Allosteric calcium sensors in synaptic plasticity

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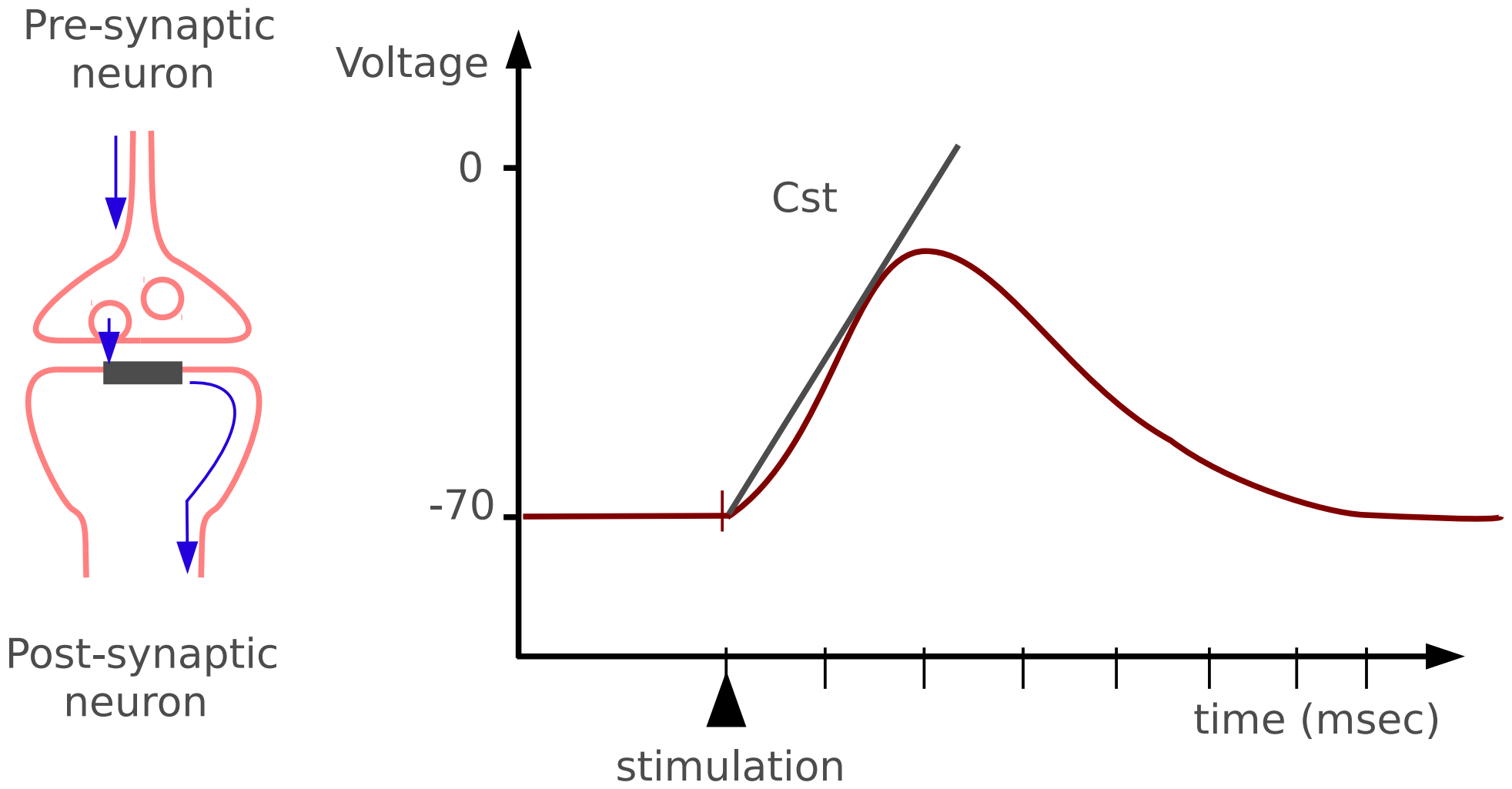
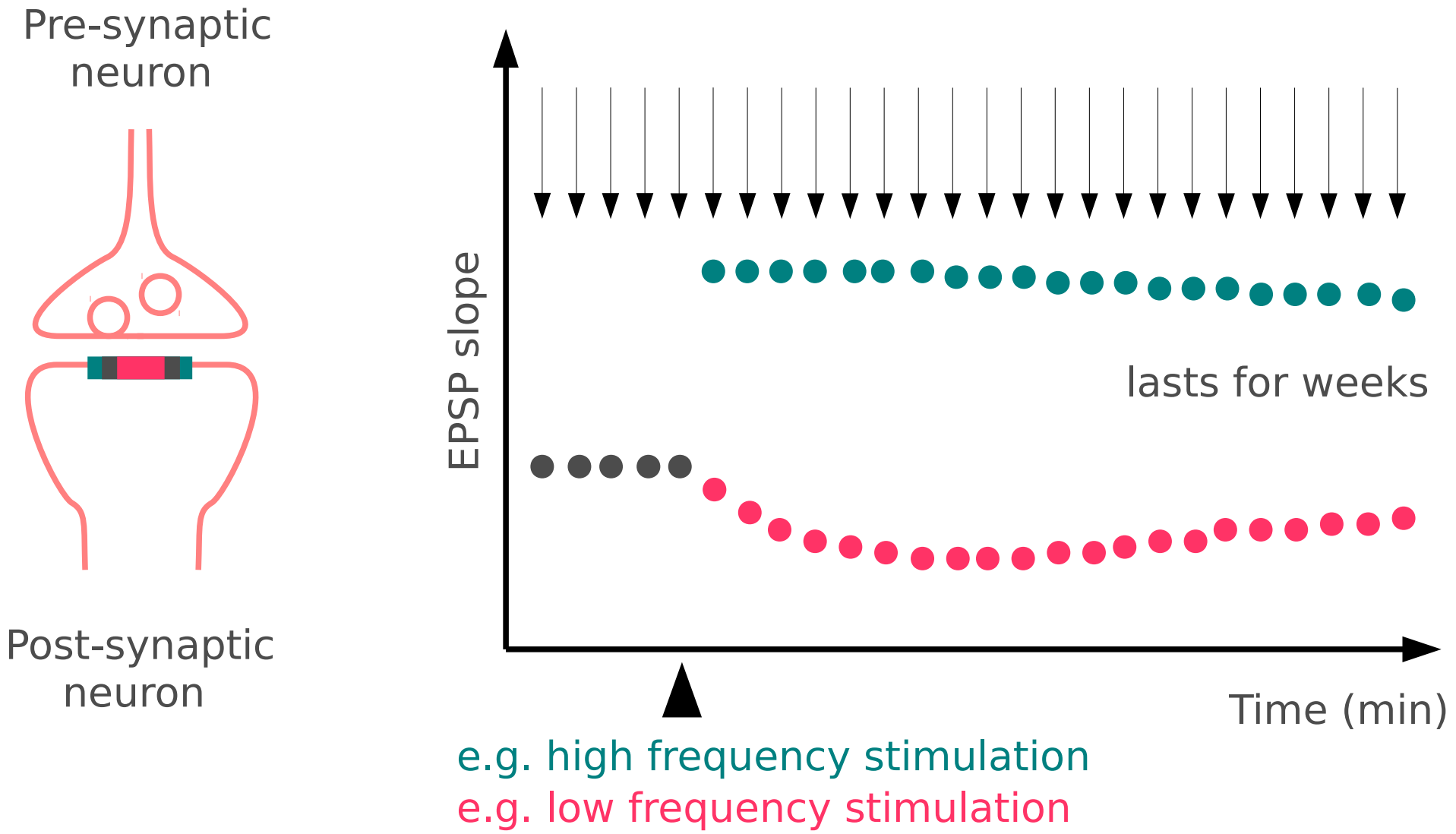
http://lenoverelab.org

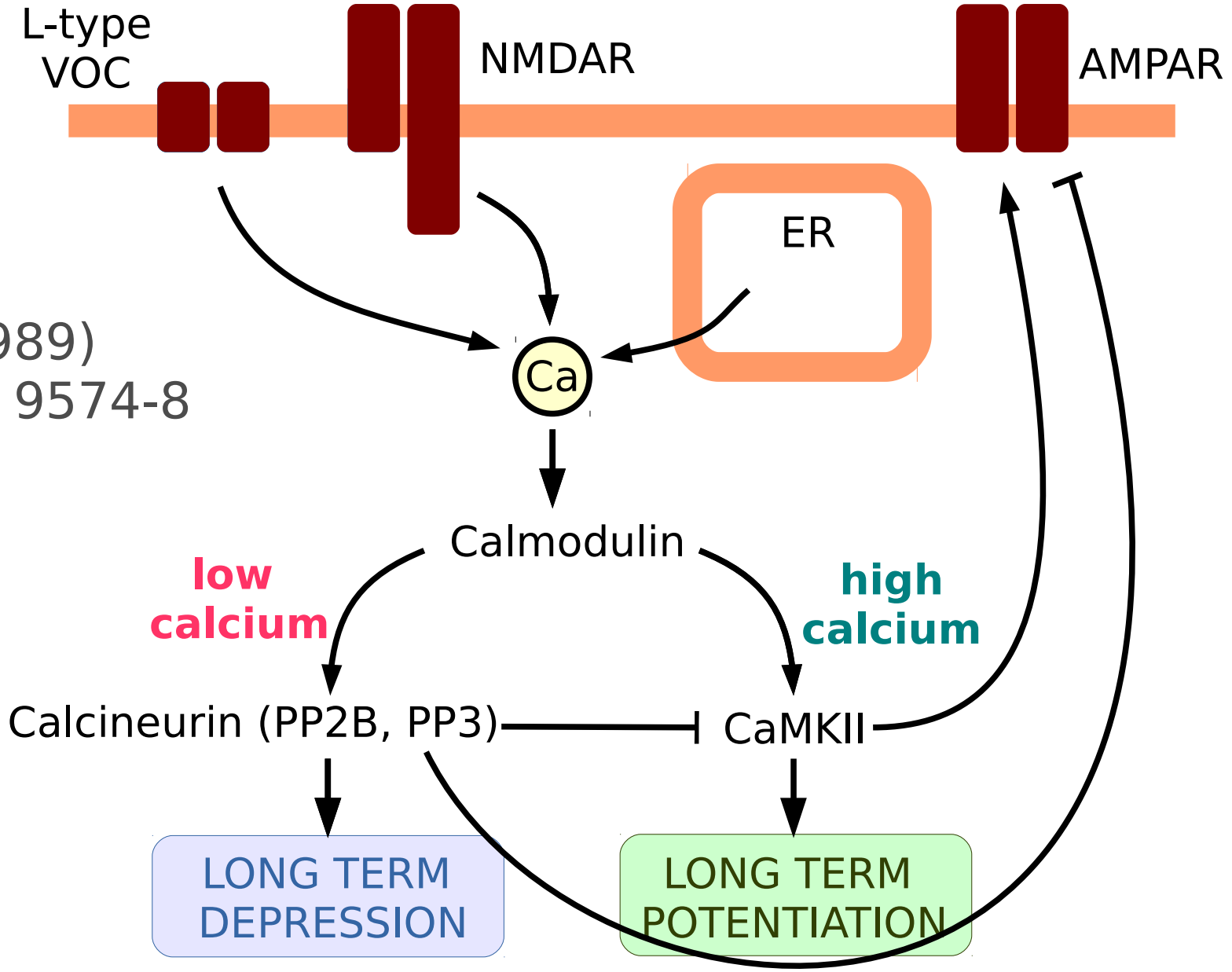
Excitatory post-synaptic potential



Bidirectional synaptic plasticity

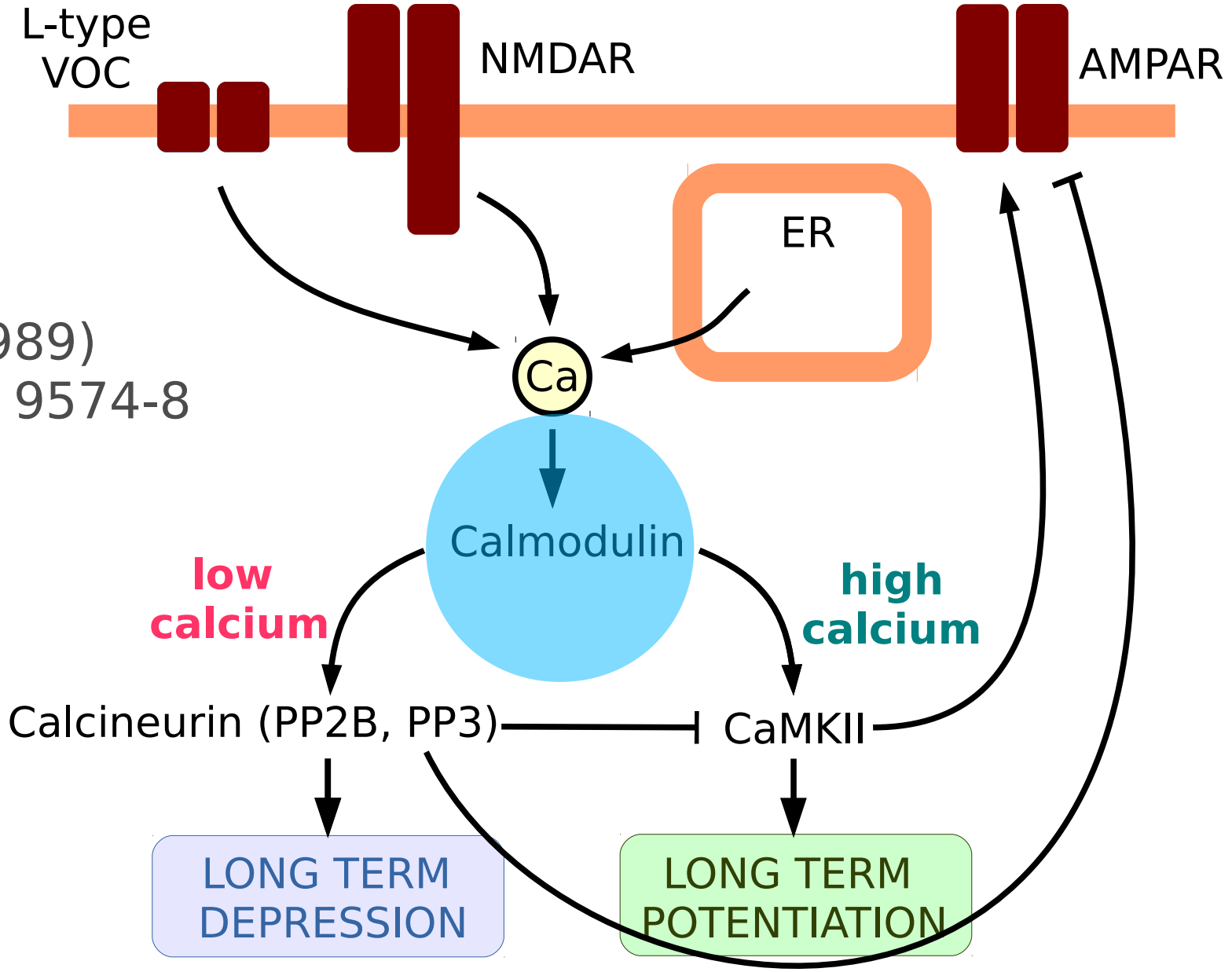


Calmodulin, the memory switch



Lisman (1989)
PNAS, 86: 9574-8

Calmodulin, the memory switch



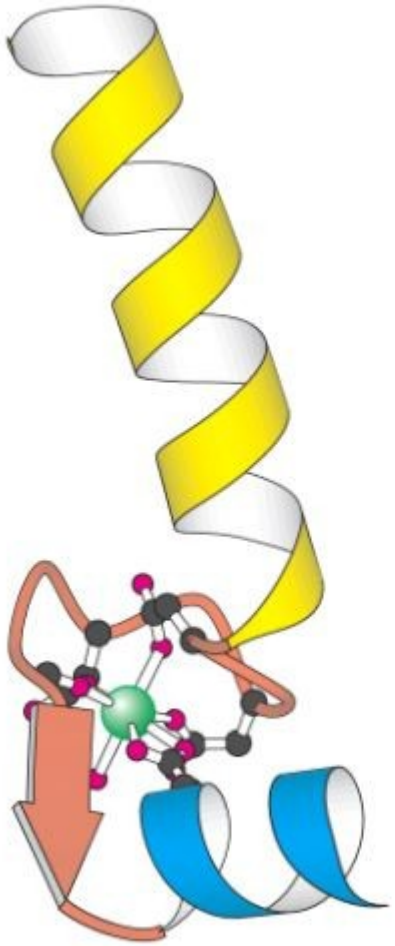
Lisman (1989)
PNAS, 86: 9574-8

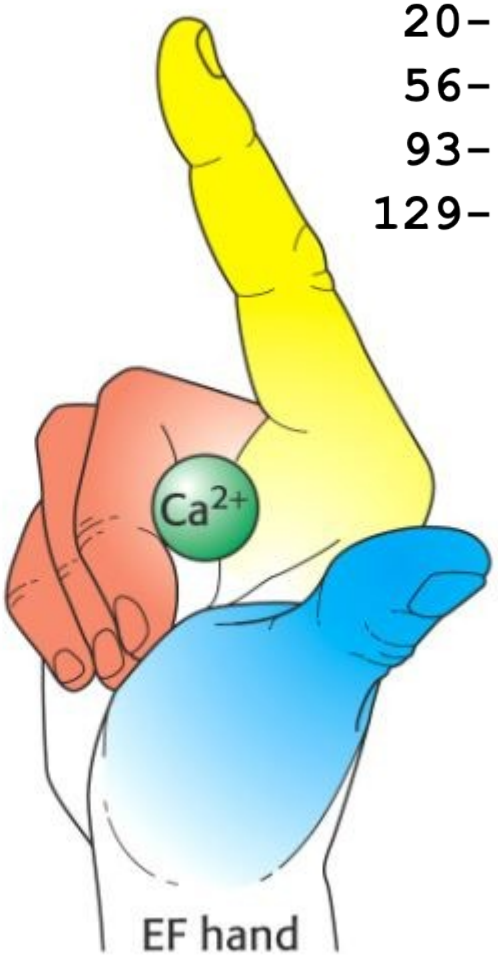
Modelling the behaviour of molecules

Modelling the behaviour of
a system of molecules

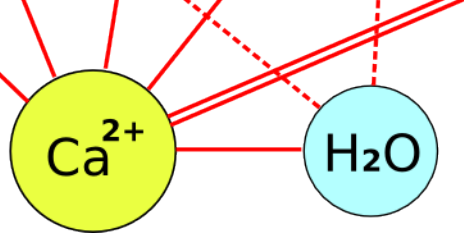
Modelling the behaviour of a cell

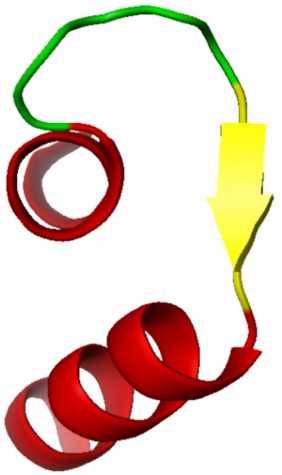
Structure of a Calmodulin Ca^{2+} binding domain

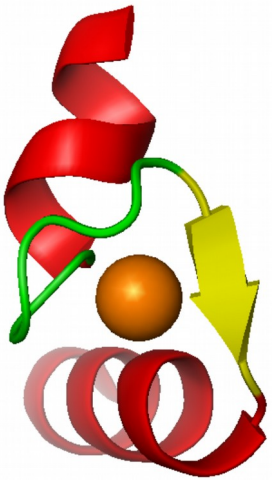




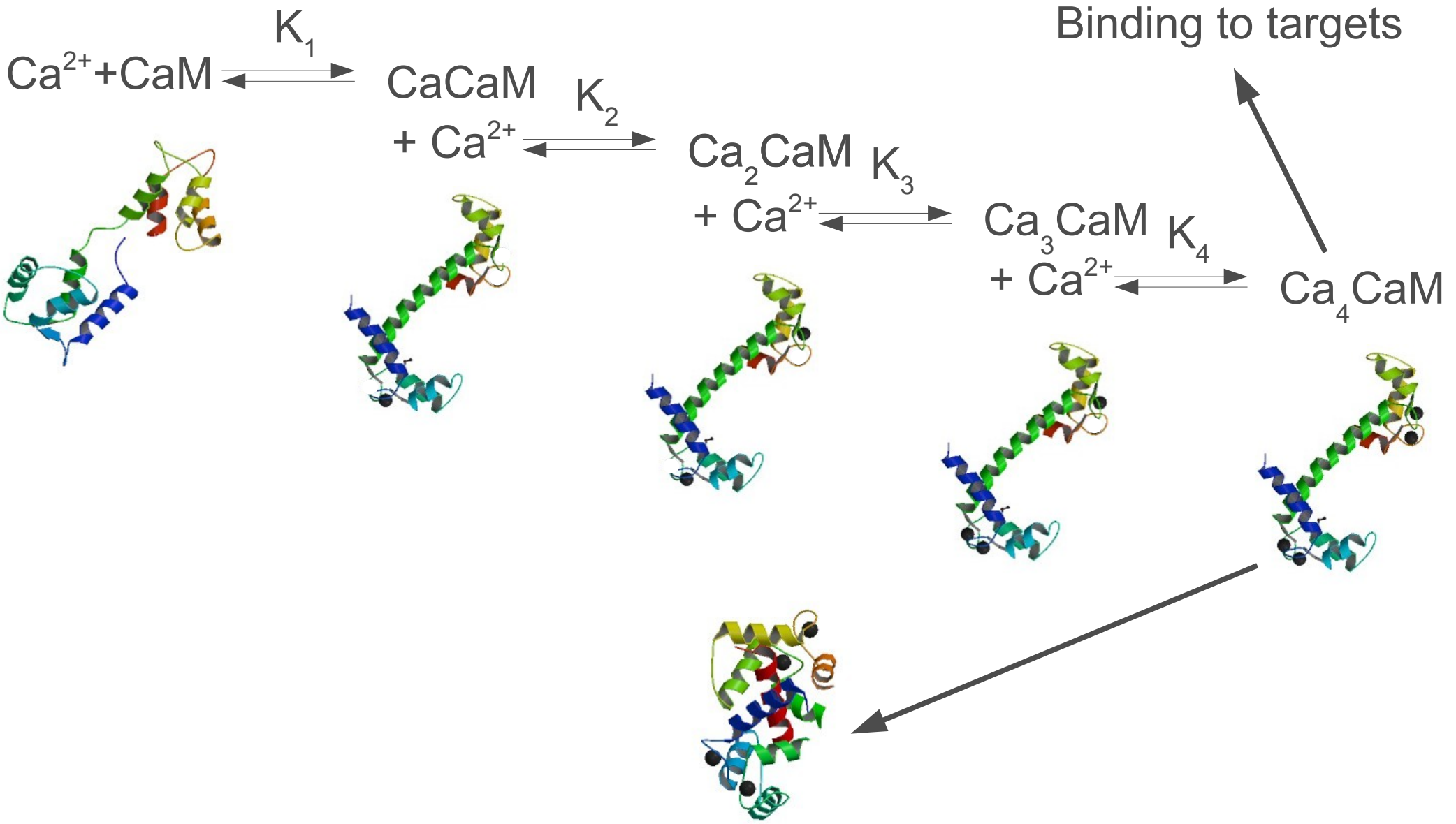
	X	Y	Z	-Y	-X	-Z	
20-	D	K	D	G	D	G	T	I	T	T	K	E	- 31
56-	D	A	D	G	N	G	T	I	D	F	P	E	- 67
93-	D	K	D	G	N	G	Y	I	S	A	A	E	-104
129-	D	I	D	G	D	G	Q	V	N	Y	E	E	-140



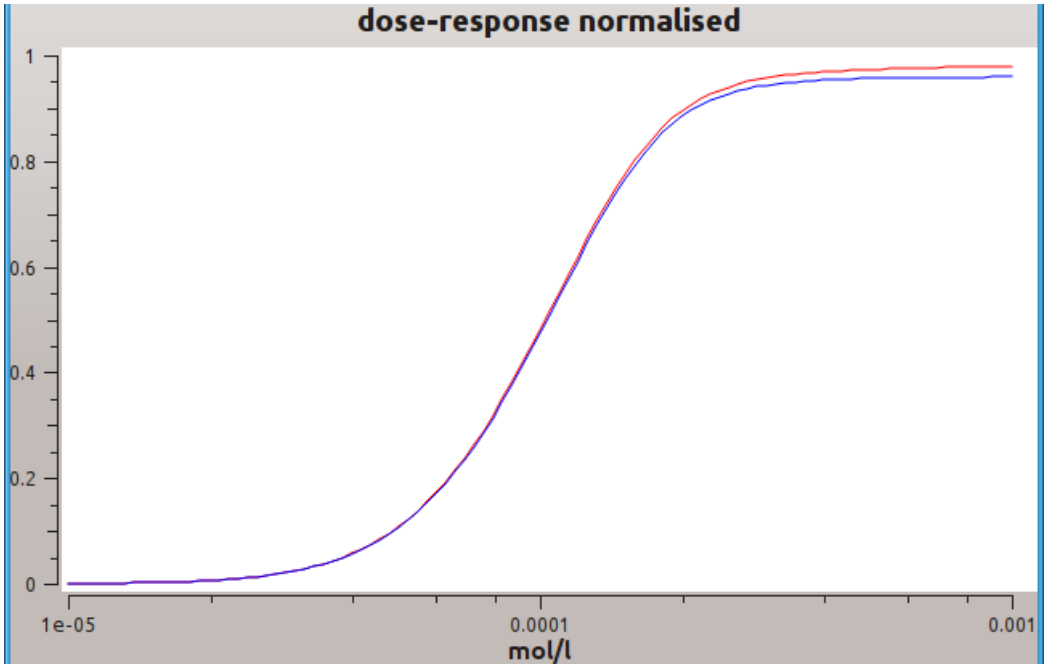




The naive view

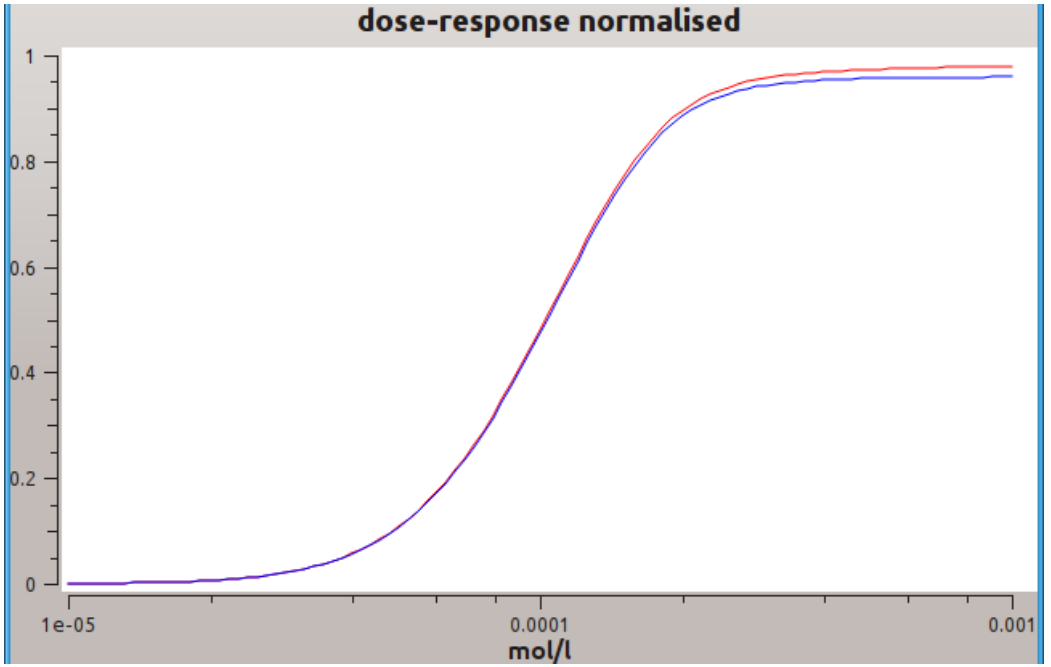


That does not work ...



[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

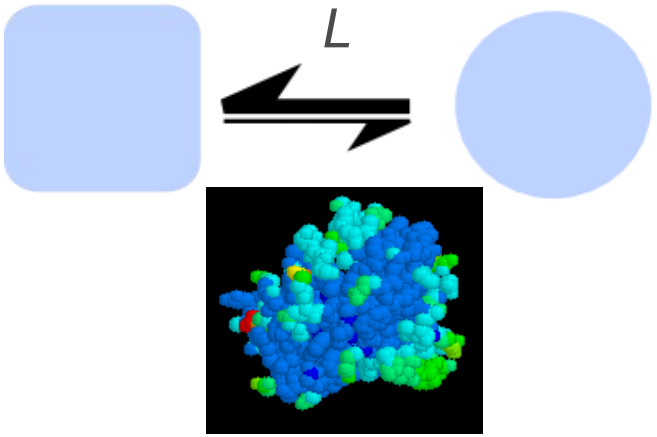
- Calmodulin can activate calcineurin with 3 Ca^{2+} (Kincaid and Vaughan (1986). *PNAS*, 83: 1193-1197)
- Calmodulin can bind CaMKII with 2 Ca^{2+} (Shifman et al (2006). *PNAS*, 103: 13968-13973)
- Calmodulin affinity for calcium increases once bound to CaMKII (Shifman et al (2006) [but many previous reports on other targets: e.g. Burger et al (1983). *JBC*, 258: 14733-14739 ; Olwin et (1984). *JBC* 259: 10949-10955])

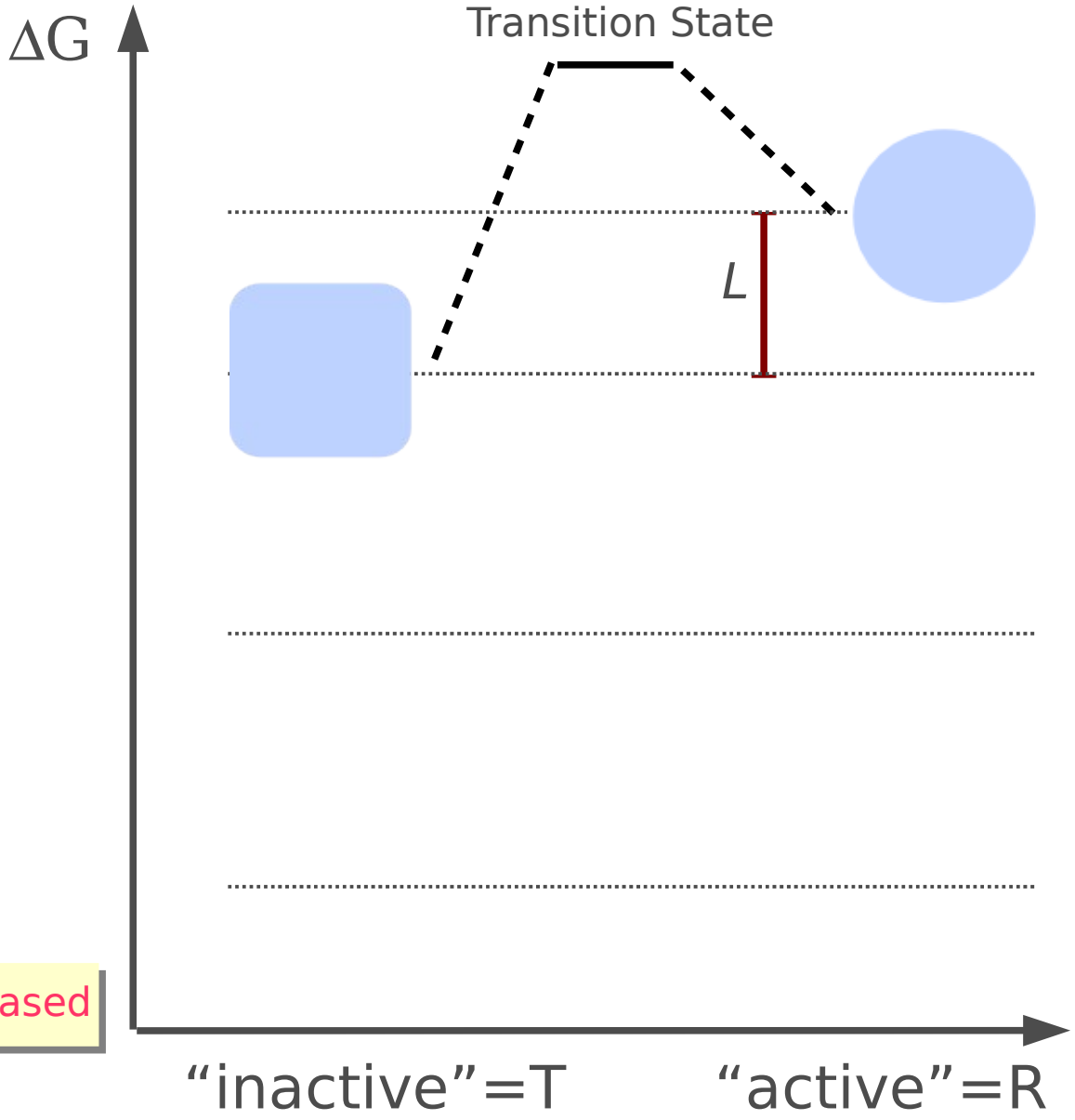
Monod, Wyman, Changeux (1965)

On the nature of allosteric transitions: a plausible model

J Mol Biol, 12: 88-118

1 Modulation of thermal equilibria ≠ induced-fit

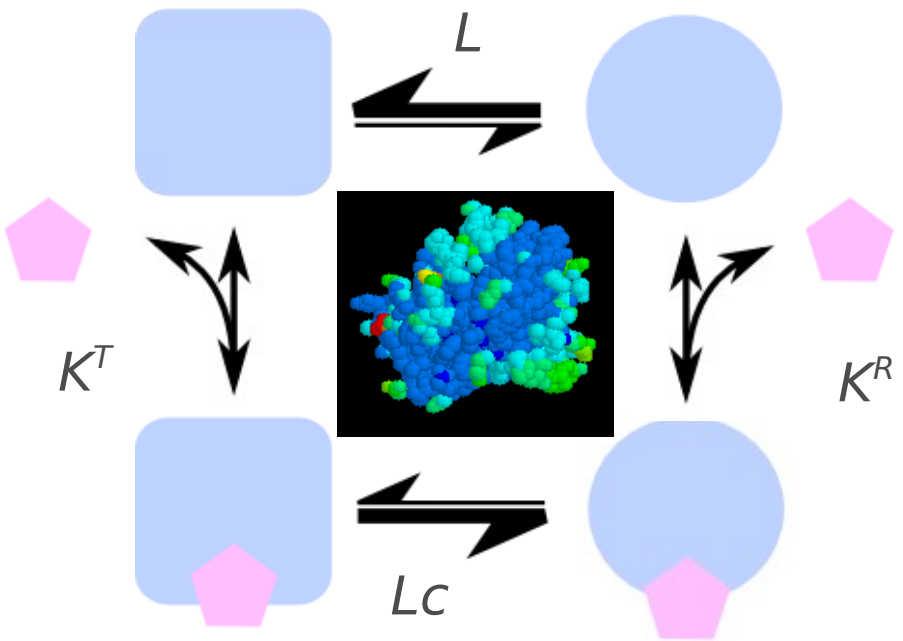




$$L = \frac{[T_0]}{[R_0]}$$

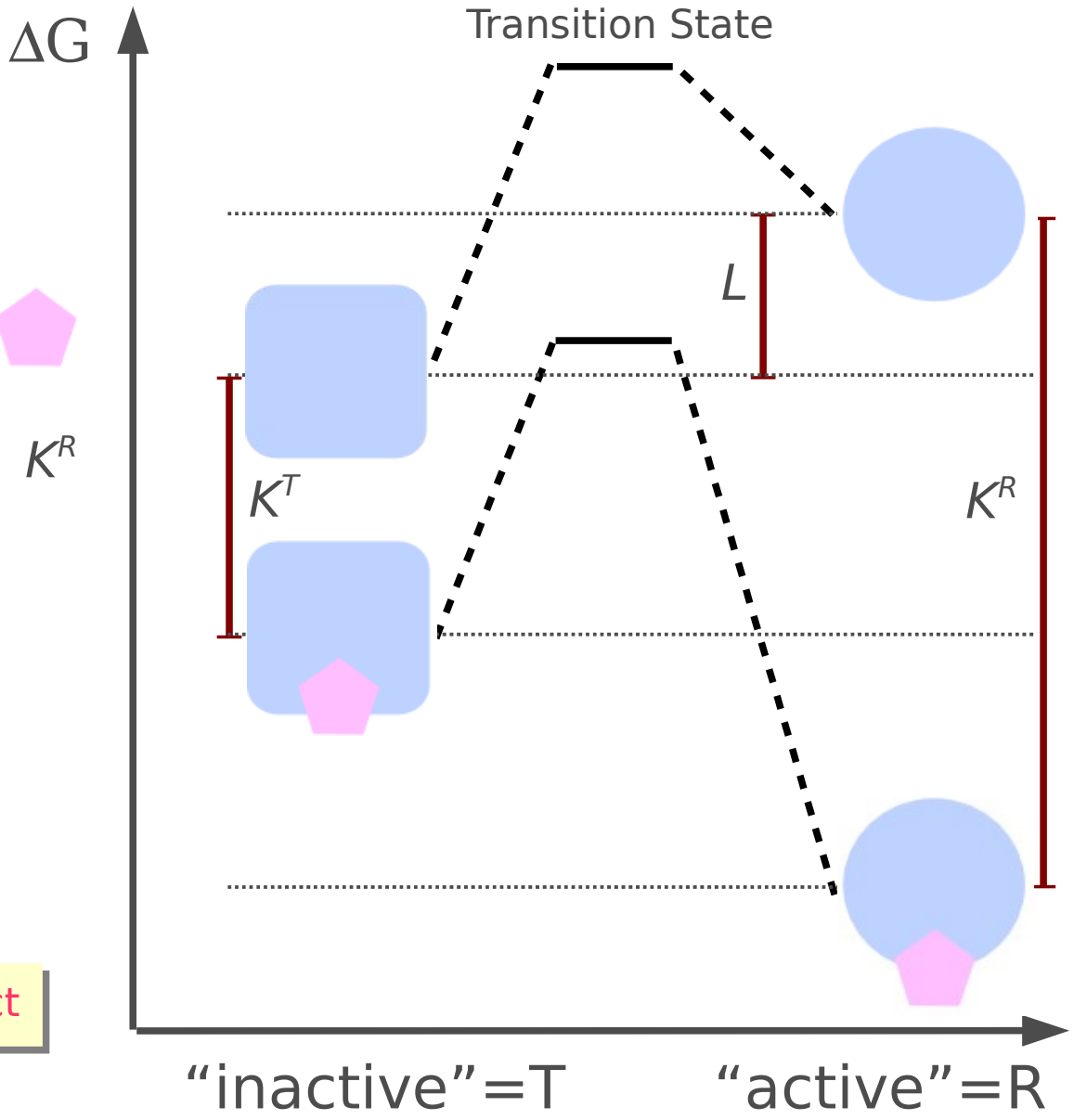
$L \gg 1 \Rightarrow$ Equilibrium strongly biased

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



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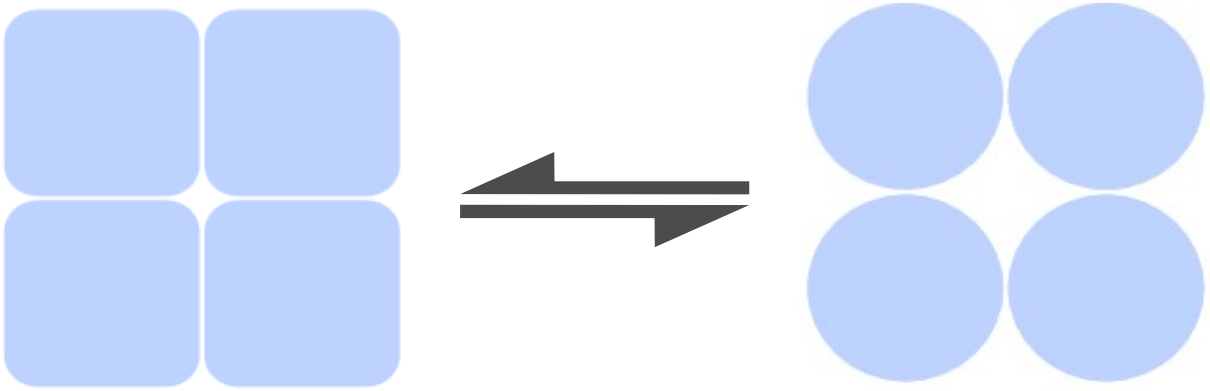
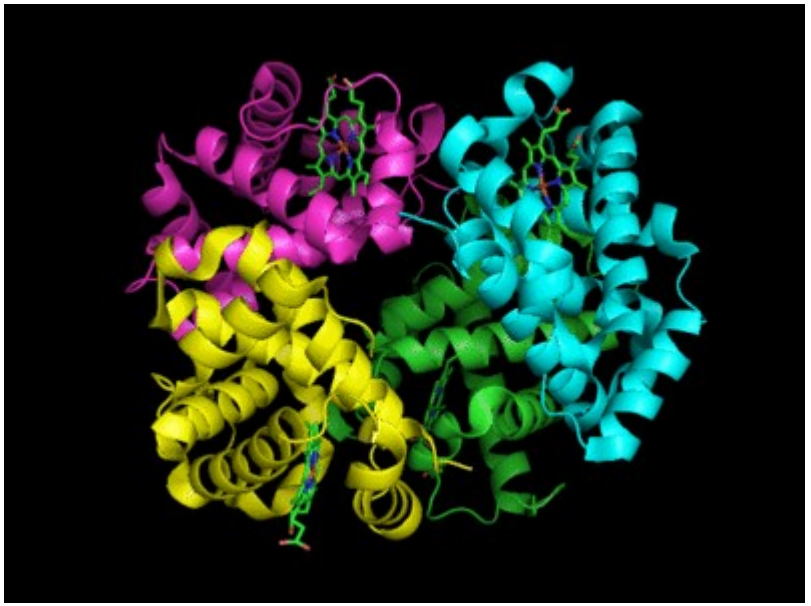
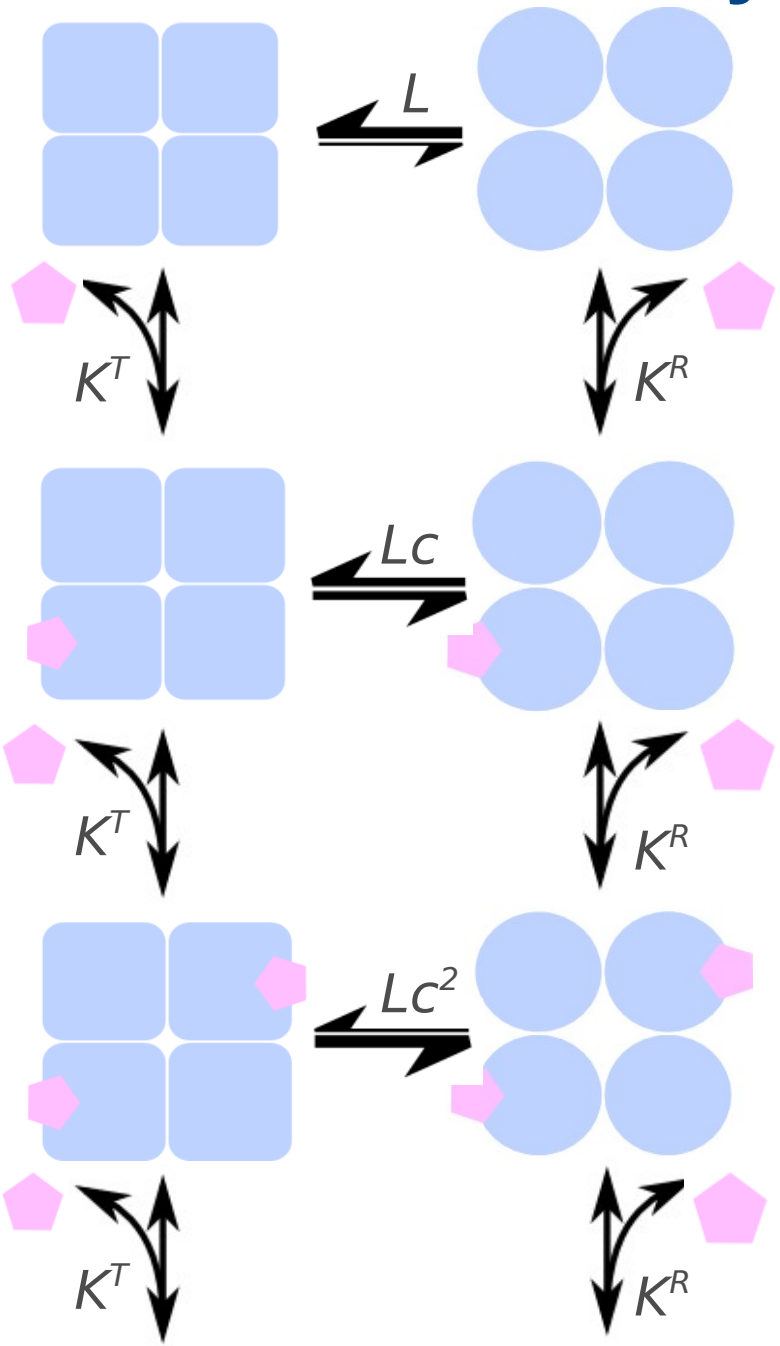
2 Concerted transitions ≠ sequential model





Monod-Wyman-Changeux model



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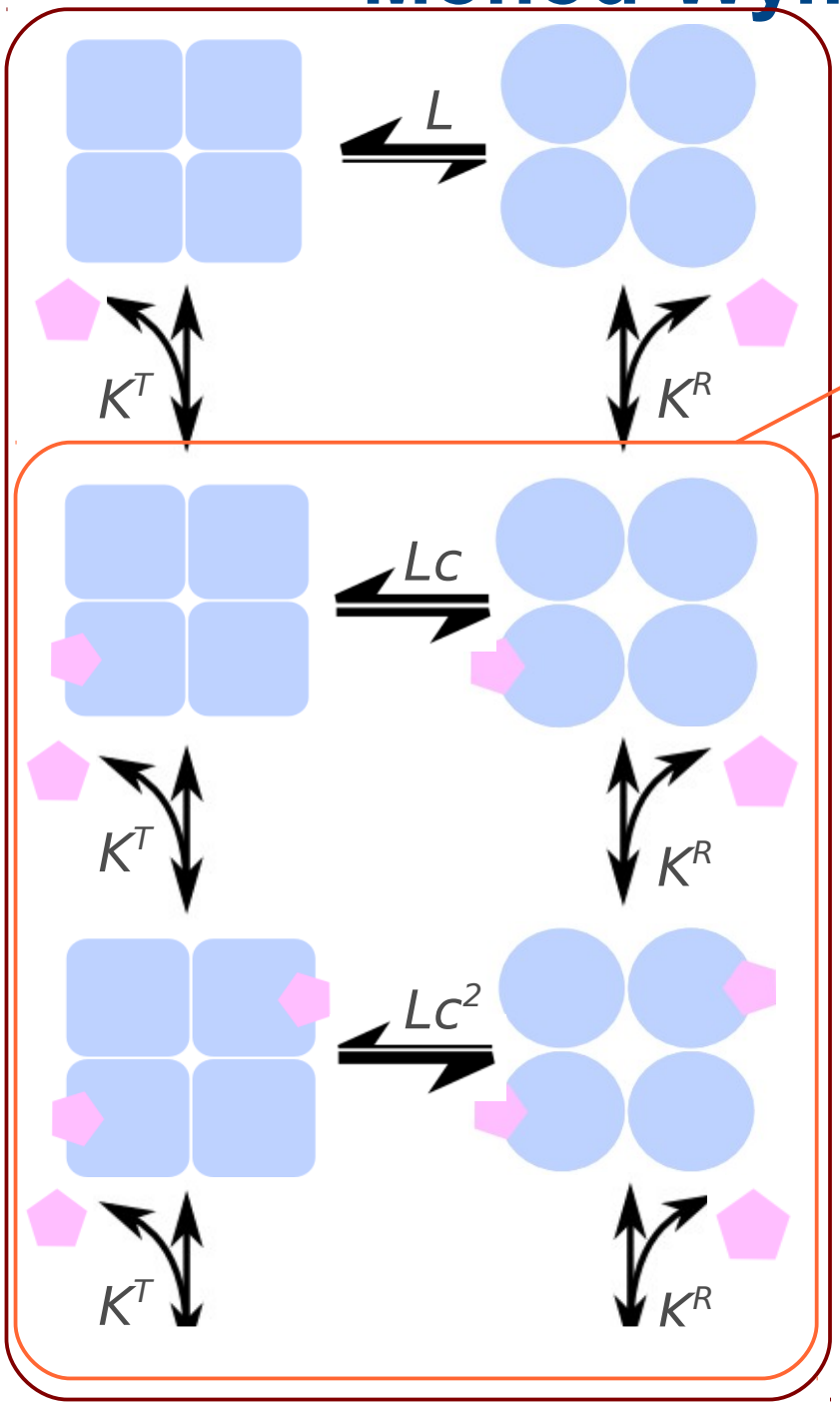
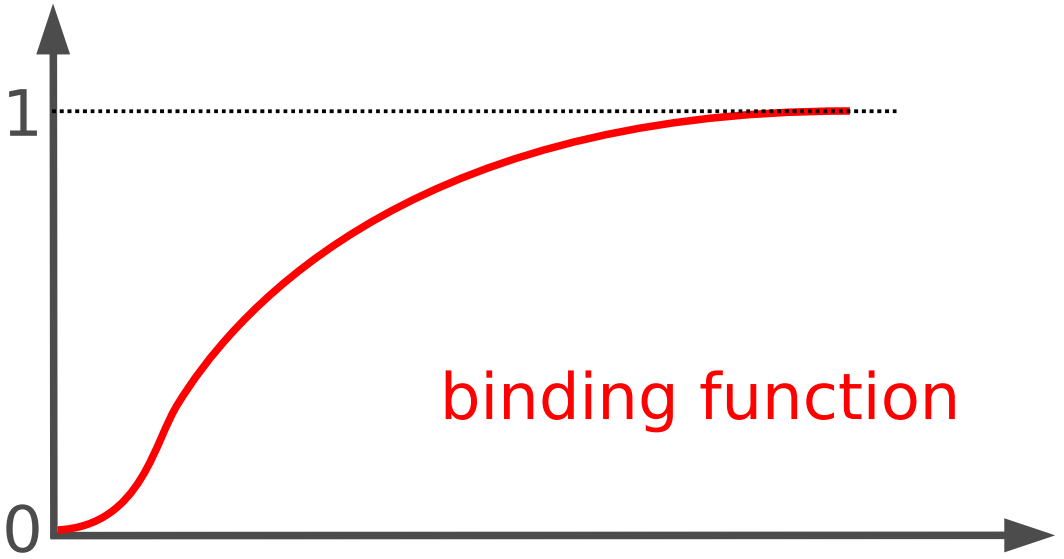
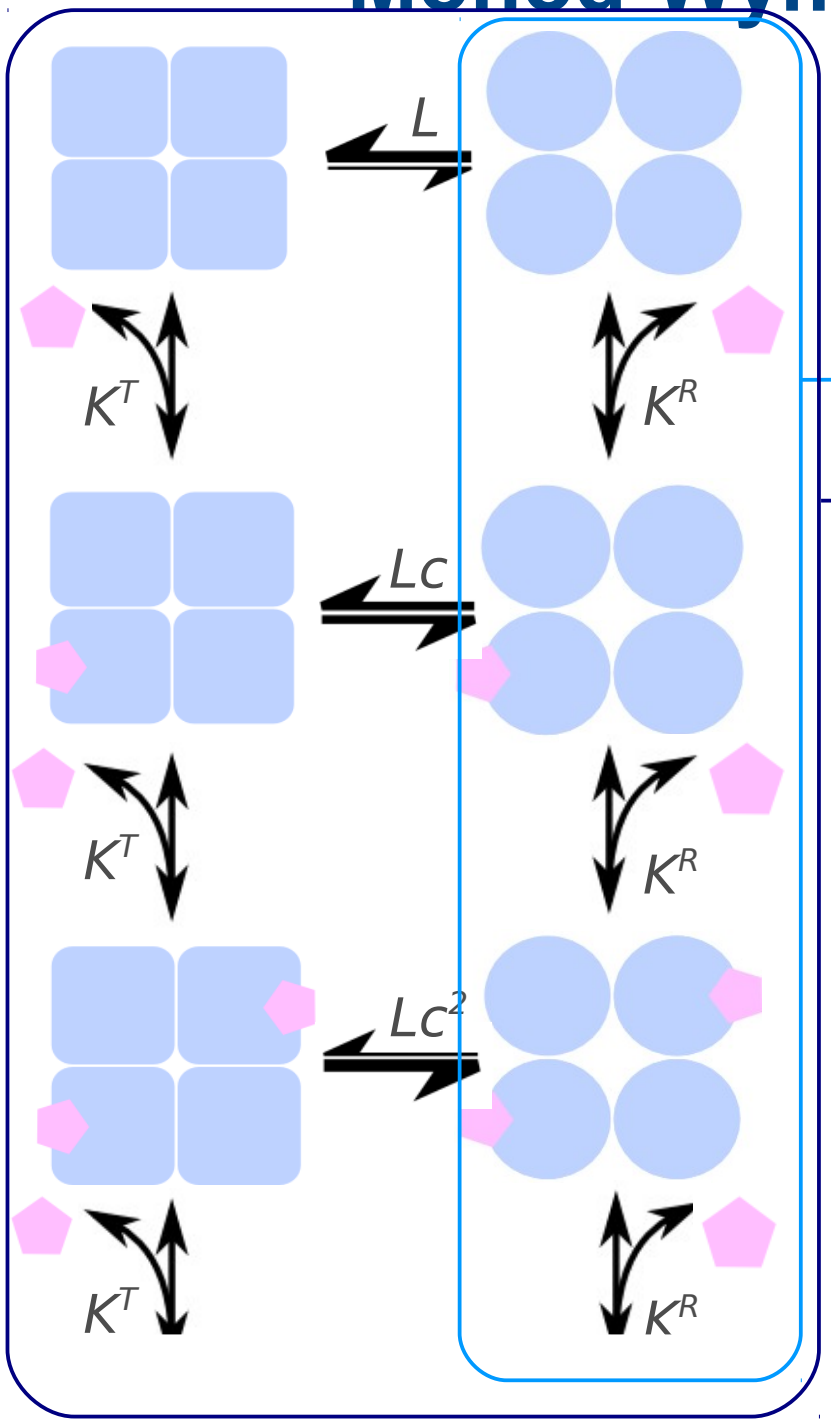


$$\frac{\text{this}}{\text{that}} = \bar{Y}$$

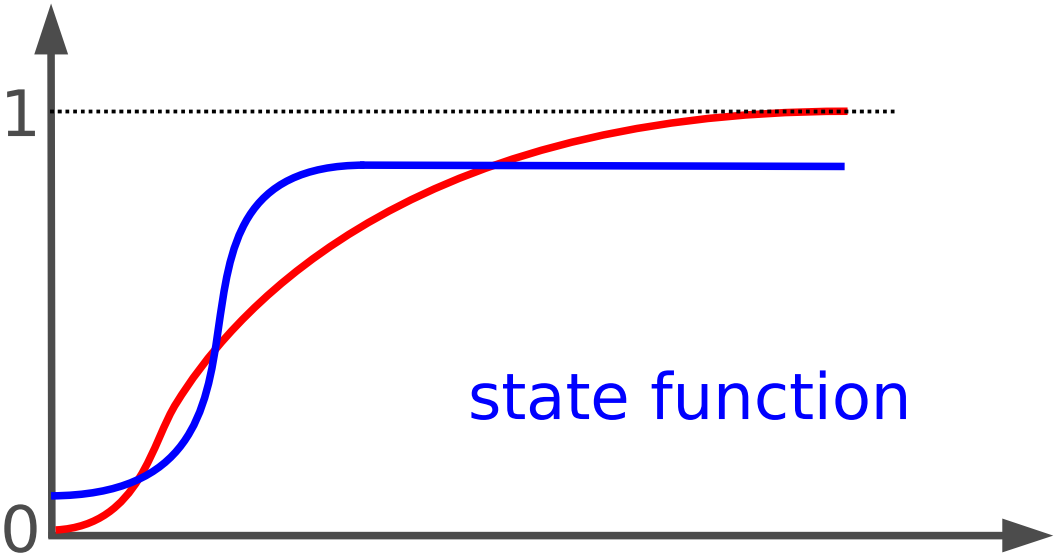


Monod-Wyman-Changeux model



$\bar{Y}$

this
—— = $\bar{R}$
that



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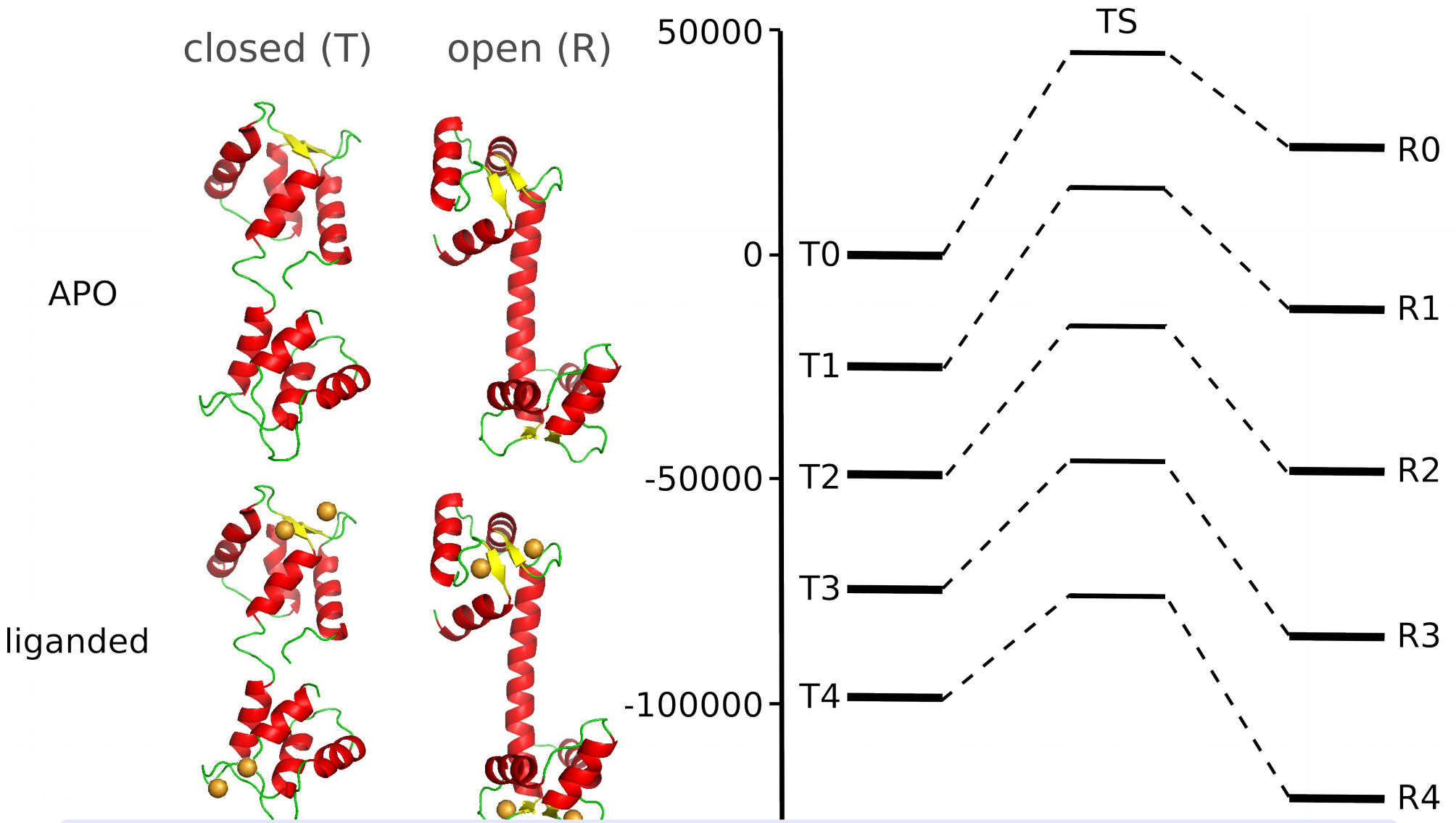
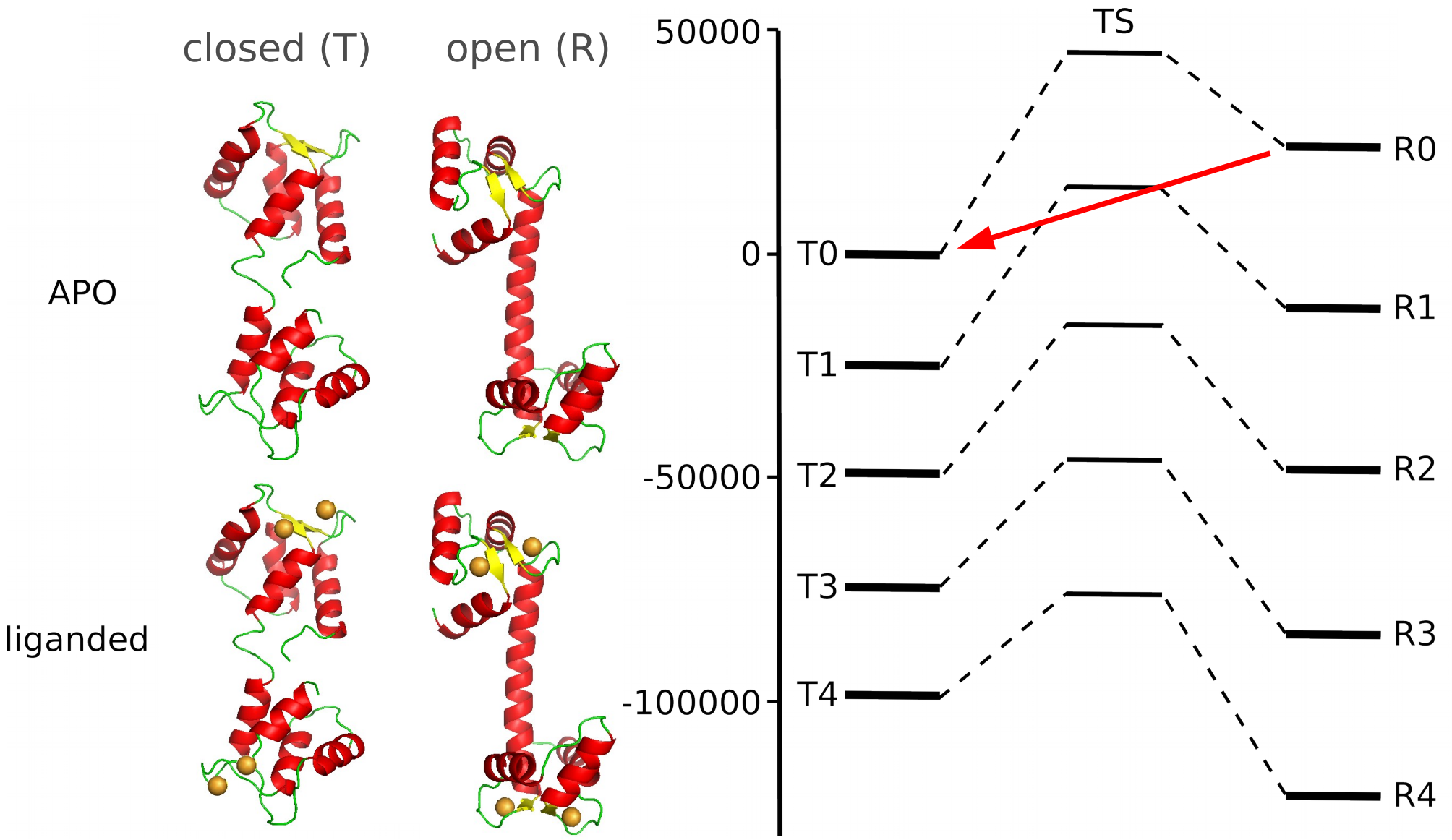
Allosteric model of Calmodulin activation



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

Allosteric model of Calmodulin activation



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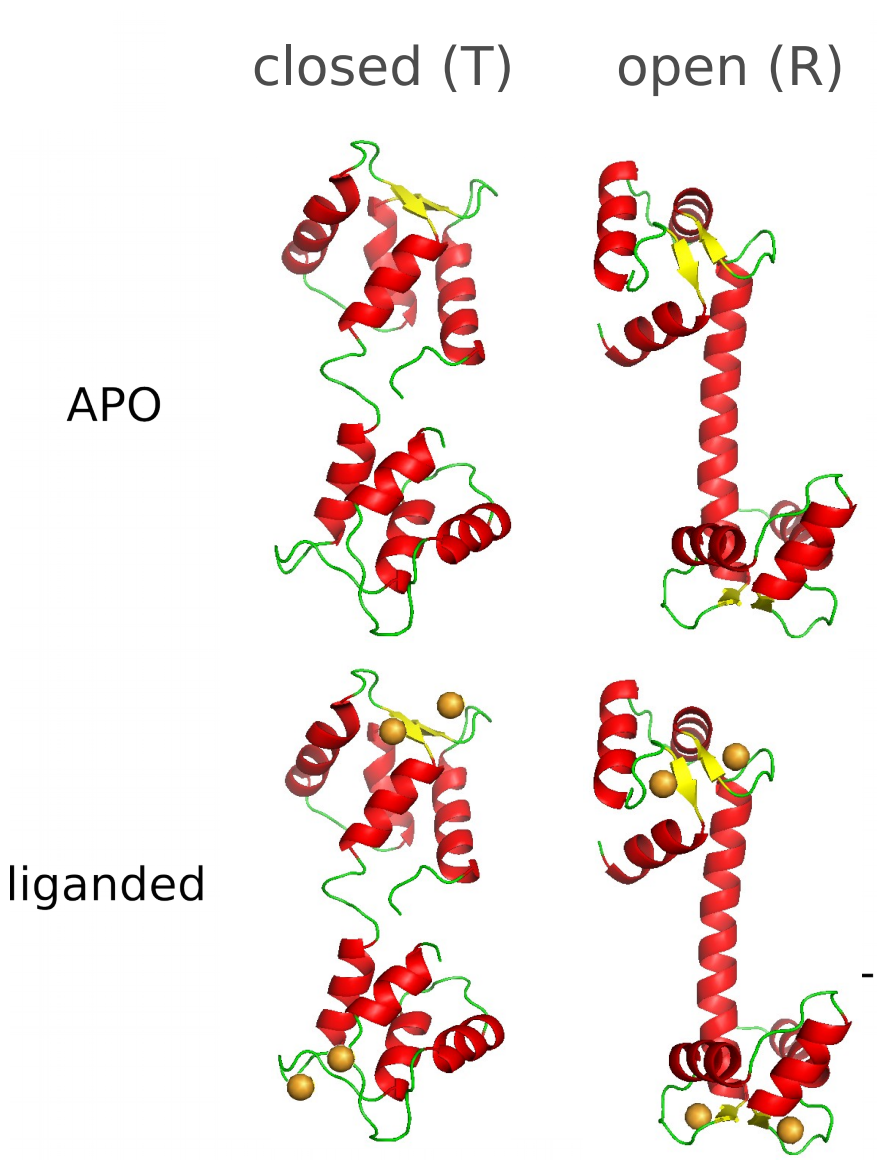

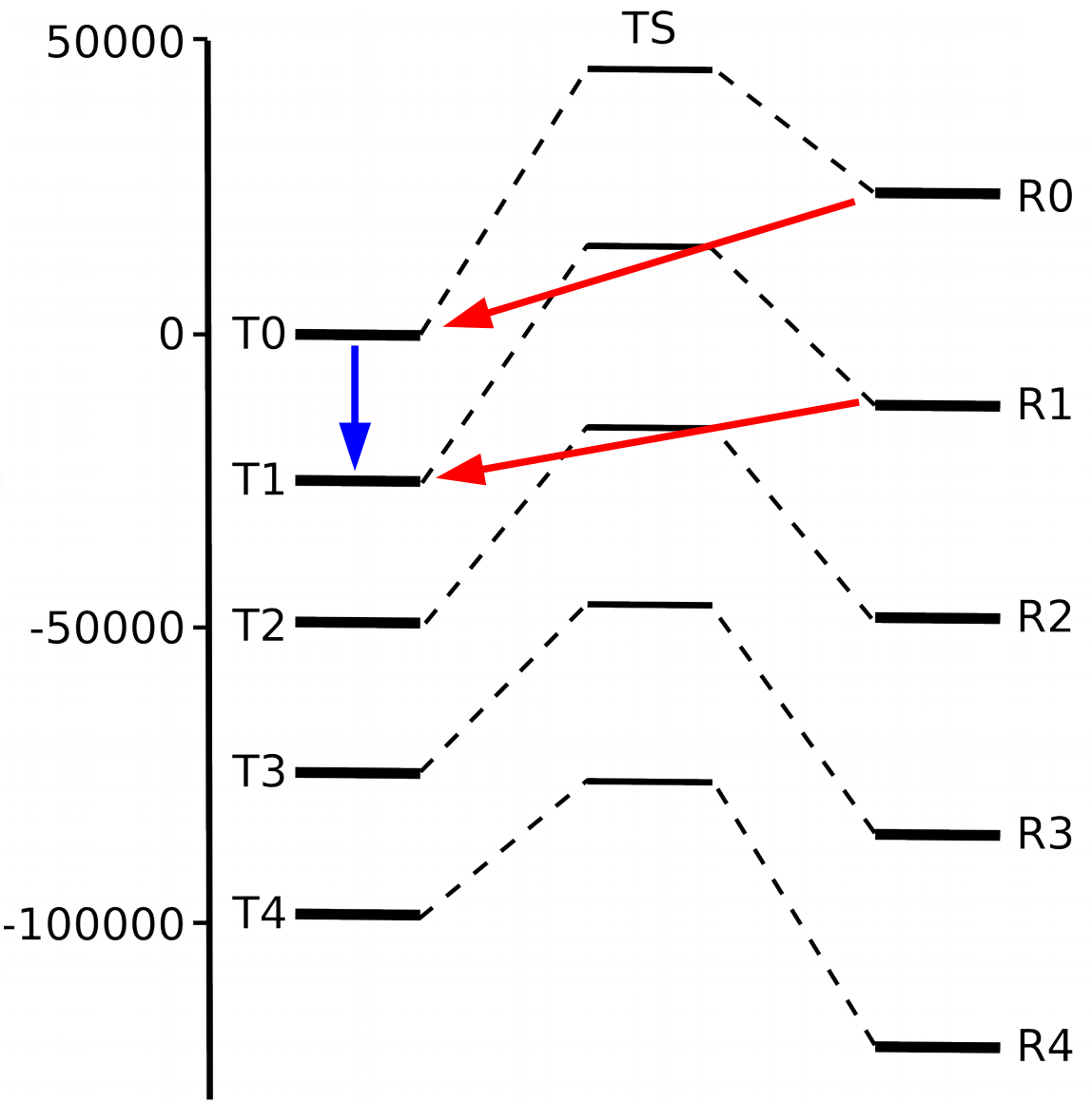

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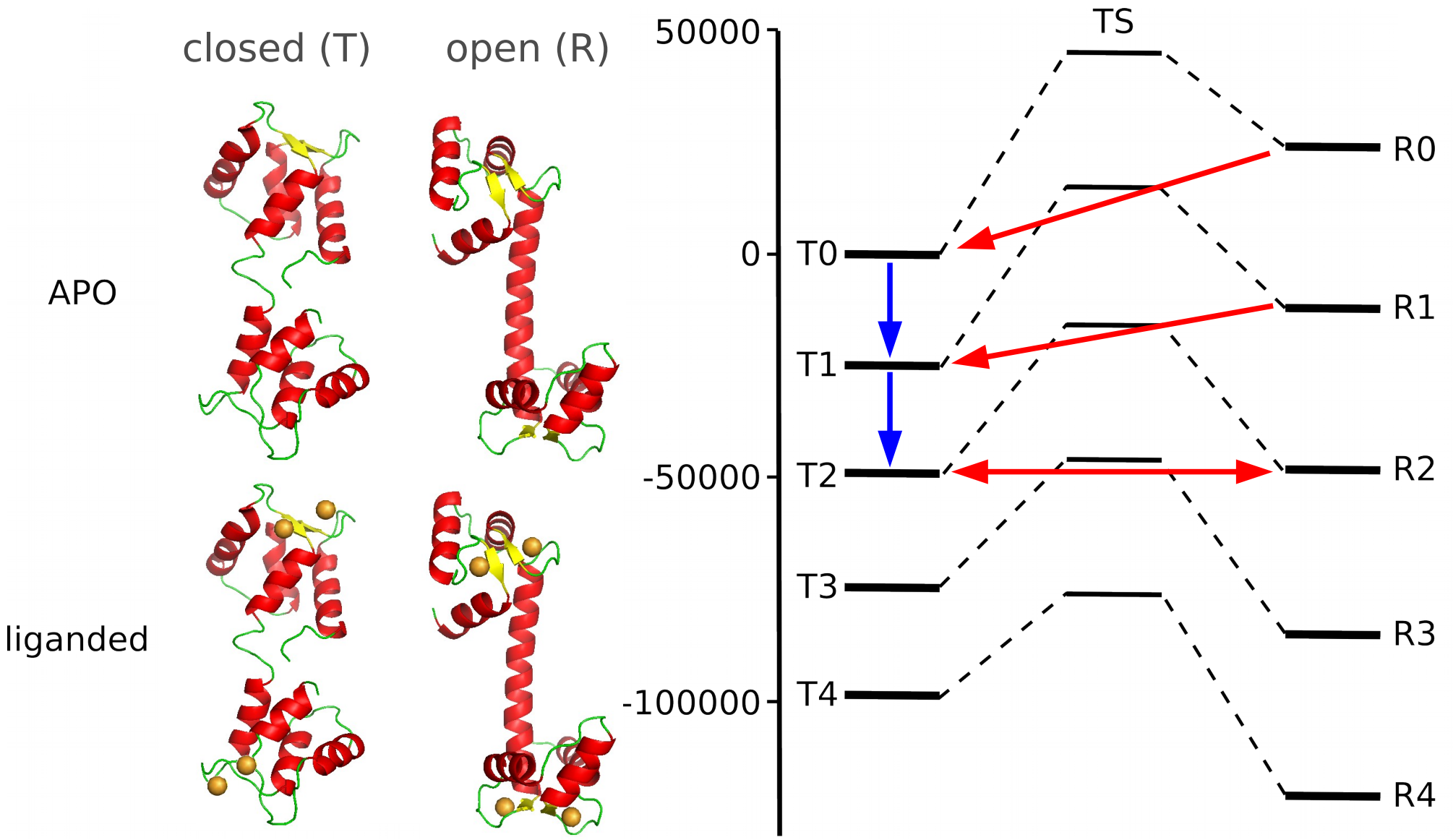

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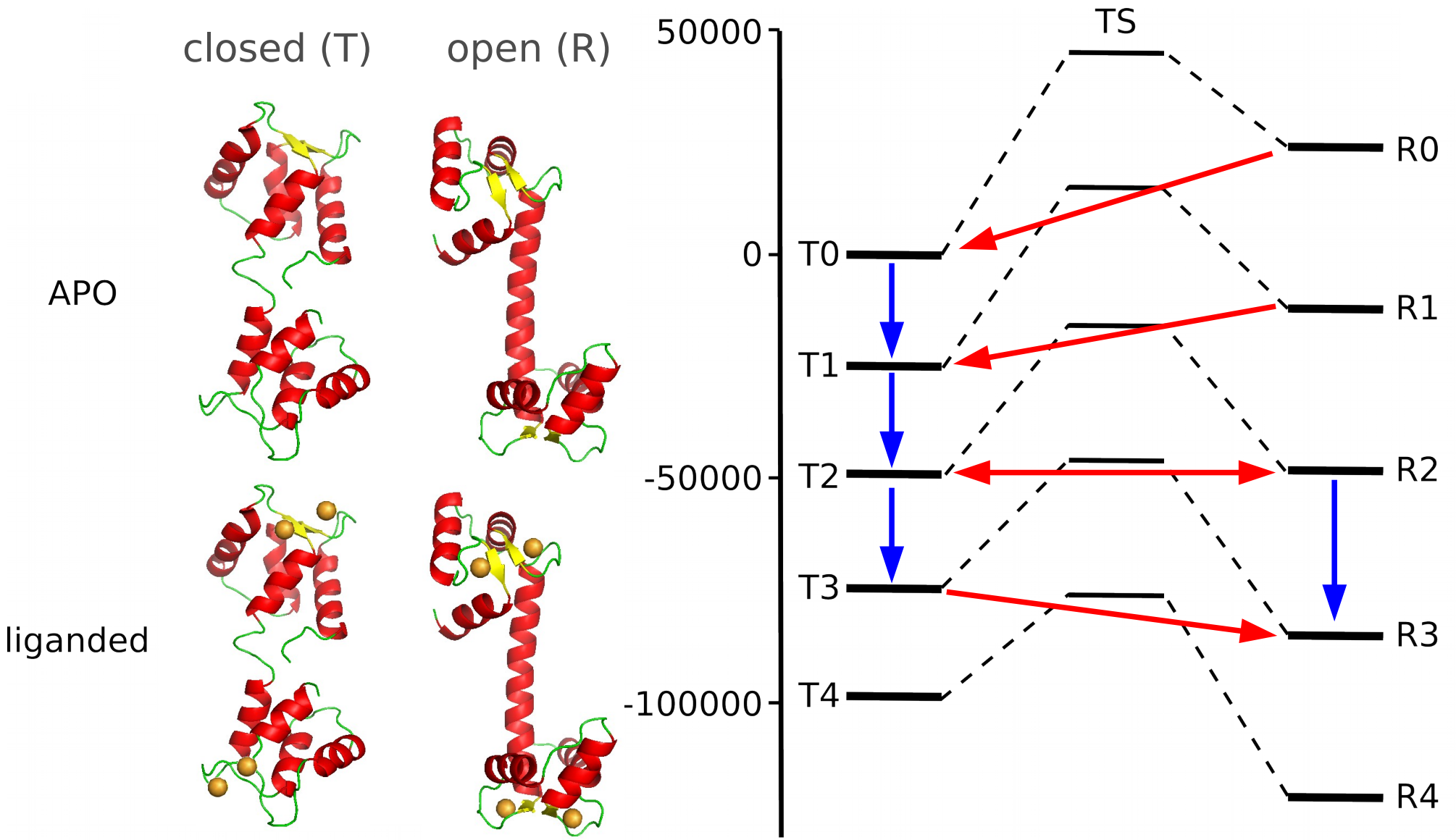
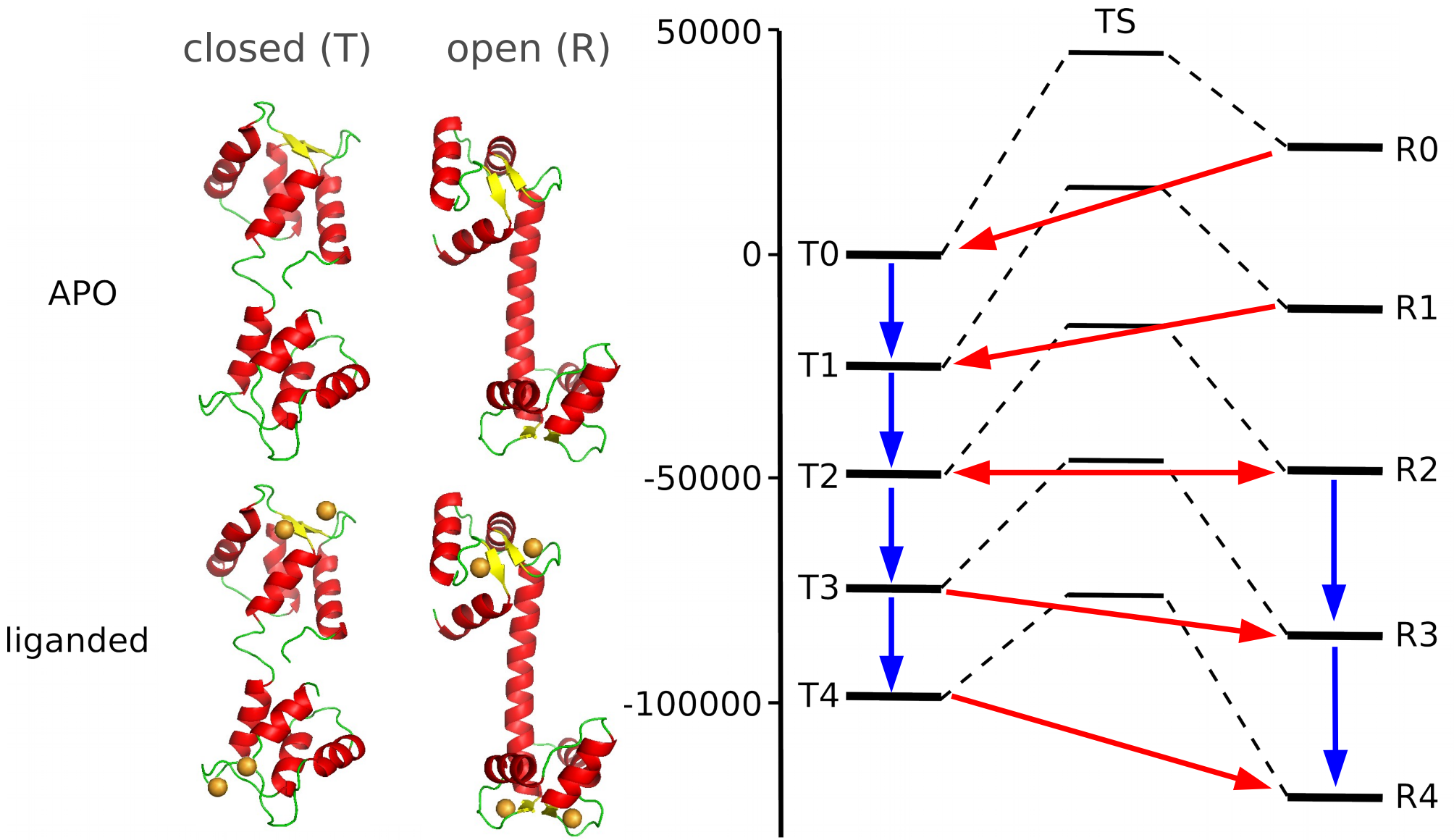


Allosteric model of Calmodulin activation



Parameter estimation using:

- Ca^{2+} binding in presence of targets: none, skMLCK, PhK5, CaATPase
- Ca^{2+} dissociation constants for complete calmodulin and N and C term mutants

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"

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

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

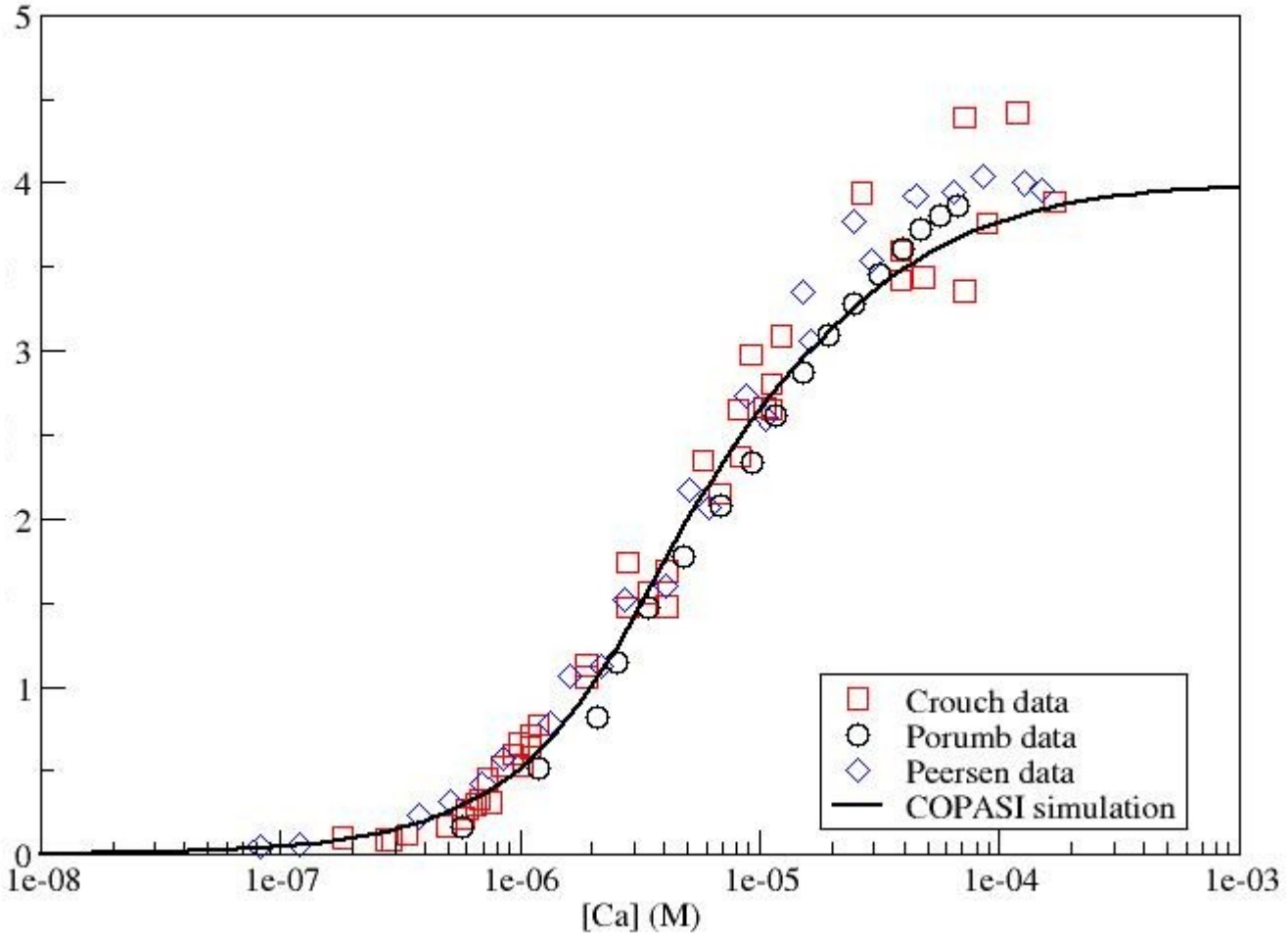
$K^R_C=1.74\ 10^{-5}$

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

2 high, 2 low, as expected

Comparison with experiments (binding function)

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



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

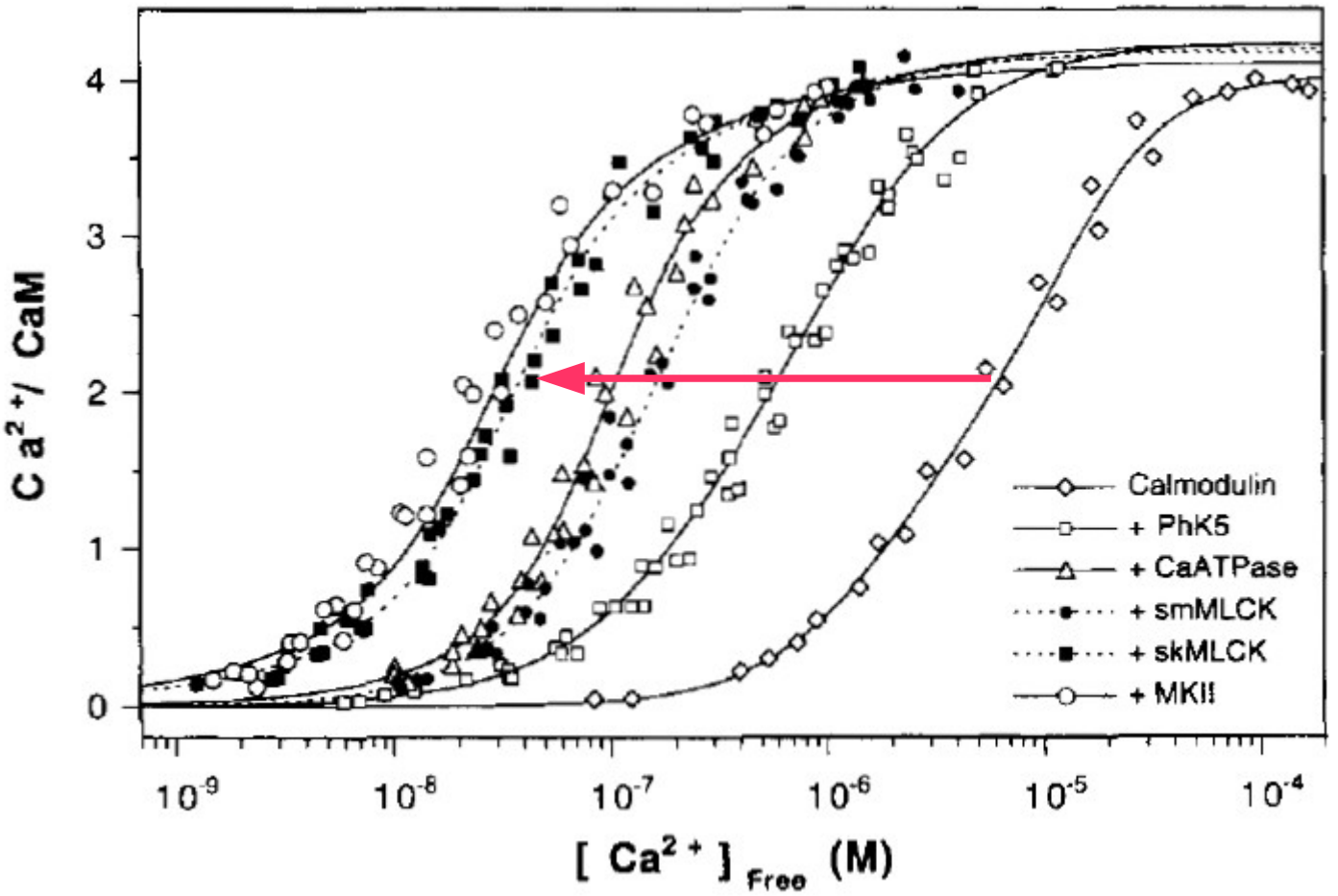
Activity of unsaturated calmodulin (state function)

- Fractional activity depends on the number of calcium ions bound

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

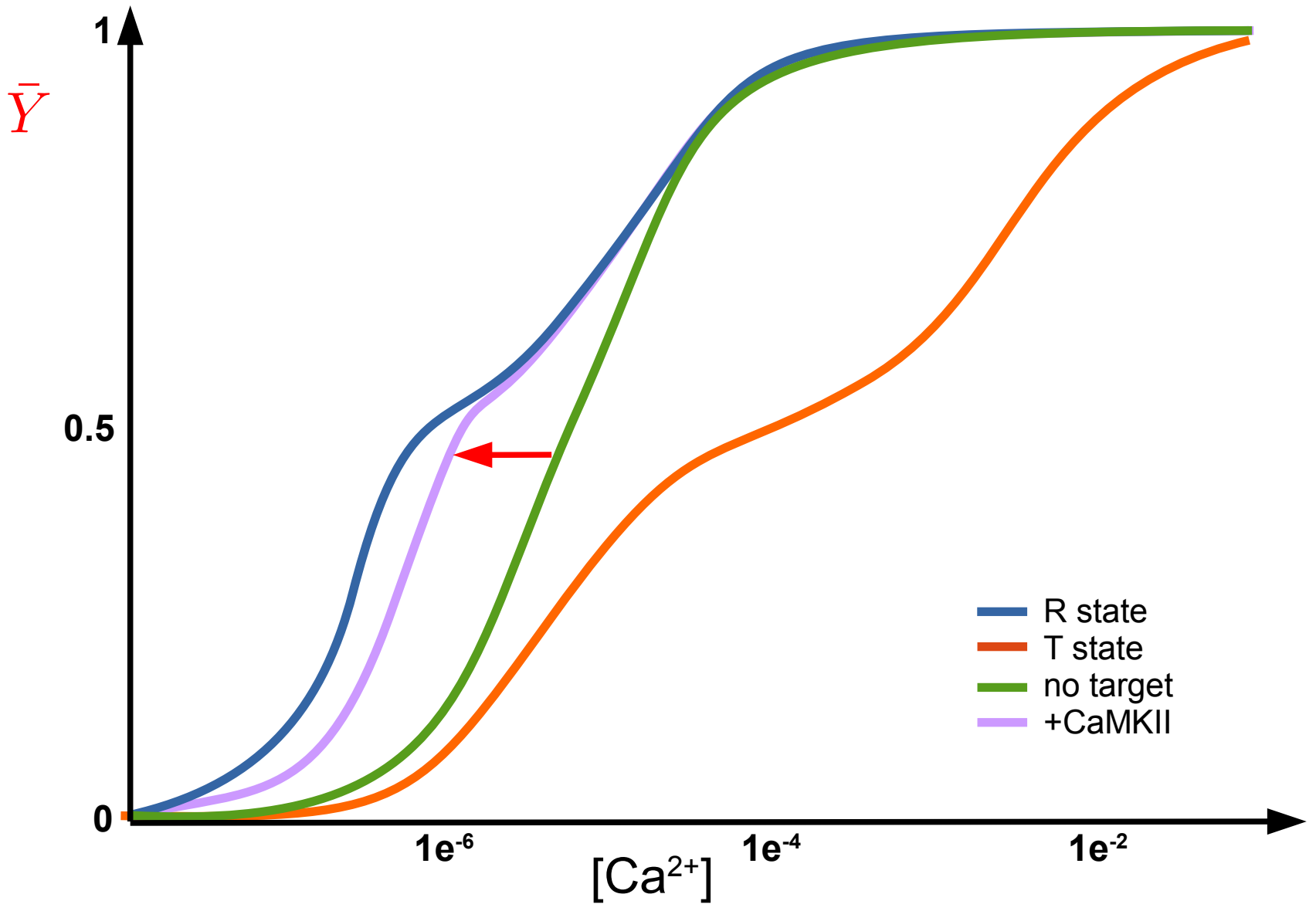
- $R_0/T_0 = 1/20000$ (1/L)
- $R_1/T_1 = 1/170$
- $R_2/T_2 = 0.69$ → half-saturation ≈ equi-probability
- $R_4/T_4 = 10000$

Targets as allosteric effectors

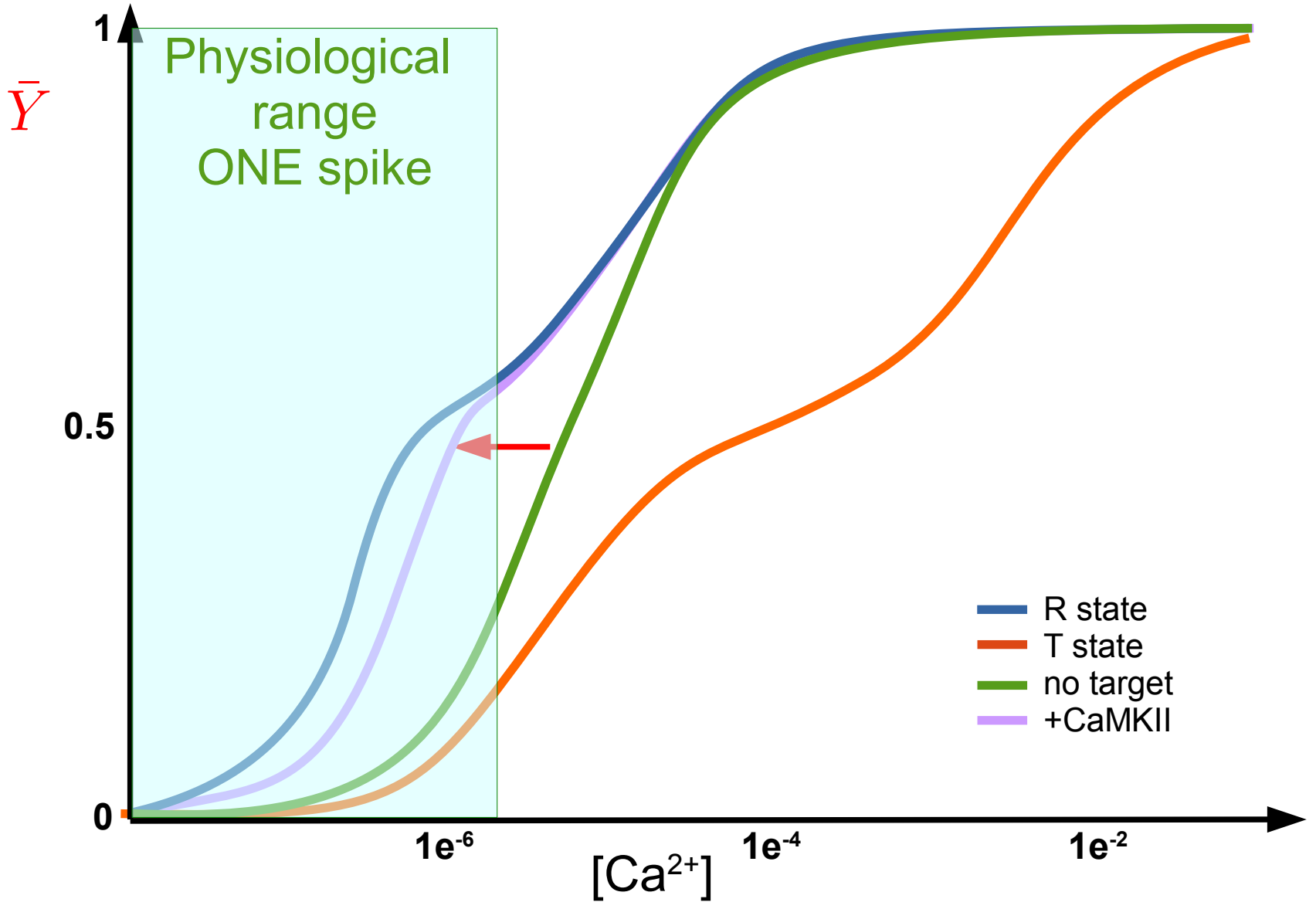


Peersen et al. (1997) *Prot Sci*, 6: 794-807

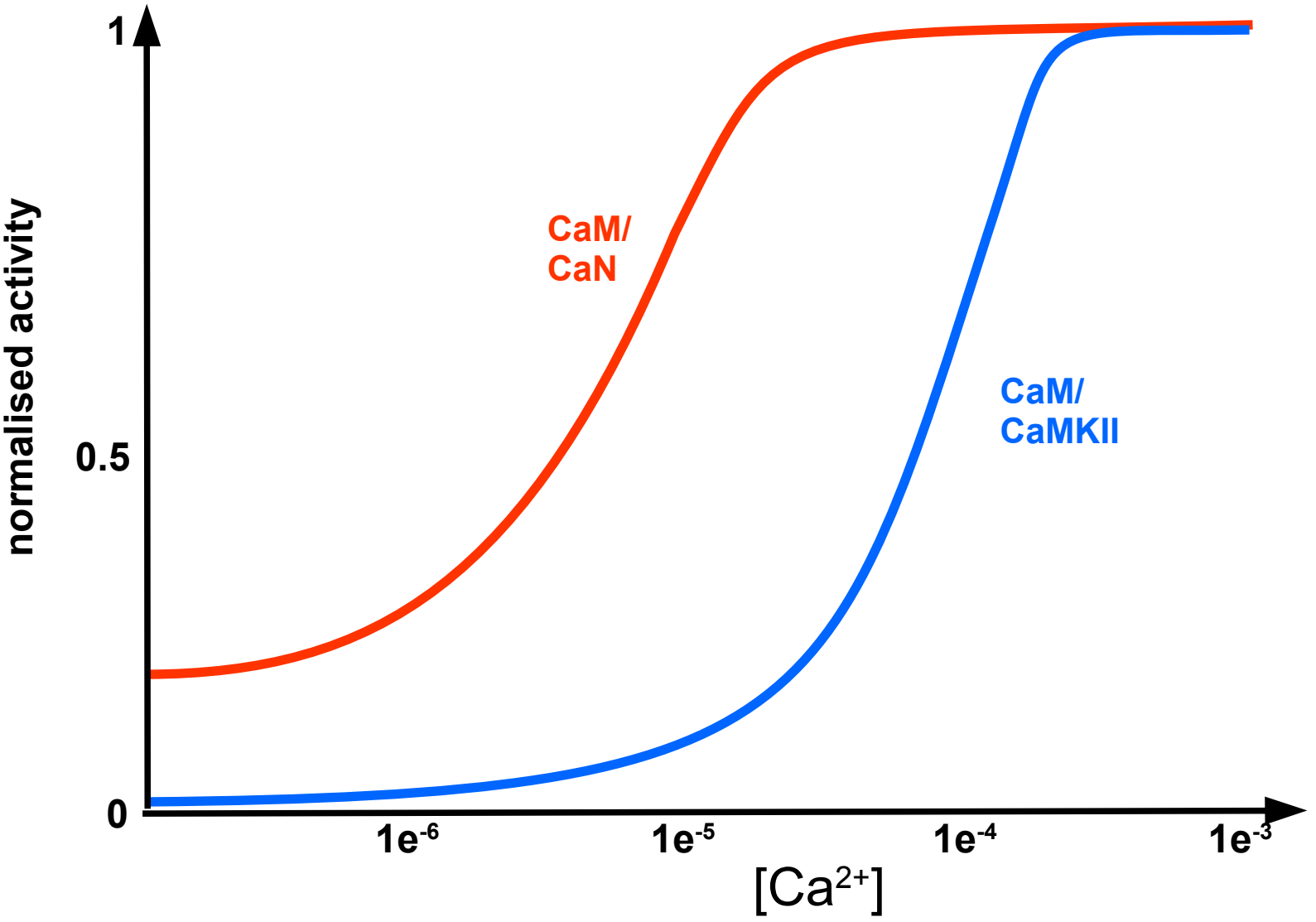
Binding to target increases the affinity for Ca^{2+}



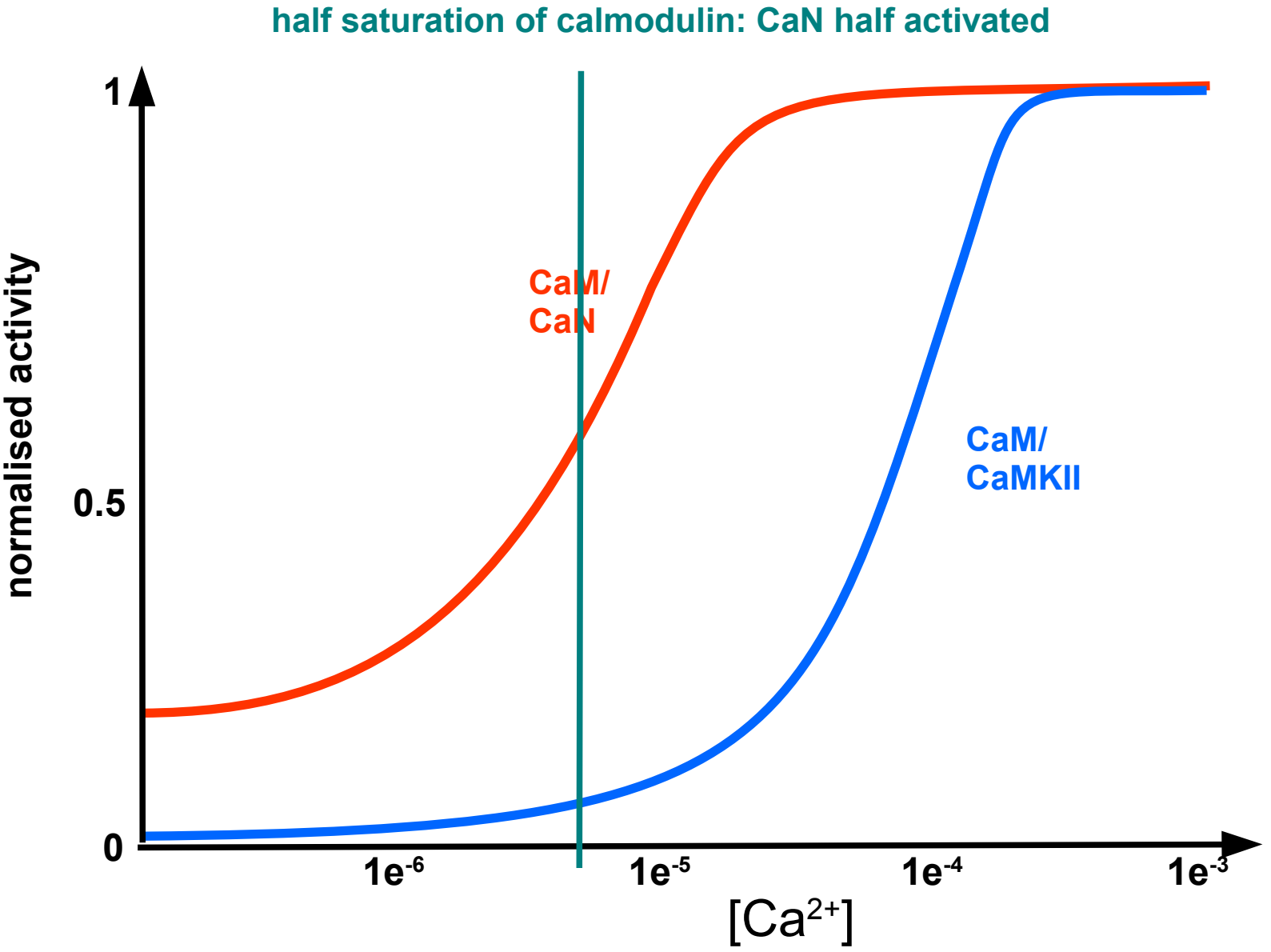
Targets stabilises Ca^{2+} binding into the physiological range



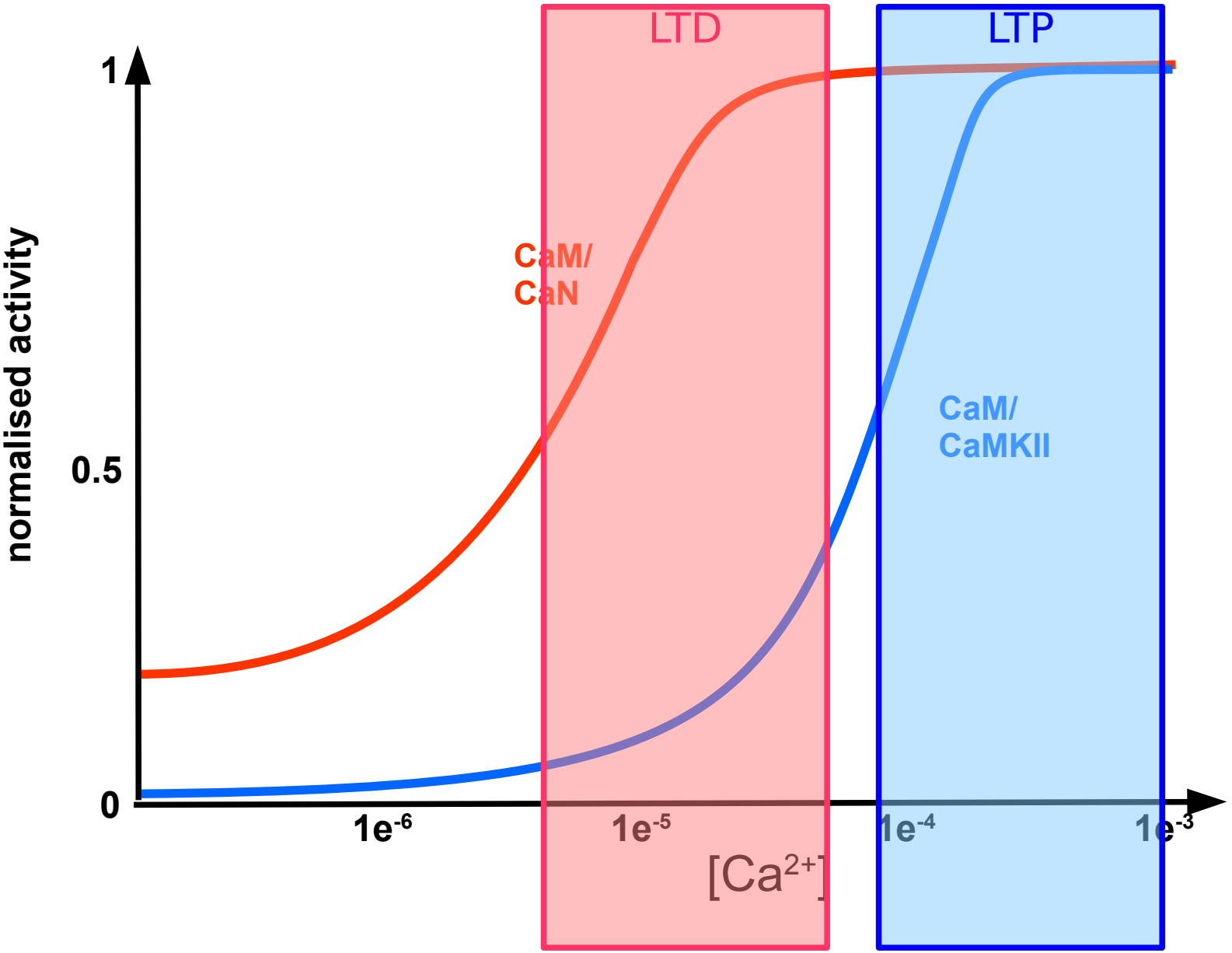
Bidirectional synaptic plasticity



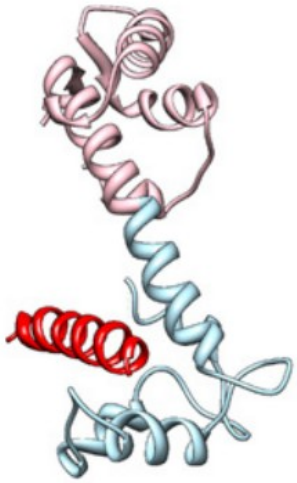
Bidirectional synaptic plasticity



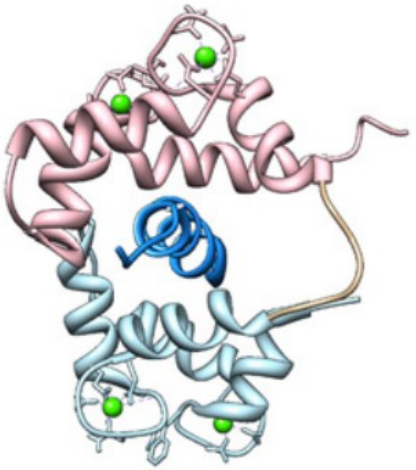
Bidirectional synaptic plasticity



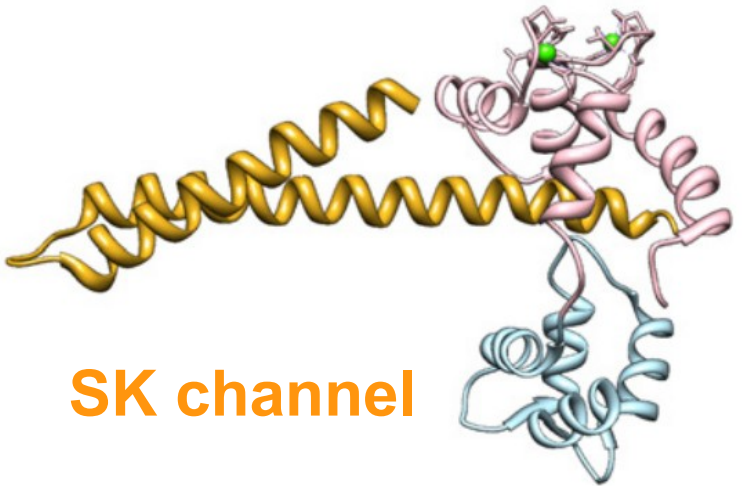
Different binding to different targets



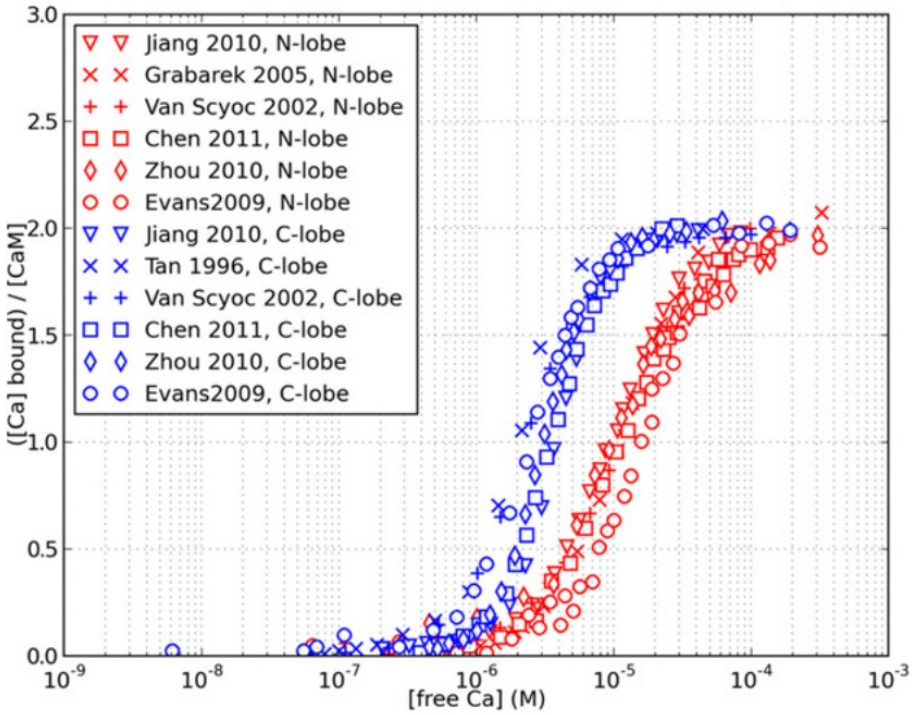
Neurogranin



MLCK

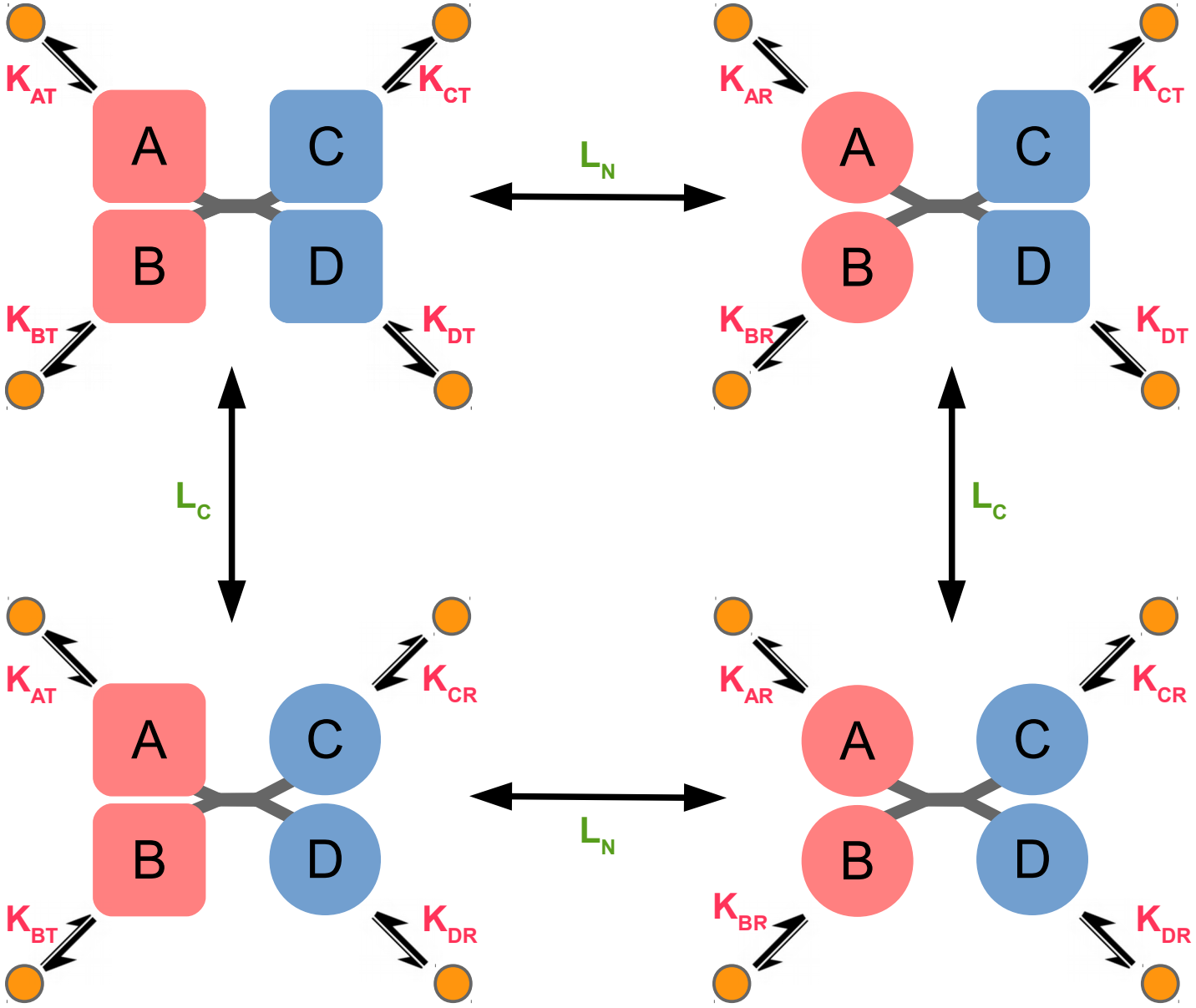


SK channel



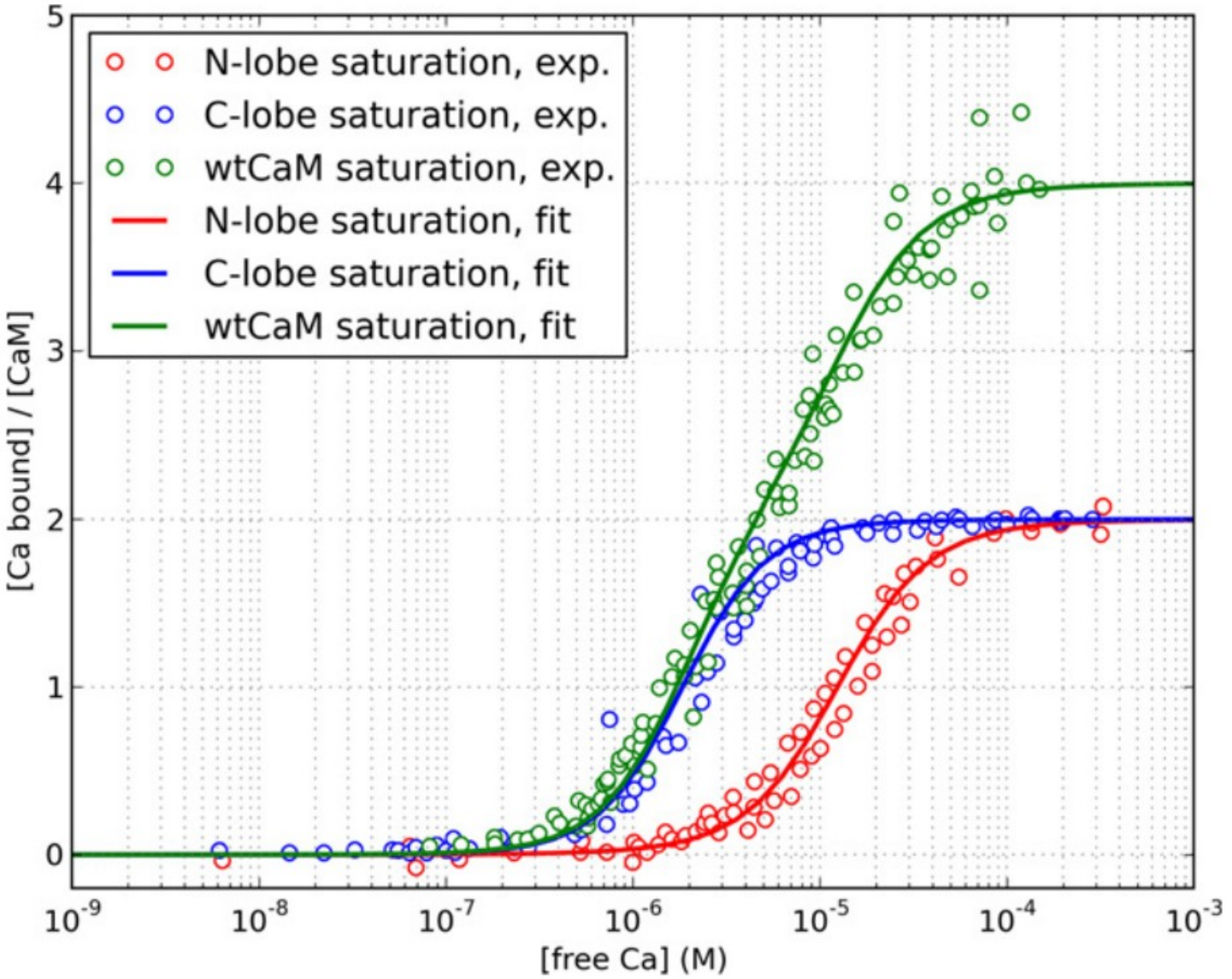
Lai M, Brun D, Edelstein SJ, Le Novère N (2015)
PloS Comput Biol, 11(1): e1004063

Hemiconcerted model of calmodulin



Lai M, Brun D, Edelstein SJ, Le Novère N (2015) *PloS Comput Biol*, 10(1):e0116616

Calcium binding to lobes and whole CaM



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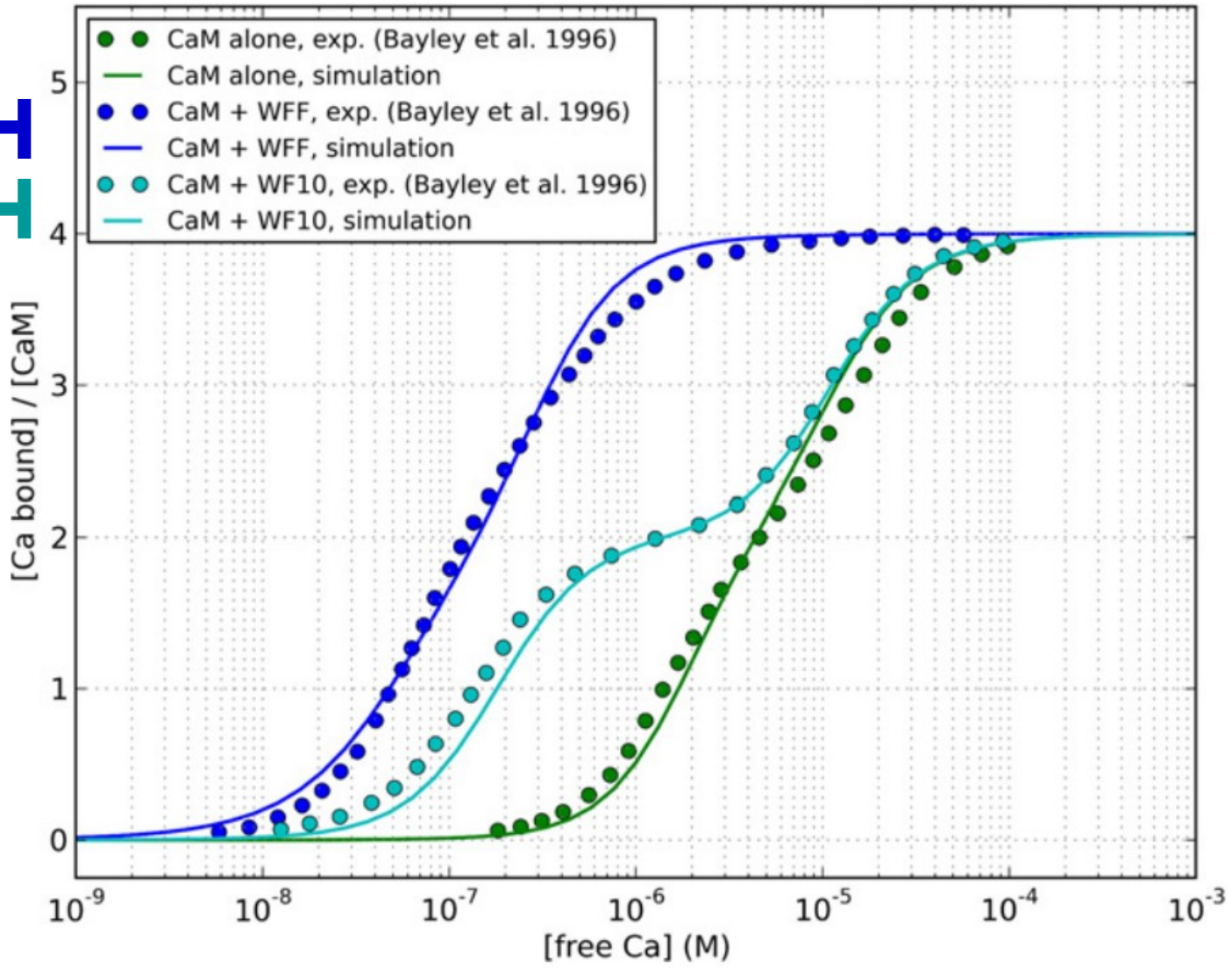
Effect of R-stabilising targets

Stabilise R state of both lobes

Stabilise R state of C lobe



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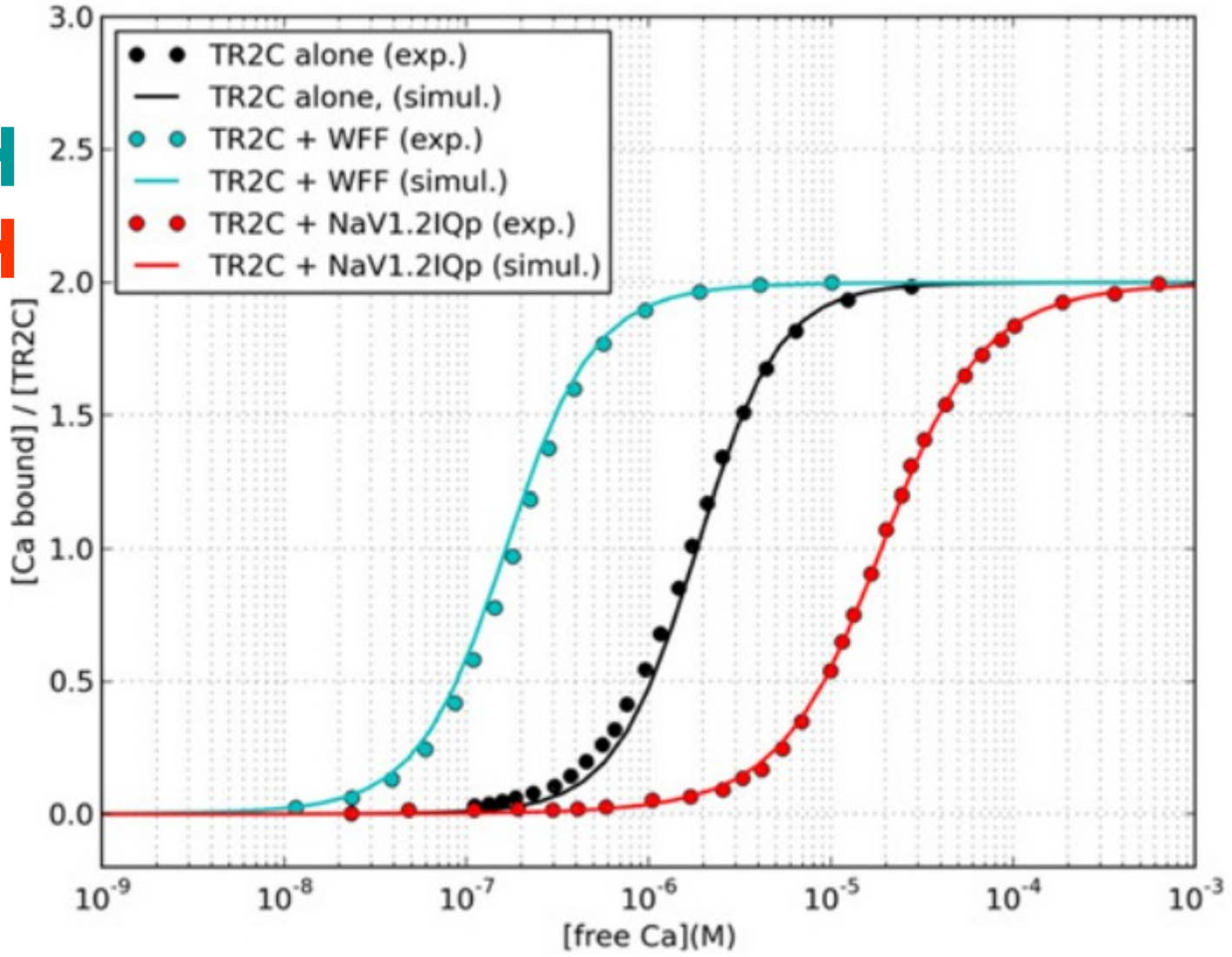
Effect of R and T stabilising targets

Peptide from skMLCK → R

IQ domain from NaV1.2 → T



Conclusions of part 1

Allosteric model of Calmodulin, with only two states for the EF hands, binding calcium with different affinities, and concerted transitions of the 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.

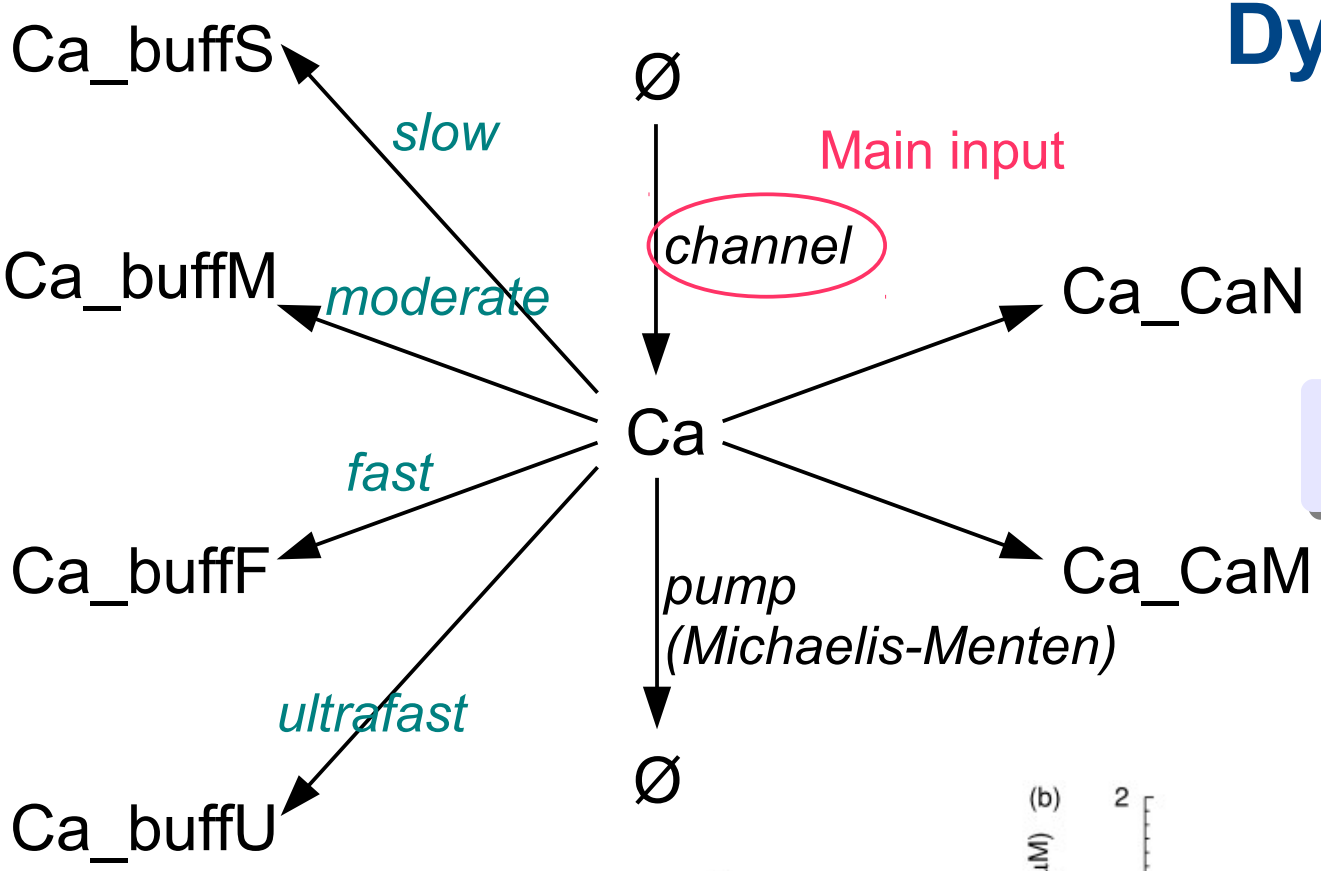
The model displays an activation of the sole CaN at low concentration of calcium, while high concentrations activate both CaN and CaMKII.

Wait a minute!
Signal transduction is not at equilibrium!

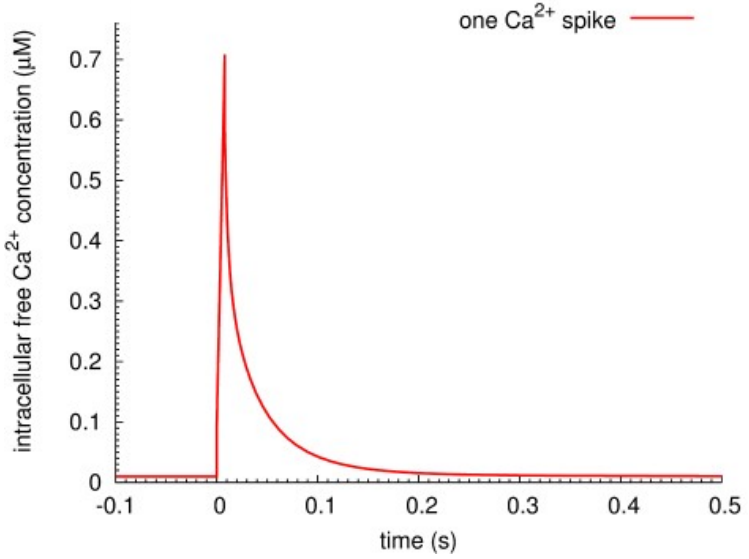
AMPAR post-synaptic potential:	5 ms
Calcium spike:	50 ms
Half saturation calmodulin (kon=1.5e6, koff=100):	5 ms
Relaxation between calmodulin states:	1 ms
autophosphorylation of CaMKII (kon=6):	100 ms

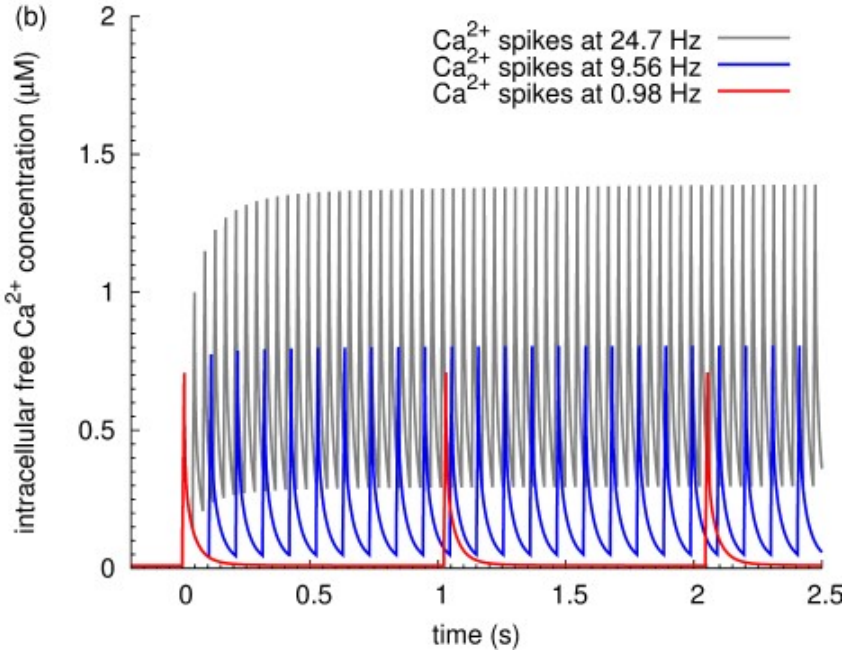
≠

Dynamic of calcium in the spine



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





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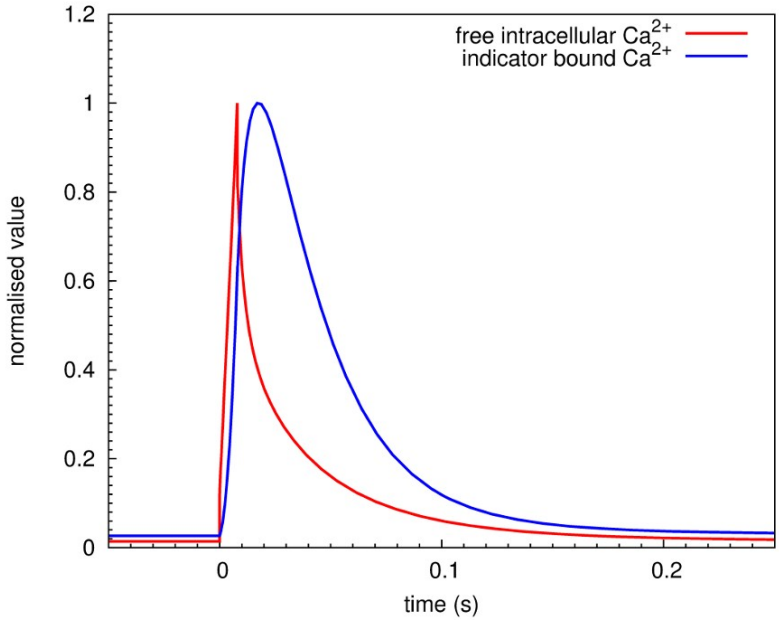
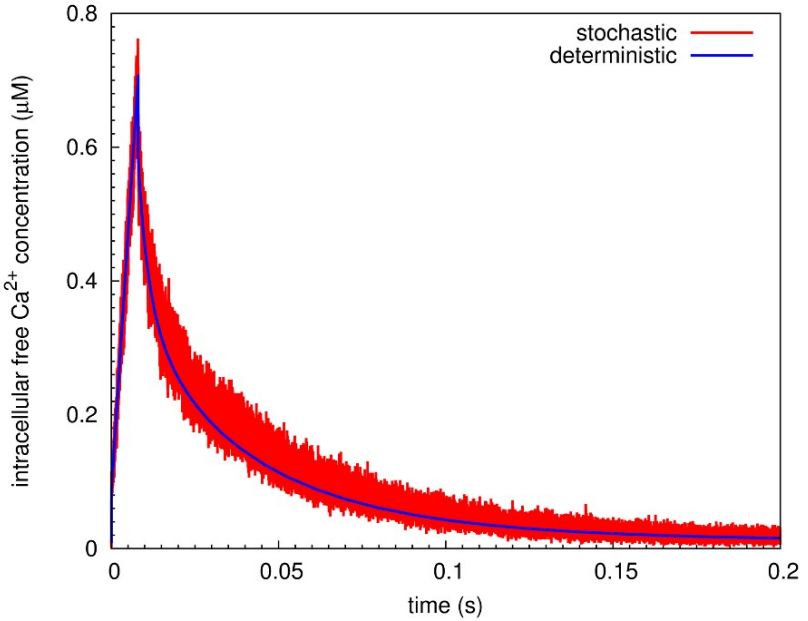
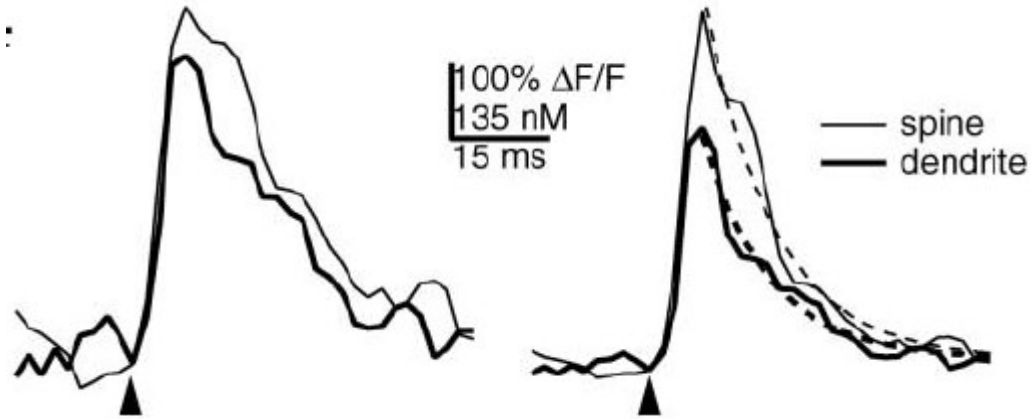
Are those spikes realistic?







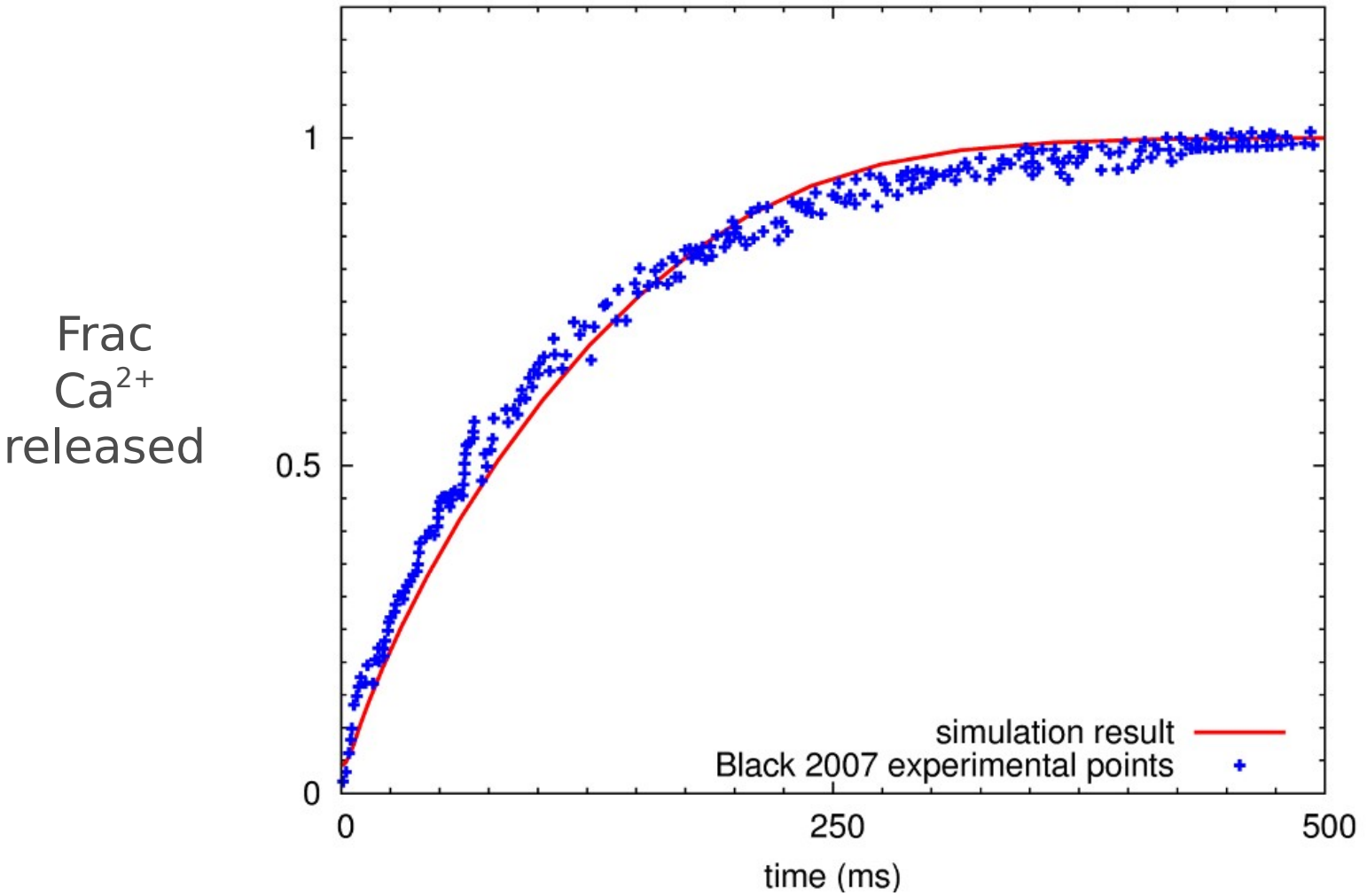
Fluorescence

Free calcium

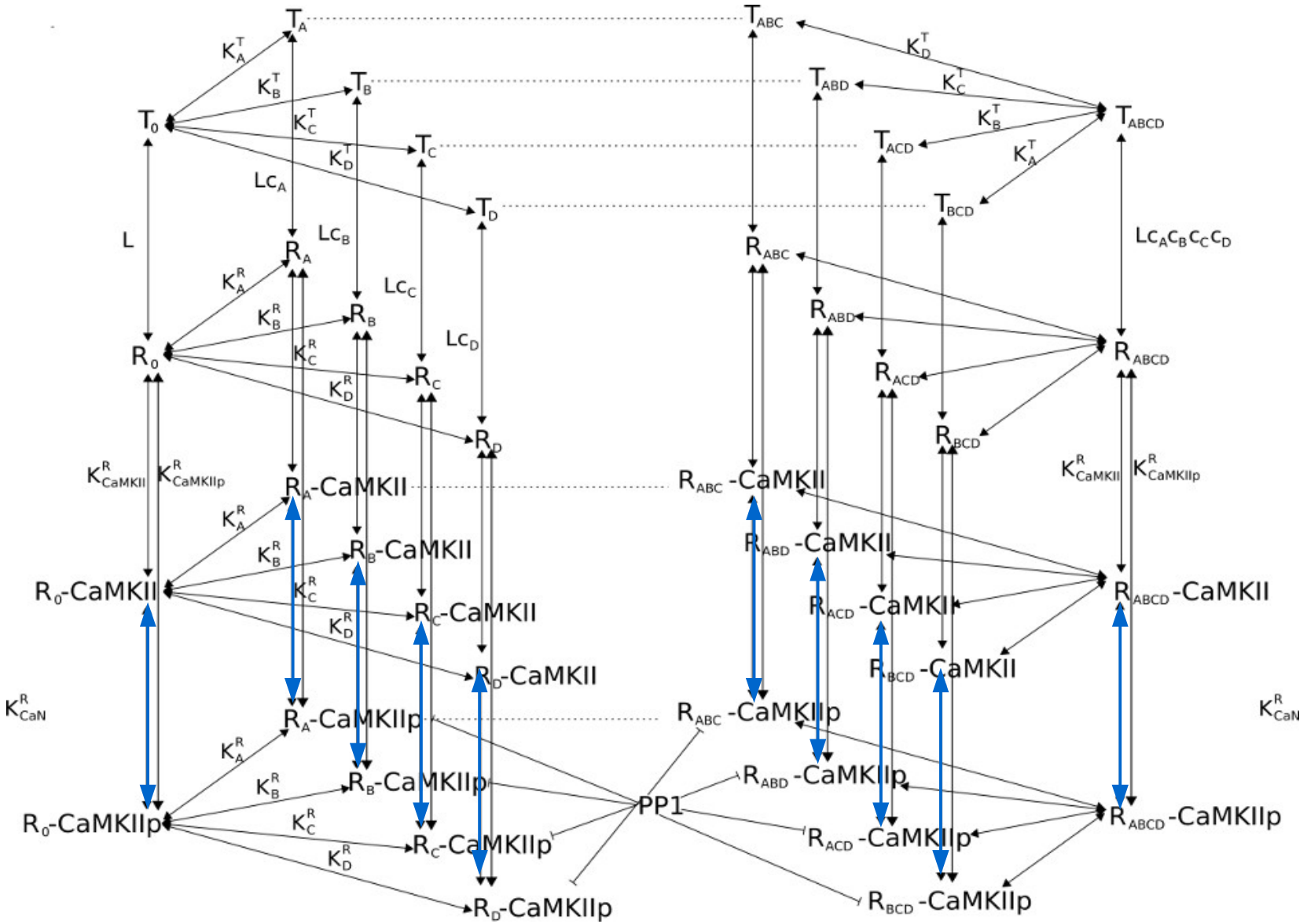
Relative uncertainty increases when concentration decreases, both in concentration and time, but no difference in dynamics.

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

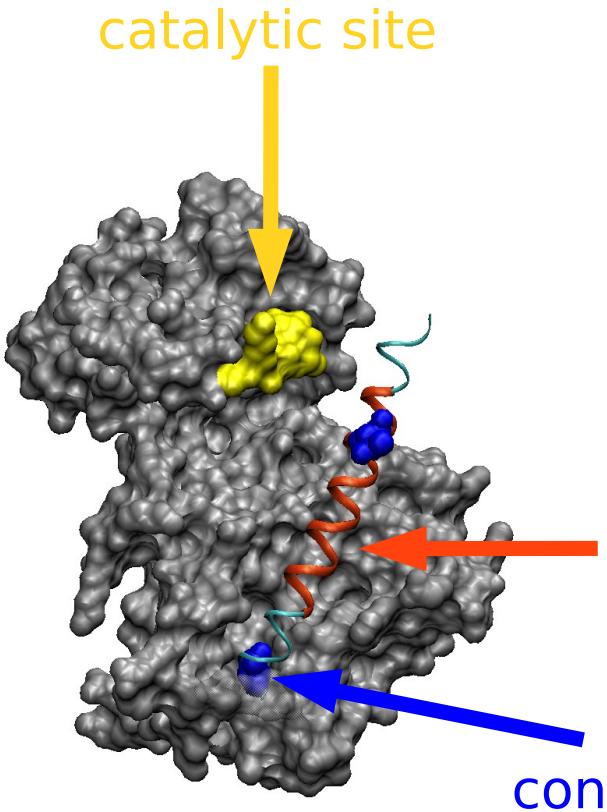
Validation of CaM kinetics



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


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

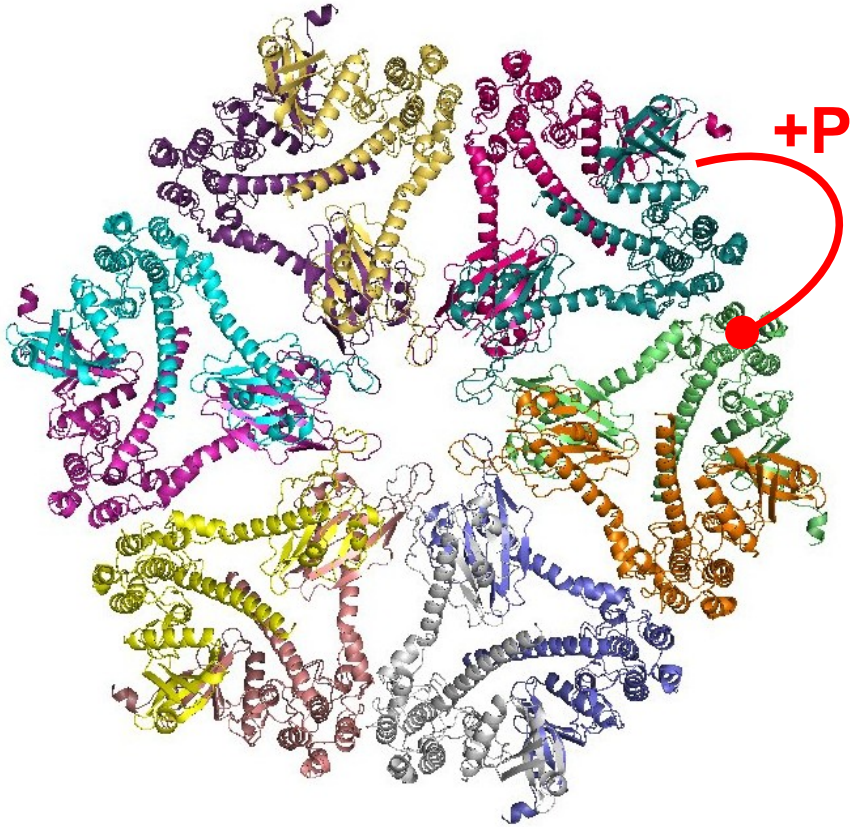
Calcium/calmodulin kinase II

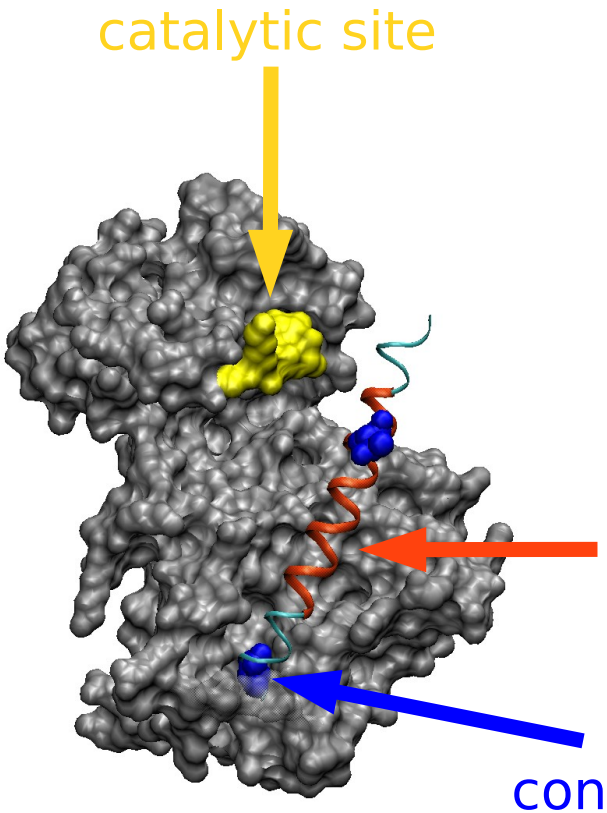


Calmodulin trapping is
an apparent increase of
affinity of CaMKII for CaM
when T286 is phosphorylated

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

Calcium/calmodulin kinase II

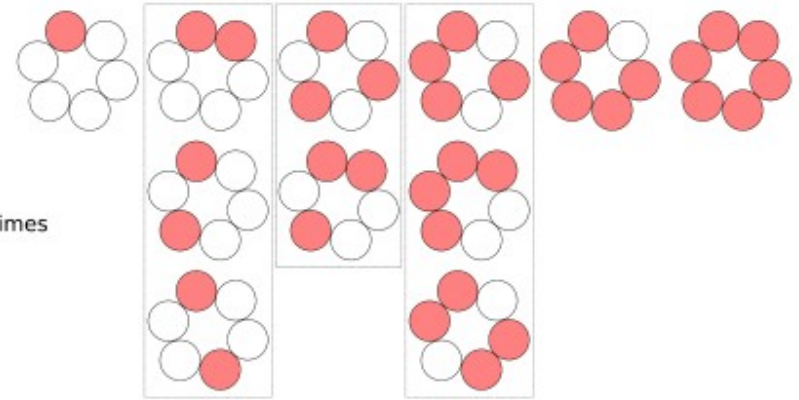




Dodecamer;
Trans-phosphorylation of T286
by neighbouring subunits

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.

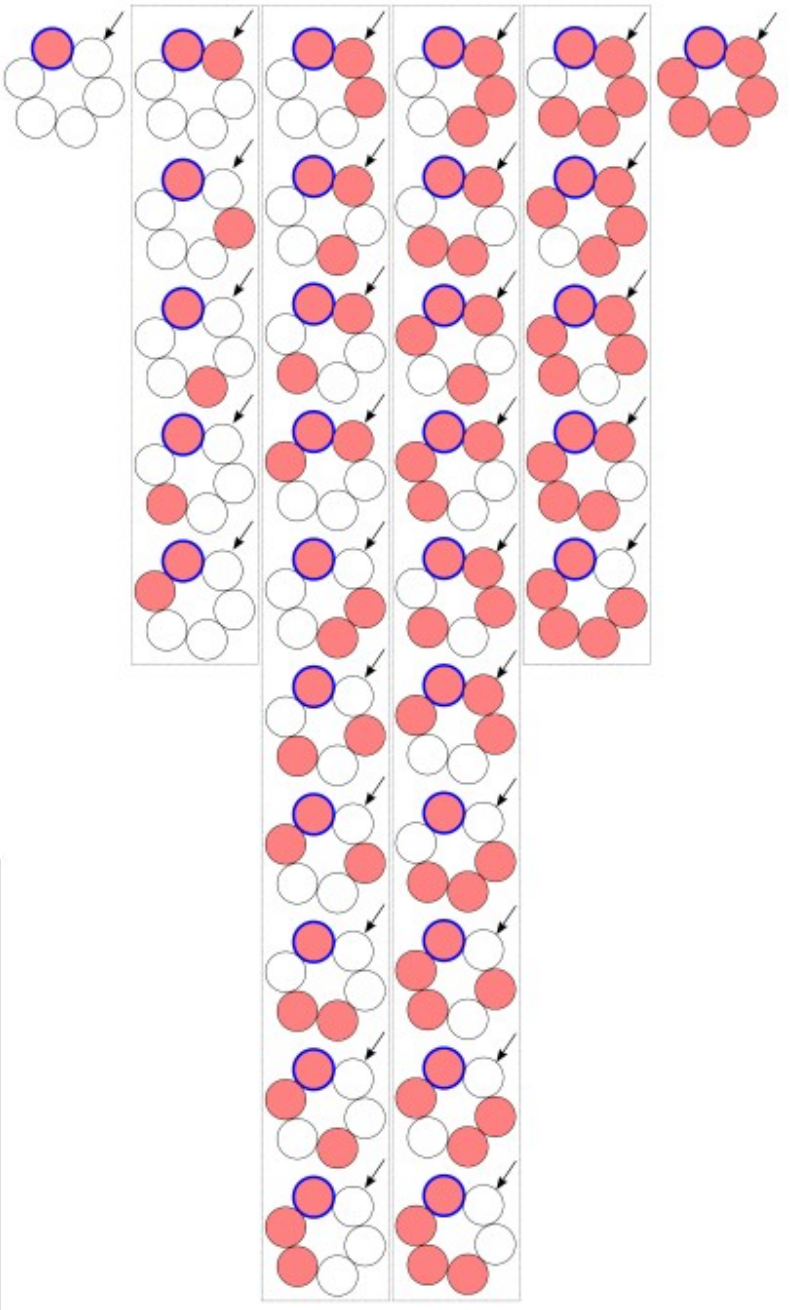
Repeat for every 1% increase of CaMKII active monomers

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

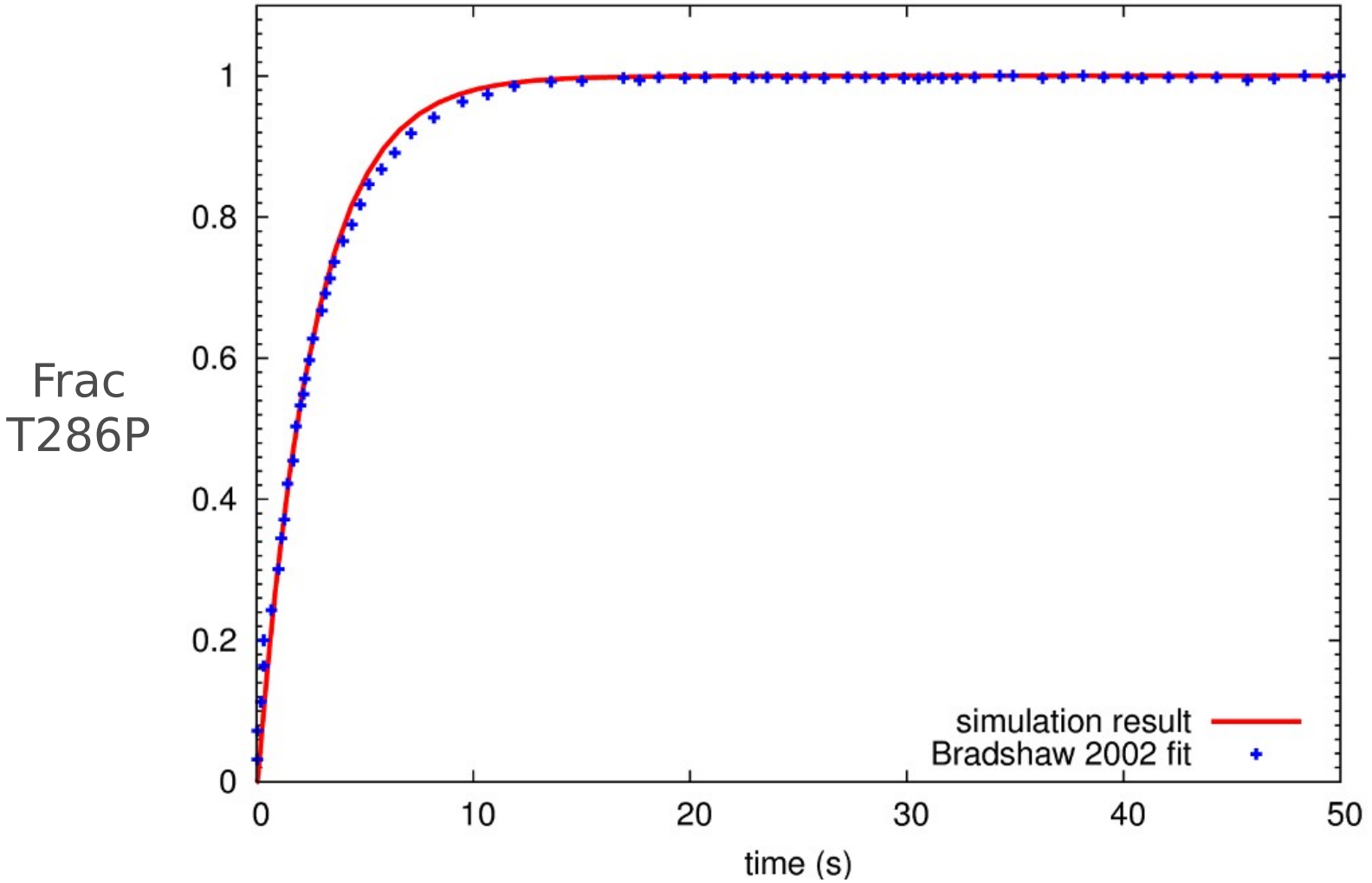
The sum of these six numbers is a coefficient that can be used to adjust CaMKII autophosphorylation rate.

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

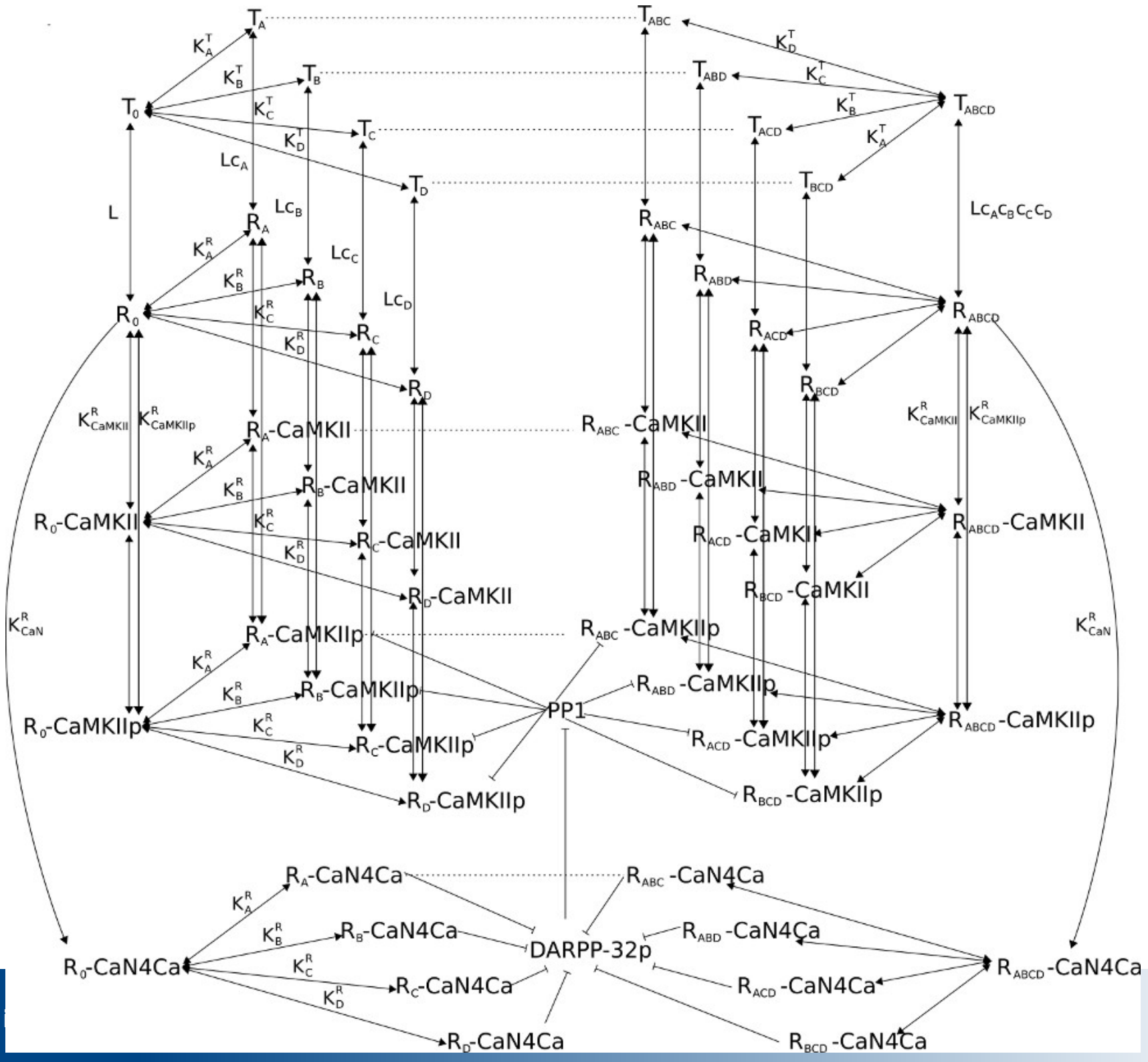
Calculate the probabilities of having an active neighbour on one specific side (indicated by the arrow) of the activated monomer of interest (blue outline) (since only the asymmetrical situation is considered). The possible positions of activated monomers are listed as the following, with corresponding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

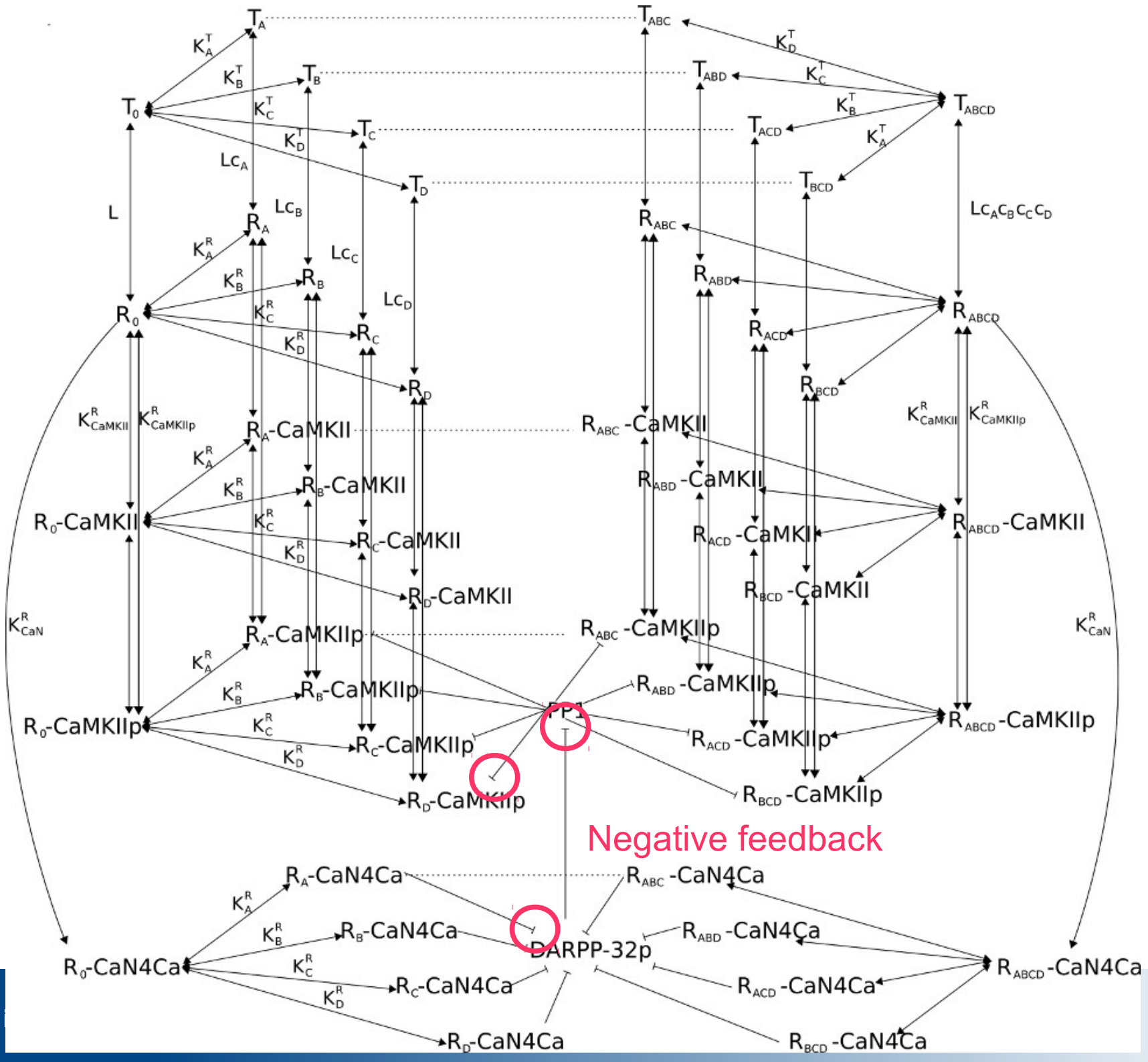


Validation of CaMKII kinetics

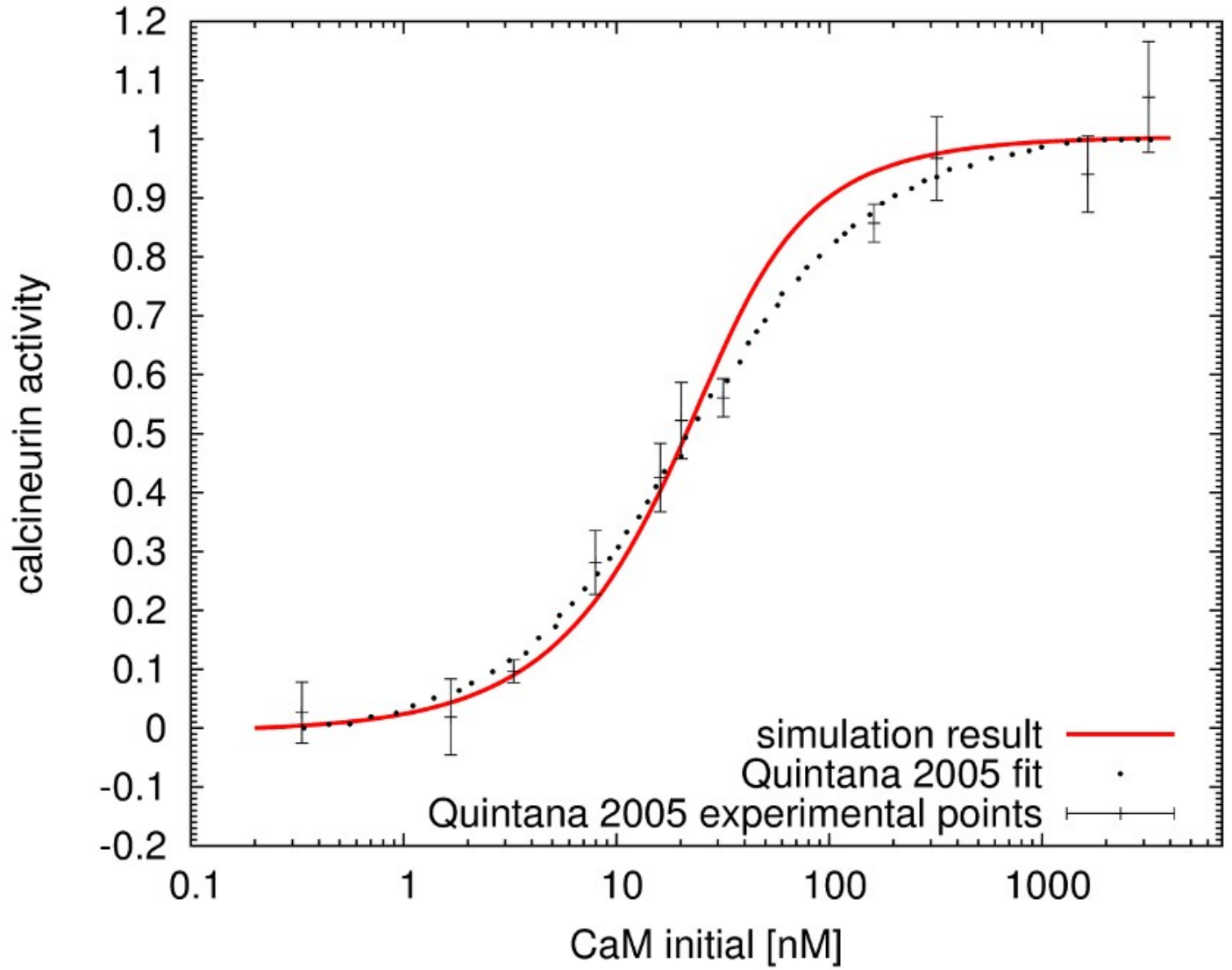


Bradshaw JM, Kubota Y, Meyer T, Schulman H (2003). *PNAS* 100: 10512–10517.



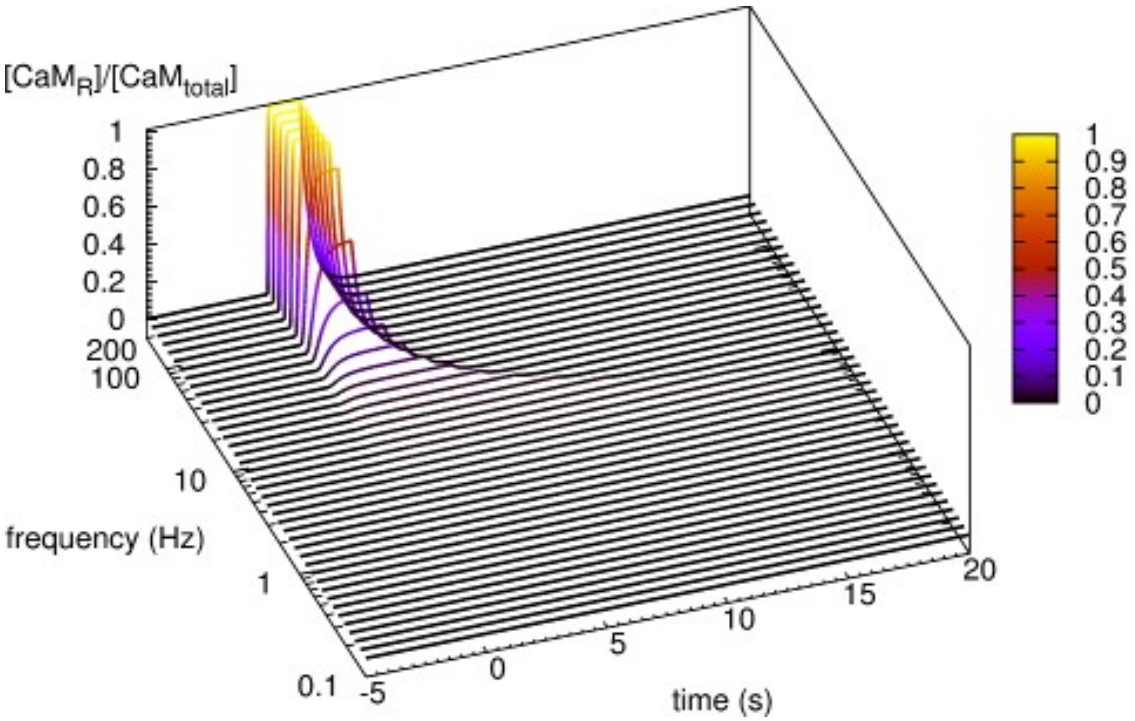

Negative feedback

Validation of calcium-activation of CaN



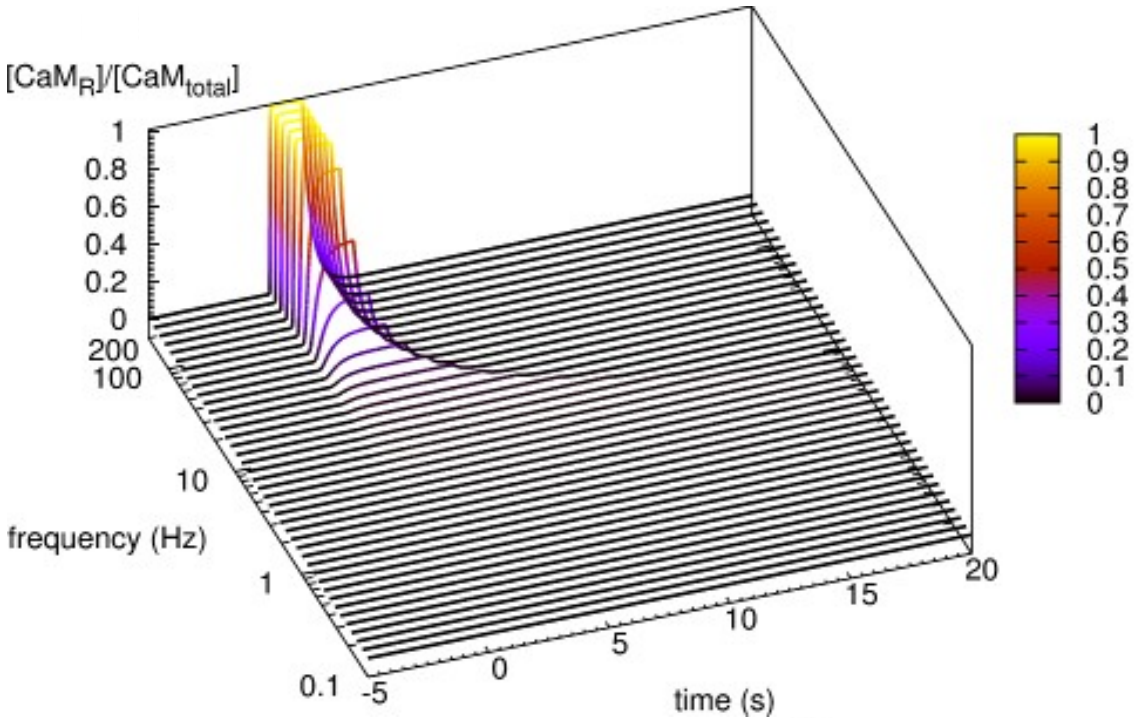
Quintana AR, Wang D, Forbes JE, Waxham MN (2005). *BBRC* 334: 674–680.

Calmodulin activation

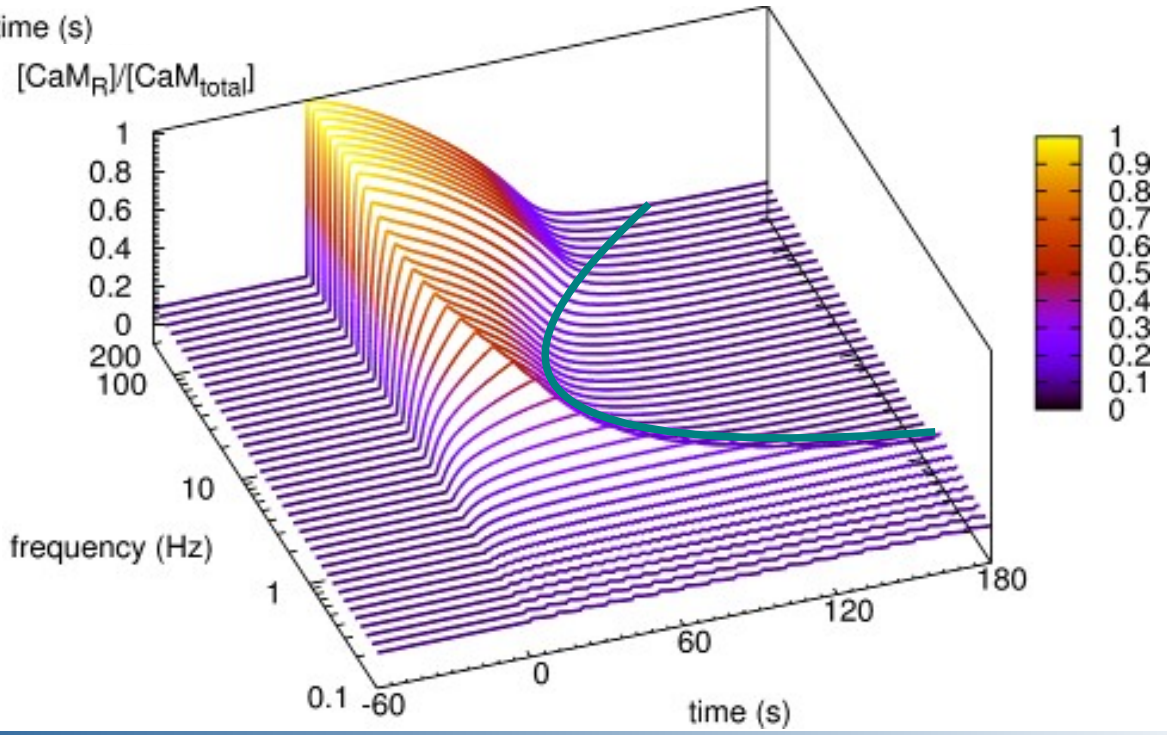


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

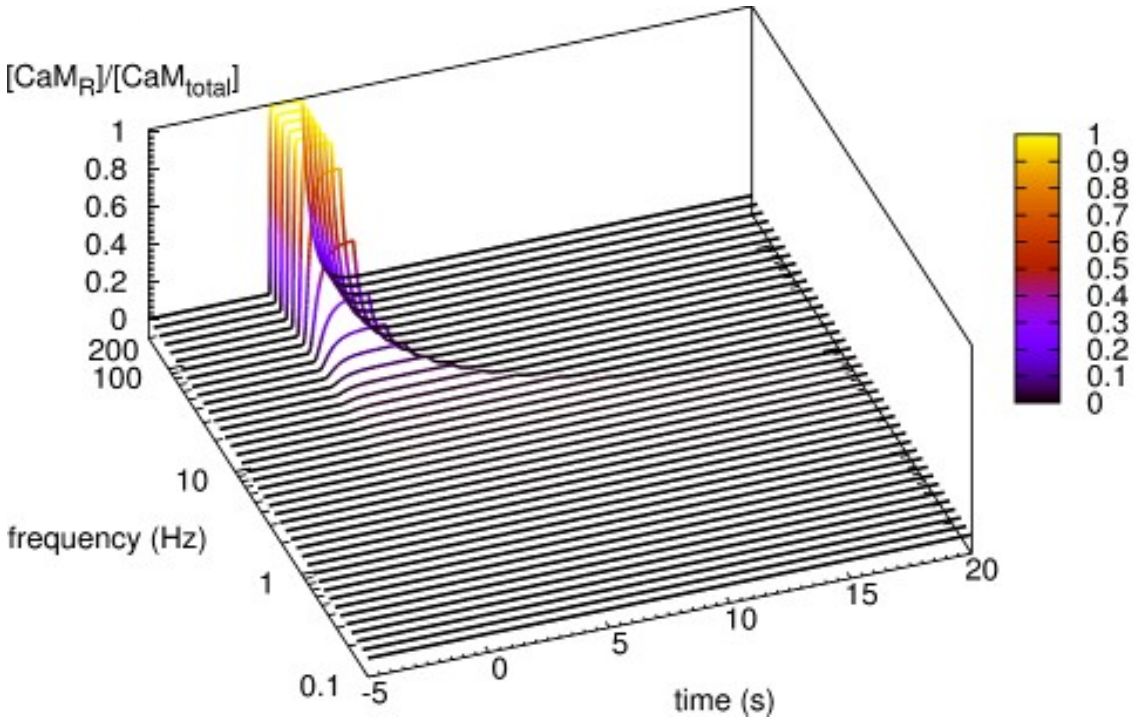


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)



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

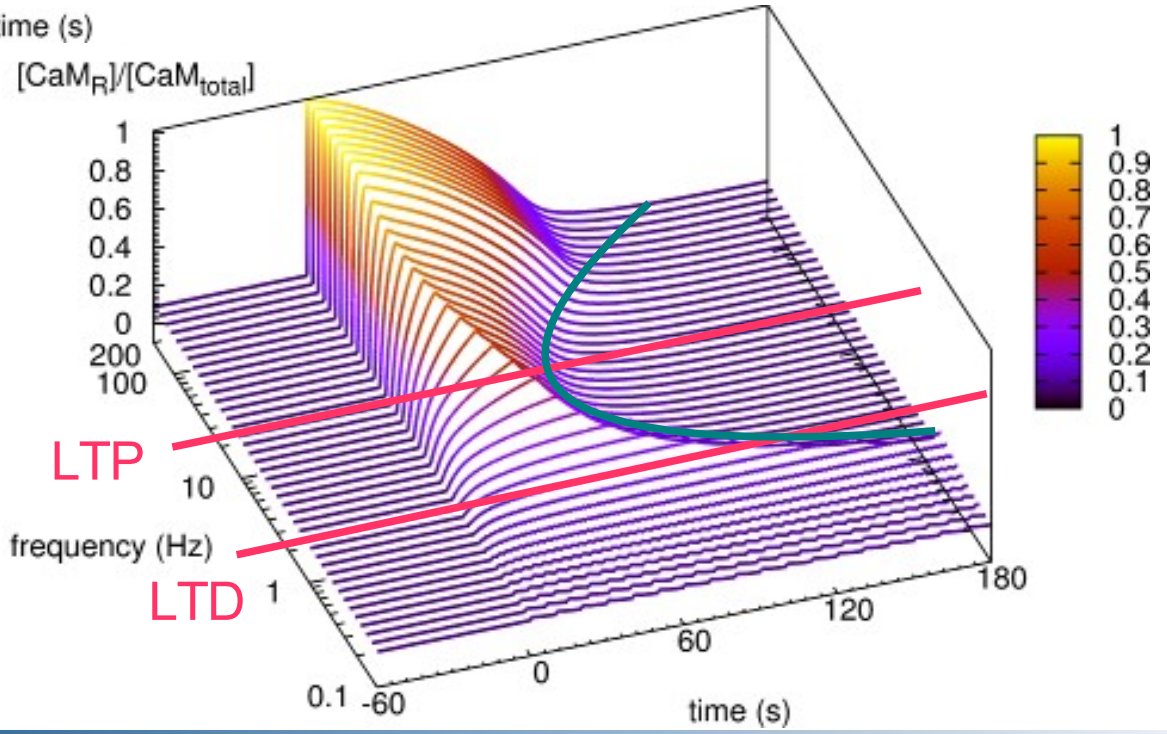
Calmodulin activation



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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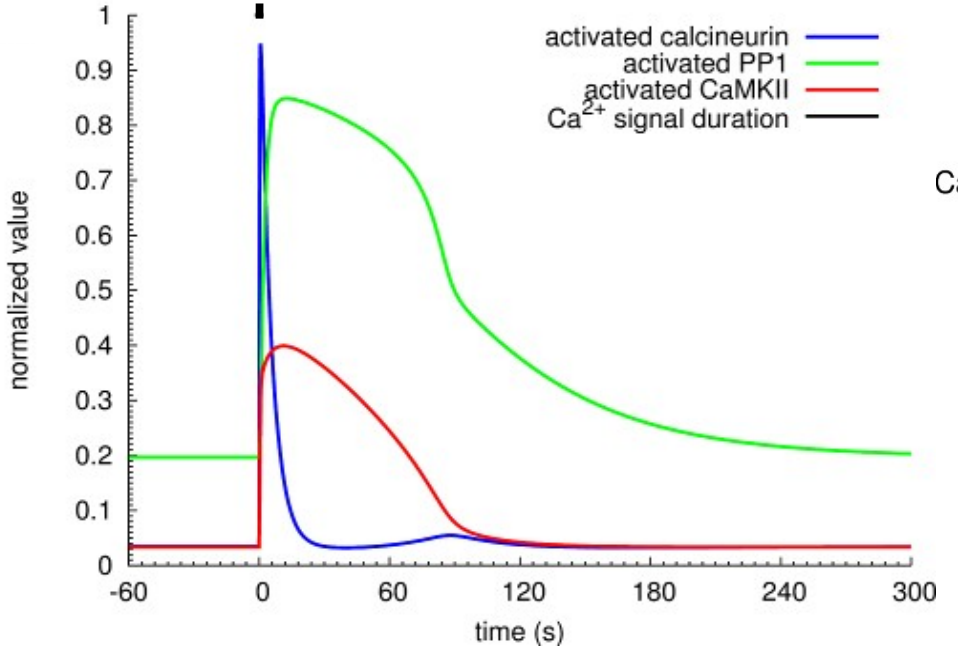
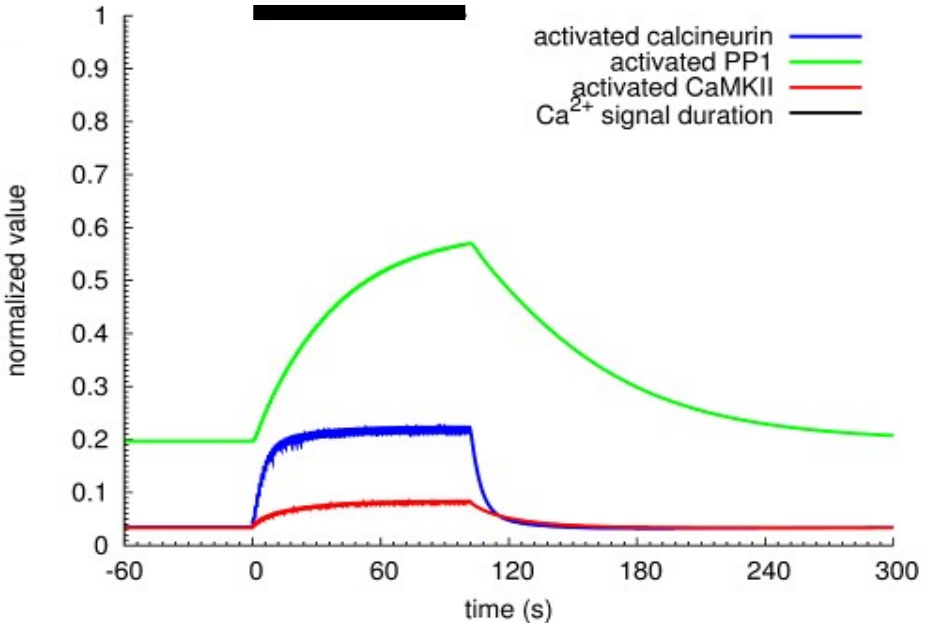
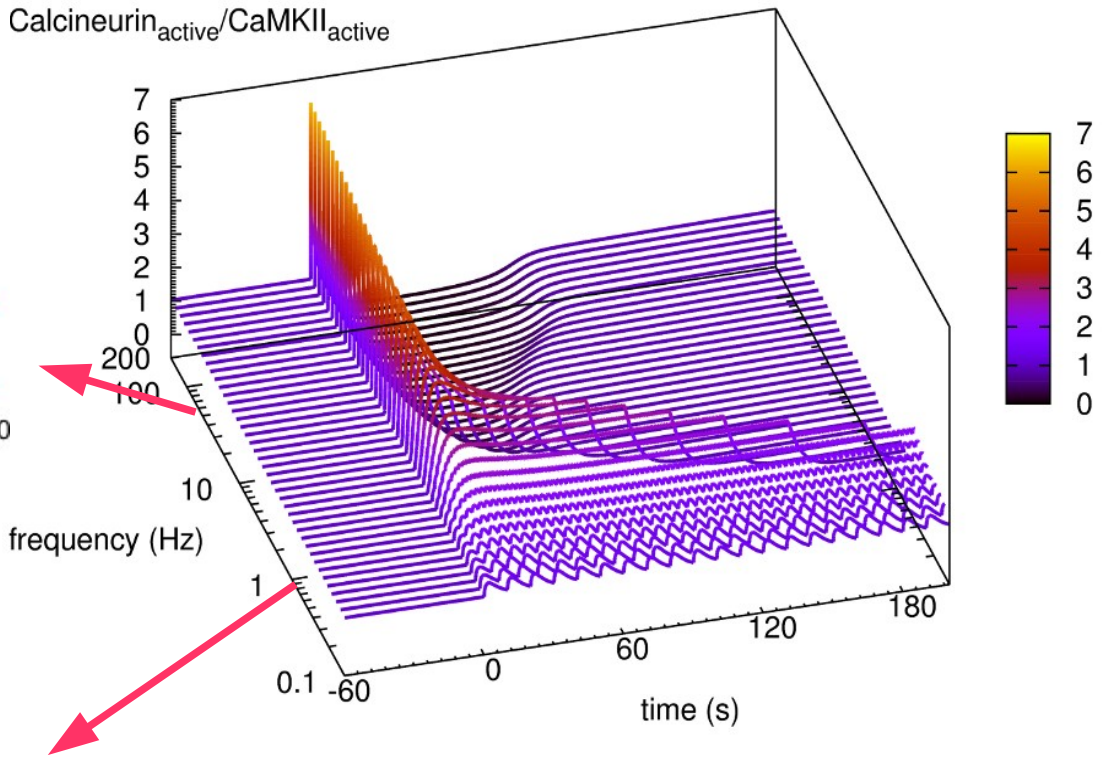
Temporal activation of CaMKII and CaN







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

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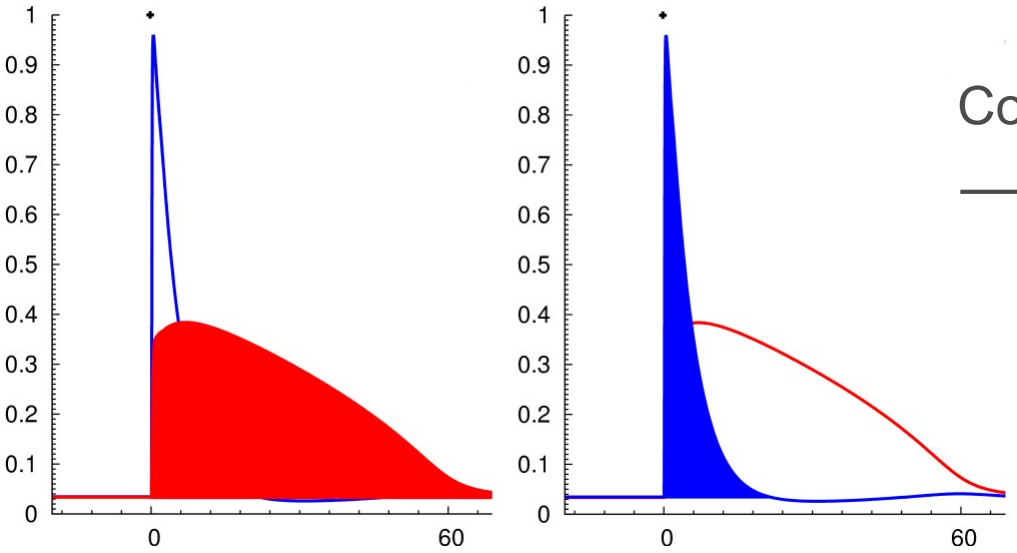
Bidirectional plasticity

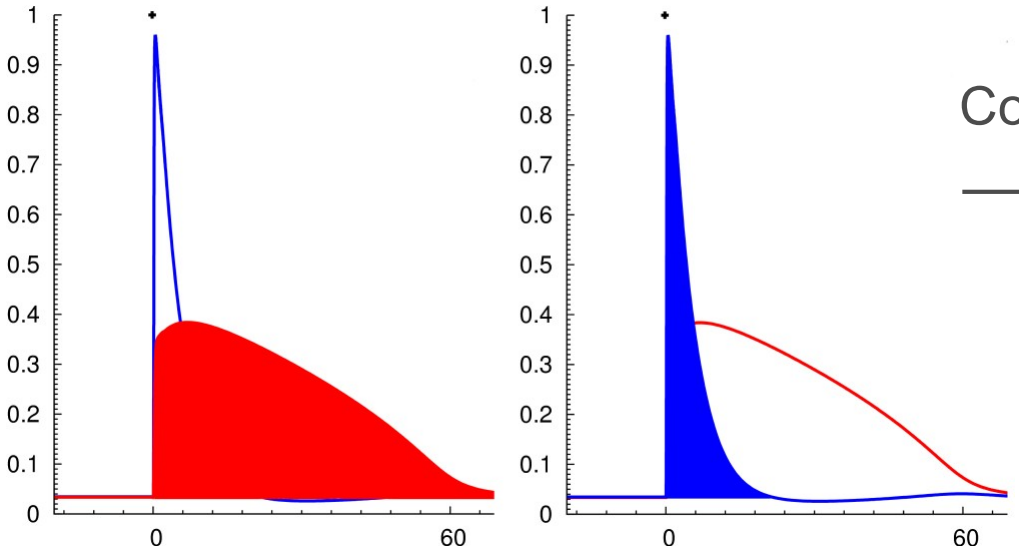


Constant catalytic rates of active enzyme

$\longrightarrow$ quantity of catalysed reaction events

μ integral of the activation curve

Bidirectional plasticity

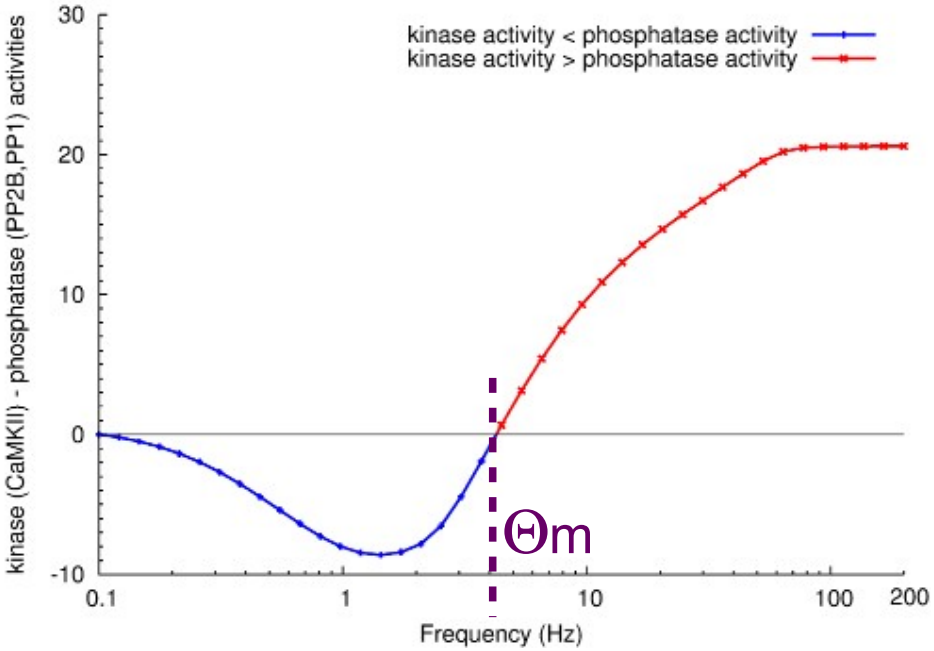


Constant catalytic rates of active enzyme

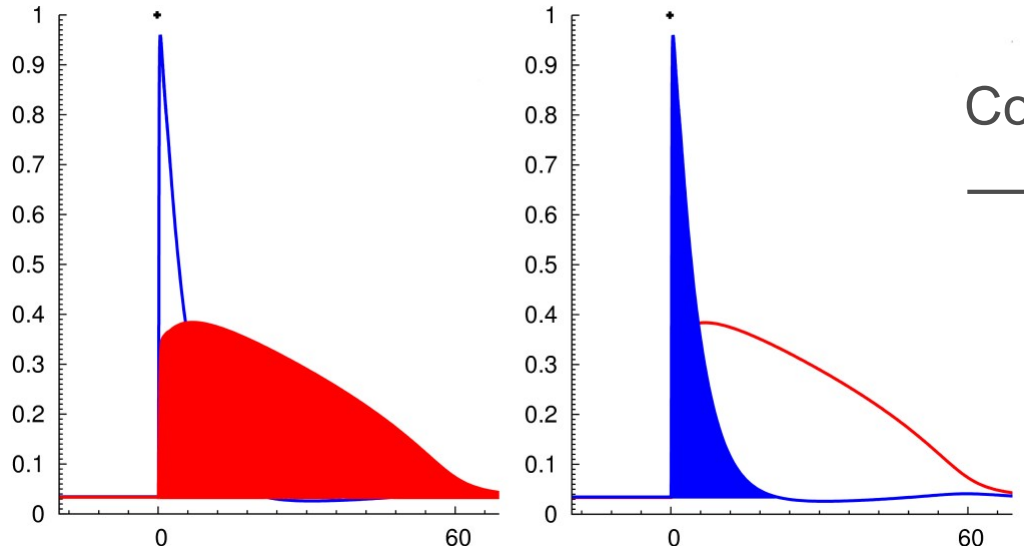
⟶ quantity of catalysed reaction events

μ integral of the activation curve

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



Bidirectional plasticity

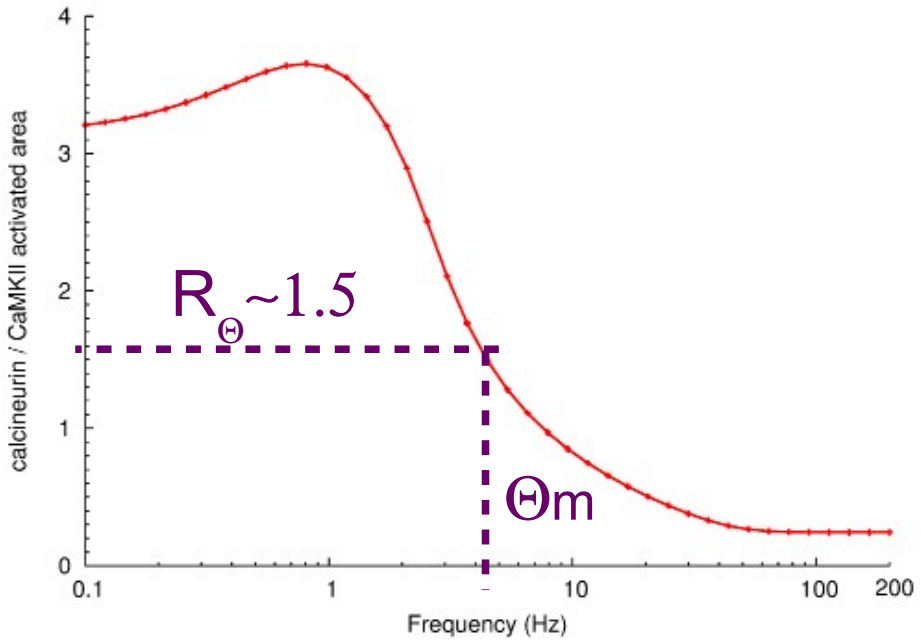


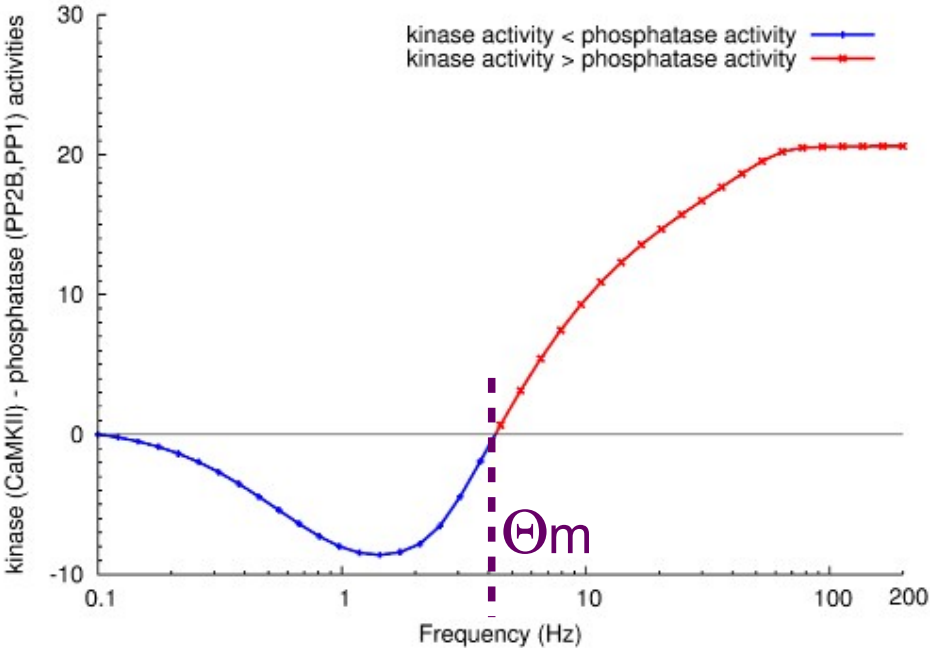
Constant catalytic rates of active enzyme

⟶ quantity of catalysed reaction events

μ integral of the activation curve

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





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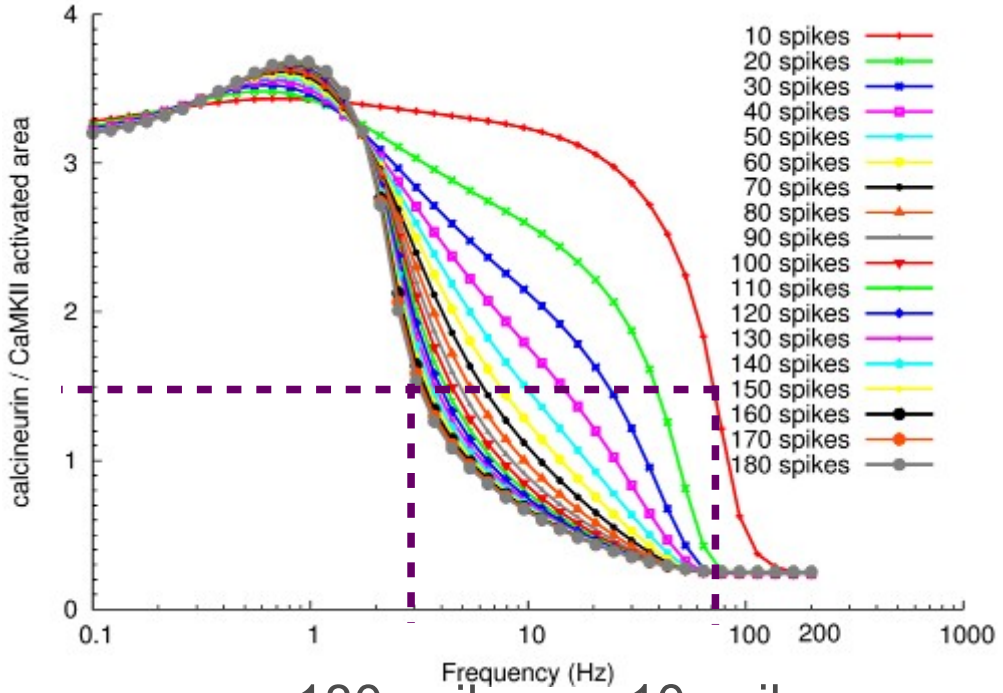
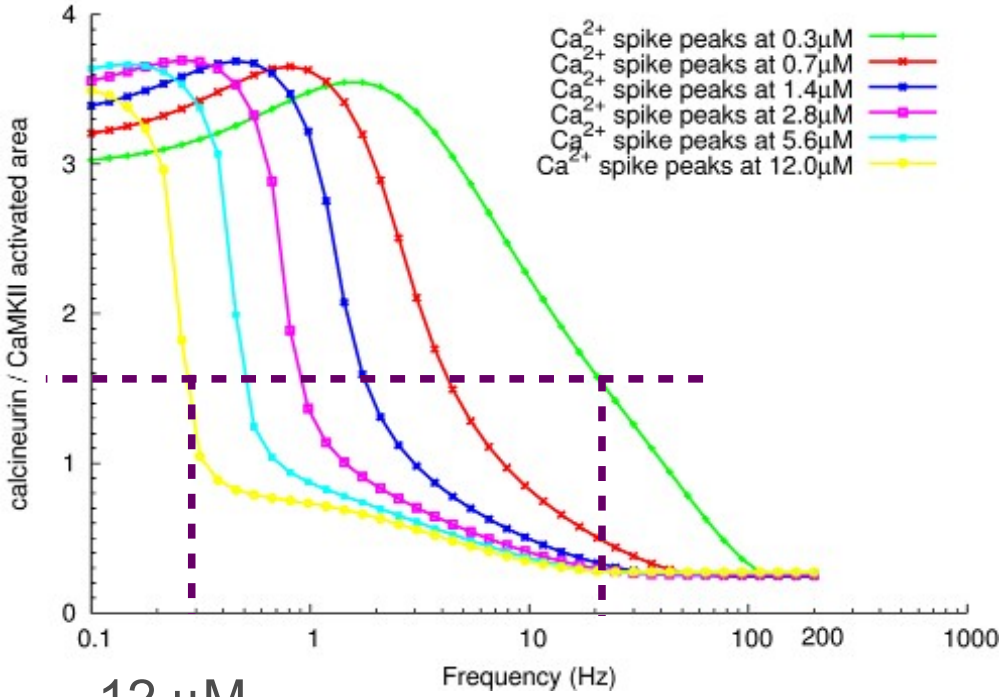
Effect of calcium duration and amount



180 spikes = 3 Hz

10 spikes = 70 Hz

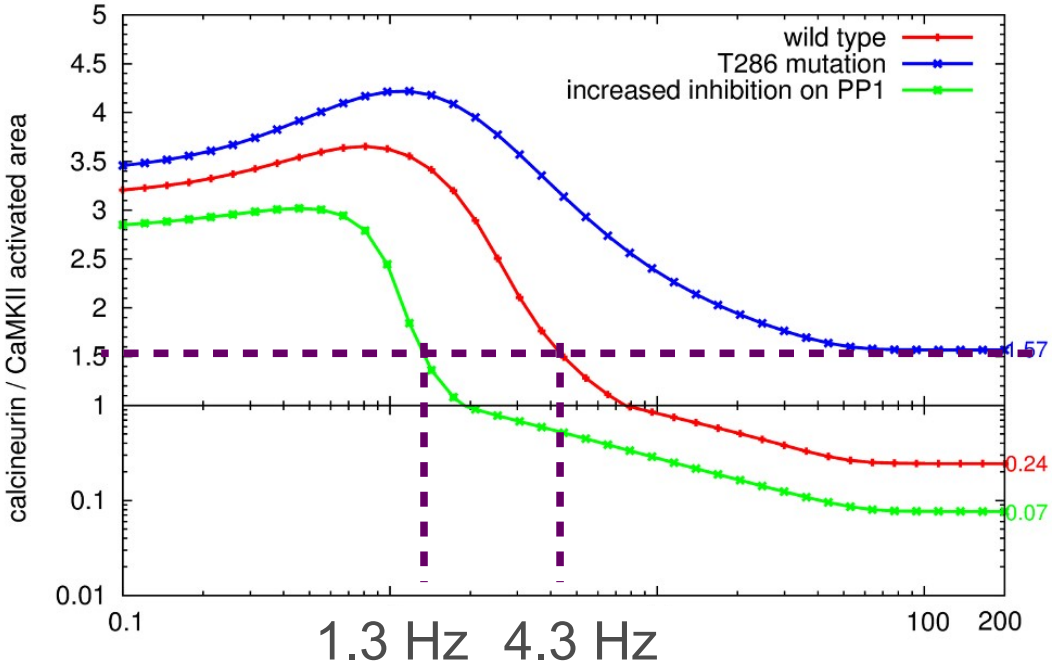


12 µM = 0.3 Hz

0.3 µM = 20 Hz

Prolonged or intense signals decrease 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

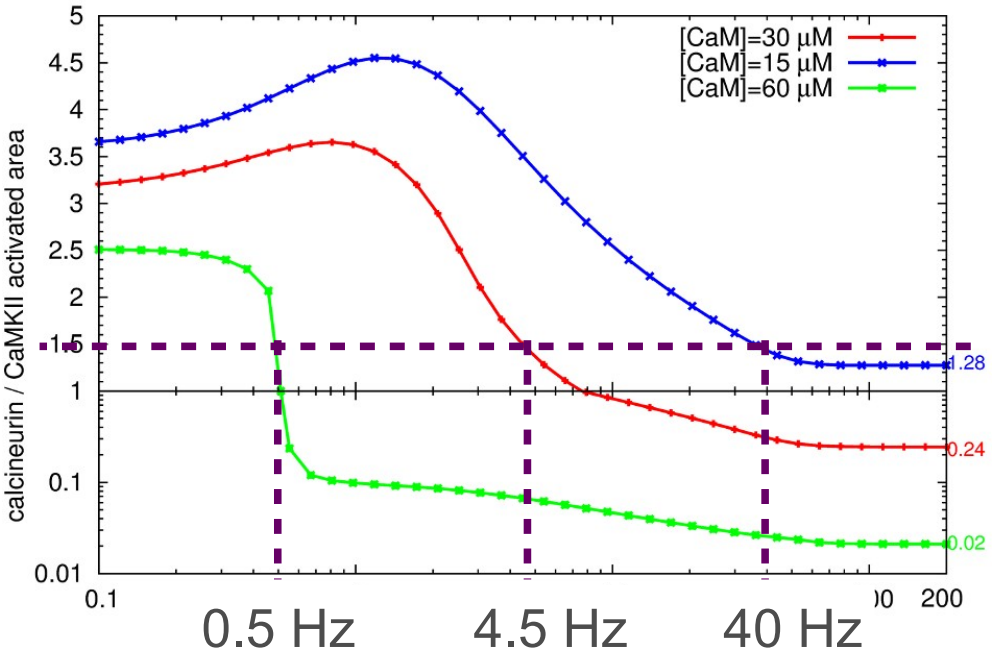
Lower deactivation of CaMKII

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

Proposed some direct interactions between CaN and CaMKII.

They got it completely wrong!



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Summary of part 2

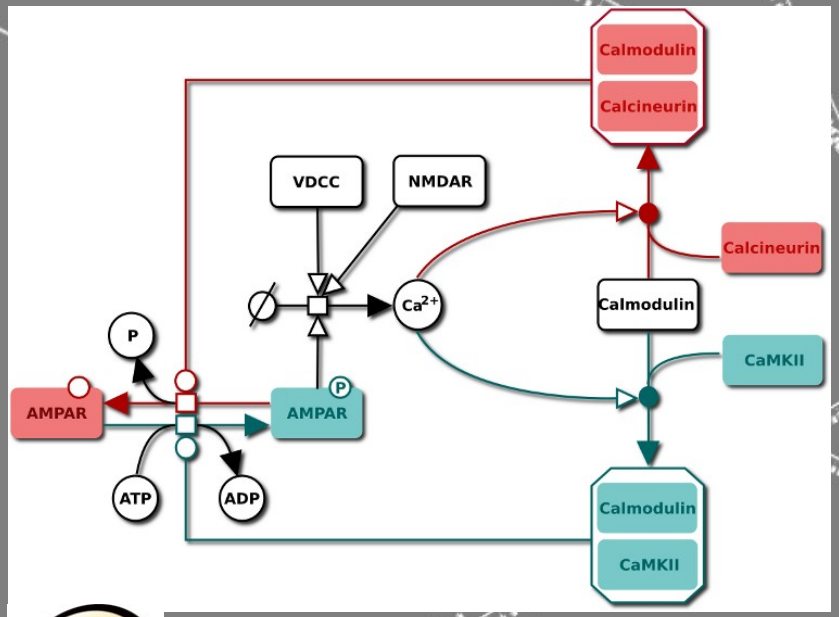
Allosteric stabilisation by targets triggers bistable CaM response to calcium. Above a certain frequency, CaM activation lasts longer than the initial signal.

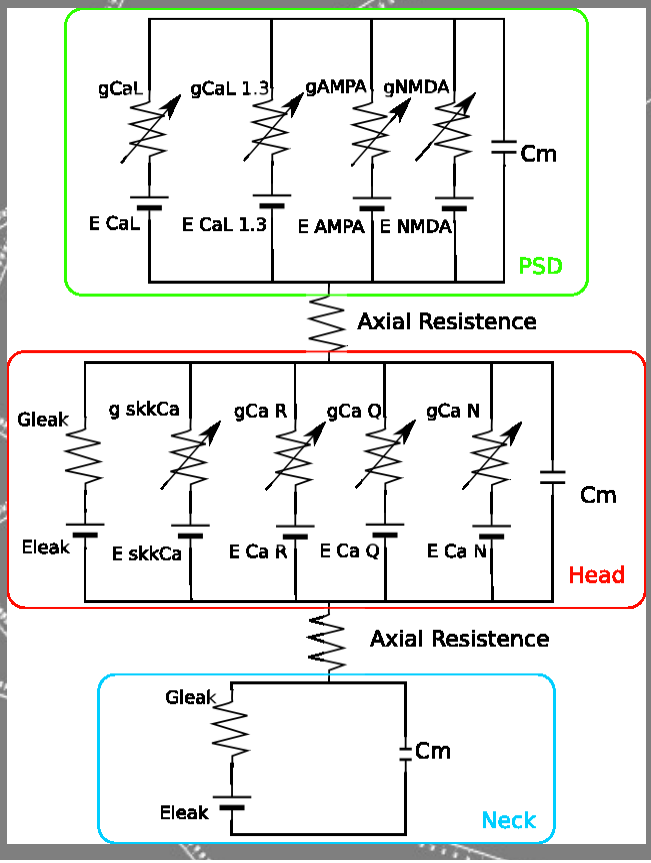
Calcium signals do not choose between CaN and CaMKII, *BOTH* enzymes are activated at *ALL* frequencies. The ratio of activity changes.

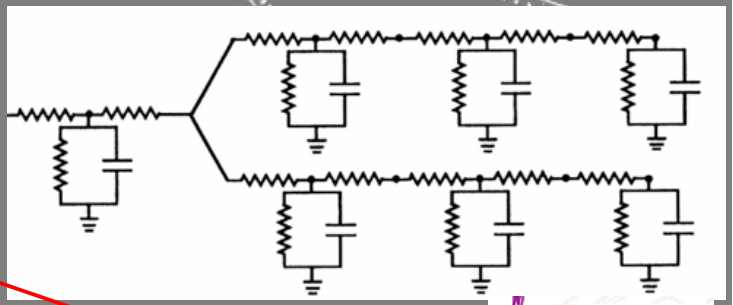
The frequency at which a synapse switches from a depression to a potentiation mode is not an intrinsic property of the synapse, but a dynamical one that depends on the length and amplitude of stimulations.

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



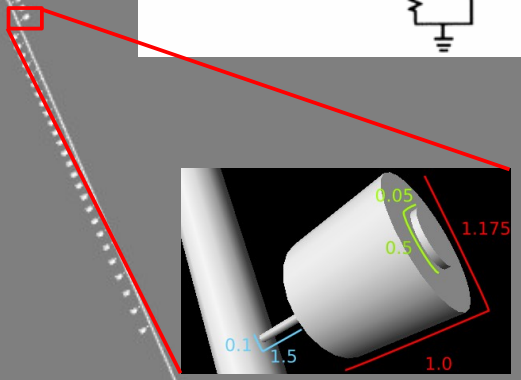




1504 spines

4761 compartments

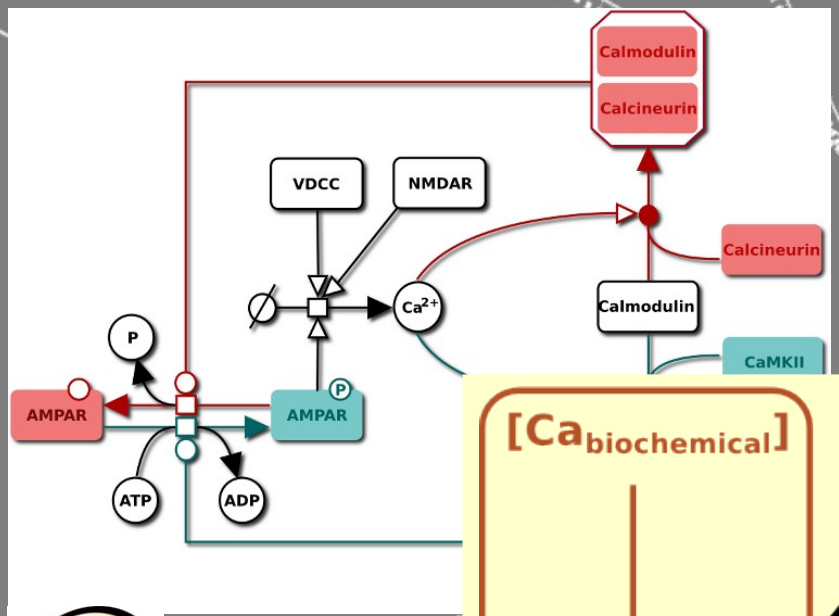
16362 channels

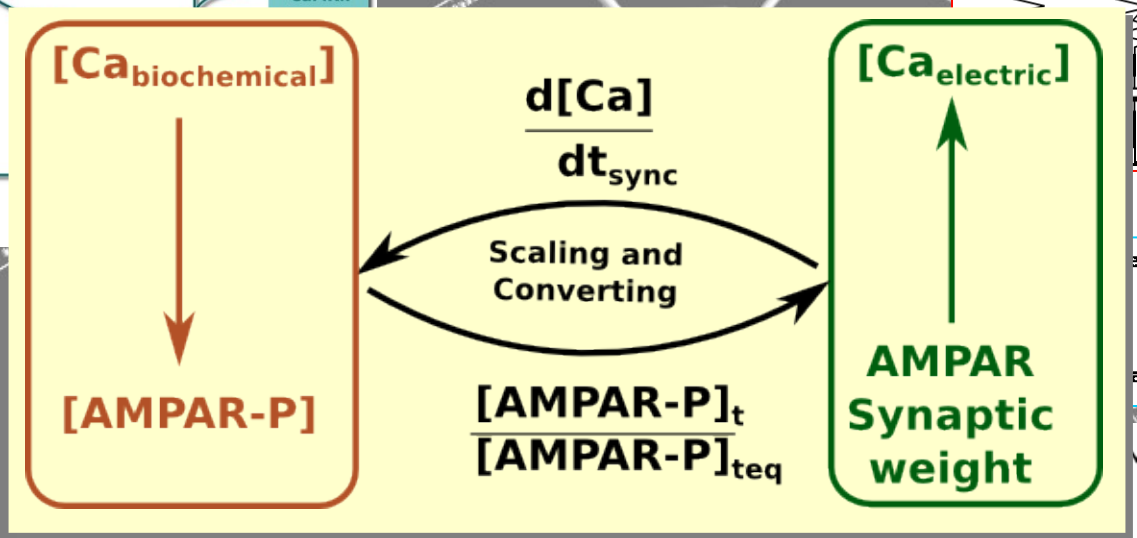


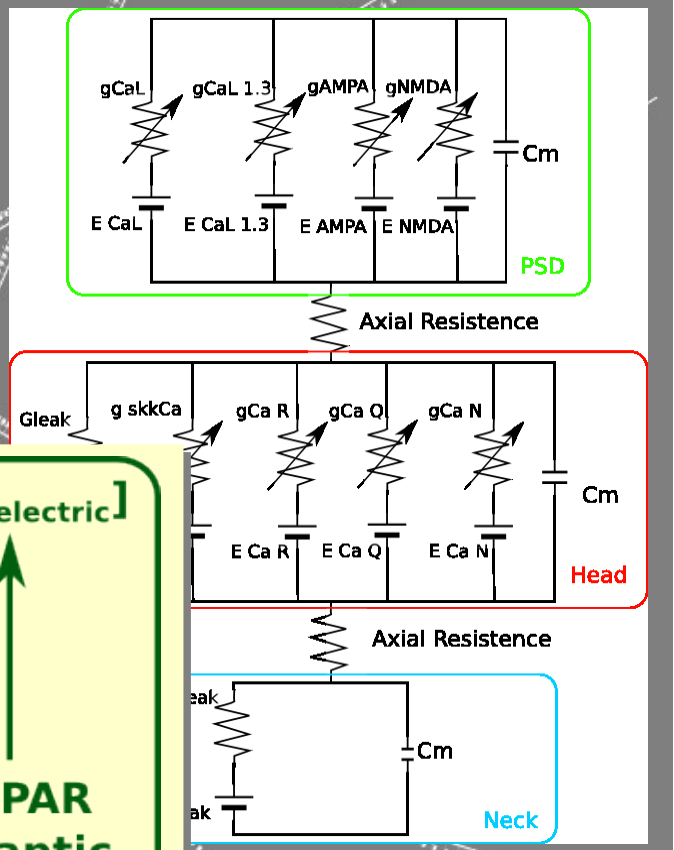
Mattioni M, Cohen U, Le Novère N (2012)
Frontiers Neuroinfo, 6:20

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

Whole cell: electro-biochemical models







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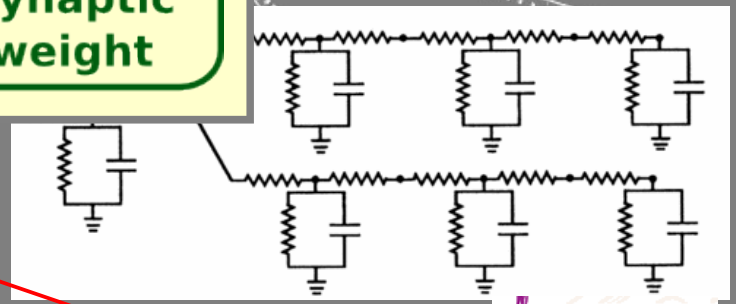
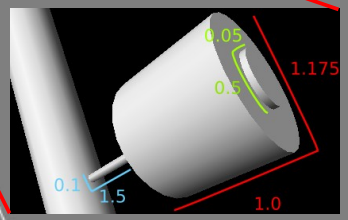




Mattioni M, Cohen U, Le Novère N (2012)
Frontiers Neuroinfo, 6:20

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

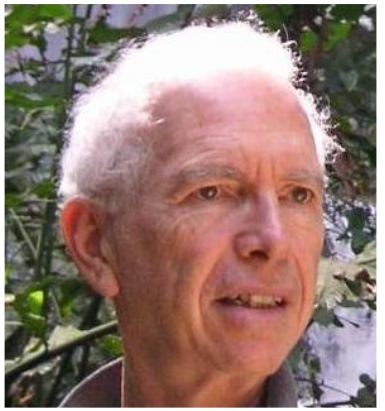
- Developers of ECell3, COPASI, Scilab

Melanie Stefan

Lu Li

Denis Brun

Michele Mattioni



Stuart Edelstein

Massimo Lai