Allosteric calcium sensors in synaptic plasticity

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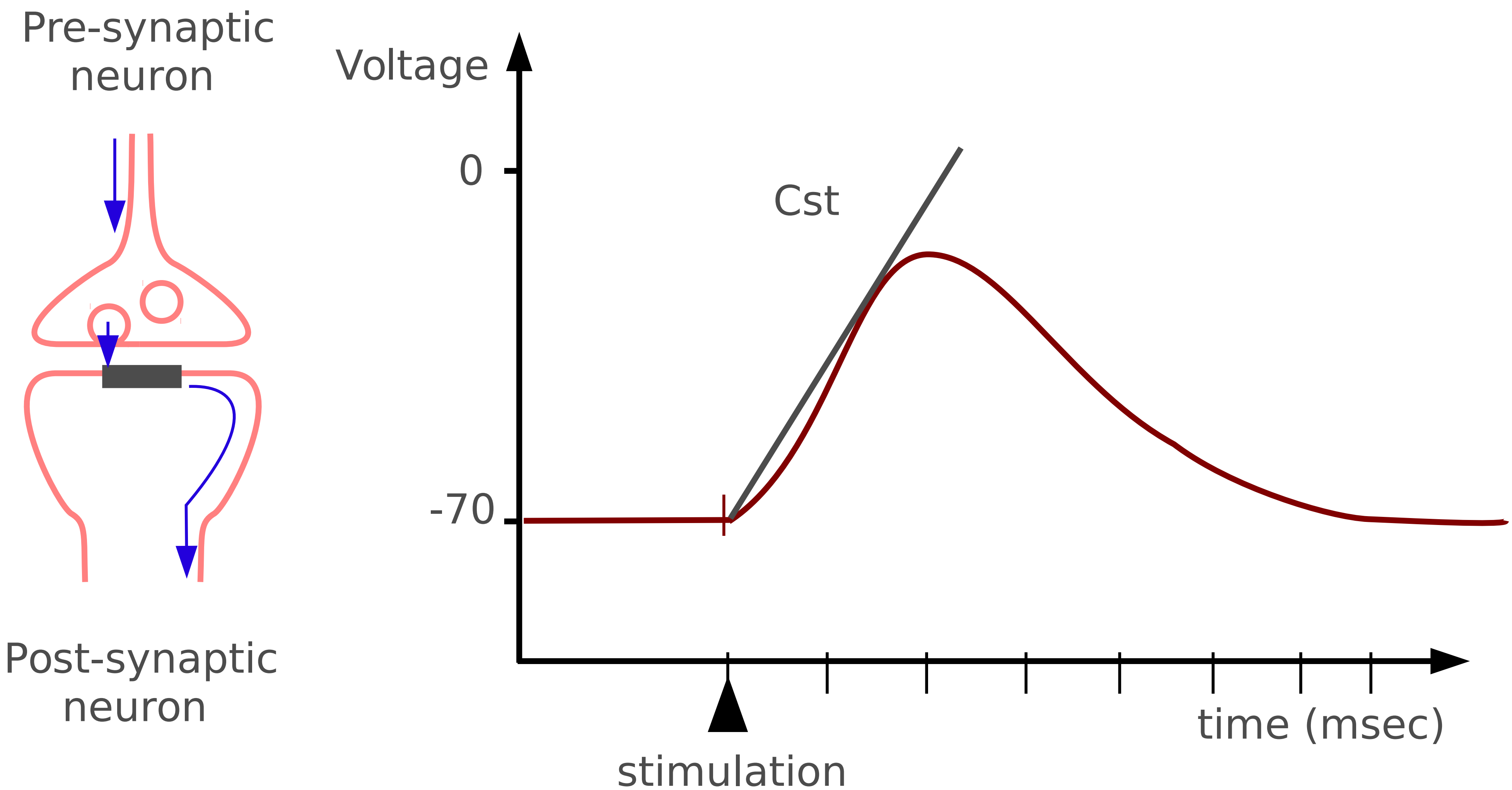
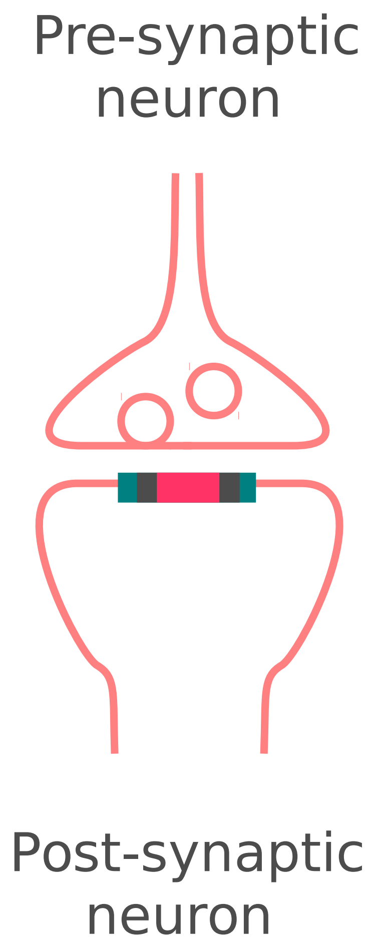
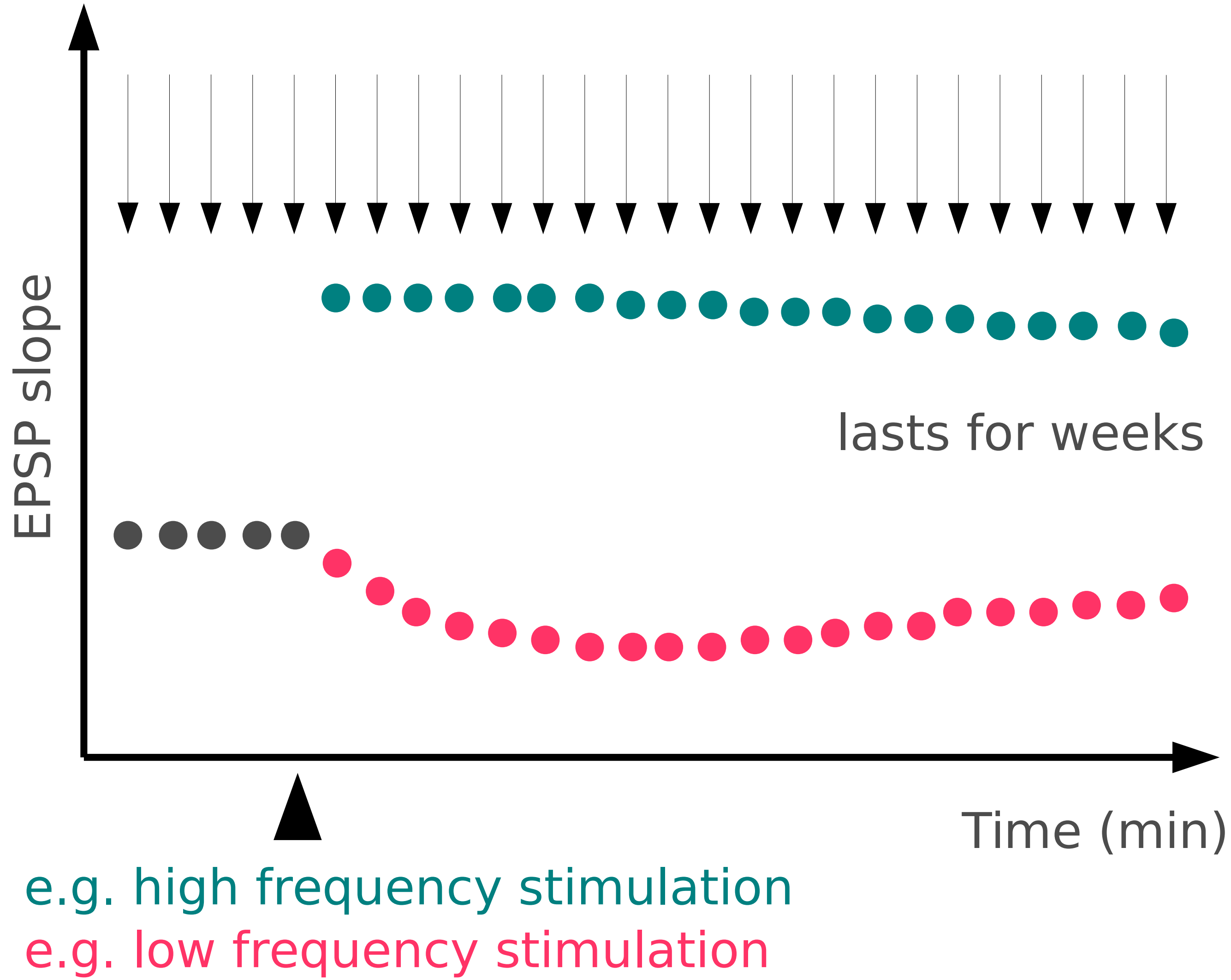
Babraham Institute
http://lenoverelab.org

Excitatory post-synaptic potential

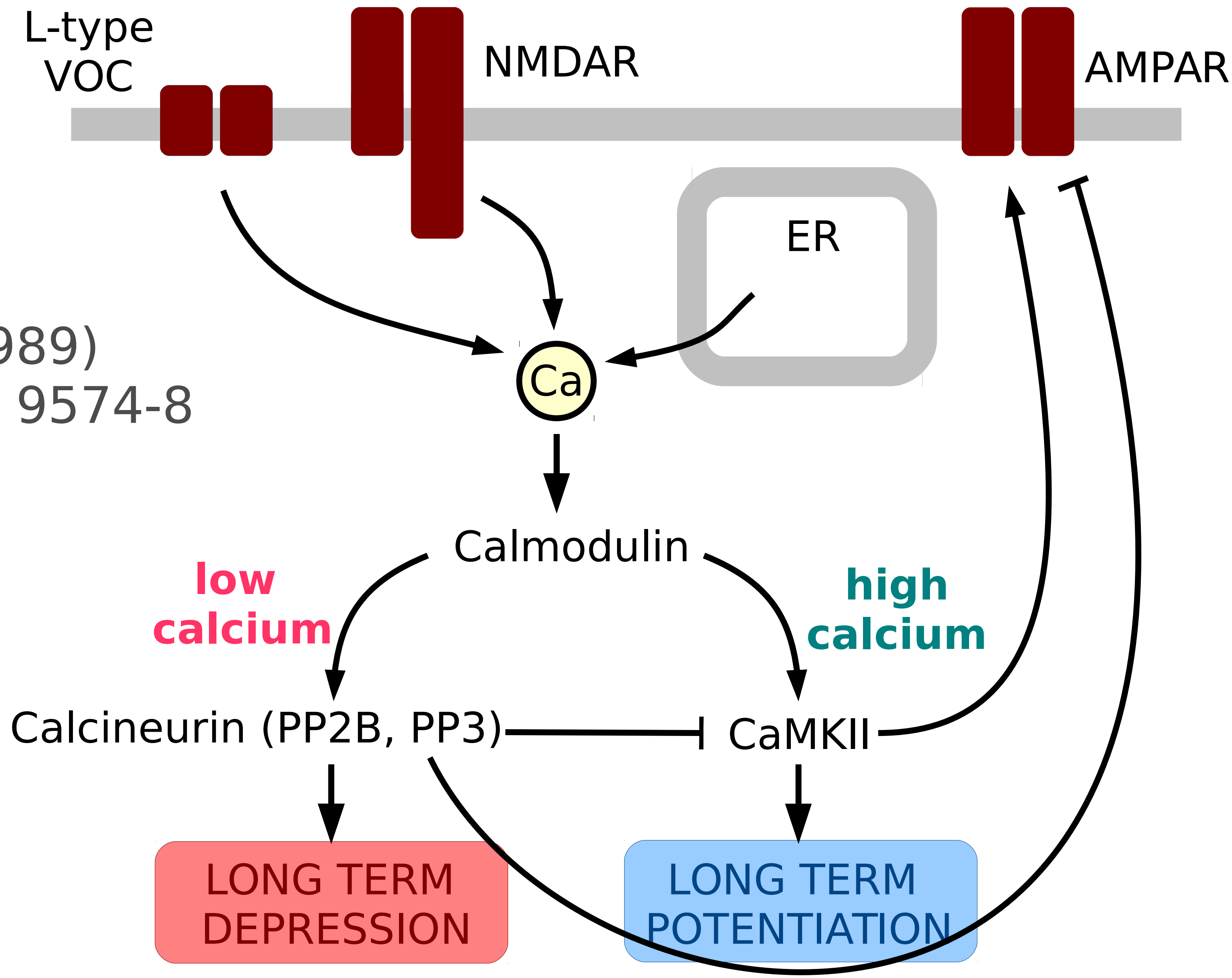


Bidirectional synaptic plasticity



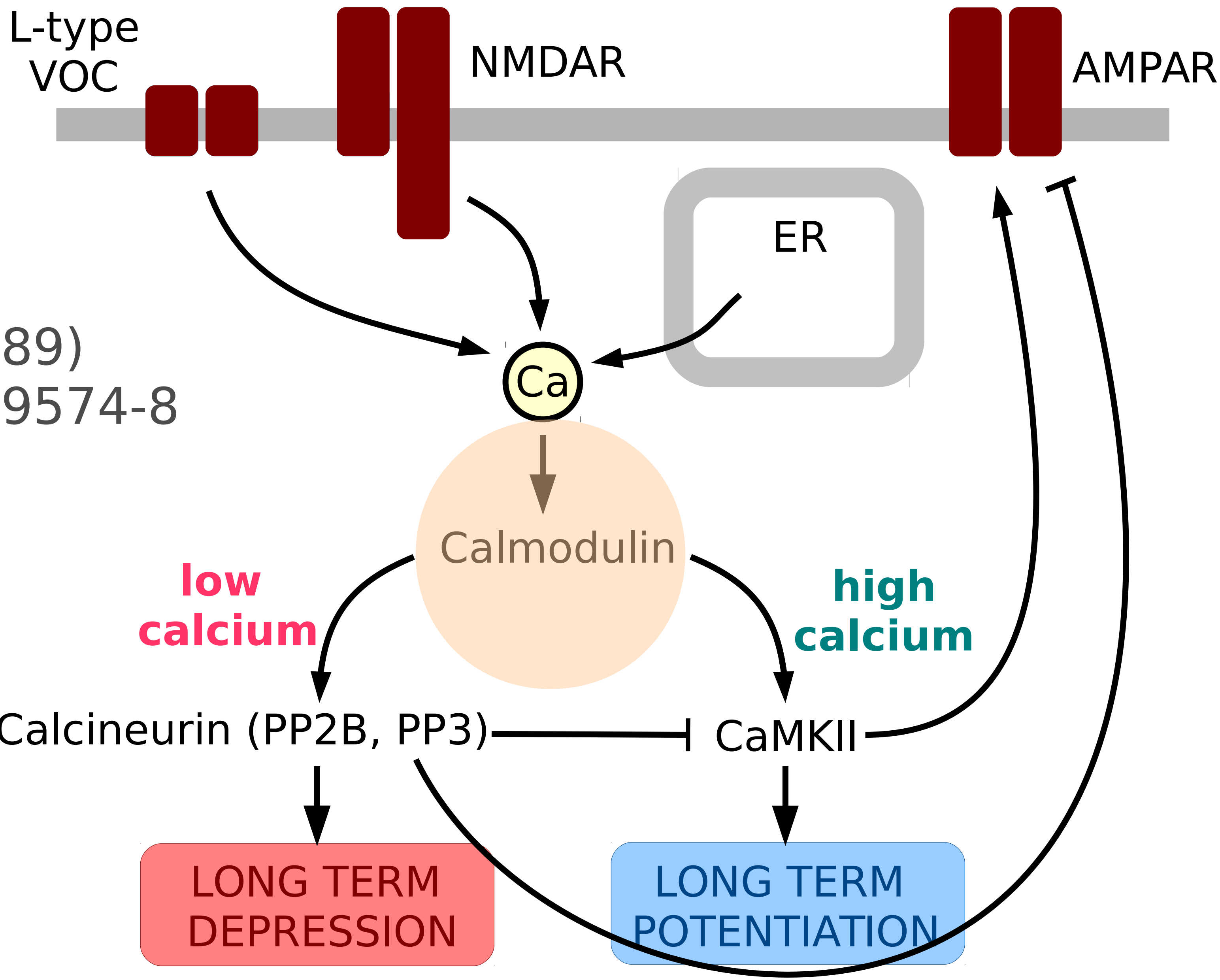


The calcium theory of synaptic plasticity



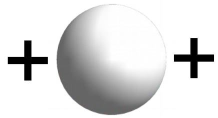
Lisman (1989)
PNAS, 86: 9574-8

Calmodulin, the memory switchboard



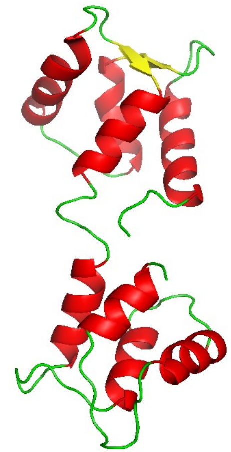
Lisman (1989)
PNAS, 86: 9574-8

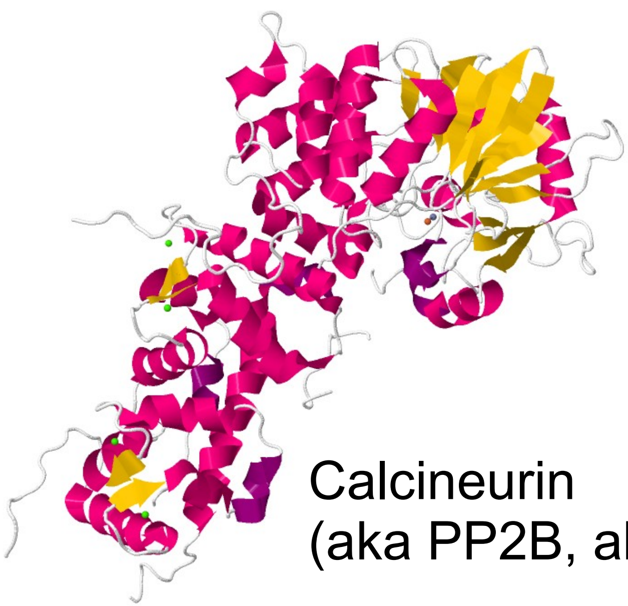
Calcium



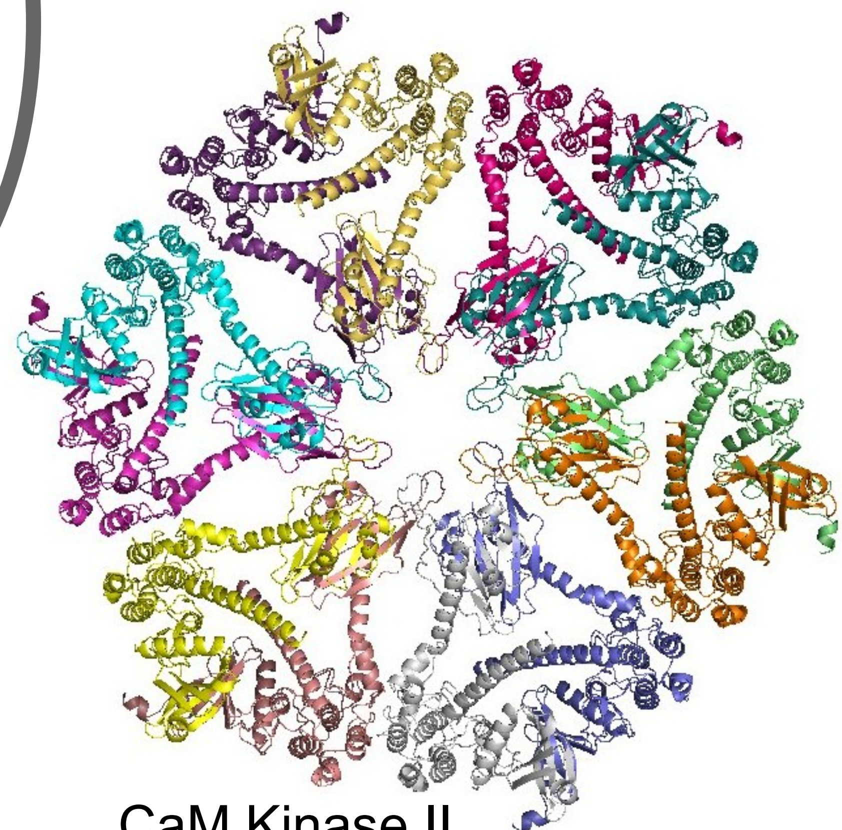
Neurogranin

Calmodulin



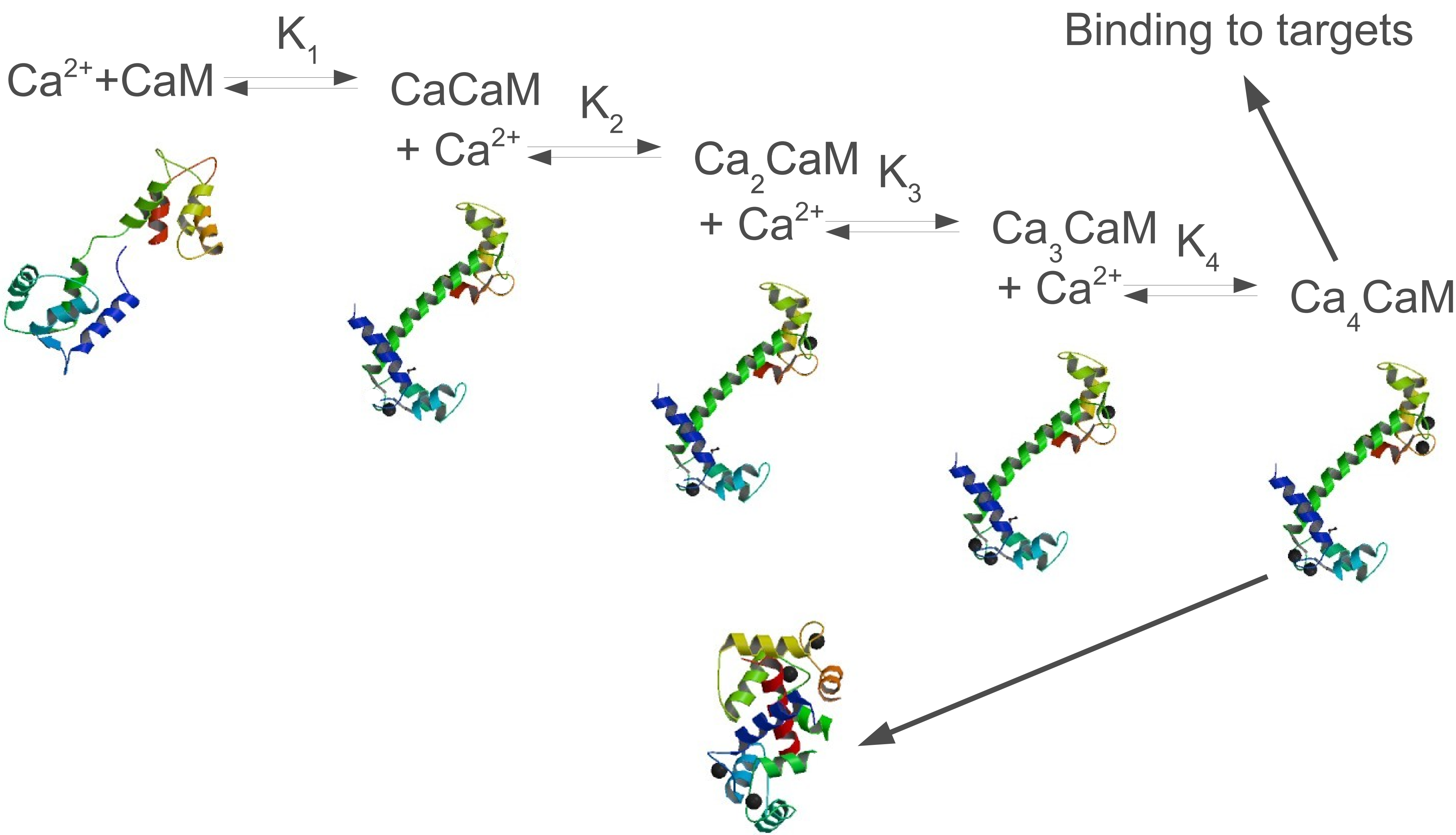


Calcineurin
(aka PP2B, aka PP3)

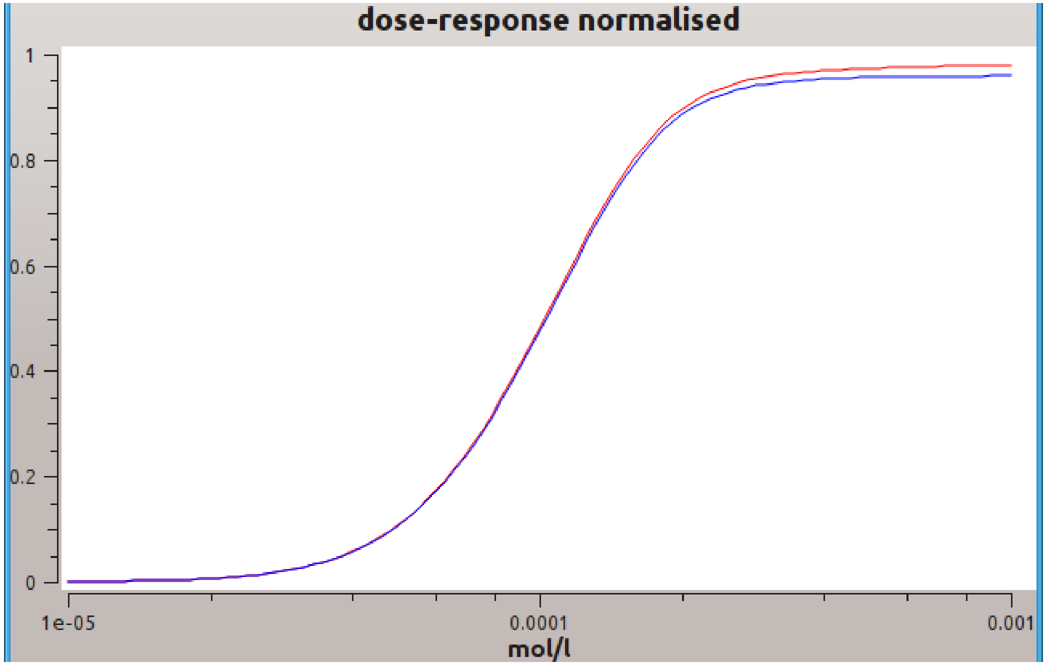


CaM Kinase II

Adair-Klotz + key-lock



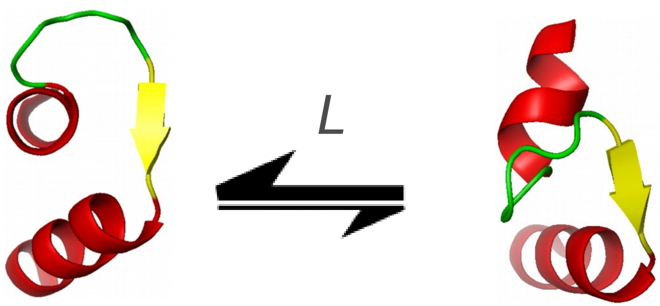
Adair-Klotz does not permit differential activations

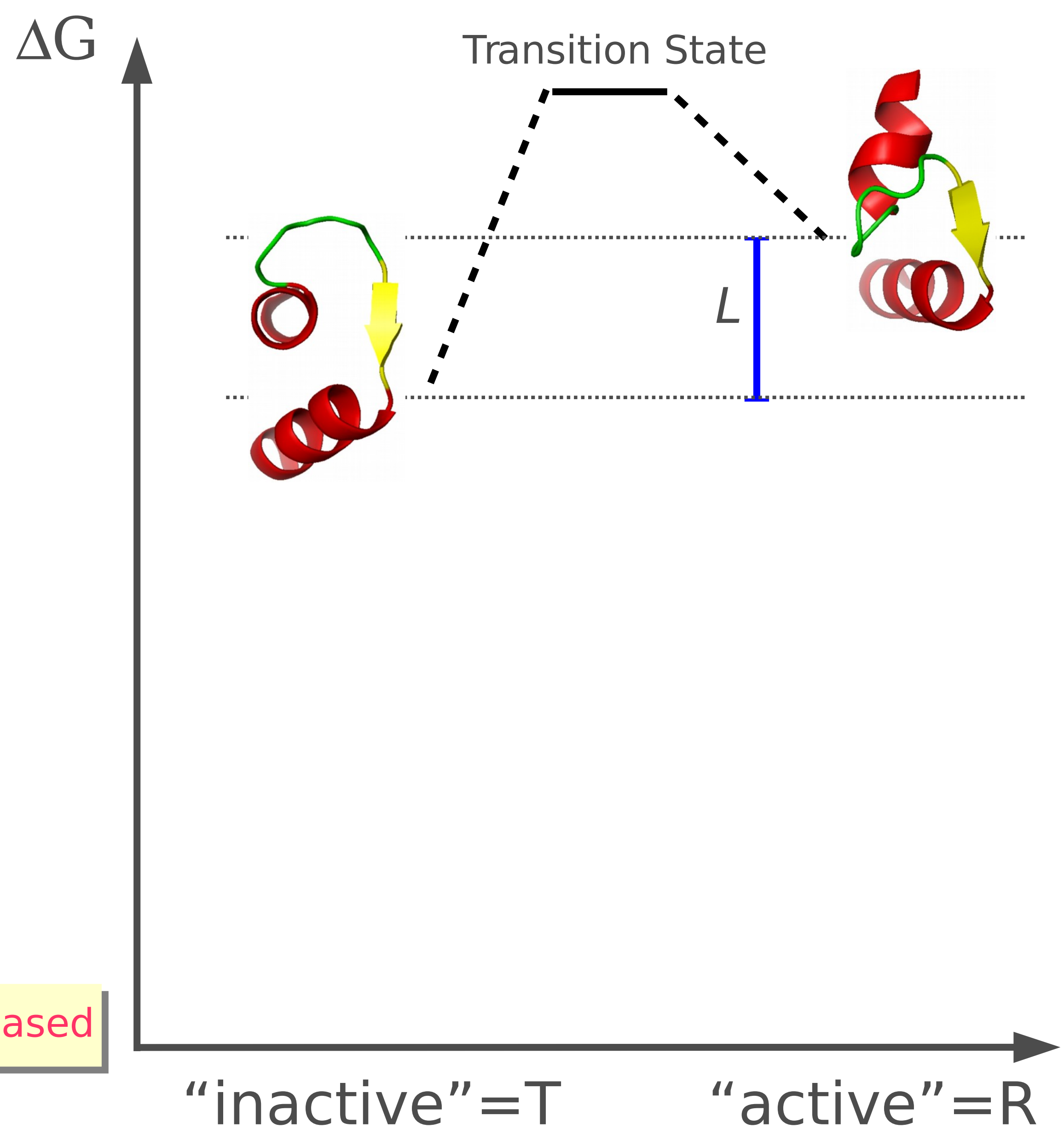


[CaN]=[CamKII]=[CaM]/10 ;
Kd_CaMKII = 10xKd_CaN;

- Calmodulin can activate calcineurin with 3 Ca^{2+} (Kincaid and Vaughan 1986)
- Calmodulin can bind CaMKII with 2 Ca^{2+} (Shifman *et al* 2006)
- Calmodulin affinity for calcium increases once bound to CaMKII (Shifman *et al* 2006; Previous reports on other targets: e.g. Burger *et al* 1983. Olwin *et al* 1984)

1 Modulation of thermal equilibria ≠ induced-fit

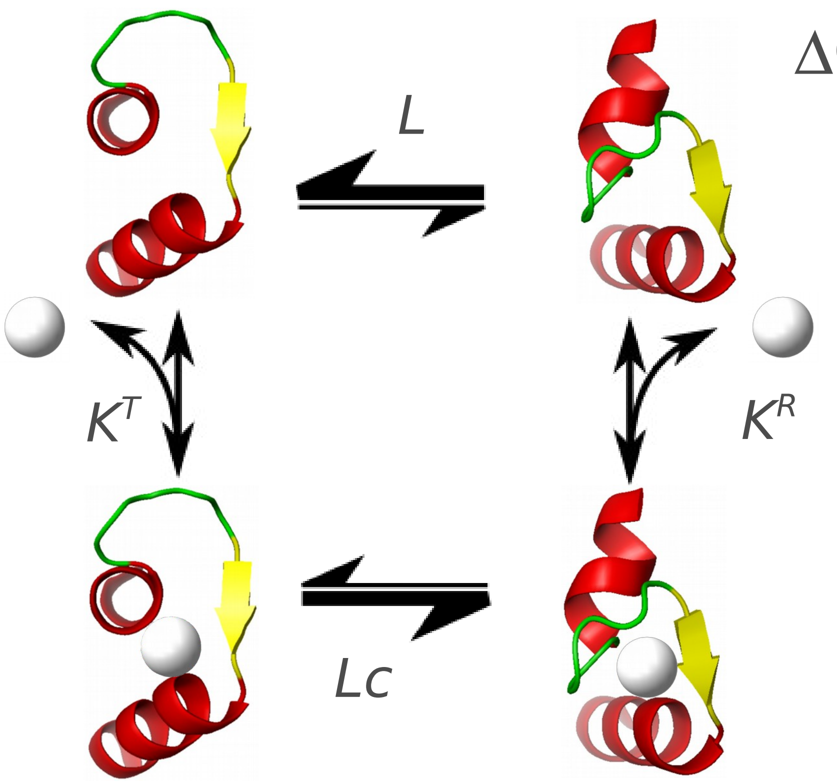


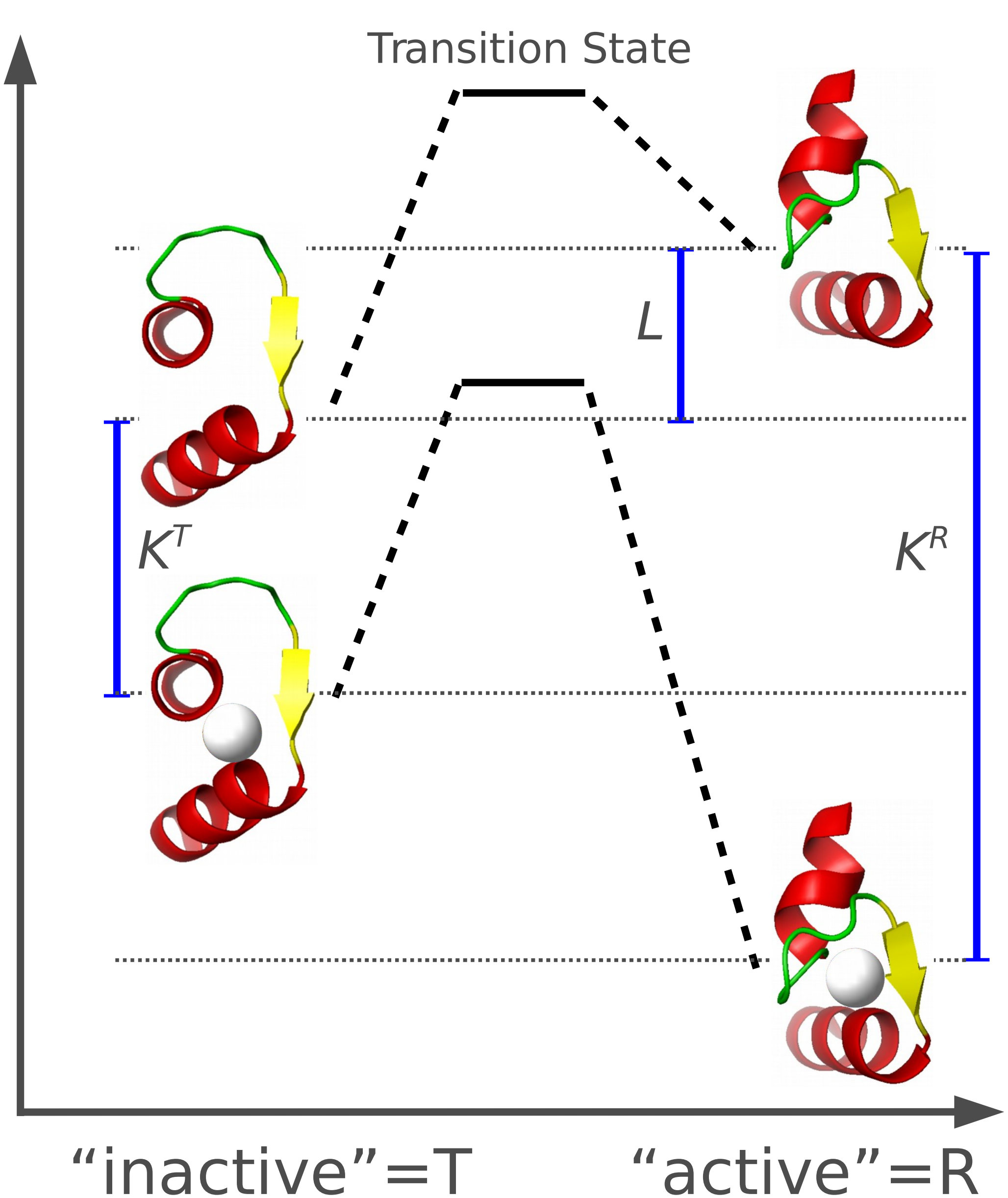


$$L = \frac{[T_0]}{[R_0]}$$

$L \gg 1 \Rightarrow$ Equilibrium strongly biased

1 Modulation of thermal equilibria ≠ induced-fit



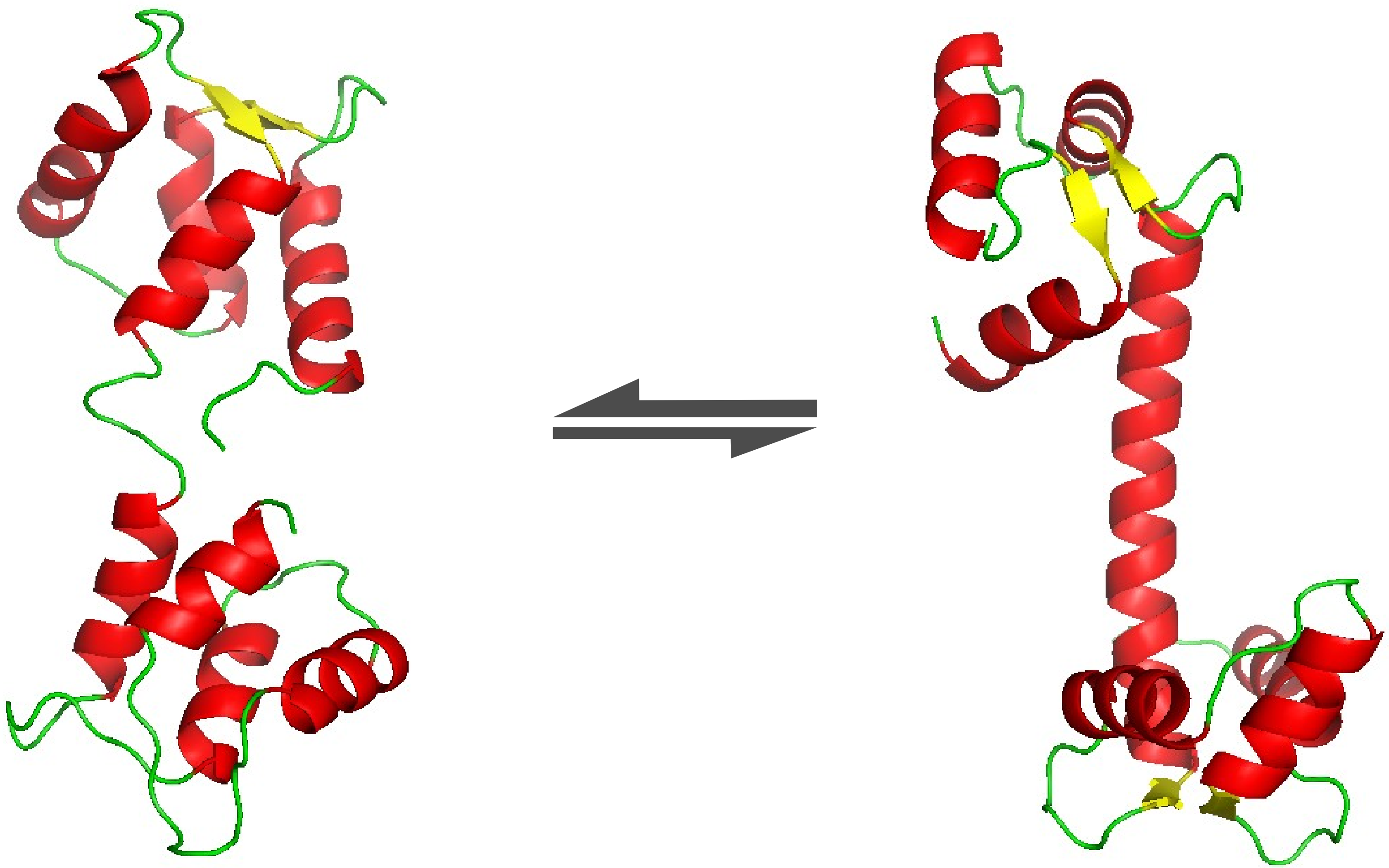


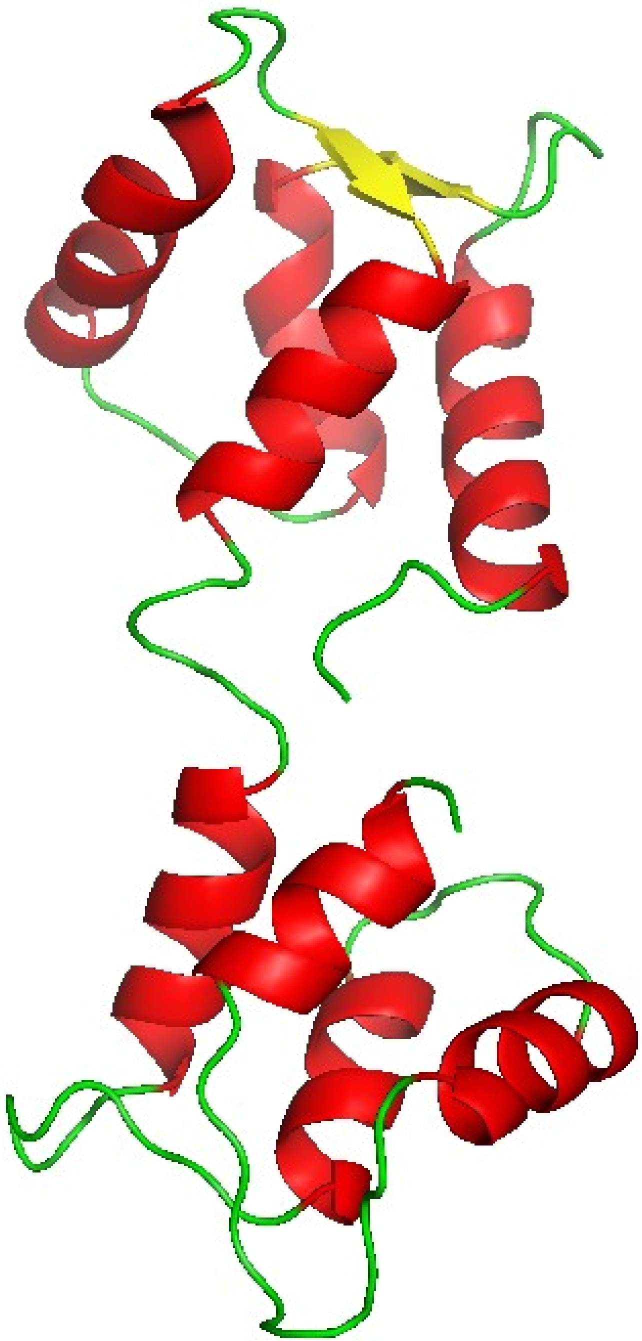
$$L = \frac{[T_0]}{[R_0]}$$

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

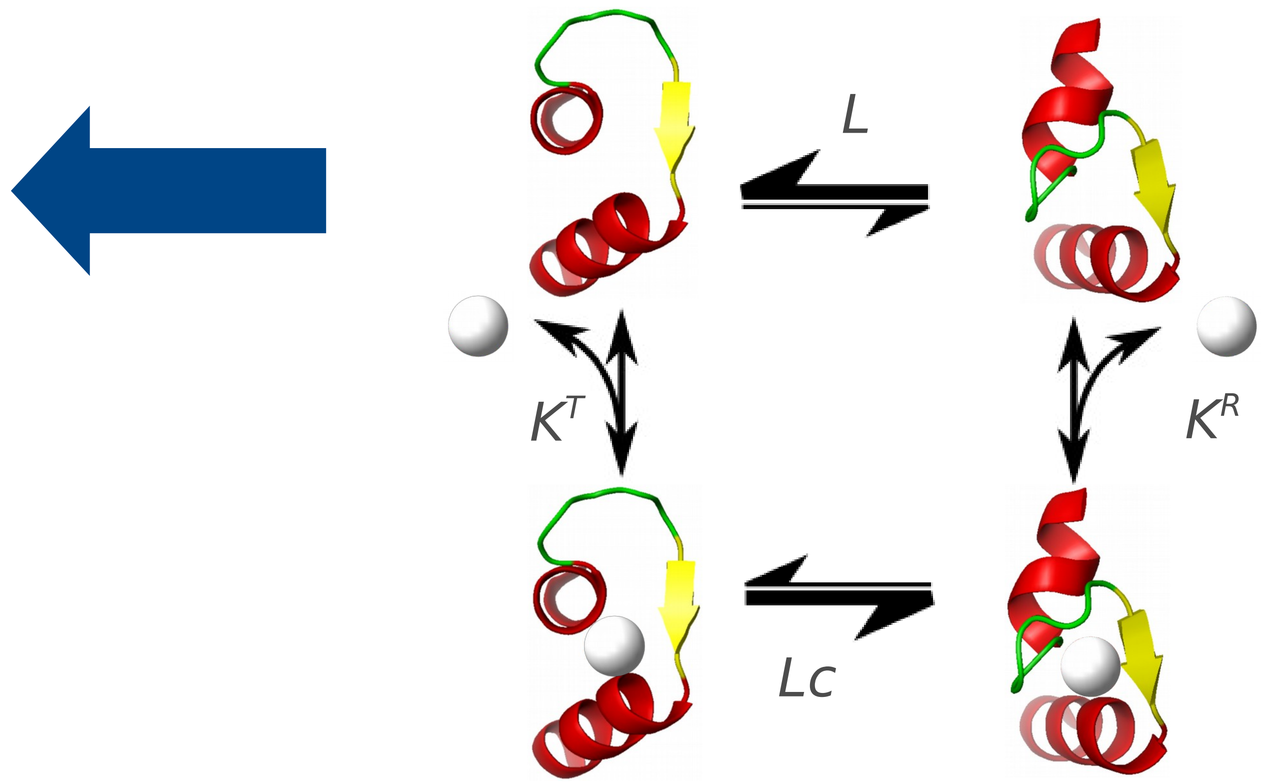
$c \ll 1 \Rightarrow$ ligand has strong effect

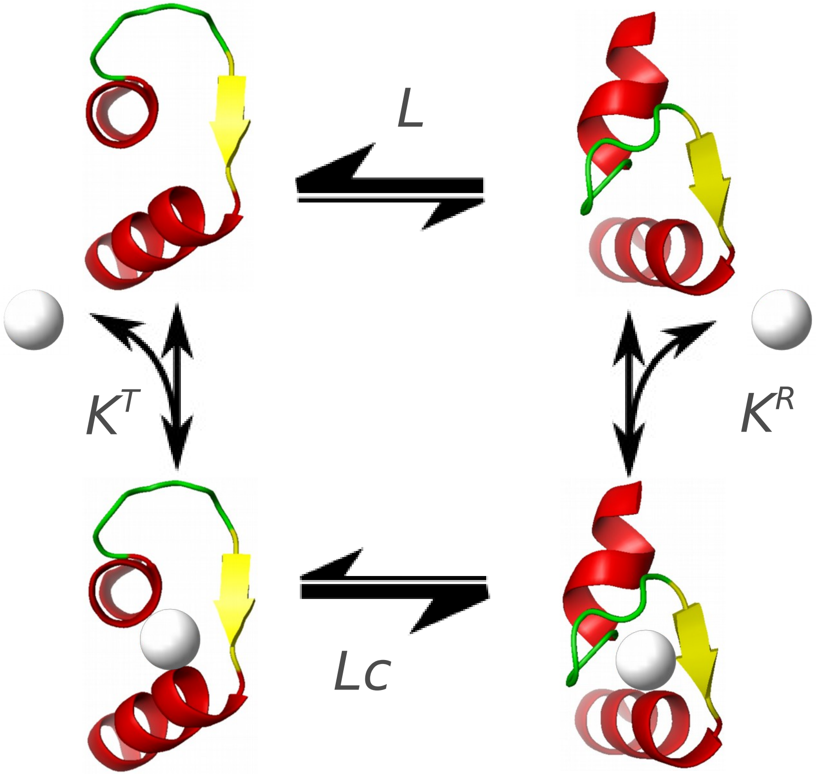
2 Concerted transitions ≠ sequential model

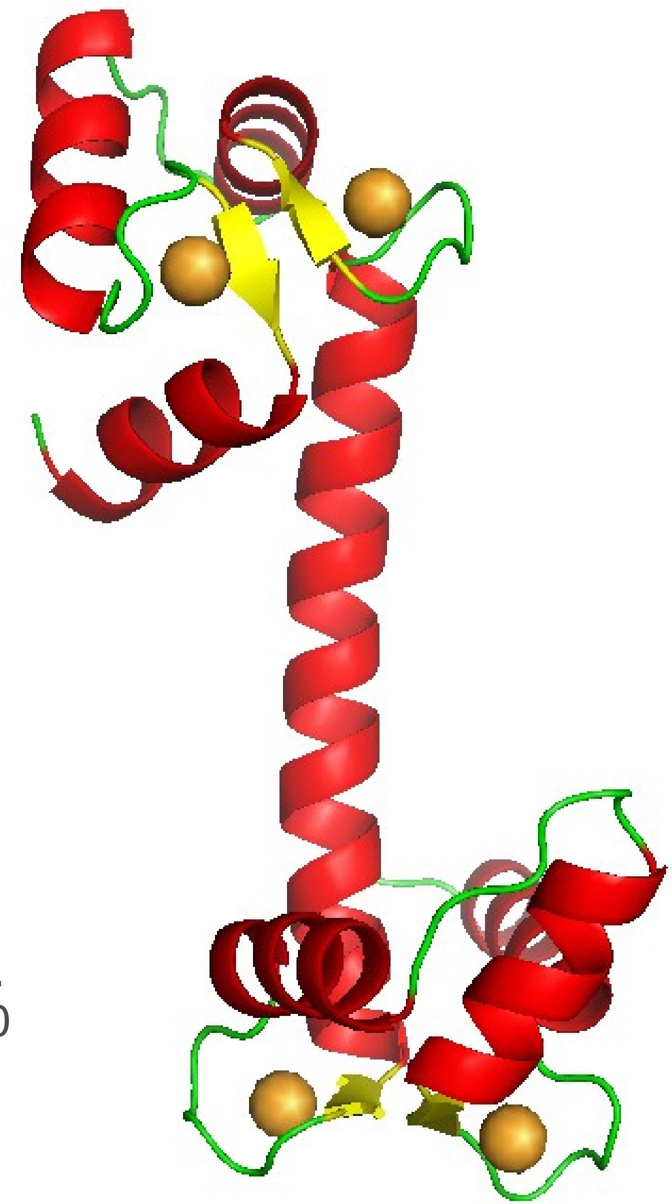




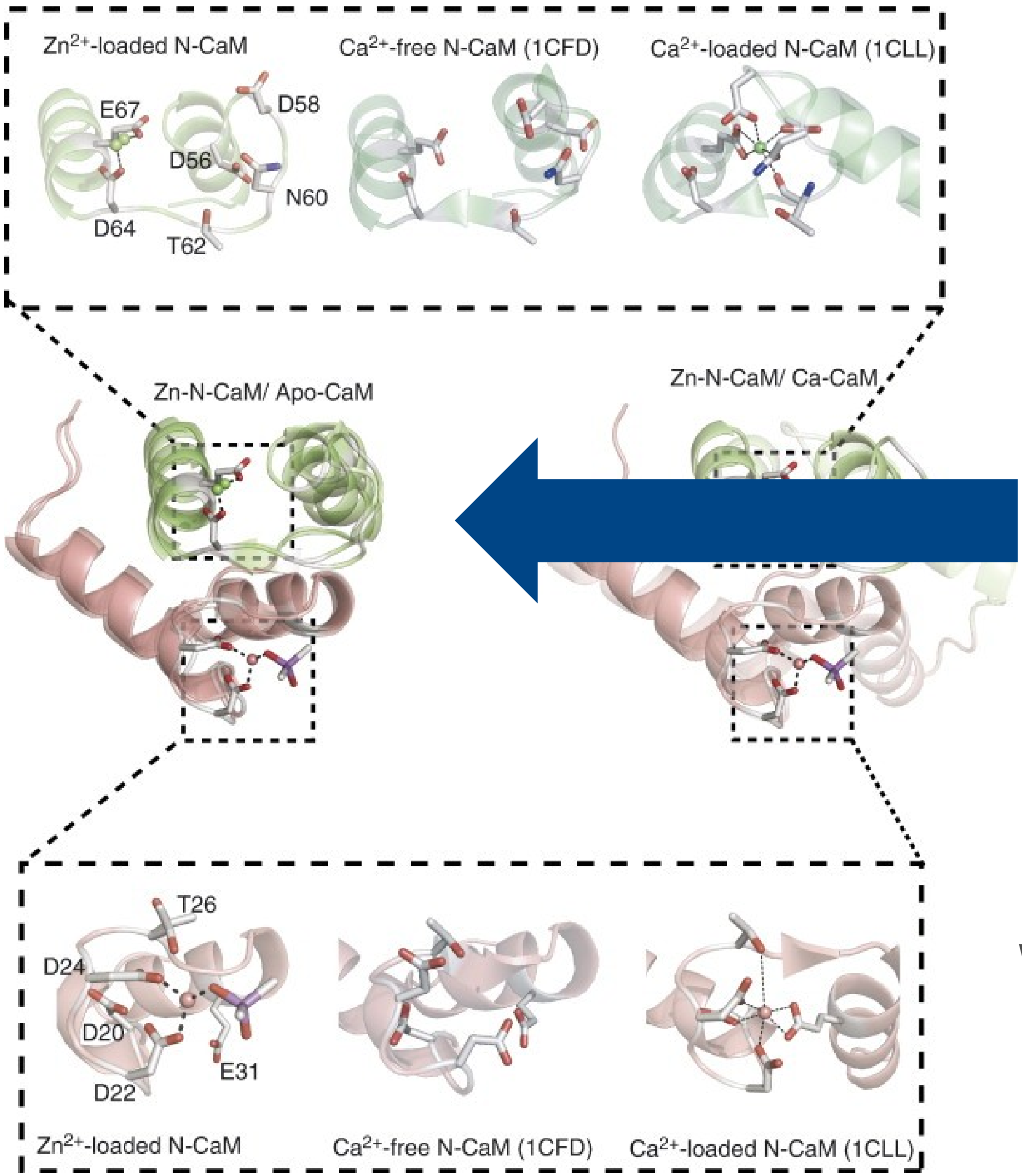
Kubiniwas *et al.* (1995).
Nat Struct Biol 2: 768-776

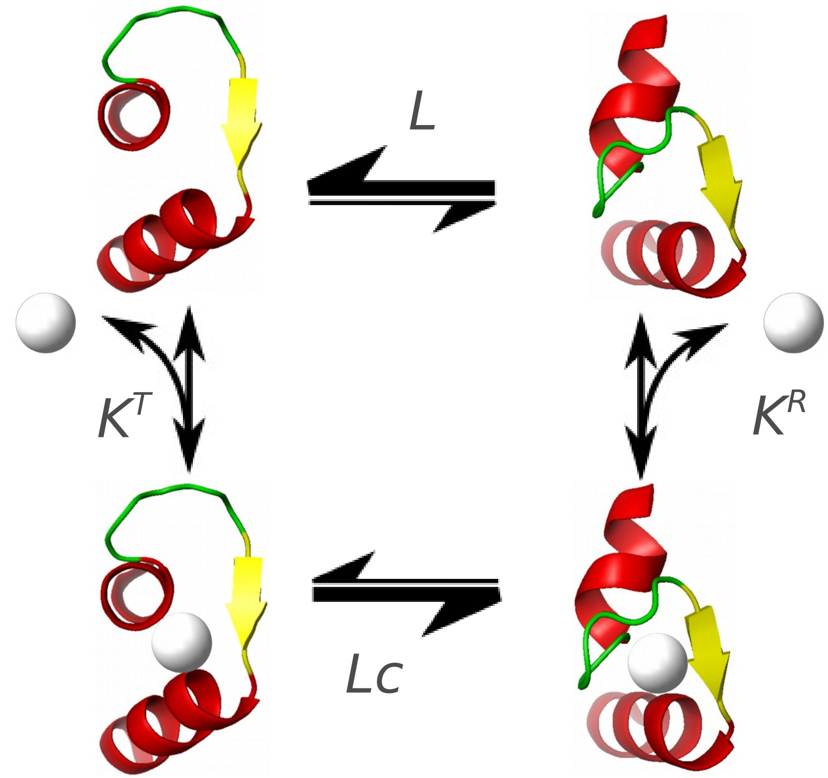




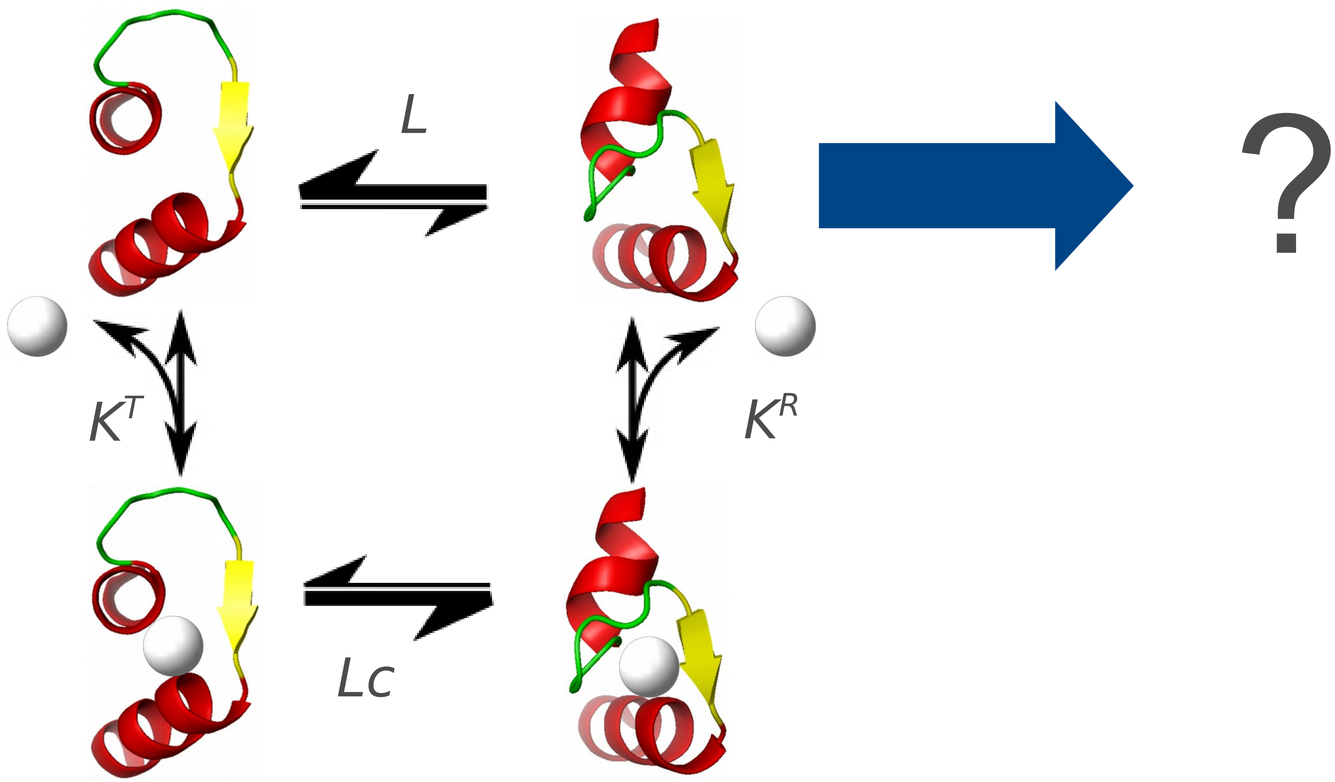


Babu *et al.* (1985).
Nature 315: 37-40

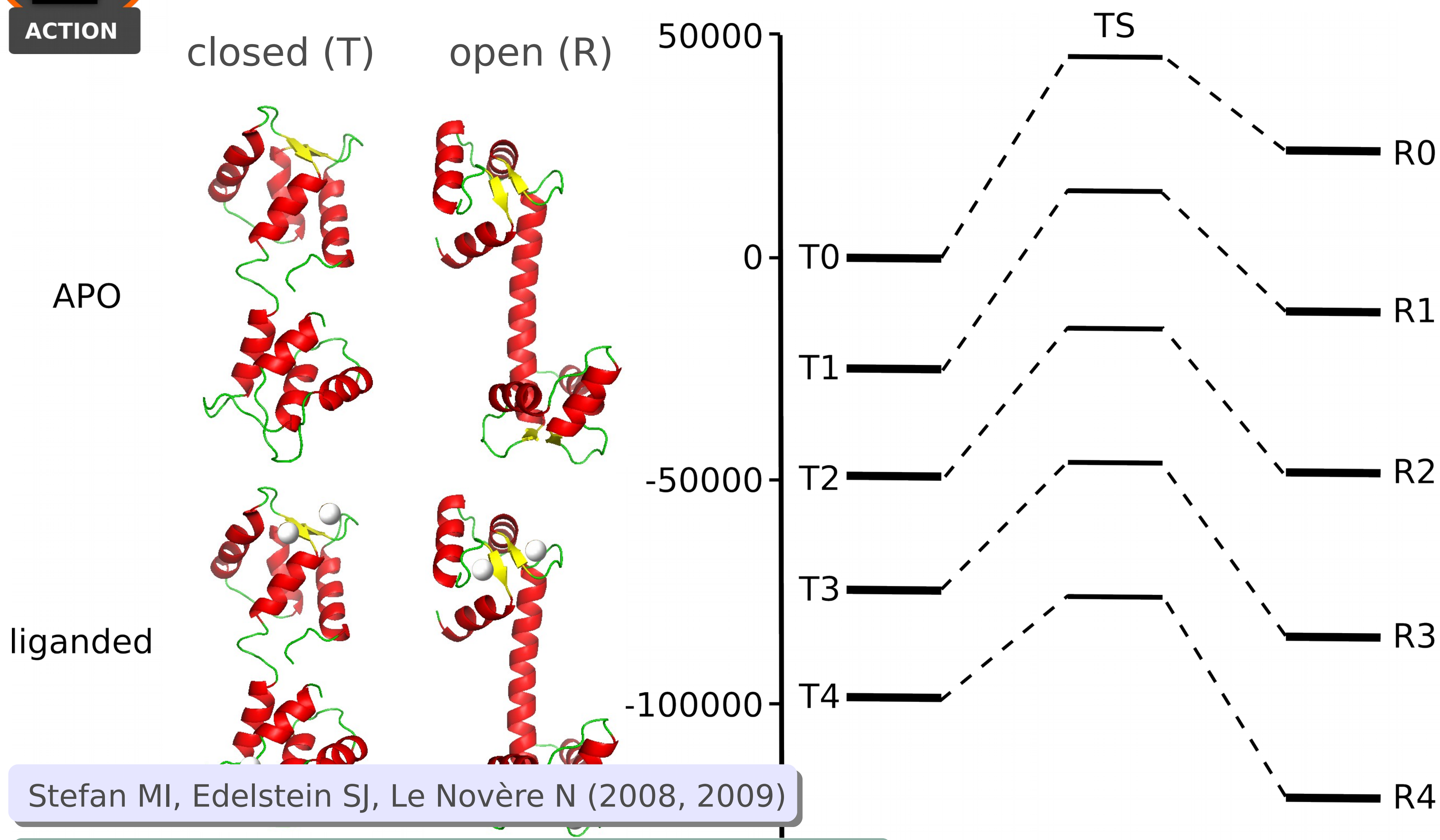




Warren *et al.* (2007).
J Mol Biol 374: 517-527


L
K^T
K^R
Lc
?

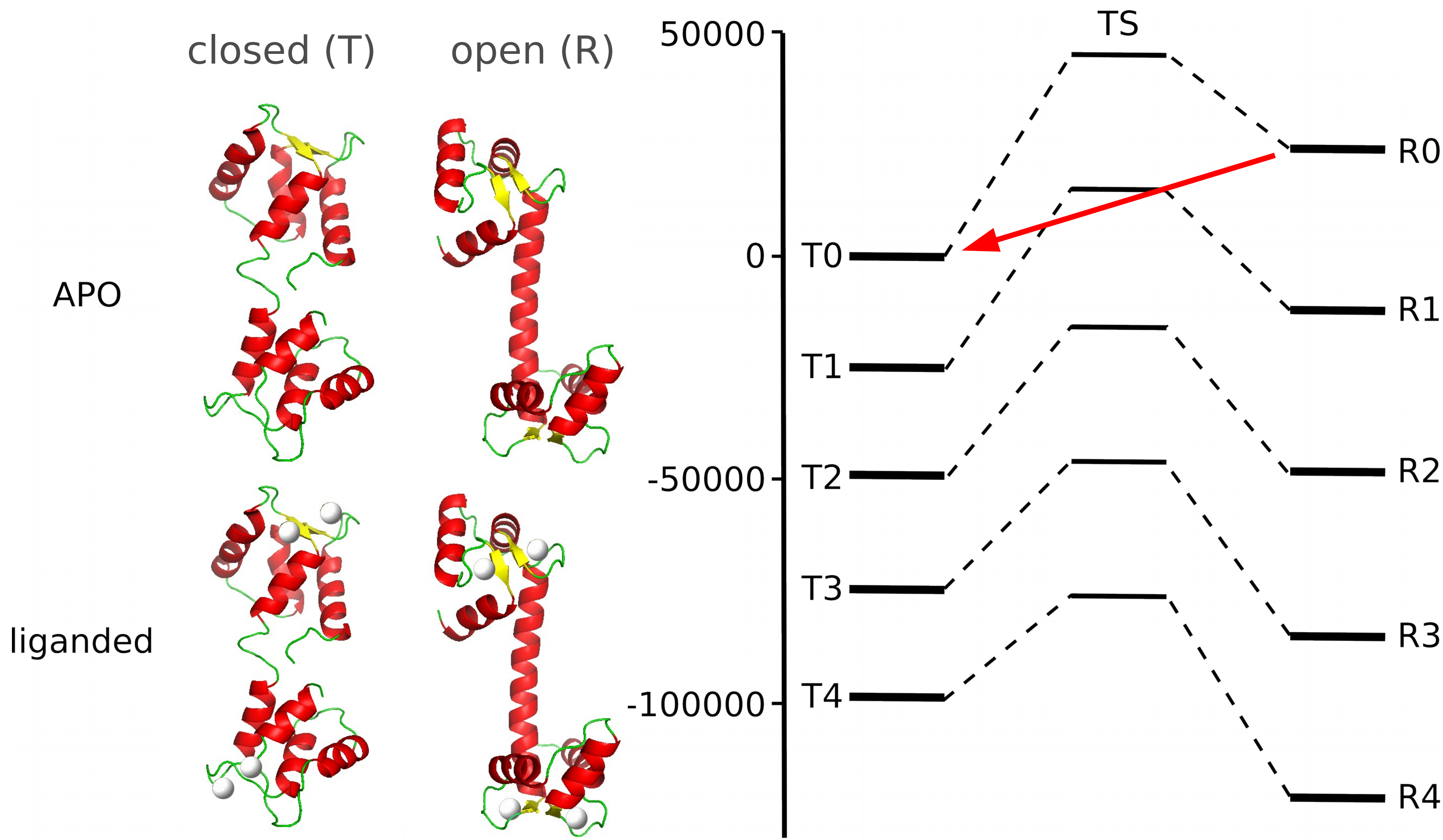
Allosteric model of Calmodulin activation



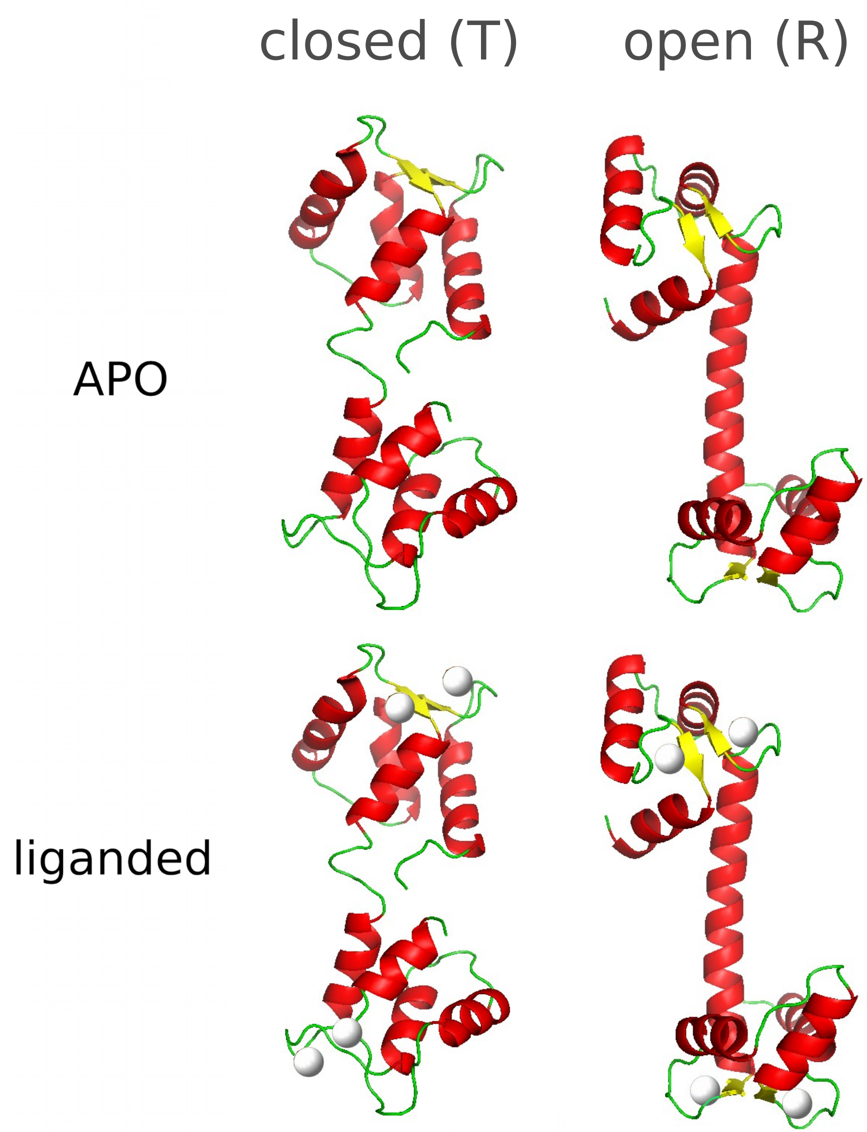
Stefan MI, Edelstein SJ, Le Novère N (2008, 2009)

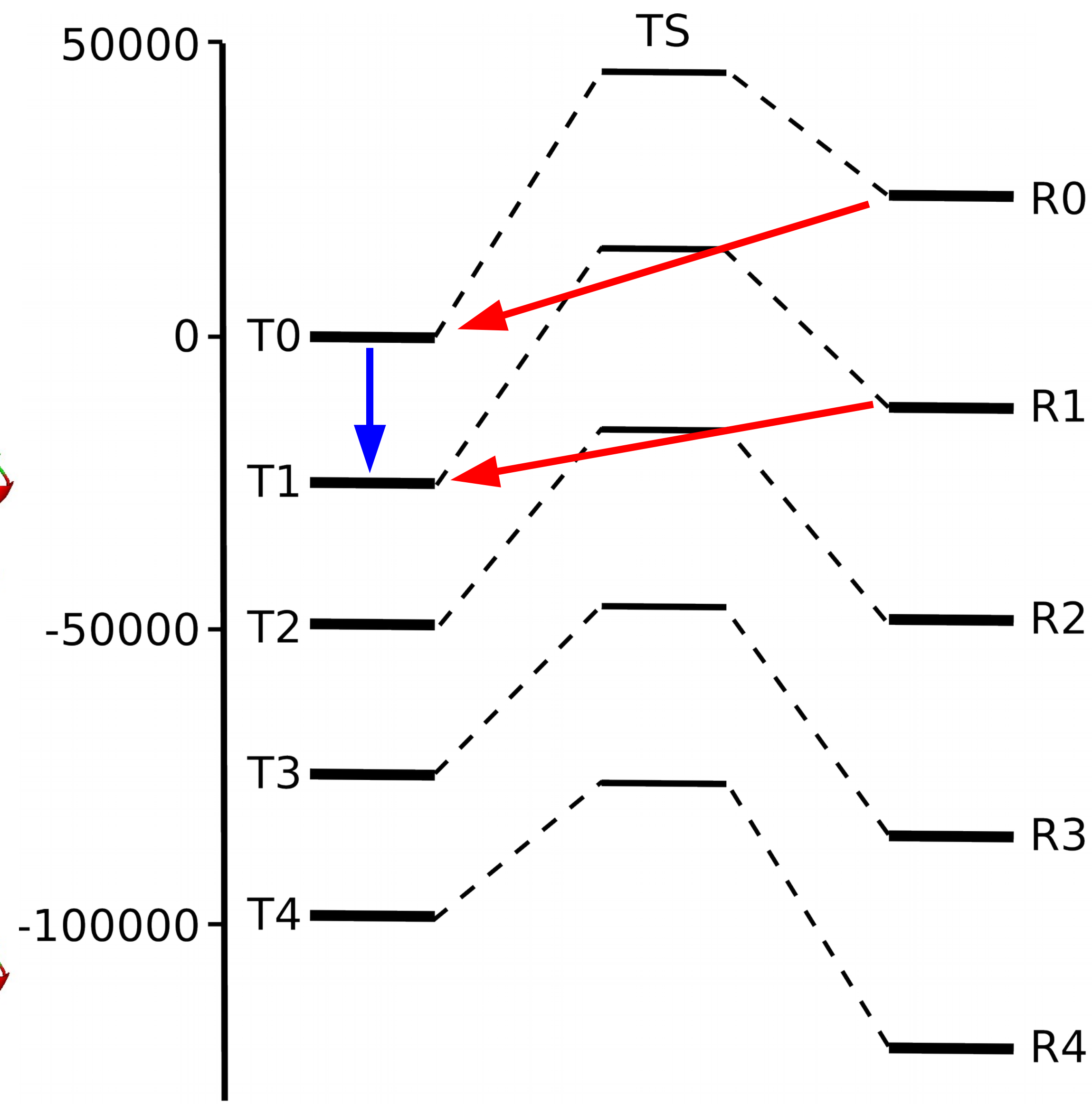
http://identifiers.org/biomodels.db/BIOMD0000000183

Allosteric model of Calmodulin activation

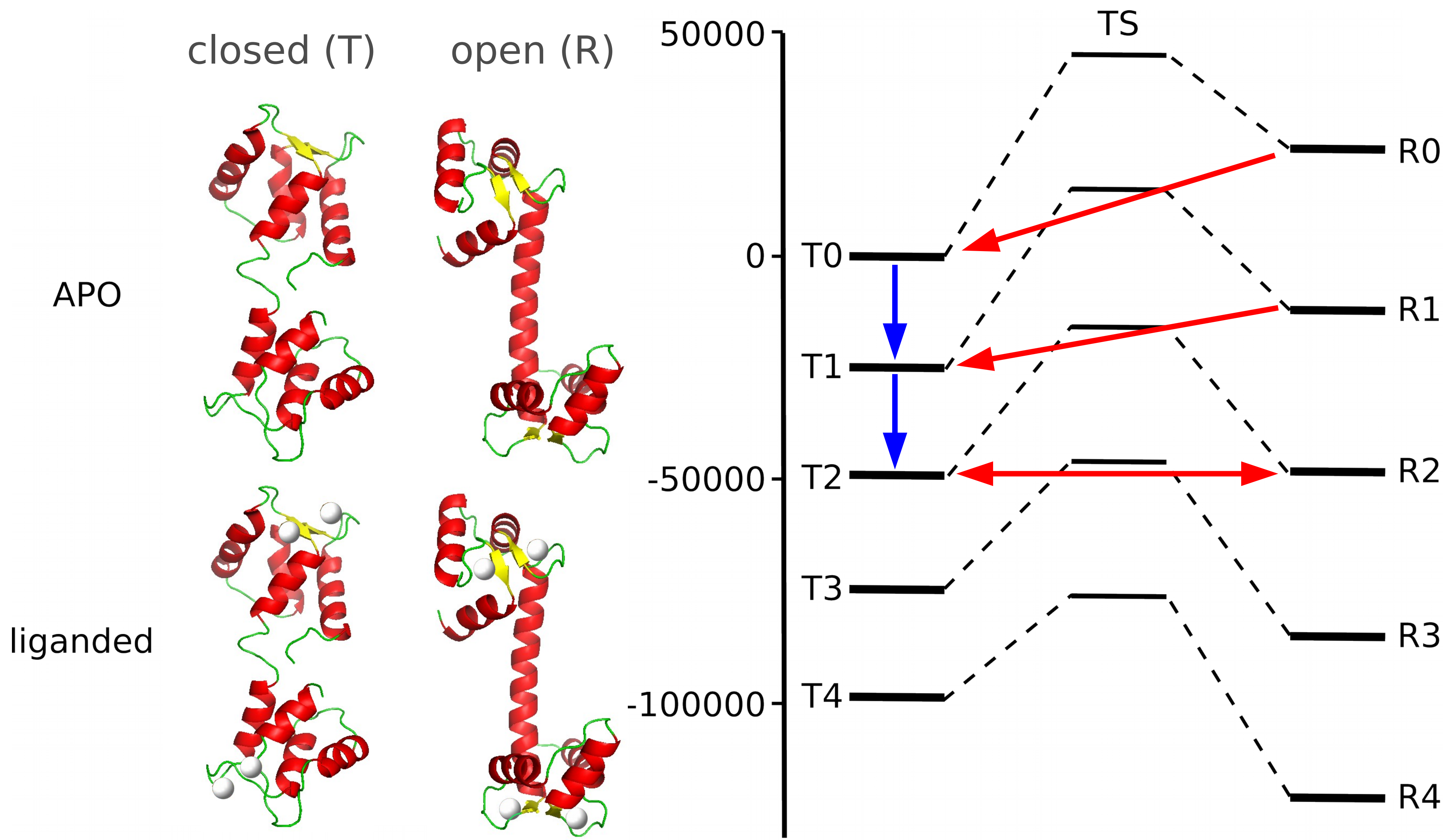


Allosteric model of Calmodulin activation

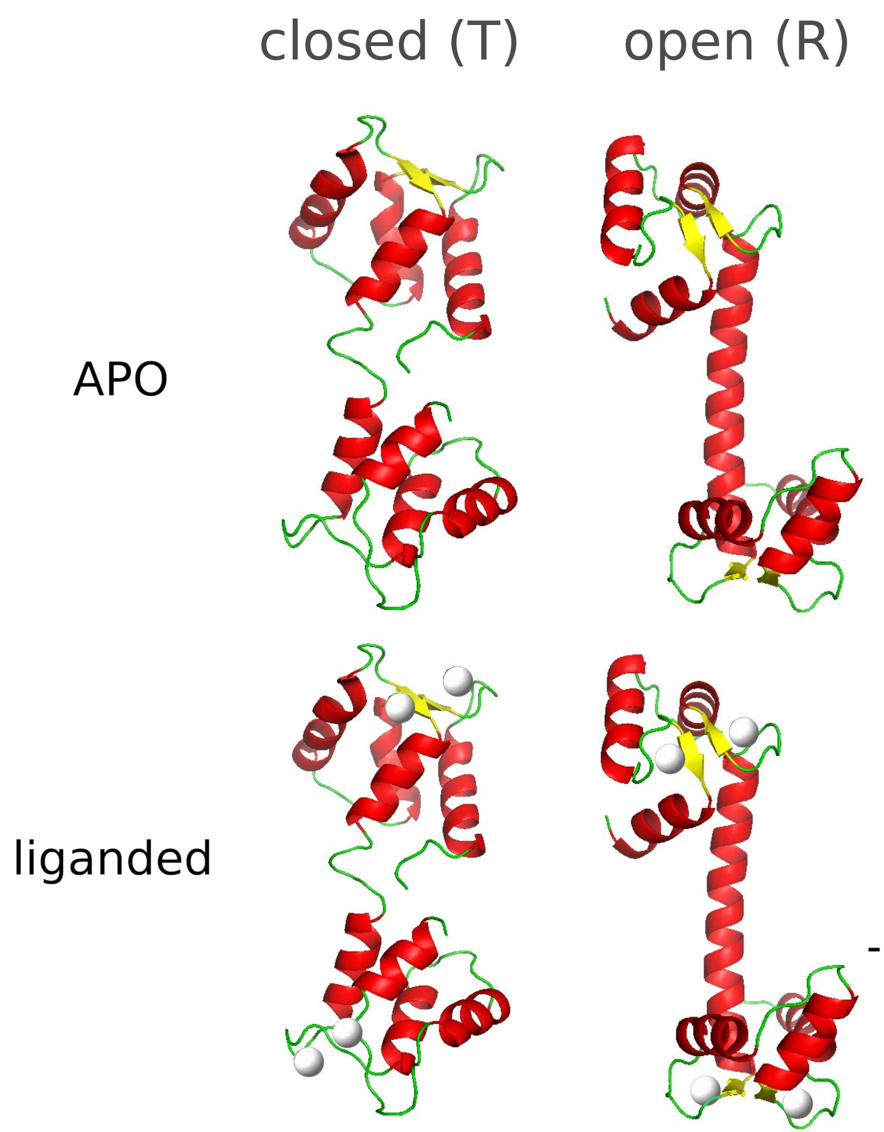


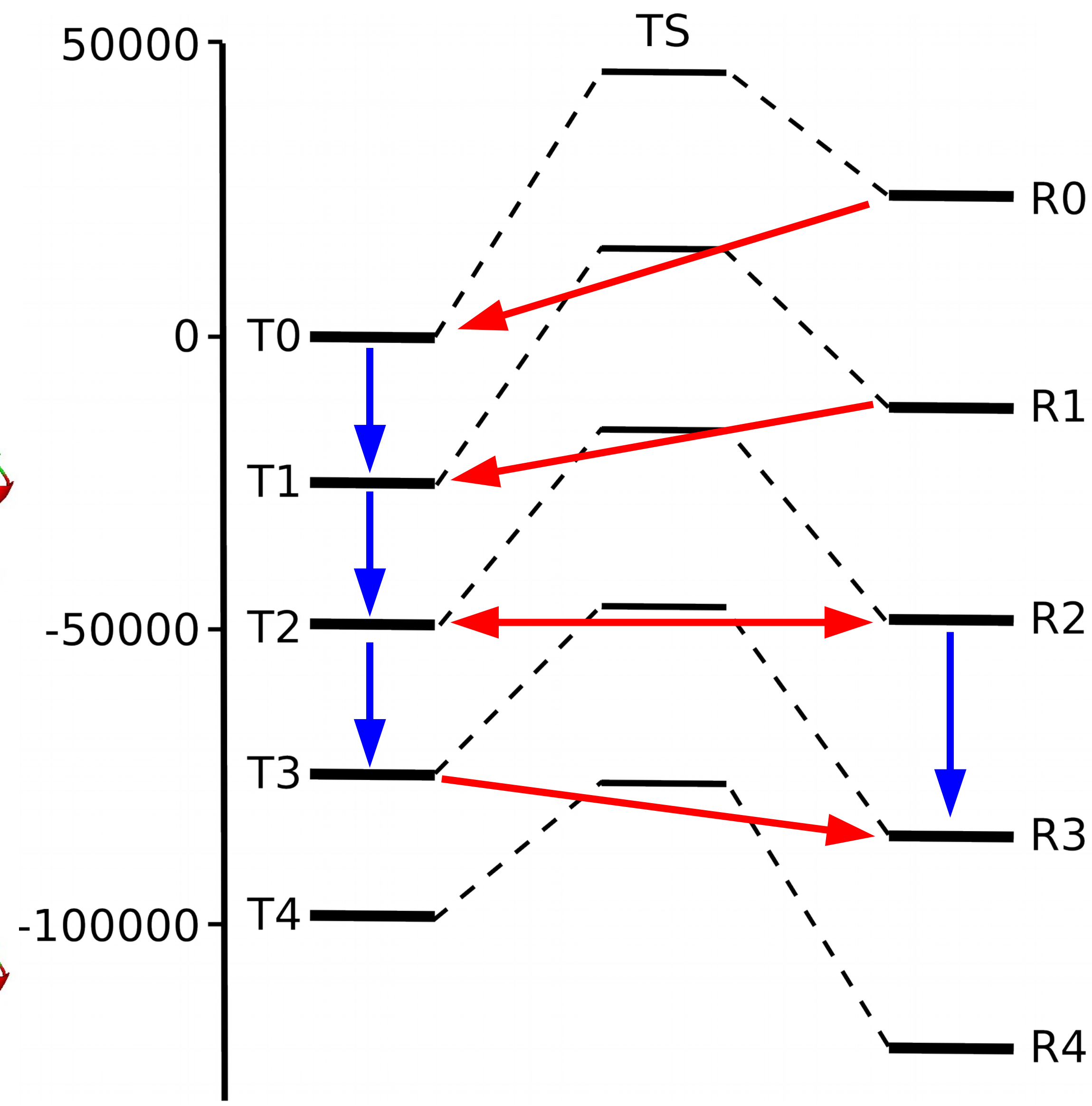


Allosteric model of Calmodulin activation

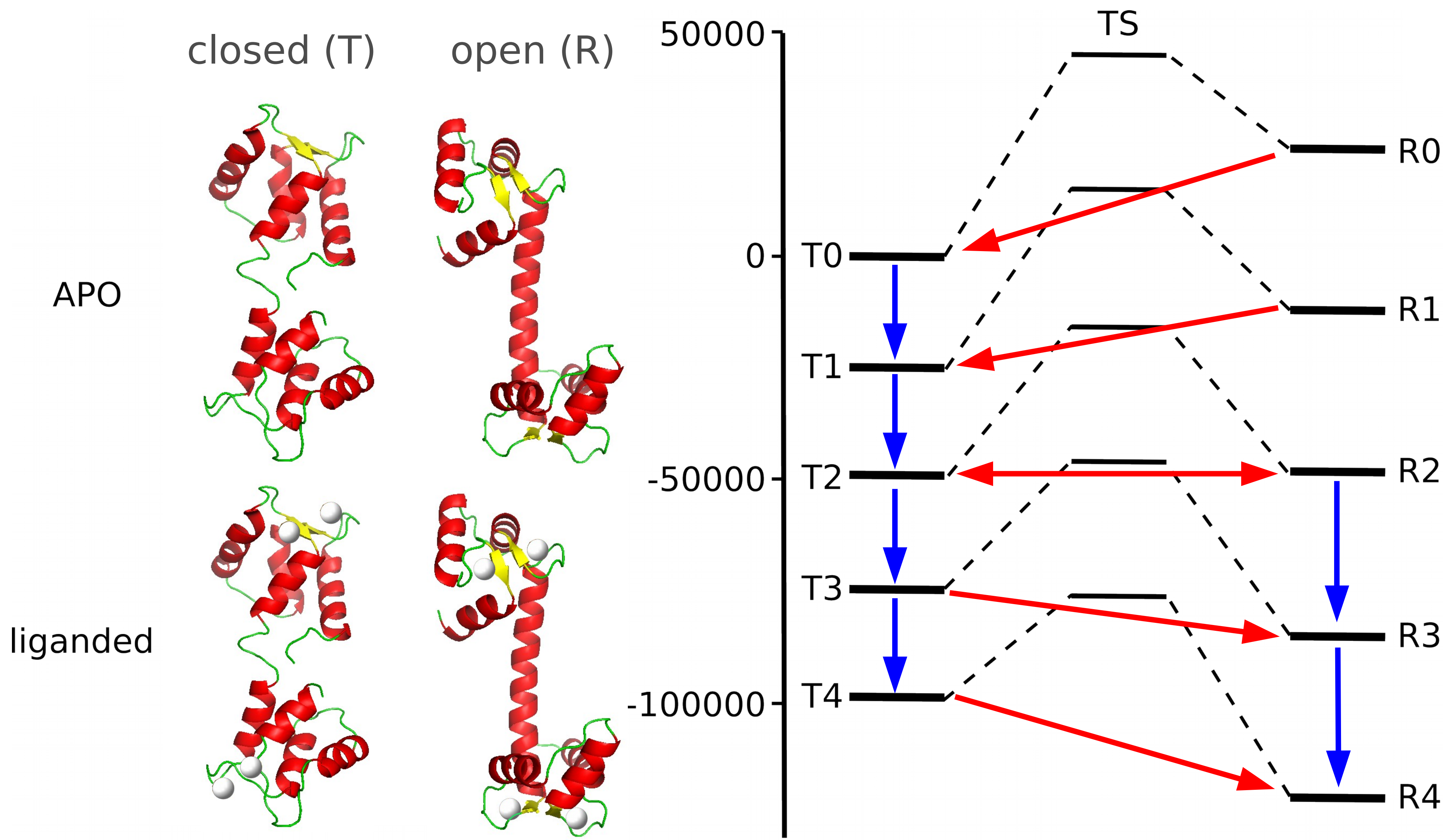


Allosteric model of Calmodulin activation

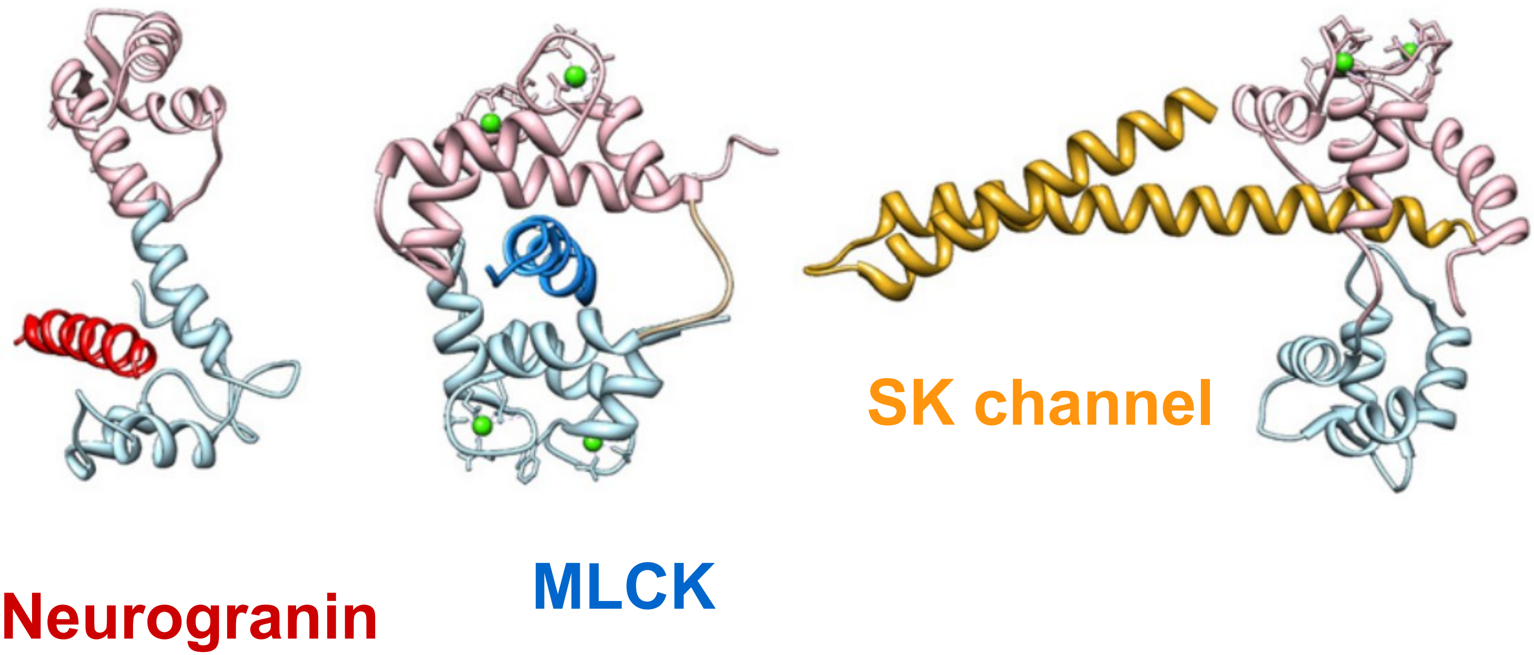




Allosteric model of Calmodulin activation



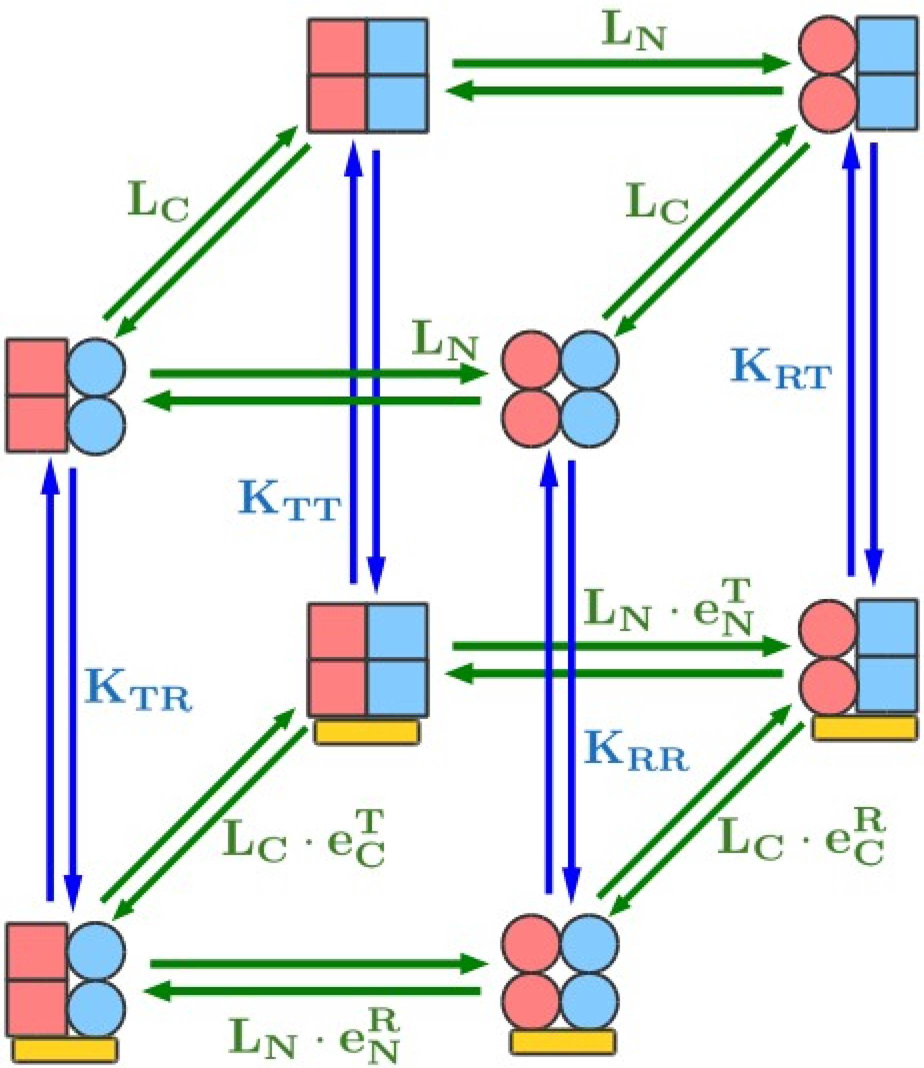
Different targets stabilise lobes in different states

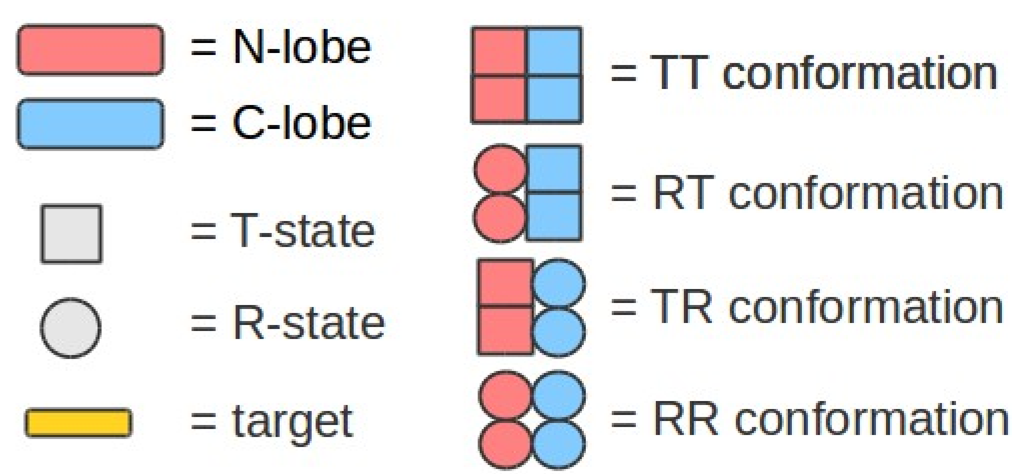


Lai M, Brun D, Edelstein SJ, Le Novère N (2015)
Lai M, Edelstein SJ, Le Novère N (in preparation)

http://identifiers.org/biomodels.db/BIOMD0000000574

Hemiconcerted model of calmodulin



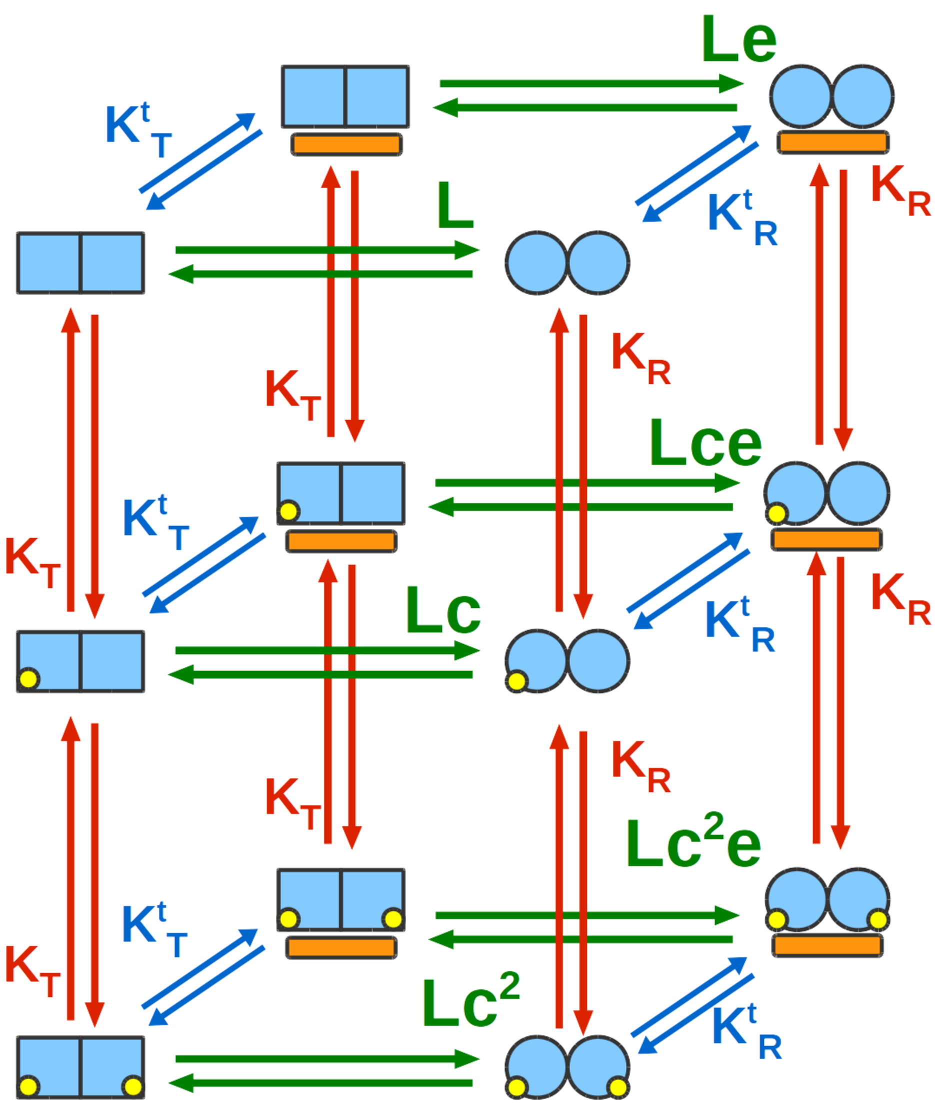


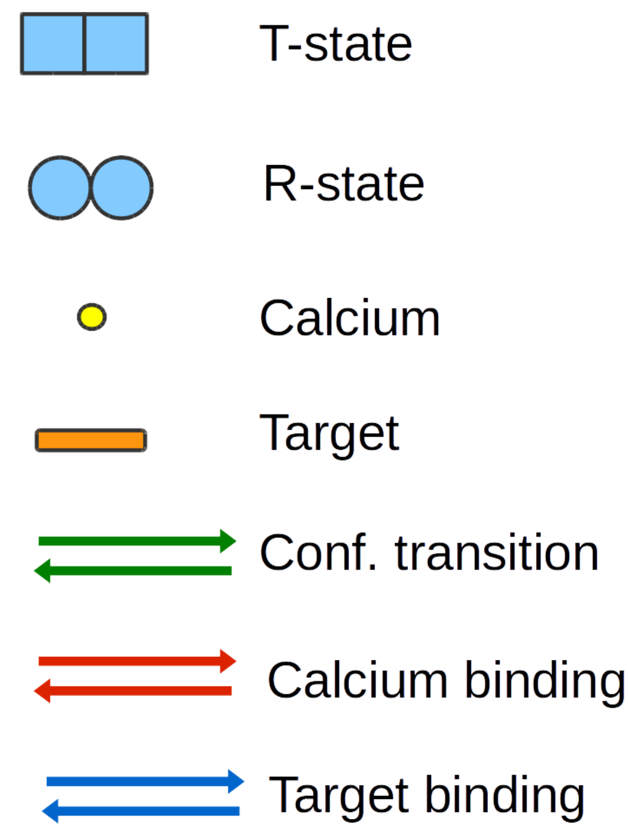
$$L_N = \frac{[TT]}{[RT]} = \frac{[TR]}{[RR]} \qquad L_C = \frac{[TT]}{[TR]} = \frac{[RT]}{[RR]}$$

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

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

Bindings of calcium and targets

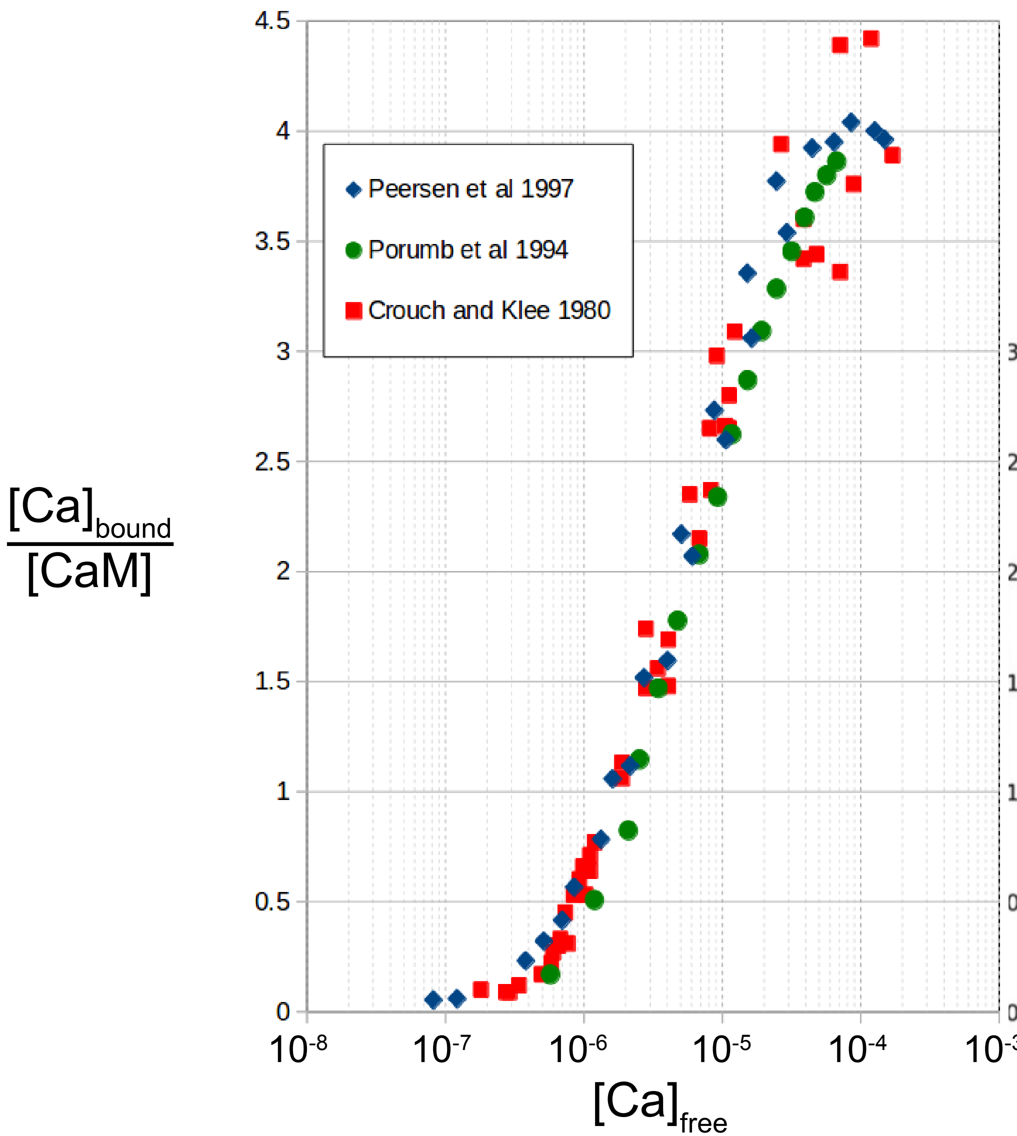


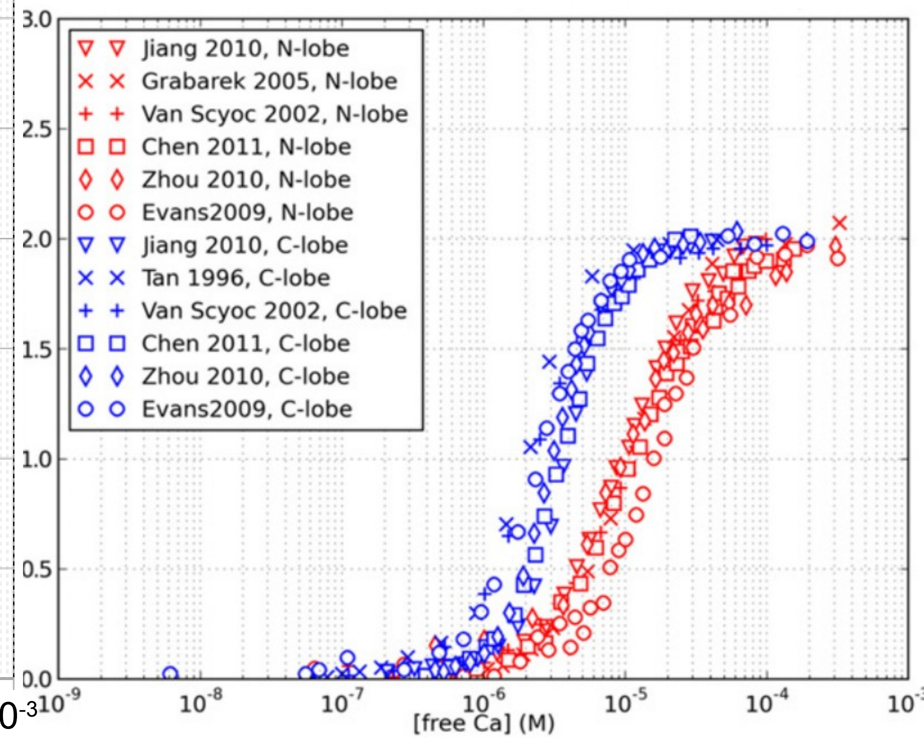


$$c = \frac{K_R}{K_T}$$

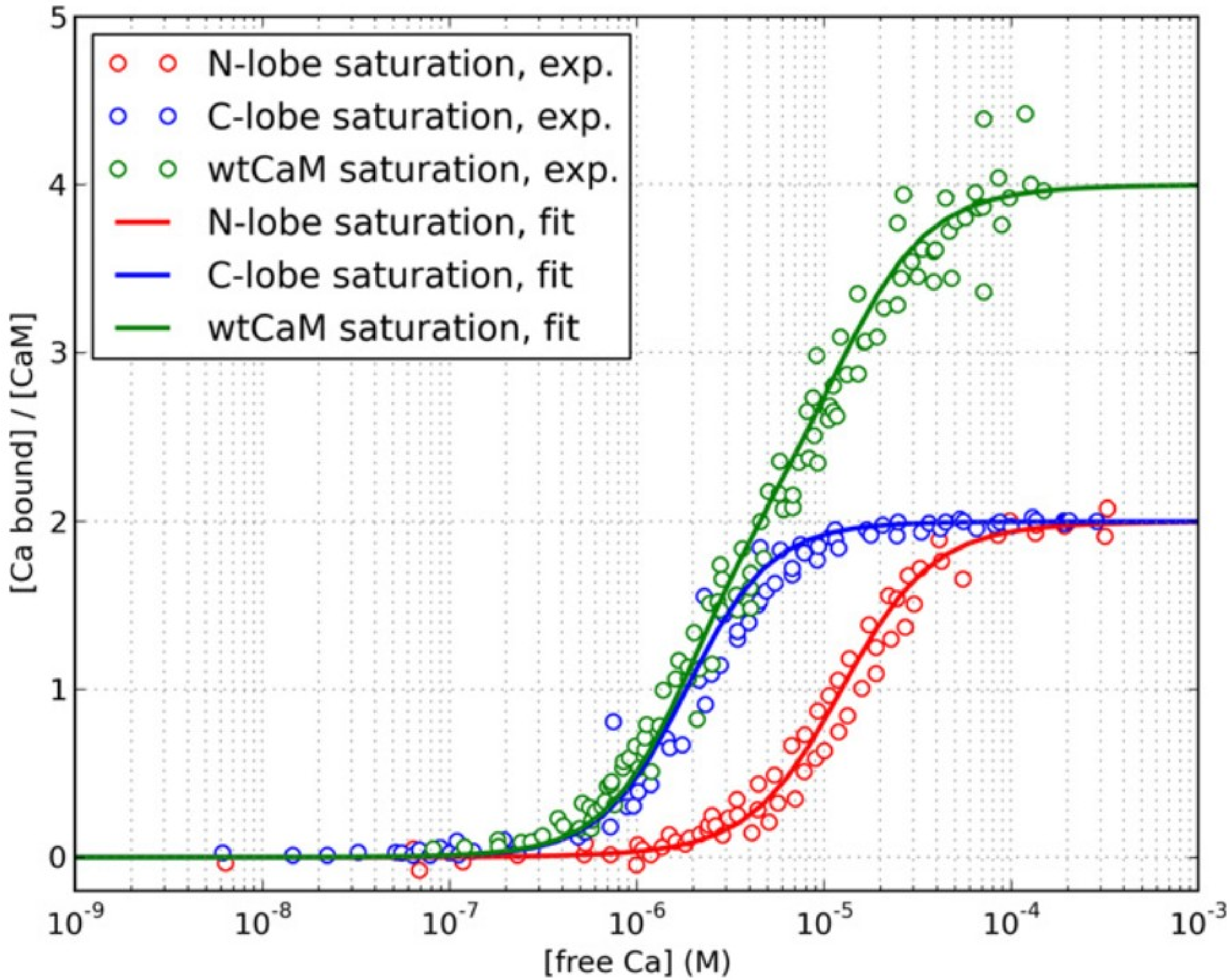
$$e = \frac{K^t_R}{K^t_T}$$

Calcium binding to lobes and whole CaM (exp)





Calcium binding to lobes and whole CaM (sim)



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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

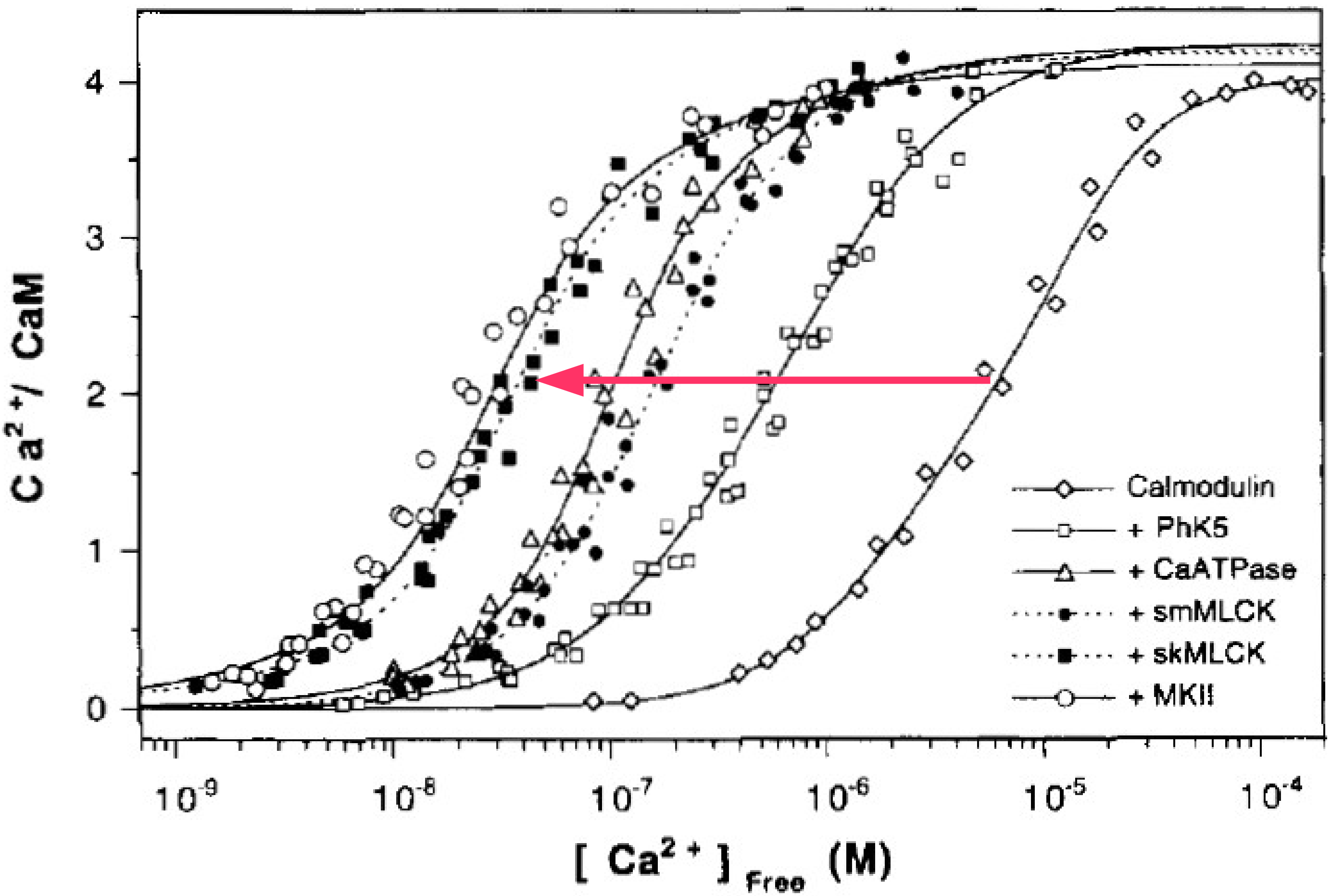
Activity of unsaturated calmodulin (state function)

- 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

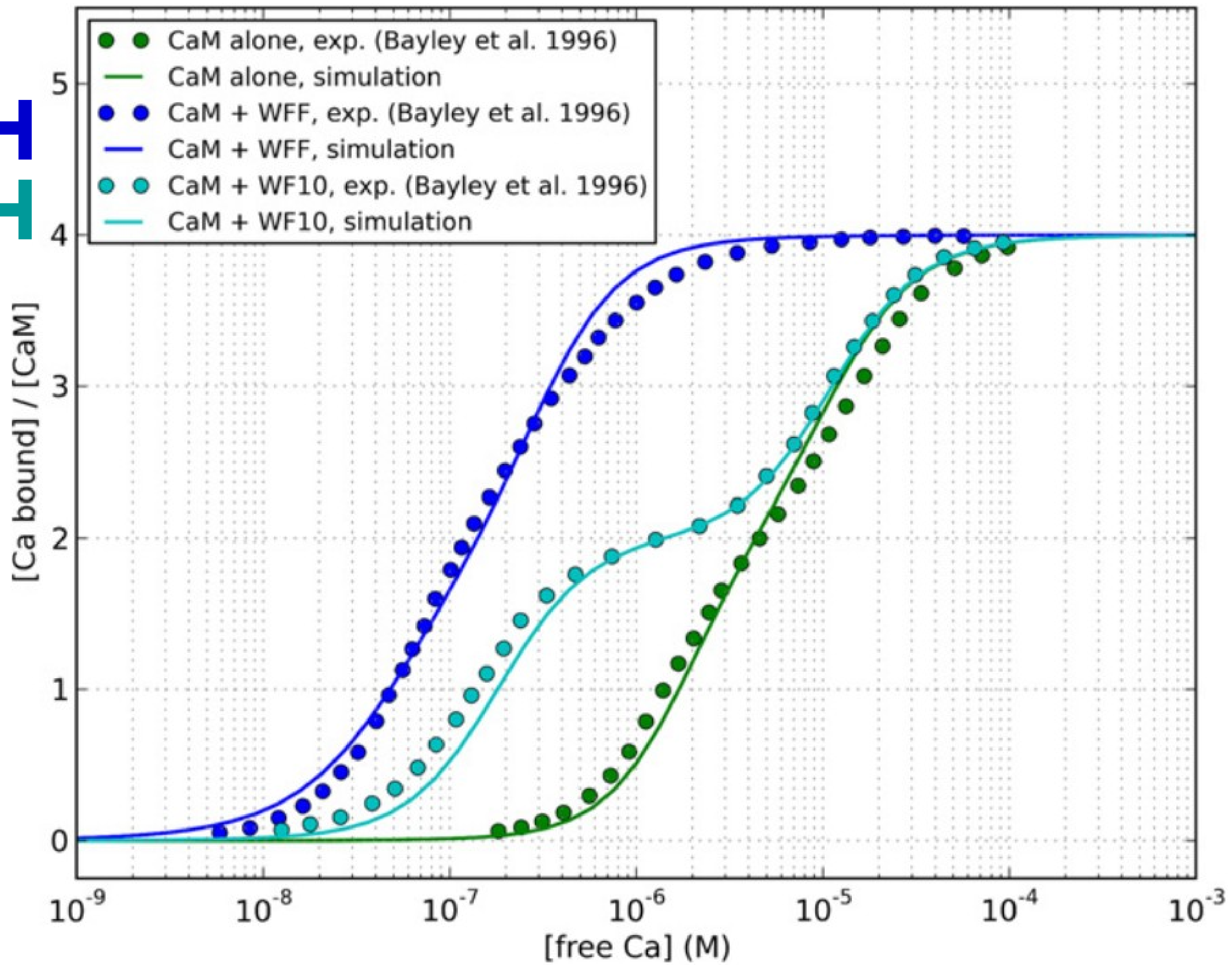


Peersen et al. (1997)

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

Stabilise R state of both lobes

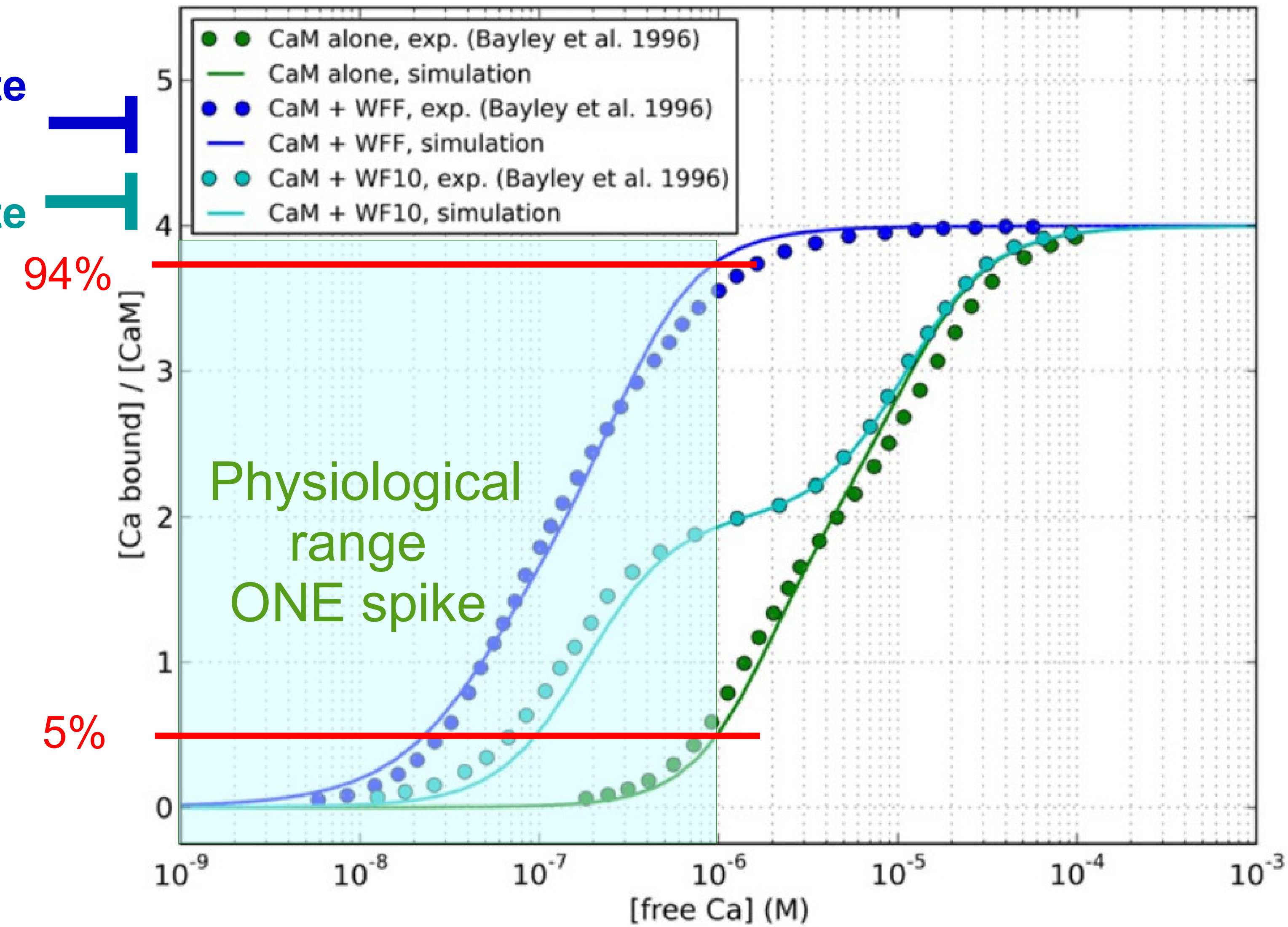
Stabilise R state of C lobe



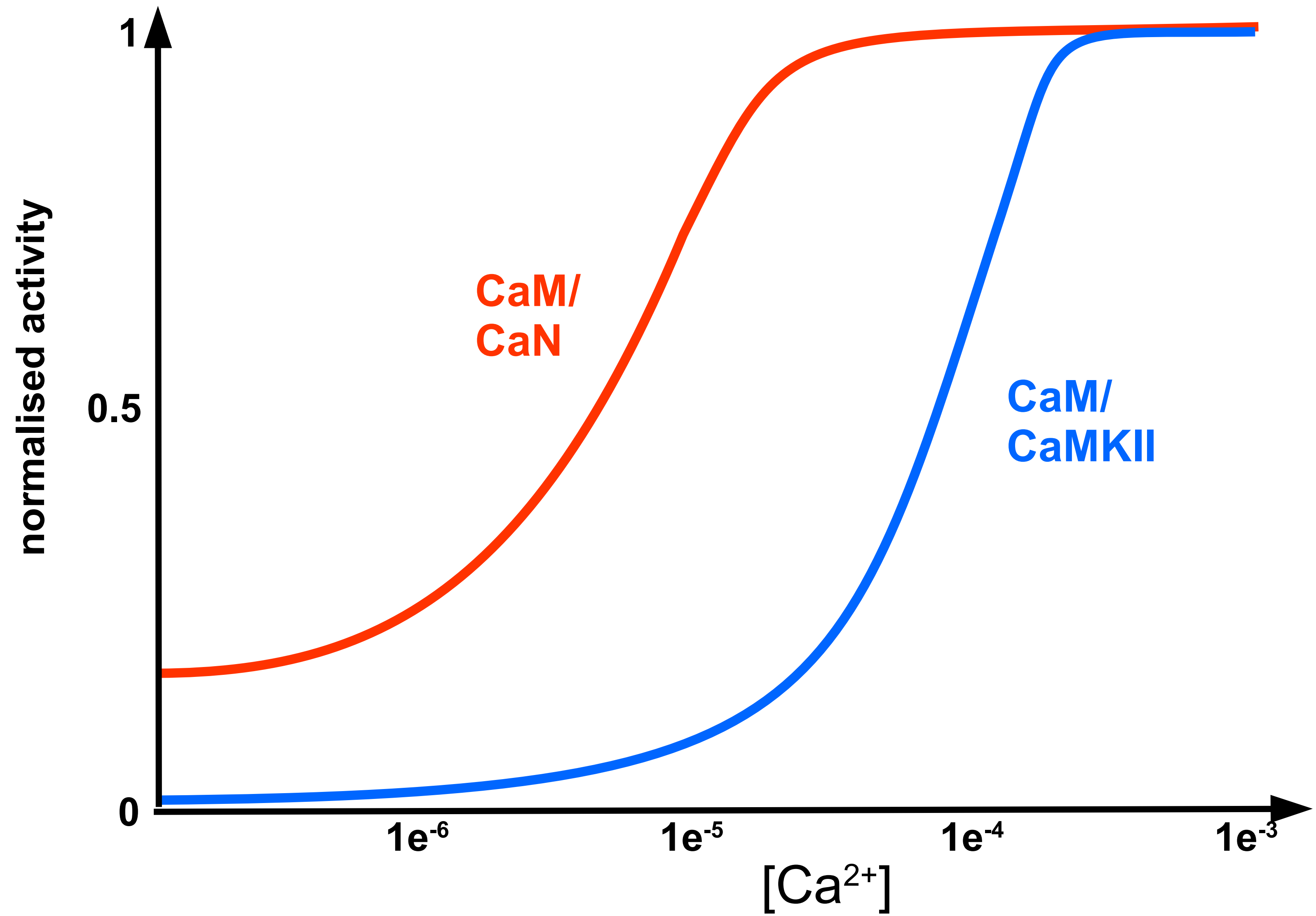
Targets stabilises Ca^{2+} binding into the physiological range

Stabilise R state of both lobes

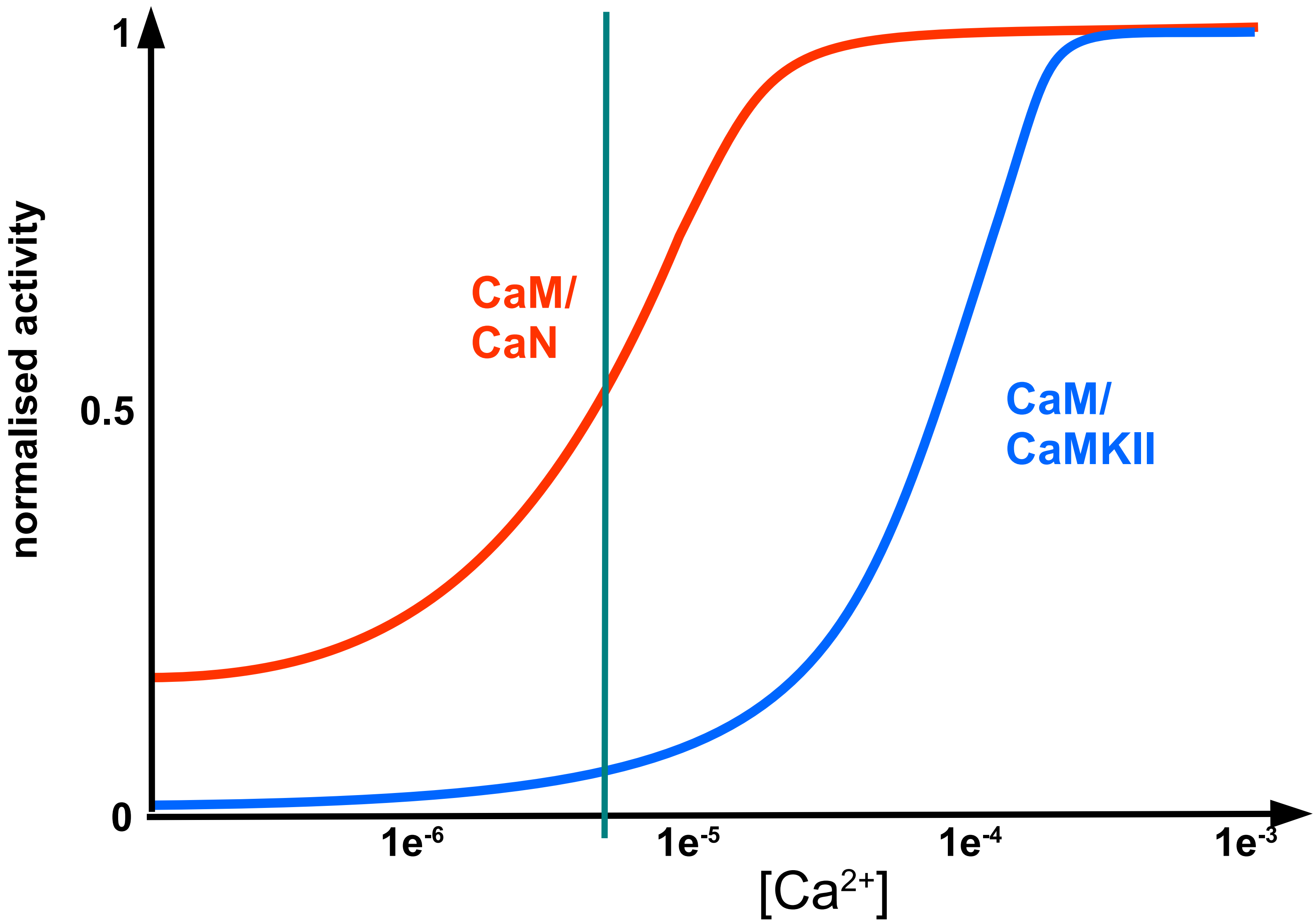
Stabilise R state of C lobe



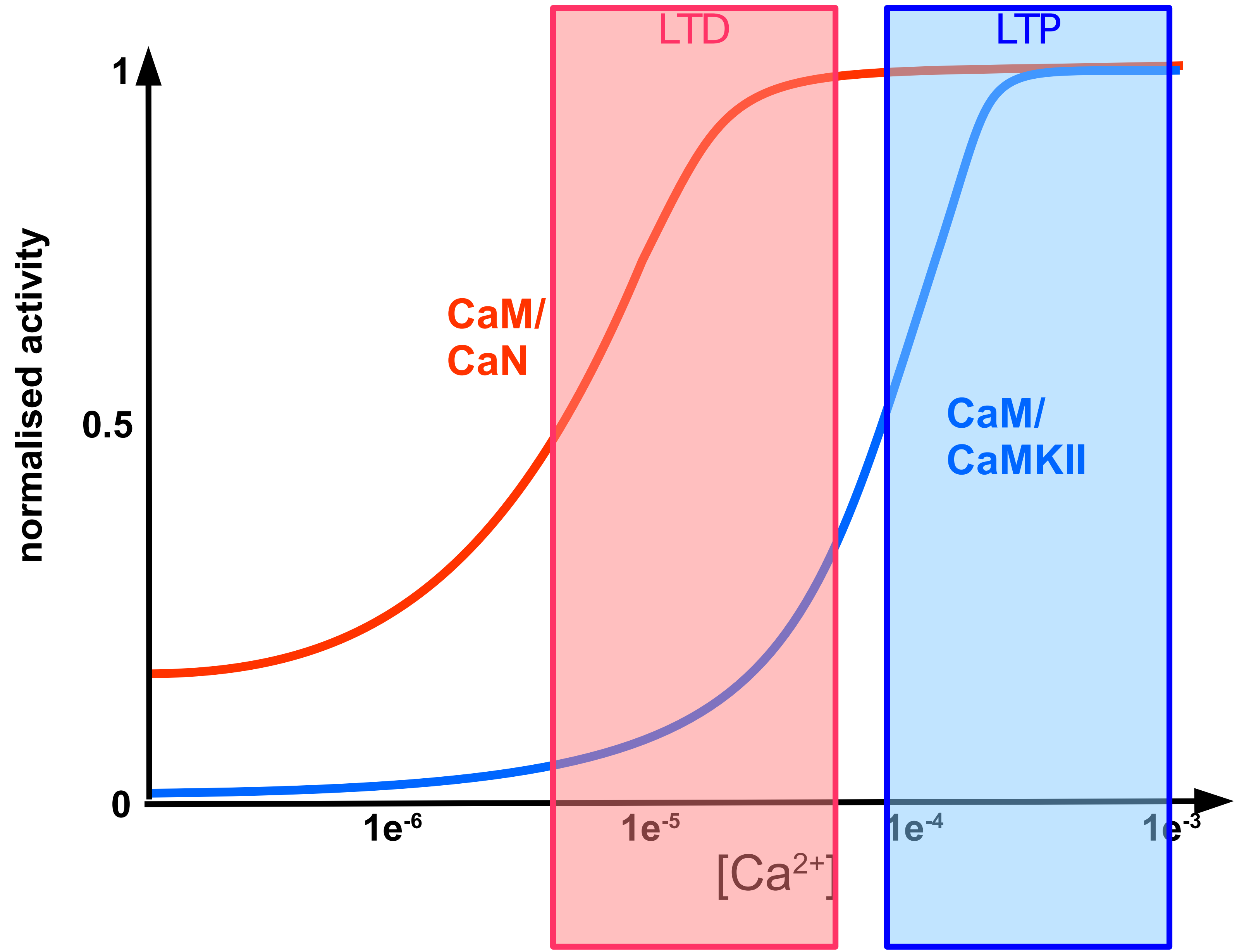
Calmodulin its ligand and its targets



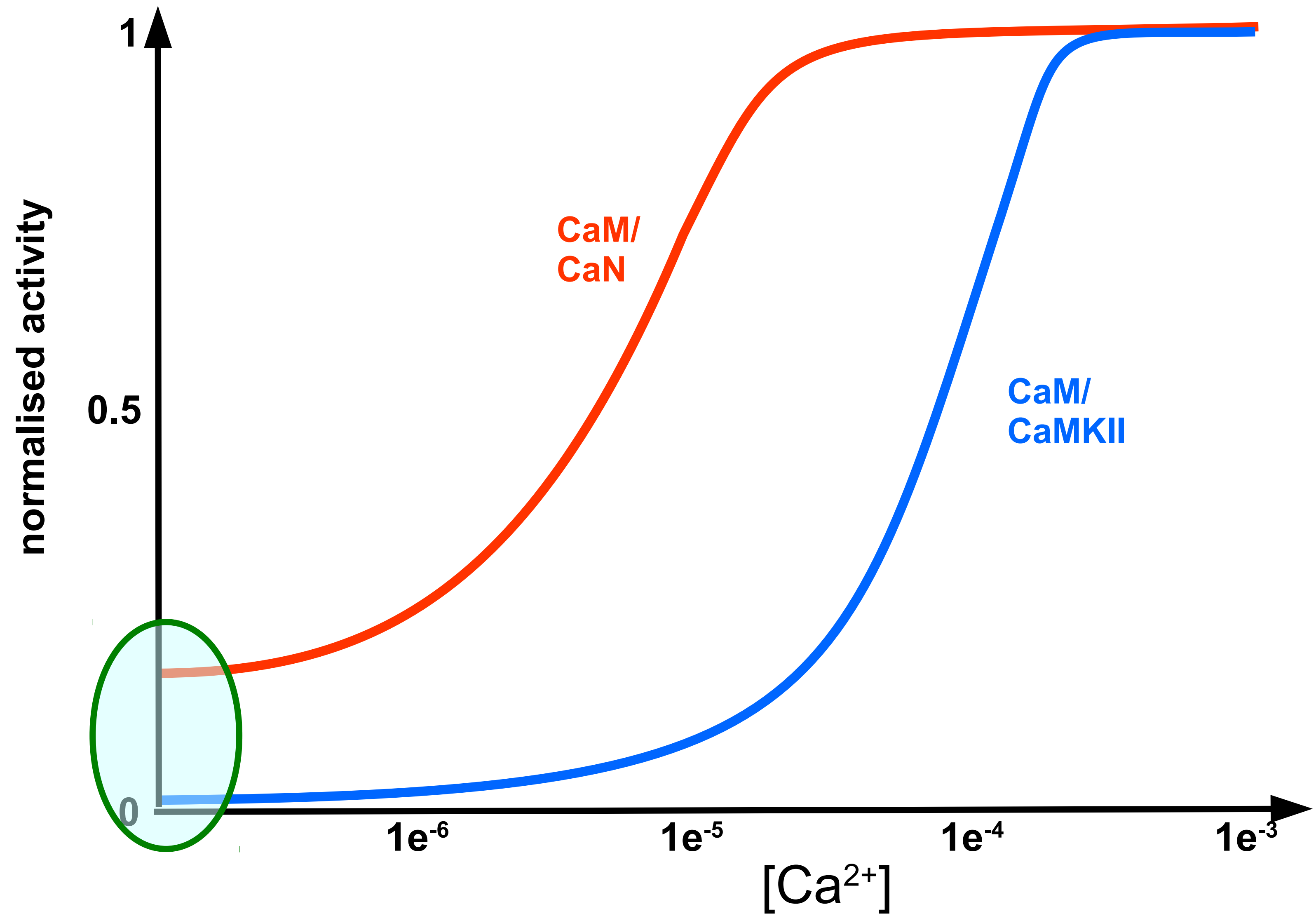
CaM half activated at half saturation of Calmodulin



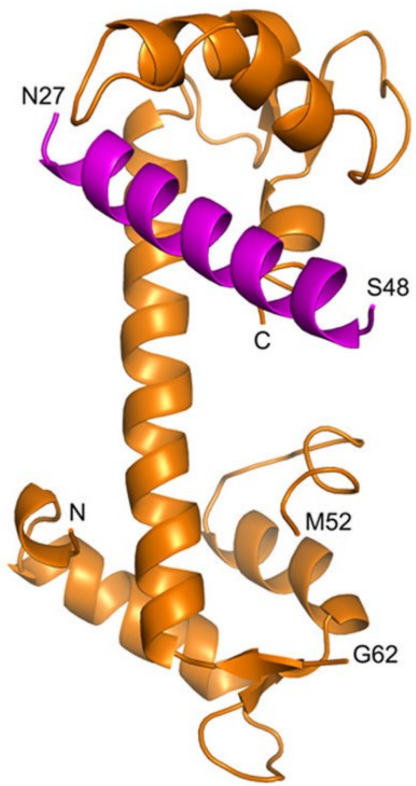
Bidirectional synaptic plasticity



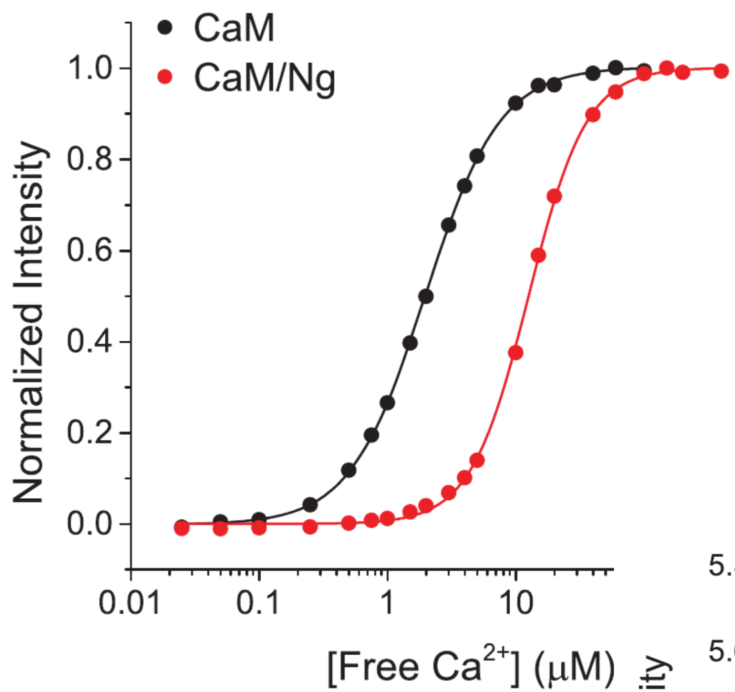
Calcineurin stabilises CaM R → no deactivation



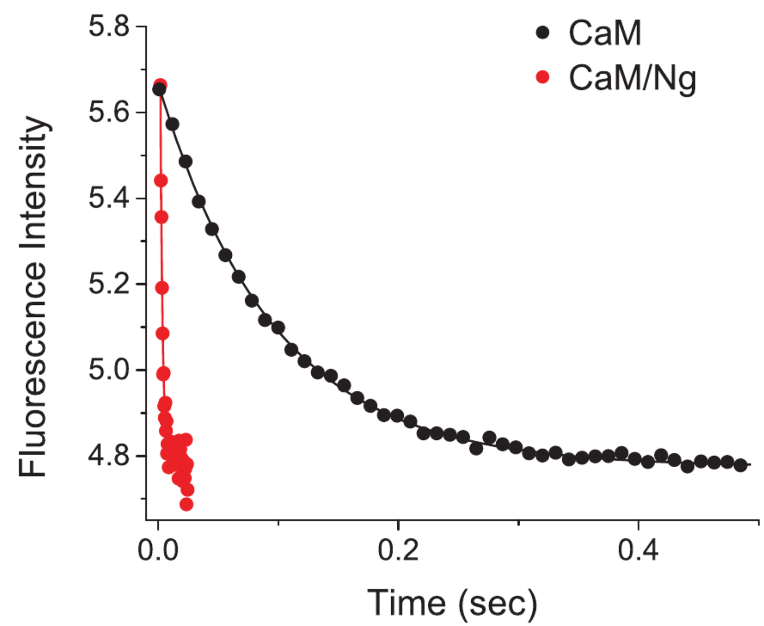
Neurogranin binds to apo-CaM, decrease affinity for [Ca^{2+}] and increase dissociation rate



Kumar *et al* (2013)



Hoffman *et al* (2014)



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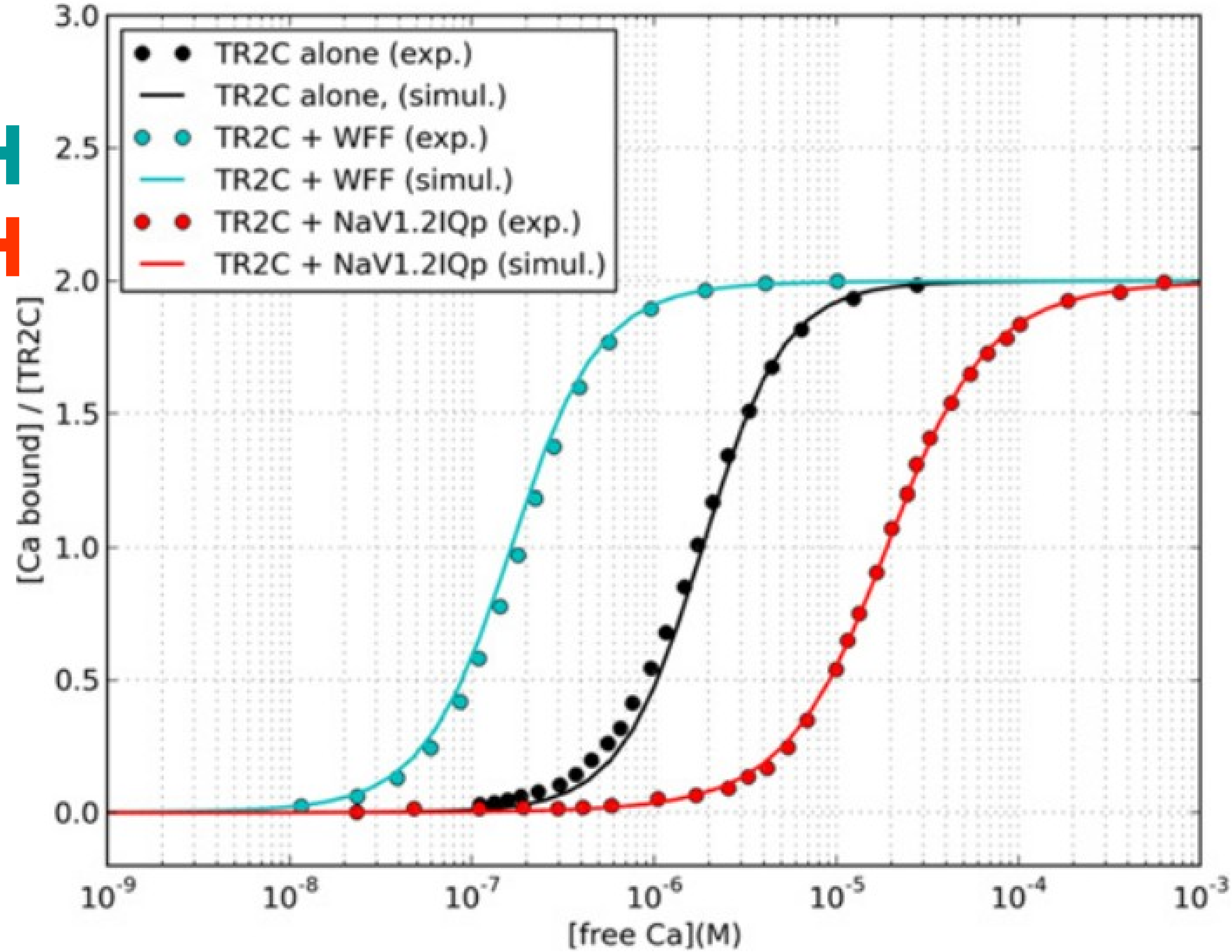
Effect of R and T stabilising targets on CaM affinity

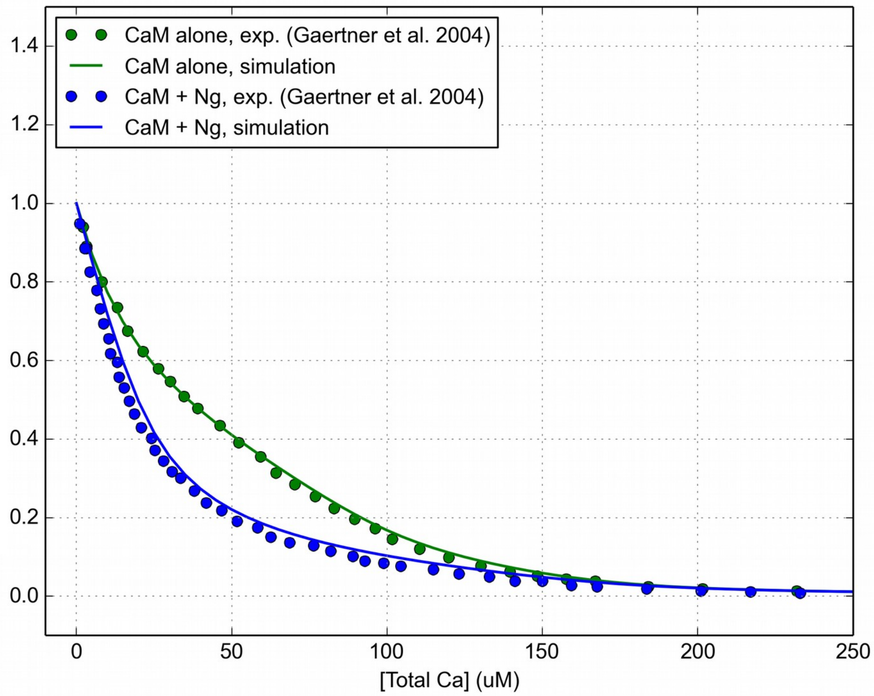
Bayley *et al* (1996)

R stabilising peptide

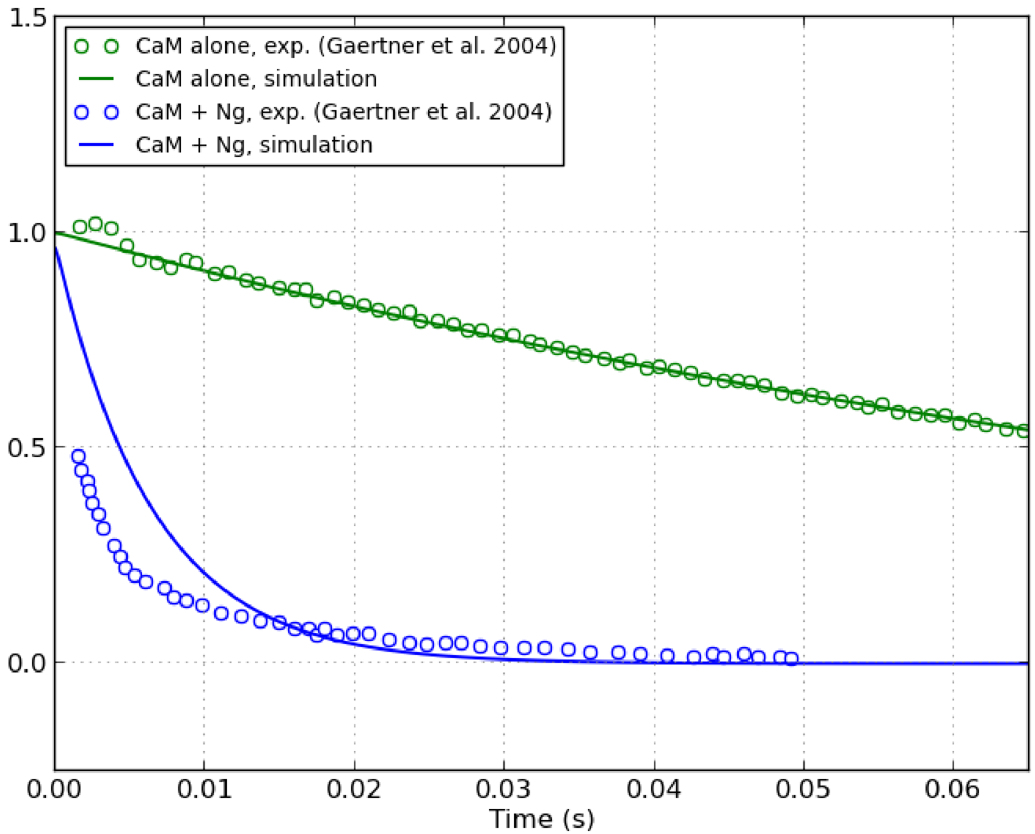
T stabilising Peptide (IQ domain)

Theoharis *et al (*2008)



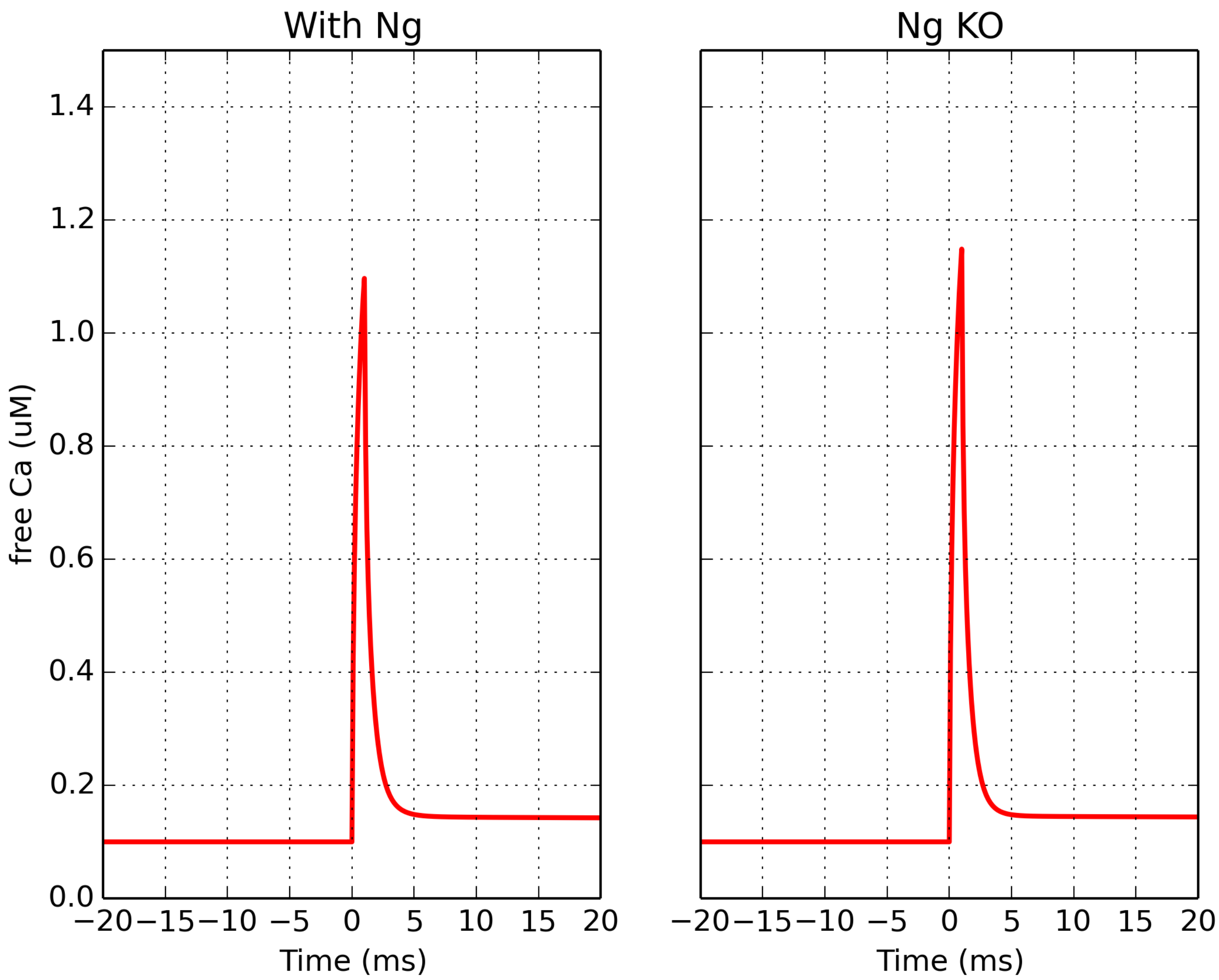


CaM alone, exp. (Gaertner et al. 2004)
CaM alone, simulation
CaM + Ng, exp. (Gaertner et al. 2004)
CaM + Ng, simulation
[Total Ca] (uM)

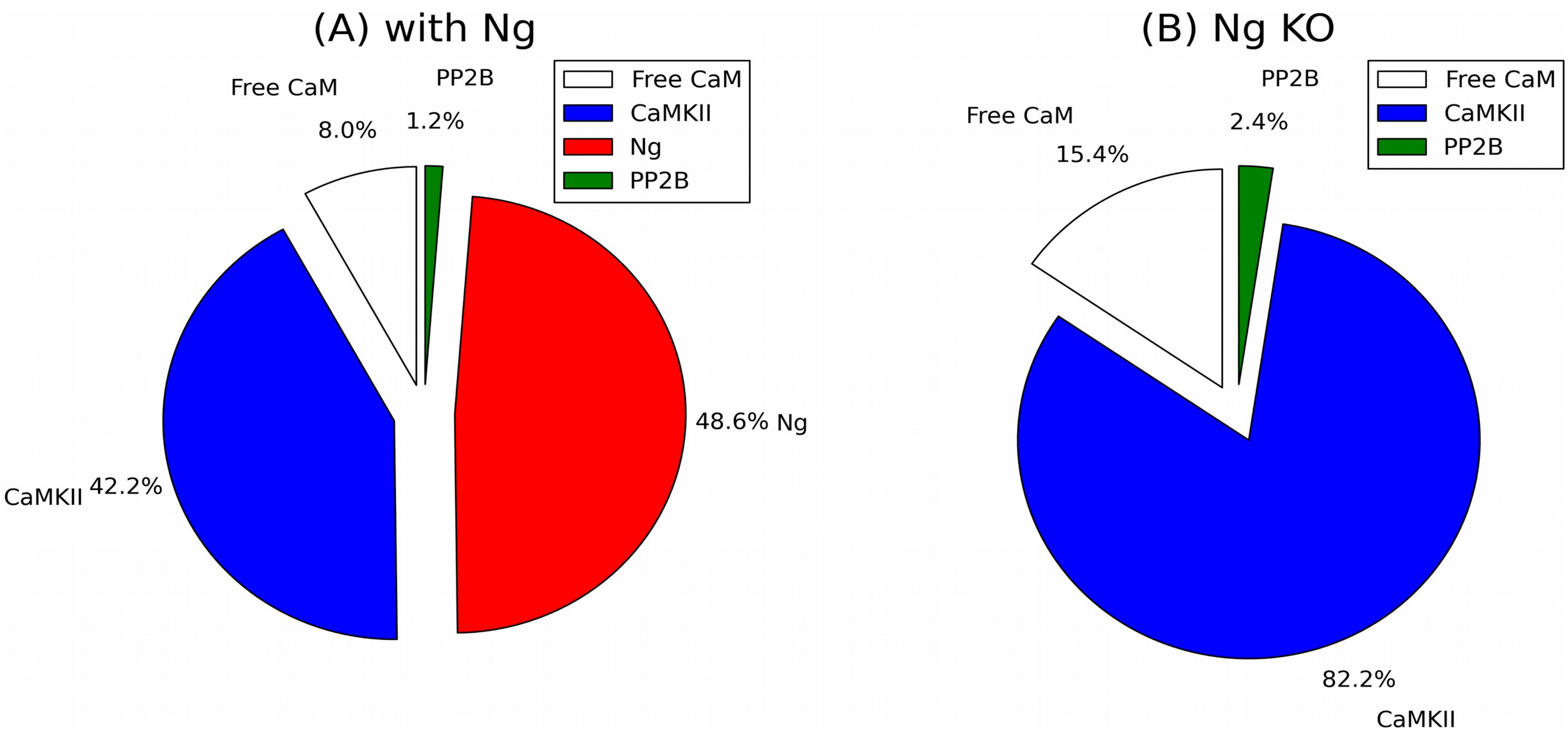


CaM alone, exp. (Gaertner et al. 2004)
CaM alone, simulation
CaM + Ng, exp. (Gaertner et al. 2004)
CaM + Ng, simulation
Time (s)

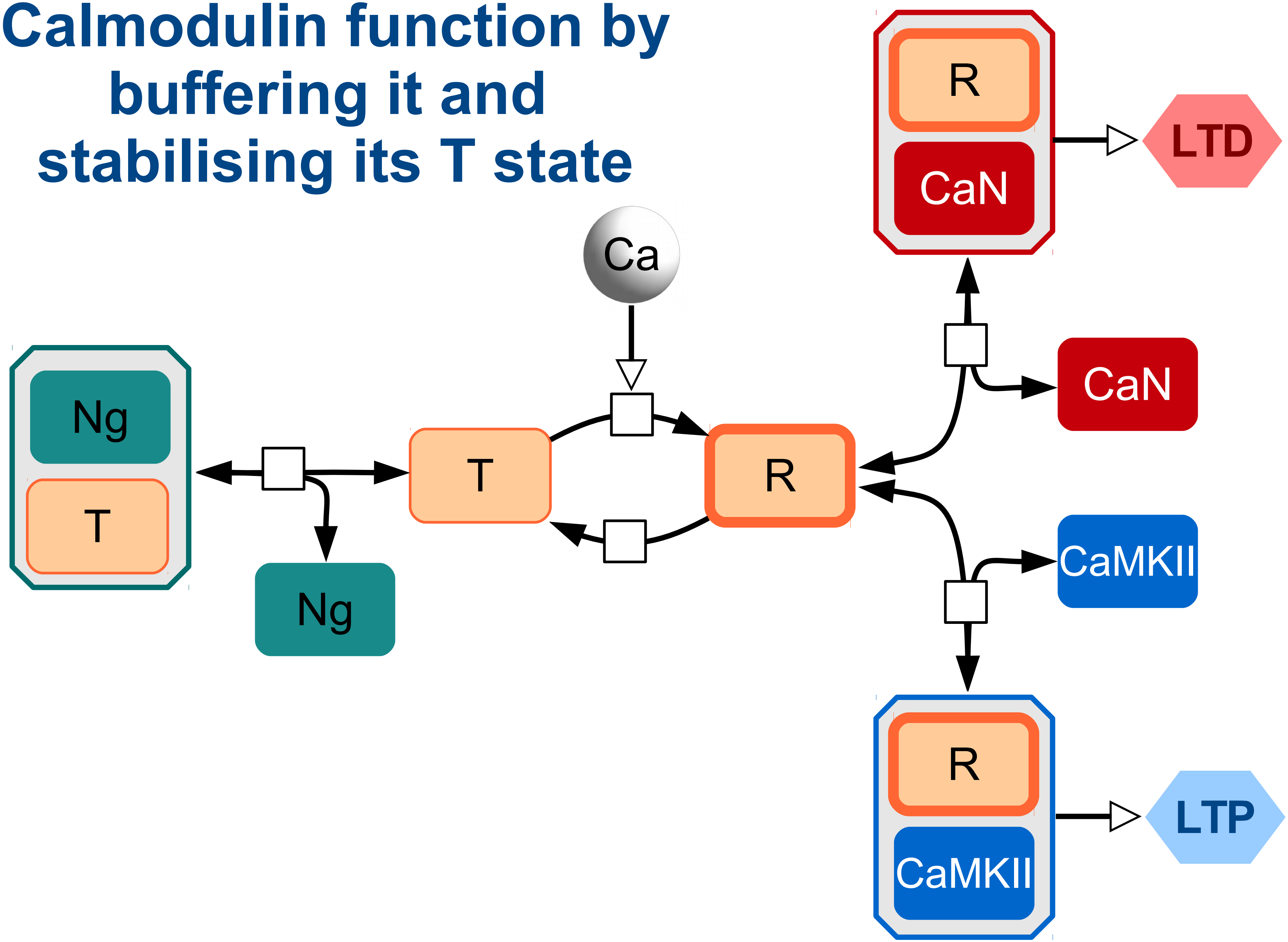
No large effect of Ng on [Ca^{2+}free]



Ng affects the distribution of CaM



Neurogranin affects Calmodulin function by buffering it and stabilising its T state



Hemi-concerted model of Calmodulin, 2 states for EF hands, binding Ca^{2+} with different affinities

Apparent **affinity for Ca^{2+} increases** when bound to target

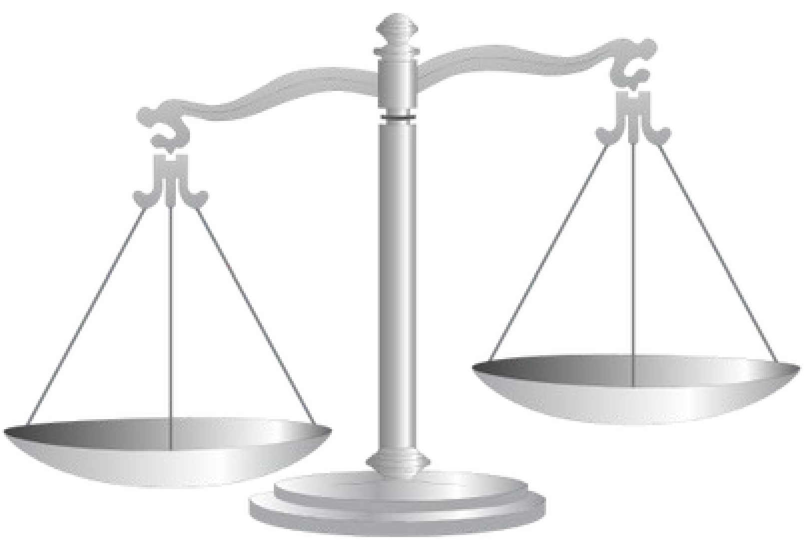
CaM able to **bind targets with less than 4 Ca^{2+}** bound

CaN can bind calmodulin at low Ca^{2+} concentration while both CaN and CaMKII bind CaM at high Ca^{2+} concentrations

Neurogranin **stabilises 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



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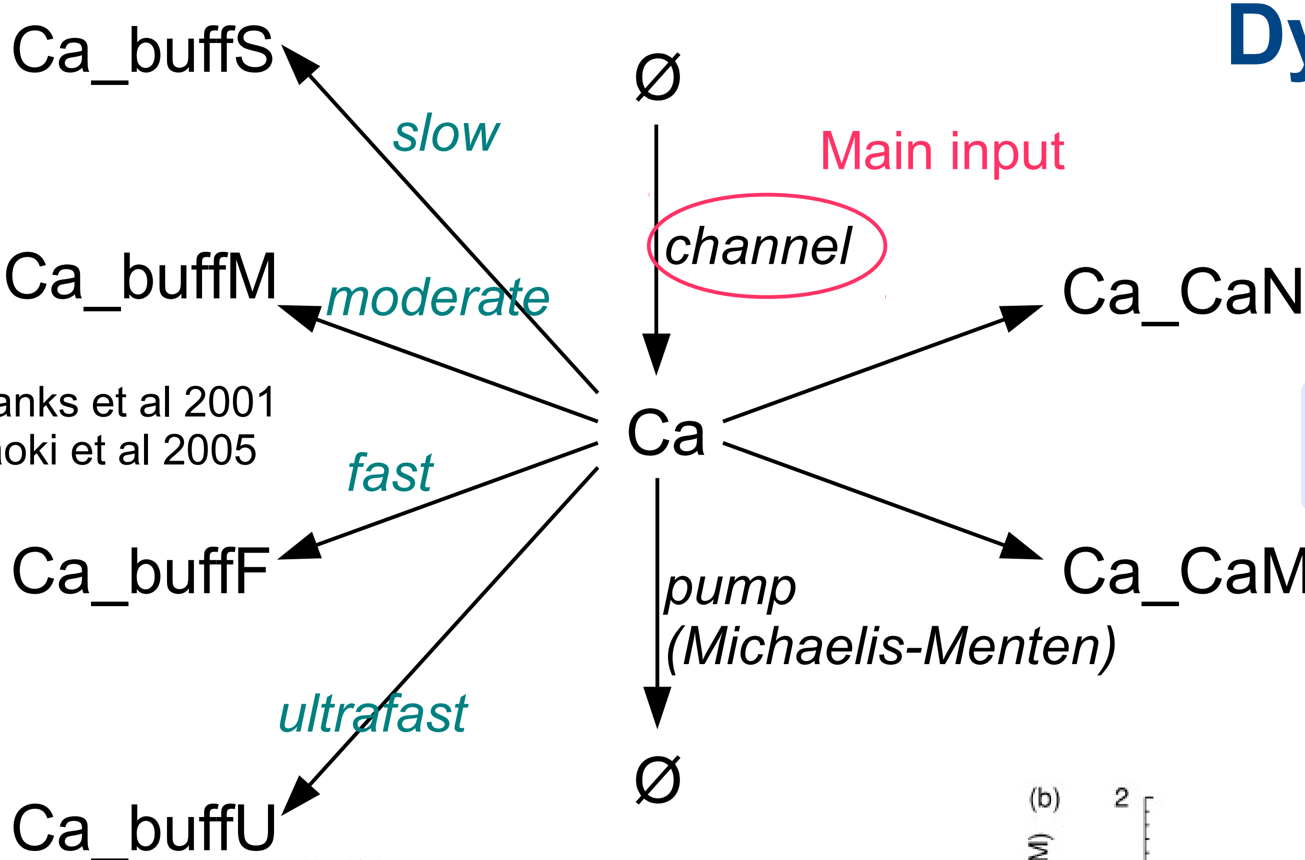
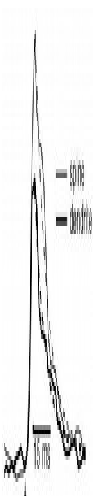
Dynamic of calcium in the spine

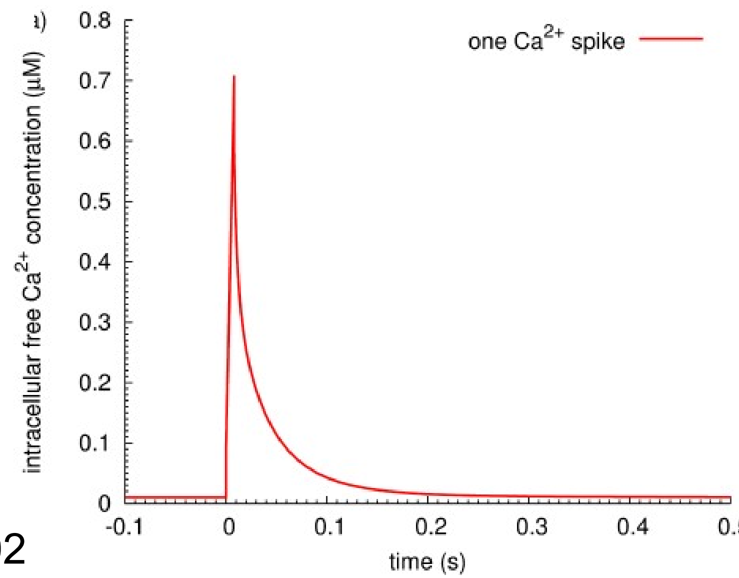


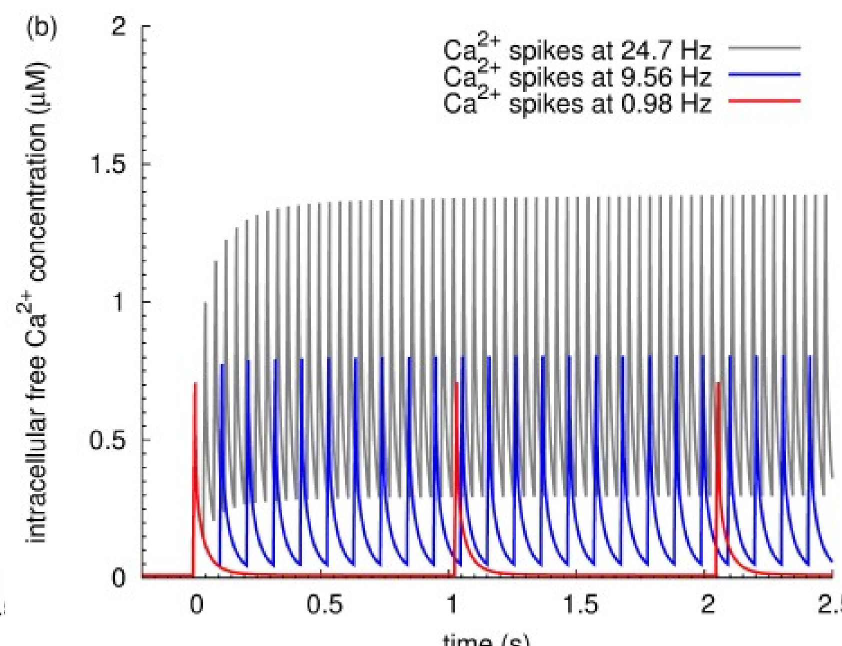
Franks et al 2001
Naoki et al 2005

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

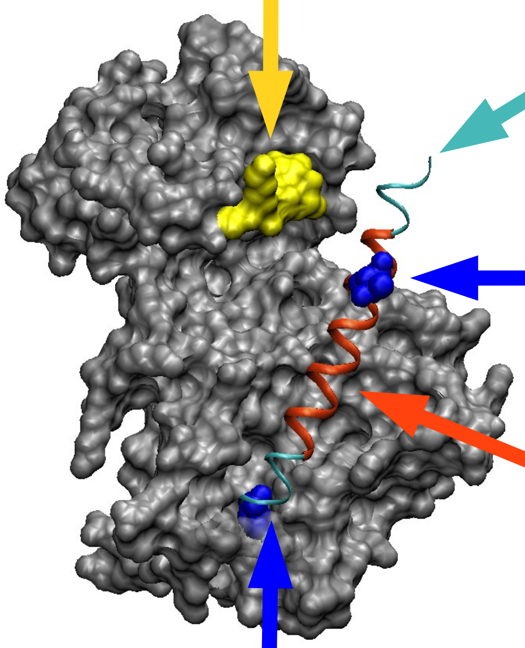


Sabatini et al 2002





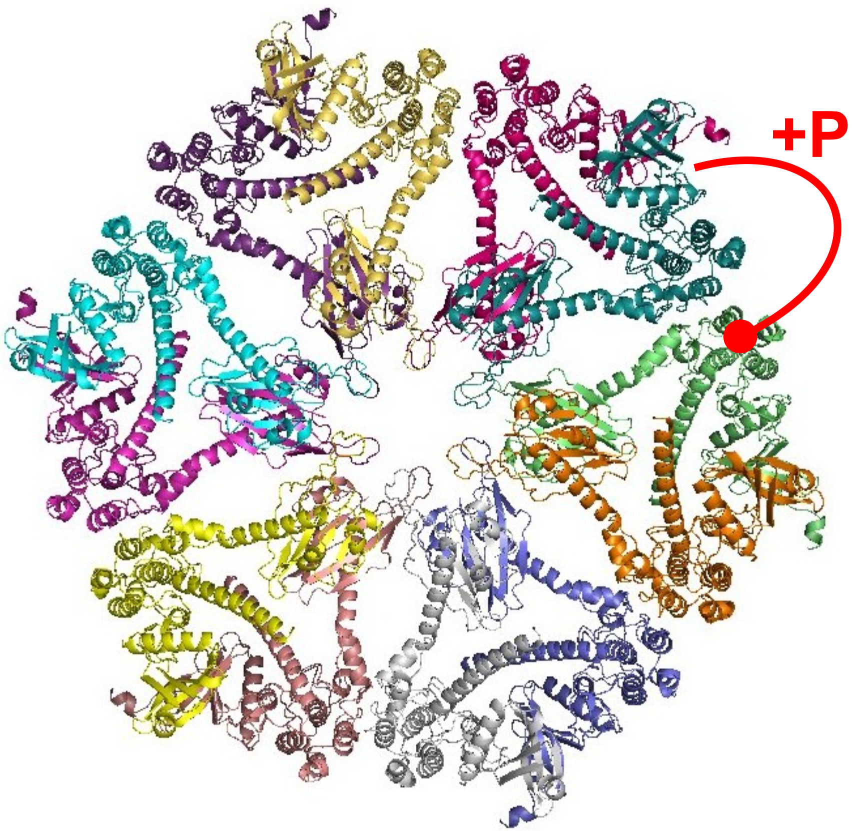
Calcium/calmodulin kinase II

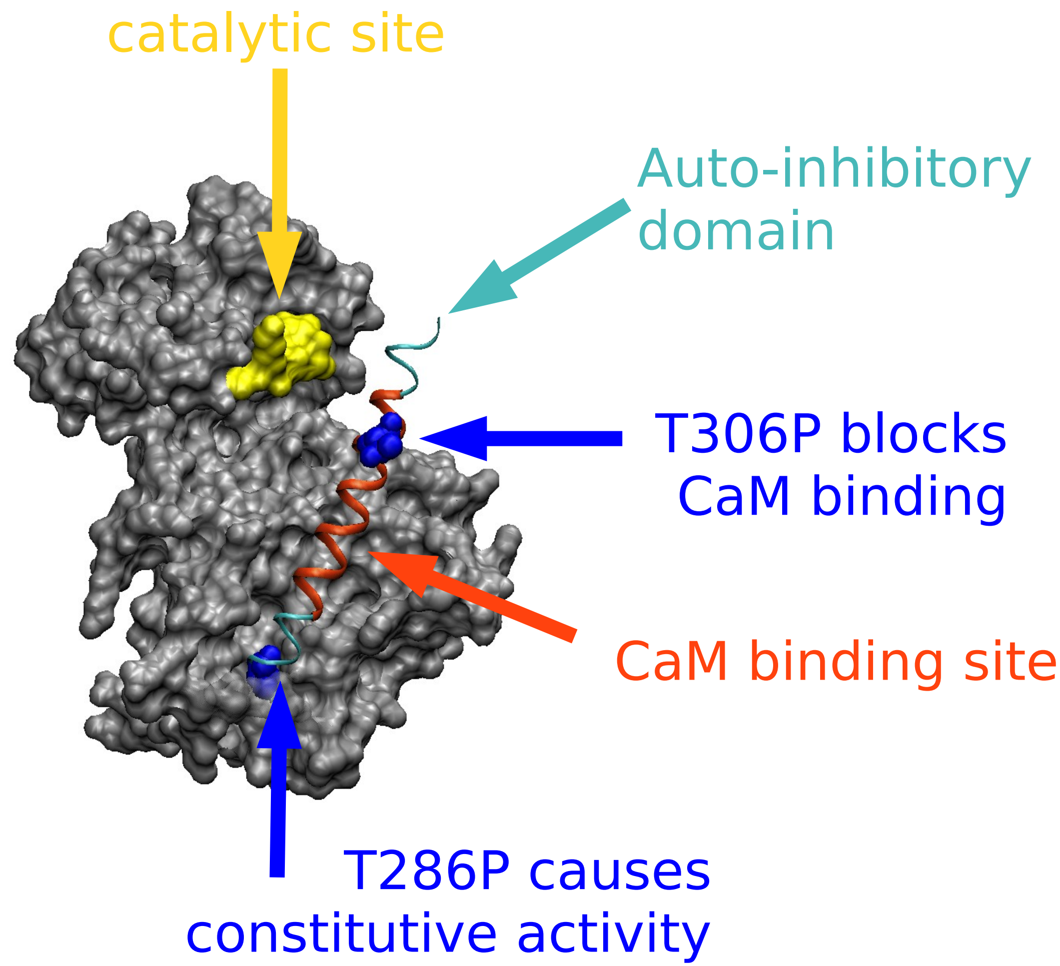


Calmodulin trapping is an apparent increase of affinity of CaMKII for CaM when T286 is phosphorylated

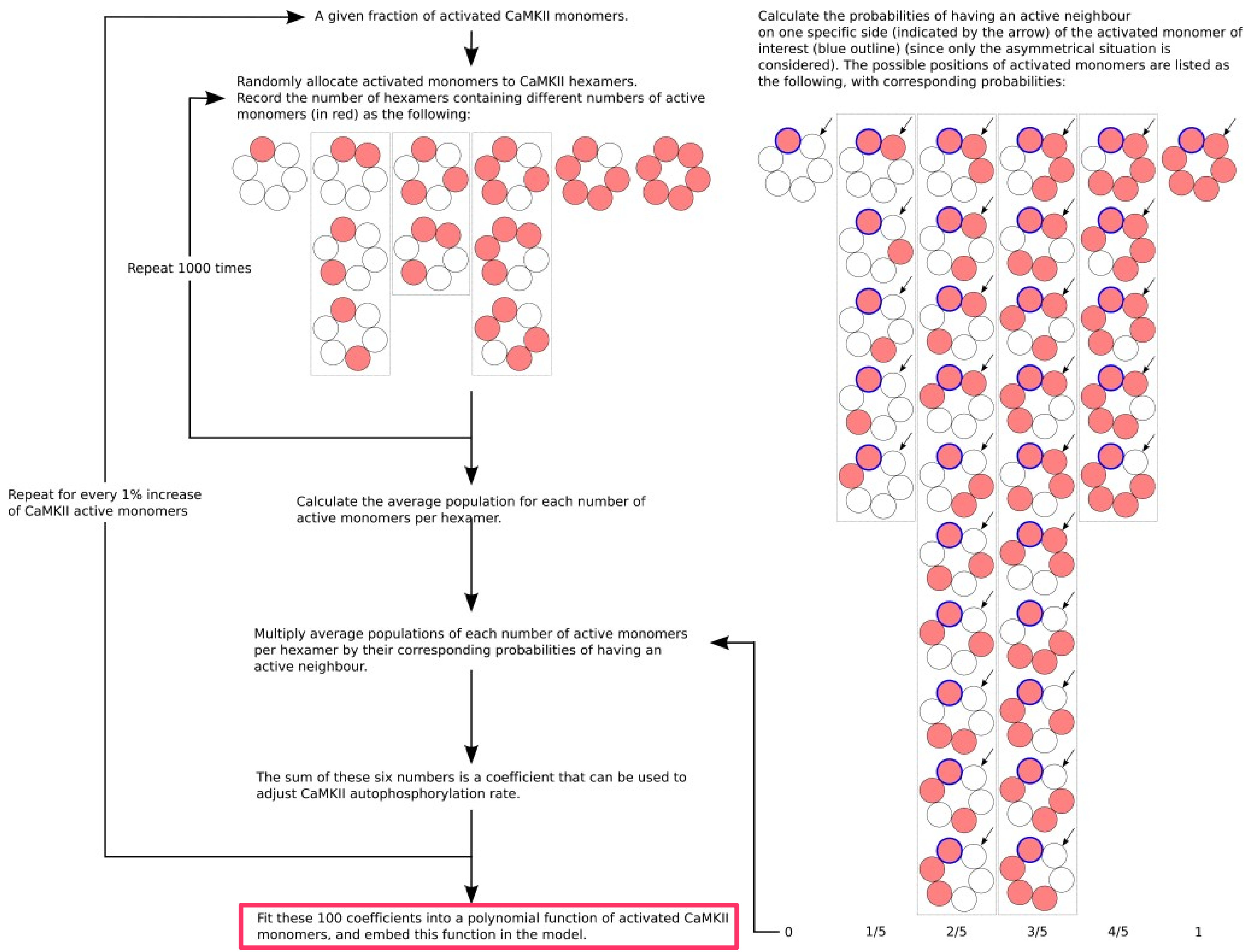
Stefan MI, Marshall D, Le Novère N (2012)

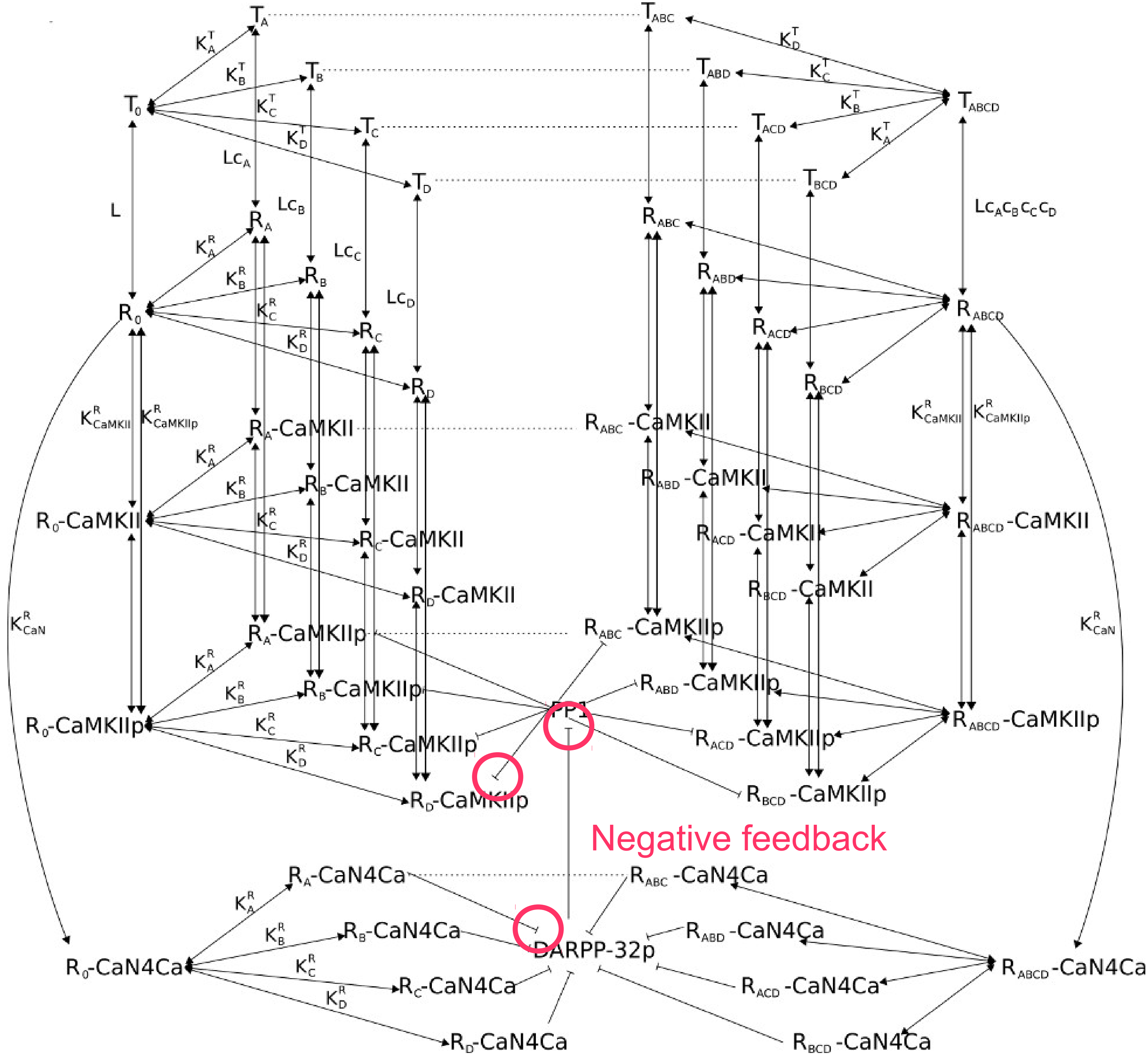
Calcium/calmodulin kinase II





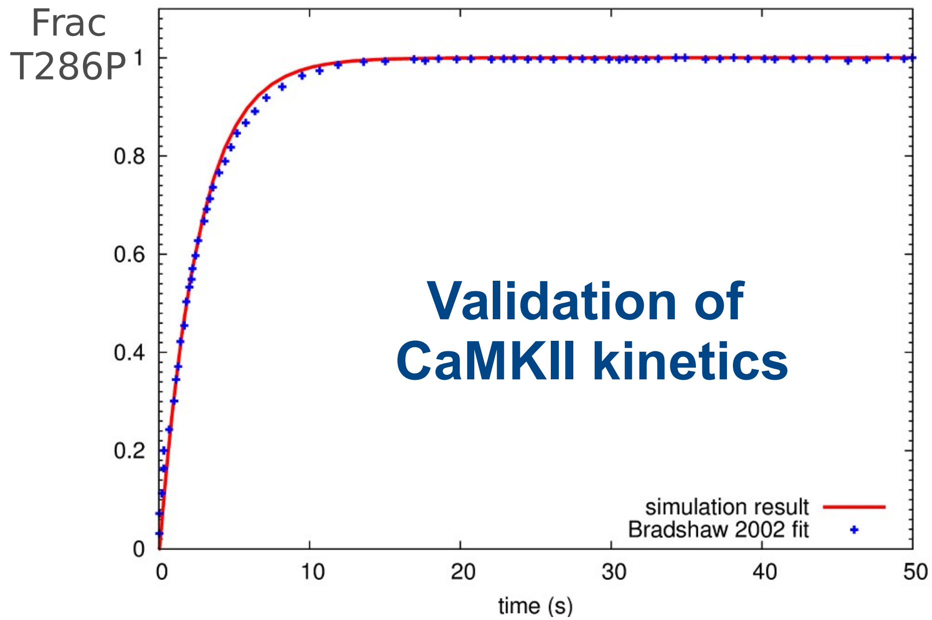
Dodecamer:
Trans-phosphorylation of T286 by neighbouring subunits
Cis-phosphorylation of T306
Most quantitative measurements made on monomers ...


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:
0
1/5
2/5
3/5
4/5
1



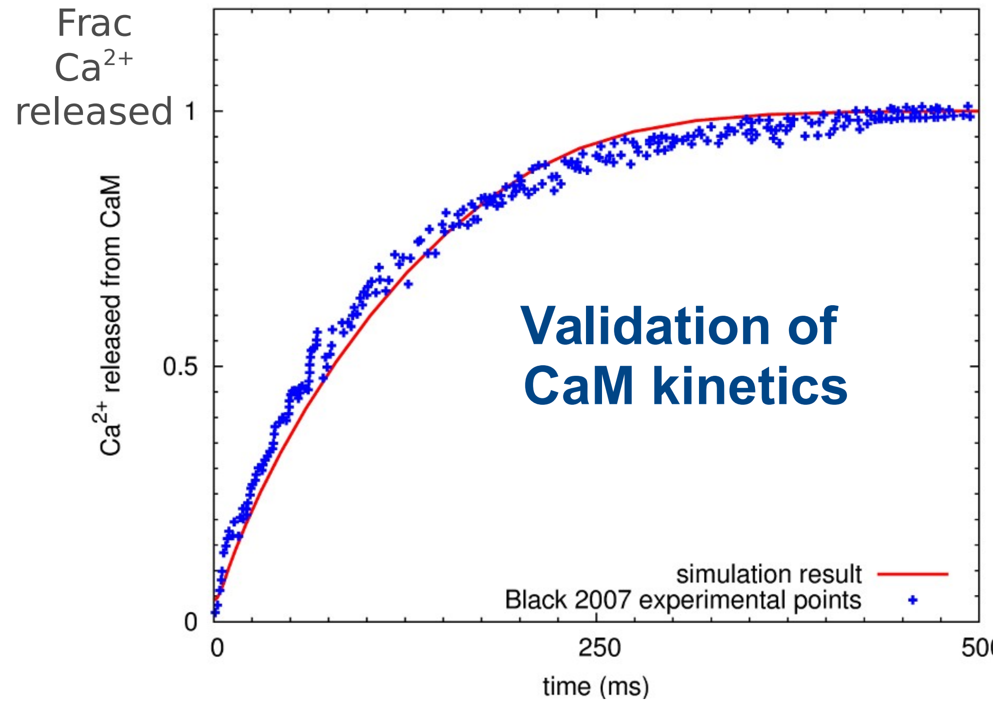
T_A
T_B
T_C
T_D
T_0
T_{ABC}
T_{ABD}
T_{ACD}
T_{BCD}
T_{ABCD}
K_A^T
K_B^T
K_C^T
K_D^T
L
Lc_A
Lc_B
Lc_C
Lc_D
$Lc_Ac_Bc_Cc_D$
R_0
R_A
R_B
R_C
R_D
R_{ABC}
R_{ABD}
R_{ACD}
R_{BCD}
R_{ABCD}
K_A^R
K_B^R
K_C^R
K_D^R
K_{CaMKII}^R
$K_{CaMKIIp}^R$
R_0-CaMKII
R_A-CaMKII
R_B-CaMKII
R_C-CaMKII
R_D-CaMKII
R_{ABC} -CaMKII
R_{ABD} -CaMKII
R_{ACD} -CaMKII
R_{BCD} -CaMKII
R_{ABCD} -CaMKII
K_{CaN}^R
R_0-CaMKIIp
R_A-CaMKIIp
R_B-CaMKIIp
R_C-CaMKIIp
R_D-CaMKIIp
R_{ABC} -CaMKIIp
R_{ABD} -CaMKIIp
R_{ACD} -CaMKIIp
R_{BCD} -CaMKIIp
R_{ABCD} -CaMKIIp
PP1
Negative feedback
R_0-CaN4Ca
R_A-CaN4Ca
R_B-CaN4Ca
R_C-CaN4Ca
R_D-CaN4Ca
R_{ABC} -CaN4Ca
R_{ABD} -CaN4Ca
R_{ACD} -CaN4Ca
R_{BCD} -CaN4Ca
R_{ABCD} -CaN4Ca
DARPP-32p

Validation of CaMKII kinetics



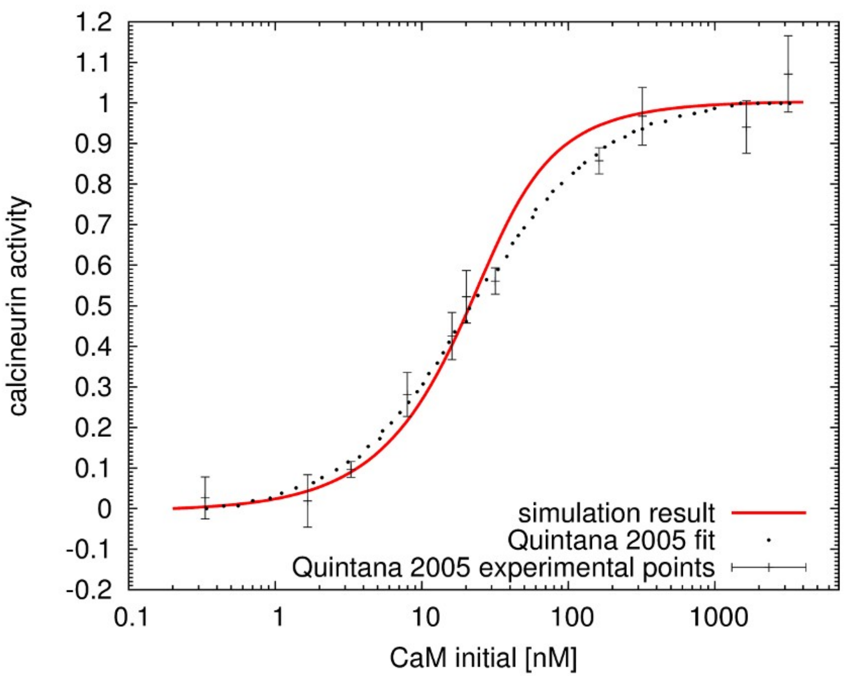
Bradshaw JM, Kubota Y, Meyer T, Schulman H (2003). *PNAS* 100: 10512–10517.

Validation of CaM kinetics



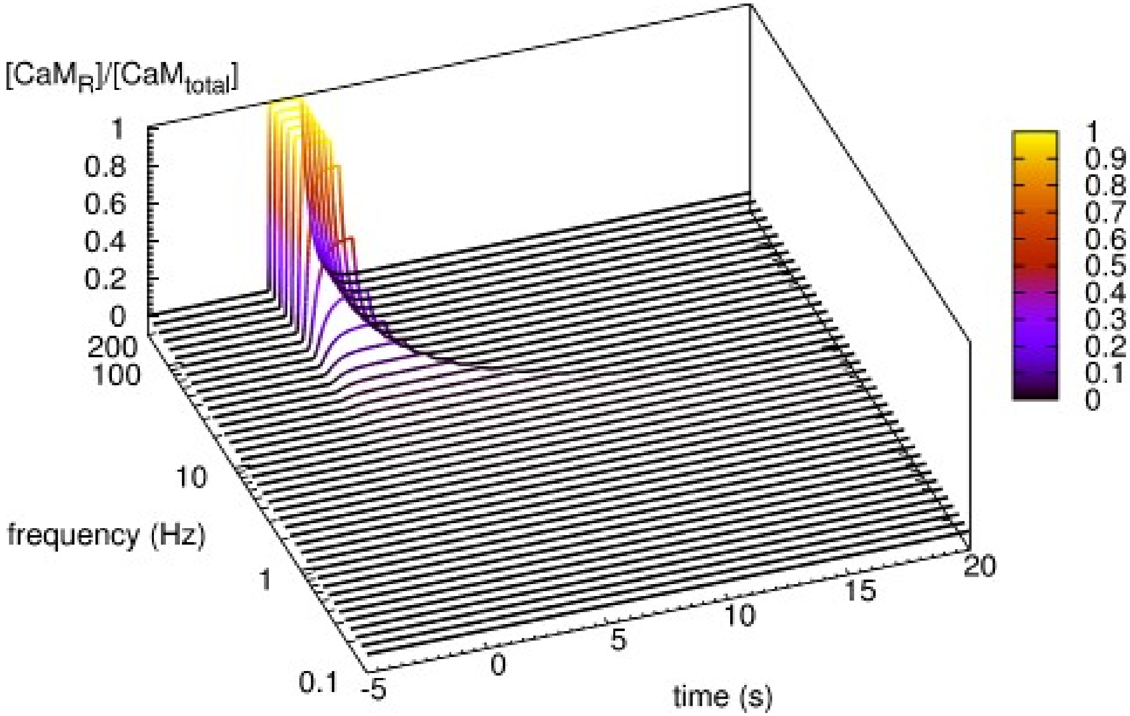
Black DJ, Selfridge JE, Persechini A (2007). *Biochemistry* 46: 13415–13424.

Validation of calcium-activation of CaN



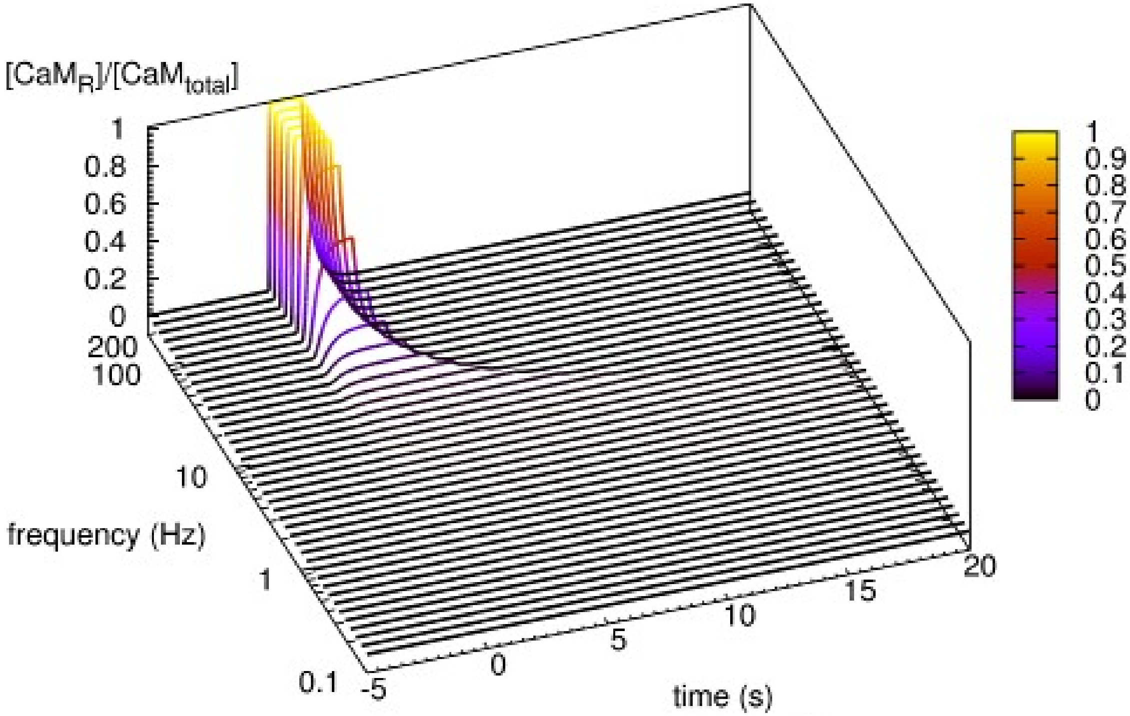
Quintana AR, Wang D, Forbes JE, Waxham MN (2005). *BBRC* 334: 674–680.

Calmodulin activation

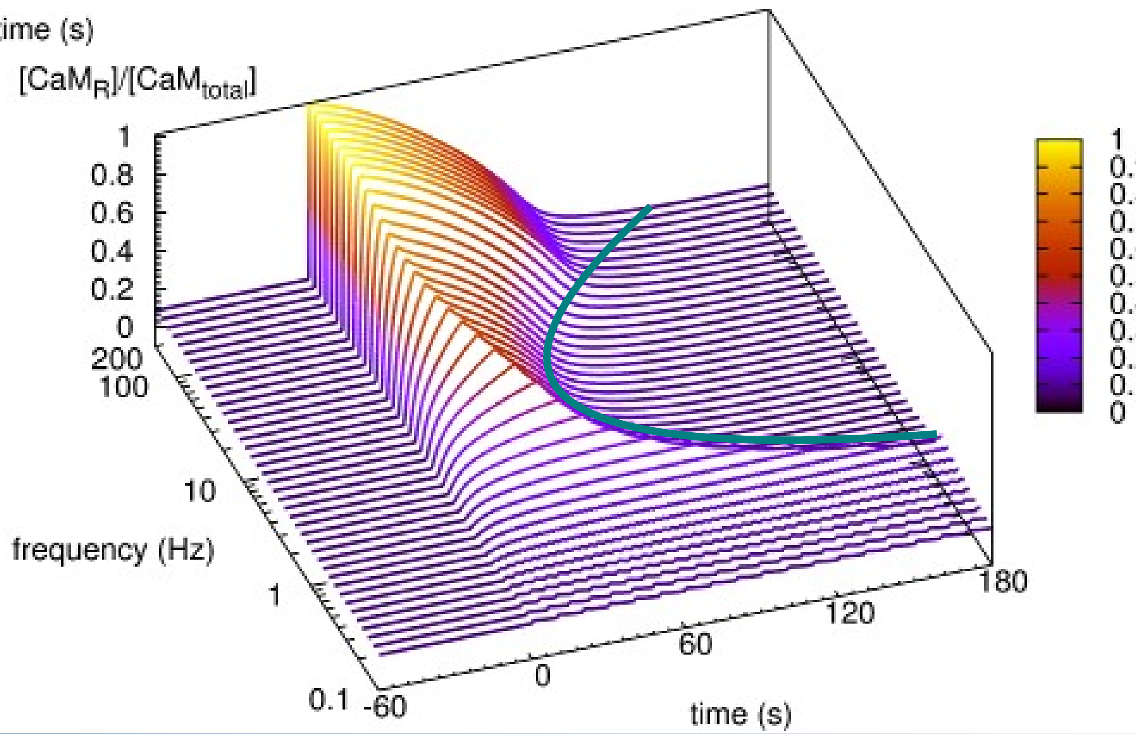


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

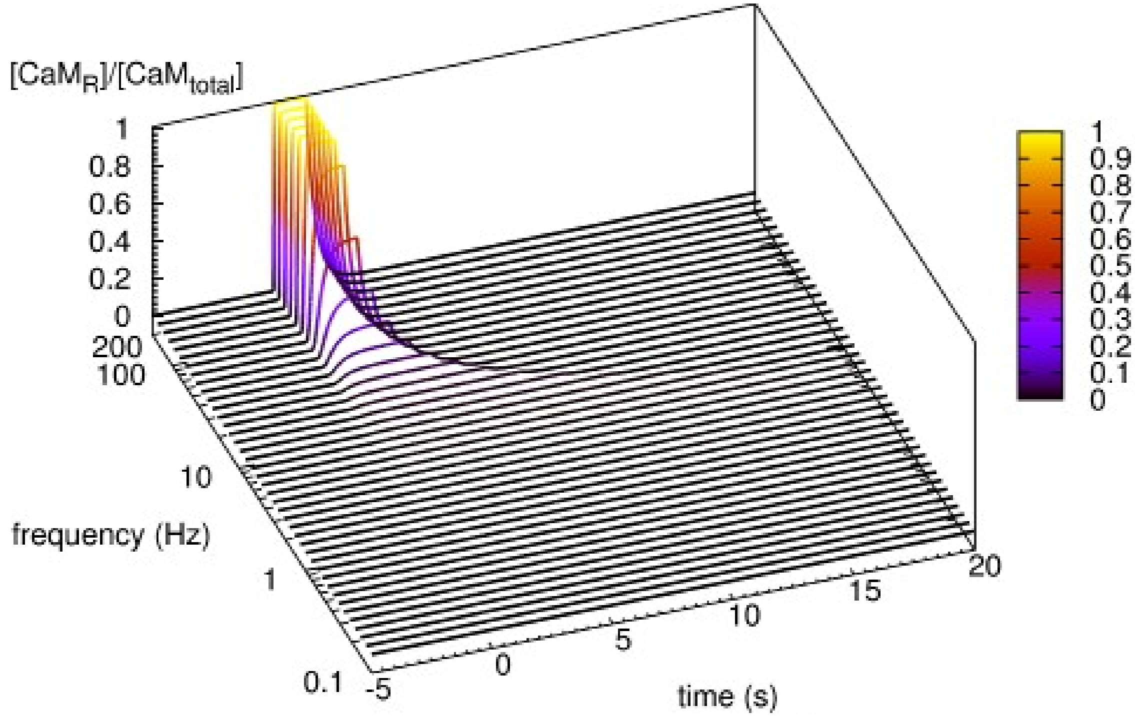


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)



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

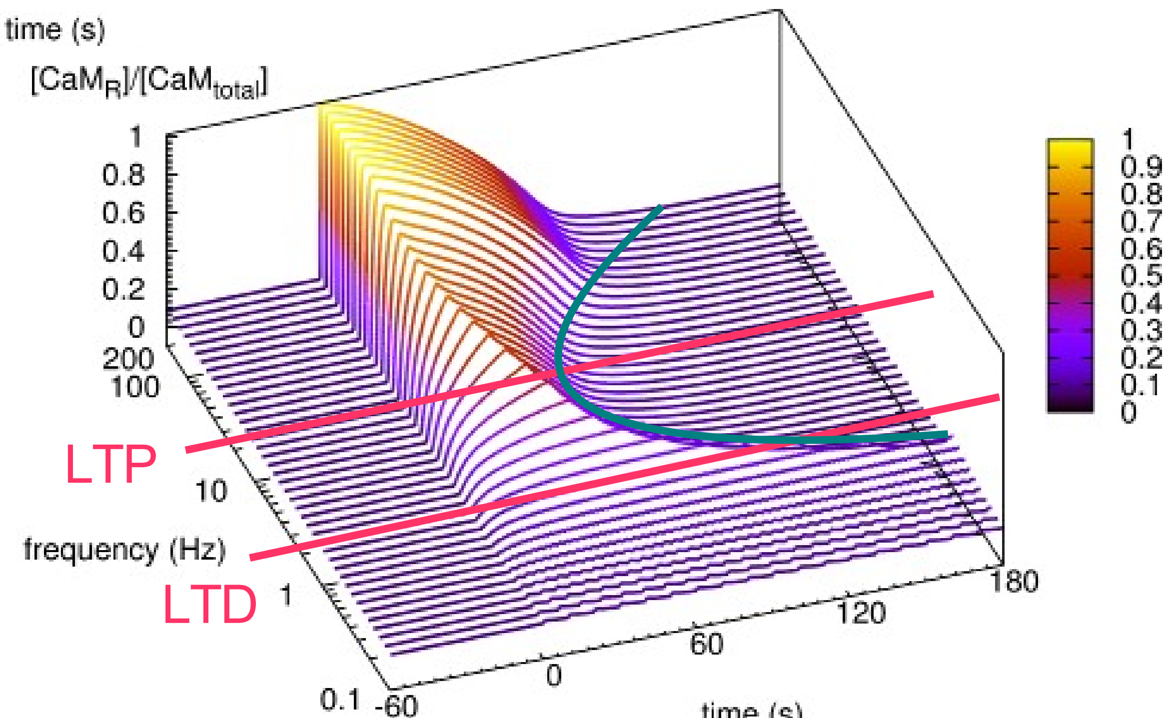
Calmodulin activation



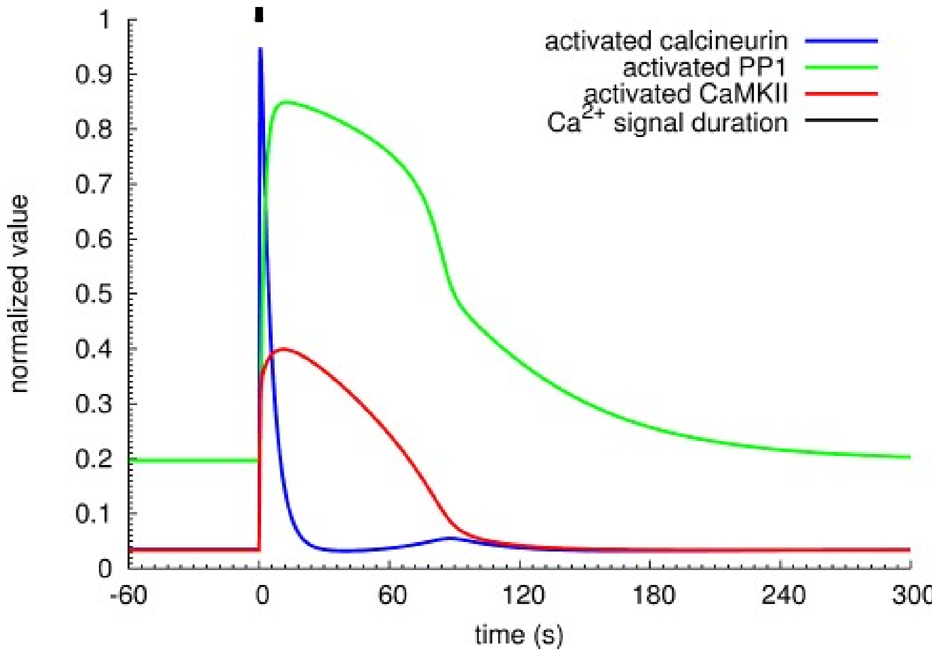
CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

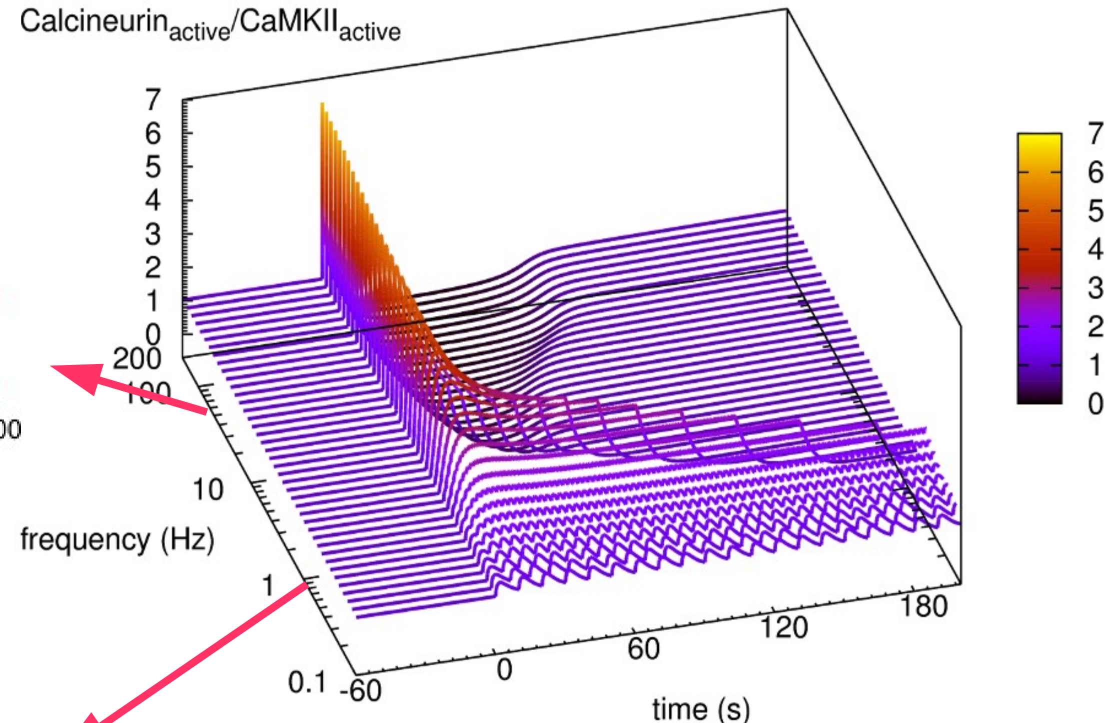
At high frequency, effects of calcium signals last much longer than the signal itself

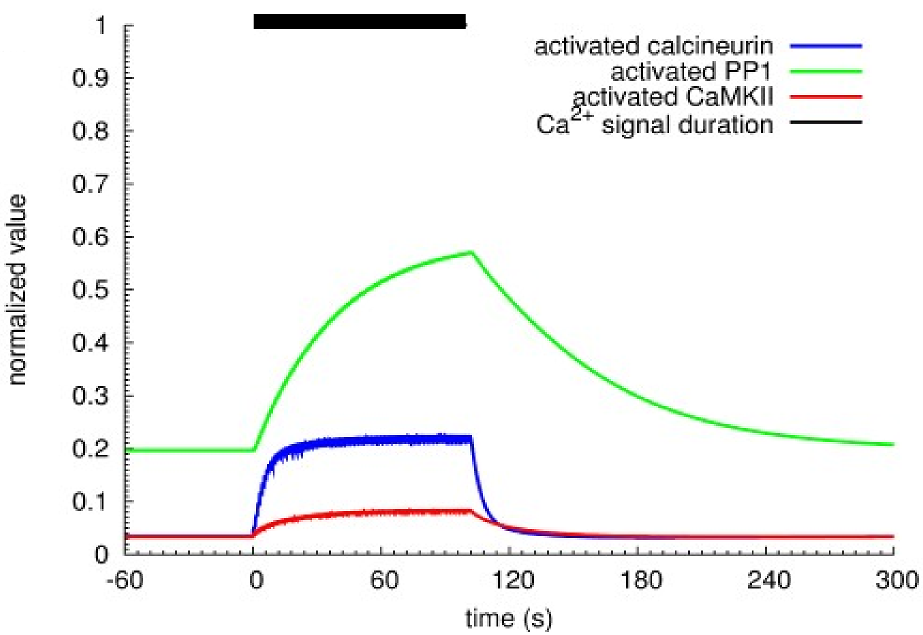
CaM with targets. Binding to CaN and CaMKII stabilises R state, with higher affinity. Positive feedback loop
➜ bistability



Temporal activation of CaMKII and CaN

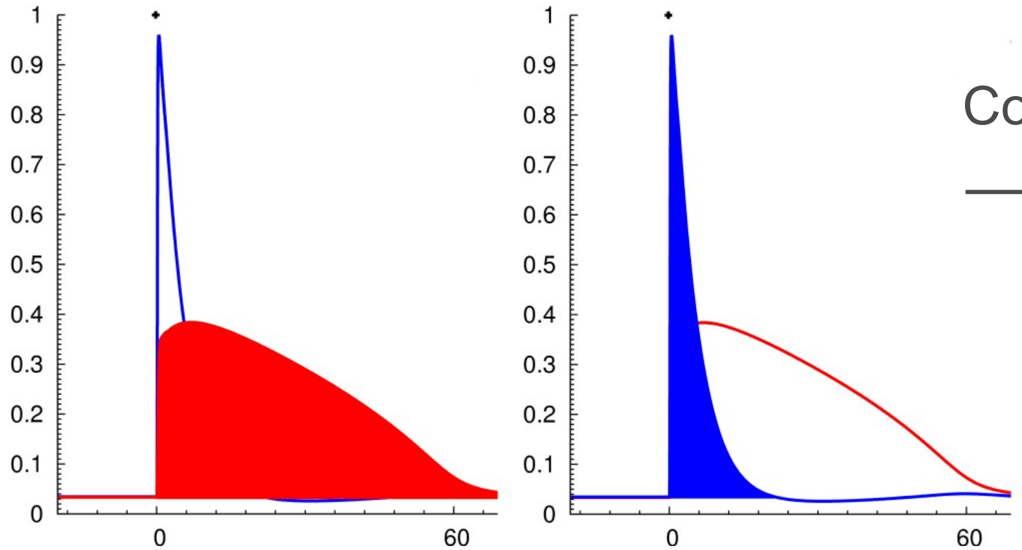






All calcium frequencies increase CaN **AND** CaMKII. It is not a Switch. But the ratio of activation changes

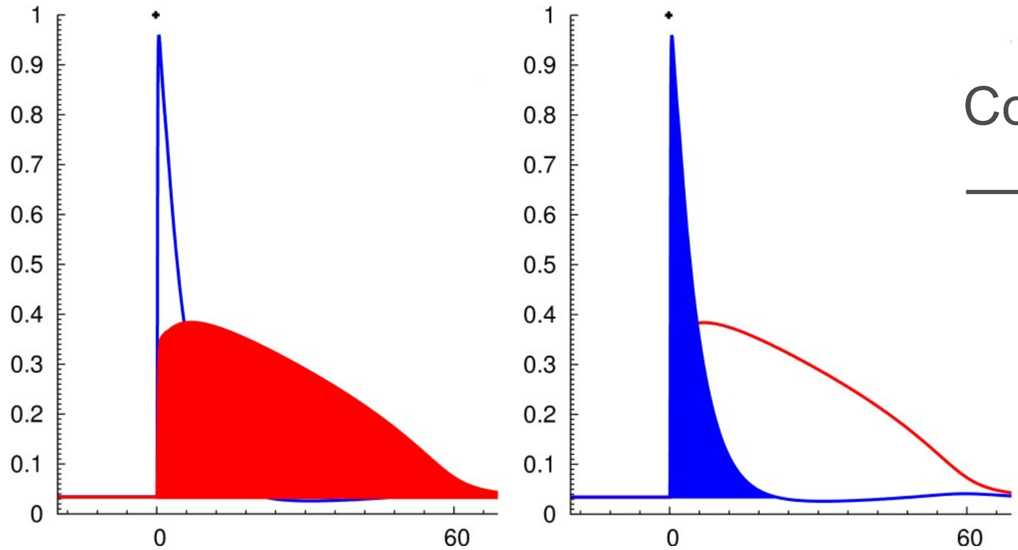
Bidirectional plasticity



Constant catalytic rates of active enzyme

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

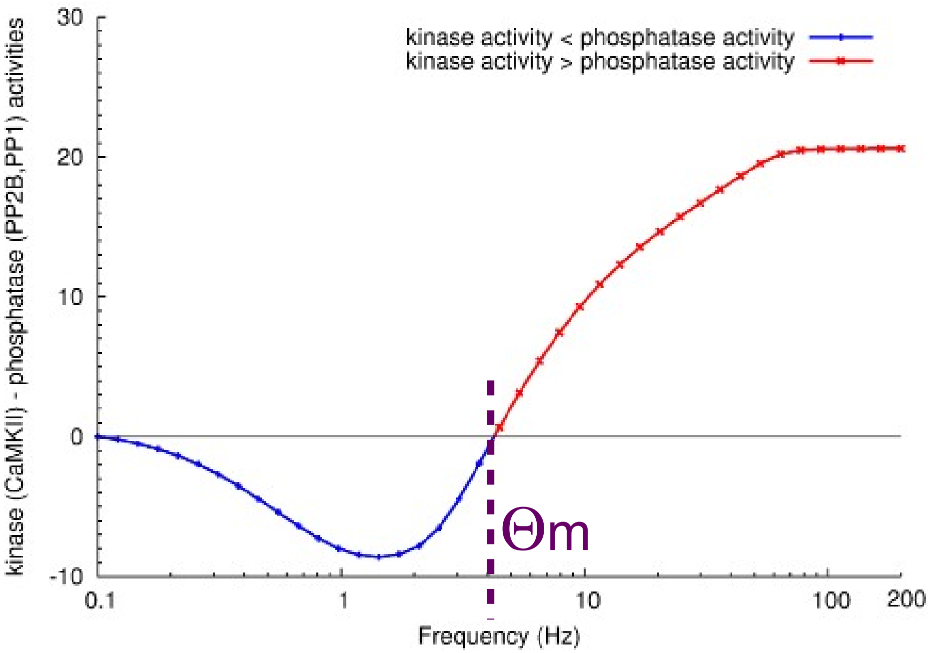
Bidirectional plasticity



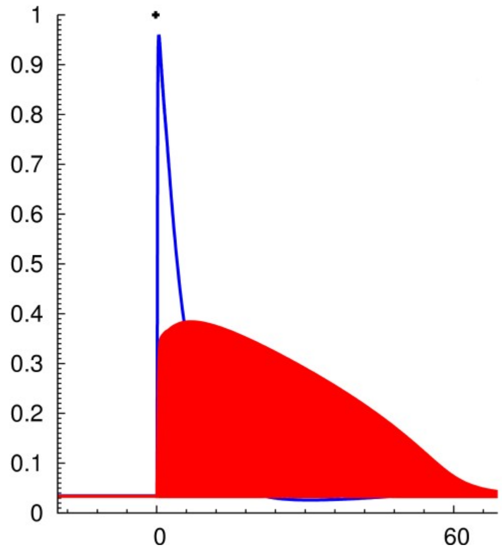
Constant catalytic rates of active enzyme

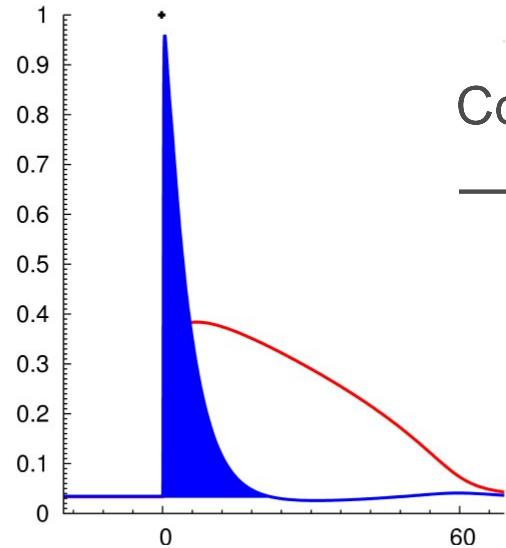
⟶ quantity of catalysed reaction events prop to integral of the activation curve

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



Bidirectional plasticity

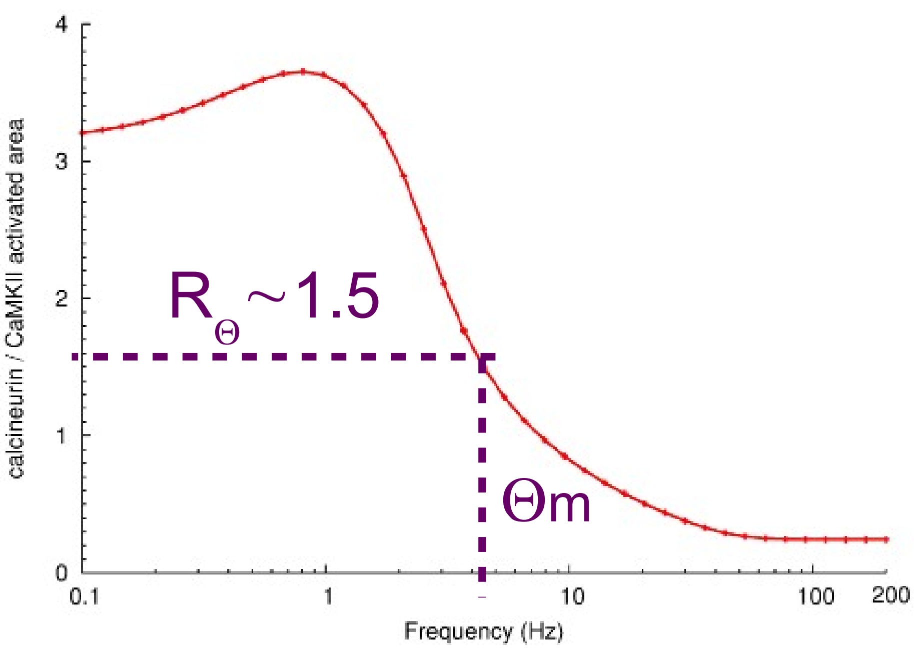


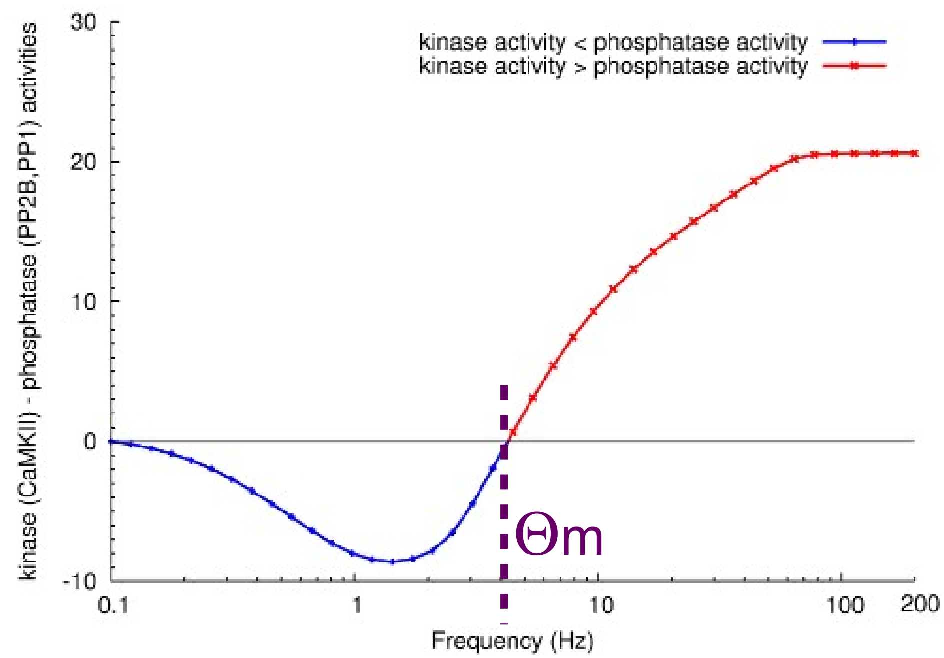


Constant catalytic rates of active enzyme

$\longrightarrow$ quantity of catalysed reaction events prop to integral of the activation curve

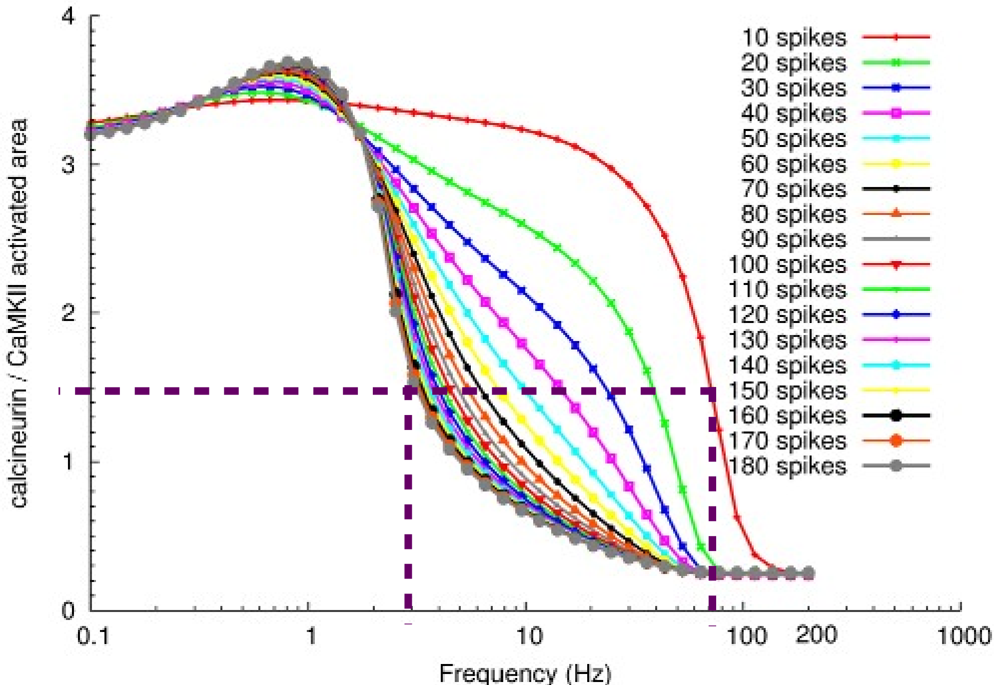
Bienestock-Cooper-Munro (BCM) curve: difference of active areas*catalytic activities





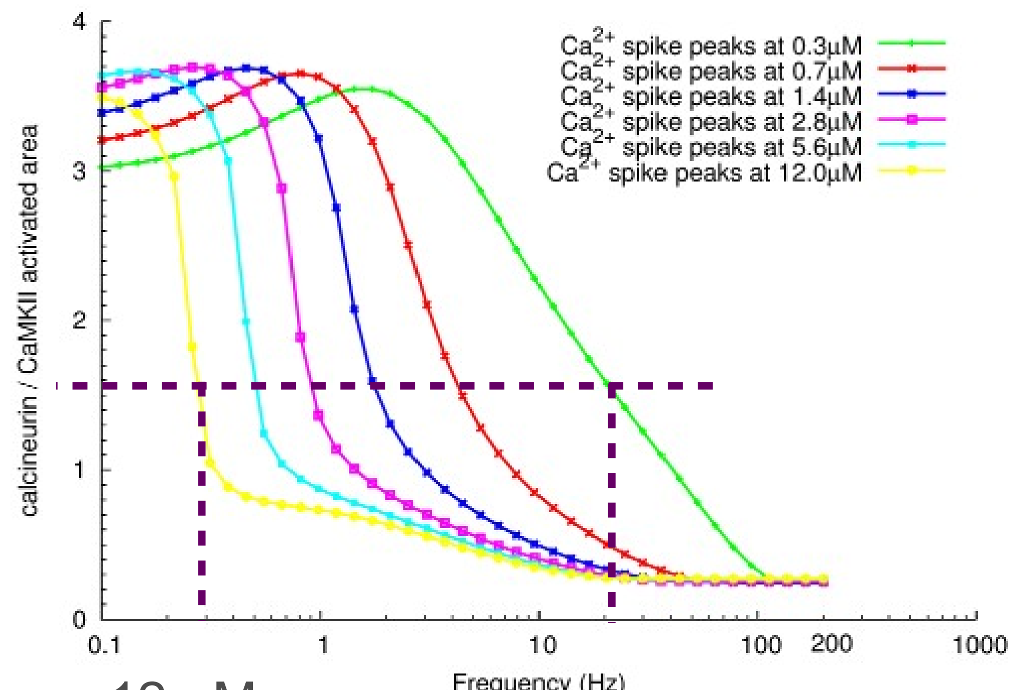
Babraham Institute

Effect of calcium duration and amount



180 spikes
= 3 Hz

10 spikes
= 70 Hz

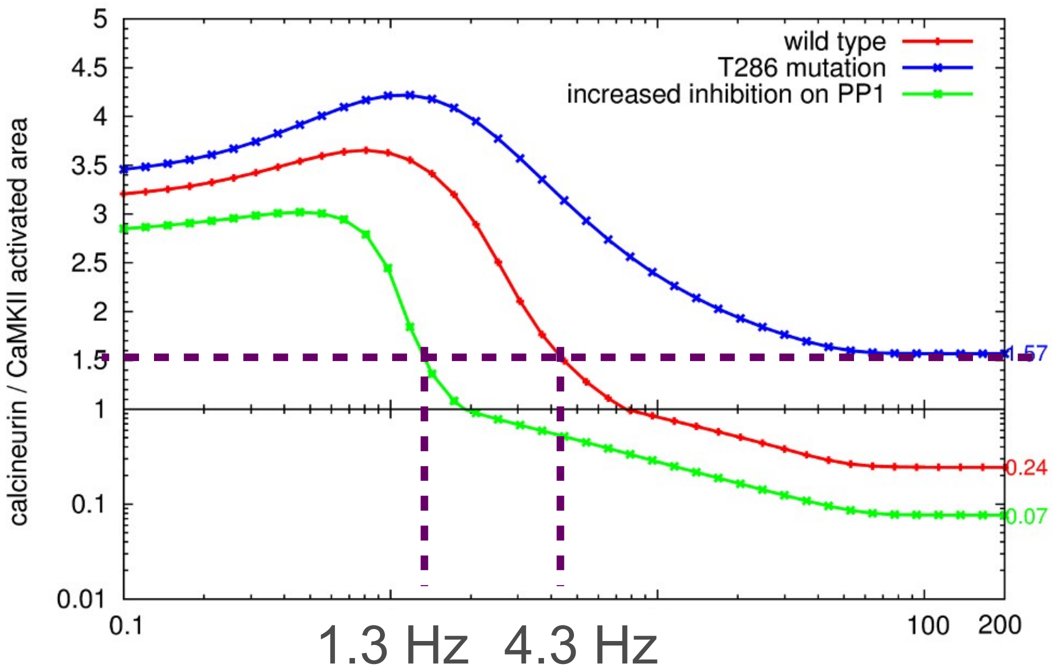


12 µM
= 0.3 Hz

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

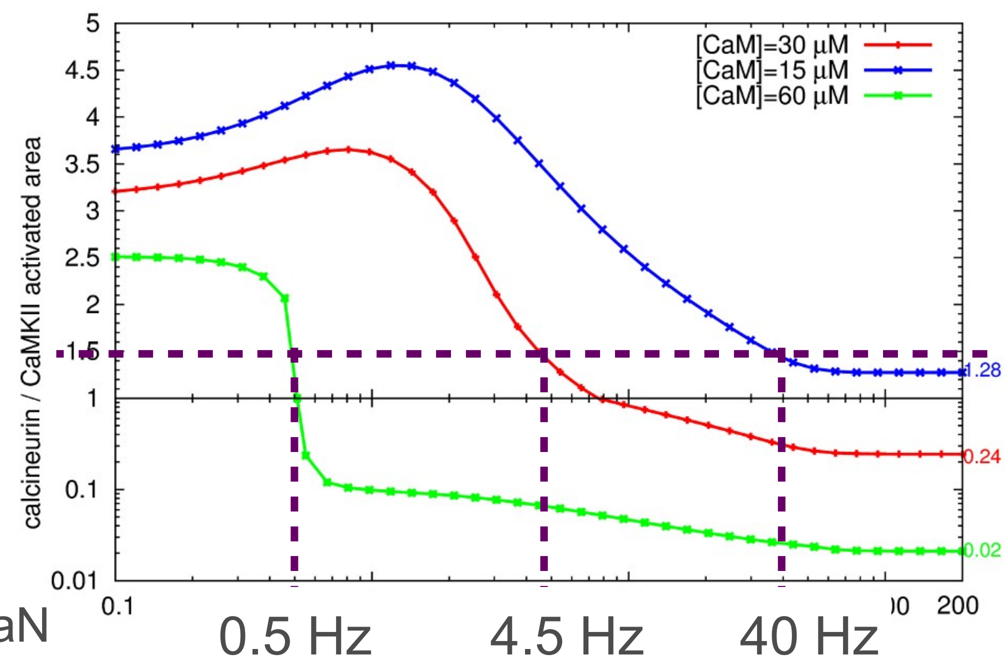


CaMKII not constitutively active
No CaM trapping

Never any positive plasticity
Giese et al (1998) *Science*, 279:870-873

Lower deactivation of CaMKII

Competition for CaM, CaN wins



Effect of Ng$^{-/-}$ (Huang *et al* 2004).
Better performance at low frequencies.

NB: 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 then activate CaN

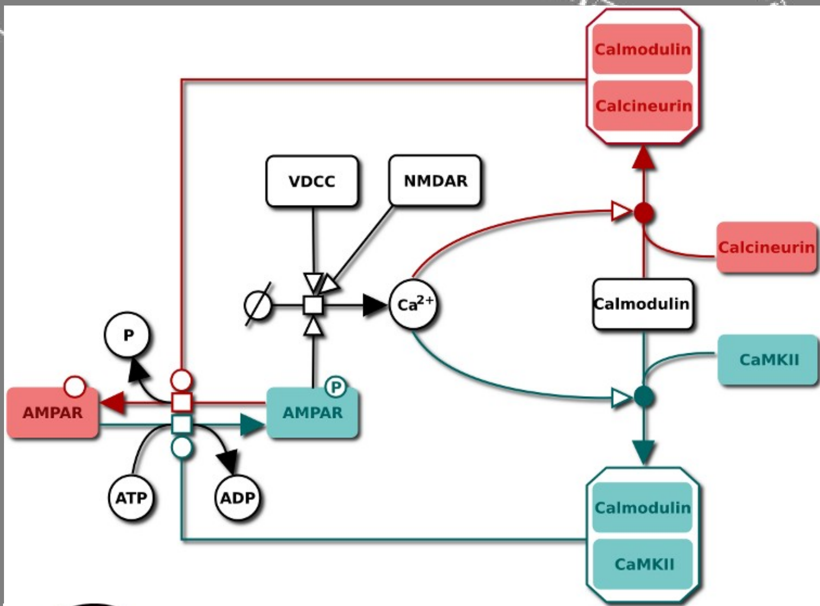
Allosteric stabilisation triggers **bistable CaM response** > certain freq, CaM activation longer than initial signal

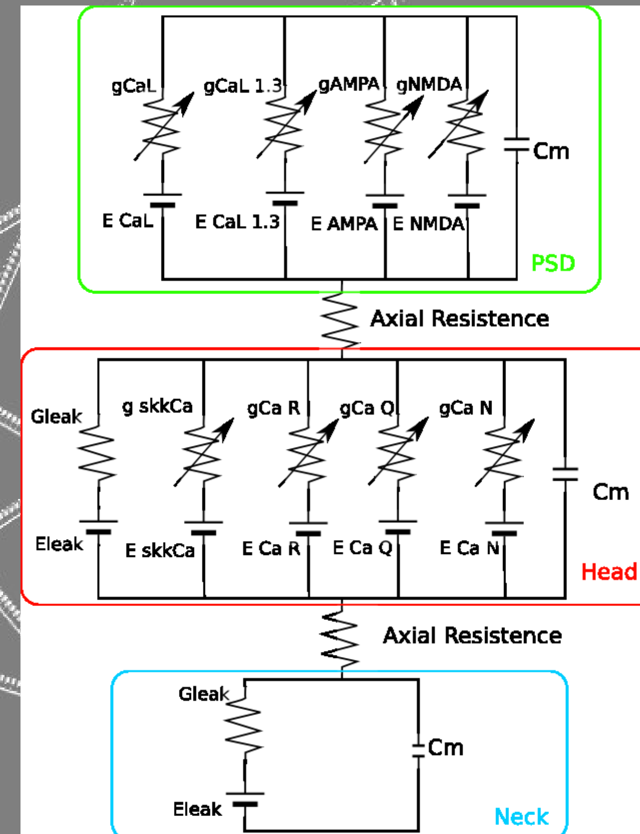
Calcium signals **activate both CaN and CaMKII** at *ALL* frequencies. The ratio of activity changes

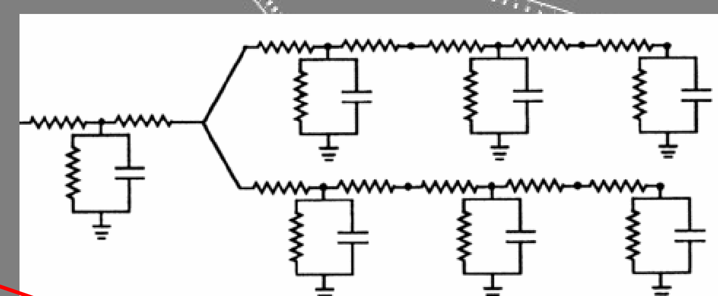
Θm is not an **intrinsic property of the synapse**, but a dynamical one that depends on the length and amplitude of stimulations

Θm and intensity affected by reactions, parameters and initial conditions. **[CaM] decides** the balance CaN/CaMKII

Whole cell: electro-biochemical models



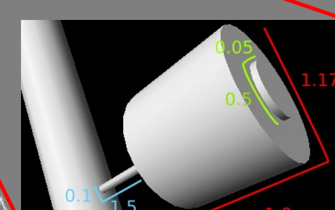




1504 spines

4761 compartments

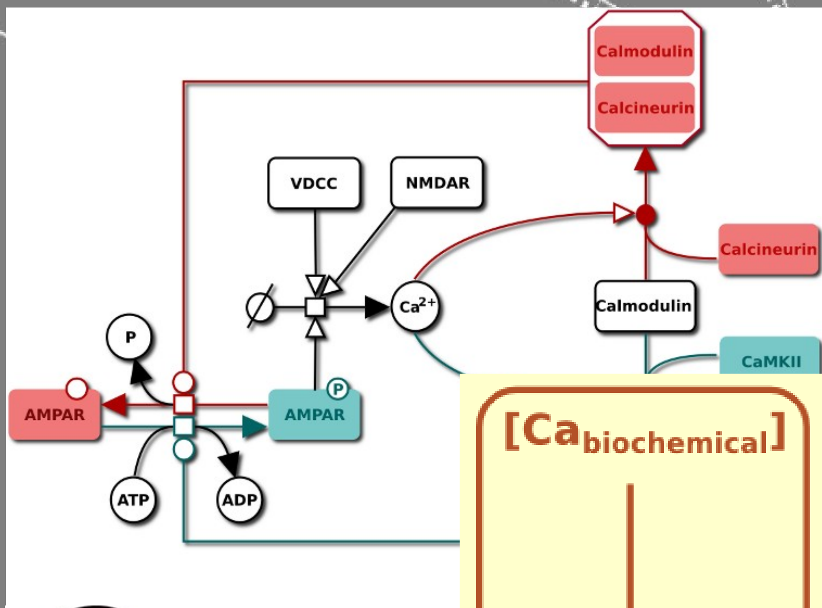
16362 channels

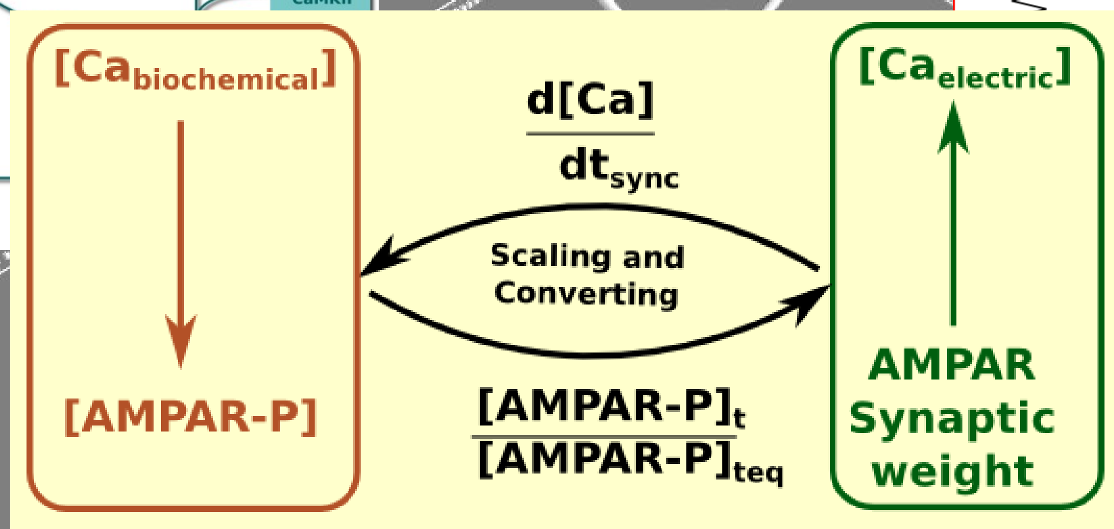


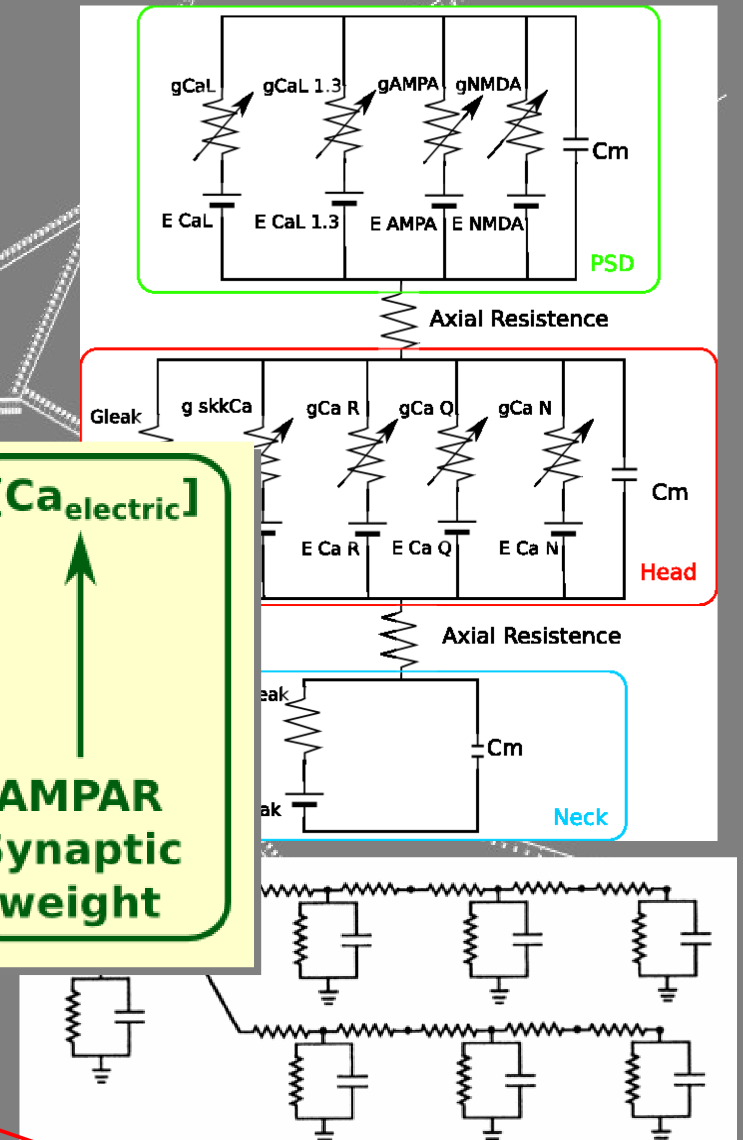
Mattioni M, Cohen U, Le Novère N (2012)

Mattioni M, Le Novère N (2013)

Whole cell: electro-biochemical models







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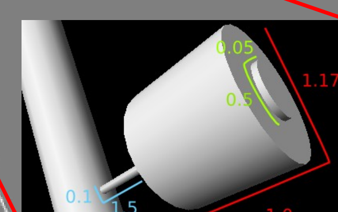


Mattioni M, Cohen U, Le Novère N (2012)

Mattioni M, Le Novère N (2013)

Developers of ECell3, NEURON, Scilab, and ...

COPASI

Melanie Stefan

Lu Li

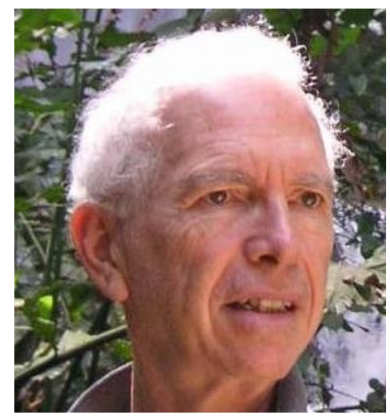
Denis Brun

EMBL

wellcome trust

SEVENTH FRAMEWORK PROGRAMME

Michele Mattioni



Stuart Edelstein

Massimo Lai

BBSRC bioscience for the future

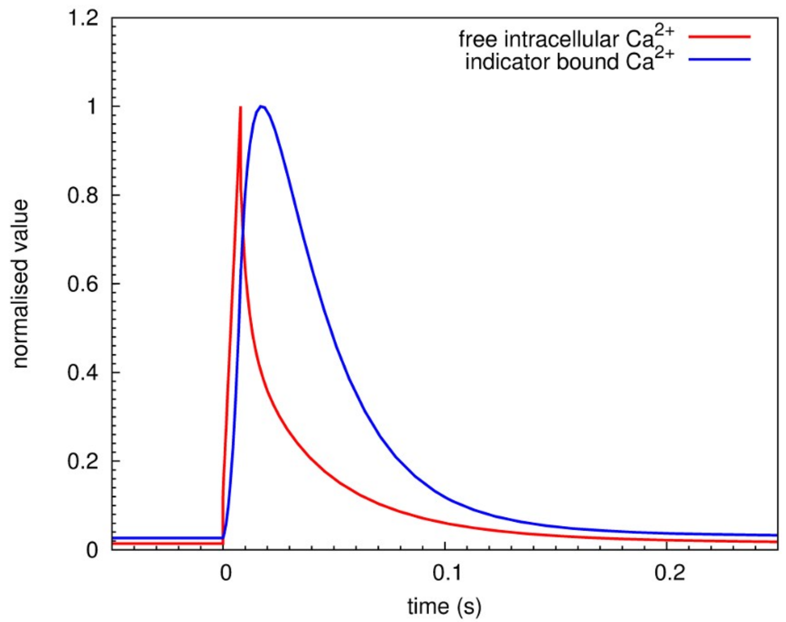
elixir Data for Life

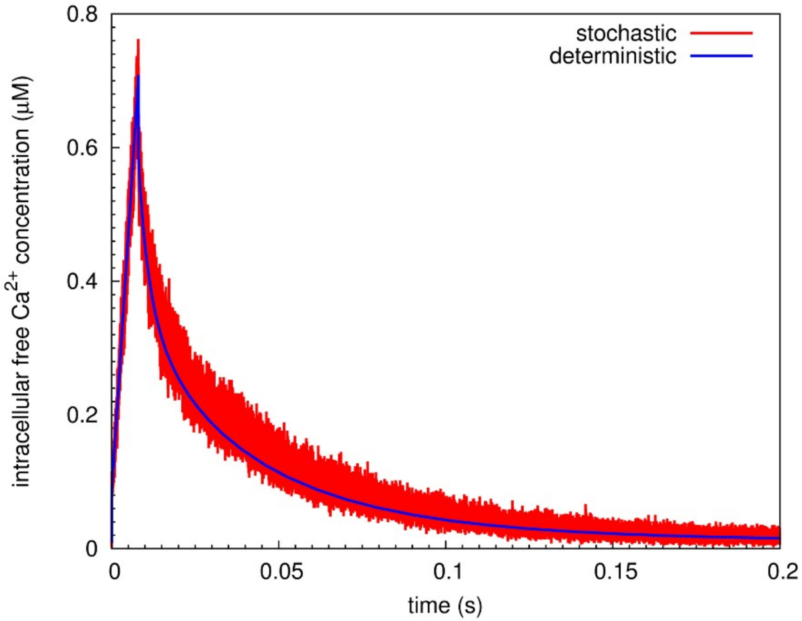
imi Innovative Medicines Initiative

NEDO

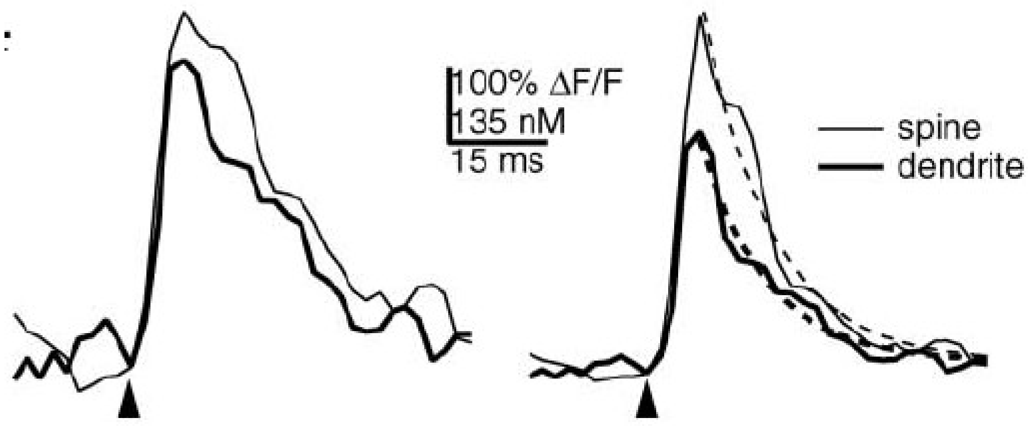
NIGMS

Are those spikes realistic?





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



Fluorescence

Free calcium

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