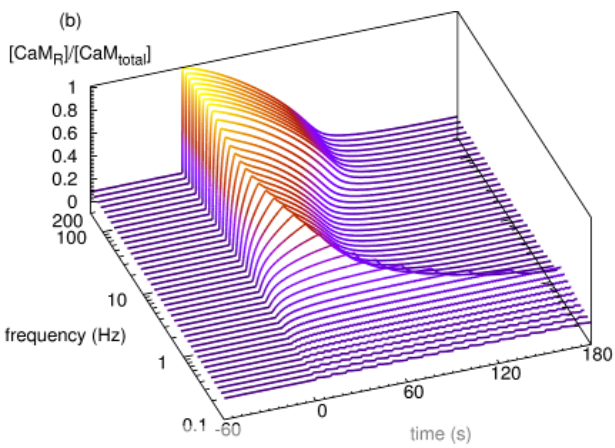
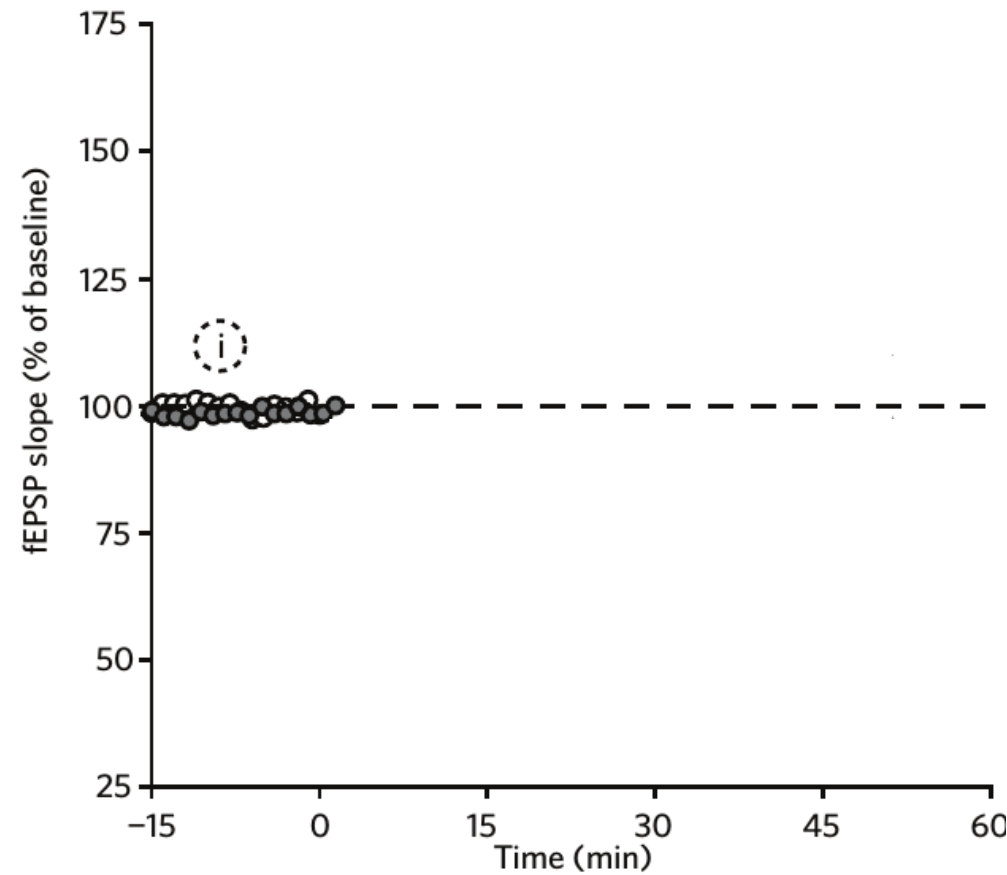
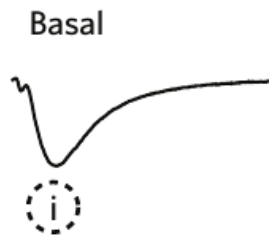
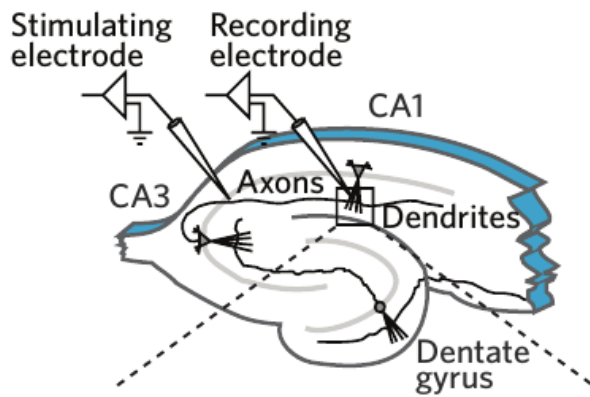
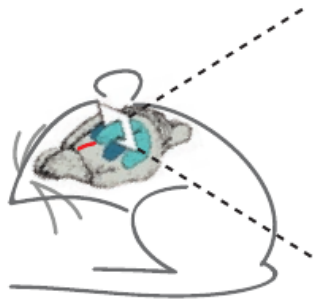
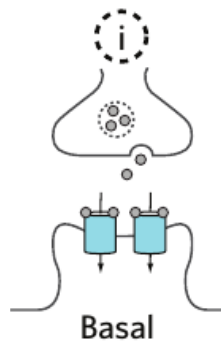




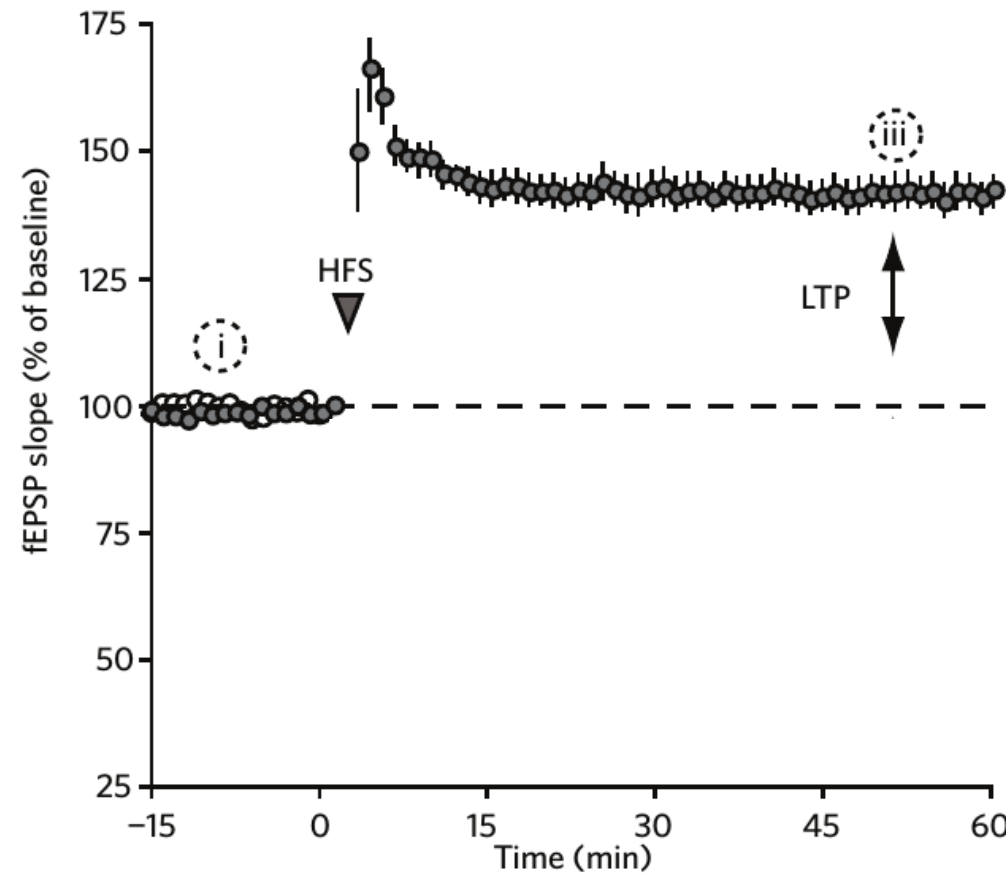
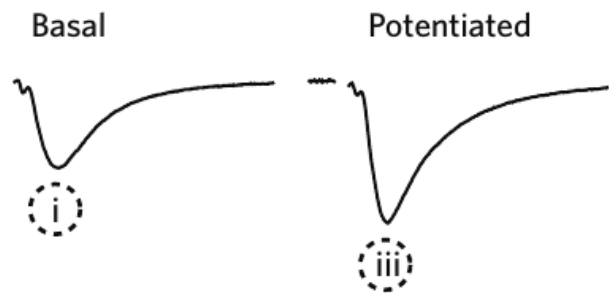
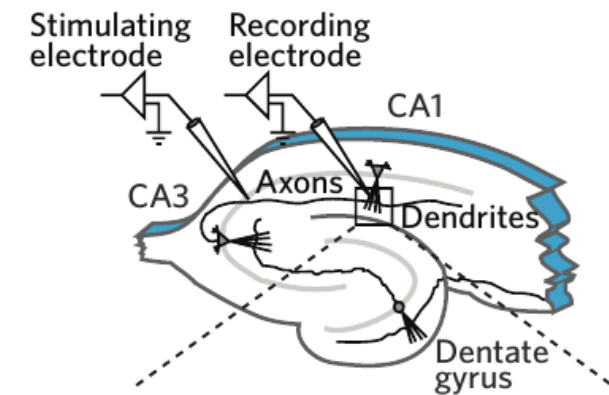
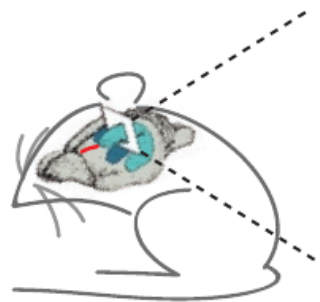
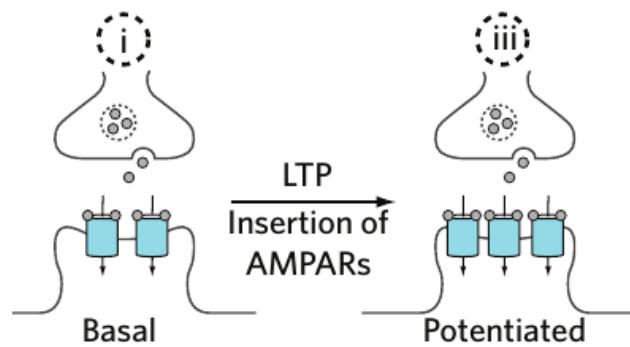
# Modelling the regulation of bidirectional synaptic plasticity by allosteric calcium sensors



Nicolas Le Novère  
Babraham Institute,  
[n.lenovere@gmail.com](mailto:n.lenovere@gmail.com)

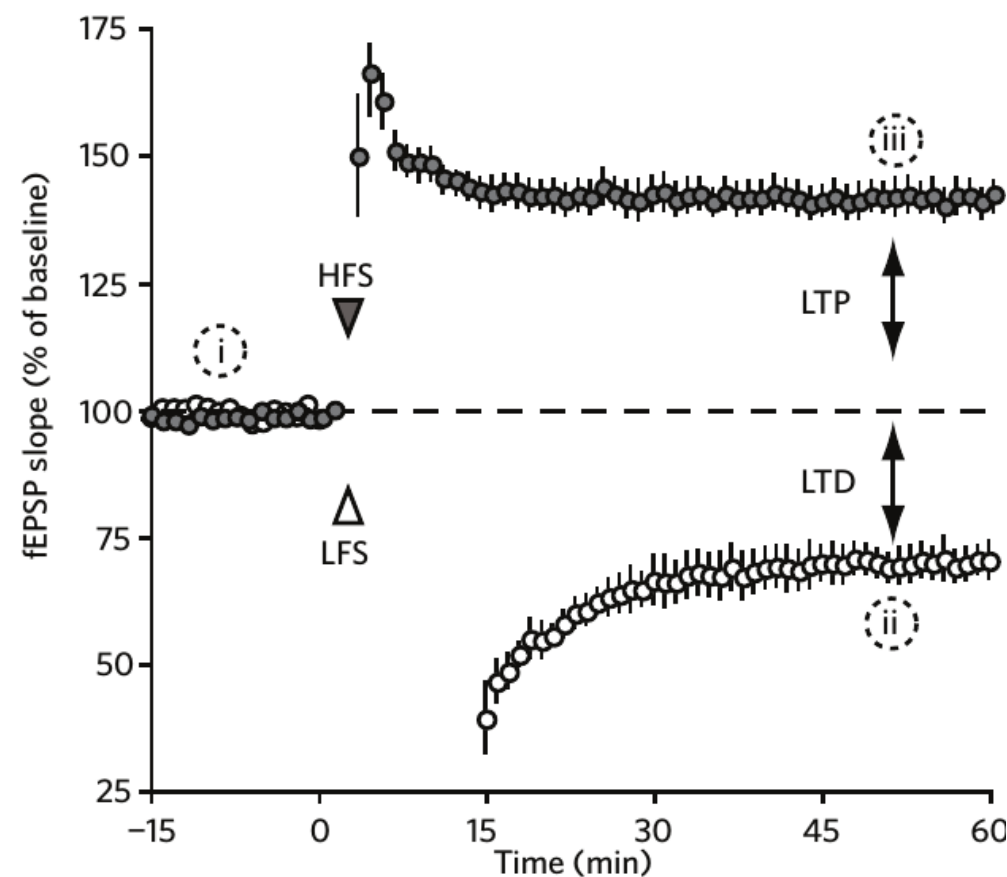
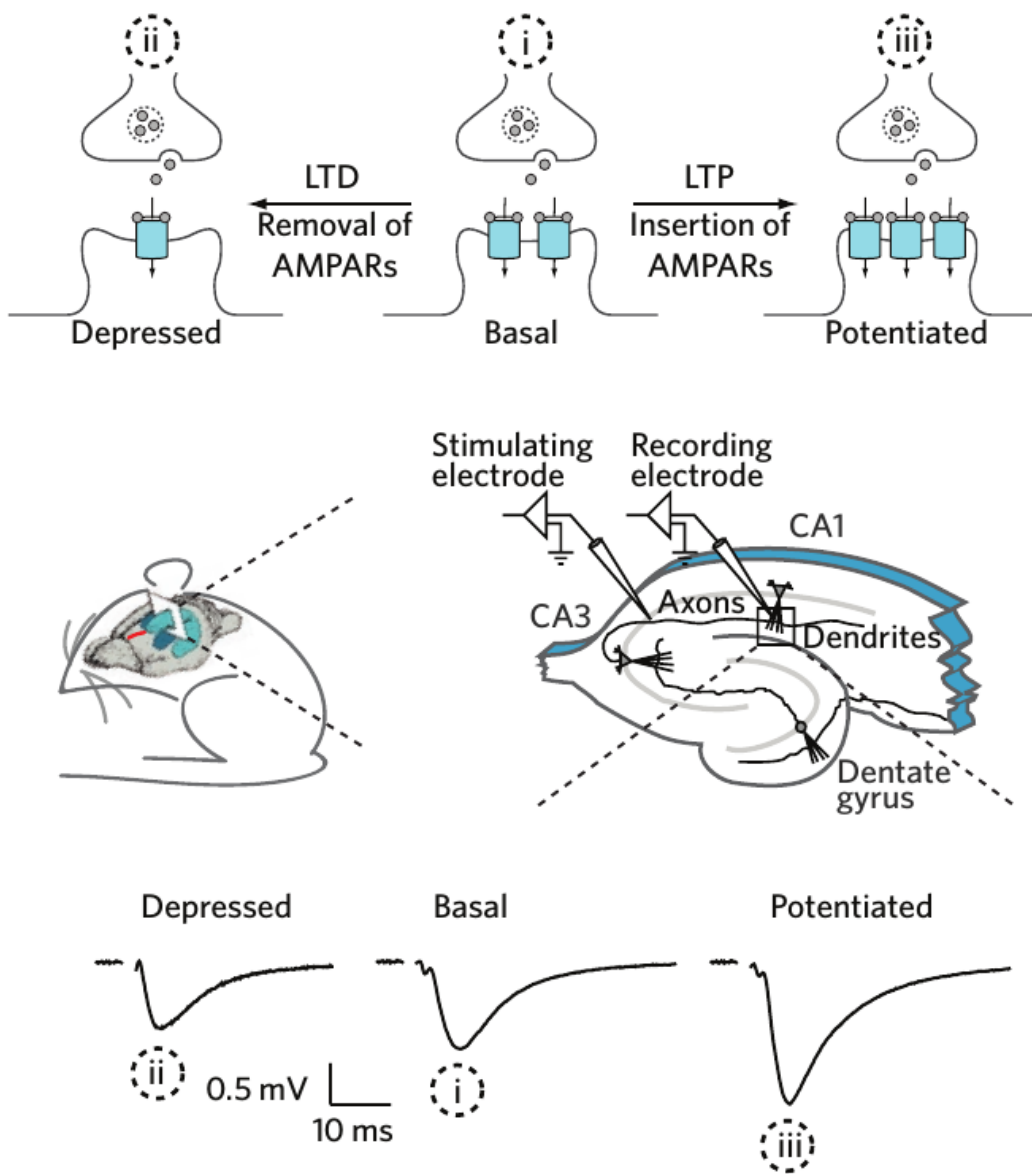


Fleming and England (2010) *Nat Chem Biol*



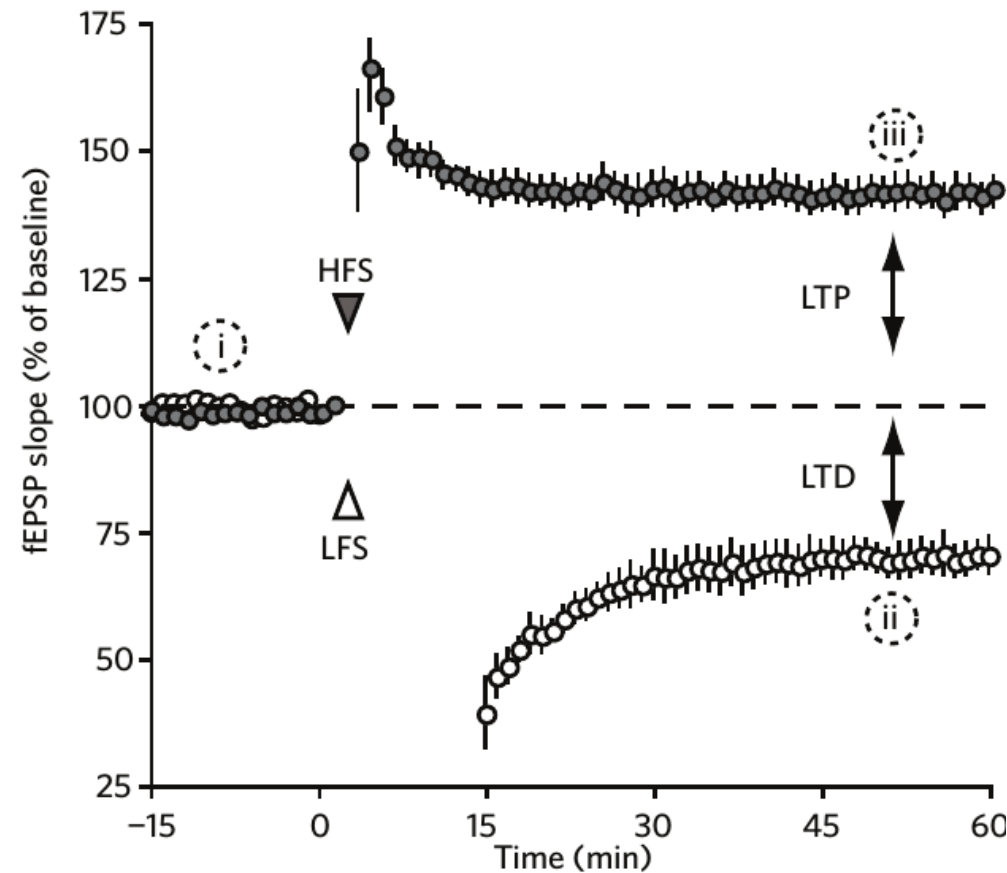
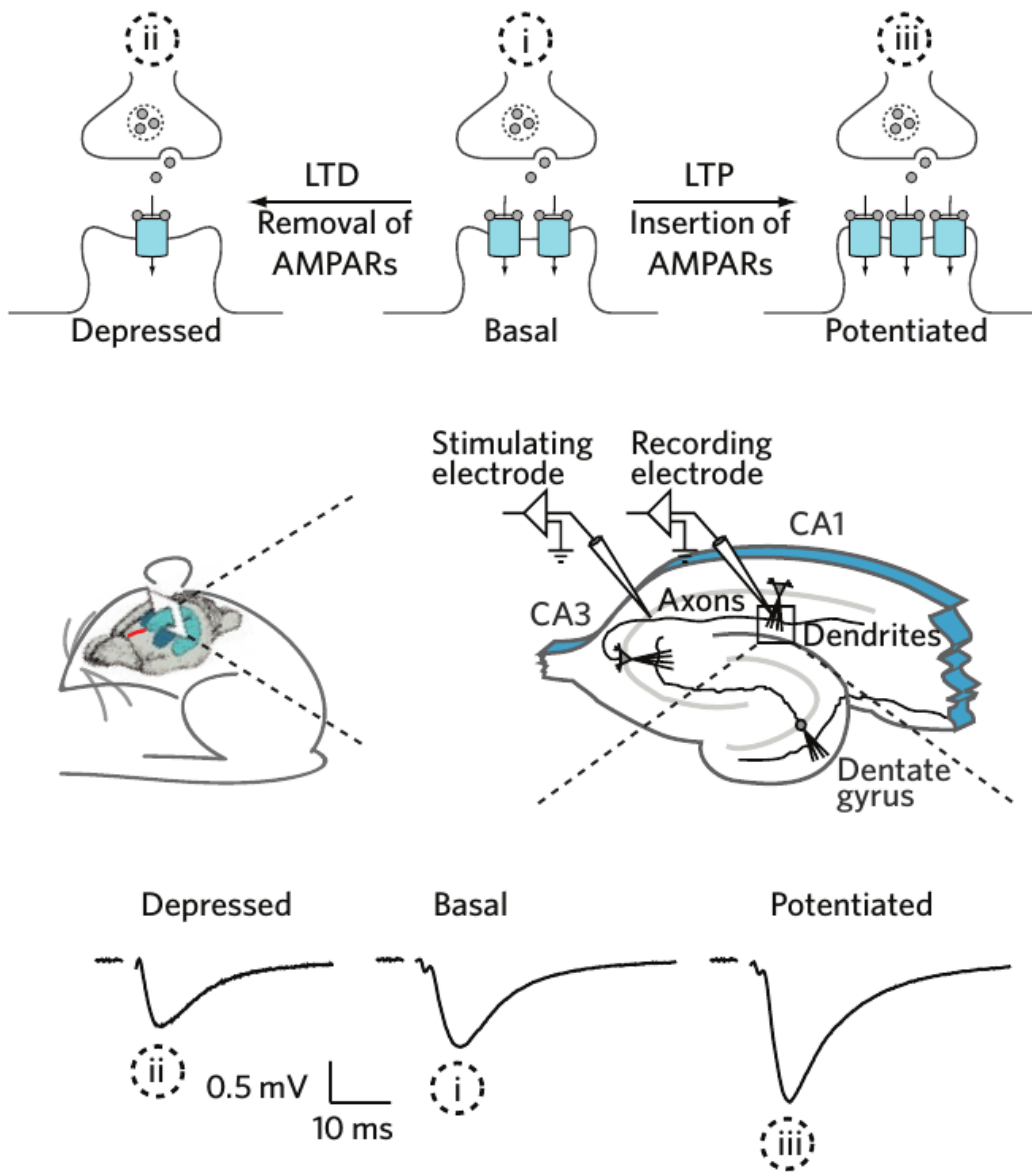
Fleming and England (2010) *Nat Chem Biol*





Fleming and England (2010) *Nat Chem Biol*





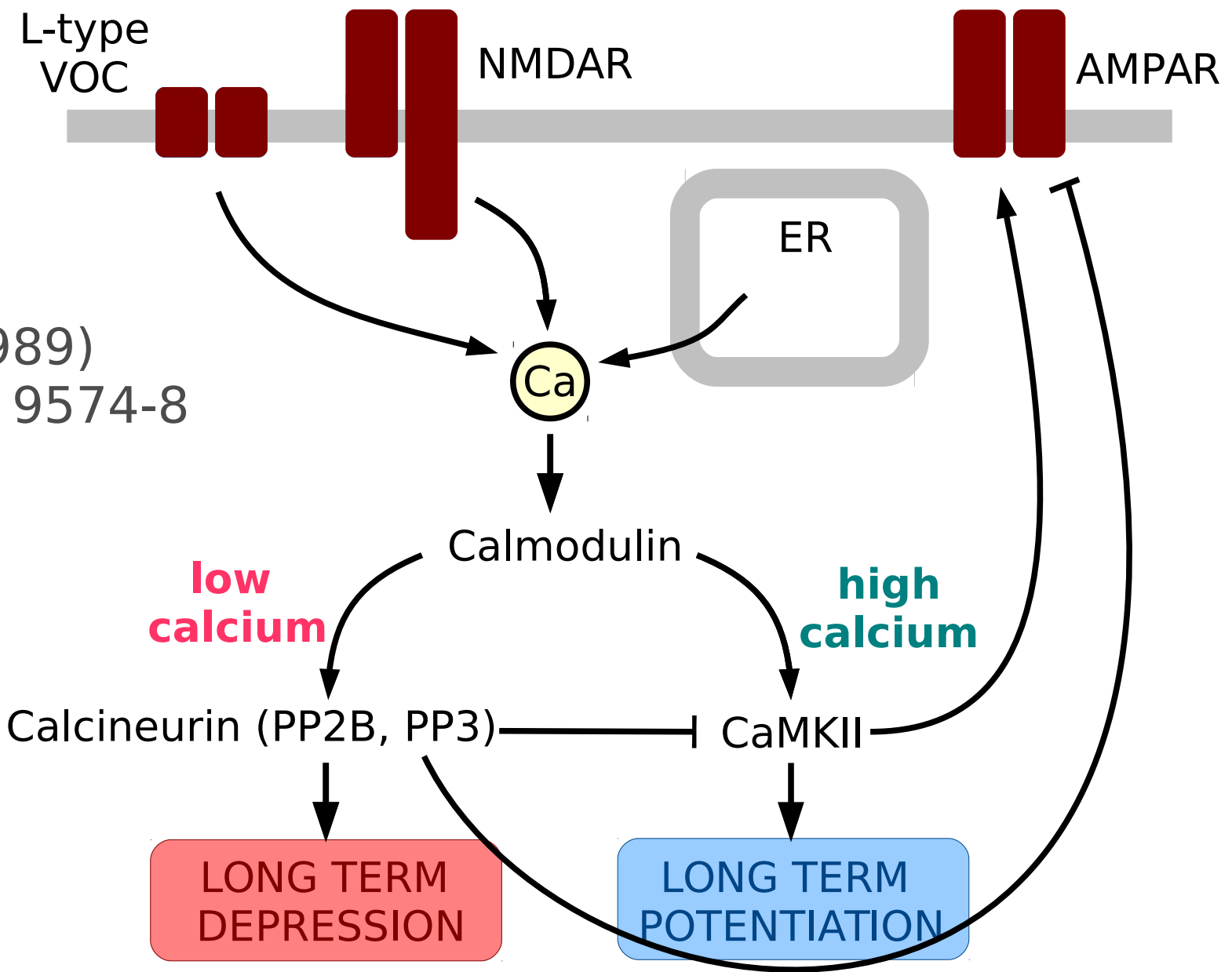
**lasts for weeks**

Fleming and England (2010) *Nat Chem Biol*

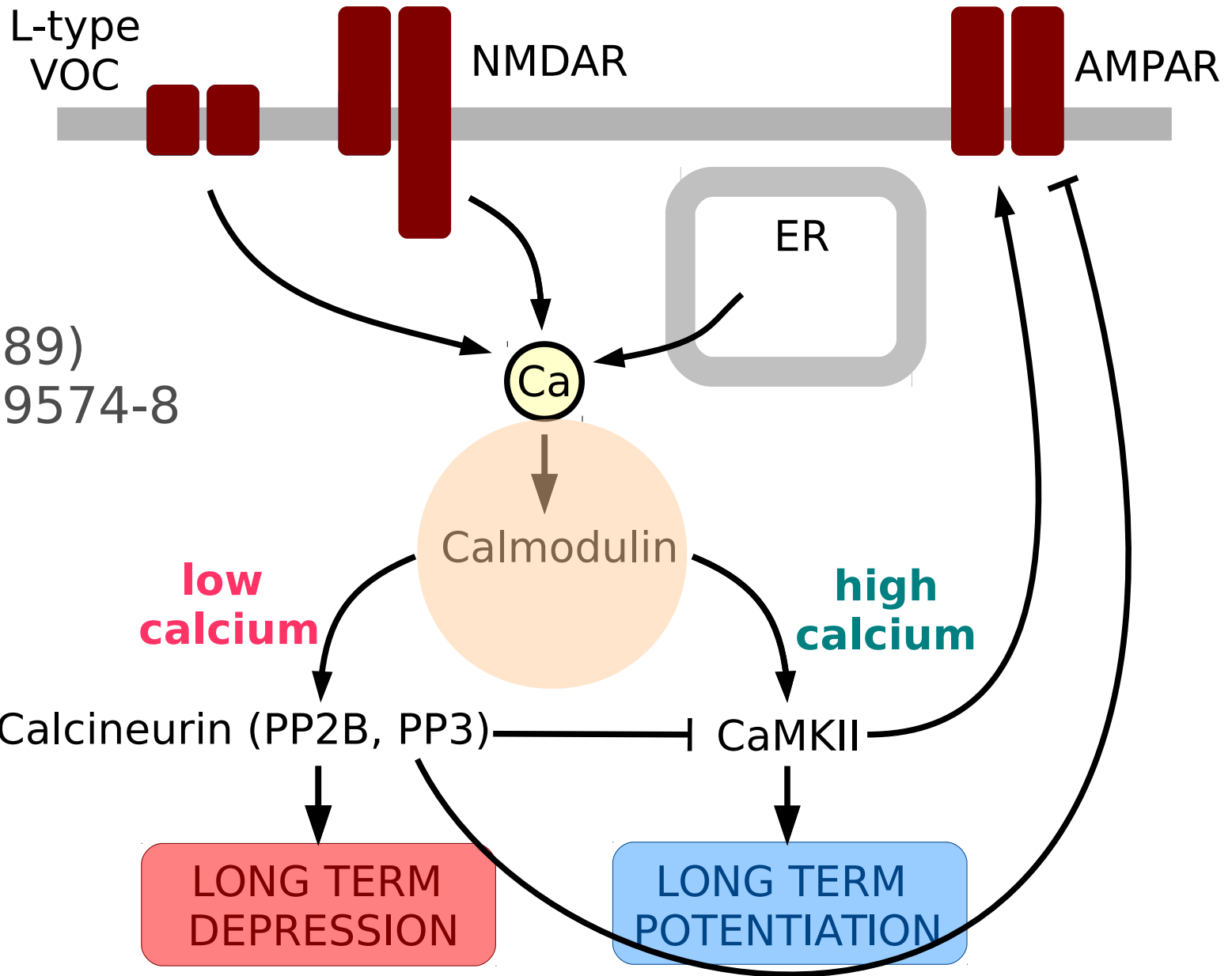


# The calcium theory of synaptic plasticity

Lisman (1989)  
*PNAS*, 86: 9574-8

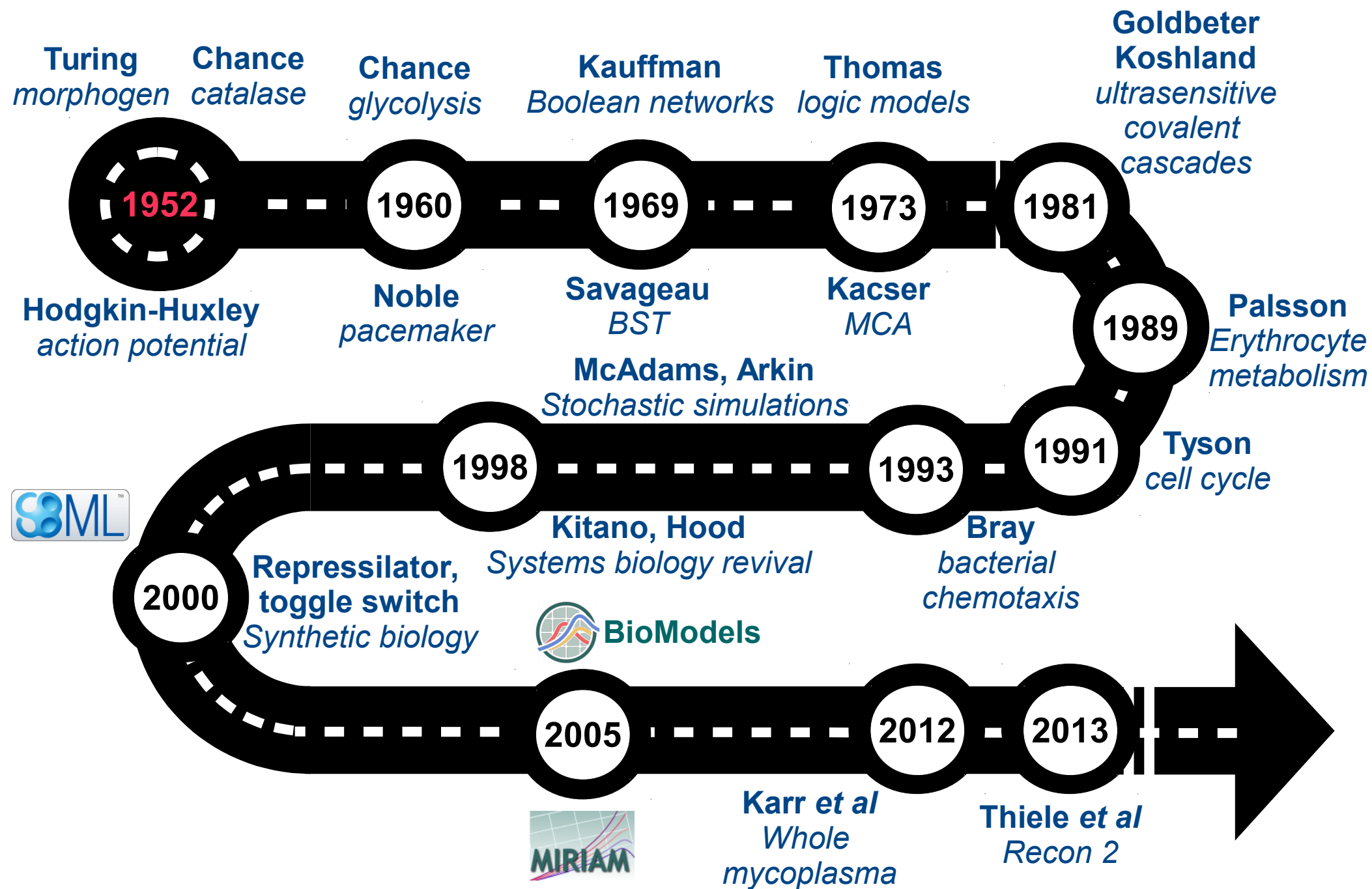


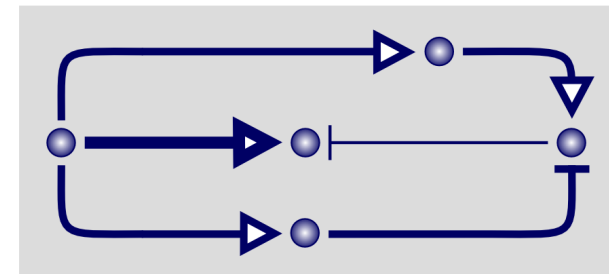
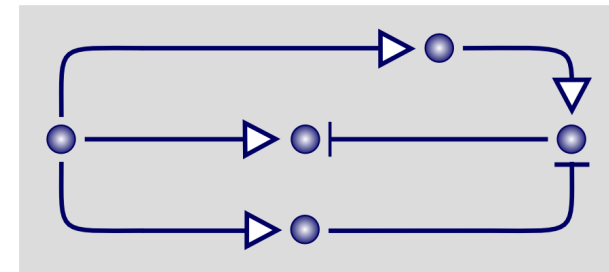
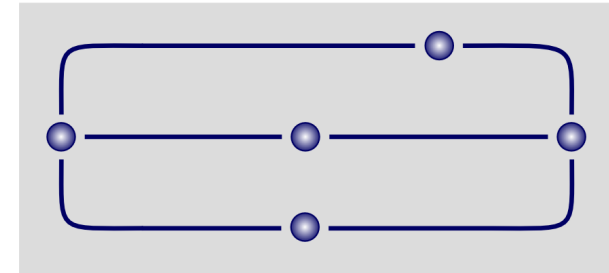
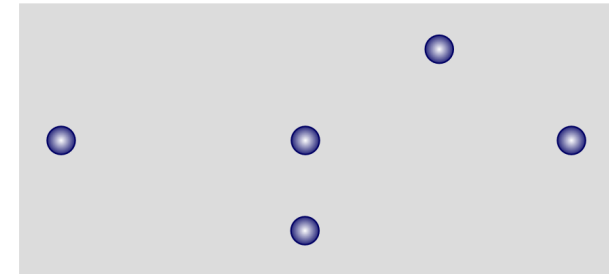
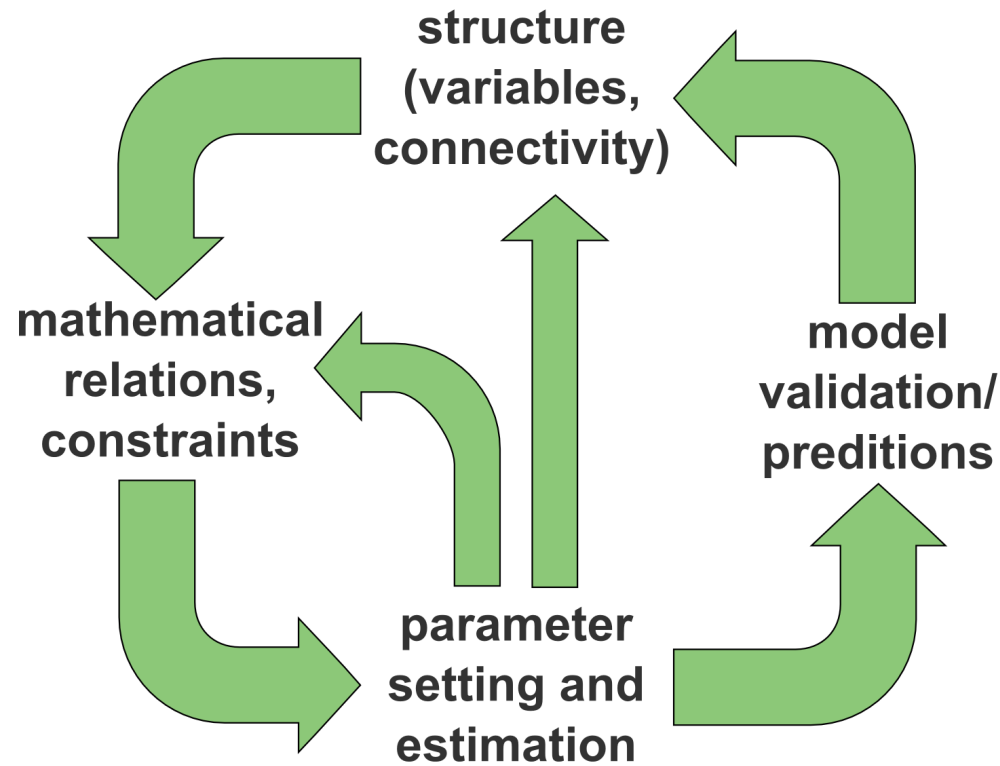
# Calmodulin, the memory switchboard



Lisman (1989)  
*PNAS*, 86: 9574-8





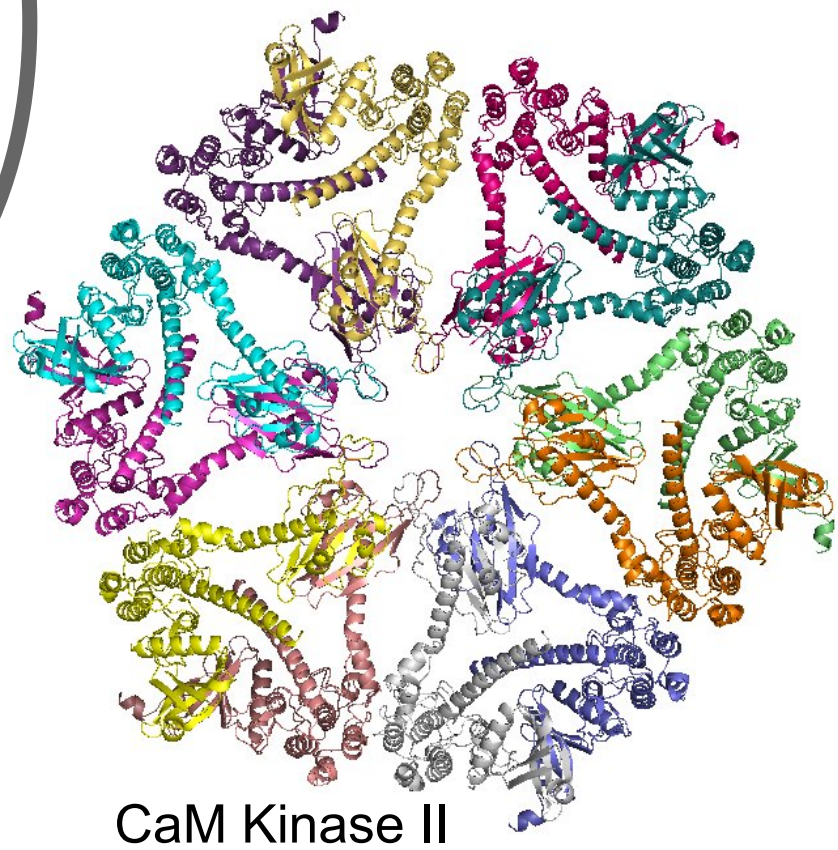
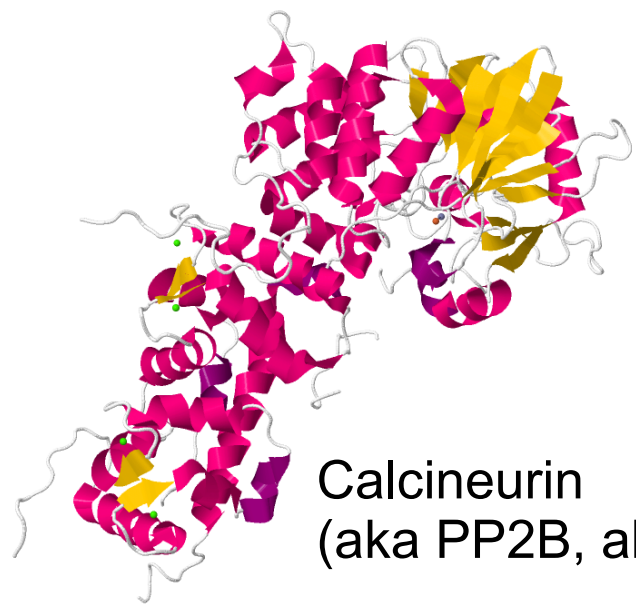
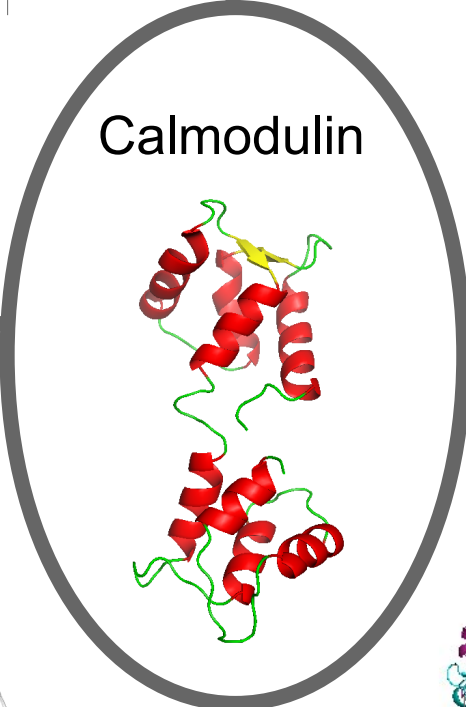


Le Novère N (2016) *Nat Rev Genet*

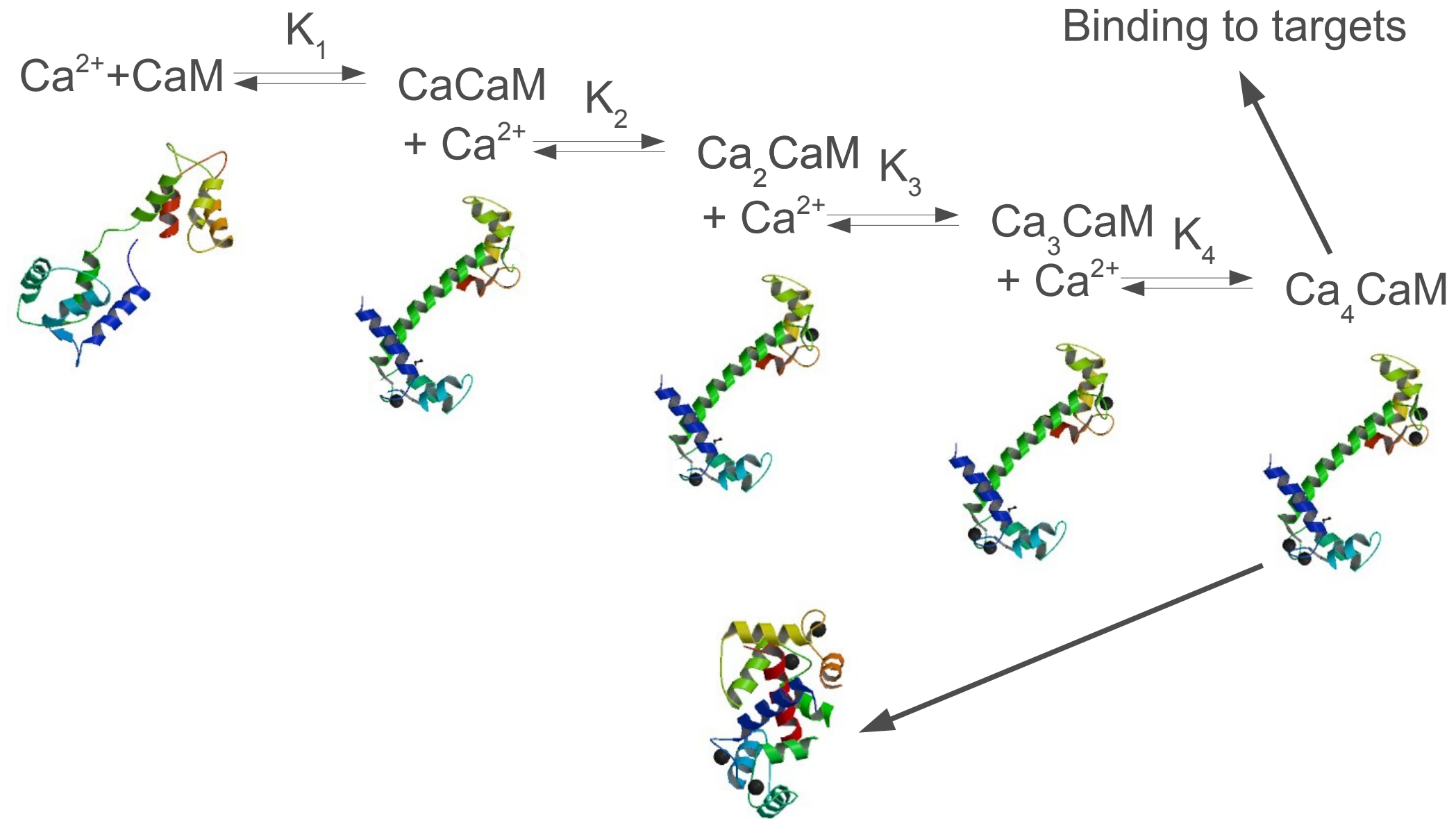


Calcium  
+  +

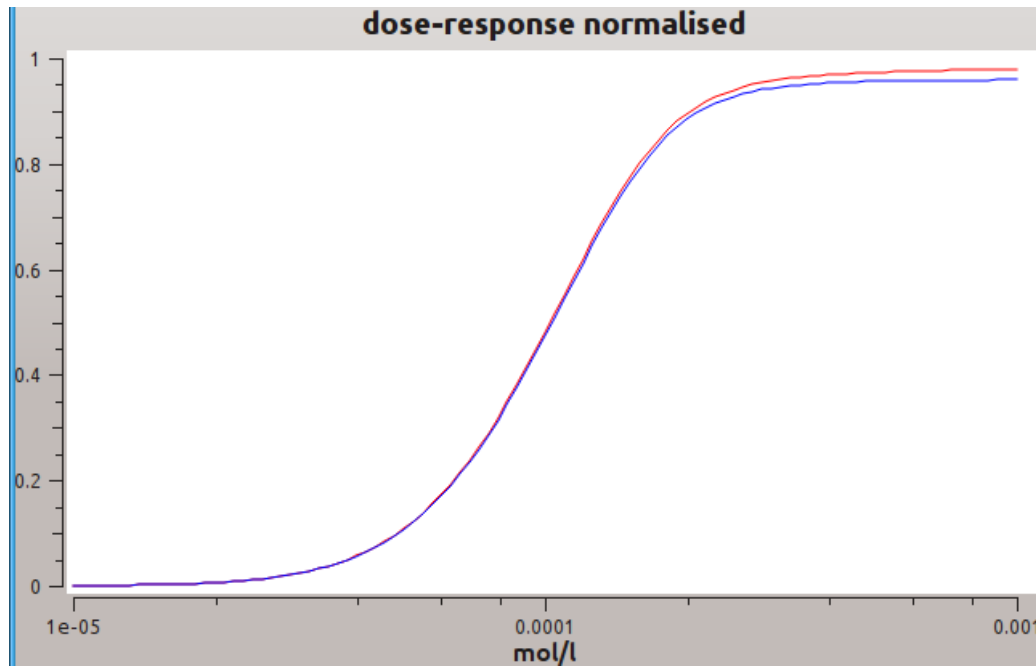
Neurogranin



# Adair-Klotz + key-lock



# Adair-Klotz does not permit differential activations

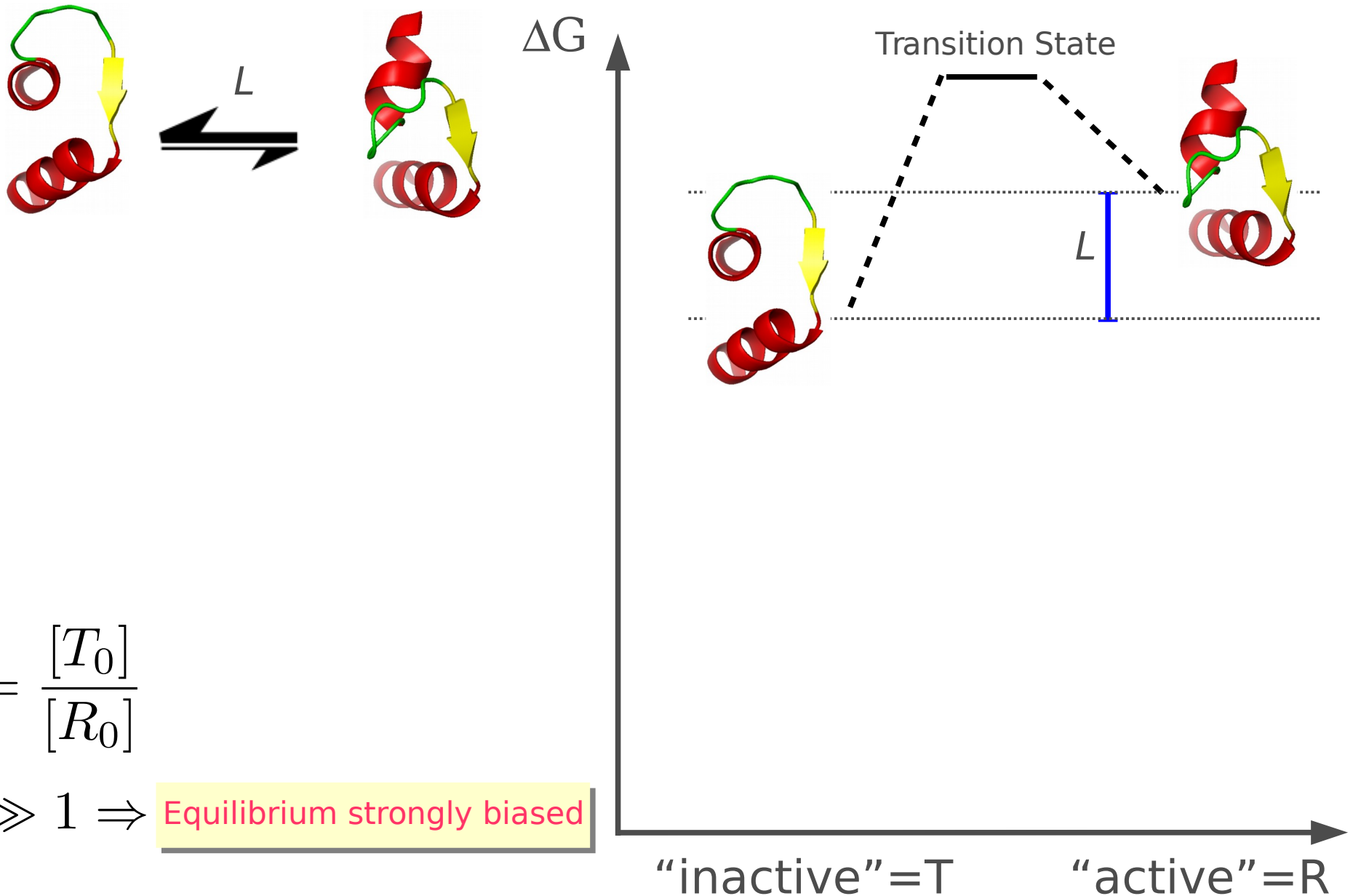


$$\begin{aligned} [\text{CaN}] &= [\text{CamKII}] = [\text{CaM}]/10 ; \\ Kd_{\text{CaMKII}} &= 10 \times Kd_{\text{CaN}} ; \end{aligned}$$

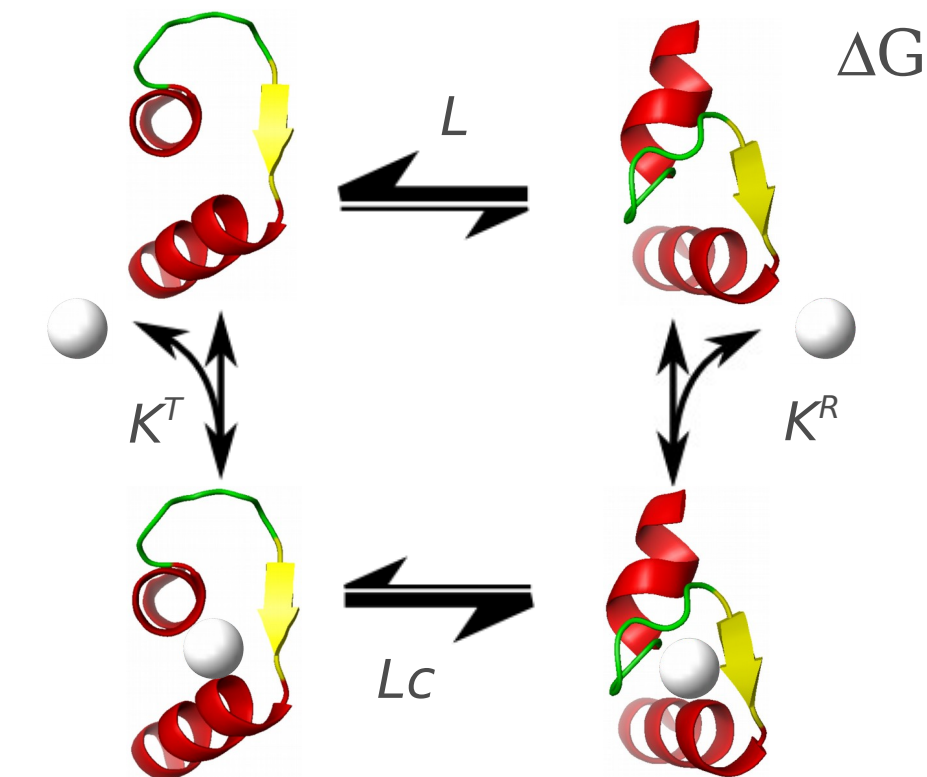
- Calmodulin can activate calcineurin with 3  $\text{Ca}^{2+}$  (Kincaid and Vaughan 1986)
- Calmodulin can bind CaMKII with 2  $\text{Ca}^{2+}$  (Shifman *et al* 2006)
- Calmodulin affinity for calcium increases once bound to CaMKII (Shifman *et al* 2006; Previous reports on other targets: e.g. Burger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



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



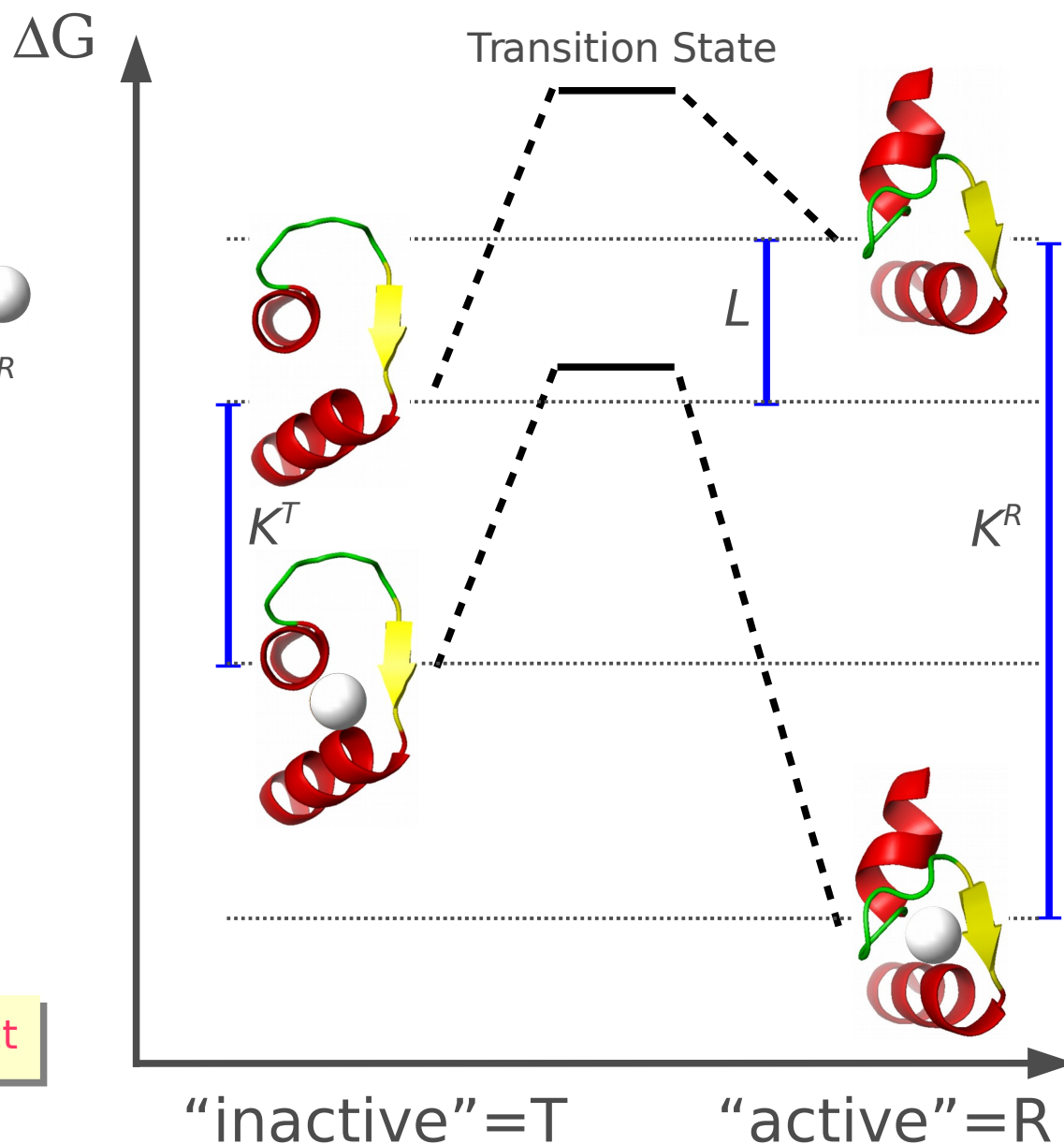
# 1 Modulation of thermal equilibria $\neq$ induced-fit



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

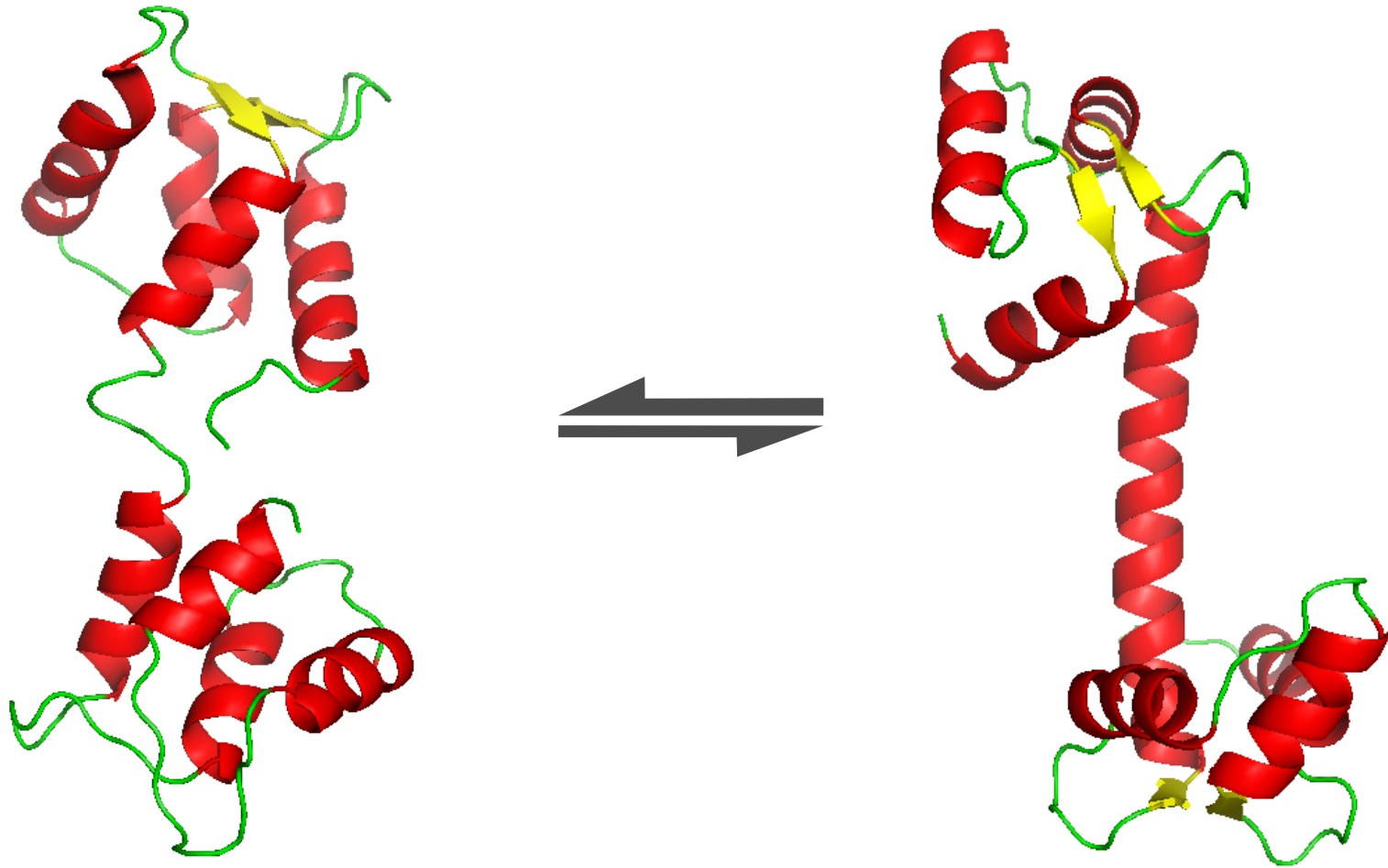
$$c = \frac{K^R}{K^T}$$

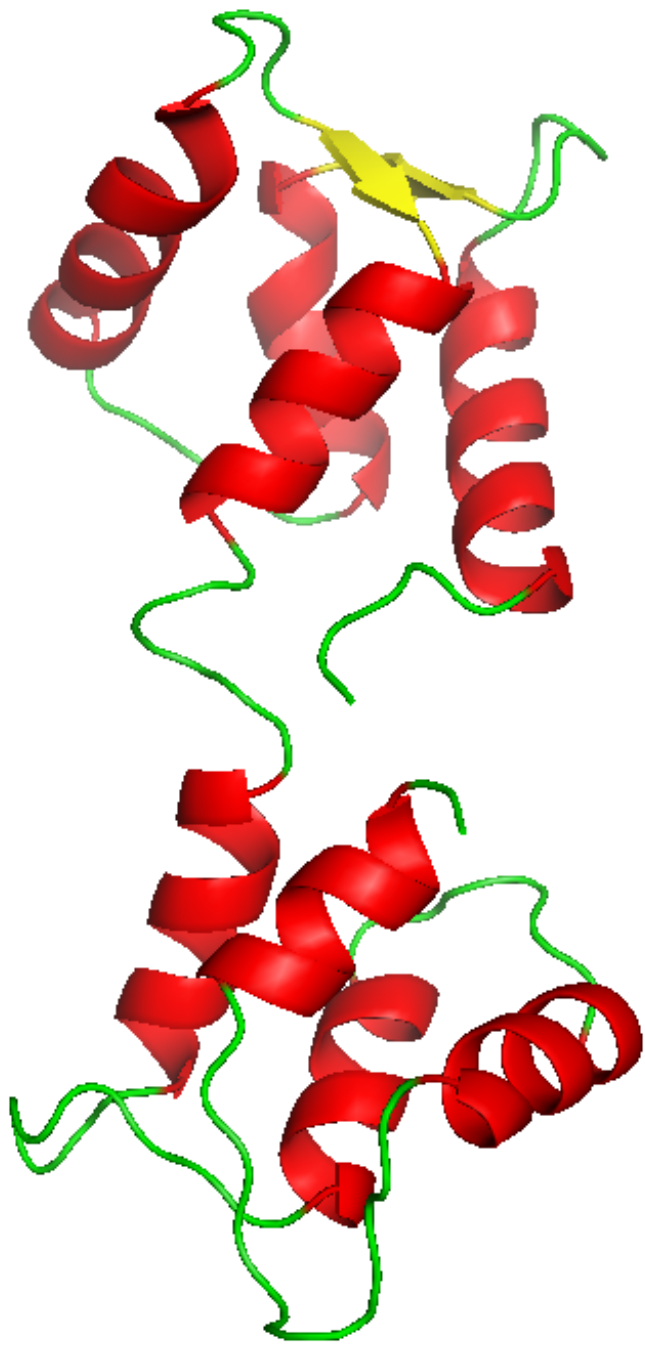
$c \ll 1 \Rightarrow$  ligand has strong effect



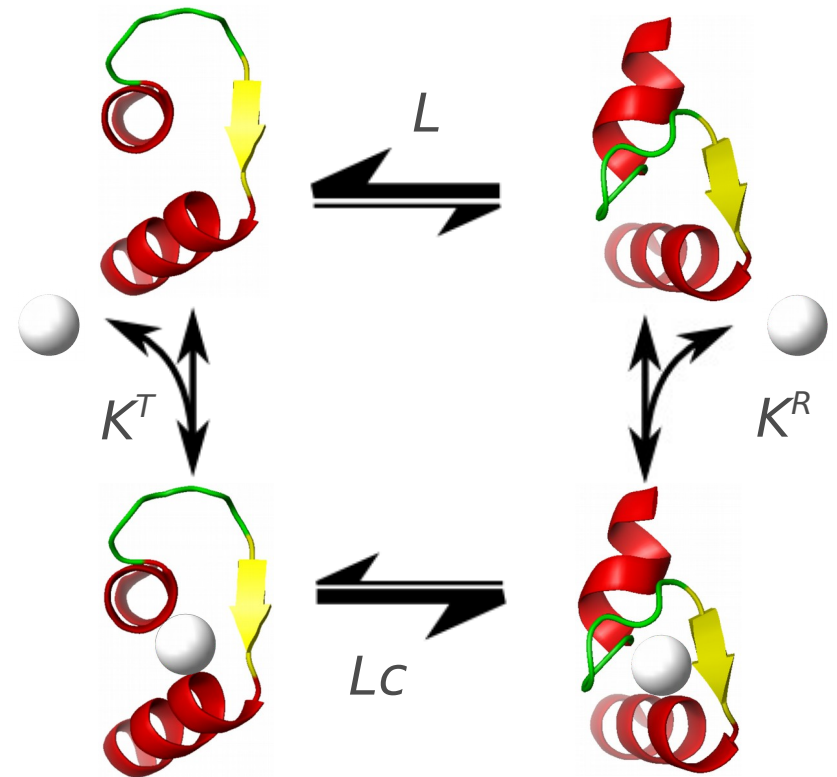
2

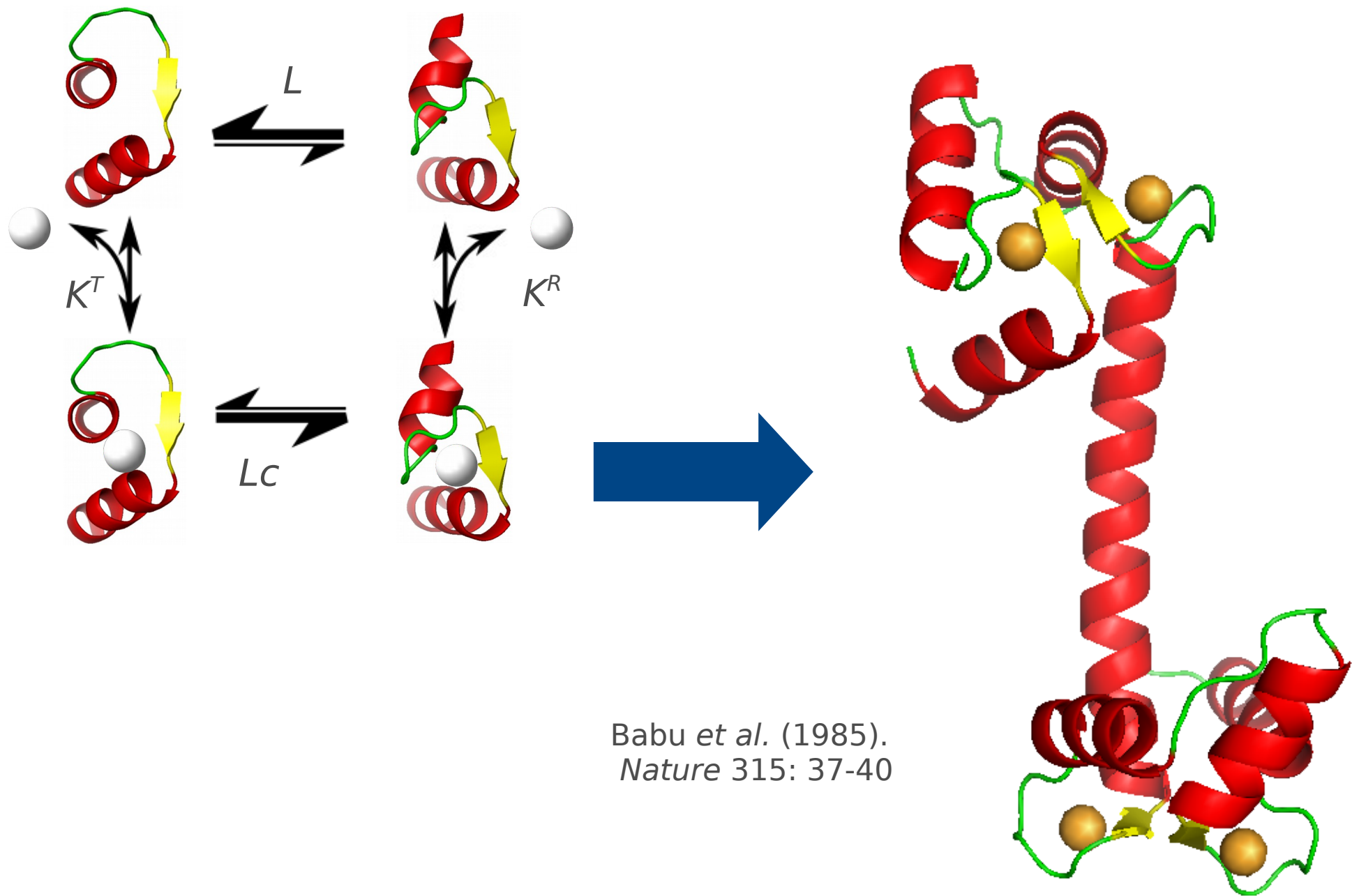
## Concerted transitions $\neq$ sequential model



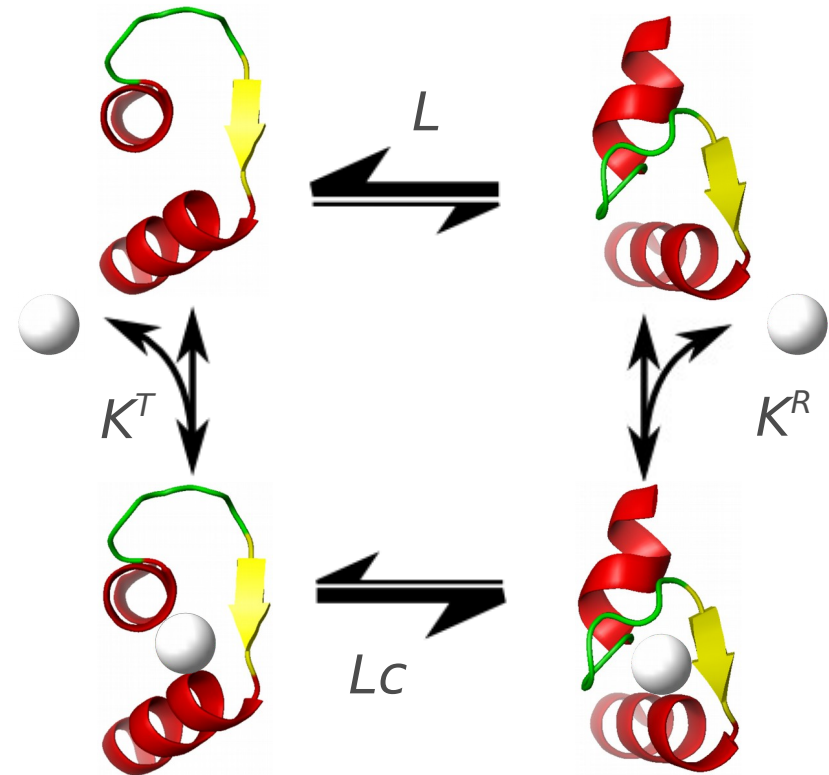
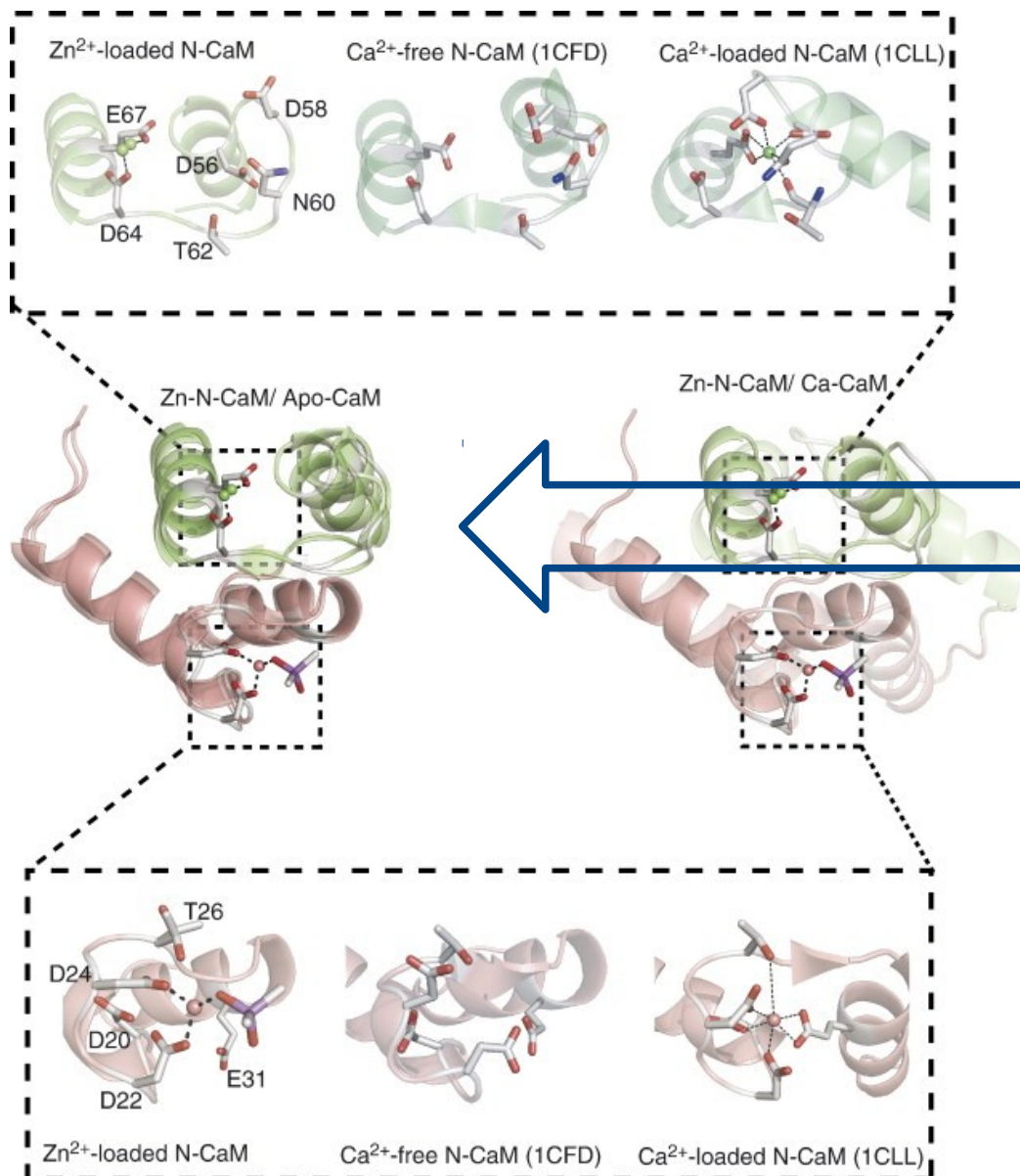


Kuboniwa *et al.* (1995).  
*Nat Struct Biol* 2: 768-776

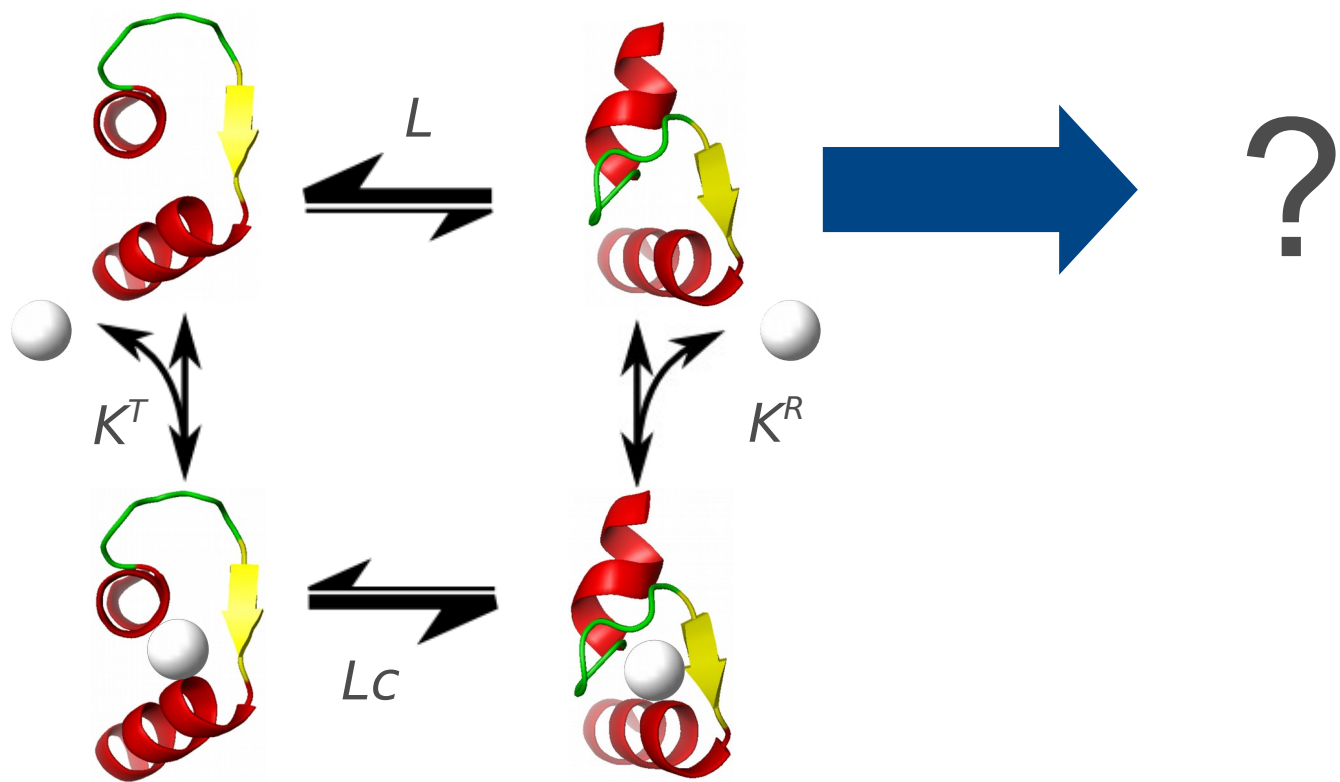


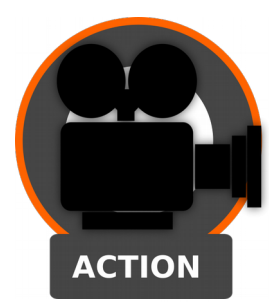




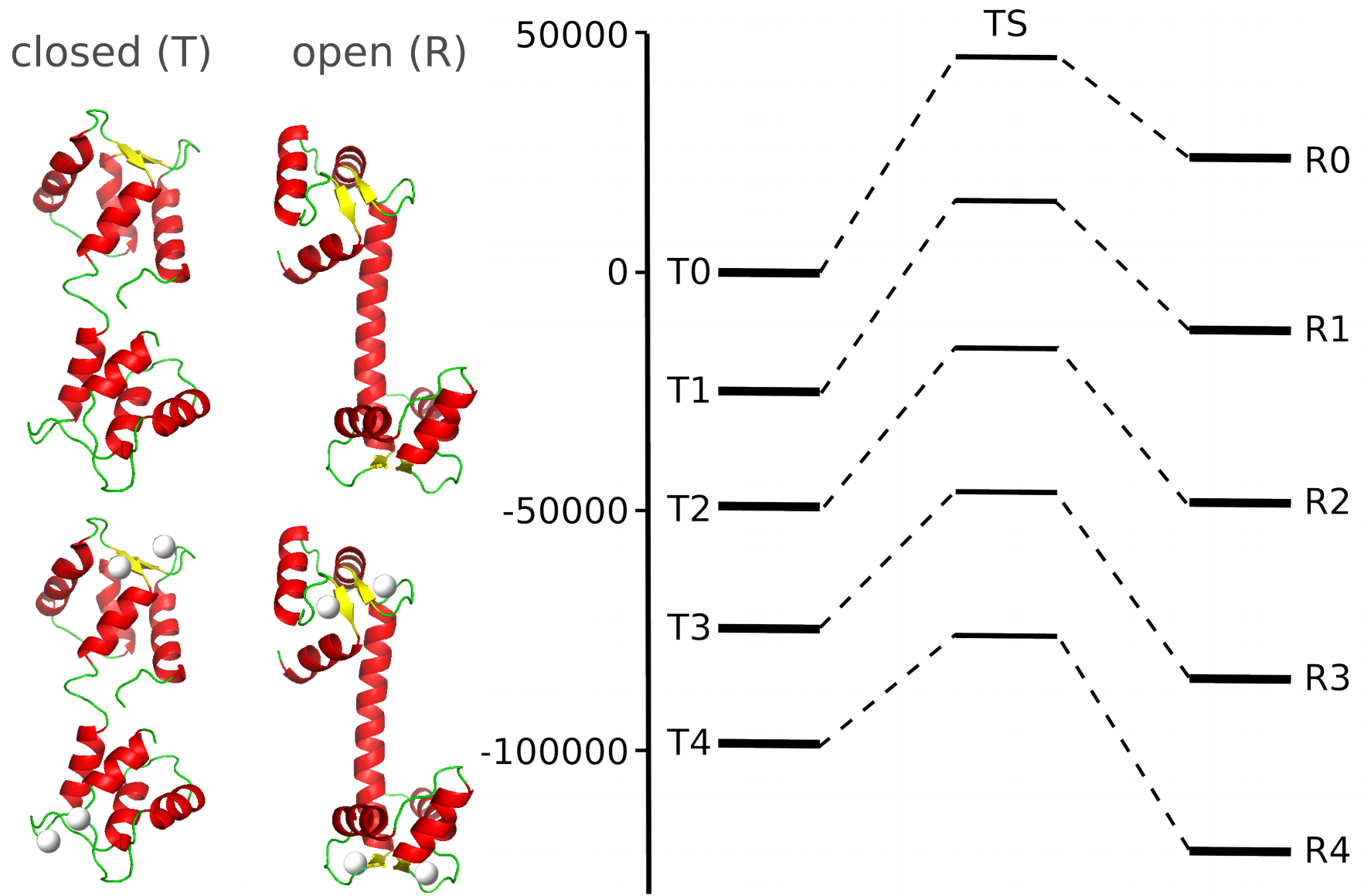


Warren et al. (2007).  
*J Mol Biol* 374: 517-527



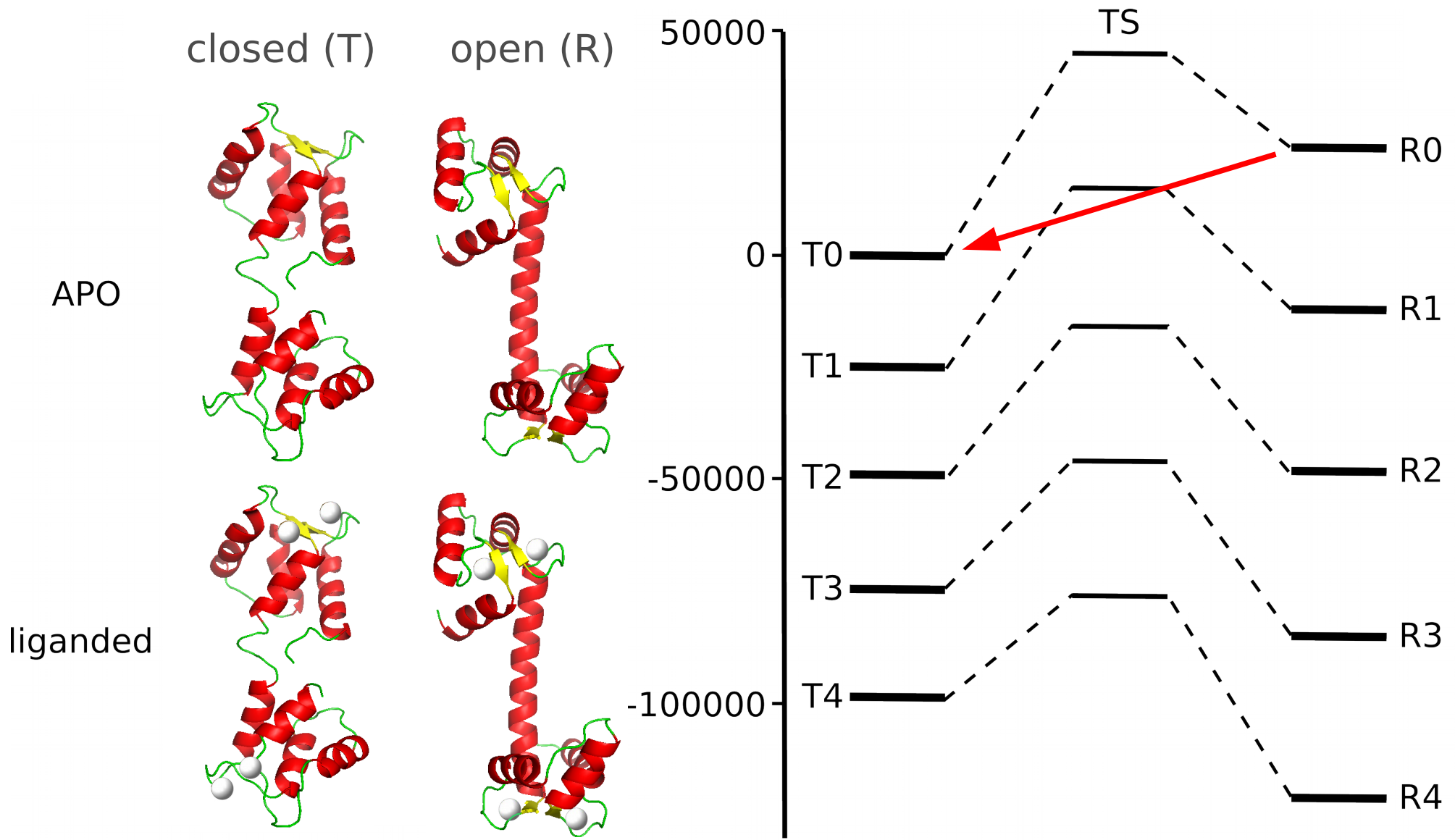


# Allosteric model of Calmodulin activation

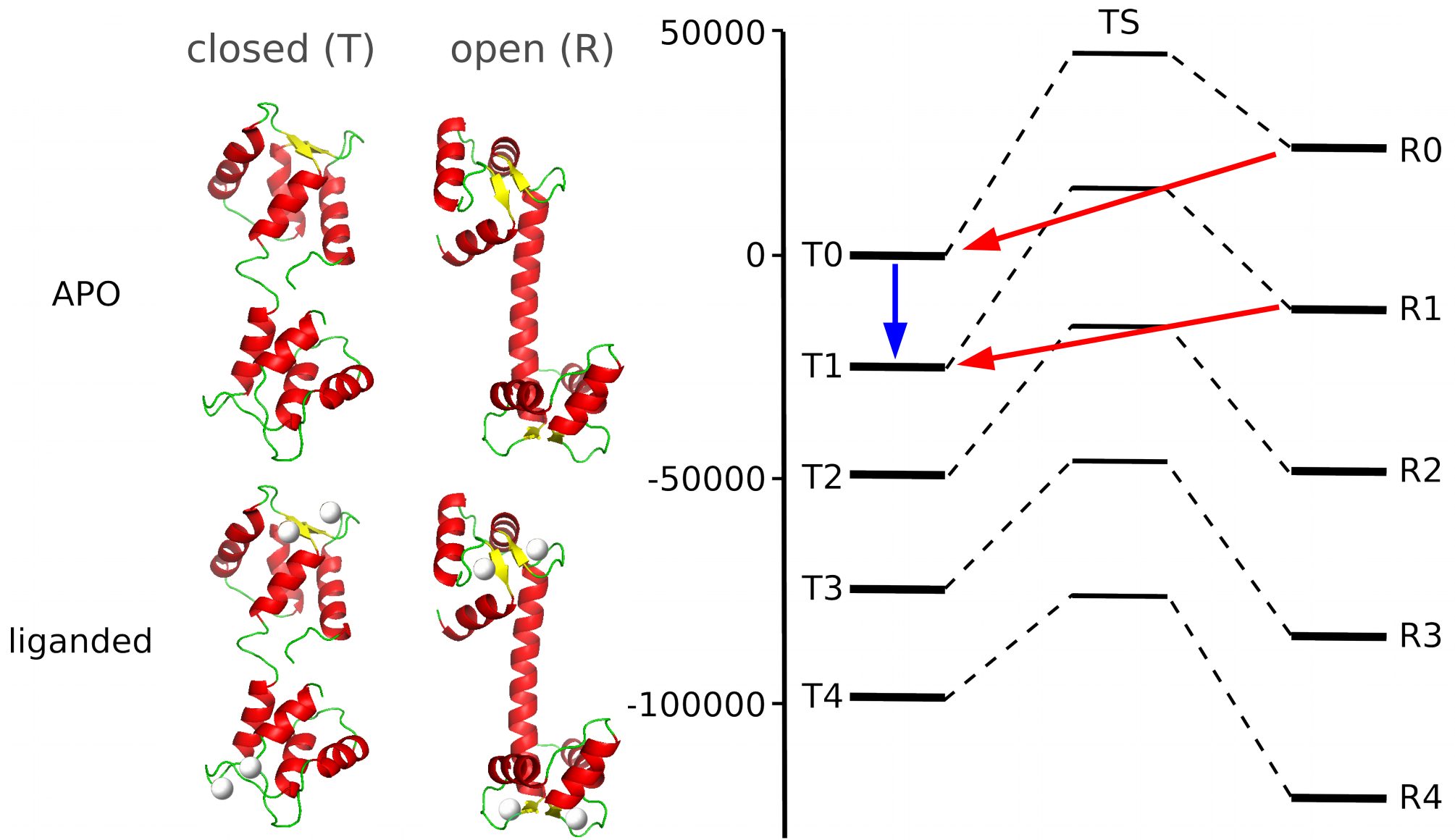


Stefan MI, Edelstein SJ, Le Novère N (2008, 2009)

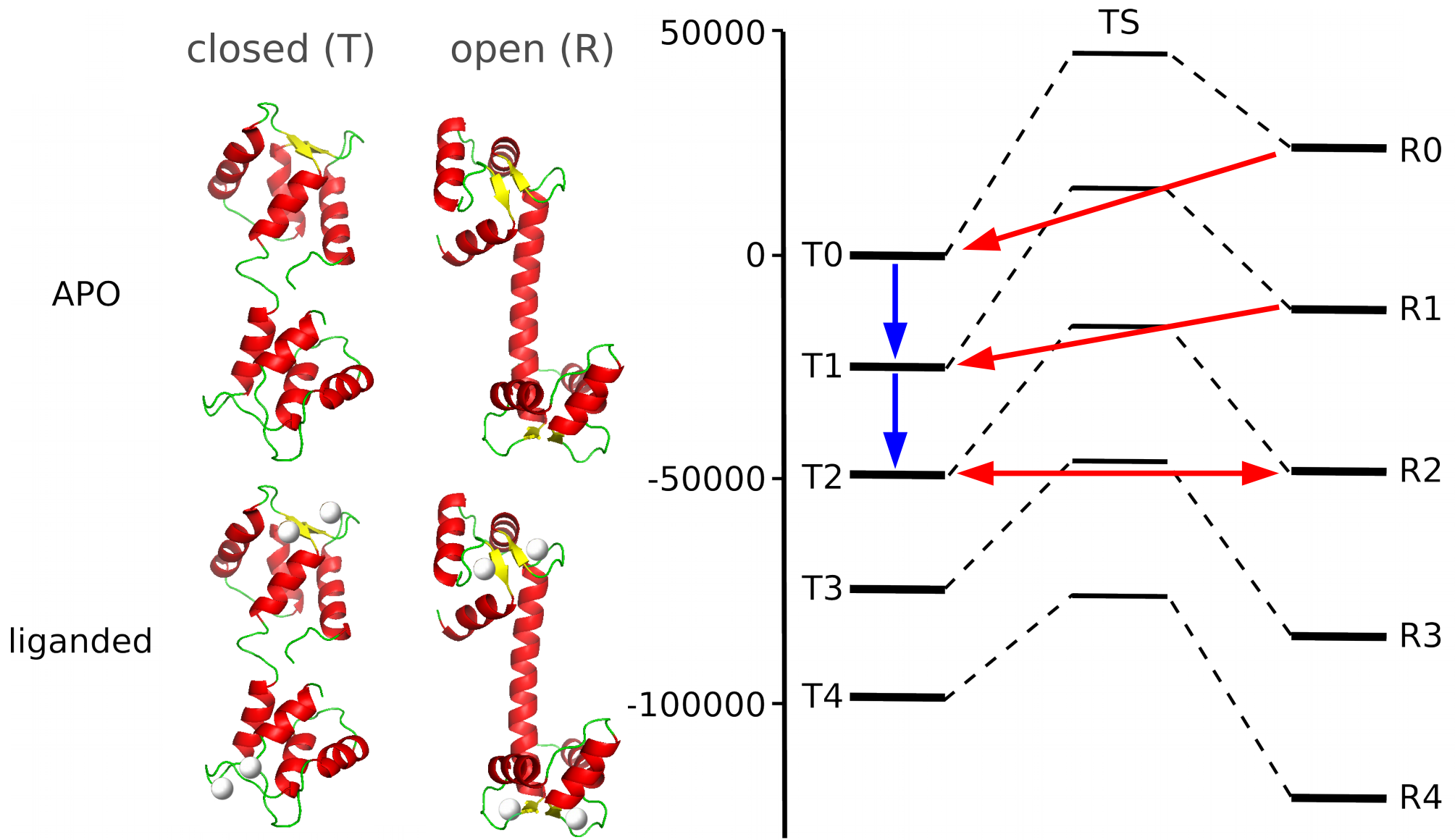
# Allosteric model of Calmodulin activation



# Allosteric model of Calmodulin activation

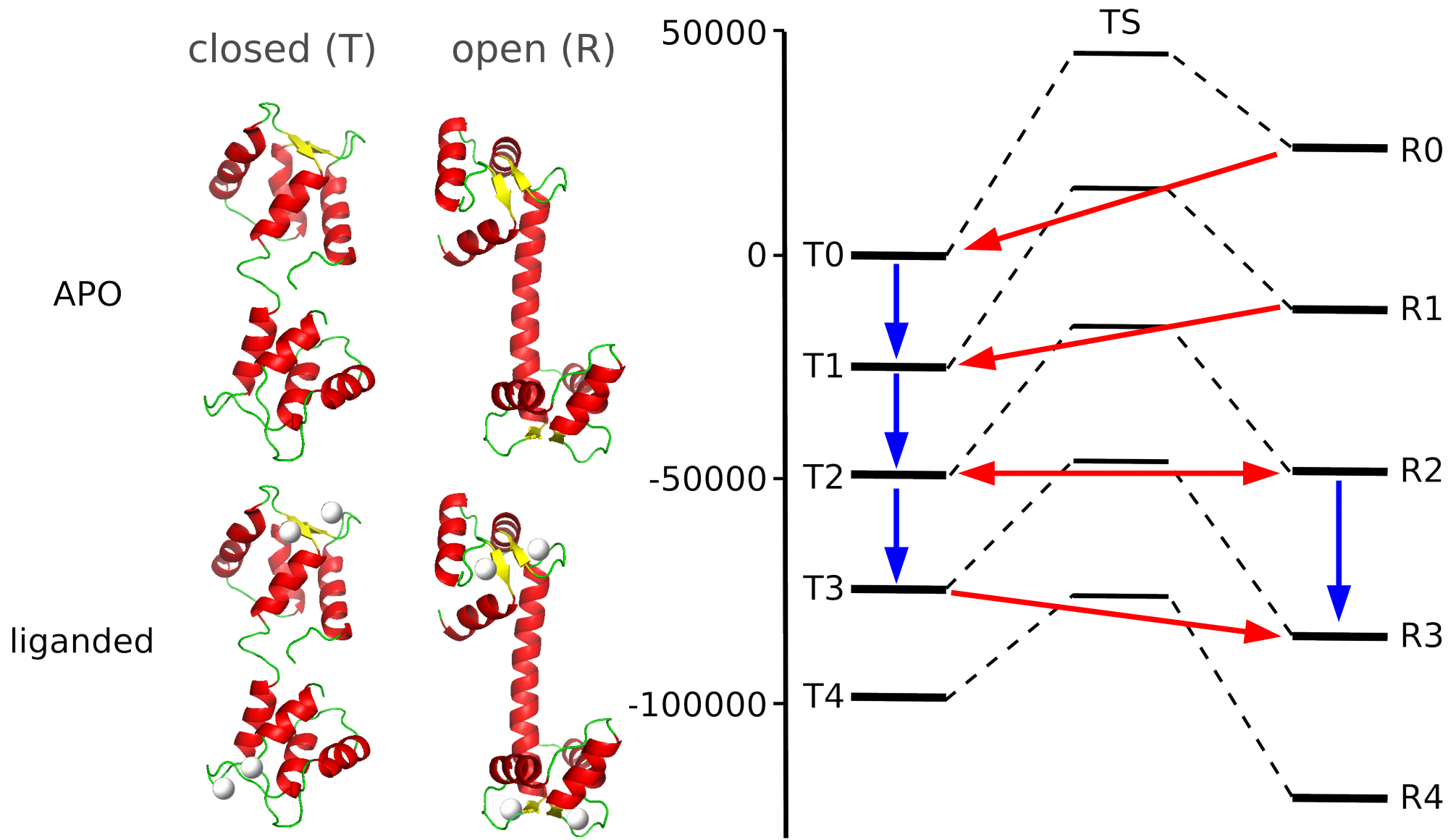


# Allosteric model of Calmodulin activation

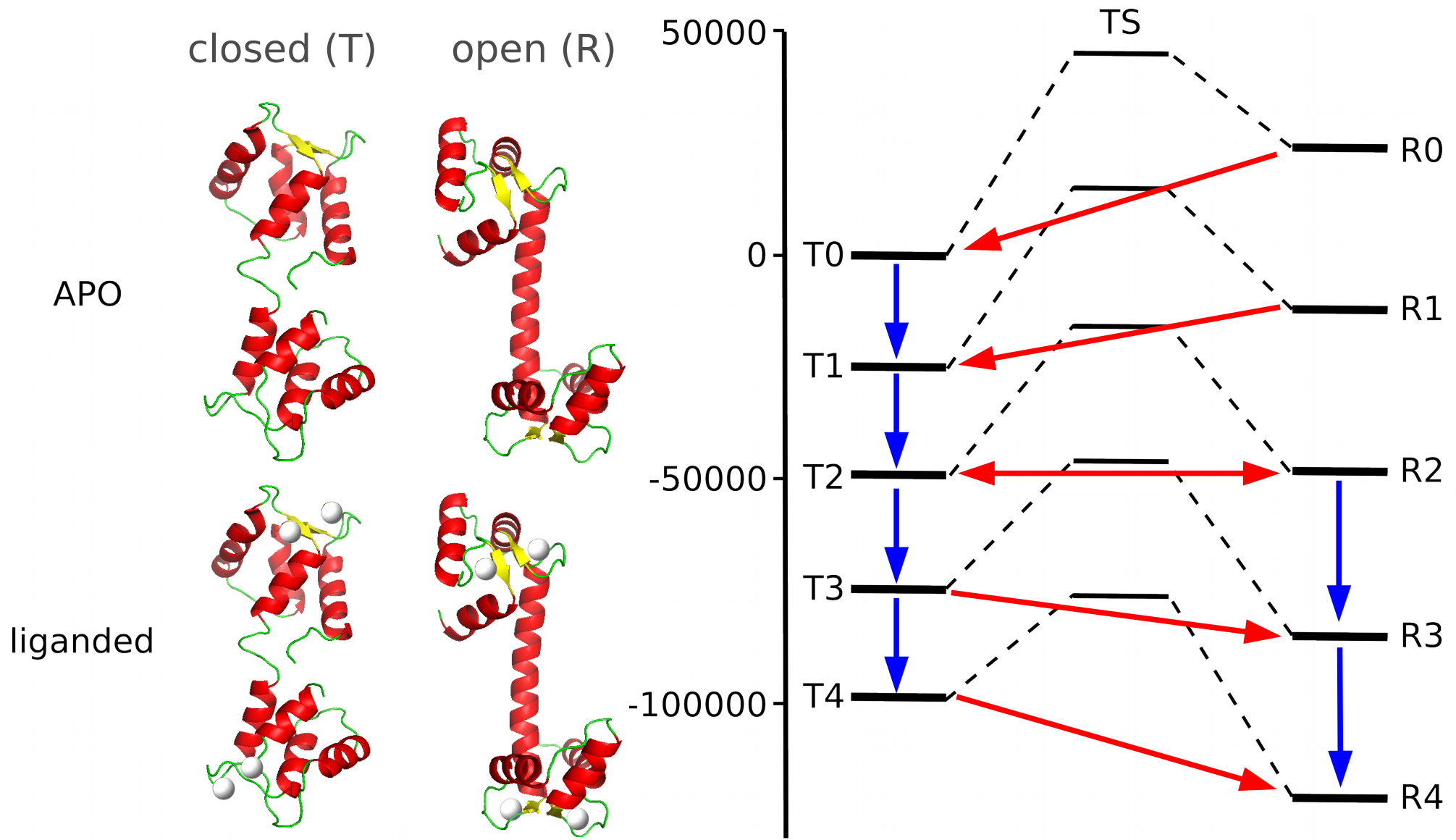




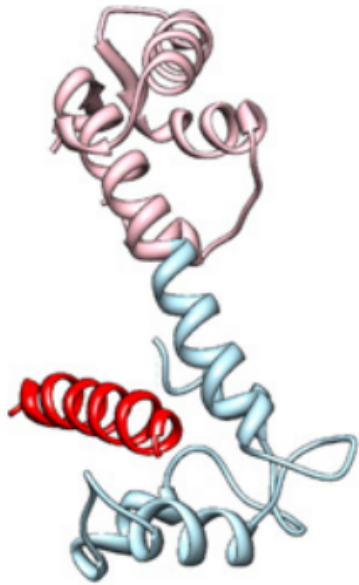
# Allosteric model of Calmodulin activation



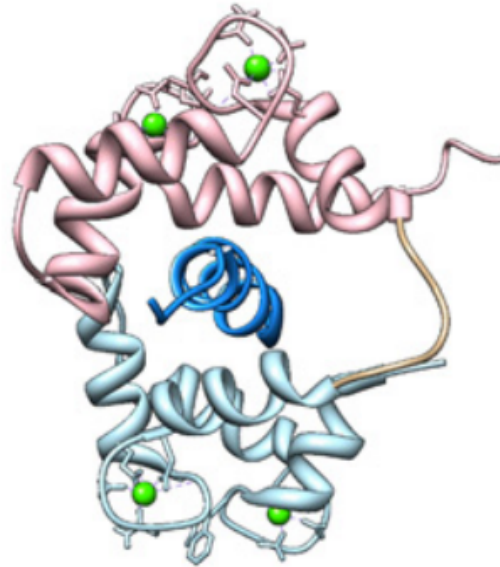
# Allosteric model of Calmodulin activation



# Different targets stabilise lobes in different states



**Neurogranin**



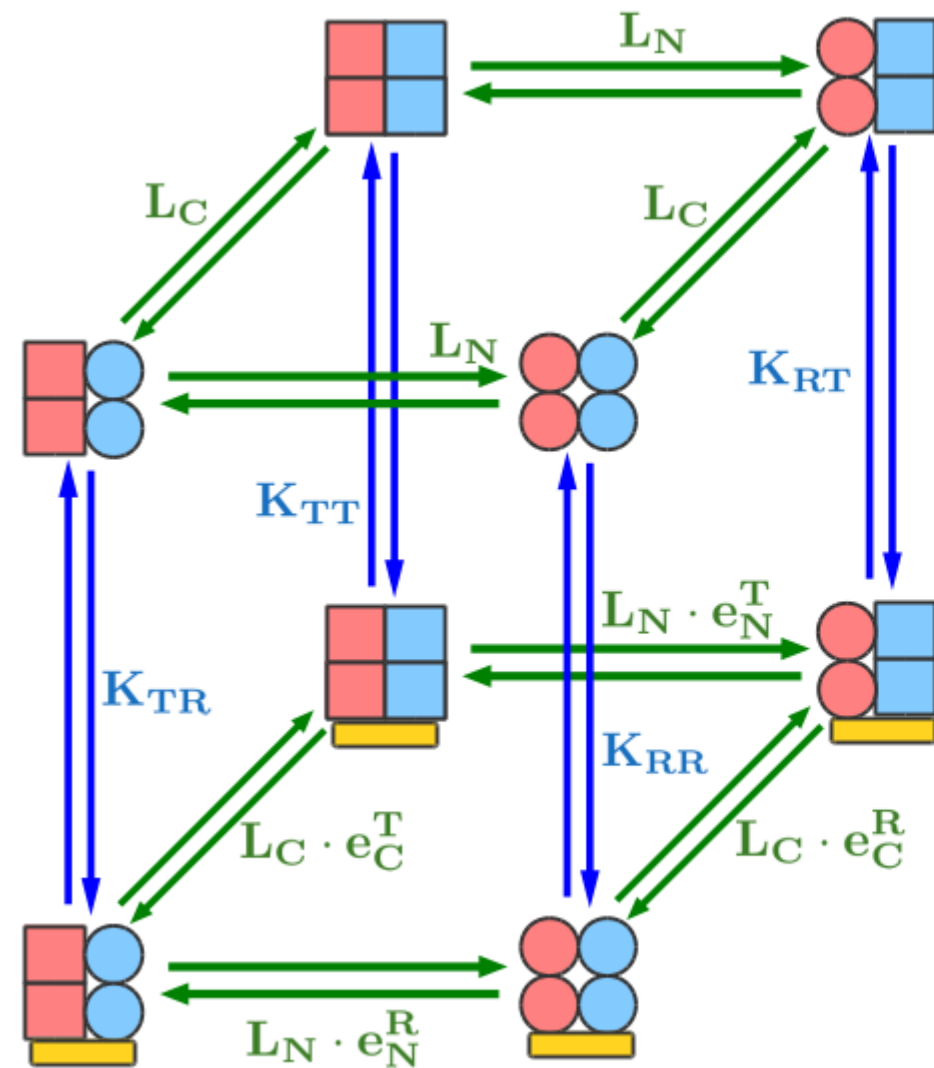
**MLCK**



**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



= N-lobe

= C-lobe



= T-state



= R-state



= target



= TT conformation



= RT conformation



= TR conformation



= RR conformation

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

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

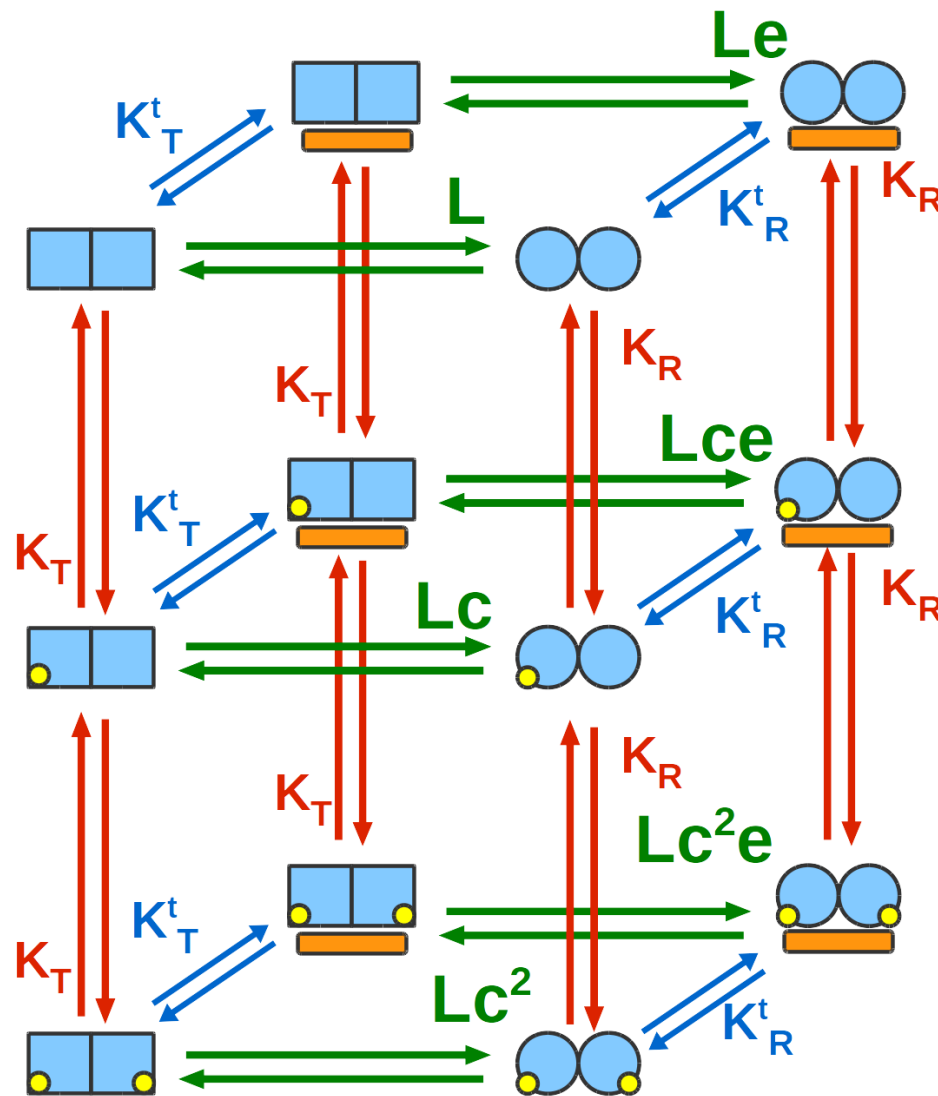
$$e_N^R = \frac{K_{RR}}{K_{TR}}$$

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

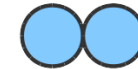
$$e_N^T = \frac{K_{RT}}{K_{TT}}$$

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

# Bindings of calcium and targets



T-state



R-state



Calcium



Target



Conf. transition



Calcium binding

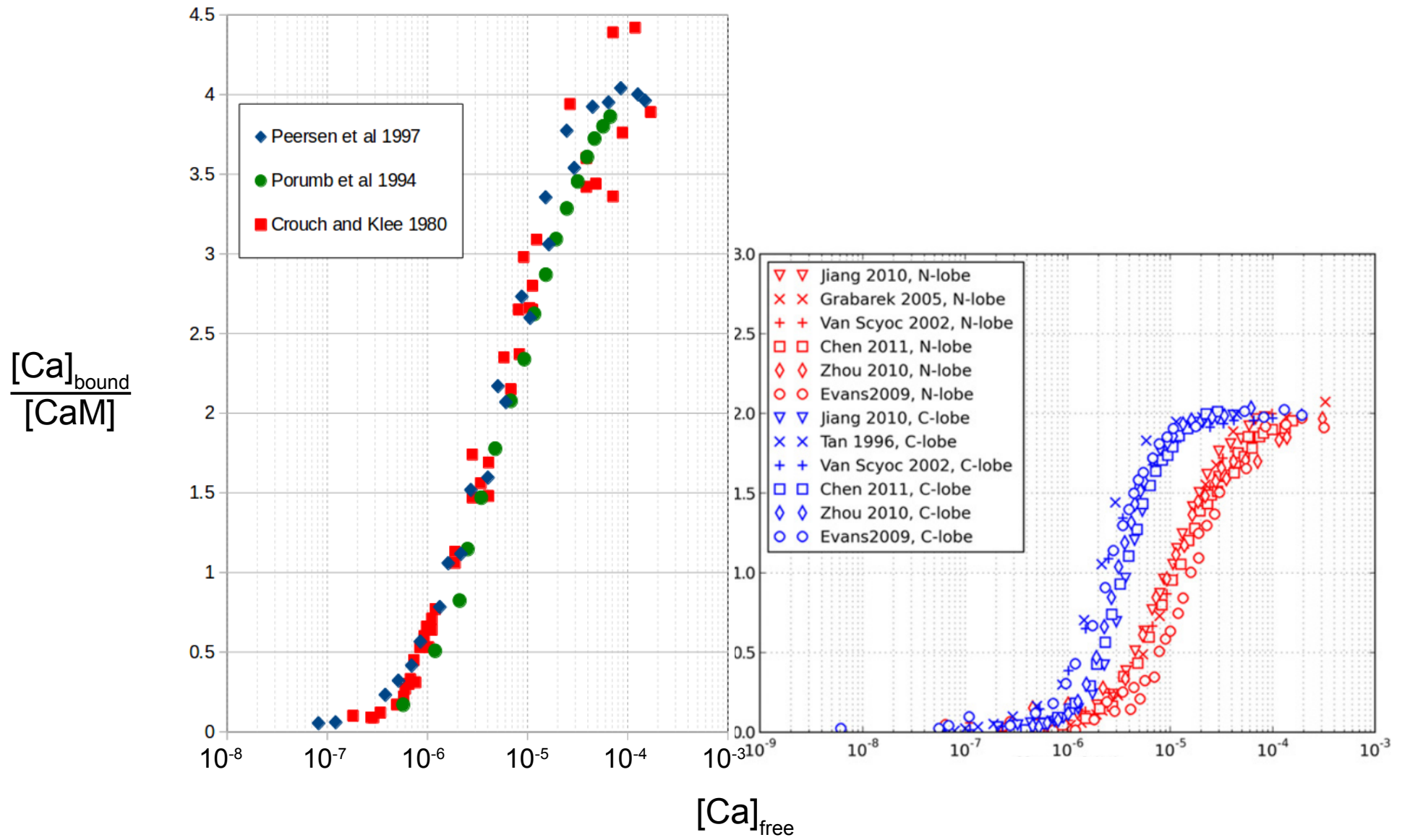


Target binding

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

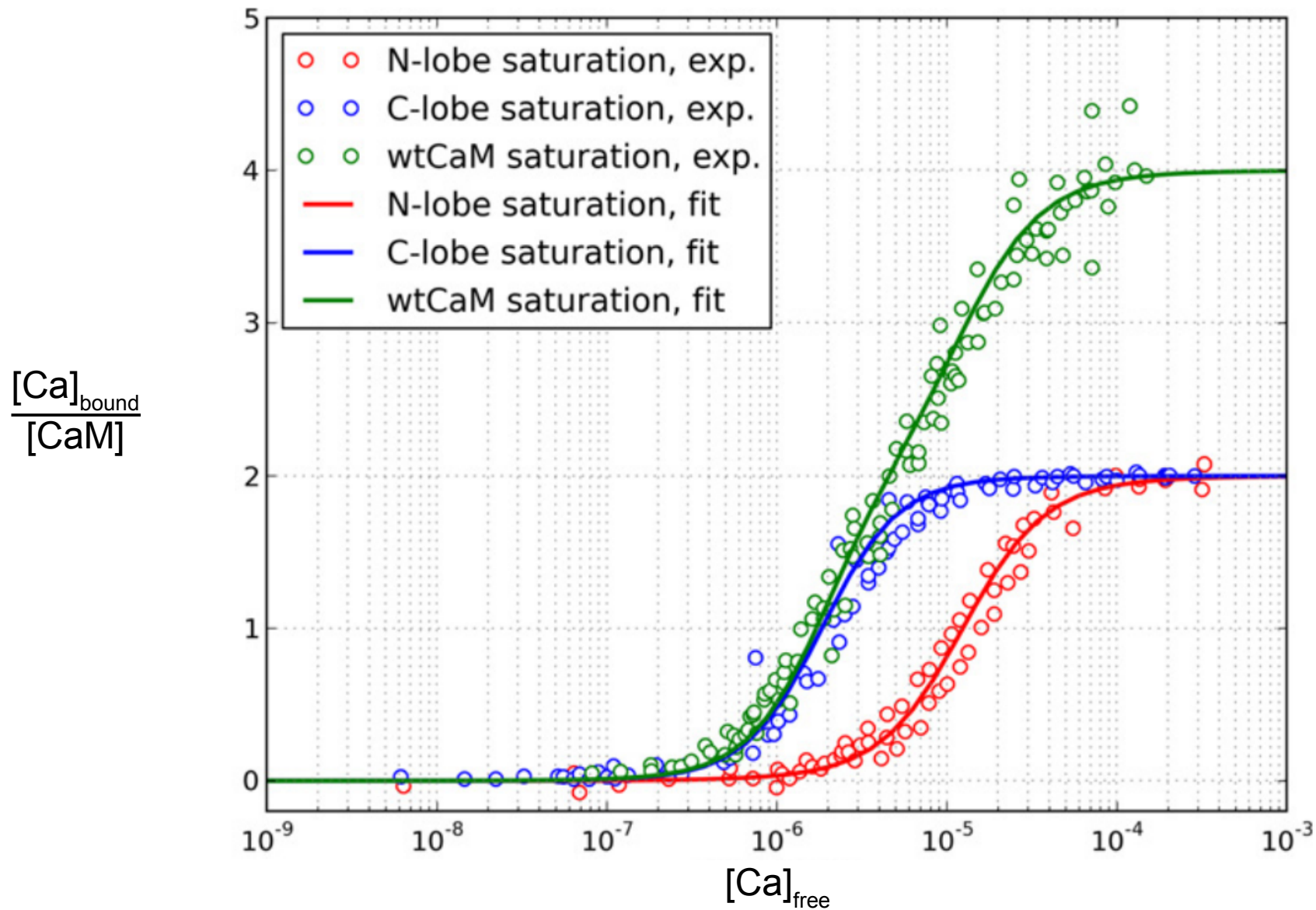
$$e = \frac{K_R^t}{K_T^t}$$

# Calcium binding to lobes and whole CaM (exp)





# Calcium binding to lobes and whole CaM (sim)



# Parametrisation using accurate measurements

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

1 in 20000 active w/o  $\text{Ca}^{2+}$

$$L=20670$$

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

Affinity of  $\text{Ca}^{2+}$  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

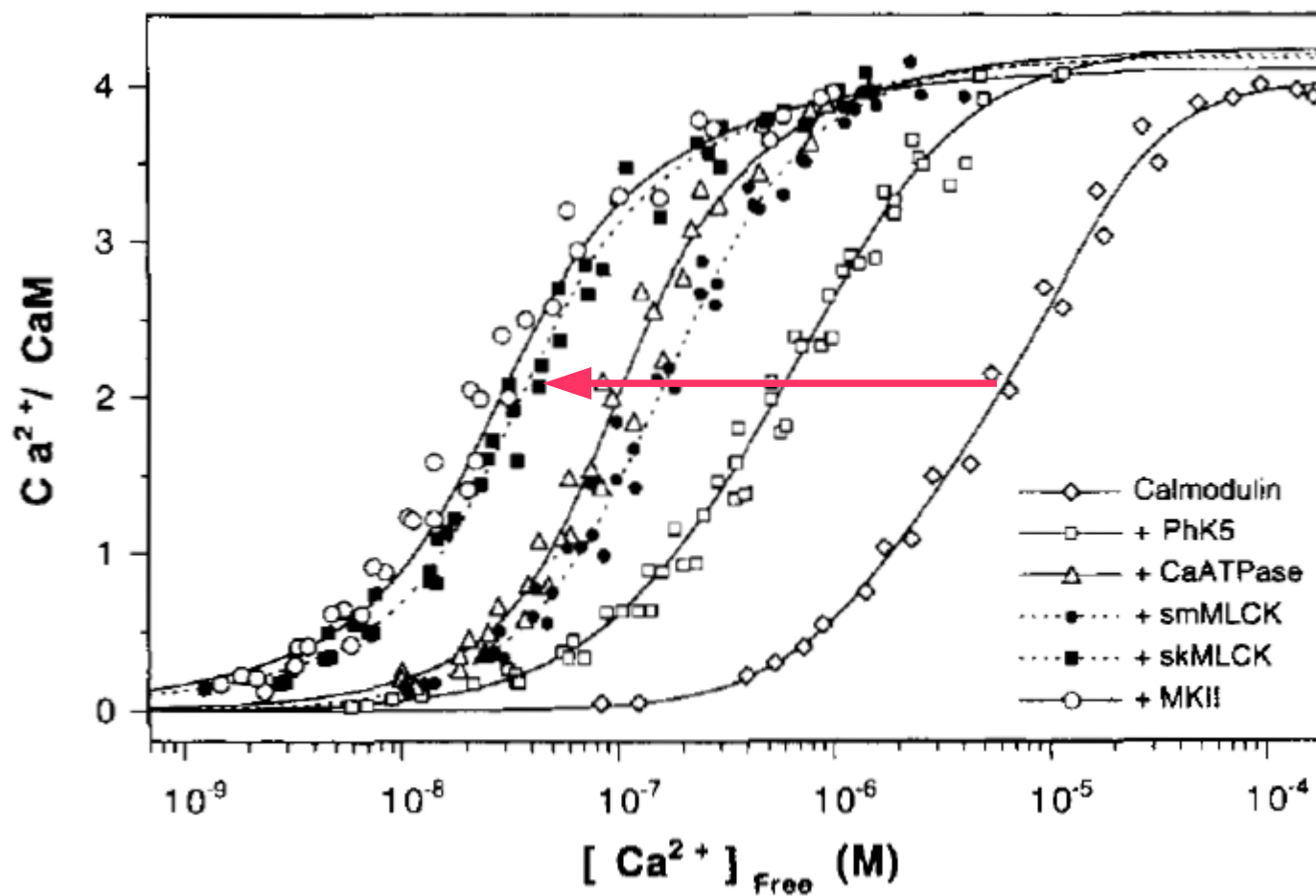
# Activity of unsaturated calmodulin (state function)

- 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

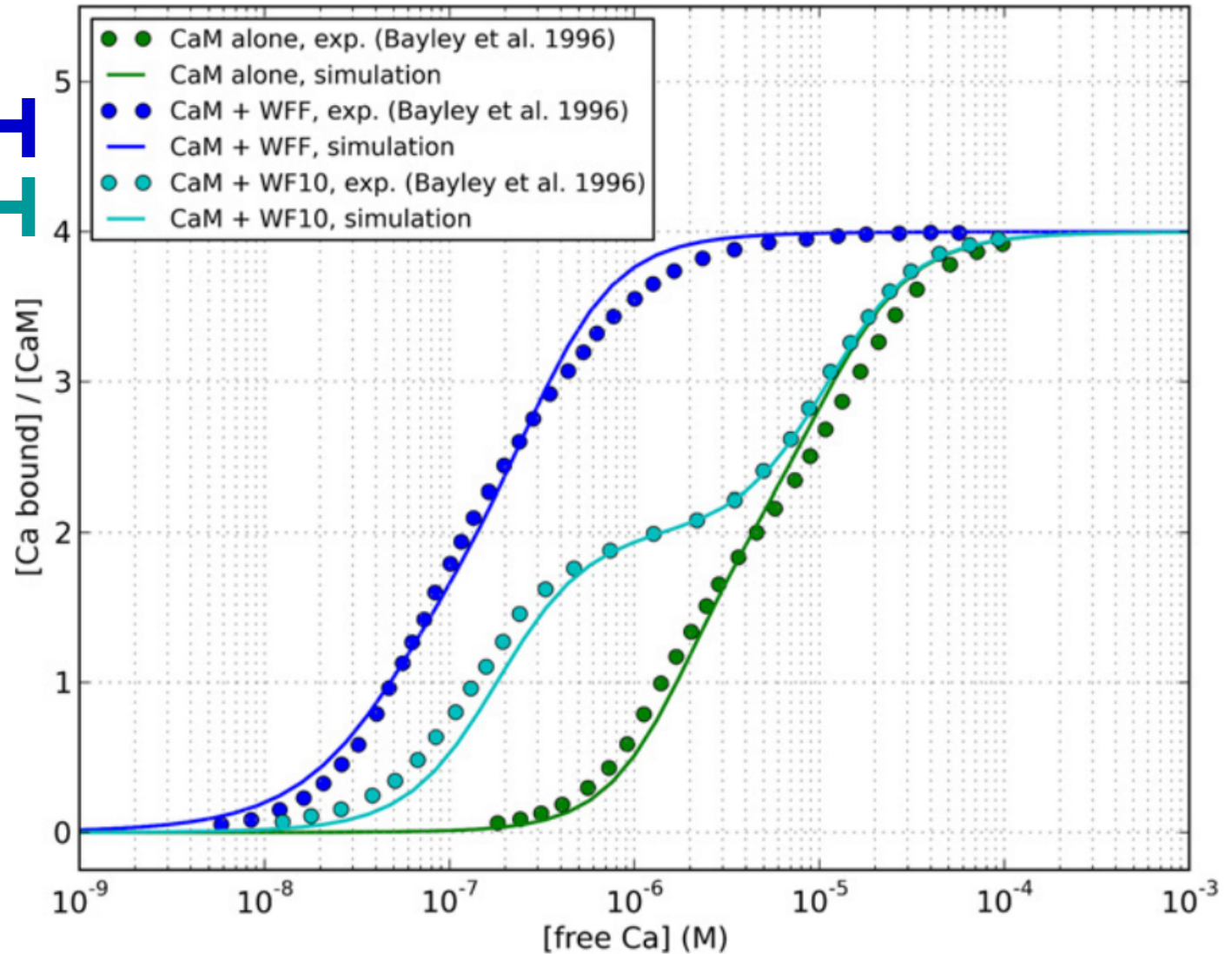


Peersen et al. (1997)

# Binding to target increases the affinity for $\text{Ca}^{2+}$

Stabilise R state  
of both lobes

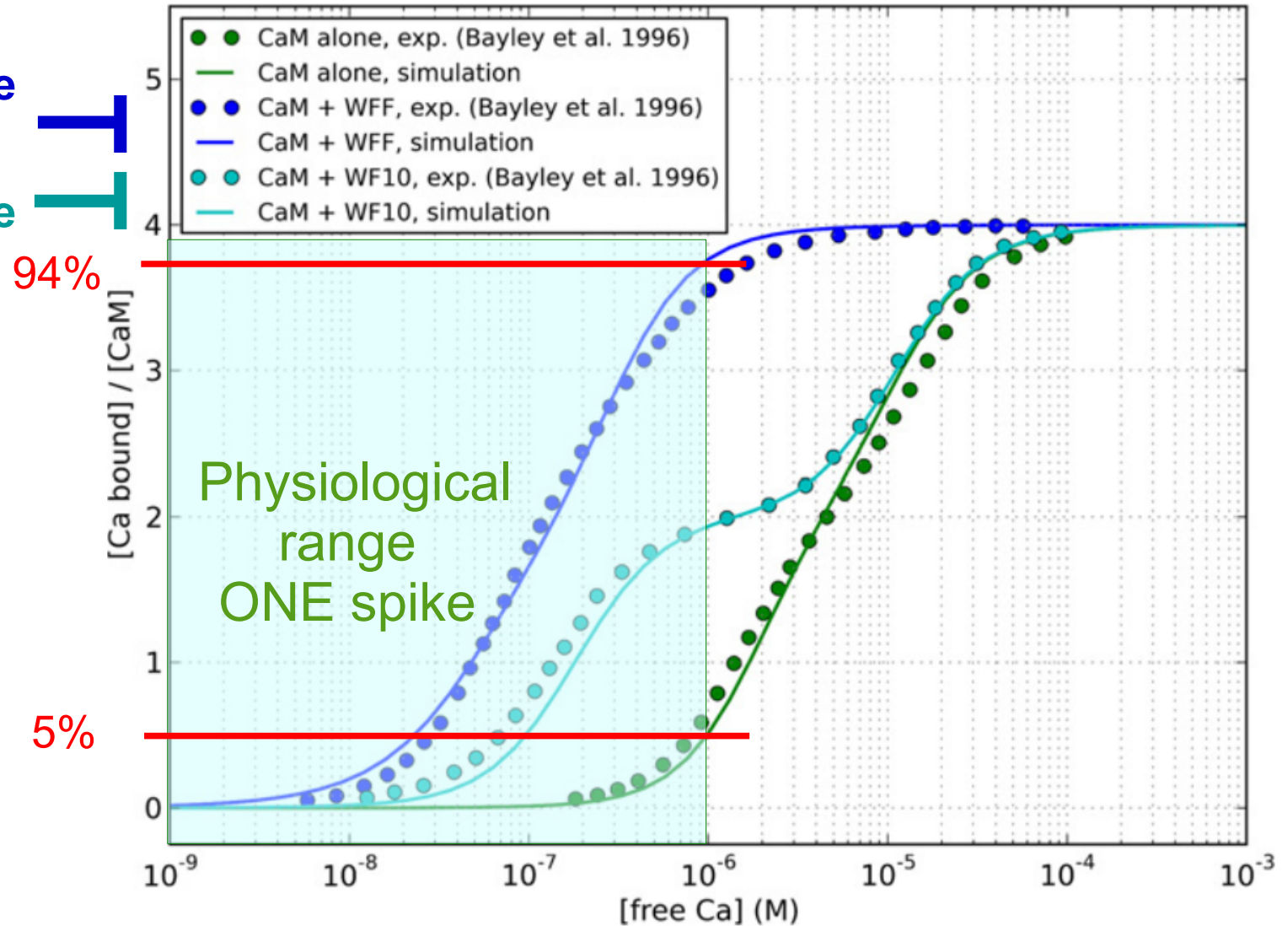
Stabilise R state  
of C lobe



# Targets stabilises $\text{Ca}^{2+}$ binding into the physiological range

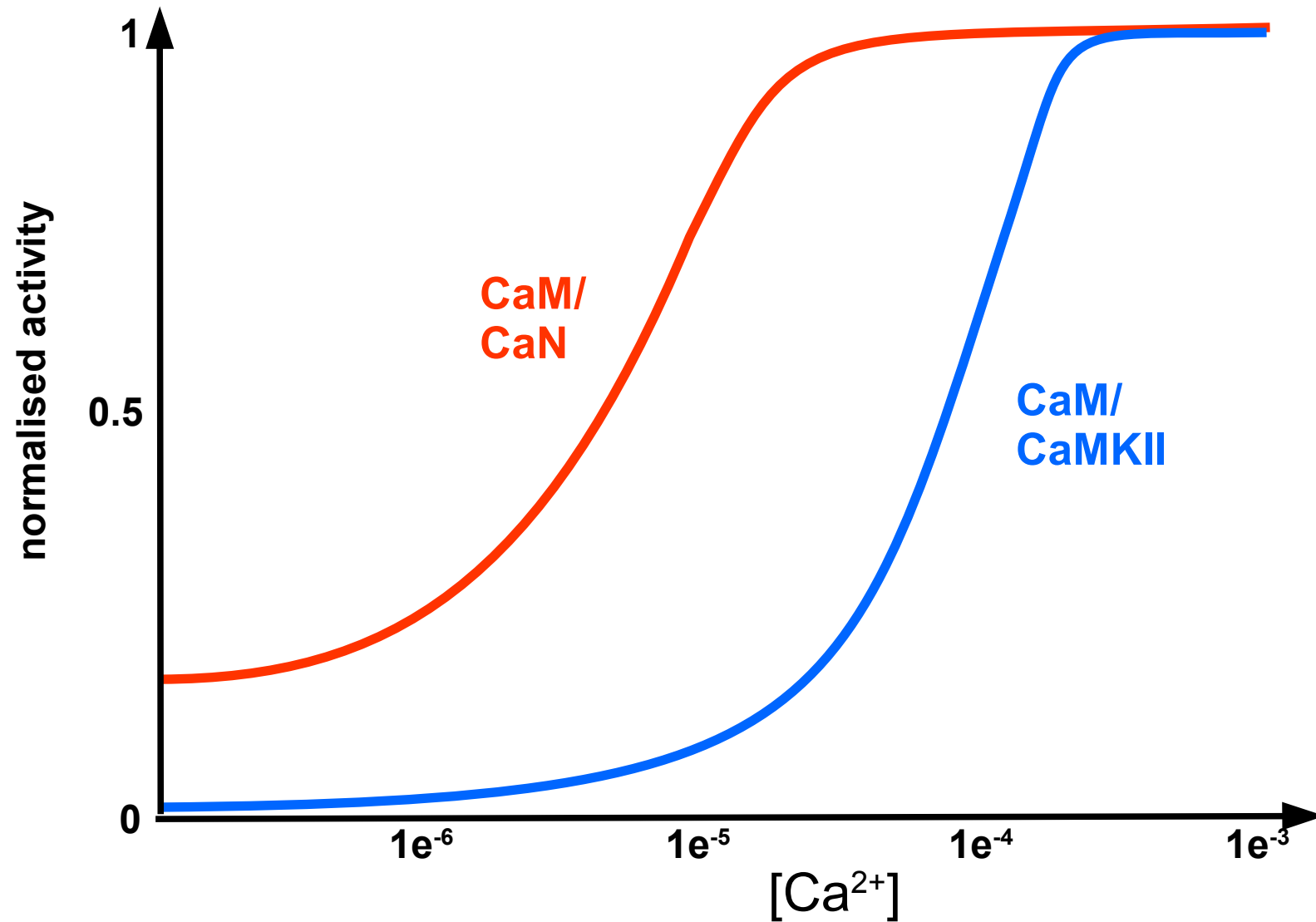
Stabilise R state of both lobes

Stabilise R state of C lobe

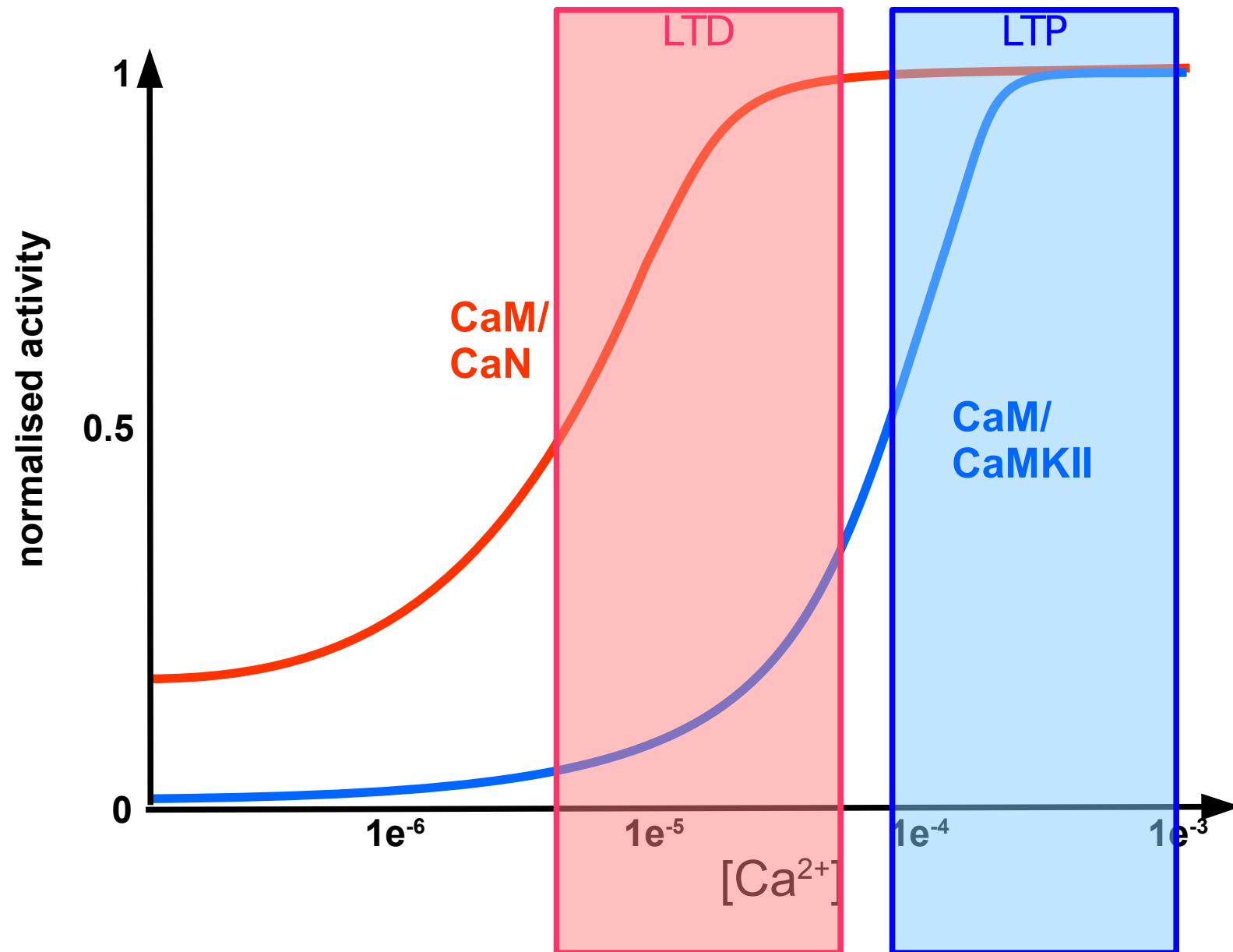




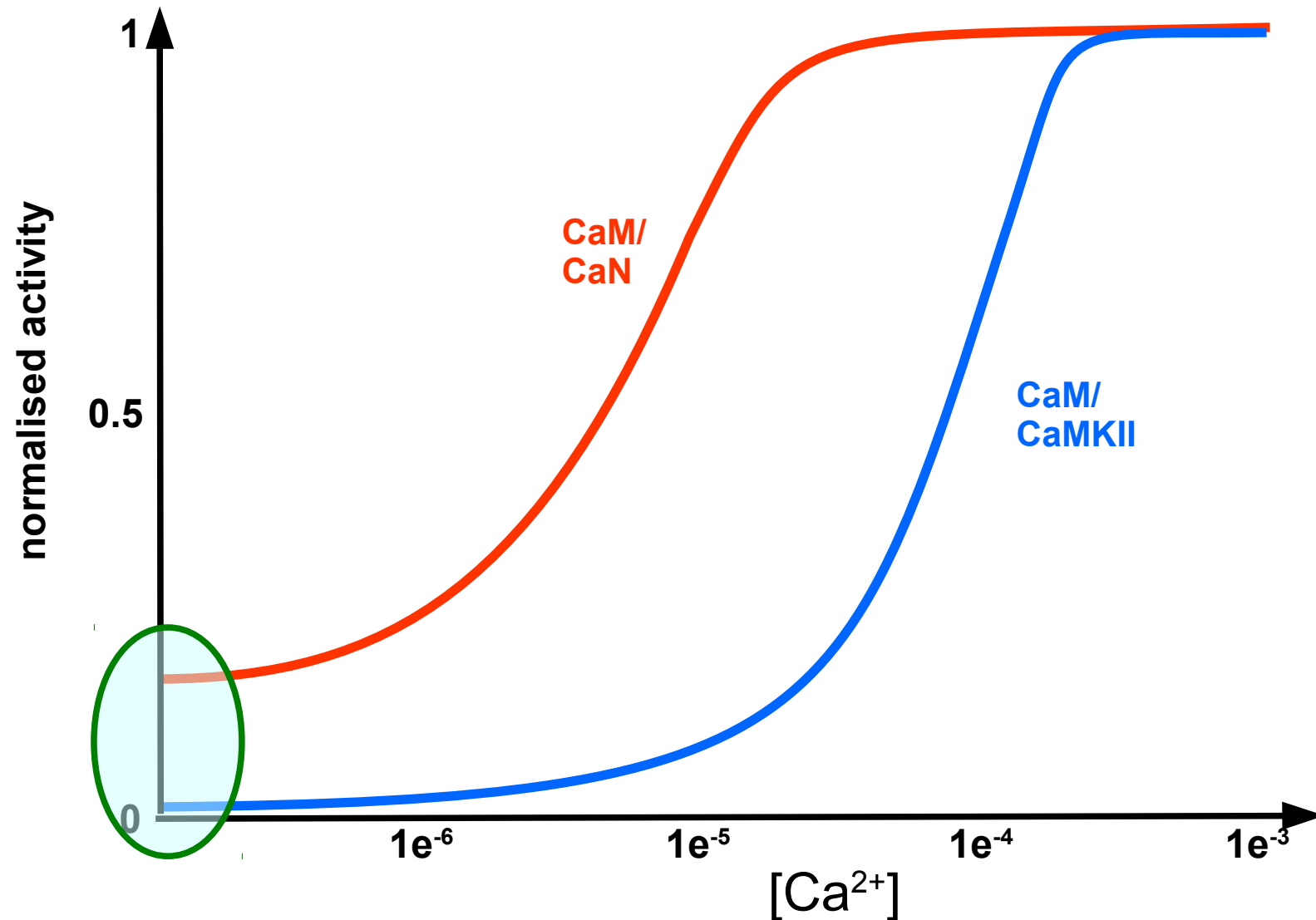
# Calmodulin, its ligand and its targets



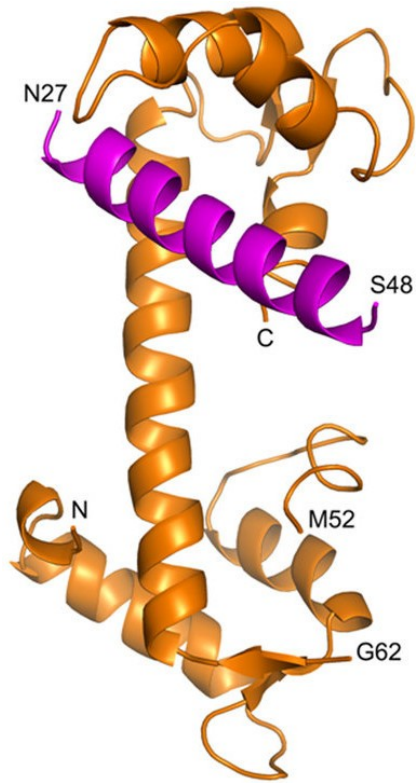
# Bidirectional synaptic plasticity



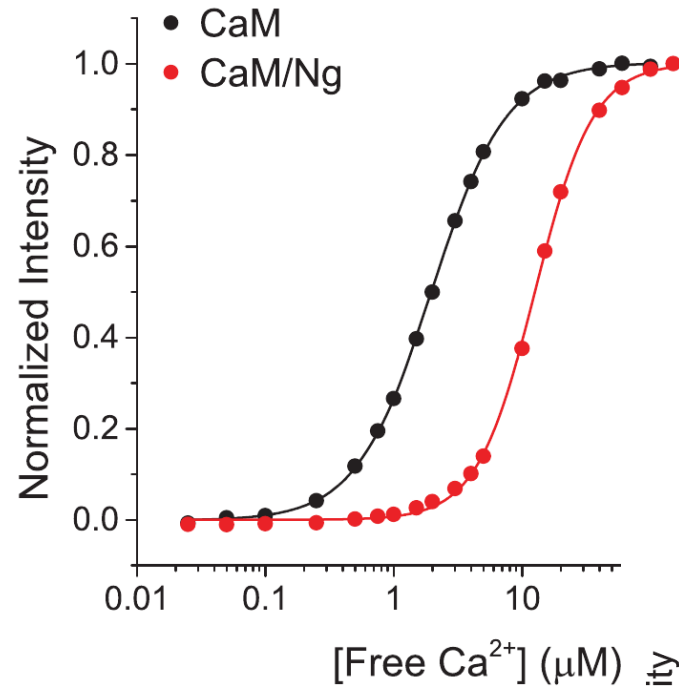
# Calcineurin stabilises CaM R → no deactivation



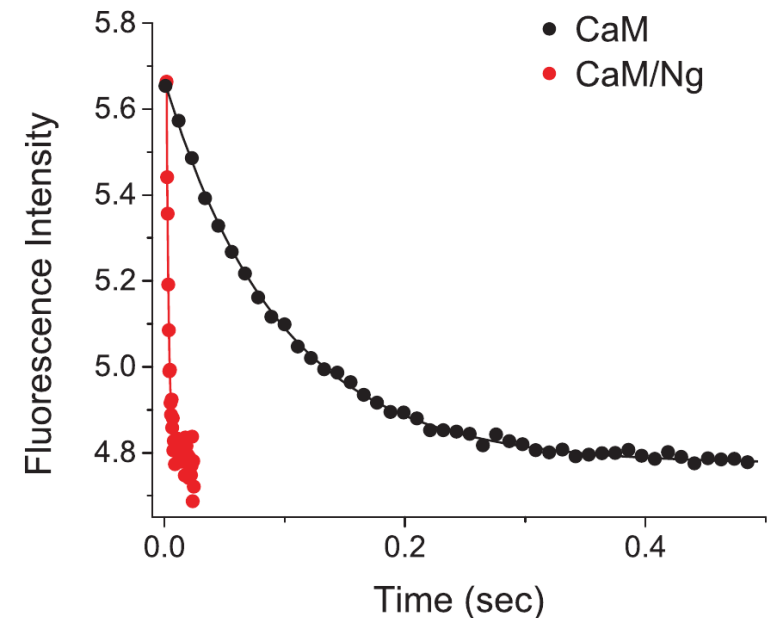
# Neurogranin binds to apo-CaM, decrease affinity for $[Ca^{2+}]$ and increase dissociation rate



Kumar *et al* (2013)



Hoffman *et al* (2014)



# Effect of R and T stabilising targets on CaM affinity

Bayley *et al* (1996)

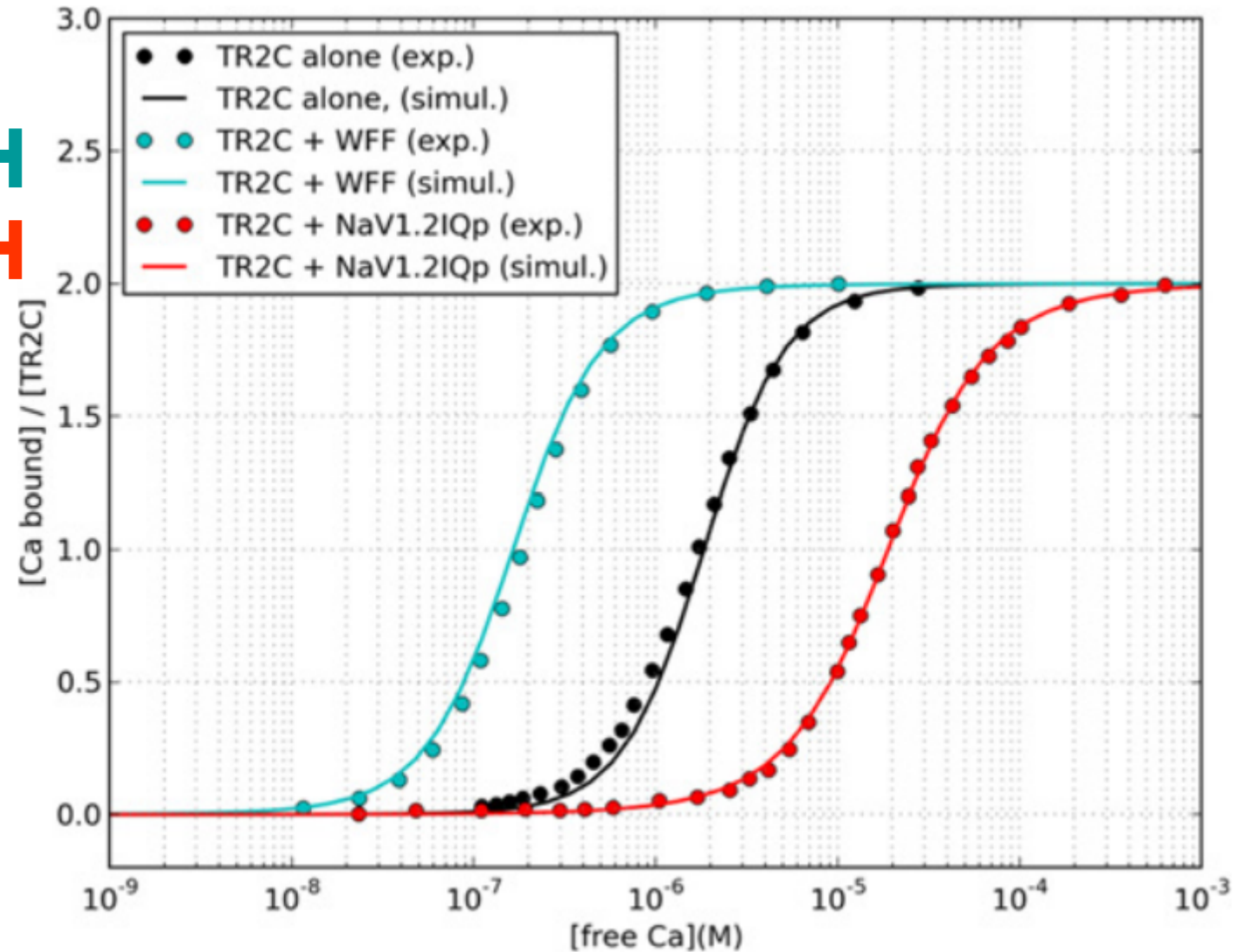
R stabilising  
peptide

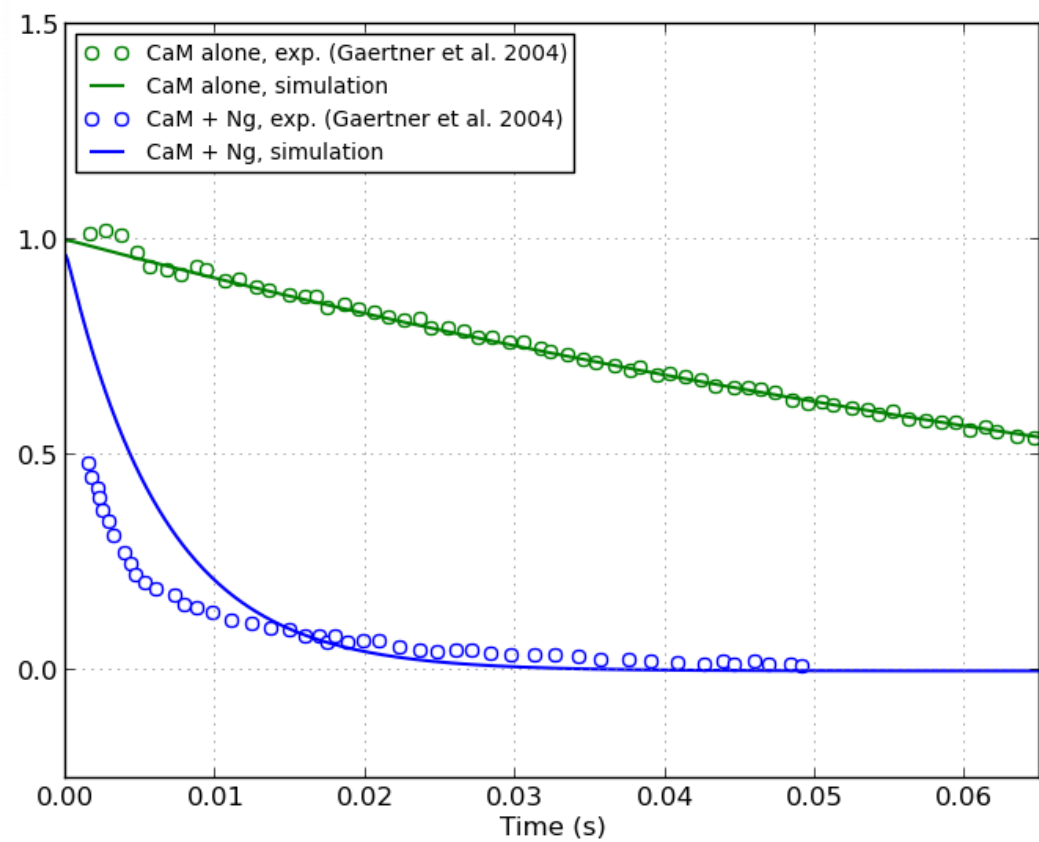
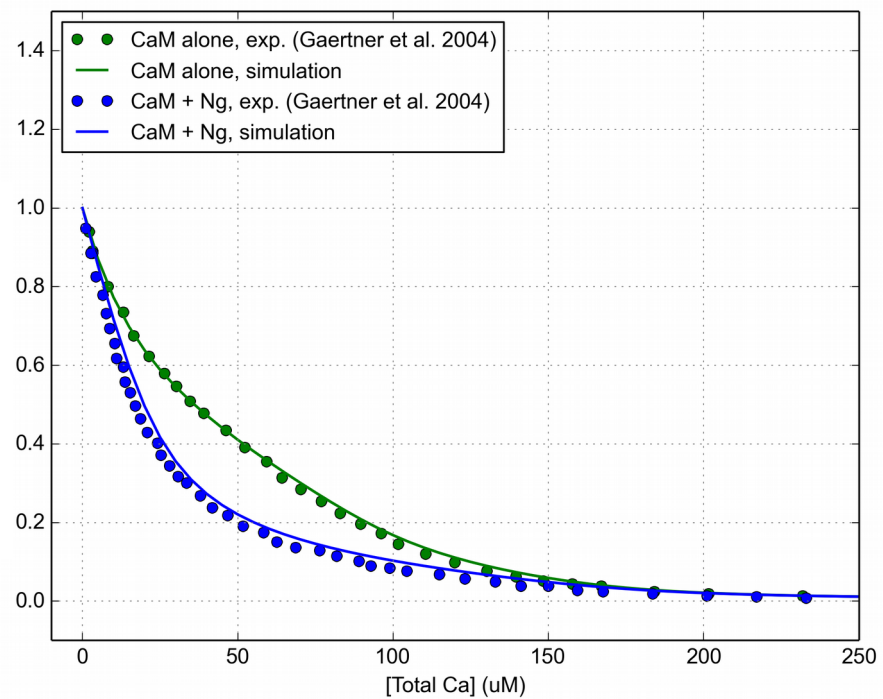


T stabilising  
Peptide (IQ domain)



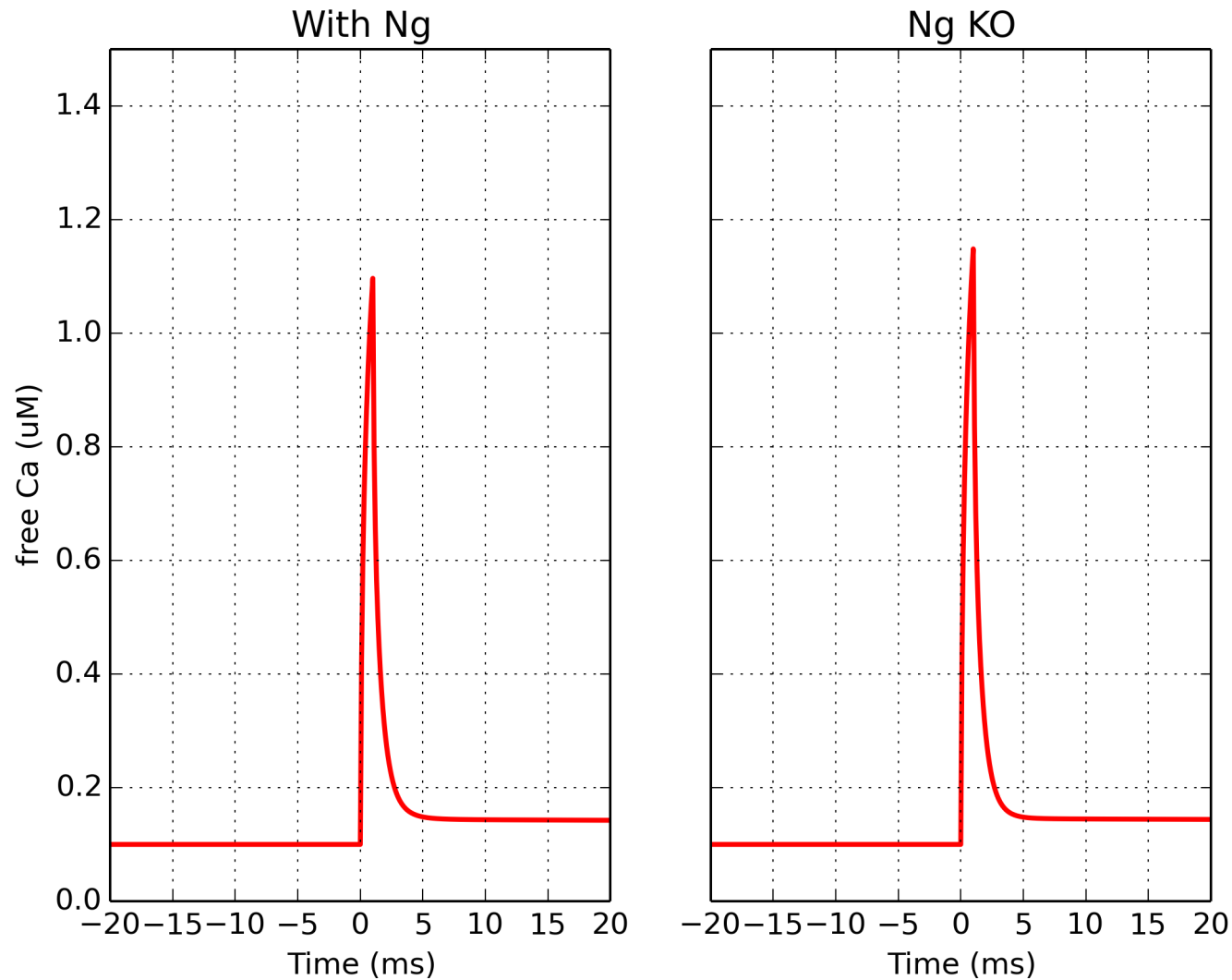
Theoharis *et al* (2008)



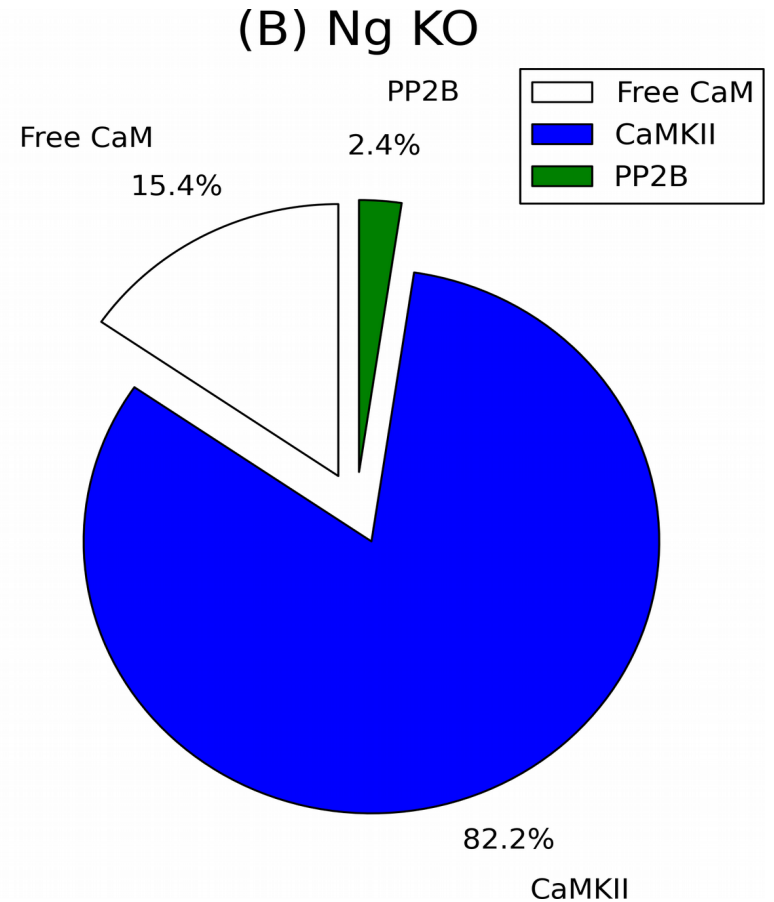
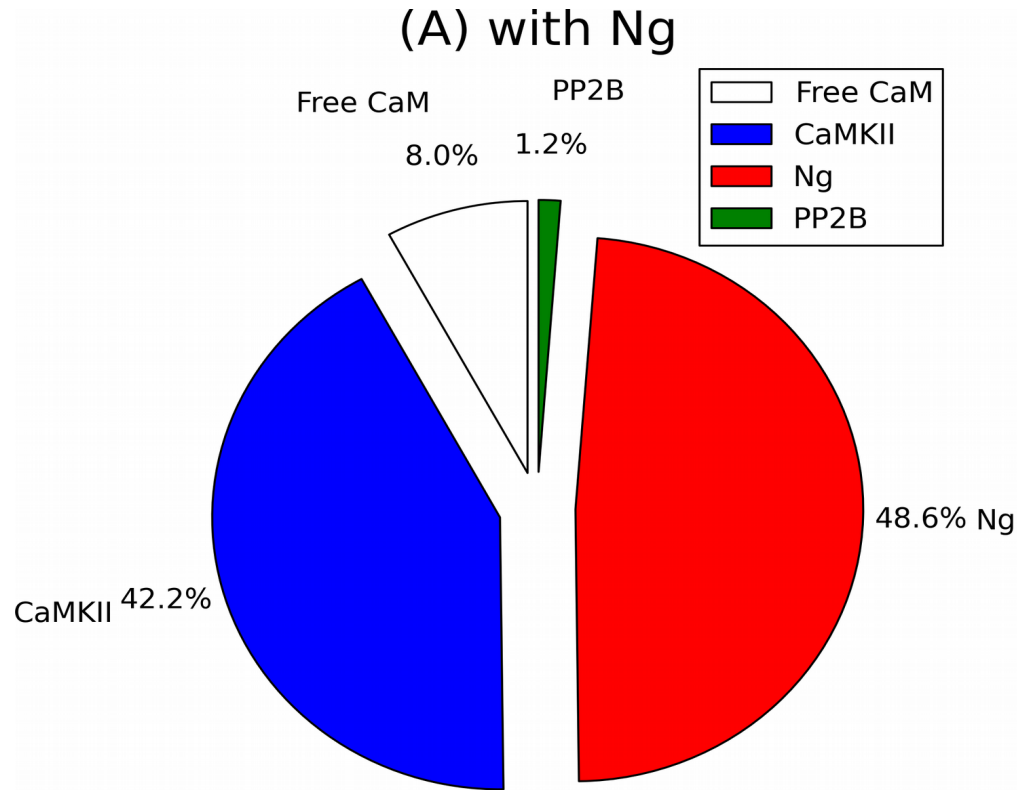


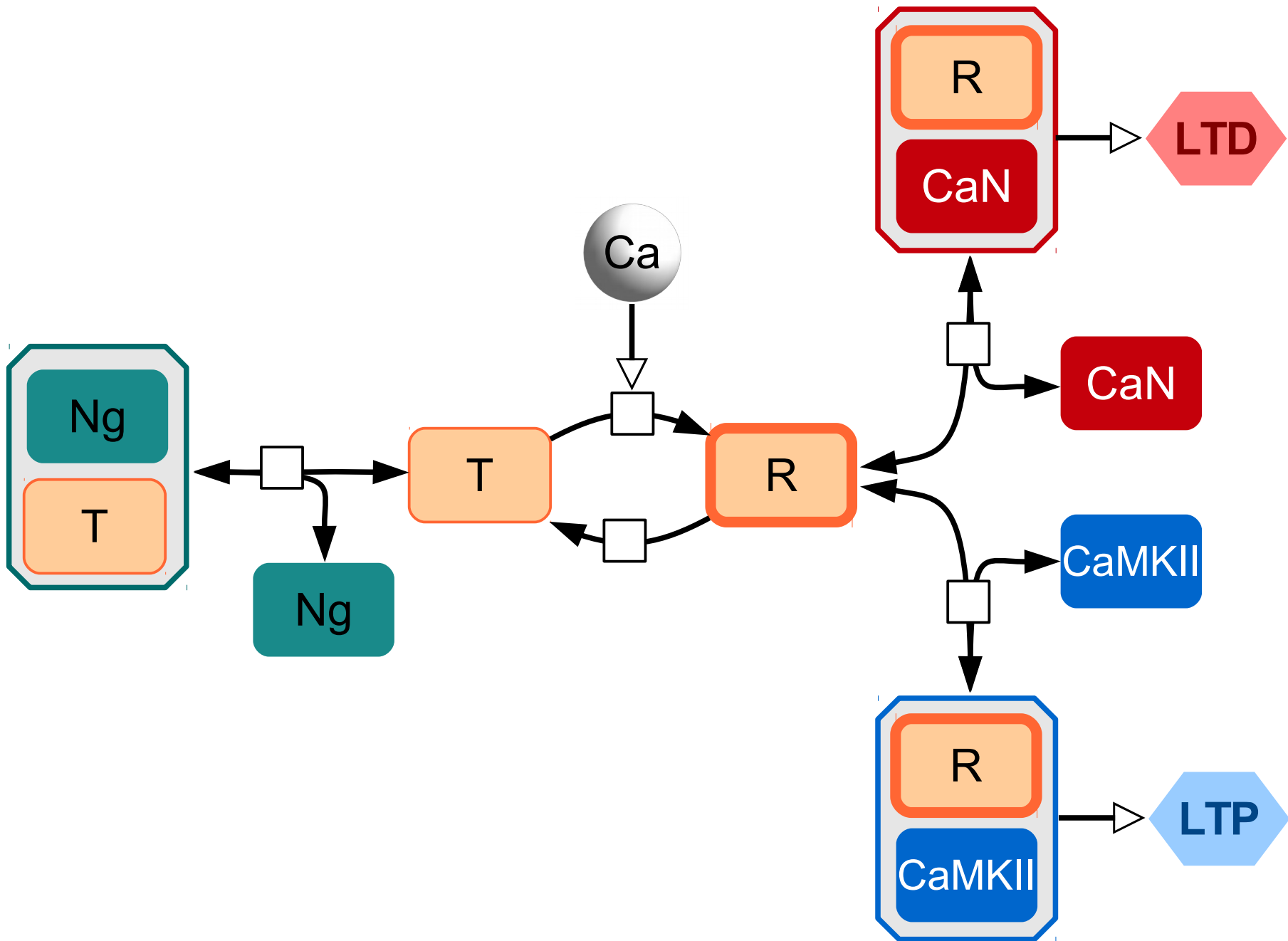


# No large effect of Ng on $[Ca^{2+}]_{free}$



# Ng affects the distribution of CaM





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

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

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

CaN can bind calmodulin at low  $\text{Ca}^{2+}$  concentration while both CaN and CaMKII bind CaM at high  $\text{Ca}^{2+}$  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!

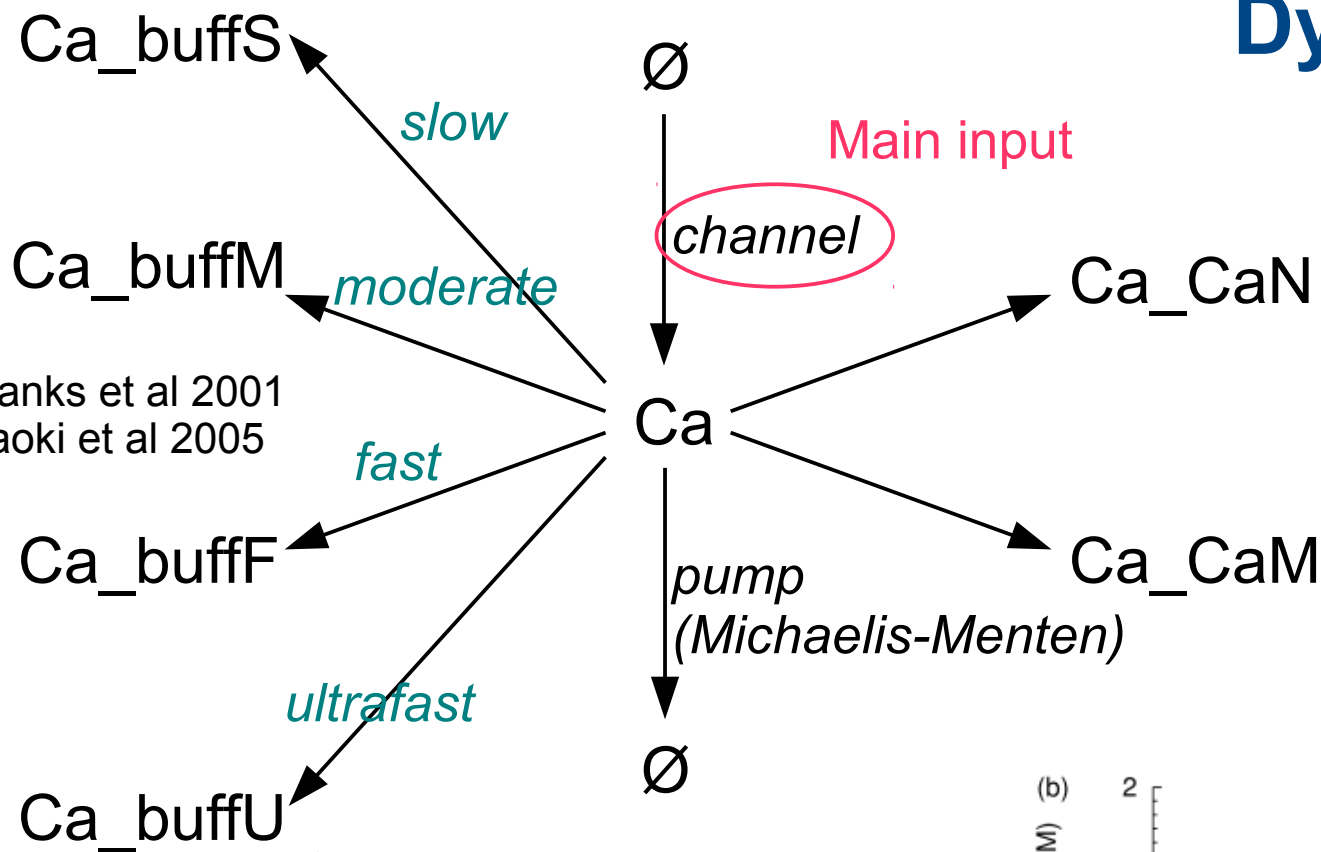
|                                                                |        |
|----------------------------------------------------------------|--------|
| AMPAR post-synaptic potential:                                 | 5 ms   |
| Calcium spike:                                                 | 50 ms  |
| 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 |



≠

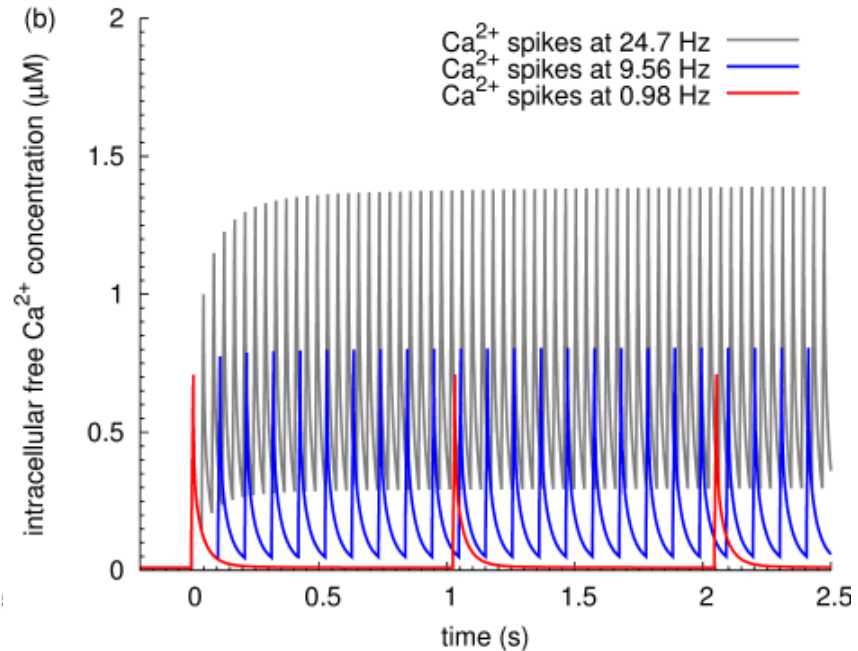
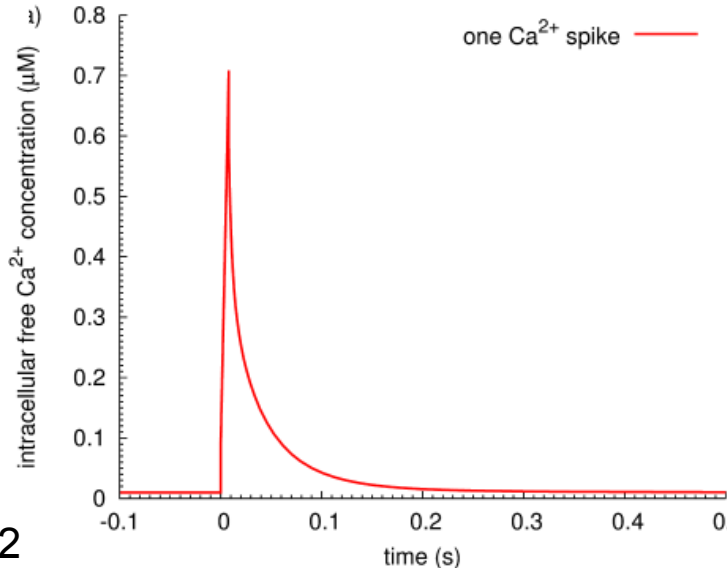


# Dynamic of calcium in the spine



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

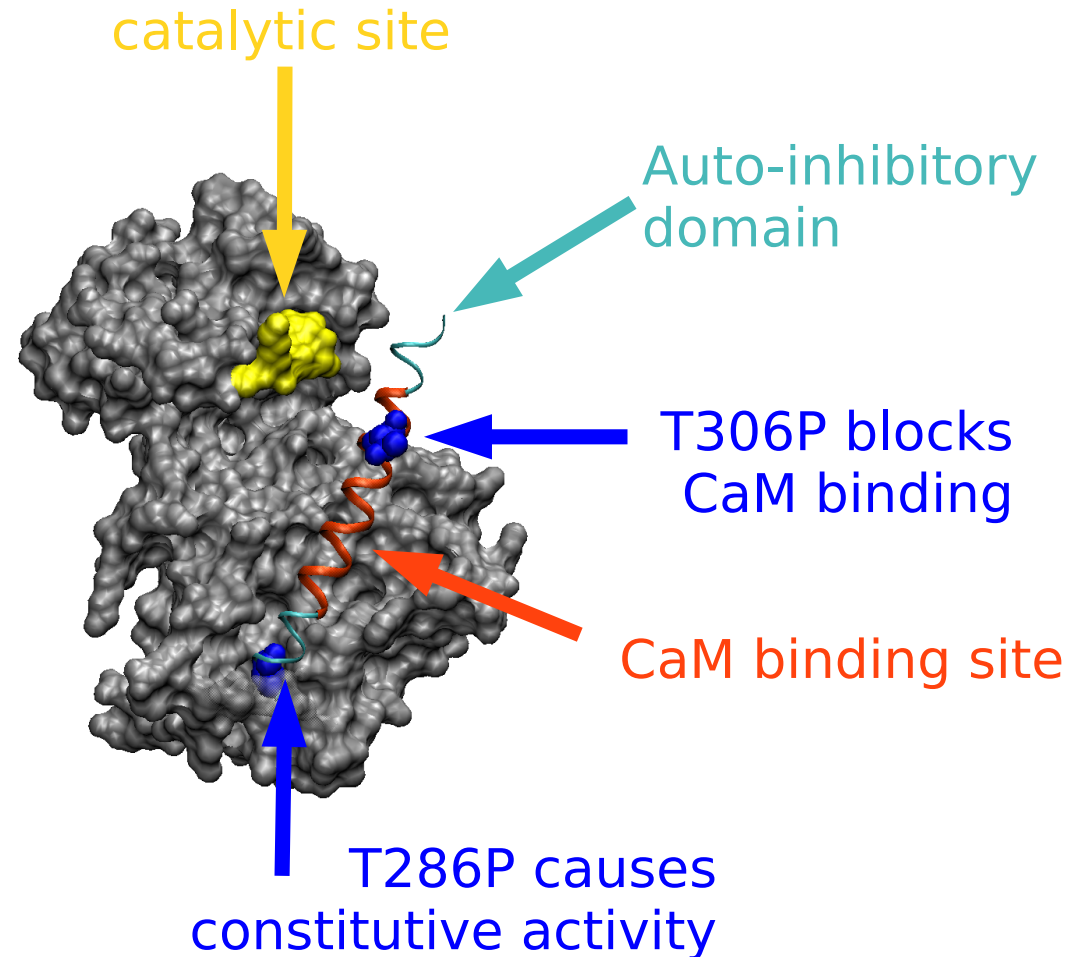
Ca\_buffU



Sabatini et al 2002



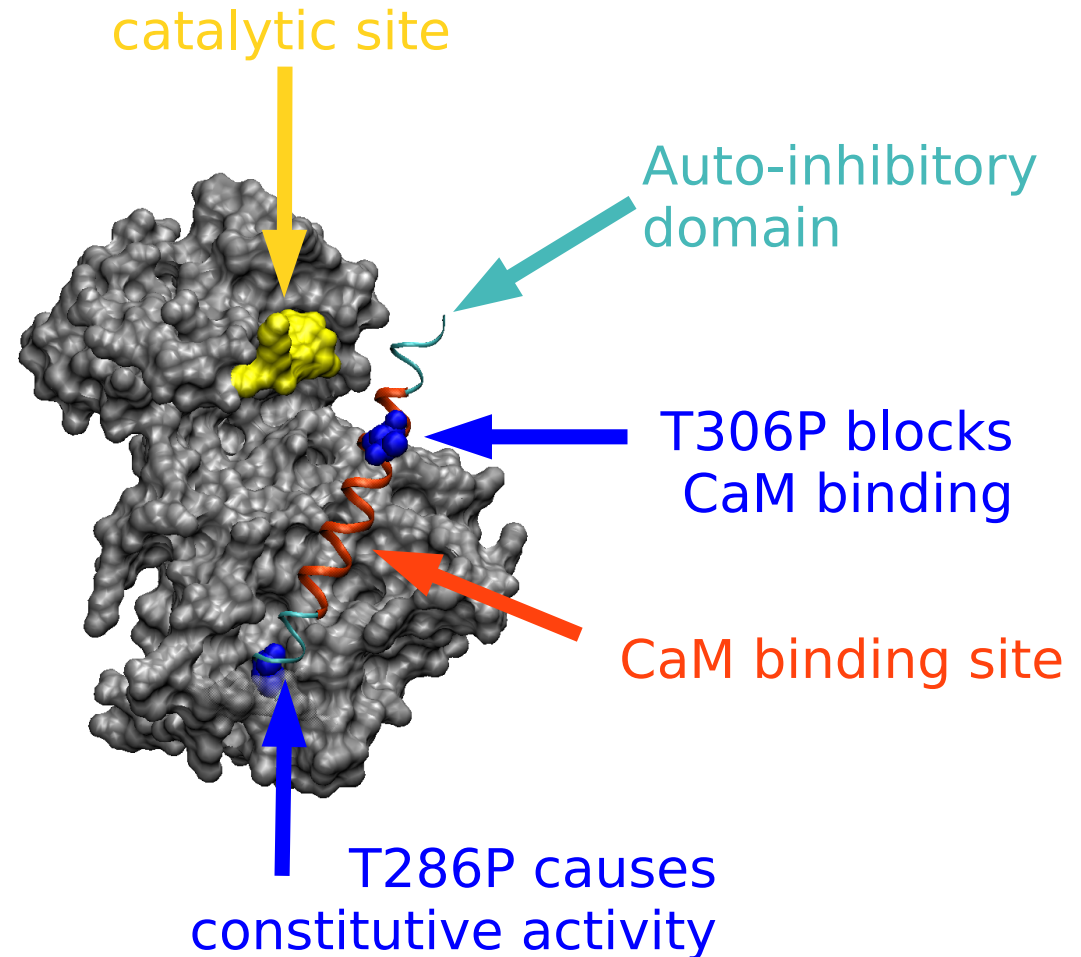
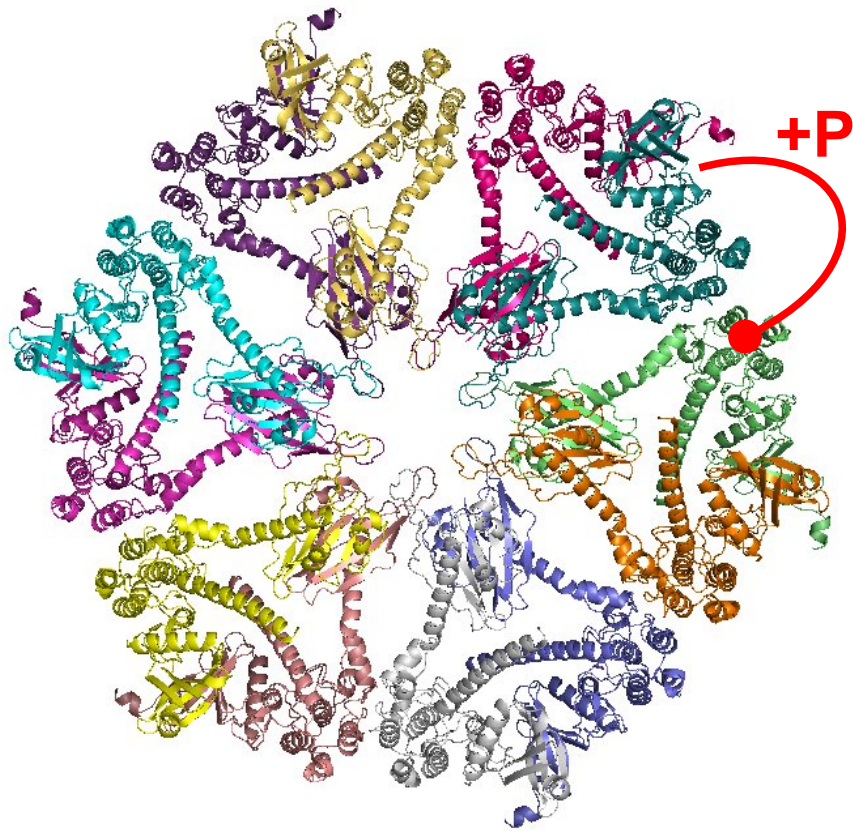
# Calcium/calmodulin kinase II



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

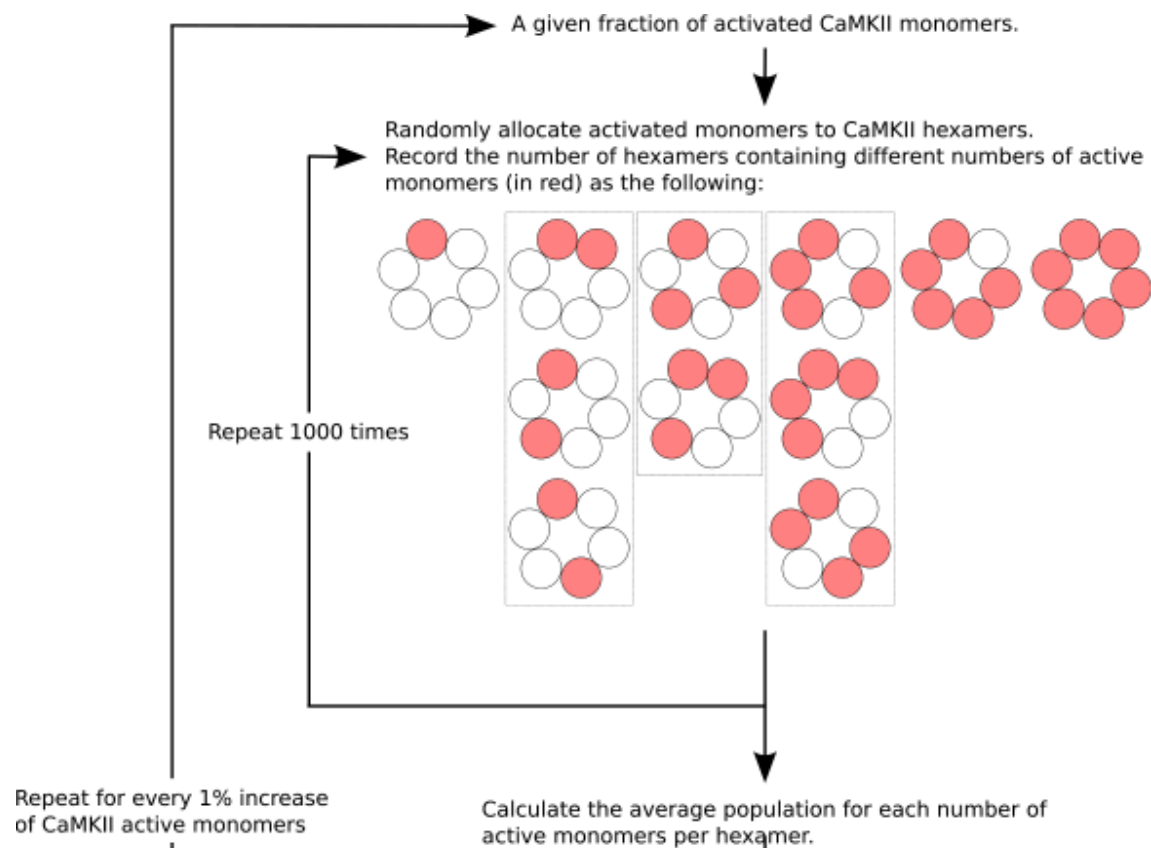


Dodecamer:

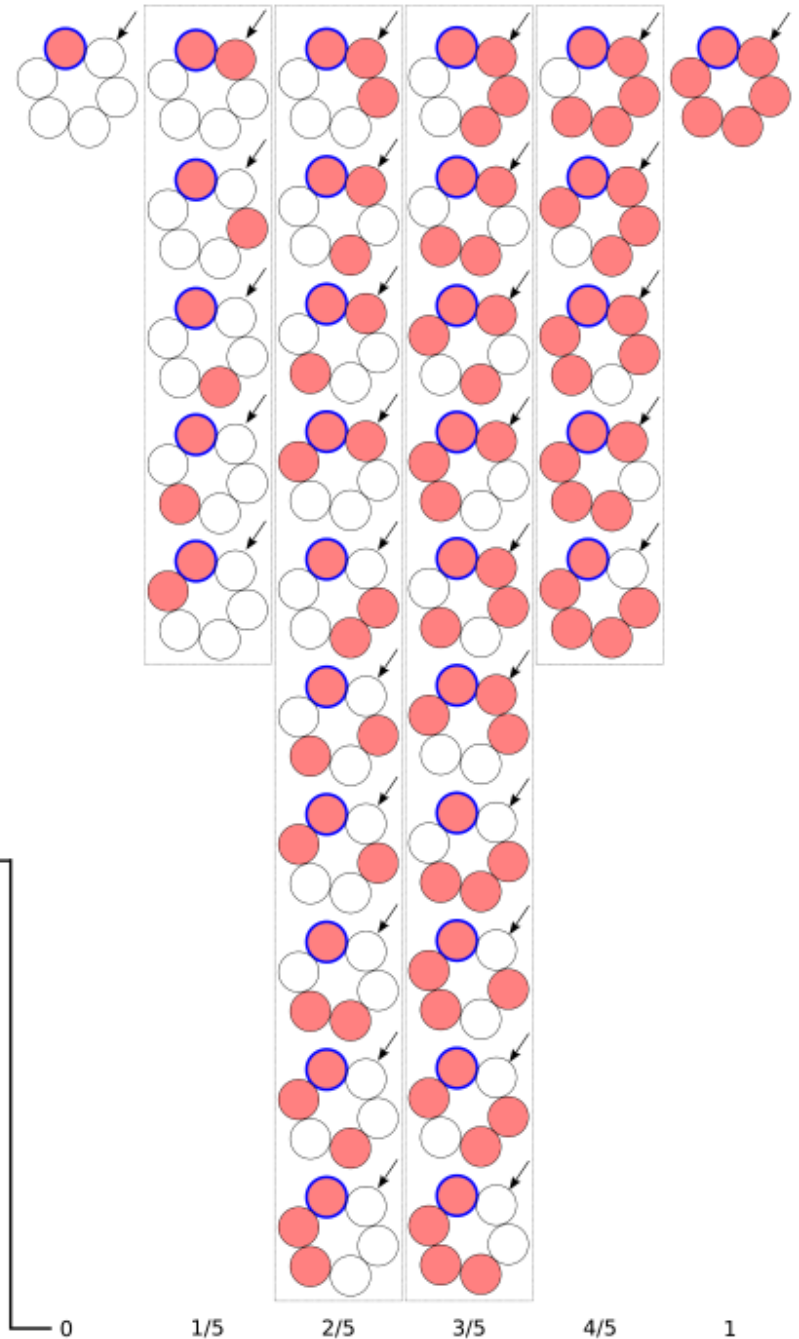
Trans-phosphorylation of T286  
by neighbouring subunits

Cis-phosphorylation of T306

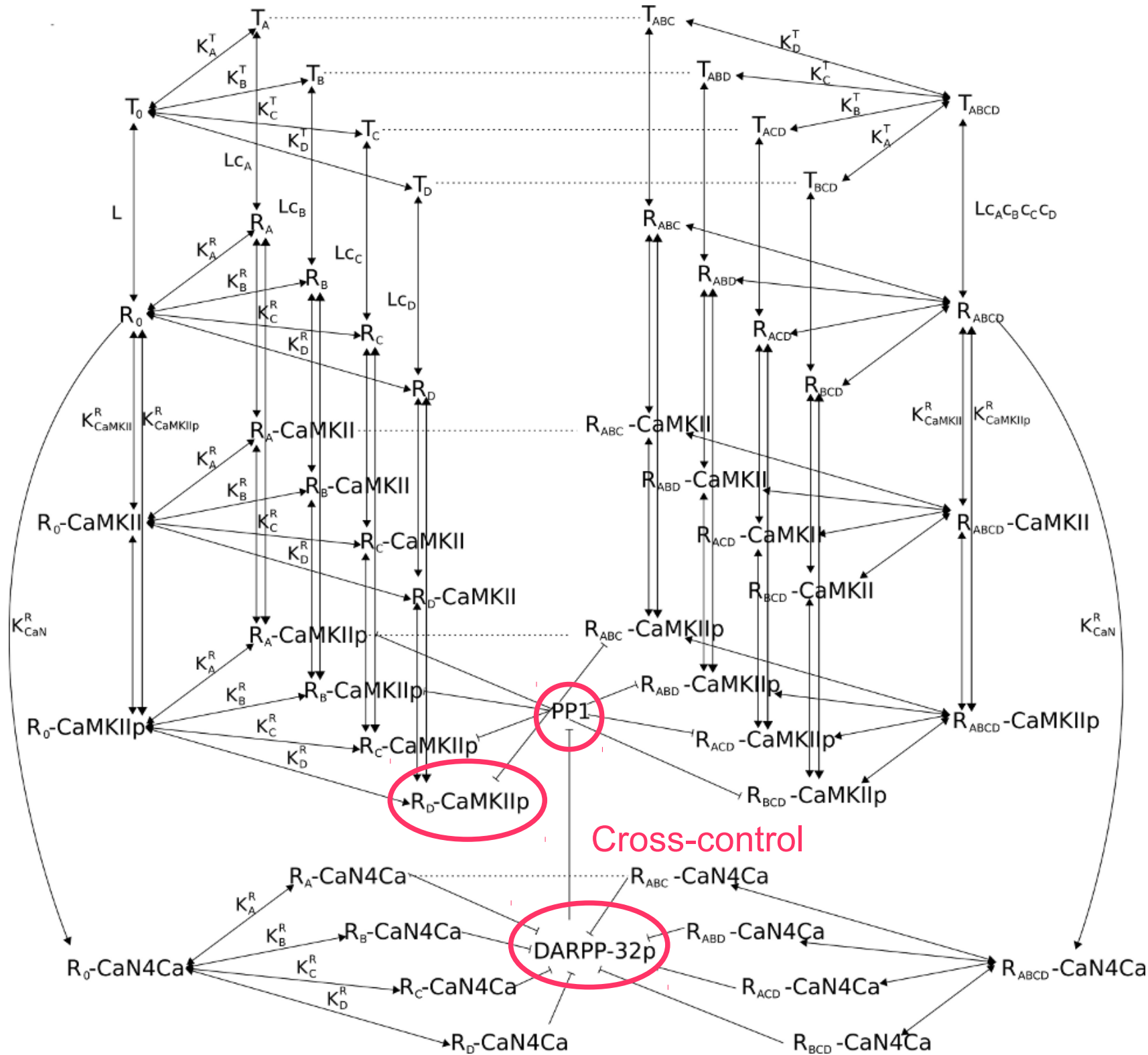
Most quantitative measurements made on monomers ...

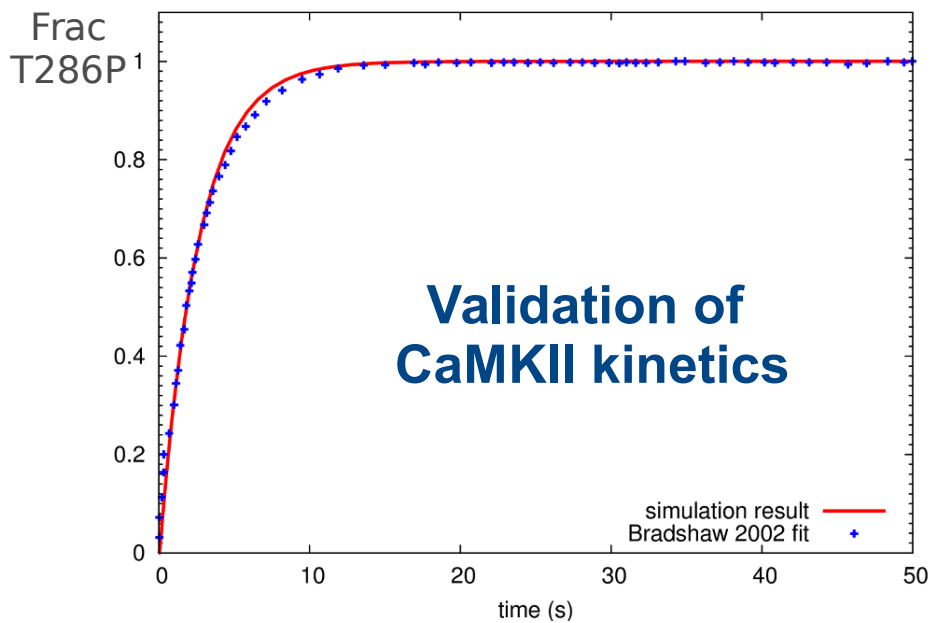


Calculate the probabilities of having an active neighbour on one specific side (indicated by the arrow) of the activated monomer of interest (blue outline) (since only the asymmetrical situation is considered). The possible positions of activated monomers are listed as the following, with corresponding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

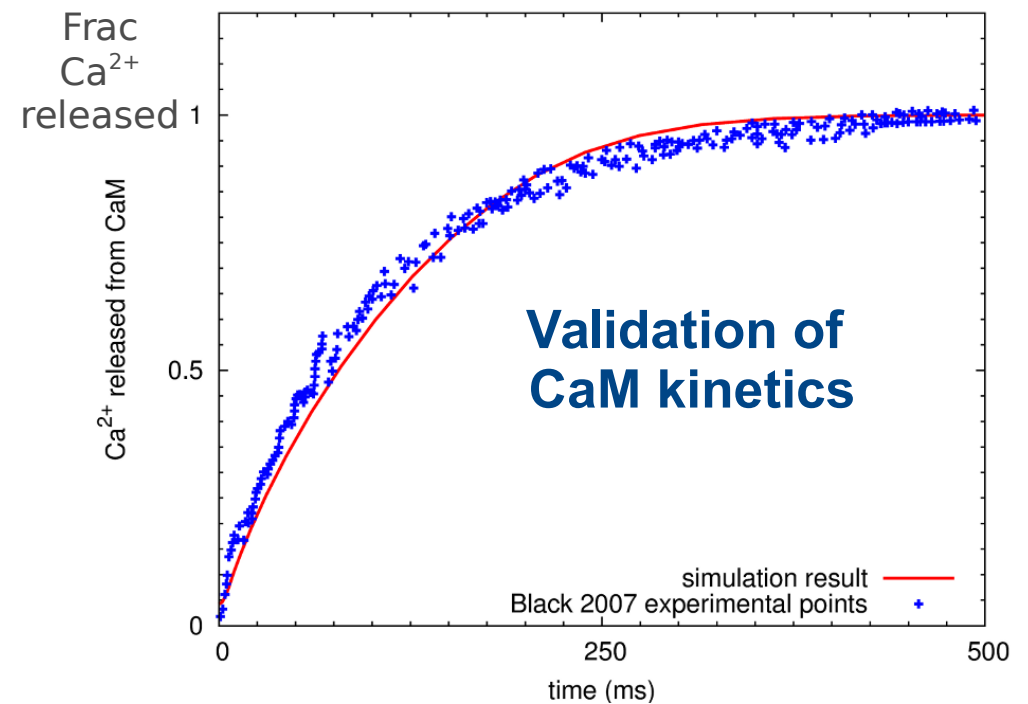


Fit these 100 coefficients into a polynomial function of activated CaMKII monomers, and embed this function in the model.

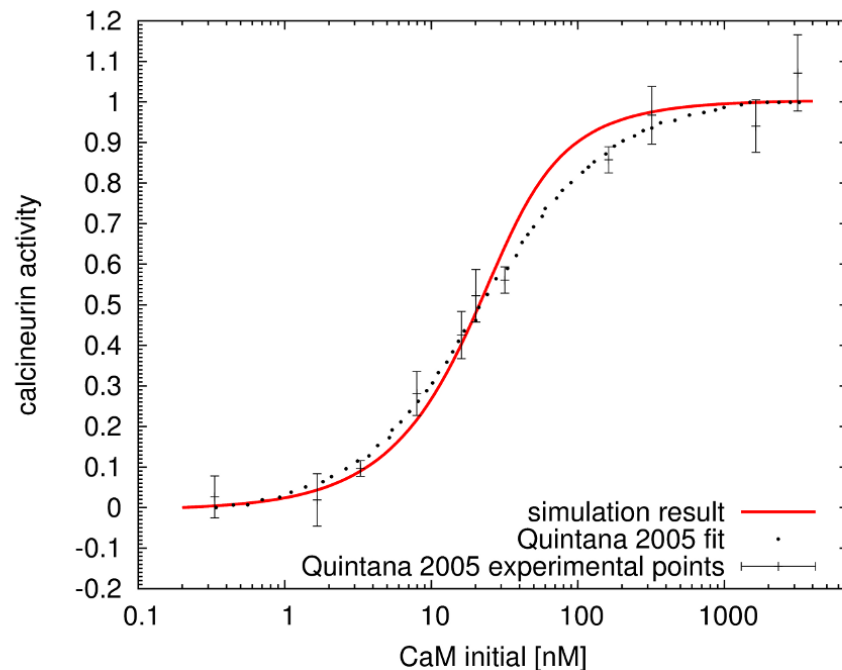




Bradshaw JM, Kubota Y, Meyer T, Schulman H (2003). *PNAS* 100: 10512–10517.



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

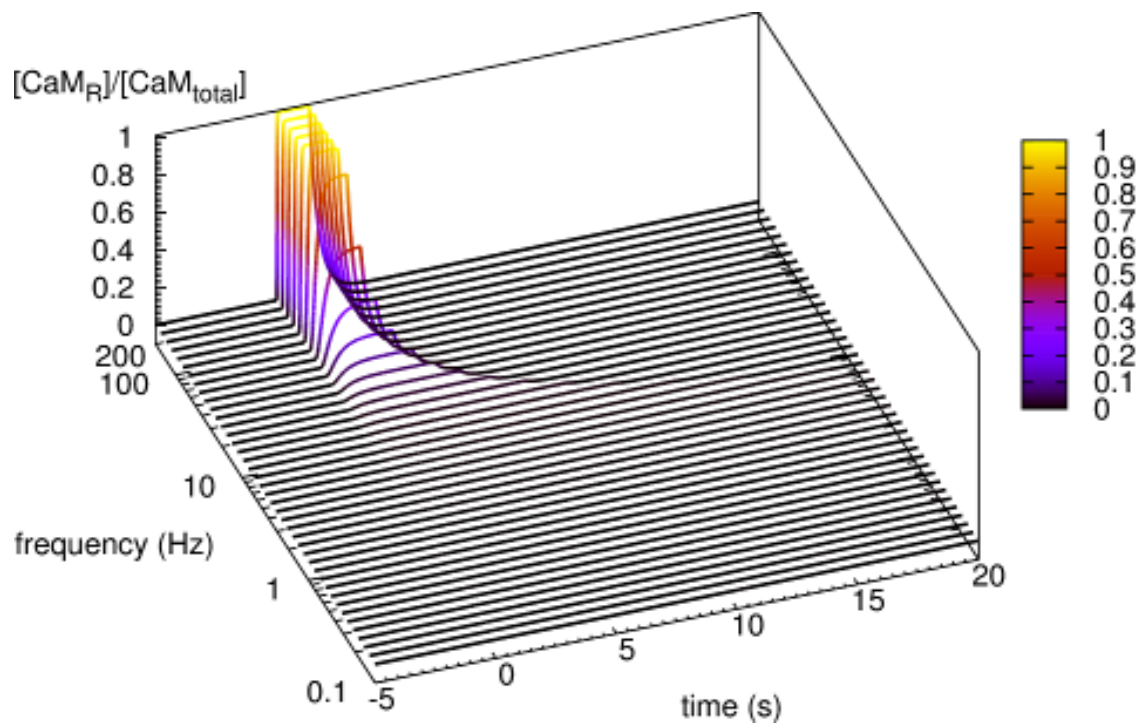


## Validation of calcium-activation of CaN

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

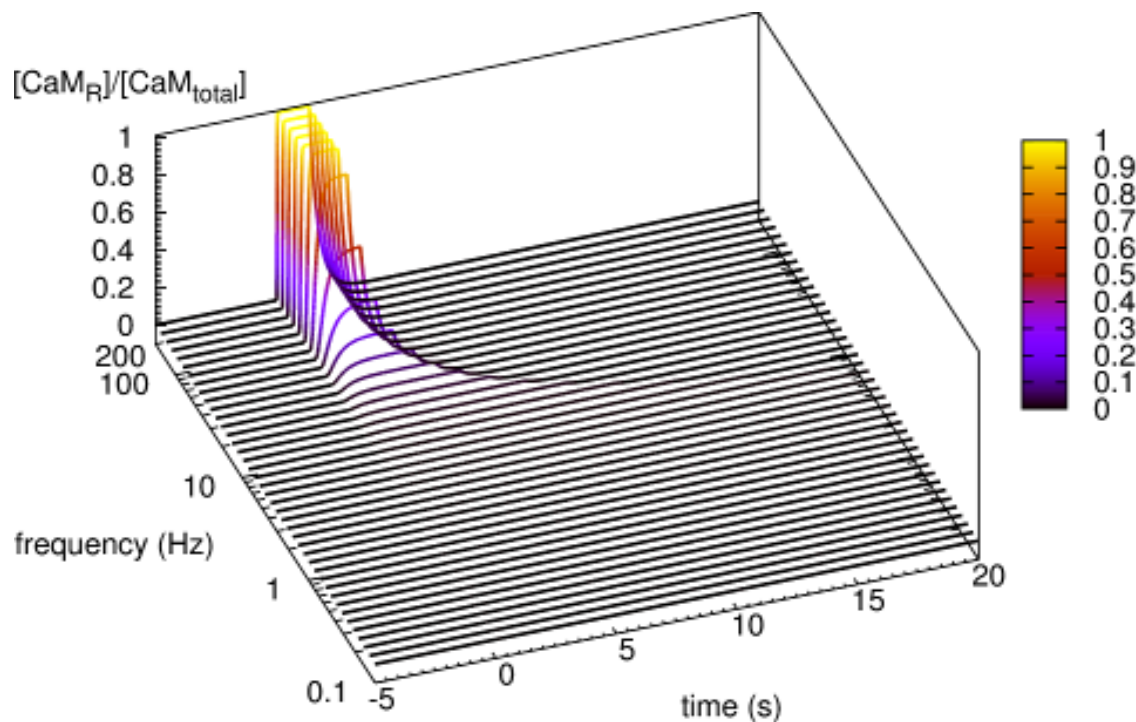


# Calmodulin activation



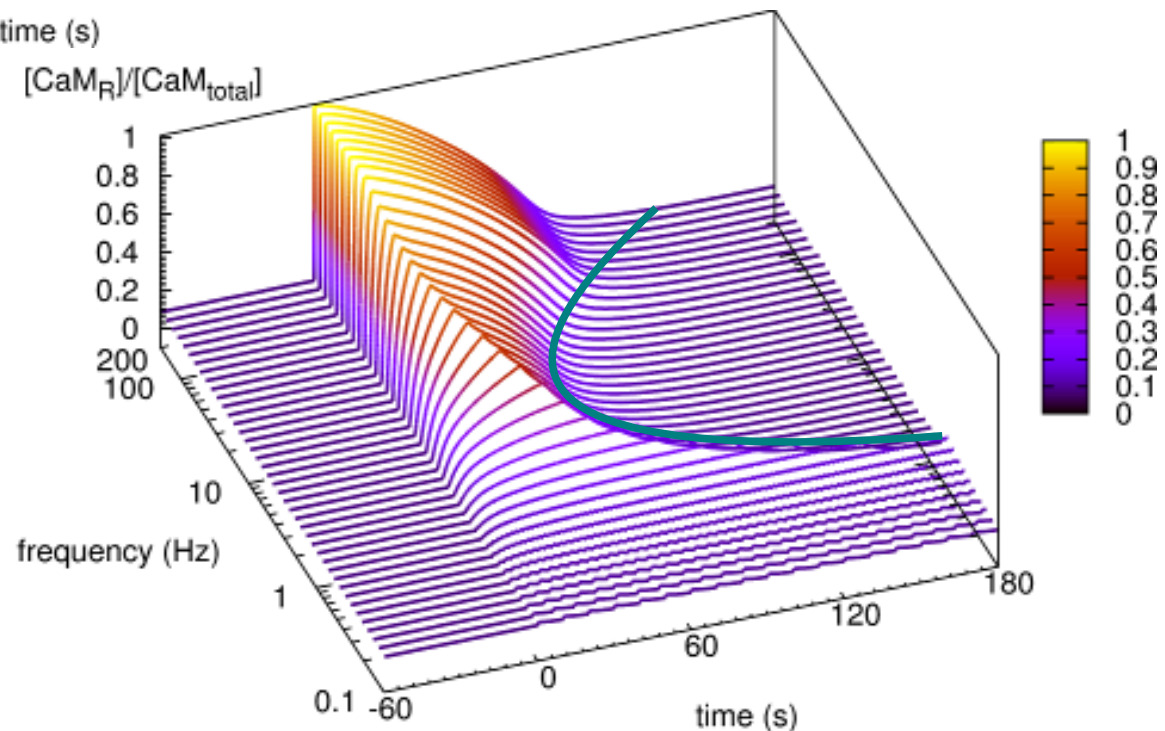
**CaM without targets.** Low frequencies do not activate calmodulin (binding events without conformational changes)

# Calmodulin activation



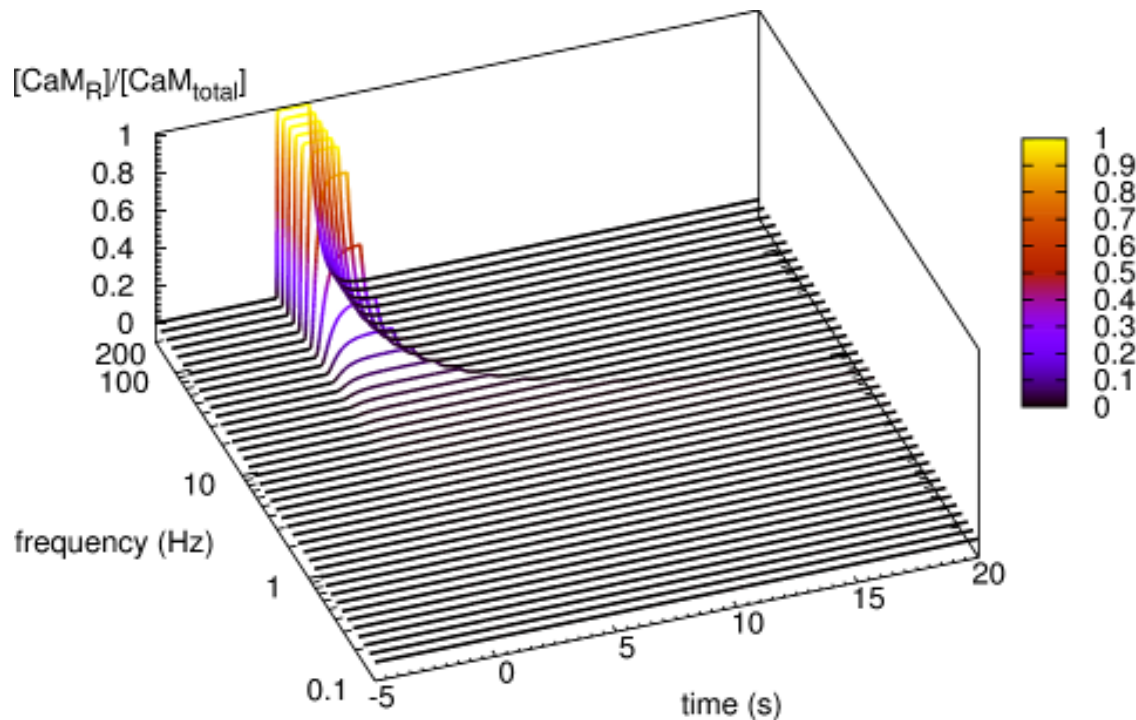
**CaM without targets.** Low frequencies do not activate calmodulin (binding events without conformational changes)

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





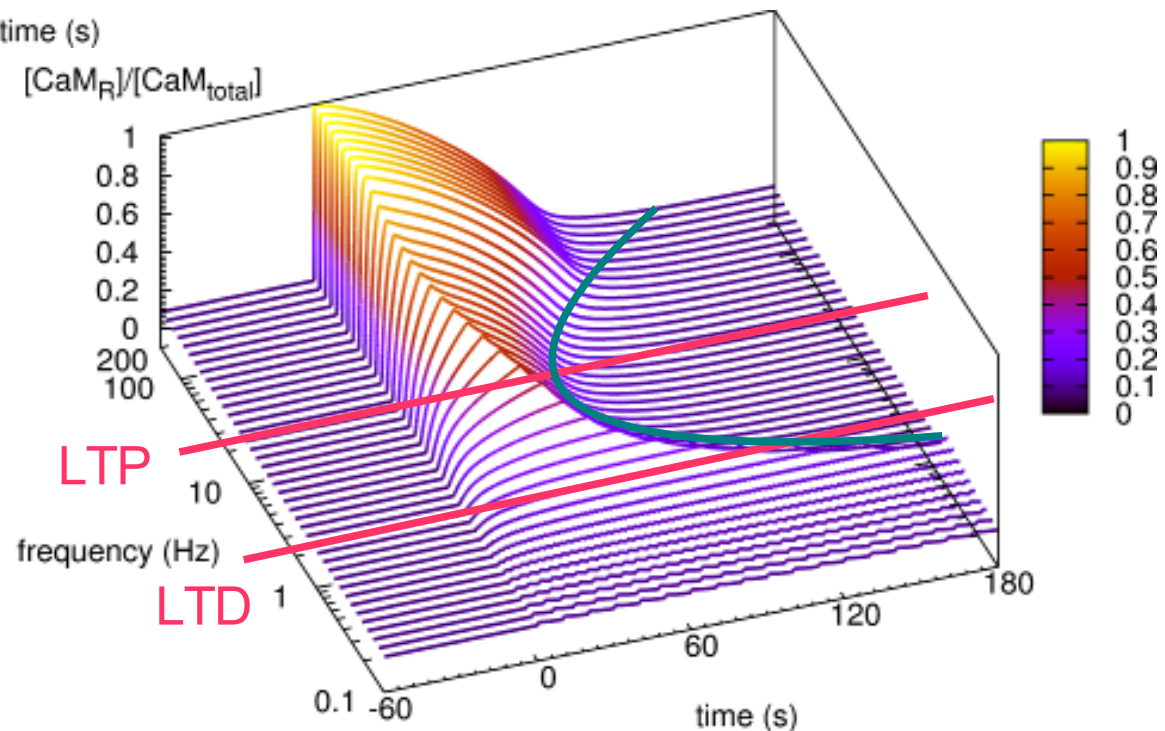
# Calmodulin activation



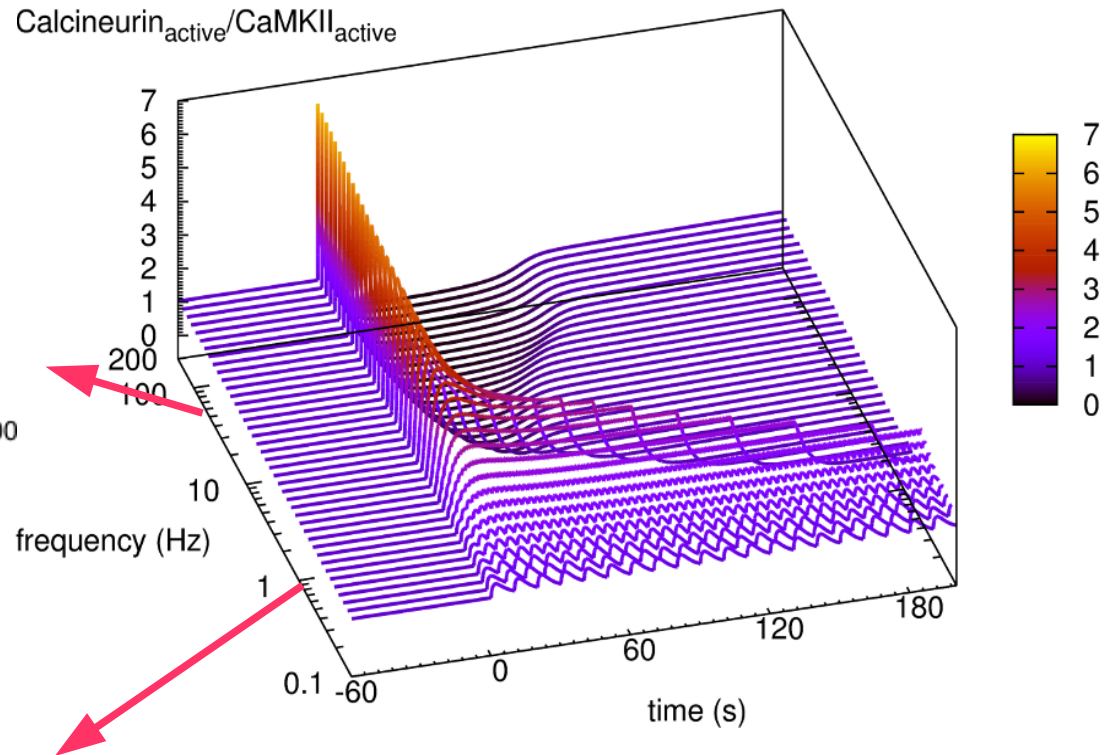
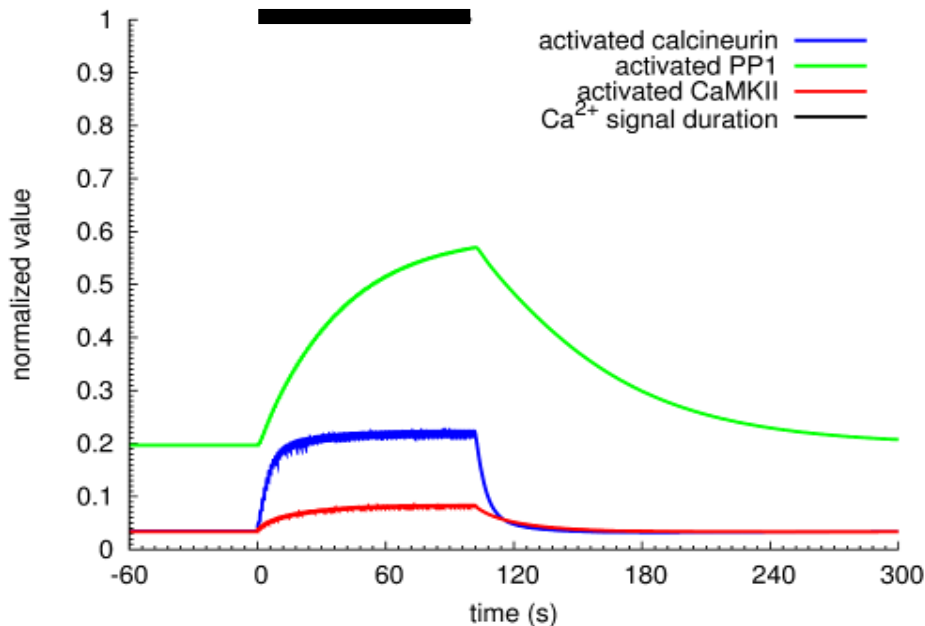
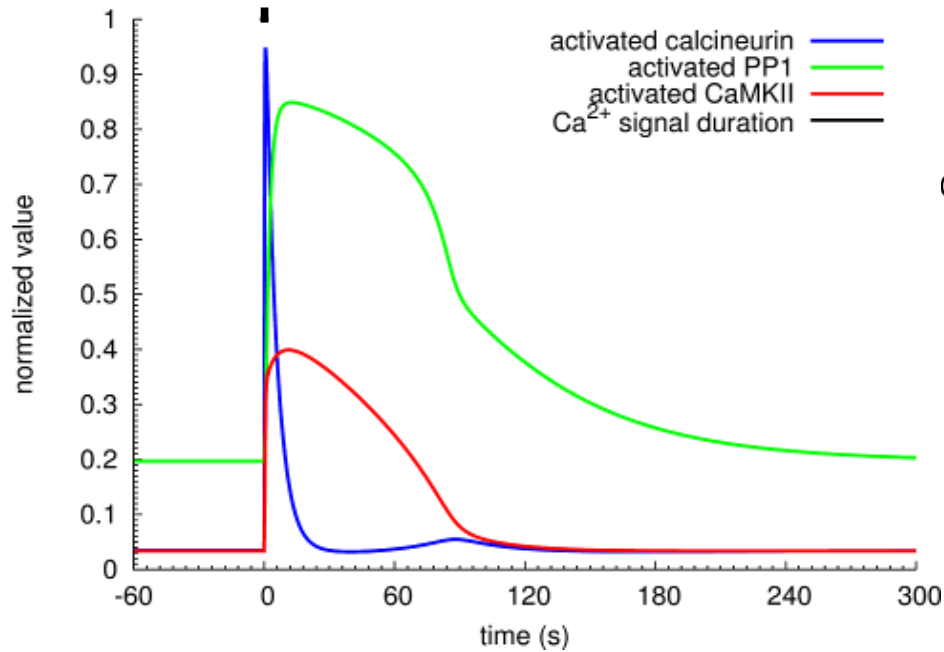
**CaM without targets.** Low frequencies do not activate calmodulin (binding events without conformational changes)

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



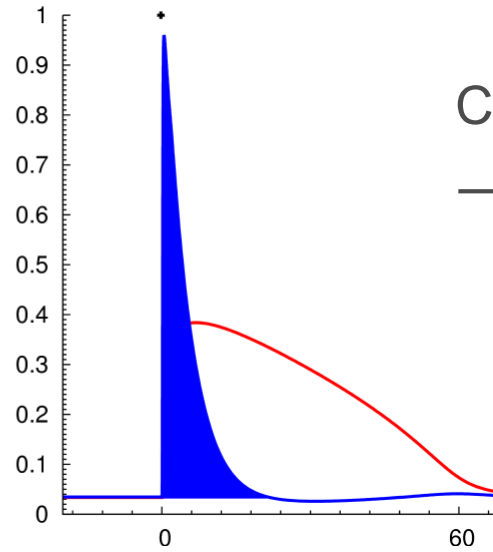
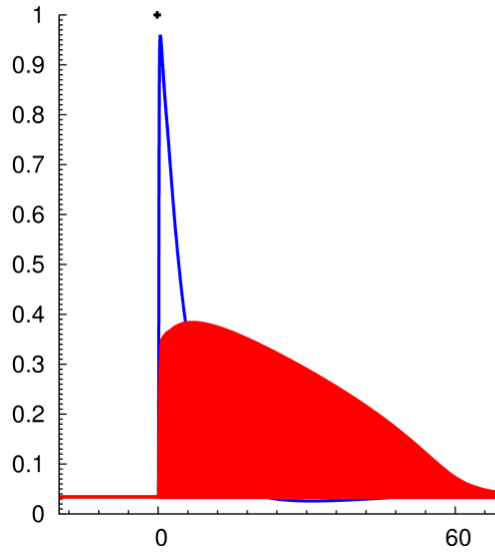
# Temporal activation of CaMKII and CaN



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

# Bidirectional plasticity

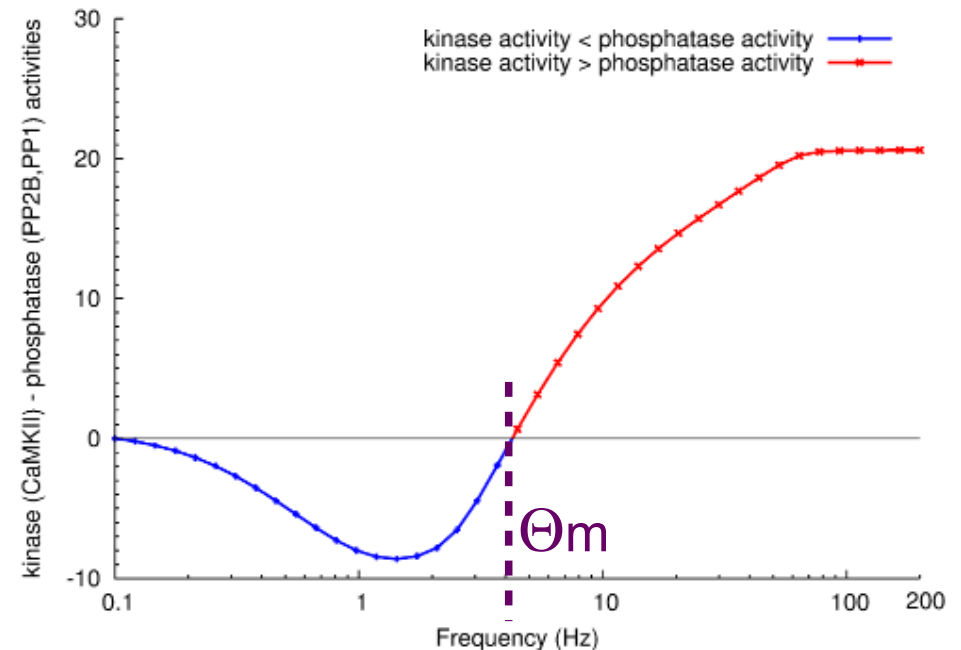
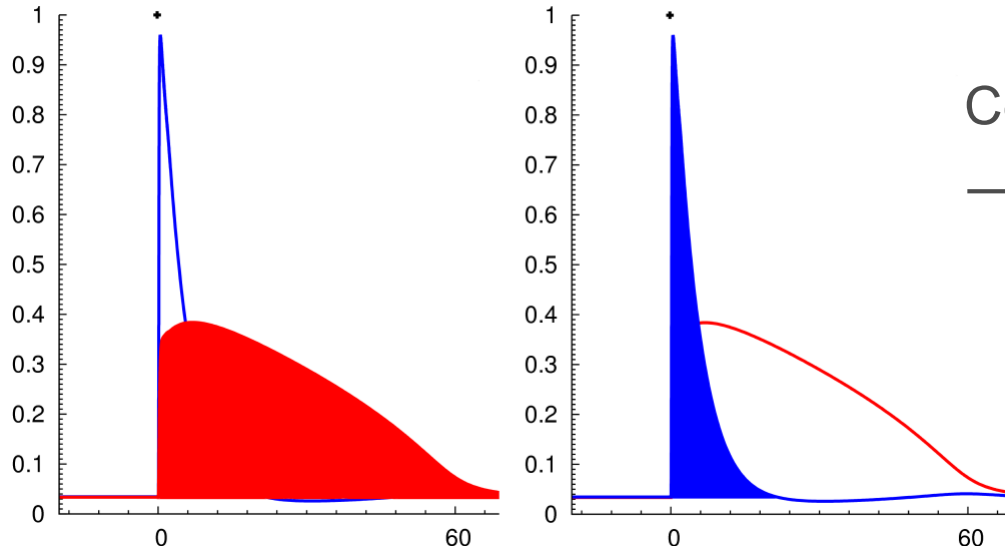
Constant catalytic rates of active enzyme  
→ quantity of catalysed reaction events  
prop to integral of the activation curve



# Bidirectional plasticity

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

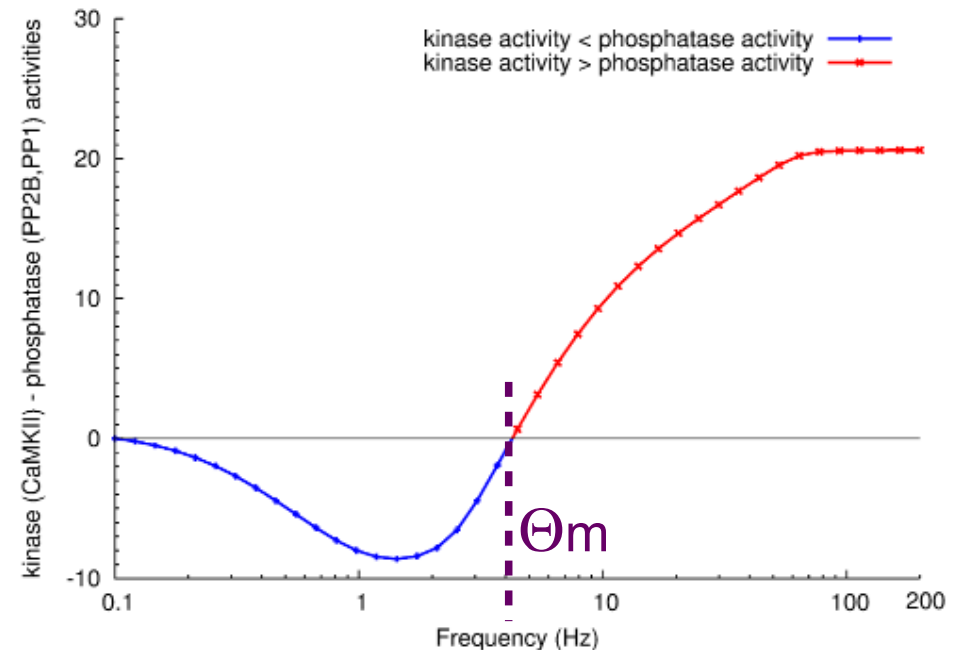
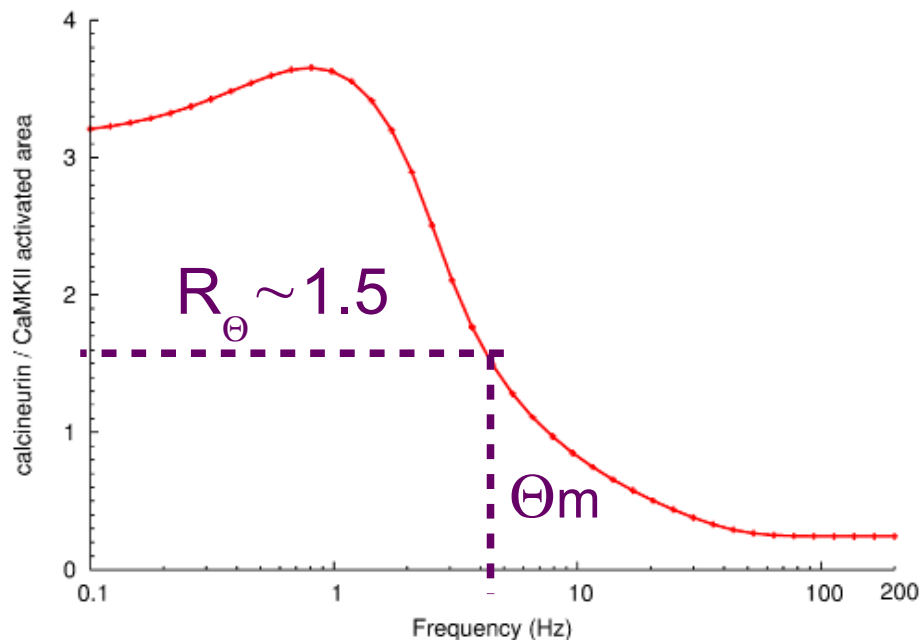
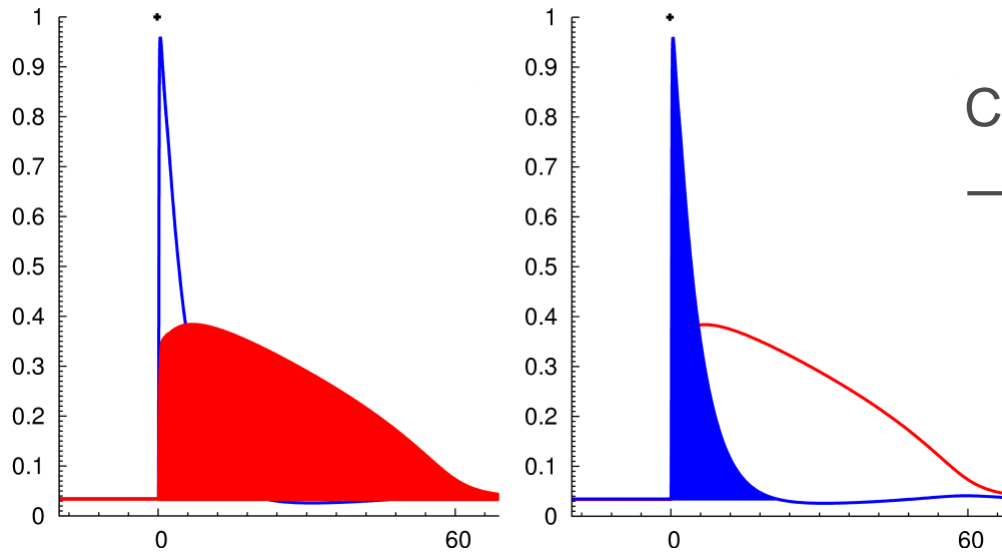
Bienestock-Cooper-Munro  
(BCM) curve: difference of  
active areas\* catalytic activities



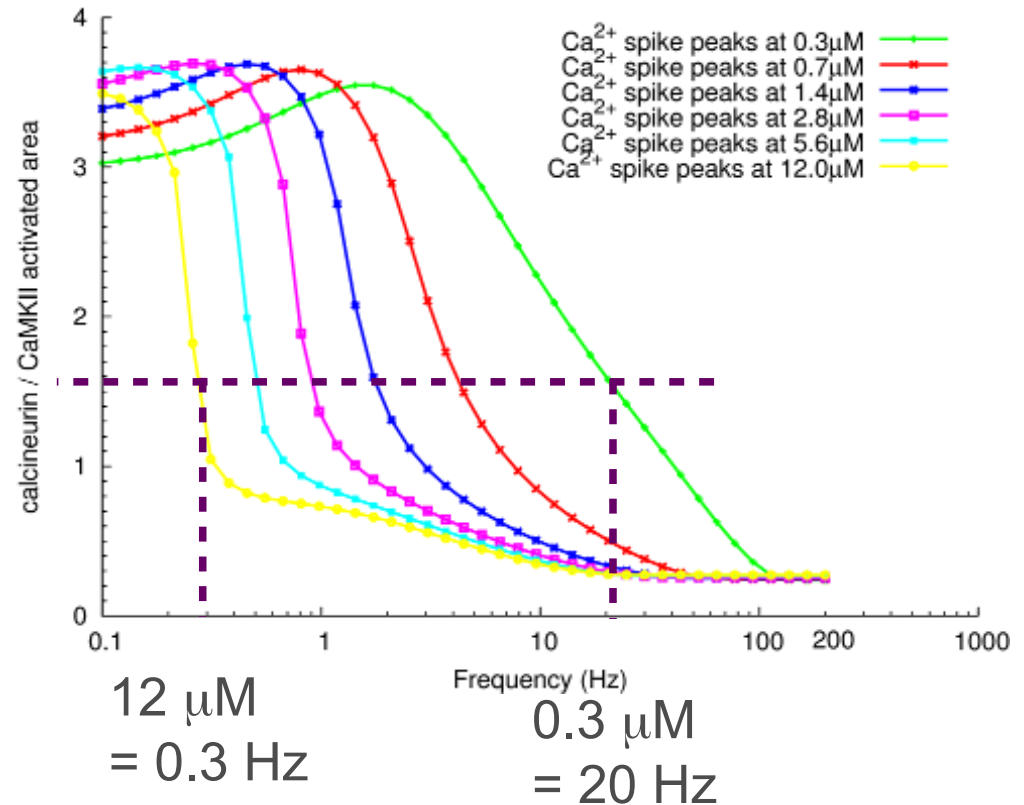
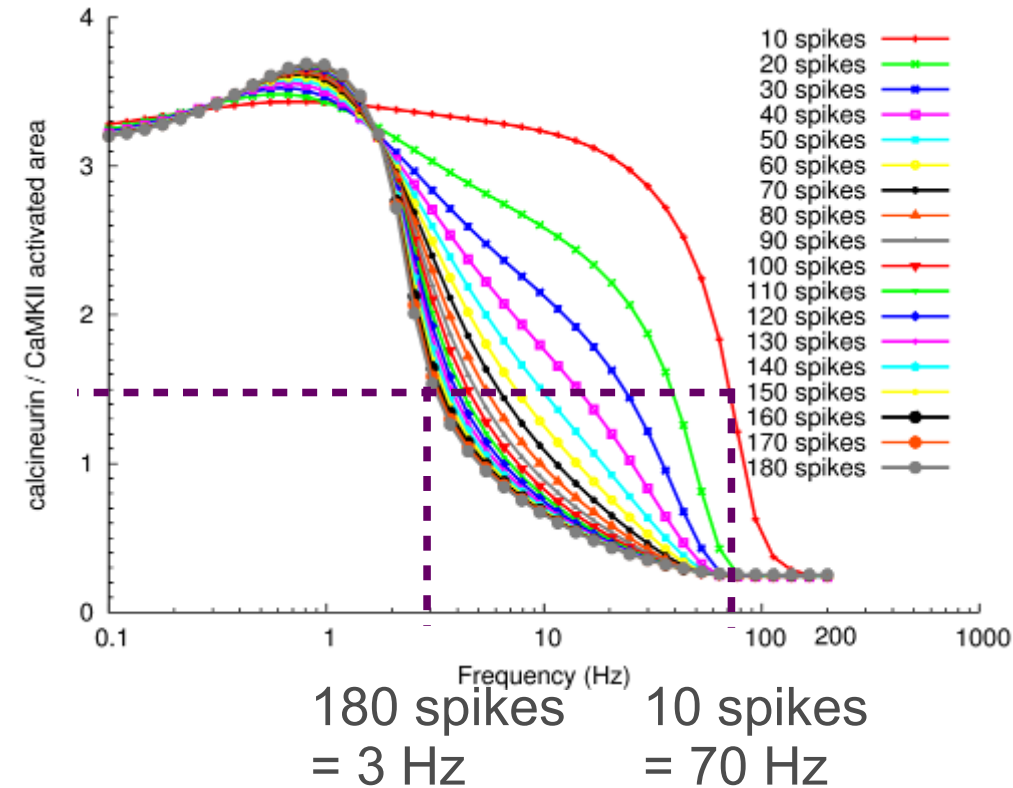
# Bidirectional plasticity

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  
 active areas\* catalytic activities



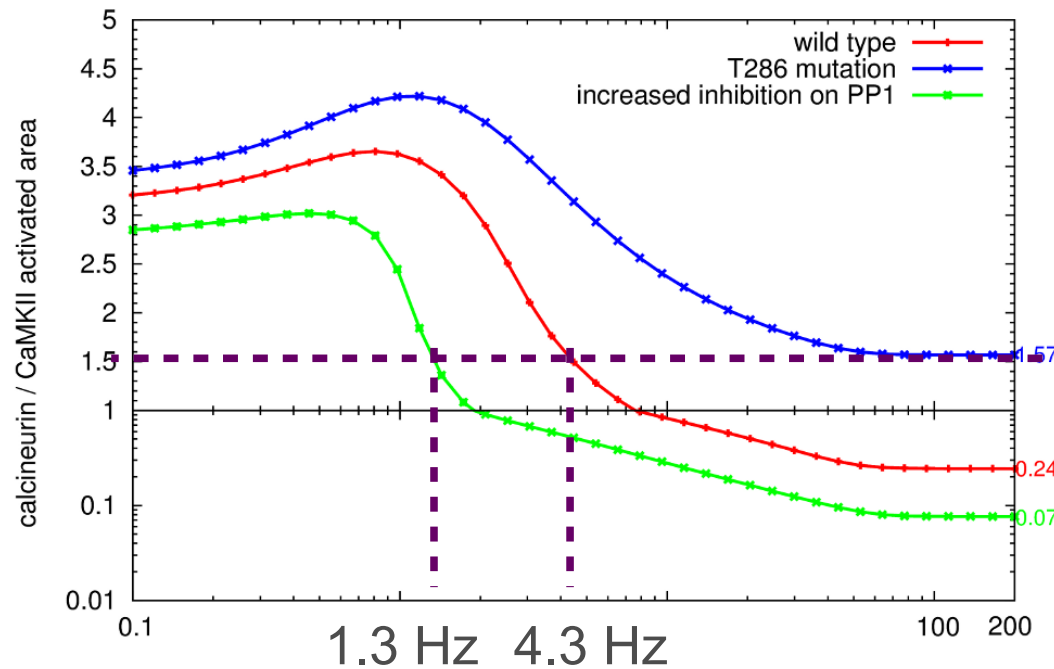
# Effect of calcium duration and amount



Prolonged or intense signals decrease  $\Theta_m$ : 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

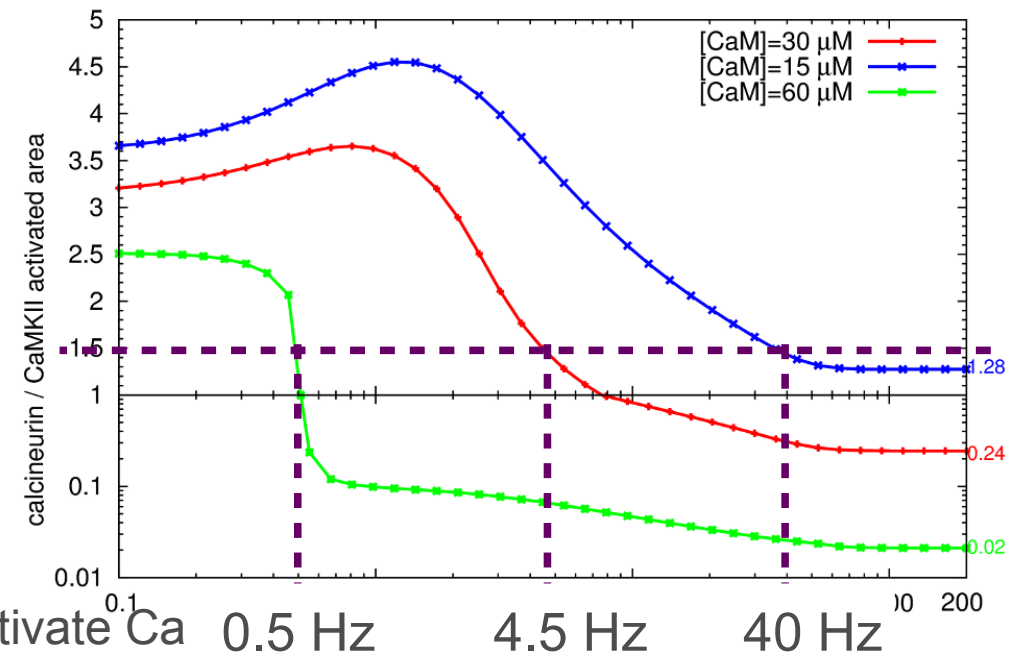
Lower deactivation of CaMKII

## 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 paradoxical effects  
of T306 phosphorylation (Pi *et al* 2010).

T206P releases limiting CaM, that can then activate



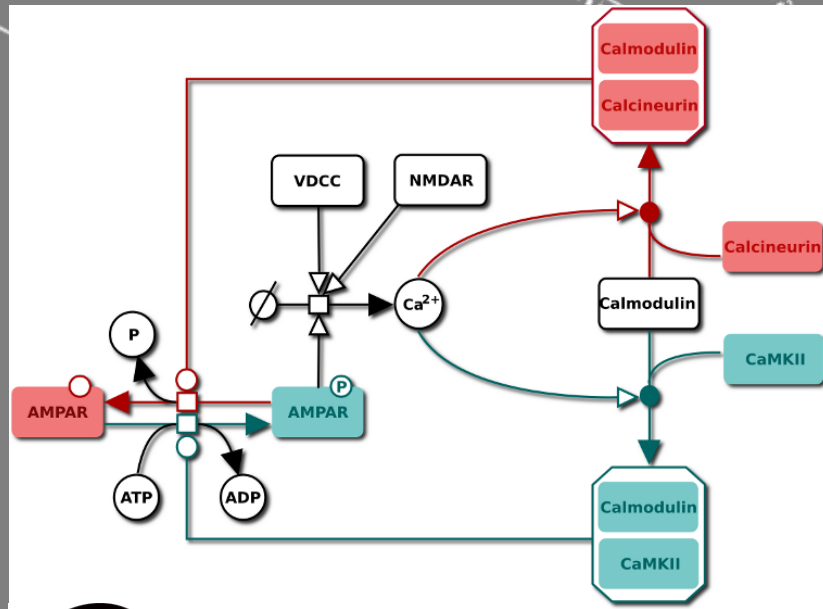
Allosteric stabilisation triggers **bistable CaM response**  
> certain freq, CaM activation longer than initial signal

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

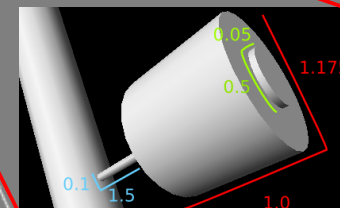
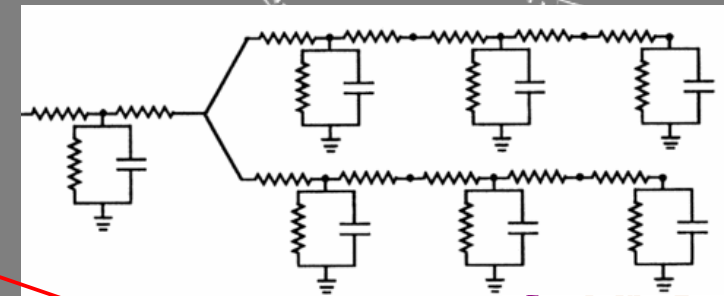
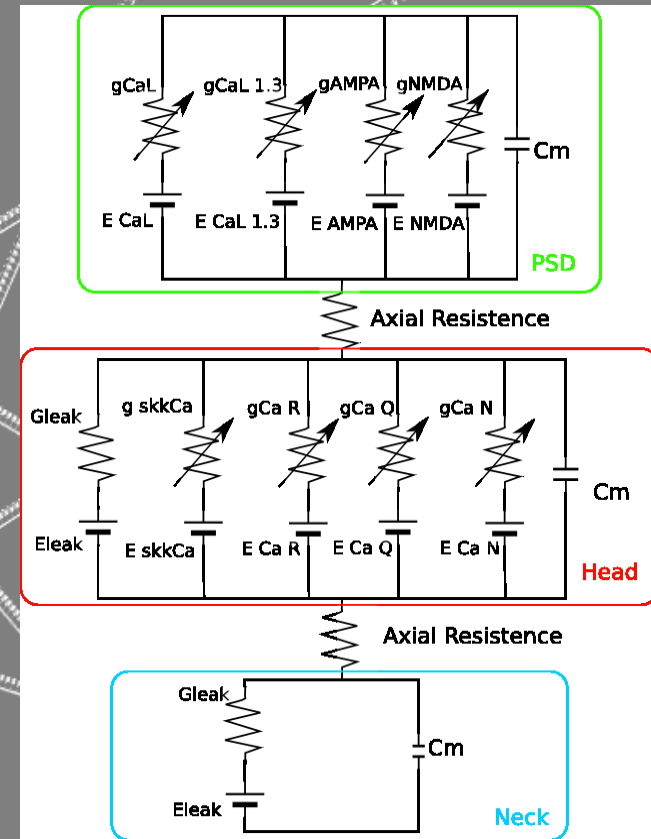
$\Theta_m$  is not an **intrinsic property of the synapse**, but a dynamical one that depends on the length and amplitude of stimulations

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

# Whole cell: electro-biochemical models



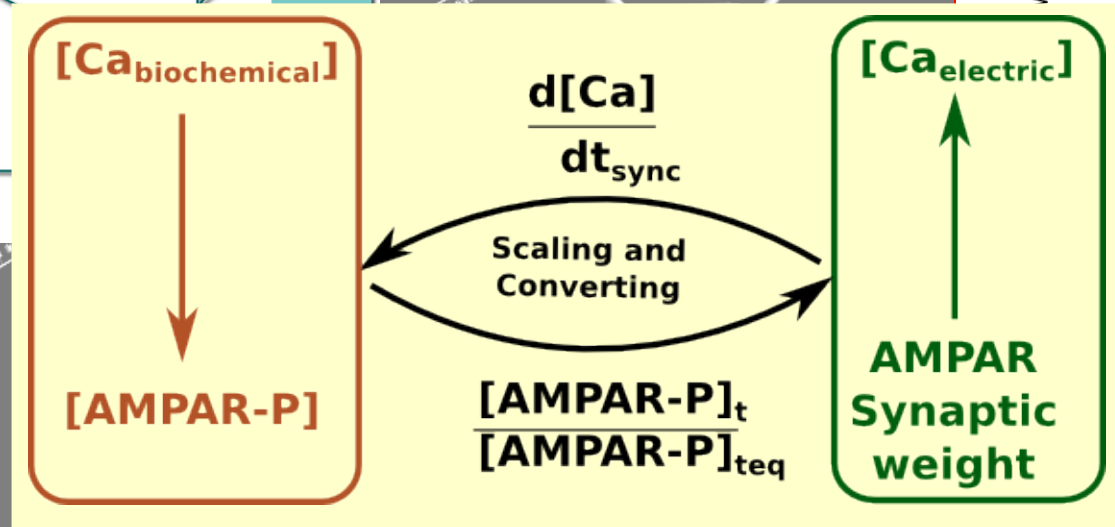
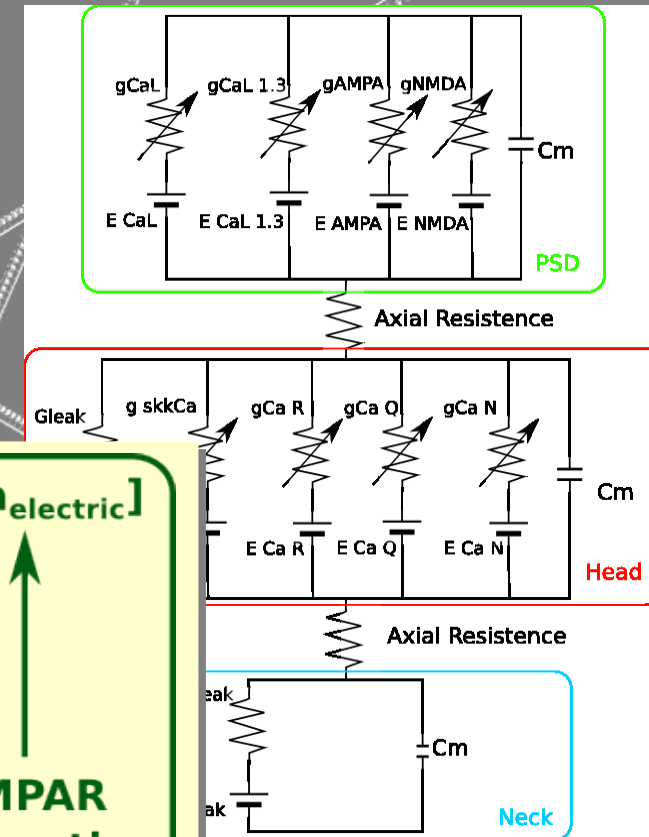
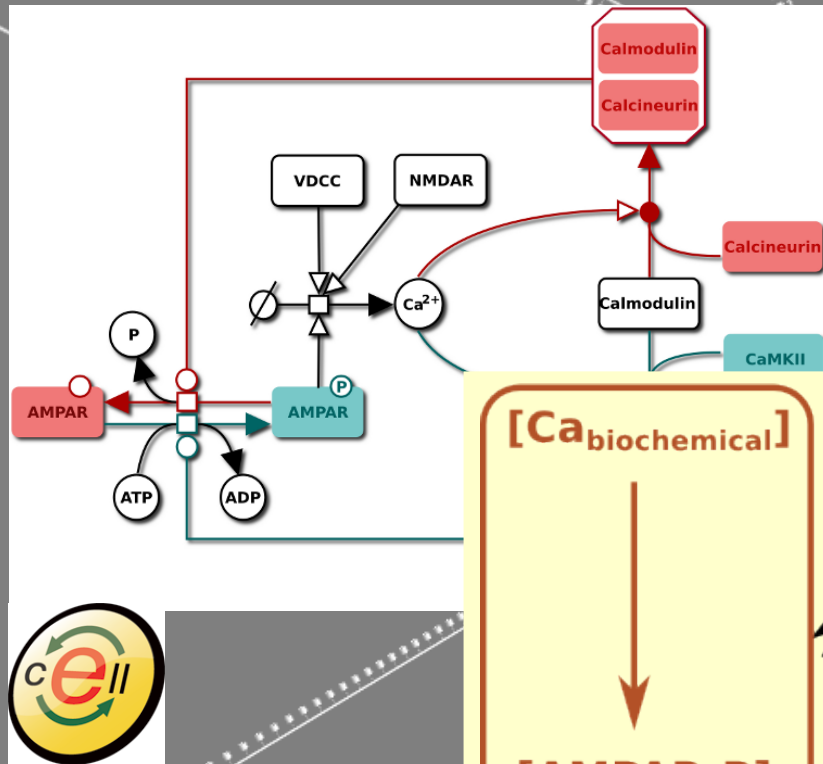
1504 spines  
4761 compartments  
16362 channels



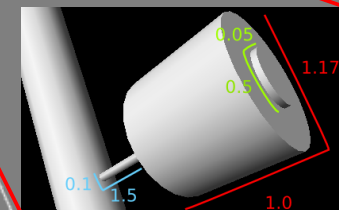
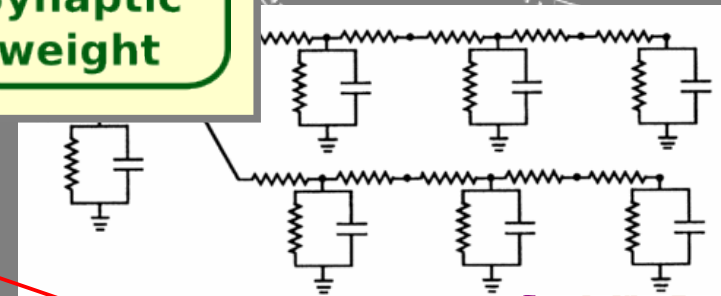
Mattioni M, Cohen U, Le Novère N (2012)

Mattioni M, Le Novère N (2013)

# Whole cell: electro-biochemical models



1504 spines  
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Mattioni M, Cohen U, Le Novère N (2012)

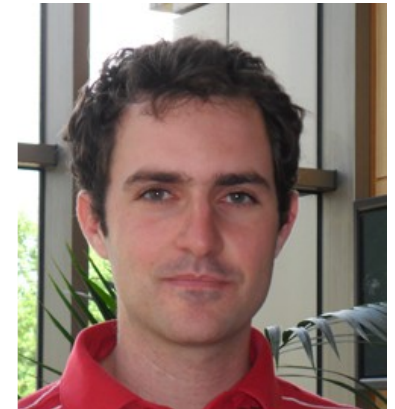
Mattioni M, Le Novère N (2013)



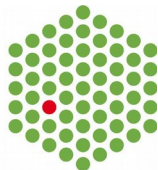
Melanie Stefan



Lu Li



Denis Brun



Michele  
Mattioni



Stuart  
Edelstein

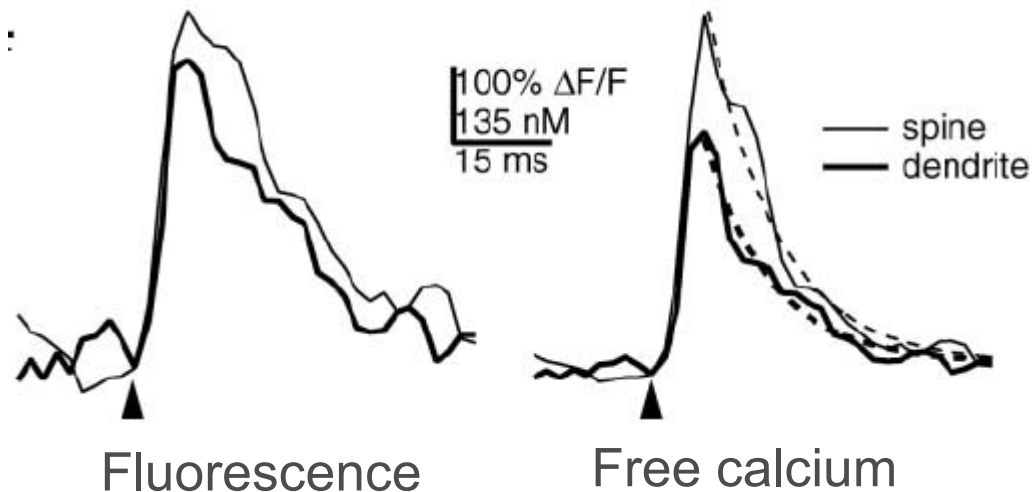
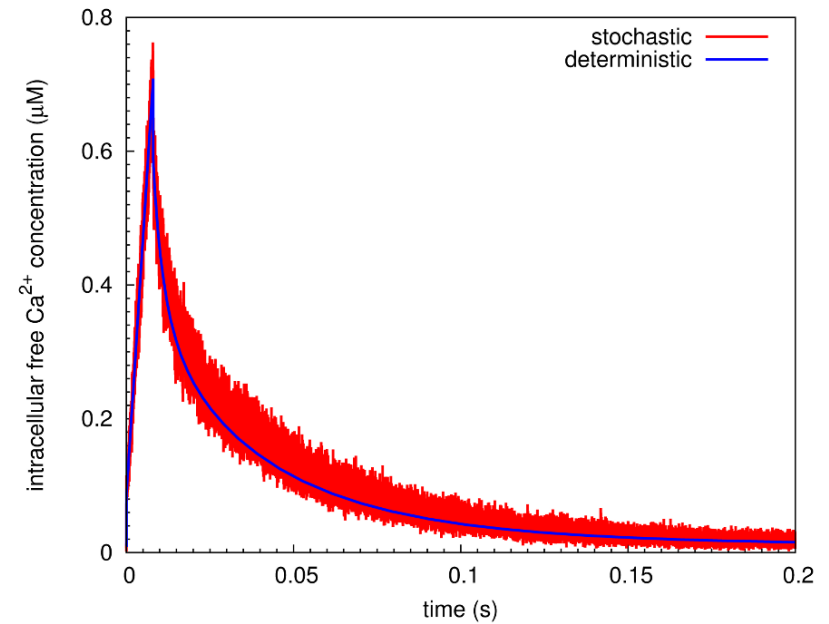
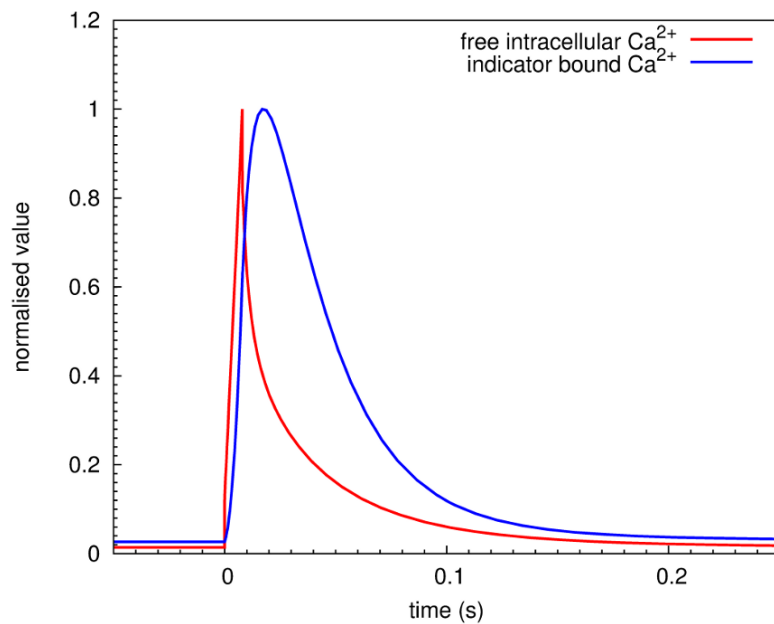


Massimo  
Lai





# 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.