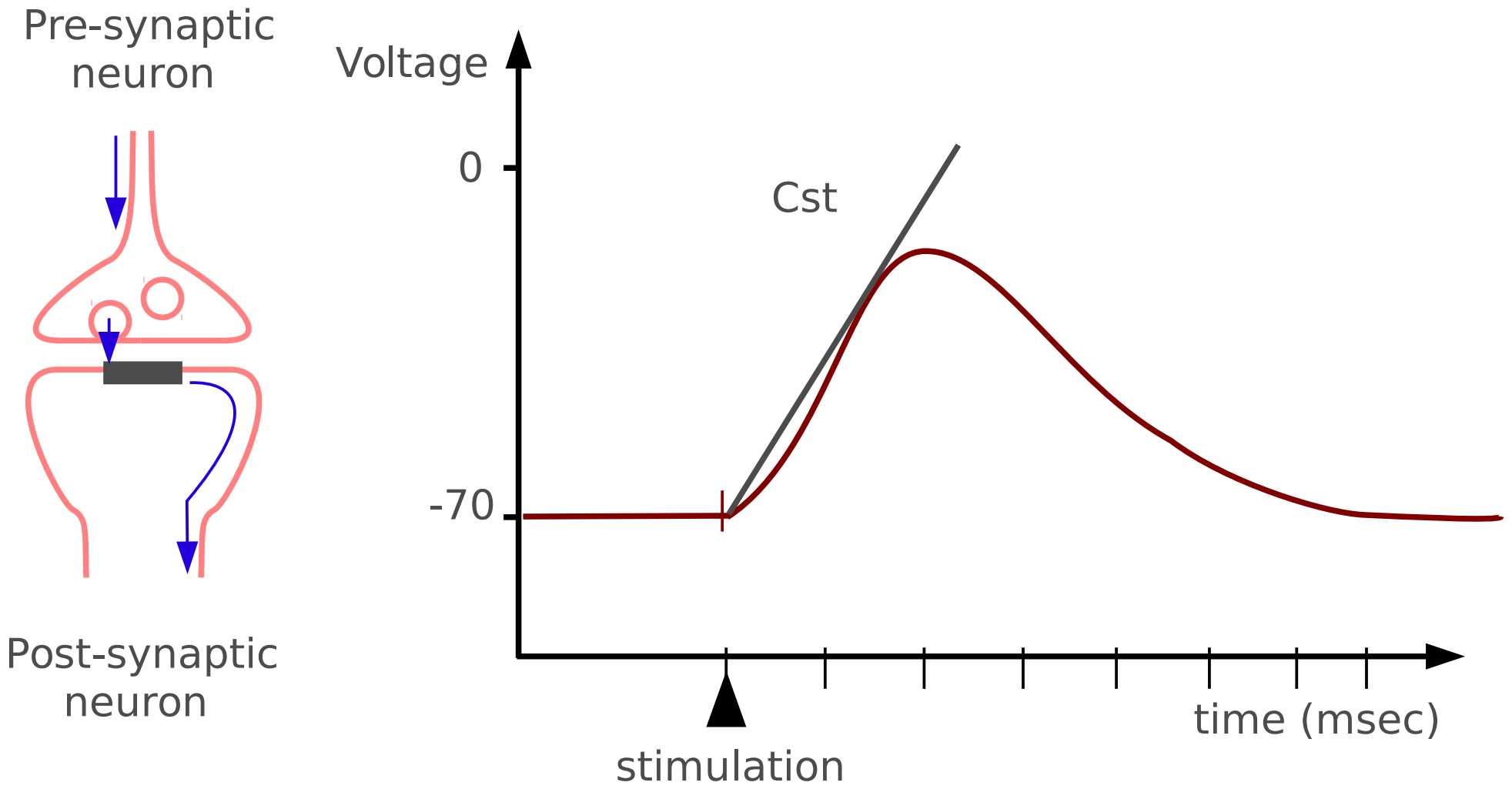
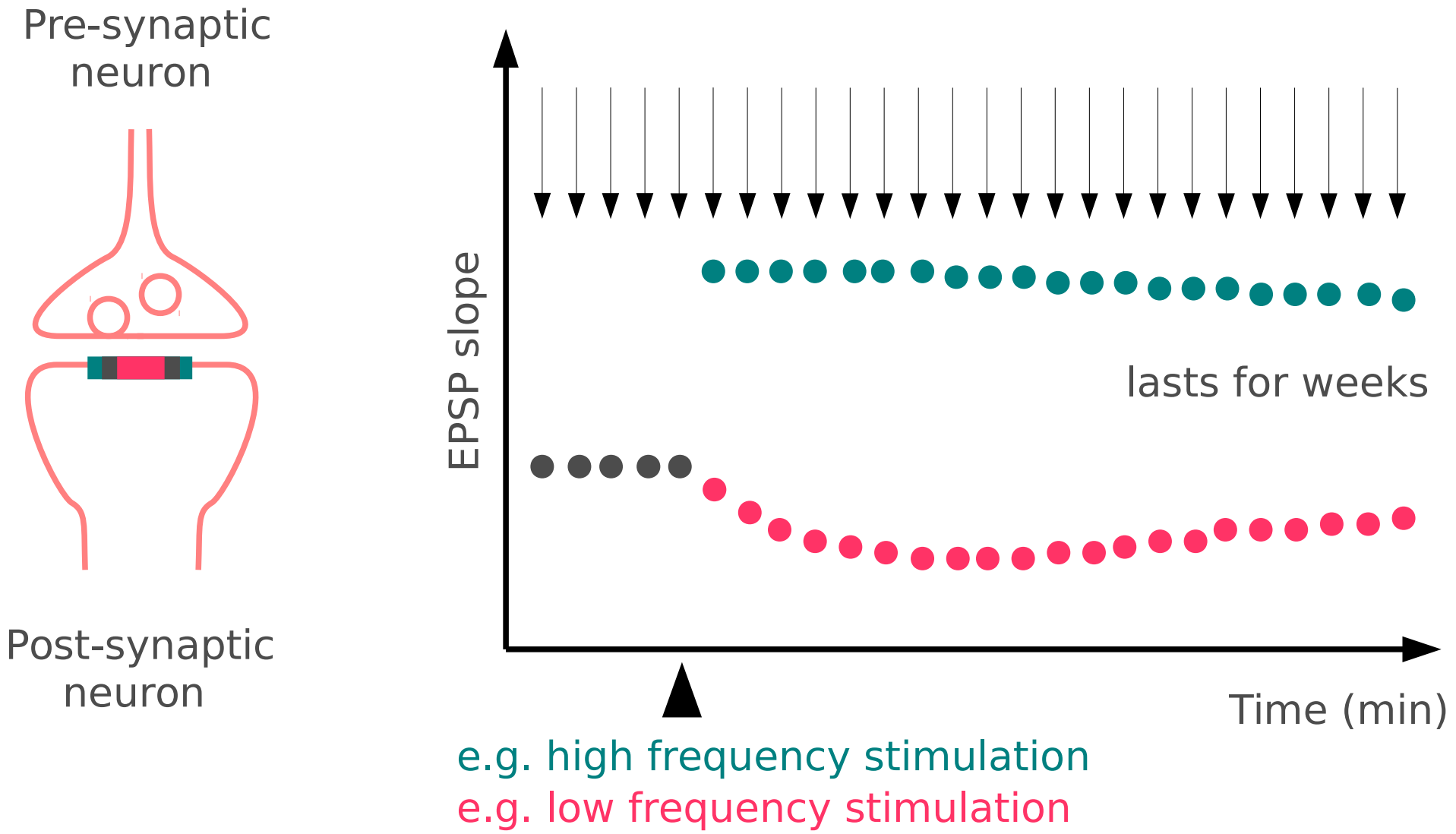
Modelling the regulation of bidirectional synaptic plasticity by allosteric calcium sensors

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

Excitatory post-synaptic potential



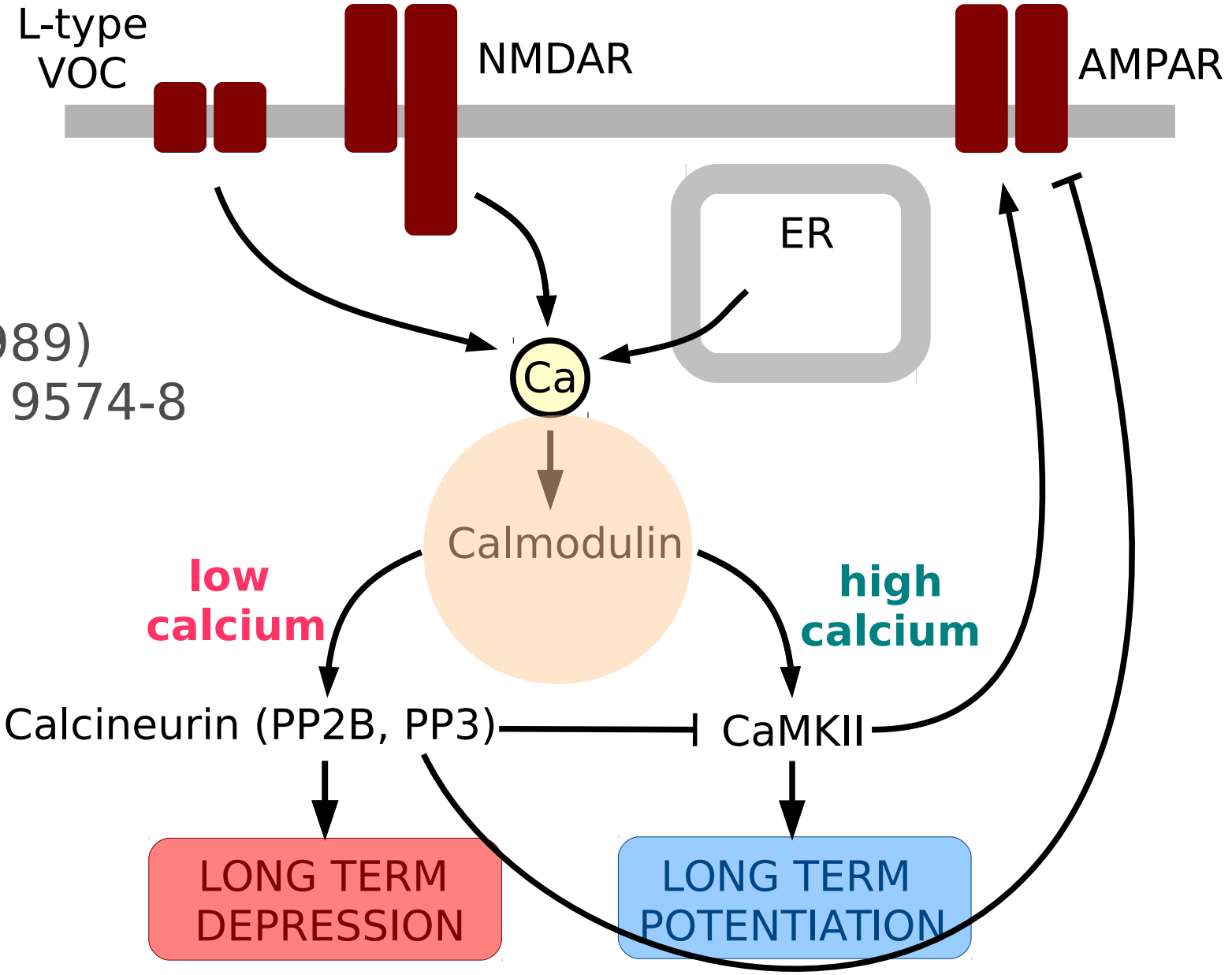
Bidirectional synaptic plasticity



The calcium theory of synaptic plasticity

Lisman (1989)
PNAS, 86: 9574-8

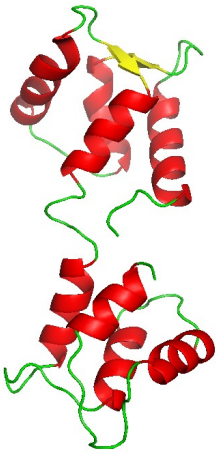
Calmodulin, the memory switchboard



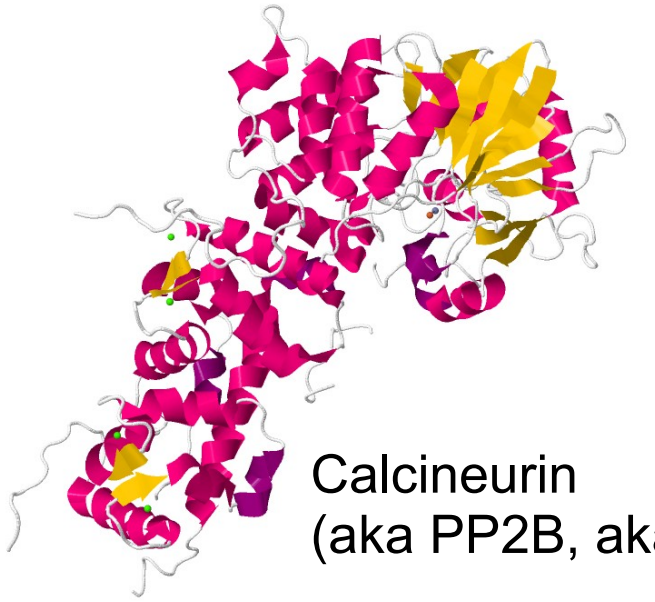
Lisman (1989)
PNAS, 86: 9574-8

Calcium

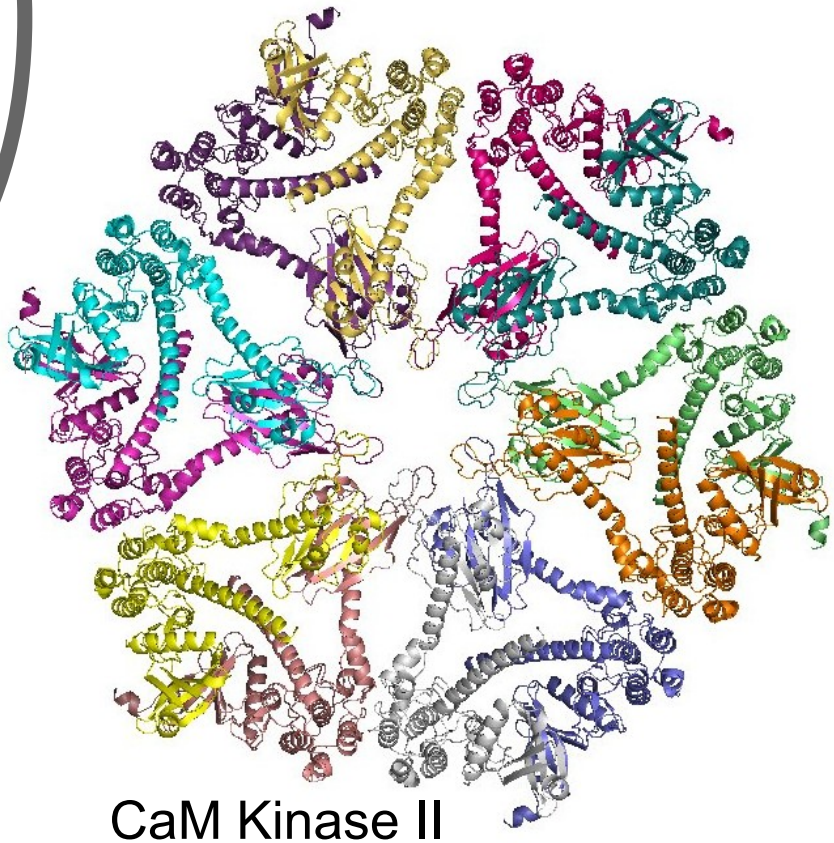
Calmodulin



Neurogranin

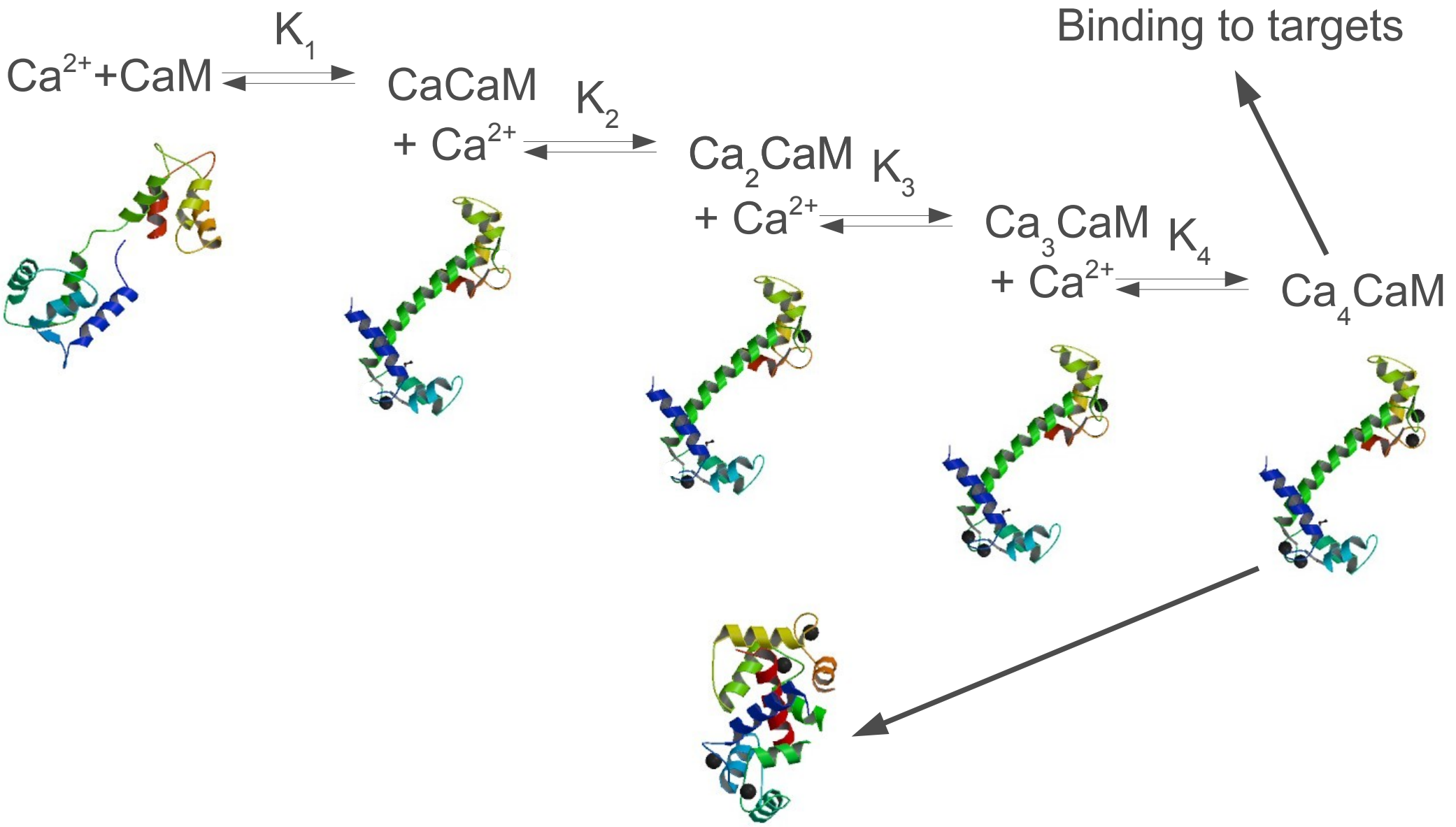


Calcineurin
(aka PP2B, aka PP3)

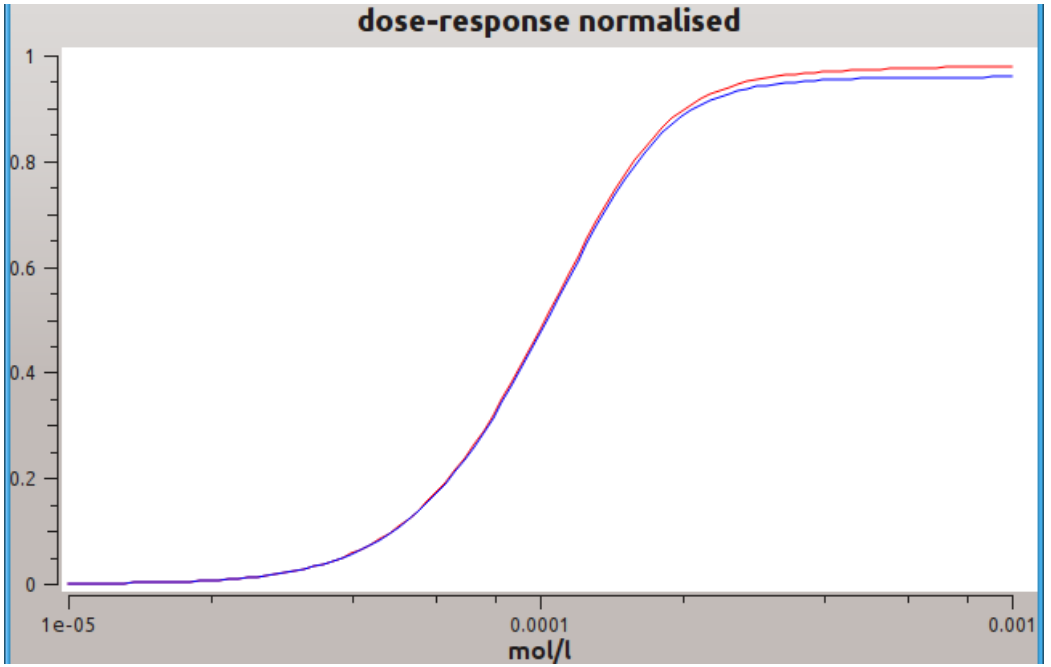


CaM Kinase II

Adair-Klotz induced-fit model

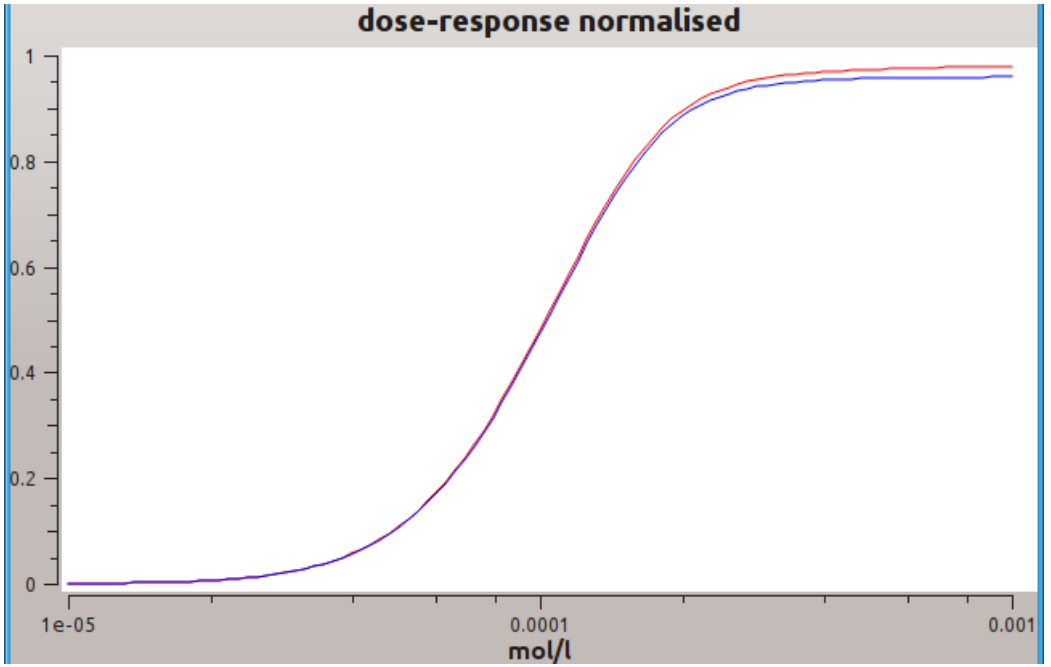


That does not work ...



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

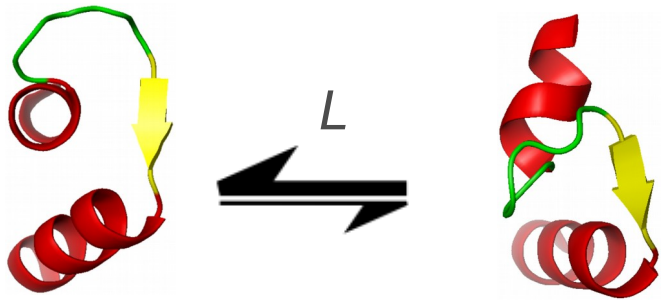
We knew it would not work

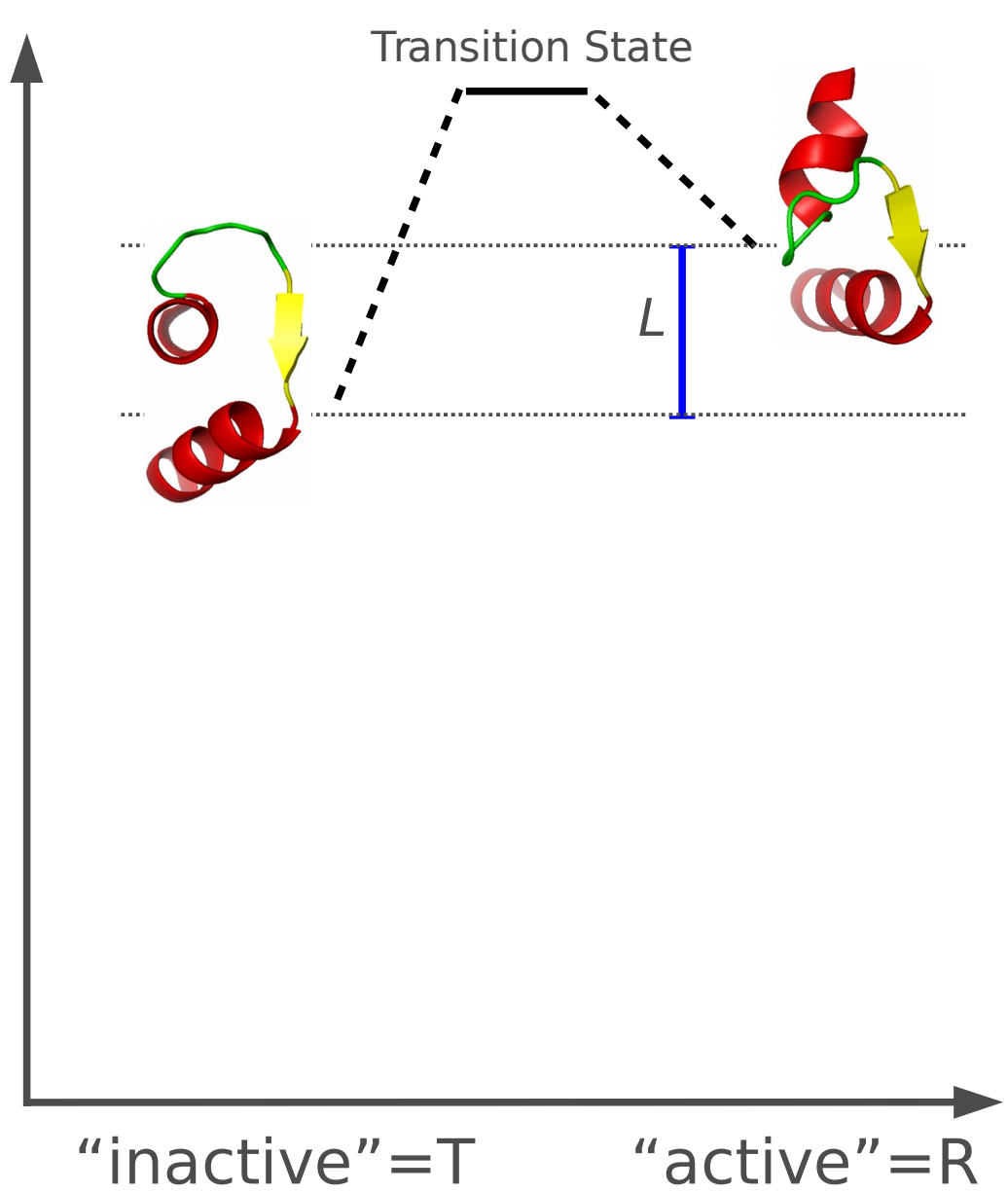


[CaN]=[CamKII]=[CaM]/10 ;
Kd_CaMKII = 10xKd_CaN;
Software COPASI

- 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])

1 Modulation of thermal equilibria ≠ induced-fit

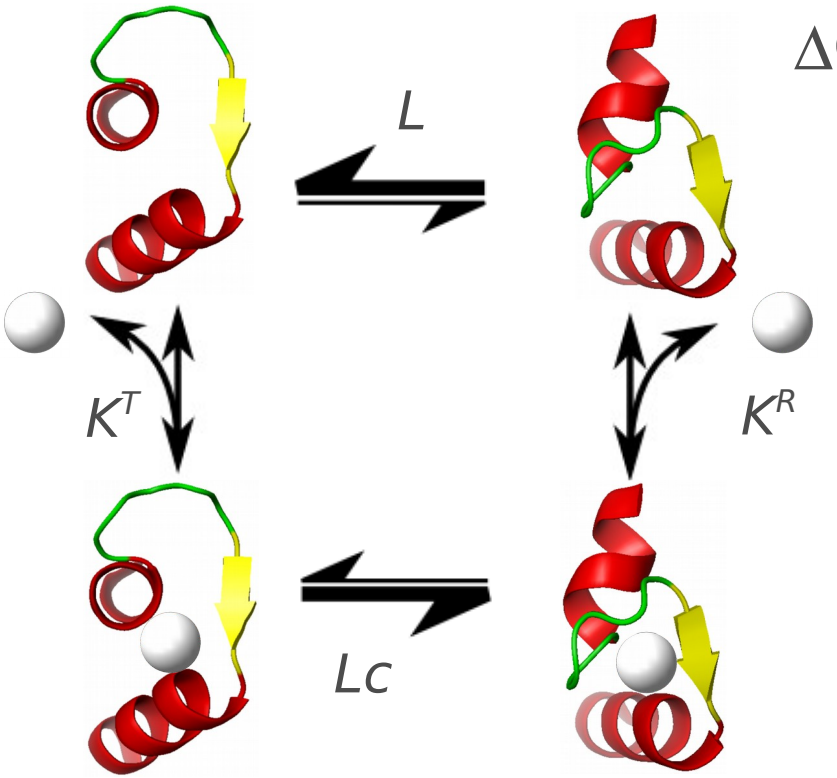




$$L = \frac{[T_0]}{[R_0]}$$

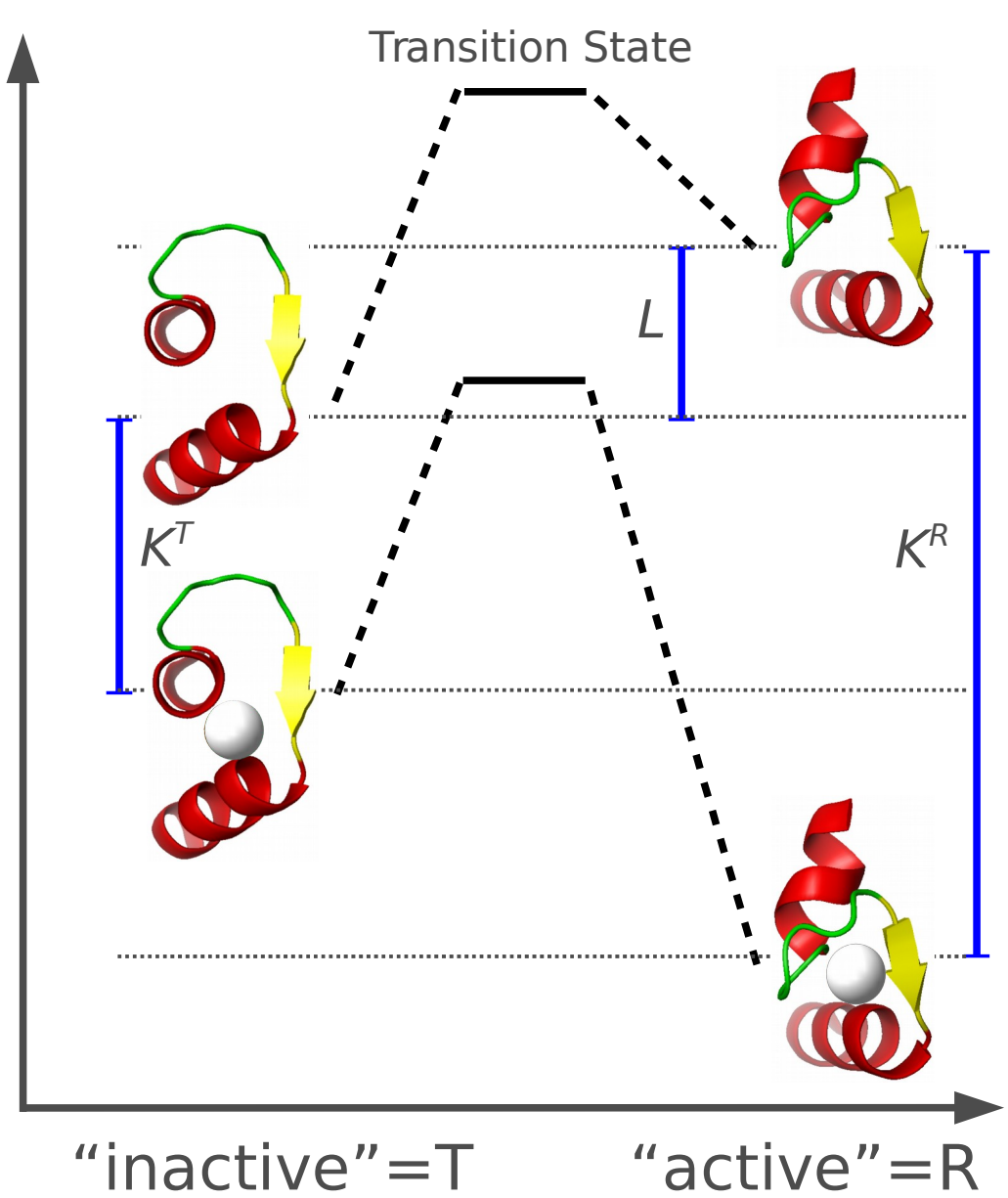
$L \gg 1 \Rightarrow$ Equilibrium strongly biased

1 Modulation of thermal equilibria ≠ induced-fit

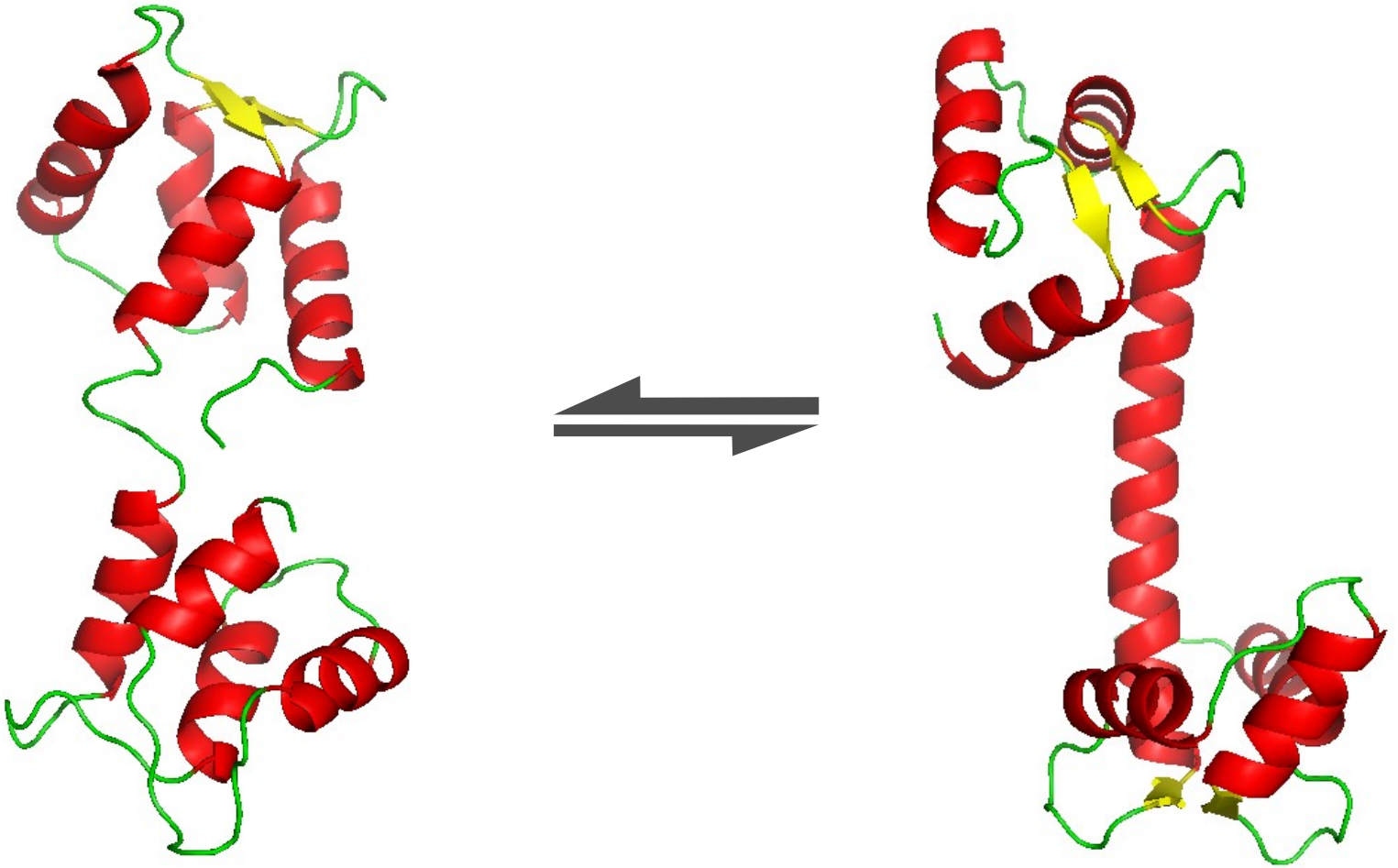


$$L = \frac{[T_0]}{[R_0]} \qquad c = \frac{K^R}{K^T}$$

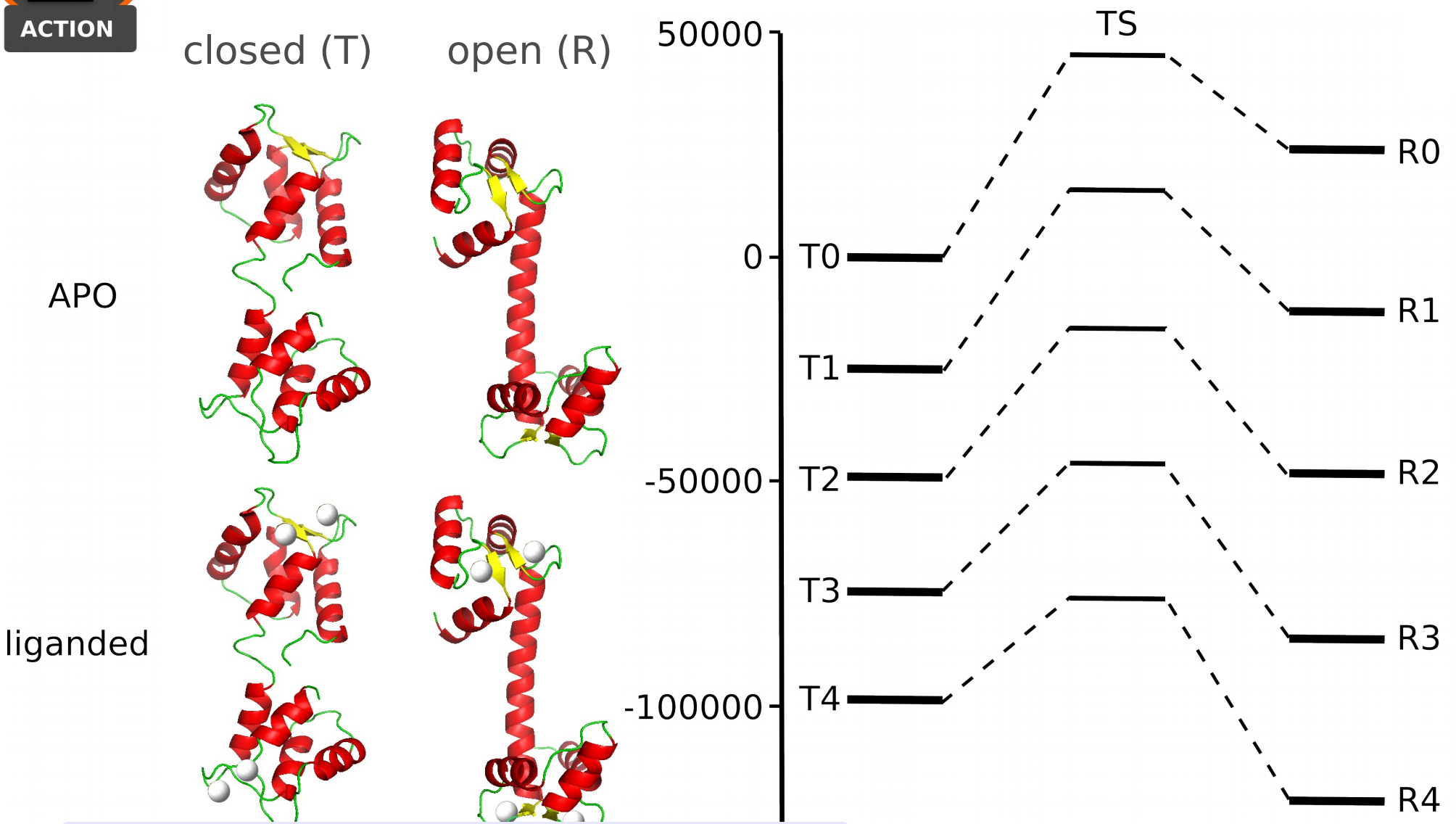
$c \ll 1 \Rightarrow$ ligand has strong effect



2 Concerted transitions ≠ sequential model

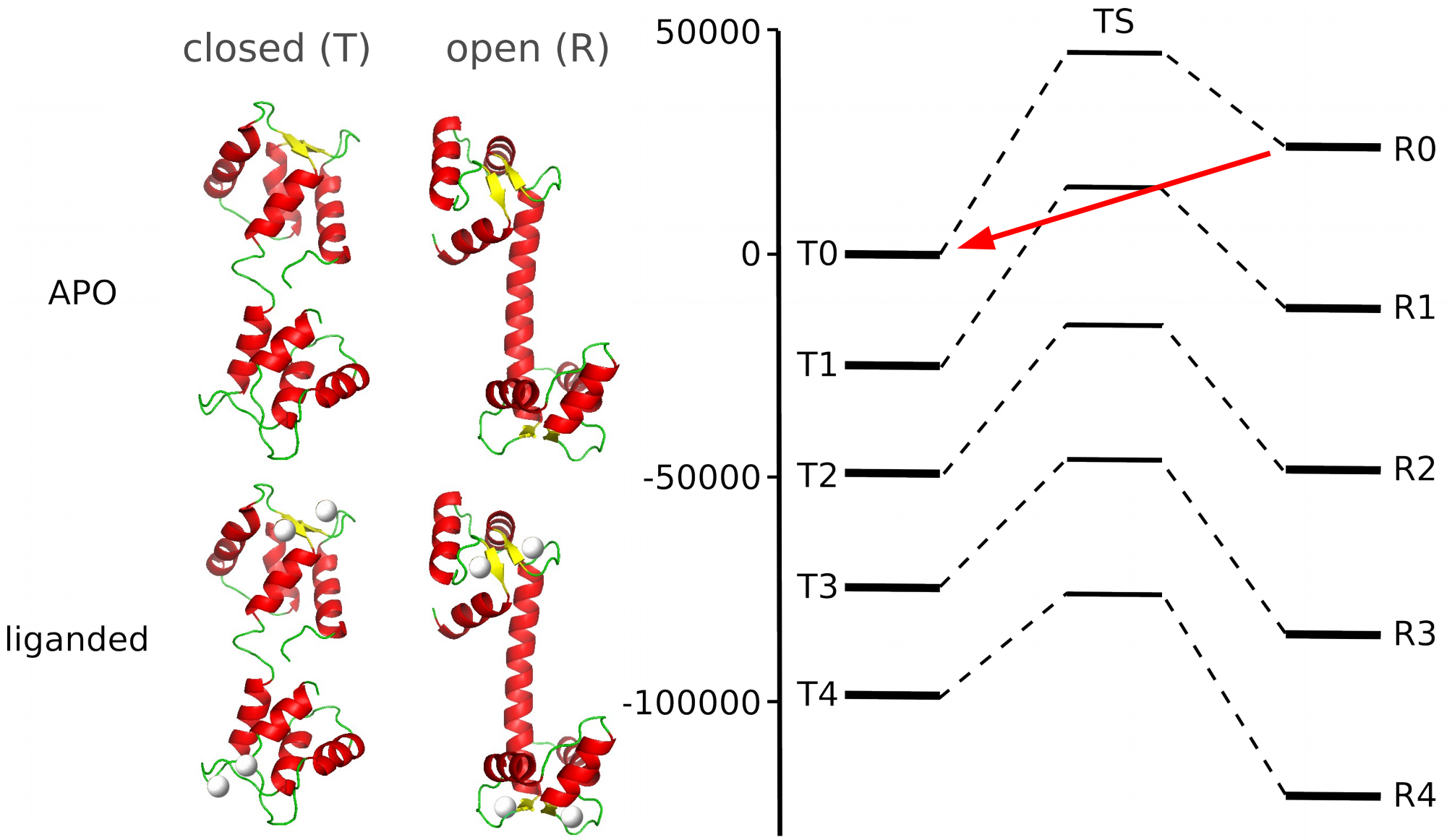


Allosteric model of Calmodulin activation

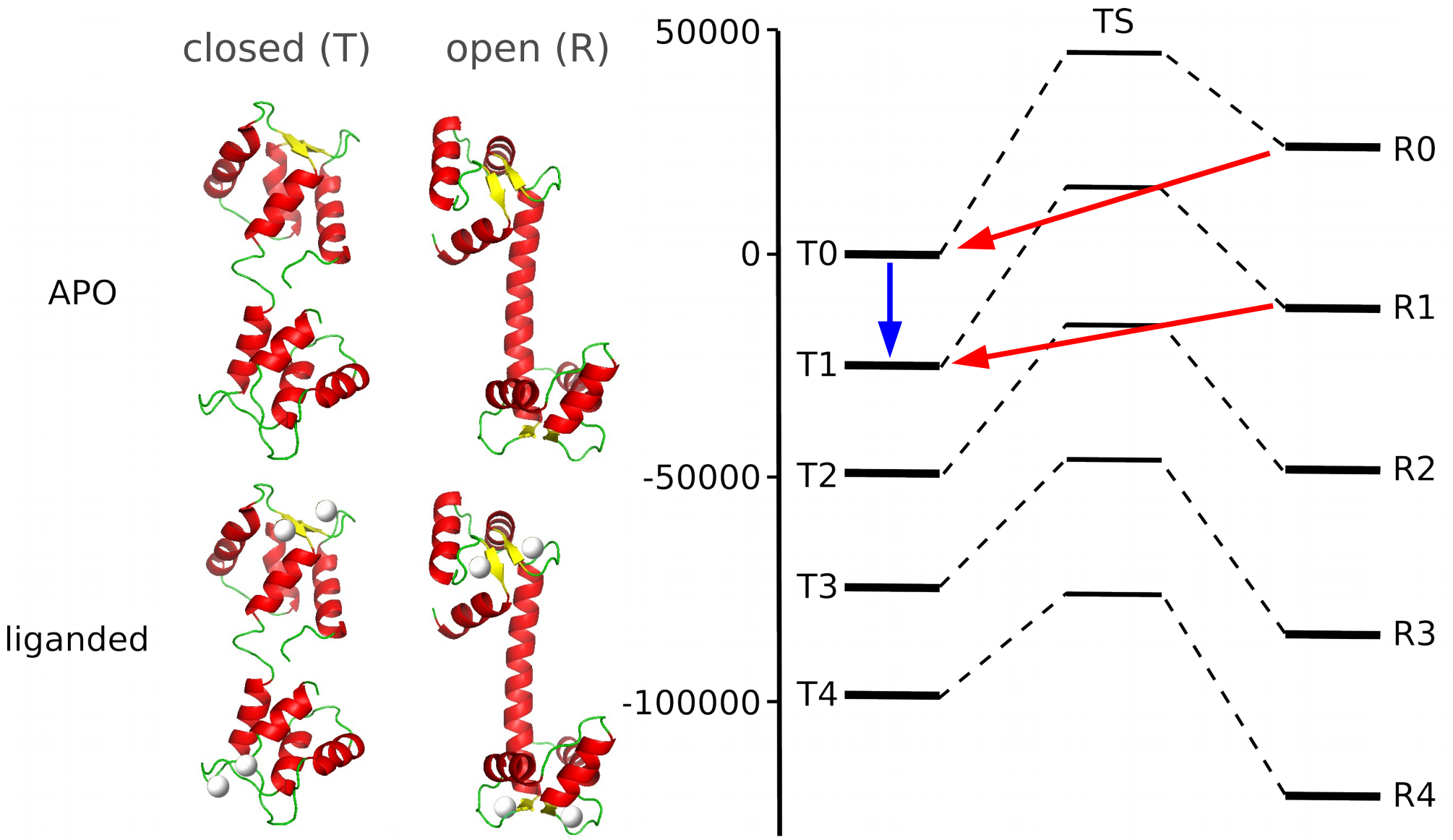


Stefan MI, Edelstein SJ, Le Novère N (2008, 2009)

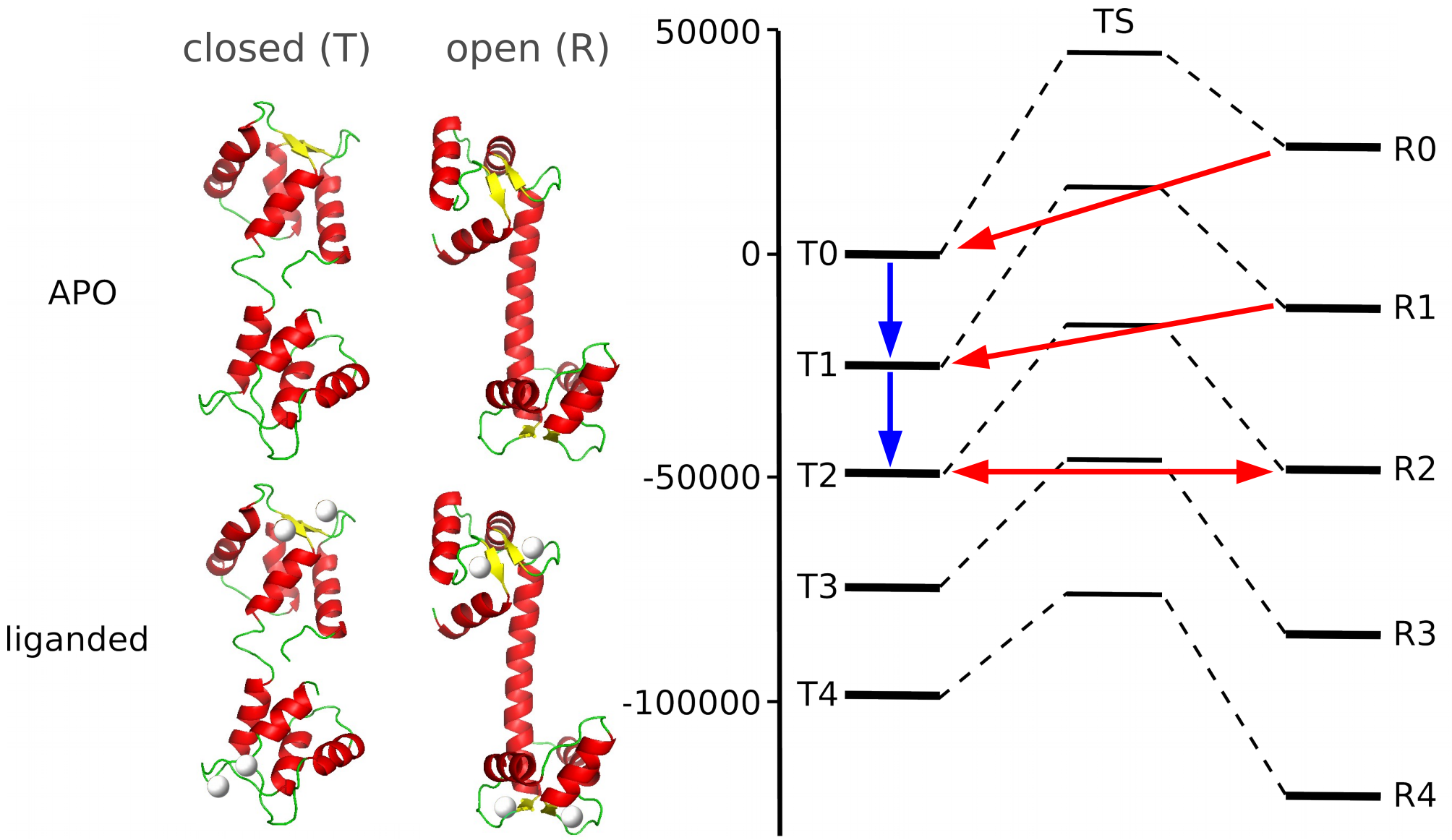
Allosteric model of Calmodulin activation



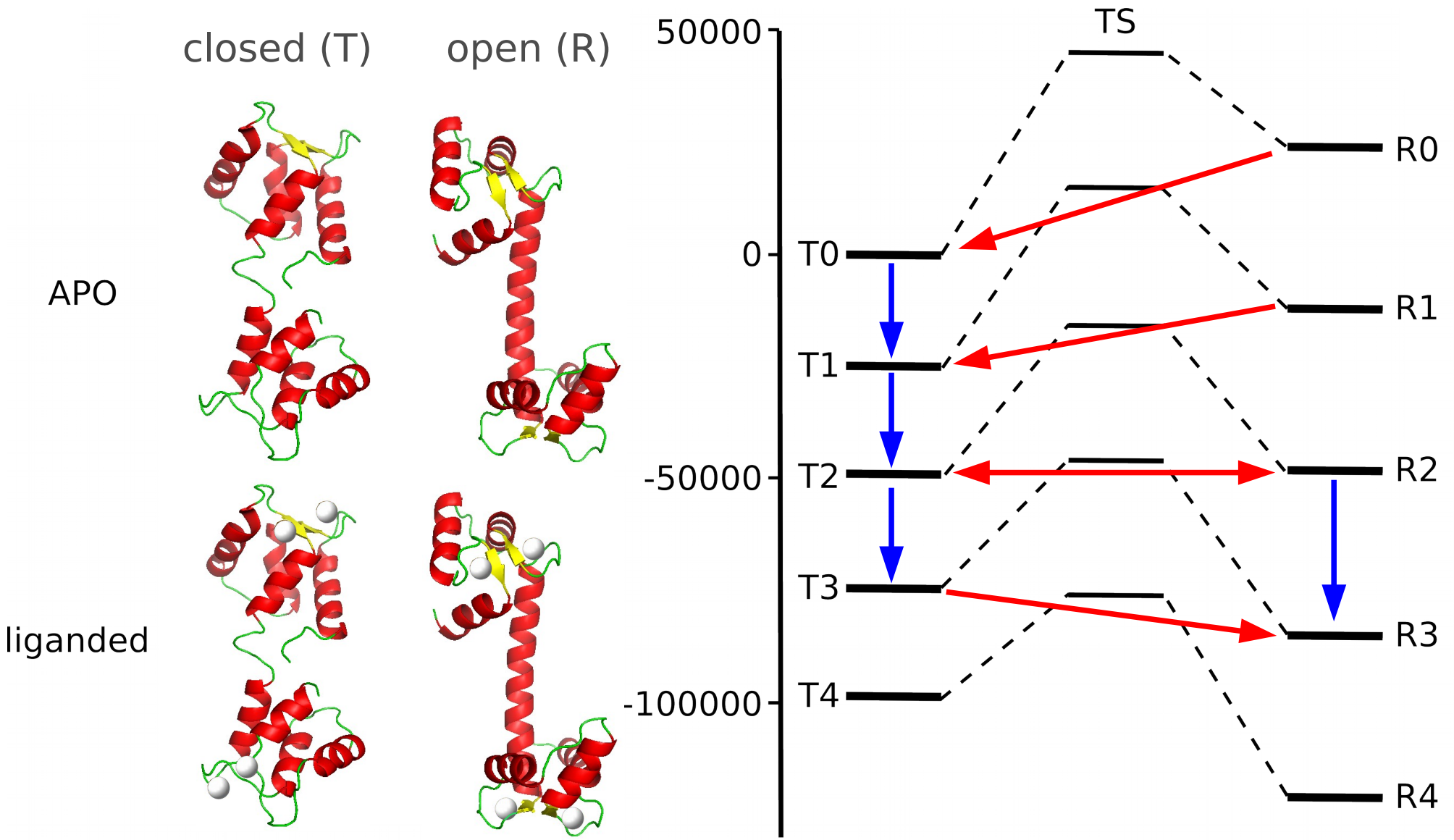
Allosteric model of Calmodulin activation



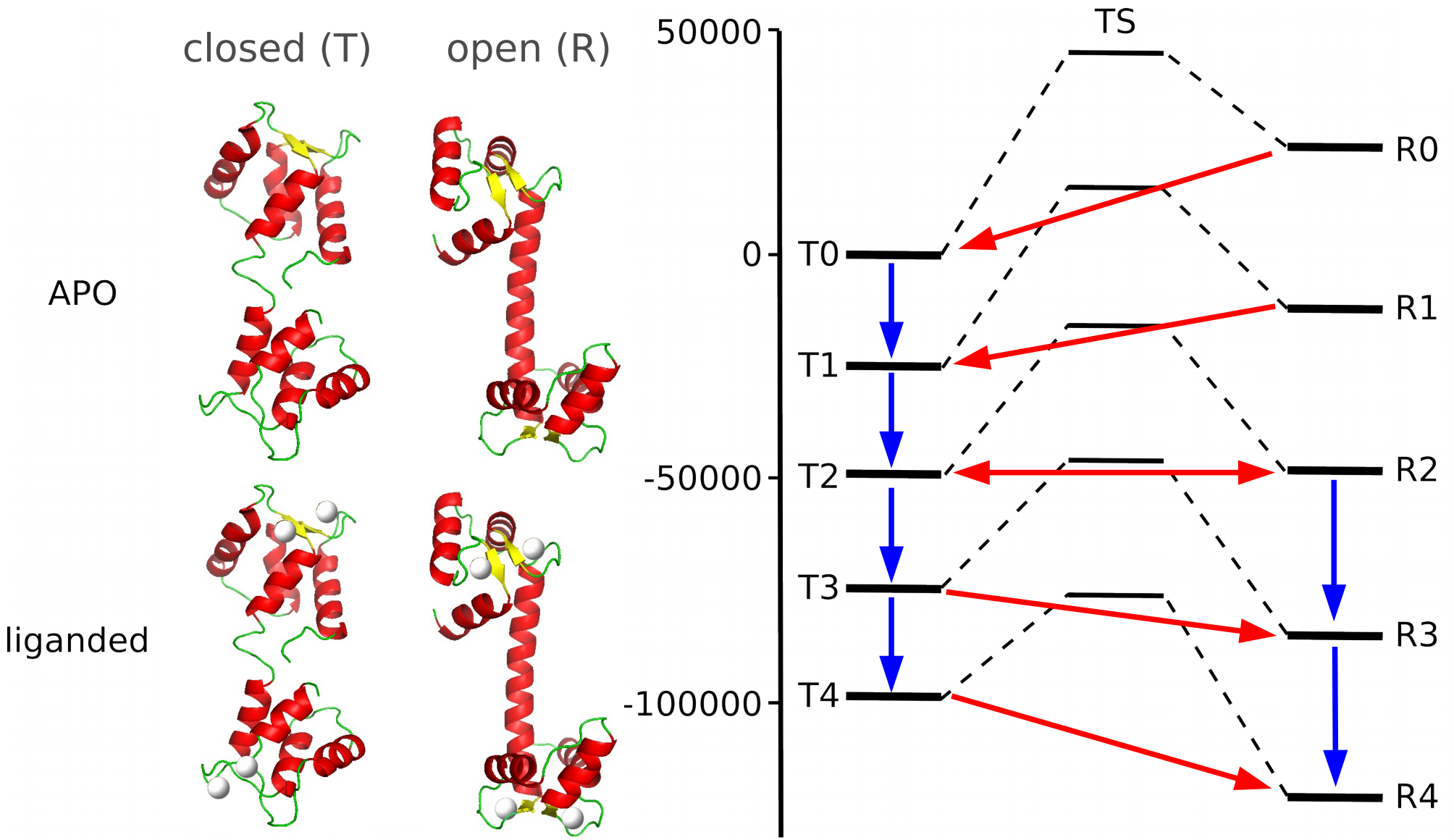
Allosteric model of Calmodulin activation



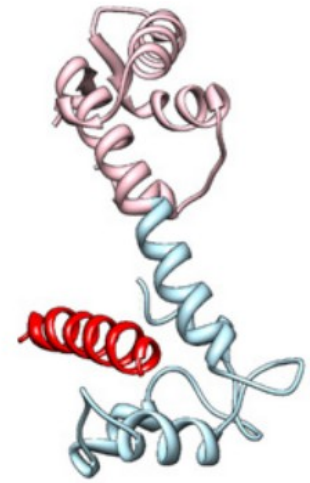
Allosteric model of Calmodulin activation



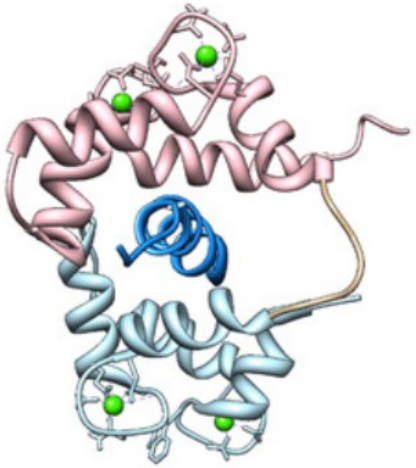
Allosteric model of Calmodulin activation



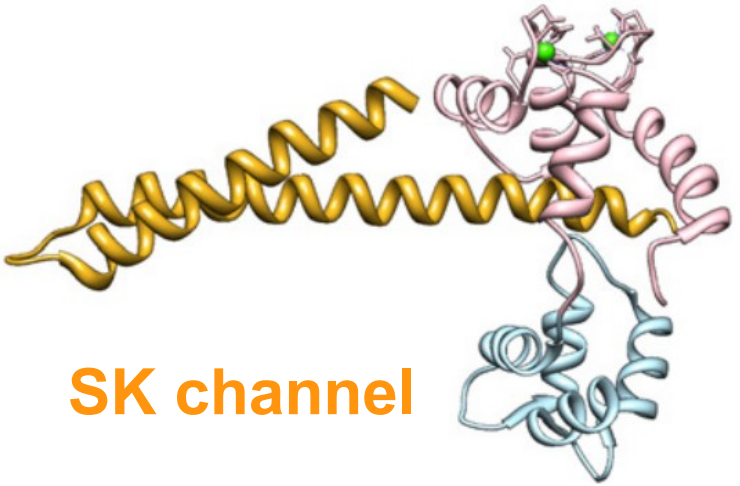
Different targets stabilise different states



Neurogranin



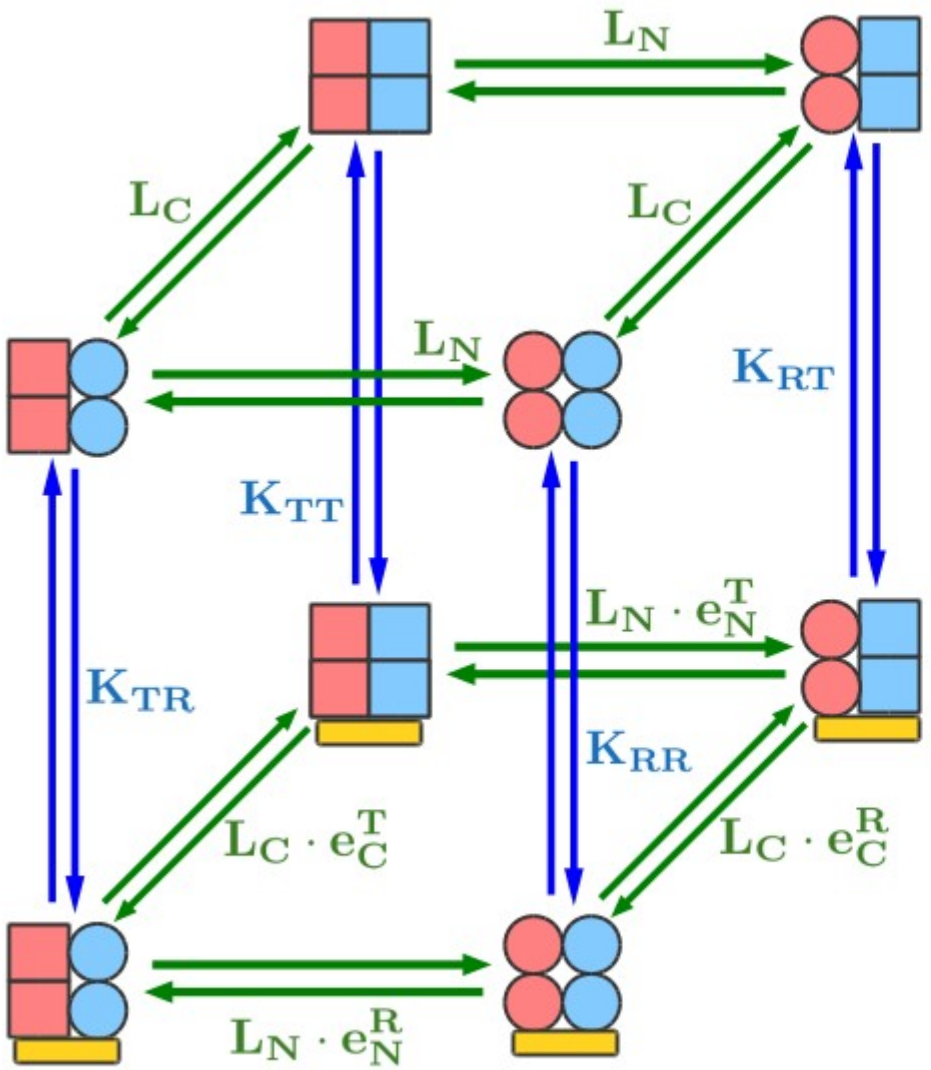
MLCK

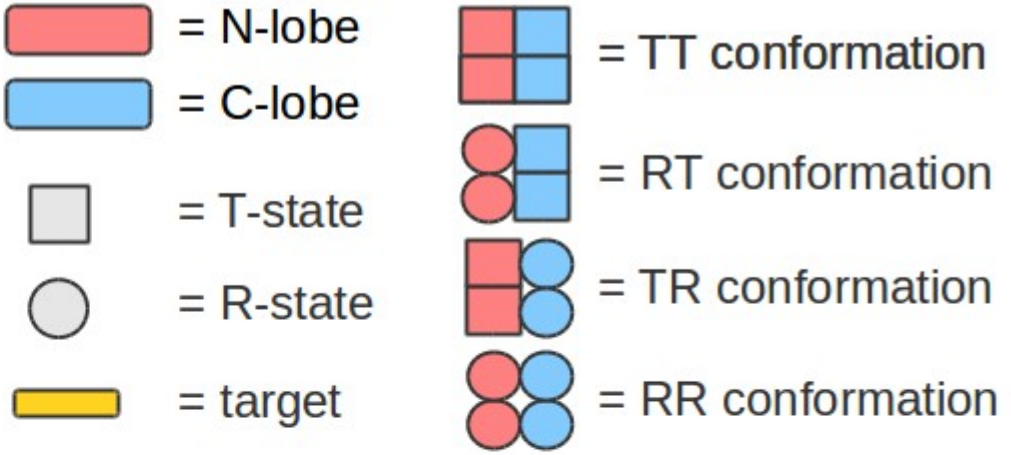


SK channel

Lai M, Brun D, Edelstein SJ, Le Novère N (2015)
Lai M, Edelstein SJ, Le Novère N (in preparation)

Hemiconcerted model of calmodulin



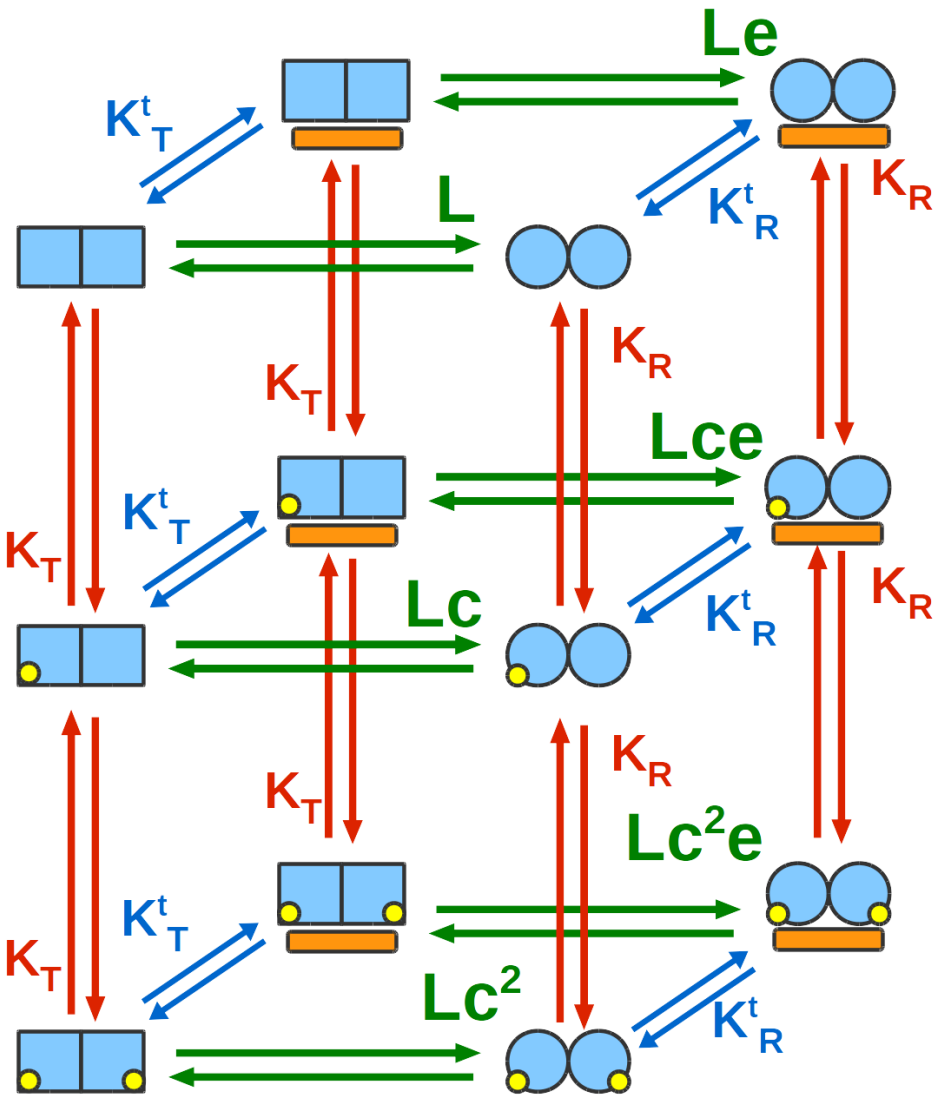


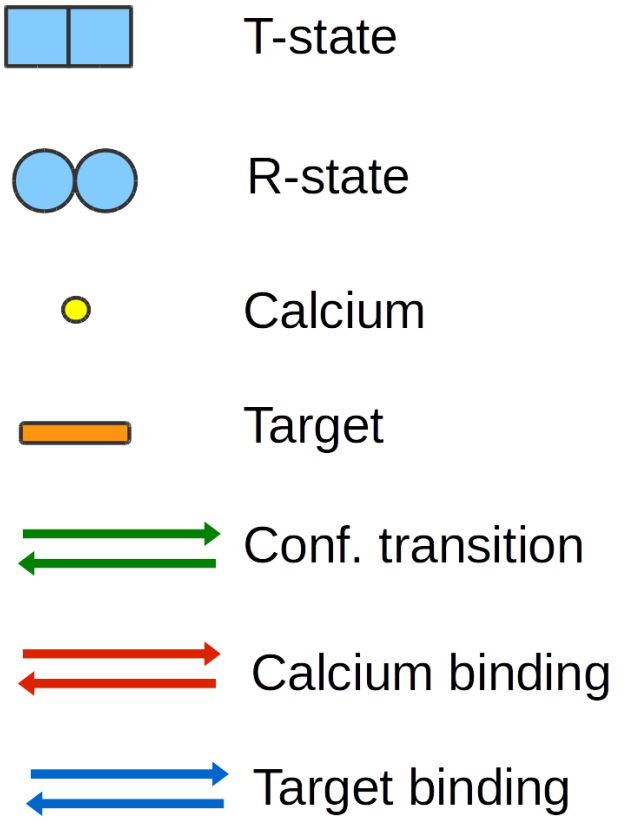
$$L_N = \frac{[TT]}{[RT]} = \frac{[TR]}{[RR]} \qquad L_C = \frac{[TT]}{[TR]} = \frac{[RT]}{[RR]}$$

$$e_N^R = \frac{K_{RR}}{K_{TR}} \qquad e_C^R = \frac{K_{RR}}{K_{RT}}$$

$$e_N^T = \frac{K_{RT}}{K_{TT}} \qquad e_C^T = \frac{K_{TR}}{K_{TT}}$$

Bindings of calcium and targets

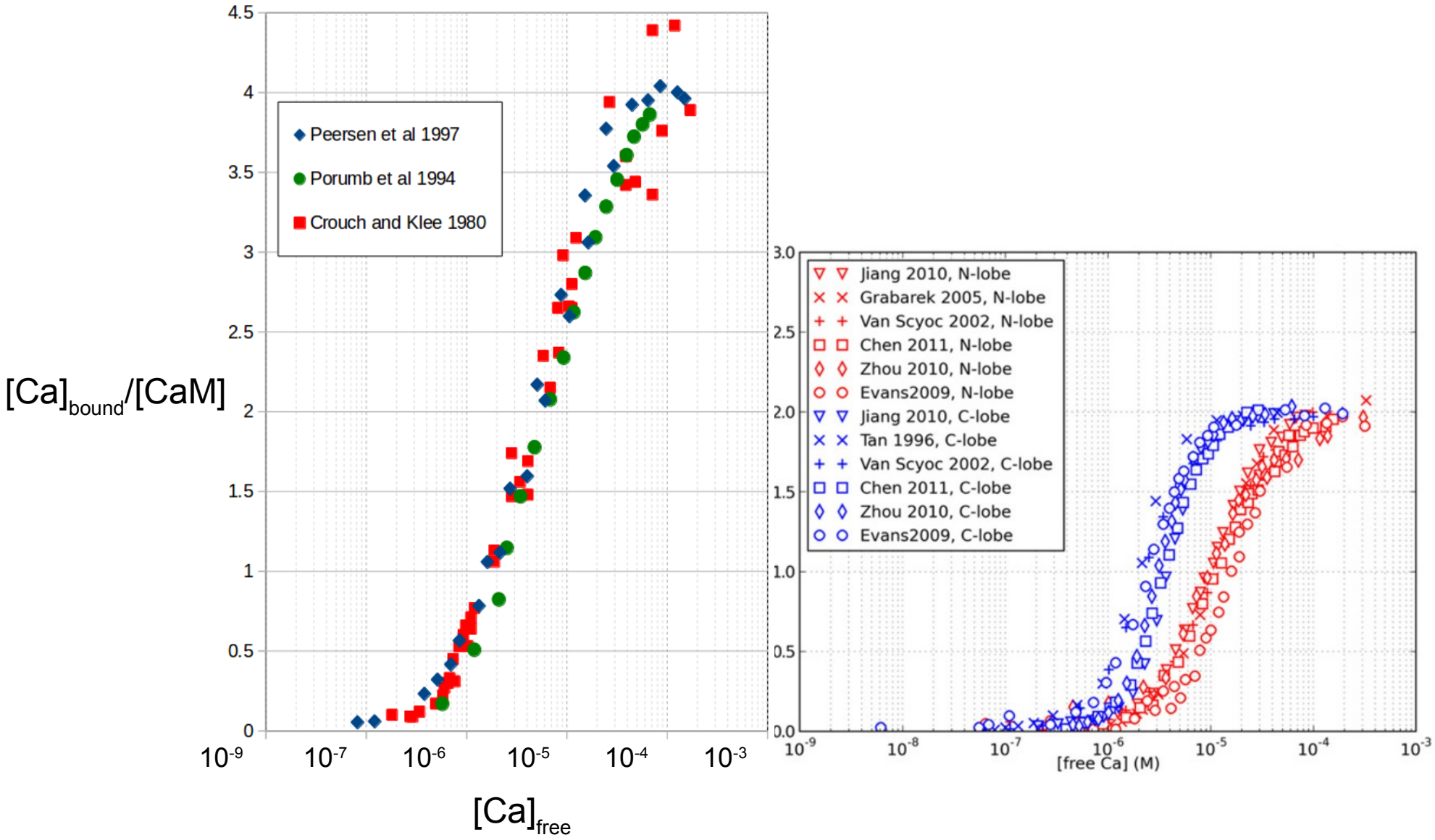




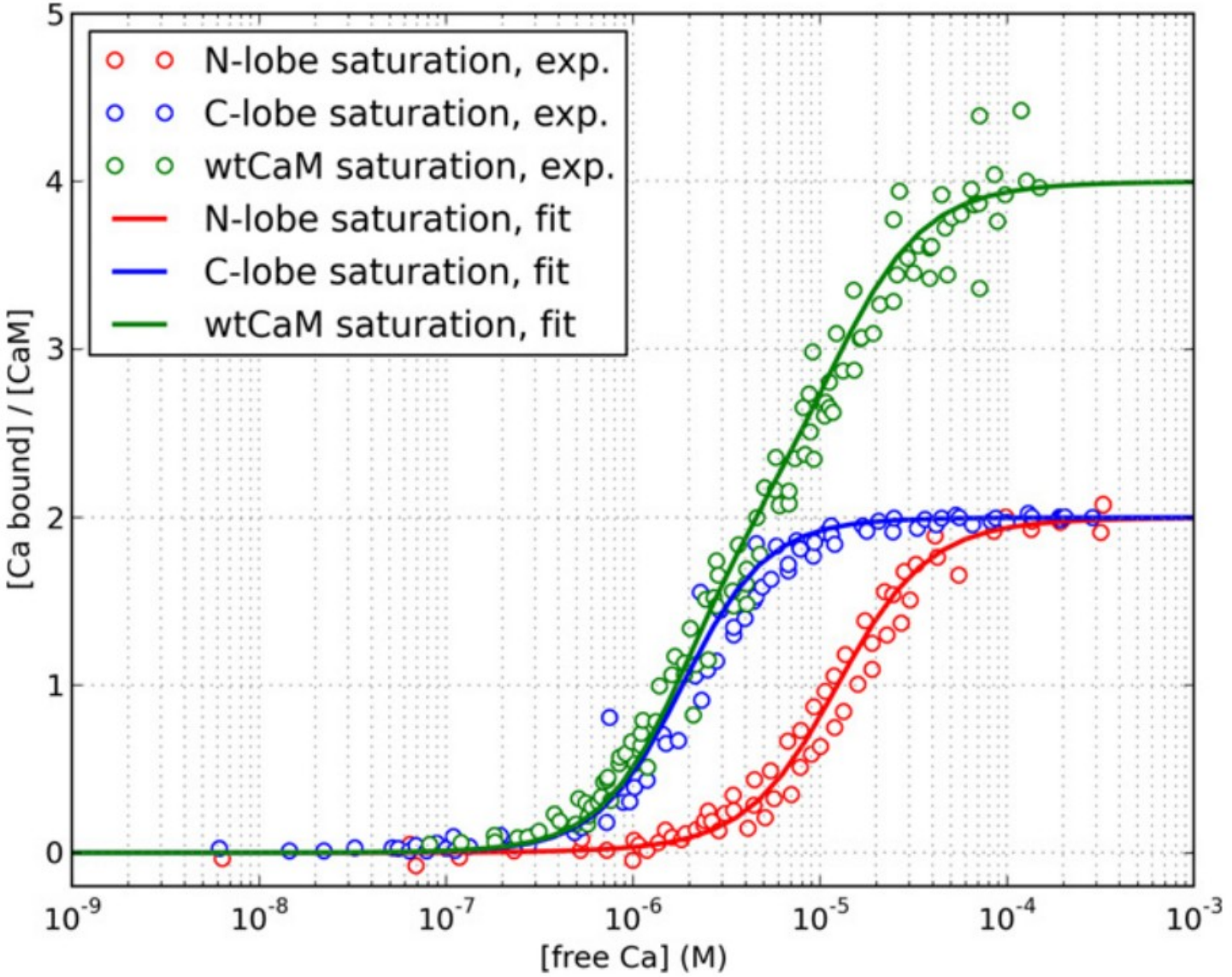
$$c = \frac{K_R}{K_T}$$

$$e = \frac{K_R^t}{K_T^t}$$

Calcium binding to lobes and whole CaM (exp)



Calcium binding to lobes and whole CaM (sim)



Parametrisation using accurate measurements

- 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

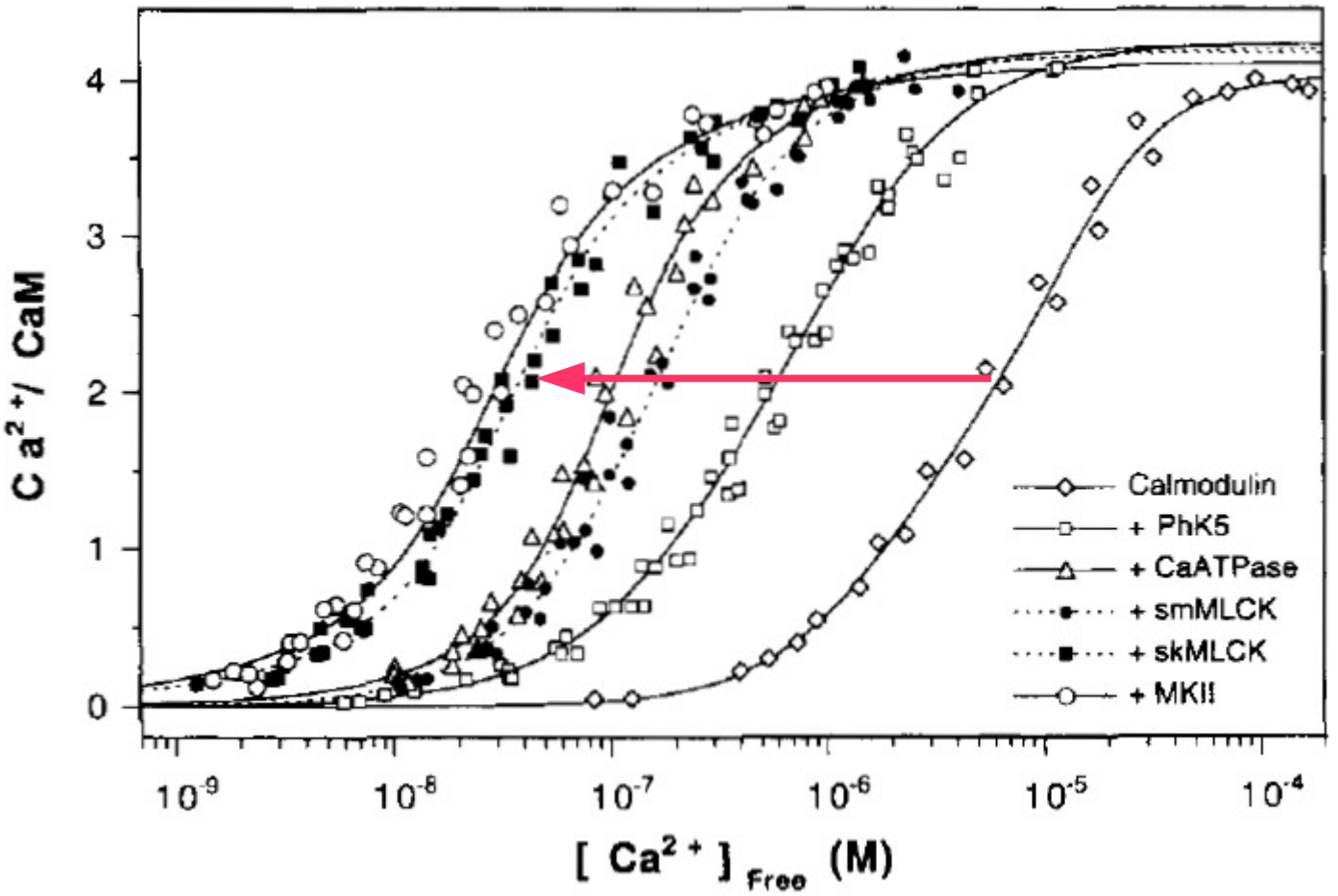
Activity of unsaturated calmodulin (state function)

- 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

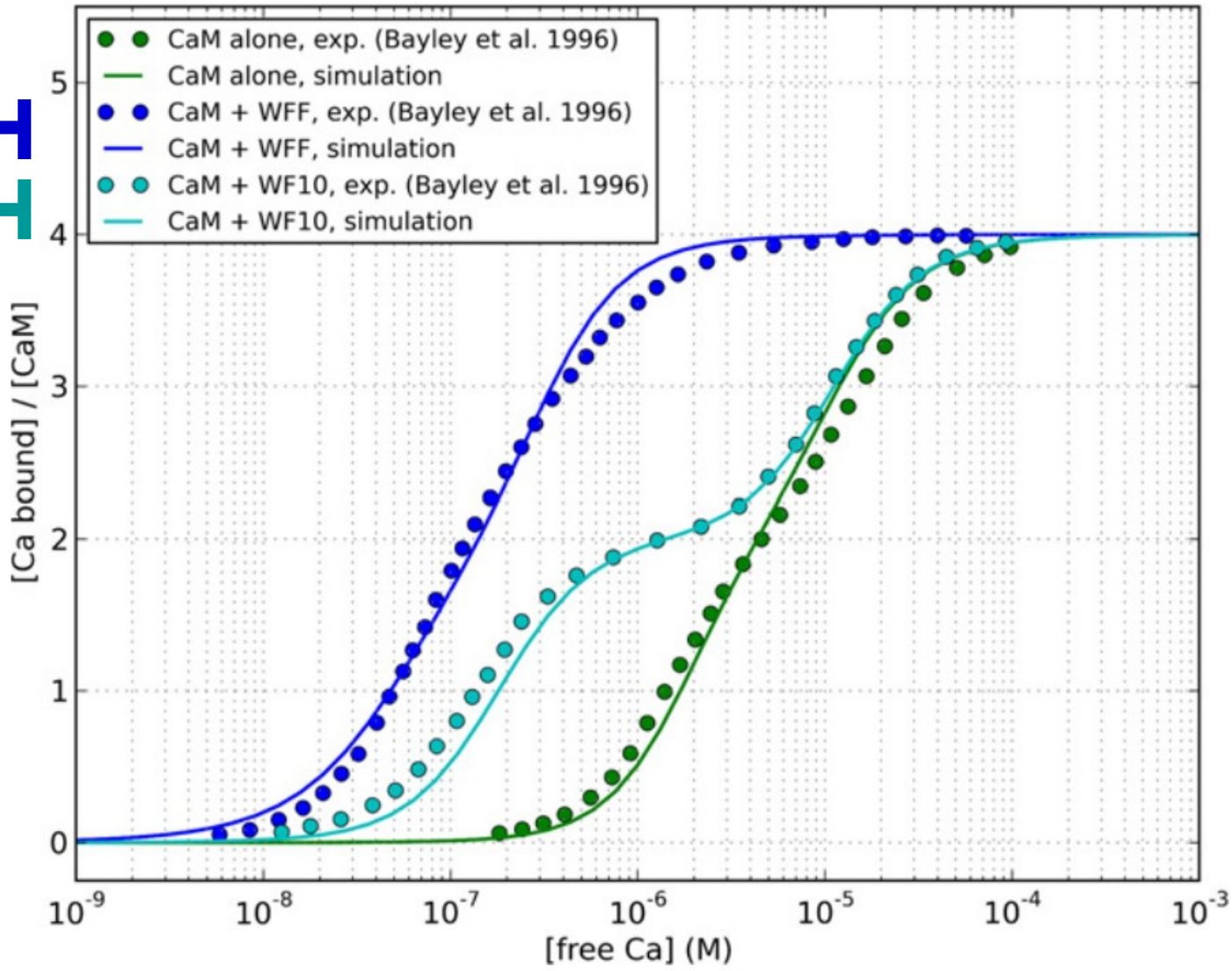


Peersen et al. (1997) *Prot Sci*, 6: 794-807

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

Stabilise R state of both lobes

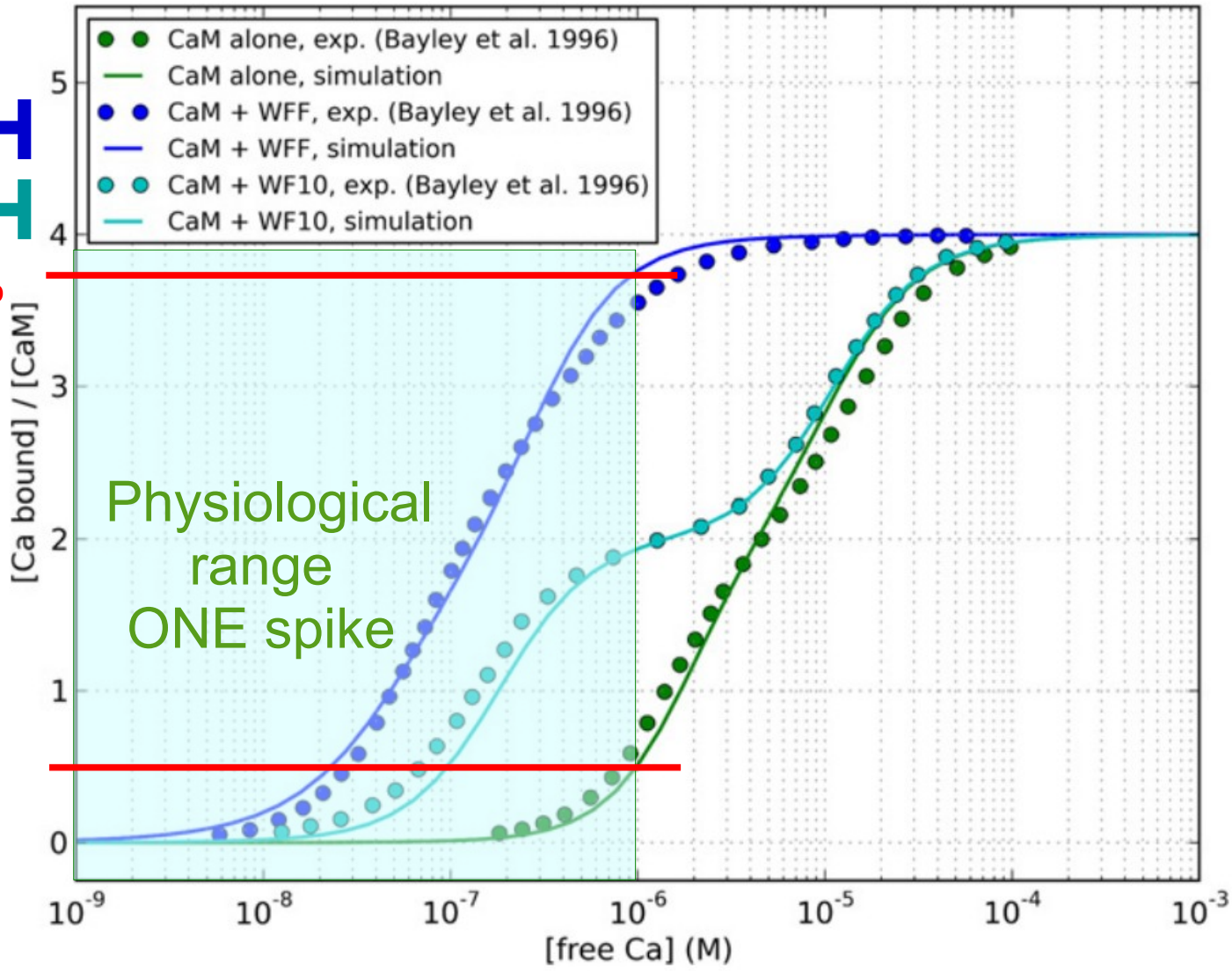
Stabilise R state of C lobe



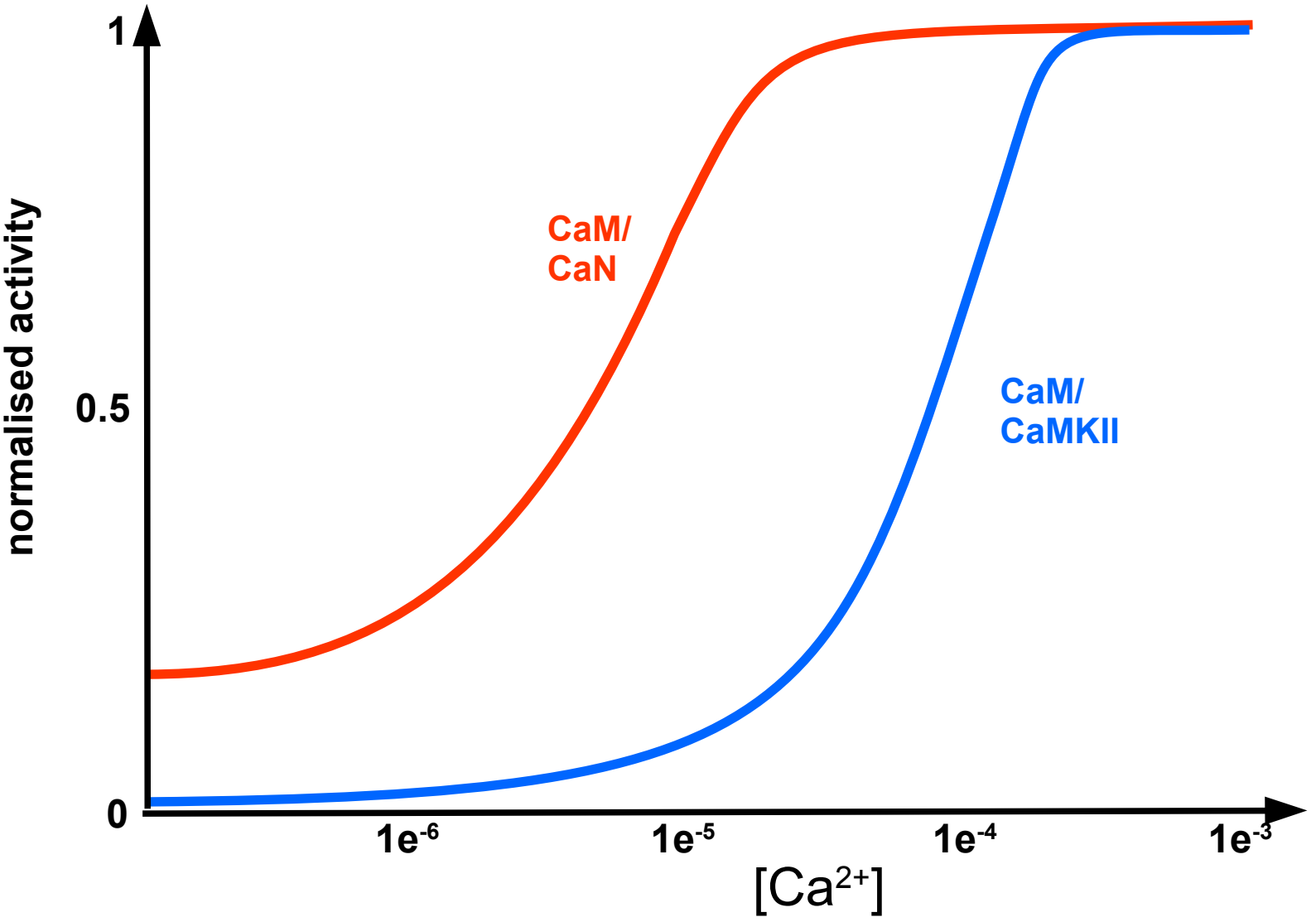
Targets stabilises Ca^{2+} binding into the physiological range

Stabilise R state of both lobes

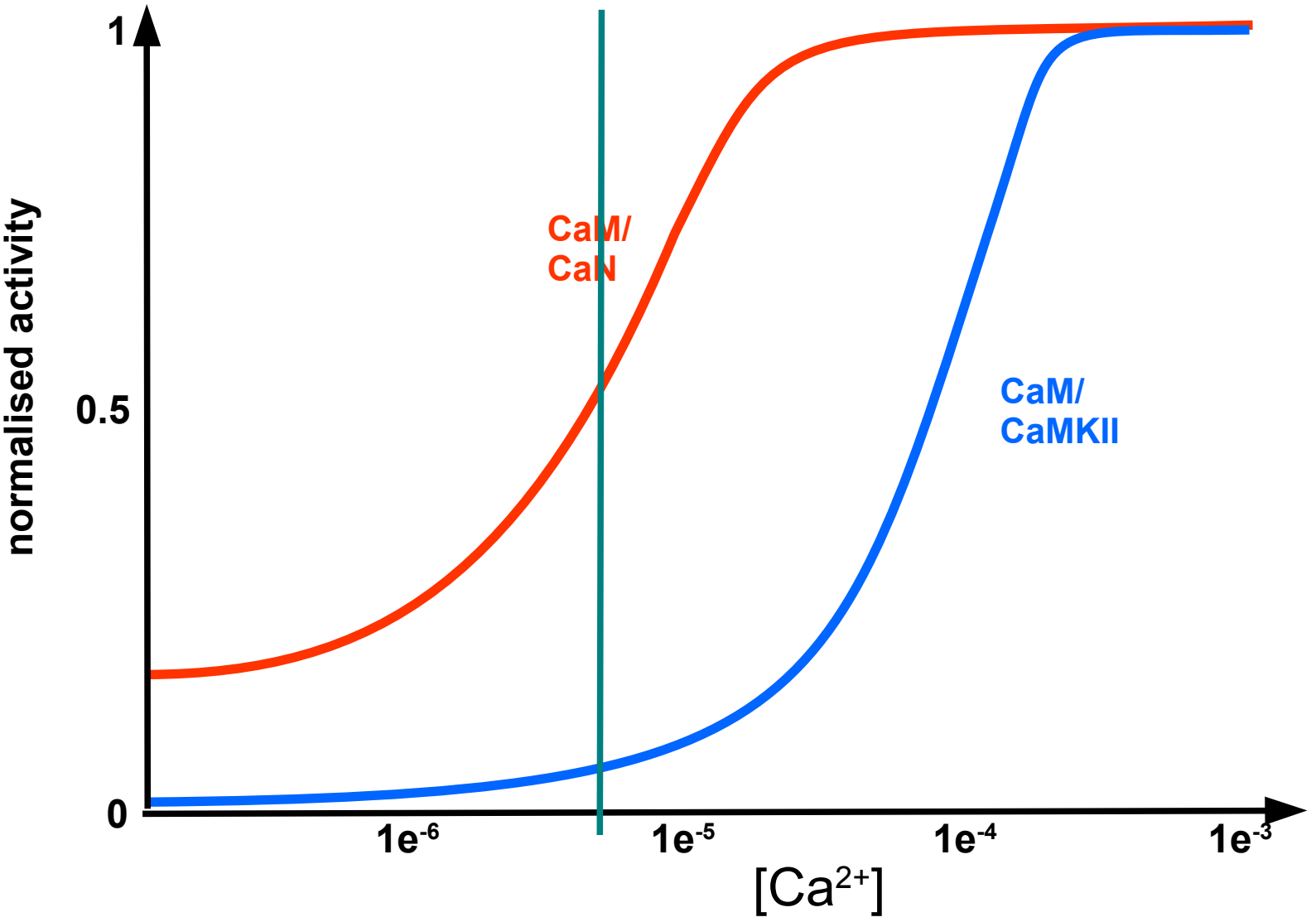
Stabilise R state of C lobe



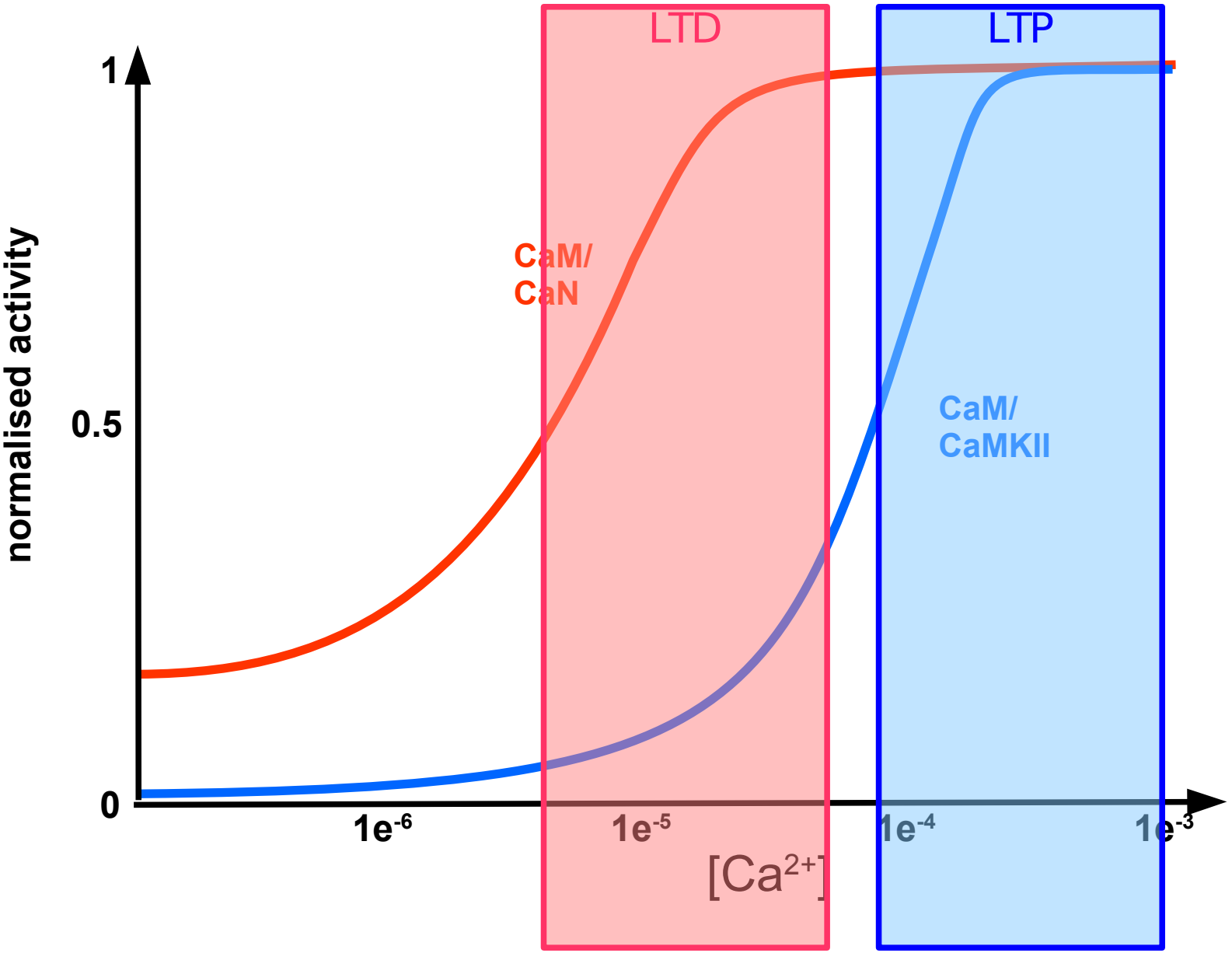
Calmodulin its ligand and its targets



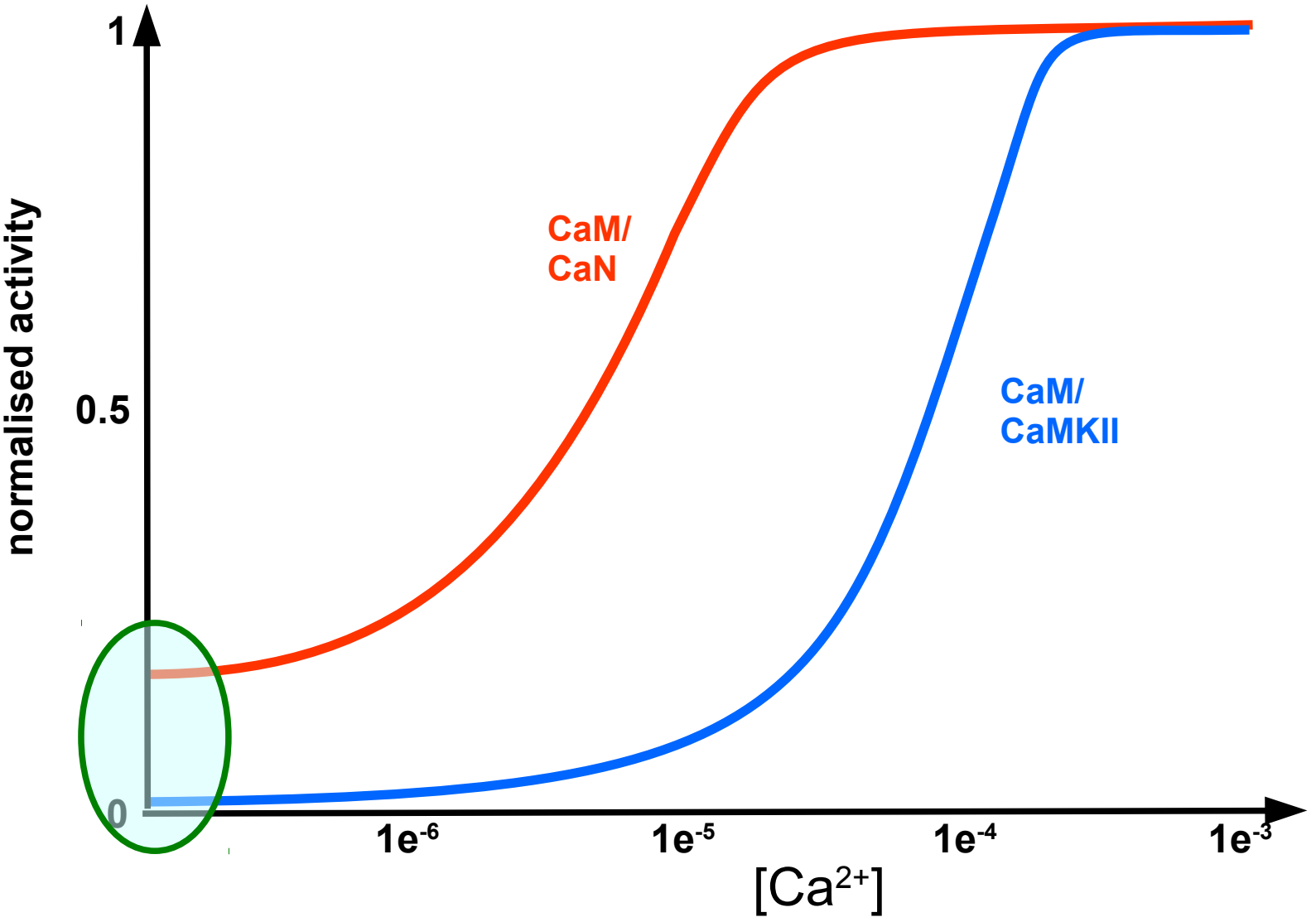
CaM half activated at half saturation of Calmodulin



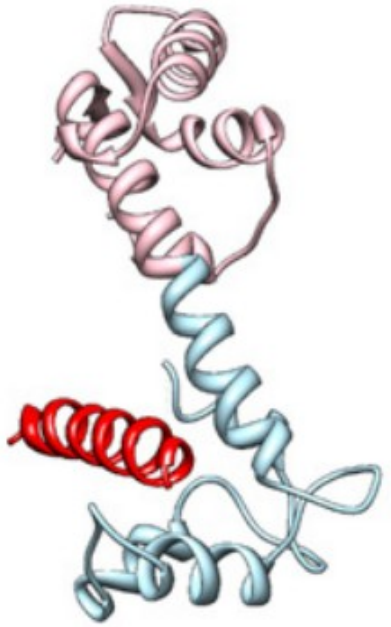
Bidirectional synaptic plasticity



Calcineurin stabilises CaM R → no deactivation



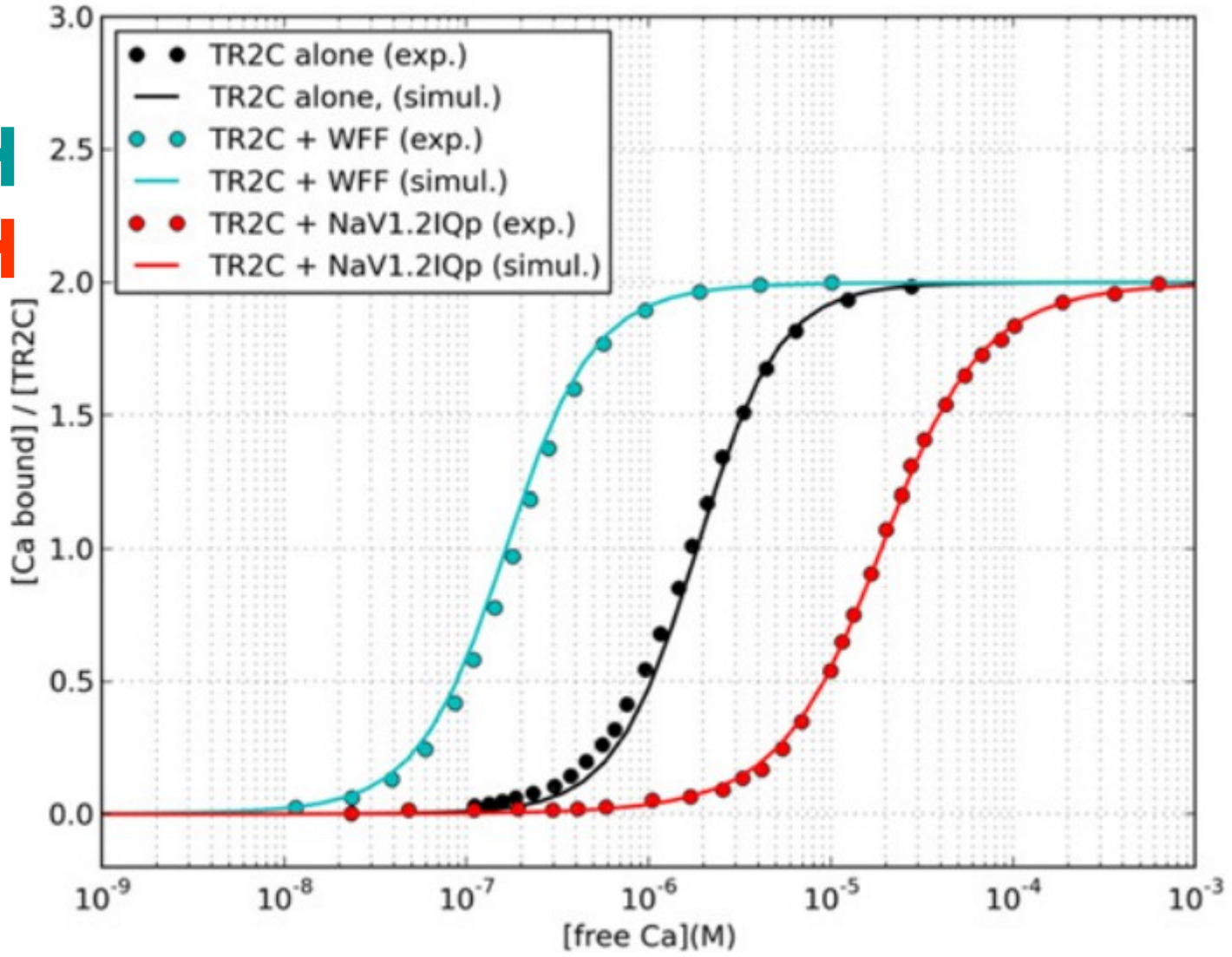
Neurogranin binds to T-state



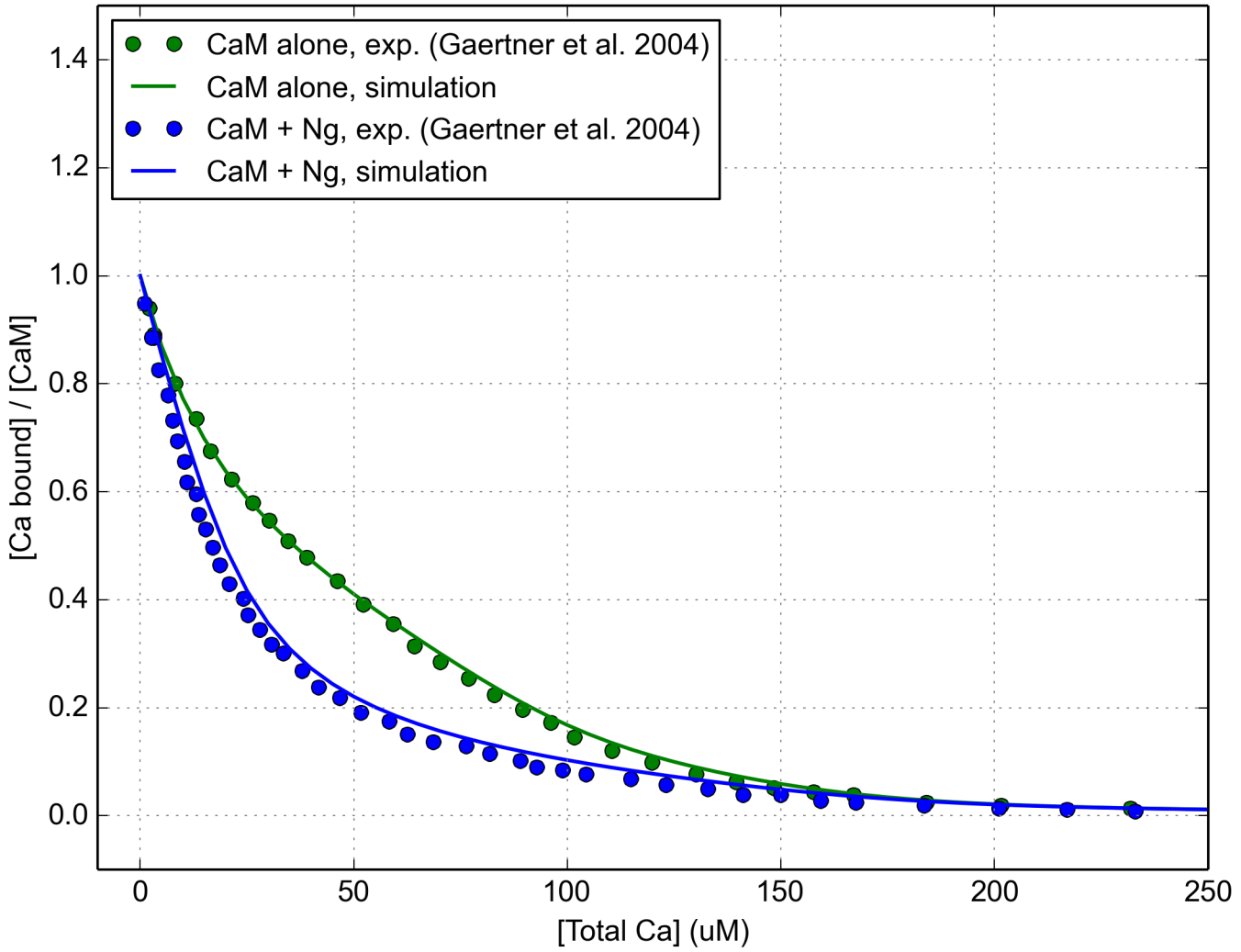
Effect of R and T stabilising targets

Peptide from skMLCK → R

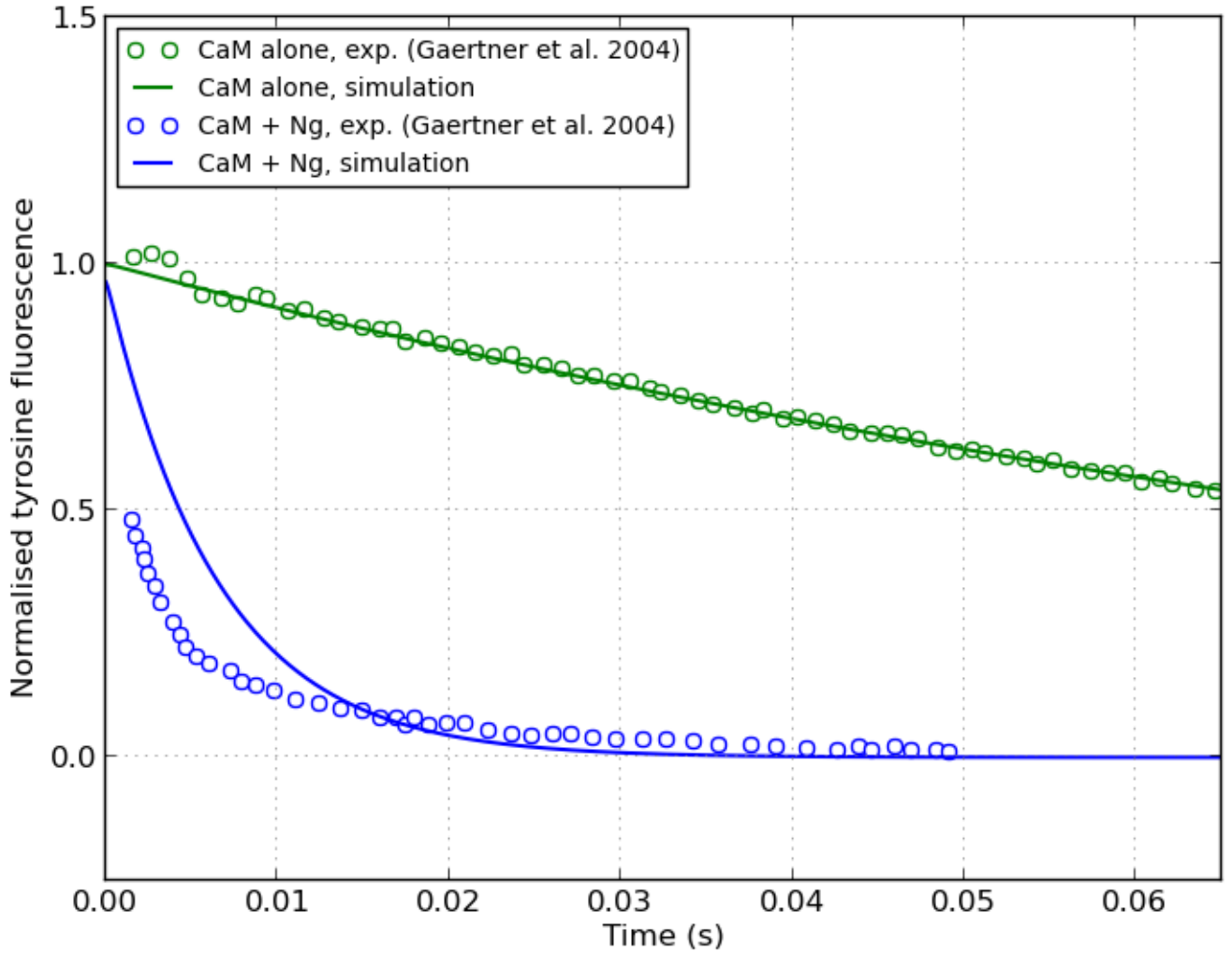
IQ domain from NaV1.2 → T



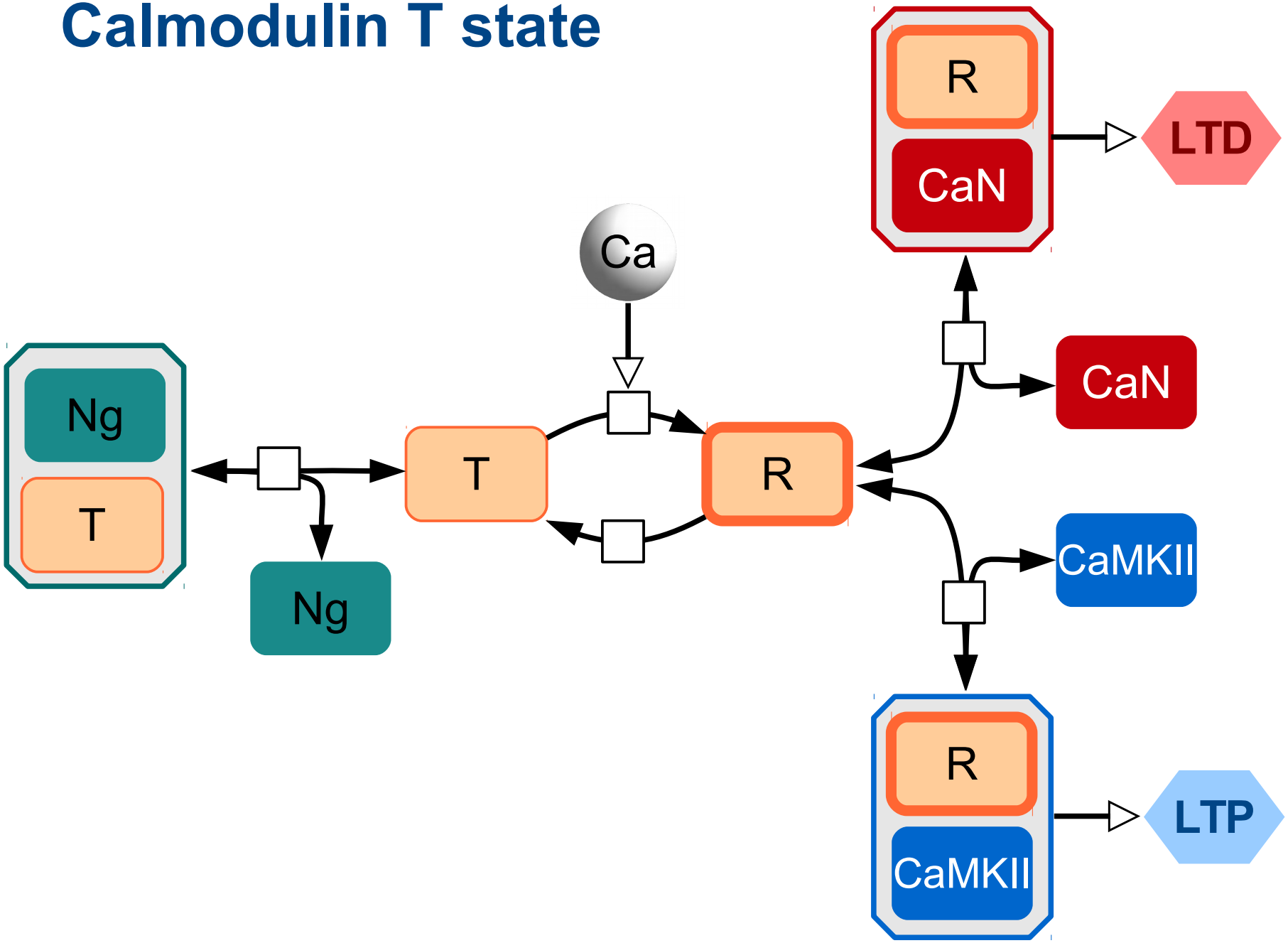
Neurogranin decreases affinity of CaM for Ca^{2+}



Neurogranin increases Ca^{2+} dissociation rate



Neurogranin stabilises Calmodulin T state



Conclusions of part 1

Hemi-concerted model of Calmodulin, with only 2 states for the EF hands, binding calcium with different affinities. Parameters estimated from experimental datasets.

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.

CaN is able to bind calmodulin at low concentration of calcium, while both CaN and CaMKII binds calmodulin at high calcium concentrations.

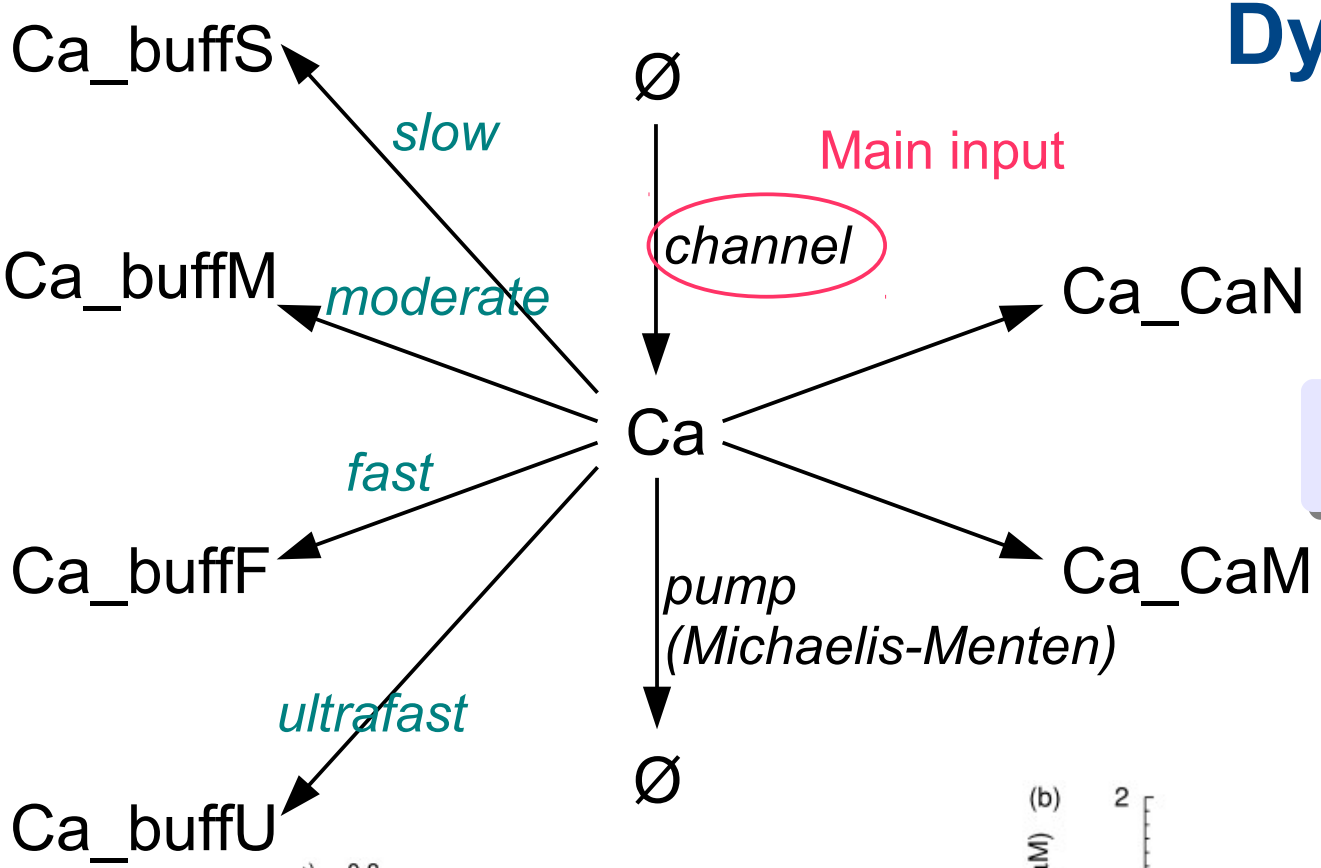
In the absence of Ca^{2+}, Neurogranin binds Calmodulin in the T state, resetting the system and acting as a Calmodulin reservoir.

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

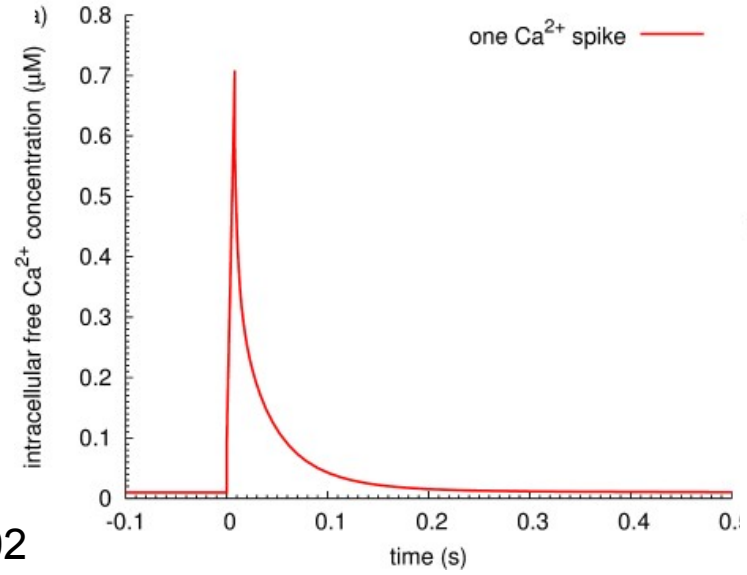
≠

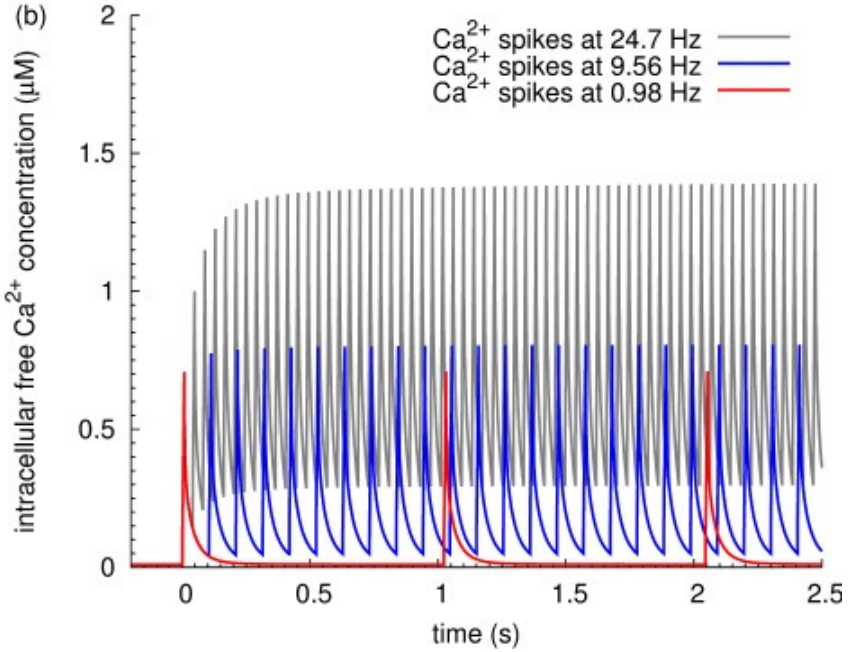
Dynamic of calcium in the spine



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

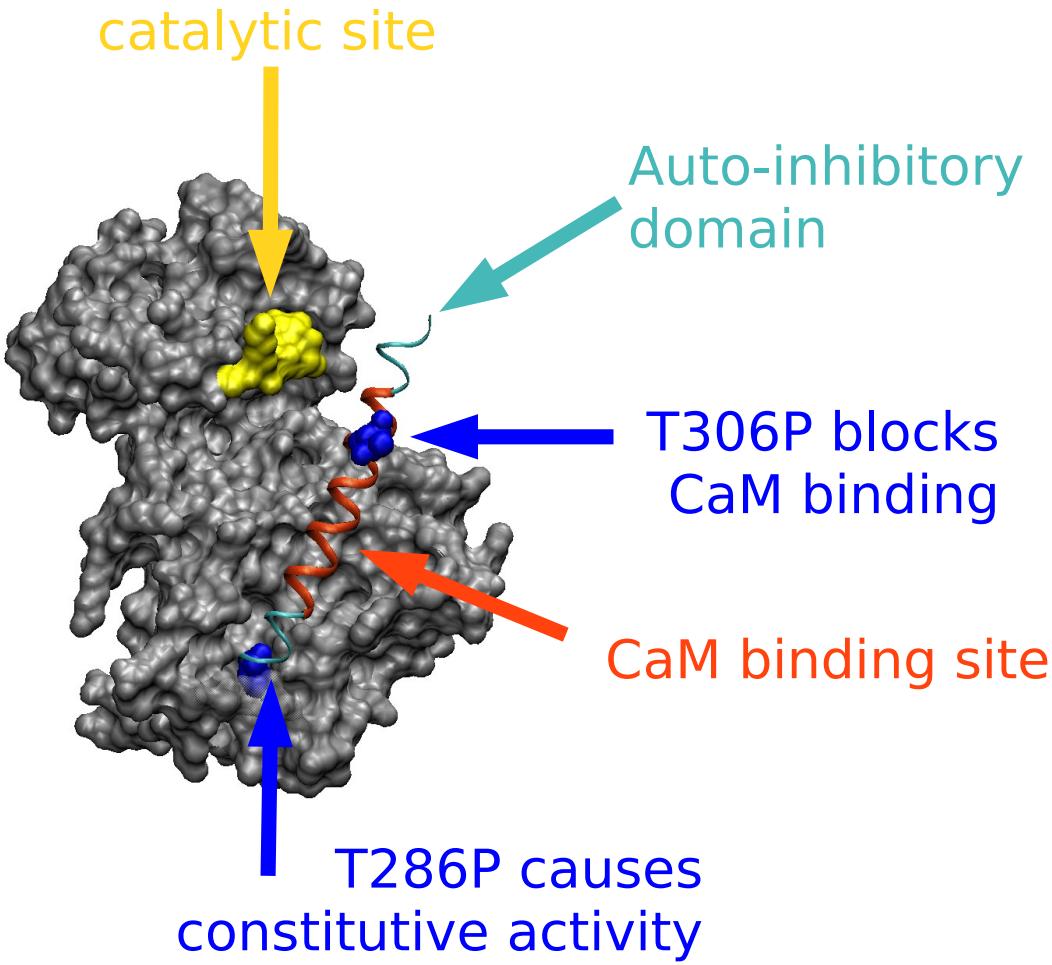
Sabatini et al 2002





Babraham Institute

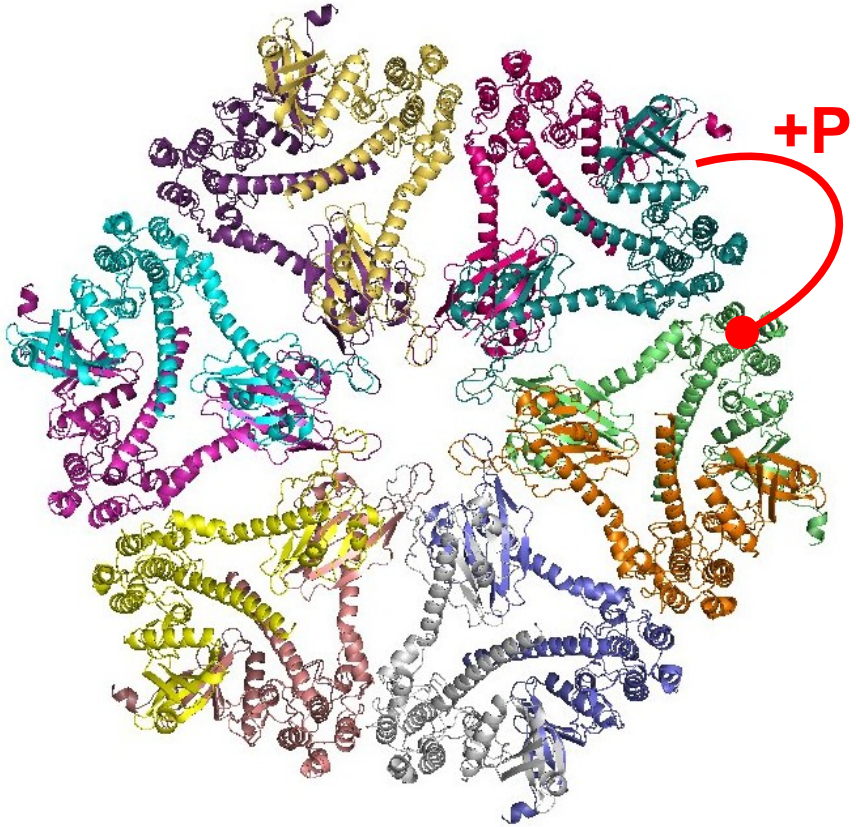
Calcium/calmodulin kinase II

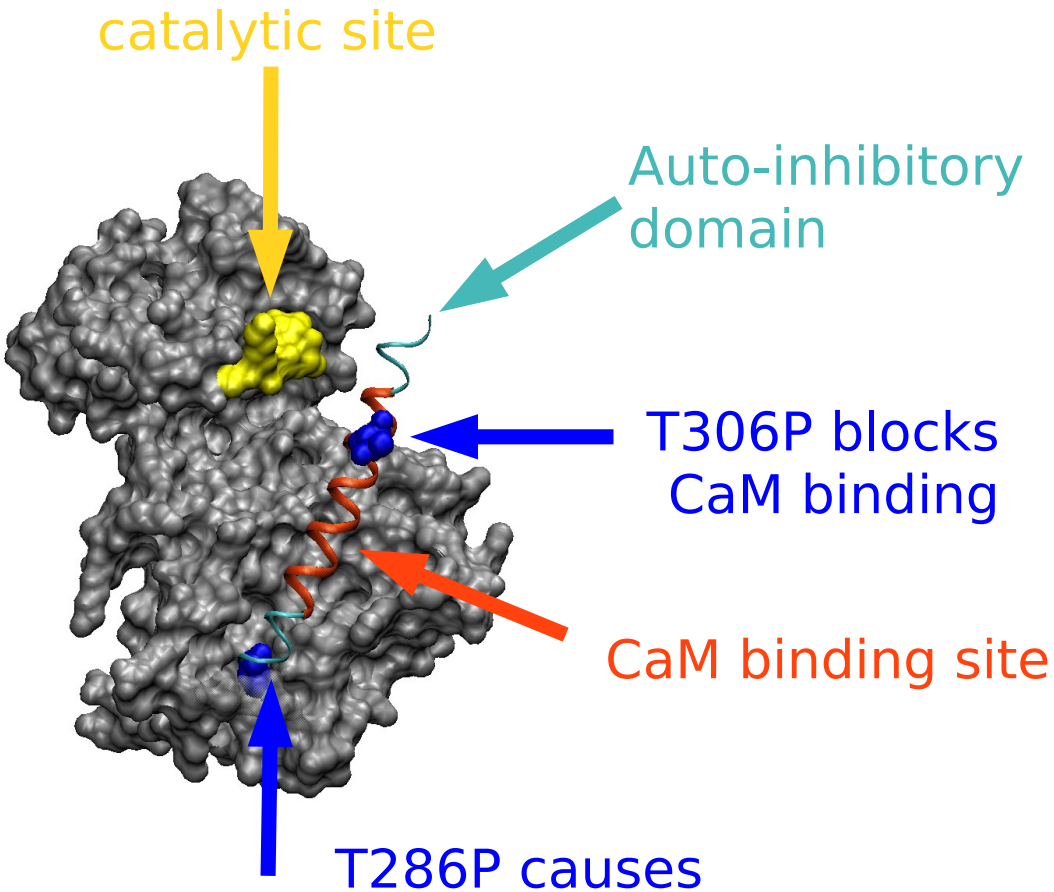


Calmodulin trapping is an apparent increase of affinity of CaMKII for CaM when T286 is phosphorylated

Stefan MI, Marshall D, Le Novère N (2012)

Calcium/calmodulin kinase II

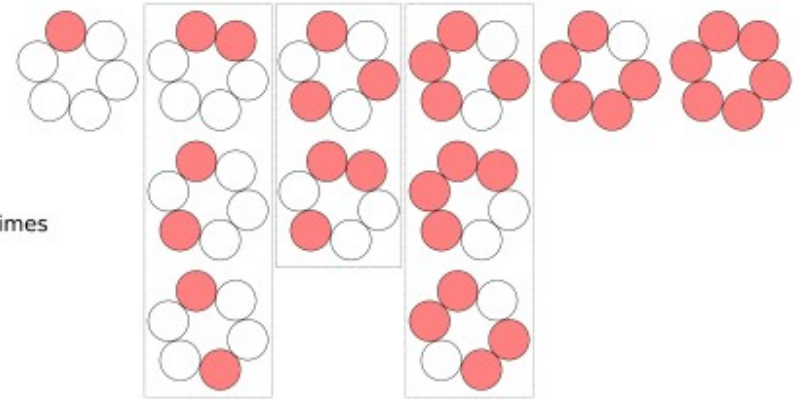




Dodecamer:
Trans-phosphorylation of T286 by neighbouring subunits
Cis-phosphorylation of T306
Most quantitative measurements made on monomers ...

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.

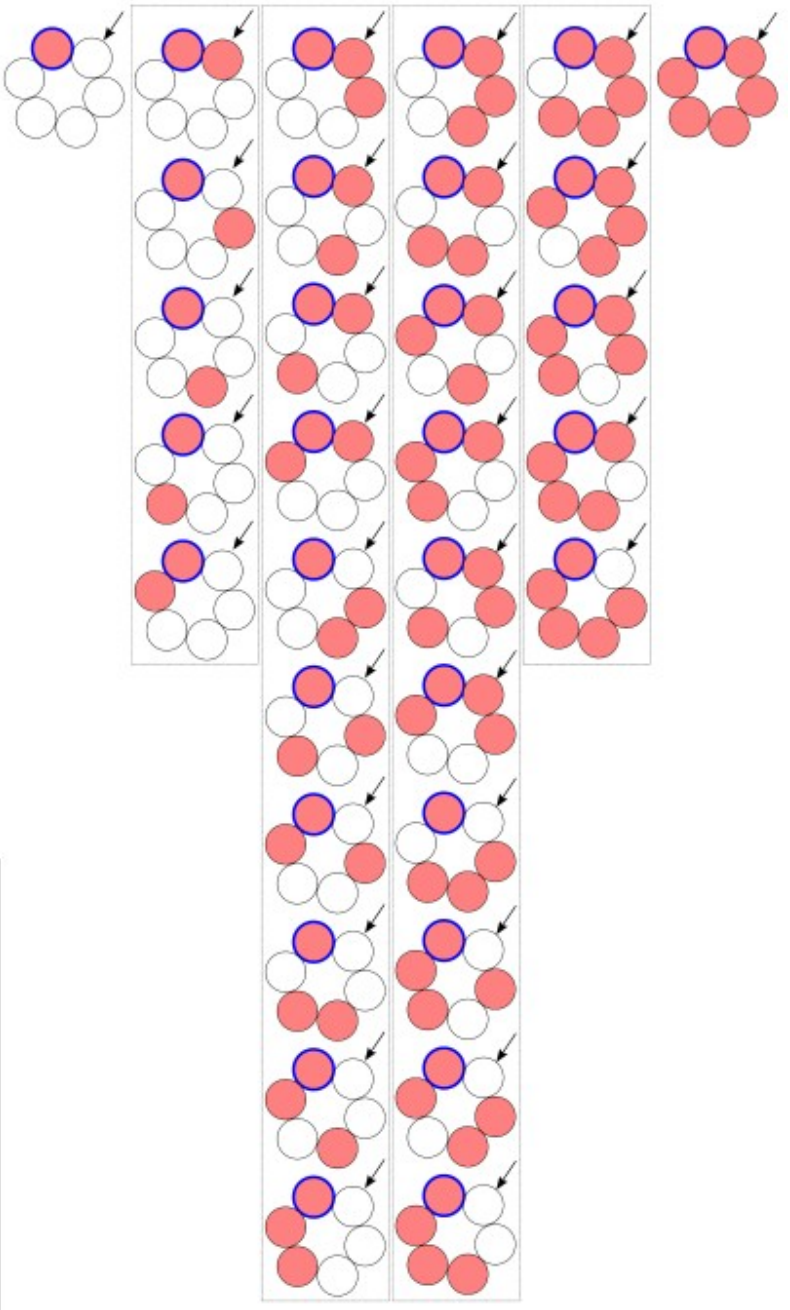
Repeat for every 1% increase of CaMKII active monomers

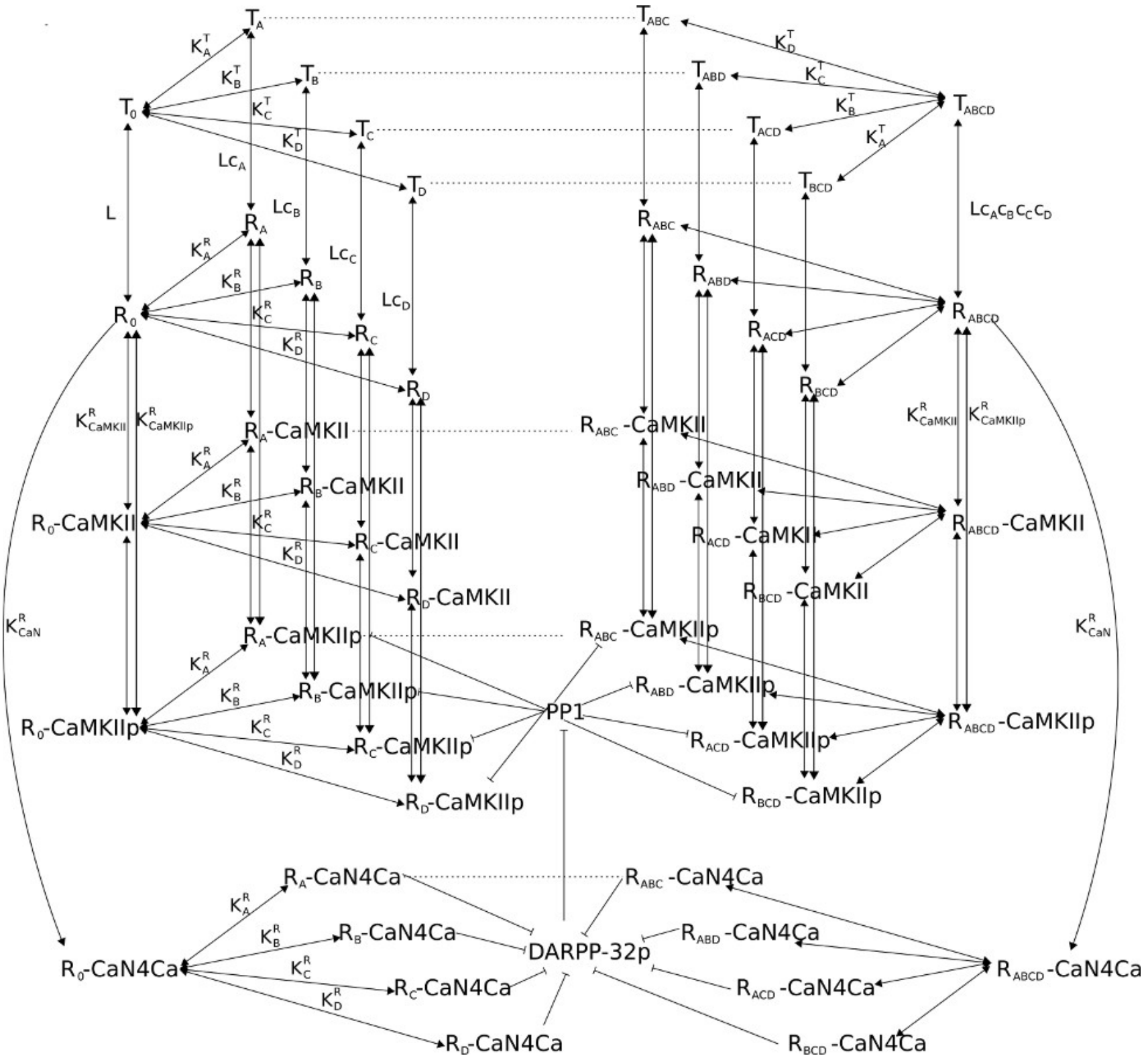
Multiply average populations of each number of active monomers per hexamer by their corresponding probabilities of having an active neighbour.

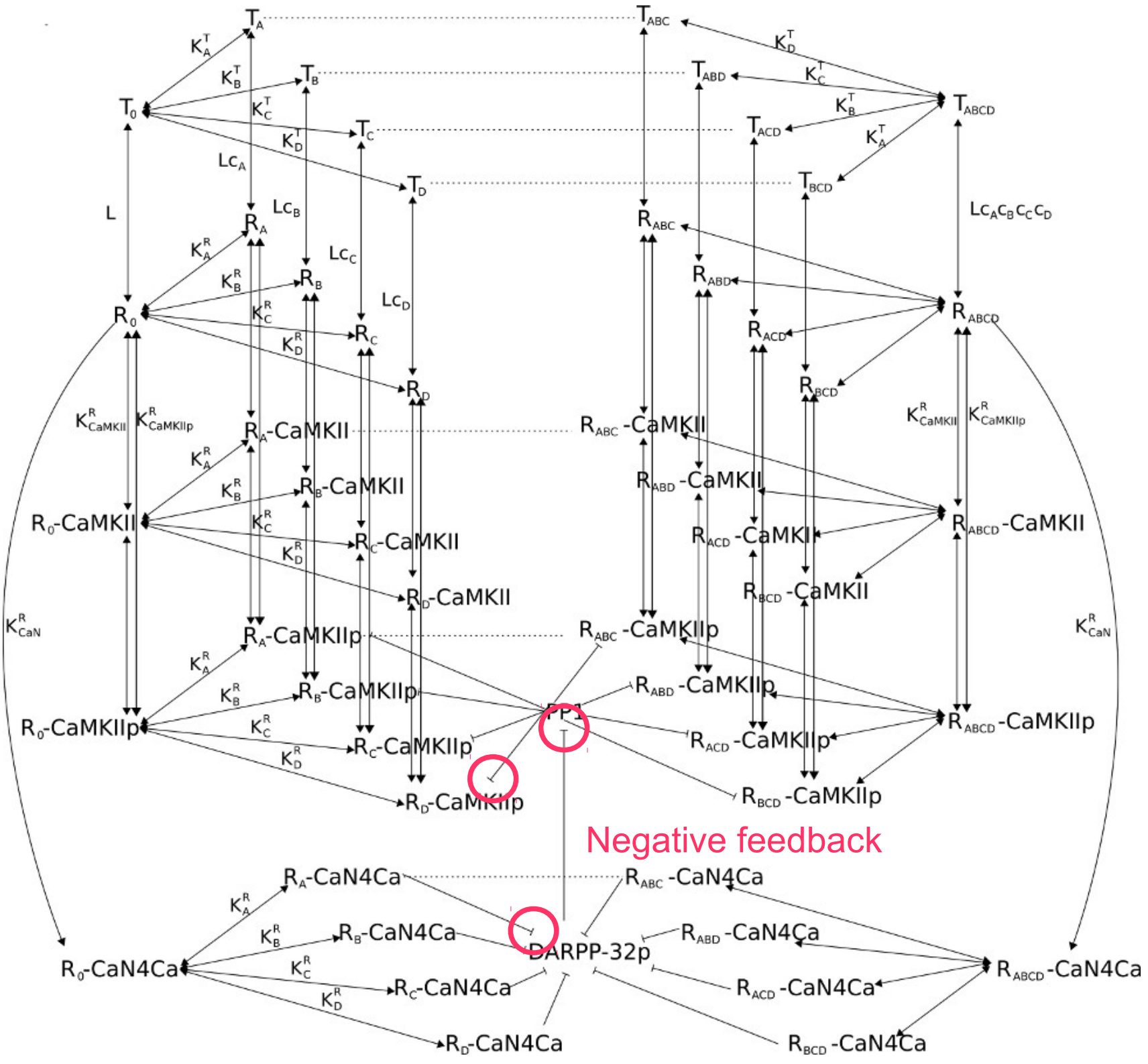
The sum of these six numbers is a coefficient that can be used to adjust CaMKII autophosphorylation rate.

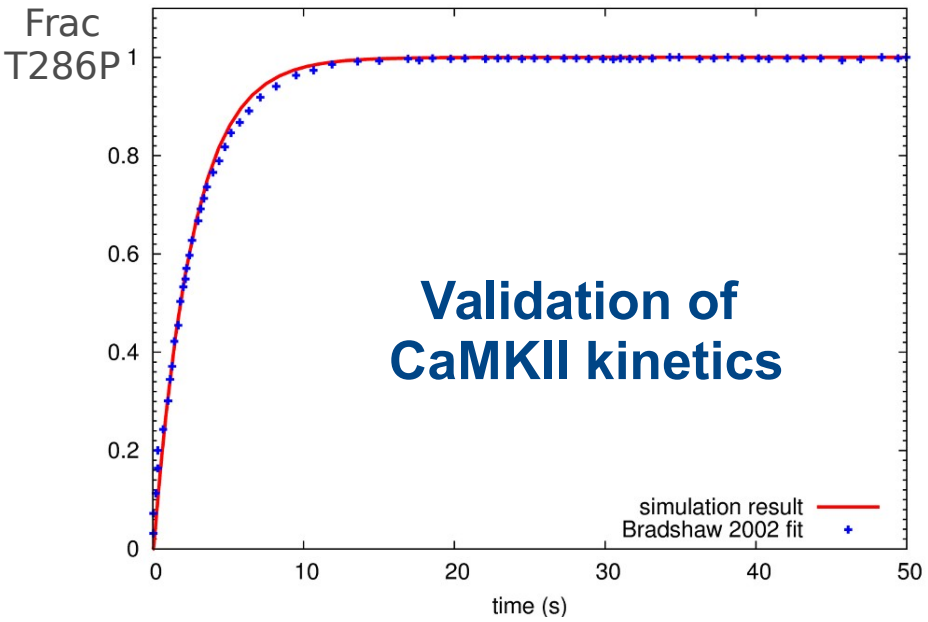
Fit these 100 coefficients into a polynomial function of activated CaMKII monomers, and embed this function in the model.

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:



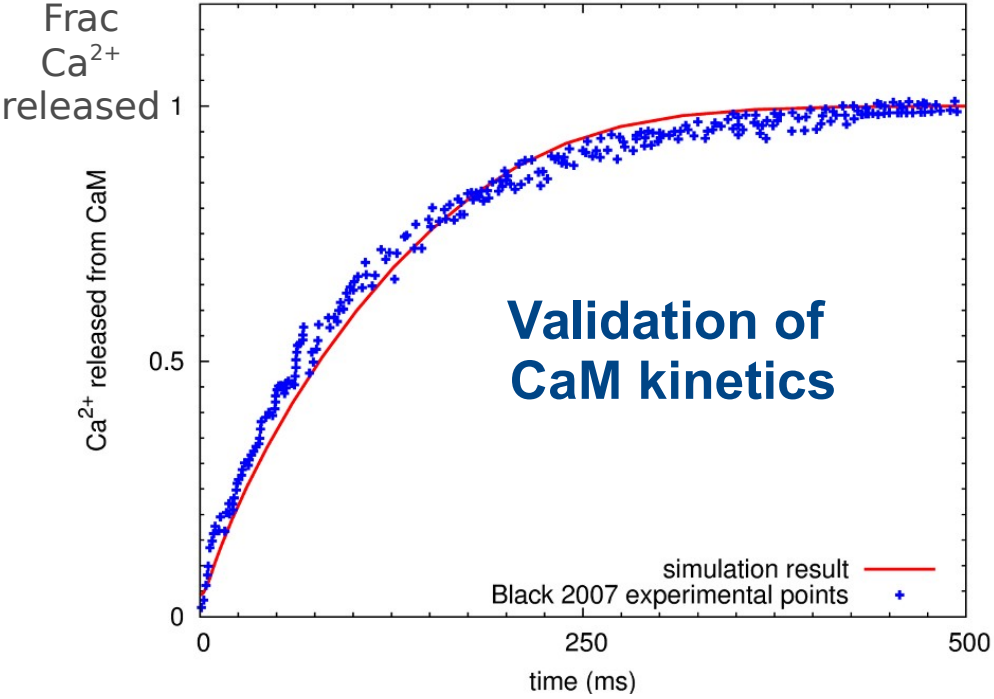

T0
TA
TB
TC
TD
TABC
TABD
TACD
TBCD
TABCD
KAT
KBT
KCT
KDT
LcA
LcB
LcC
LcD
L
LcAcBcCcD
R0
RA
RB
RC
RD
RABC
RABD
RACD
RBCD
RABCD
KAR
KBR
KCR
KDR
KCaMKII
KCaMKIIp
KCaN
R0-CaMKII
RA-CaMKII
RB-CaMKII
RC-CaMKII
RD-CaMKII
RABC-CaMKII
RABD-CaMKII
RACD-CaMKII
RBCD-CaMKII
RABCD-CaMKII
R0-CaMKIIp
RA-CaMKIIp
RB-CaMKIIp
RC-CaMKIIp
RD-CaMKIIp
RABC-CaMKIIp
RABD-CaMKIIp
RACD-CaMKIIp
RBCD-CaMKIIp
RABCD-CaMKIIp
PP1
R0-CaN4Ca
RA-CaN4Ca
RB-CaN4Ca
RC-CaN4Ca
RD-CaN4Ca
RABC-CaN4Ca
RABD-CaN4Ca
RACD-CaN4Ca
RBCD-CaN4Ca
RABCD-CaN4Ca
DARPP-32p


Negative feedback



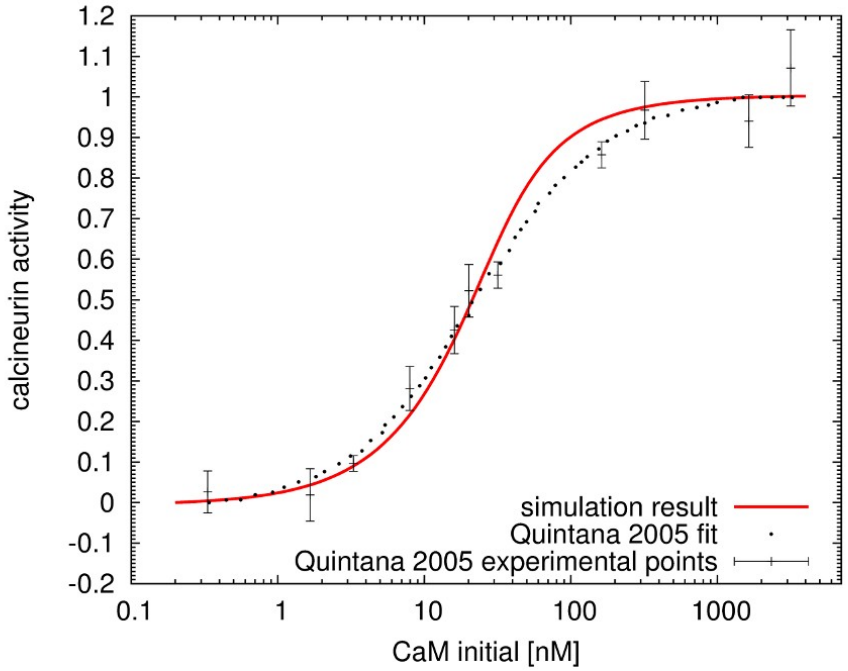
Validation of CaMKII kinetics

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



Validation of CaM kinetics

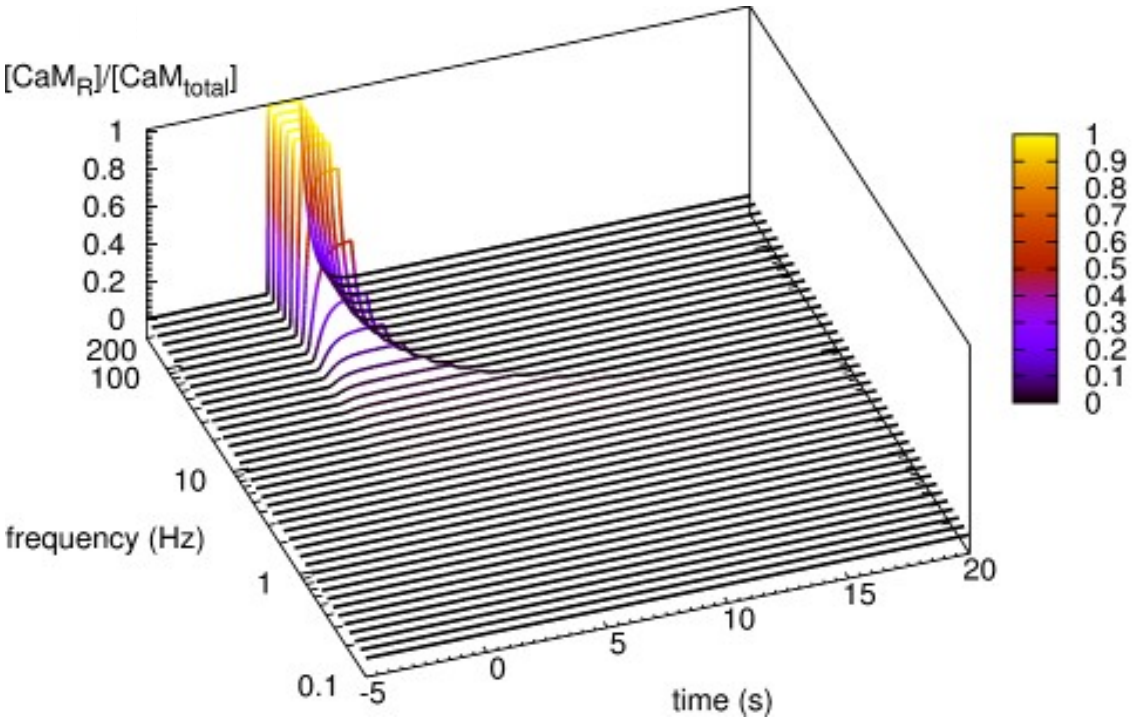
Black DJ, Selfridge JE, Persechini A (2007). *Biochemistry* 46: 13415–13424.



Validation of calcium-activation of CaN

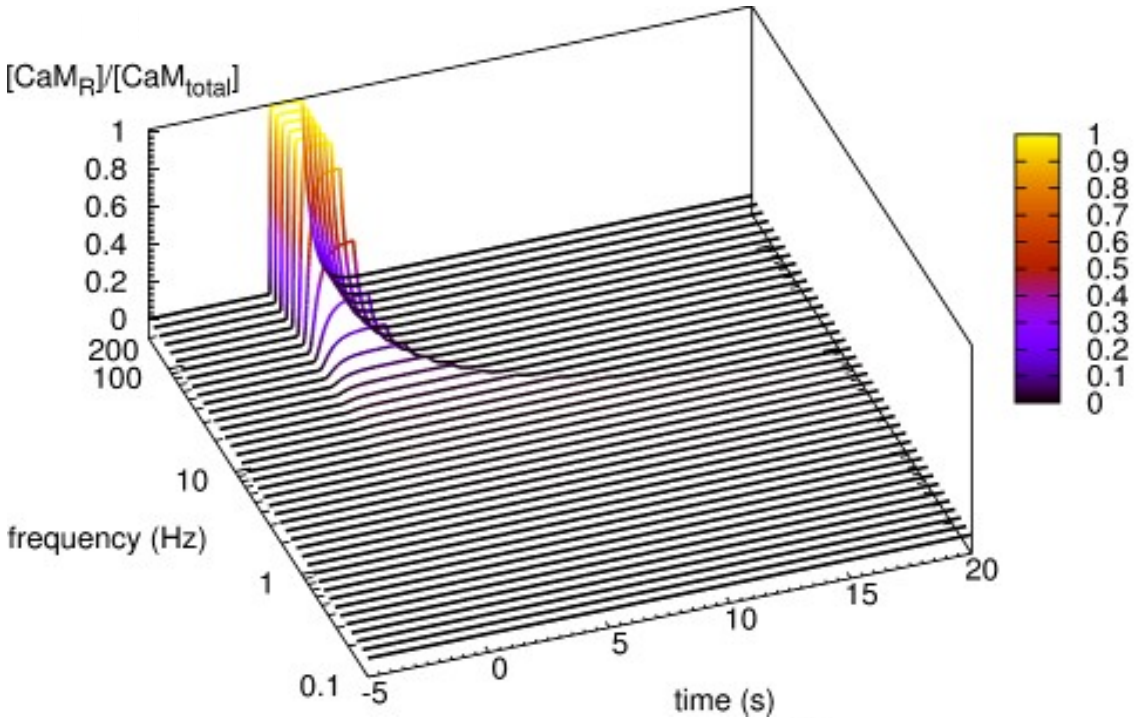
Quintana AR, Wang D, Forbes JE, Waxham MN (2005). *BBRC* 334: 674–680.

Calmodulin activation

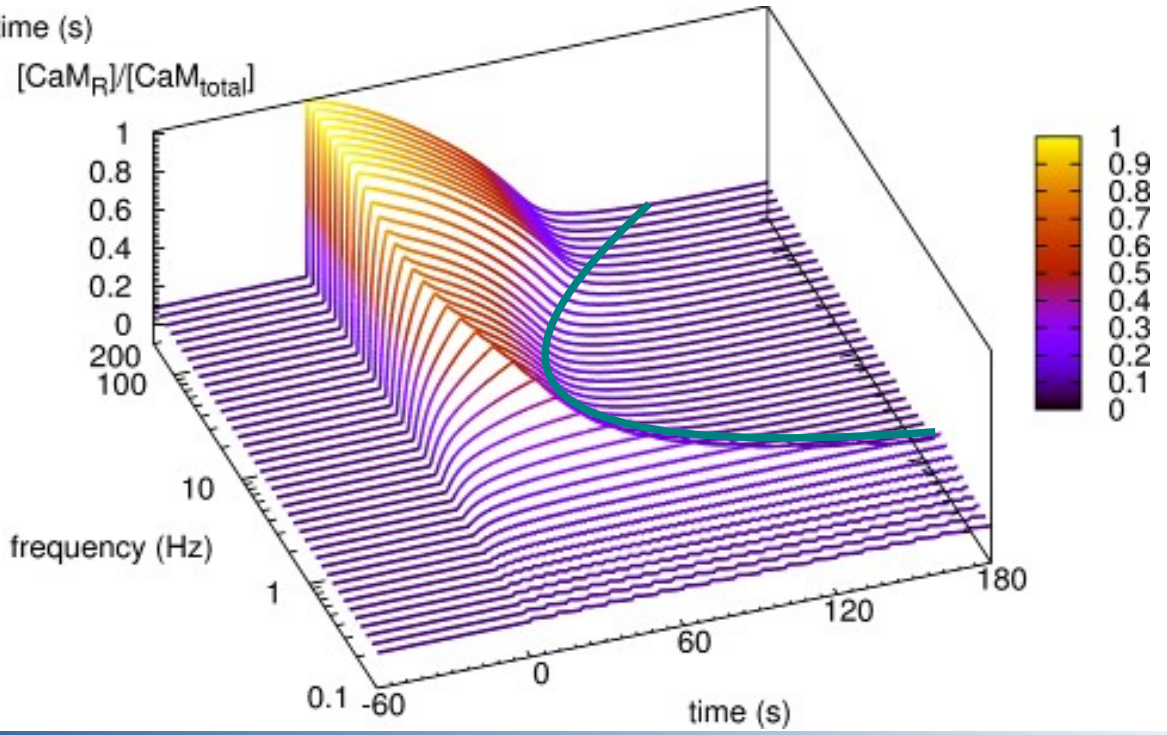


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

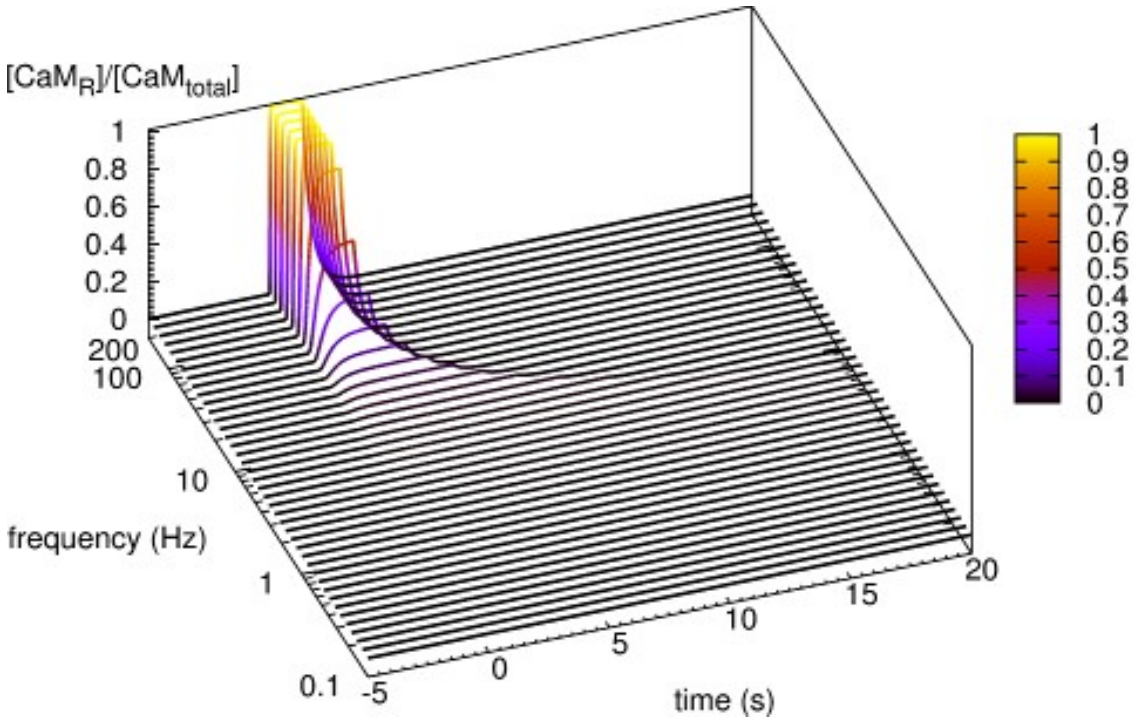


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)



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

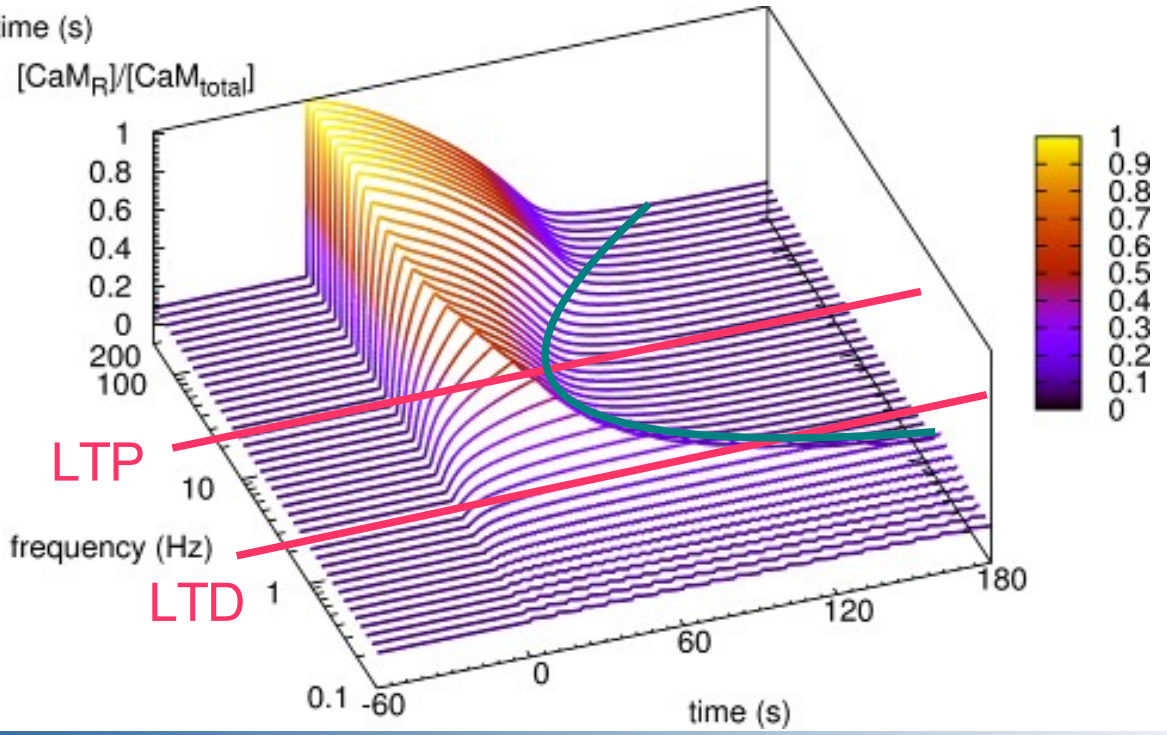
Calmodulin activation



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

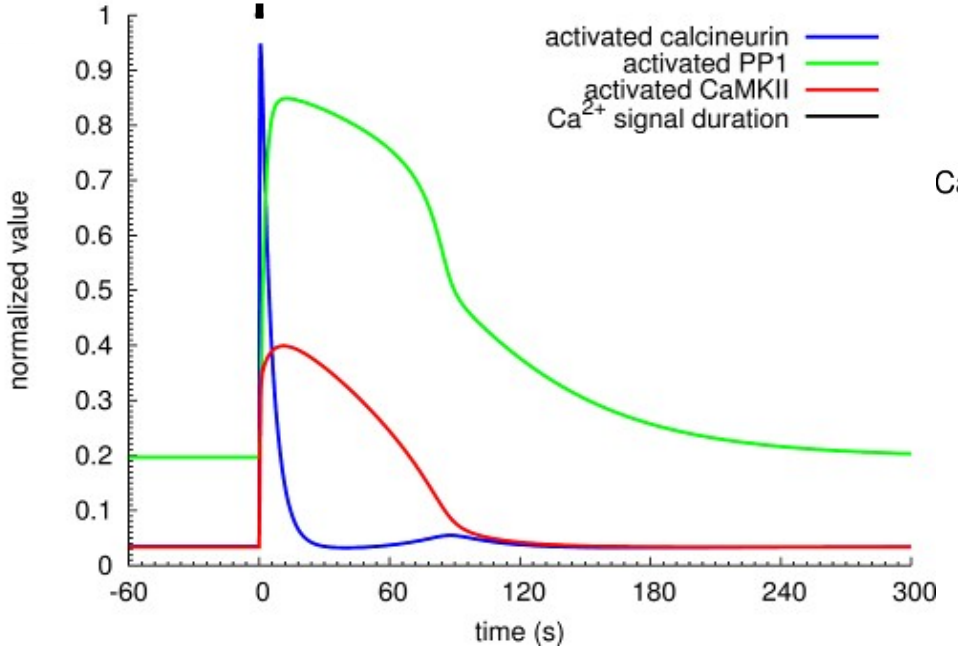
At high frequency, effects of calcium signals last much longer than the signal itself

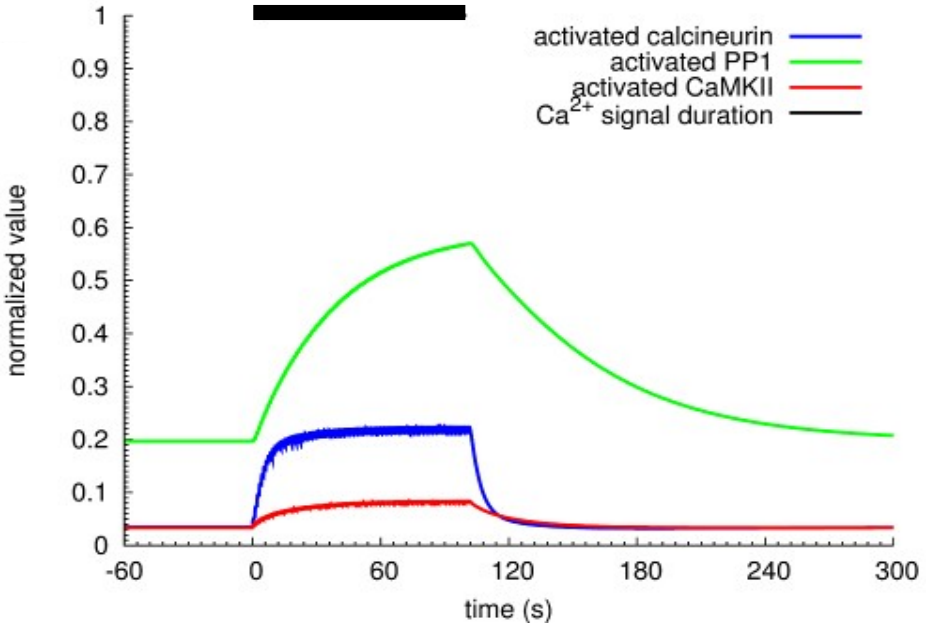
CaM with targets. Binding to CaN and CaMKII stabilises R state, with higher affinity. Positive feedback loop
➜ bistability

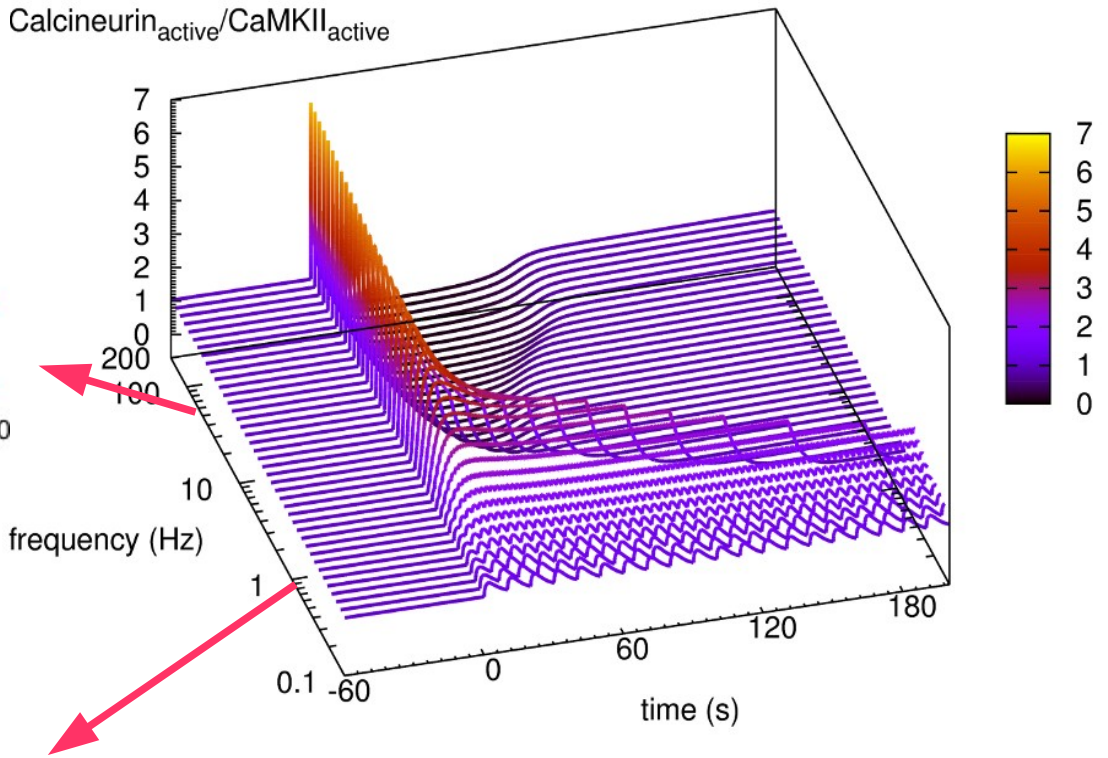


Babraham Institute

Temporal activation of CaMKII and CaN



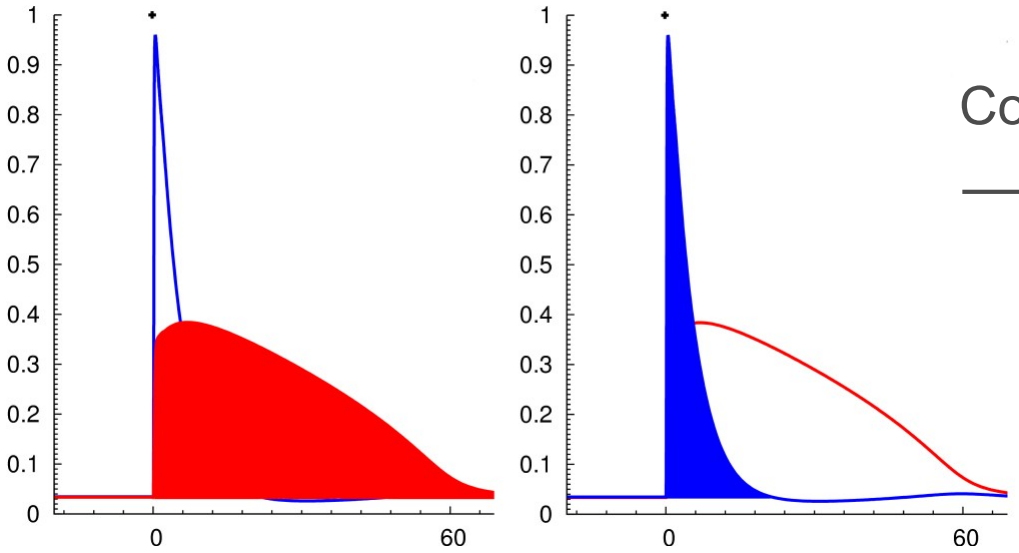




All calcium frequencies increase CaN **AND** CaMKII. It is not a Switch. But the ratio of activation changes

Babraham Institute

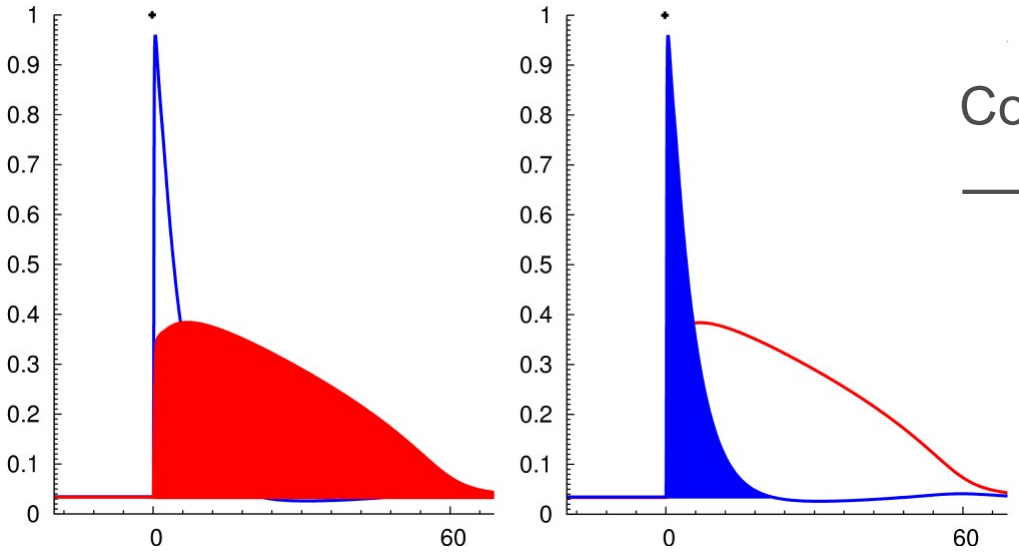
Bidirectional plasticity



Constant catalytic rates of active enzyme

⟶ quantity of catalysed reaction events prop to integral of the activation curve

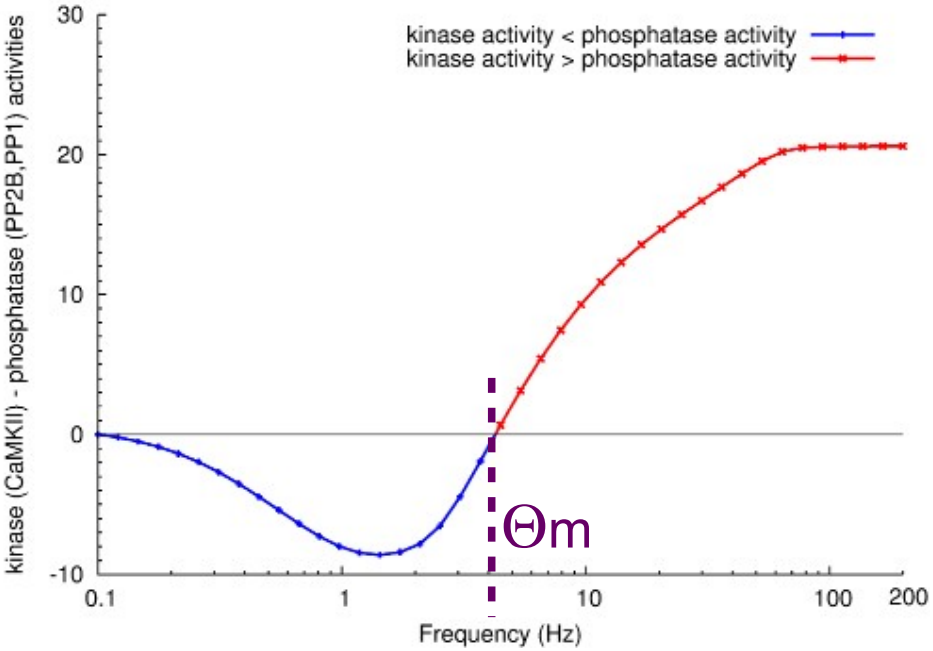
Bidirectional plasticity



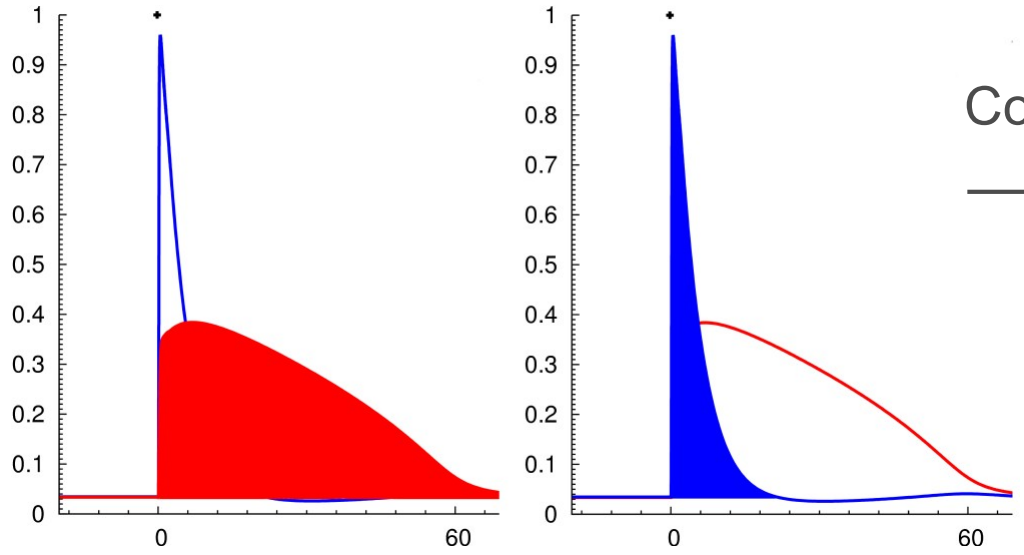
Constant catalytic rates of active enzyme

⟶ quantity of catalysed reaction events prop to integral of the activation curve

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



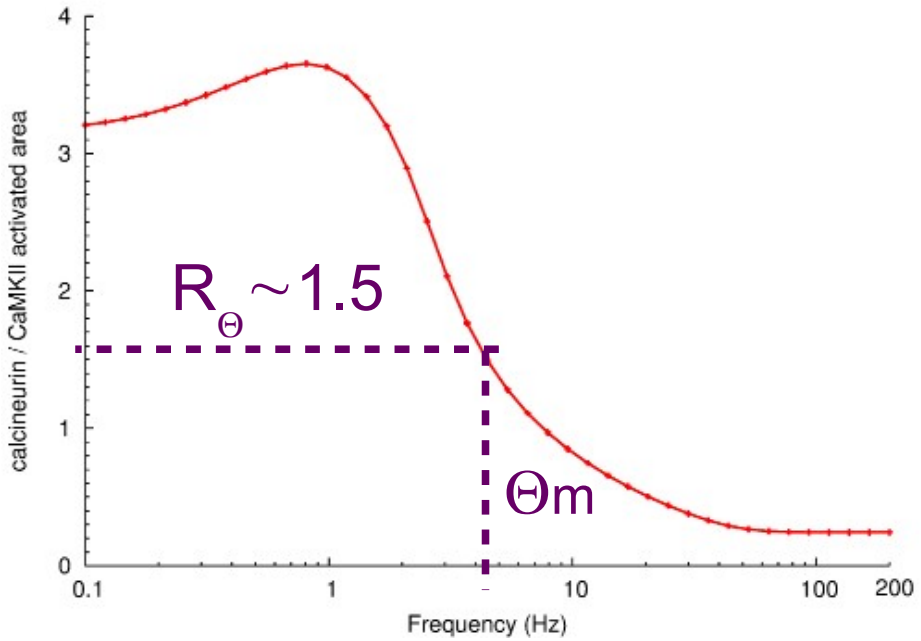
Bidirectional plasticity

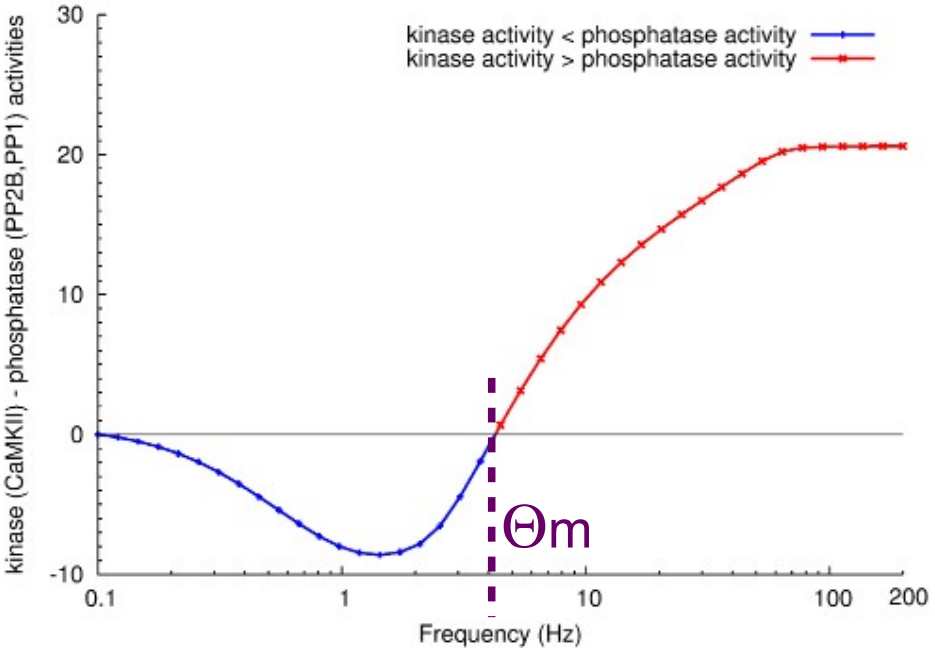


Constant catalytic rates of active enzyme

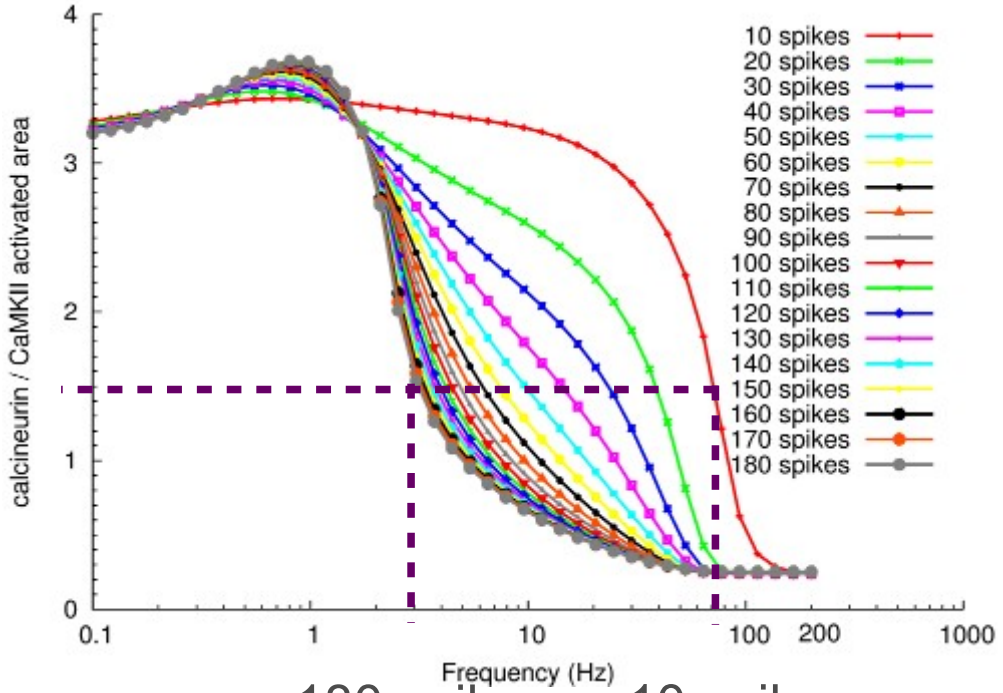
⟶ quantity of catalysed reaction events prop to integral of the activation curve

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



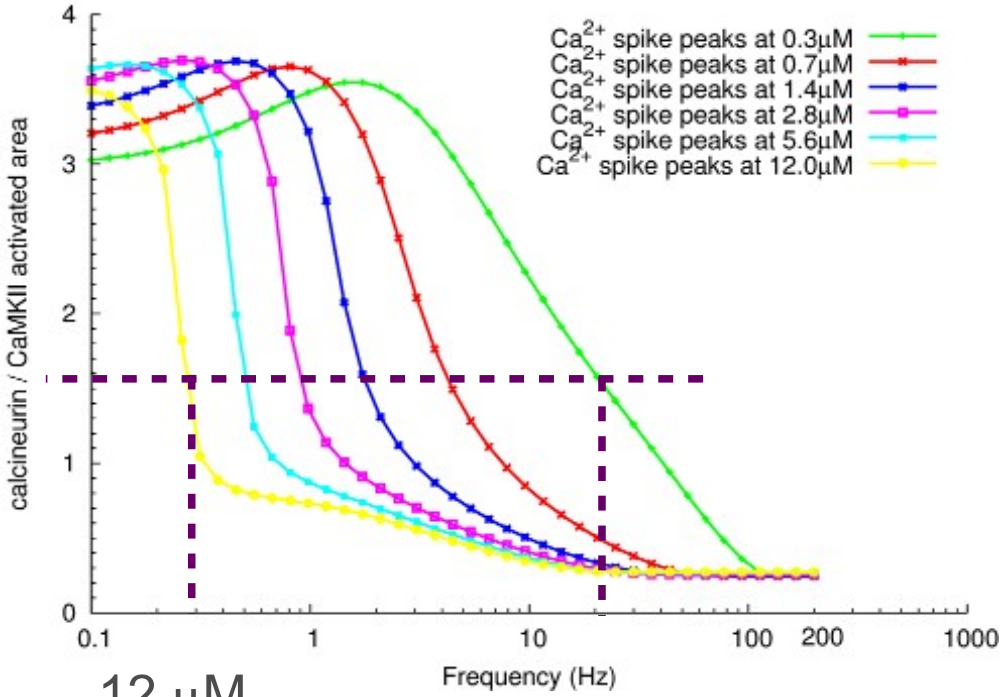


Effect of calcium duration and amount



180 spikes = 3 Hz

10 spikes = 70 Hz

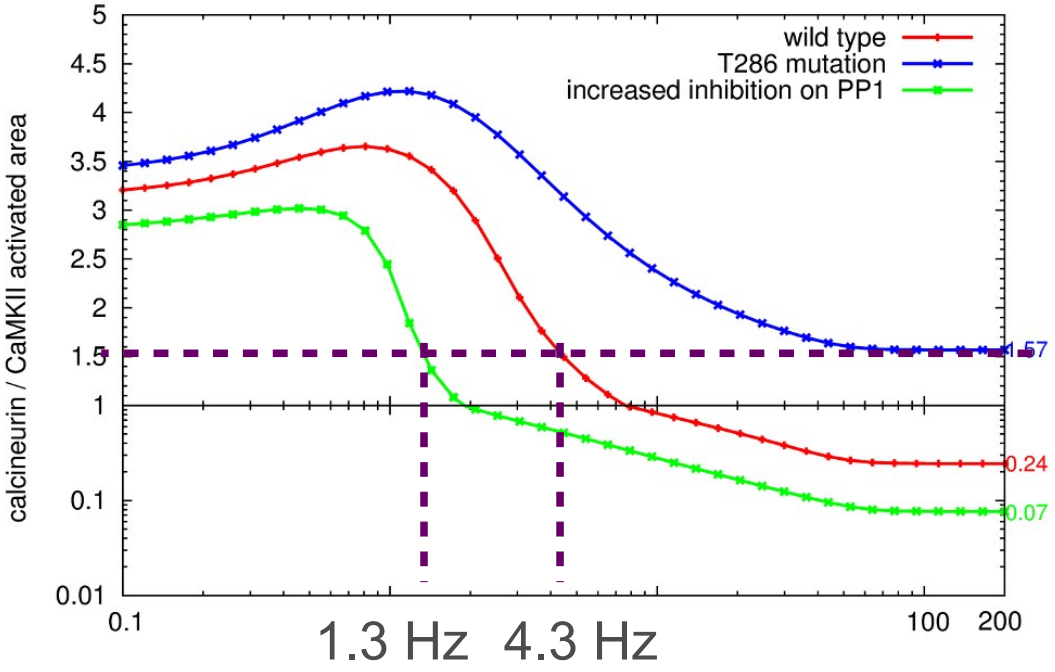


12 µM = 0.3 Hz

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

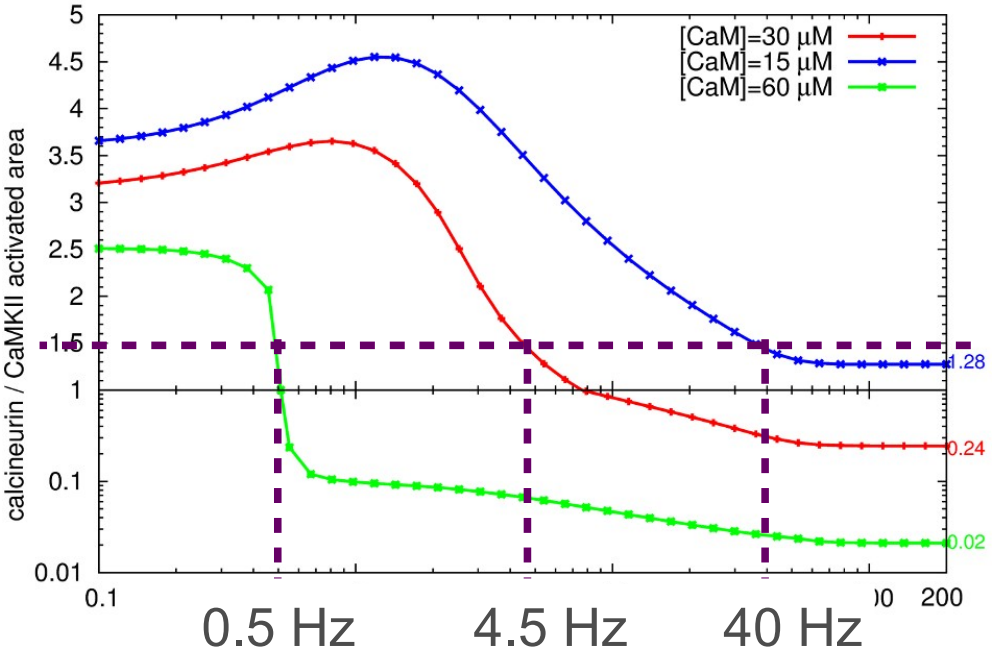
Never any positive plasticity

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

Lower deactivation of CaMKII

Competition for CaM, and CaN wins

No need of direct interactions between CaN and CaMKII to explain effect of T306 phosphorylation (Pi *et al* 2010).
T206P releases limiting CaM, that can Activate CaN



Babraham Institute

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

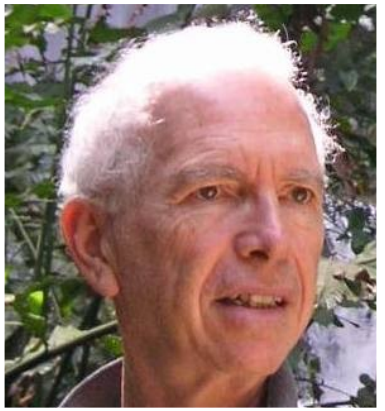
- Developers of ECell3, COPASI, Scilab

Melanie Stefan

Lu Li

Denis Brun

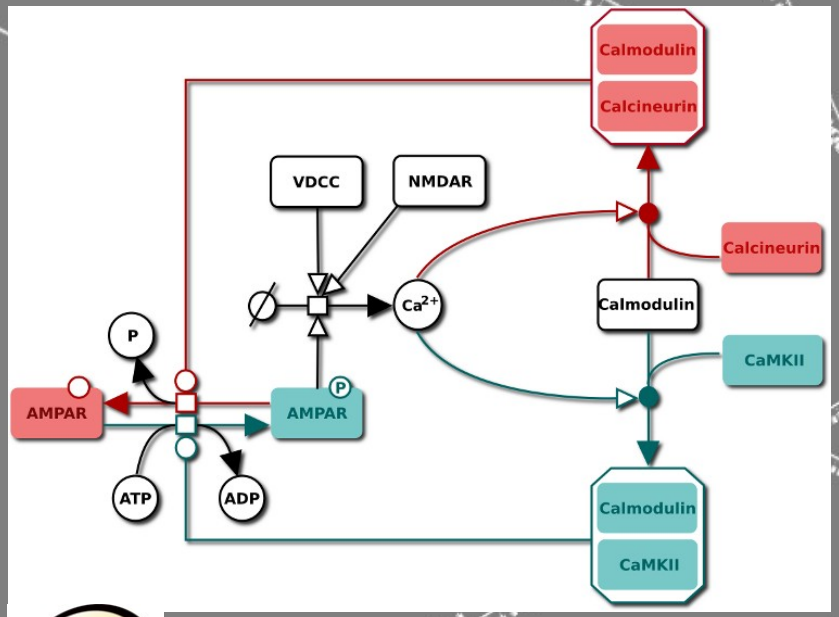
Michele Mattioni

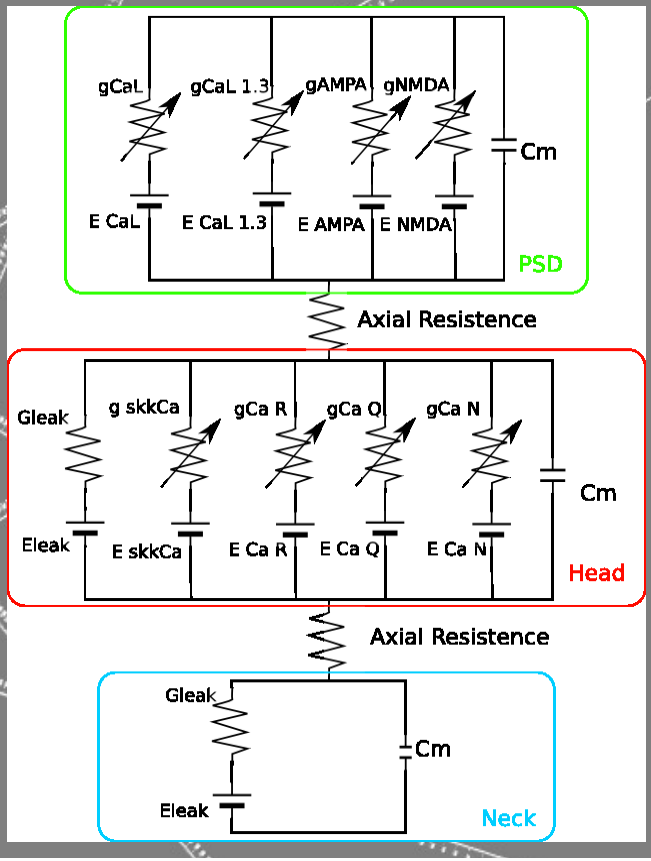


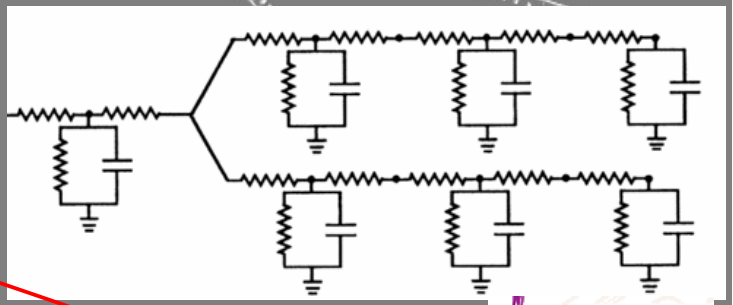
Stuart Edelstein

Massimo Lai

Whole cell: electro-biochemical models



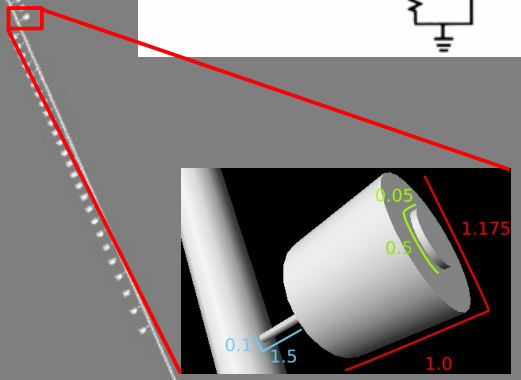




1504 spines

4761 compartments

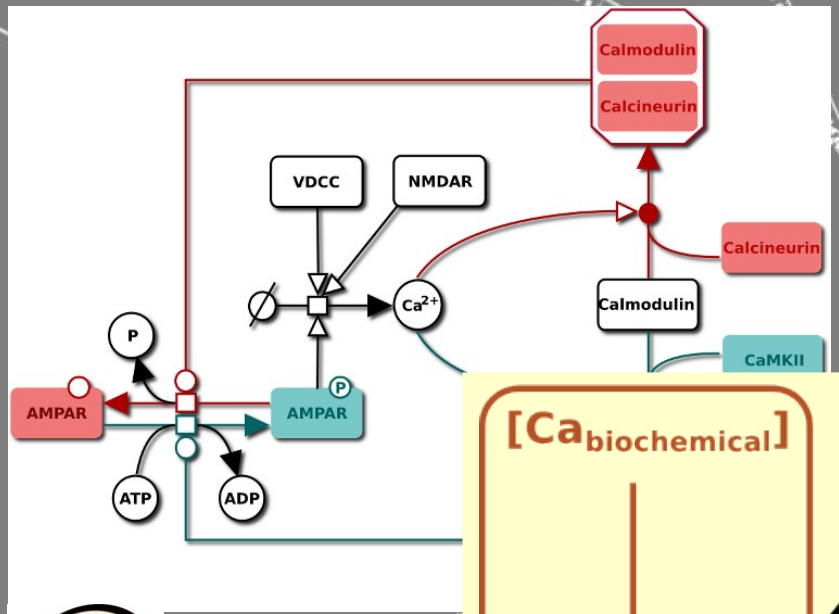
16362 channels

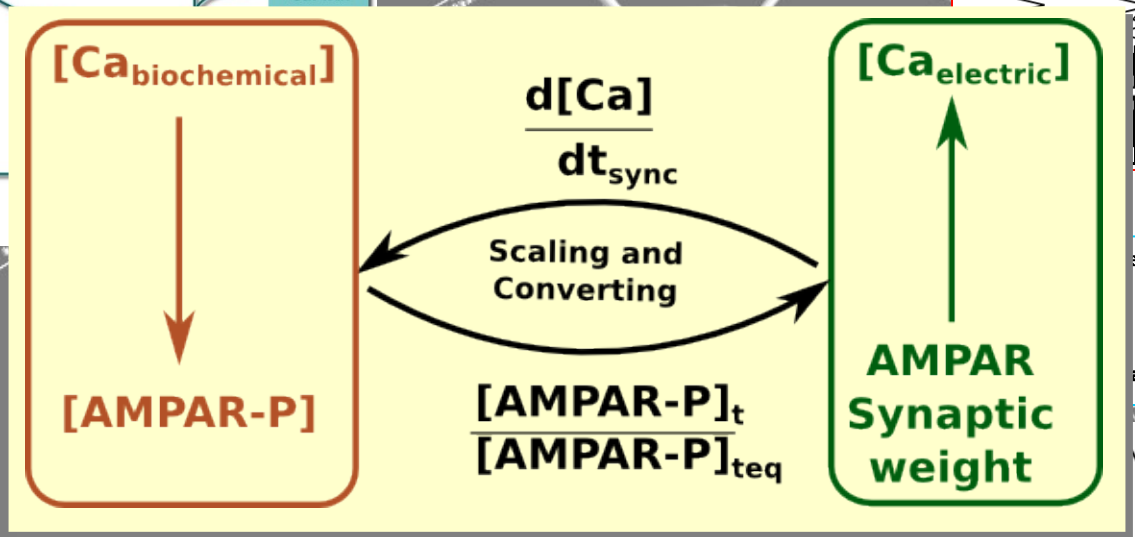


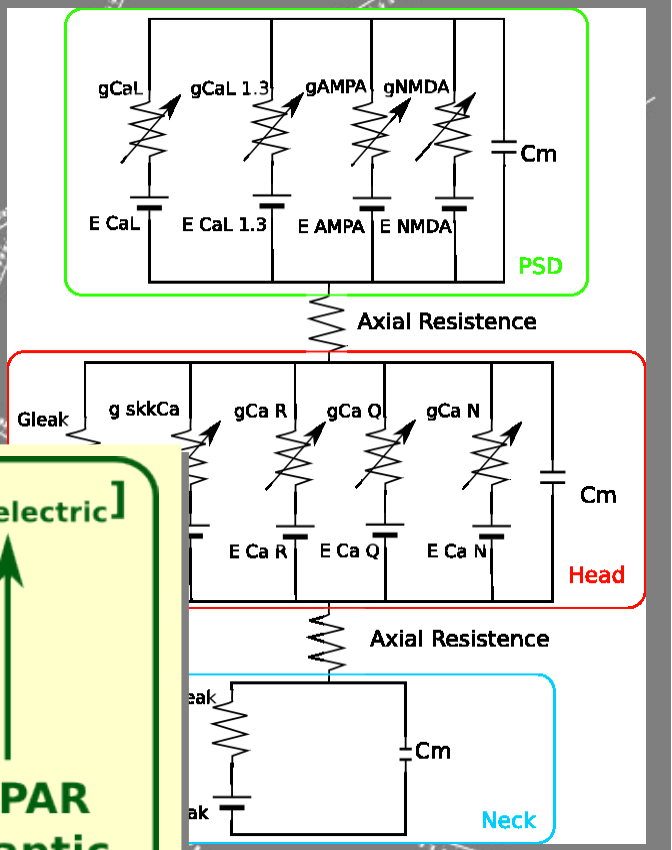
Mattioni M, Cohen U, Le Novère N (2012)

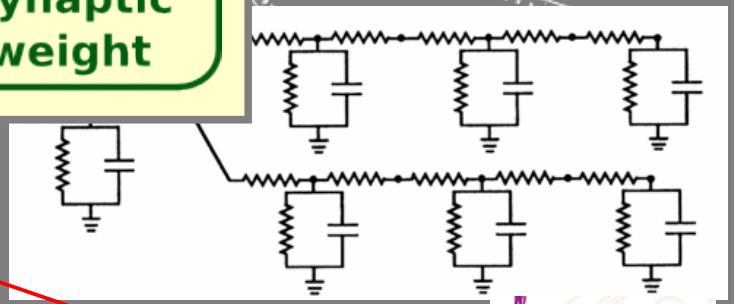
Mattioni M, Le Novère N (2013)

Whole cell: electro-biochemical models





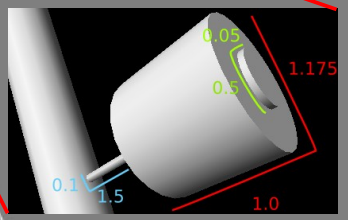




1504 spines

4761 compartments

16362 channels



Mattioni M, Cohen U, Le Novère N (2012)

Mattioni M, Le Novère N (2013)