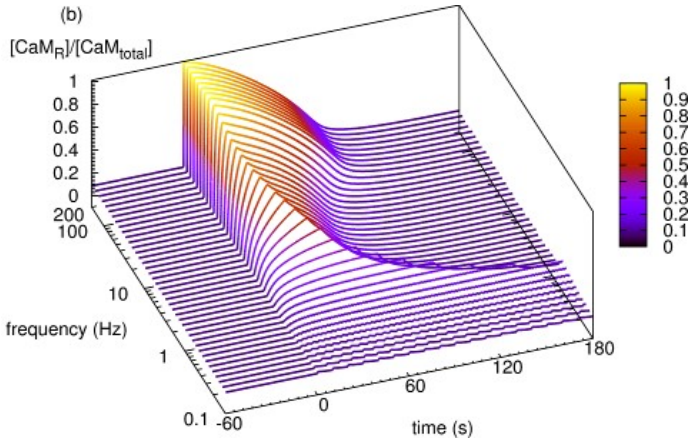
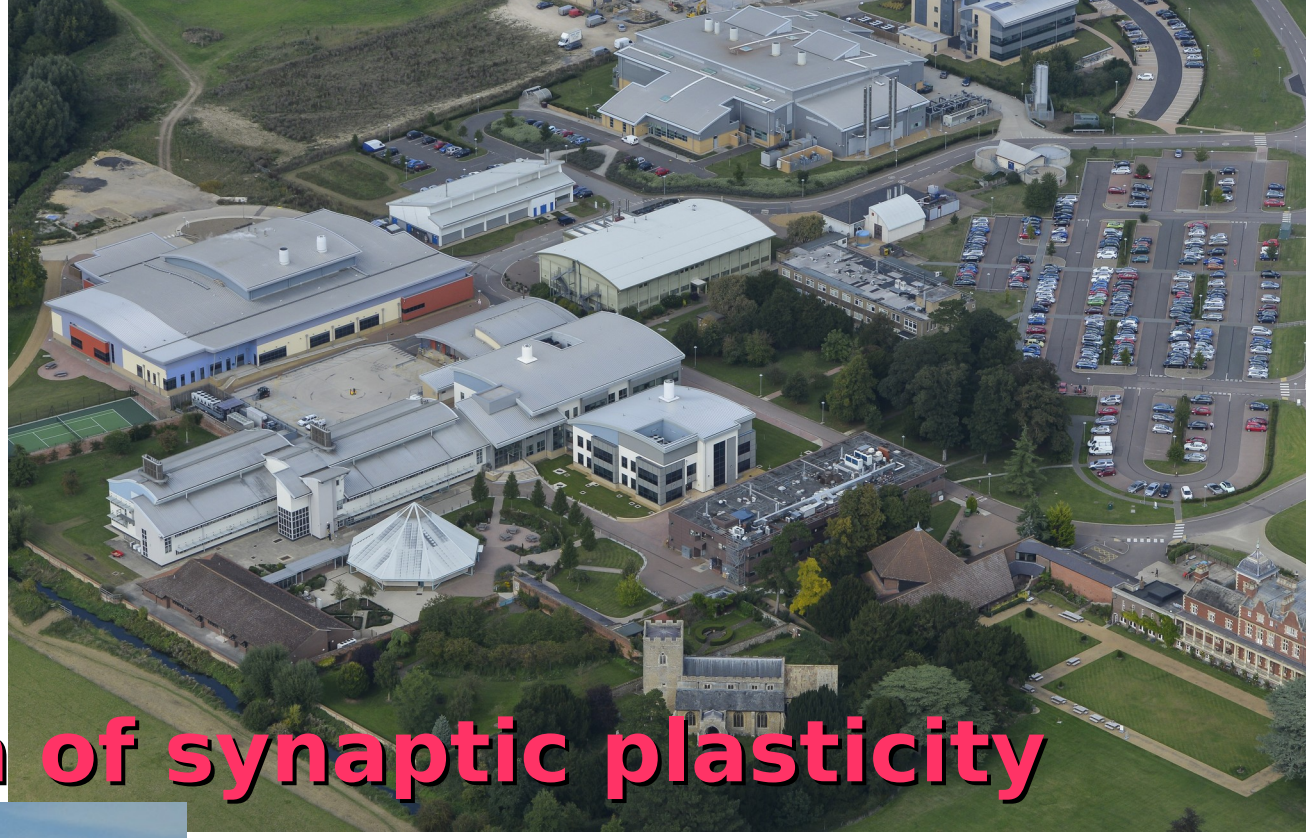




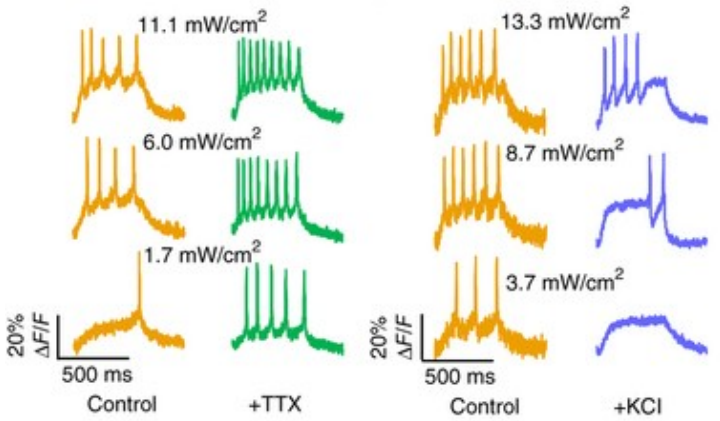
Regulation of synaptic plasticity by allosteric calcium sensors

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

How do we learn?

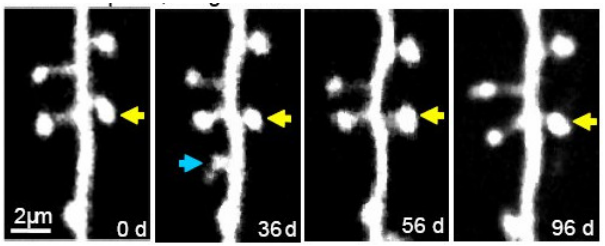
We learn by modifying the activity of pre-existing neuronal networks:

- Properties of neurons:
  Intrinsic plasticity – e.g. neuronal excitability



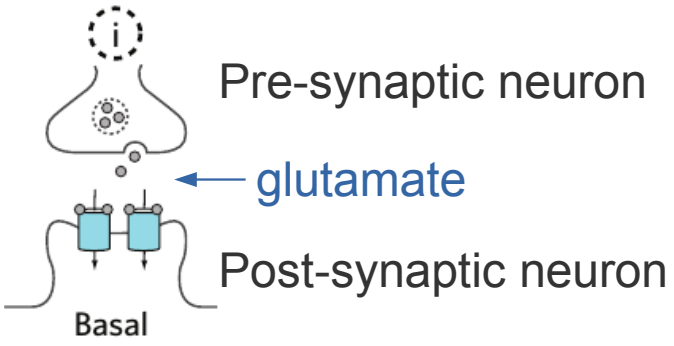
Hochbaum et al (2014) *Nat Meth*

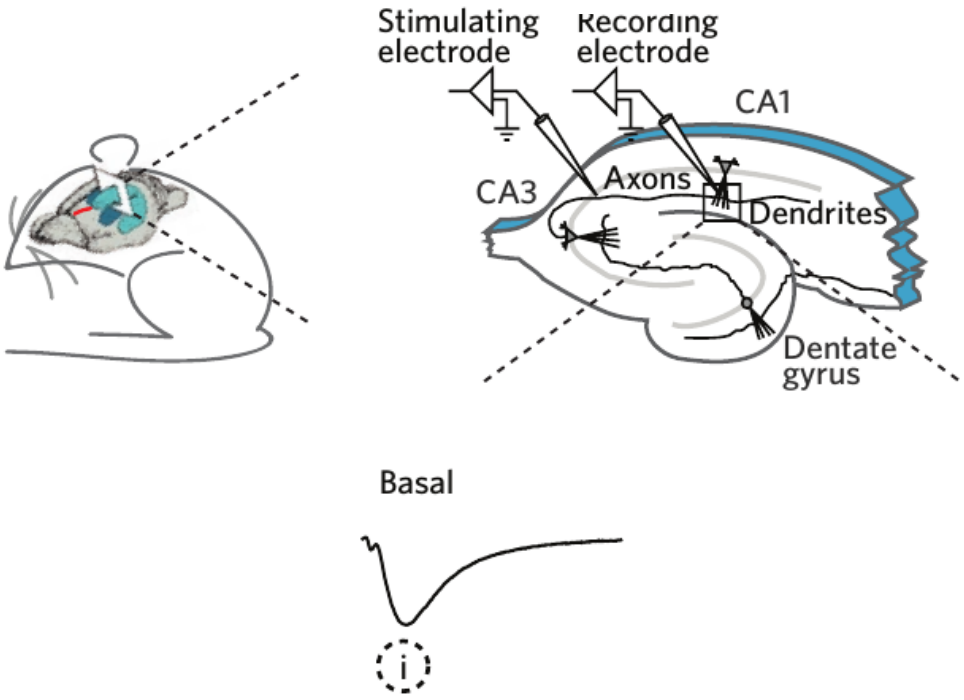
- Connectivity:
  Dendritic plasticity - remodelling, creation, growth and removal of inter-neuronal connections

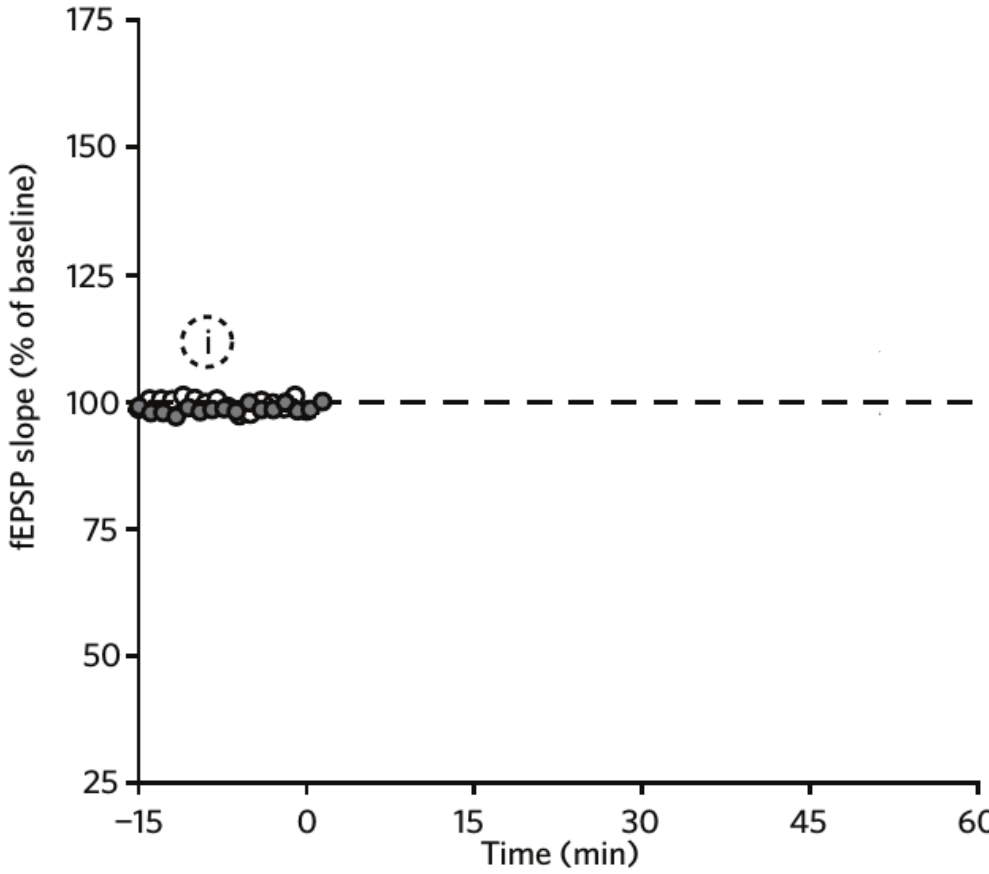


Holmaat et al (2005) *Neuron*

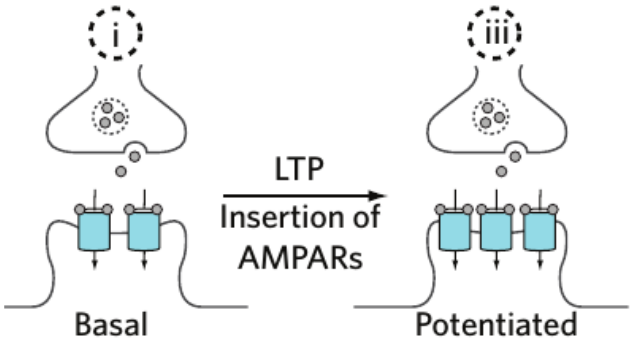
- Communication:
  Synaptic plasticity - Increase or decrease of synaptic weight

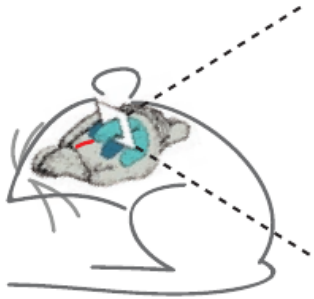


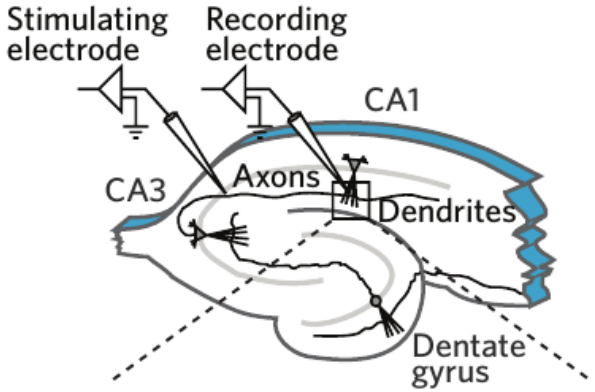


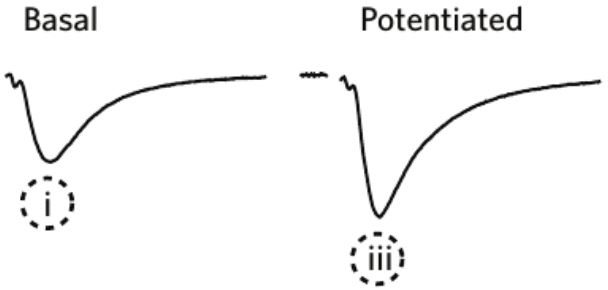


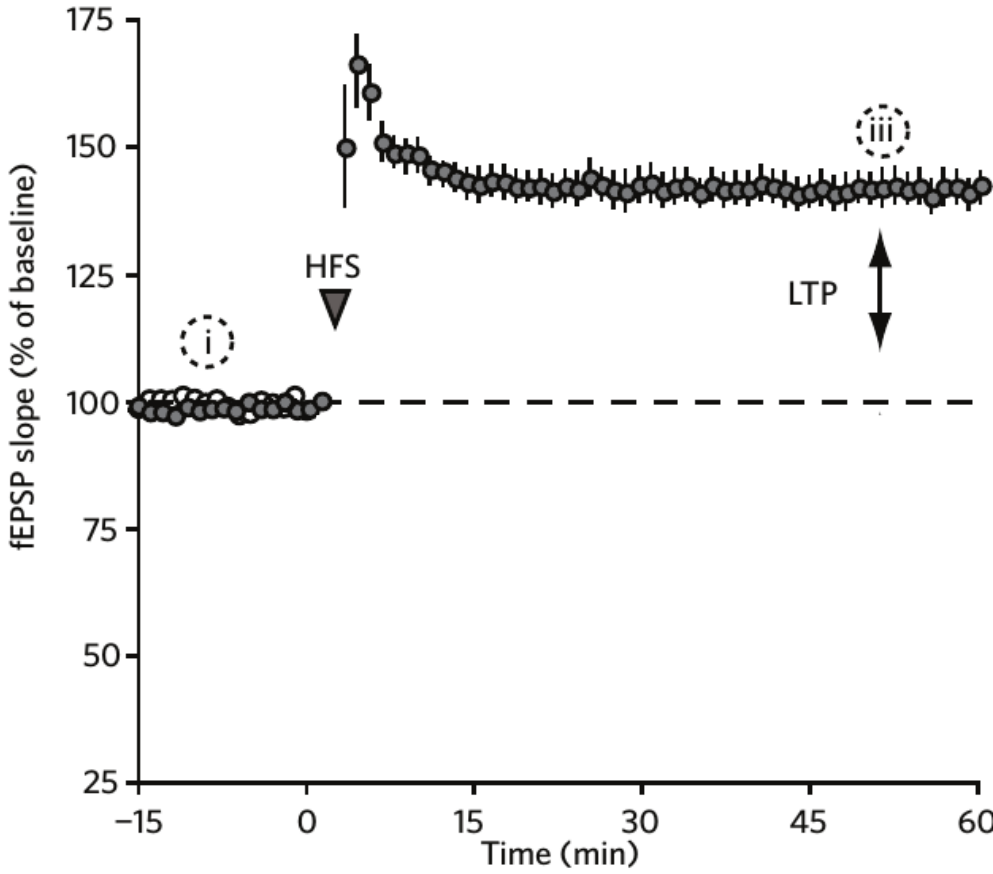
Fleming and England (2010) *Nat Chem Biol*



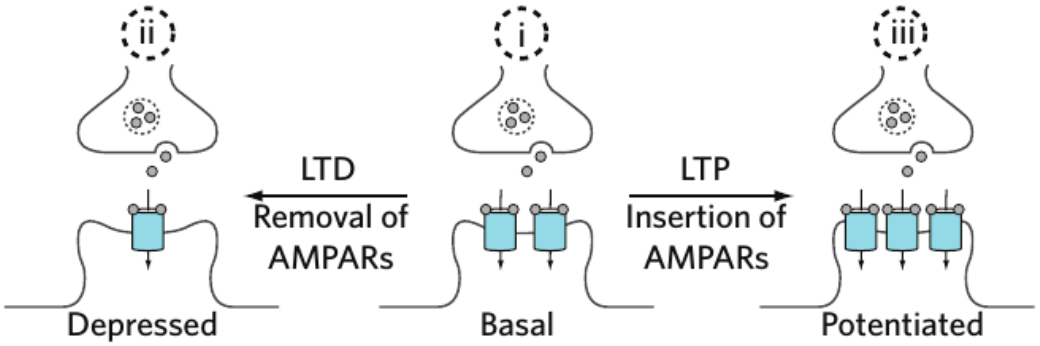


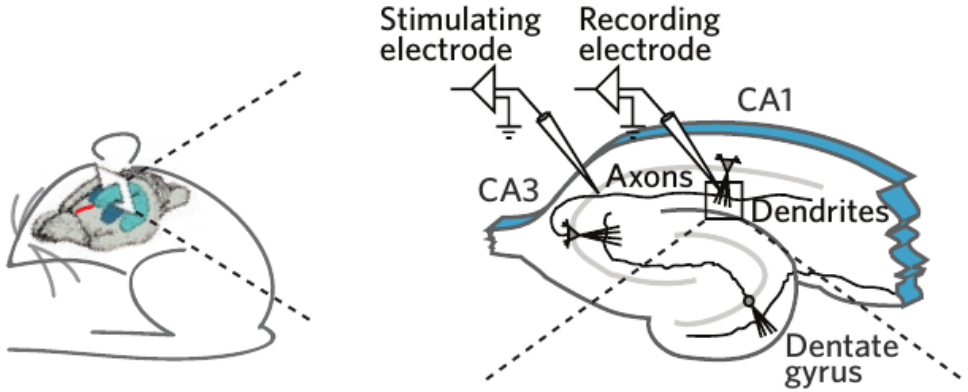


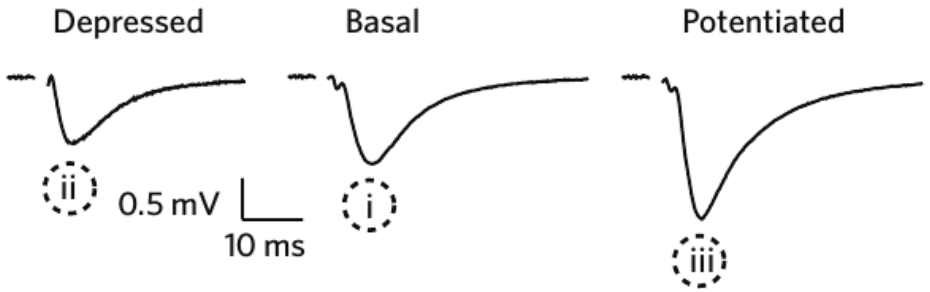


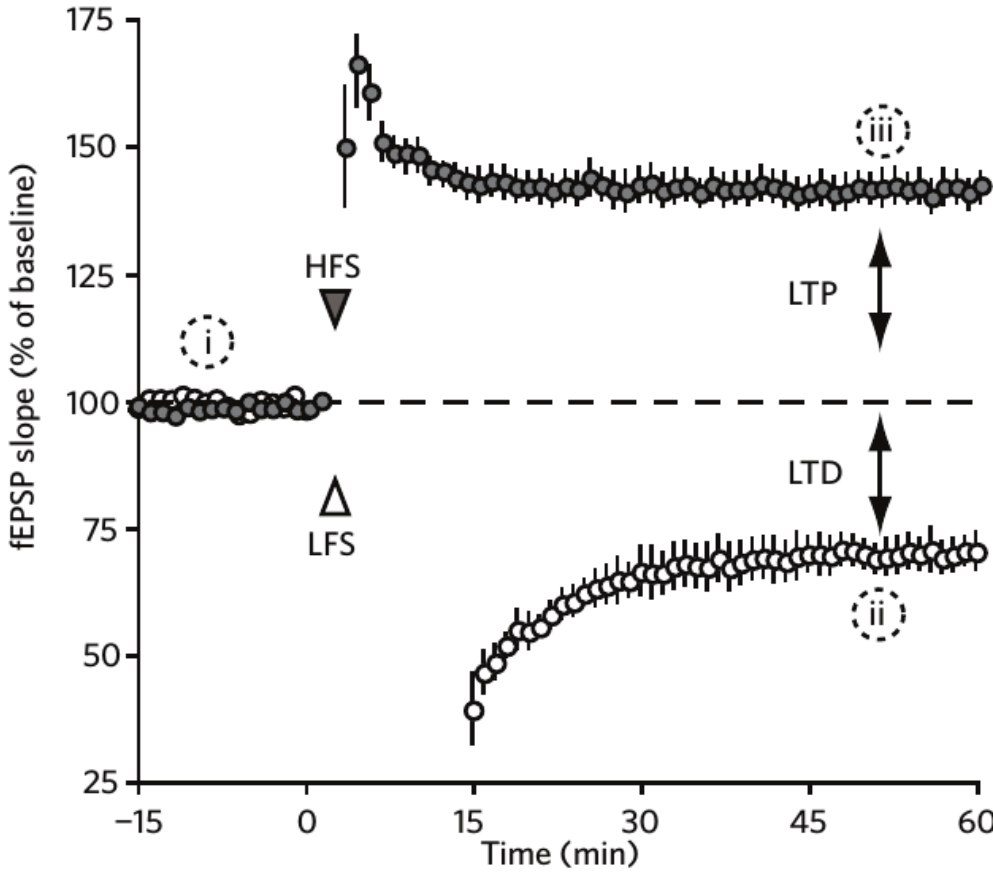


Fleming and England (2010) *Nat Chem Biol*





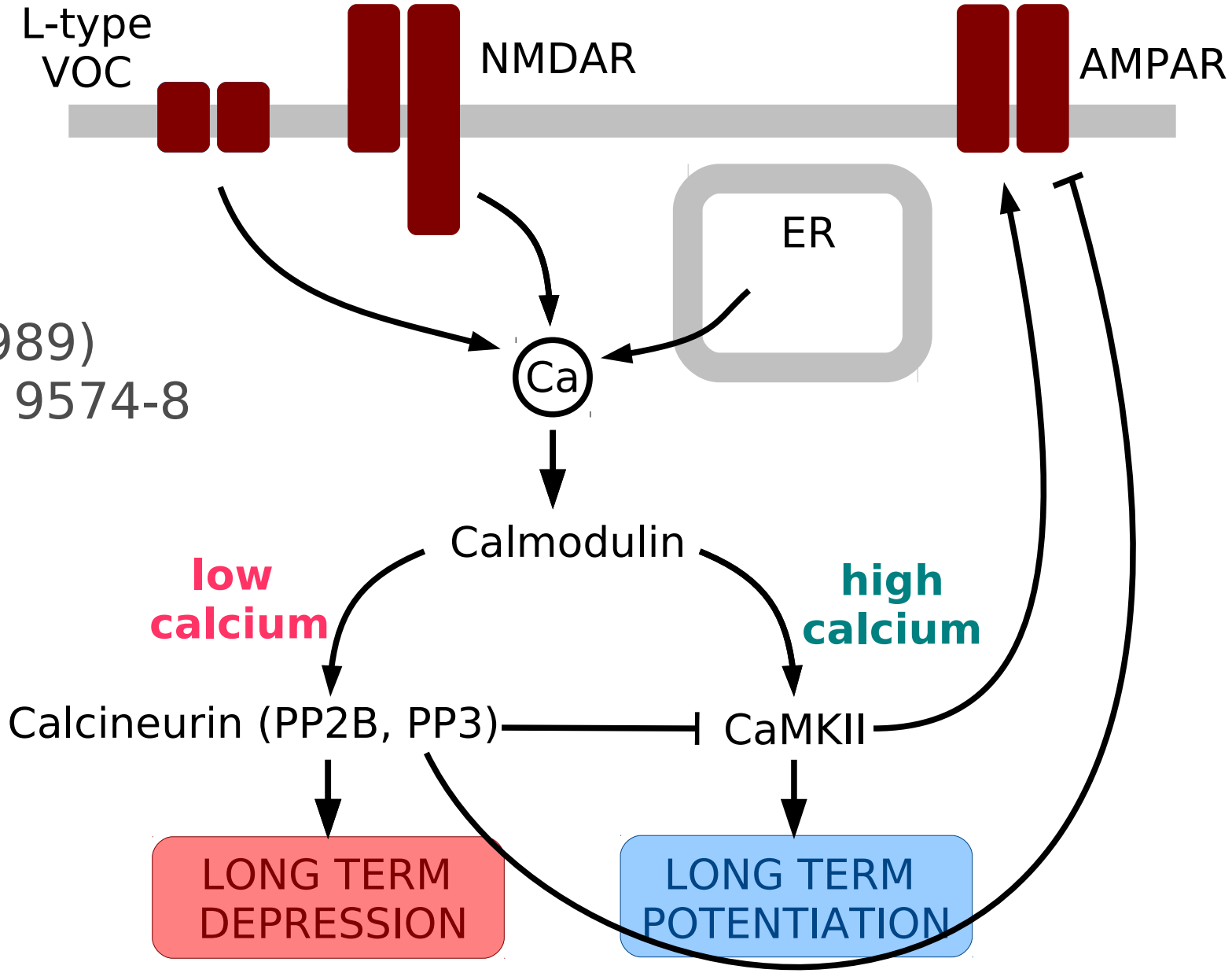




lasts for weeks

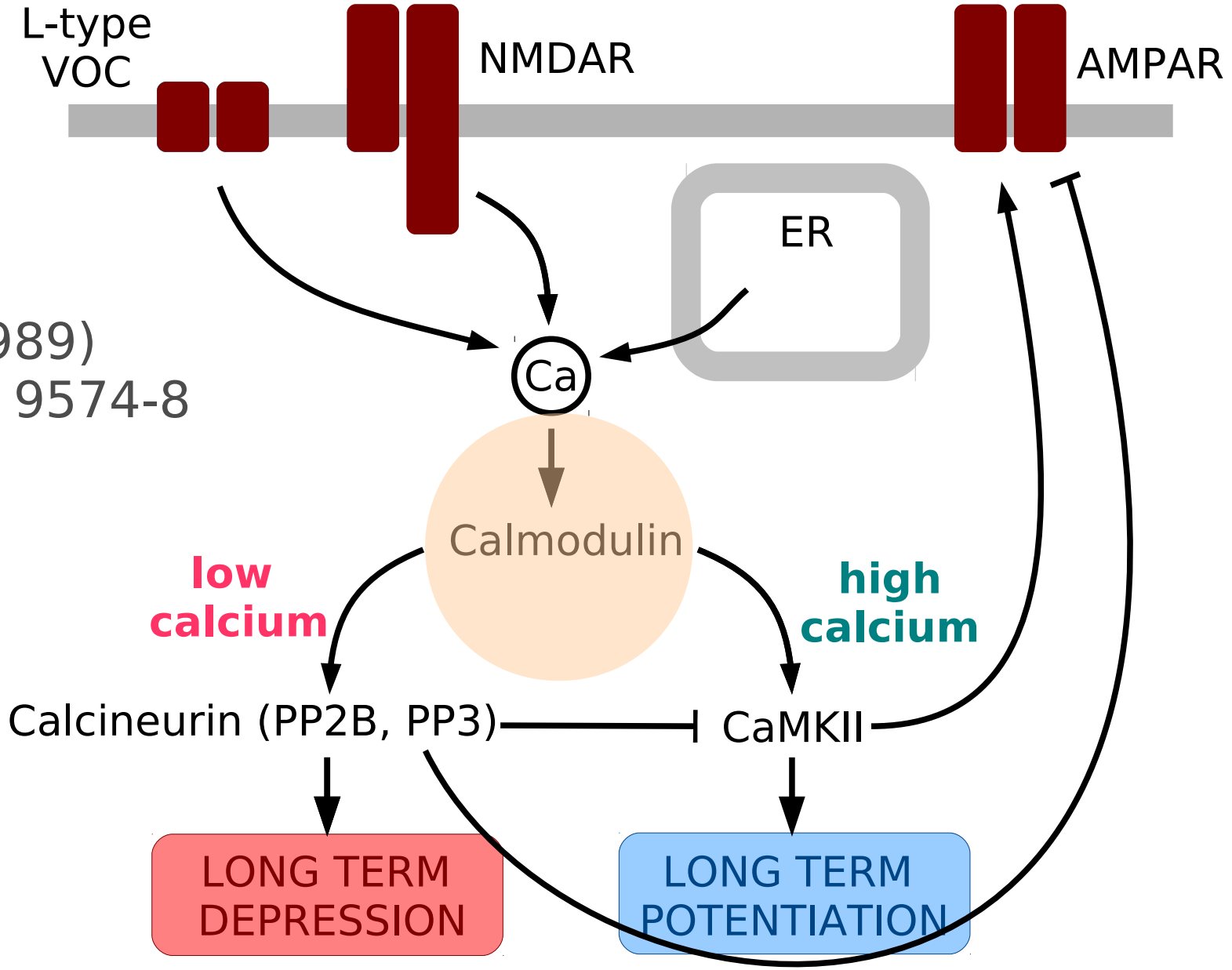
Fleming and England (2010) *Nat Chem Biol*

The calcium theory of synaptic plasticity



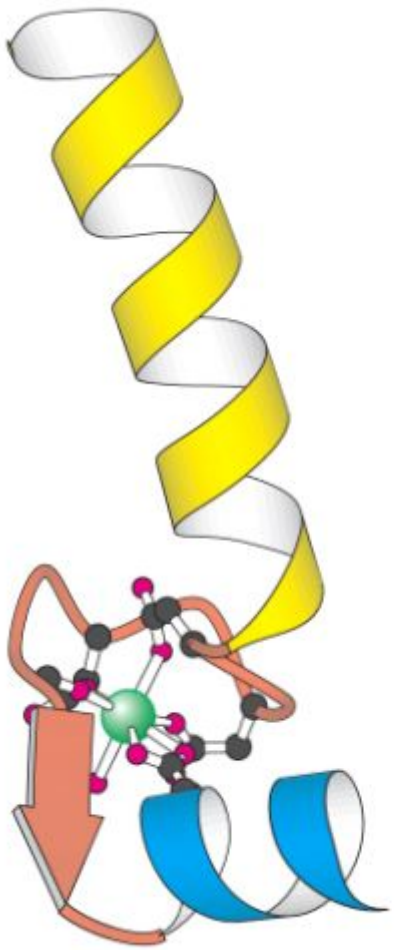
Lisman (1989)
PNAS, 86: 9574-8

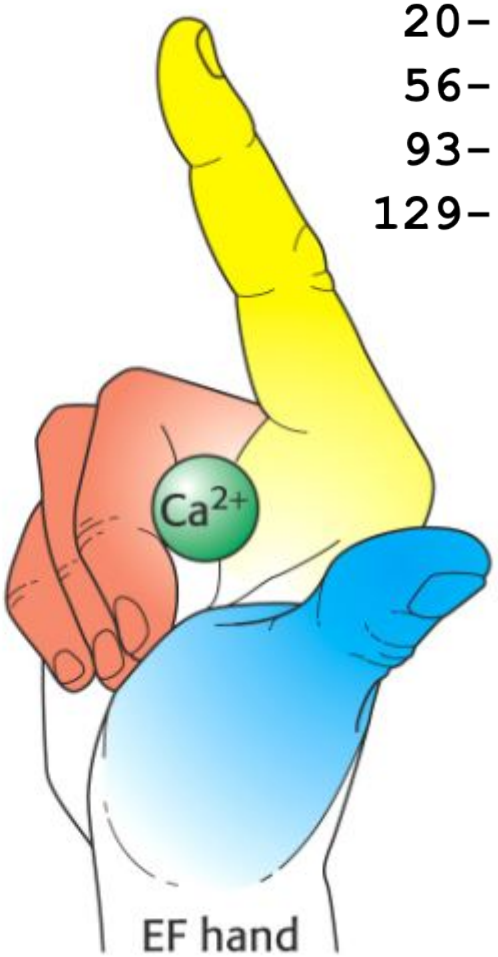
Calmodulin, the memory switchboard



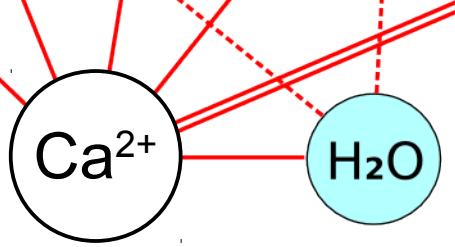
Lisman (1989)
PNAS, 86: 9574-8

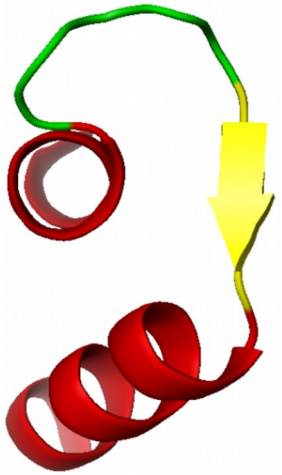
Calmodulin carries 4 Ca^{2+} binding domains

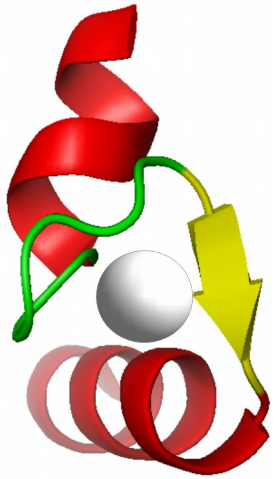


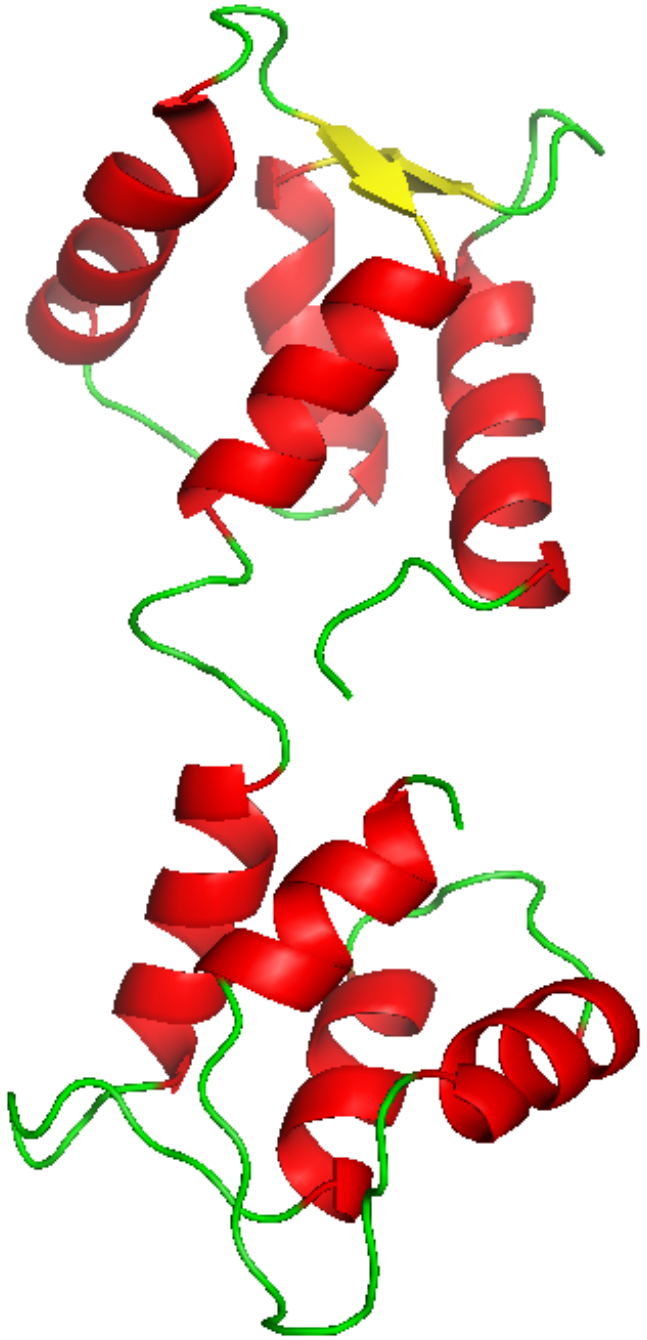


	X	Y	Z	-Y	-X	-Z	
20-	D	K	D	G	D	G	T	I	T	T	K	E	- 31
56-	D	A	D	G	N	G	T	I	D	F	P	E	- 67
93-	D	K	D	G	N	G	Y	I	S	A	A	E	-104
129-	D	I	D	G	D	G	Q	V	N	Y	E	E	-140

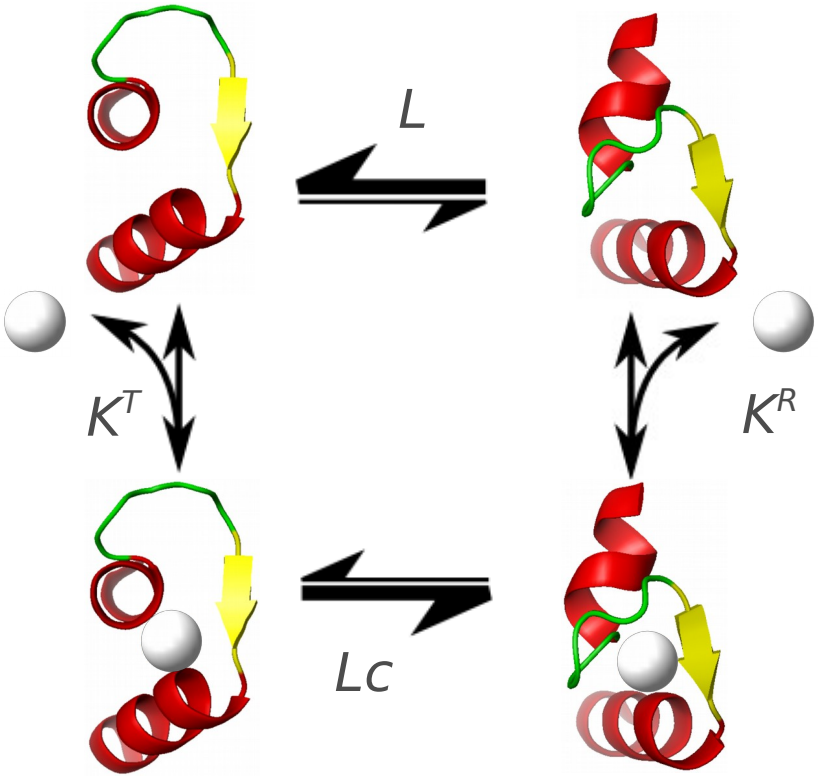


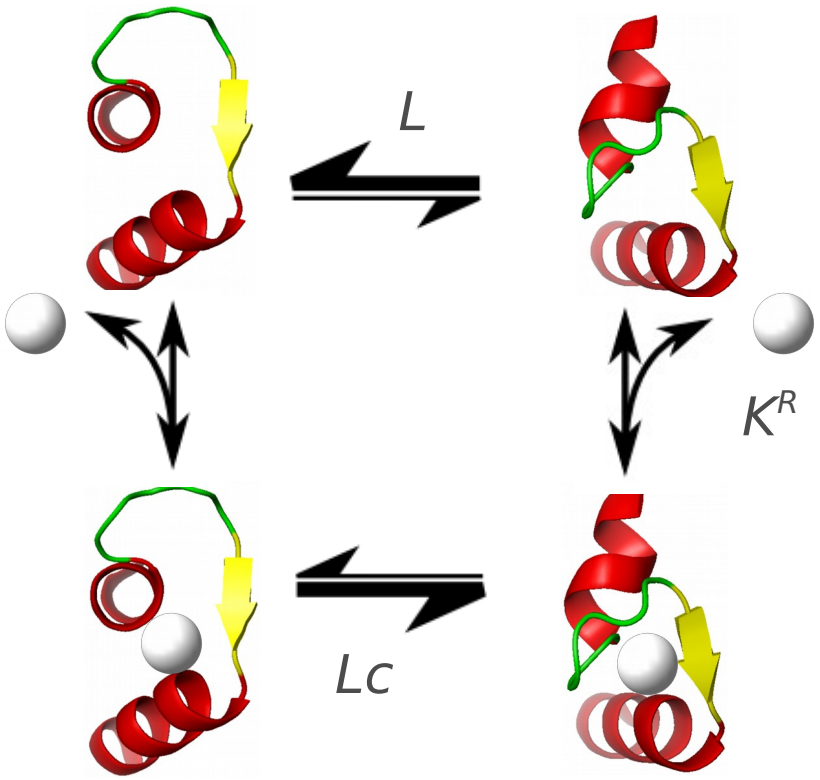


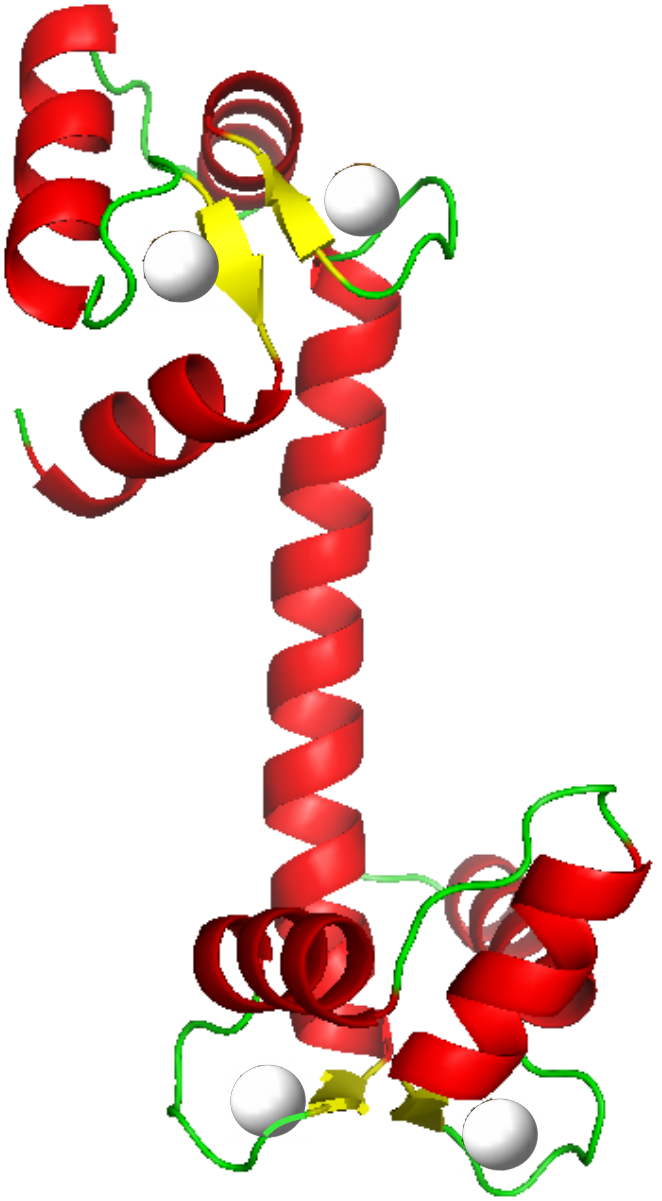




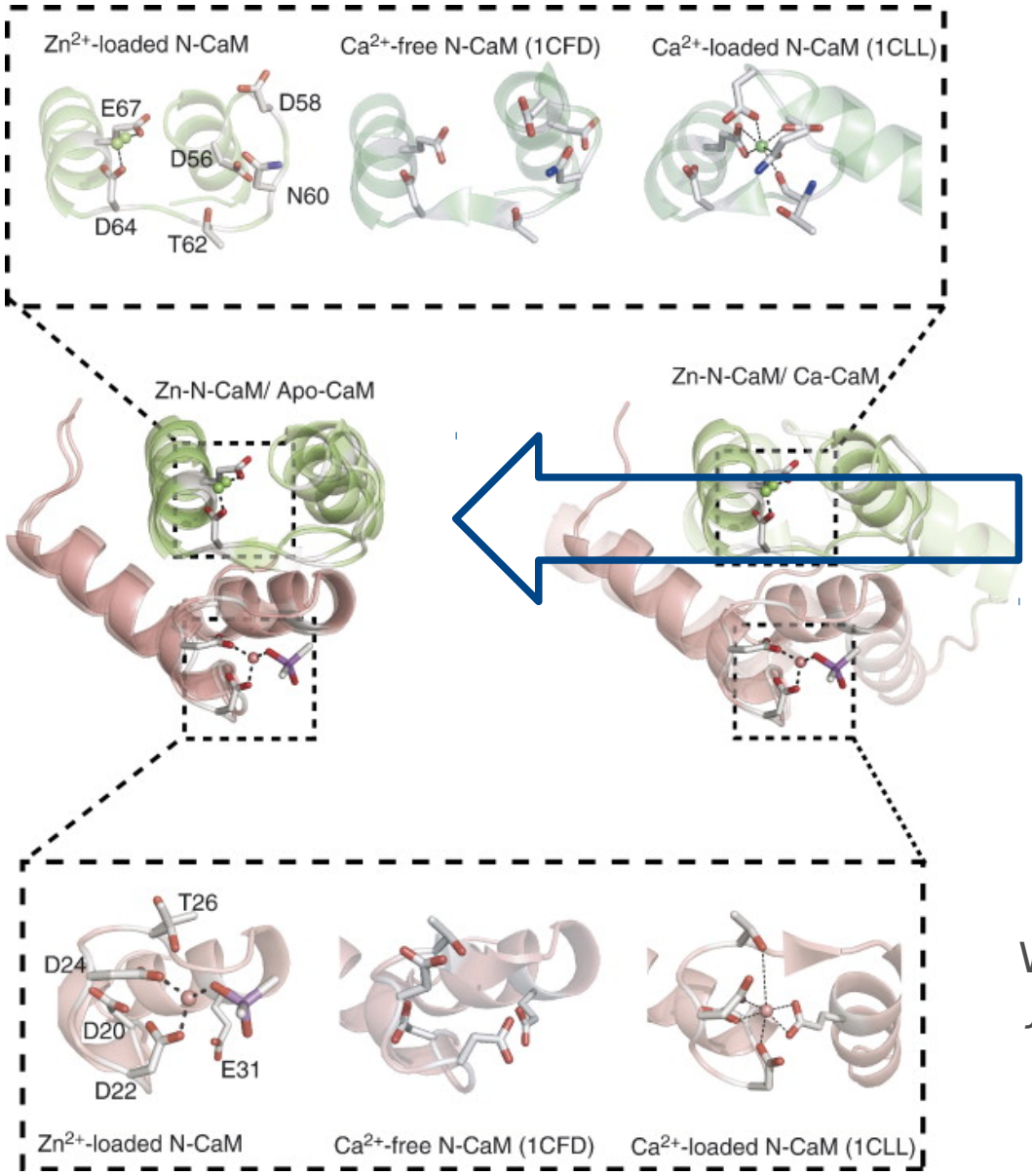
Kuboniwa *et al.* (1995).
Nat Struct Biol 2: 768-776

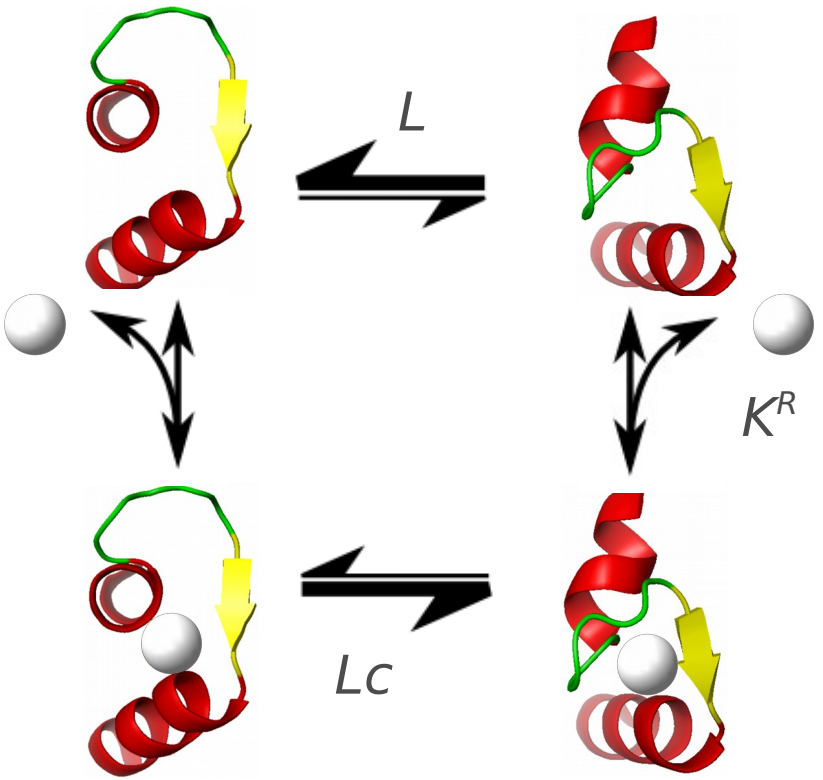






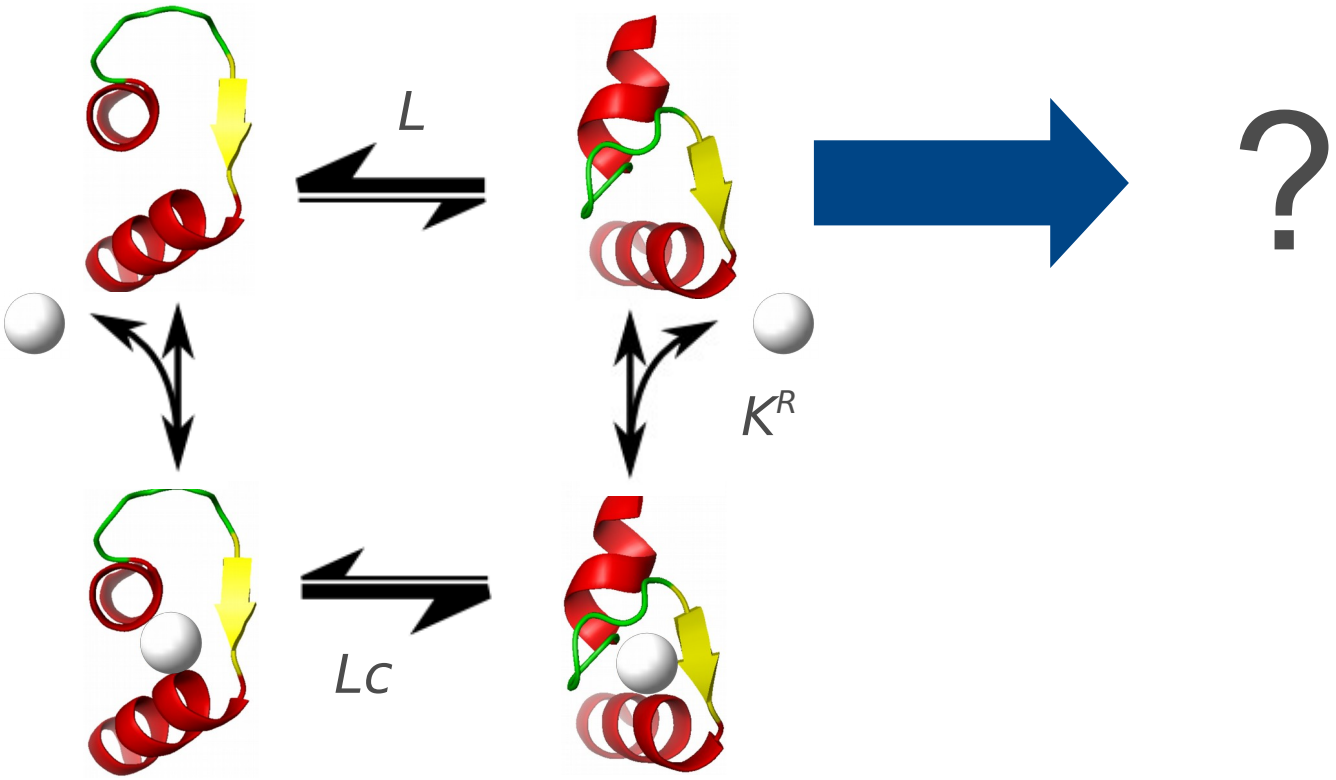
Babu *et al.* (1985).
Nature 315: 37-40





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

Babraham Institute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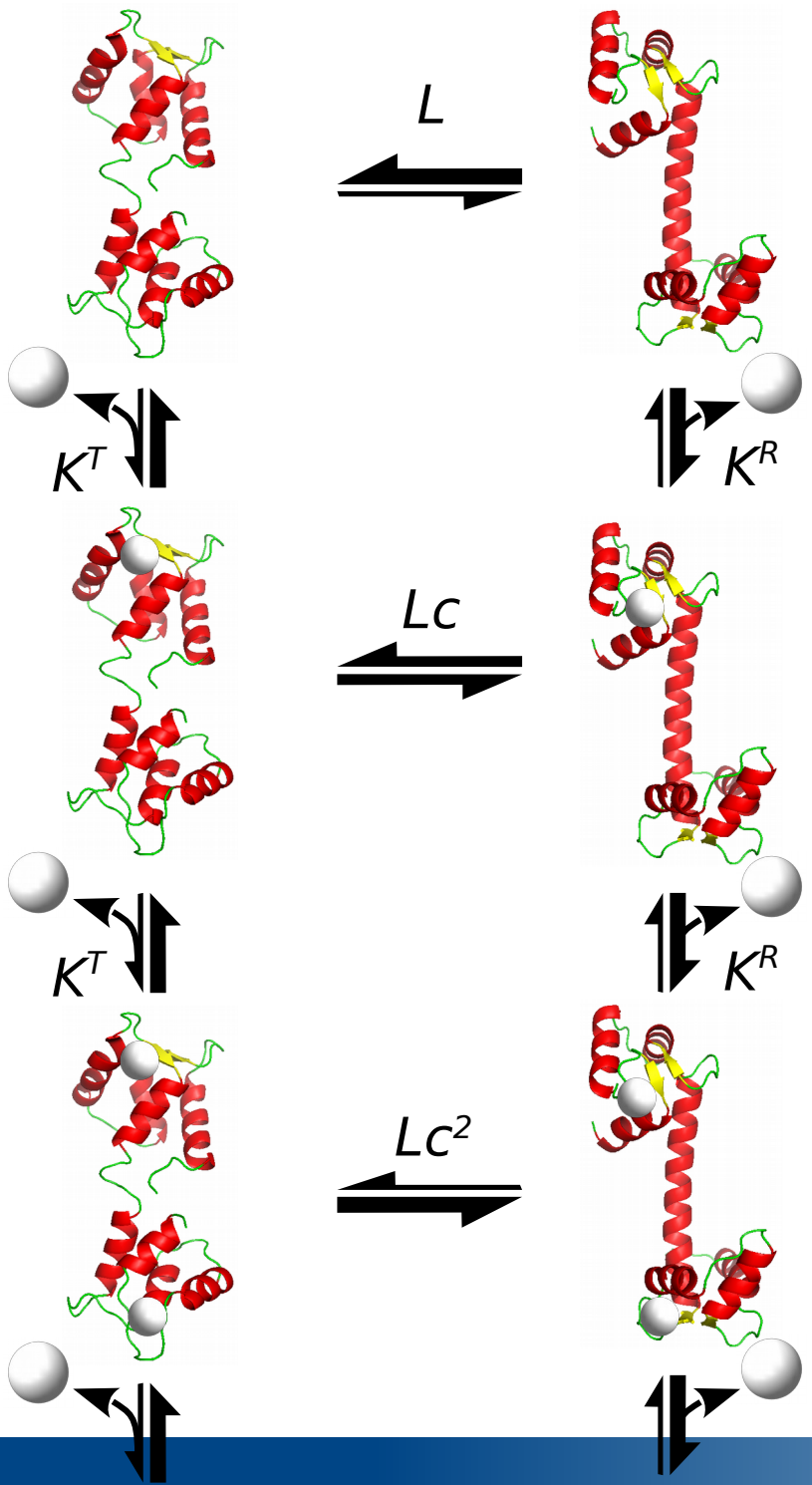
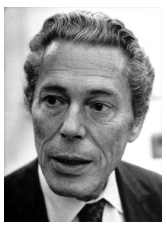
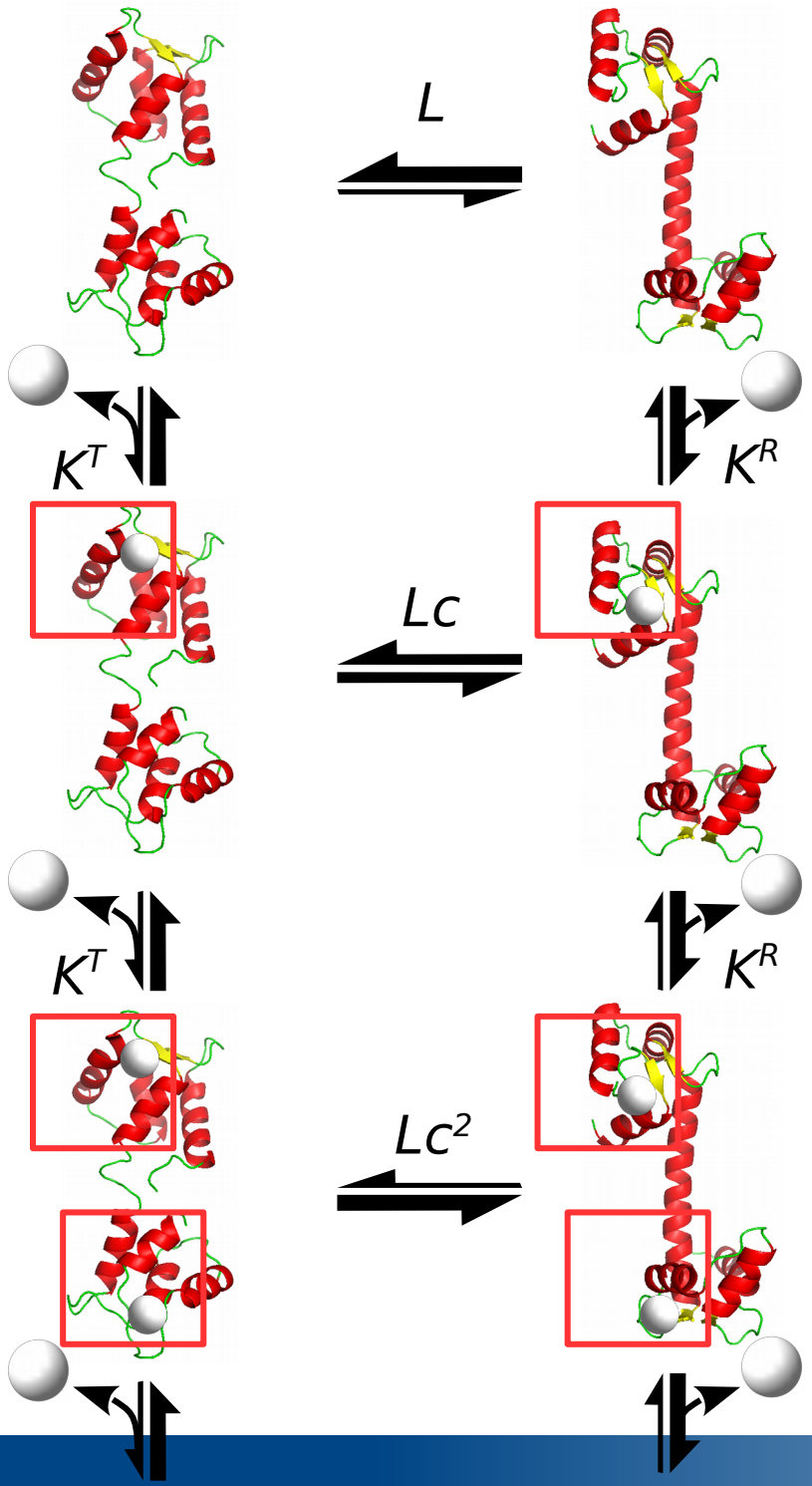




Monod, Wyman, Changeux (1965)
On the nature of allosteric transitions: a plausible model.
J Mol Biol, 12: 88-118

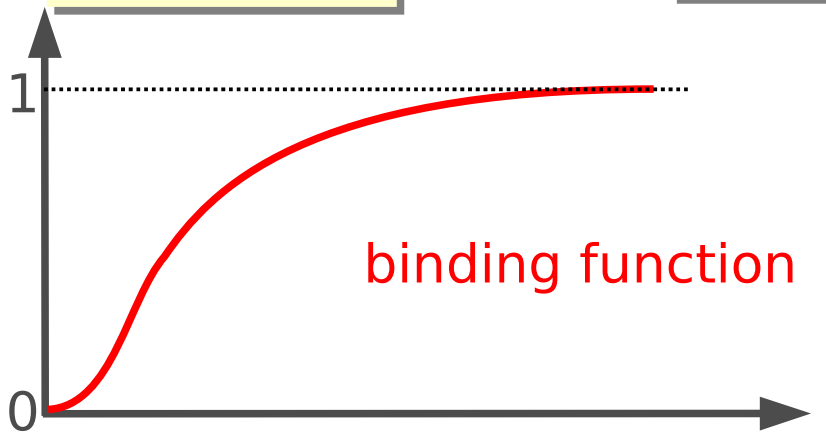


$$\bar{Y} = \frac{\alpha(1+\alpha)^{n-1} + Lc\alpha(1+c\alpha)^{n-1}}{(1+\alpha)^n + L(1+c\alpha)^n}$$

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

$L \gg 1 \Rightarrow$ Equilibrium strongly biased

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



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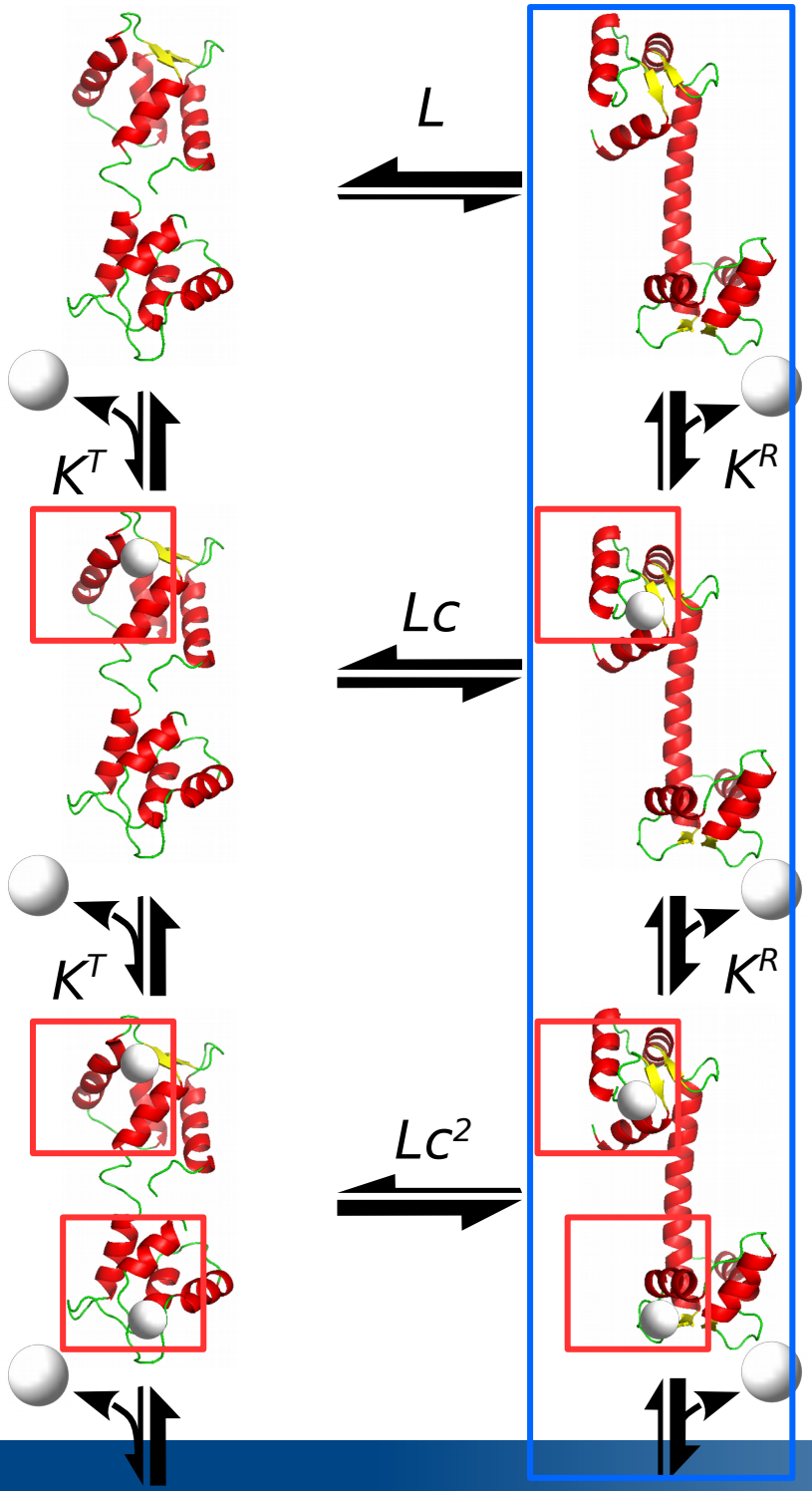

$$\bar{Y} = \frac{\alpha(1+\alpha)^{n-1} + Lc\alpha(1+c\alpha)^{n-1}}{(1+\alpha)^n + L(1+c\alpha)^n}$$

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

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

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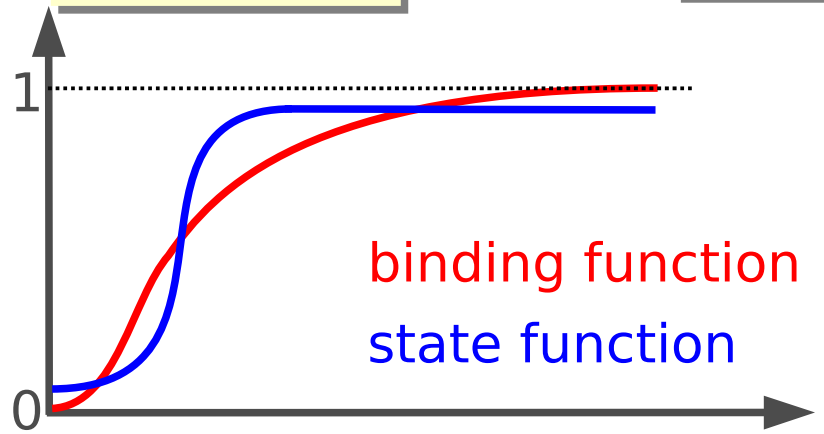

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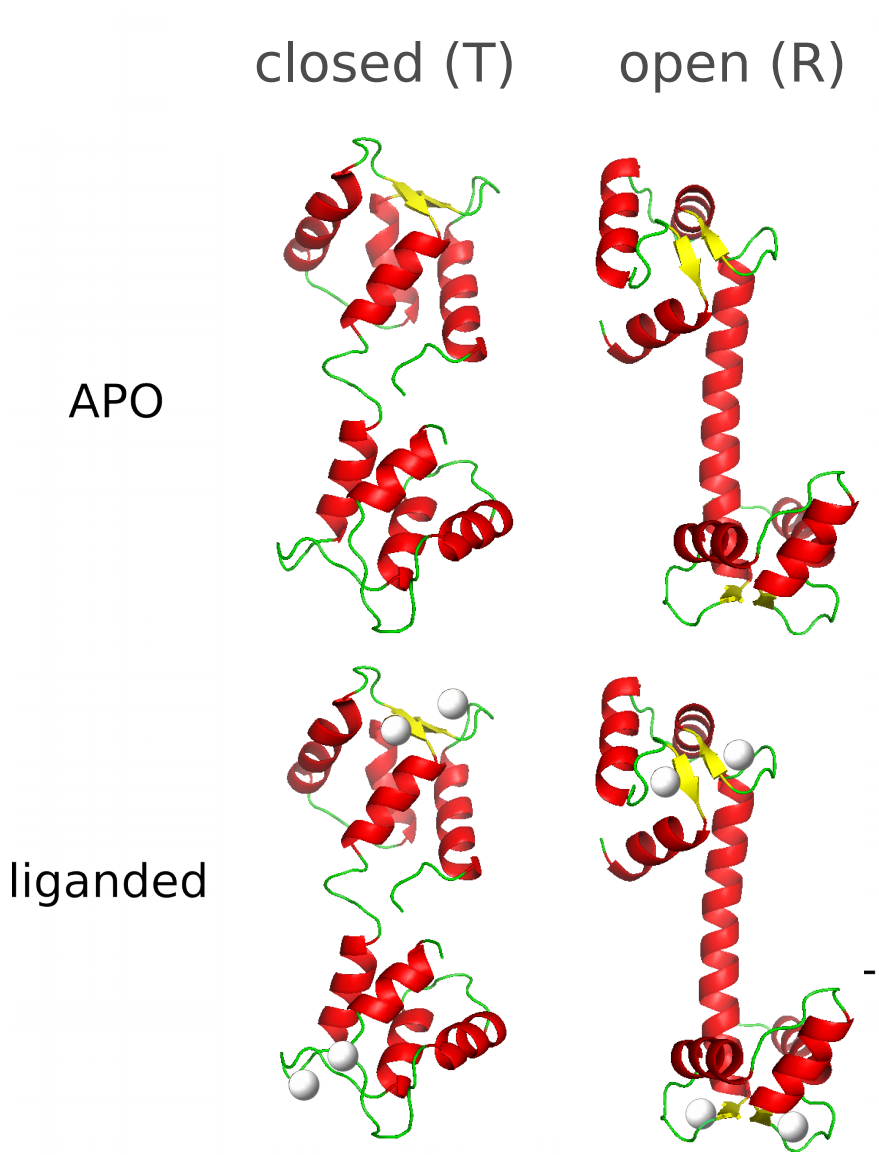

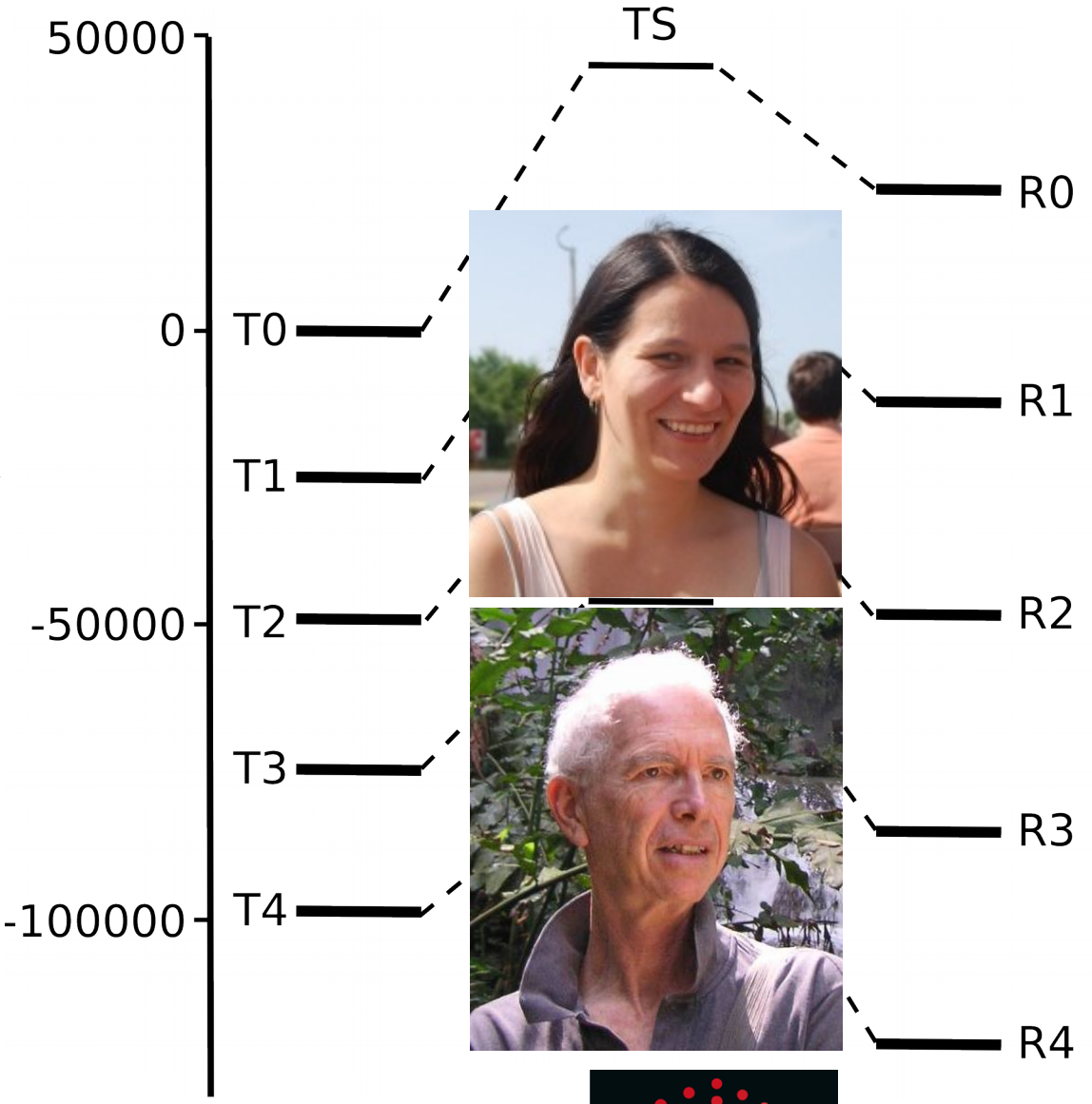

Stefan MI, Edelstein SJ, Le Novère N (2008)
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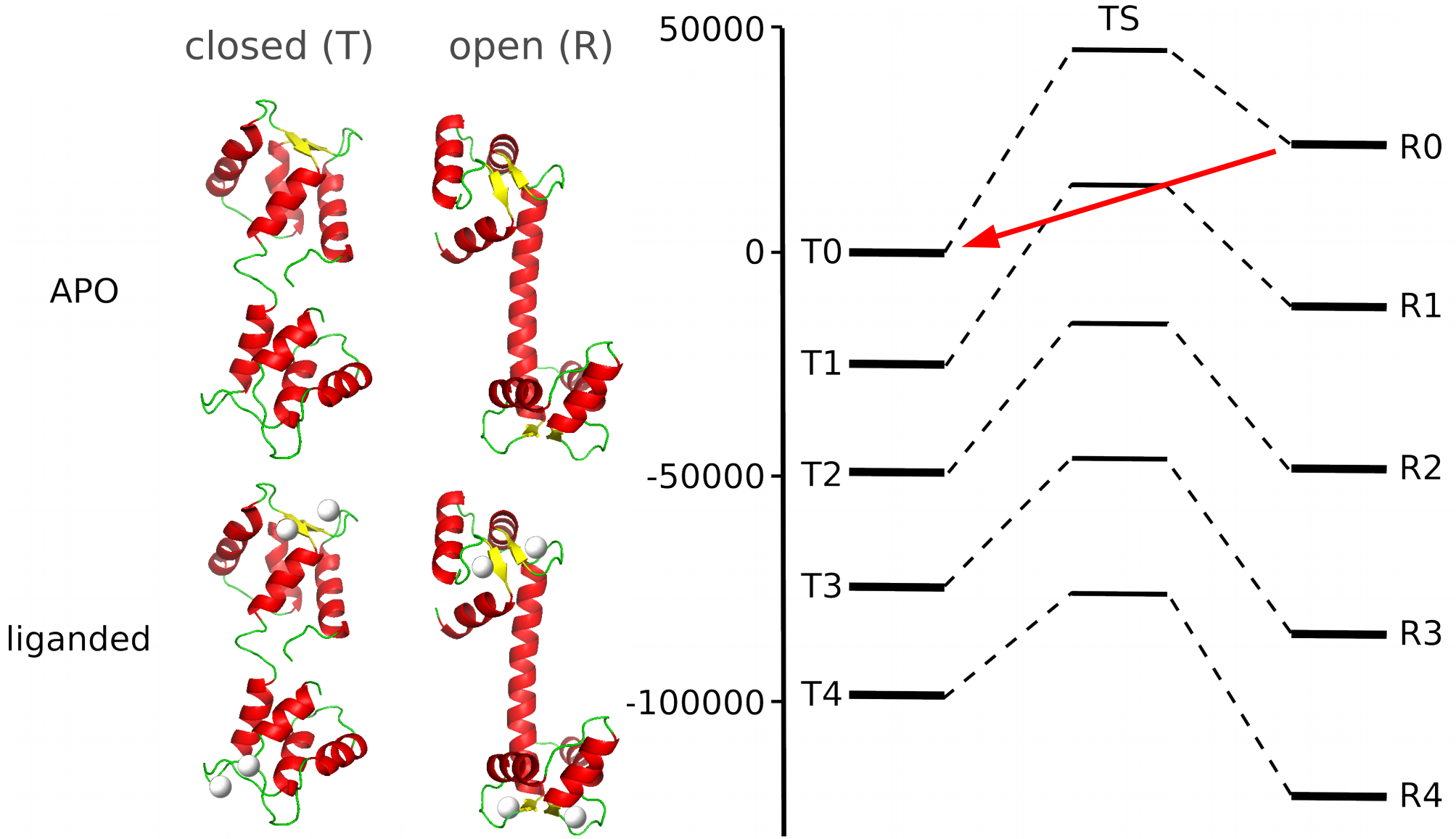
Edelstein SJ, Stefan MI, Le Novère N (2010)



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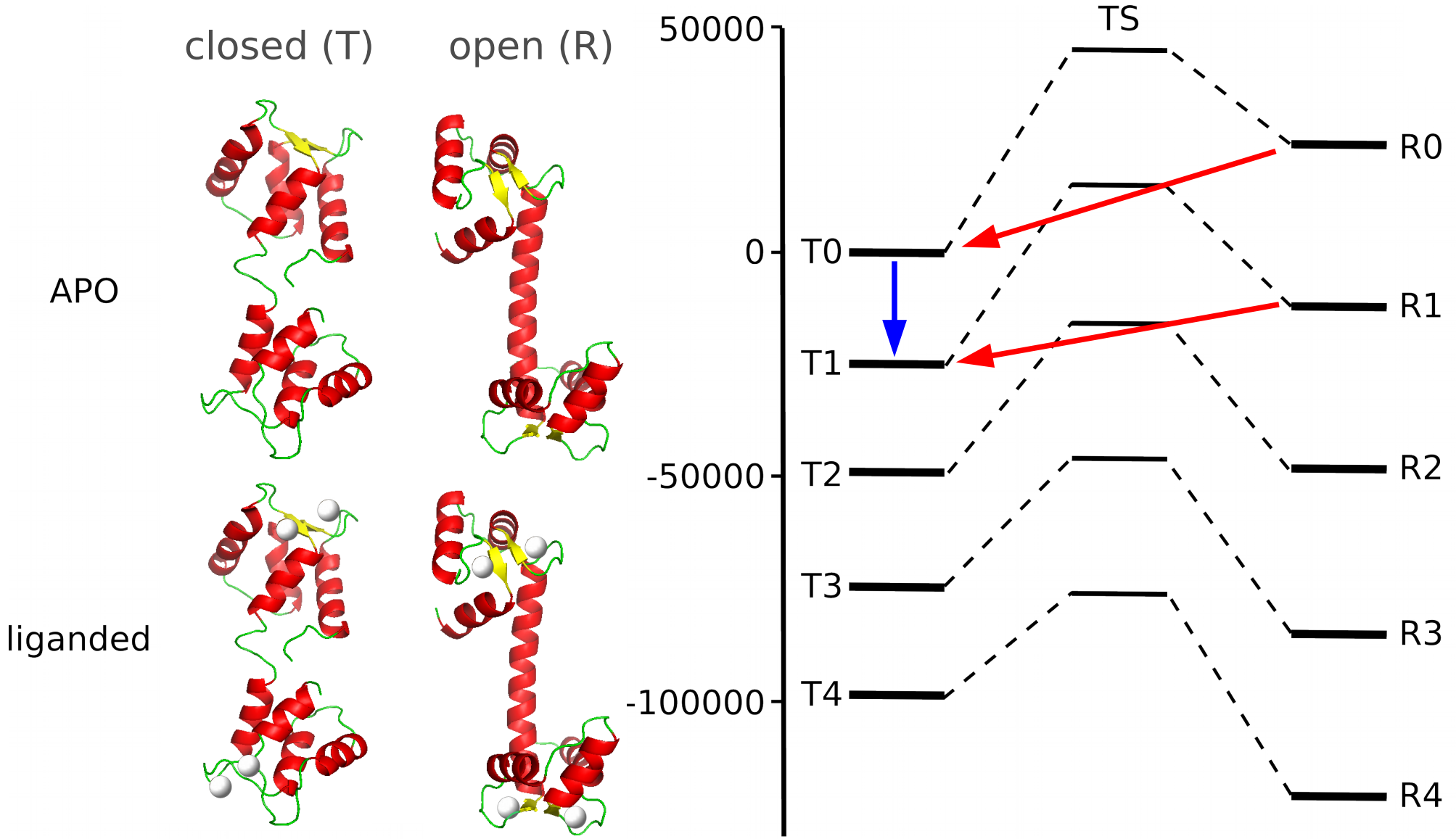

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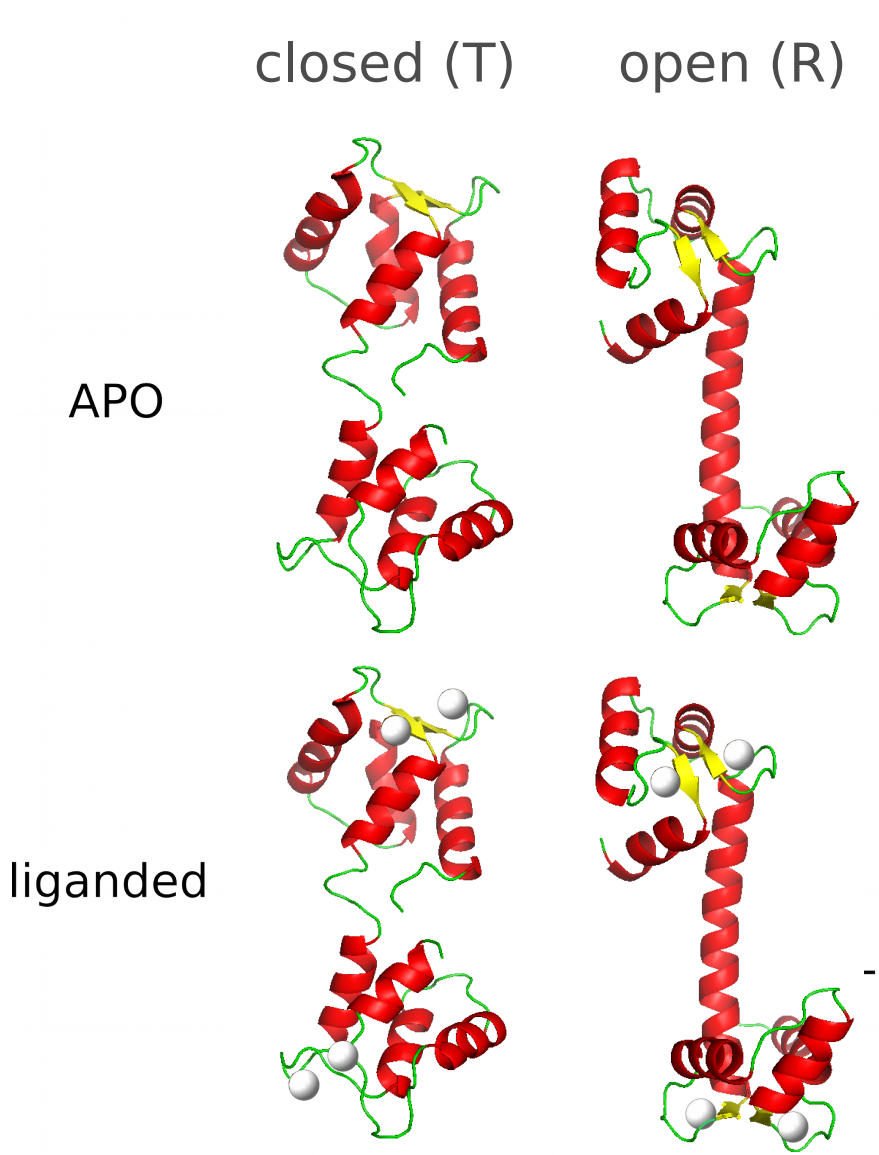

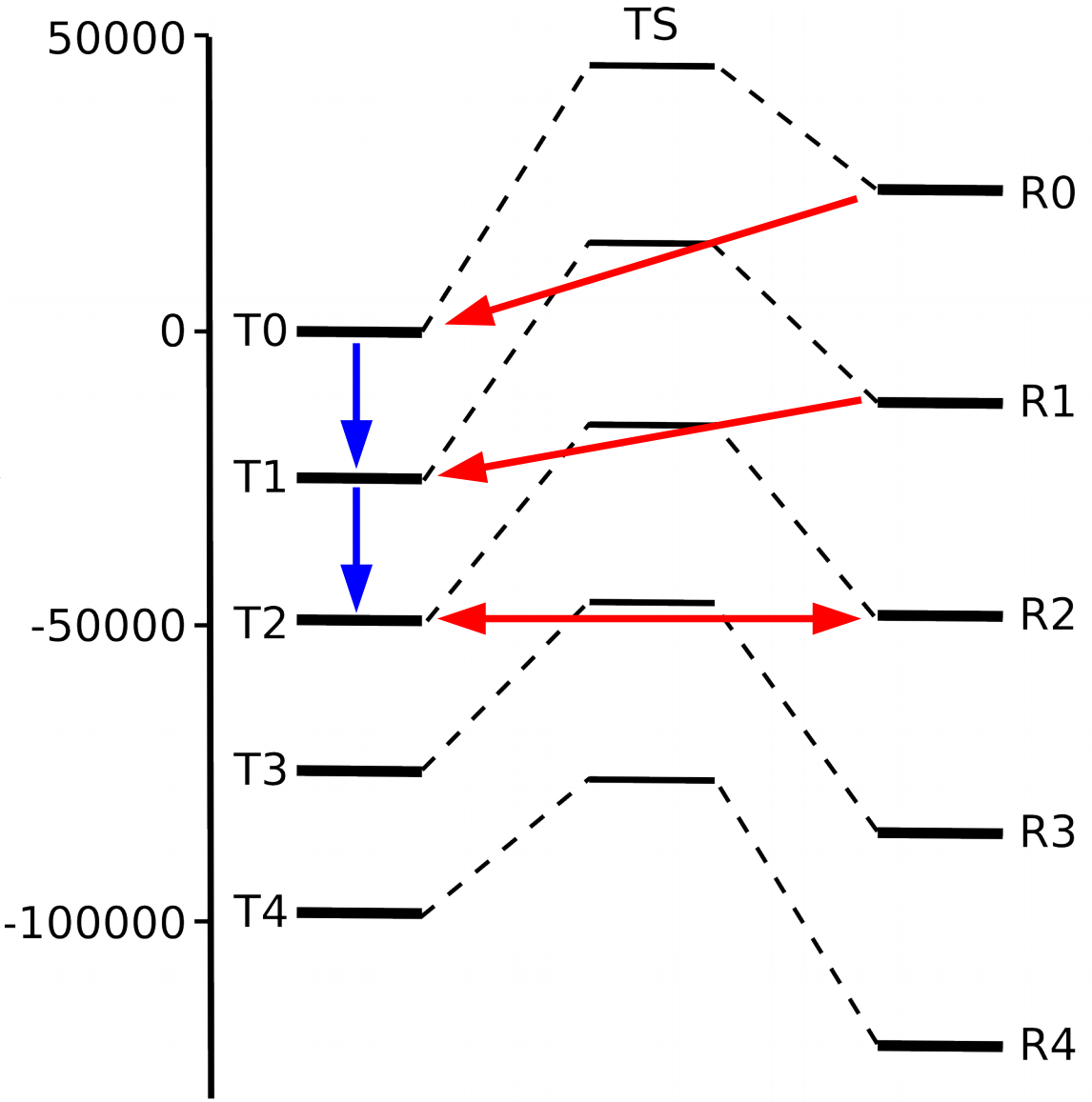

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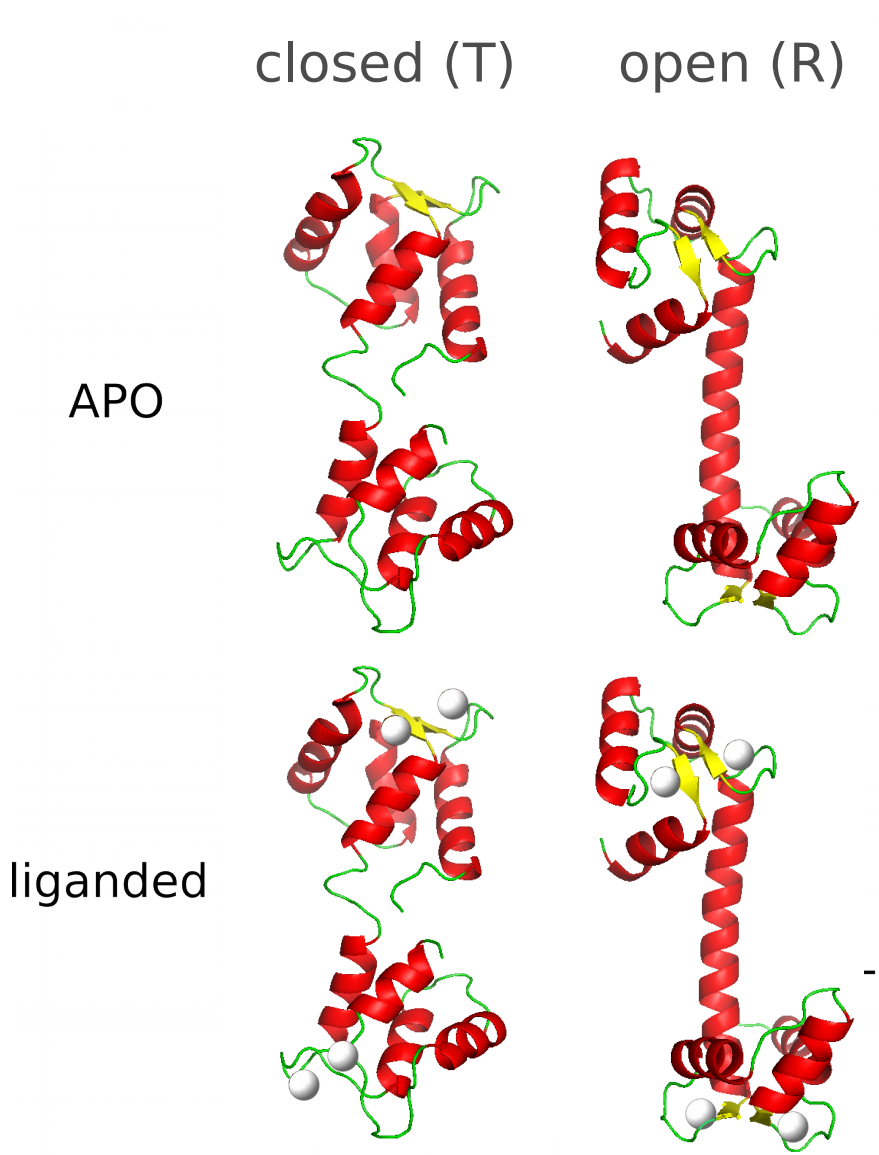

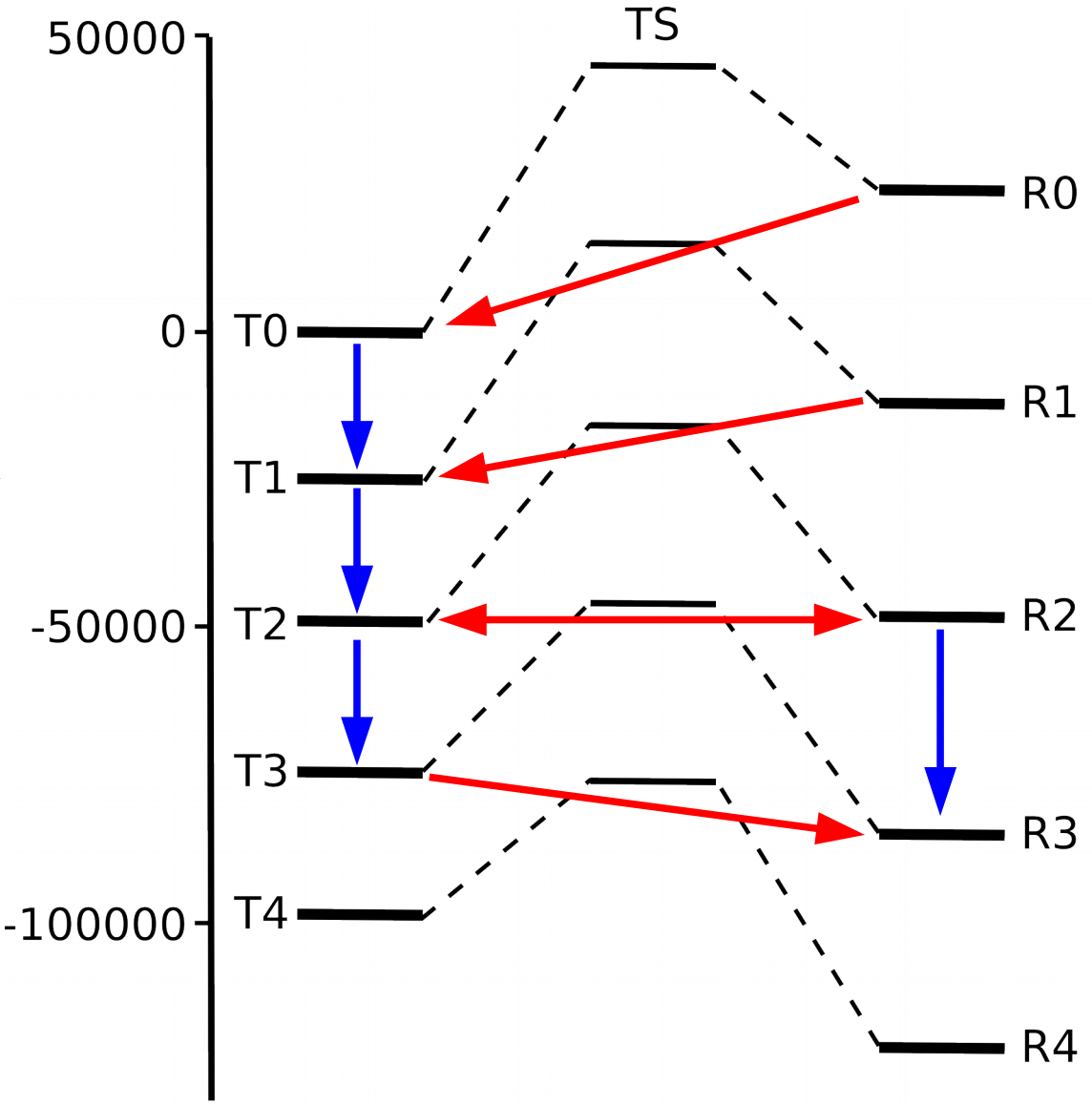

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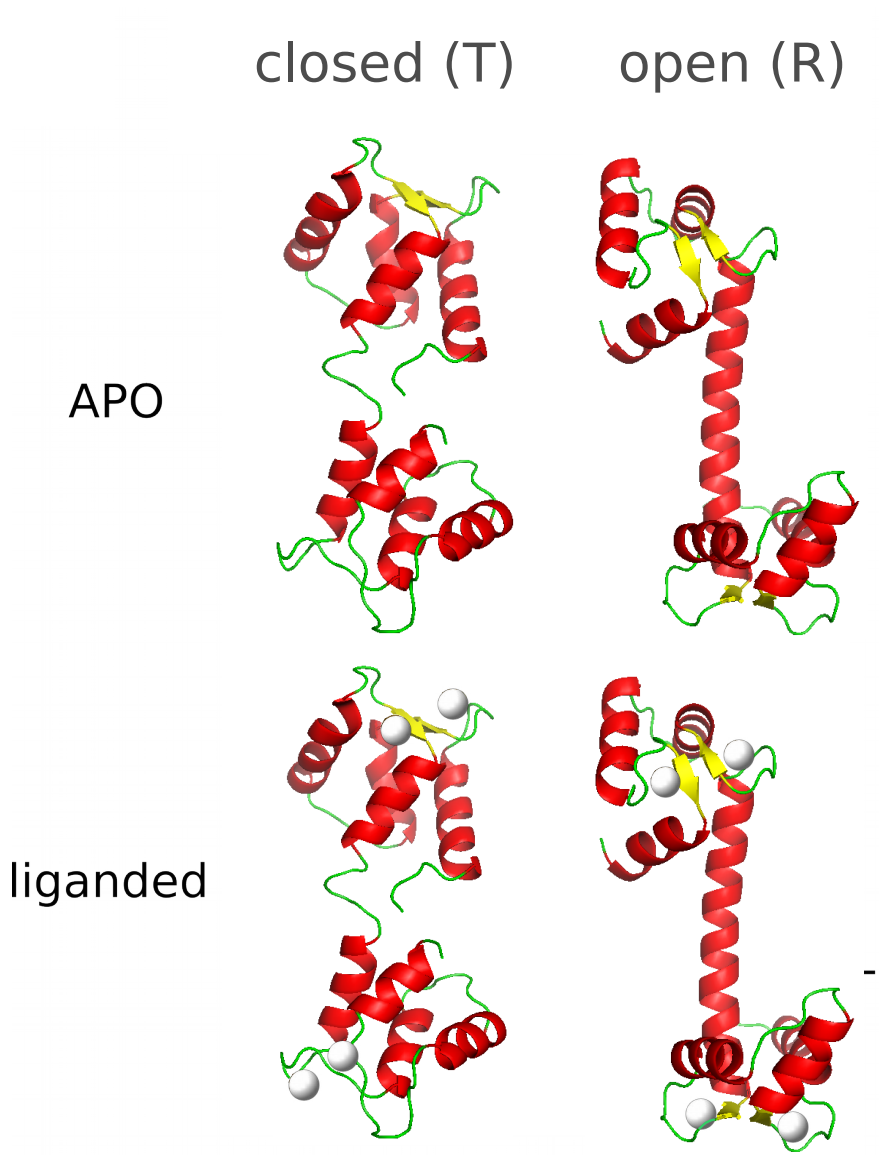

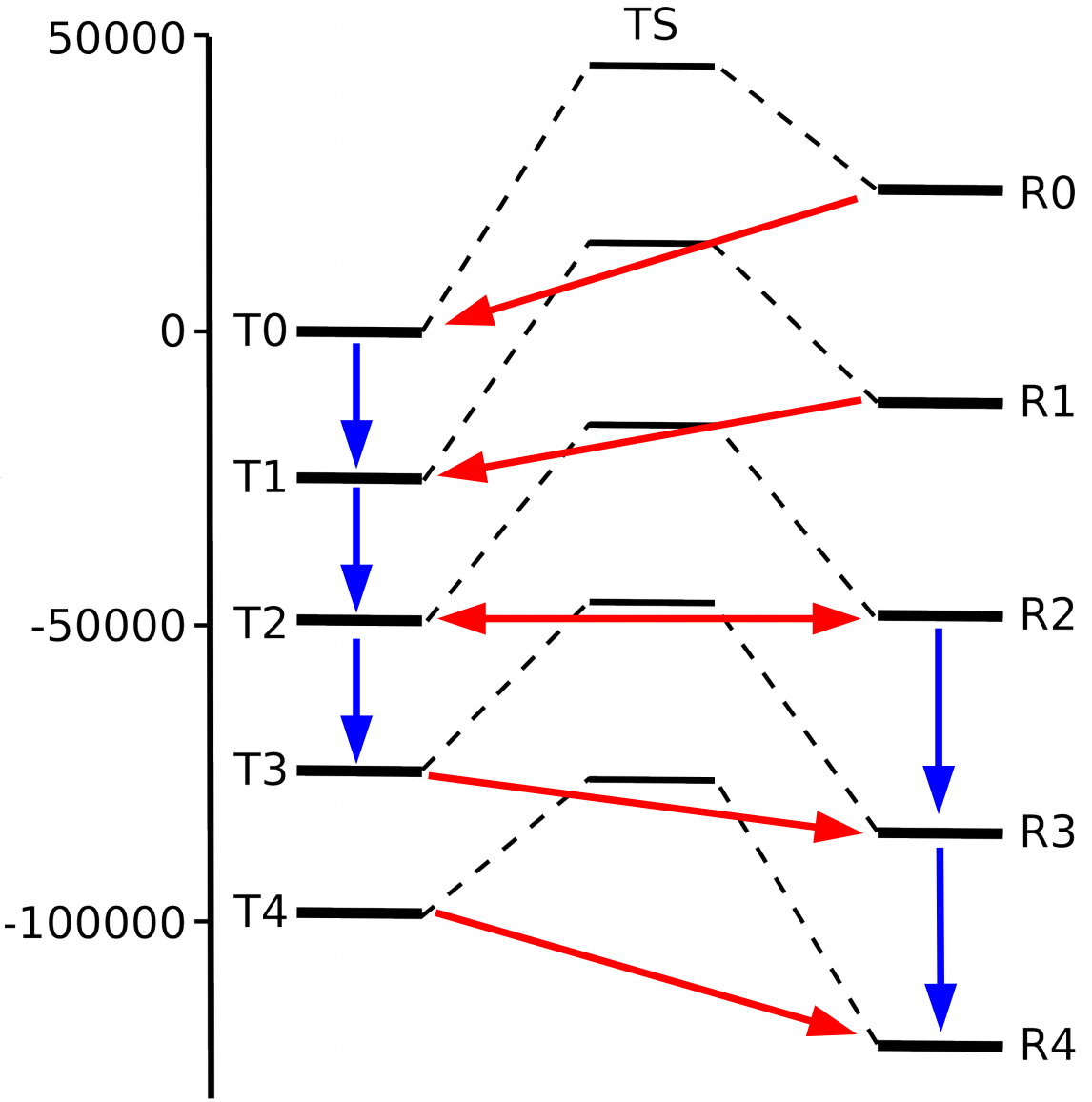

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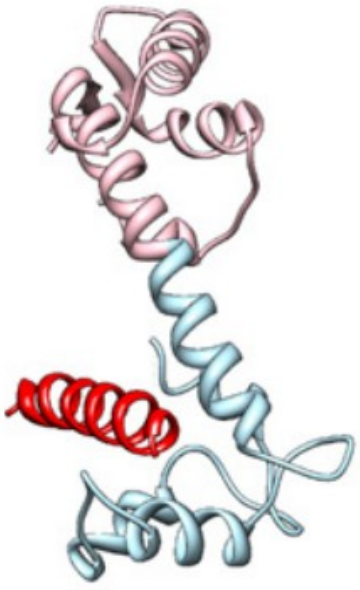
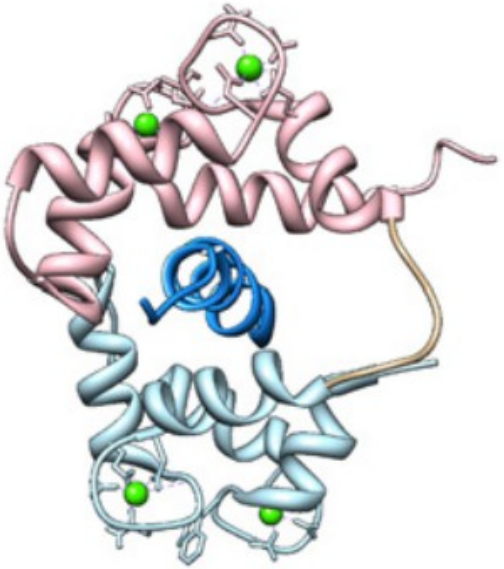
Stefan MI, Edelstein SJ, Le Novère N (2009)
Edelstein SJ, Stefan MI, Le Novère N (2010)

Different targets stabilise lobes in different states

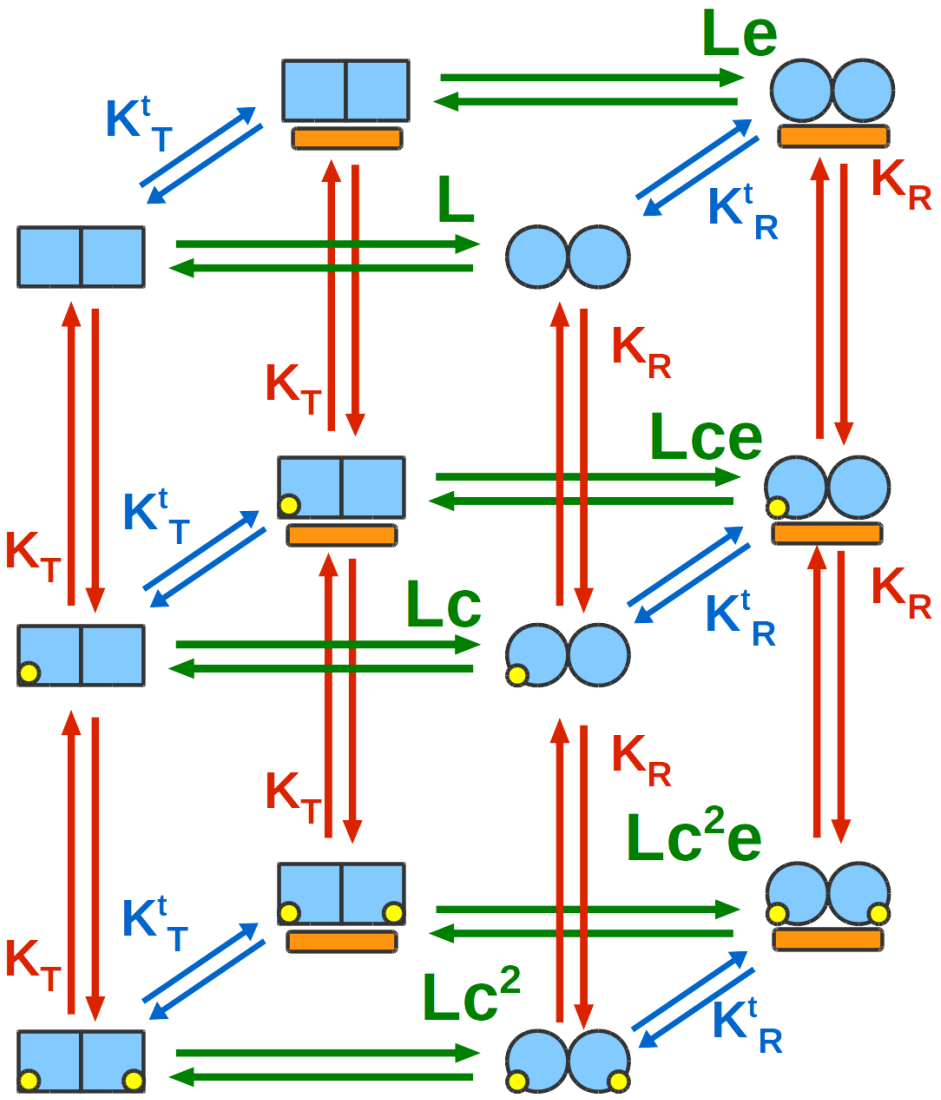


Neurogranin



MLCK

Bindings of calcium and targets



T-state

R-state

Calcium

Target

Conf. transition

Calcium binding

Target binding

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

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

Targets as allosteric effectors: theory

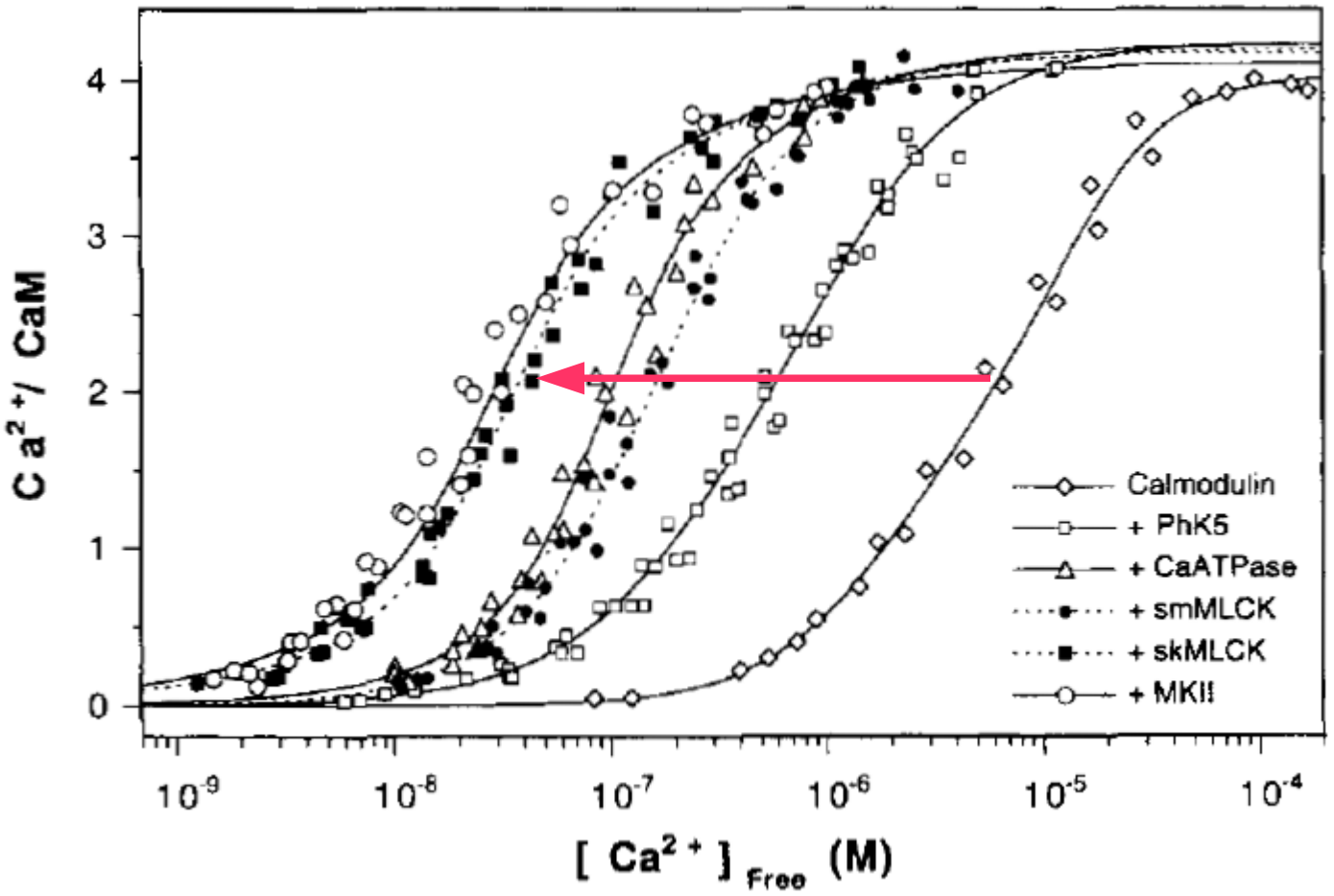
$$\bar{Y}_{\mathrm{Ca^{2+}}} = \frac{\alpha(1+\alpha)^{n-1} + L'c\alpha(1+c\alpha)^{n-1}}{(1+\alpha)^n + L'(1+c\alpha)^n}$$

$$L = \frac{[T_0]}{[R_0]} \qquad L' = L\left(\frac{1+\gamma e}{1+\gamma}\right)^n$$

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

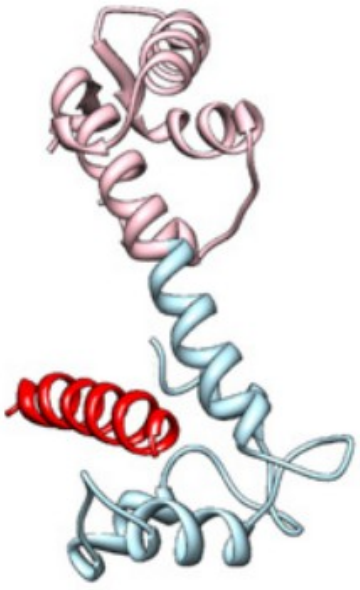
$$\alpha = \frac{[Ca^{2+}]}{[K^R]} \qquad \gamma = \frac{[Target]}{[K_t^R g]}$$

Targets as allosteric effectors: experiments

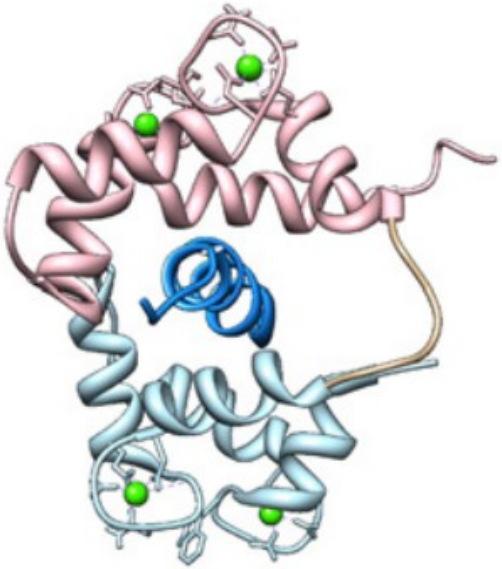


Peersen et al. (1997)

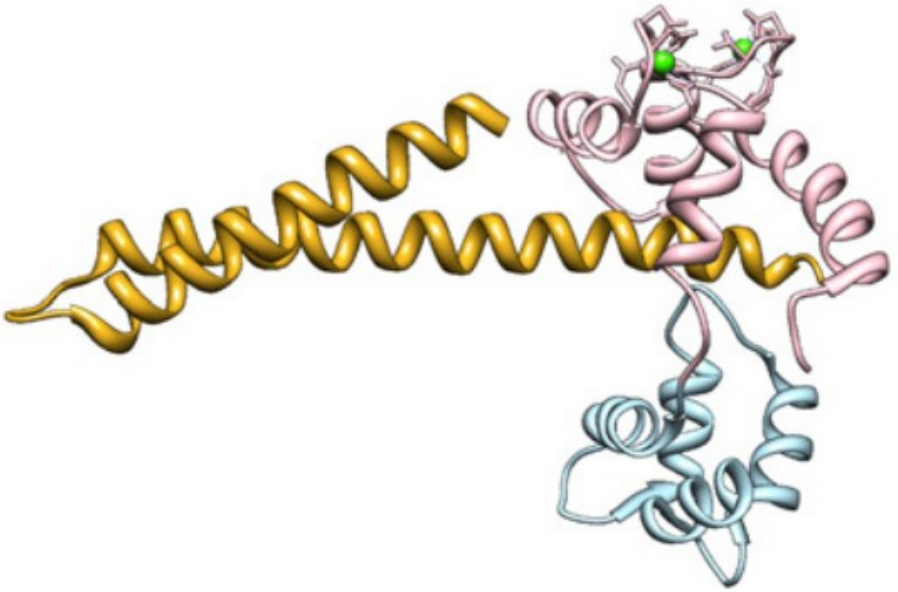
Different targets stabilise lobes in different states



Neurogranin



MLCK



SK channel

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

COPASI

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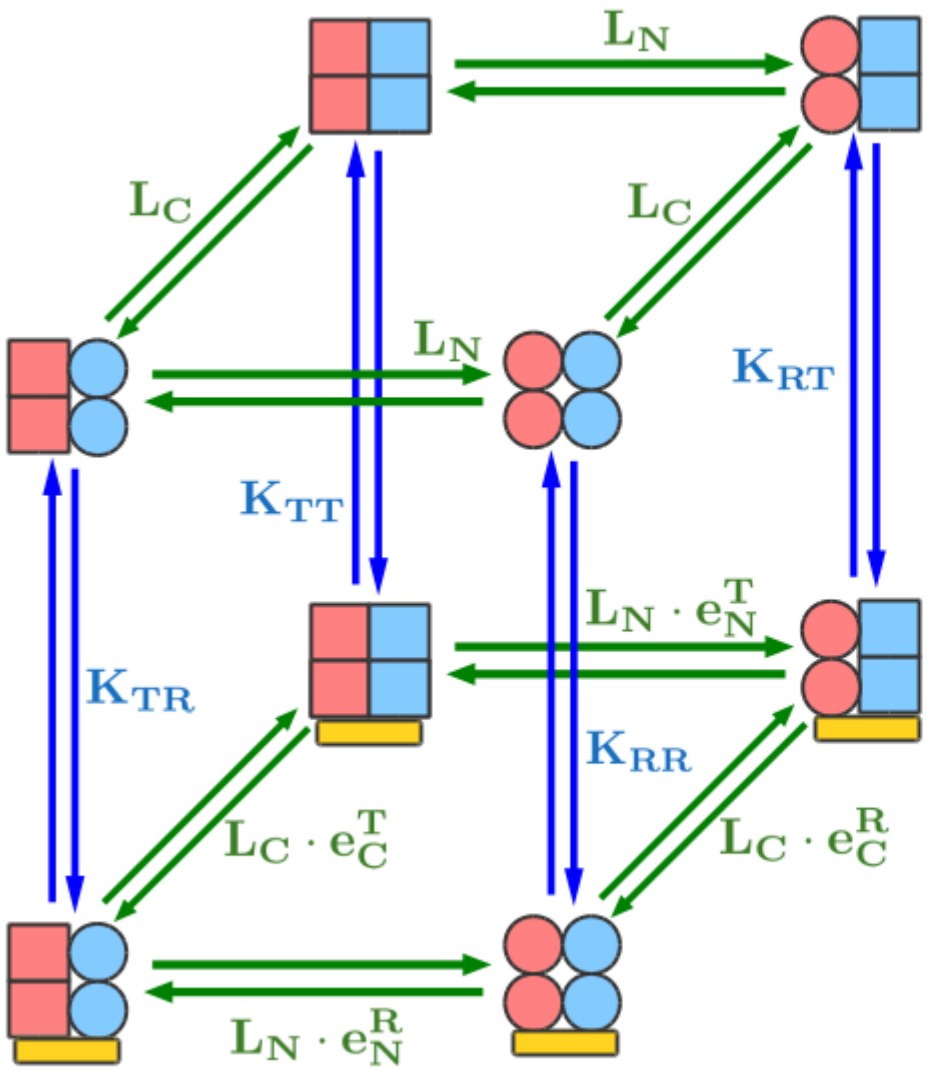
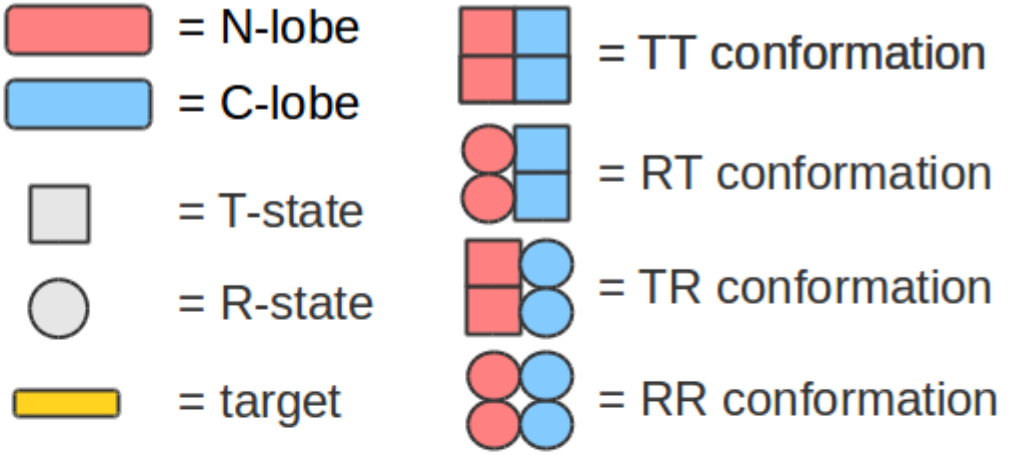
Hemiconcerted model of calmodulin





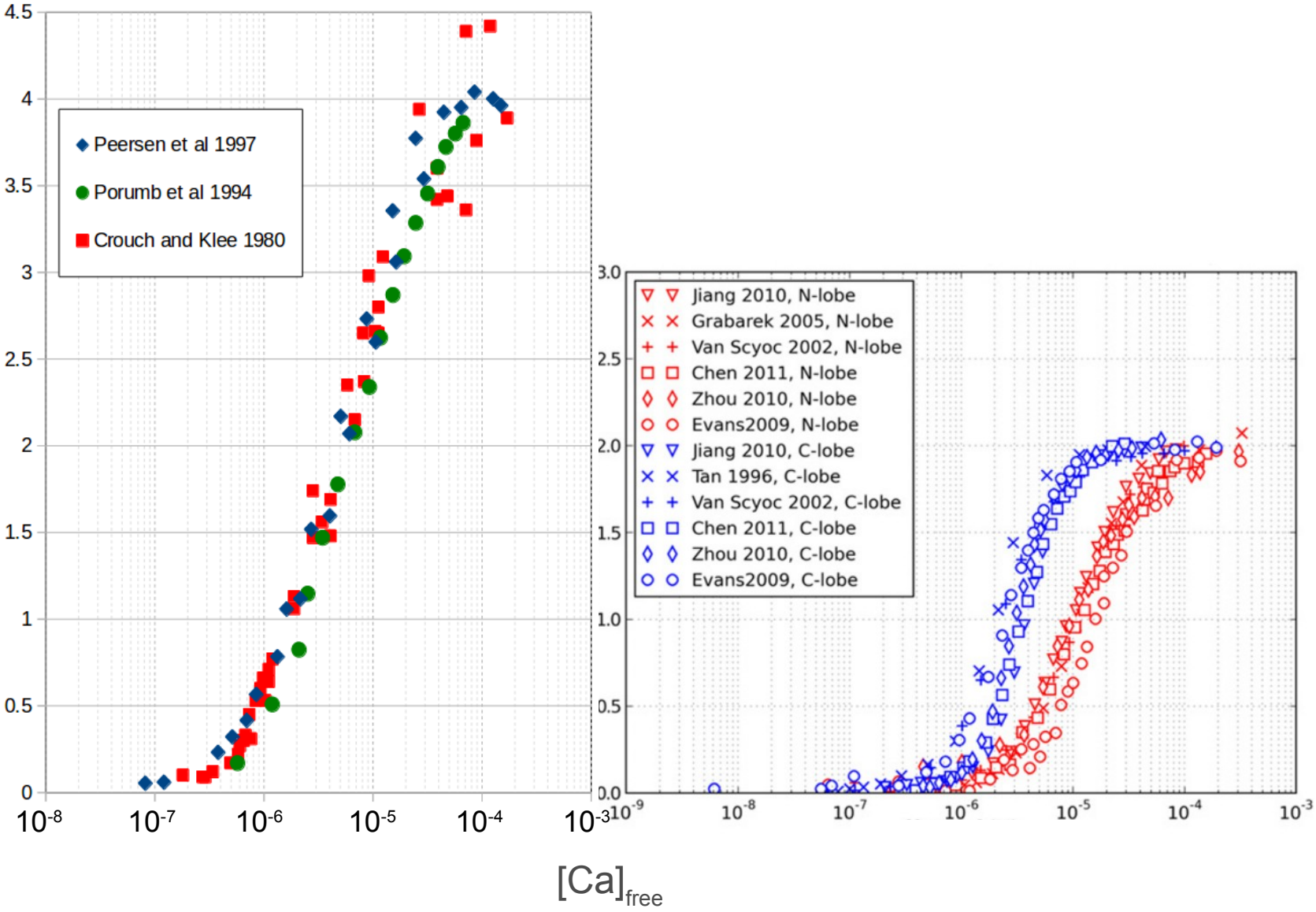
$$L_N = \frac{[TT]}{[RT]} = \frac{[TR]}{[RR]} \qquad L_C = \frac{[TT]}{[TR]} = \frac{[RT]}{[RR]}$$

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

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

Calcium binding to lobes and whole CaM (exp)

$\frac{[Ca]_{bound}}{[CaM]}$



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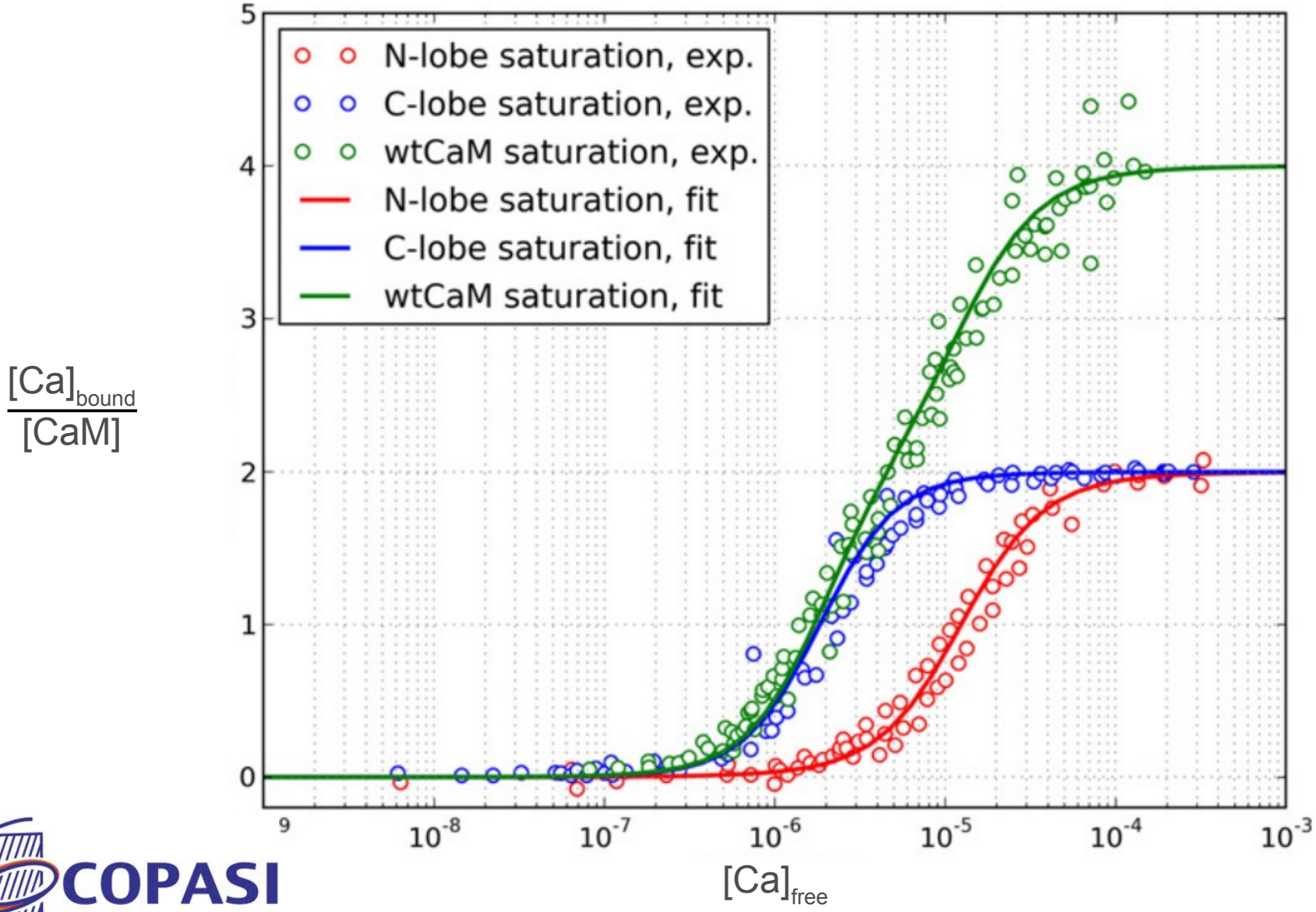
Calcium binding to lobes and whole CaM (sim)



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+}

$L=20670$

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

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

$K^R_A=8.32\ 10^{-6}$

$K^R_B=1.66\ 10^{-8}$

$K^R_C=1.74\ 10^{-5}$

$K^R_D=1.45\ 10^{-8}$

2 high, 2 low, as expected

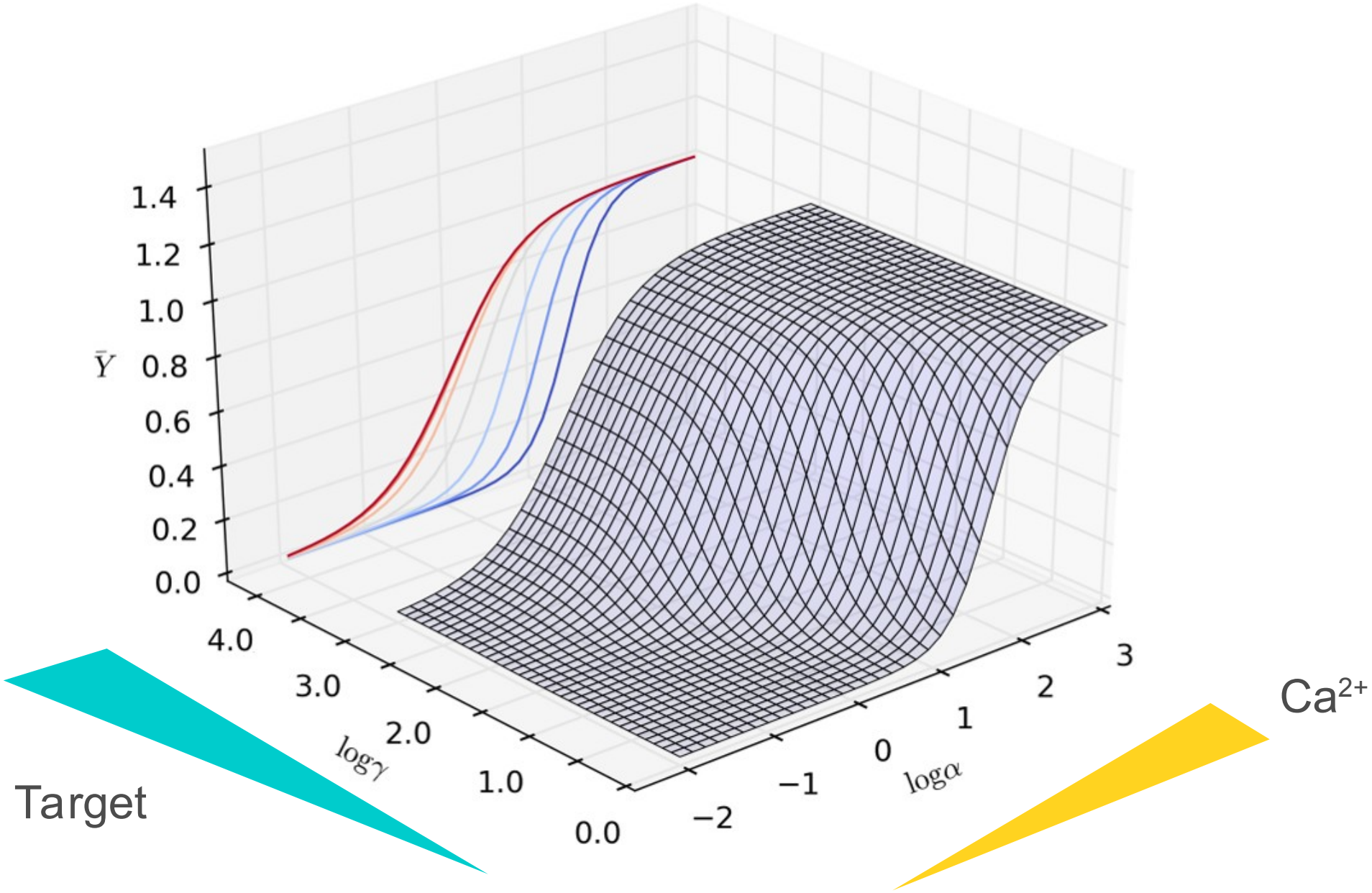
Activity of unsaturated calmodulin (state function)

- 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 ≈ equi-probability
- $R_4/T_4 = 10000$

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



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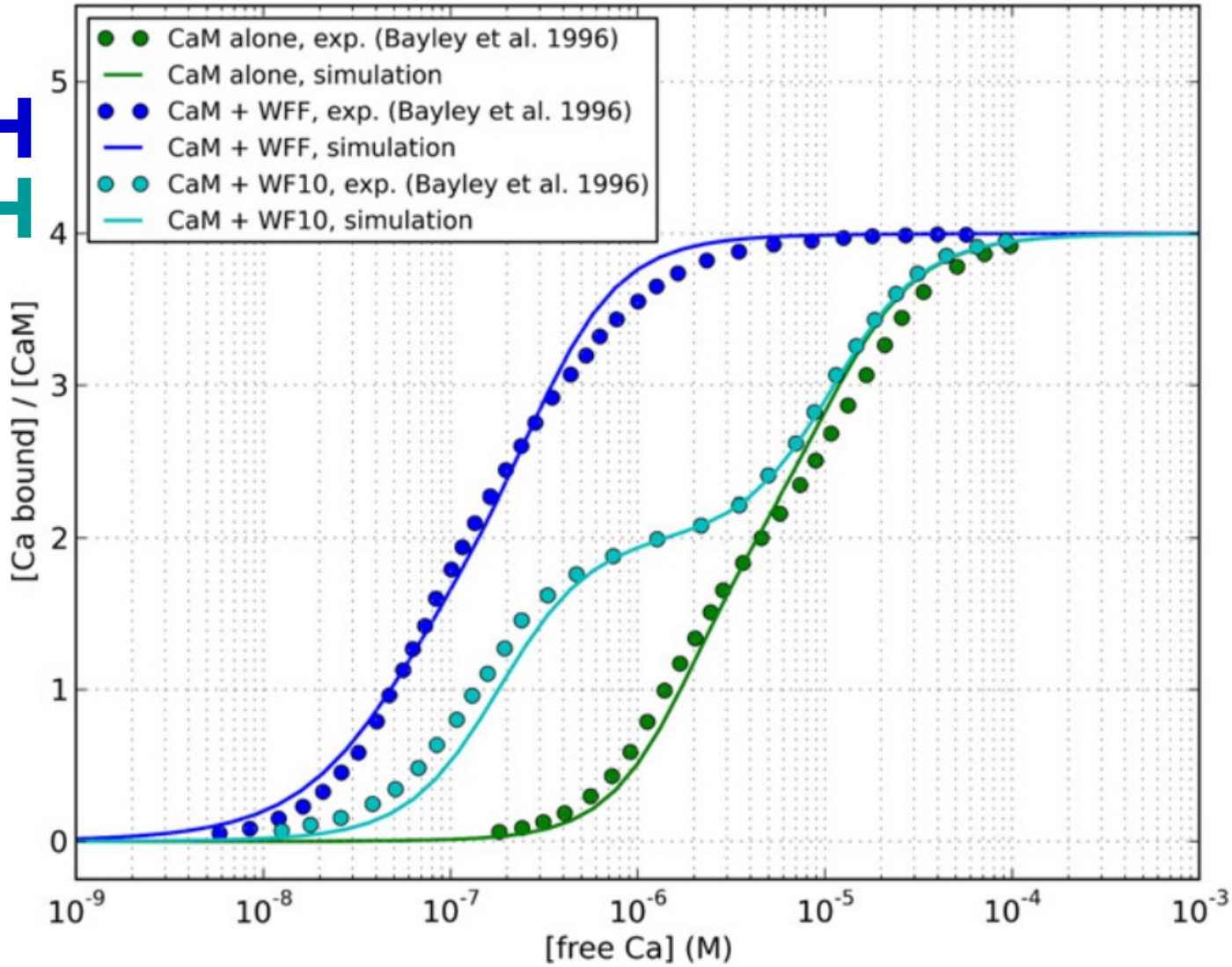
Binding to target increases the affinity for Ca^{2+}

Stabilise R state of both lobes

Stabilise R state of C lobe



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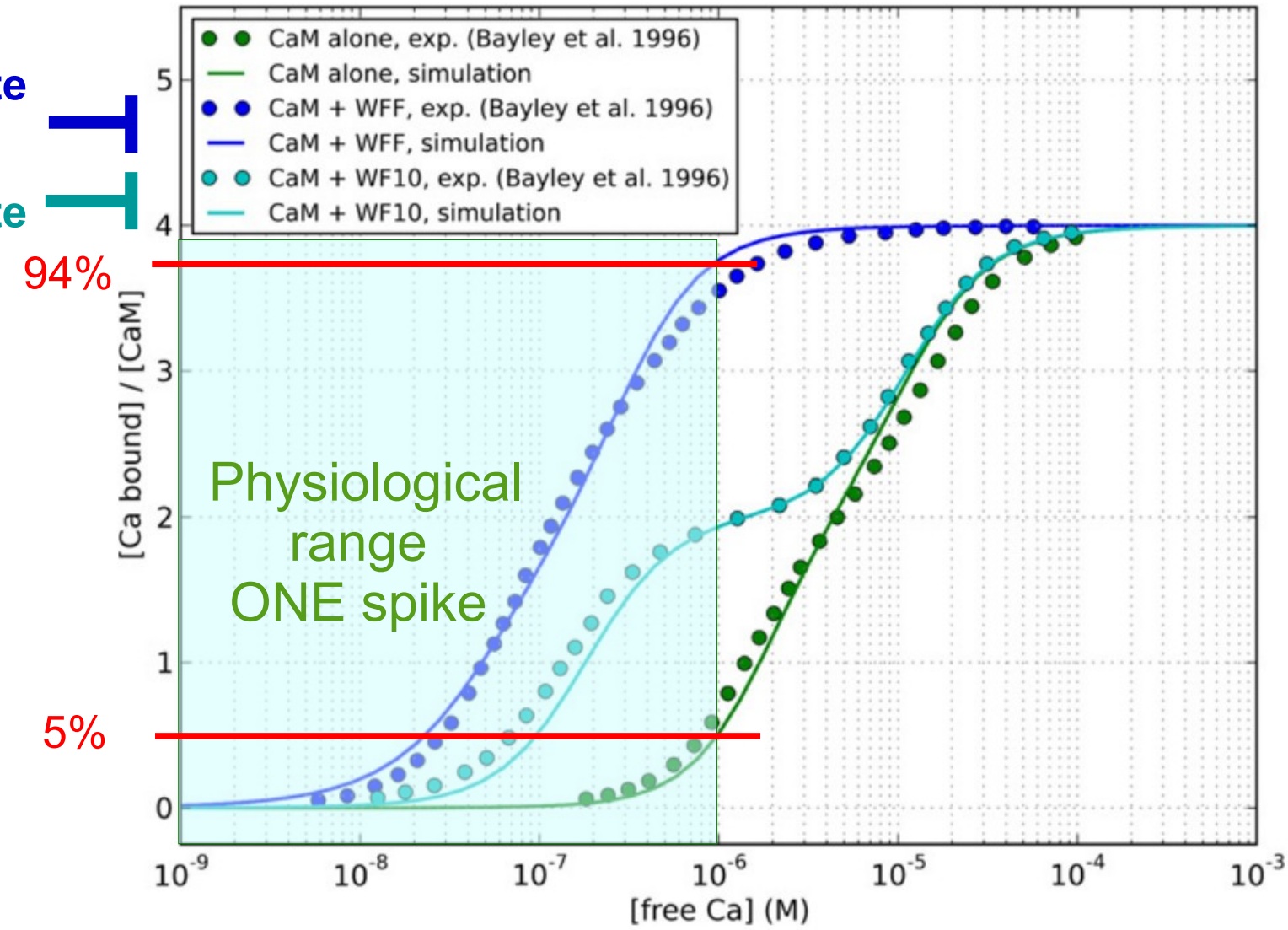
Targets stabilises Ca^{2+} binding into the physiological range

Stabilise R state of both lobes

Stabilise R state of C lobe



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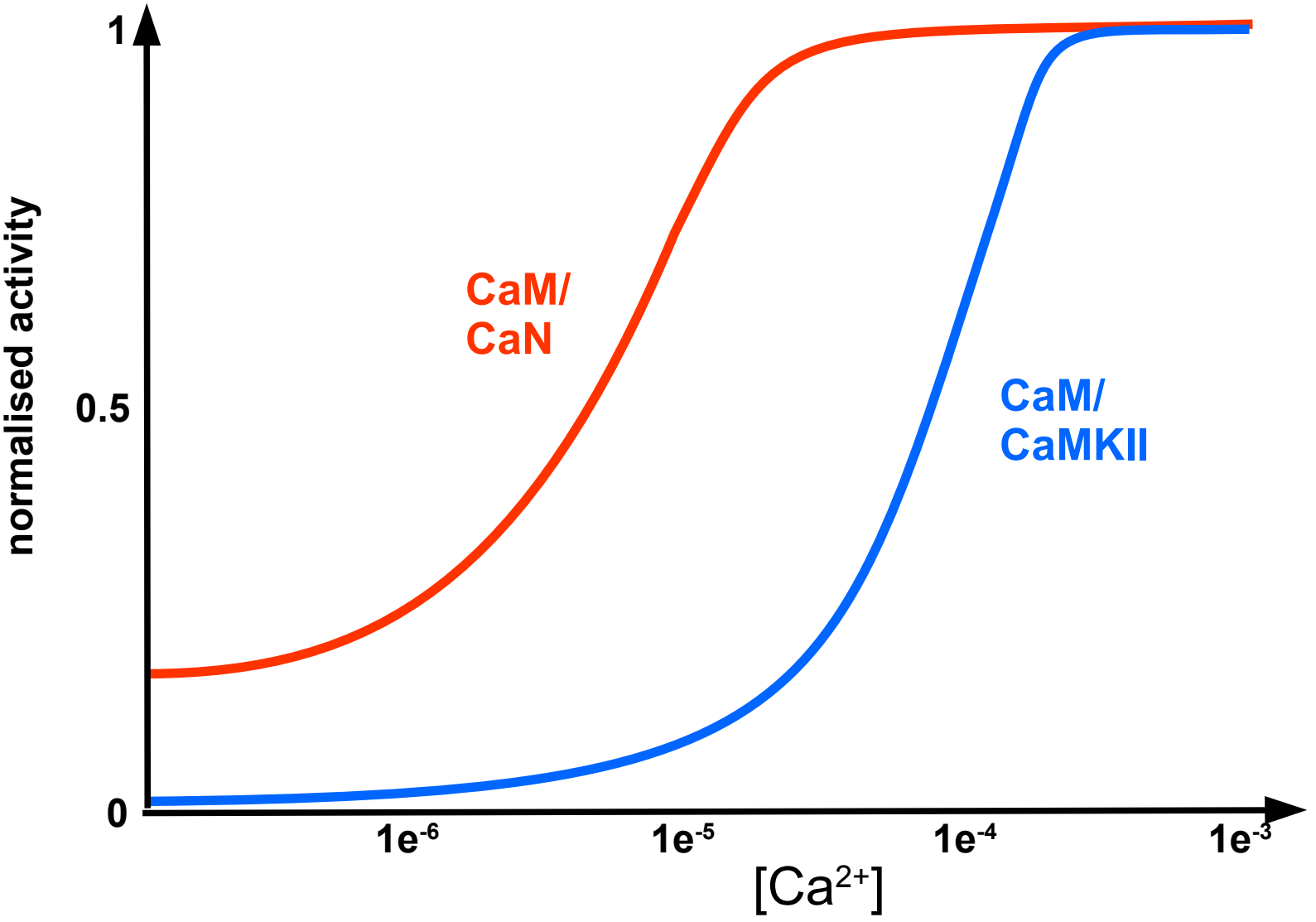
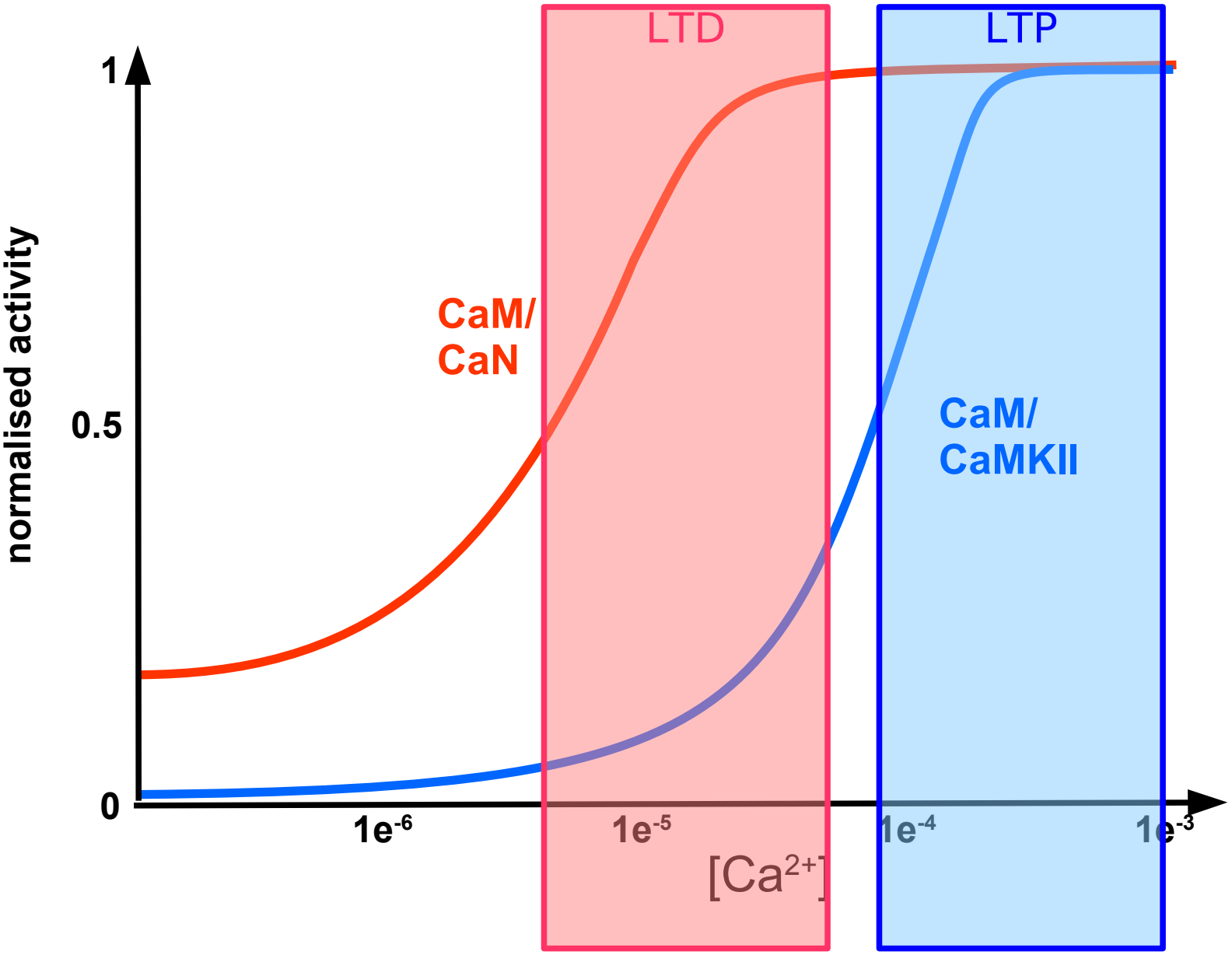
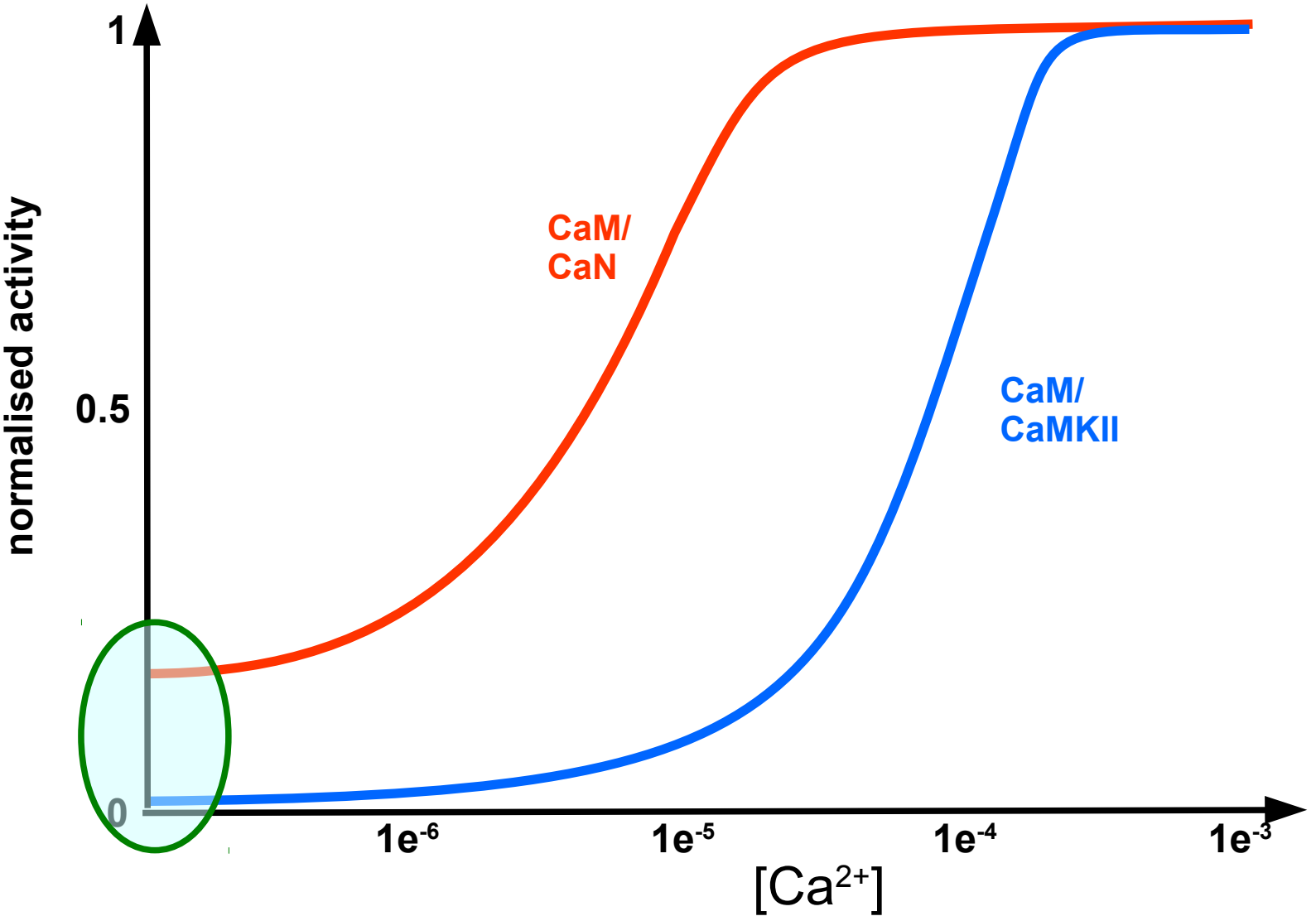
Calmodulin, its ligand and its targets



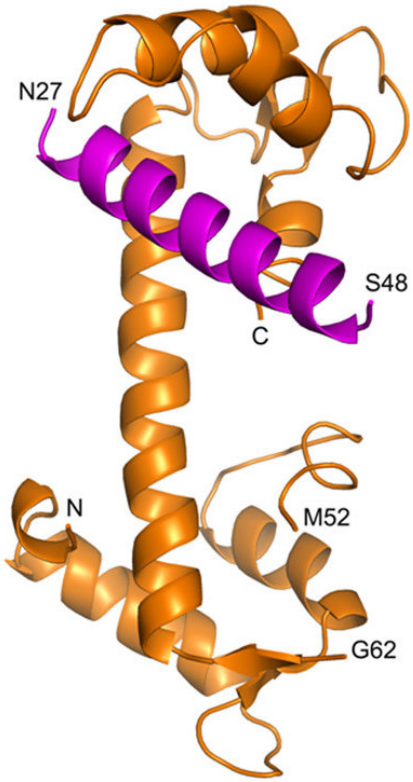
Bidirectional synaptic plasticity



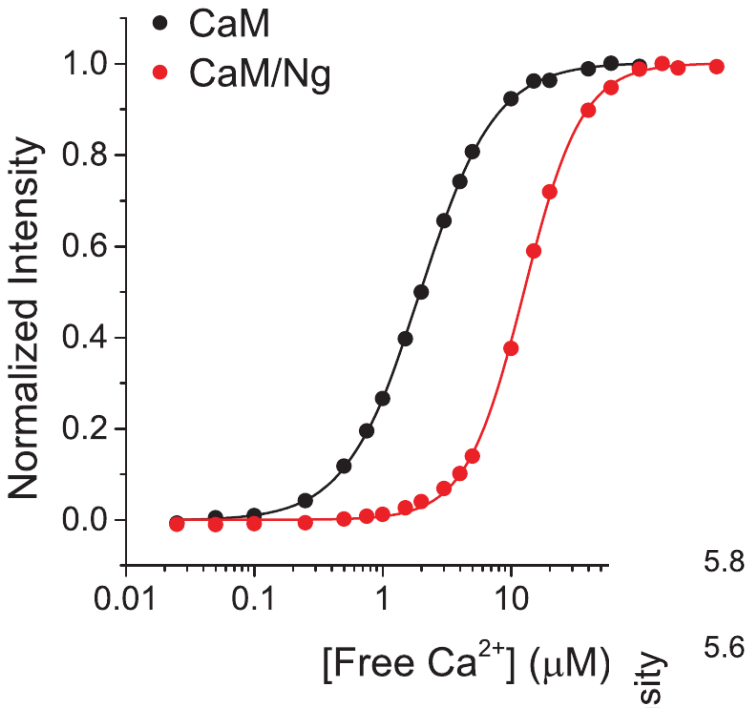
Calcineurin stabilises CaM R → no deactivation



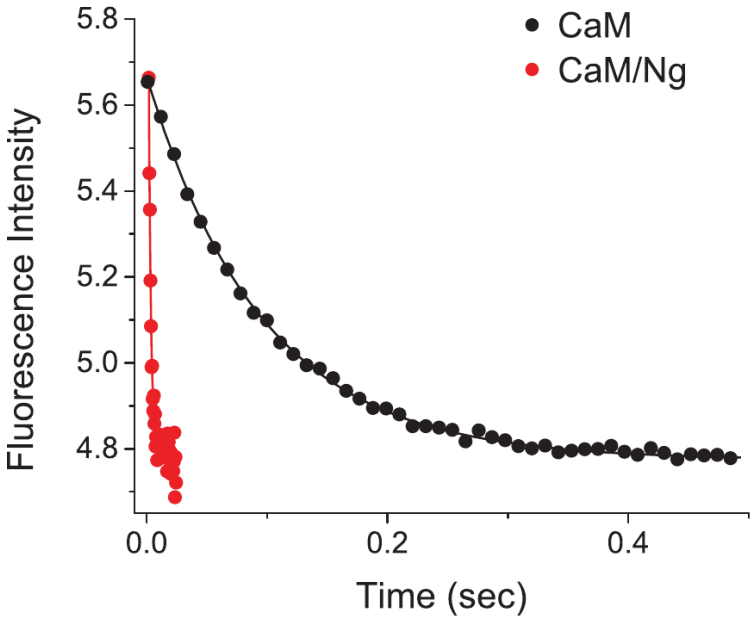
Neurogranin binds apo-CaM, decreases affinity for [Ca^{2+}] and increases dissociation rate



Kumar *et al* (2013)



Hoffman *et al* (2014)



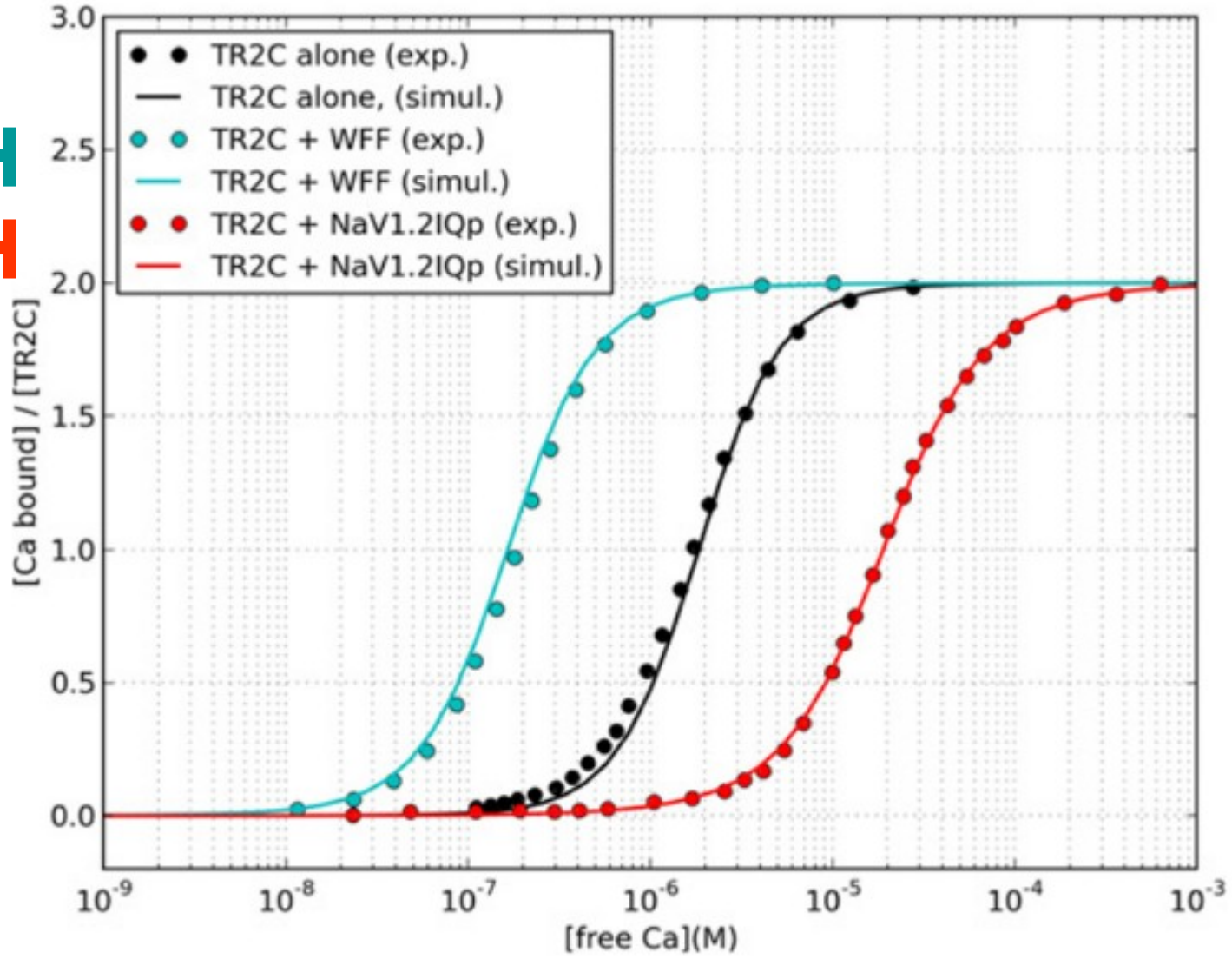
Effect of R and T stabilising targets on CaM affinity

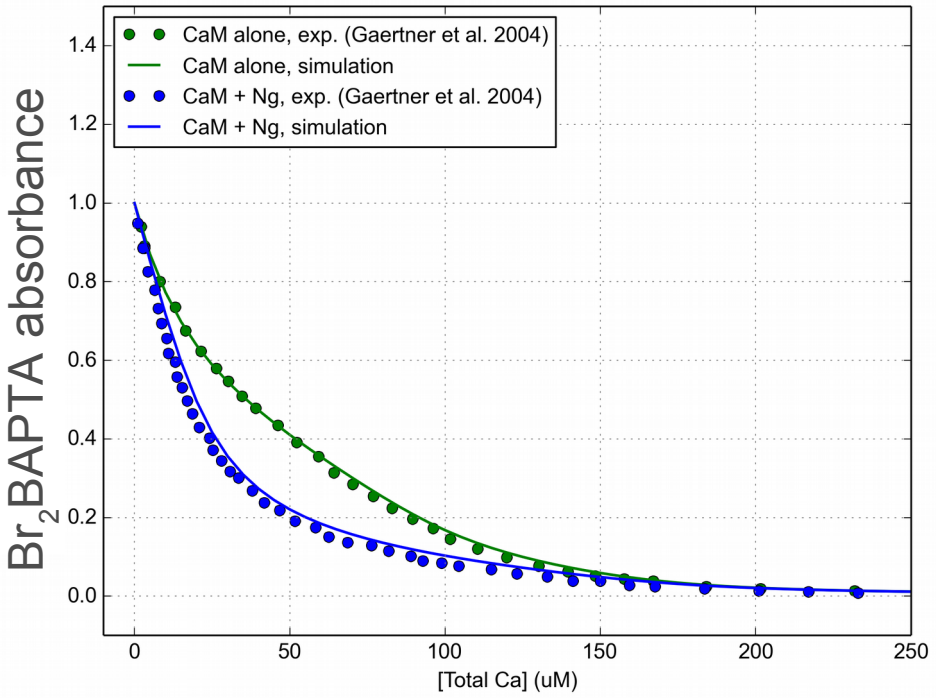
Bayley *et al* (1996)

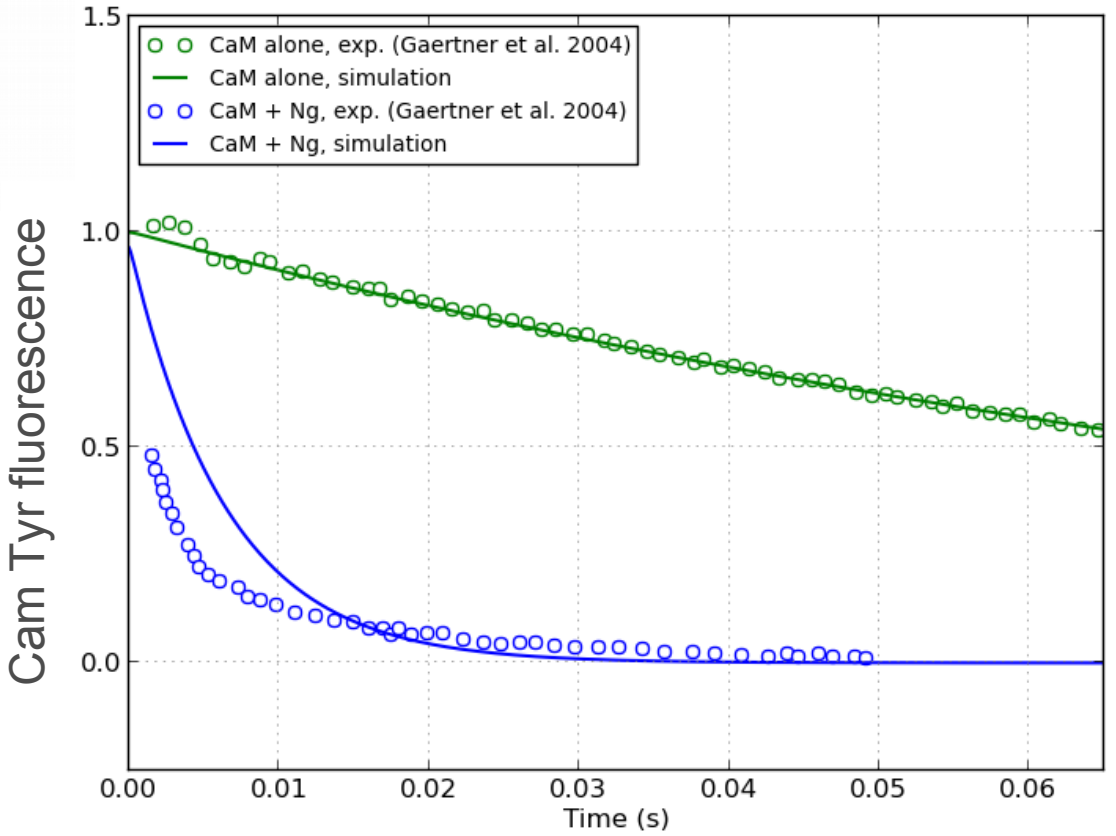
R stabilising peptide

T stabilising Peptide (IQ domain)

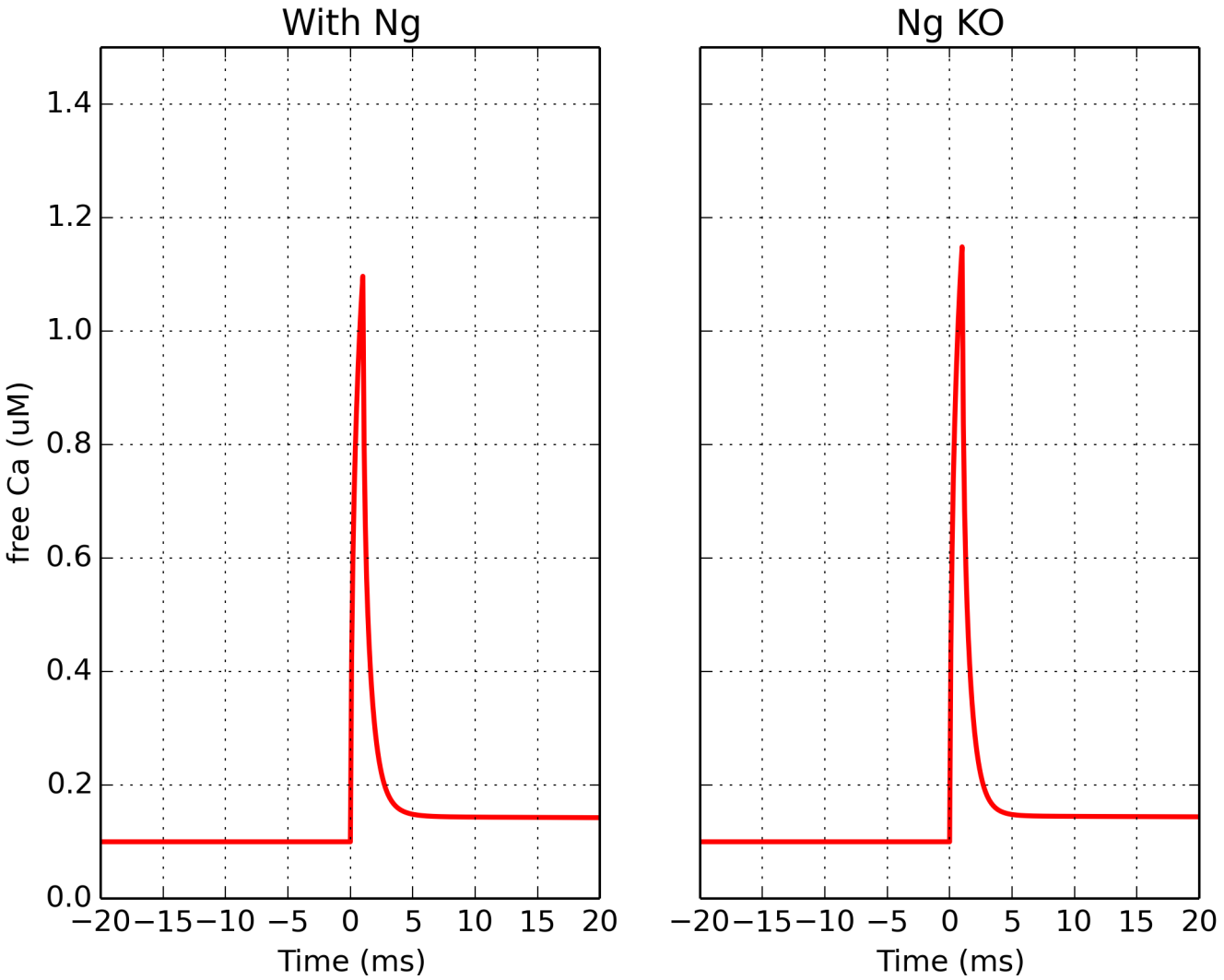
Theoharis *et al* (2008)




CaM alone, exp. (Gaertner et al. 2004)
CaM alone, simulation
CaM + Ng, exp. (Gaertner et al. 2004)
CaM + Ng, simulation
Br2BAPTA absorbance
[Total Ca] (uM)


CaM alone, exp. (Gaertner et al. 2004)
CaM alone, simulation
CaM + Ng, exp. (Gaertner et al. 2004)
CaM + Ng, simulation
Cam Tyr fluorescence
Time (s)

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



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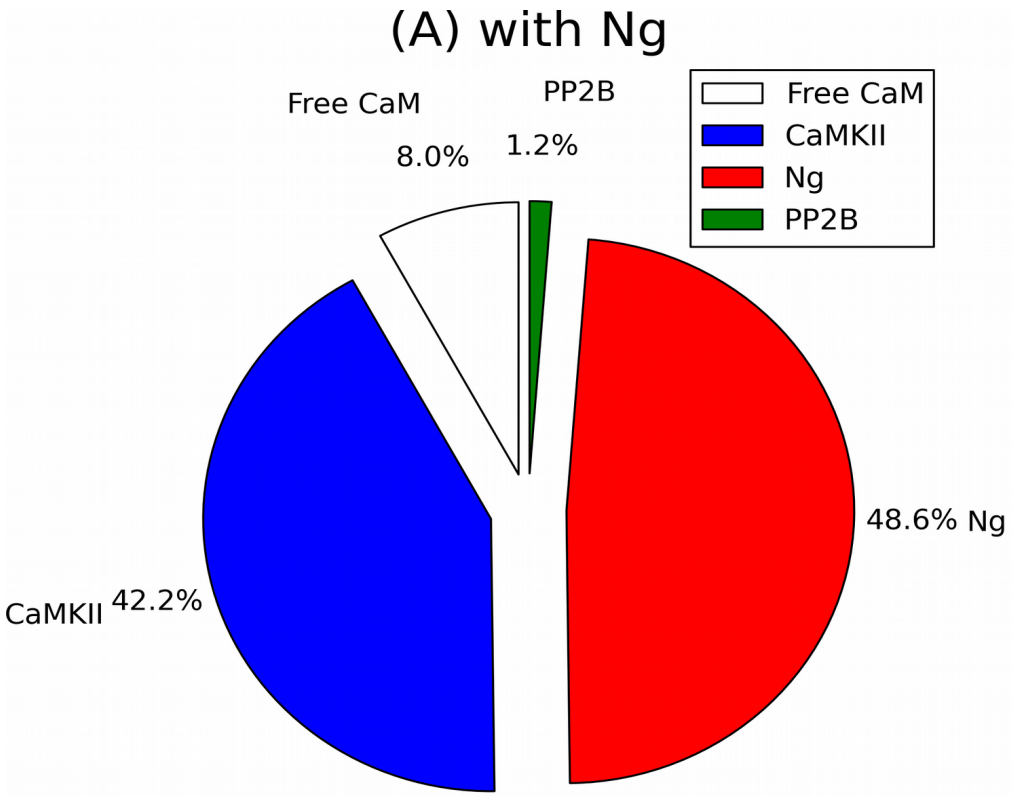
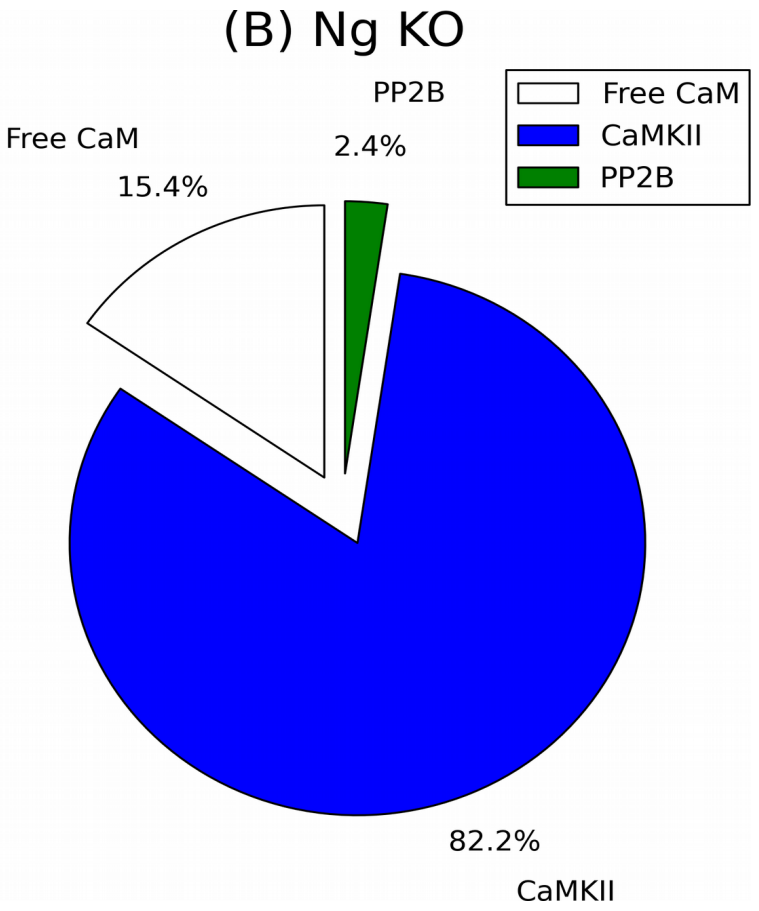
Ng affects the distribution of CaM





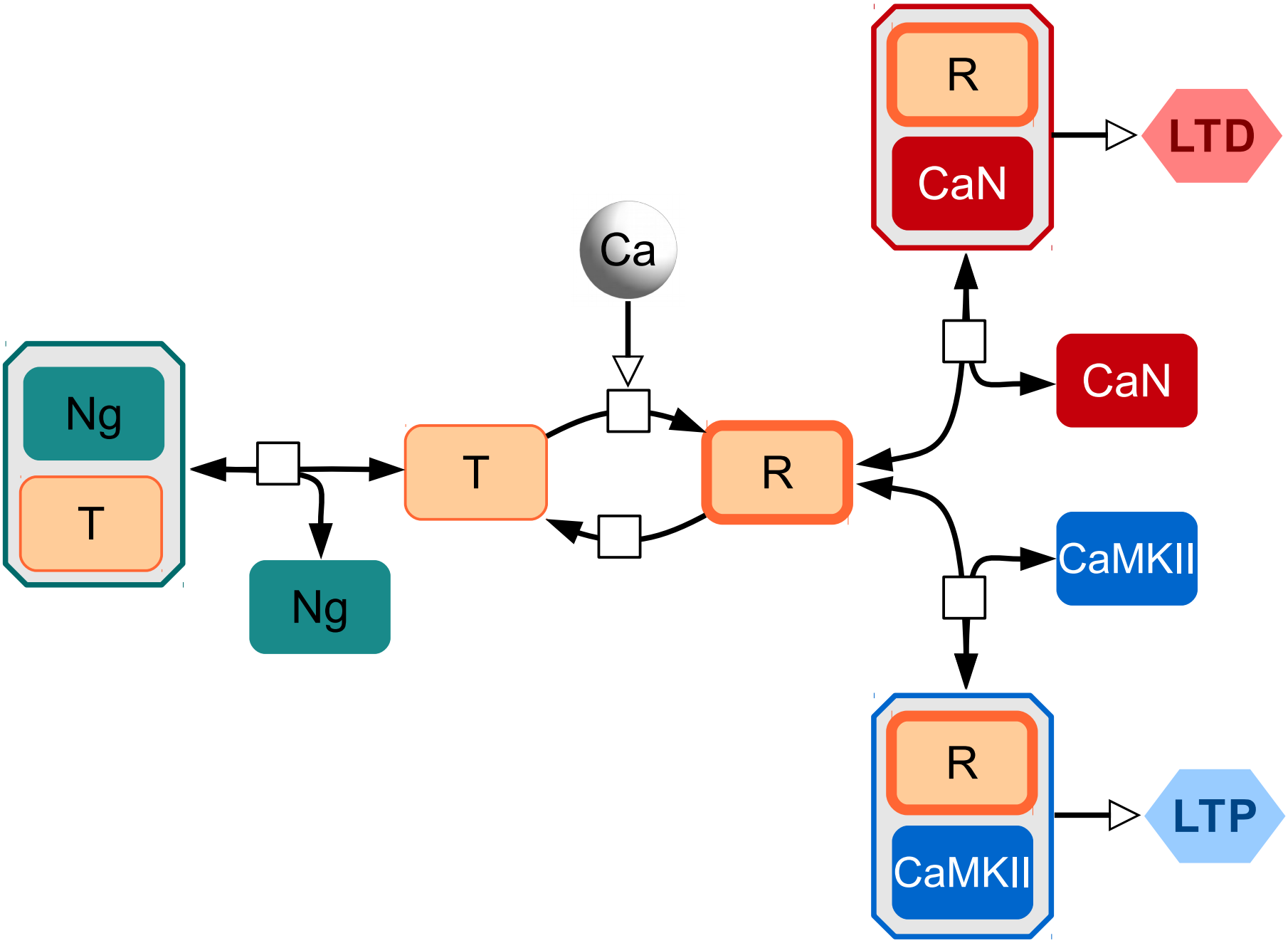
Hemi-concerted model of Calmodulin, 2 states for EF hands, binding Ca^{2+} with different affinities

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

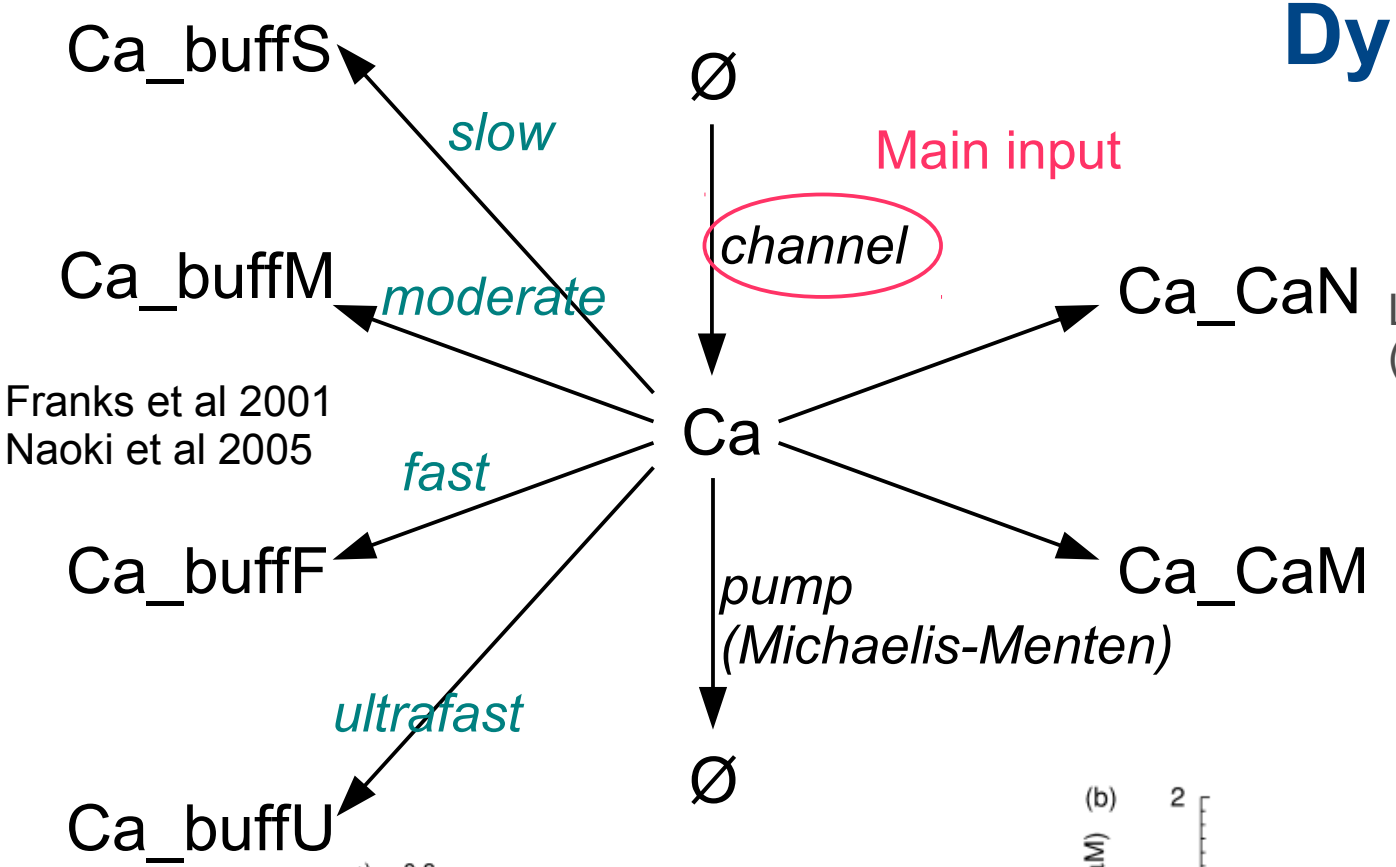

R
CaN
LTD
Ca
Ng
T
CaN
T
R
Ng
CaMKII
R
CaMKII
LTP

Wait a minute!
Signal transduction is not at equilibrium!

AMPAR post-synaptic potential:	5 ms
Calcium spike:	50 ms
Half saturation calmodulin (kon=1.5e6, koff=100):	5 ms
Relaxation between calmodulin states:	1 ms
autophosphorylation of CaMKII (kon=6):	100 ms

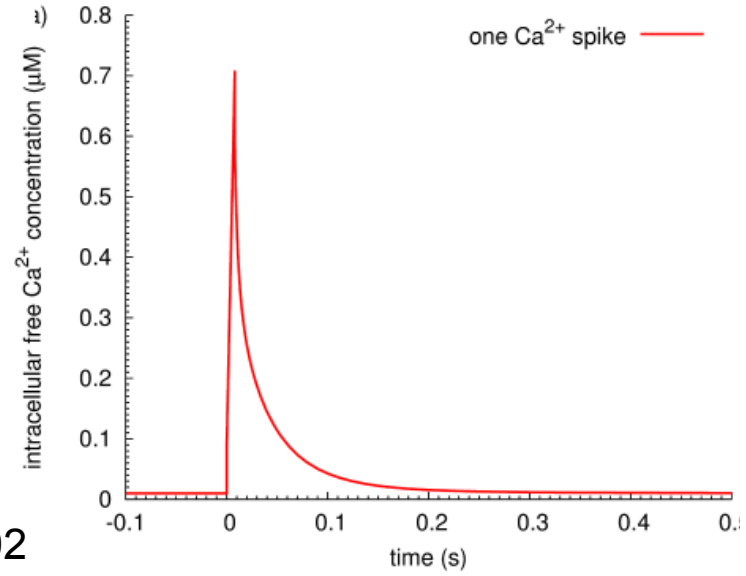
≠

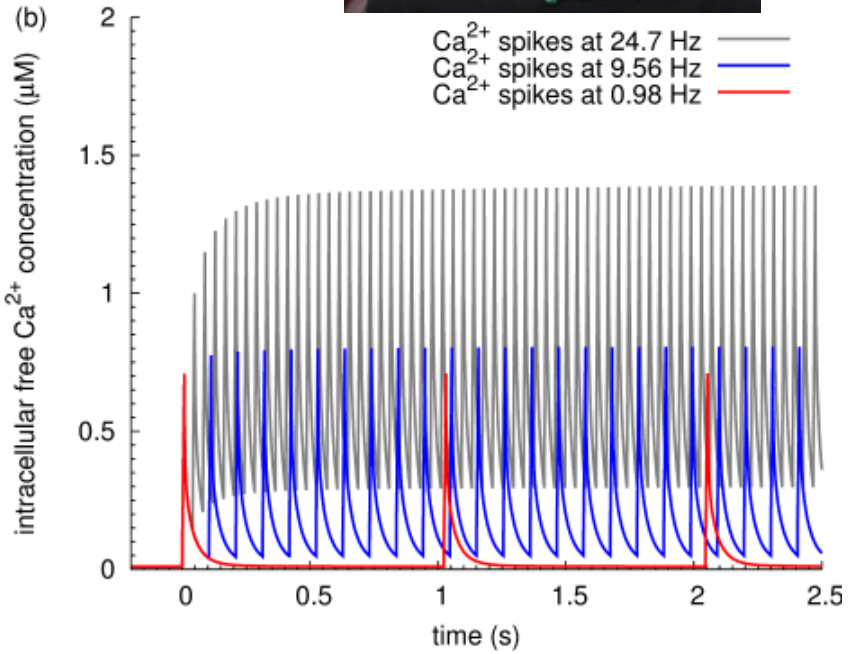
Dynamic of calcium in the spine



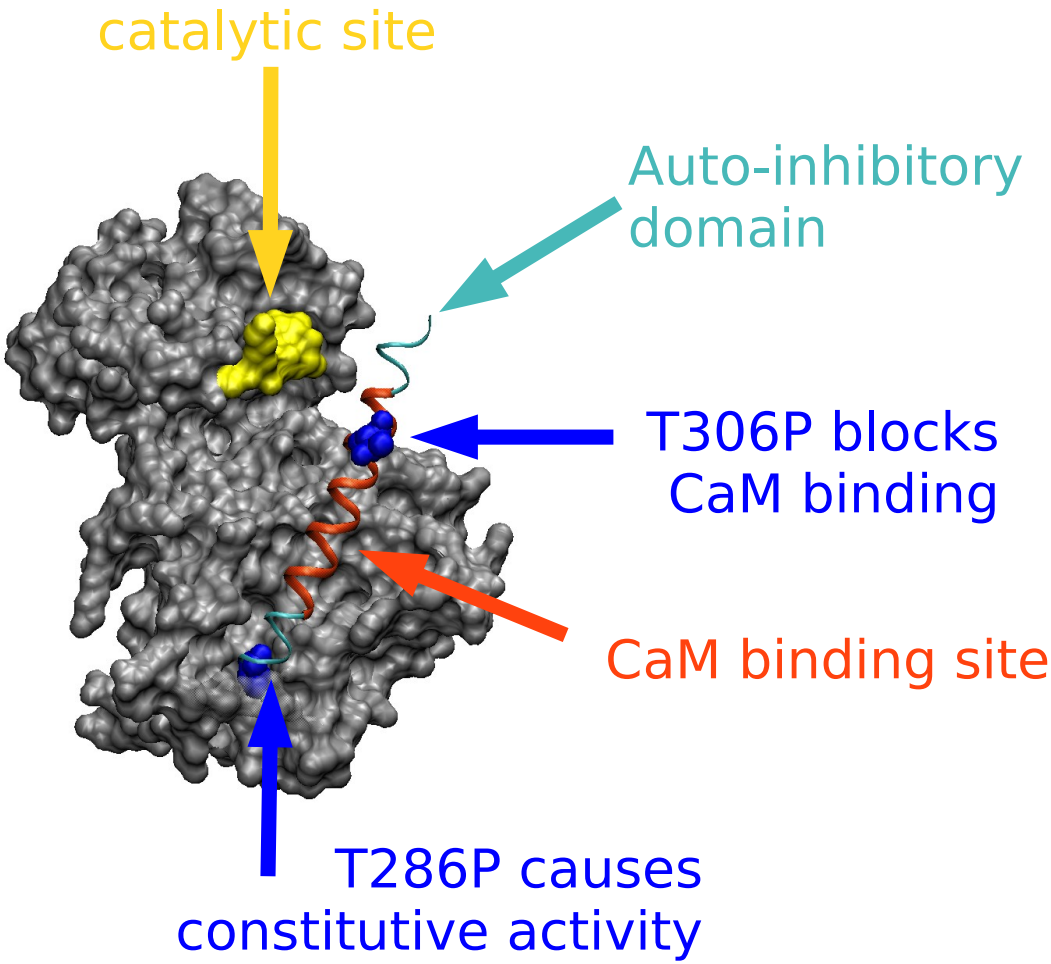
Li L, Stefan MI, Le Novère N (2012) *PLoS ONE*, 7(9): e43810

Sabatini et al 2002





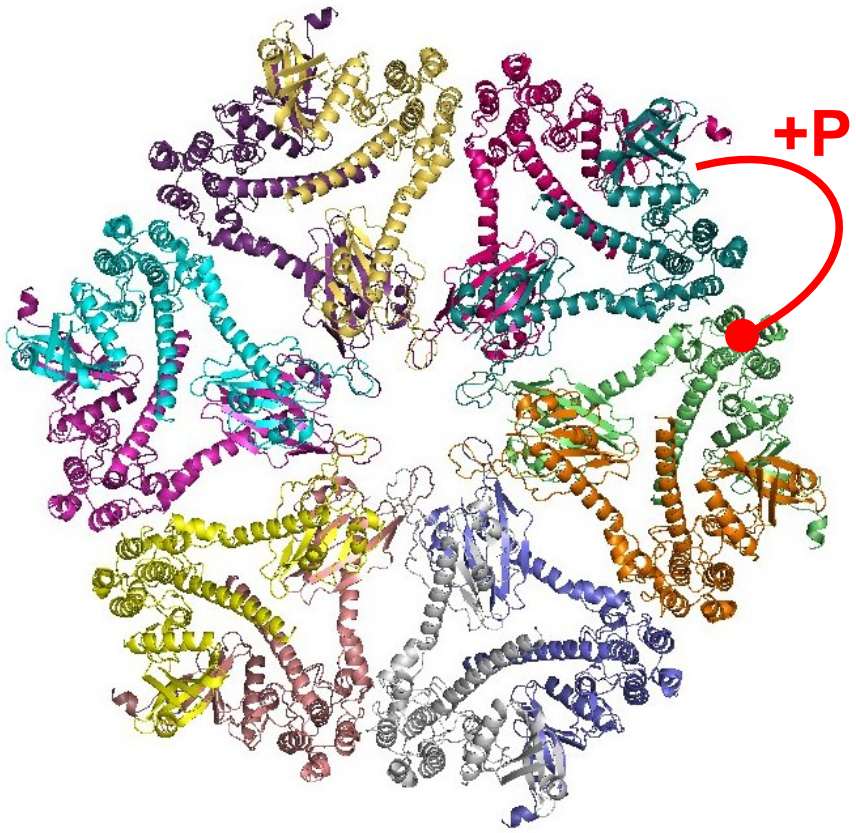
Calcium/calmodulin kinase II

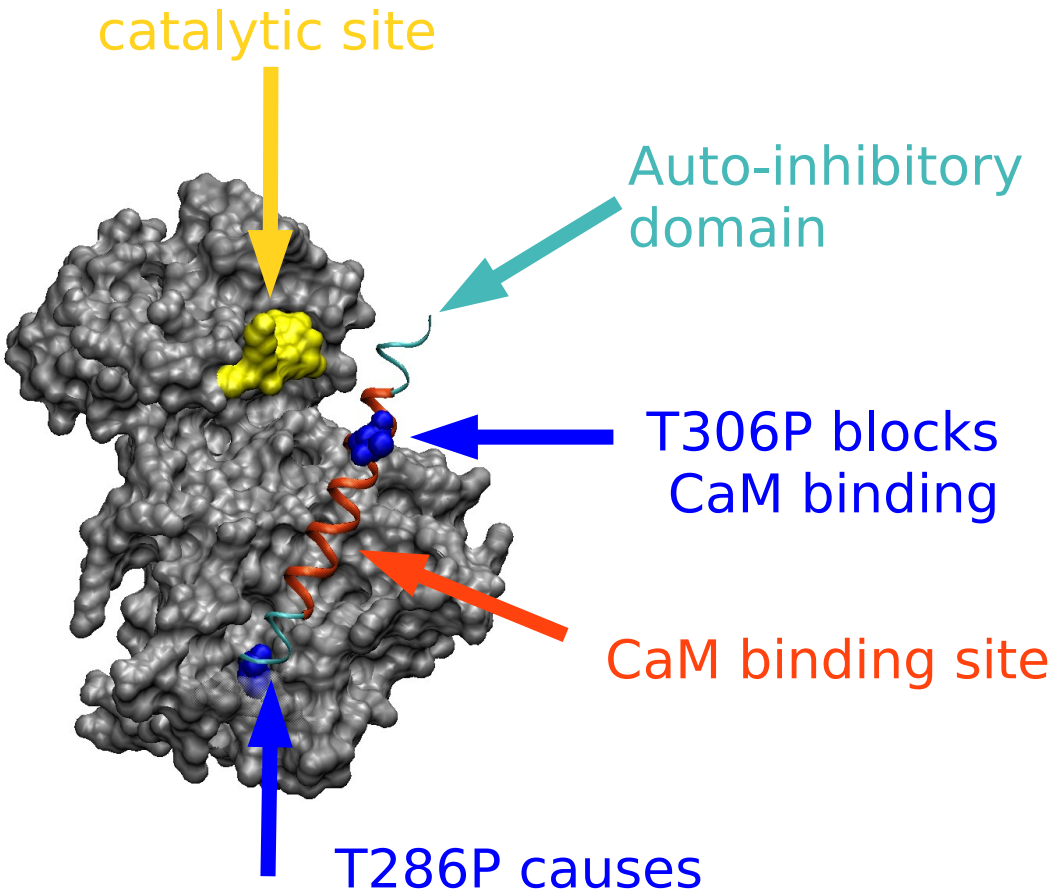


Calmodulin trapping is an apparent increase of affinity of CaMKII for CaM when T286 is phosphorylated

Stefan MI, Marshall D, Le Novère N (2012)

Calcium/calmodulin kinase II

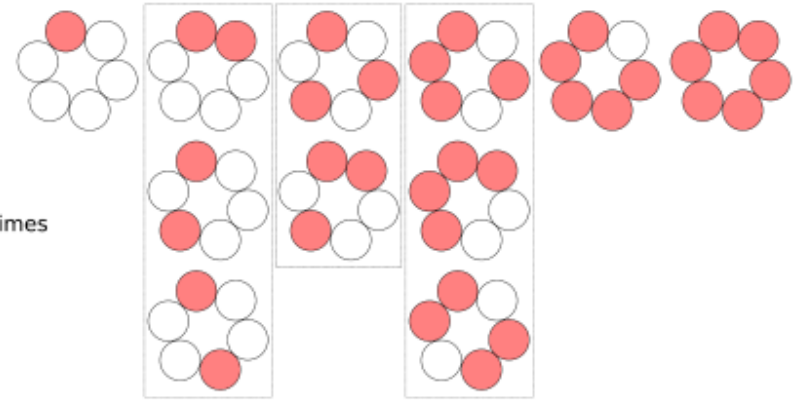




Dodecamer:
Trans-phosphorylation of T286 by neighbouring subunits
Cis-phosphorylation of T306
Most quantitative measurements made on monomers ...

A given fraction of activated CaMKII monomers.

Randomly allocate activated monomers to CaMKII hexamers.
Record the number of hexamers containing different numbers of active monomers (in red) as the following:



Repeat 1000 times

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

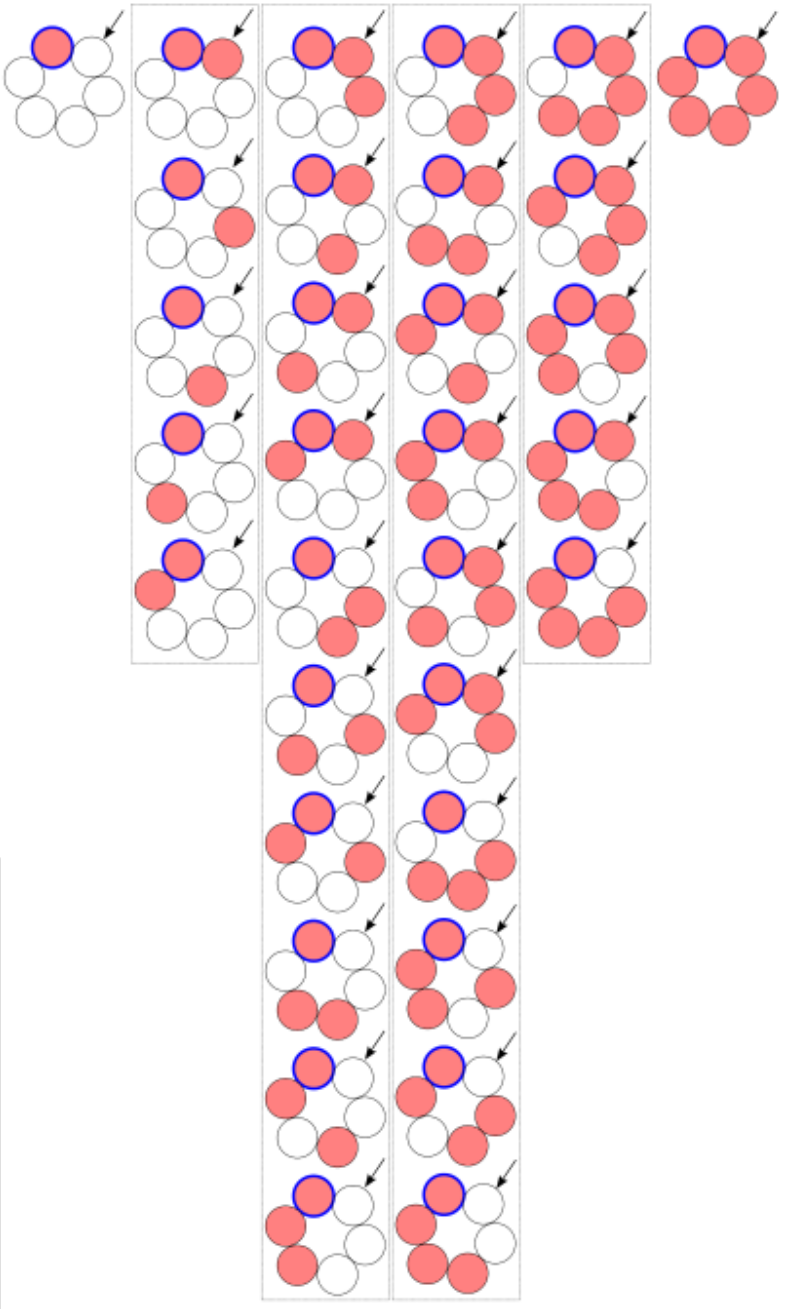
Multiply average populations of each number of active monomers per hexamer by their corresponding probabilities of having an active neighbour.

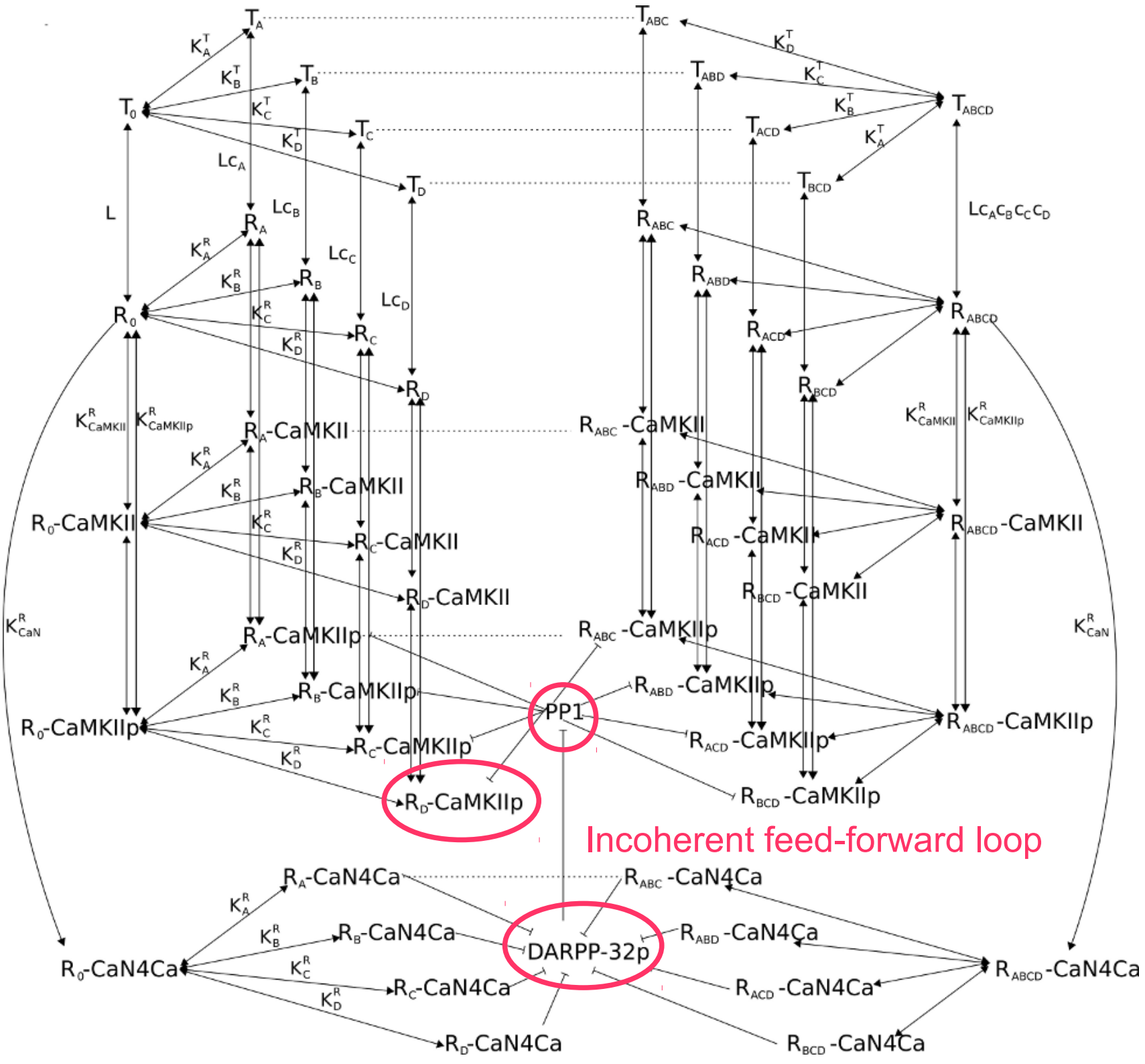
The sum of these six numbers is a coefficient that can be used to adjust CaMKII autophosphorylation rate.

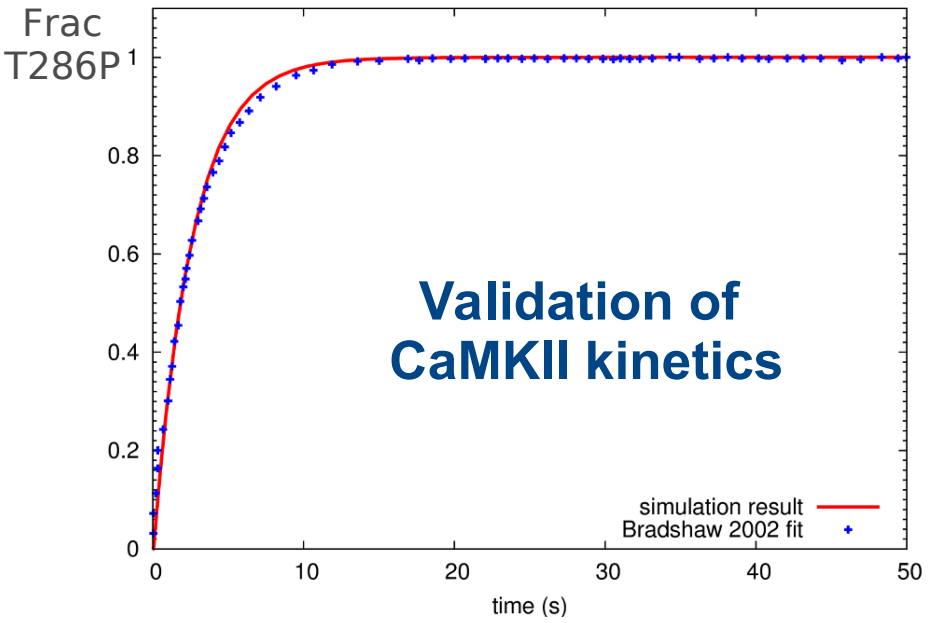
Repeat for every 1% increase of CaMKII active monomers

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:

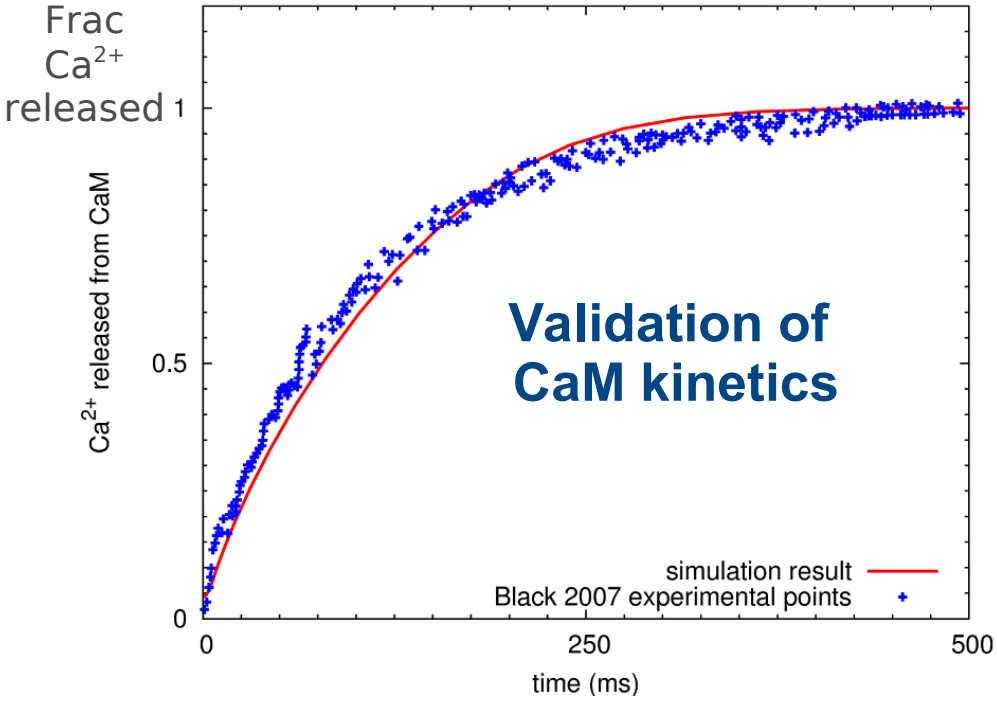



T0
TA
TB
TC
TD
TABC
TABD
TACD
TBCD
TABCD
KAT
KBT
KCT
KDT
LcA
LcB
LcC
LcD
LcAcBcCcD
L
R0
RA
RB
RC
RD
RABC
RABD
RACD
RBCD
RABCD
KRCaMKII
KRCaMKIIp
KRCaN
KAR
KBR
KCR
KDR
R0-CaMKII
RA-CaMKII
RB-CaMKII
RC-CaMKII
RD-CaMKII
RABC-CaMKII
RABD-CaMKII
RACD-CaMKII
RBCD-CaMKII
RABCD-CaMKII
R0-CaMKIIp
RA-CaMKIIp
RB-CaMKIIp
RC-CaMKIIp
RD-CaMKIIp
RABC-CaMKIIp
RABD-CaMKIIp
RACD-CaMKIIp
RBCD-CaMKIIp
RABCD-CaMKIIp
PP1
Incoherent feed-forward loop
R0-CaN4Ca
RA-CaN4Ca
RB-CaN4Ca
RC-CaN4Ca
RD-CaN4Ca
RABC-CaN4Ca
RABD-CaN4Ca
RACD-CaN4Ca
RBCD-CaN4Ca
RABCD-CaN4Ca
DARPP-32p



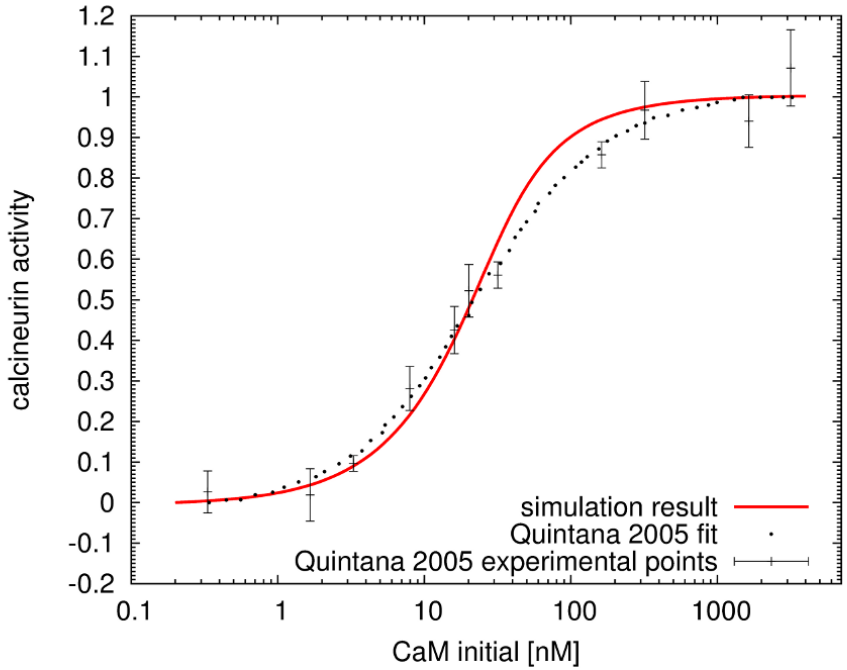
Validation of CaMKII kinetics

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



Validation of CaM kinetics

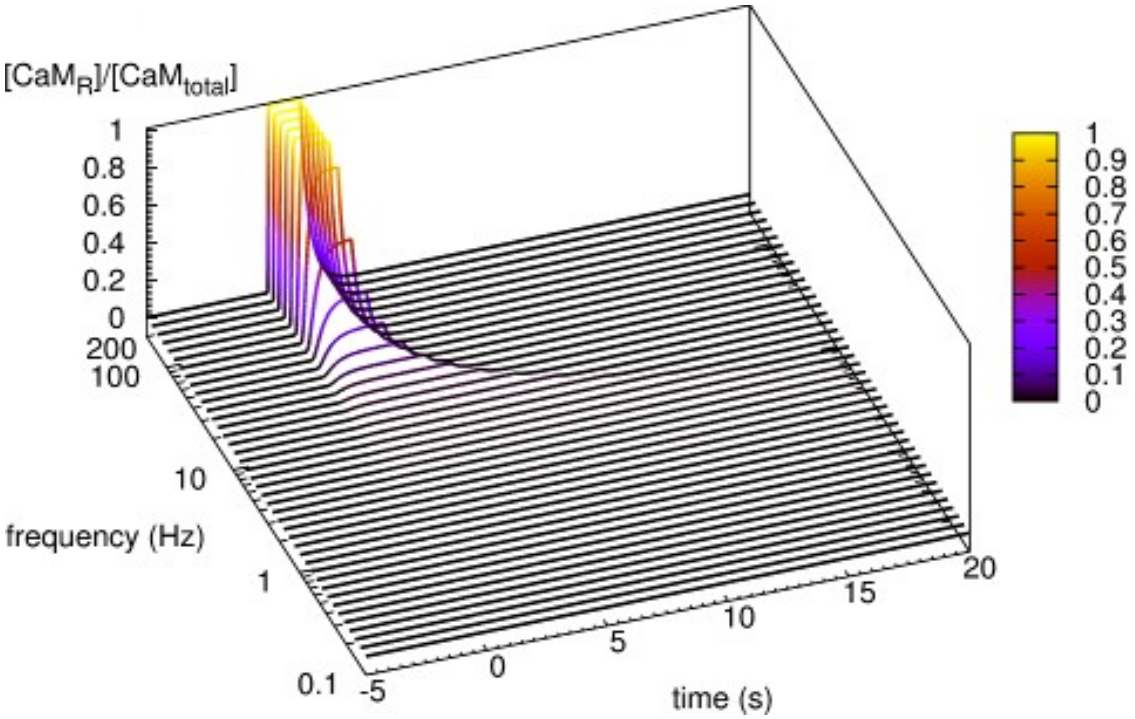
Black DJ, Selfridge JE, Persechini A (2007). *Biochemistry* 46: 13415–13424.



Validation of calcium-activation of CaN

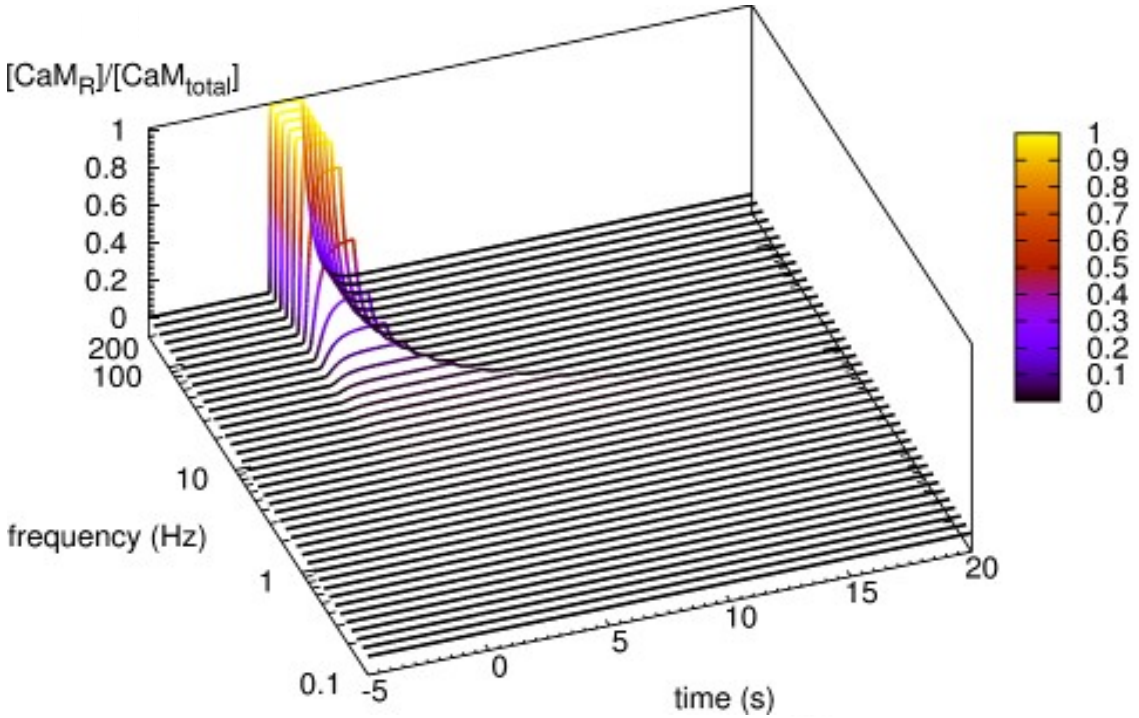
Quintana AR, Wang D, Forbes JE, Waxham MN (2005). *BBRC* 334: 674–680.

Calmodulin activation

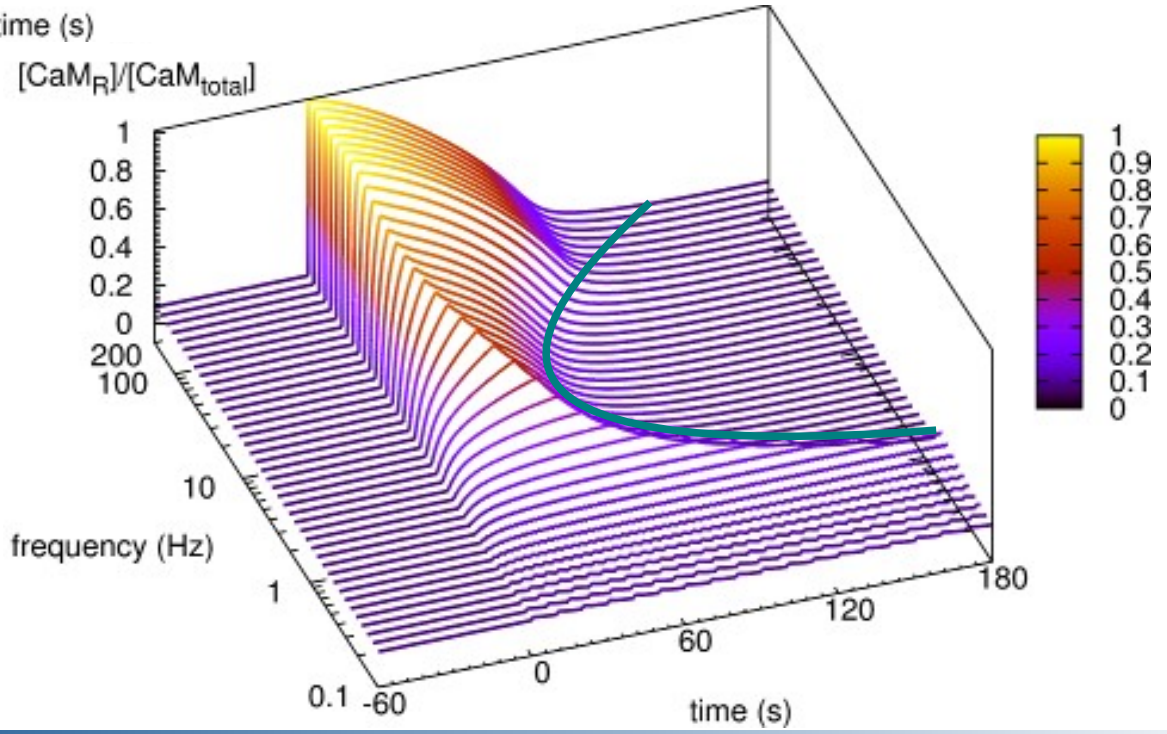


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

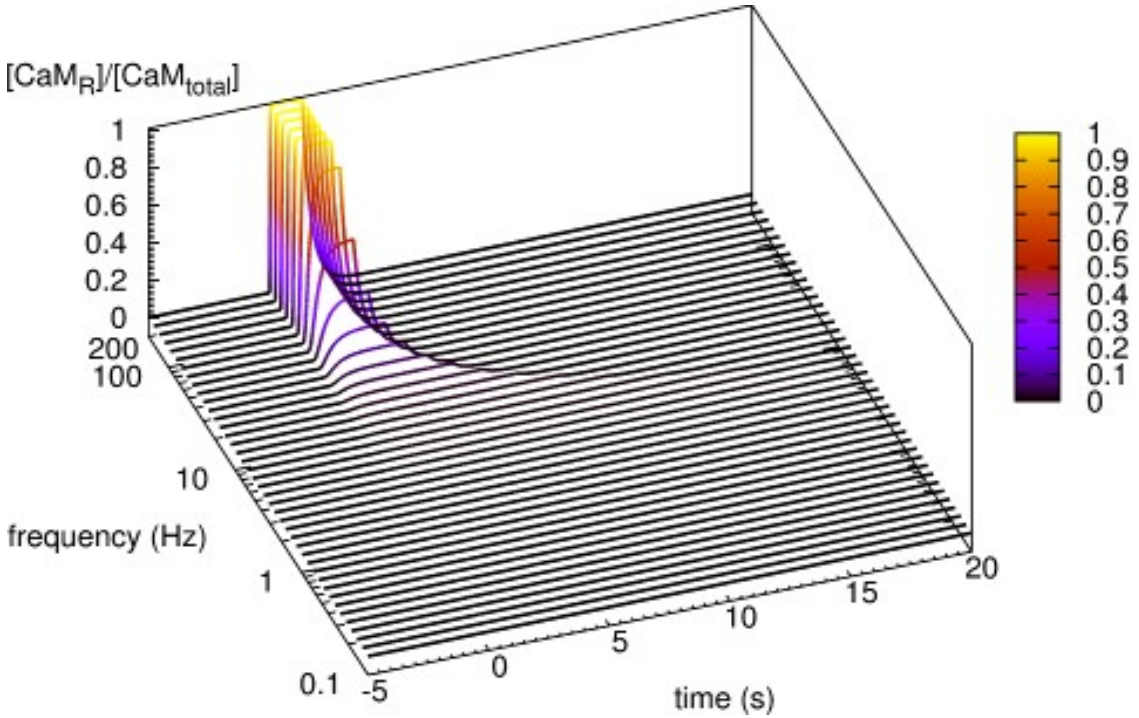


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)



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

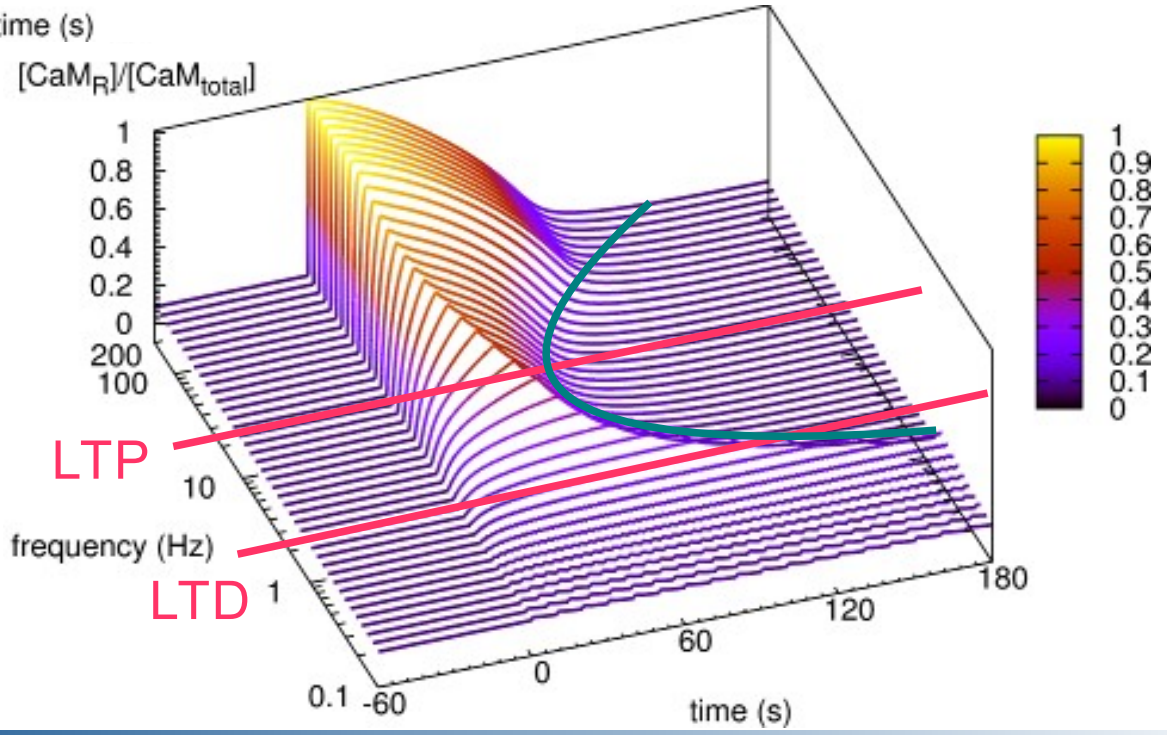
Calmodulin activation



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



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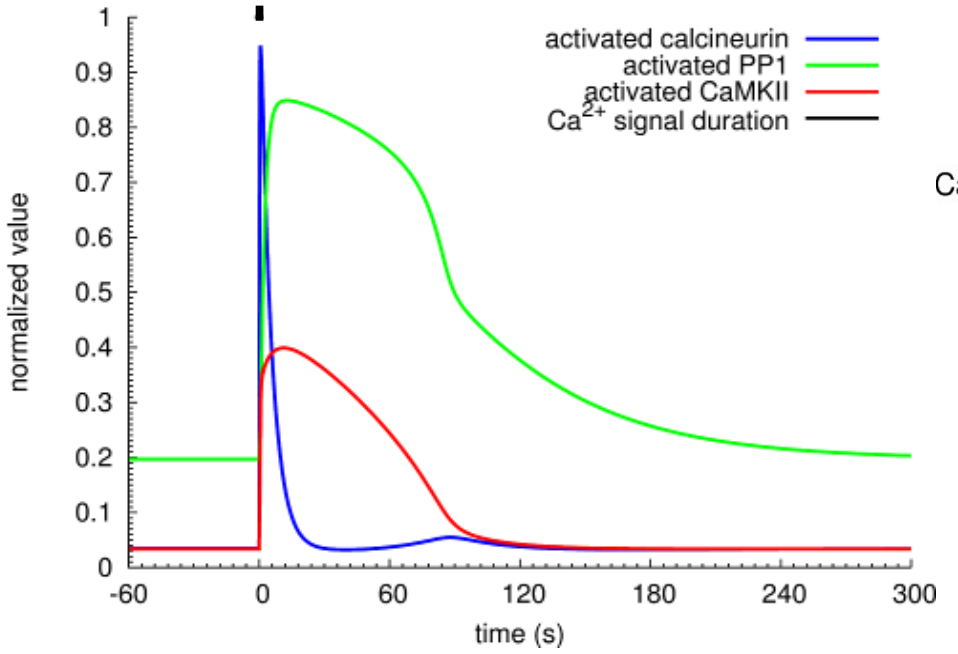
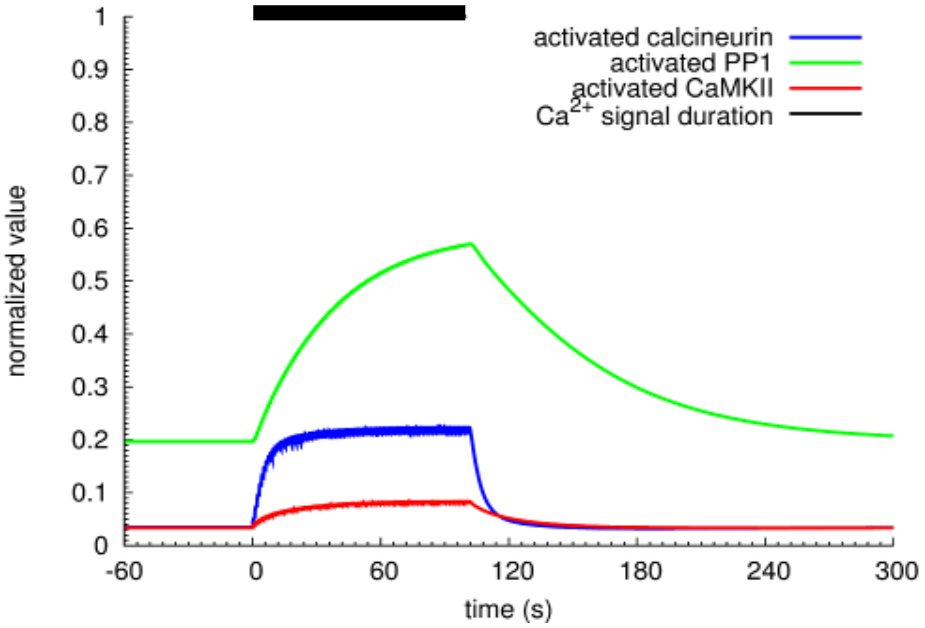
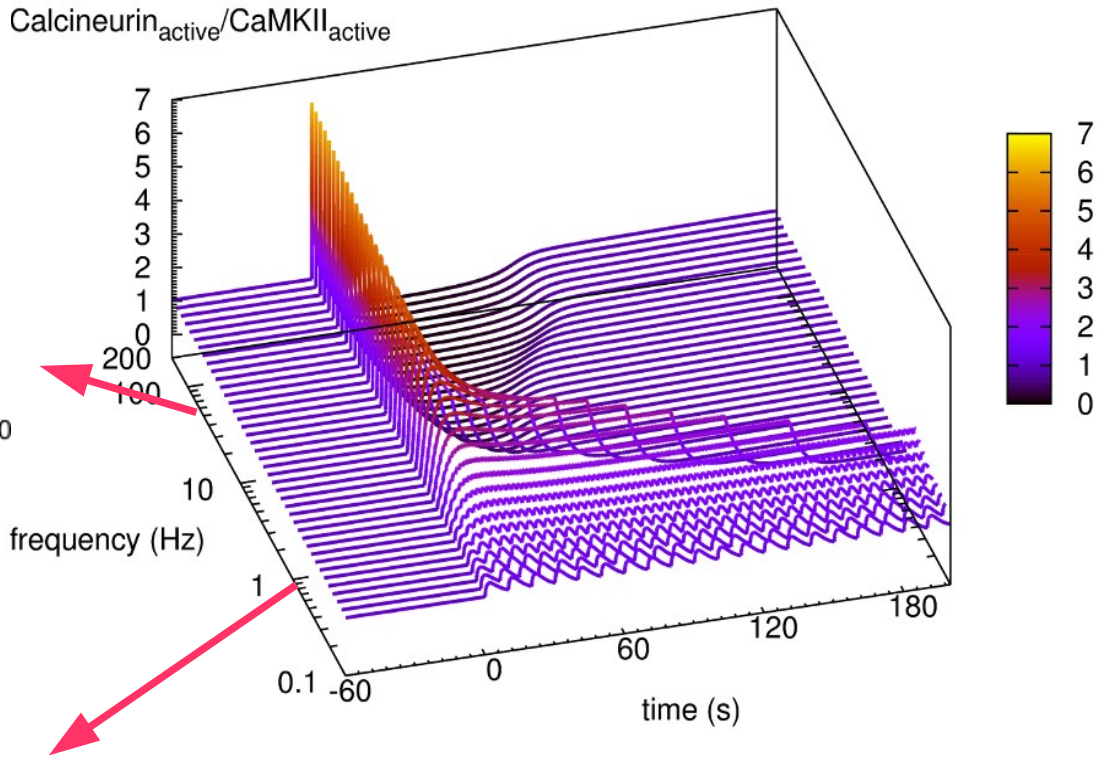
Temporal activation of CaMKII and CaN







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

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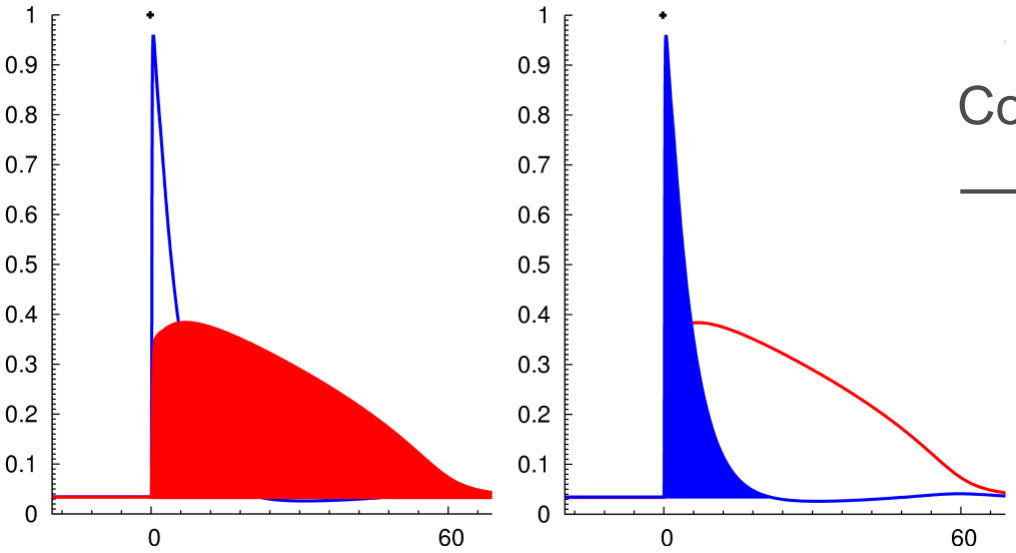
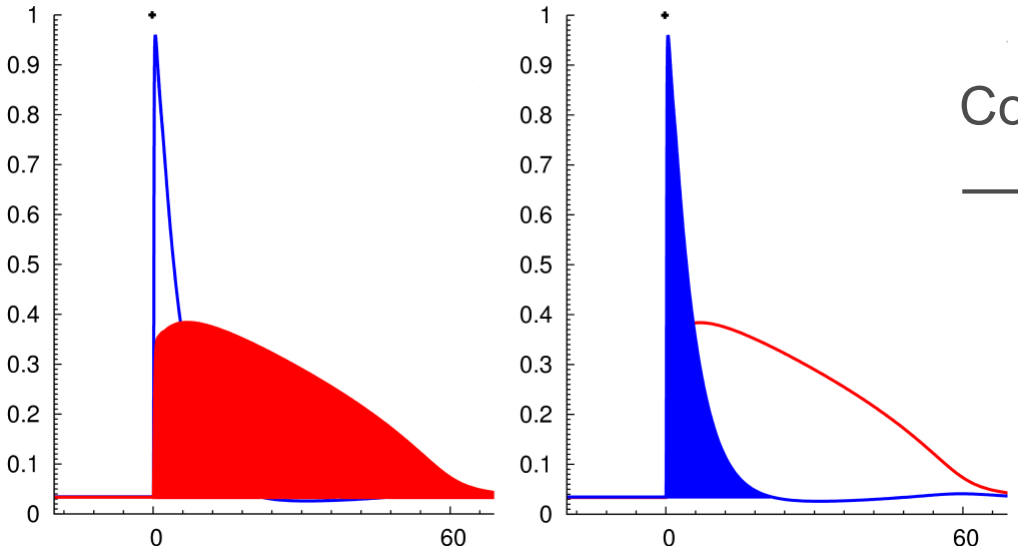
Bidirectional plasticity



Constant catalytic rates of active enzyme

$\longrightarrow$ quantity of catalysed reaction events prop to integral of the activation curve

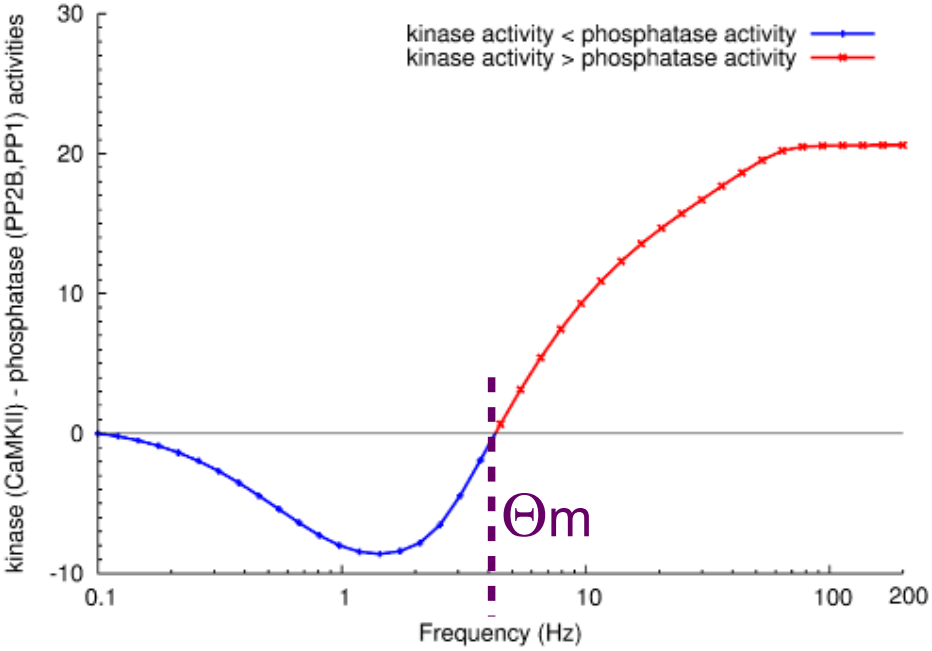
Bidirectional plasticity



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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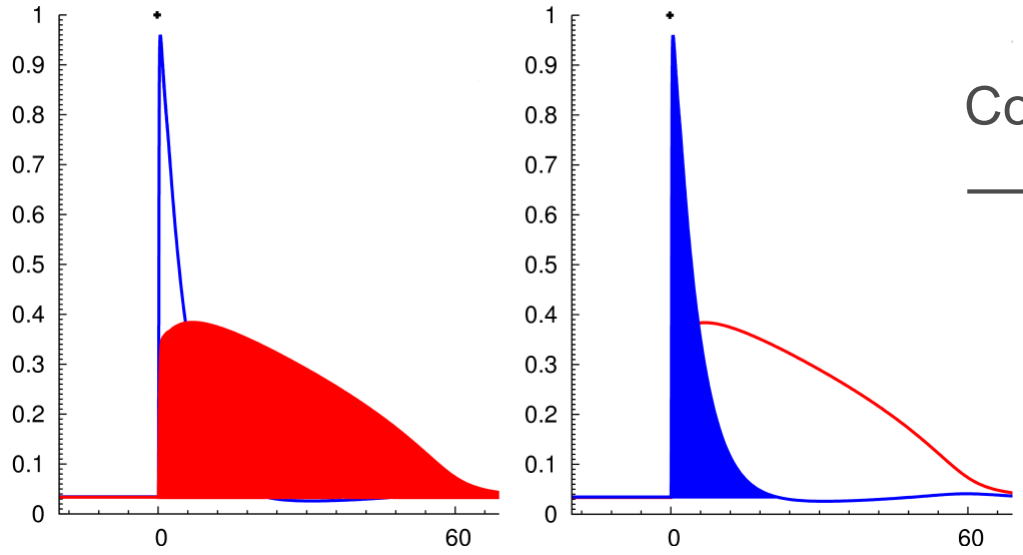
Bidirectional plasticity



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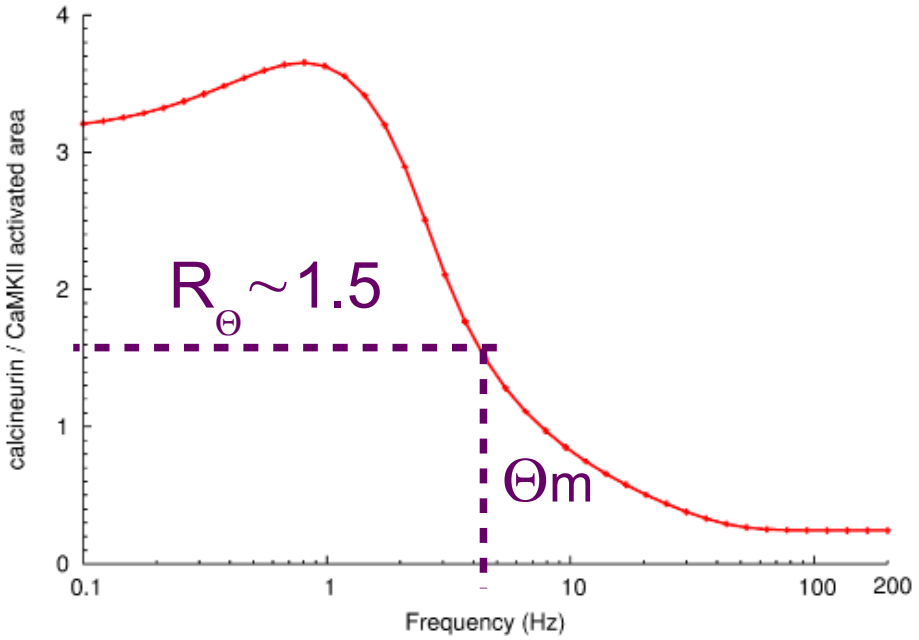

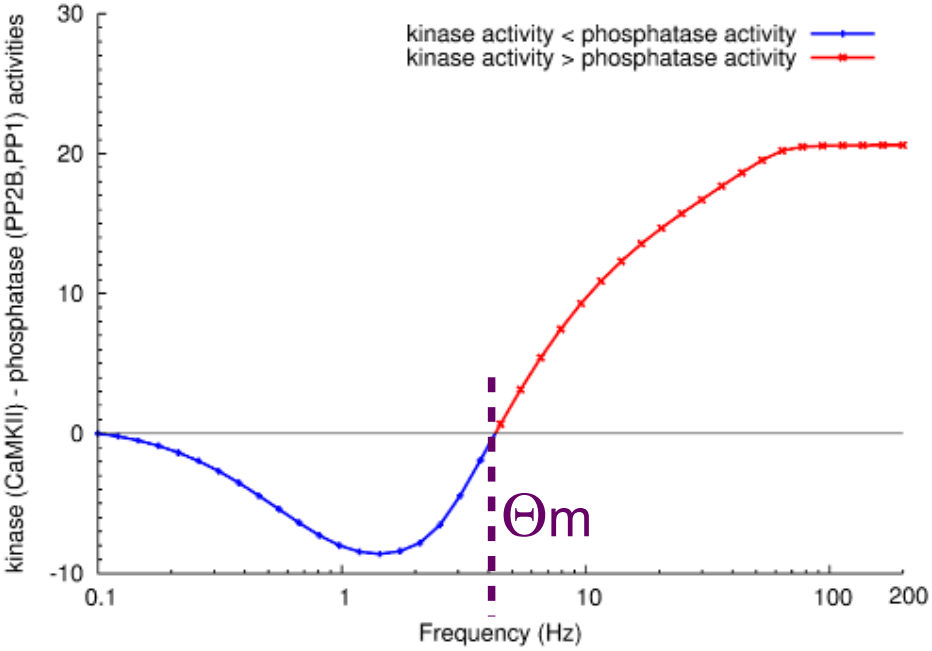

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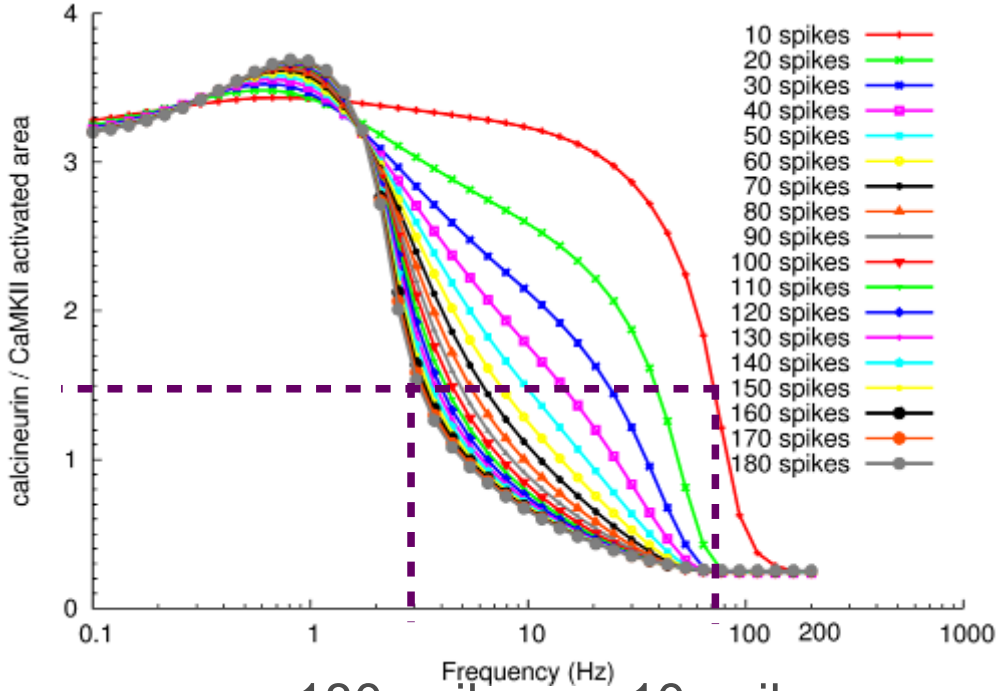
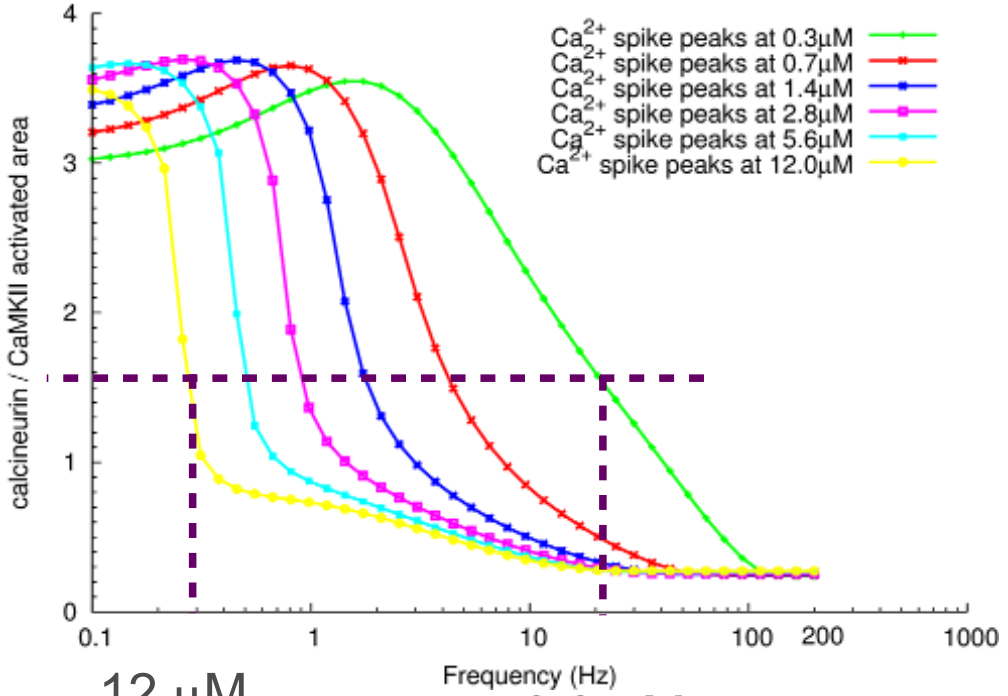
Effect of calcium duration and amount



180 spikes = 3 Hz

10 spikes = 70 Hz

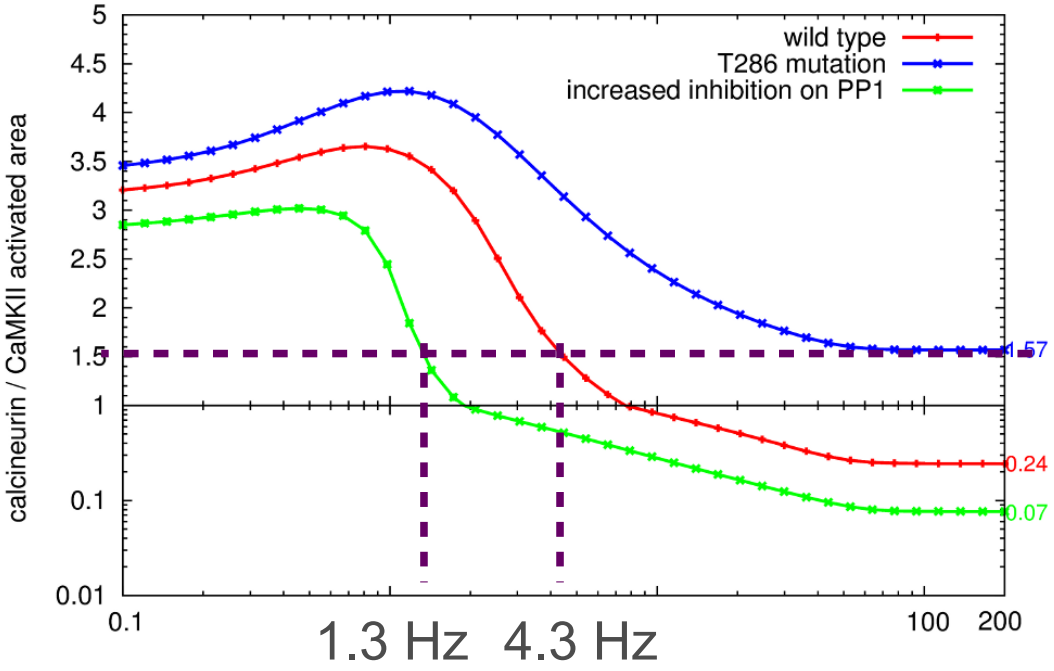


12 μM = 0.3 Hz

0.3 μM = 20 Hz

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

Effect of intrinsic system perturbations



CaMKII not constitutively active
No CaM trapping

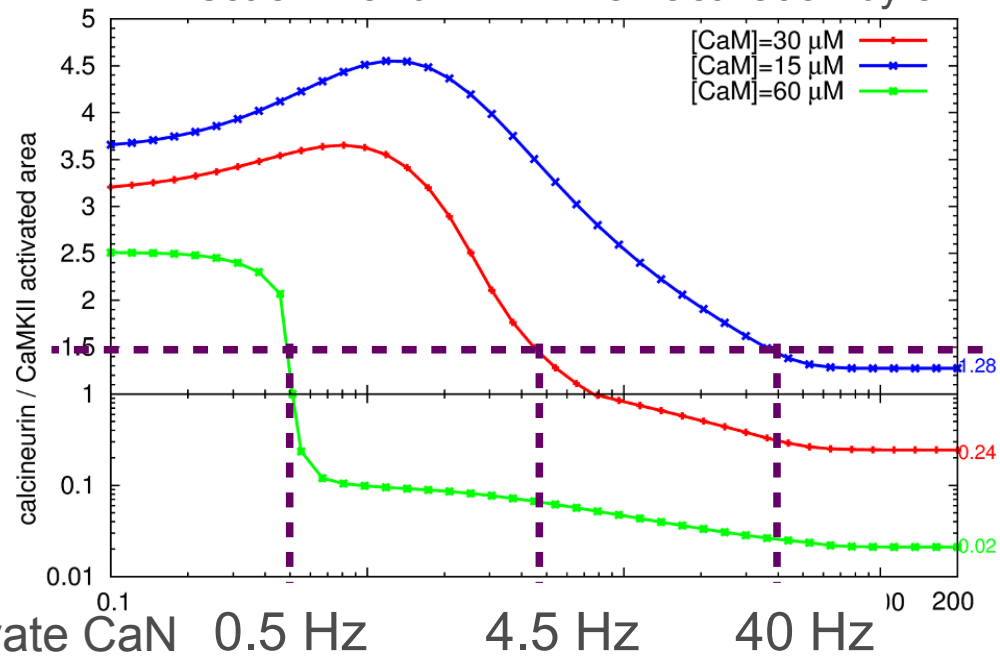
Never any positive plasticity

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

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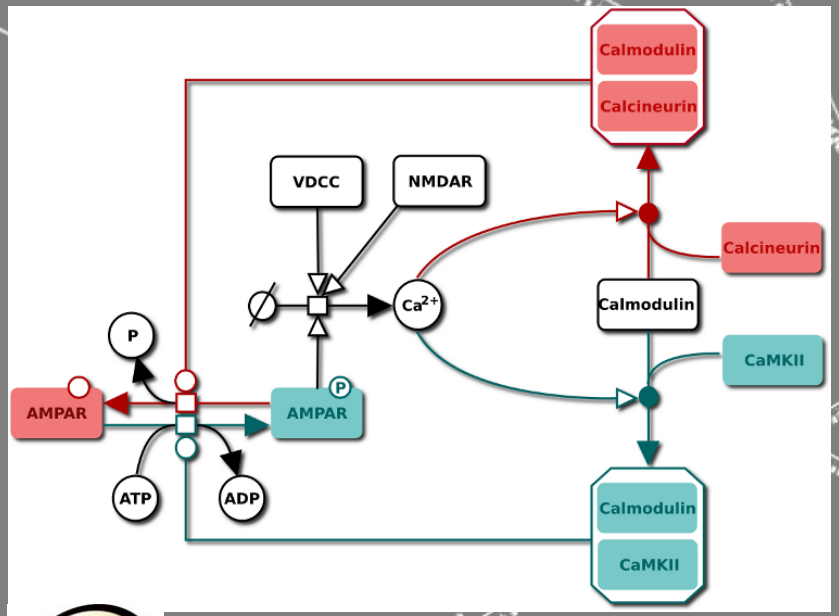
Allosteric stabilisation triggers **bistable CaM response**
> certain freq, CaM activation longer than initial signal

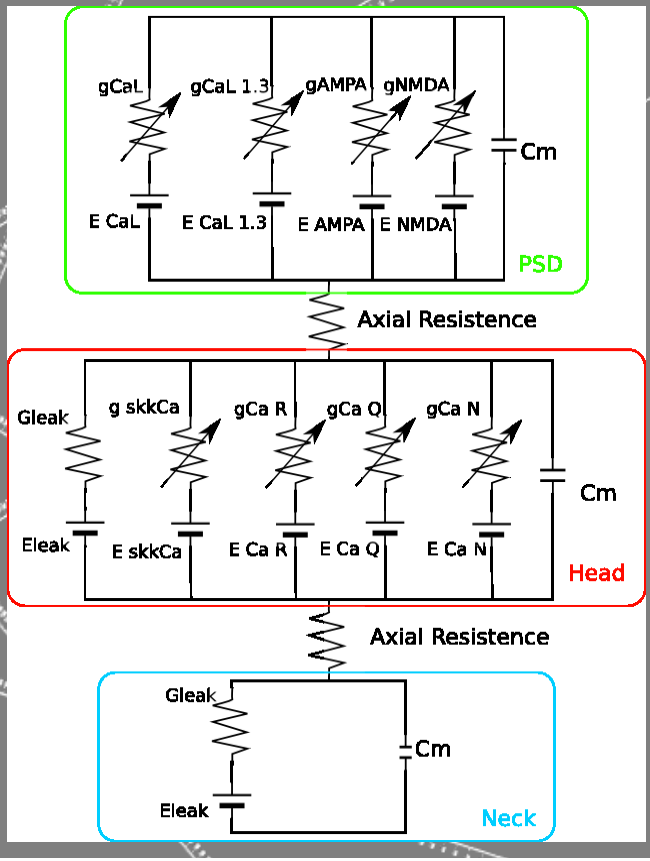
Calcium signals **activate both CaN and CaMKII** at *ALL* frequencies. The ratio of activity changes

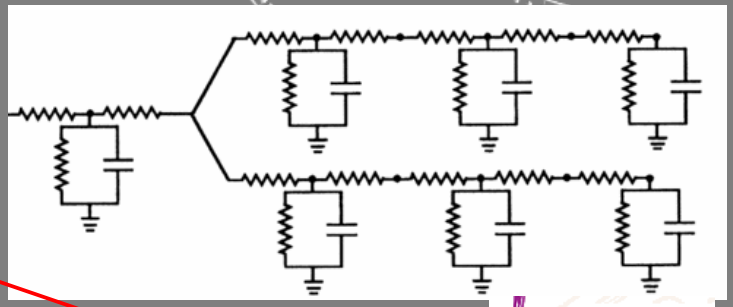
Θm is not an **intrinsic property of the synapse**, but a dynamical one that depends on the length and amplitude of stimulations

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

Whole cell: electro-biochemical models



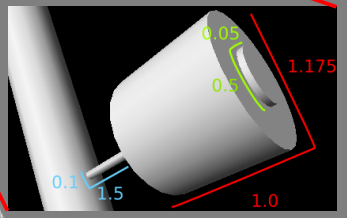




1504 spines

4761 compartments

16362 channels

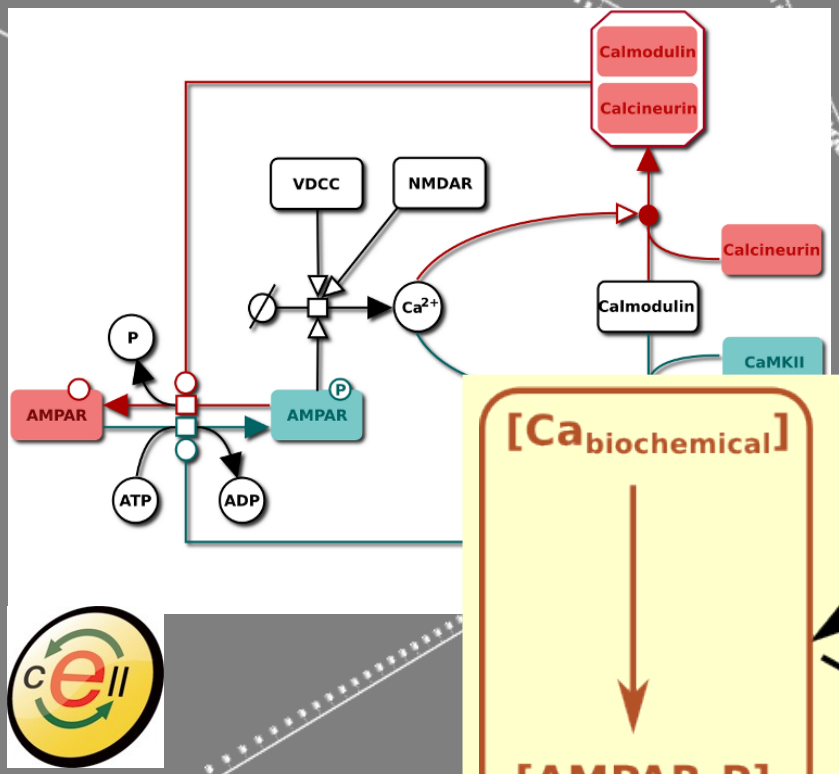


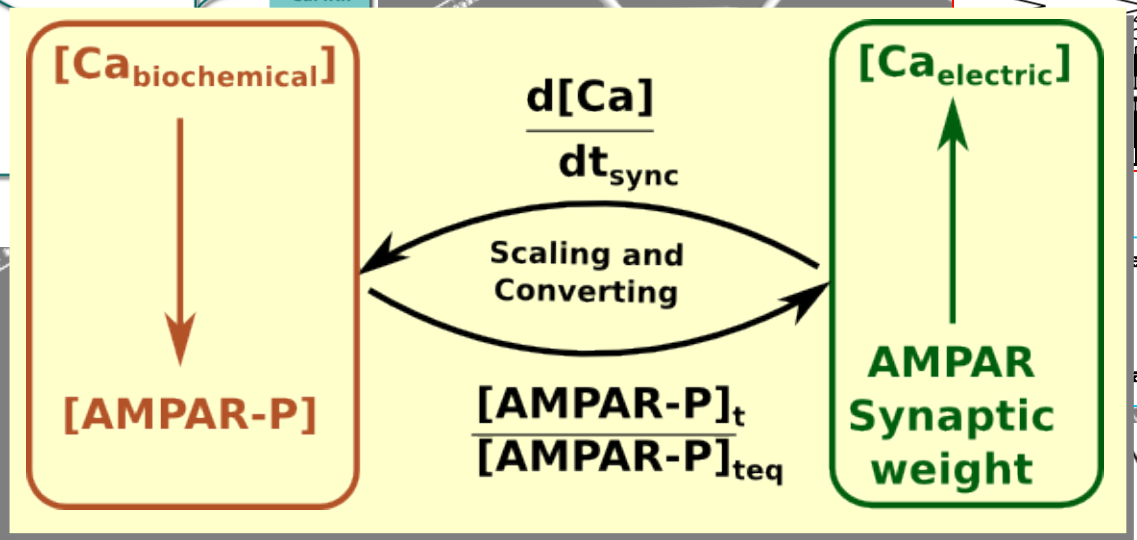
NEURON

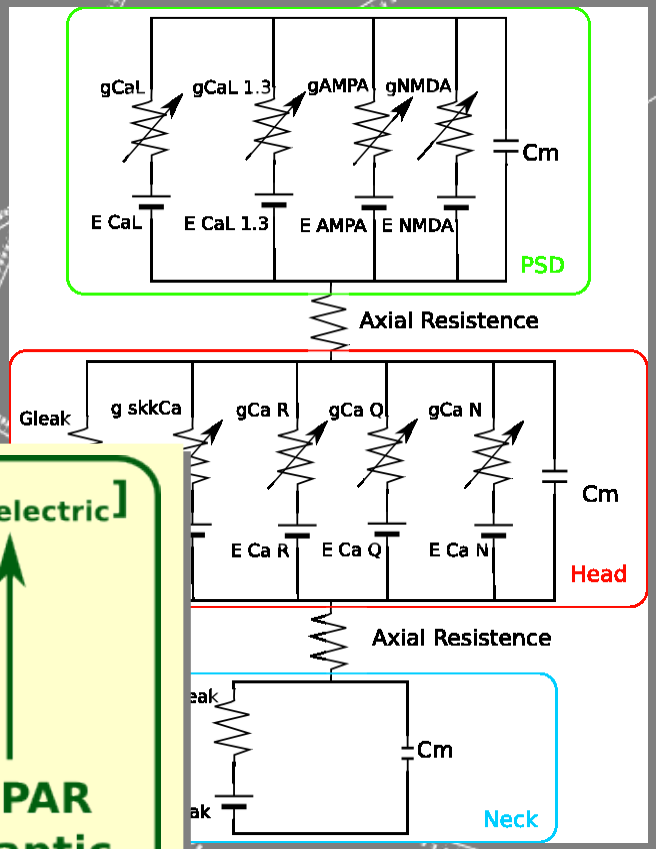
Mattioni M, Cohen U, Le Novère N (2012)

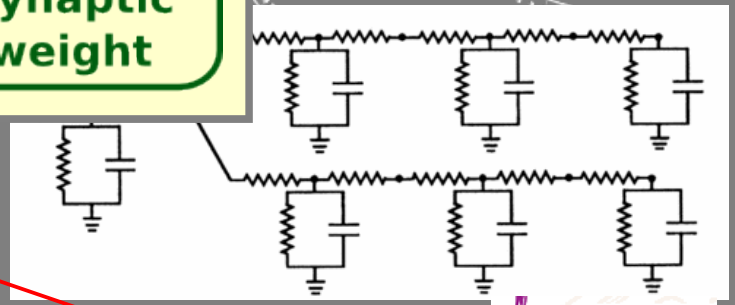
Mattioni M, Le Novère N (2013)

Whole cell: electro-biochemical models









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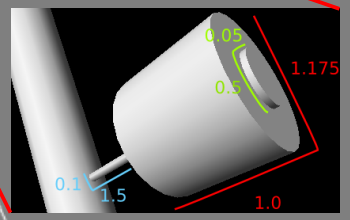
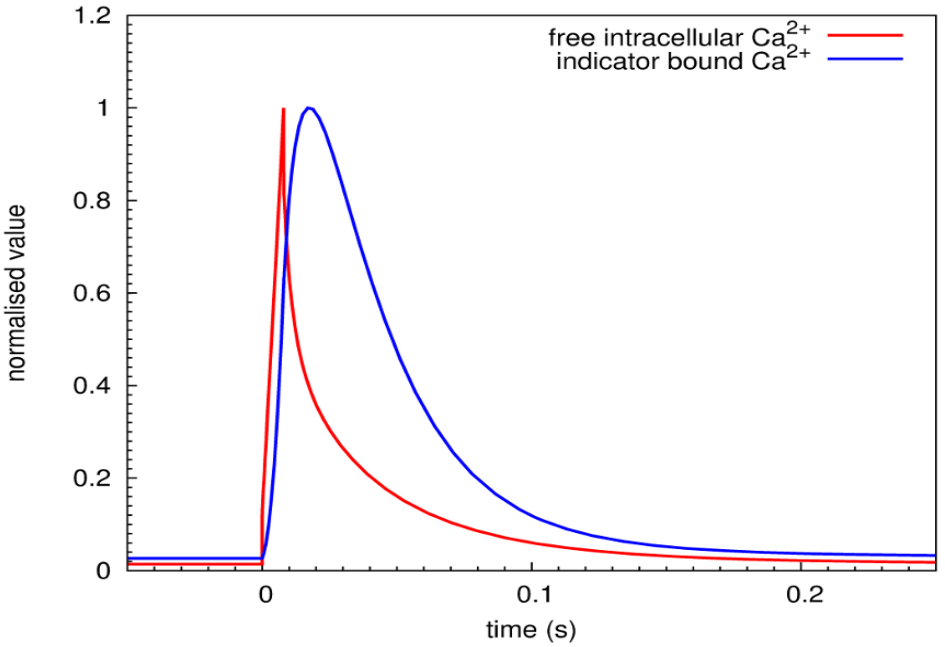


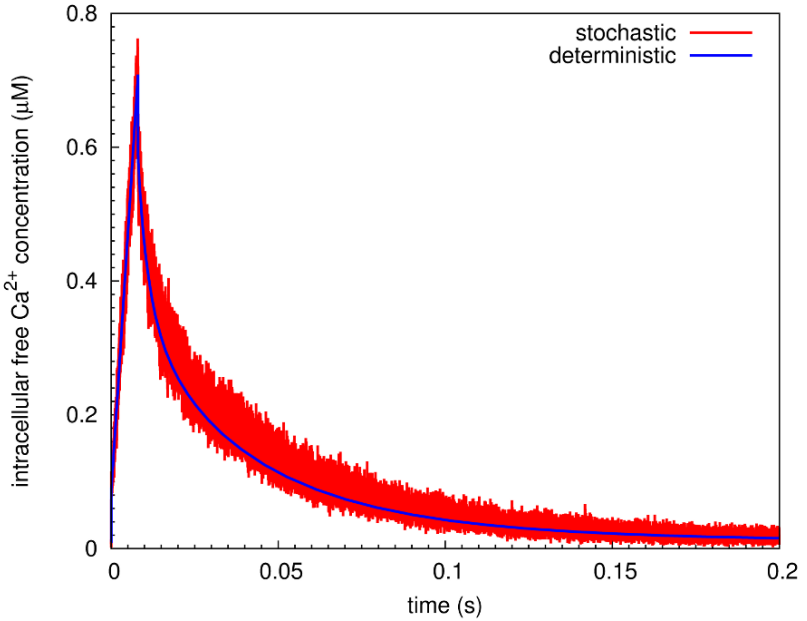
Mattioni M, Cohen U, Le Novère N (2012)

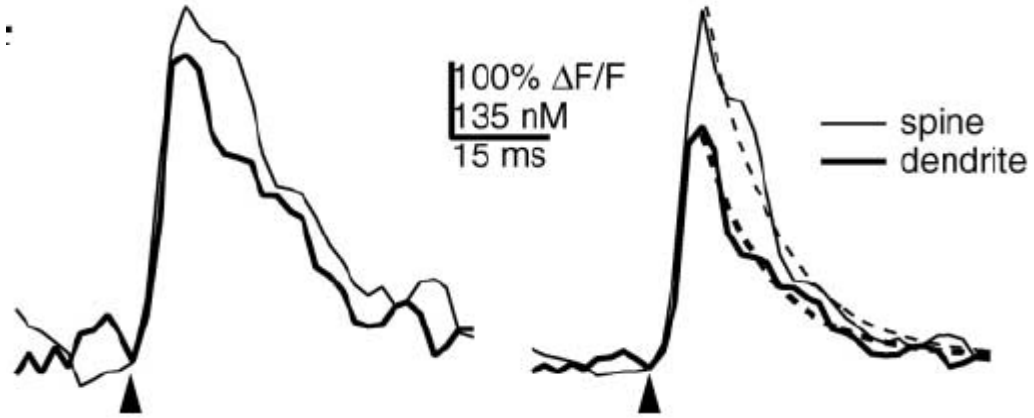
Mattioni M, Le Novère N (2013)

Pecunia est nervus belli

Are those spikes realistic?







Fluorescence

Free calcium

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

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