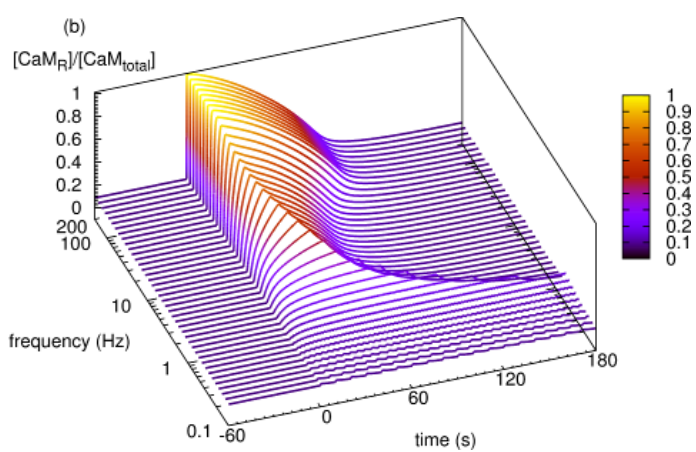
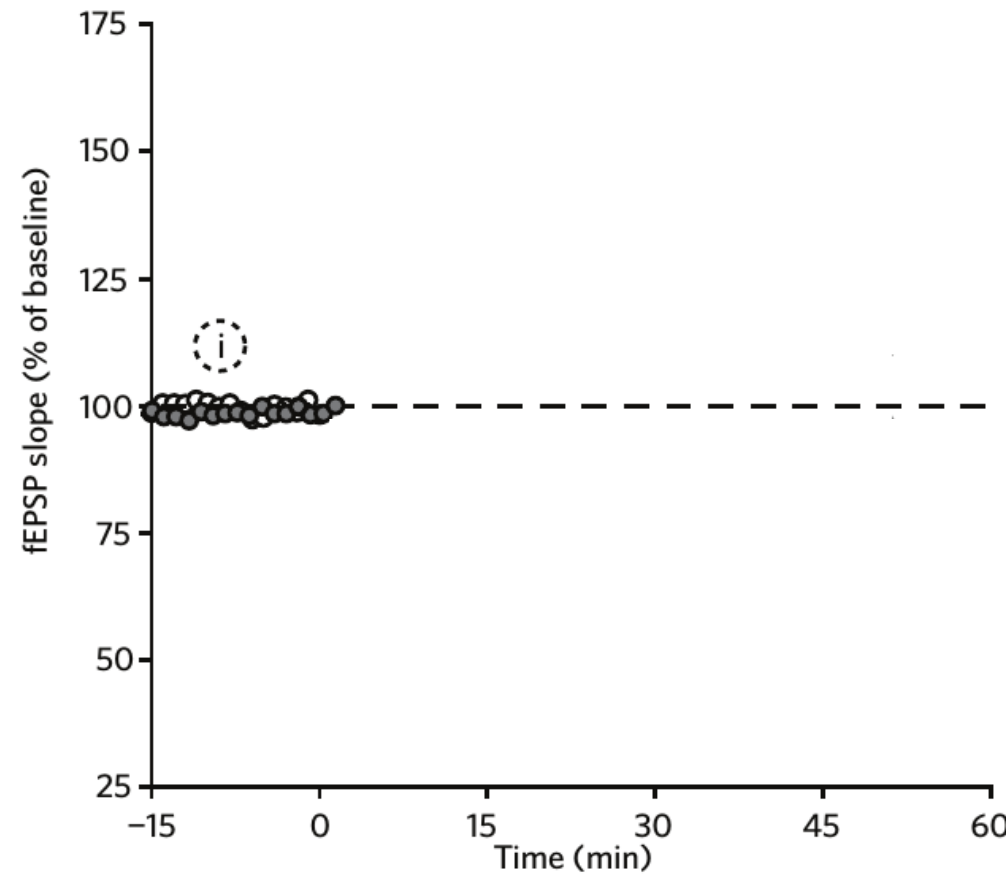
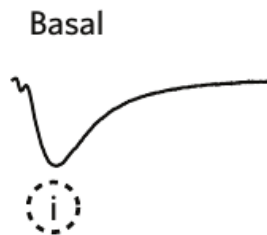
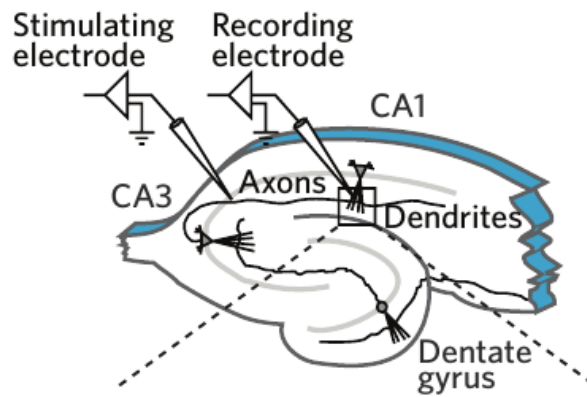
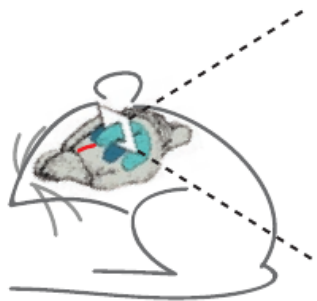
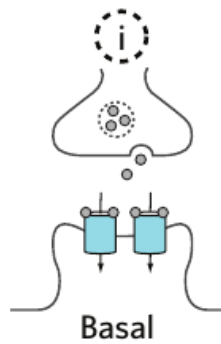




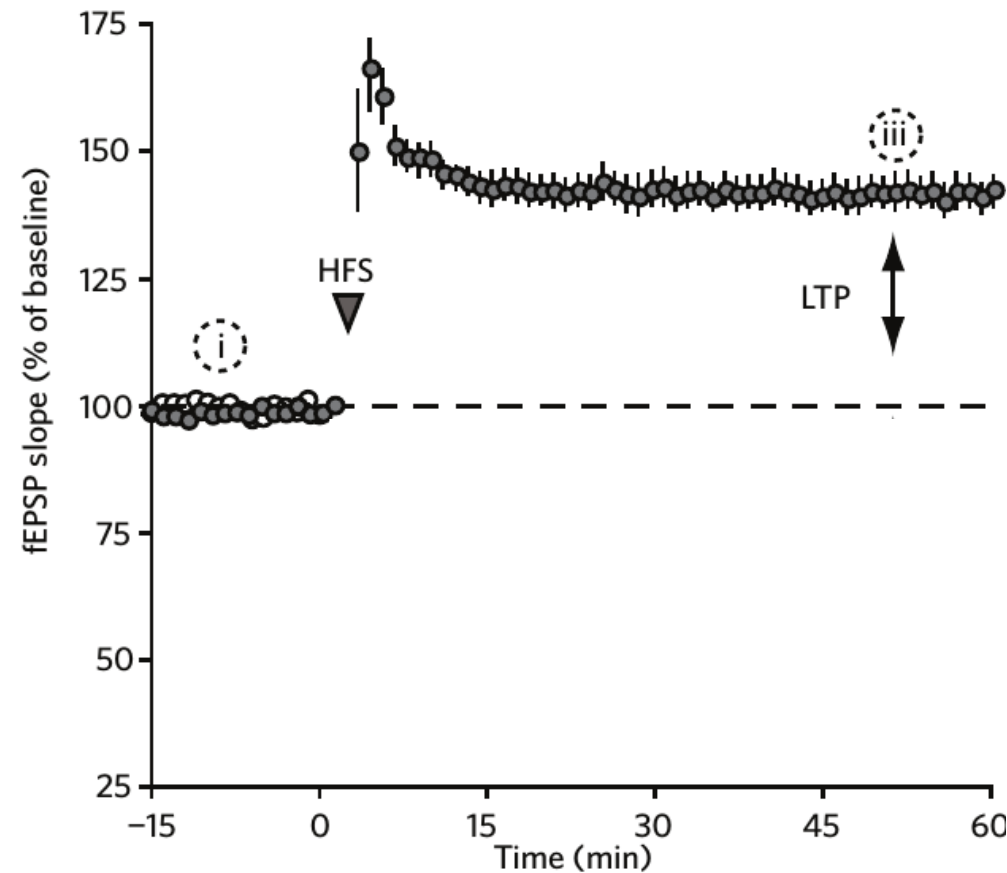
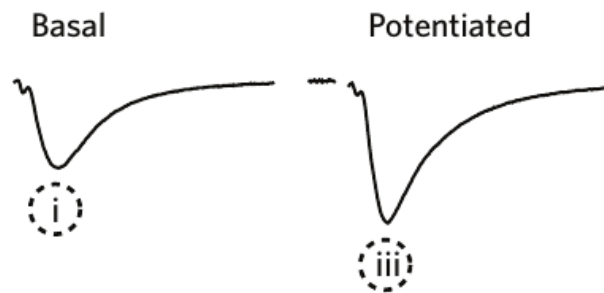
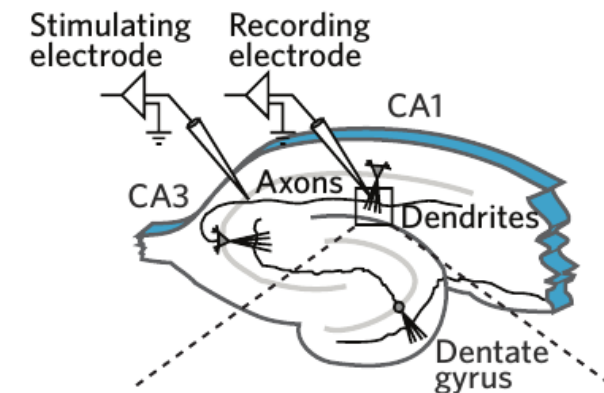
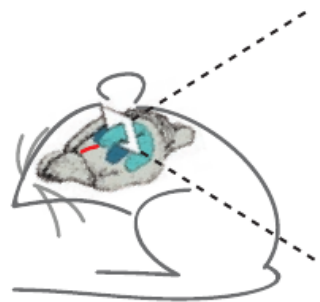
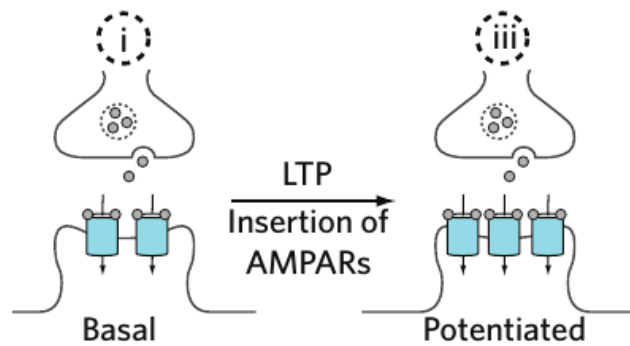
Regulation of synaptic plasticity by allosteric calcium sensors



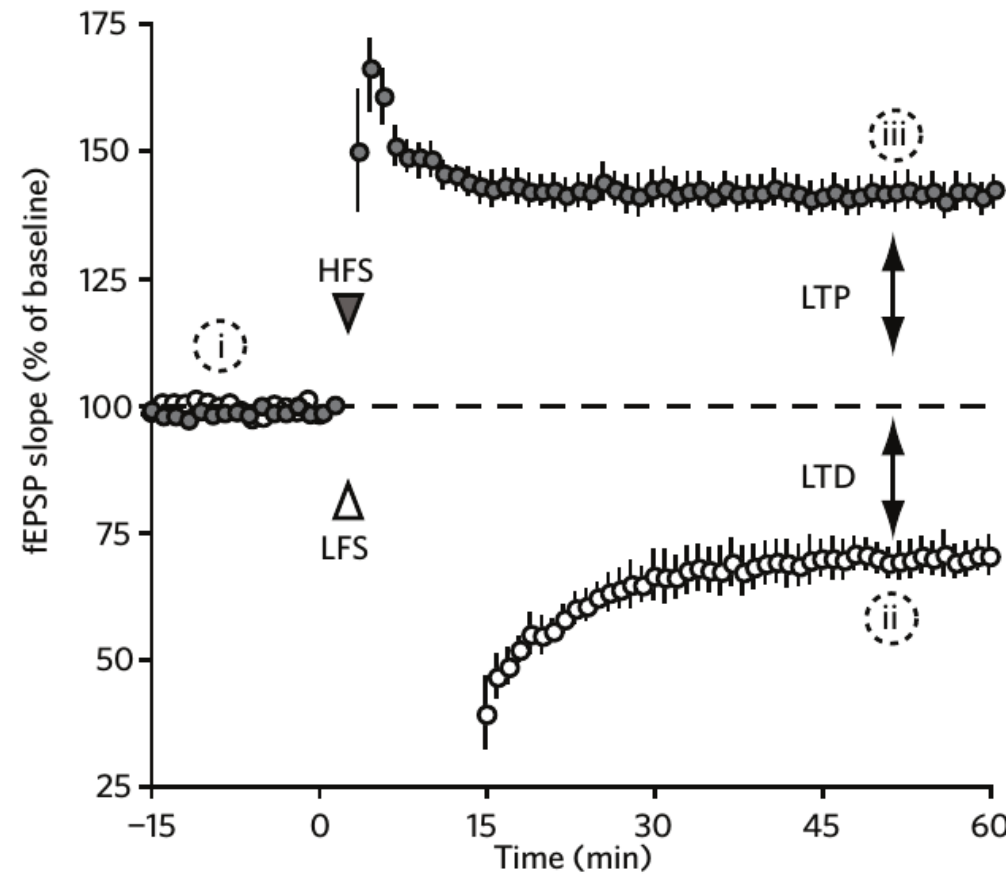
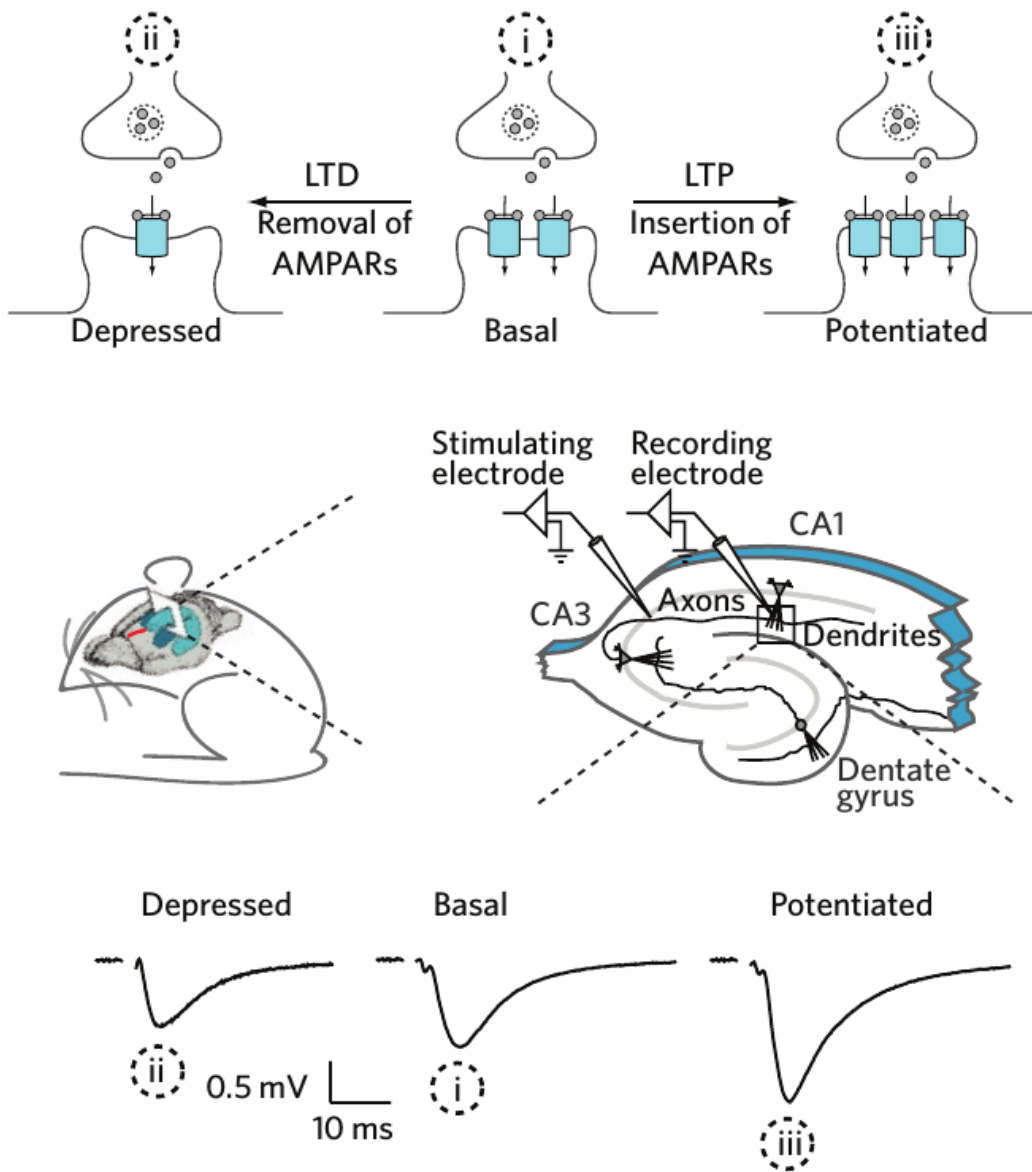
Nicolas Le Novère
Babraham Institute,
n.lenovere@gmail.com



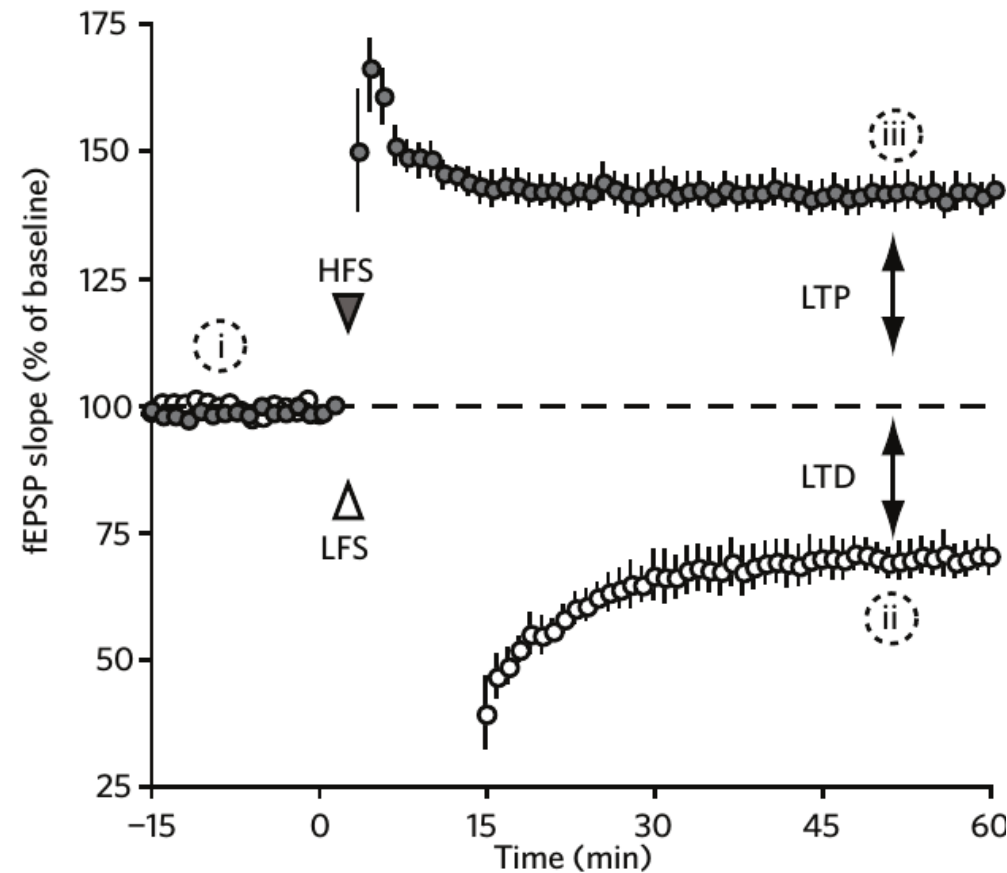
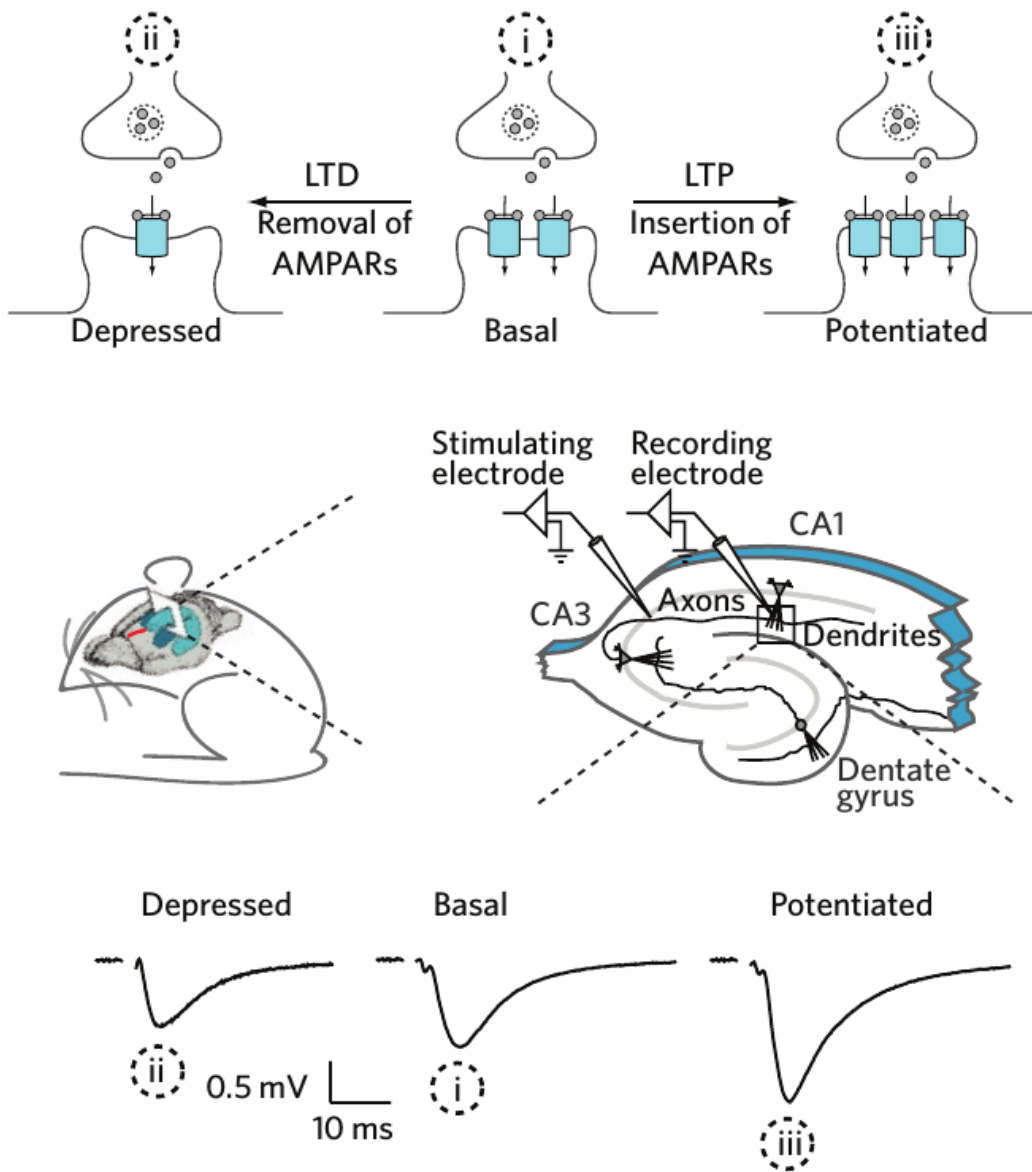
Fleming and England (2010) *Nat Chem Biol*



Fleming and England (2010) *Nat Chem Biol*



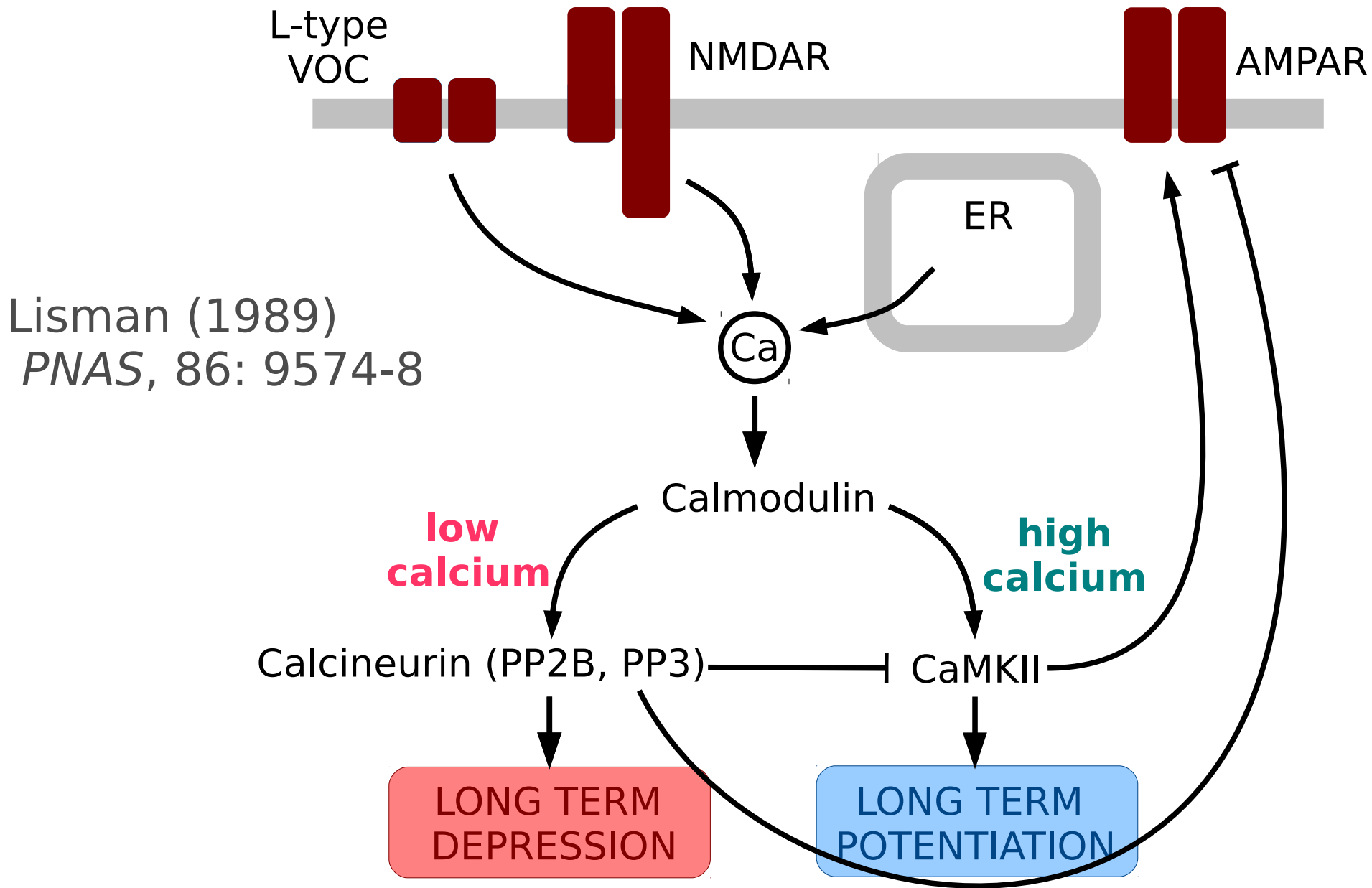
Fleming and England (2010) *Nat Chem Biol*



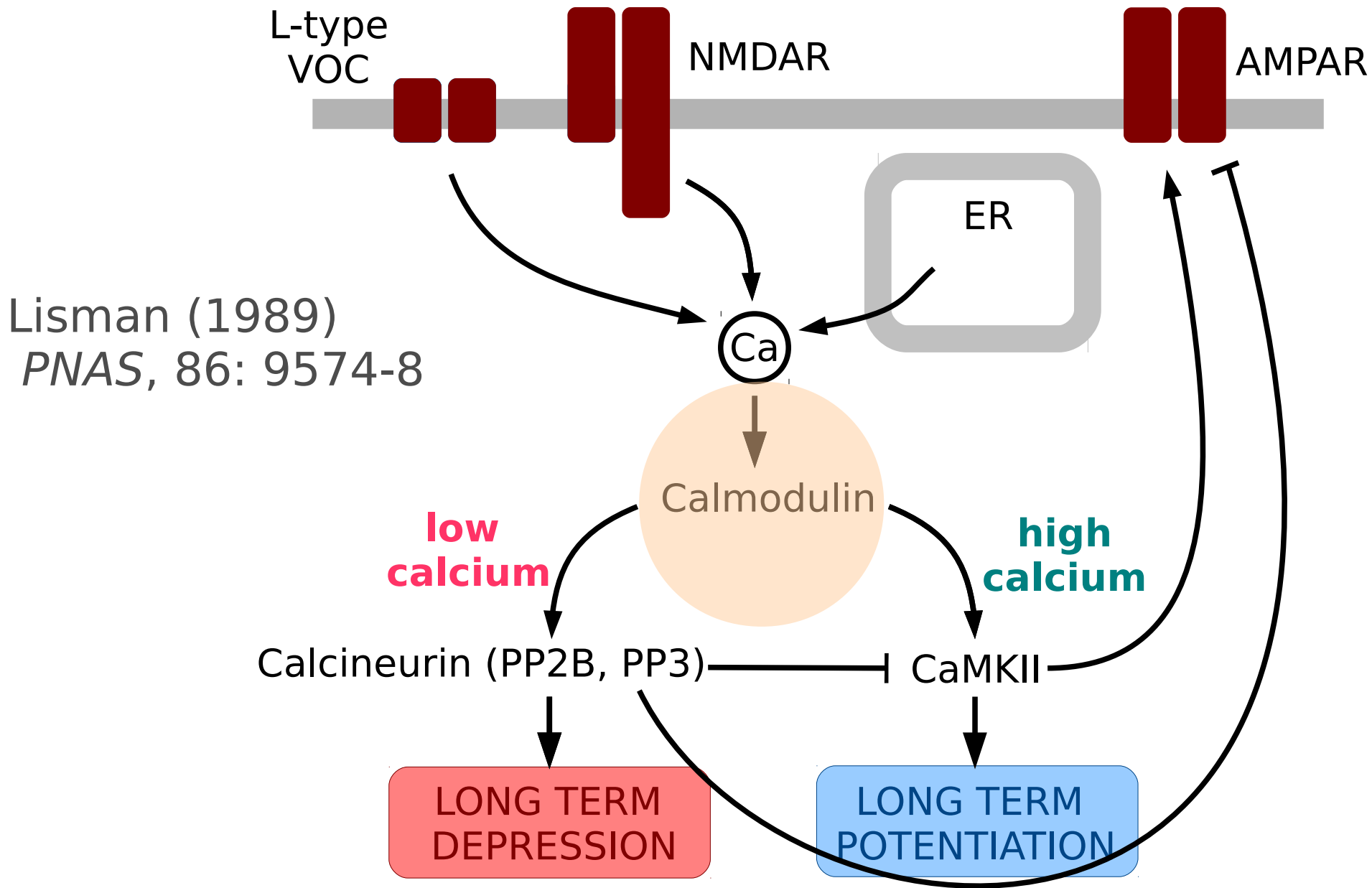
lasts for weeks

Fleming and England (2010) *Nat Chem Biol*

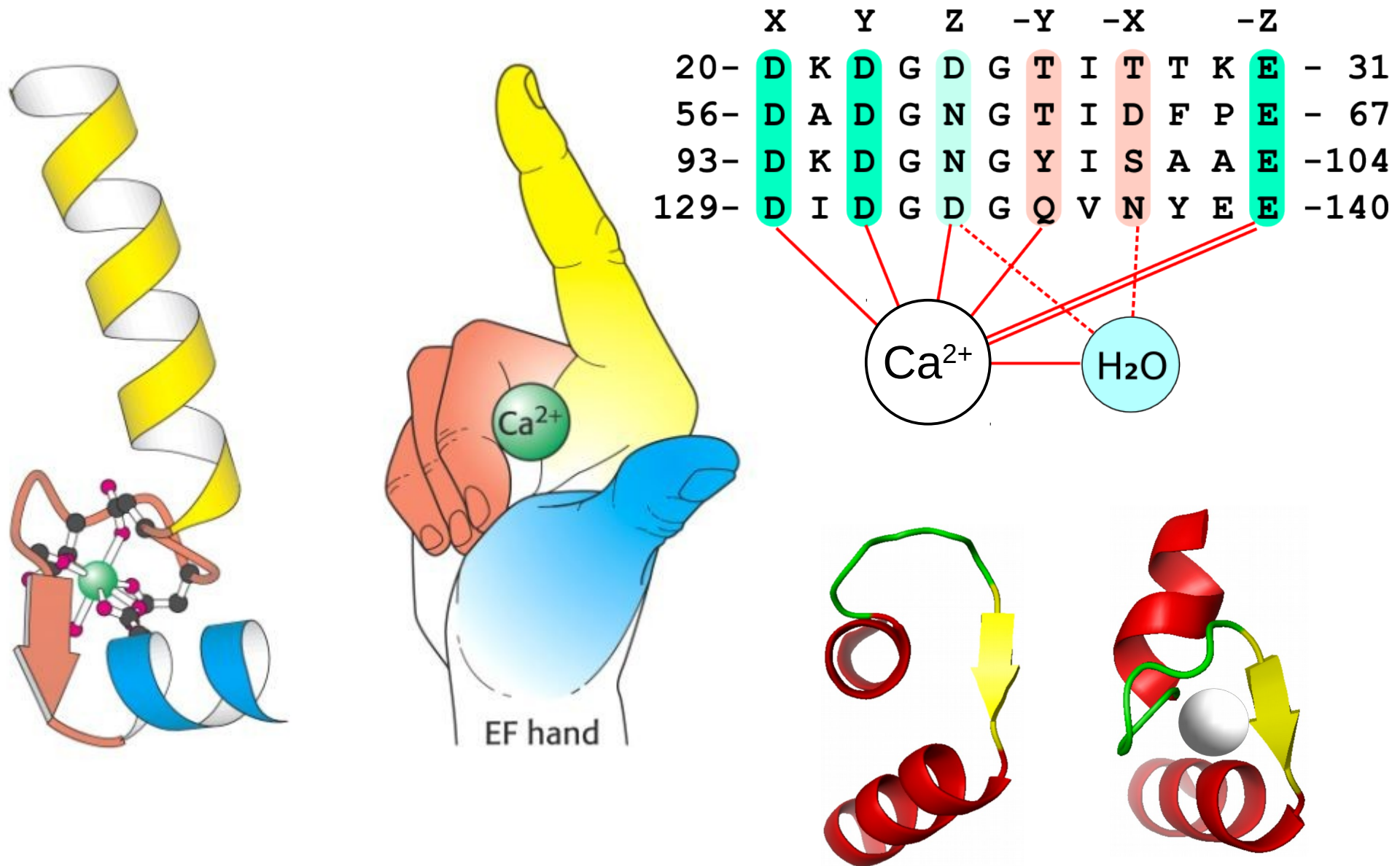
The calcium theory of synaptic plasticity

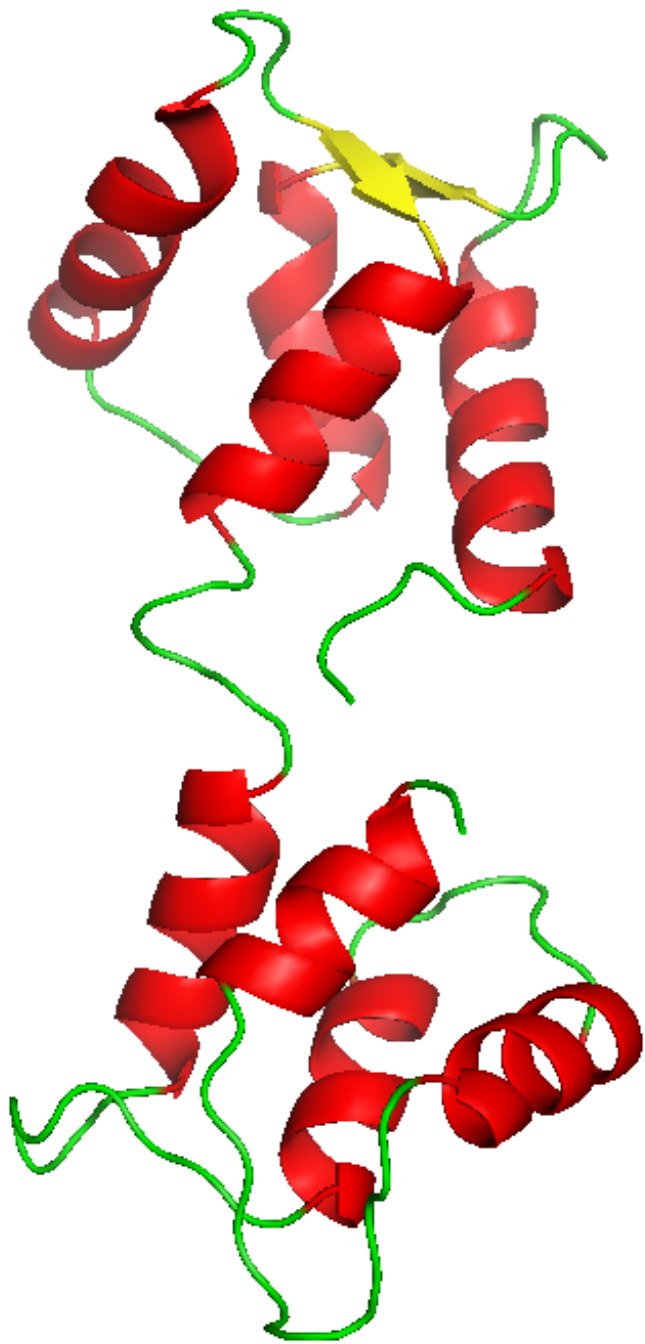


Calmodulin, the memory switchboard

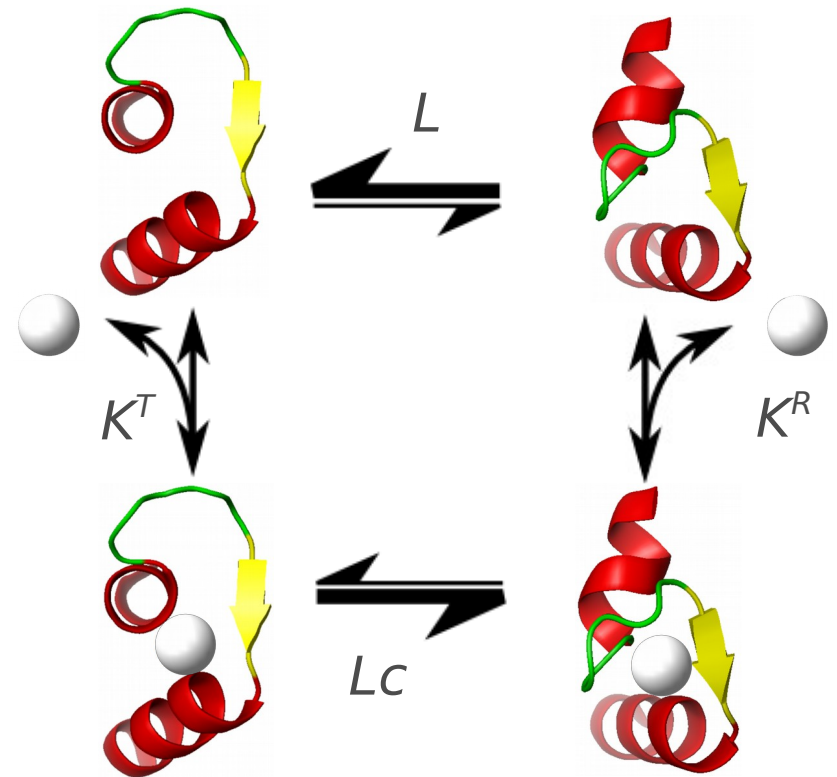


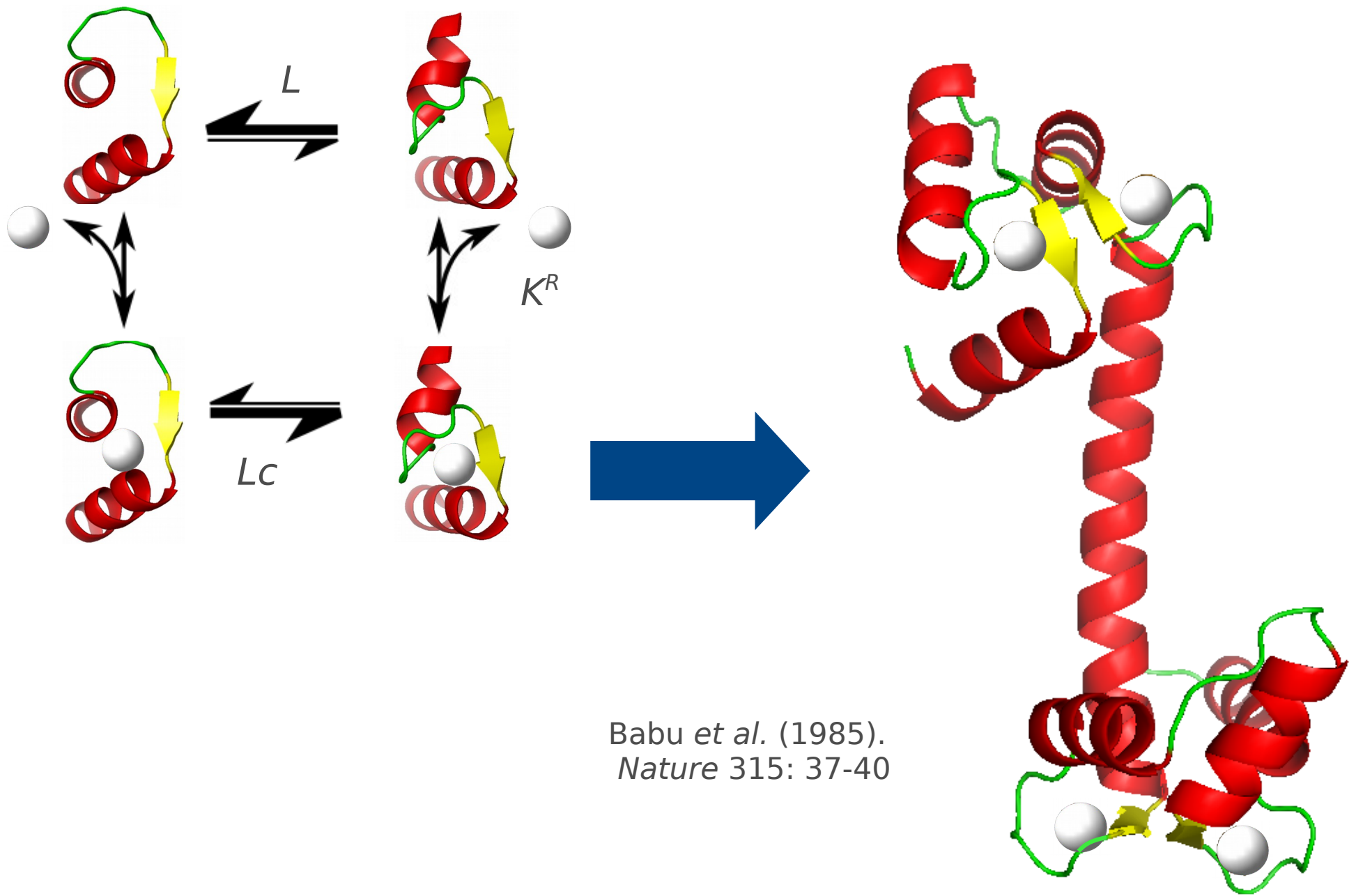
Calmodulin carries 4 Ca^{2+} binding domains

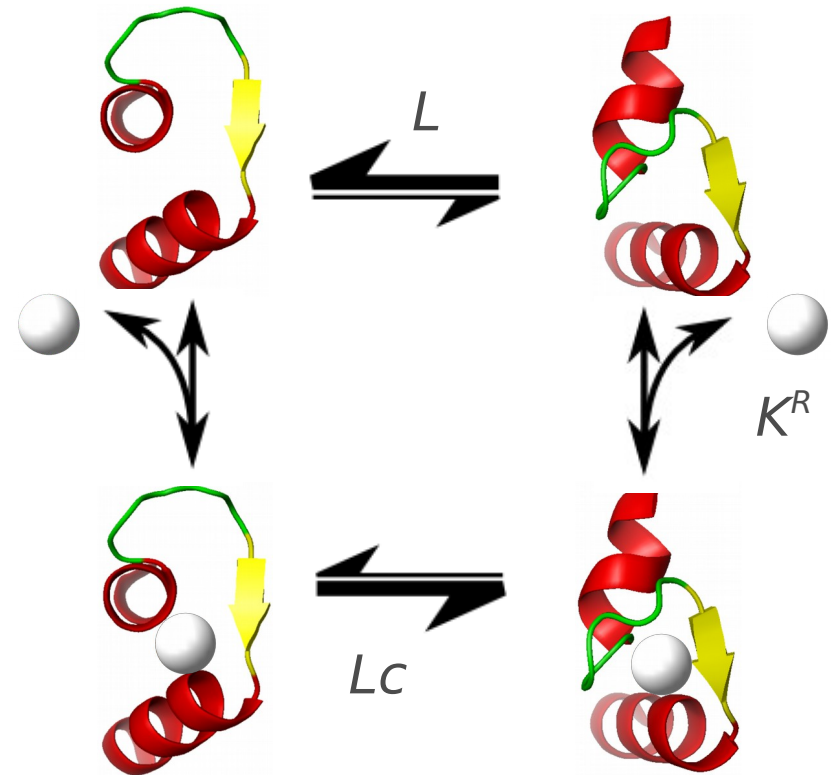
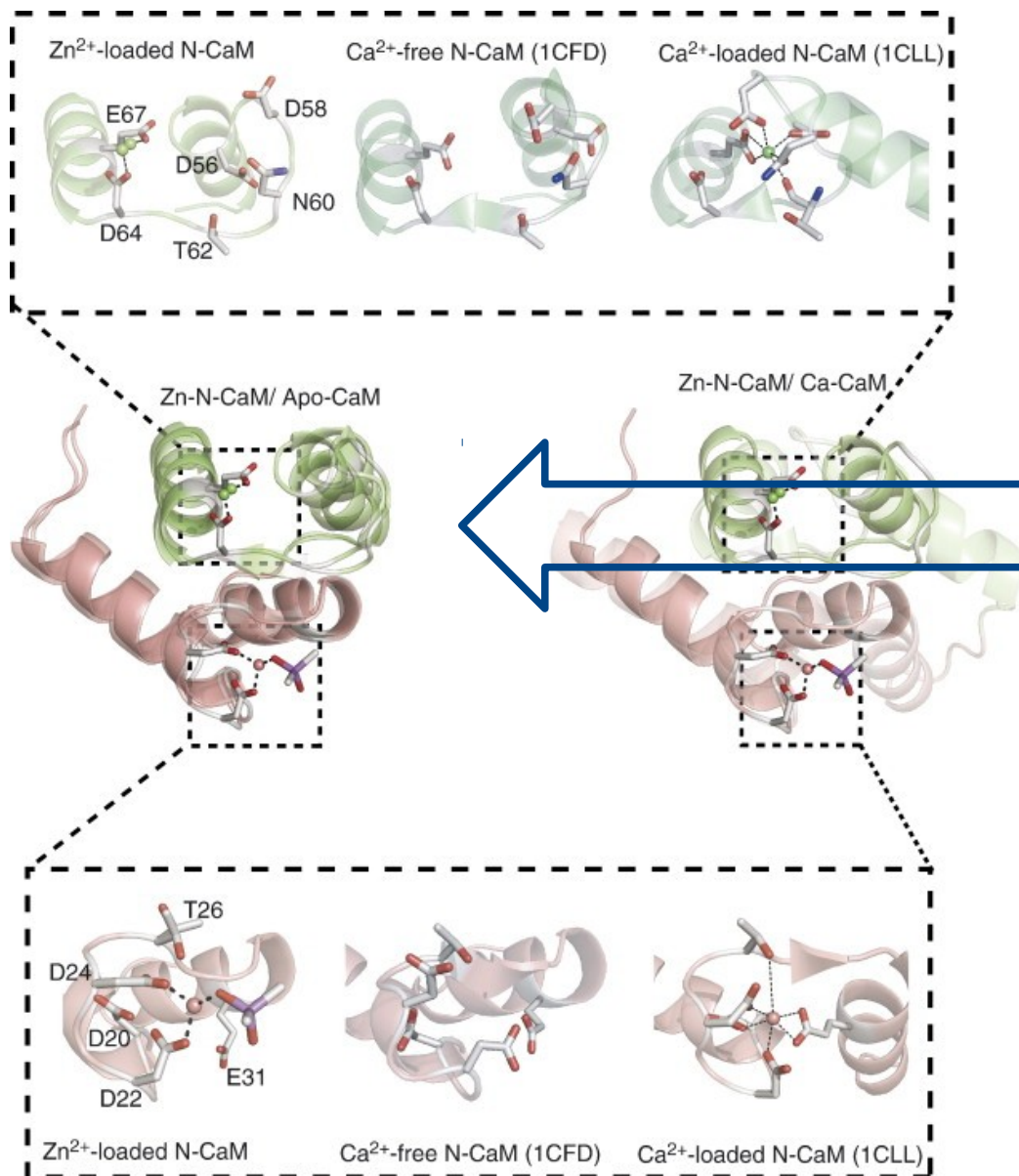




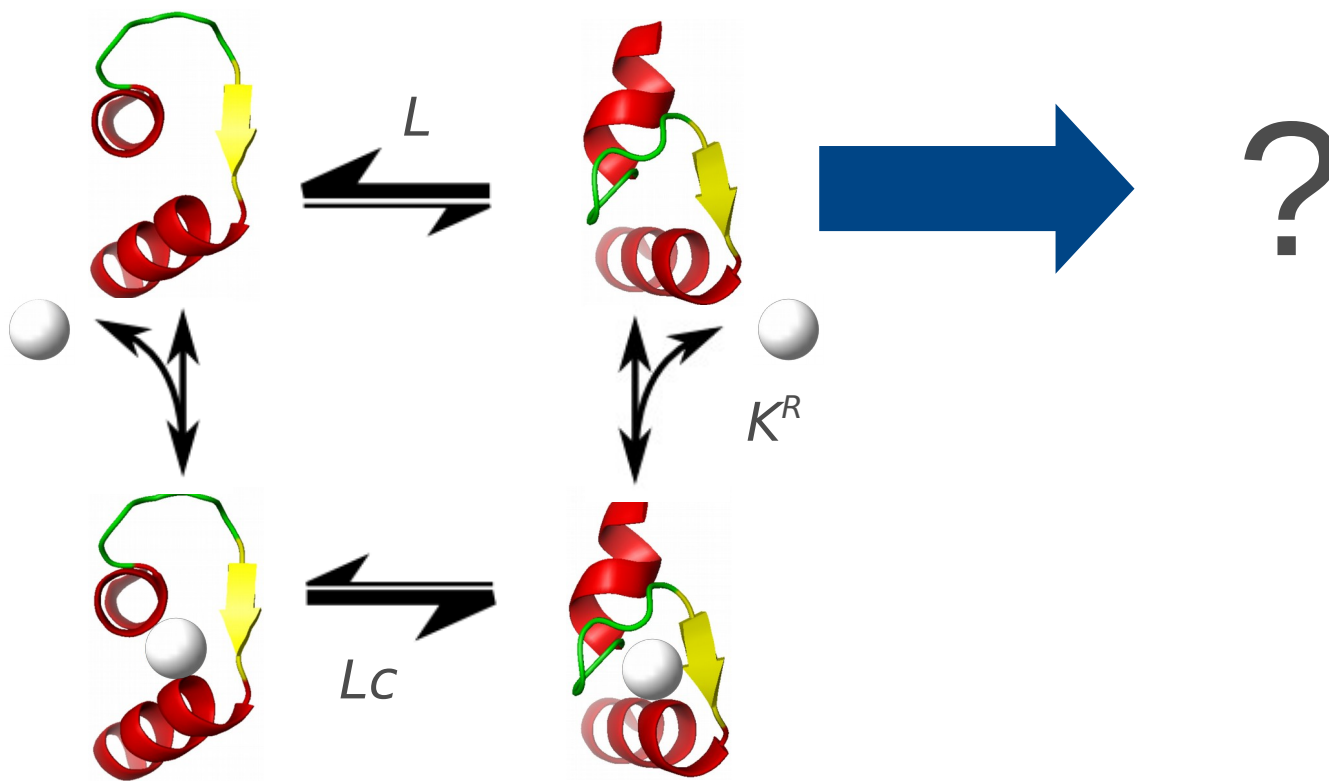
Kuboniwa *et al.* (1995).
Nat Struct Biol 2: 768-776

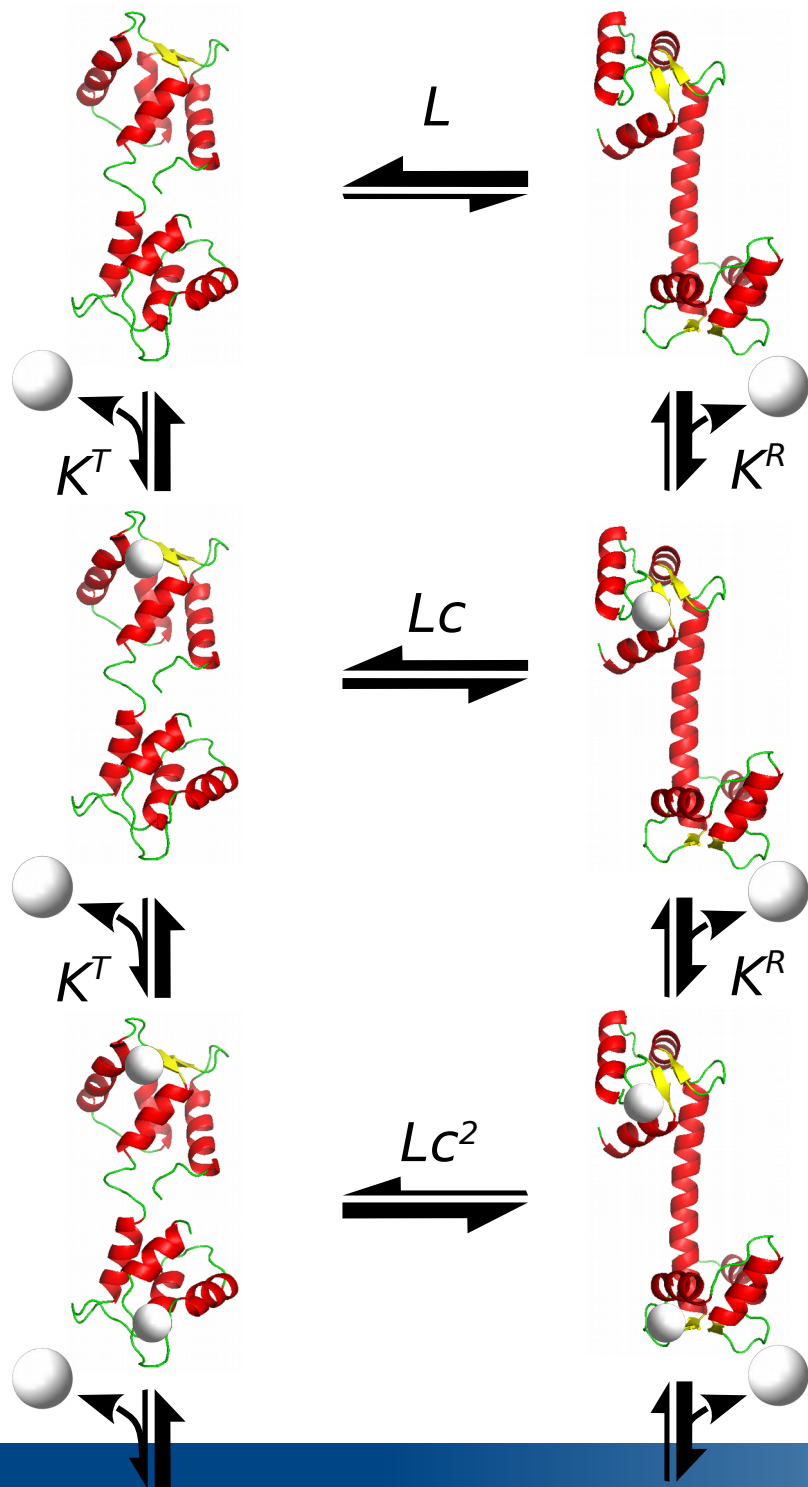






Warren *et al.* (2007).
J Mol Biol 374: 517-527



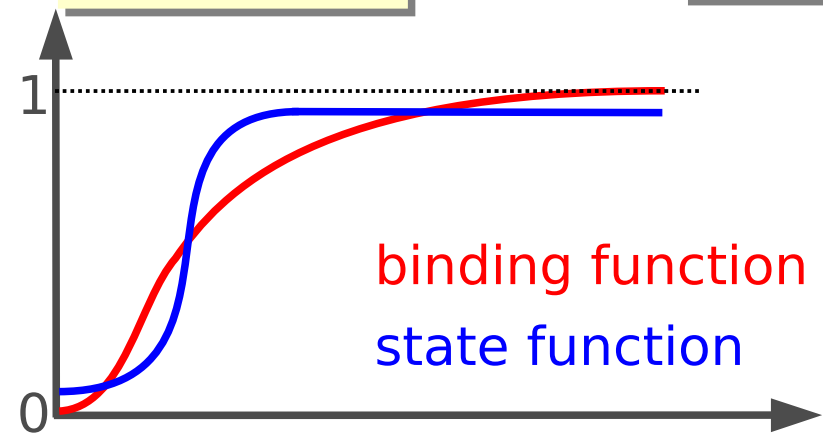


$$\bar{Y} = \frac{\alpha(1 + \alpha)^{n-1} + Lc\alpha(1 + c\alpha)^{n-1}}{(1 + \alpha)^n + L(1 + c\alpha)^n}$$

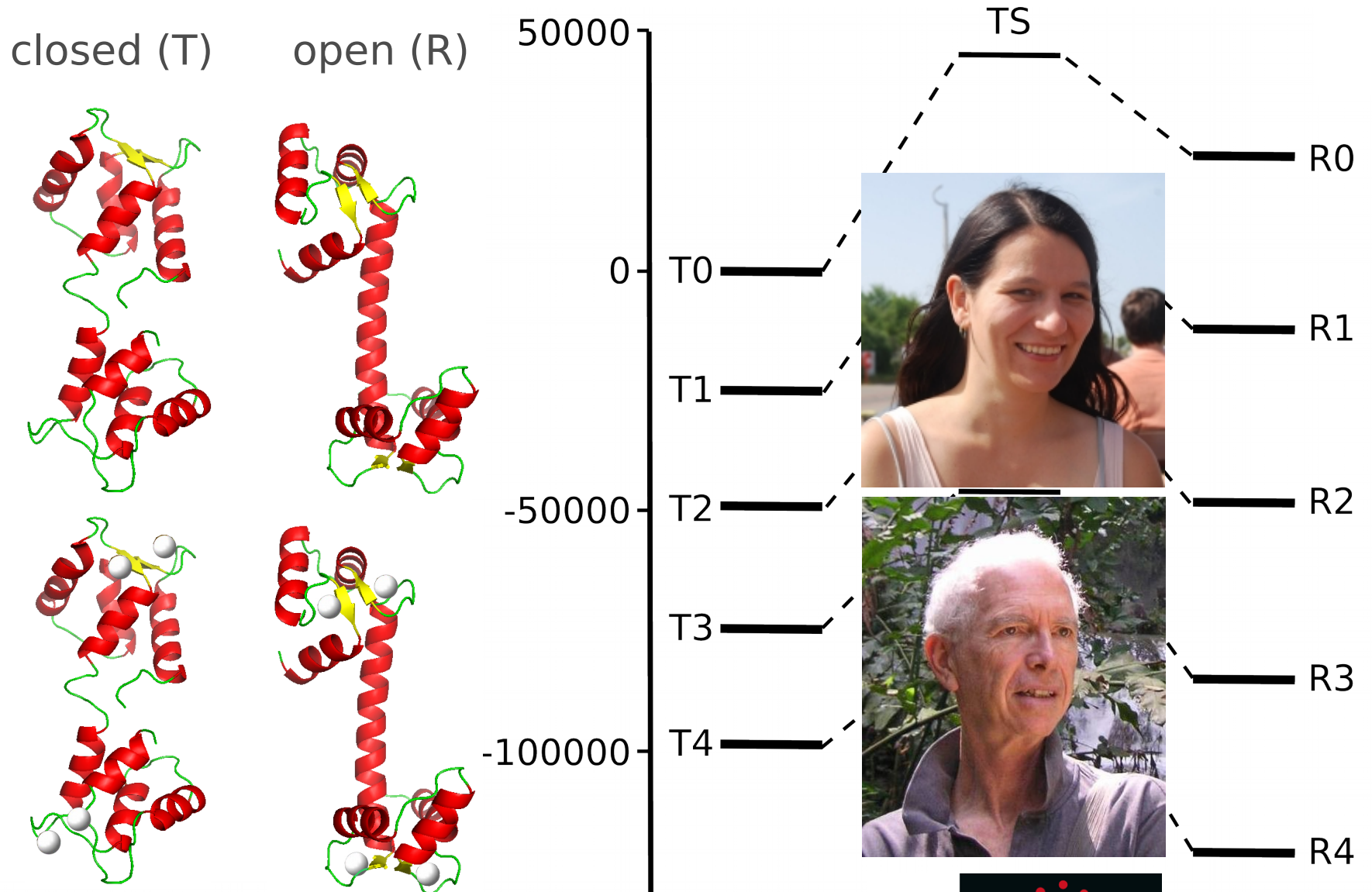
$$\bar{R} = \frac{(1 + \alpha)^n}{(1 + \alpha)^n + L(1 + c\alpha)^n}$$

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

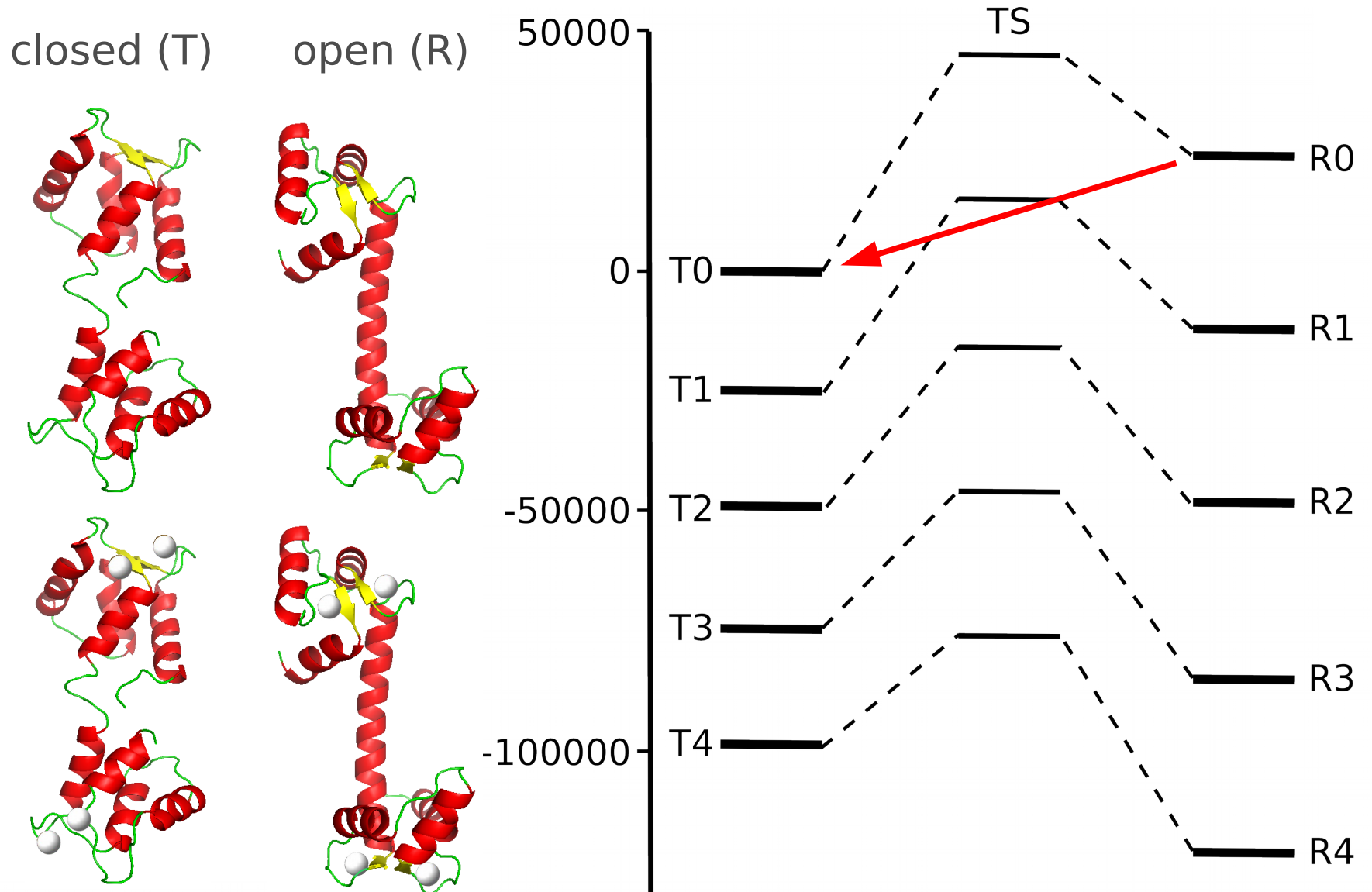
$L \gg 1 \Rightarrow$ Equilibrium strongly biased $c \ll 1 \Rightarrow$ strong effect of ligand



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



Stefan MI, Edelstein SJ, Le Novère N (2008)
 Stefan MI, Edelstein SJ, Le Novère N (2009)
 Edelstein SJ, Stefan MI, Le Novère N (2010)



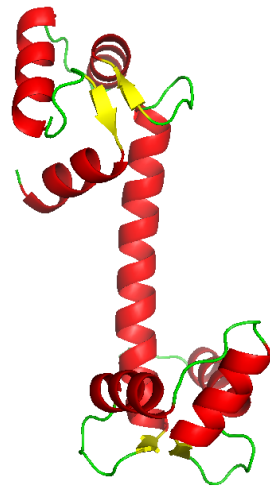
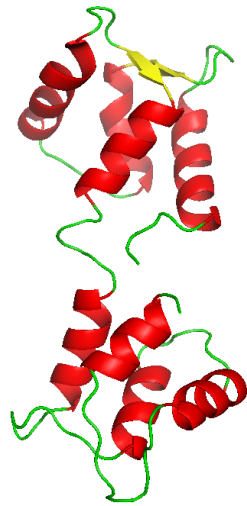
Stefan MI, Edelstein SJ, Le Novère N (2008)
 Stefan MI, Edelstein SJ, Le Novère N (2009)
 Edelstein SJ, Stefan MI, Le Novère N (2010)

closed (T)

open (R)

50000

APO



0

T0

T1

-50000

T2

T3

T4

-100000

TS

R0

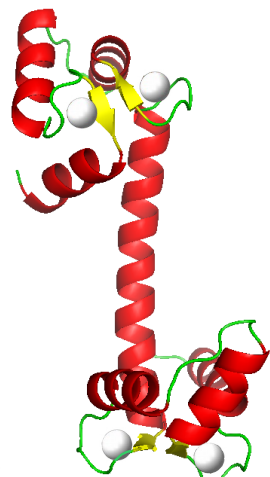
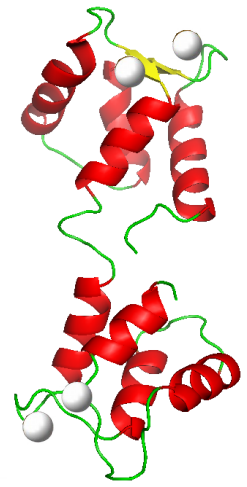
R1

R2

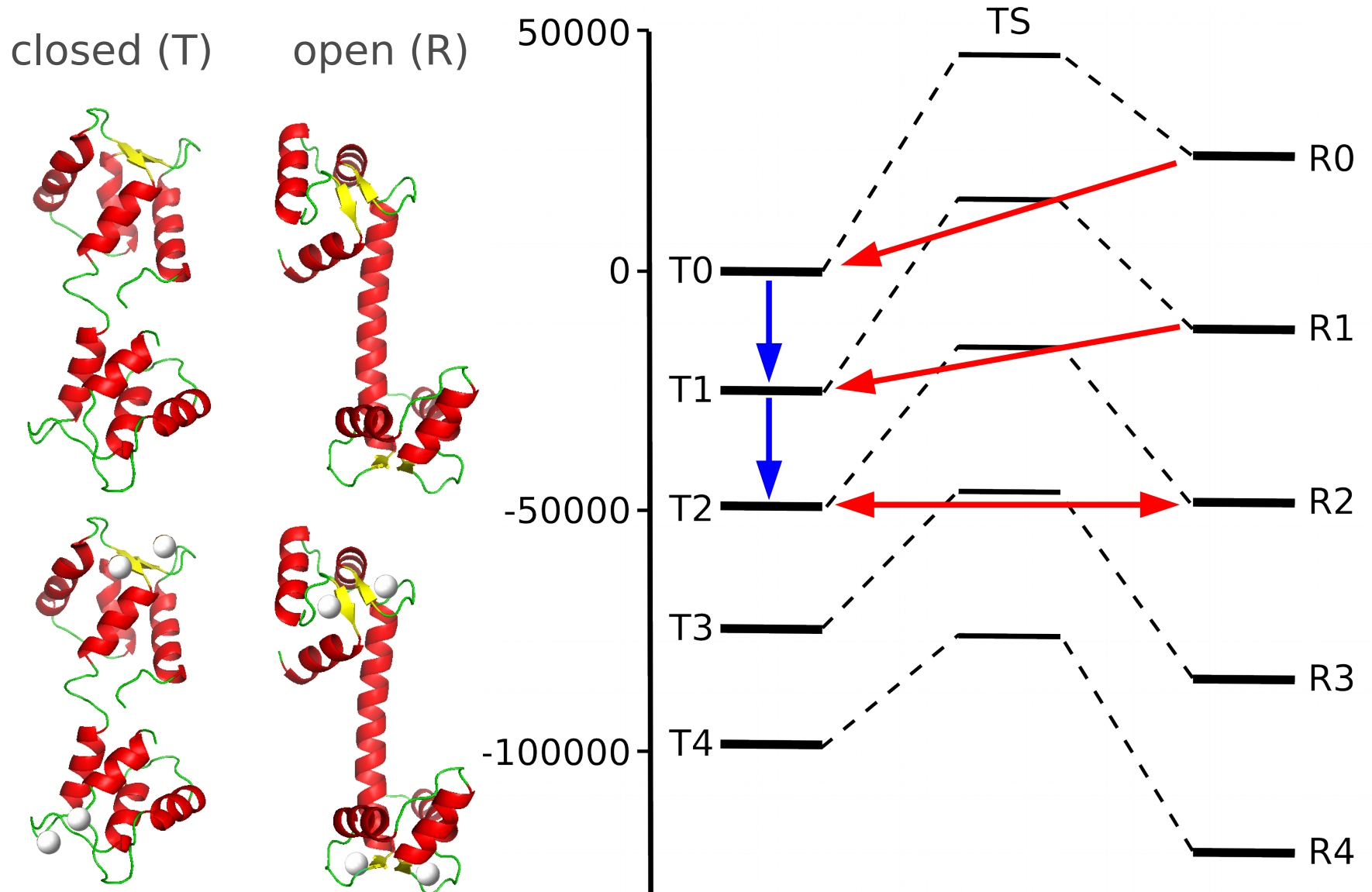
R3

R4

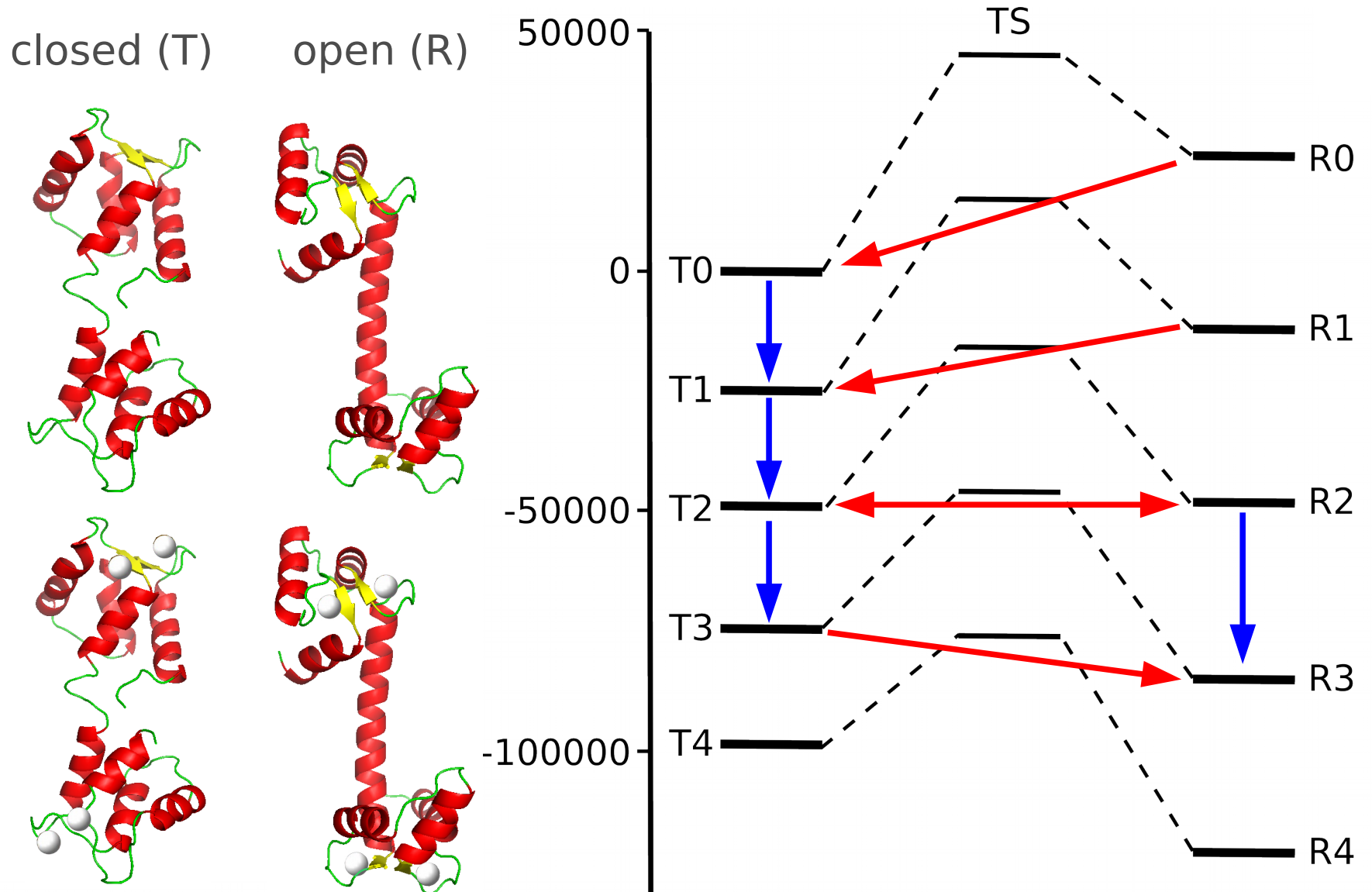
liganded



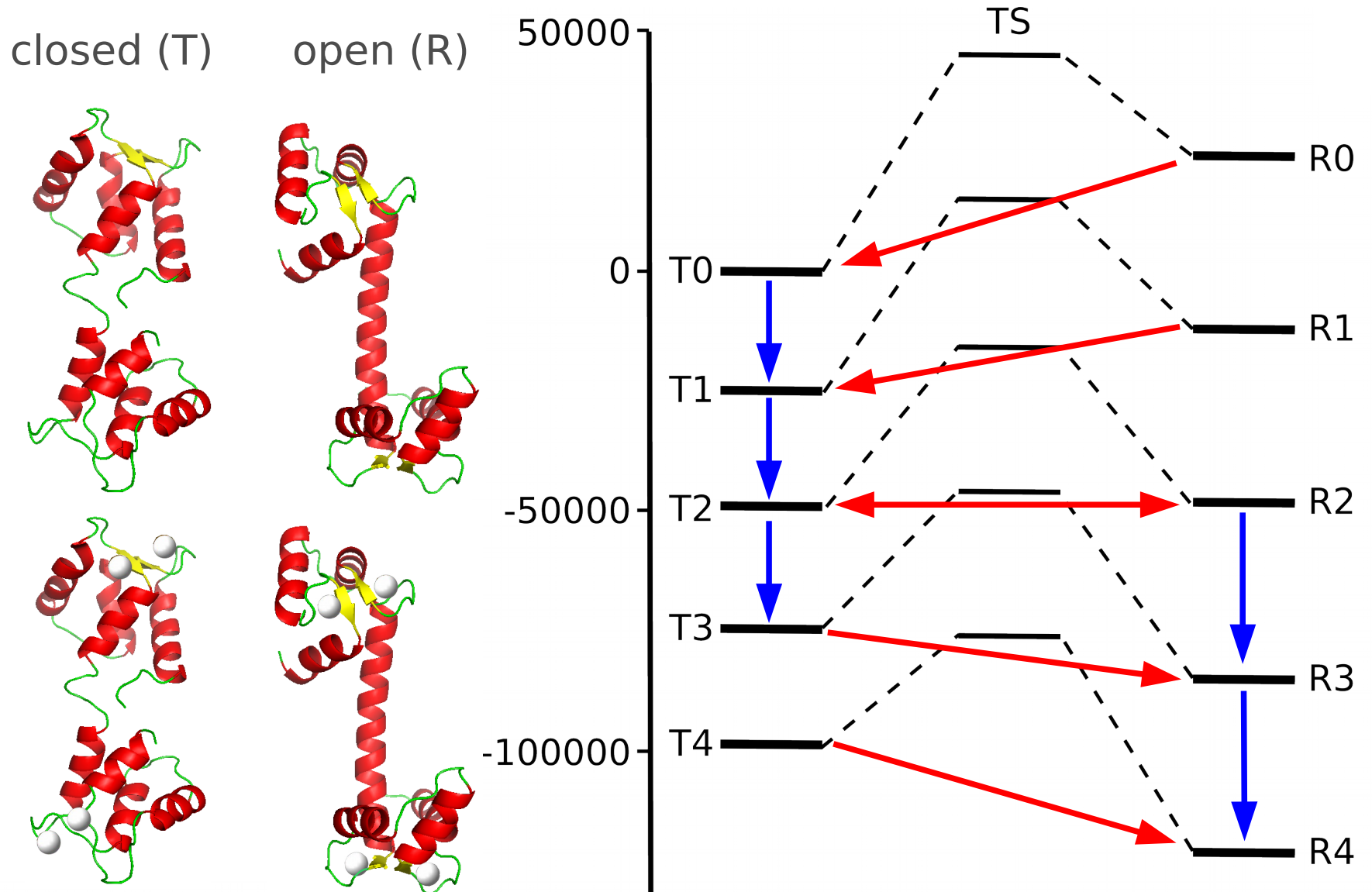
Stefan MI, Edelstein SJ, Le Novère N (2008)
 Stefan MI, Edelstein SJ, Le Novère N (2009)
 Edelstein SJ, Stefan MI, Le Novère N (2010)



Stefan MI, Edelstein SJ, Le Novère N (2008)
 Stefan MI, Edelstein SJ, Le Novère N (2009)
 Edelstein SJ, Stefan MI, Le Novère N (2010)

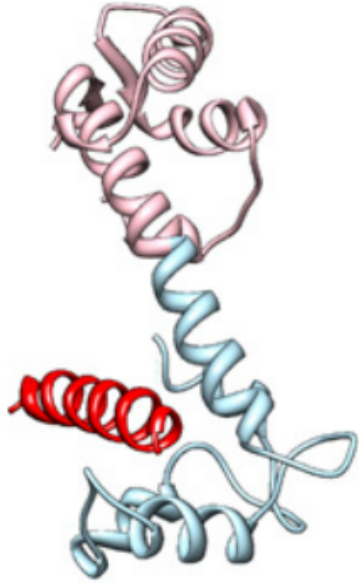


Stefan MI, Edelstein SJ, Le Novère N (2008)
 Stefan MI, Edelstein SJ, Le Novère N (2009)
 Edelstein SJ, Stefan MI, Le Novère N (2010)

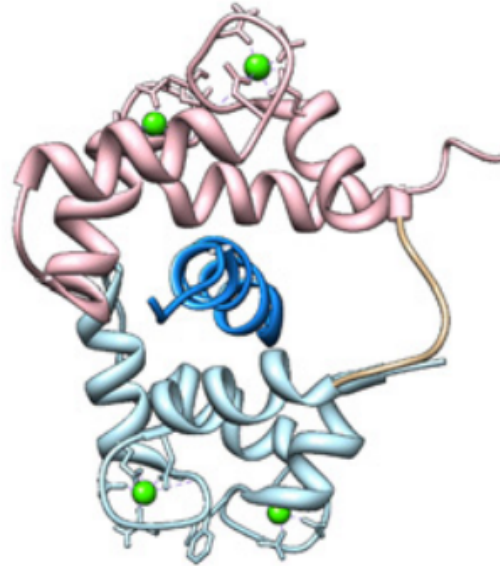


Stefan MI, Edelstein SJ, Le Novère N (2008)
 Stefan MI, Edelstein SJ, Le Novère N (2009)
 Edelstein SJ, Stefan MI, Le Novère N (2010)

Different targets stabilise lobes in different states

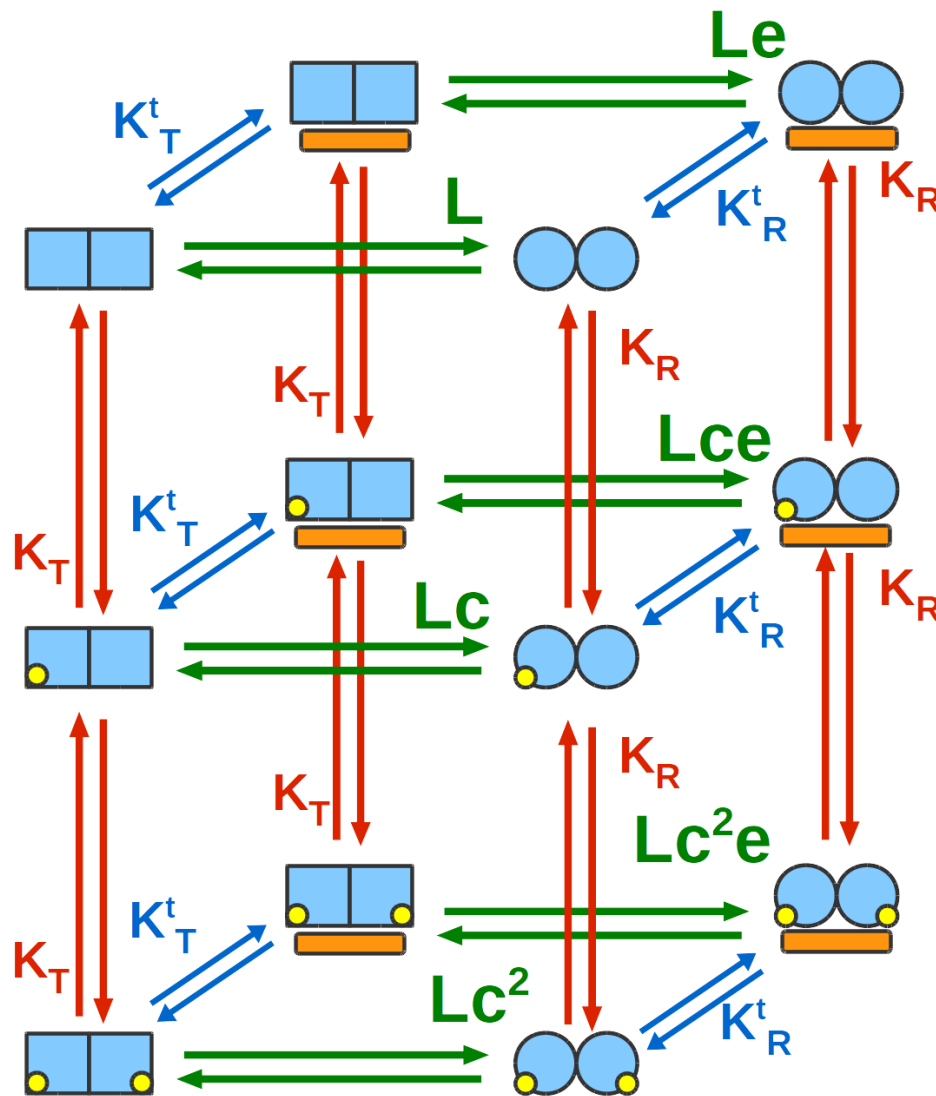


Neurogranin

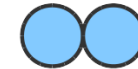


MLCK

Bindings of calcium and targets



T-state



R-state



Calcium



Target



Conf. transition



Calcium binding

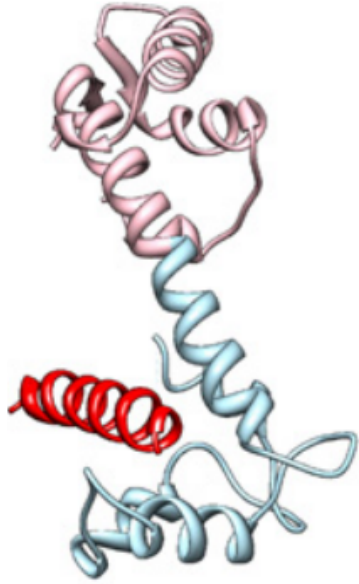


Target binding

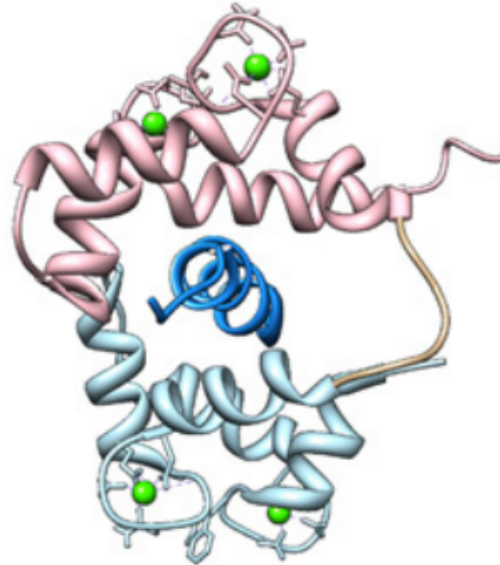
$$c = \frac{K_R}{K_T}$$

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

Different targets stabilise lobes in different states



Neurogranin



MLCK

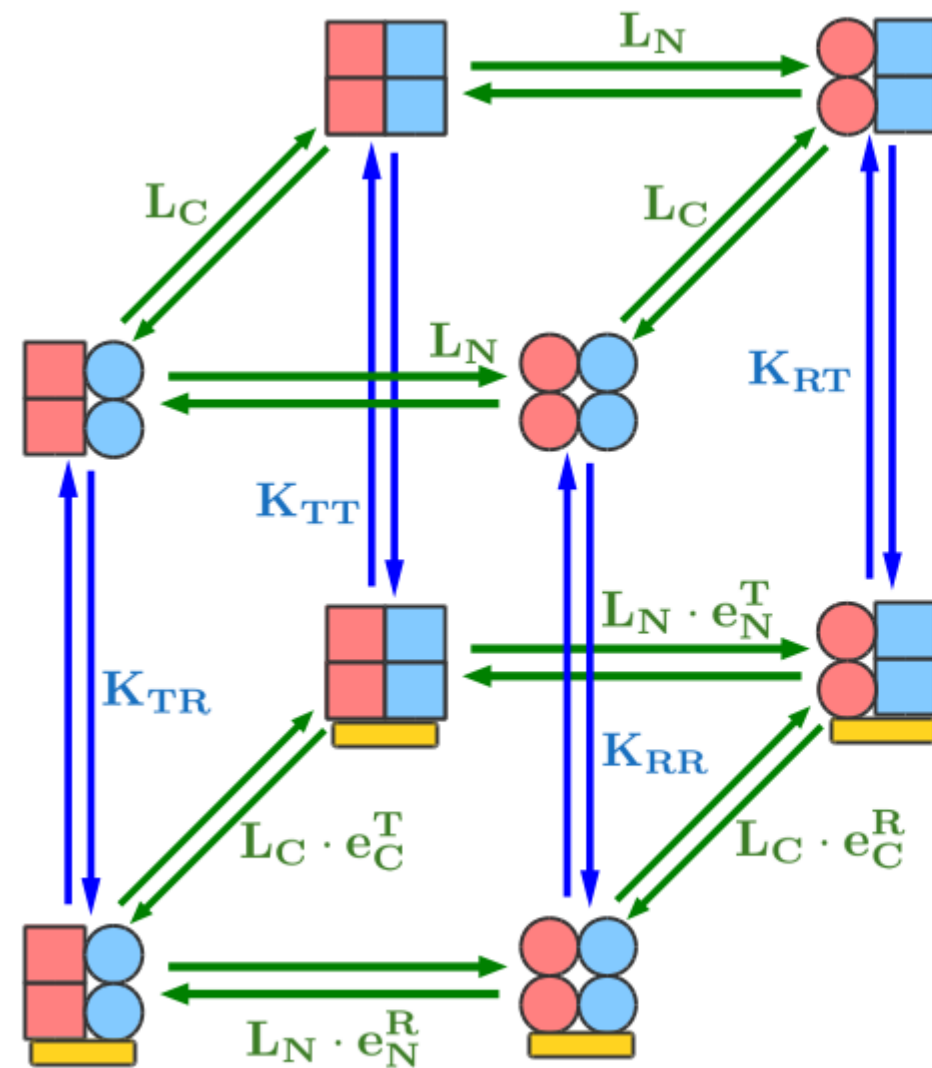


SK channel

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



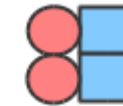
= R-state



= target



= TT conformation



= RT conformation



= TR conformation



= RR conformation

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

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

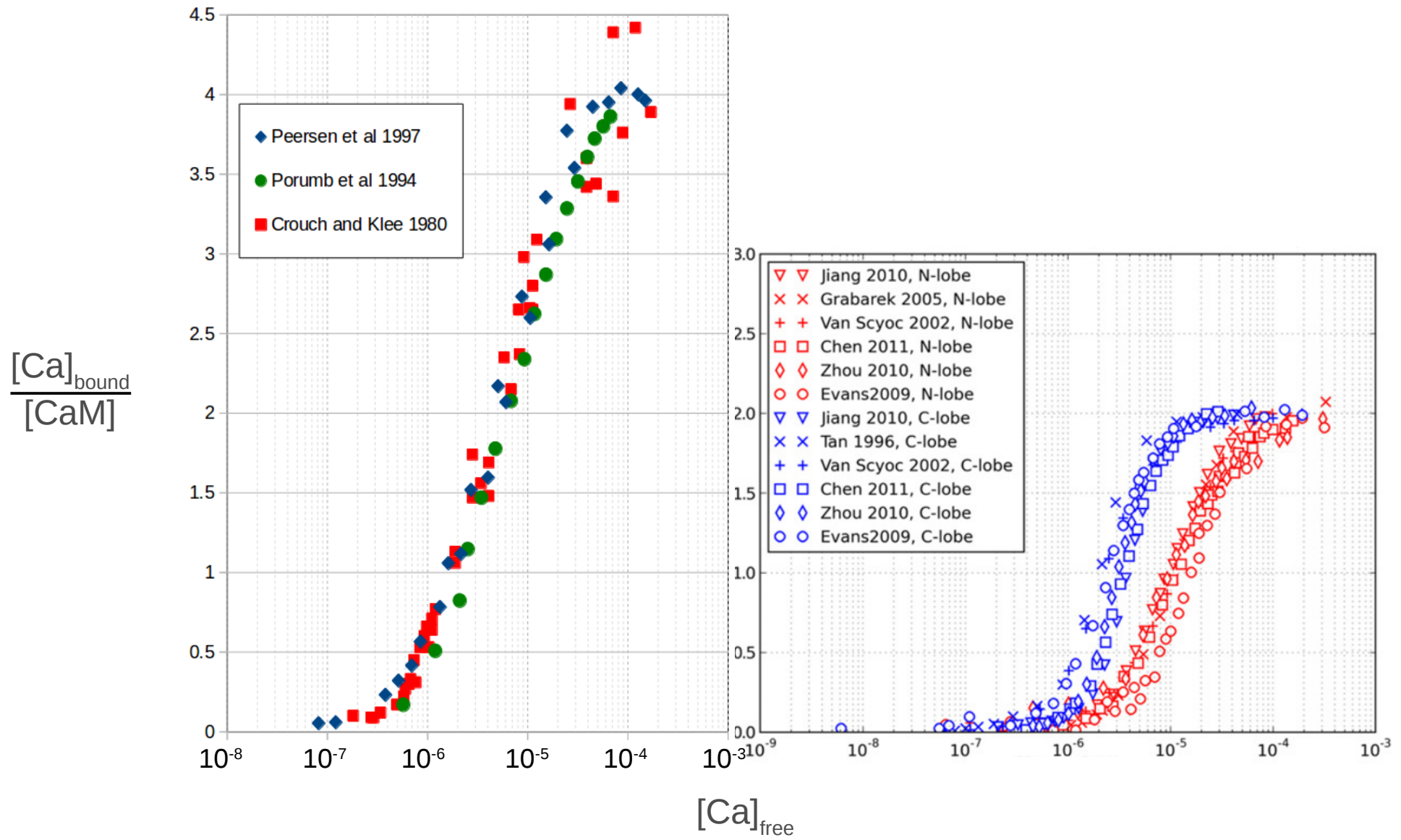
$$e_N^R = \frac{K_{RR}}{K_{TR}}$$

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

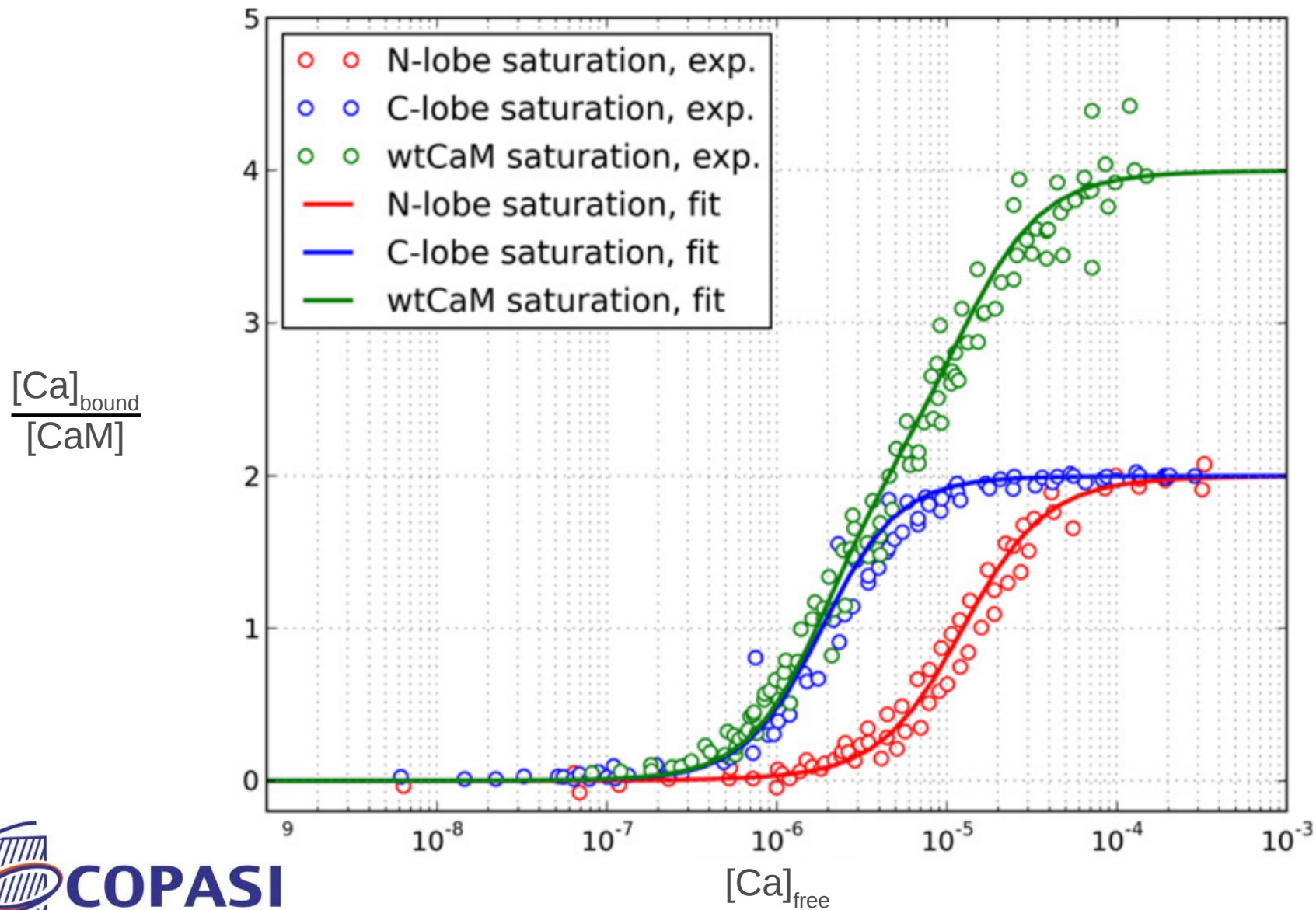
$$e_N^T = \frac{K_{RT}}{K_{TT}}$$

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

Calcium binding to lobes and whole CaM (exp)



Calcium binding to lobes and whole CaM (sim)



Parametrisation using accurate measurements

- 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

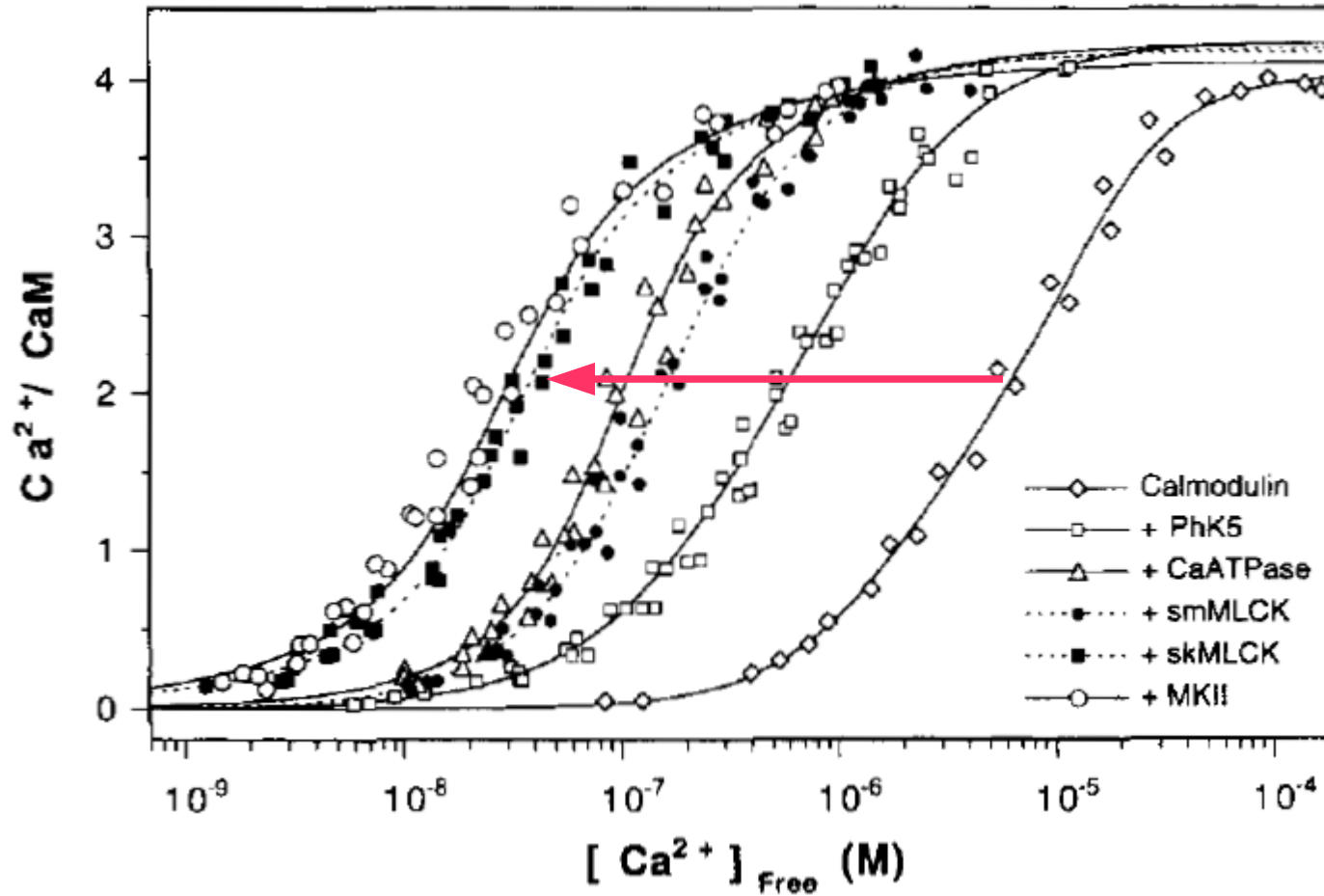
Activity of unsaturated calmodulin (state function)

- Fractional activity depends on the number of calcium ions bound

$$\frac{R_i}{T_i} = \frac{1}{L \cdot c^i}$$

- $R_0/T_0 = 1/20000$ (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: experiments



Peersen et al. (1997)

Targets as allosteric effectors: theory

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

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

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

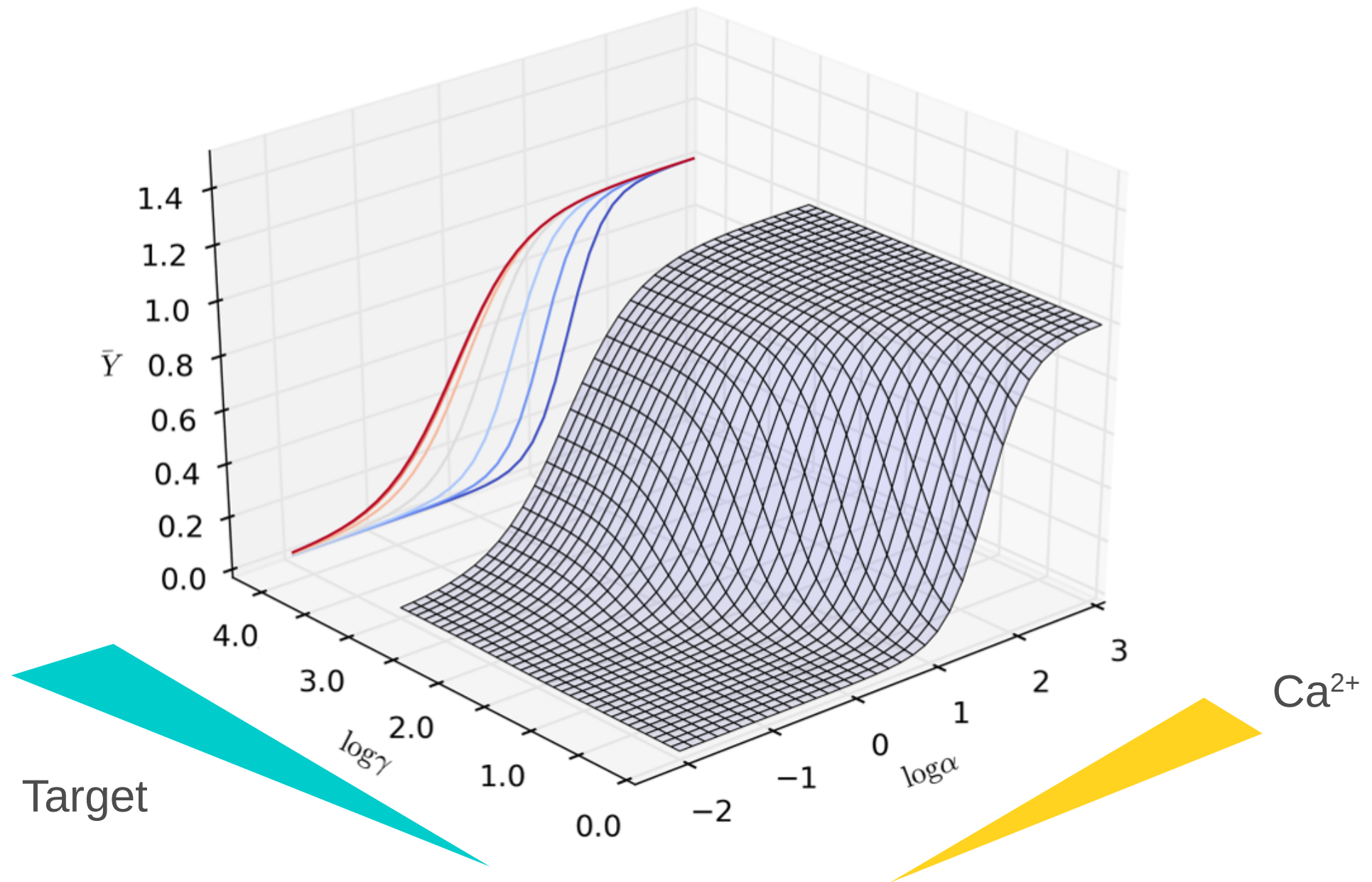
$$c = \frac{K^R}{K^T}$$

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

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

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

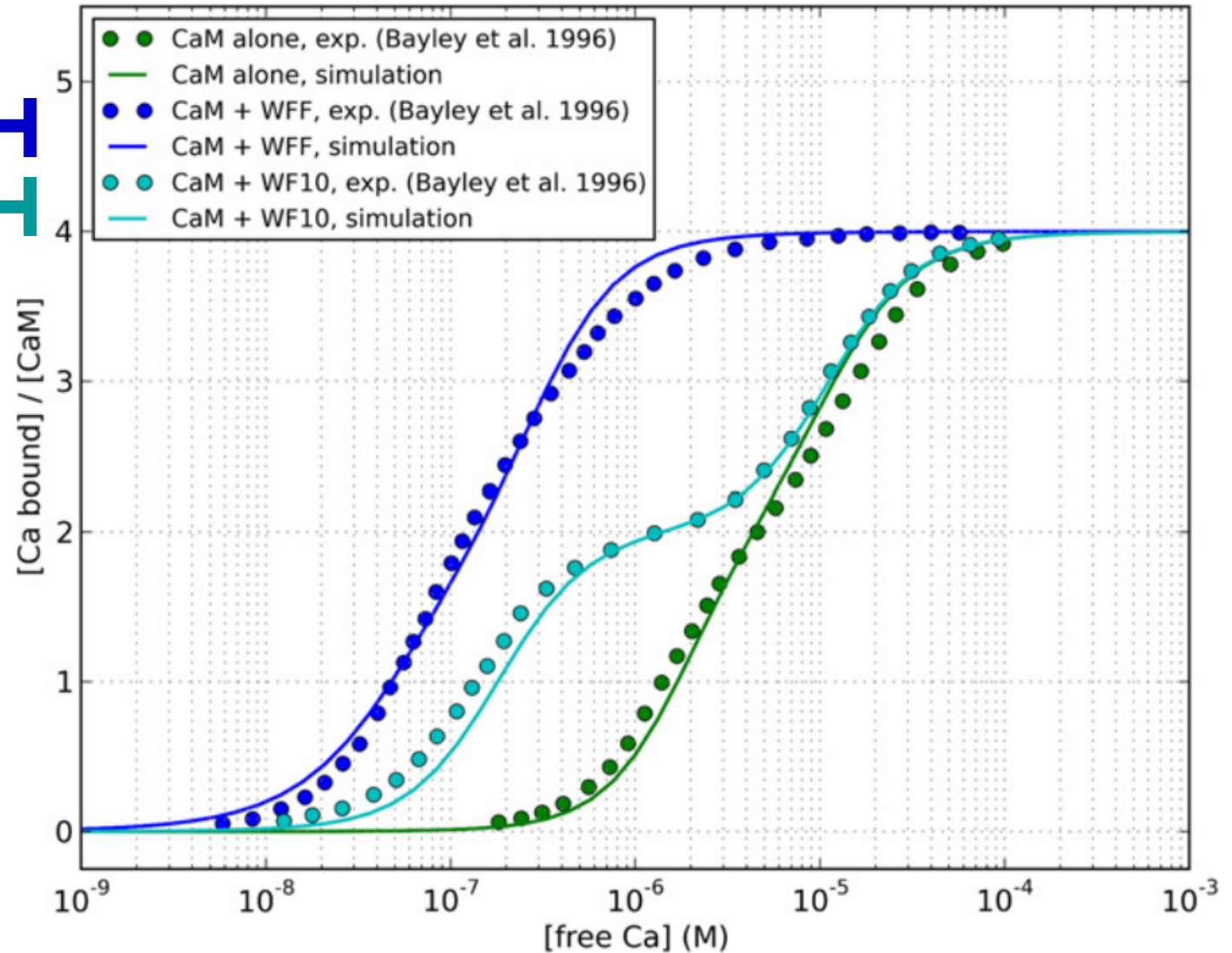
Effect of an R-stabilizing target on Ca^{2+} affinity



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

Stabilise R state
of both lobes

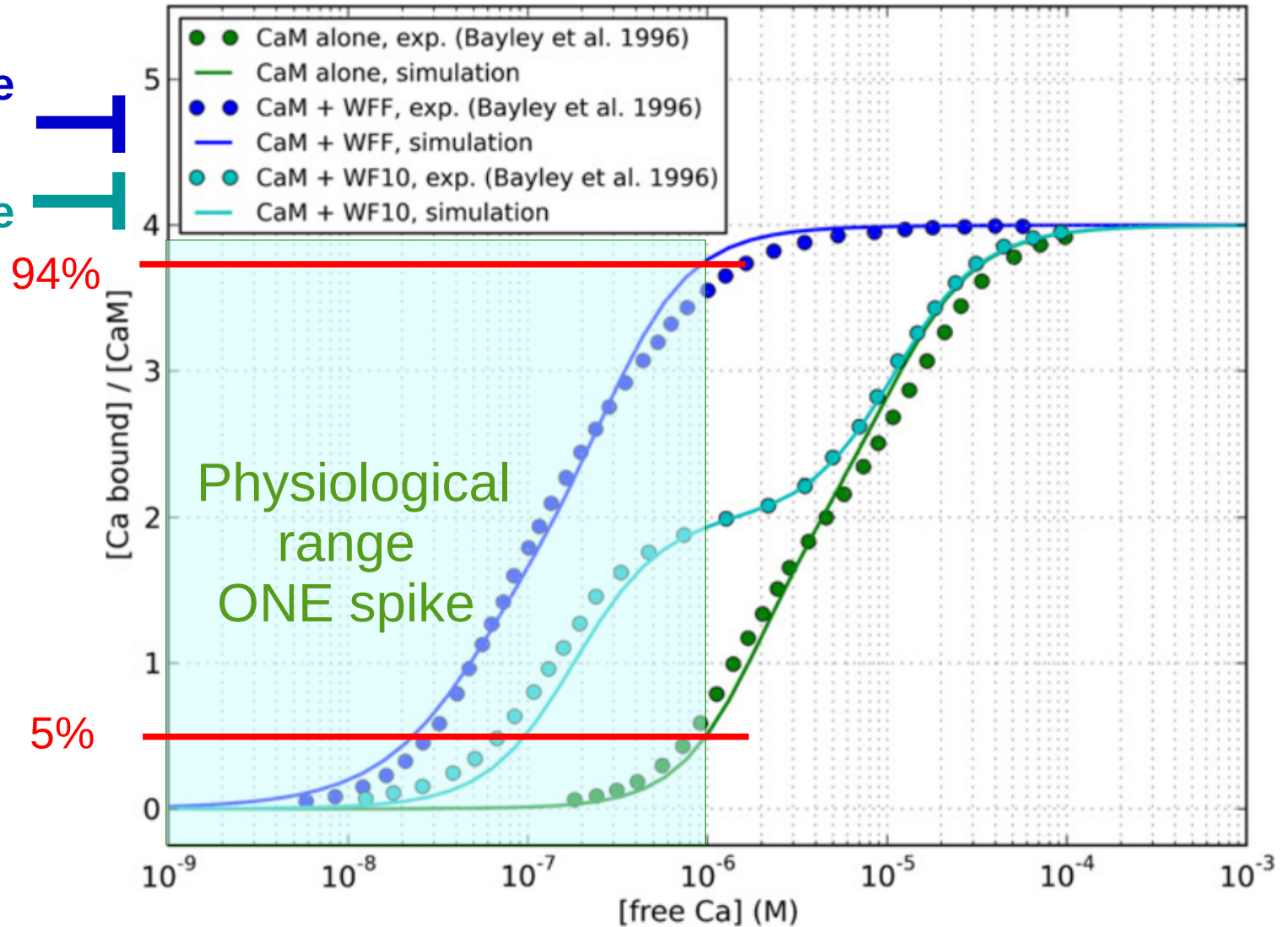
Stabilise R state
of C lobe



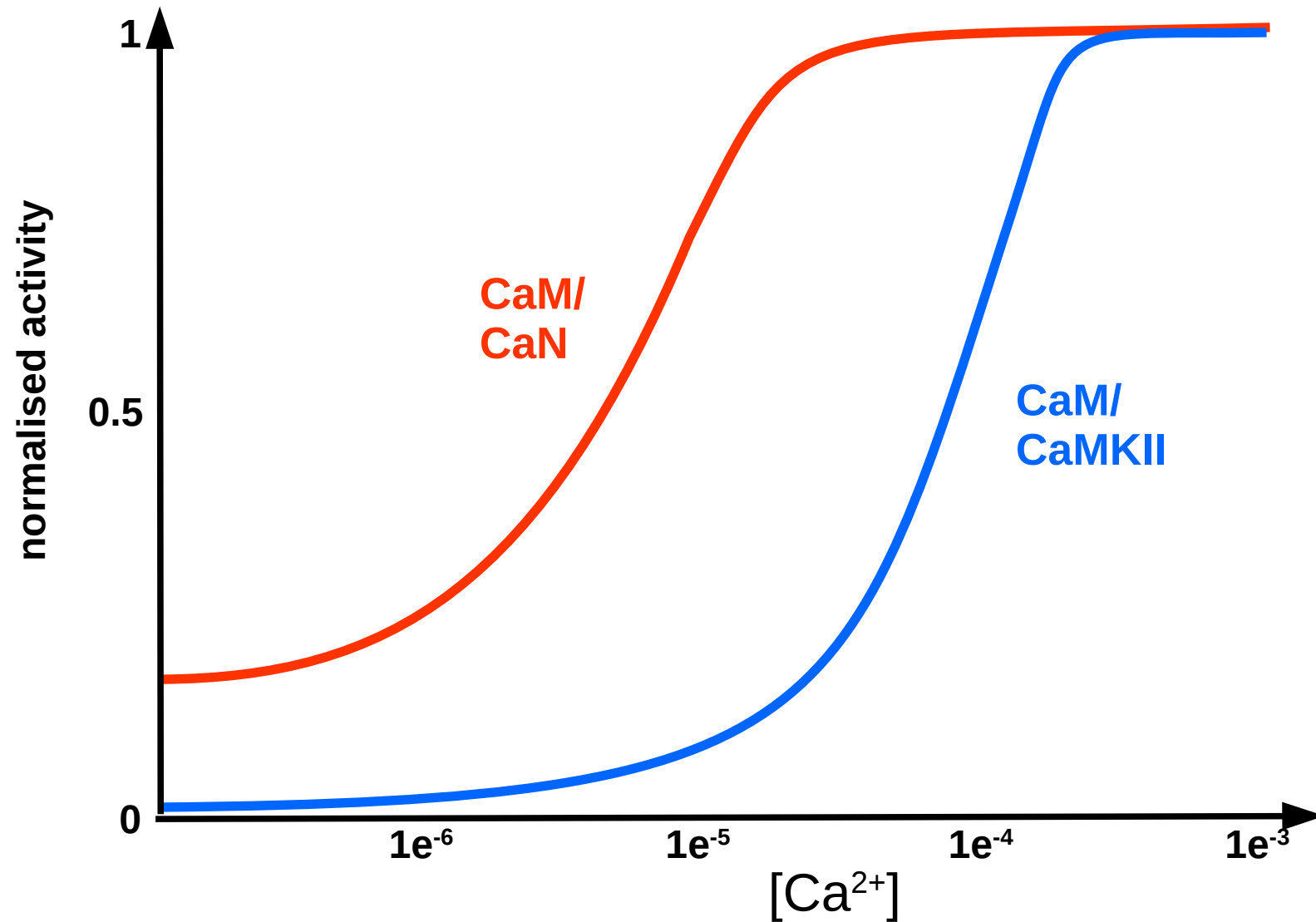
Targets stabilises Ca^{2+} binding into the physiological range

Stabilise R state of both lobes

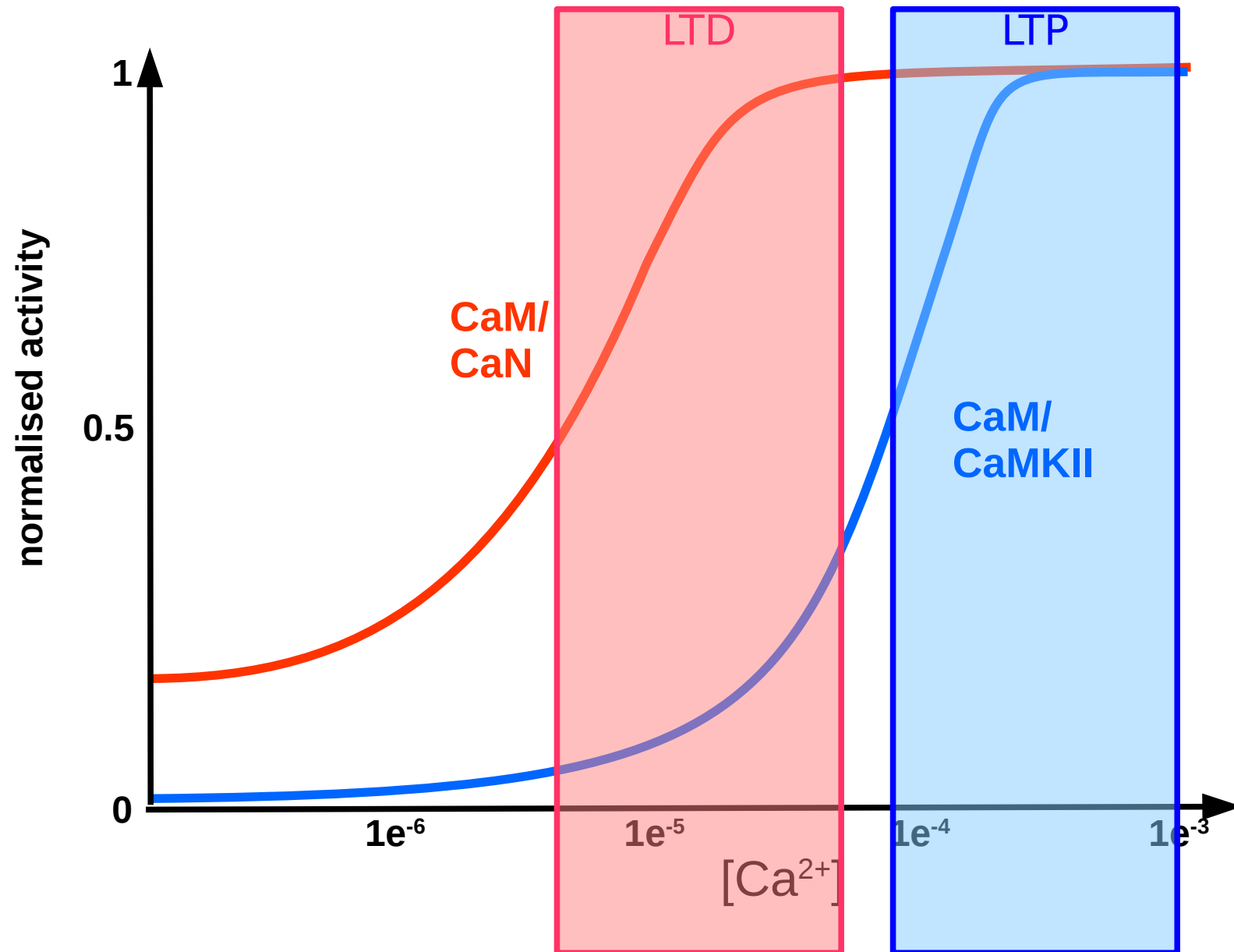
Stabilise R state of C lobe



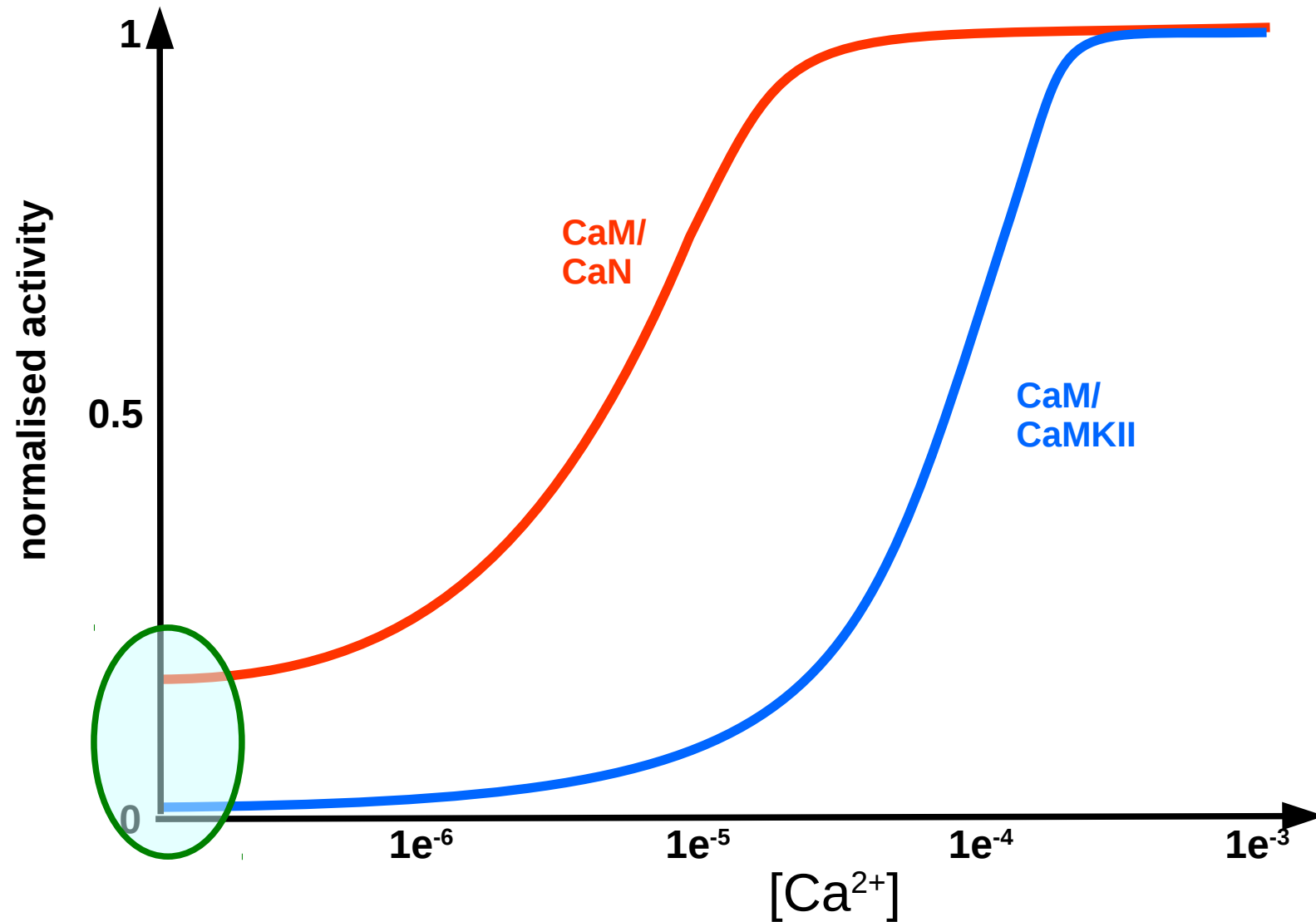
Calmodulin, its ligand and its targets



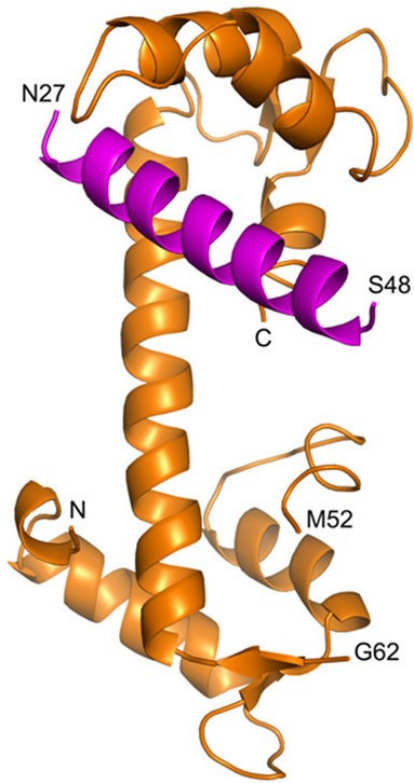
Bidirectional synaptic plasticity



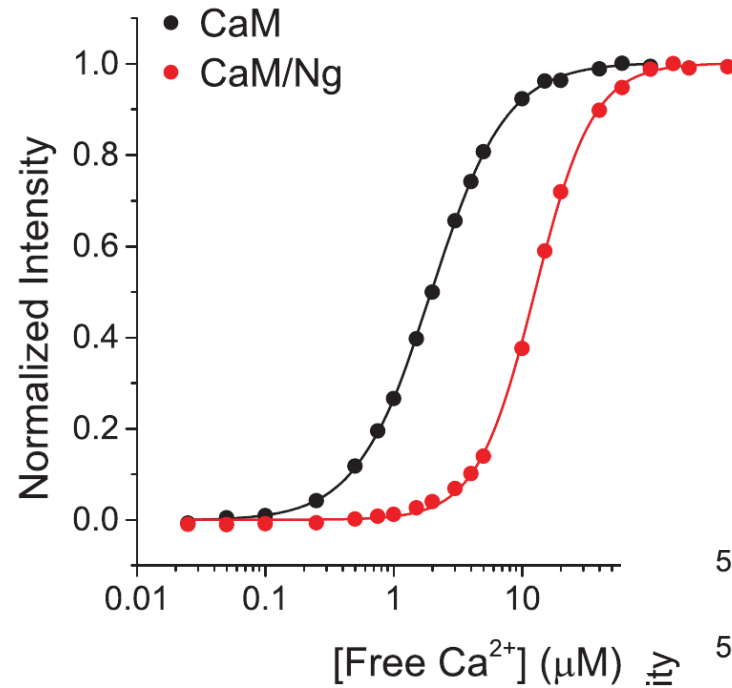
Calcineurin stabilises CaM R → no deactivation



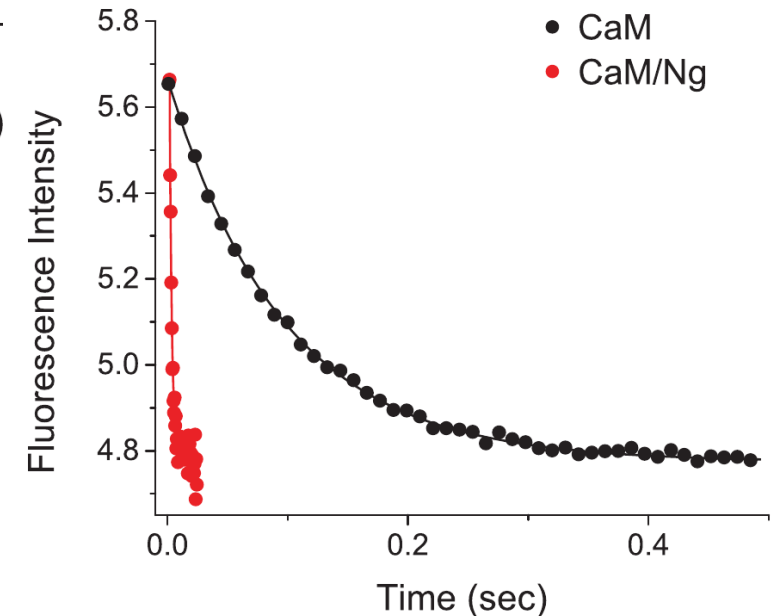
Neurogranin binds apo-CaM, decreases affinity for $[Ca^{2+}]$ and increases dissociation rate



Kumar *et al* (2013)



Hoffman *et al* (2014)



Effect of R and T stabilising targets on CaM affinity

Bayley *et al* (1996)

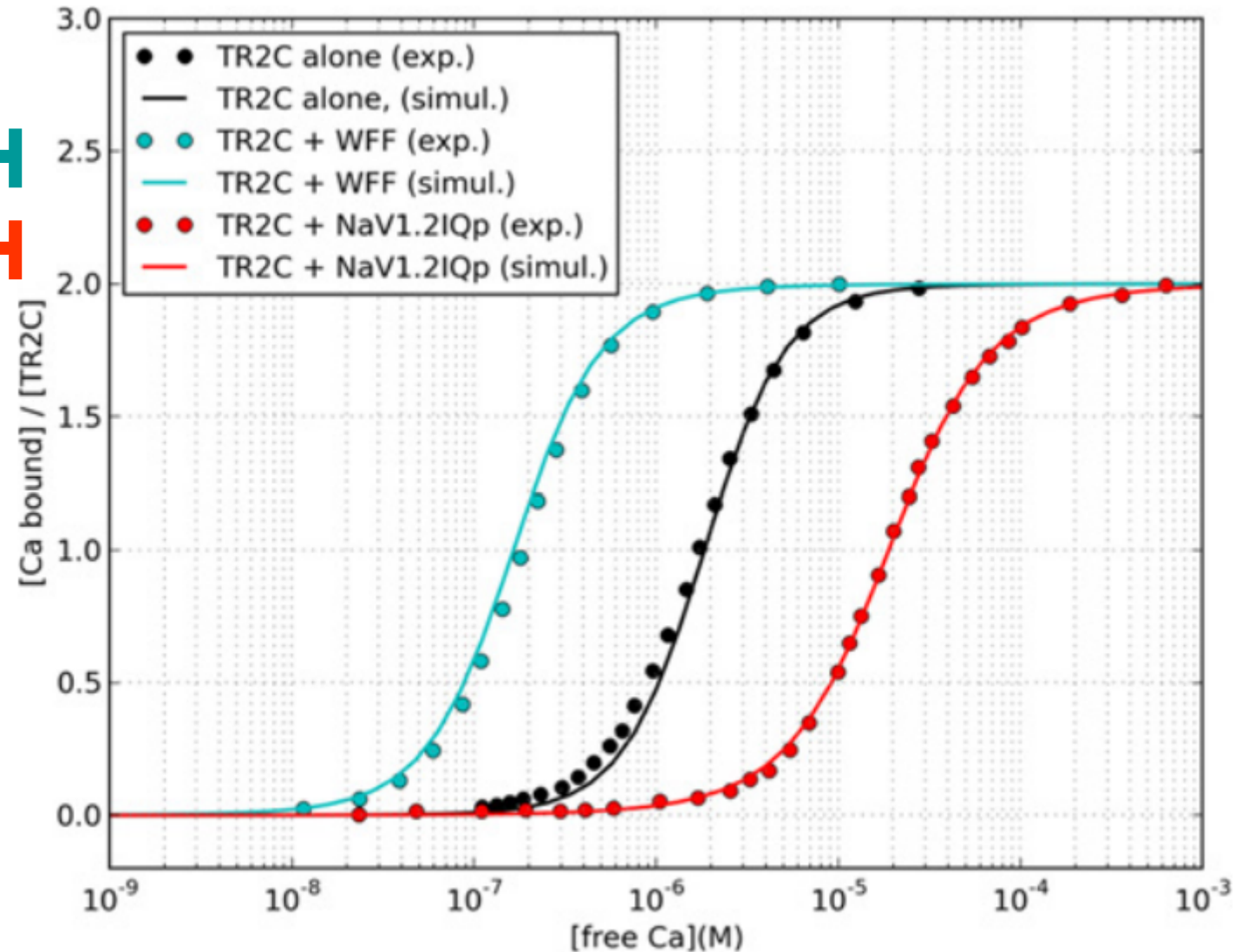
R stabilising
peptide

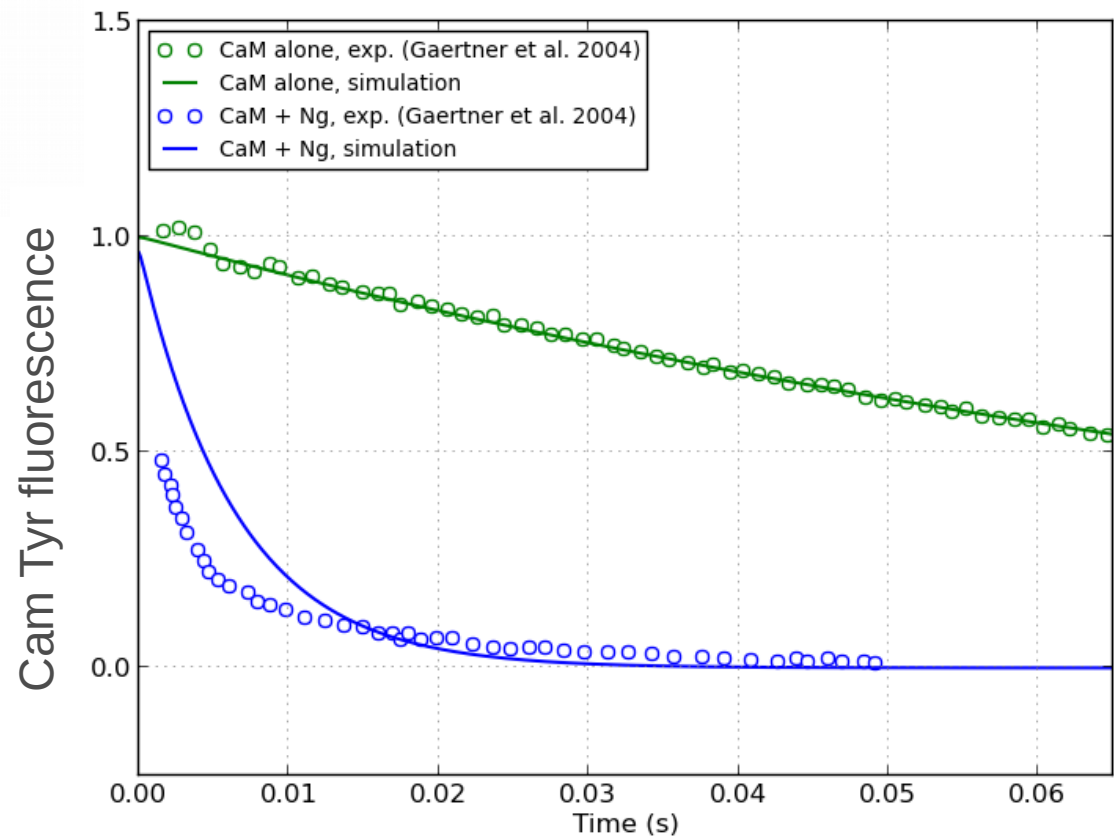
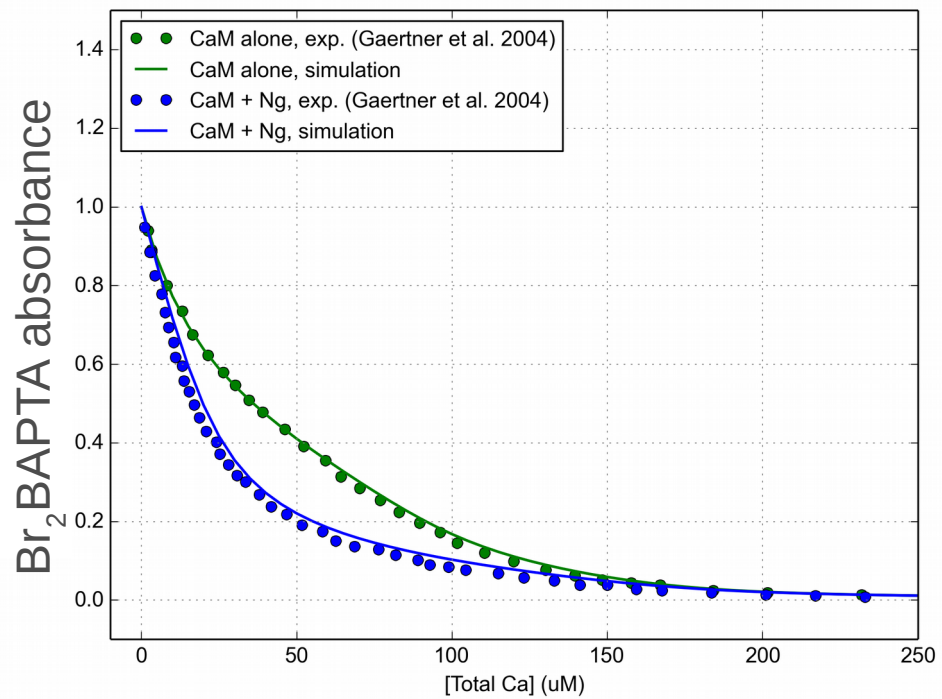


T stabilising
Peptide (IQ domain)

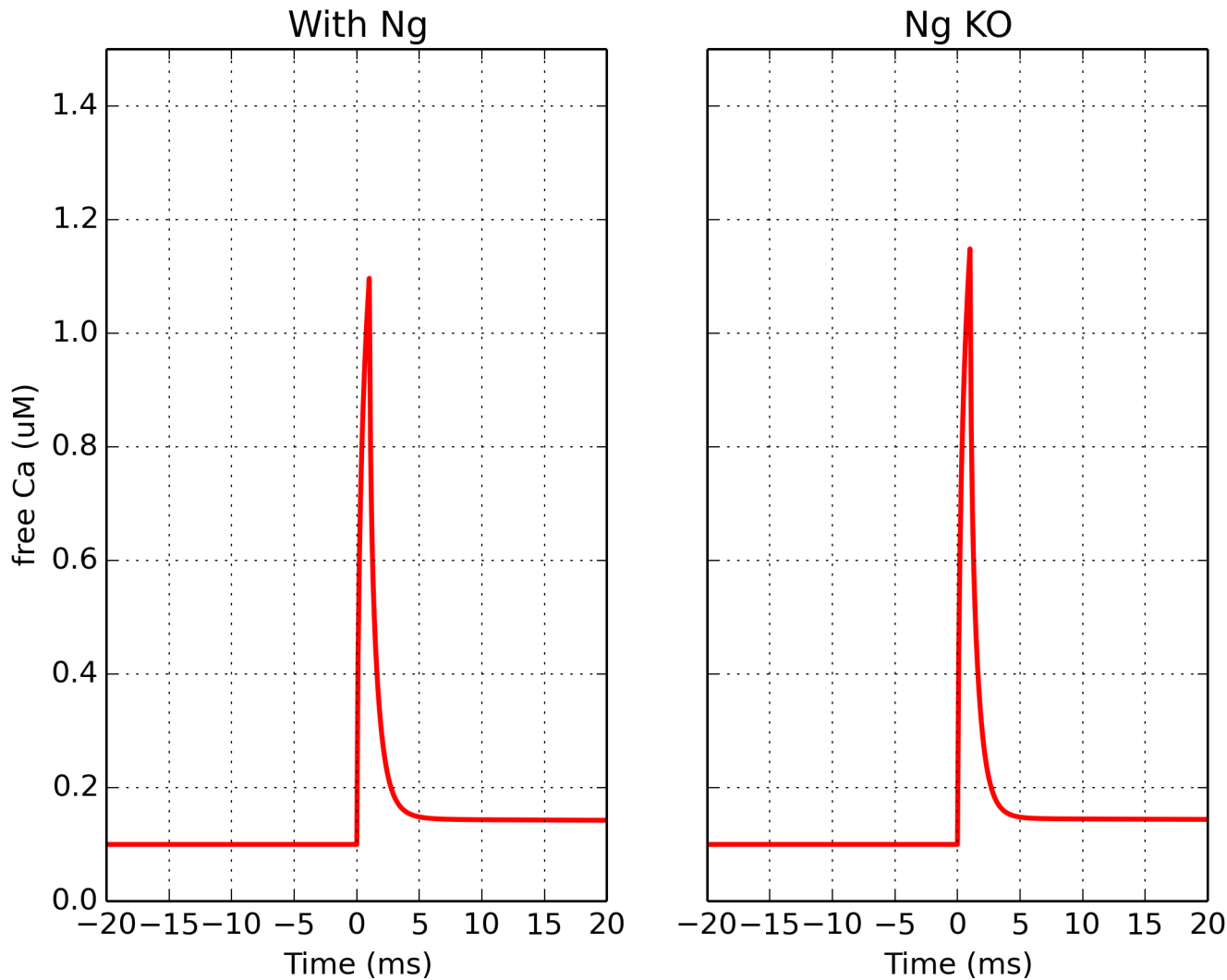


Theoharis *et al* (2008)

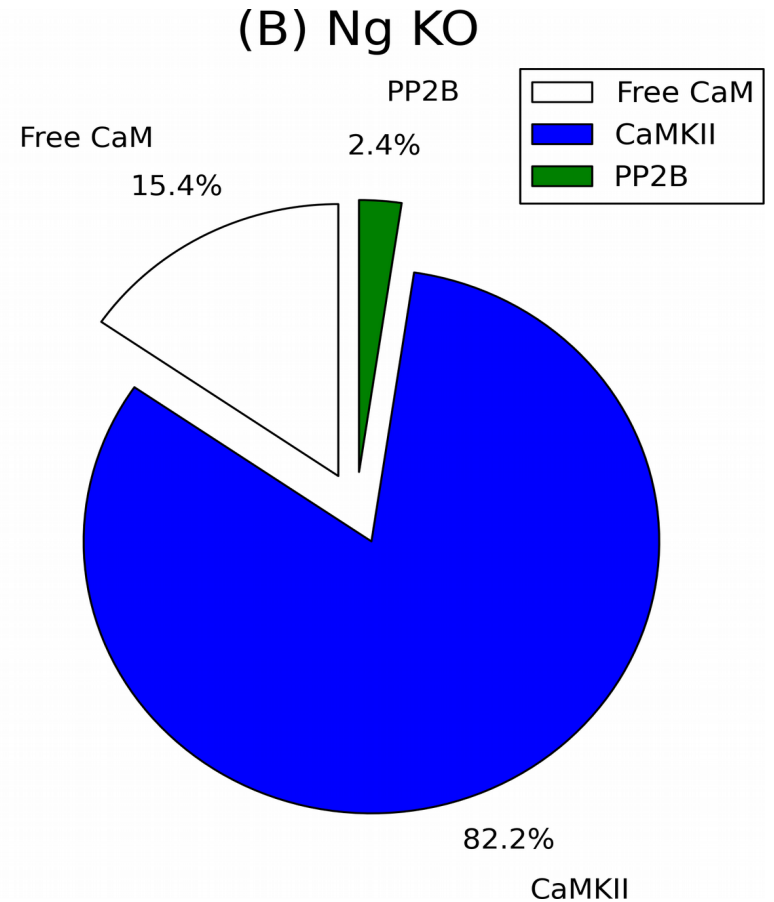
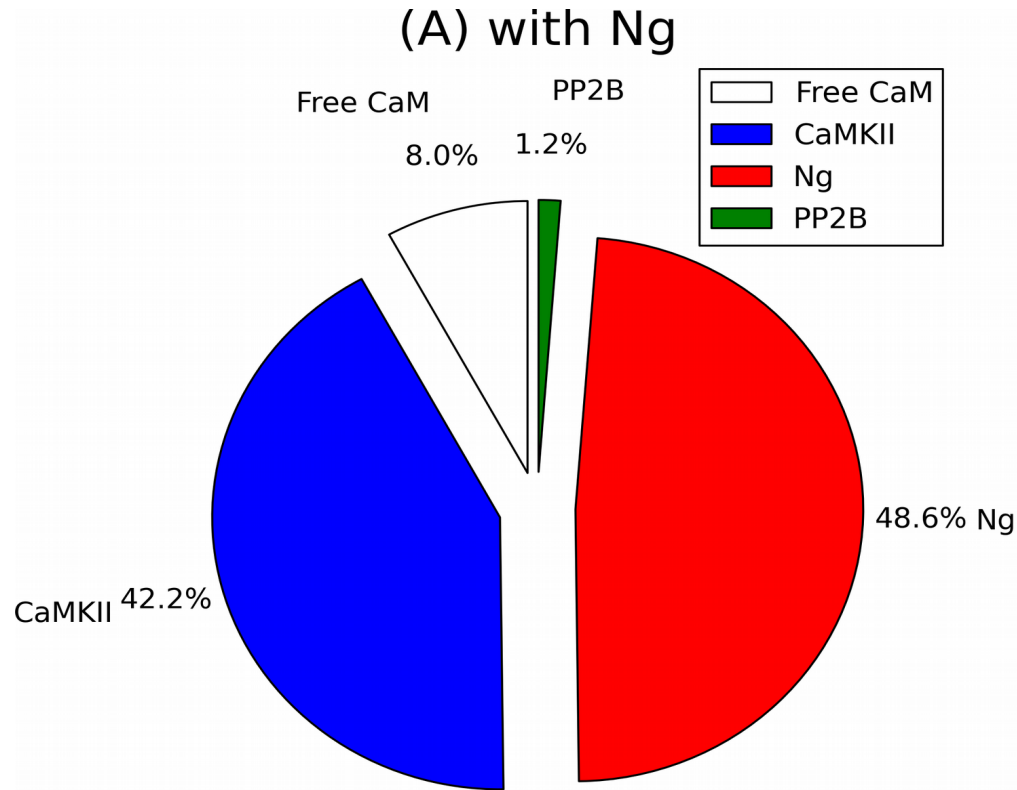




No large effect of Neurogranin on $[Ca^{2+}]_{free}$



Ng affects the distribution of CaM



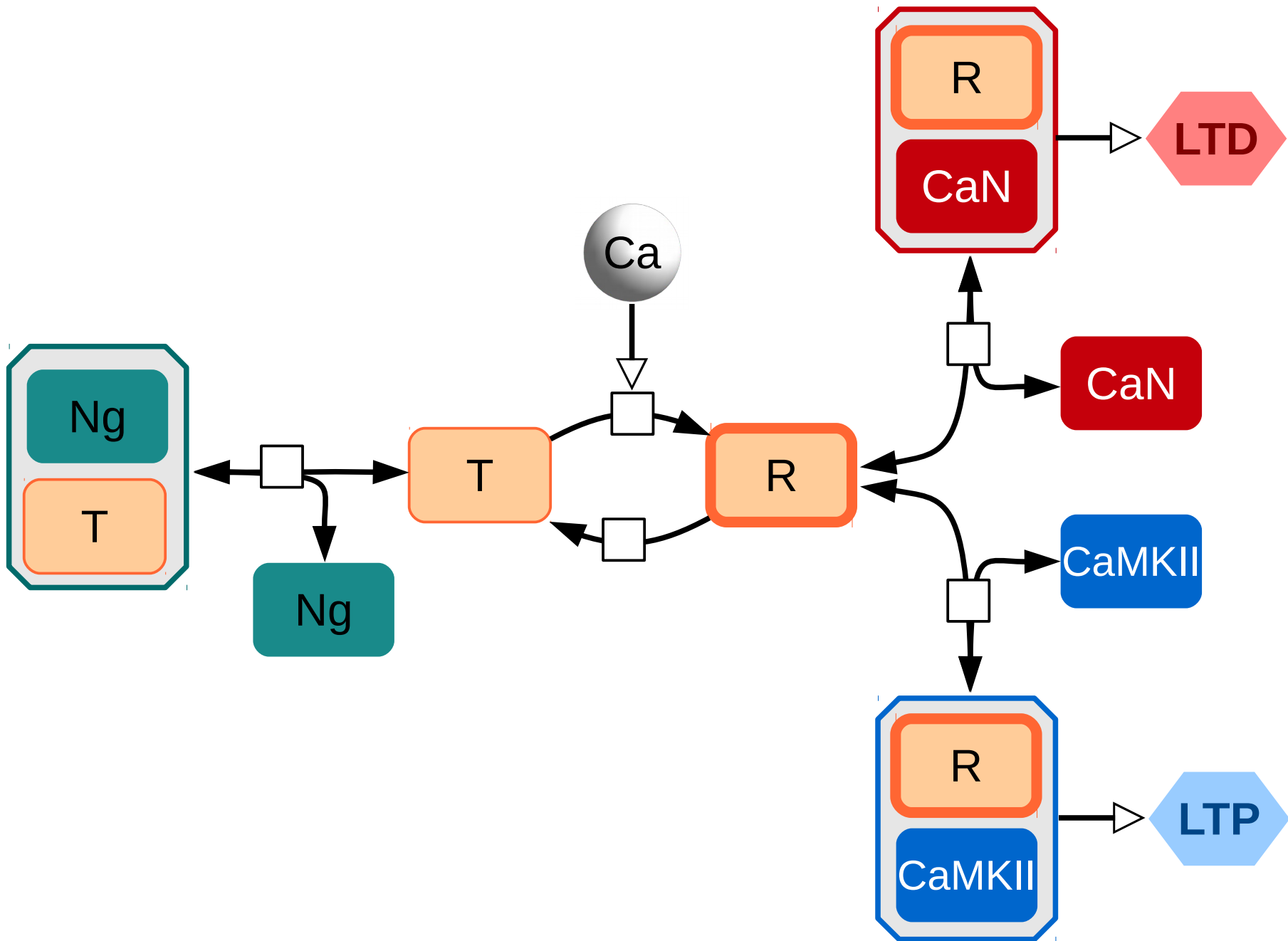
Hemi-concerted model of Calmodulin, 2 states for EF hands, binding Ca^{2+} with different affinities

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

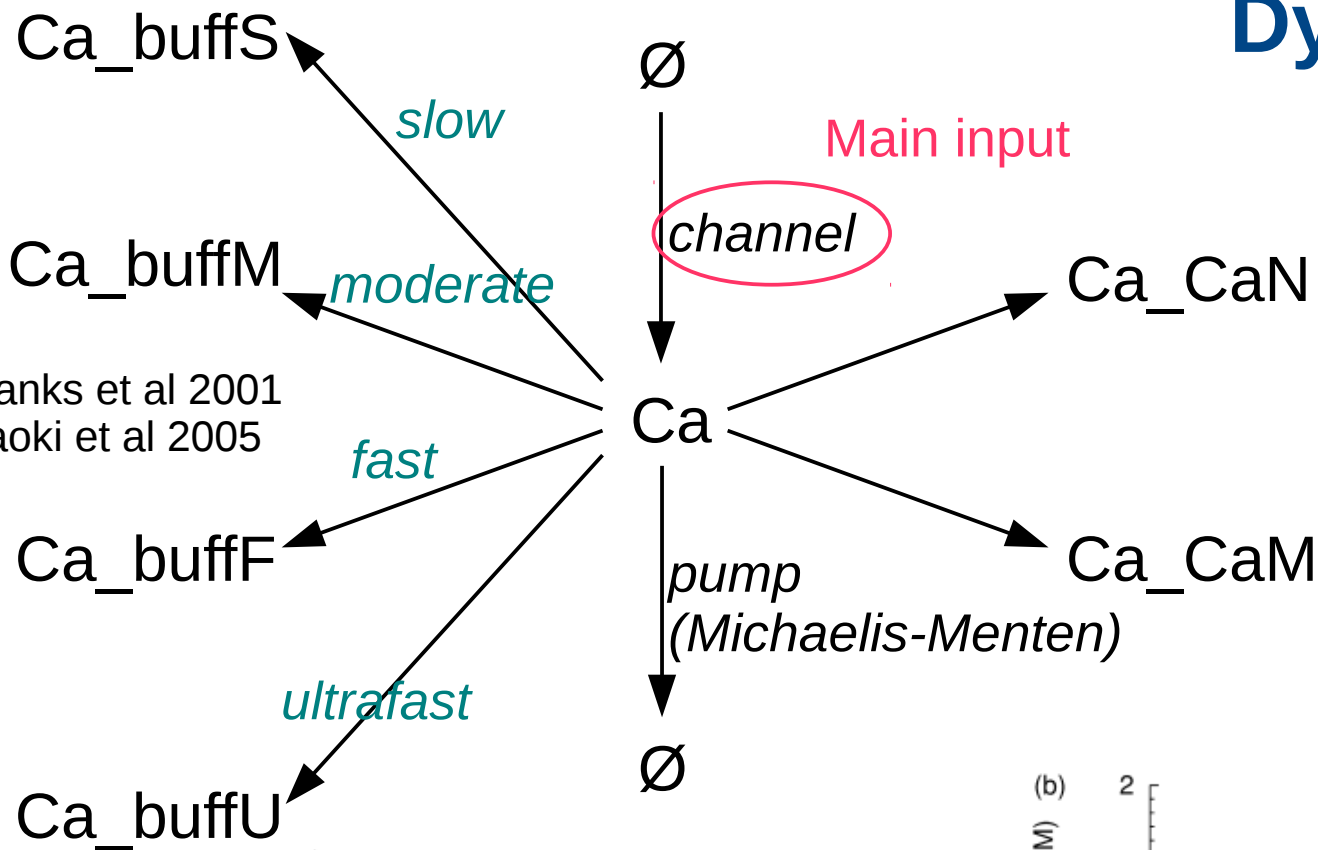


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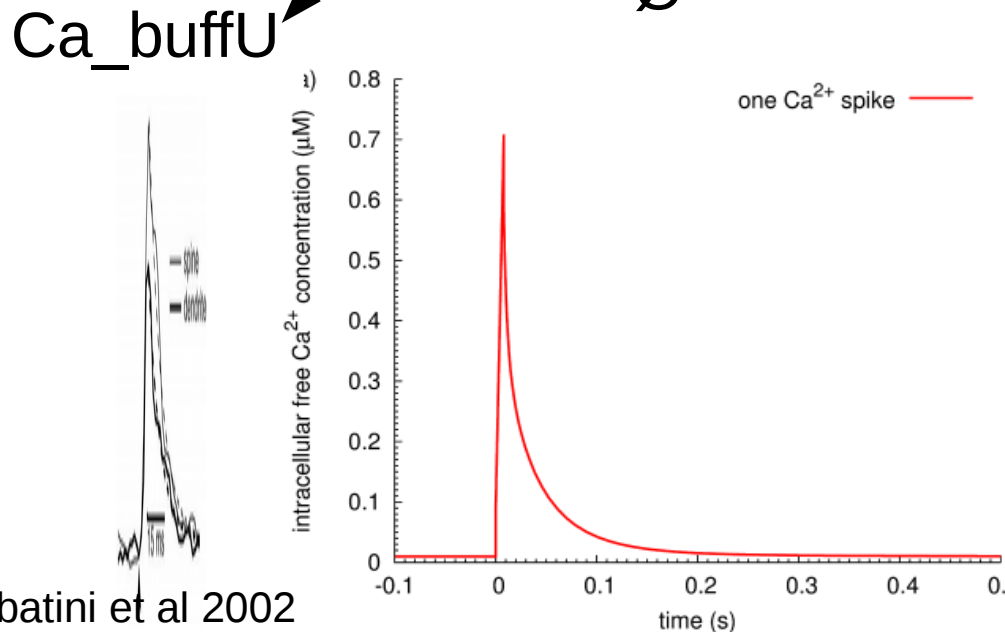


Dynamic of calcium in the spine

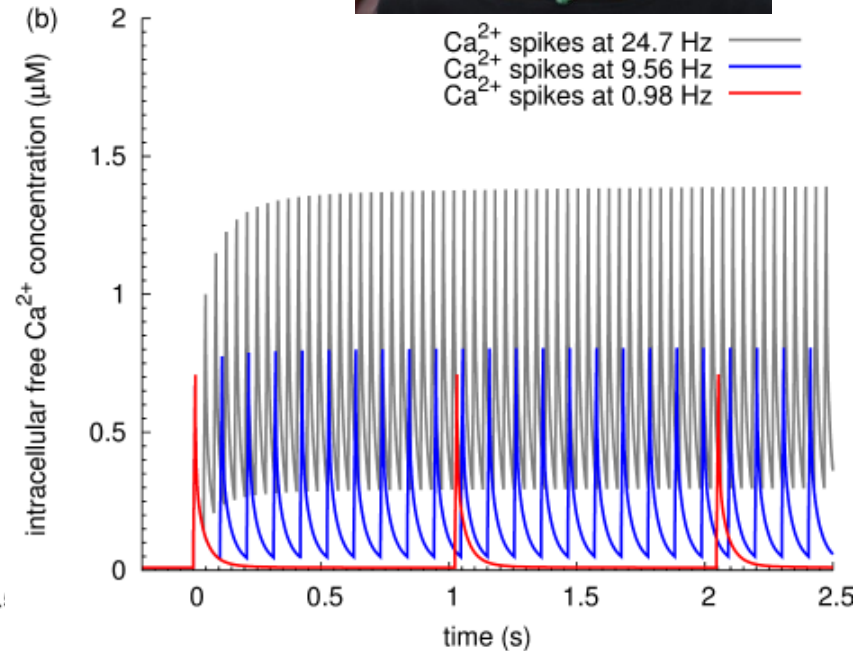
Li L, Stefan MI, Le Novère N
(2012) *PLoS ONE*, 7(9): e43810



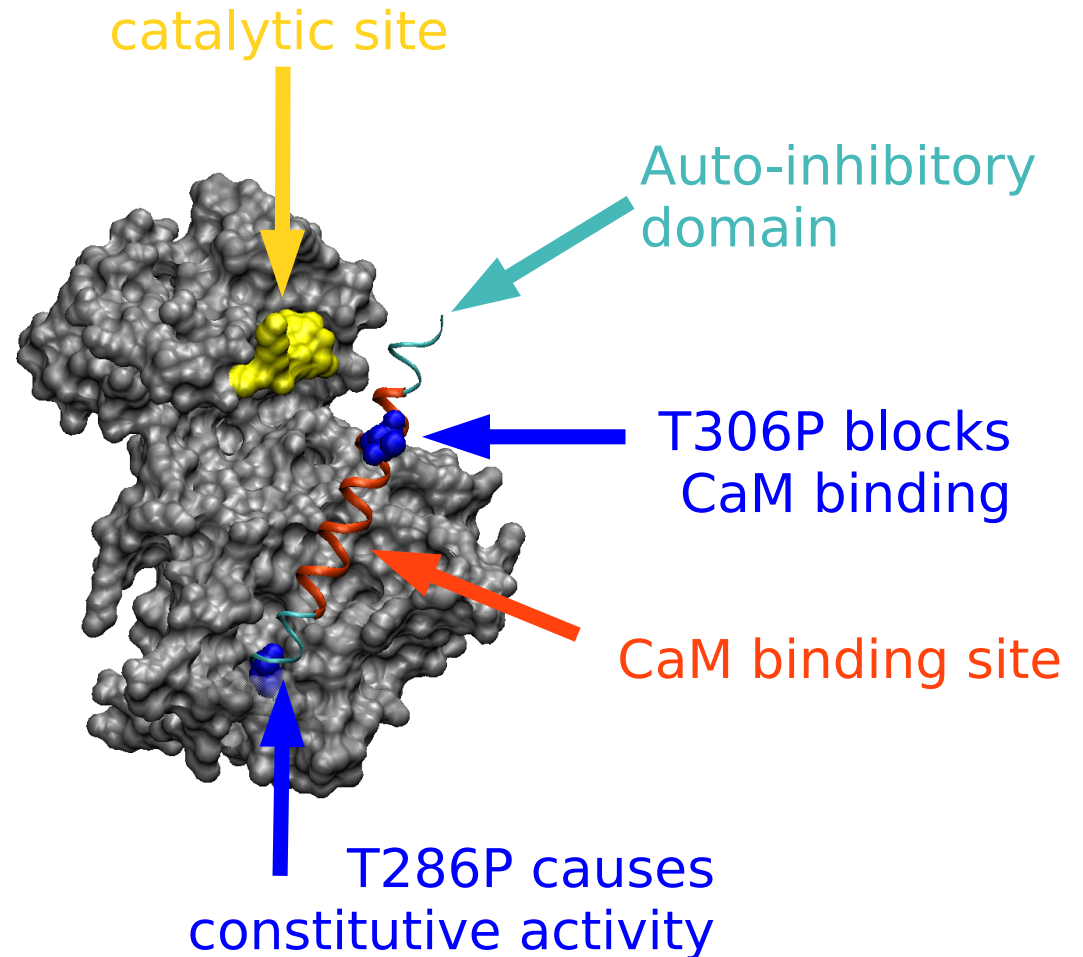
Franks et al 2001
Naoki et al 2005



Sabatini et al 2002



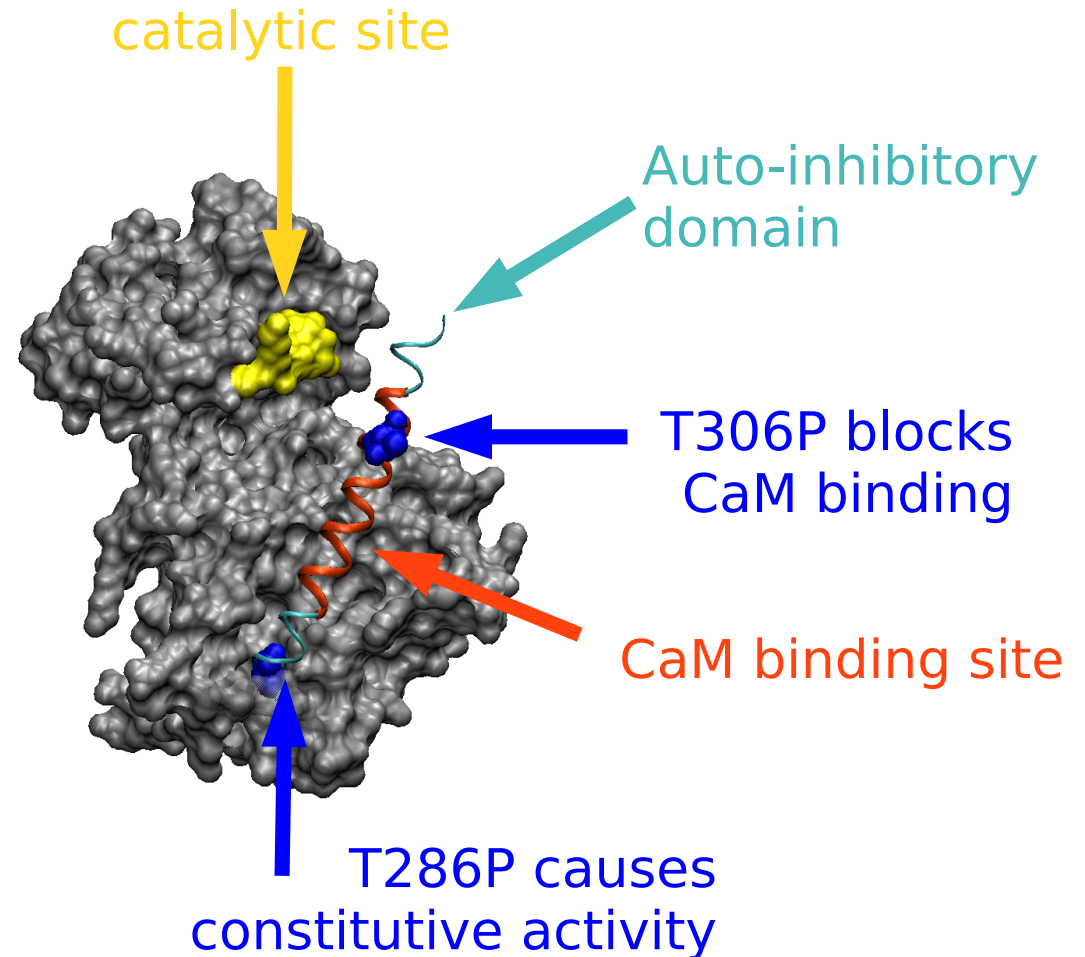
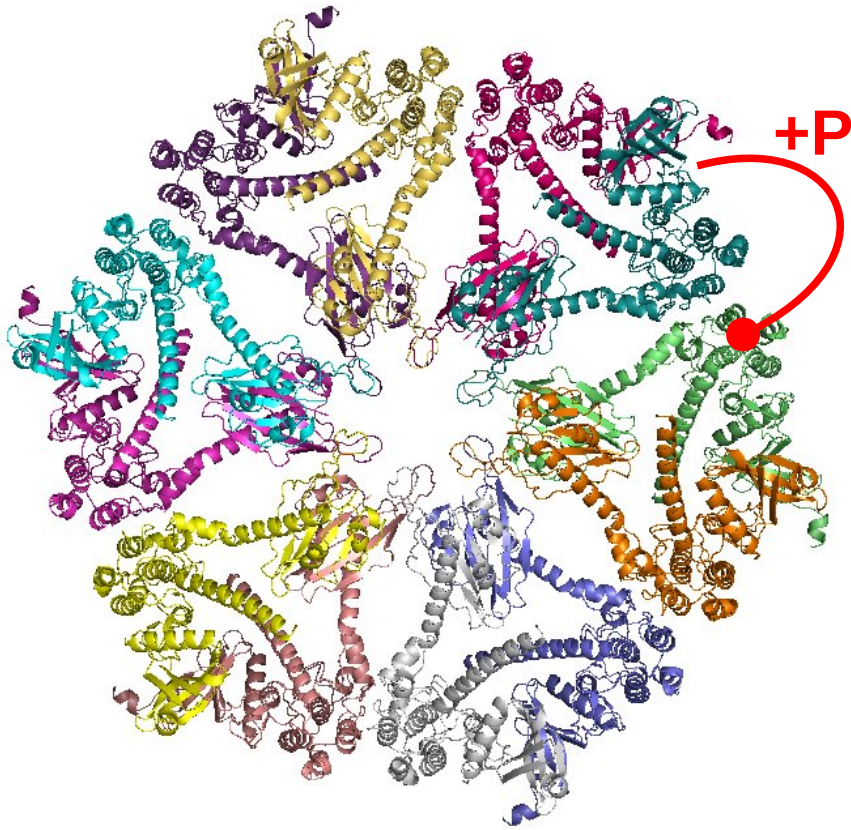
Calcium/calmodulin kinase II



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

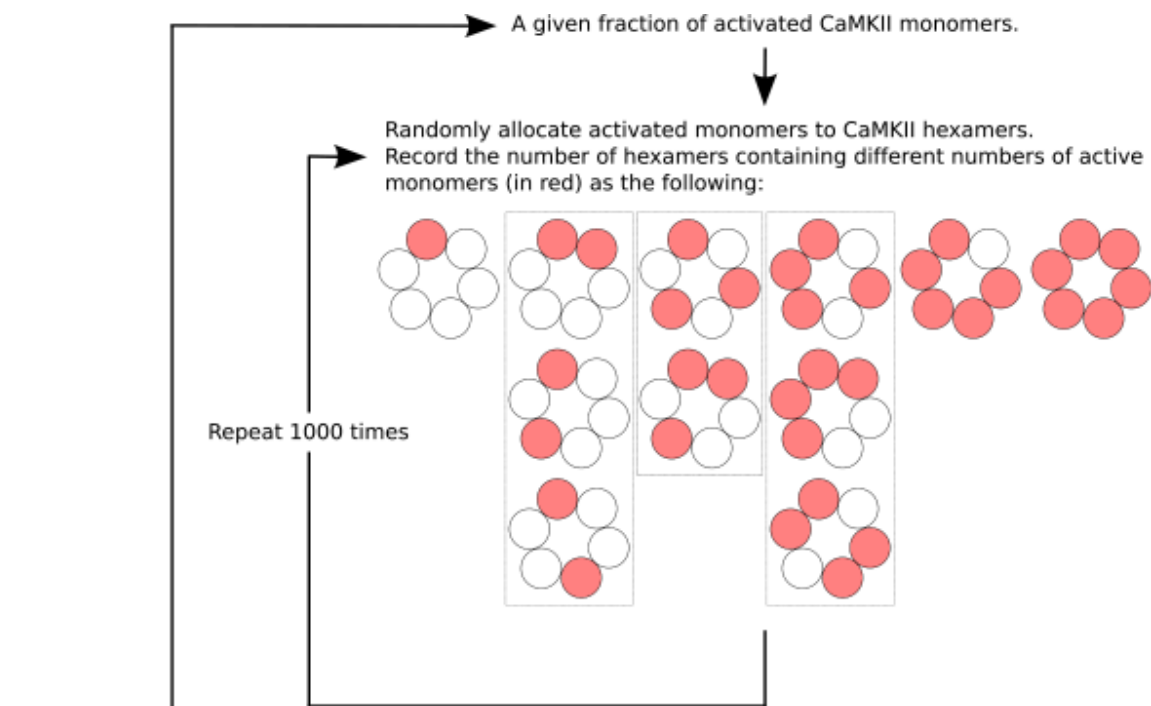


Dodecamer:

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

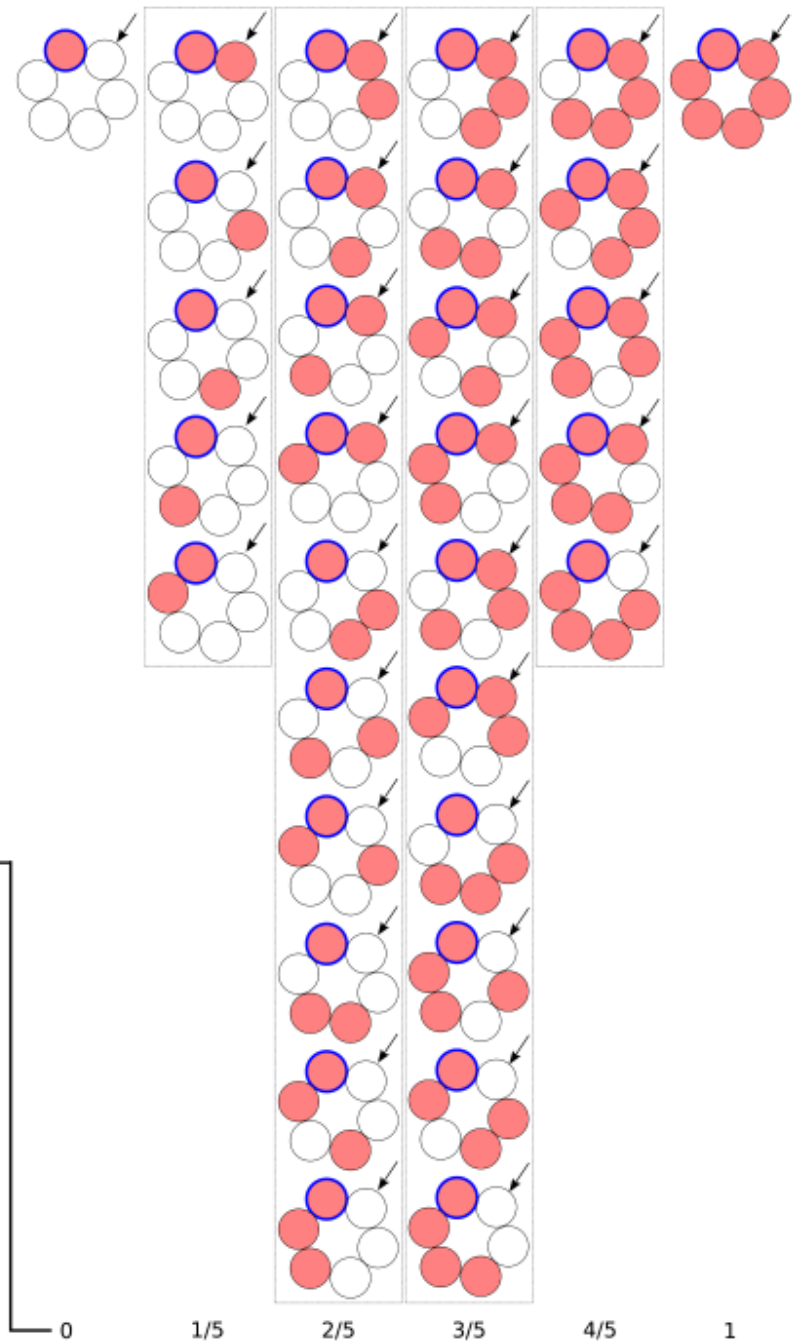
Calculate the average population for each number of active monomers per hexamer.

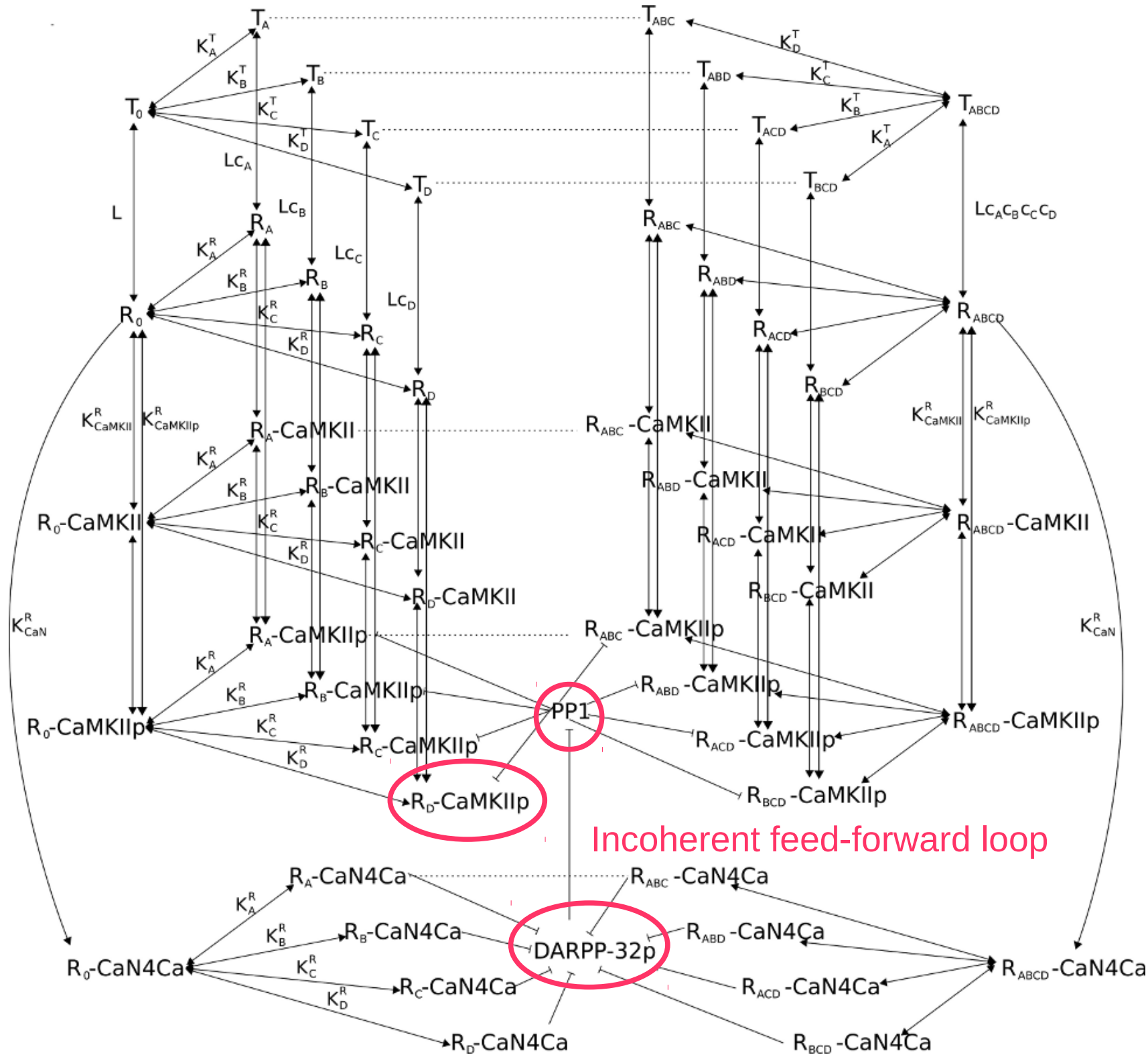
Multiply average populations of each number of active monomers per hexamer by their corresponding probabilities of having an active neighbour.

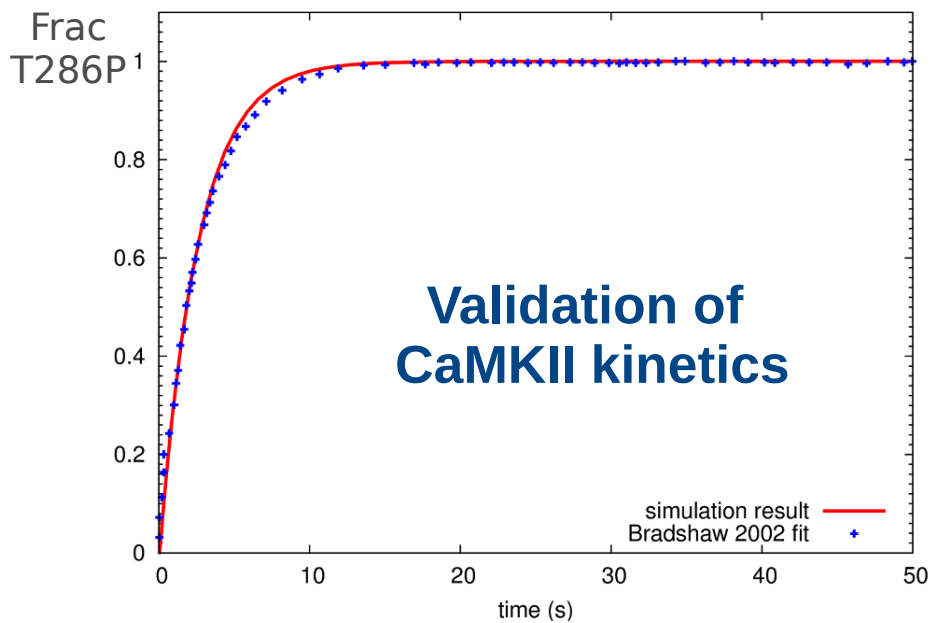
The sum of these six numbers is a coefficient that can be used to adjust CaMKII autophosphorylation rate.

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

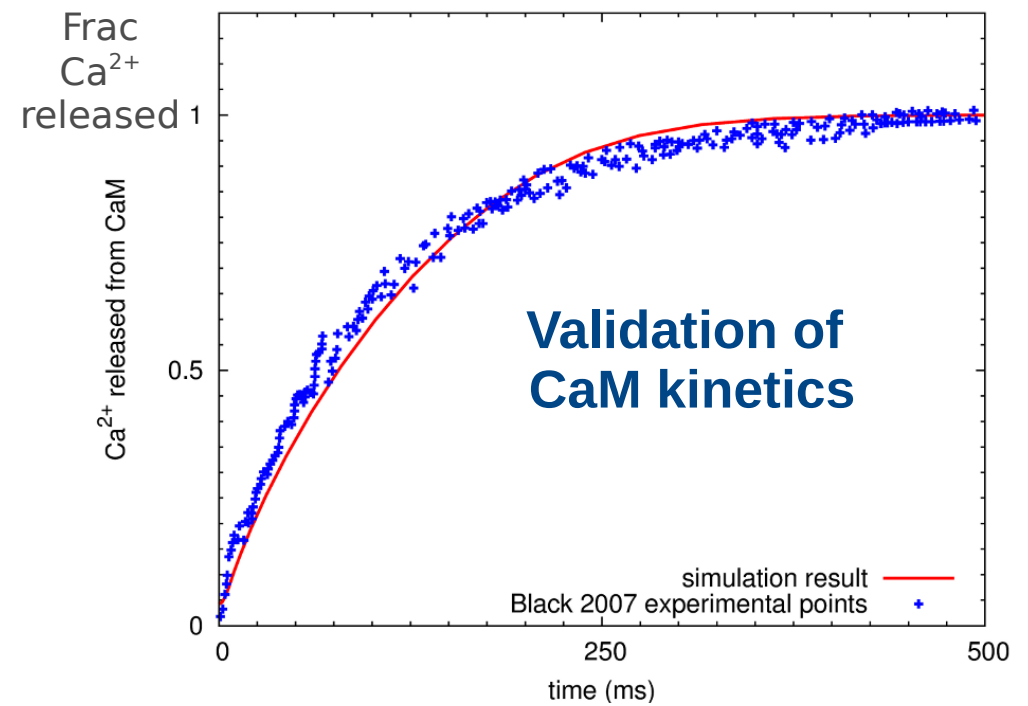
Calculate the probabilities of having an active neighbour on one specific side (indicated by the arrow) of the activated monomer of interest (blue outline) (since only the asymmetrical situation is considered). The possible positions of activated monomers are listed as the following, with corresponding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



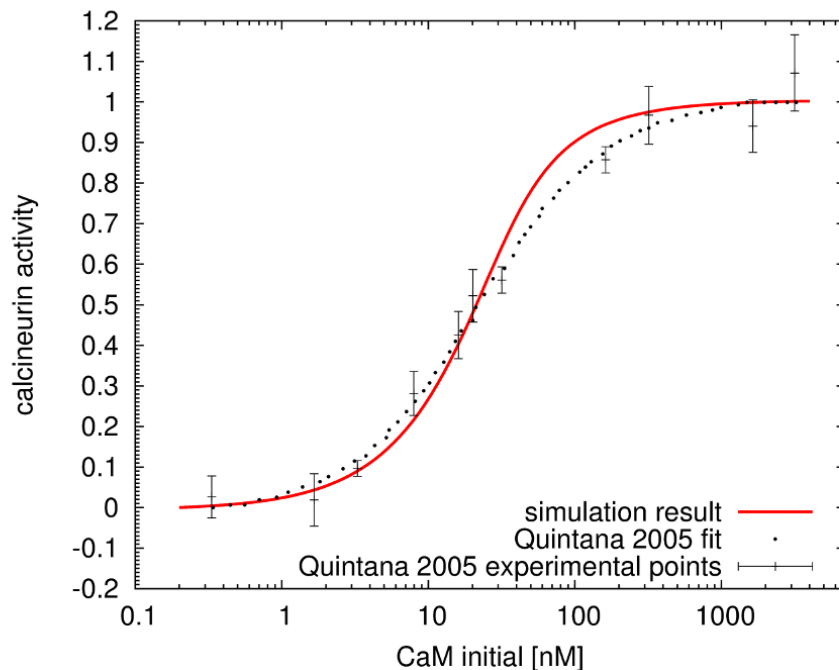




Bradshaw JM, Kubota Y, Meyer T, Schulman H (2003). *PNAS* 100: 10512-10517.



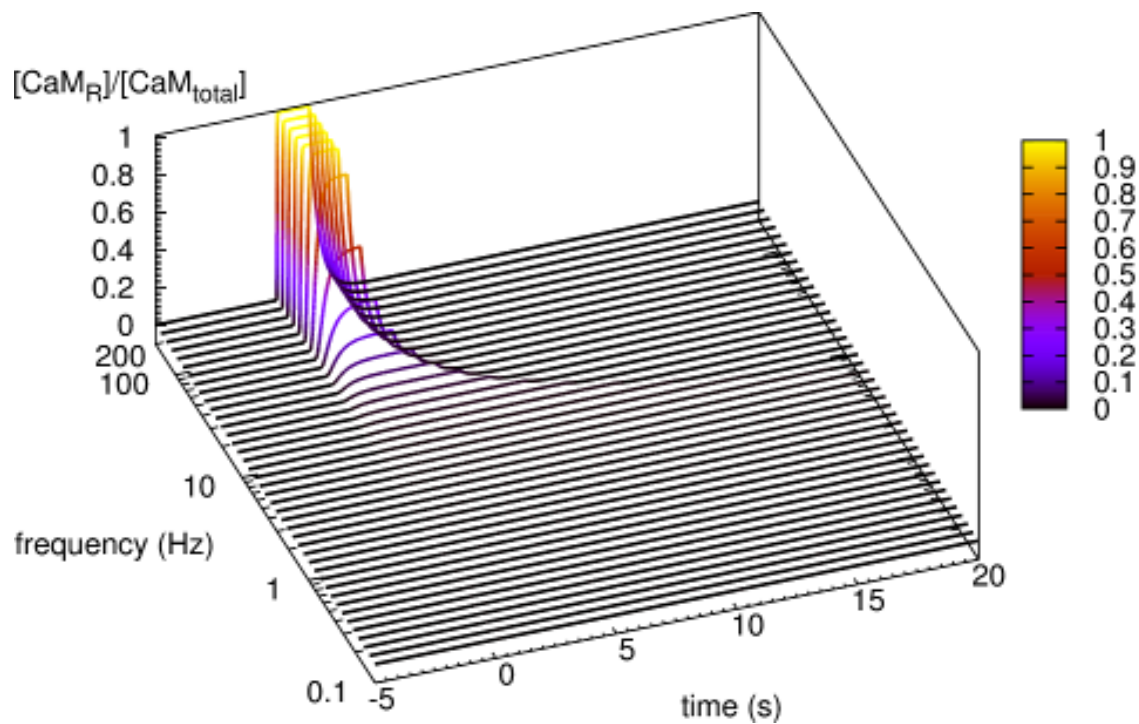
Black DJ, Selfridge JE, Persechini A (2007). *Biochemistry* 46: 13415-13424.



Validation of calcium-activation of CaN

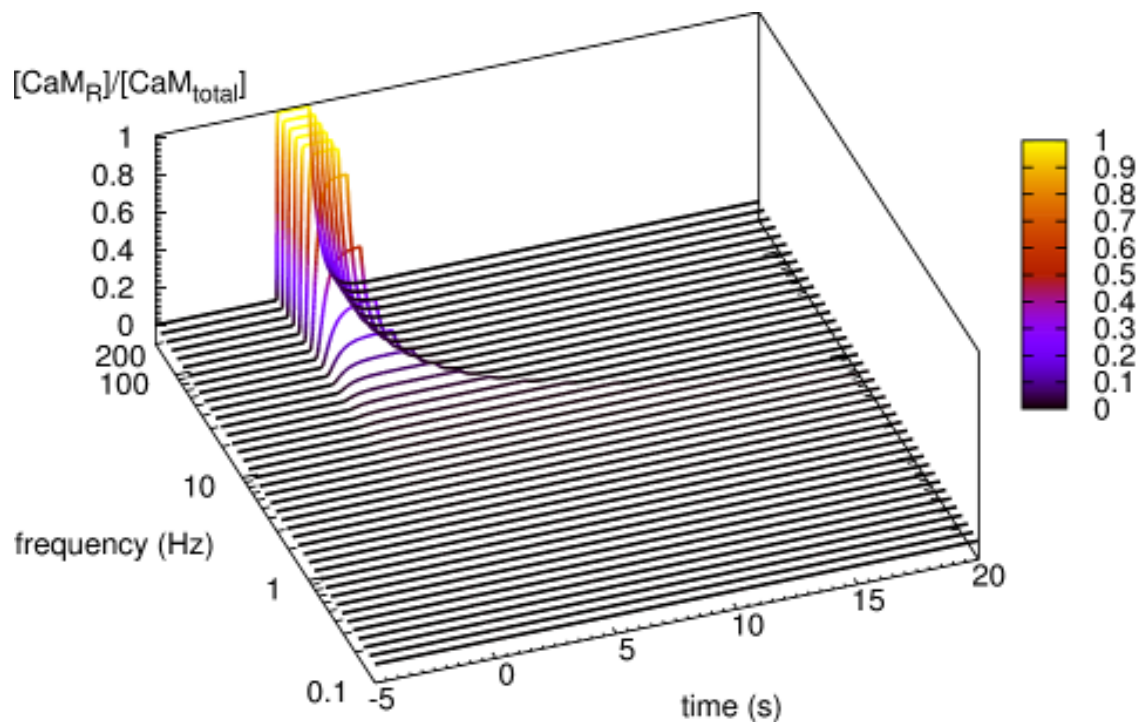
Quintana AR, Wang D, Forbes JE, Waxham MN (2005). *BBRC* 334: 674-680.

Calmodulin activation



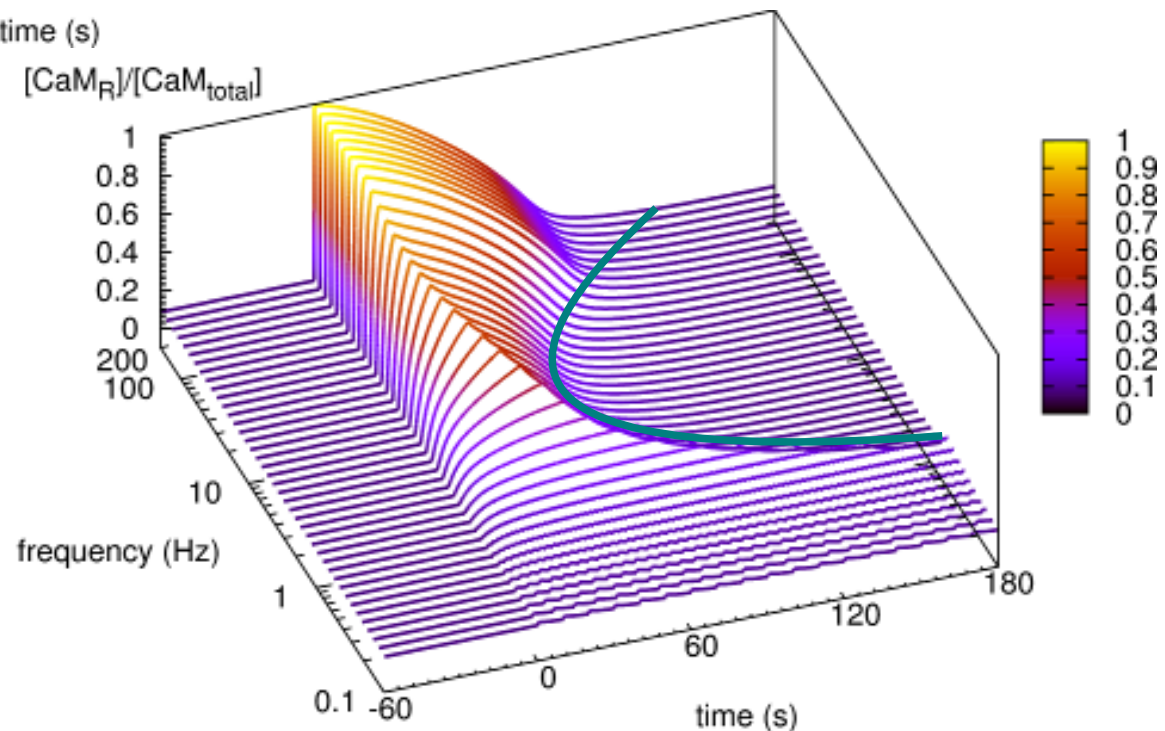
CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

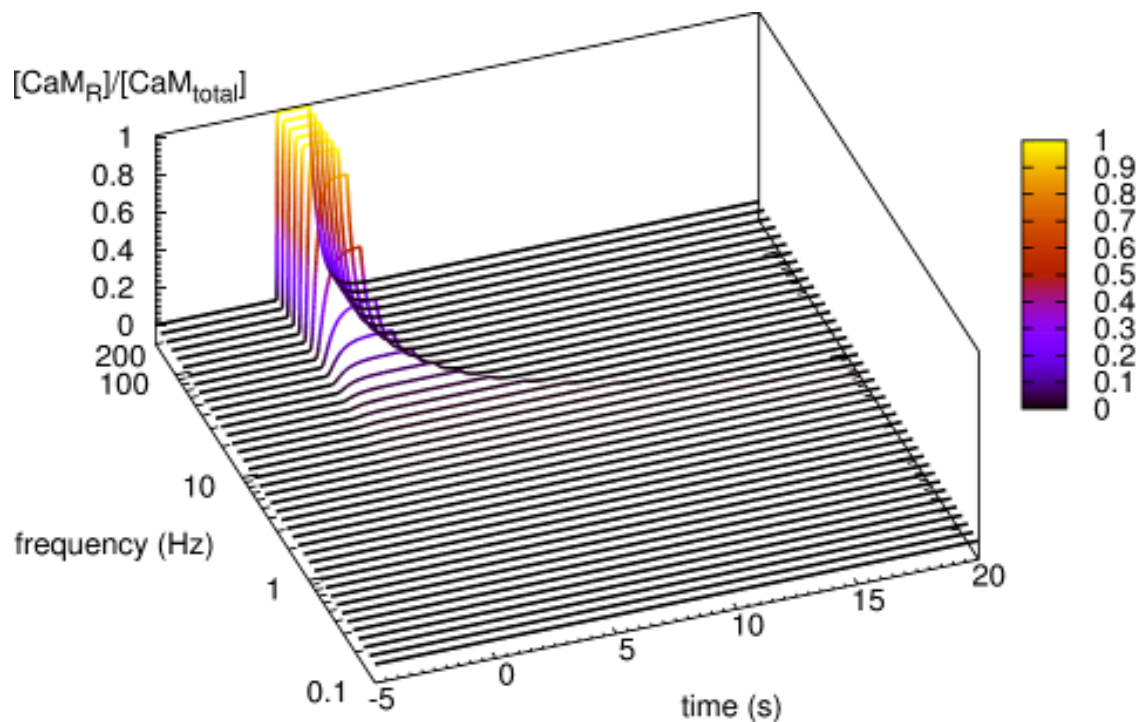


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

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



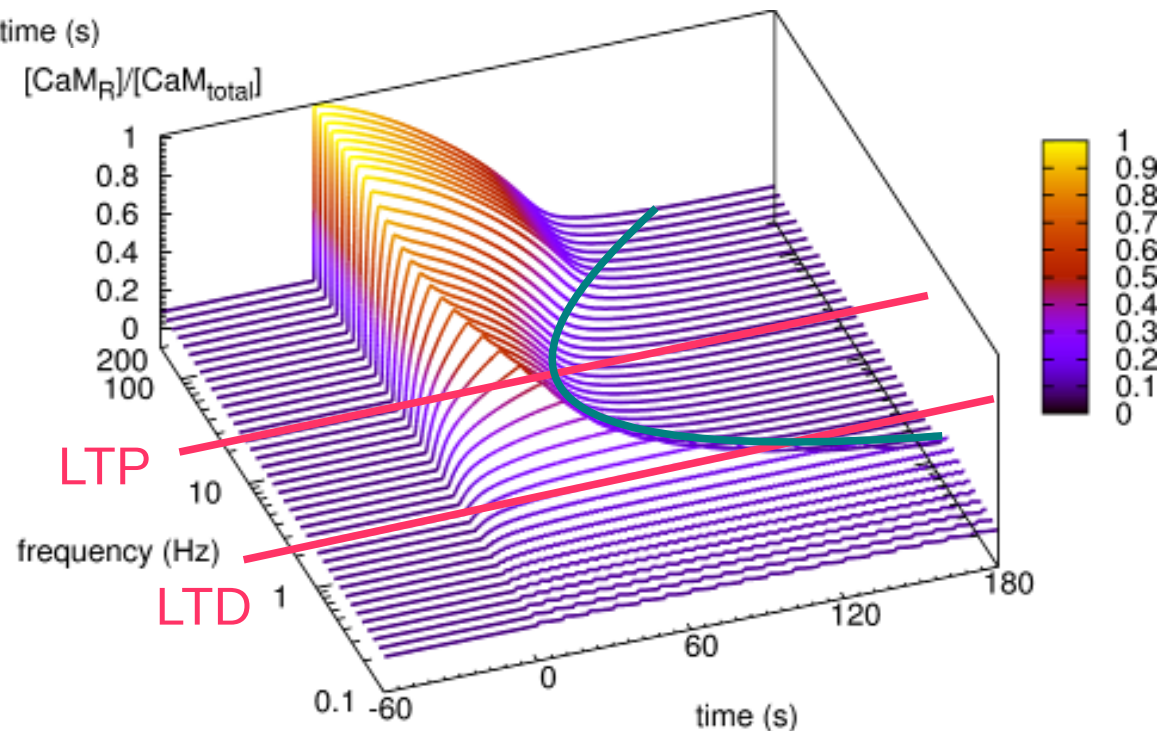
Calmodulin activation



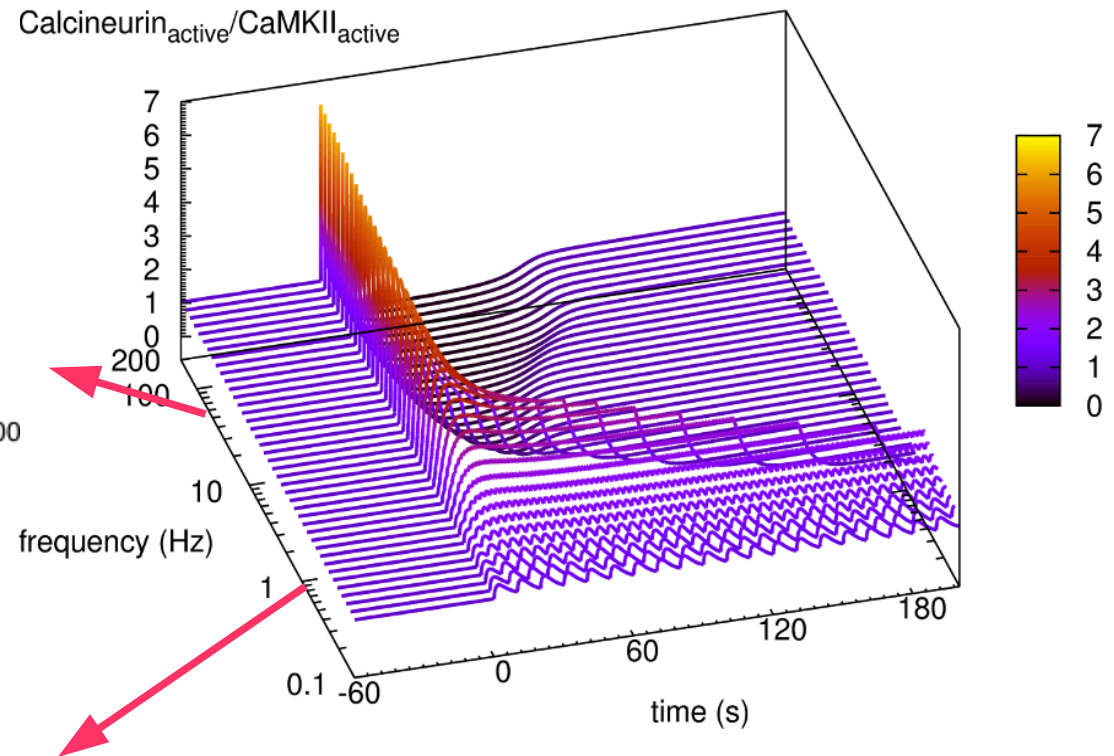
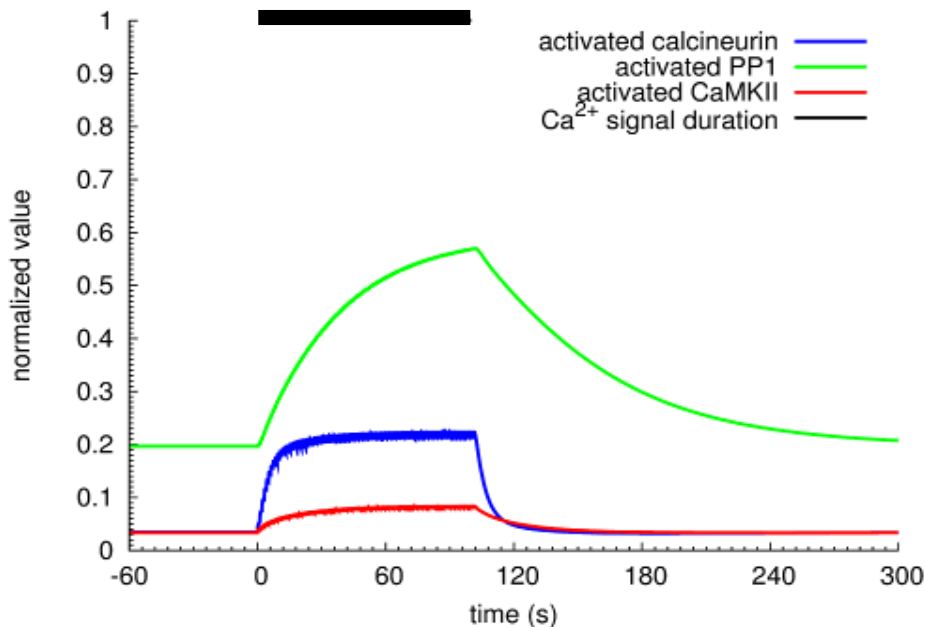
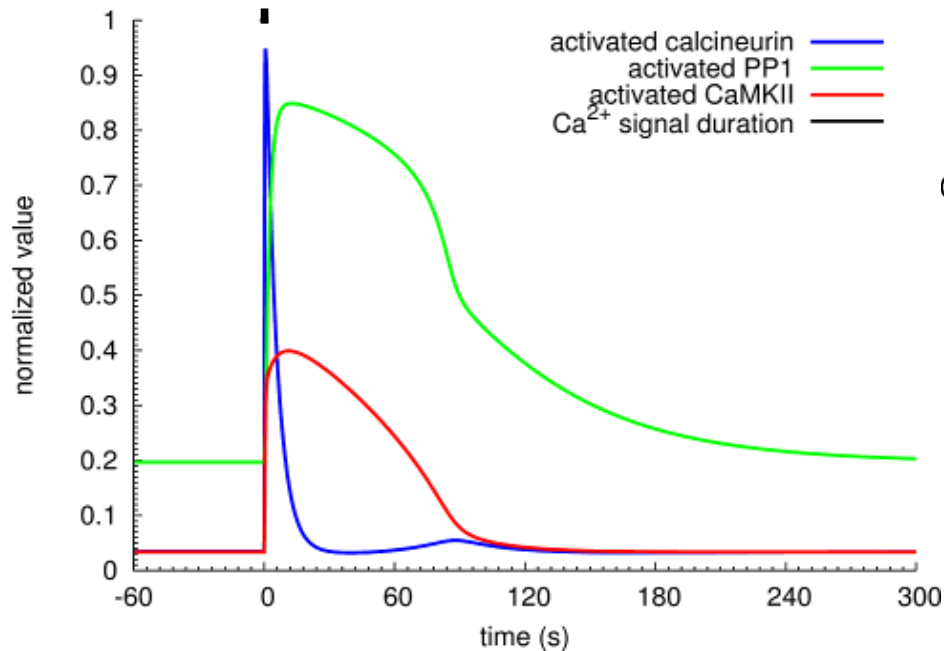
CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

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

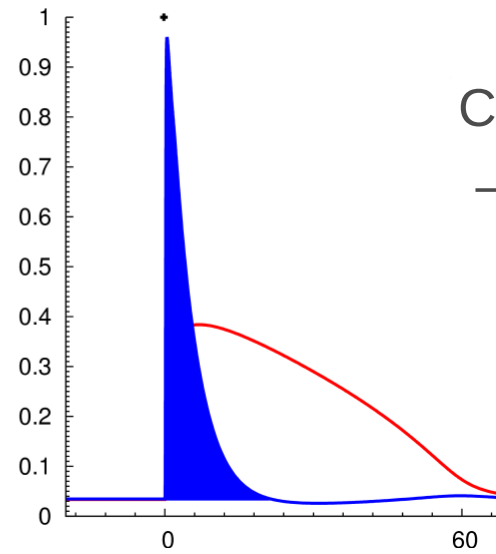
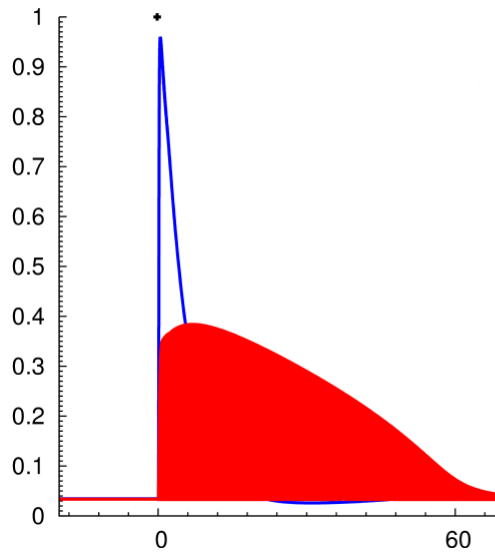


All calcium frequencies increase CaN **AND** CaMKII. It is not a Switch. But the ratio of activation changes

Bidirectional plasticity

Constant catalytic rates of active enzyme

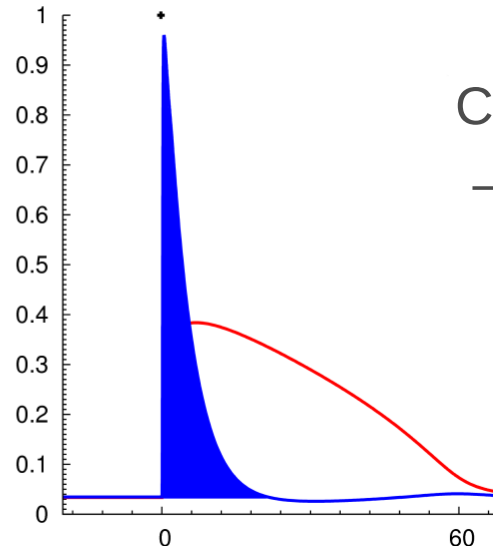
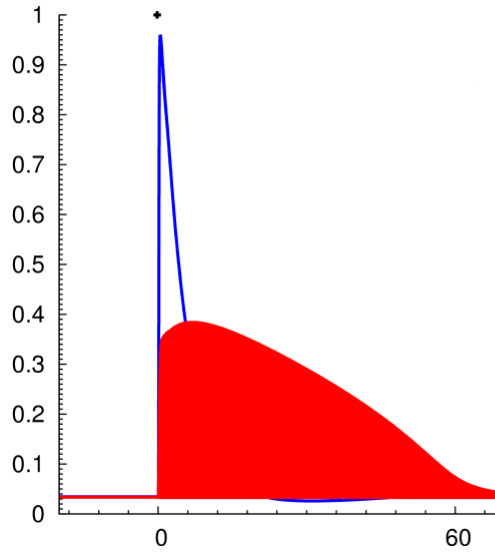
→ quantity of catalysed reaction events
prop to integral of the activation curve



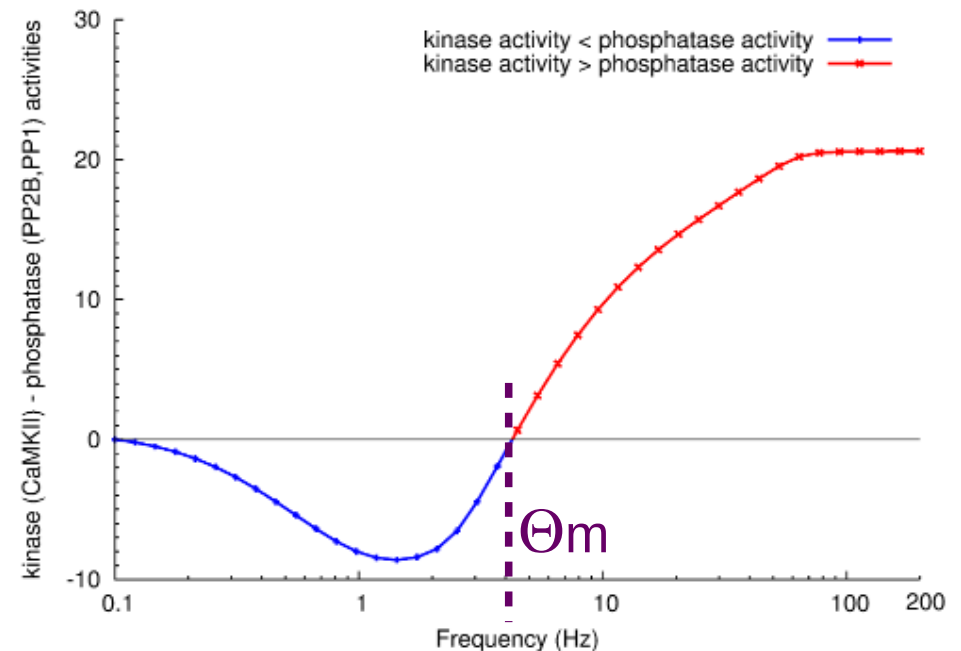
Bidirectional plasticity

Constant catalytic rates of active enzyme

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



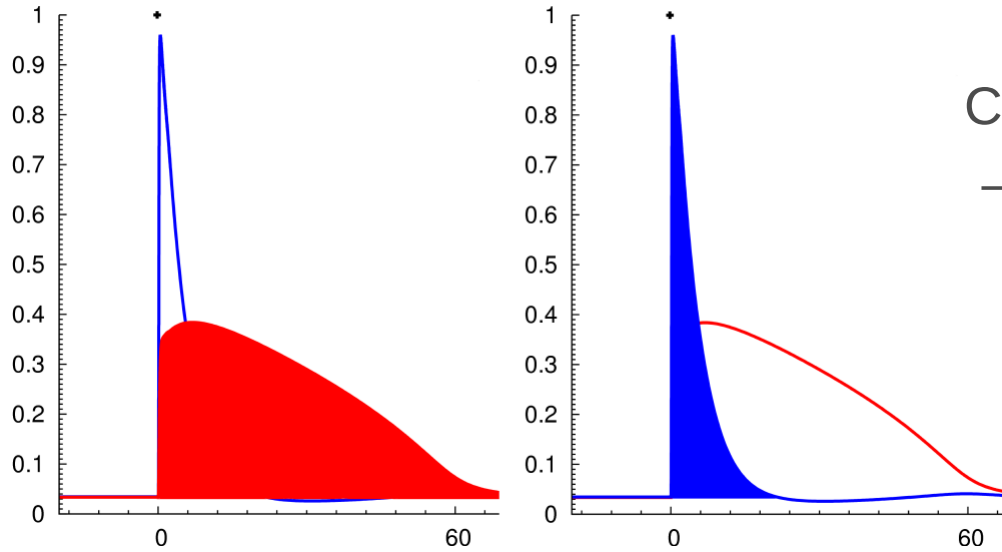
Bienestock-Cooper-Munro
(BCM) curve: difference of
active areas*catalytic activities



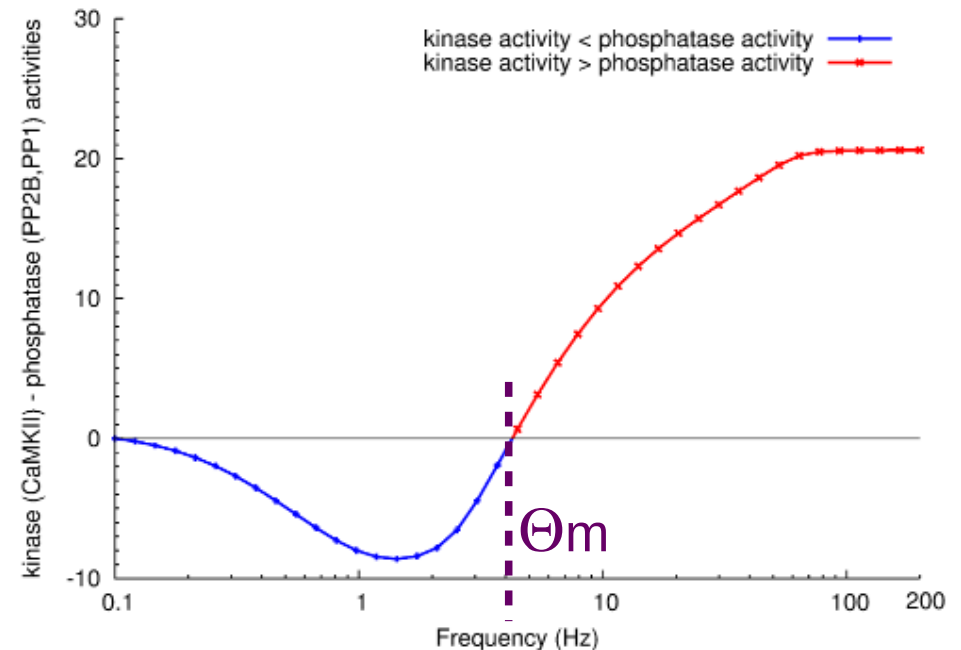
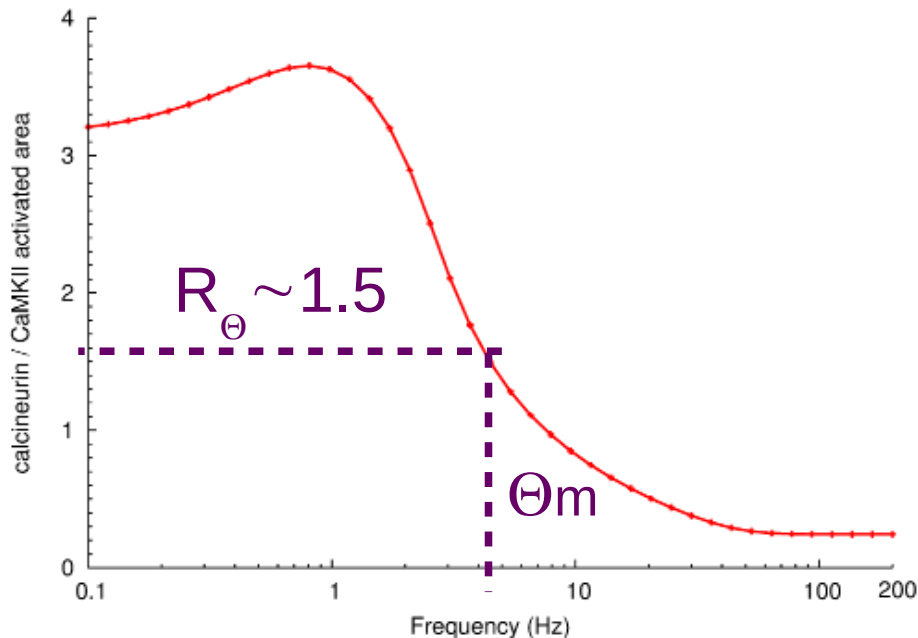
Bidirectional plasticity

Constant catalytic rates of active enzyme

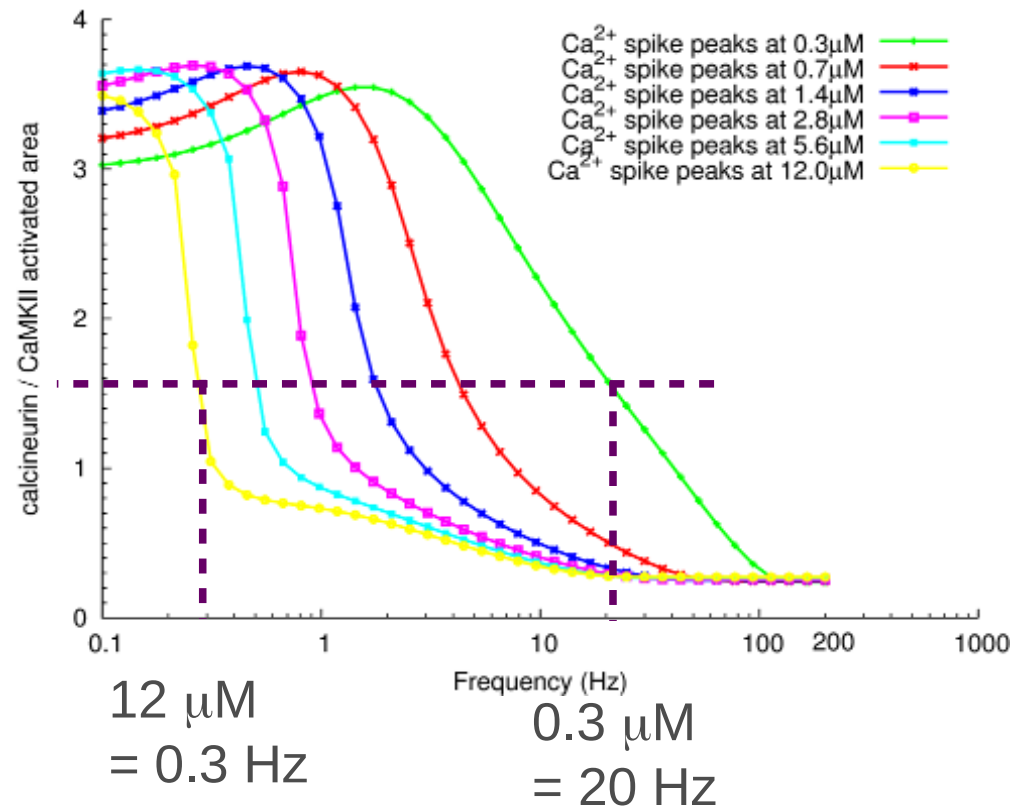
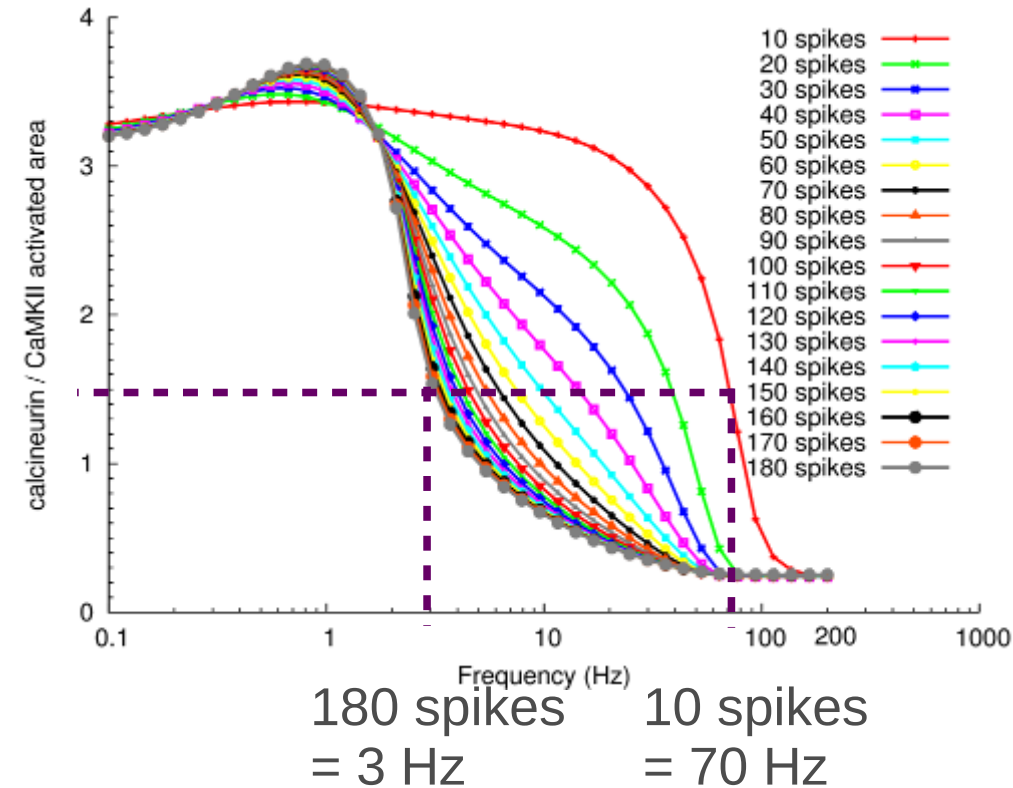
→ quantity of catalysed reaction events
prop to integral of the activation curve



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

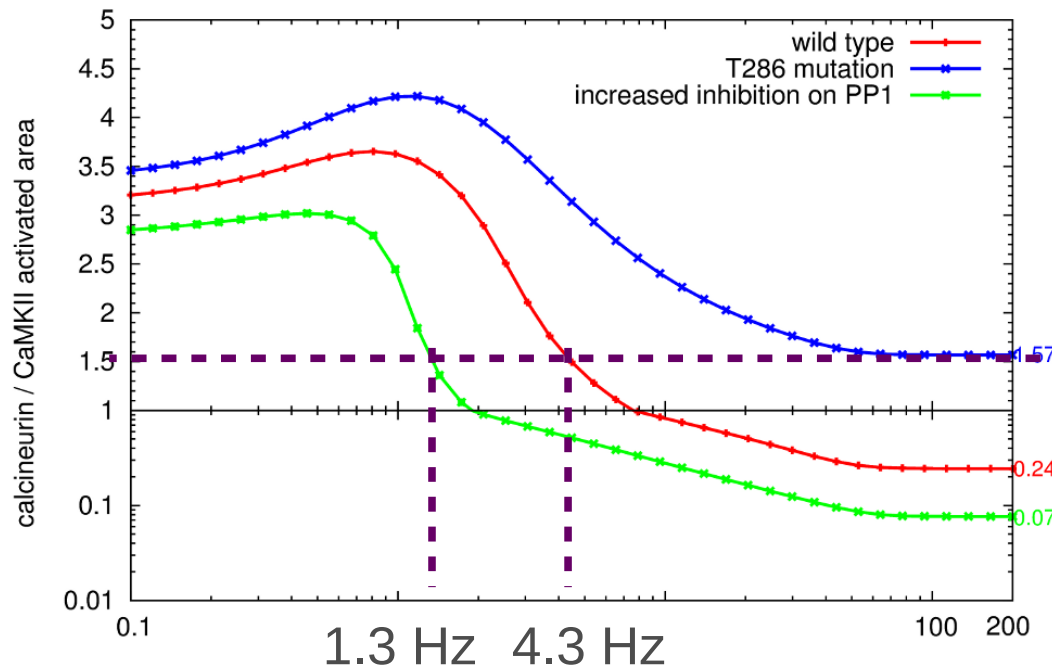


Effect of calcium duration and amount



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

Effect of intrinsic system perturbations

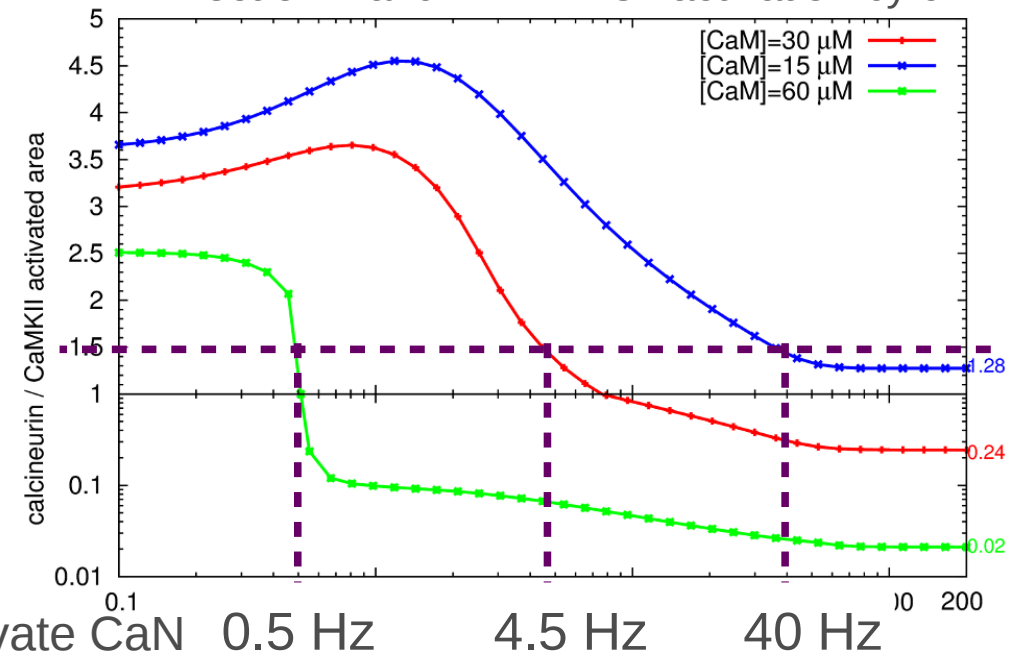


CaMKII not constitutively active
No CaM trapping

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

Lower deactivation of CaMKII

Effect of I1 and DARPP-32 activation by cAMP



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) on CaN.
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

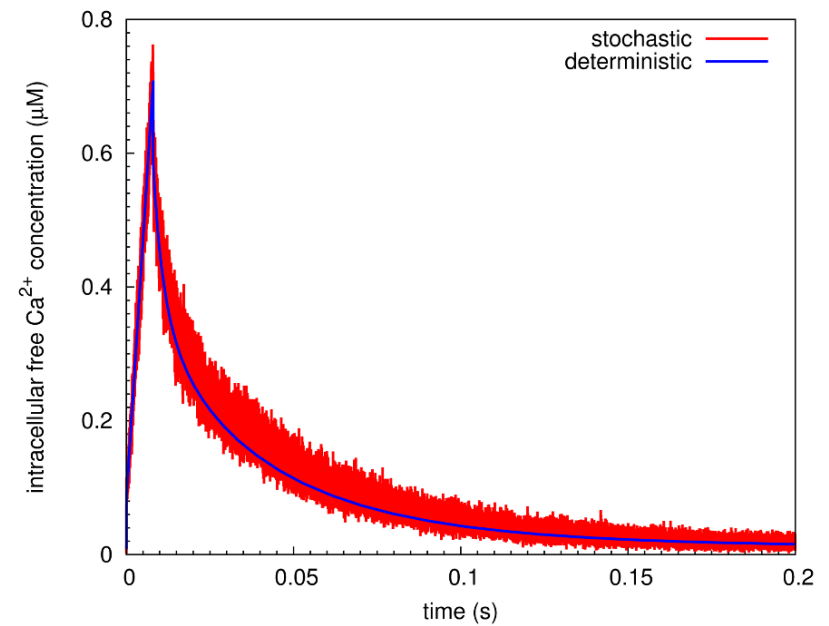
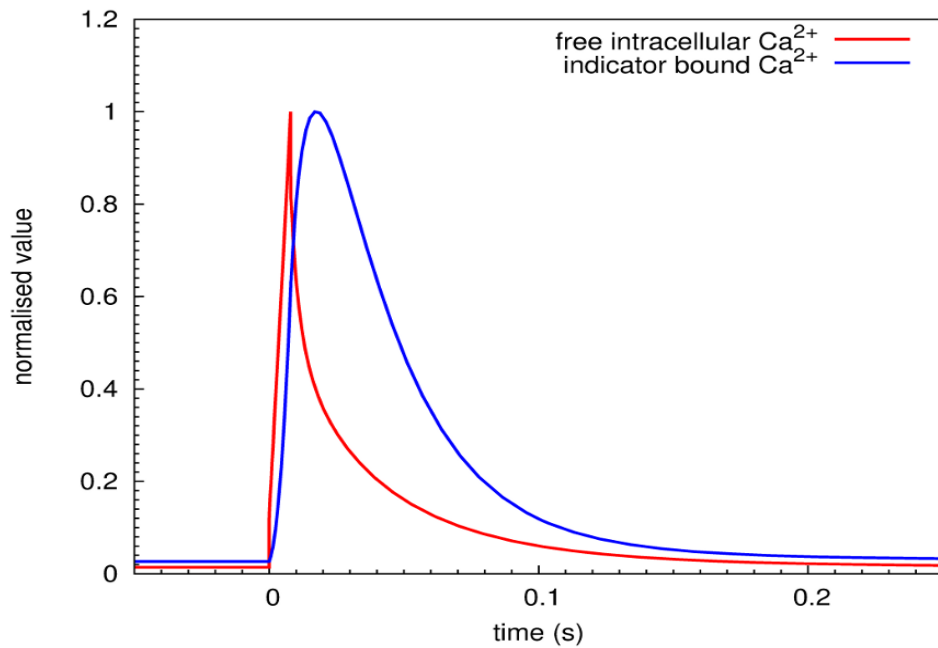
Θ_m is not an **intrinsic property of the synapse**, but a dynamical one that depends on the length and amplitude of stimulations

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

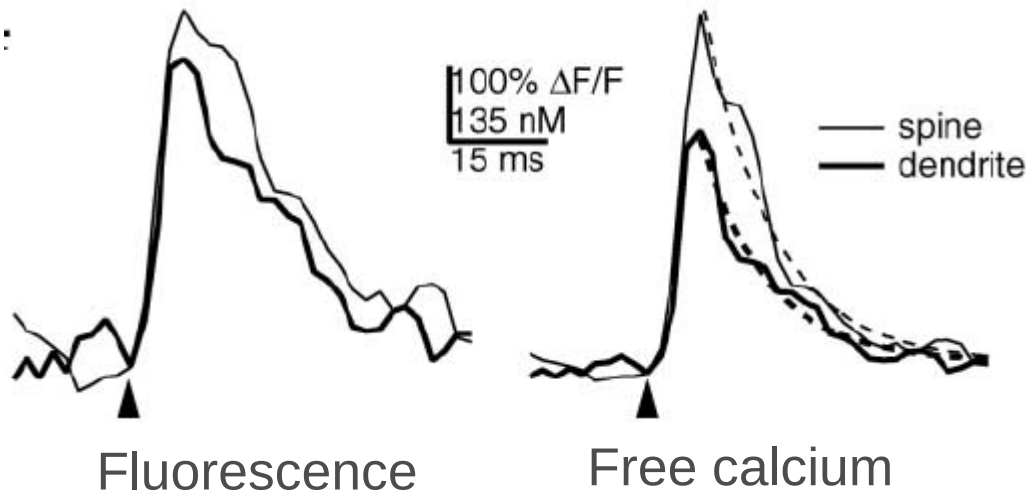
Pecunia est nervus belli



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.