Allosteric calcium sensors and signalling switching

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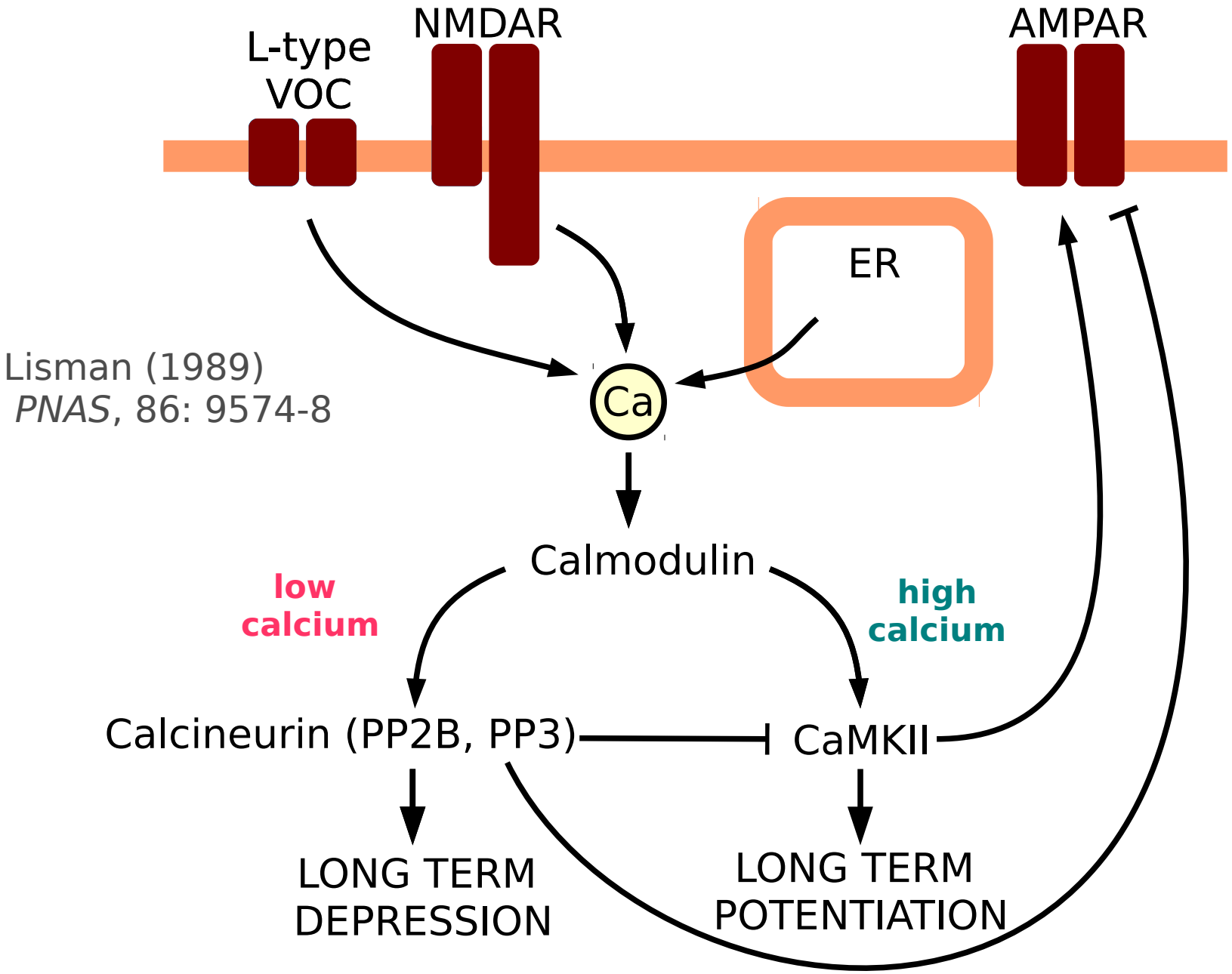
Babraham Institute

Modelling the behaviour of interacting molecules

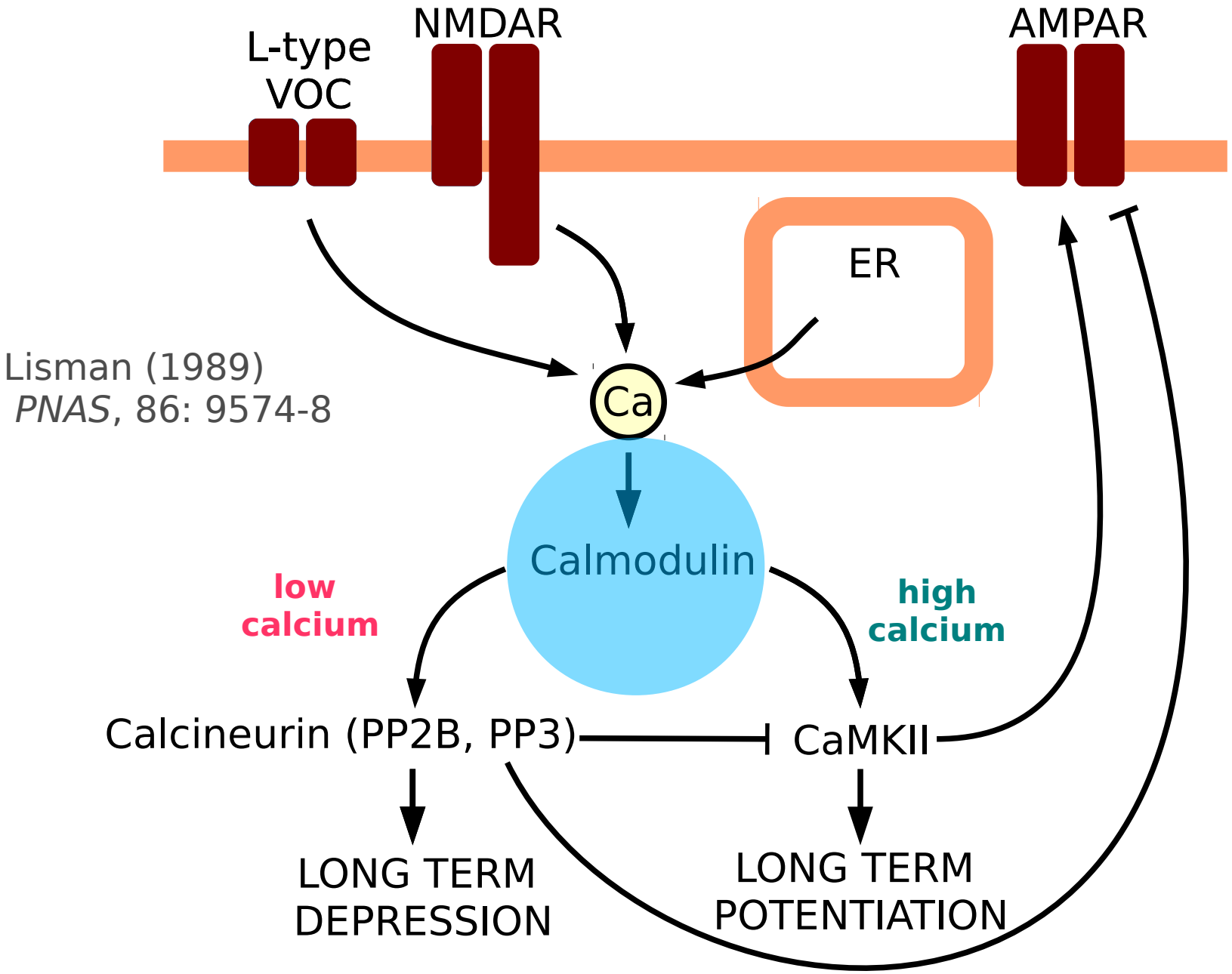
Modelling the behaviour of a system of molecules

Modelling the behaviour of a cell

Calmodulin, the memory switch

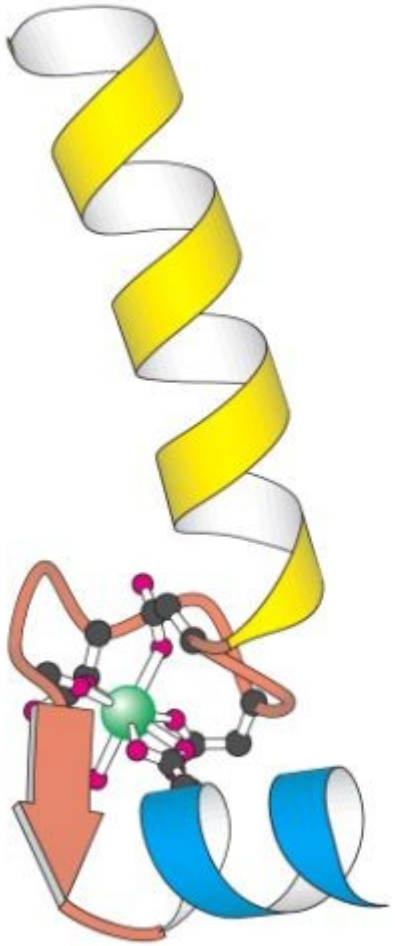


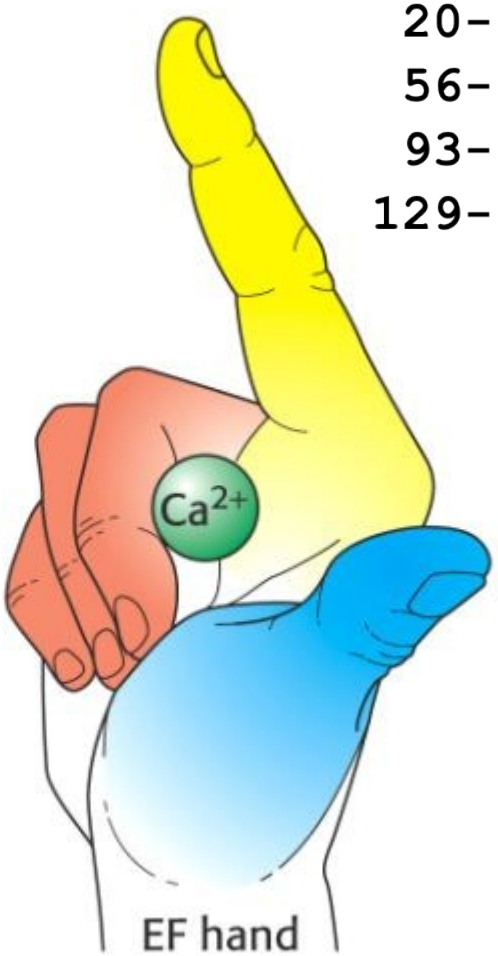
Calmodulin, the memory switch



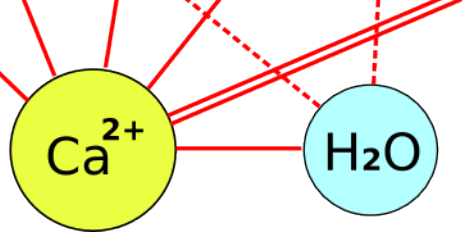
Lisman (1989)
PNAS, 86: 9574-8

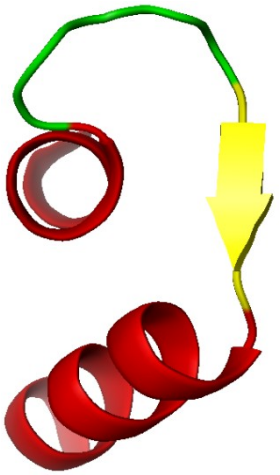
Structure of Calmodulin

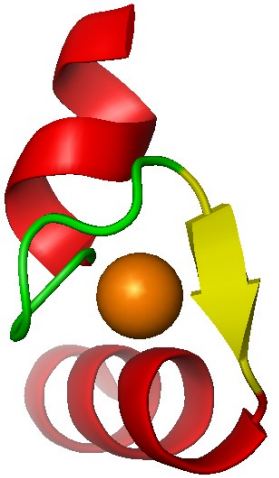




	X	Y	Z	-Y	-X	-Z	
20-	D	K	D	G	D	G	T	I	T	T	K	E	- 31
56-	D	A	D	G	N	G	T	I	D	F	P	E	- 67
93-	D	K	D	G	N	G	Y	I	S	A	A	E	-104
129-	D	I	D	G	D	G	Q	V	N	Y	E	E	-140



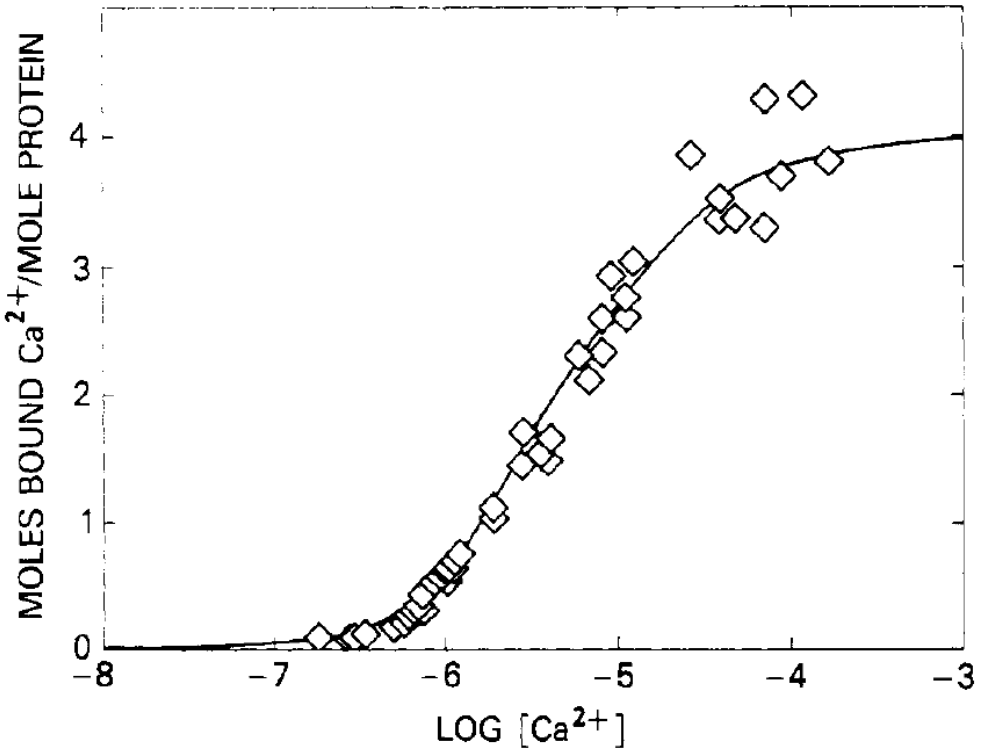




Adair-Klotz model applied to Calmodulin

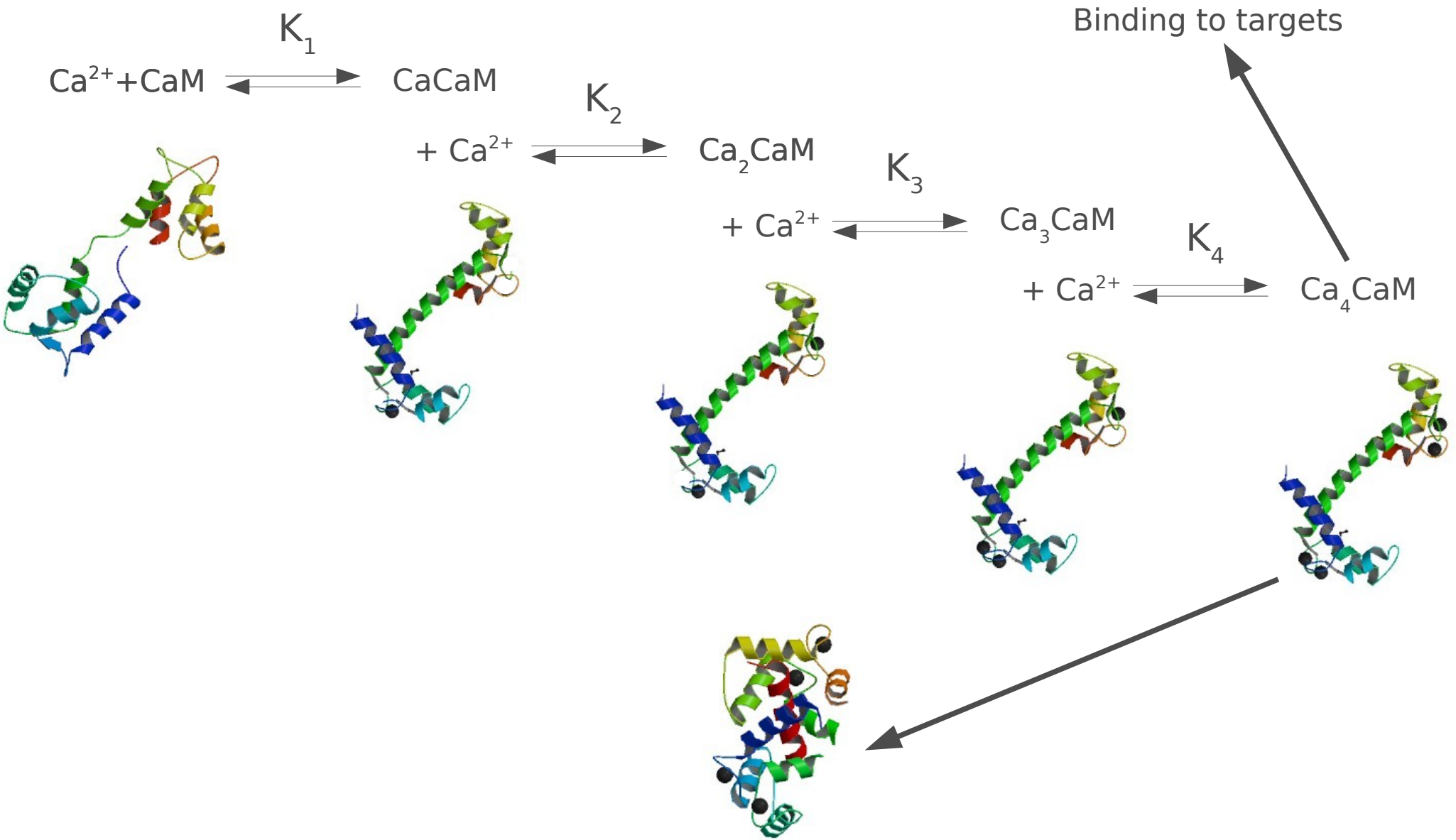
Klotz (1946) The Application of the Law of Mass Action to Binding by Proteins. Interactions with Calcium. *Arch Biochem*, 9:109–117.

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

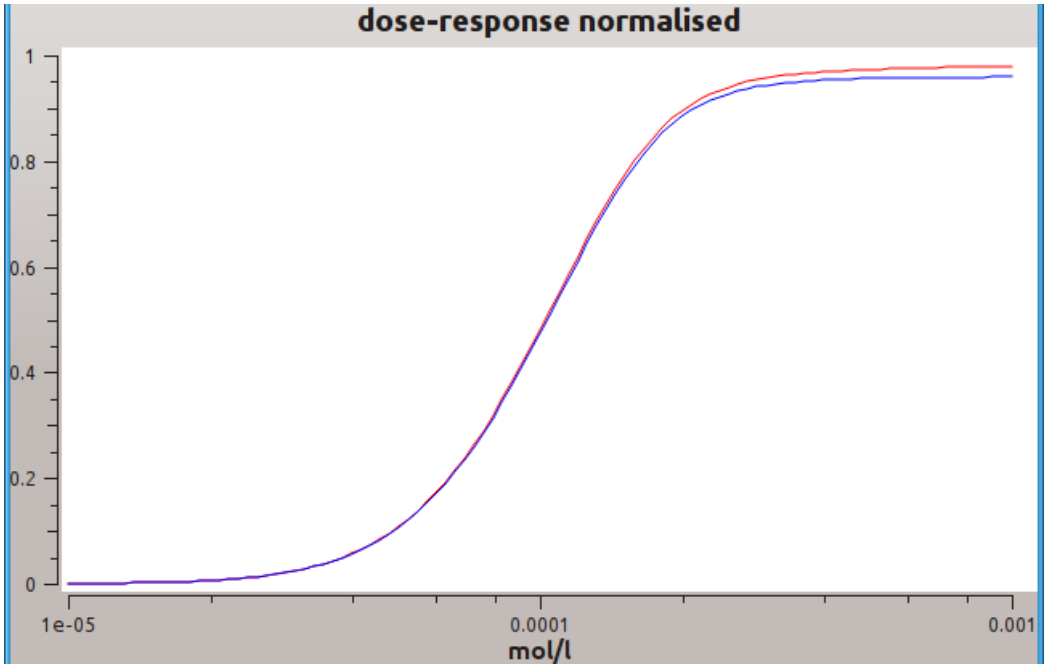


Crouch and Klee (1980) *Biochemistry*, 19: 3692-3698

Corresponding induced-fit model

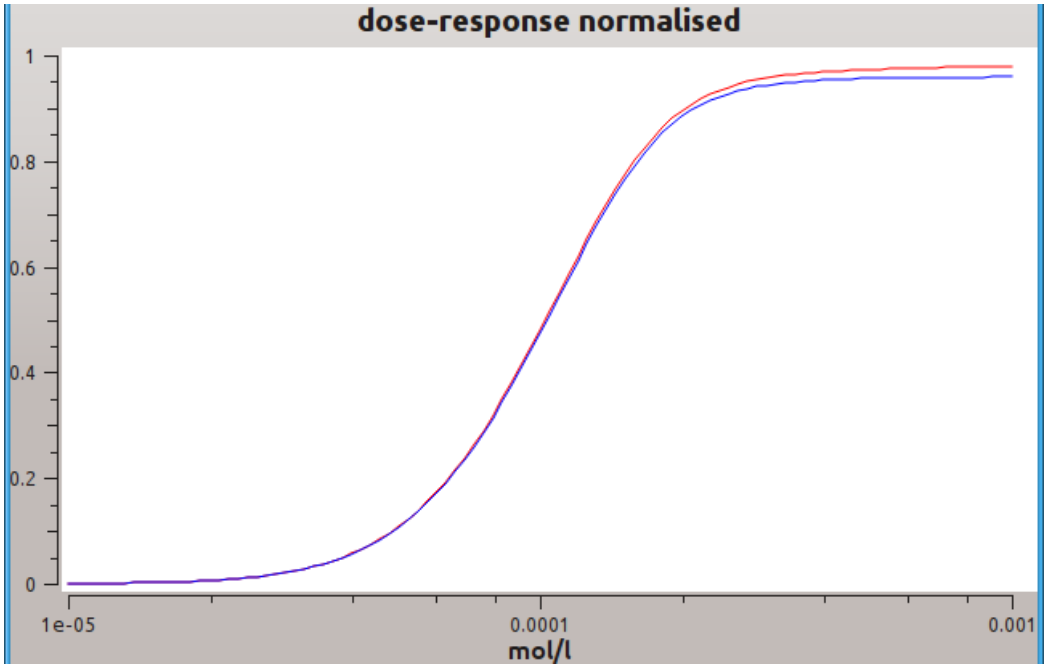


That does not work ...



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

We knew it would not work

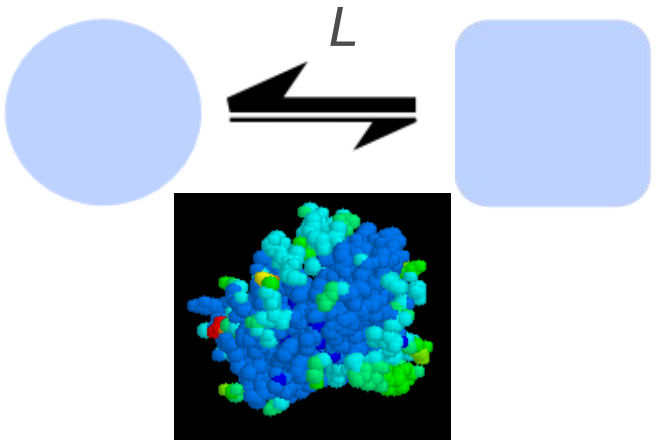


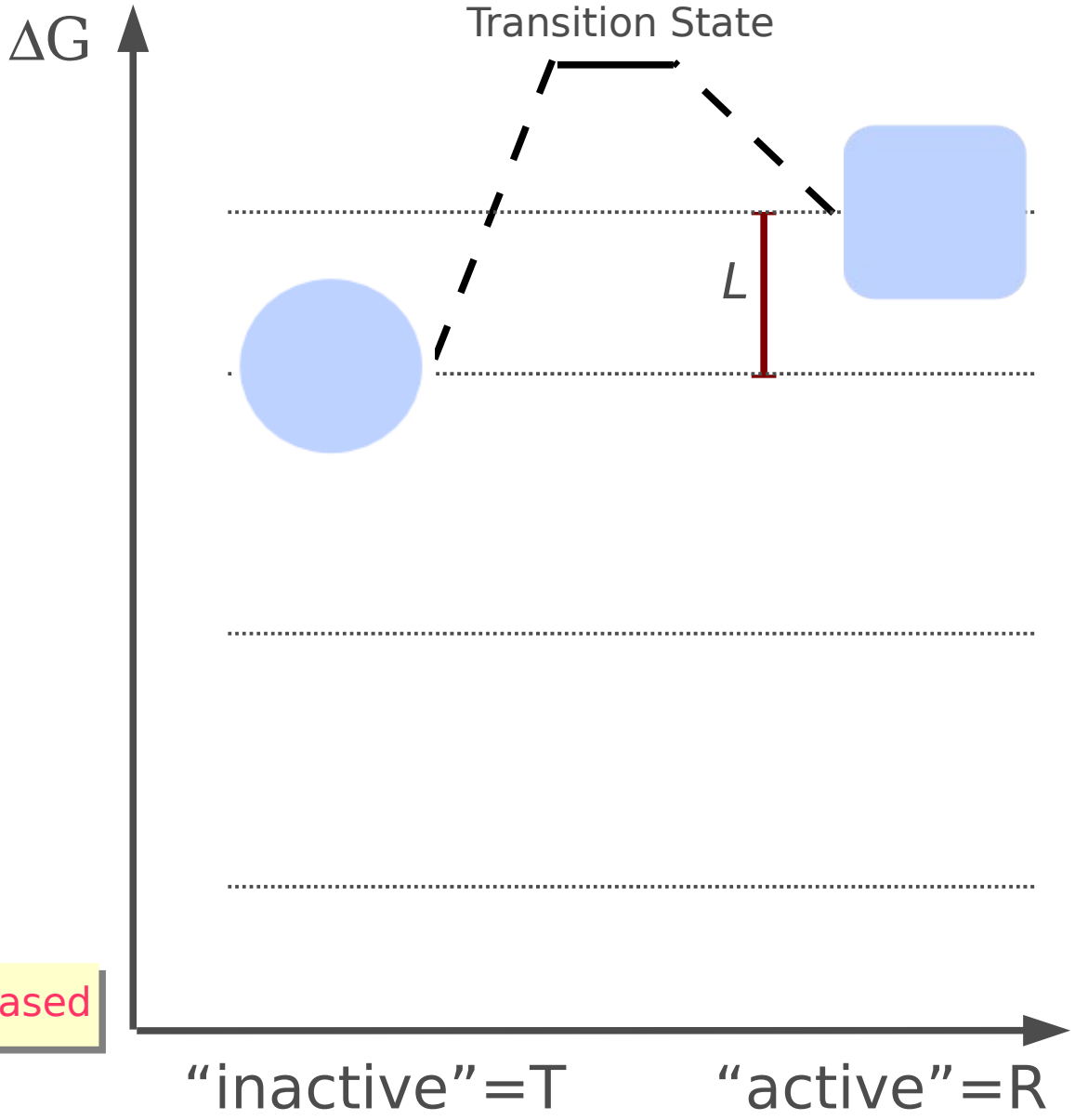
[CaN]=[CamKII]=[CaM]/10 ;
Kd_CaMKII = 10xKd_CaN;
Software COPASI

- Calmodulin can activate calcineurin with 3 Ca^{2+} (Kincaid and Vaughan (1986). *PNAS*, 83: 1193-1197)
- Calmodulin can bind CaMKII with 2 Ca^{2+} (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])

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

1 Modulation of thermal equilibria ≠ induced-fit

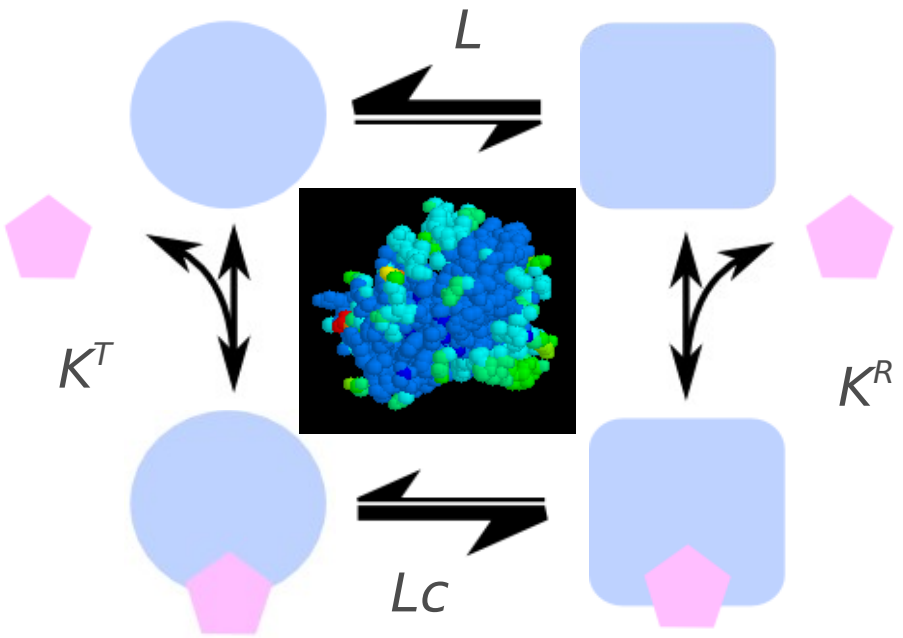


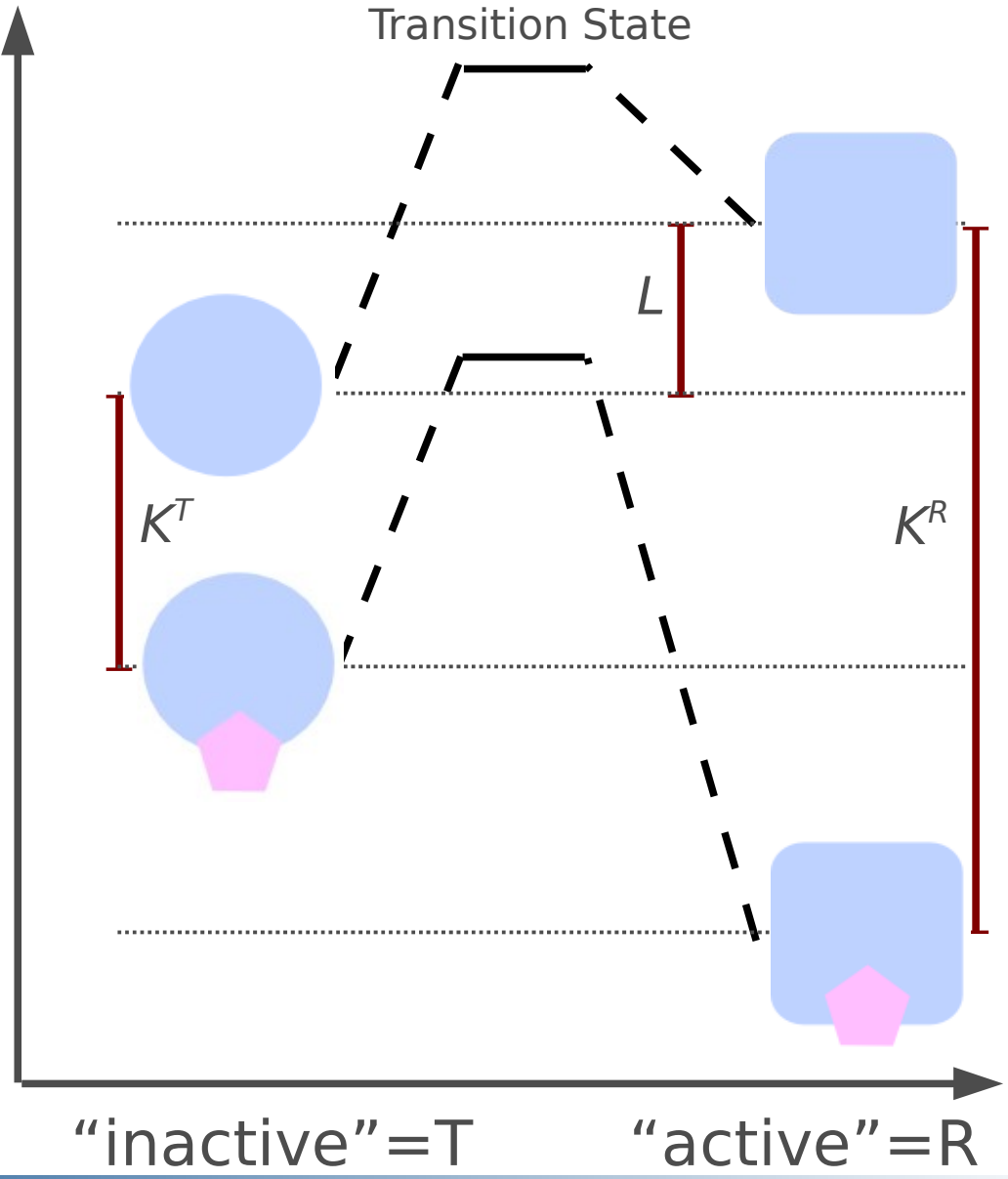


$$L = \frac{[T_0]}{[R_0]}$$

$L \gg 1 \Rightarrow$ Equilibrium strongly biased

1 Modulation of thermal equilibria ≠ induced-fit

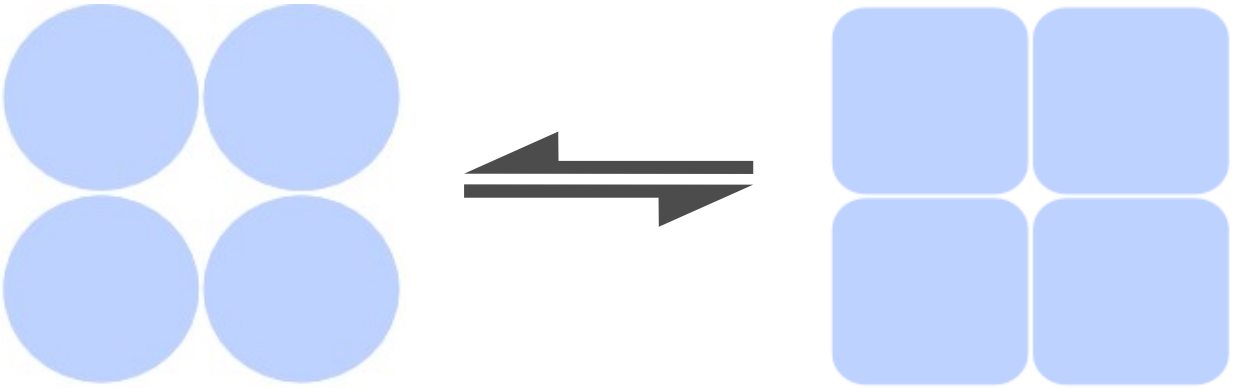


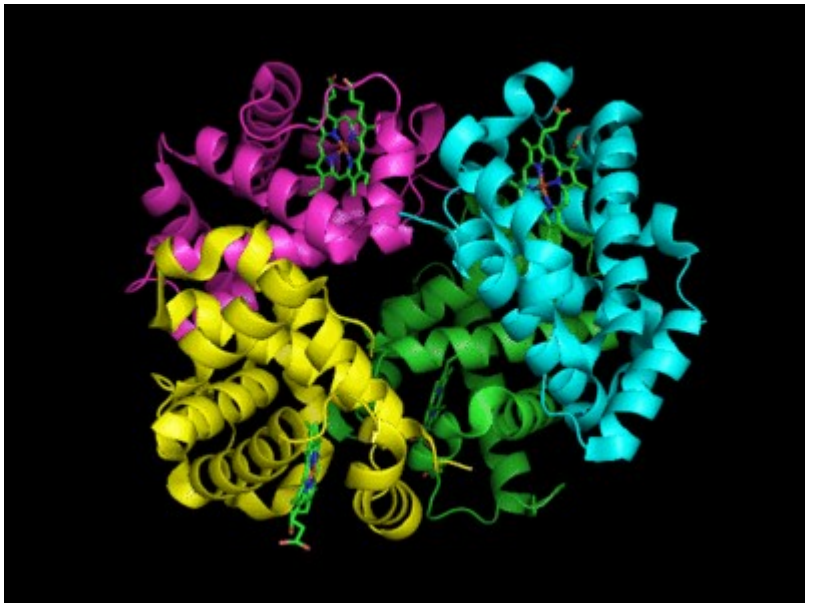


$$L = \frac{[T_0]}{[R_0]} \qquad c = \frac{K^R}{K^T}$$

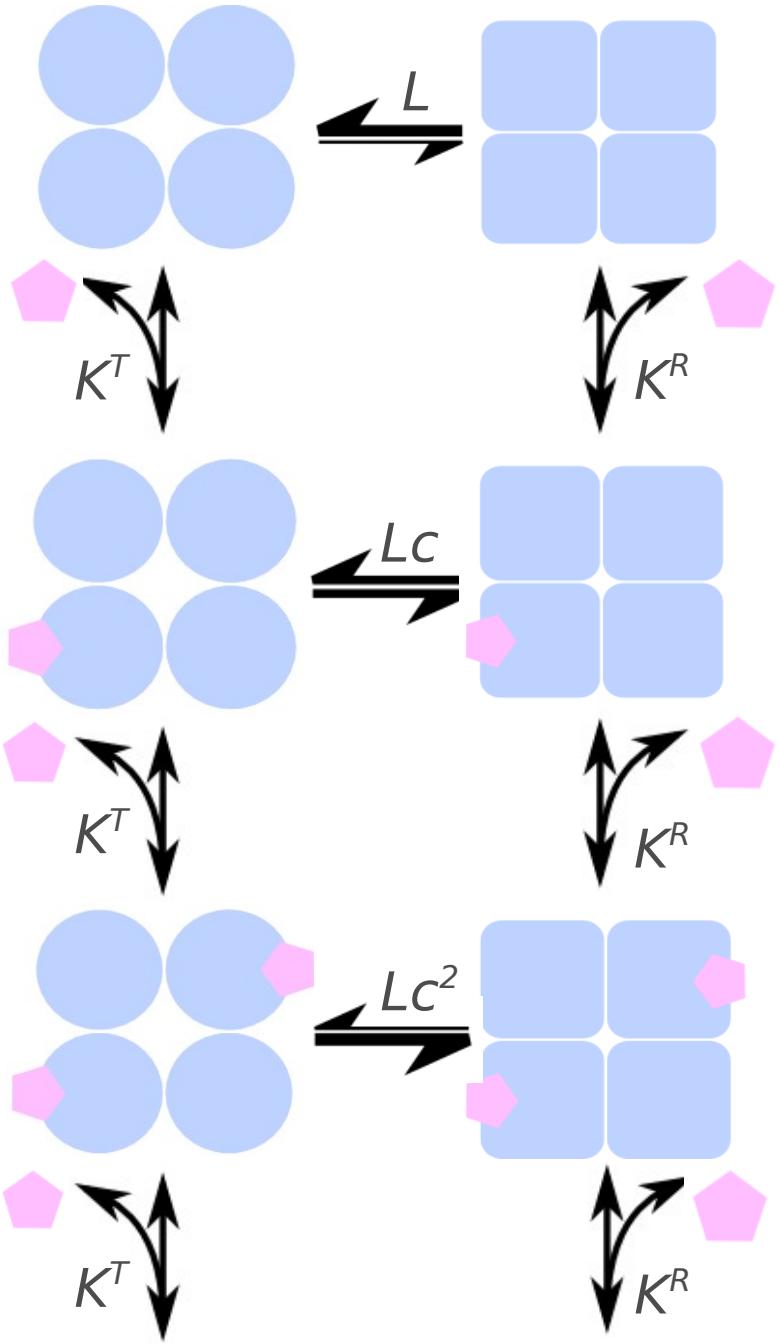
$c \ll 1 \Rightarrow$ ligand has strong effect

Concerted transitions ≠ sequential model

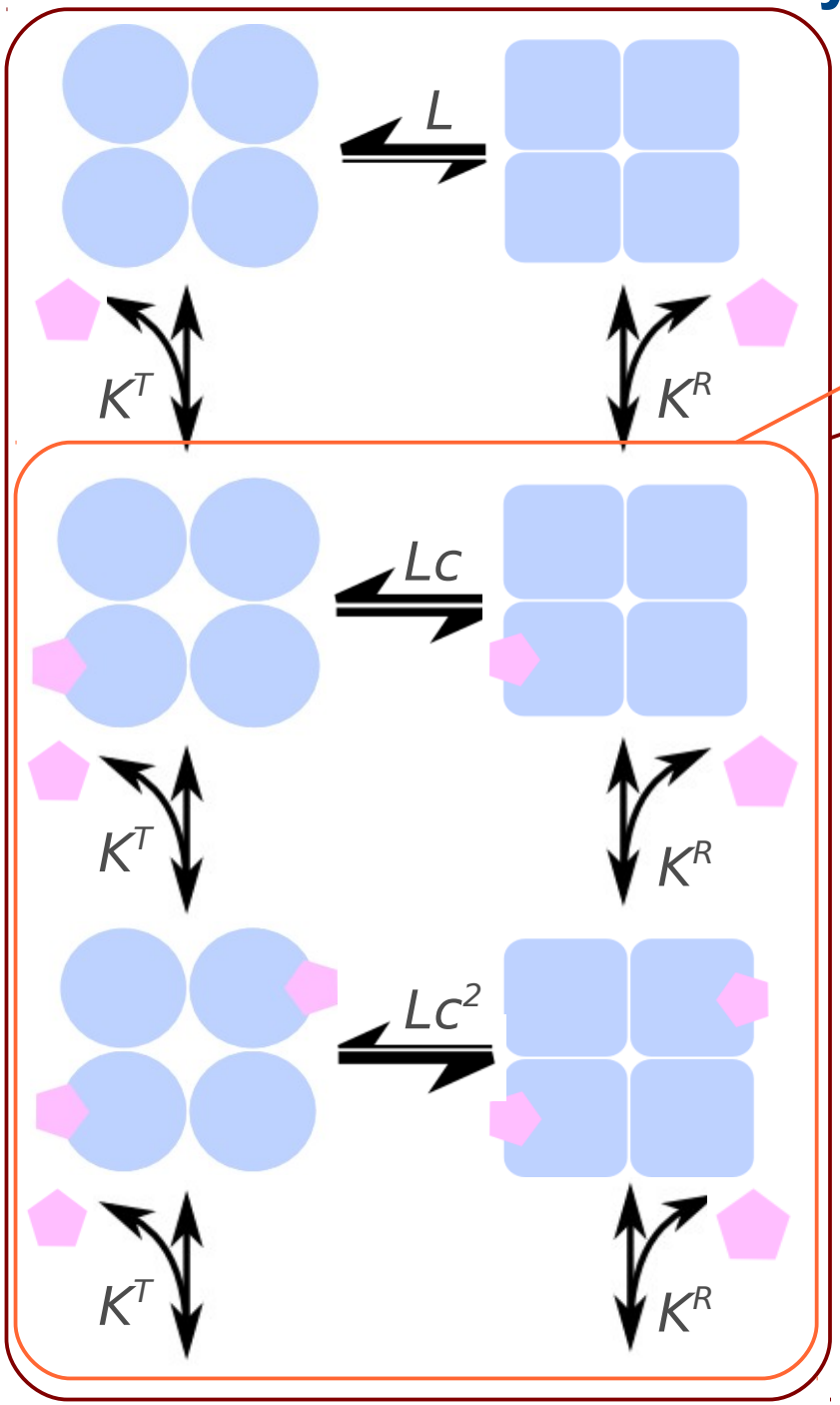




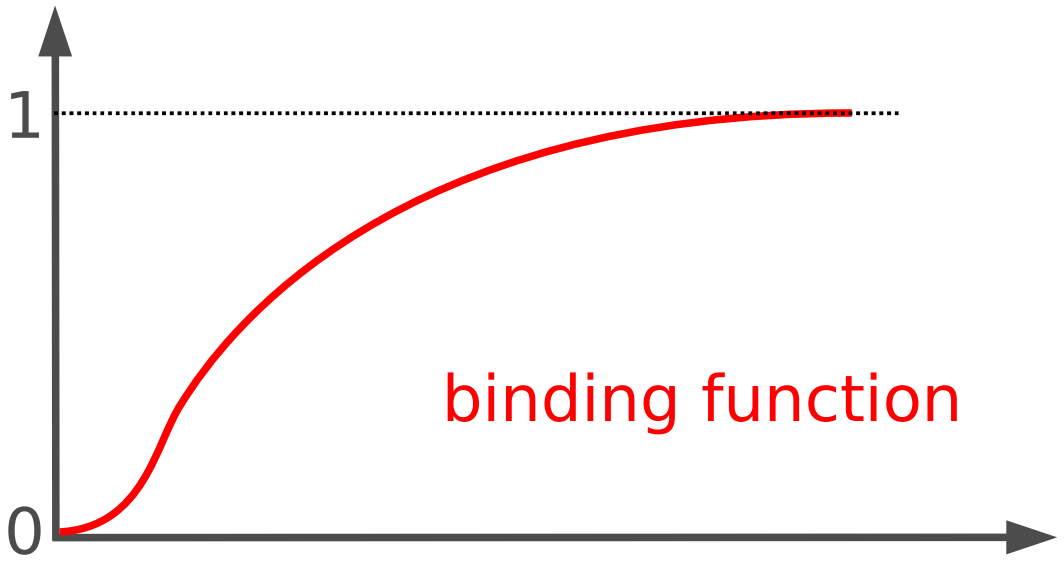
Monod-Wyman-Changeux model



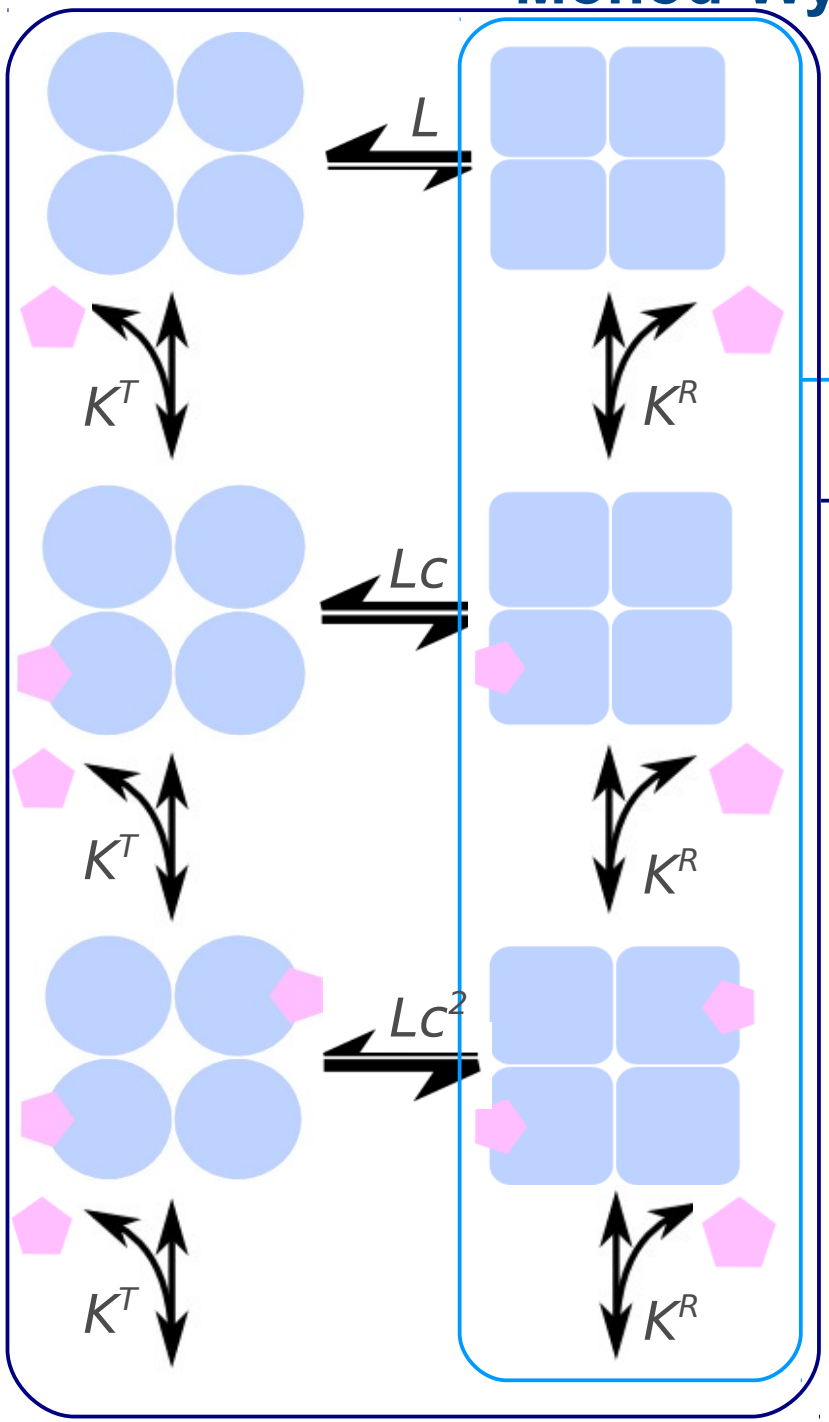
Monod-Wyman-Changeux model



$$\frac{\text{this}}{\text{that}} = \bar{Y}$$

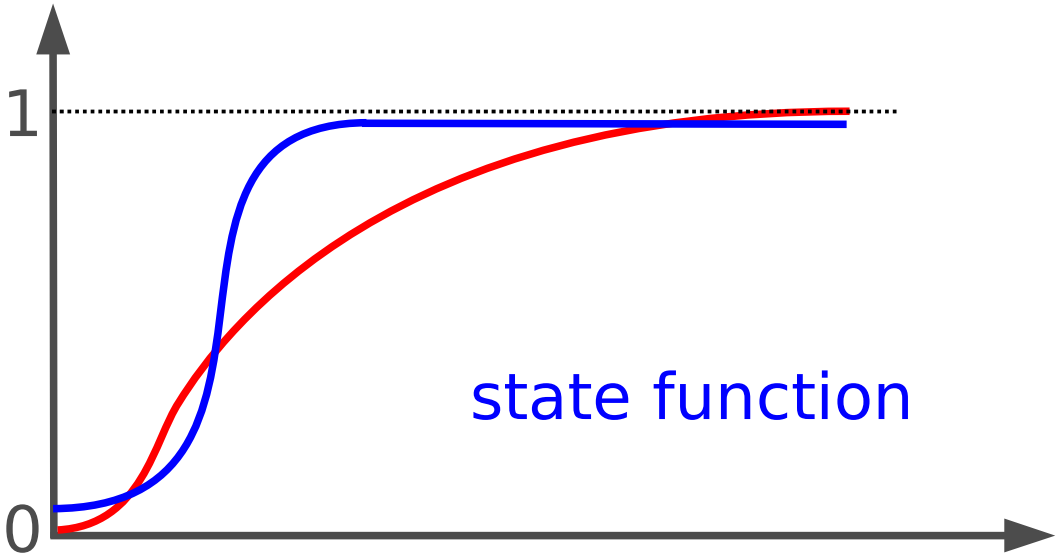


Monod-Wyman-Changeux model



$\bar{Y}$

this / that $= \bar{R}$



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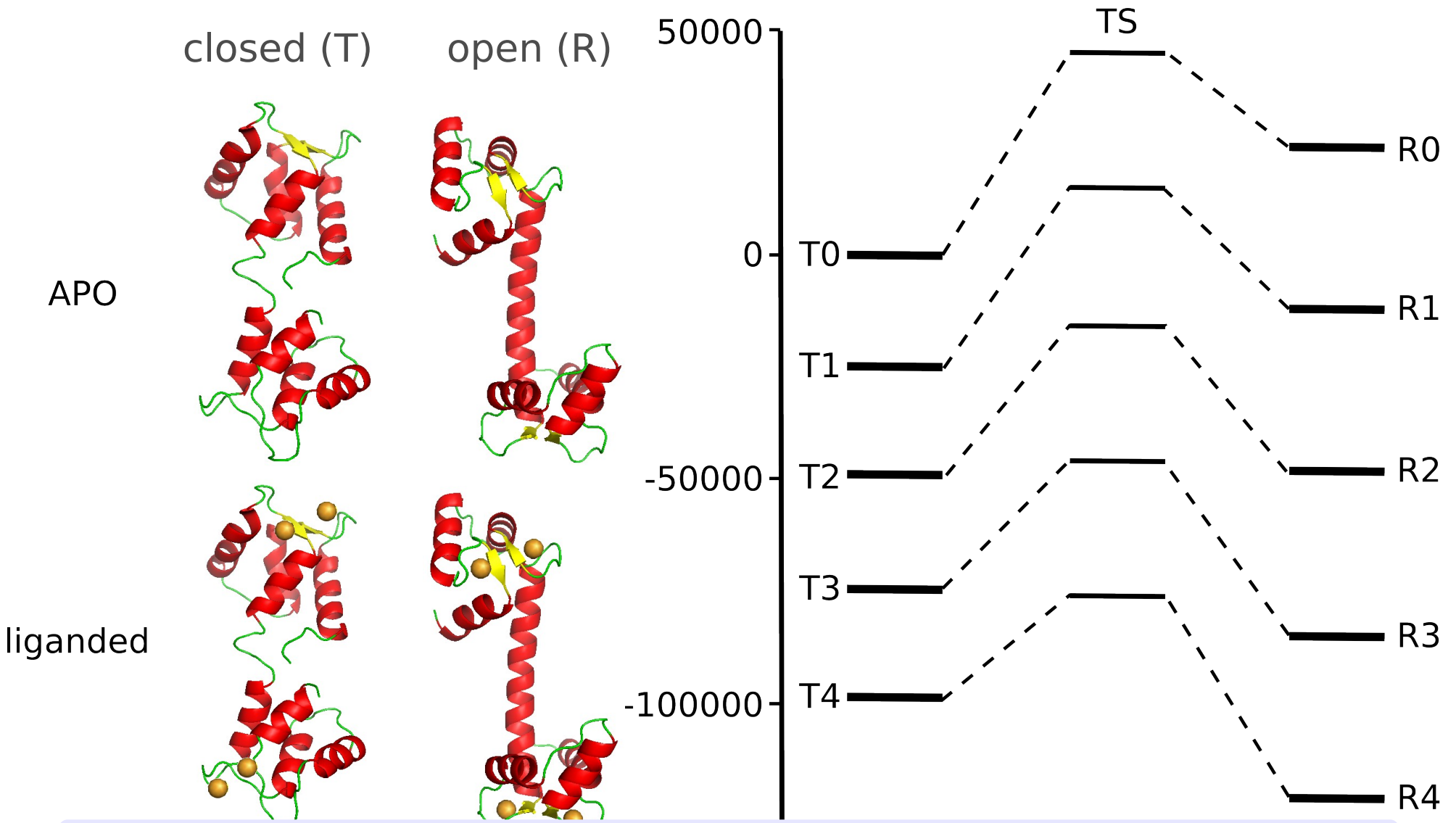
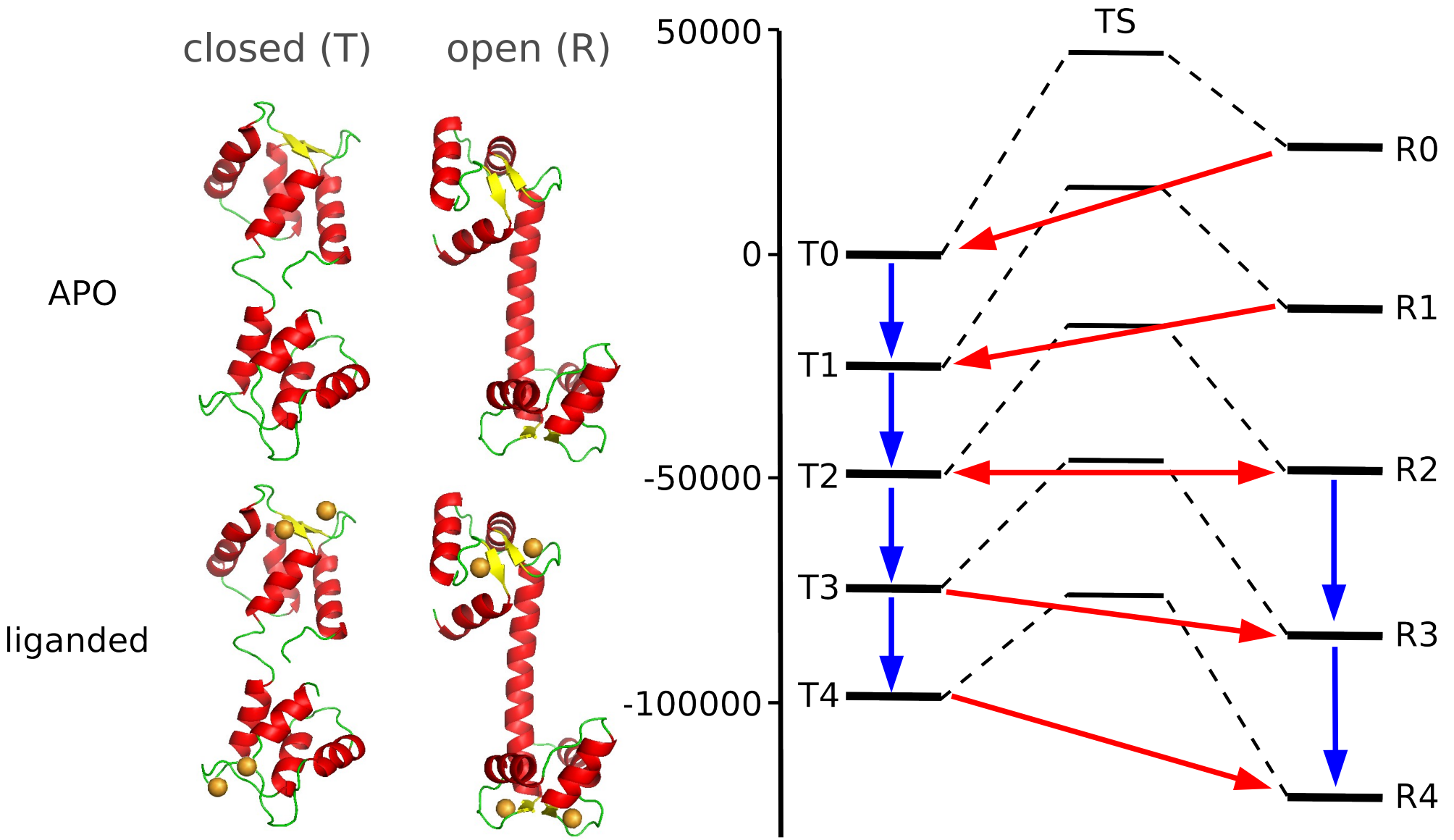
Allosteric model of Calmodulin activation

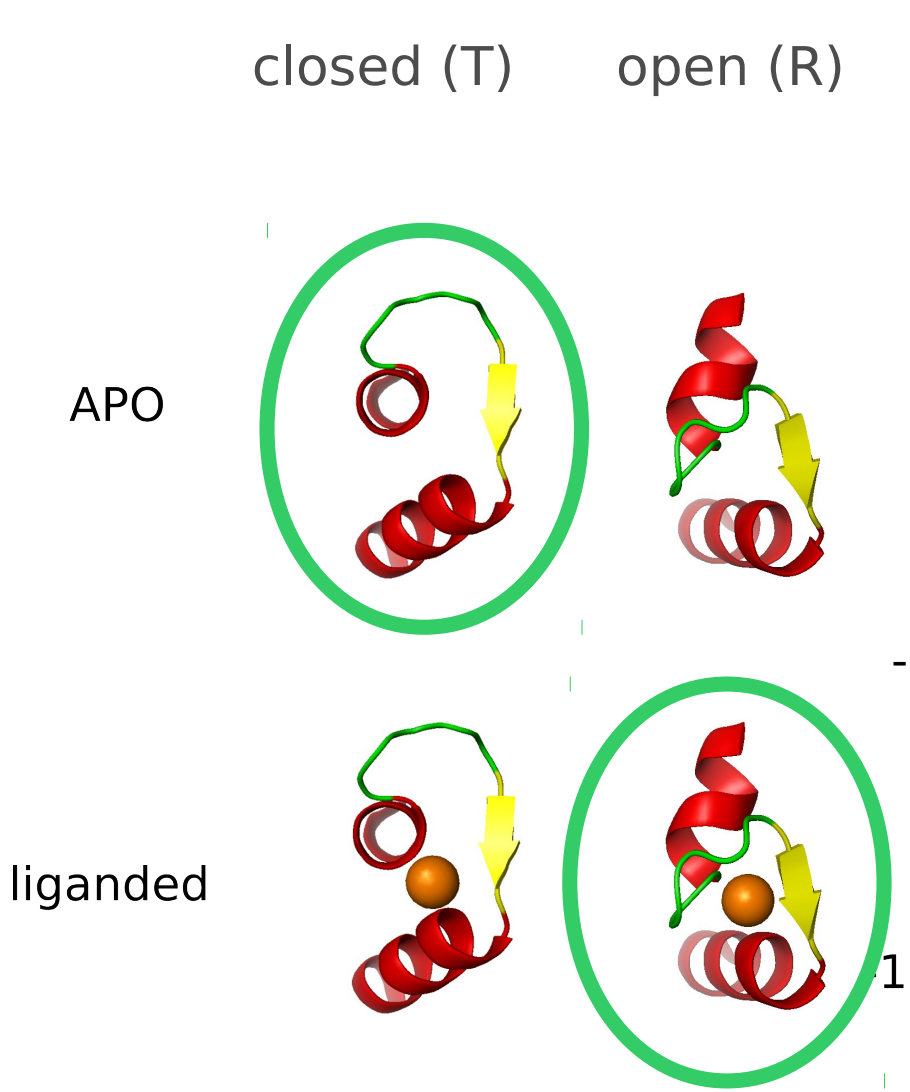


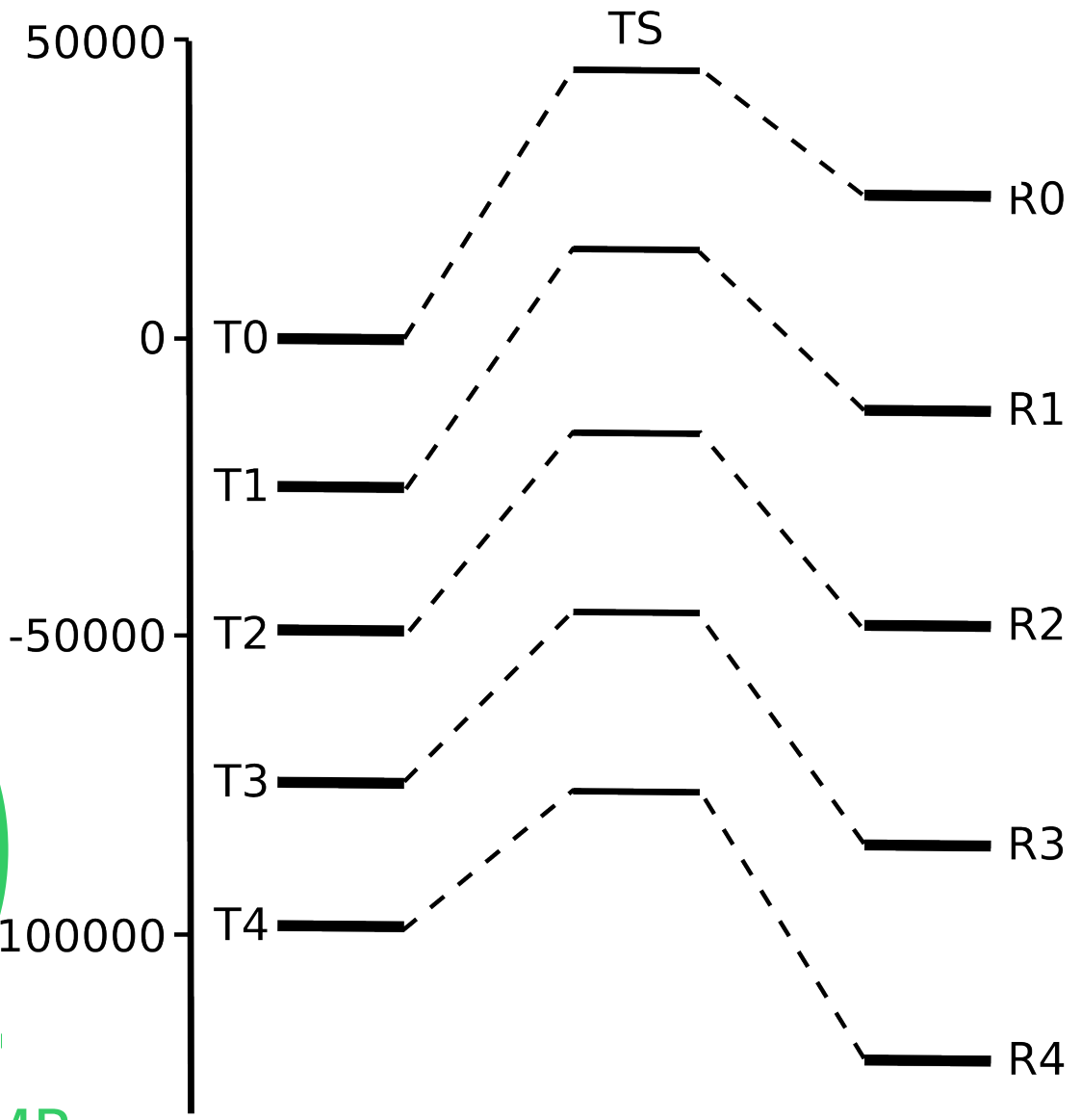
Stefan MI, Edelstein SJ, Le Novère N (2008) *Proc Natl Acad Sci USA*, 105:10768-10773

Allosteric model of Calmodulin activation



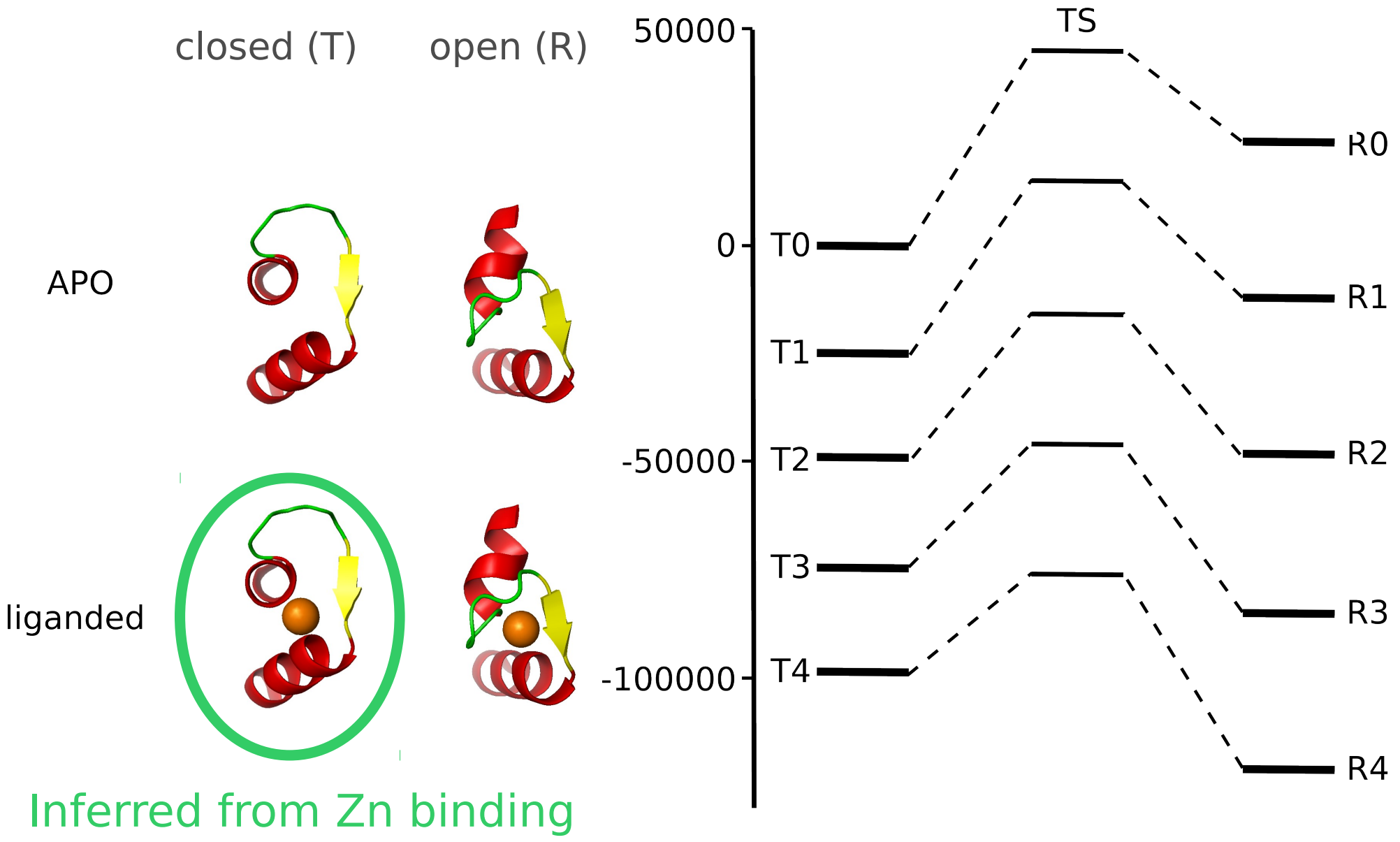
Observation Vs. Prediction





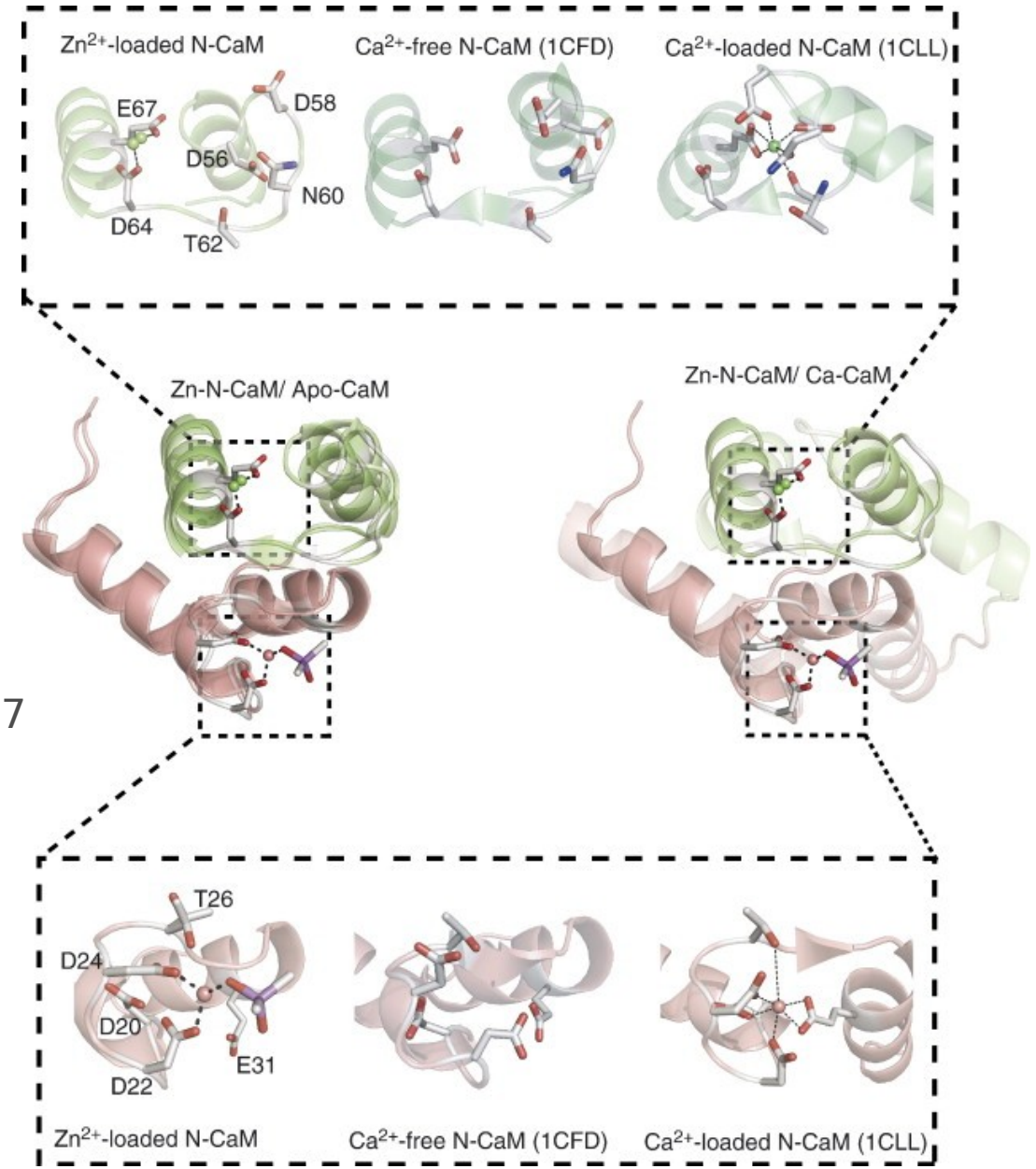
Observed by X-Ray and NMR

Observation Vs. Prediction

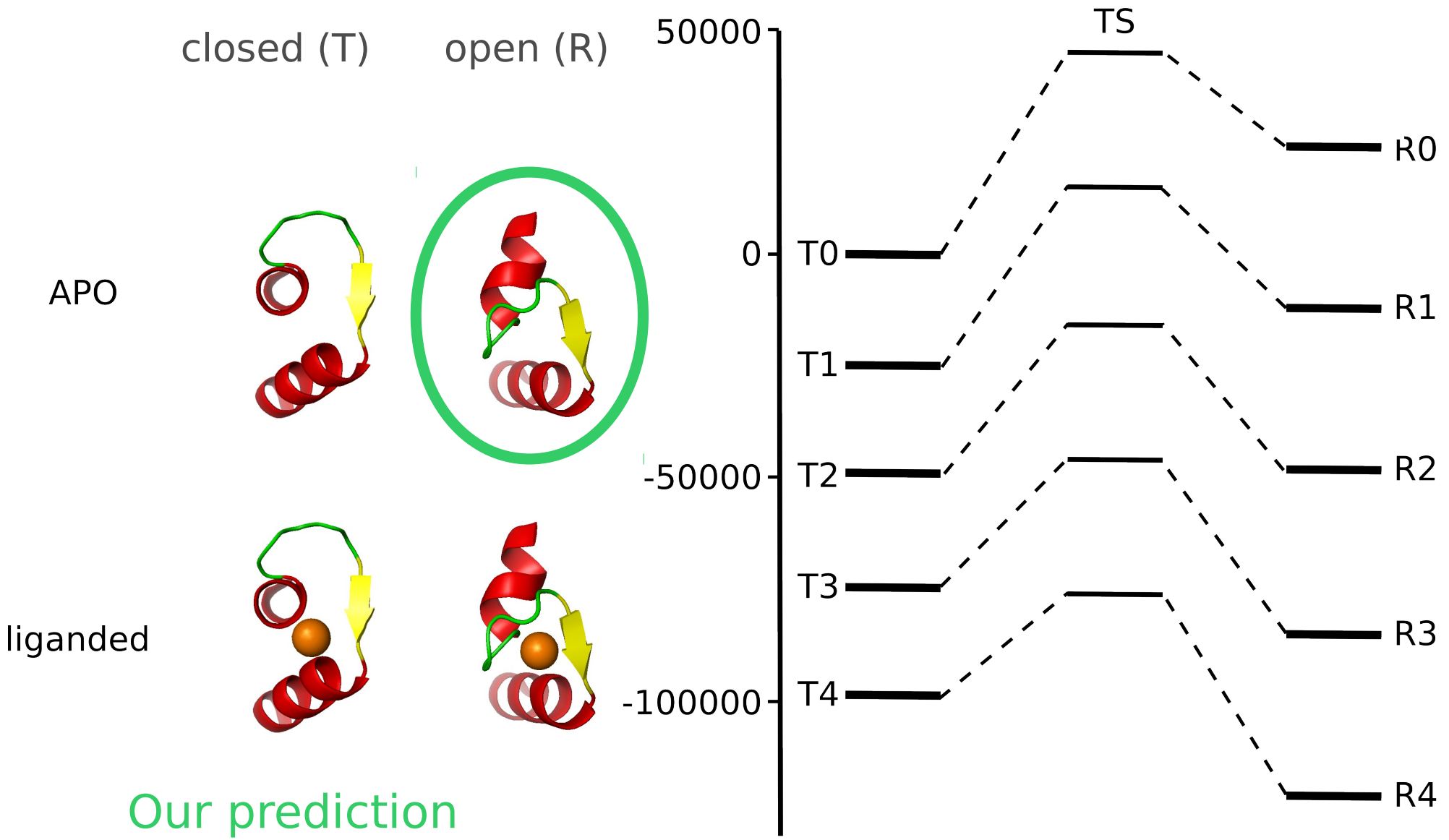


Inferred from Zn binding

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



Observation Vs. Prediction



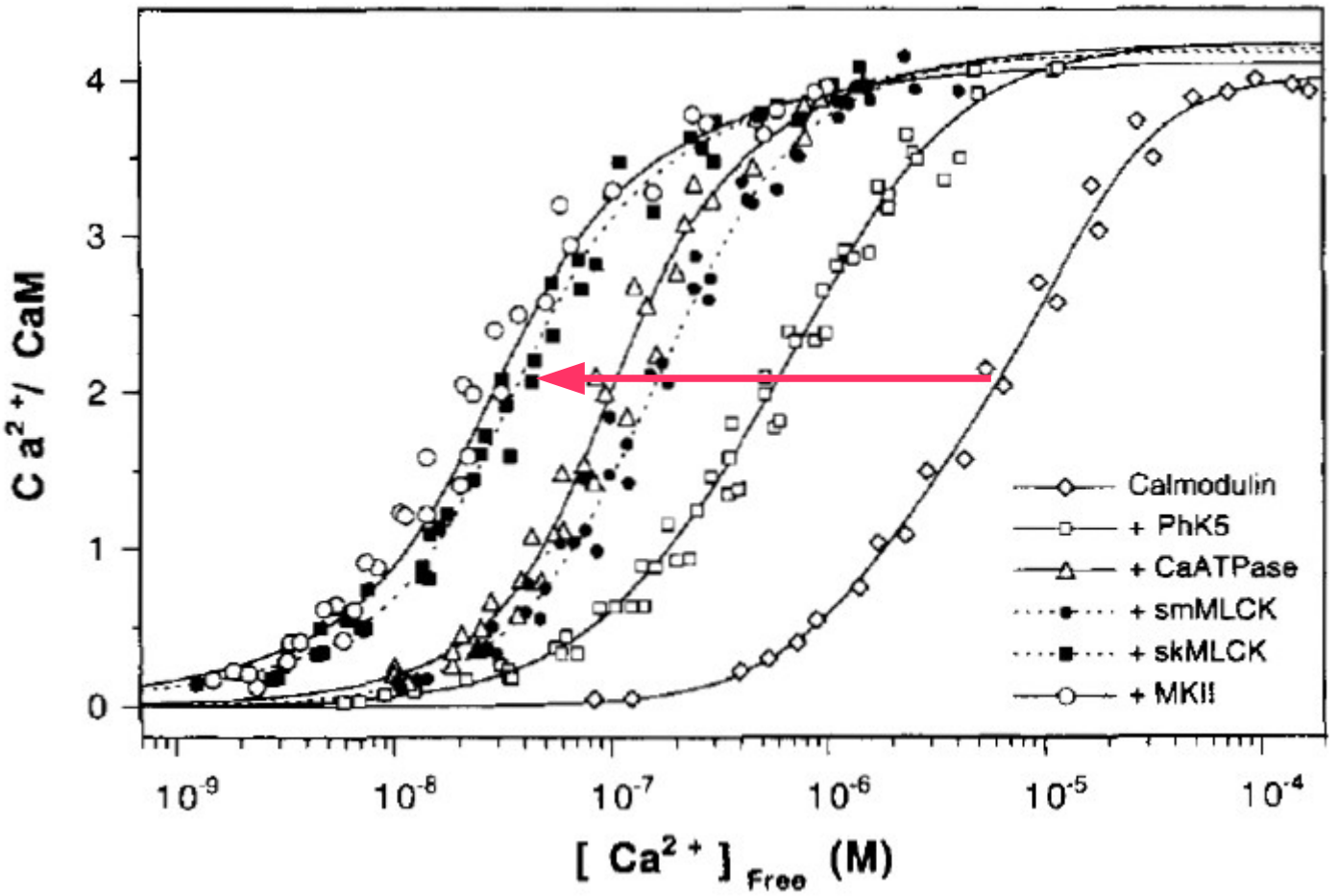
Parameter estimations: L and c

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

  Stefan MI, Edelstein SJ, Le Novère N (2009) *BMC Syst Biol*, 3: 68

- Hypothesis: isotropic 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

Parameter estimations: Ki

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

- Hypothesis: isotropic 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"

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

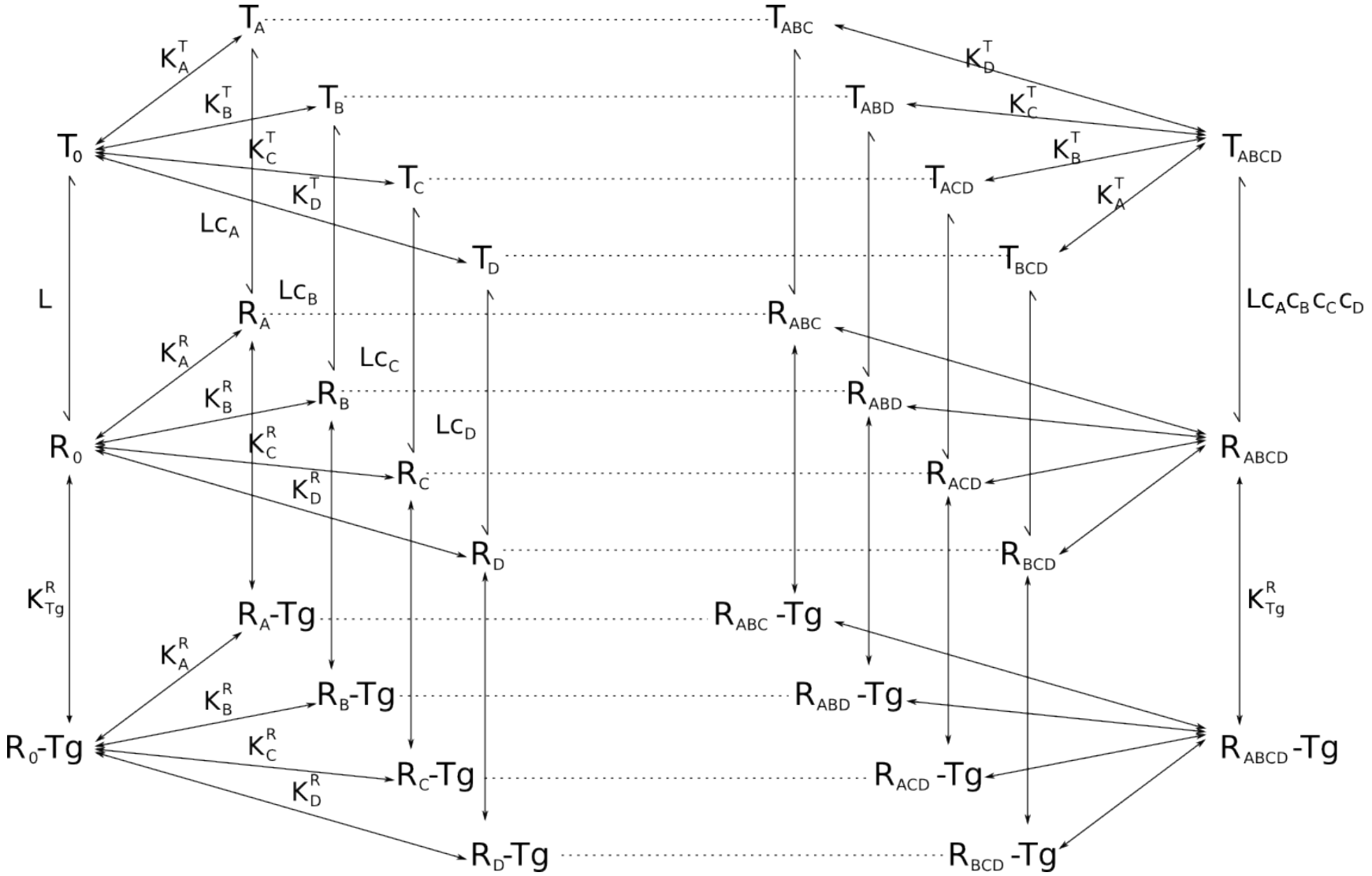
$K^R_B=1.66\ 10^{-8}$

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

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

2 high, 2 low, as anticipated

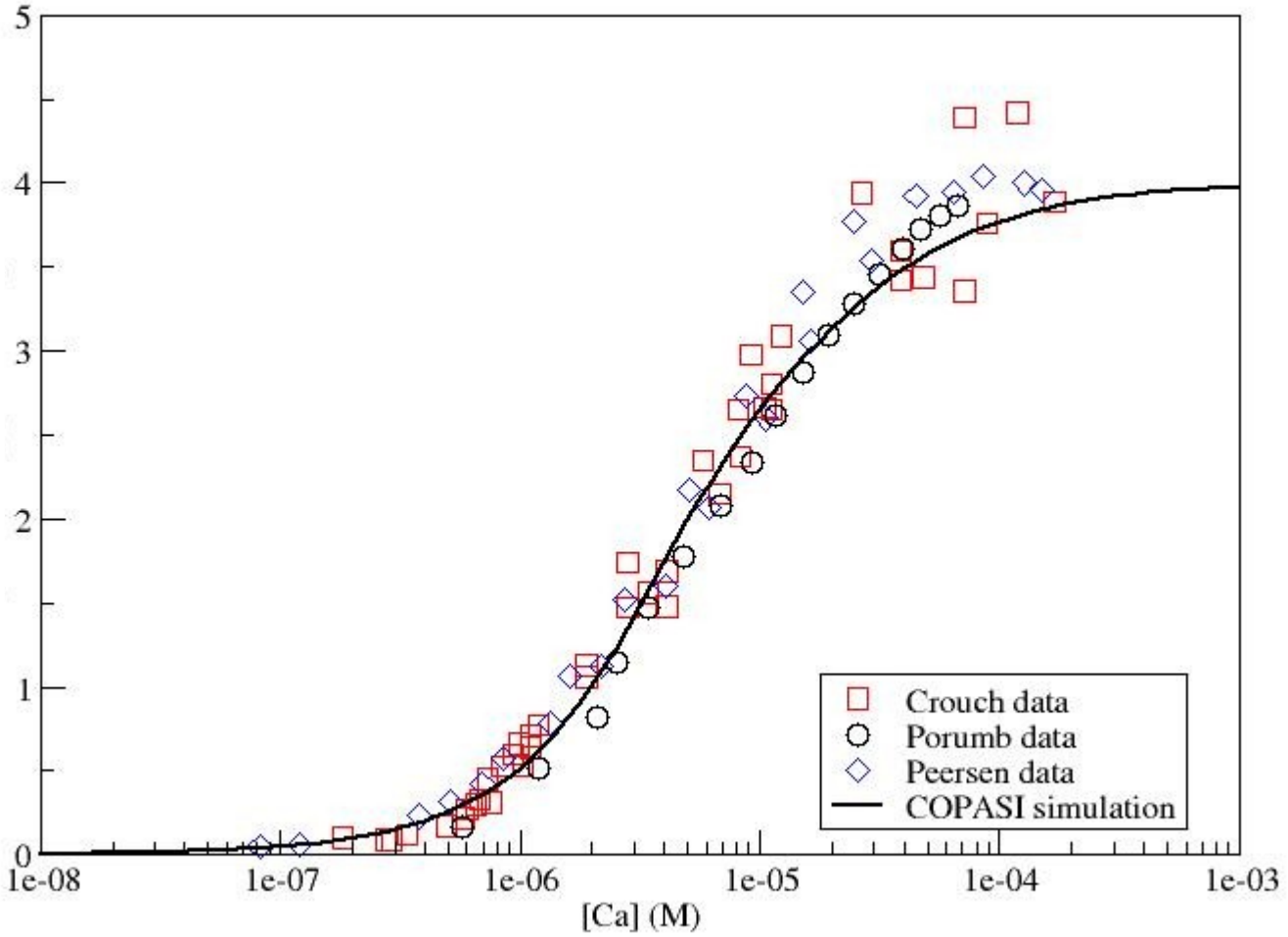
Full mechanistic model



320 reactions

Comparison with experiments

$\frac{Ca^{2+}\ bound}{CaM}$



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

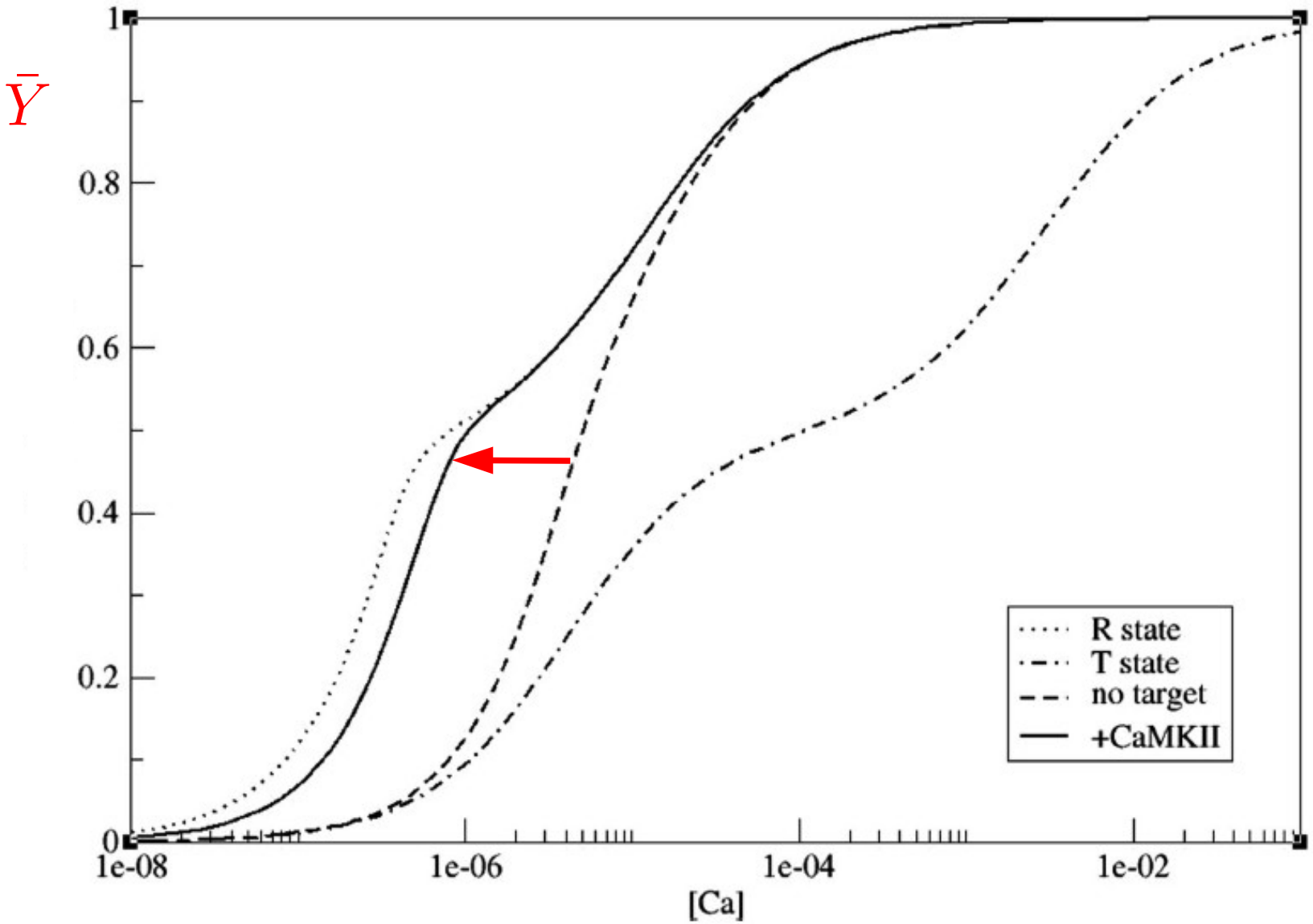
Activity of unsaturated calmodulin

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

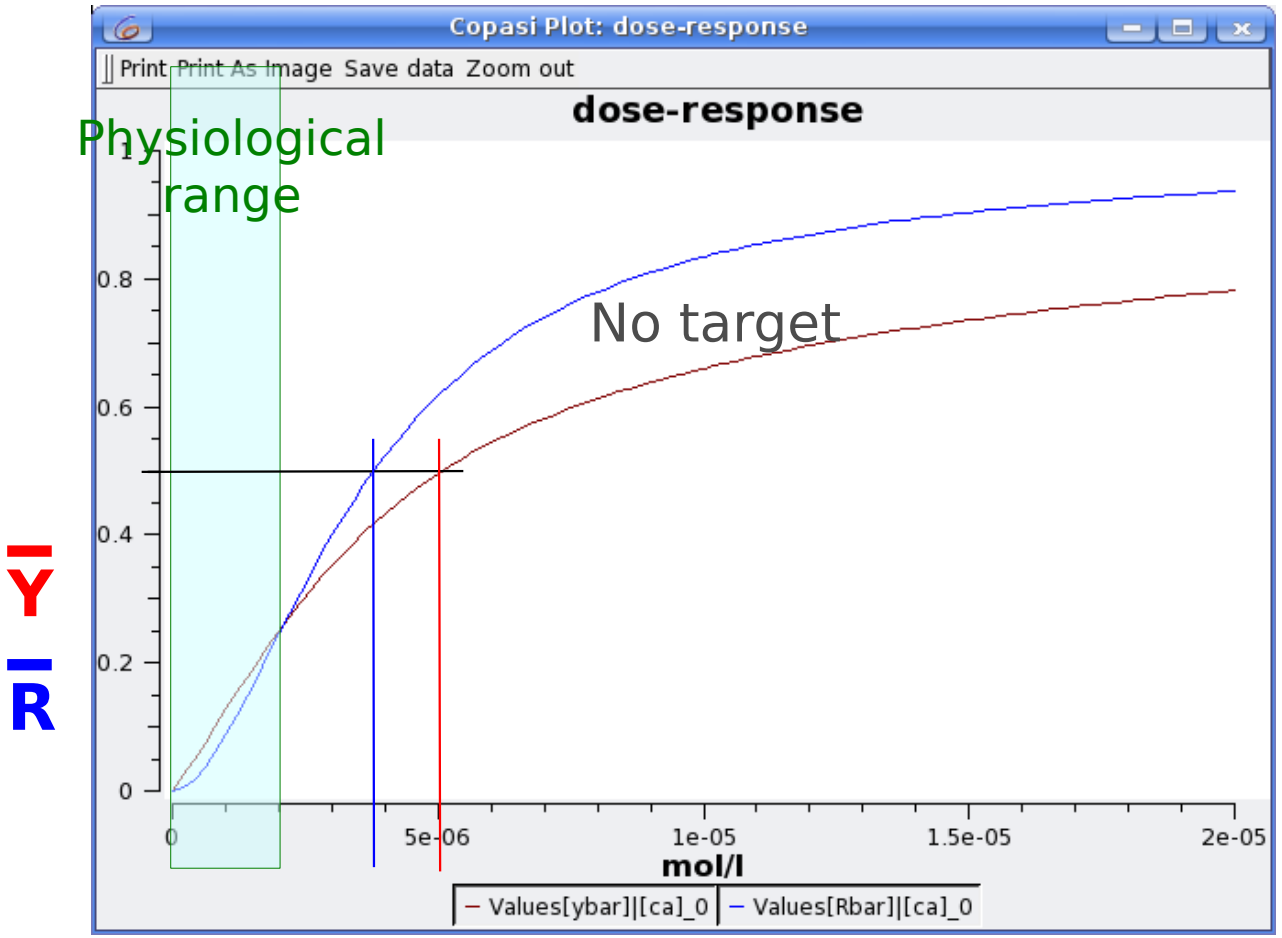
$$\frac{R_2}{T_2} = \frac{1}{L \cdot c^2}$$

- 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

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



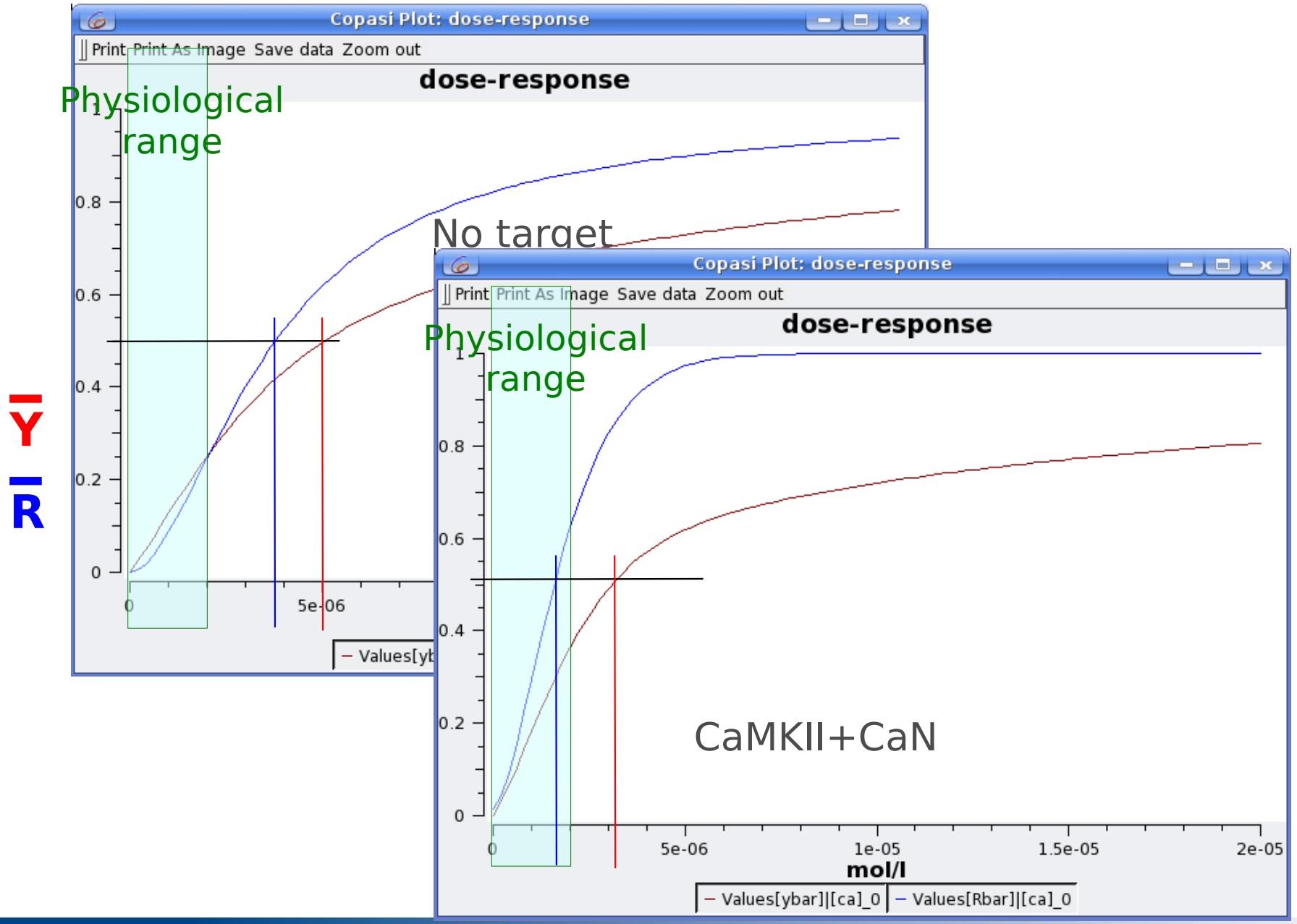
But ... we're out of the physiological range?



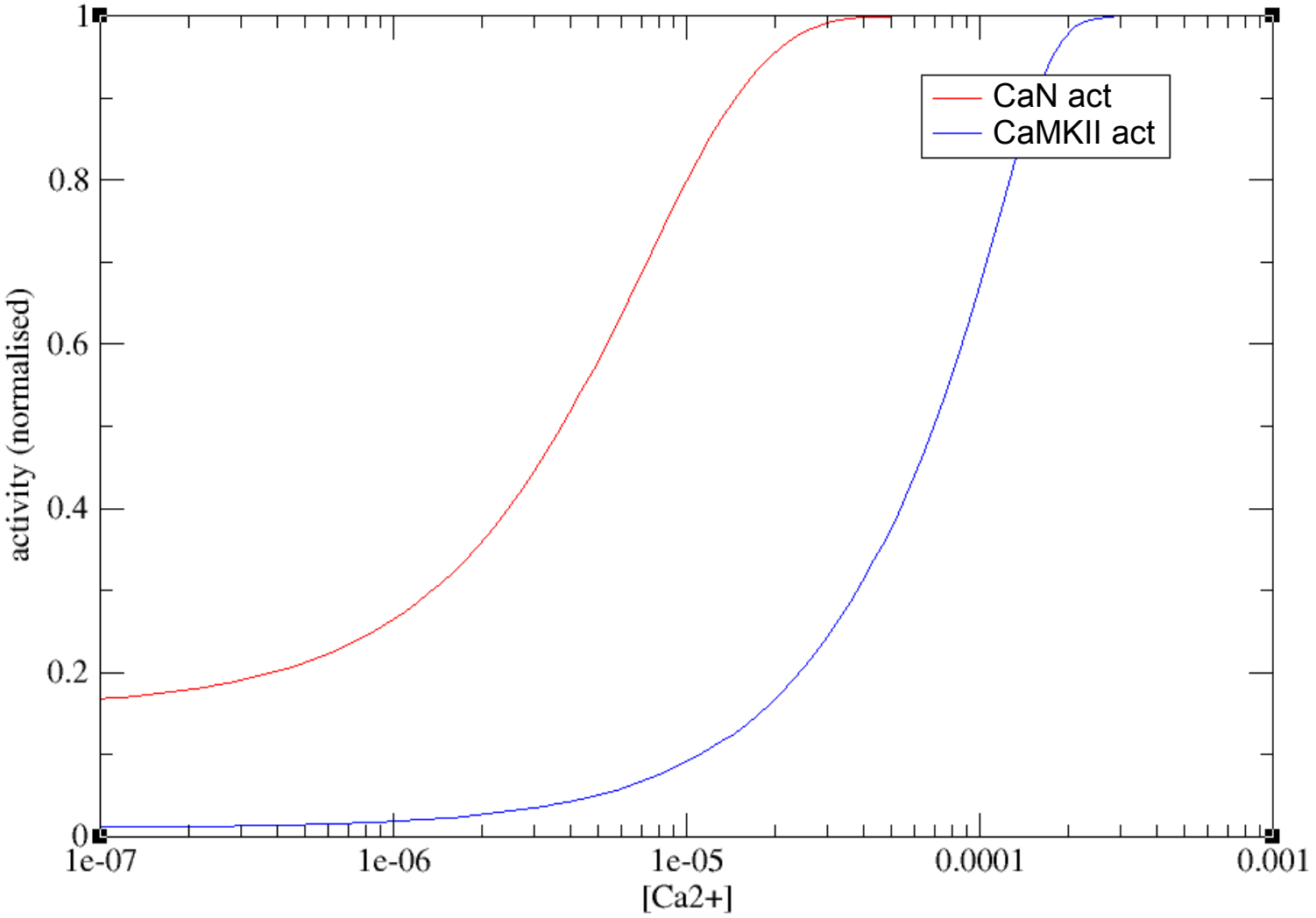
$\bar{Y}$

$\bar{R}$

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

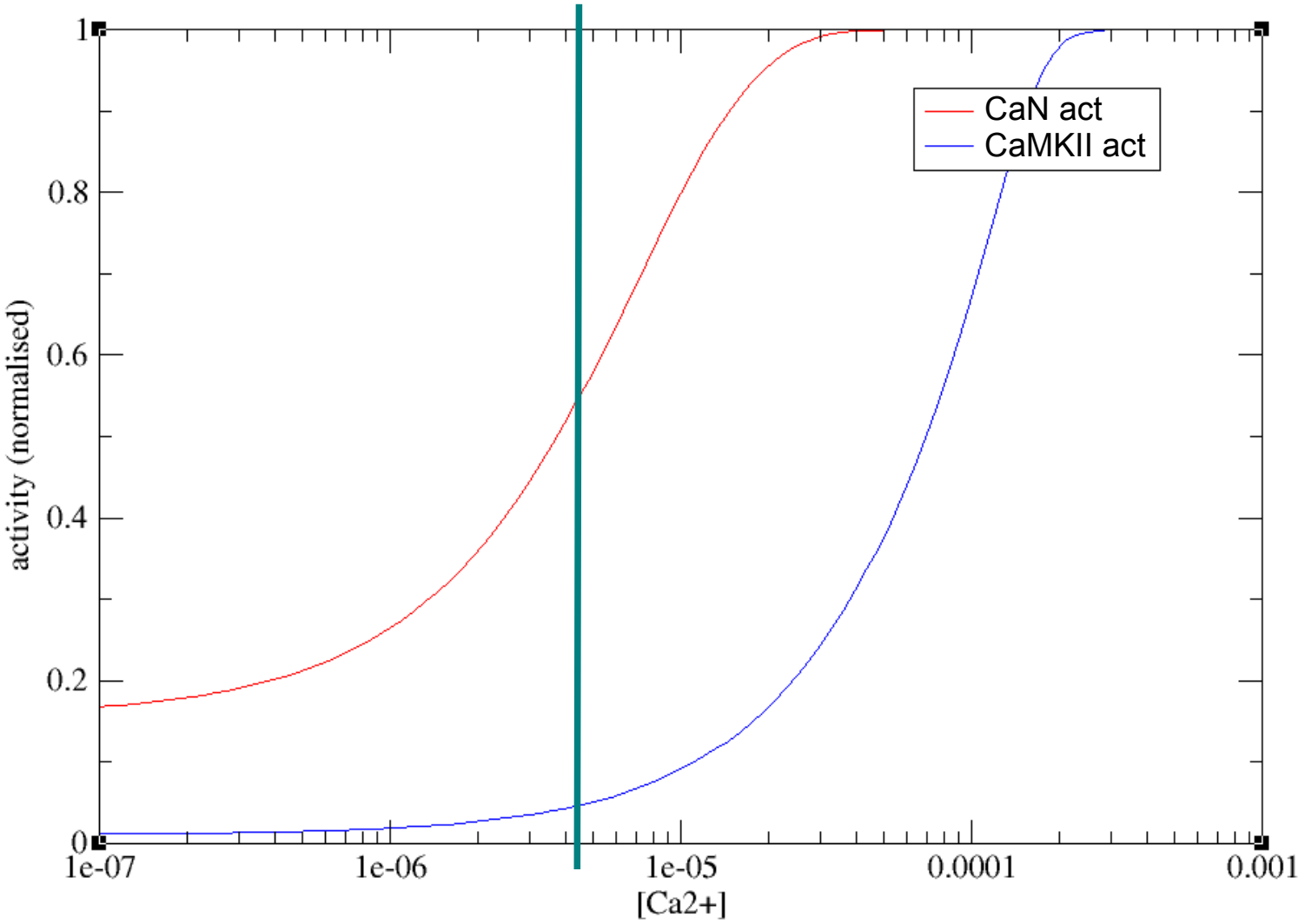


Bidirectional synaptic plasticity

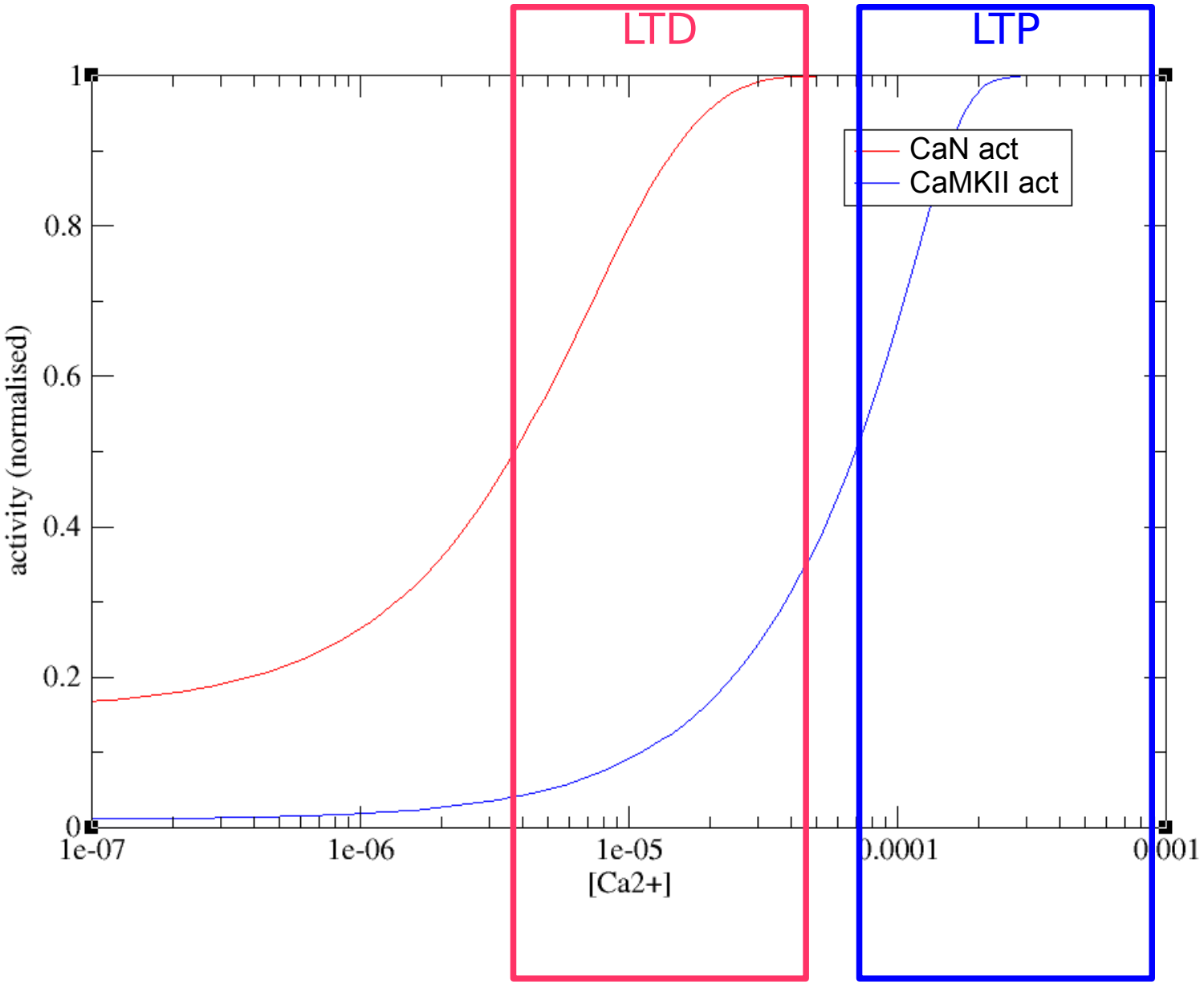


Bidirectional synaptic plasticity

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.

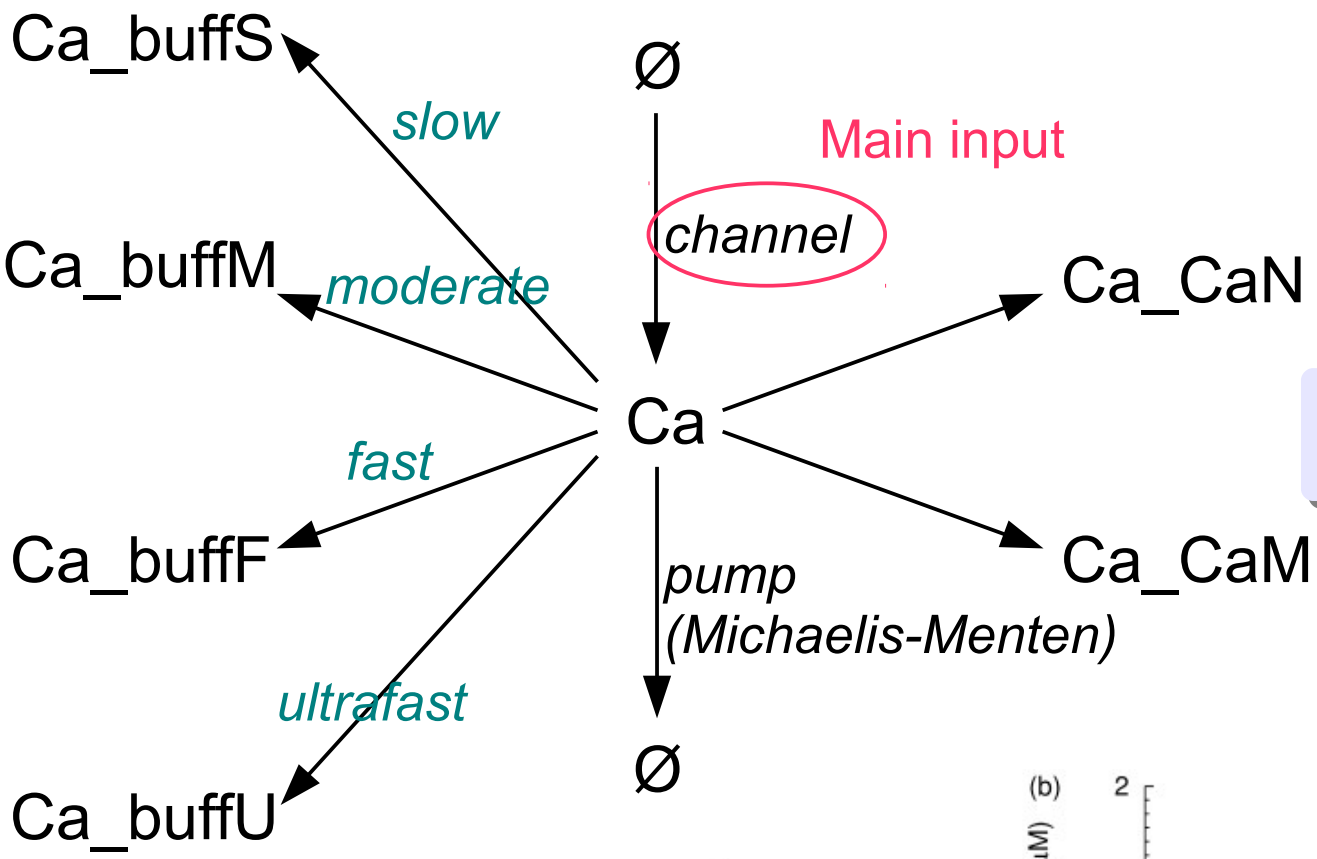
The model displays an activation of the sole CaN at low concentration of calcium, while high concentrations activate both CaN and CaMKII.

Wait a minute!
Signal transduction is not at equilibrium!

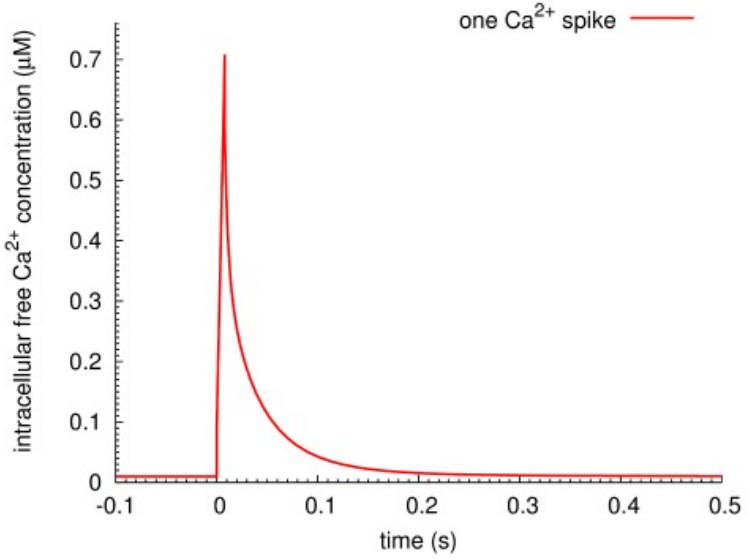
AMPAR post-synaptic potential:	5 ms
Calcium spike:	50 ms
Half saturation calmodulin (kon=1.5e6, koff=100):	5 ms
Relaxation between calmodulin states:	1 ms
autophosphorylation of CaMKII (kon=6):	100 ms

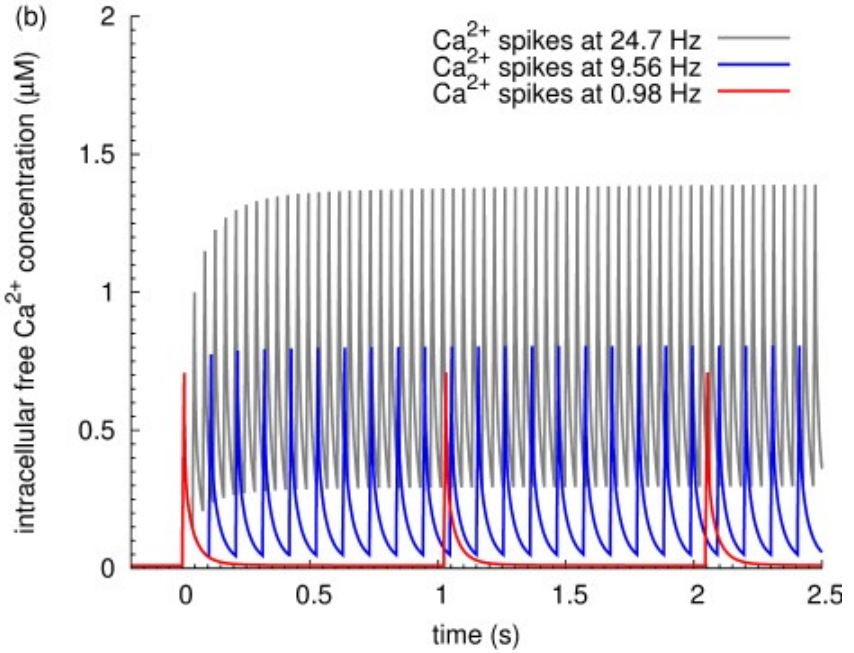
≠

Dynamic of calcium in the spine



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





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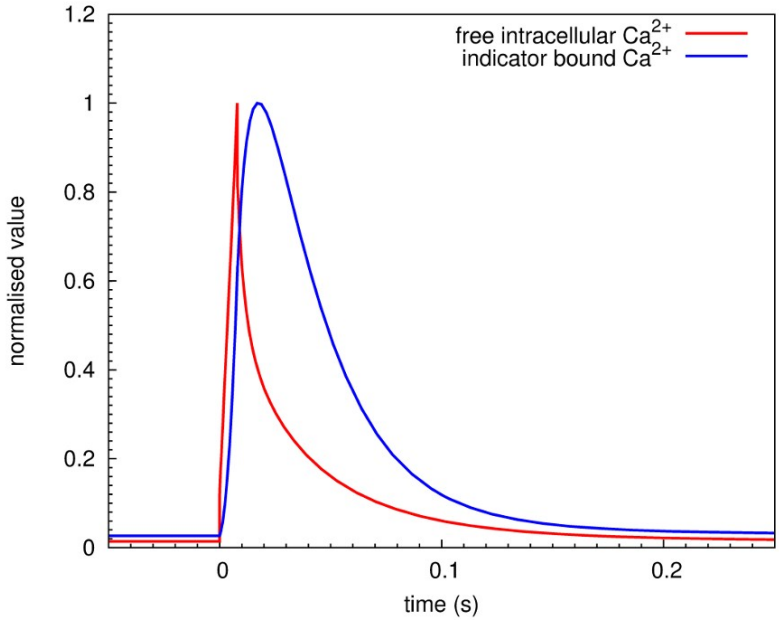
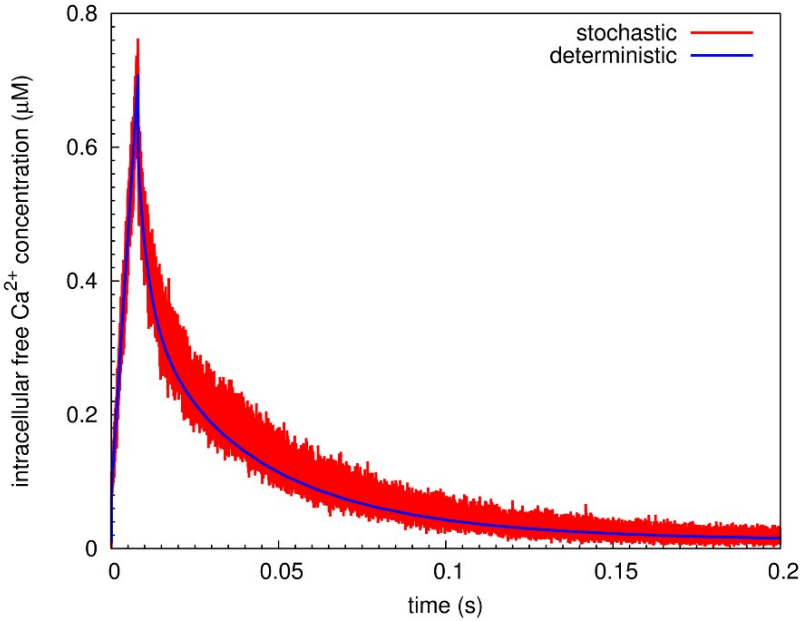
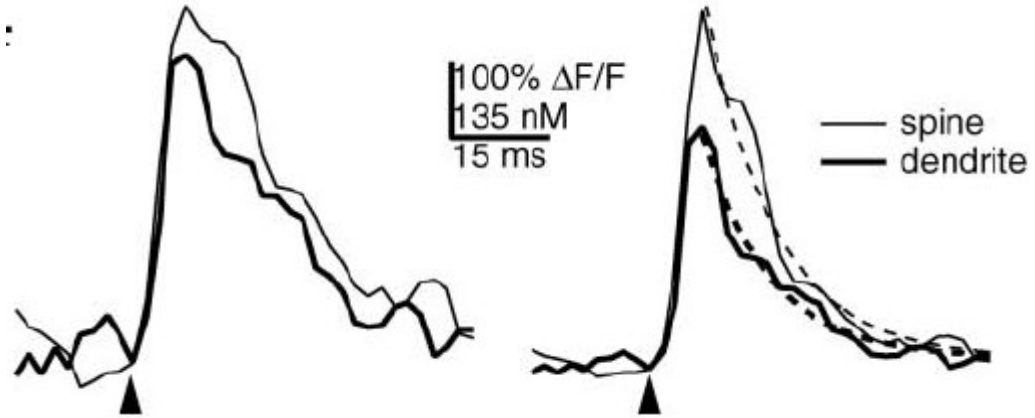
Are those spikes realistic?







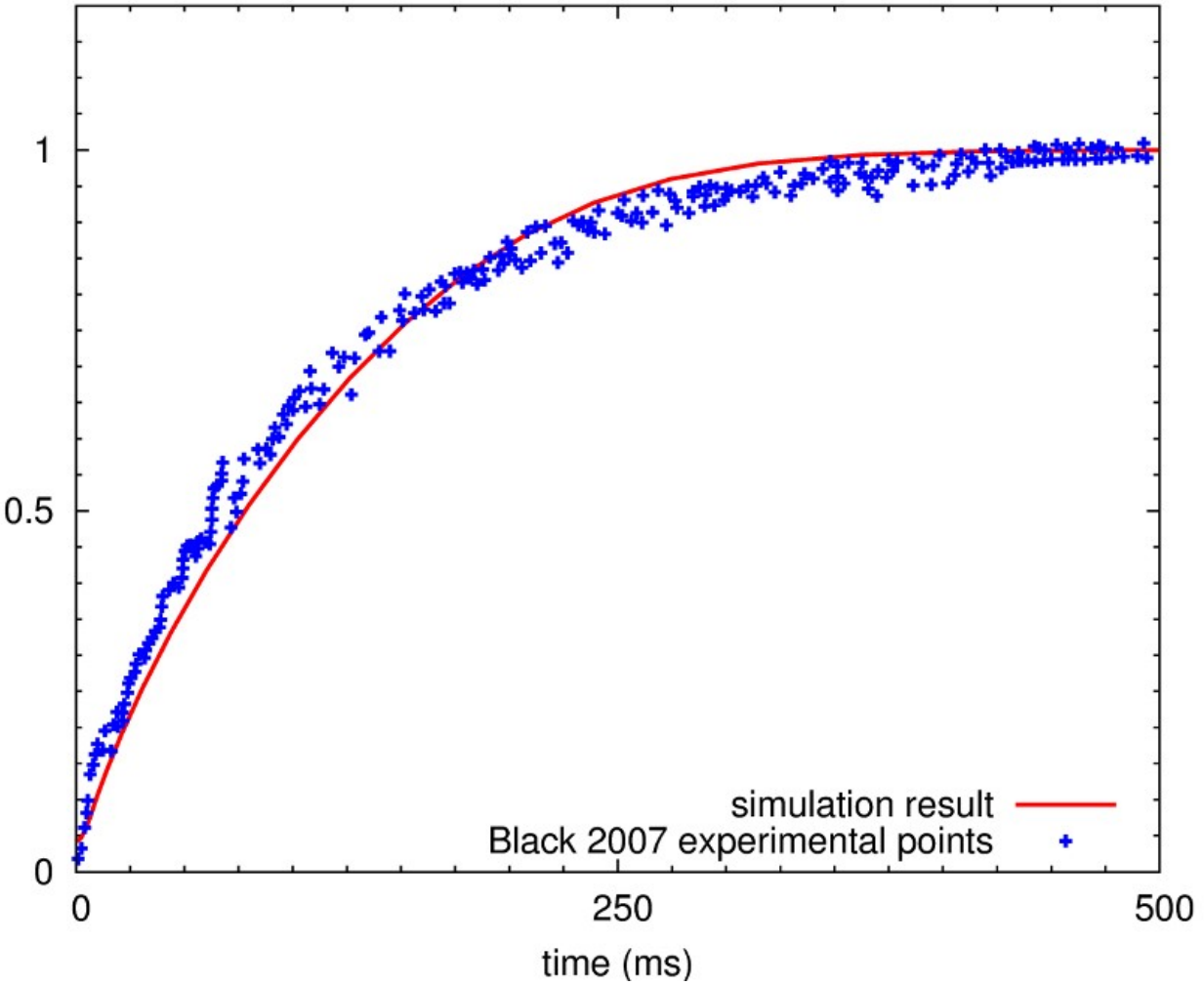
Fluorescence

Free calcium

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

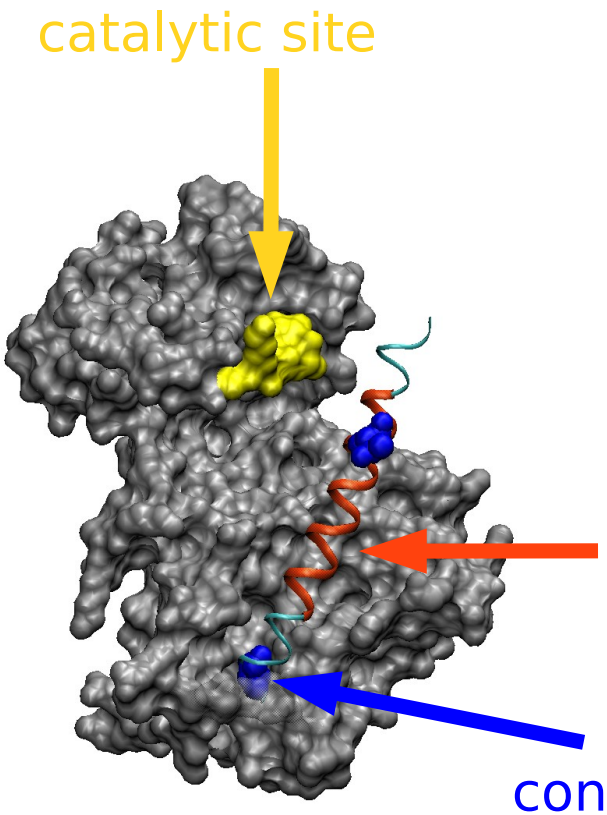
Sabatini et al (2002)
Neuron 33: 439–452.

Validation of CaM kinetics



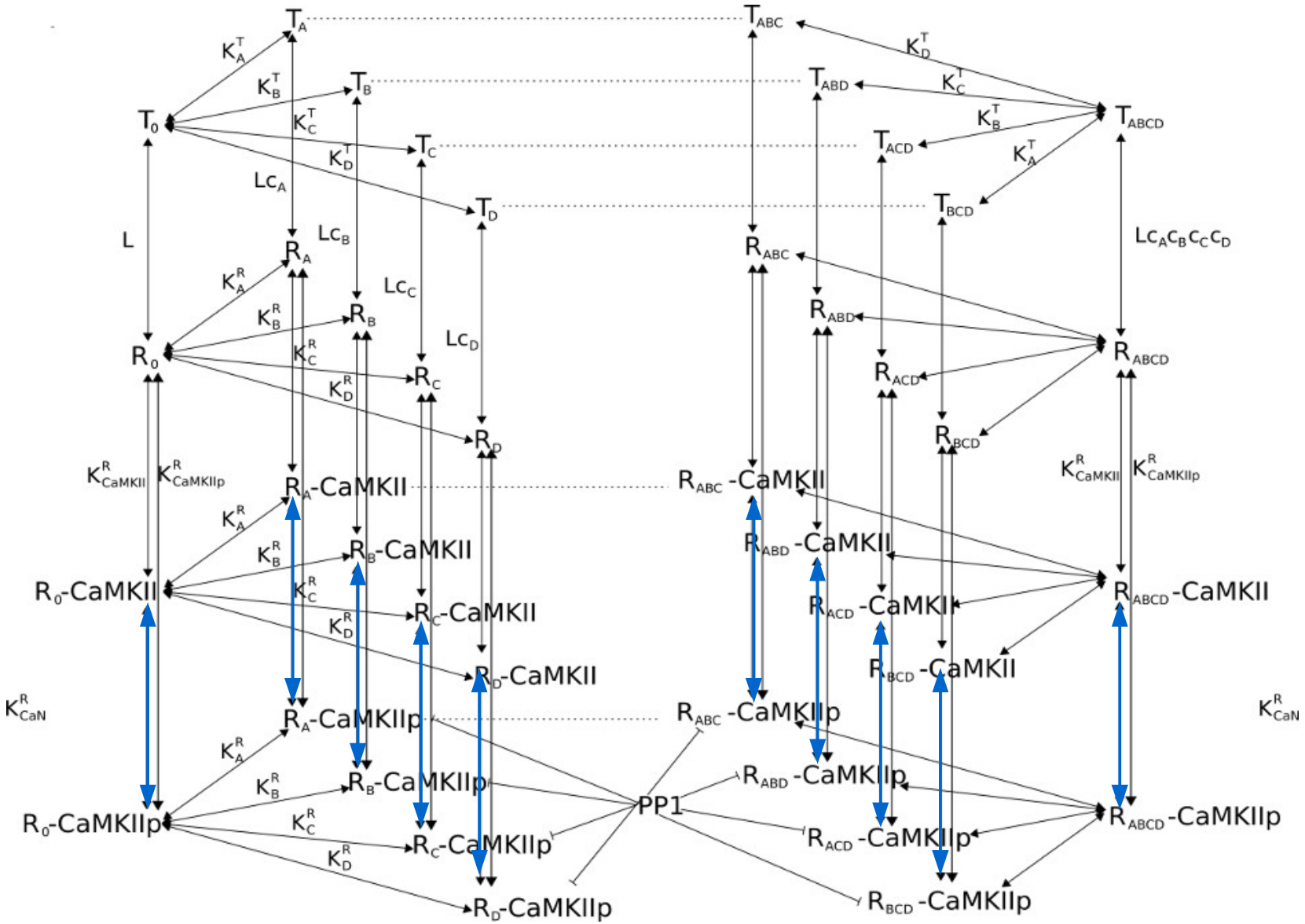
Black DJ, Selfridge JE, Persechini A (2007). *Biochemistry* 46: 13415–13424.

Calcium/calmodulin kinase II

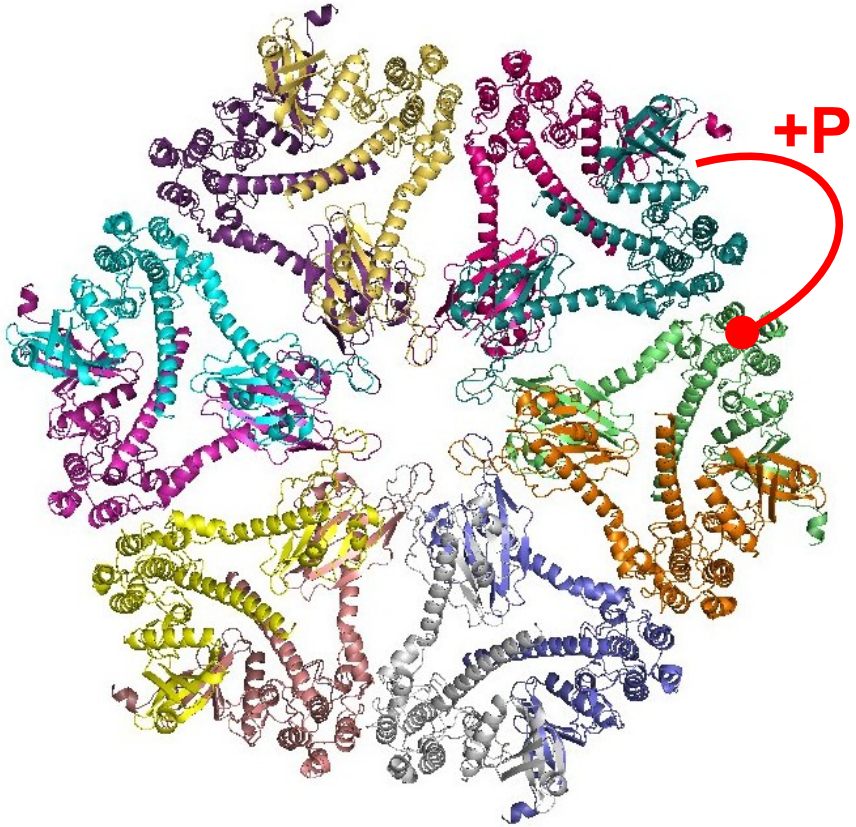


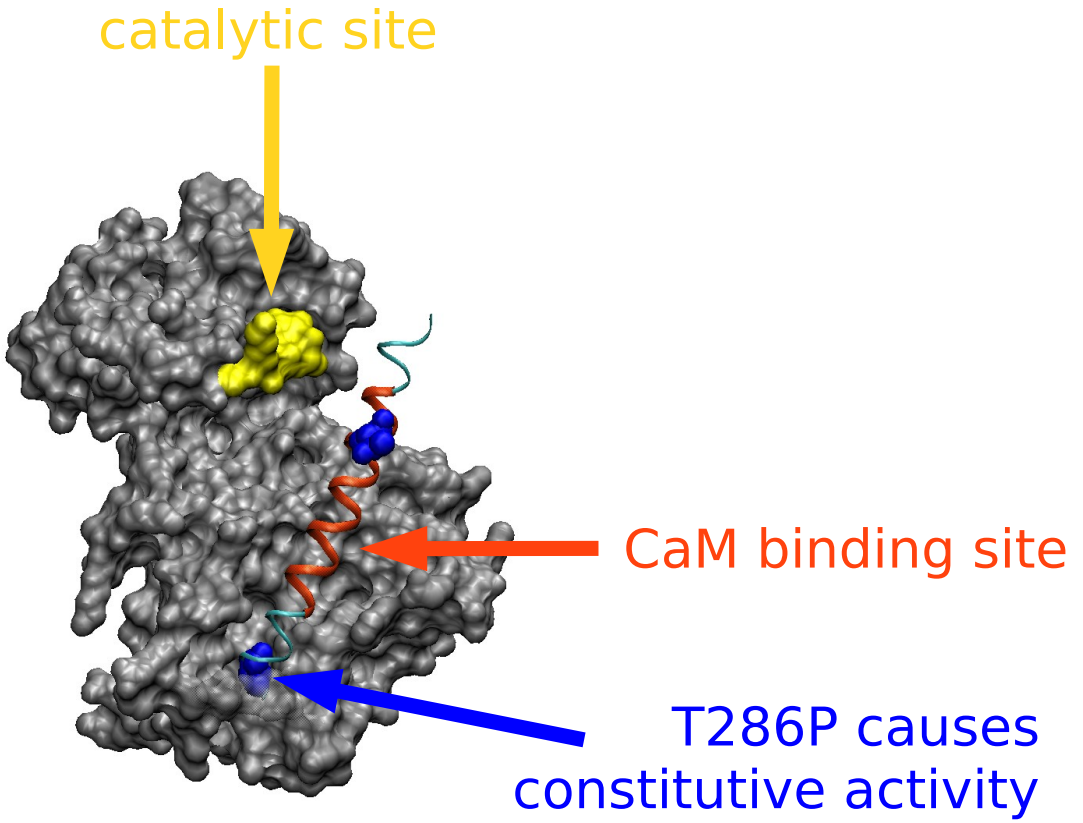
Calmodulin trapping is
an apparent increase of
affinity of CaMKII for CaM
when T286 is phosphorylated

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



Calcium/calmodulin kinase II

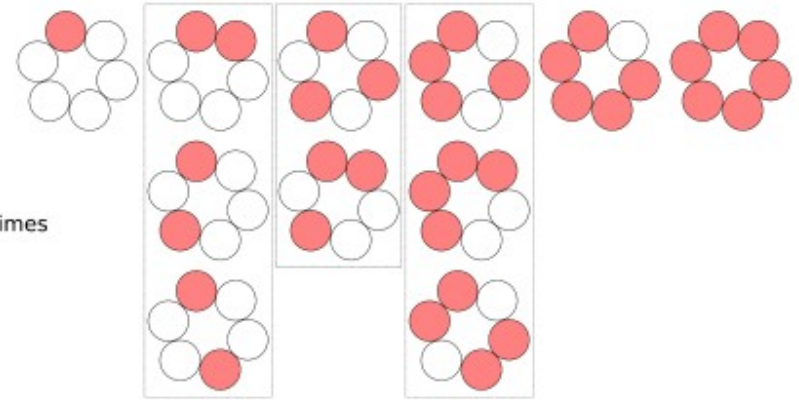




Dodecamer;
Trans-phosphorylation of T286
by neighbouring subunits

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.

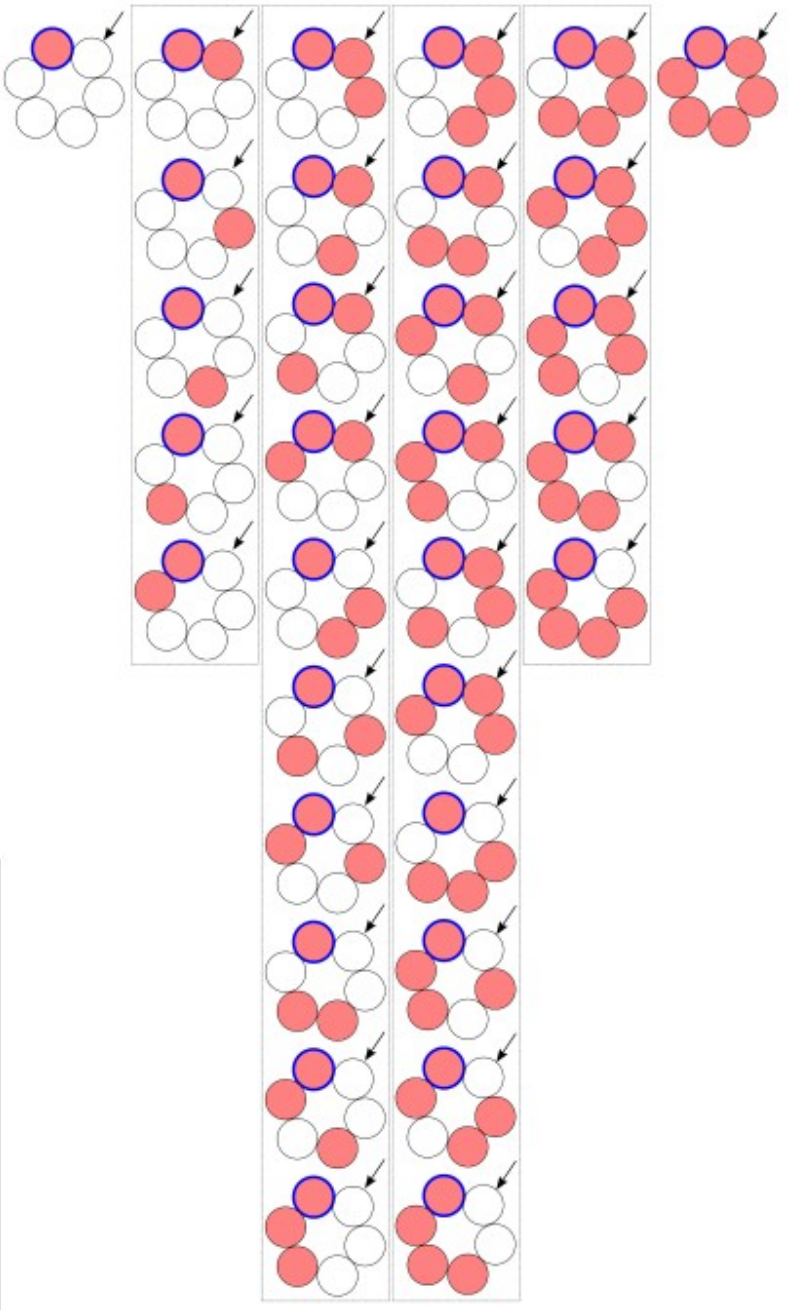
Repeat for every 1% increase of CaMKII active monomers

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

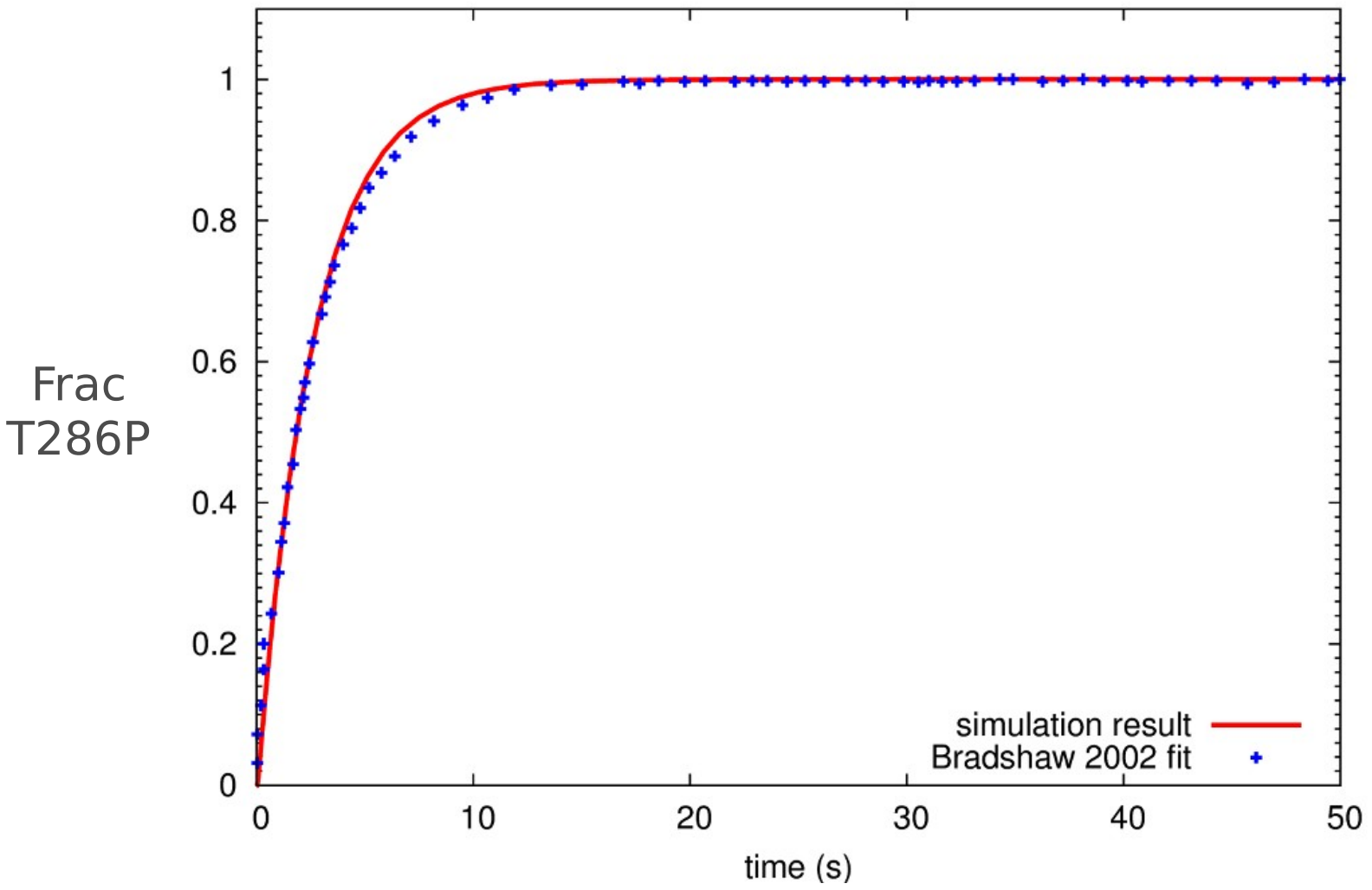
The sum of these six numbers is a coefficient that can be used to adjust CaMKII autophosphorylation rate.

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

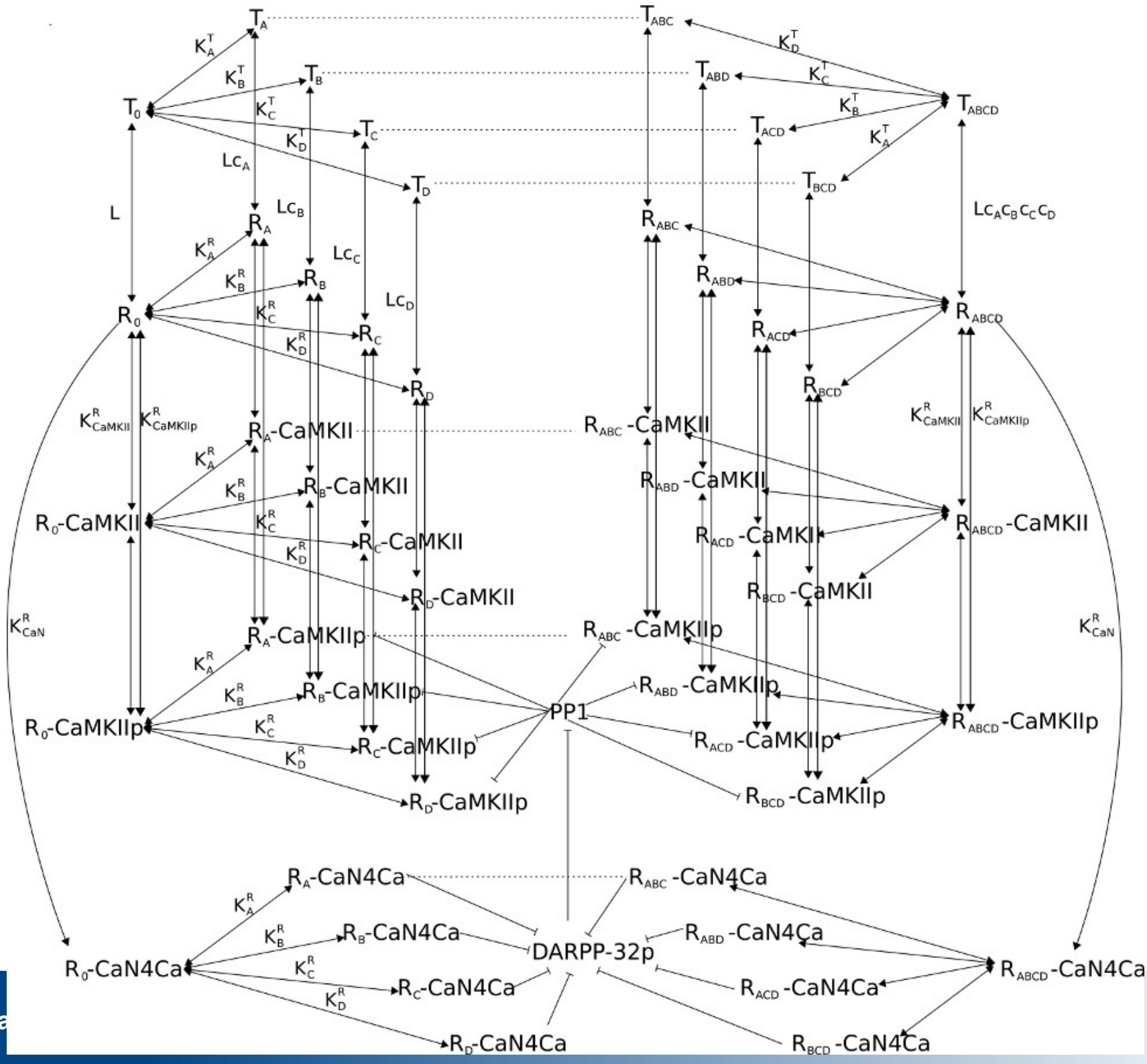
Calculate the probabilities of having an active neighbour on one specific side (indicated by the arrow) of the activated monomer of interest (blue outline) (since only the asymmetrical situation is considered). The possible positions of activated monomers are listed as the following, with corresponding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

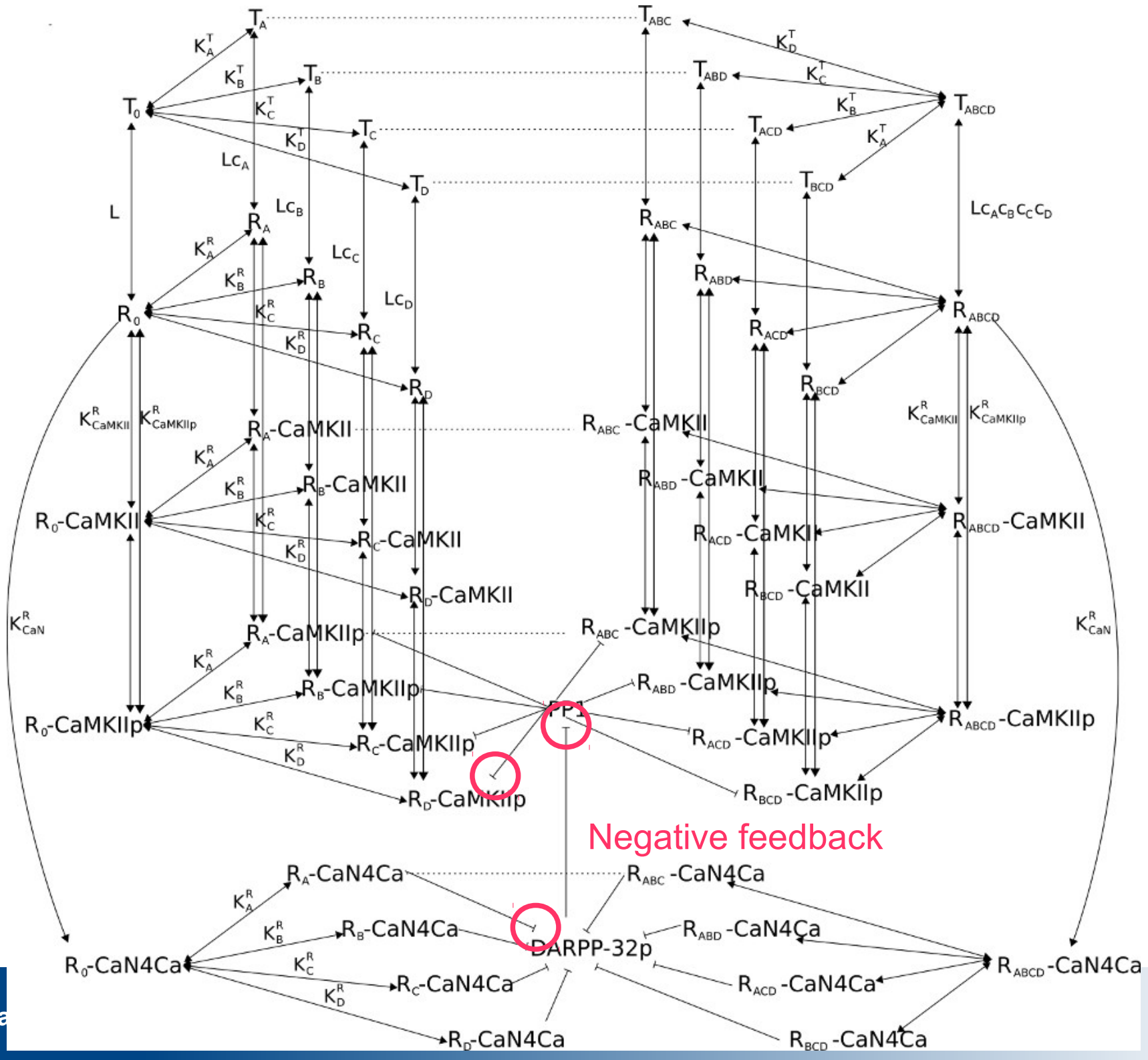


Validation of CaMKII kinetics

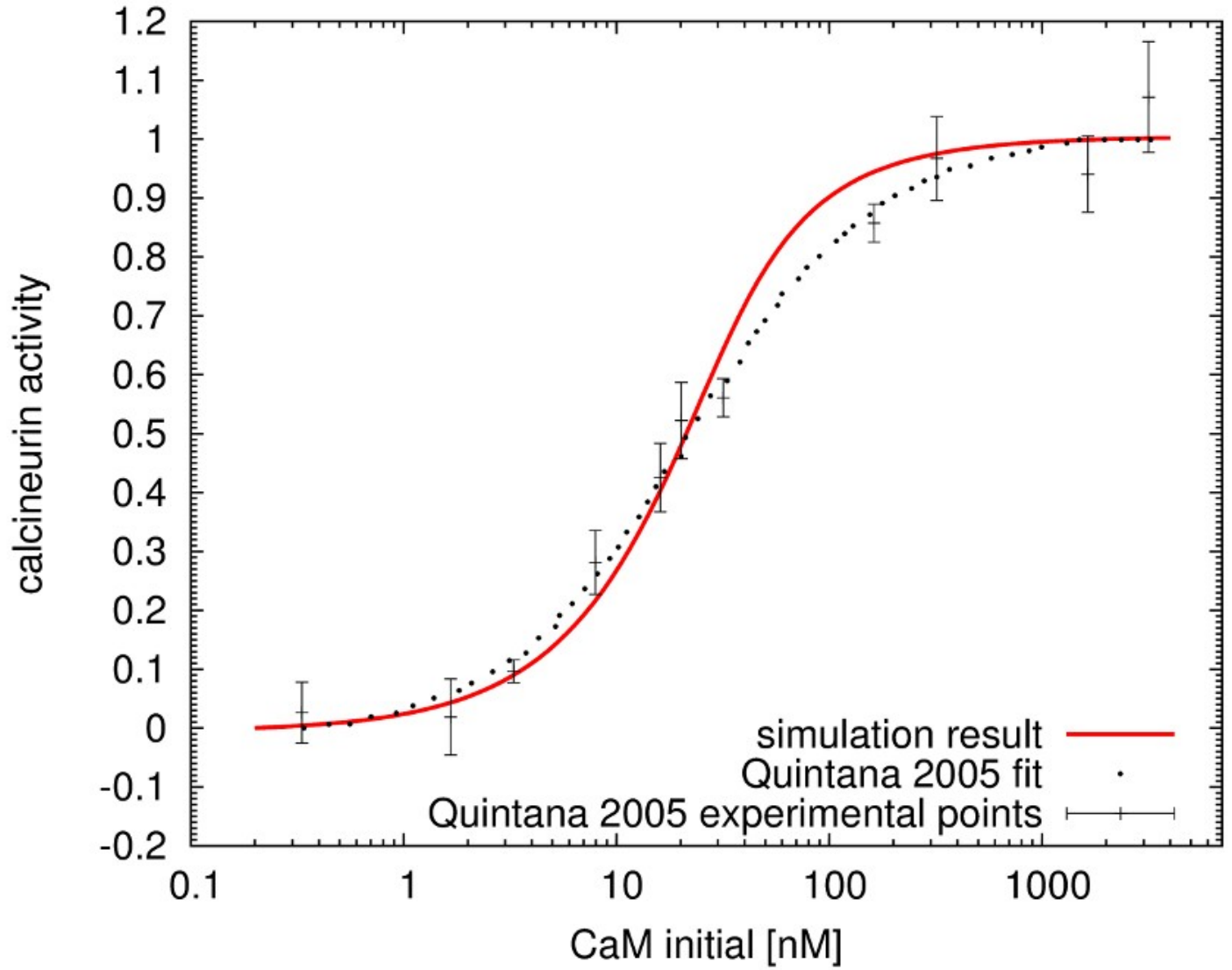


Bradshaw JM, Kubota Y, Meyer T, Schulman H (2003). *PNAS* 100: 10512–10517.


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

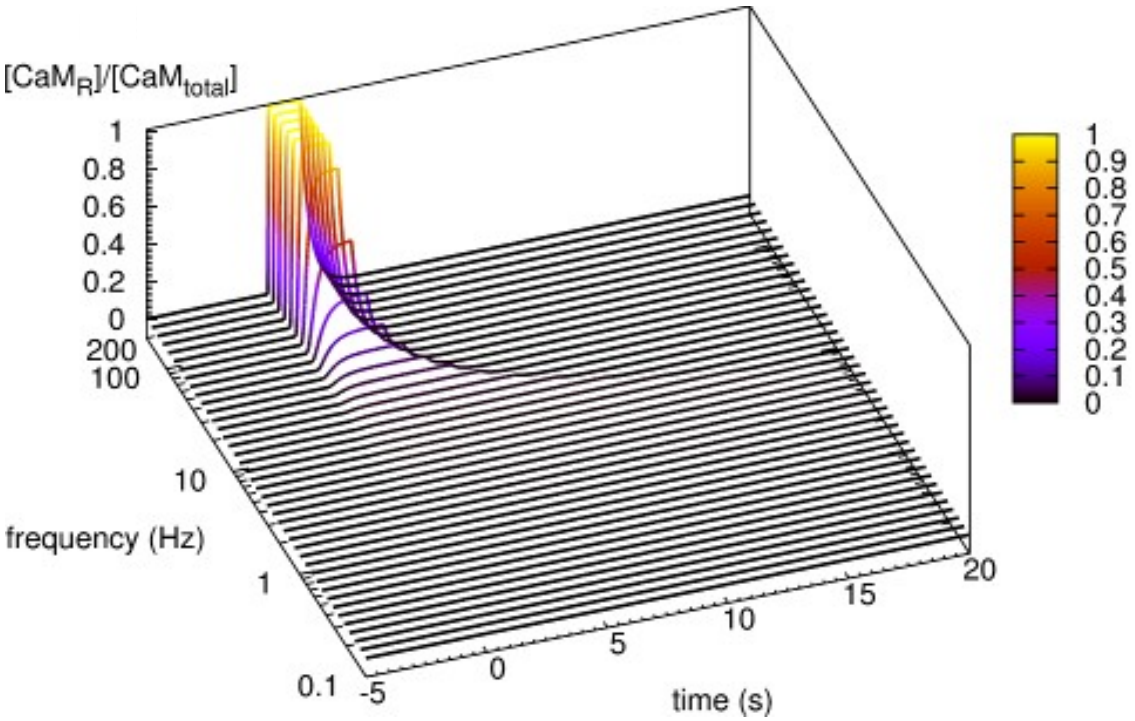

Negative feedback

Validation of calcium-activation of CaN



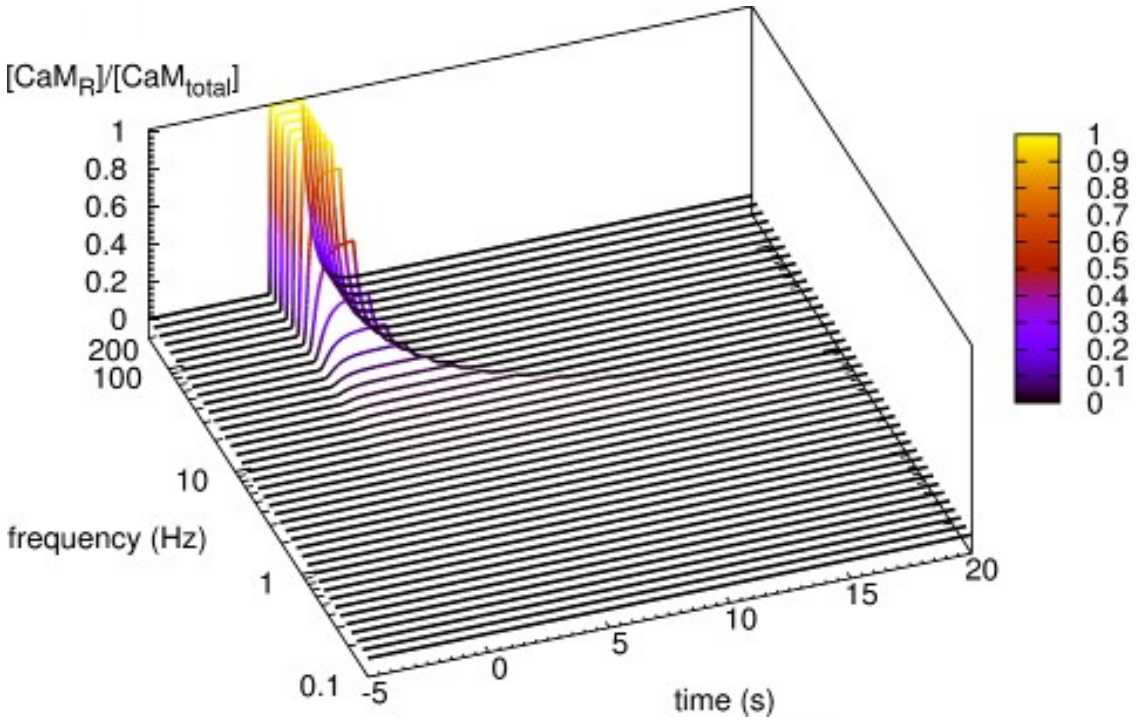
Quintana AR, Wang D, Forbes JE, Waxham MN (2005). *BBRC* 334: 674–680.

Calmodulin activation

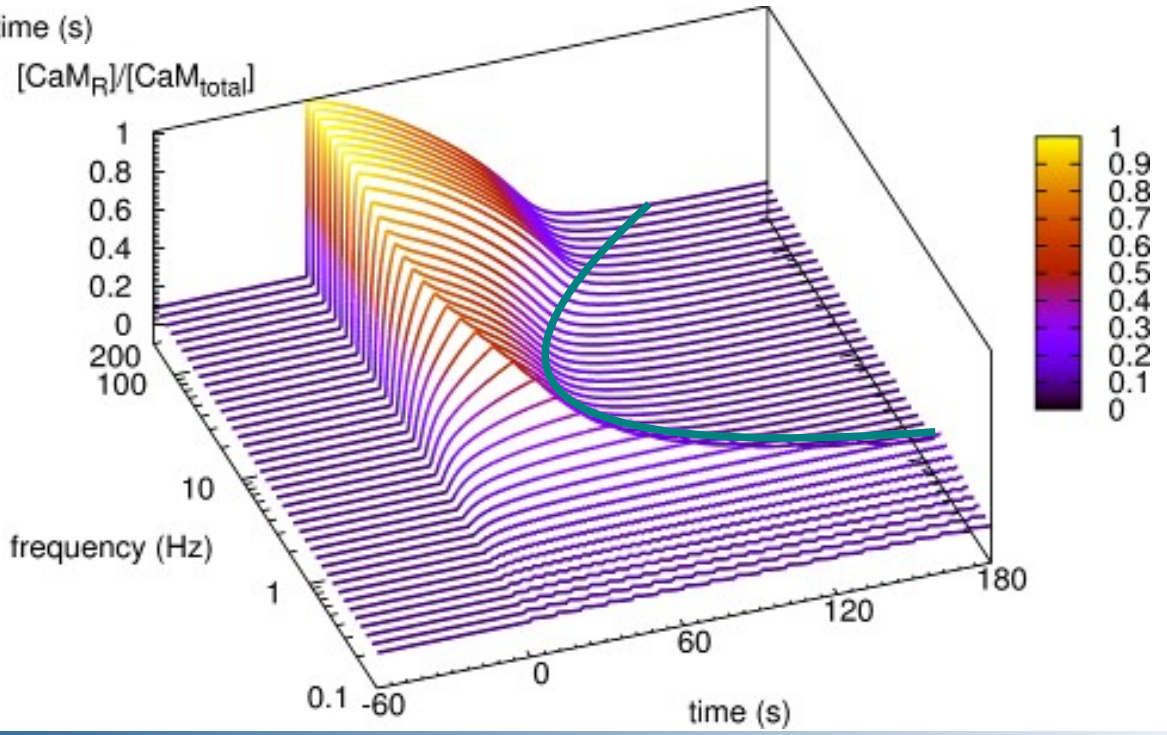


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

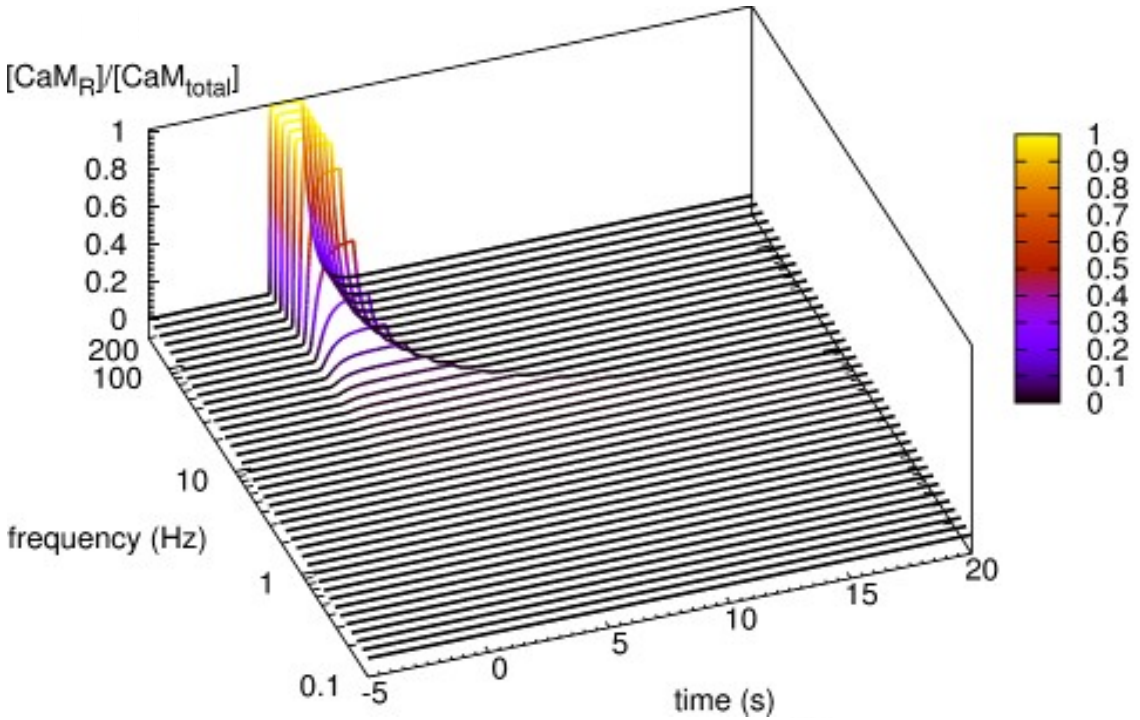


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)



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

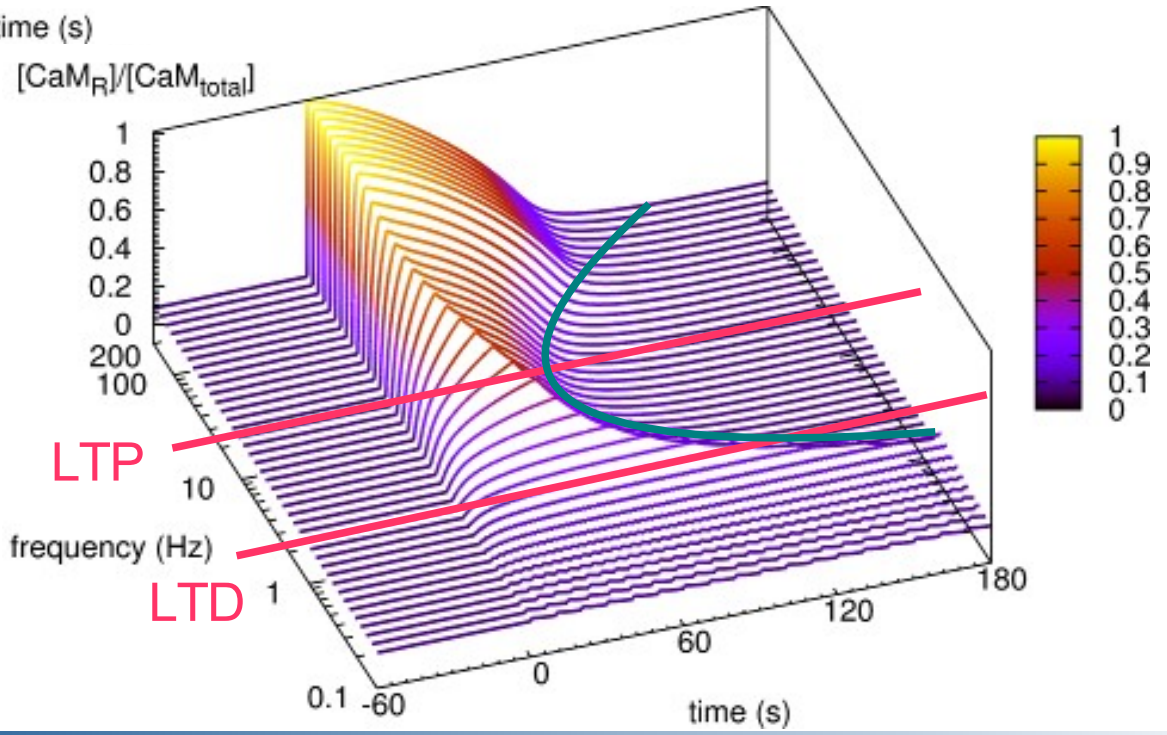
Calmodulin activation



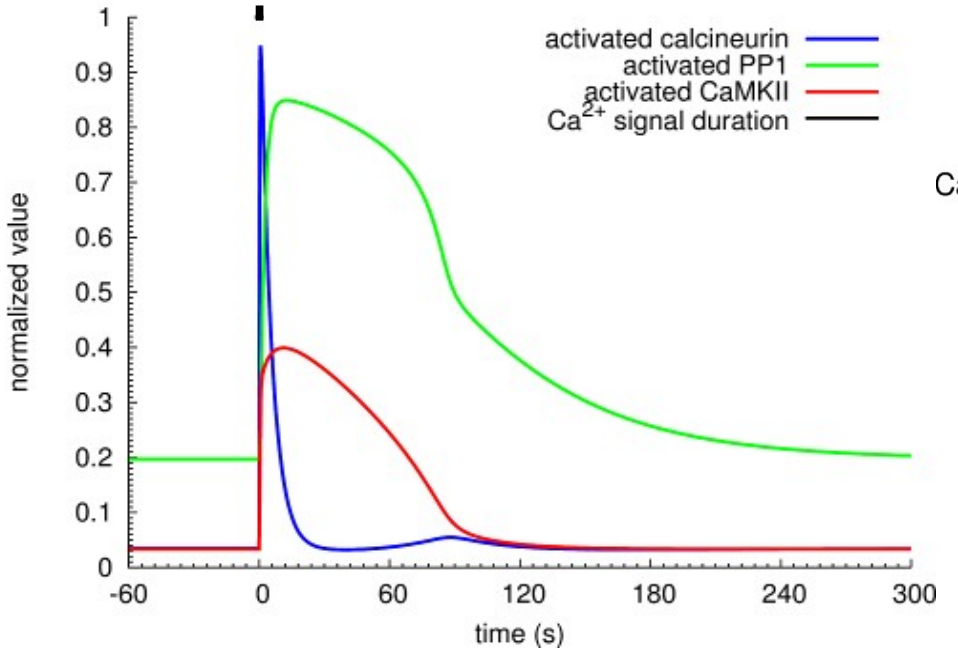
CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

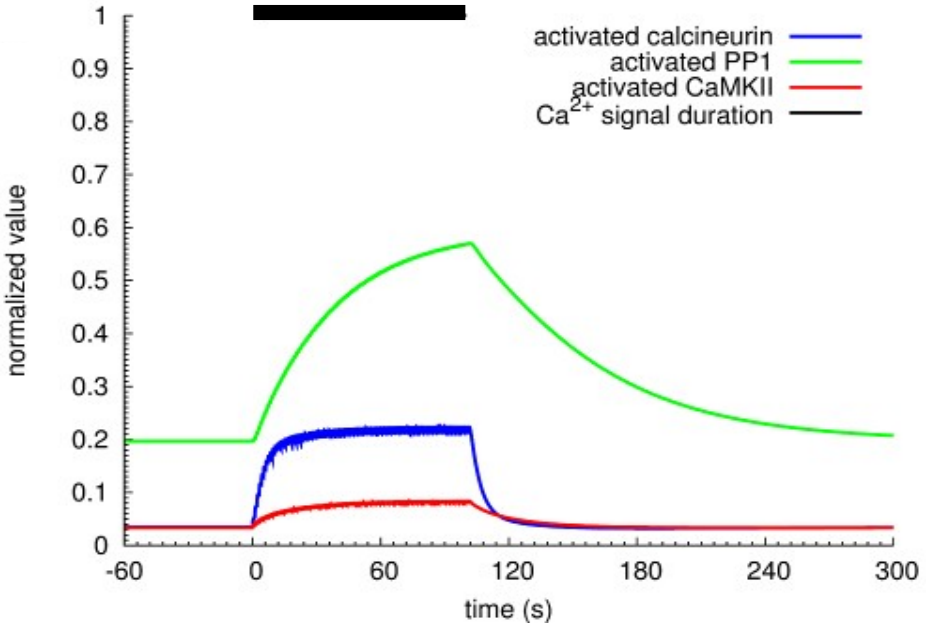
At high frequency, effects of calcium signals last much longer than the signal itself

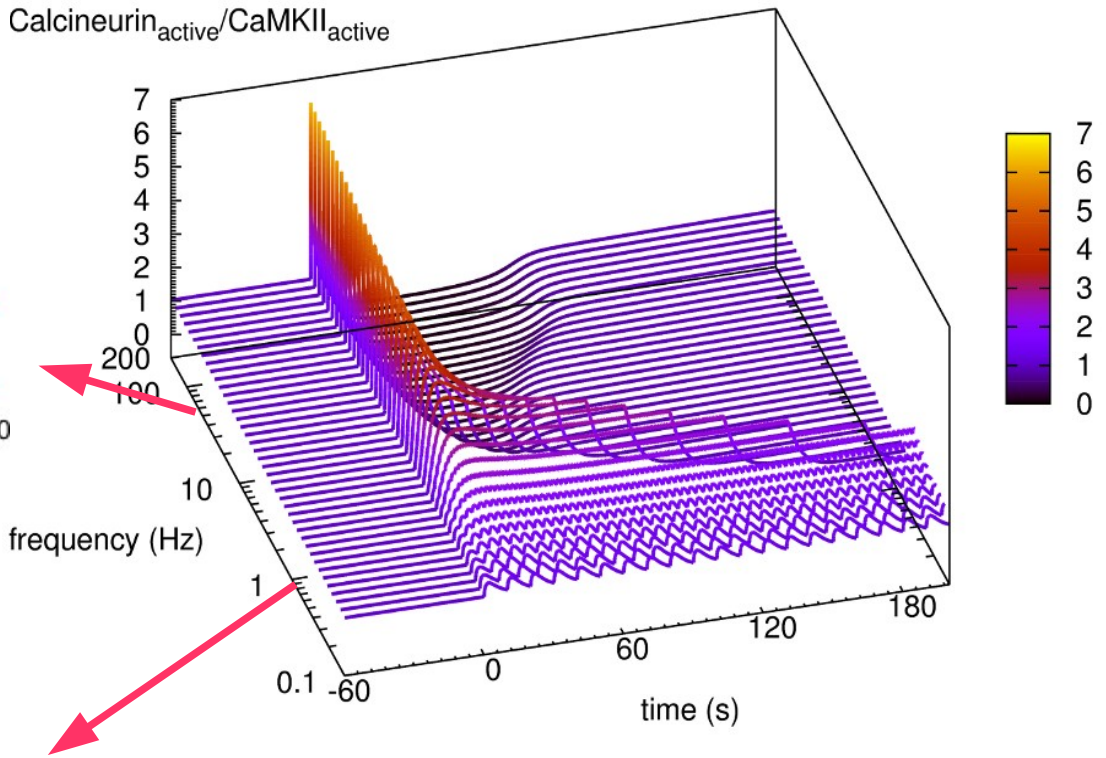
CaM with targets. Binding to CaN and CaMKII stabilises R state, with higher affinity. Positive feedback loop
➜ bistability



Temporal activation of CaMKII and CaN

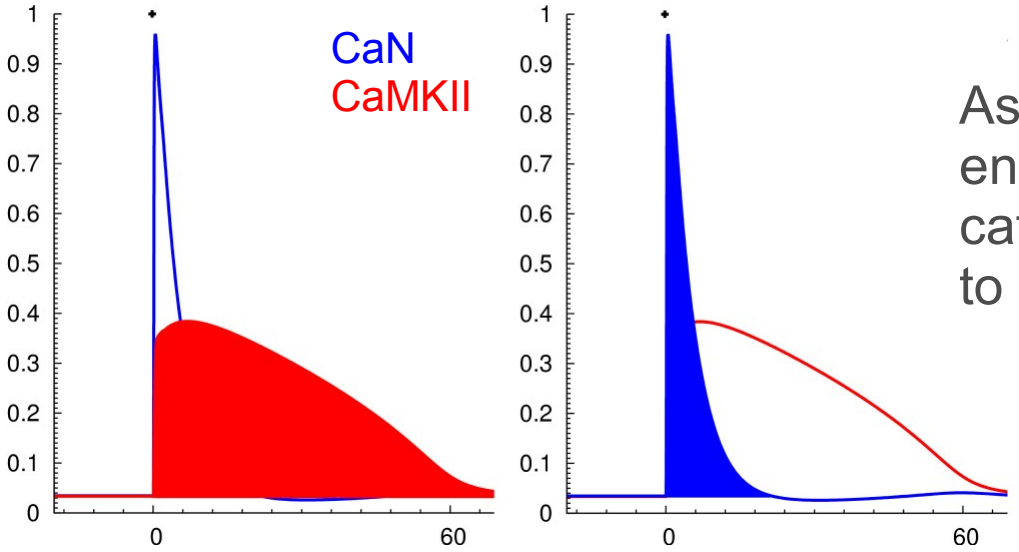






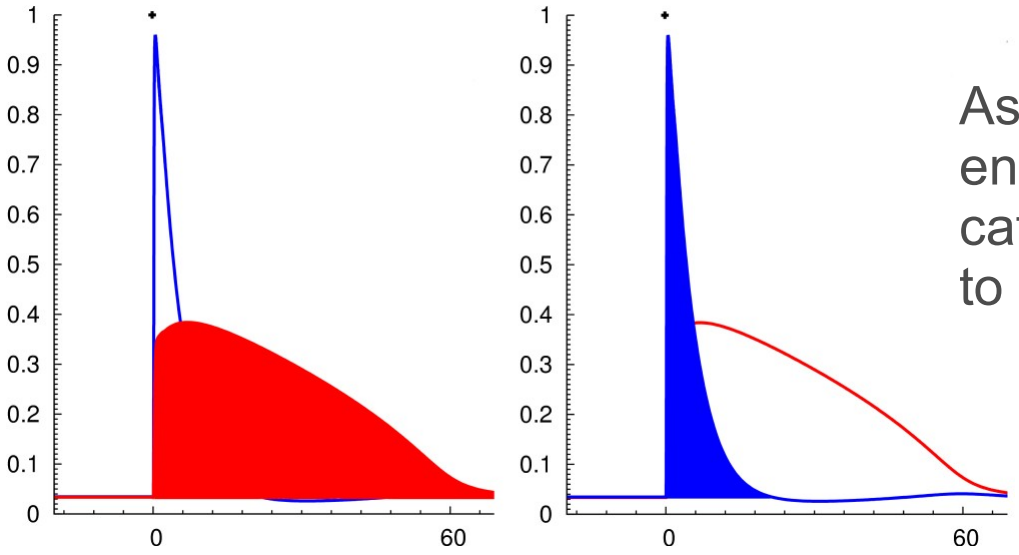
All calcium frequencies increase CaN **AND** CaMKII. It is not a Switch. But the ratio of activation changes

Bidirectional plasticity



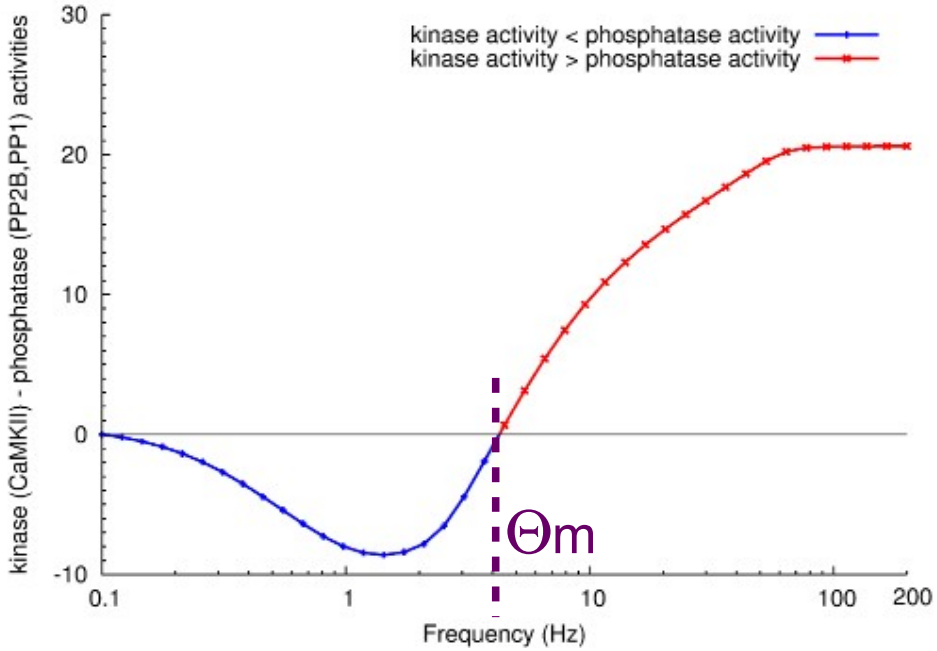
Assuming that catalytic rates of active enzyme do no change, the quantity of catalysed reaction events is proportional to the integral of the activation curve

Bidirectional plasticity

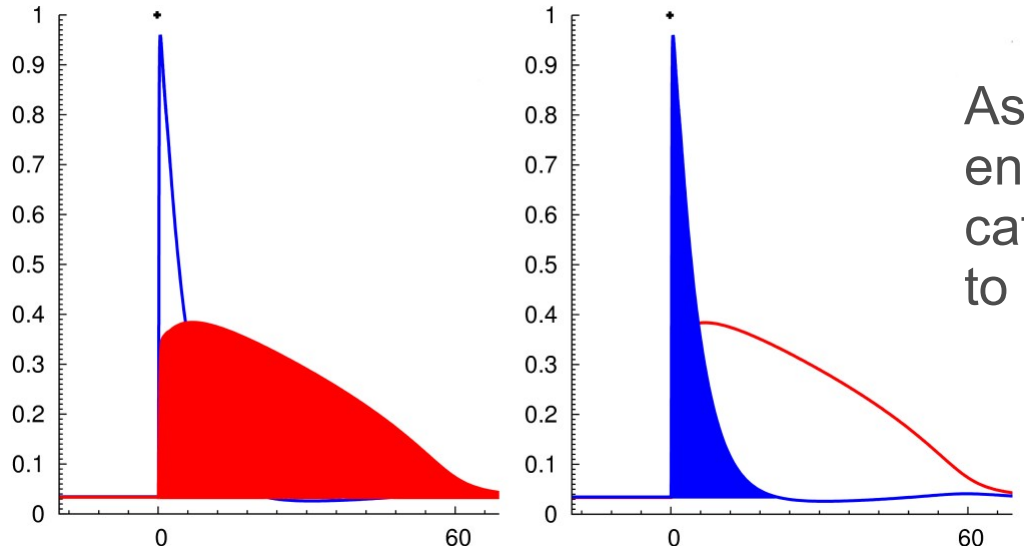


Assuming that catalytic rates of active enzyme do no change, the quantity of catalysed reaction events is proportional to the integral of the activation curve

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

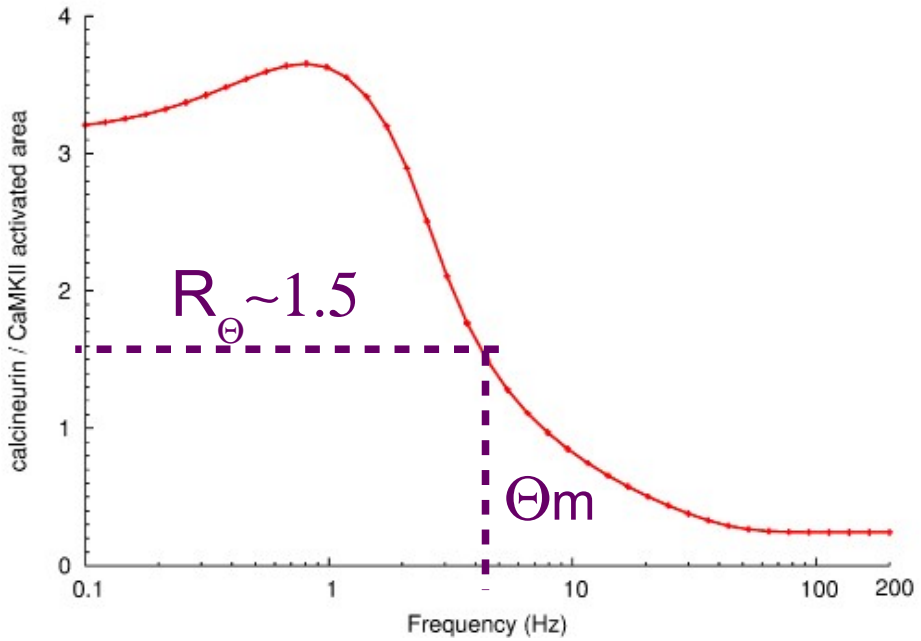


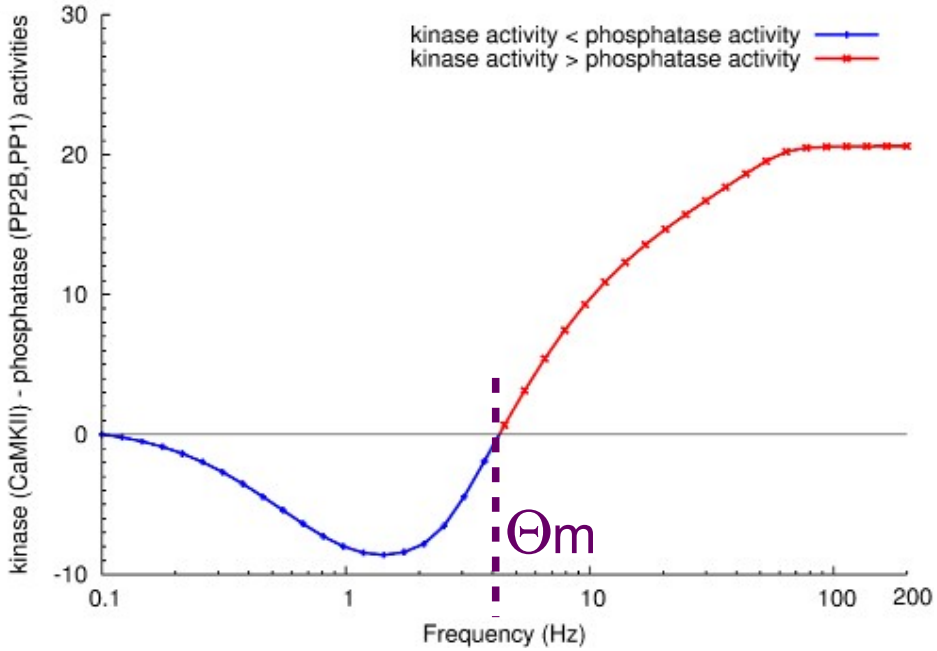
Bidirectional plasticity



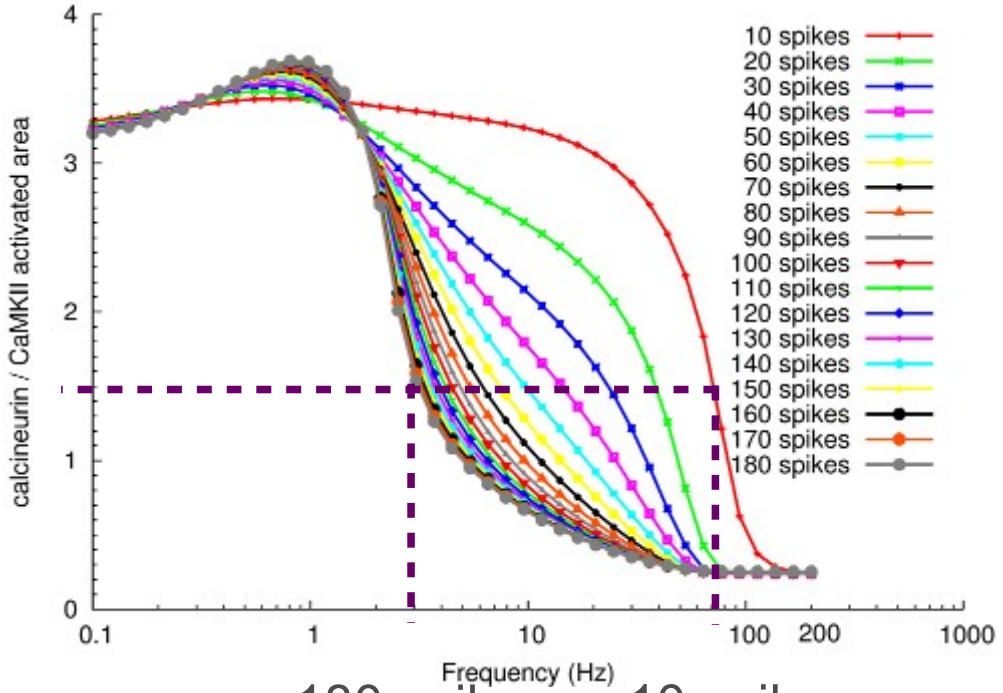
Assuming that catalytic rates of active enzyme do no change, the quantity of catalysed reaction events is proportional to the integral of the activation curve

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



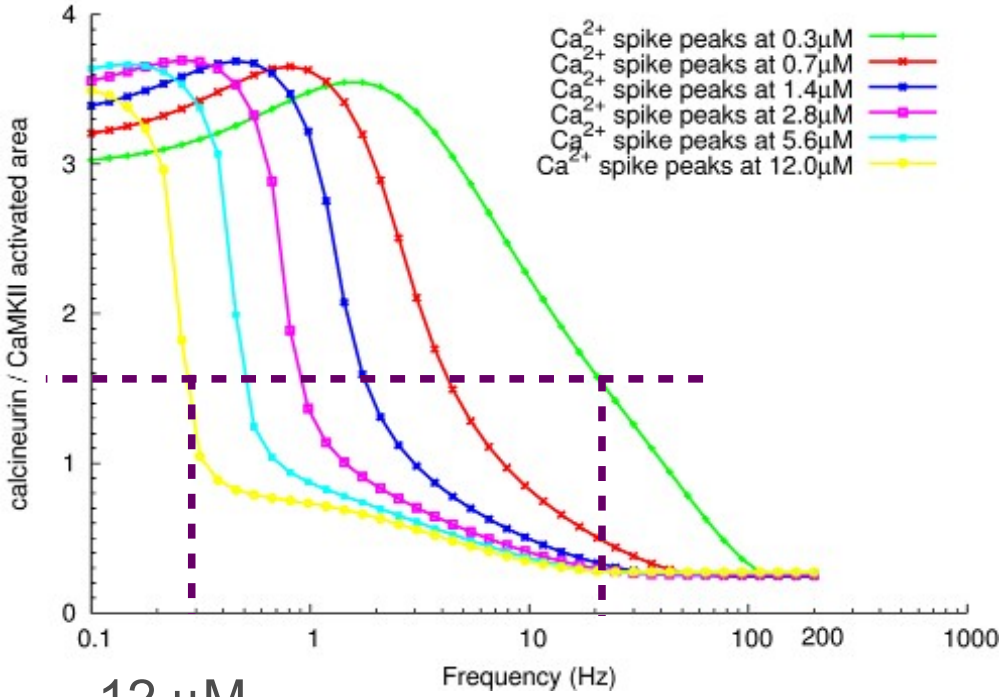


Effect of calcium duration and amount



180 spikes = 3 Hz

10 spikes = 70 Hz

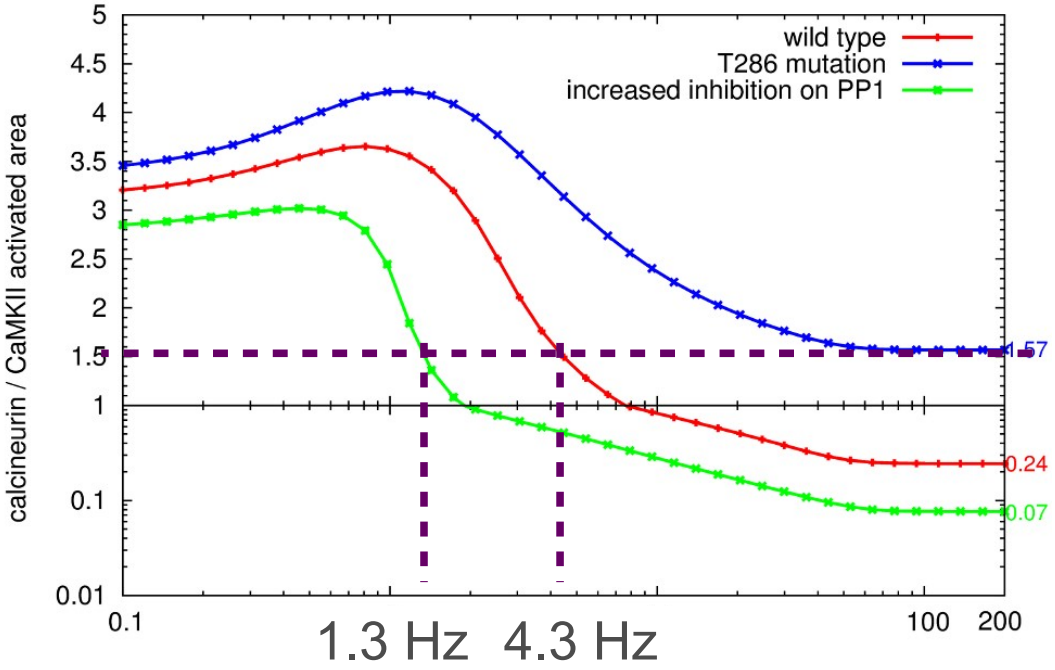


12 µM = 0.3 Hz

0.3 µM = 20 Hz

Prolonged or intense signals decrease Θm: 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

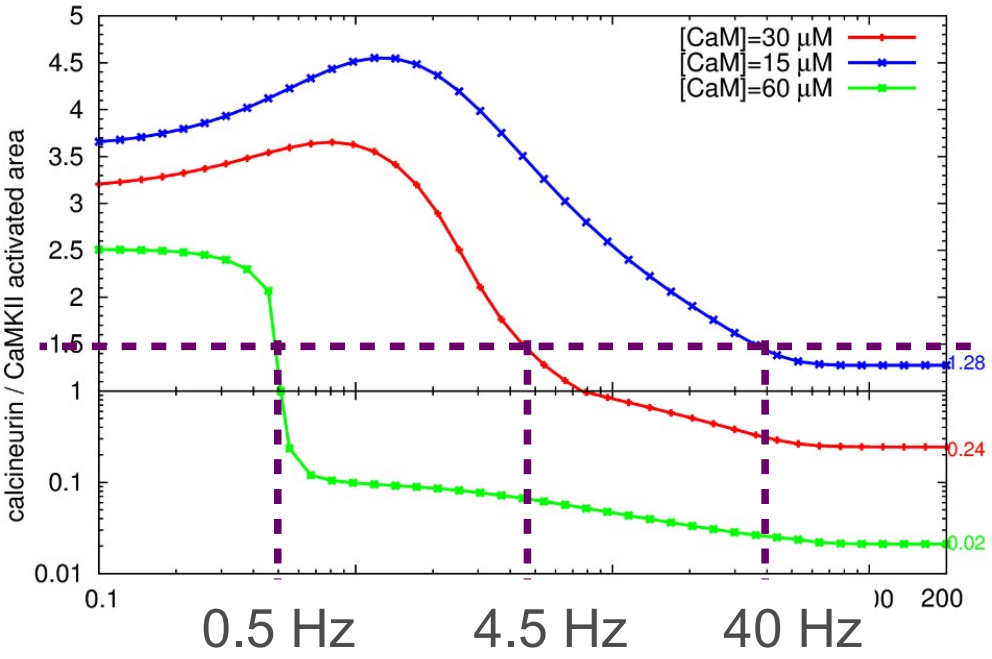
Giese et al (1998) *Science,* 279:870-873

Lower deactivation of CaMKII

Competition for CaM, and CaN wins

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

got it completely wrong)



Summary of part 2

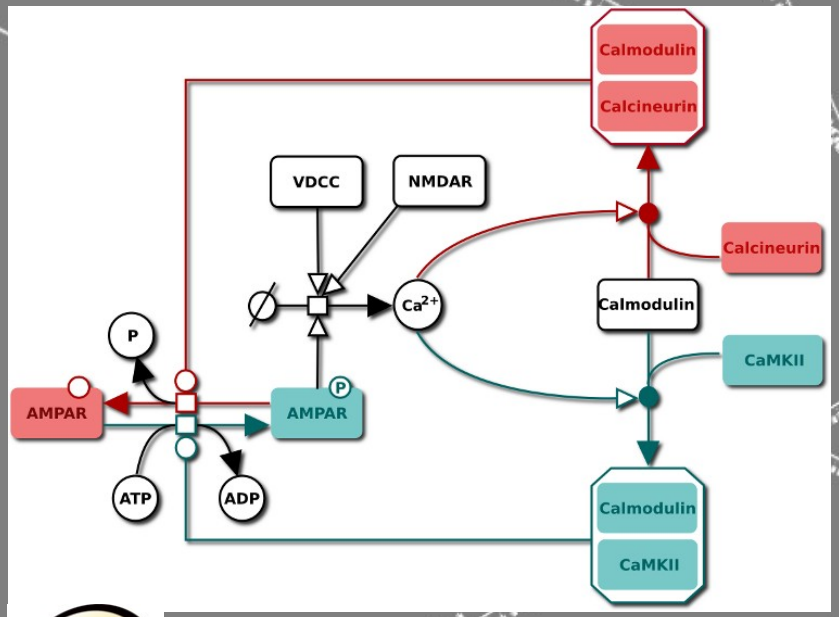
Allosteric stabilisation by targets triggers bistable CaM response to calcium. Above a certain frequency, CaM activation lasts longer than the initial signal.

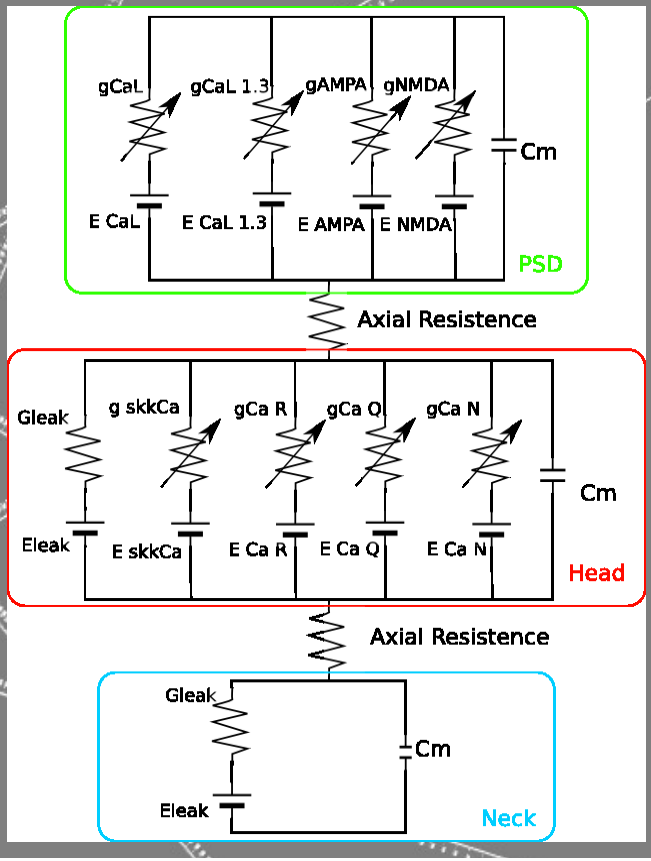
Calcium signals do not choose between CaN and CaMKII, *BOTH* enzymes are activated at *ALL* frequencies. The ratio of activity changes.

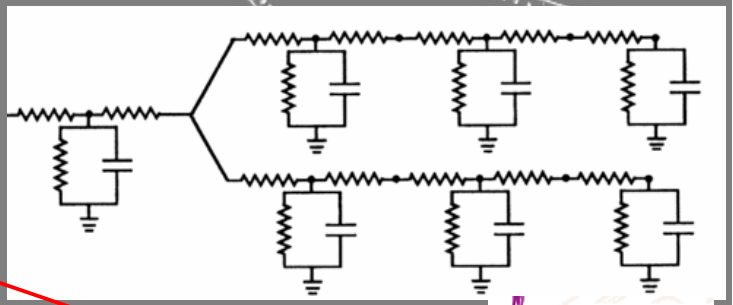
The frequency at which a synapse switches from a depression to a potentiation mode is not an intrinsic property of the synapse, but a dynamical one that depends on the length and amplitude of stimulations.

Modifications of topology, parameters and initial conditions affect both response intensity and threshold frequency. Some mutants can't have positive plasticity for any stimulation. [CaM] decides of the balance CaN/KII

Whole cell: electro-biochemical models



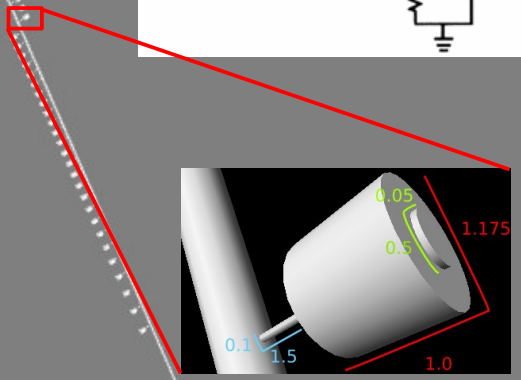




1504 spines

4761 compartments

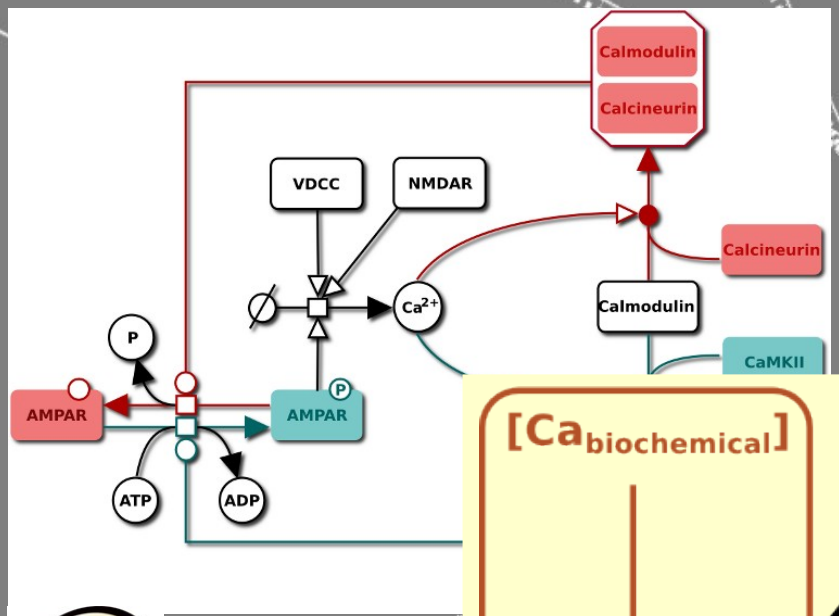
16362 channels

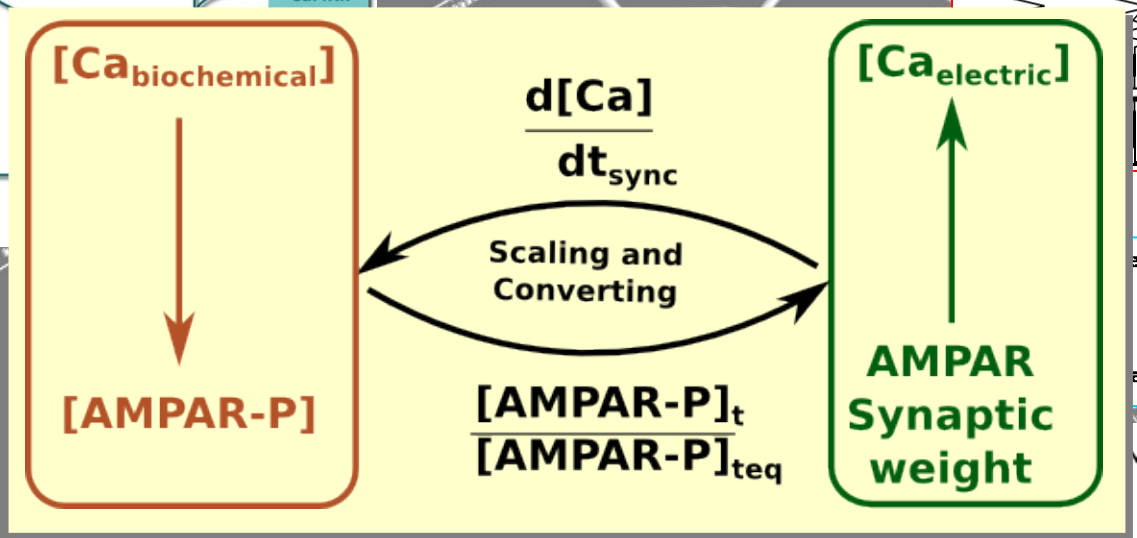


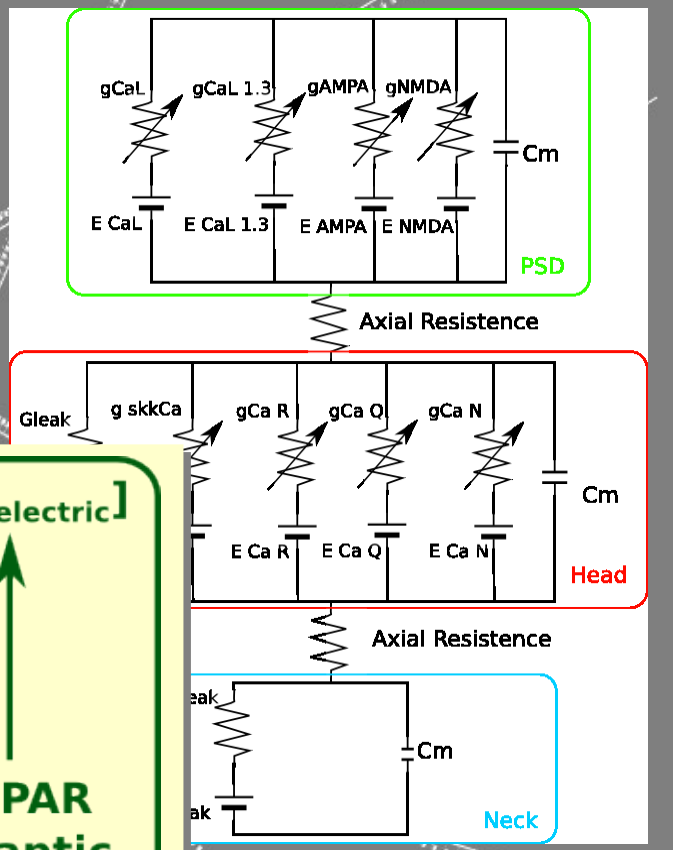
Mattioni M, Cohen U, Le Novère N (2012)
Frontiers Neuroinfo, 6:20

Mattioni M, Le Novère N (2013)
PLoS ONE, 8(7): e66811

Whole cell: electro-biochemical models







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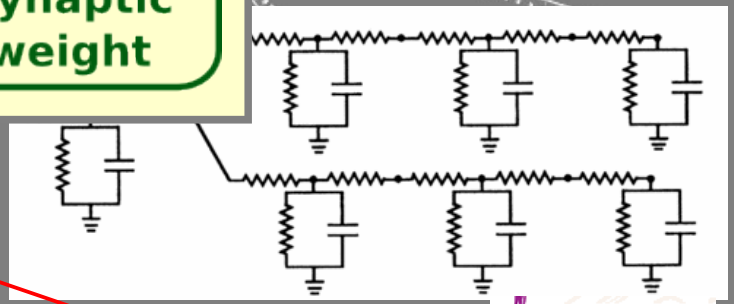
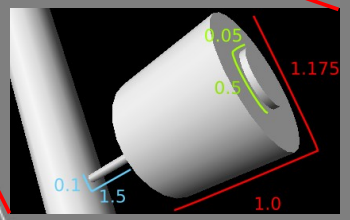


NEURON



Mattioni M, Cohen U, Le Novère N (2012)
Frontiers Neuroinfo, 6:20

Mattioni M, Le Novère N (2013)
PLoS ONE, 8(7): e66811

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

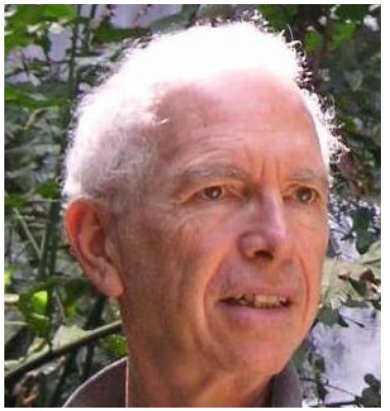
Melanie Stefan

Lu Li

EMBL

wellcome trust

Michele Mattioni



Stuart Edelstein

BBSRC bioscience for the future

elixir Data for Life

SEVENTH FRAMEWORK PROGRAMME

imi Innovative Medicines Initiative

NEDO

NIGMS