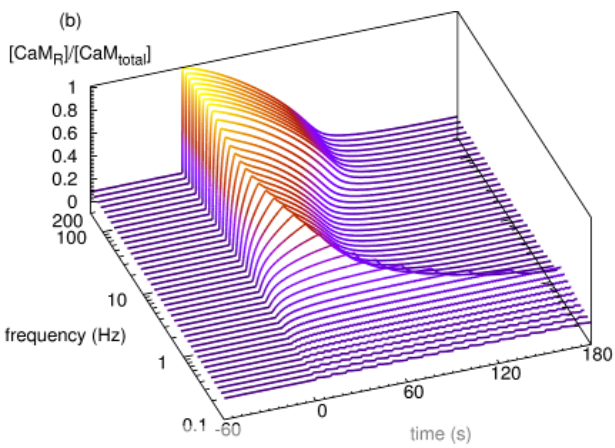




Modelling the regulation of bidirectional synaptic plasticity by allosteric calcium sensors

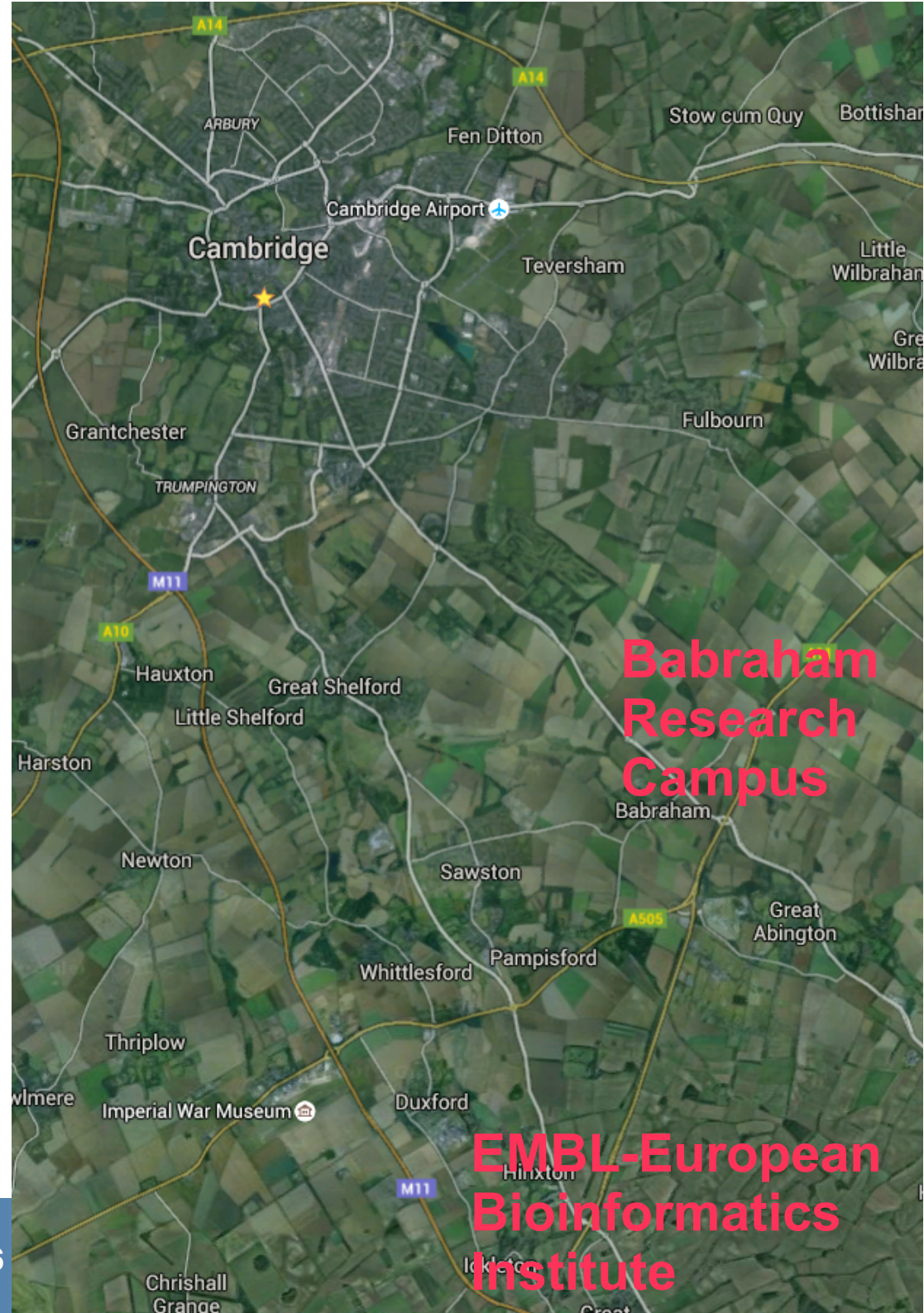


Nicolas Le Novère
Babraham Institute,
n.lenovere@gmail.com



Programmes in:
Signalling
Immunology
Epigenetics
Nuclear Dynamics

Platforms including:
Bioinformatics
Imaging
FACS
Lipidomics
Mouse facilities
Sequencing



Some of Babraham Institute's historical fame

- **Discovery of the liposome**

Bangham AD, Standish MM, Watkins JC (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol* 13, 238–252

- **Discovery of IP3 signalling and release of internal calcium stores**

H Streb, RF Irvine, MJ Berridge, I Schulz H Streb, RF Irvine, MJ Berridge, I Schulz (1983) Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1, 4, 5-trisphosphate. *Nature* 306, 67-69

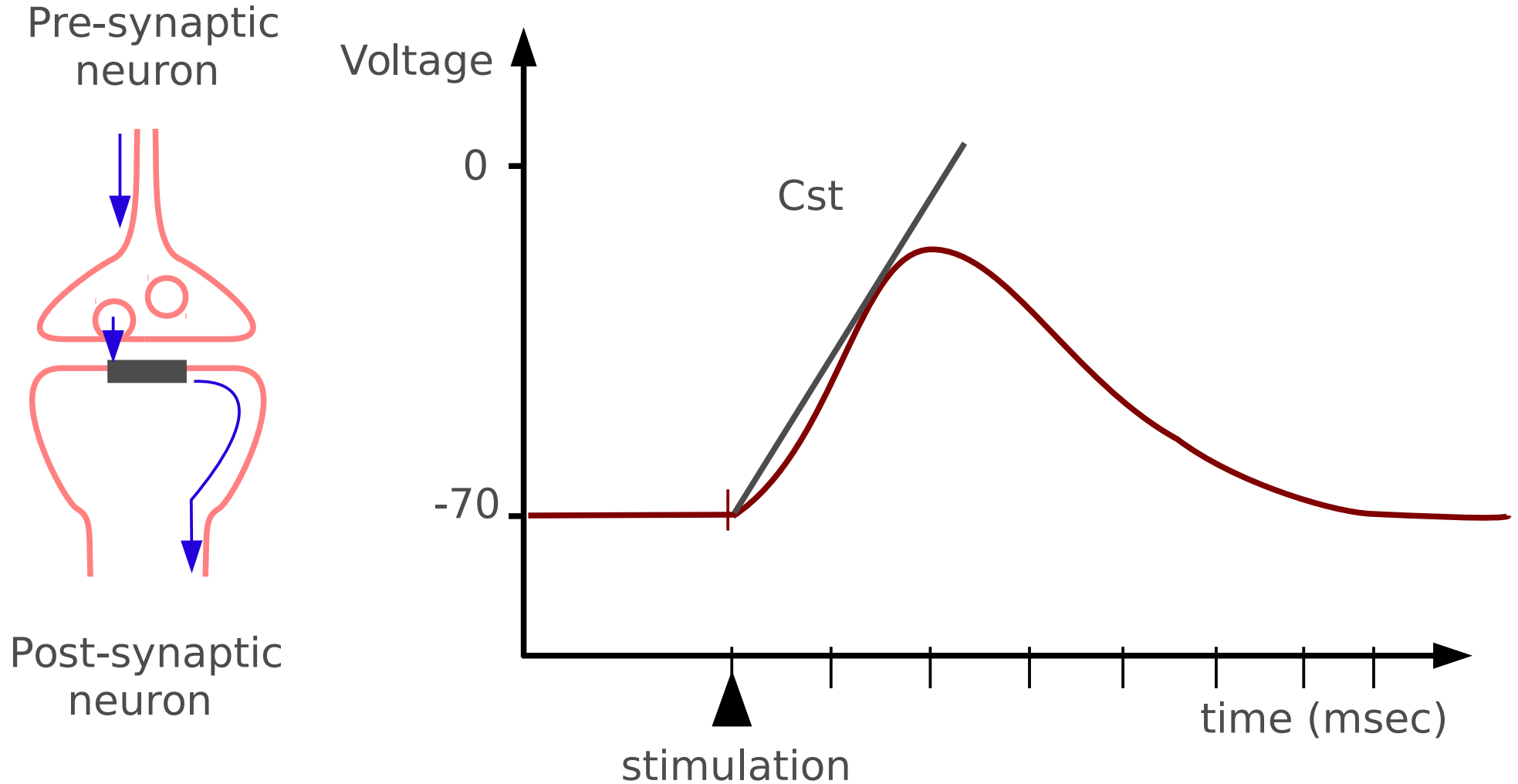
- **DNA methylation responsible of parental imprinting**

W Reik, A Collick, ML Norris, SC Barton, MA Surani (1987) Genomic imprinting determines methylation of parental alleles in transgenic mice. *Nature* 328, 248-251

- **Phosphorylation of PIP2 into PIP3 by PI3K**

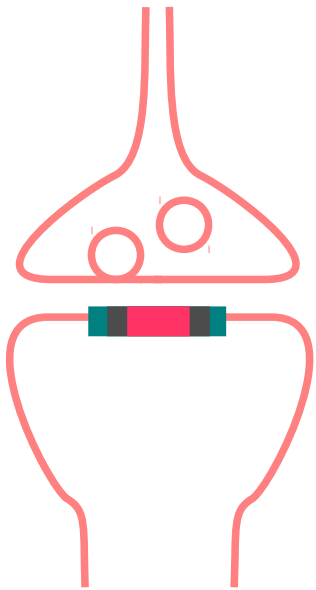
PT Hawkins, TR Jackson, LR Stephens (1992) Platelet-derived growth factor stimulates synthesis of $\text{PtdIns}(3,4,5)\text{P}_3$ by activating a $\text{PtdIns}(4,5)\text{P}_2$ 3-OH kinase. *Nature* 358, 157-159

Excitatory post-synaptic potential

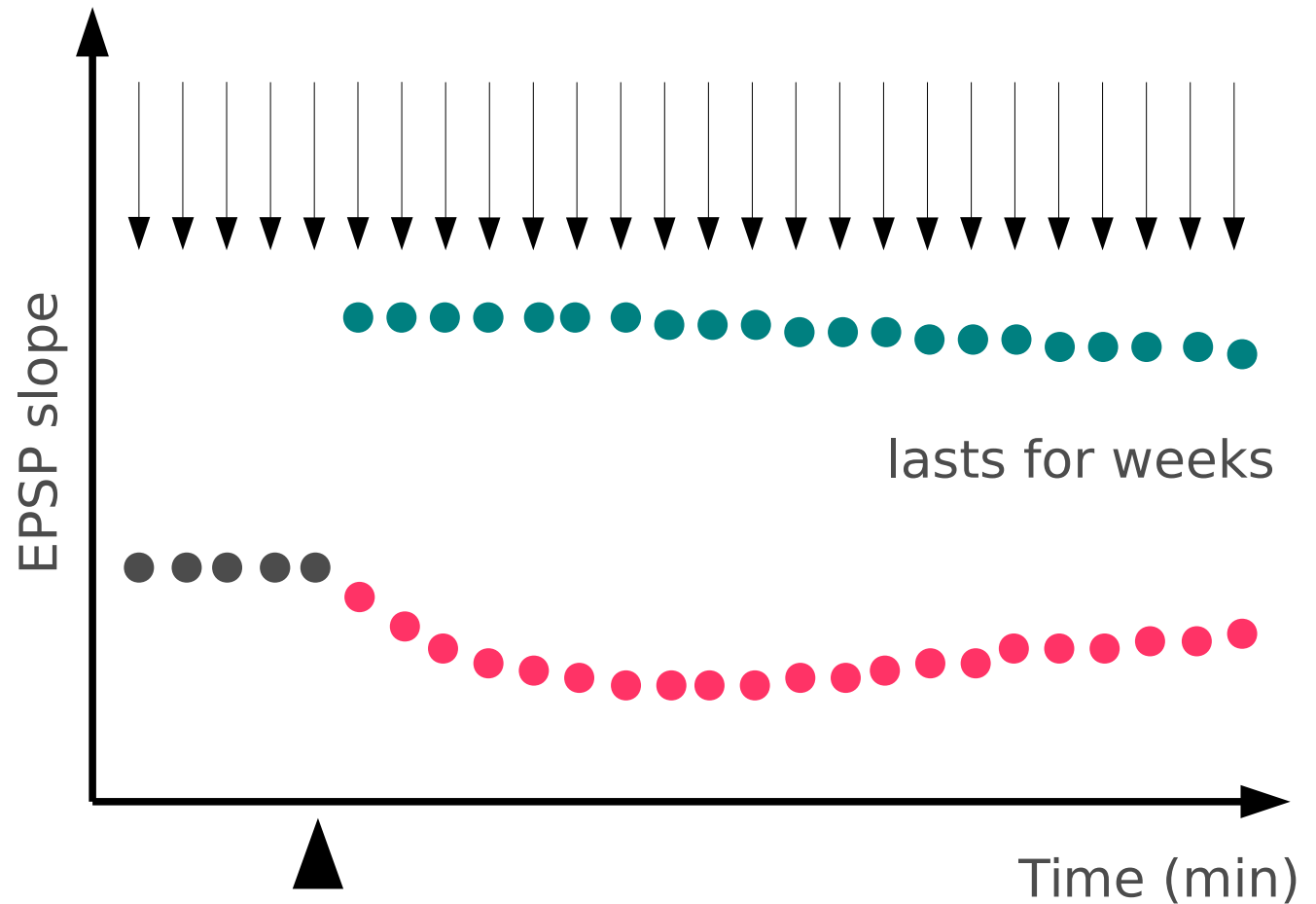


Bidirectional synaptic plasticity

Pre-synaptic neuron



Post-synaptic neuron



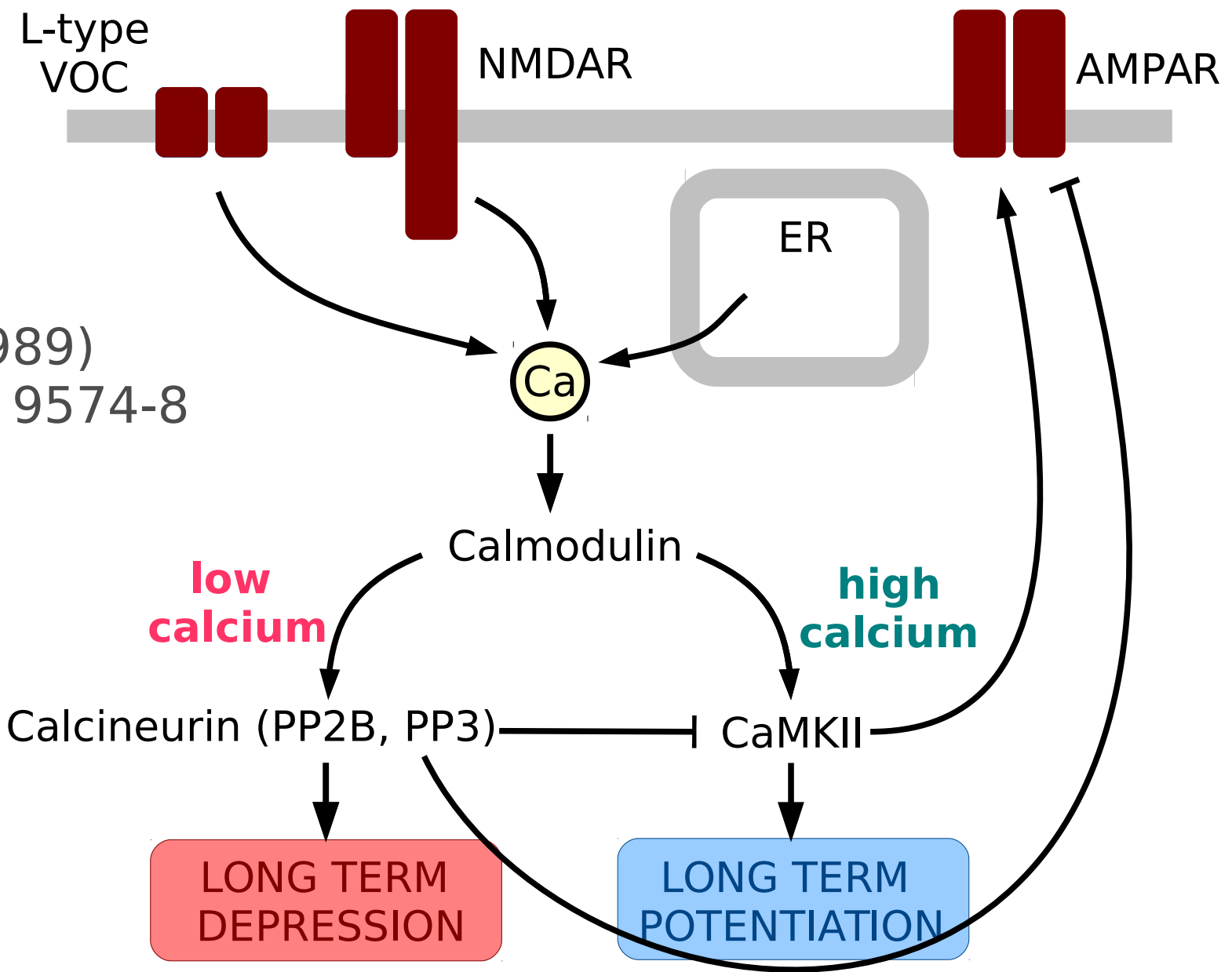
e.g. high frequency stimulation

e.g. low frequency stimulation

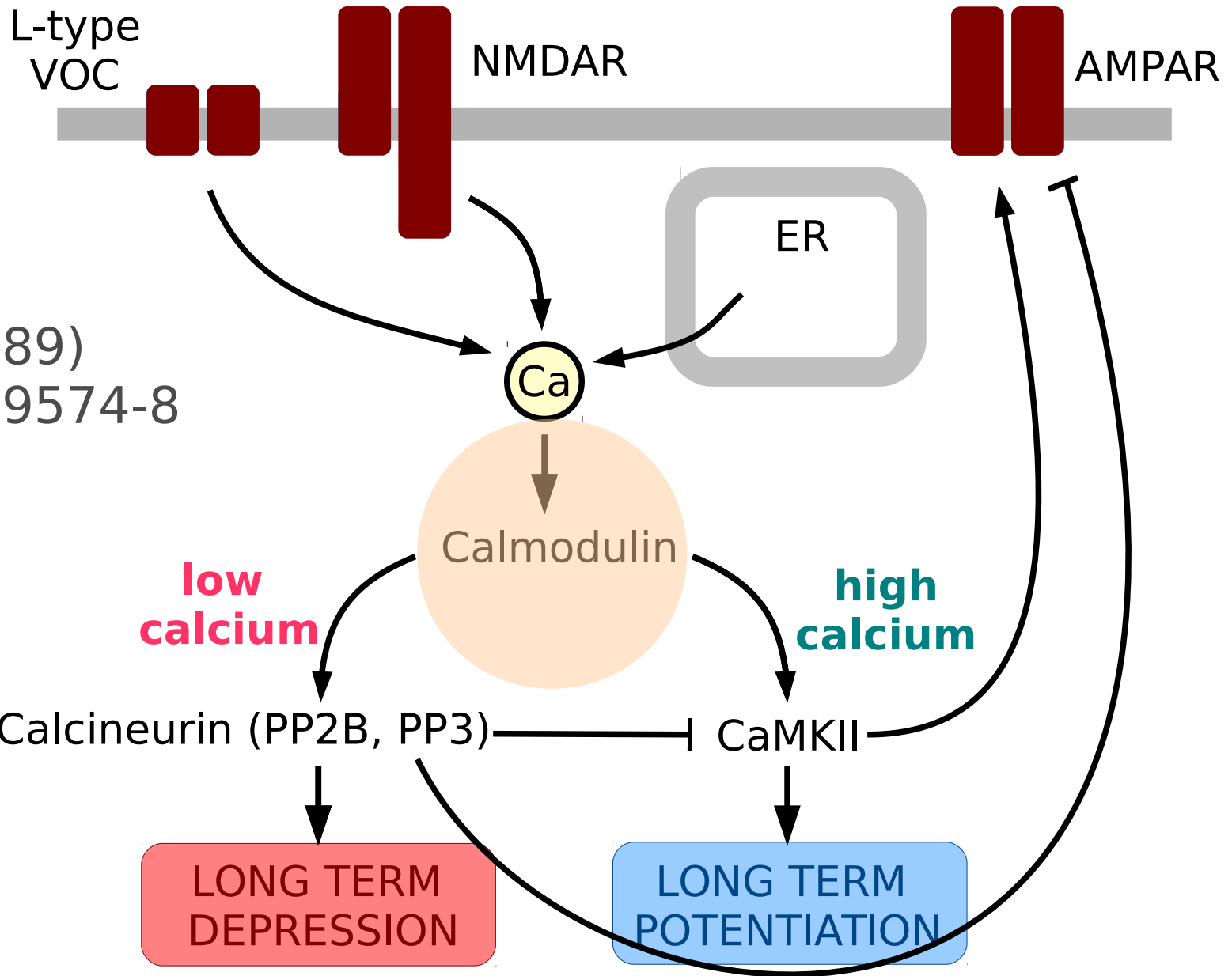


The calcium theory of synaptic plasticity

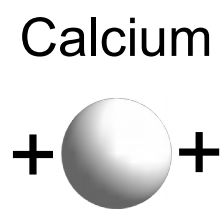
Lisman (1989)
PNAS, 86: 9574-8



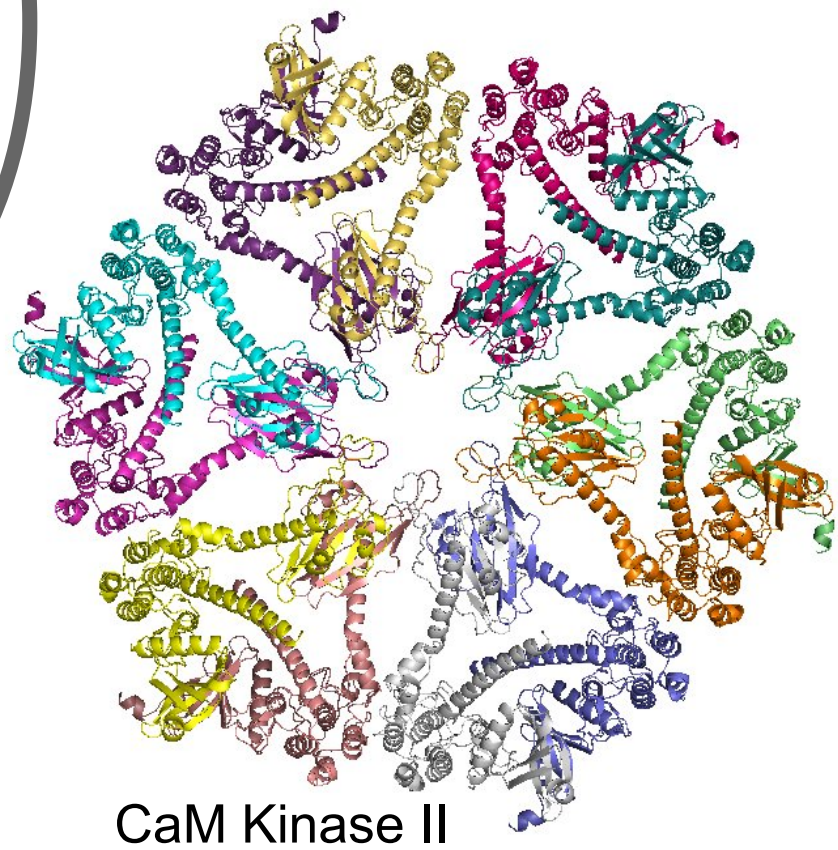
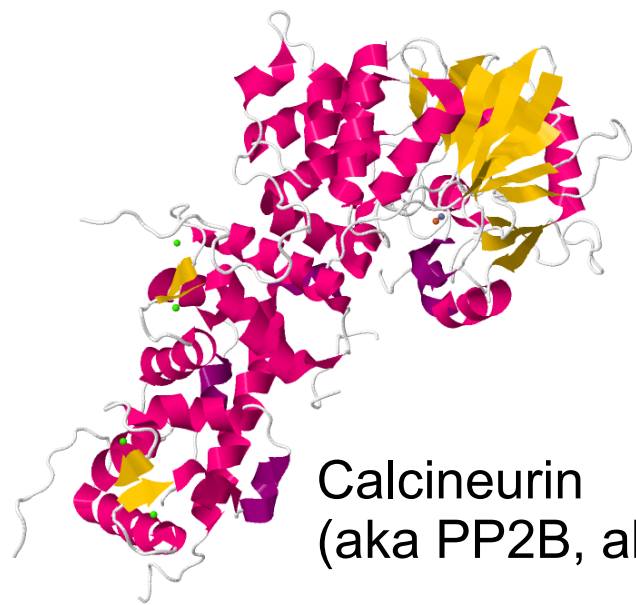
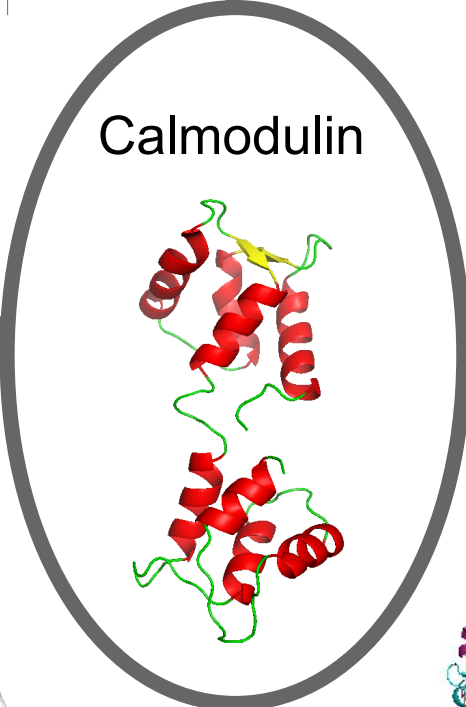
Calmodulin, the memory switchboard



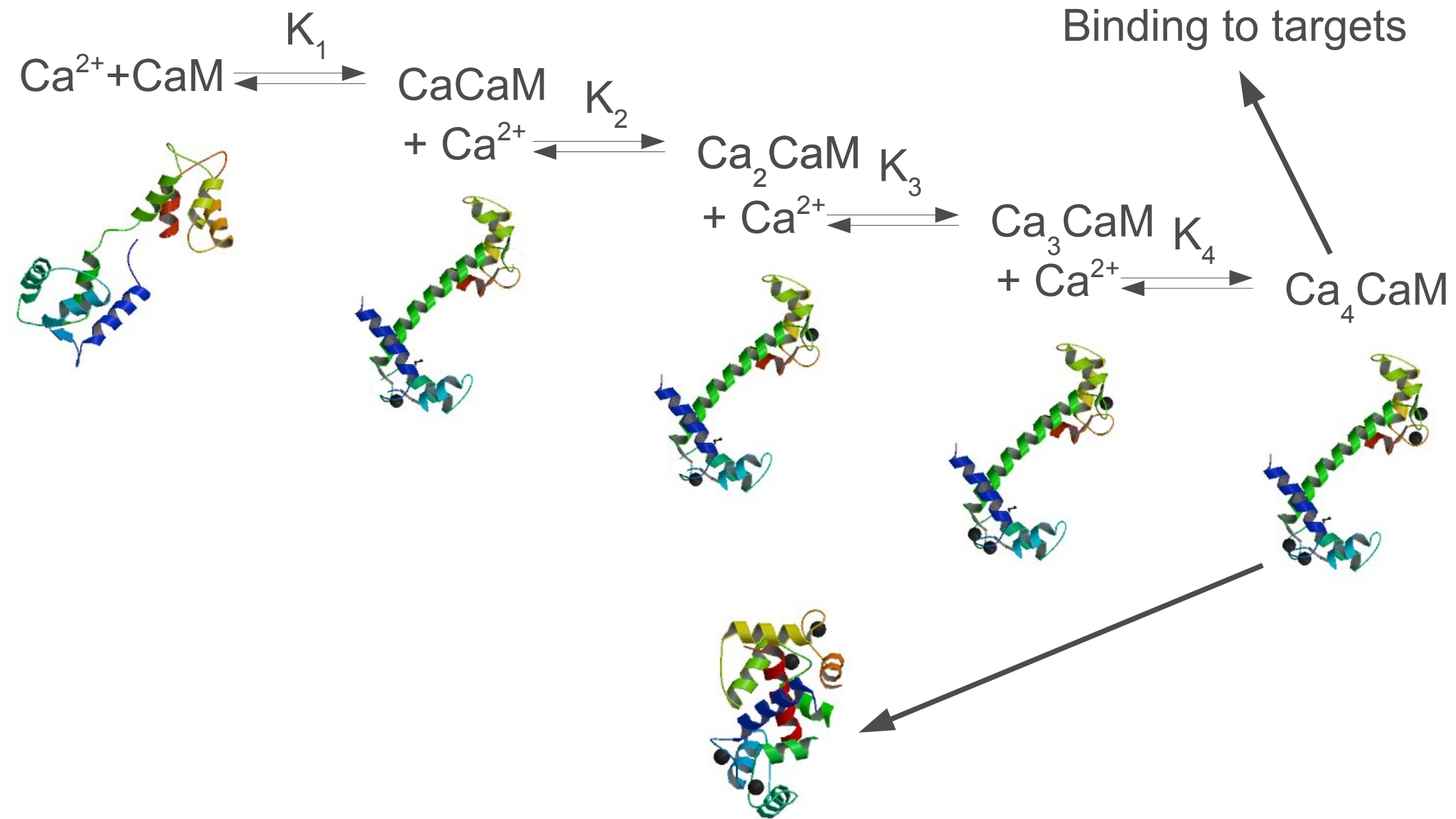
Lisman (1989)
PNAS, 86: 9574-8



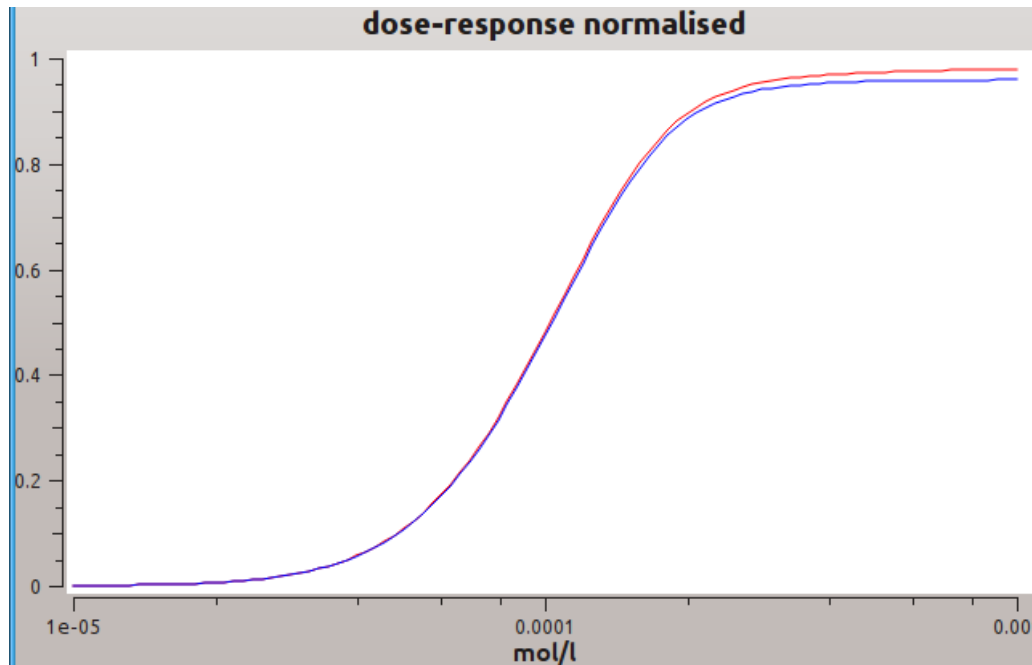
Neurogranin



Adair-Klotz + key-lock



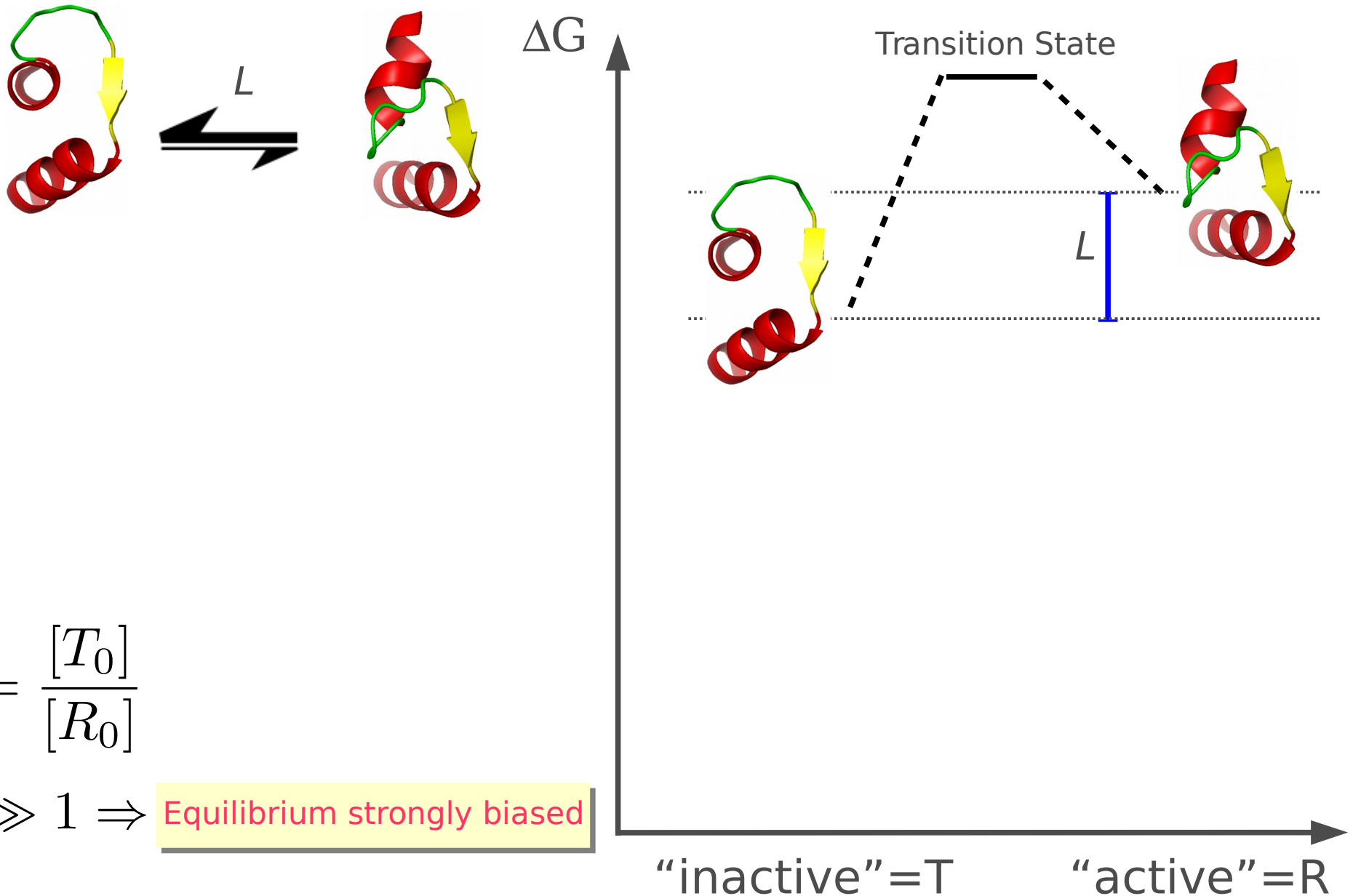
Adair-Klotz does not permit differential activations



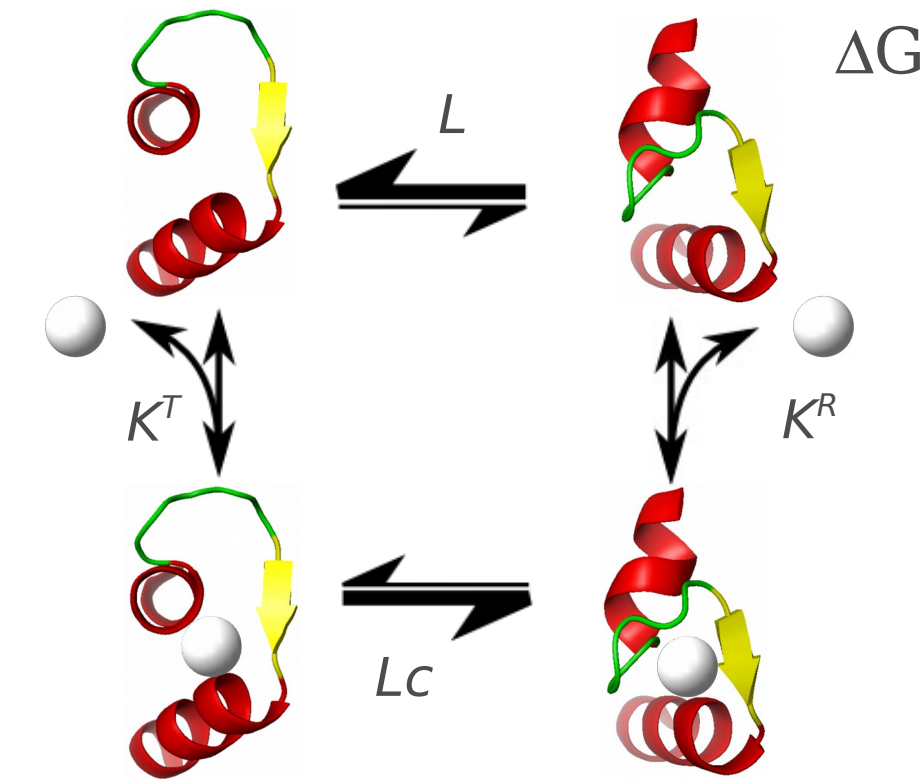
$$[\text{CaN}] = [\text{CamKII}] = [\text{CaM}]/10 ;$$
$$Kd_{\text{CaMKII}} = 10 \times Kd_{\text{CaN}} ;$$

- 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 $\neq$ induced-fit



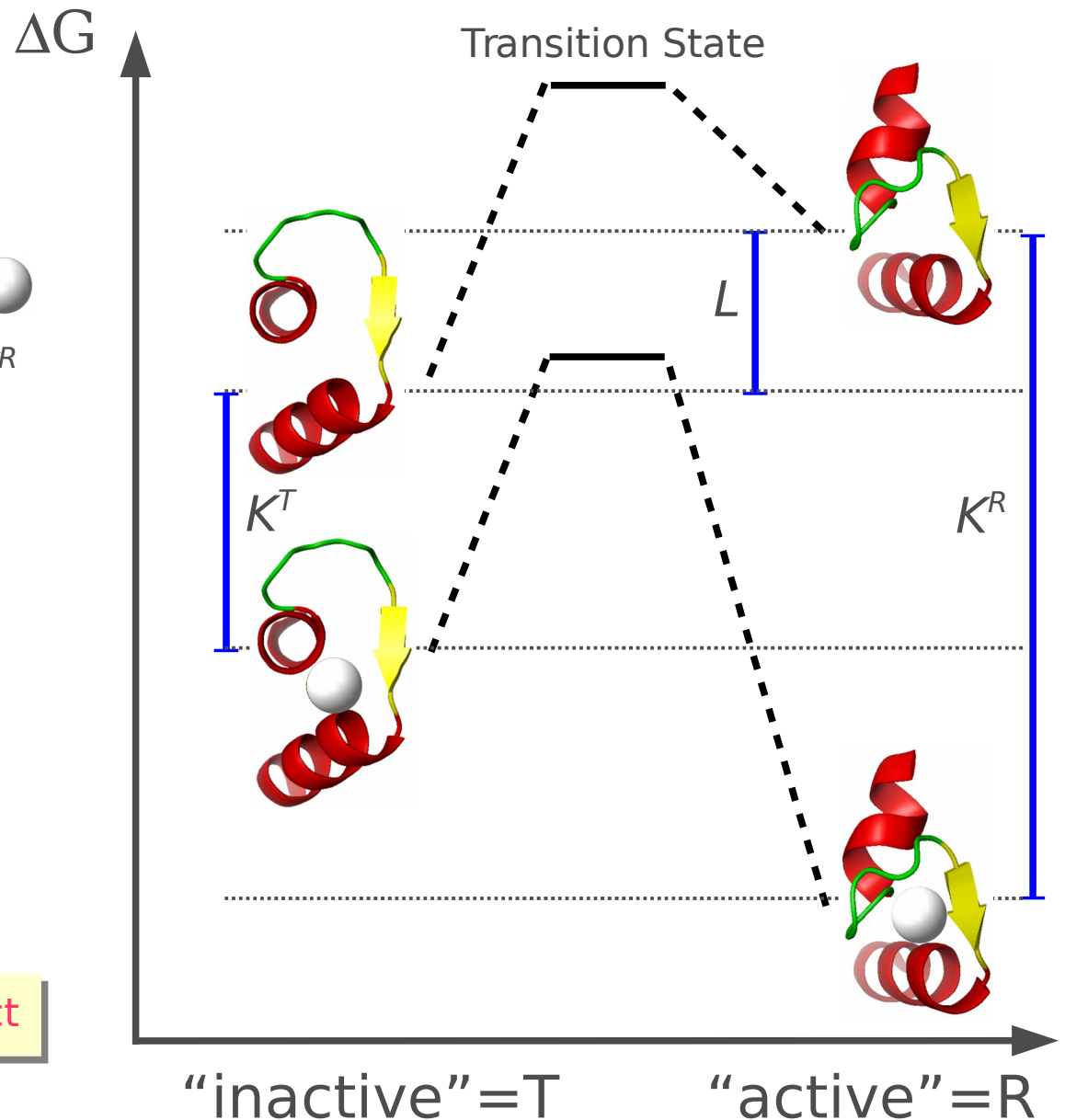
① Modulation of thermal equilibria $\neq$ induced-fit



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

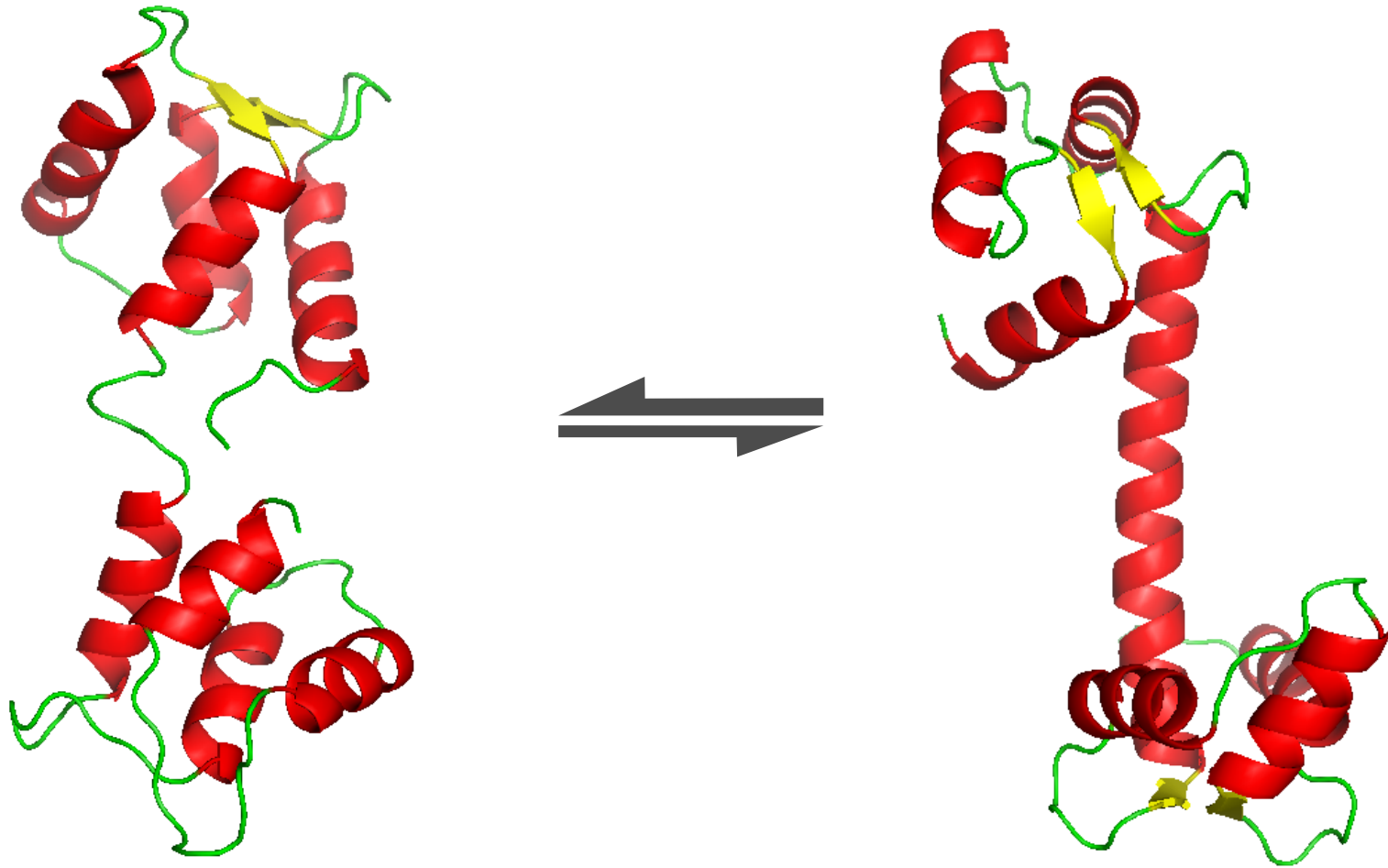
$$c = \frac{K^R}{K^T}$$

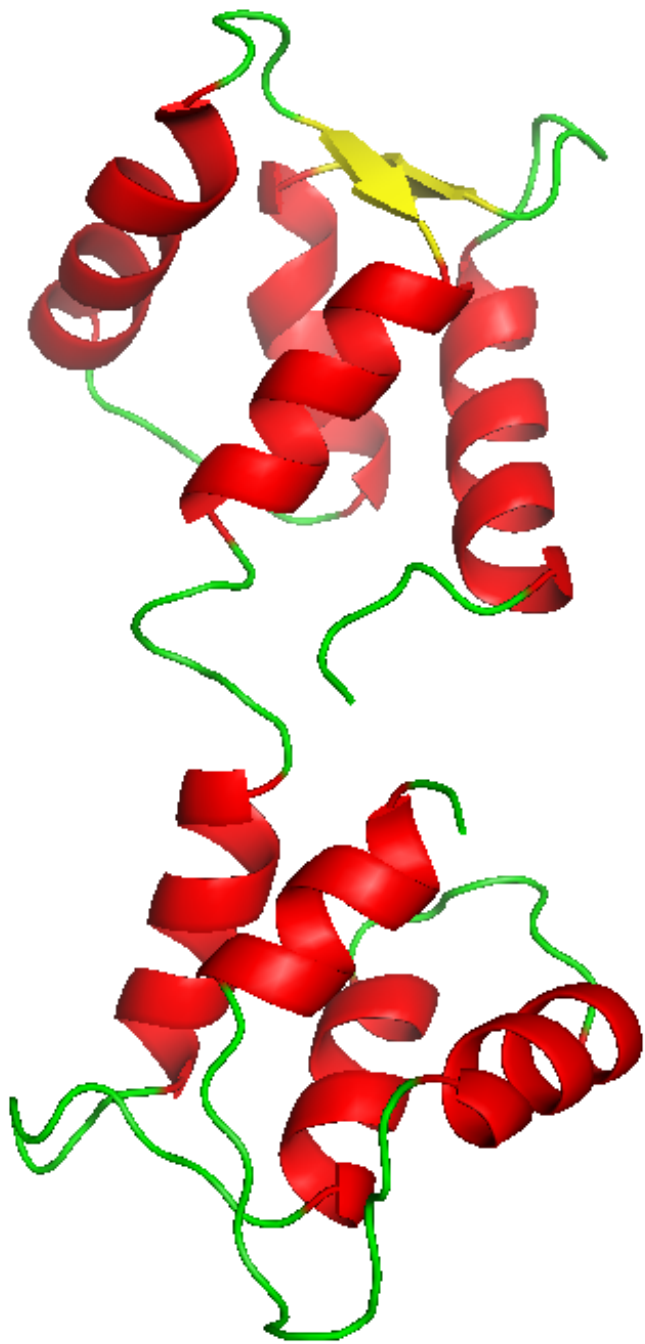
$c \ll 1 \Rightarrow$ ligand has strong effect



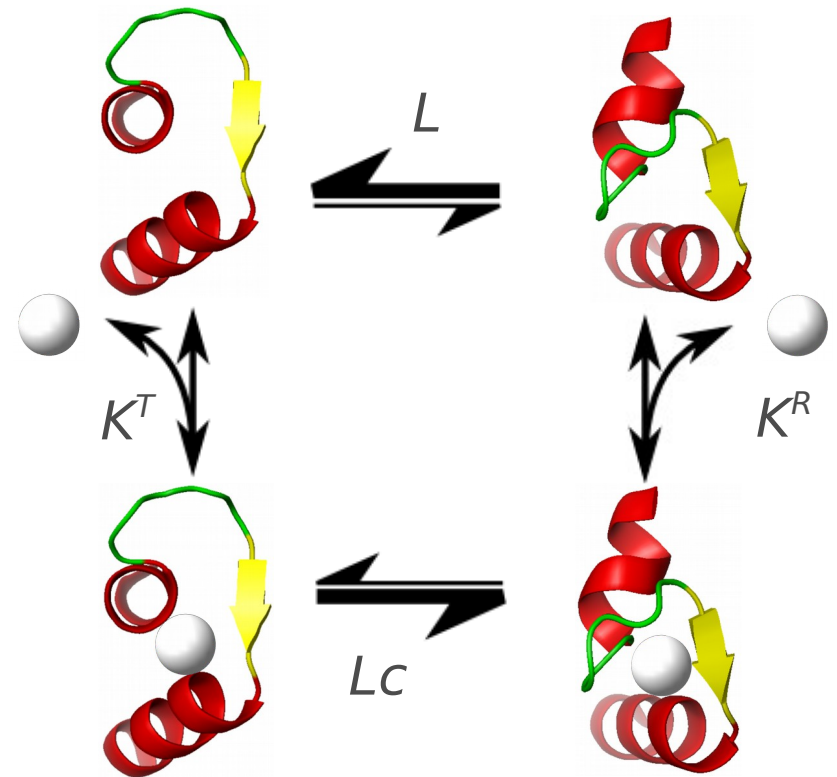
2

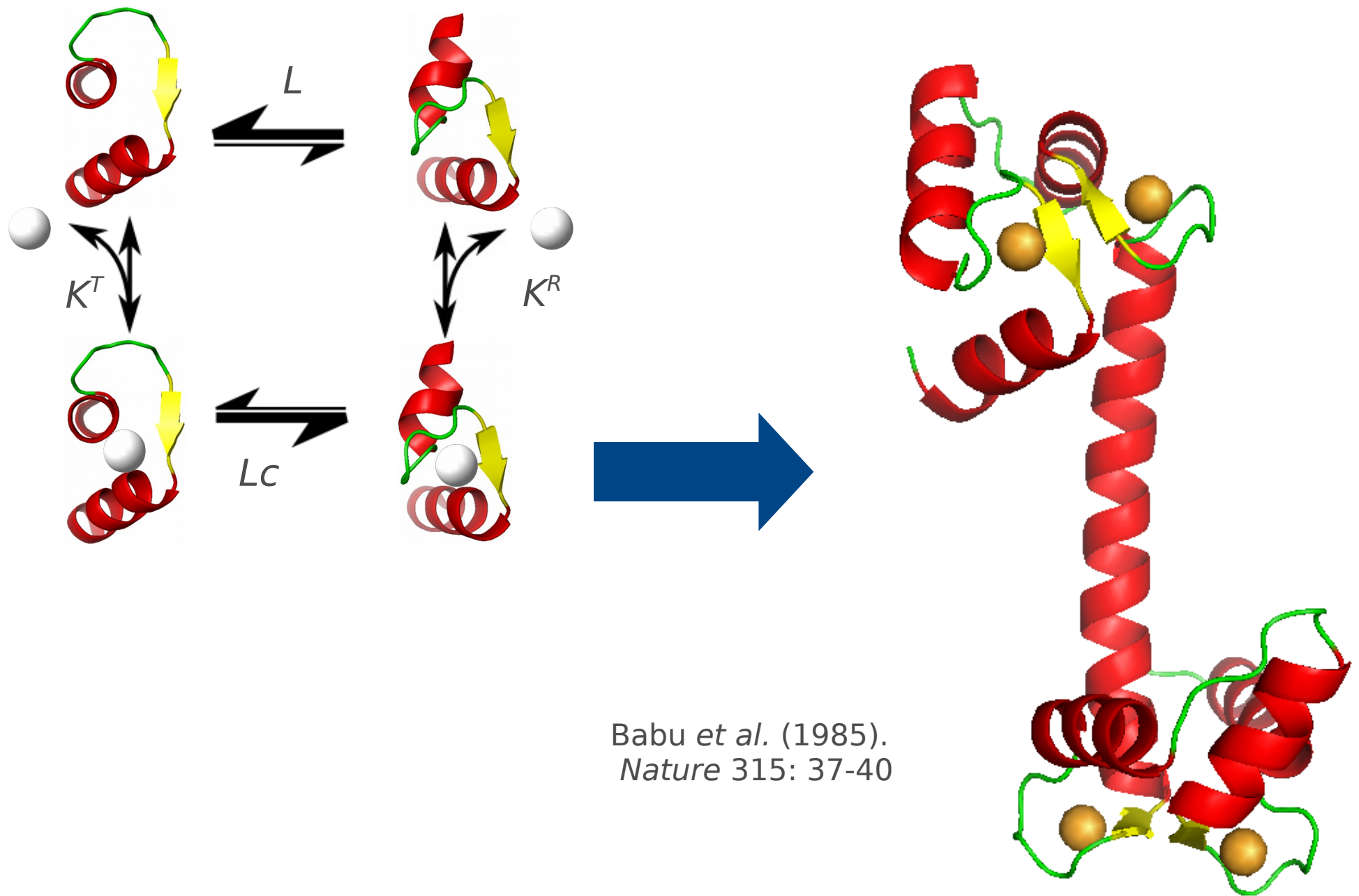
Concerted transitions $\neq$ sequential model

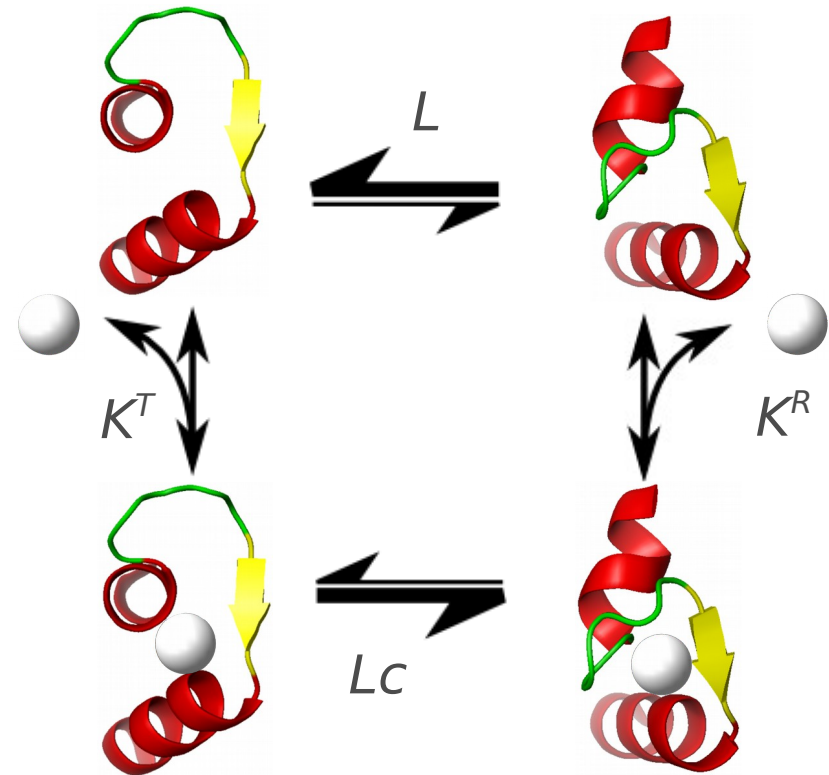
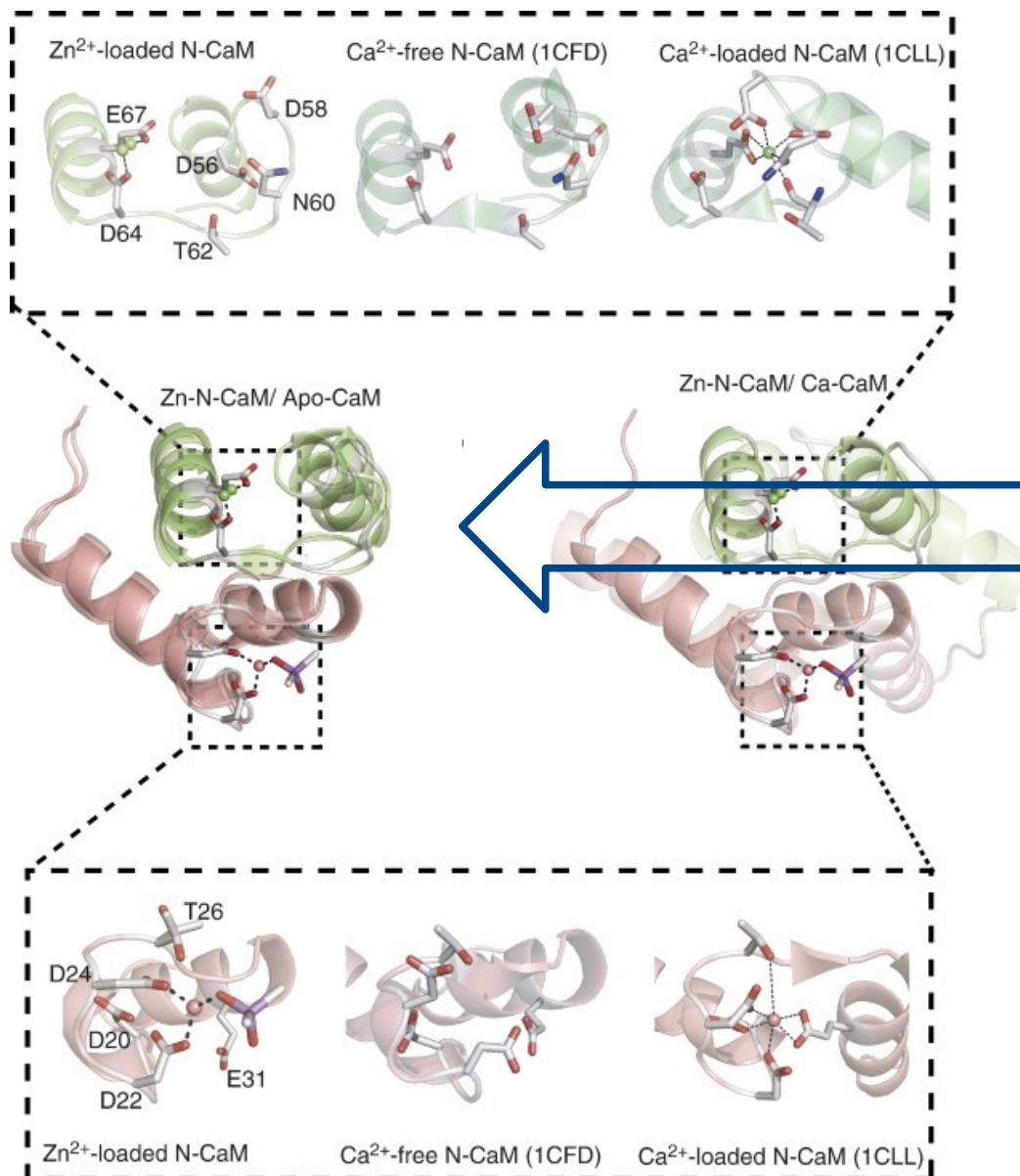




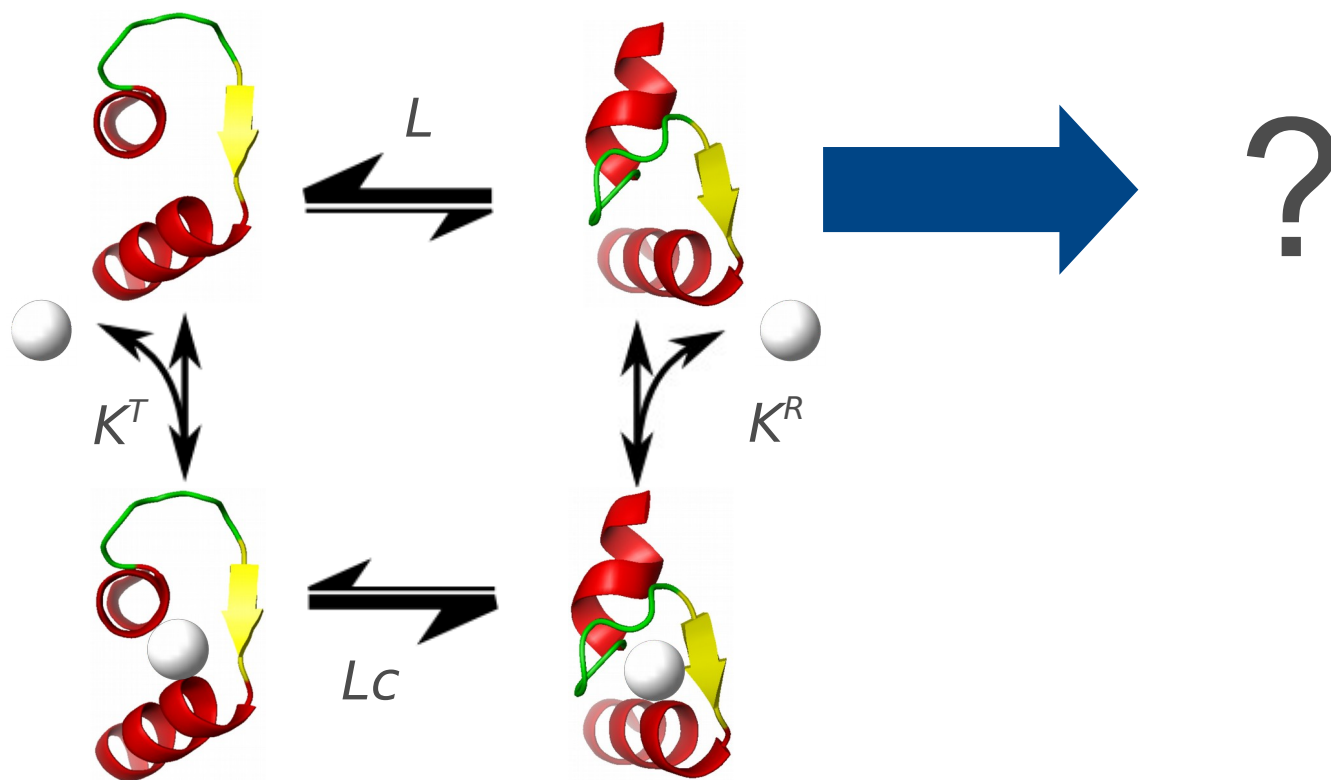
Kuboniwa *et al.* (1995).
Nat Struct Biol 2: 768-776

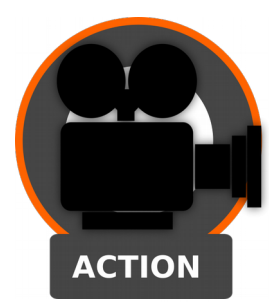




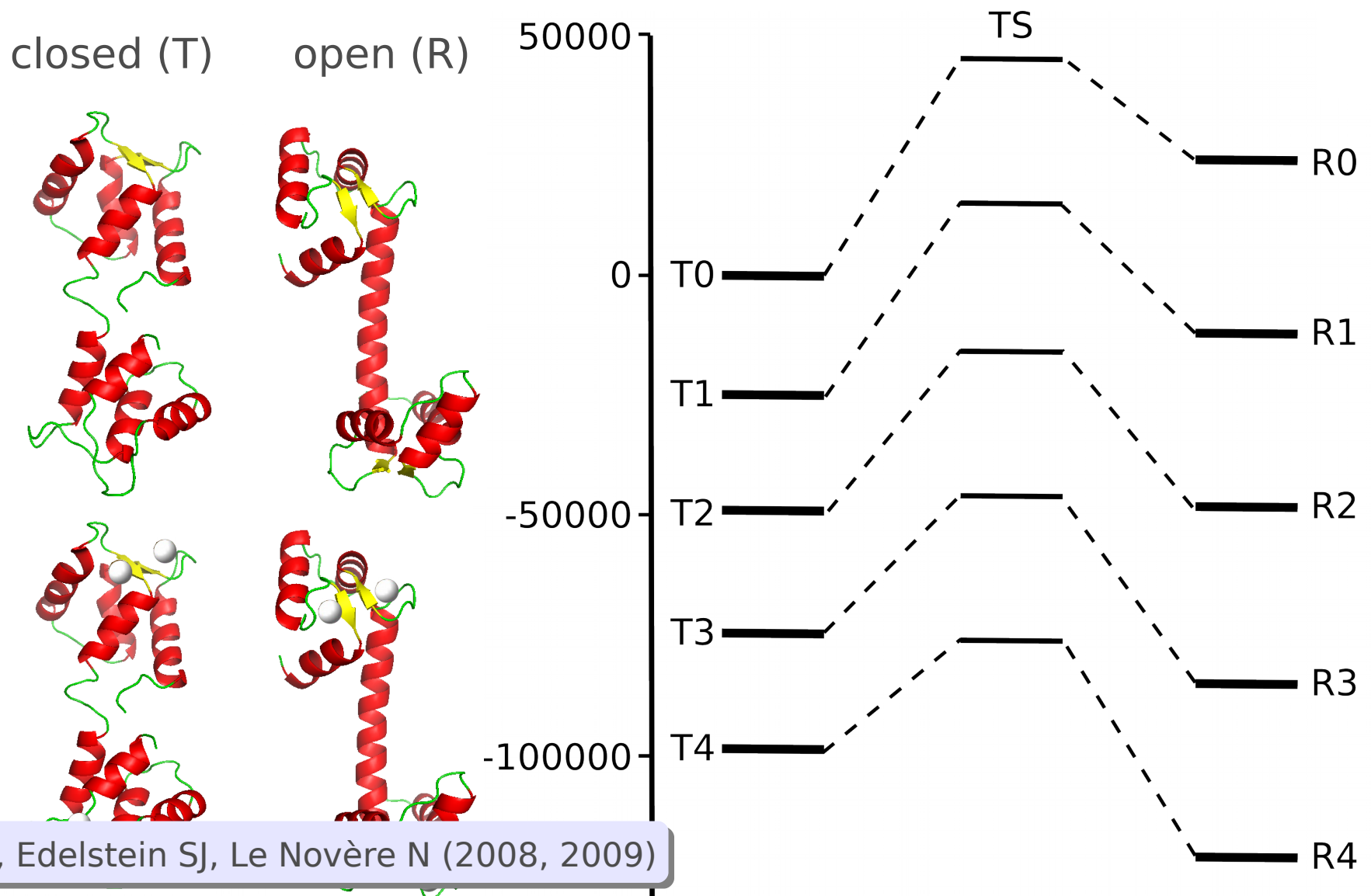


Warren *et al.* (2007).
J Mol Biol 374: 517-527





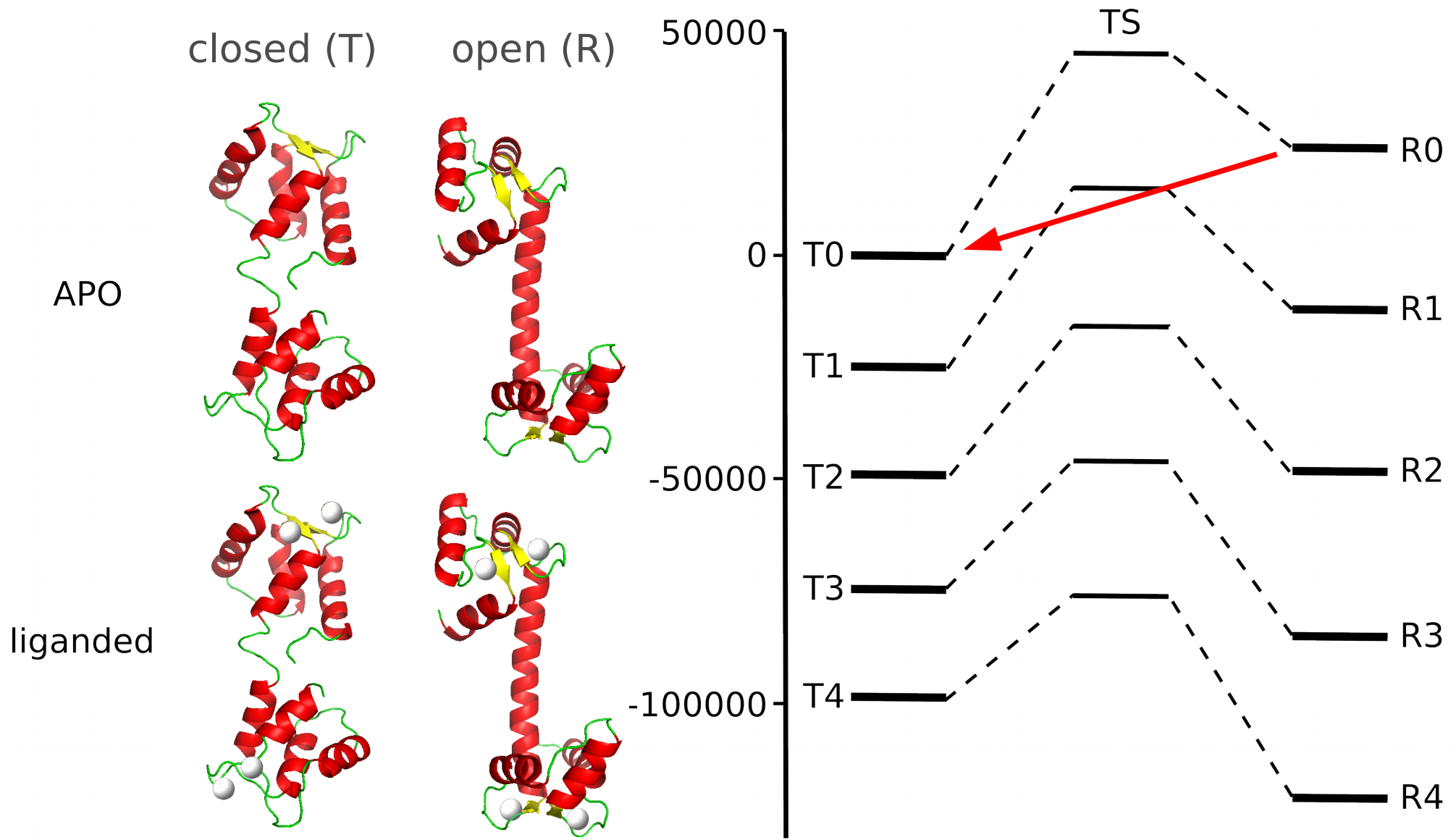
Allosteric model of Calmodulin activation



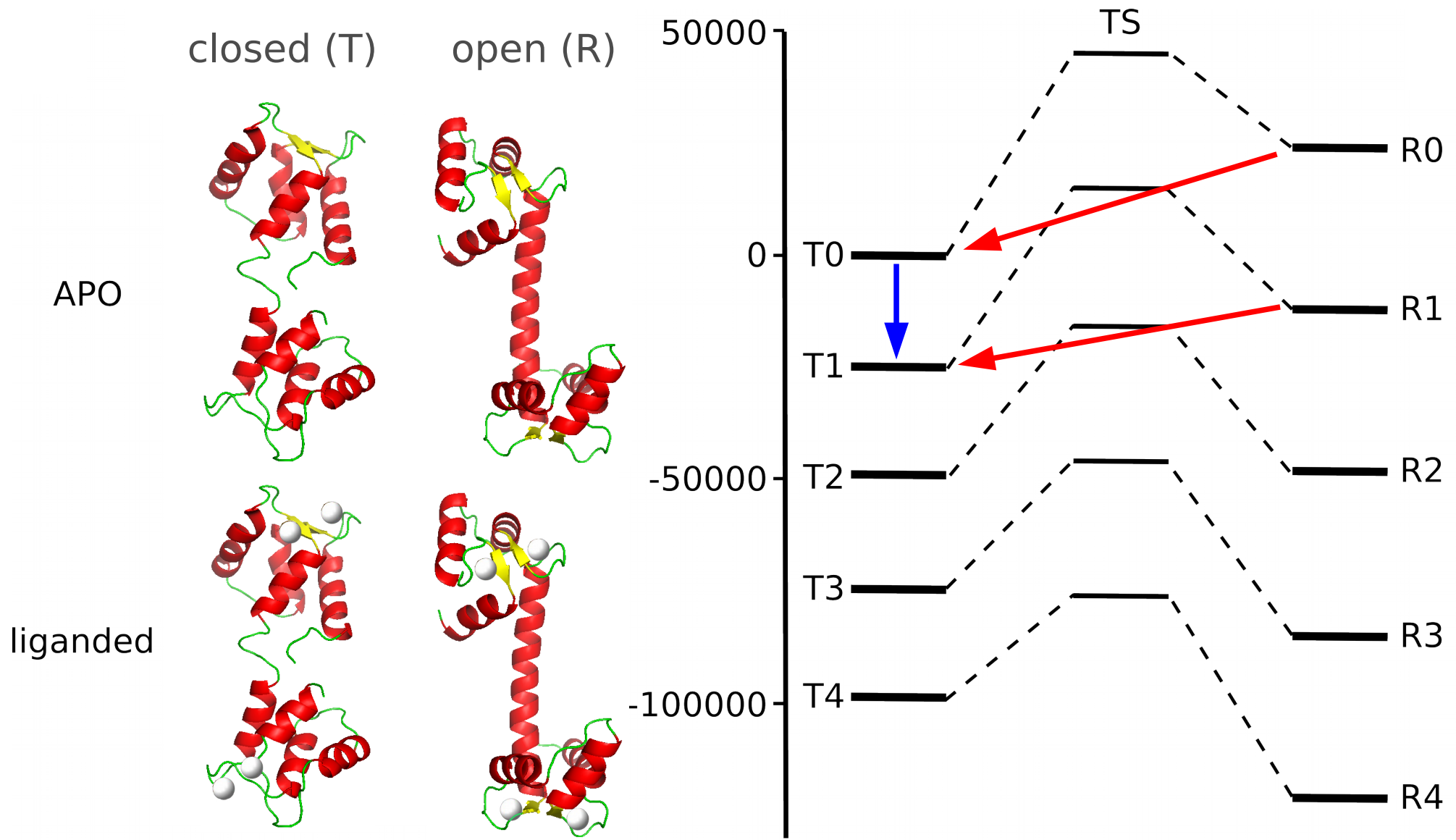
Stefan MI, Edelstein SJ, Le Novère N (2008, 2009)

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

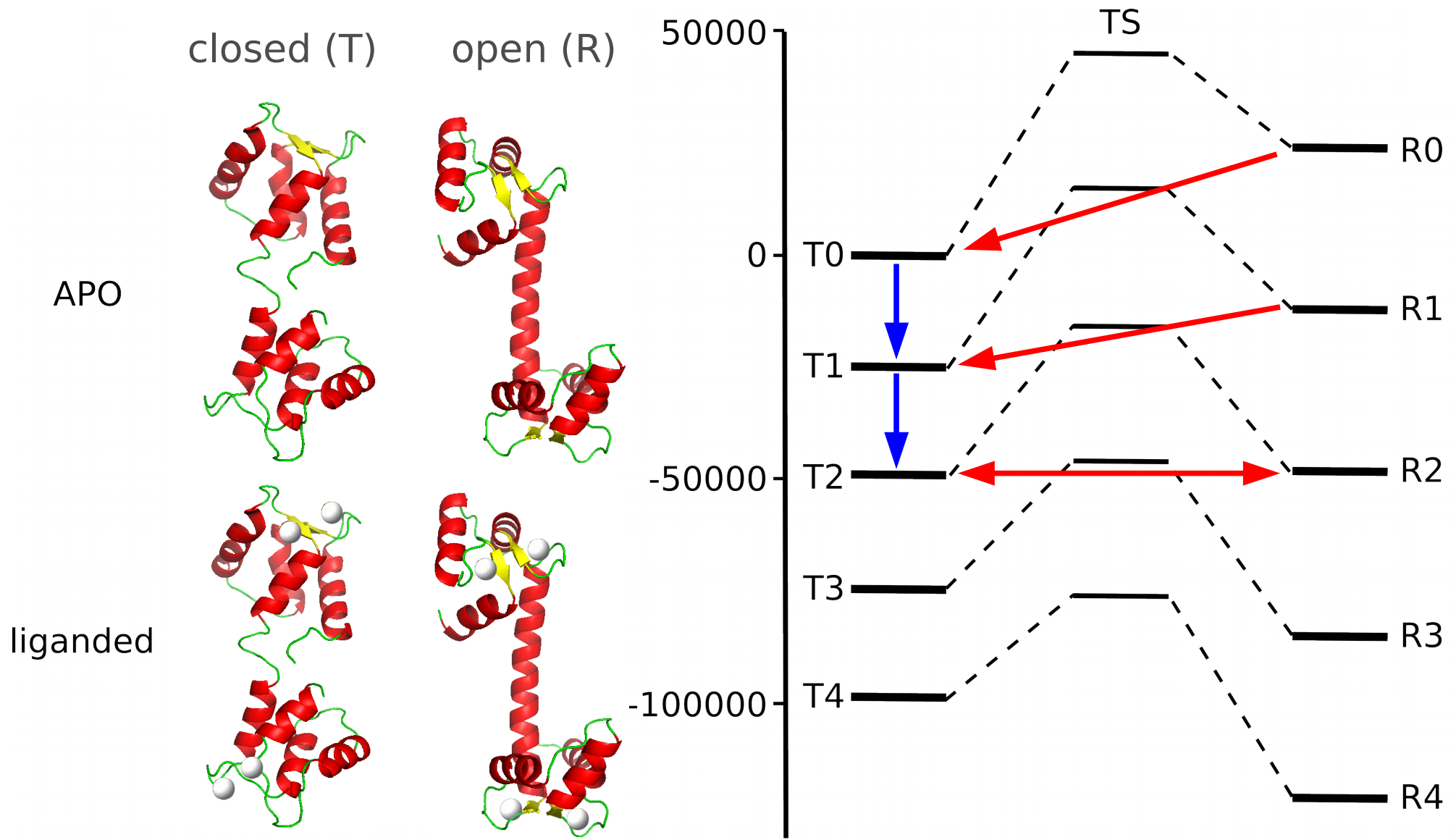
Allosteric model of Calmodulin activation



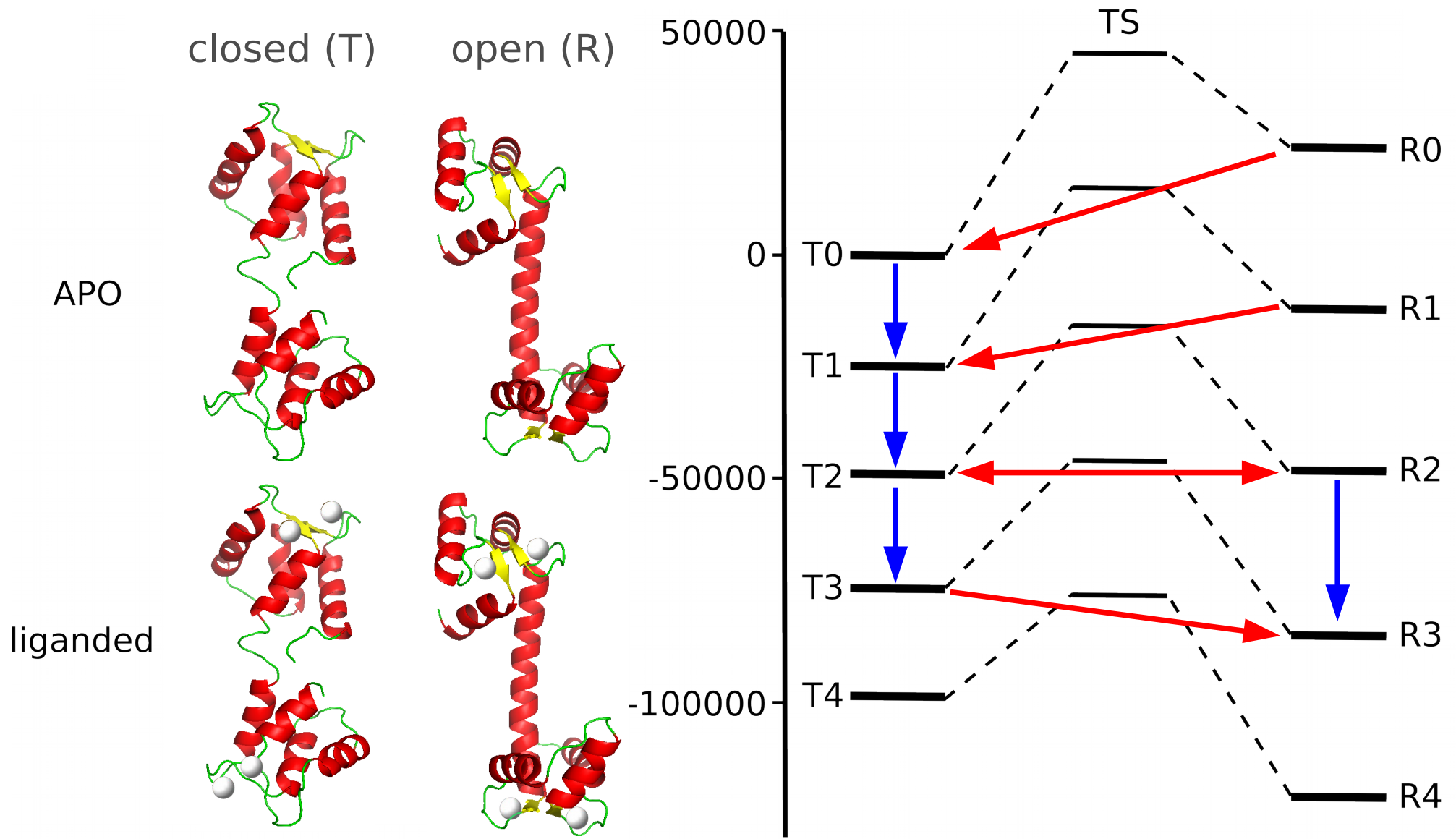
Allosteric model of Calmodulin activation



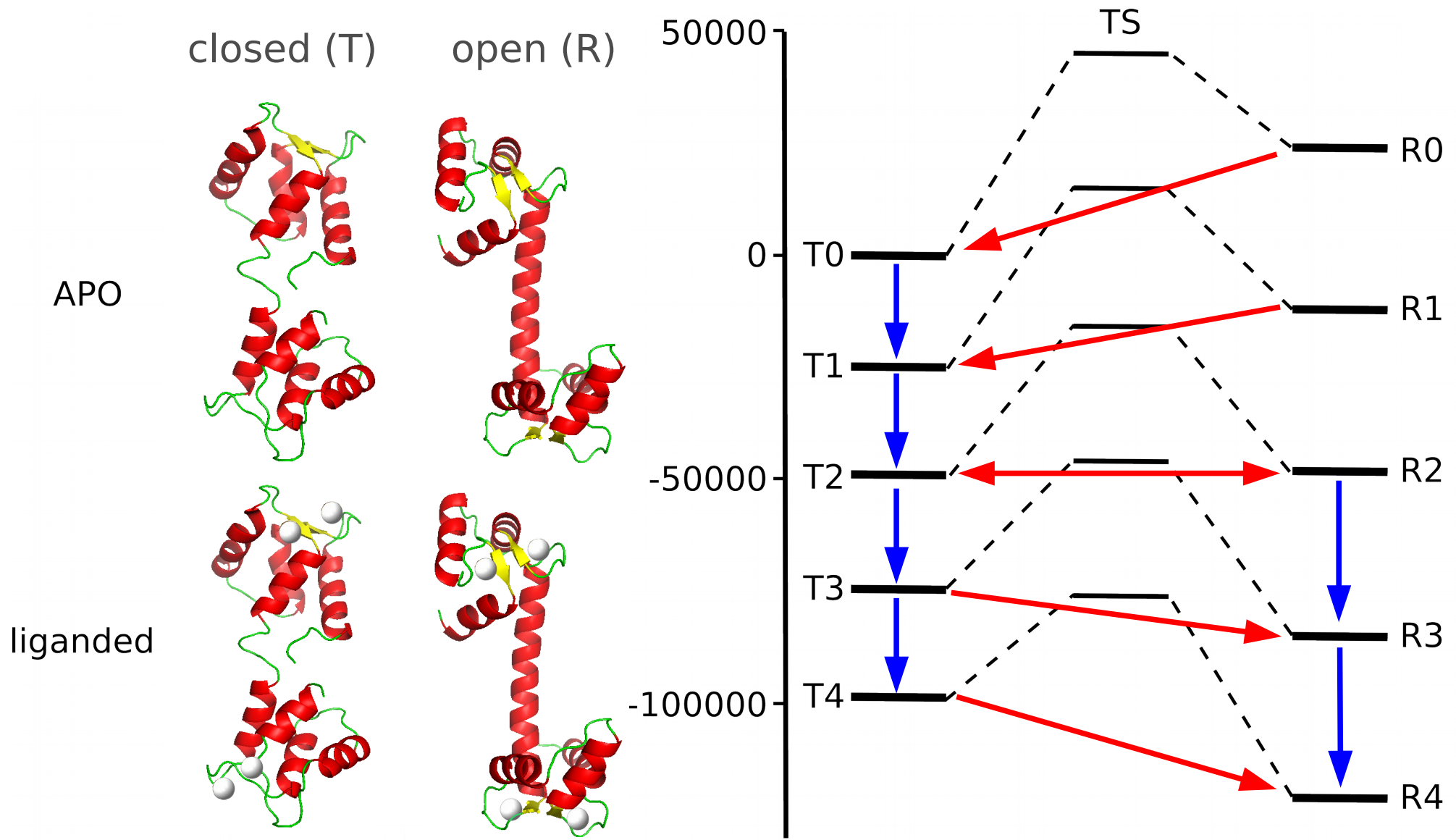
Allosteric model of Calmodulin activation



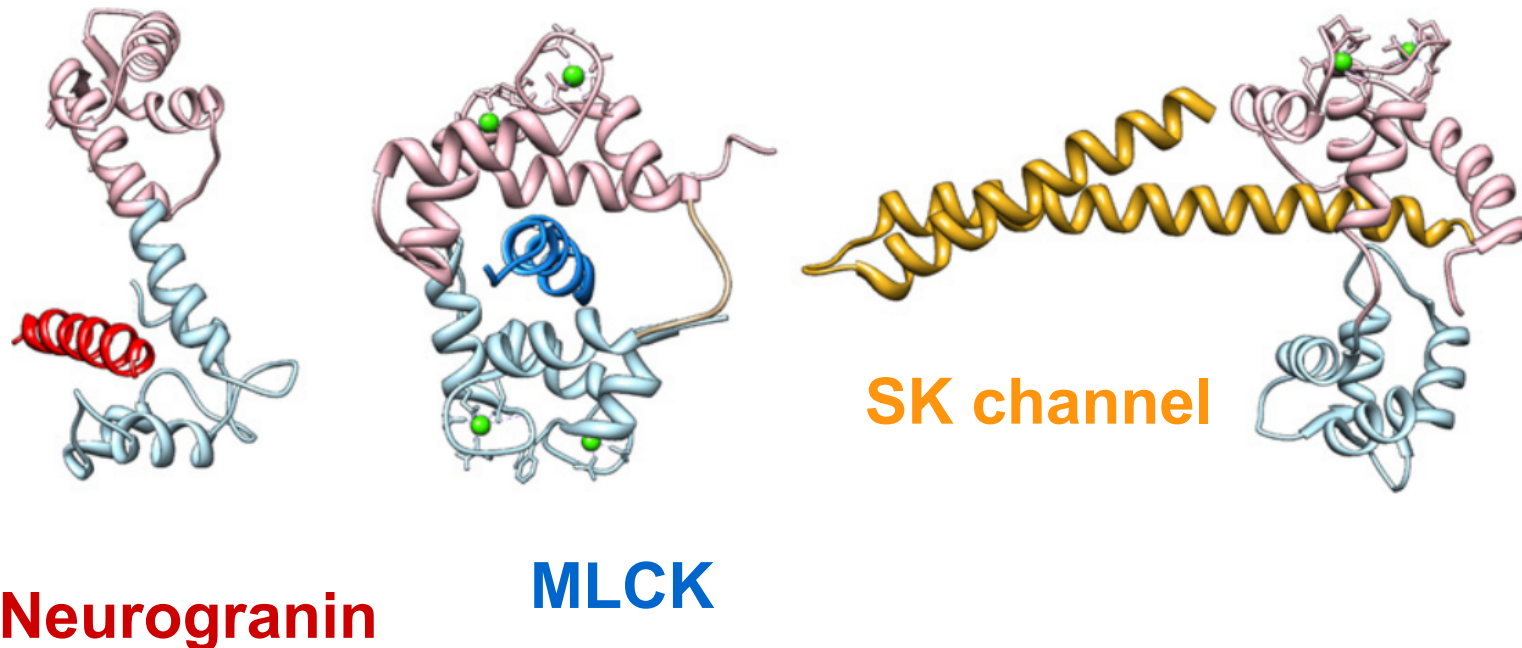
Allosteric model of Calmodulin activation



Allosteric model of Calmodulin activation



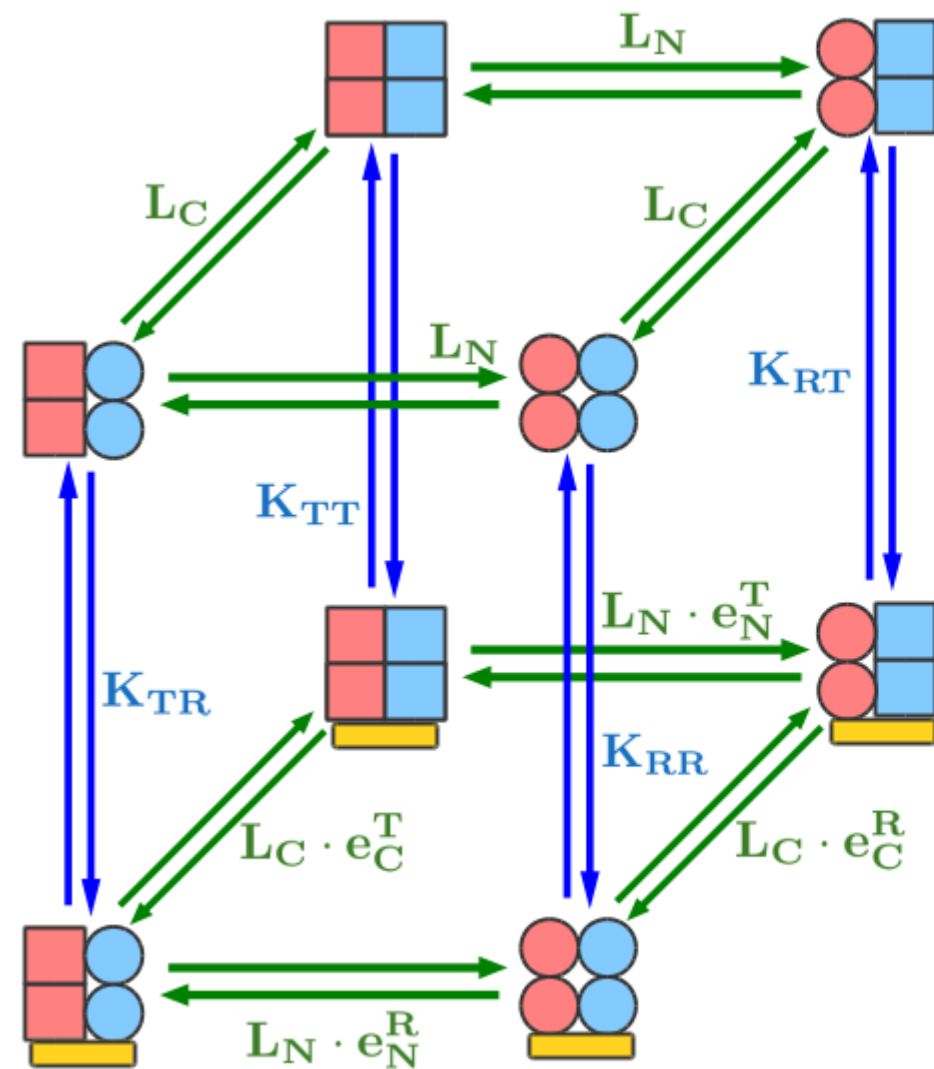
Different targets stabilise lobes in different states



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



= N-lobe

= C-lobe



= T-state



= R-state



= target



= TT conformation



= RT conformation



= TR conformation



= RR conformation

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

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

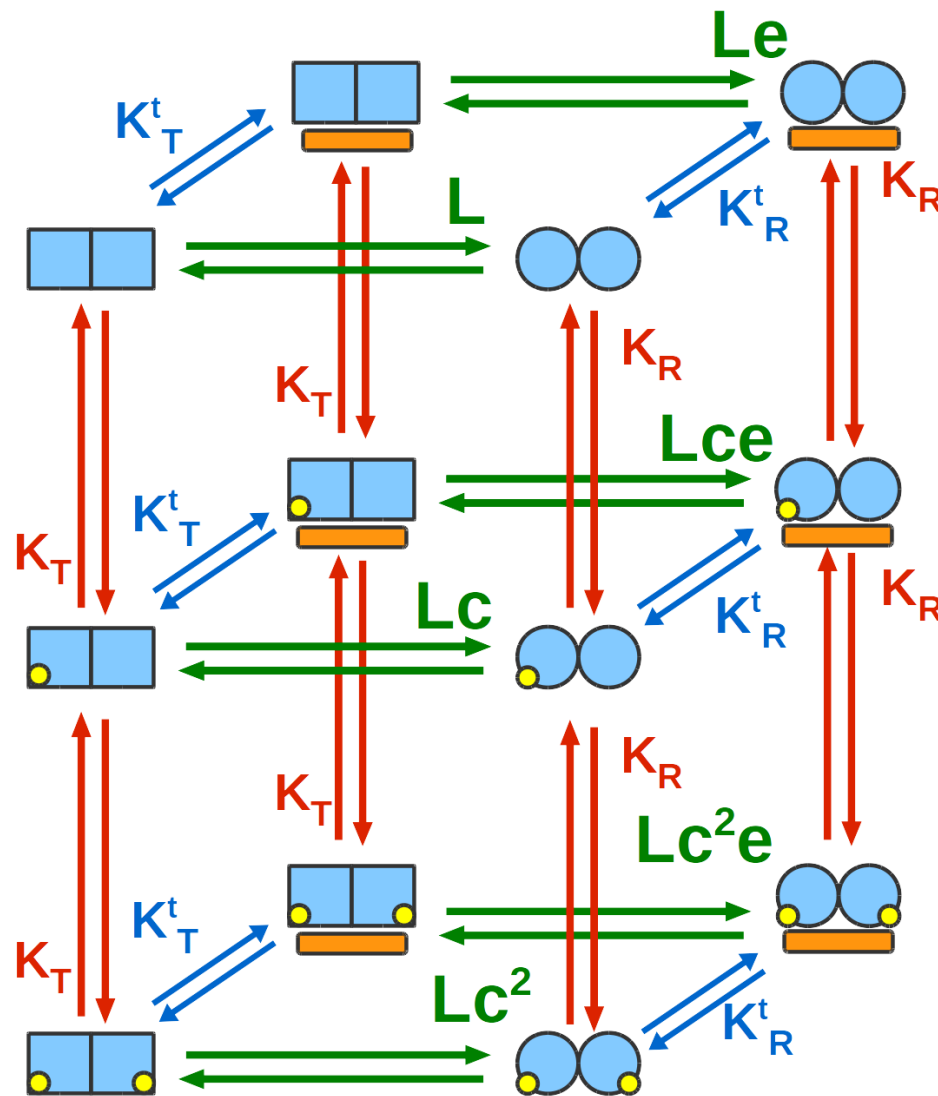
$$e_N^R = \frac{K_{RR}}{K_{TR}}$$

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

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

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

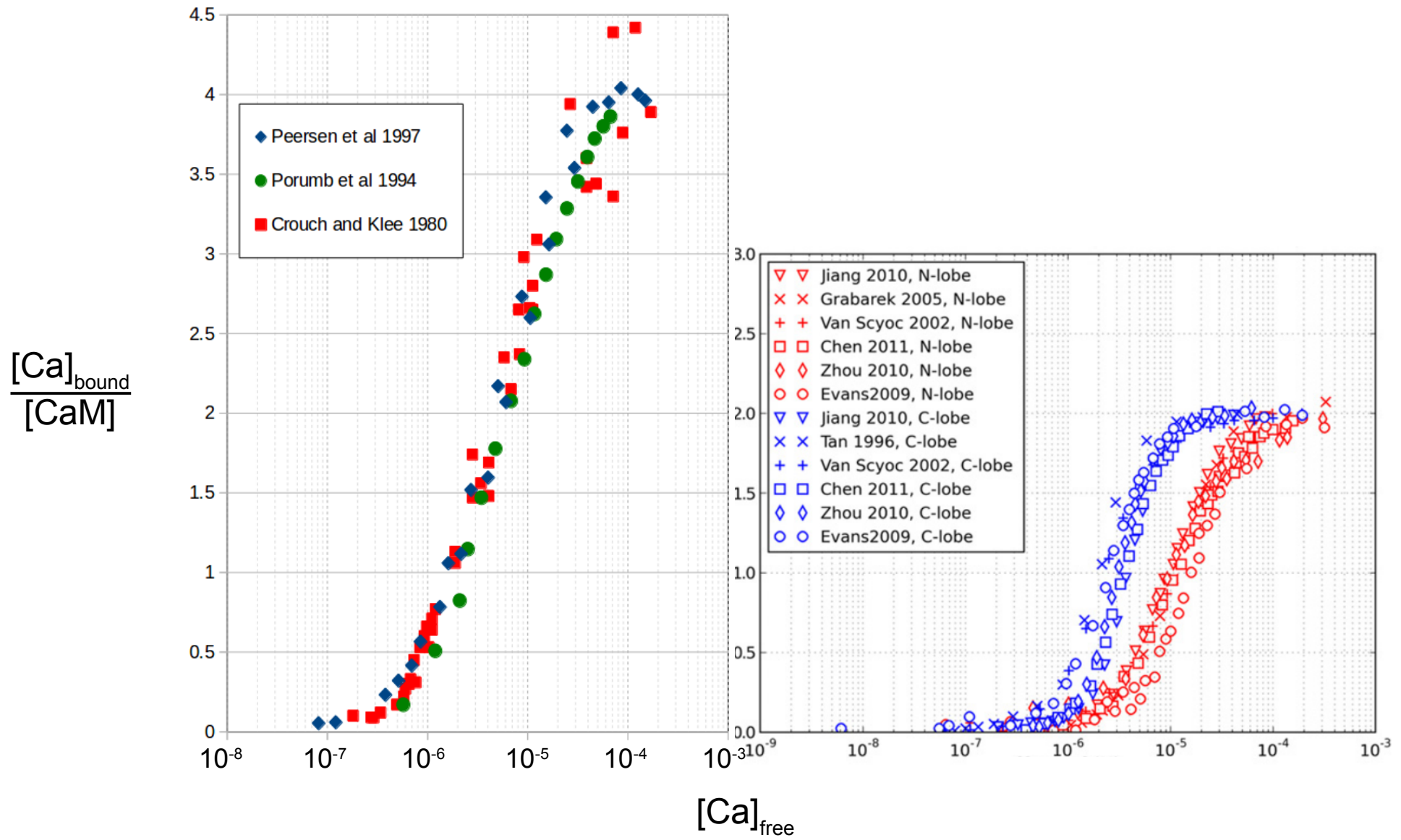
Bindings of calcium and targets



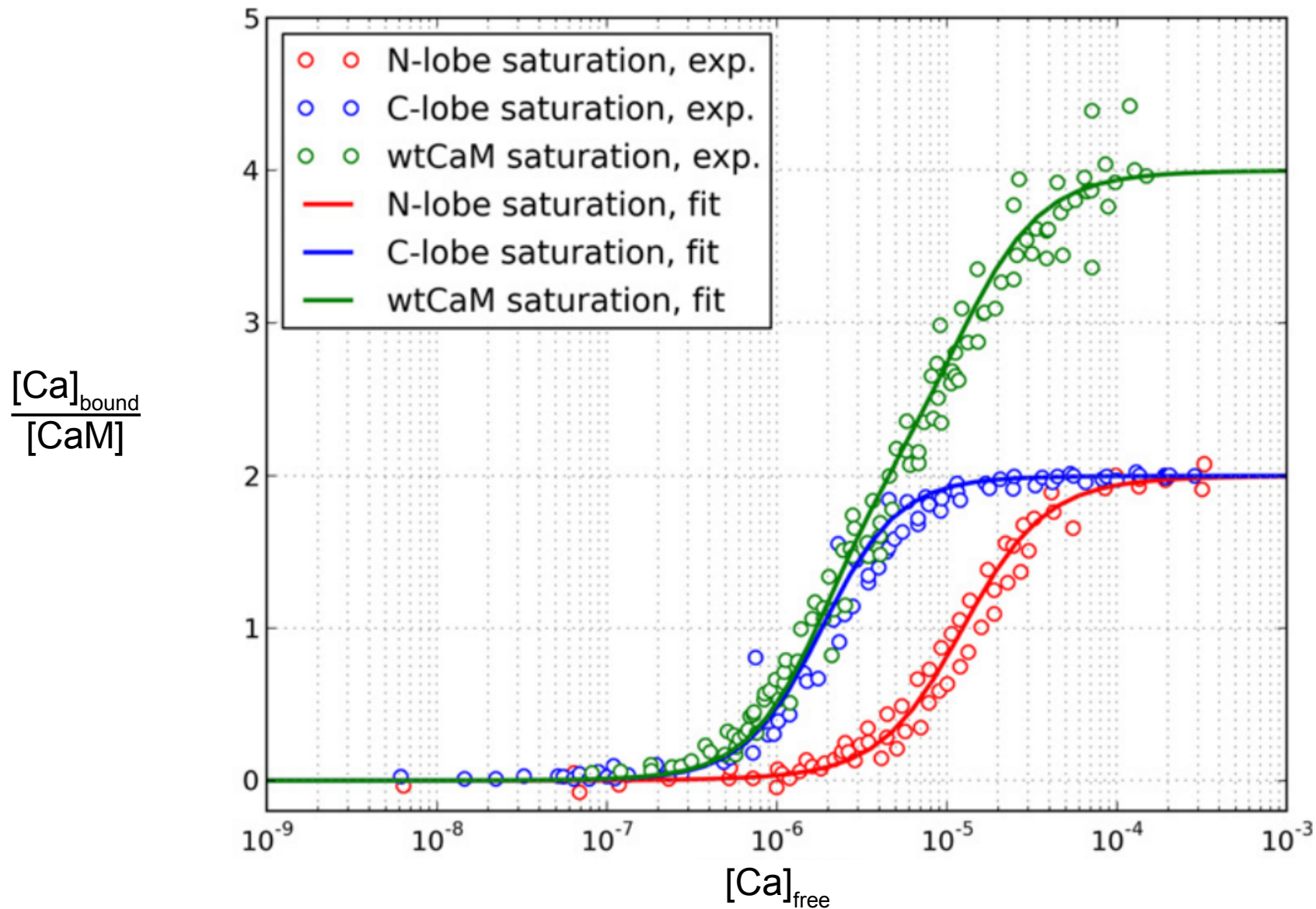
$$c = \frac{K_R}{K_T}$$

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

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+}

$$L=20670$$

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

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

$$K_A^R = 8.32 \cdot 10^{-6}$$

$$K_B^R = 1.66 \cdot 10^{-8}$$

$$K_C^R = 1.74 \cdot 10^{-5}$$

$$K_D^R = 1.45 \cdot 10^{-8}$$

2 high, 2 low, as expected

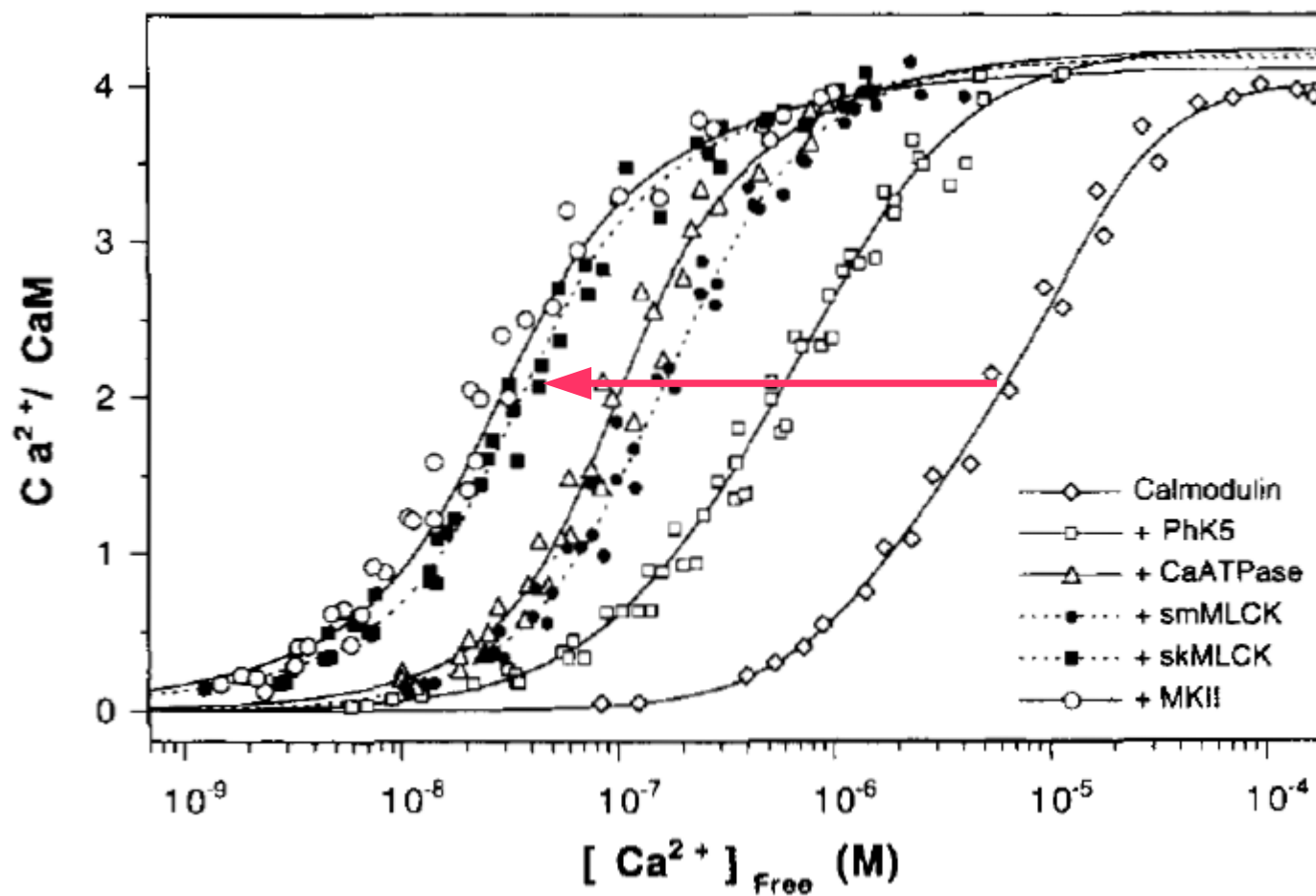
Activity of unsaturated calmodulin (state function)

- 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 $\approx$ equi-probability
- $R_4/T_4 = 10000$

Targets as allosteric effectors

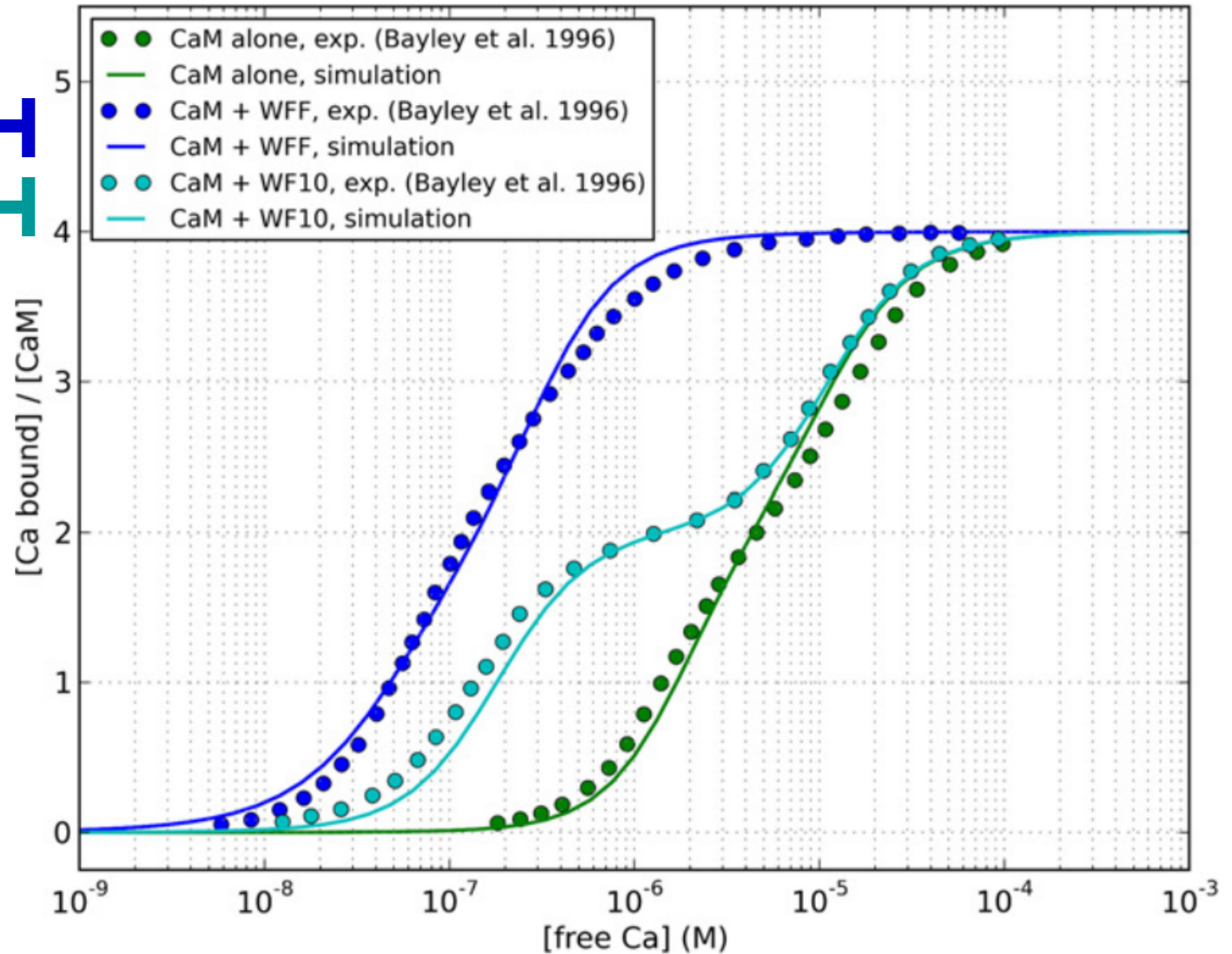


Peersen et al. (1997)

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

Stabilise R state
of both lobes

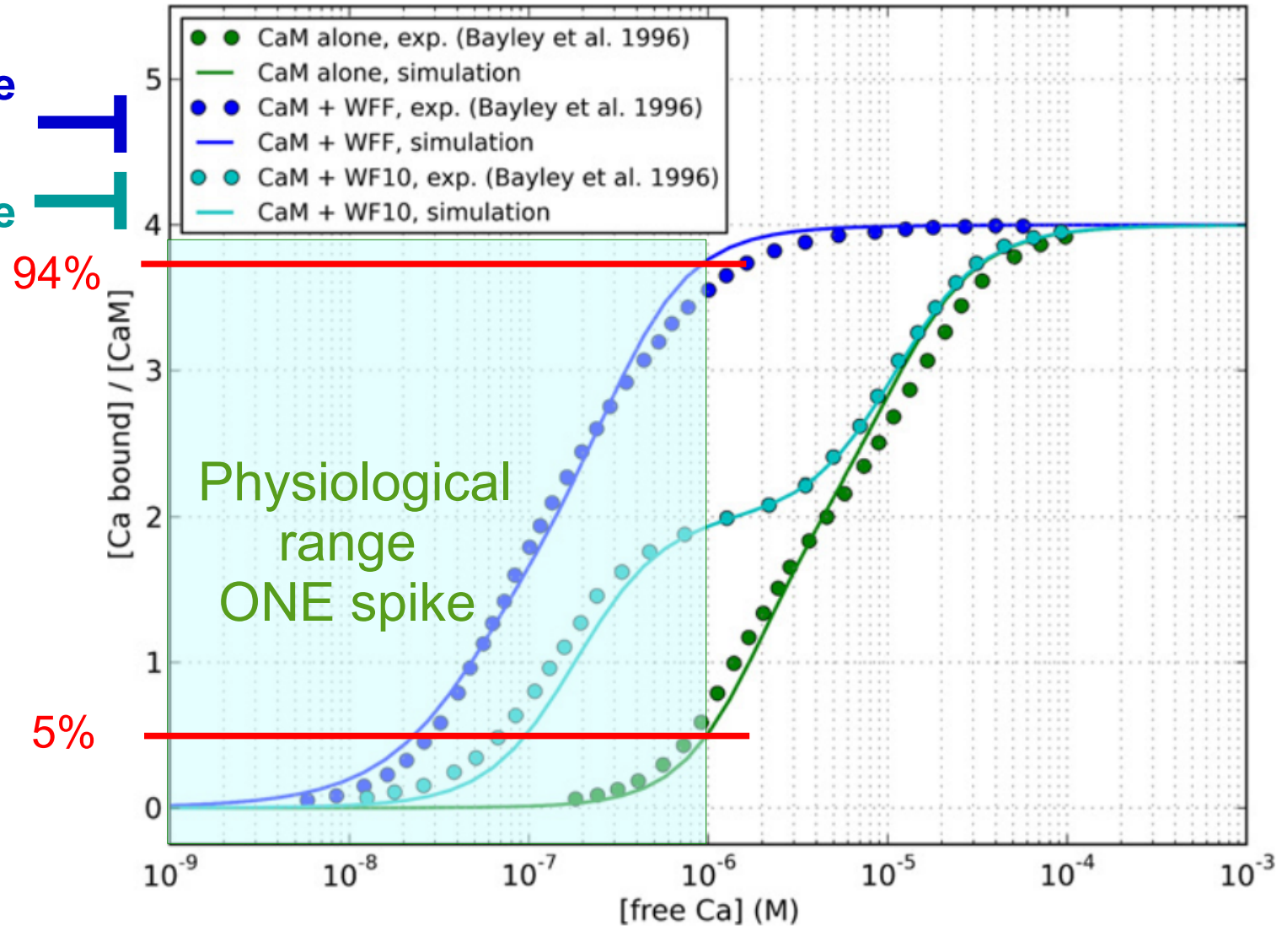
Stabilise R state
of C lobe



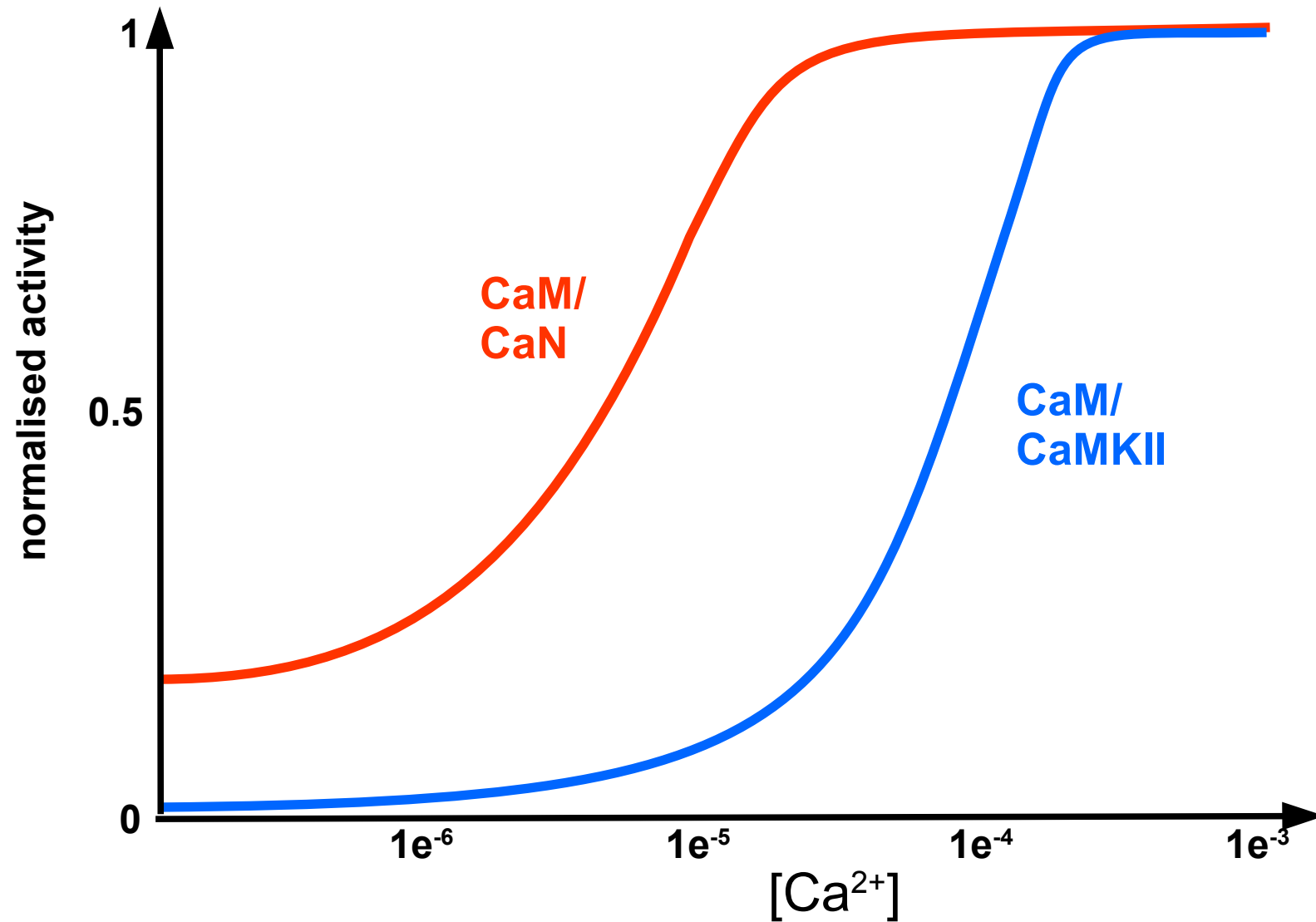
Targets stabilises Ca^{2+} binding into the physiological range

Stabilise R state of both lobes

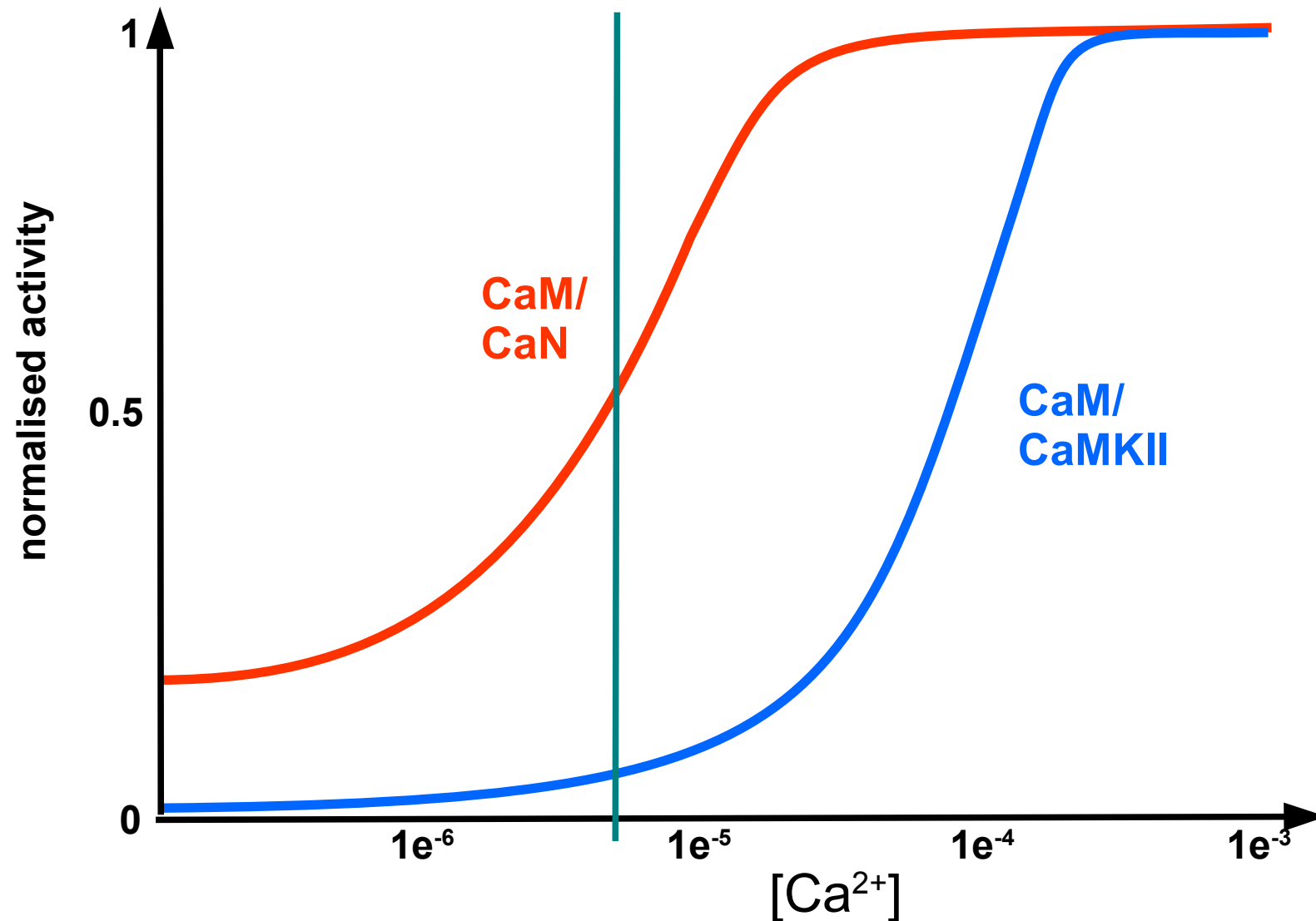
Stabilise R state of C lobe



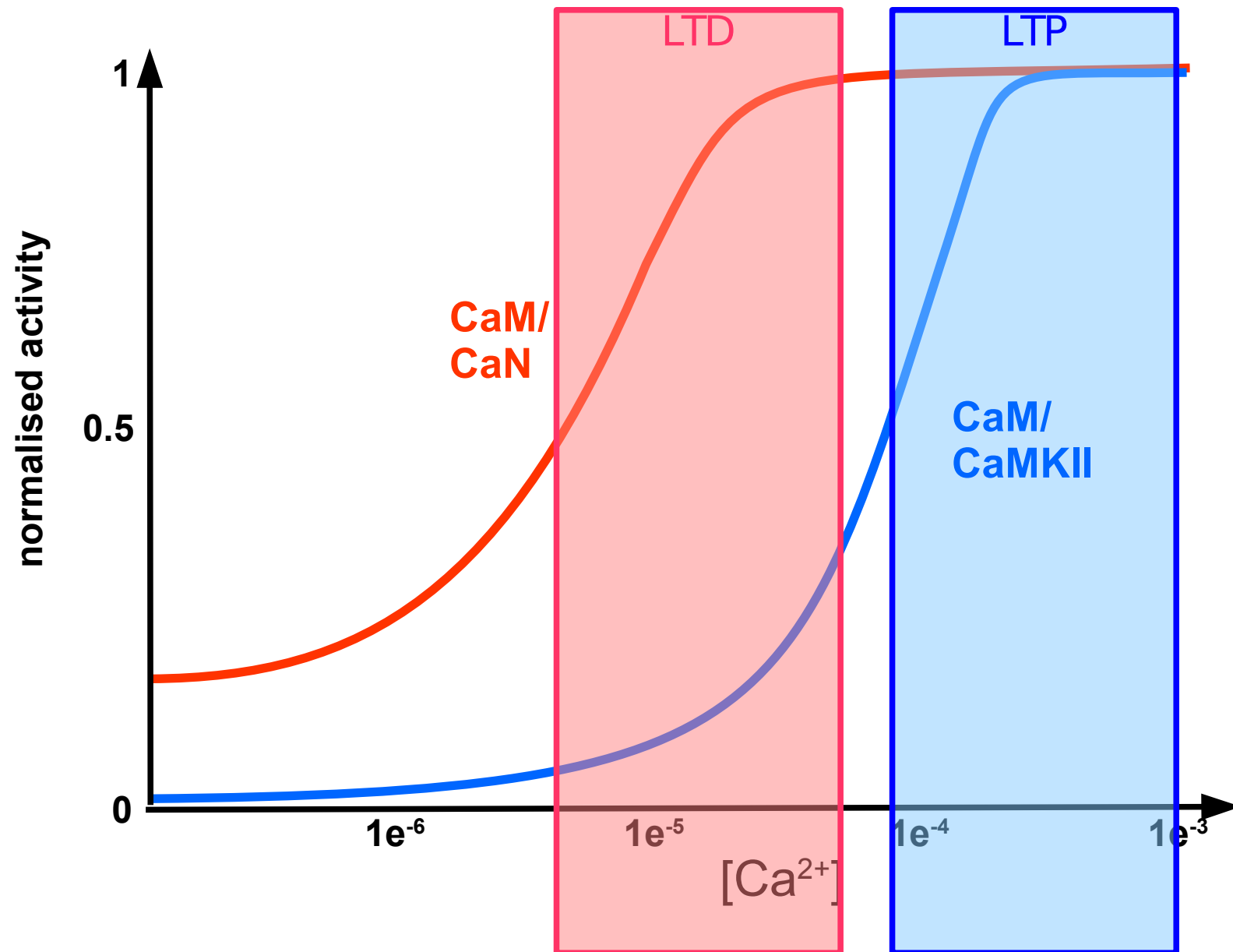
Calmodulin, its ligand and its targets



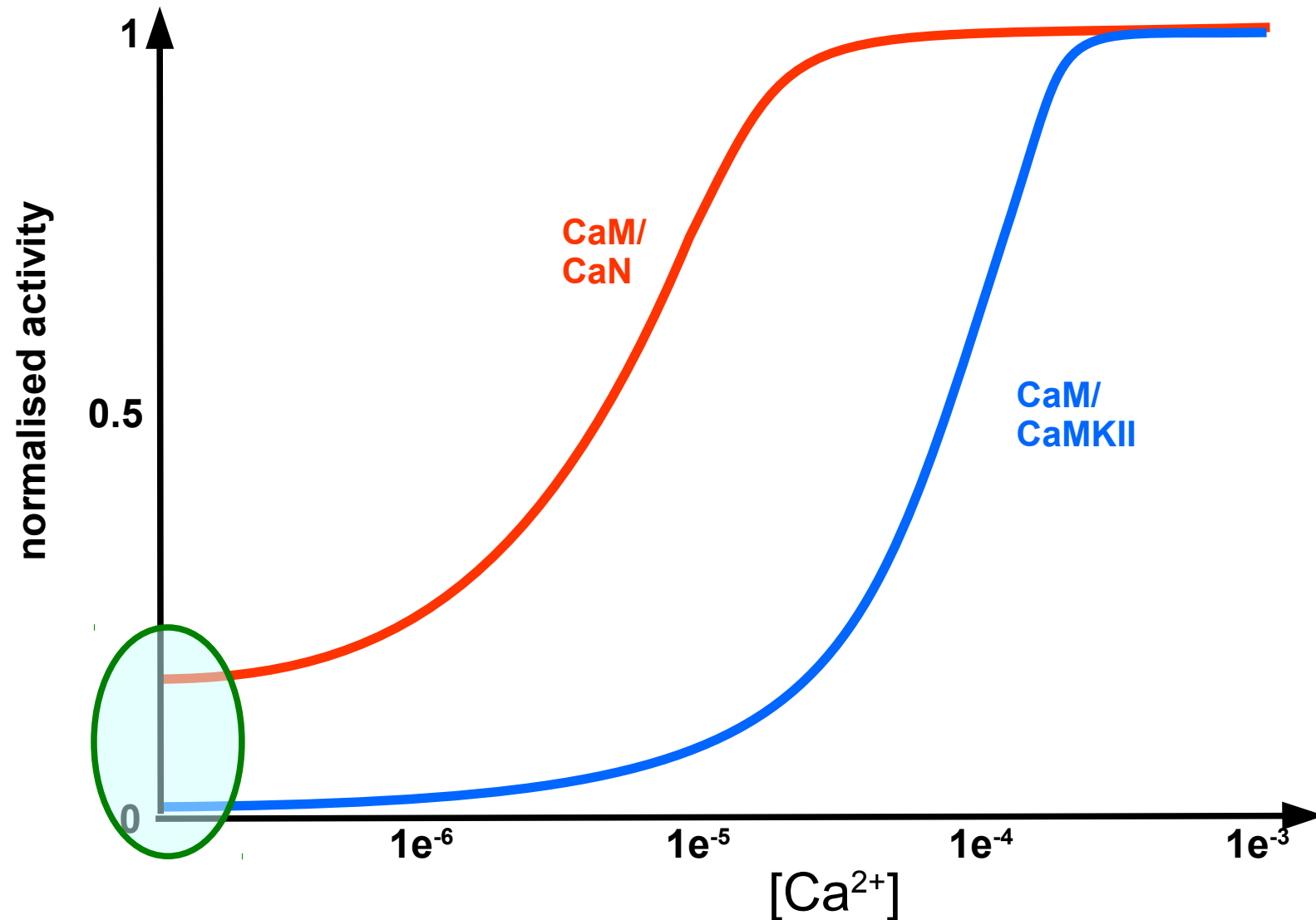
CaM half-activated at half-saturation of Calmodulin



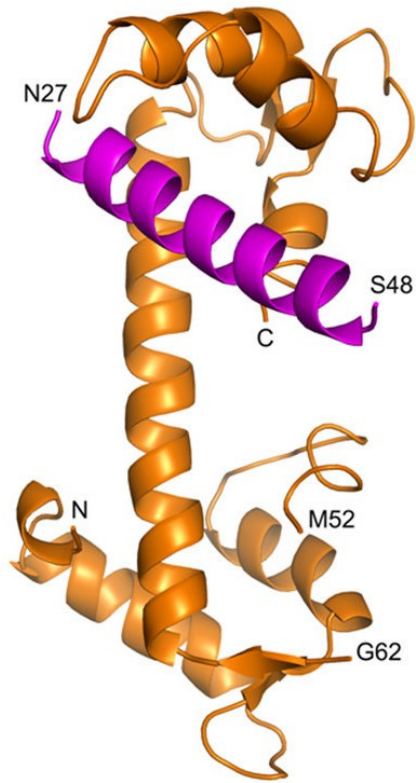
Bidirectional synaptic plasticity



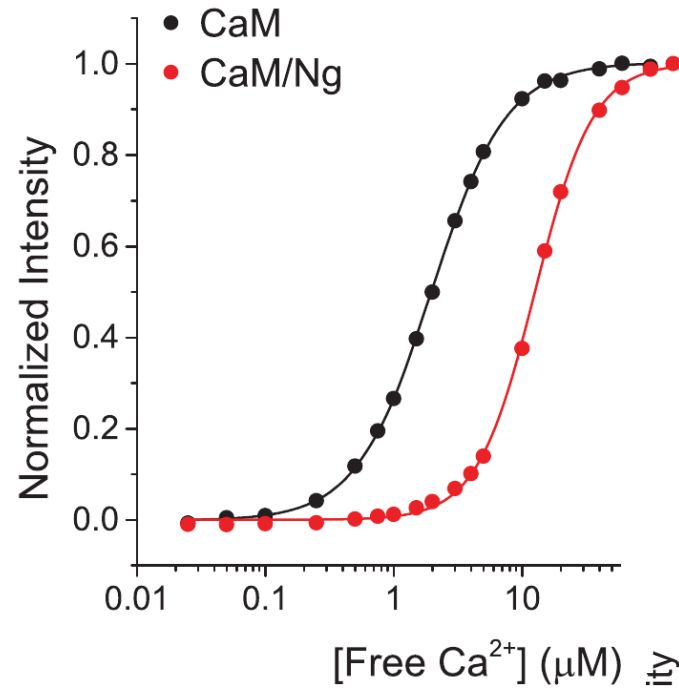
Calcineurin stabilises CaM R → no deactivation



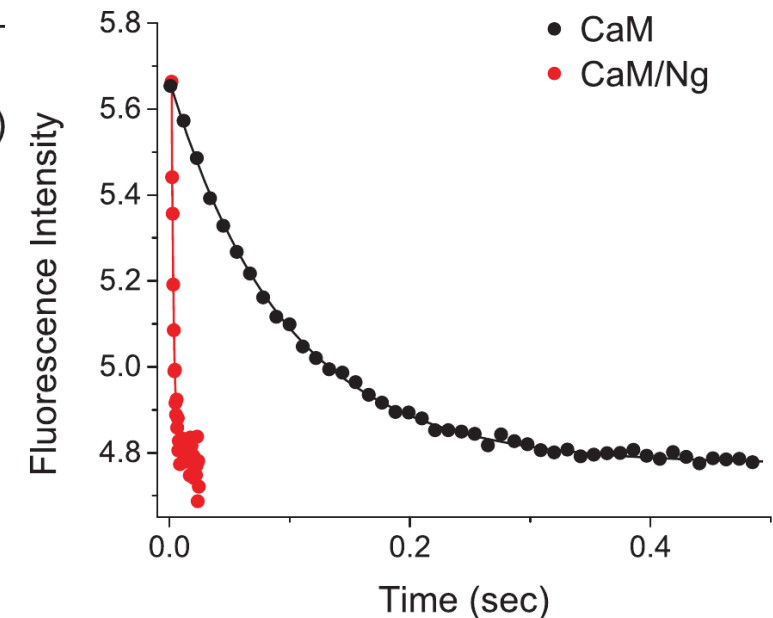
Neurogranin binds to apo-CaM, decrease affinity for $[Ca^{2+}]$ and increase dissociation rate



Kumar *et al* (2013)



Hoffman *et al* (2014)



Effect of R and T stabilising targets on CaM affinity

Bayley *et al* (1996)

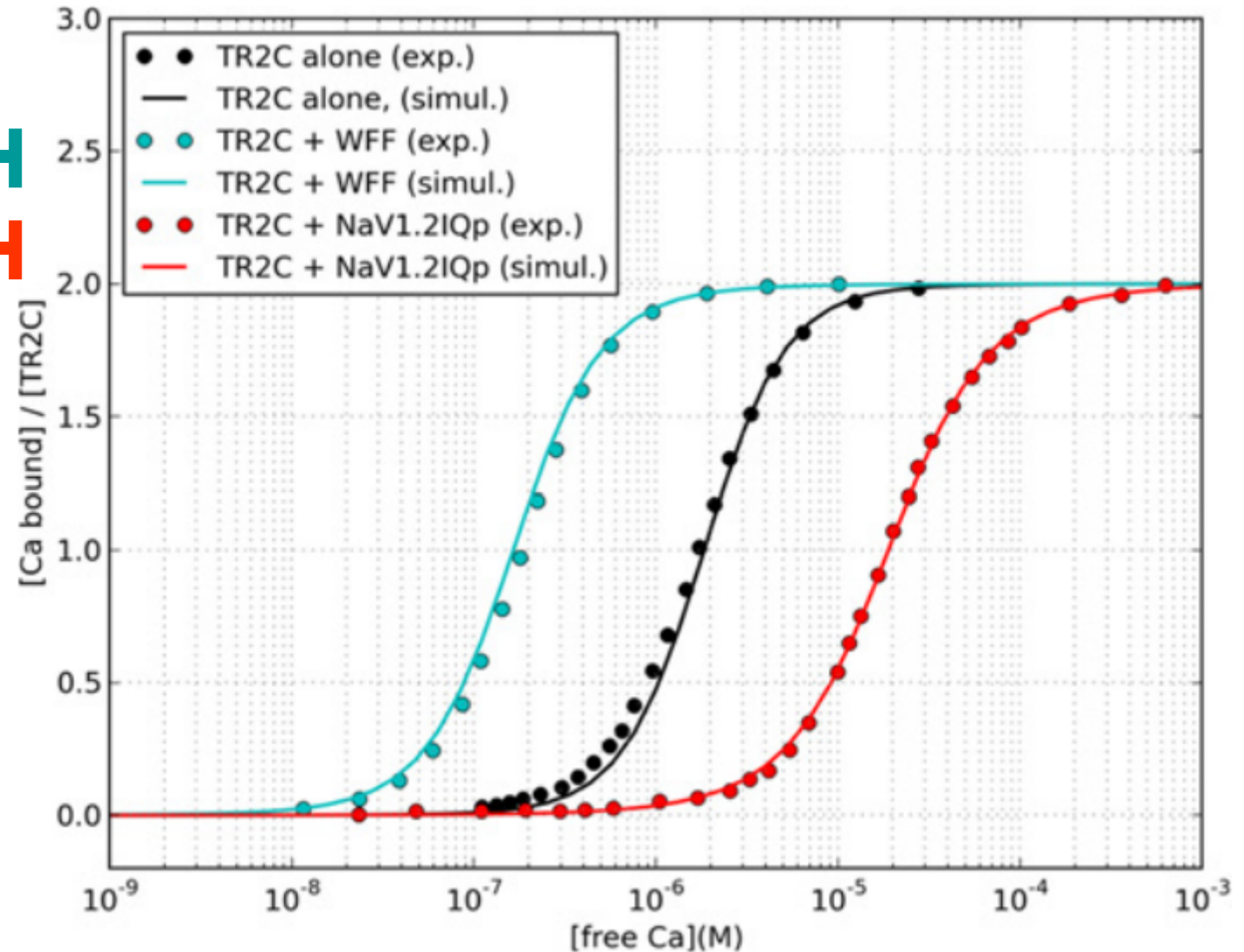
R stabilising
peptide

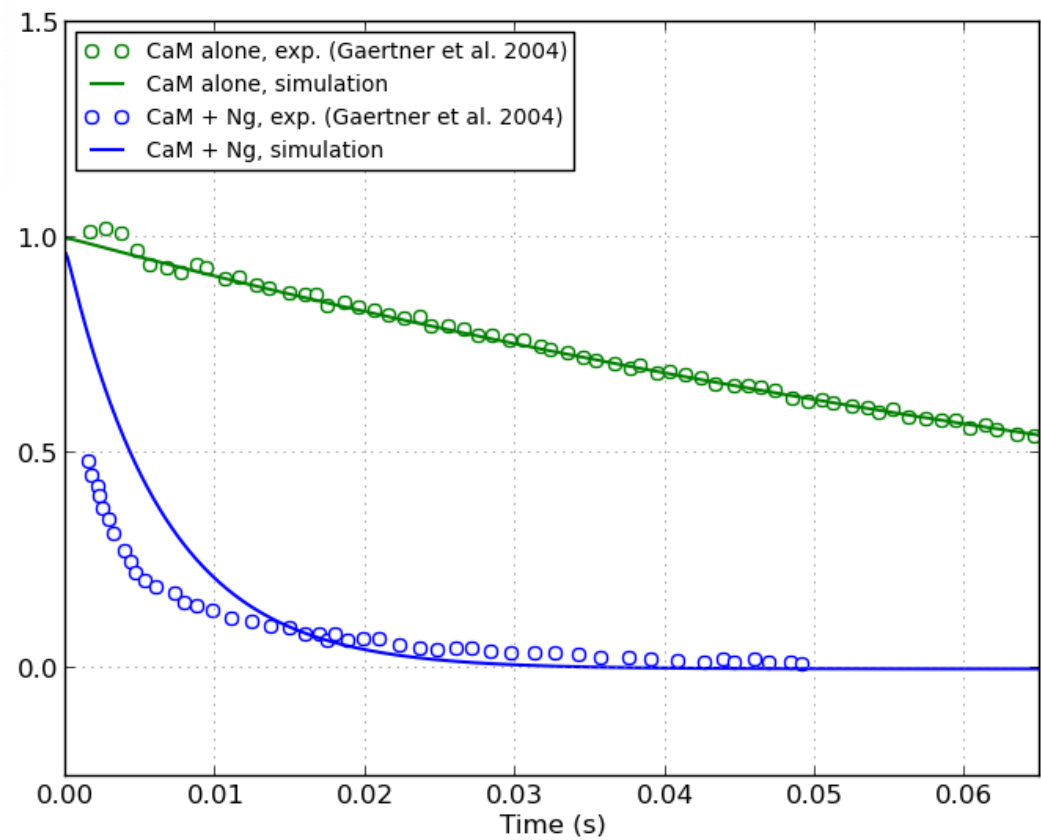
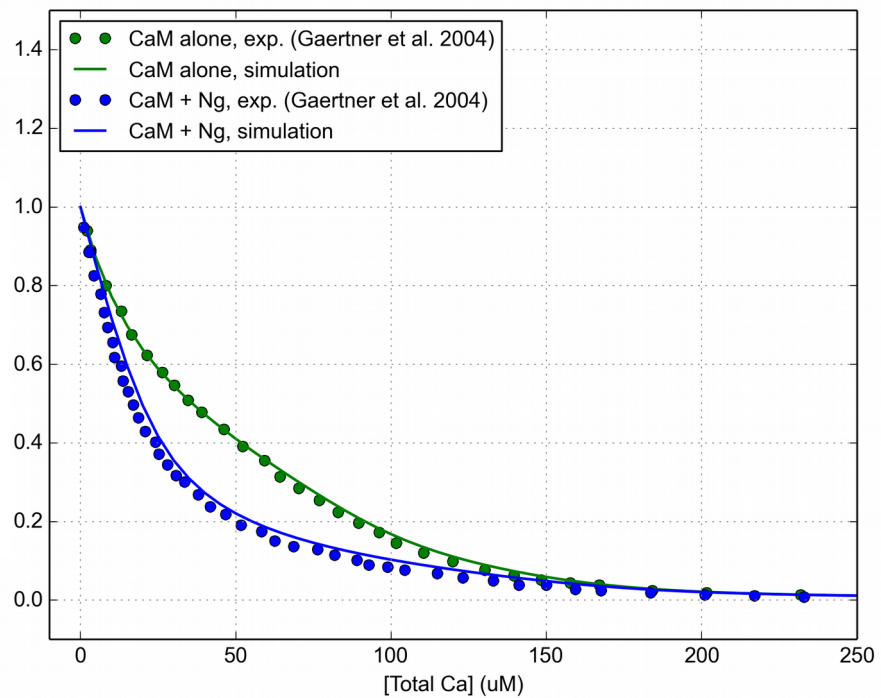


T stabilising
Peptide (IQ domain)

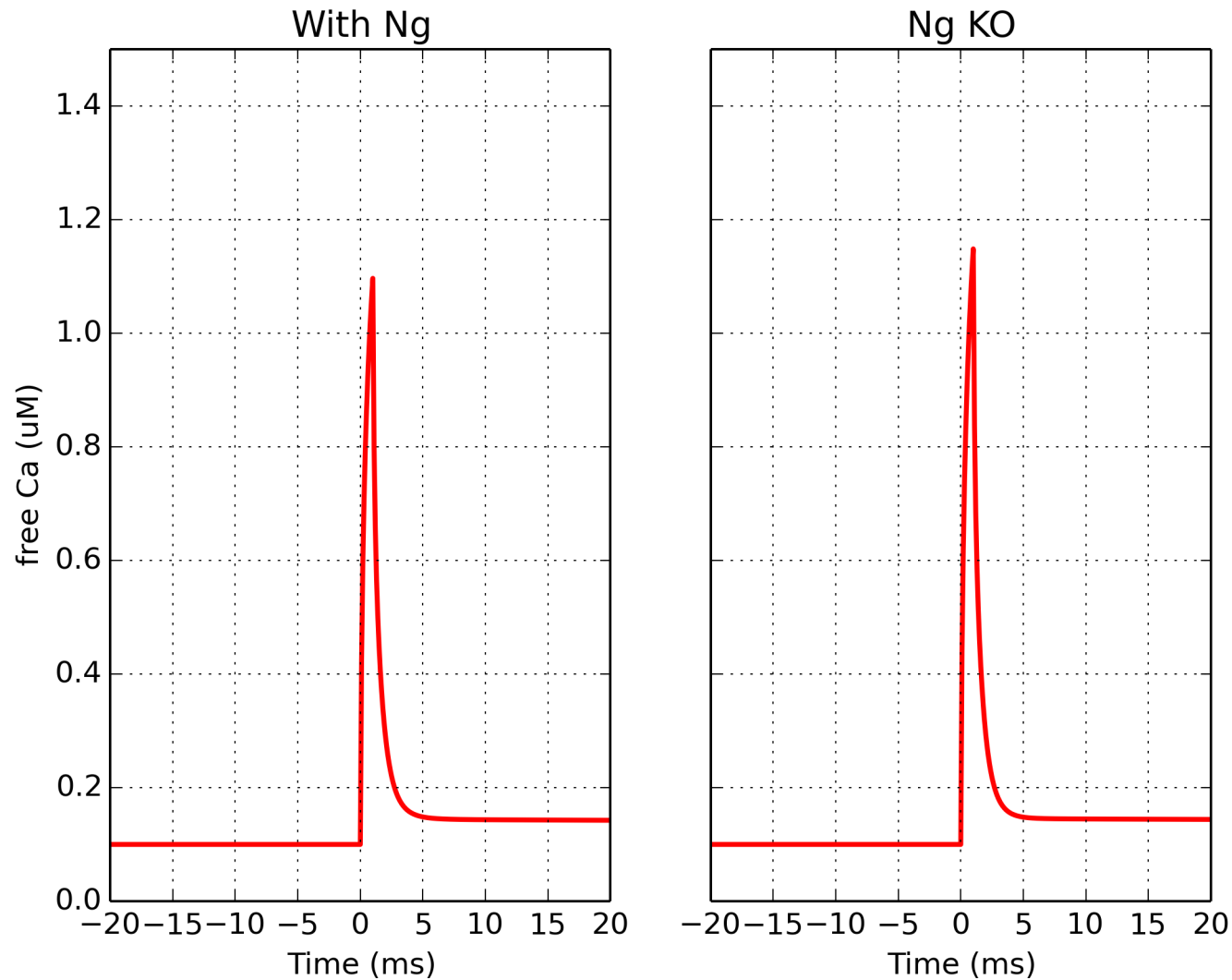


Theoharis *et al* (2008)

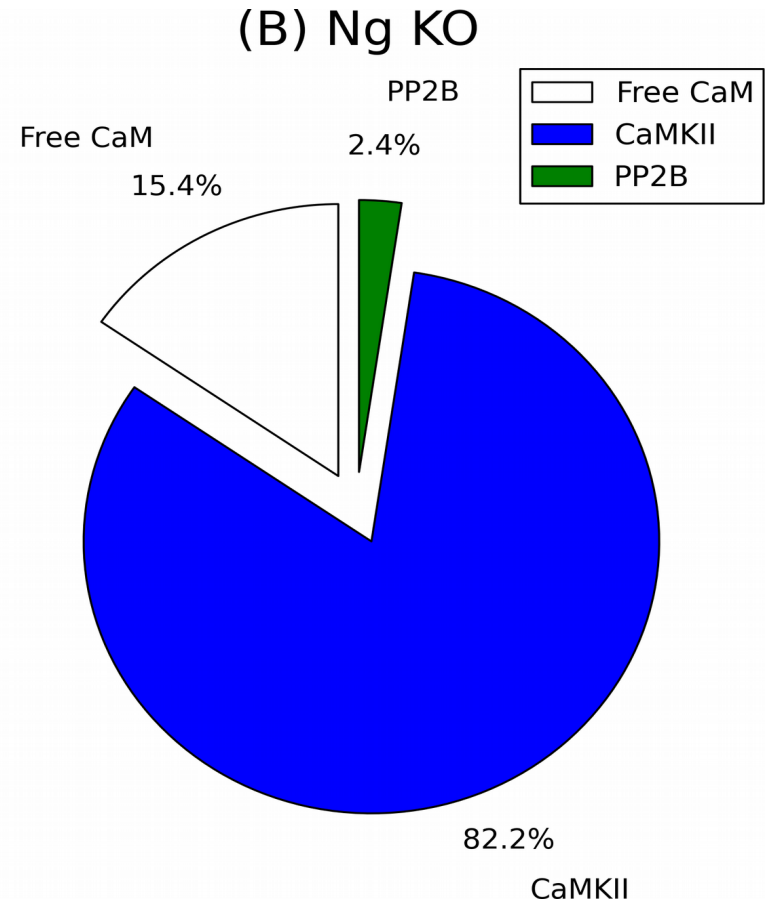
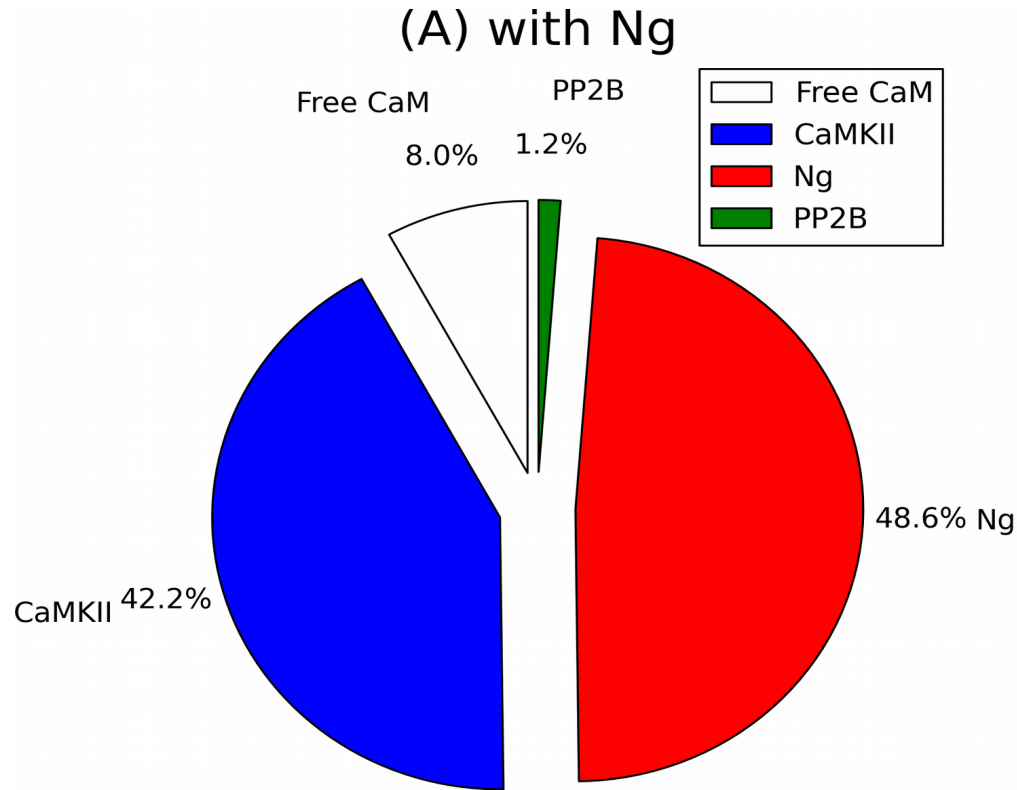


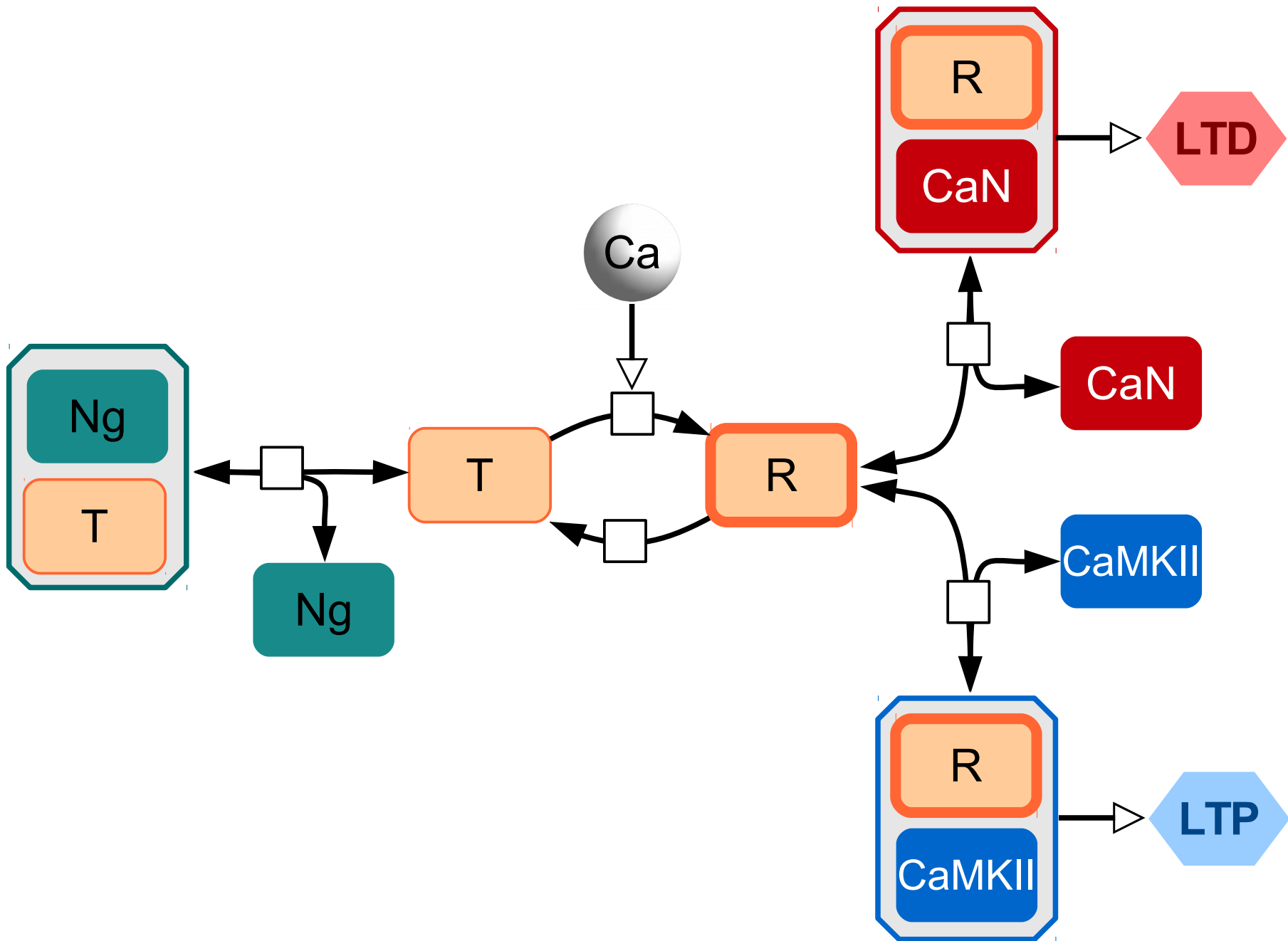


No large effect of Ng on $[Ca^{2+}]_{free}$



Ng affects the distribution of CaM





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!

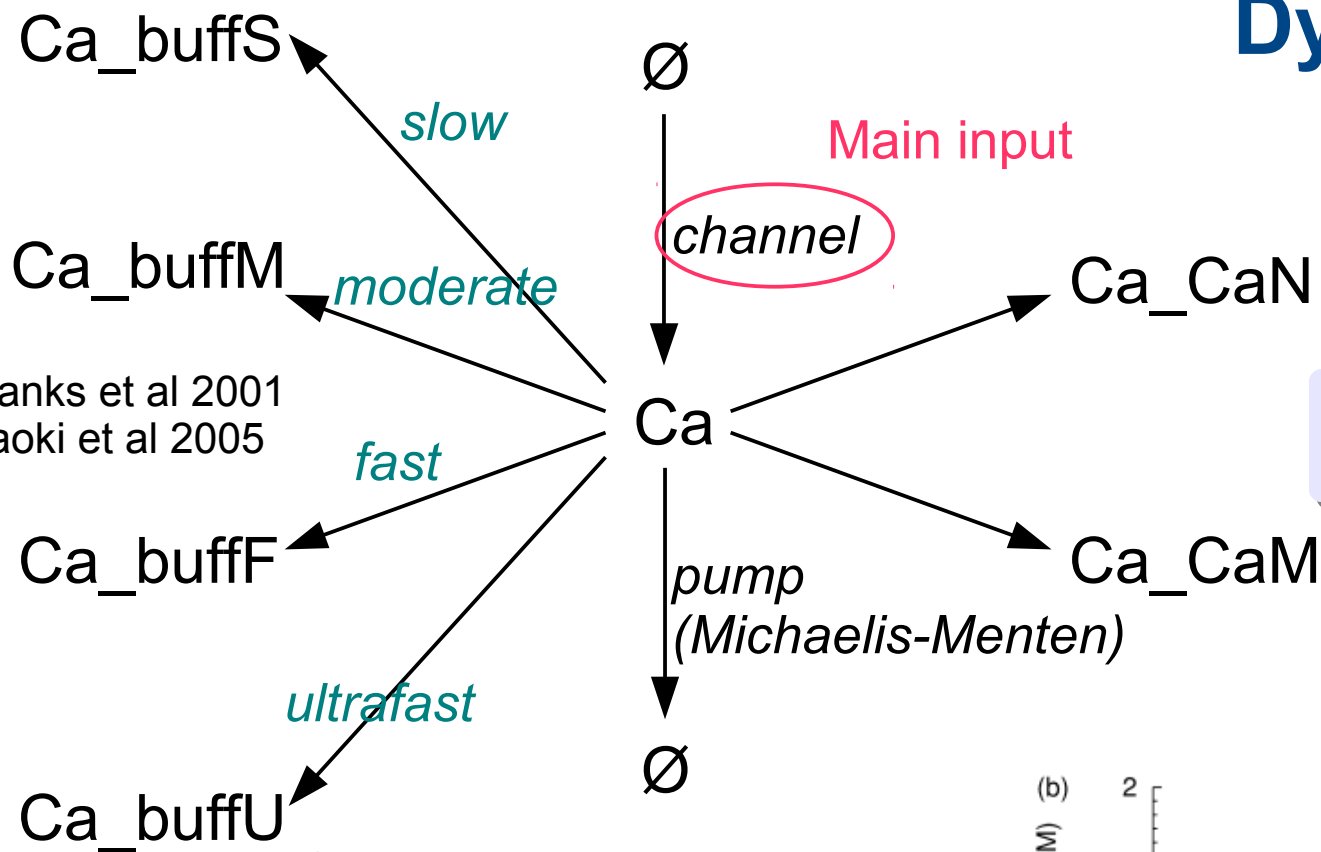
AMPAR post-synaptic potential:	5 ms
Calcium spike:	50 ms
Half saturation calmodulin ($k_{on}=1.5e6$, $k_{off}=100$):	5 ms
Relaxation between calmodulin states:	1 ms
autophosphorylation of CaMKII ($k_{on}=6$):	100 ms



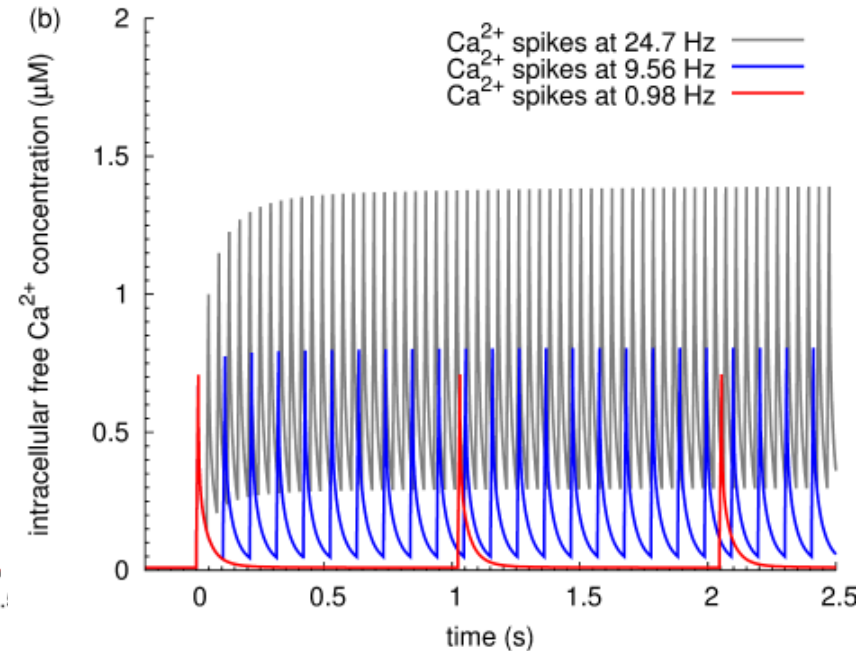
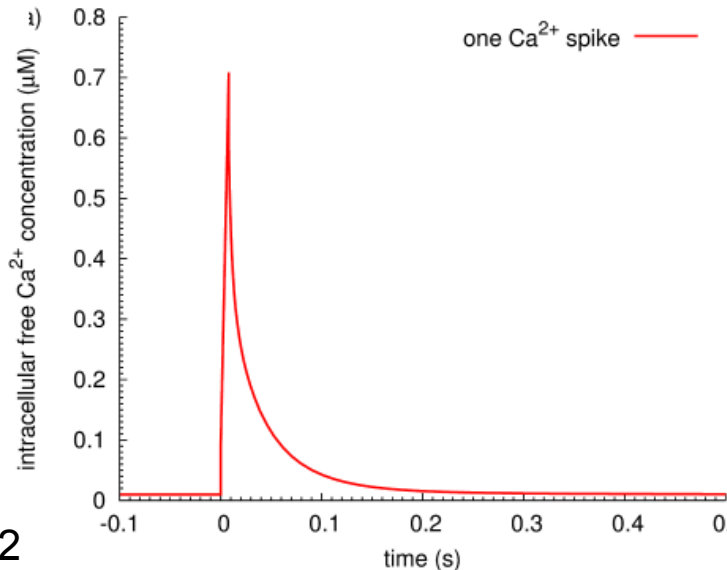
≠



Dynamic of calcium in the spine

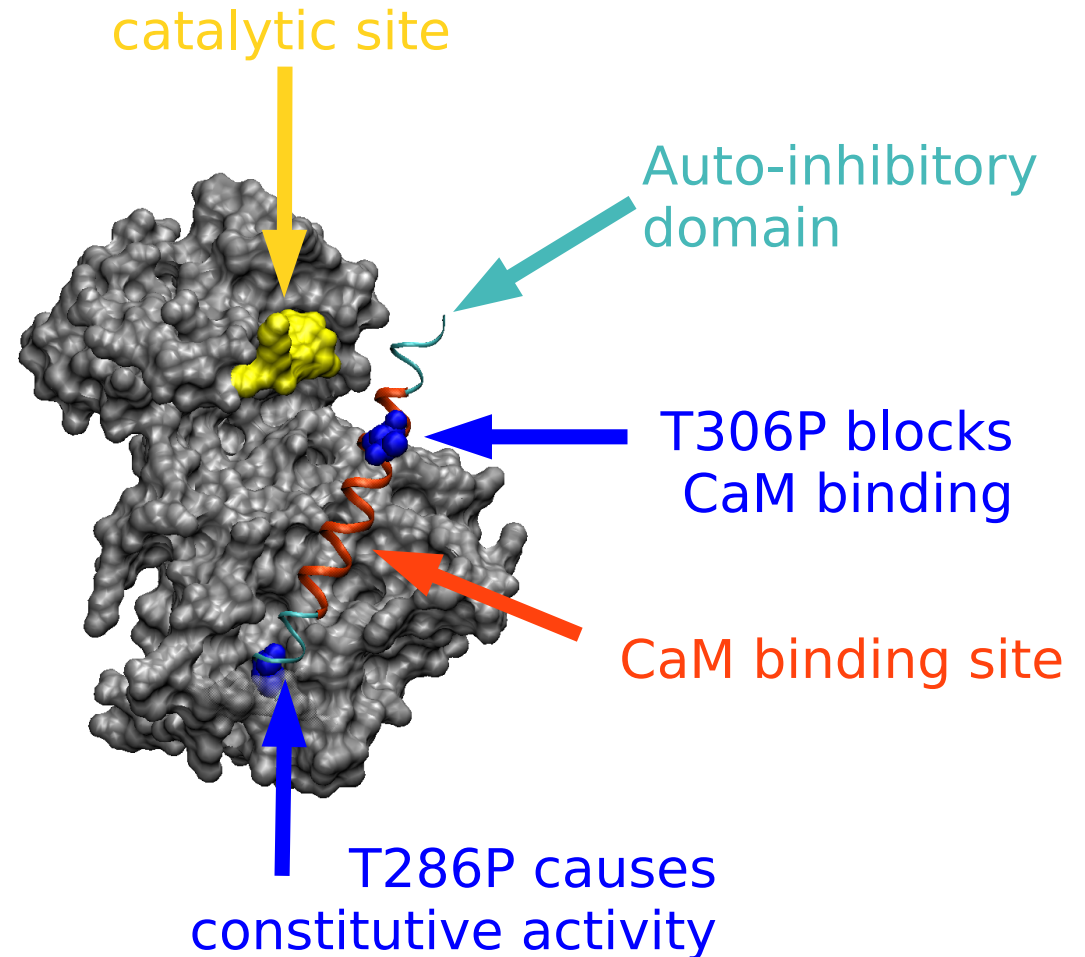


Li L, Stefan MI, Le Novère N
(2012) *PLoS ONE*, 7(9): e43810



Sabatini et al 2002

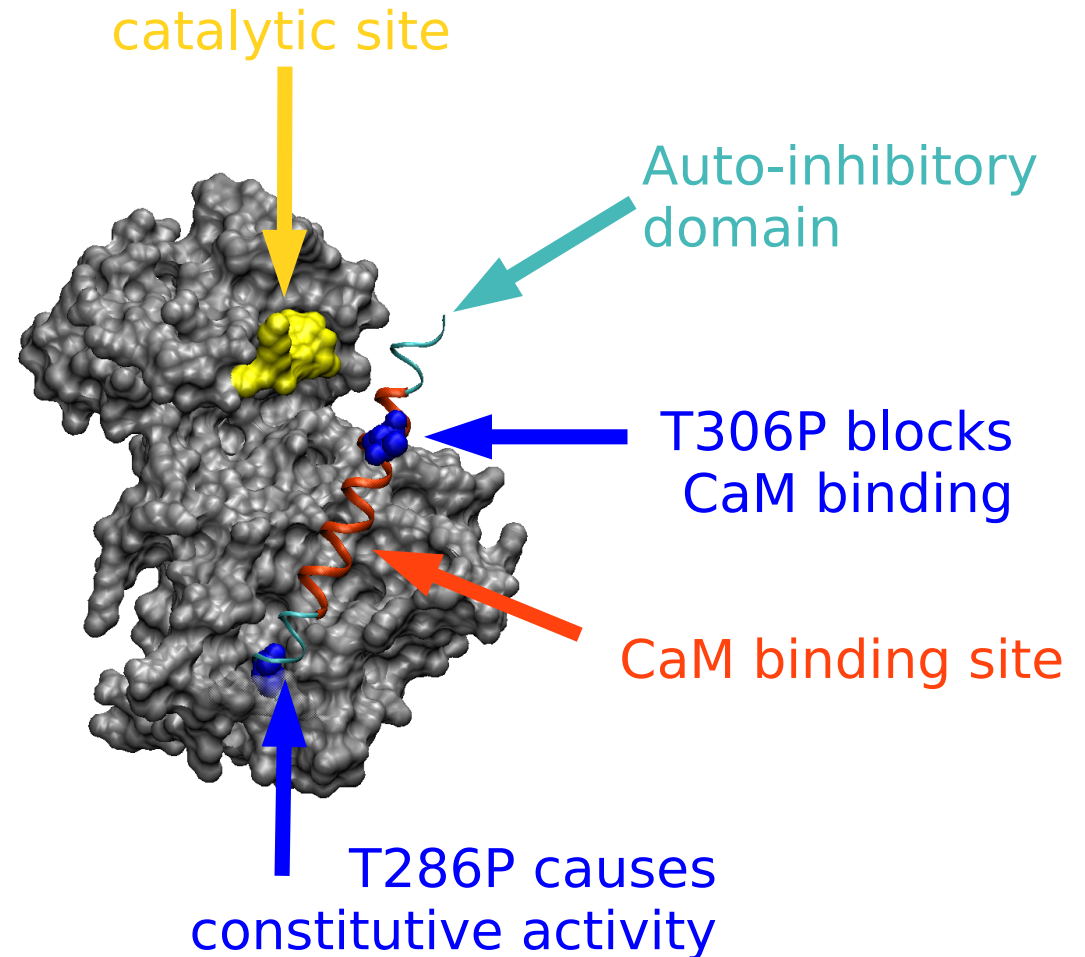
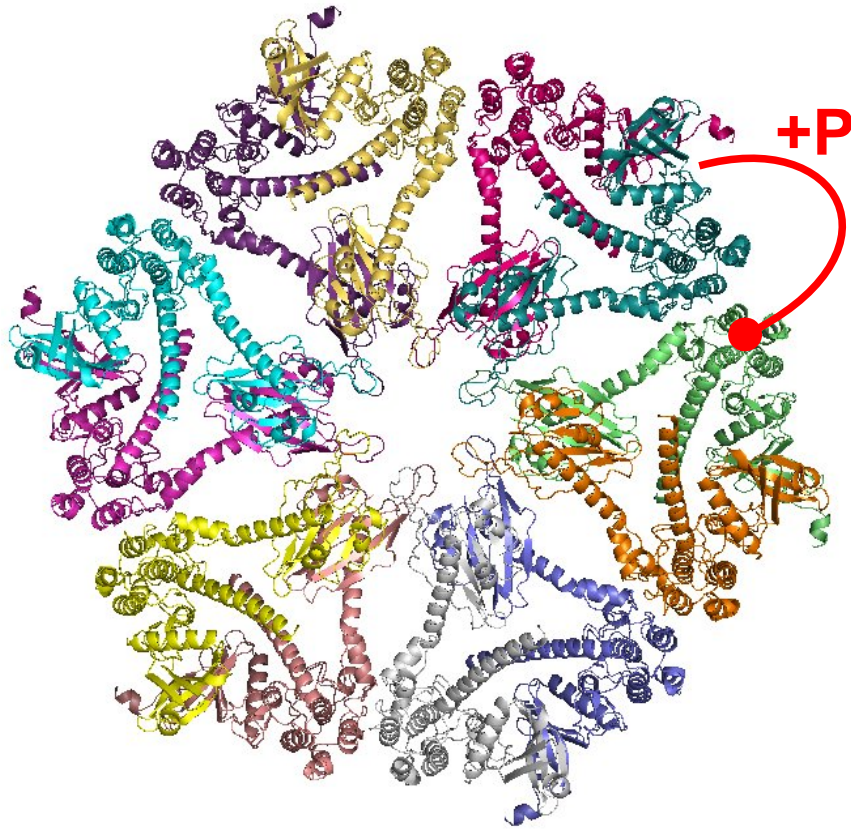
Calcium/calmodulin kinase II



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

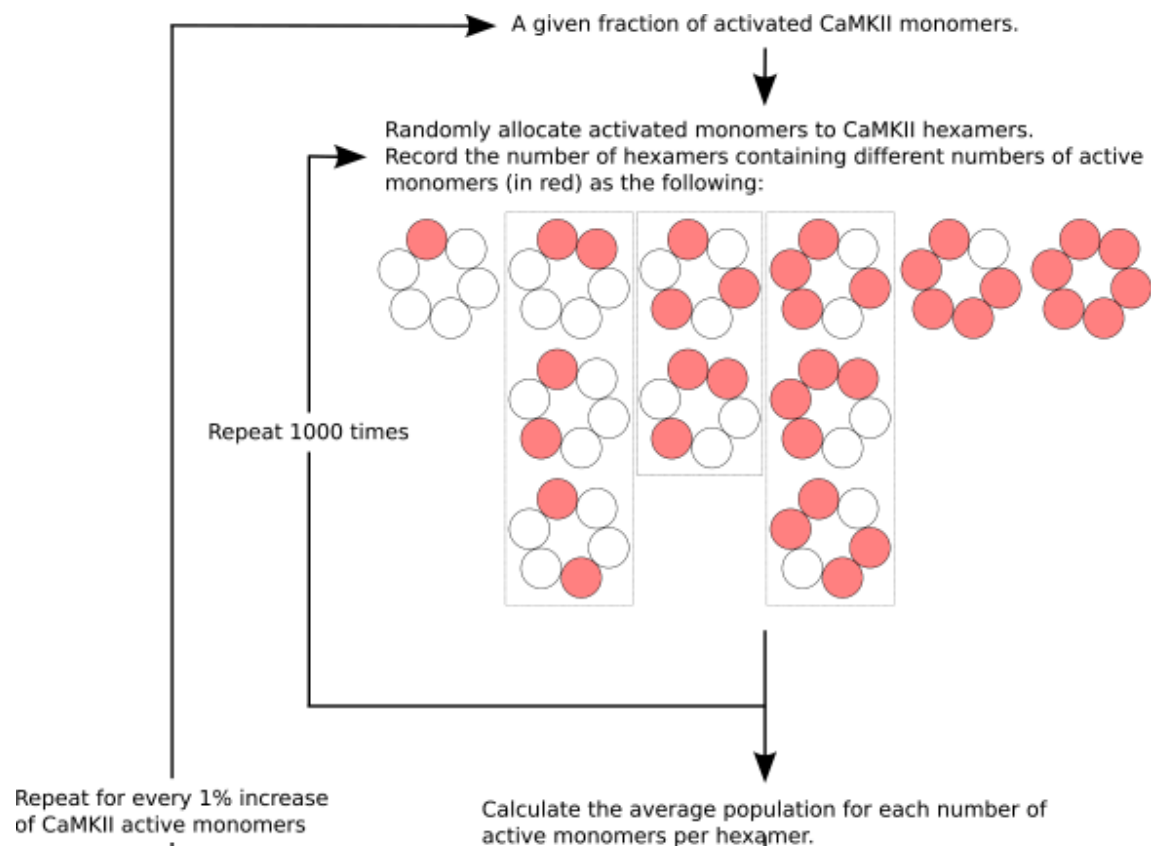


Dodecamer:

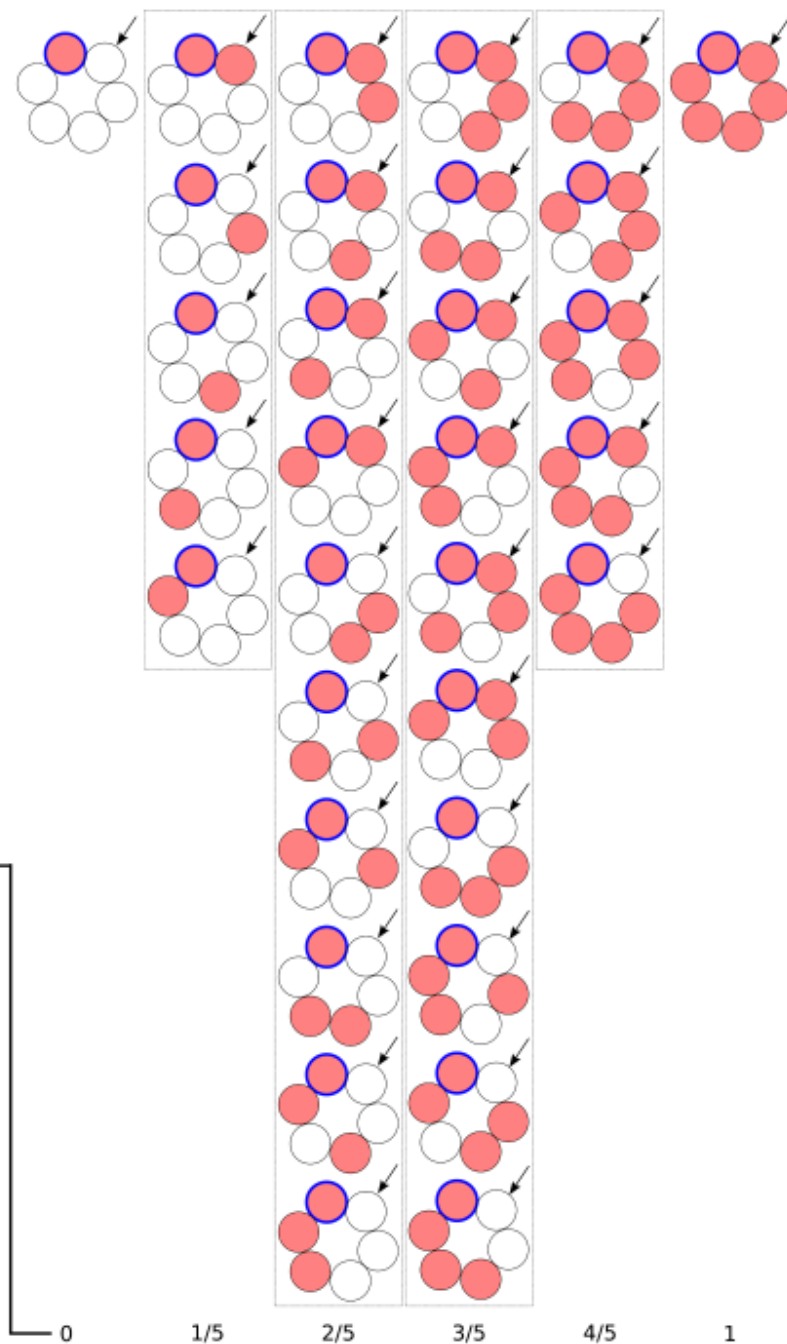
Trans-phosphorylation of T286
by neighbouring subunits

Cis-phosphorylation of T306

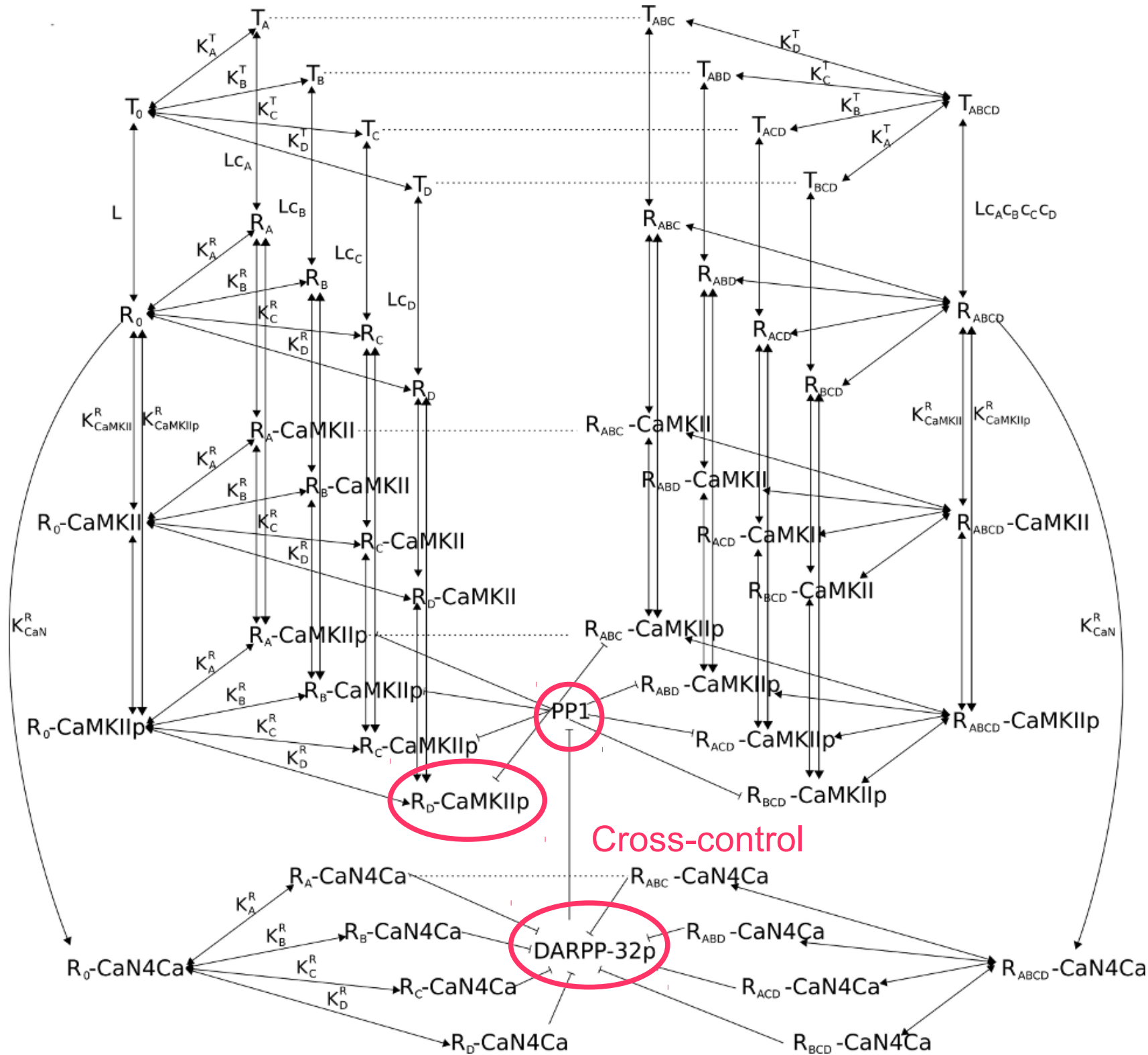
Most quantitative measurements made on monomers ...

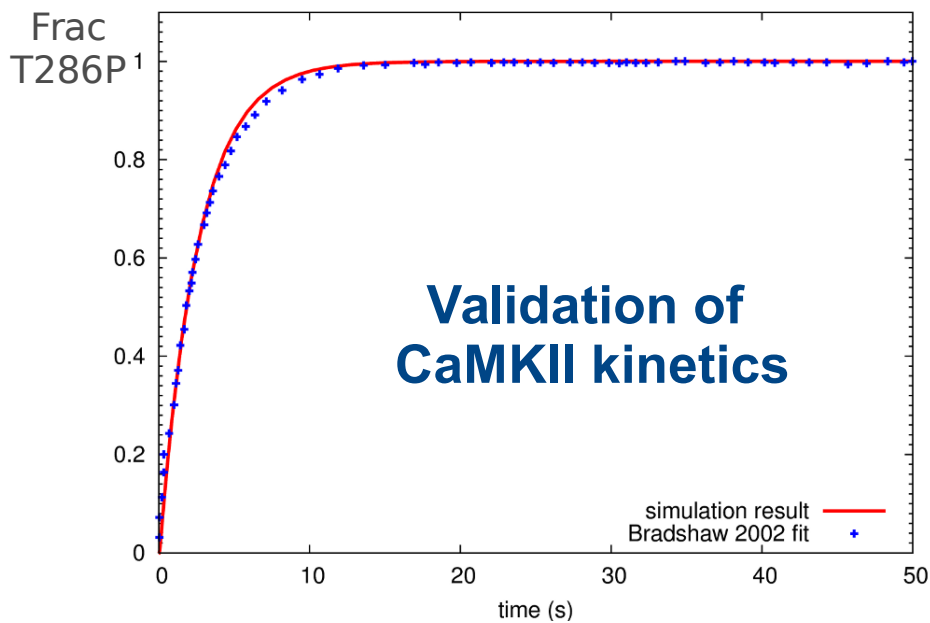


Calculate the probabilities of having an active neighbour on one specific side (indicated by the arrow) of the activated monomer of interest (blue outline) (since only the asymmetrical situation is considered). The possible positions of activated monomers are listed as the following, with corresponding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

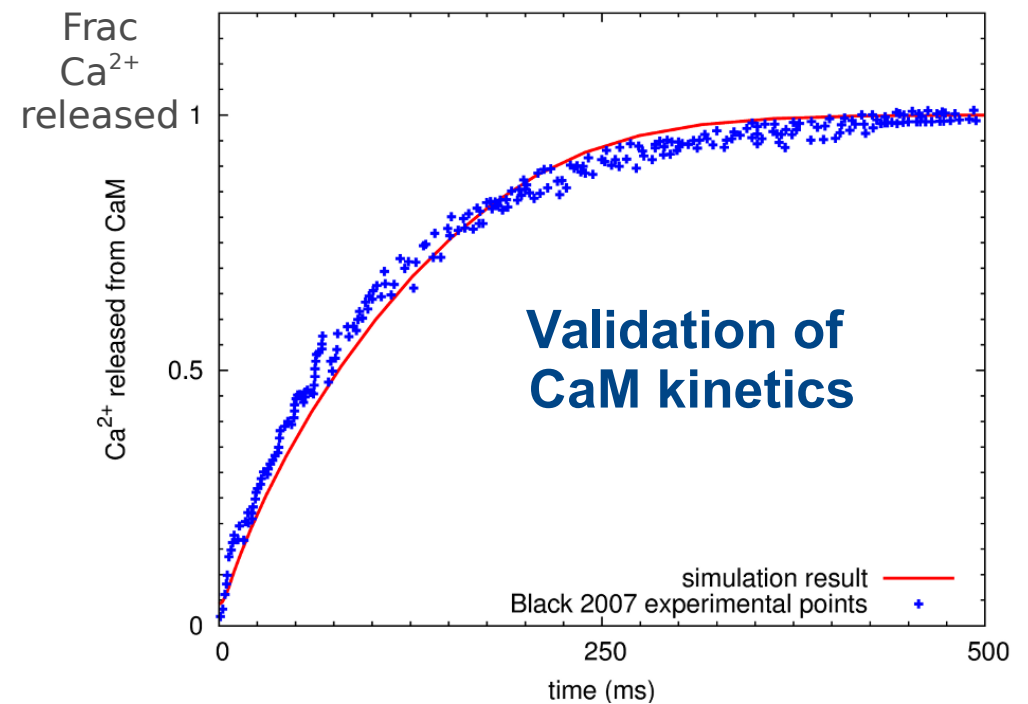


Fit these 100 coefficients into a polynomial function of activated CaMKII monomers, and embed this function in the model.

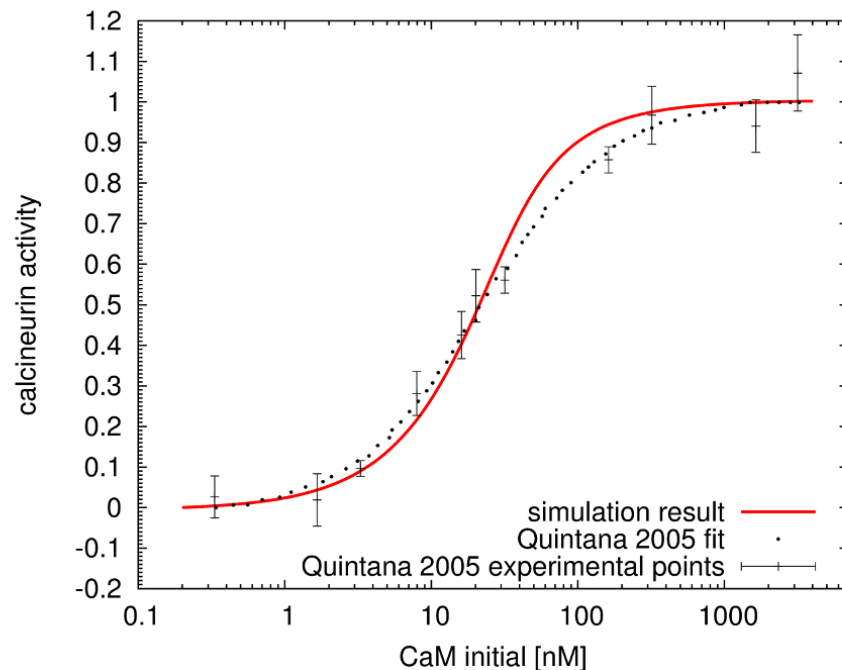




Bradshaw JM, Kubota Y, Meyer T, Schulman H (2003). *PNAS* 100: 10512–10517.



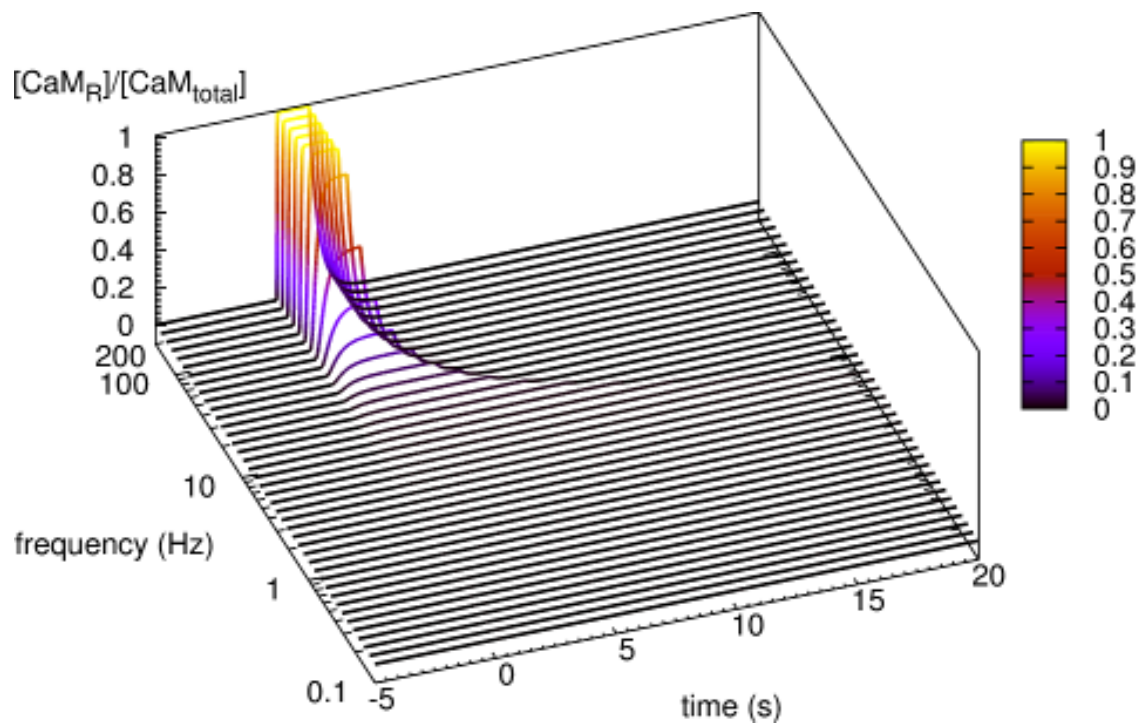
Black DJ, Selfridge JE, Persechini A (2007). *Biochemistry* 46: 13415–13424.



Validation of calcium-activation of CaN

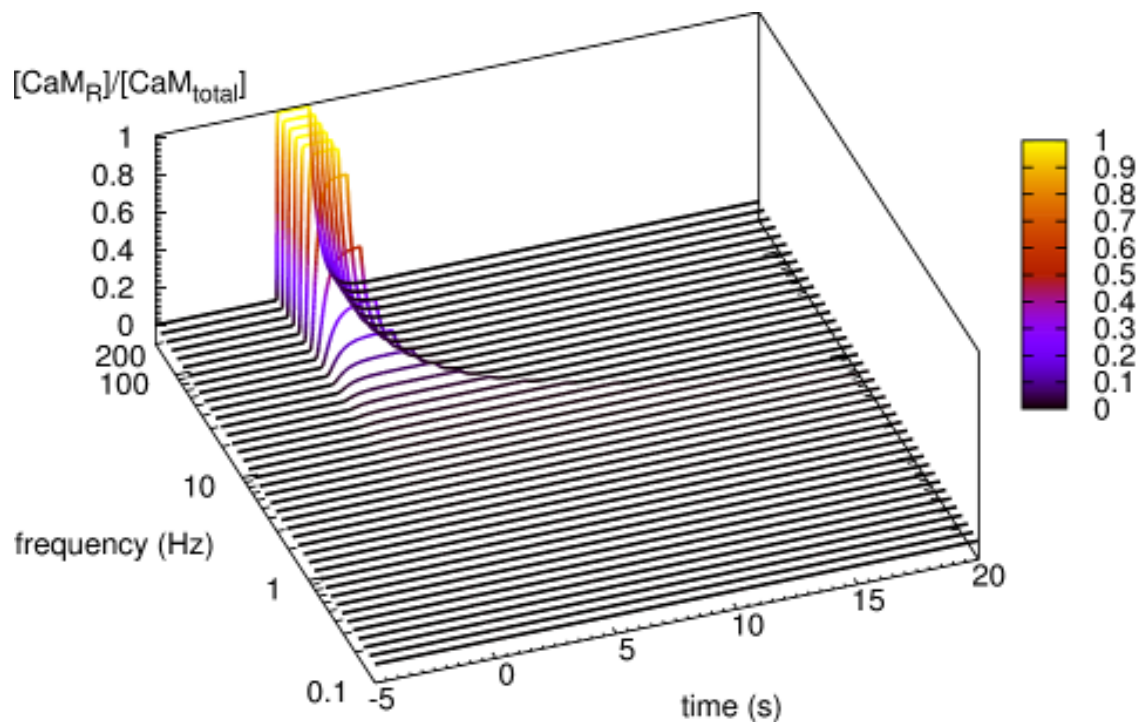
Quintana AR, Wang D, Forbes JE, Waxham MN (2005). *BBRC* 334: 674–680.

Calmodulin activation



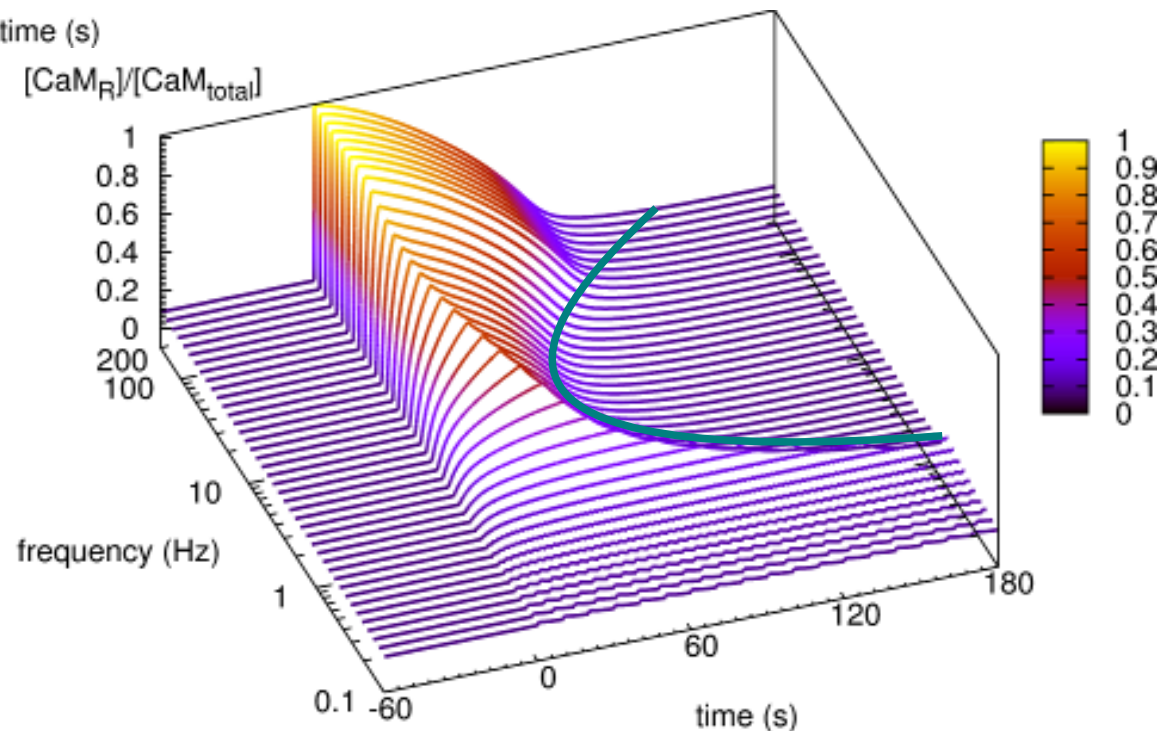
CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

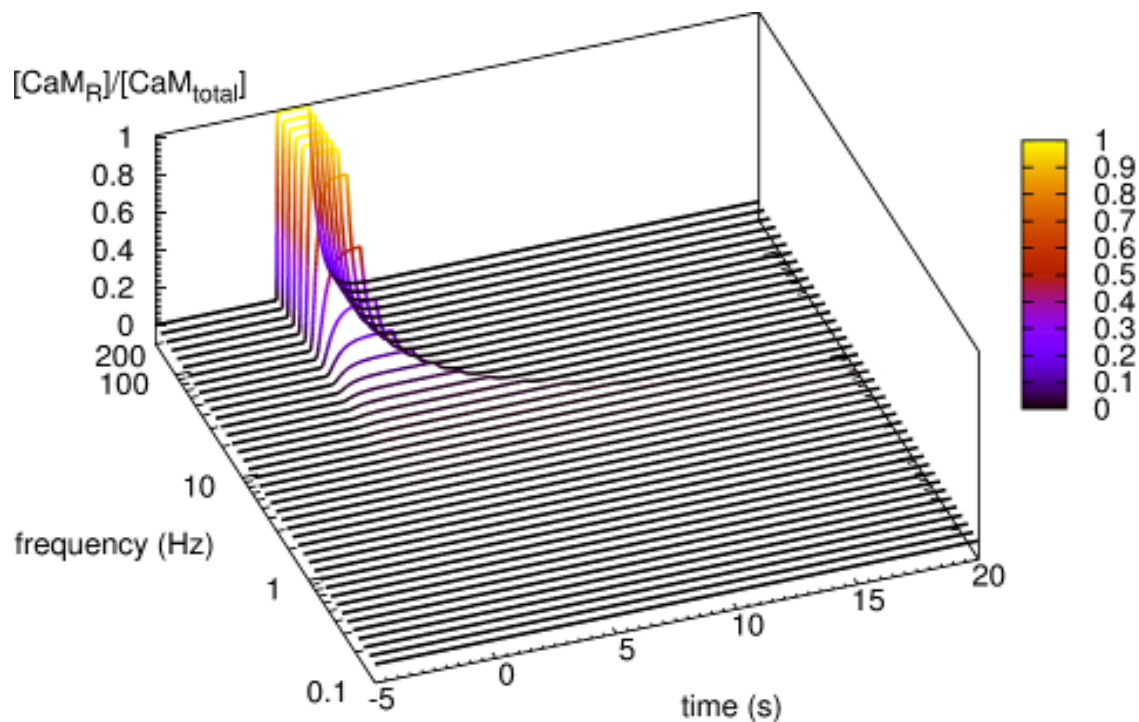


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

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



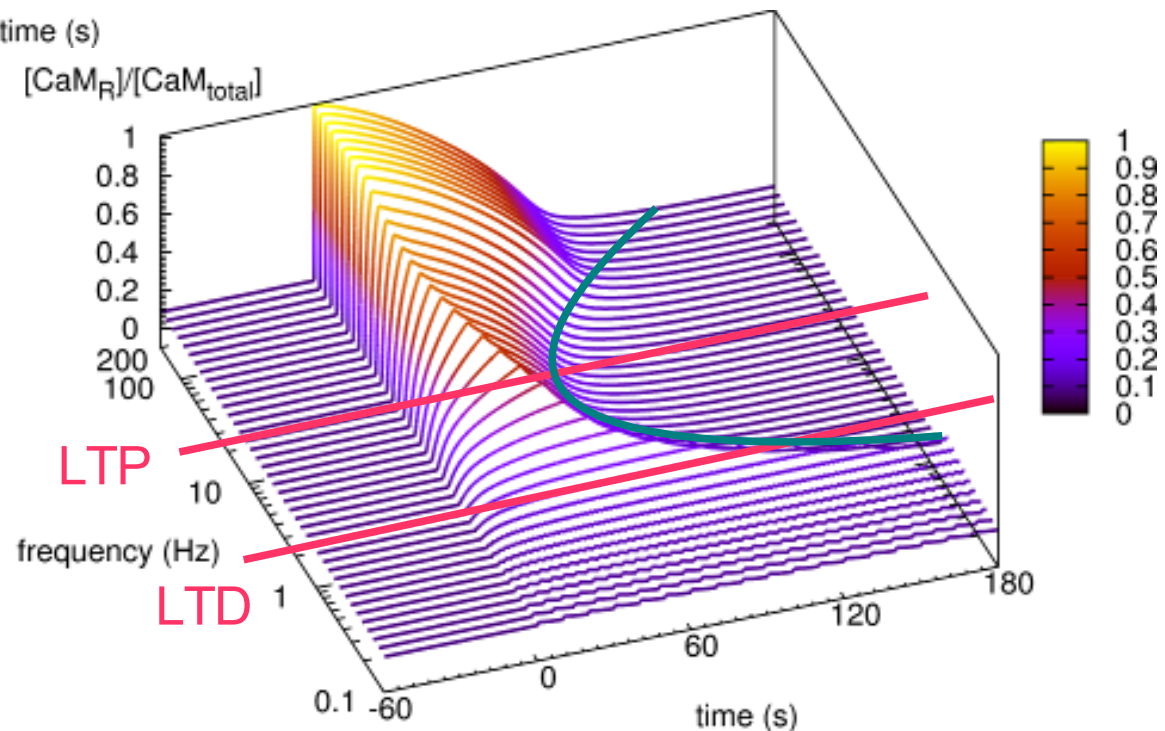
Calmodulin activation



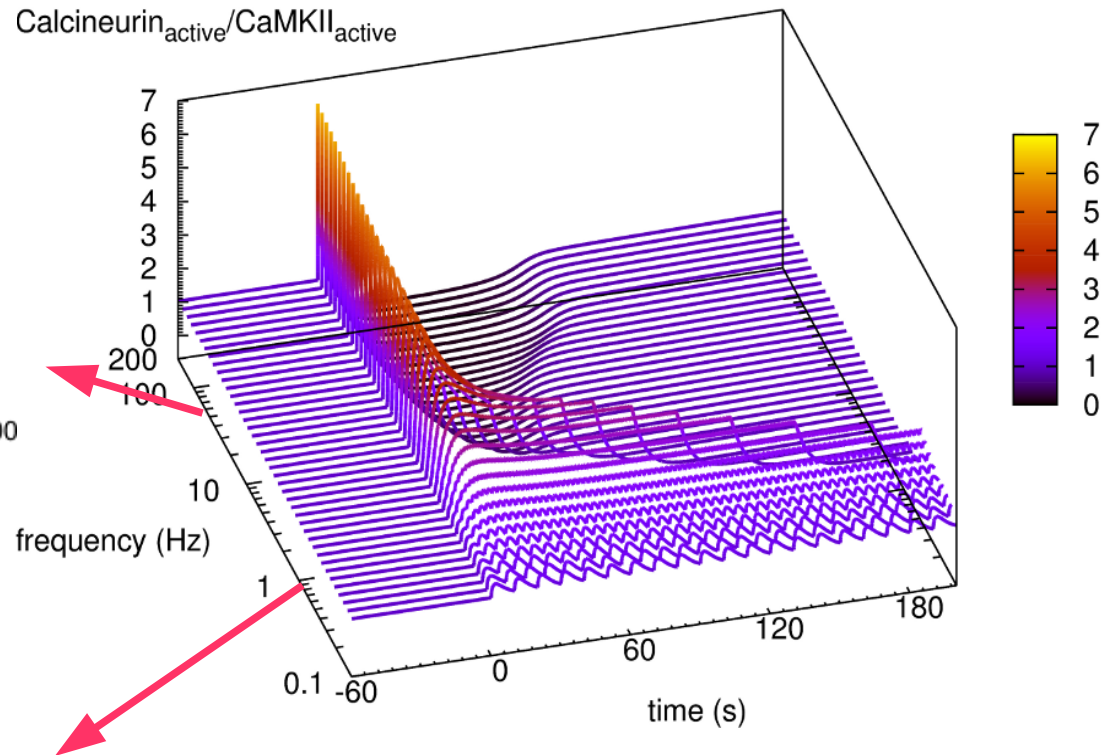
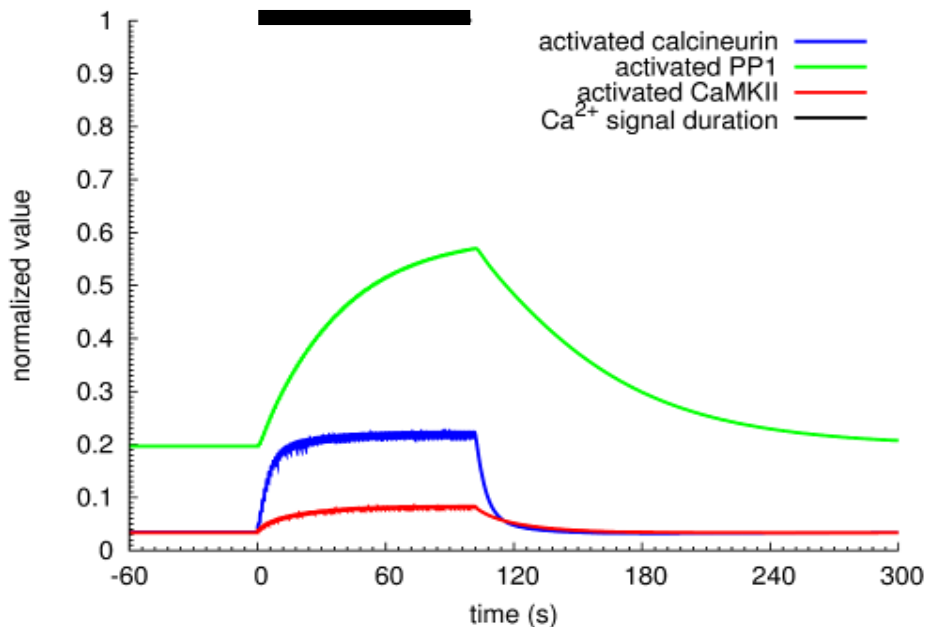
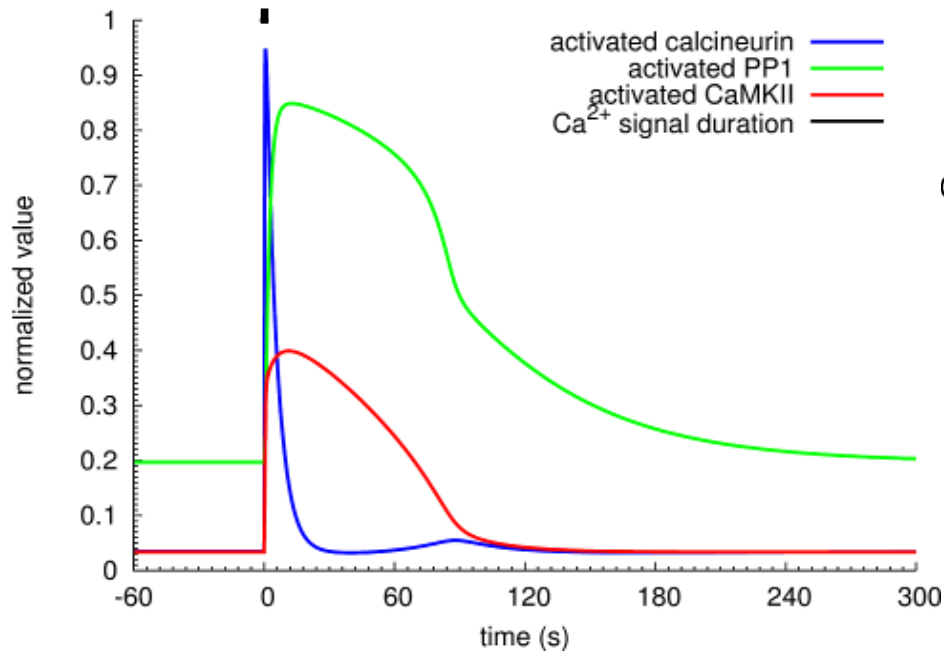
CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

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

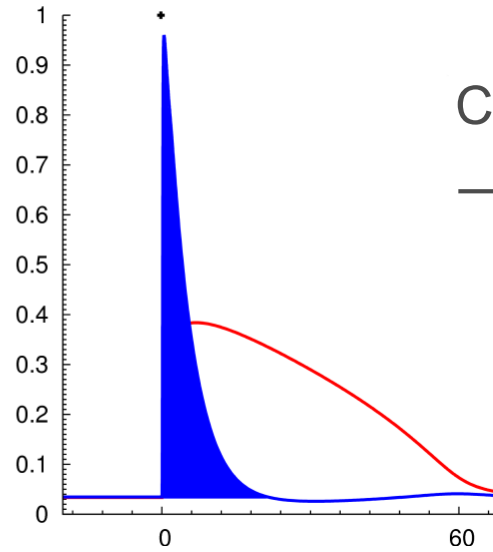
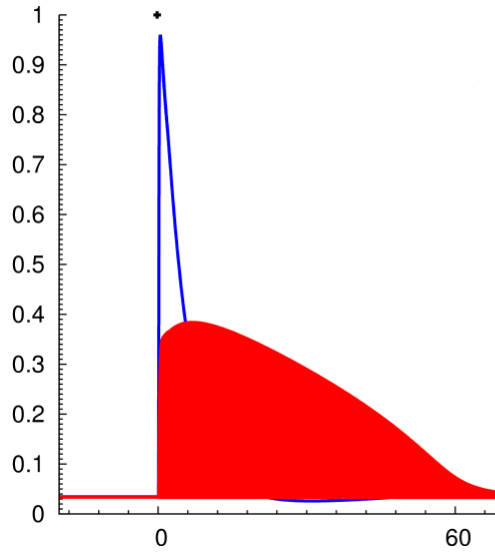


All calcium frequencies increase CaN **AND** CaMKII. It is not a Switch. But the ratio of activation changes

Bidirectional plasticity

Constant catalytic rates of active enzyme

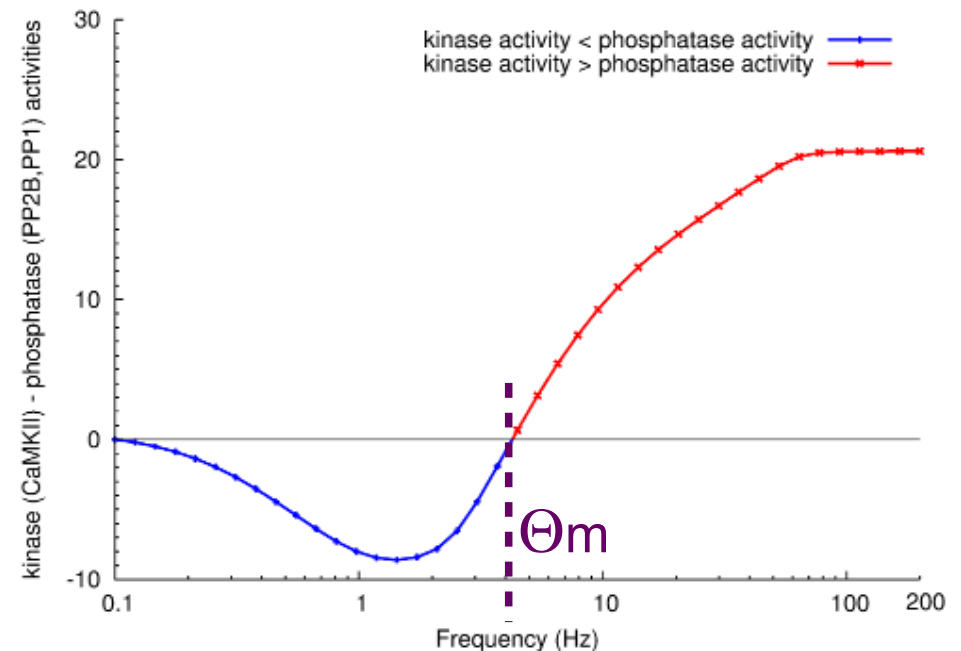
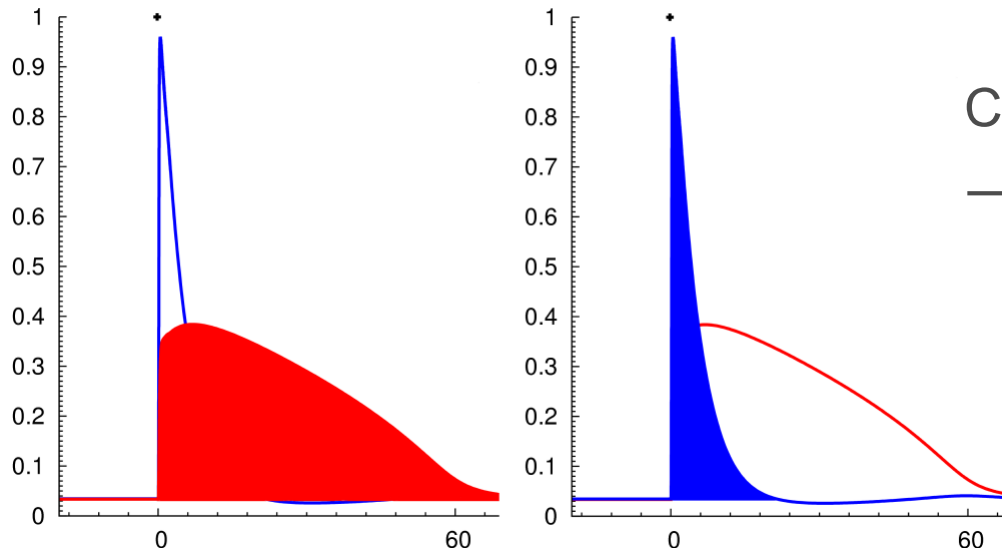
→ quantity of catalysed reaction events
prop to integral of the activation curve



Bidirectional plasticity

Constant catalytic rates of active enzyme
→ quantity of catalysed reaction events
prop to integral of the activation curve

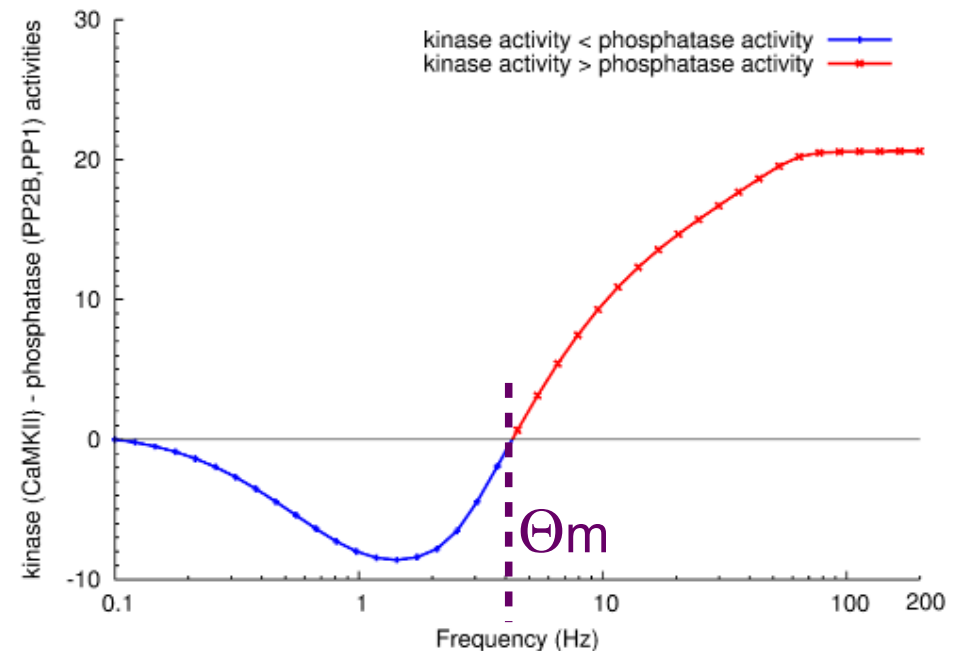
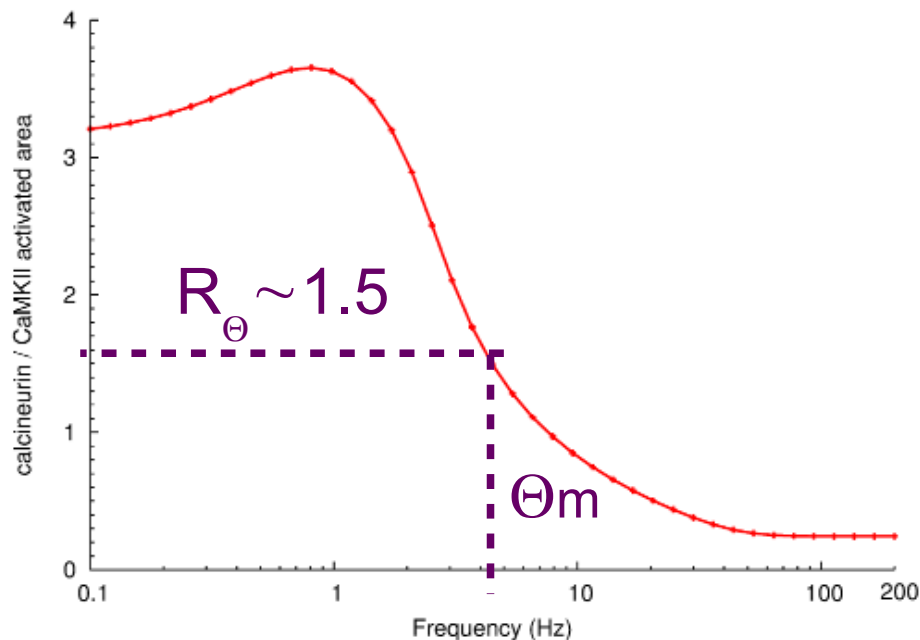
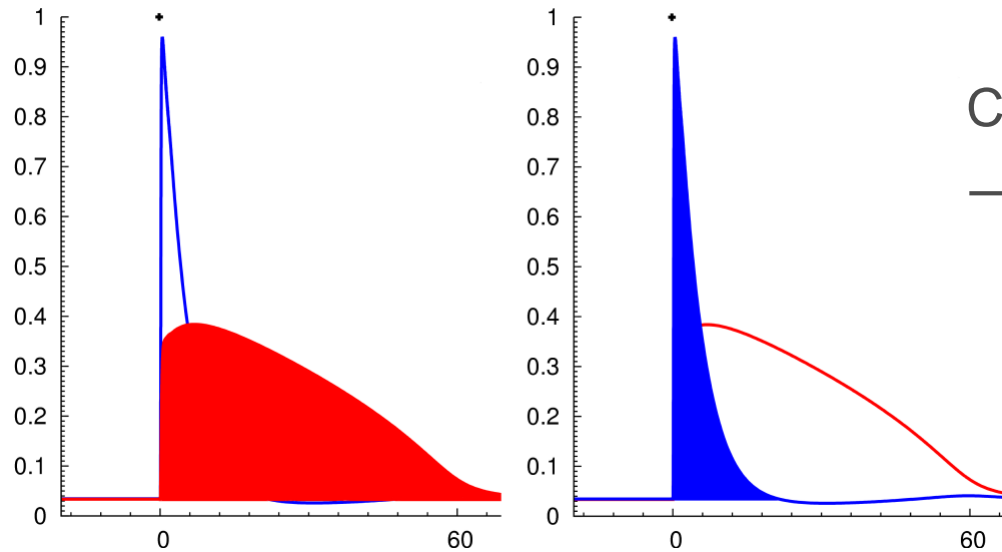
Bienestock-Cooper-Munro
(BCM) curve: difference of
active areas* catalytic activities



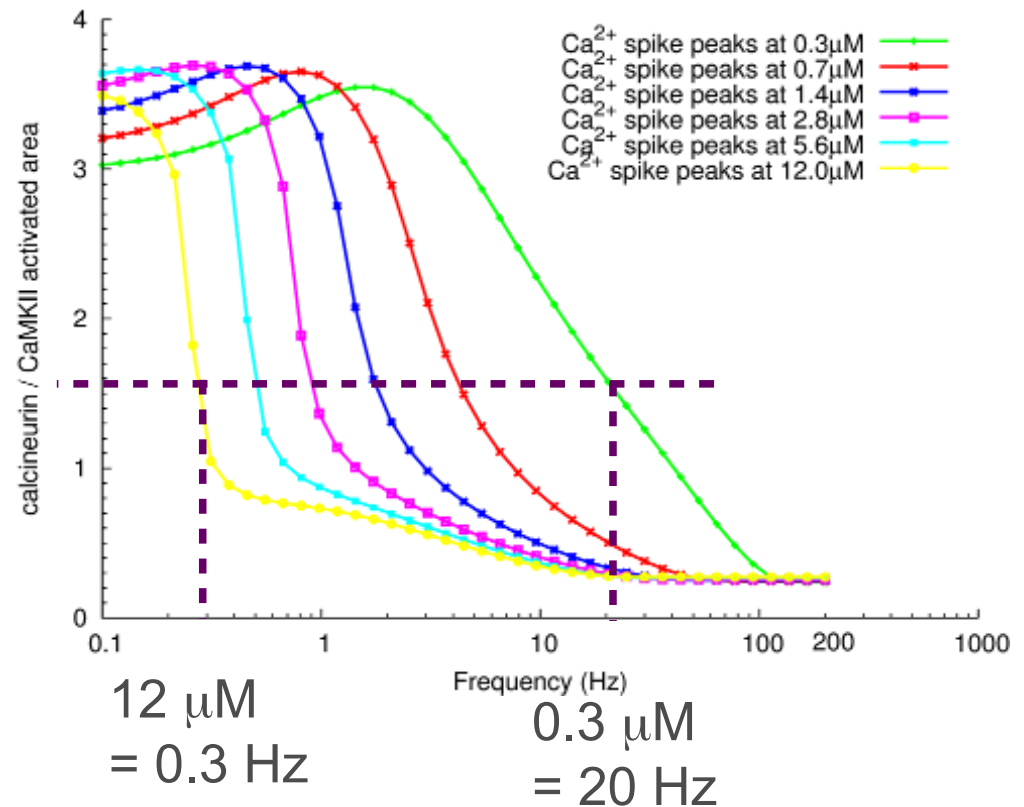
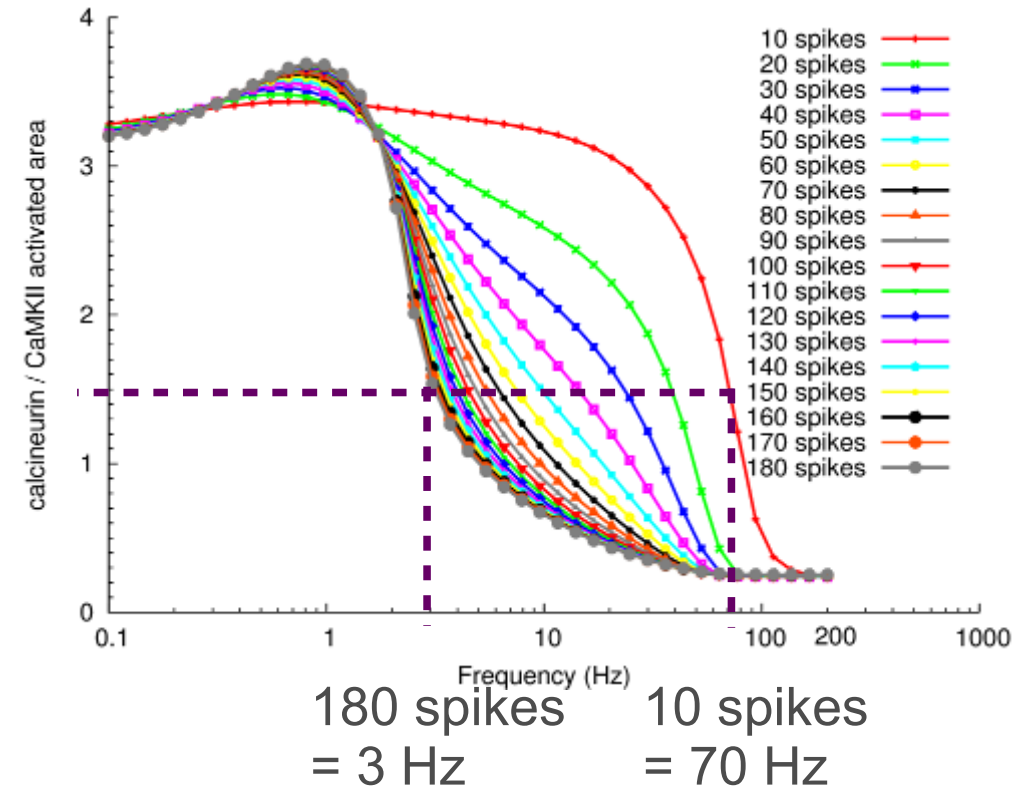
Bidirectional plasticity

Constant catalytic rates of active enzyme
 → quantity of catalysed reaction events
 prop to integral of the activation curve

Bienestock-Cooper-Munro
 (BCM) curve: difference of
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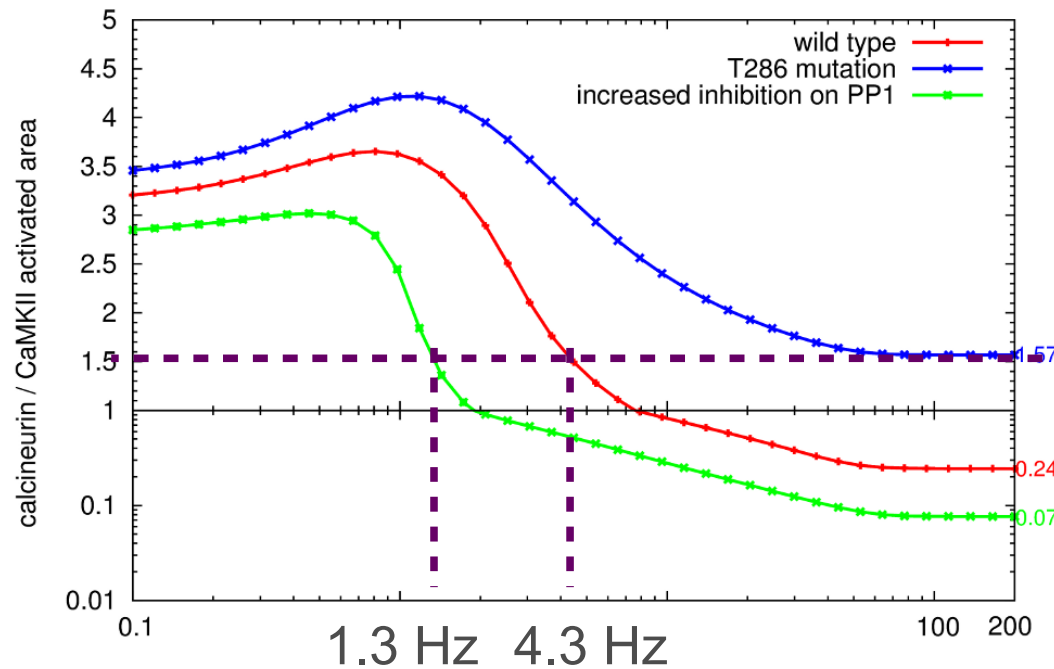


Effect of calcium duration and amount



Prolonged or intense signals decrease Θ_m : It is not an intrinsic property of the synapse

Effect of intrinsic system perturbations



CaMKII not constitutively active
 No CaM trapping

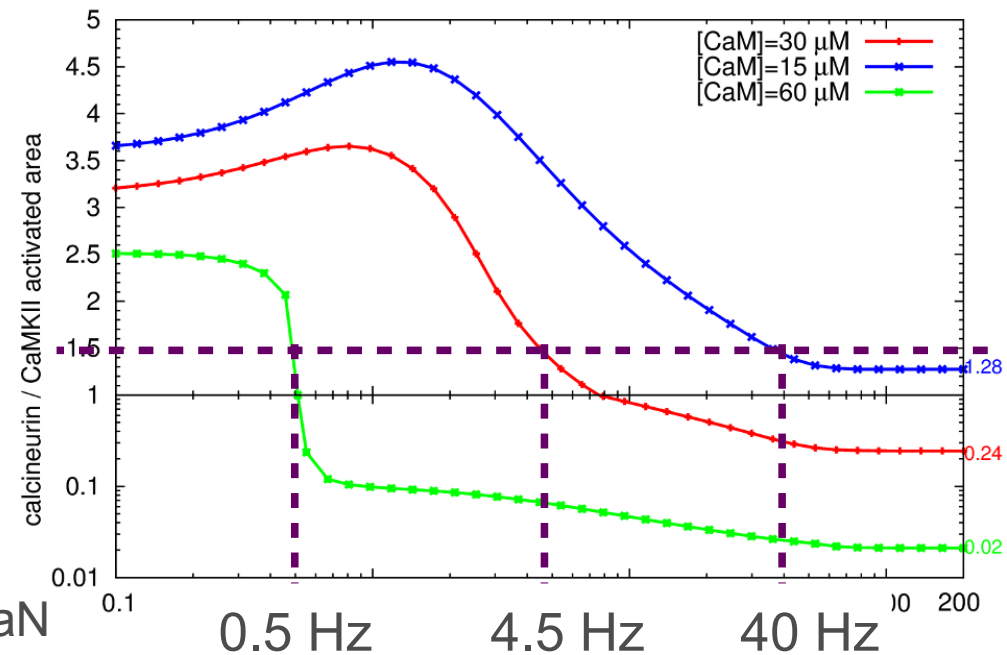
Never any positive plasticity
 Giese et al (1998) *Science*, 279:870-873

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

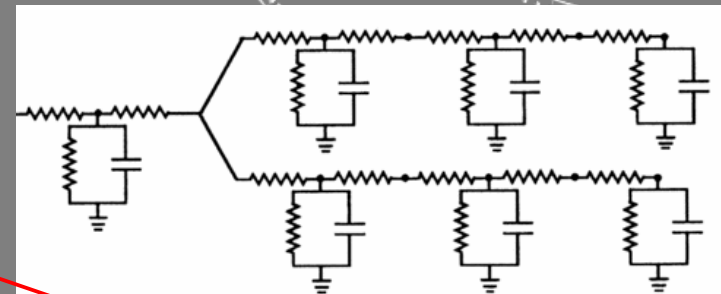


Allosteric stabilisation triggers **bistable CaM response**
> certain freq, CaM activation longer than initial signal

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

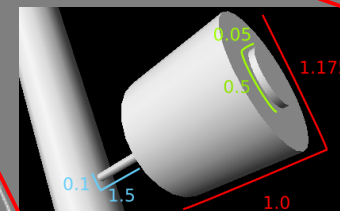
Θ_m is not an **intrinsic property of the synapse**, but a dynamical one that depends on the length and amplitude of stimulations

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

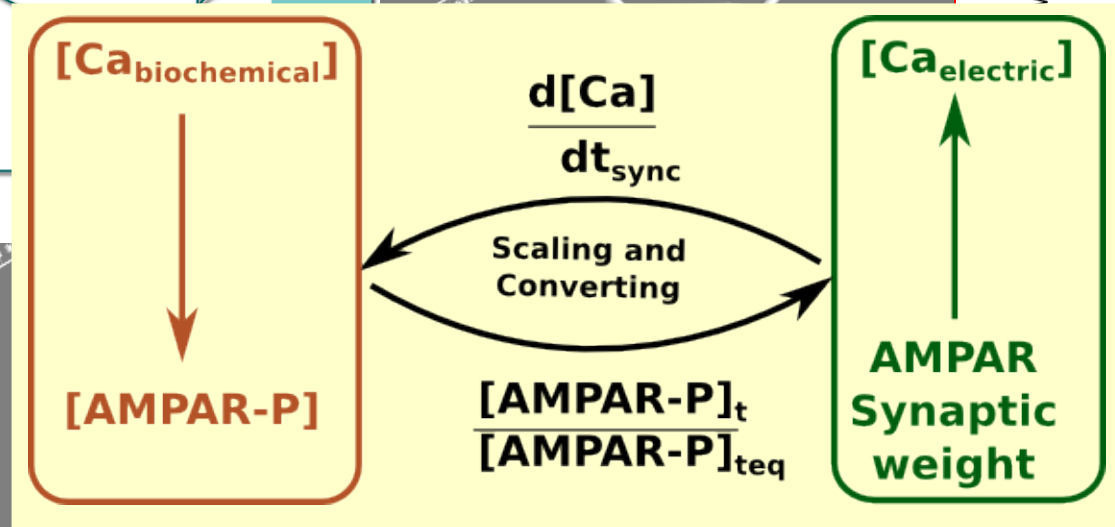
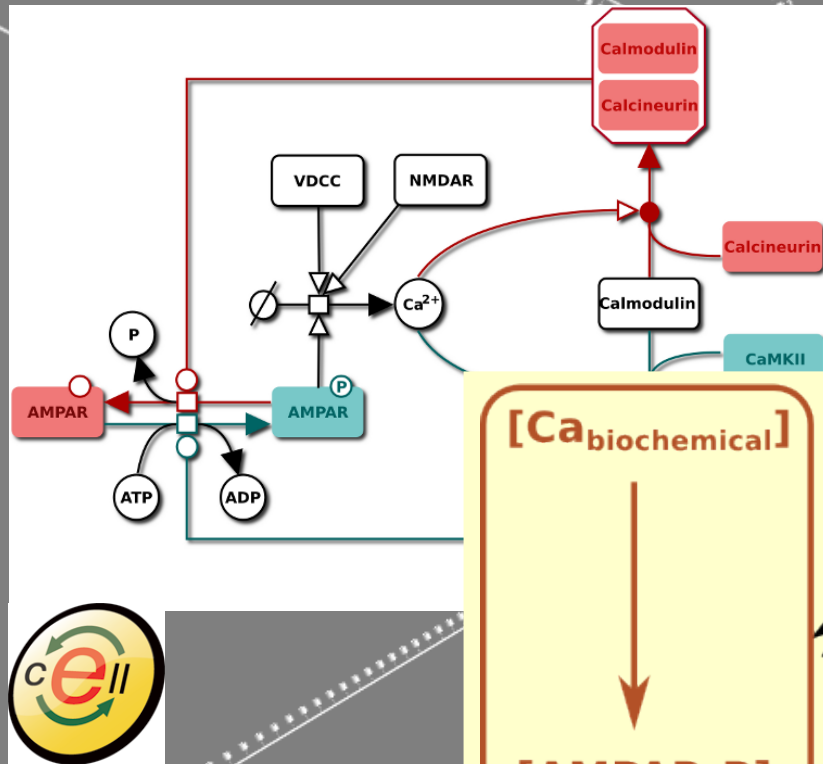


NEURON

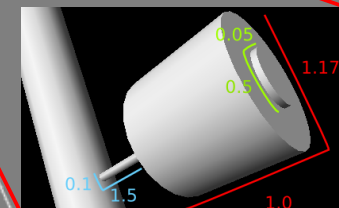
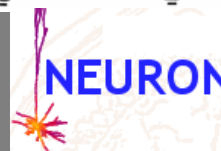
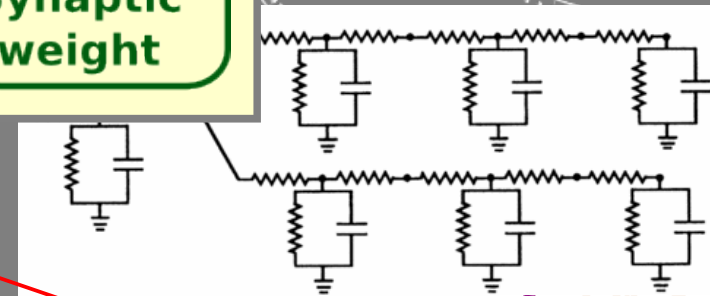
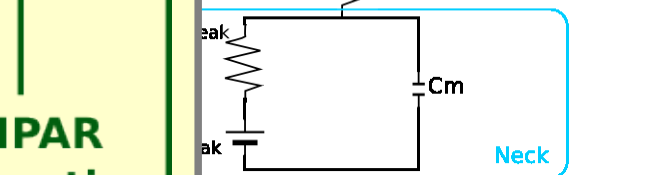
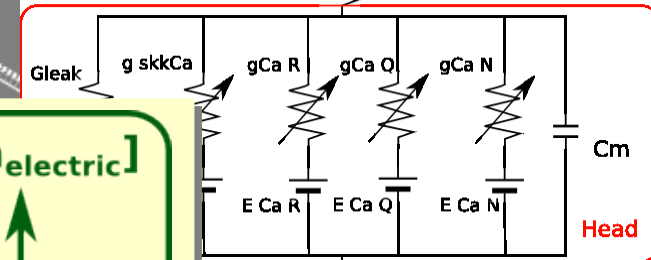
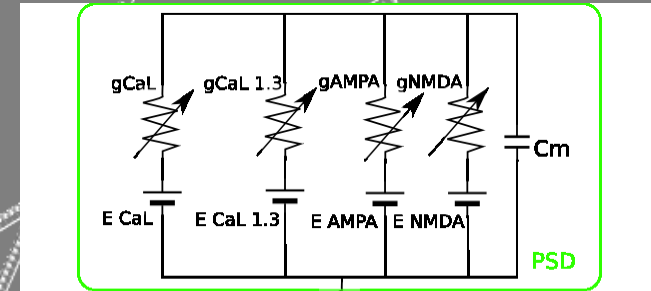
Mattioni M, Le Novère N (2013)



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)

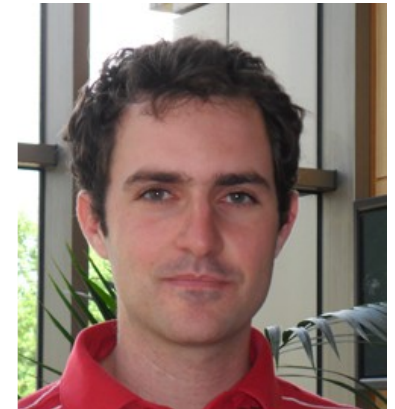
Developers of ECell3,
NEURON, Scilab, and ...



Melanie Stefan



Lu Li



Denis Brun



Michele
Mattioni



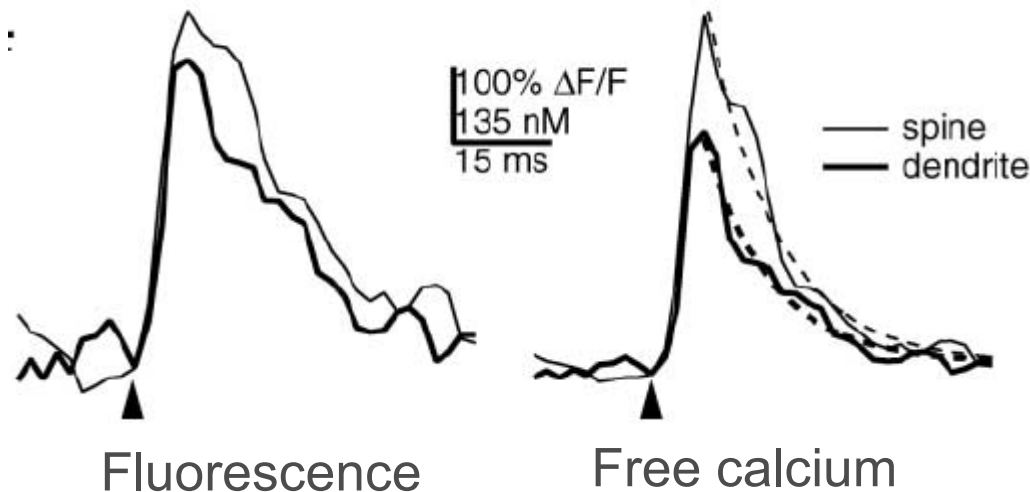
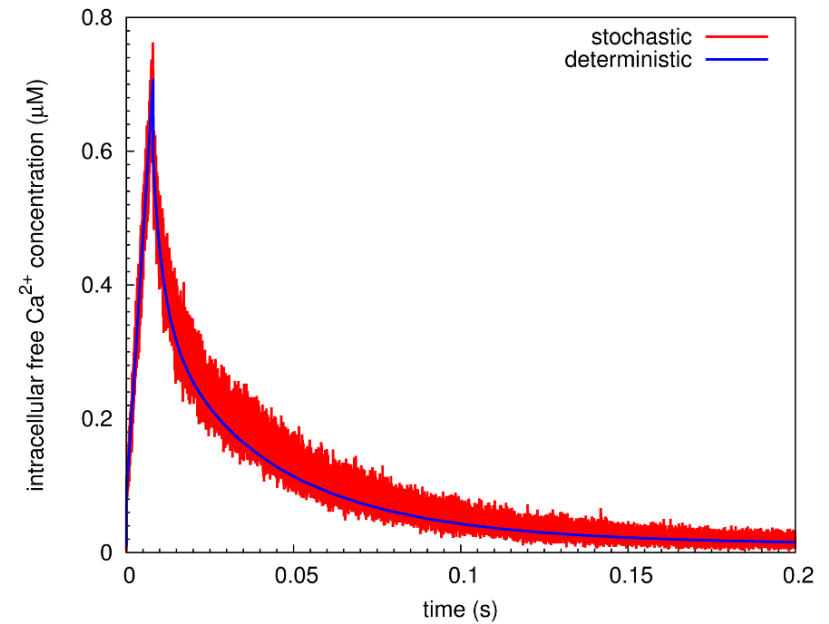
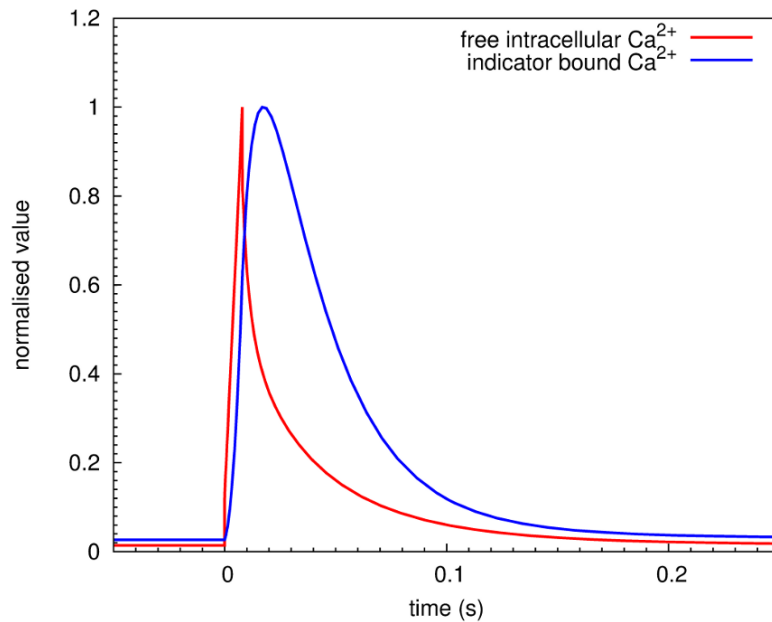
Stuart
Edelstein



Massimo
Lai



Are those spikes realistic?



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

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