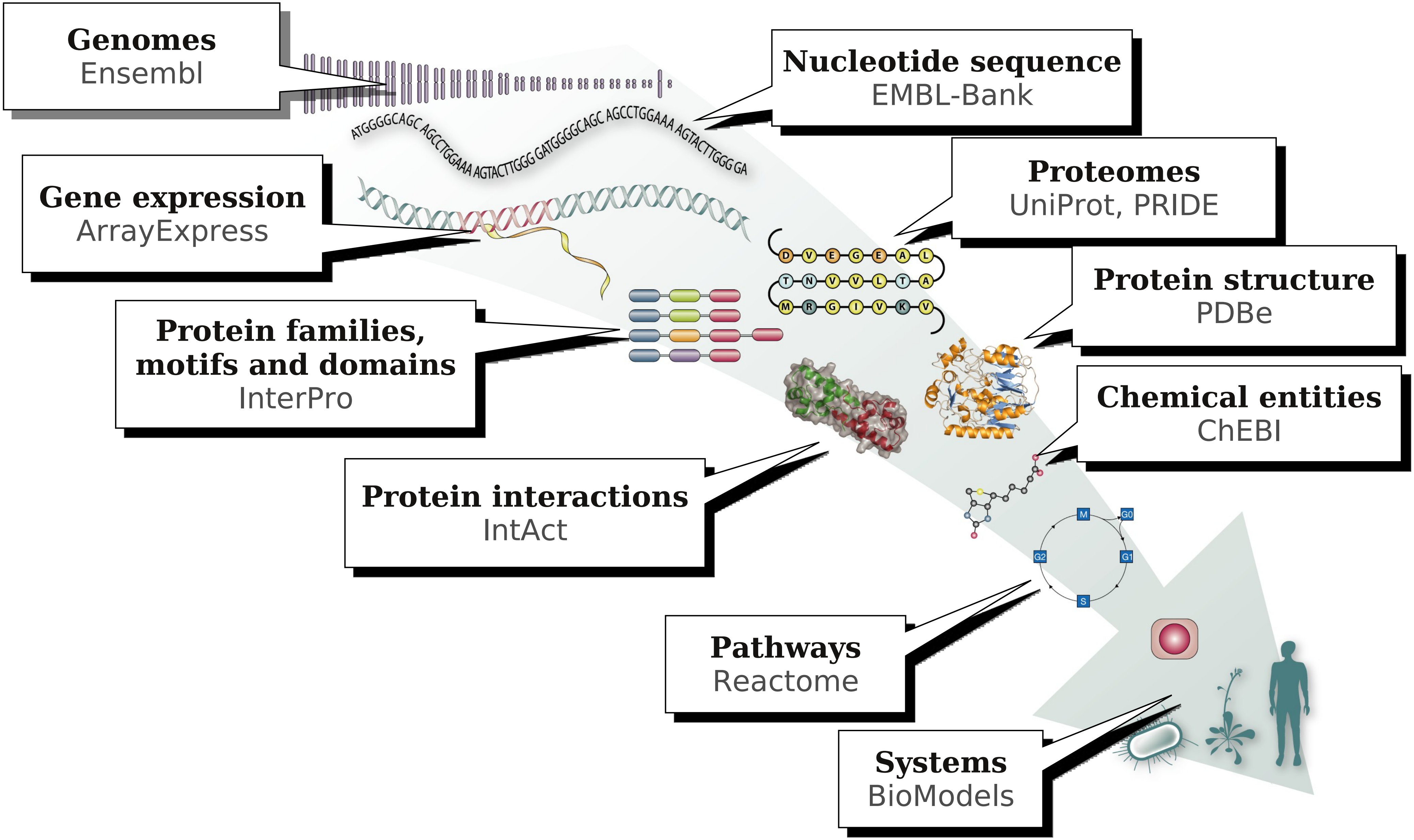
EBI is part of EMBL

- Non-profit organization
- Part of the European Molecular Biology Laboratory (EMBL), a basic research institute funded by public research monies from 20 member states.



- Based on the Wellcome Trust Genome Campus near Cambridge, UK

EBI Data resources: molecules to systems



Genomes
Ensembl

Nucleotide sequence
EMBL-Bank

Gene expression
ArrayExpress

Proteomes
UniProt, PRIDE

Protein structure
PDBe

Protein families, motifs and domains
InterPro

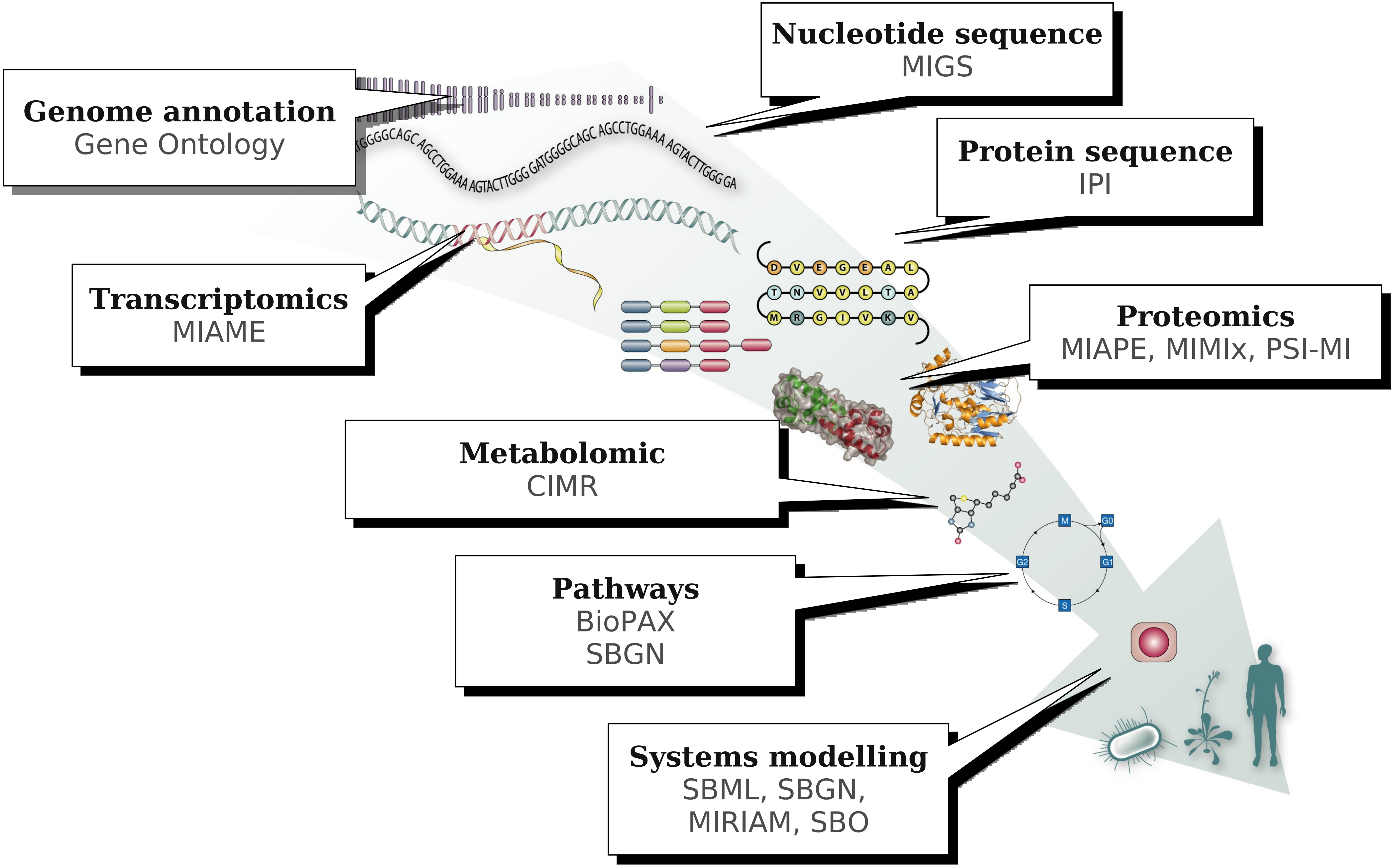
Chemical entities
ChEBI

Protein interactions
IntAct

Pathways
Reactome

Systems
BioModels

Standards development



Nucleotide sequence
MIGS

Genome annotation
Gene Ontology

Protein sequence
IPI

Transcriptomics
MIAME

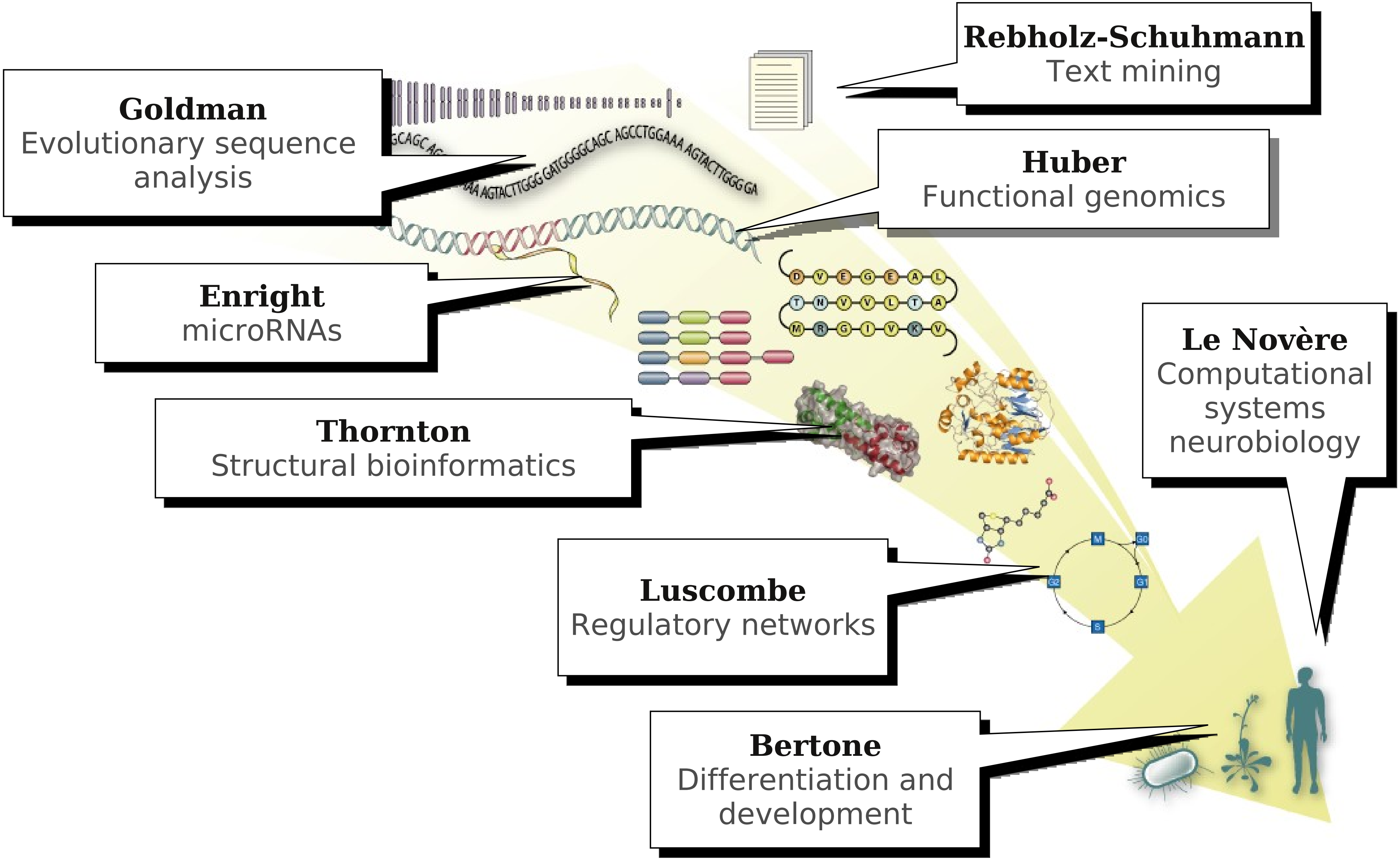
Proteomics
MIAPE, MIMIx, PSI-MI

Metabolomic
CIMR

Pathways
BioPAX
SBGN

Systems modelling
SBML, SBGN,
MIRIAM, SBO

Research groups



Rebholz-Schuhmann
Text mining

Goldman
Evolutionary sequence analysis

Huber
Functional genomics

Enright
microRNAs

Le Novère
Computational systems neurobiology

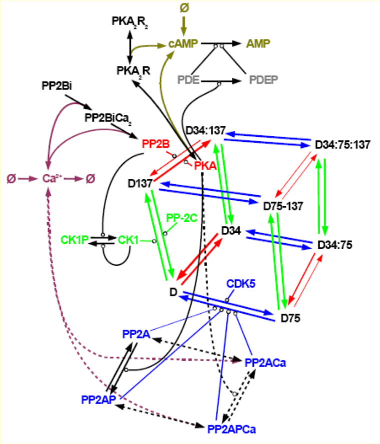
Thornton
Structural bioinformatics

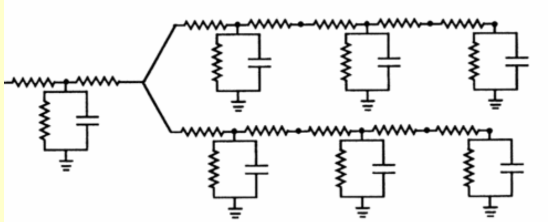
Luscombe
Regulatory networks

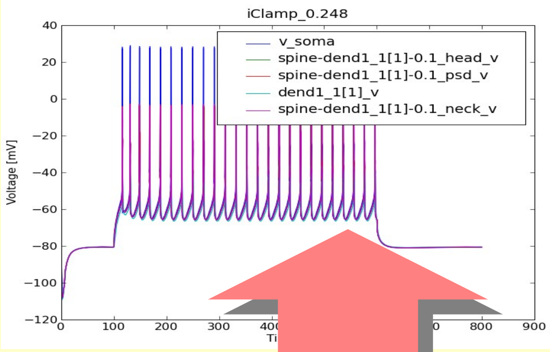
Bertone
Differentiation and development

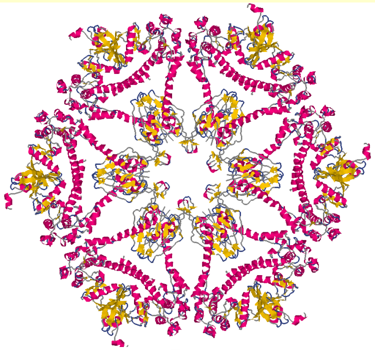
compneur group's themes and projects

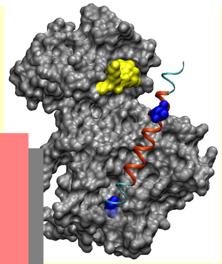
Computational Neurobiology

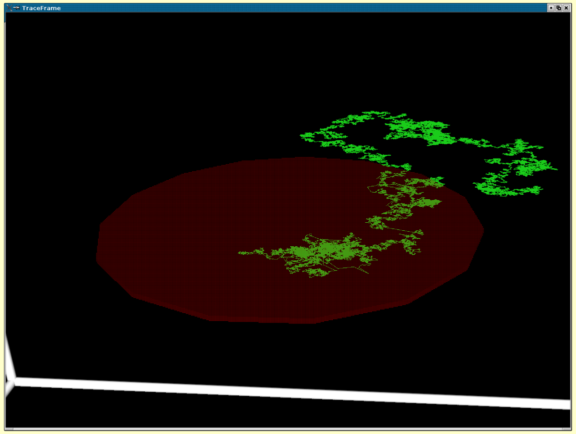












SBML™

SBGN

SBO

TEDDY

KISAO

e-MeP

Ligand-Gated Ion Channel database

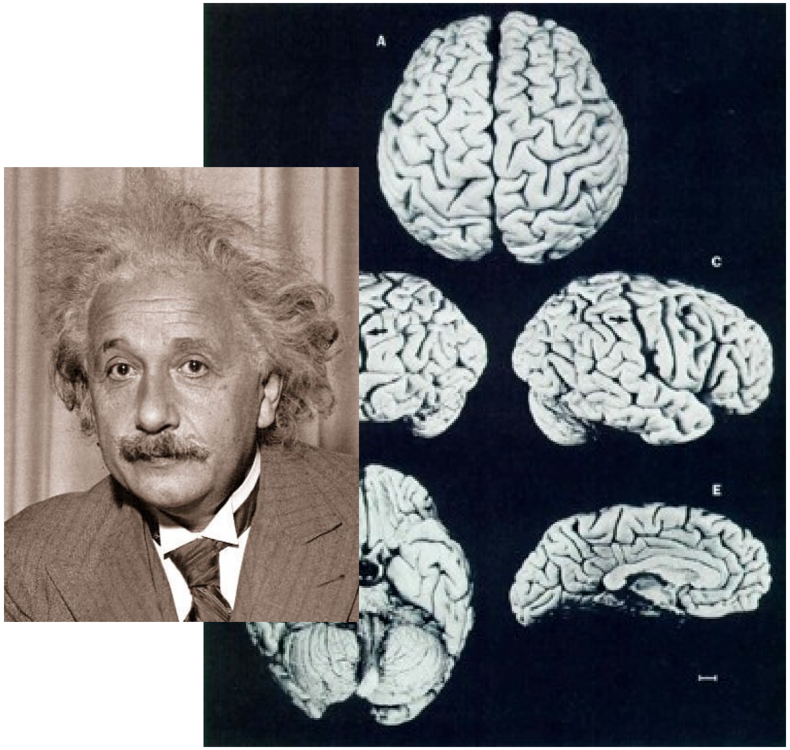
Computational Systems Biology

MIRIAM

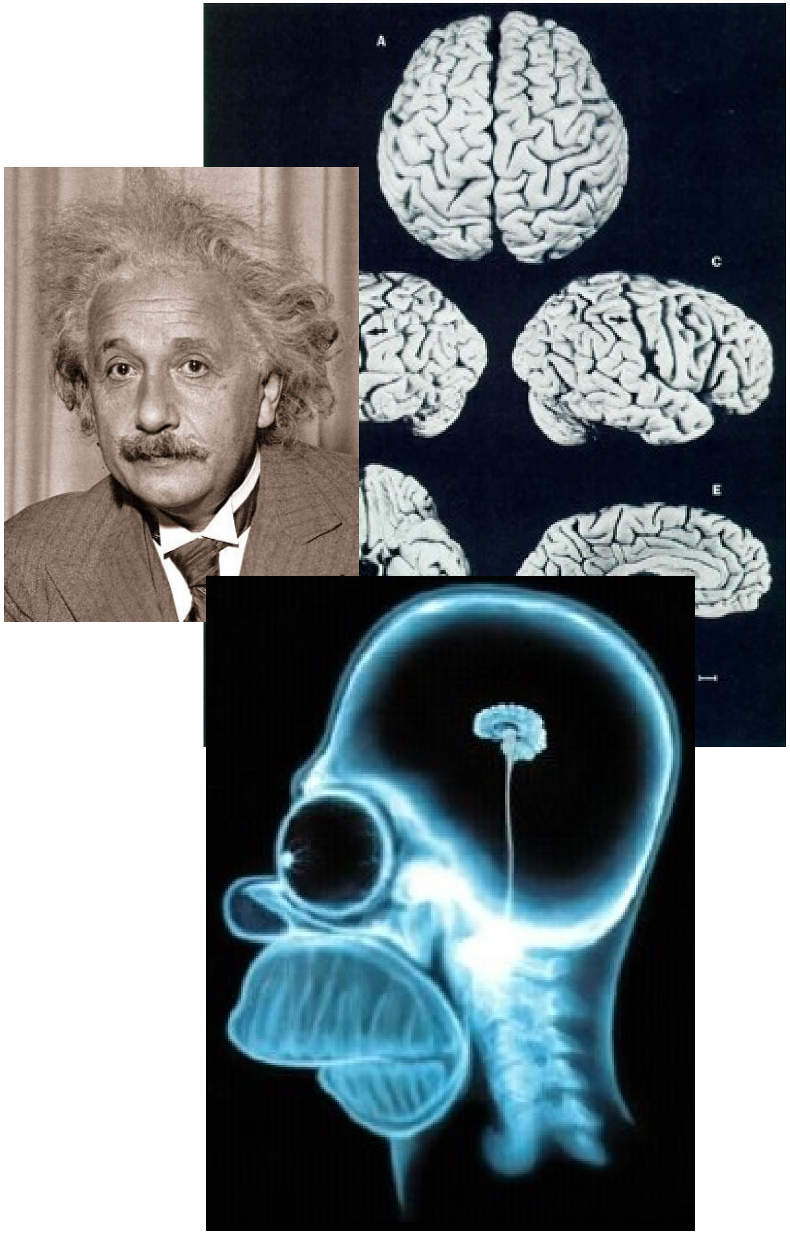
MIASE

BioModels

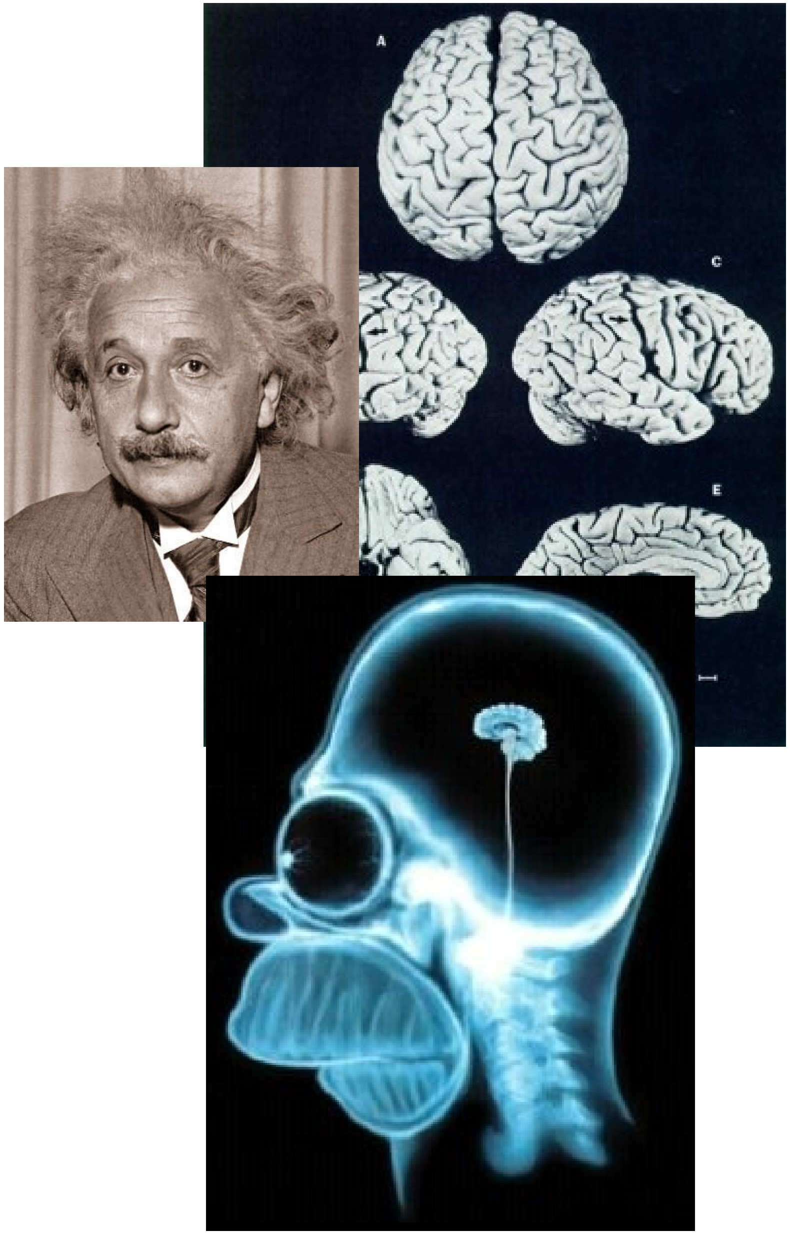
The brain

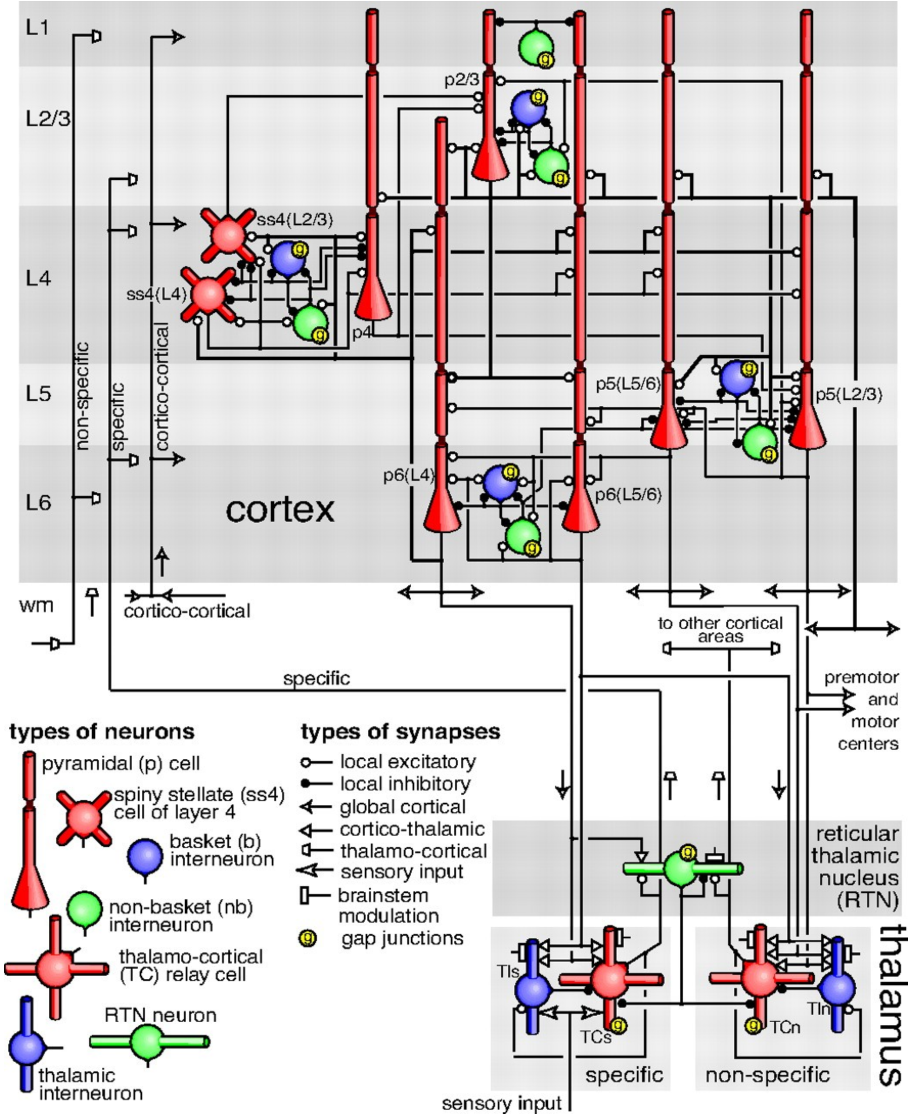


The brain



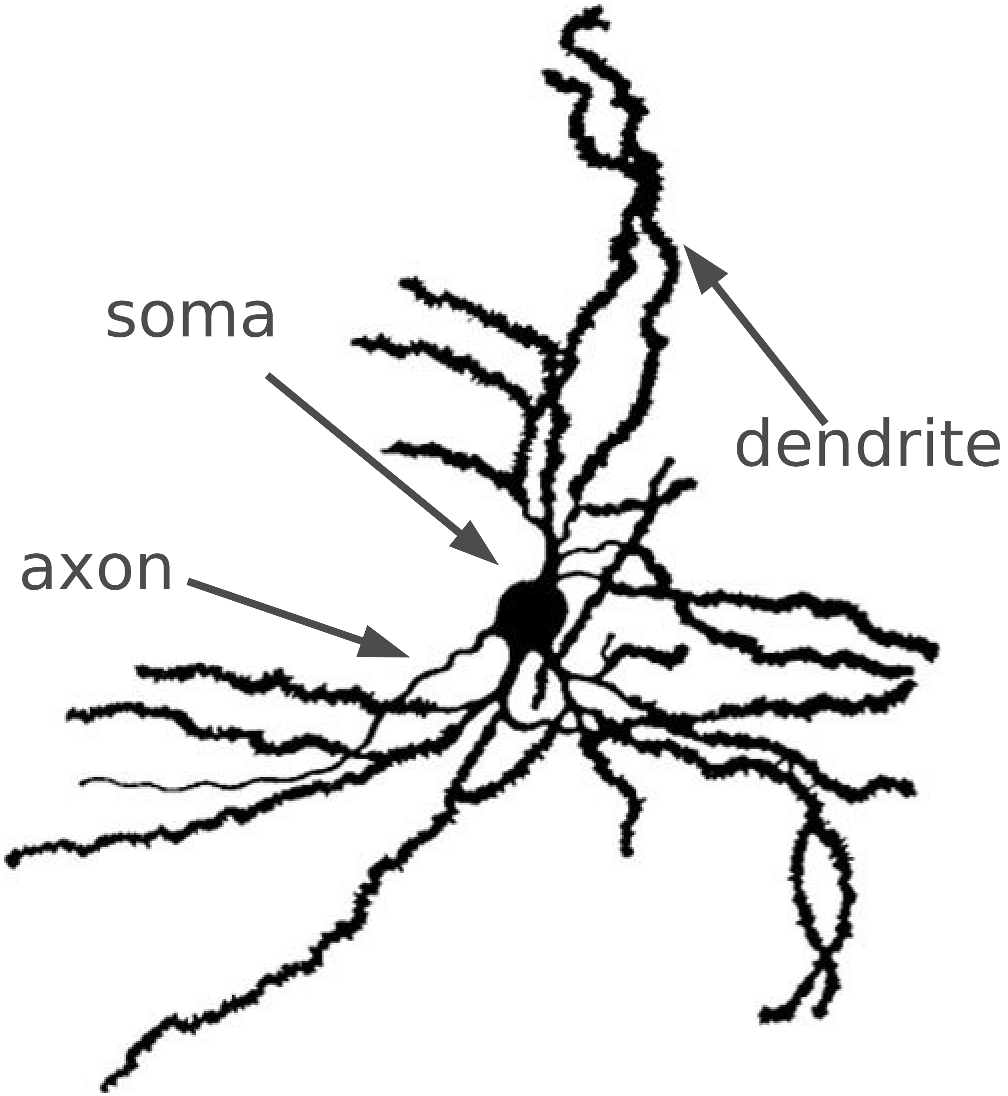
The brain

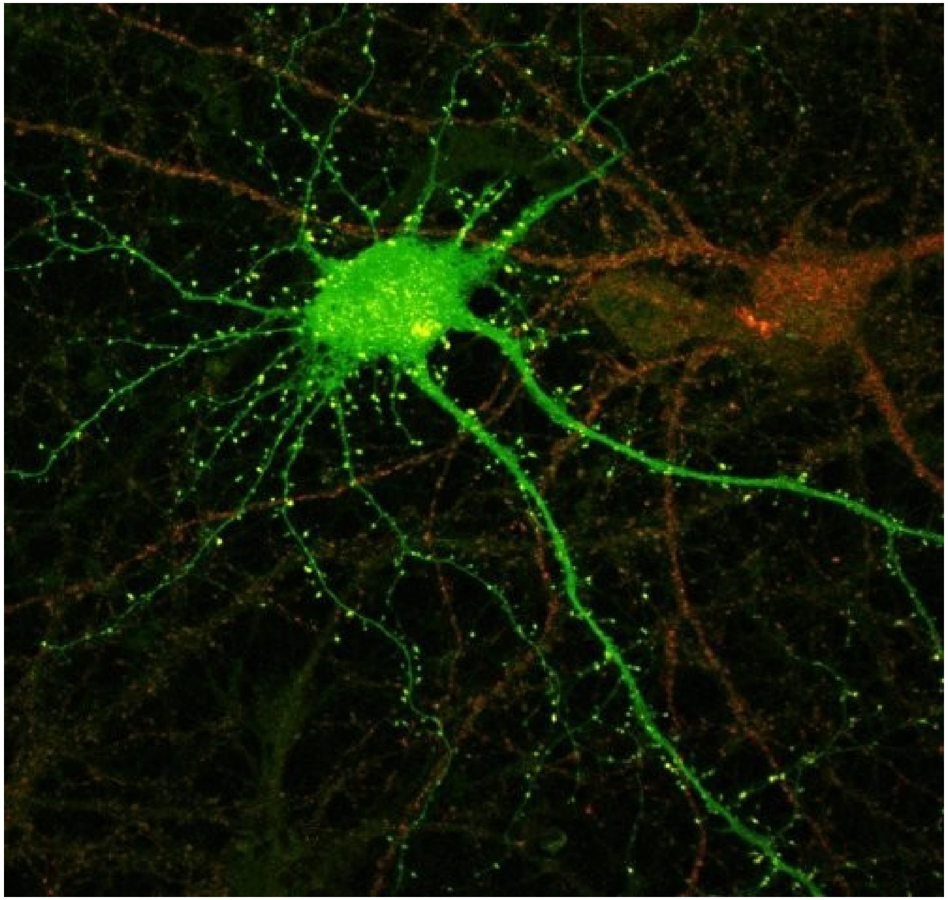




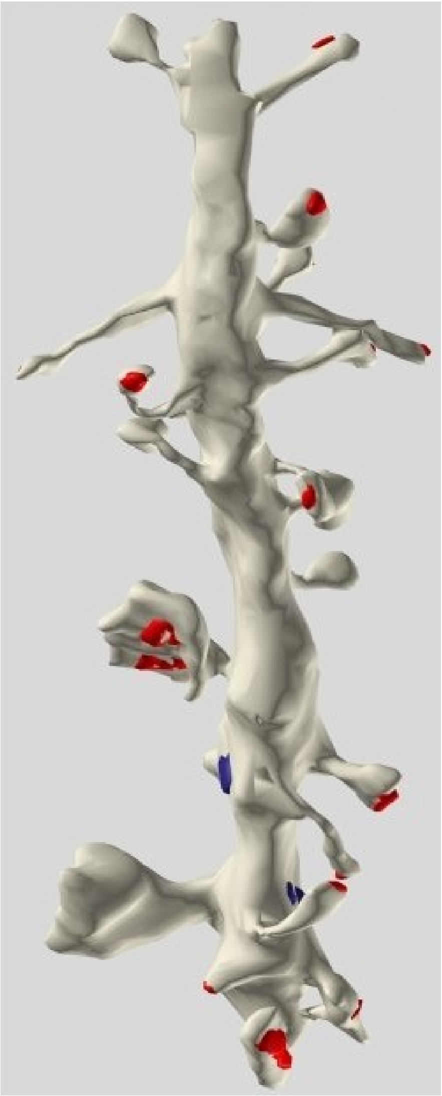
Izhikevich, Edelman (2008)
PNAS 105: 3593-3598

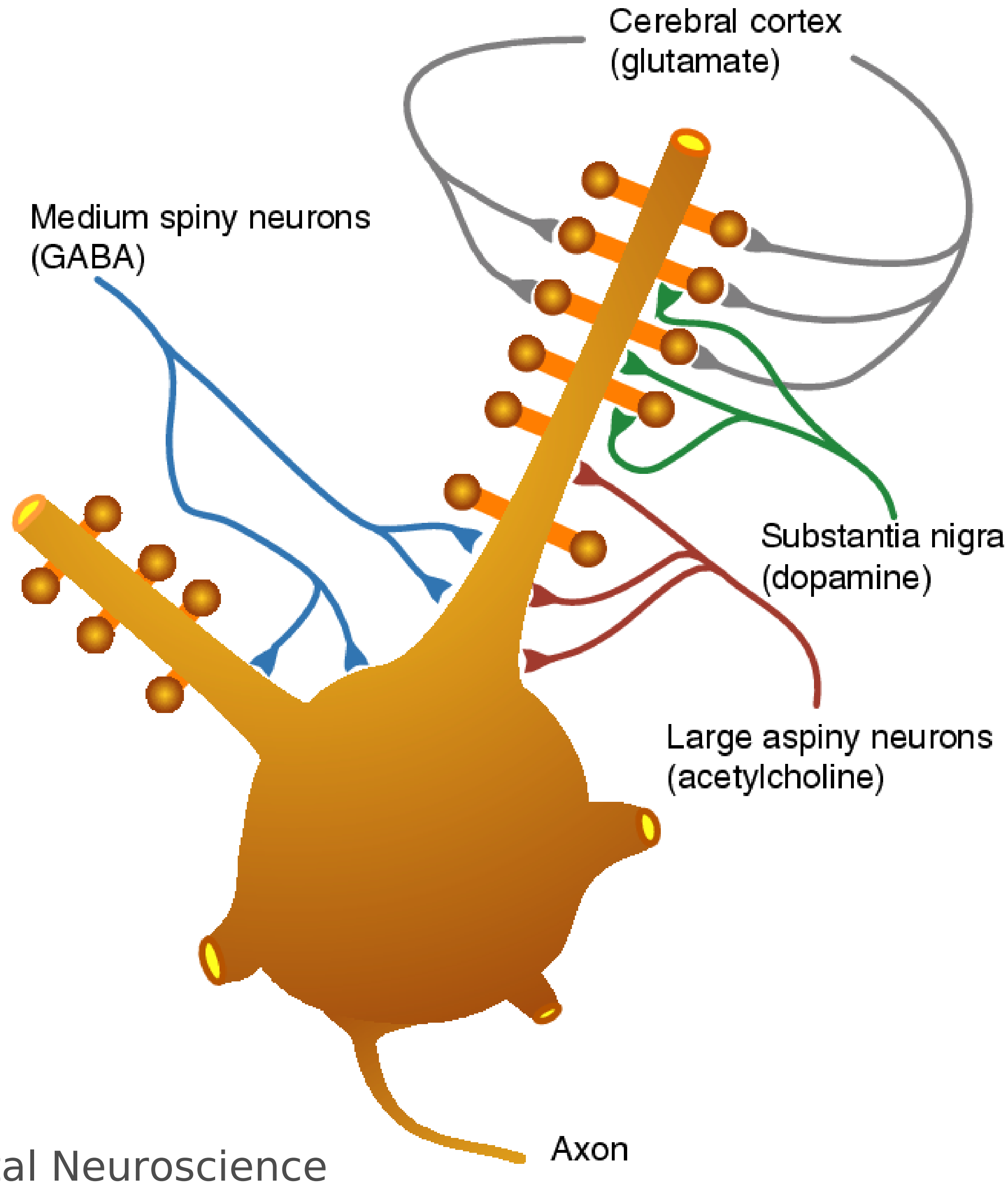
The neuron





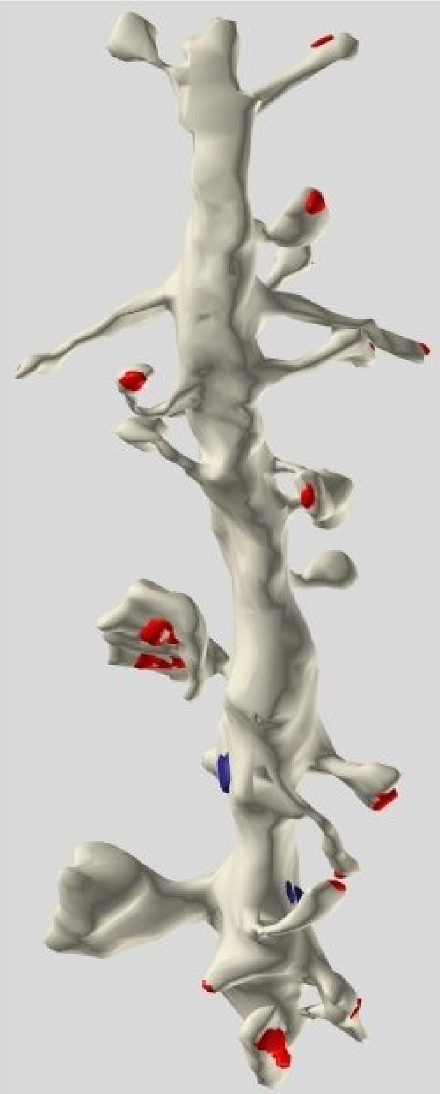
The dendrite

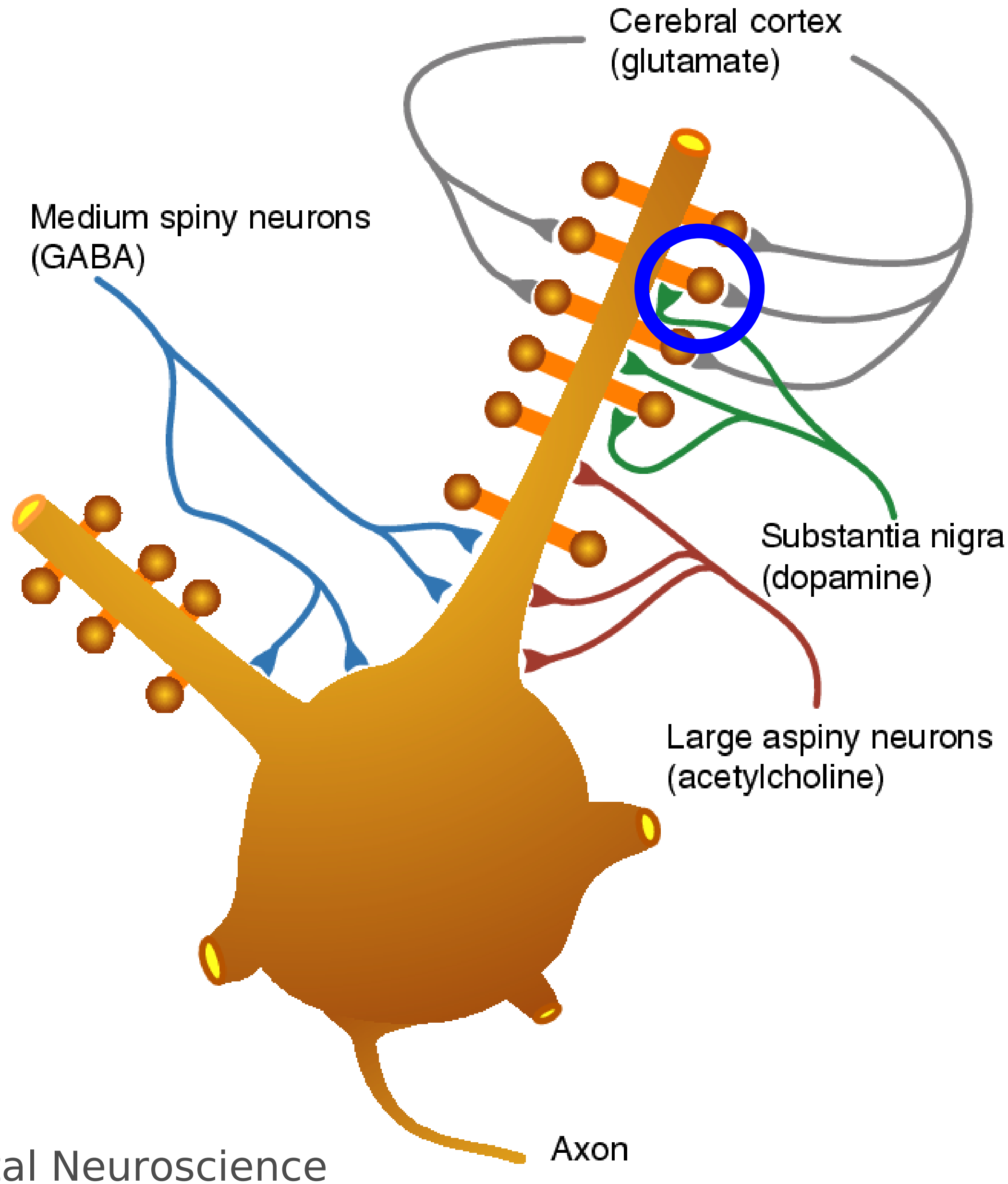




Fundamental Neuroscience
Squire et al. 2nd ed(2003)

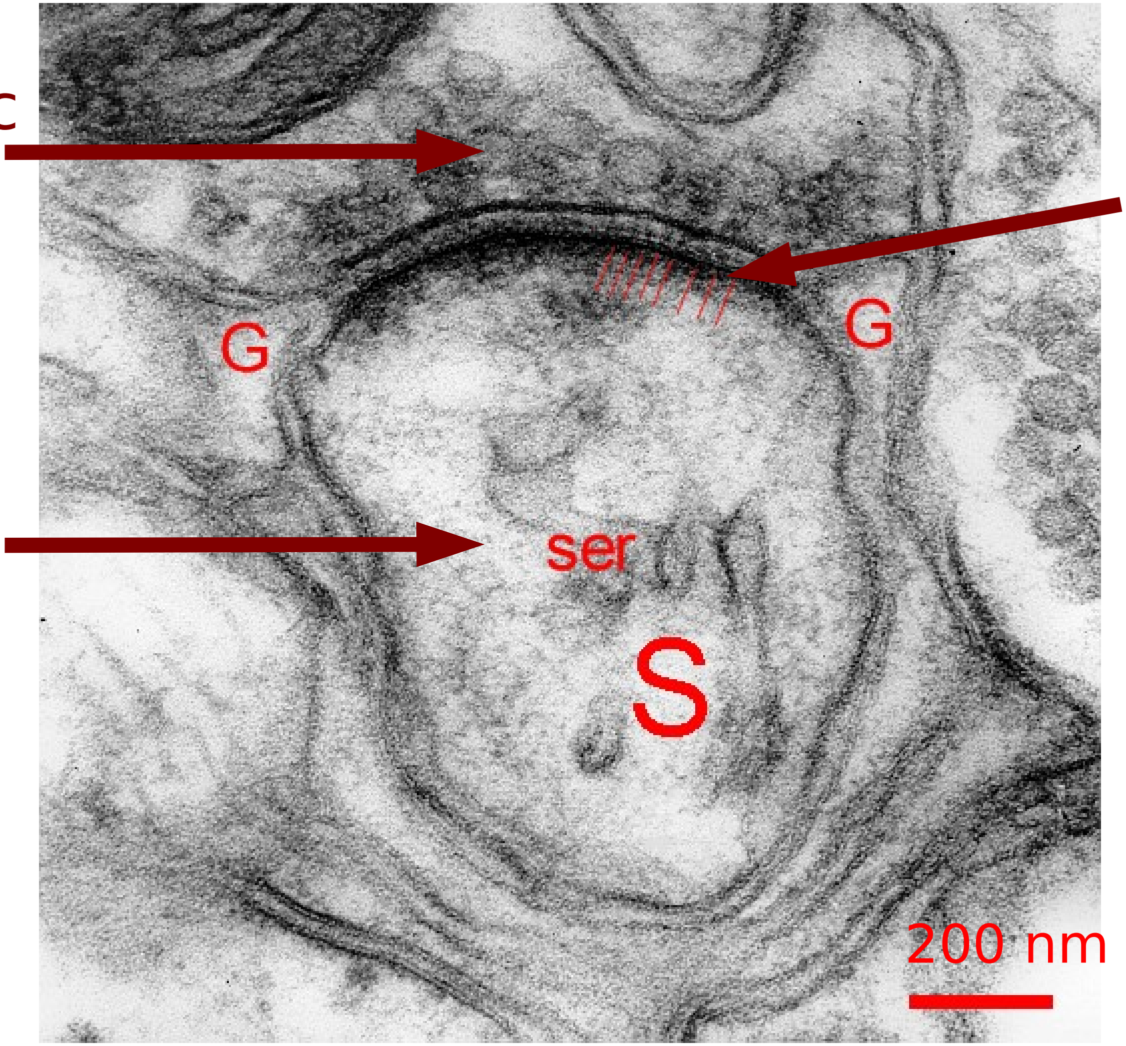
The dendrite





Fundamental Neuroscience
Squire et al. 2nd ed(2003)

The spine



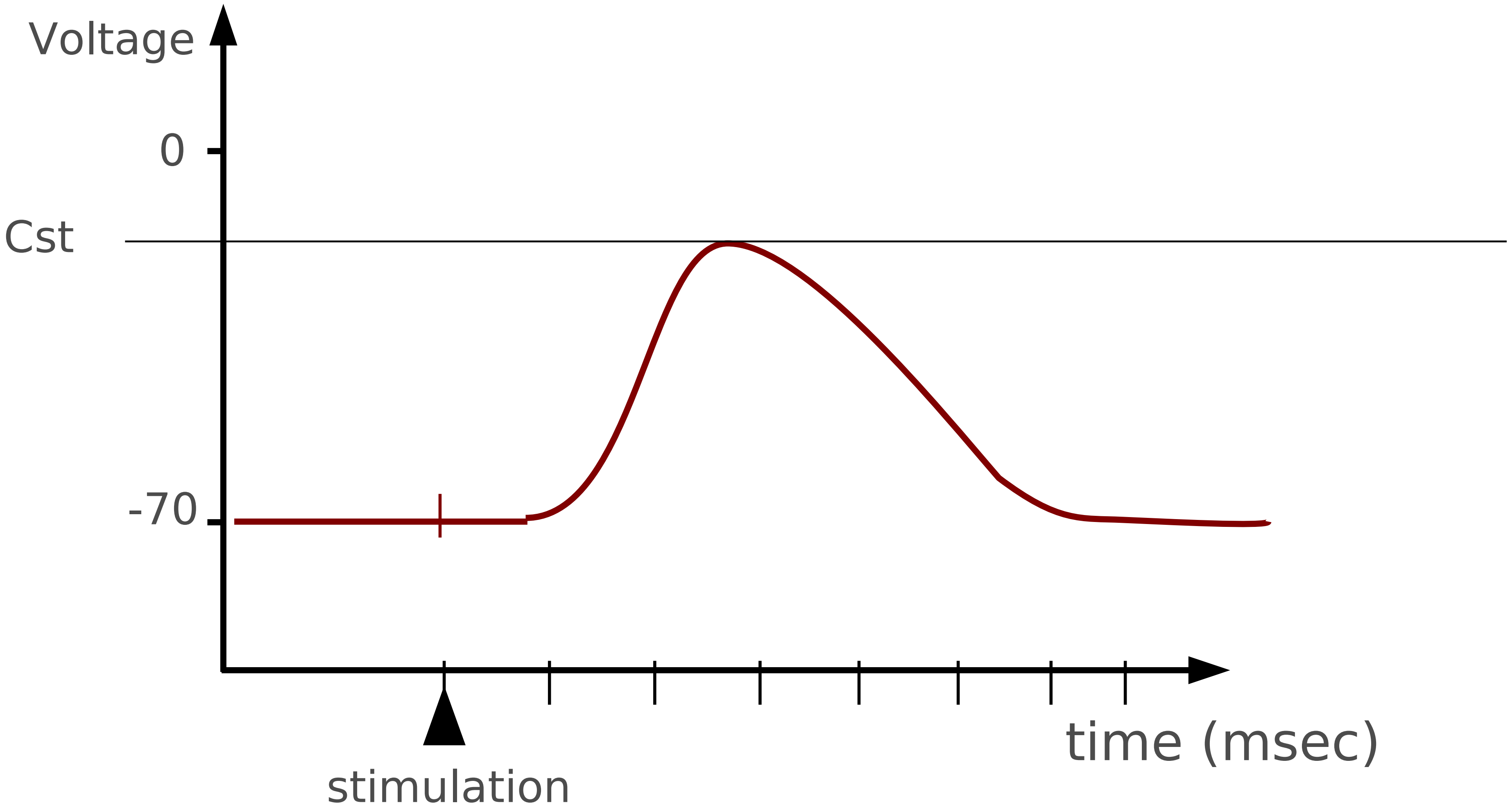
pre-synaptic neuron

post-synaptic density

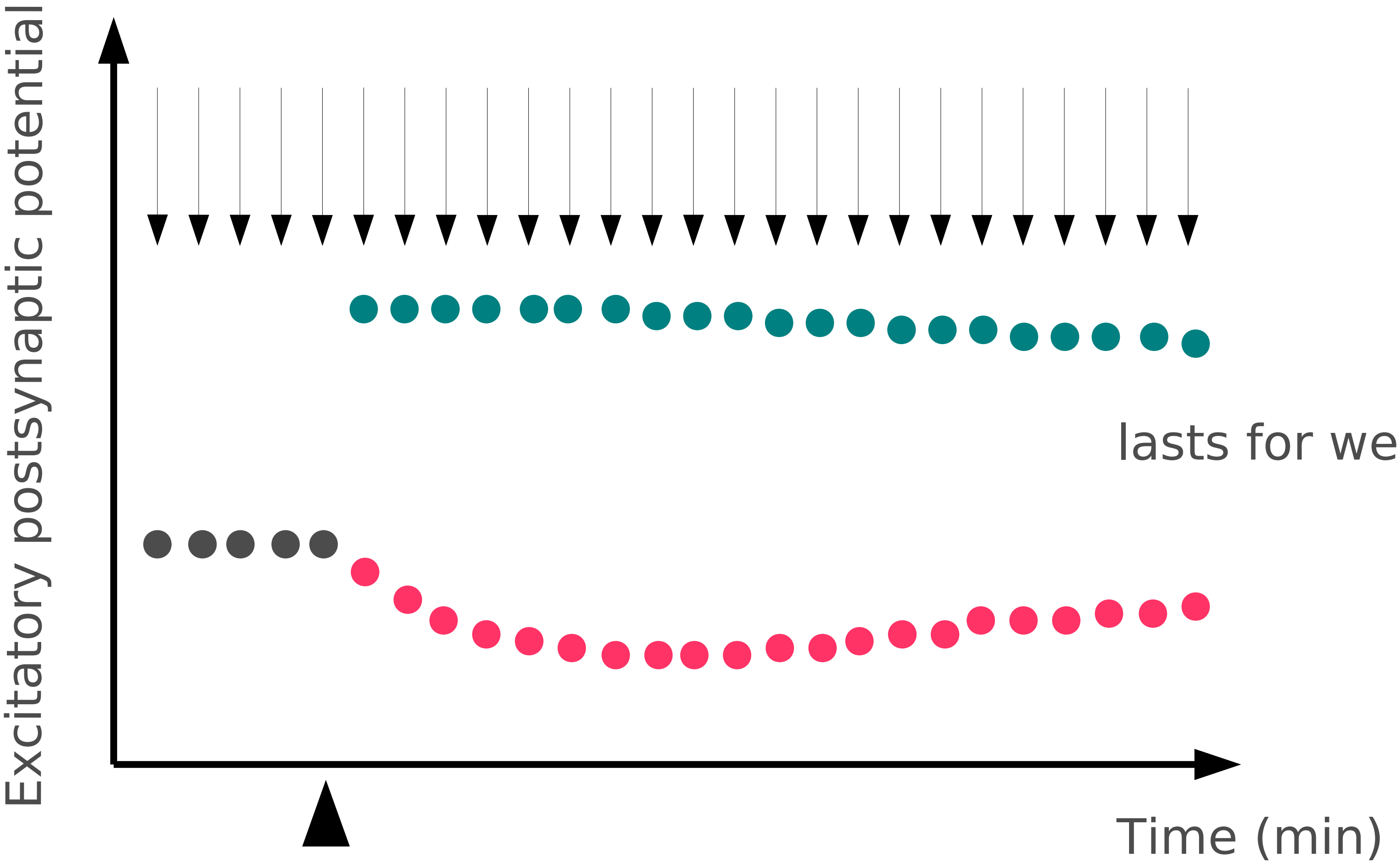
Dendritic spine

Atlas of ultrastructural neurocytology (http://synapse-web.org/)

Excitatory post-synaptic potential

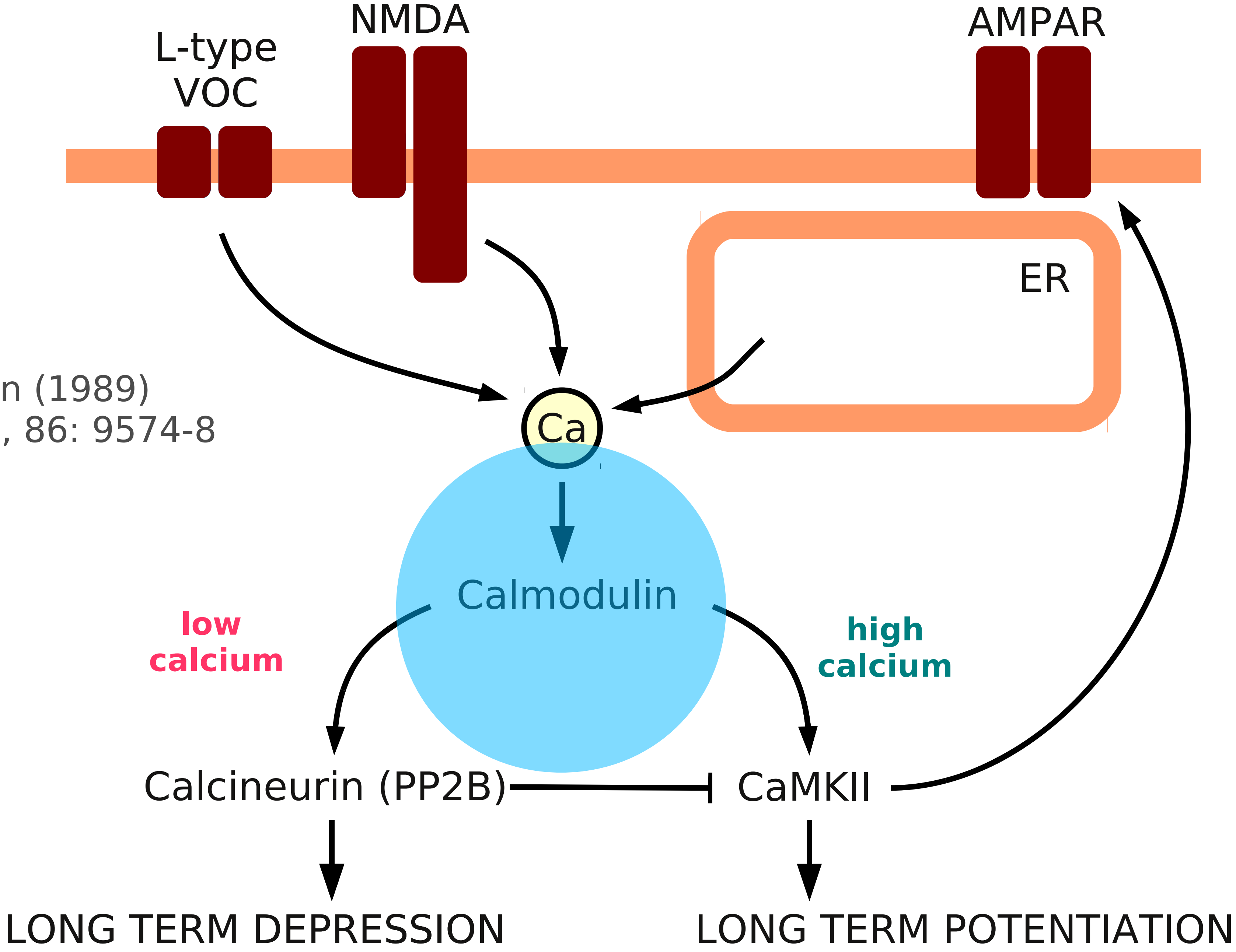


Bidirectional synaptic plasticity



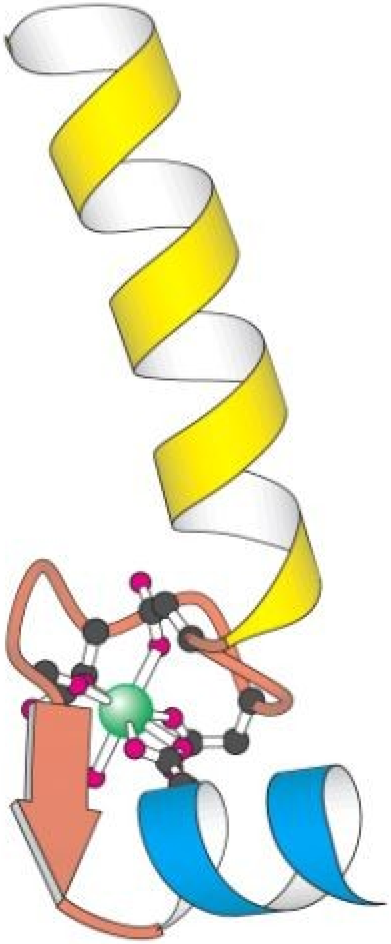
High frequency stimulation
Low frequency stimulation

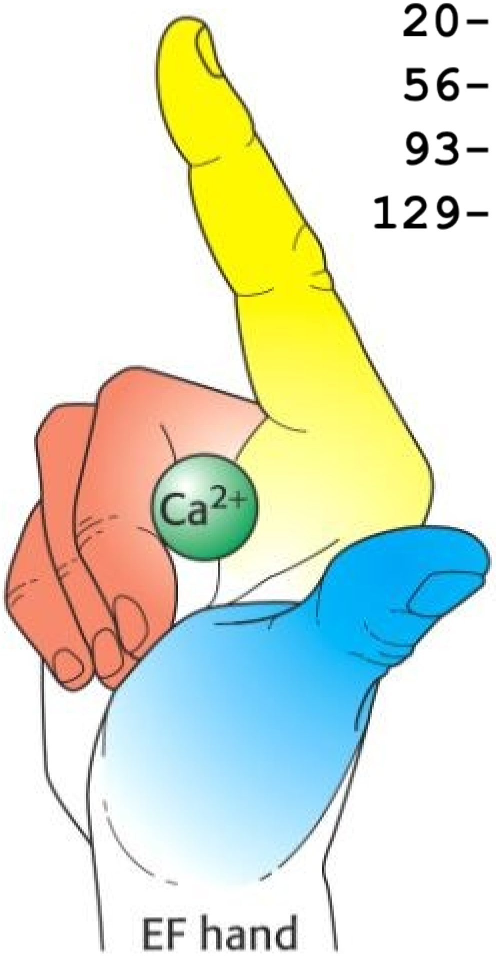
Calmodulin, the memory switch



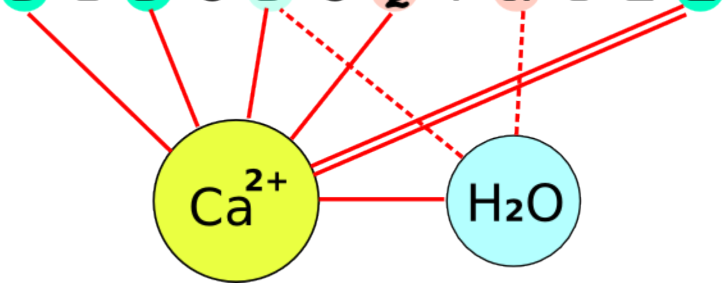
Lisman (1989)
PNAS, 86: 9574-8

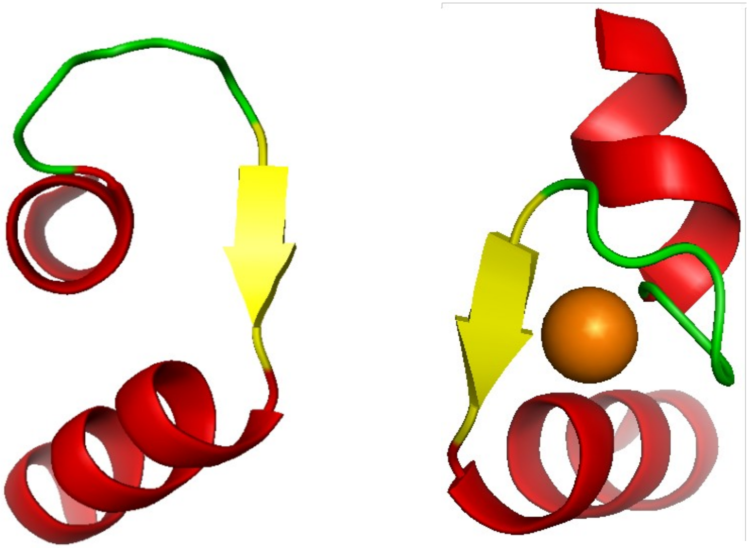
Structure of Calmodulin



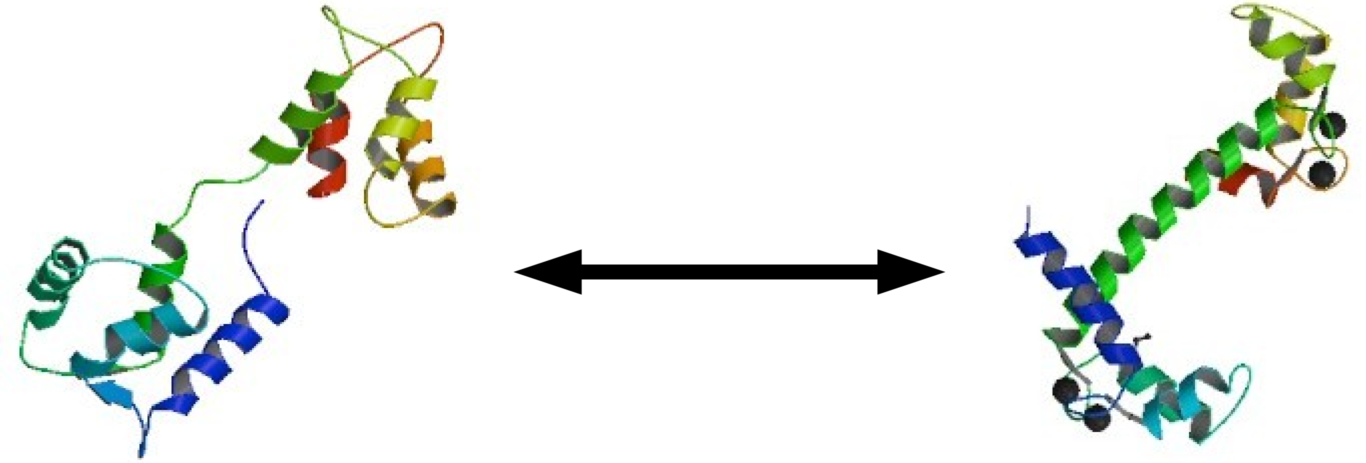


	X		Y		Z		-Y		-X			-Z	
20-	**D**	K	**D**	G	**D**	G	**T**	I	**T**	T	K	**E**	- 31
56-	**D**	A	**D**	G	**N**	G	**T**	I	**D**	F	P	**E**	- 67
93-	**D**	K	**D**	G	**N**	G	**Y**	I	**S**	A	A	**E**	-104
129-	**D**	I	**D**	G	**D**	G	**Q**	V	**N**	Y	E	**E**	-140





State transitions of calmodulin



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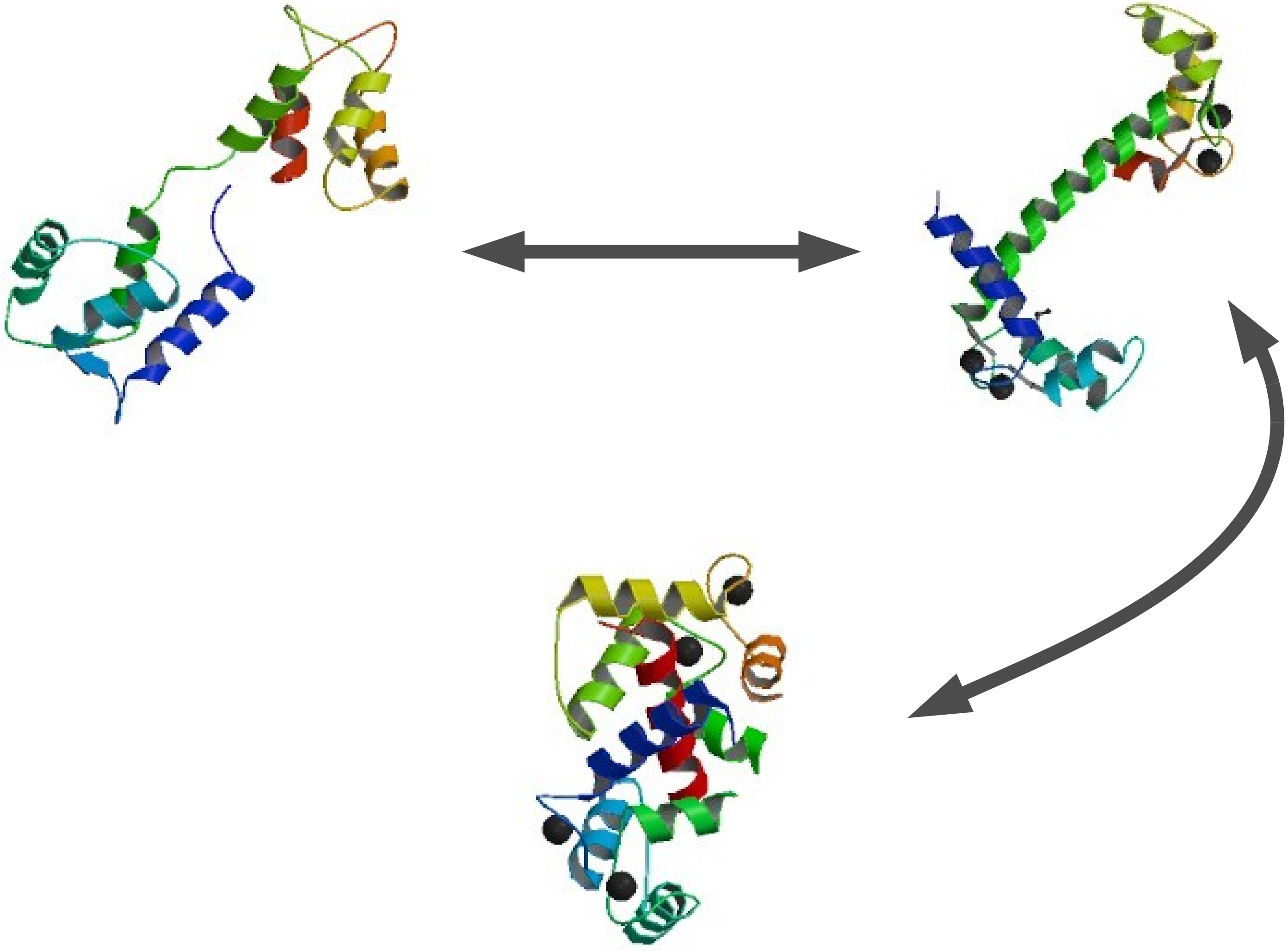
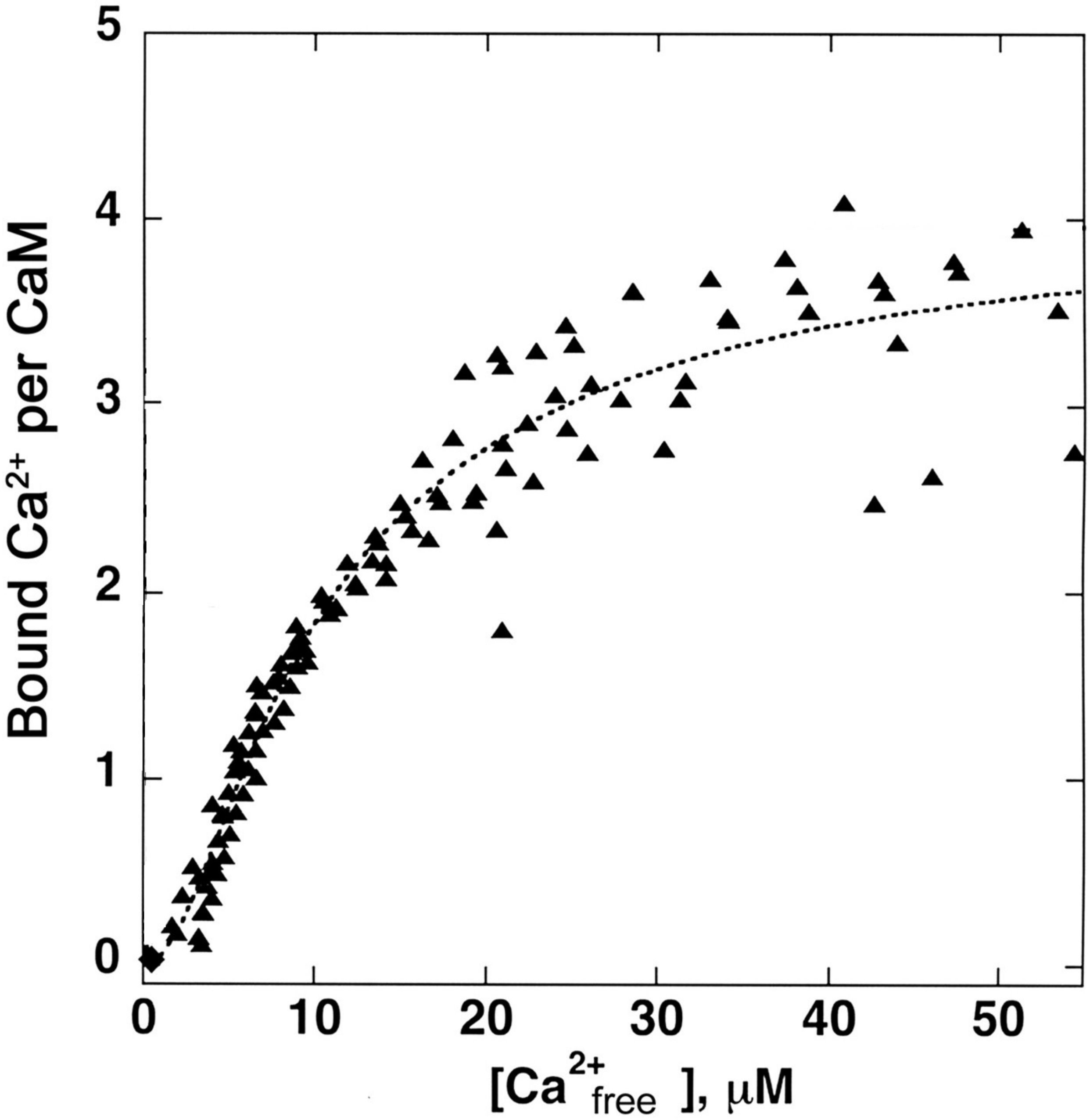
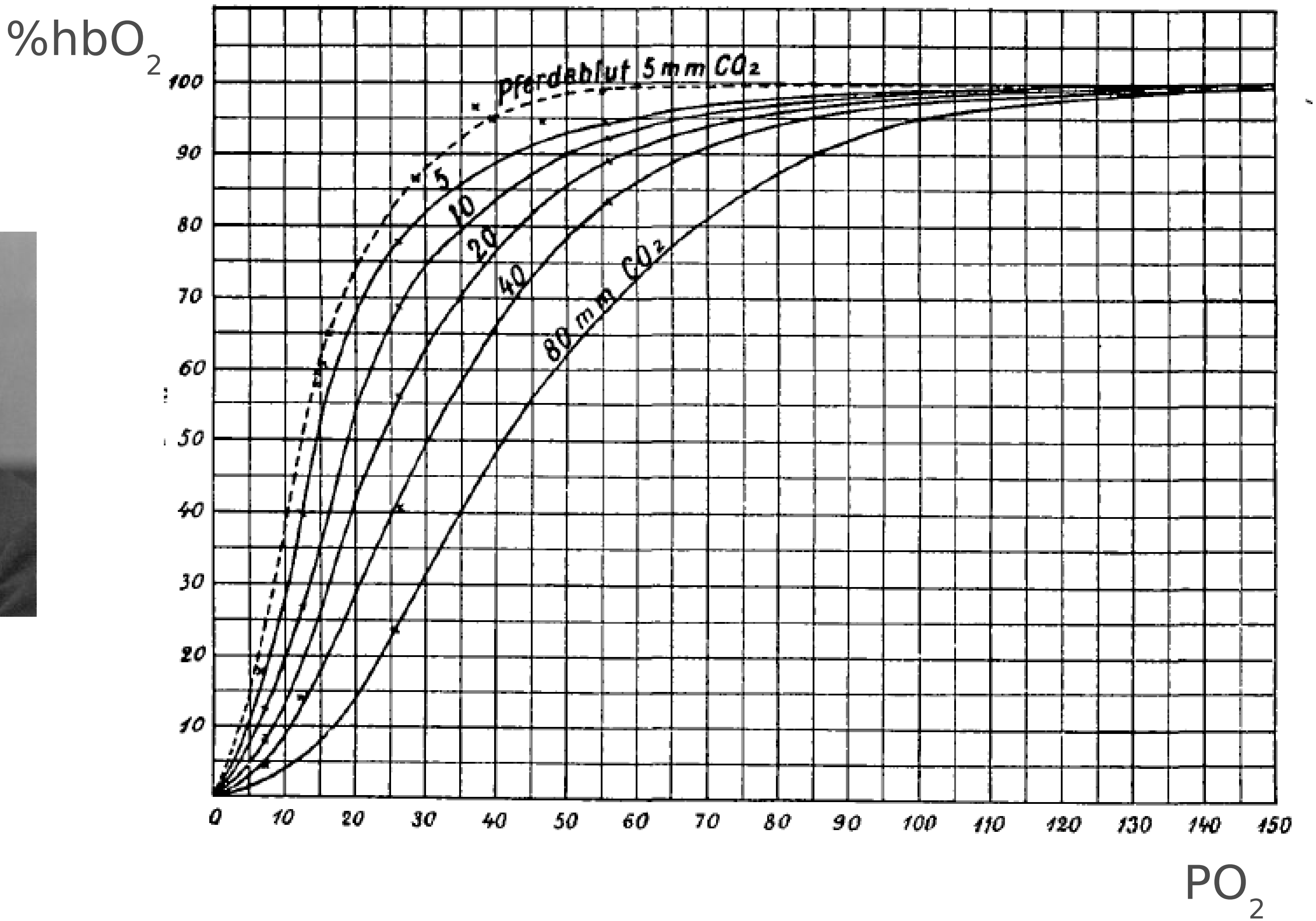


Calmodulin is ultra-sensitive



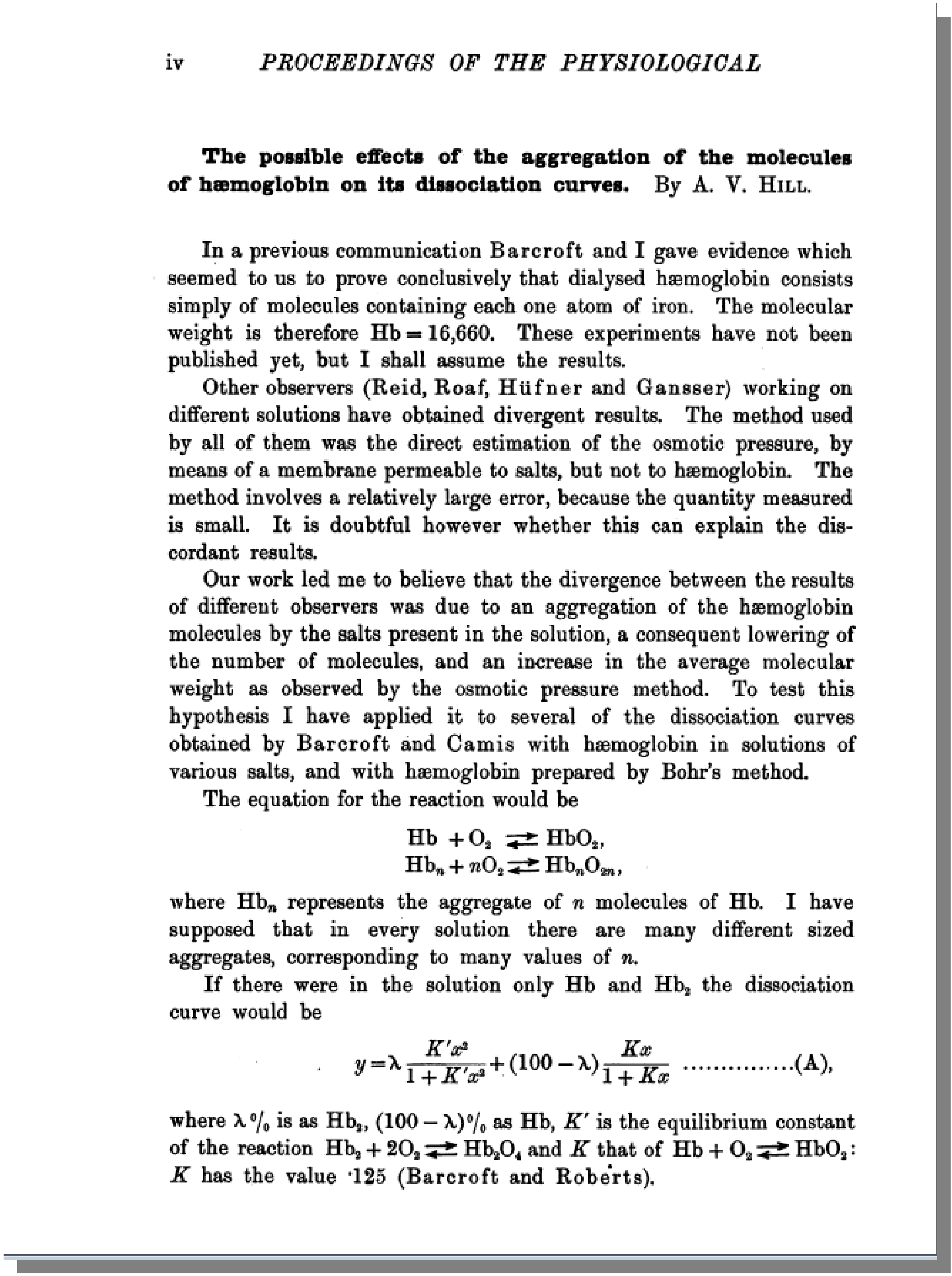
from:
Shifman et al (2006)
PNAS, 103: 13968-13973

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



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

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

where λ °/₀ is as $\mathrm{Hb_2}$, $(100-\lambda)$°/₀ 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 (1910) *J Physiol* 40: iv-vii.

Origins of cooperativity: Hill

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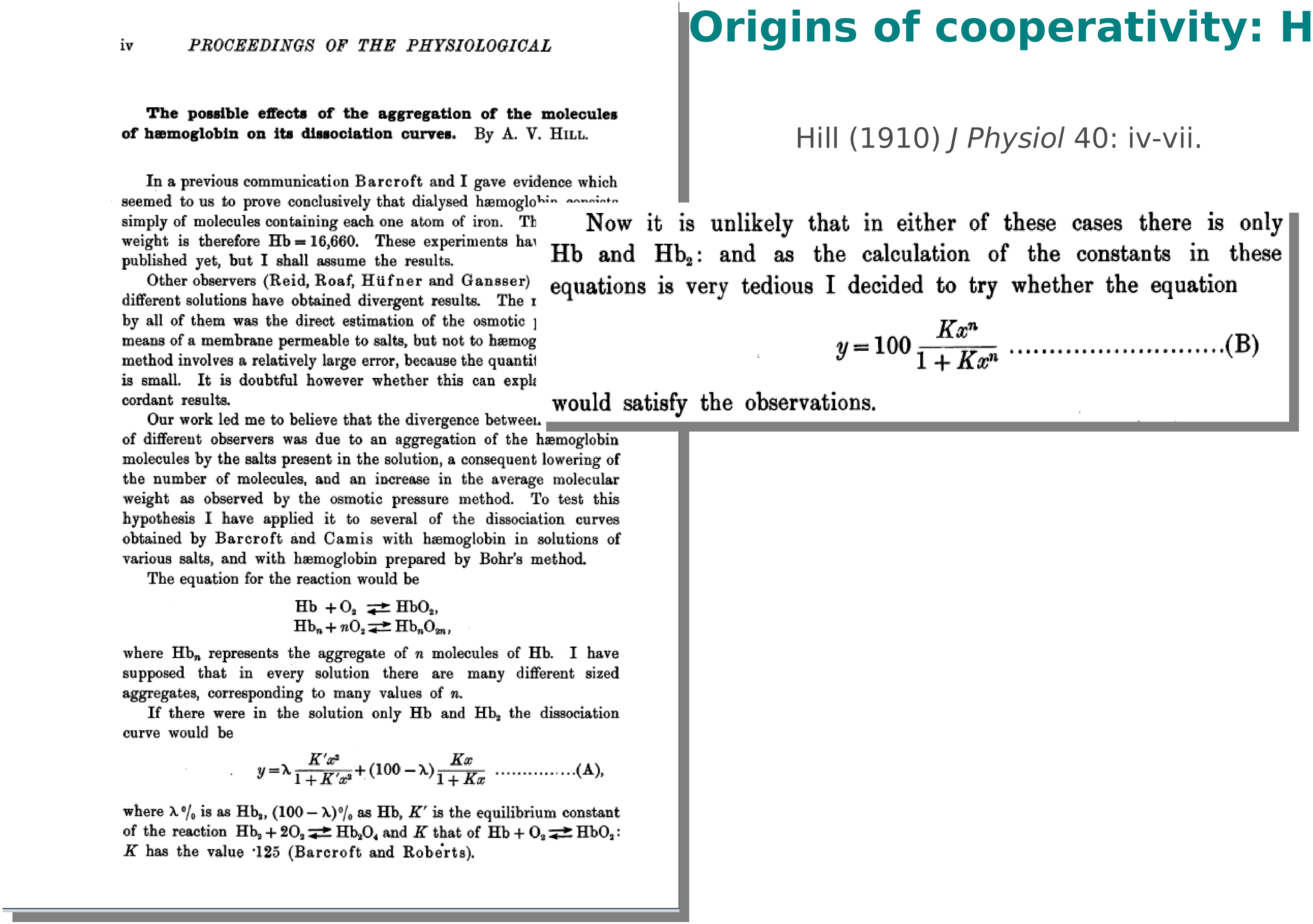

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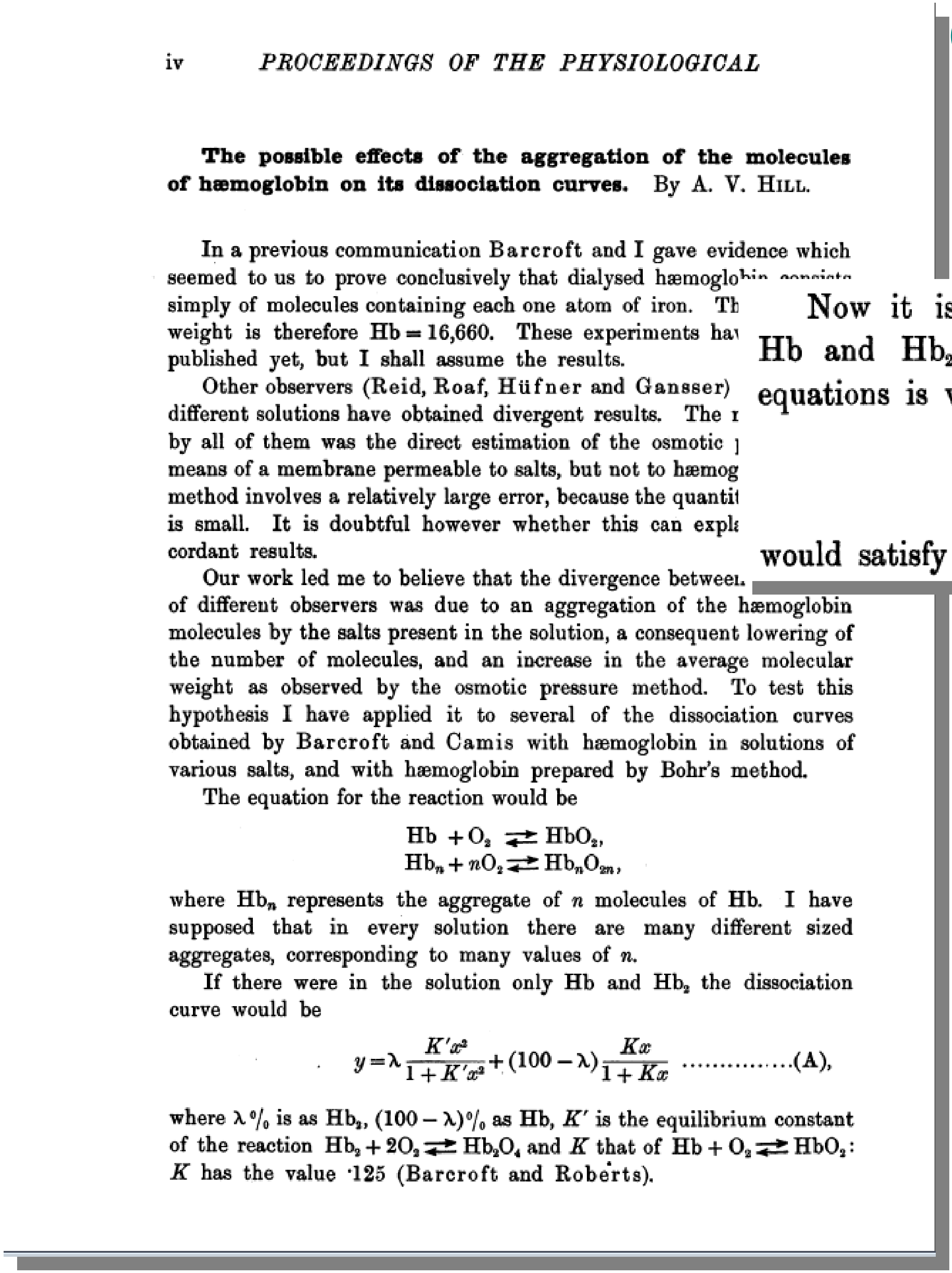
Now it is unlikely that in either of these cases there is only Hb and $\mathrm{Hb_2}$: and as the calculation of the constants in these equations is very tedious I decided to try whether the equation

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

would satisfy the observations.

Origins of cooperativity: Hill

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



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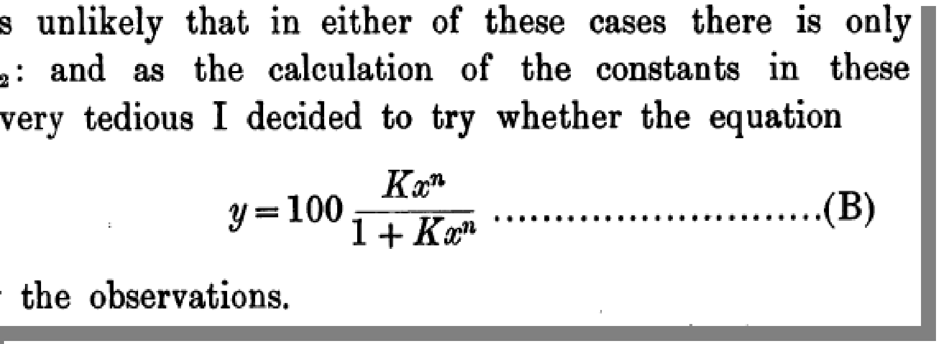

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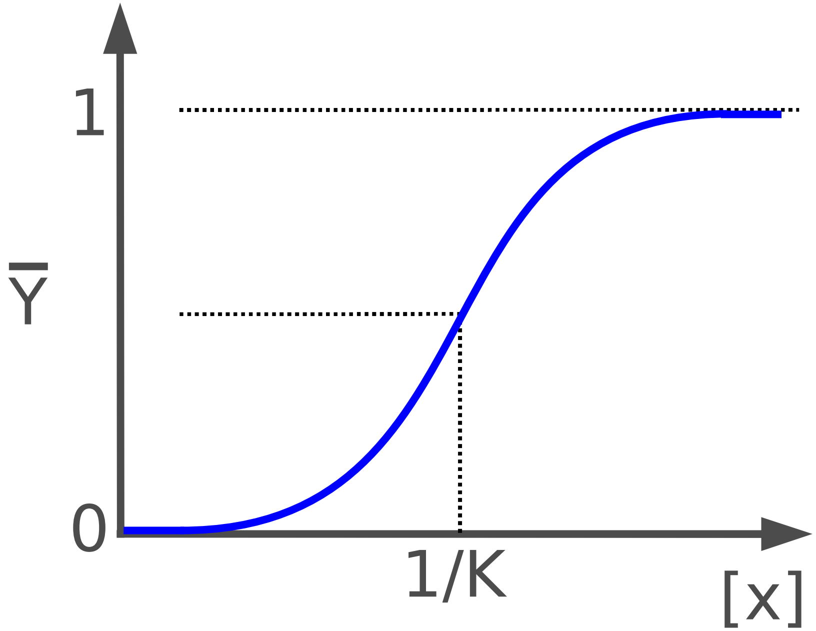


Hill Plot

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

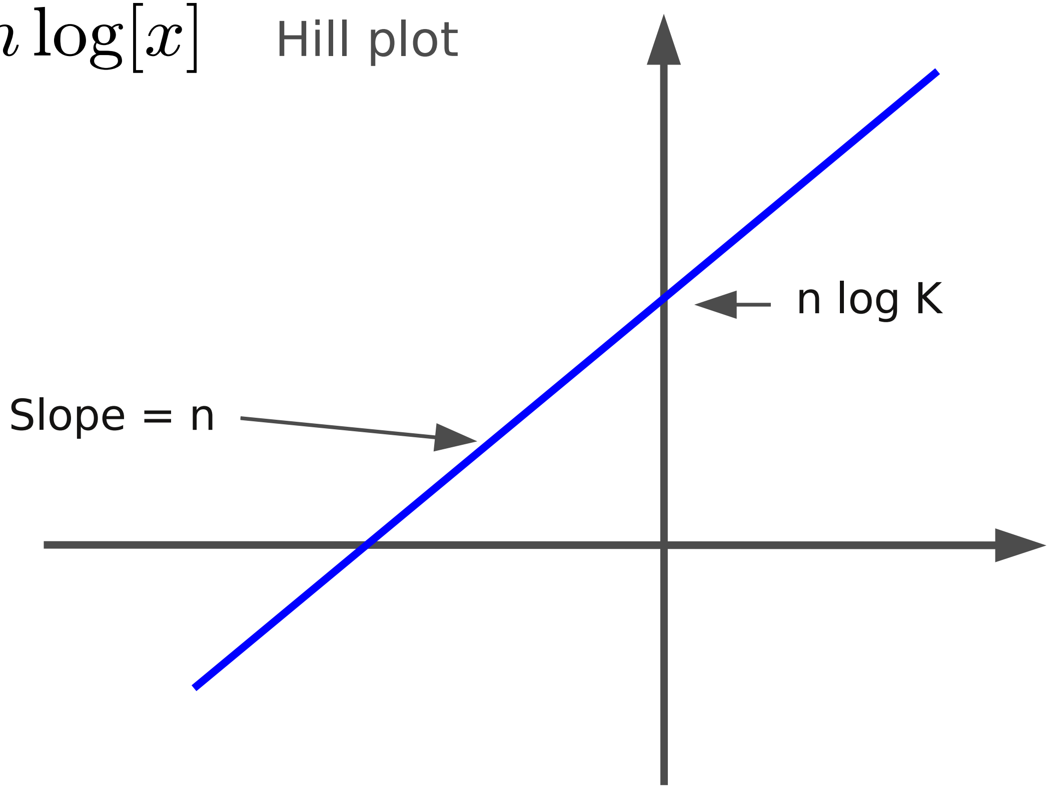
Hill equation

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

Hill plot

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

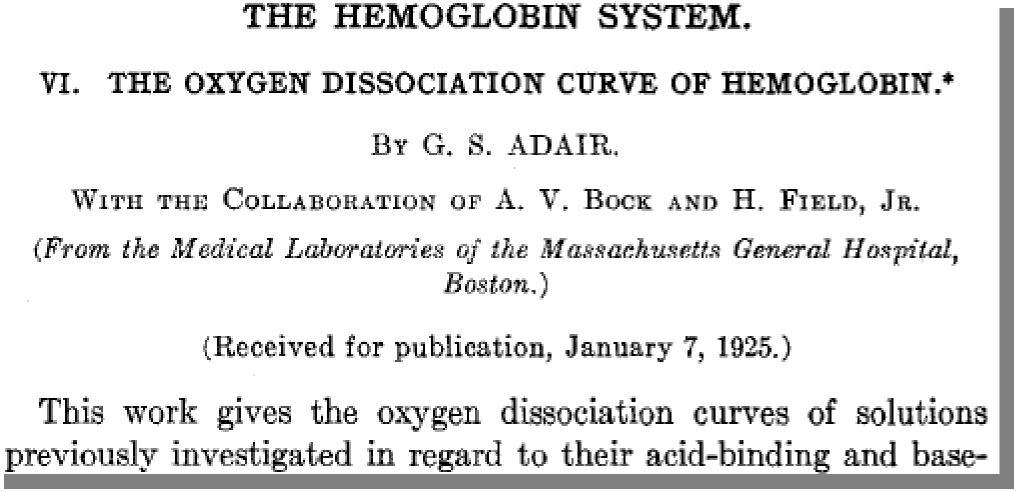
BUT cooperativity of ligand,
not of binding sites: unique affinity



n log K

Slope = n

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.

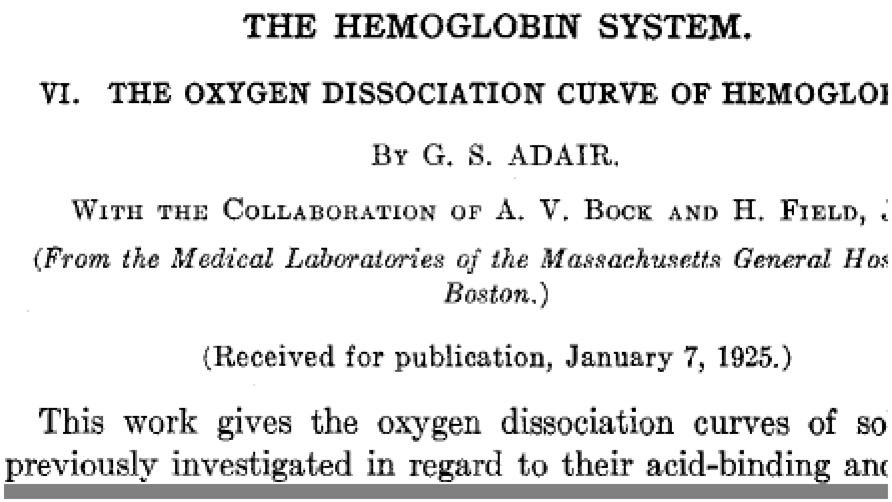
(From the Medical Laboratories of the Massachusetts General Hospital, Boston.)

(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

Origins of cooperativity: Adair-Klotz



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

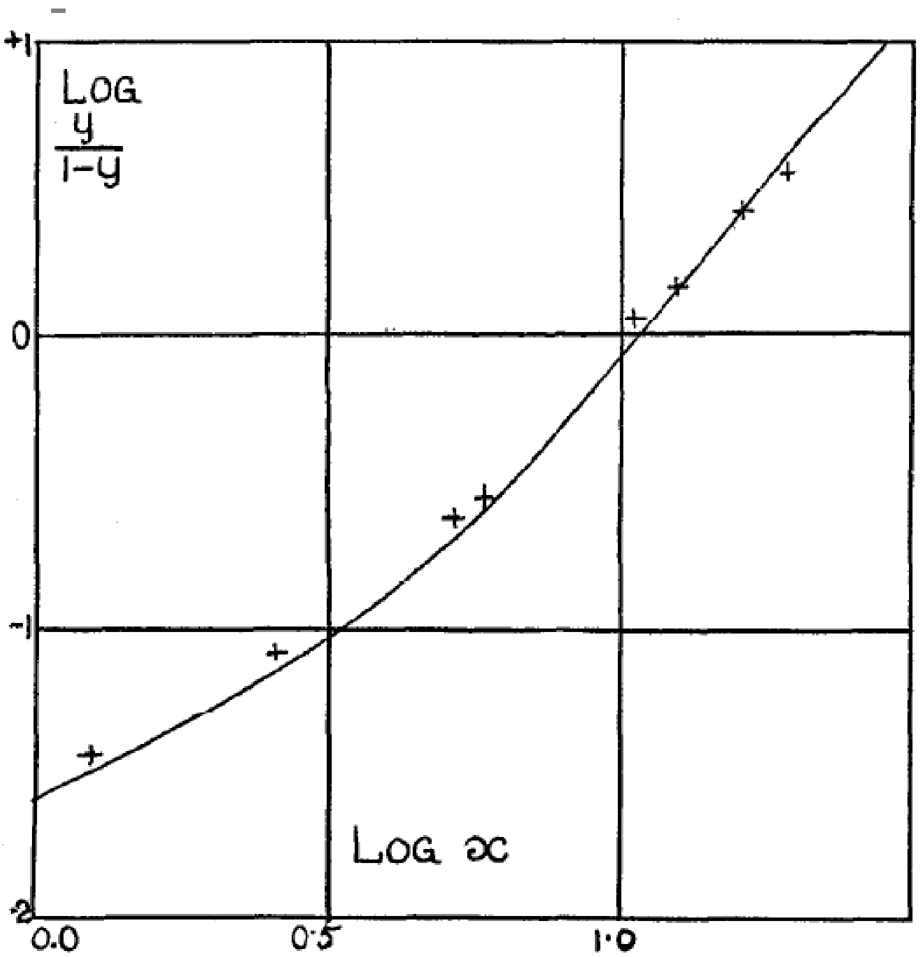
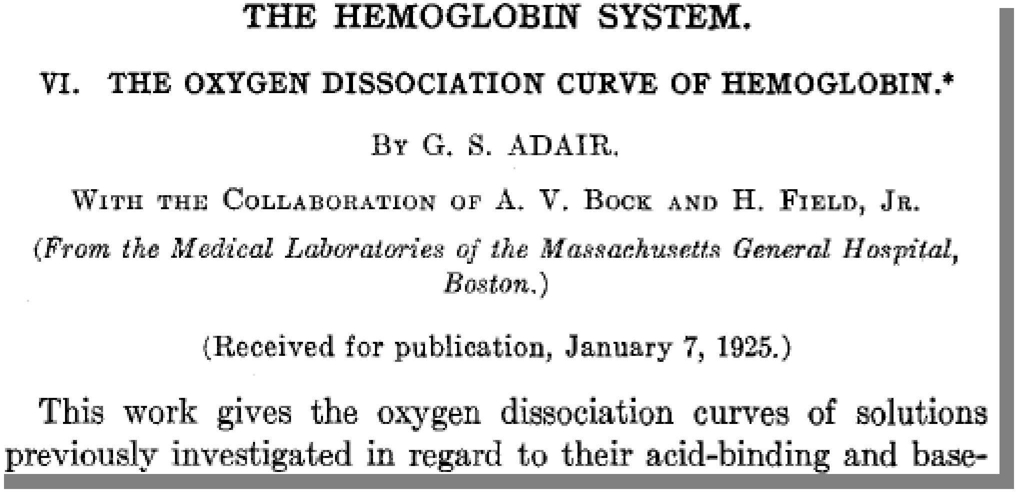


FIG. 2. Test of formula (6). Curve drawn from 6 experimental points from Table IV.

Origins of cooperativity: Adair-Klotz



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

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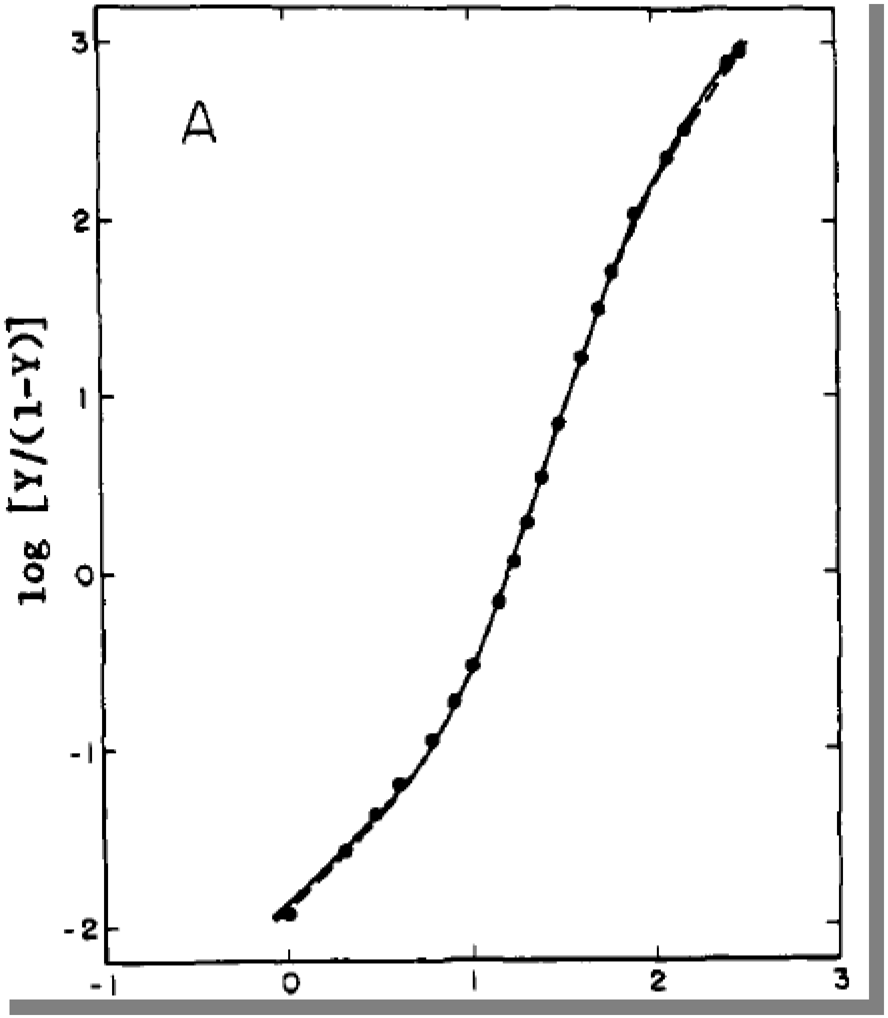
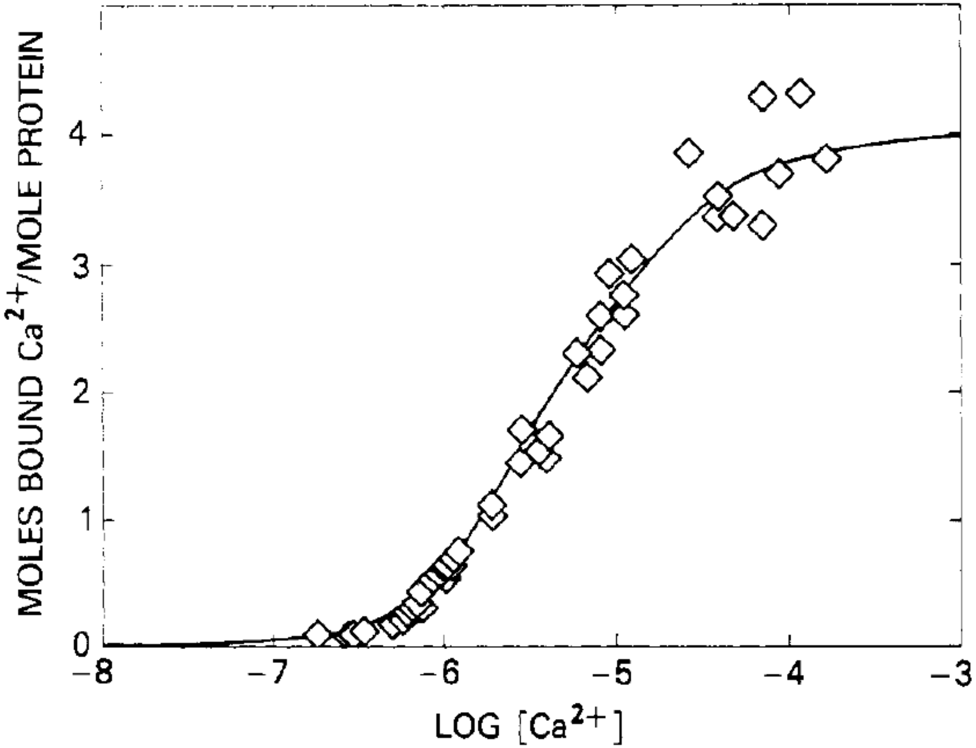


Imai (1973) *Biochemistry* 12: 798-808

Adair-Klotz model applied to Calmodulin

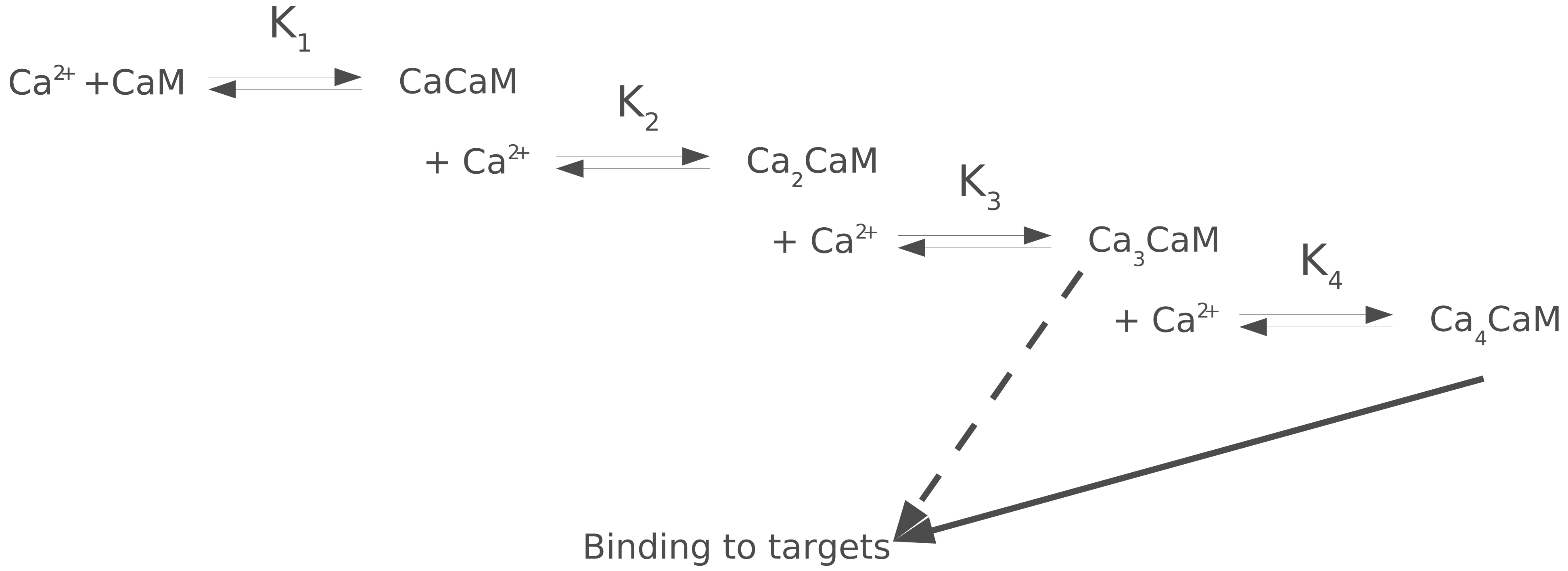
Klotz (1946) The Application of the Law of Mass Action to Binding by Proteins. Interactions with Calcium. *Arch Biochem*, 9:109–117.

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



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

Corresponding induced-fit model



$Ca^{2+} + CaM \overset{K_1}{\rightleftharpoons} CaCaM$

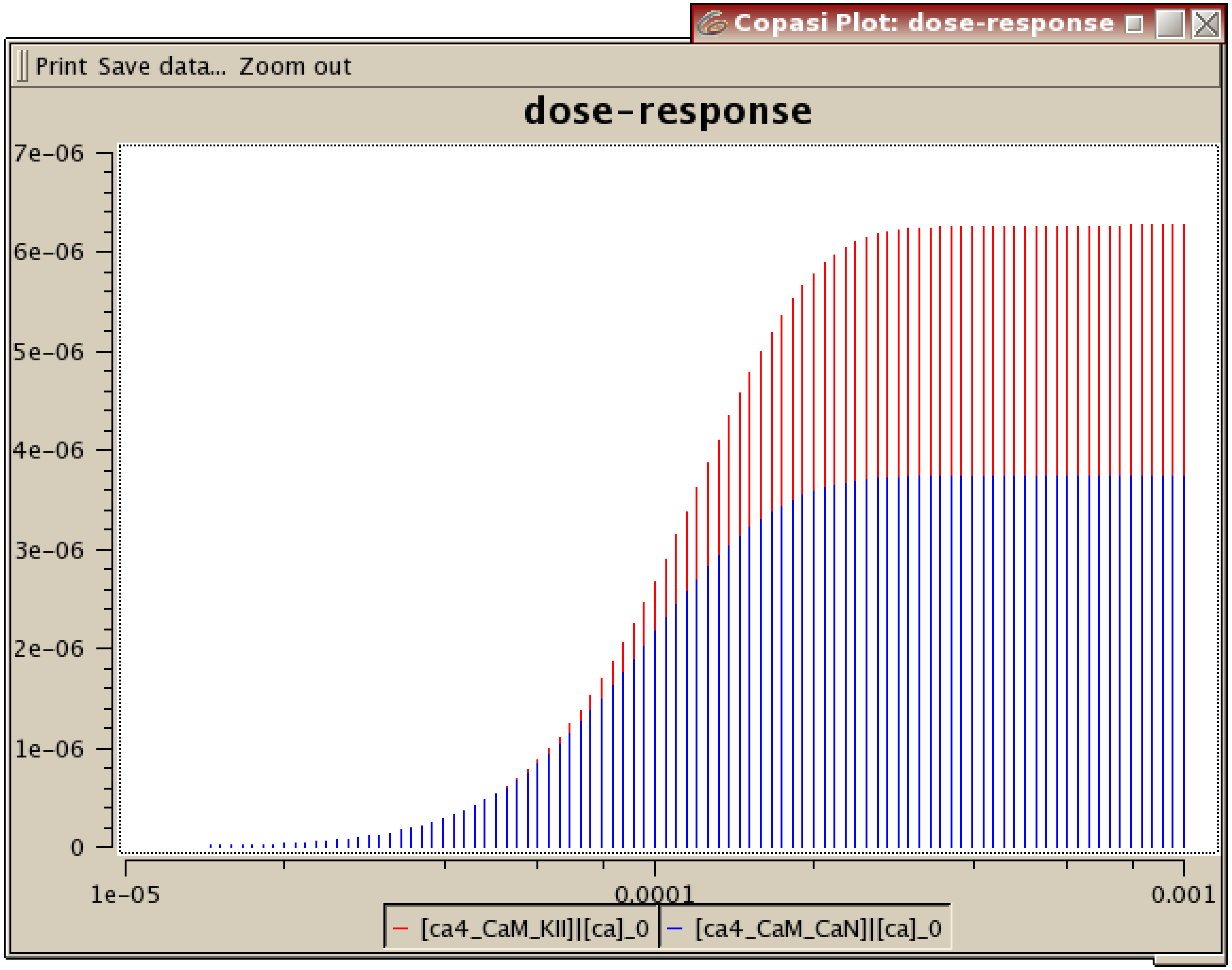
$+ Ca^{2+} \overset{K_2}{\rightleftharpoons} Ca_2CaM$

$+ Ca^{2+} \overset{K_3}{\rightleftharpoons} Ca_3CaM$

$+ Ca^{2+} \overset{K_4}{\rightleftharpoons} Ca_4CaM$

Binding to targets

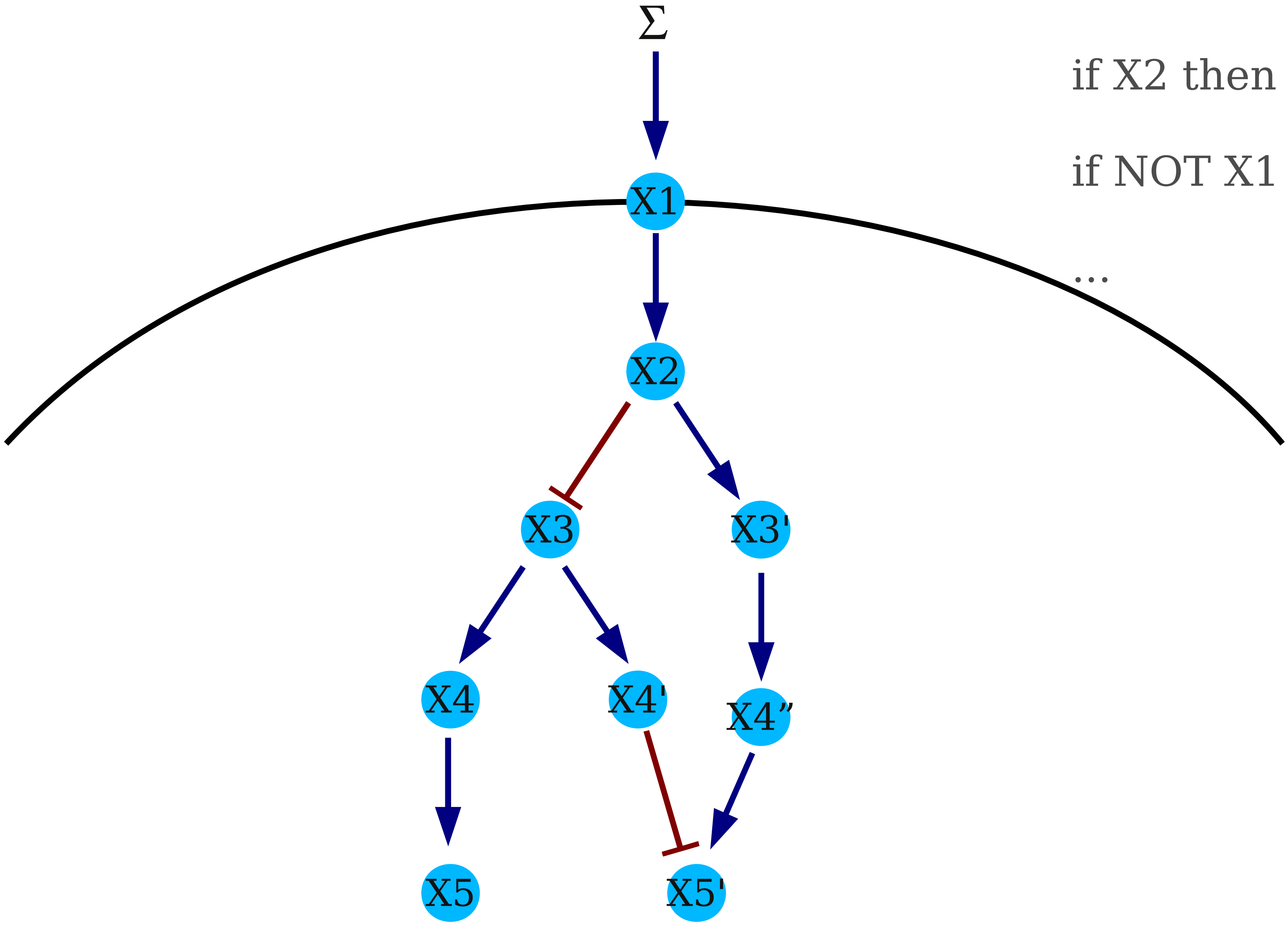
That does not work ...



That does not work ...

- 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) ≅ CaMKII ; Low [Ca^{2+}] (low freq) ≅ Calcineurin

Mistake: signals, activity flow and induction



if X1 then X2

if X2 then not X3

if NOT X1 then NOT X2

...

Induction Vs Selection

- Induction = BAD
  (Lamarck's first law, instructive theory of antibody formation, directed axonal growth, induced-fit ...)

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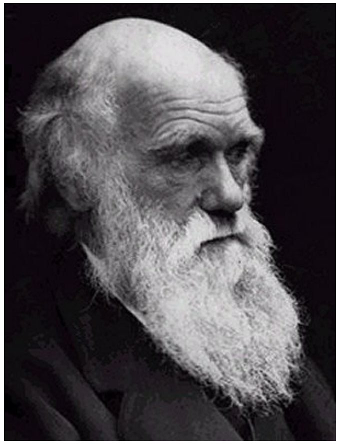
- Physically meaningless. Calcium has no inertia. Calcium cannot “trigger” a conformational change

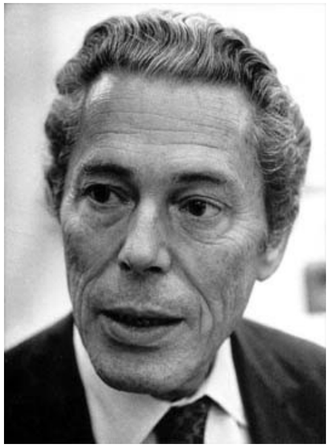
Induction Vs Selection

- Induction = BAD
  (Lamarck's first law, instructive theory of antibody formation, directed axonal growth, induced-fit ...)
- Physically meaningless. Calcium has no inertia. Calcium cannot “trigger” a conformational change
- Selection = GOOD
  (natural selection, clonal selection, synapse stabilisation, conformation stabilisation ...)

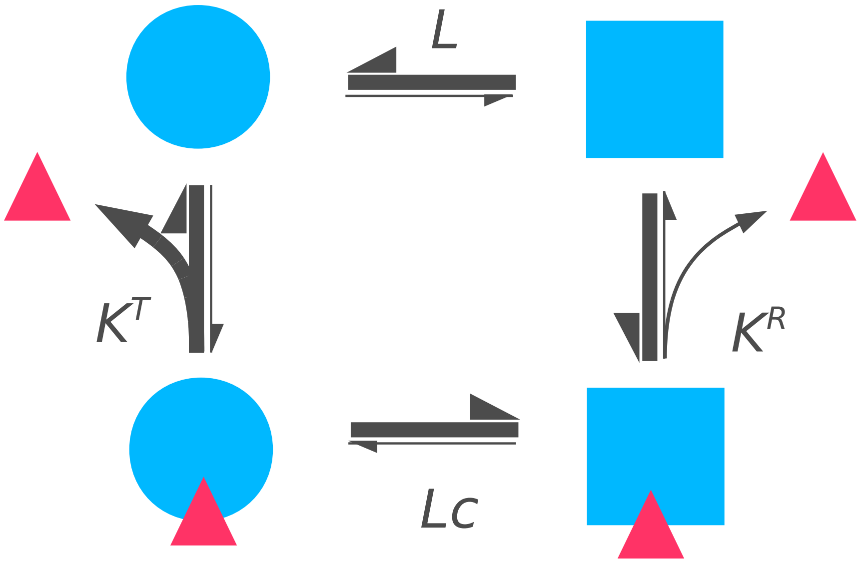


Allostery and state selection

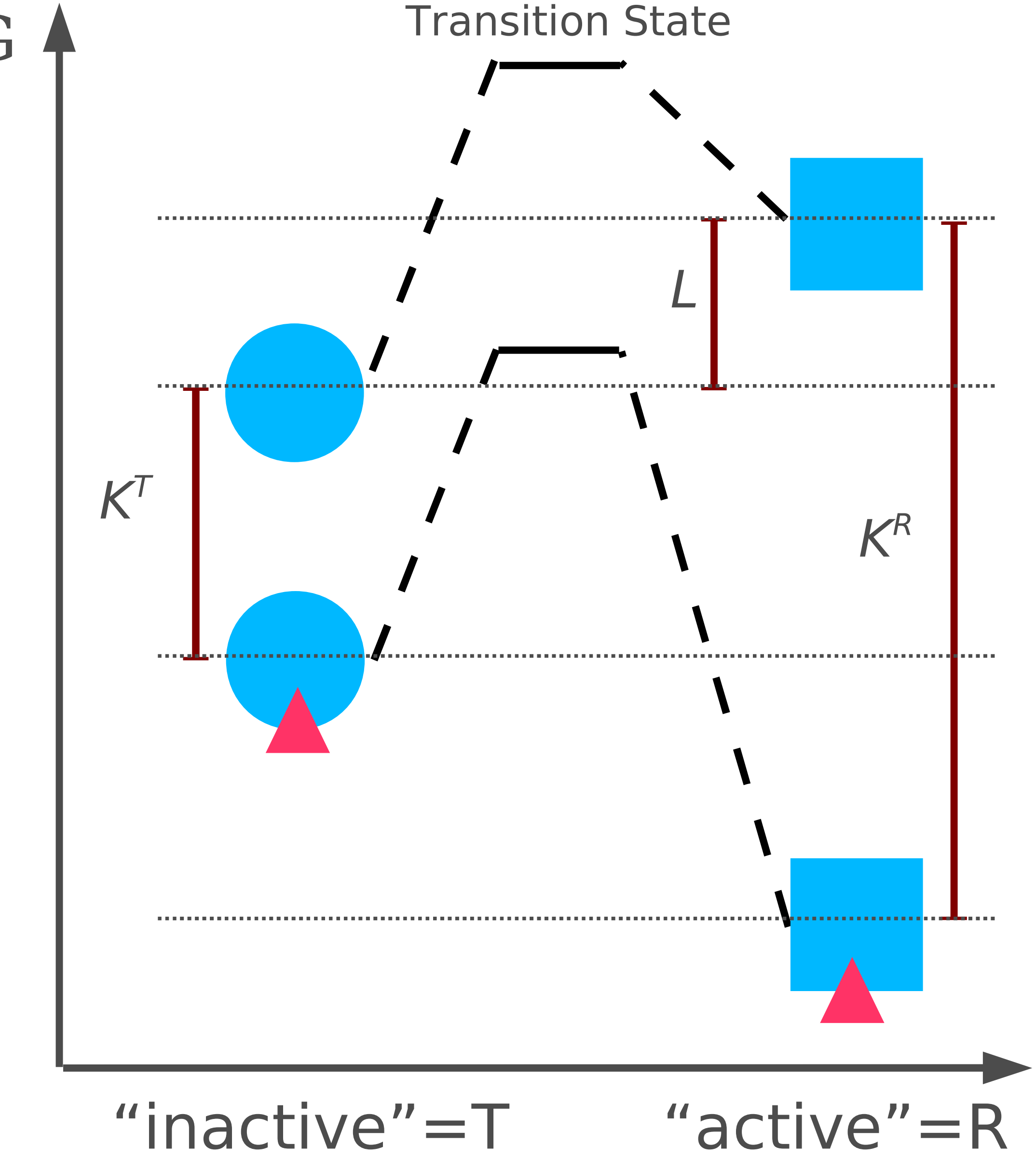
- Monod, Wyman, Changeux (1965). On the nature of allosteric transitions: a plausible model. *J Mol Biol*, 12: 88-118



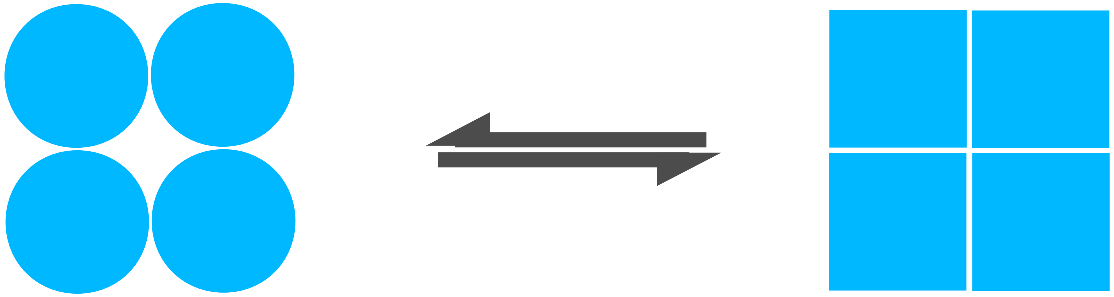
Modulation of thermal equilibria ≠ induced-fit



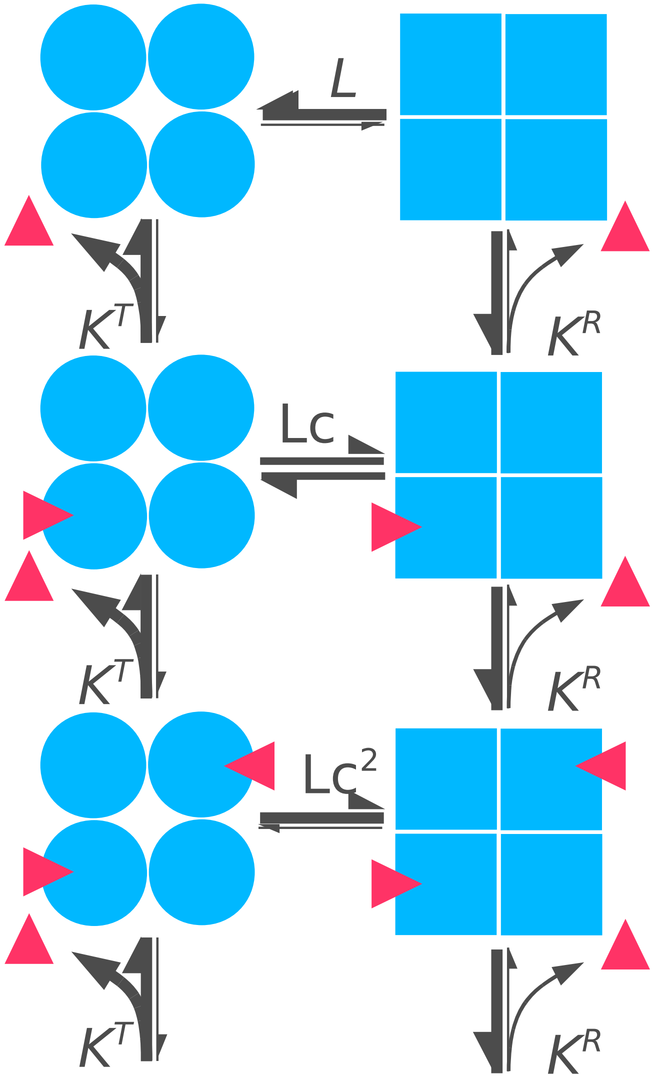
$$L = \frac{[T_0]}{[R_0]} \qquad c = \frac{K^R}{K^T}$$



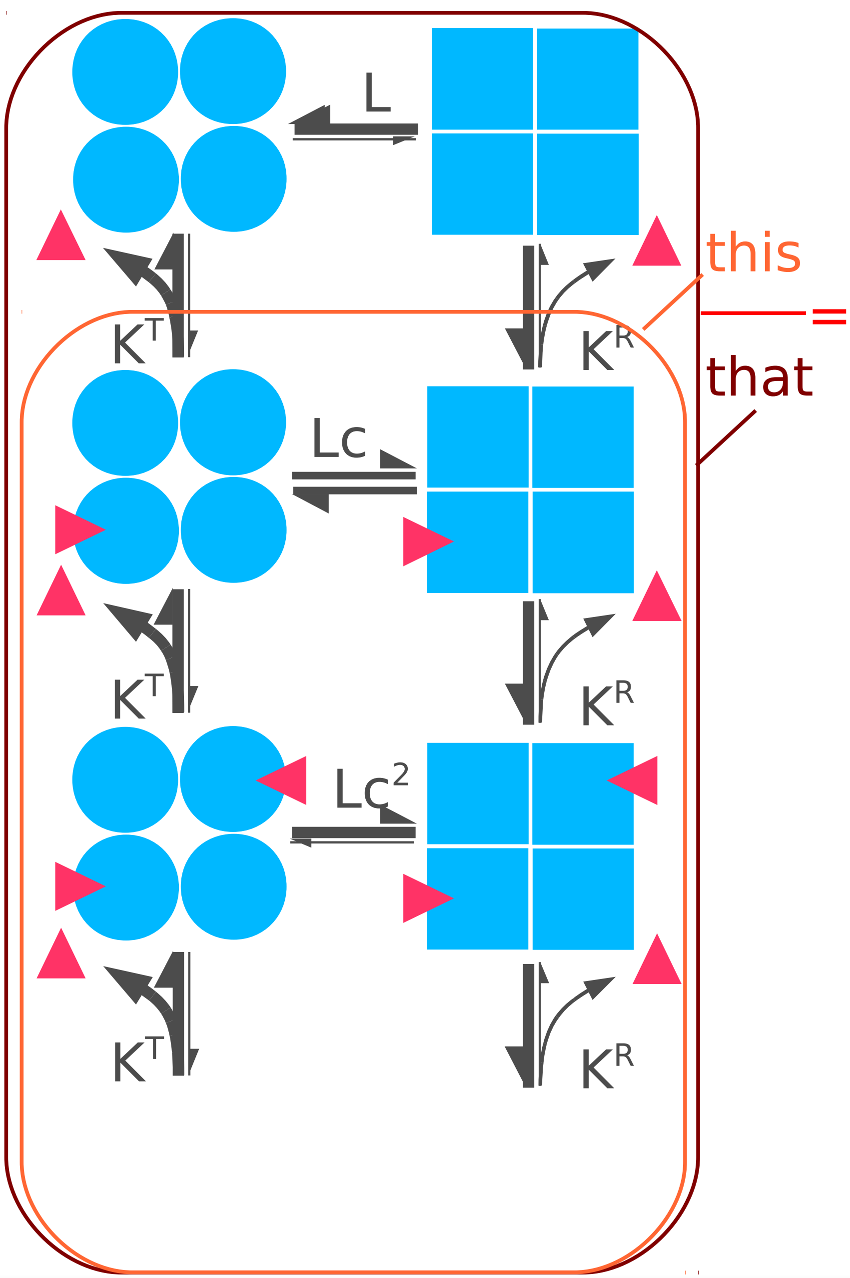
Concerted transitions ≠ sequential model



Monod-Wyman-Changeux model

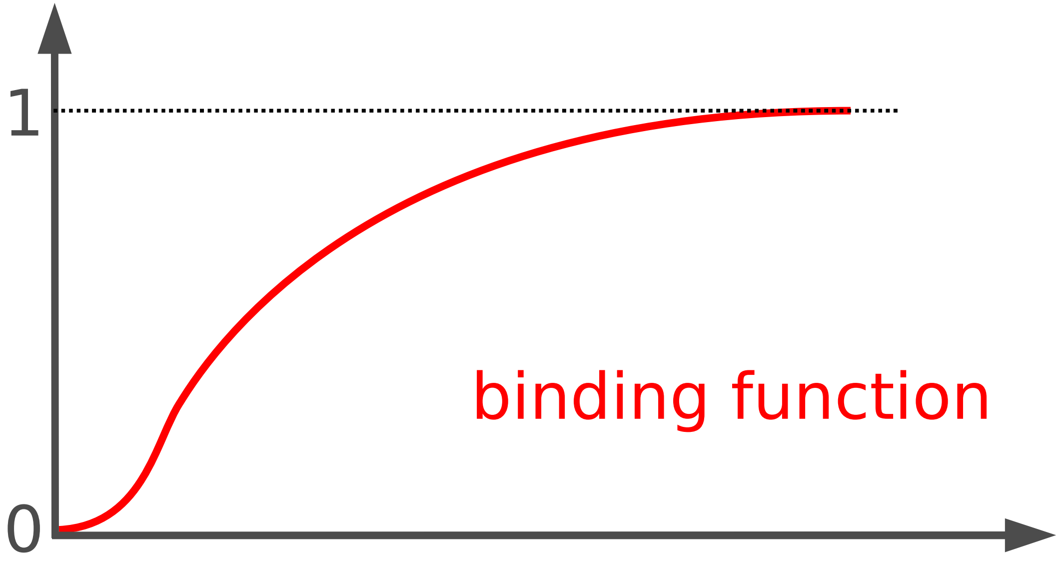


Monod-Wyman-Changeux model

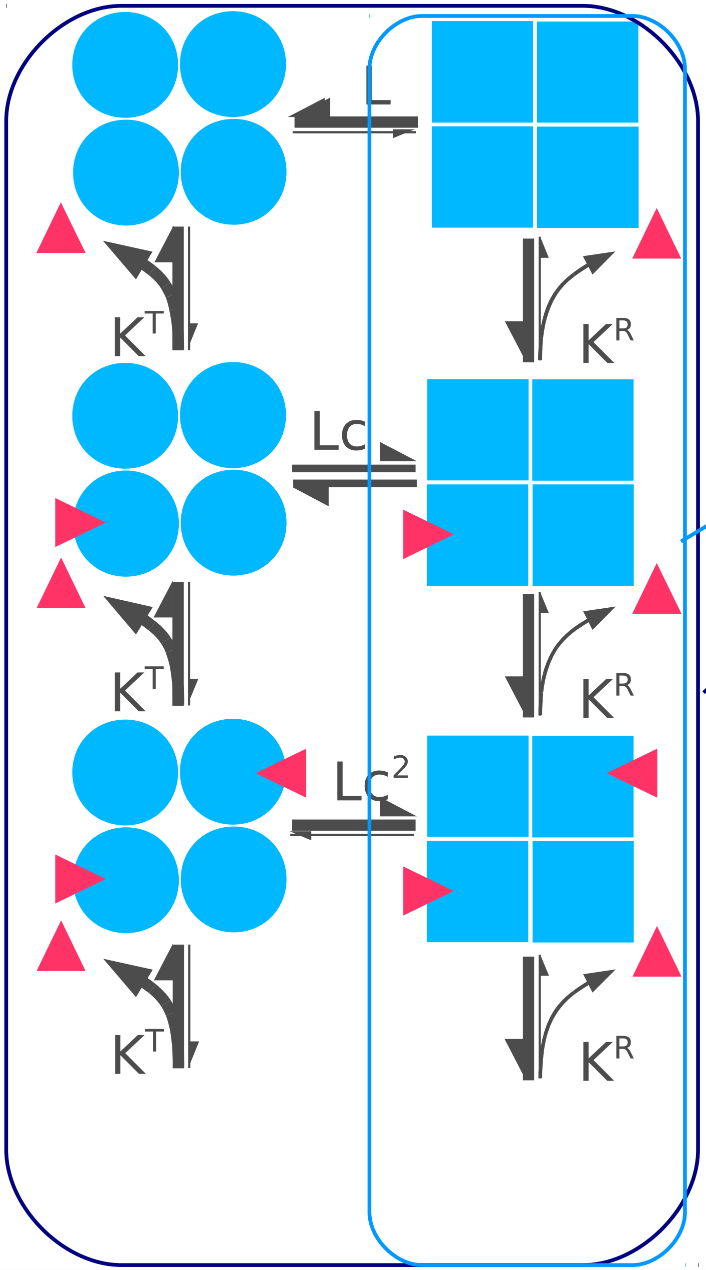


$$\alpha = \frac{[x]}{K^R}$$

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



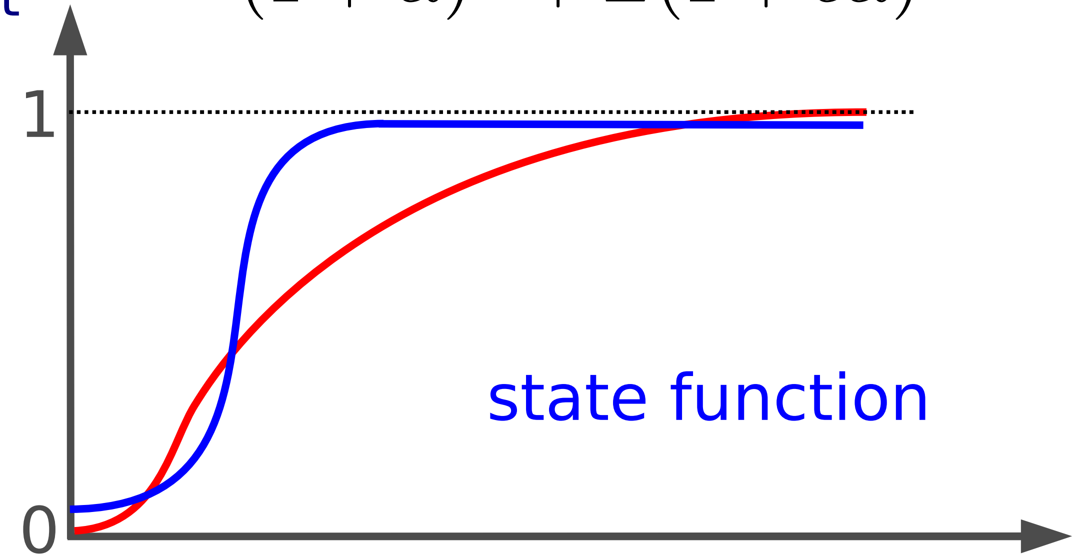
Monod-Wyman-Changeux model



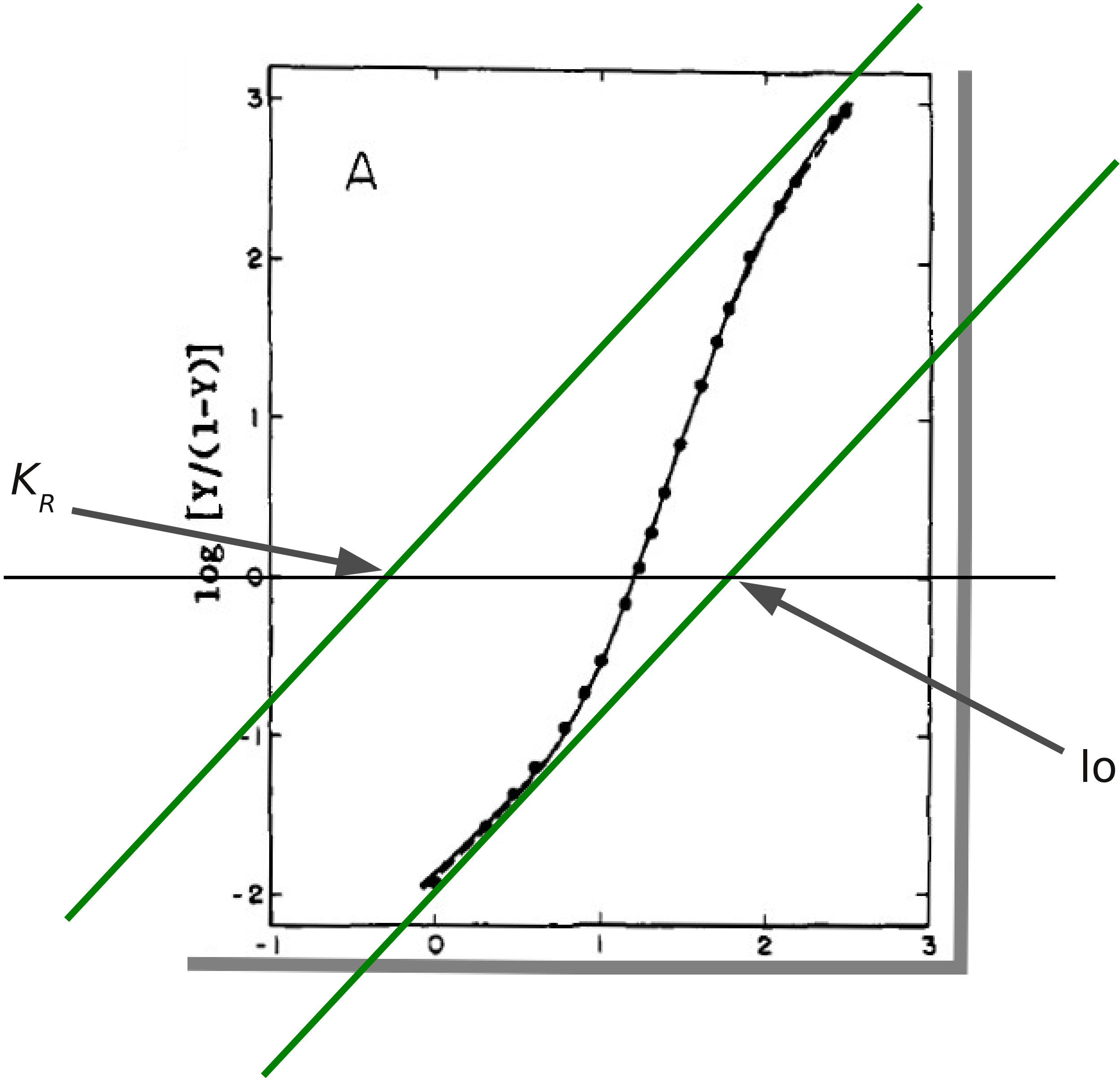
$$\alpha = \frac{[x]}{K^R}$$

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

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



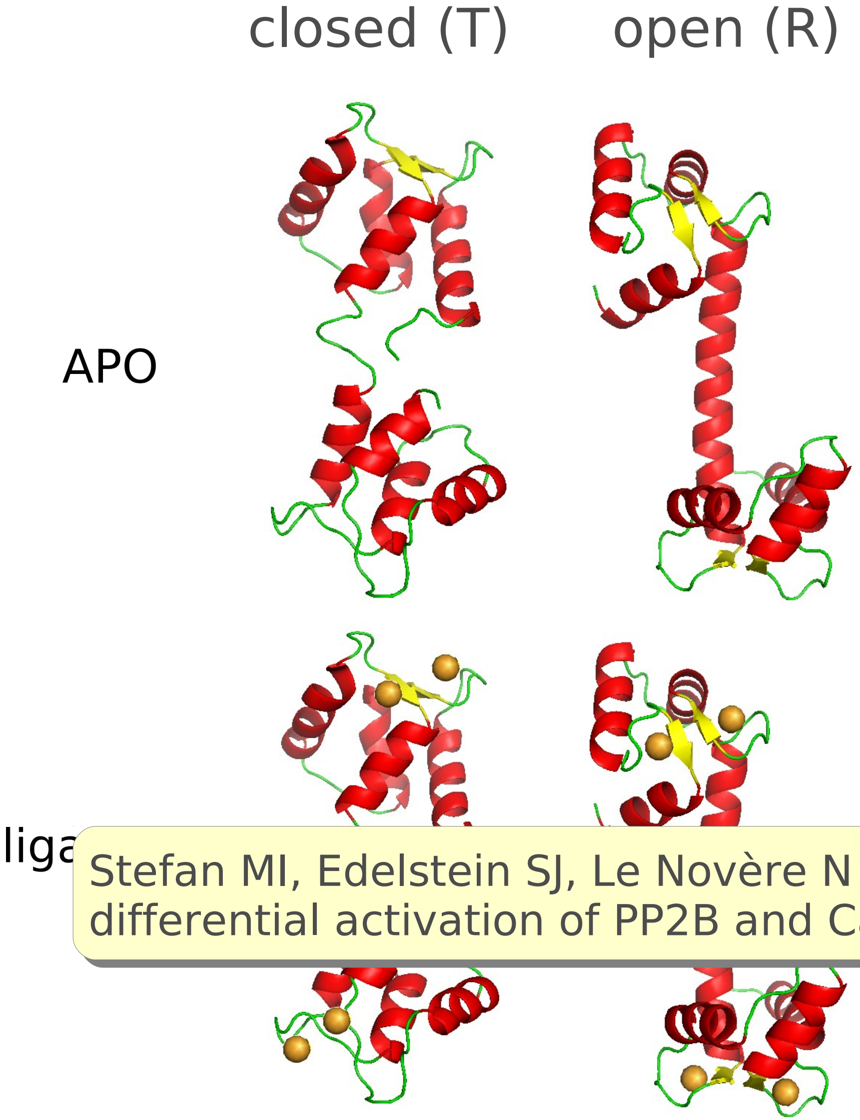
“Hill” Plot for MWC model

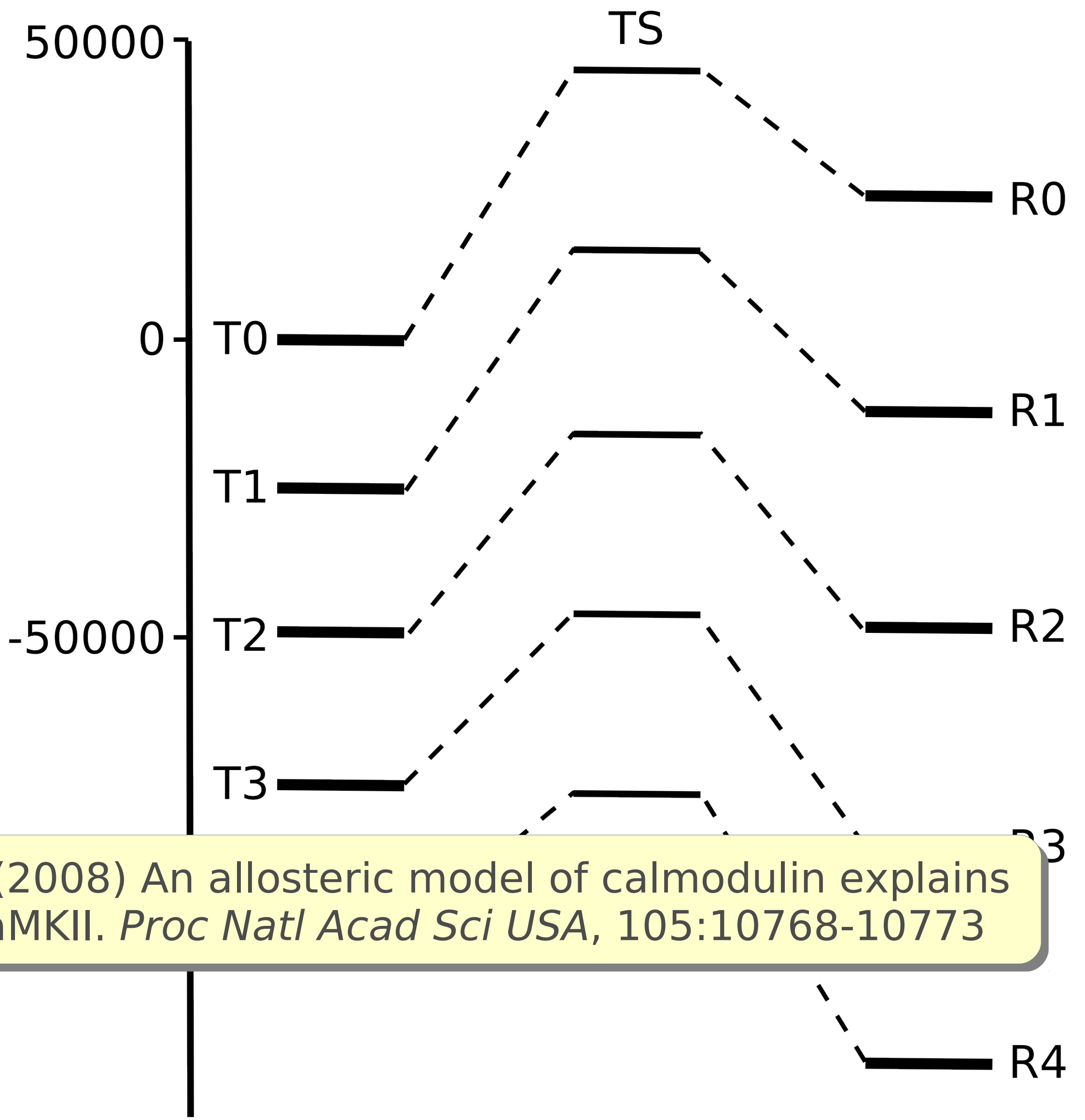


log K_R

log K_T

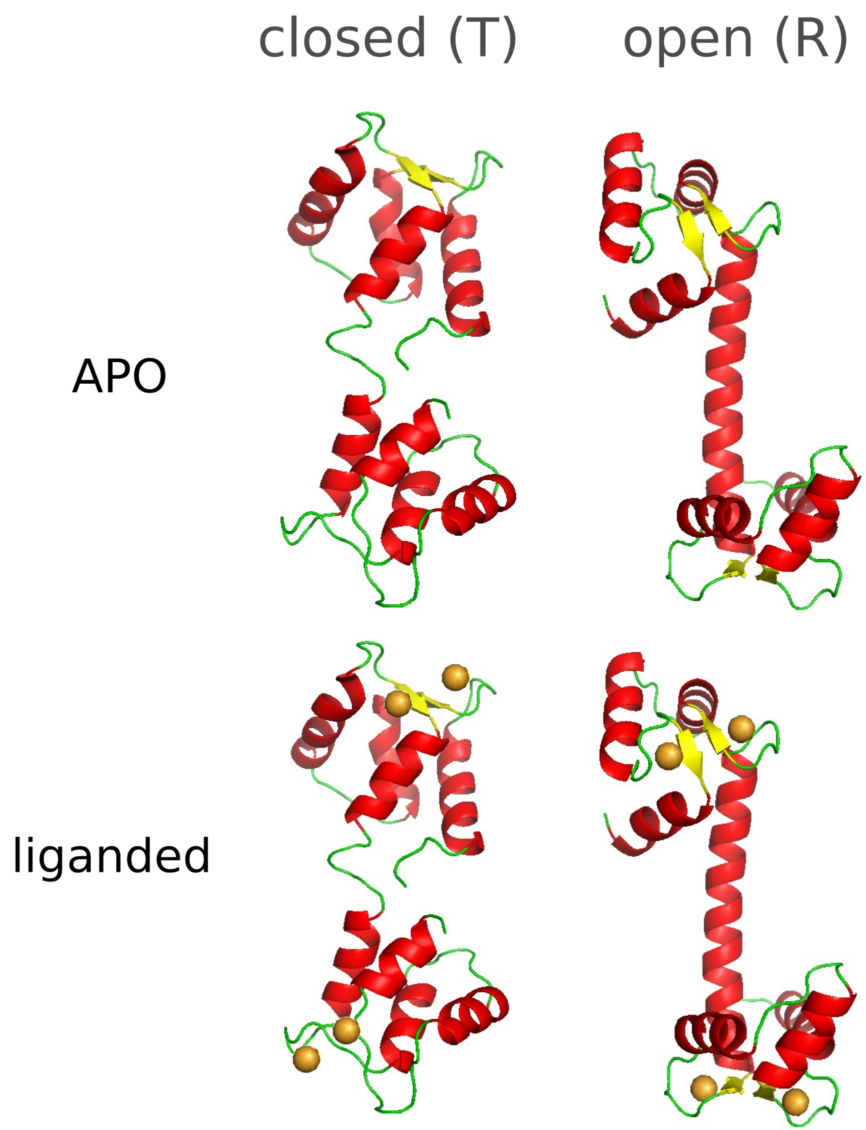
Concerted transition

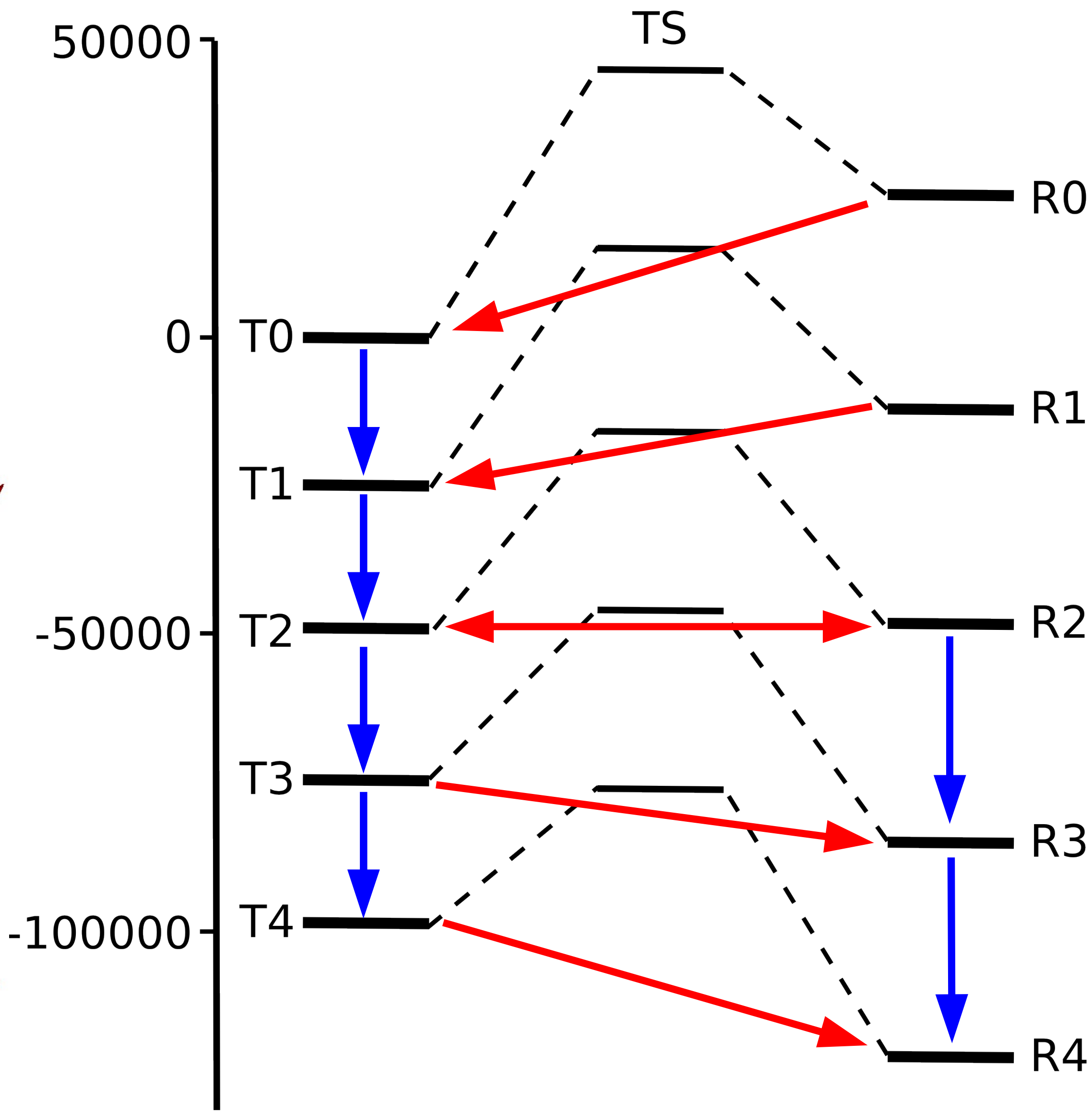




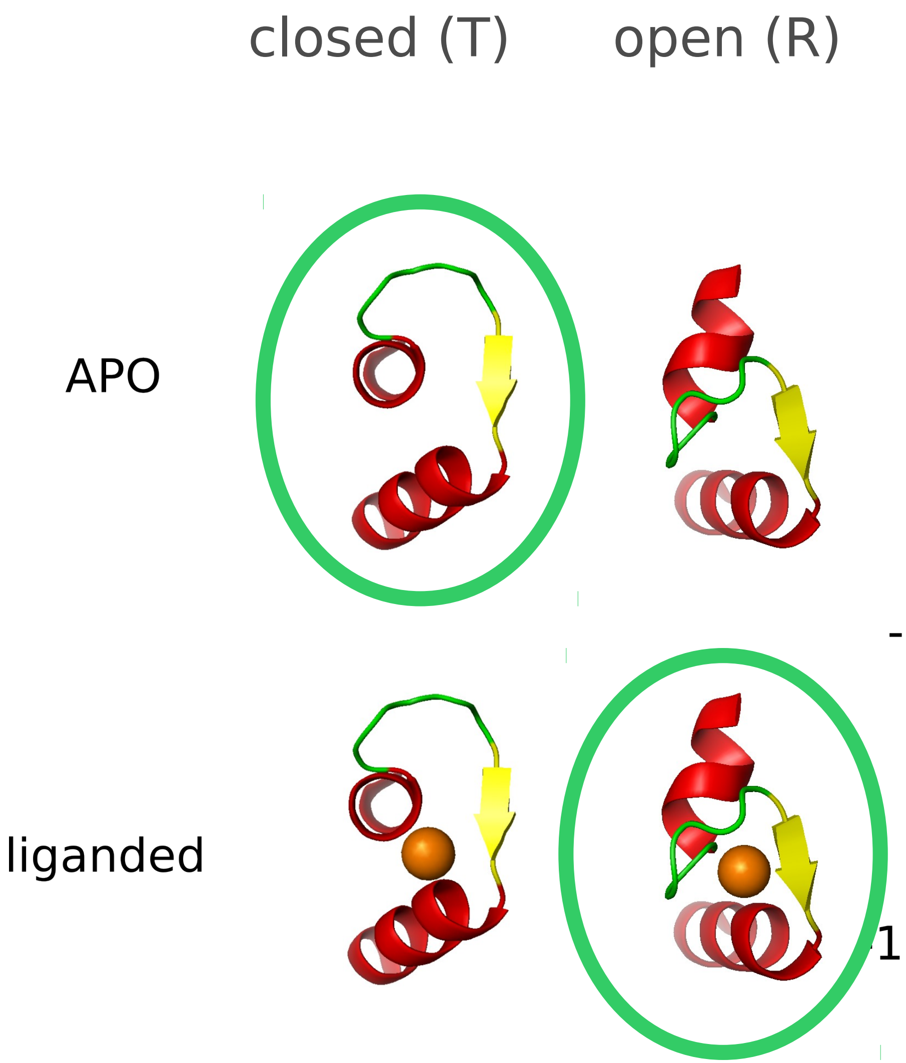
Stefan MI, Edelstein SJ, Le Novère N (2008) An allosteric model of calmodulin explains differential activation of PP2B and CaMKII. *Proc Natl Acad Sci USA*, 105:10768-10773

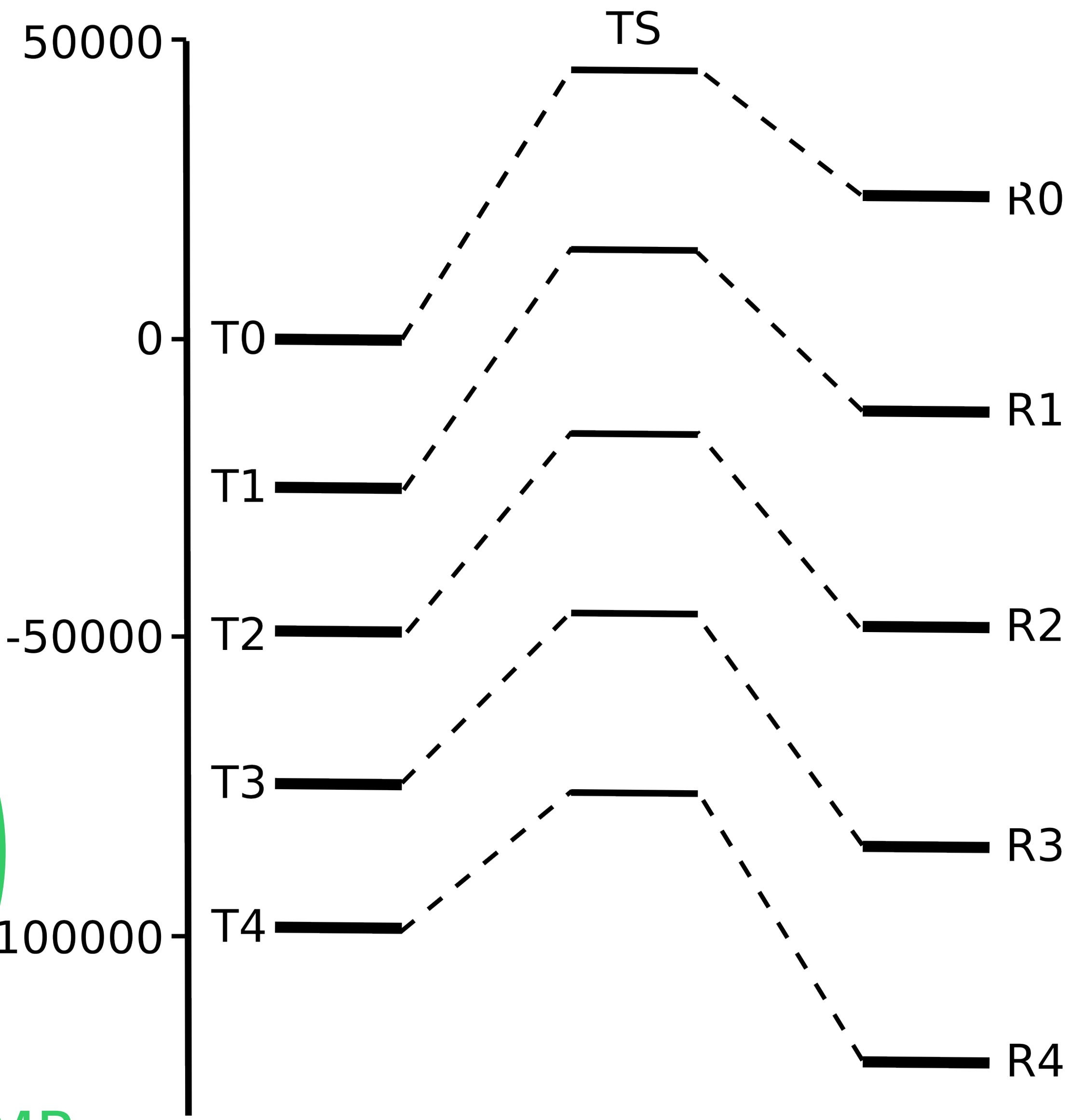
Concerted transition





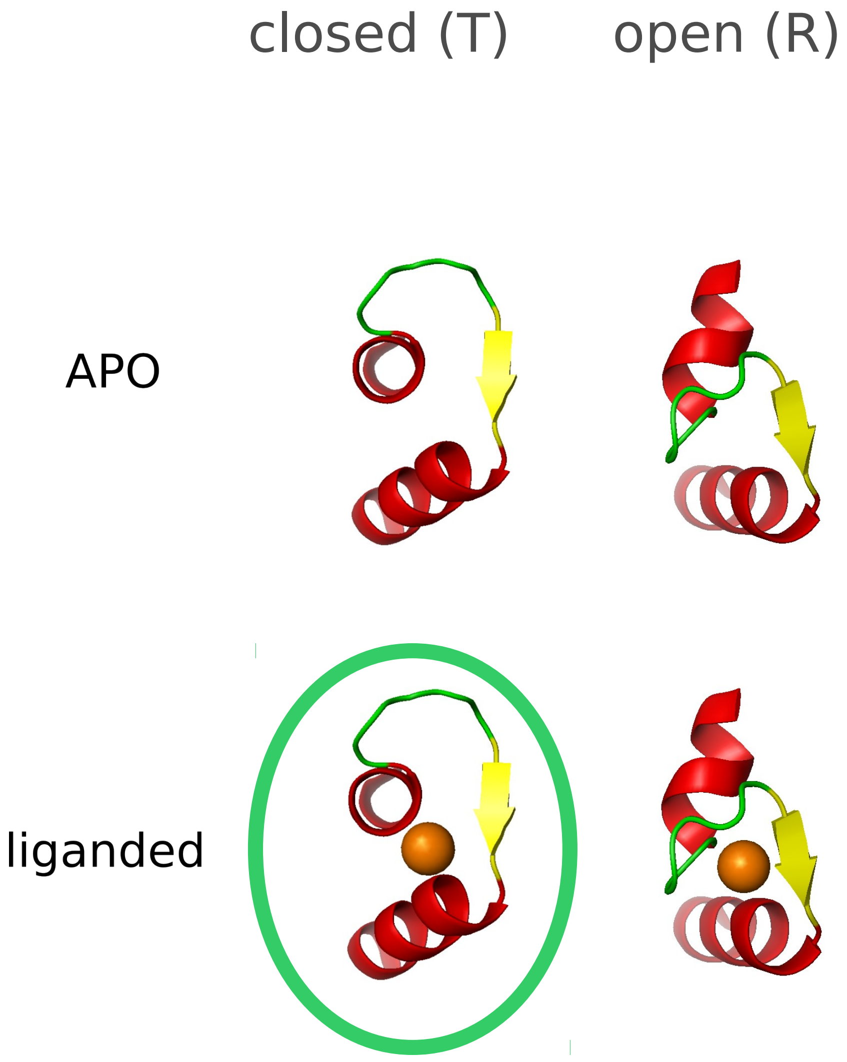
Observation Vs. Prediction

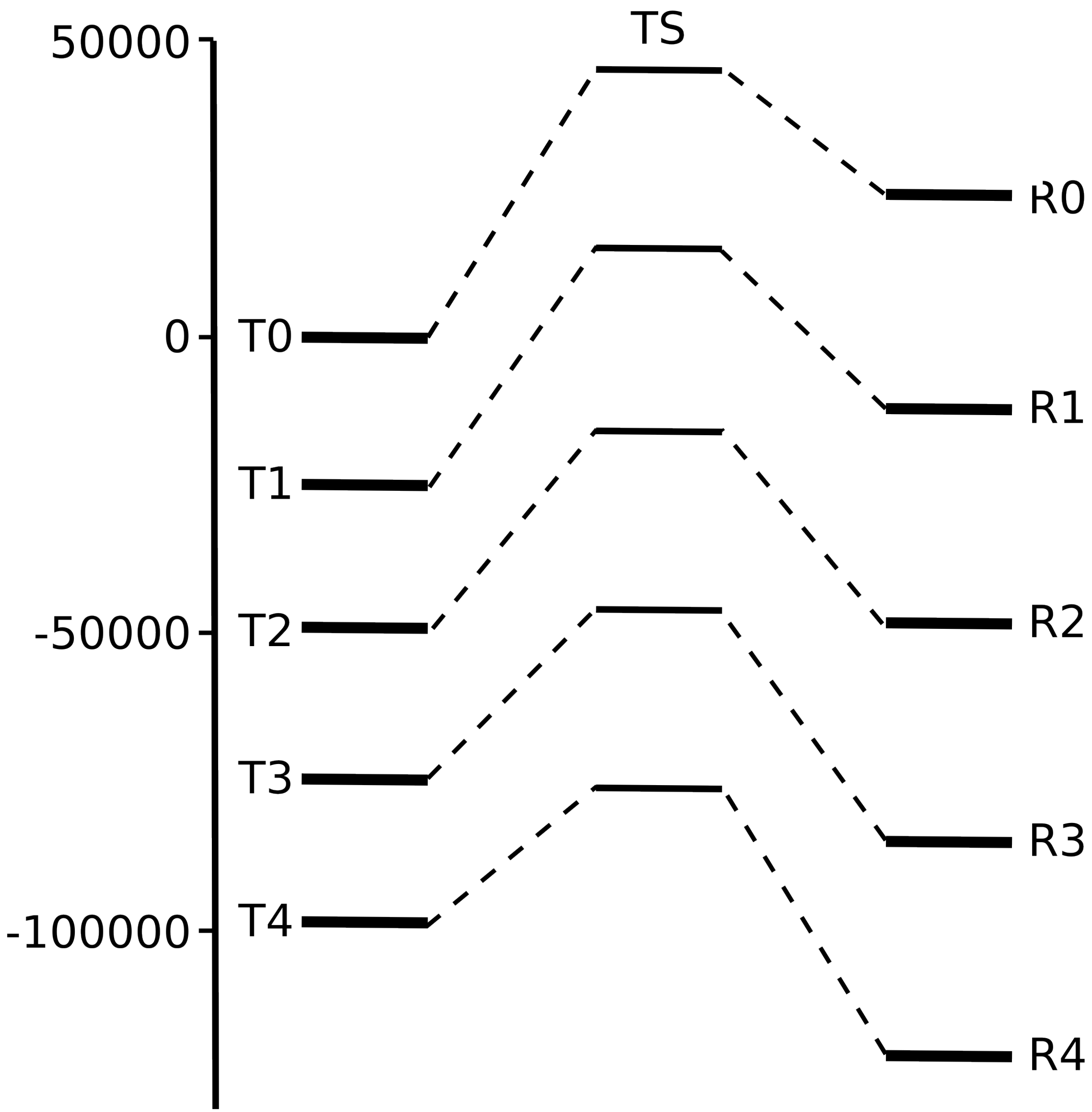




Observed by X-Ray and NMR

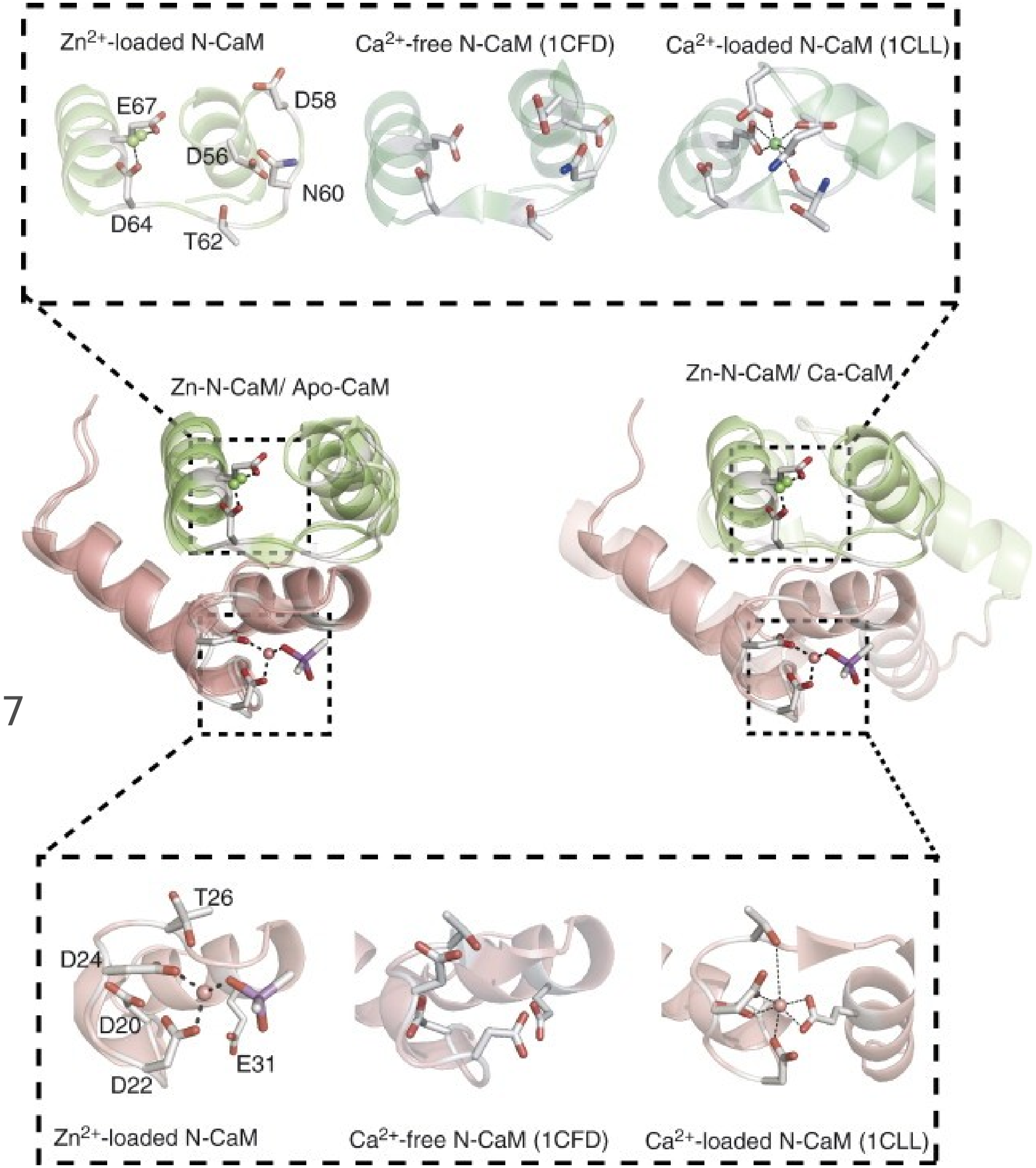
Observation Vs. Prediction





Inferred from Zn binding

Warren *et al.* (2007).
J Mol Biol 374: 517-527



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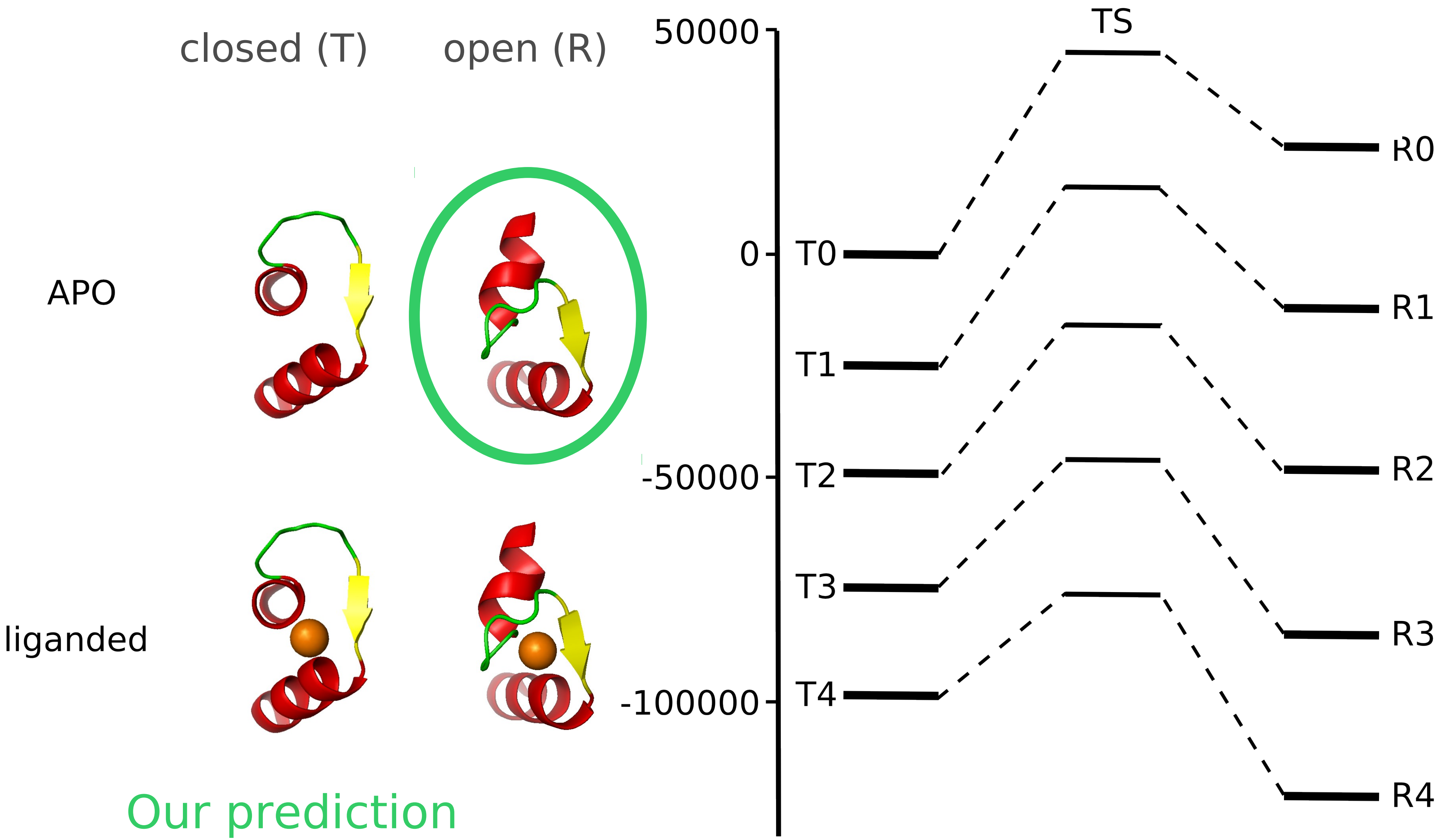
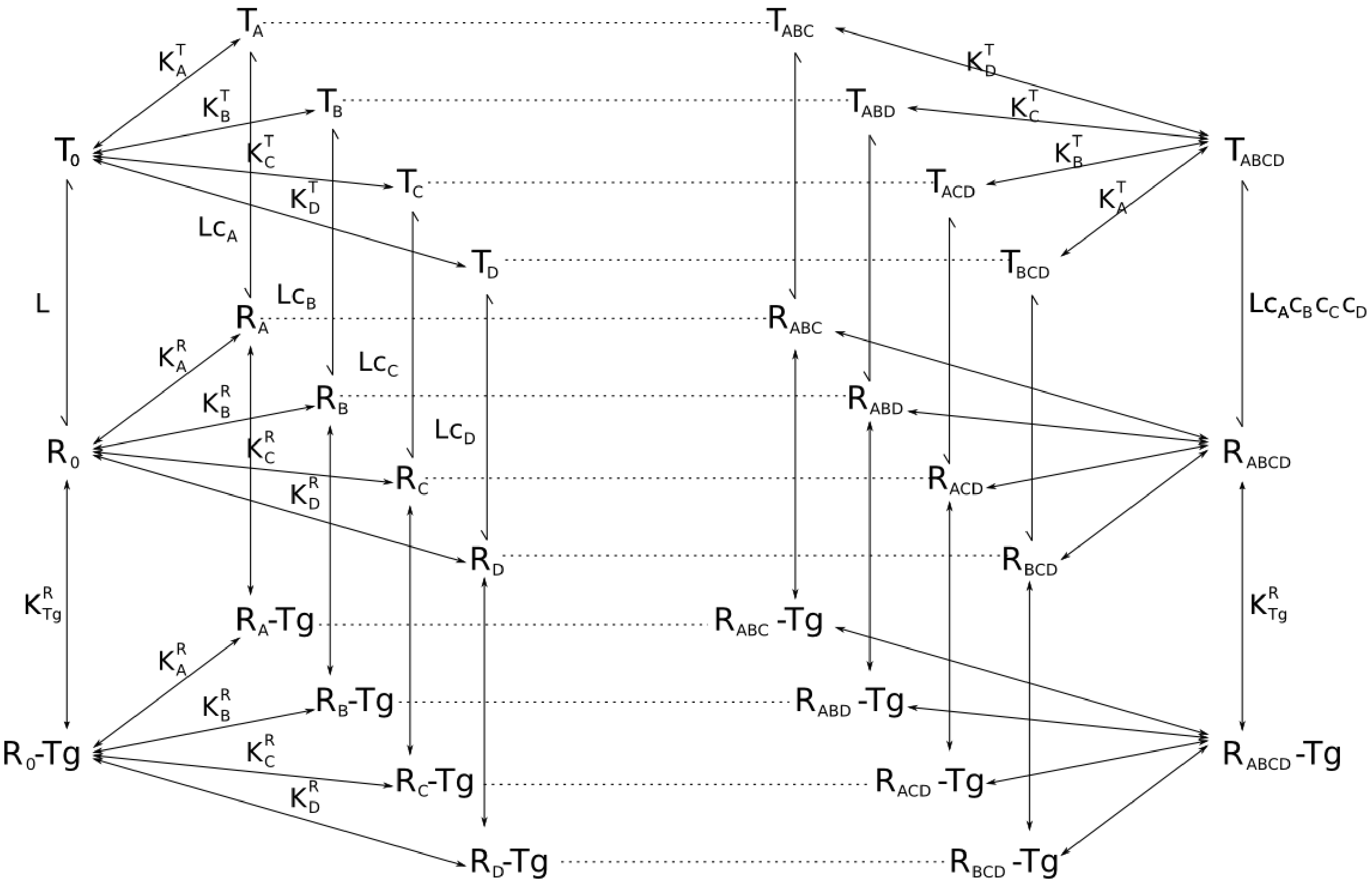


Full mechanistic thermodynamic model



320 reactions

Extended MWC model necessary for Calmodulin

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

$$\bar{Y} = \frac{\alpha(1+\alpha)^{n-1} + L\left(\frac{1+d\beta}{1+\beta}\frac{1+e\gamma}{1+\gamma}\right)^n c\alpha(1+c\alpha)^{n-1}}{(1+\alpha)^n + L\left(\frac{1+d\beta}{1+\beta}\frac{1+e\gamma}{1+\gamma}\right)^n (1+c\alpha)^n}$$

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

- $\alpha_i = [\text{ligand}]/K^{R}_{i,\,\text{lig}}$
- $\gamma_k = [\text{modulator}]/K^{R}_{k,\text{mod}}$
- $c_i = K^{R}_{i,\,\text{lig}} / K^{T}_{i,\,\text{lig}}$
- $e_k = K^{R}_{k,\text{mod}} / K^{T}_{k,\text{mod}}$

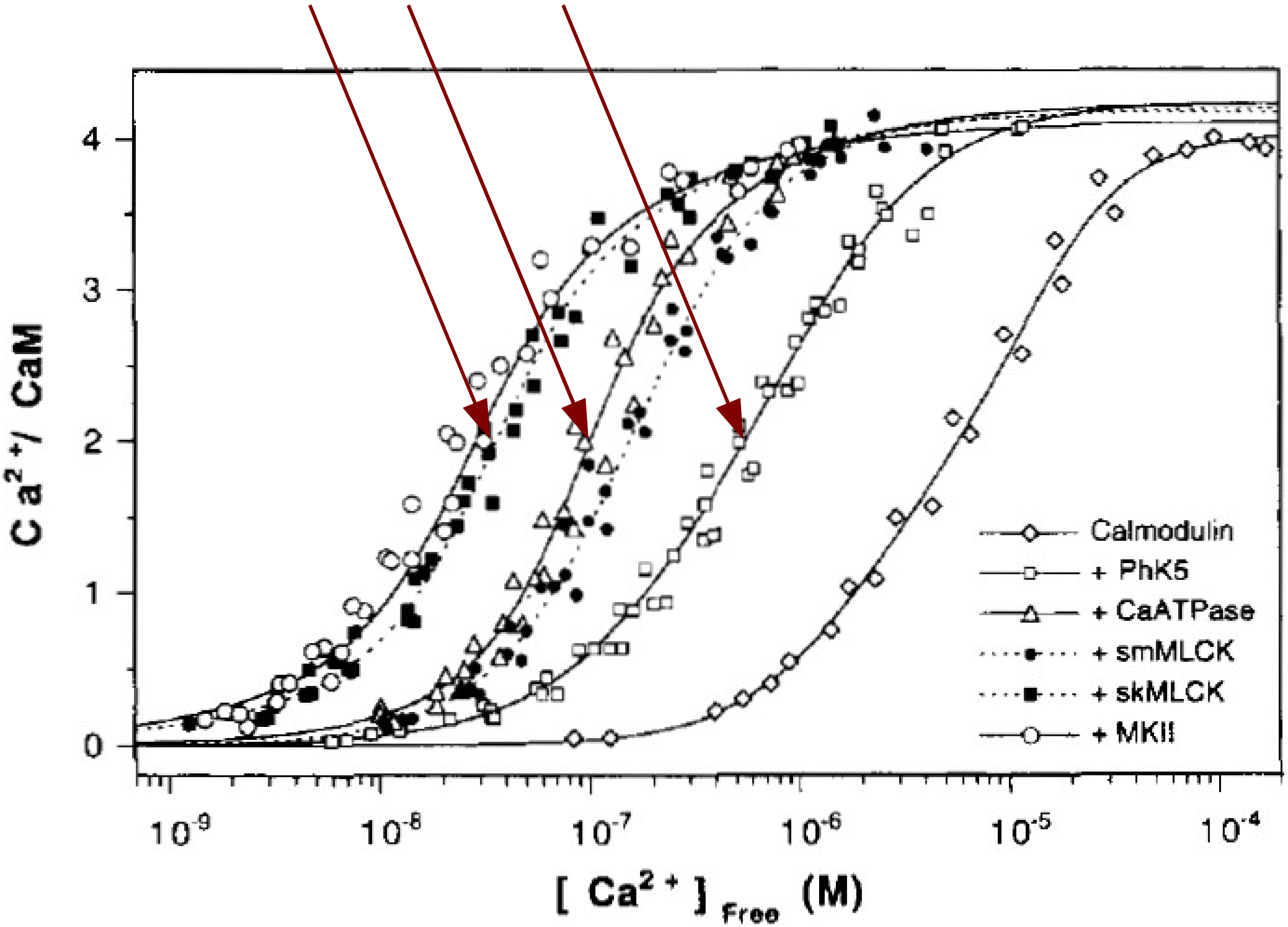
Stefan M.I., Edelstein S.J., Le Novère N.
Computing phenomenologic Adair-Klotz constants from microscopic MWC parameters.
BMC Systems Biology (2009), 3: 68

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

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

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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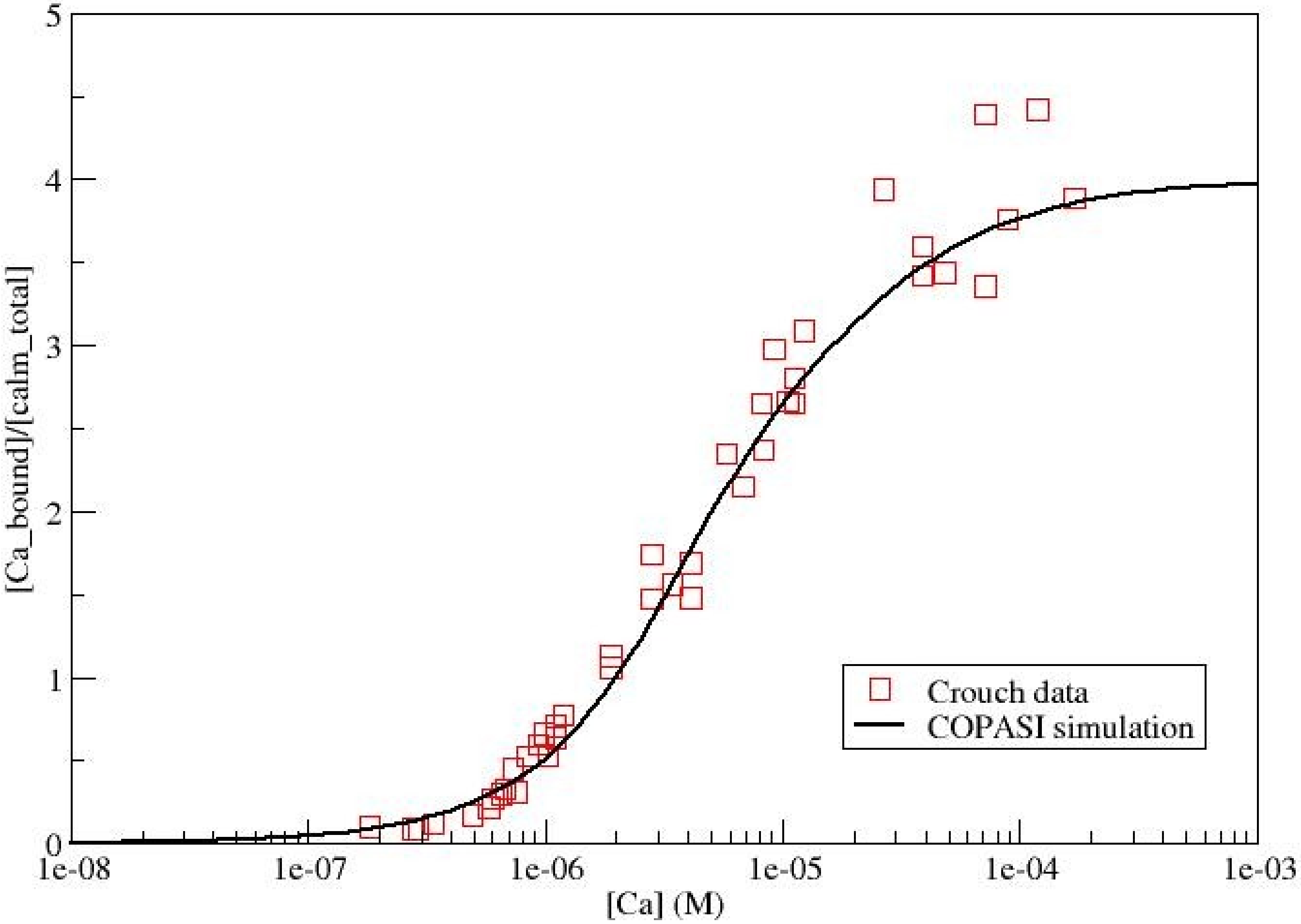
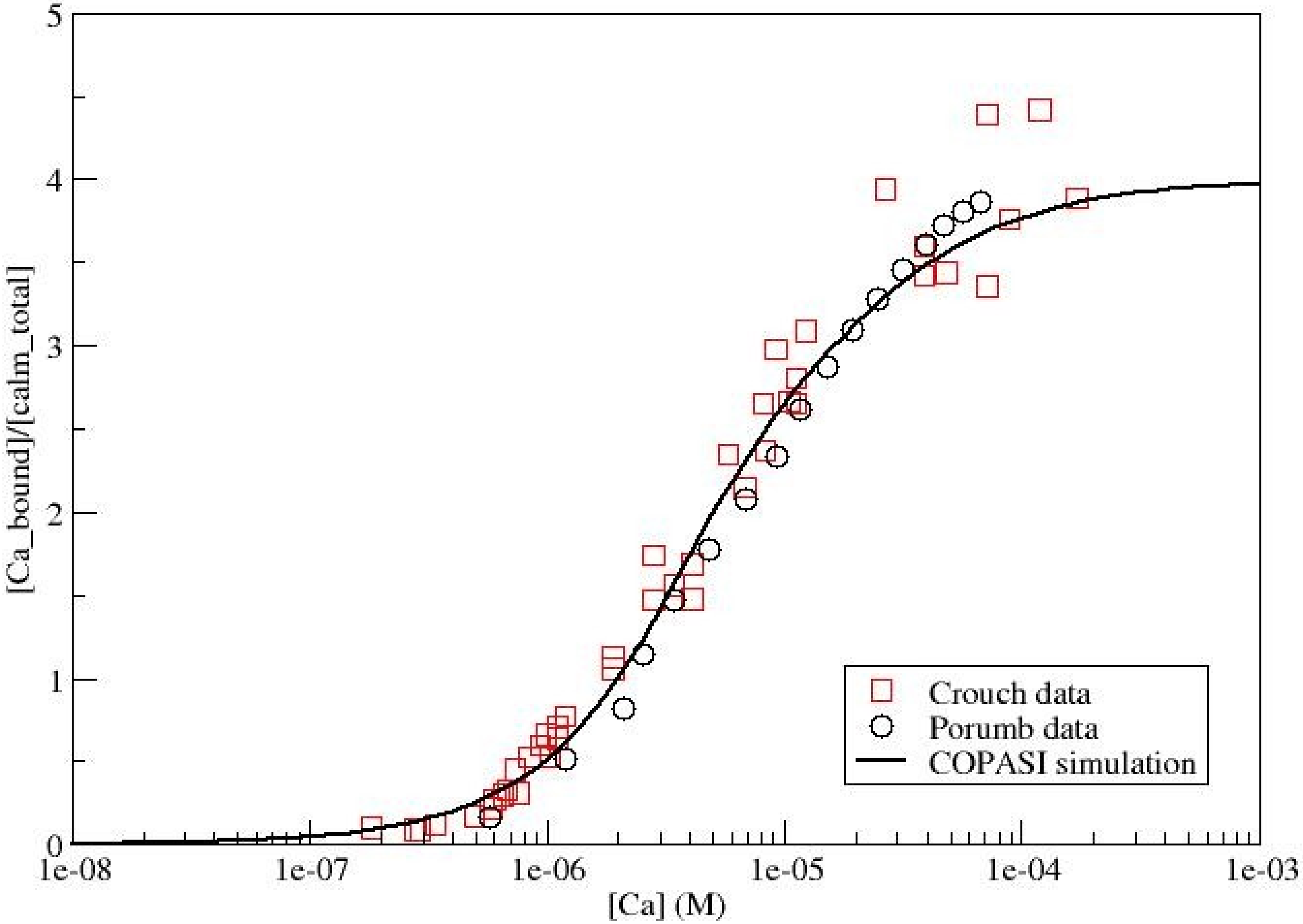
Comparison with experiments



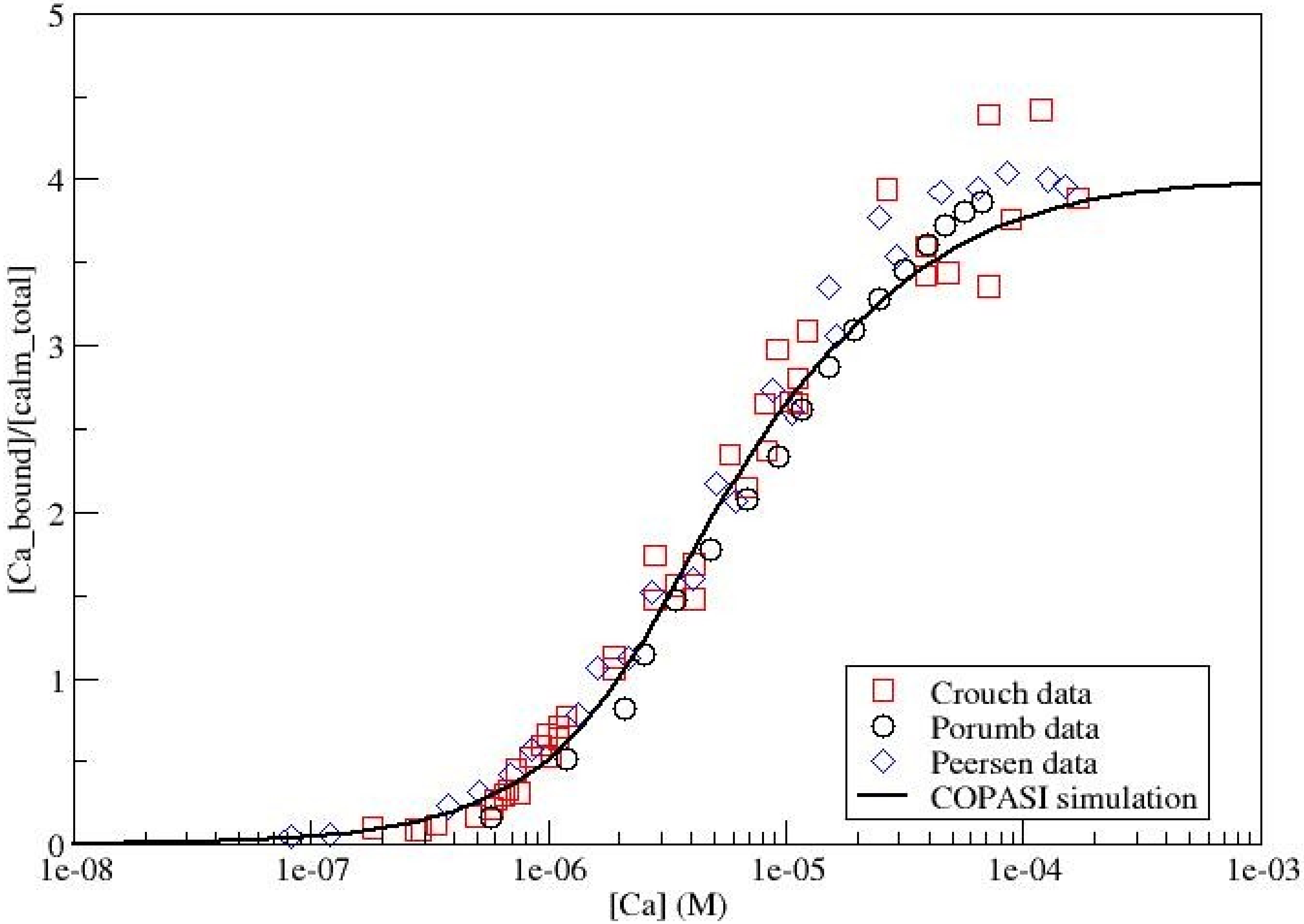
Crouch and Klee (1980) *Biochemistry*, 19: 3692-3698c

Comparison with experiments



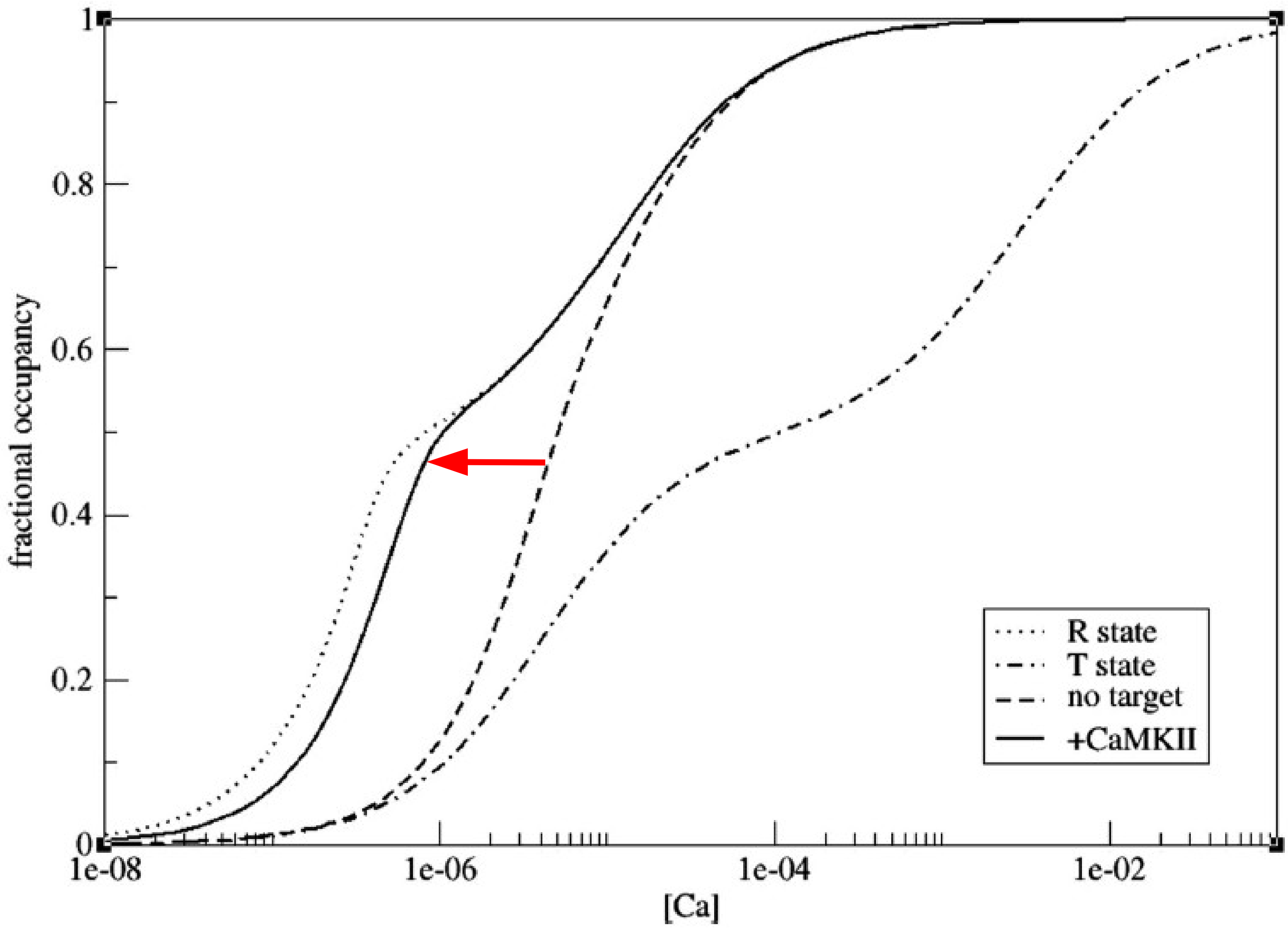
Porumb et al (1994) Anal Biochem 220: 227-237

Comparison with experiments



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

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



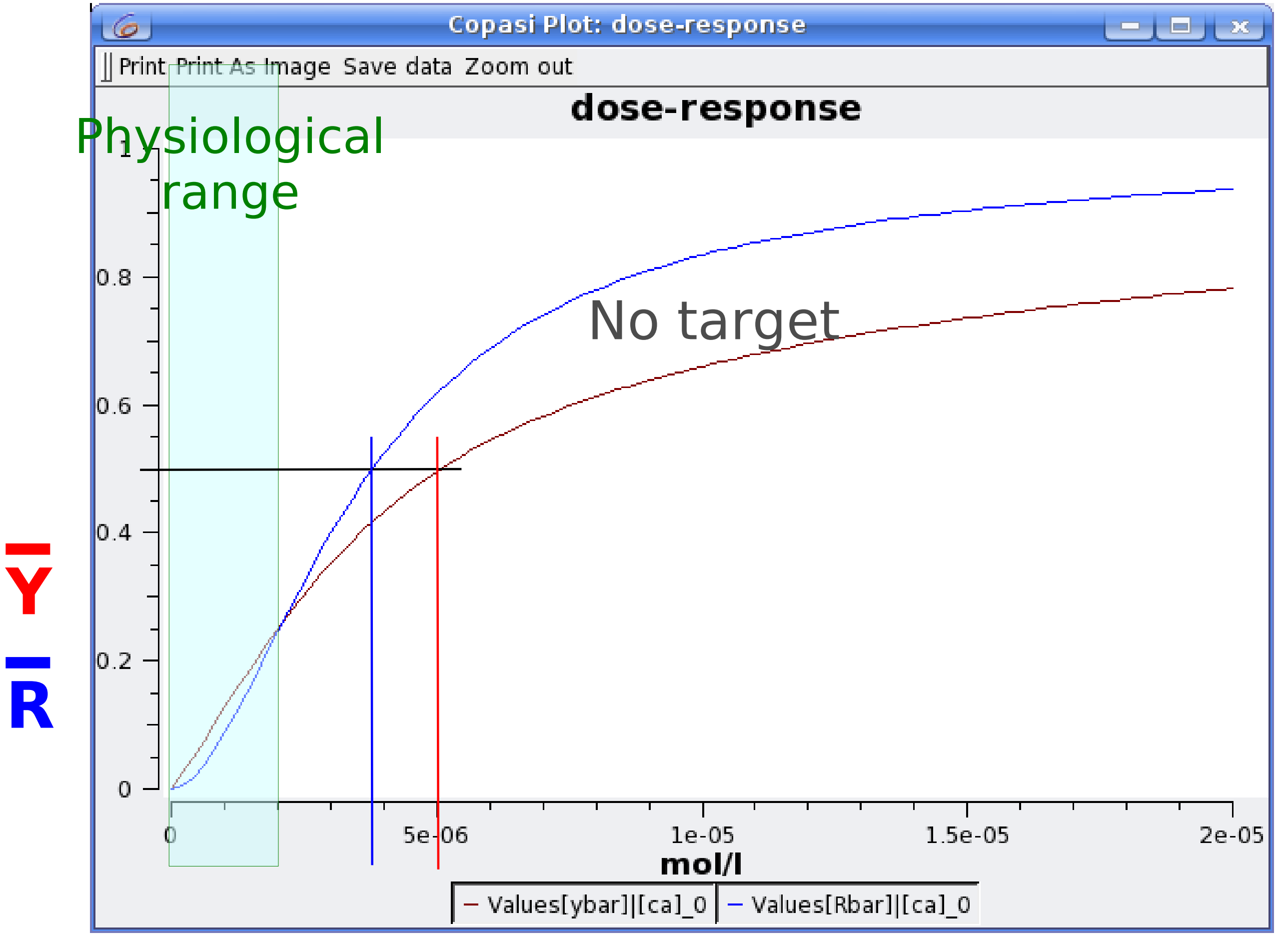
Activity of unsaturated calmodulin

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

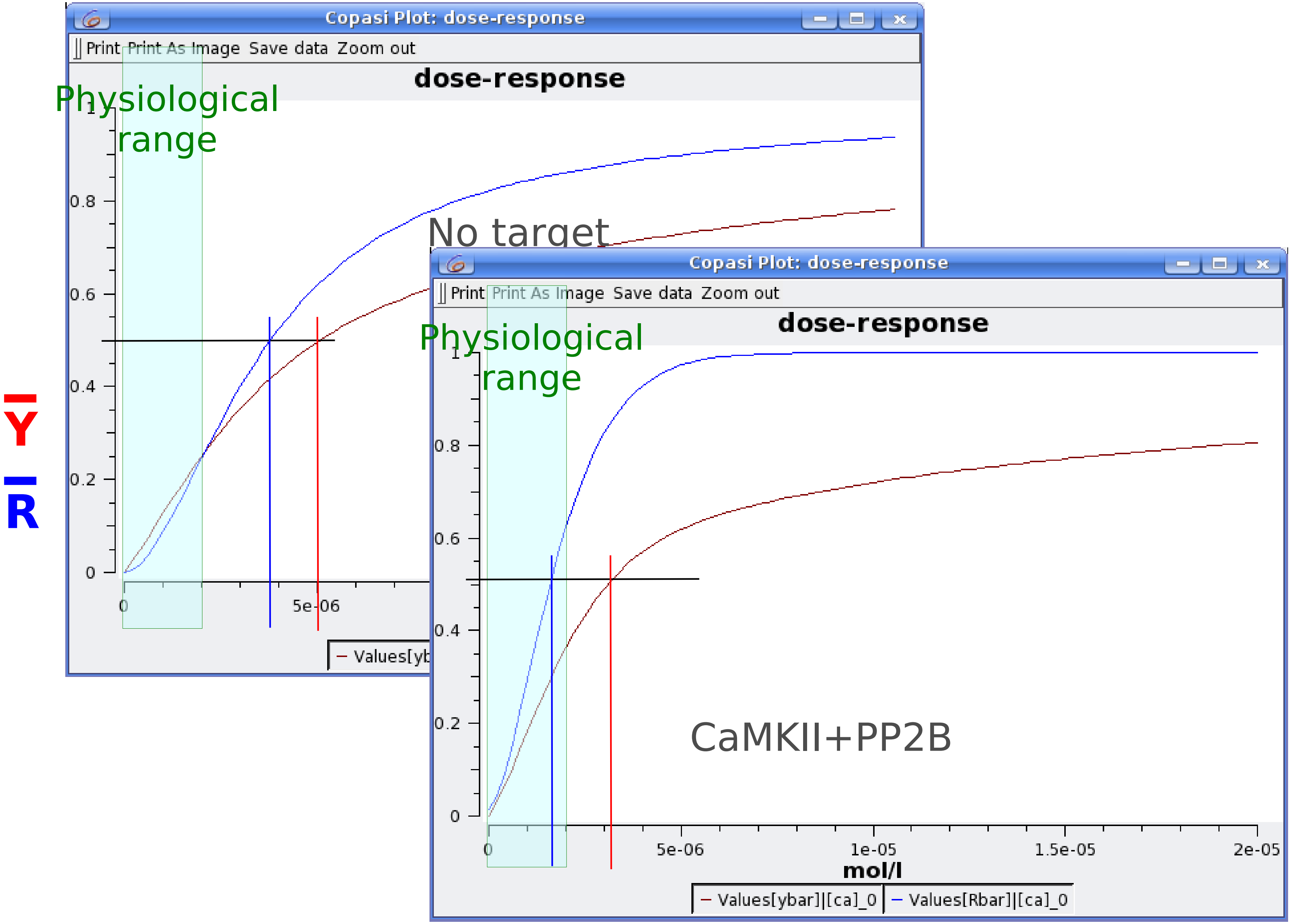
$$\frac{R_2}{T_2} = \frac{1}{L \cdot c^2}$$

- 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

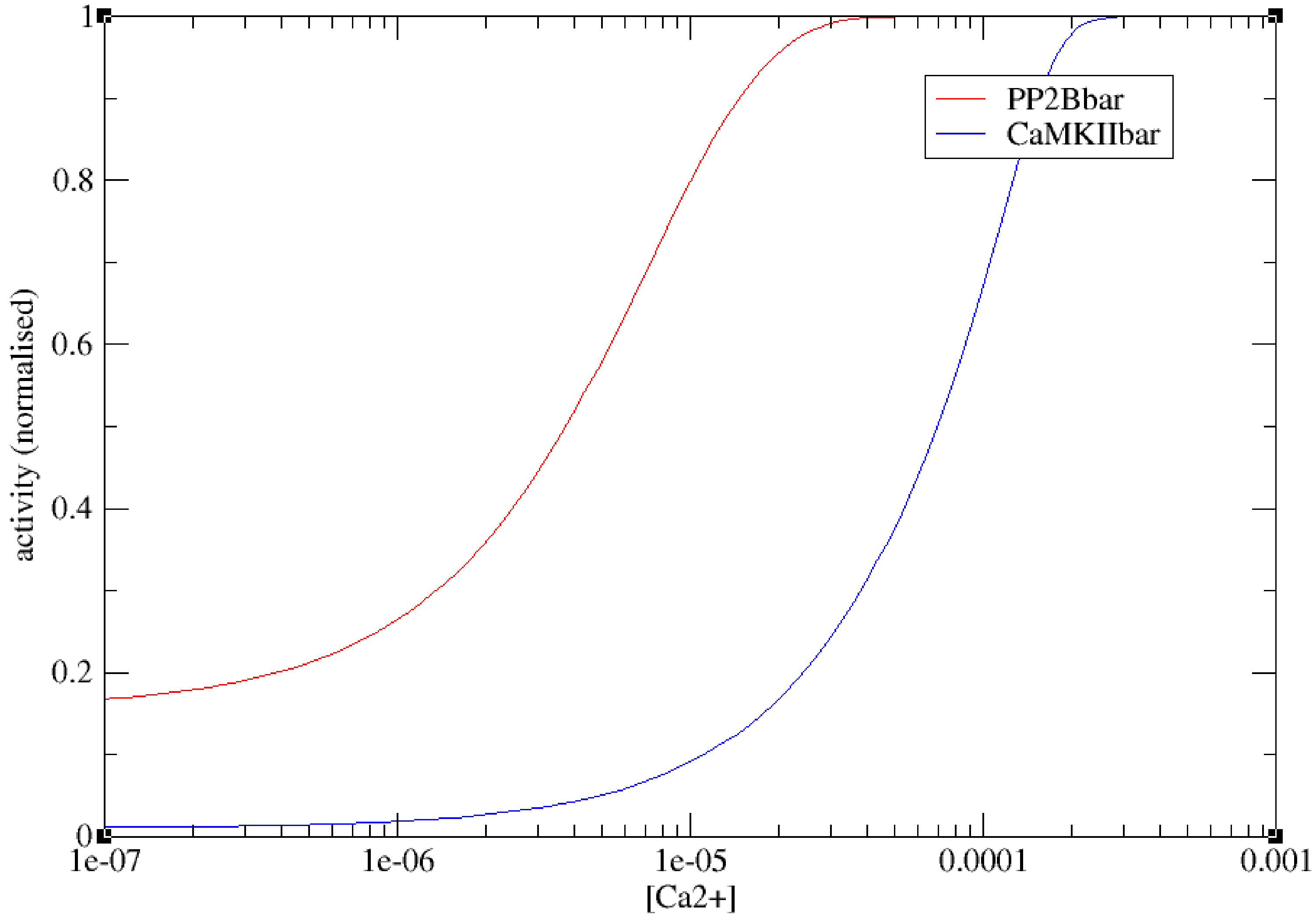
But ... we're out of the physiological range?



This is Systems Biology!



Bidirectional synaptic plasticity



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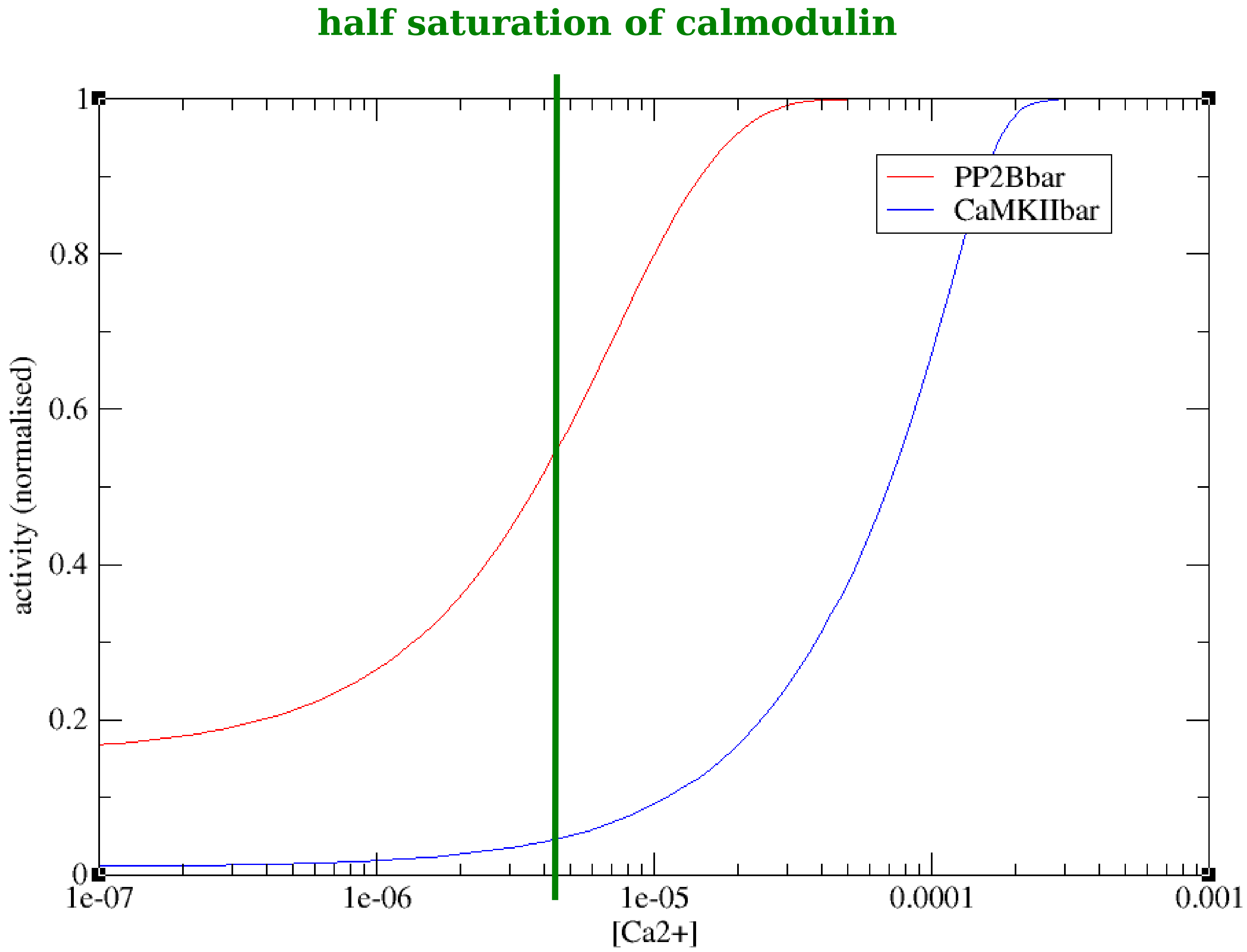

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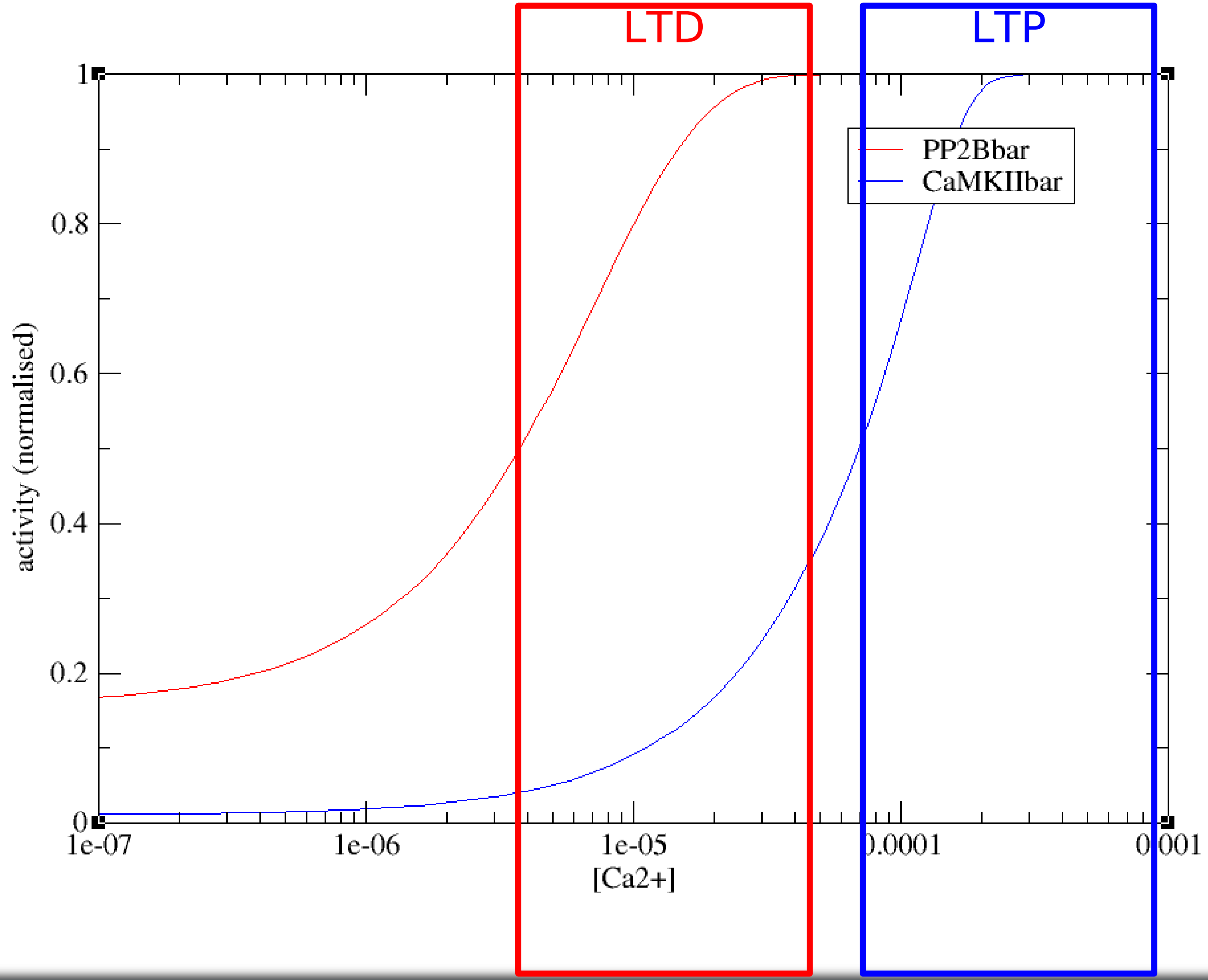
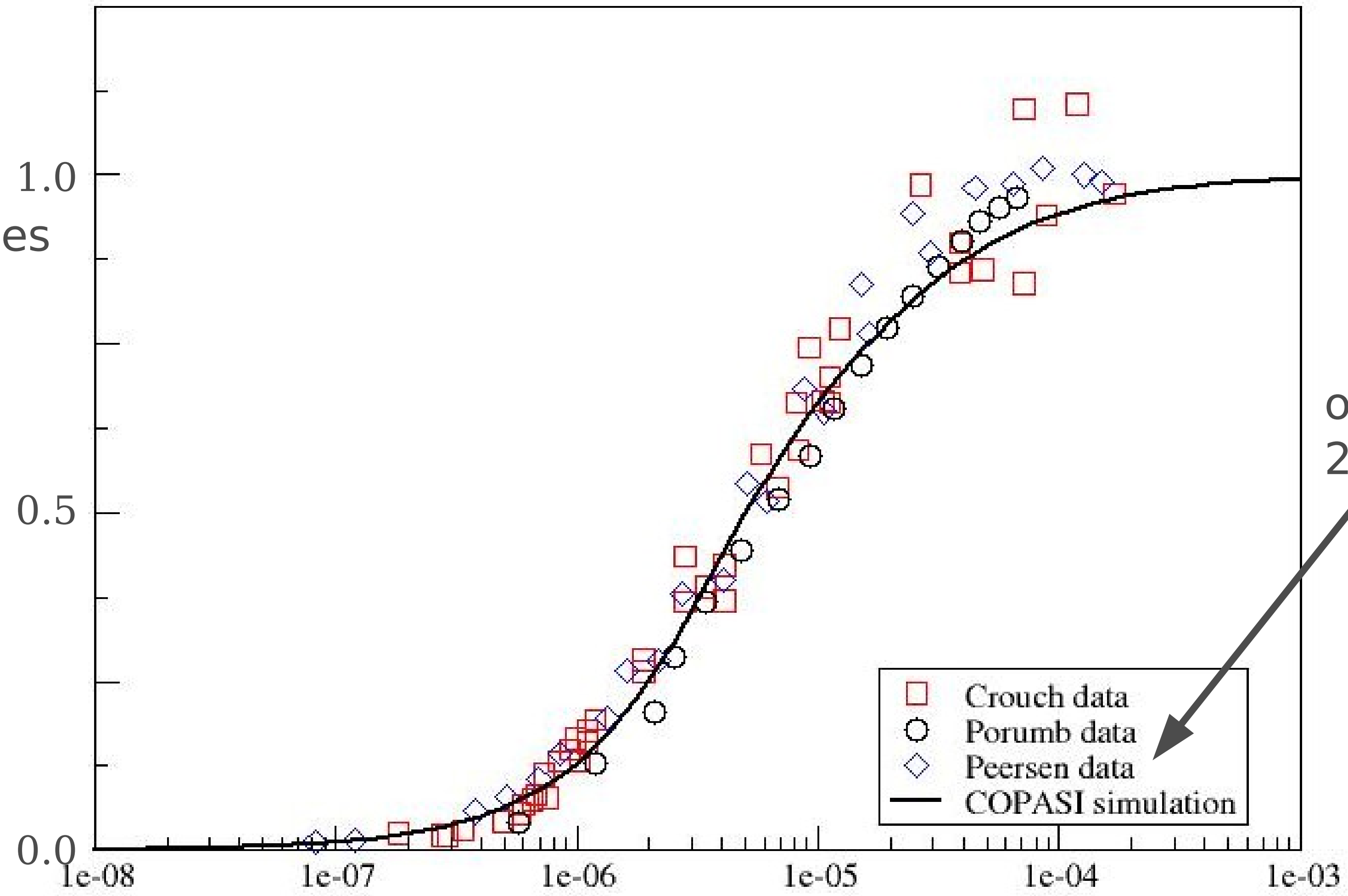


Conclusions

- 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. We parametrised the model with experimental data-sets.
    - The model fits independent experimental datasets.
    - The affinitity of CaM for calcium increases upon binding of the target.
    - CaM can be significantly in the open state even with less than 4 calcium bounds.
    - CaM can bind its targets even when 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 PP2N and CaMKII.

Allosteric model of Calmodulin function

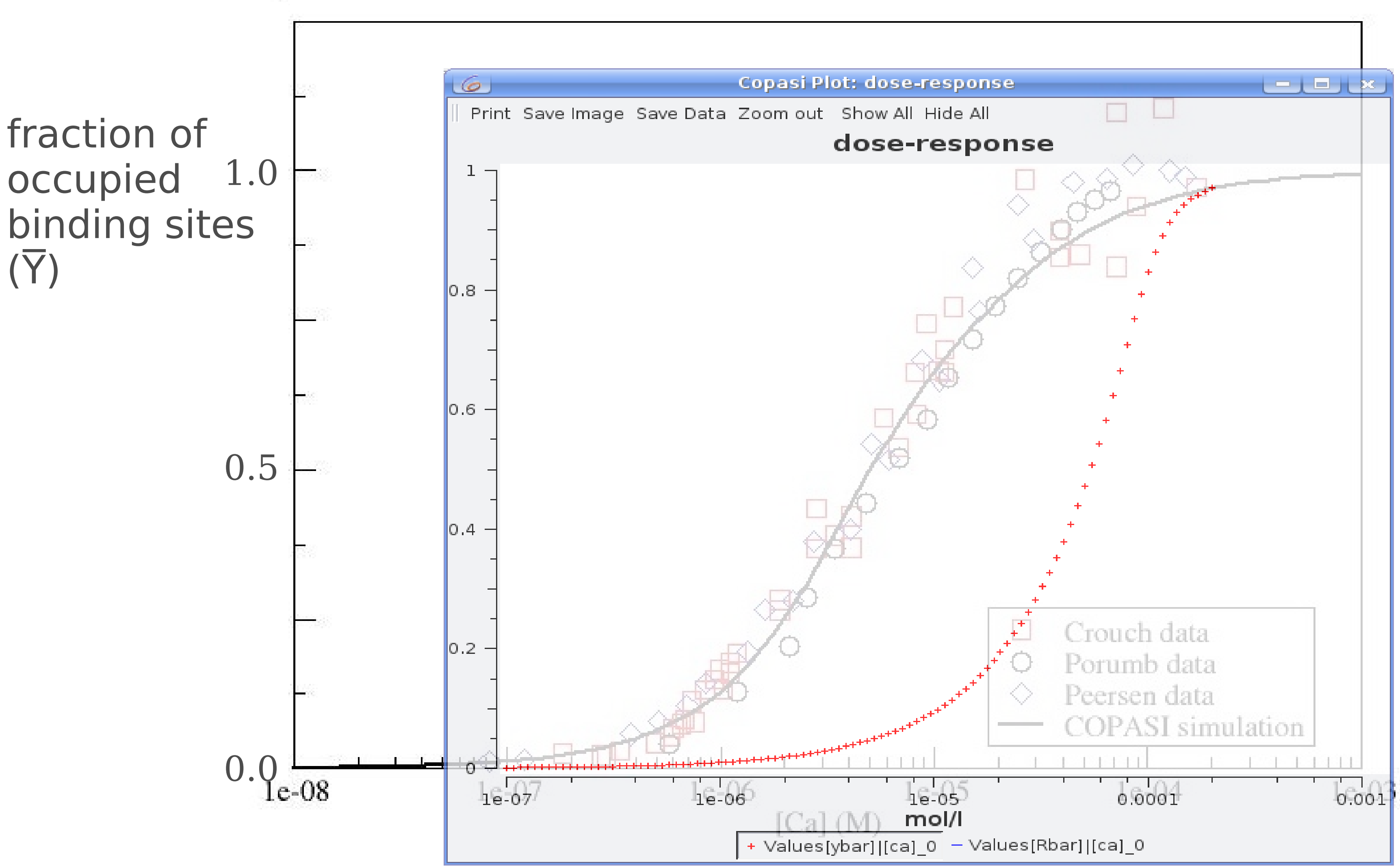
fraction of occupied binding sites $(\bar{Y})$



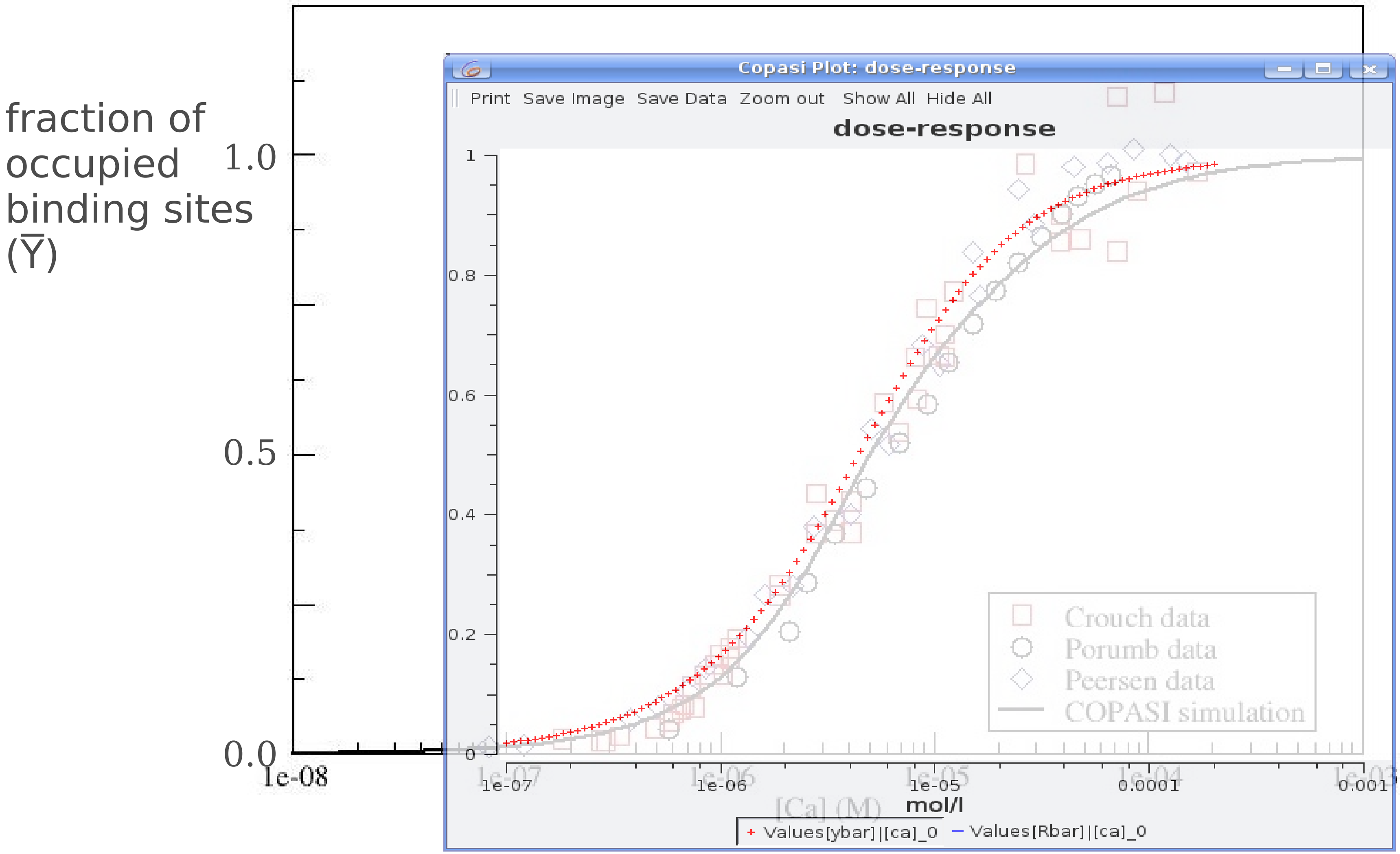
[free Ca^{2+}] (M)

obtained with 25 µM CaM

Calcium dose-response on 25 μM Calmodulin



Calcium dose-response on 0.1 μM Calmodulin



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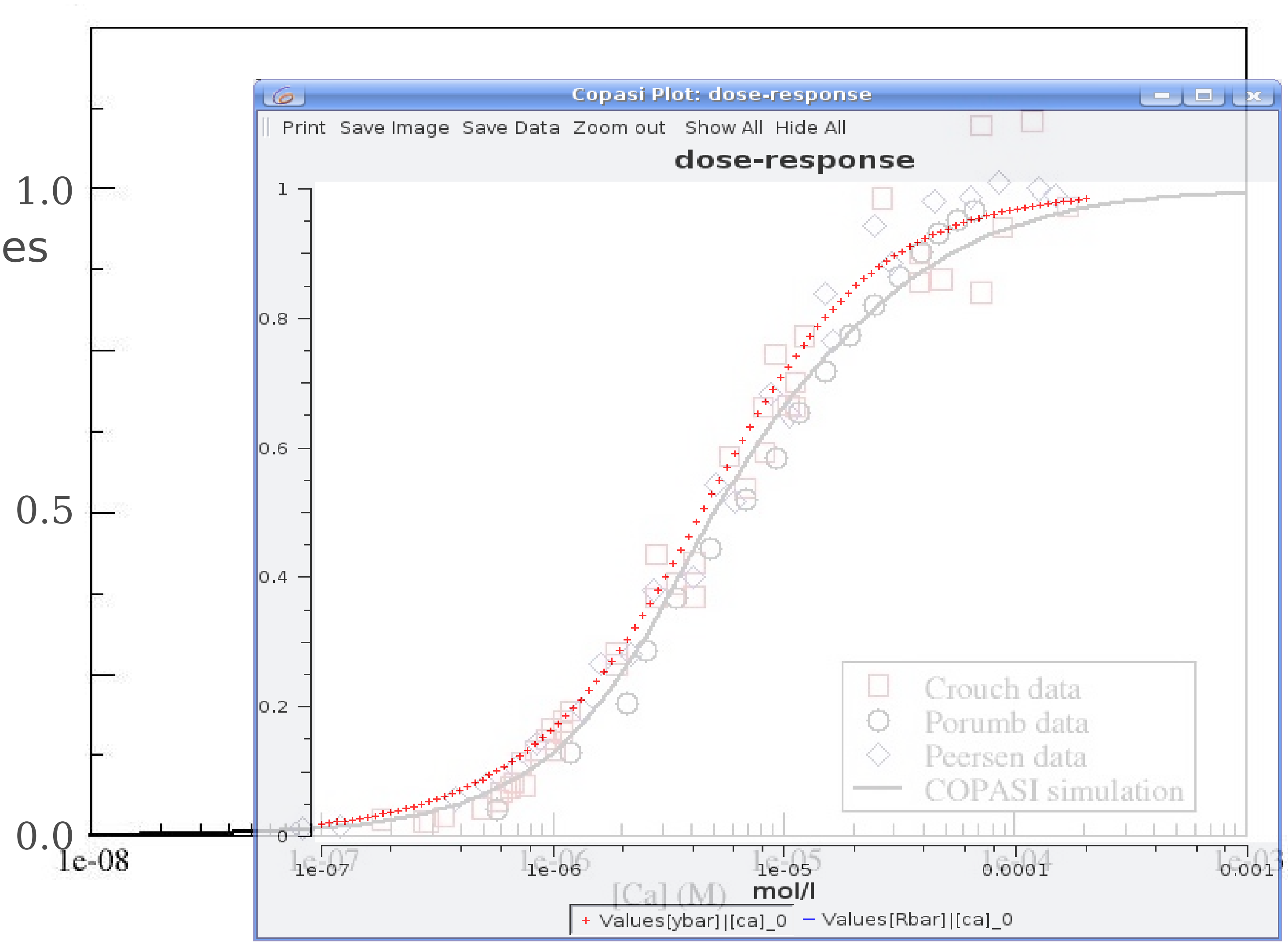
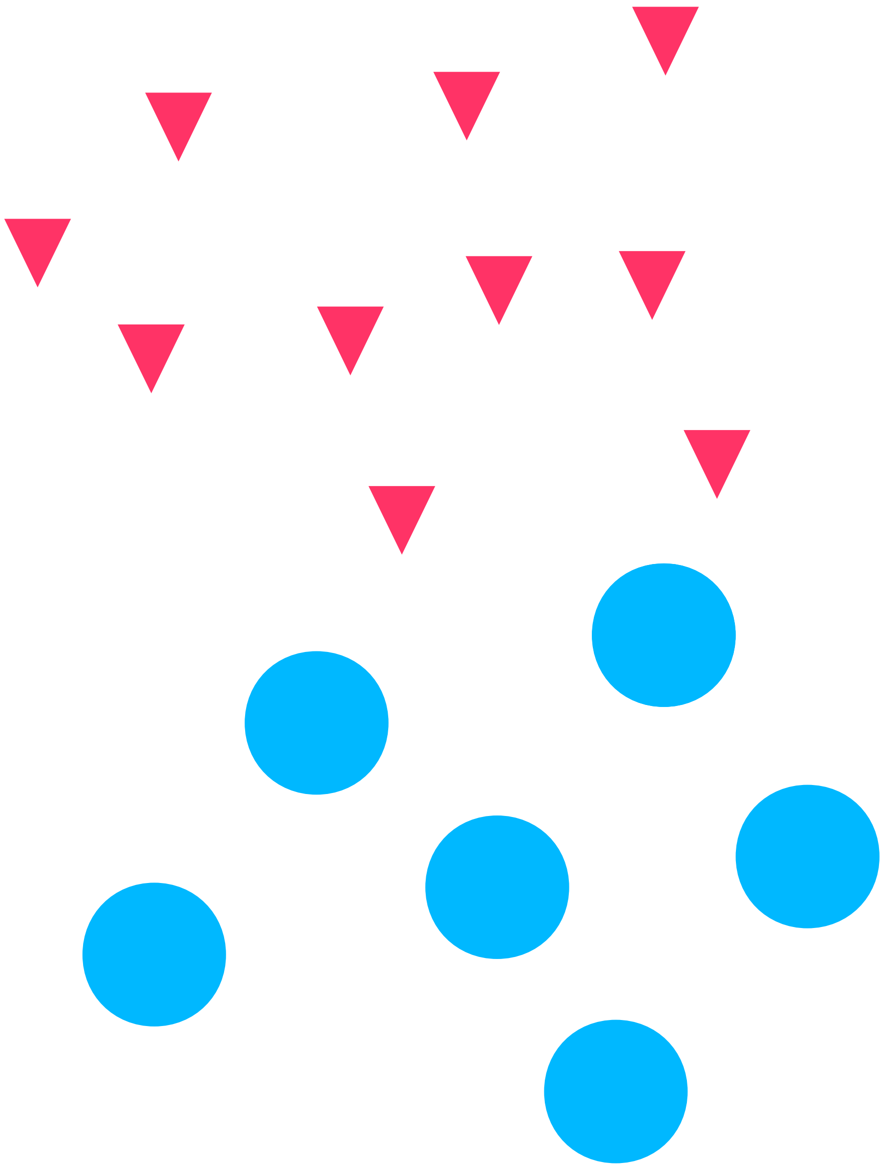


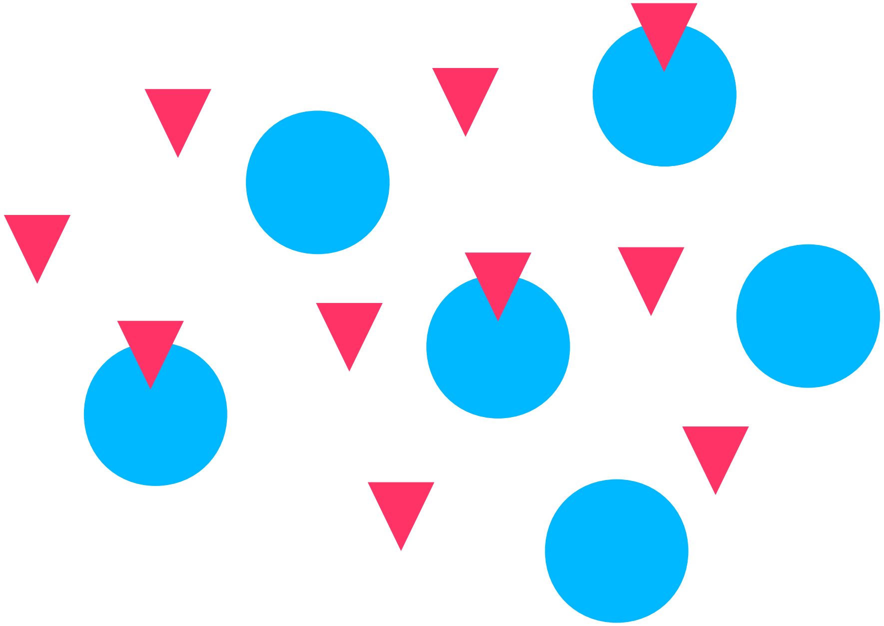
Edelstein S.J., Stefan M.I, Le Novère N. Ligand depletion in vivo modulates the dynamic range and cooperativity of signal transduction. PLoS One (2010), 5(1): e8449

What is ligand depletion?

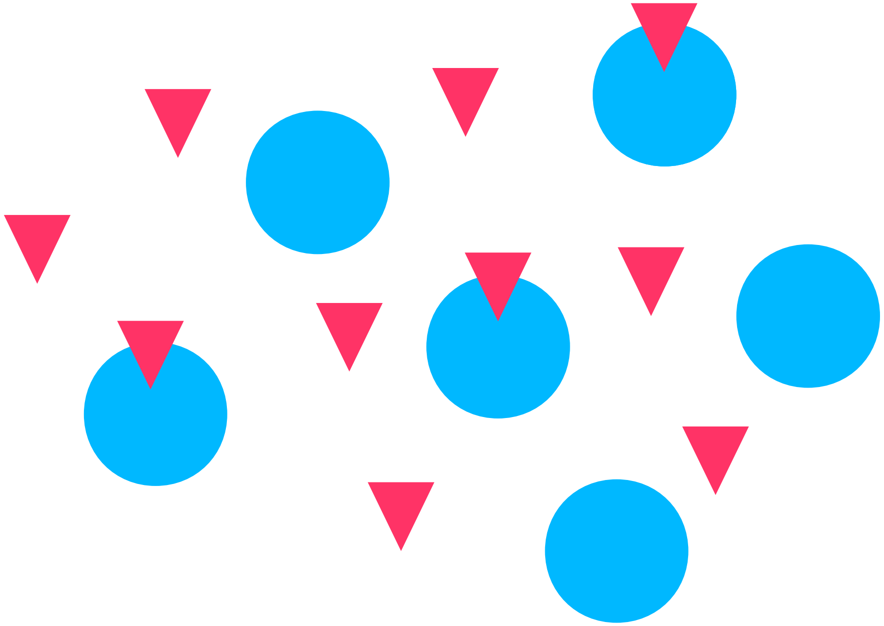


What is ligand depletion?

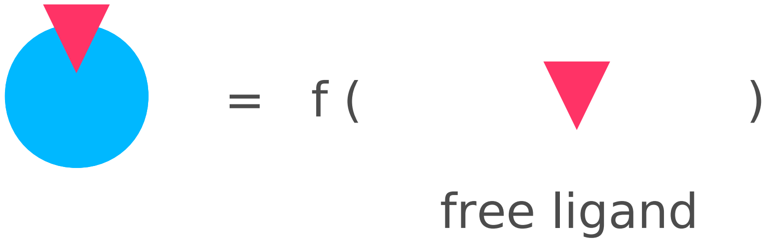
Chemistry (mass-action law)



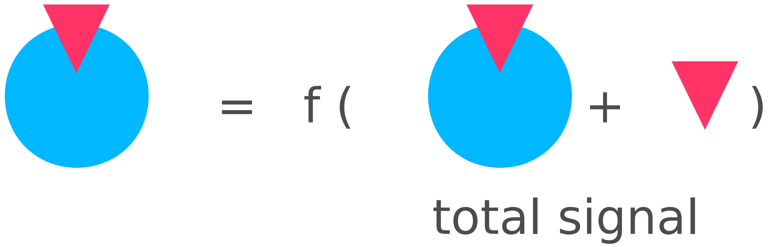
What is ligand depletion?



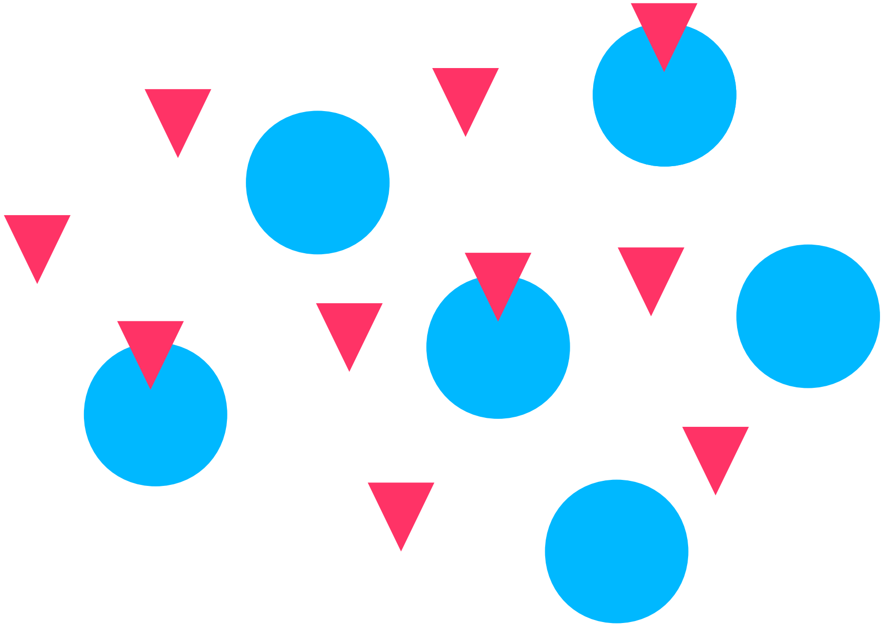
Chemistry (mass-action law)

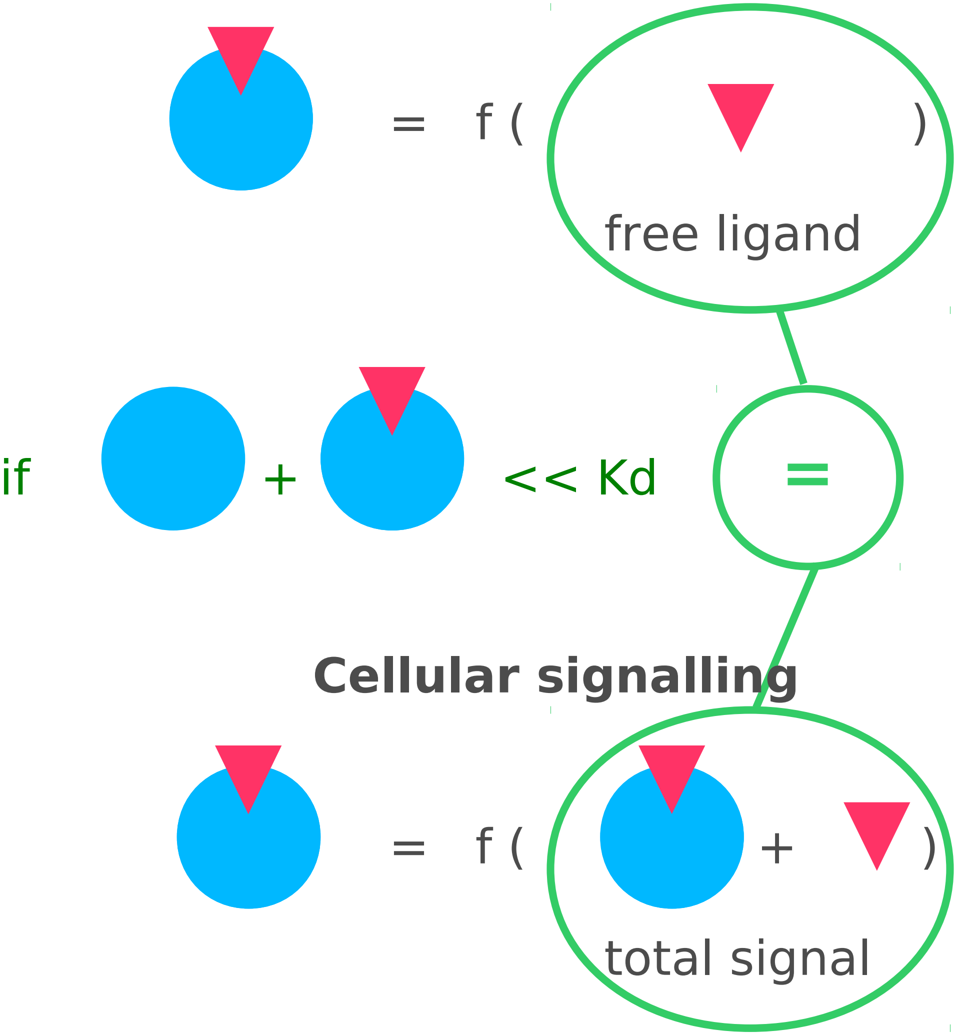


Cellular signalling



What is ligand depletion?





Chemistry (mass-action law)

= f ( )

free ligand

if + << Kd

=

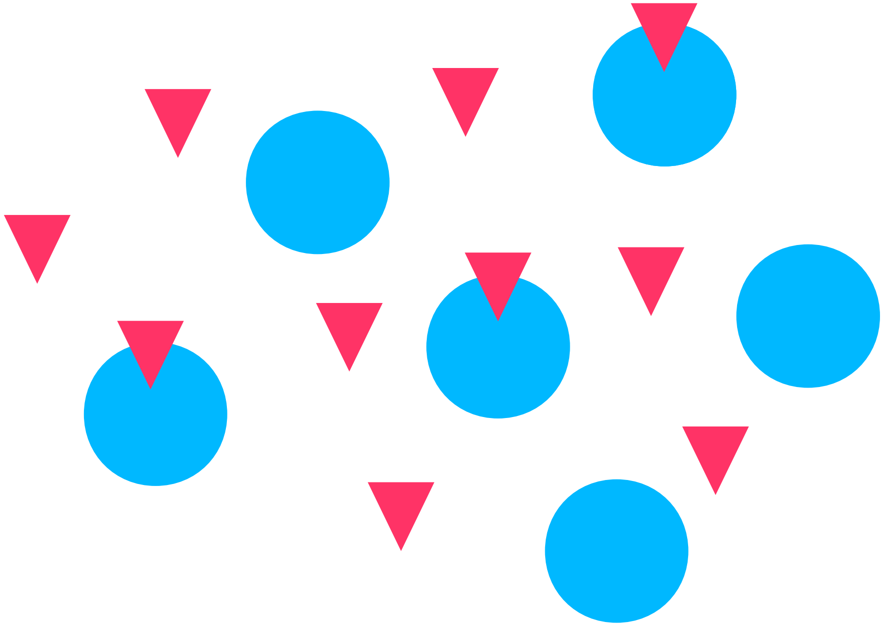
Cellular signalling

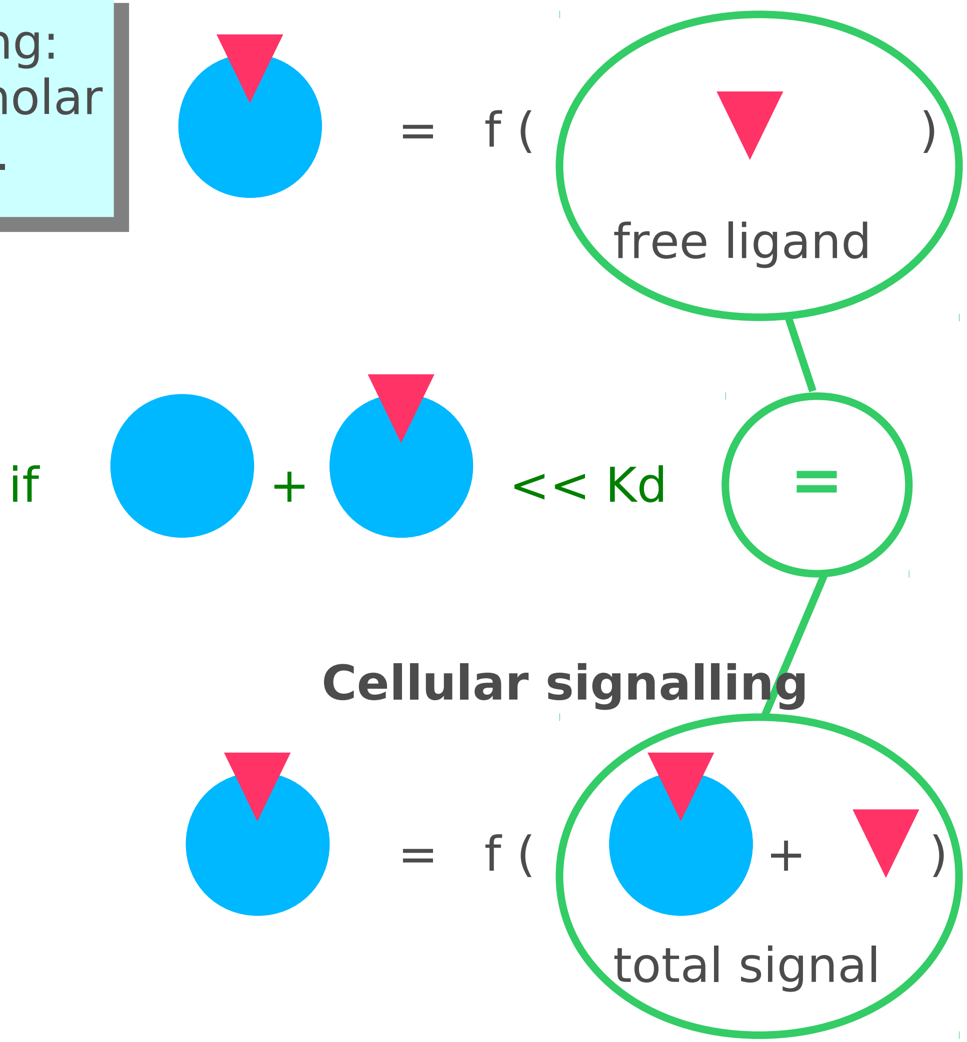
= f (+)

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.





Chemistry (mass-action law)

= f ( )

free ligand

if + << Kd

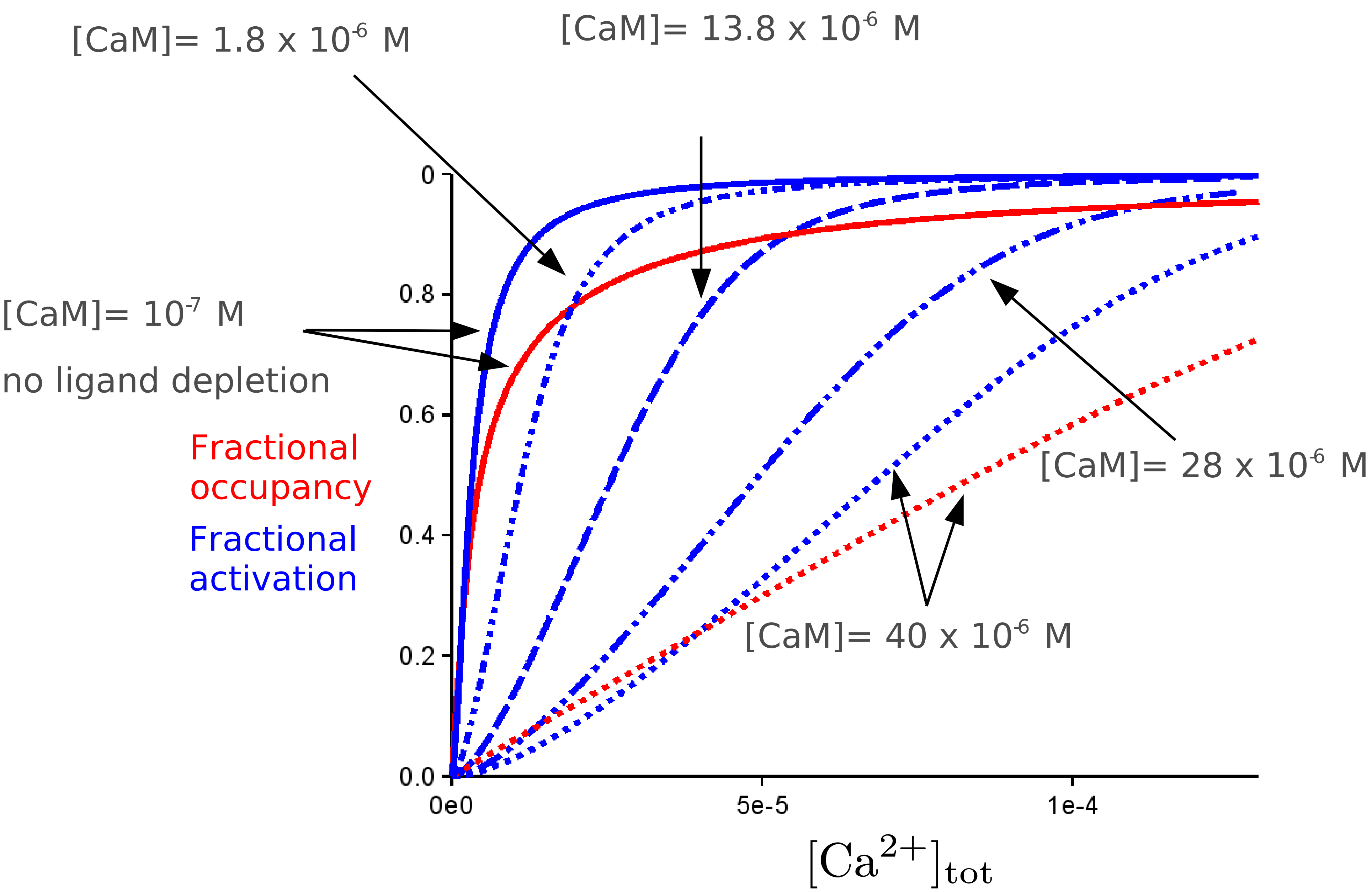
=

Cellular signalling

= f (+)

total signal

Dose-response depends on Calmodulin concentration



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

$[\mathrm{CaM}] = 13.8 \times 10^{-6}$ M

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

no ligand depletion

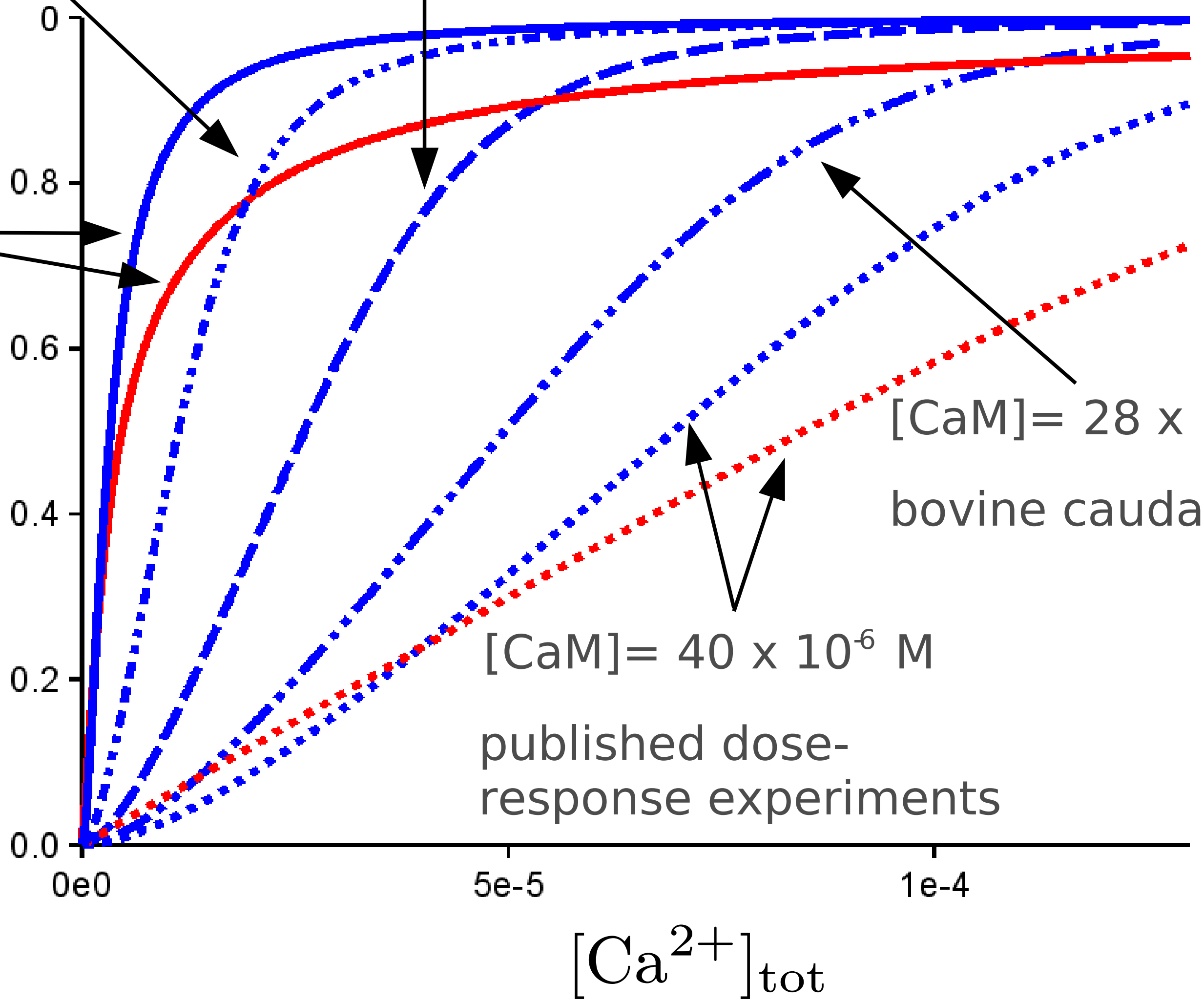
Fractional occupancy

Fractional activation

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

$[\mathrm{CaM}] = 40 \times 10^{-6}$ M

Dose-response depends on Calmodulin concentration



[CaM]= 1.8×10^{-6} M

rat spleen

[CaM]= 13.8×10^{-6} M

bovine hypothalamus

[CaM]= 10^{-7} M

no ligand depletion

Fractional occupancy

Fractional activation

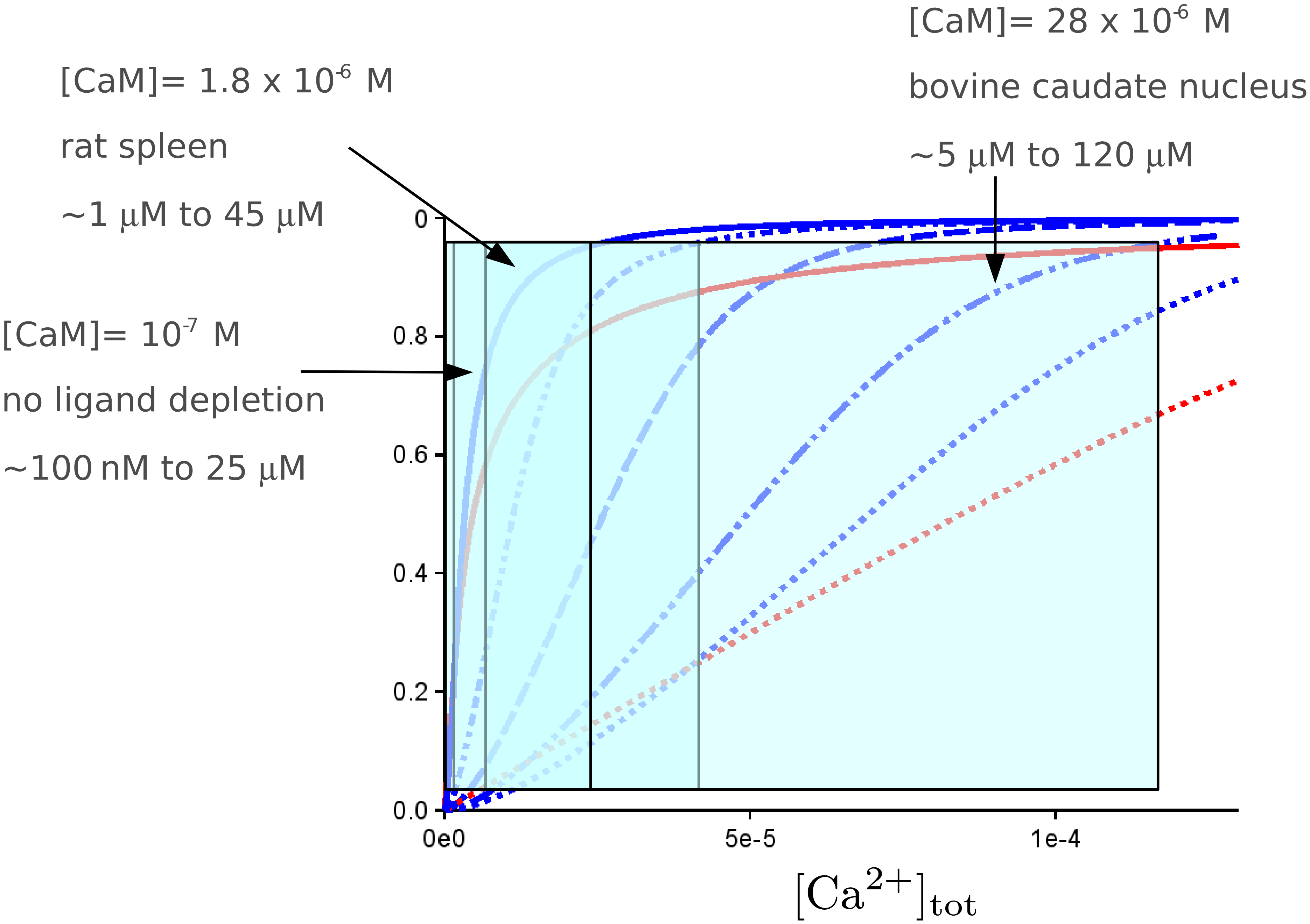
[CaM]= 28×10^{-6} M

bovine caudate nucleus

[CaM]= 40×10^{-6} M

published dose-response experiments

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

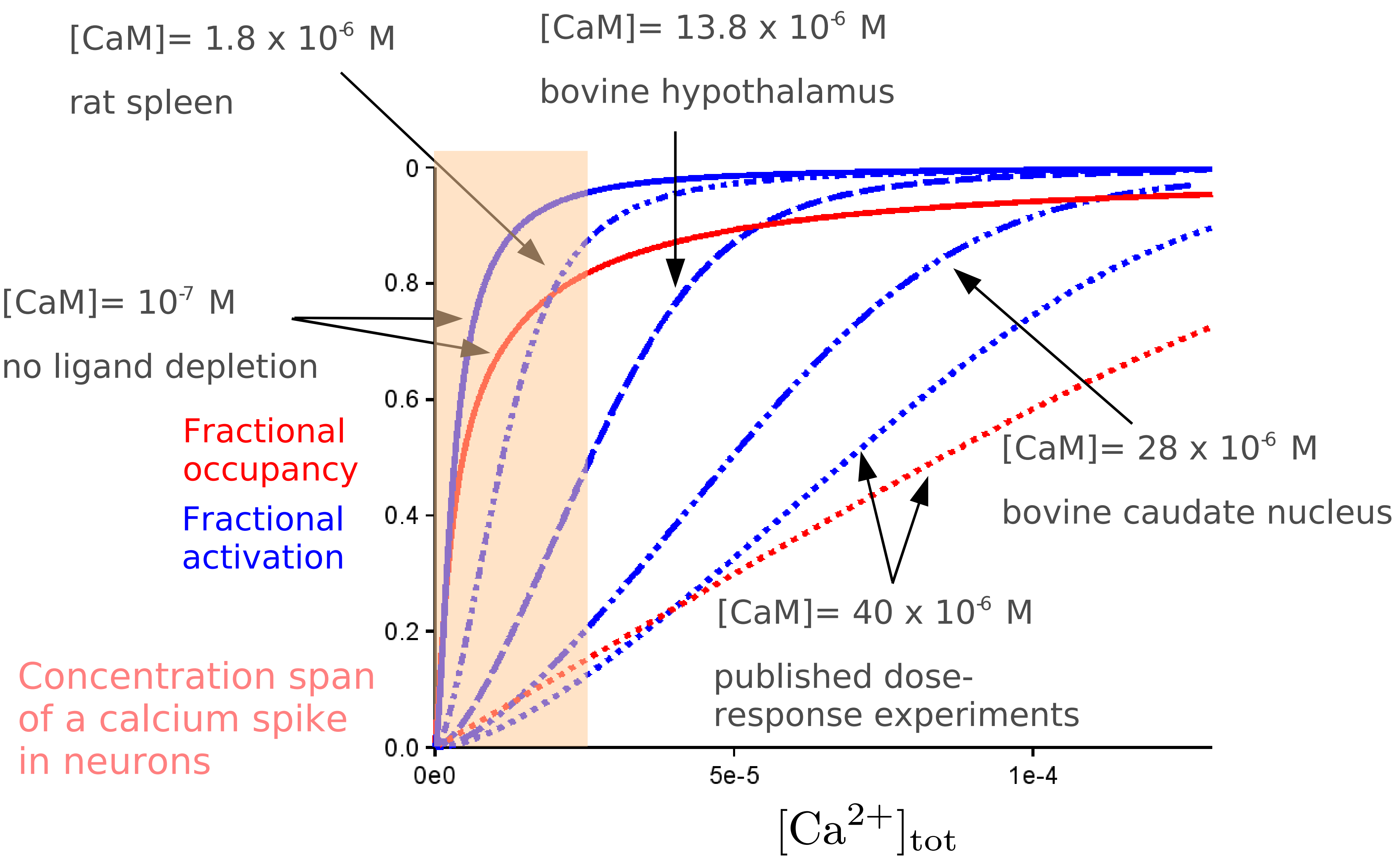
~1 μM to 45 μM

[CaM]= 10^{-7} M

no ligand depletion

~100 nM to 25 μM

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



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

rat spleen

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

bovine hypothalamus

[CaM]= 10^{-7} M

no ligand depletion

Fractional occupancy

Fractional activation

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

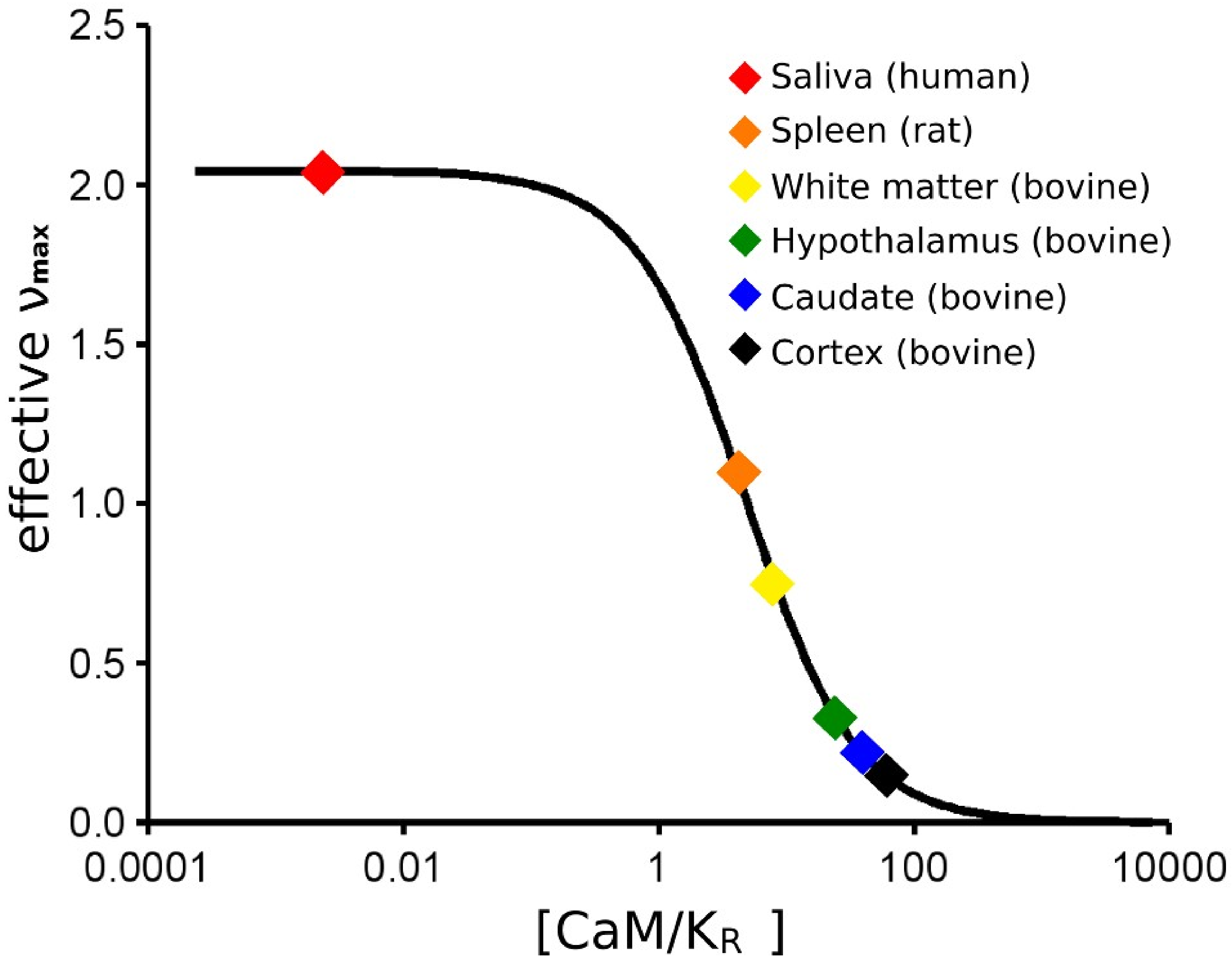
bovine caudate nucleus

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

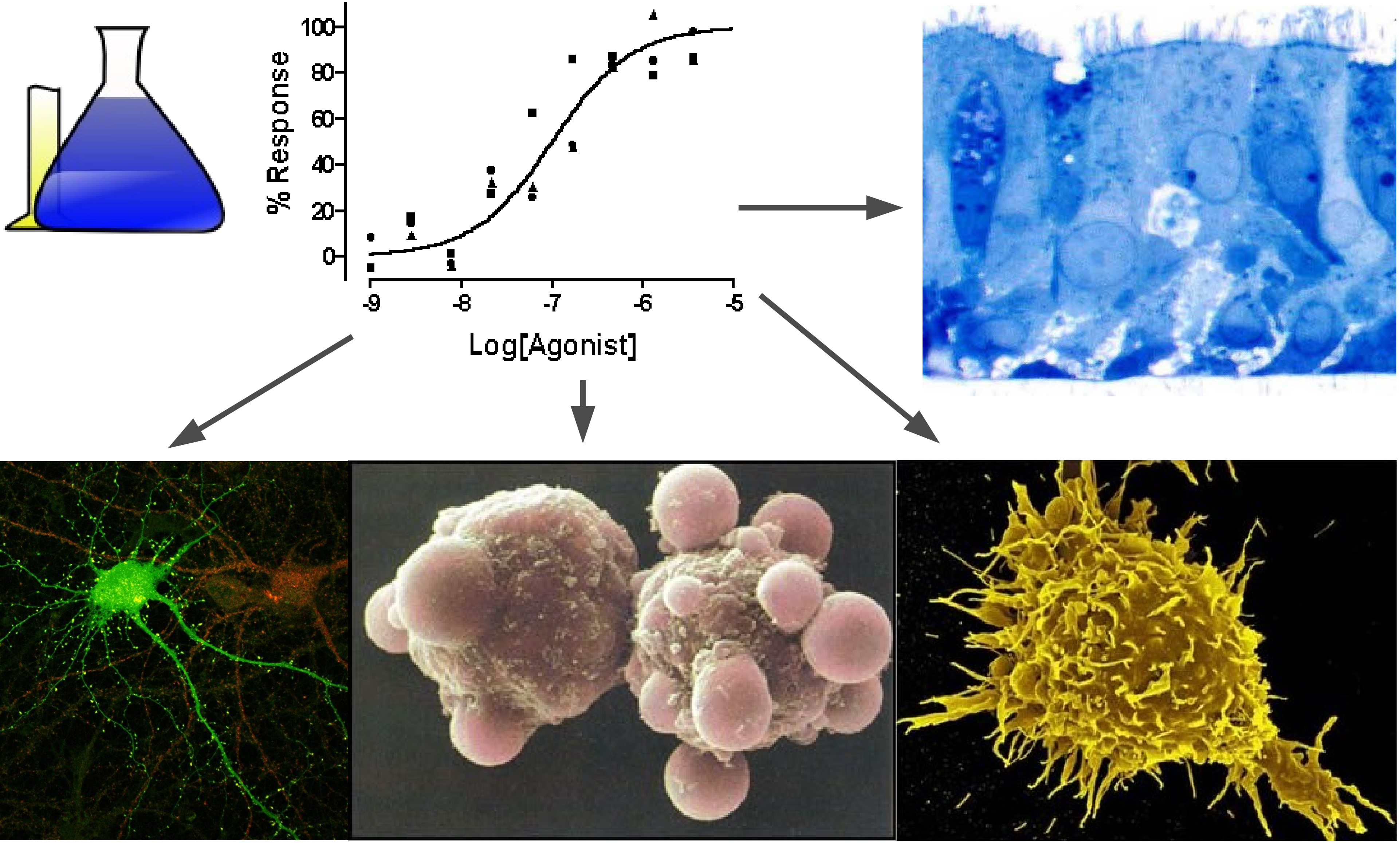
published dose-response experiments

Concentration span of a calcium spike in neurons

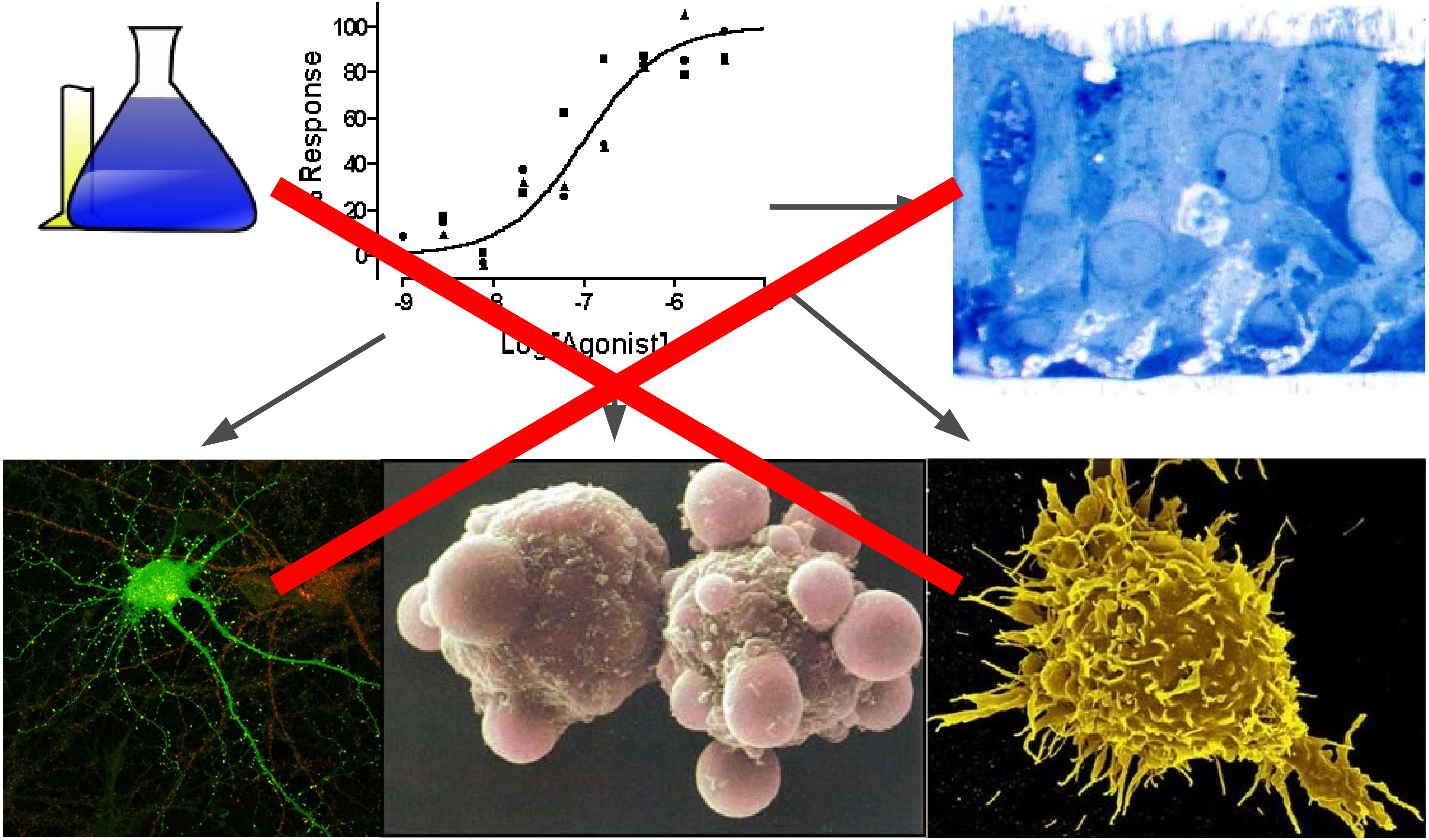
Ligand-depletion decreases effective cooperativity



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

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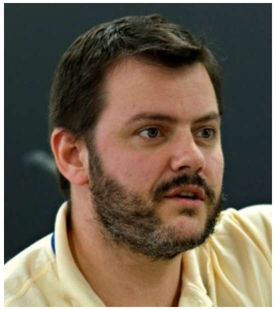
  - Stefan Hoops
  - Ursula Kummer
  - Pedro Mendes
- Developers of Scilab
- Annalisa Pastore
- Stephen Martins

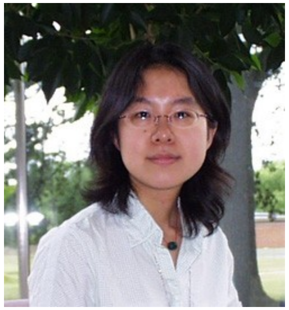
Melanie Stefan

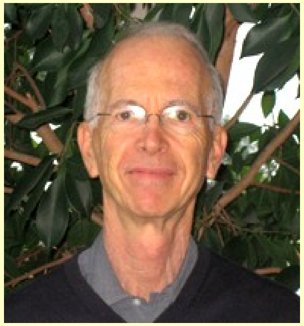
Stuart Edelstsein

Compneur group
Current members


Nicolas Le Novère
group leader


Melanie Stefan, postdoc


Lu Li, predoc


Stuart Edelstein, visitor


Benedetta Baldi, predoc


Michele Mattioni, predoc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