

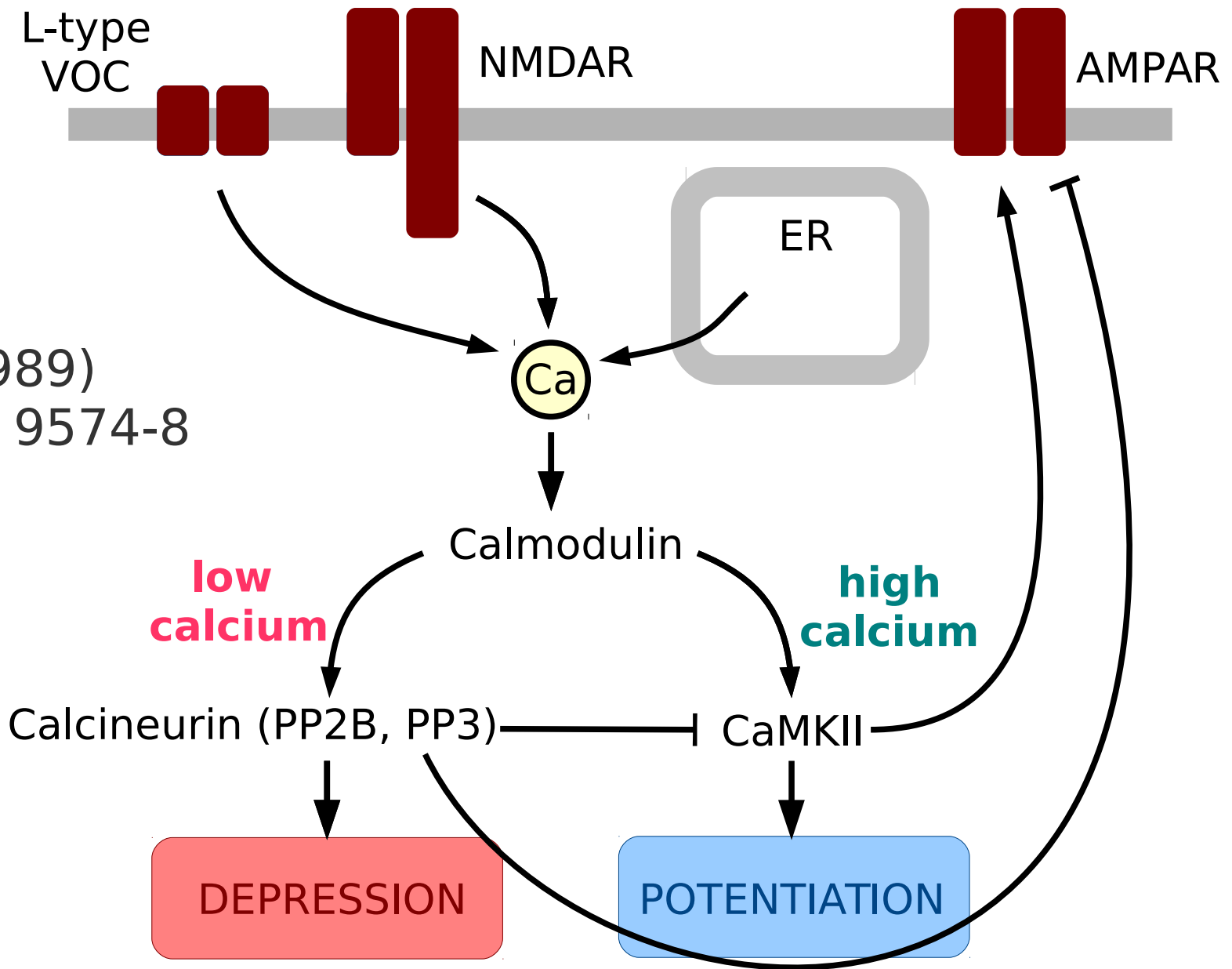
Allosteric calcium sensors in synaptic plasticity

*Nicolas Le Novère
Babraham Institute*

Feel free to

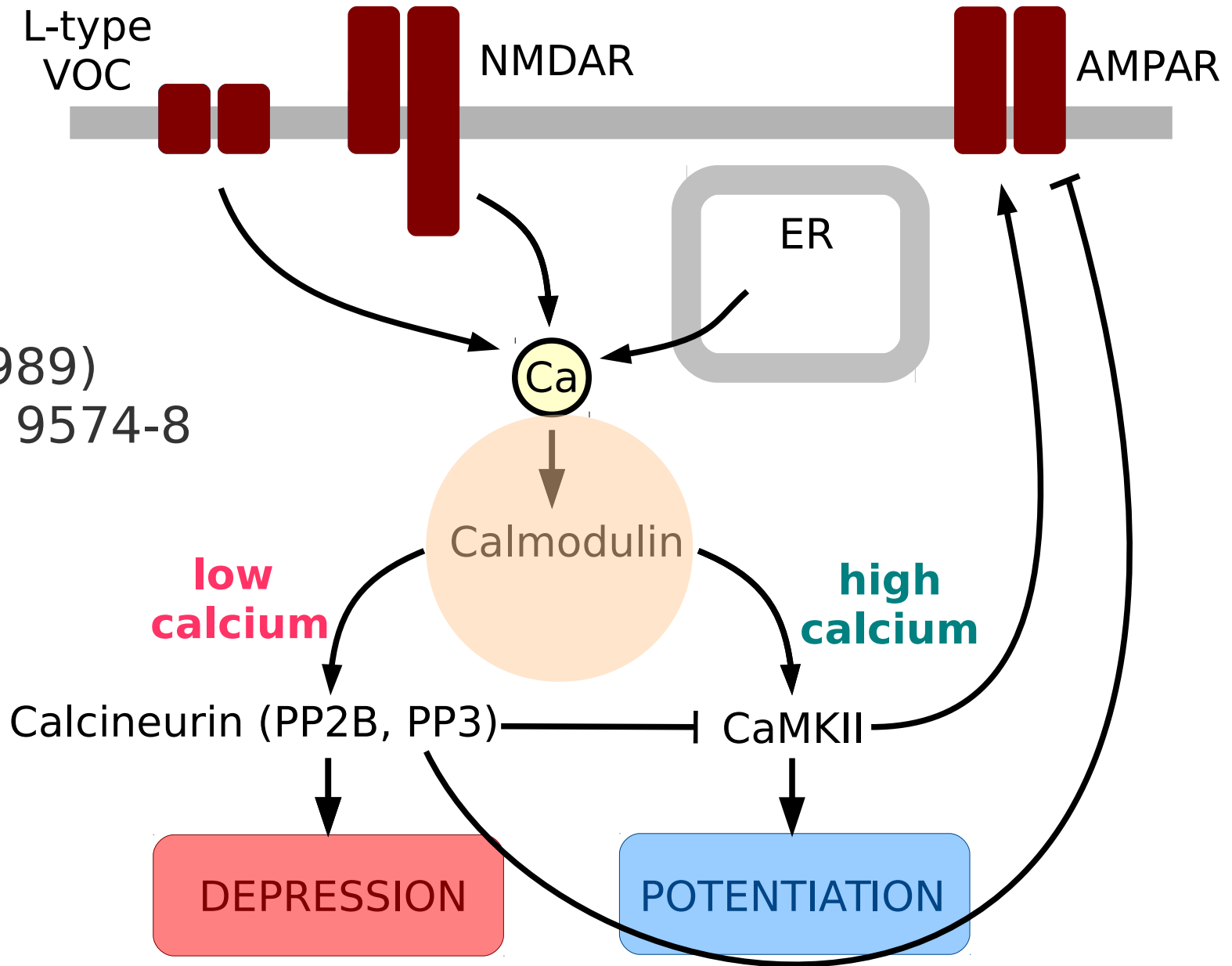


Calmodulin, the memory switchboard



Lisman (1989)
PNAS, 86: 9574-8

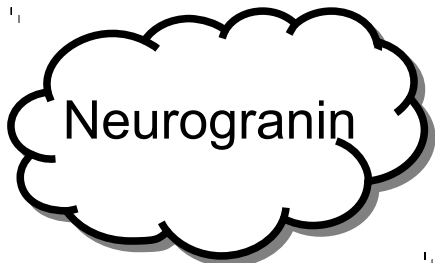
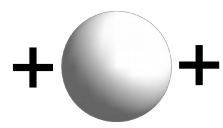
Calmodulin, the memory switchboard



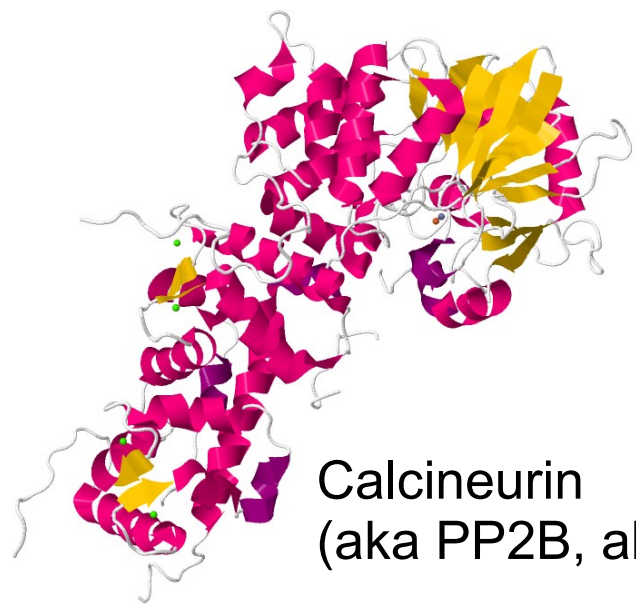
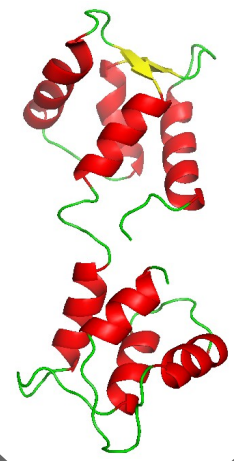
Lisman (1989)
PNAS, 86: 9574-8



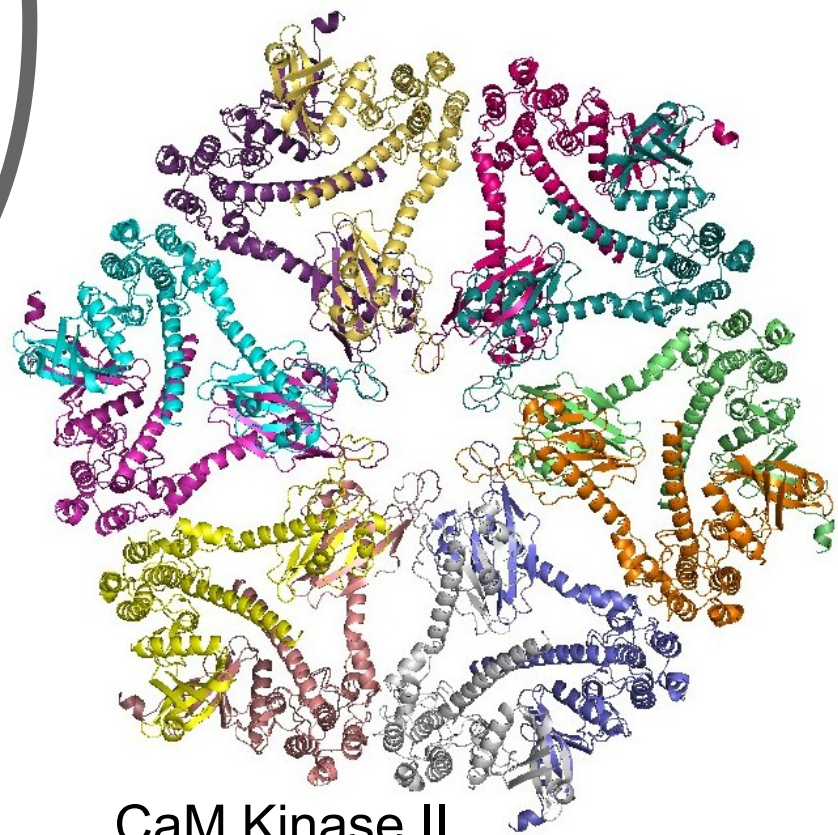
Calcium



Calmodulin

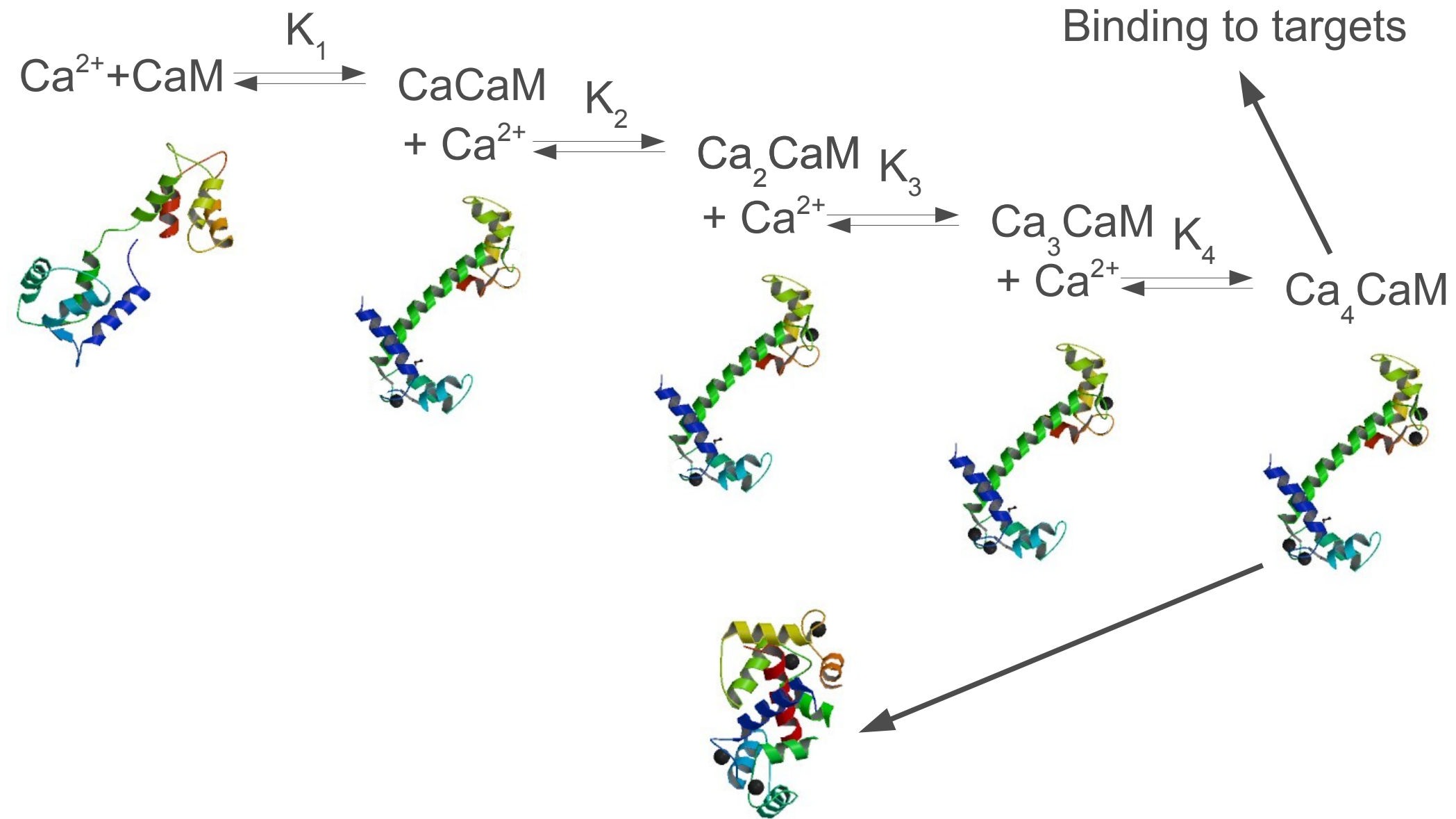


Calcineurin
(aka PP2B, aka PP3)

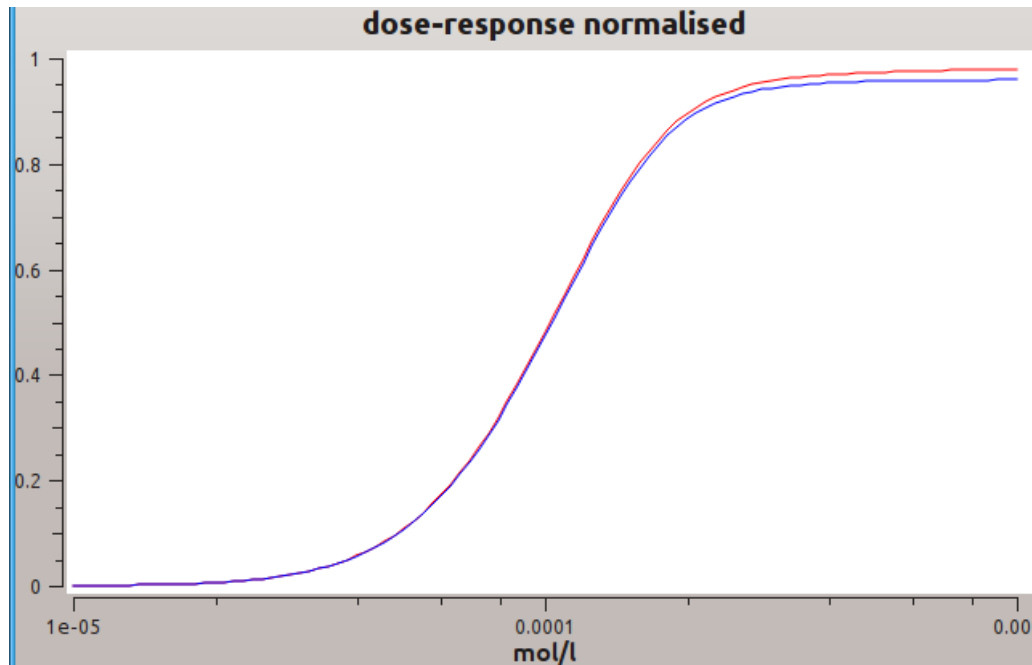


CaM Kinase II

Adair-Klotz induced-fit model



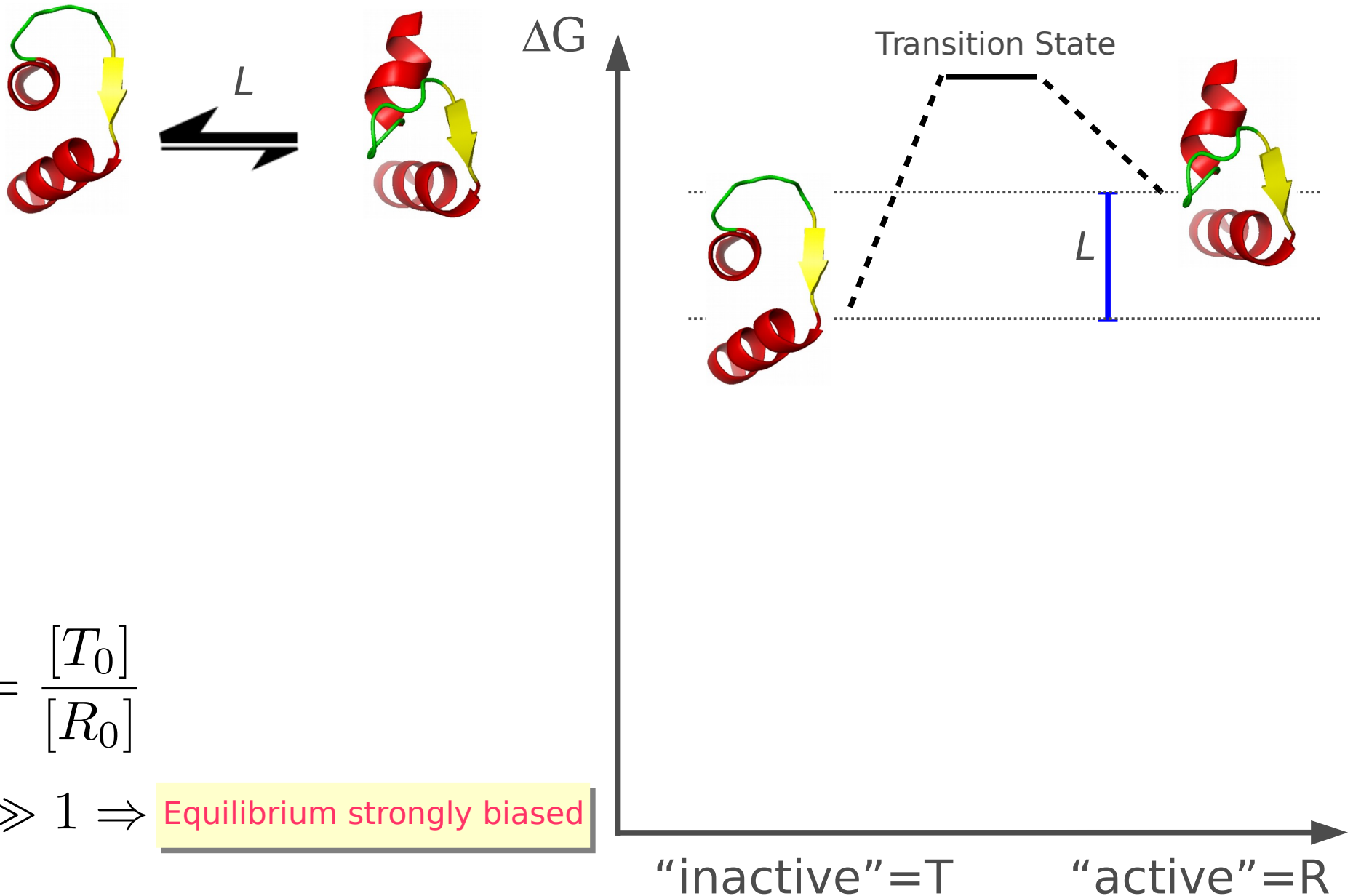
Adair-Klotz does not permit differential activations



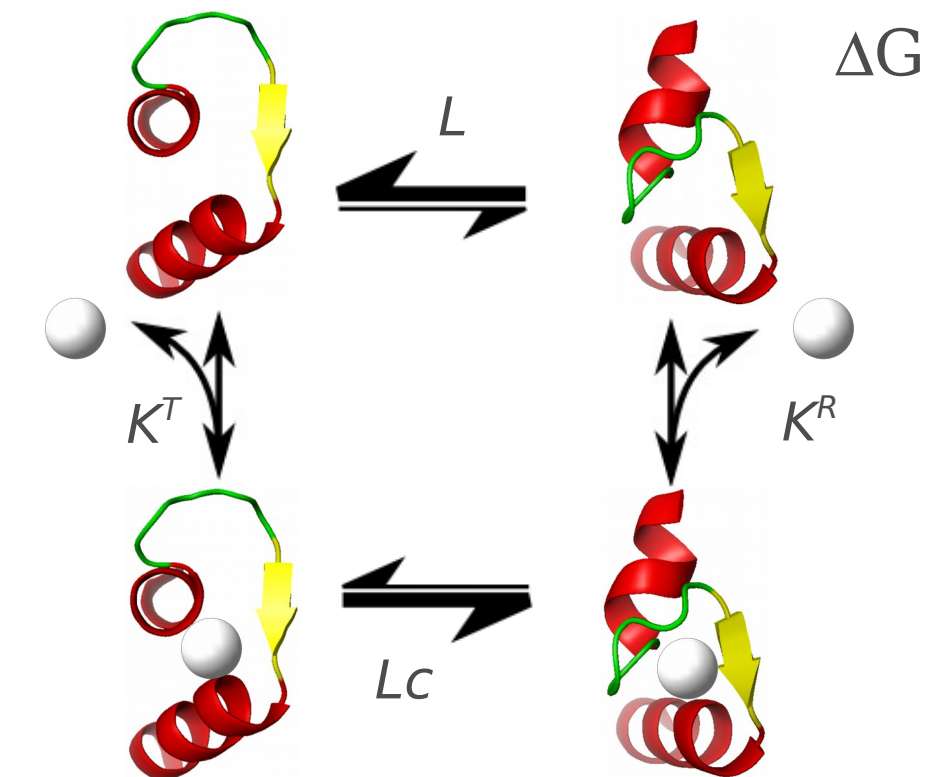
$$\begin{aligned} [\text{CaN}] &= [\text{CamKII}] = [\text{CaM}]/10 ; \\ Kd_{\text{CaMKII}} &= 10 \times Kd_{\text{CaN}} ; \end{aligned}$$

- 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



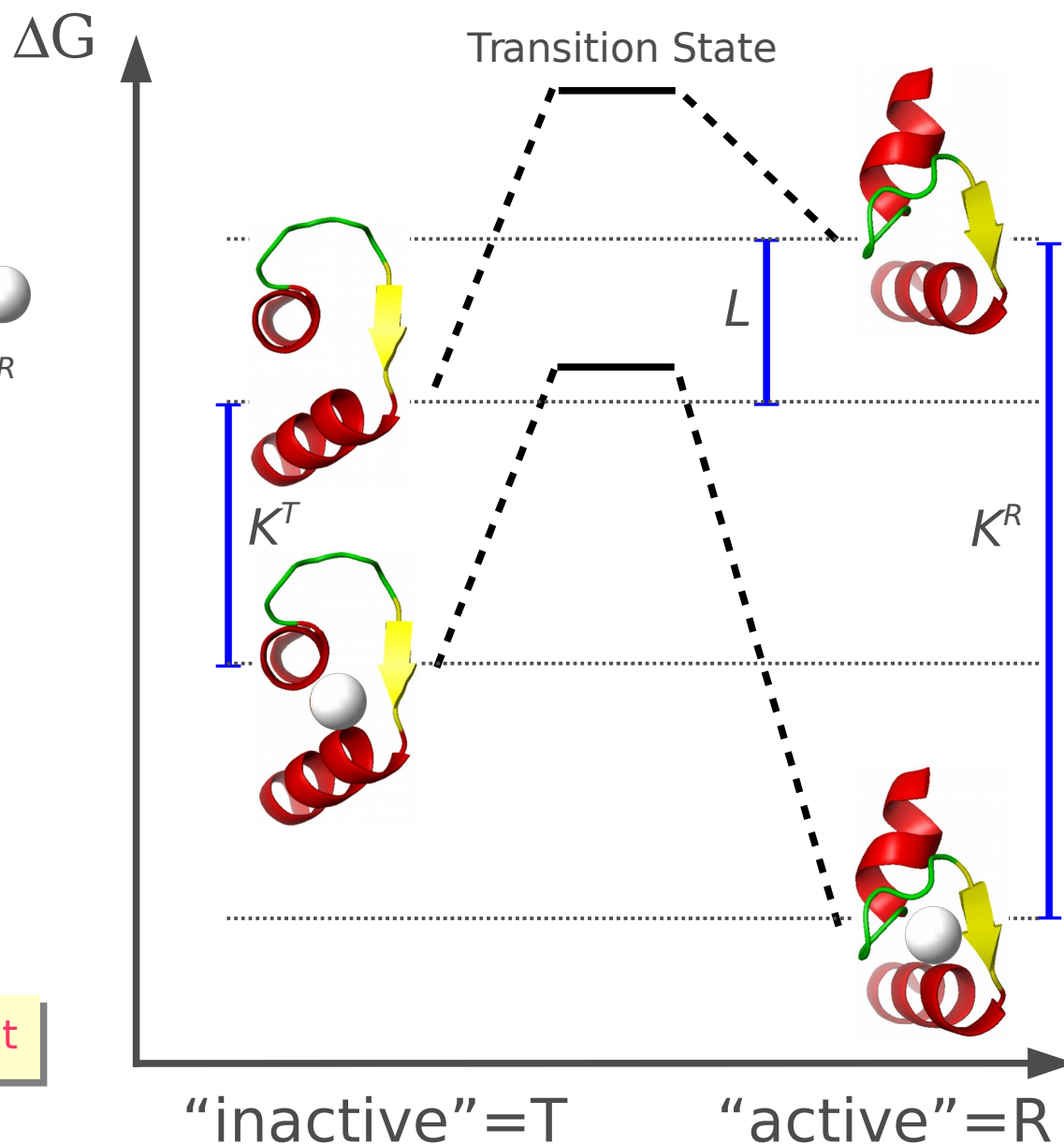
① Modulation of thermal equilibria $\neq$ induced-fit



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

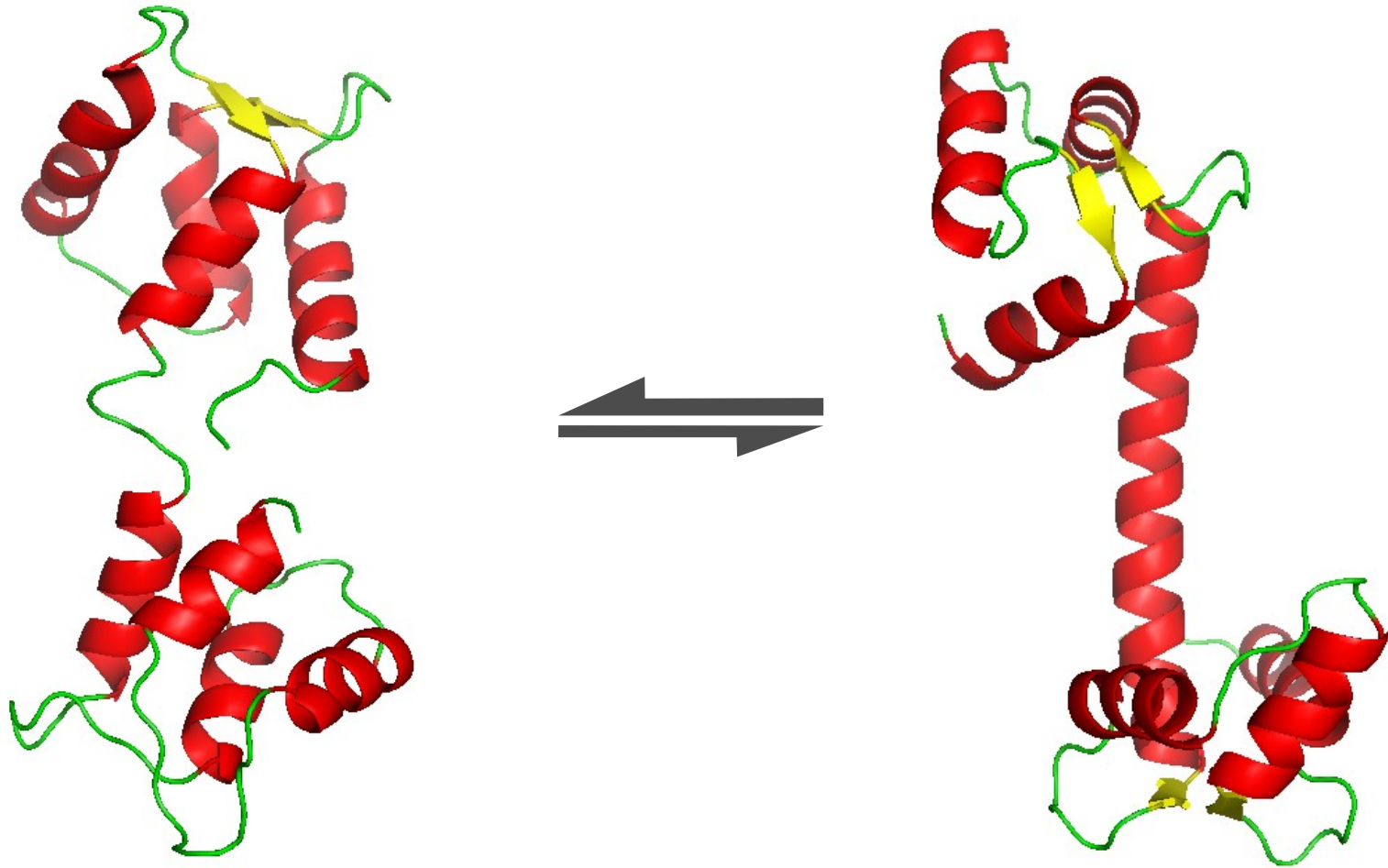
$$c = \frac{K^R}{K^T}$$

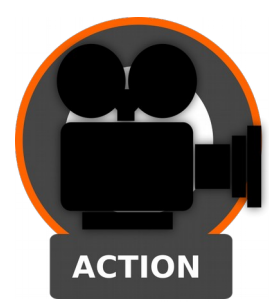
$c \ll 1 \Rightarrow$ ligand has strong effect



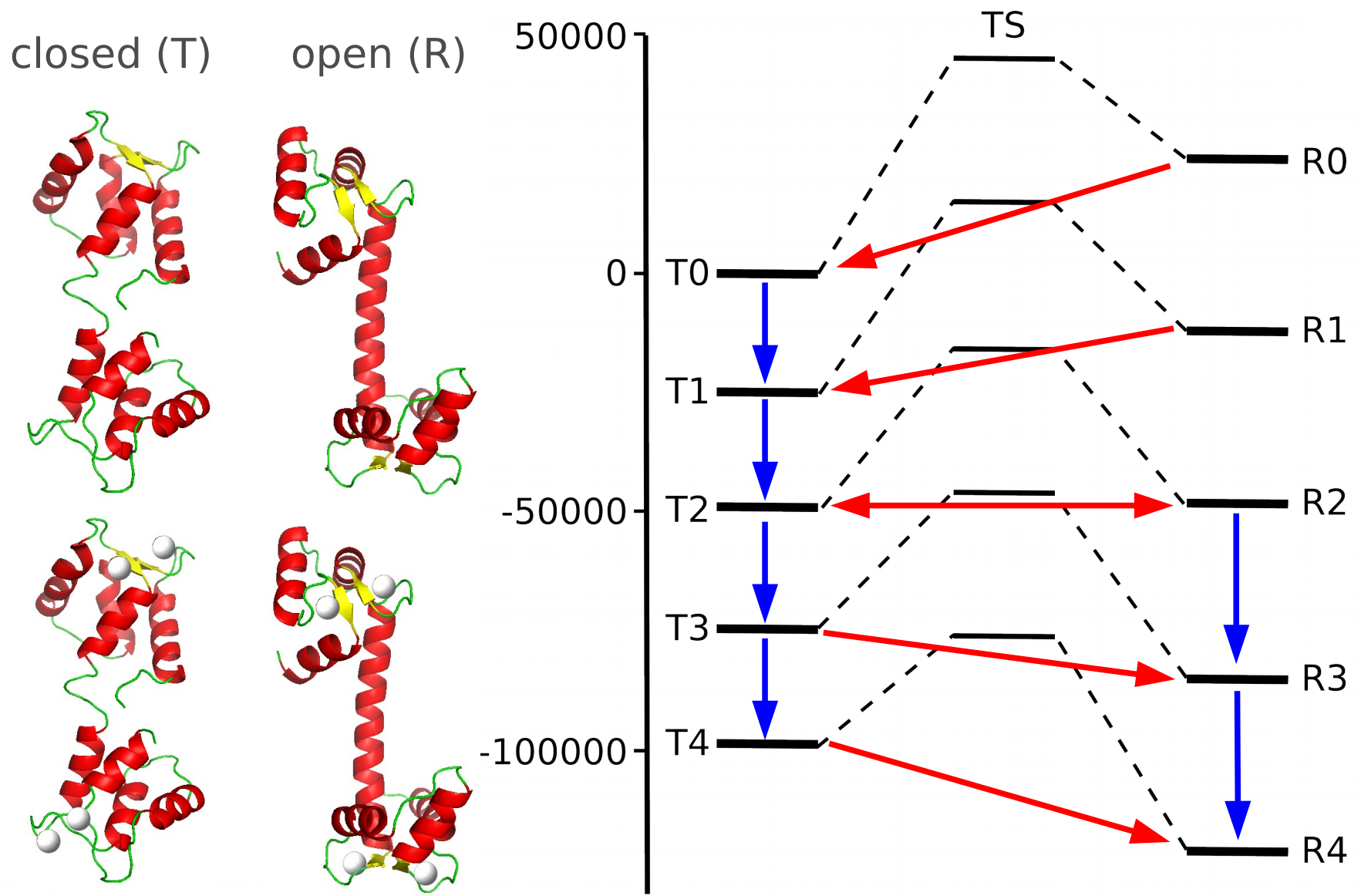
2

Concerted transitions $\neq$ sequential model

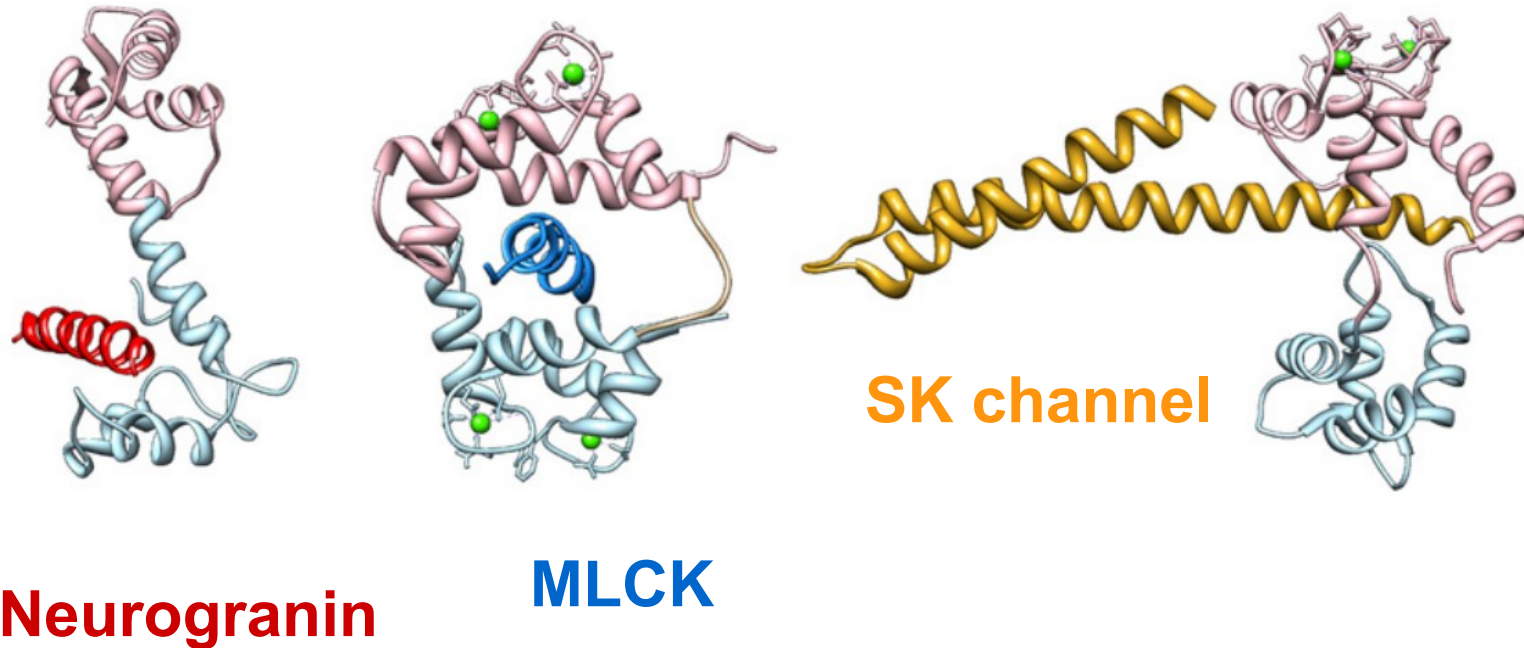




Allosteric model of Calmodulin activation

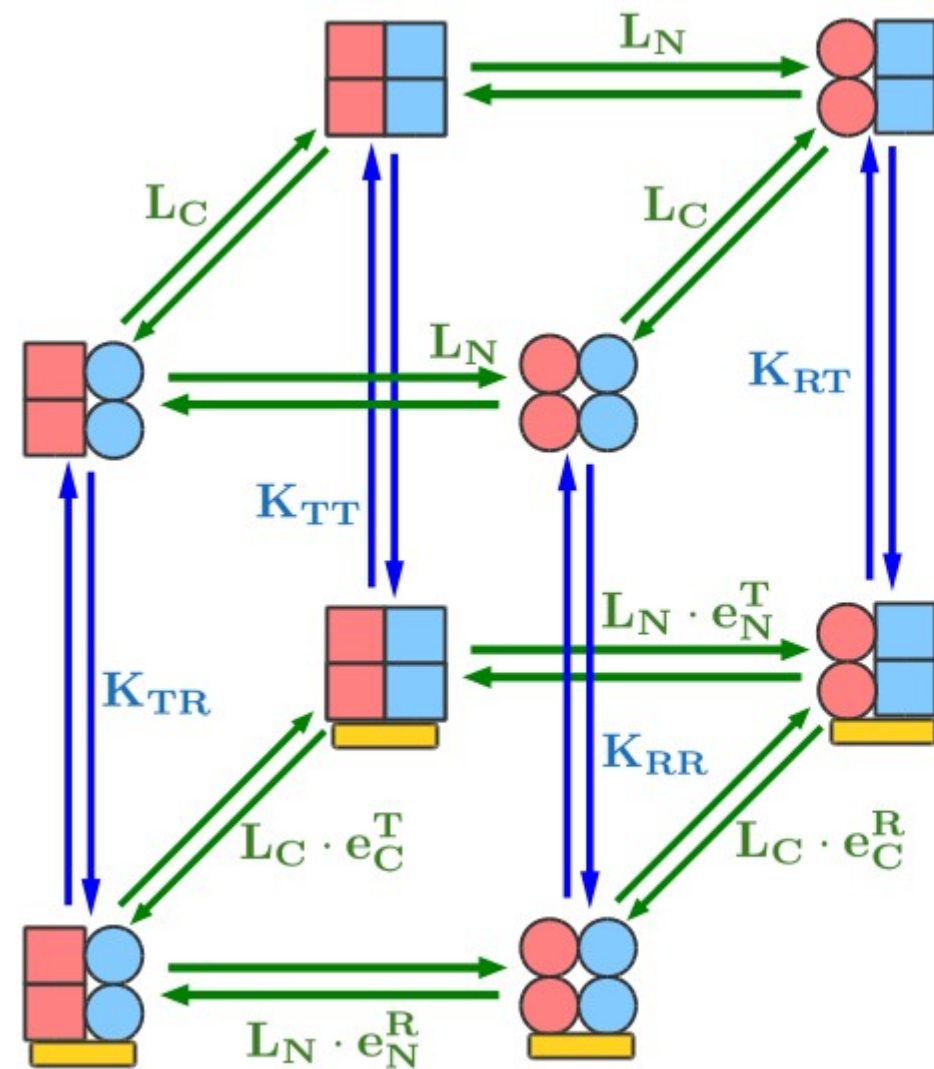


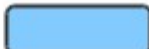
Different targets stabilise lobes in different states



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

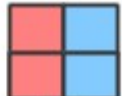
Hemiconcerted model of calmodulin

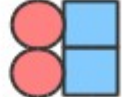


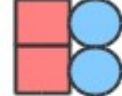
 = N-lobe
 = C-lobe

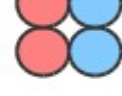
 = T-state
 = R-state

 = target

 = TT conformation

 = RT conformation

 = TR conformation

 = RR conformation

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

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

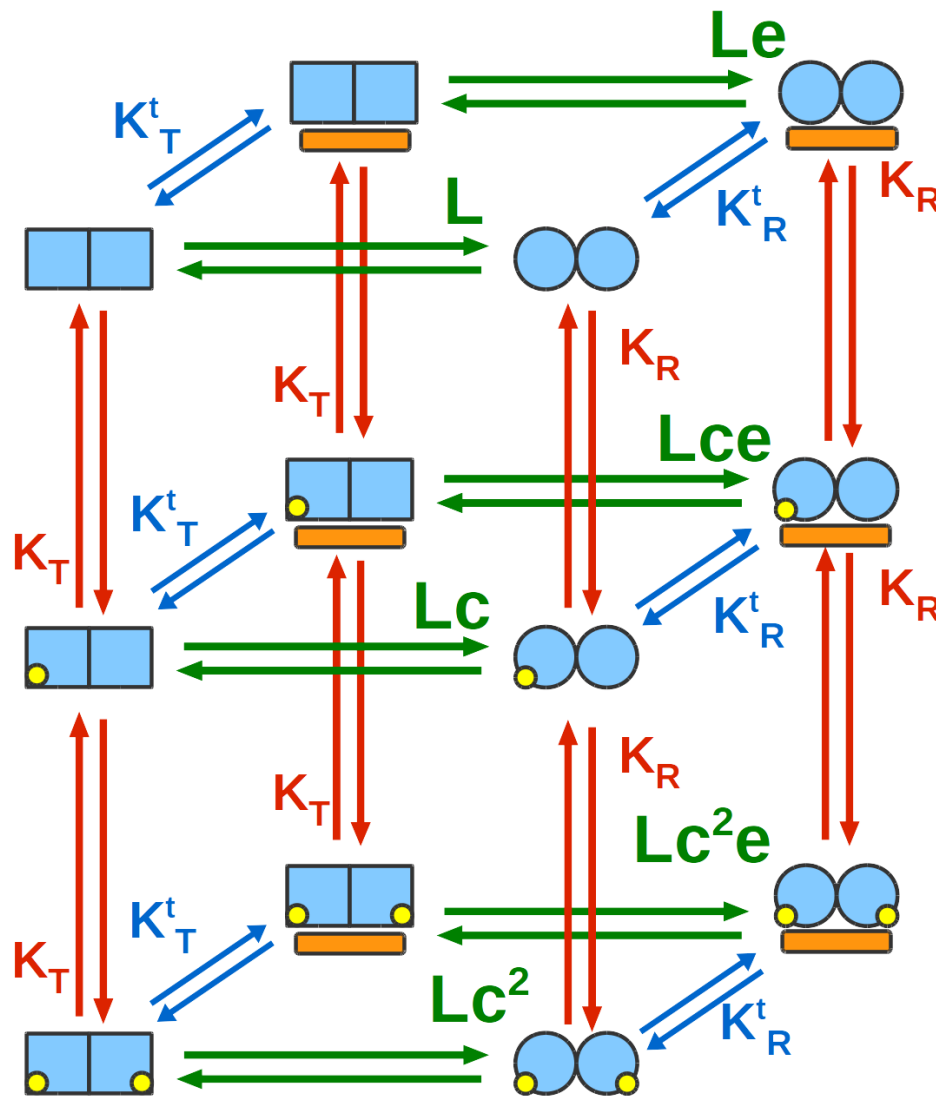
$$e_N^R = \frac{K_{RR}}{K_{TR}}$$

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

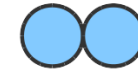
$$e_N^T = \frac{K_{RT}}{K_{TT}}$$

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

Bindings of calcium and targets



T-state



R-state



Calcium



Target



Conf. transition



Calcium binding

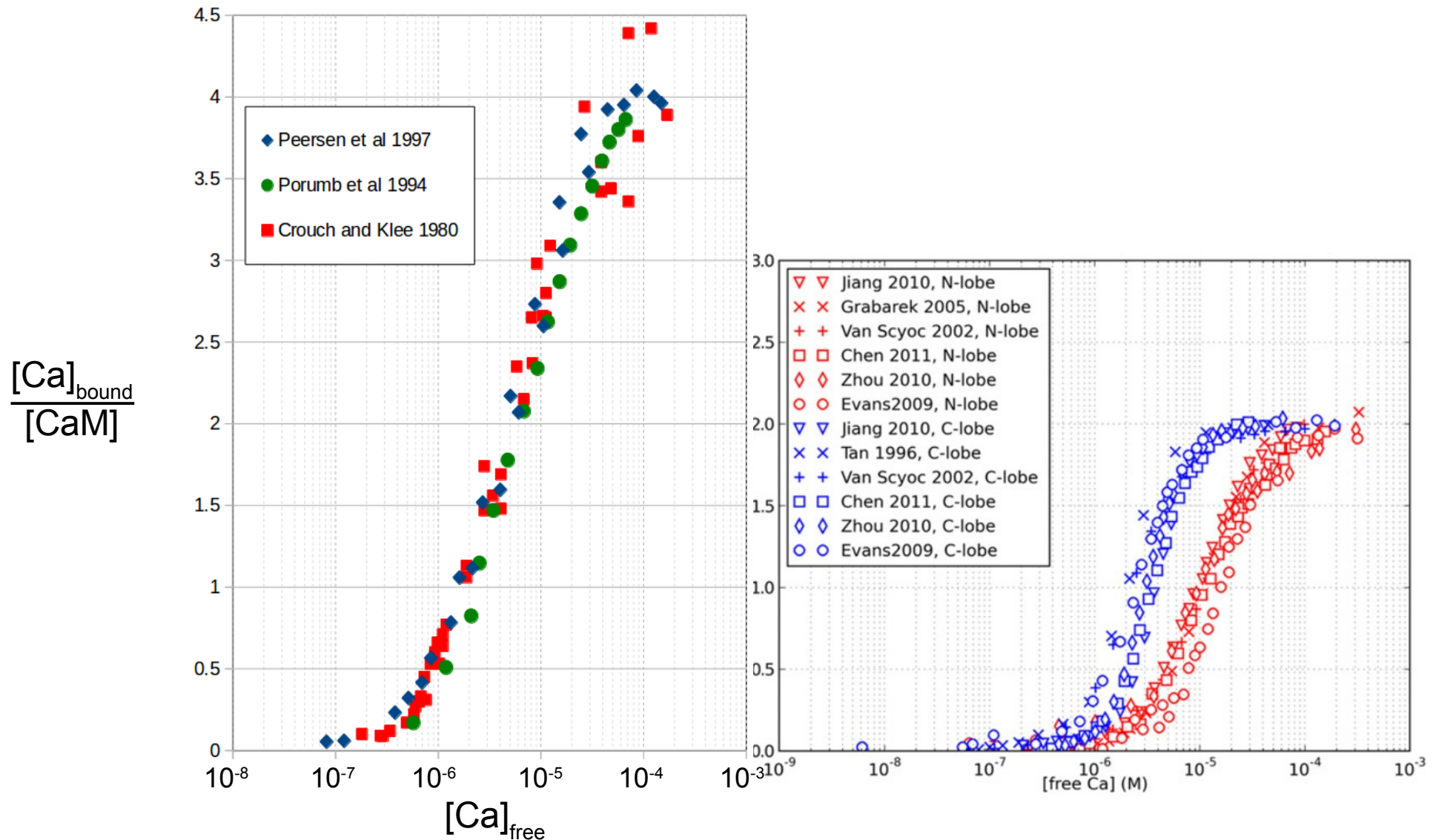


Target binding

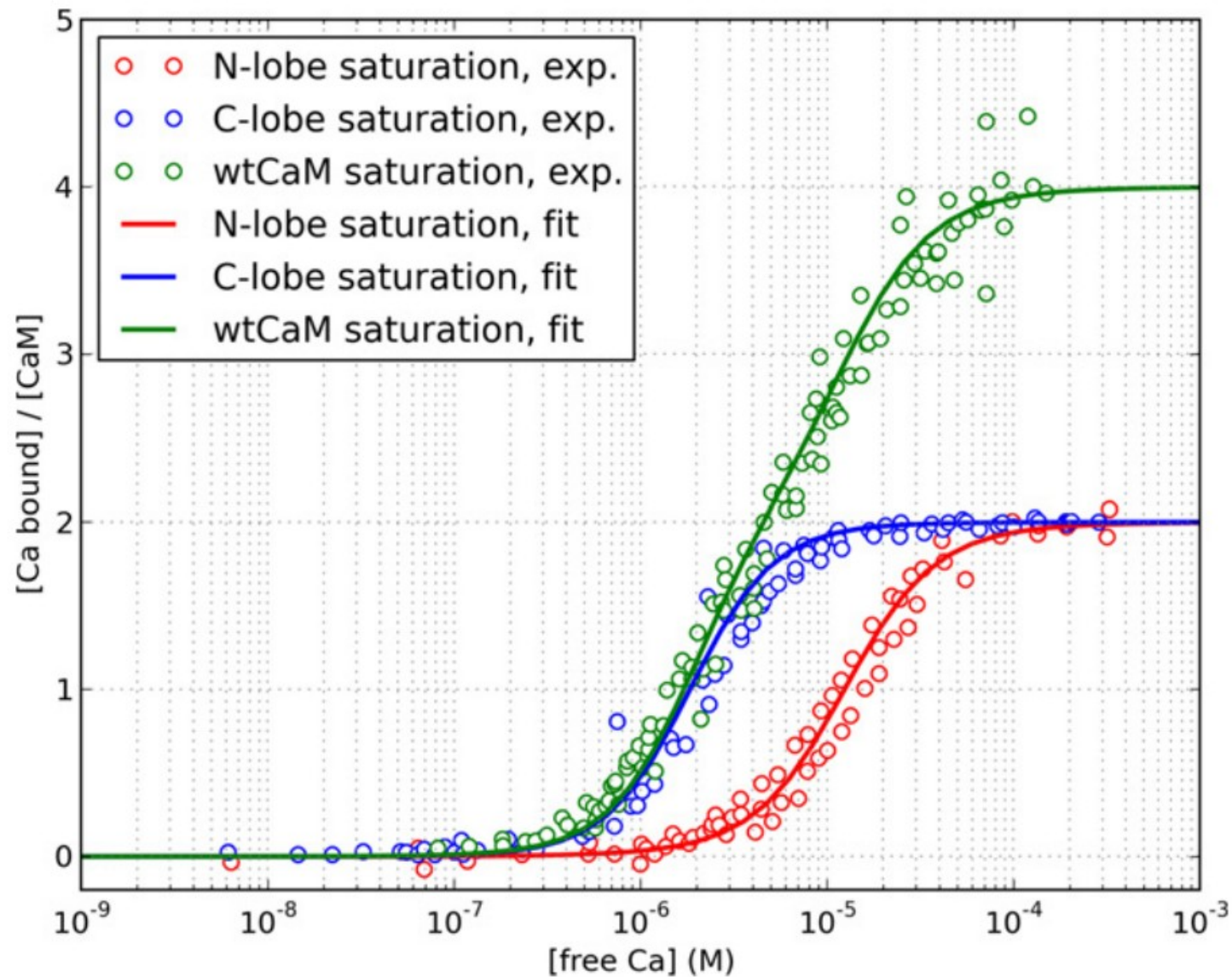
$$c = \frac{K_R}{K_T}$$

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

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

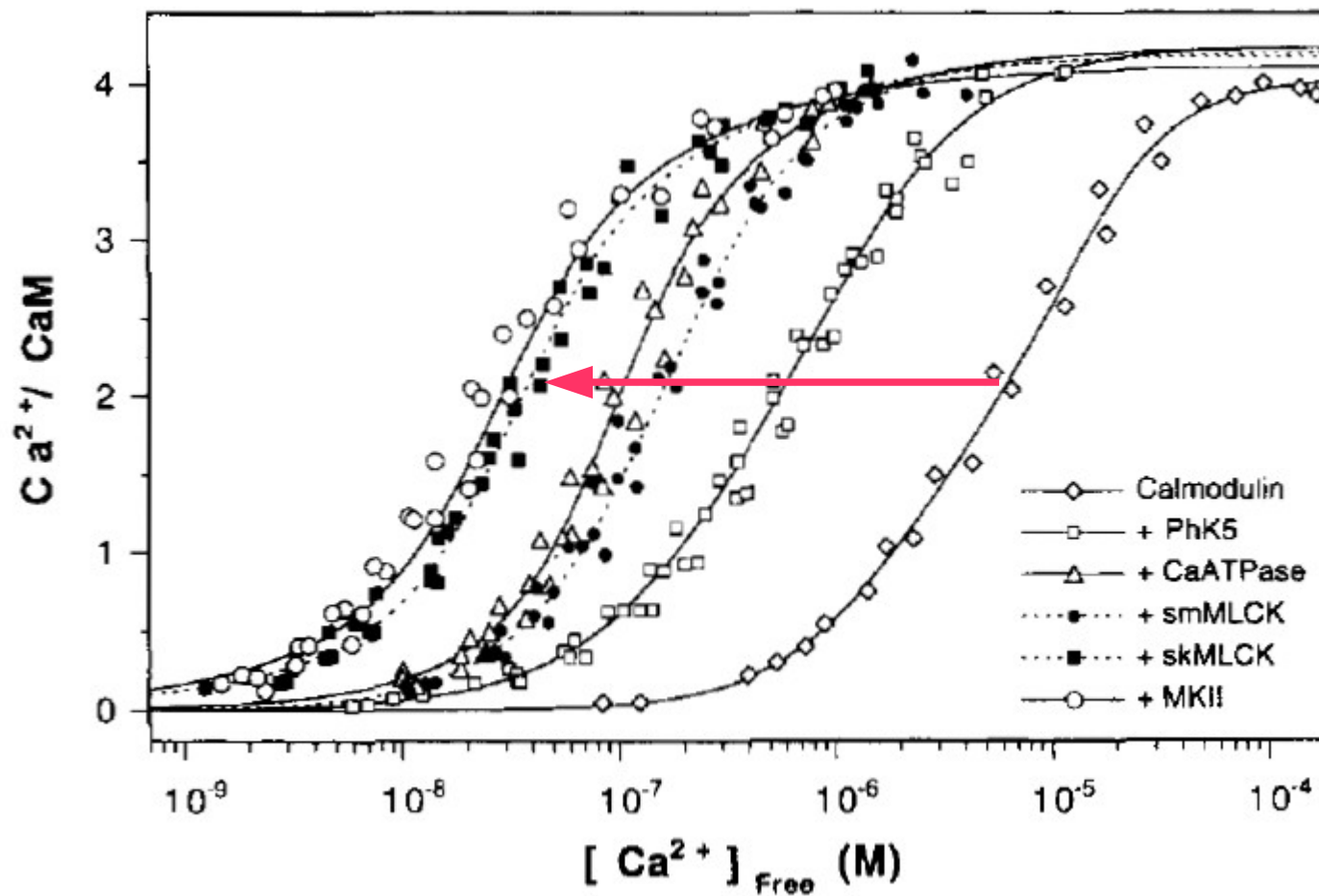
Activity of unsaturated calmodulin (state function)

- 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

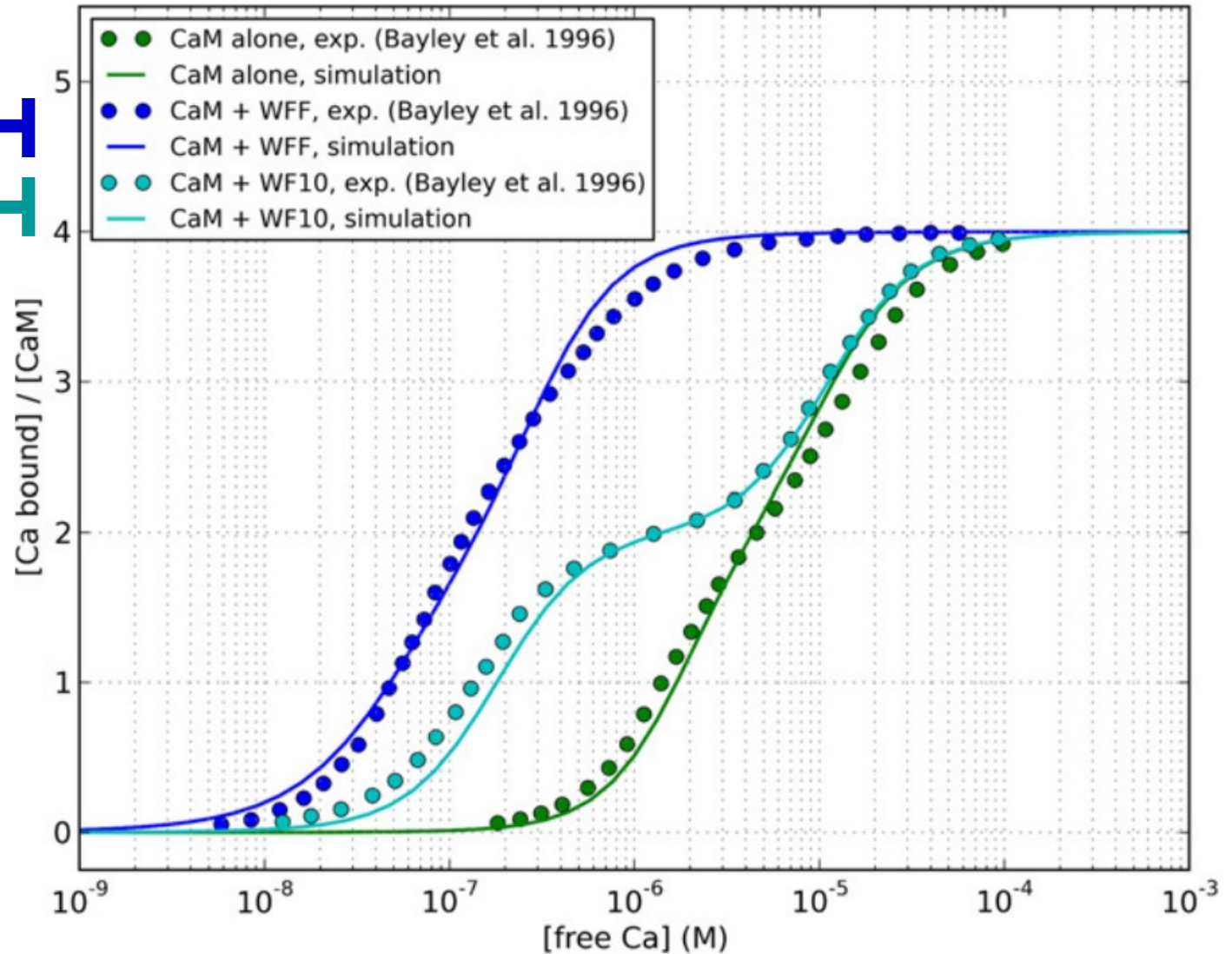


Peersen et al. (1997)

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

Stabilise R state
of both lobes

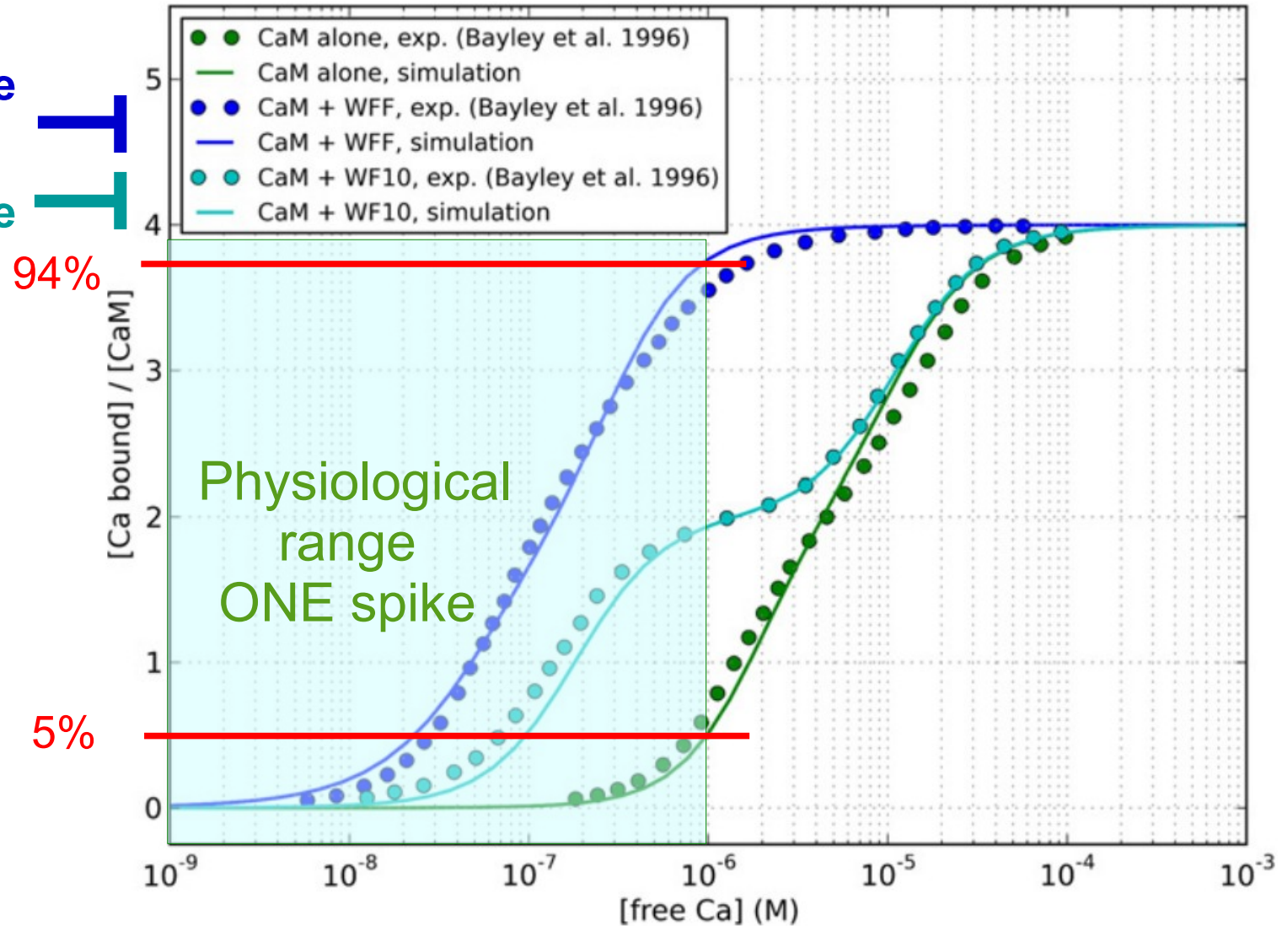
Stabilise R state
of C lobe



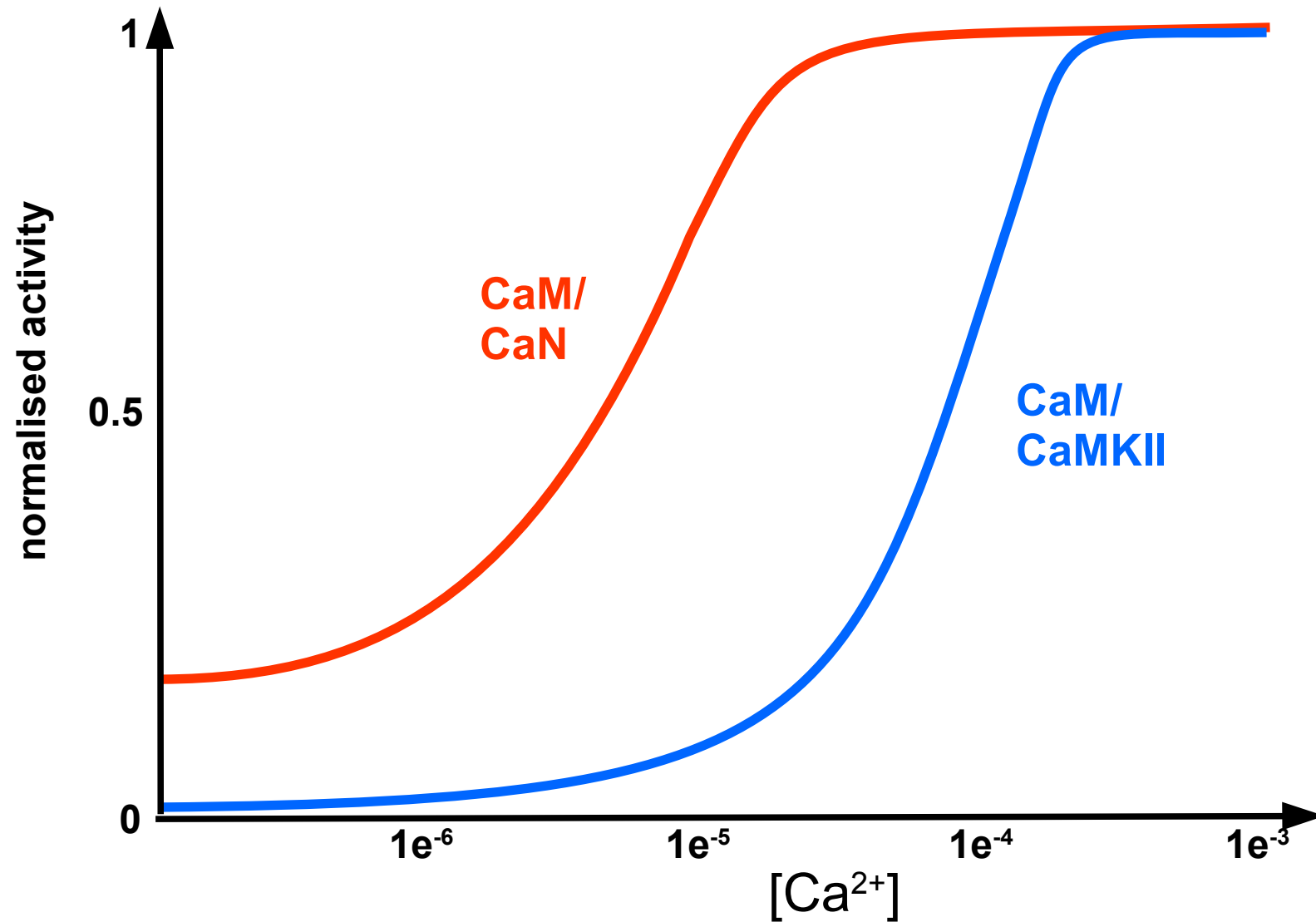
Targets stabilises Ca^{2+} binding into the physiological range

Stabilise R state of both lobes

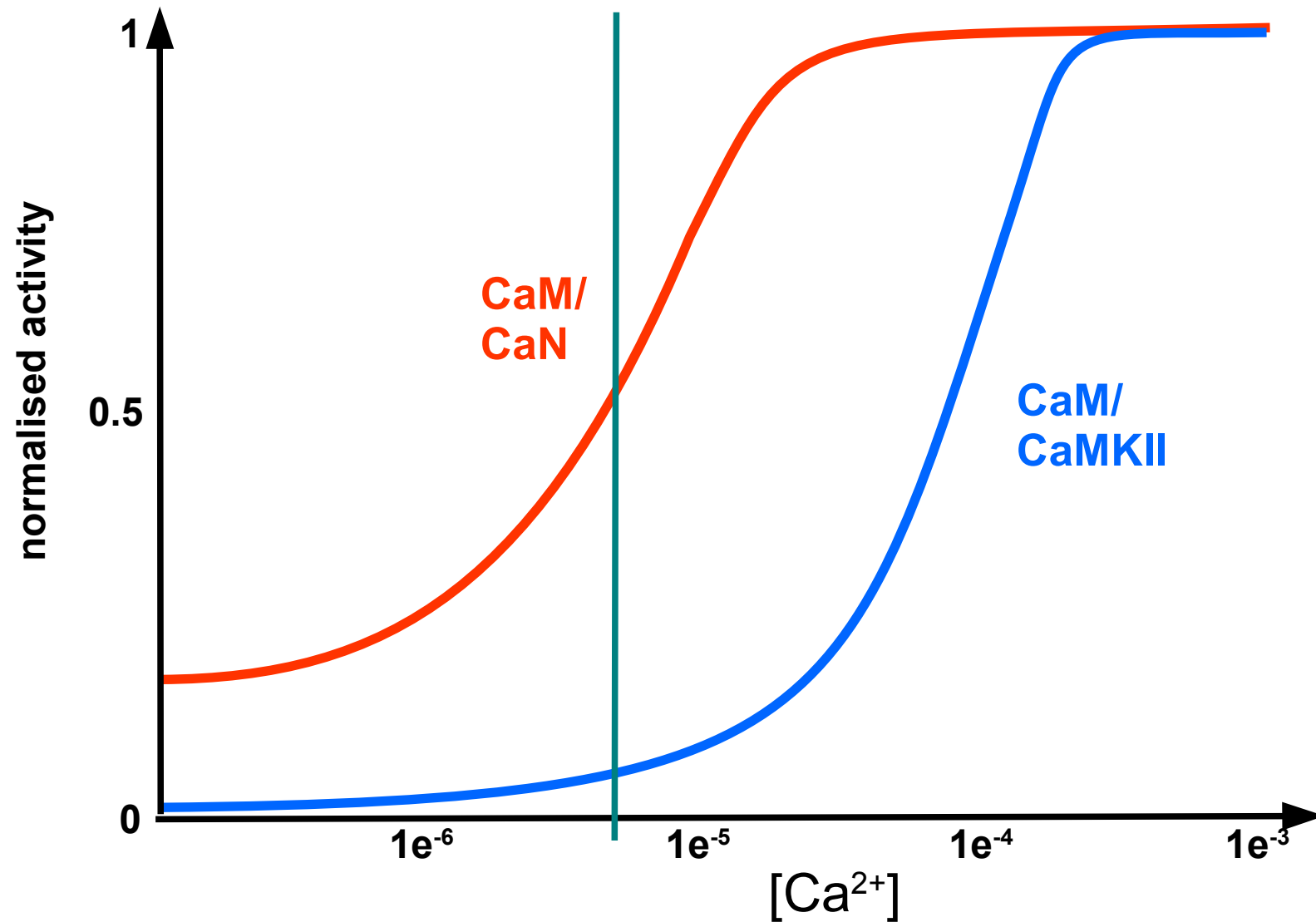
Stabilise R state of C lobe



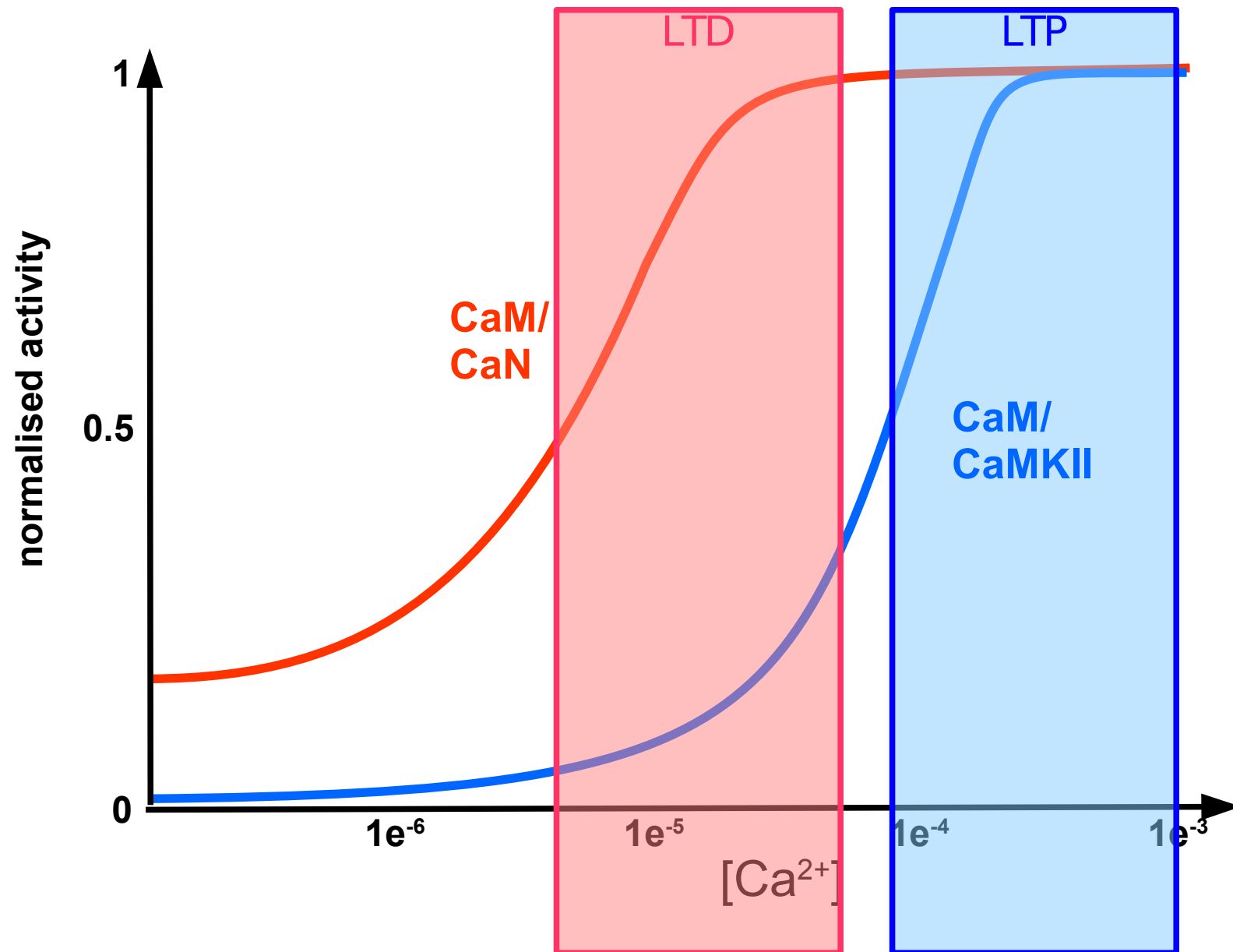
Calmodulin its ligand and its targets



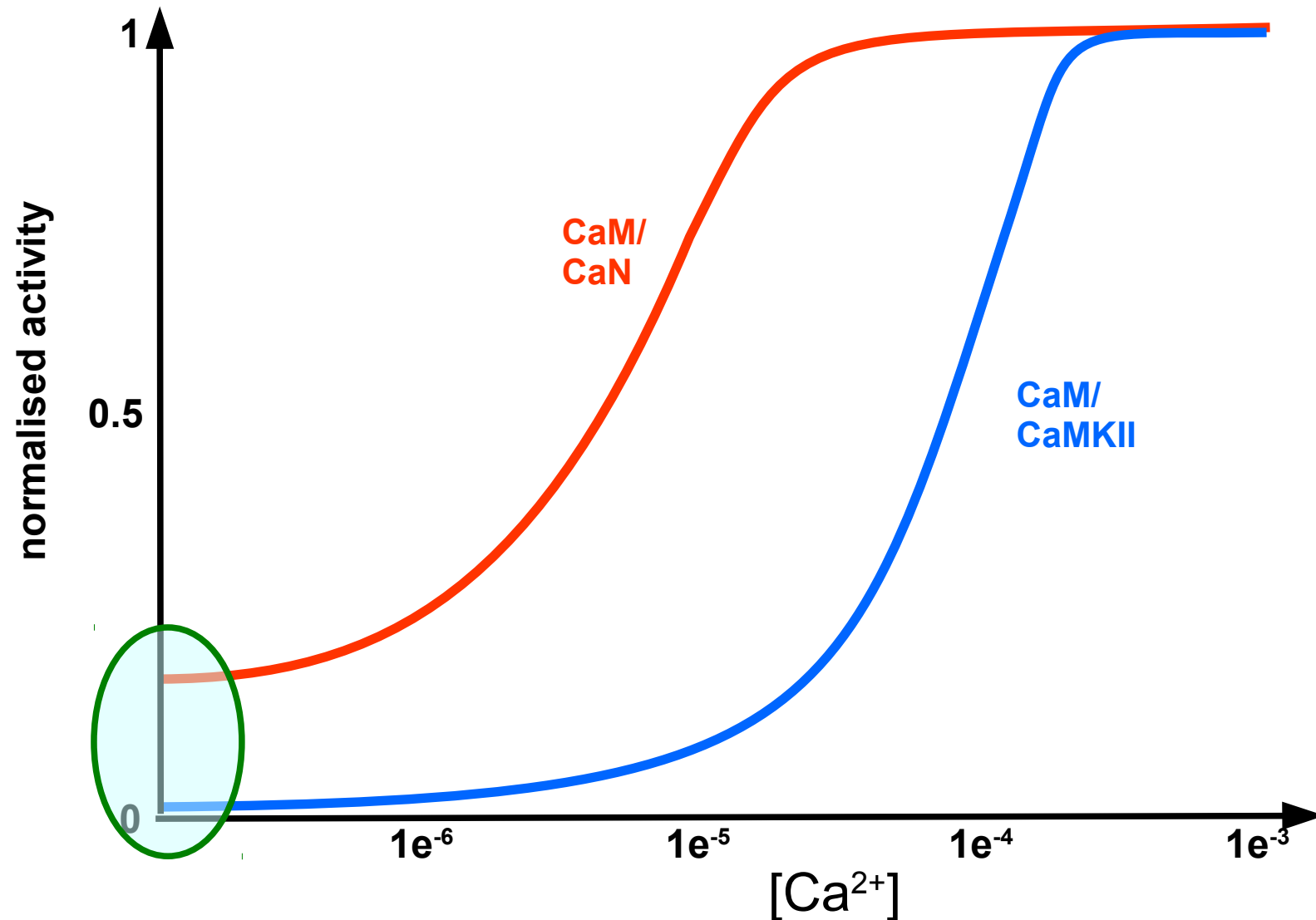
CaM half activated at half saturation of Calmodulin



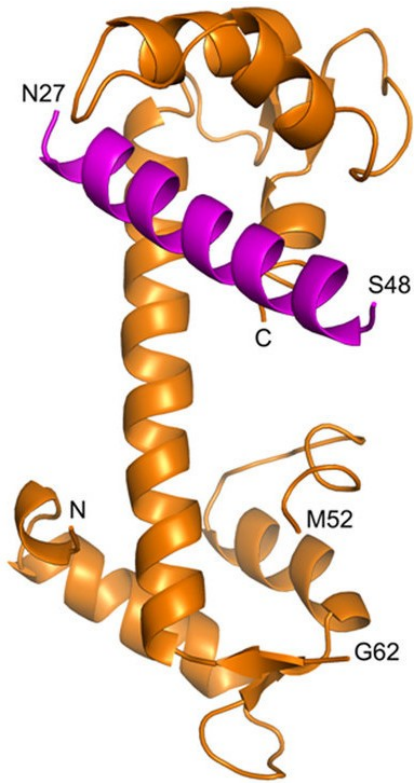
Bidirectional synaptic plasticity



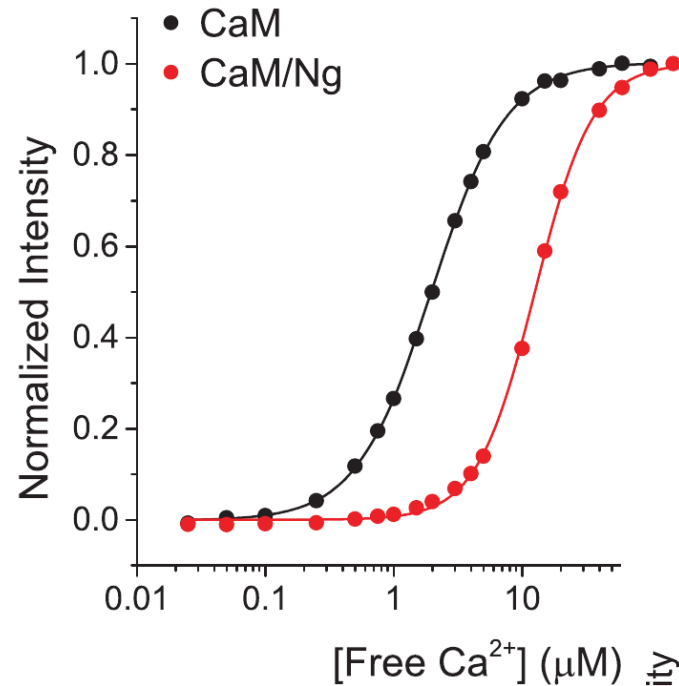
Calcineurin stabilises CaM R → no deactivation



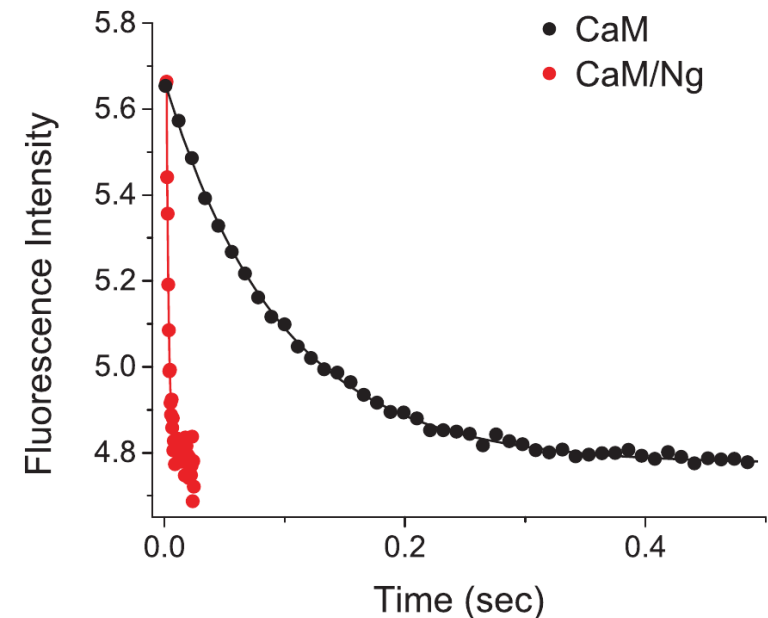
Neurogranin binds to apo-CaM, decrease affinity for $[Ca^{2+}]$ and increase dissociation rate



Kumar *et al* (2013)



Hoffman *et al* (2014)



Effect of R and T stabilising targets on CaM affinity

Bayley *et al* (1996)

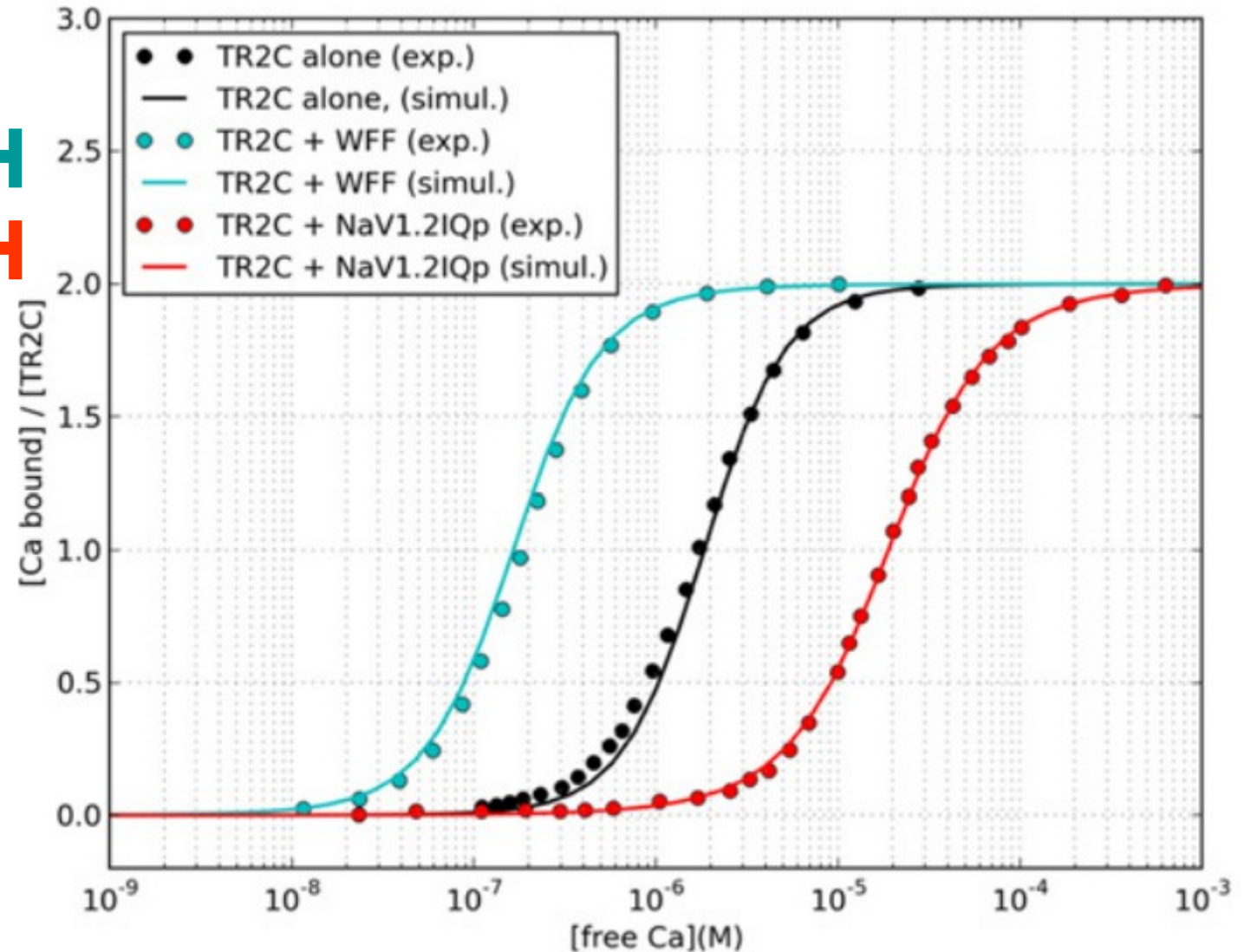
R stabilising
peptide

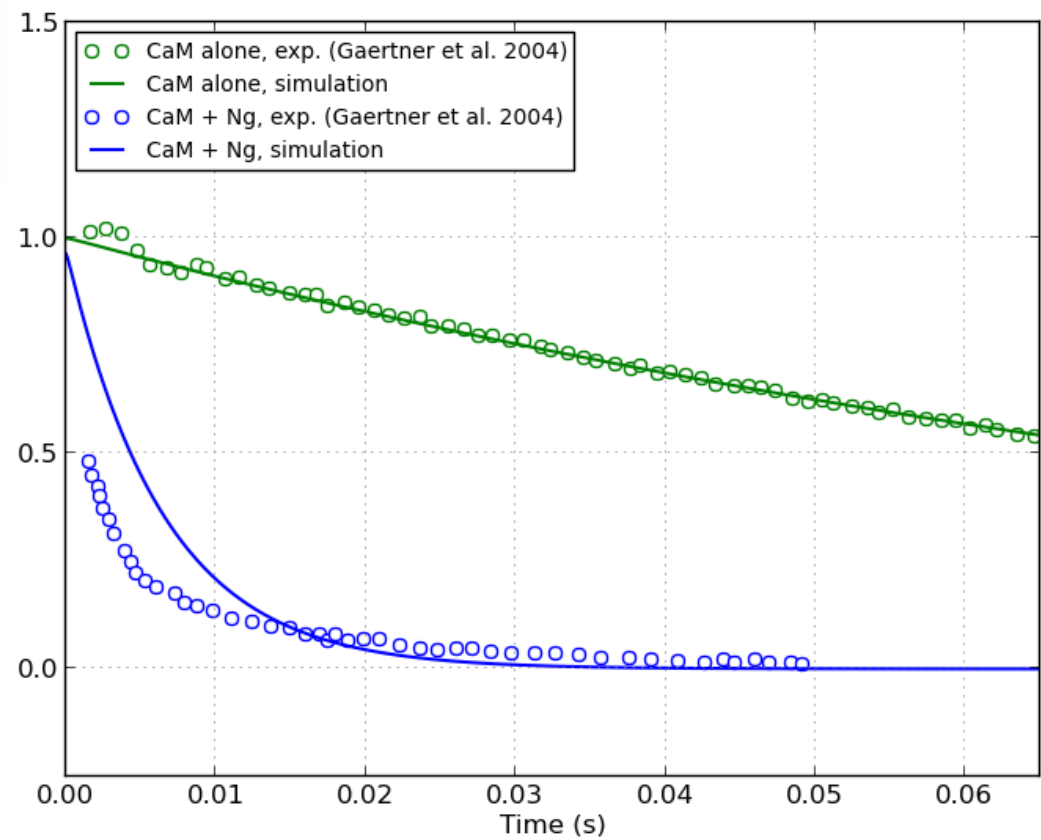
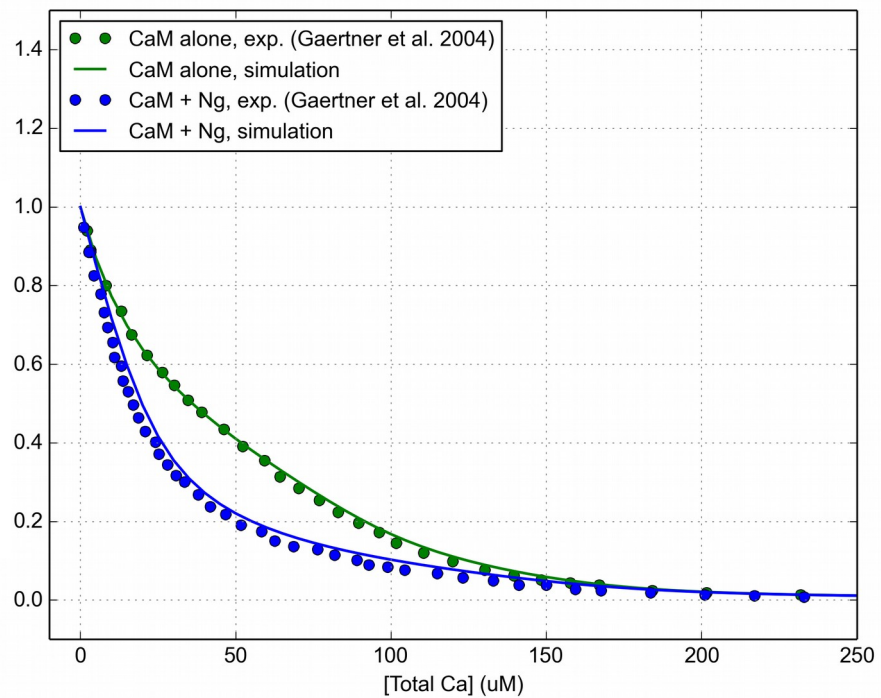


T stabilising
Peptide (IQ domain)

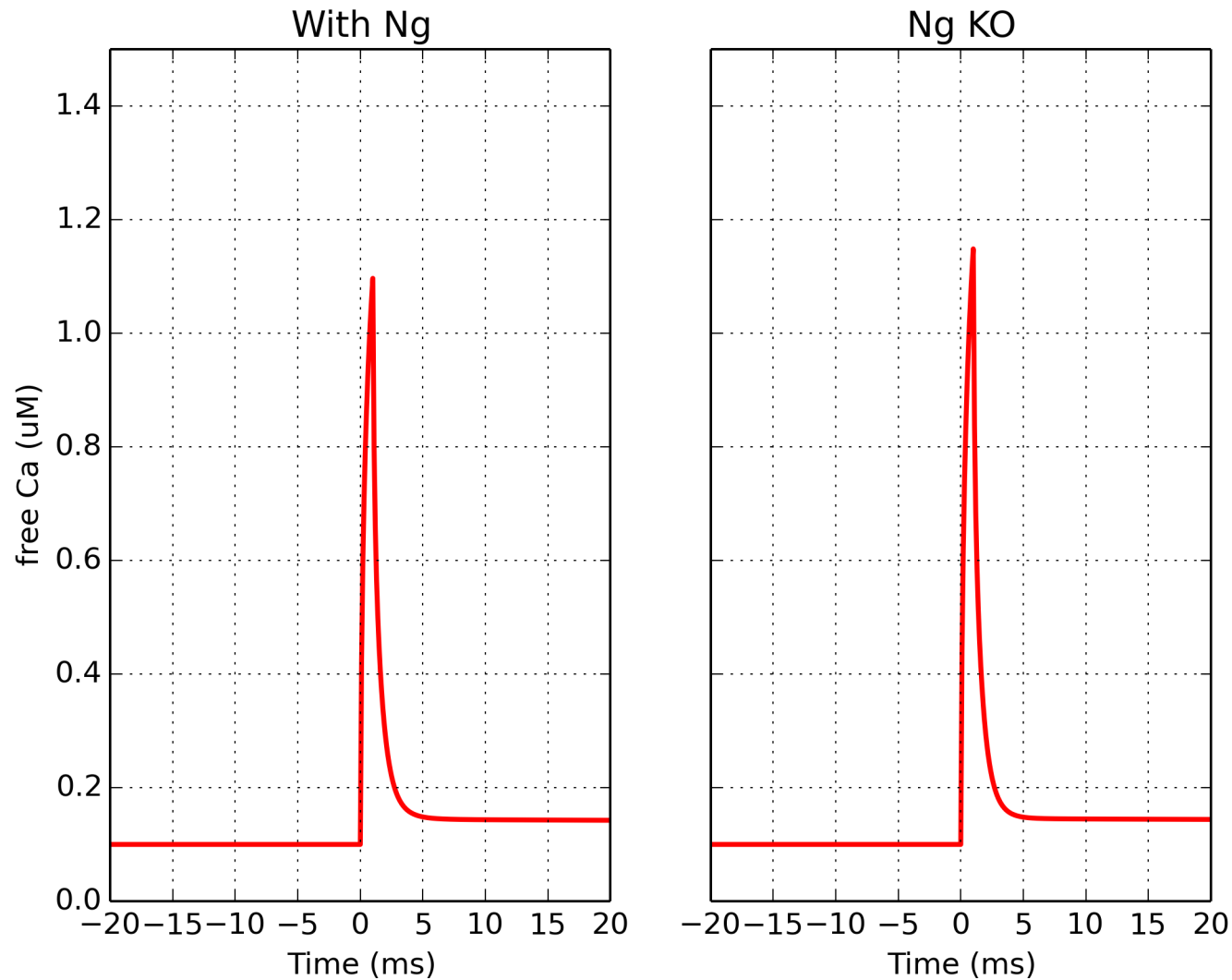


Theoharis *et al* (2008)

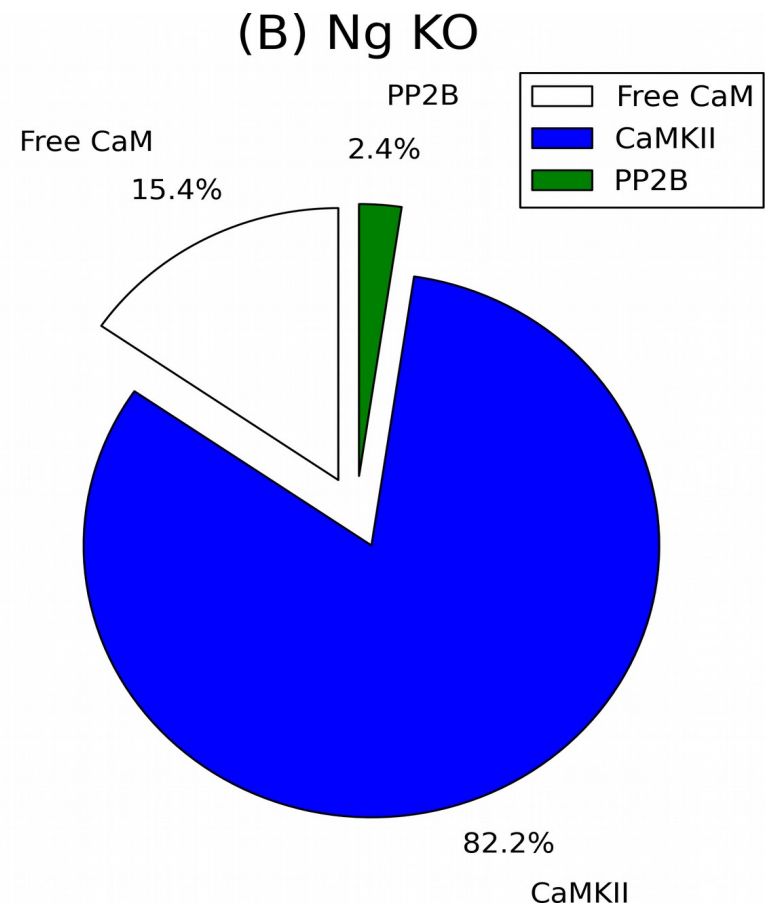
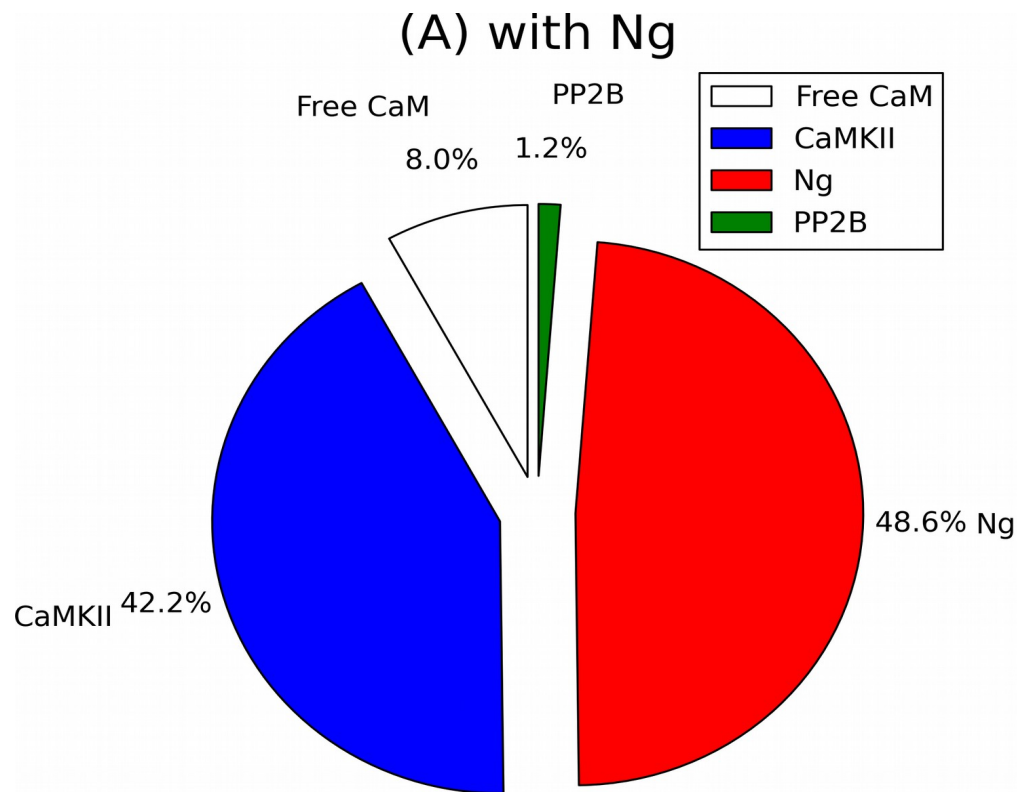




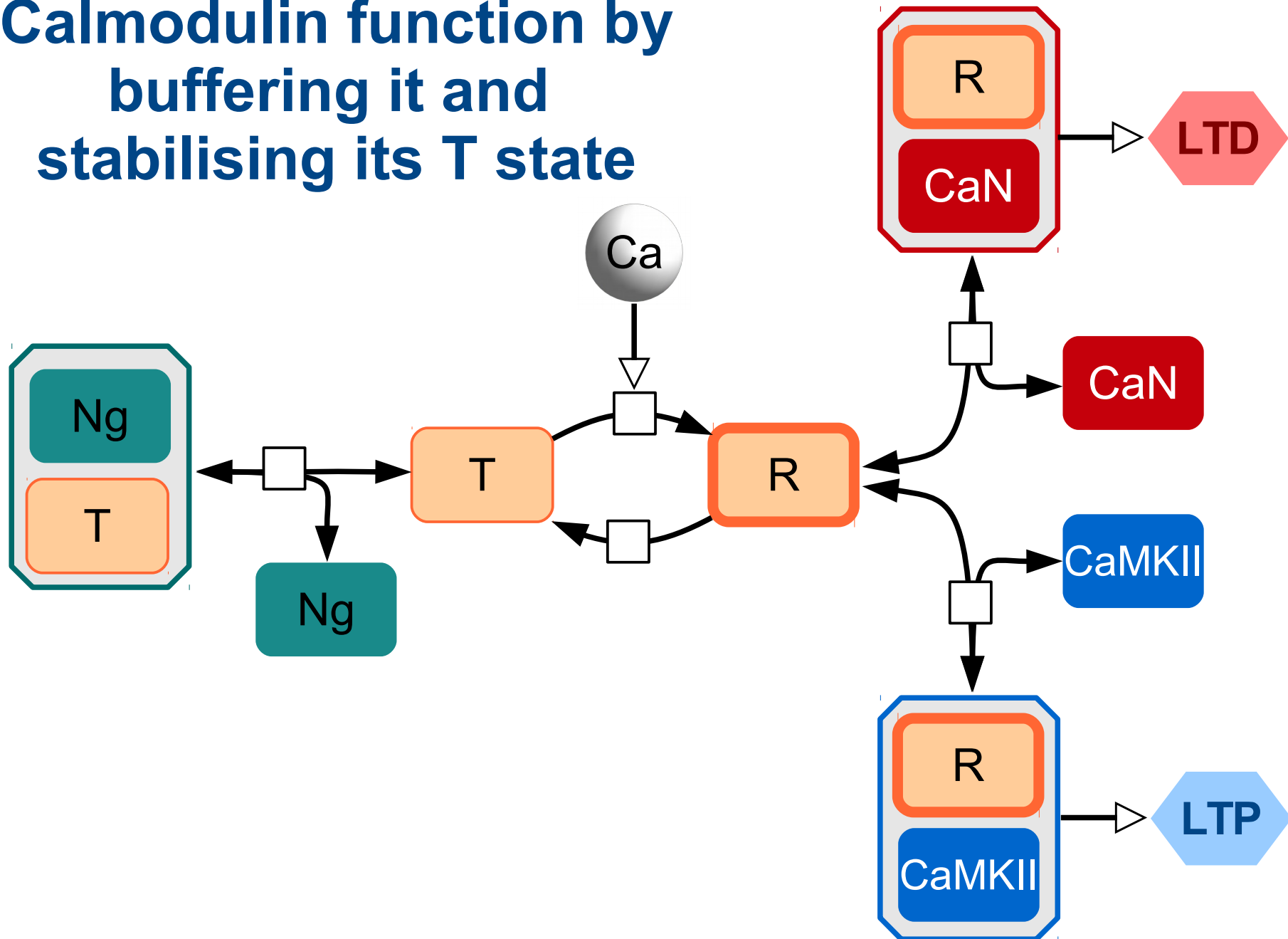
No large effect of Ng on $[Ca^{2+}]_{free}$



Ng affects the distribution of CaM



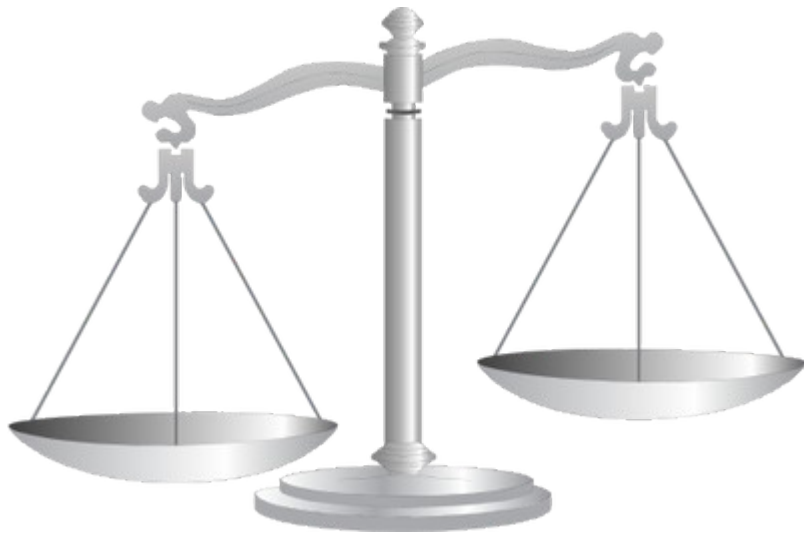
Neurogranin affects Calmodulin function by buffering it and stabilising its T state



Wait a minute!

Signal transduction is not at equilibrium!

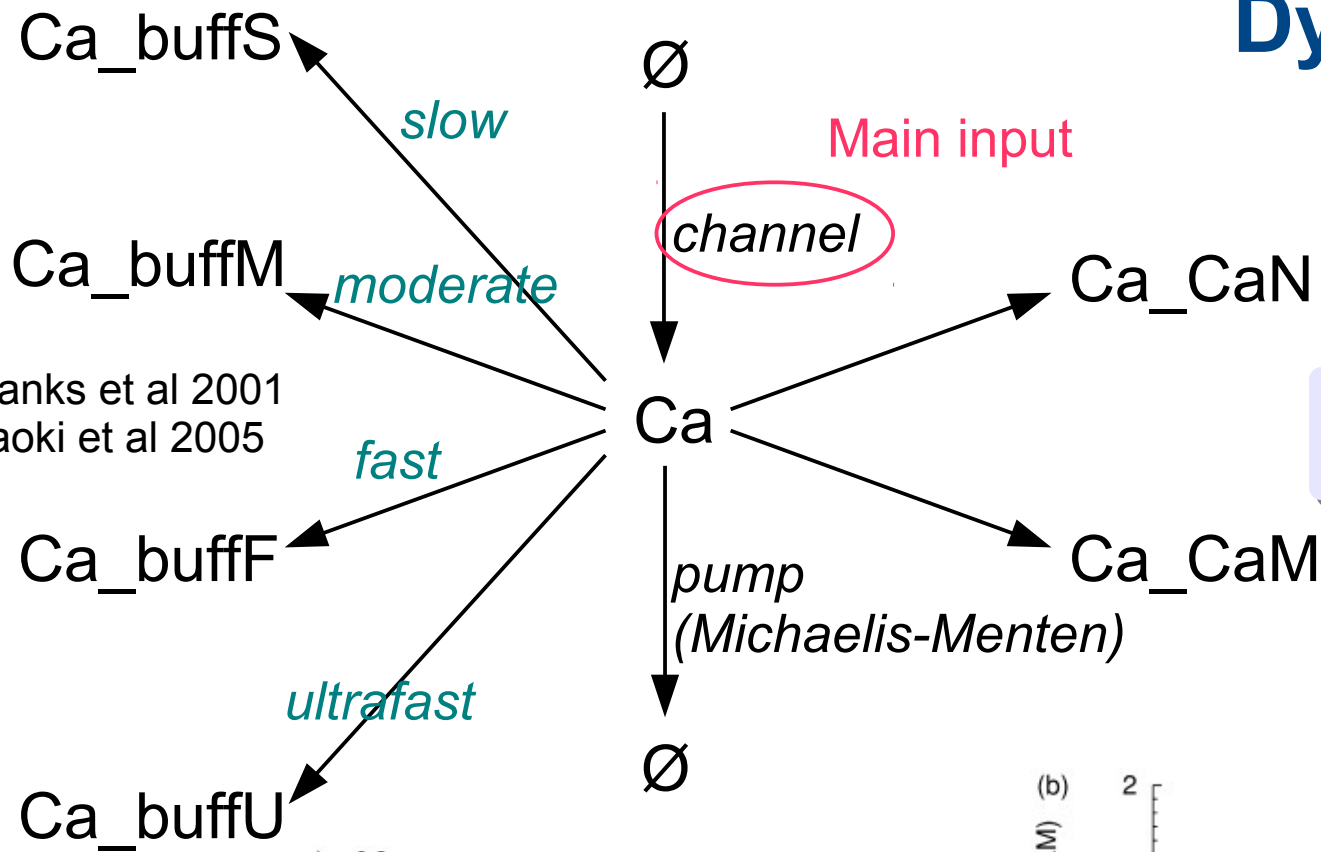
AMPAR post-synaptic potential:	5 ms
Calcium spike:	50 ms
Half saturation calmodulin ($k_{on}=1.5e6$, $k_{off}=100$):	5 ms
Relaxation between calmodulin states:	1 ms
autophosphorylation of CaMKII ($k_{on}=6$):	100 ms



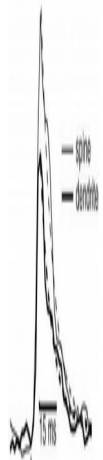
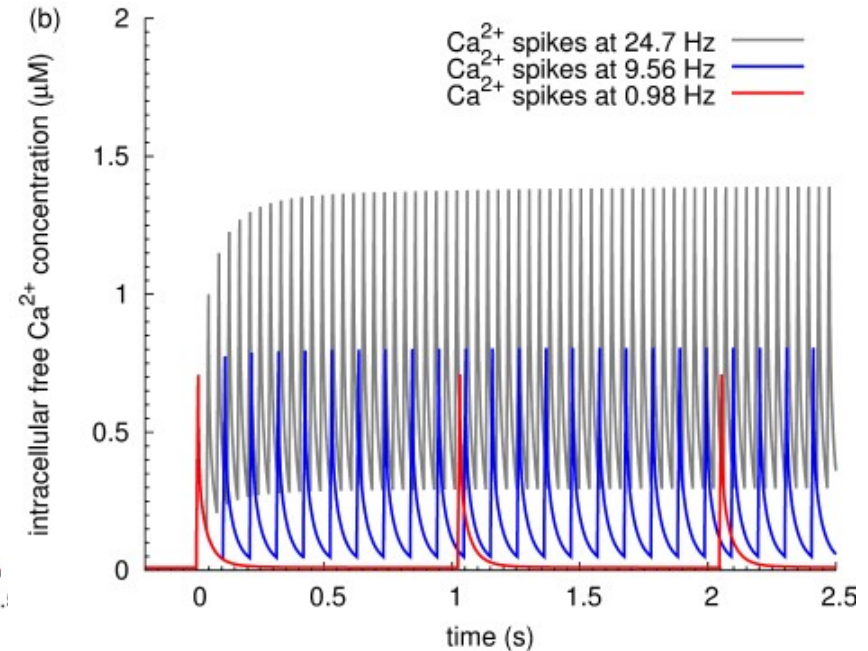
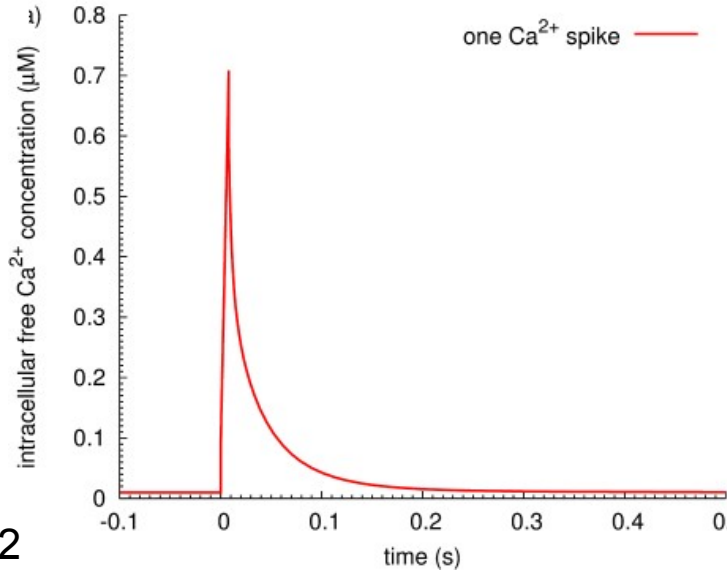
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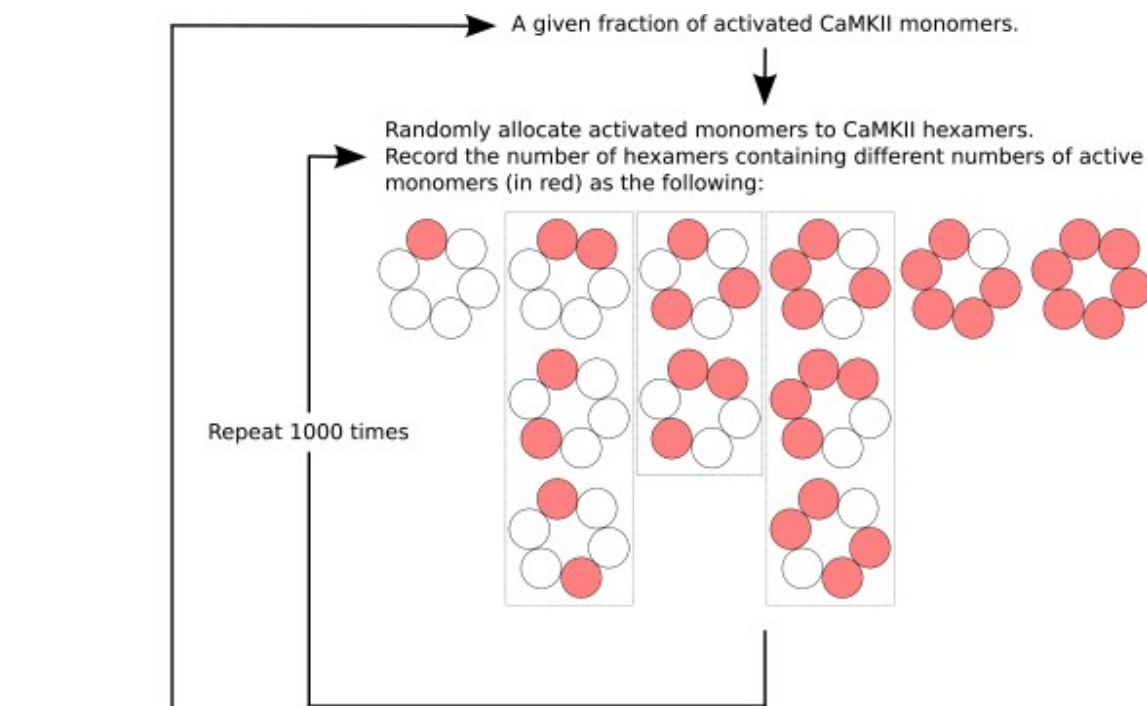
Dynamic of calcium in the spine



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



Sabatini et al 2002



Repeat for every 1% increase of CaMKII active monomers

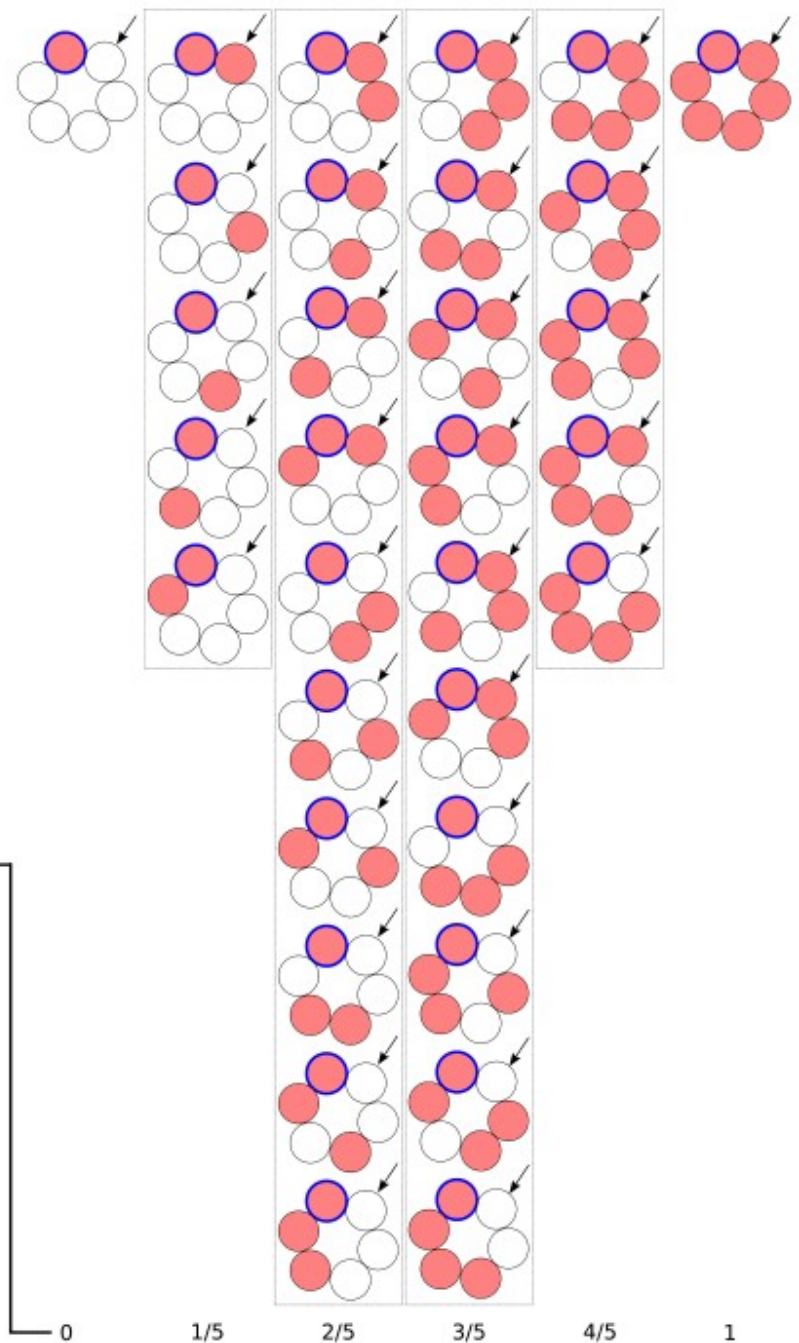
Calculate the average population for each number of active monomers per hexamer.

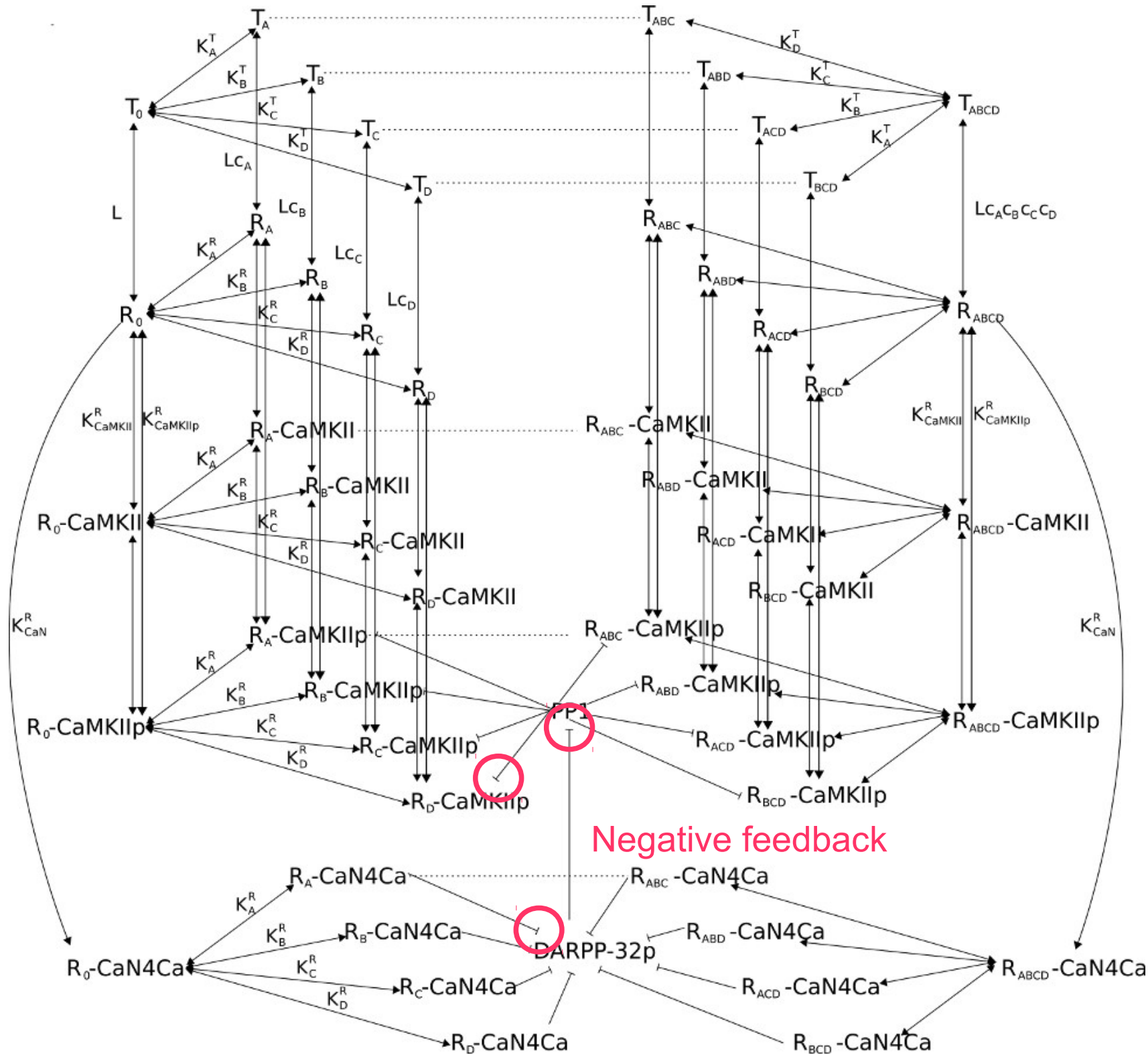
Multiply average populations of each number of active monomers per hexamer by their corresponding probabilities of having an active neighbour.

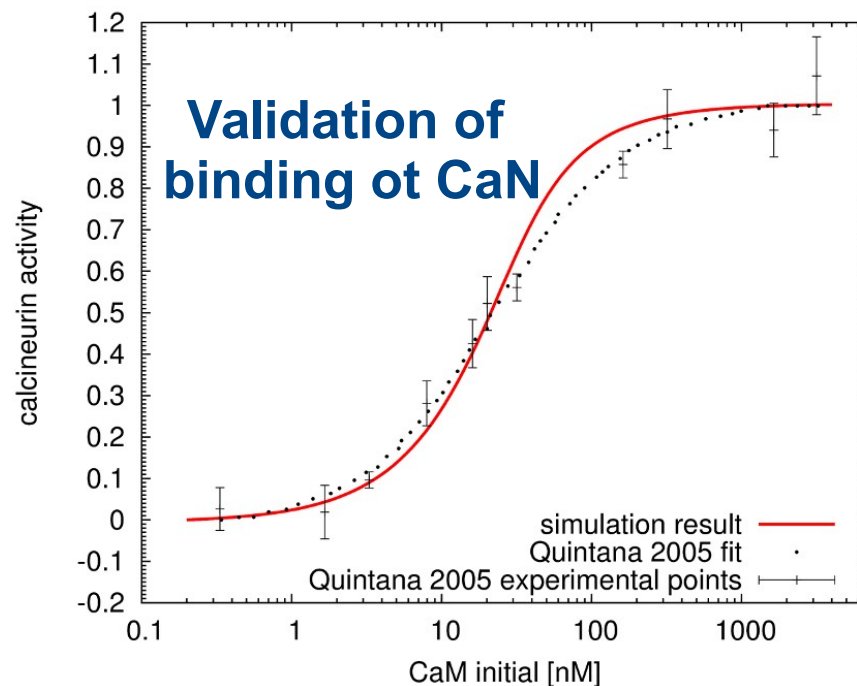
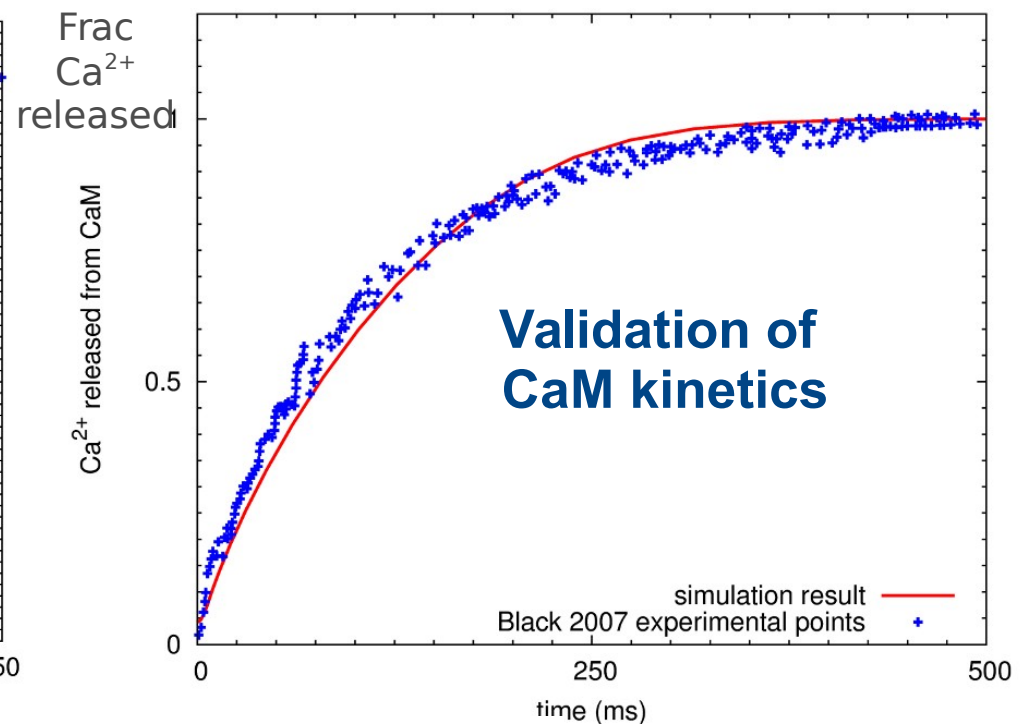
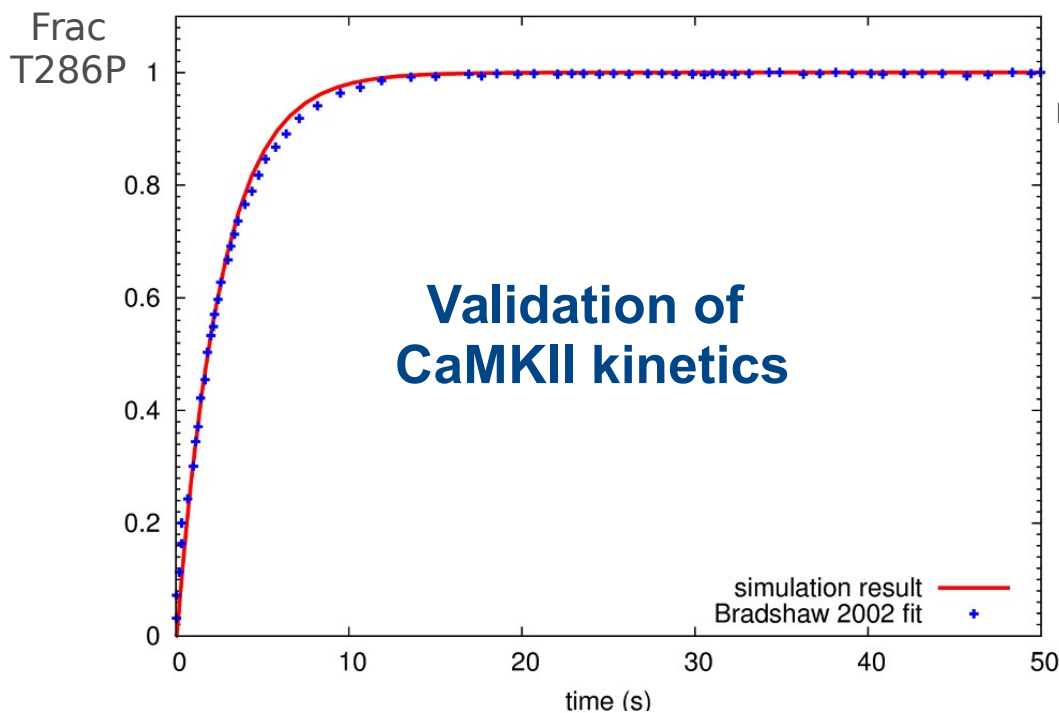
The sum of these six numbers is a coefficient that can be used to adjust CaMKII autophosphorylation rate.

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

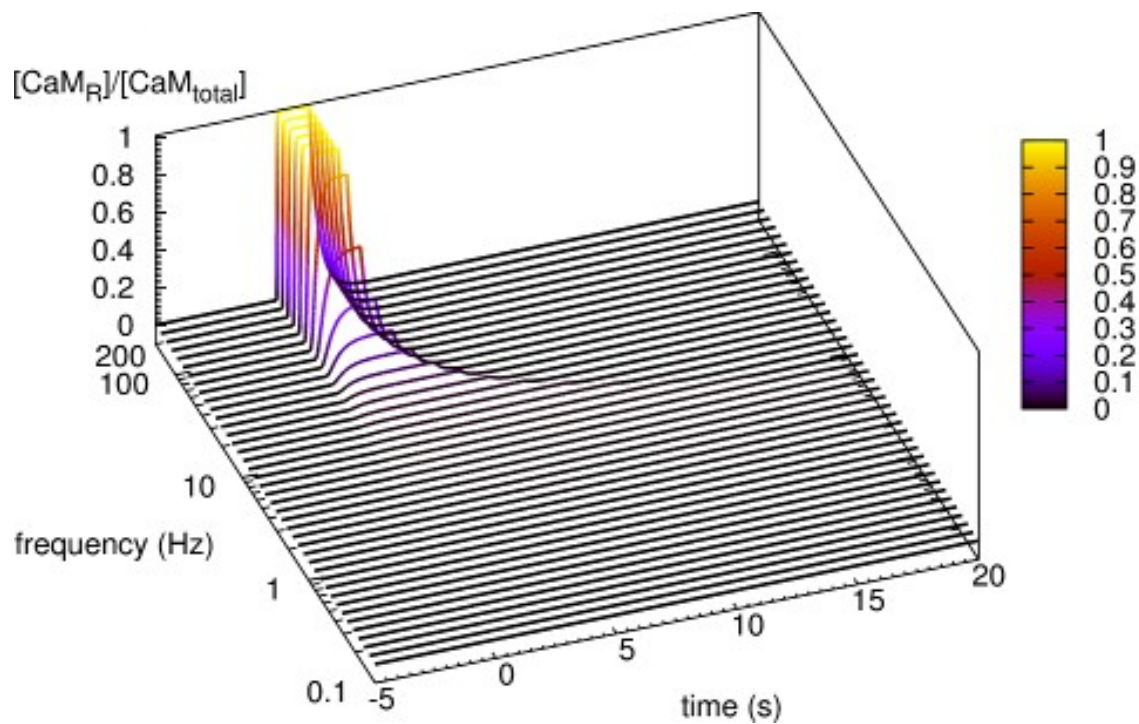
Calculate the probabilities of having an active neighbour on one specific side (indicated by the arrow) of the activated monomer of interest (blue outline) (since only the asymmetrical situation is considered). The possible positions of activated monomers are listed as the following, with corresponding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





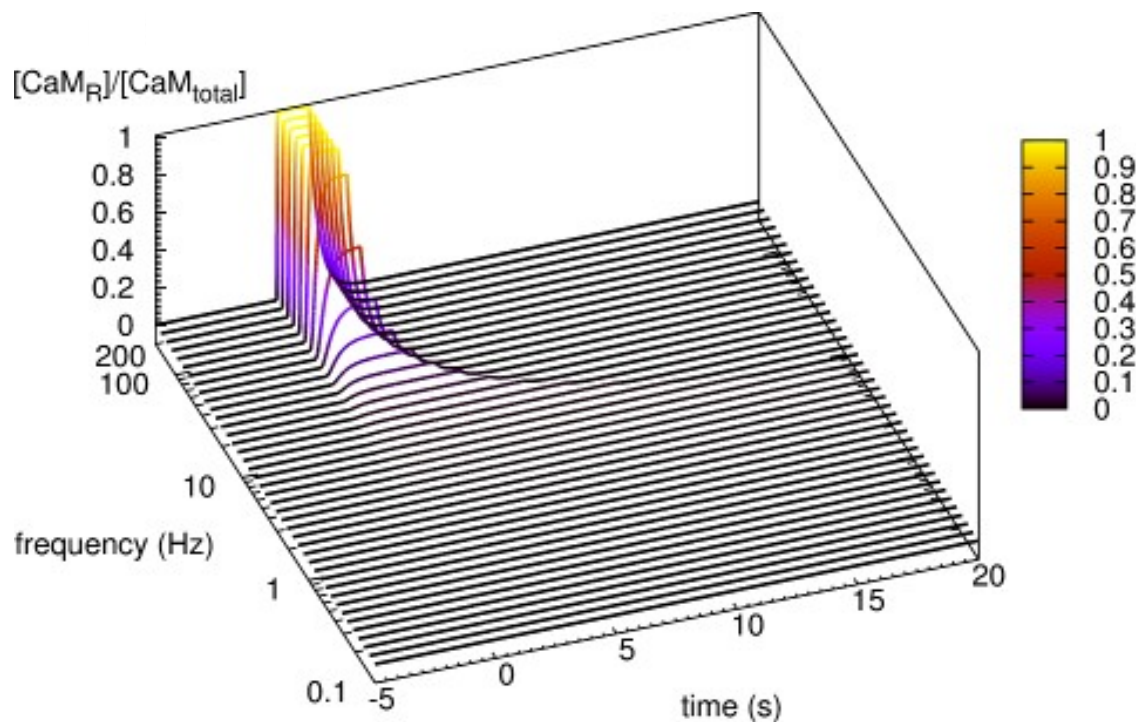


Calmodulin activation



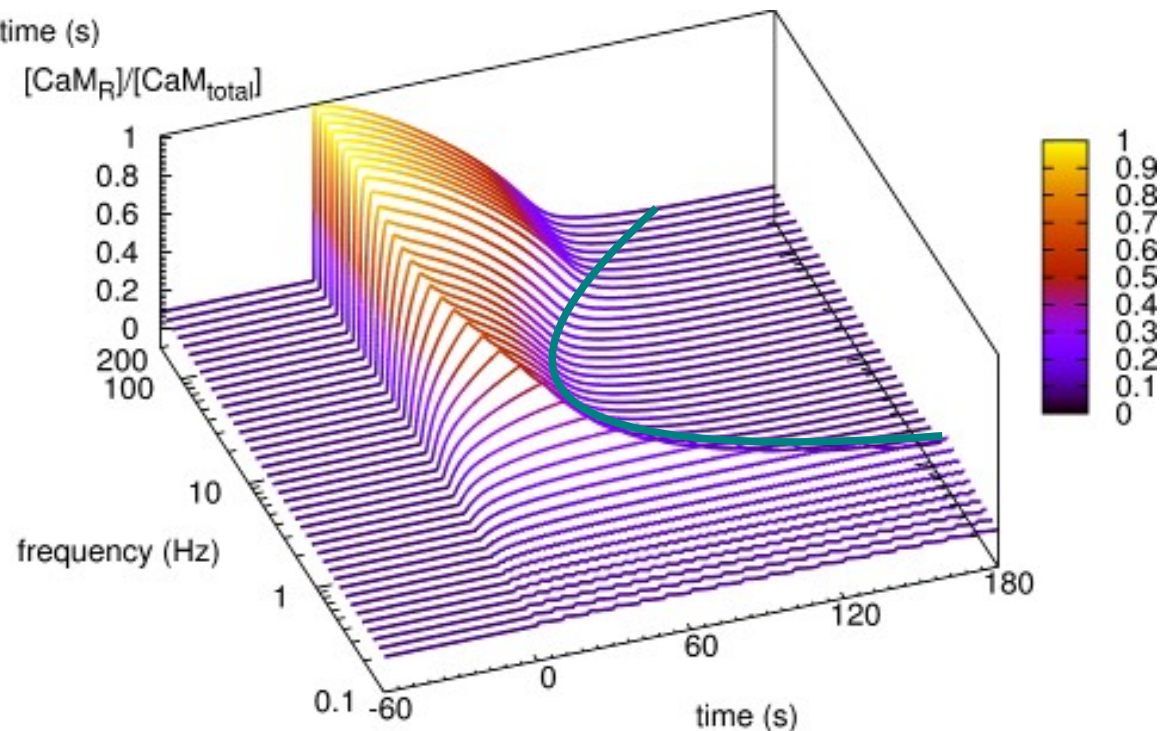
CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

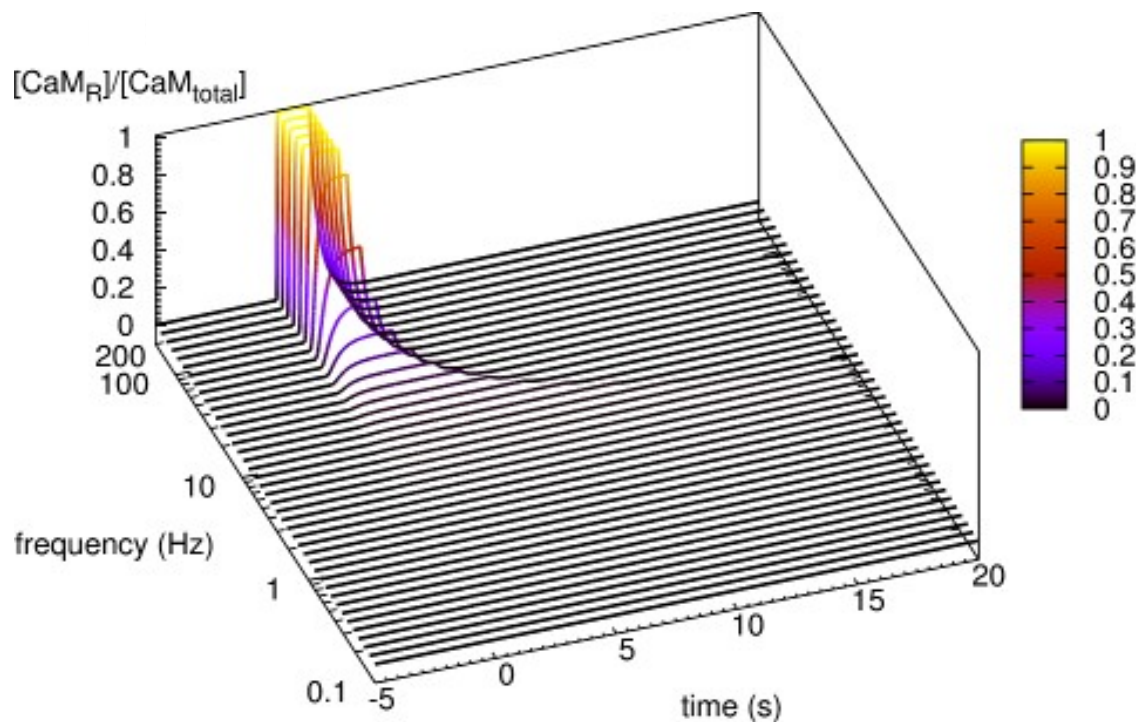


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

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



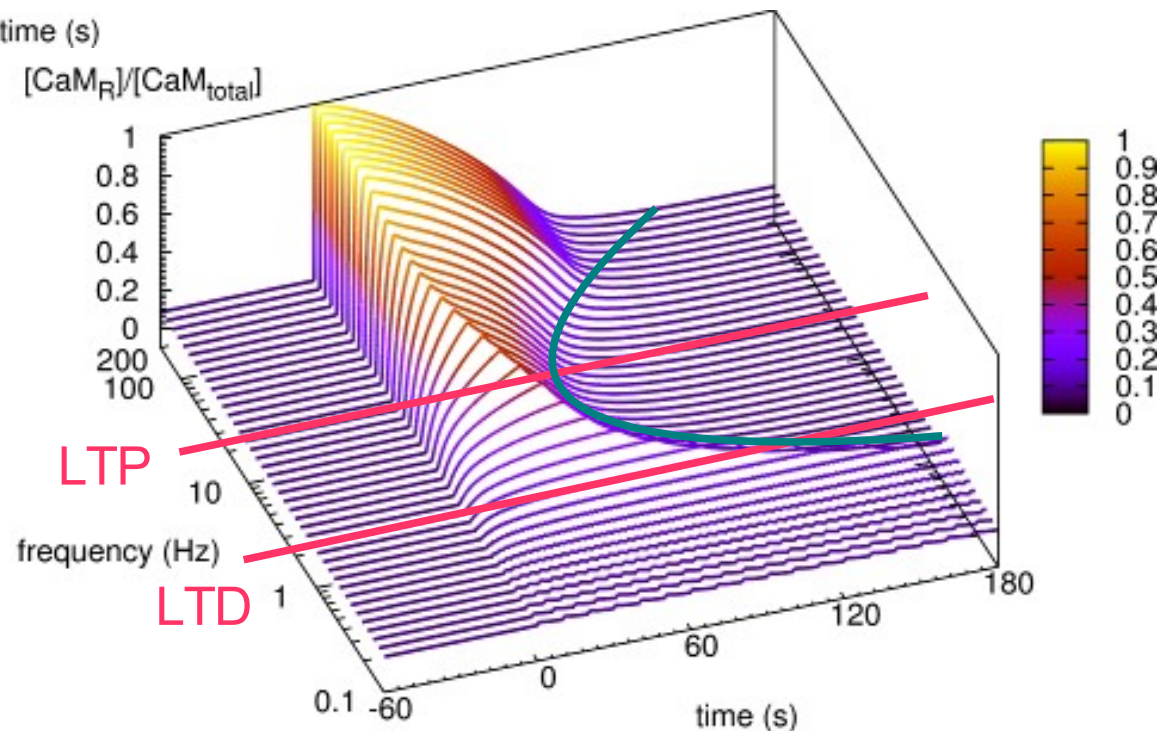
Calmodulin activation



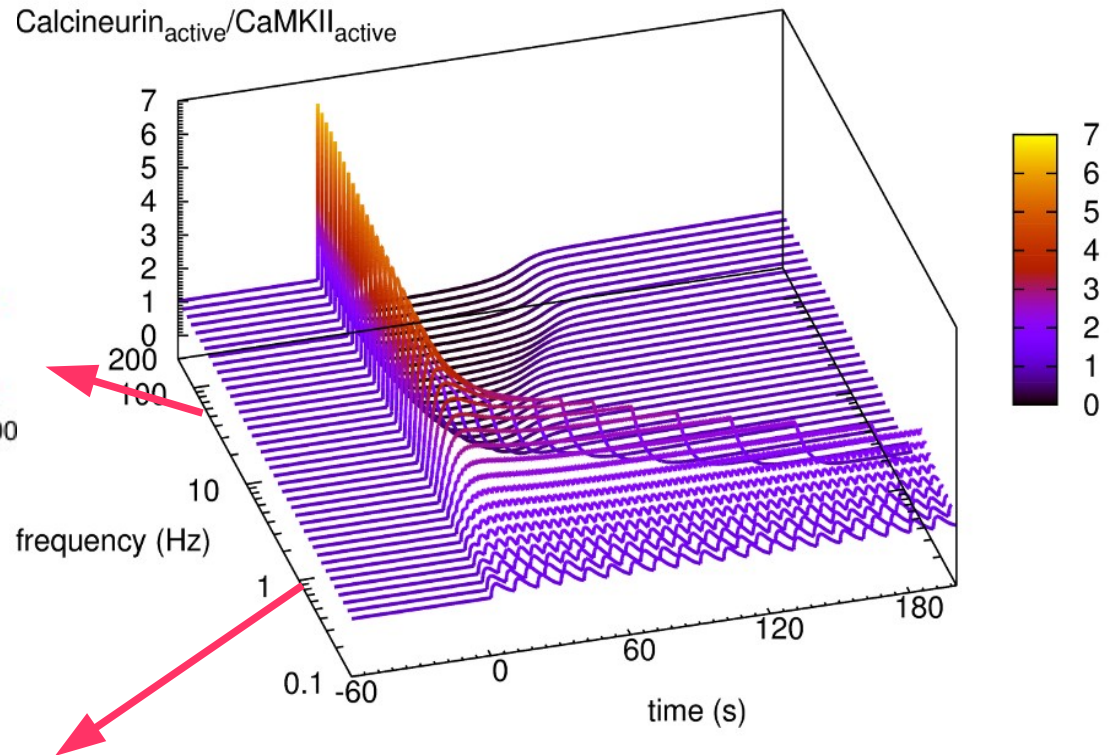
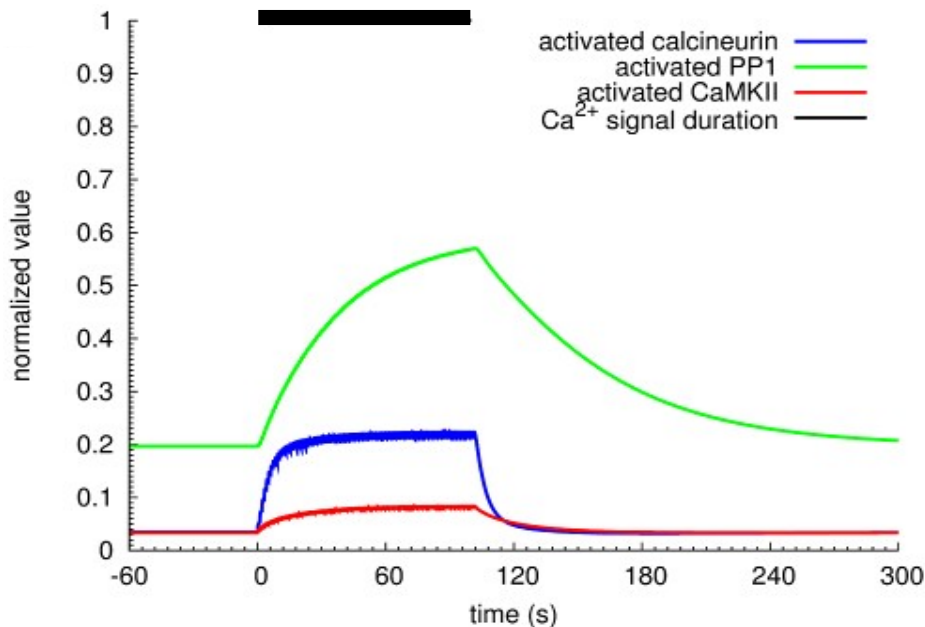
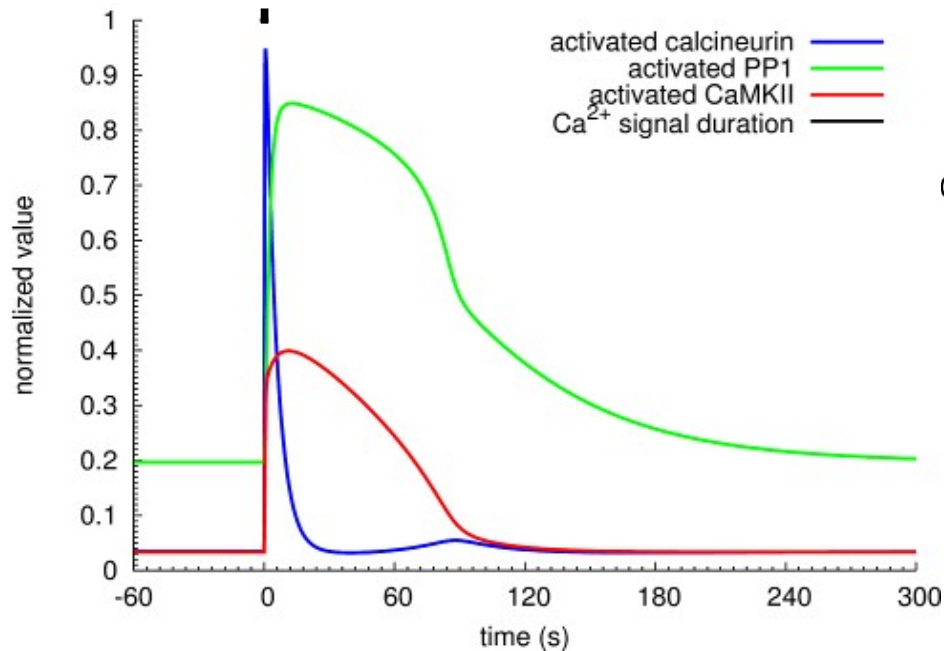
CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

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

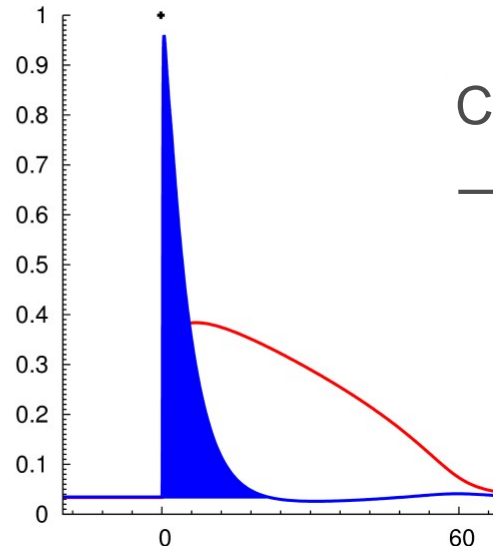
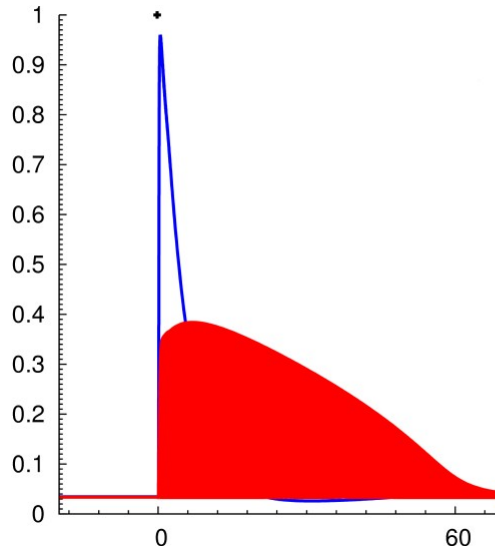


All calcium frequencies increase CaN **AND** CaMKII. It is not a Switch. But the ratio of activation changes

Bidirectional plasticity

Constant catalytic rates of active enzyme

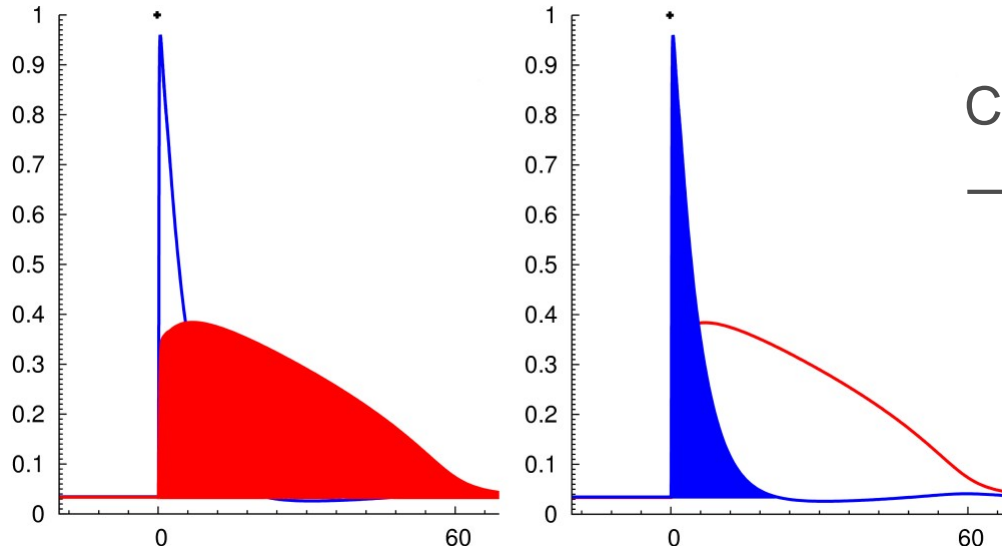
→ quantity of catalysed reaction events
prop to integral of the activation curve



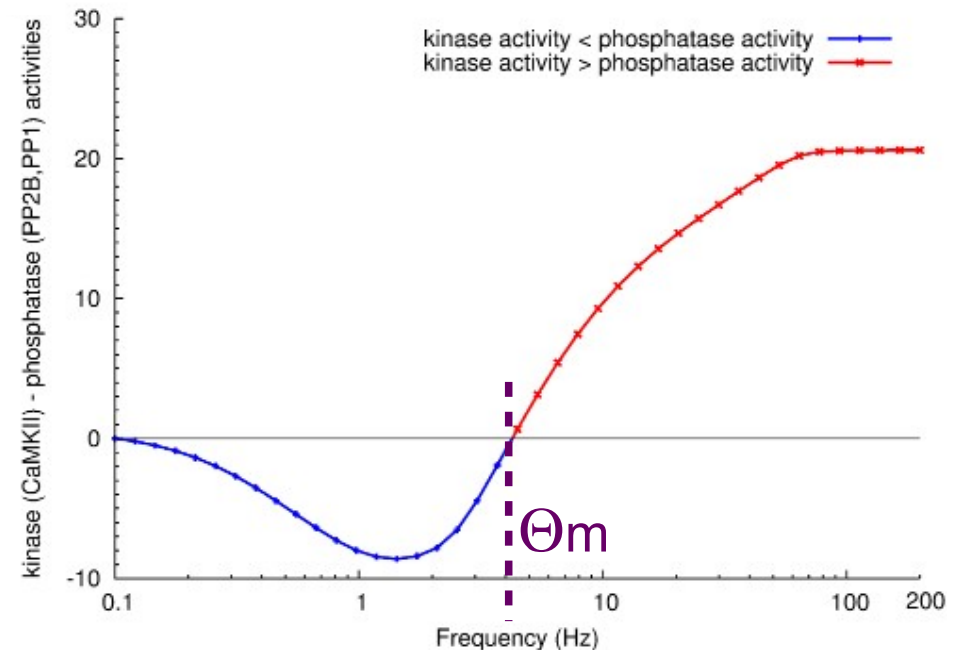
Bidirectional plasticity

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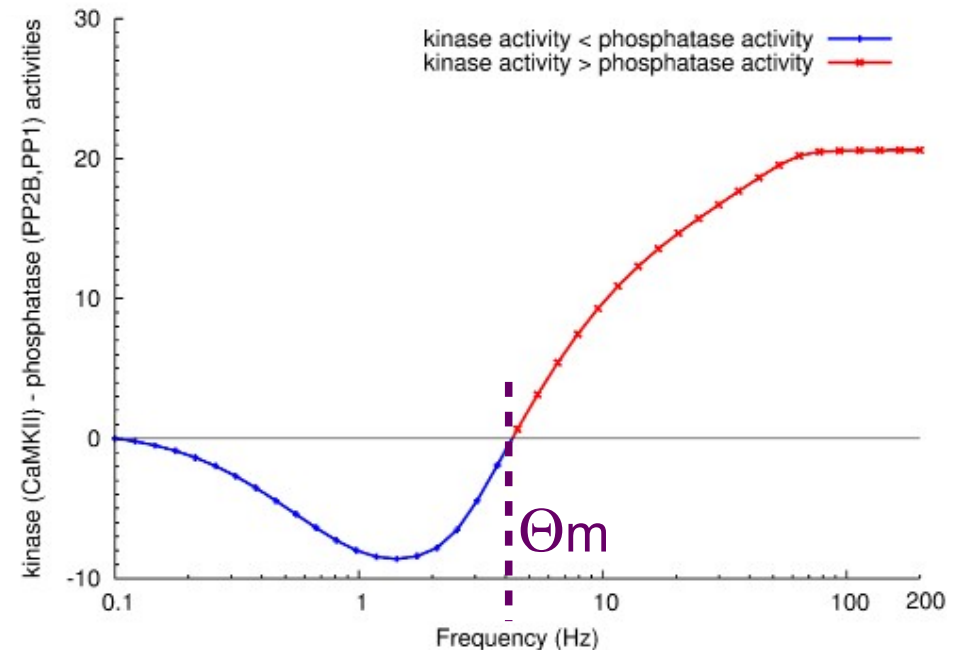
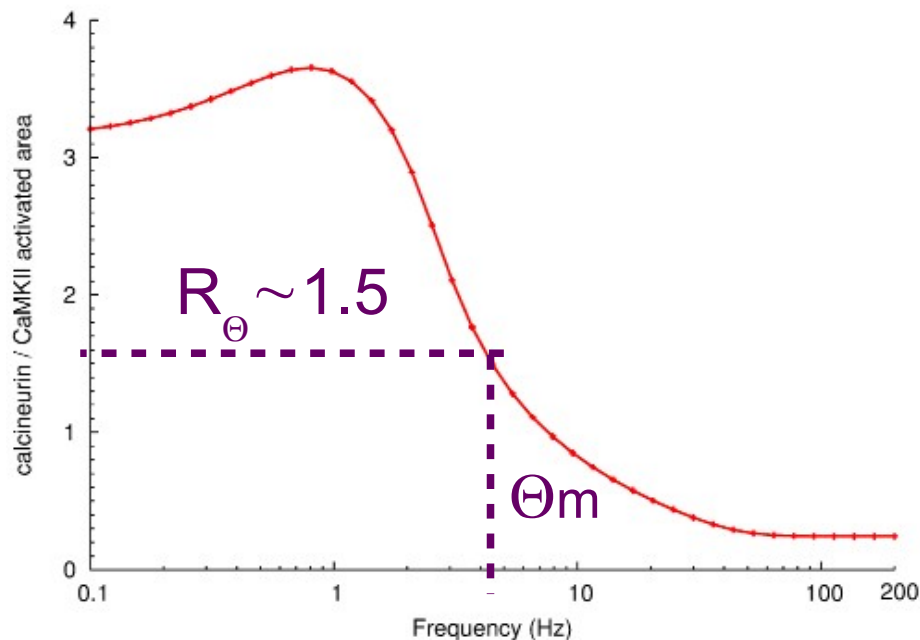
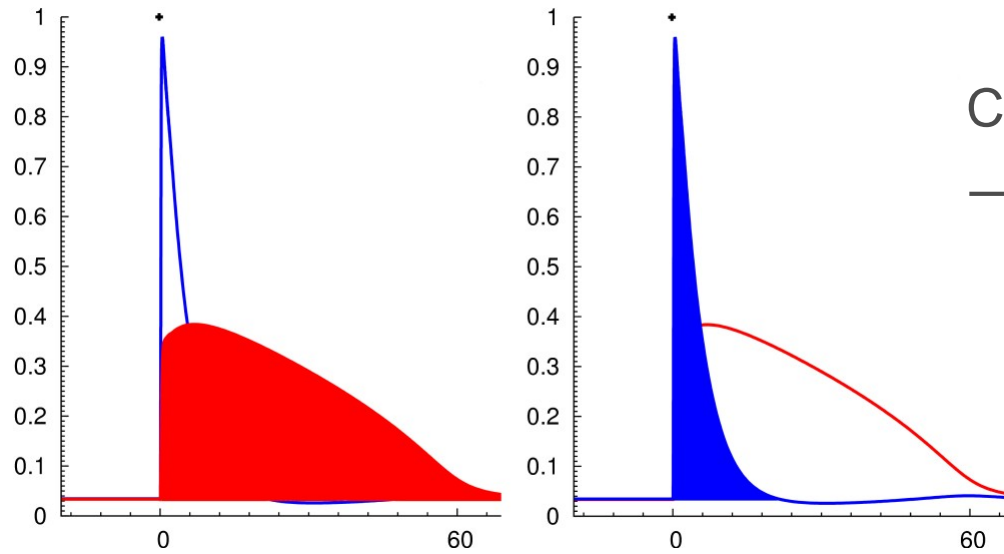
Bienestock-Cooper-Munro
(BCM) curve: difference of
active areas* catalytic activities



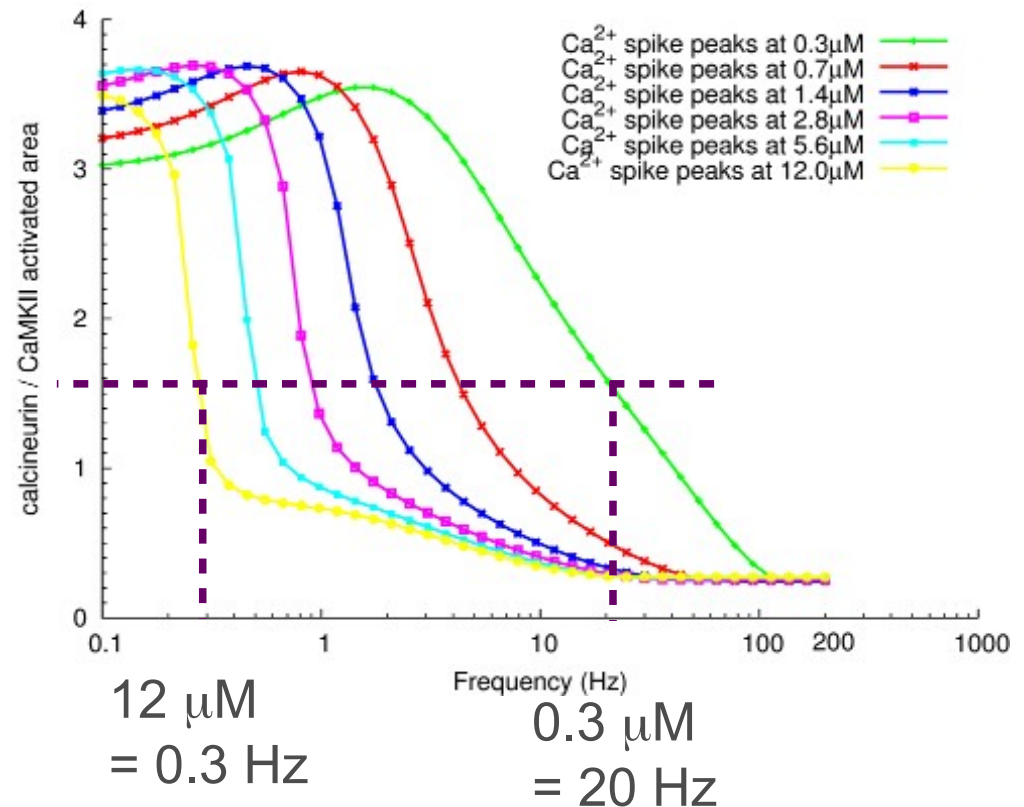
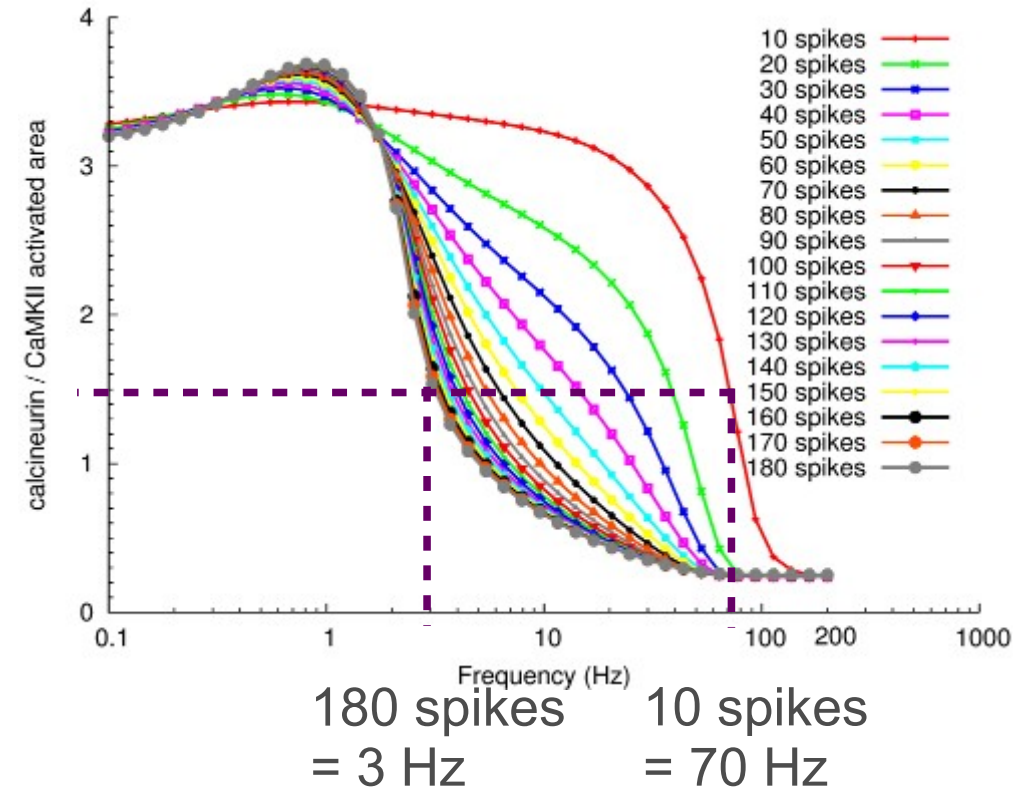
Bidirectional plasticity

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

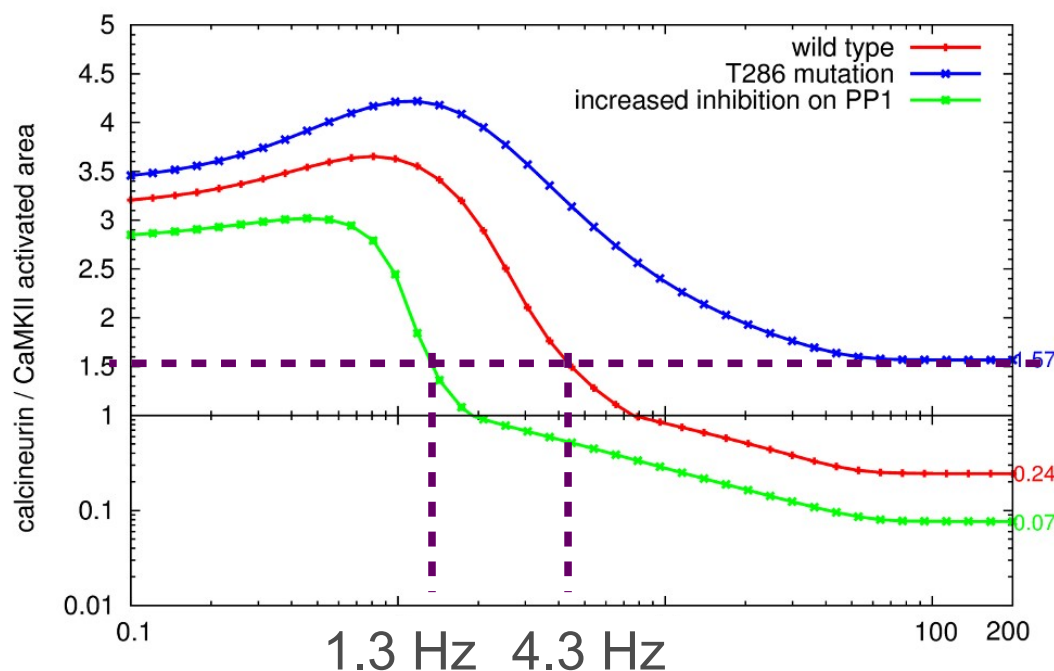


Effect of calcium duration and amount



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

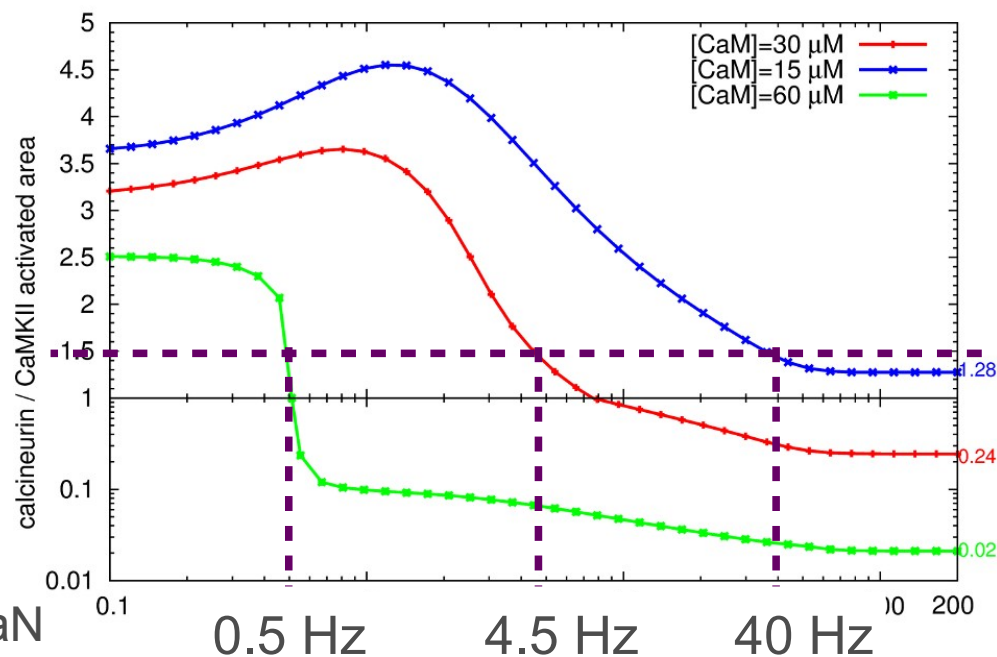
Never any positive plasticity
Giese et al (1998) *Science*, 279:870-873

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



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**.

Allosteric stabilisation triggers **bistable CaM response** to Ca^{2+} . Above a certain freq, CaM activation longer than initial signal.

CaN can bind calmodulin at low concentration of calcium, while both CaN and CaMKII binds CaM at high calcium concentrations. Calcium signals **activate both CaN and CaMKII** at *ALL* frequencies. The ratio of activity changes.

Neurogranin **stabilises Calmodulin in the T state**, resetting the system and acting as a Calmodulin reservoir.

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

- Developers of ECell3, COPASI, Scilab



wellcome trust



Melanie Stefan



Lu Li



Denis Brun



Michele
Mattioni



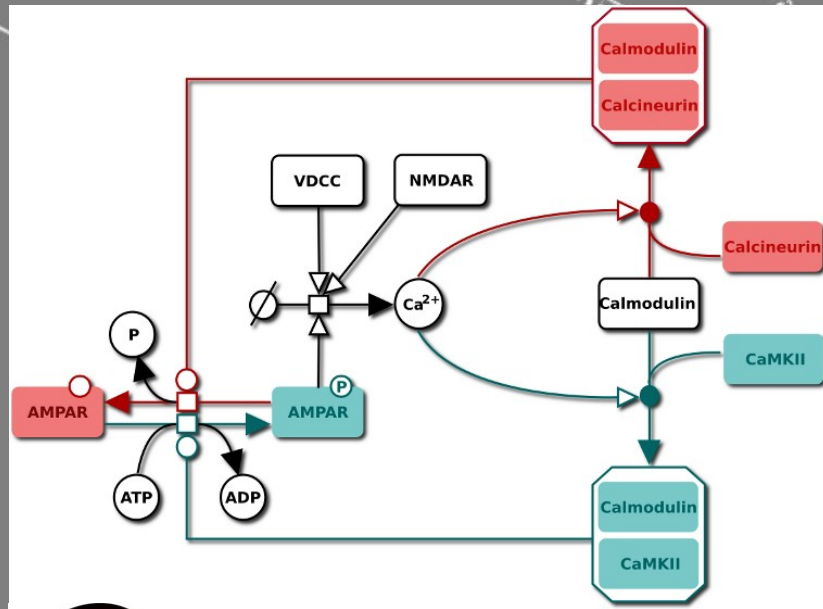
Stuart
Edelstein



Massimo
Lai



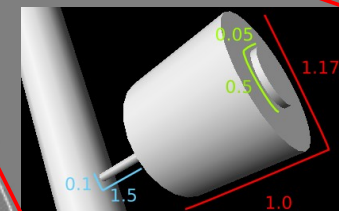
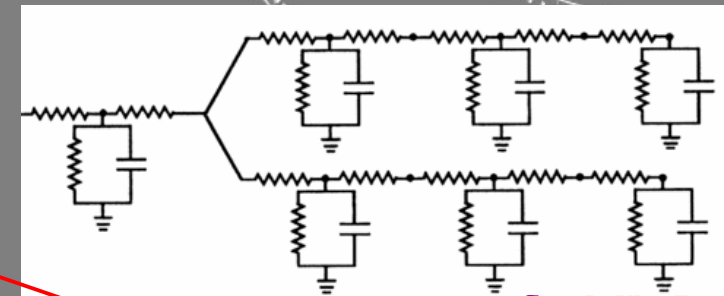
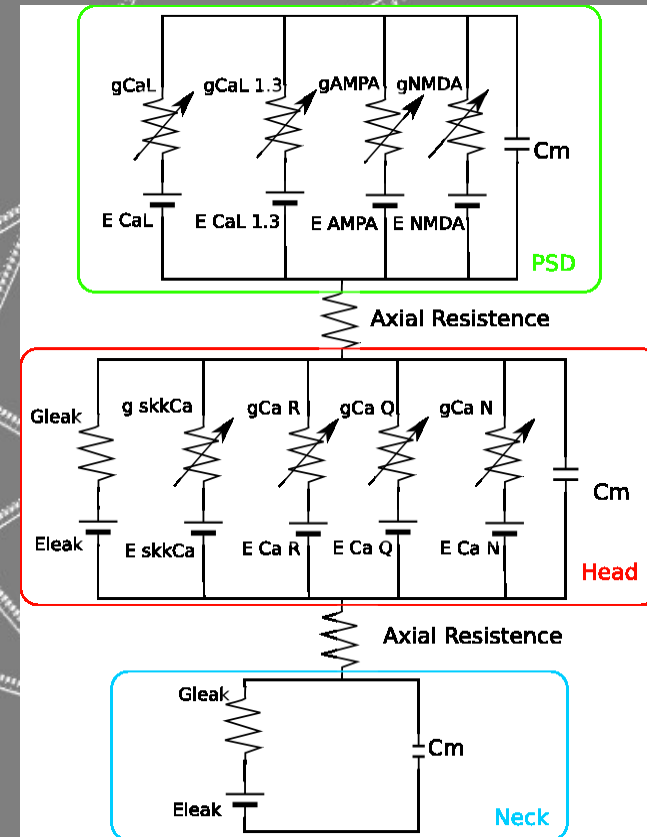
Whole cell: electro-biochemical models



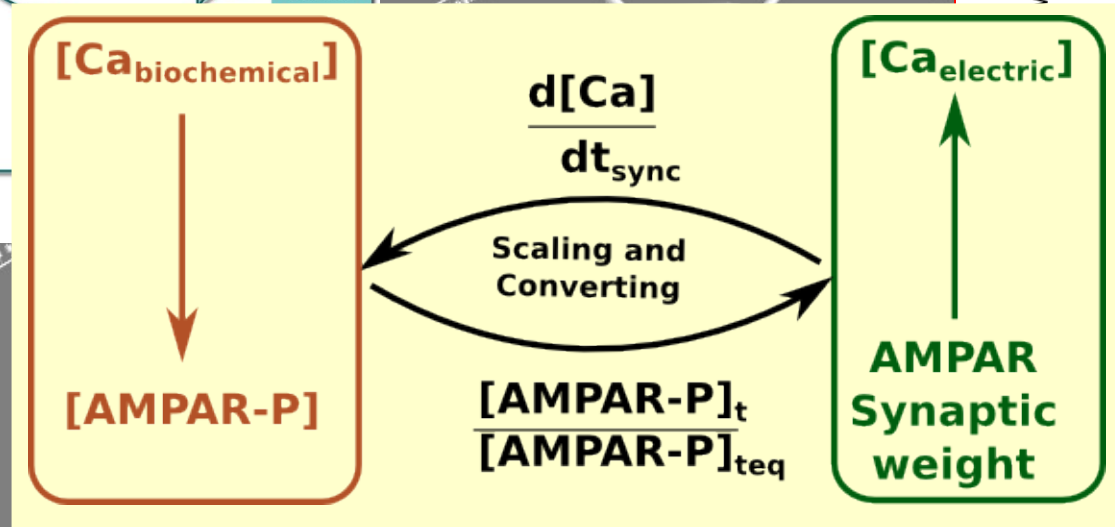
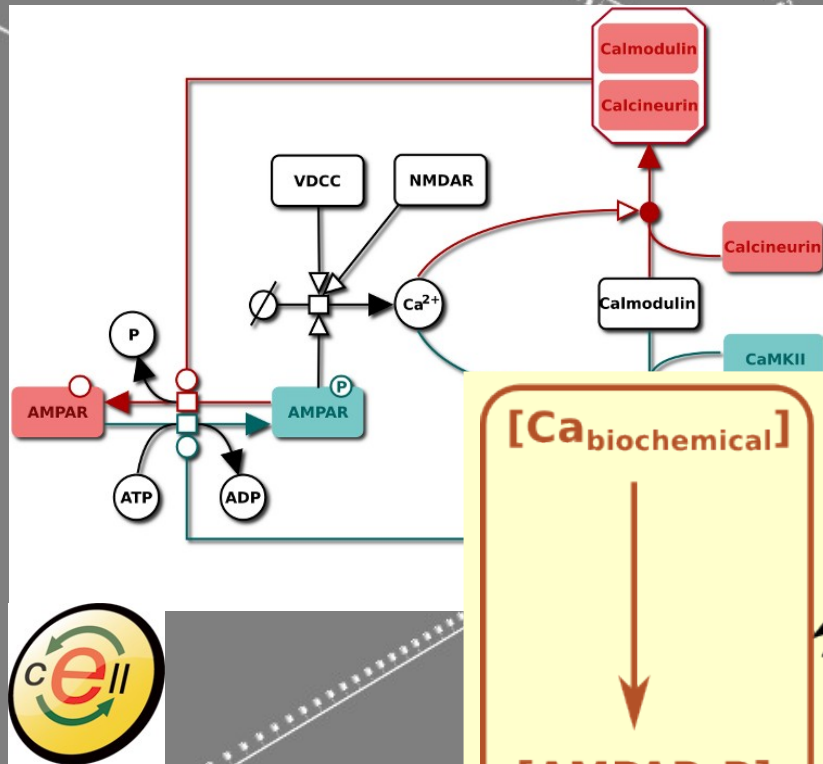
1504 spines
4761 compartments
16362 channels

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

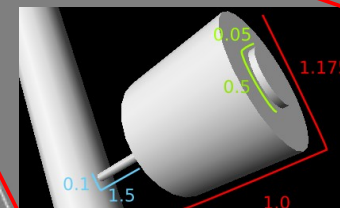
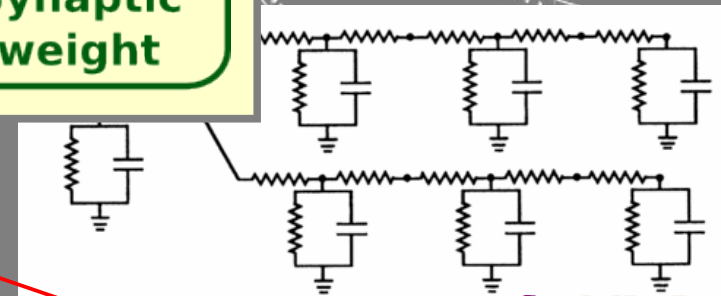
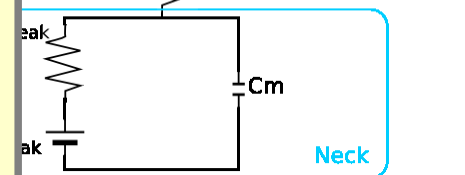
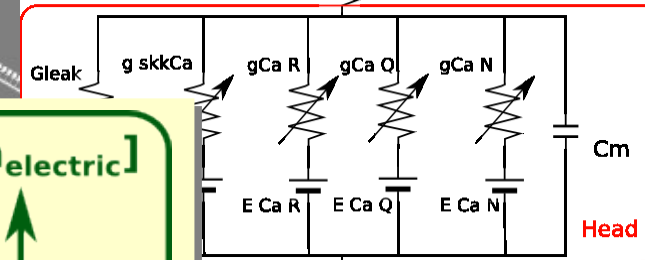
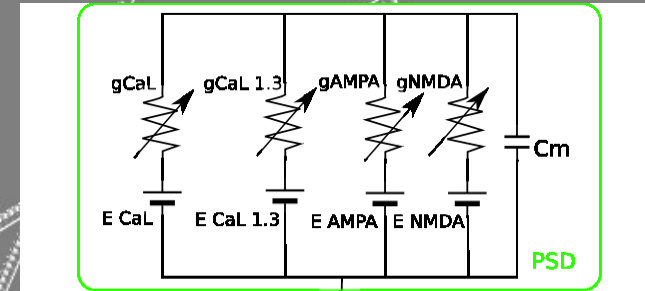
Mattioni M, Le Novère N (2013)



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1504 spines
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