1992 Institut Pasteur 1999

Student (Msc, PhD)
(Jean-Pierre Changeux)
Nicotinic receptors:
Bioinformatics,
Neuroanatomy
Behaviour

2001 Institut Pasteur 2003

CNRS Researcher
(Jean-Pierre Changeux)
Nicotinic receptors:
Bioinformatics

"A tale of two cities"

1999 2001

EMBO post-doc
(Dennis Bray)
Bacterial chemotaxis:
Bioinformatics,
Mathematical Modelling

2003 EMBL-EBI 2012

Group leader
Systems Biology
Synaptic signalling:
Modelling, knowledge
Representation, databases

2012 Babraham Institute

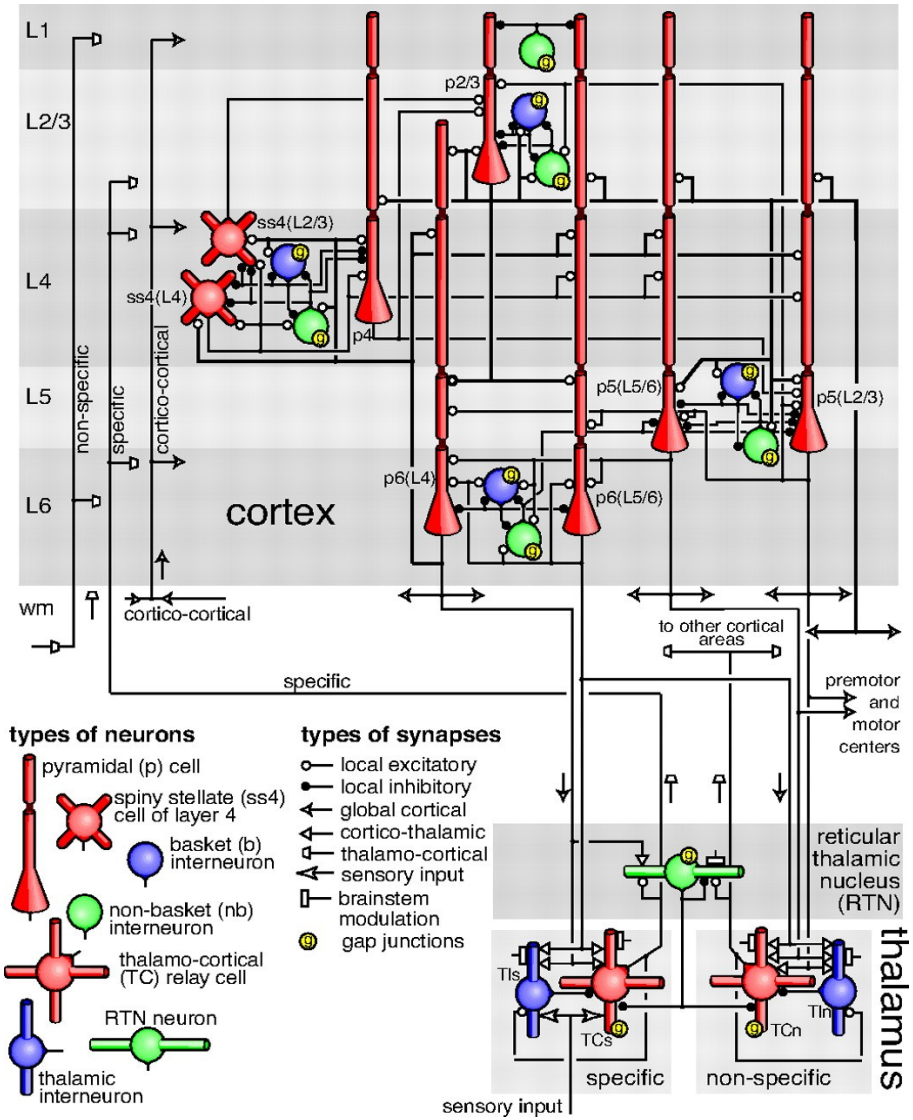
Senior group leader
Signalling
neurodegeneration
Stem cells

Allosteric calcium sensors and signalling switching

Nicolas Le Novère, Babraham Institute
n.lenovere@gmail.com
http://lenoverelab.org

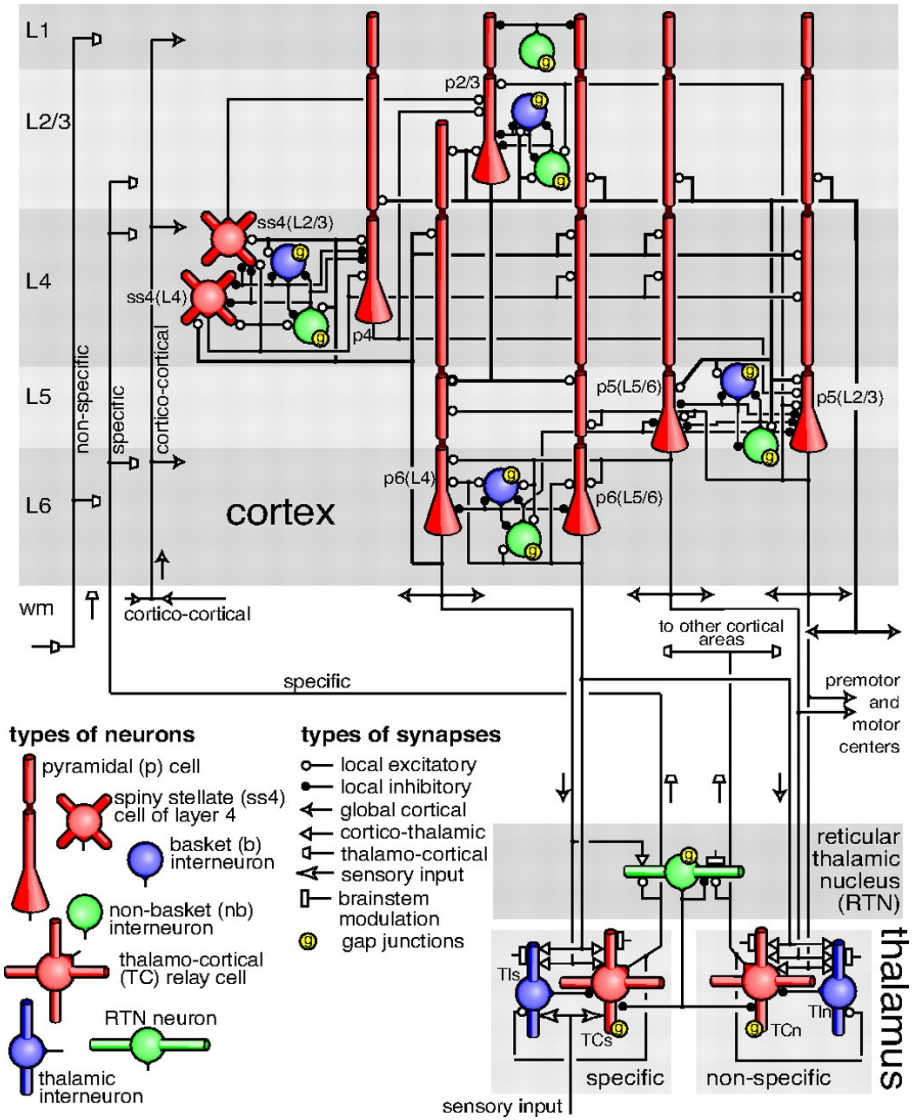
Babraham Institute

The brain as an electrical circuit?



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

The brain as an electrical circuit?



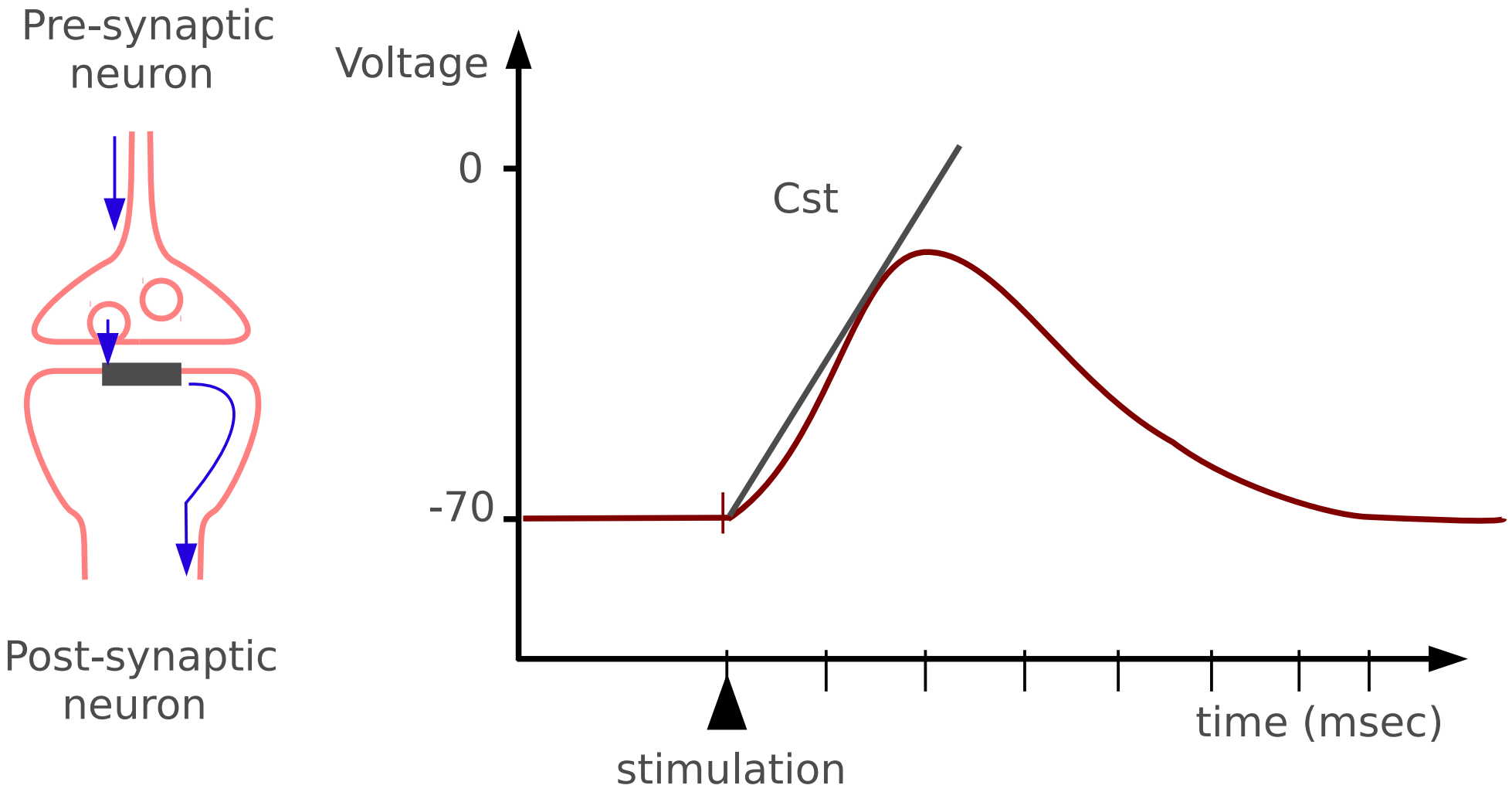
BUT

With variable topology

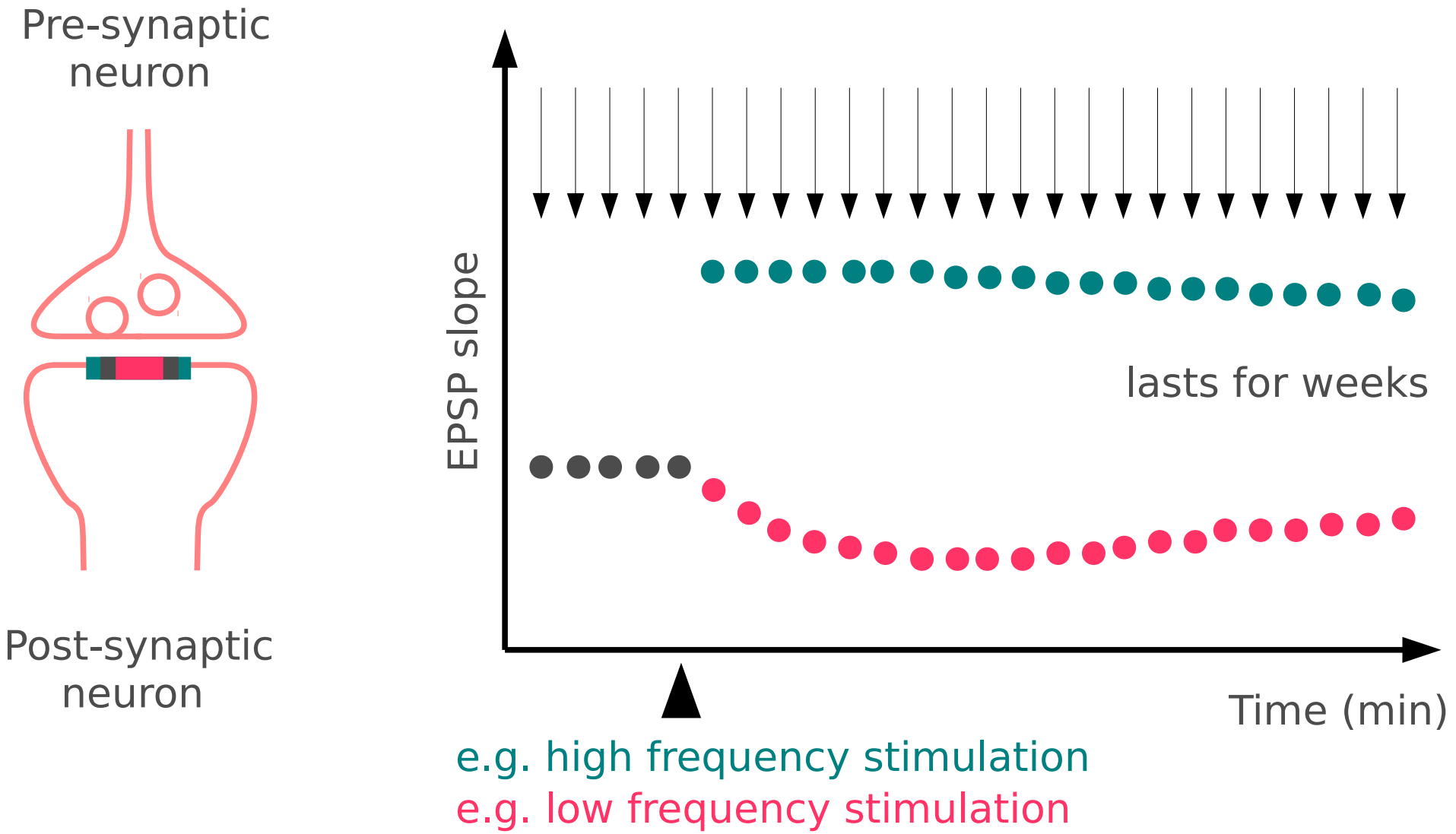
With variable connexions strength

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

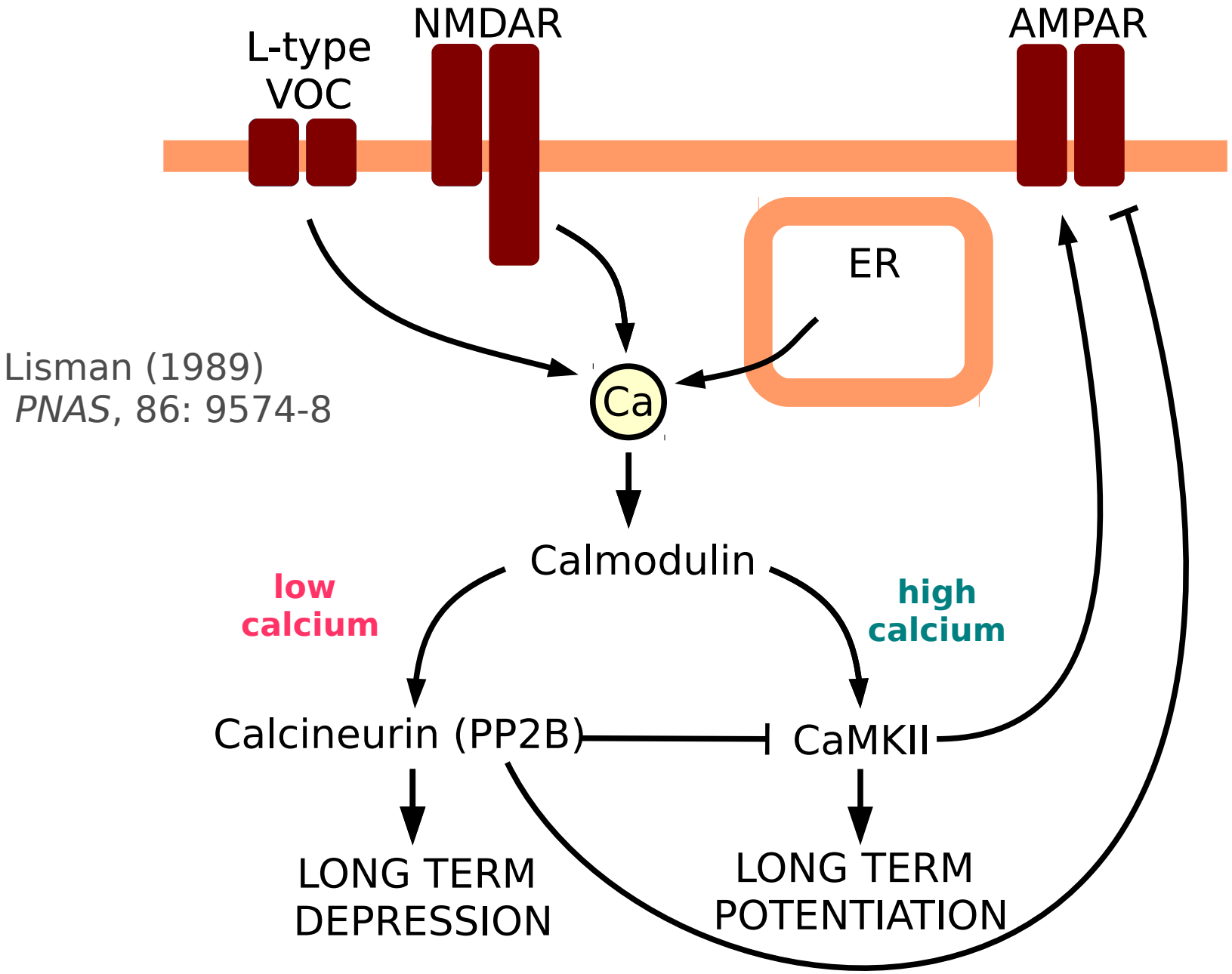
Excitatory post-synaptic potential



Bidirectional synaptic plasticity



Calmodulin, the memory switch



Lisman (1989)
PNAS, 86: 9574-8

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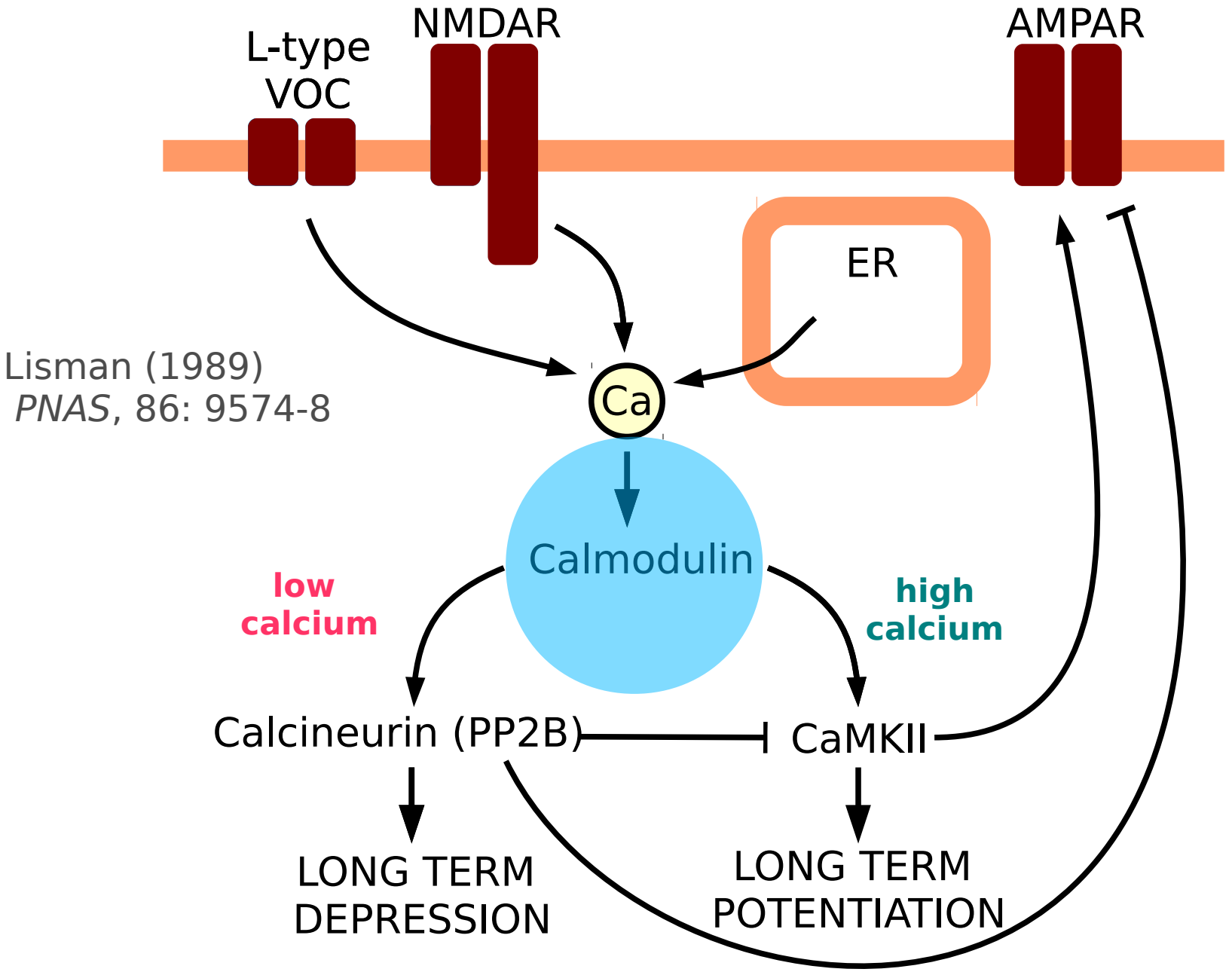
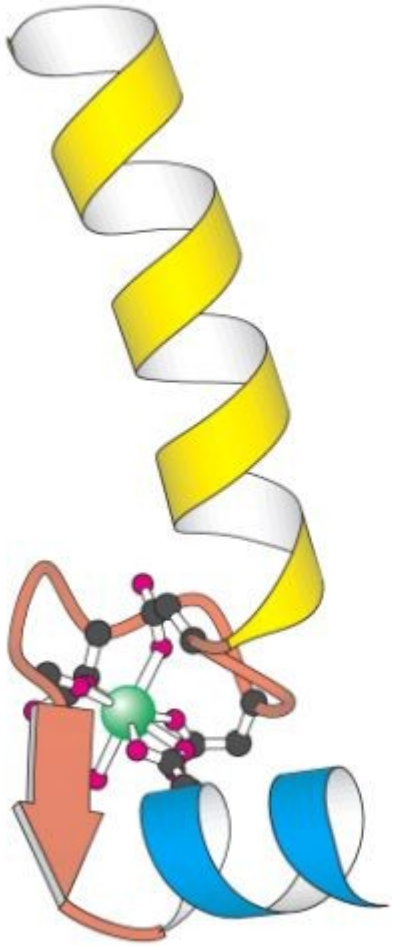
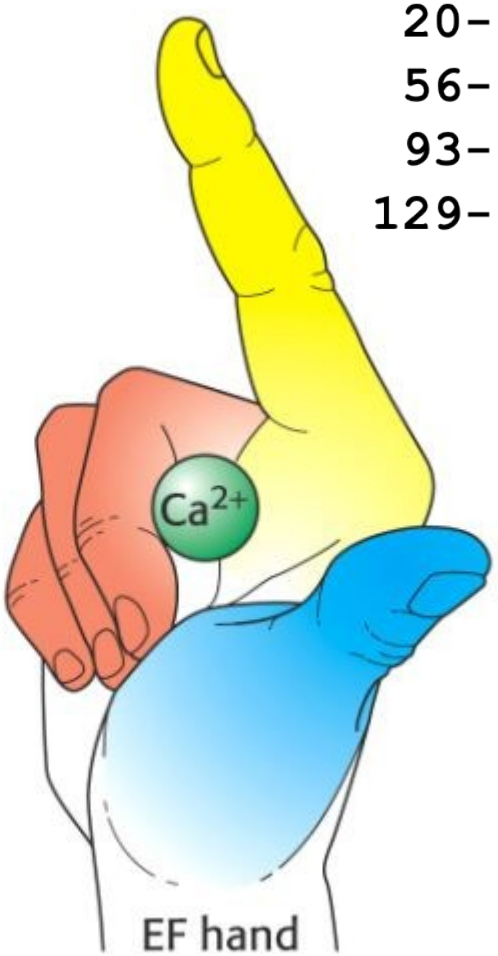


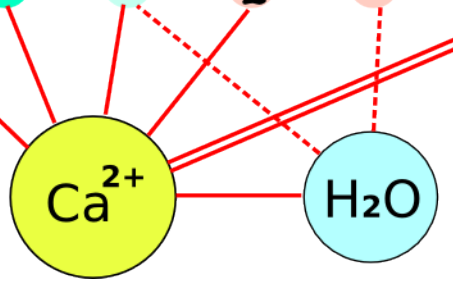
Lisman (1989)
PNAS, 86: 9574-8

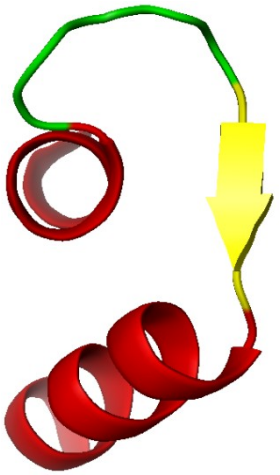
Structure of Calmodulin

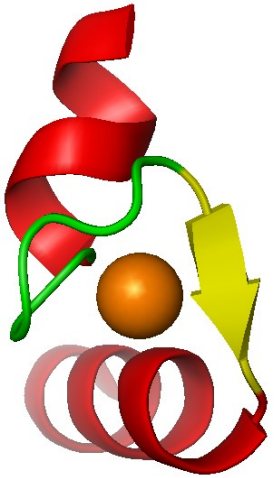




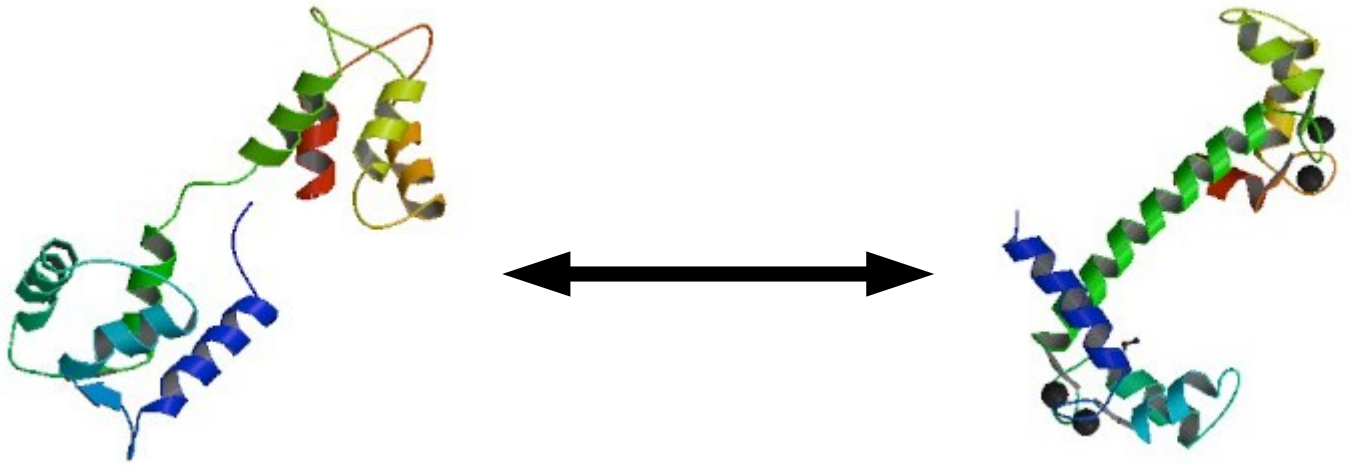
	X	Y	Z	-Y	-X	-Z	
20-	D	K	D	G	D	G	T	I	T	T	K	E	- 31
56-	D	A	D	G	N	G	T	I	D	F	P	E	- 67
93-	D	K	D	G	N	G	Y	I	S	A	A	E	-104
129-	D	I	D	G	D	G	Q	V	N	Y	E	E	-140







State transitions of calmodulin



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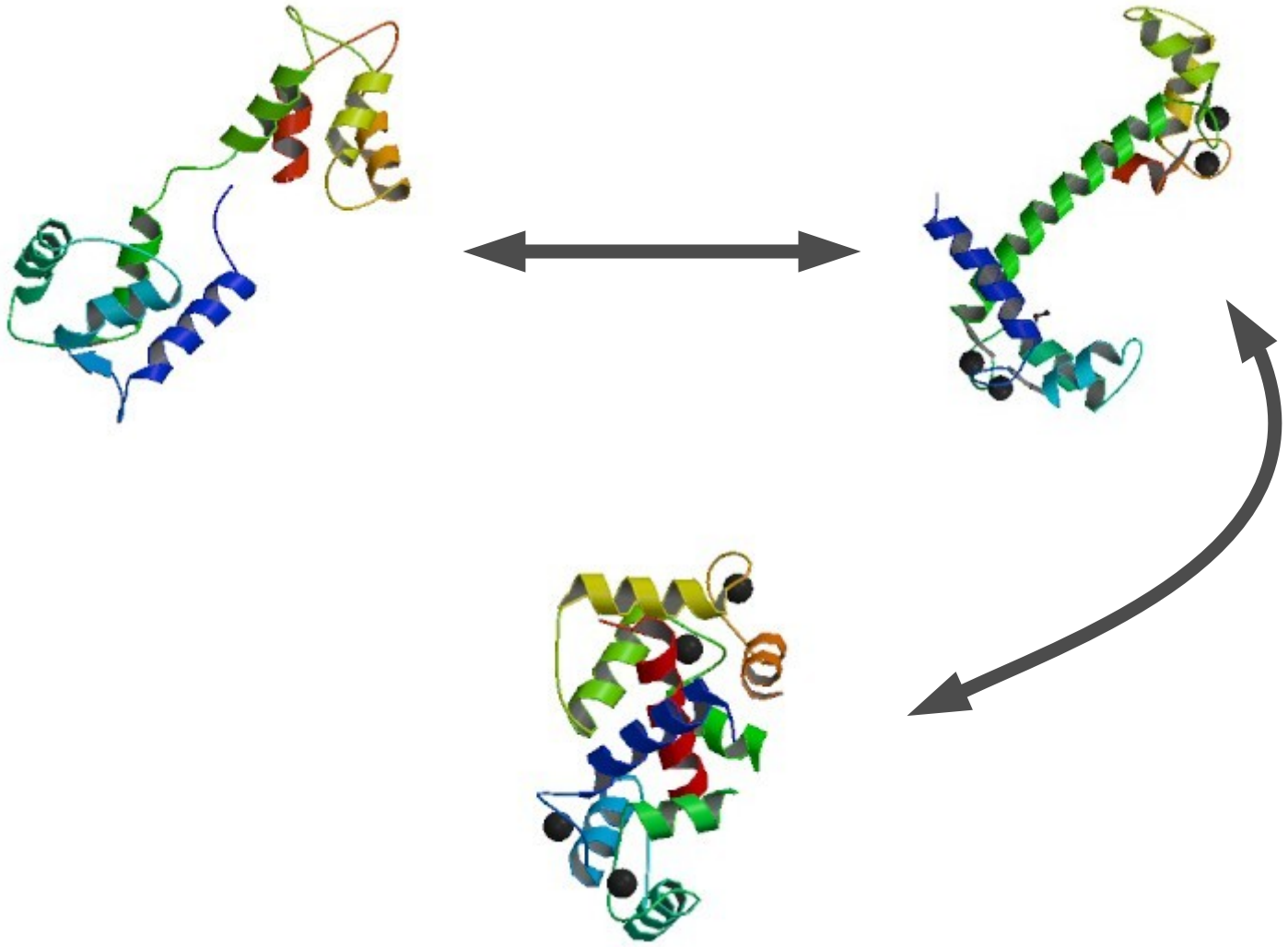
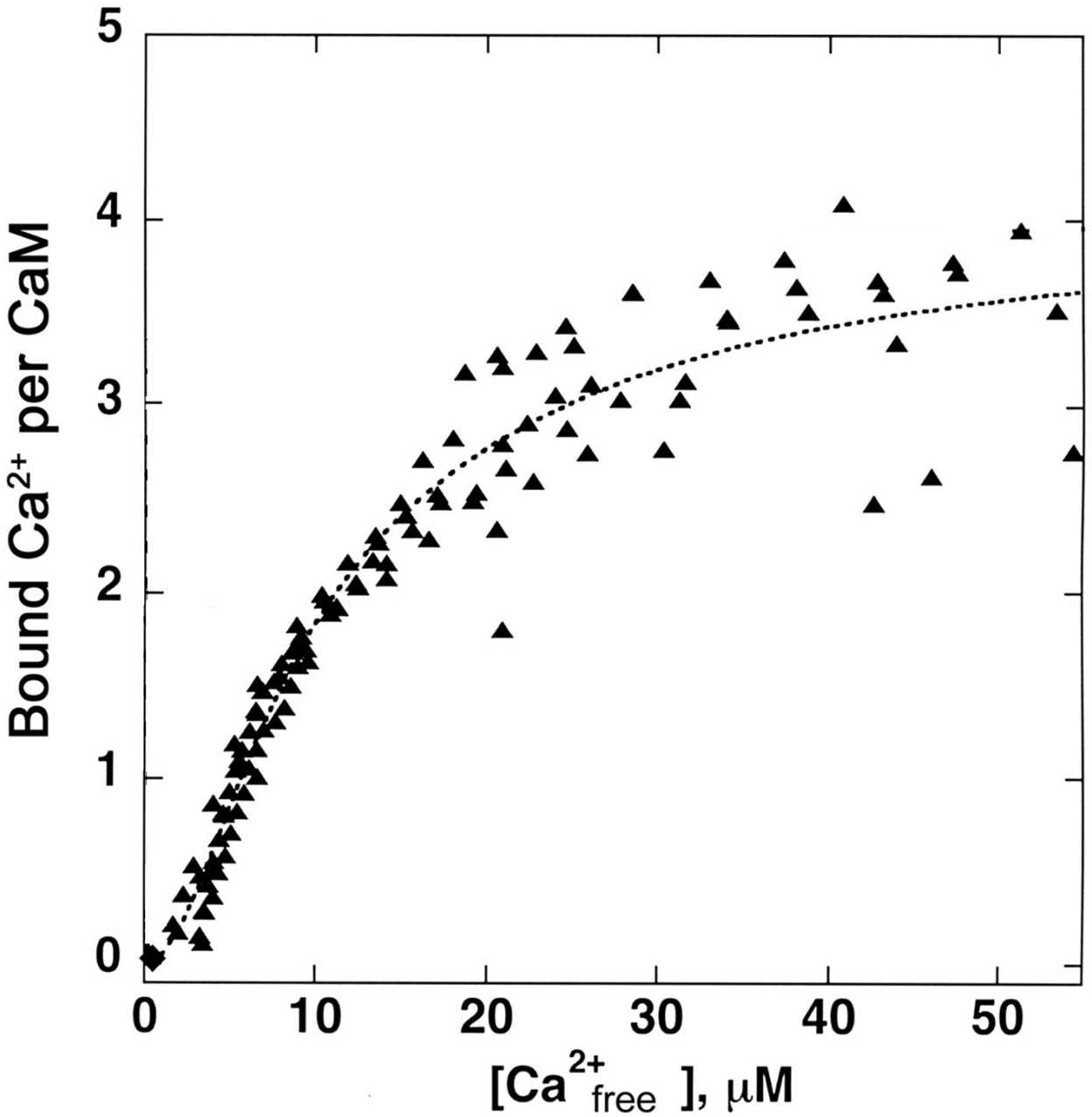
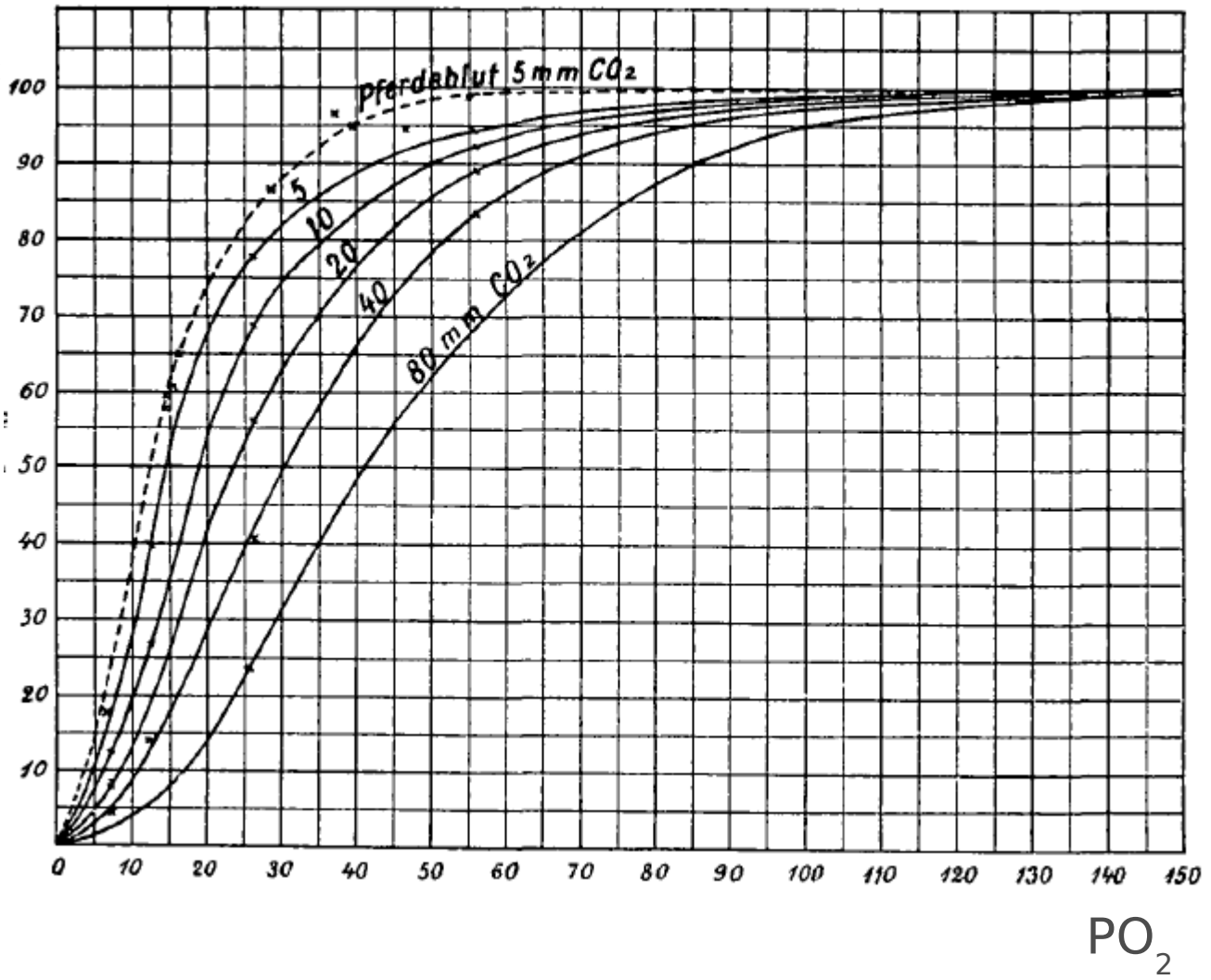


Calmodulin is ultra-sensitive



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

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

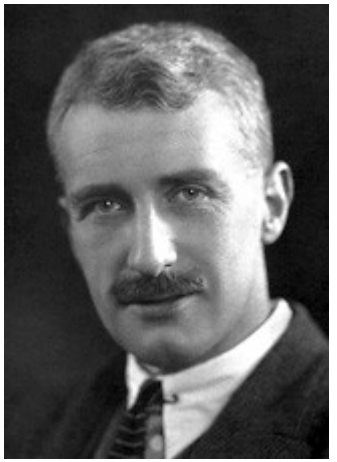
where Hb_n represents the aggregate of n molecules of Hb. I have supposed that in every solution there are many different sized aggregates, corresponding to many values of n.

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

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

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

Hill (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 $\mathrm{Hb_2}$: and as the calculation of the constants in these equations is very tedious I decided to try whether the equation

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

would satisfy the observations.

Origins of cooperativity: Hill

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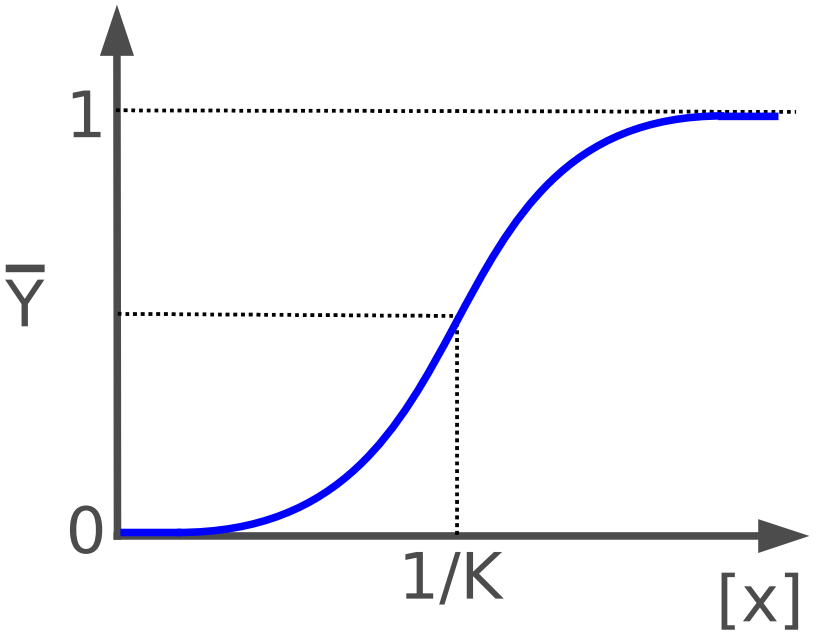


Babraham Institute

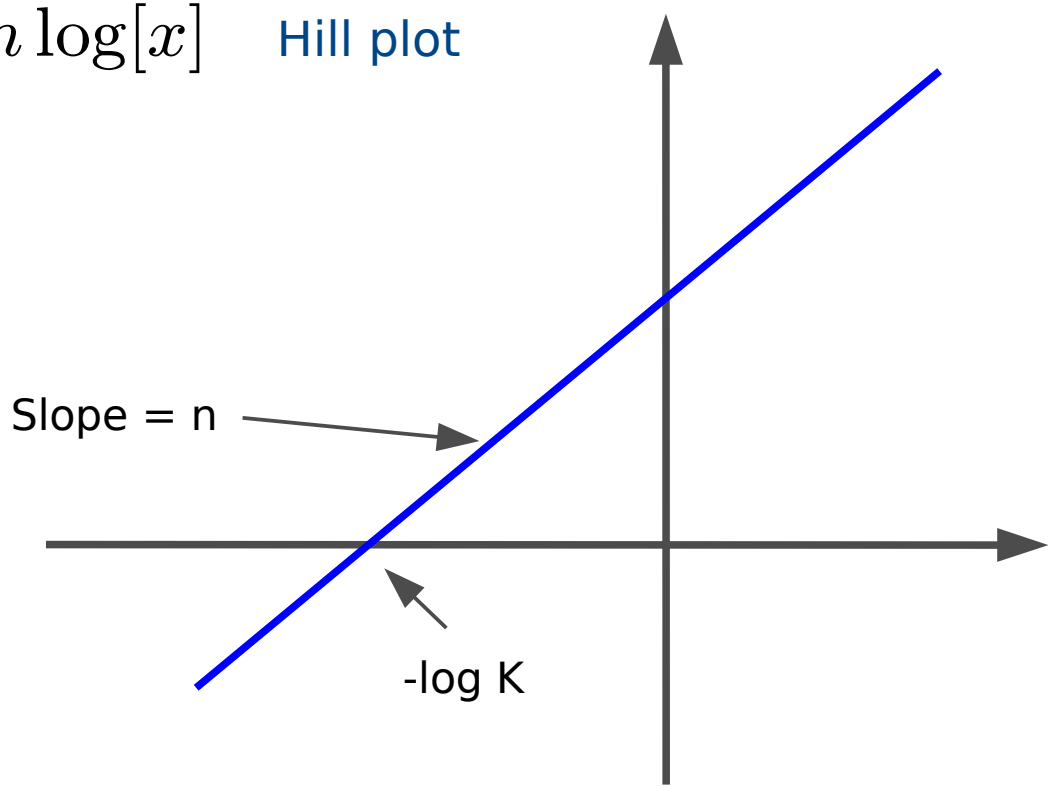
Hill equation can be linearised

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

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

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

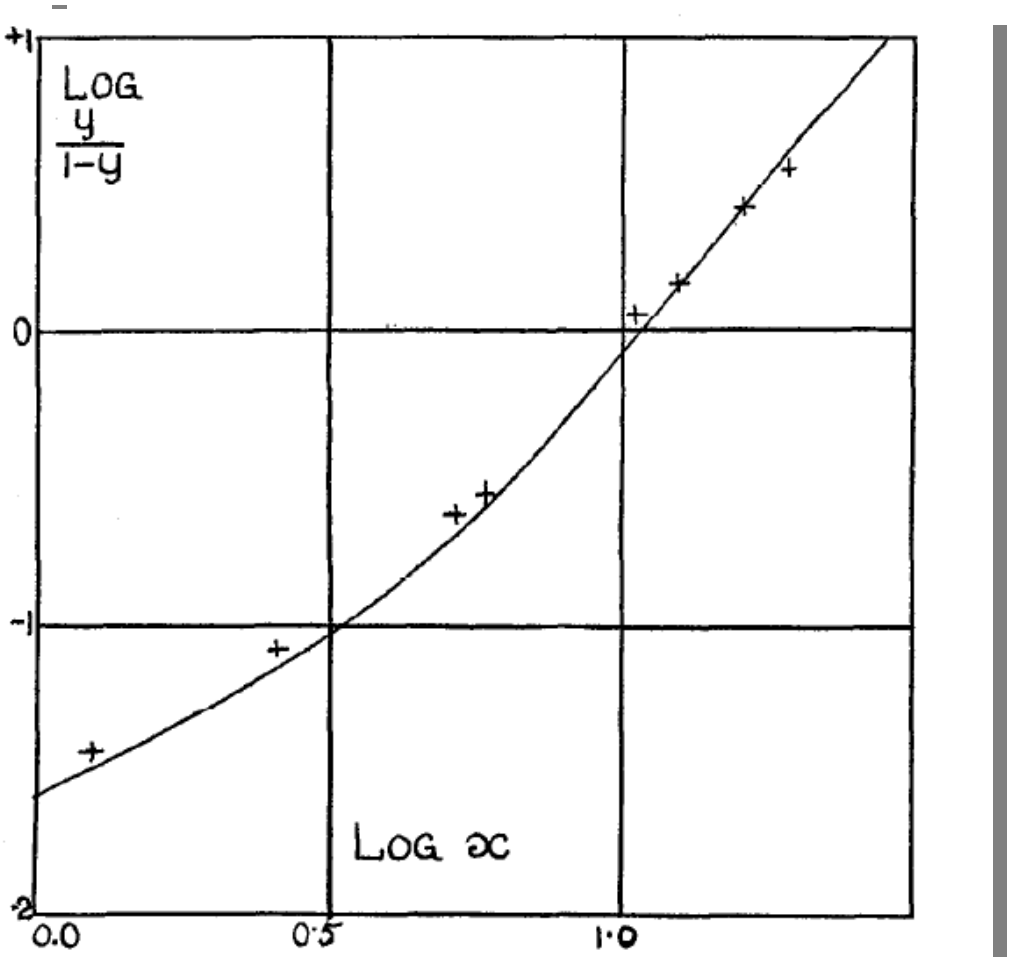


FIG. 2. Test of formula (6). Curve drawn from 6 experimental points from Table IV.

Origins of cooperativity: Adair-Klotz

THE HEMOGLOBIN SYSTEM.

VI. THE OXYGEN DISSOCIATION CURVE OF HEMOGLOBIN.*

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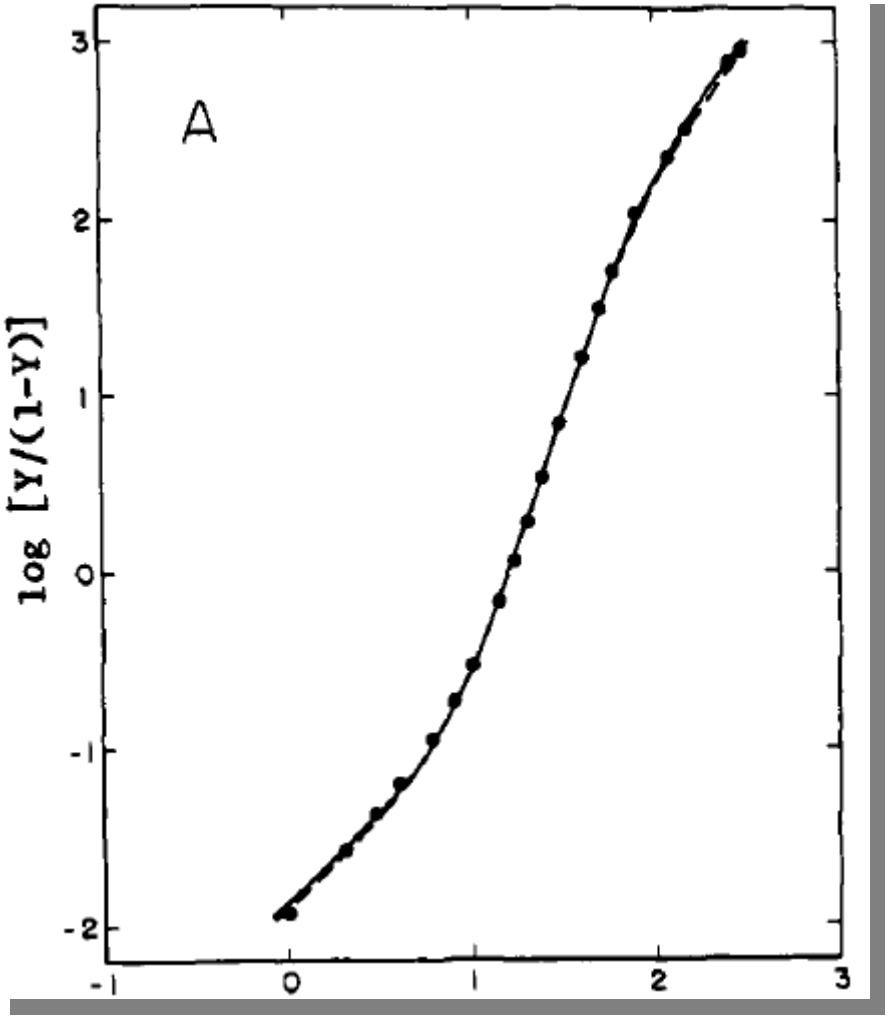
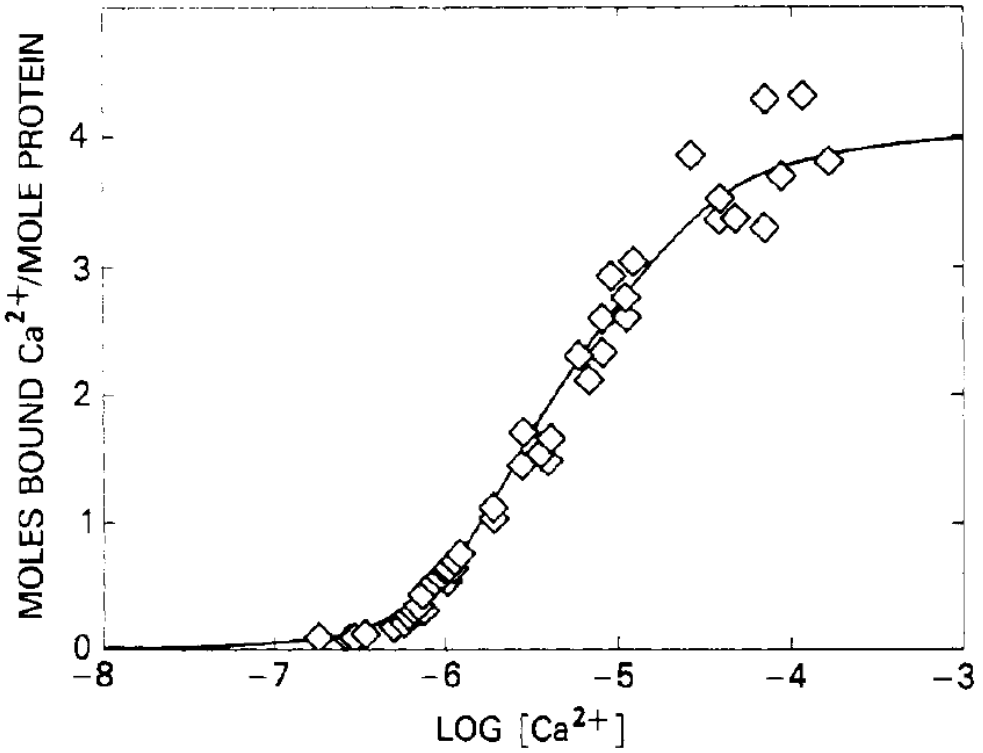


Imai (1973) *Biochemistry* 12: 798-808

Adair-Klotz model applied to Calmodulin

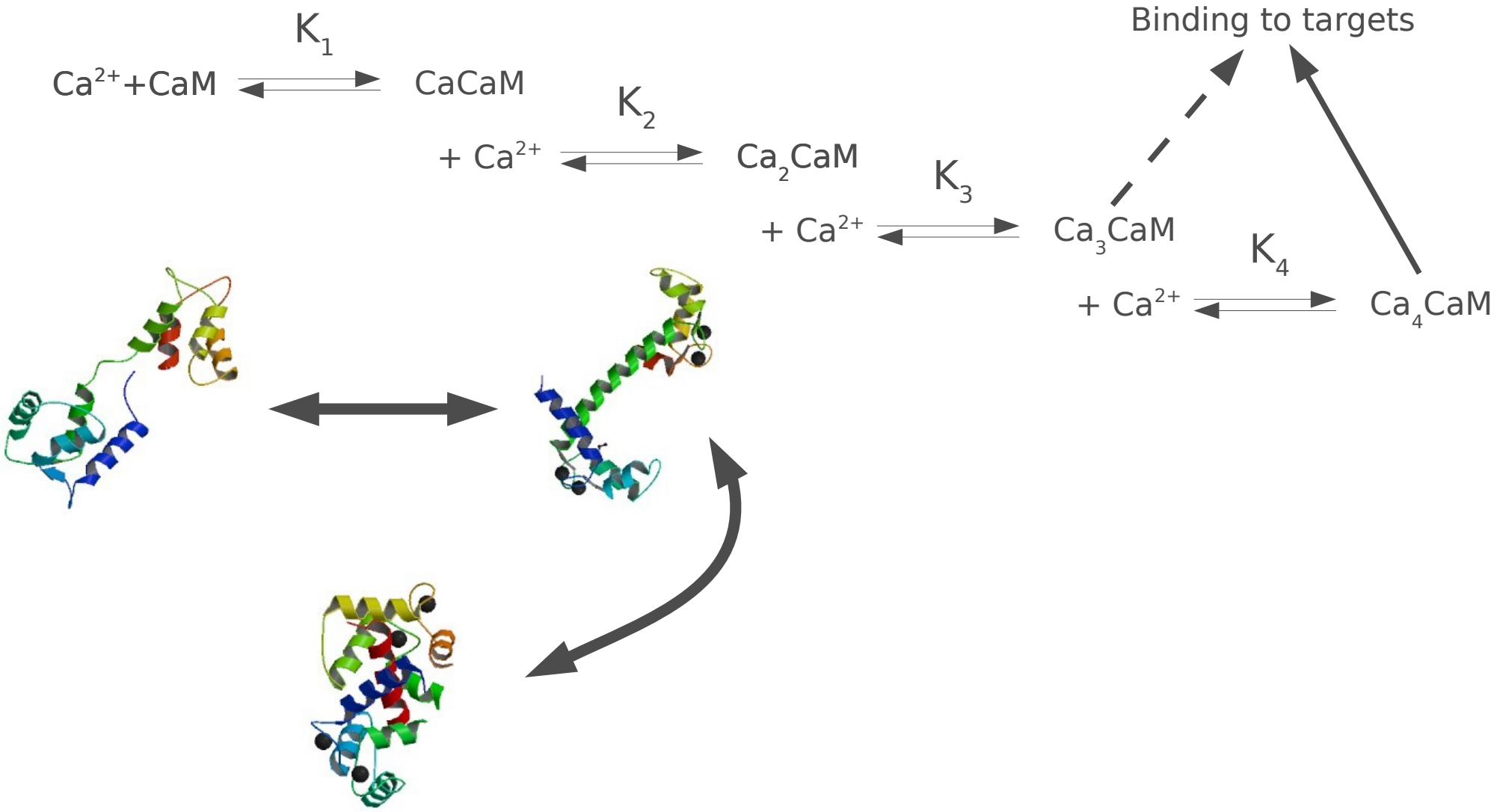
Klotz (1946) The Application of the Law of Mass Action to Binding by Proteins. Interactions with Calcium. *Arch Biochem*, 9:109–117.

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

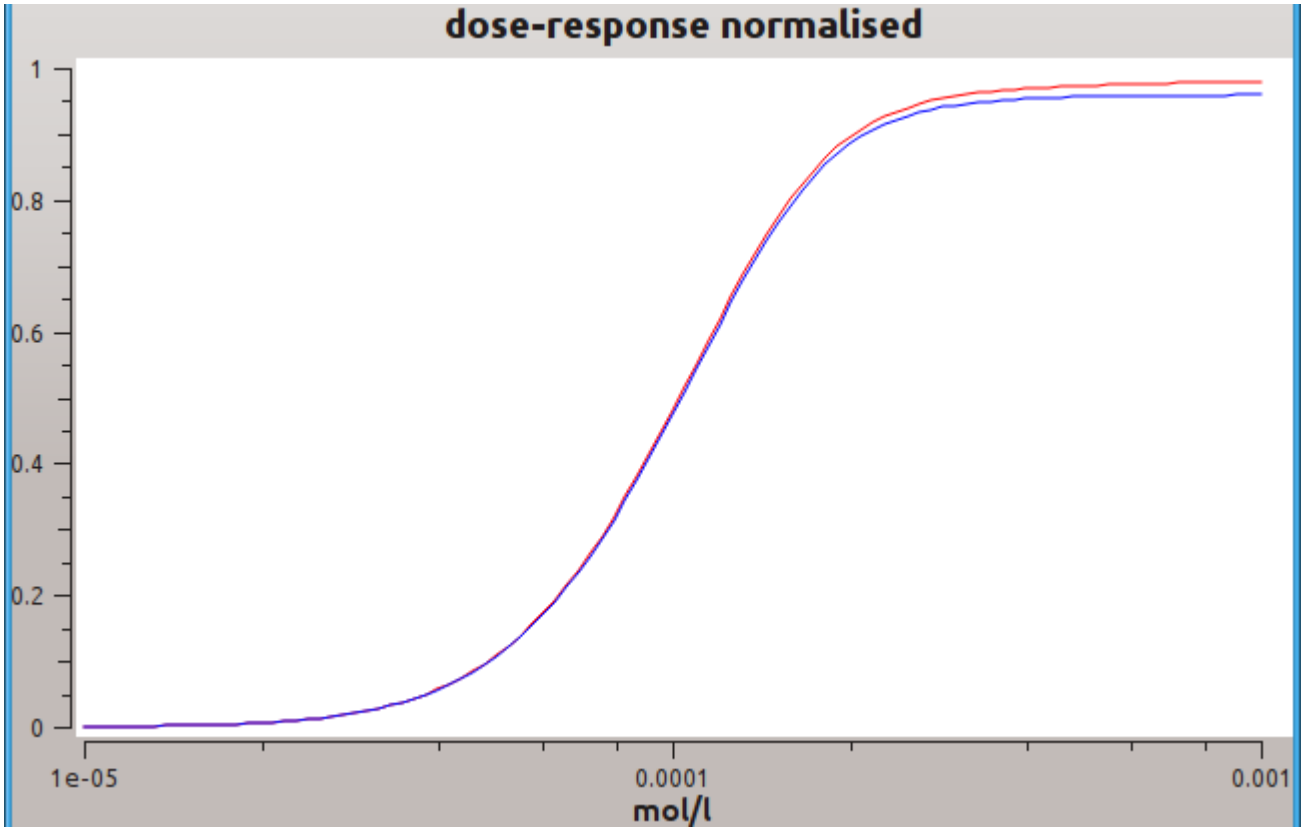


Crouch and Klee (1980) *Biochemistry*, 19: 3692-3698

Corresponding induced-fit model



That does not work ...



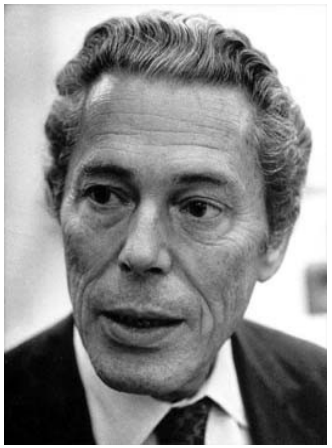
[CaN]=[CamKII]=[CaM]/10 ; Kd_CaMKII = 10xKd_CaN; Software COPASI

We knew it would not work

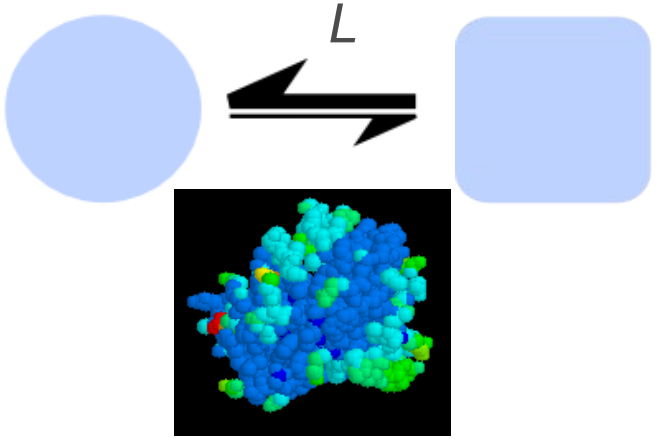
- Calmodulin bound to 3 calcium ions can activate calcineurin
  - Kincaid and Vaughan (1986). *PNAS*, 83: 1193-1197
- Calmodulin bound to 2 calcium ions can bind CaMKII
  - Shifman et al (2006). *PNAS*, 103: 13968-13973
- Calmodulin affinity for calcium increases once bound to CaMKII
  - Shifman et al (2006) [but many previous reports on other targets: e.g. Burger et al (1983). *JBC*, 258: 14733-14739 ; Olwin et (1984). *JBC* 259: 10949-10955]
- Calcium activates both LTP and LTD through calmodulin
  - Lisman (1989) *PNAS*, 86: 9574-9578
  - High $[Ca^{2+}]$ (high freq) ≅ CaMKII ; Low $[Ca^{2+}]$ (low freq) ≅ Calcineurin

Allostery and state selection

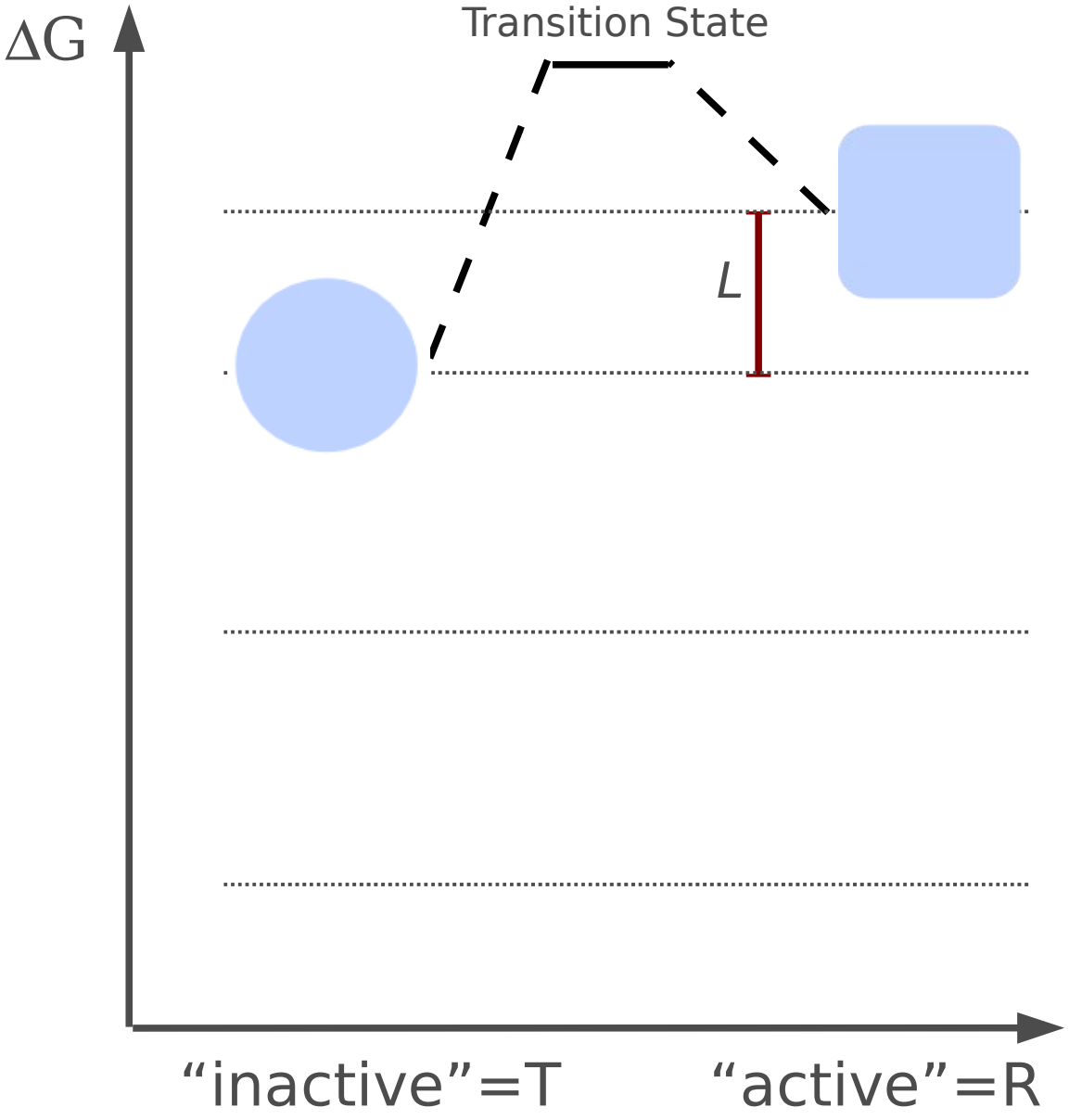
- Monod, Wyman, Changeux (1965). On the nature of allosteric transitions: a plausible model. *J Mol Biol*, 12: 88-118



Modulation of thermal equilibria ≠ induced-fit



$$L = \frac{[T_0]}{[R_0]}$$



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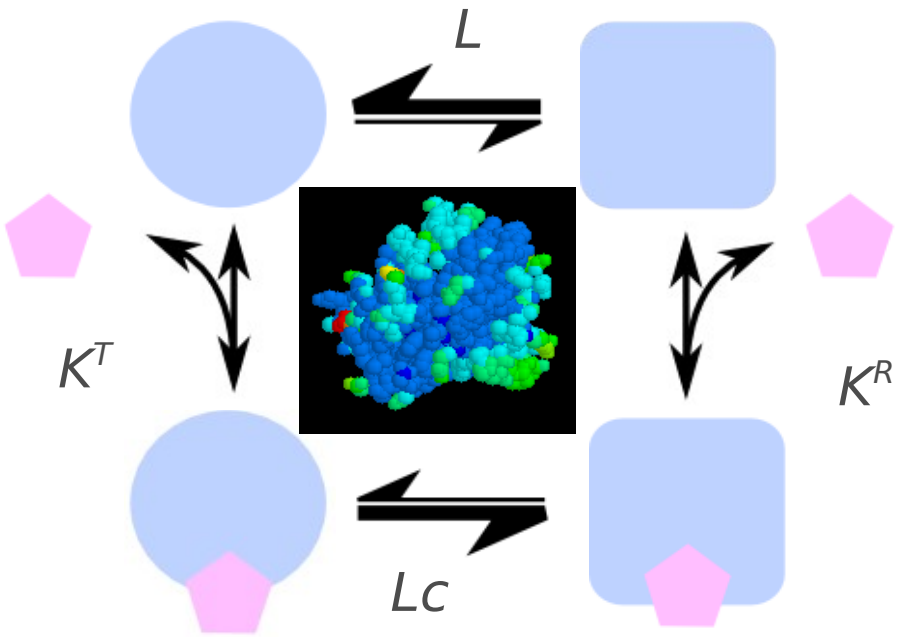
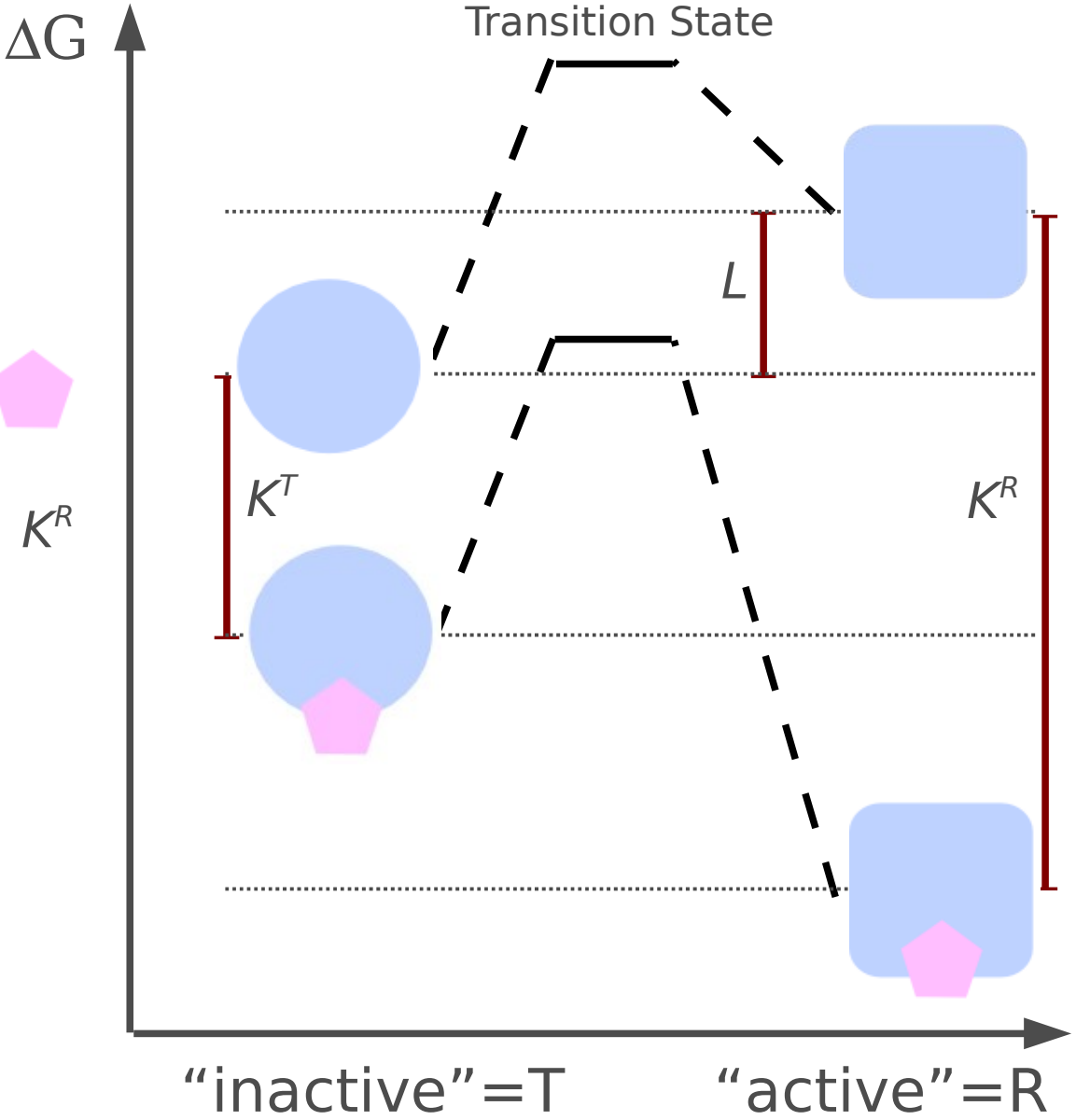
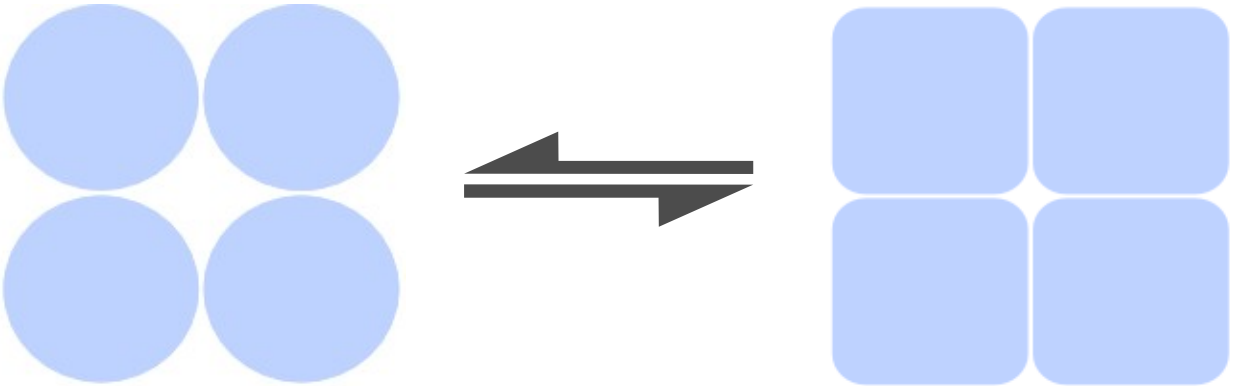
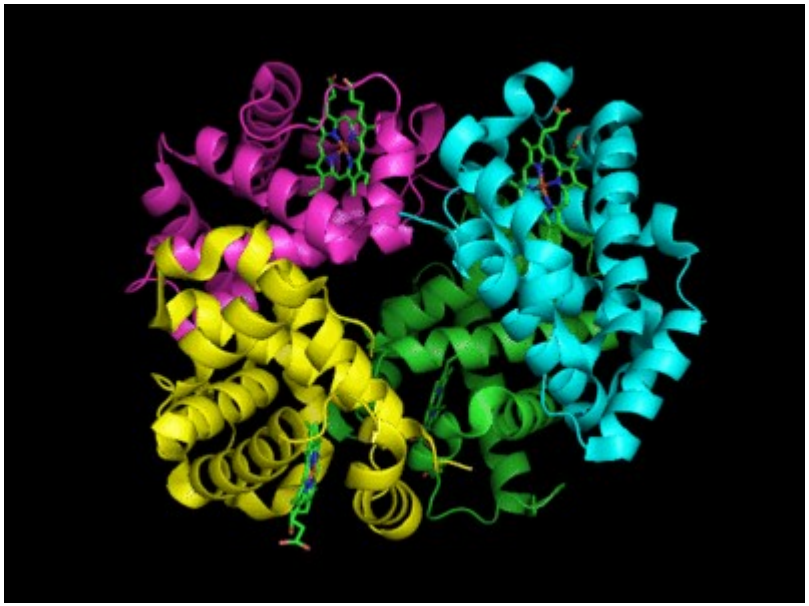


$$L = \frac{[T_0]}{[R_0]} \qquad c = \frac{K^R}{K^T}$$

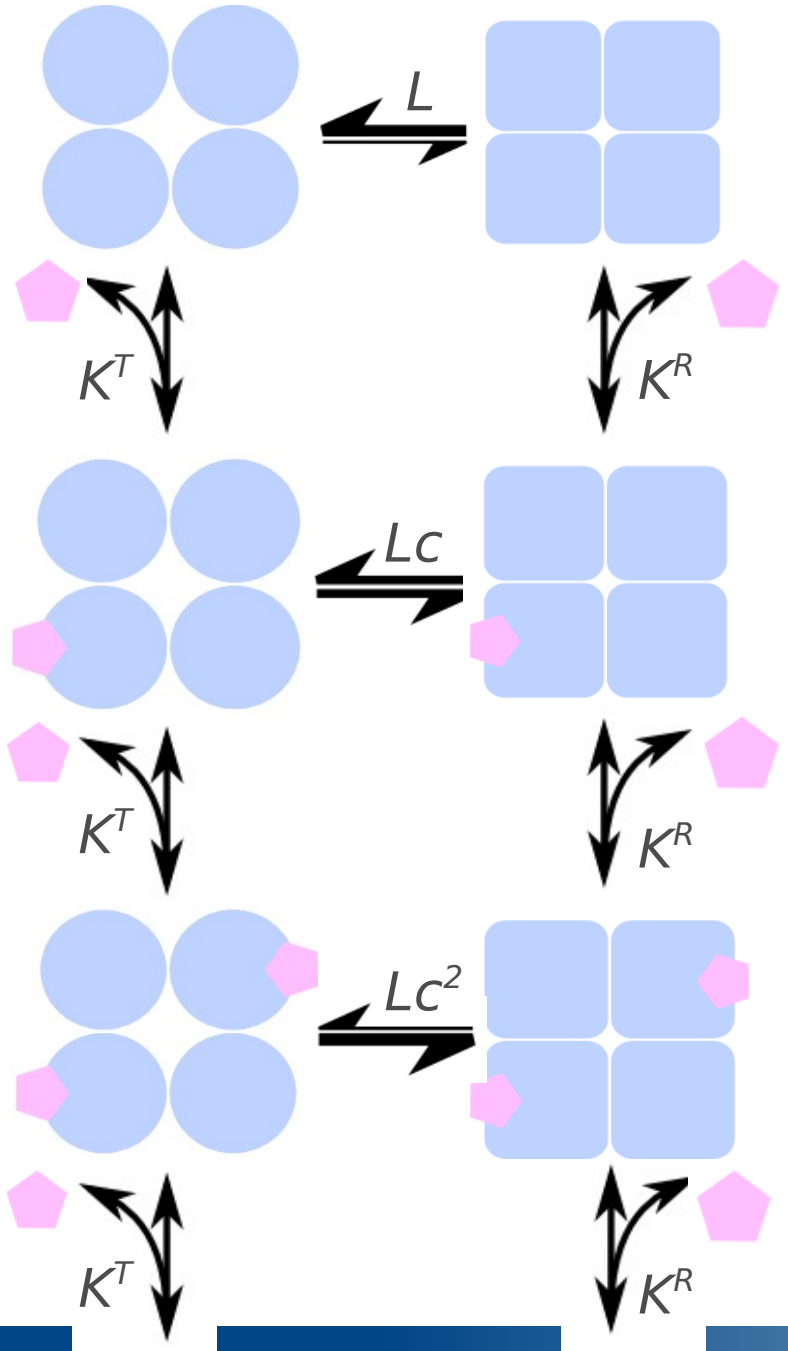


Concerted transitions ≠ sequential model





Monod-Wyman-Changeux model



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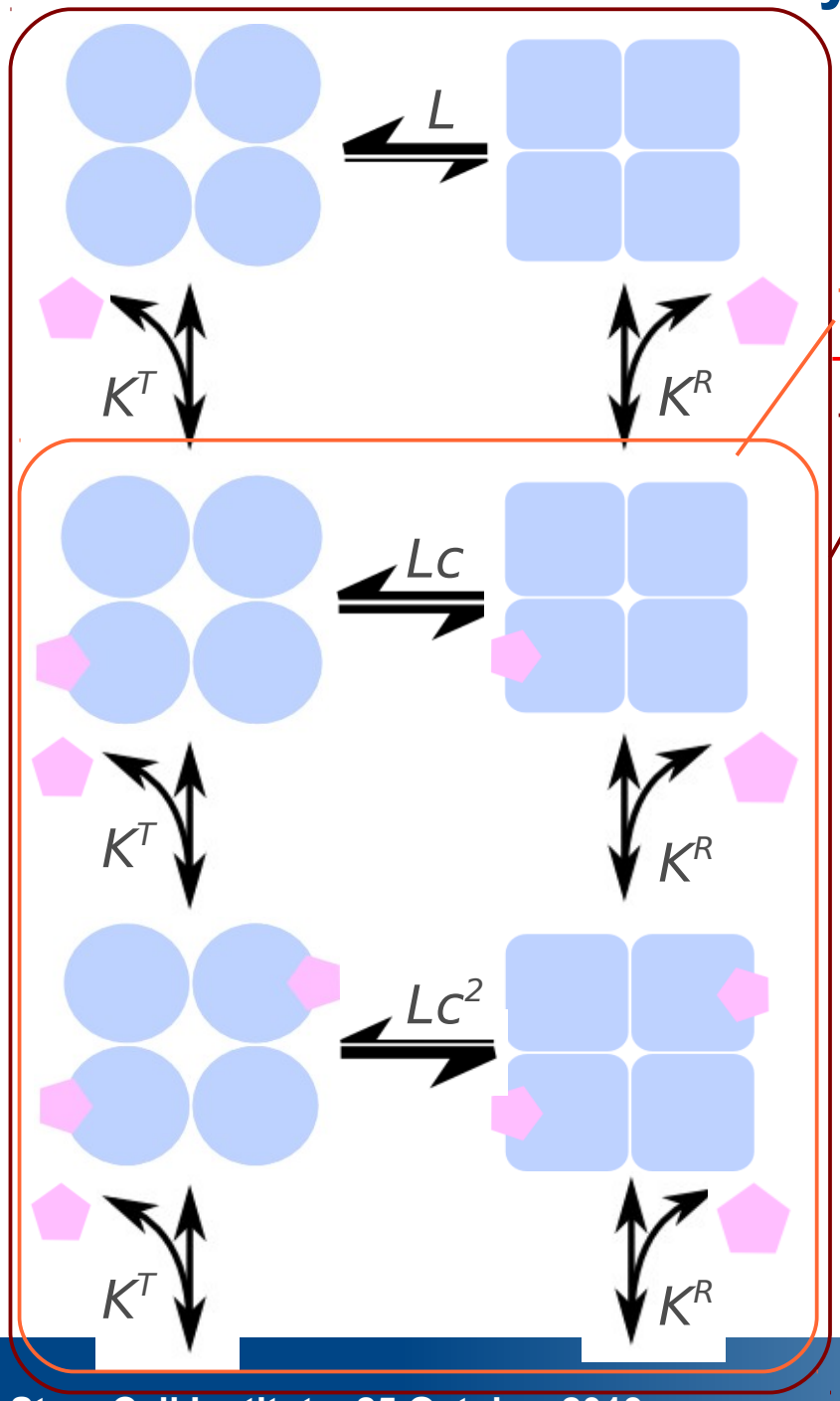
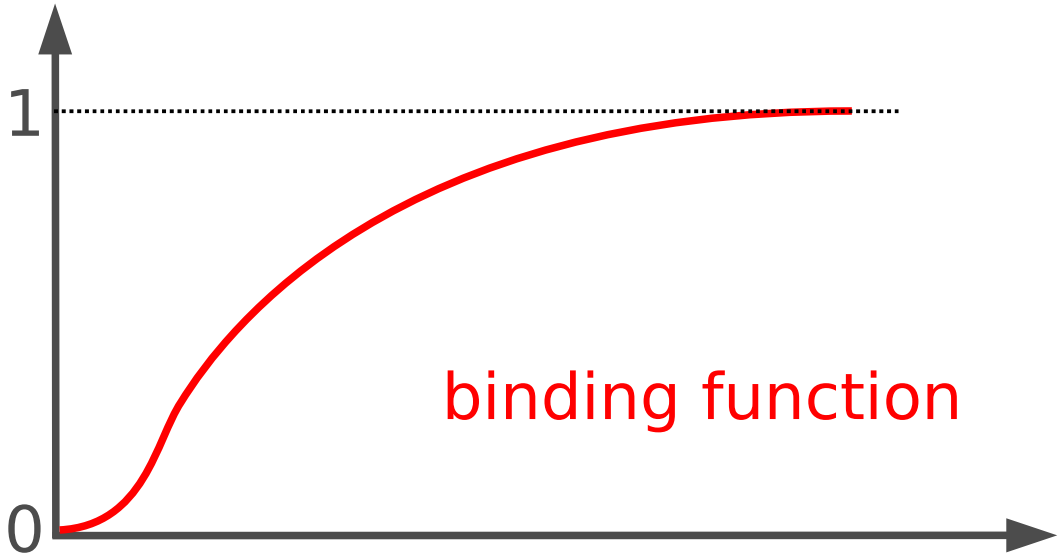


$$\alpha = \frac{[x]}{K^R}$$

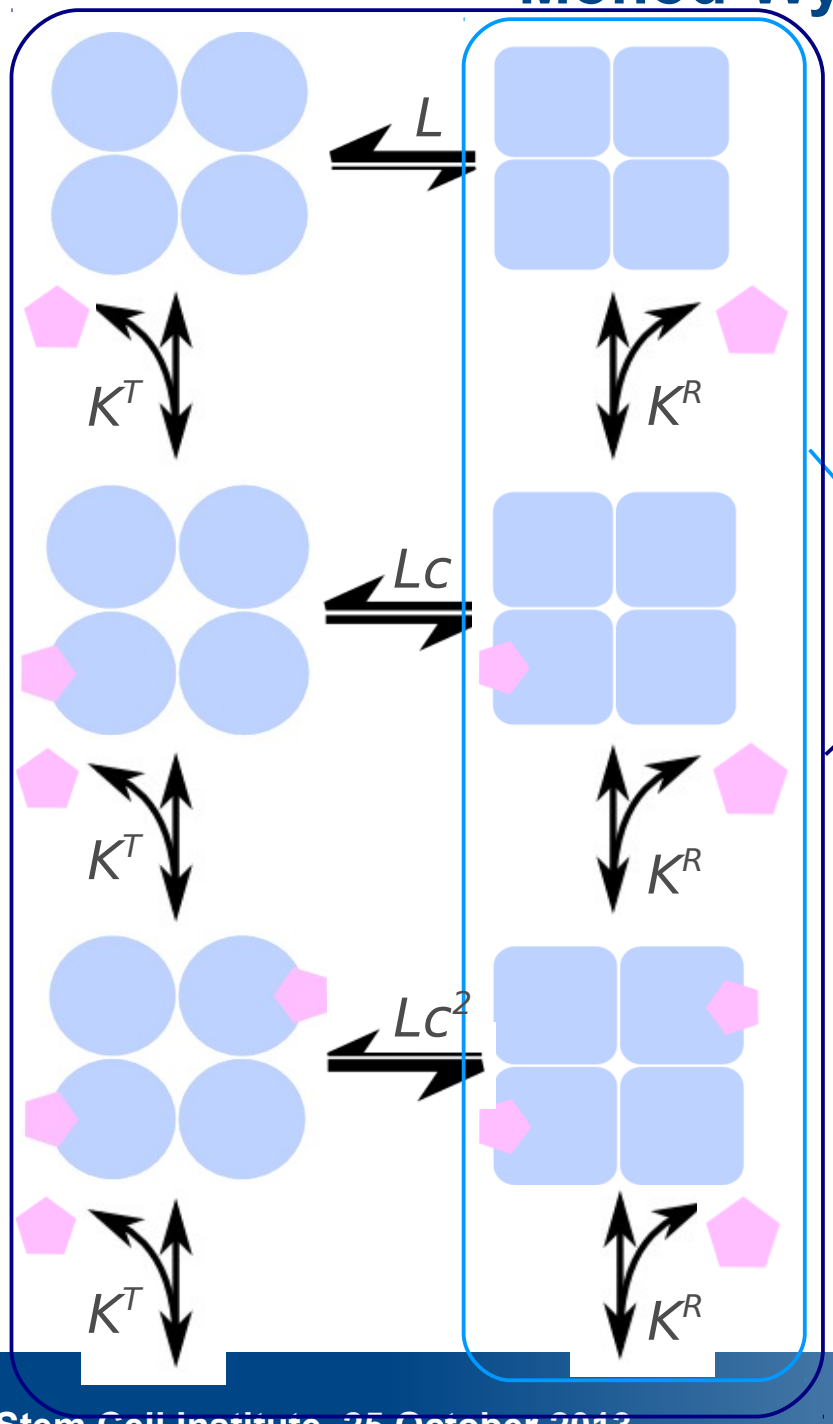
this

that

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



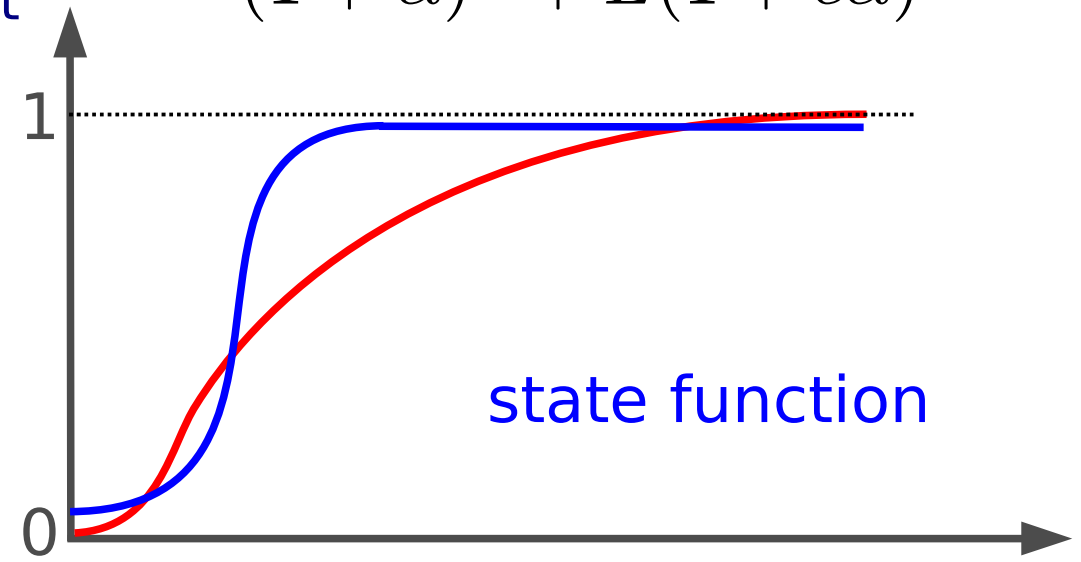
Monod-Wyman-Changeux model



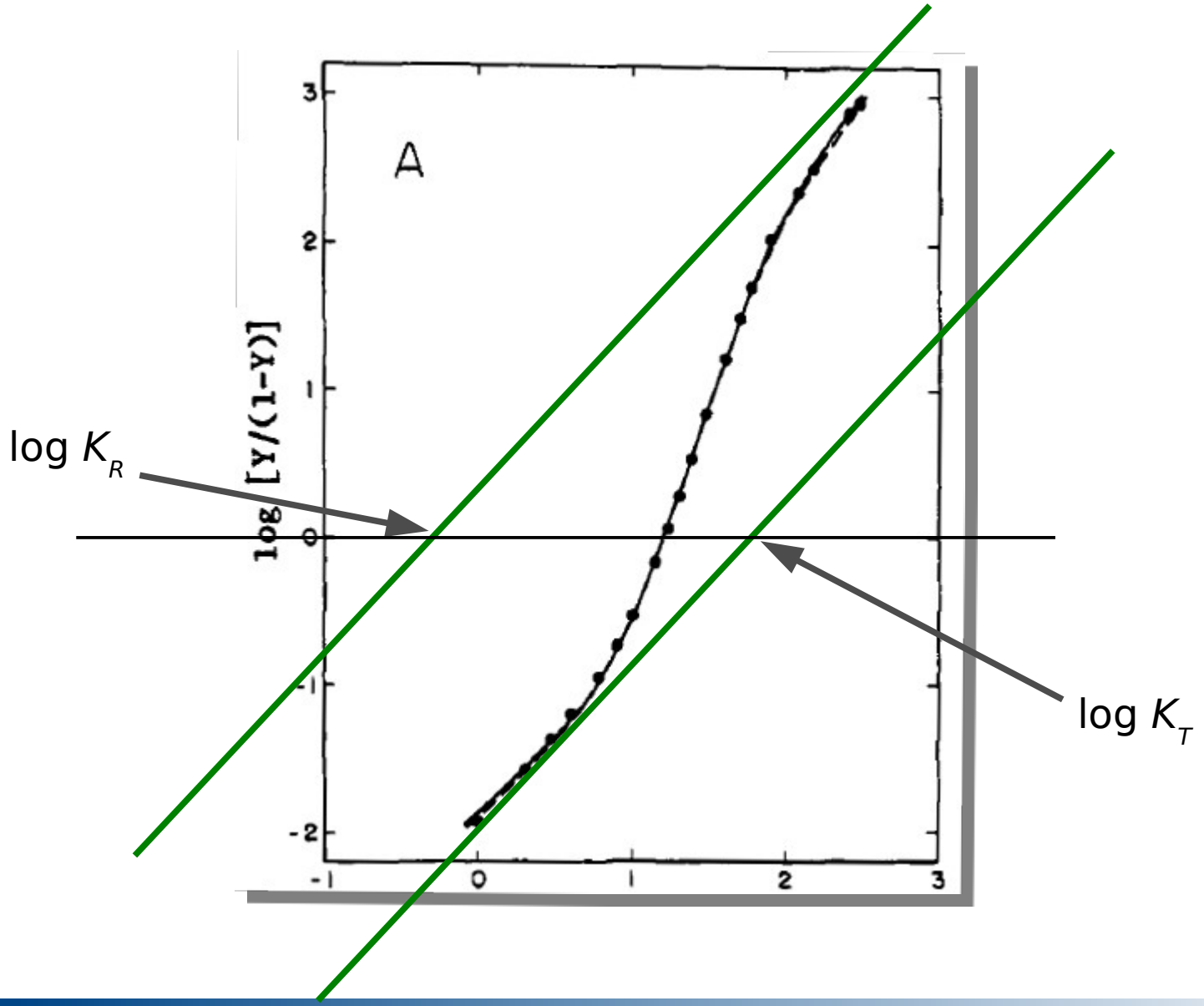
$$\alpha = \frac{[x]}{K^R}$$

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

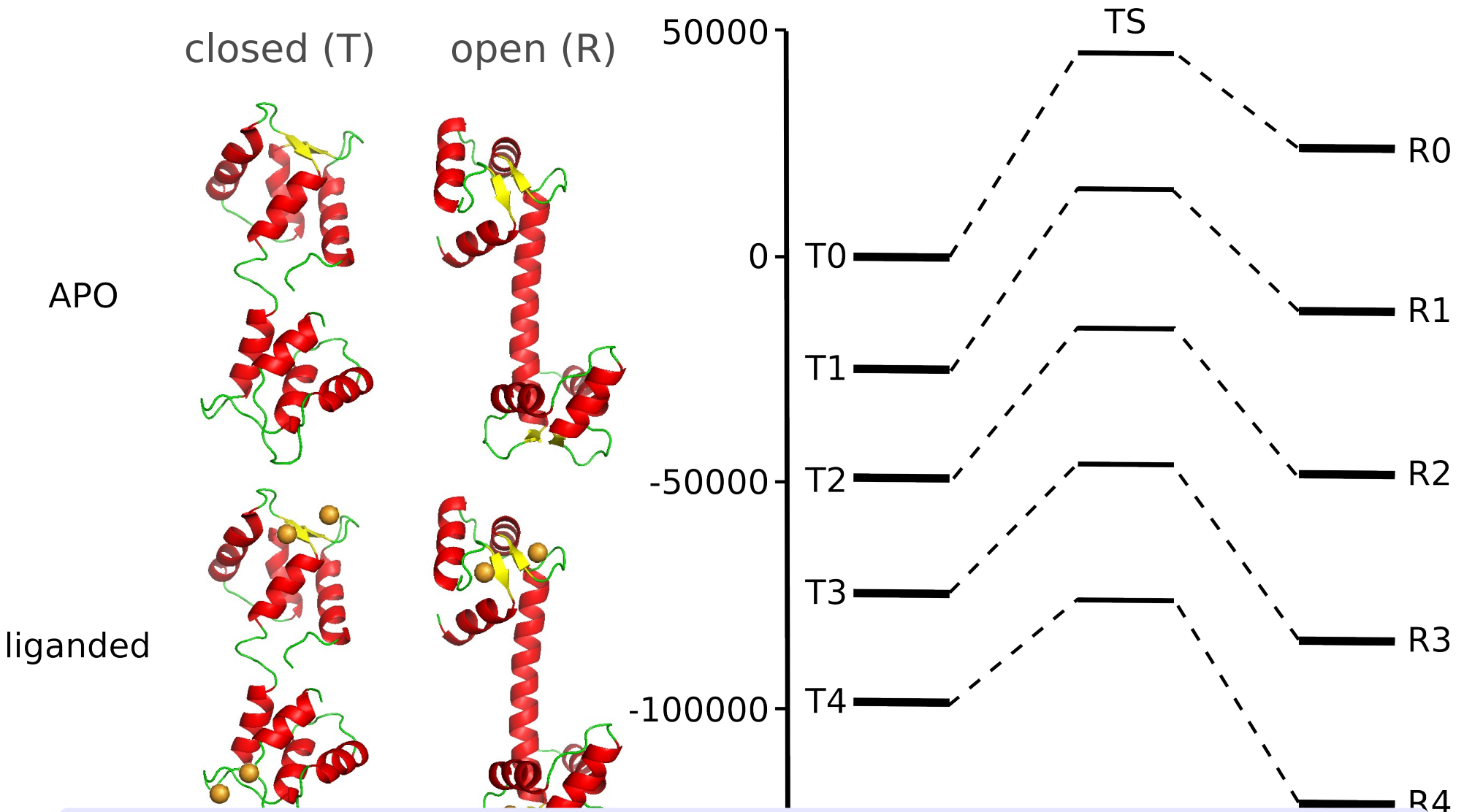
this = that $\bar{R} = \dfrac{(1+\alpha)^n}{(1+\alpha)^n + L(1+c\alpha)^n}$



"Hill" Plot for MWC model

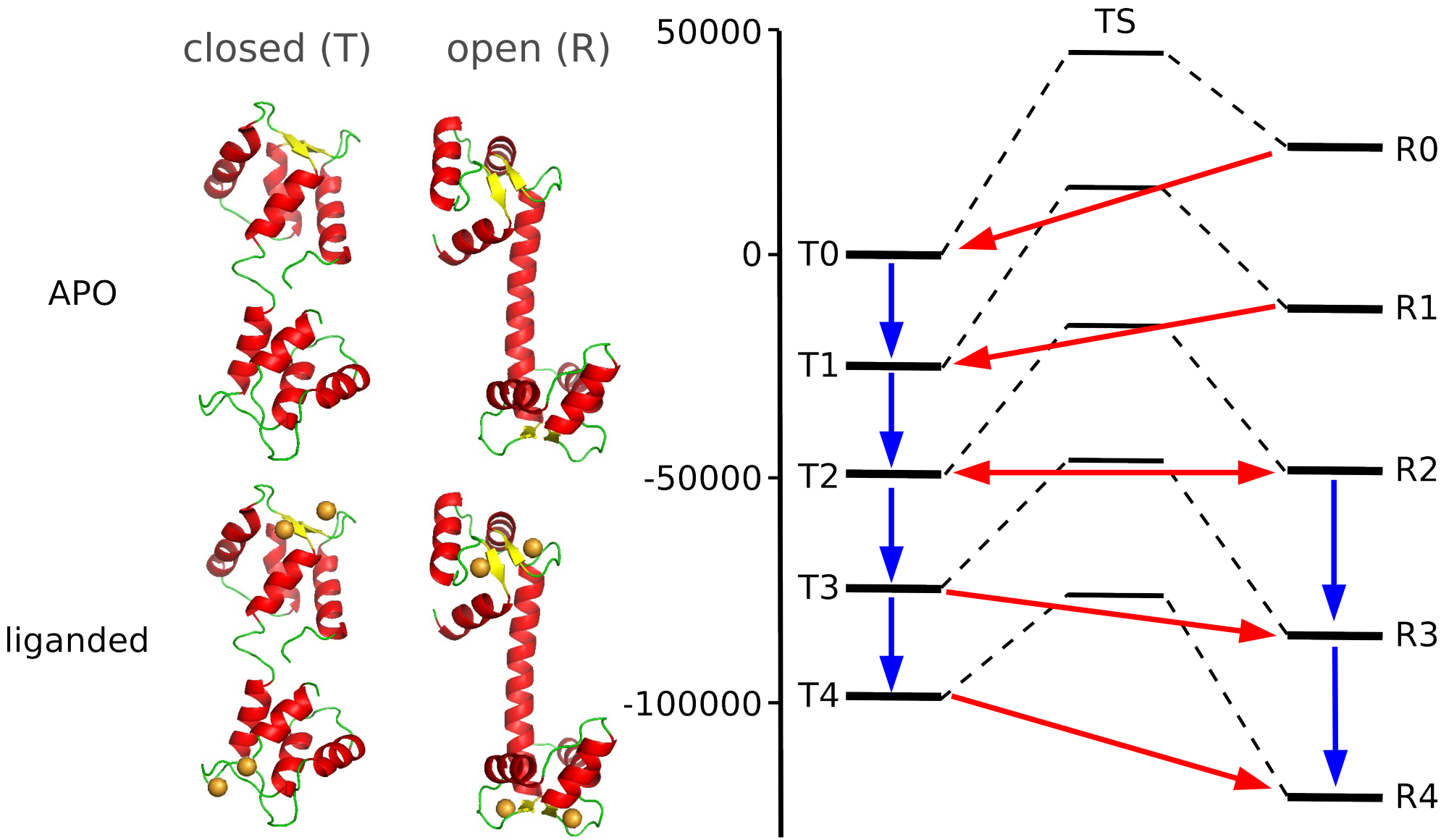


Allosteric model of Calmodulin activation

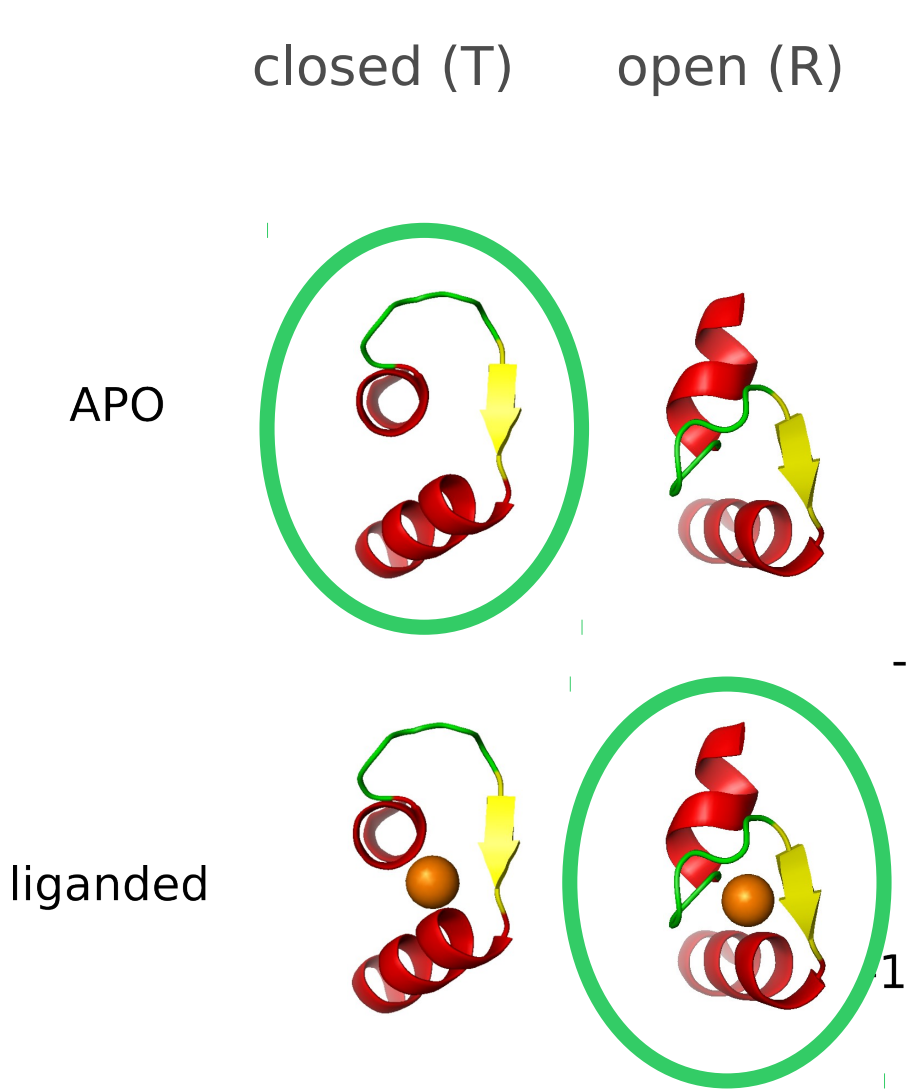


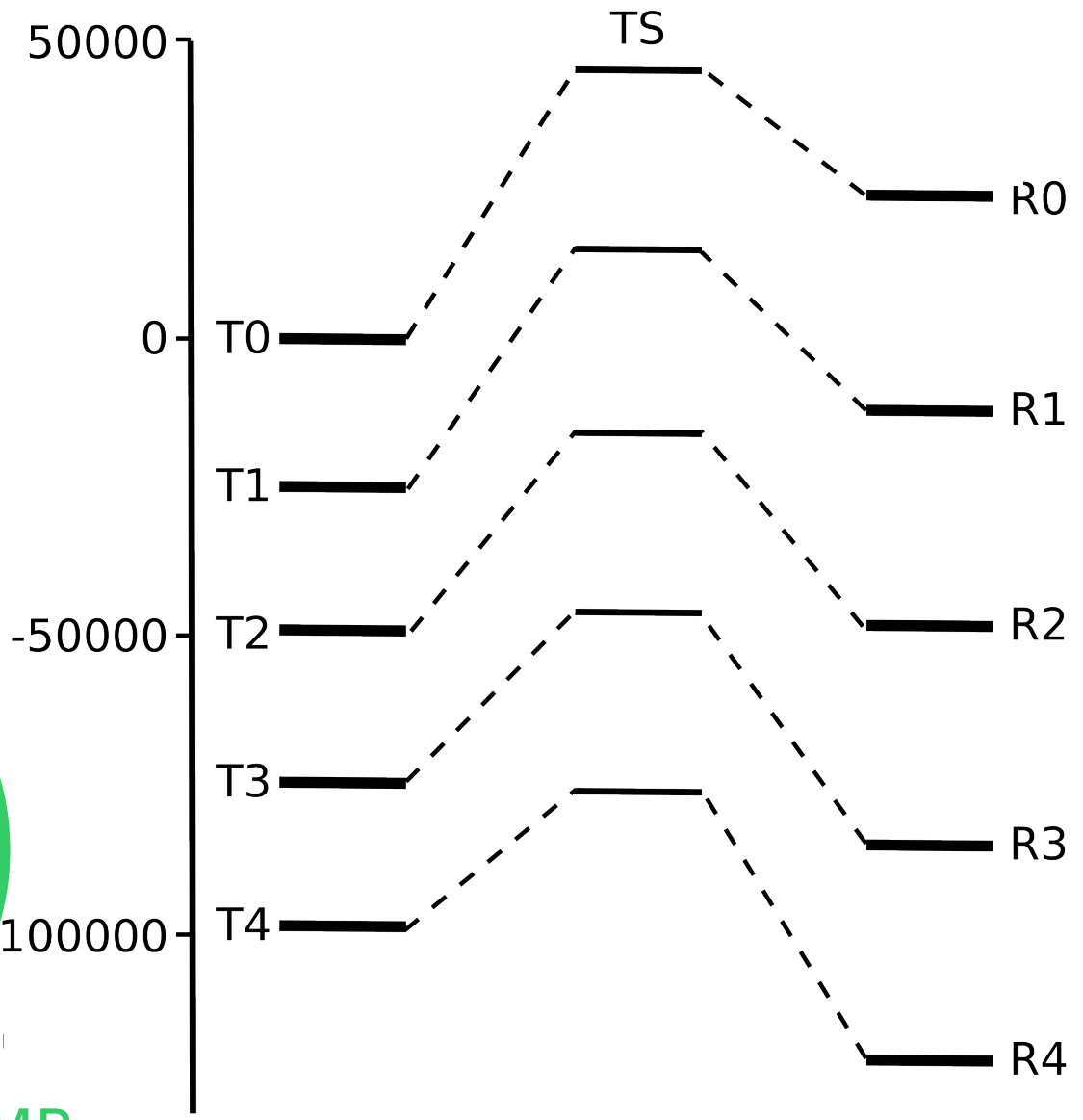
Stefan MI, Edelstein SJ, Le Novère N (2008) *Proc Natl Acad Sci USA*, 105:10768-10773

Allosteric model of Calmodulin activation



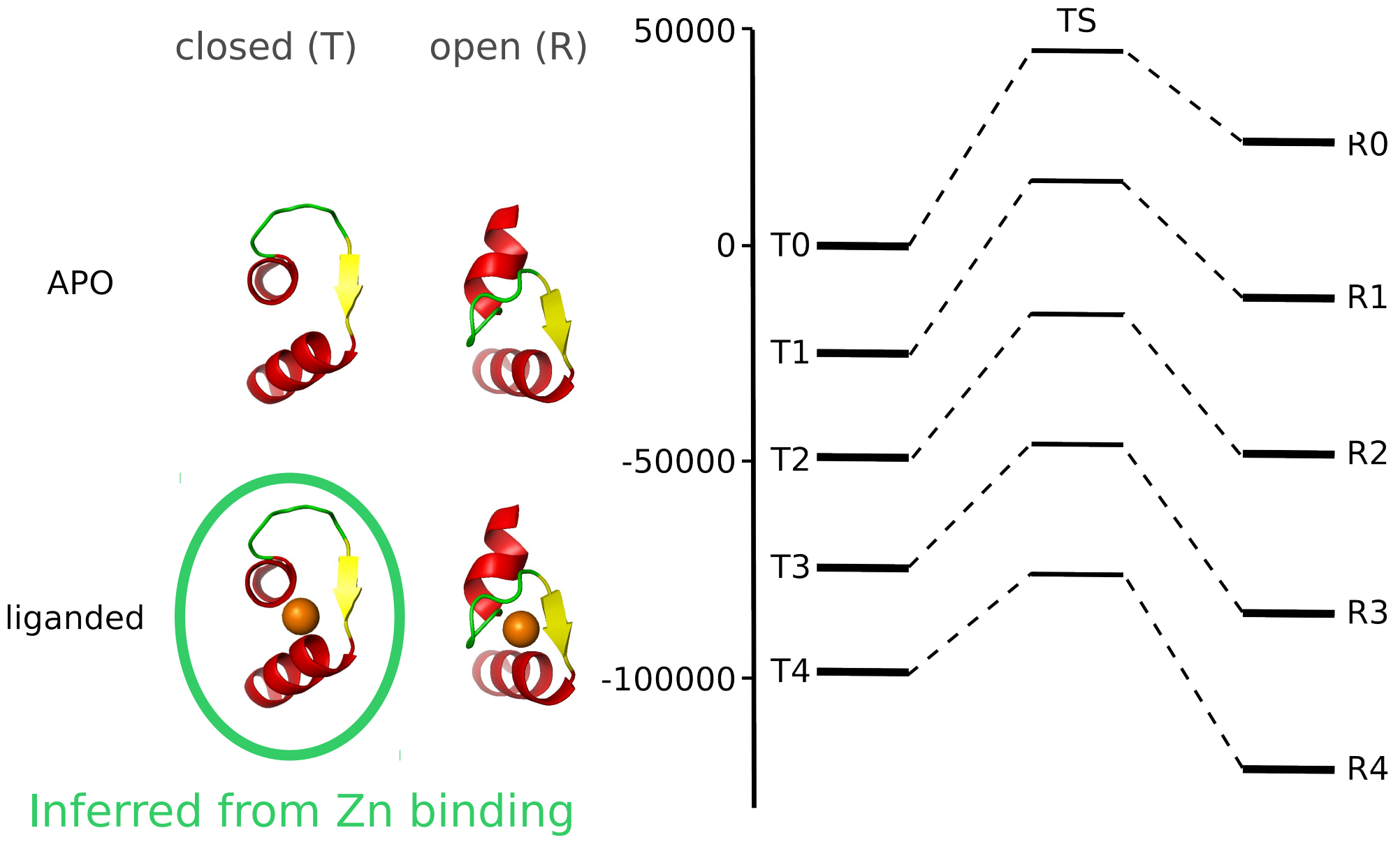
Observation Vs. Prediction





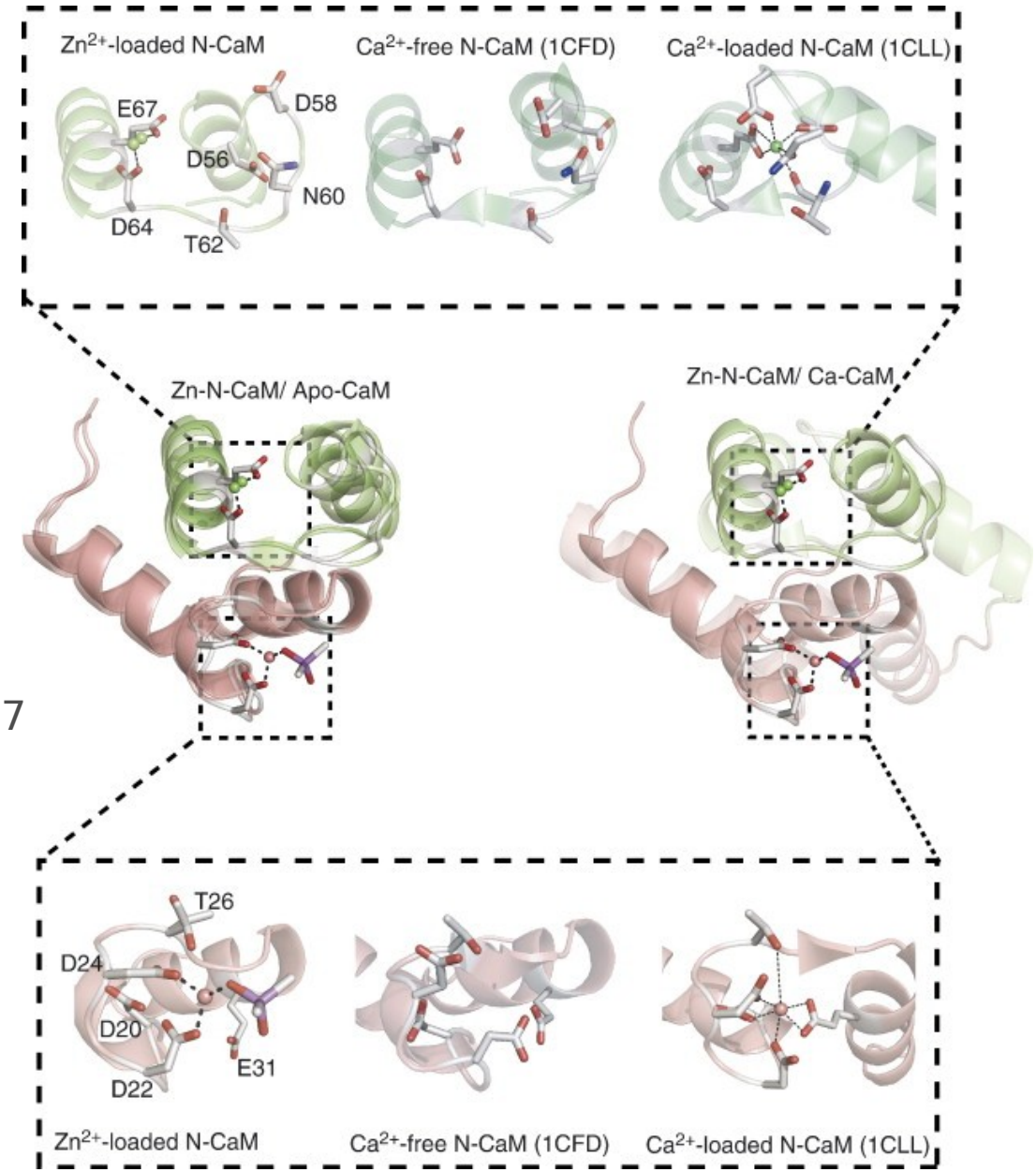
Observed by X-Ray and NMR

Observation Vs. Prediction

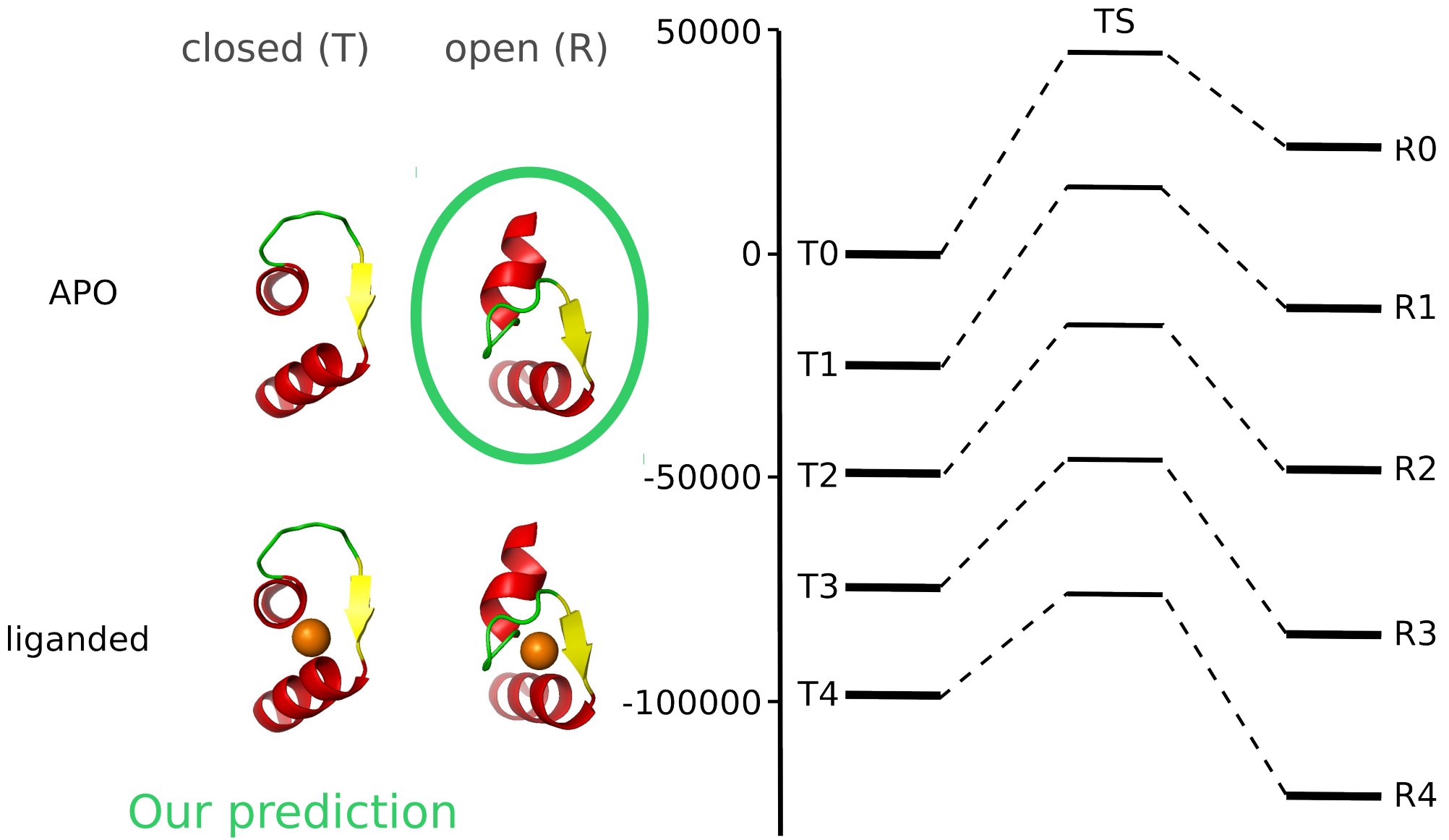


Inferred from Zn binding

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



Observation Vs. Prediction



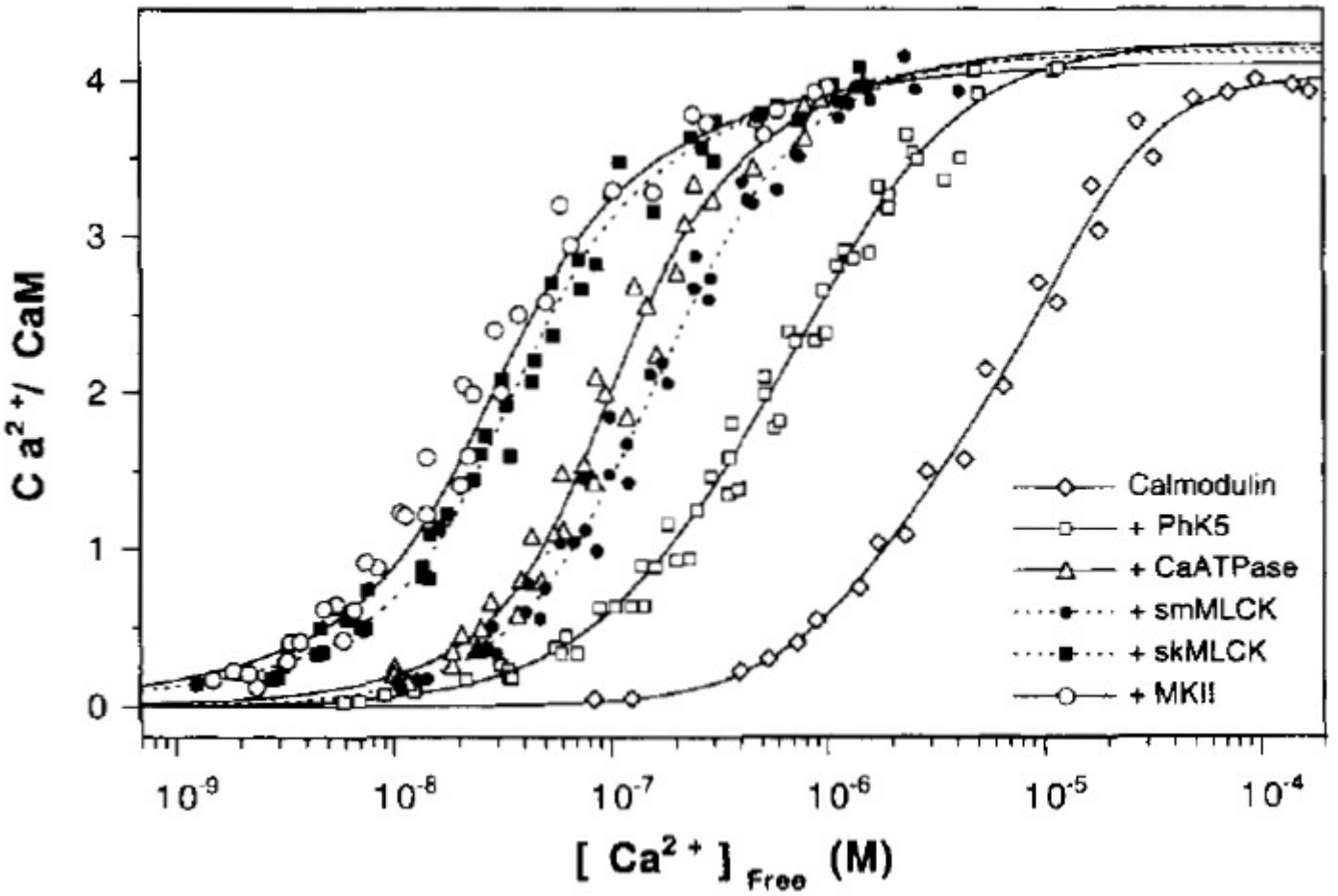
Procedure for parameter estimations

- Extension of the MWC model to support any number of sites, with different affinities, per “protomer”; several protomers per subunit

  Stefan M.I., Edelstein S.J., Le Novère N. *BMC Systems Biology* (2009), 3: 68

- Hypothesis: homogeneous distribution of transition energy = unique *c*. Estimation of L and c based on Ca^{2+} binding in presence of targets: none, skMLCK, PhK5, CaATPase (Peersen et al (1997) Prot Sci 6: 794-807). 100 000 parameter sets. 13 identical minima.

Targets as allosteric effectors



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

Procedure for parameter estimations

- Extension of the MWC model to support any number of sites with different affinities per “protomer” and several protomers per subunit

  Stefan M.I., Edelstein S.J., Le Novère N. *BMC Systems Biology* (2009), 3: 68

- Hypothesis: homogeneous distribution of transition energy = unique *c*. Estimation of L and c based on Ca^{2+} binding in presence of targets: none, skMLCK, PhK5, CaATPase (Peersen et al (1997) Prot Sci 6: 794-807). 100 000 parameter sets. 13 identical minima.

- Estimation of affinities for the four sites using calcium dissociation constants for 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). 25 millions parameter sets.

1 in 20000 active w/o Ca^{2+}

$L=20670$

$C=3.96\ 10^{-3}$

Affinity of Ca^{2+} for "open state" 250 times higher than for "closed state"

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

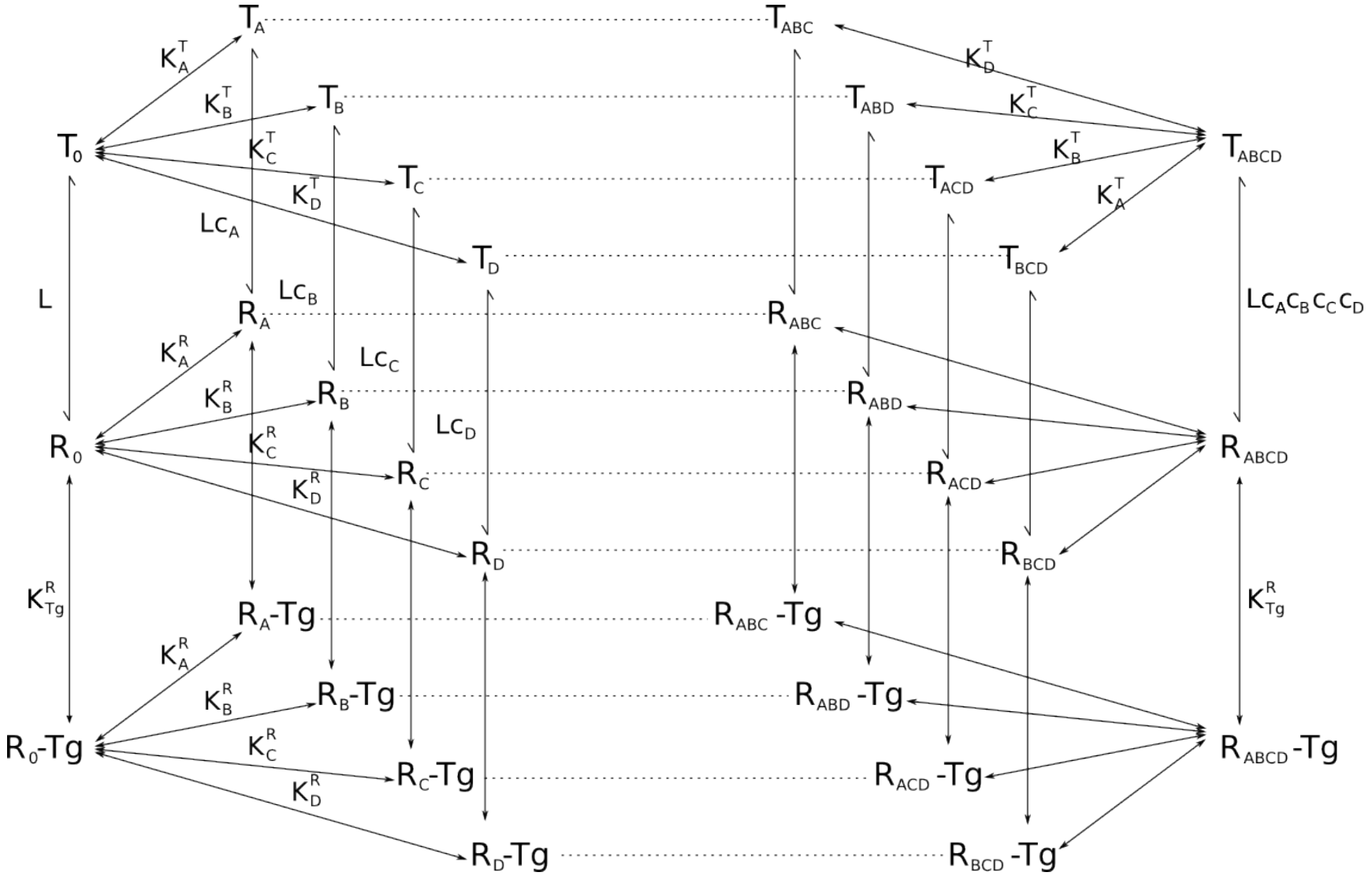
$K^R_B=1.66\ 10^{-8}$

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

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

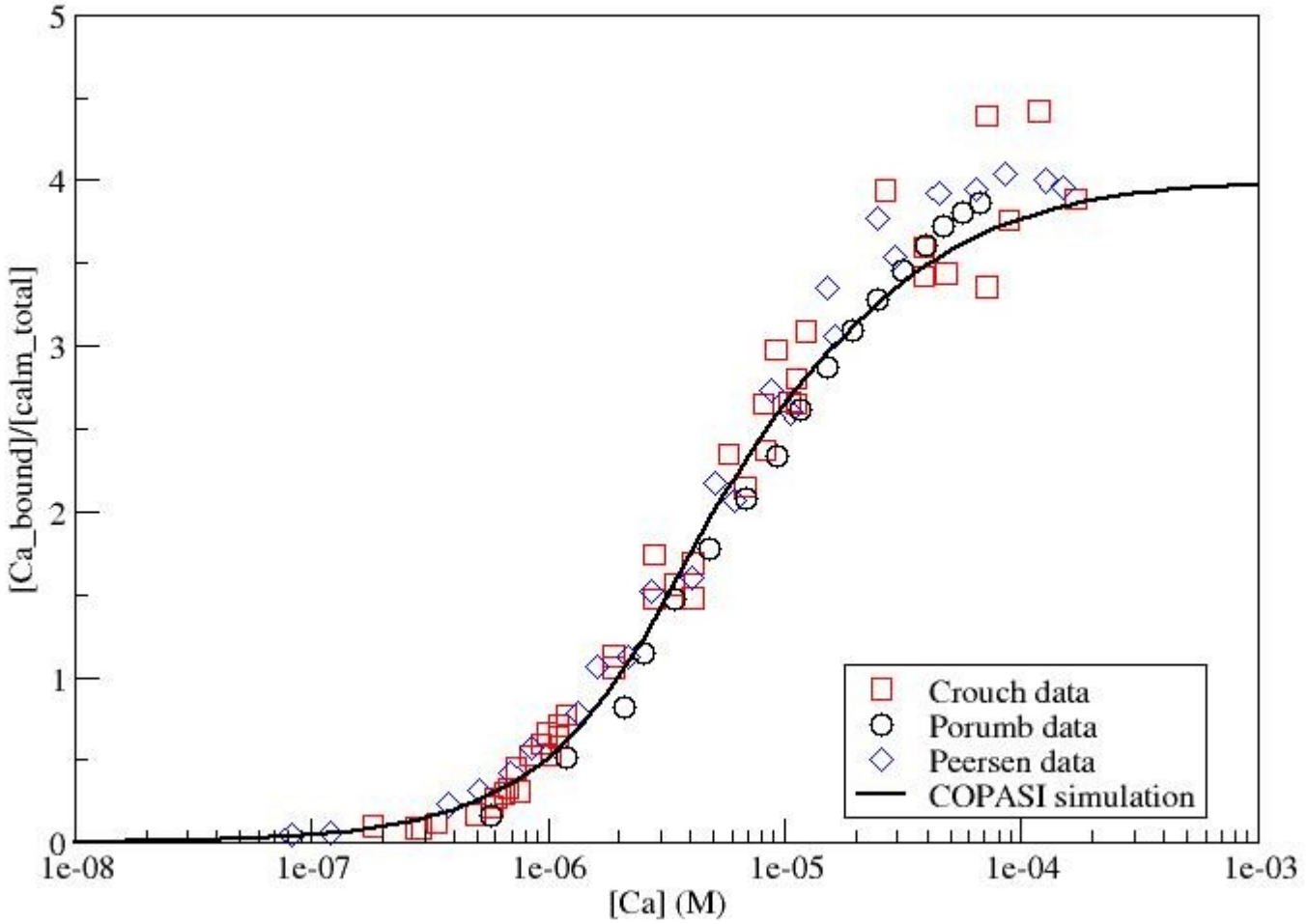
2 high, 2 low, as anticipated

Full mechanistic thermodynamic model



320 reactions

Comparison with experiments

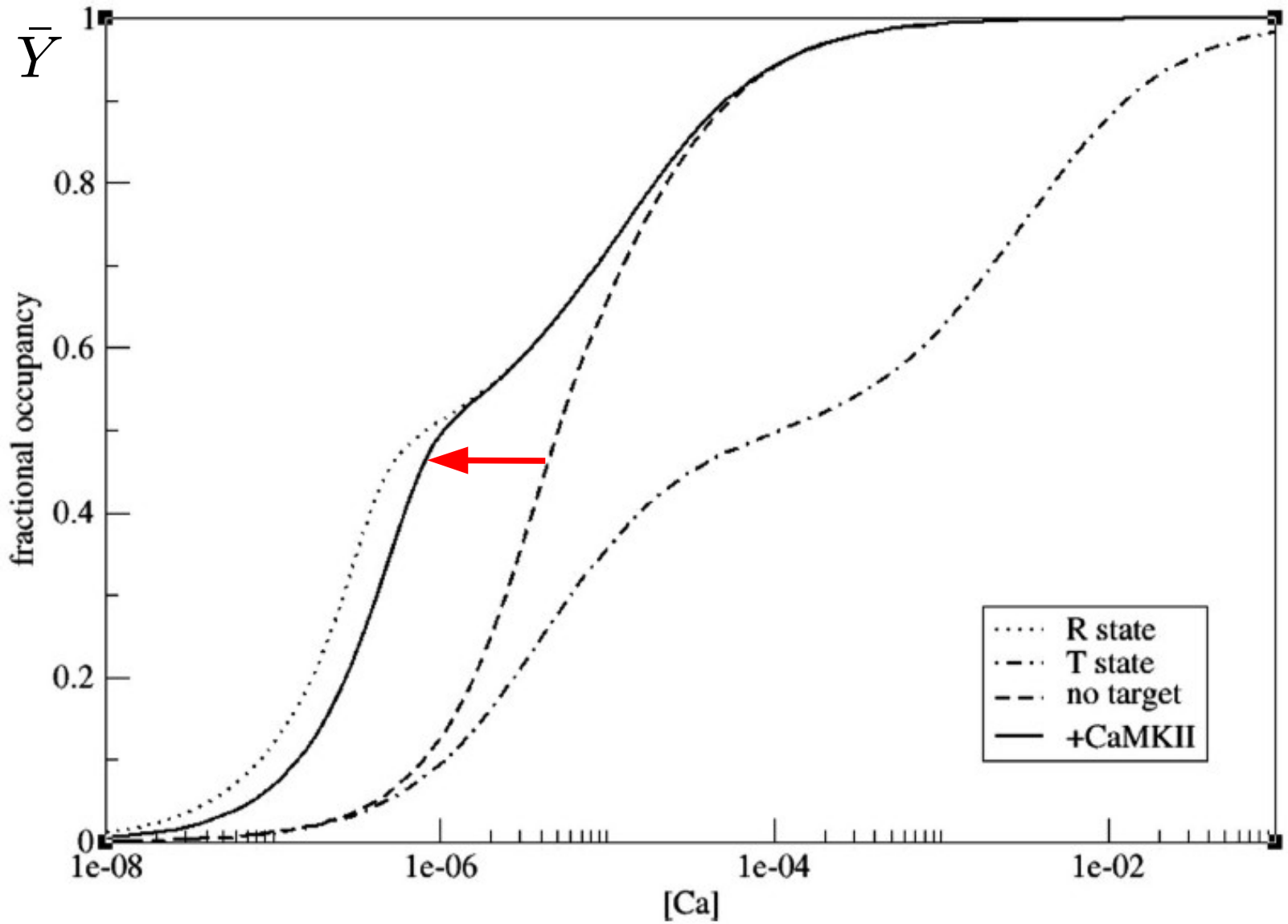


Crouch and Klee (1980) *Biochemistry*, 19: 3692-3698c

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

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

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



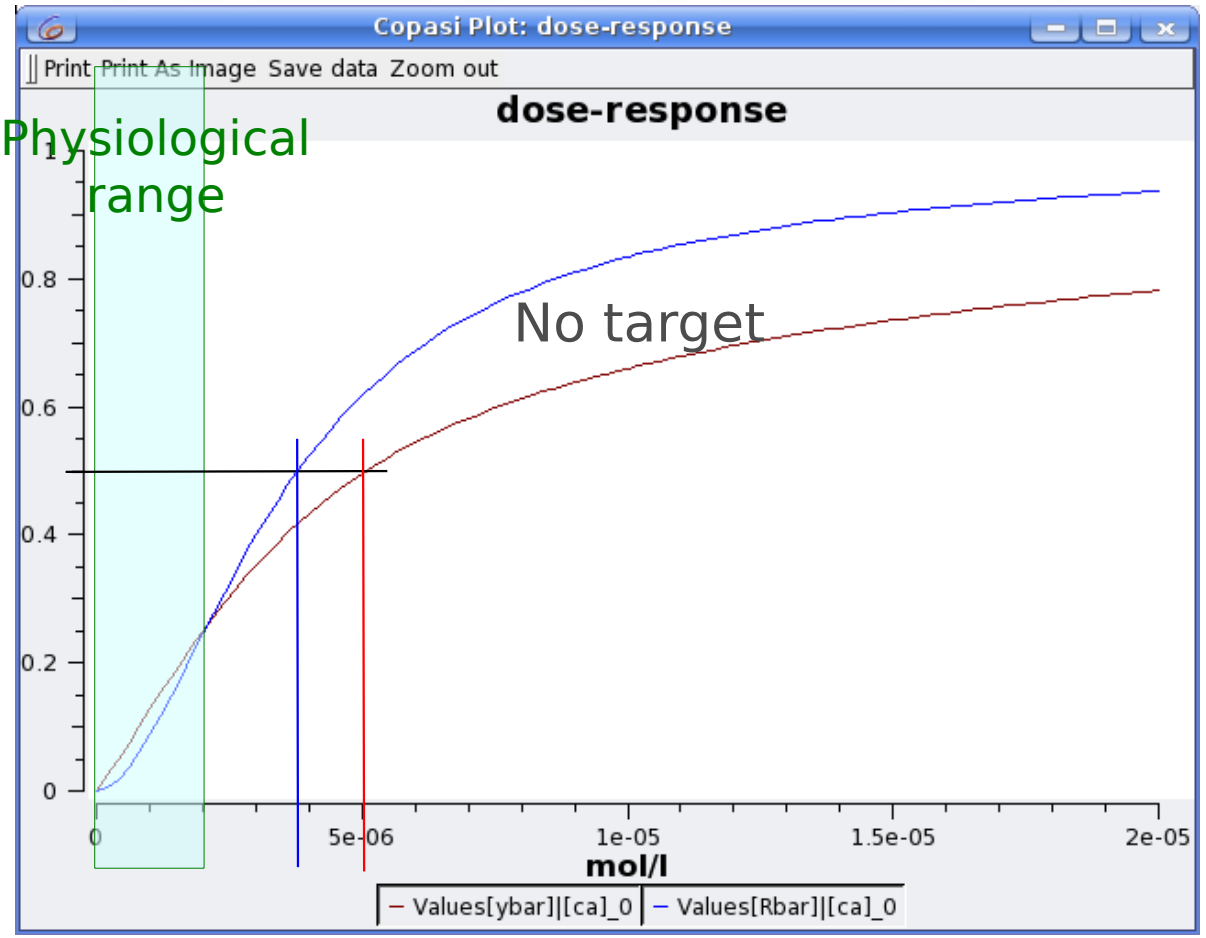
Activity of unsaturated calmodulin

- 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

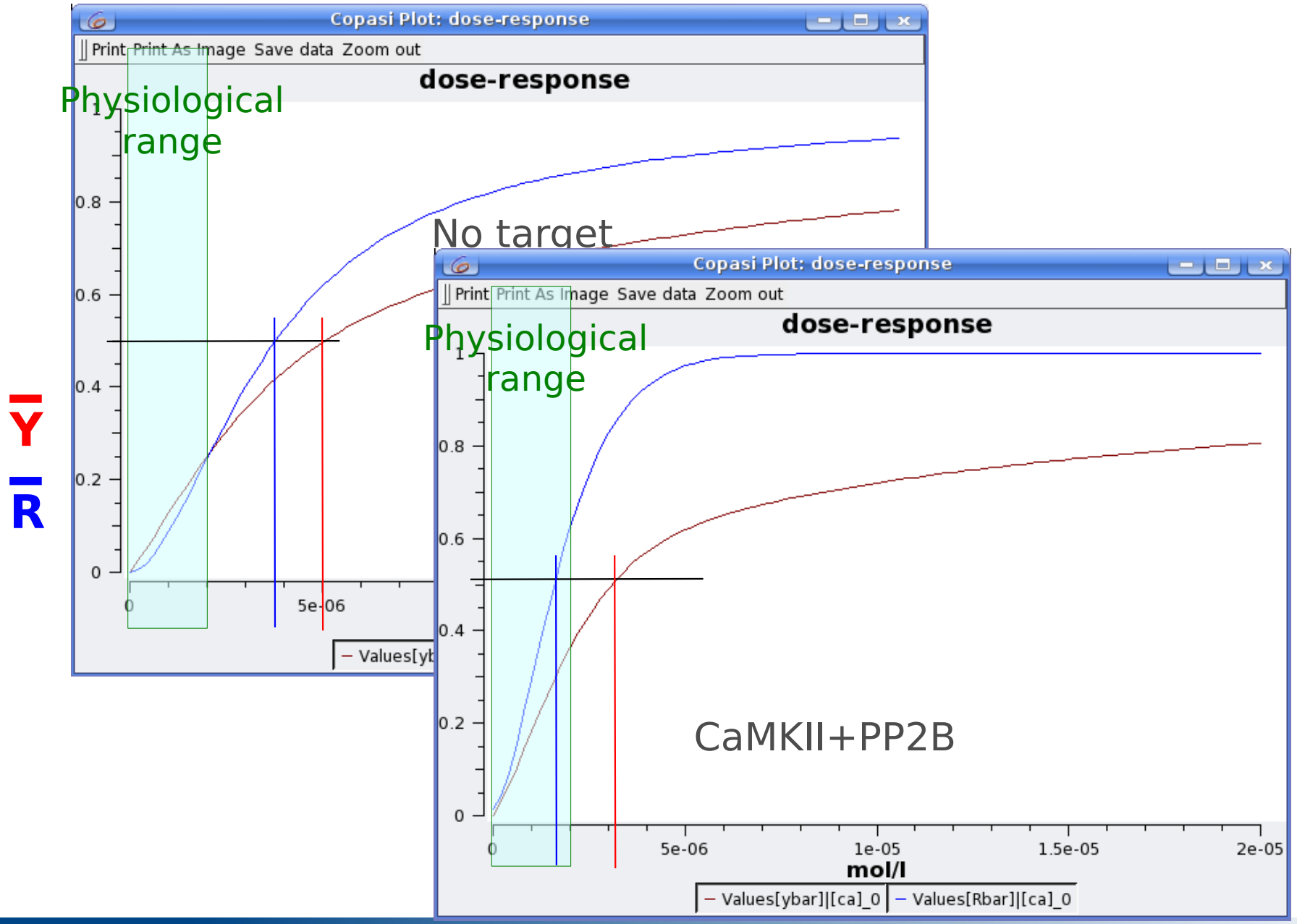
But ... we're out of the physiological range?



$\bar{Y}$

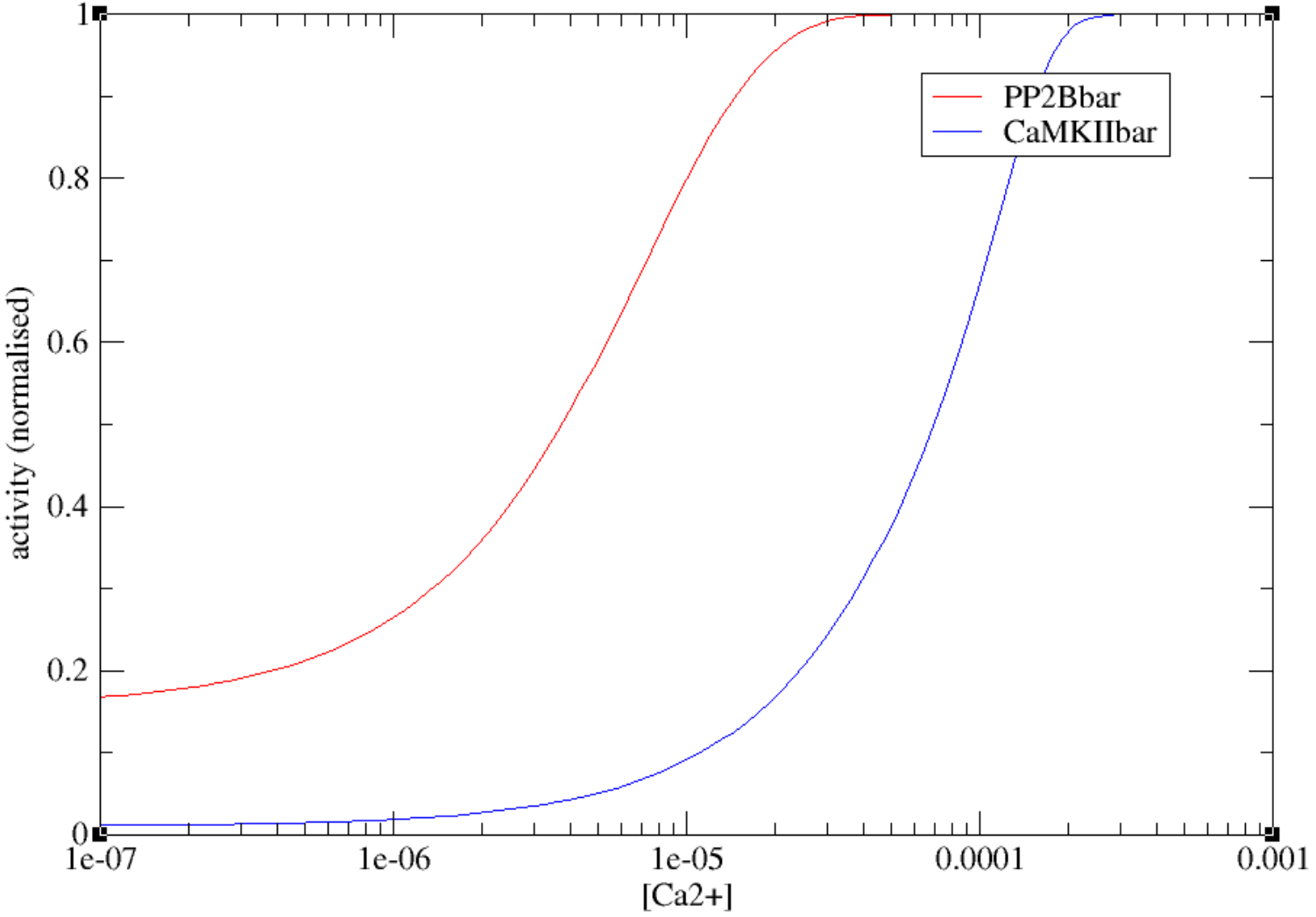
$\bar{R}$

Targets stabilises Ca^{2+} binding: This is systems biology!



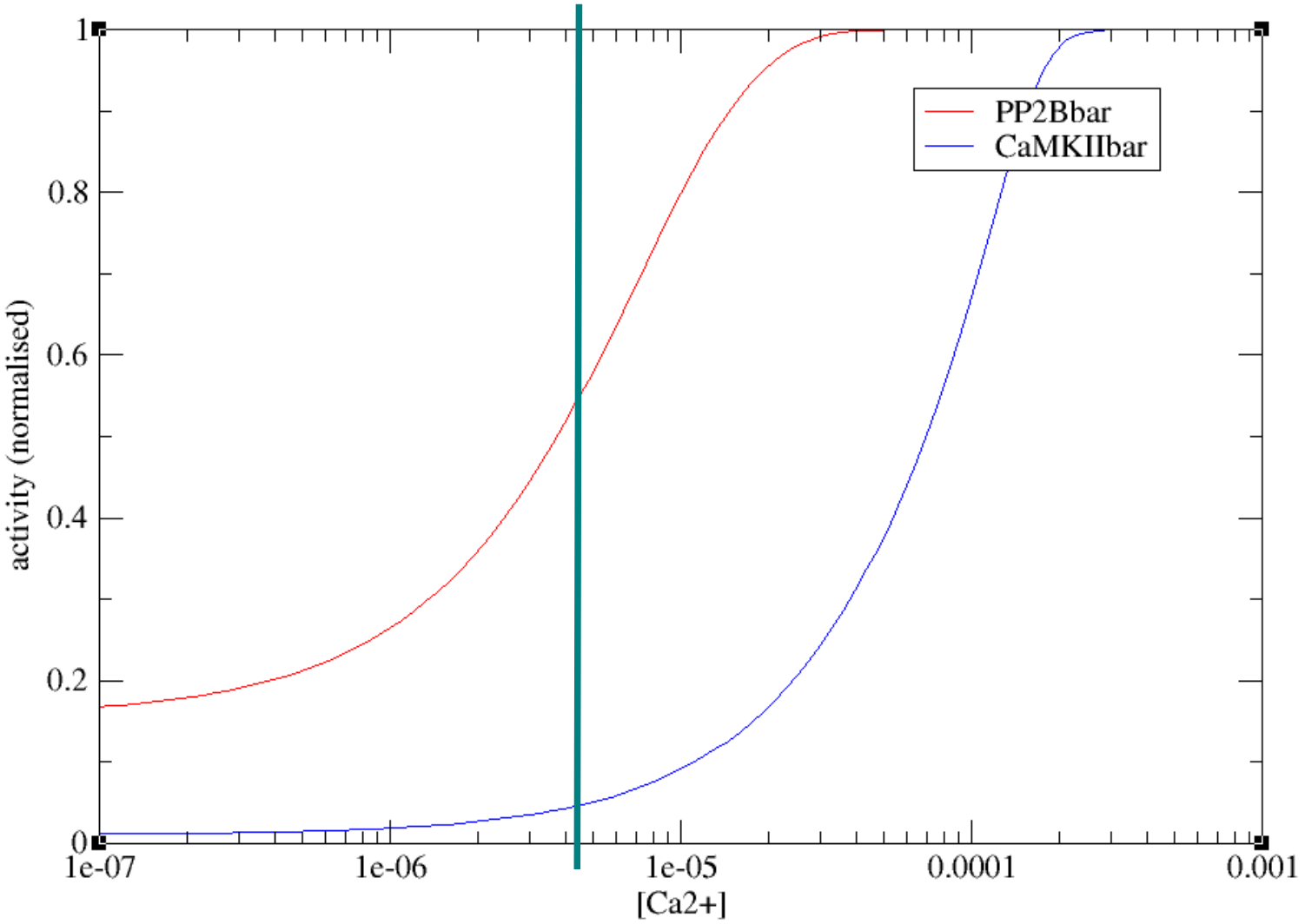
Babraham Institute

Bidirectional synaptic plasticity

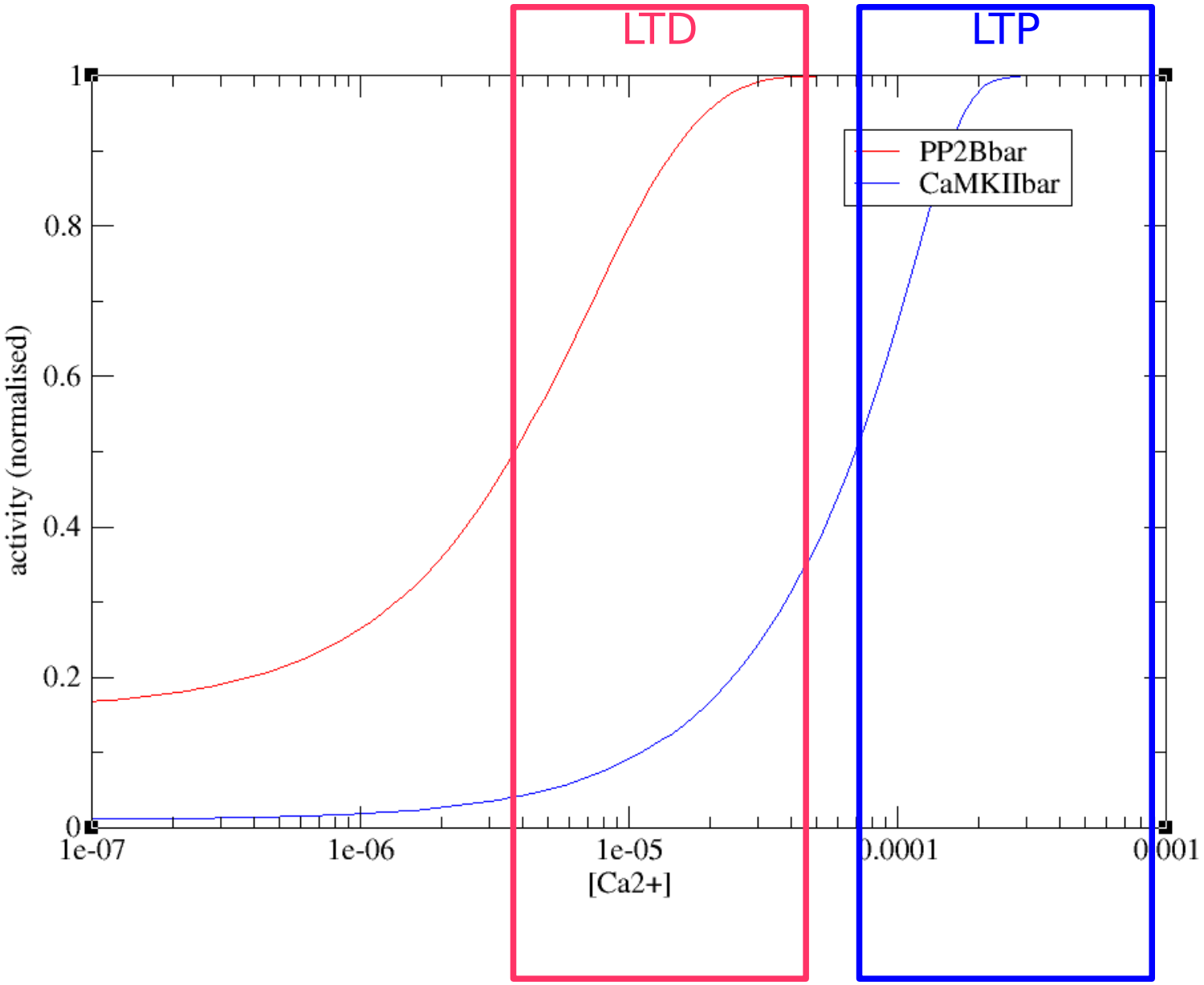


Bidirectional synaptic plasticity

half saturation of calmodulin: CaN half activated



Bidirectional synaptic plasticity



Conclusions of part 1

Allosteric model of Calmodulin, with only two states for the EF hands, binding calcium with different affinities, and a concerted transition for all 4 EF hands. Parameters estimated from experimental data-sets.

Model fits independent experimental datasets.

Affinity for calcium increases upon binding of the target.

CaM significantly “active” with less than 4 Ca^{2+} bound.

CaM bind its targets with less than 4 Ca^{2+} bounds.

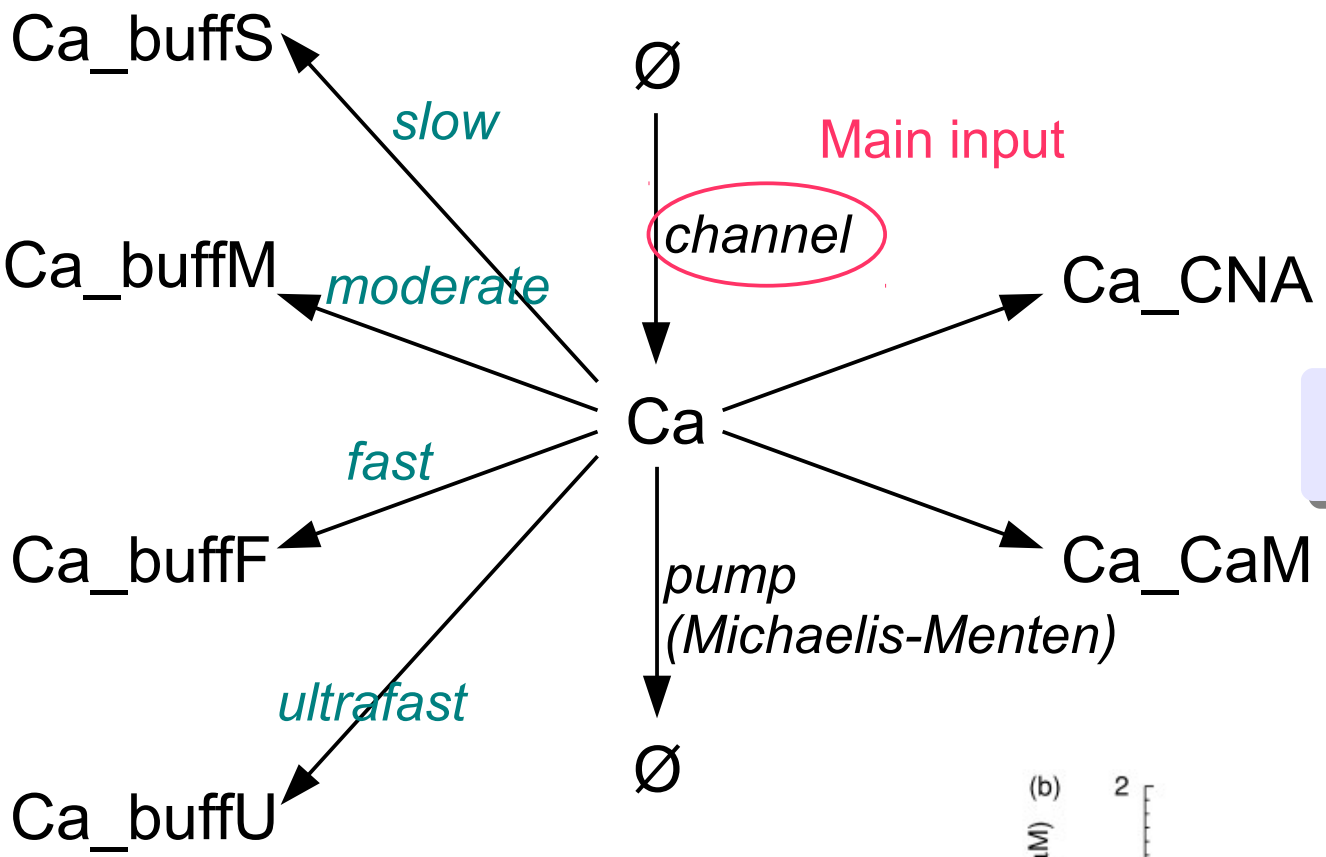
The model displays an activation of the sole PP2B at low concentration of calcium, while high concentrations activate both PP2B and CaMKII.

Wait a minute!
Signal transduction is not at equilibrium!

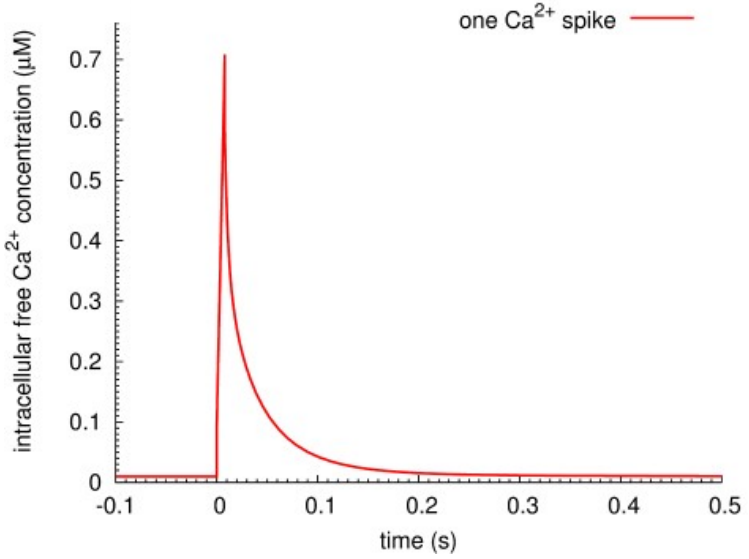
AMPAR post-synaptic potential:	5 ms
Calcium spike:	50 ms
Half saturation calmodulin (kon=1.5e6, koff=100):	5 ms
Relaxation between calmodulin states:	1 ms
autophosphorylation of CaMKII (kon=6):	100 ms

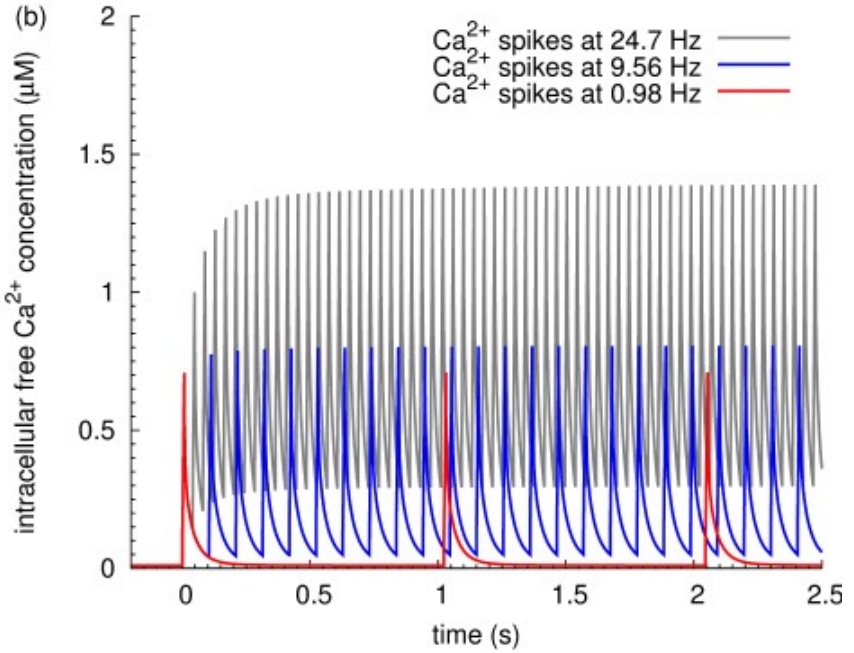
≠

Dynamic of calcium in the spine



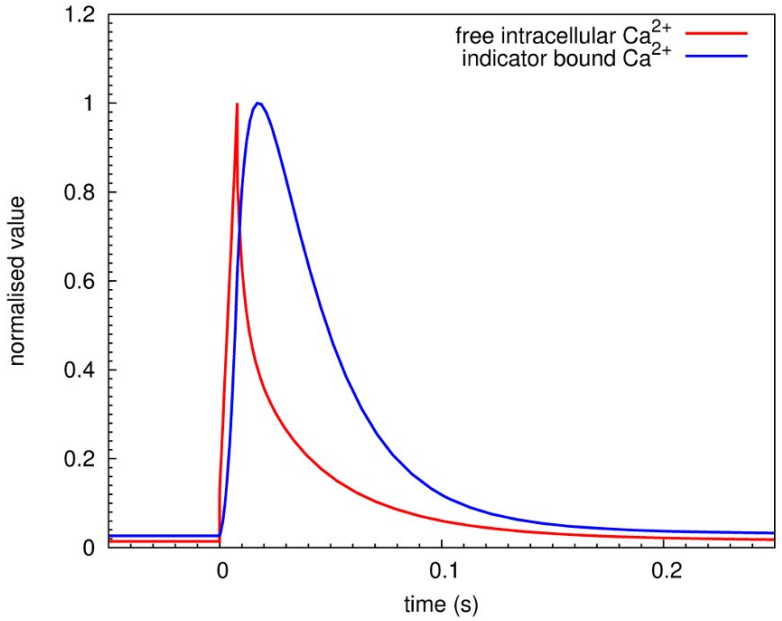
Li L, Stefan MI, Le novère N.
PLoS ONE (2012), 7(9): e43810

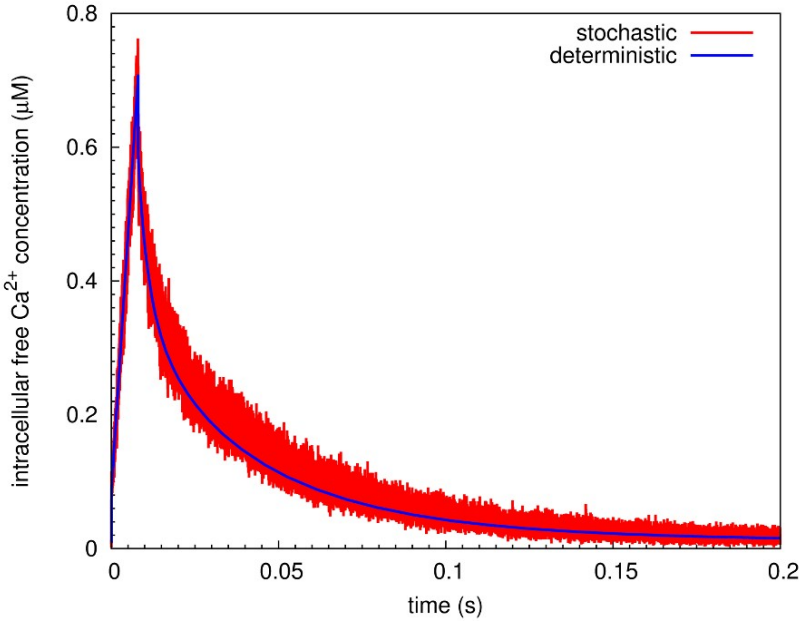


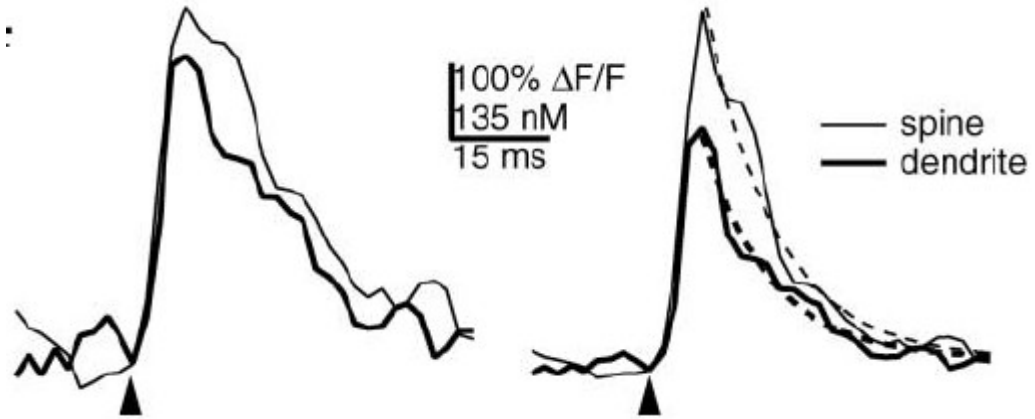


Babraham Institute

Are those spikes realistic?







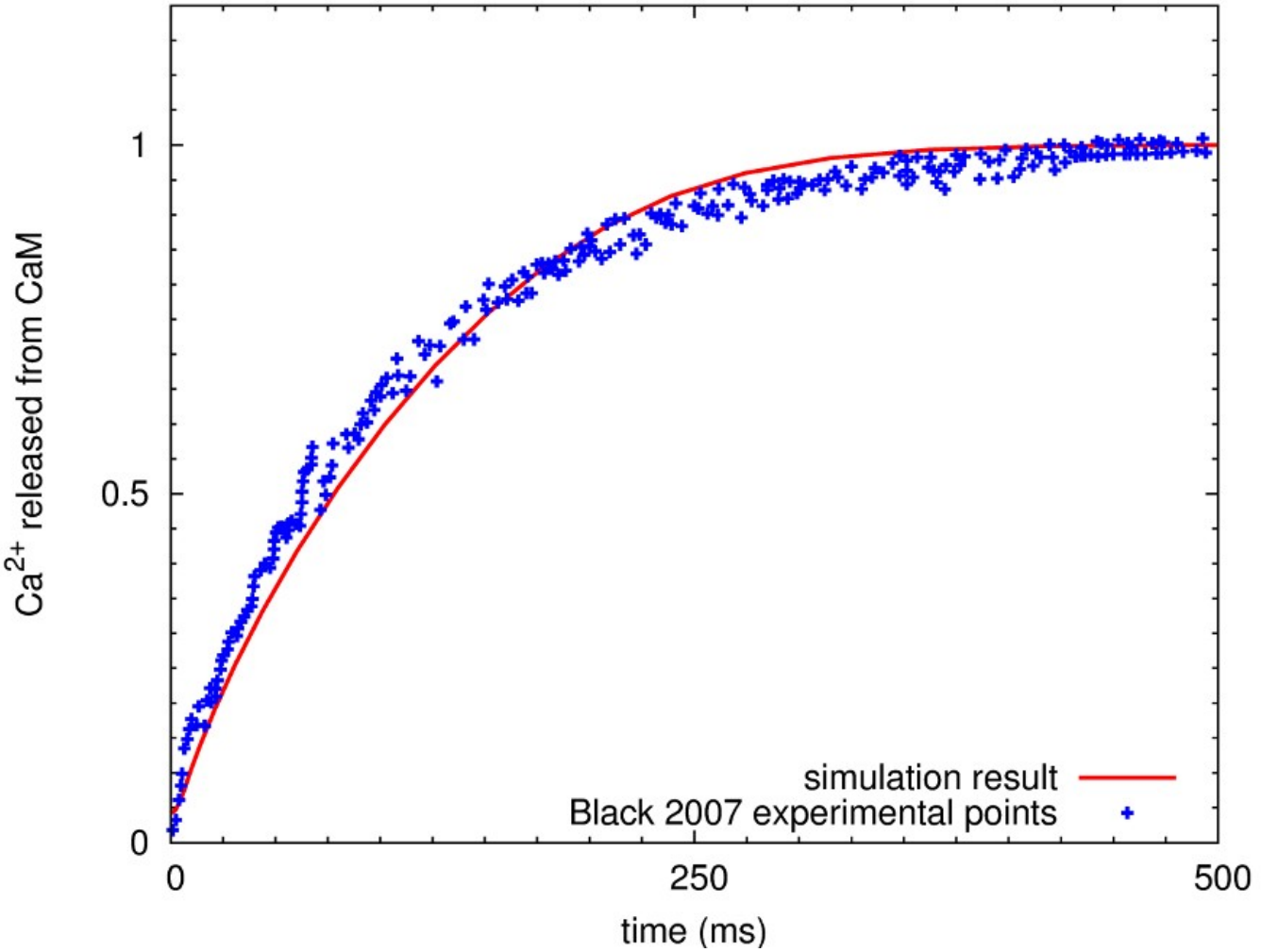
Fluorescence

Free calcium

Relative uncertainty increases when concentration decreases, both in concentration and time, but no difference in dynamics.

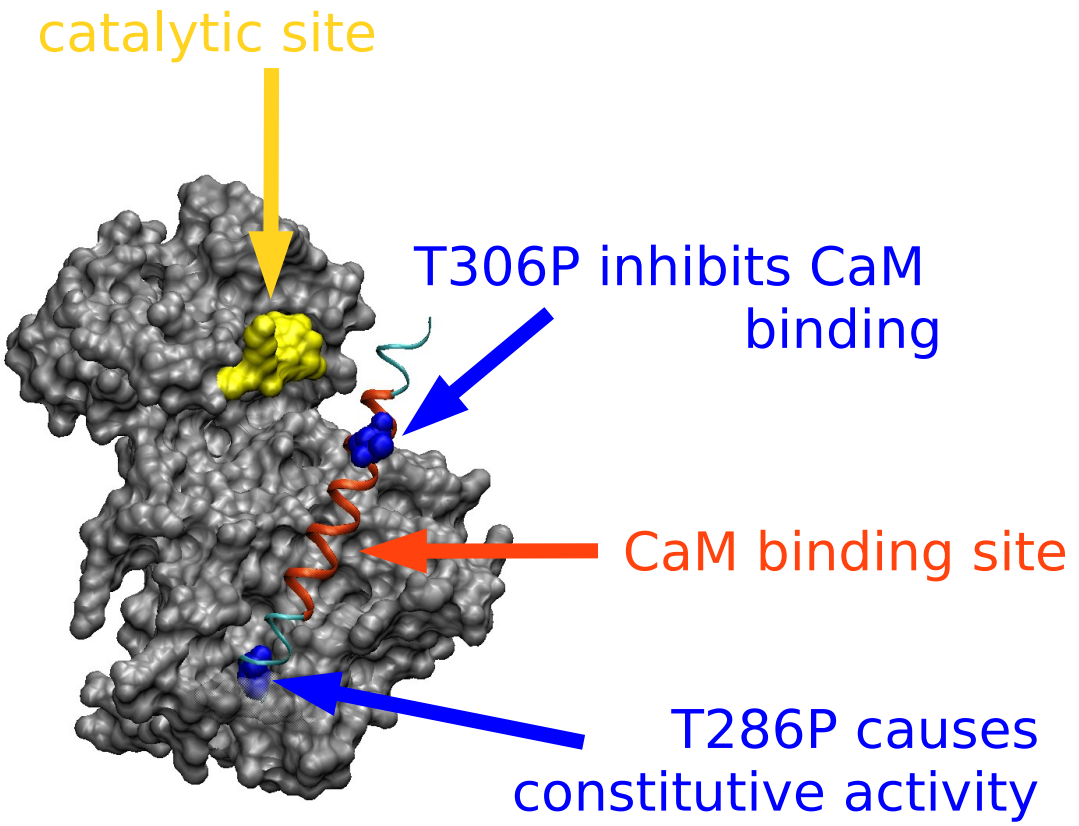
Sabatini et al (2002)
Neuron 33: 439–452.

Validation of CaM kinetics



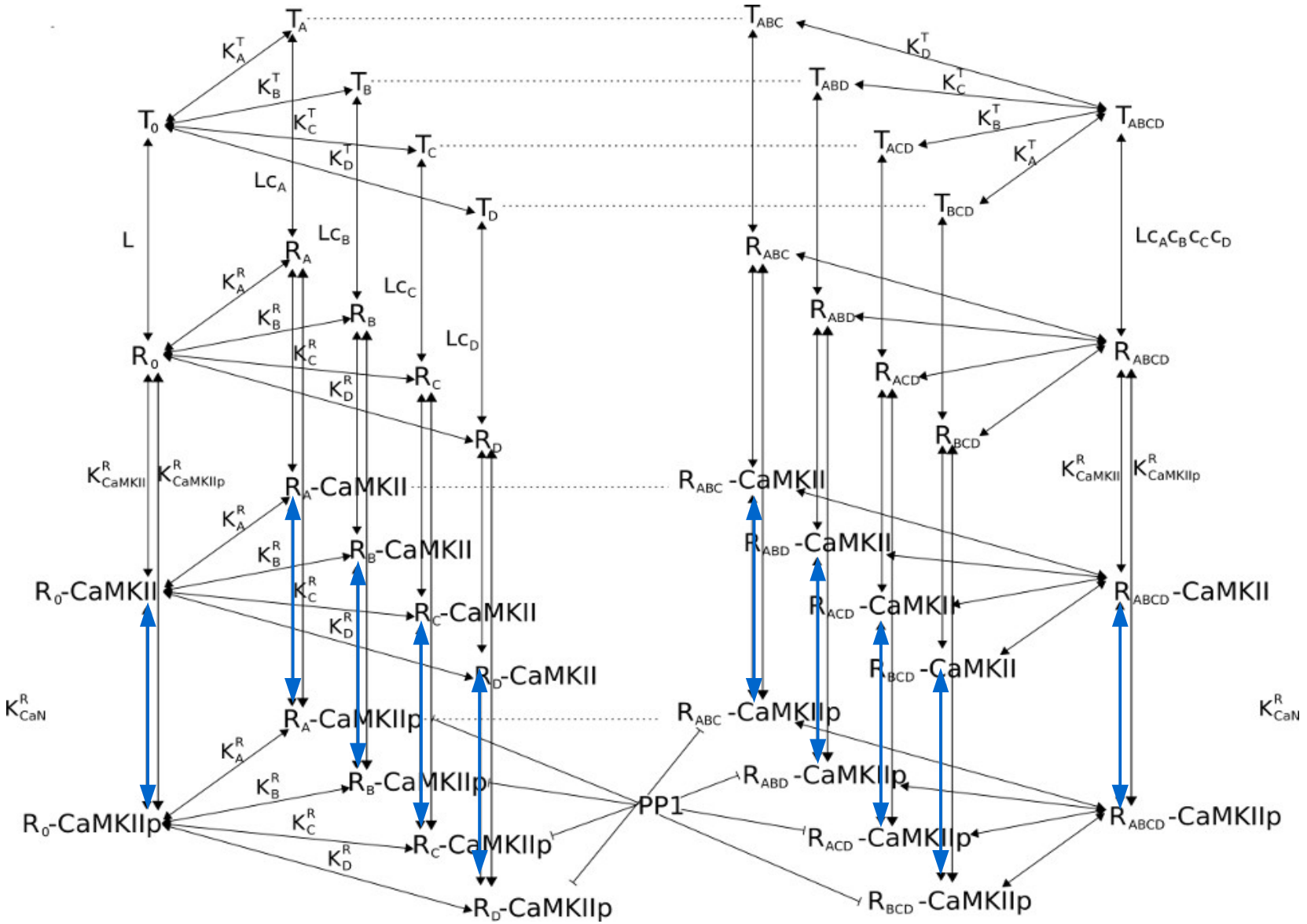
Black DJ, Selfridge JE, Persechini A (2007). *Biochemistry* 46: 13415–13424.

Calcium/calmodulin kinase II

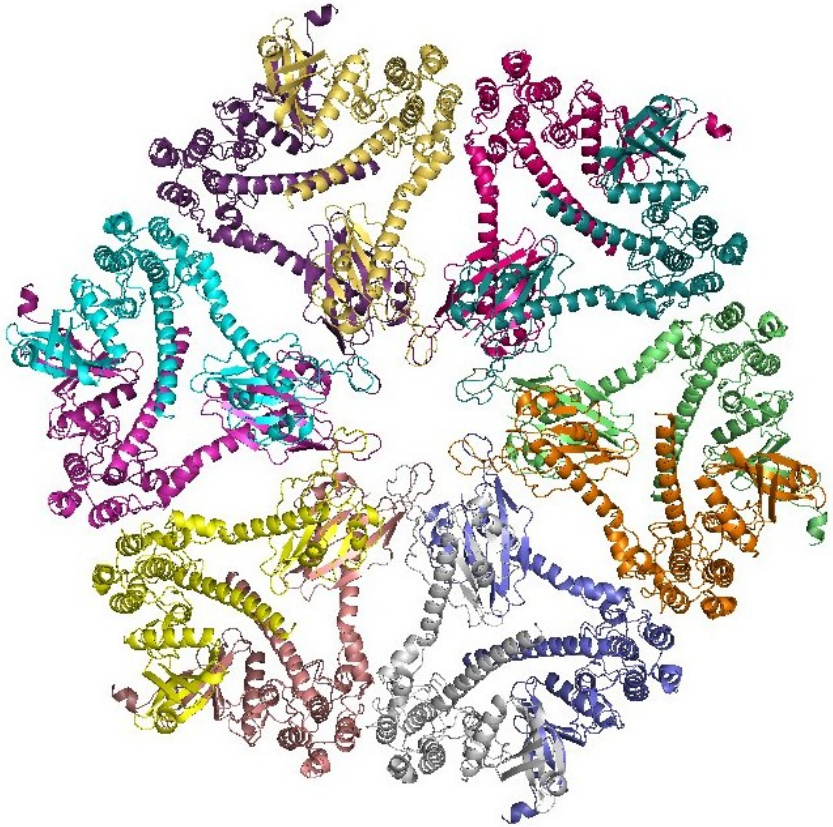


Calmodulin trapping is an apparent increase of affinity of CaMKII for CaM when T286 is phosphorylated

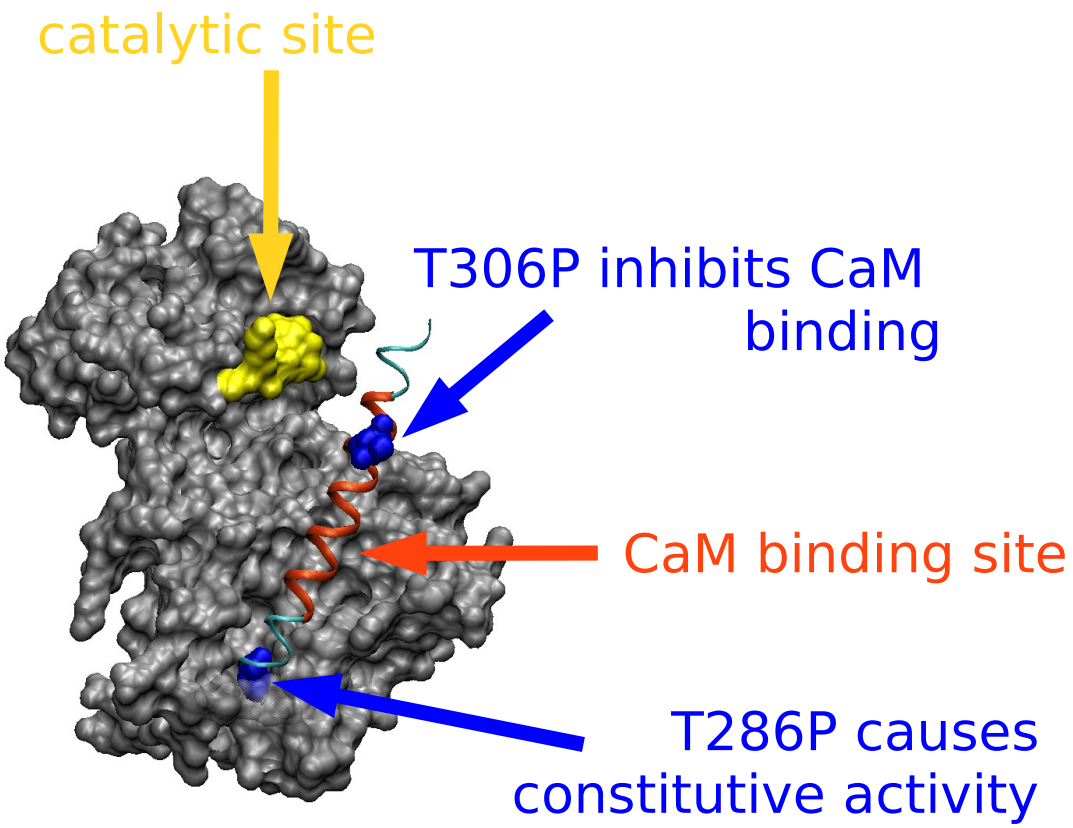
Stefan MI, Marshall D, Le Novère N. *PLoS ONE* (2012), 7(1): e29406


T_A
T_B
T_C
T_D
T_0
K_A^T
K_B^T
K_C^T
K_D^T
Lc_A
Lc_B
Lc_C
Lc_D
L
R_A
R_B
R_C
R_D
R_0
K_A^R
K_B^R
K_C^R
K_D^R
K_CaMKII^R
K_CaMKIIp^R
R_A-CaMKII
R_B-CaMKII
R_C-CaMKII
R_D-CaMKII
R_0-CaMKII
R_A-CaMKIIp
R_B-CaMKIIp
R_C-CaMKIIp
R_D-CaMKIIp
R_0-CaMKIIp
K_CaN^R
PP1
T_ABC
T_ABD
T_ACD
T_BCD
T_ABCD
K_D^T
K_C^T
K_B^T
K_A^T
R_ABC
R_ABD
R_ACD
R_BCD
R_ABCD
Lc_A Lc_B Lc_C Lc_D
R_ABC -CaMKII
R_ABD -CaMKII
R_ACD -CaMKII
R_BCD -CaMKII
R_ABCD -CaMKII
R_ABC -CaMKIIp
R_ABD -CaMKIIp
R_ACD -CaMKIIp
R_BCD -CaMKIIp
R_ABCD -CaMKIIp

Calcium/calmodulin kinase II



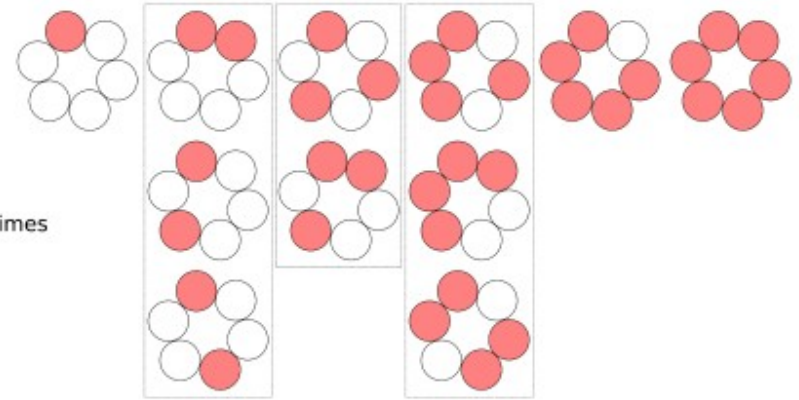
Dodecamer;
Trans-phosphorylation of T286
by neighbouring subunits



Stefan MI, Marshall D, Le Novère N. *PLoS ONE* (2012), 7(1): e29406

A given fraction of activated CaMKII monomers.

Randomly allocate activated monomers to CaMKII hexamers.
Record the number of hexamers containing different numbers of active monomers (in red) as the following:



Repeat 1000 times

Calculate the average population for each number of active monomers per hexamer.

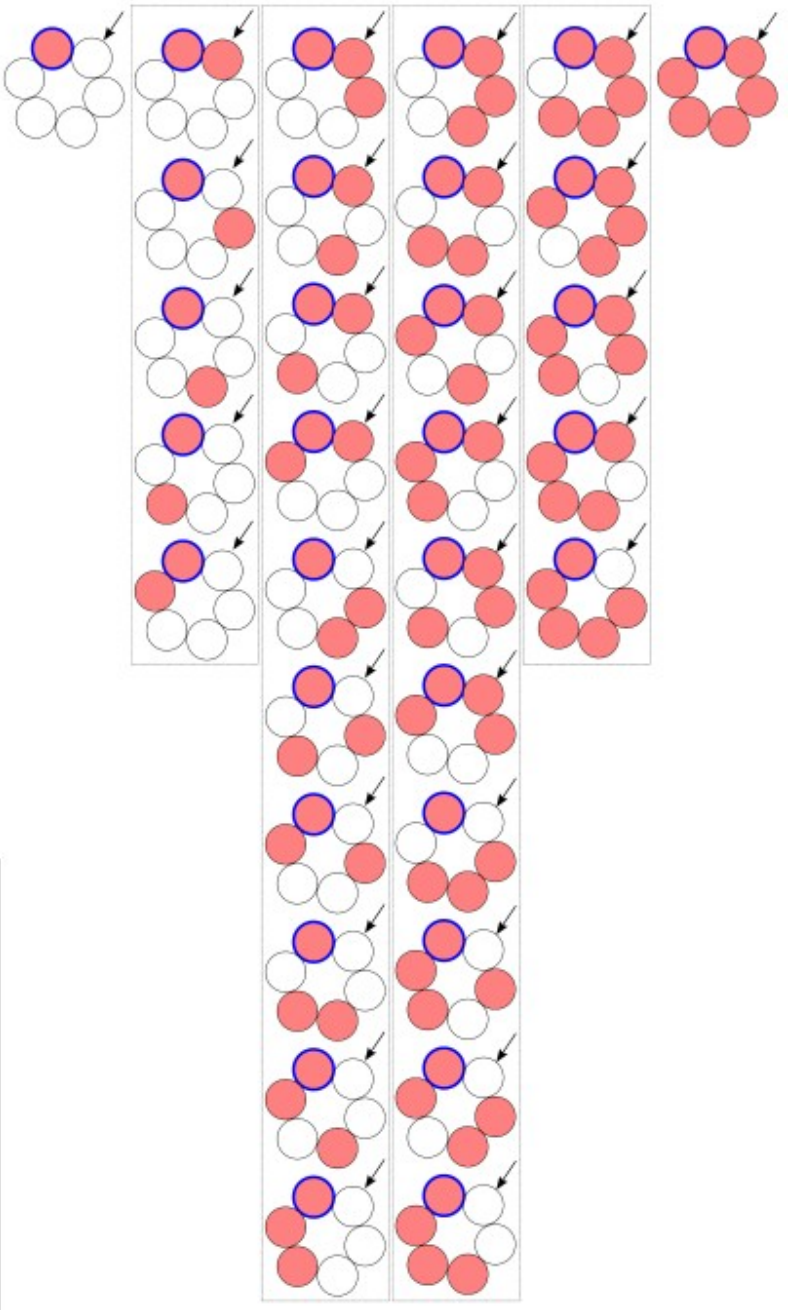
Repeat for every 1% increase of CaMKII active monomers

Multiply average populations of each number of active monomers per hexamer by their corresponding probabilities of having an active neighbour.

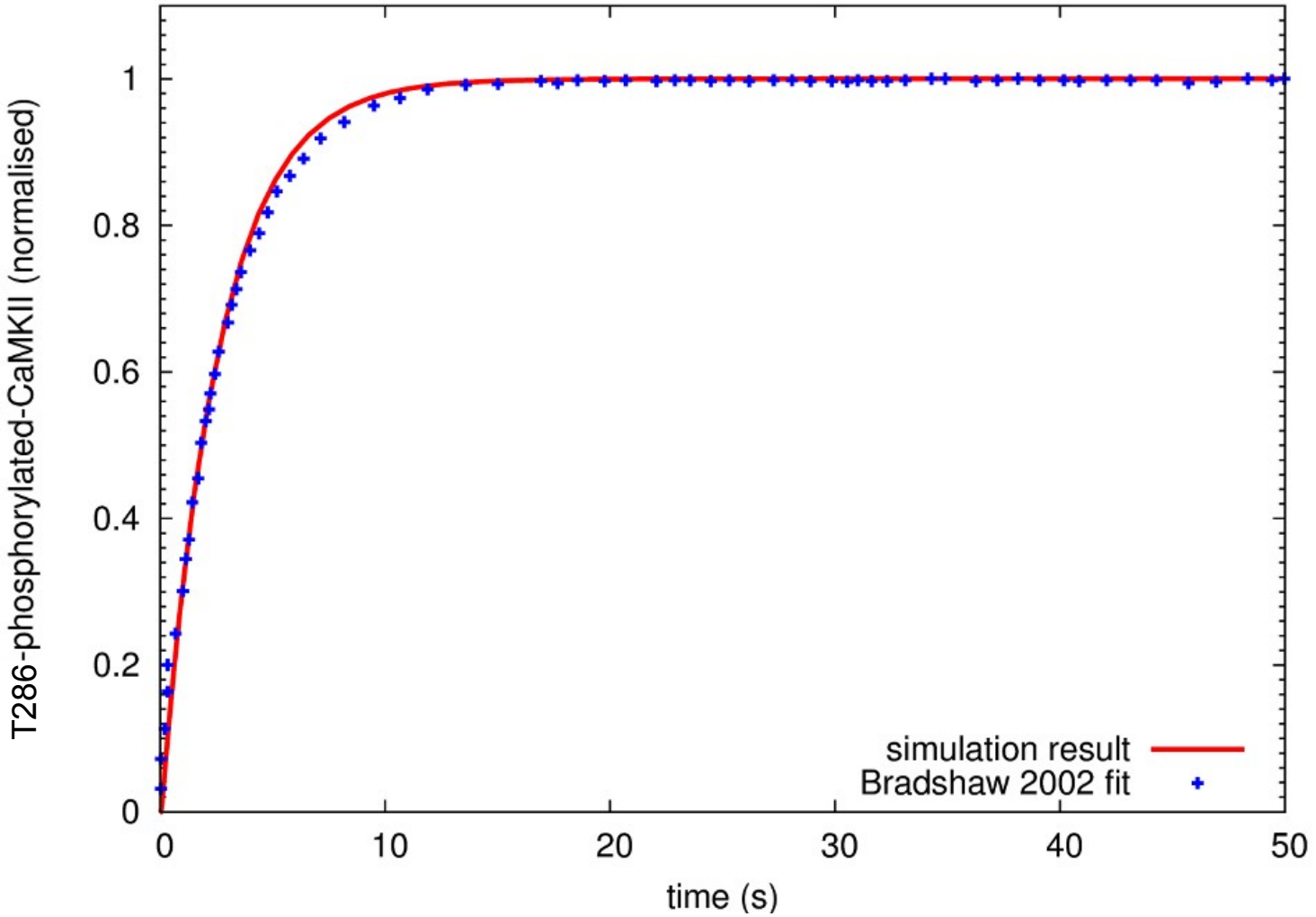
The sum of these six numbers is a coefficient that can be used to adjust CaMKII autophosphorylation rate.

Fit these 100 coefficients into a polynomial function of activated CaMKII monomers, and embed this function in the model.

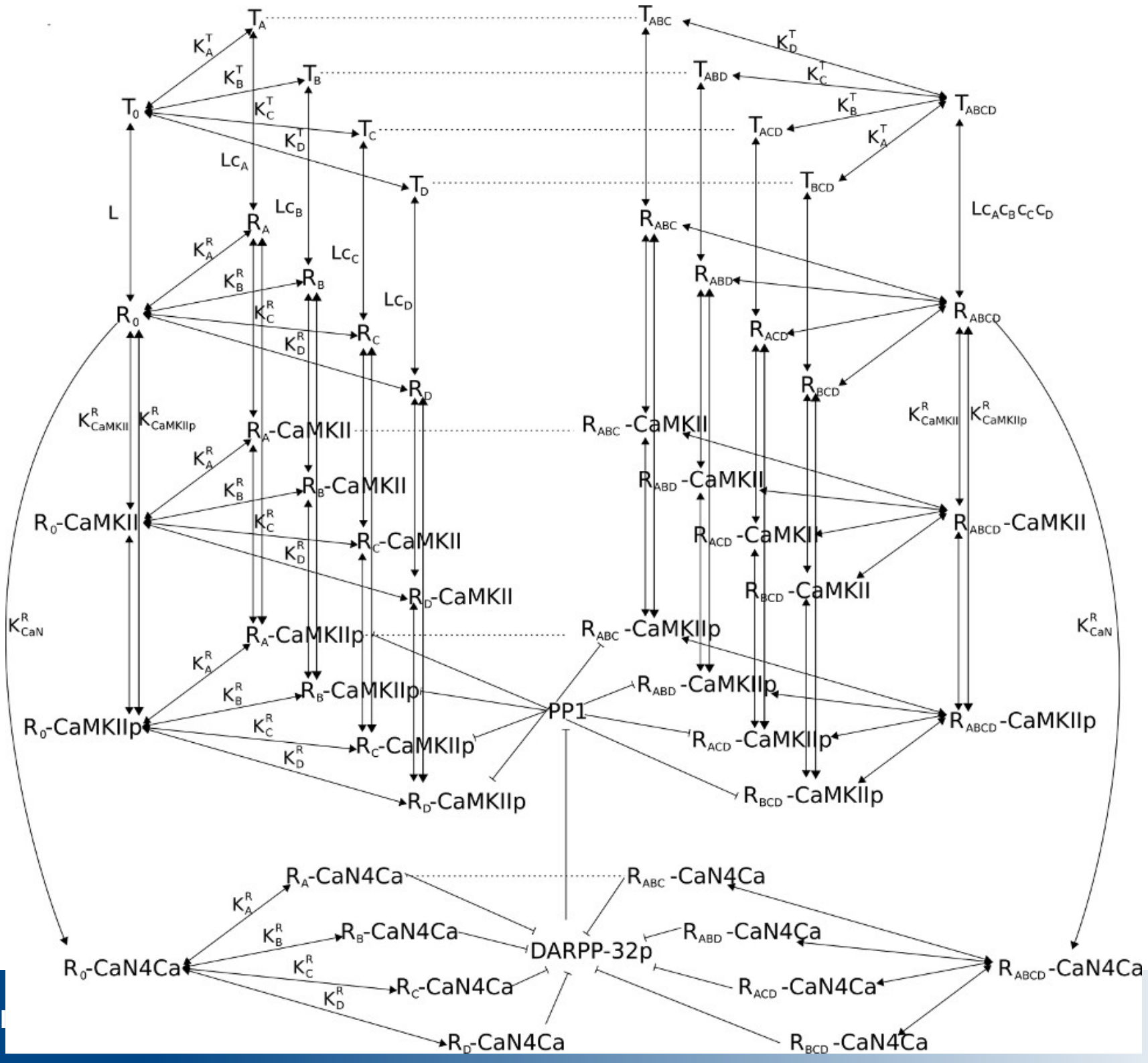
Calculate the probabilities of having an active neighbour on one specific side (indicated by the arrow) of the activated monomer of interest (blue outline) (since only the asymmetrical situation is considered). The possible positions of activated monomers are listed as the following, with corresponding probabilities:

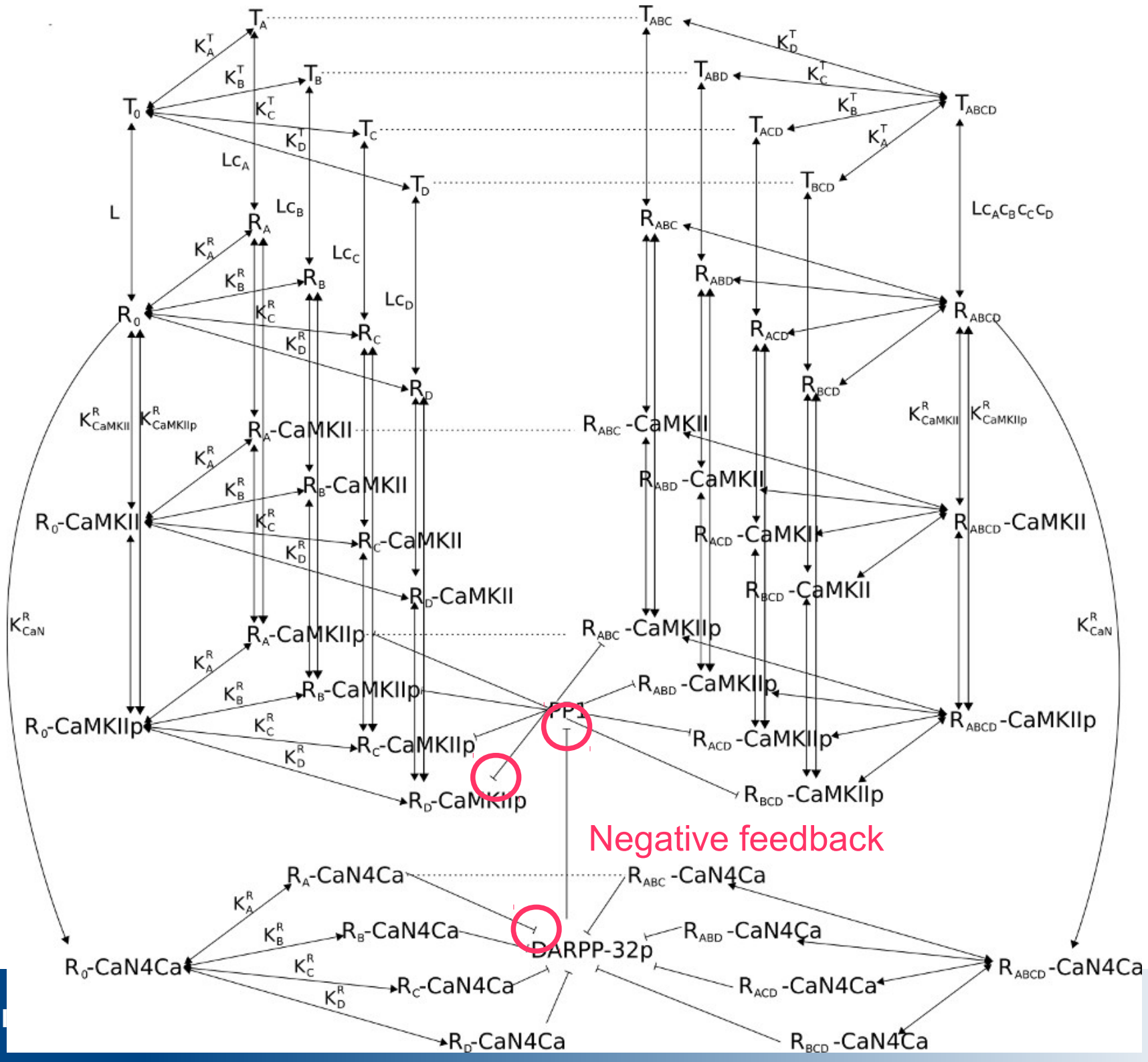


Validation of CaMKII kinetics

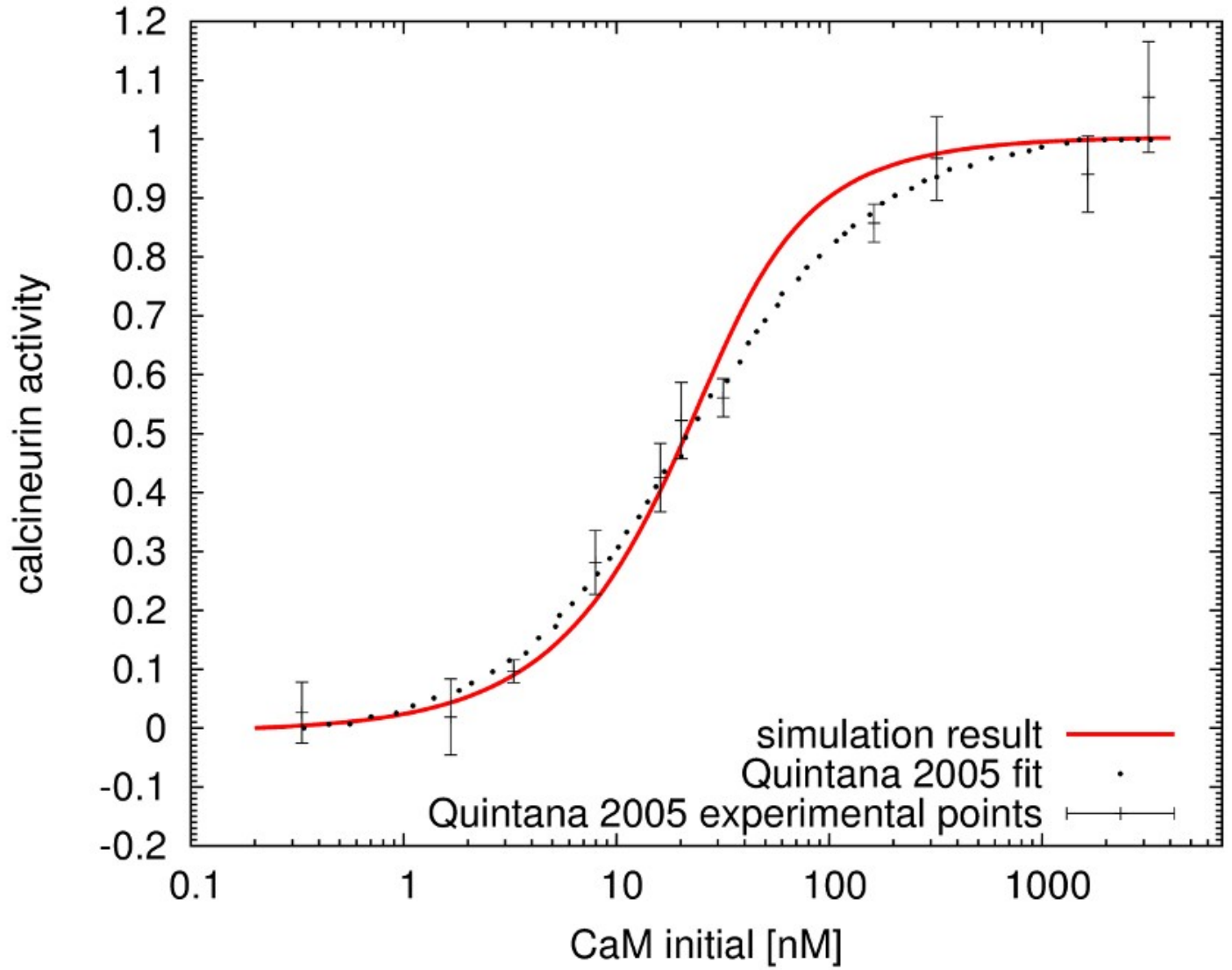


Bradshaw JM, Kubota Y, Meyer T, Schulman H (2003). *PNAS* 100: 10512–10517.


T_A
T_ABC
K_A^T
K_D^T
T_0
T_B
T_ABD
K_B^T
K_C^T
K_C^T
T_ABCD
T_C
T_ACD
K_D^T
K_B^T
Lc_A
K_A^T
T_D
T_BCD
L
R_A
R_ABC
Lc_B
Lc_A Lc_B Lc_C Lc_D
K_A^R
Lc_C
R_B
R_ABD
K_B^R
R_0
K_C^R
R_C
R_ACD
R_ABCD
K_D^R
Lc_D
R_D
R_BCD
K_CaMKII^R
K_CaMKIIp^R
R_A-CaMKII
R_ABC-CaMKII
K_A^R
R_B-CaMKII
R_ABD-CaMKII
K_B^R
R_0-CaMKII
R_ABCD-CaMKII
K_C^R
R_C-CaMKII
R_ACD-CaMKII
K_D^R
R_D-CaMKII
R_BCD-CaMKII
K_CaN^R
R_A-CaMKIIp
R_ABC-CaMKIIp
K_A^R
R_B-CaMKIIp
R_ABD-CaMKIIp
K_B^R
PP1
R_0-CaMKIIp
R_ABCD-CaMKIIp
K_C^R
R_C-CaMKIIp
R_ACD-CaMKIIp
K_D^R
R_D-CaMKIIp
R_BCD-CaMKIIp
R_A-CaN4Ca
R_ABC-CaN4Ca
K_A^R
K_B^R
R_B-CaN4Ca
R_ABD-CaN4Ca
R_0-CaN4Ca
DARPP-32p
R_ABCD-CaN4Ca
K_C^R
R_C-CaN4Ca
R_ACD-CaN4Ca
K_D^R
R_D-CaN4Ca
R_BCD-CaN4Ca

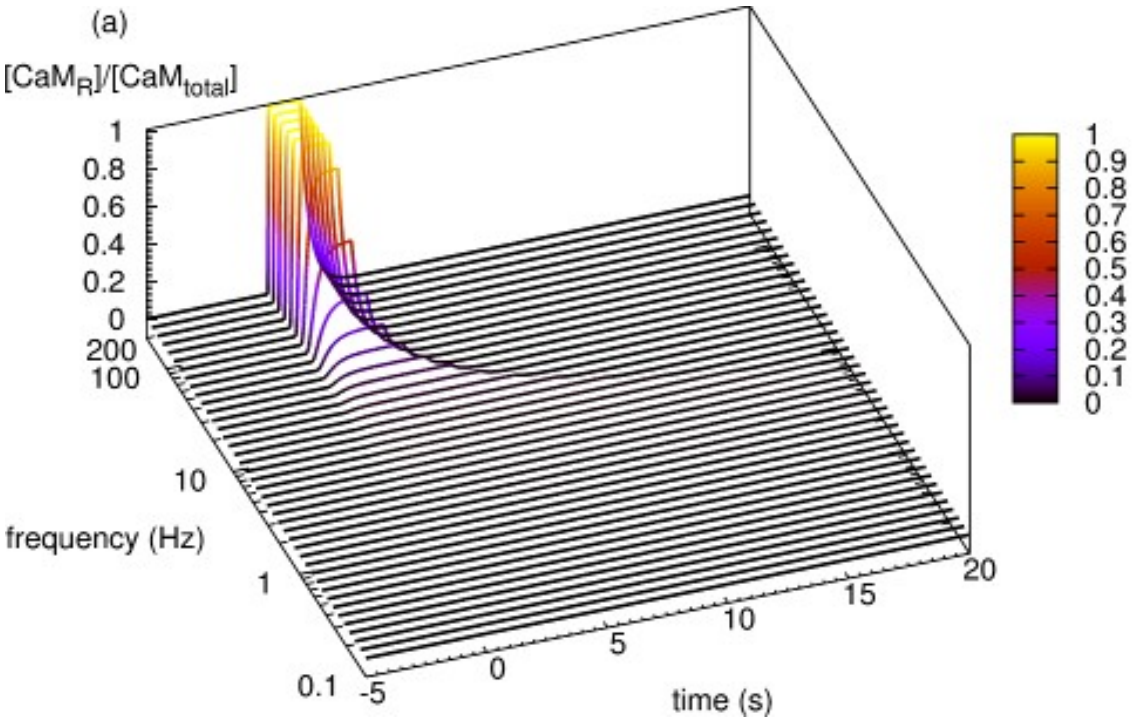

Negative feedback

Validation of calcium-activation of CaN



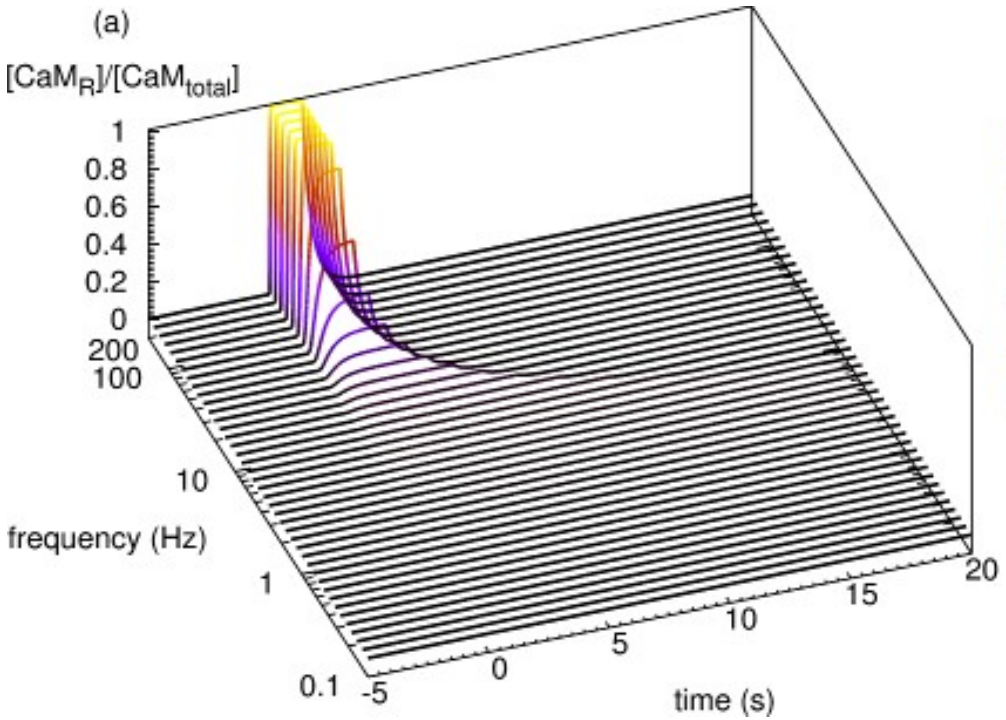
Quintana AR, Wang D, Forbes JE, Waxham MN (2005). *BBRC* 334: 674–680.

Calmodulin activation

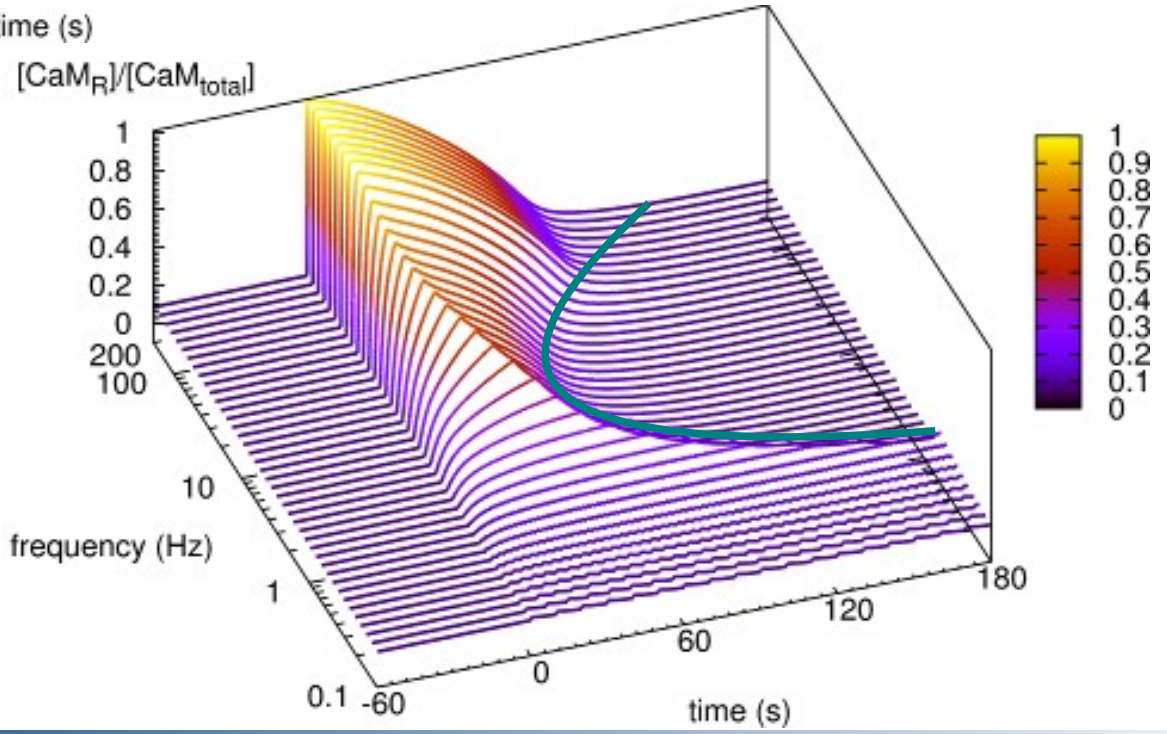


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

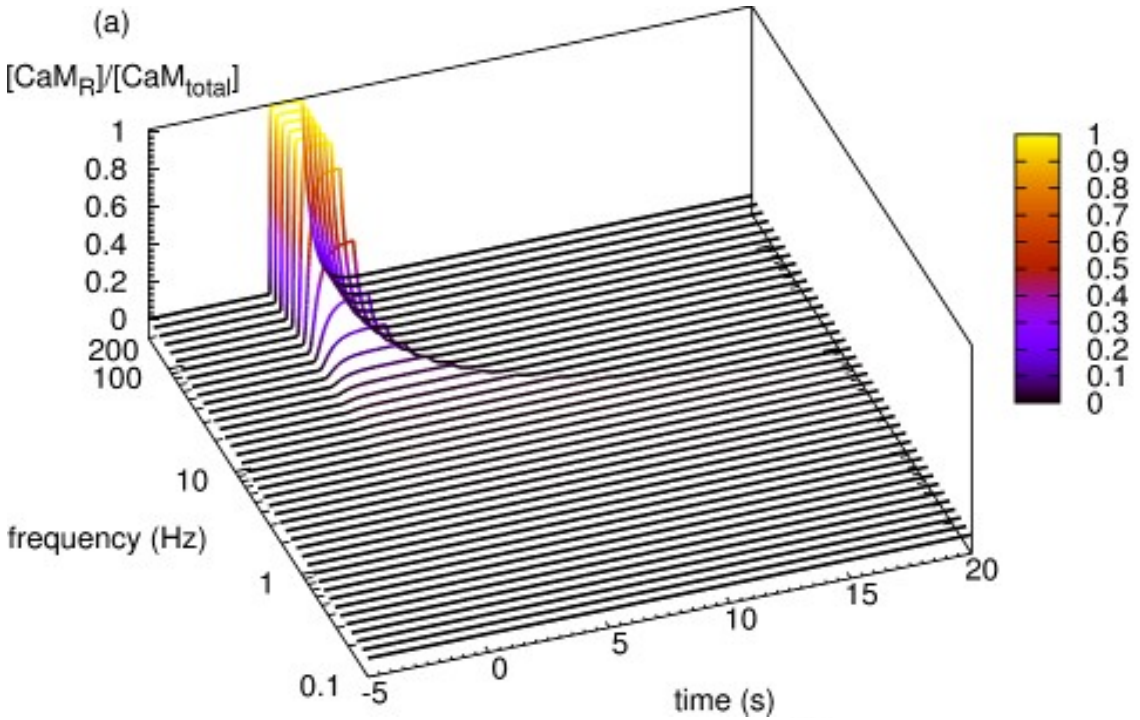


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)



CaM with targets. Binding to CaN and CaMKII stabilises R state, with higher affinity. Positive feedback loop
➜ bistability

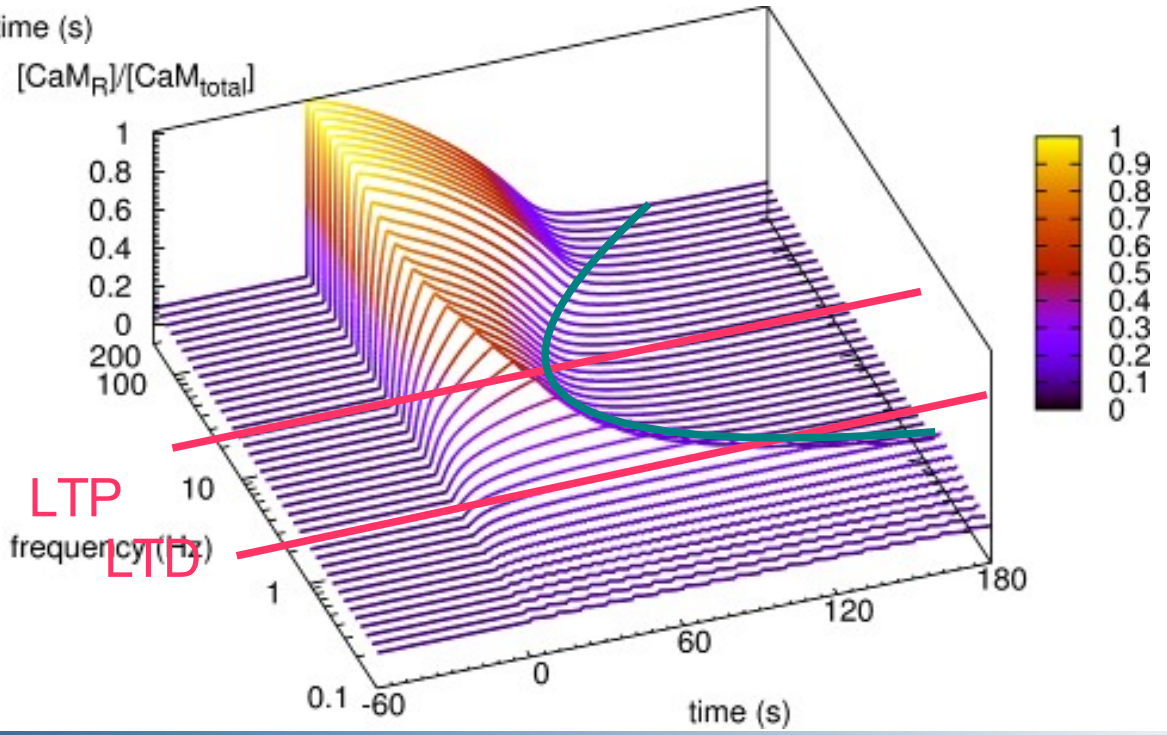
Calmodulin activation



CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

At high frequency, effects of calcium signals last much longer than the signal itself

CaM with targets. Binding to CaN and CaMKII stabilises R state, with higher affinity. Positive feedback loop
➜ bistability



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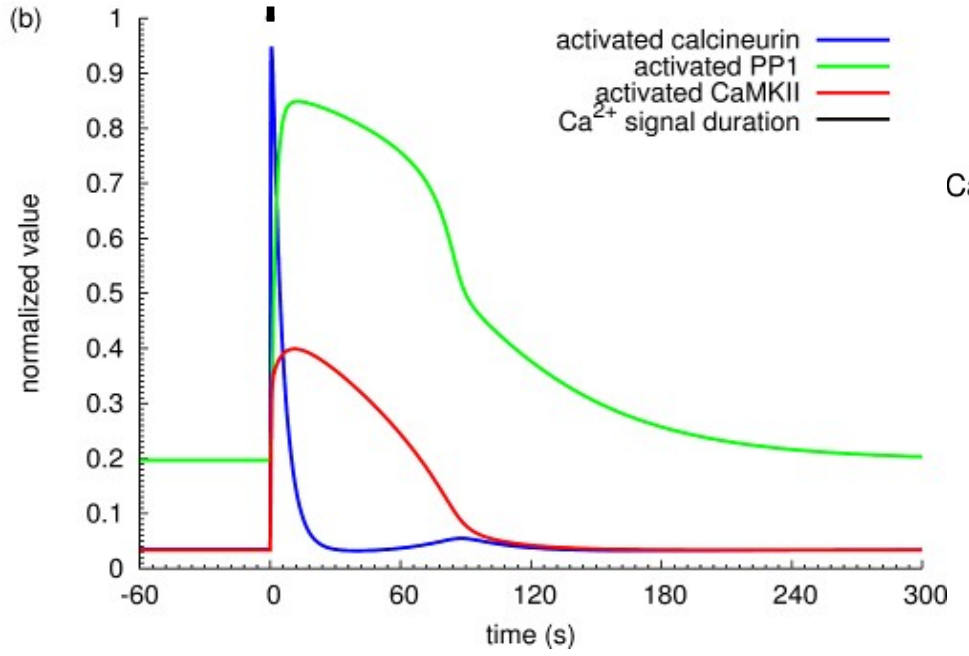
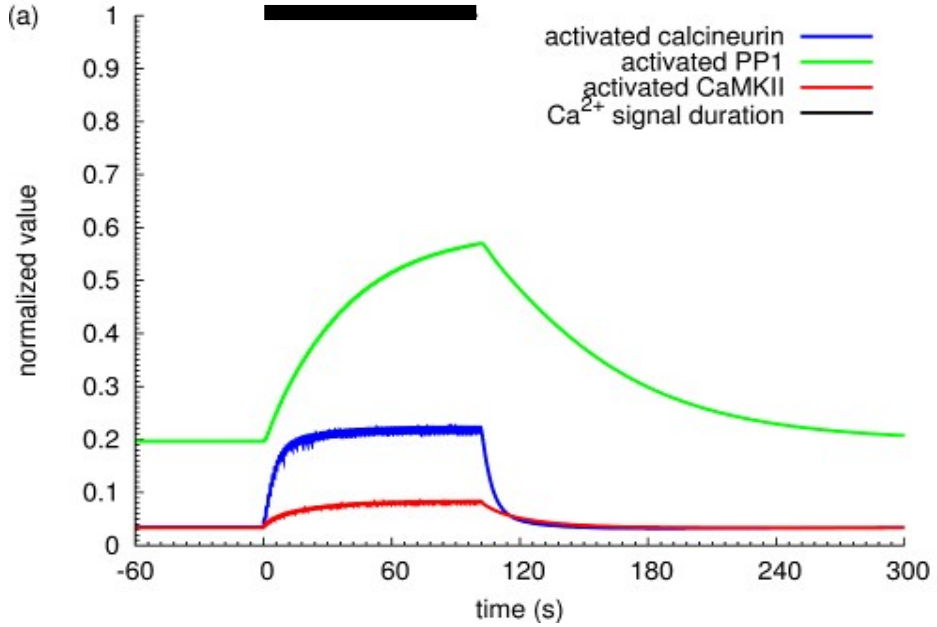
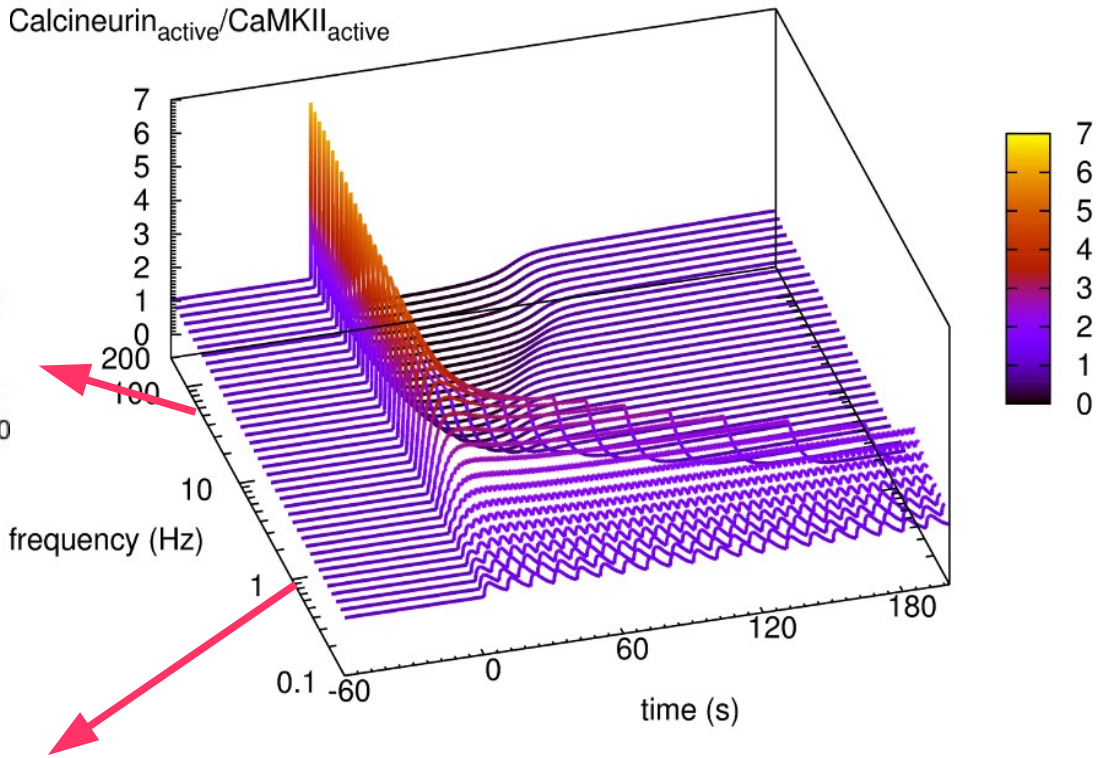
Temporal activation of CaMKII and CaN







All calcium frequencies increase CaN **AND** CaMKII. It is not a Switch. But the ratio of activation changes

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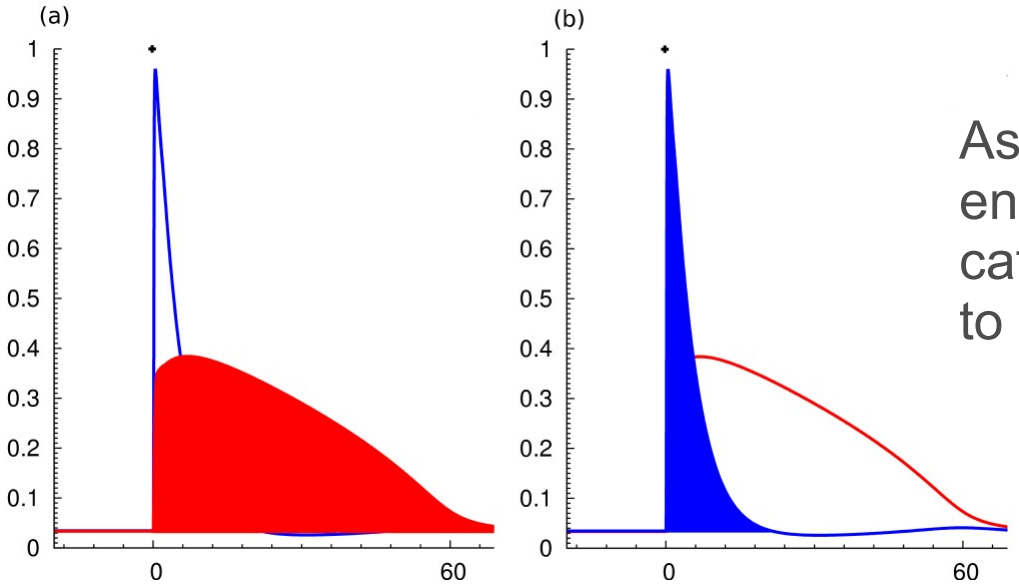
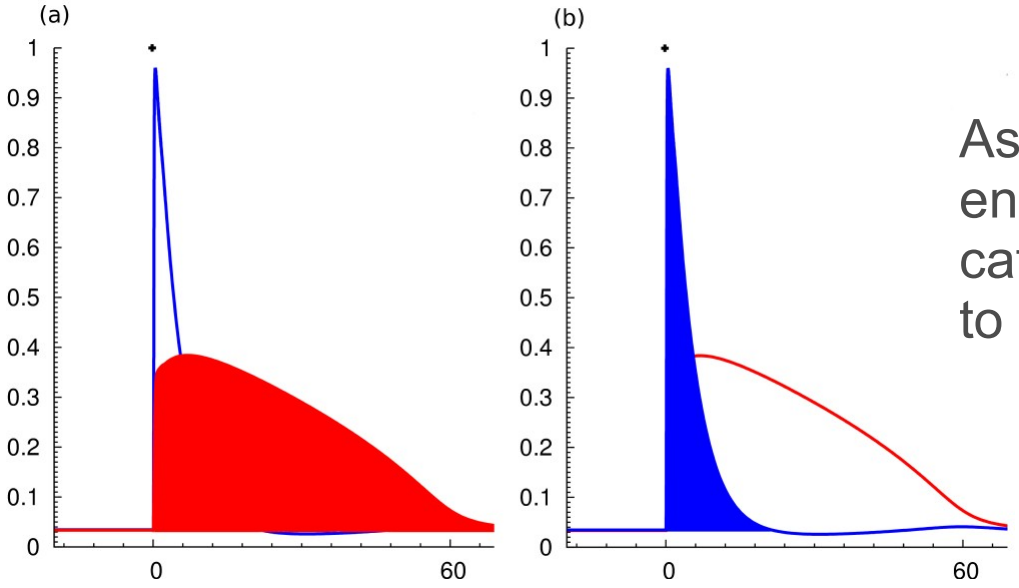
Bidirectional plasticity



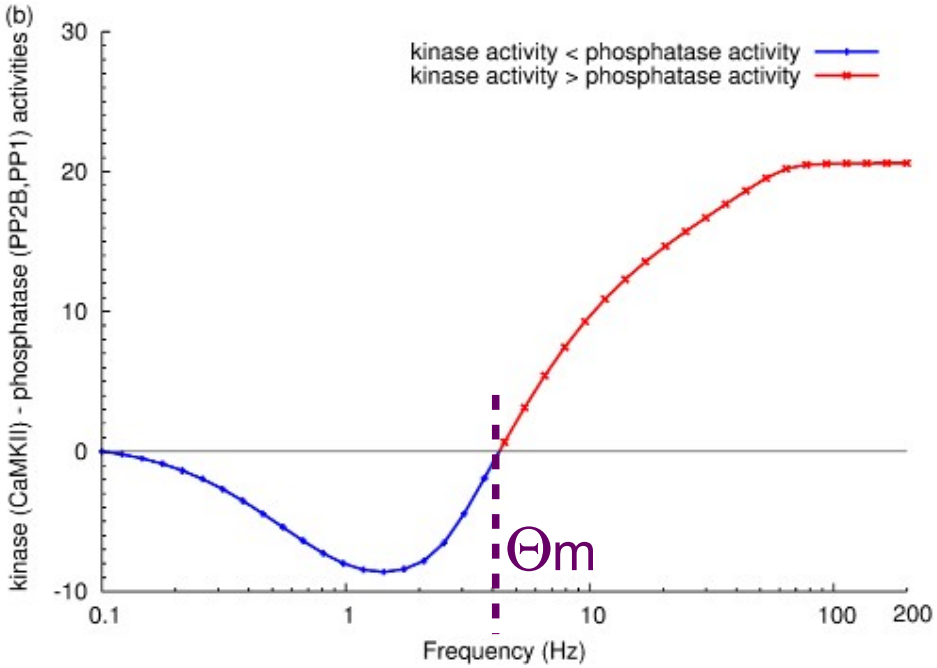
Assuming that catalytic rates of active enzyme do no change, the quantity of catalysed reaction events is proportional to the integral of the activation curve

Bidirectional plasticity

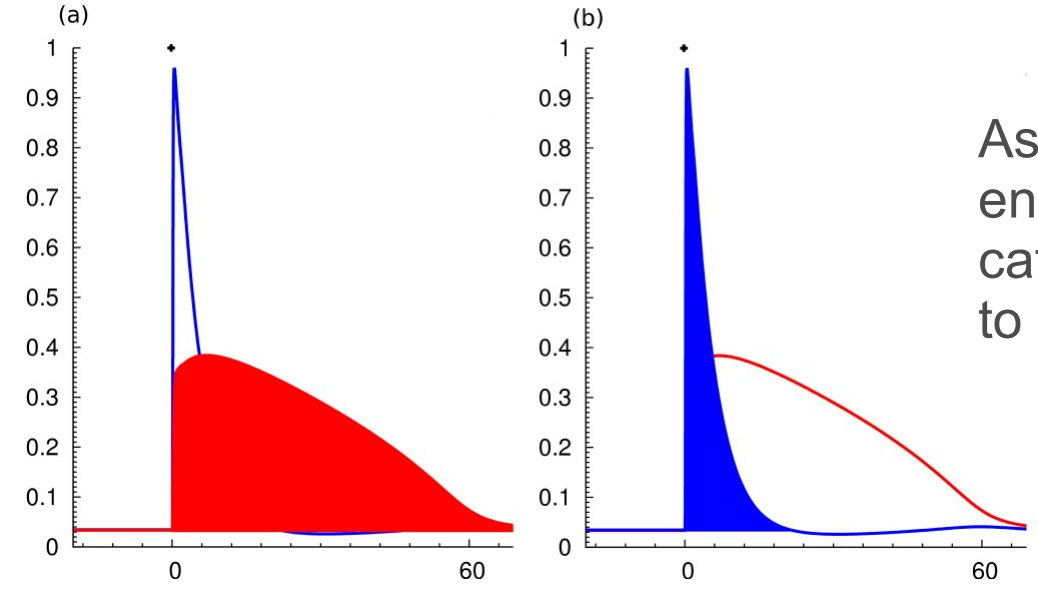


Assuming that catalytic rates of active enzyme do no change, the quantity of catalysed reaction events is proportional to the integral of the activation curve

Bienestock-Cooper-Munro (BCM) curve: difference of active areas*catalytic activities



Bidirectional plasticity



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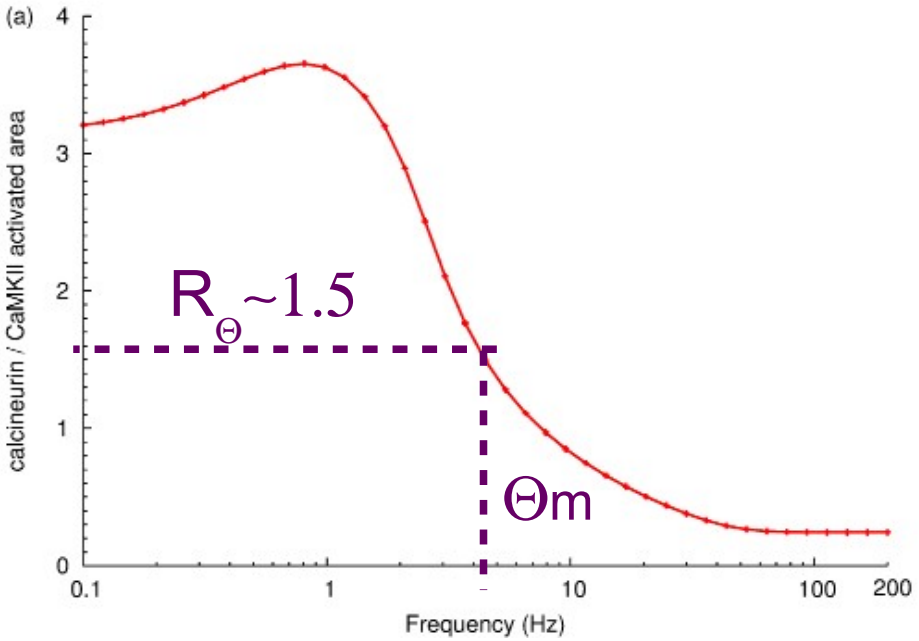
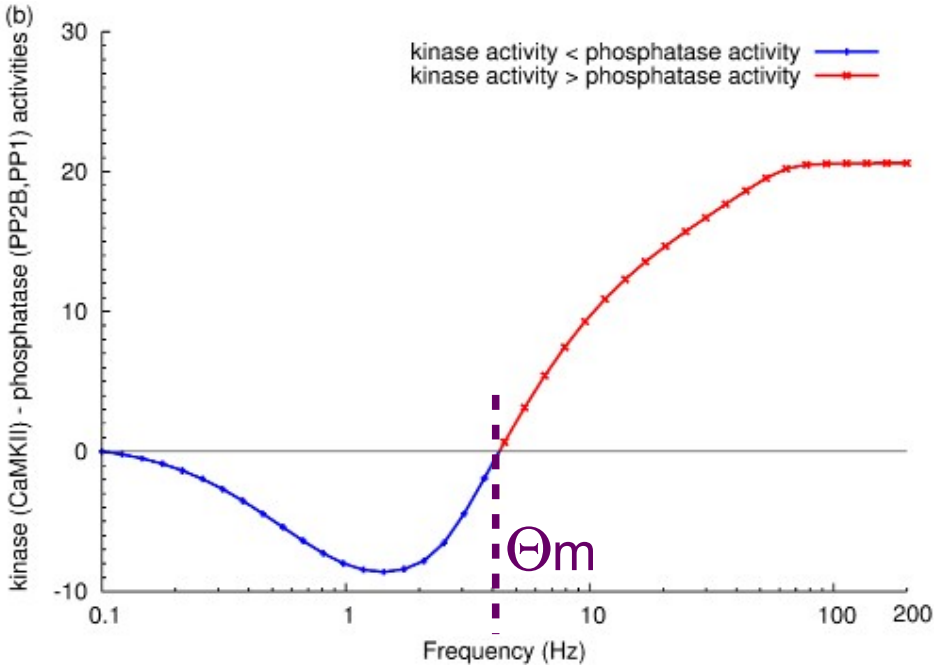
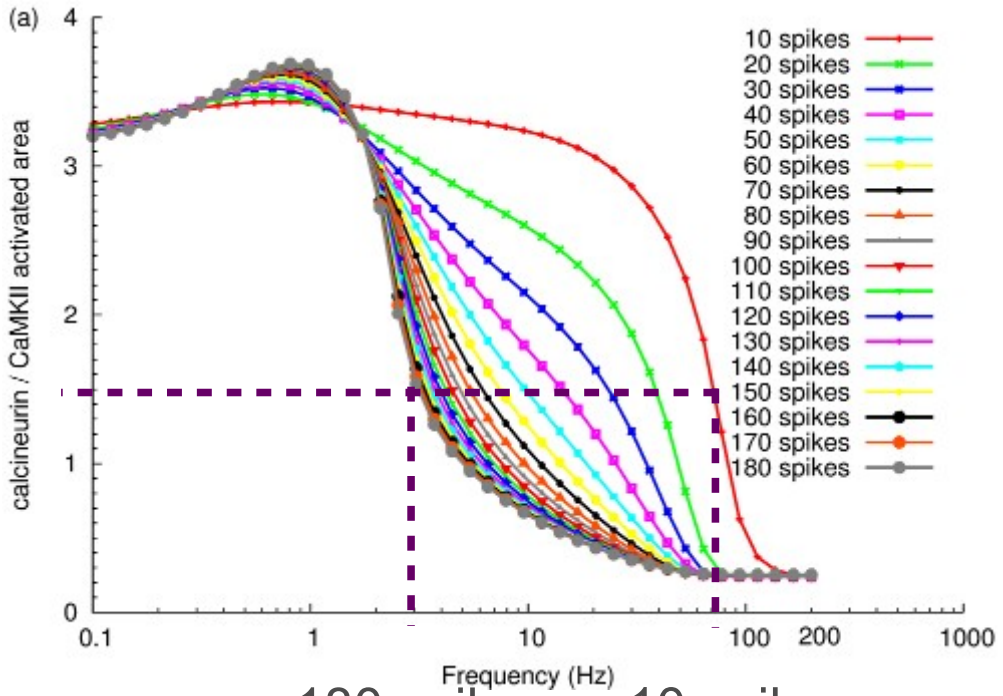


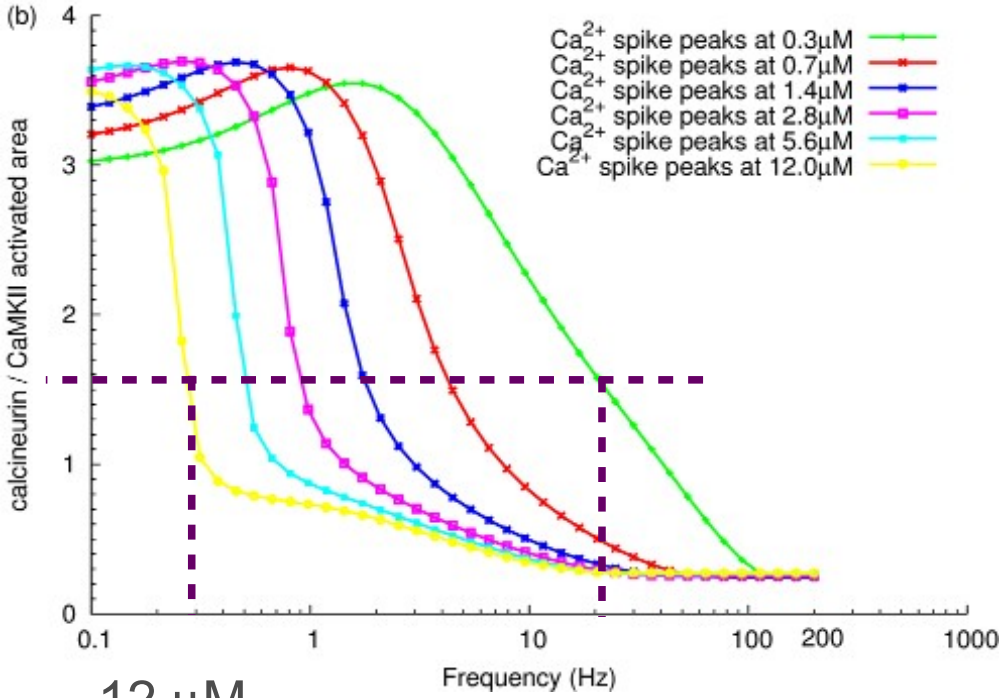


Effect of calcium duration and amount



180 spikes = 3 Hz

10 spikes = 70 Hz

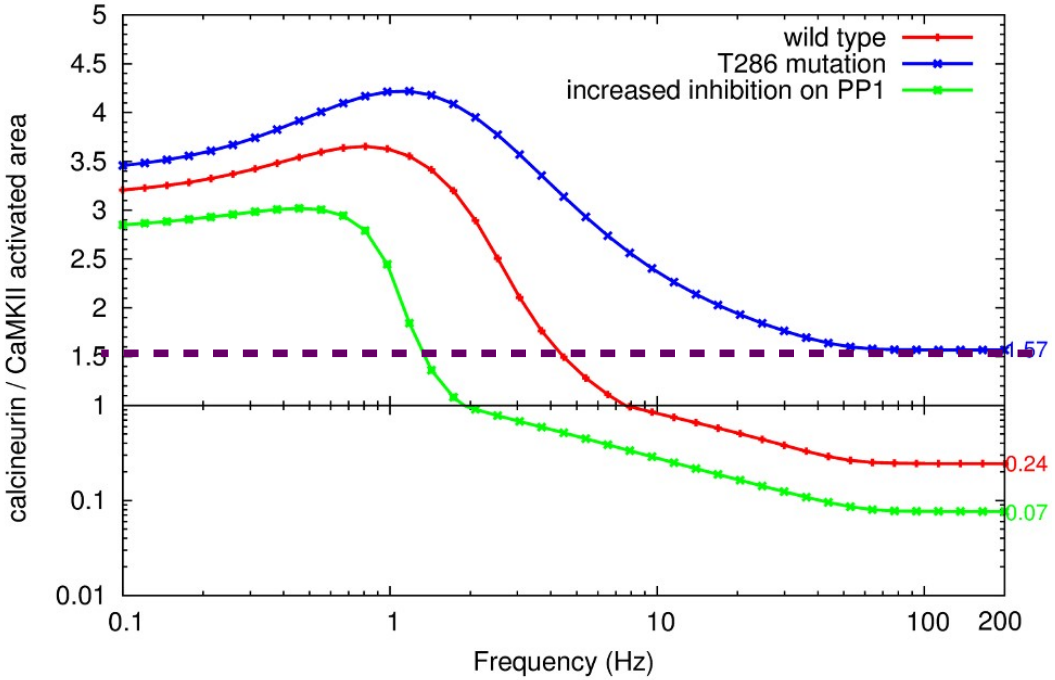


12 µM = 0.3 Hz

0.3 µM = 20 Hz

Prolonged or intense signals Decrease the threshold frequency: It is not an intrinsic property of the synapse

Effect of intrinsic system perturbations

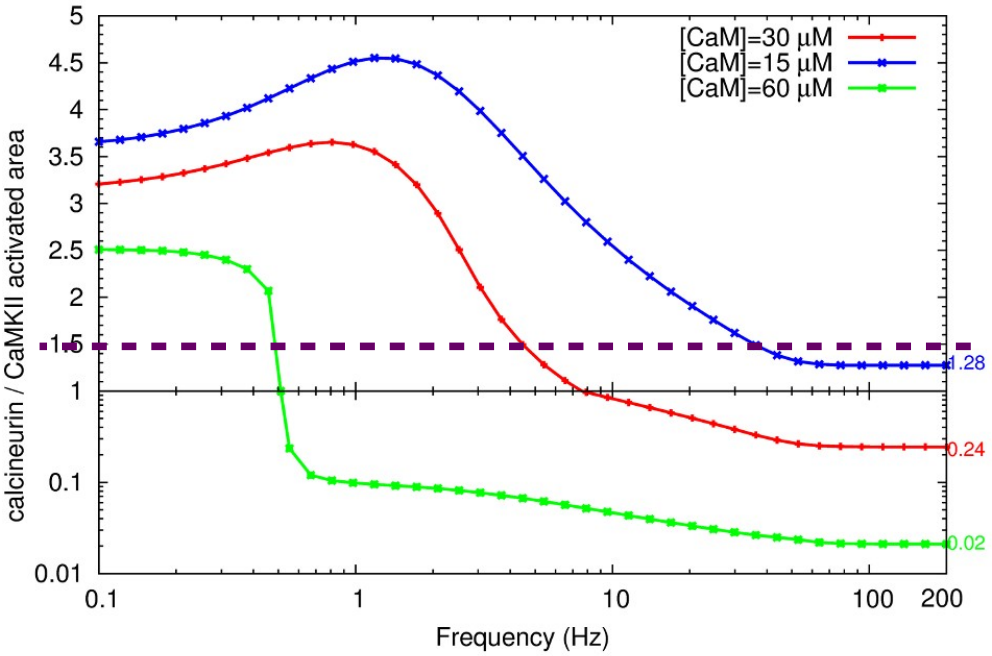


CaMKII not constitutively active
No CaM trapping

Never any positive plasticity

Lower deactivation of CaMKII

Competition for CaM, and CaN wins



(Pi, Otmakhov, Lemelin, De Koninck, Lisman. Autonomous CaMKII can promote either long-term potentiation or long-term depression, depending on the state of T305/T306 phosphorylation. *J Neurosci.* 2010 Jun 30;30(26):8704-9)

Summary of part 2

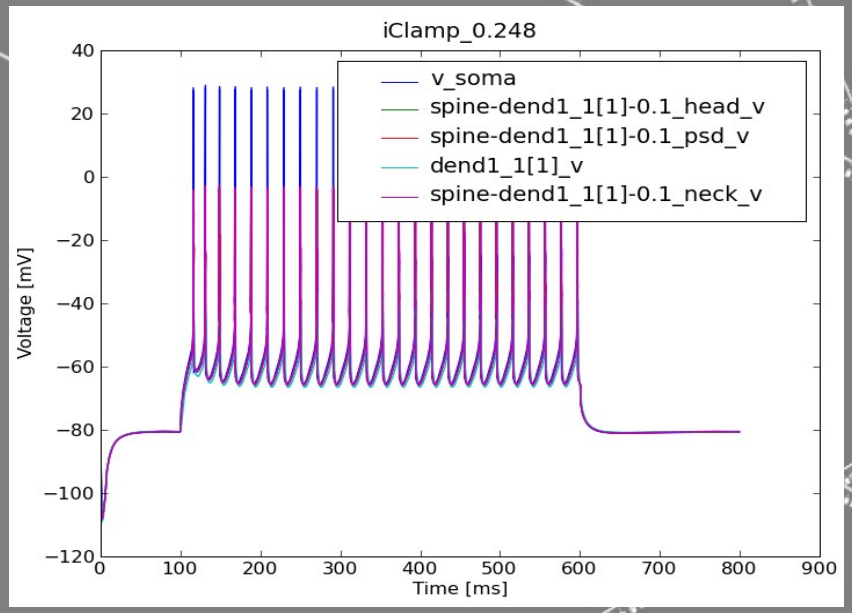
Allosteric stabilisation by targets triggers bistable CaM response to calcium. Above a certain frequency, CaM activation lasts longer than the initial signal.

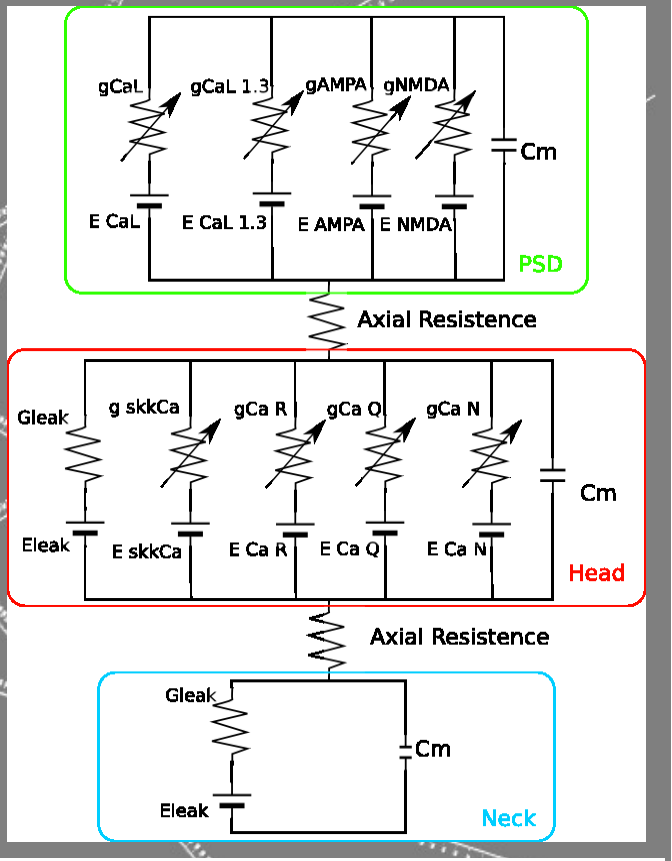
Calcium signals do not choose between CaN and CaMKII, *BOTH* enzymes are activated at *ALL* frequencies. The ratio of activity changes.

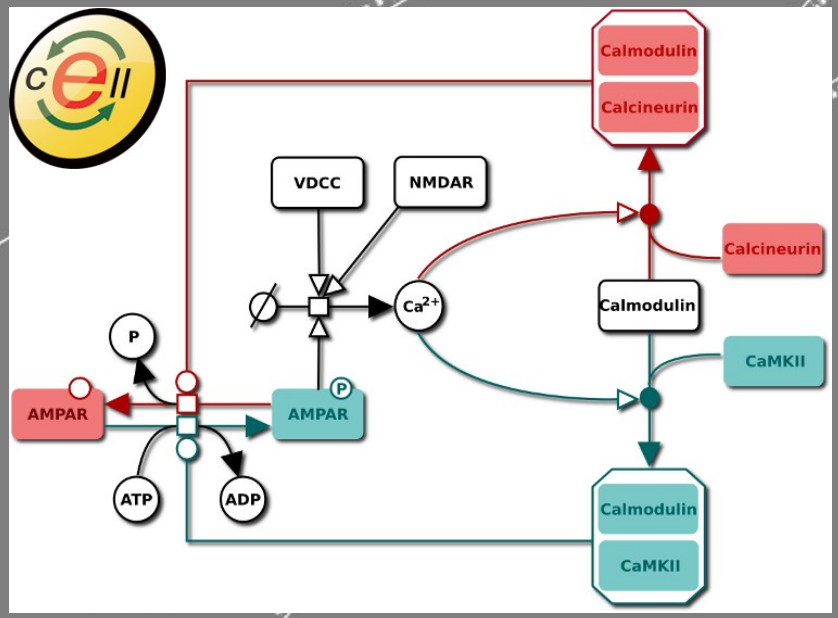
The frequency at which a synapse switches from a depression to a potentiation mode is not an intrinsic property of the synapse, but depends on the length and amplitude of stimulations.

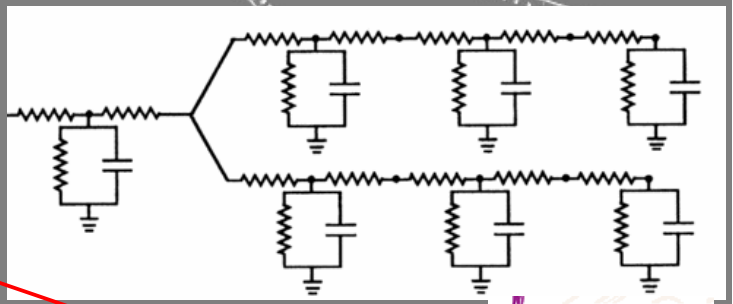
Modifications of topology (T286A), parameters (PP1 inhibition) and initial conditions ([CaM]) affect both response intensity and threshold frequency. Some mutants can't have positive plasticity for any stimulation.

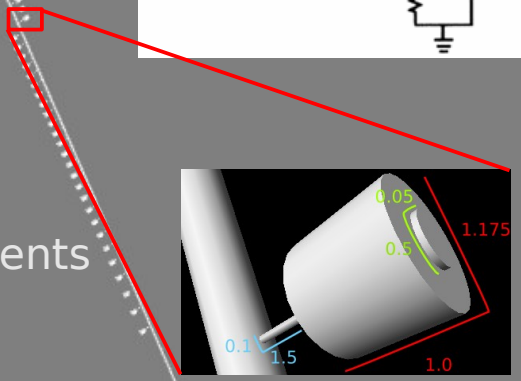
Whole cell: electro-biochemical models











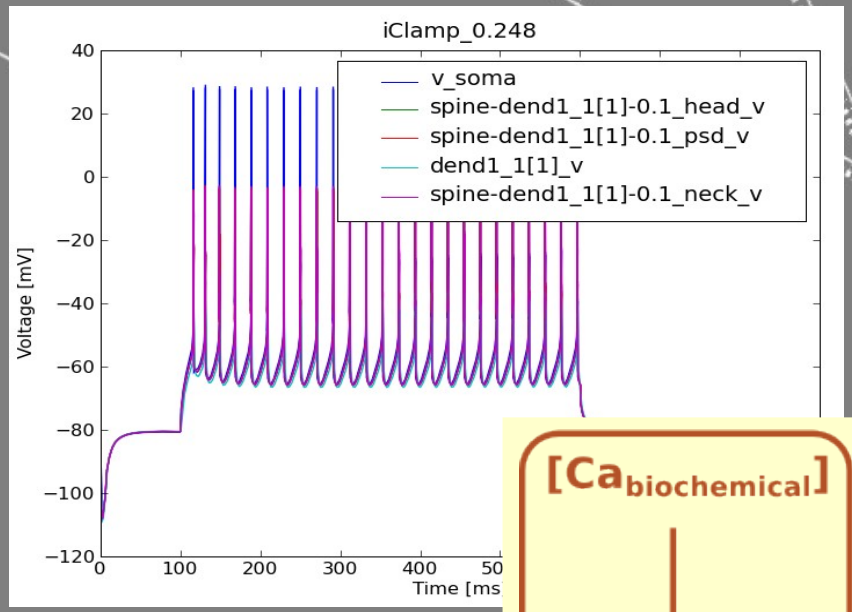
1504 spines

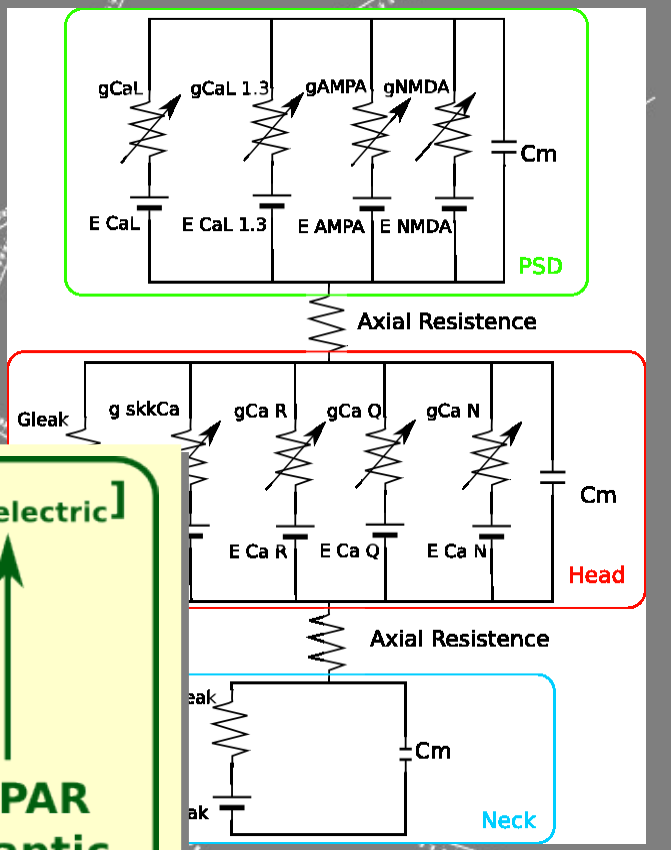
4761 compartments

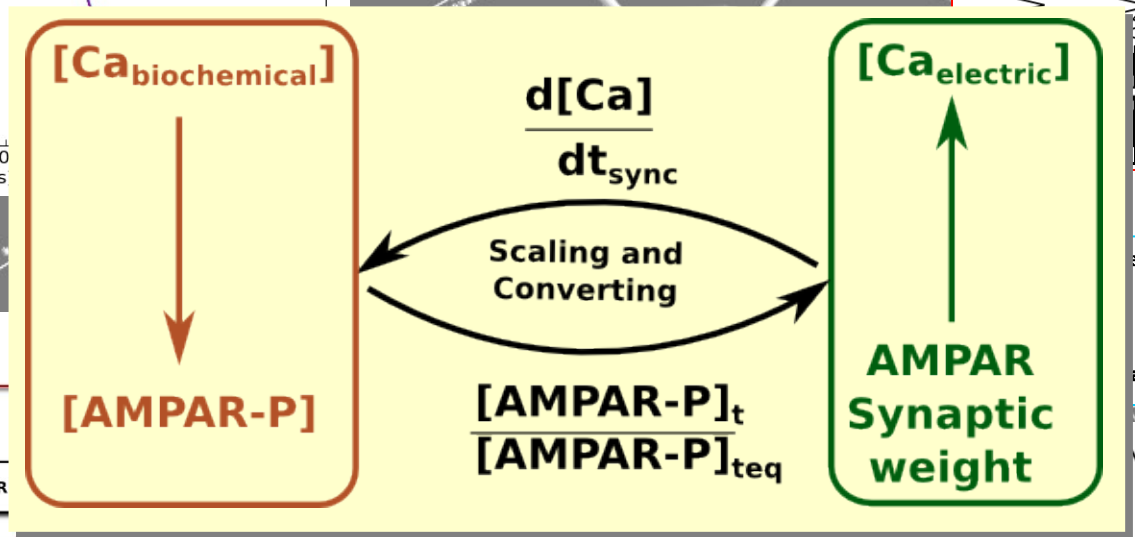
16362 channels

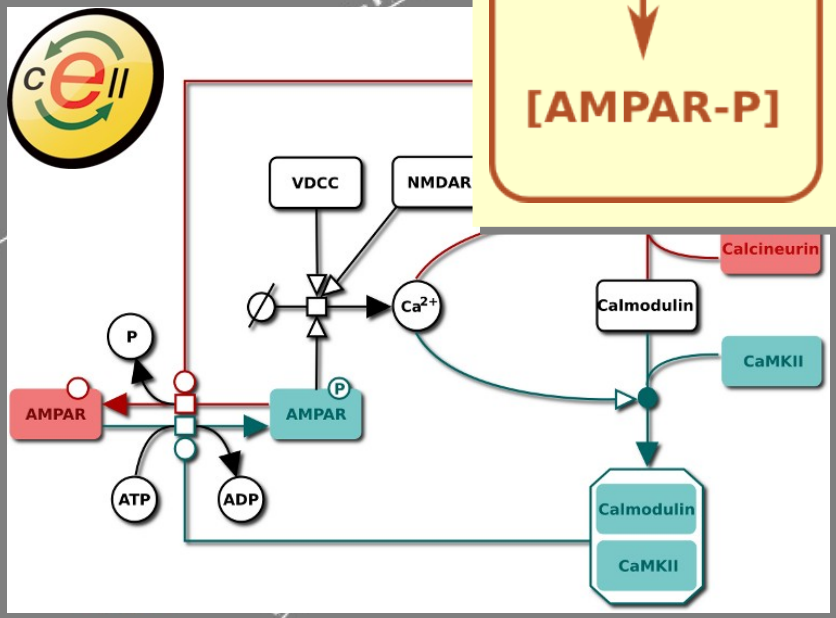
Mattioni & Le Novère (2013)

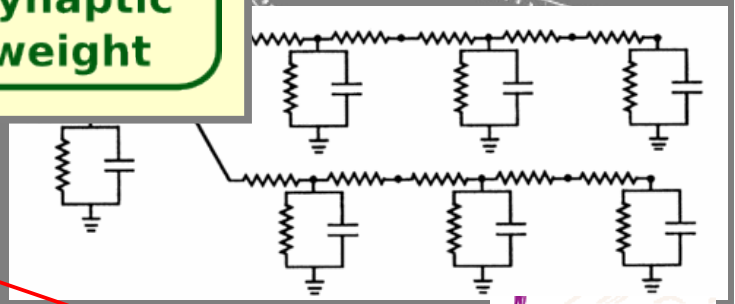
Whole cell: electro-biochemical models

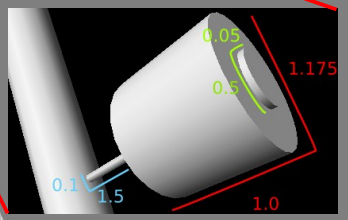












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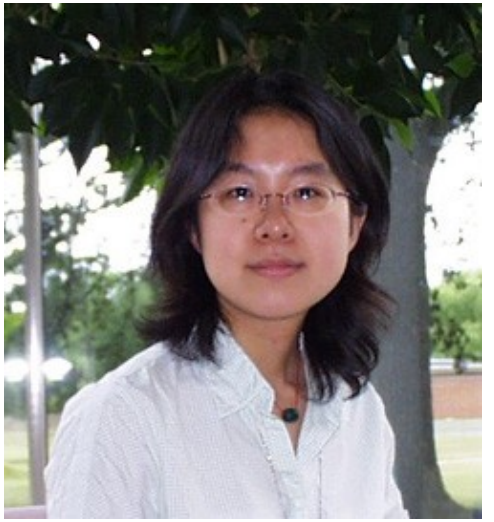
Mattioni & Le Novère (2013)

- Developers of ECell3
- Developers of COPASI
- Developers of Scilab
- Developers of Gnuplot

Melanie Stefan



Lu Li

EMBL

wellcome trust

Stuart Edelstein

BBSRC bioscience for the future

elixir Data for Life

SEVENTH FRAMEWORK PROGRAMME

imi Innovative Medicines Initiative

NEDO

NIGMS

Extended MWC model necessary for Calmodulin

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

1) Any number of different sites per protomer

2) Several protomers can be carried by one subunit

- αi = [ligand]/$K^R_{i,lig}$
- γk = [modulator]/$K^R_{k,mod}$
- $c i$ = $K^R_{i,lig}$ / $K^T_{i,lig}$
- $e k$ = $K^R_{k,mod}$ / $K^T_{k,mod}$

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

Stefan M.I., Edelstein S.J., Le Novère N. *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

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

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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.

Relaxation of the model for finding individual *Kd*

- 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