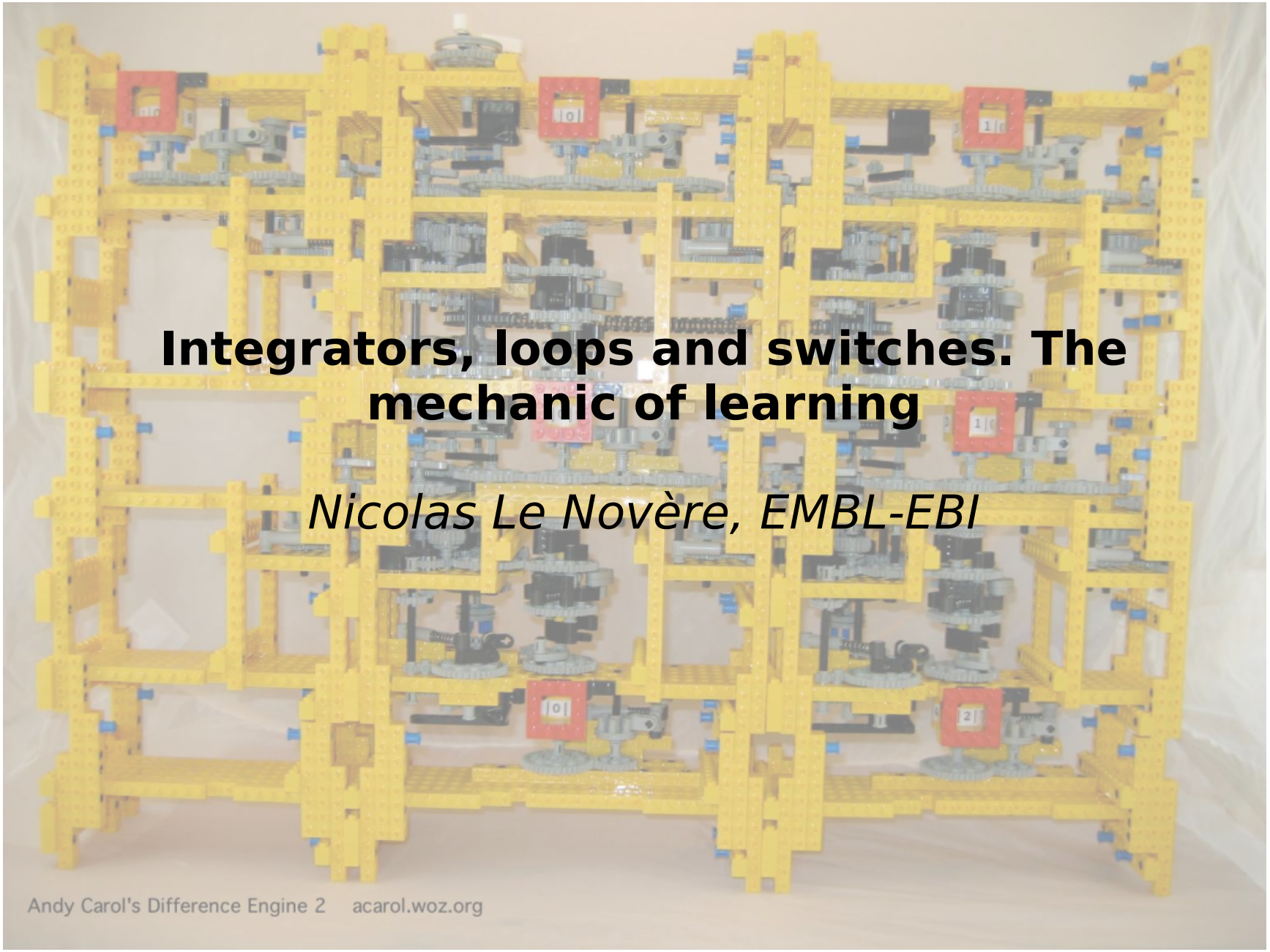
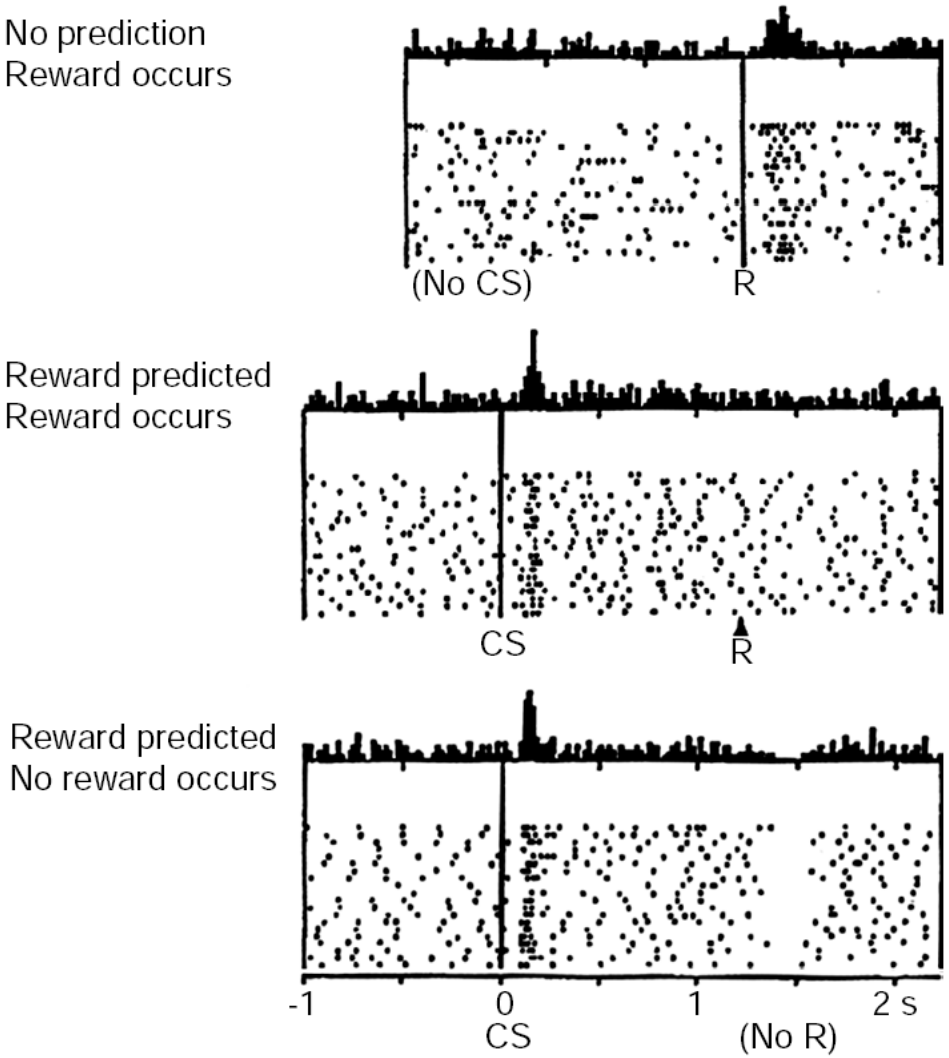
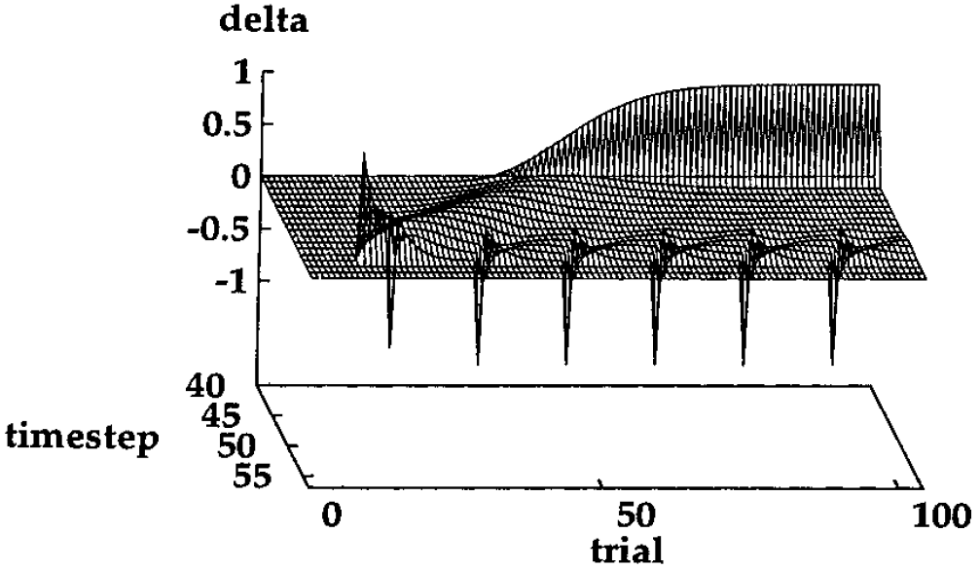


Integrators, loops and switches. The mechanic of learning

Nicolas Le Novère, EMBL-EBI

Dopamine, reward and learning





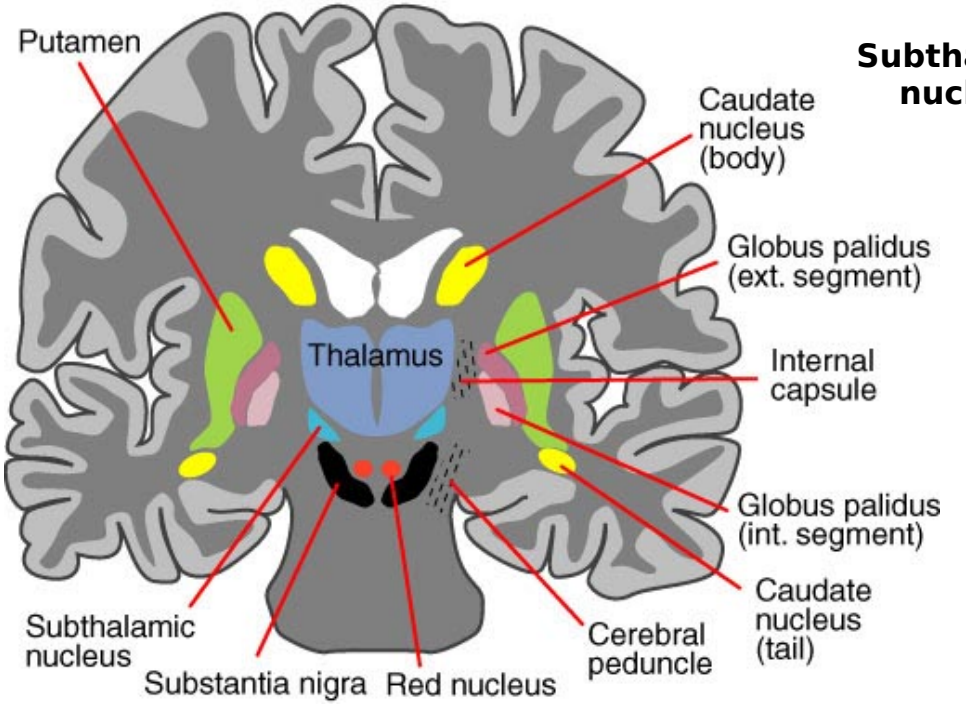
Schultz W, Dayan P, Montague PR (1997)
A neural substrate of prediction and reward.
Science 275:1593-1599.

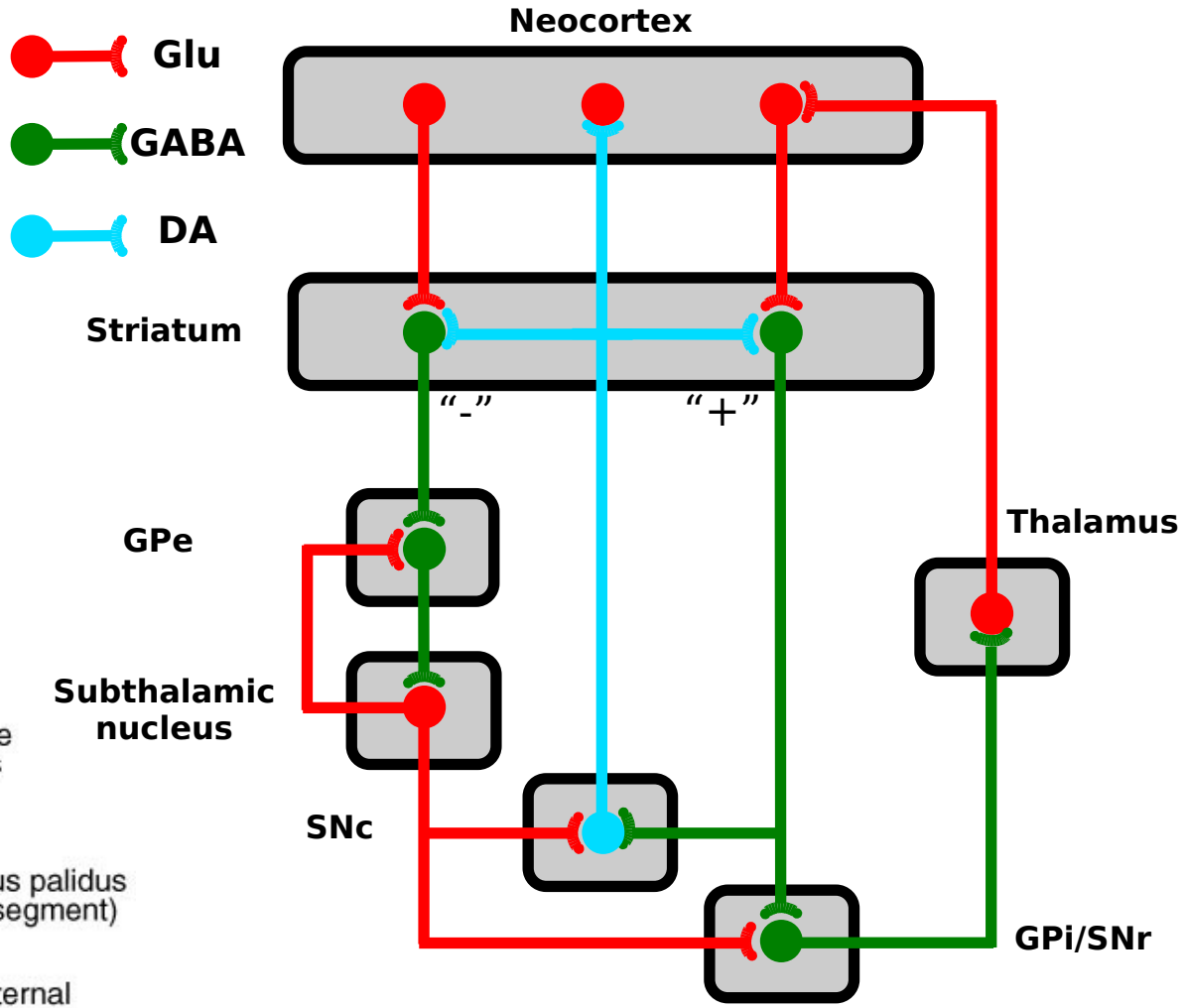
Montague PR, Dayan P, Sejnowski TJ (1996)
A framework for mesencephalic dopamine systems based on predictive Hebbian learning.
J Neurosci 16: 1936-1947

Dopamine mesotelencephalic pathway

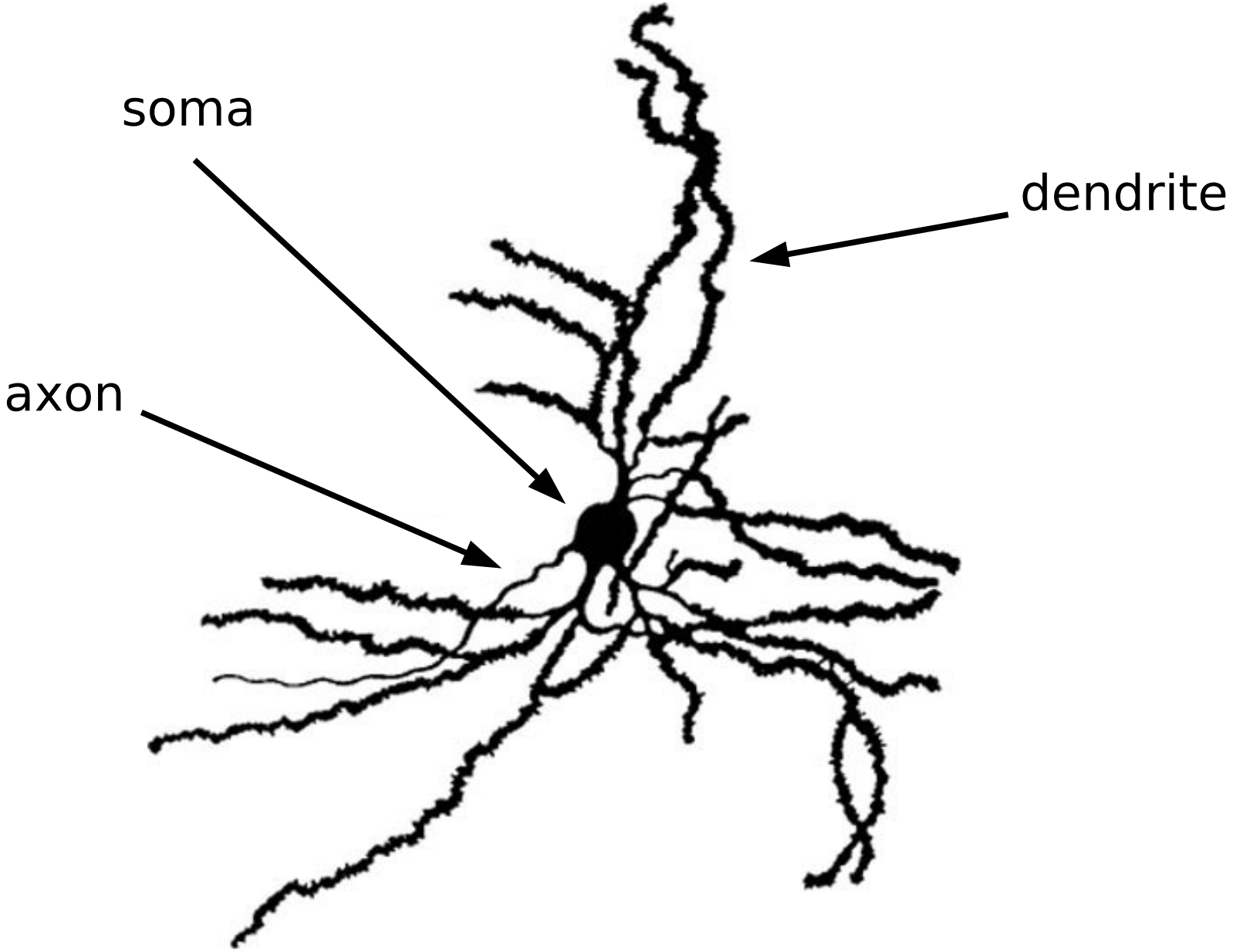
Voluntary motion
Emotional control
Reward
Learning

Parkinson's disease
Huntington's chorea
Hemibalism
Schizophrenia
Drug addiction
Depression
Obsessive-Compulsive Disorder
Tourette syndrome
ADHD

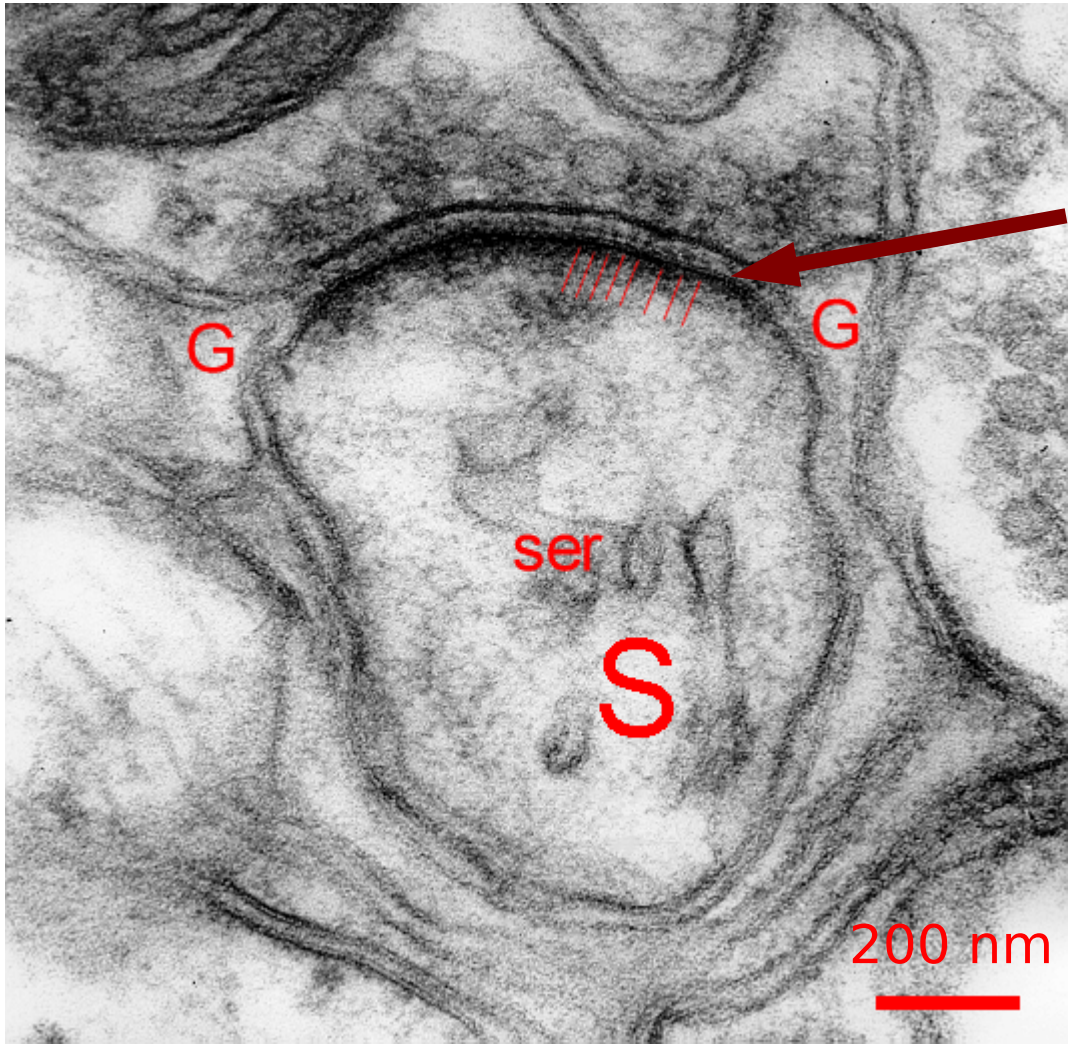




Structure of a neuron

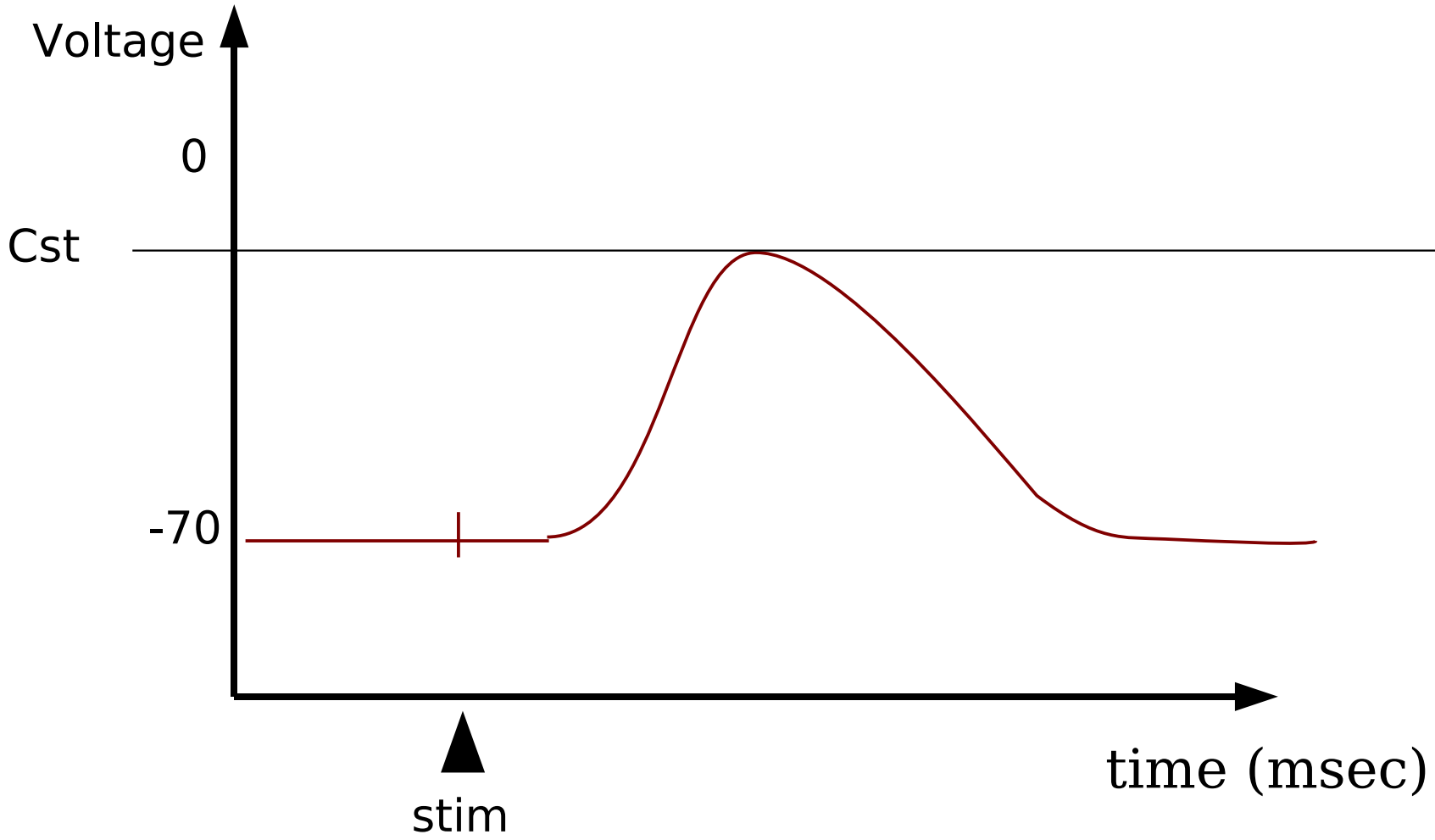


The spine as a “mini-cell”

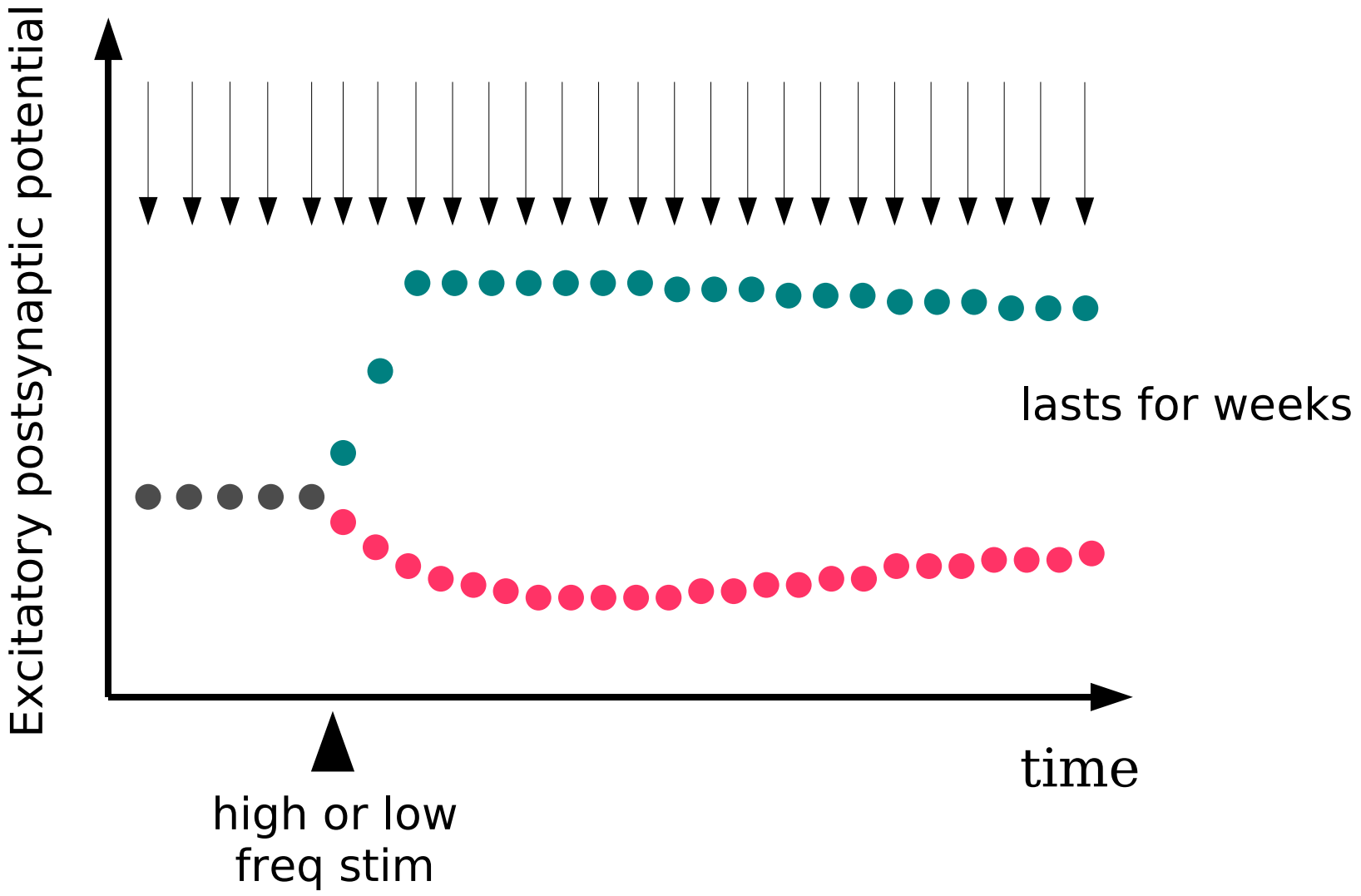


Atlas of ultrastructural neurocytology (http://synapse-web.org/)

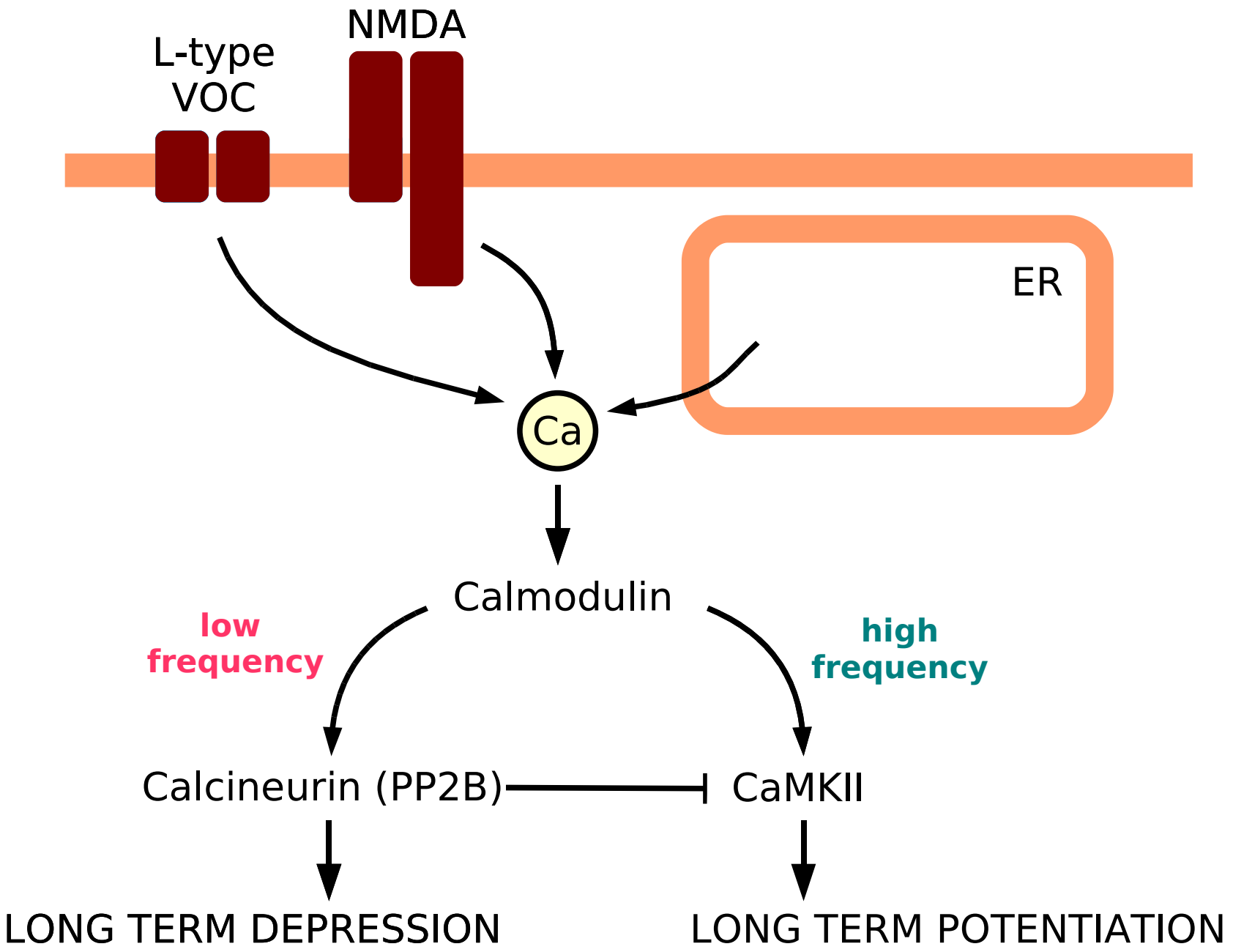
Excitatory post-synaptic potential



Bidirectional synaptic plasticity



Calcium theory of Synaptic Plasticity



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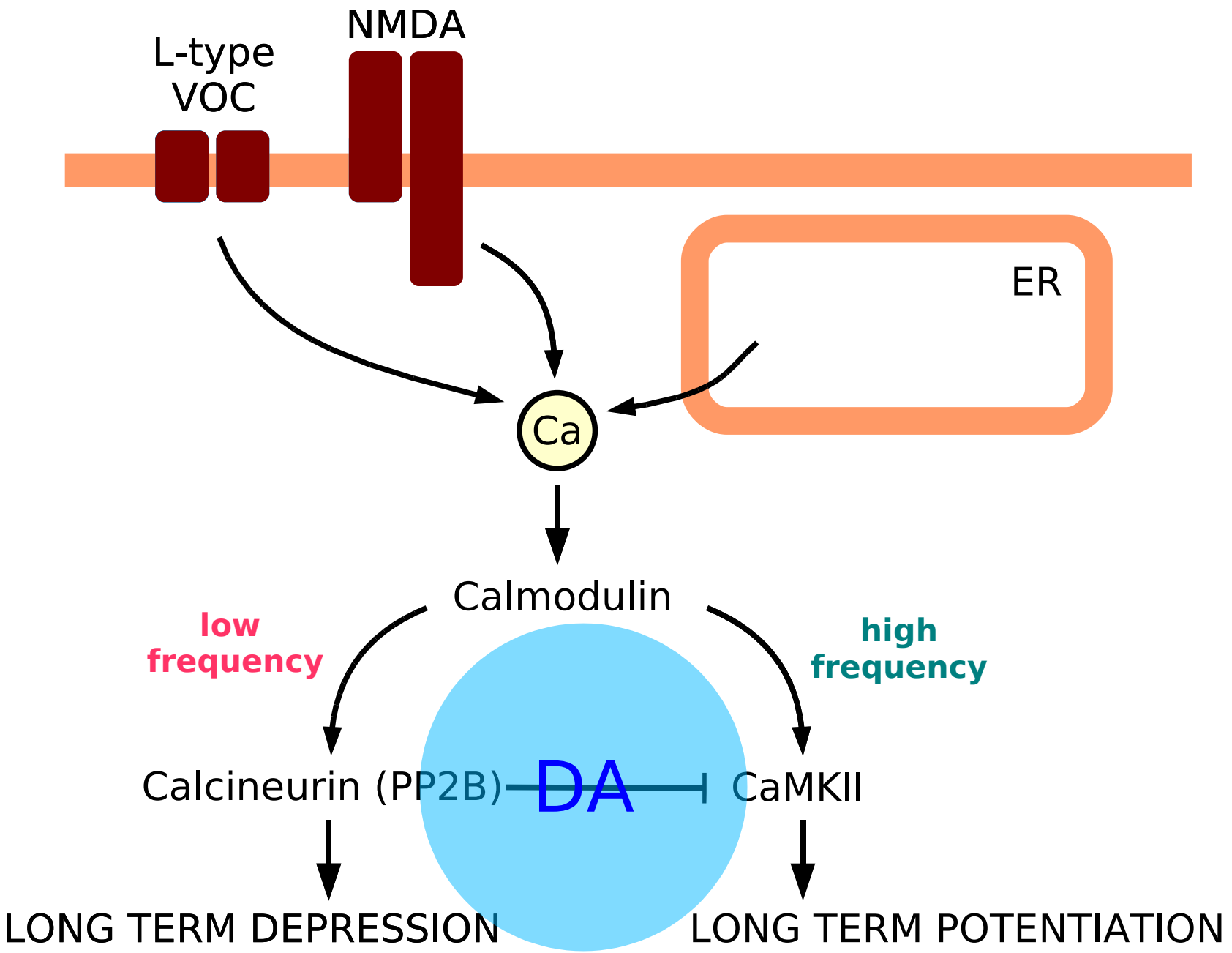
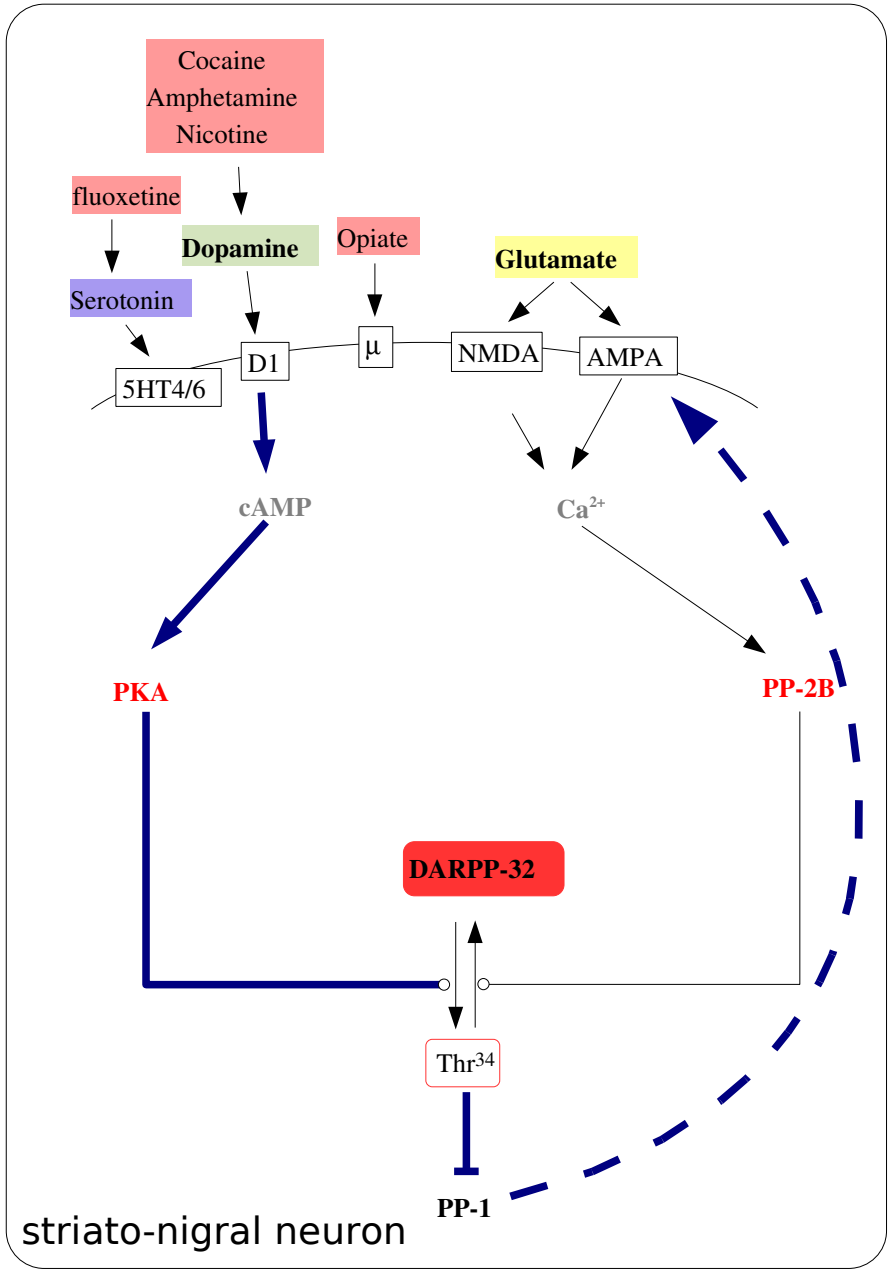


Is DARPP-32 a switch?

Fernandez E, Schiappa R, Girault JA, Le Novère N (2006) DARPP-32 is a robust integrator of dopamine and glutamate signals. *PLoS Computational Biology*, 2(12): e176.



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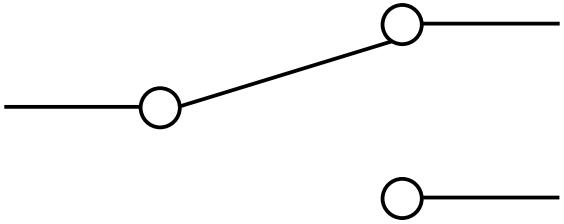

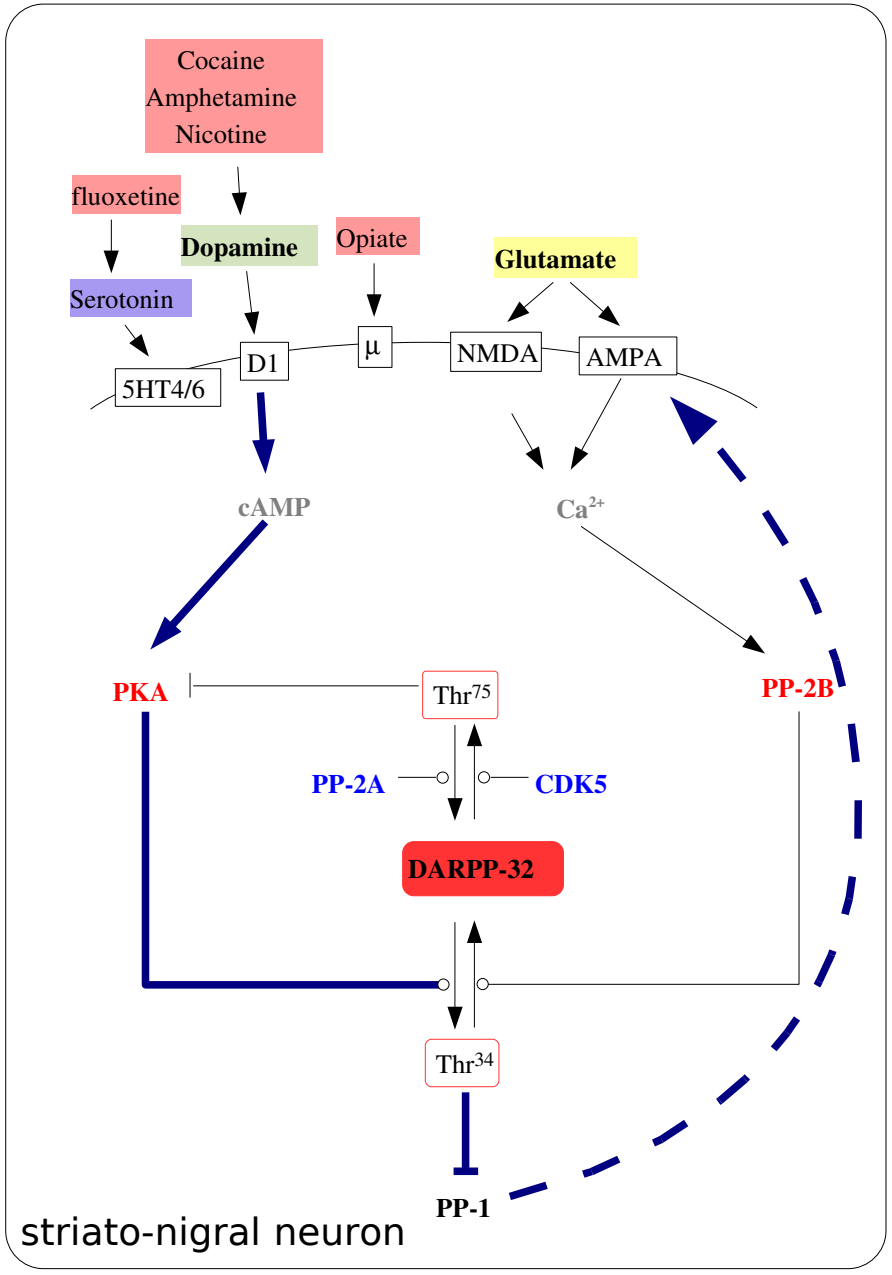

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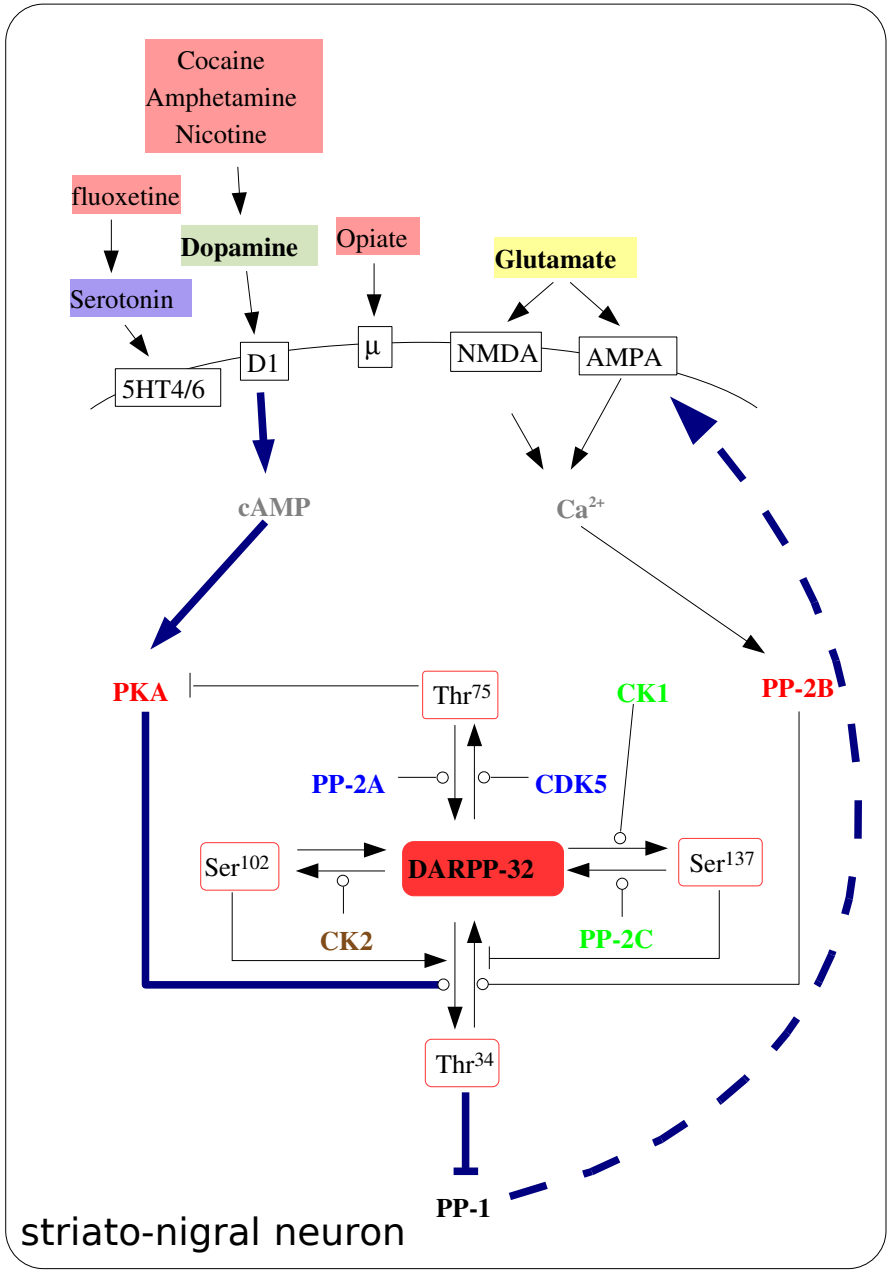

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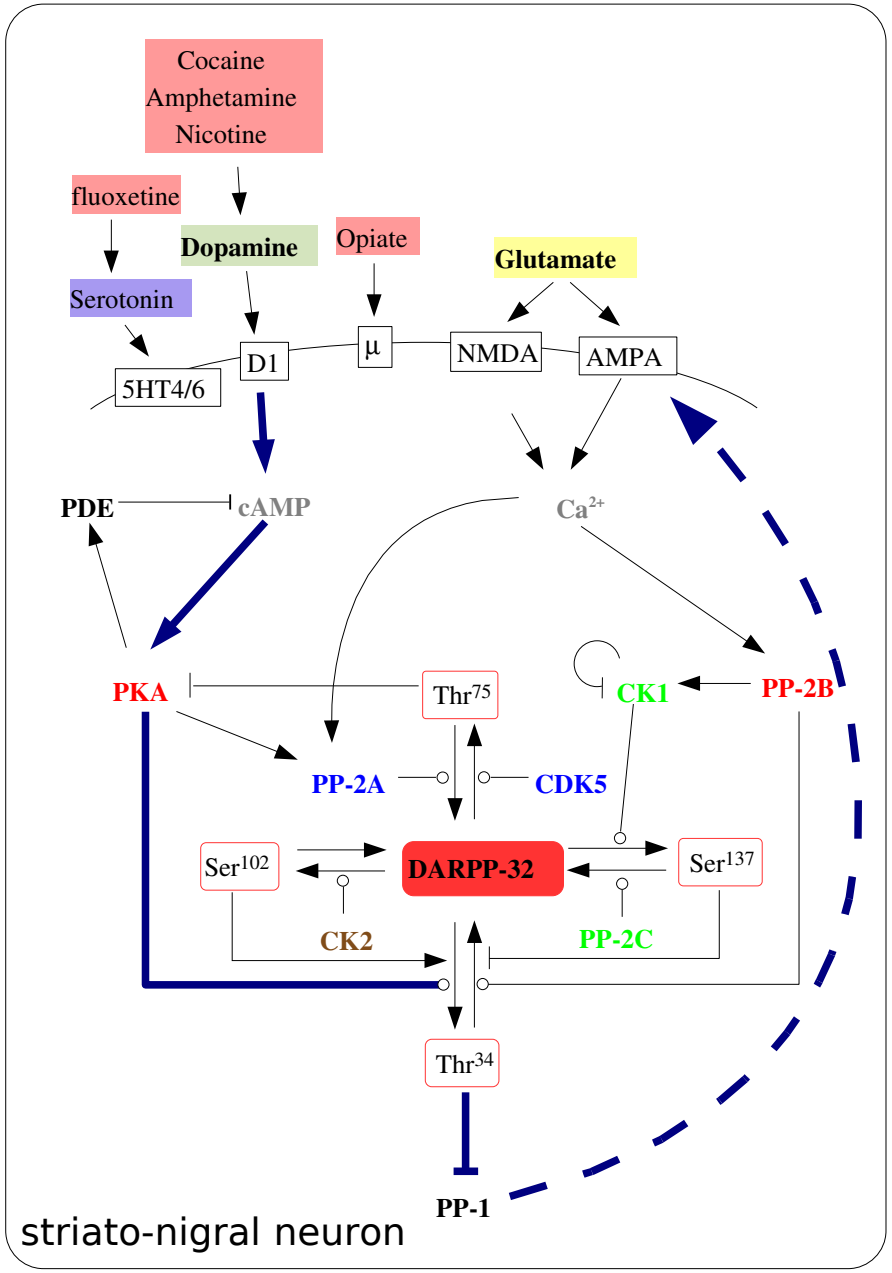


Chemical model of DARPP-32 regulation

79 species, 151 reactions

- D + CDK5 ⇔ D_CDK5 → D75 + CDK5
- D75 + PP2A ⇔ D75_PP2A → D + PP2A
- D75 + PP2ACa ⇔ D75_PP2ACa → D + PP2ACa
- D75 + PP2AP ⇔ D75_PP2AP → D + PP2AP
- D75 + PP2APCa ⇔ D75_PP2APCa → D + PP2APCa
- D137 + CDK5 ⇔ D137_CDK5 → D75-137 + CDK5
- D75-137 + PP2A ⇔ D75-137_PP2A → D137 + PP2A
- D75-137 + PP2ACa ⇔ D75-137_PP2ACa → D137 + PP2ACa
- D75-137 + PP2AP ⇔ D75-137_PP2AP → D137 + PP2AP
- D75-137 + PP2APCa ⇔ D75-137_PP2APCa → D137 + PP2APCa
- D34 + CDK5 ⇔ D34_CDK5 → D34-75 + CDK5
- D34-75 + PP2A ⇔ D34-75_PP2A → D34 + PP2A
- D34-75 + PP2ACa ⇔ D34-75_PP2ACa → D34 + PP2ACa
- D34-75 + PP2AP ⇔ D34-75_PP2AP → D34 + PP2AP
- D34-75 + PP2APCa ⇔ D34-75_PP2APCa → D34 + PP2APCa
- D34-137 + CDK5 ⇔ D34-137_CDK5 → D34-75_137 + CDK5
- D34-75-137 + PP2A ⇔ D34-75-137_PP2A → D34-137 + PP2A
- D34-75-137 + PP2ACa ⇔ D34-75-137_PP2ACa → D34-137 + PP2ACa
- D34-75-137 + PP2AP ⇔ D34-75-137_PP2AP → D34-137 + PP2AP
- D34-75-137 + PP2APCa ⇔ D34-75-137_PP2APCa → D34-137 + PP2APCa

- D + CK1 ⇔ D_CK1 → D137 + CK1
- D137 + PP2C ⇔ D137_PP2C → D + PP2C
- D75 + CK1 ⇔ D75_CK1 → D75-137 + CK1
- D75-137 + PP2C ⇔ D75-137_PP2C → D75 + PP2C
- D34 + CK1 ⇔ D34_CK1 → D34-137 + CK1
- D34-75 + PP2C ⇔ D34-75_PP2C → D75 + PP2C
- D34-75 + CK1 ⇔ D34-75_CK1 → D34-75-137 + CK1
- D34-75-137 + PP2C ⇔ D34-75-137_PP2C → D34-75 + PP2C
- CK1 + CK1 ⇔ CK1_CK1 → CK1P + CK1
- CK1P + PP2B ⇔ CK1P_PP2B → CK1 + PP2B

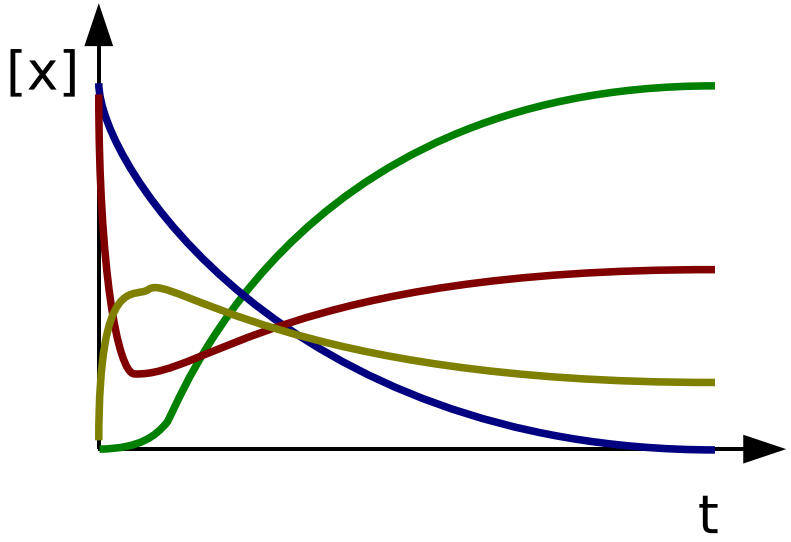
- D + PKA ⇔ D_PKA → D34 + PKA
- D34 + PP2B ⇔ D34_PP2B → D + PP2B
- D75 + PKA ⇔ D75_PKA → D34-75 + PKA
- D34-75 + PP2B ⇔ D34-75_PP2B → D75 + PP2B
- D137 + PKA ⇔ D137_PKA → D34-137 + PKA
- D34-137 + PP2B ⇔ D34-135_PP2B → D137 + PP2B
- D75-137 + PKA ⇔ D75-137_PKA → D34-75-137 + PKA
- D34-75-137 + PP2B ⇔ D34-75-135_PP2B → D75-137 + PP2B
- PP2A + PKA ⇔ PP2A_PKA → PP2AP + PKA
- PP2ACa + PKA ⇔ PP2ACa_PKA → PP2APCa + PKA
- Ø → Ca^{2+}
- Ca^{2+} → Ø
- PP2Bi + 2Ca ⇔ PP2Bi_Ca2
- PP2Bi_Ca + 2Ca ⇔ PP2B
- PP2A + Ca ⇔ PP2ACa
- PP2AP + Ca ⇔ PP2APCa

- R2_PKA2 + cAMP ⇔ cAMP_R2_PKA2
- cAMP_R2_PKA2 + cAMP ⇔ cAMP2_R2_PKA2
- cAMP2_R2_PKA2 + cAMP ⇔ cAMP3_R2_PKA2
- cAMP3_R2_PKA2 + cAMP ⇔ cAMP4_R2_PKA2
- cAMP4_R2_PKA2 ⇔ cAMP4_R2_PKA + PKA
- cAMP4_R2_PKA ⇔ cAMP4_R2 + PKA
- PKA + PDE ⇔ PKA_PDE → PKA + PDEP
- cAMP + PDE ⇔ cAMP_PDE → AMP + PDE
- cAMP + PDEP ⇔ cAMP_PDEP → AMP + PDEP

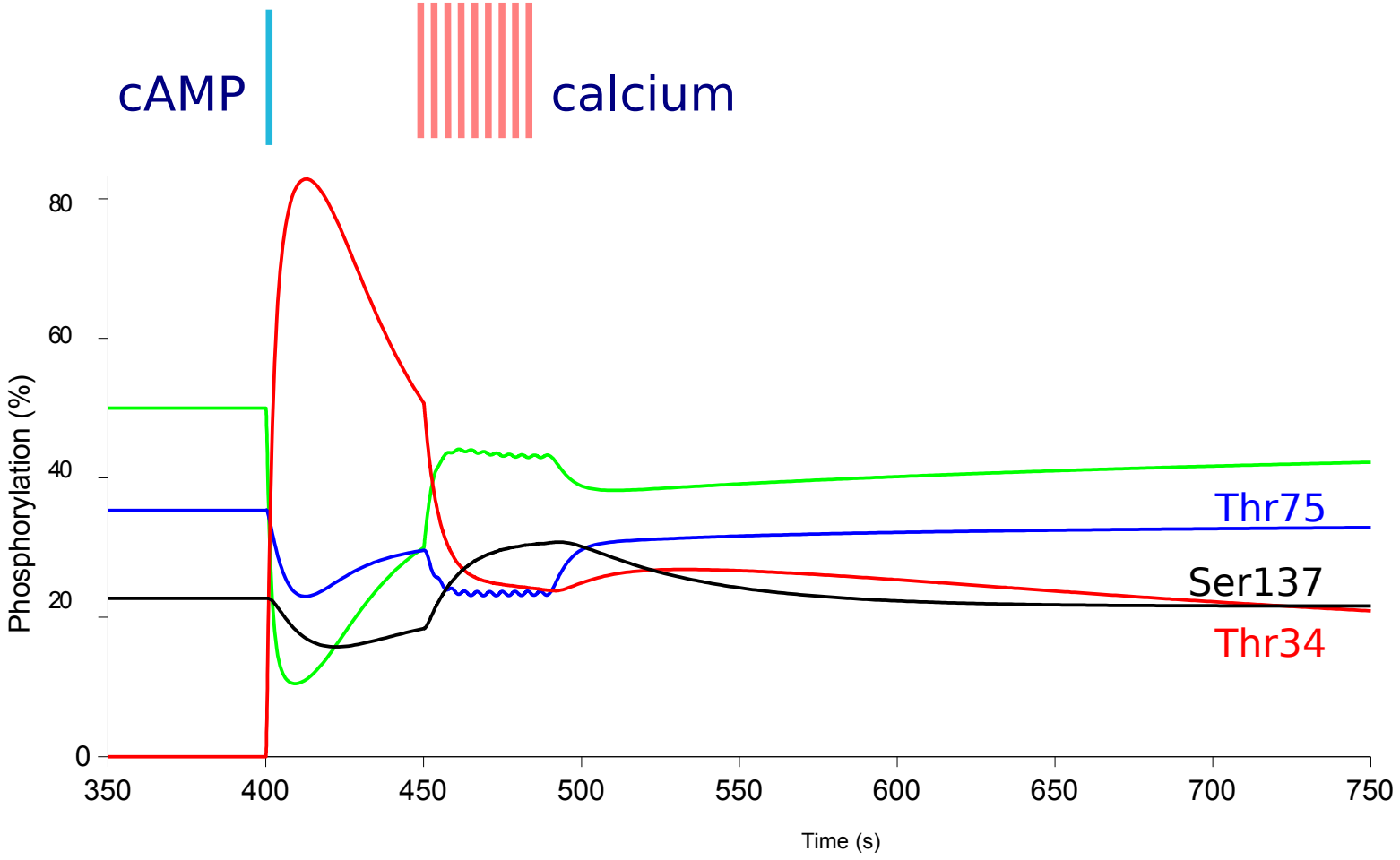
Mathematical model

$$D + CDK5 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} D\_CDK5 \xrightarrow{k_3} D\text{-}75P + CDK5$$

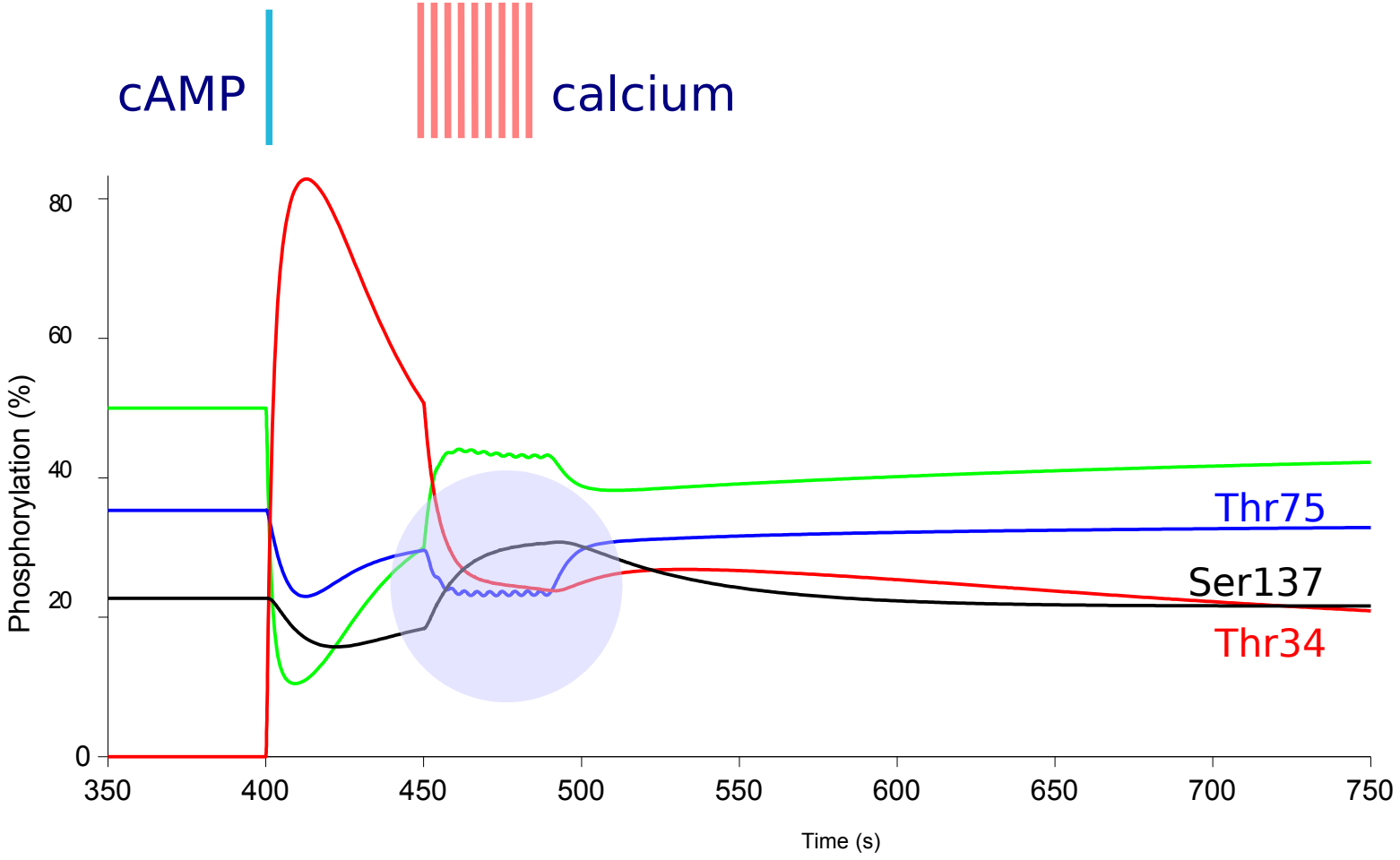
$$\begin{aligned}
d[D]/dt &= -k_1[CDK5][D] + k_2[D_CDK5] \\
d[D\text{-}75P]/dt &= + k_3[D_CDK5] \\
d[CDK5]/dt &= -k_1[CDK5][D] + k_2[D_CDK5] + k_3[D_CDK5] \\
d[D_CDK5]/dt &= +k_1[CDK5][S] - k_2[D_CDK5] - k_3[D_CDK5]
\end{aligned}$$



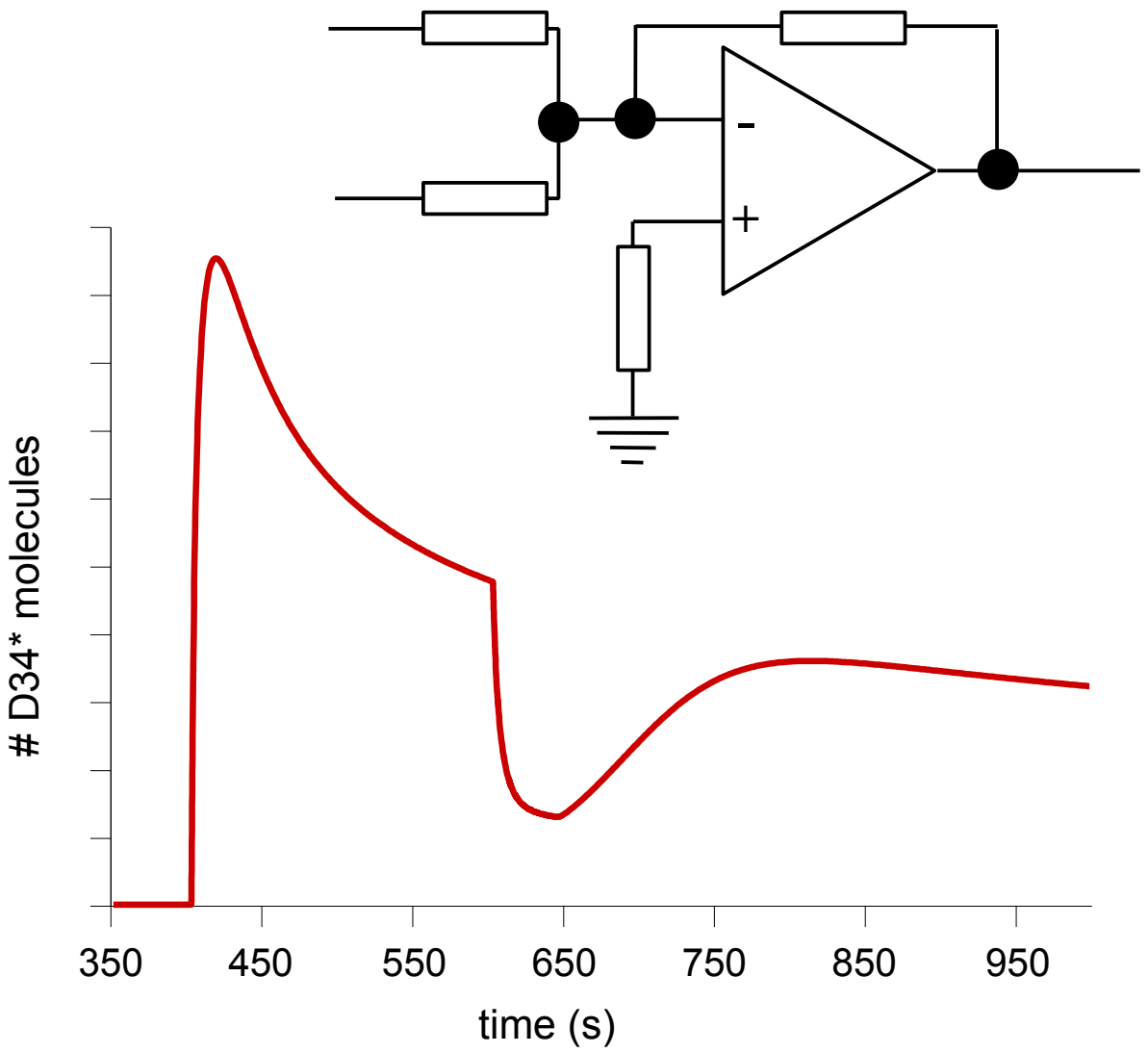
Dynamic simulations of DARPP-32 function



Dynamic simulations of DARPP-32 function



DARPP-32 is an integrator



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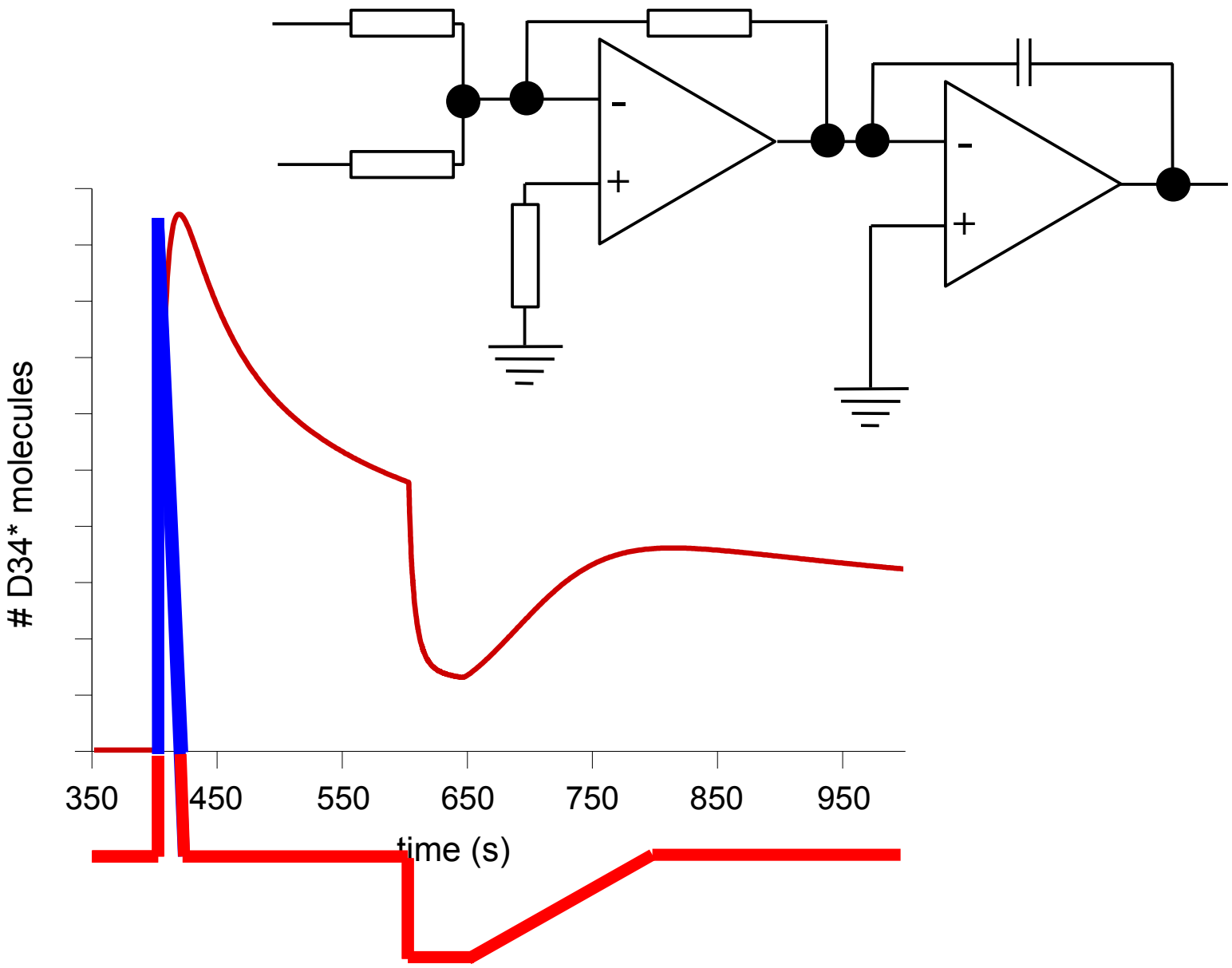
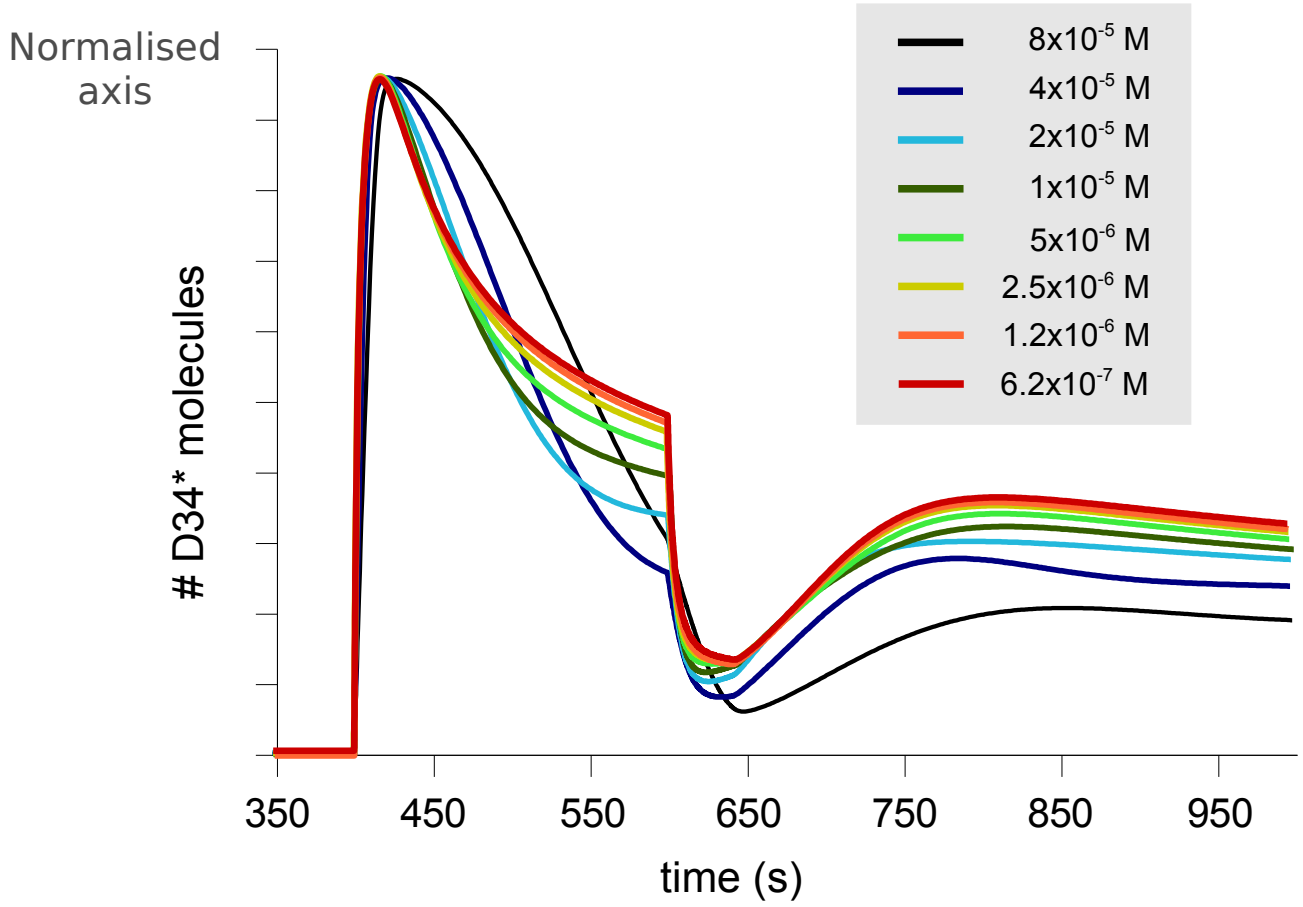
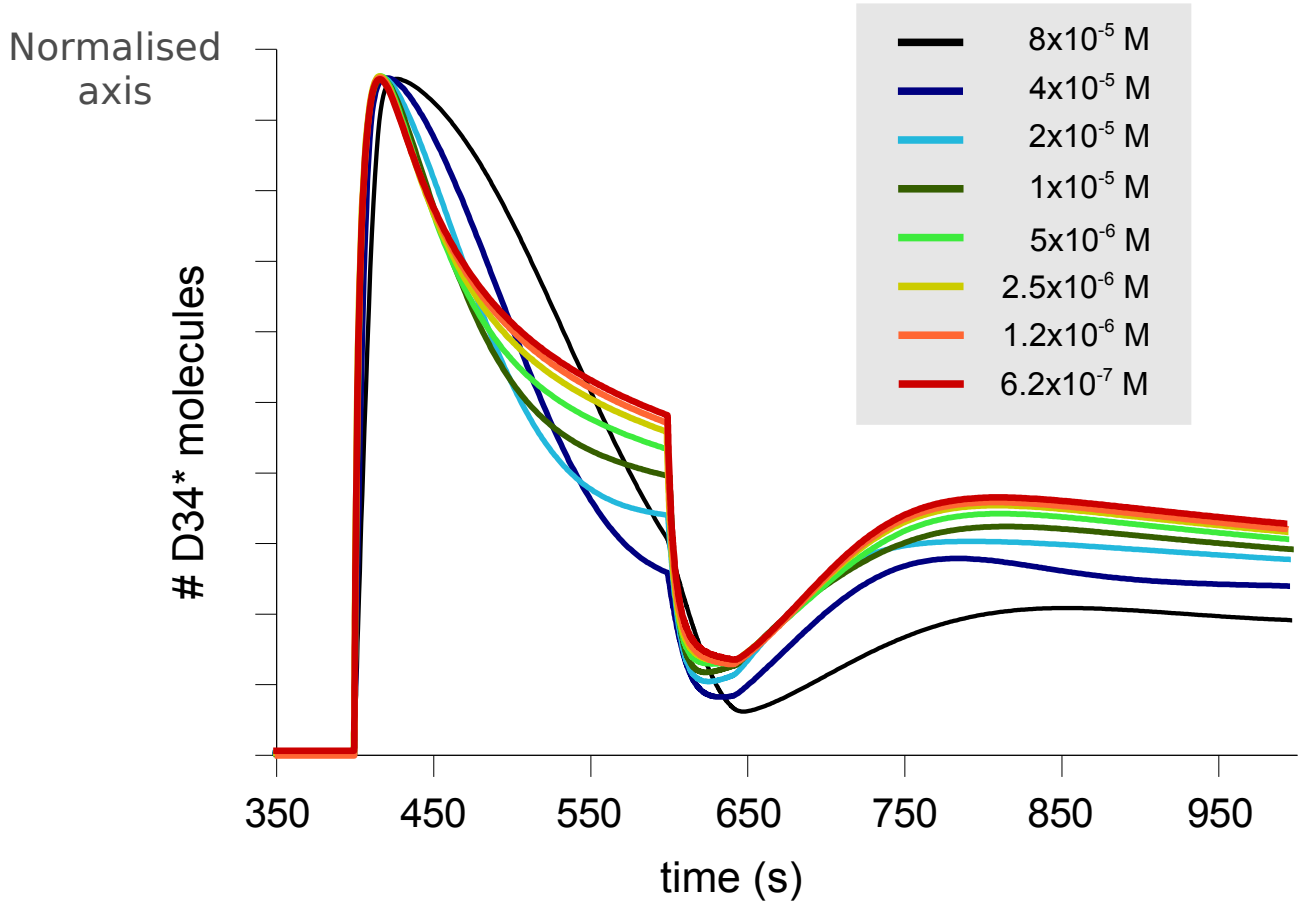


DARPP-32 as a robust integrator

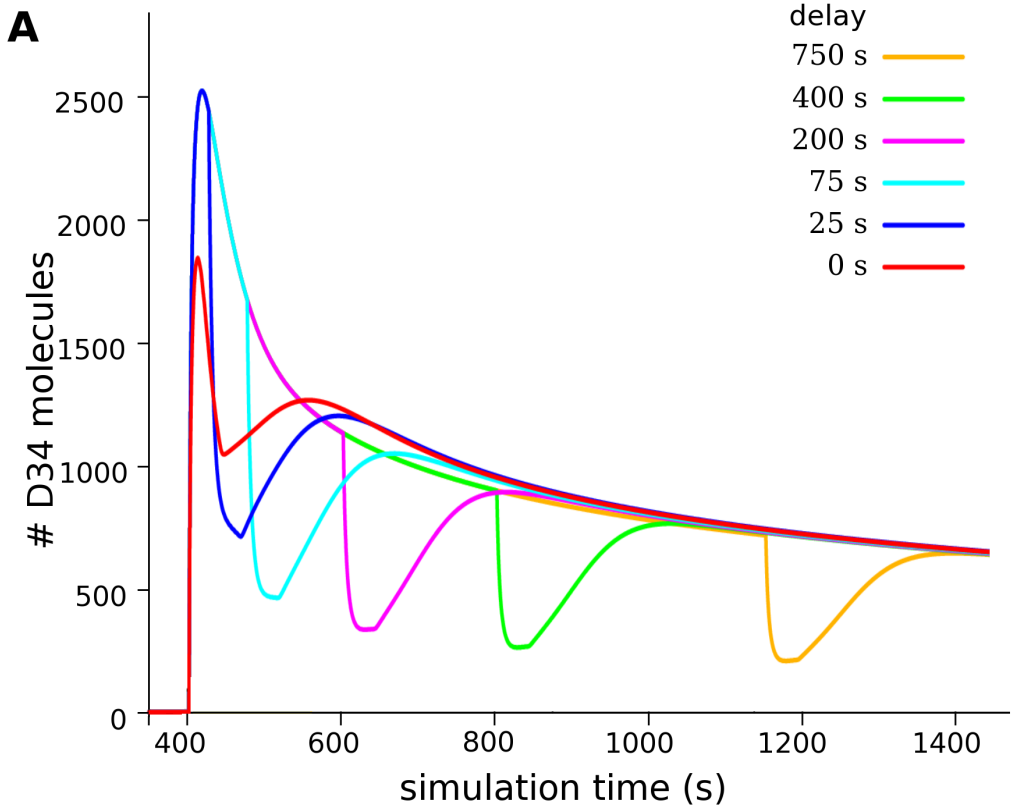


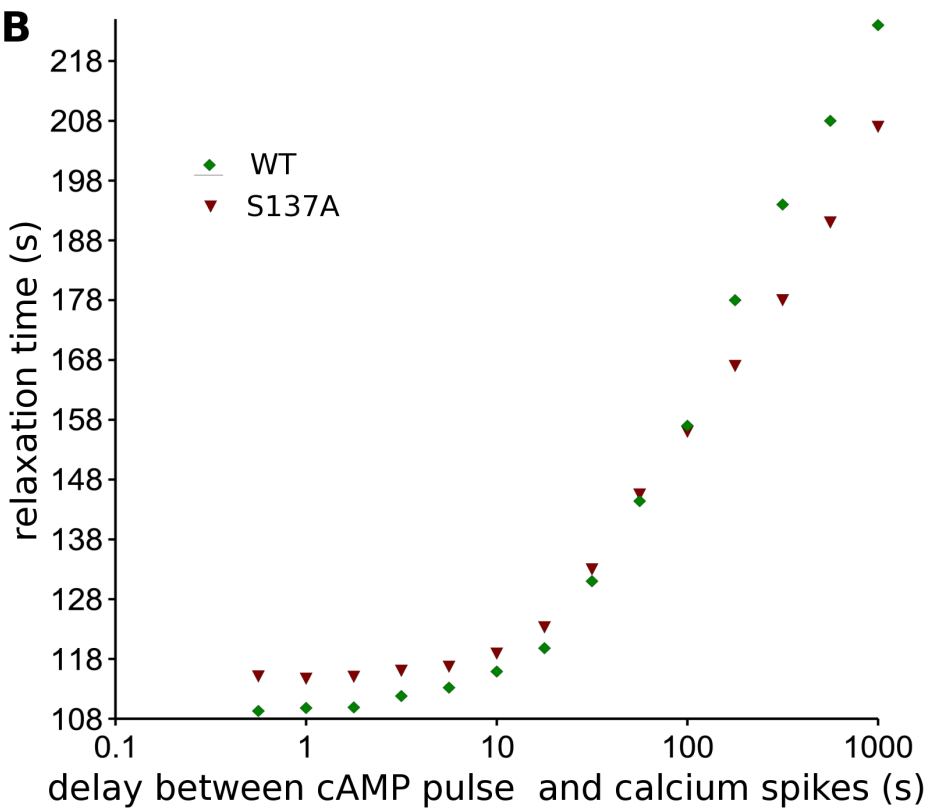
DARPP-32 as a robust integrator



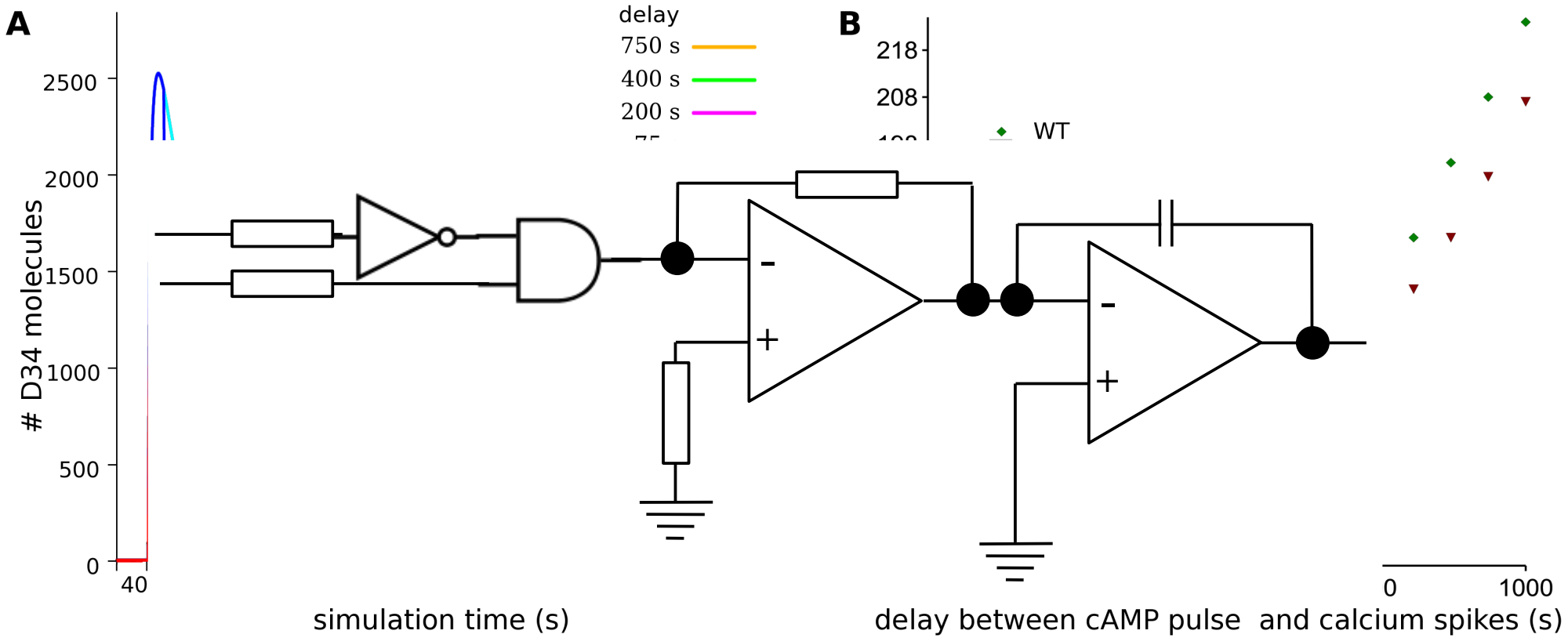
Heterozygous DARPP-32 +/- do not display phenotypes

Only an integrator?

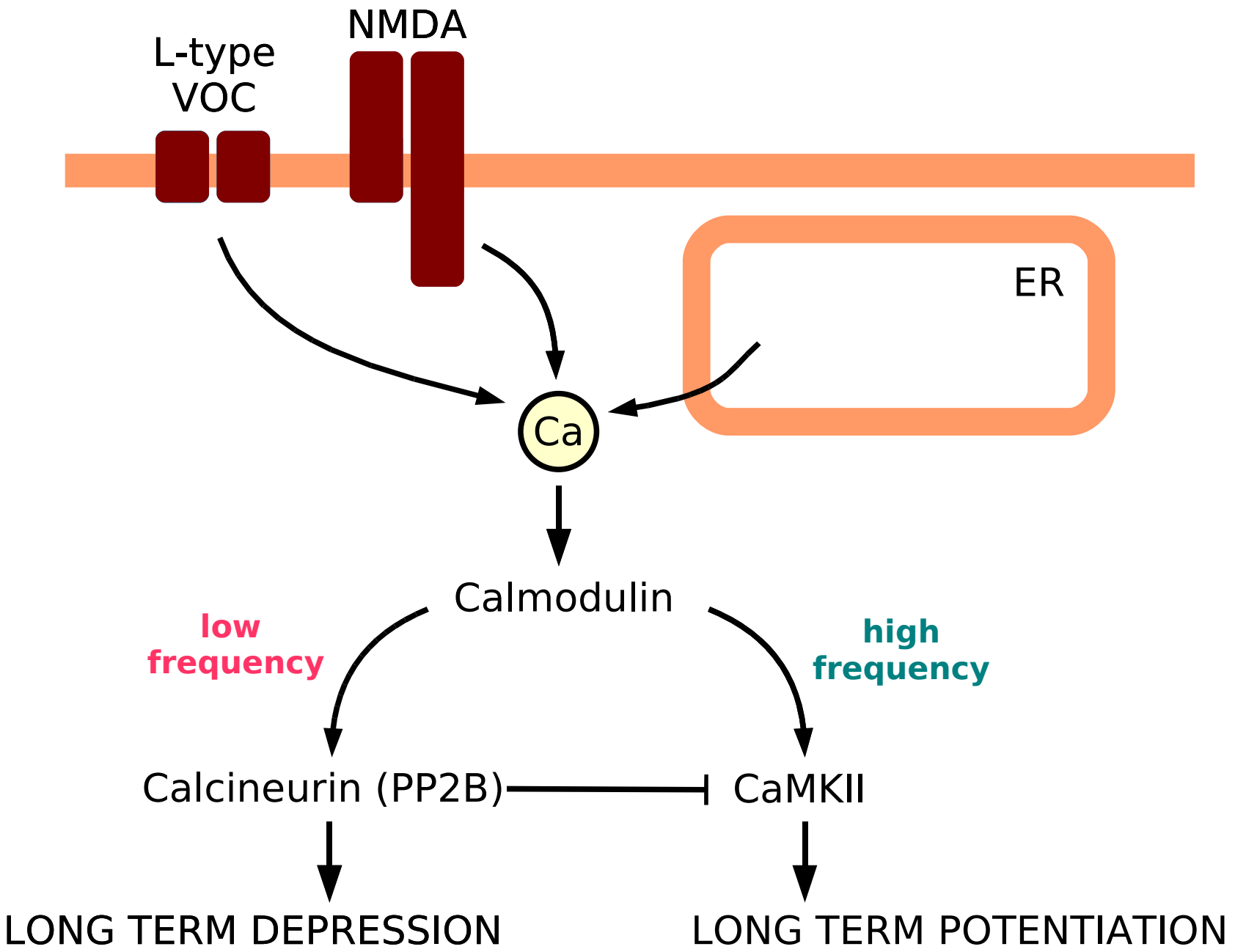




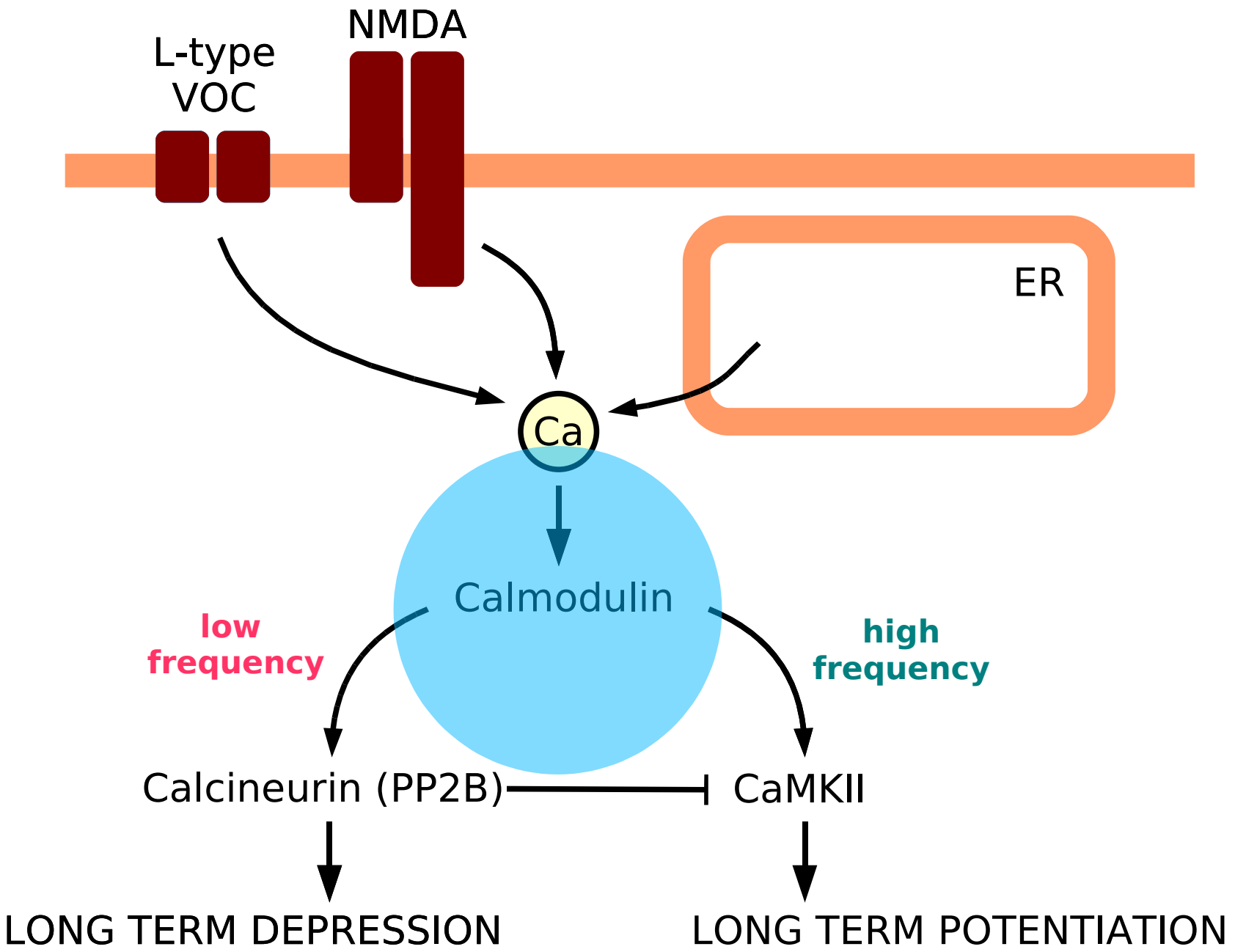
DARPP-32 is a coincidence detector



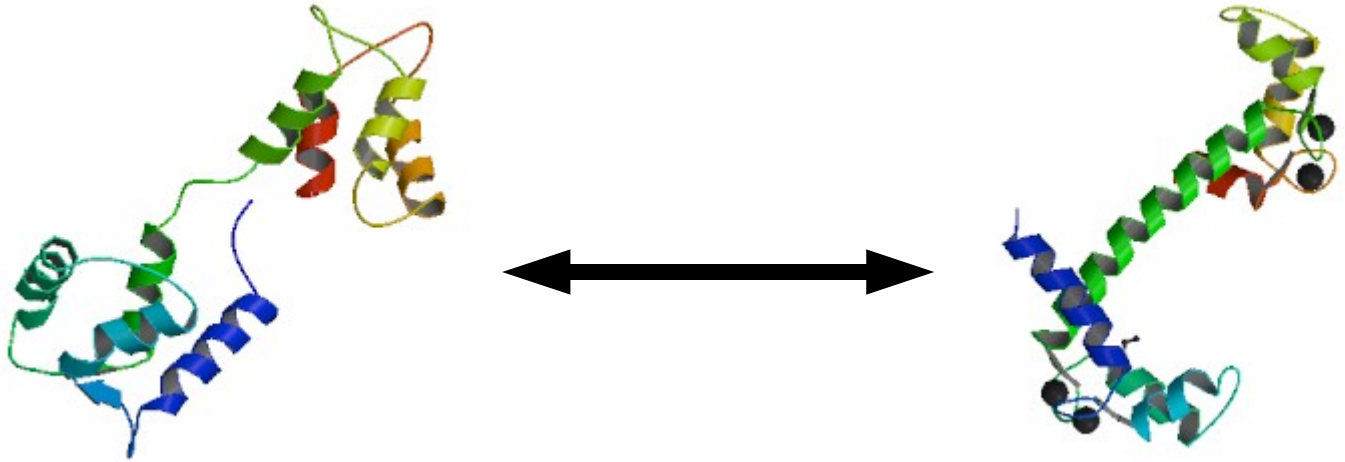
Calmodulin as a switch



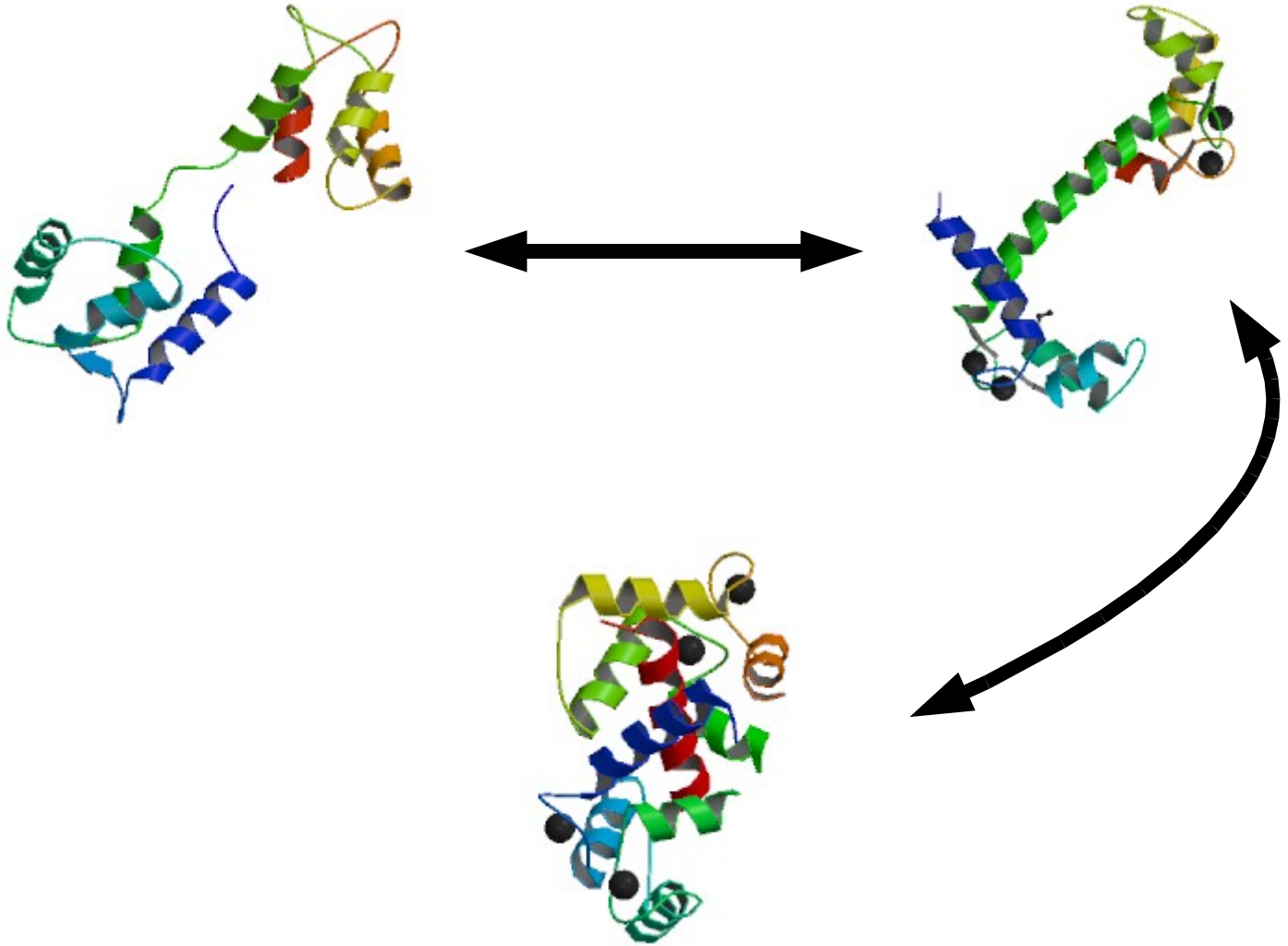
Calmodulin as a switch



Alternative structures of calmodulin



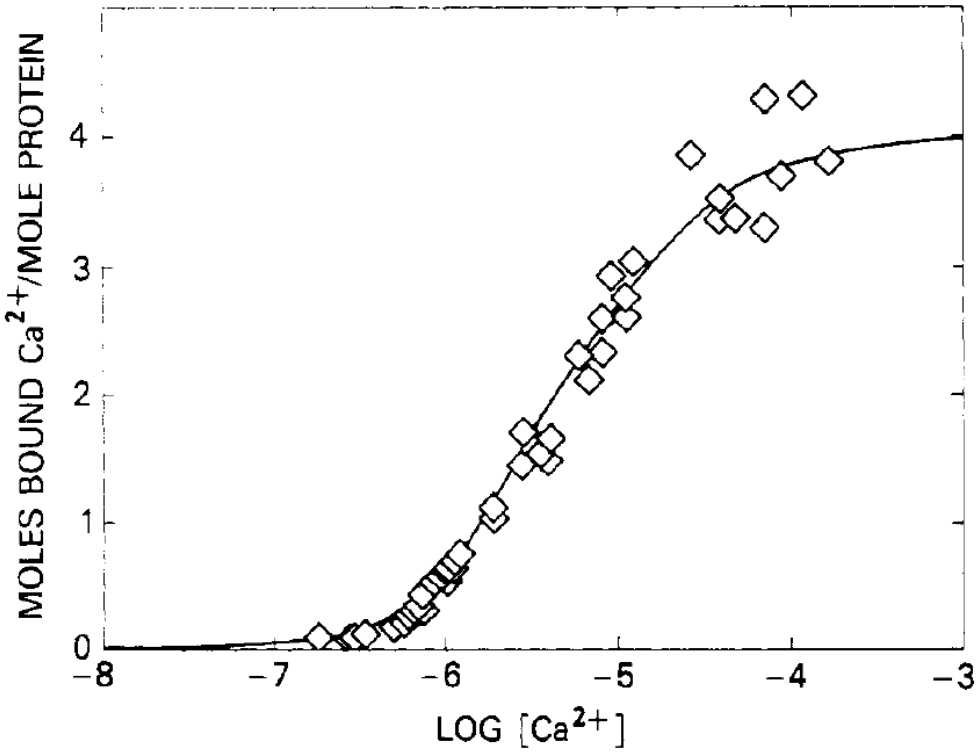
Alternative structures of calmodulin



Fitting of experimental results

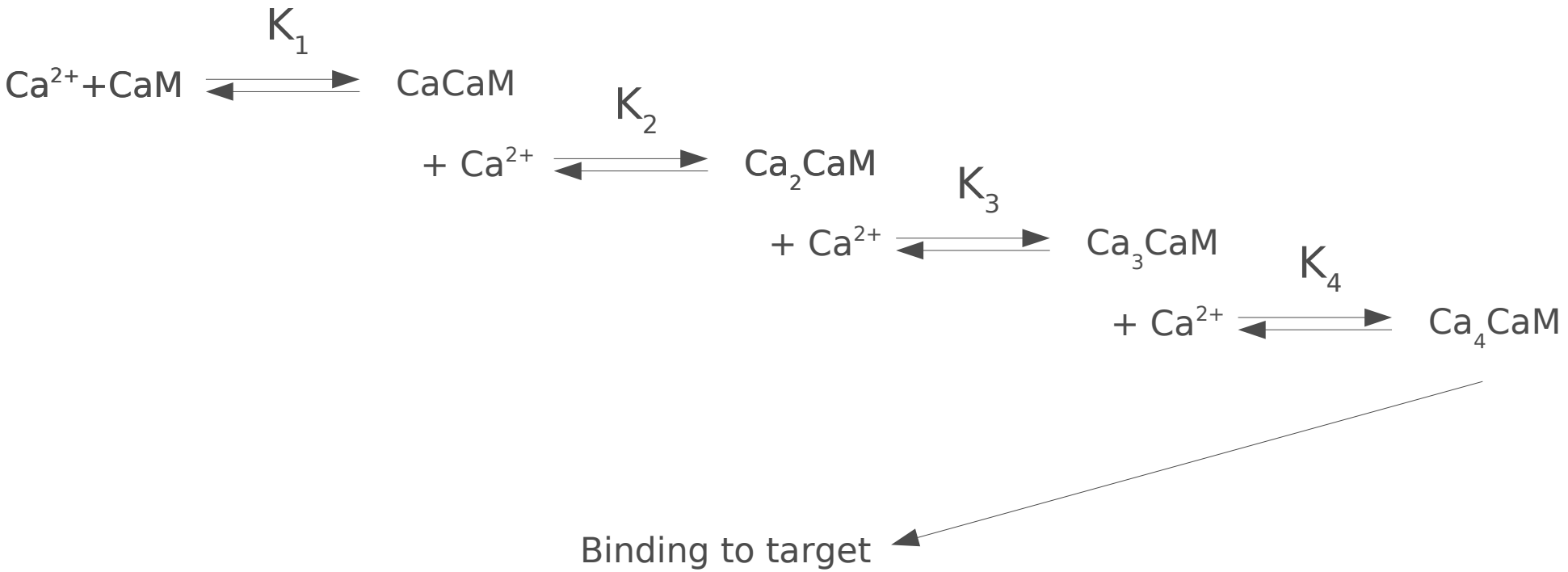
$$\overline{Y} = \frac{K_1[Ca]+2K_1K_2[Ca]^2+3K_1K_2K_3[Ca]^3+4K_1K_2K_3K_4[Ca]^4}{1+K_1[Ca]+K_1K_2[Ca]^2+K_1K_2K_3[Ca]^3+K_1K_2K_3K_4[Ca]^4}$$

Adair (1925)
J Biol Chem, 63: 529-545



Crouch and Klee (1980)
Biochemistry, 19: 3692-3698

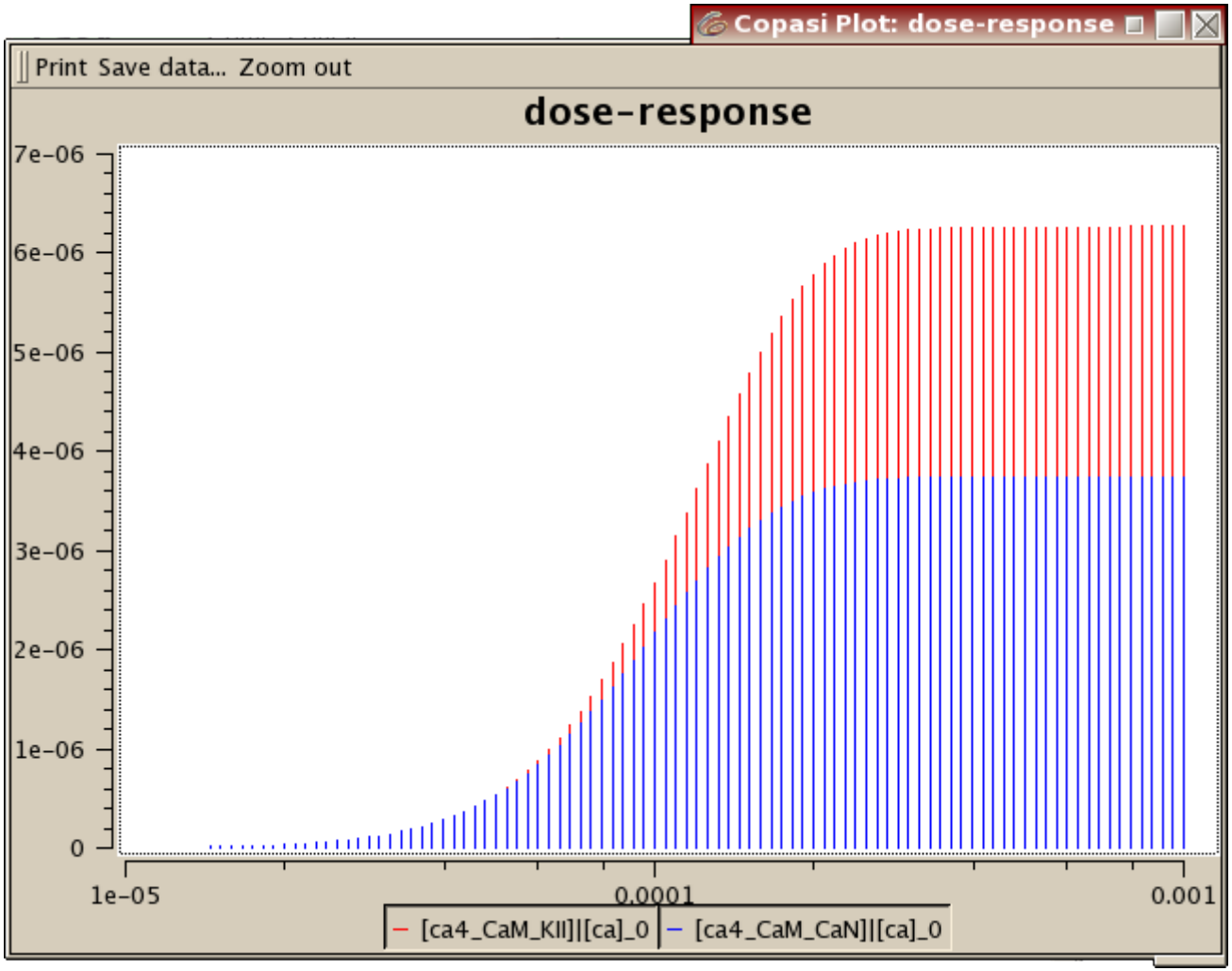
Phenomenological models



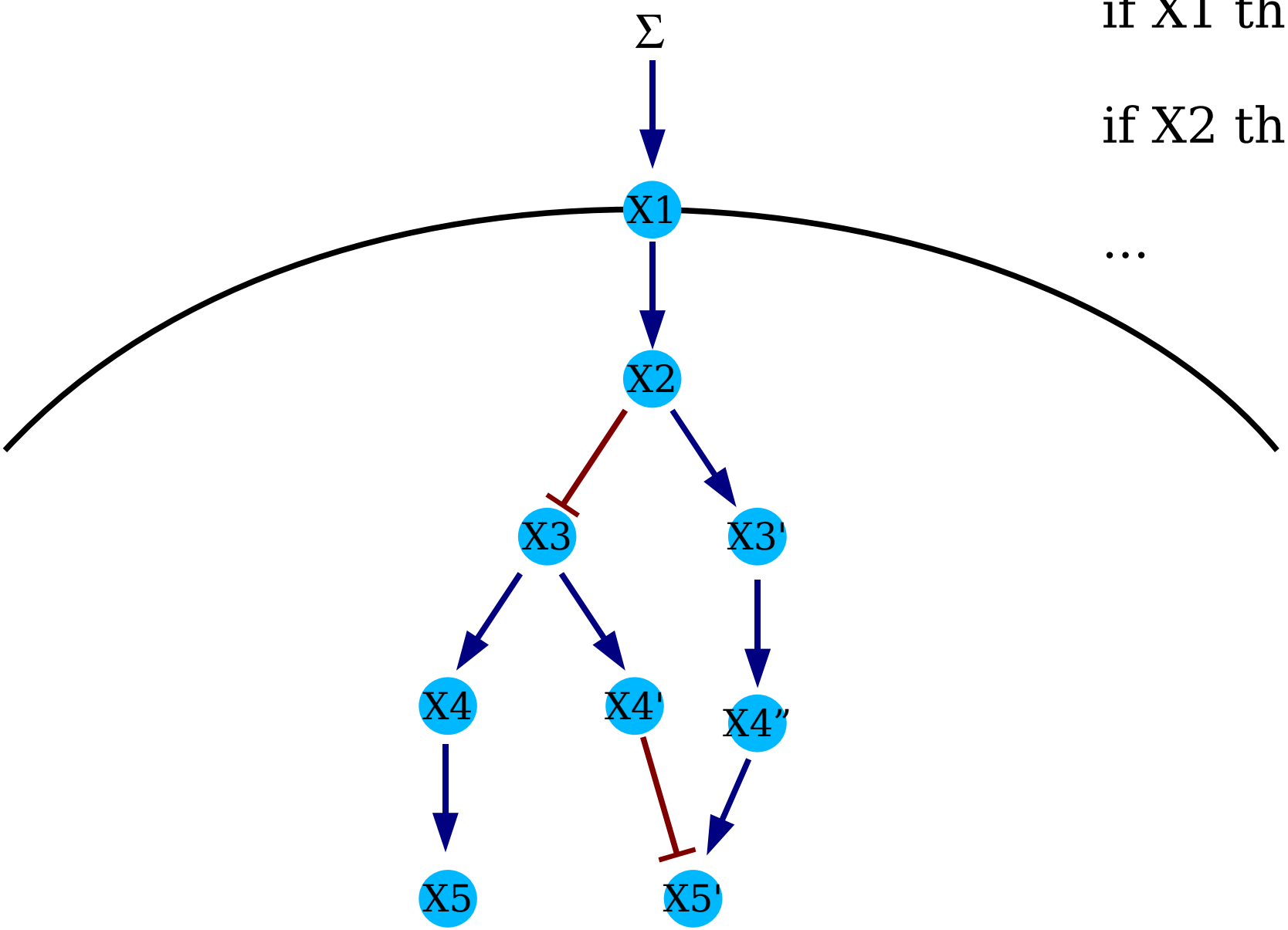
That does not work ...

- Calmodulin bound to three calcium activates calcineurin
  - Kincaid and Vaughan (1986). *PNAS*, 83: 1193-1197
- Calmodulin bound to two calcium can bind CaMKII
  - Shifman et al (2006). *PNAS*, 103: 13968-13973
- Calmodulin affinity for calcium increases once bound to CaMKII
  - Shifman et al (2006) [but many previous reports on other targets: e.g. Burger et al (1983). *JBC*, 258: 14733-14739 ; Olwin et (1984). *JBC* 259: 10949-10955]
- Calcium activates both LTP and LTD through calmodulin
  - Lisman (1989) *PNAS*, 86: 9574-9578
  - High [Ca^{2+}] (high freq) ≅ CaMKII ; Low [Ca^{2+}] (low freq) ≅ Calcineurin

That does not work ...



Signals, fluxes and induction



if X1 then X2

if X2 then not X3

…

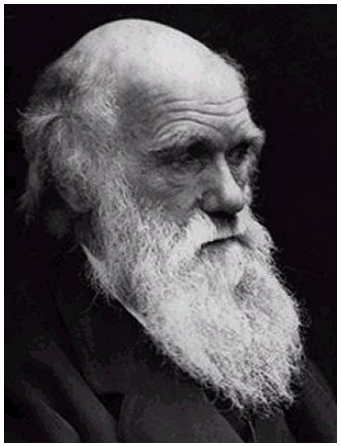
Induction Vs Selection

- Induction = BAD
  (intelligent design, Lamarck's first law, antibody moulding, directed axonal growth, induced-fit ...)

Induction Vs Selection

- Induction = BAD
  (intelligent design, Lamarck's first law, antibody moulding, directed axonal growth, induced-fit ...)

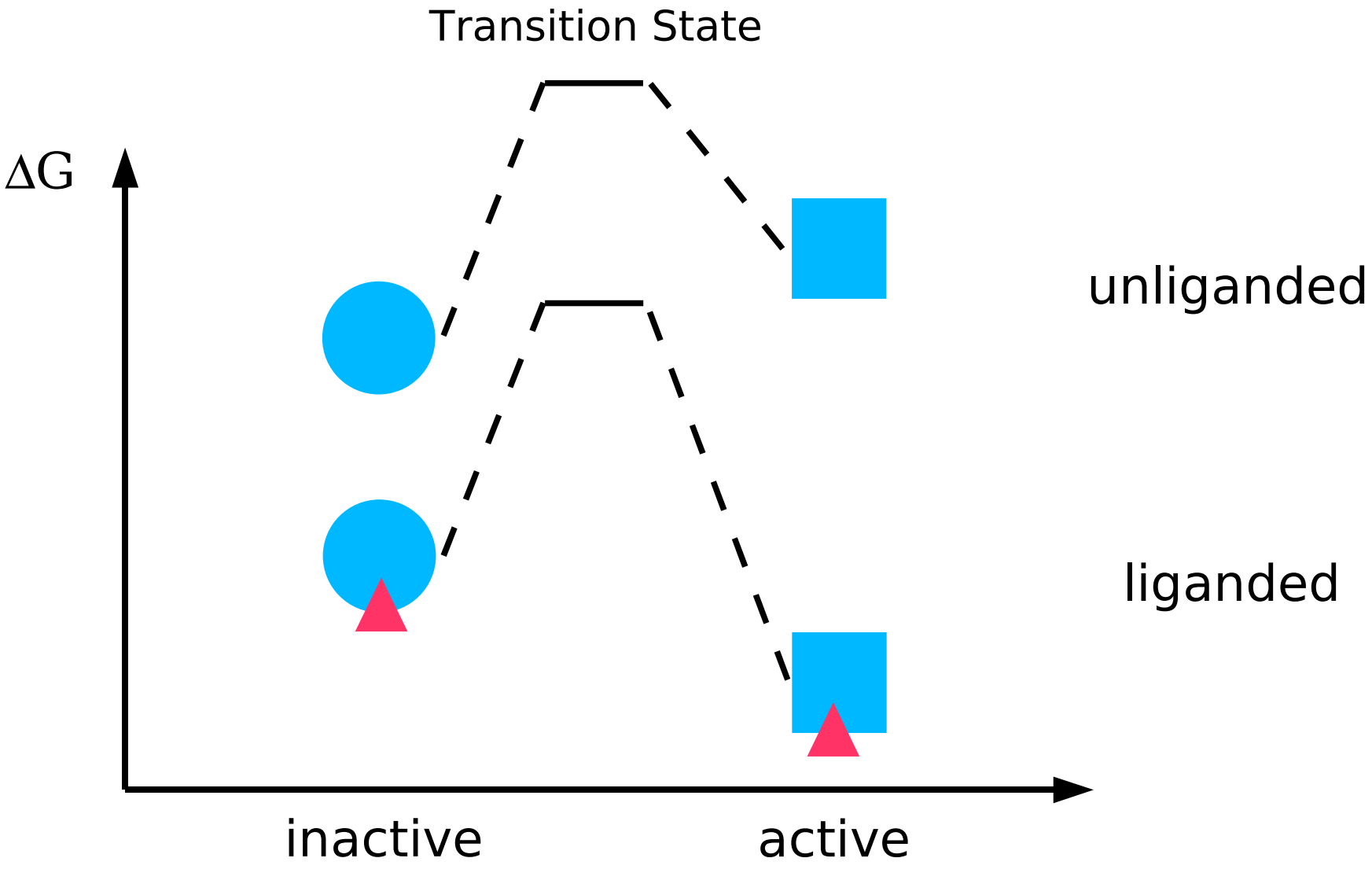
- Selection = GOOD
  (natural selection, clonal selection, synapse stabilisation, concerted transitions ...)



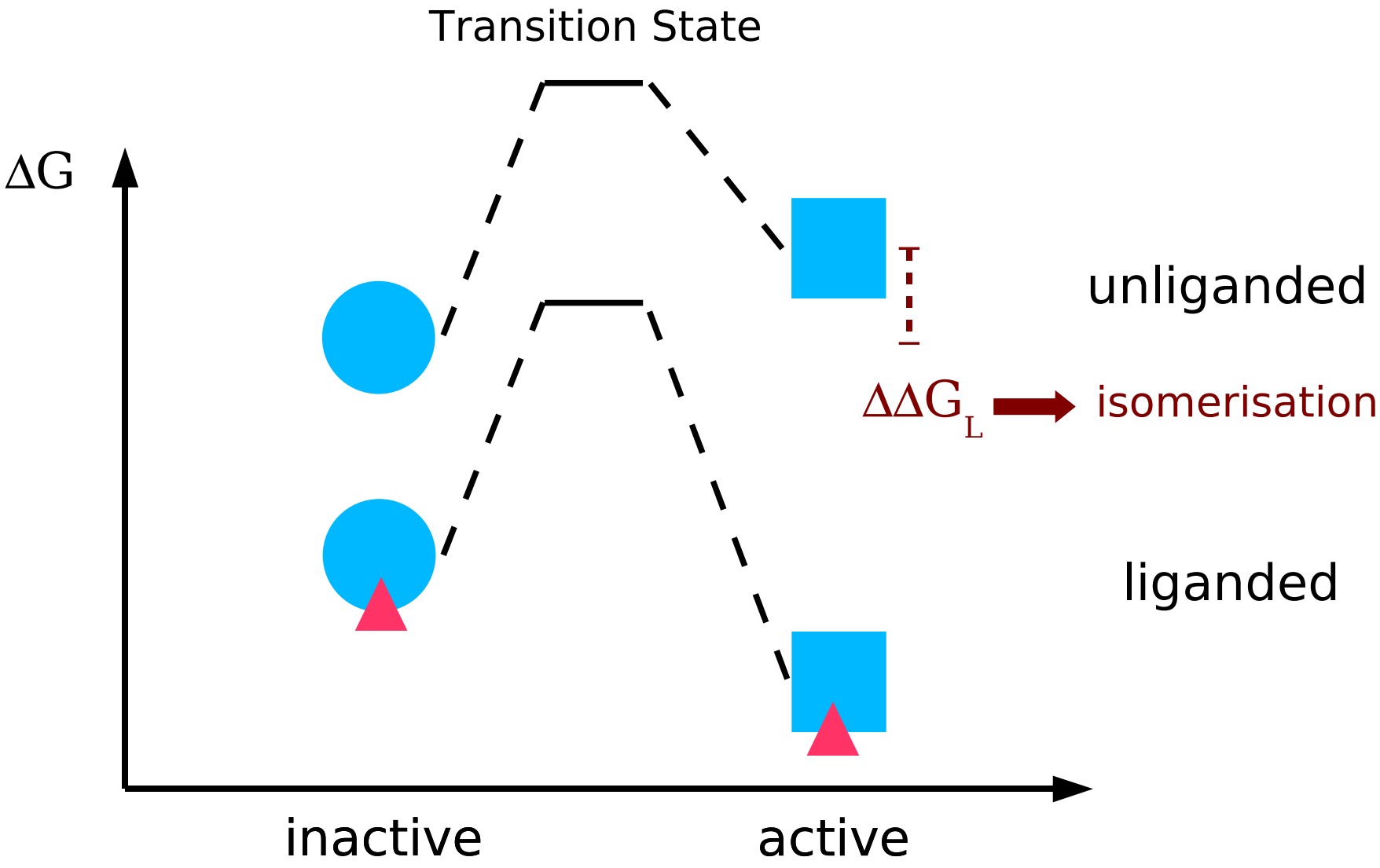
Allostery and state selection

- Monod, Wyman, Changeux (1965). *J Mol Biol*, 12: 88-118

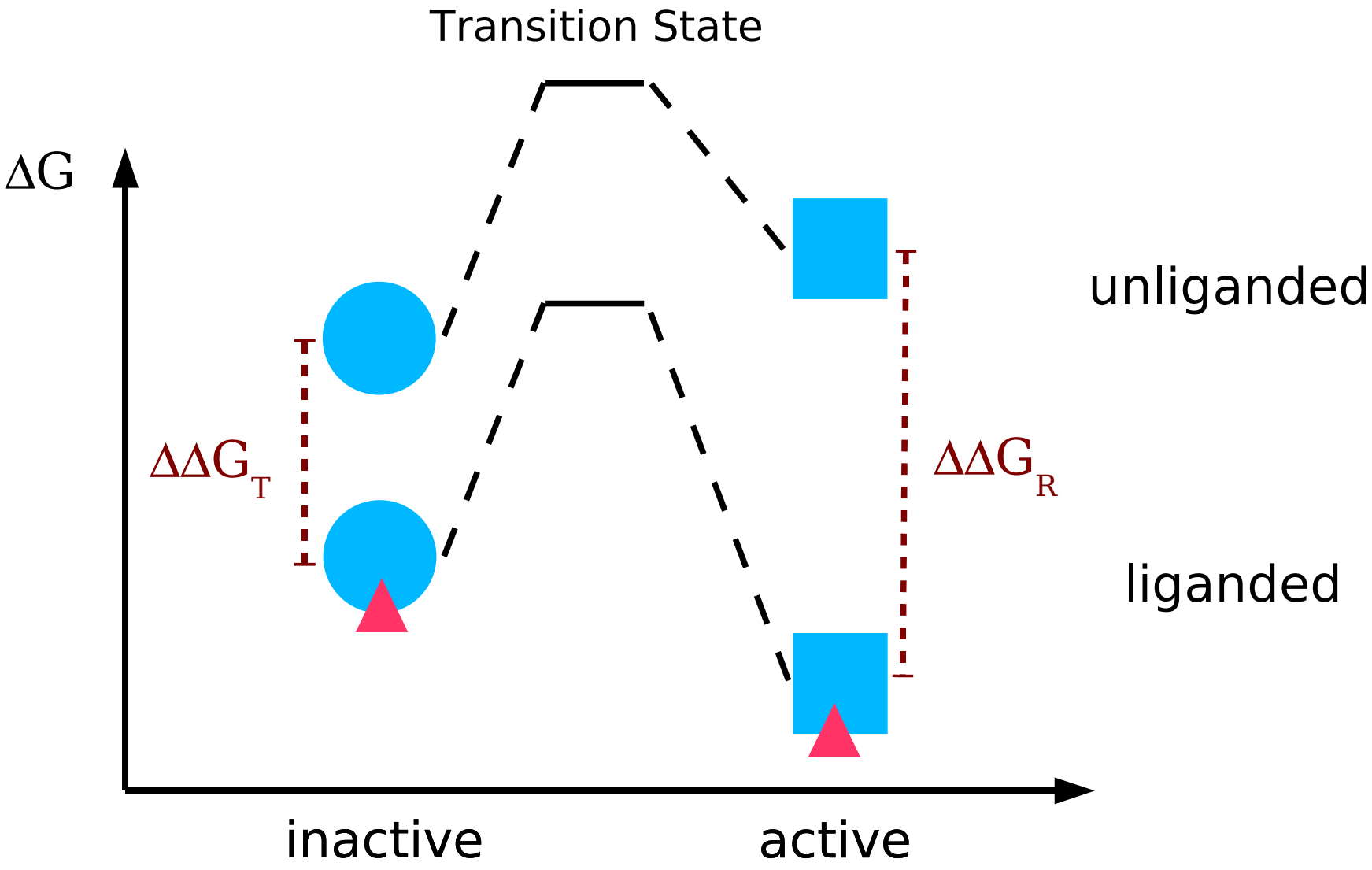
Free energy, ligand binding and isomerisation



Free energy, ligand binding and isomerisation

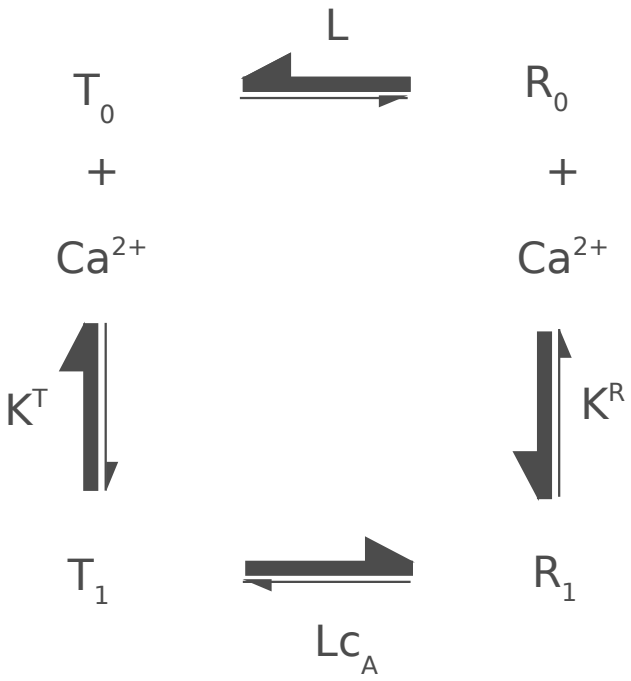


Free energy, ligand binding and isomerisation



$$K \propto e^{-(\Delta\Delta G/RT)}$$

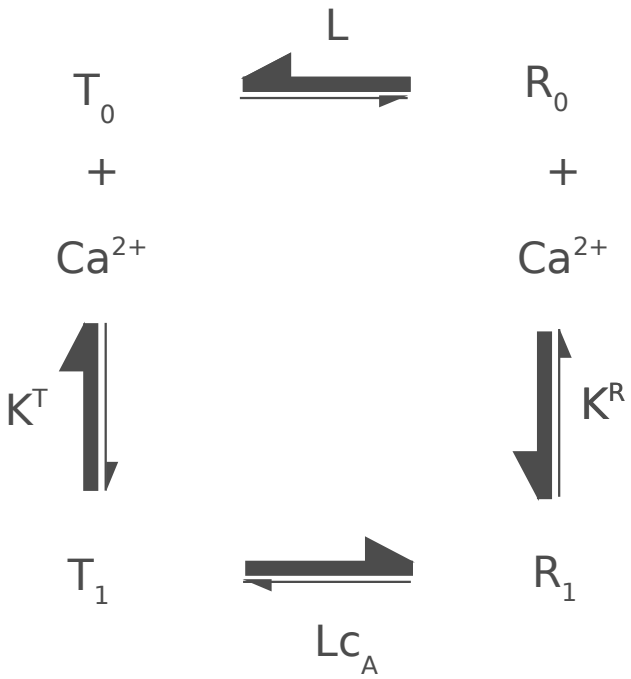
Allosteric regulation of calmodulin

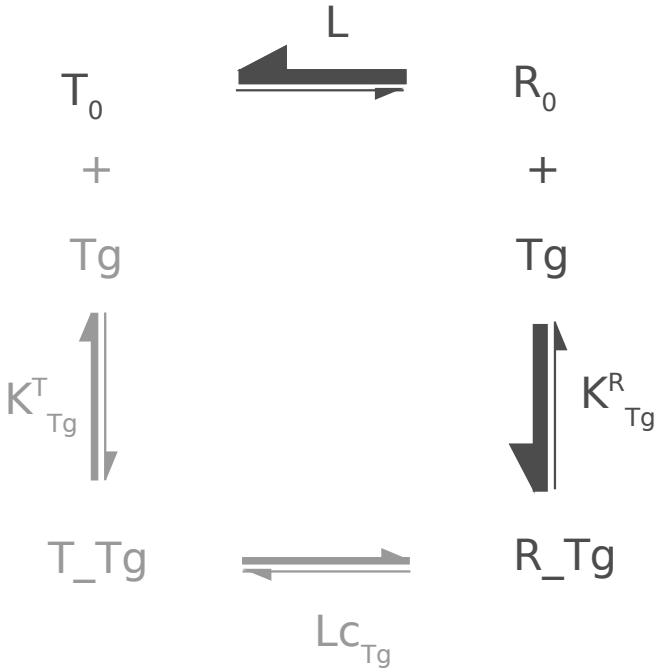


$$L = \frac{[T0]}{[R0]}$$

$$C_I = \frac{K^R_I}{K^T_I}$$

Allosteric regulation of calmodulin

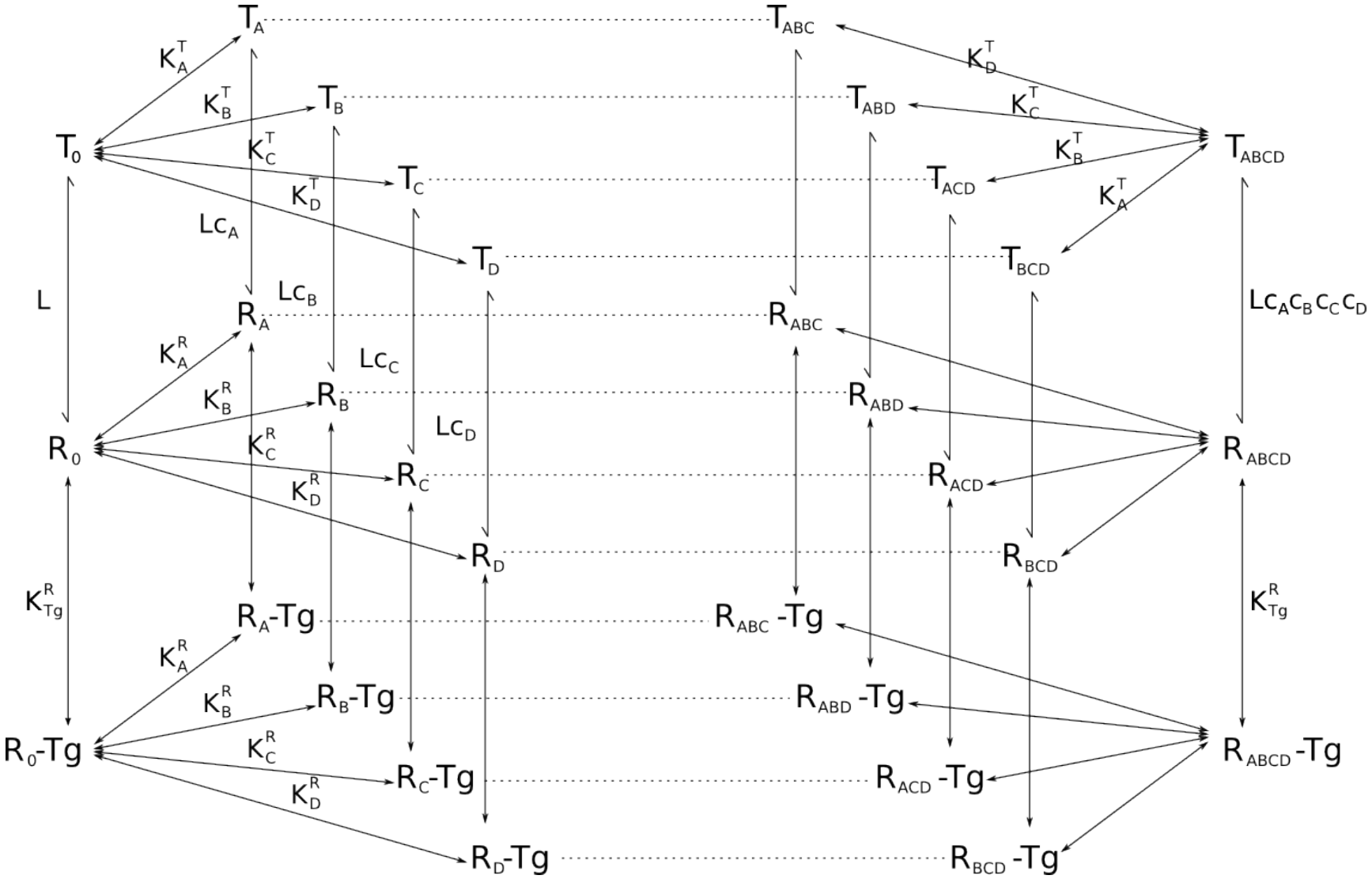




$$L = \frac{[T0]}{[R0]}$$

$$C_I = \frac{K^R_I}{K^T_I}$$

Full mechanistic thermodynamic model



$$L = \frac{[T0]}{[R0]} \qquad C_I = \frac{K^R_I}{K^T_I}$$

Model constraints

Parameter estimation constrained by:

- Calcium binding R and T states
  - Crouch and Klee (1980) *Biochemistry*, 19: 3692-3698
- Calcium binding R state only (locked by troponin) and isomerisation constant
  - Keller et al (1982). *Biochemistry*, 21: 156-162
- Calcium binding of N and C terminals
  - Shifman et al (2006) *PNAS*, 103: 13968-13973

- CaN affinity for CaM R state >> CaMKII one
- [CaMKII] >> [CaN]

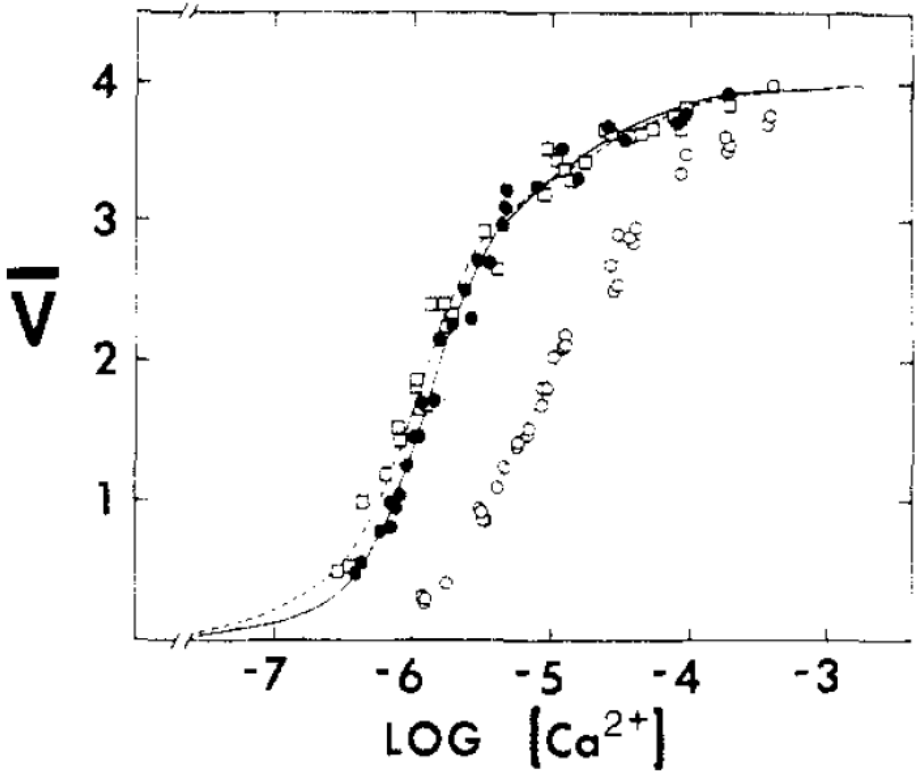


FIGURE 2: Ca^{2+} binding to CaM in the absence (○) and in the presence of stoichometric ● and higher (□) concentrations of TnI.

Keller et al (1982) *Biochemistry*, 21: 156-162

Monod, Wyman, Changeux (1965). *J Mol Biol*, 12: 88-118

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

- α = [ligand]/K^R_{lig}
- γ = [activator]/K^R_{act}
- n : number of ligand binding sites
- m : number of activator binding sites

- Make $\bar{Y}$ = 0.5 and replace α by $\alpha_{½}$ for both [troponin]

Getting L

Monod, Wyman, Changeux (1965). *J Mol Biol*, 12: 88-118

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

- α = [ligand]/K^R_{lig}
- γ = [activator]/K^R_{act}
- n : number of ligand binding sites
- m : number of activator binding sites

$L \approx 20\,000$

- Make $\bar{Y} = 0.5$ and replace α by $\alpha_{½}$ for both [troponin]

Getting c and K^R

$$\bar{Y} = \frac{\sum \alpha_i + 2\sum \alpha_i\alpha_j + 3\sum \alpha_i\alpha_j\alpha_k + 4\alpha_A\alpha_B\alpha_C\alpha_D}{4\left(\prod(1+\alpha_i) + L\prod(1+c_i\alpha_i)\right)}$$

$$+L\frac{\left(\sum c_i\alpha_i + 2\sum c_i\alpha_i c_j\alpha_j + 3\sum c_i\alpha_i c_j\alpha_j c_k\alpha_k + 4c_A\alpha_A c_B\alpha_B c_C\alpha_C c_D\alpha_D\right)}{4\left(\prod(1+\alpha_i) + L\prod(1+c_i\alpha_i)\right)}$$

$\alpha_i = [Ca]/K^R_i$

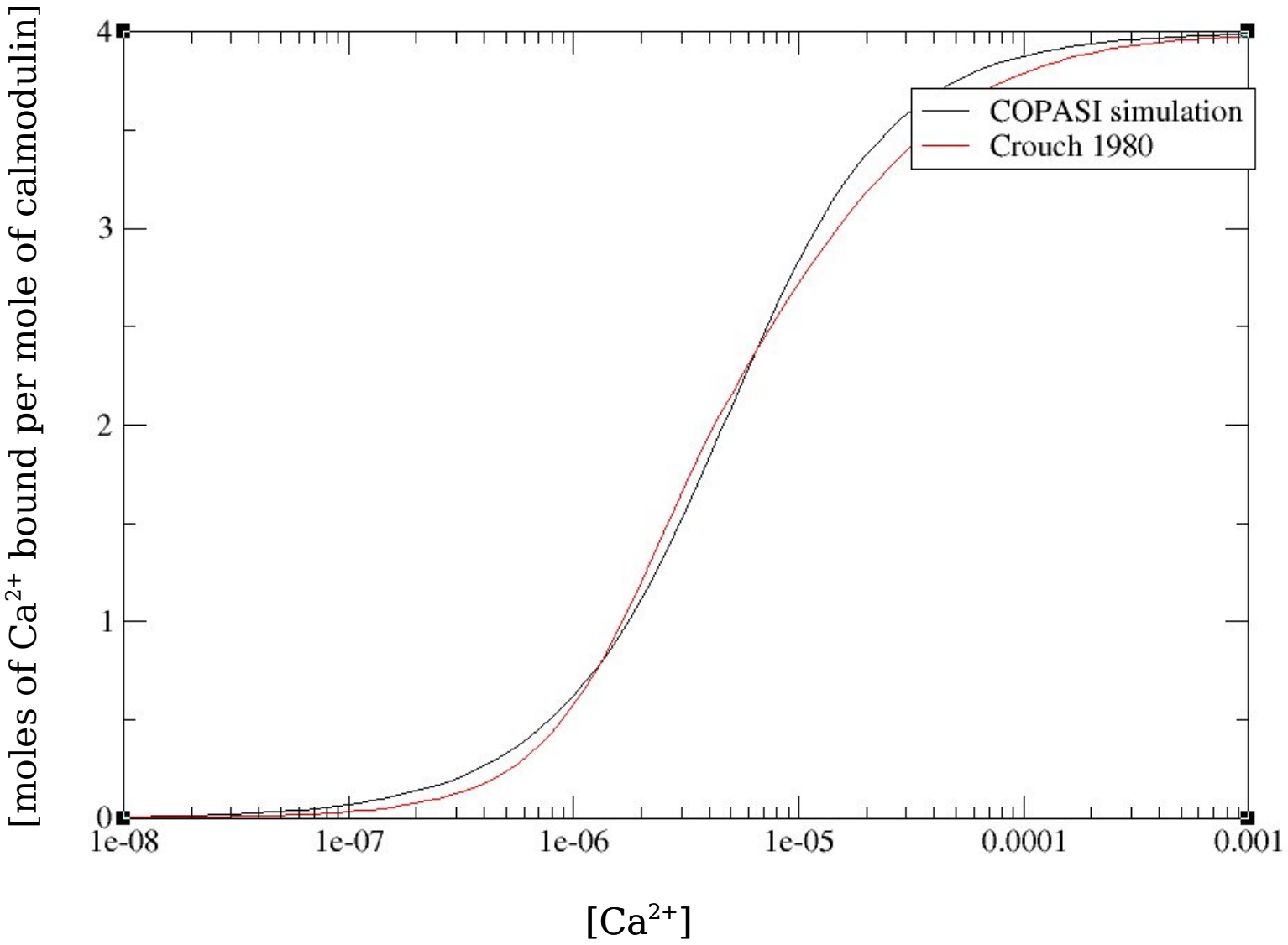
Use calmodulin with mutated Ca binding sites
(Shifman et al (2006) *PNAS*, 103: 13968-13973)

→ Reduced equation

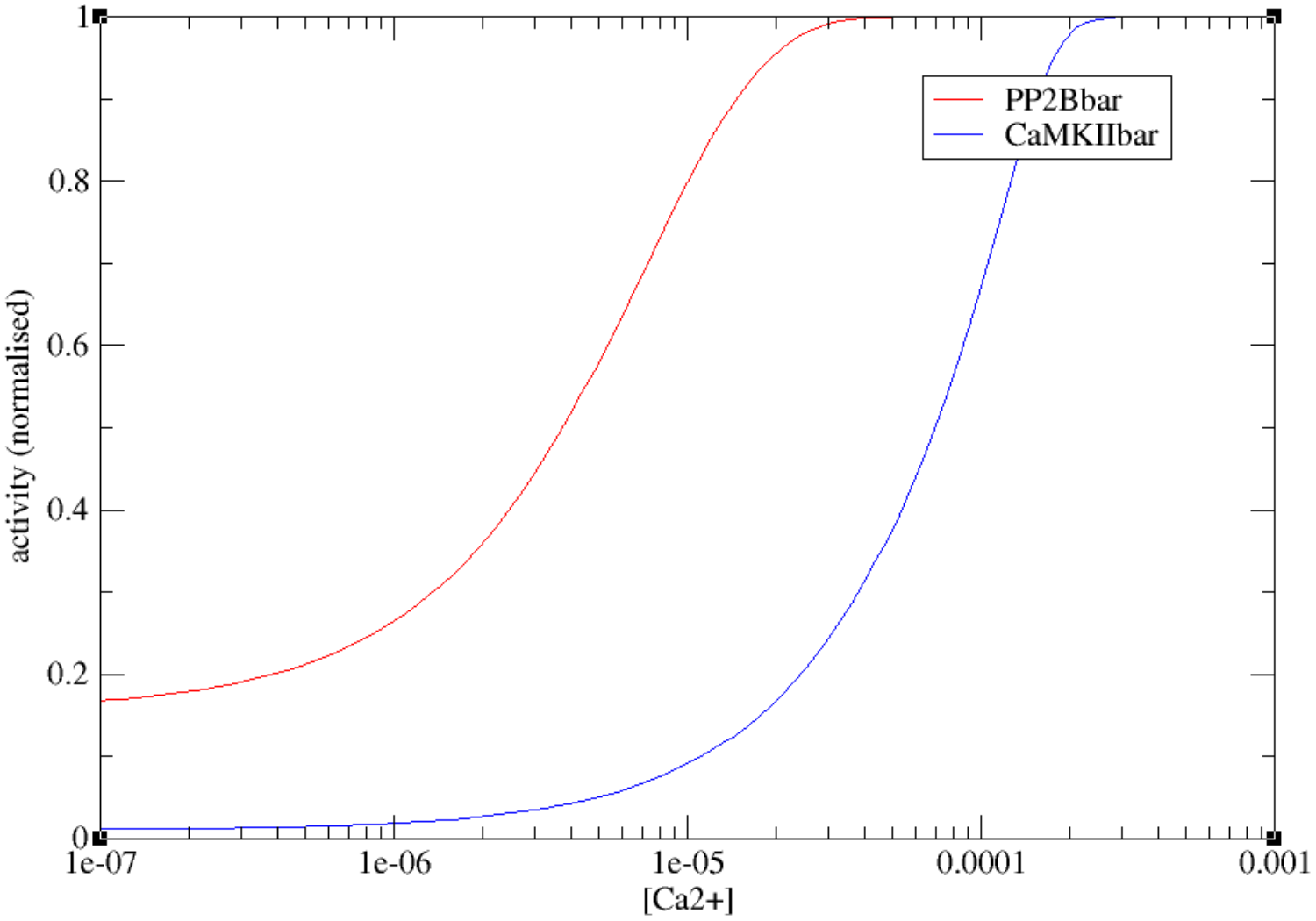
Derivation of Adair's constants

$$K_1 = 4\frac{1+L}{\frac{1}{K_A^R}+\frac{1}{K_B^R}+\frac{1}{K_C^R}+\frac{1}{K_D^R}+L\left(\frac{1}{K_A^T}+\frac{1}{K_B^T}+\frac{1}{K_C^T}+\frac{1}{K_D^T}\right)}$$

Parameter scan on kinetic simulations

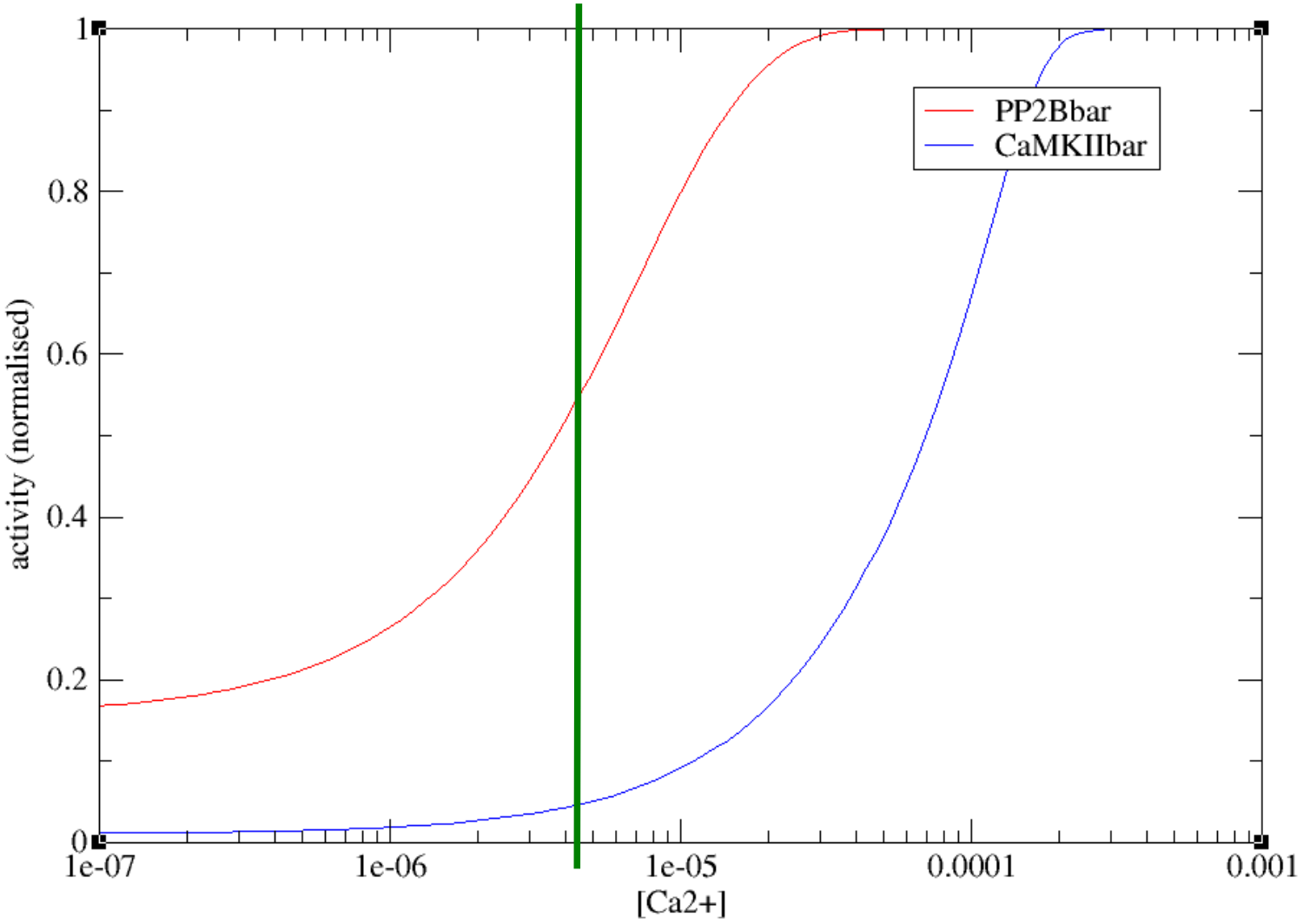


Bidirectional synaptic plasticity

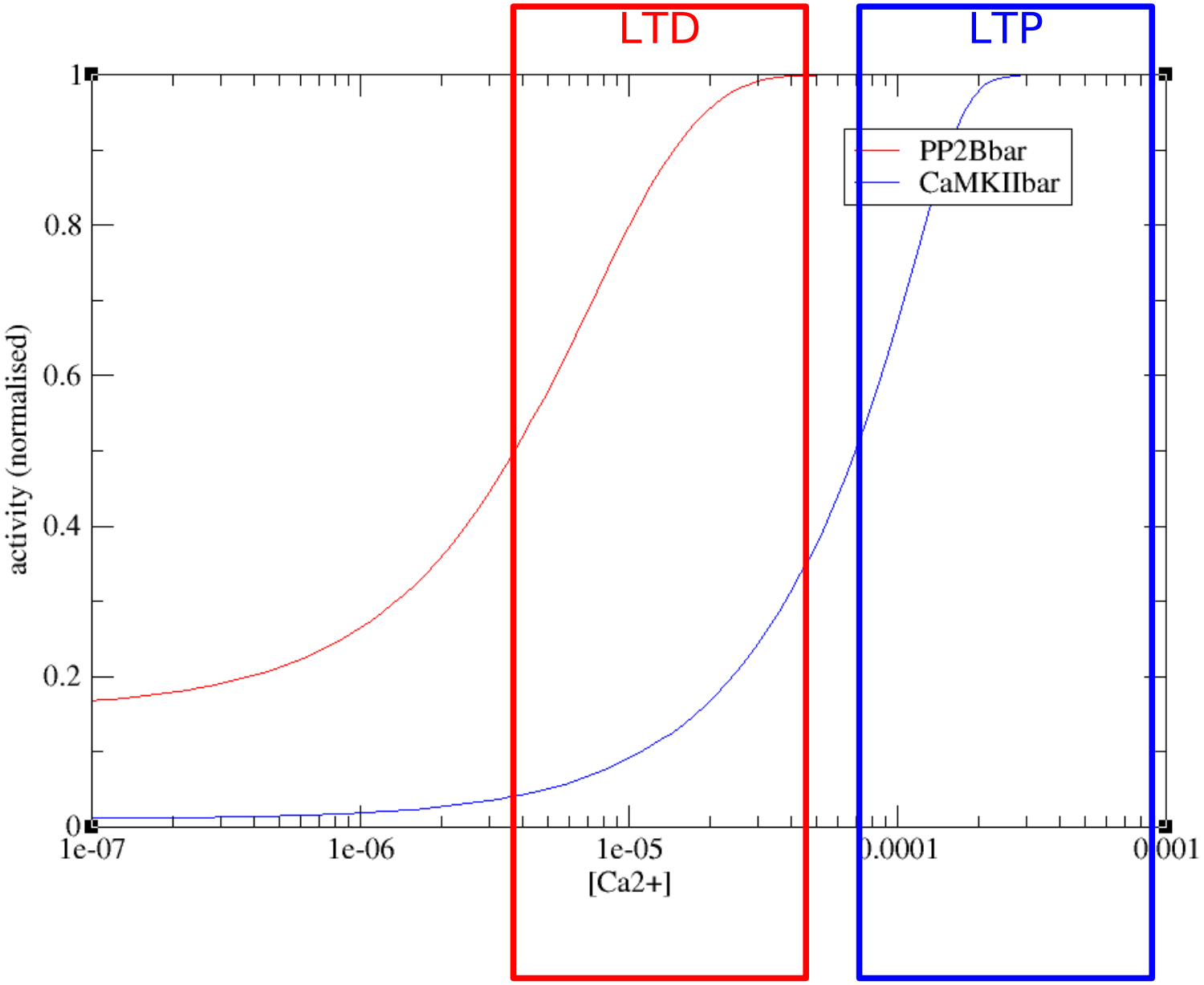


Bidirectional synaptic plasticity

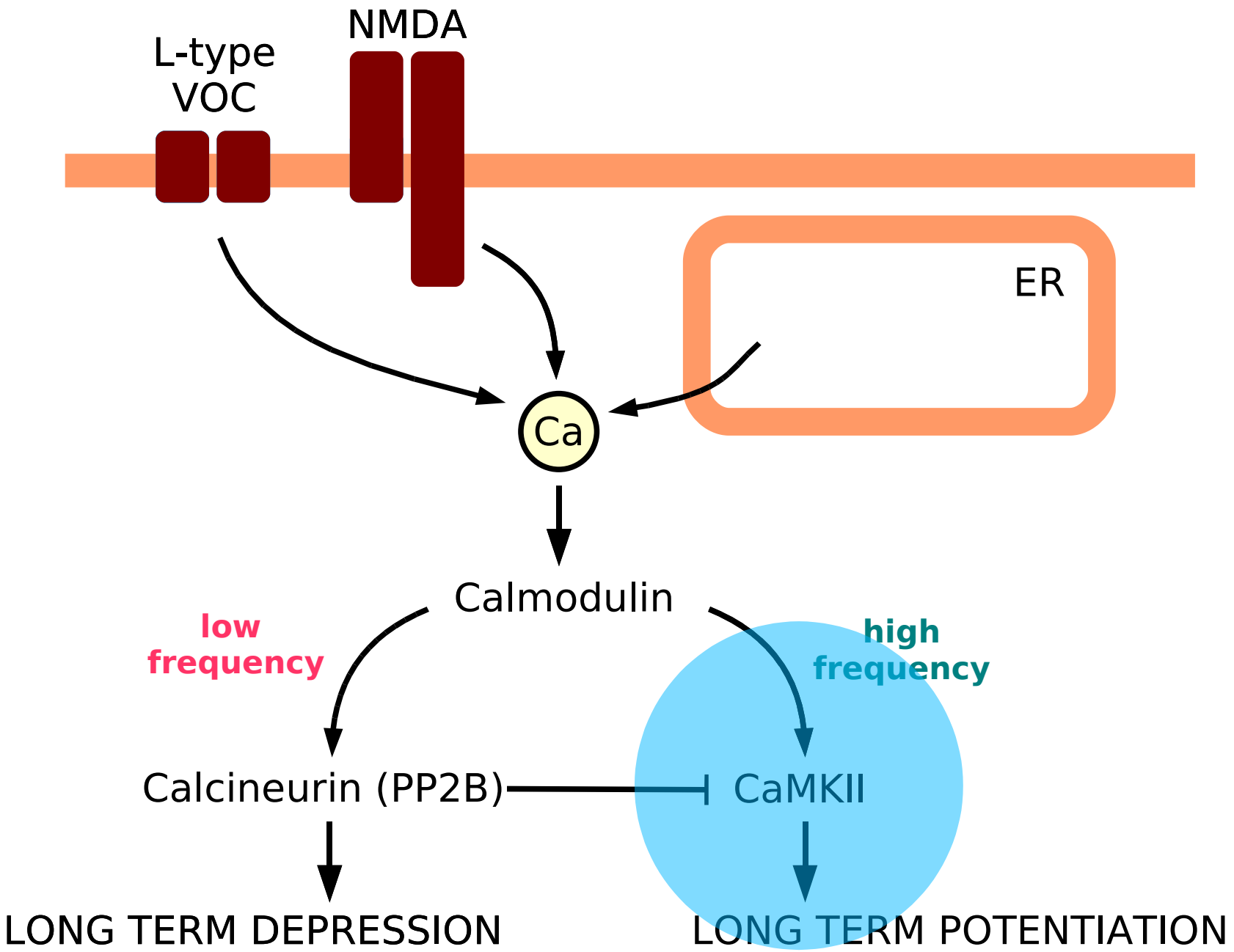
half saturation of calmodulin



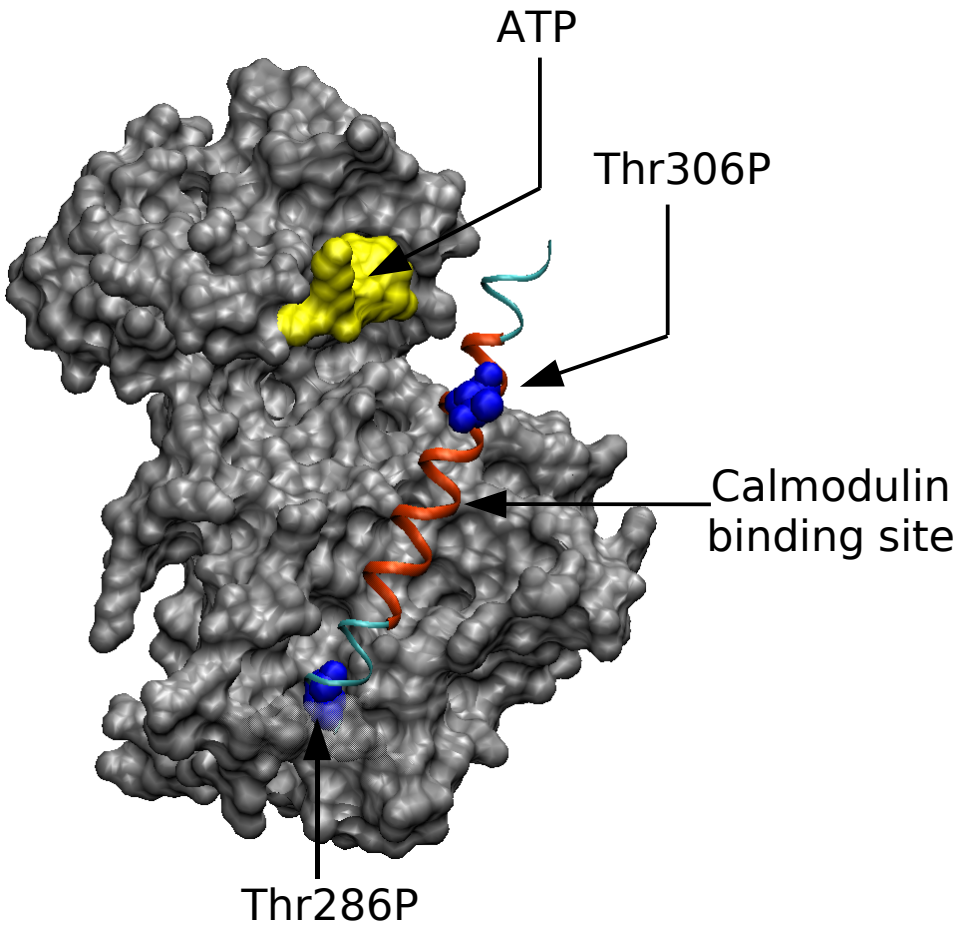
Bidirectional synaptic plasticity

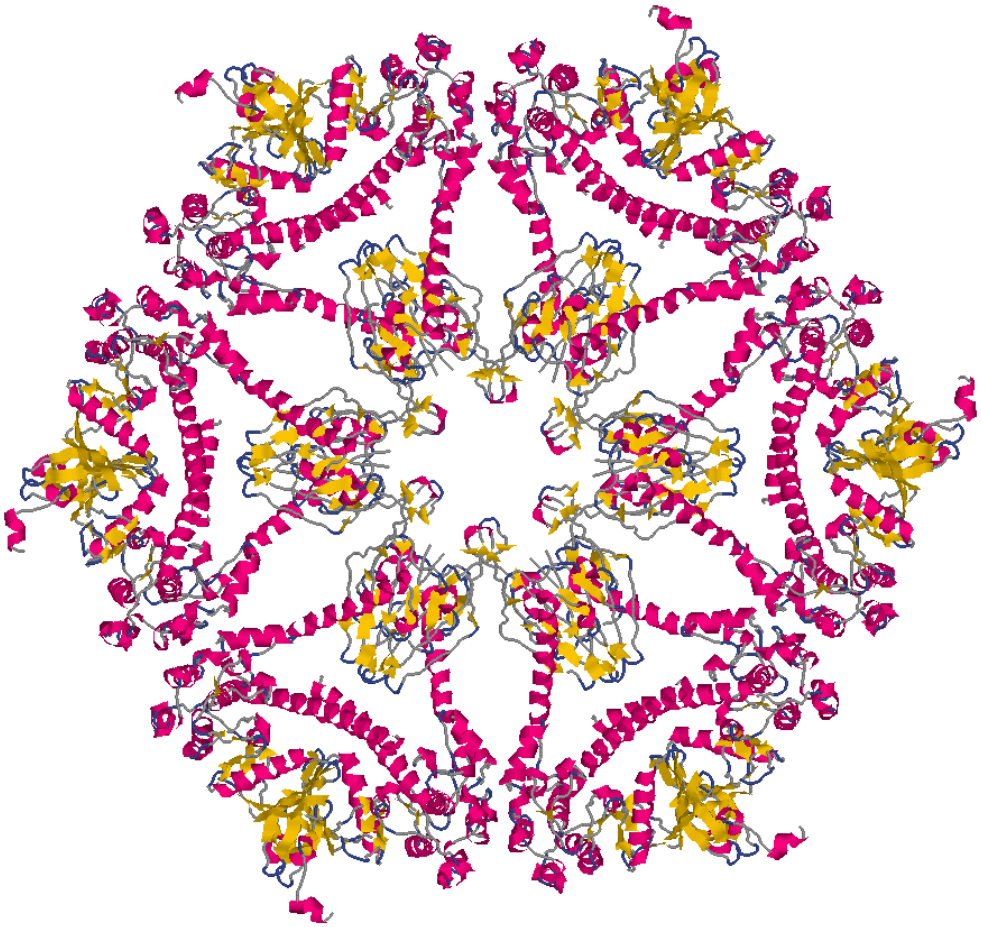


What next?


L-type
VOC
NMDA
ER
Ca
Calmodulin
low
frequency
high
frequency
Calcineurin (PP2B)
CaMKII
LONG TERM DEPRESSION
LONG TERM POTENTIATION

What next?





5x12 state variables=

1 152 900 000 000 000 000 states

(1 billion of billion)

Acknowledgements

- E-cell developers
  (Institute of Advanced Biosciences, Tsuruoka)

  Kouichi Takahashi
  Kazunari Kaizu
  Gabor Bereczki

- COPASI developers
  (Virginia Bioinformatics Institute
  EML-research)

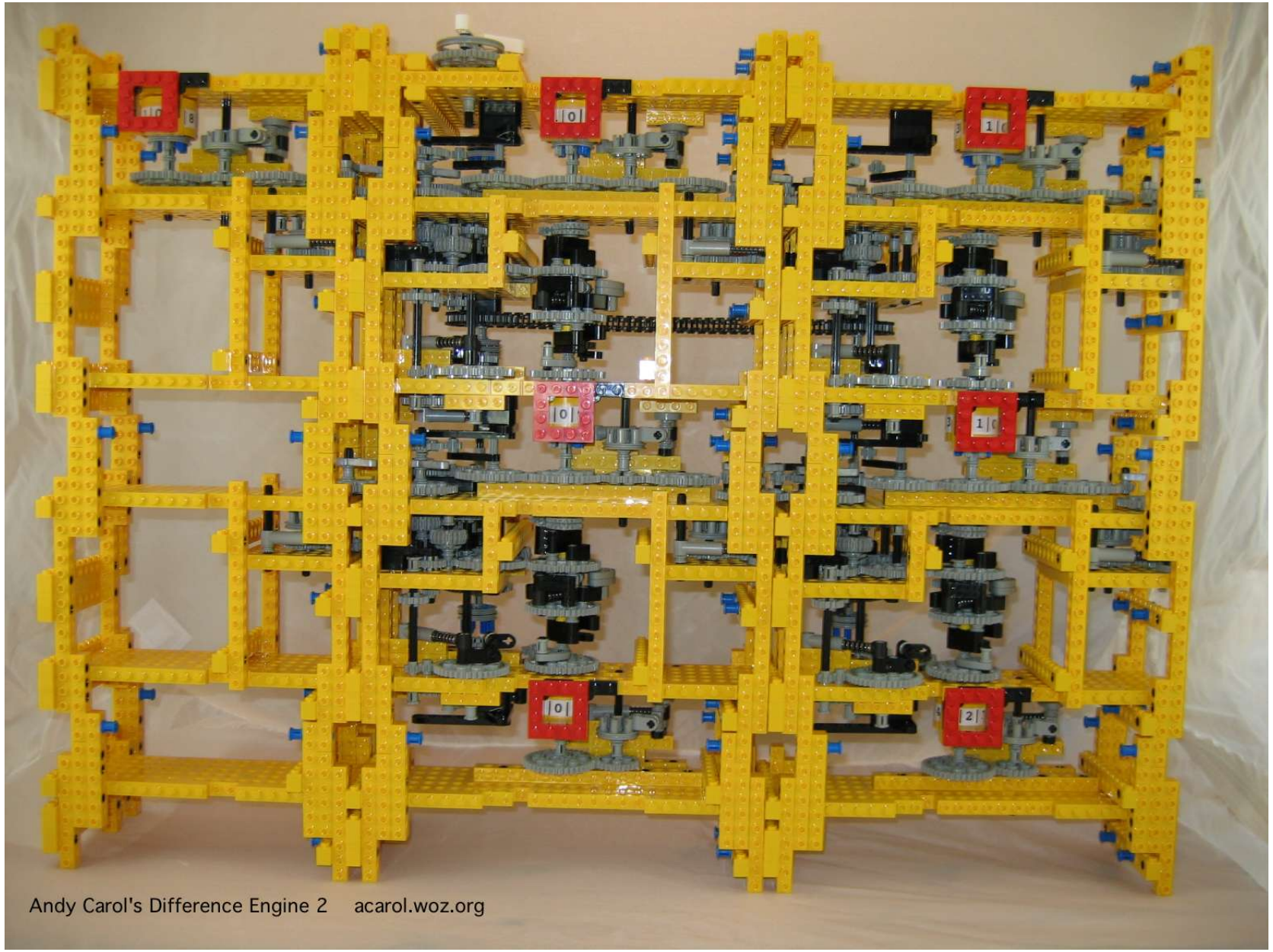
  Ursula Kummer
  Pedro Mendes
  Stefan Hoops
  Sven Sahle
  Ralf Gauges

- Compneur group
  (EBI)

  Prof Stuart Edelstein, visitor
  Eric Fernandez, post-doc
  Renaud Schiappa, undergraduate
  Melanie Stefan, PhD student

- Collaborators
  (INSERM U536, Paris)

  Jean-Antoine Girault
  Denis Herve



Andy Carol's Difference Engine 2 acarol.woz.org

Derivation of Adair's constants

$$K_1 = \frac{\text{concentration of ligand binding sites} \times \text{ligand concentration}}{\text{concentration of protein bound to exactly one ligand molecule}}$$

$$= \frac{4[P_0][X]}{[P_1]}$$

Derivation of Adair's constants

$$K_1 = \frac{4([R_0] + [T_0])[X]}{[R_1] + [T_1]}$$

Derivation of Adair's constants

$$K_1 = 4\frac{([R_0] + [T_0])[X]}{([R_A] + [R_B] + [R_C] + [R_D]) + ([T_A] + [T_B] + [T_C] + [T_D])}$$

Derivation of Adair's constants

$$K_1 = 4\frac{([R_0] + L[R_0])[X]}{\left(\frac{[R_0][X]}{K_A^R} + \frac{[R_0][X]}{K_B^R} + \frac{[R_0][X]}{K_C^R} + \frac{[R_0][X]}{K_D^R}\right) + \left(\frac{[T_0][X]}{K_A^T} + \frac{[T_0][X]}{K_B^T} + \frac{[T_0][X]}{K_C^T} + \frac{[T_0][X]}{K_D^T}\right)}$$

$$= 4\frac{[R_0][X](1+L)}{[R_0][X]\left(\frac{1}{K_A^R} + \frac{1}{K_B^R} + \frac{1}{K_C^R} + \frac{1}{K_D^R}\right) + [T_0][X]\left(\frac{1}{K_A^T} + \frac{1}{K_B^T} + \frac{1}{K_C^T} + \frac{1}{K_D^T}\right)} =$$

$$= 4\frac{[R_0][X](1+L)}{[R_0][X]\left(\frac{1}{K_A^R} + \frac{1}{K_B^R} + \frac{1}{K_C^R} + \frac{1}{K_D^R}\right) + L[R_0][X]\left(\frac{1}{K_A^T} + \frac{1}{K_B^T} + \frac{1}{K_C^T} + \frac{1}{K_D^T}\right)}$$

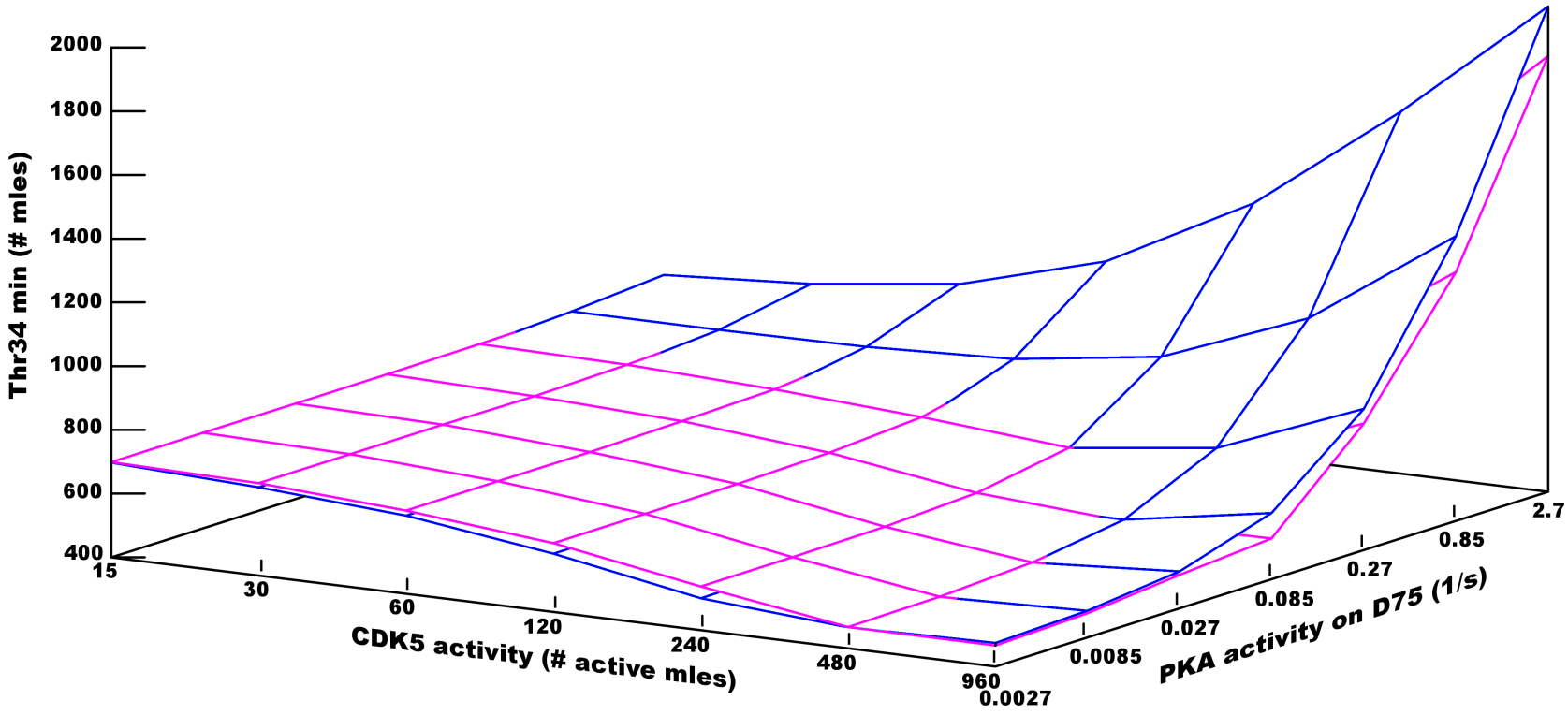
Derivation of Adair's constants

$$K_i = \frac{N - i + 1}{i} \frac{S_{i-1}^R + LS_{i-1}^T}{S_i^R + LS_i^T}$$

$$S_i^R := \sum_{K_j^R \in \{K_A^R, K_B^R, \dots\}, j_1 < j_2 < \dots < j_i} \frac{1}{K_{j_1}^R} \cdots \frac{1}{K_{j_i}^R}$$

$$S_i^T := \sum_{K_j^T \in \{K_A^T, K_B^T, \dots\}, j_1 < j_2 < \dots < j_i} \frac{1}{K_{j_1}^T} \cdots \frac{1}{K_{j_i}^T}$$

Some parameters are sensitive



Some parameters are robust