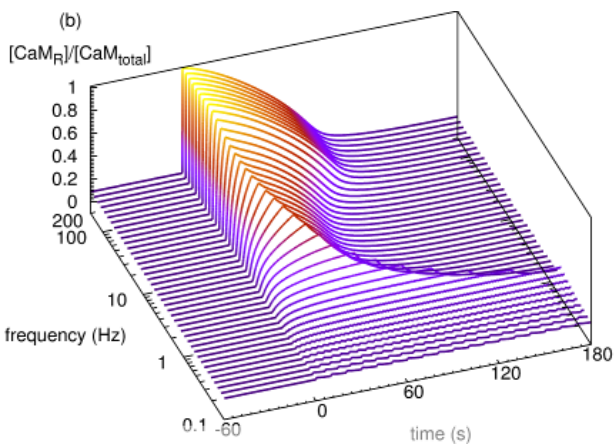


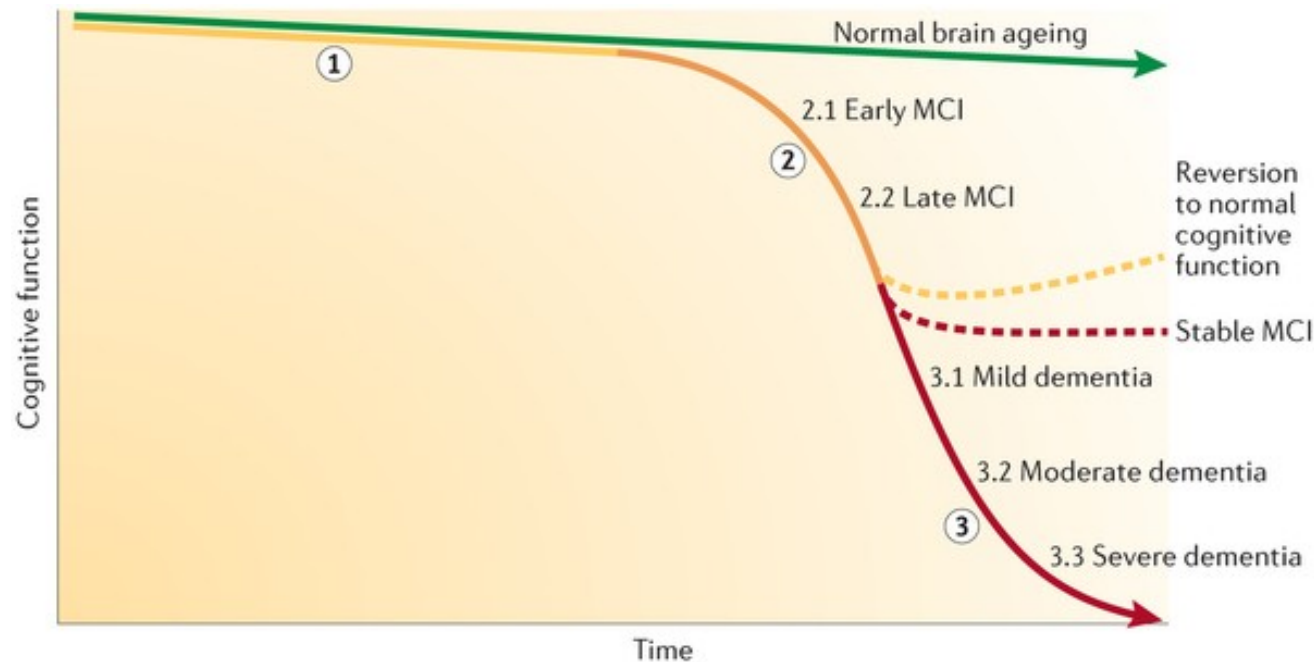


# Synapses, Systems and Simulations



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





**1. Preclinical stage:  
asymptomatic, at-risk**

Duration: decades

- Amyloid- $\beta$  accumulates in the brain
- Tau hyperphosphorylation gradually leads to neuronal loss
- Pathology does not yet noticeably affect cognition
- Biomarkers and genetic profile can indicate the risk of disease progression and reveal underlying AD

**2. Prodromal stage: MCI**

Duration: ~7 years

Subtypes:

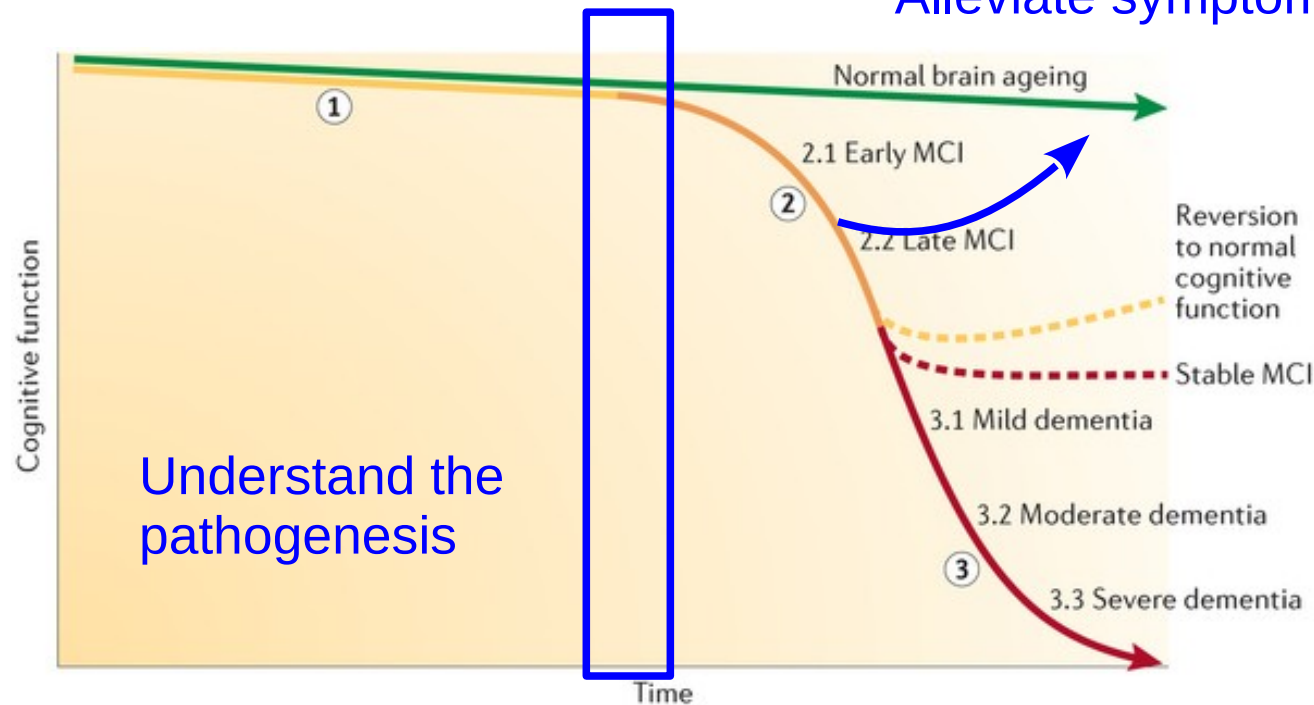
- Progressive MCI
- MCI caused by AD
- Amnesic syndrome of the hippocampal type
- Deficits in memory and/or other cognitive domains noticeable to the person affected and/or others, but not severe enough to interfere with activities of daily living
- Biomarkers can determine the aetiological diagnosis

**3. Syndromal stage:  
dementia**

Duration: ~7 years; followed by total loss of independent function

- Notable loss of intellectual ability affecting memory and at least one other cognitive domain
- The impairment interferes with activities of daily living

Hampel and Lista (2016) *Nature Reviews | Neurology*



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Duration: decades

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Duration: ~7 years

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- Progressive MCI
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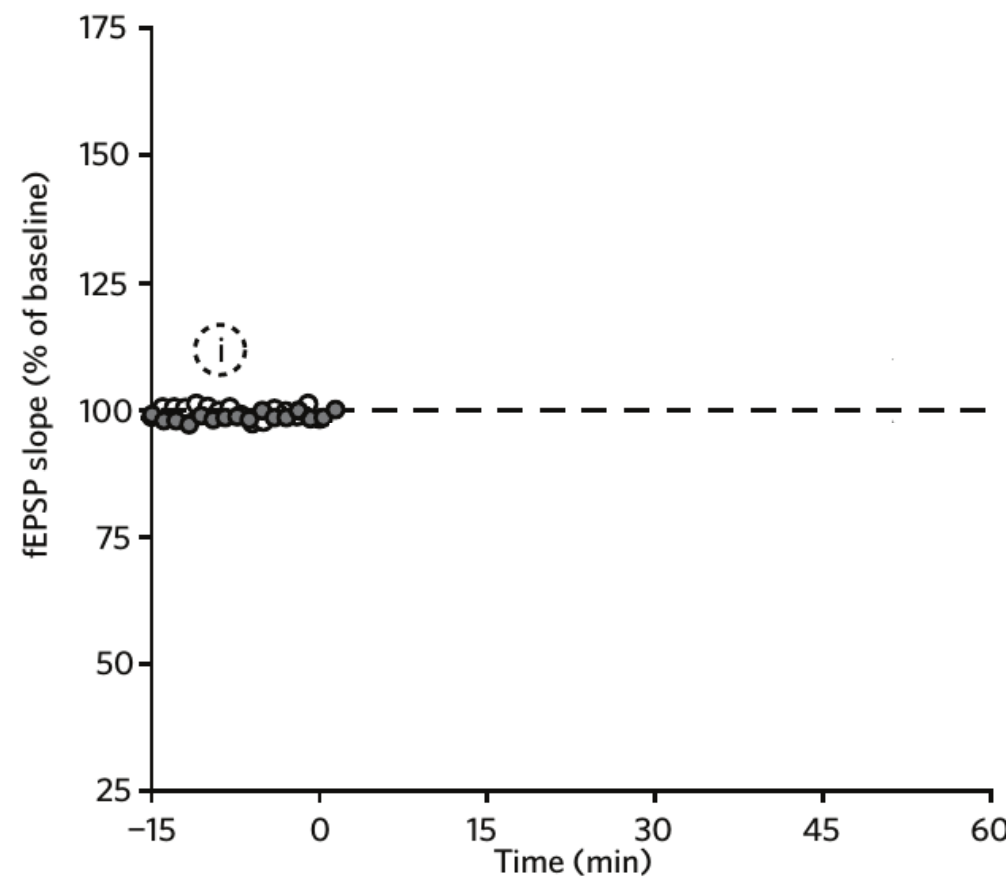
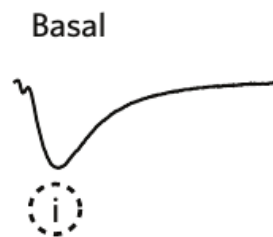
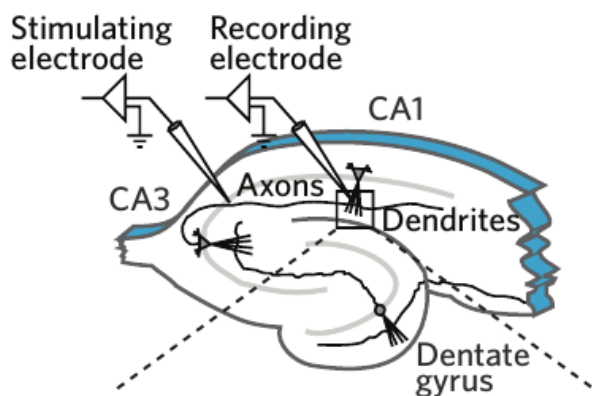
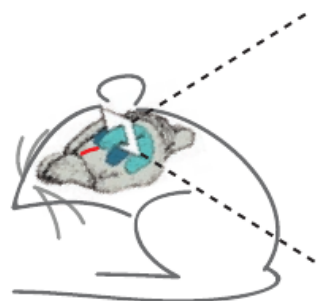
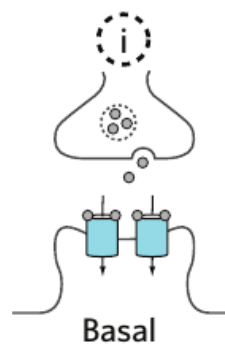
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Hampel and Lista (2016) *Nature Reviews | Neurology*

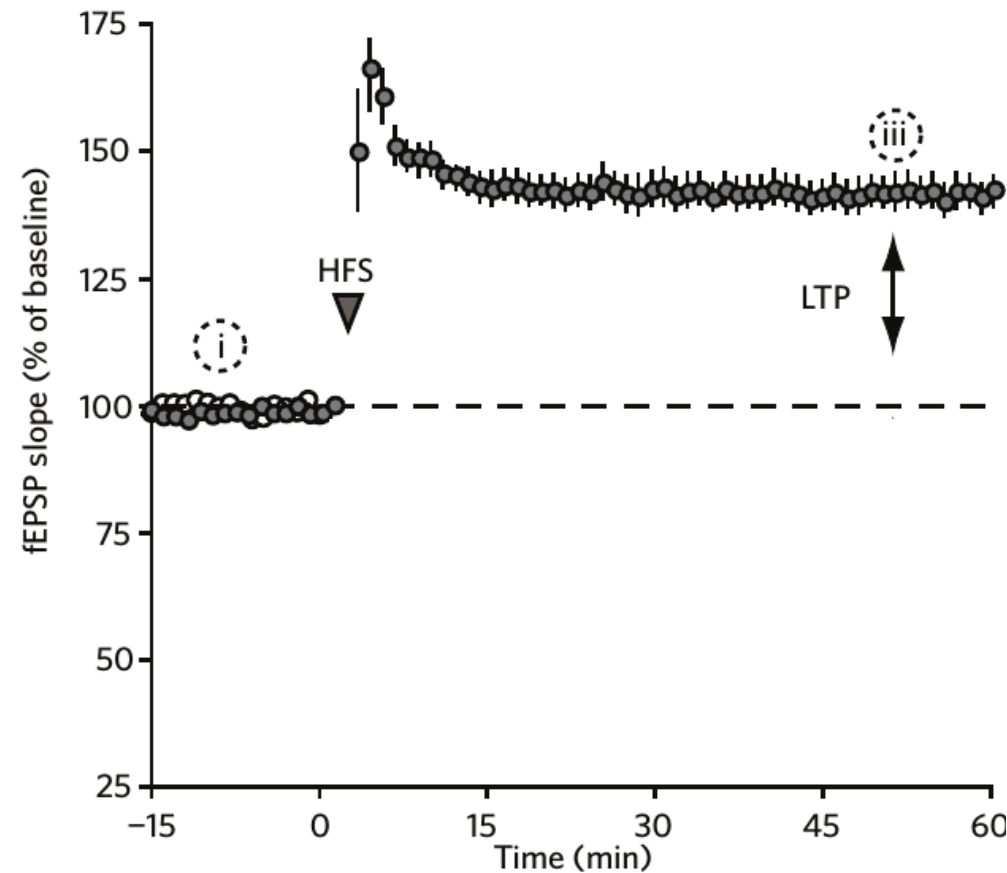
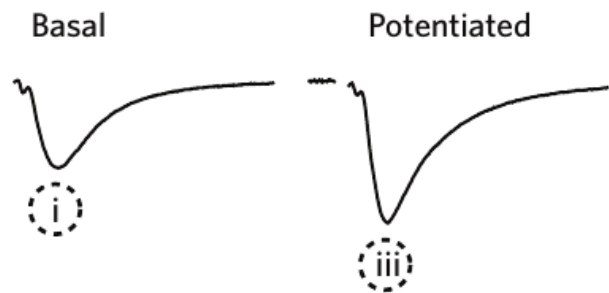
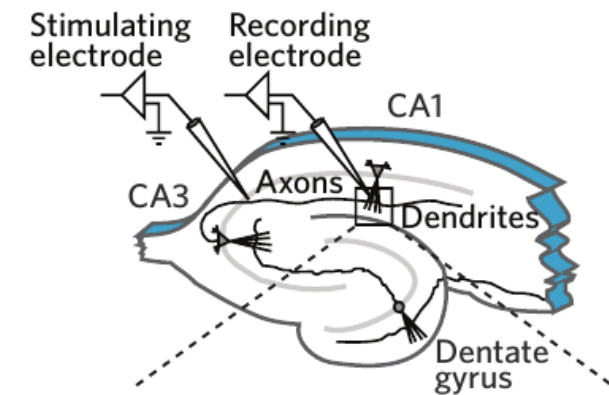
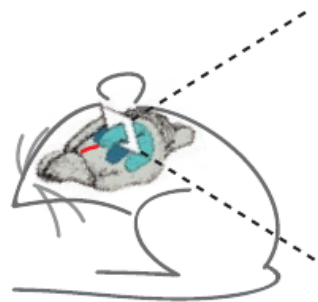
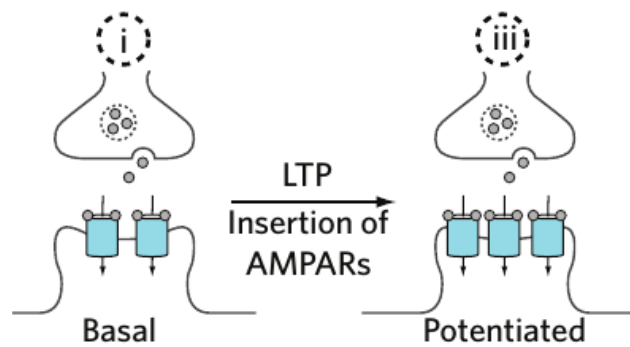
“Rather than neuronal loss, it is now widely accepted that synaptic impairments underlie the decrease performance”

Federico Grillo - 2016

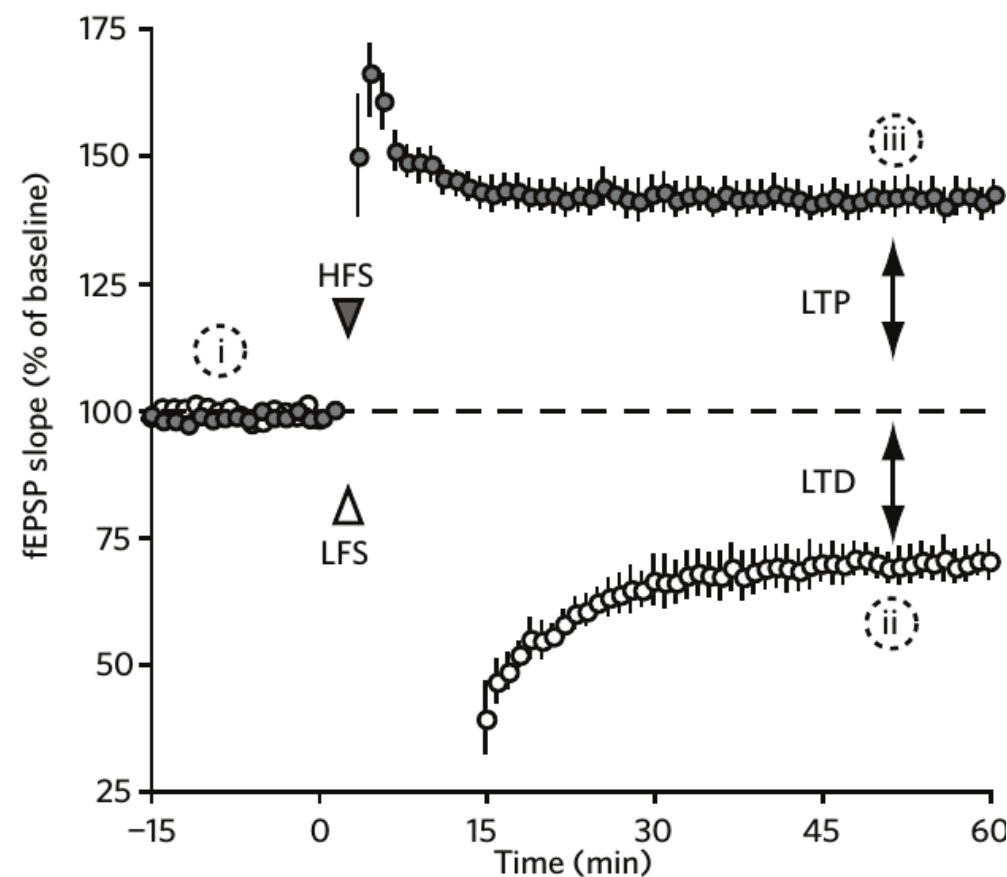
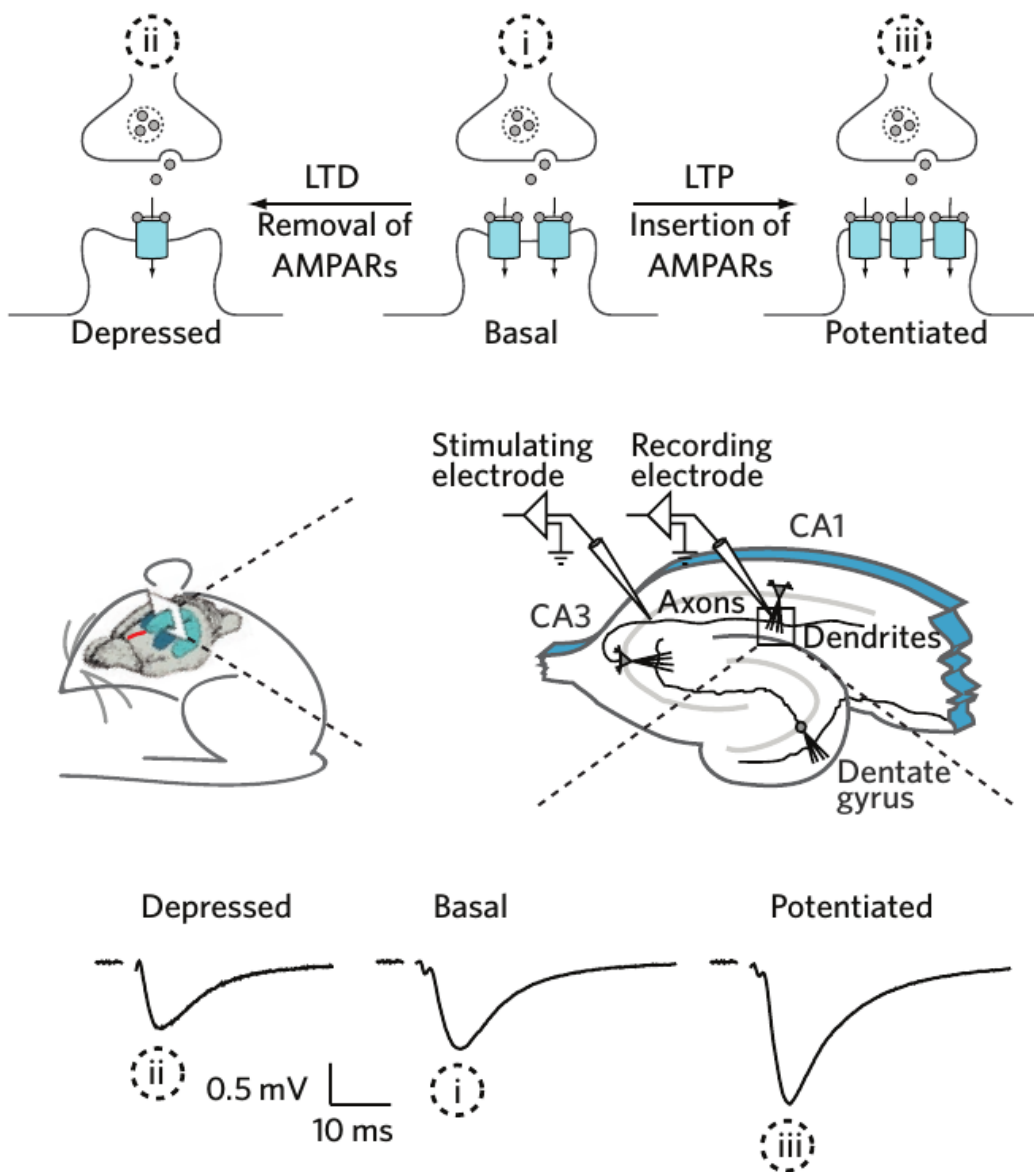




Fleming and England (2010) *Nat Chem Biol*

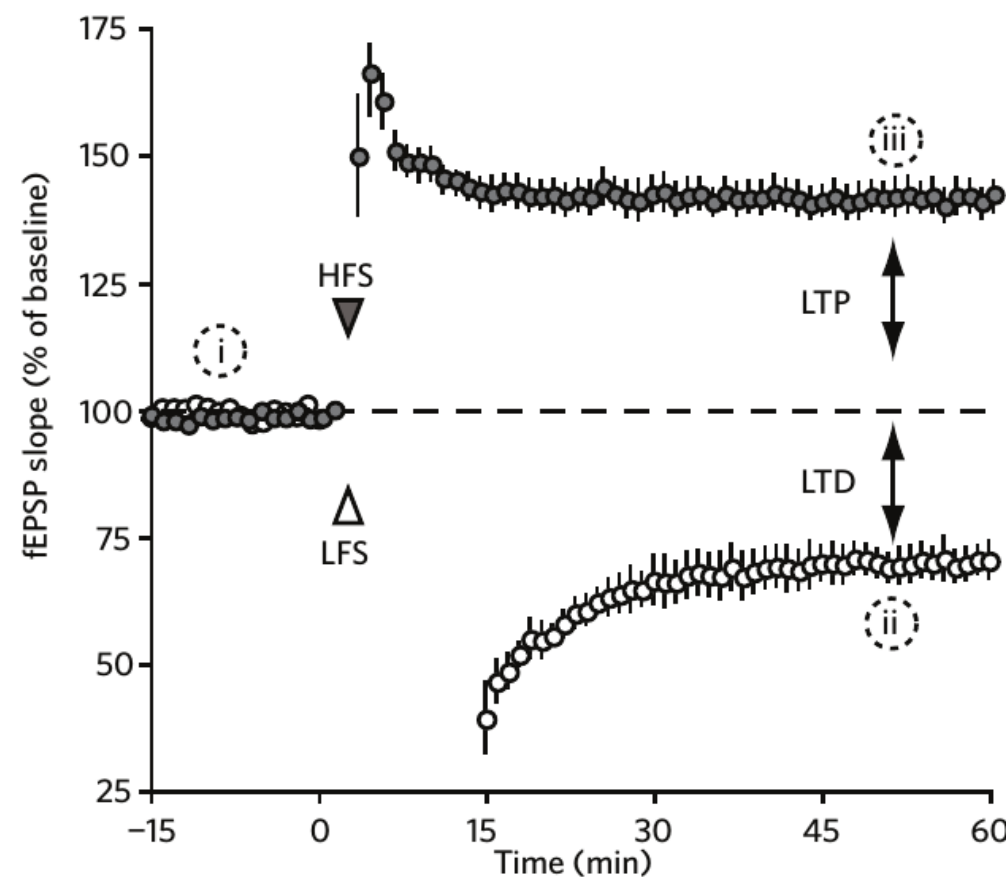
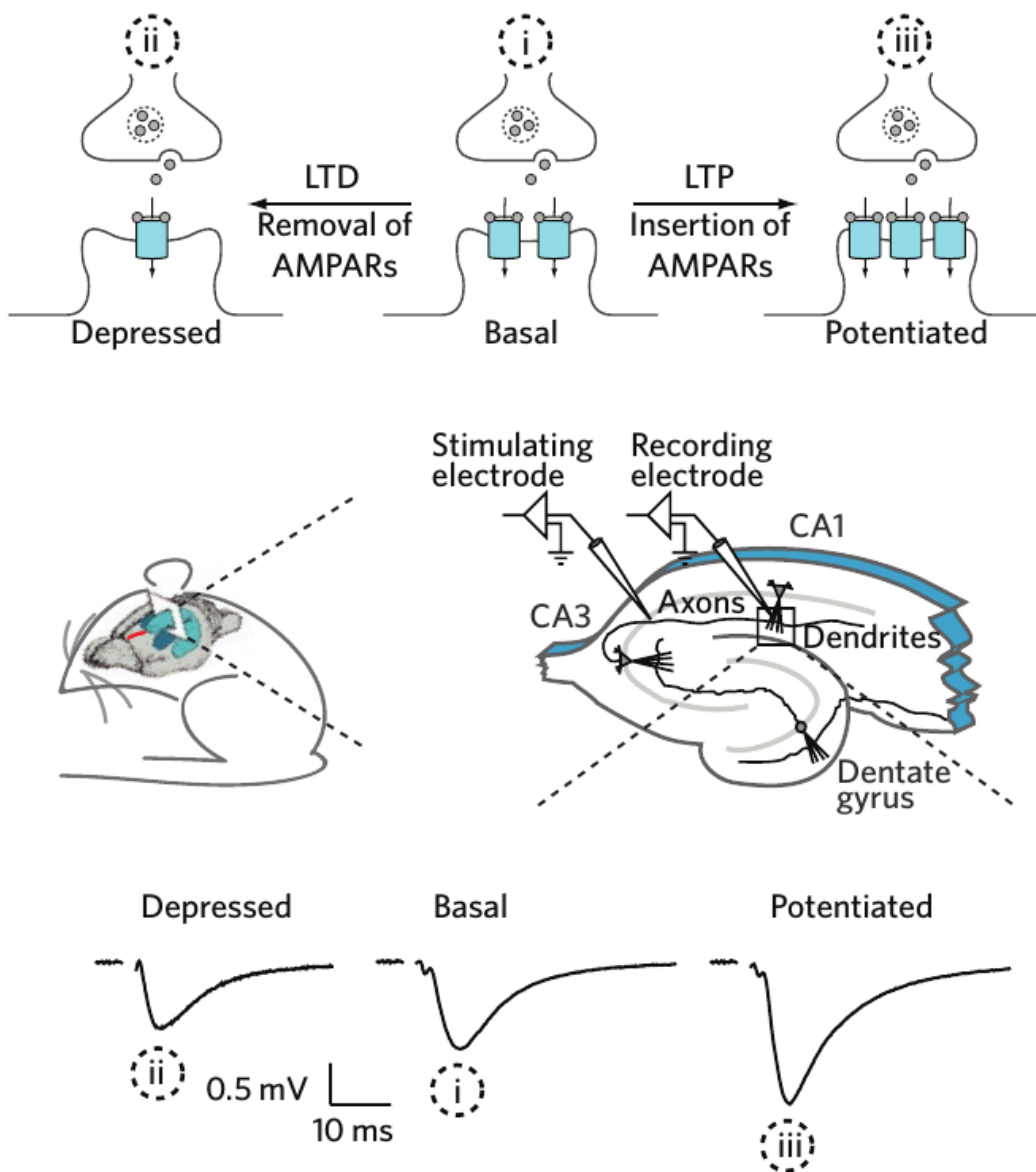


Fleming and England (2010) *Nat Chem Biol*



Fleming and England (2010) *Nat Chem Biol*





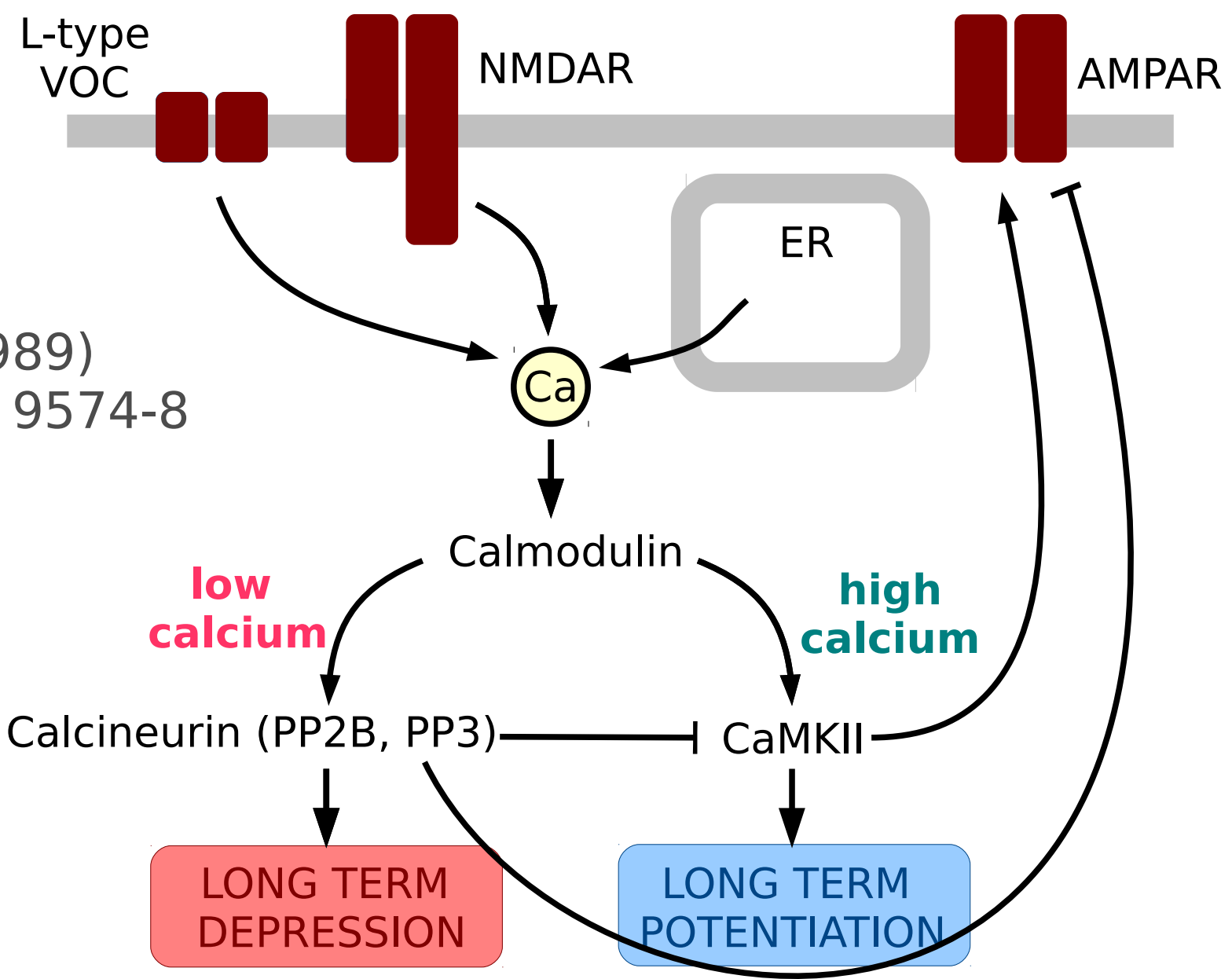
**lasts for weeks**

Fleming and England (2010) *Nat Chem Biol*

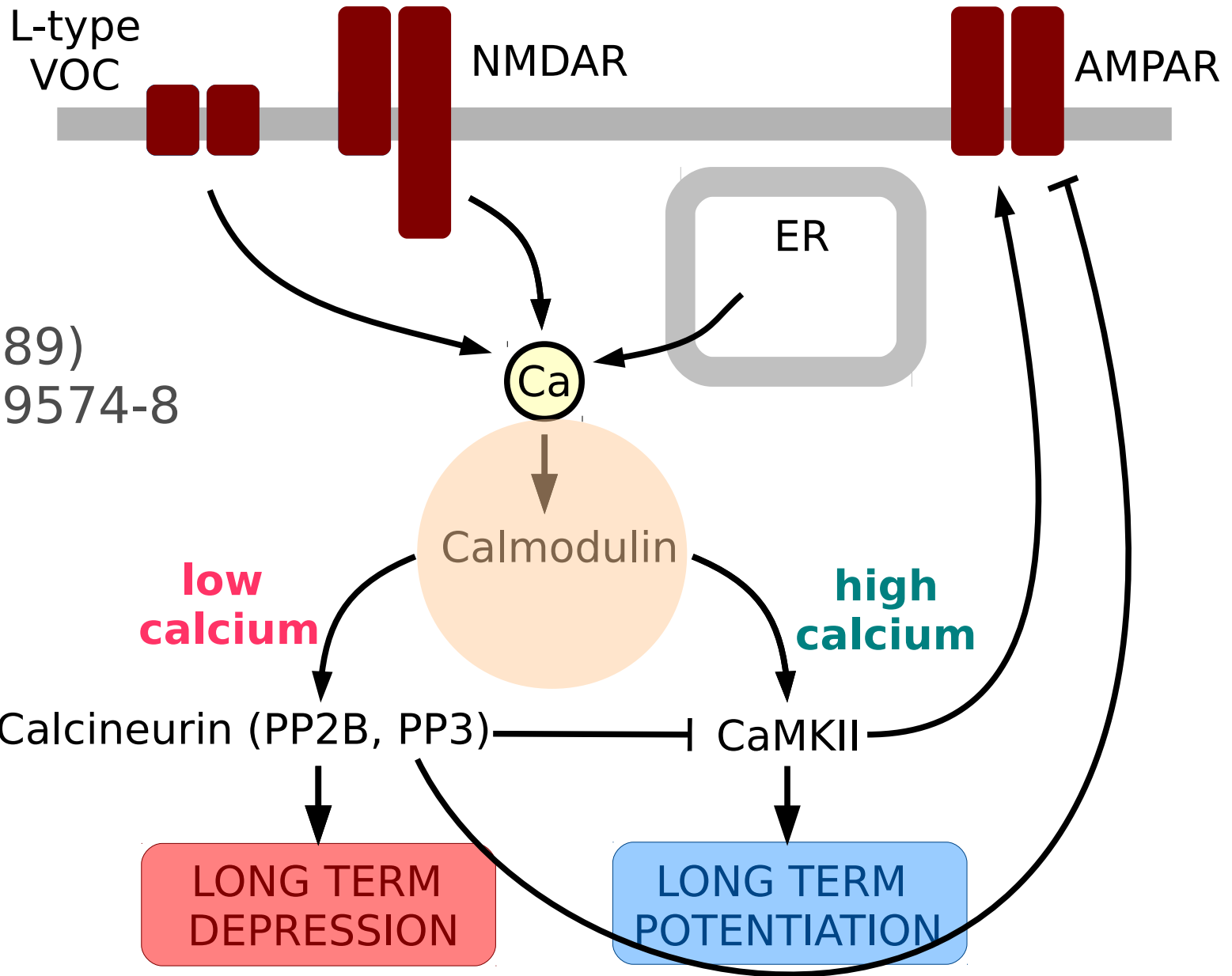


# The calcium theory of synaptic plasticity

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



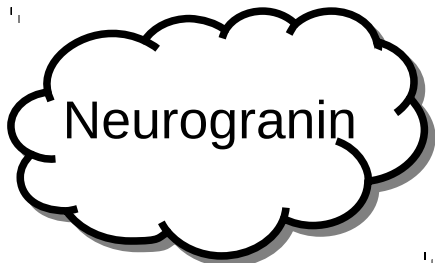
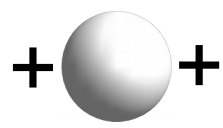
# Calmodulin, the memory switchboard



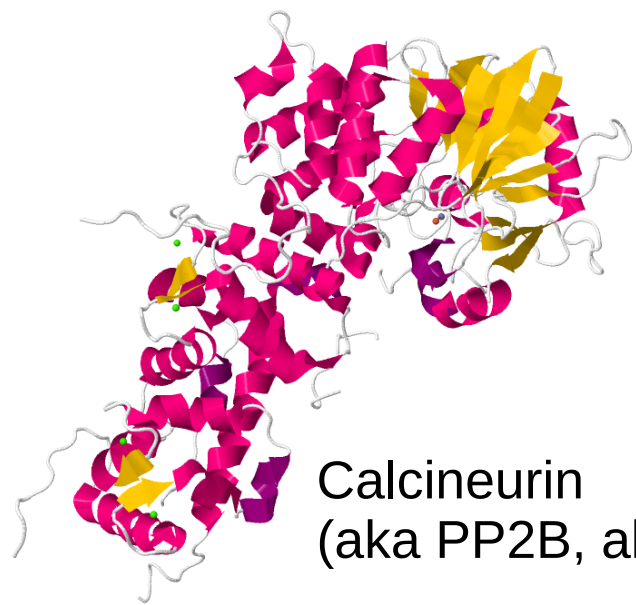
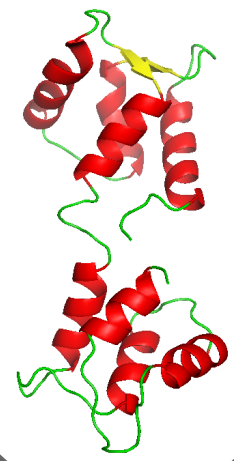
Lisman (1989)  
*PNAS*, 86: 9574-8



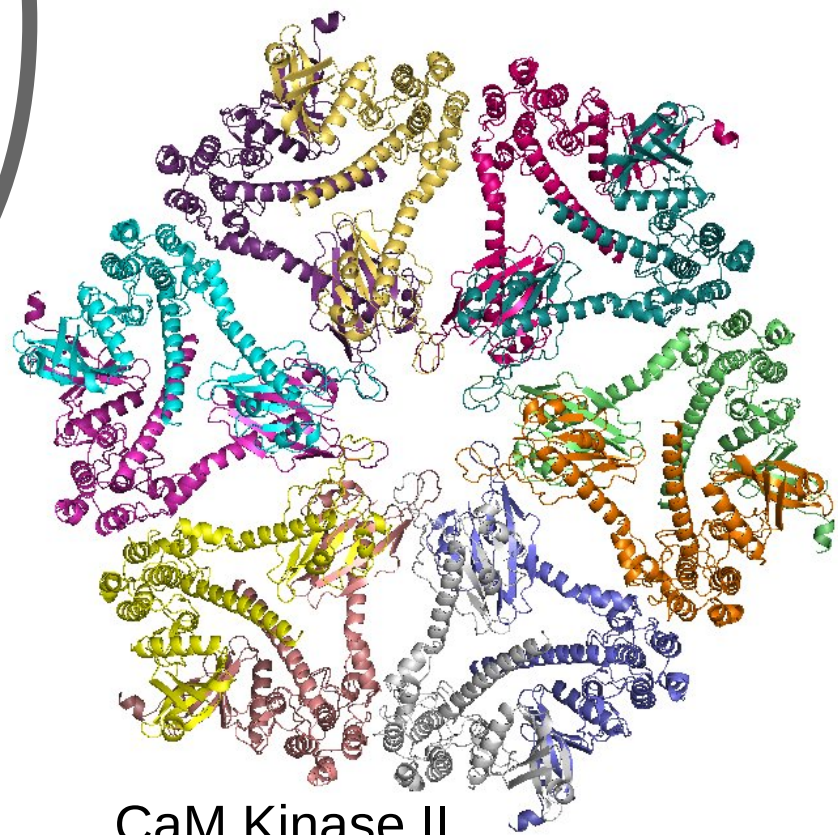
Calcium



Calmodulin



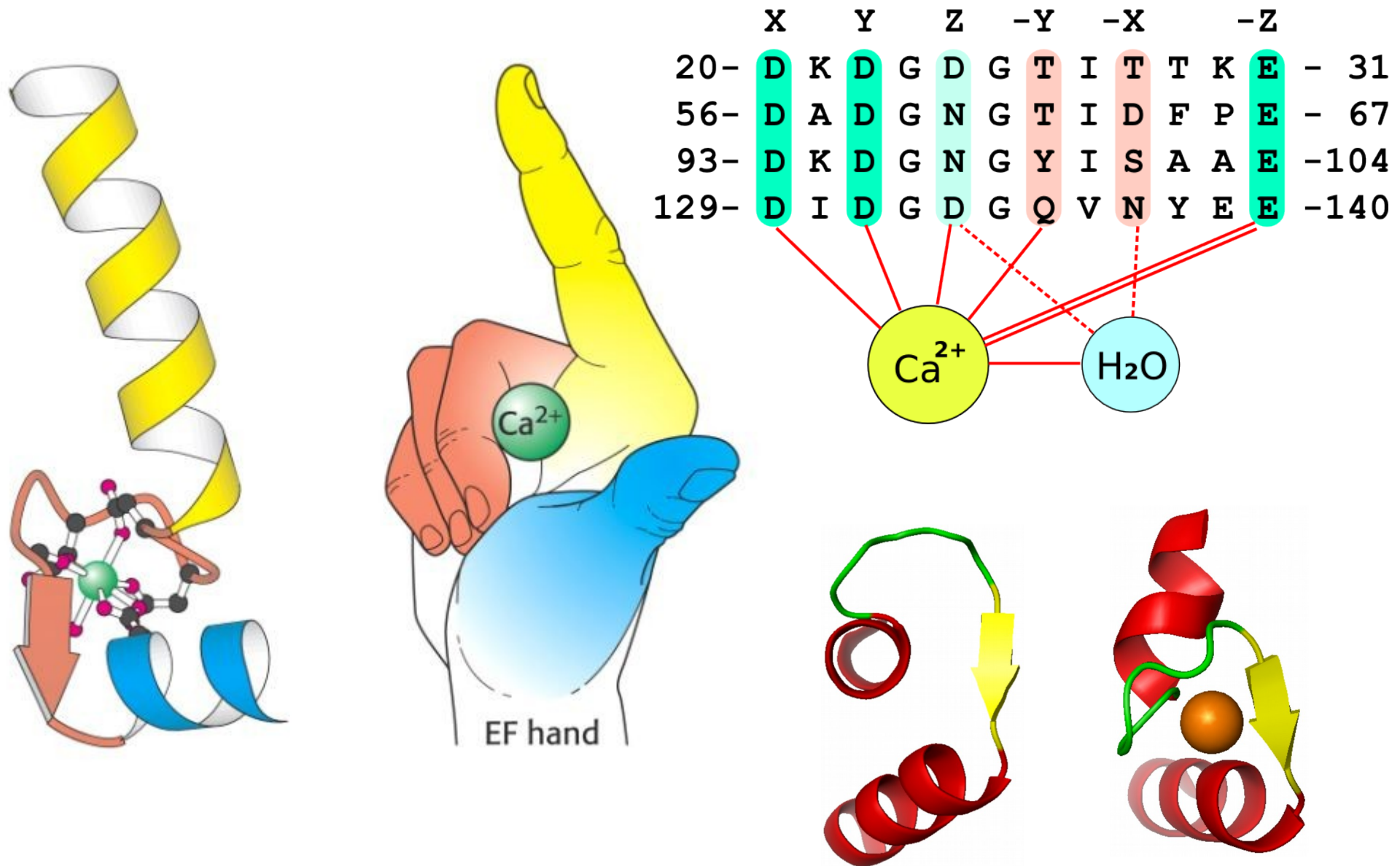
Calcineurin  
(aka PP2B, aka PP3)

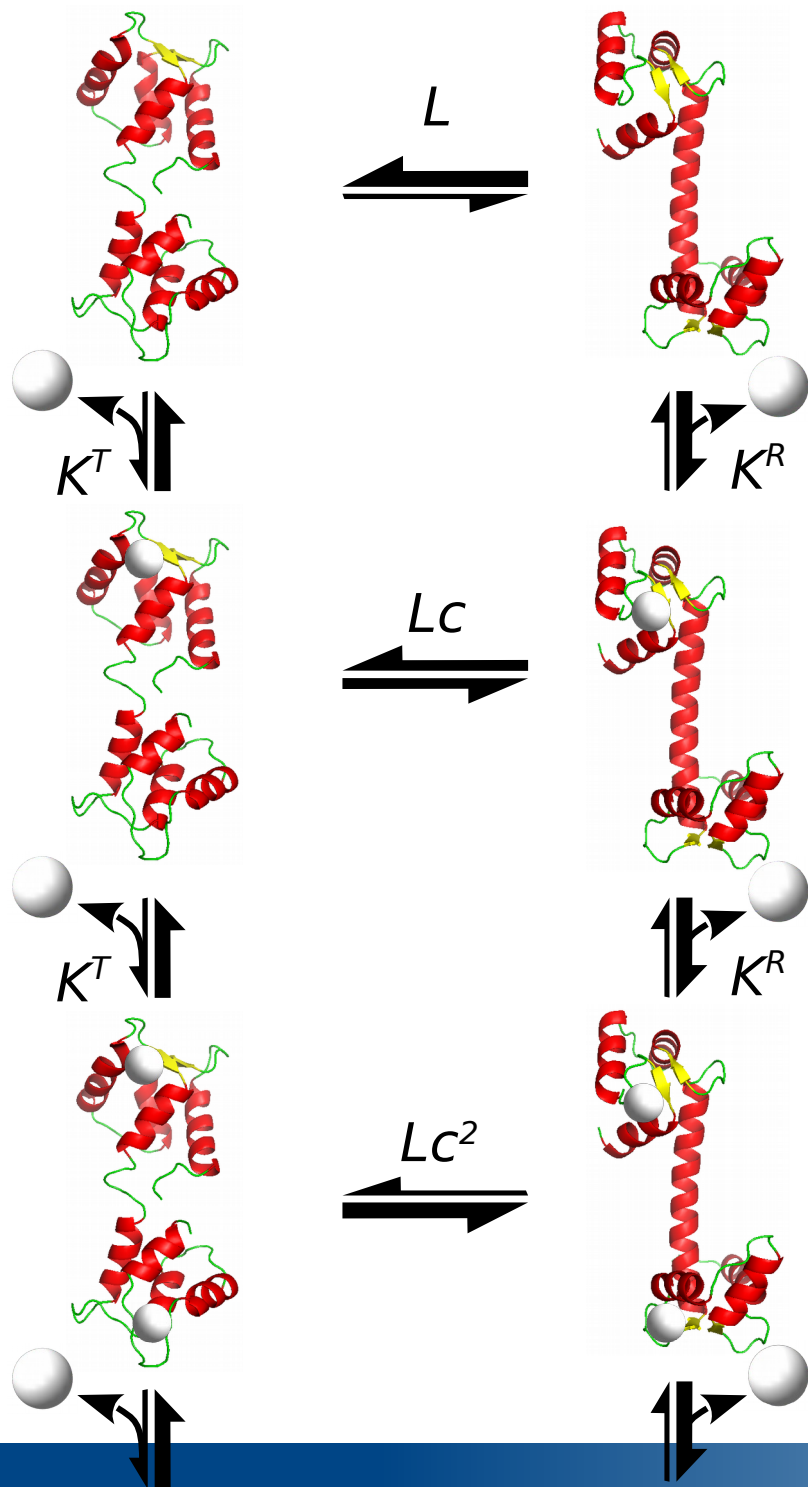


CaM Kinase II



# Calmodulin carries 4 $\text{Ca}^{2+}$ binding domains





$$\bar{Y} = \frac{\alpha(1 + \alpha)^{n-1} + Lc\alpha(1 + c\alpha)^{n-1}}{(1 + \alpha)^n + L(1 + c\alpha)^n}$$

$$\bar{R} = \frac{(1 + \alpha)^n}{(1 + \alpha)^n + L(1 + c\alpha)^n}$$

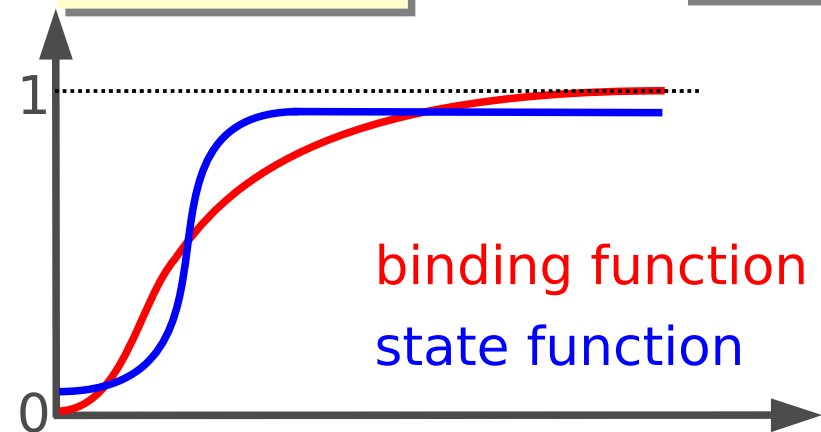
$$L = \frac{[T_0]}{[R_0]} \quad c = \frac{K^R}{K^T} \quad \alpha = \frac{[Ca^{2+}]}{[K^R]}$$

$L \gg 1 \Rightarrow$

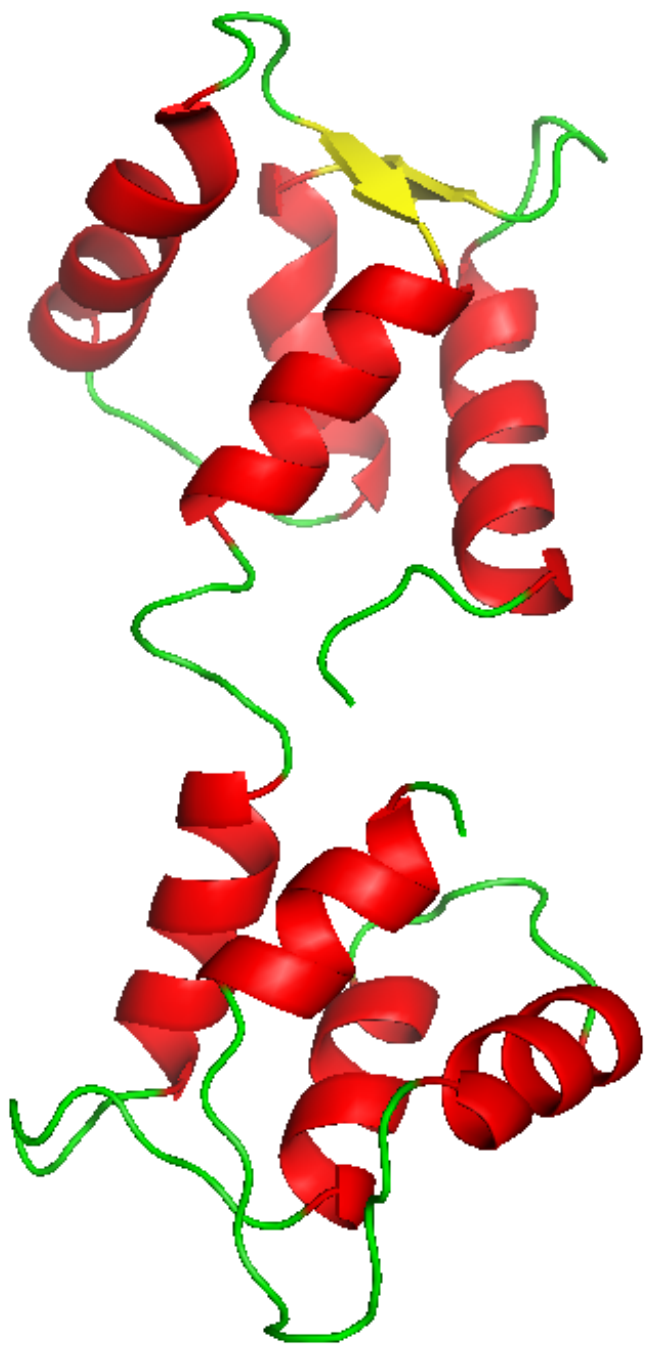
Equilibrium  
strongly biased

$c \ll 1 \Rightarrow$

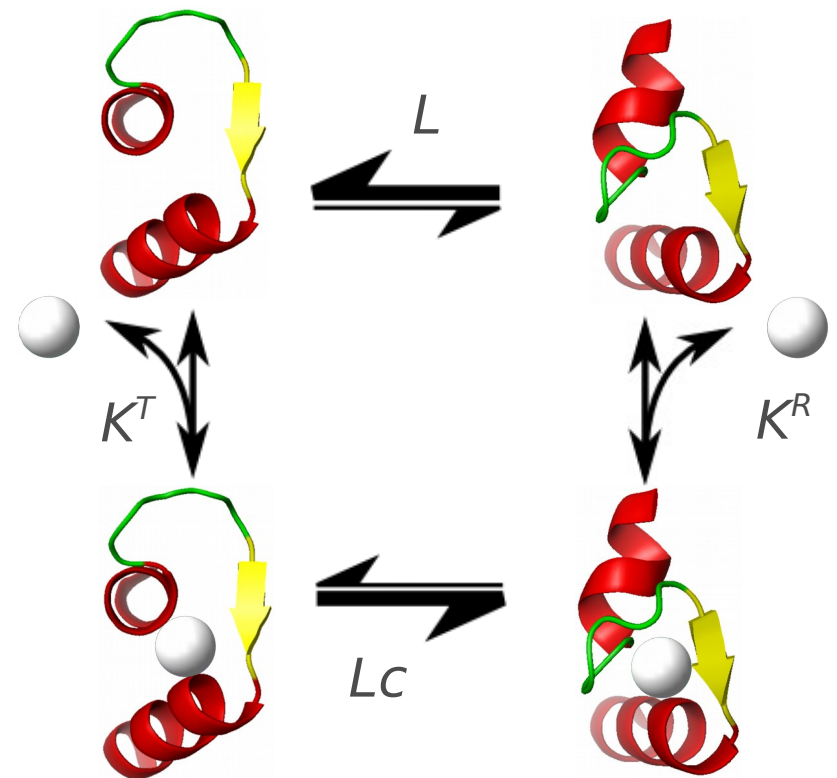
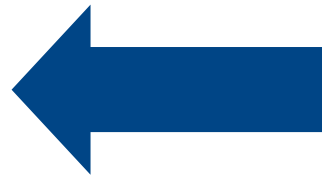
strong effect  
of ligand

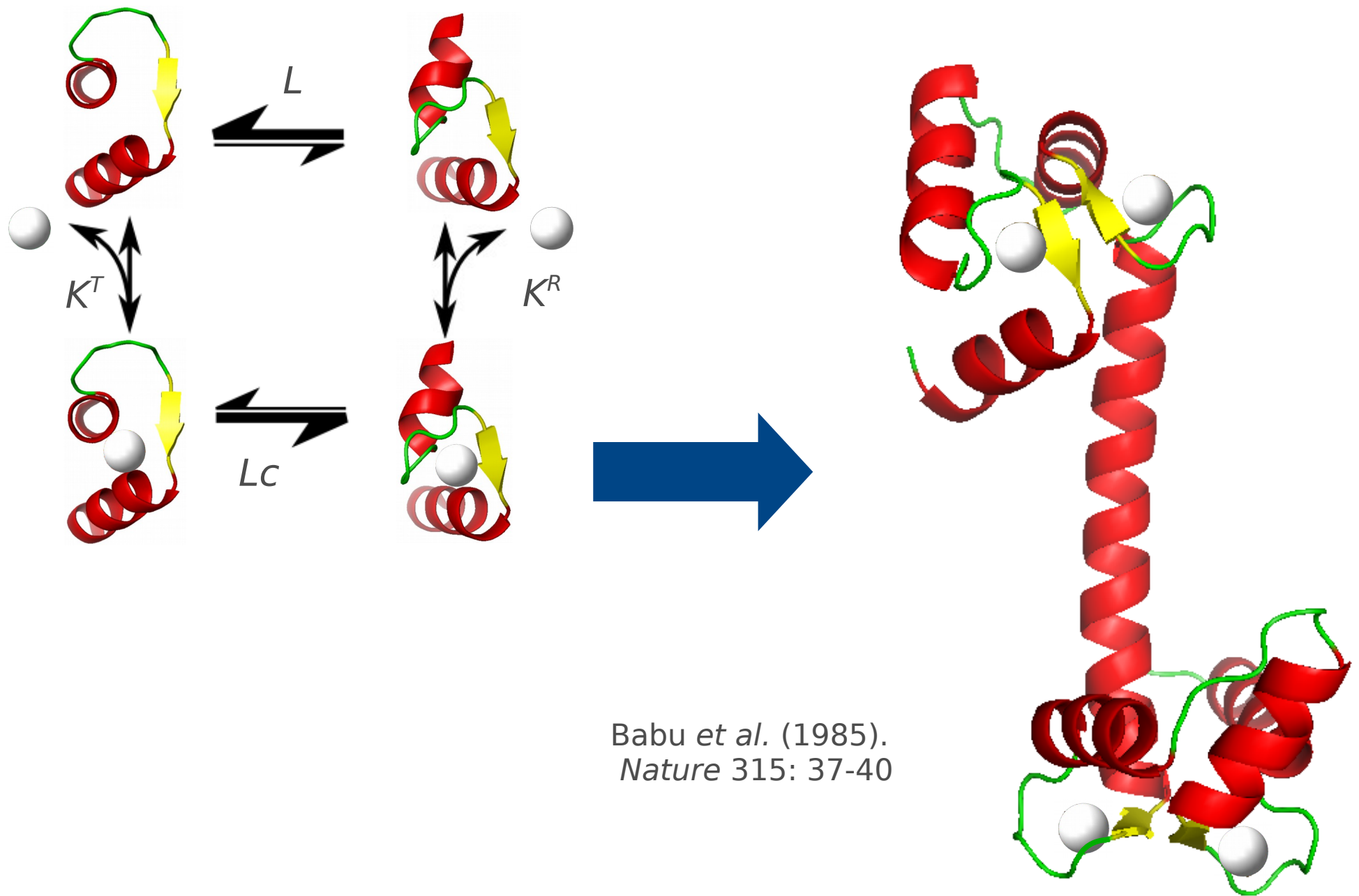


Monod, Wyman, Changeux (1965)  
**On the nature of allosteric transitions: a plausible model.**  
*J Mol Biol*, 12: 88-118

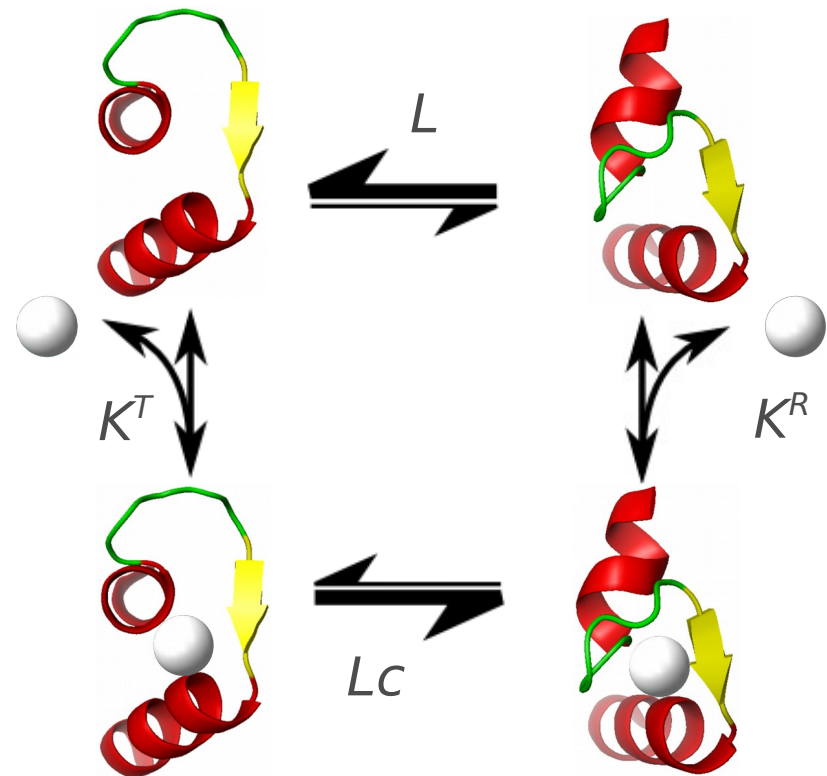
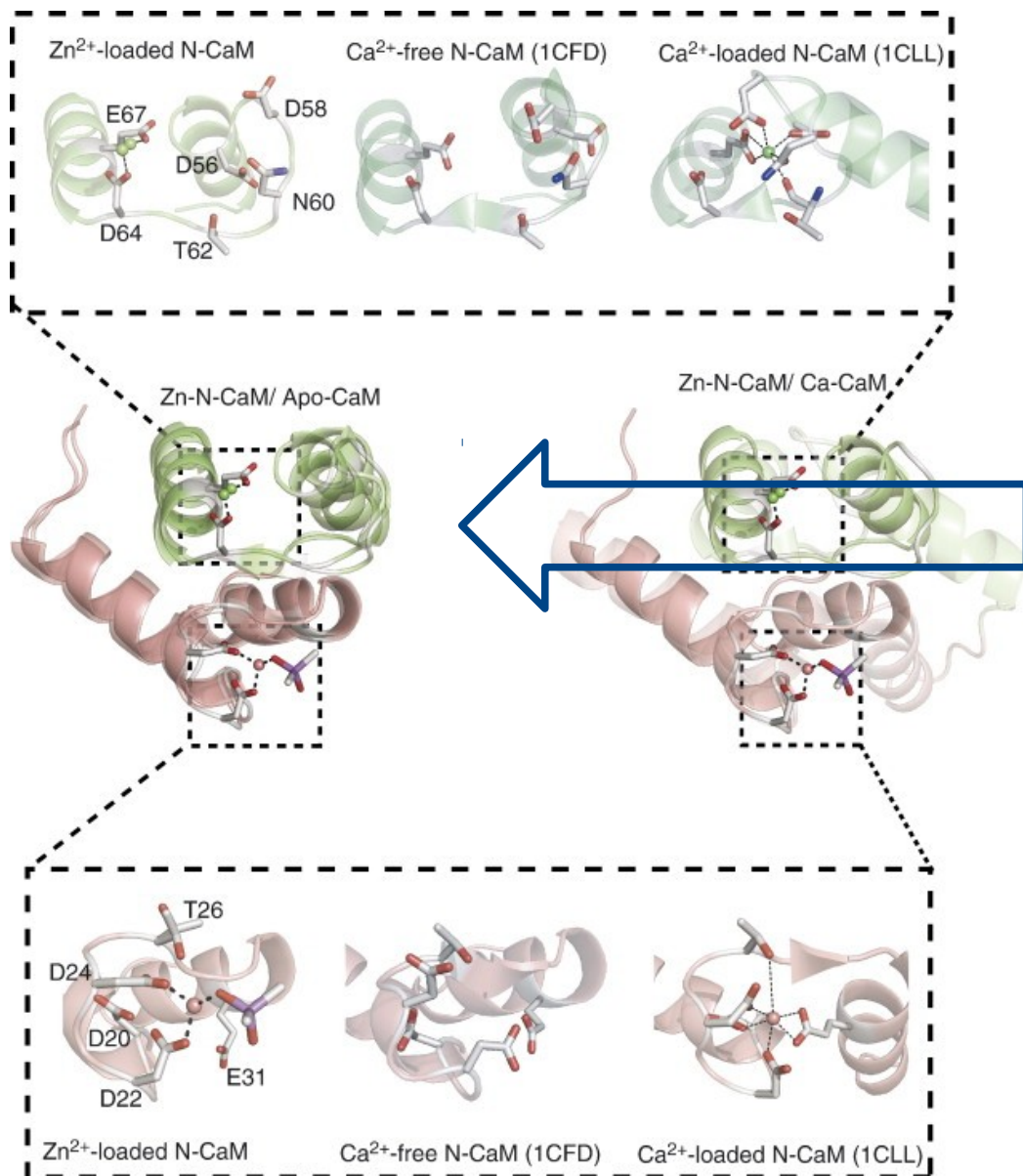


Kuboniwa *et al.* (1995).  
*Nat Struct Biol* 2: 768-776

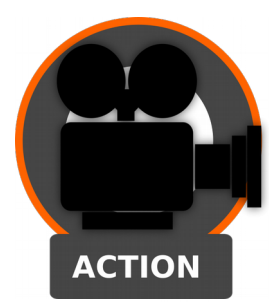




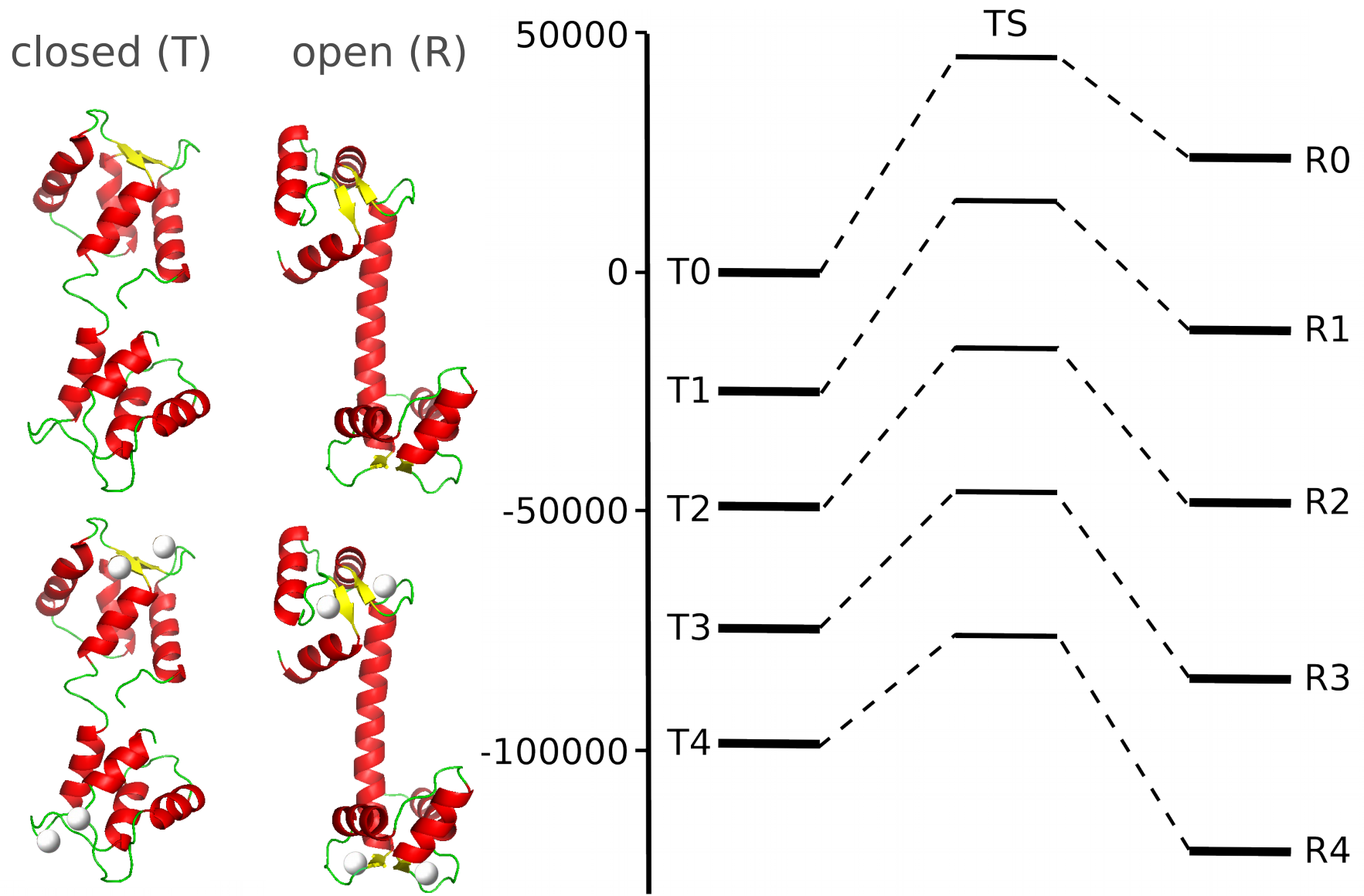




Warren *et al.* (2007).  
*J Mol Biol* 374: 517-527

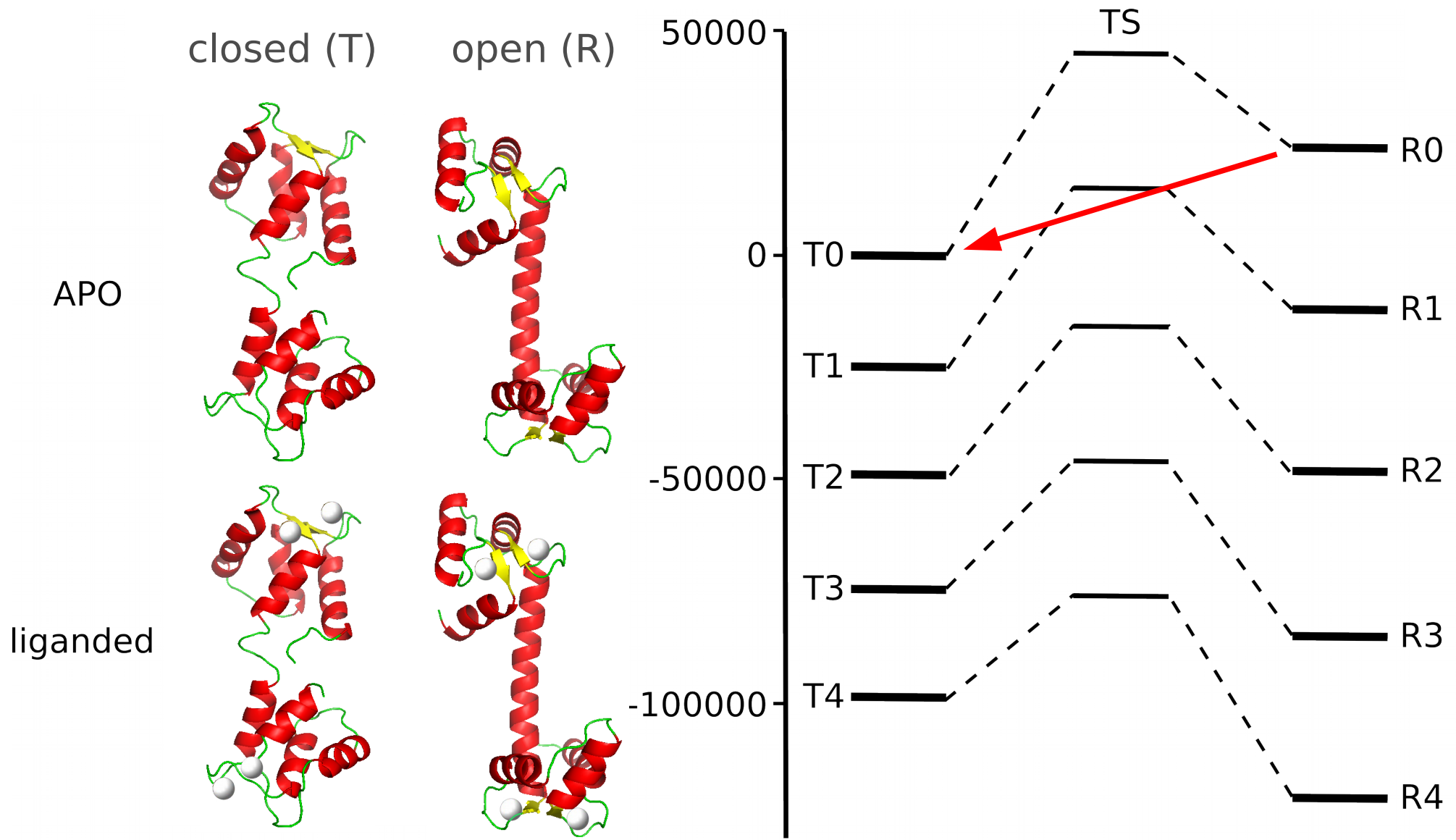


# Allosteric model of Calmodulin activation

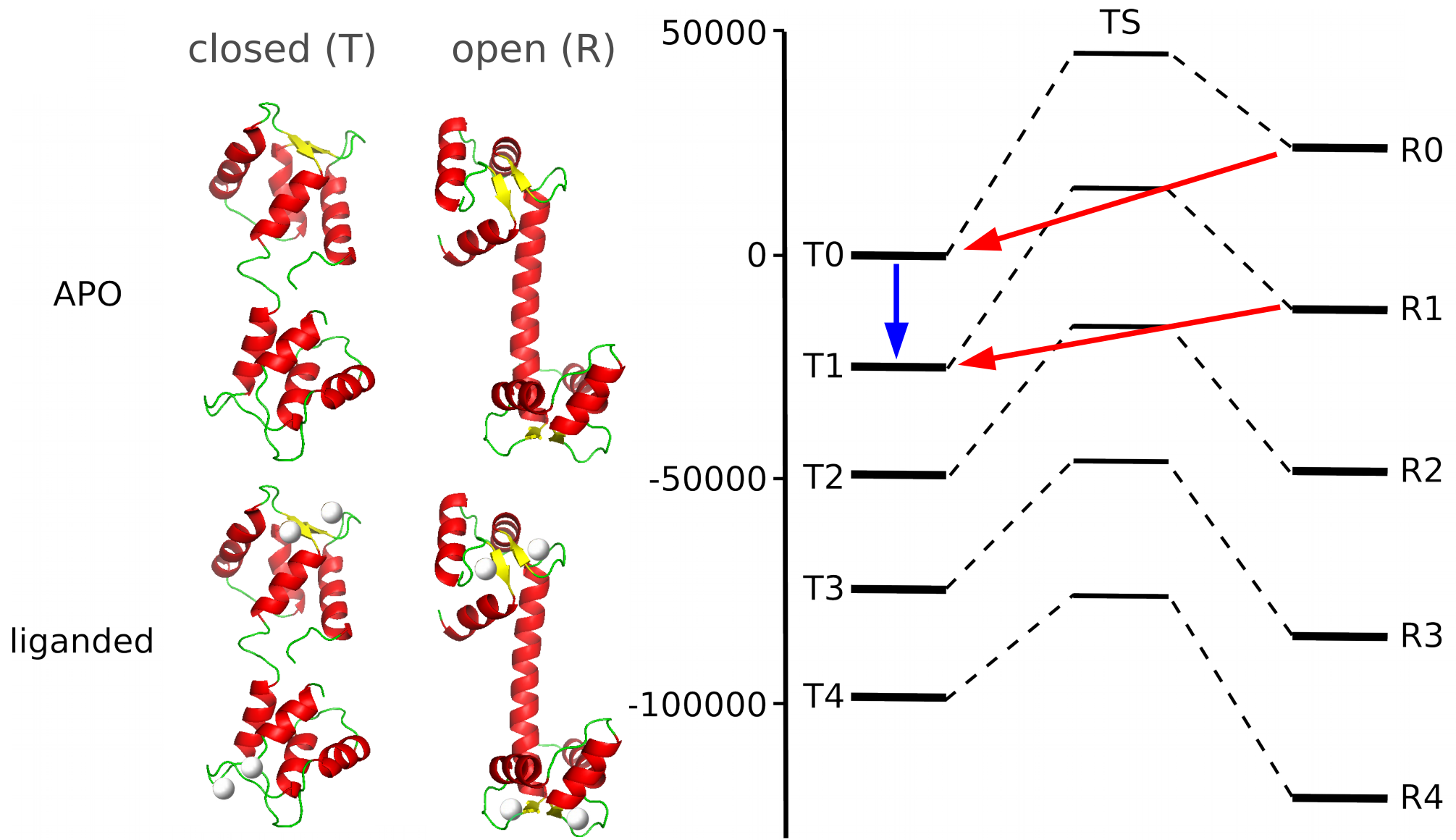


Stefan MI, Edelstein SJ, Le Novère N (2008, 2009)

# Allosteric model of Calmodulin activation

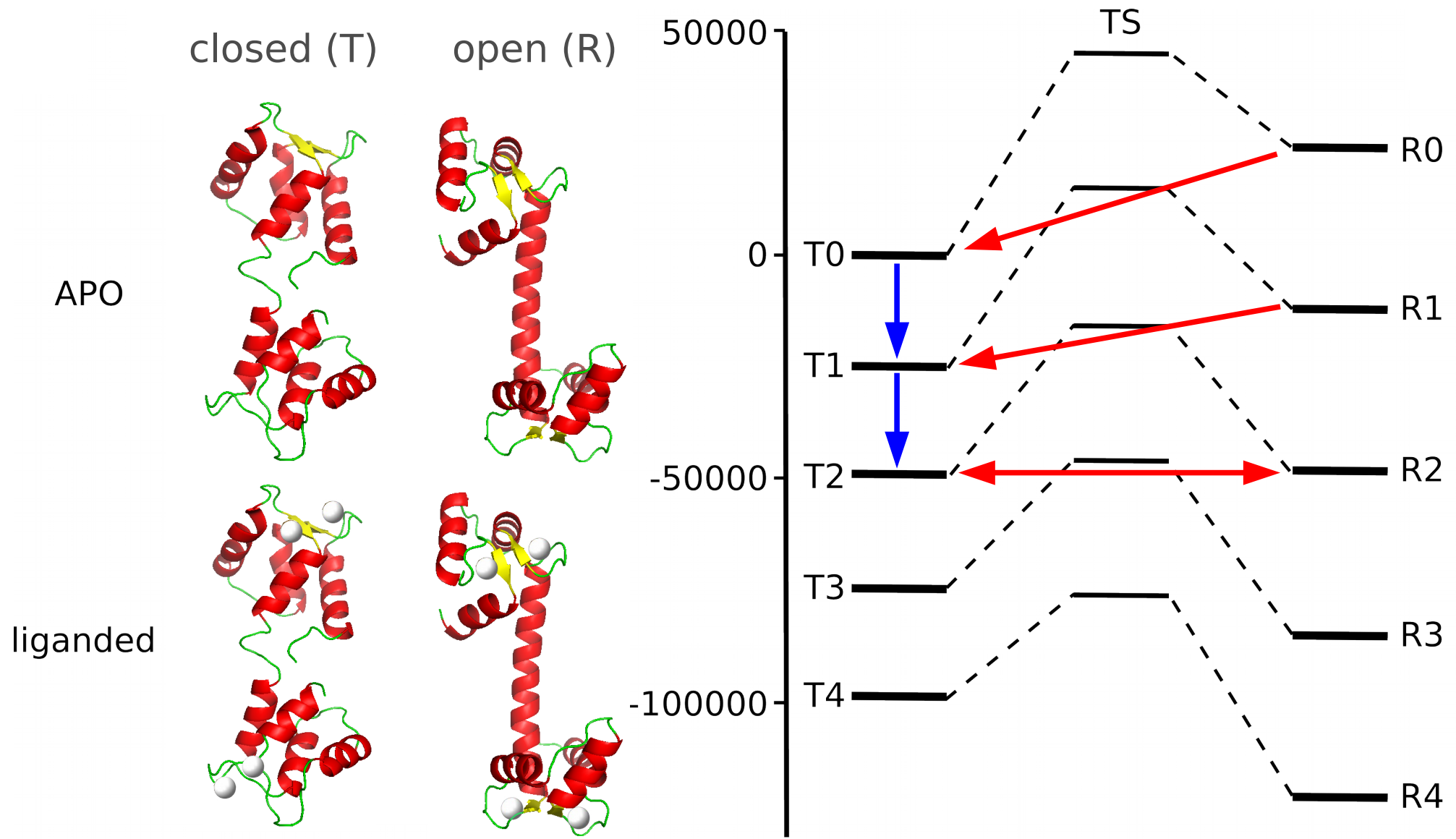


# Allosteric model of Calmodulin activation

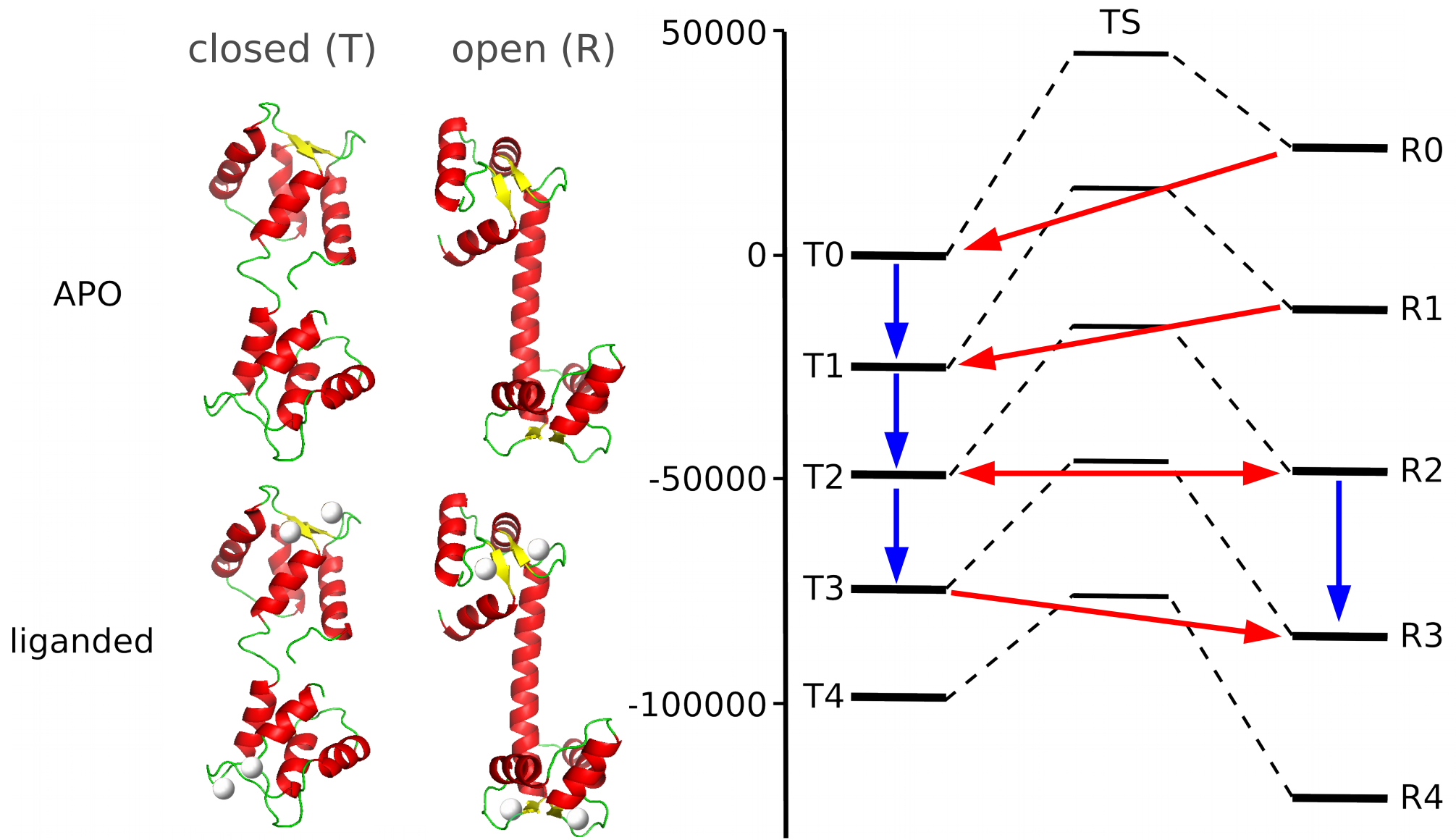




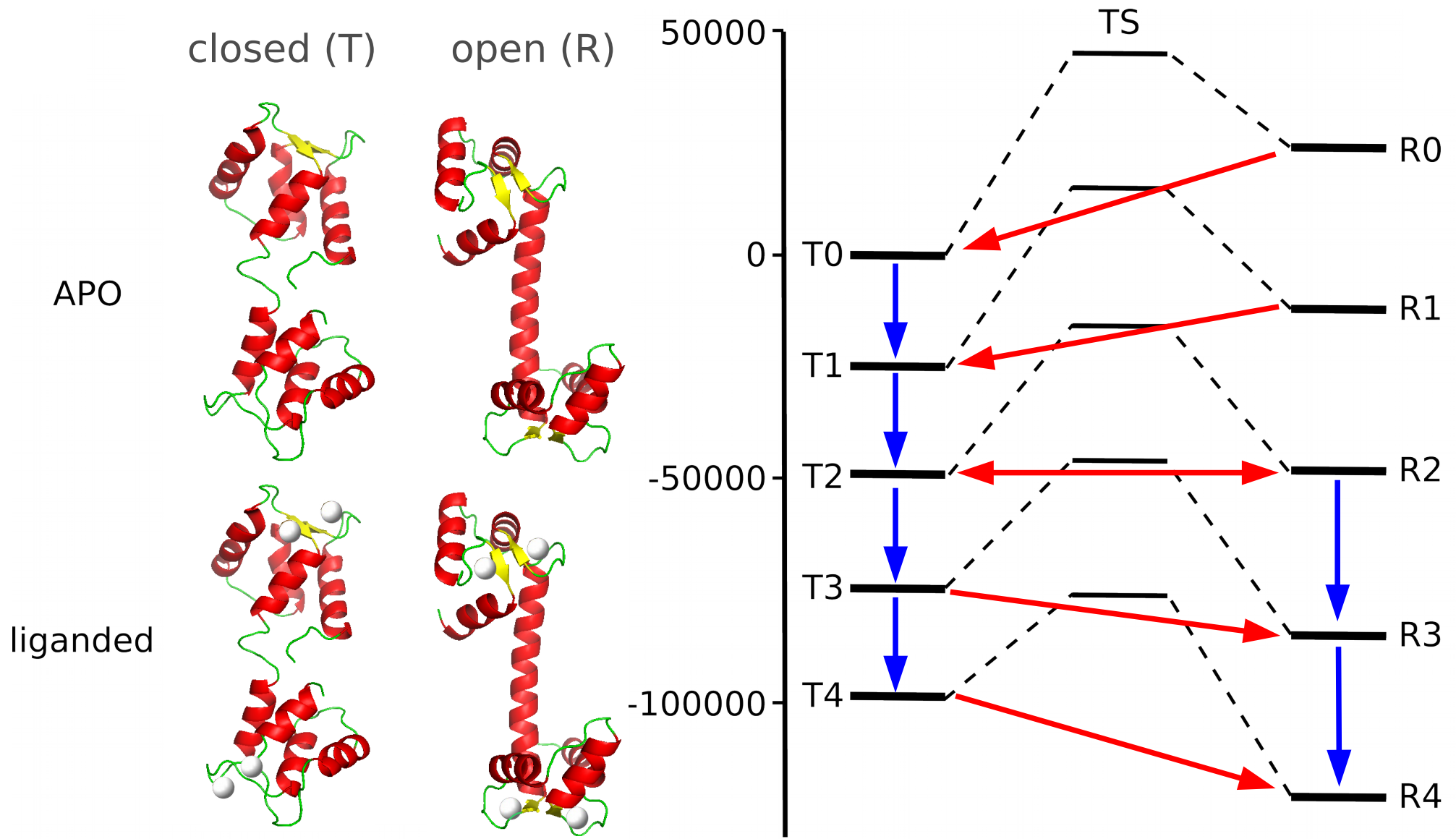
# Allosteric model of Calmodulin activation



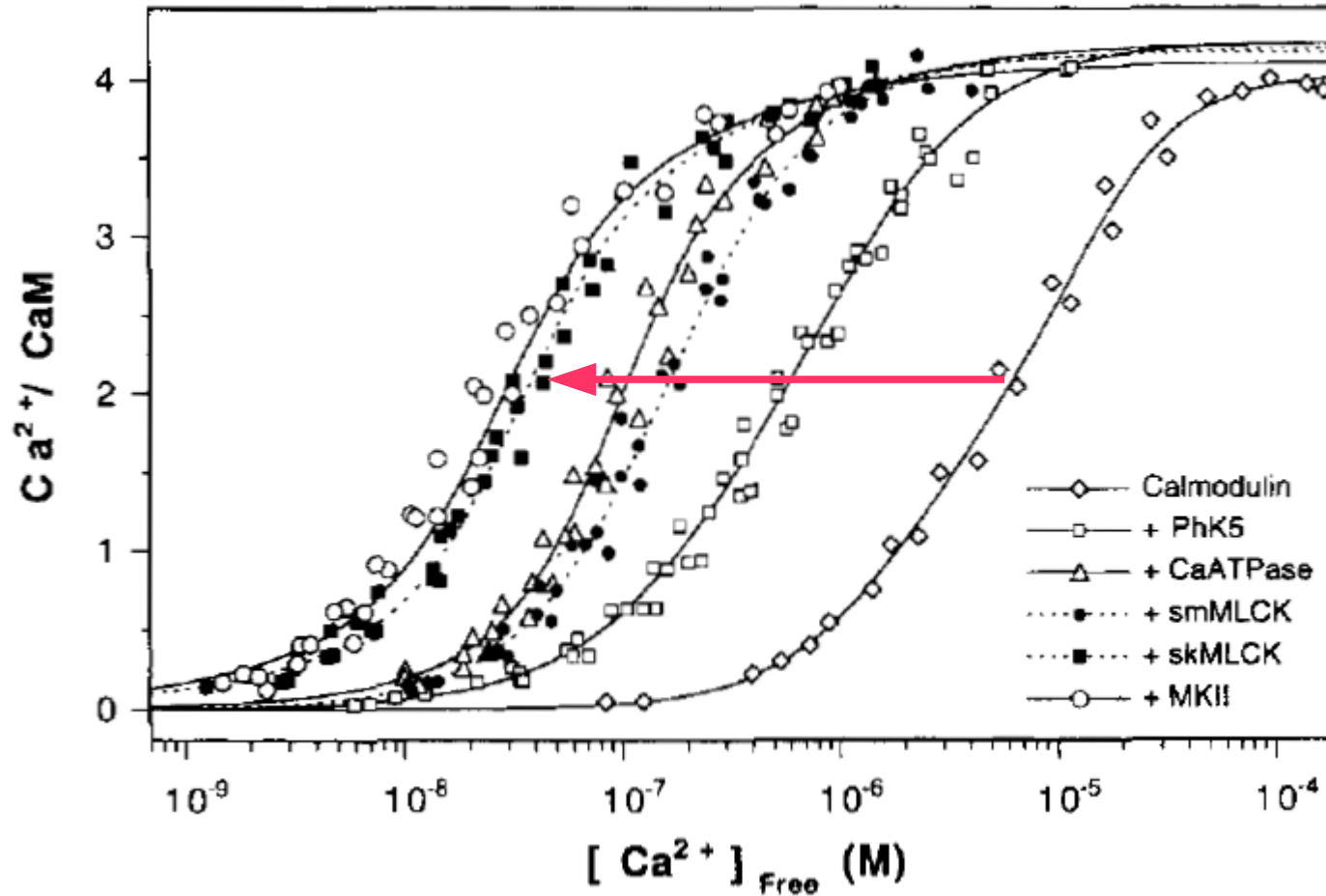
# Allosteric model of Calmodulin activation



# Allosteric model of Calmodulin activation

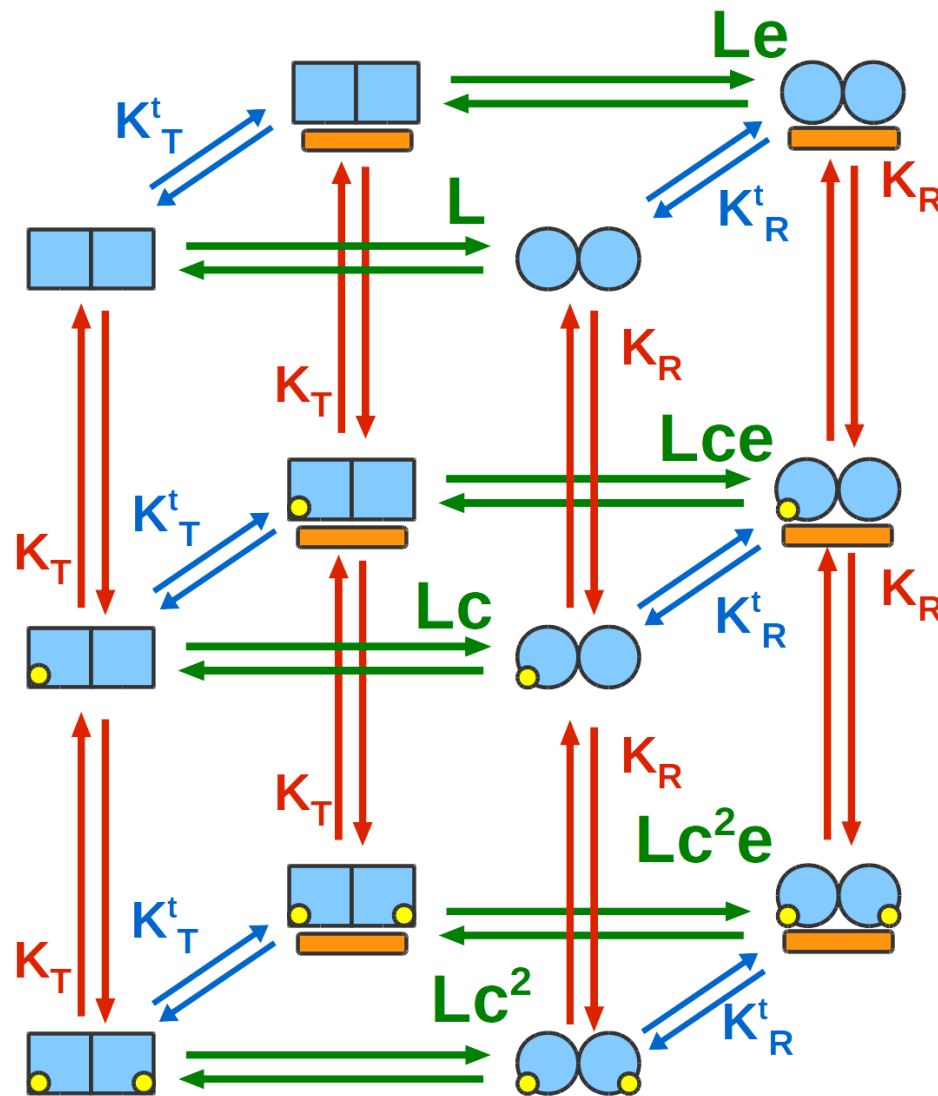


# Targets as allosteric effectors: the evidence

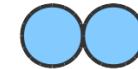


Peersen et al. (1997)

# Bindings of calcium and protein targets



T-state



R-state



Calcium



Target



Conf. transition



Calcium binding



Target binding

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

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



# Targets as allosteric effectors: the theory

$$\bar{Y}_{Ca^{2+}} = \frac{\alpha(1 + \alpha)^{n-1} + L'c\alpha(1 + c\alpha)^{n-1}}{(1 + \alpha)^n + L'(1 + c\alpha)^n}$$

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

$$L' = L \left( \frac{1 + \gamma e}{1 + \gamma} \right)^n$$

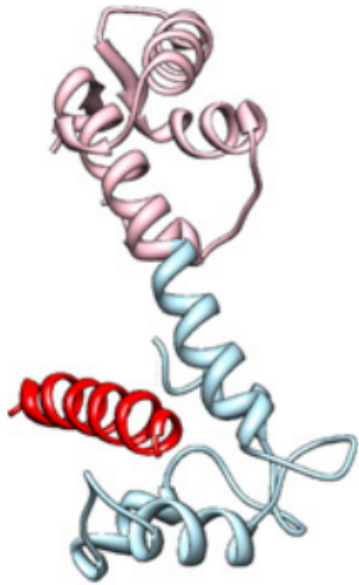
$$c = \frac{K^R}{K^T}$$

$$e = \frac{K_t^R g}{K_t^T g}$$

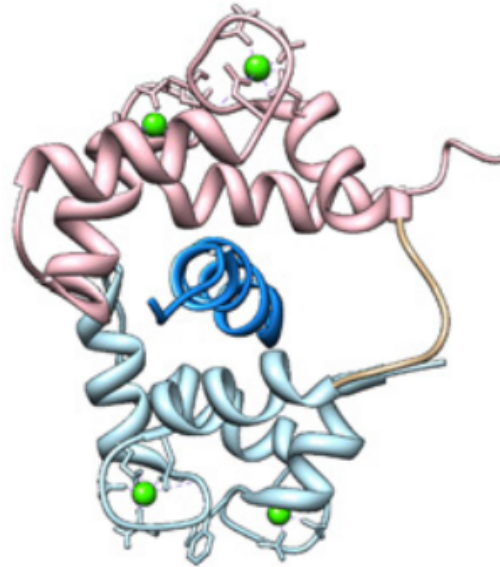
$$\alpha = \frac{[Ca^{2+}]}{[K^R]}$$

$$\gamma = \frac{[Target]}{[K_t^R g]}$$

# Different targets stabilise lobes in different states



**Neurogranin**



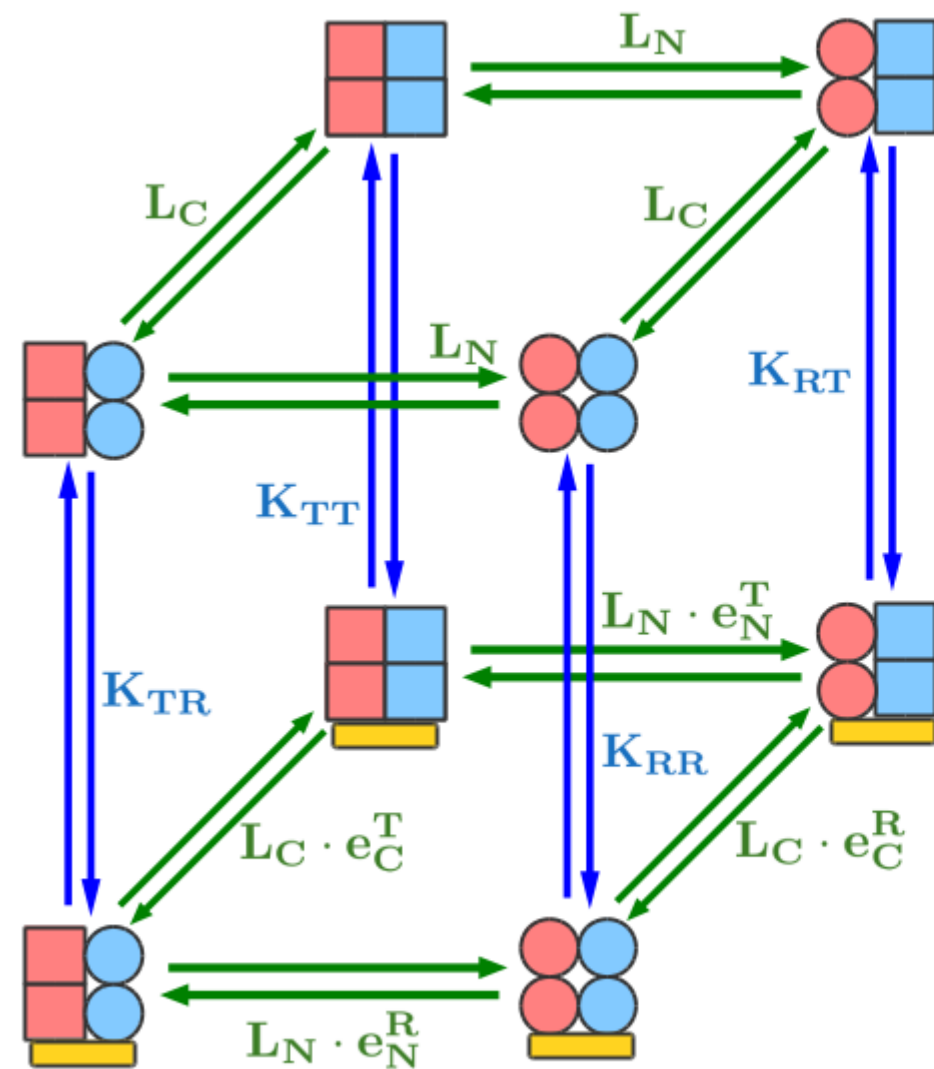
**MLCK**



**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

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

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

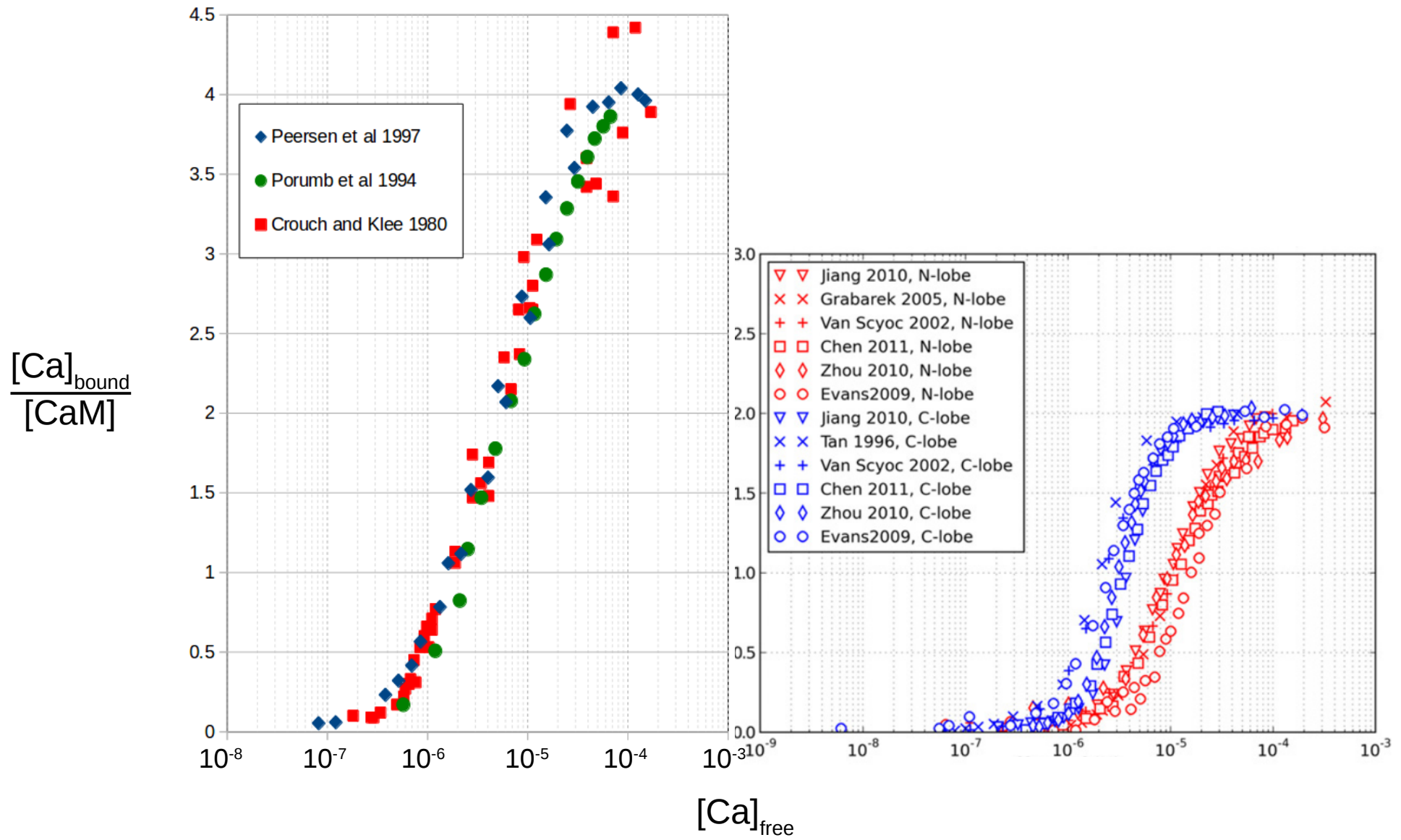
$$e_N^R = \frac{K_{RR}}{K_{TR}}$$

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

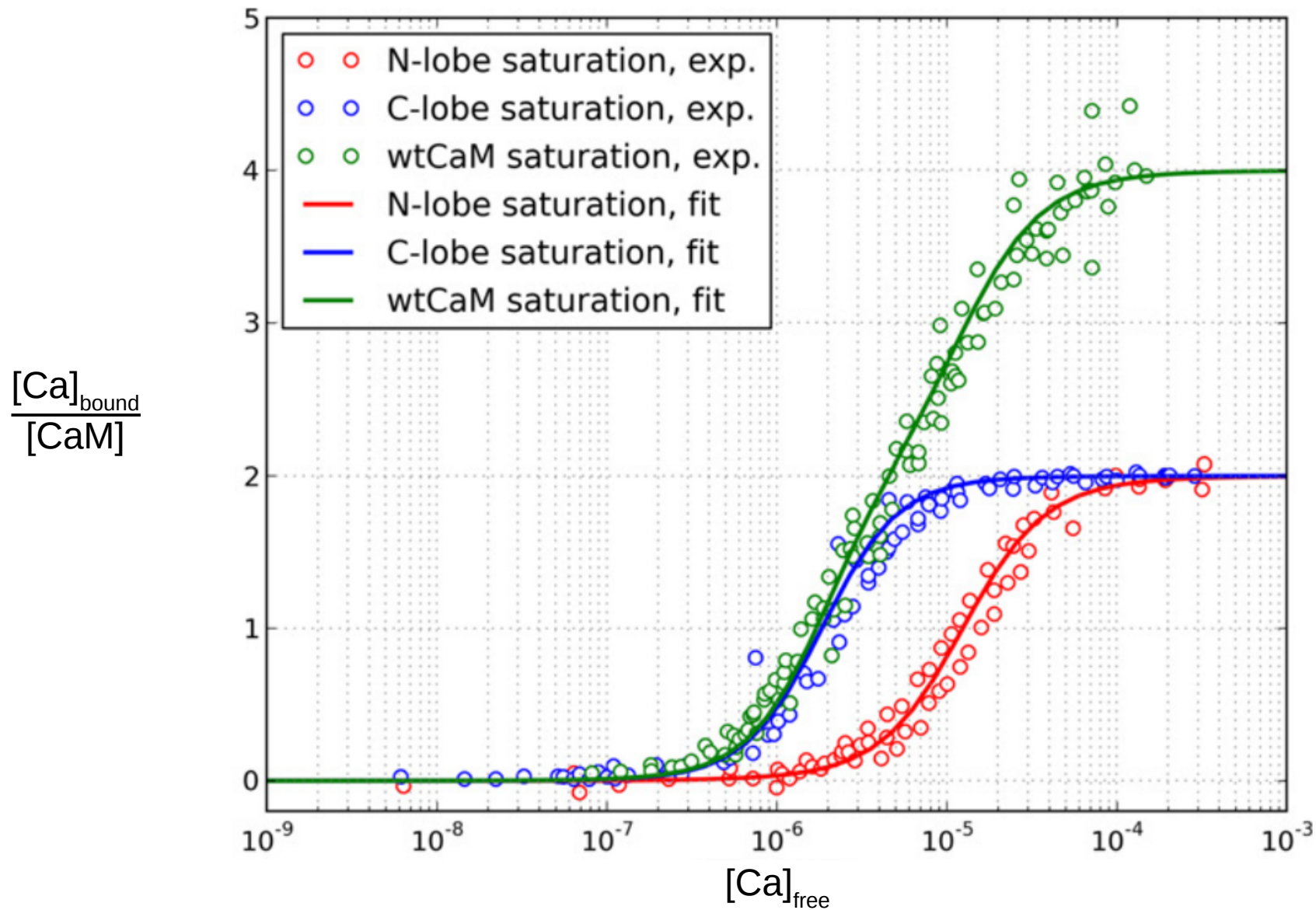
$$e_N^T = \frac{K_{RT}}{K_{TT}}$$

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

# 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

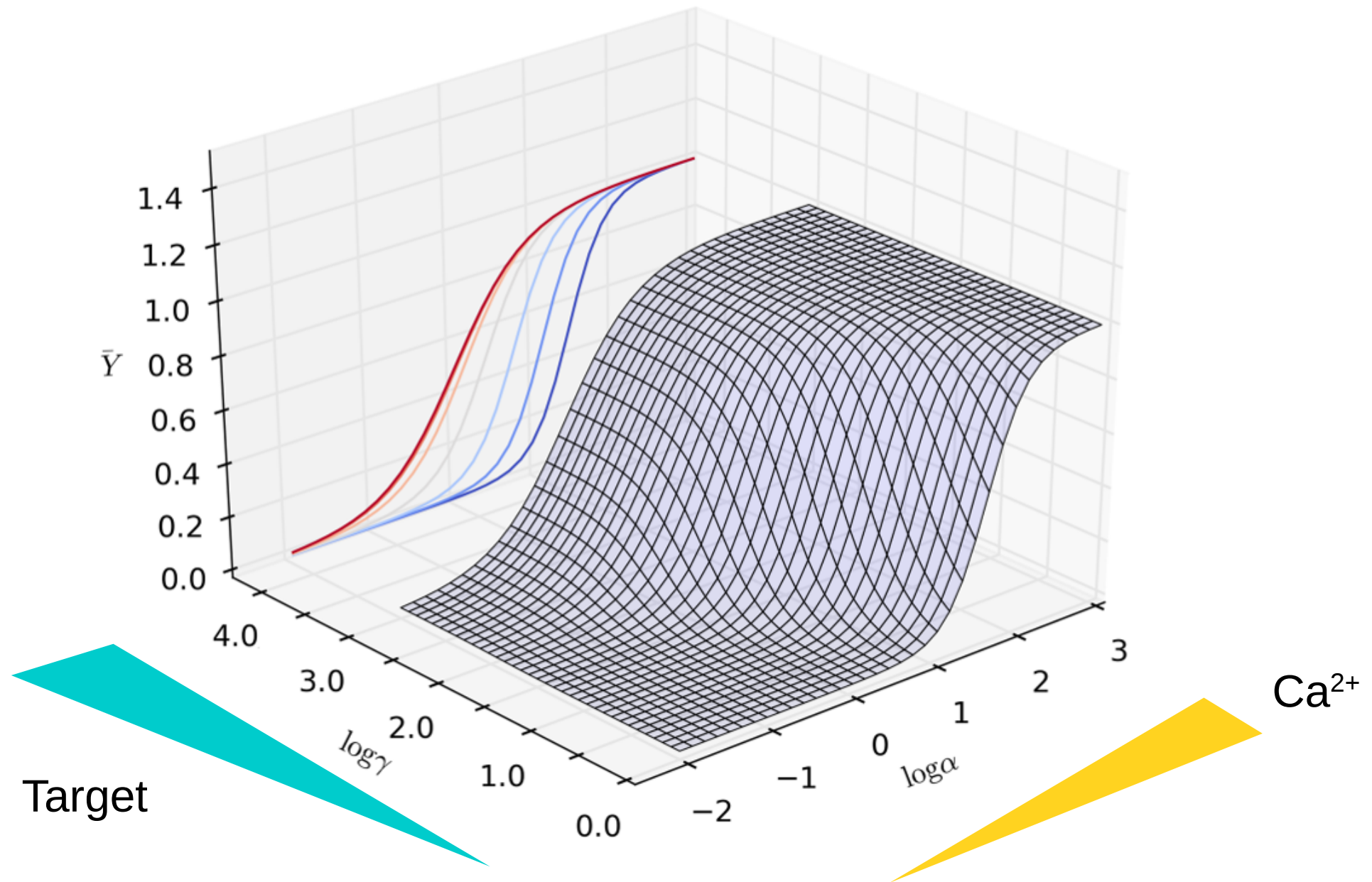
# Activity of unsaturated calmodulin (state function)

- 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
- $R_4/T_4 = 10000$

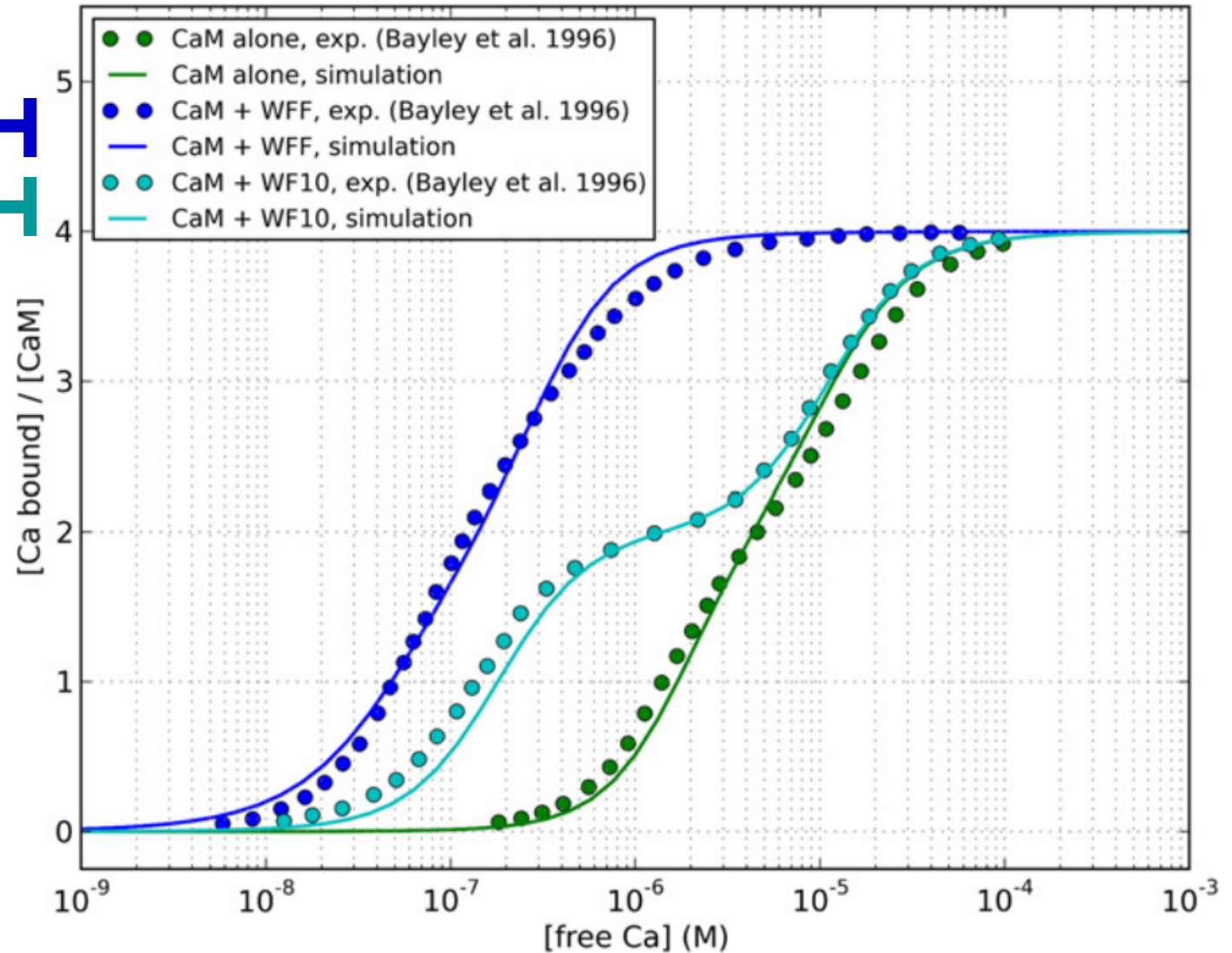
# Effect of an R-stabilizing target on $\text{Ca}^{2+}$ affinity



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

Stabilise R state  
of both lobes

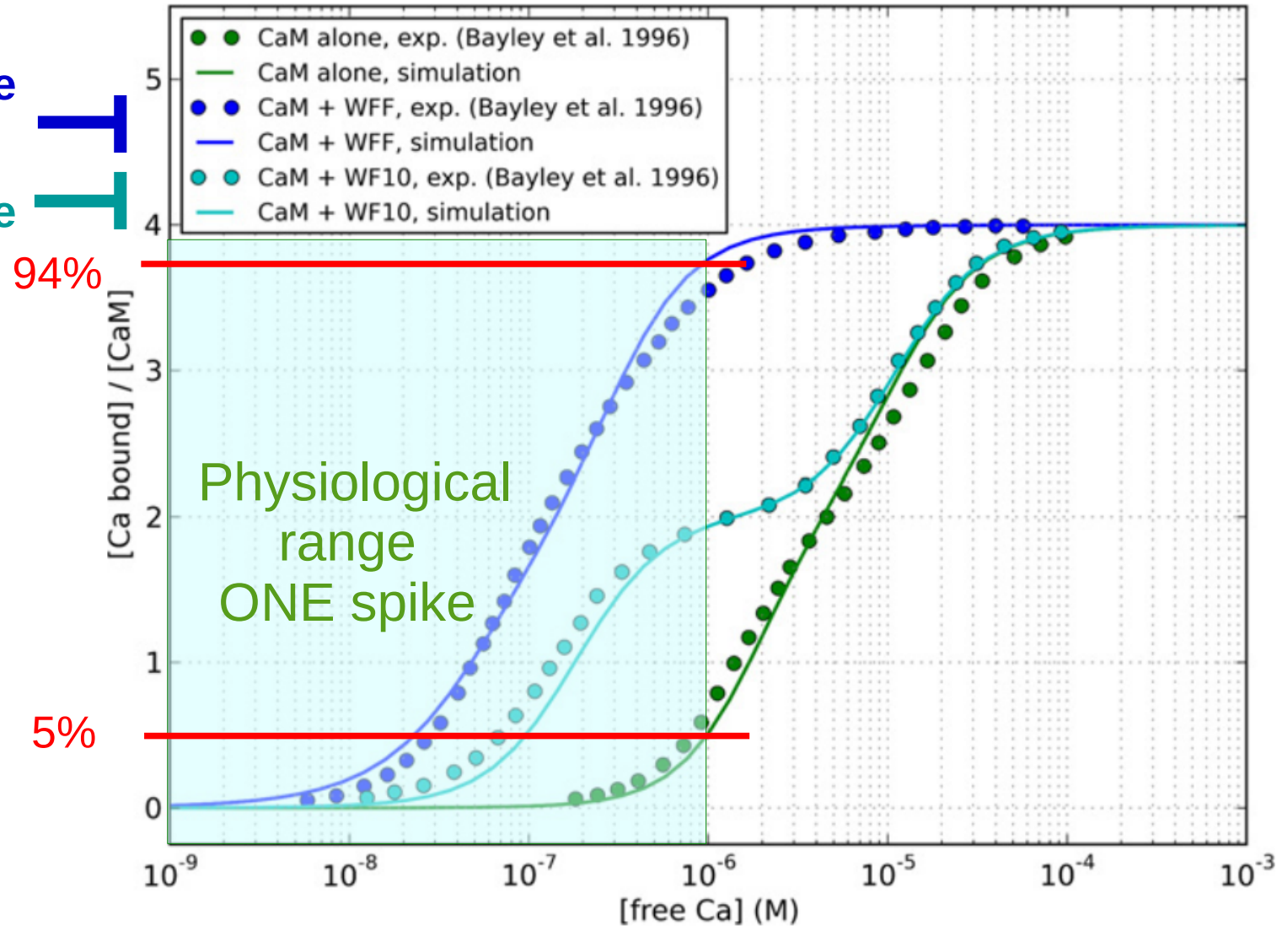
Stabilise R state  
of C lobe



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

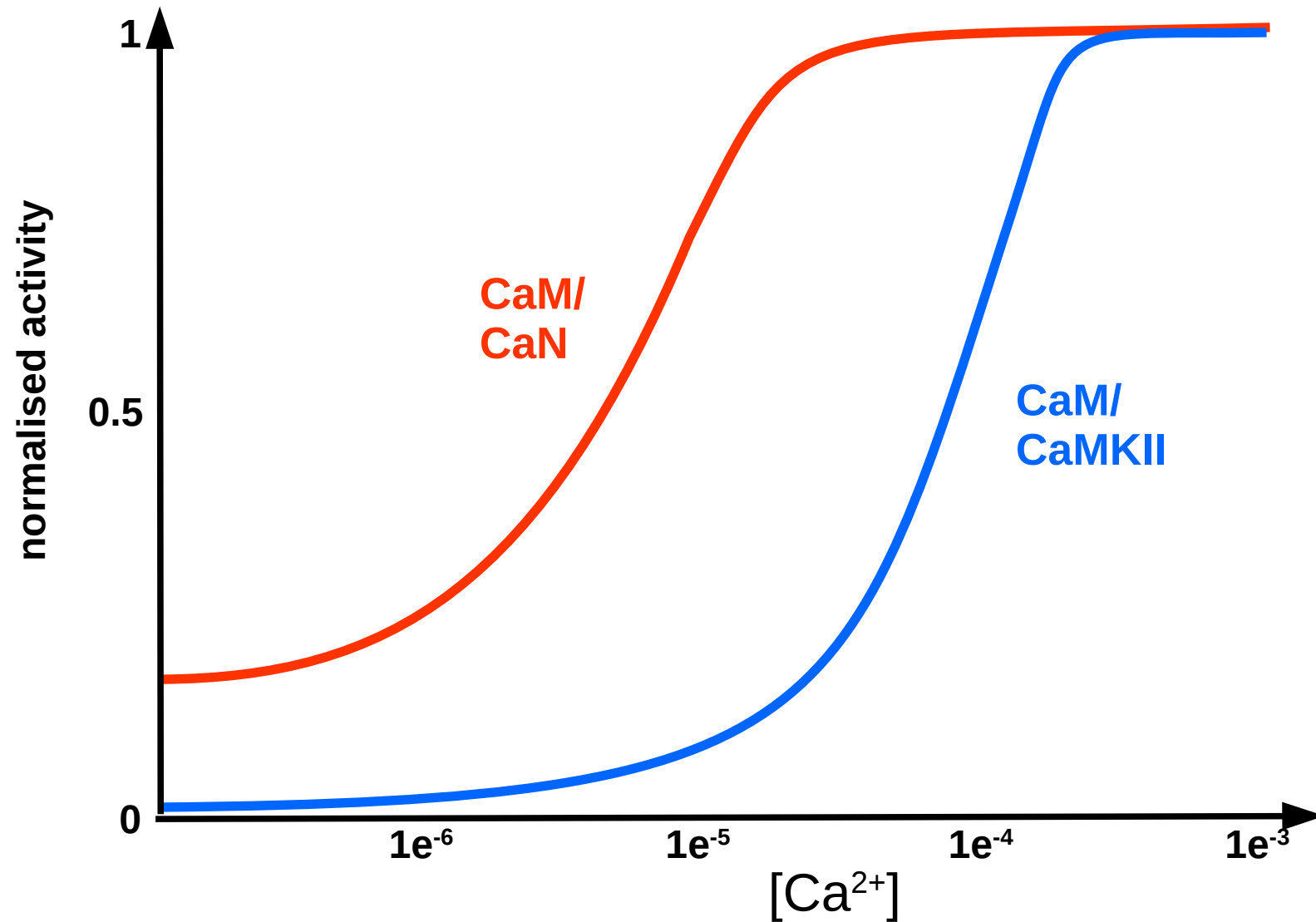
Stabilise R state of both lobes

Stabilise R state of C lobe

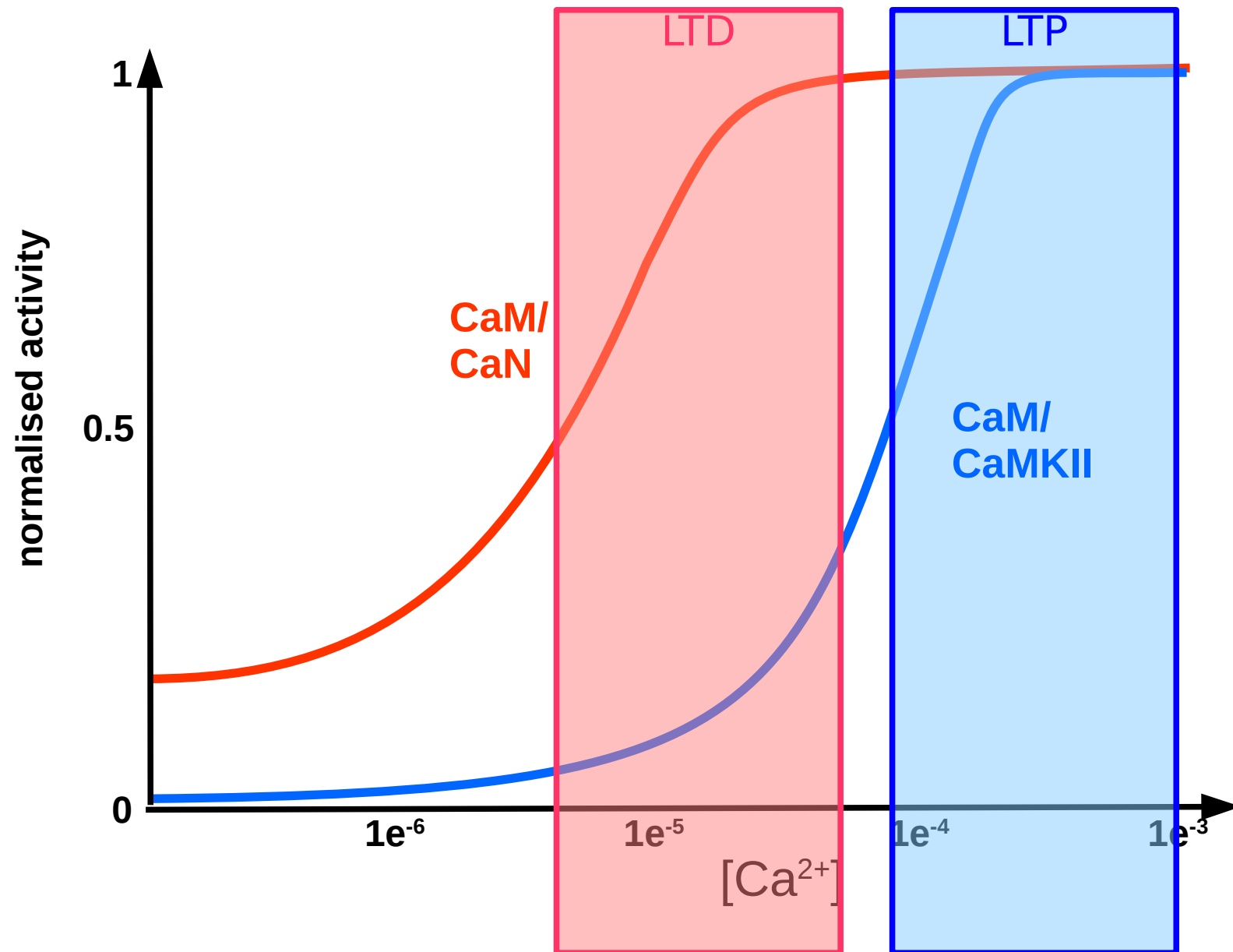




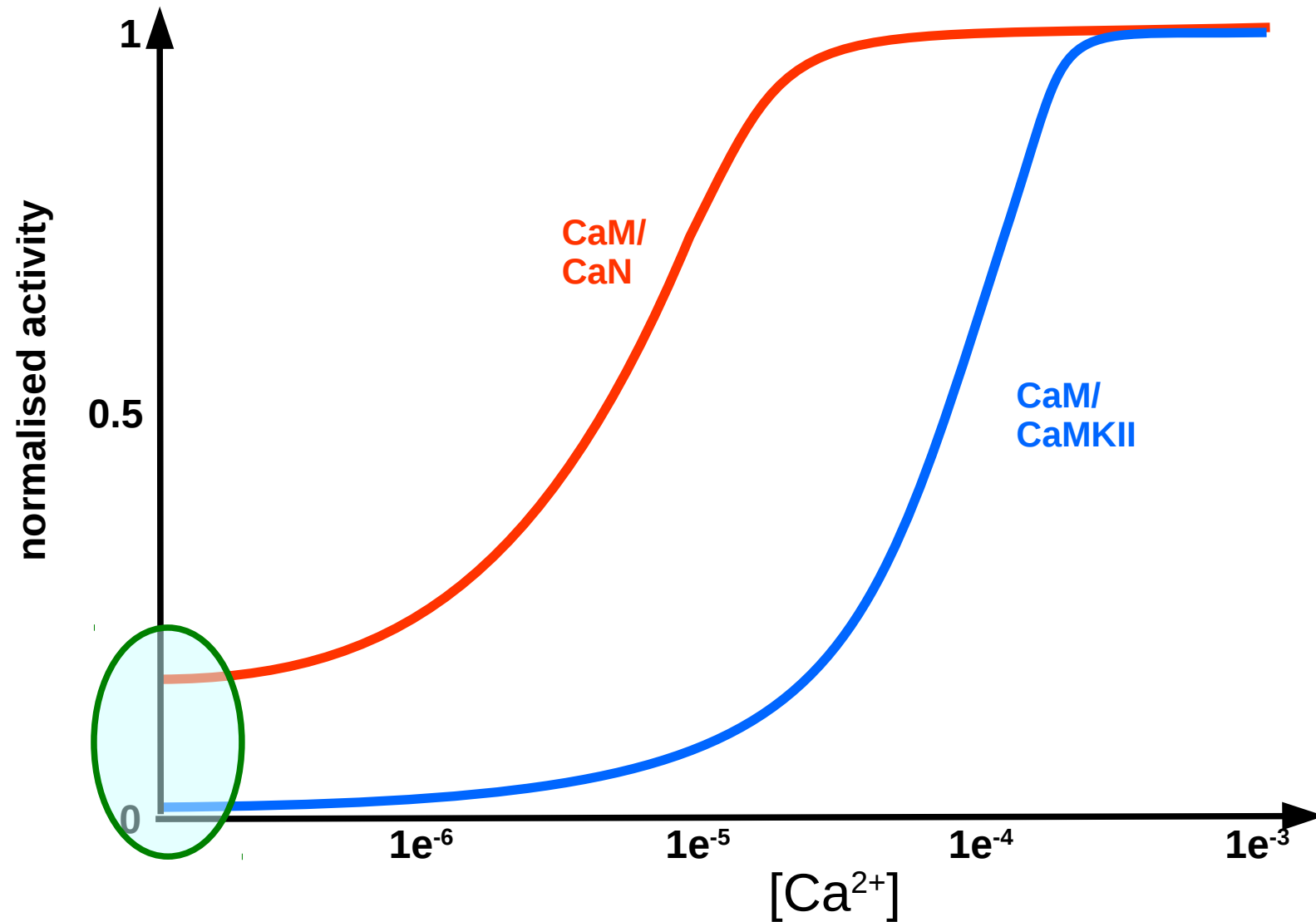
# Calmodulin, its ligand and its targets



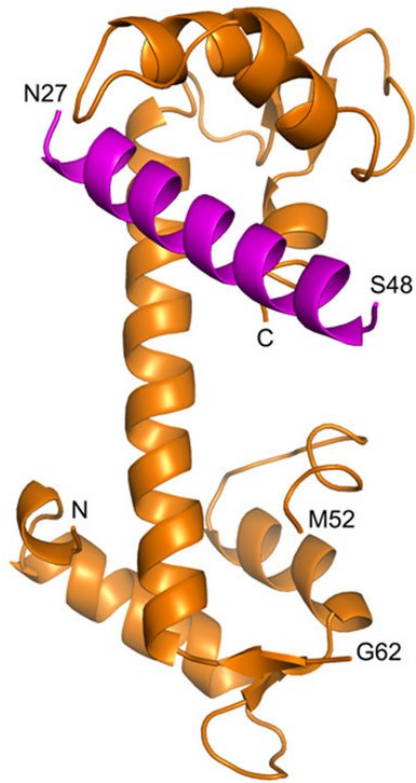
# Bidirectional synaptic plasticity



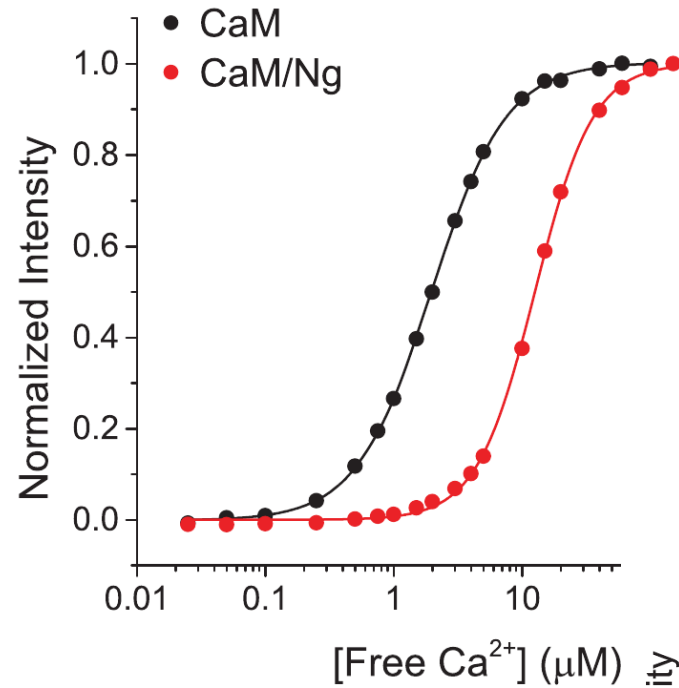
# Calcineurin stabilises CaM R → no deactivation



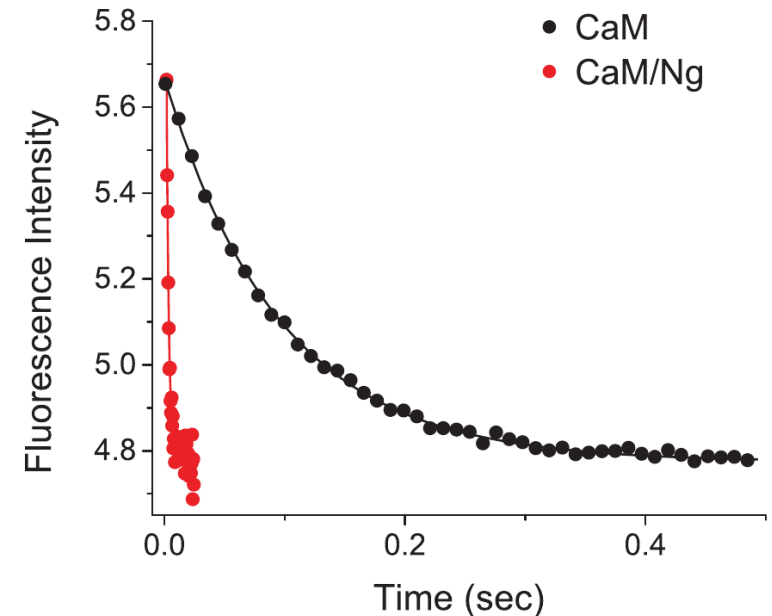
# Neurogranin binds to apo-CaM, decrease affinity for $[Ca^{2+}]$ and increase dissociation rate



Kumar *et al* (2013)



Hoffman *et al* (2014)



# Effect of R and T stabilising targets on CaM affinity

Bayley *et al* (1996)

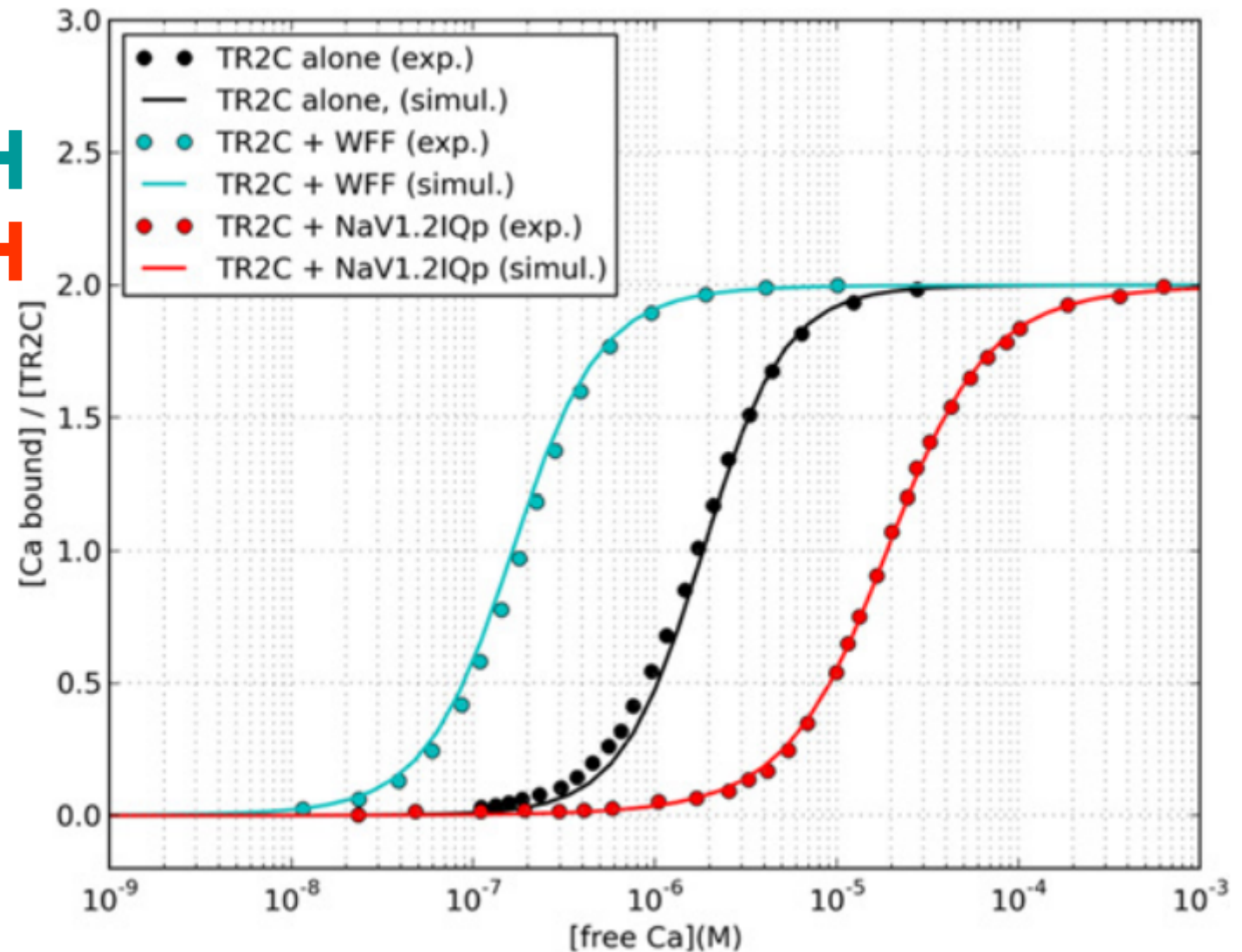
R stabilising  
peptide



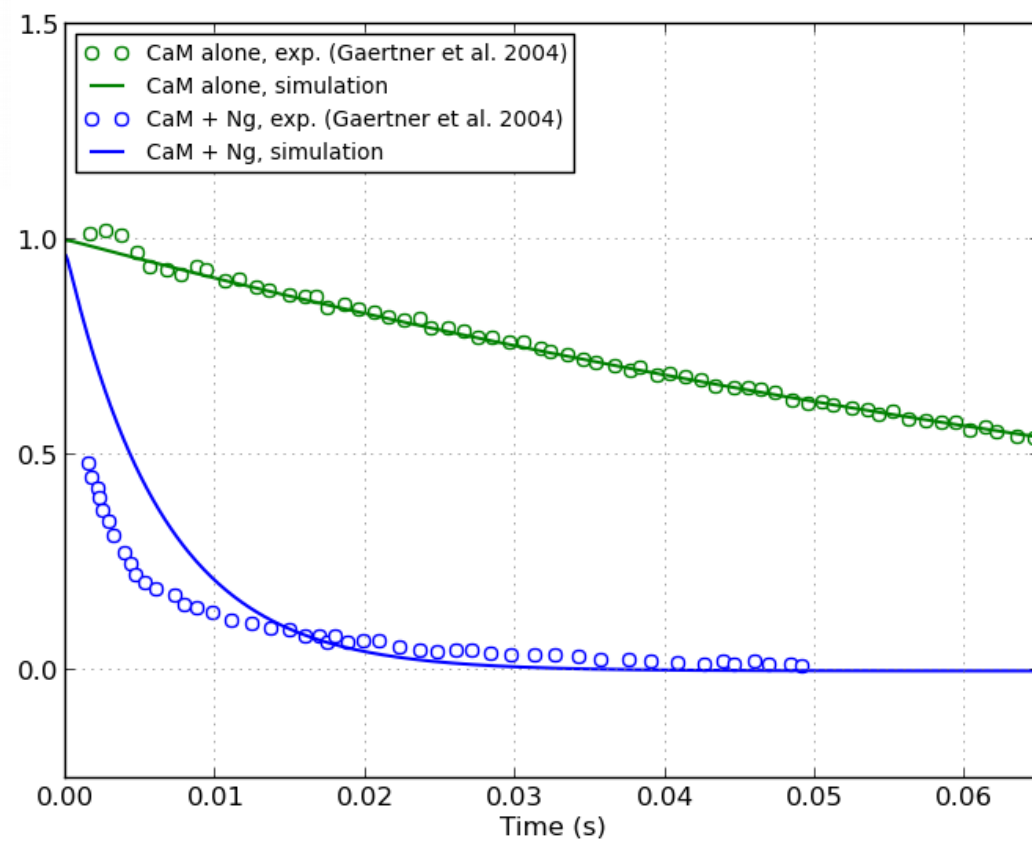
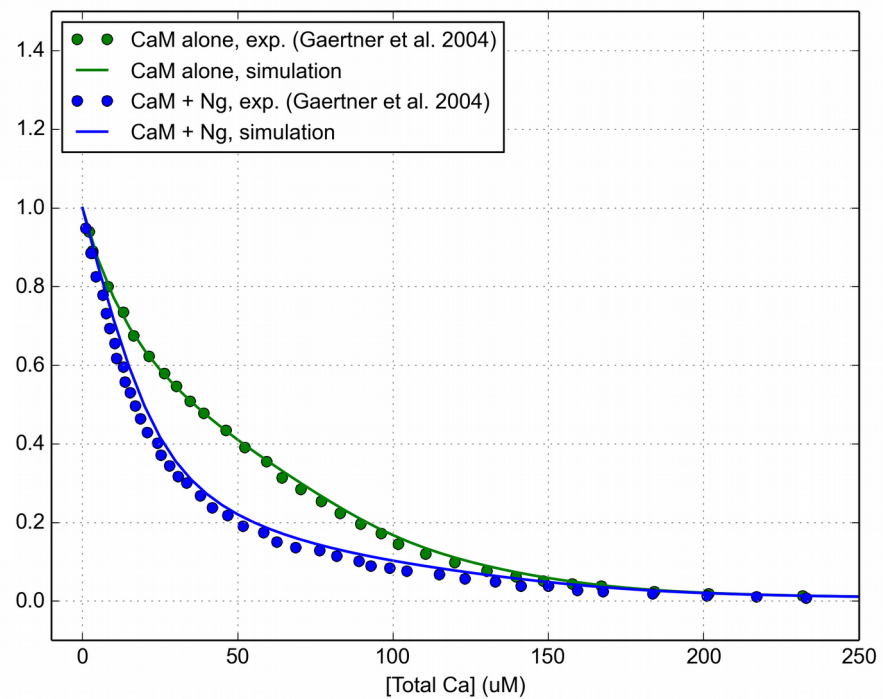
T stabilising  
Peptide (IQ domain)



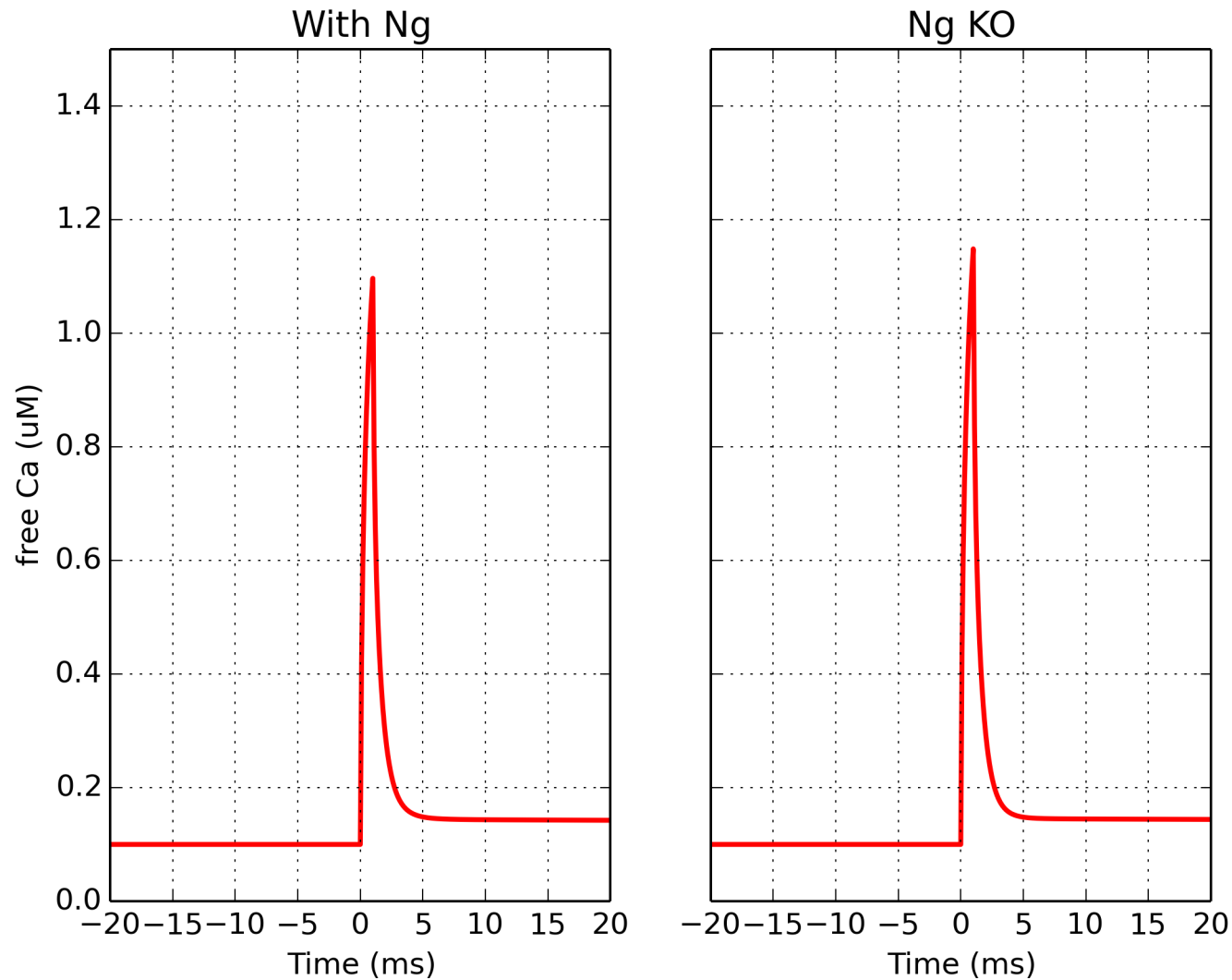
Theoharis *et al* (2008)



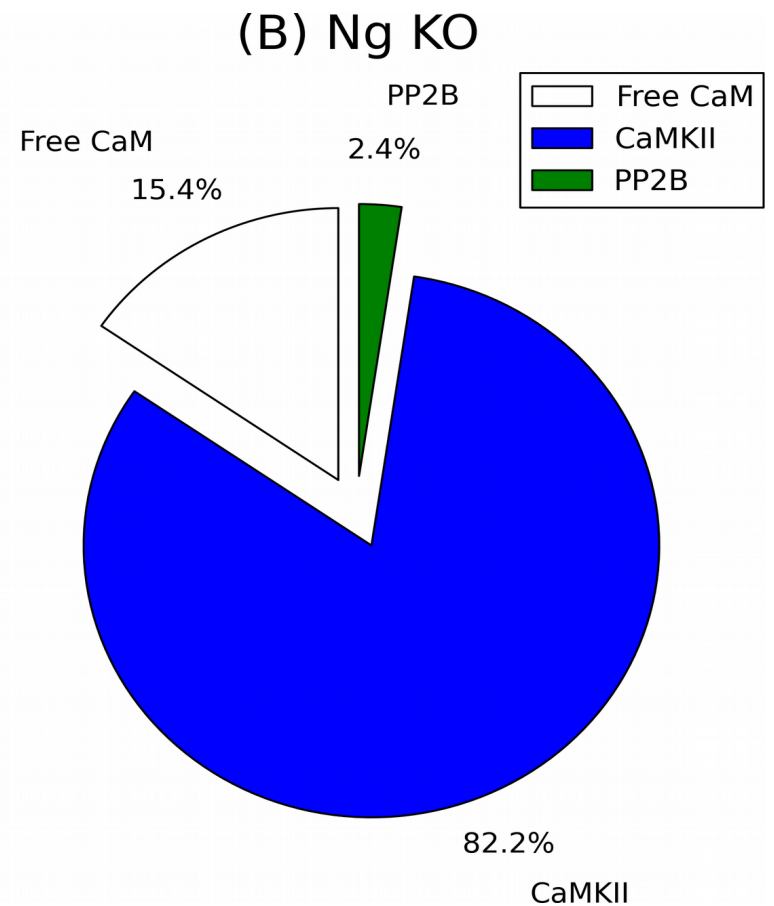
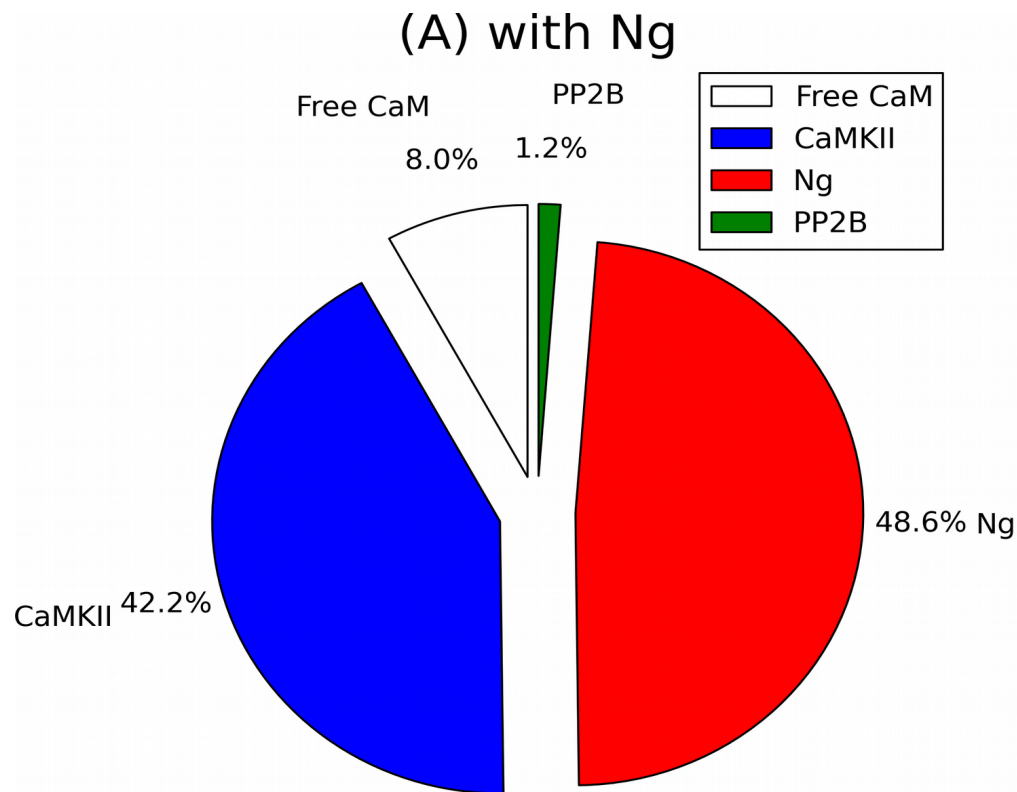


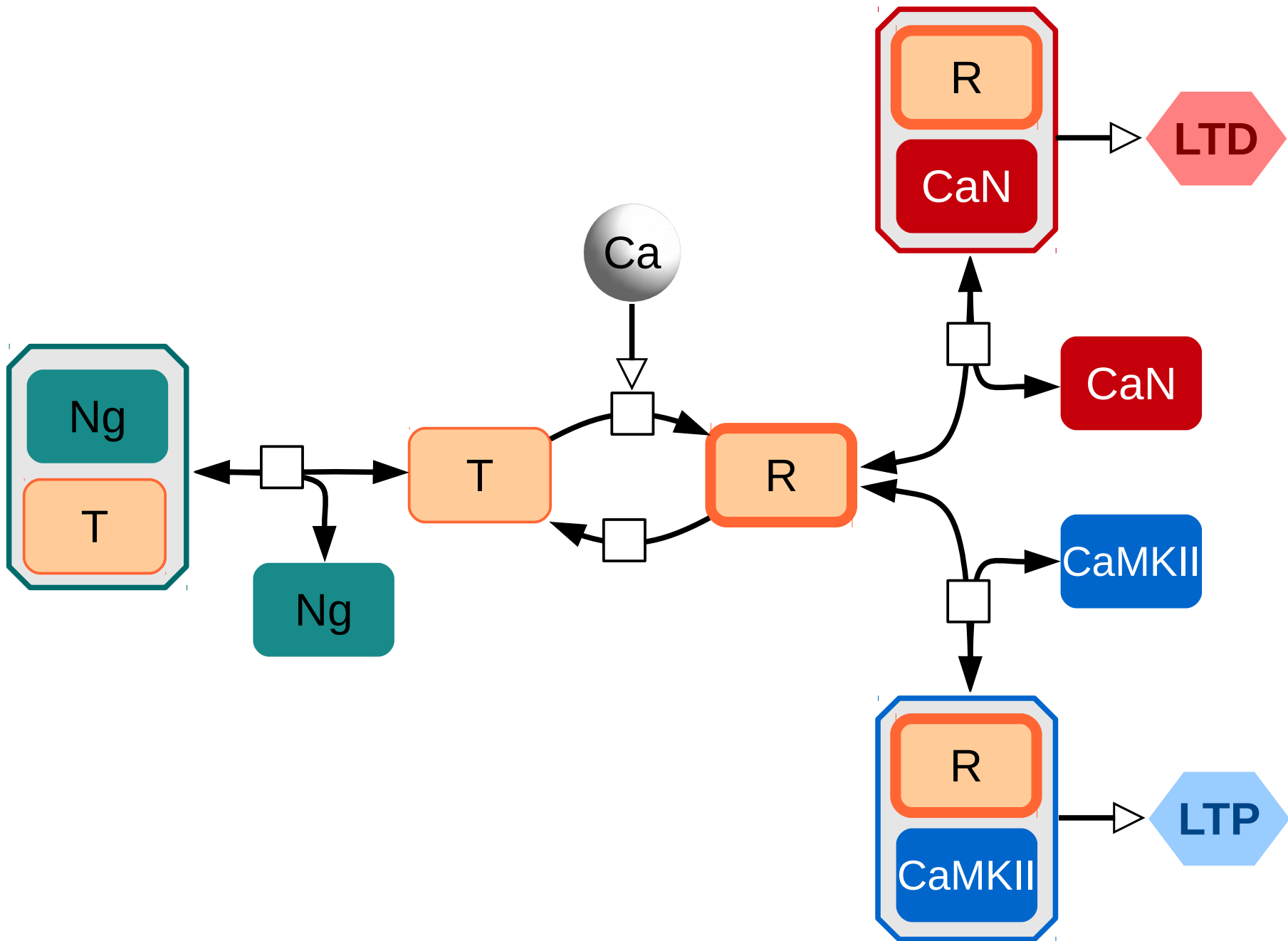


# No large effect of Ng on $[Ca^{2+}]_{free}$



# Ng affects the distribution of CaM





# 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 |

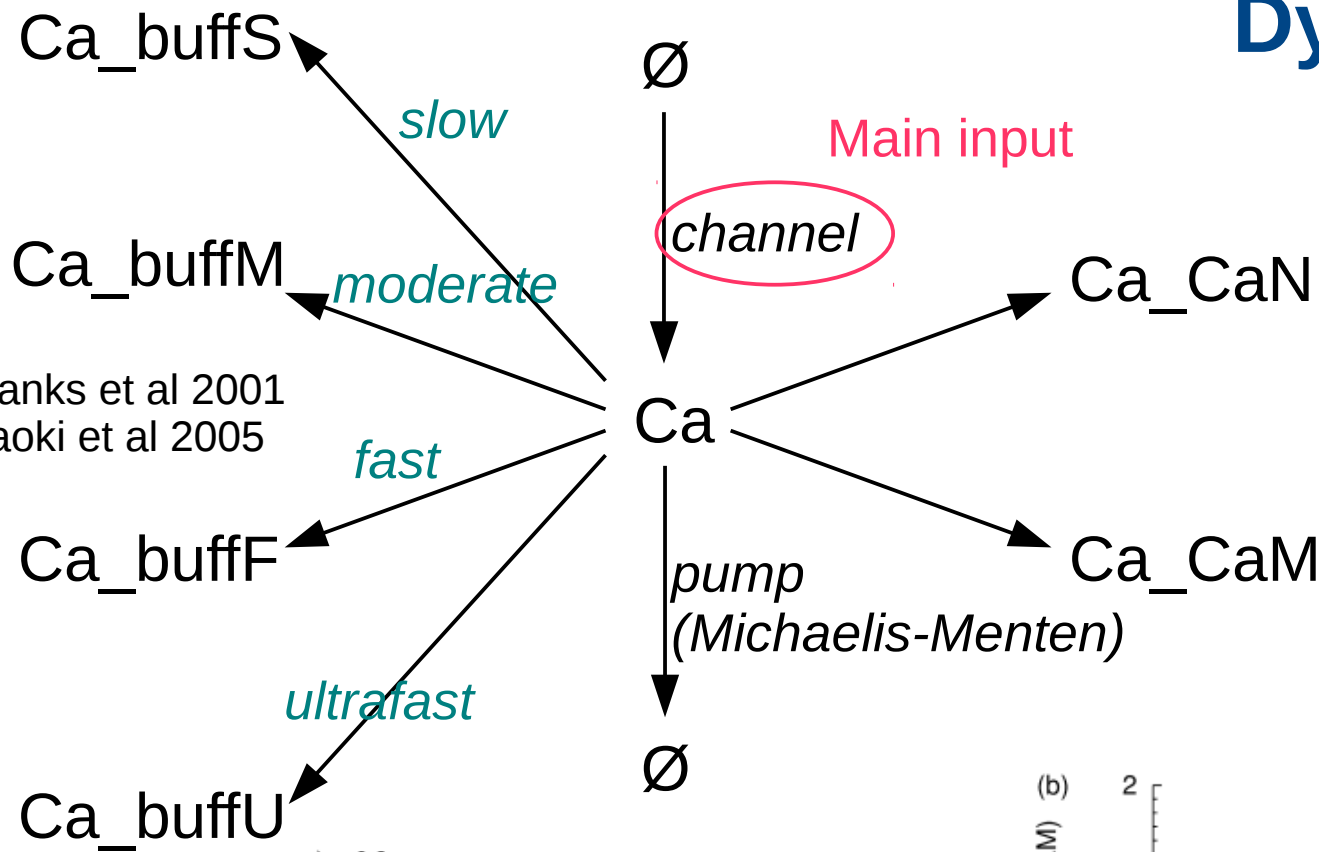


≠



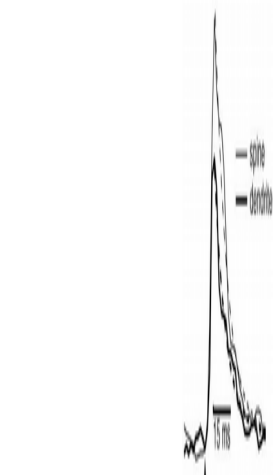


# Dynamic of calcium in the spine

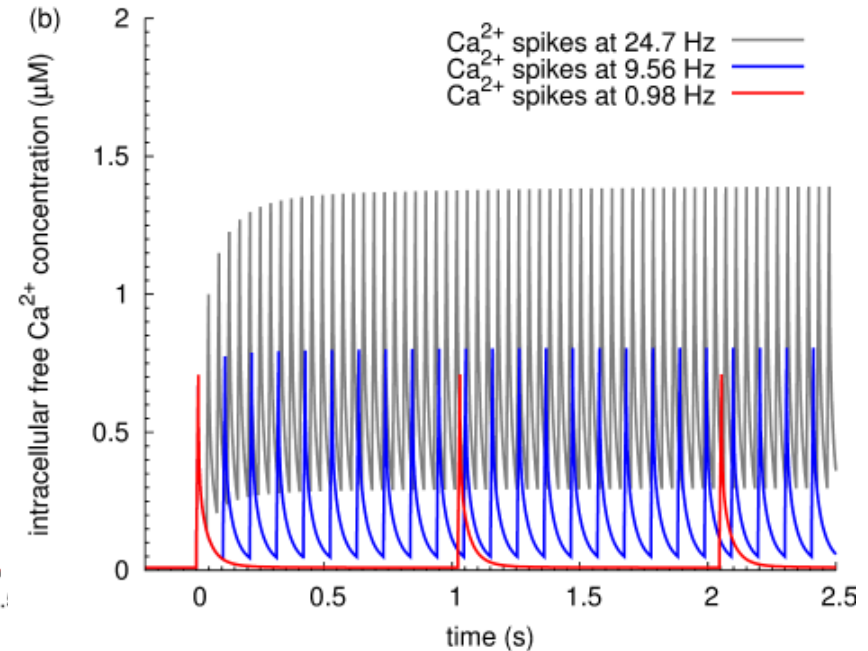
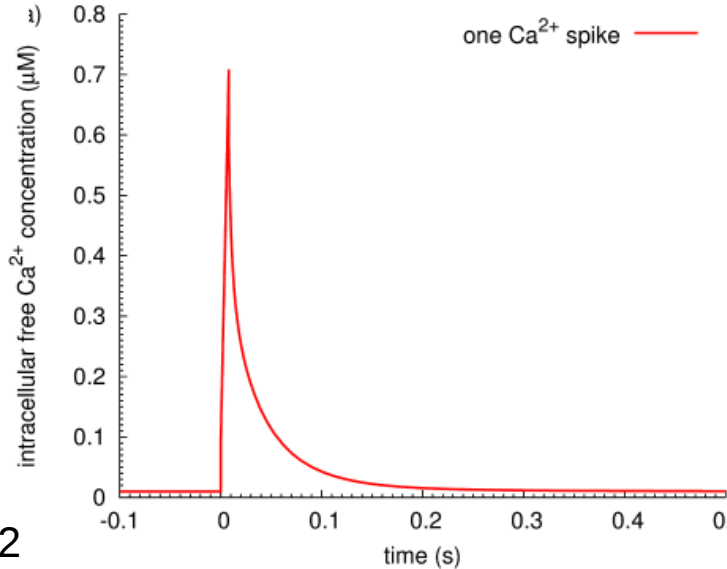


Li L, Stefan MI, Le Novère N  
(2012) *PLoS ONE*, 7(9): e43810

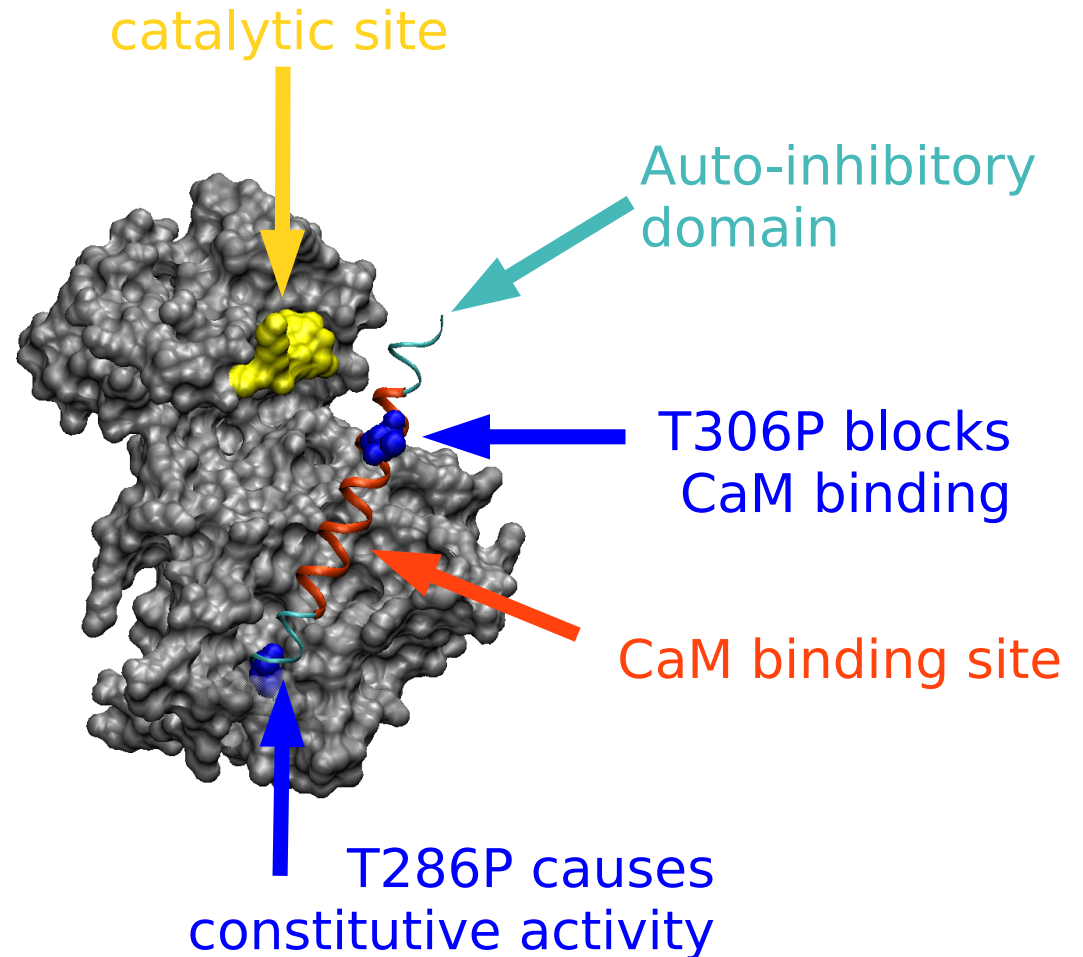
Franks et al 2001  
Naoki et al 2005



Sabatini et al 2002



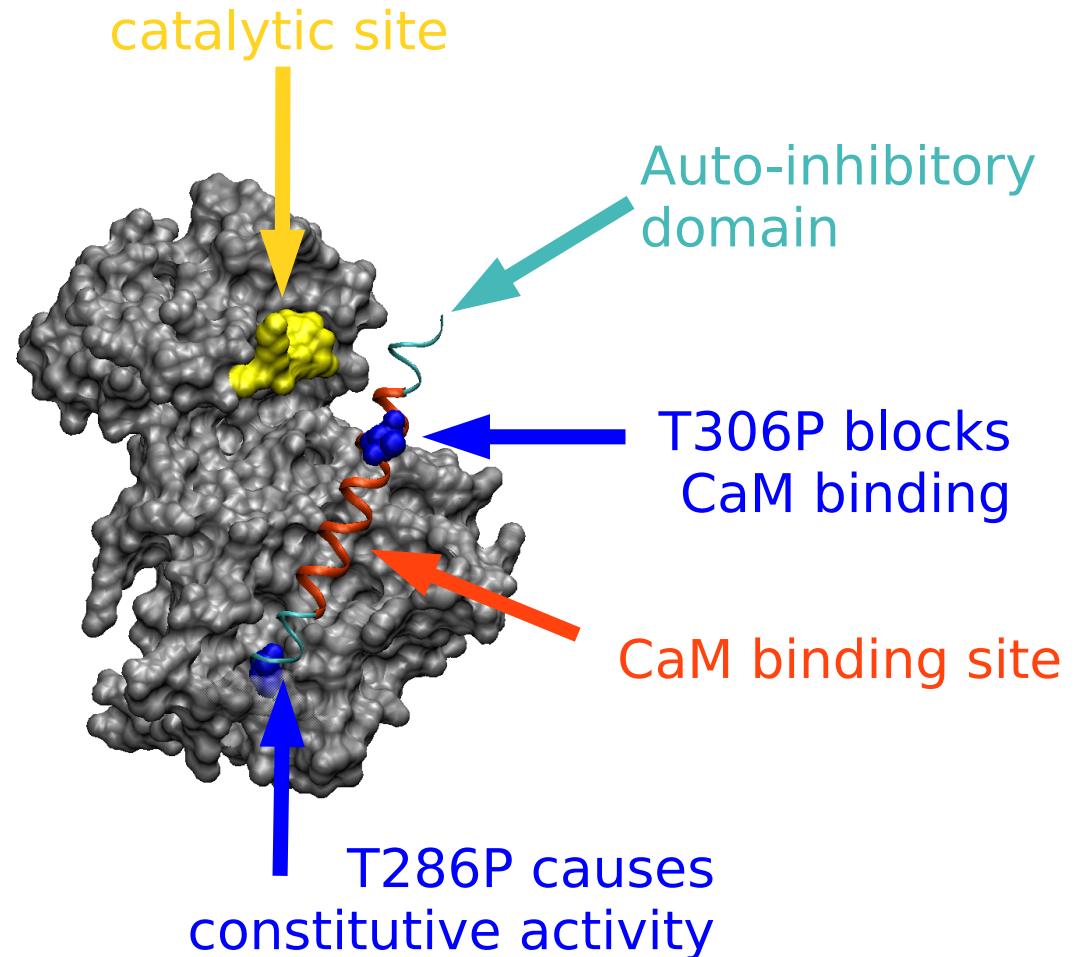
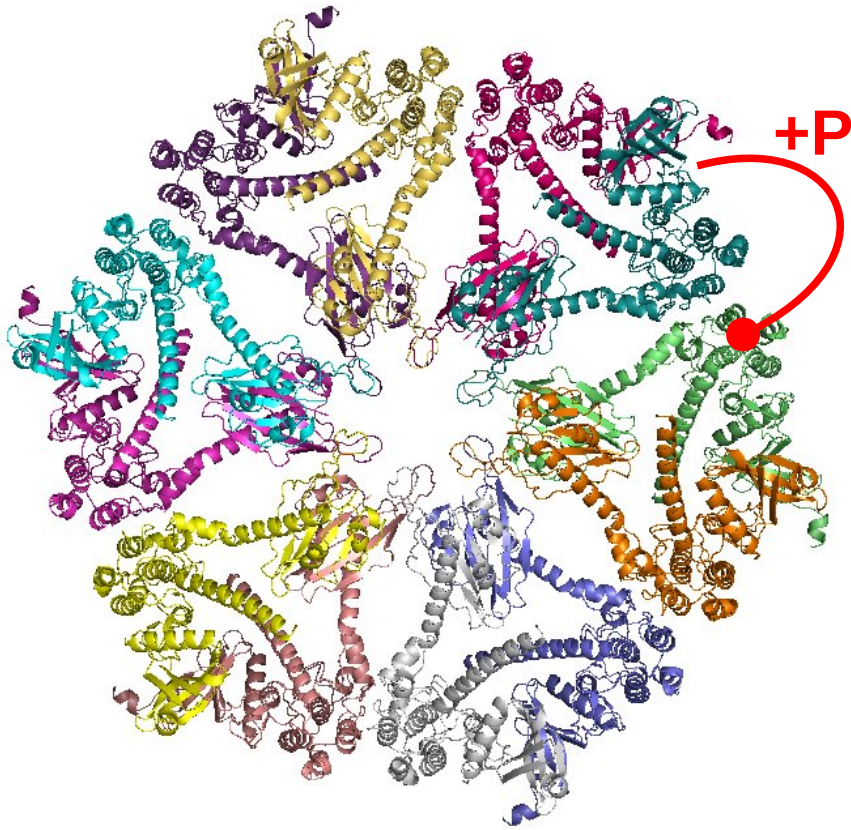
# Calcium/calmodulin kinase II



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

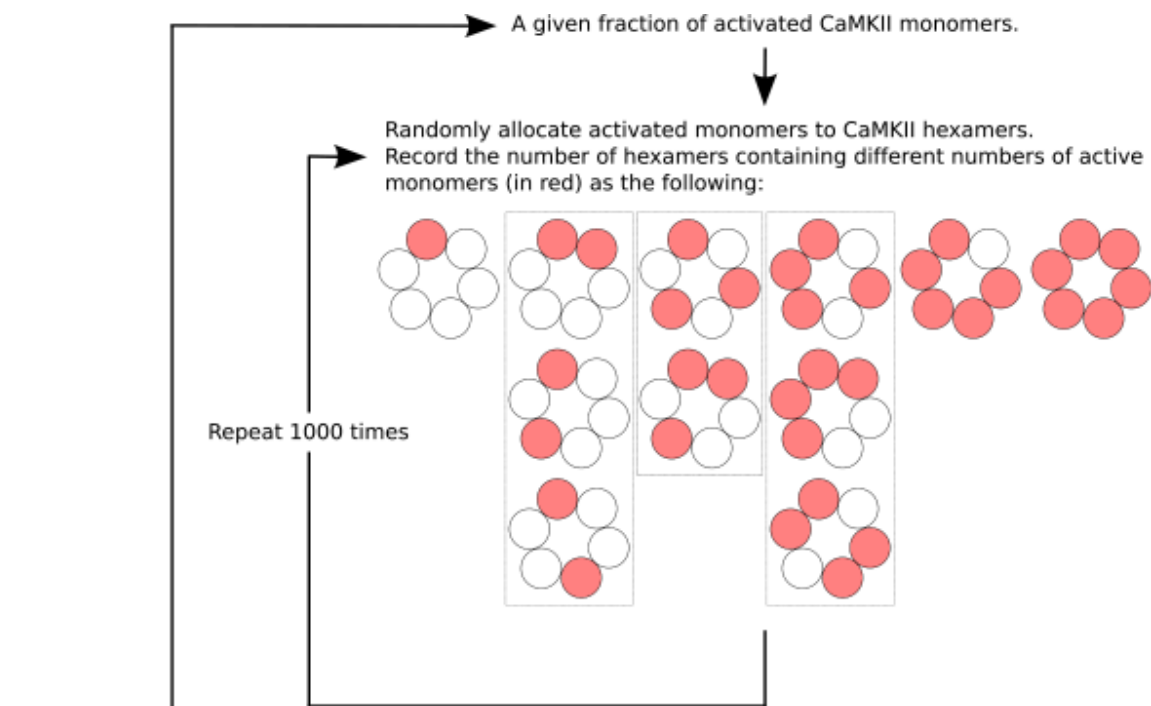


Dodecamer:

Trans-phosphorylation of T286  
by neighbouring subunits

Cis-phosphorylation of T306

Most quantitative measurements made on monomers ...



Repeat for every 1% increase of CaMKII active monomers

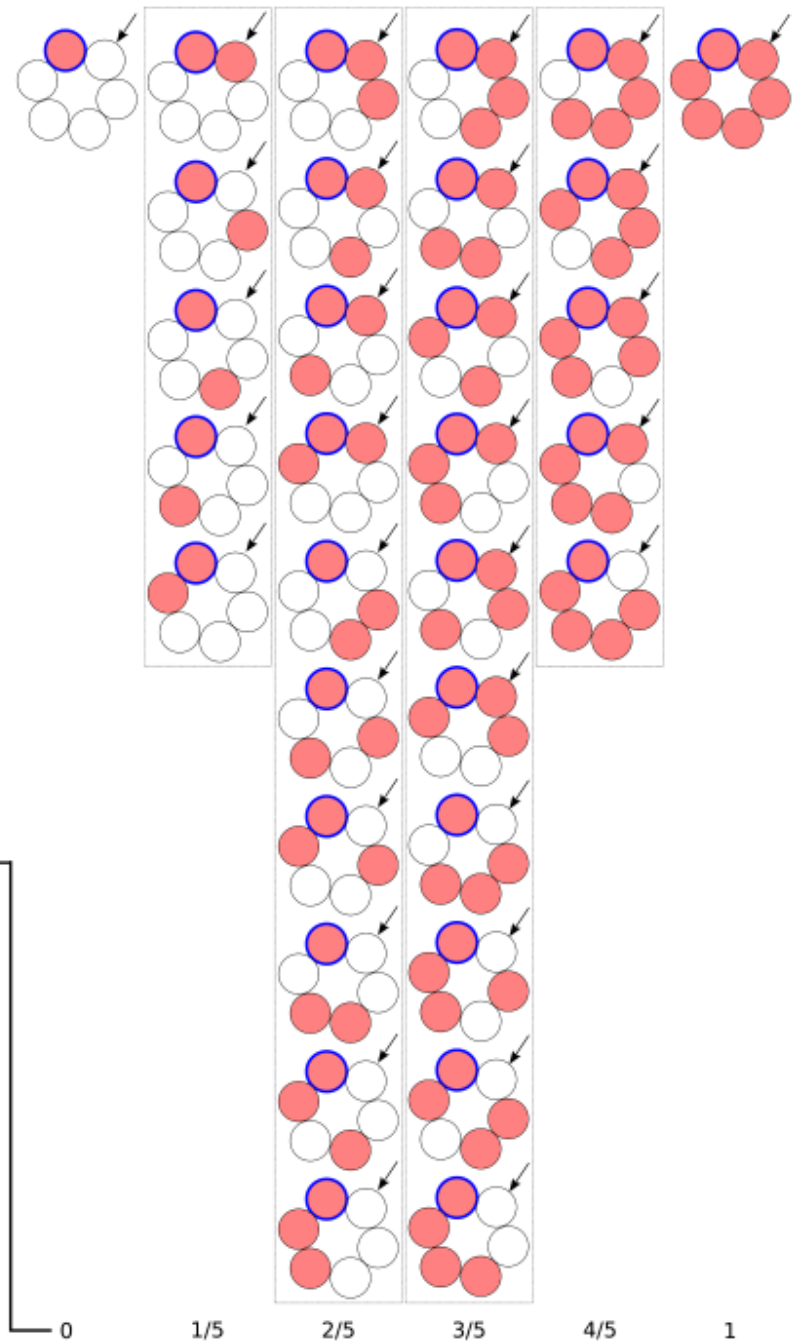
Calculate the average population for each number of active monomers per hexamer.

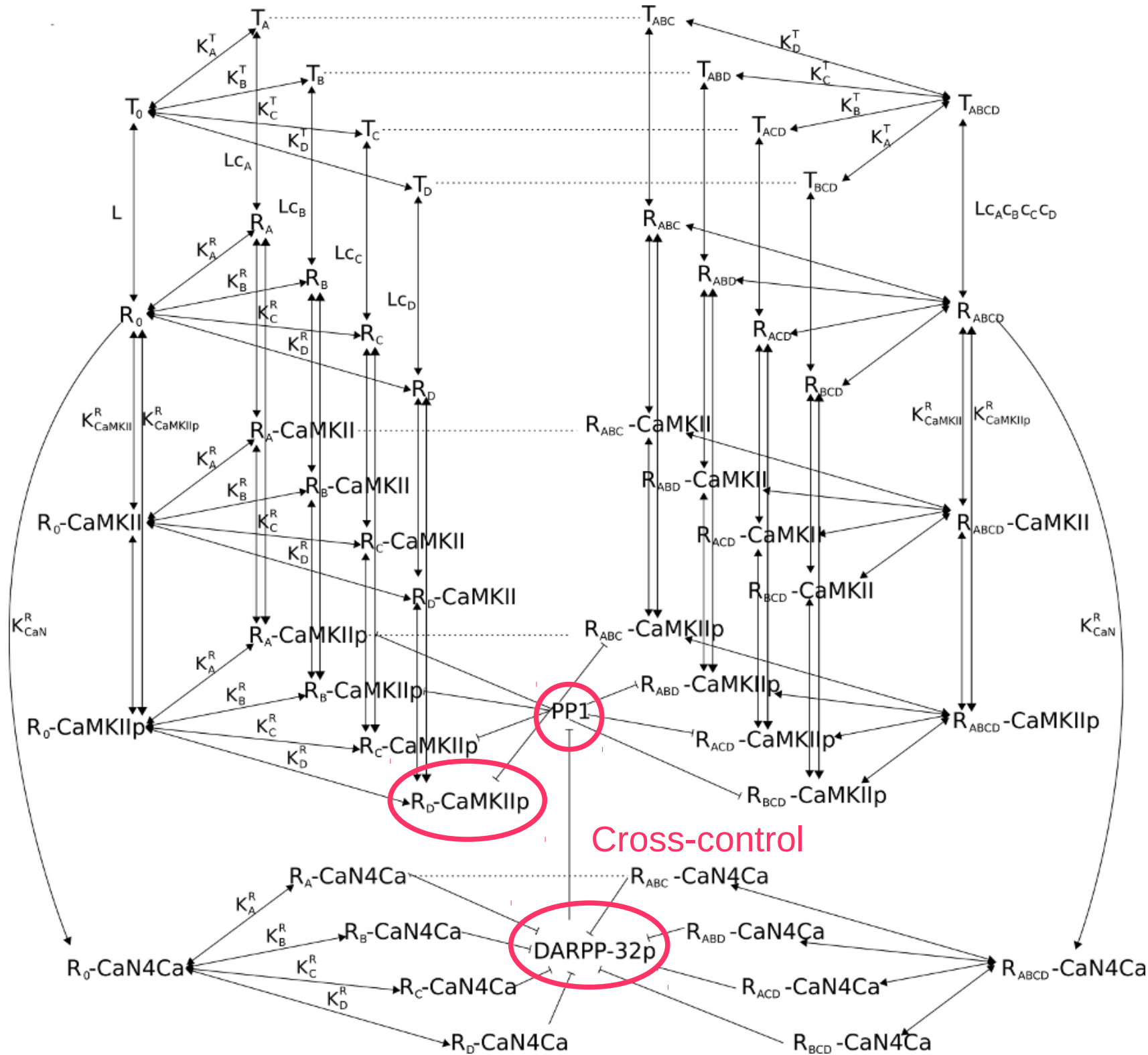
Multiply average populations of each number of active monomers per hexamer by their corresponding probabilities of having an active neighbour.

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

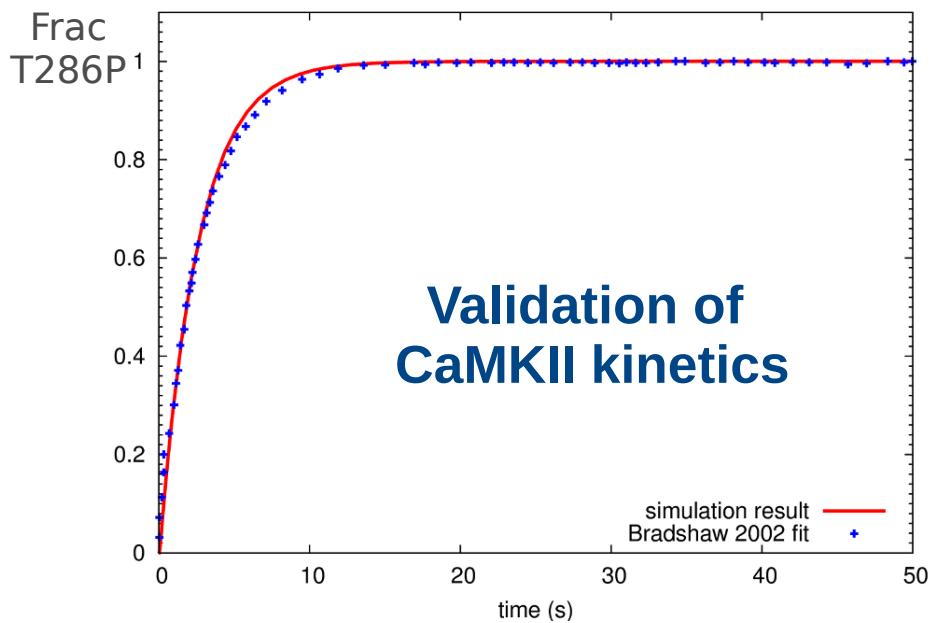
Fit these 100 coefficients into a polynomial function of activated CaMKII monomers, and embed this function in the model.

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:

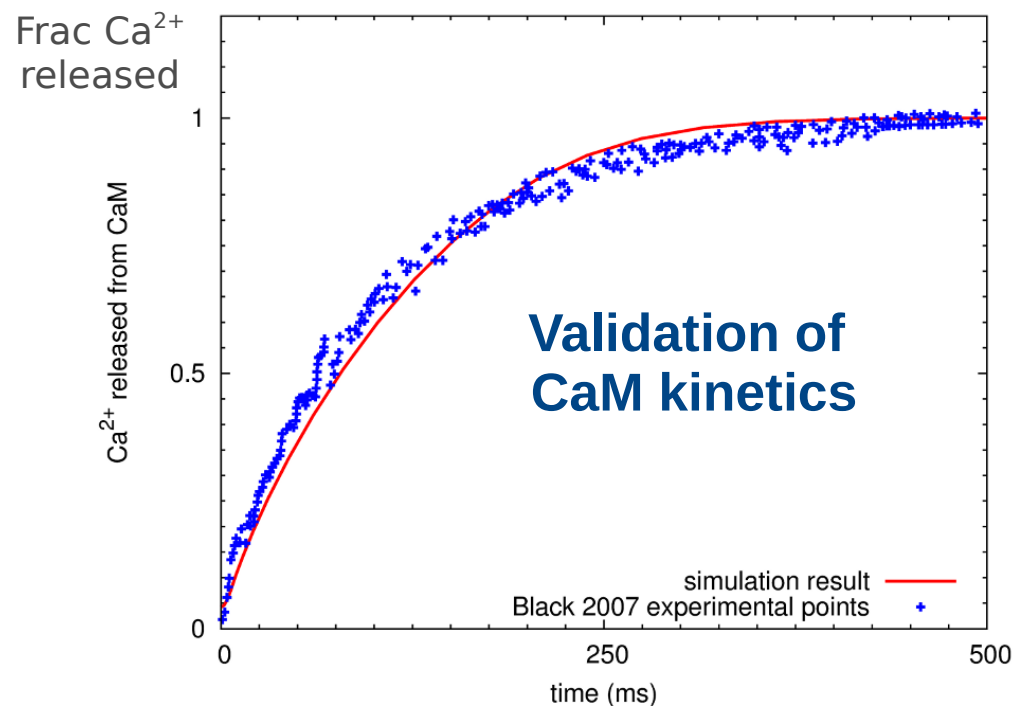




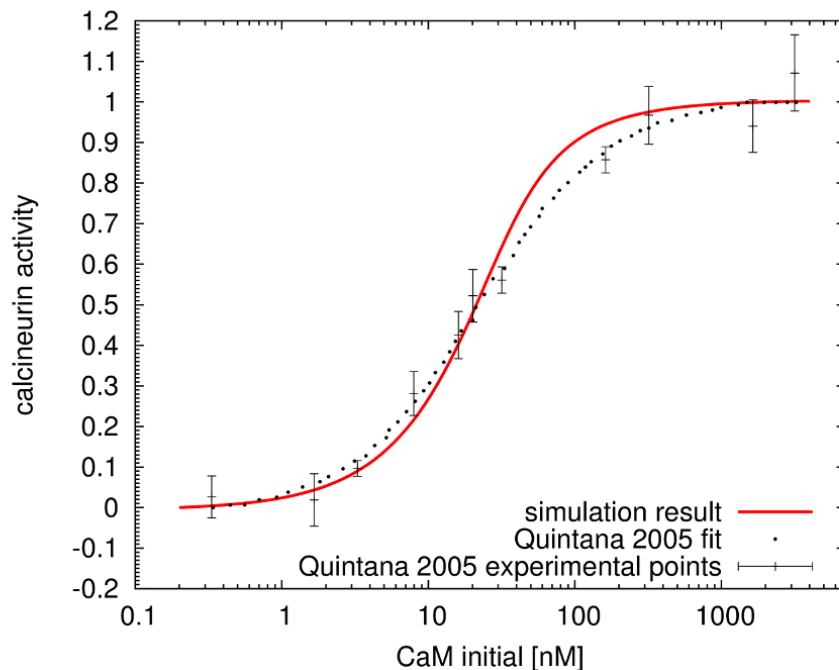




Bradshaw JM, Kubota Y, Meyer T, Schulman H (2003). *PNAS* 100: 10512-10517.



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

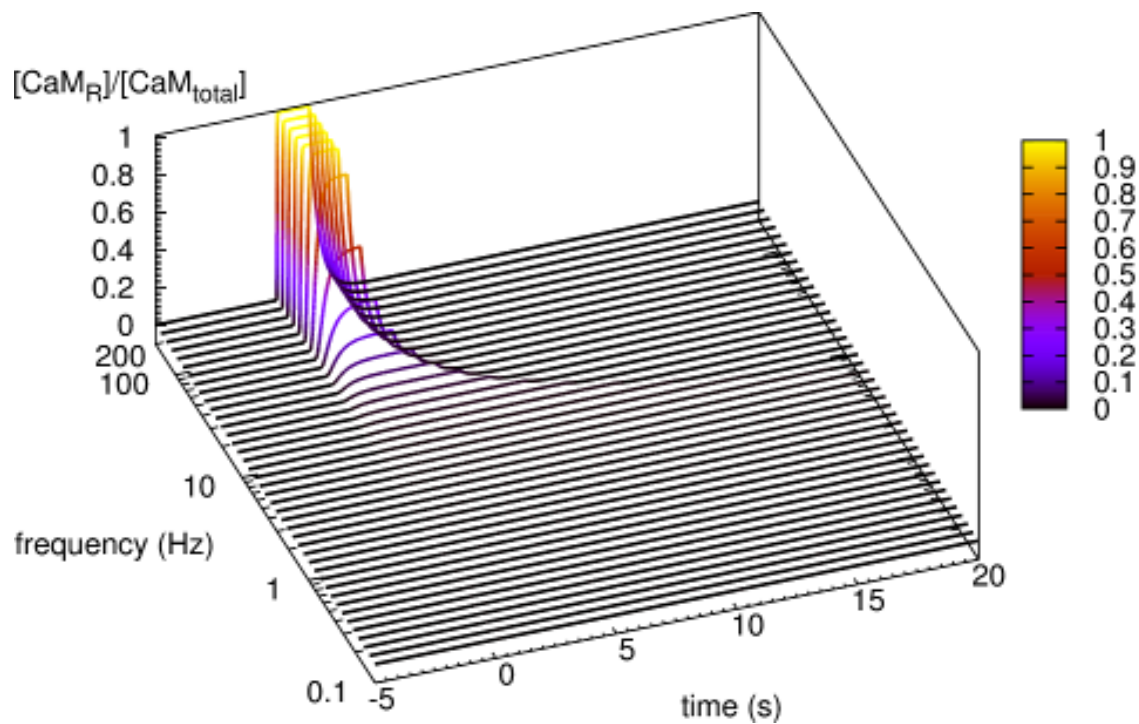


## Validation of calcium-activation of CaN

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

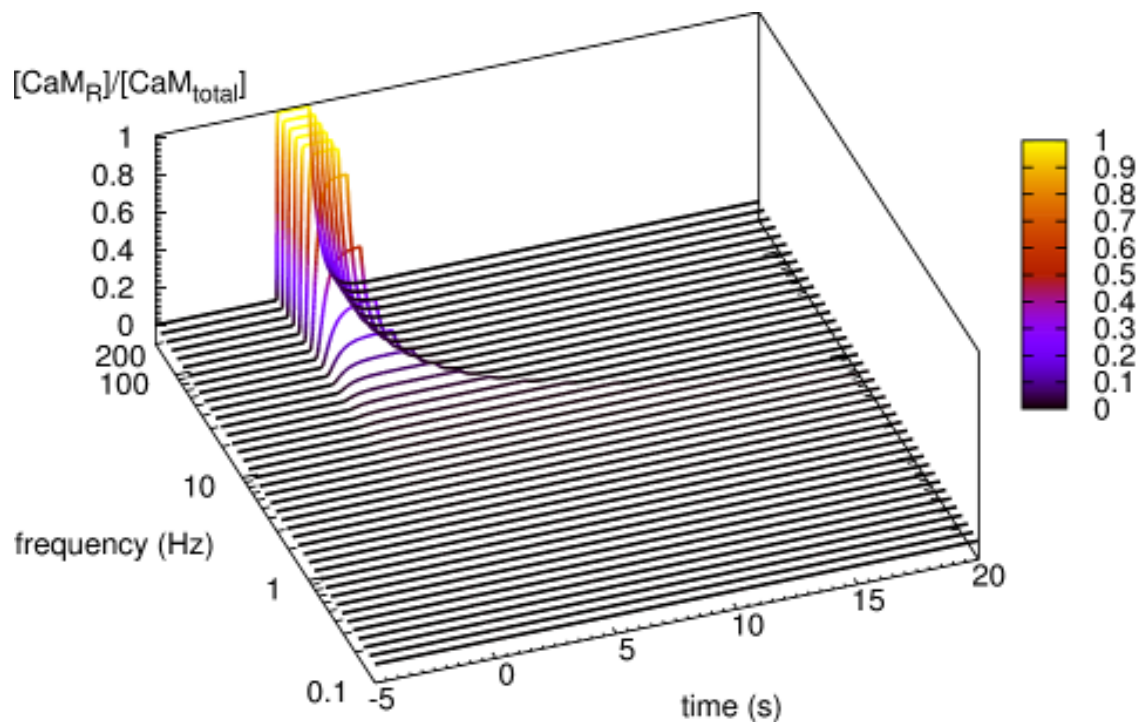


# Calmodulin activation



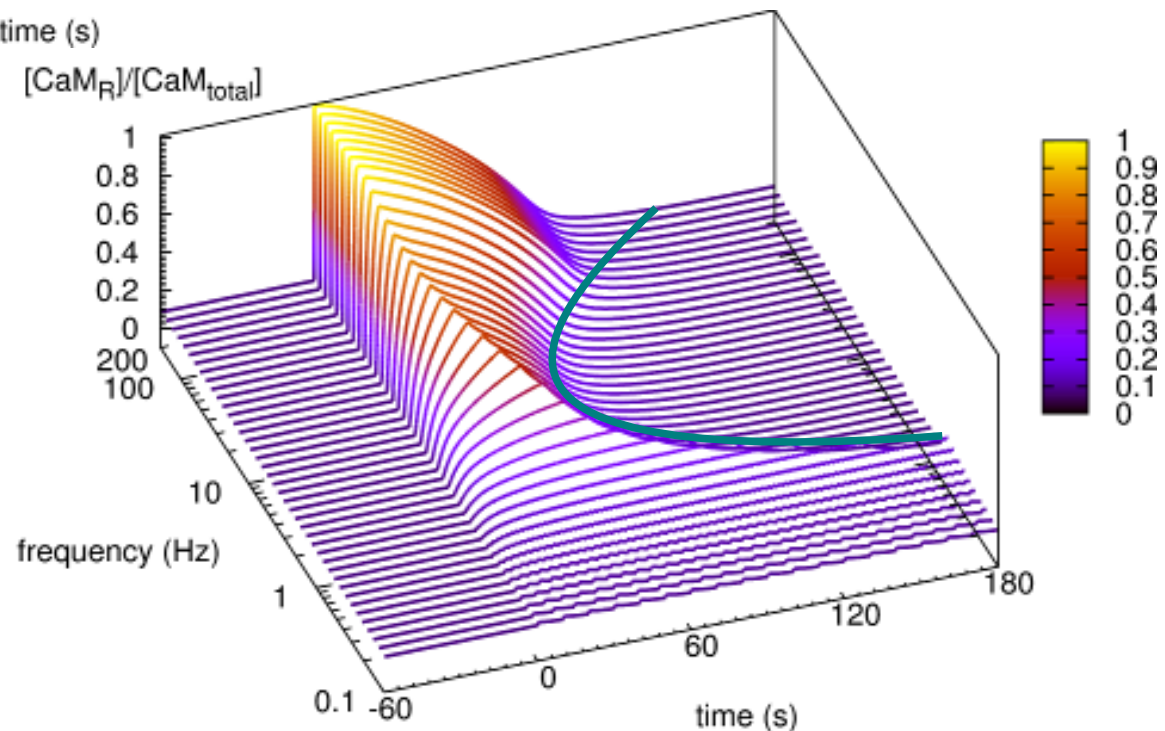
**CaM without targets.** Low frequencies do not activate calmodulin (binding events without conformational changes)

# Calmodulin activation

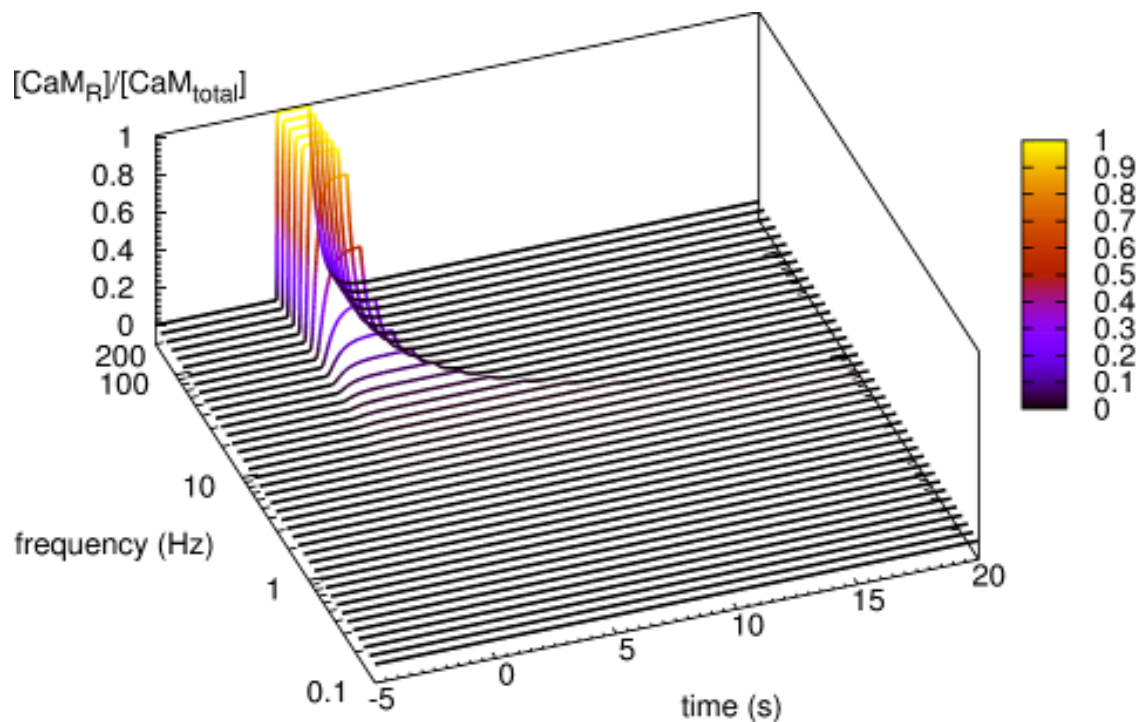


**CaM without targets.** Low frequencies do not activate calmodulin (binding events without conformational changes)

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



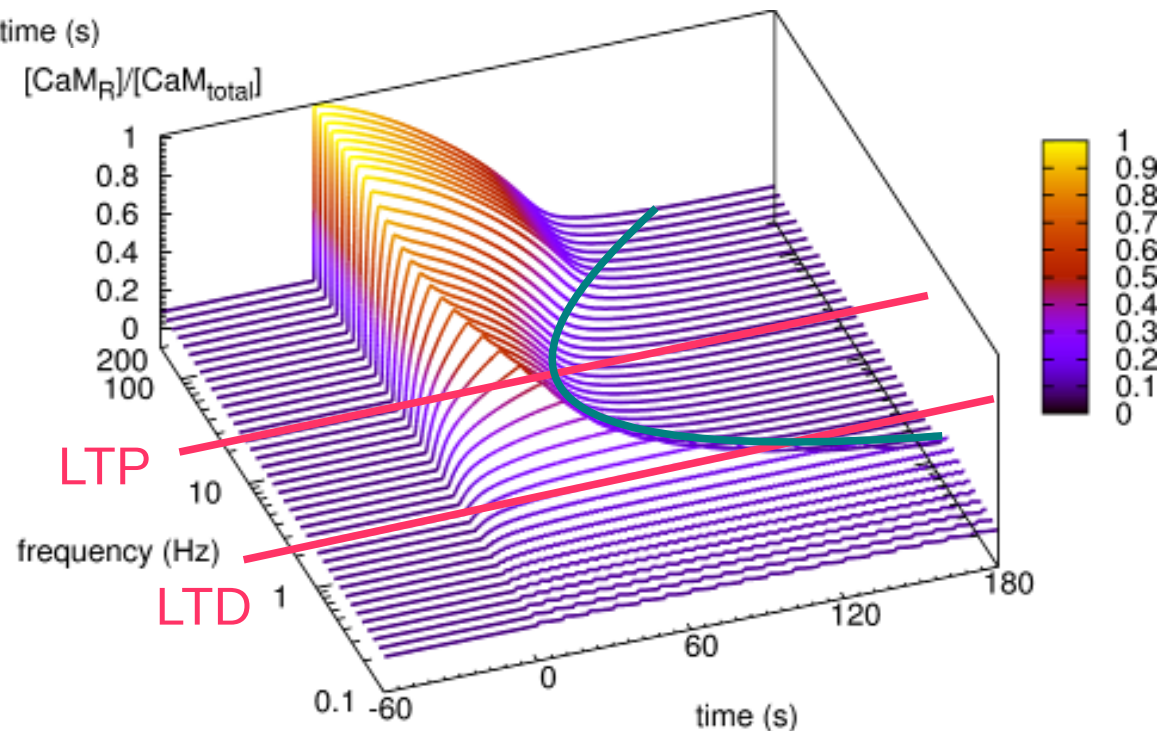
# Calmodulin activation



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

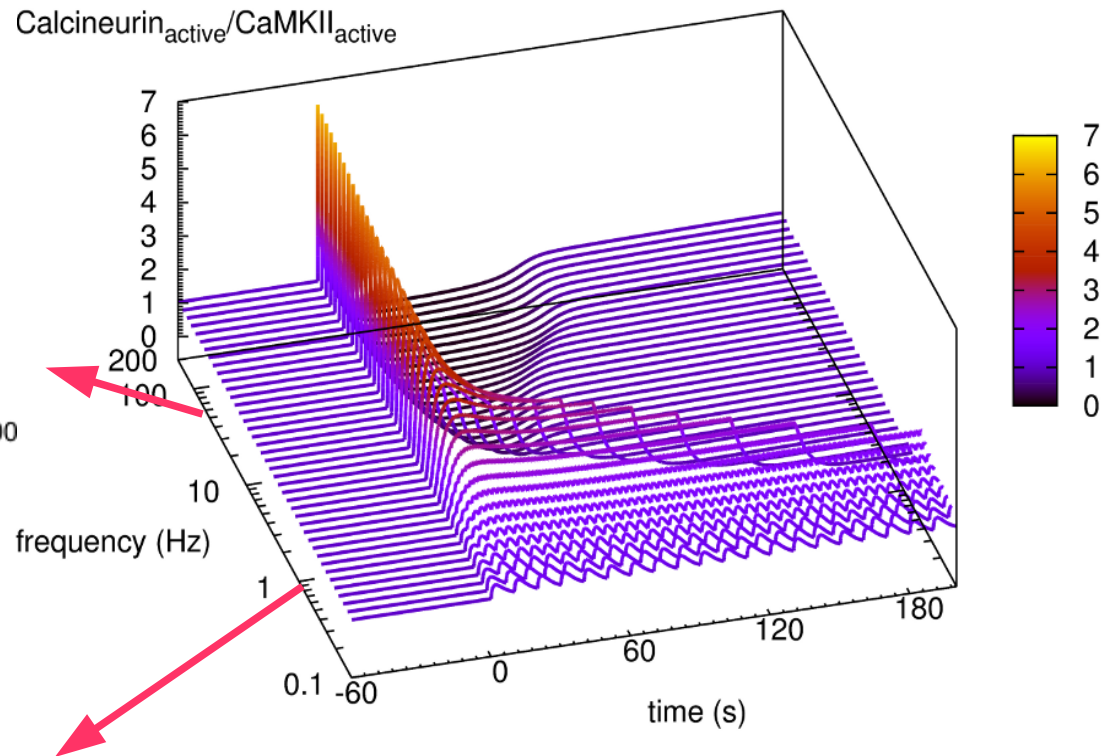
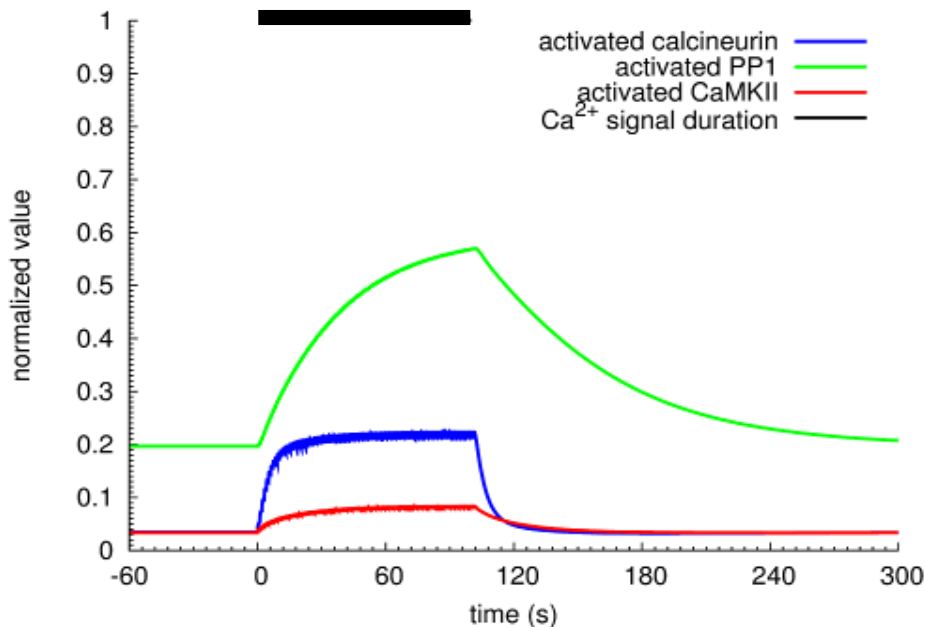
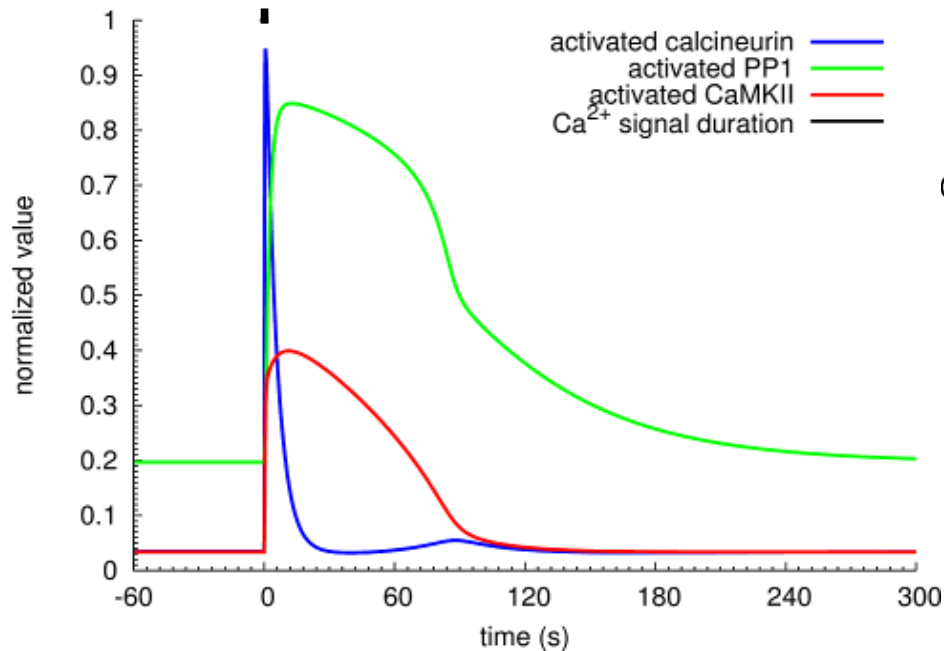
At high frequency, effects of calcium signals last much longer than the signal itself

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





# Temporal activation of CaMKII and CaN

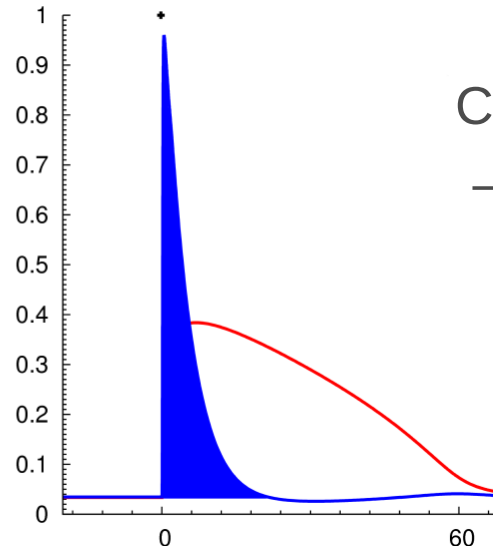
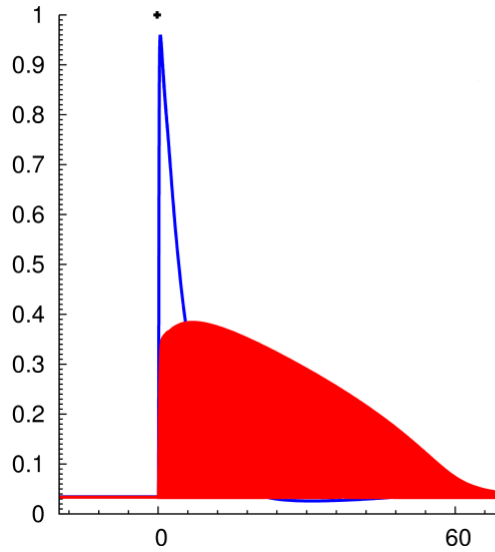


All calcium frequencies increase CaN **AND** CaMKII. It is not a Switch. But the ratio of activation changes

# Bidirectional plasticity

Constant catalytic rates of active enzyme

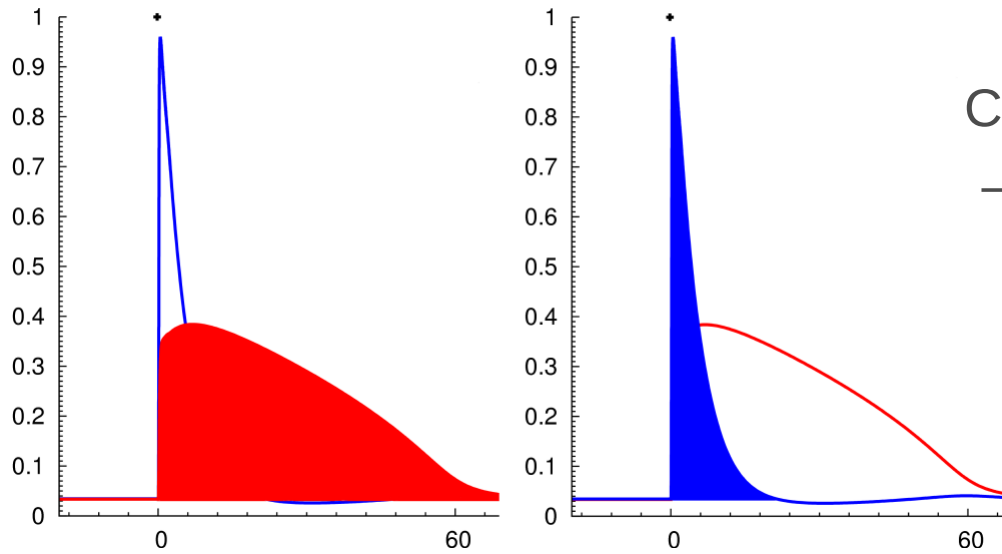
→ quantity of catalysed reaction events  
prop to integral of the activation curve



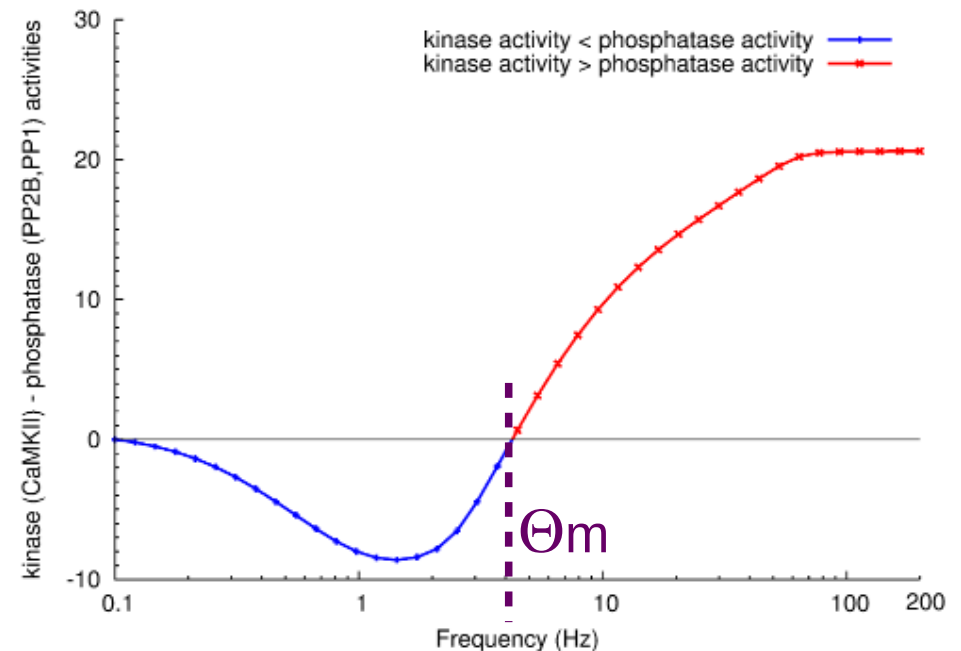
# Bidirectional plasticity

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

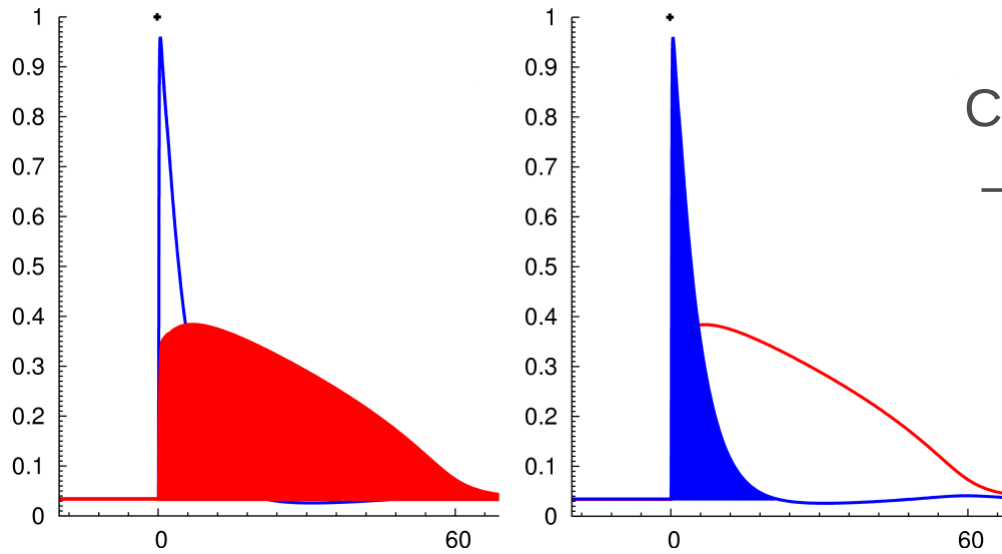




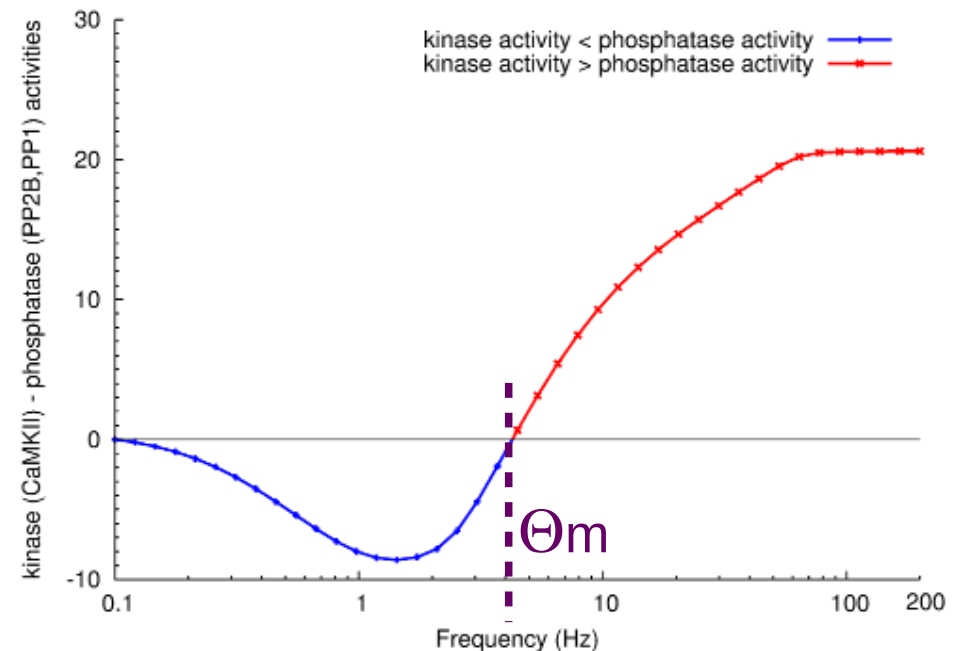
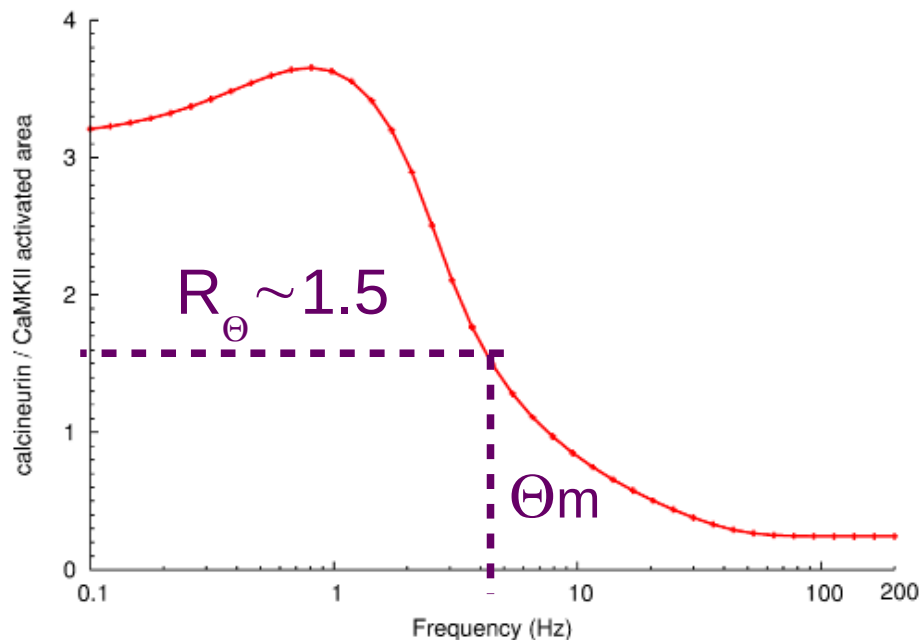
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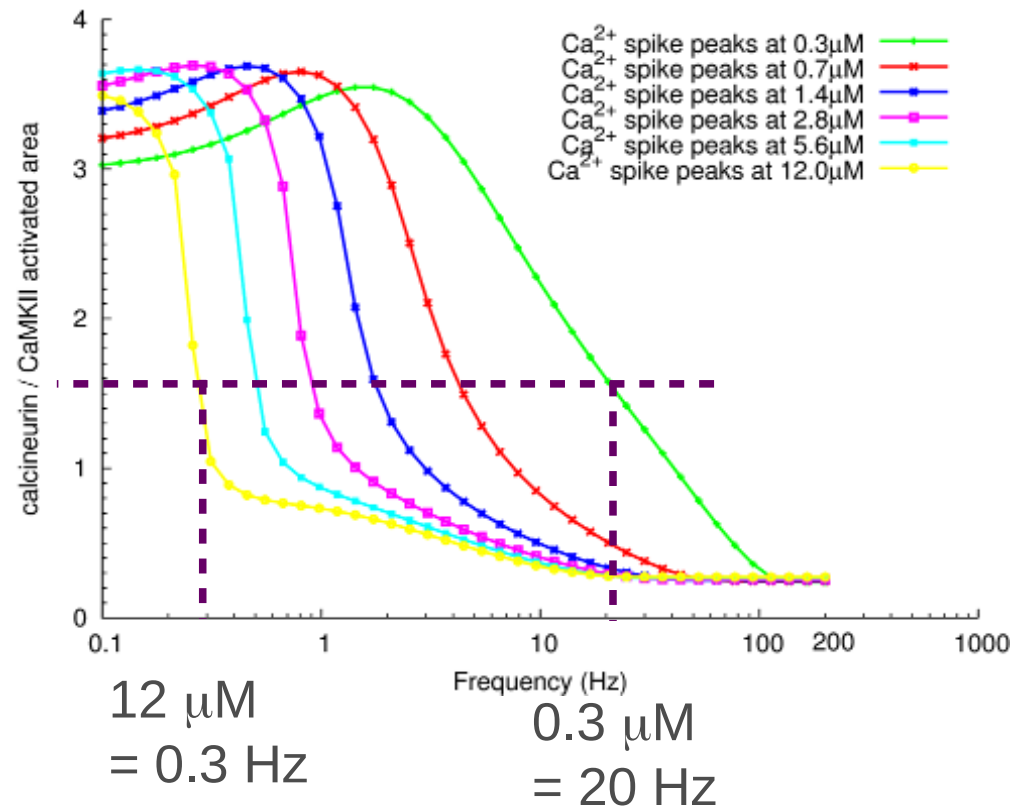
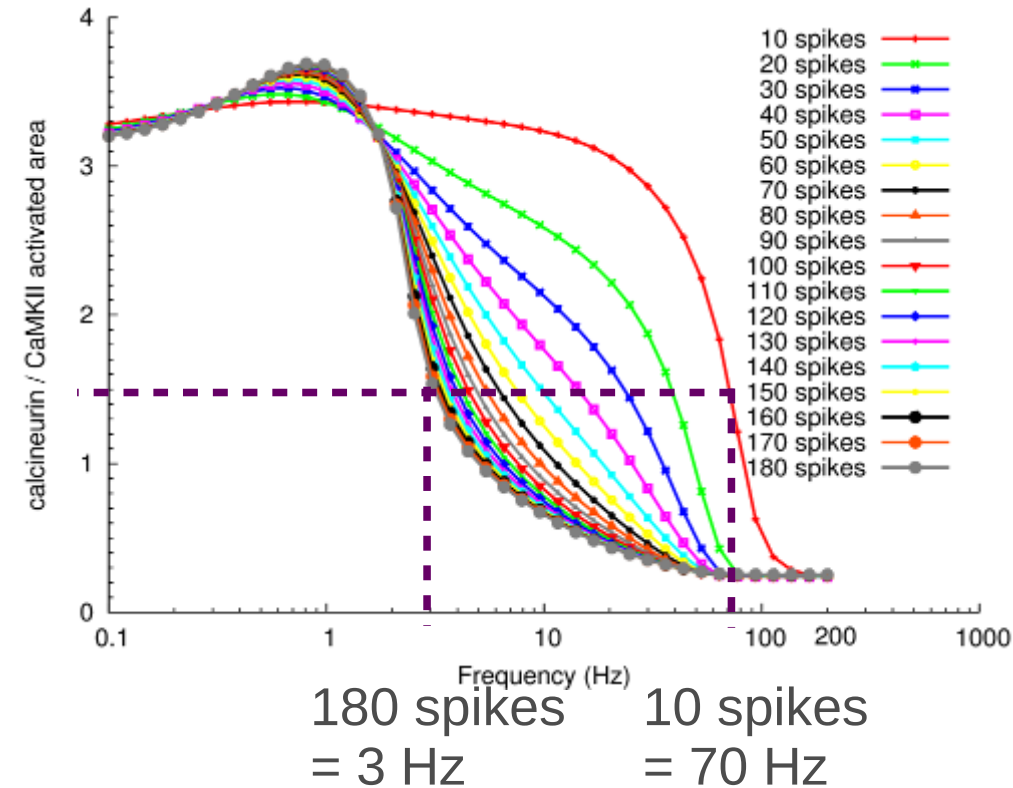
→ quantity of catalysed reaction events  
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Bienestock-Cooper-Munro  
(BCM) curve: difference of  
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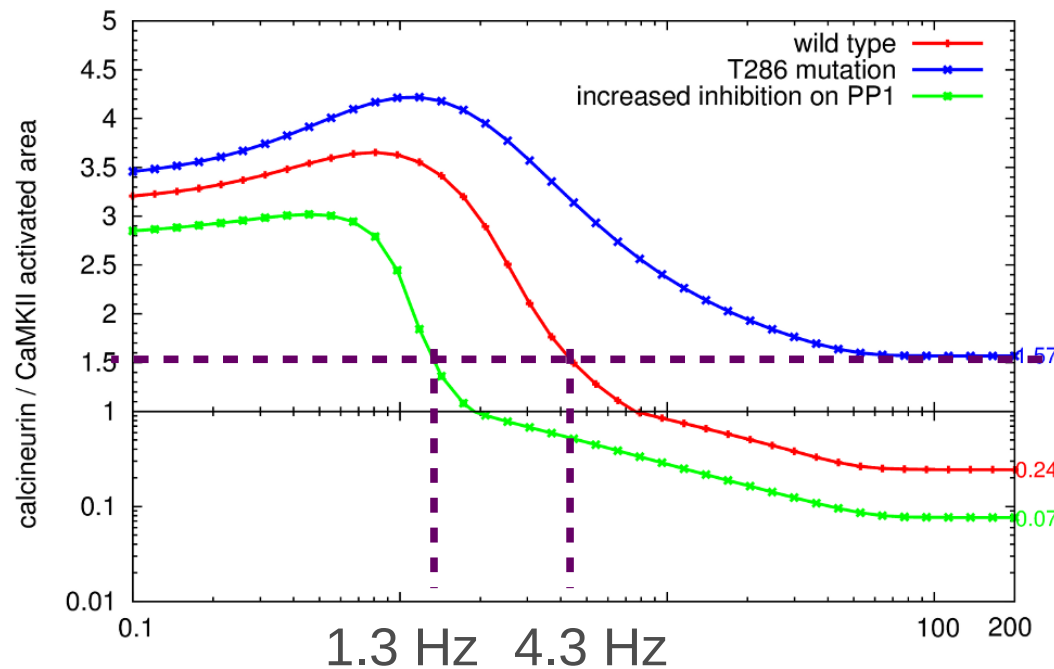


# Effect of calcium duration and amount



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

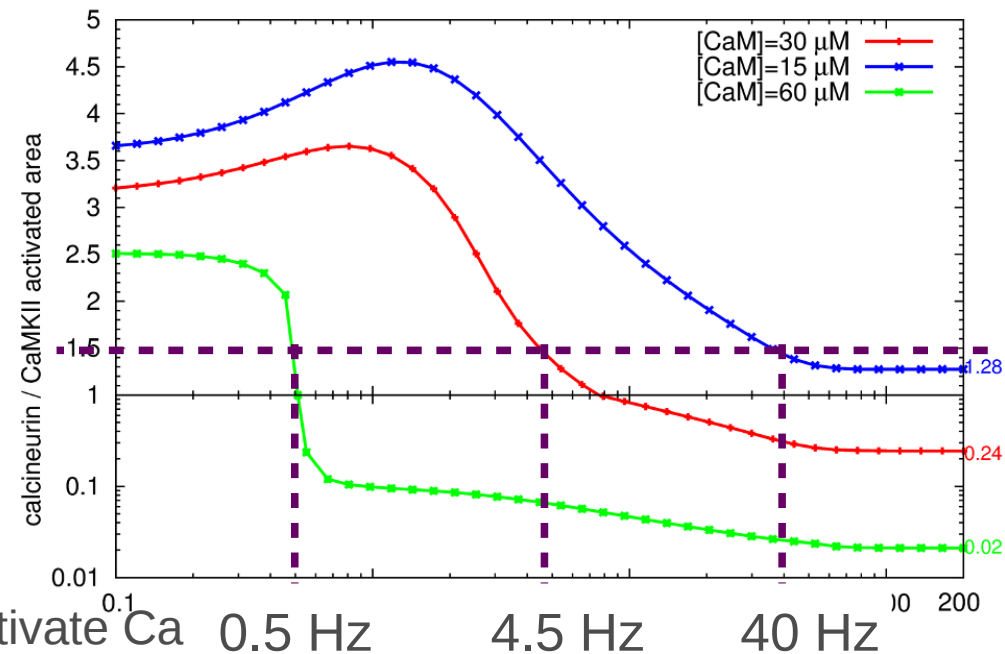
Lower deactivation of CaMKII

## 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





Melanie Stefan



Lu Li

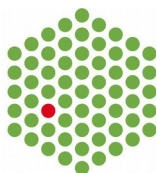


Denis Brun

**wellcome**trust



EMBL



AgedBrainSYSBIO



Stuart  
Edelstein



Massimo  
Lai



