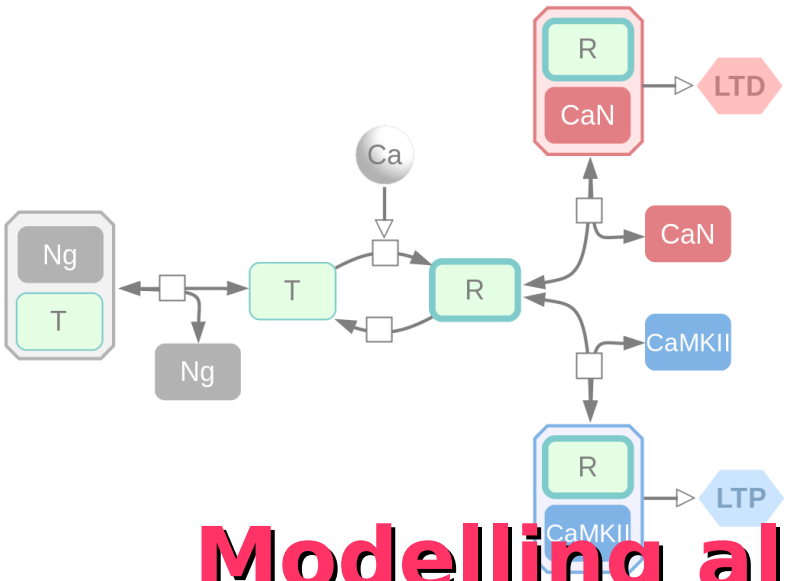
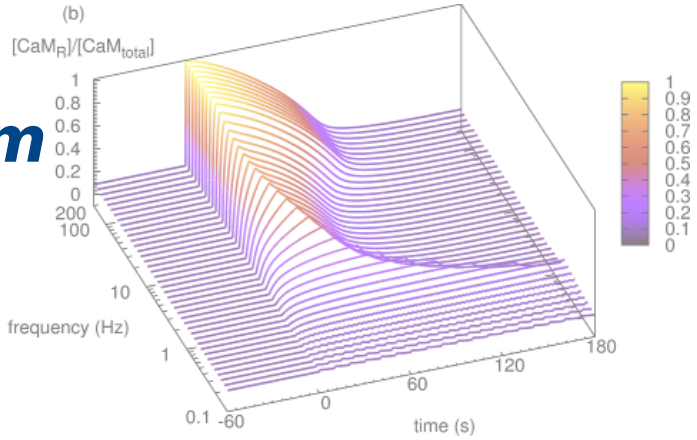
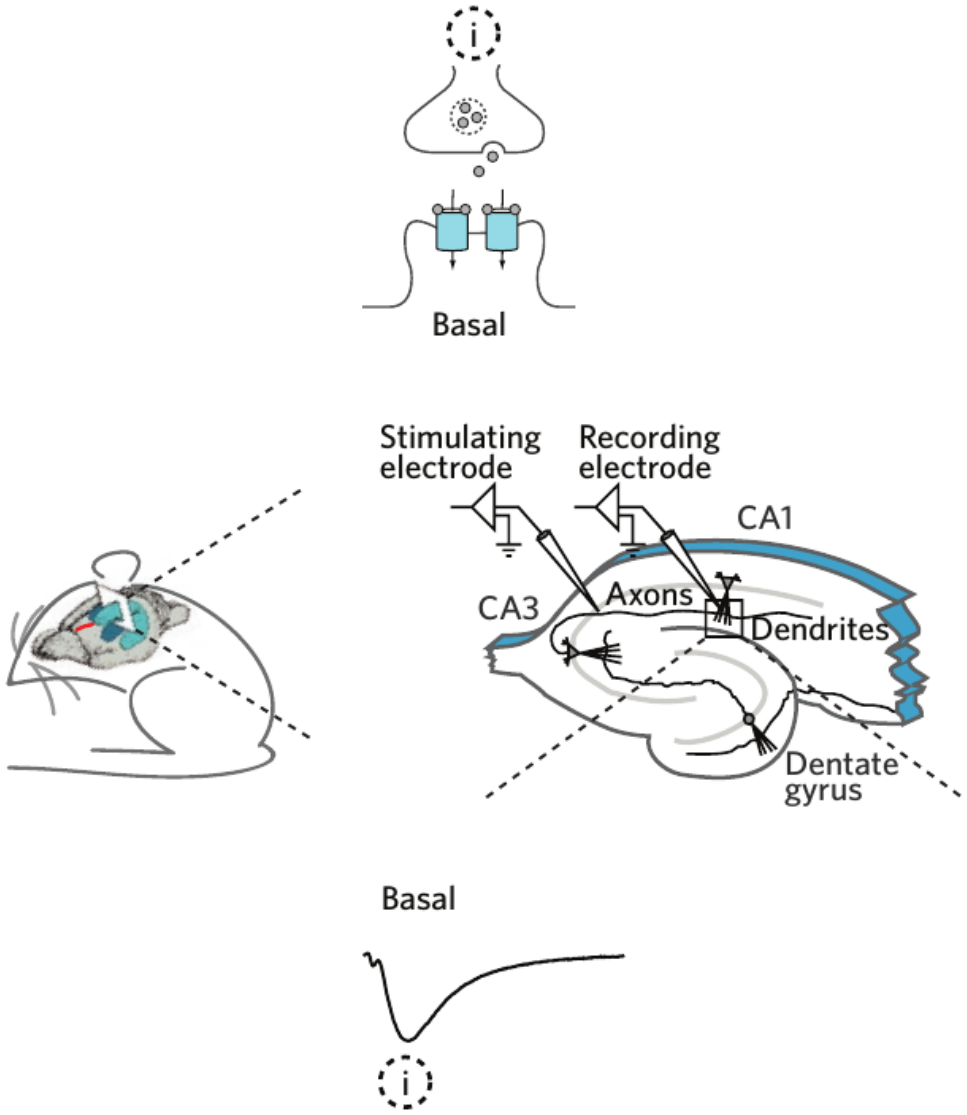


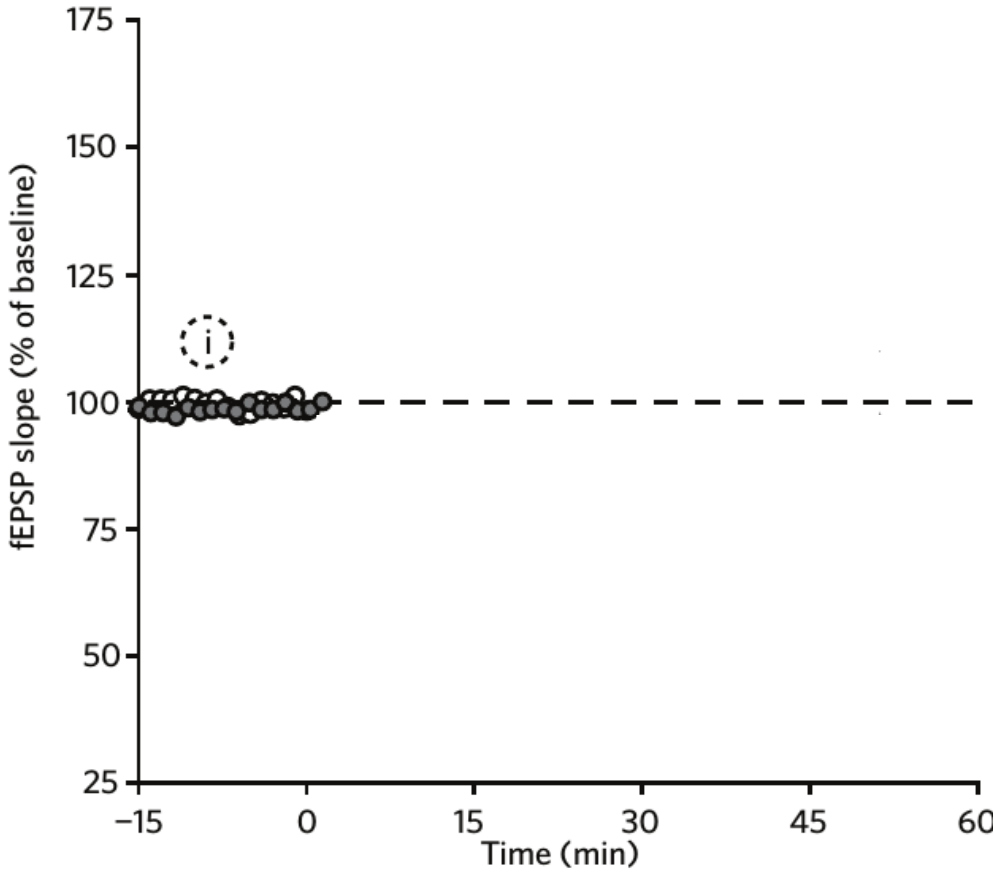
Modelling allosteric calcium sensors: The curse of combinatorial explosion

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

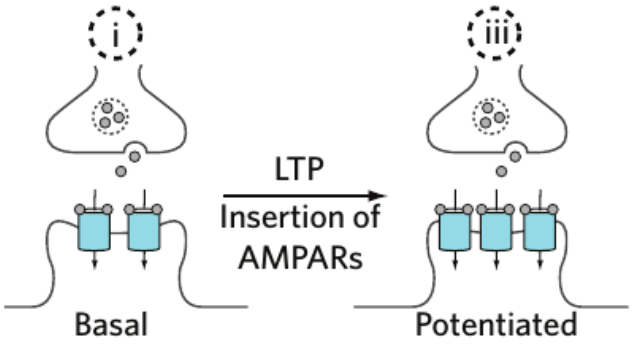


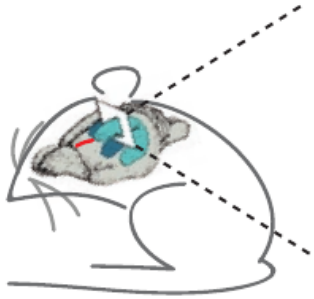
Babraham Institute

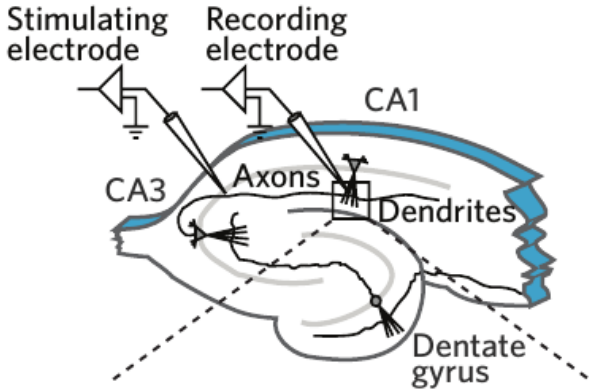


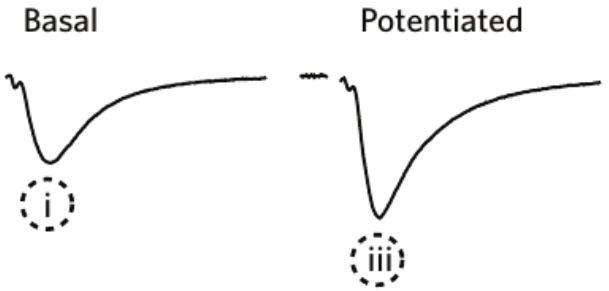


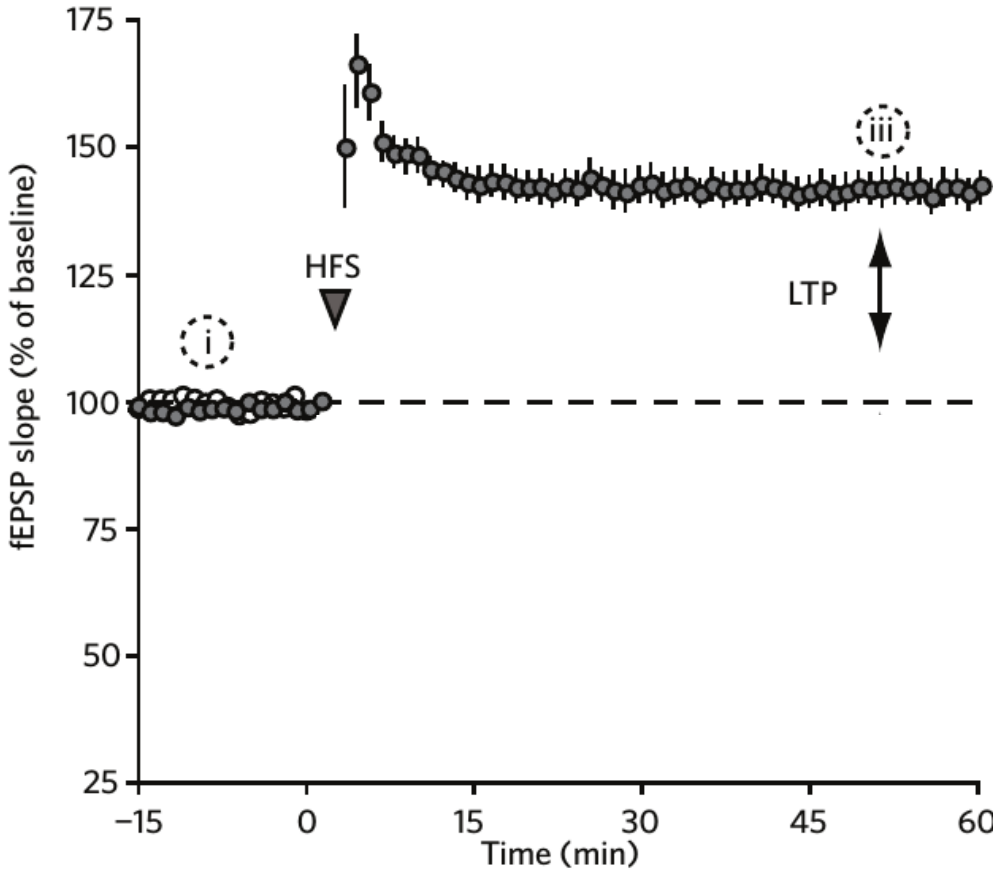
Fleming and England (2010) *Nat Chem Biol*



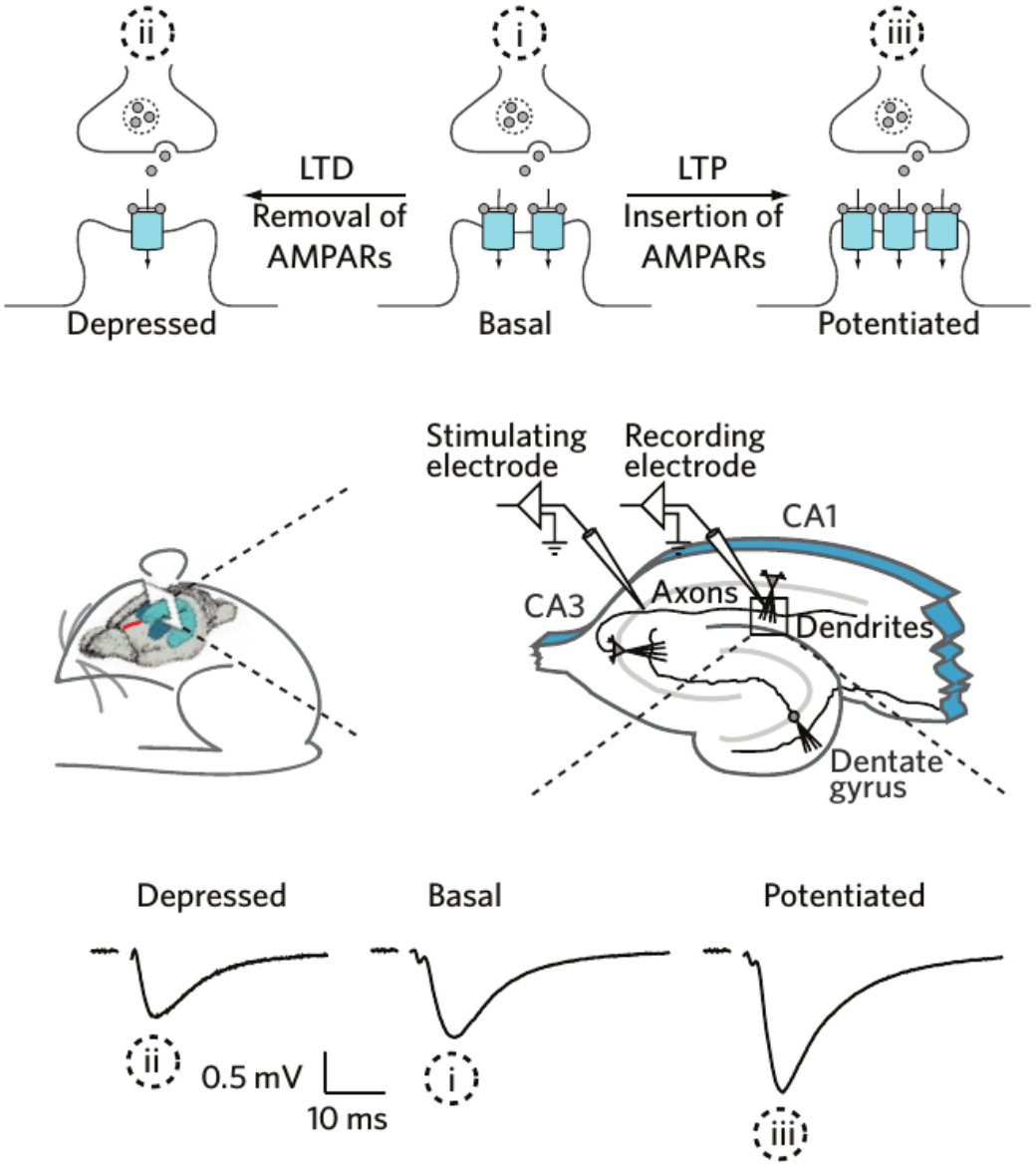


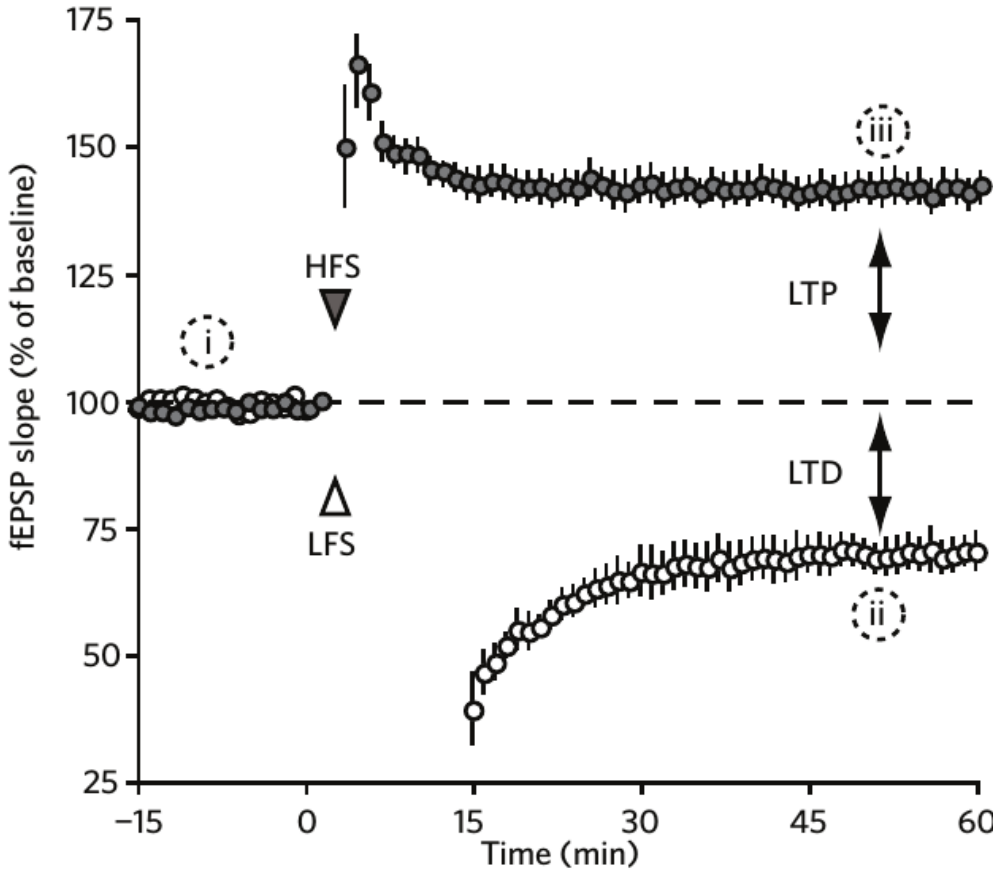






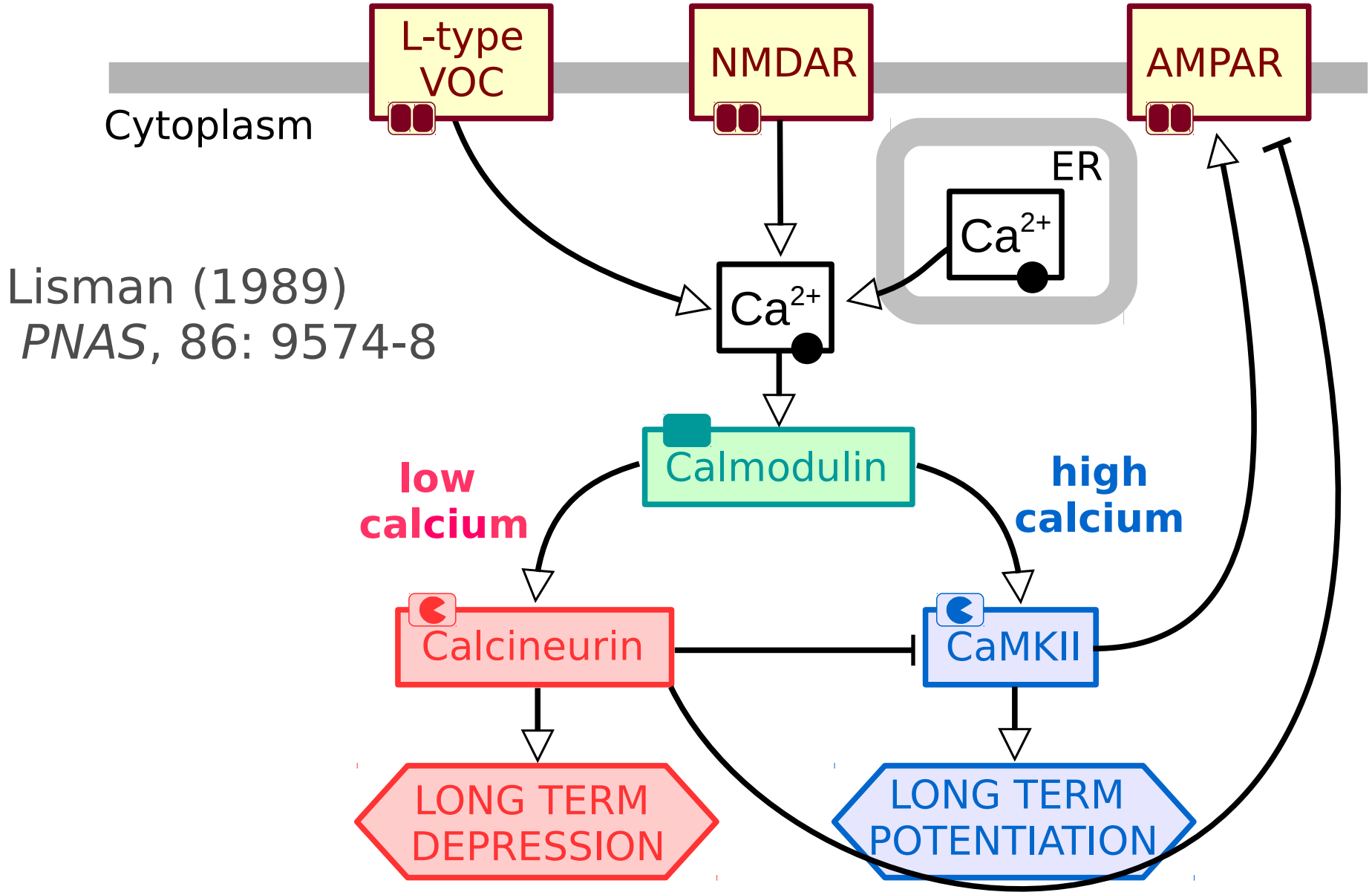
Fleming and England (2010) *Nat Chem Biol*

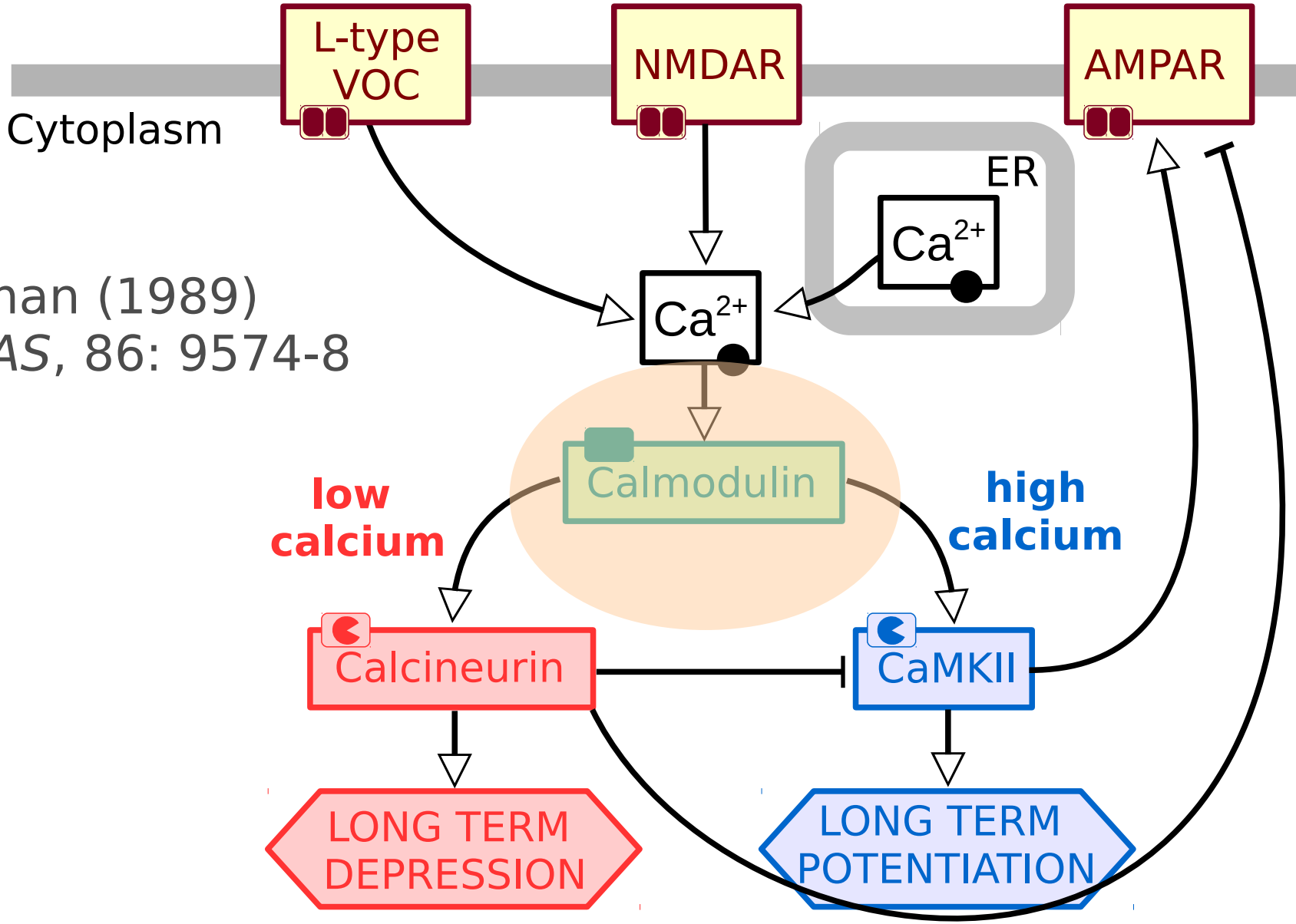




lasts for weeks

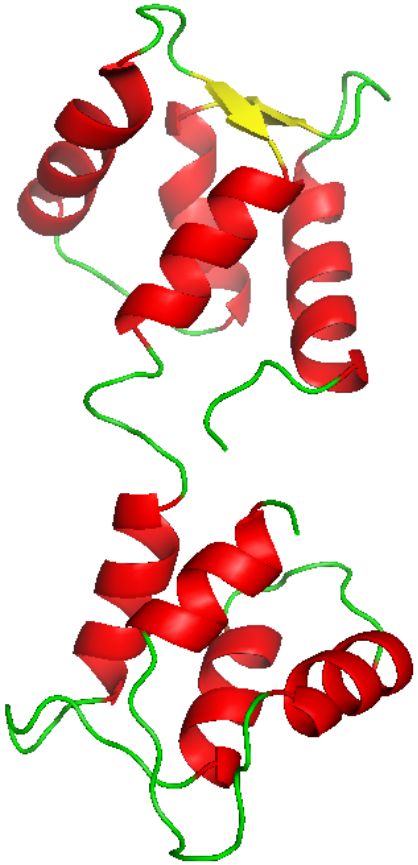
Fleming and England (2010) *Nat Chem Biol*


L-type VOC
NMDAR
AMPAR
Cytoplasm
ER
Ca^{2+}
Ca^{2+}
Lisman (1989)
PNAS, 86: 9574-8
Calmodulin
low calcium
high calcium
Calcineurin
CaMKII
LONG TERM DEPRESSION
LONG TERM POTENTIATION

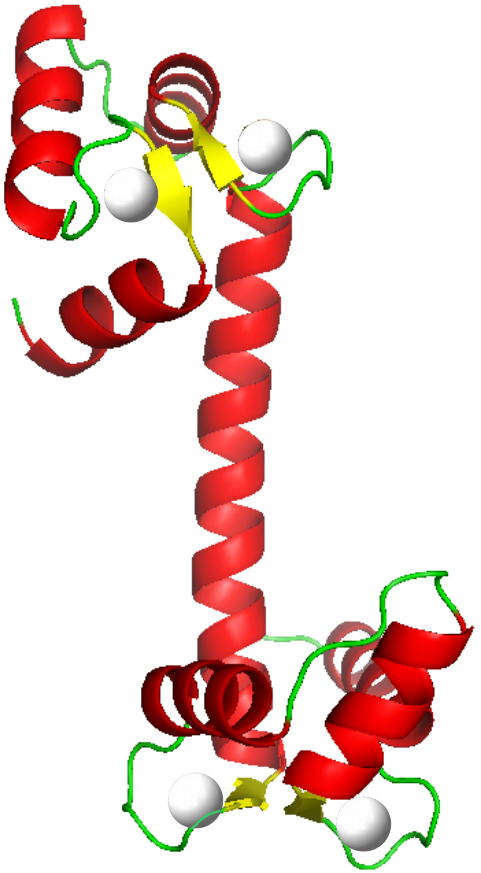


Lisman (1989)
PNAS, 86: 9574-8

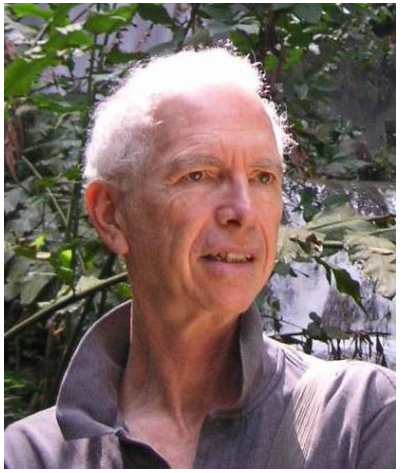
Closed (T)



Open (R)



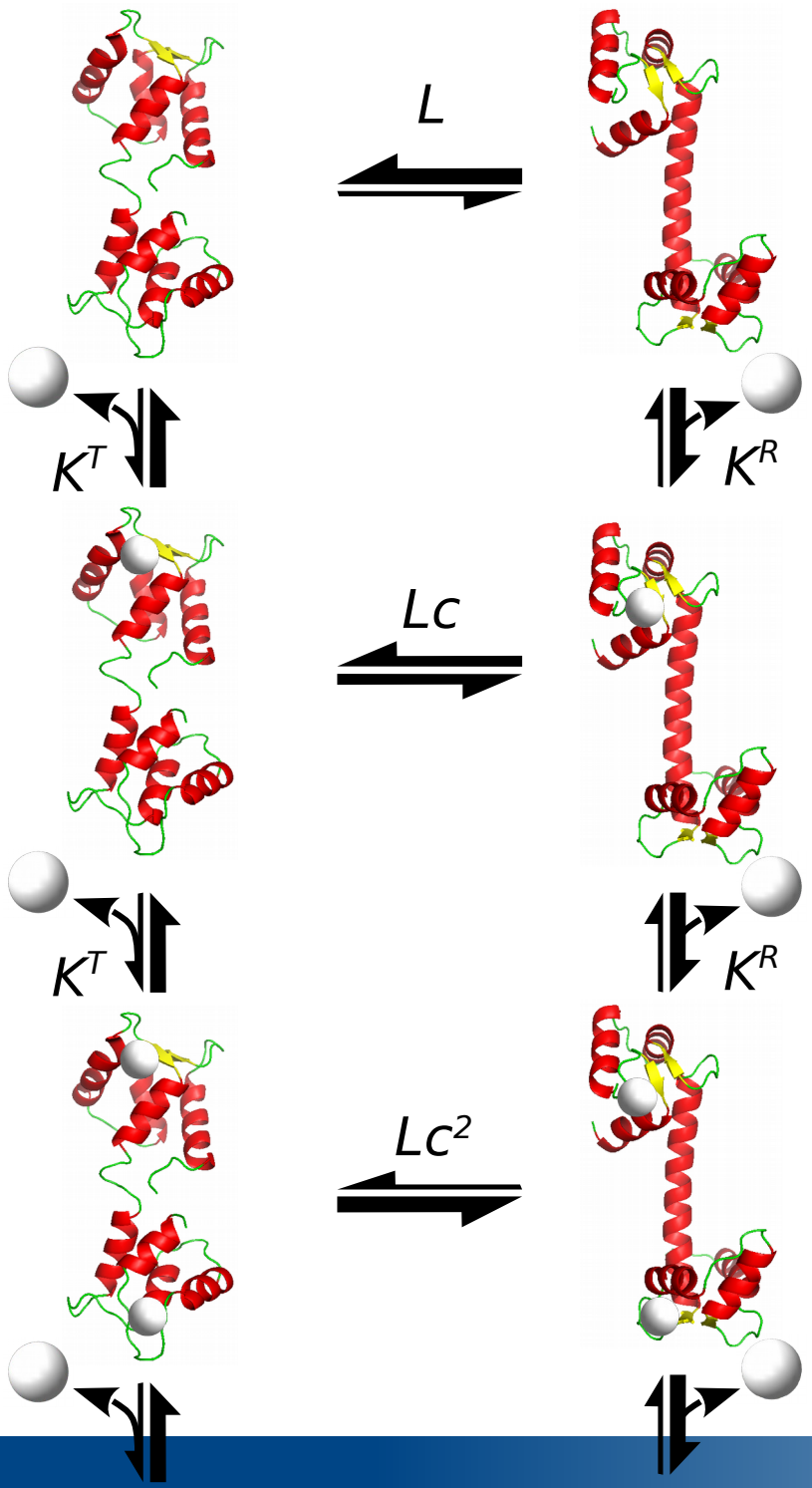
Melanie Stefan

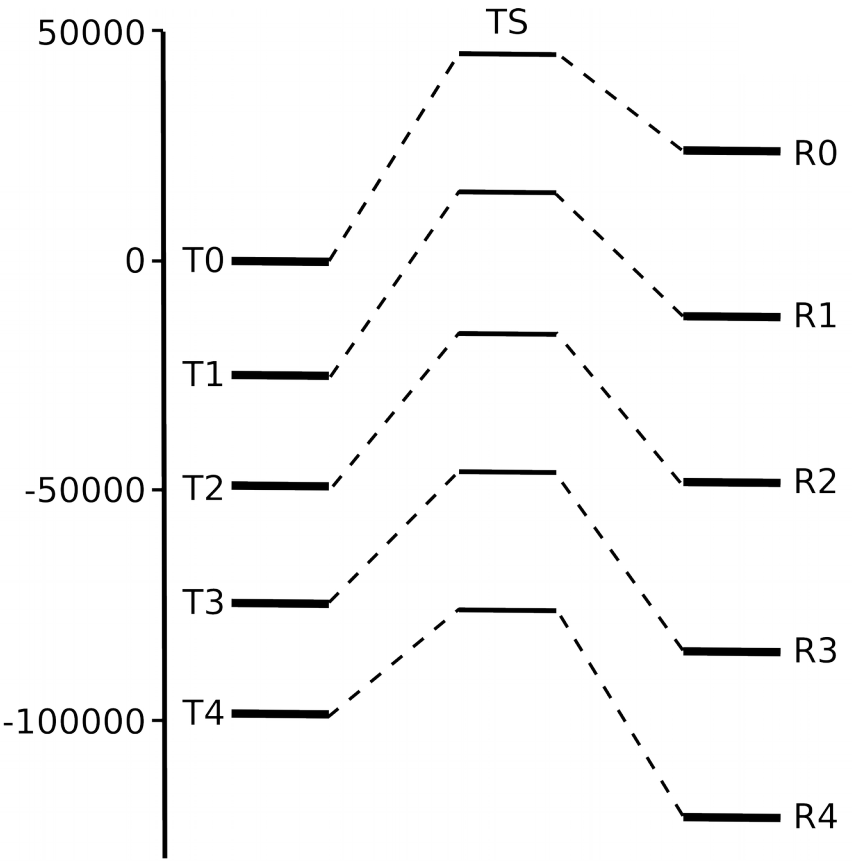


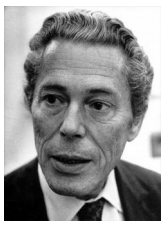
Stuart Edelstein

Scilab

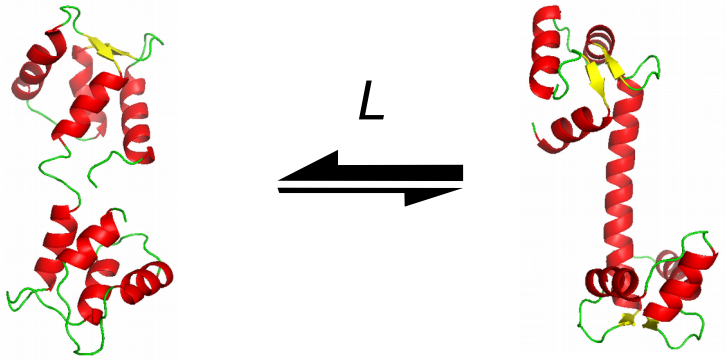
Stefan MI, Edelstein SJ, Le Novère N (2008)
Stefan MI, Edelstein SJ, Le Novère N (2009)
Edelstein SJ, Stefan MI, Le Novère N (2010)

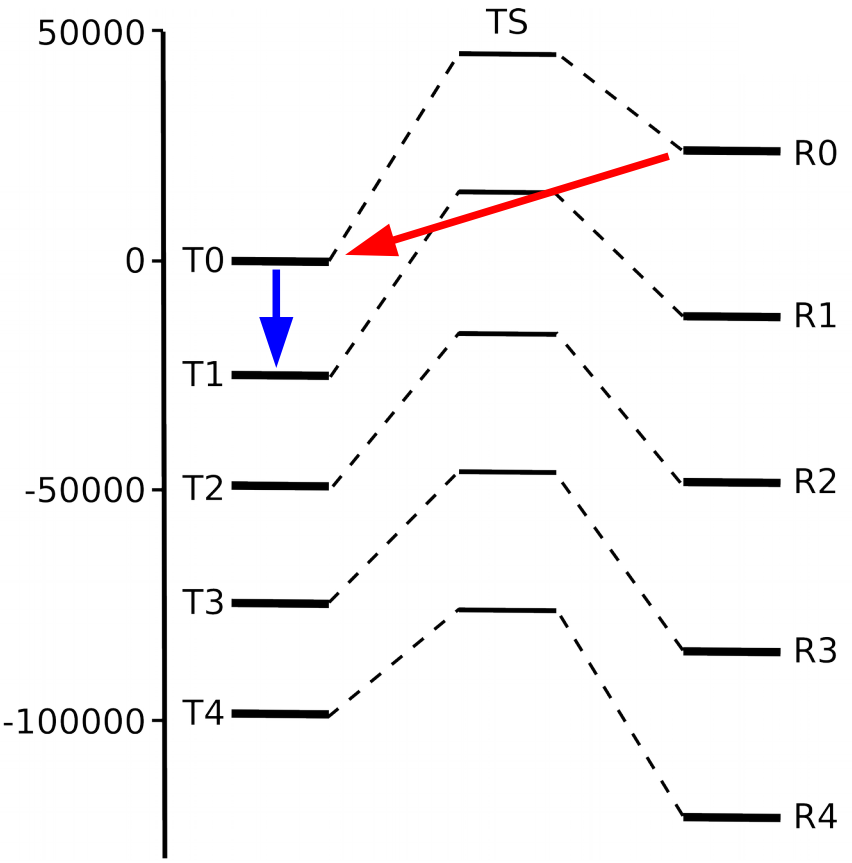


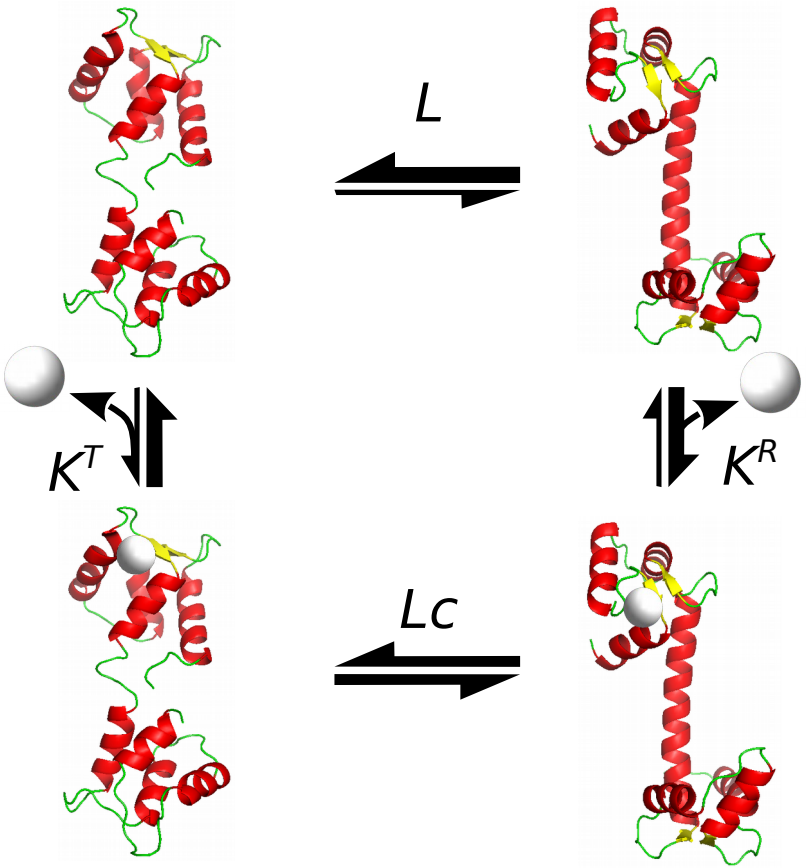


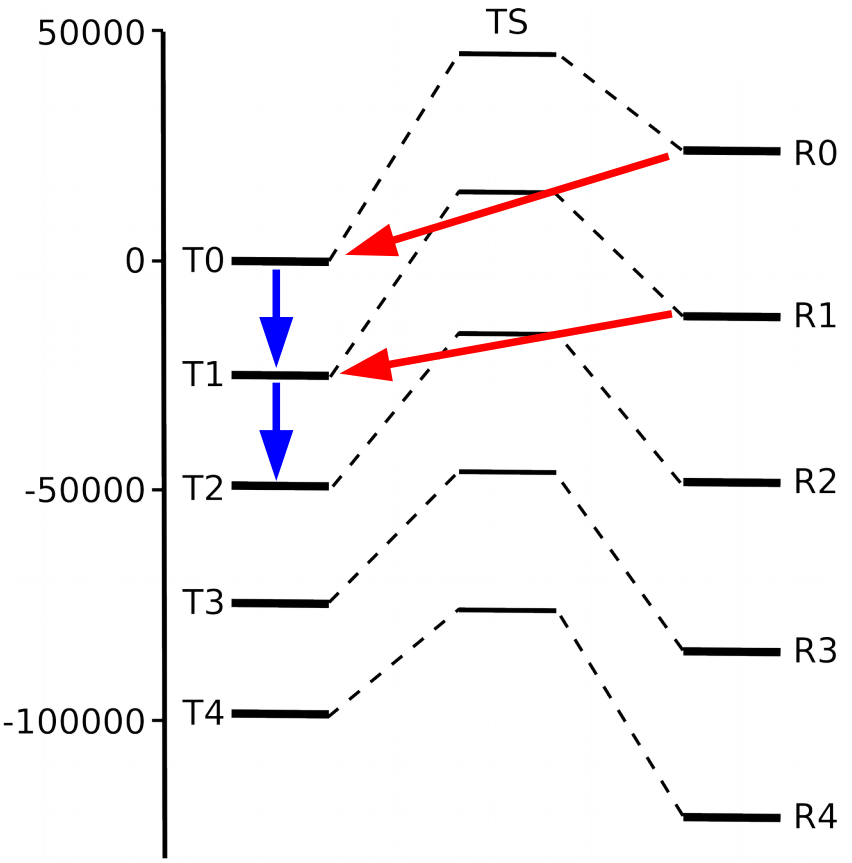


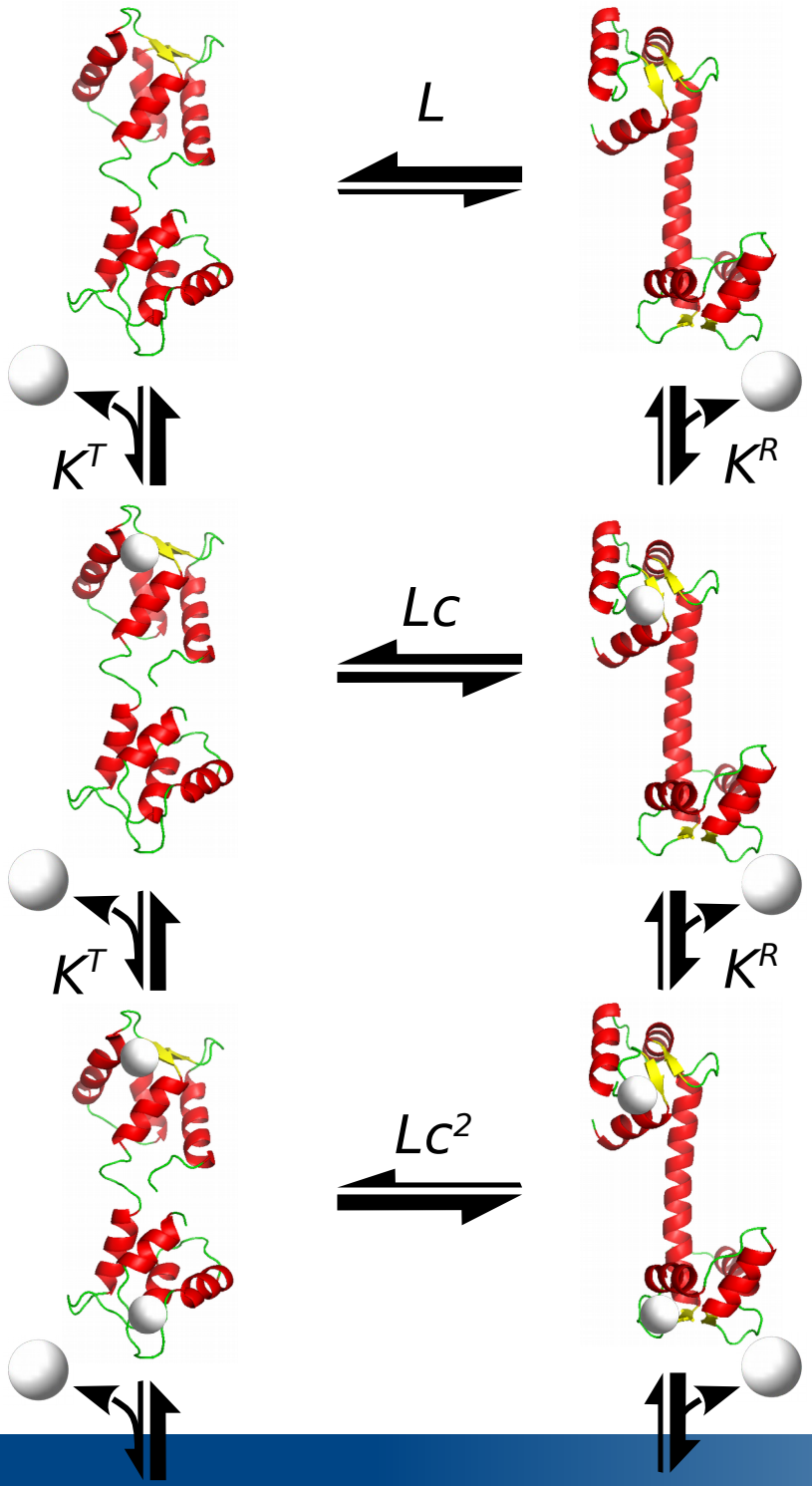
Monod, Wyman, Changeux (1965)
On the nature of allosteric transitions: a plausible model.
J Mol Biol, 12: 88-118

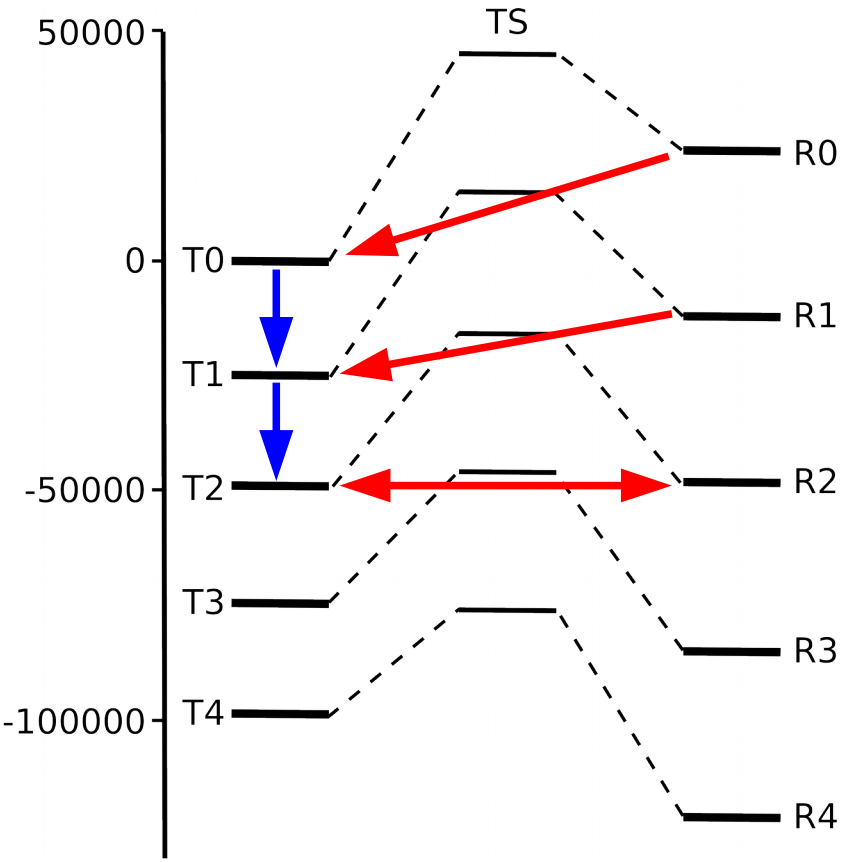

L

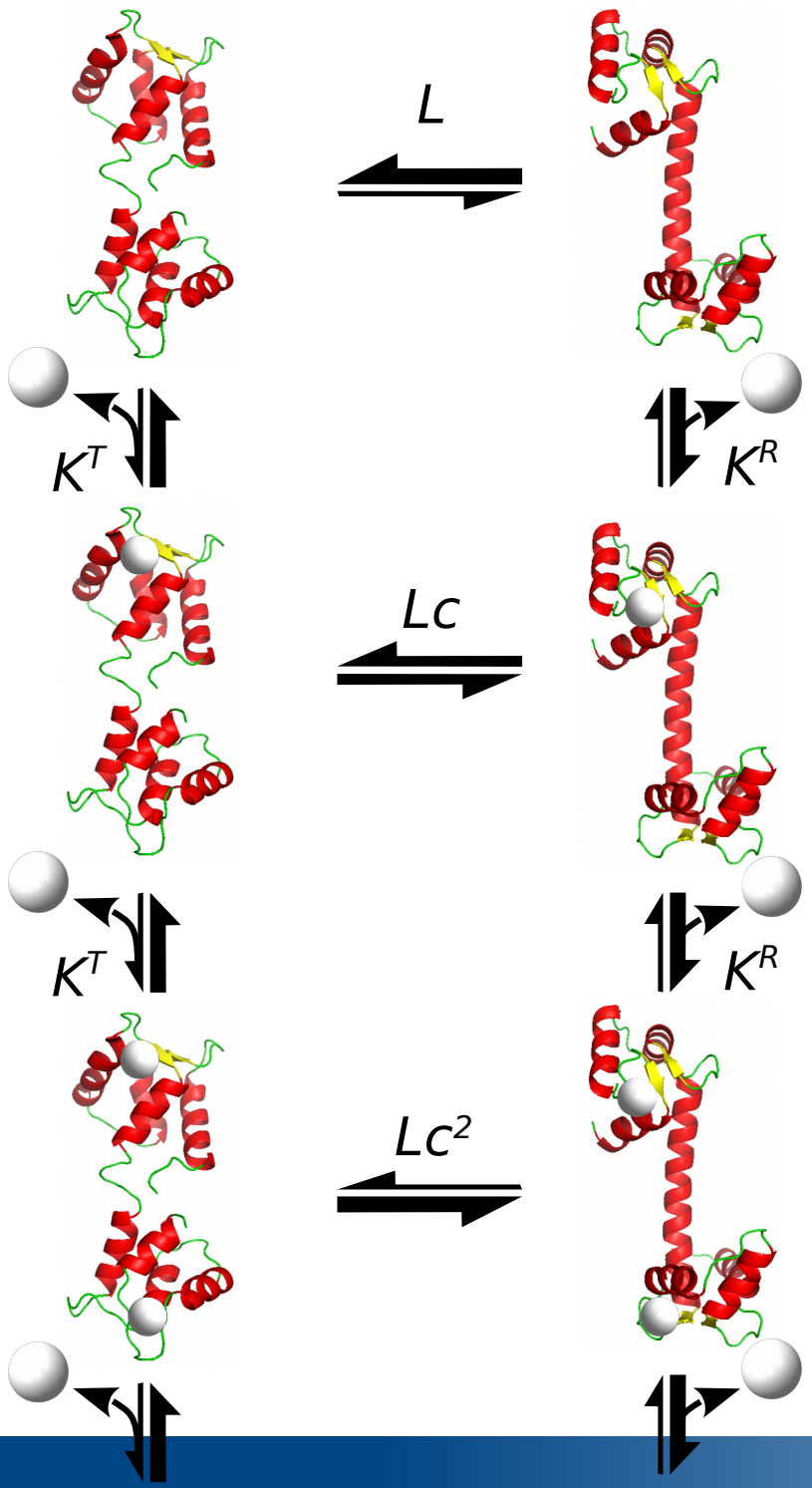

50000
0
-50000
-100000
TS
T0
T1
T2
T3
T4
R0
R1
R2
R3
R4

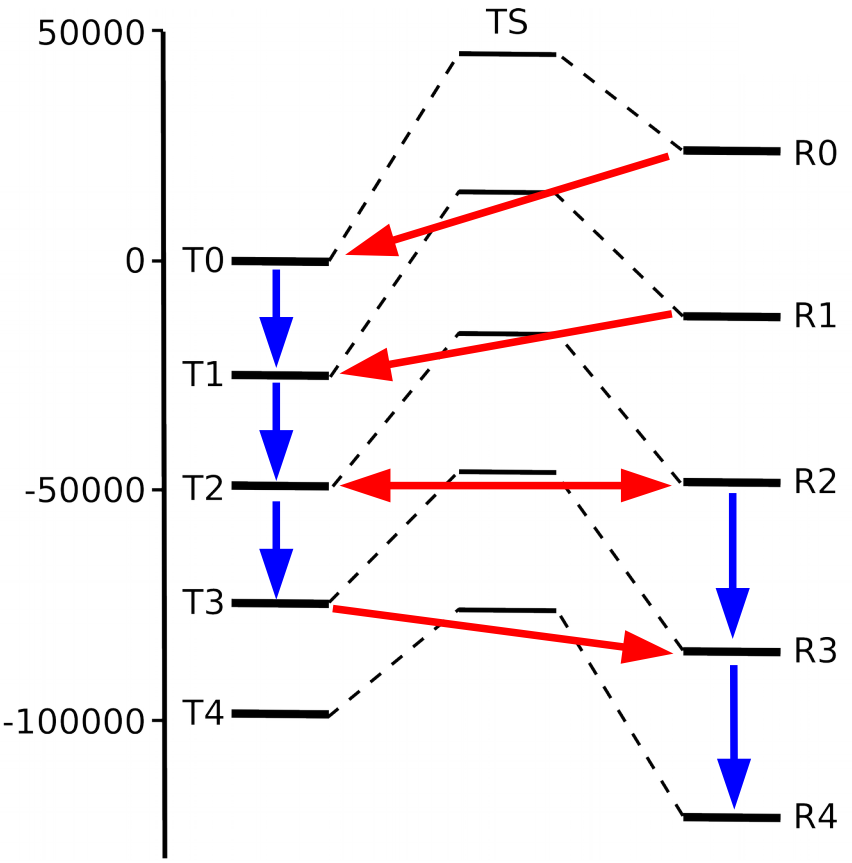

L
K^T
K^R
Lc

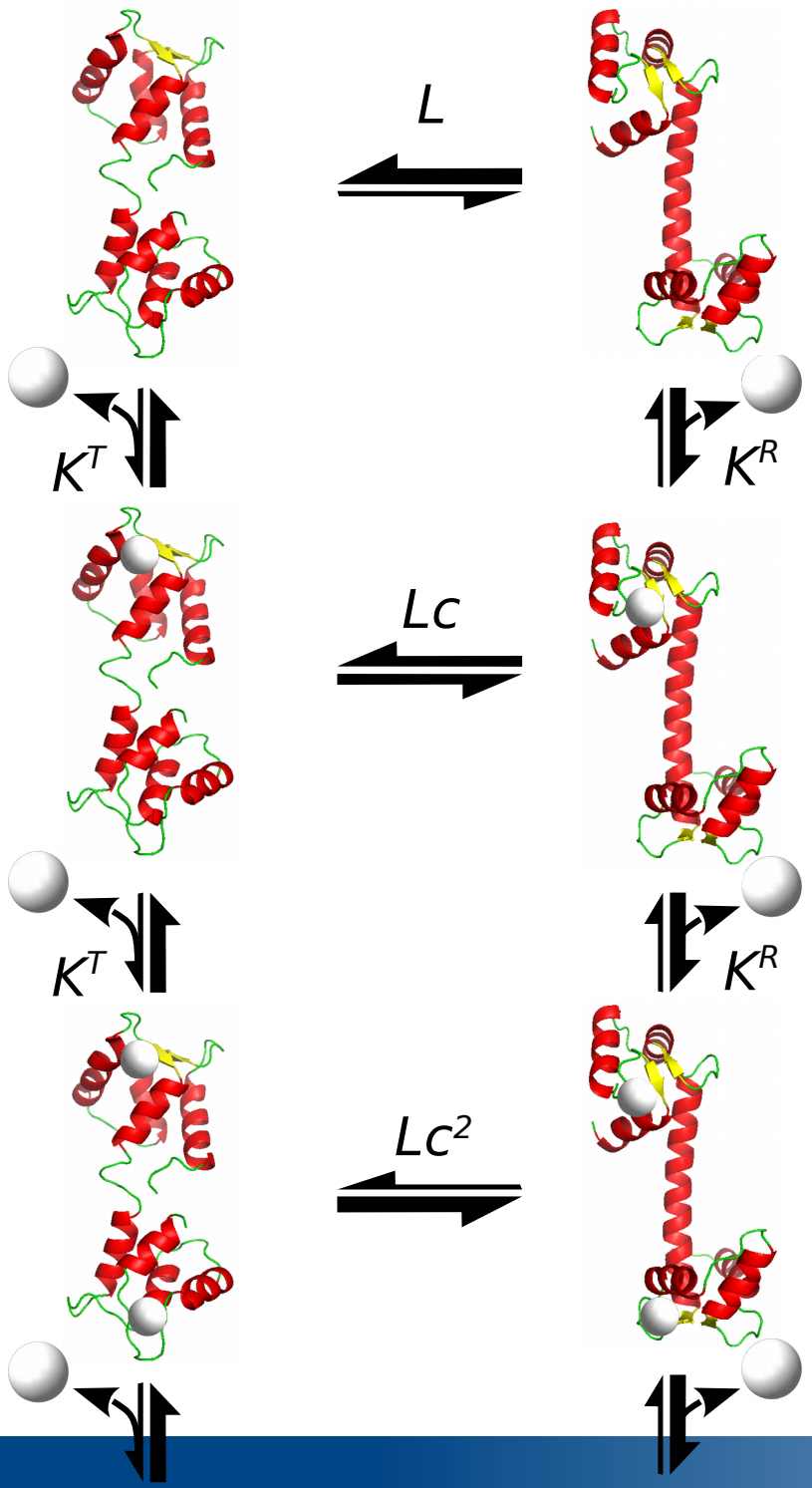

50000
0
-50000
-100000
TS
T0
T1
T2
T3
T4
R0
R1
R2
R3
R4

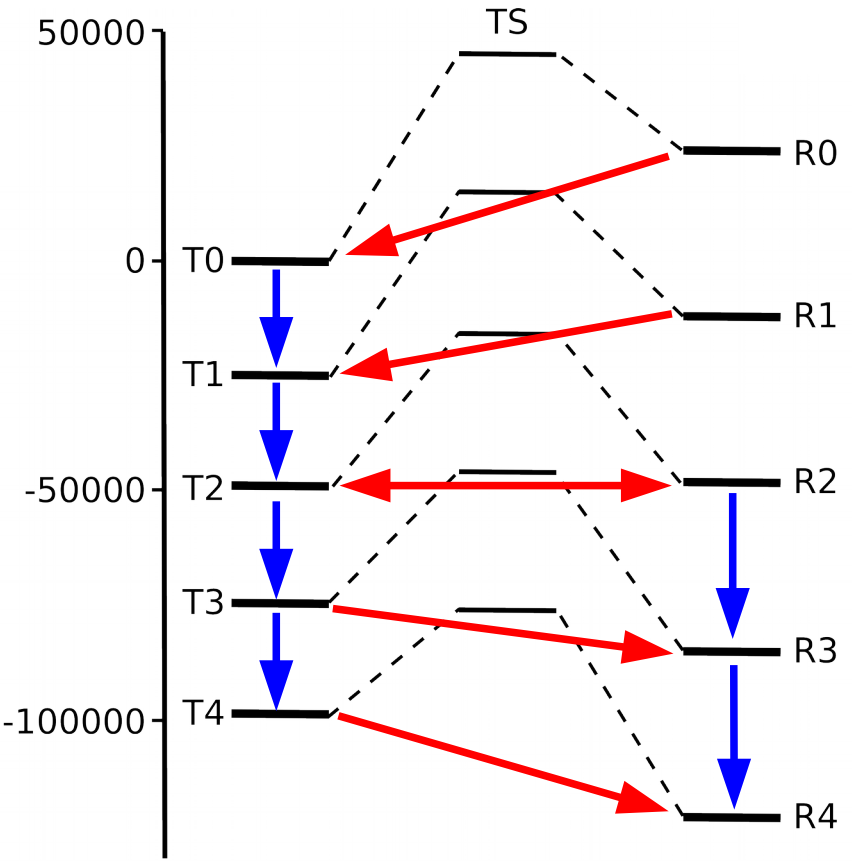

L
K^T
K^R
Lc
K^T
K^R
Lc^2

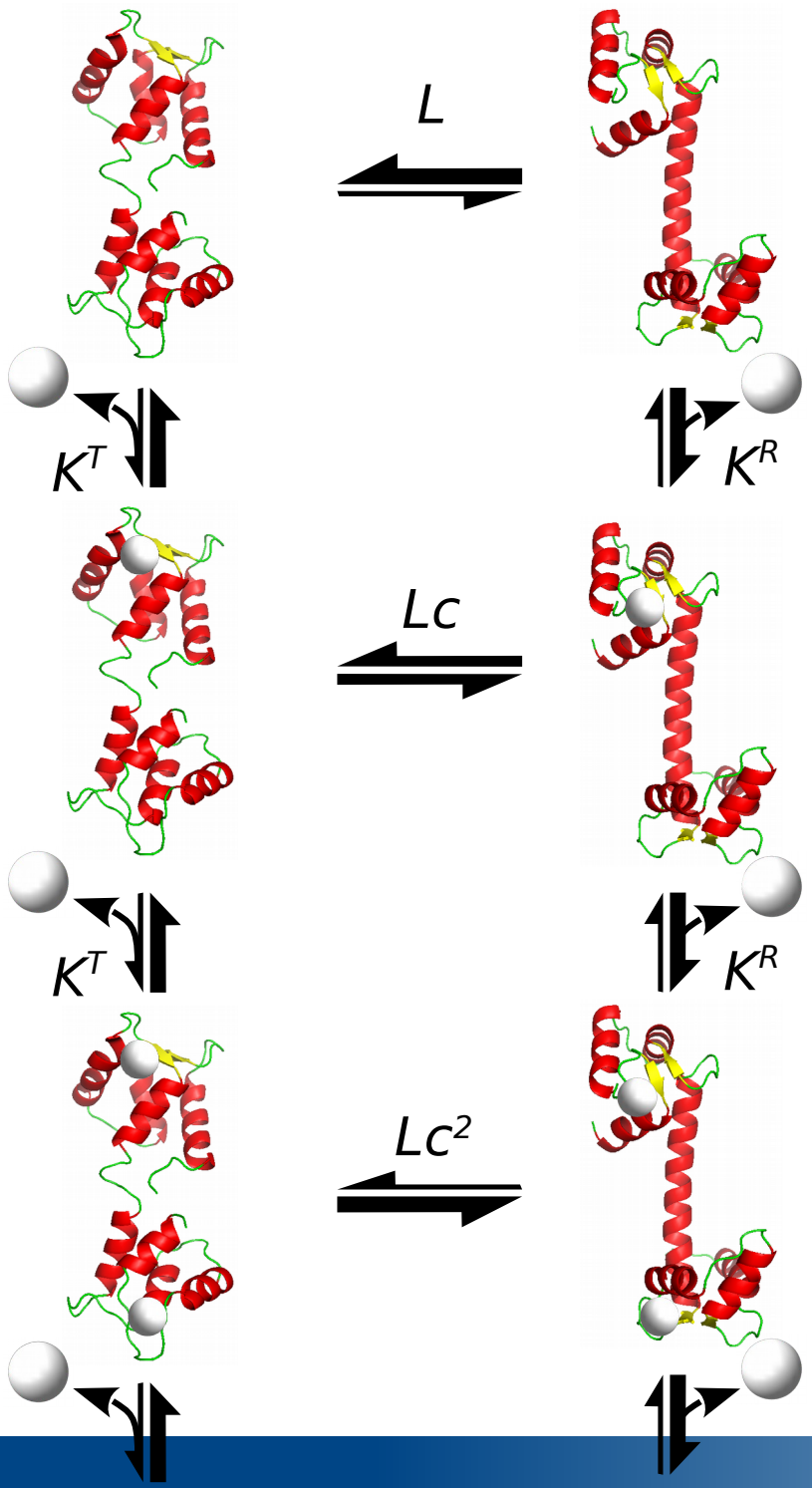

50000
0
-50000
-100000
TS
T0
T1
T2
T3
T4
R0
R1
R2
R3
R4


L
K^T
K^R
Lc
K^T
K^R
Lc^2


50000
0
-50000
-100000
TS
T0
T1
T2
T3
T4
R0
R1
R2
R3
R4


L
K^T
K^R
Lc
K^T
K^R
Lc^2


50000
0
-50000
-100000
TS
T0
T1
T2
T3
T4
R0
R1
R2
R3
R4



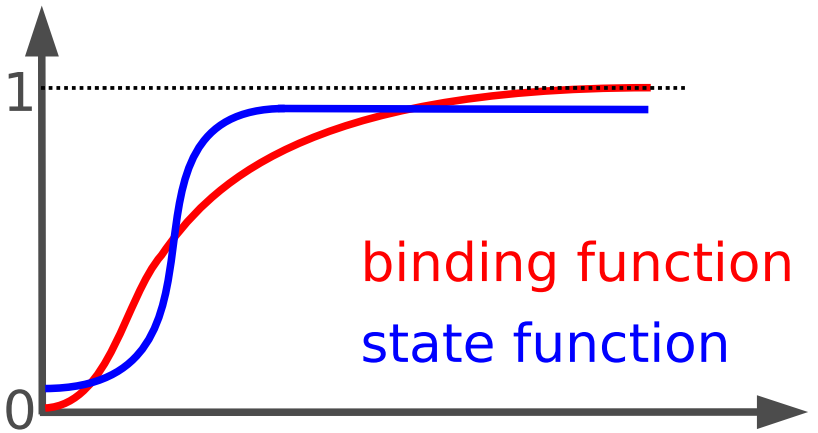
$$\bar{Y} = \frac{\alpha(1+\alpha)^{n-1} + Lc\alpha(1+c\alpha)^{n-1}}{(1+\alpha)^n + L(1+c\alpha)^n}$$

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

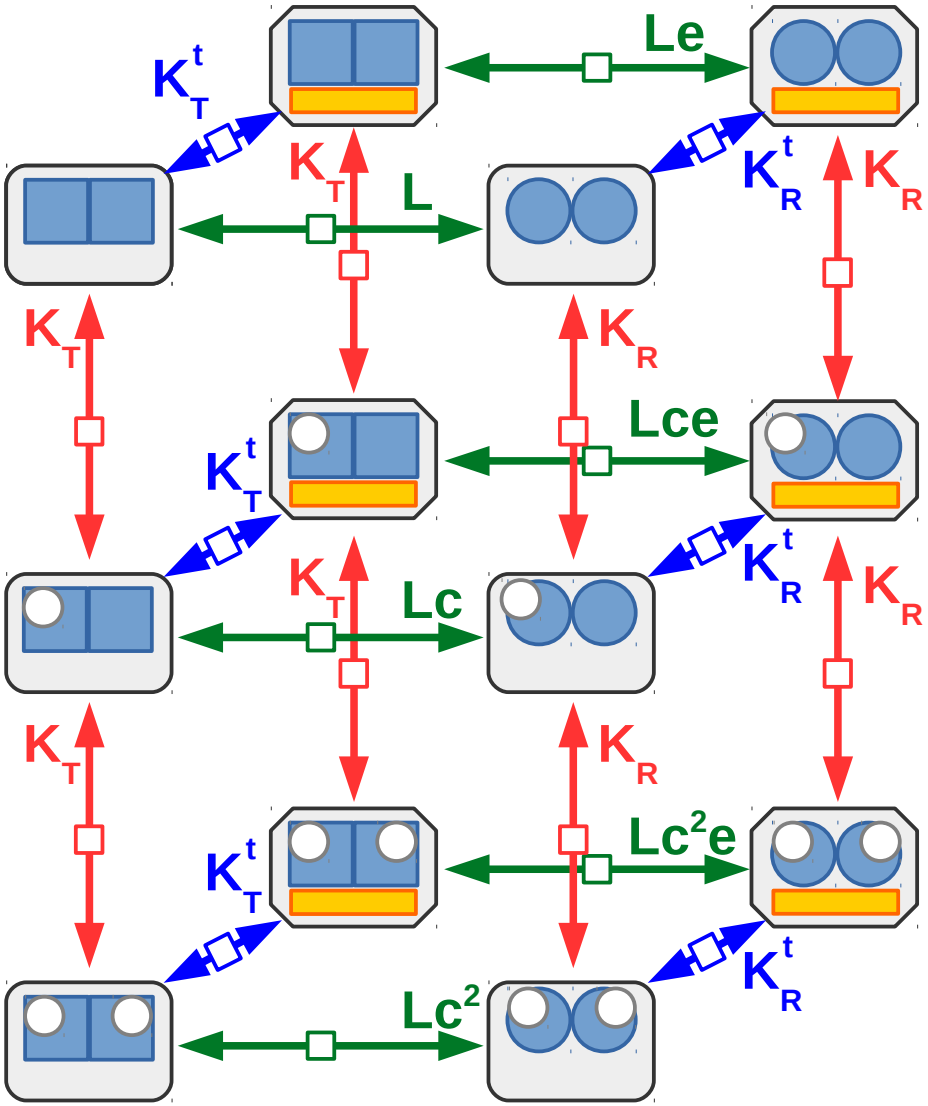
$$L = \frac{[T_0]}{[R_0]} \quad c = \frac{K^R}{K^T} \quad \alpha = \frac{[Ca^{2+}]}{[K^R]}$$

$L \gg 1 \Rightarrow$ Equilibrium strongly biased

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



Bindings of calcium and targets (one lobe)



T-state

R-state

Calcium

Target

Conf. transition

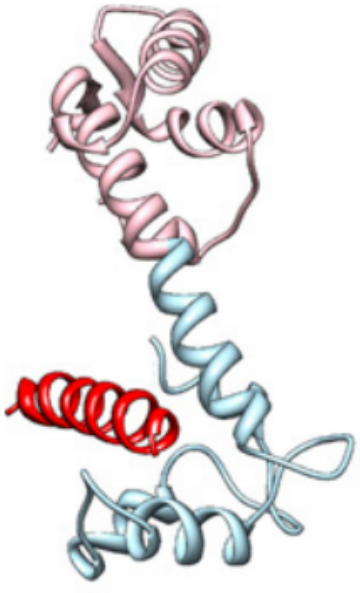
Calcium binding

Target binding

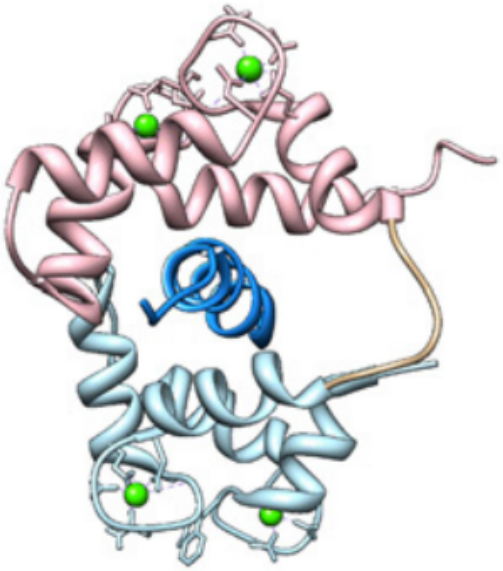
$$c = \frac{K_R}{K_T}$$

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

Different targets stabilise CaM in different states

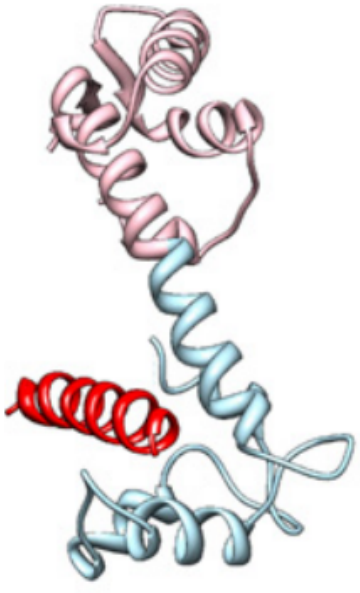


Neurogranin

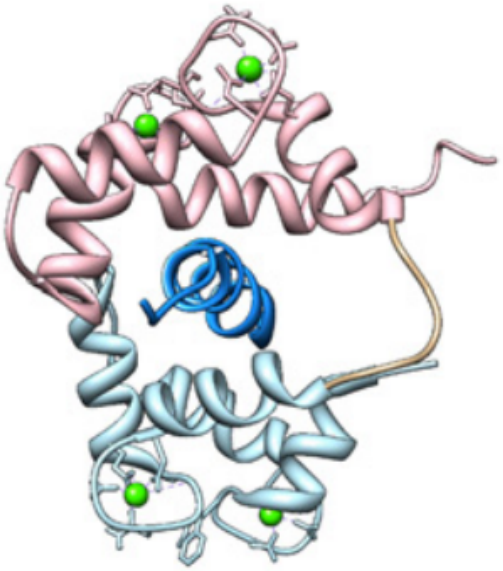


MLCK

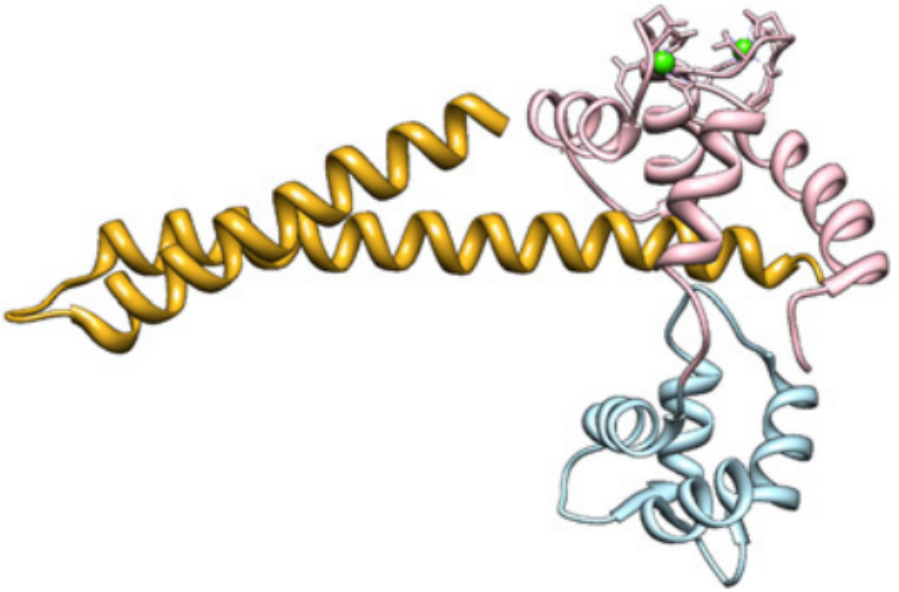
Different targets stabilise lobes in different states



Neurogranin



MLCK



SK channel

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

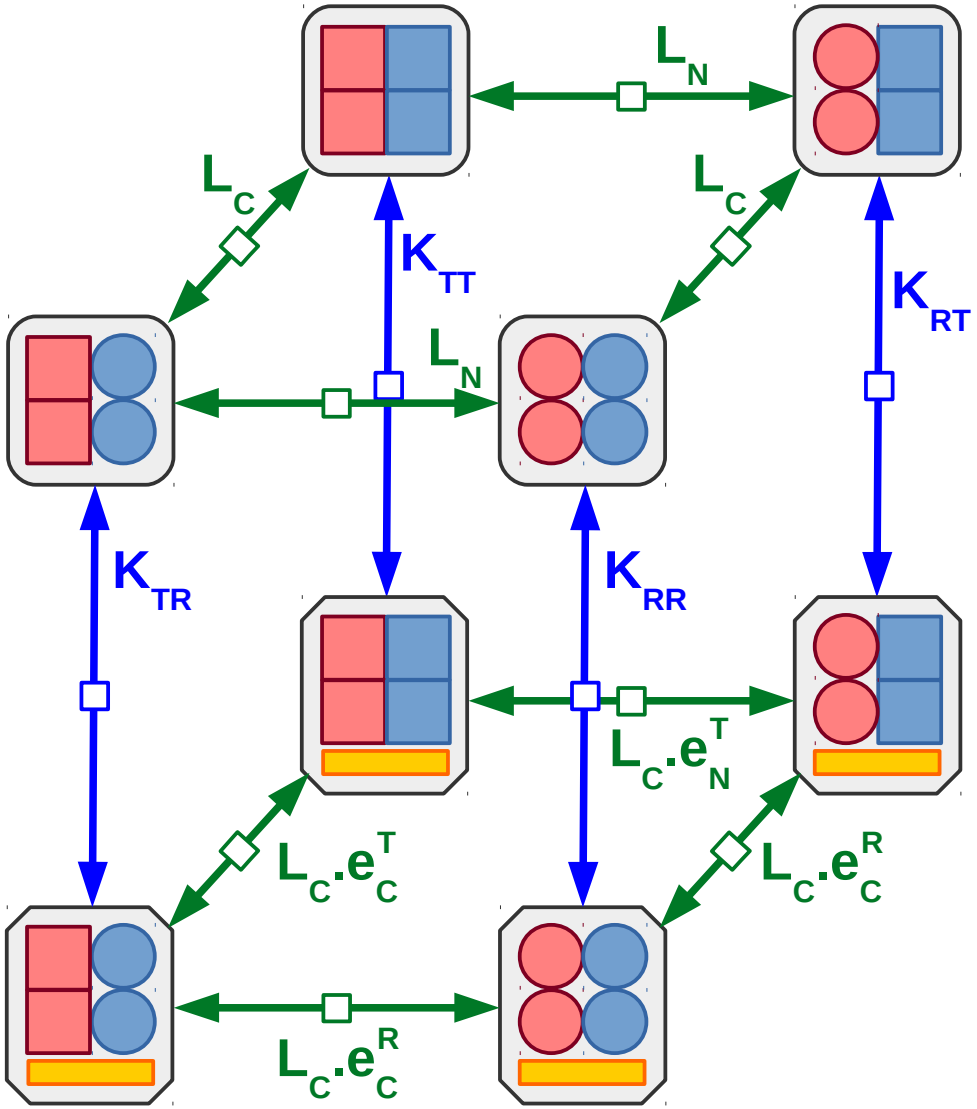
Massimo Lai

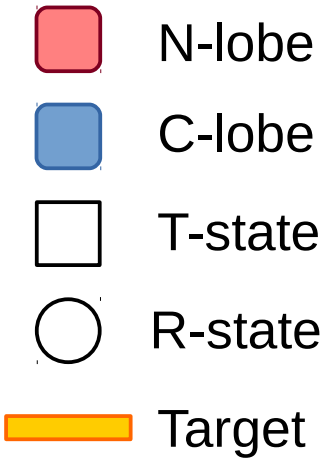
Denis Brun

COPASI

Babraham Institute

Hemiconcerted model of calmodulin



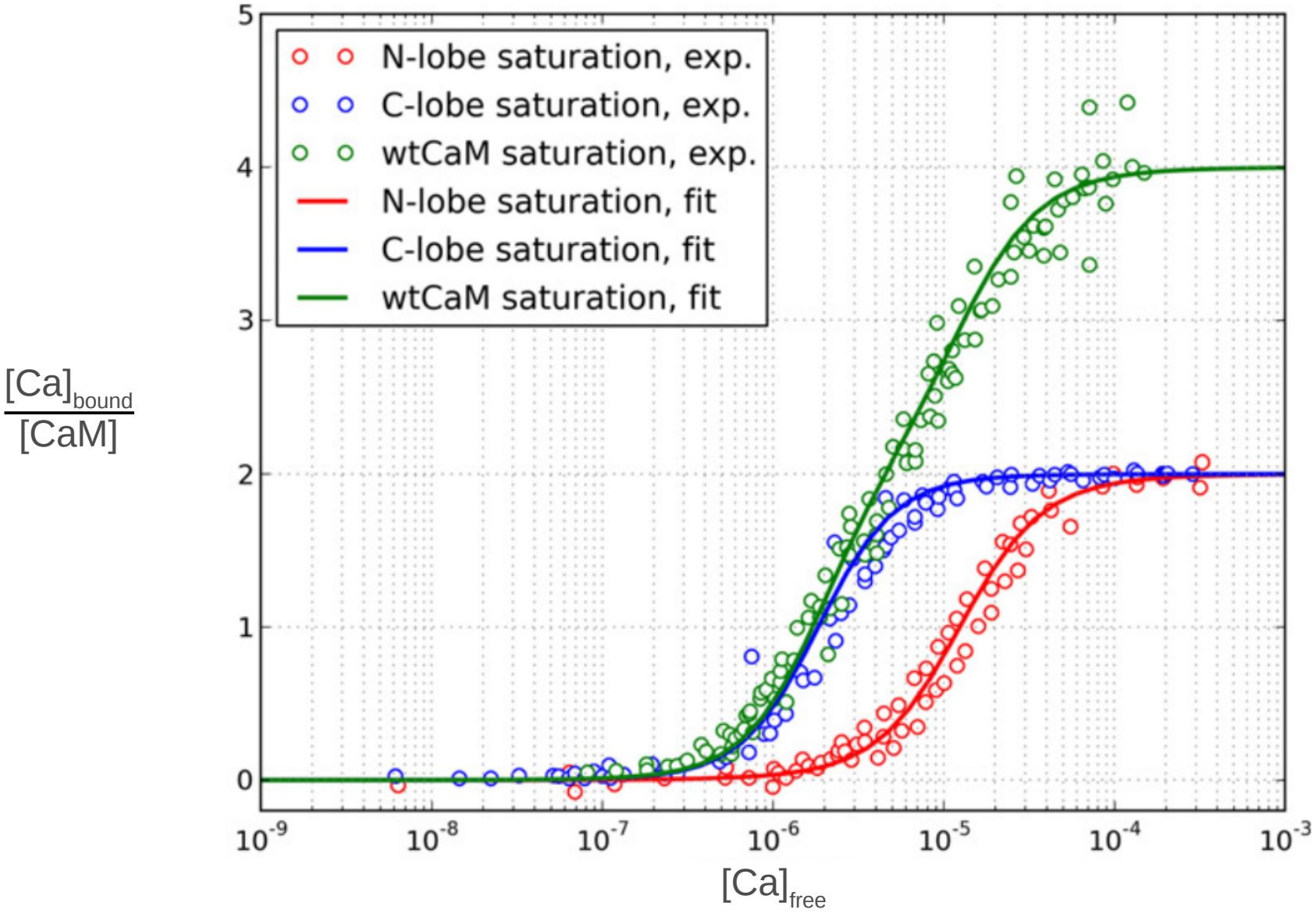


$$L_N = \frac{[TT]}{[RT]} = \frac{[TR]}{[RR]} \qquad L_C = \frac{[TT]}{[TR]} = \frac{[RT]}{[RR]}$$

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

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

Calcium binding to lobes and whole CaM



Babraham Institute

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)

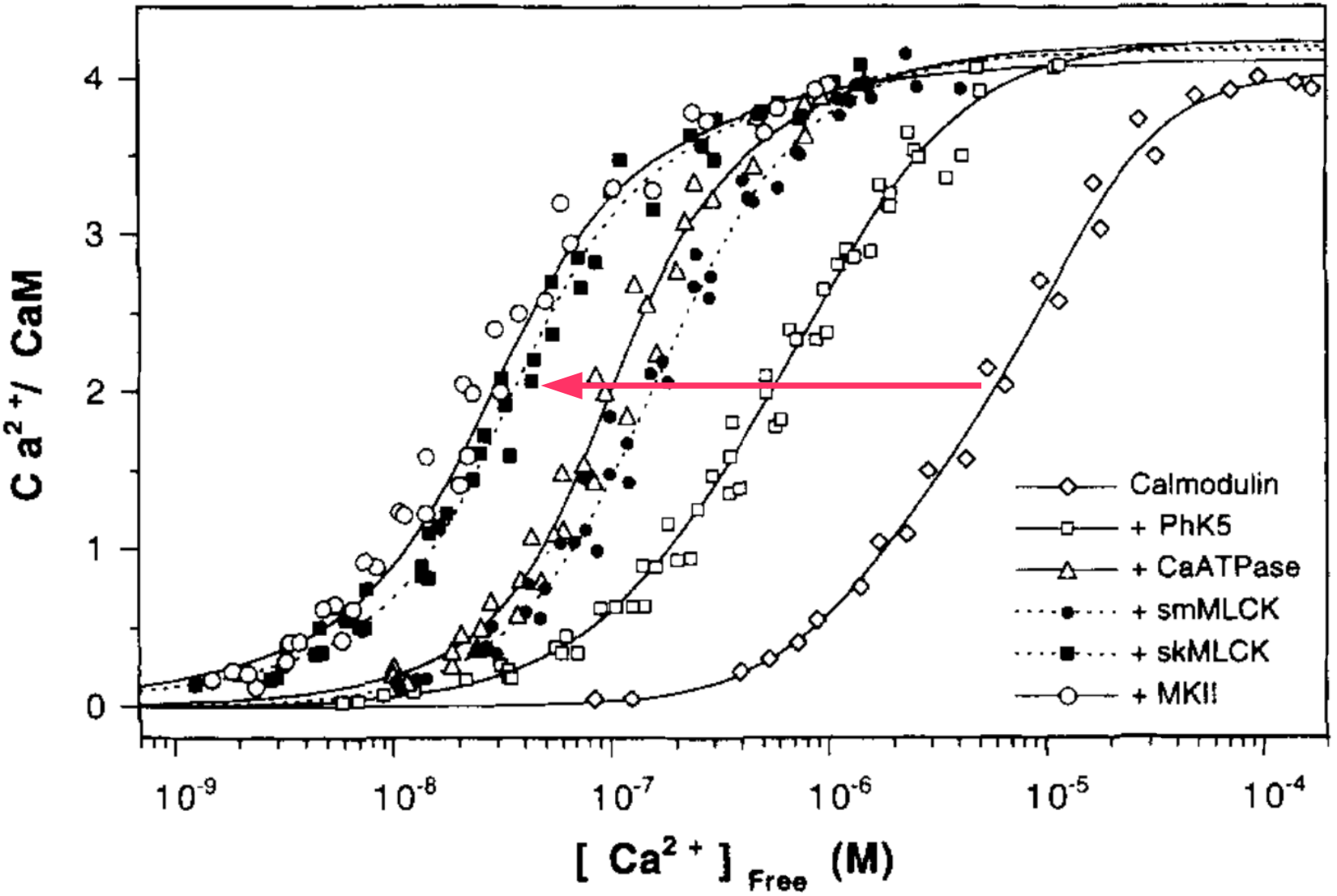
- Fractional activity depends on the number of calcium ions bound

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

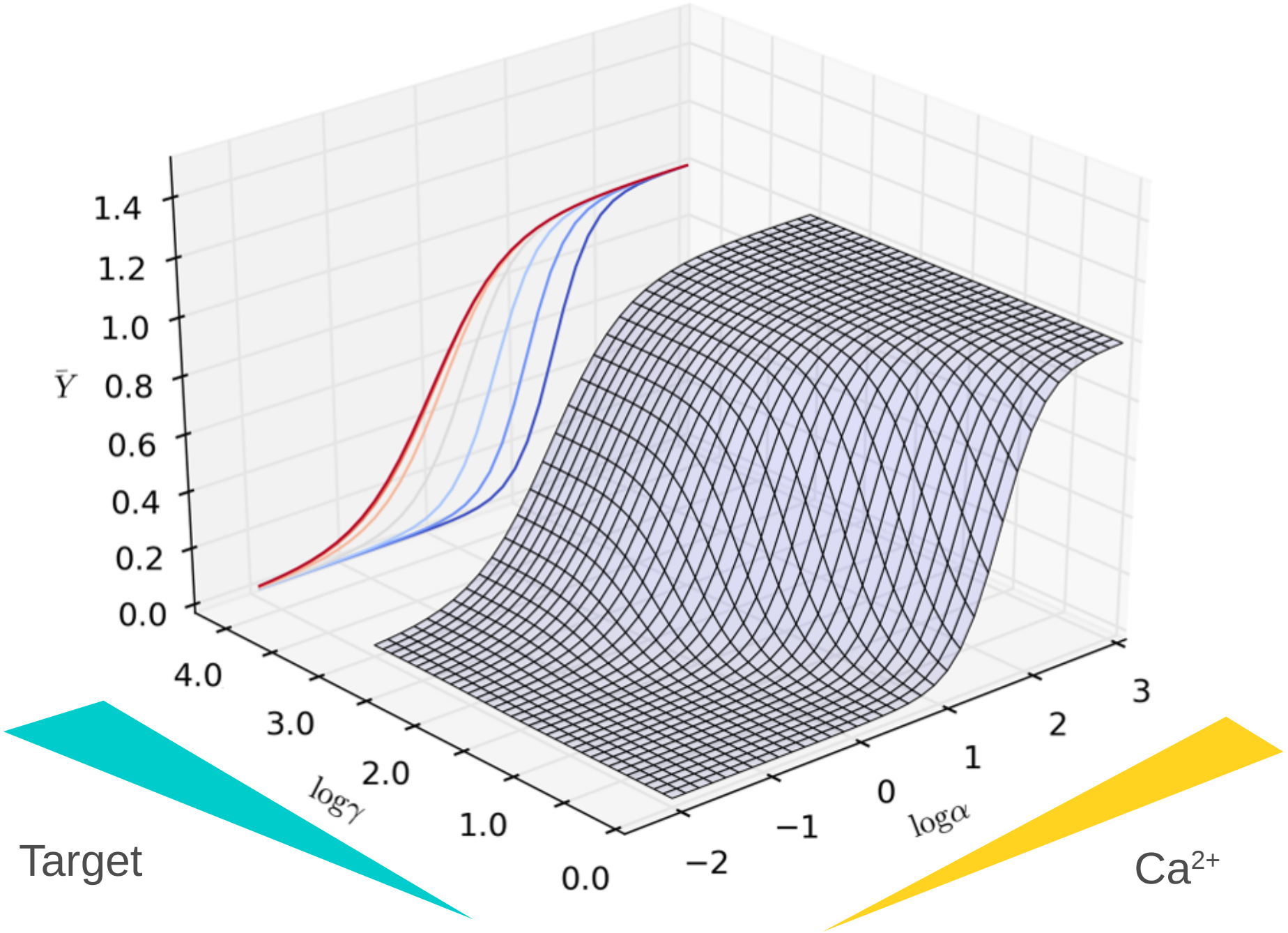
- $R_0/T_0 = 1/20000$ (1/L)
- $R_1/T_1 = 1/170$
- $R_2/T_2 = 0.69$ → half-saturation ≈ equi-probability
- $R_3/T_3 = 780$
- $R_4/T_4 = 10000$

Do we need to represent the four calcium bindings to understand Calmodulin activation?

Targets are allosteric effectors

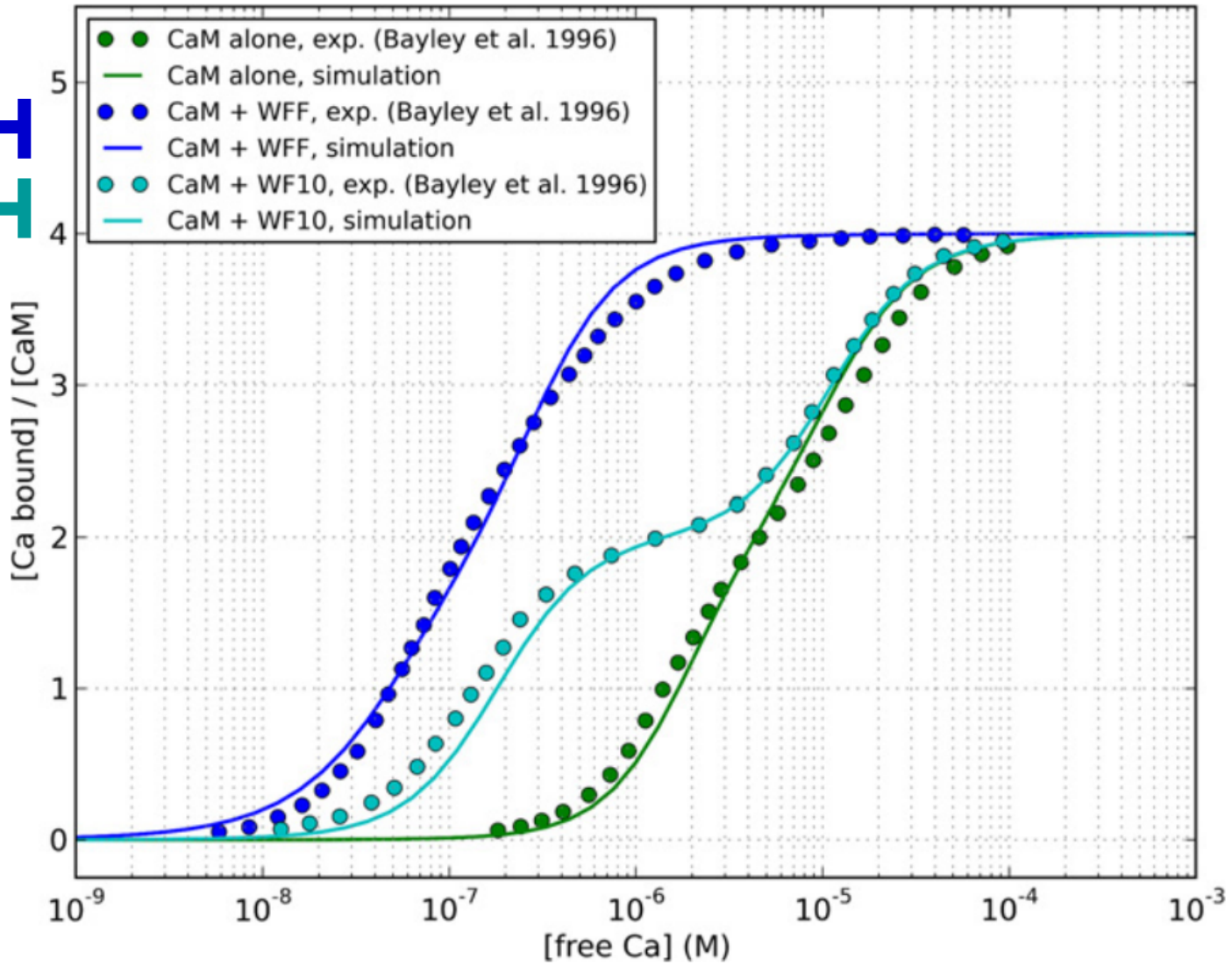


Peersen et al. (1997)


1.4
1.2
1.0
$\bar{Y}$ 0.8
0.6
0.4
0.2
0.0
4.0
3.0
2.0
1.0
0.0
$\log\gamma$
−2
−1
0
1
2
3
$\log\alpha$
Target
Ca^{2+}

Stabilise R state of both lobes

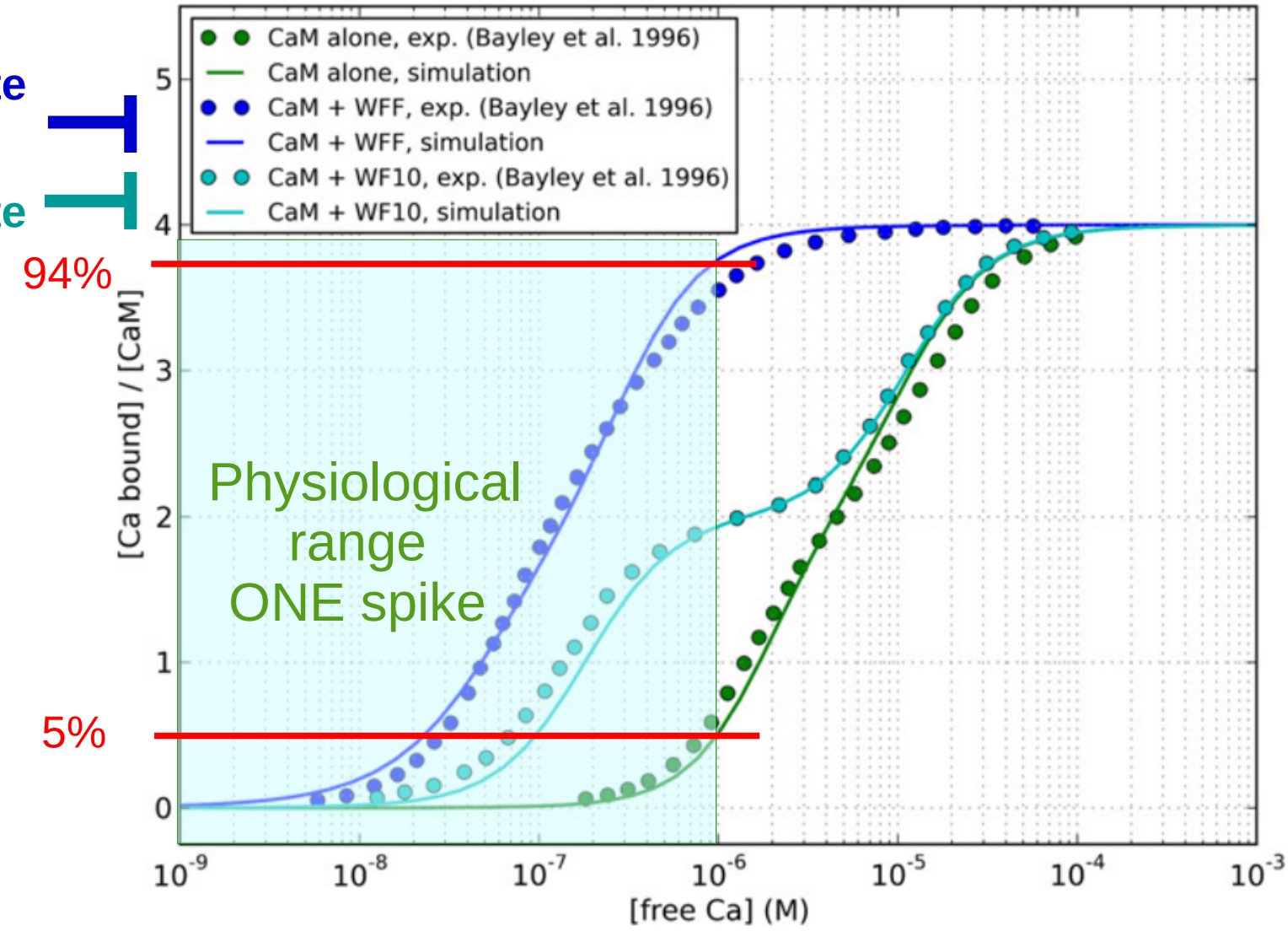
Stabilise R state of C lobe



Targets move Ca^{2+} binding into physiological range

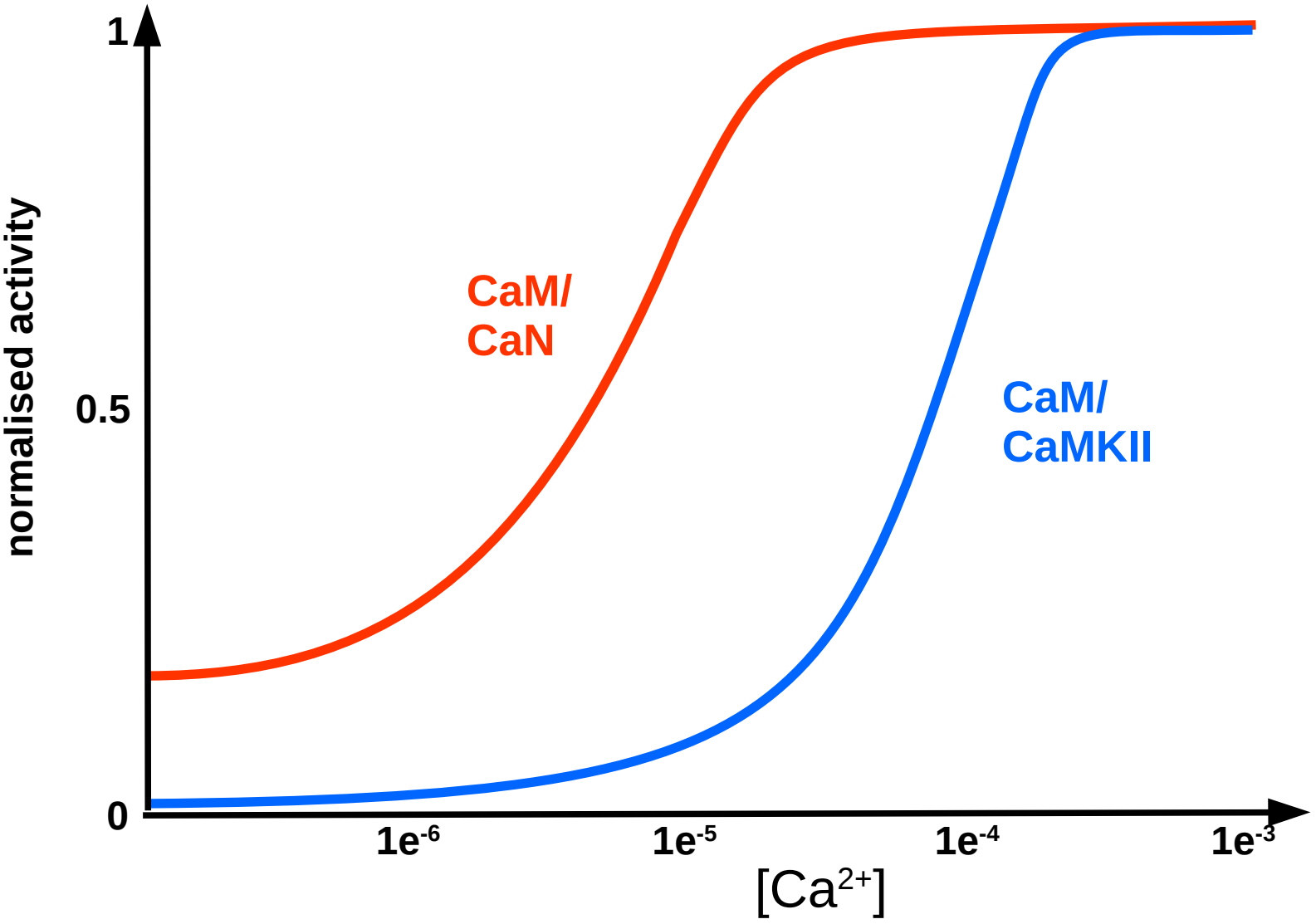
Stabilise R state of both lobes

Stabilise R state of C lobe

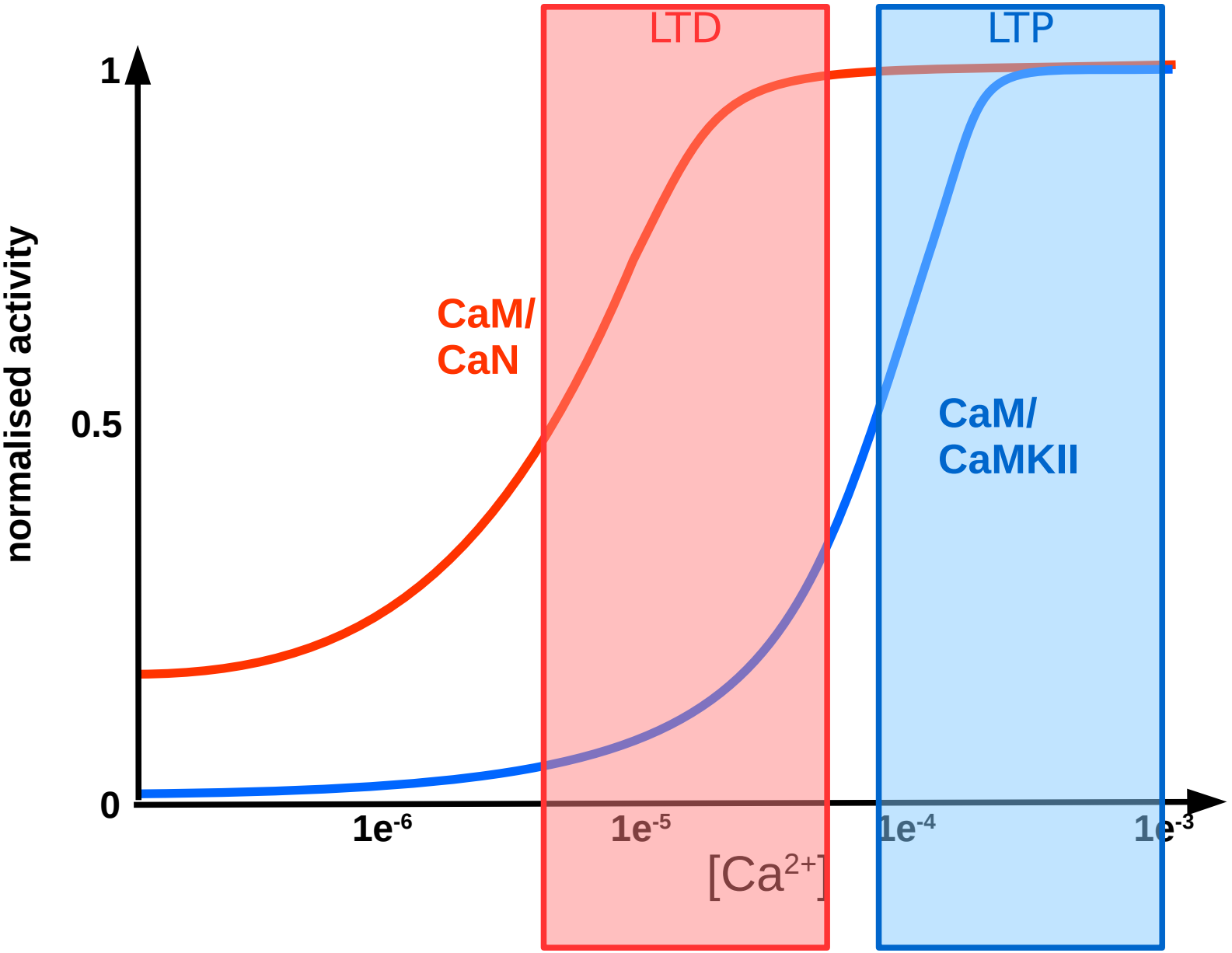


Babraham Institute

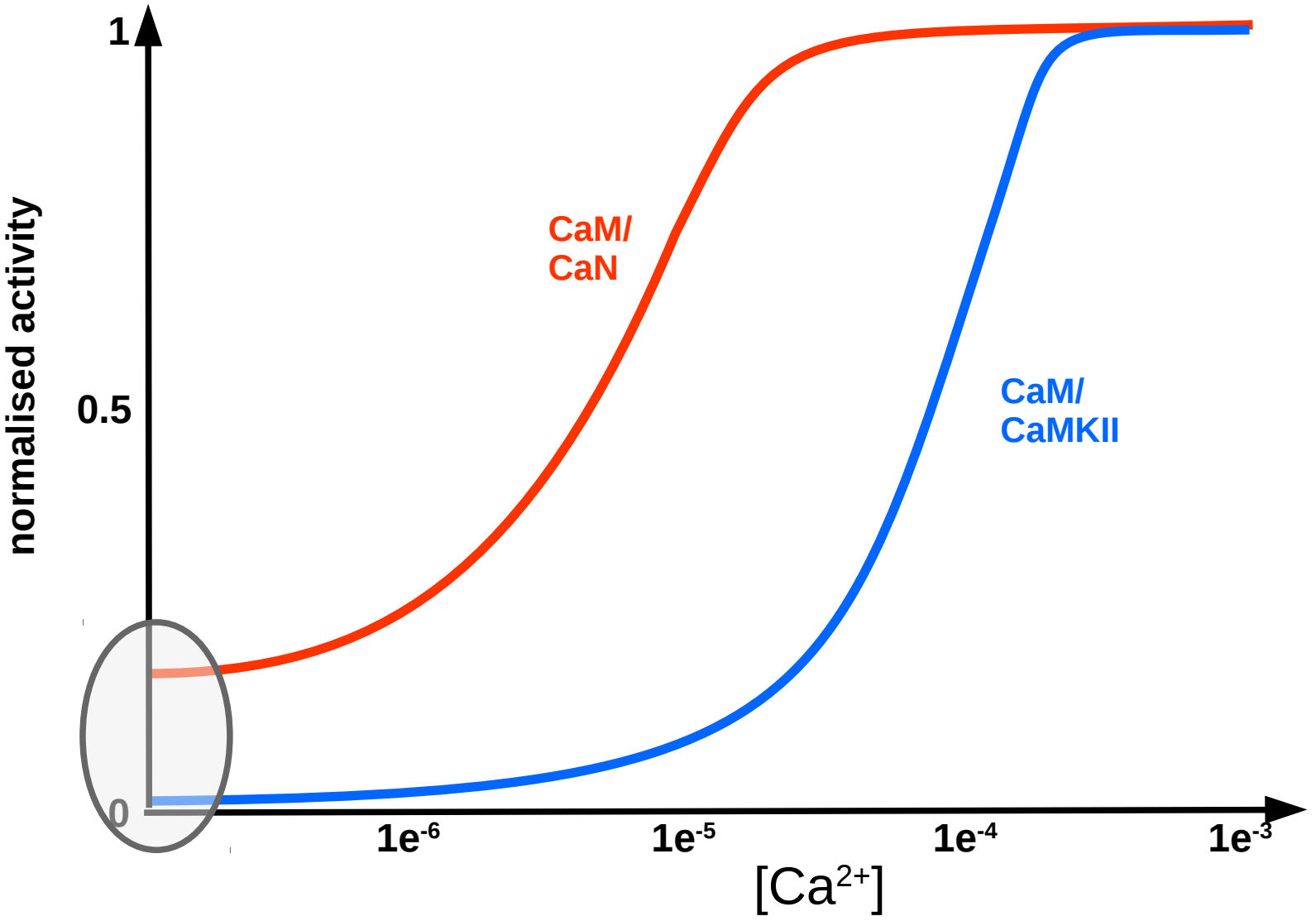
Calmodulin, its ligand and its targets



Bidirectional synaptic plasticity

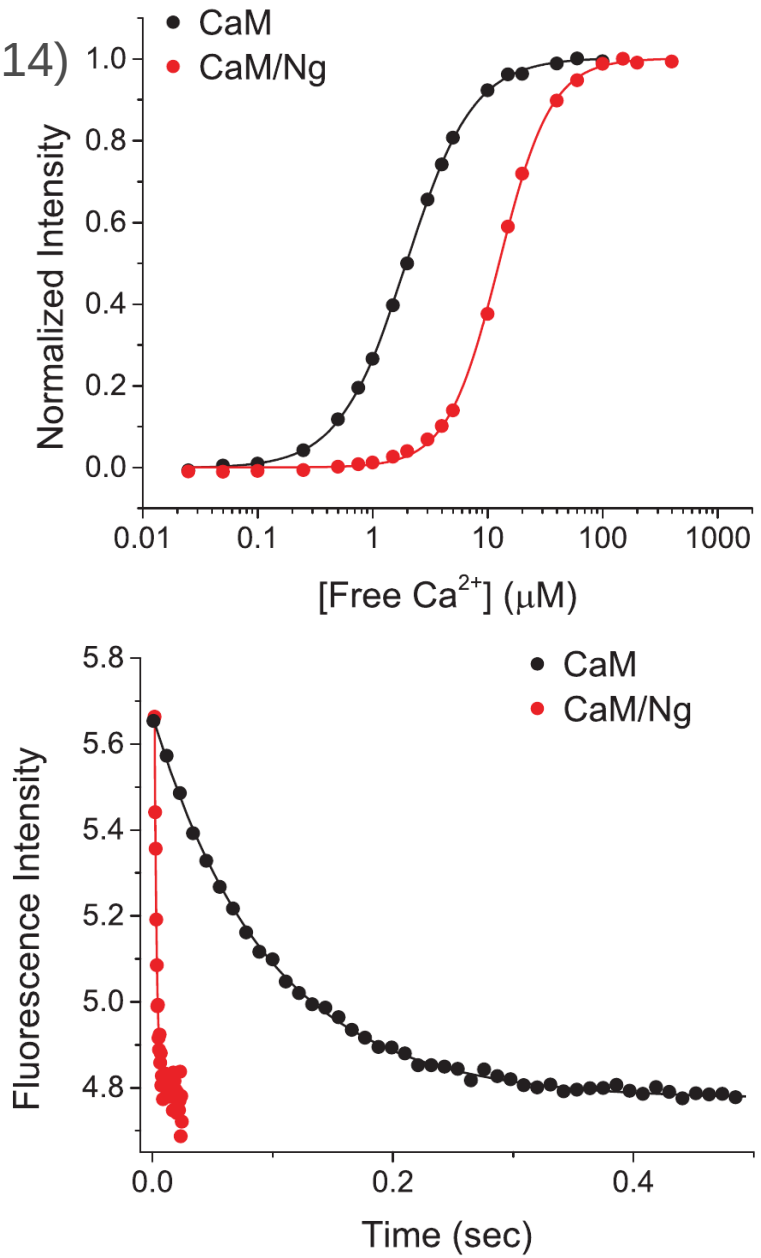


Calcineurin stabilises CaM R → no deactivation

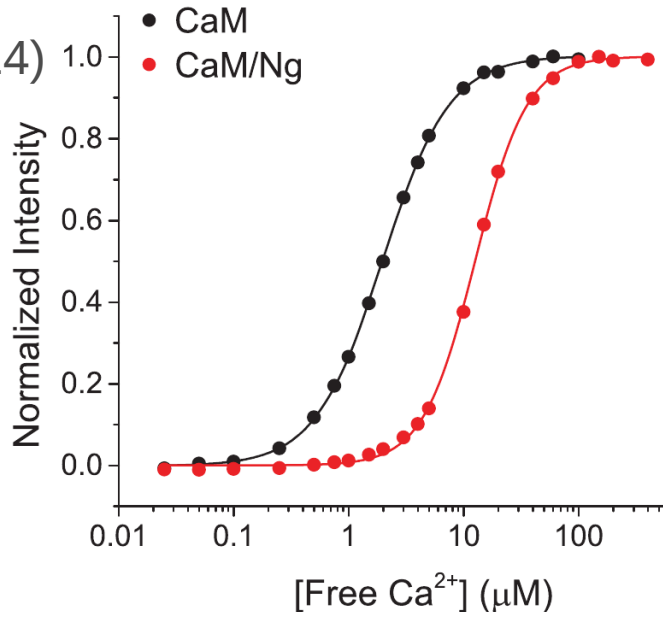


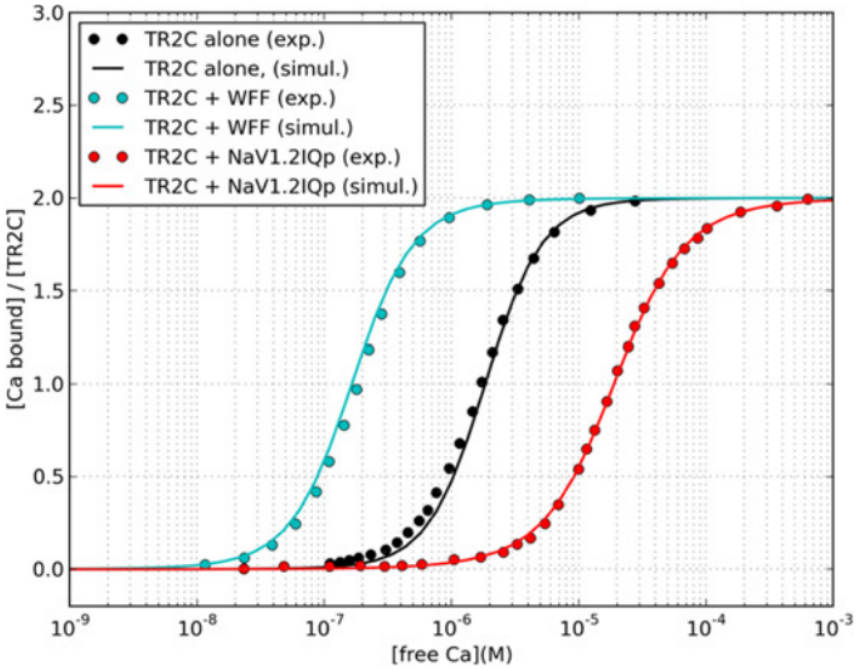
Neurogranin affects Ca^{2+} binding to CaM

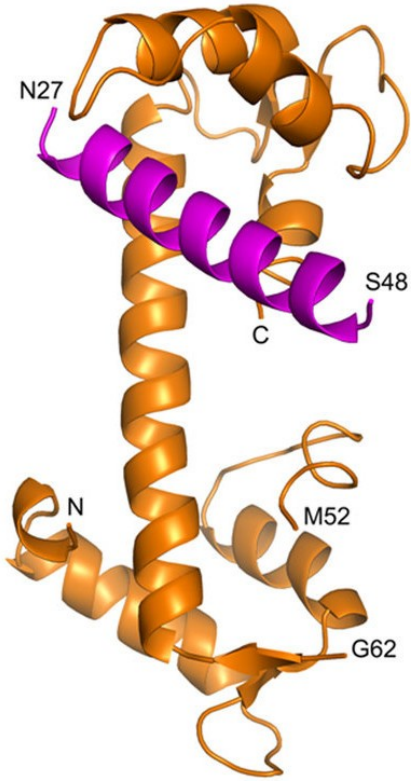
Hoffman *et al* (2014)



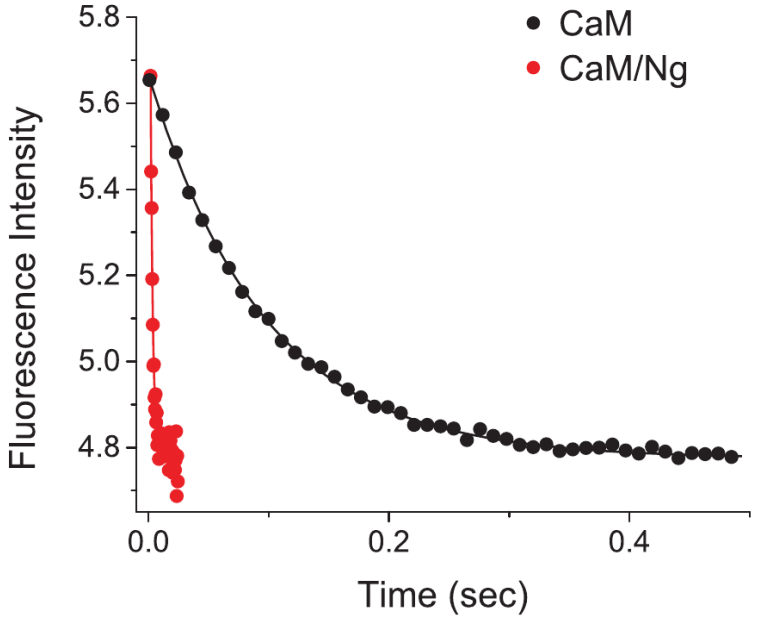
Hoffman *et al* (2014)

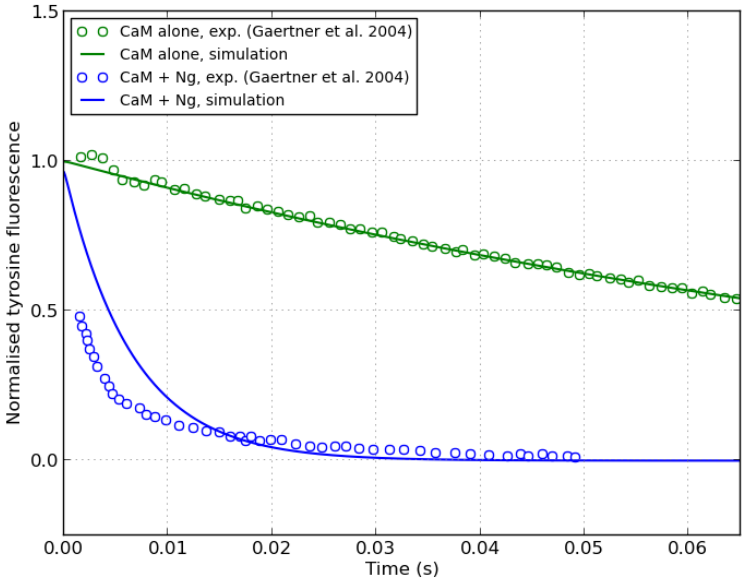




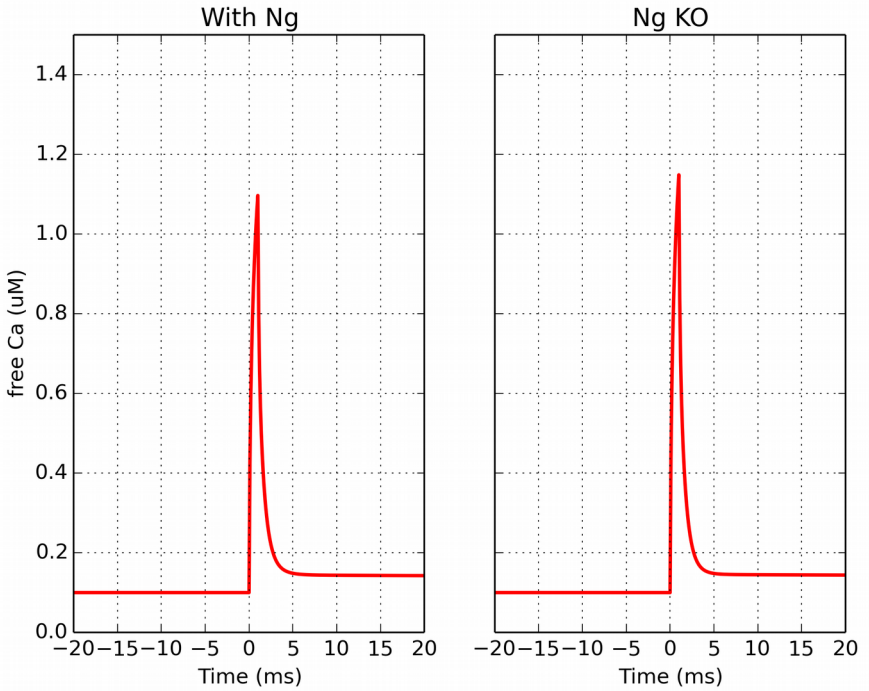


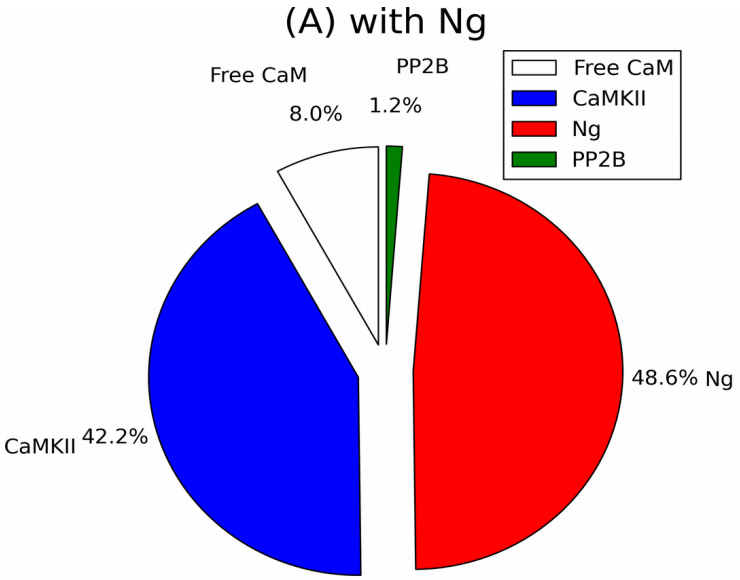
Kumar *et al* (2013)

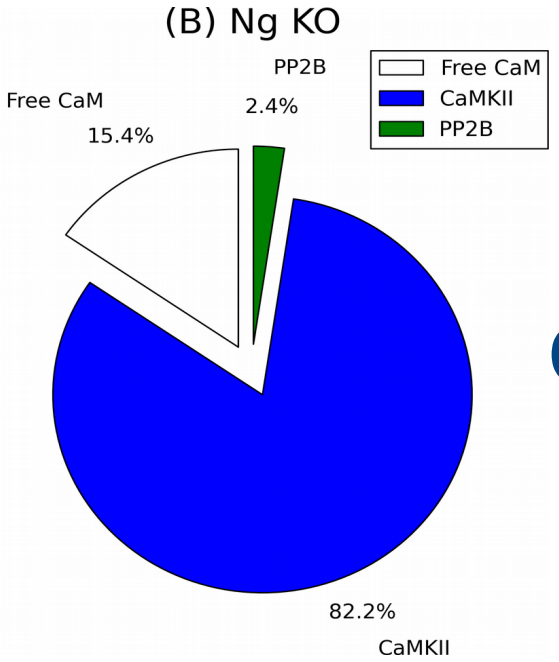




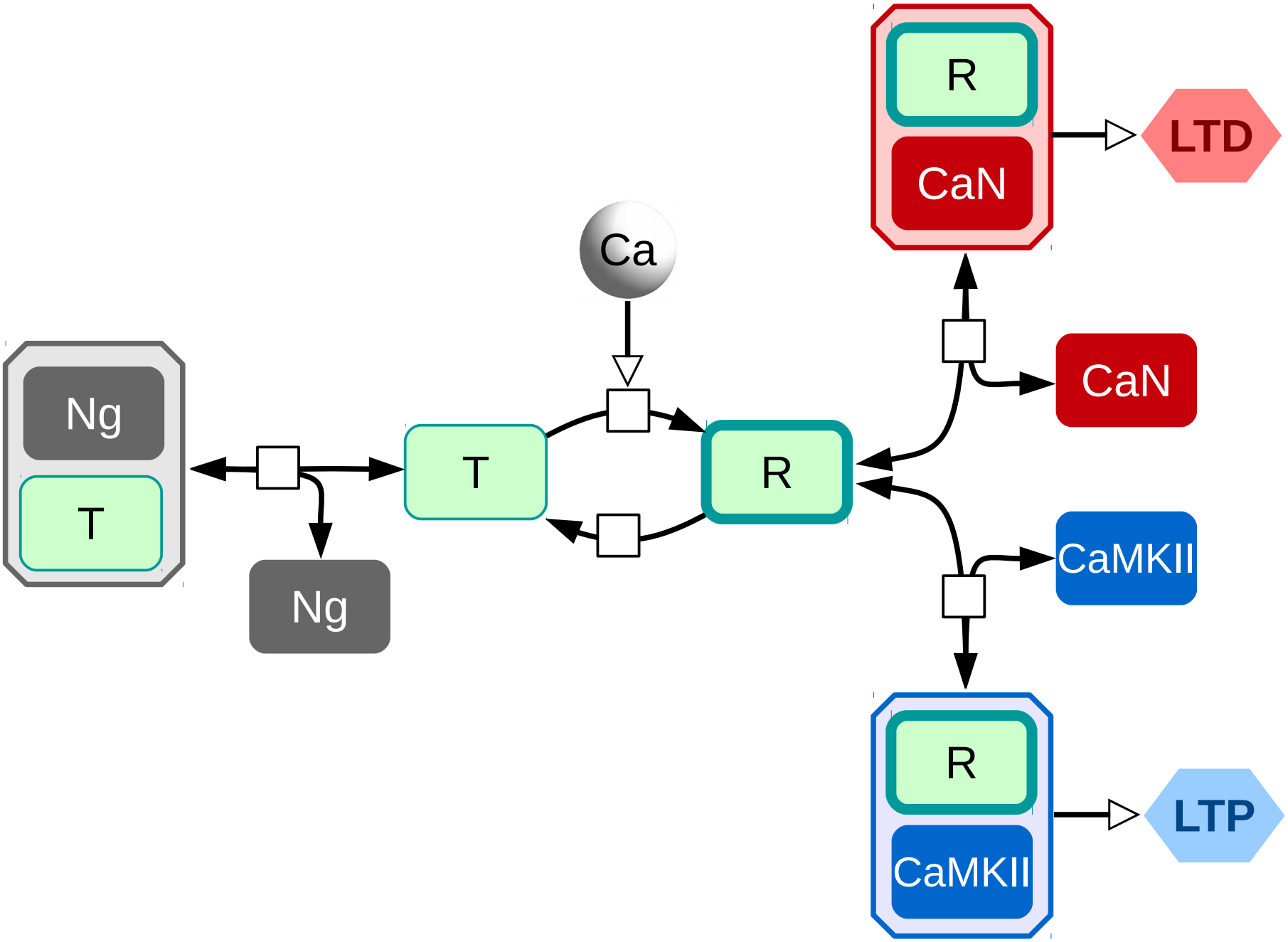
No large effect of Ng on [Ca^{2+}free]







Ng affects CaM distribution

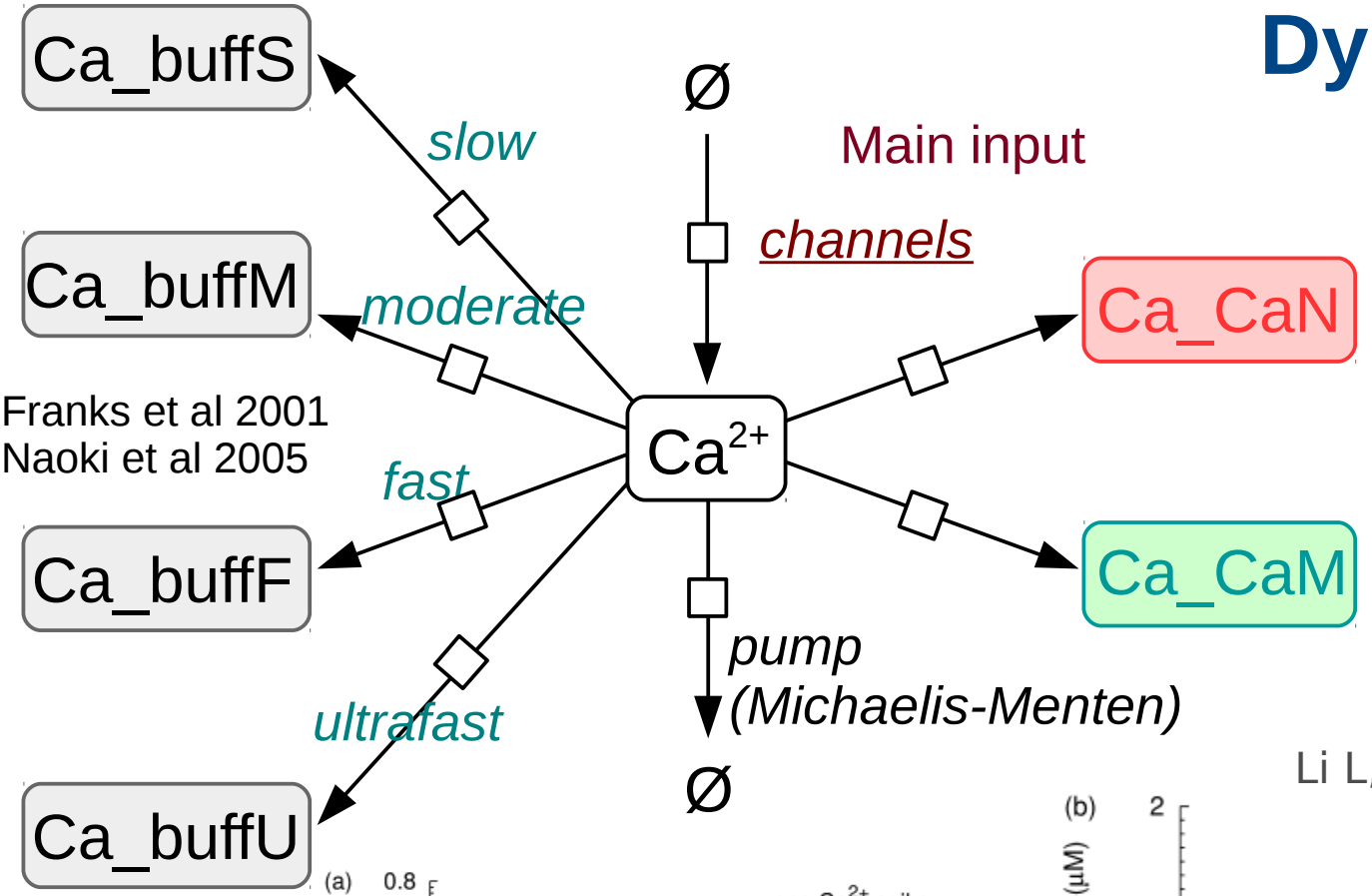

R
CaN
LTD
Ca
Ng
T
CaN
T
R
Ng
CaMKII
R
CaMKII
LTP

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

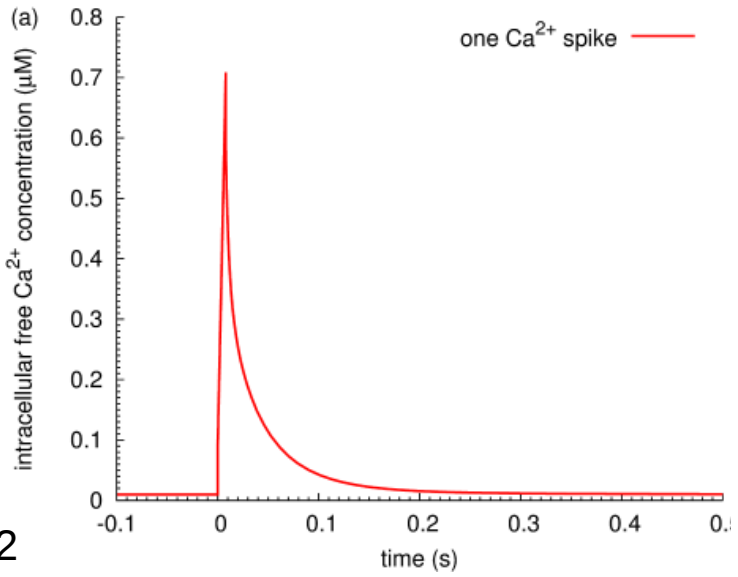


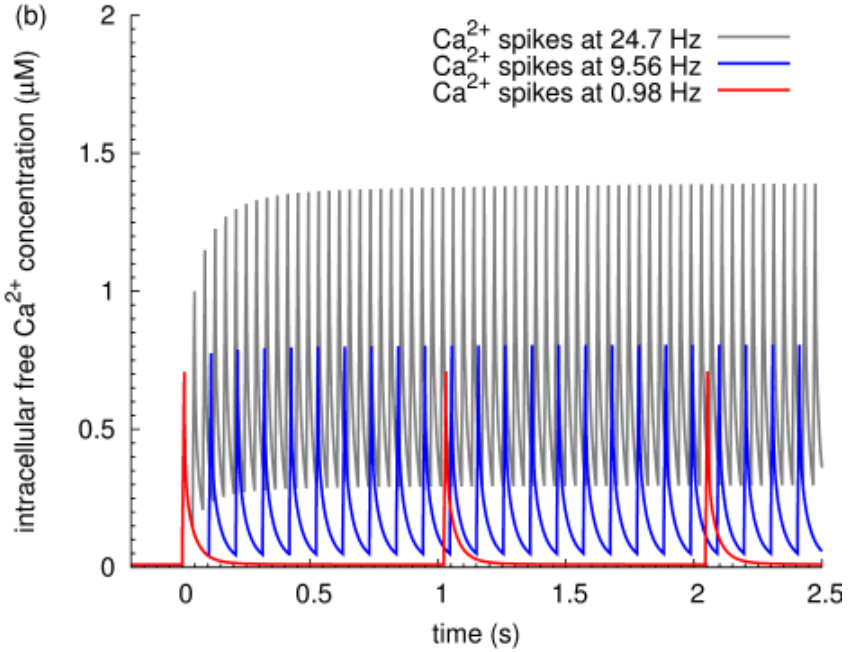
Franks et al 2001
Naoki et al 2005

Lu Li

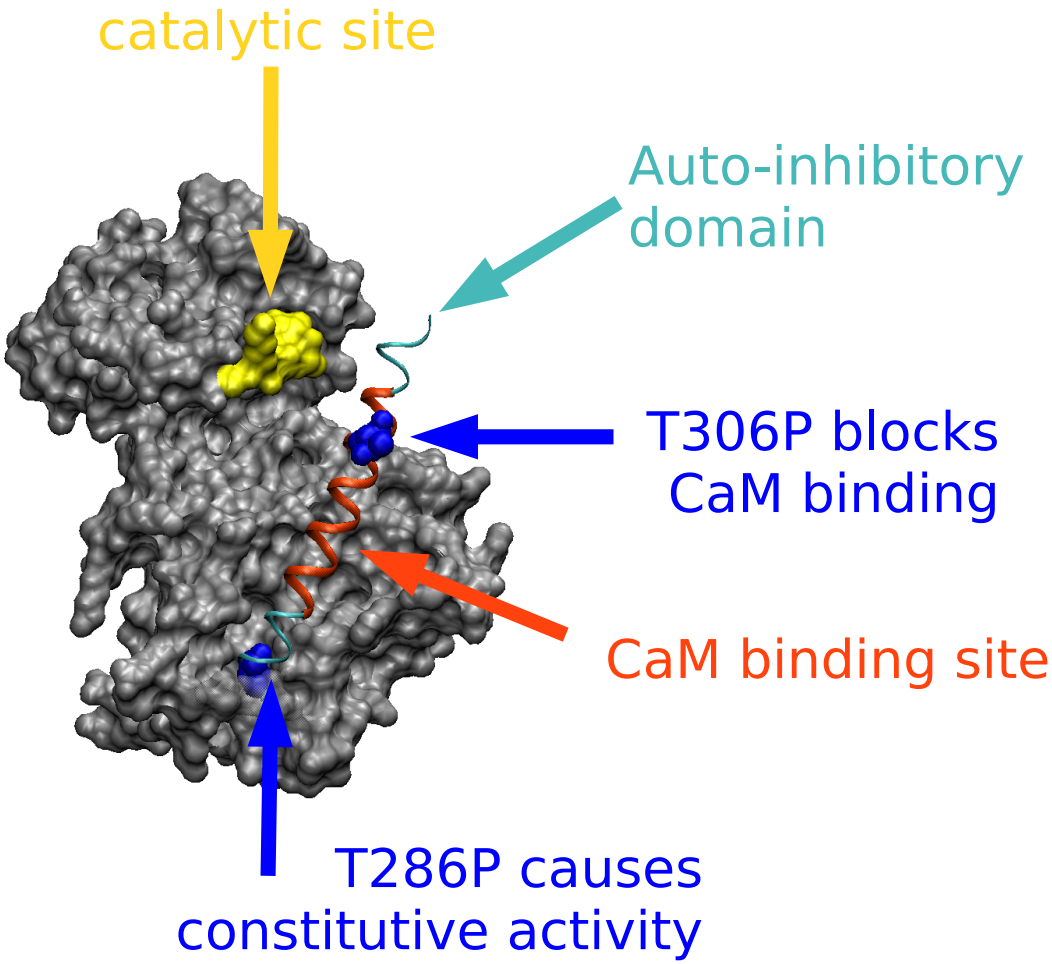
Li L, Stefan MI, Le Novère N (2012)

Sabatini et al 2002





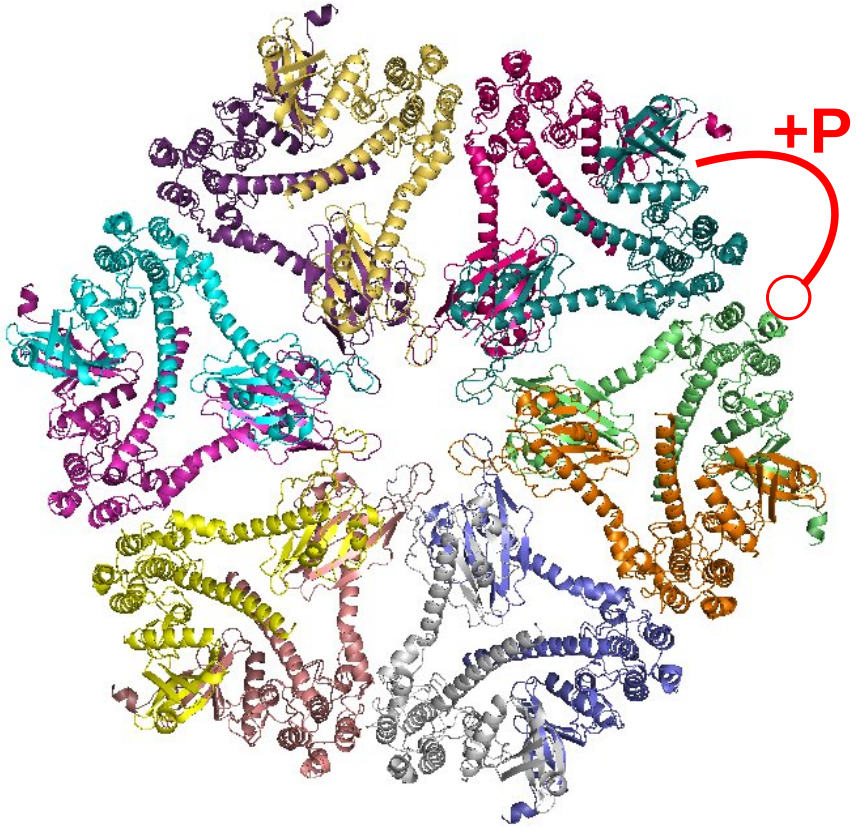
Calcium/calmodulin kinase II

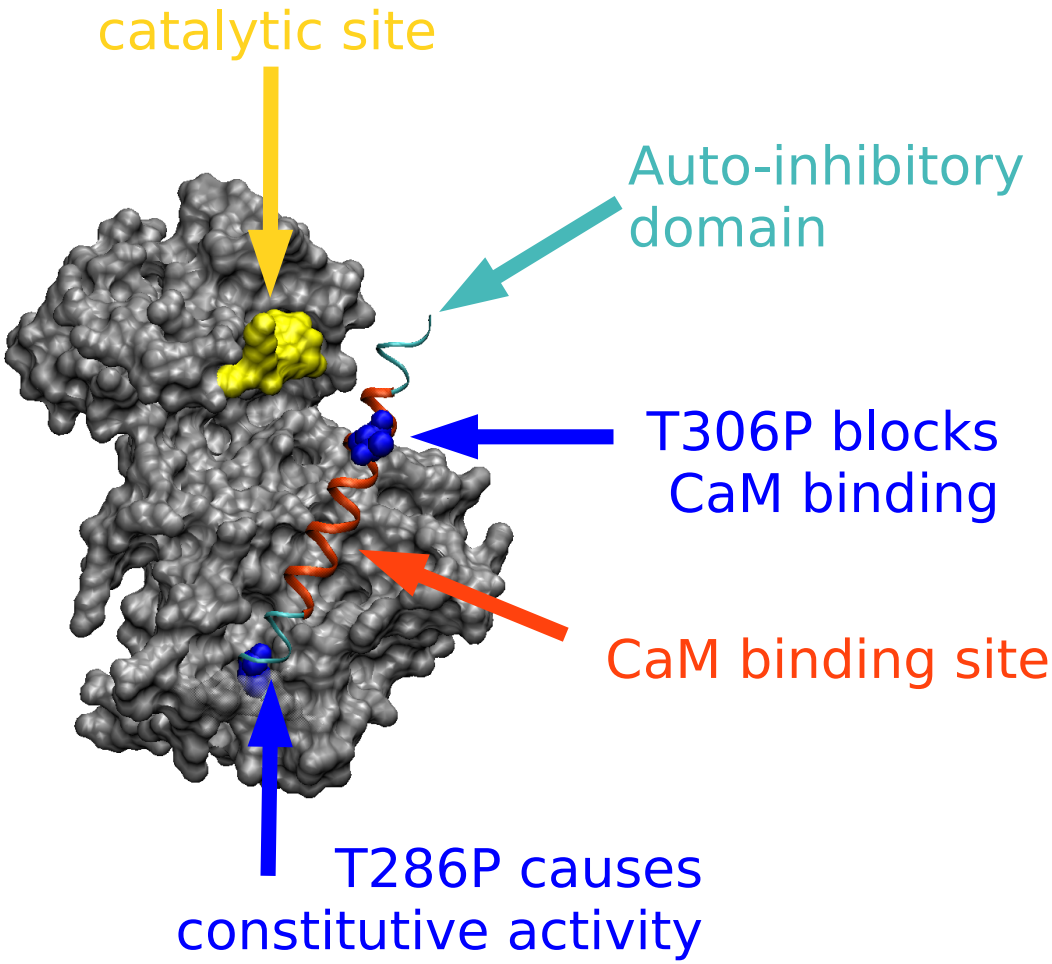


Calmodulin trapping is an apparent increase of affinity of CaMKII for CaM when T286 is phosphorylated

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

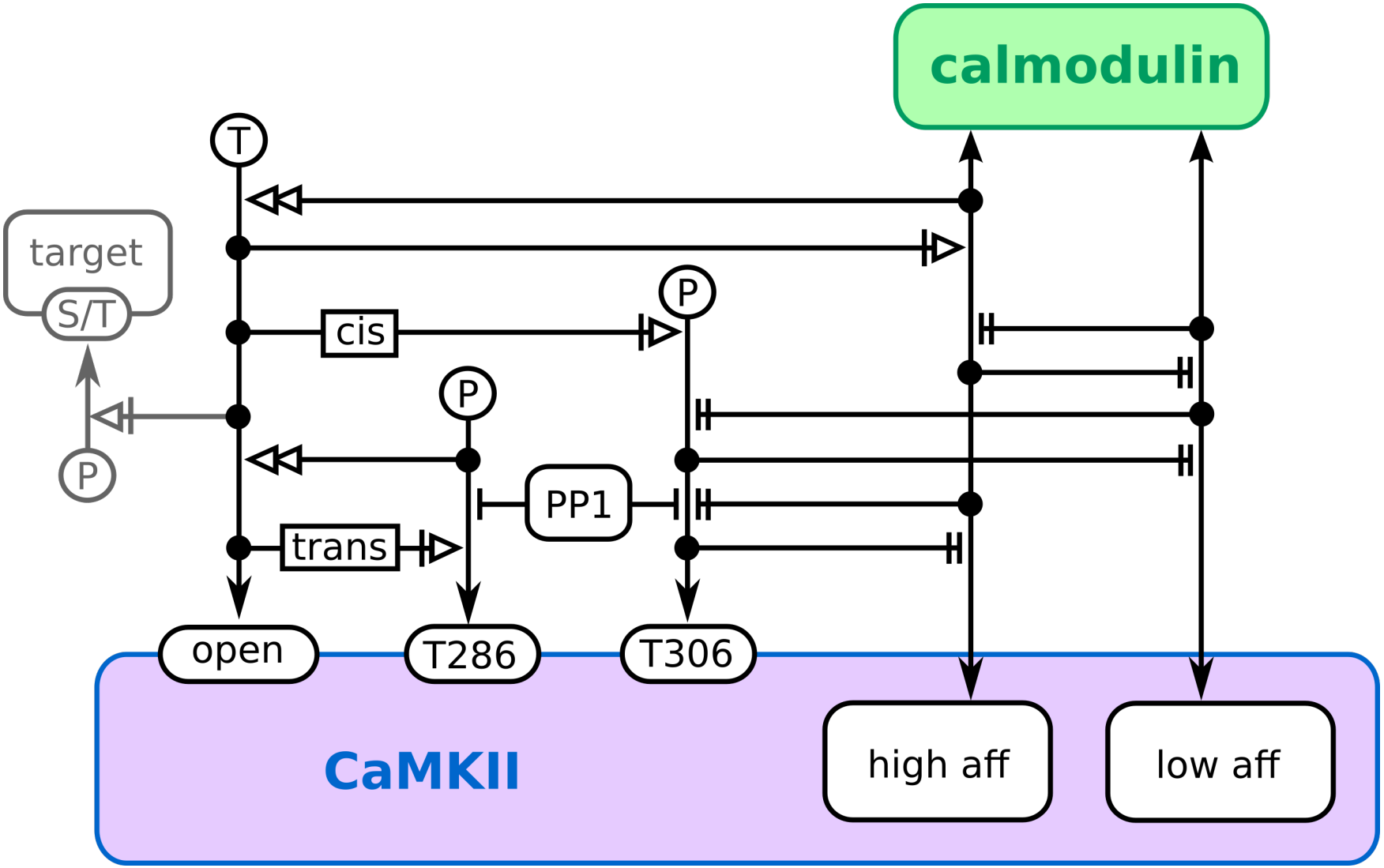
Calcium/calmodulin kinase II





Dodecamer:
Trans-phosphorylation of T286 by neighbouring subunits
Cis-phosphorylation of T306
Billions of billions of reactions
Plus, most quantitative measurements made on monomers ...

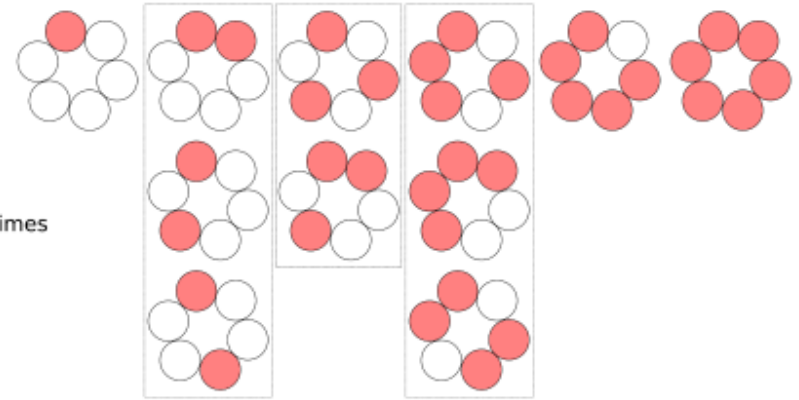
SBGN ER and rule-based modelling



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

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.

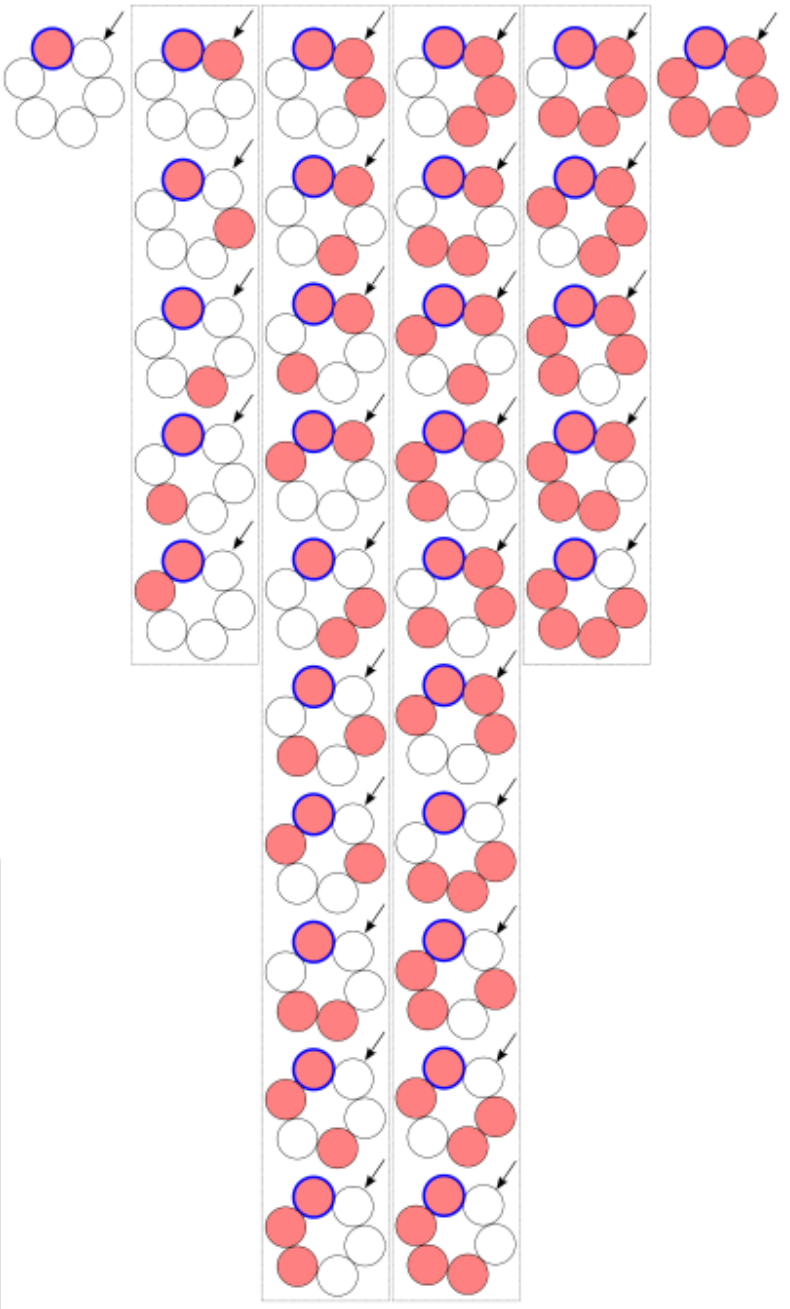
Repeat for every 1% increase of CaMKII active monomers

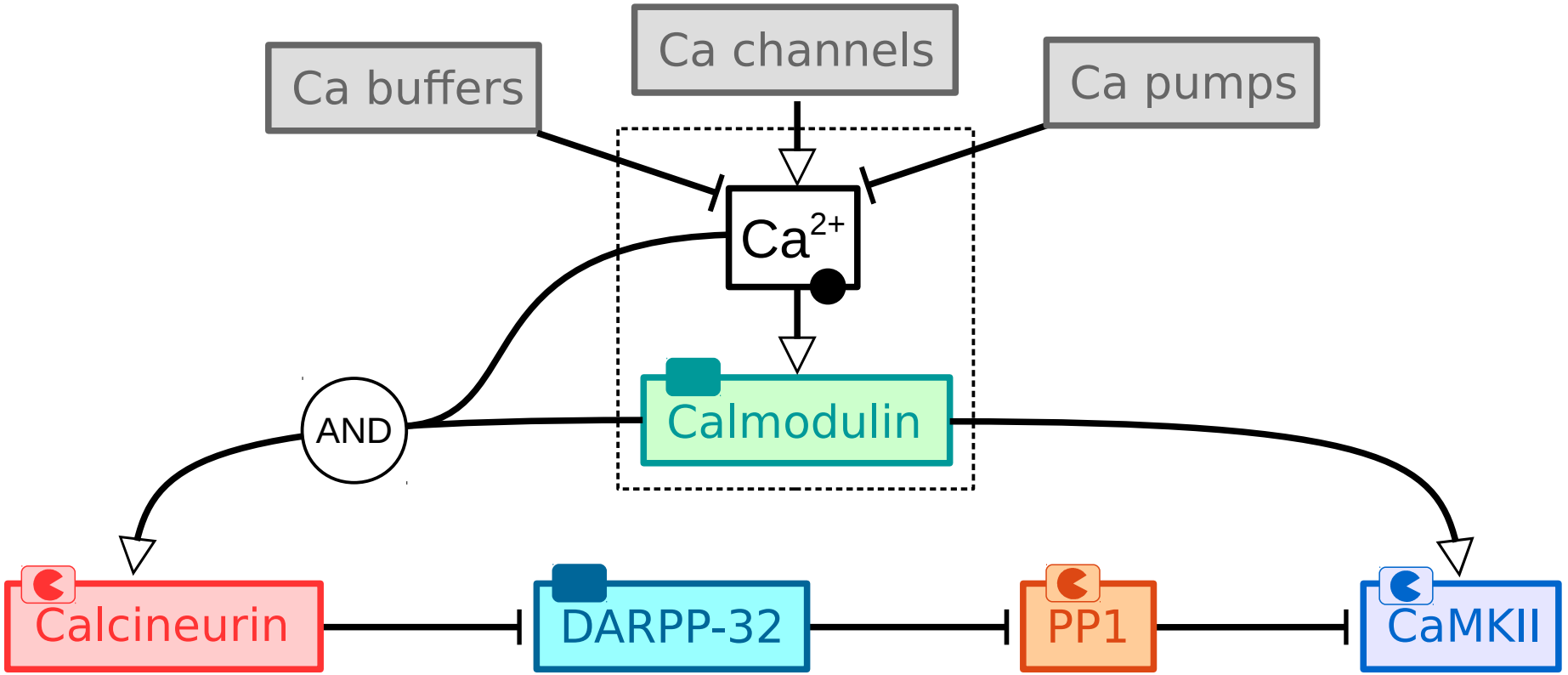
Multiply average populations of each number of active monomers per hexamer by their corresponding probabilities of having an active neighbour.

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.

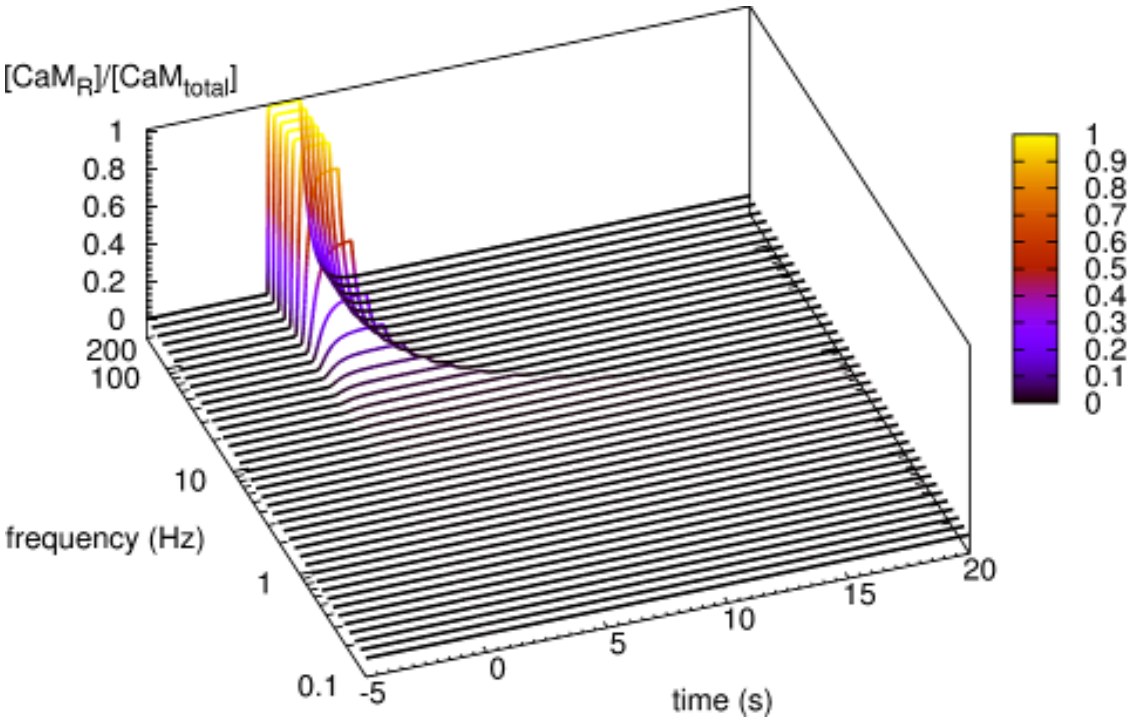
Calculate the probabilities of having an active neighbour on one specific side (indicated by the arrow) of the activated monomer of interest (blue outline) (since only the asymmetrical situation is considered). The possible positions of activated monomers are listed as the following, with corresponding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





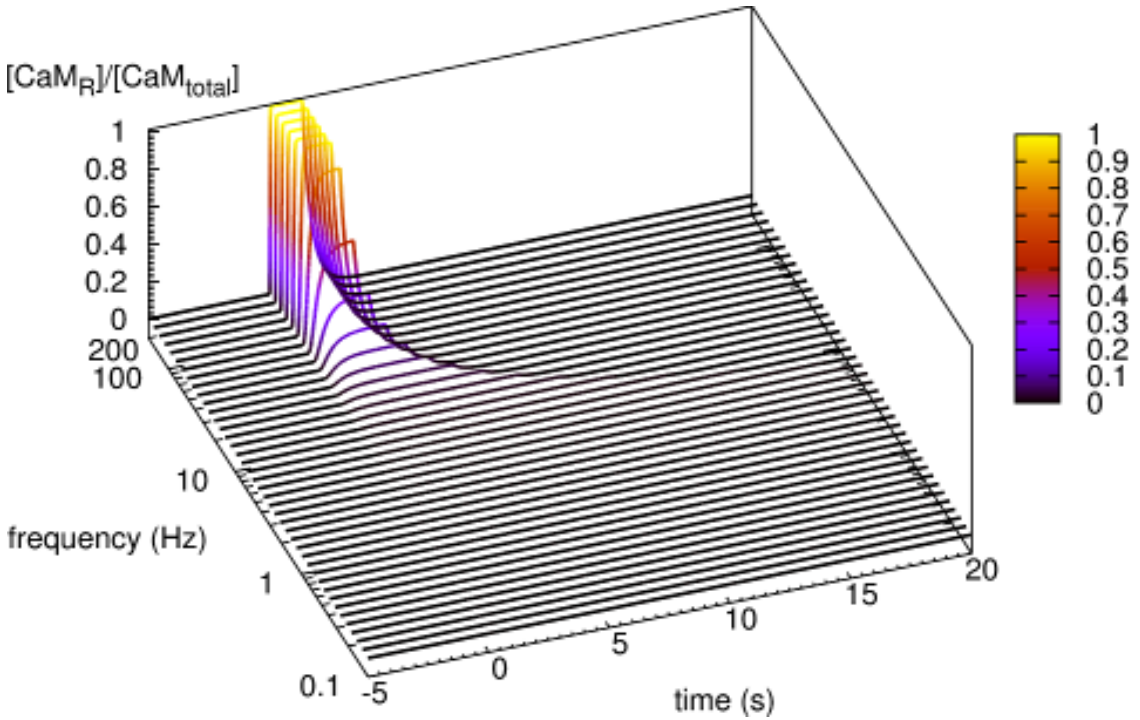
342 “molecular species”, representing 7 actual molecular entities
1295 reactions
184 mathematical rules
7 conditional discrete events

Calmodulin activation

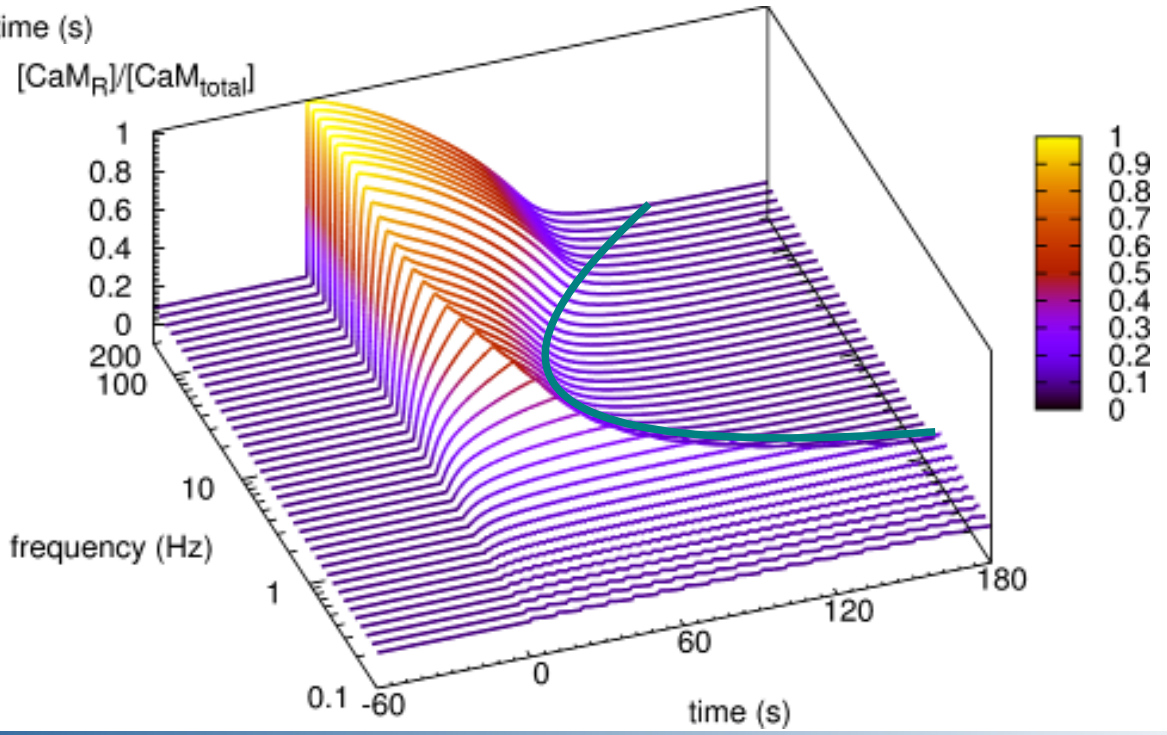


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)

Calmodulin activation

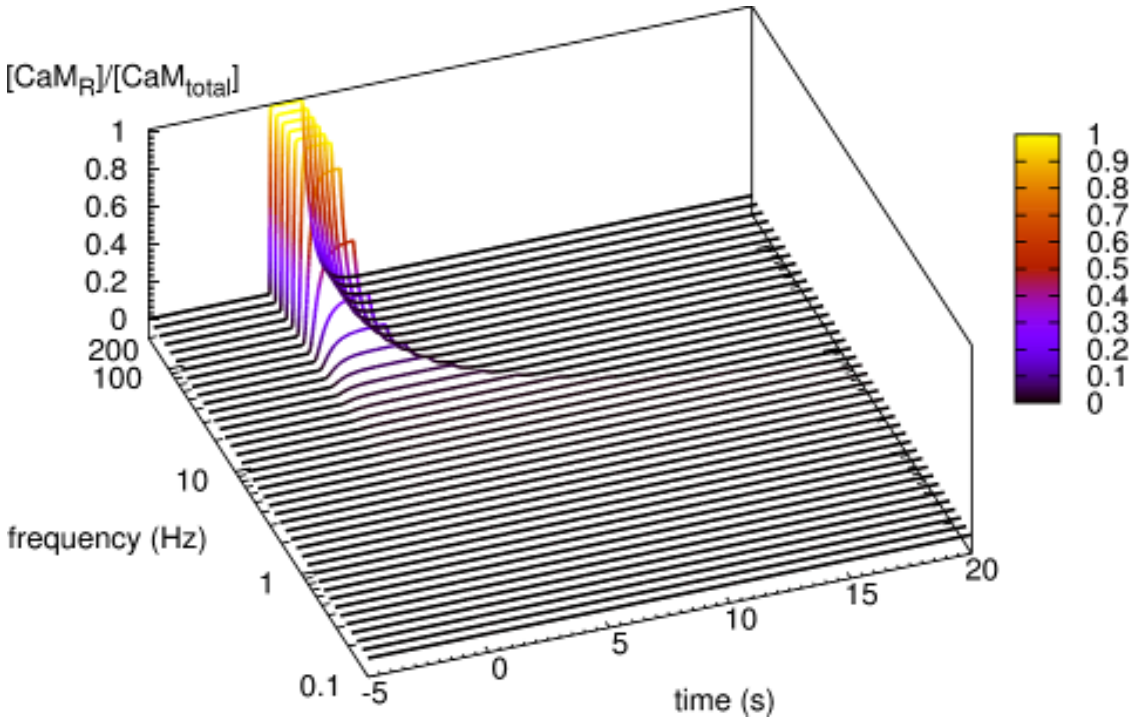


CaM without targets. Low frequencies do not activate calmodulin (binding events without conformational changes)



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

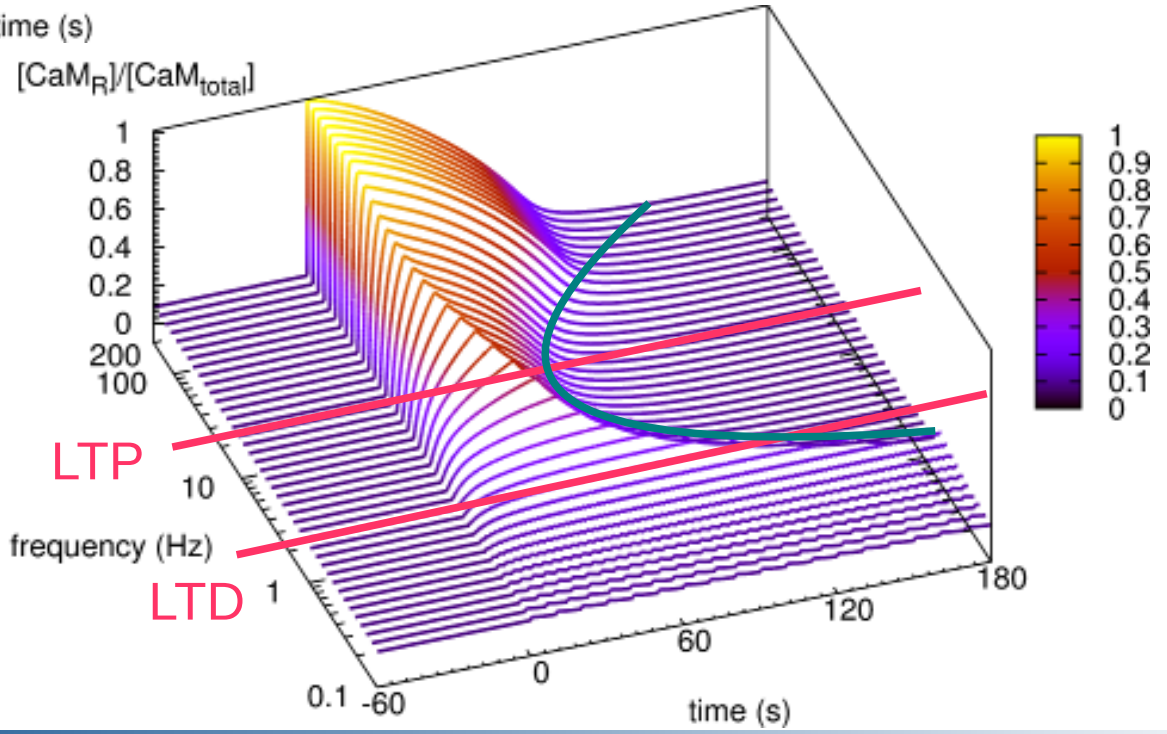
Calmodulin activation



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

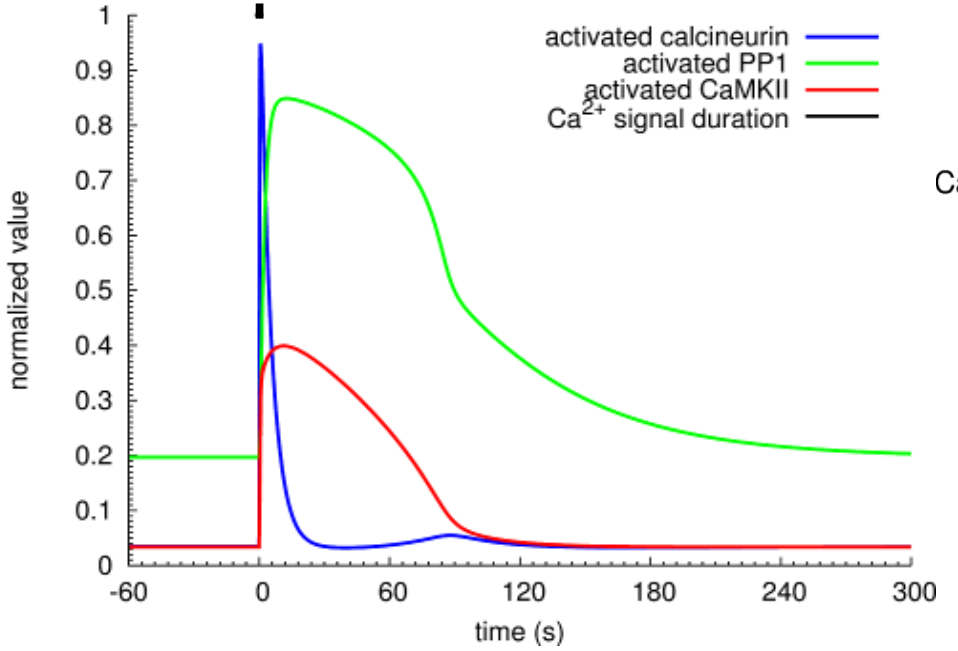
At high frequency, effects of calcium signals last much longer than the signal itself

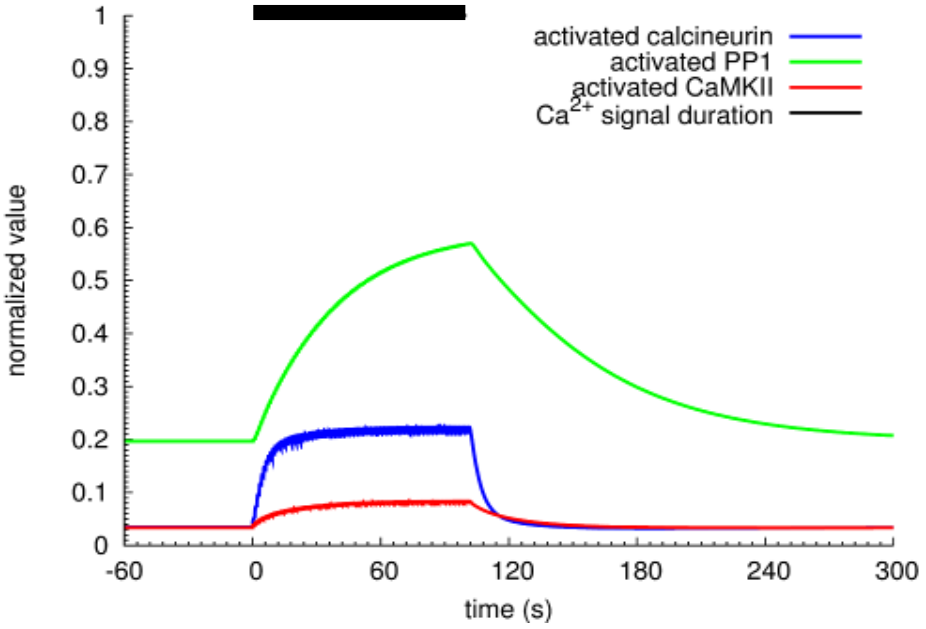
CaM with targets. Binding to CaN and CaMKII stabilises R state, with higher affinity. Positive feedback loop
➜ bistability

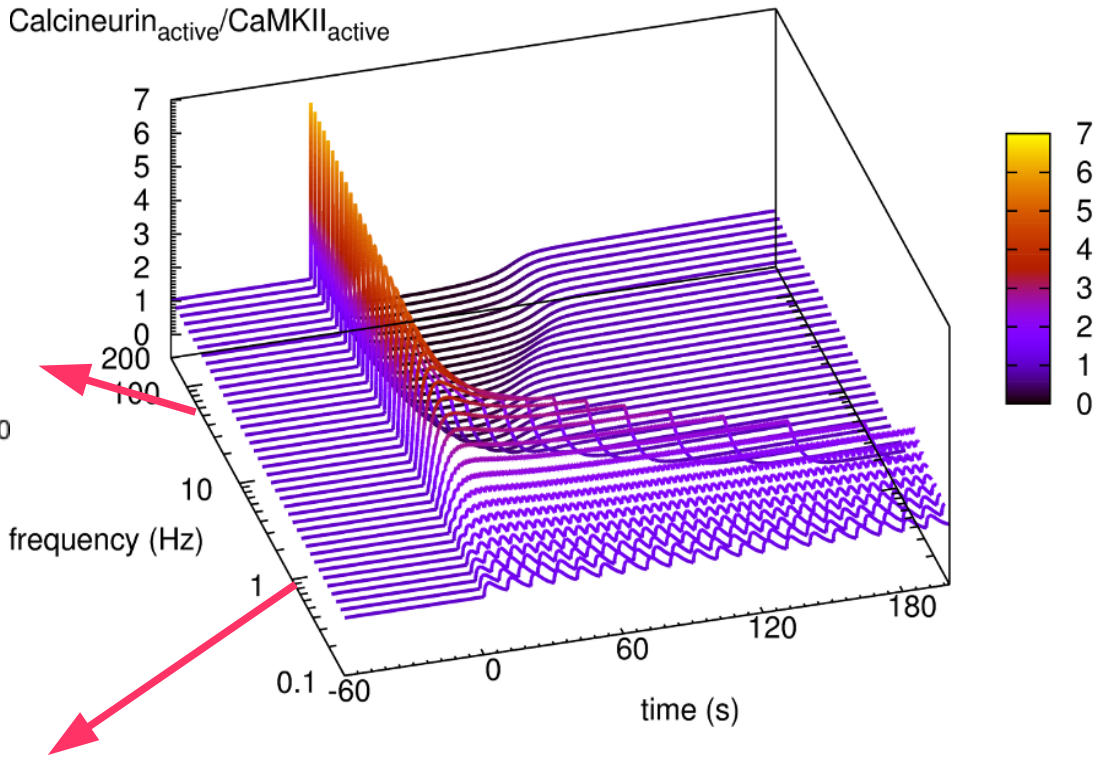


Babraham Institute

CaMKII and CaN activation

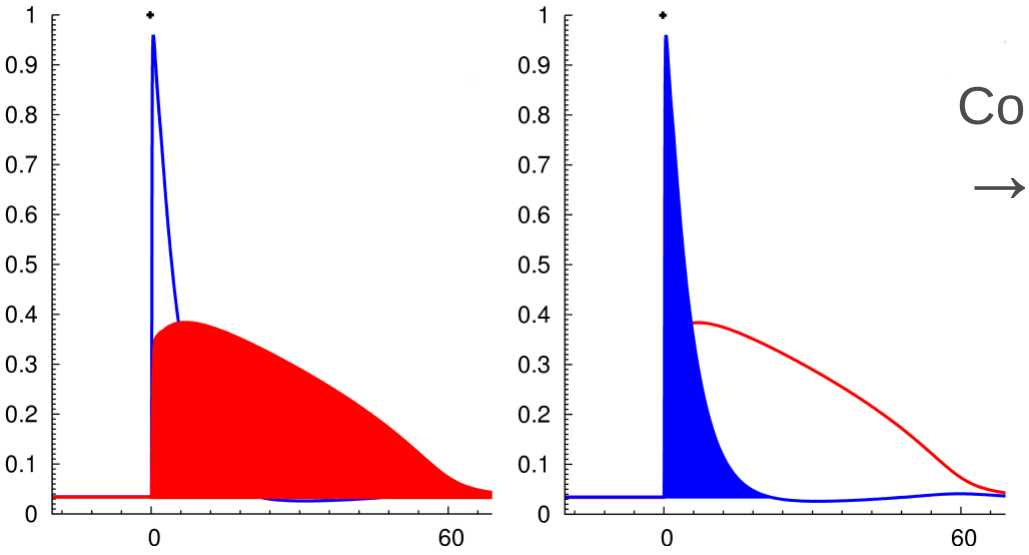






All calcium frequencies increase CaN **AND** CaMKII. It is not a Switch. But the ratio of activation changes

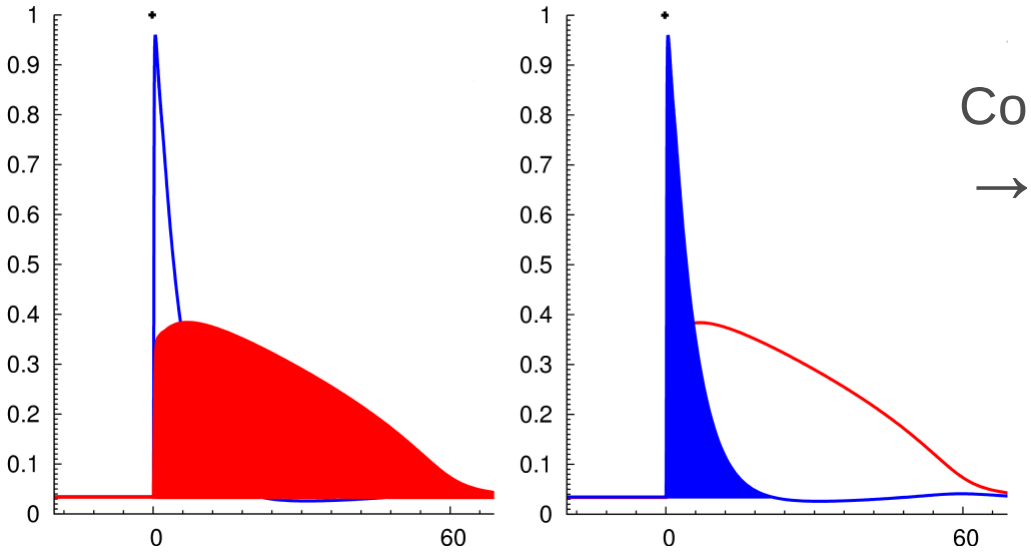
Bidirectional plasticity



Constant catalytic rates of active enzyme

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

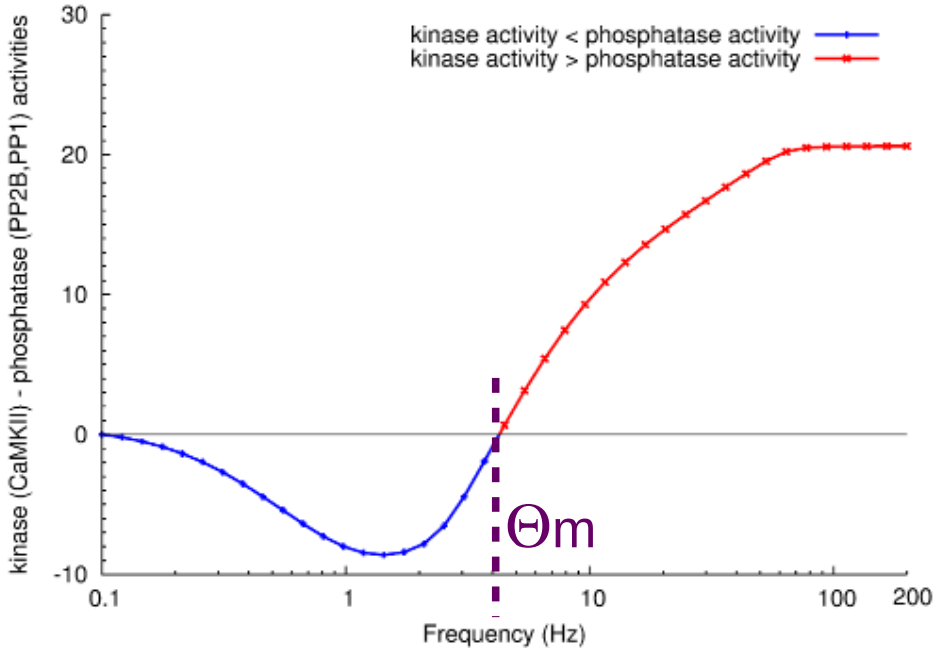
Bidirectional plasticity



Constant catalytic rates of active enzyme

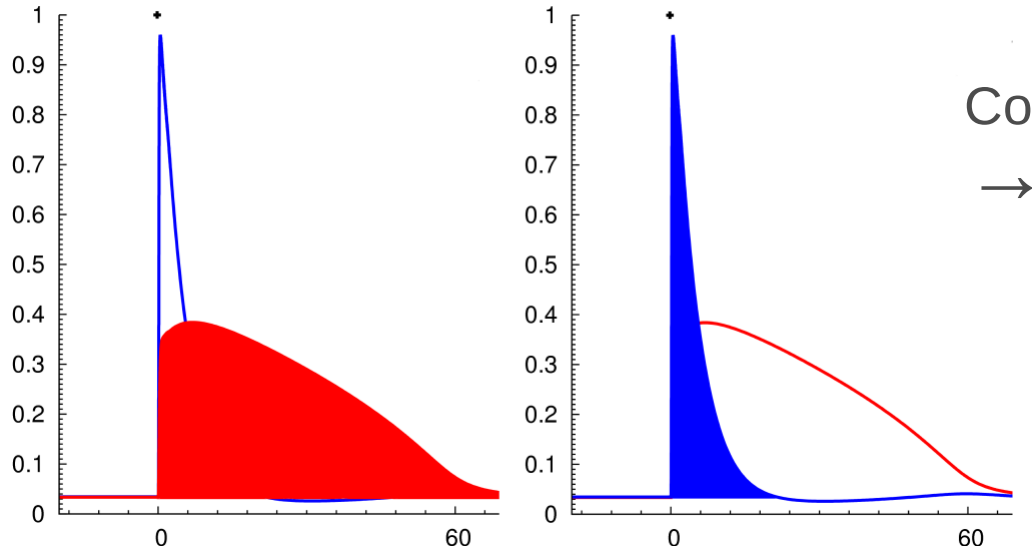
→ quantity of catalysed reaction events prop to integral of the activation curve

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



Babraham Institute

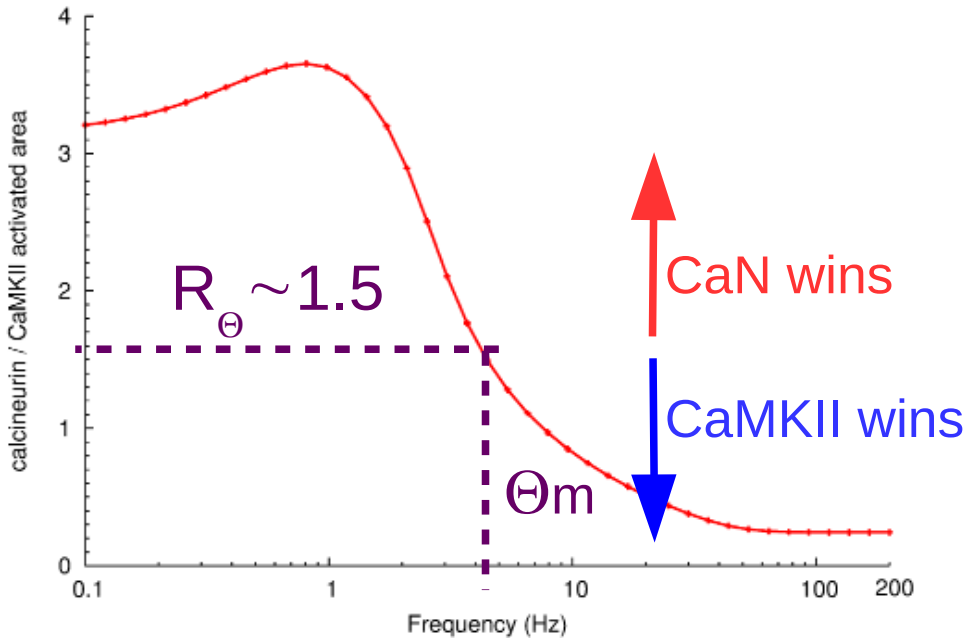
Bidirectional plasticity

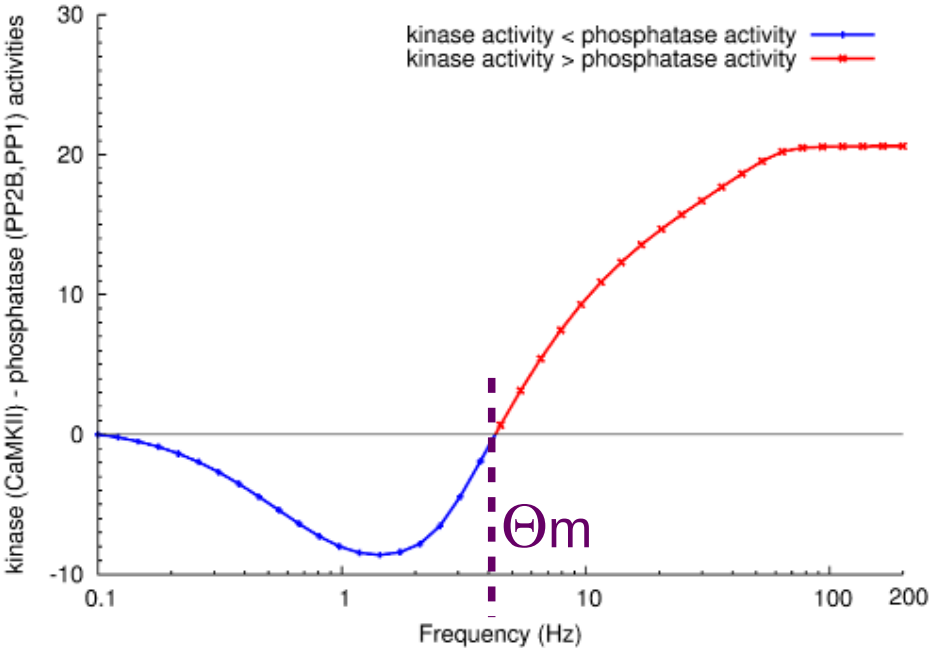


Constant catalytic rates of active enzyme

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

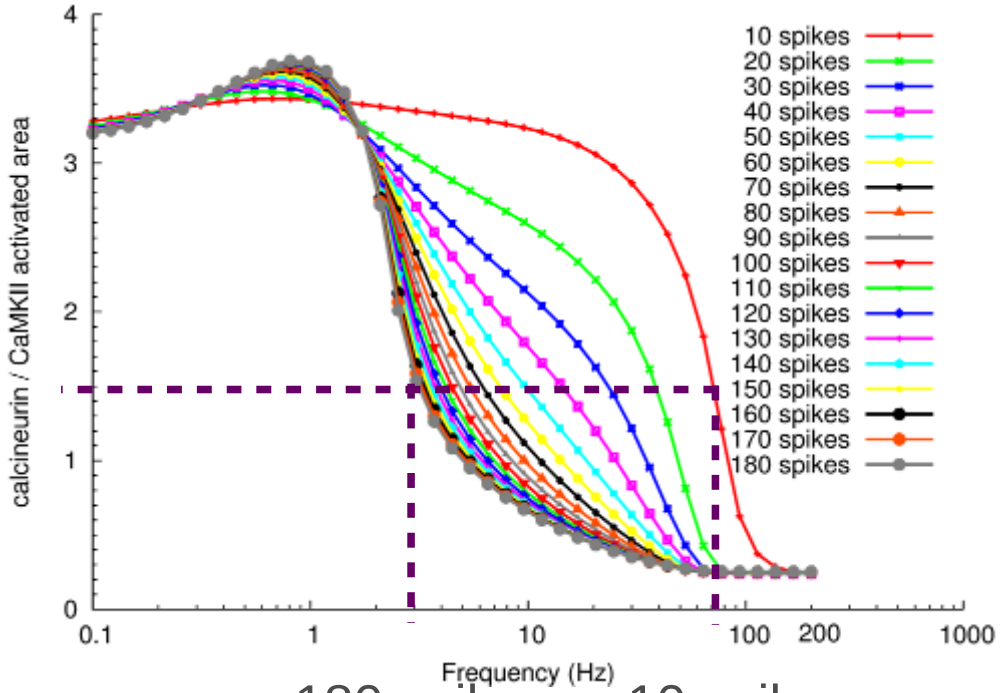
Bienestock-Cooper-Munro (BCM) curve: difference of active areas*catalytic activities





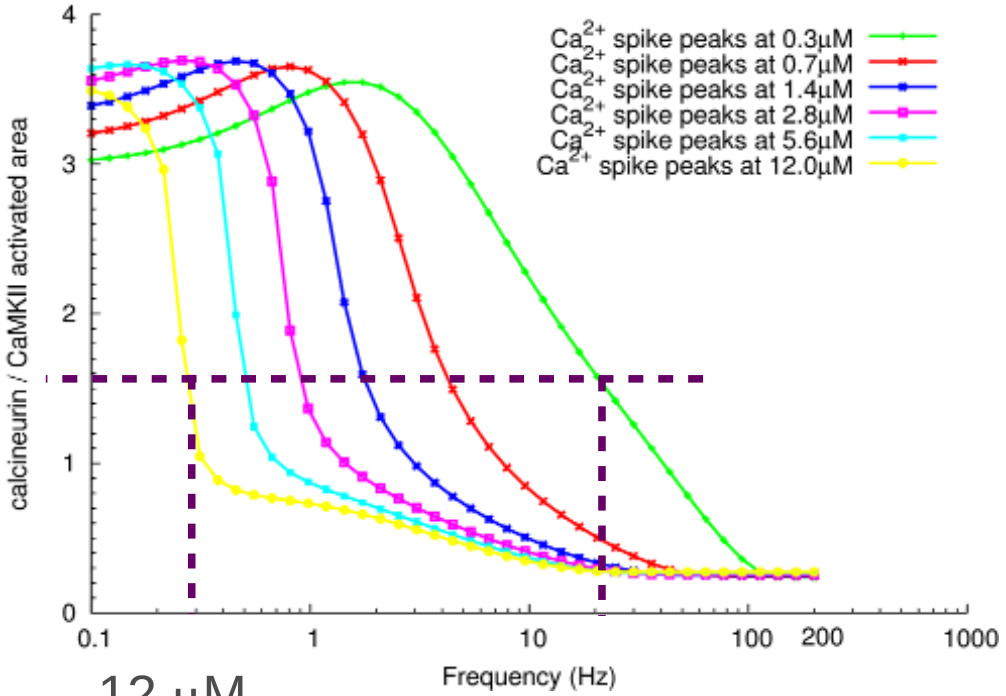
Babraham Institute

Effect of calcium duration and amount



180 spikes = 3 Hz

10 spikes = 70 Hz

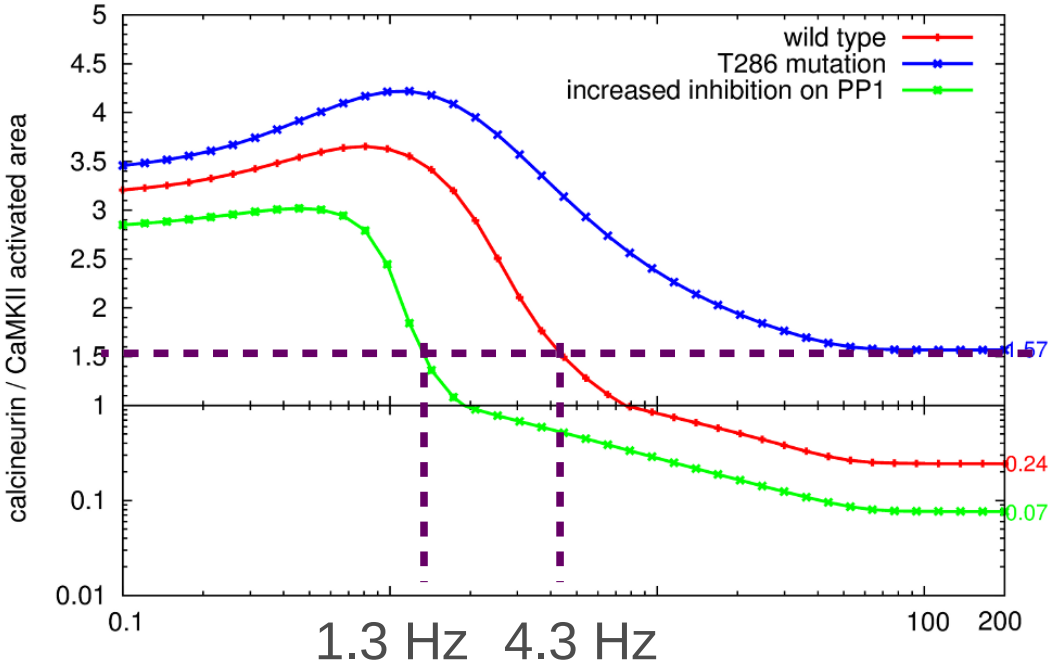


12 µM = 0.3 Hz

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



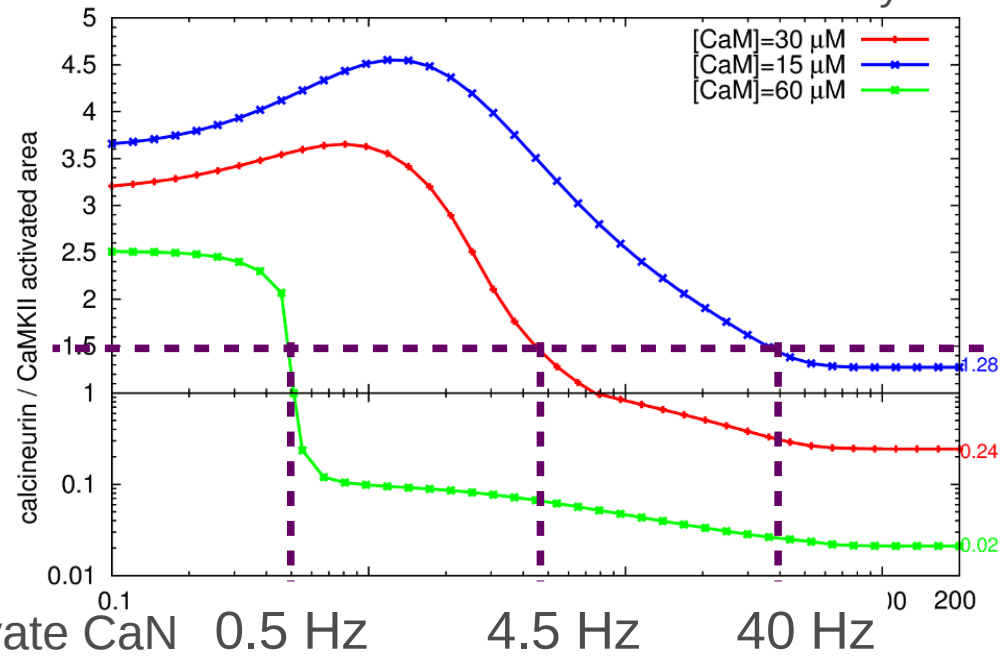
CaMKII not constitutively active
No CaM trapping

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

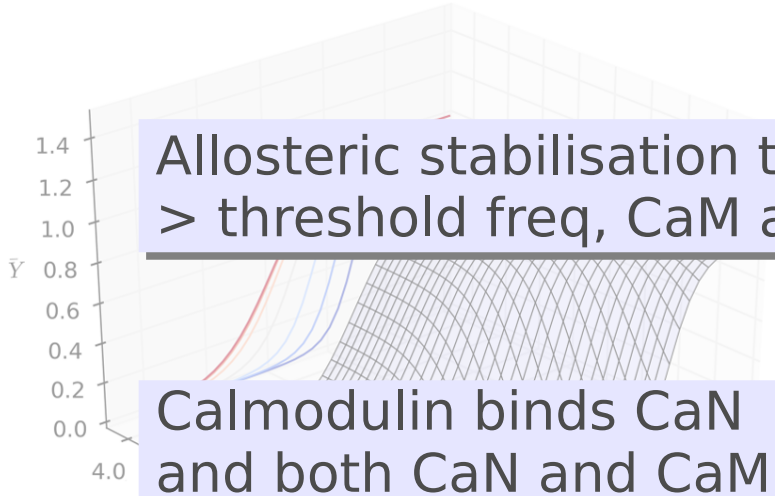


Competition for CaM, CaN wins

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

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

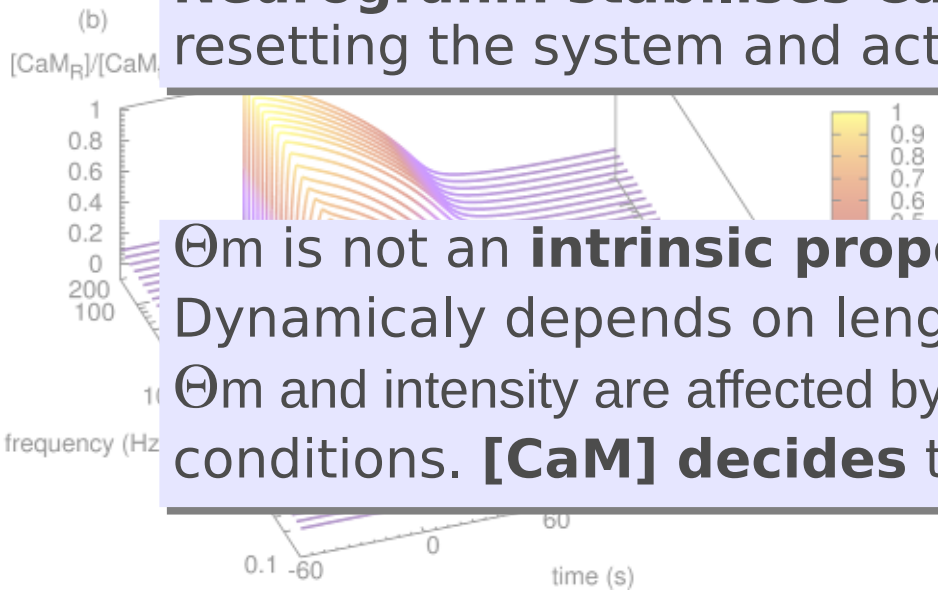


Allosteric stabilisation triggers **bistable CaM response** to Ca^{2+}
> threshold freq, CaM activation lasts longer than initial signal

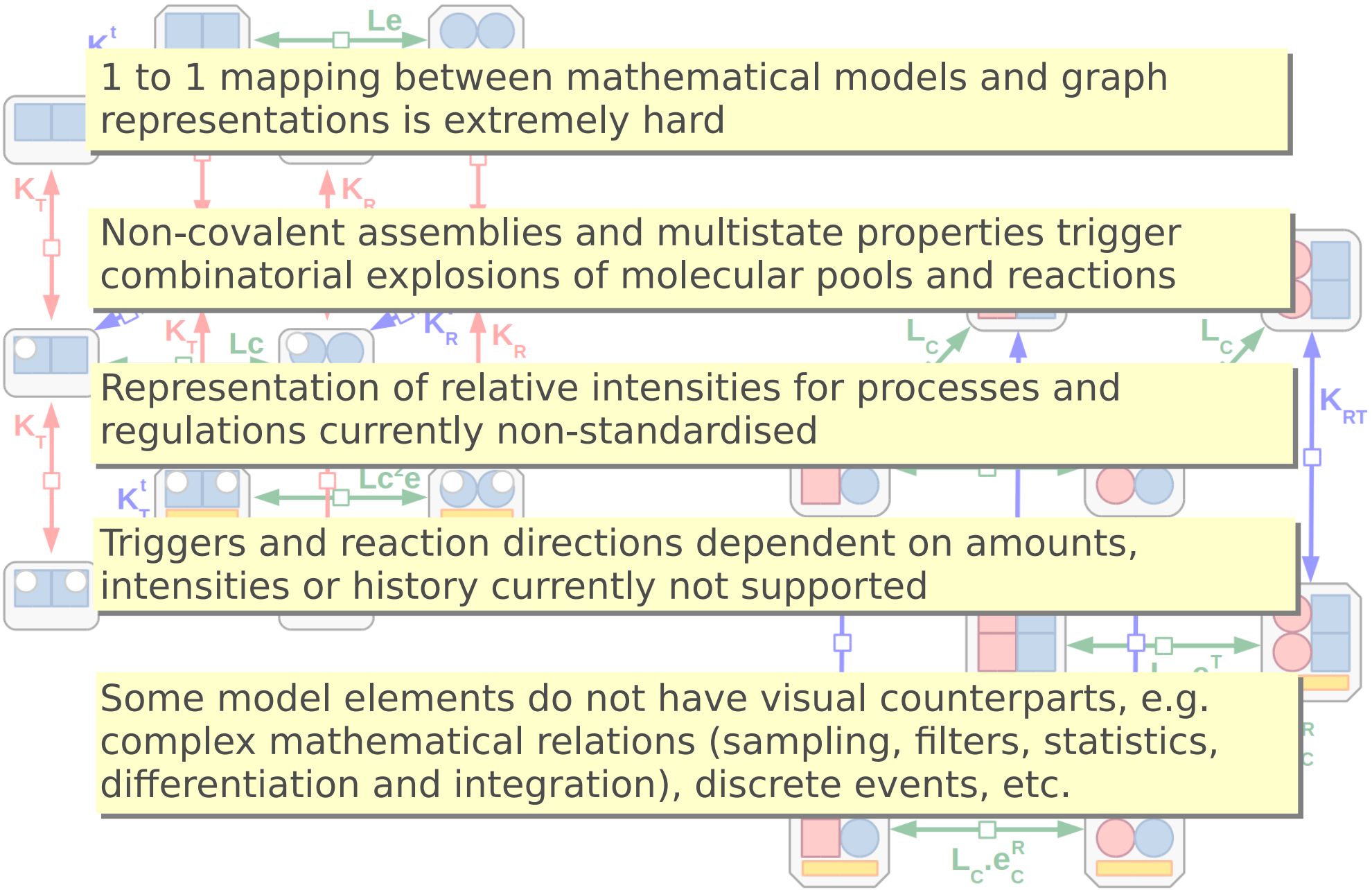
$$\bar{R} = \frac{(1+\alpha)}{(1+\alpha)^n + L(1+c\alpha)^n}$$

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

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



Θm is not an **intrinsic property of the synapse.**
Dynamicaly depends on length and amplitude of stimulations.
Θm and intensity are affected by reactions, parameters and initial conditions. **[CaM] decides** the balance CaN/CaMKII



1 to 1 mapping between mathematical models and graph representations is extremely hard

Non-covalent assemblies and multistate properties trigger combinatorial explosions of molecular pools and reactions

Representation of relative intensities for processes and regulations currently non-standardised

Triggers and reaction directions dependent on amounts, intensities or history currently not supported

Some model elements do not have visual counterparts, e.g. complex mathematical relations (sampling, filters, statistics, differentiation and integration), discrete events, etc.

Pecunia est nervus belli

Rbar, Edelstein and Le Novère version

$$L = \frac{[T_0]}{[R_0]} \qquad c = \frac{K^R}{K^T}$$

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

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

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

$$\bar{R} = \frac{1}{1 + L\Omega^n}$$

M ligands binding on different sites

$$\bar{R} = \frac{1}{1 + L\prod_{i=1}^{i=m}\Omega_i^{n_i}}$$