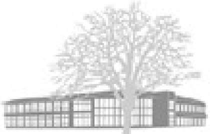
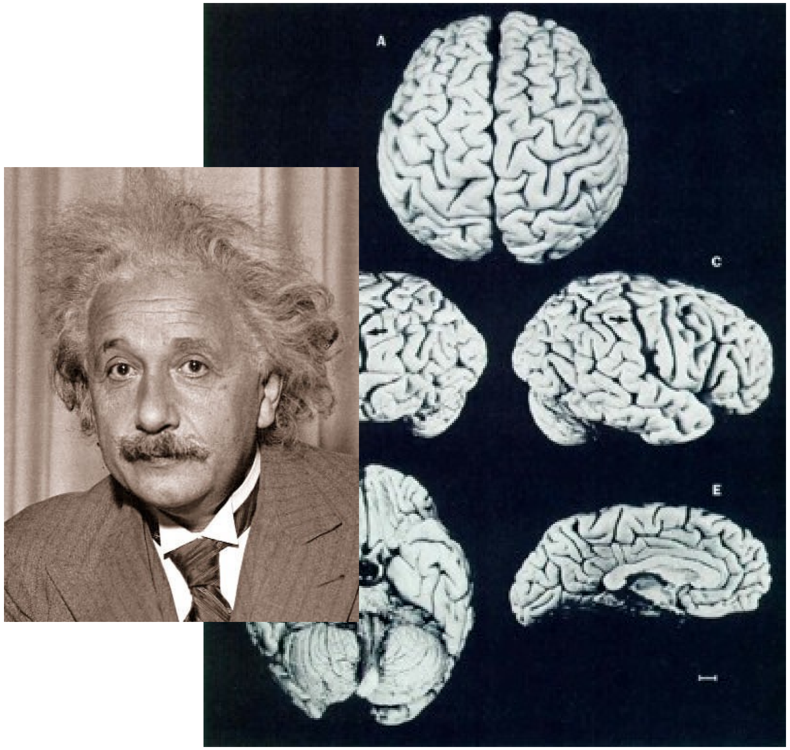
Remember or forget. Allosteric switches and molecular memory

Nicolas Le Novère, EMBL-EBI

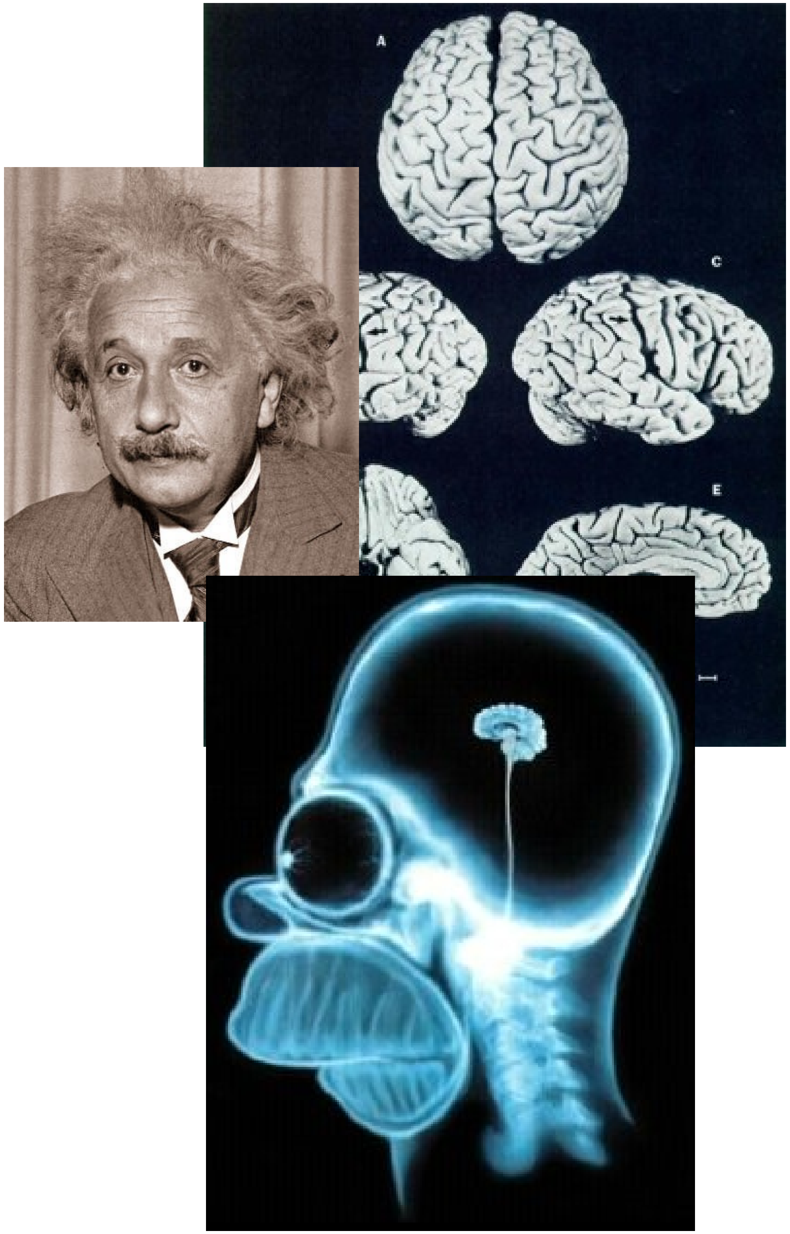
10th International EMBL PhD Student Symposium. Decision making in Biology
Heidelberg, 23-25 Oct 2008



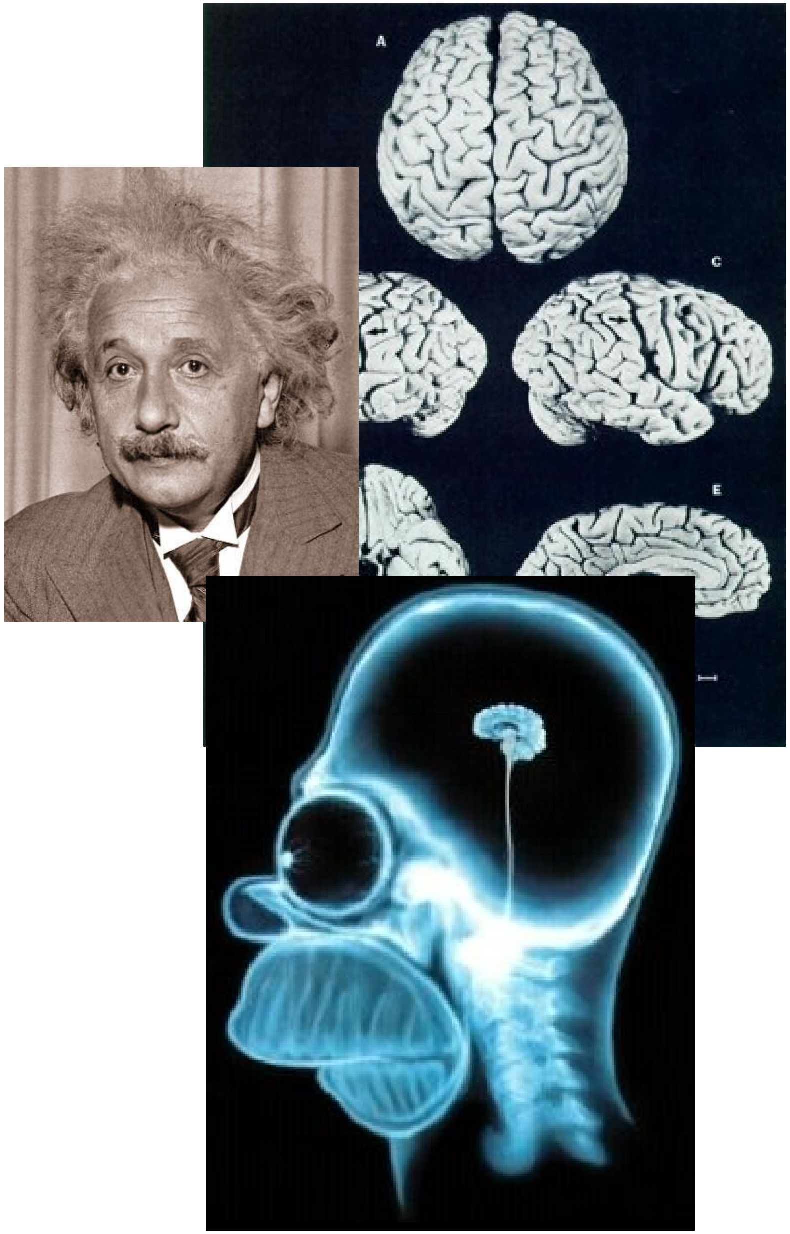
The brain

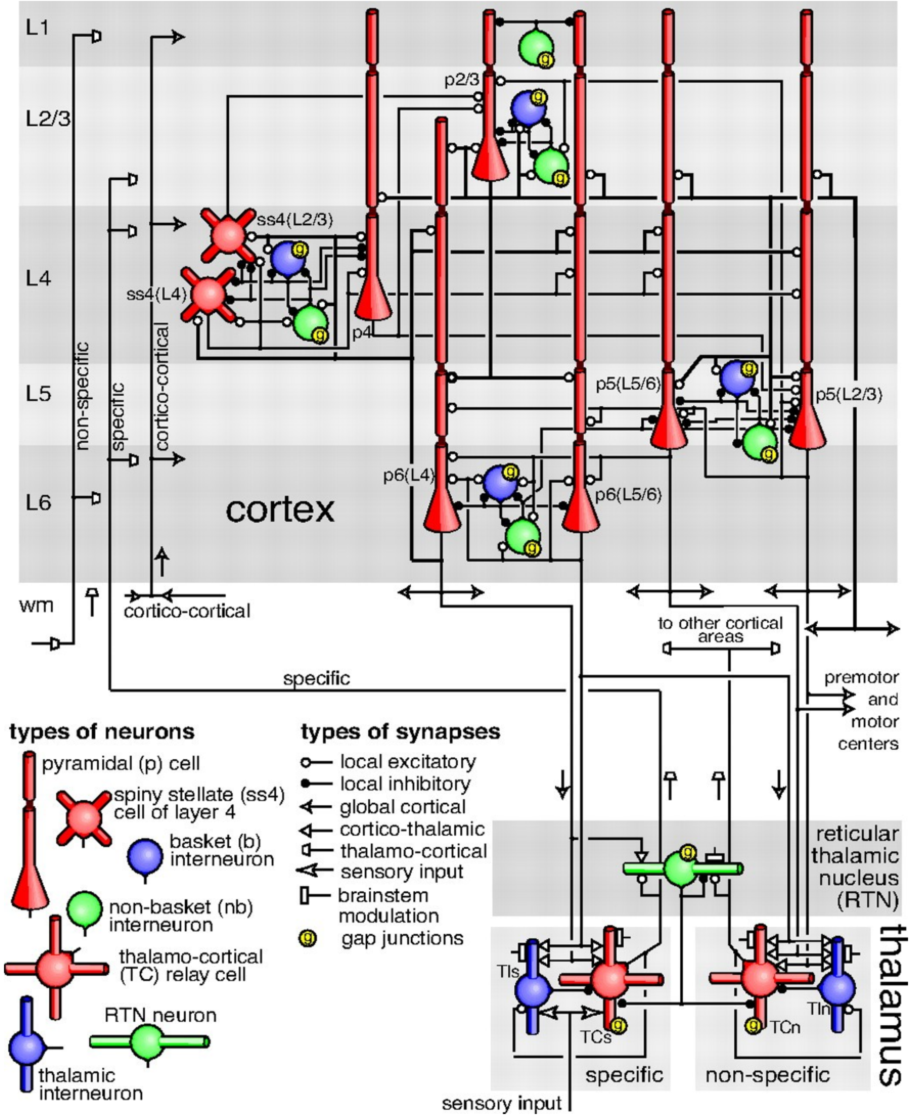


The brain



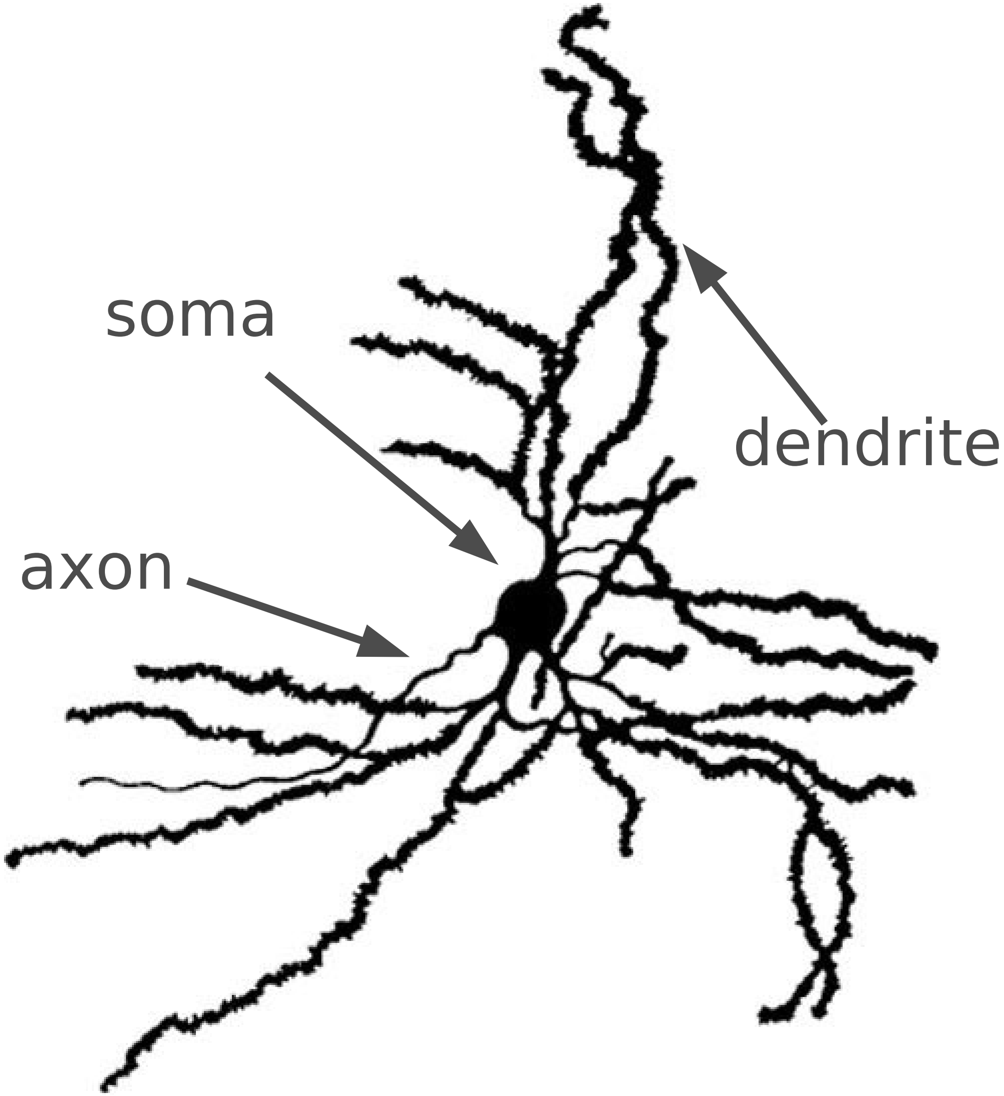
The brain

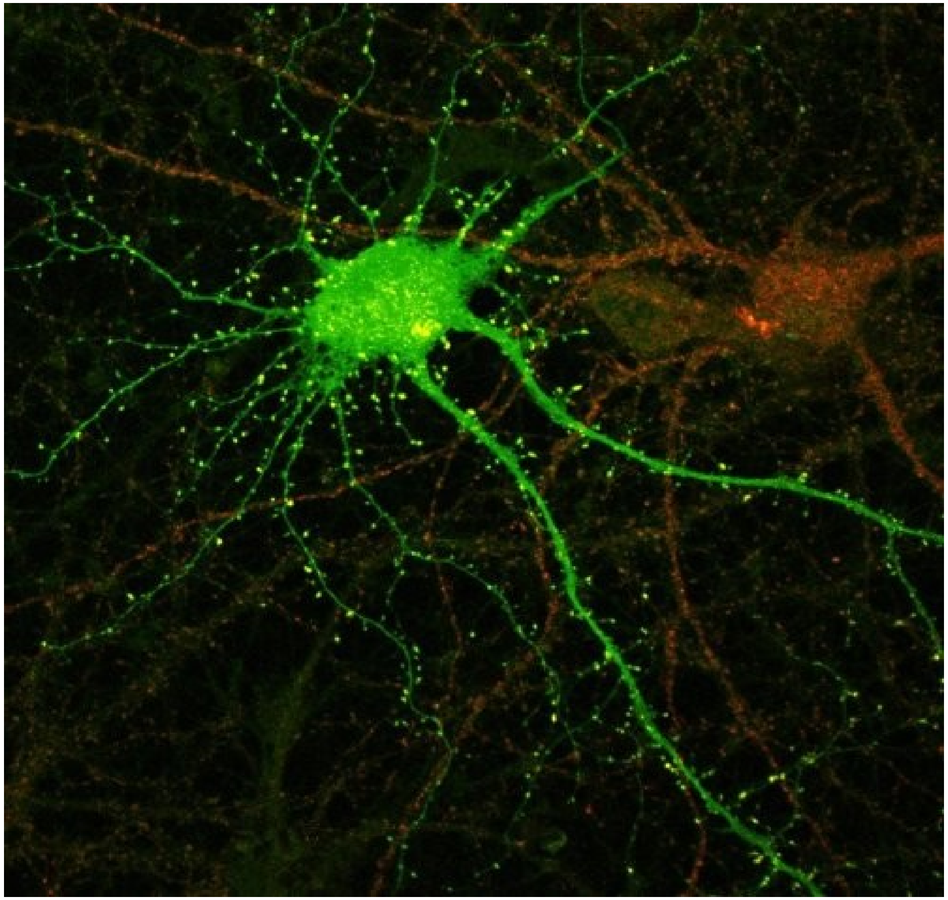




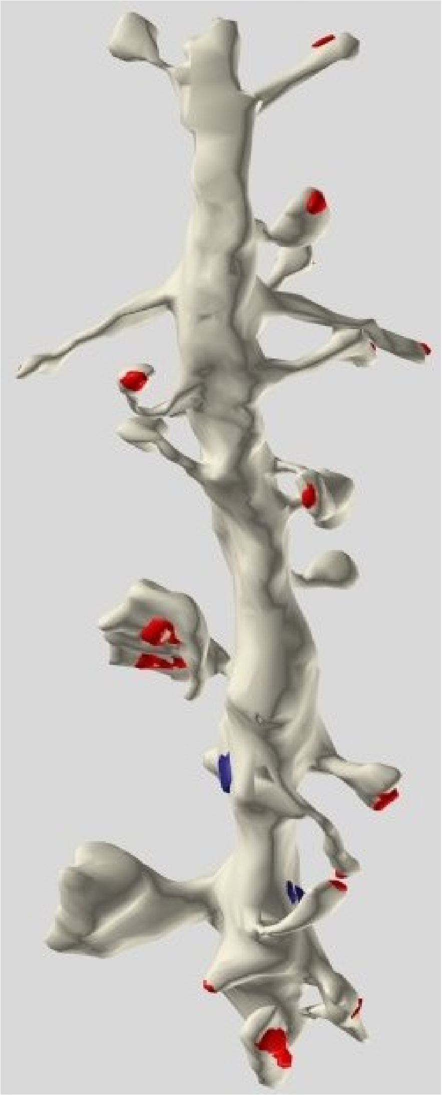
Izhikevich, Edelman (2008)
PNAS 105: 3593-3598

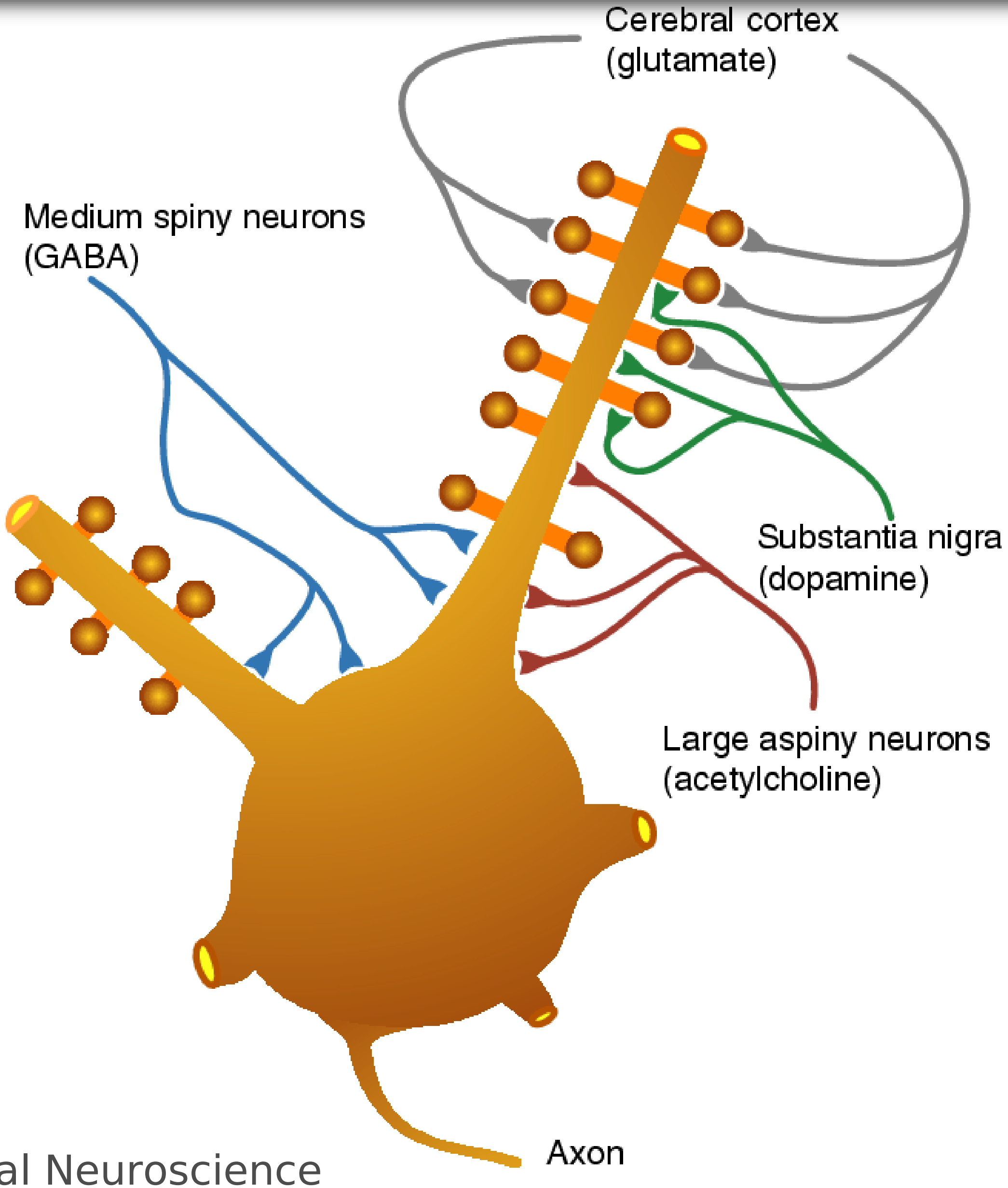
The neuron





The dendrite

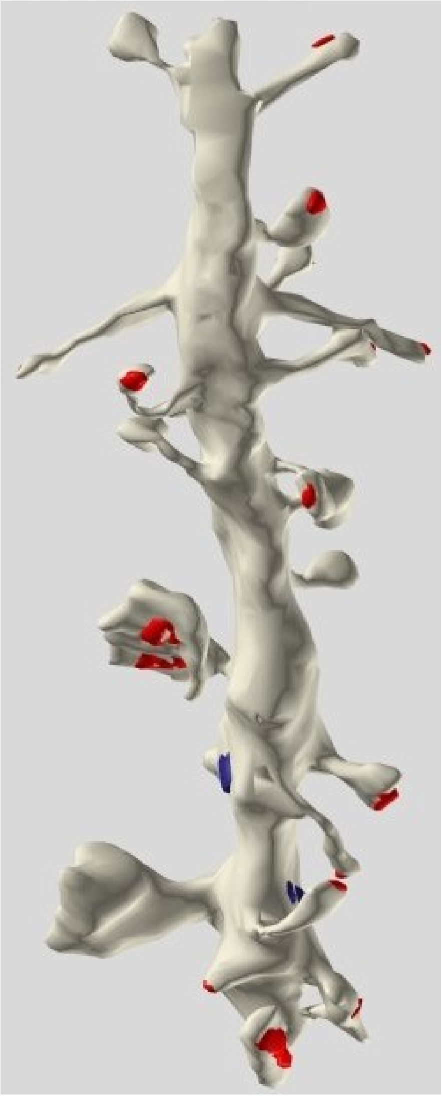


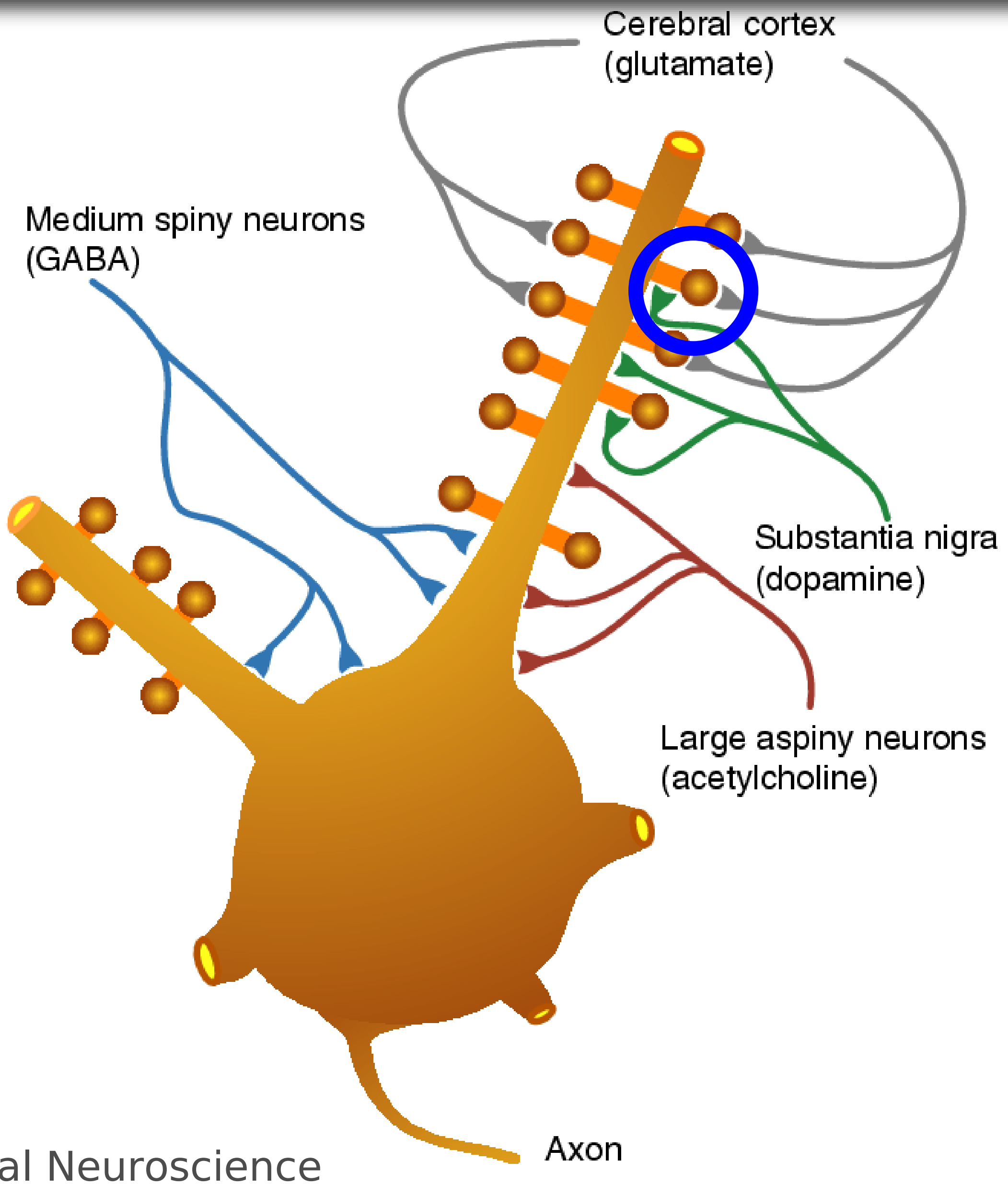


Fundamental Neuroscience
Squire et al. 2nd ed(2003)

EMBL-EBI

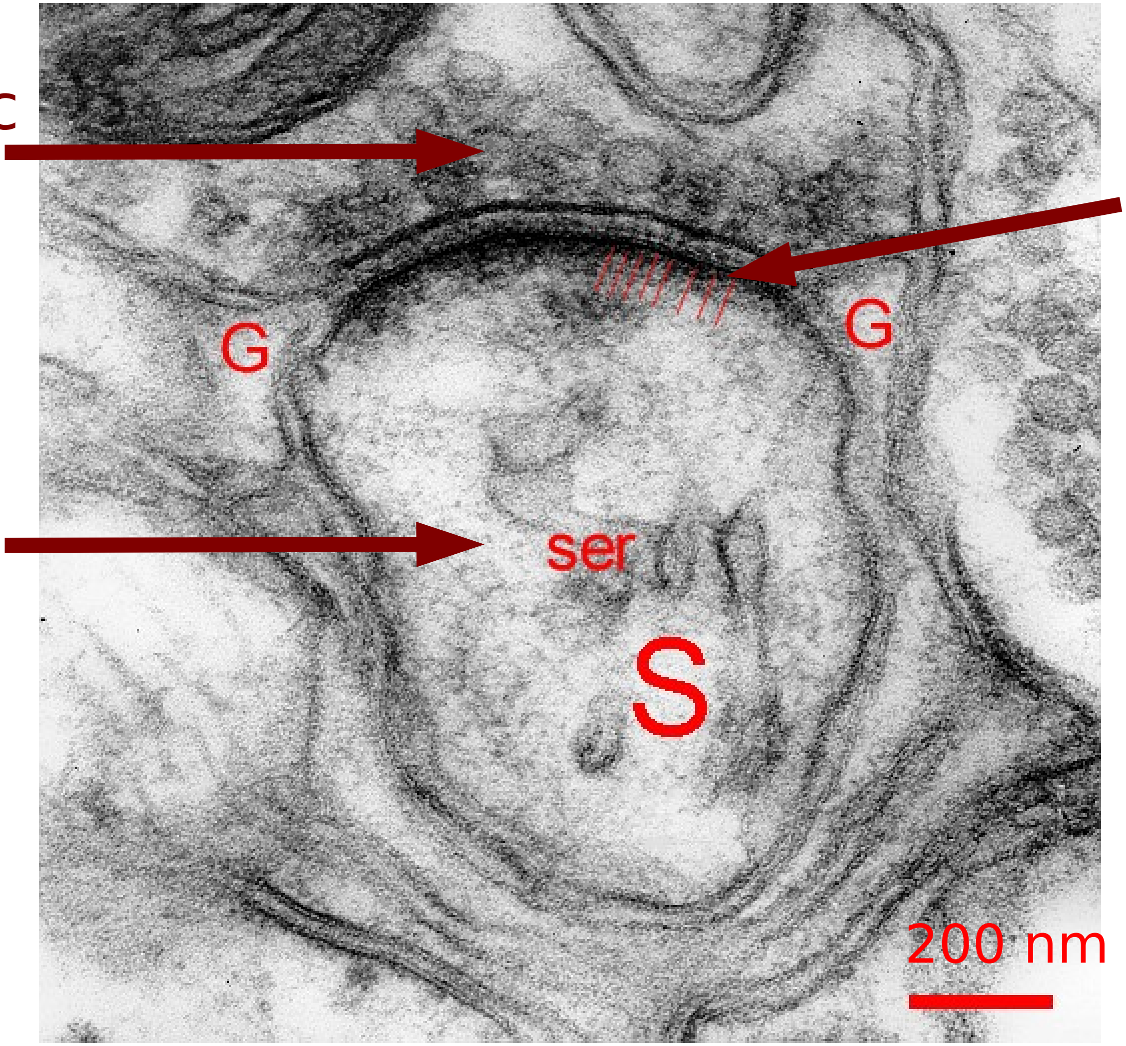
The dendrite





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

The spine



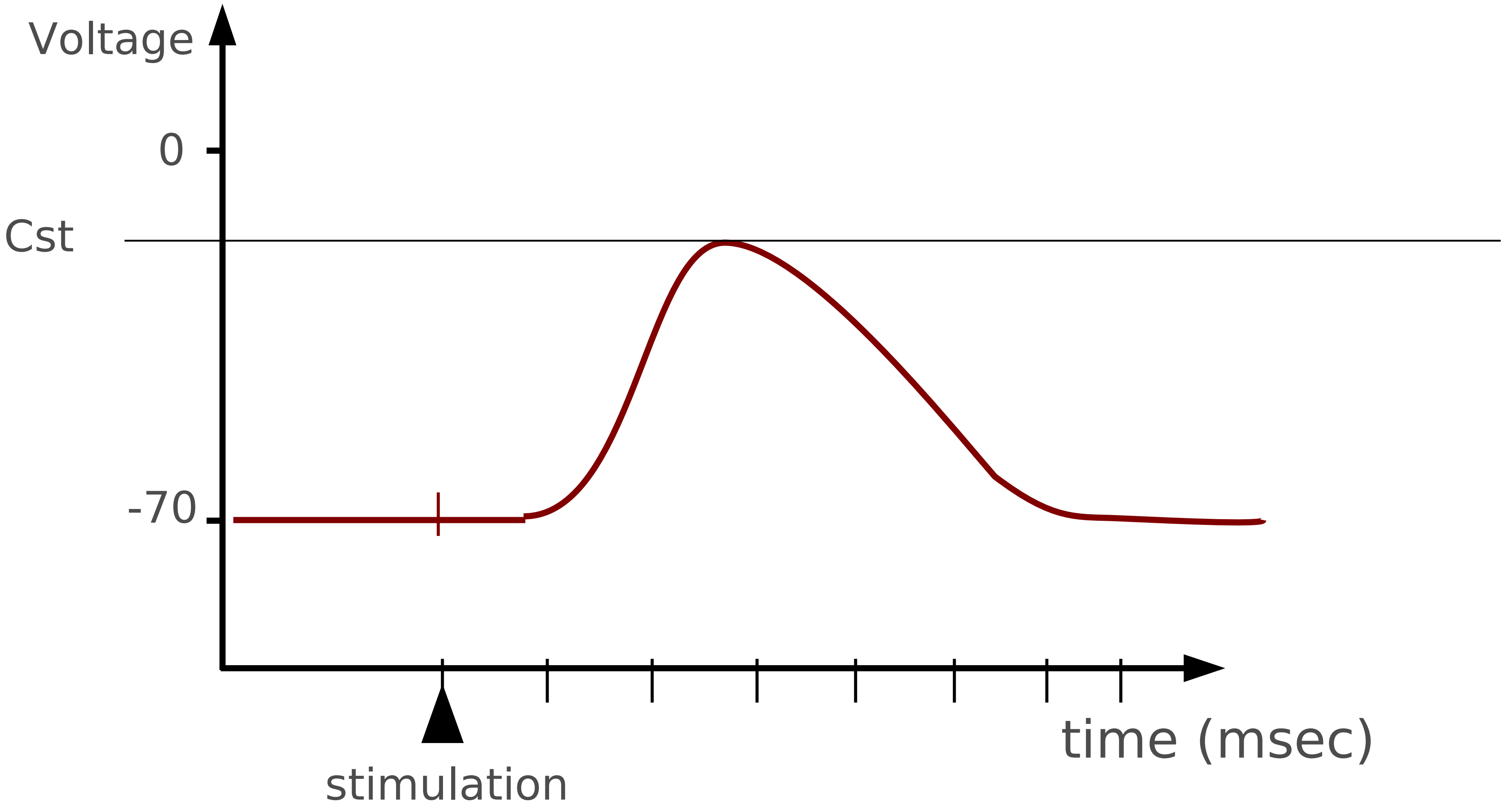
pre-synaptic neuron

post-synaptic density

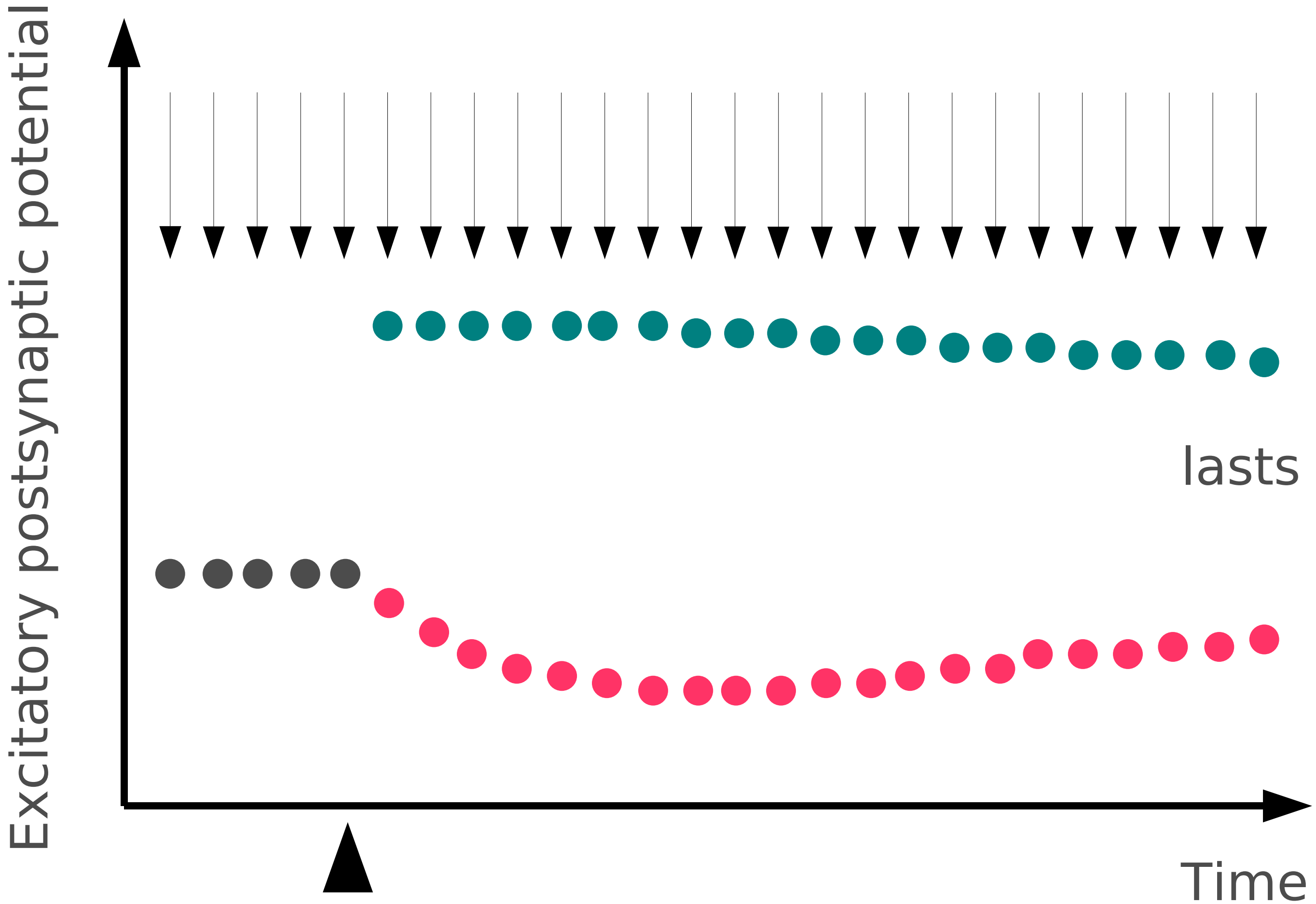
Dendritic spine

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

Excitatory post-synaptic potential

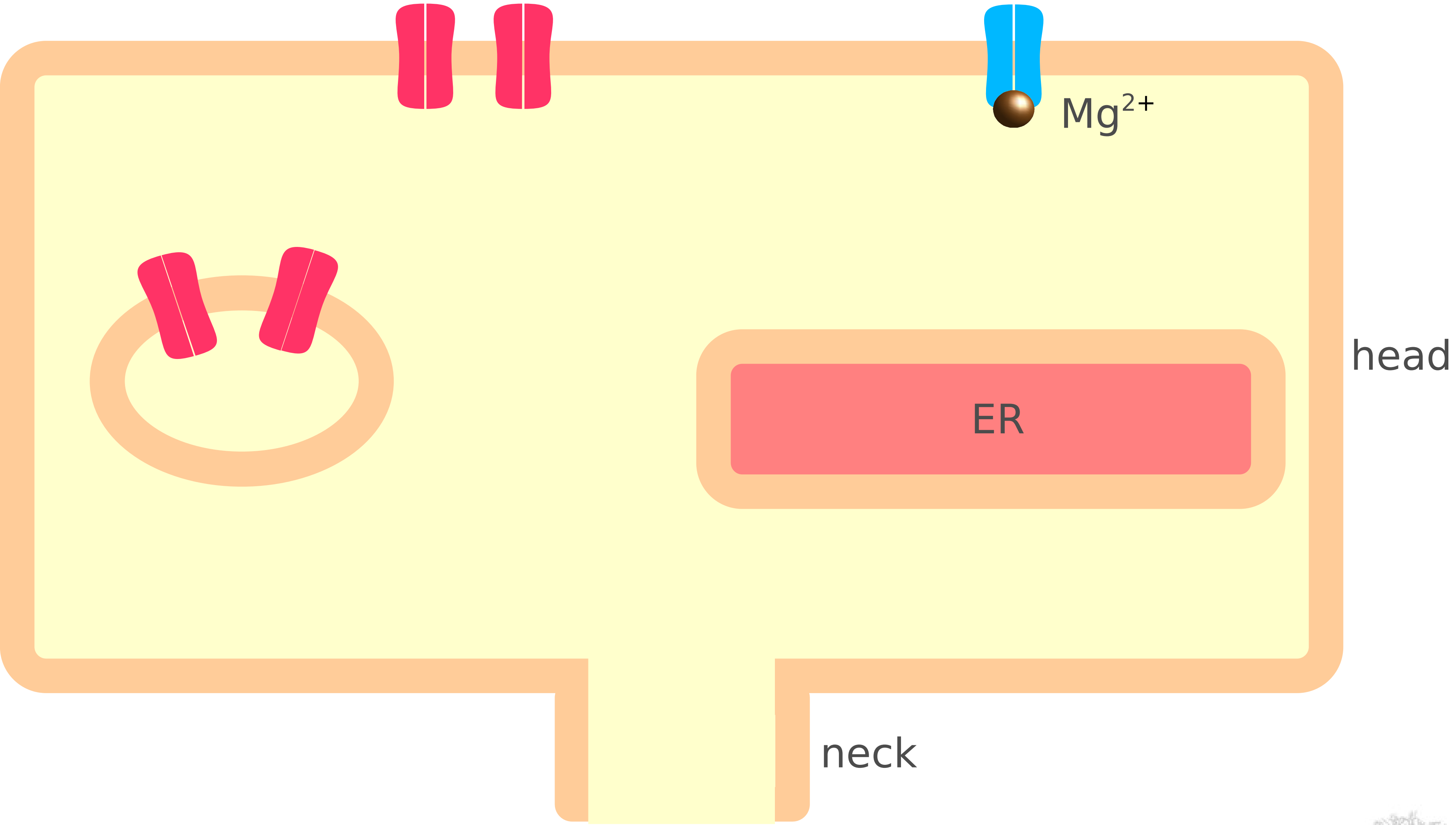


Bidirectional synaptic plasticity

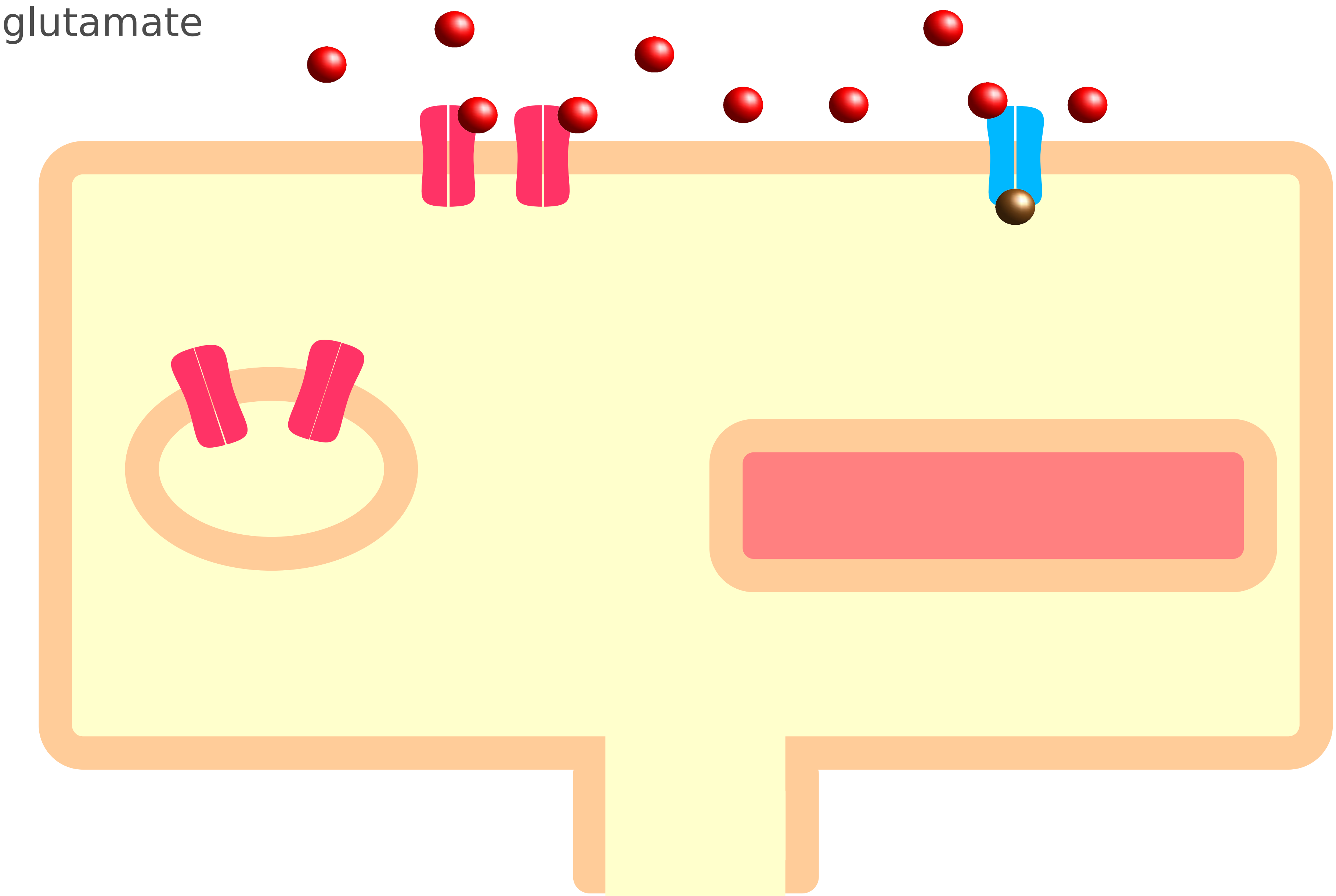


High frequency stimulation
Low frequency stimulation

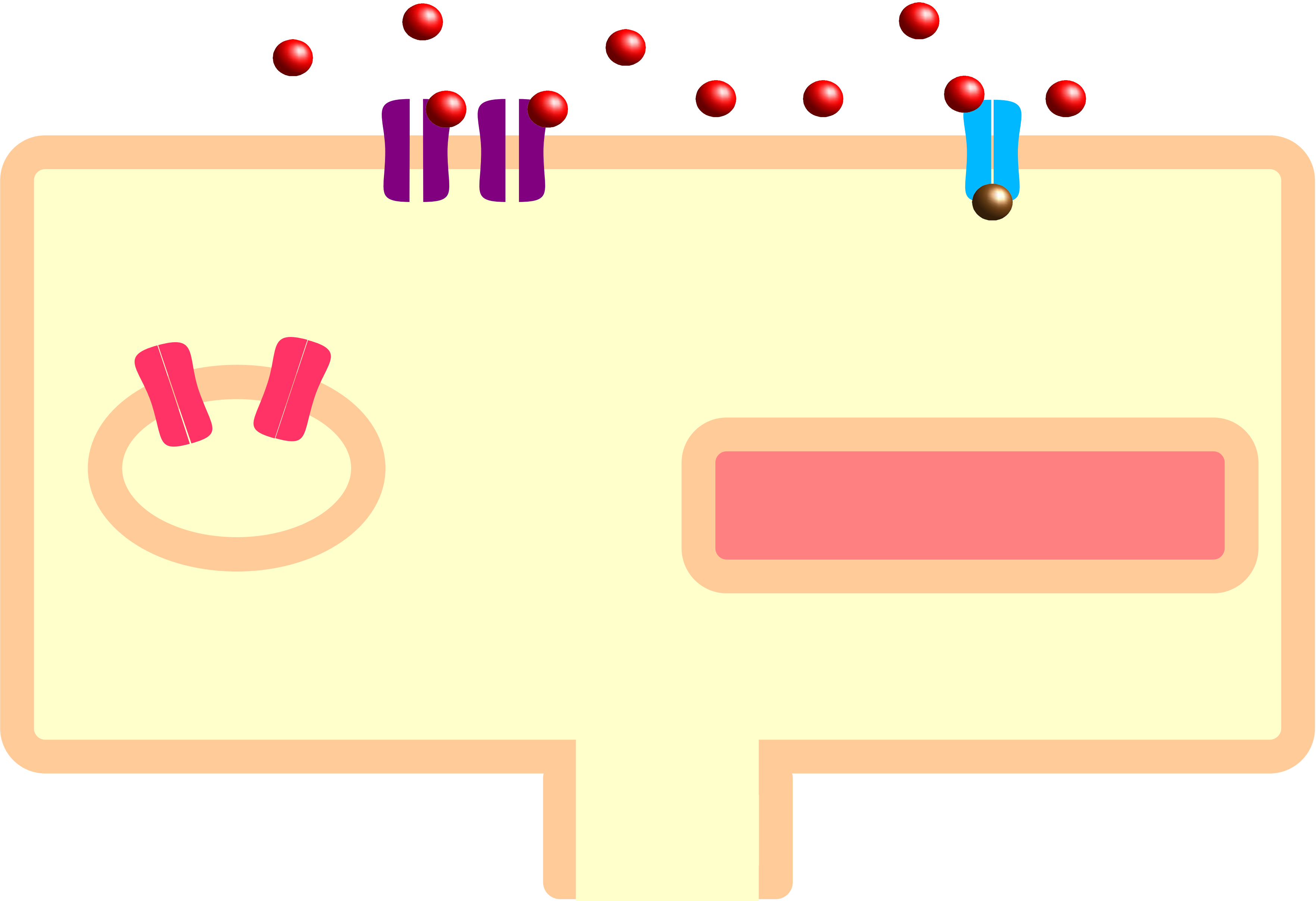
Signalling at the dendritic spine



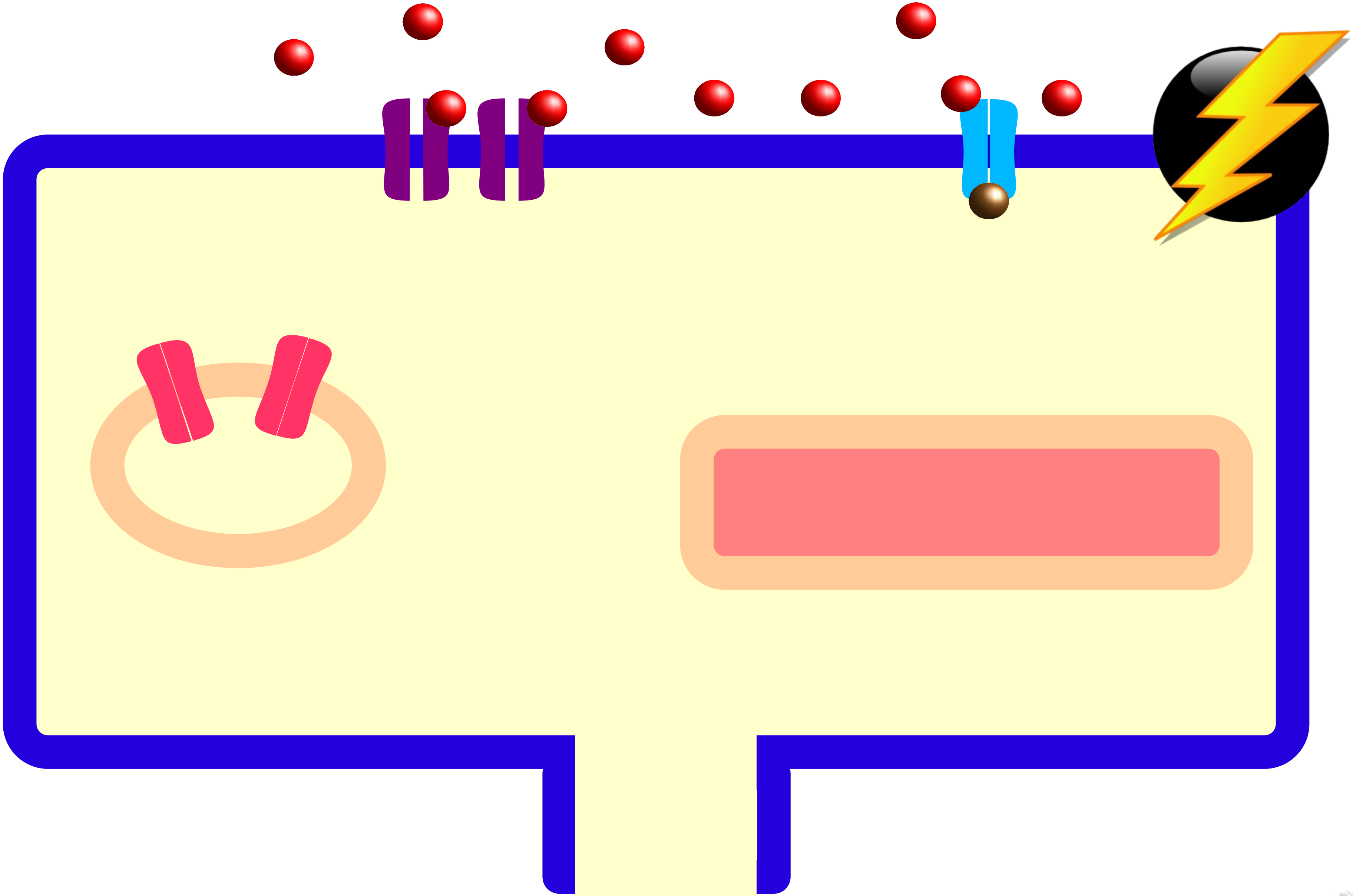
Signalling at the dendritic spine



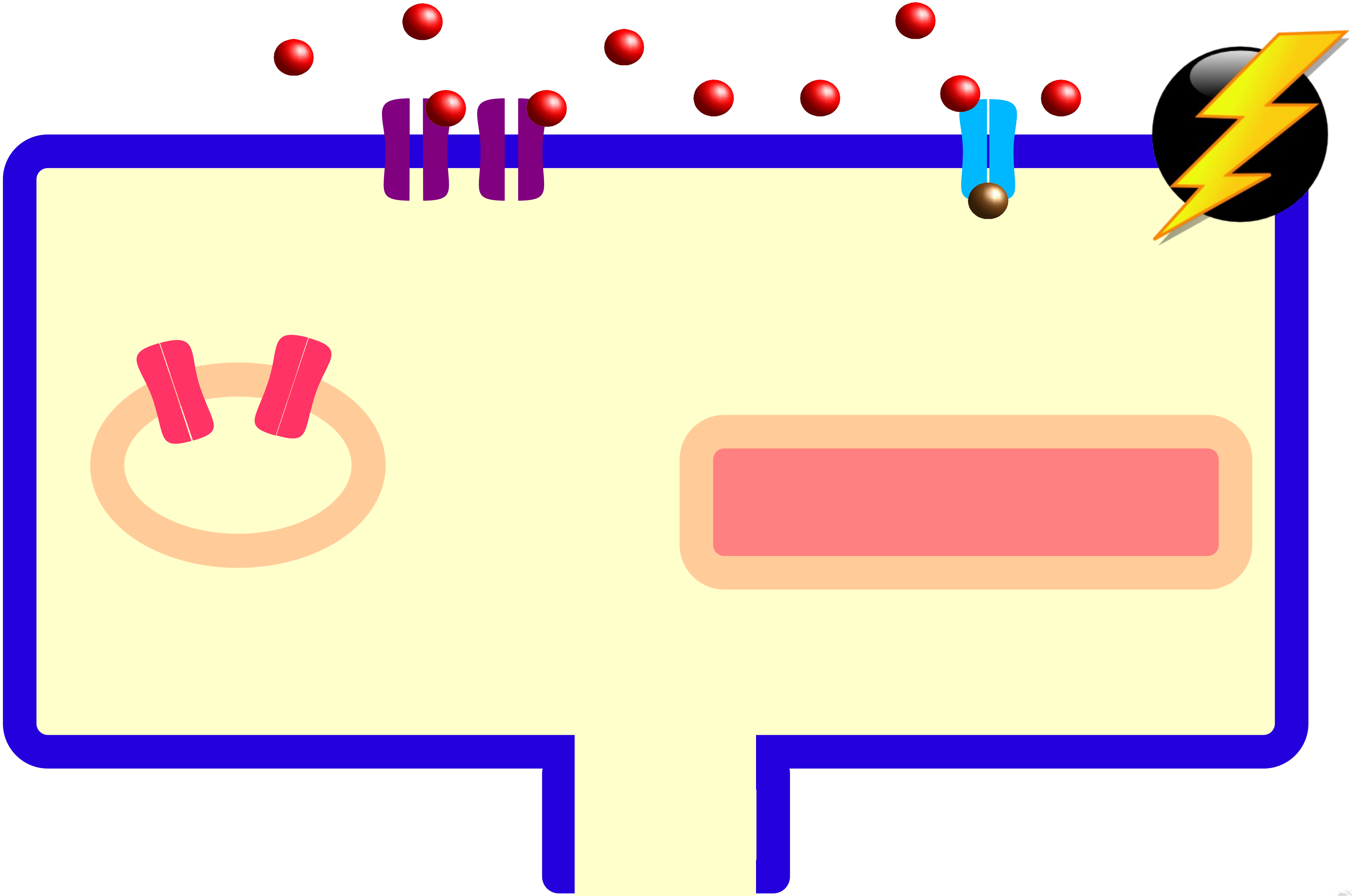
Signalling at the dendritic spine



Signalling at the dendritic spine

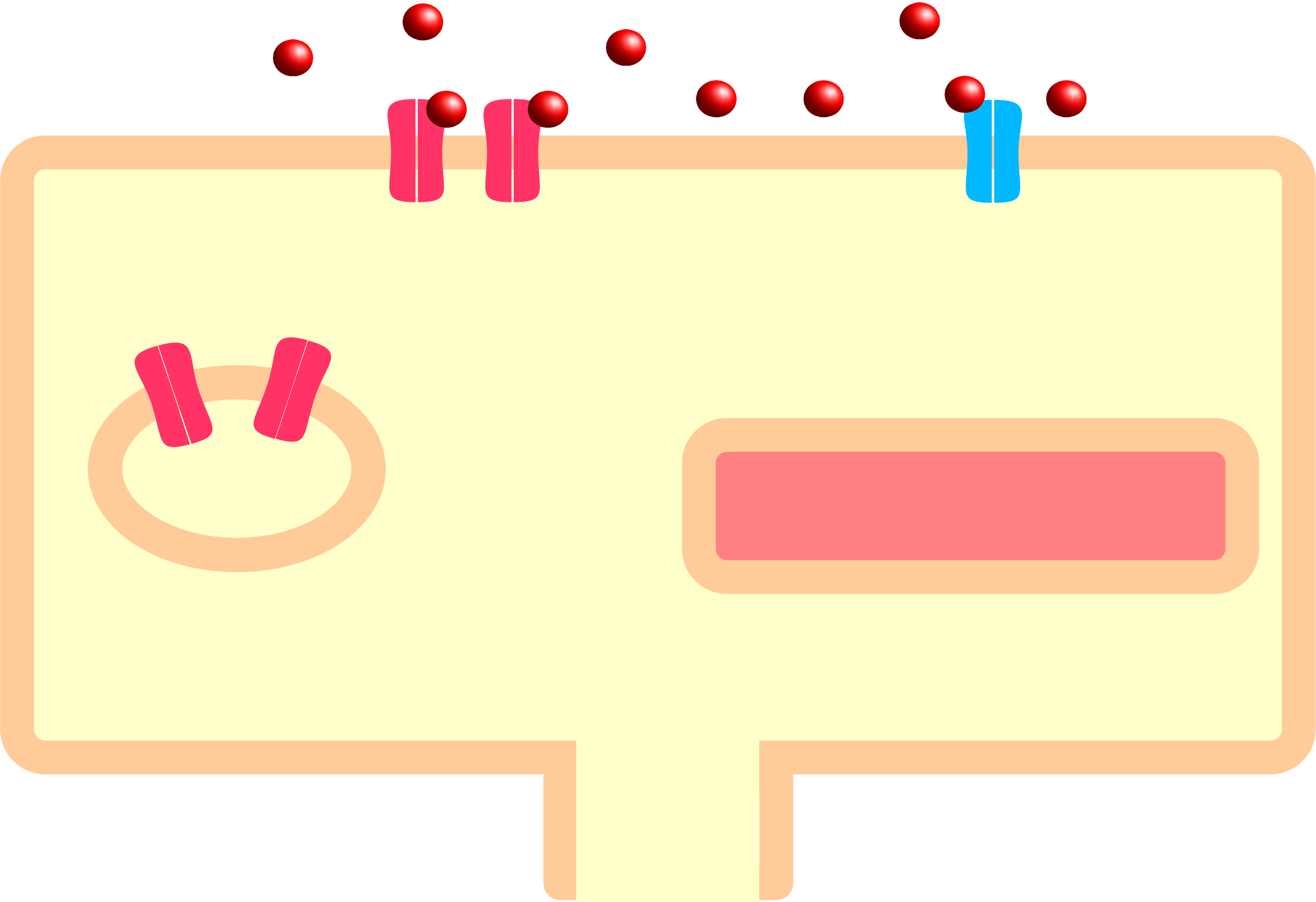


Signalling at the dendritic spine



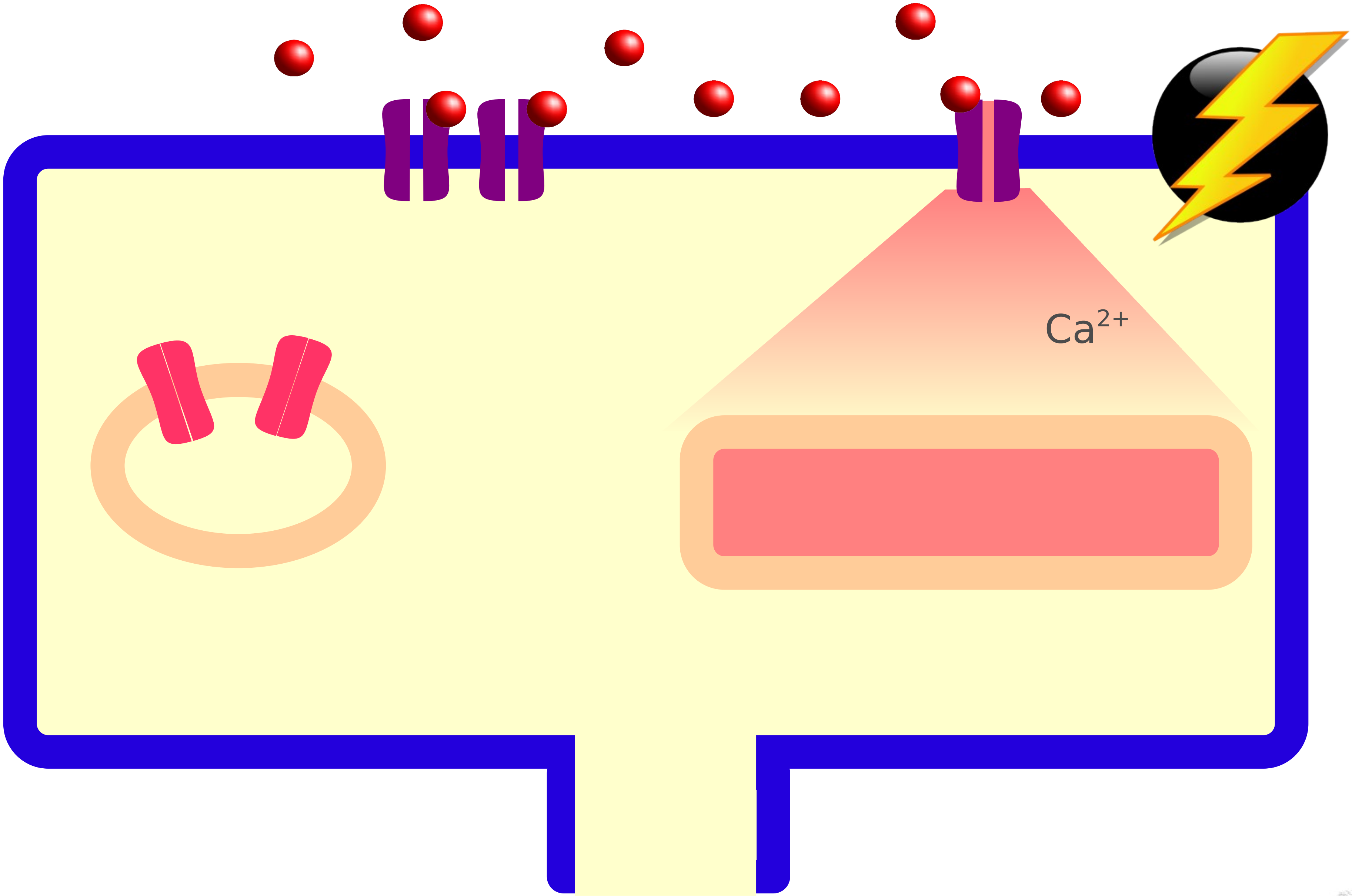
EMBL-EBI

Signalling at the dendritic spine

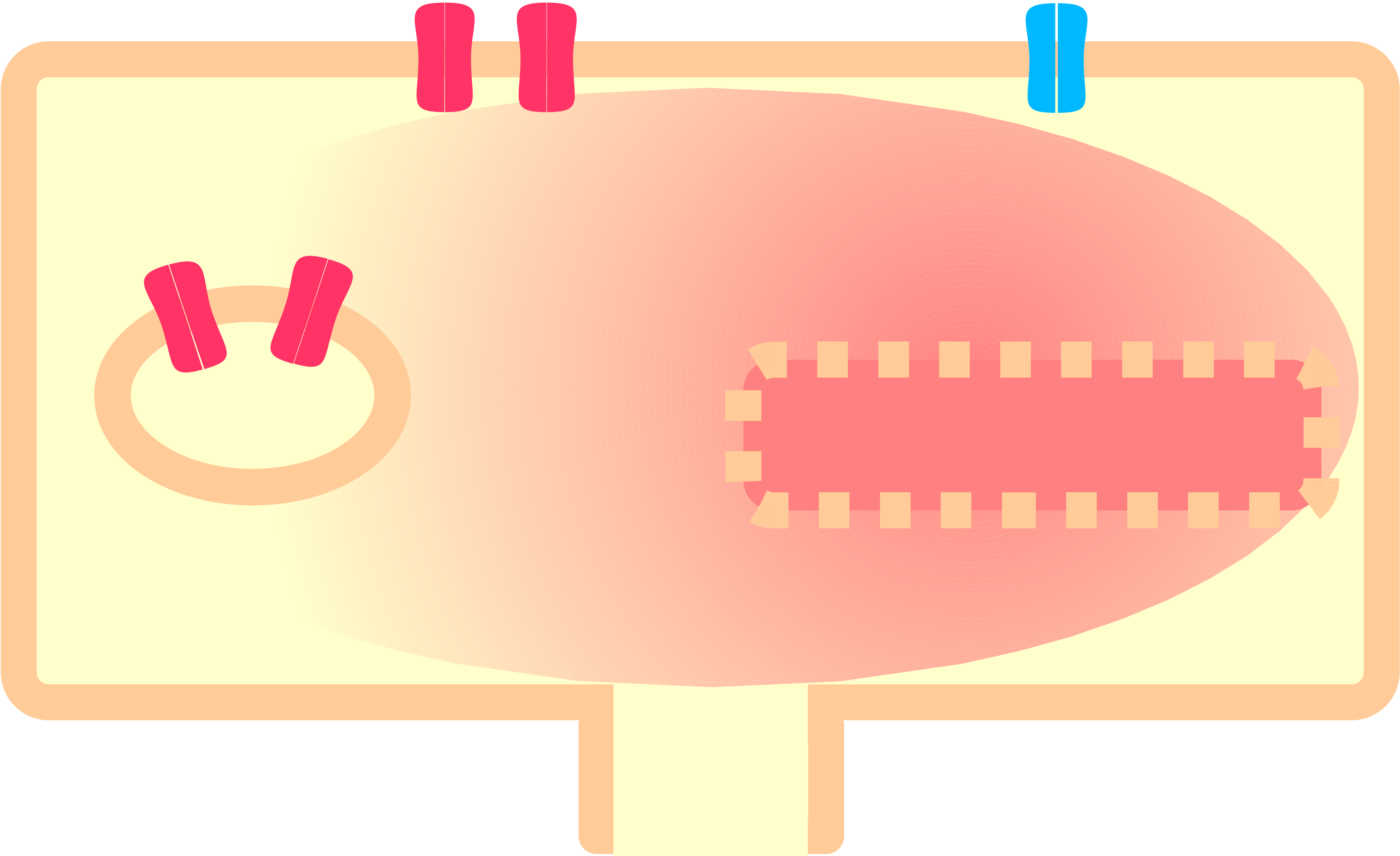


10th International EMBL PhD Student Symposium. Decision making in Biology
Heidelberg, 23-25 Oct 2008

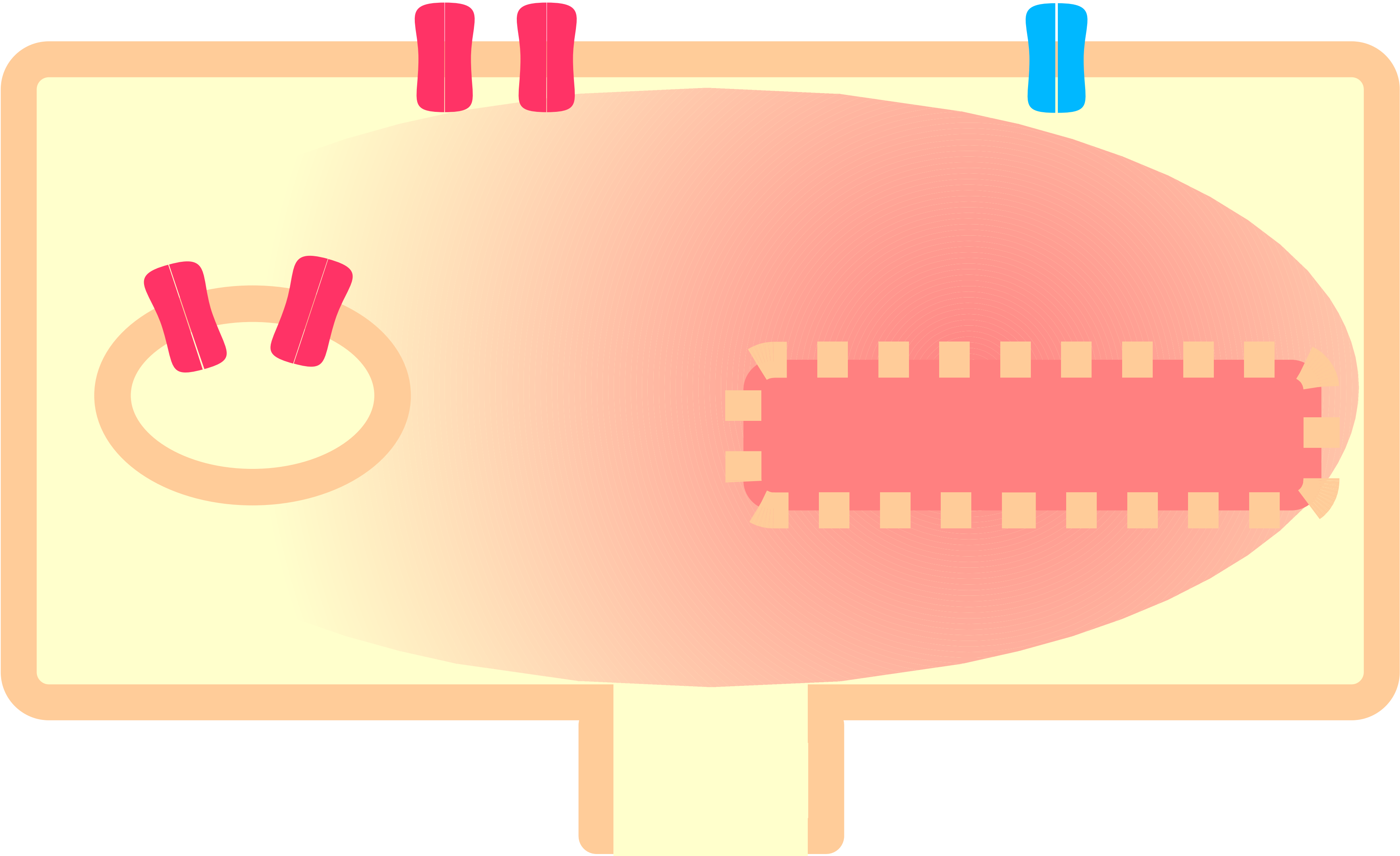
Signalling at the dendritic spine



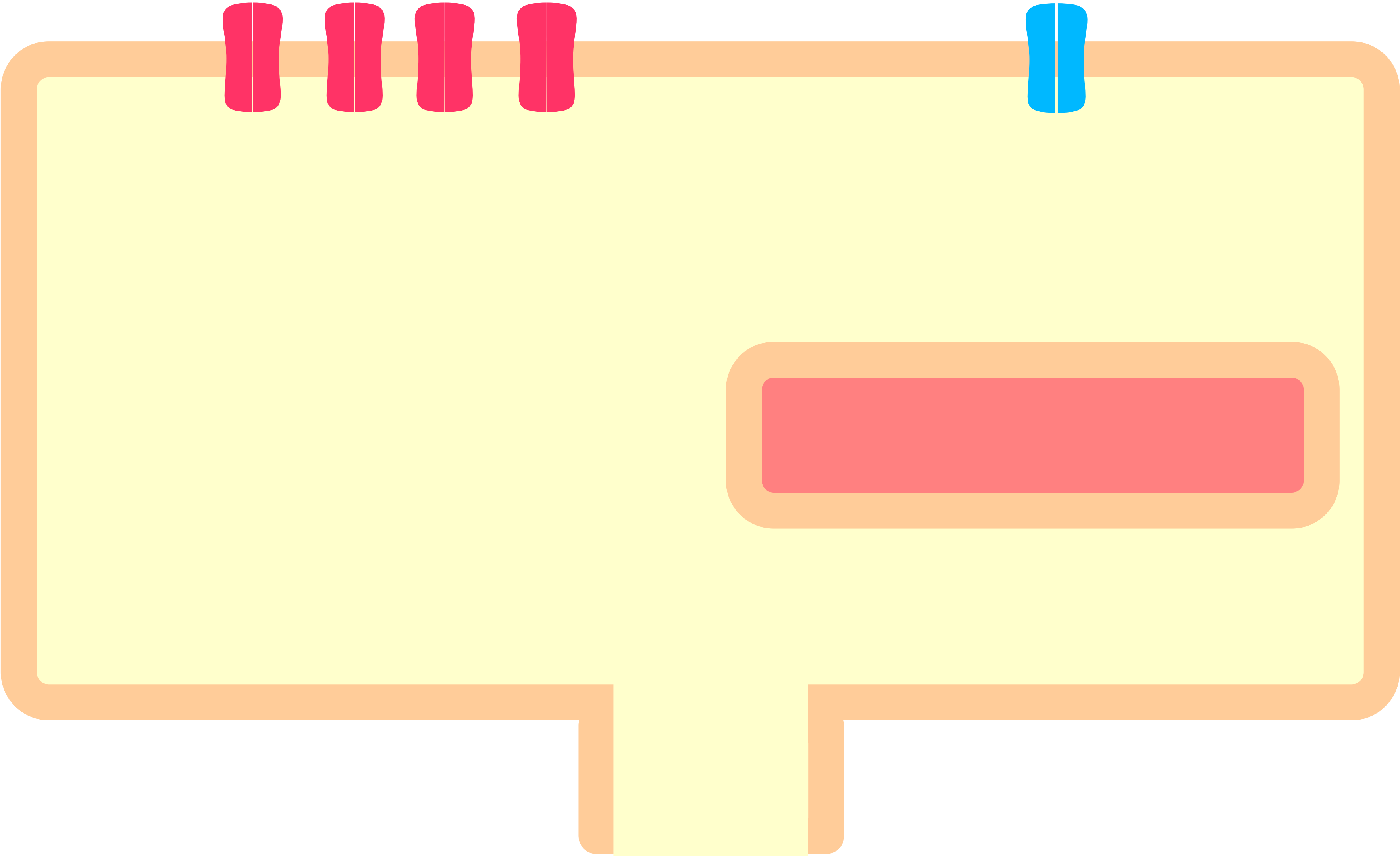
Signalling at the dendritic spine



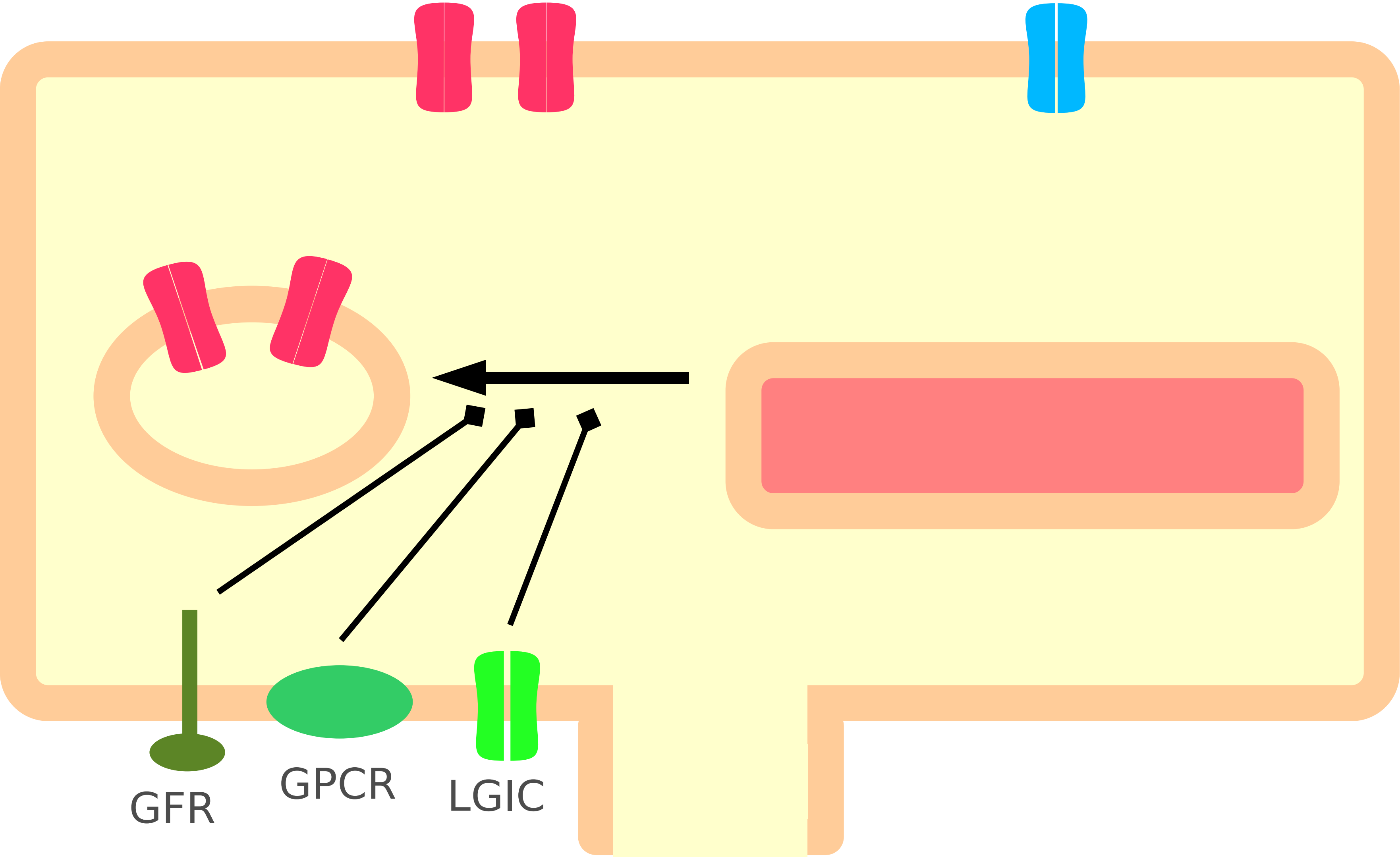
Signalling at the dendritic spine



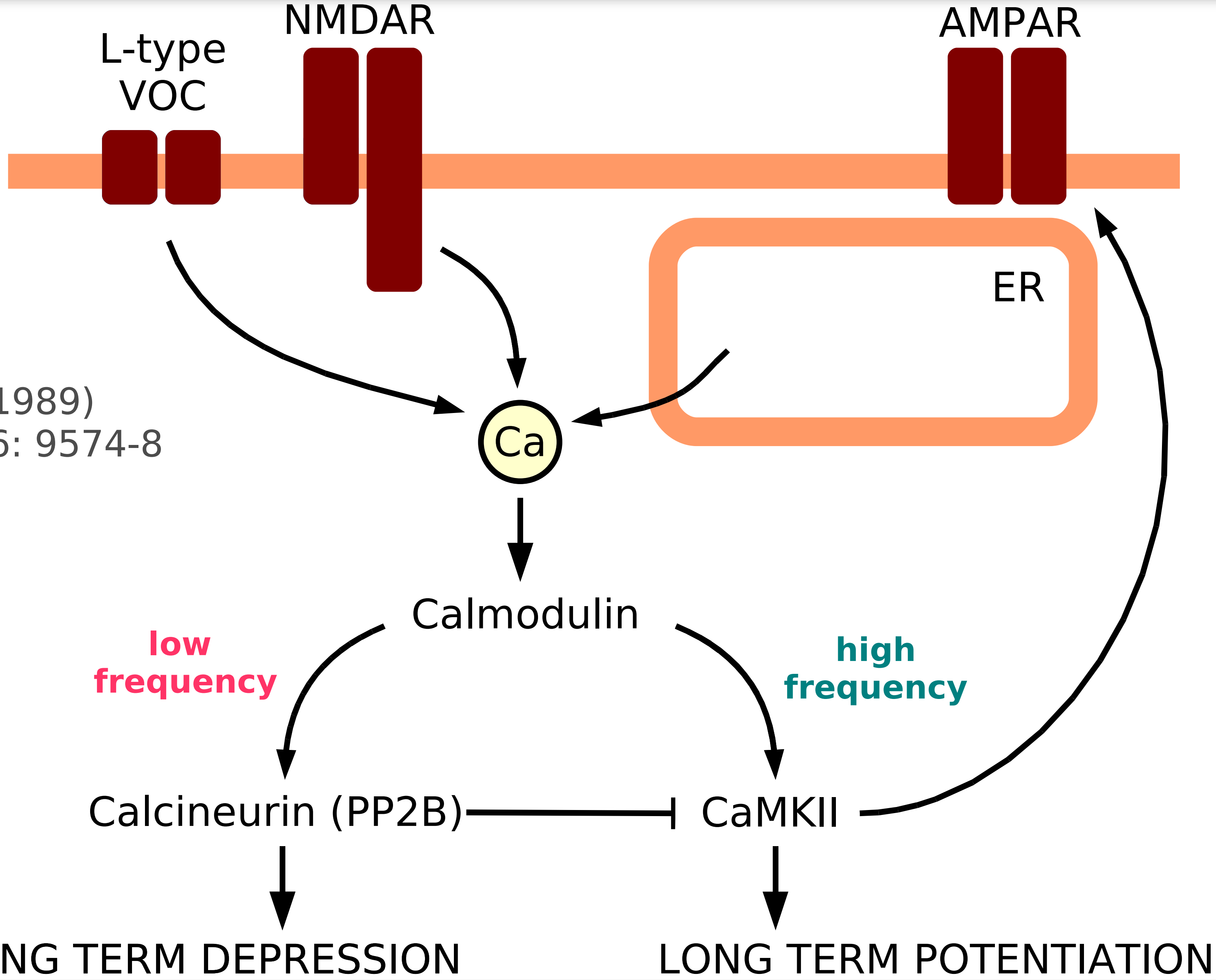
Signalling at the dendritic spine



Signalling at the dendritic spine

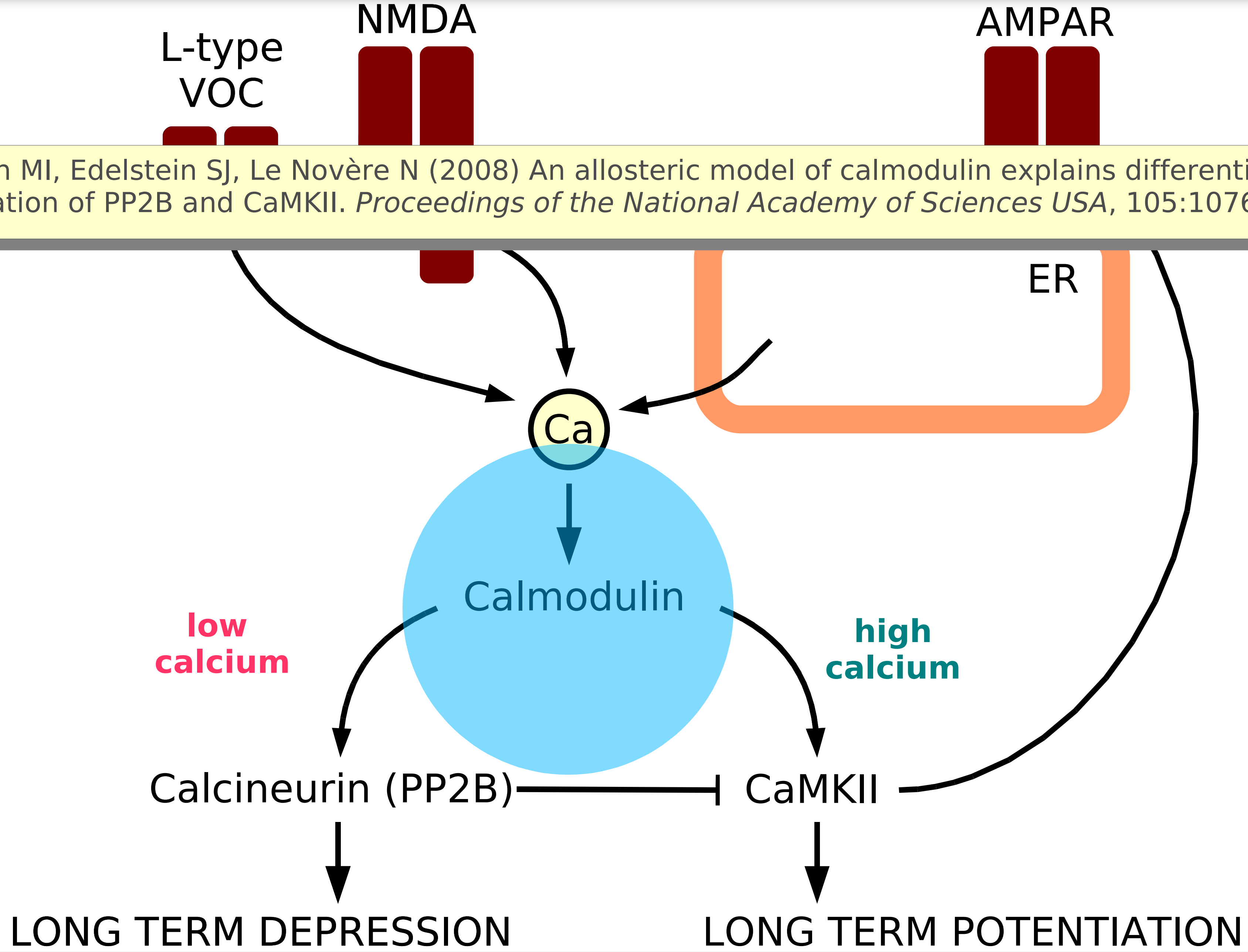


Calcium theory of Synaptic Plasticity



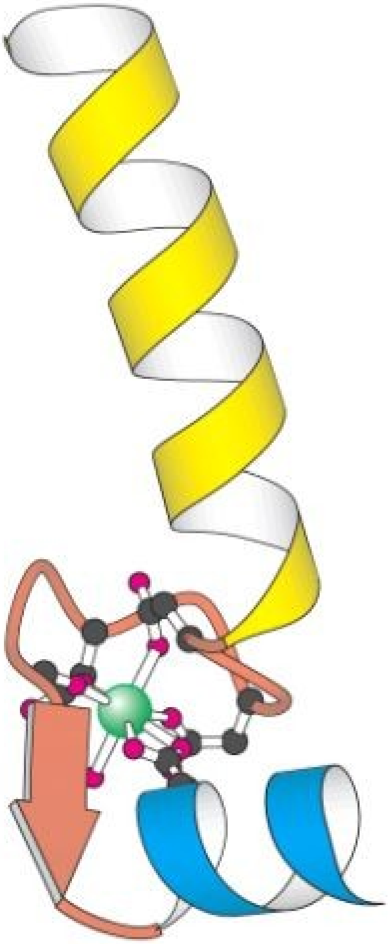
Lisman (1989)
PNAS, 86: 9574-8

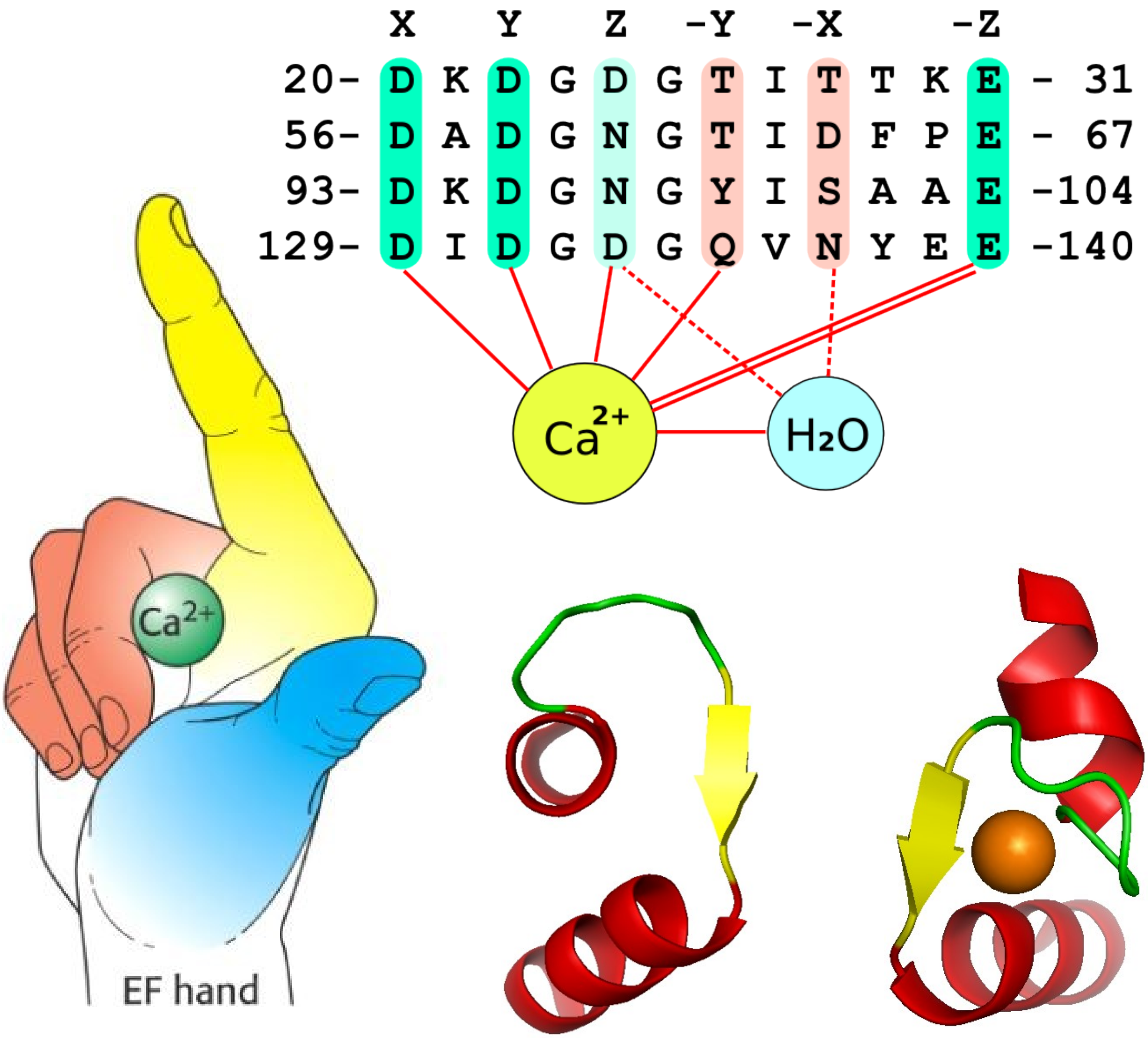
Calmodulin, the memory switch



Stefan MI, Edelstein SJ, Le Novère N (2008) An allosteric model of calmodulin explains differential activation of PP2B and CaMKII. *Proceedings of the National Academy of Sciences USA*, 105:10768-10773

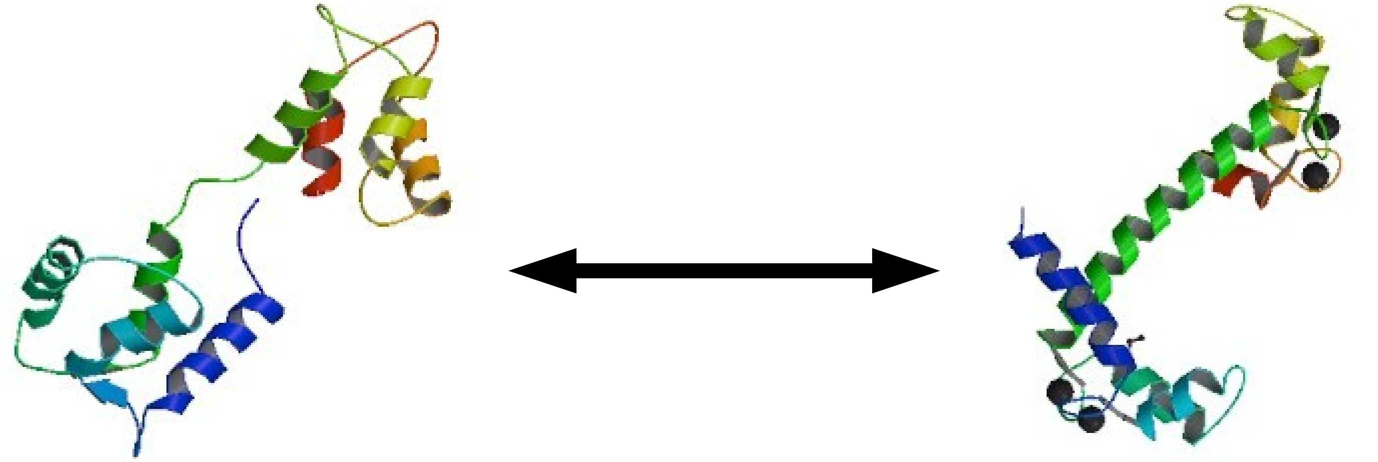
Structure of Calmodulin



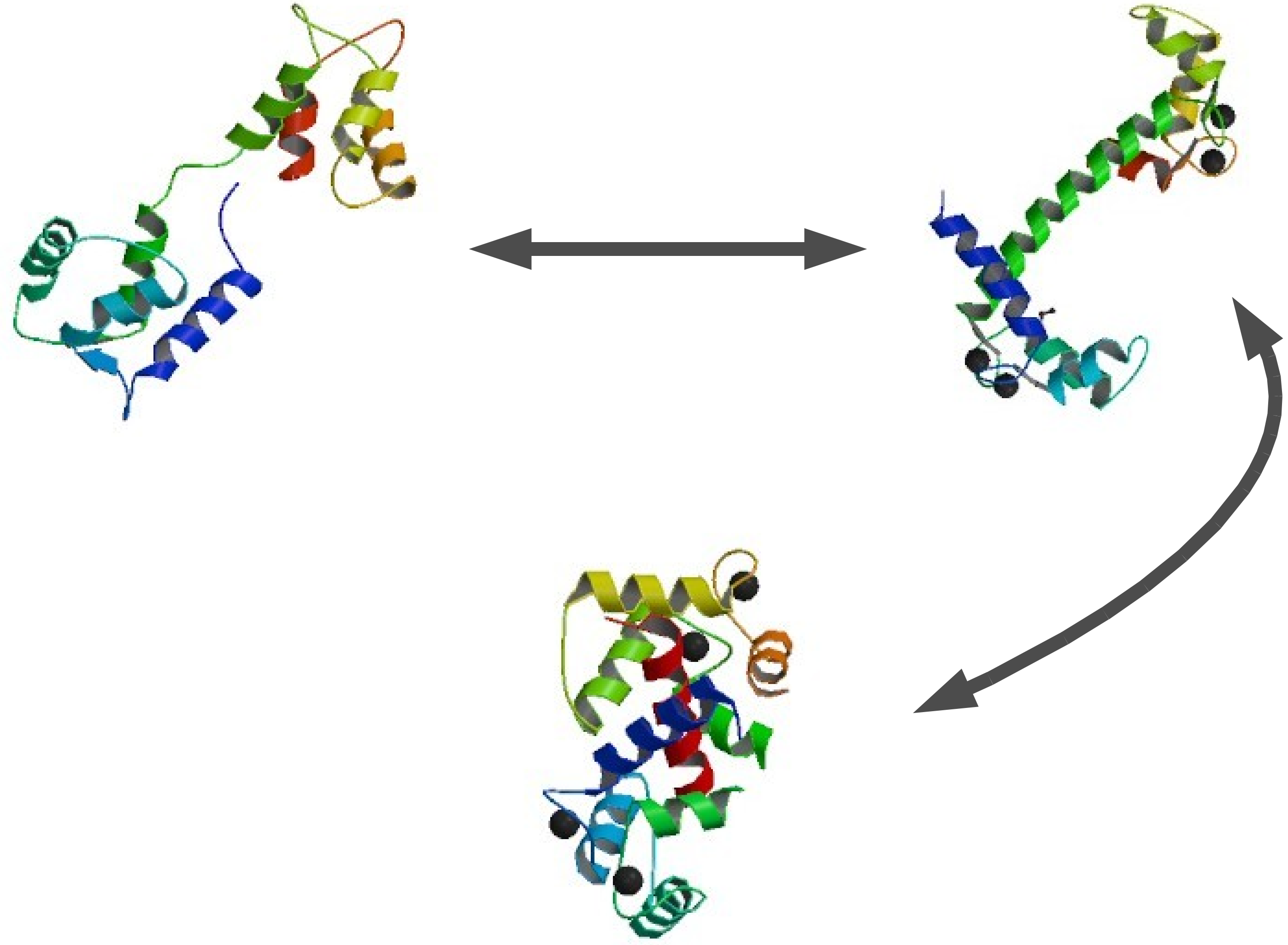


	X		Y		Z		-Y		-X			-Z	
20-	D	K	D	G	D	G	T	I	T	T	K	E	- 31
56-	D	A	D	G	N	G	T	I	D	F	P	E	- 67
93-	D	K	D	G	N	G	Y	I	S	A	A	E	-104
129-	D	I	D	G	D	G	Q	V	N	Y	E	E	-140

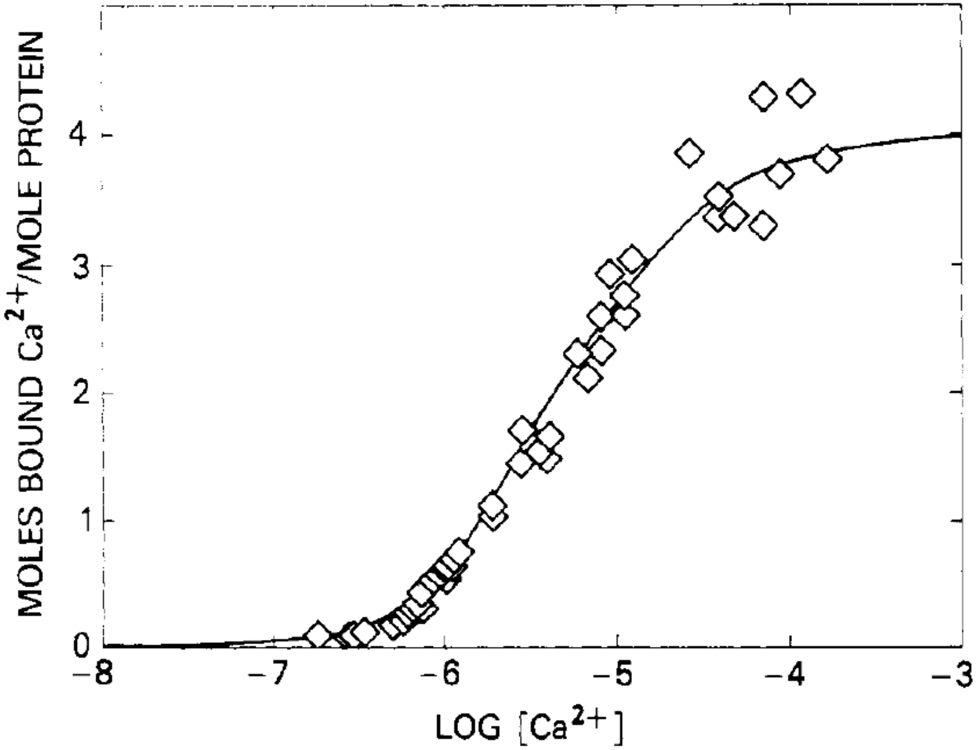
State transitions of calmodulin



State transistions of calmodulin

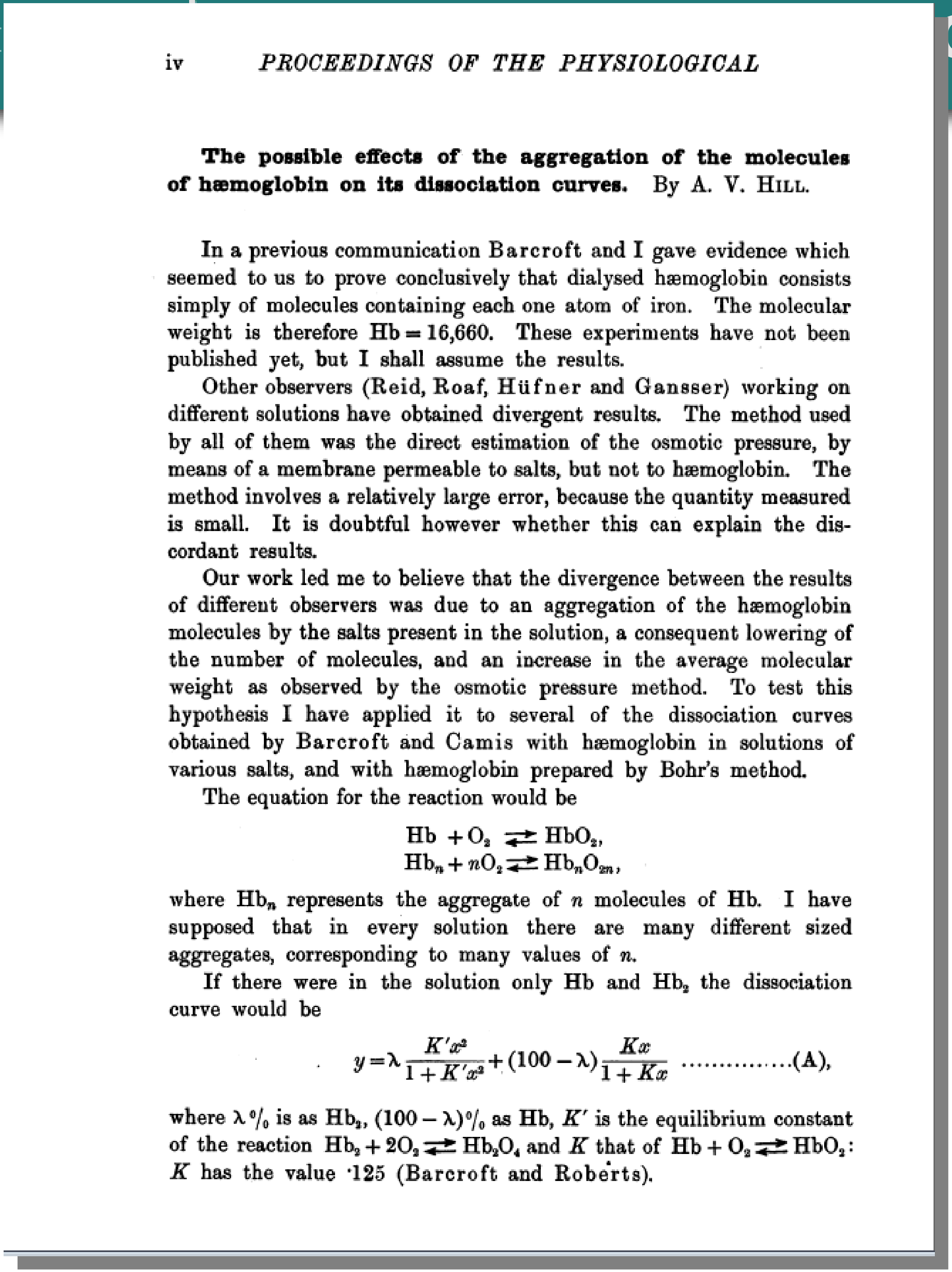


Calmodulin is ultra-sensitive



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

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

$$\begin{aligned} Hb + O_2 &\rightleftarrows HbO_2, \\ Hb_n + nO_2 &\rightleftarrows Hb_nO_{2n}, \end{aligned}$$

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

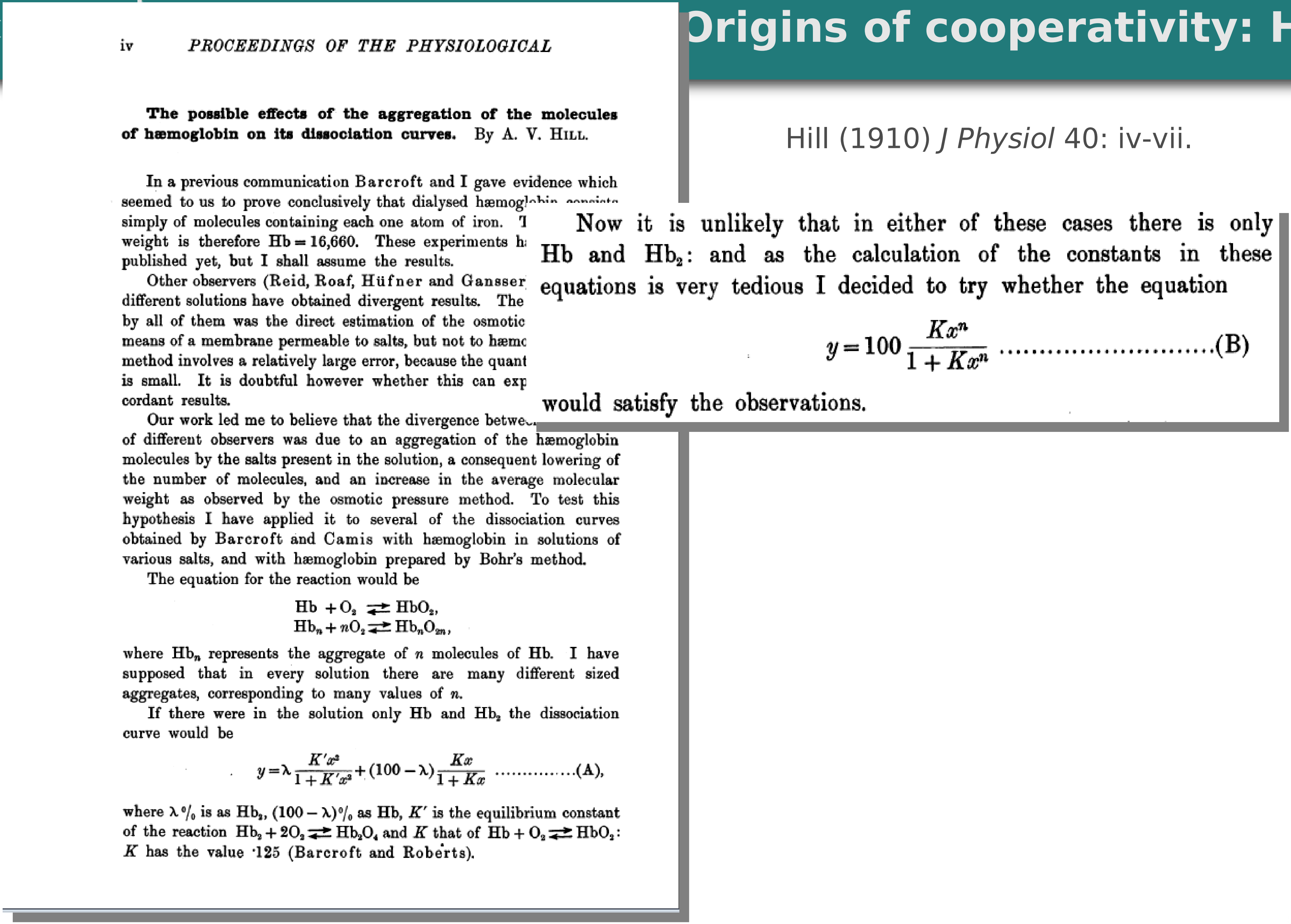
$$y = \lambda \frac{K'x^2}{1+K'x^2} + (100-\lambda)\frac{Kx}{1+Kx} \quad \ldots\ldots\ldots\ldots\text{(A)},$$

where $\lambda\,\%$ 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.

Origins of cooperativity: Hill

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



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. T
weight is therefore Hb = 16,660. These experiments h
published yet, but I shall assume the results.

Other observers (Reid, Roaf, Hüfner and Gansser
different solutions have obtained divergent results. The
by all of them was the direct estimation of the osmotic
means of a membrane permeable to salts, but not to hæmo
method involves a relatively large error, because the quant
is small. It is doubtful however whether this can exp
cordant results.

Our work led me to believe that the divergence betwe
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

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

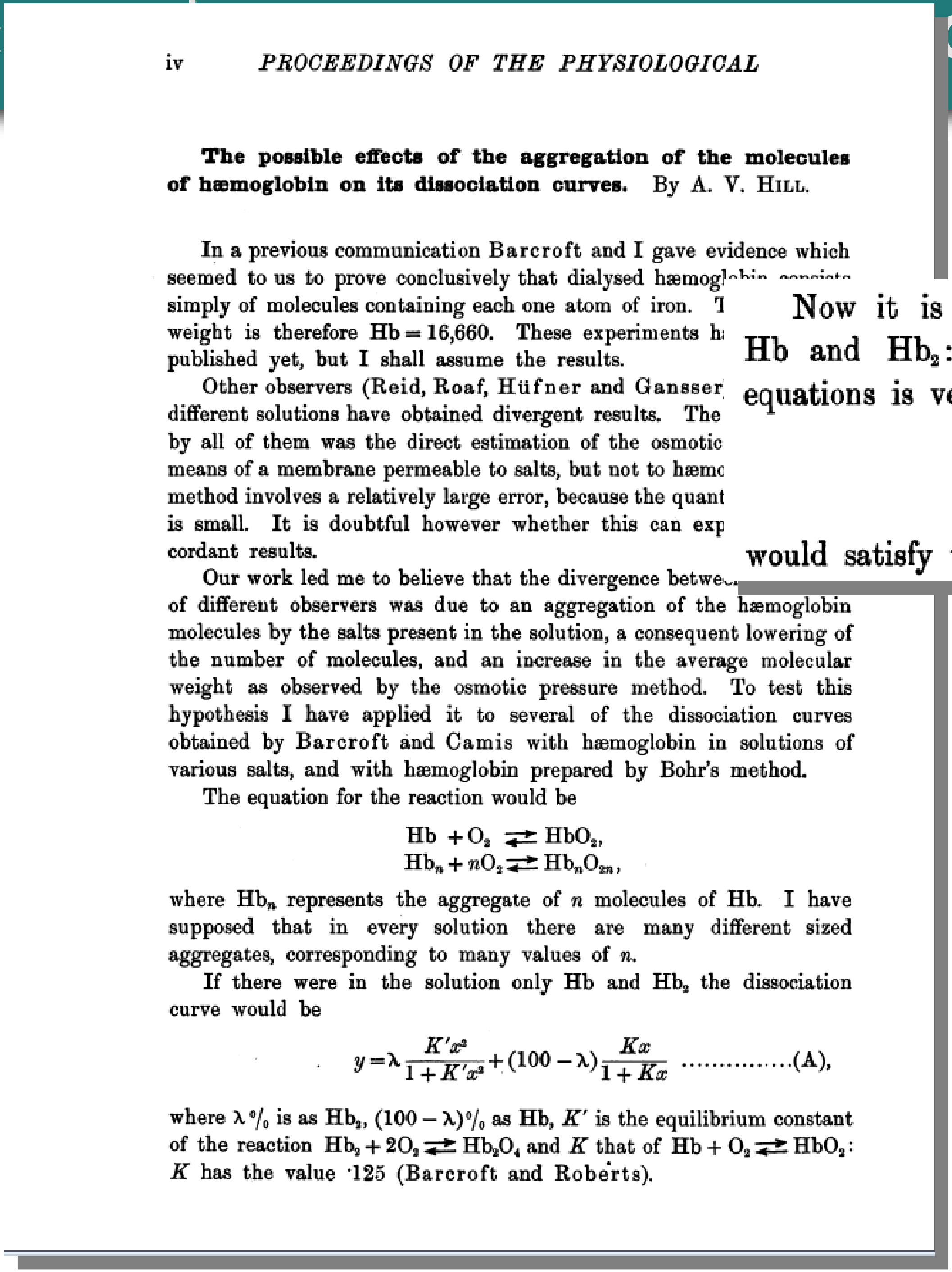
where λ % is as Hb_2, $(100-\lambda)$ % as Hb, K' is the equilibrium constant of the reaction $Hb_2 + 2O_2 \rightleftarrows Hb_2O_4$ and K that of $Hb + O_2 \rightleftarrows HbO_2$: K has the value ·125 (Barcroft and Roberts).

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

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

would satisfy the observations.

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. T weight is therefore Hb = 16,660. These experiments h published yet, but I shall assume the results.

Other observers (Reid, Roaf, Hüfner and Gansser different solutions have obtained divergent results. The by all of them was the direct estimation of the osmotic means of a membrane permeable to salts, but not to hæmo method involves a relatively large error, because the quant is small. It is doubtful however whether this can exp cordant results.

Our work led me to believe that the divergence betwe 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

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

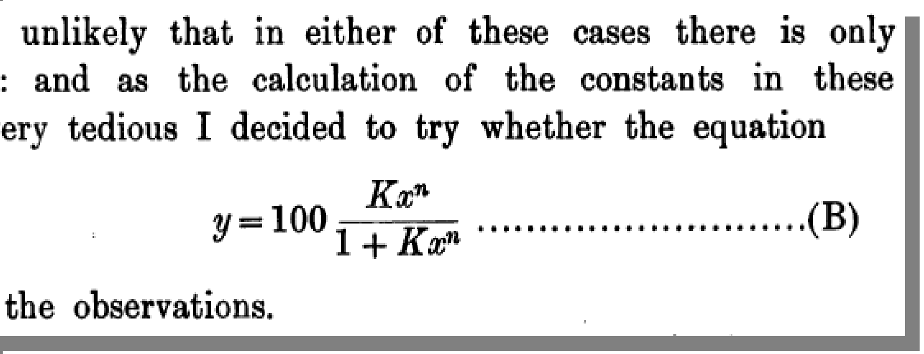
where Hb_n represents the aggregate of n molecules of Hb. I have supposed that in every solution there are many different sized aggregates, corresponding to many values of n.

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

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

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).

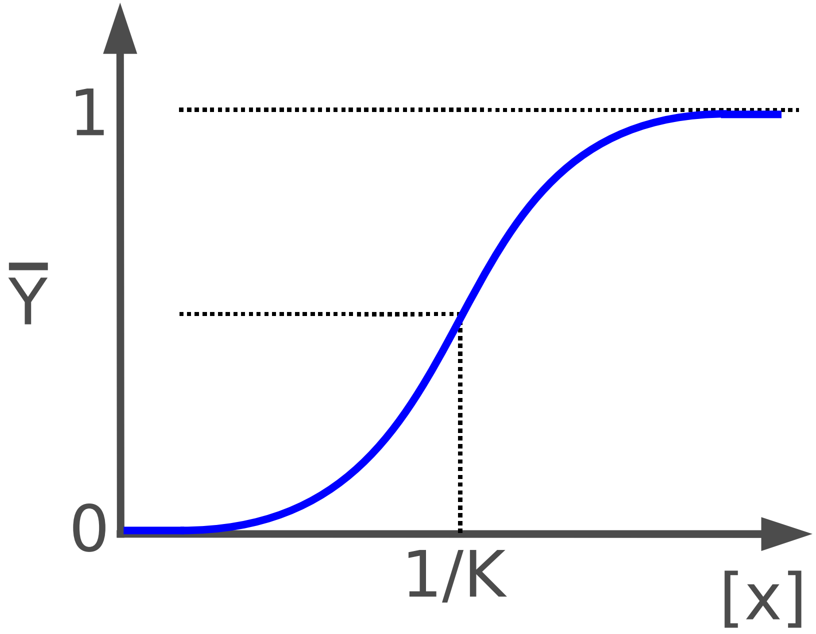
Hill (1910) *J Physiol* 40: iv-vii.



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

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

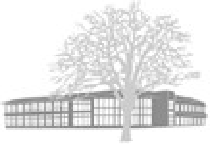
would satisfy the observations.



Hill Plot

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

Hill equation



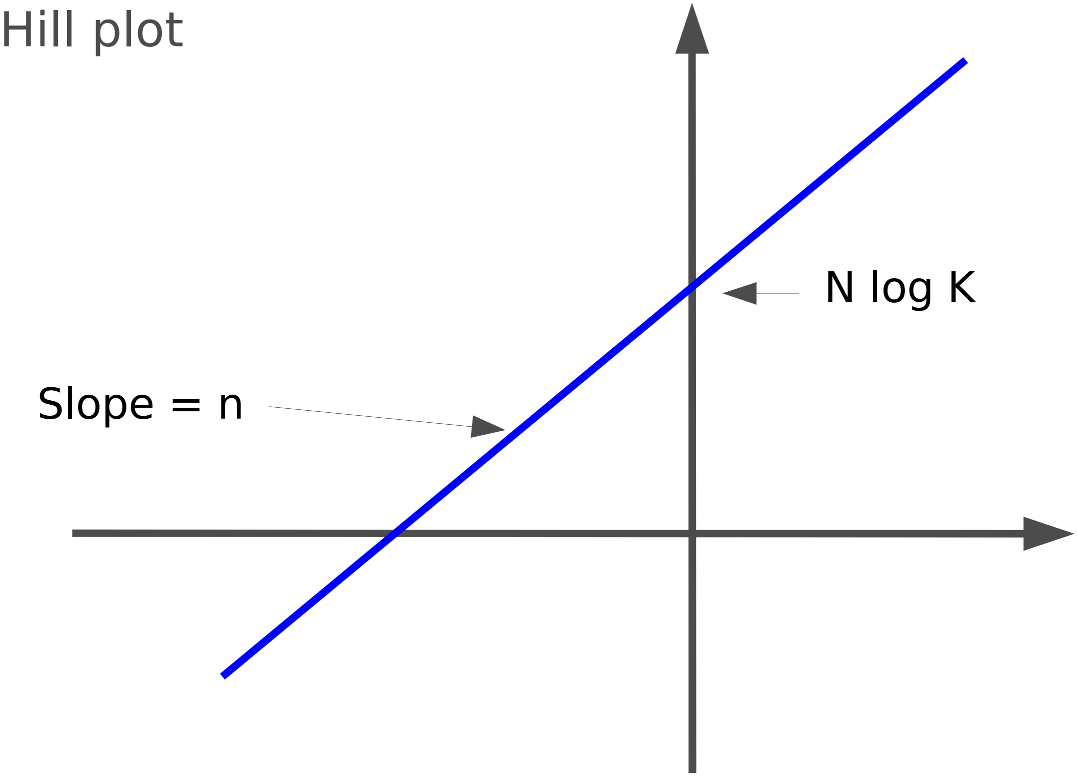
Hill Plot

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

Hill equation

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

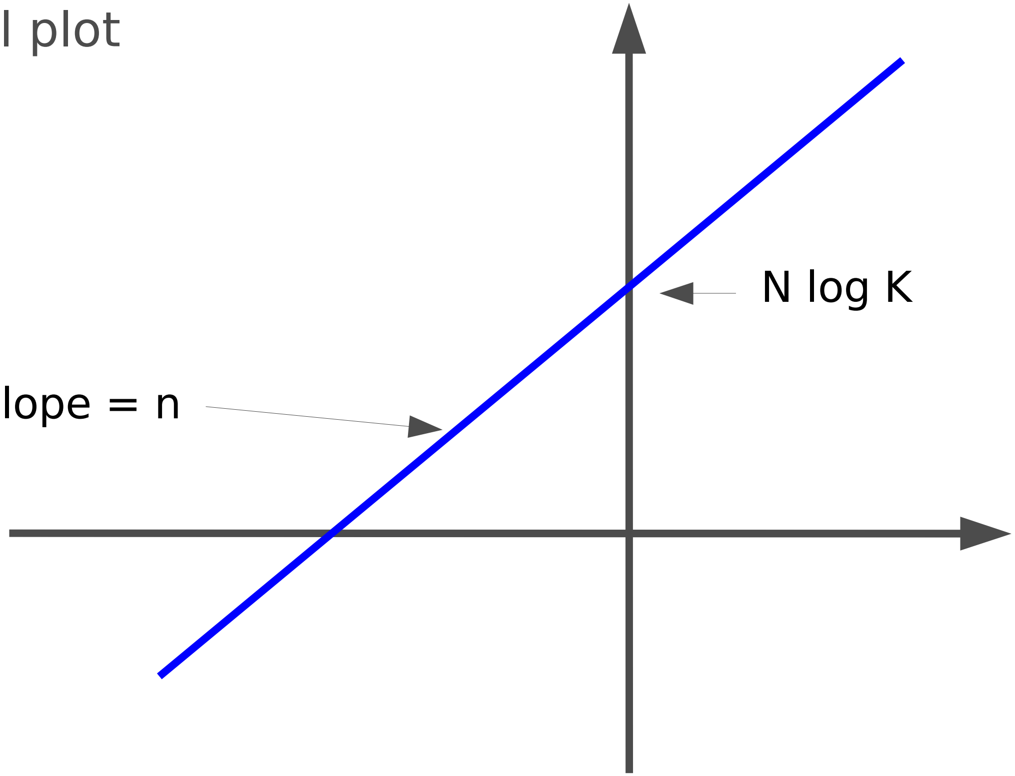
Hill plot



Hill Plot

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

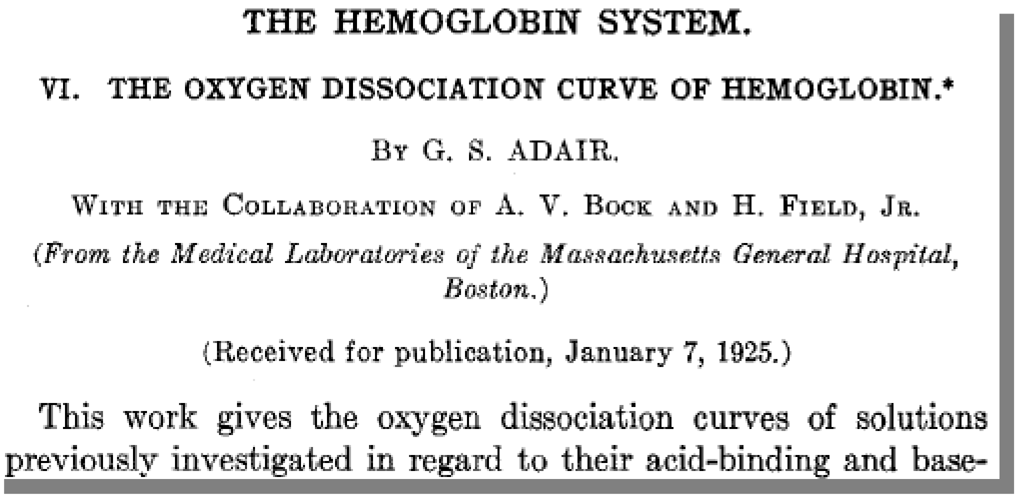
$$\log\left(\frac{\bar{Y}}{1-\bar{Y}}\right) = n \log K + n \log [x]$$ Hill plot



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.

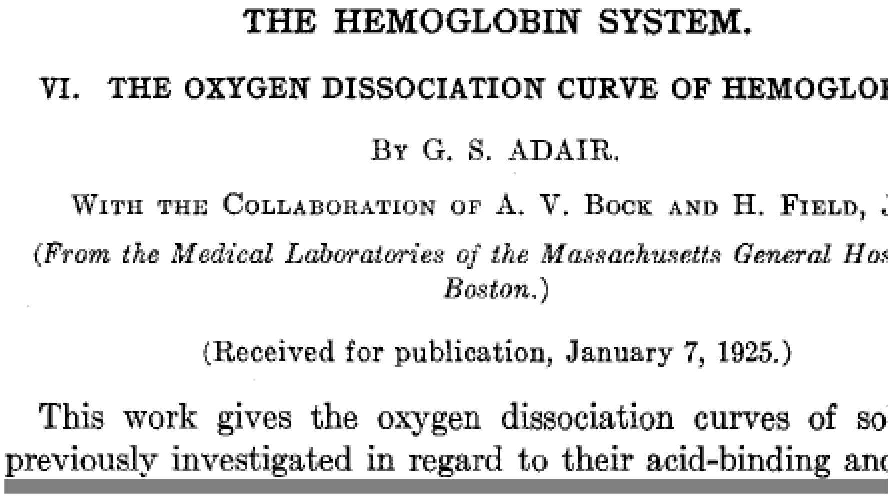
(From the Medical Laboratories of the Massachusetts General Hospital, Boston.)

(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

Origins of cooperativity: Adair-Klotz



THE HEMOGLOBIN SYSTEM.

VI. THE OXYGEN DISSOCIATION CURVE OF HEMOGLO

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 an

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

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

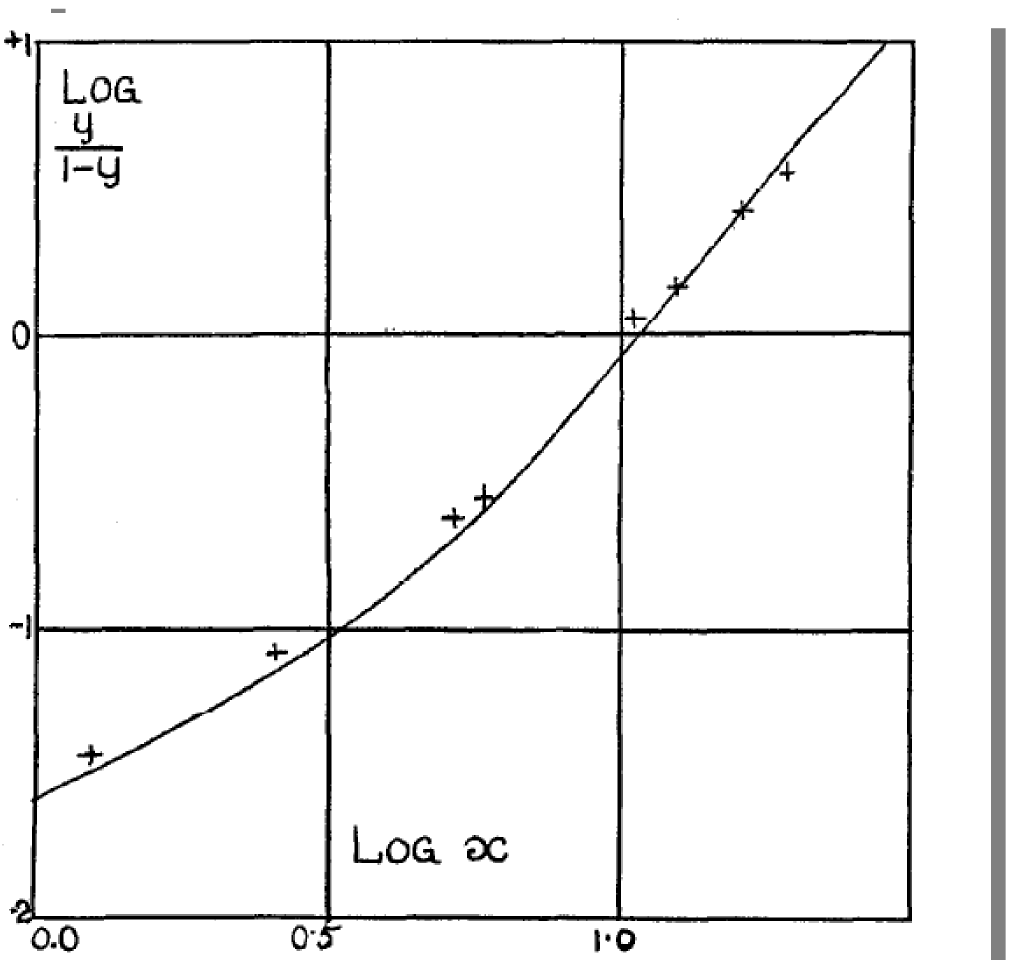
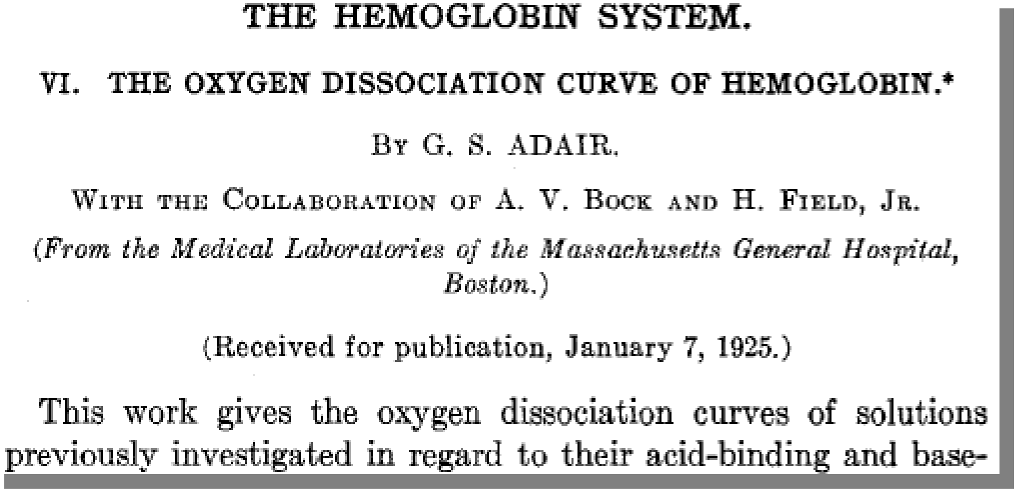


FIG. 2. Test of formula (6). Curve drawn from 6 experimental points from Table IV.

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.

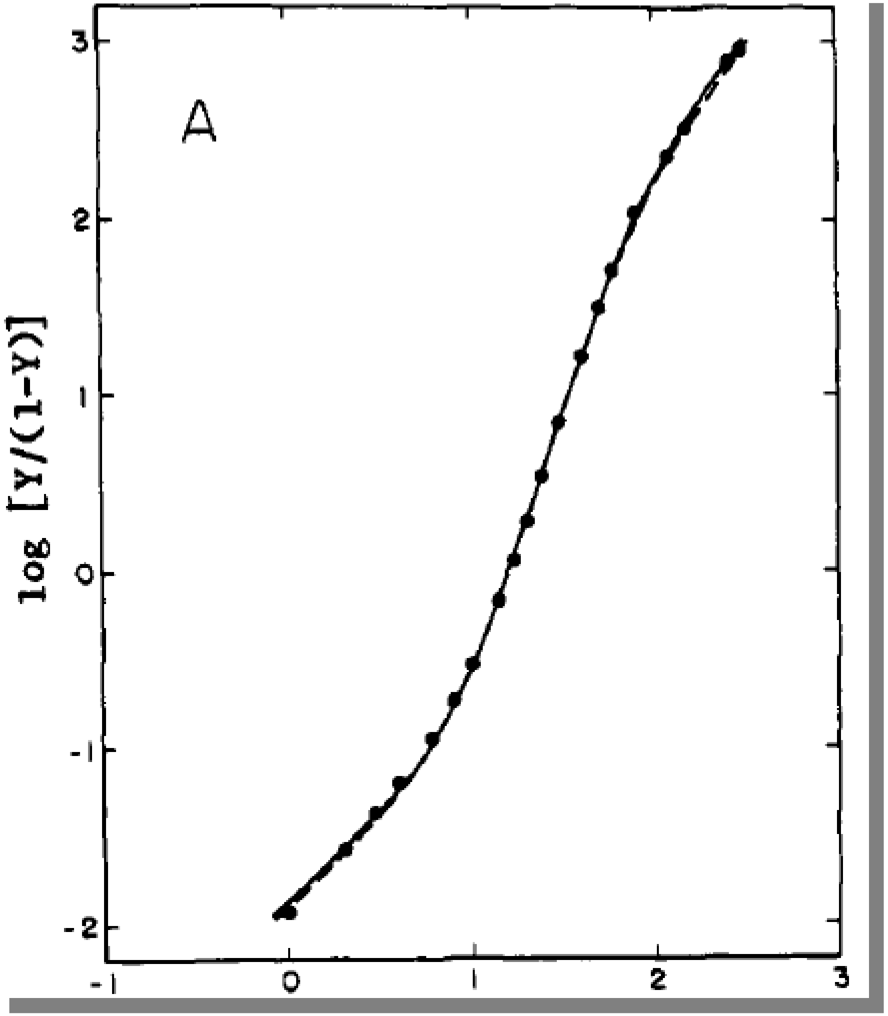
(From the Medical Laboratories of the Massachusetts General Hospital, Boston.)

(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

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



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

Adair-Klotz model applied to Calmodulin

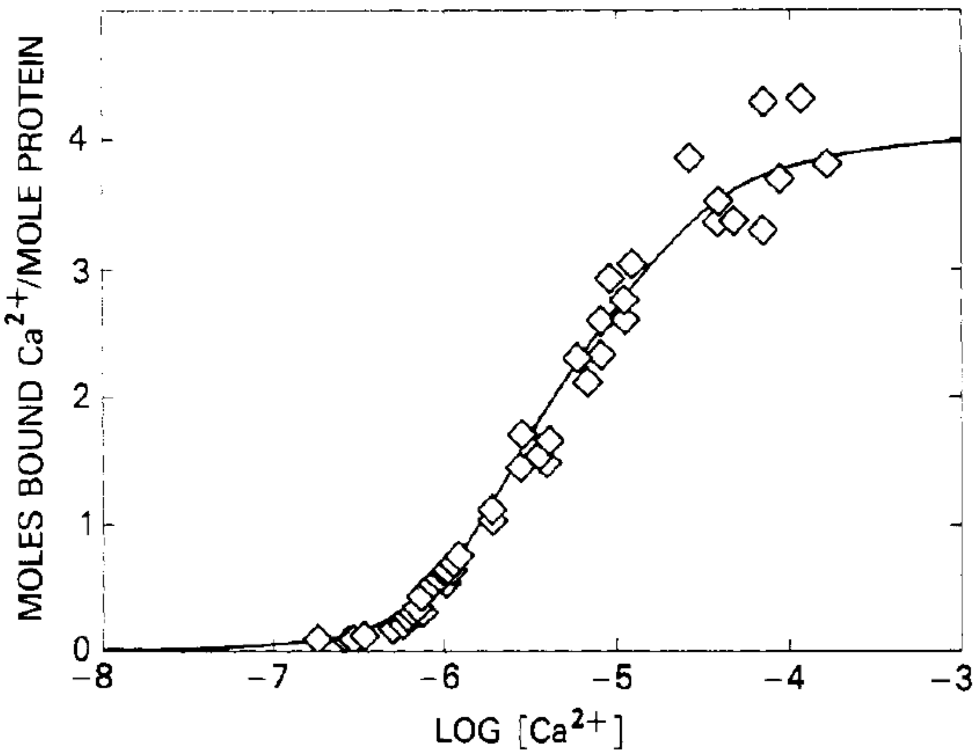
Klotz (1946) The Application of the Law of Mass Action to Binding by Proteins. Interactions with Calcium. *Arch Biochem*, 9:109–117.

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

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

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

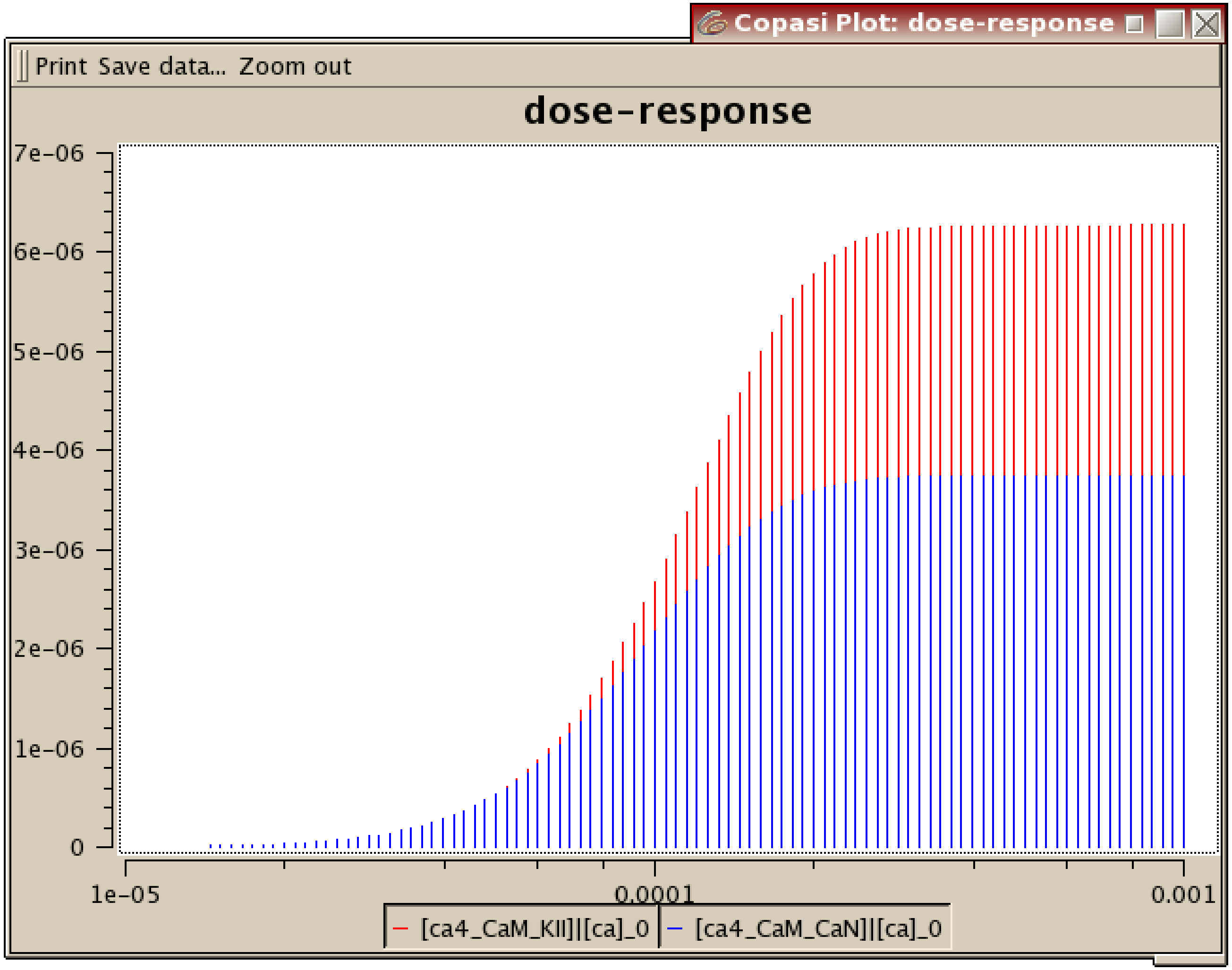
$$+ Ca^{2+} \overset{K_2}{\rightleftharpoons} Ca_2CaM$$

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

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

$$Ca_4CaM \rightarrow \text{Binding to target}$$

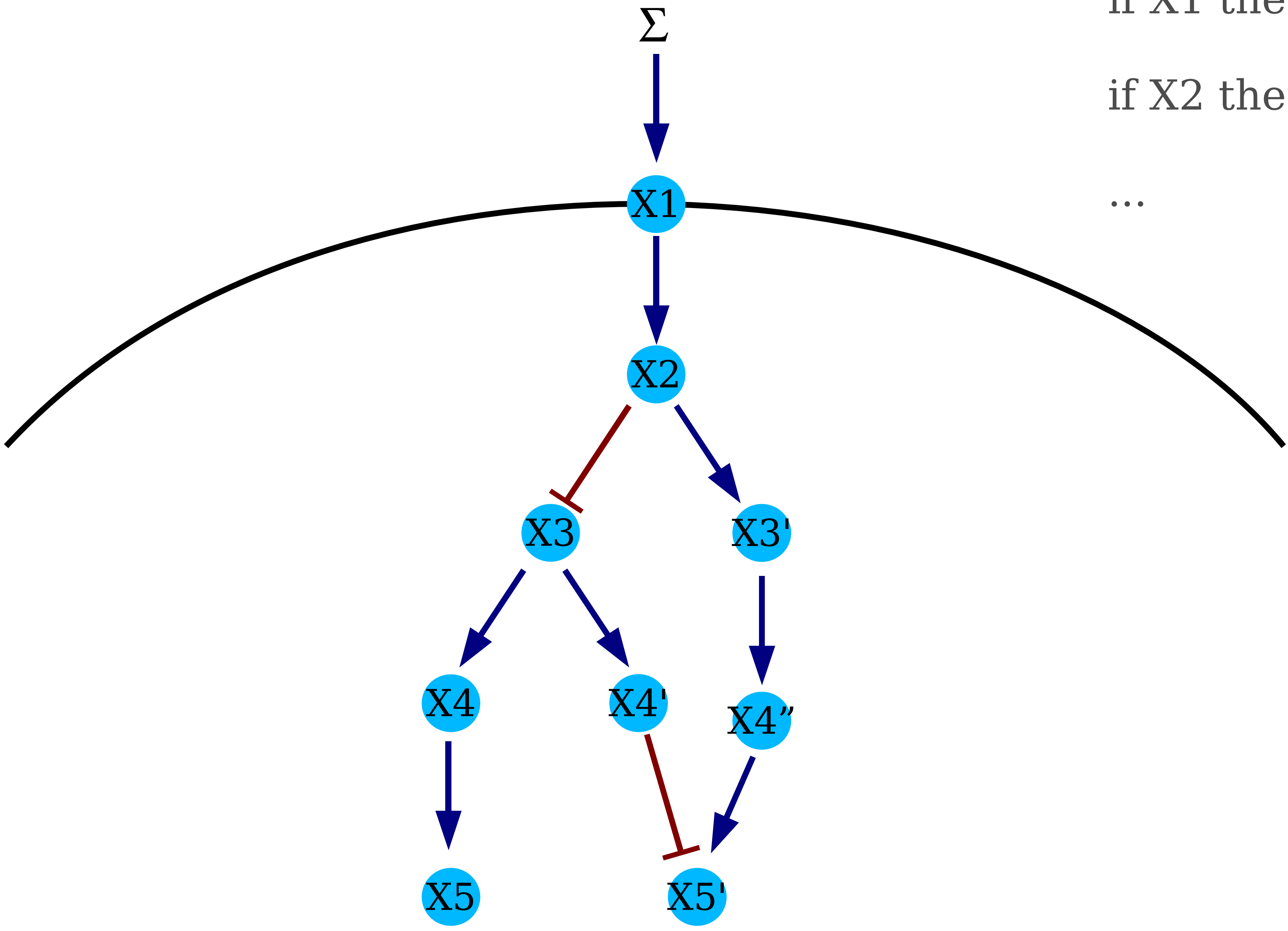
That does not work ...



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) $\cong$ CaMKII ; Low [Ca^{2+}] (low freq) $\cong$ Calcineurin

Mistake: signals, activity flow and induction



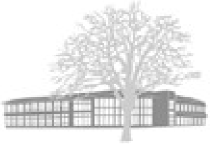
if X1 then X2

if X2 then not X3

...

Induction Vs Selection

- Induction = BAD
  (Lamarck's first law, antibody moulding on the antigen, directed axonal growth, induced-fit ...)

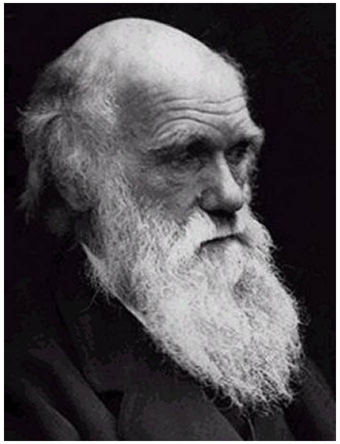


Induction Vs Selection

- Induction = BAD
  (Lamarck's first law, antibody moulding on the antigen, directed axonal growth, induced-fit ...)
- Physically meaningless. Calcium has no inertia. Calcium cannot “trigger” a conformational change

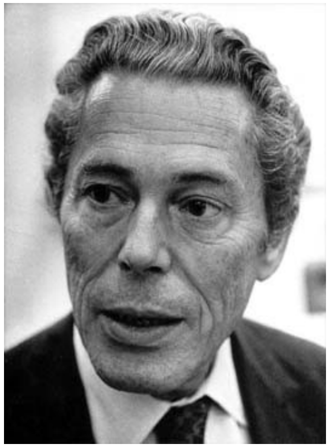
Induction Vs Selection

- Induction = BAD
  (Lamarck's first law, antibody moulding on the antigen, 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 ...)

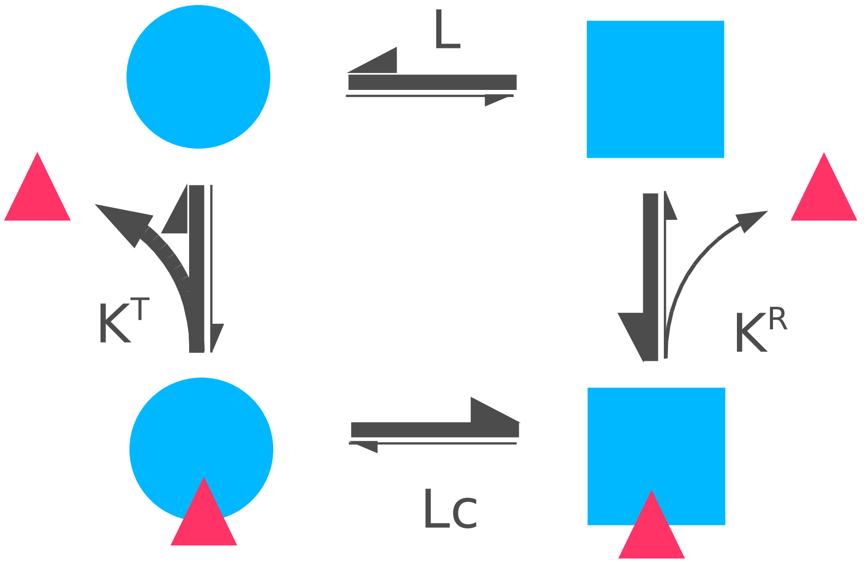


Allostery and state selection

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

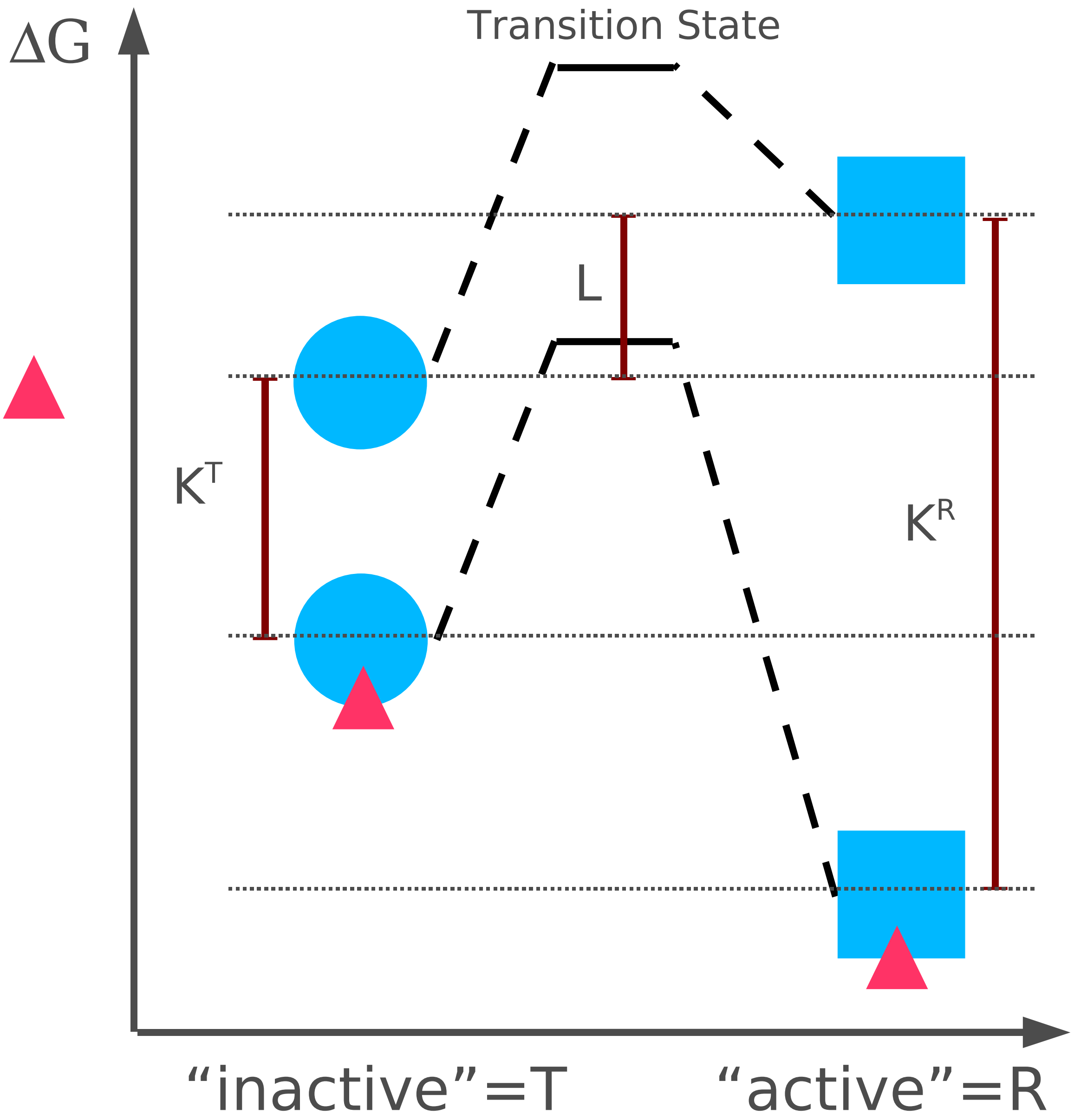


Modulation of thermal equilibria ≠ induced-fit

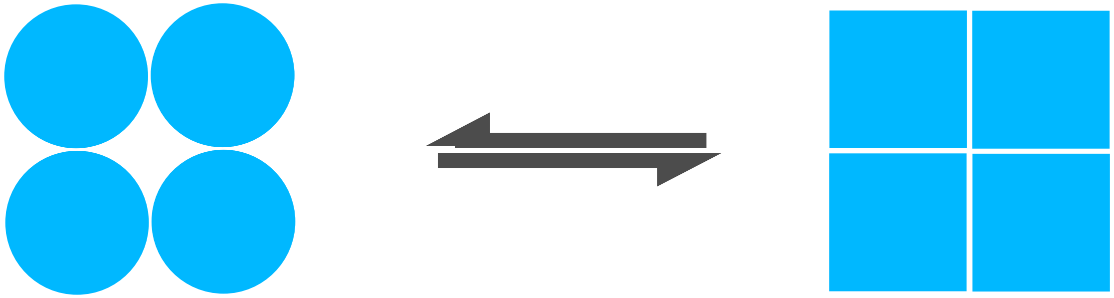


$$L = \frac{[T0]}{[R0]}$$

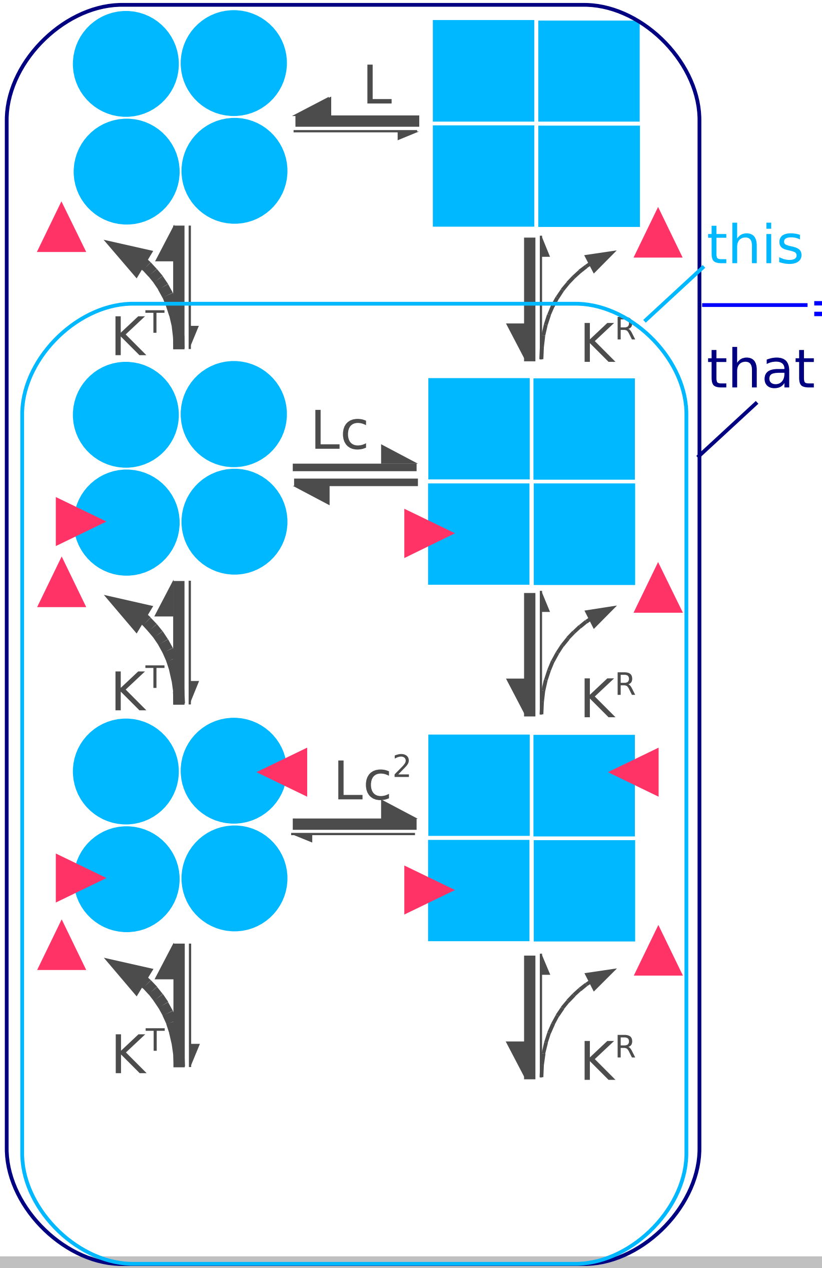
$$C = \frac{K^R}{K^T}$$



Concerted transi tions ≠ sequential model

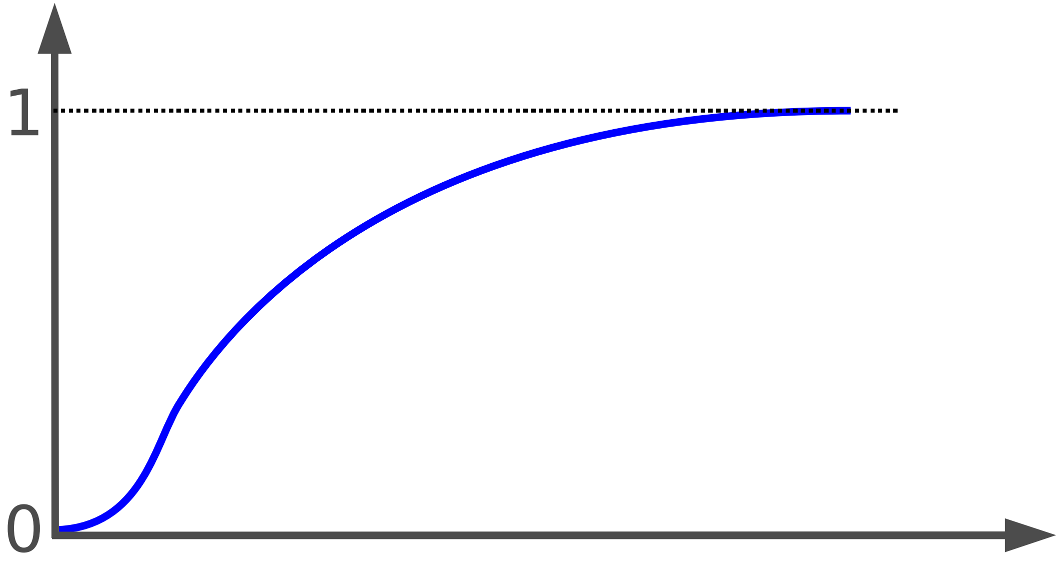


Monod-Wyman-Changeux model

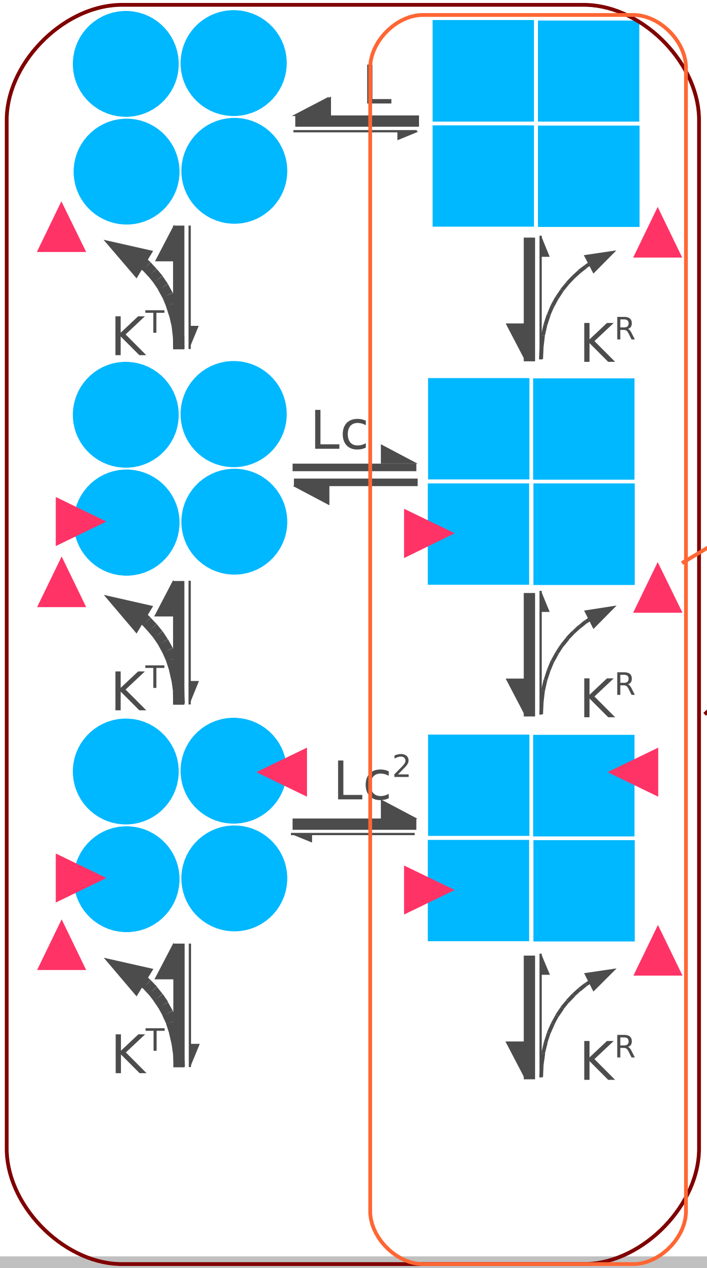


$$\alpha = \frac{[x]}{K^R}$$

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



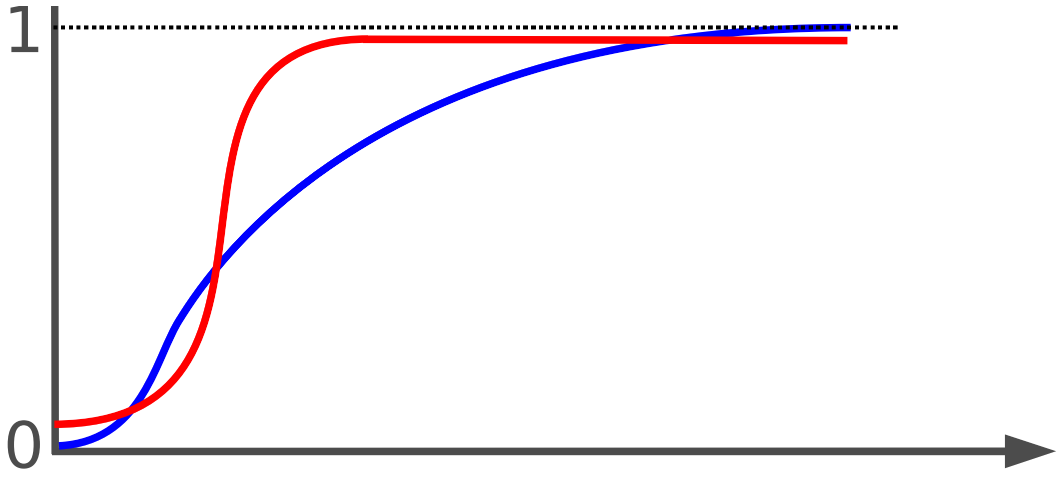
Monod-Wyman-Changeux model



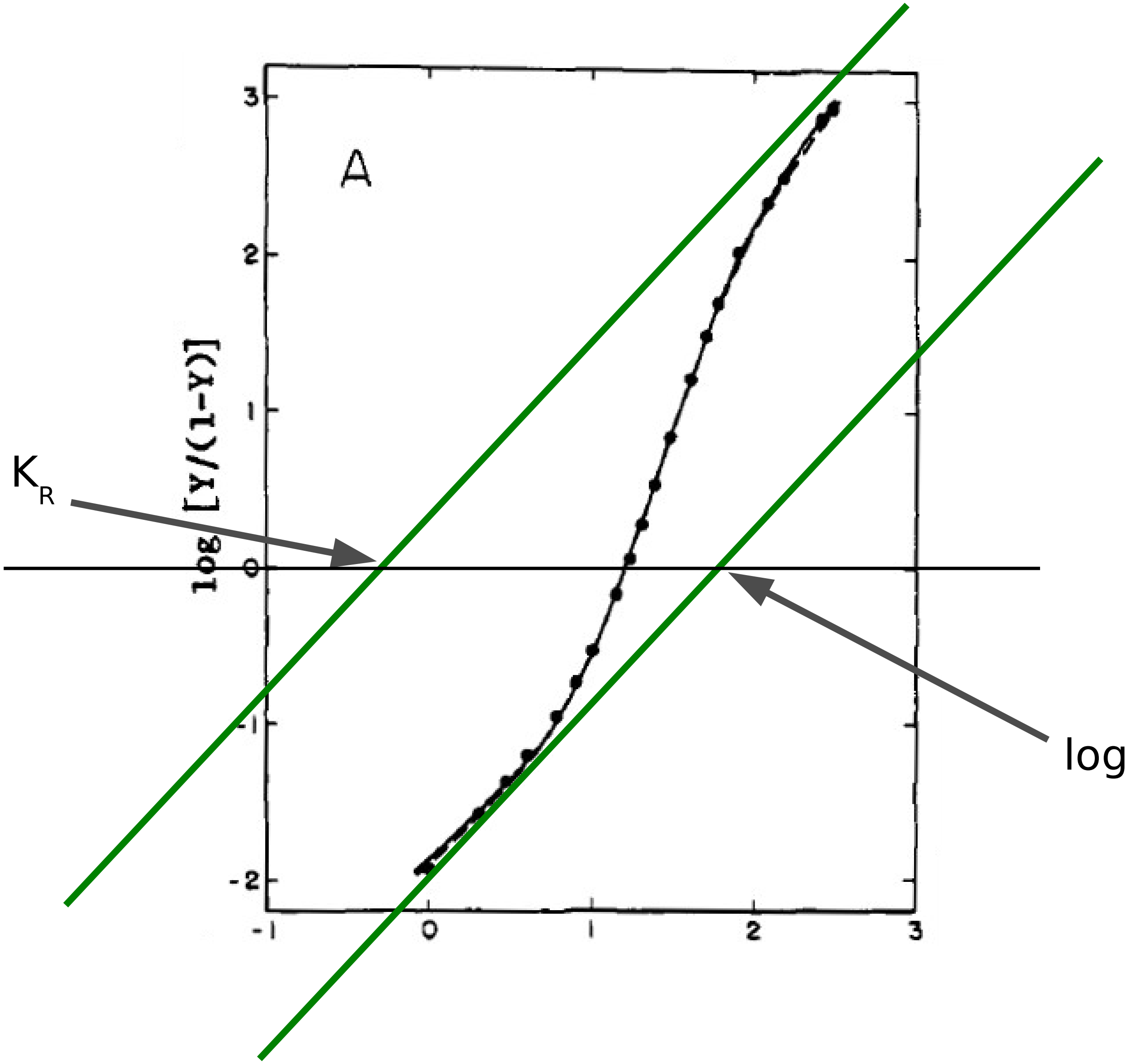
$$\alpha = \frac{[x]}{K^R}$$

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

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



“Hill” Plot for MWC model



log K_R

log K_T

Extended MWC model

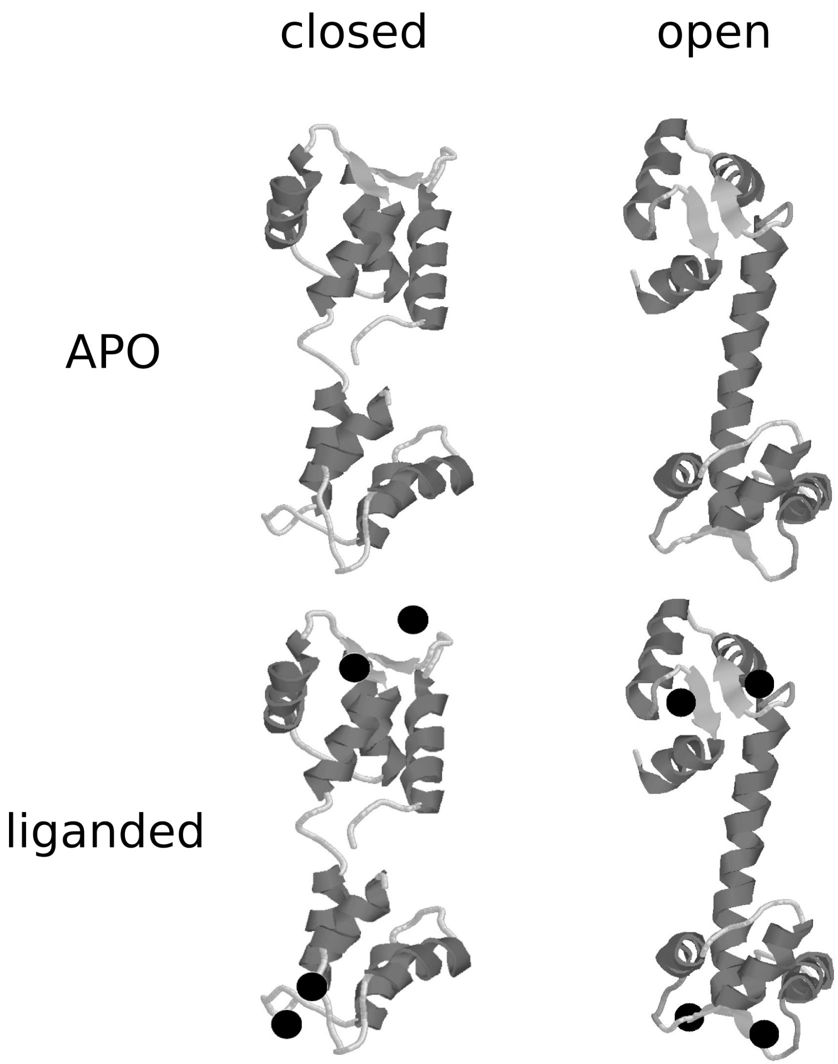
$$\bar{Y} = \frac{1}{n}\frac{\sum_i\left(\alpha_i\prod_{j\neq i}(1+\alpha_j)\right) + L\prod_k\left(\frac{1+e_k\gamma_k}{1+\gamma_k}\right)\sum_i\left(c_i\alpha_i\prod_{j\neq i}(1+c_j\alpha_j)\right)}{\prod_i(1+\alpha_i) + L\prod_k\left(\frac{1+e_k\gamma_k}{1+\gamma_k}\right)\prod_i(1+c_i\alpha_i)}$$

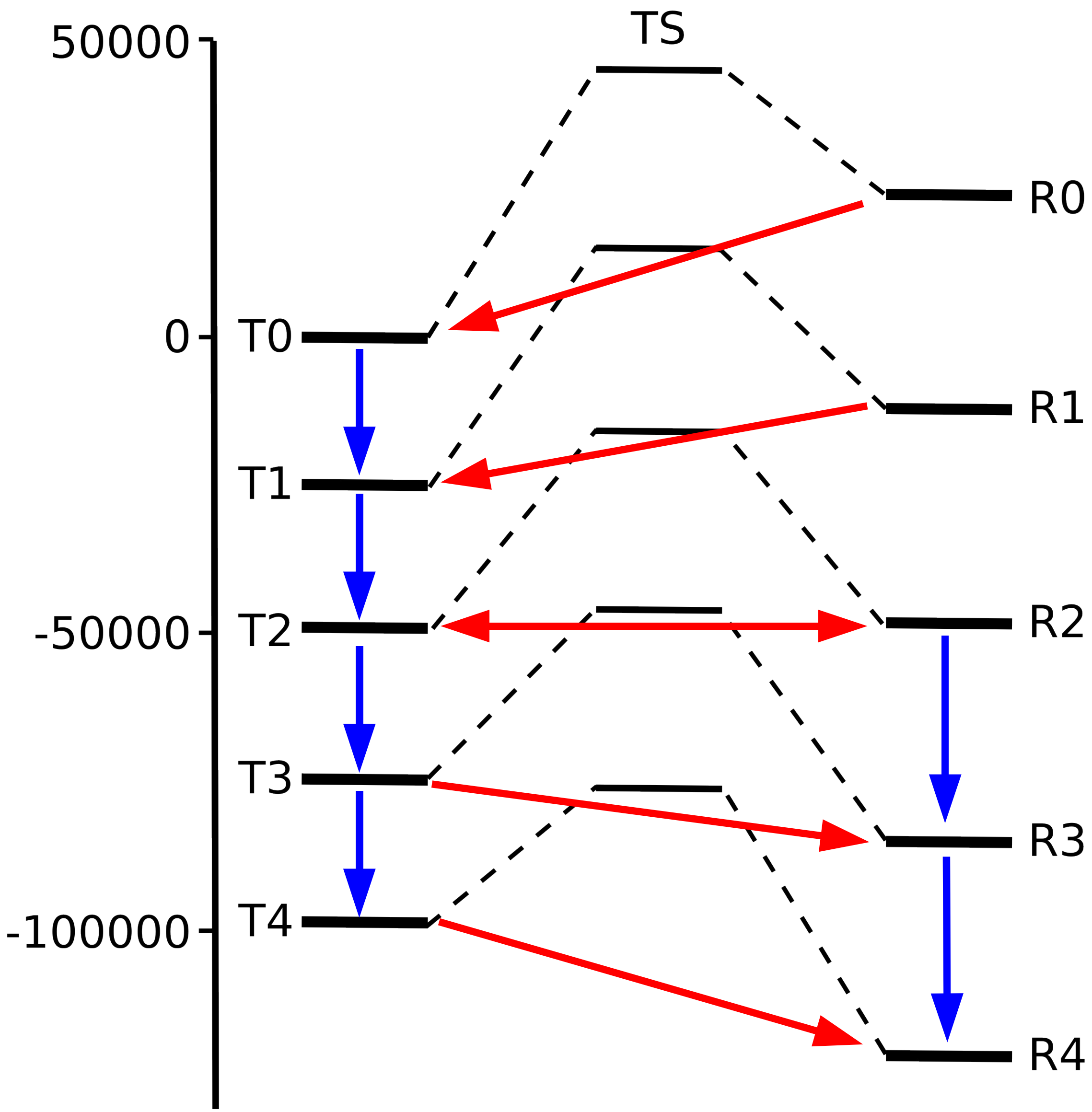
Based on Rubin and Changeux (1966) *J Mol Biol*, 21: 265-274

- αi = [ligand]/$K^{R}_{i,\mathrm{lig}}$
- γk = [modulator]/$K^{R}_{k,\mathrm{mod}}$
- ci = $K^{R}_{i,\mathrm{lig}}$/ $K^{T}_{i,\mathrm{lig}}$
- ek = $K^{R}_{k,\mathrm{mod}}$ / $K^{T}_{k,\mathrm{mod}}$

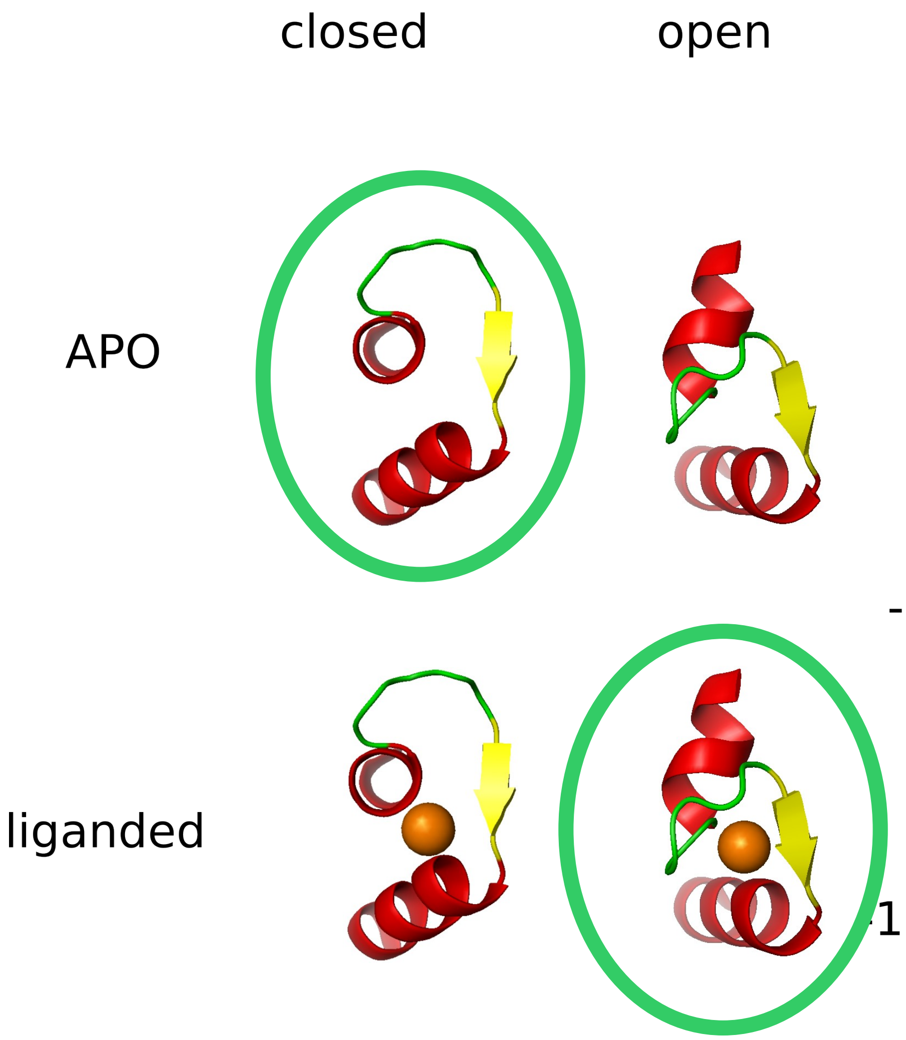
Stefan MI, Edelstein SJ, Le Novère N. Computing phenomenologic Adair-Klotz constants from microscopic MWC parameters. *submitted*

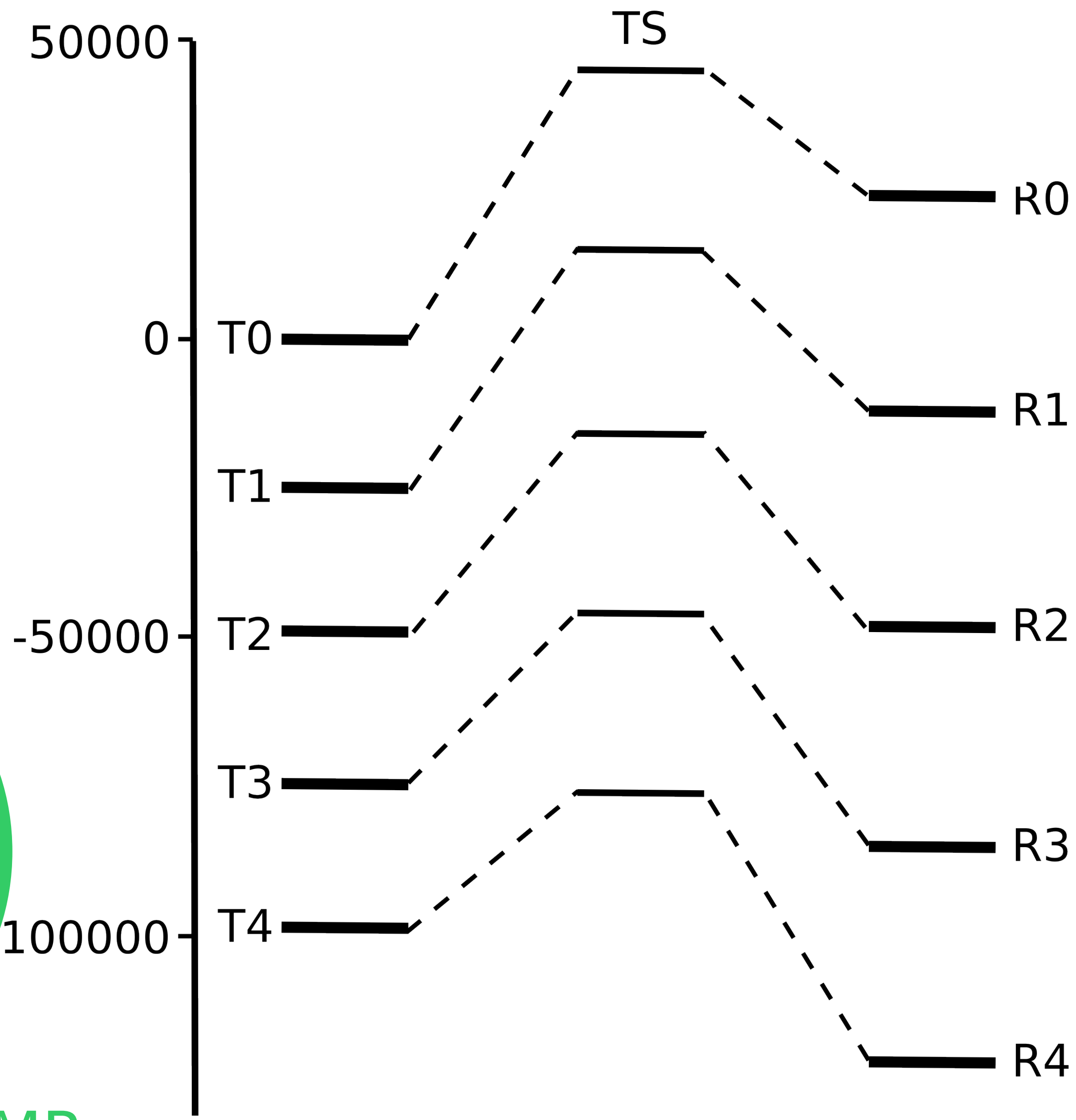
Concerted transition





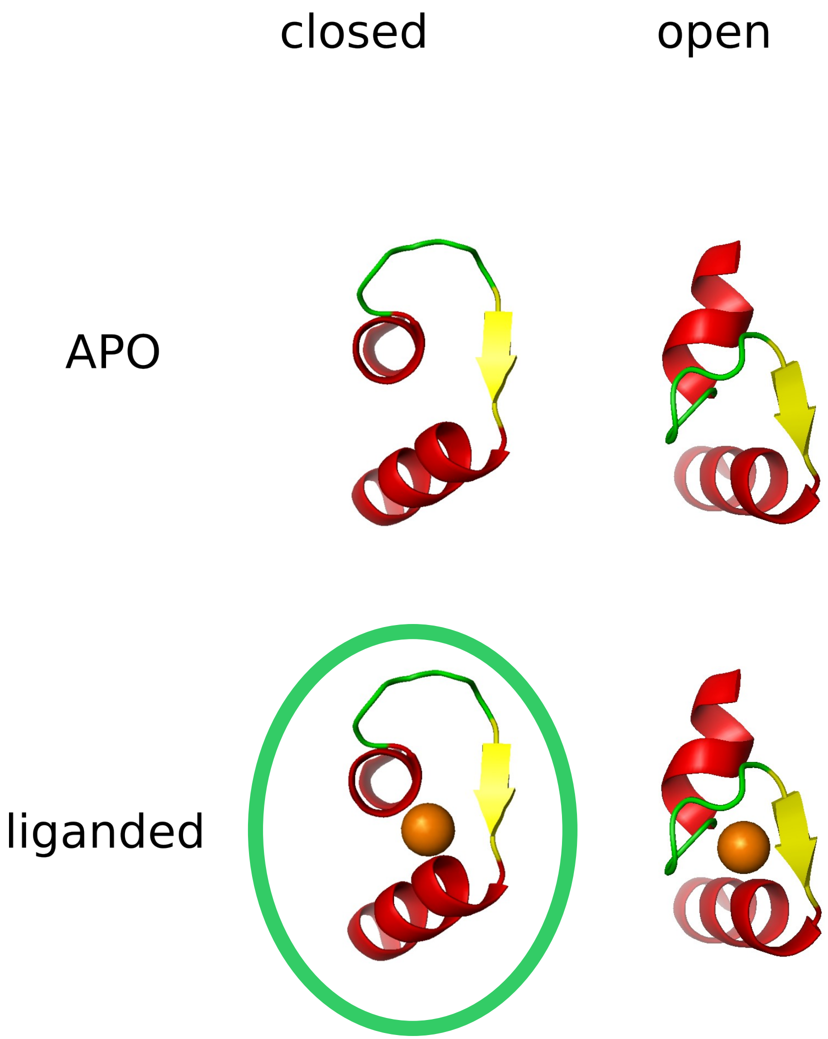
Observation Vs. Prediction

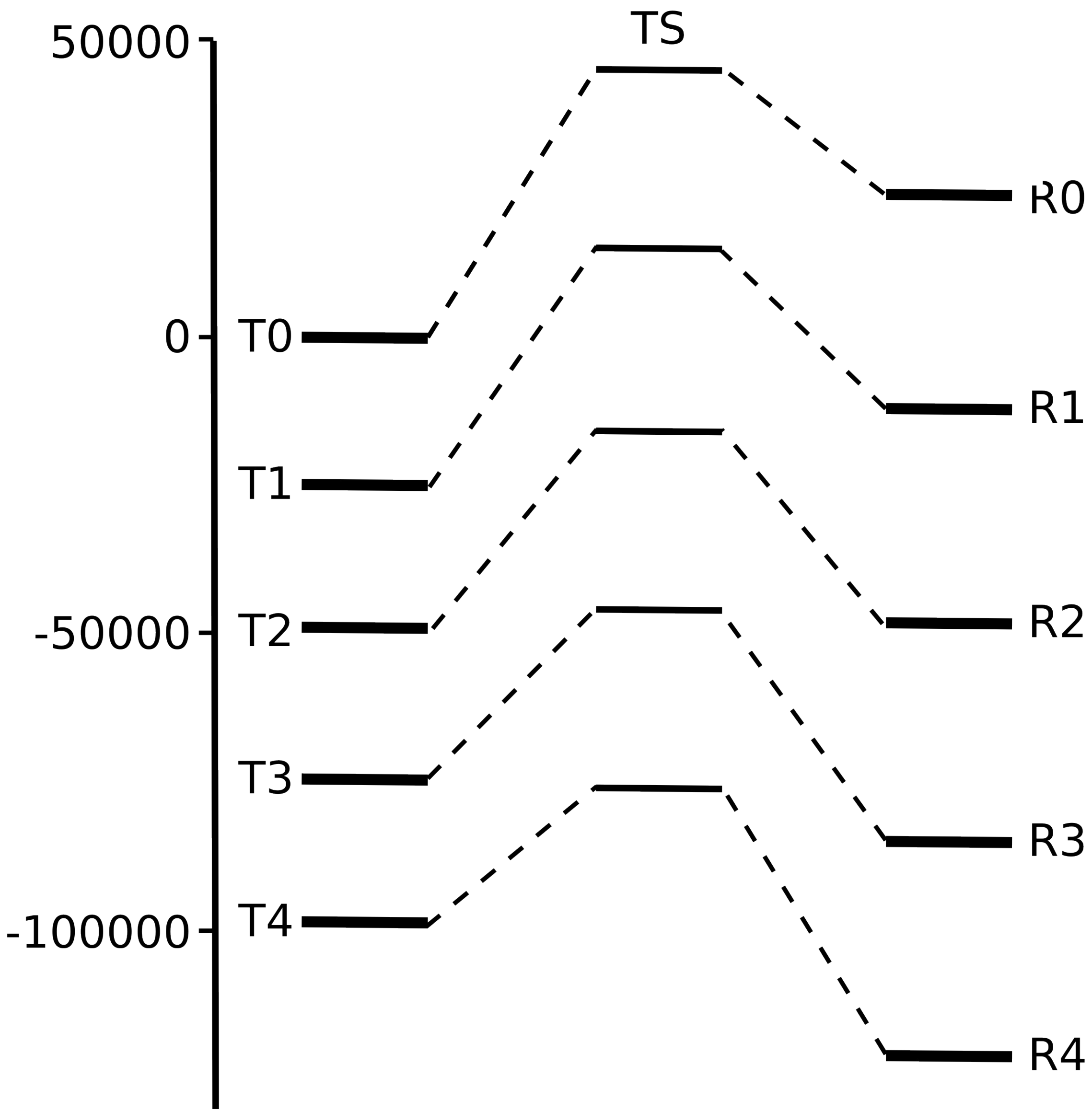




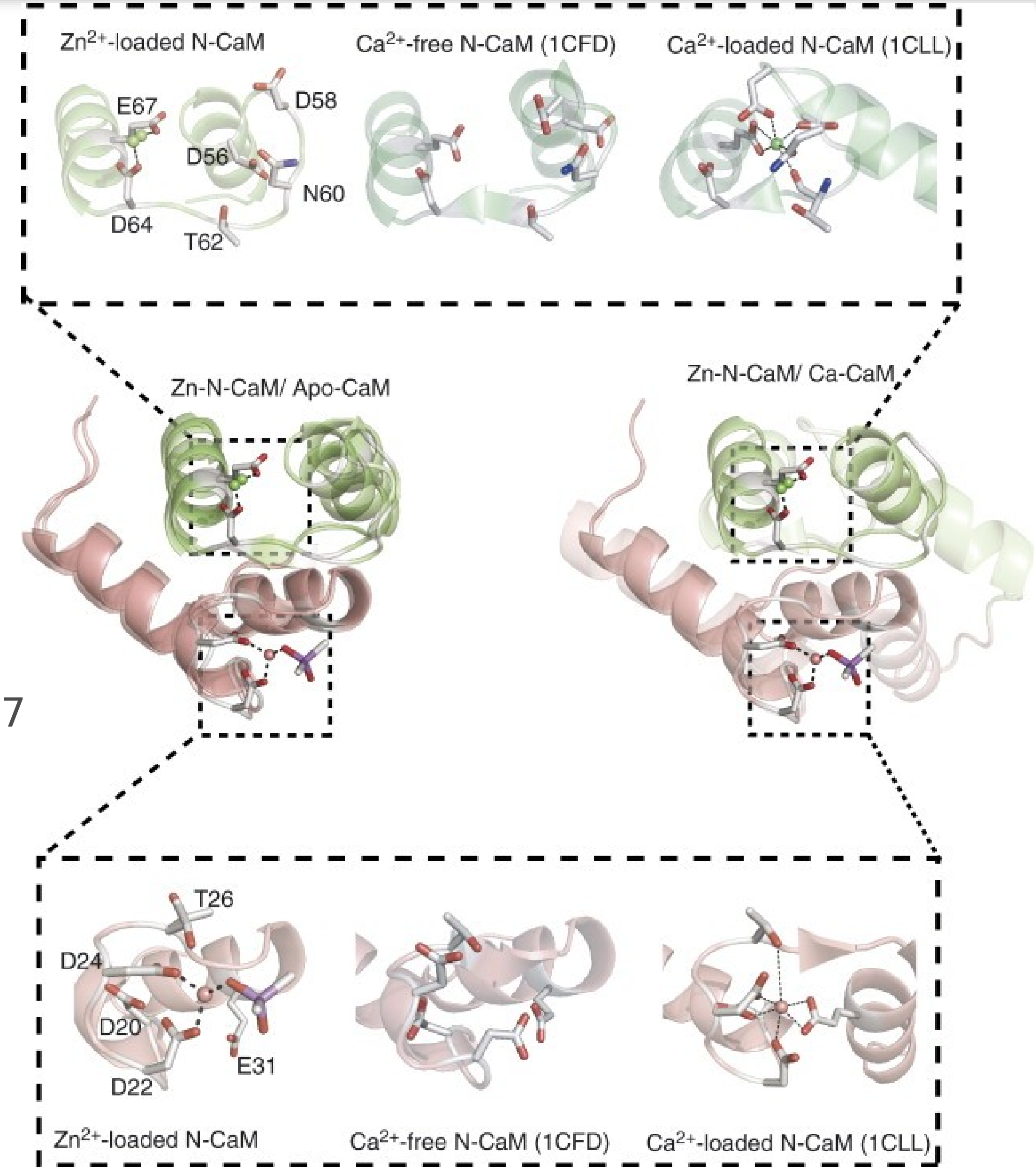
Observed by X-Ray and NMR

Observation Vs. Prediction



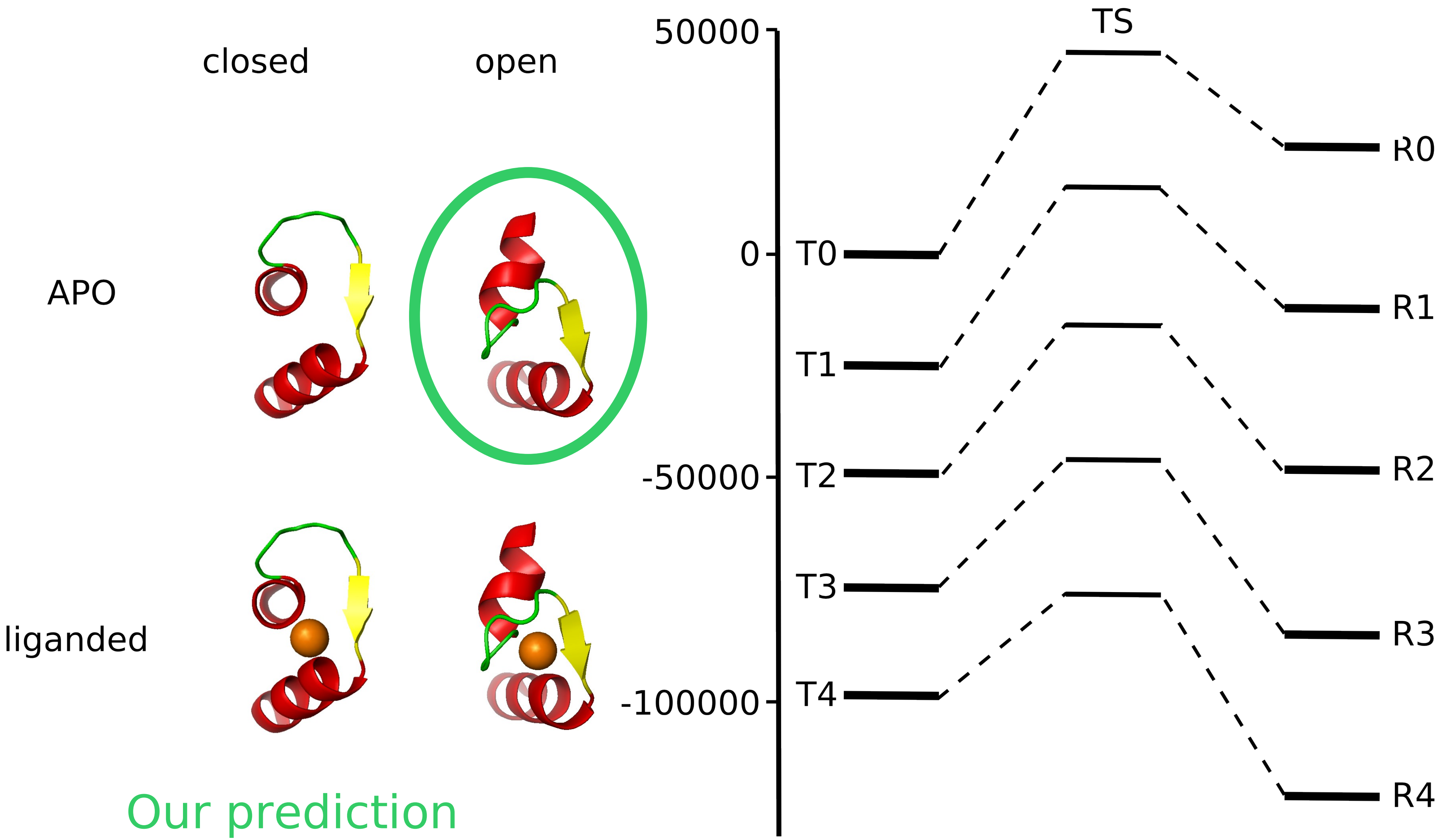


Inferred from Zn binding

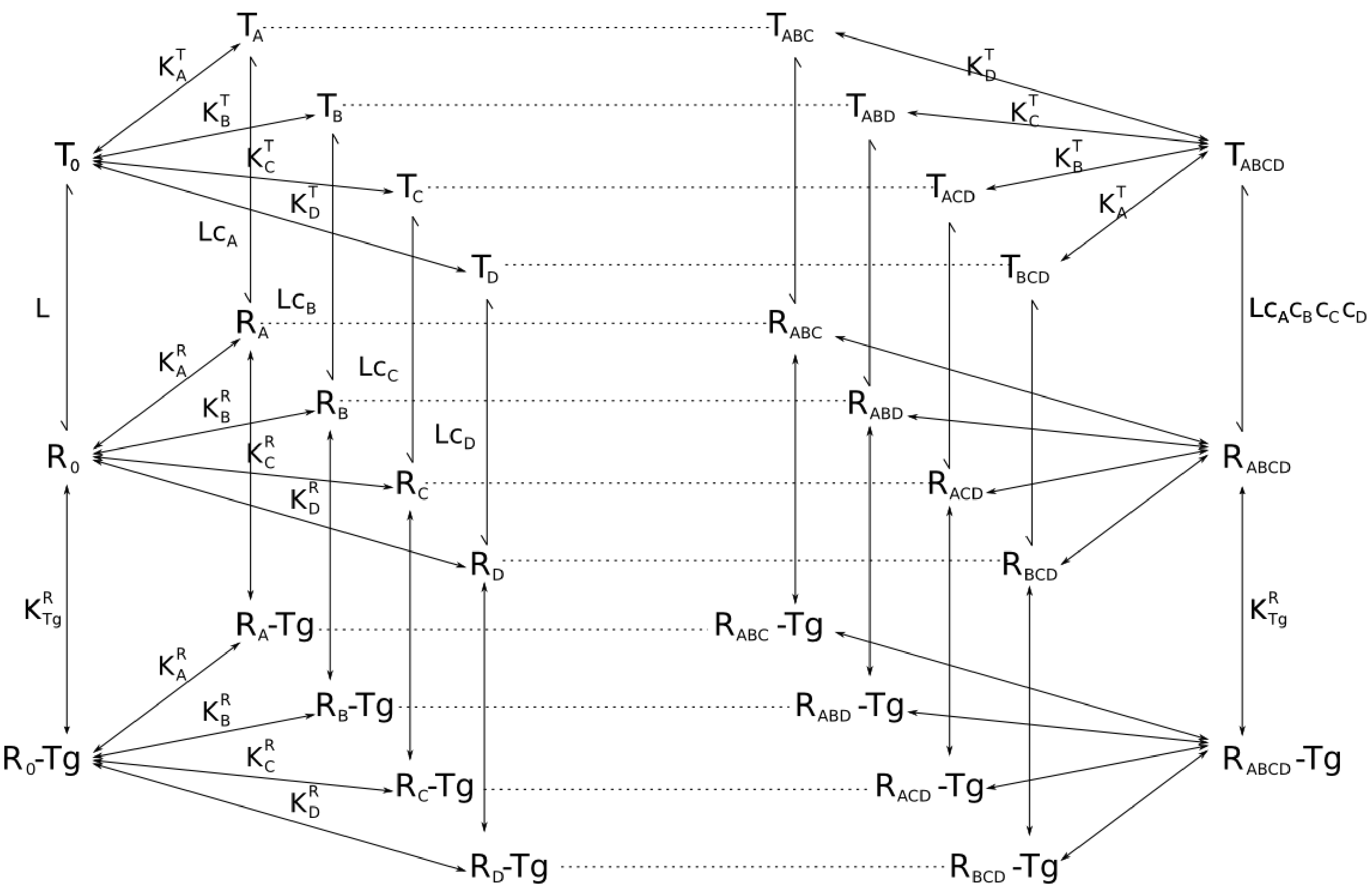


Warren *et al.* (2007).
J Mol Biol 374: 517-527

Observation Vs. Prediction



Full mechanistic thermodynamic model



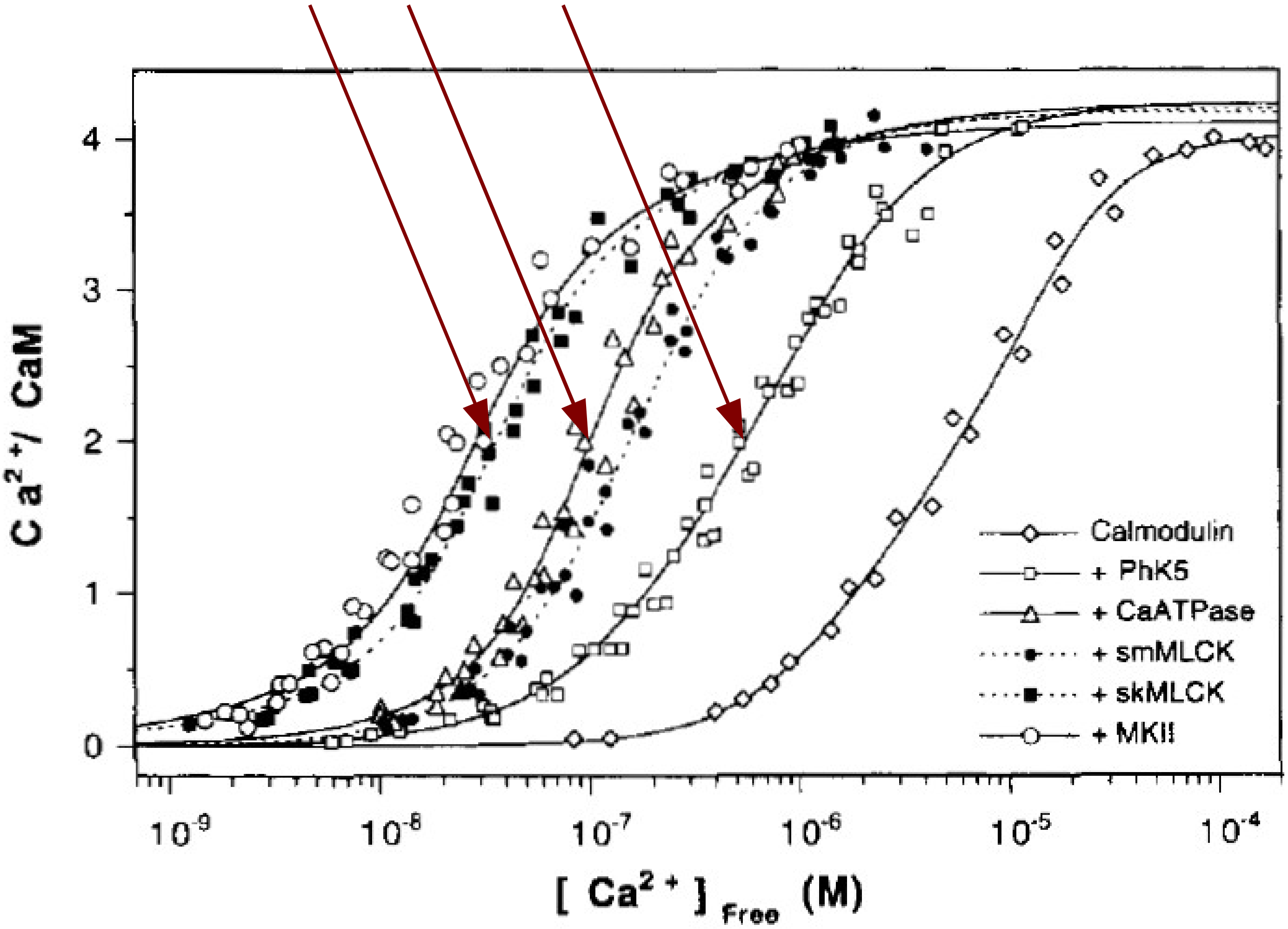
320 reactions

Restriction of the generalised model

- 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



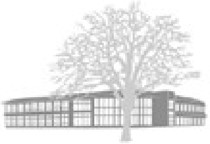
Peersen et al. (1997) *Prot Sci*, 6: 794-807

Restriction of the generalised model

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

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

- 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 e-15, which can be taken as skMLCK binding only to R state.



Restriction of the generalised model

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

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

L=20670

$c=3.96.10^{-3}$

- 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 e-15, which can be taken as skMLCK binding only to R state.

Restriction of the generalised model

- Determination of individual affinities:

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

Restriction of the generalised model

- Determination of individual affinities:

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

- 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

Restriction of the generalised model

- Determination of individual affinities:

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

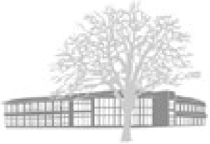
$K^R_A = 8.32\ 10^{-6}$

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

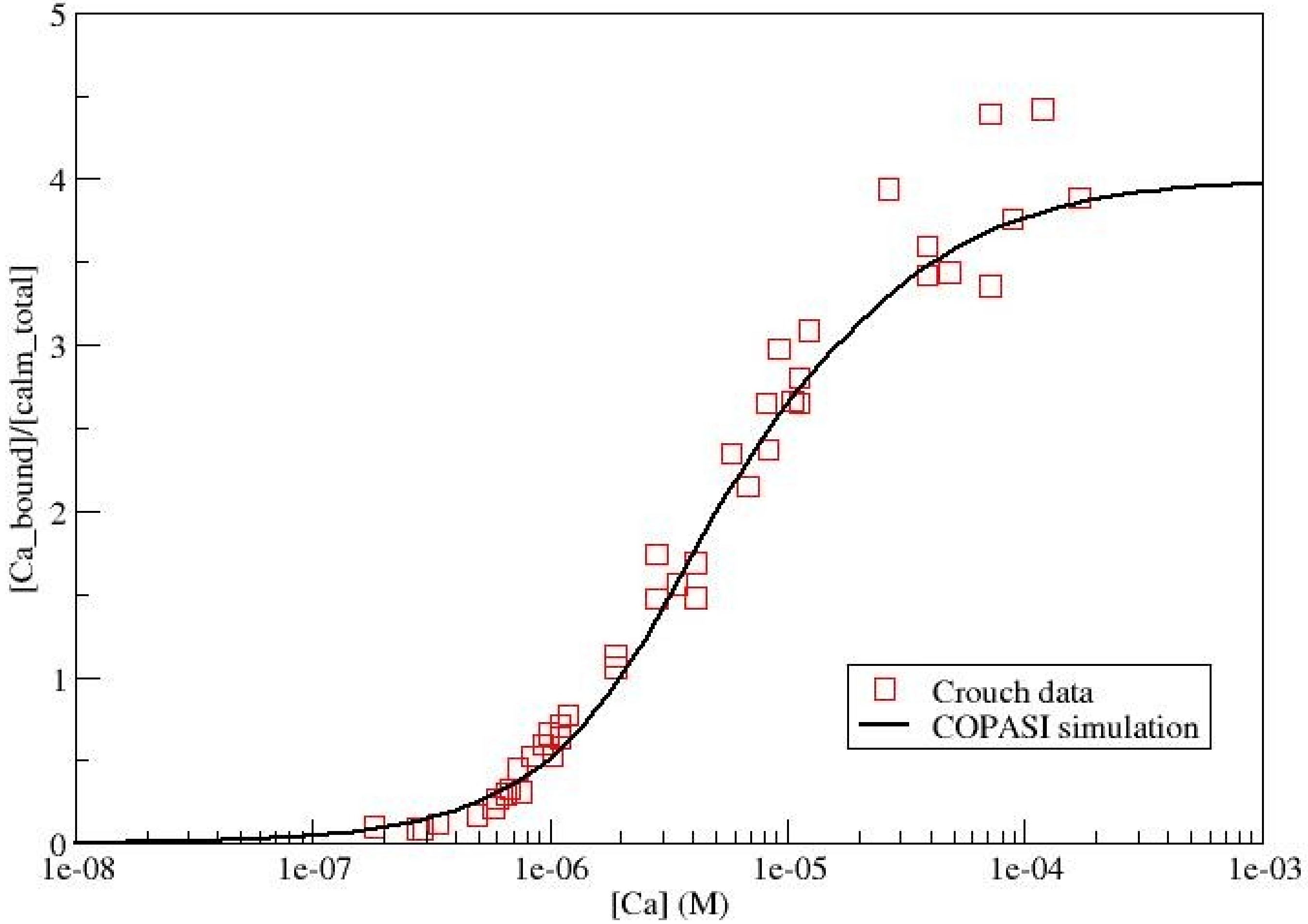
$K^R_C = 1.74\ 10^{-5}$

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

- 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

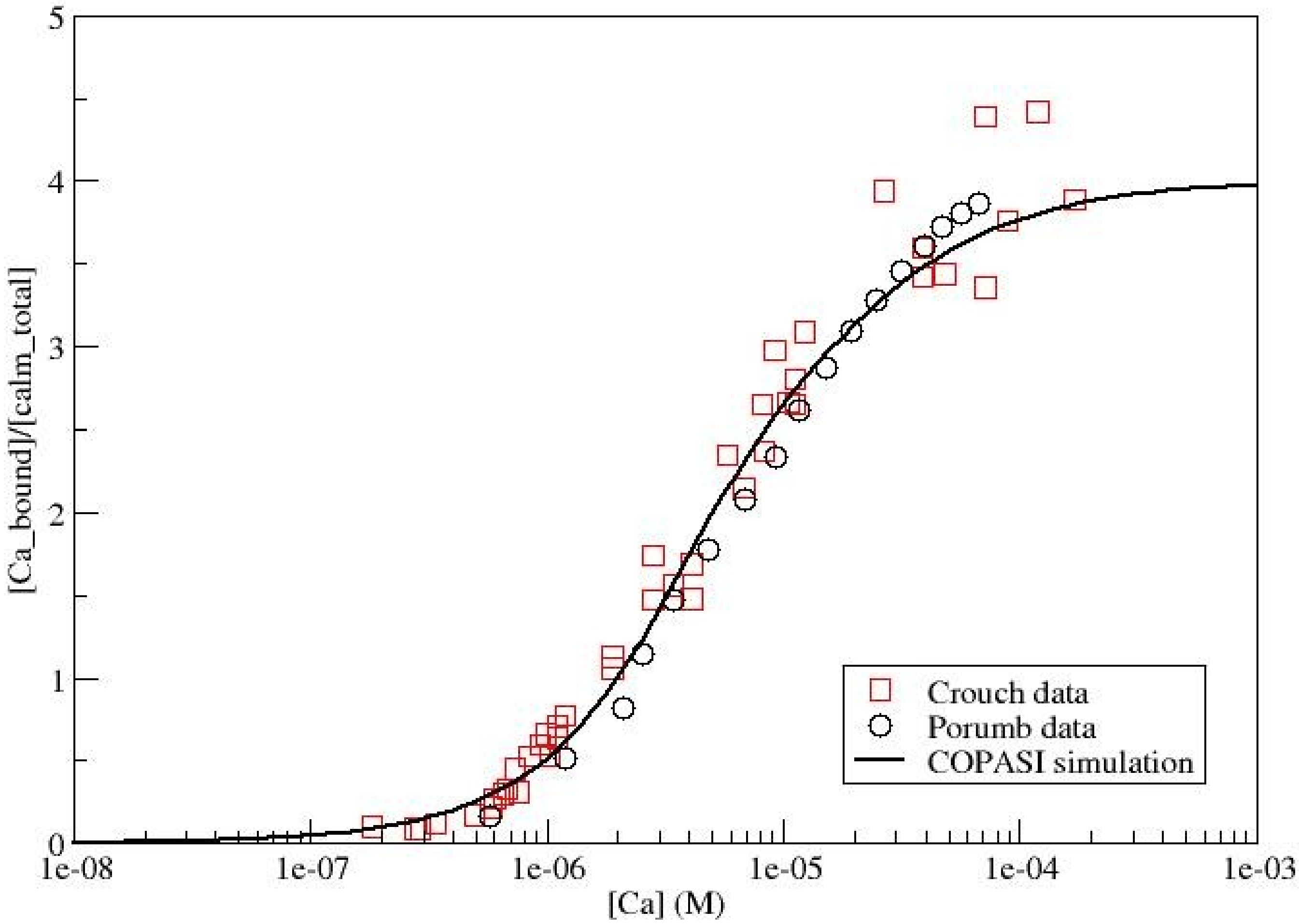


Comparison with experiments



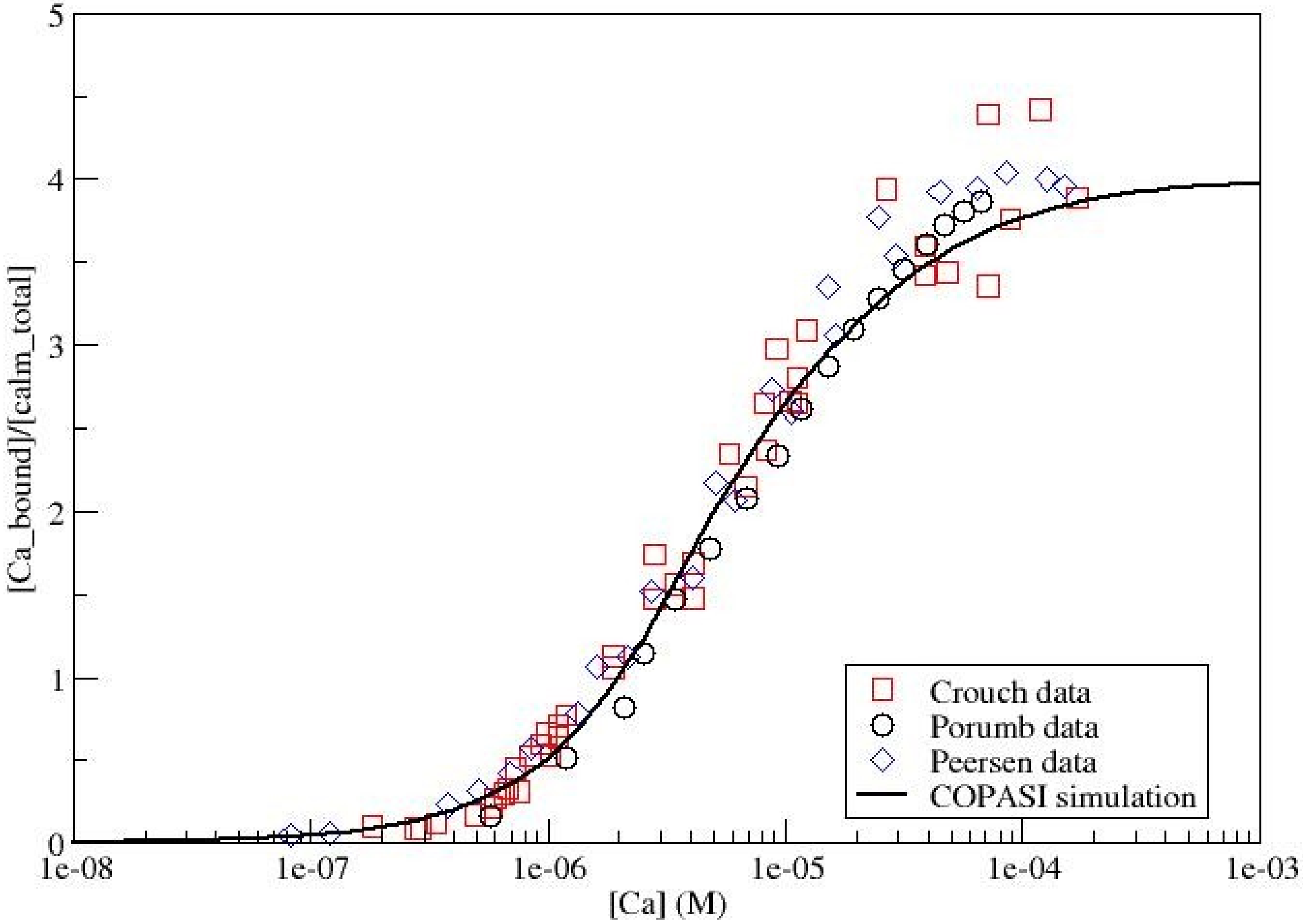
Crouch and Klee (1980) *Biochemistry*, 19: 3692-3698c

Comparison with experiments



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

Comparison with experiments



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

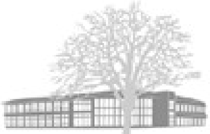
Derivation of Klotz's constants from MWC

$$K_1 = 4\frac{1+L}{\frac{1}{K_A^R}+\frac{1}{K_B^R}+\frac{1}{K_C^R}+\frac{1}{K_D^R}+L\left(\frac{1}{K_A^T}+\frac{1}{K_B^T}+\frac{1}{K_C^T}+\frac{1}{K_D^T}\right)}$$

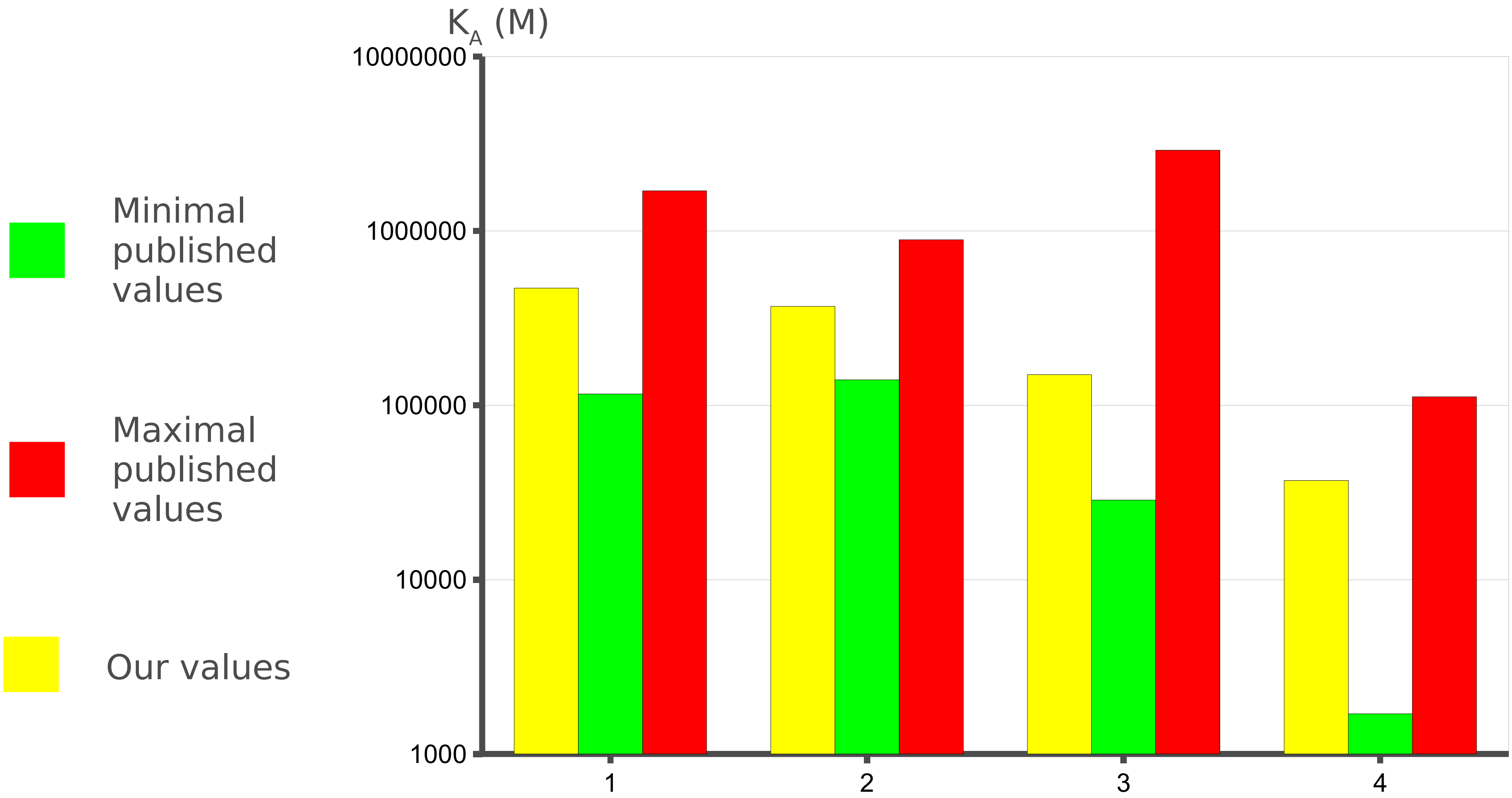
$$K_2 = \frac{3}{2}\frac{\frac{1}{K_A^R}+\frac{1}{K_B^R}+\frac{1}{K_C^R}+\frac{1}{K_D^R}+L\left(\frac{1}{K_A^T}+\frac{1}{K_B^T}+\frac{1}{K_C^T}+\frac{1}{K_D^T}\right)}{\frac{1}{K_A^RK_B^R}+\frac{1}{K_A^RK_C^R}+\frac{1}{K_A^RK_D^R}+\frac{1}{K_B^RK_C^R}+\frac{1}{K_B^RK_D^R}+\frac{1}{K_C^RK_D^R}+L\left(\frac{1}{K_A^TK_B^T}+\frac{1}{K_A^TK_C^T}+\frac{1}{K_A^TK_D^T}+\frac{1}{K_B^TK_C^T}+\frac{1}{K_B^TK_D^T}+\frac{1}{K_C^TK_D^T}\right)}$$

$$K_3 = \frac{2}{3}\frac{\frac{1}{K_A^RK_B^R}+\frac{1}{K_A^RK_C^R}+\frac{1}{K_A^RK_D^R}+\frac{1}{K_B^RK_C^R}+\frac{1}{K_B^RK_D^R}+\frac{1}{K_C^RK_D^R}+L\left(\frac{1}{K_A^TK_B^T}+\frac{1}{K_A^TK_C^T}+\frac{1}{K_A^TK_D^T}+\frac{1}{K_B^TK_C^T}+\frac{1}{K_B^TK_D^T}+\frac{1}{K_C^TK_D^T}\right)}{\frac{1}{K_A^RK_B^RK_C^R}+\frac{1}{K_A^RK_B^RK_D^R}+\frac{1}{K_A^RK_C^RK_D^R}+\frac{1}{K_B^RK_C^RK_D^R}+L\left(\frac{1}{K_A^TK_B^TK_C^T}+\frac{1}{K_A^TK_B^TK_D^T}+\frac{1}{K_A^TK_C^TK_D^T}+\frac{1}{K_B^TK_C^TK_D^T}\right)}$$

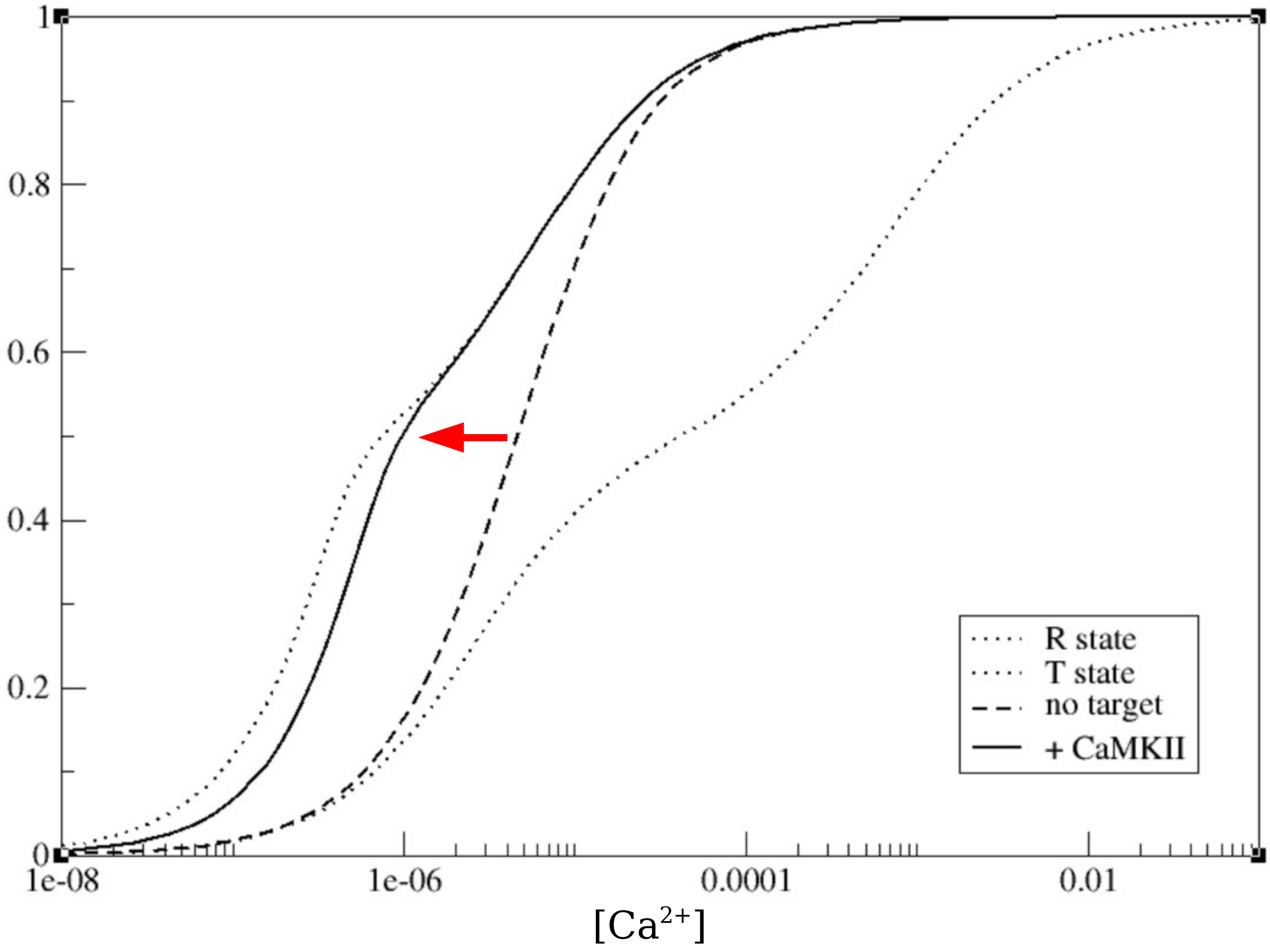
$$K_4 = \frac{1}{4}\frac{\frac{1}{K_A^RK_B^RK_C^R}+\frac{1}{K_A^RK_B^RK_D^R}+\frac{1}{K_A^RK_C^RK_D^R}+\frac{1}{K_B^RK_C^RK_D^R}+L\left(\frac{1}{K_A^TK_B^TK_C^T}+\frac{1}{K_A^TK_B^TK_D^T}+\frac{1}{K_A^TK_C^TK_D^T}+\frac{1}{K_B^TK_C^TK_D^T}\right)}{\frac{1}{K_A^RK_B^RK_C^RK_D^R}+L\frac{1}{K_A^TK_B^TK_C^TK_D^T}}$$



Comparison with measured K_{ASSOC}



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



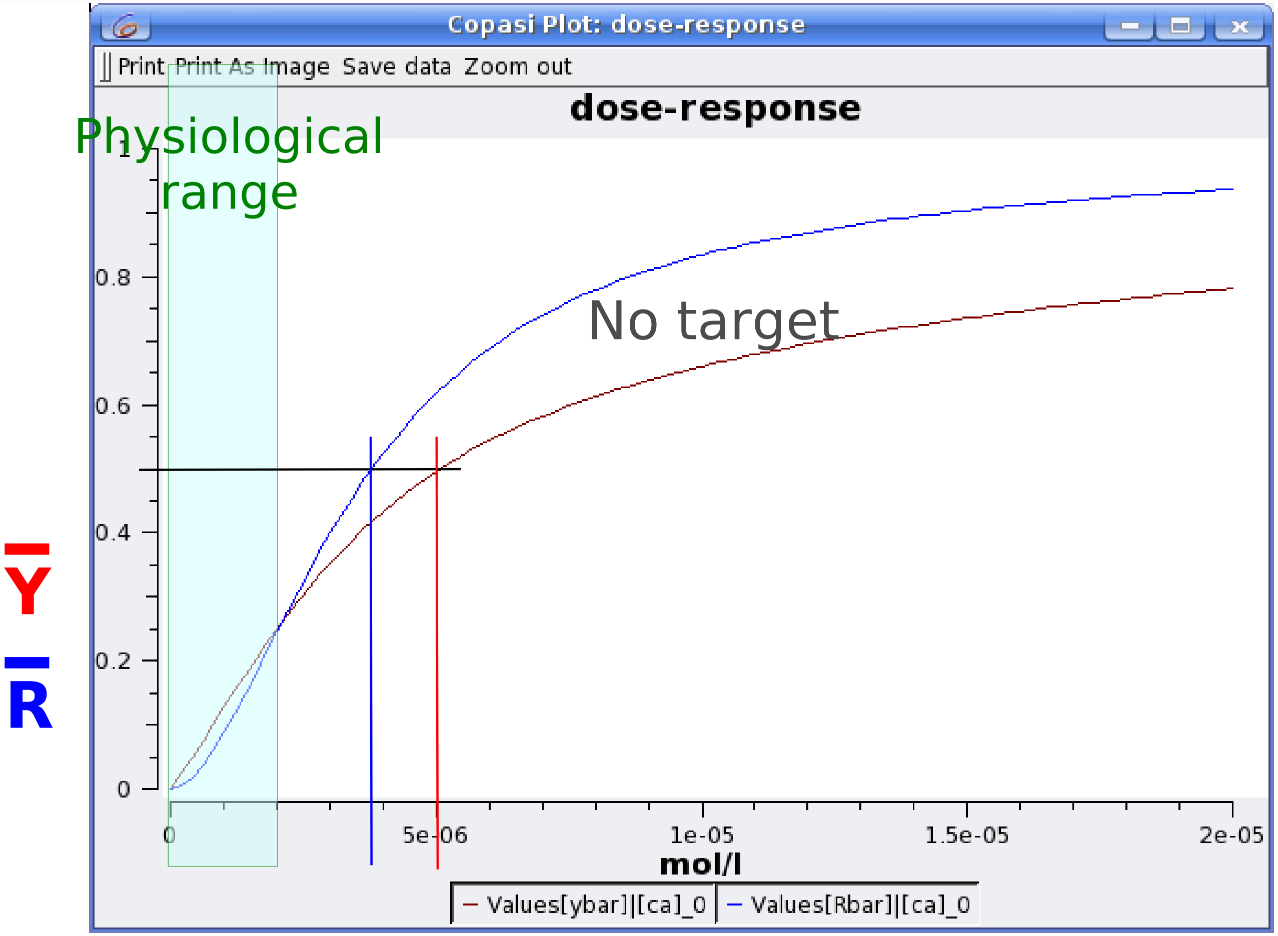
Activity of unsaturated calmodulin

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

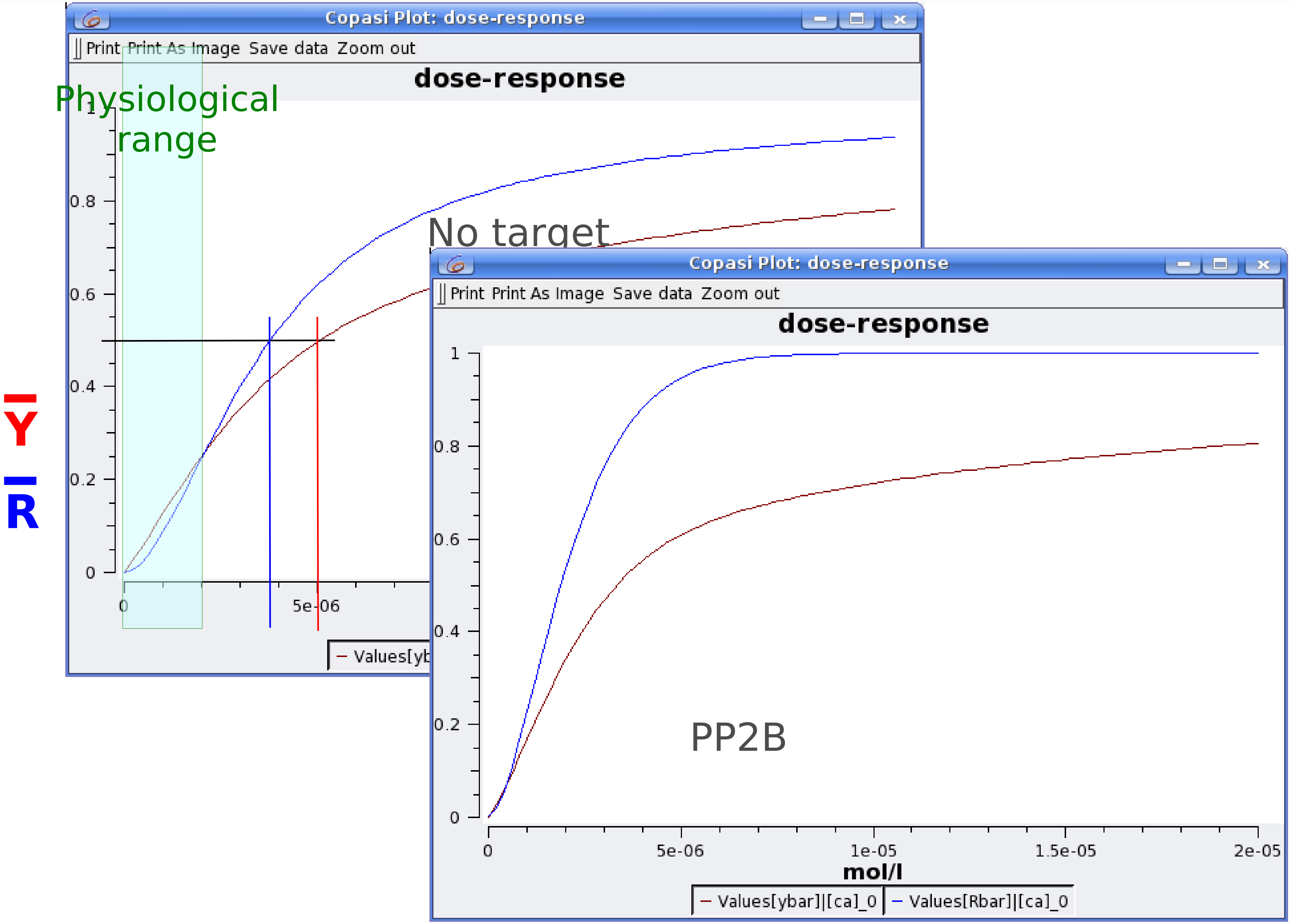
$$\frac{R_2}{T_2} = \frac{1}{L \cdot c^2}$$

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

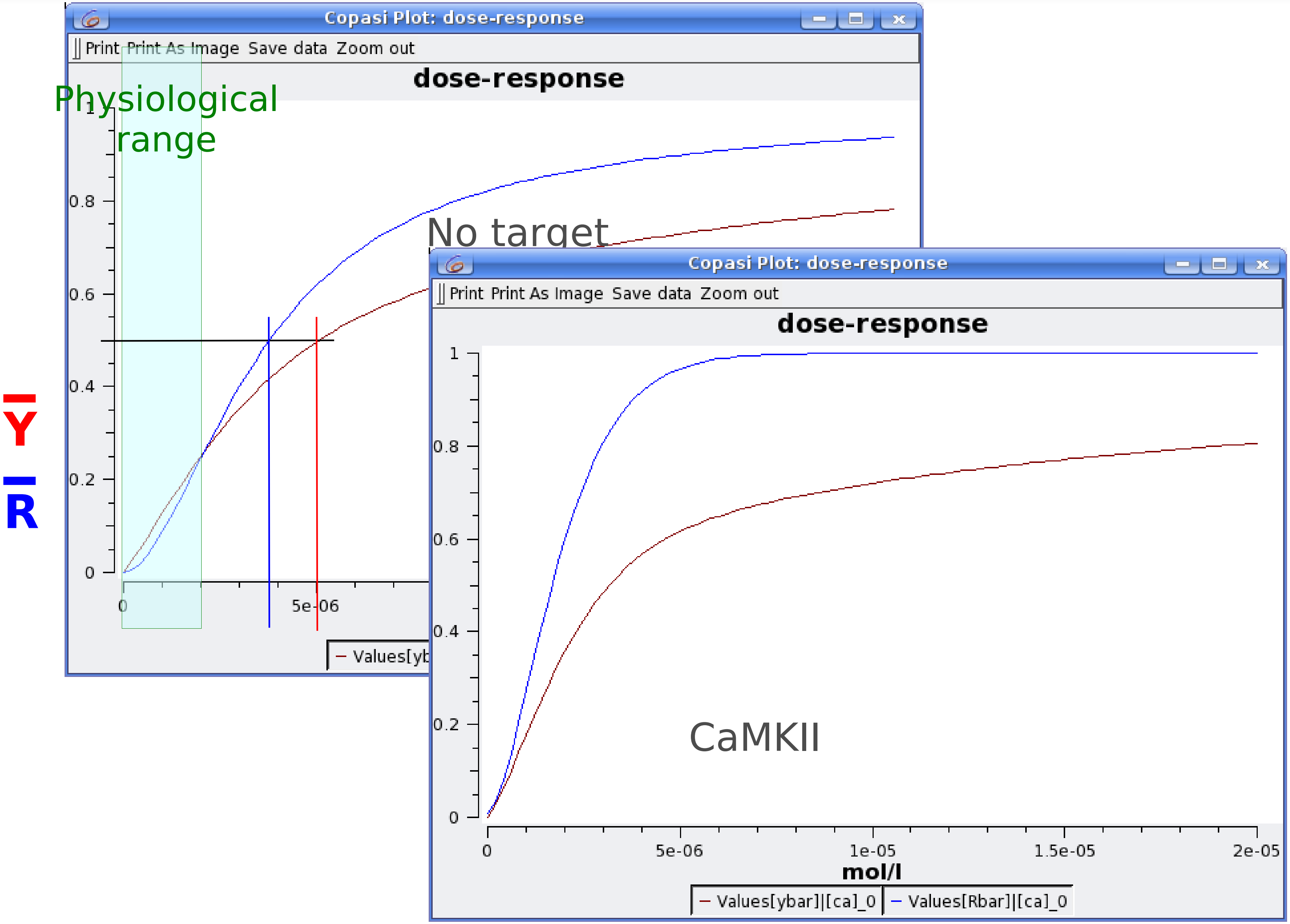
But ... we're out of the physiological range?



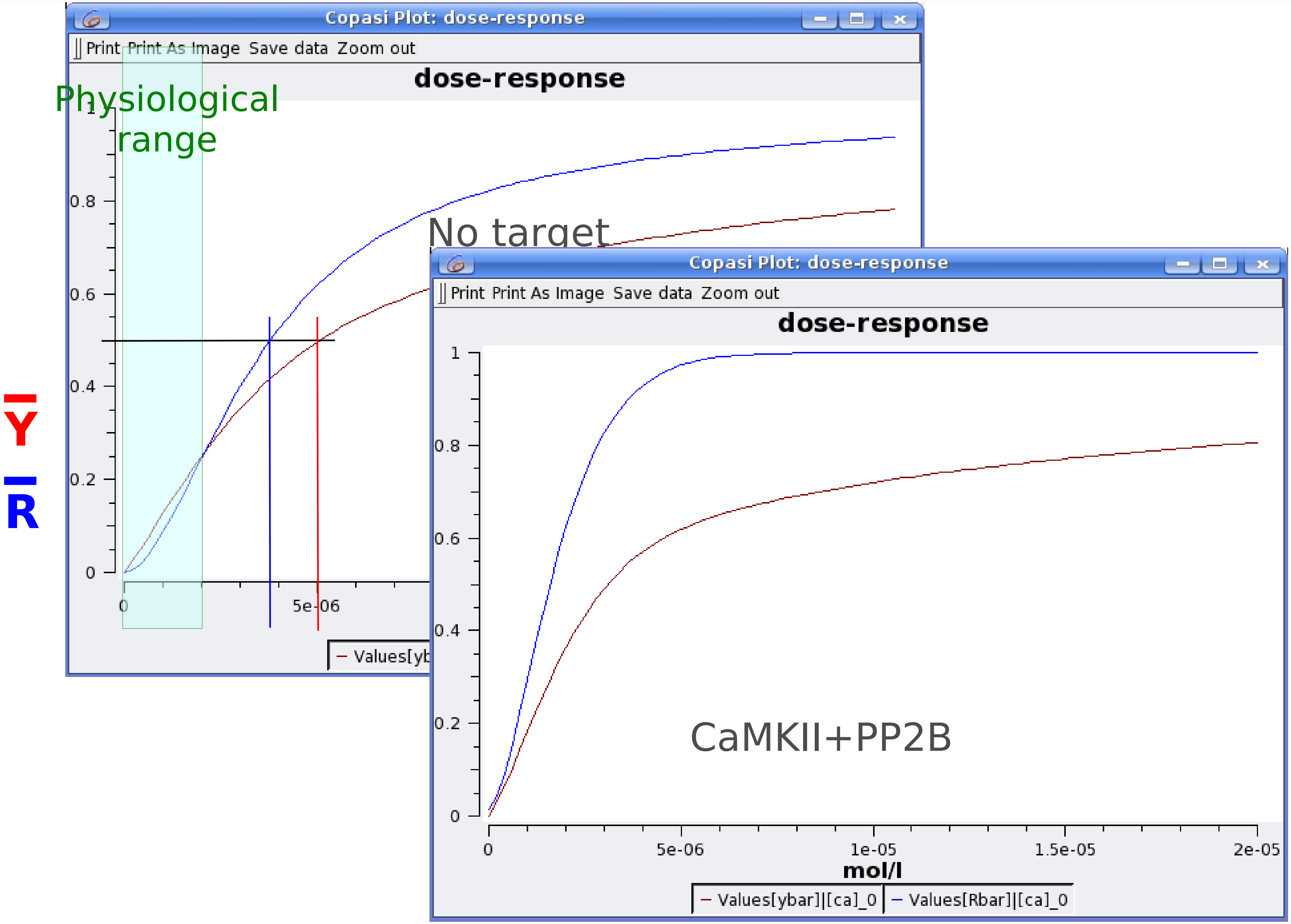
But … we're out of the physiological range?



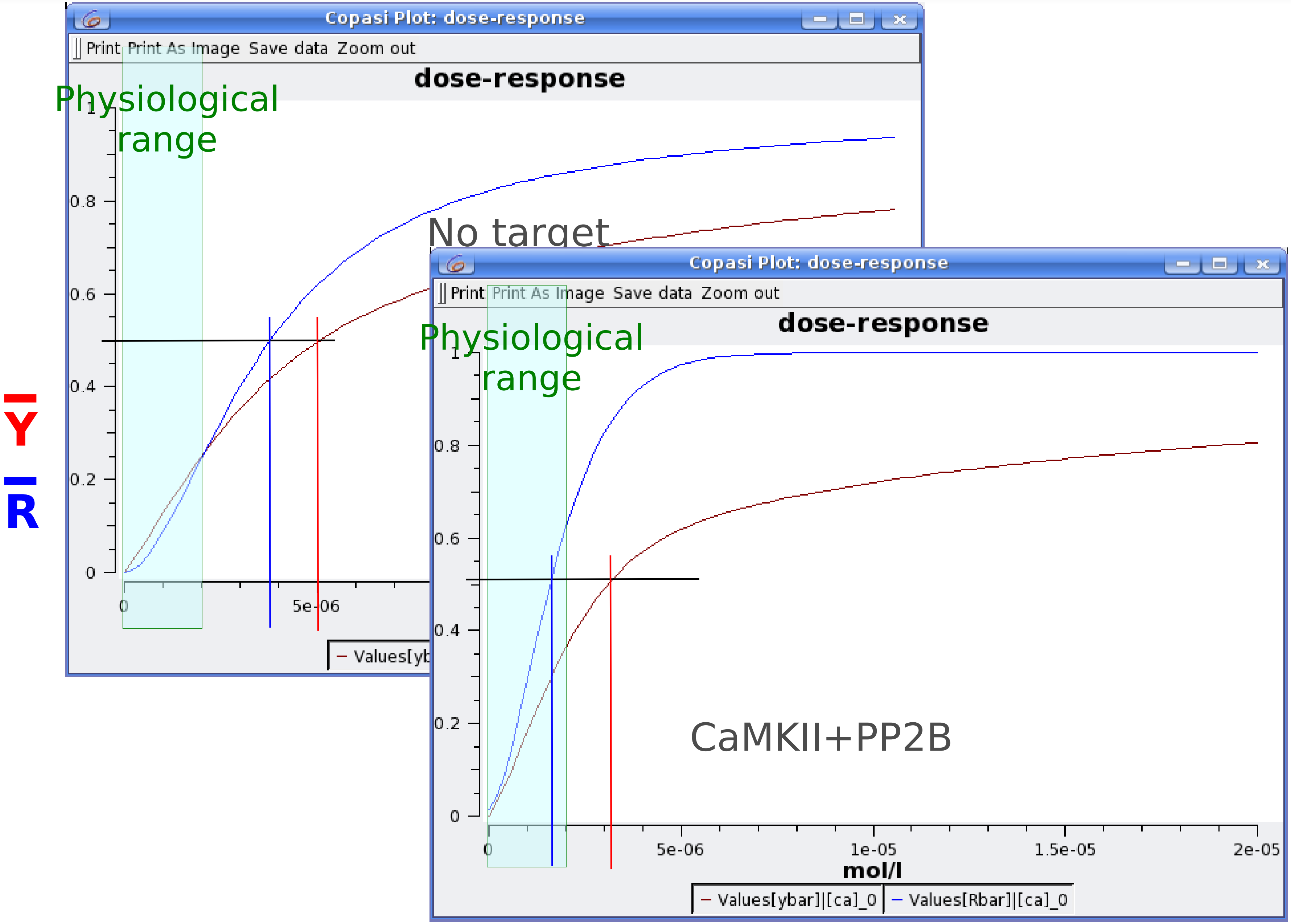
But ... we're out of the physiological range?



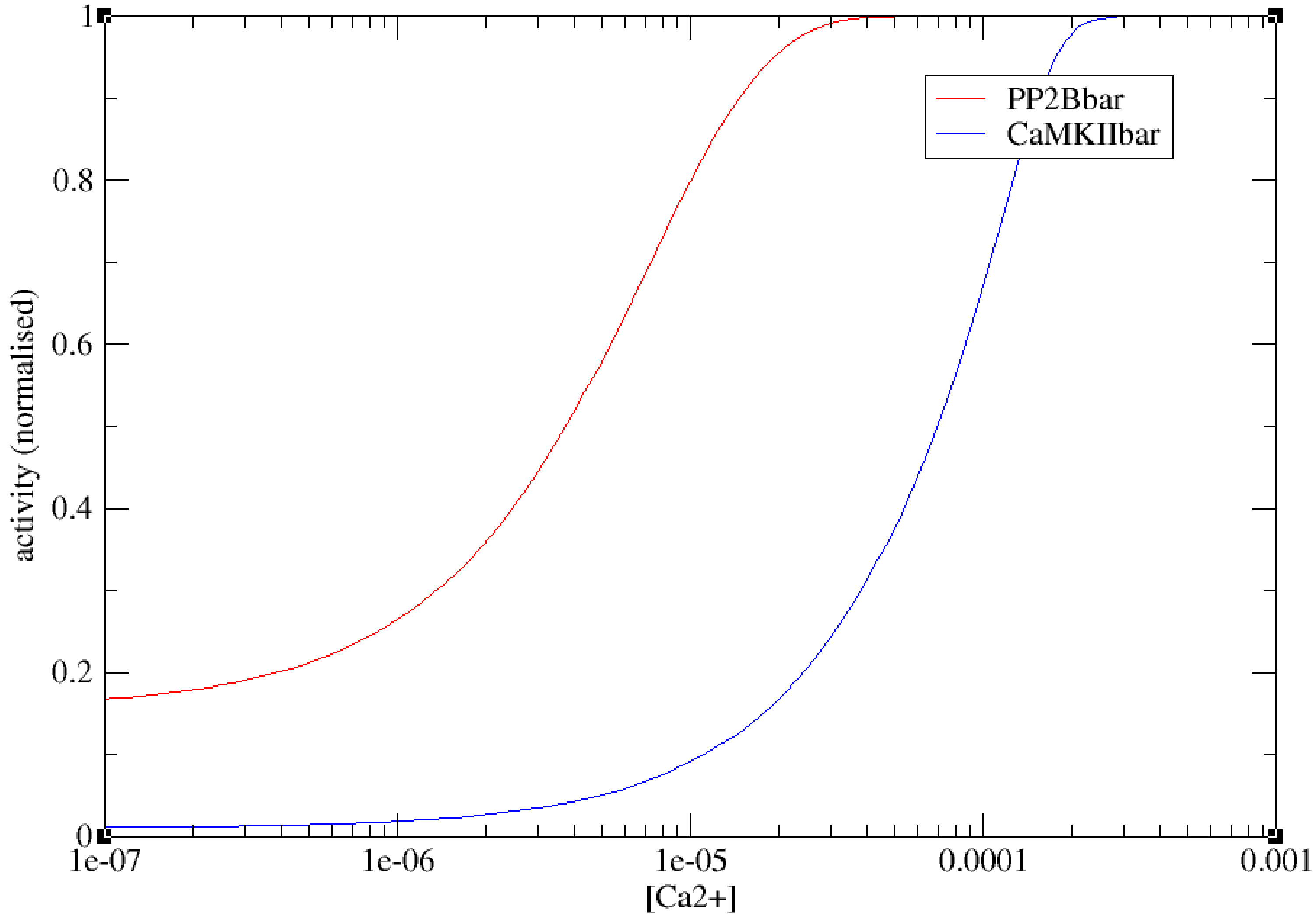
But … we're out of the physiological range?



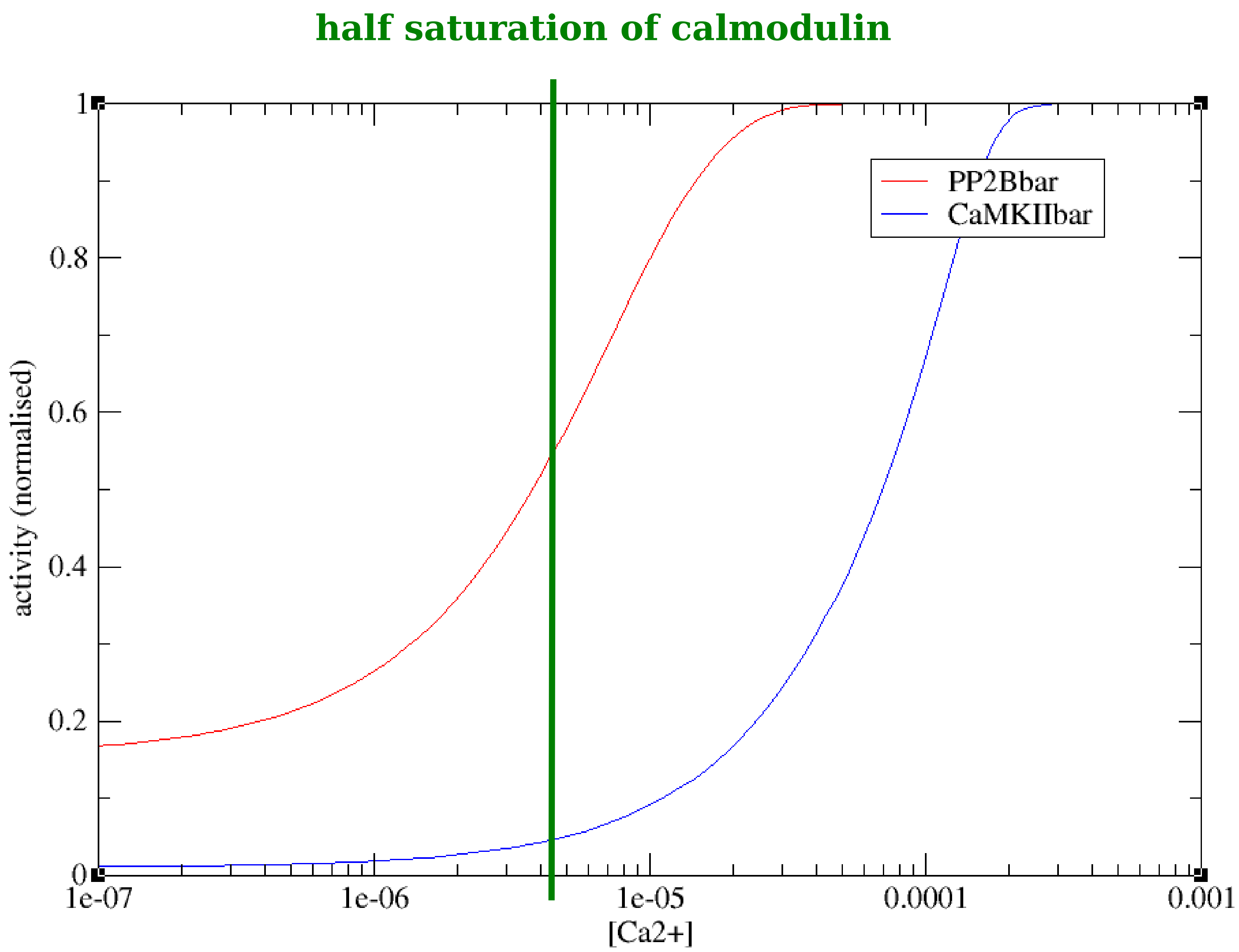
This is Systems Biology!



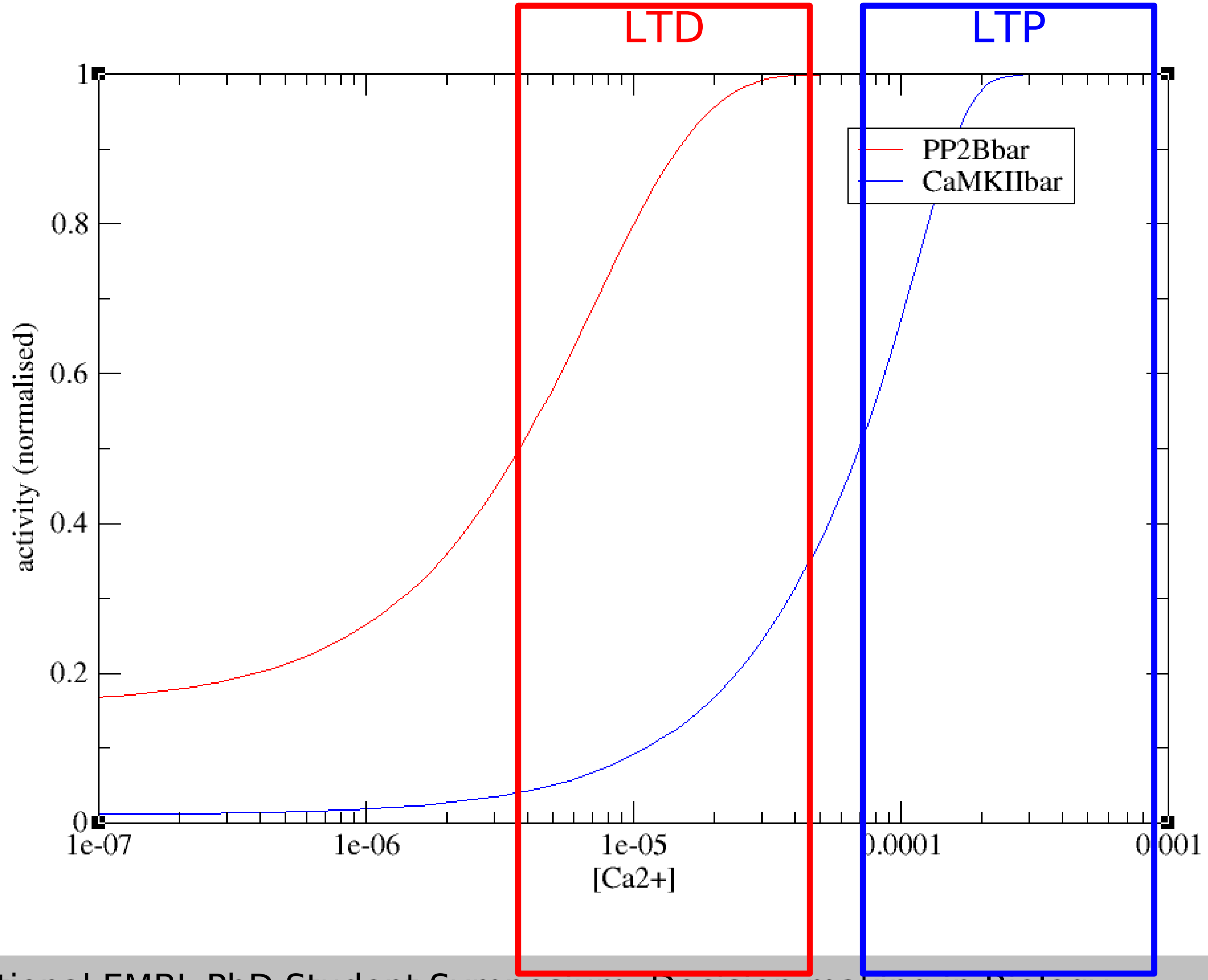
Bidirectional synaptic plasticity



Bidirectional synaptic plasticity



Bidirectional synaptic plasticity



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

Melanie Stefan

Stuart Edelstsein

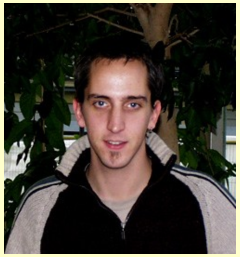
The team


Ranjita Dutta-Roy, SE


Melanie Stefan, AU

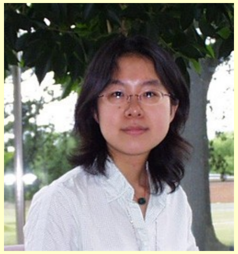

Stuart Edelstein, CH, US

Cooperativity crew


Dominic Tolle, EI, DE


Michele Mattioni, IT

Spatial simulation crew


Lu Li, CN


Noriko Hiroi, JP

Signalling pathways crew


Nick Juty, UK


Camille Laibe, FR


Christian Knüpfer, DE


Dagmar Köhn,DE

Standard and ontology crew

BioModels DB crew


Viji Chelliah


Lukas Endler


Chen Li

SBML crew


Nicolas Rodriguez, FR


Duncan Berenguier, FR


Anika Oellrich, DE


Kedar, IN