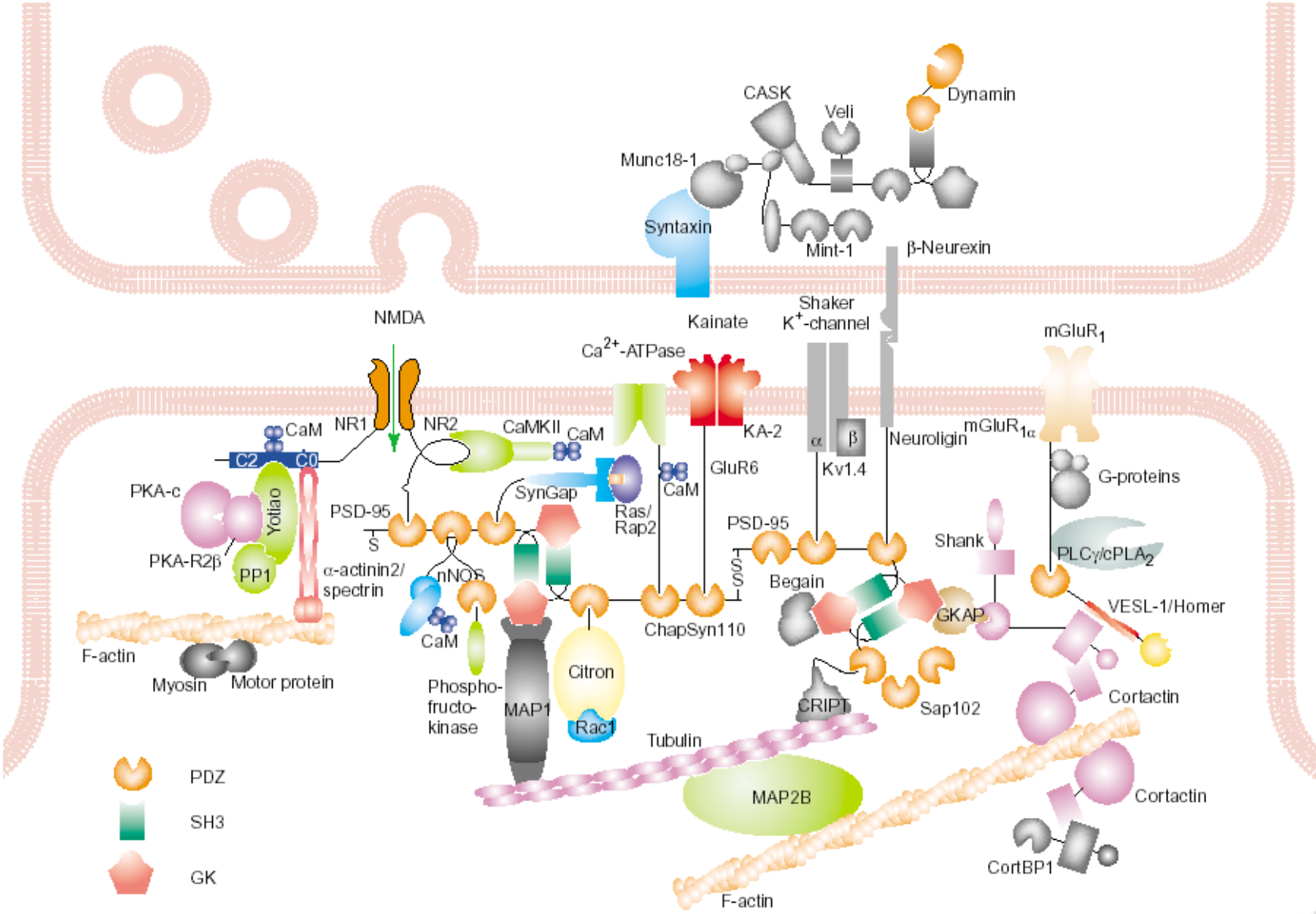
Mesoscopic modelling of receptive membranes

Nicolas Le Novère, EMBL-EBI

Complex post-synaptic machinery



Husi & Grant (2001) *TINS, 24*: 259-266

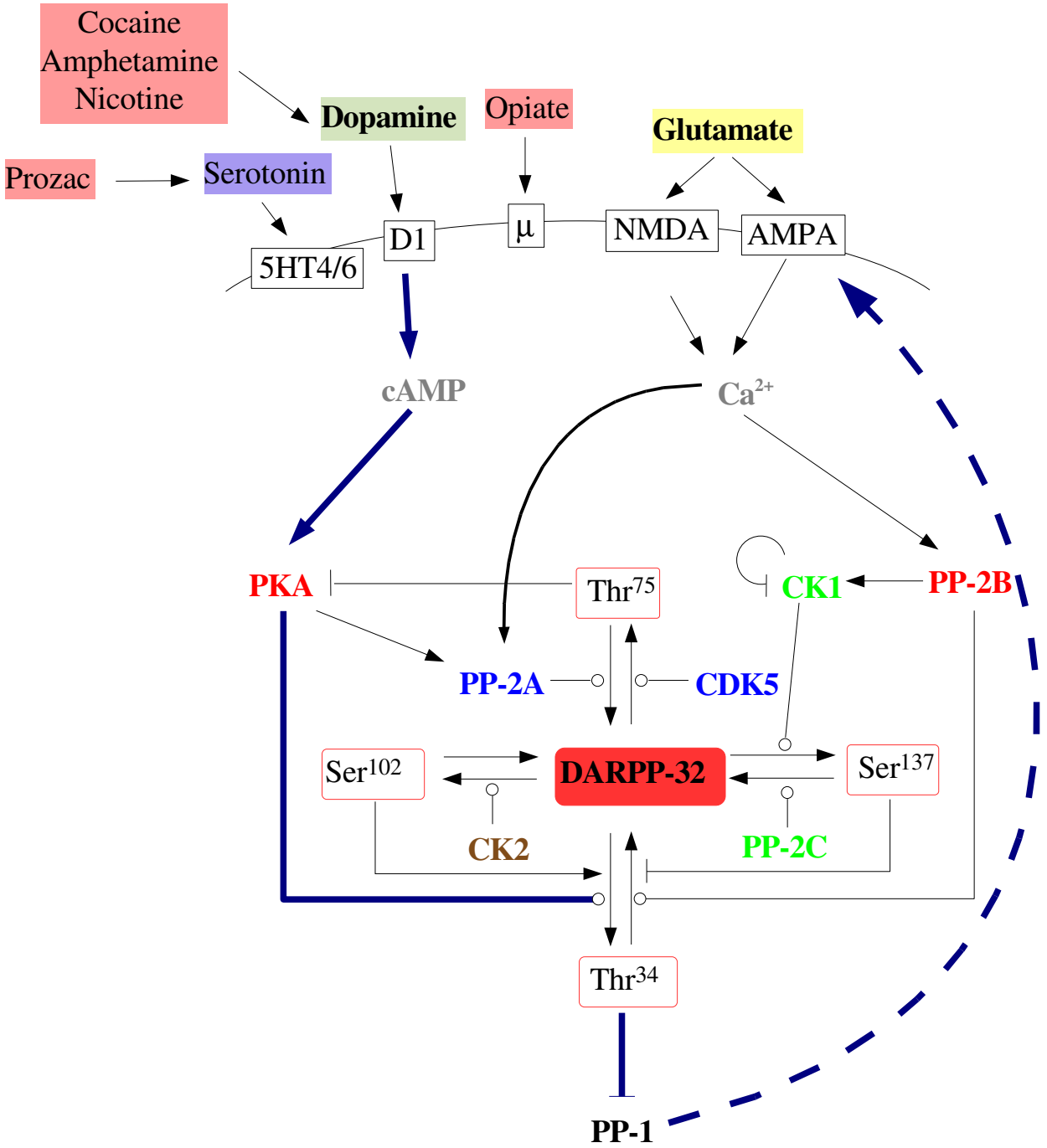
Microscopic: Molecular dynamics

You are the experts.

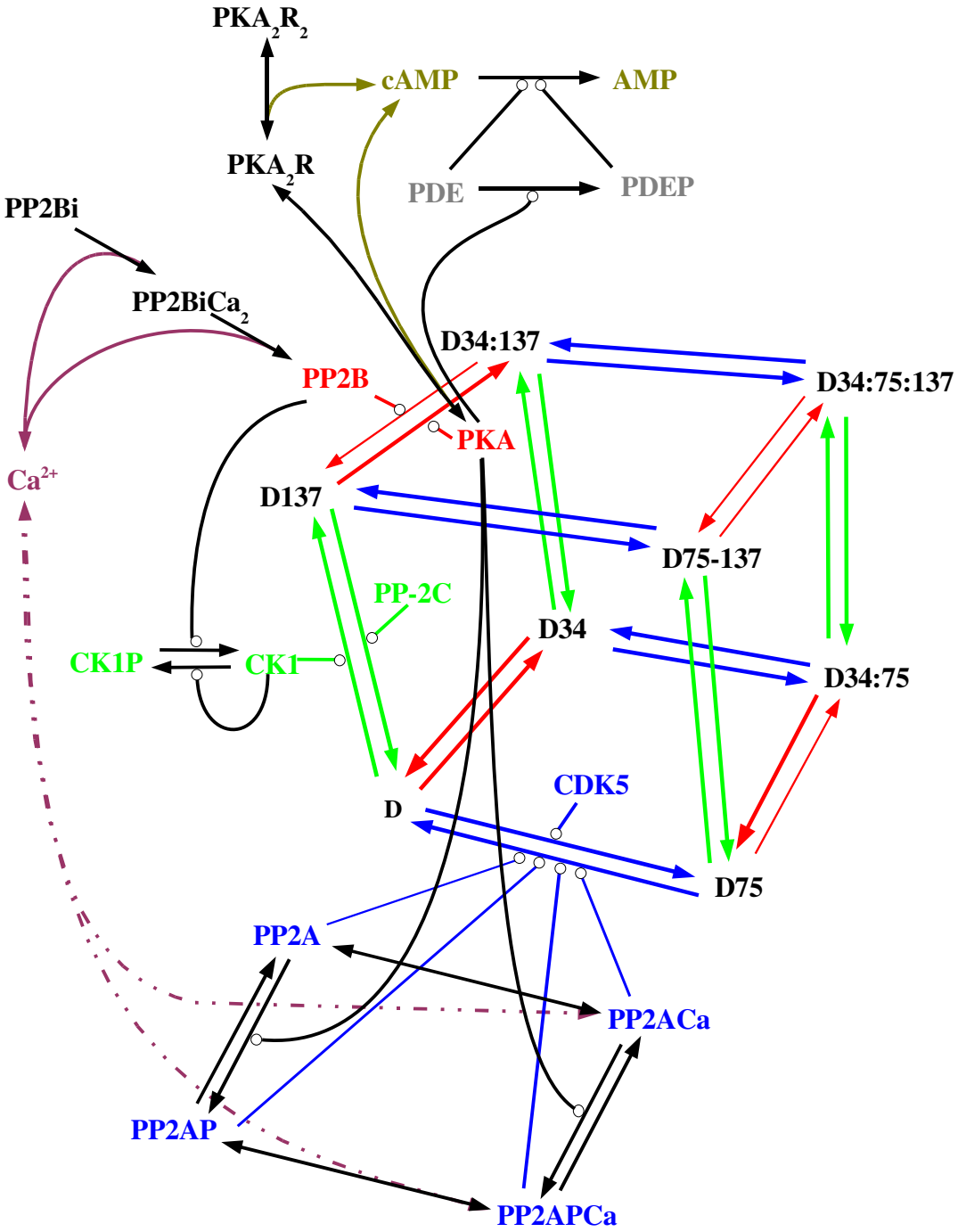
Macroscopic: Chemical Kinetics

- Continuous representation of populations
- Generally deterministic algorithms to simulate the evolution of populations (but not always: SSA aka “Gillespie”)
- Generally no representation of space (but not always: finite elements)
- No movements (but not always: PDE or reaction-diffusion)
- Molecules under different states are represented by different pools

Biological model of DARPP-32 regulation



Chemical model of DARPP-32 regulation



Chemical model of DARPP-32 regulation

79 species, 156 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

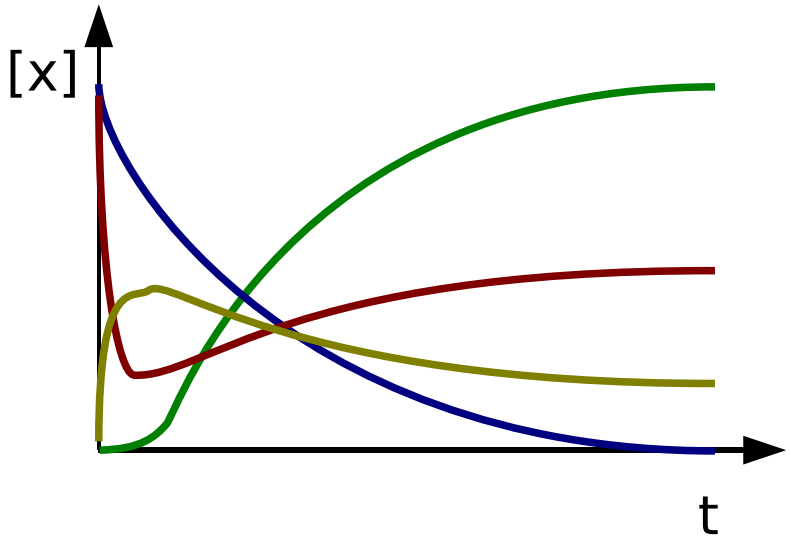
- D + PKA ⇔ D_PKA → D34 + PKA
- D34 + PP2B ⇔ D34_PP2B → D + PP2B
- D75 + PKA ⇔ D75_PKA → D34-75 + PKA
- D34-75 + PP2B ⇔ D34-75_PP2B → D75 + PP2B
- D137 + PKA ⇔ D137_PKA → D34-137 + PKA
- D34-137 + PP2B ⇔ D34-135_PP2B → D137 + PP2B
- D75-137 + PKA ⇔ D75-137_PKA → D34-75-137 + PKA
- D34-75-137 + PP2B ⇔ D34-75-135_PP2B → D75-137 + PP2B
- PP2A + PKA ⇔ PP2A_PKA → PP2AP + PKA
- PP2ACa + PKA ⇔ PP2ACa_PKA → PP2APCa + PKA
- Ø →
- → 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

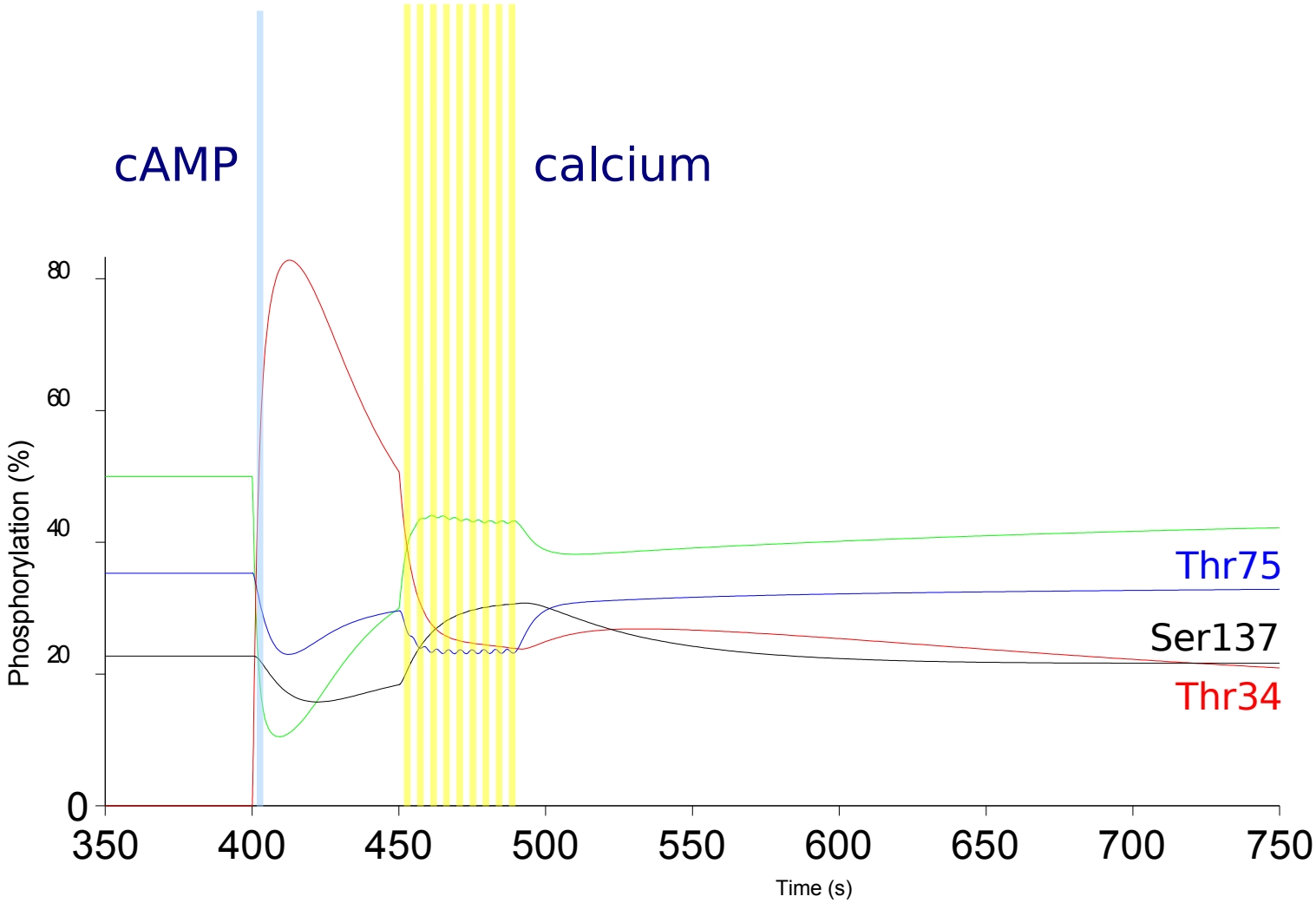
Mathematical model

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

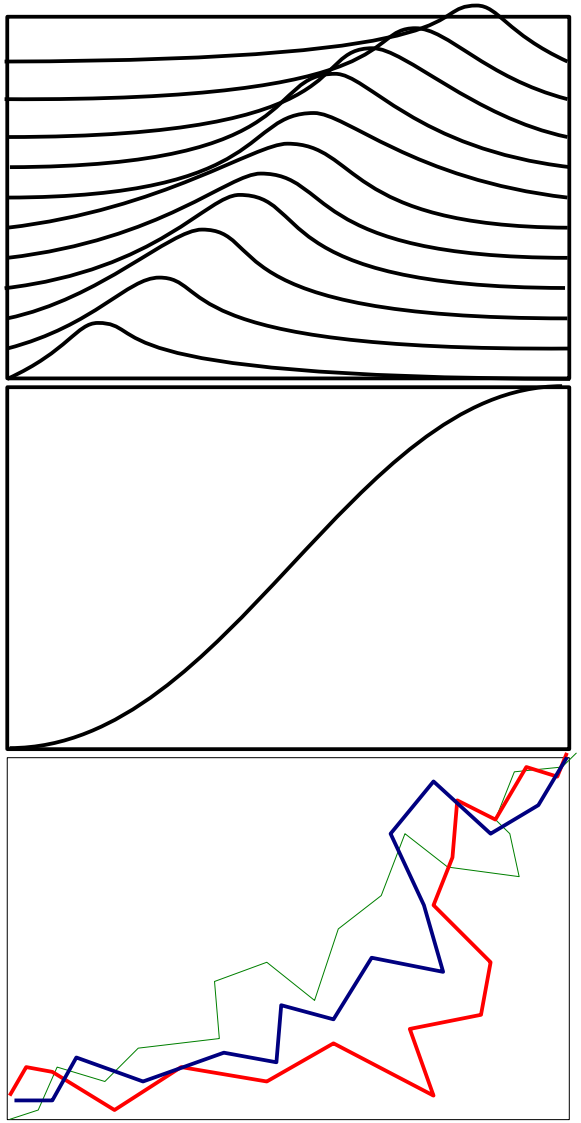
$$\begin{aligned}
d[D]/dt &= -k_1[CDK5][D] + k_2[D_CDK5] \\
d[D\text{-}75P]/dt &= + k_3[D_CDK5] \\
d[CDK5]/dt &= -k_1[CDK5][D] + k_2[D_CDK5] + k_3[D_CDK5] \\
d[D_CDK5]/dt &= +k_1[CDK5][S] - k_2[D_CDK5] - k_3[D_CDK5]
\end{aligned}$$



Dynamic simulations of DARPP-32 function



Different modelling approaches



Grand Probability function: P(X,t)

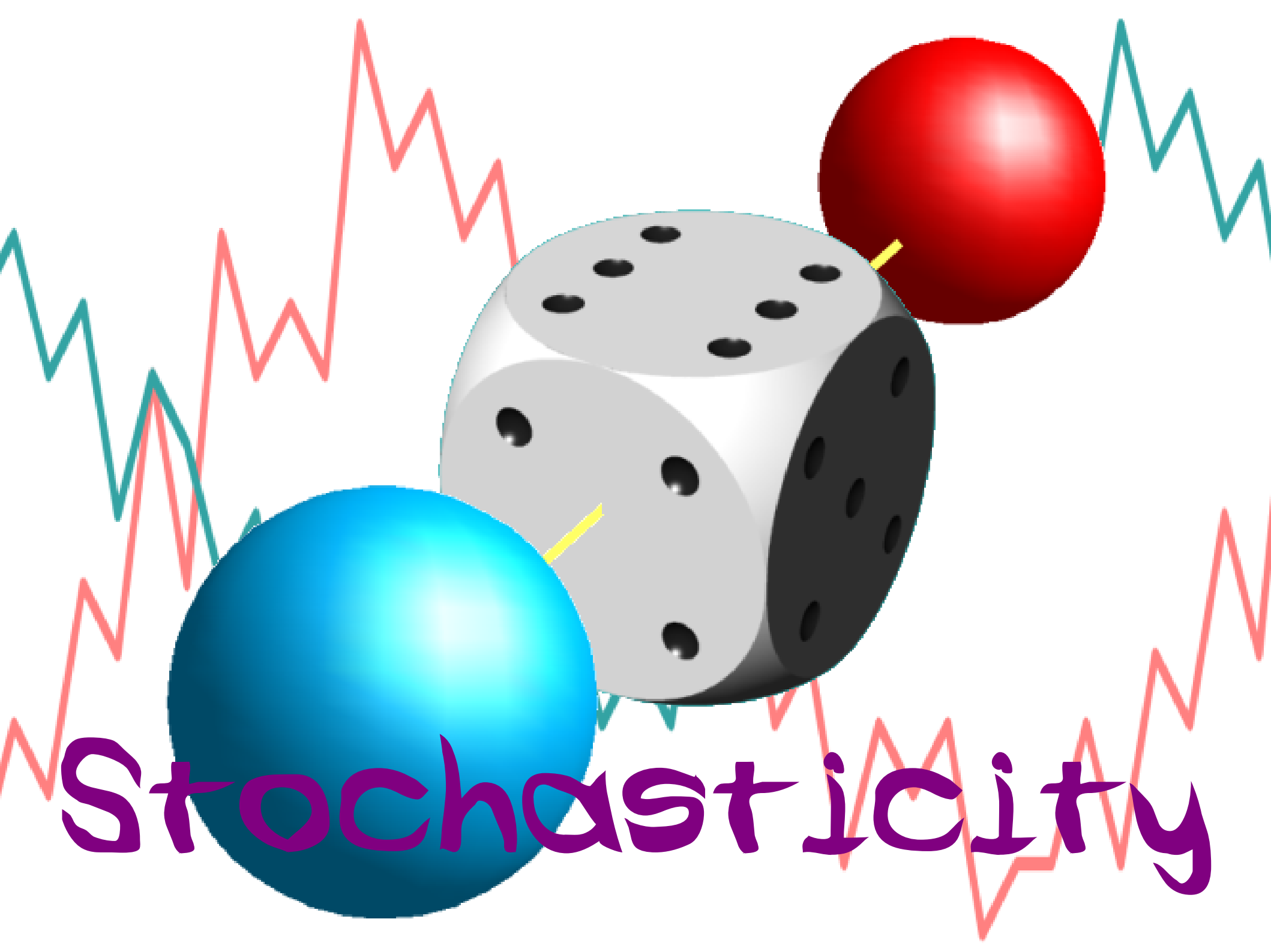
deterministic approach: (X,t)=f(X',t-1)

stochastic approach: P(X,t)/(X',t-1)

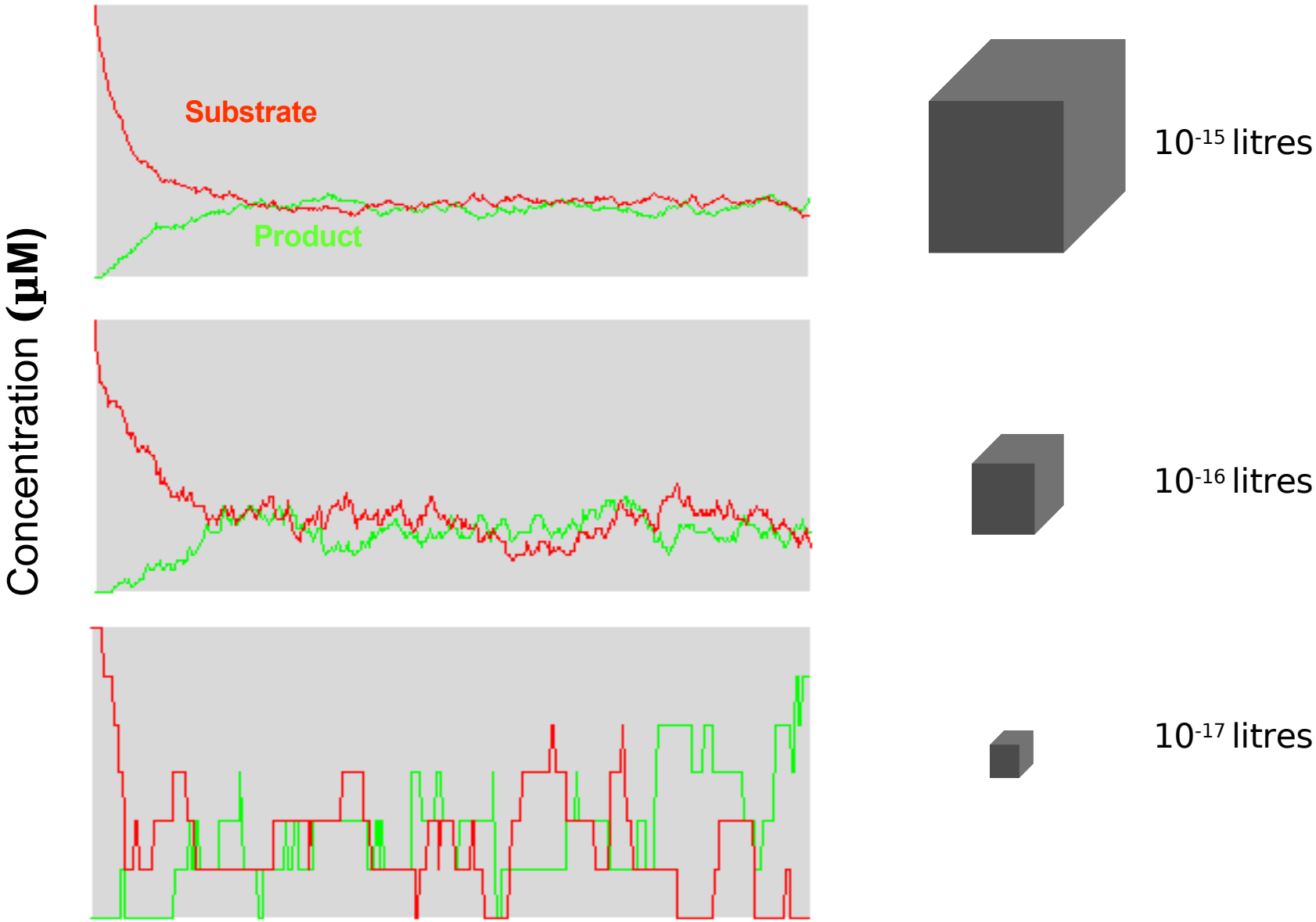
Limitation of deterministic approaches

Continuous, deterministic models can’t cope with:

1. Sensitivity to a very small number of molecules
2. Protein complexes with many states
3. Spatial heterogeneity

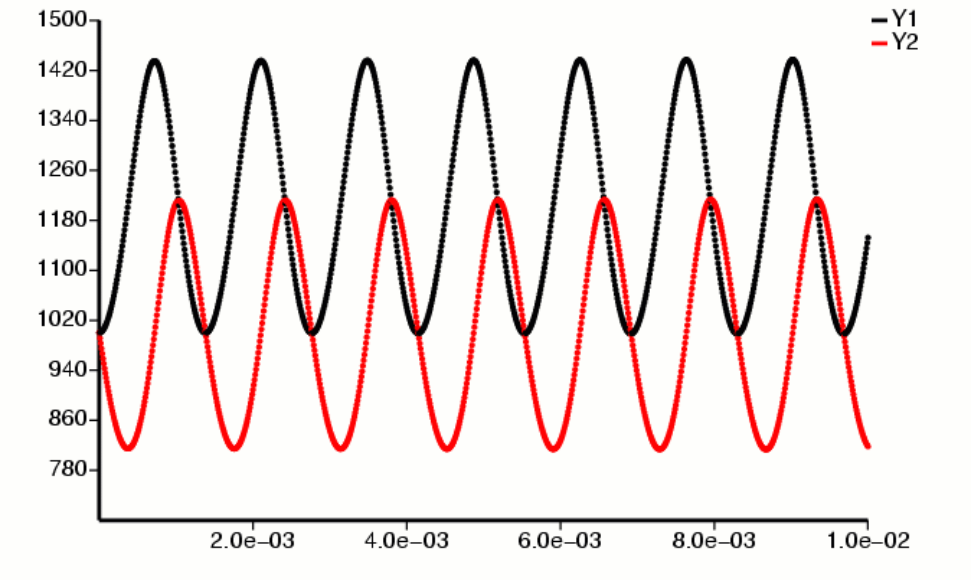

Stochasticity

On small numbers

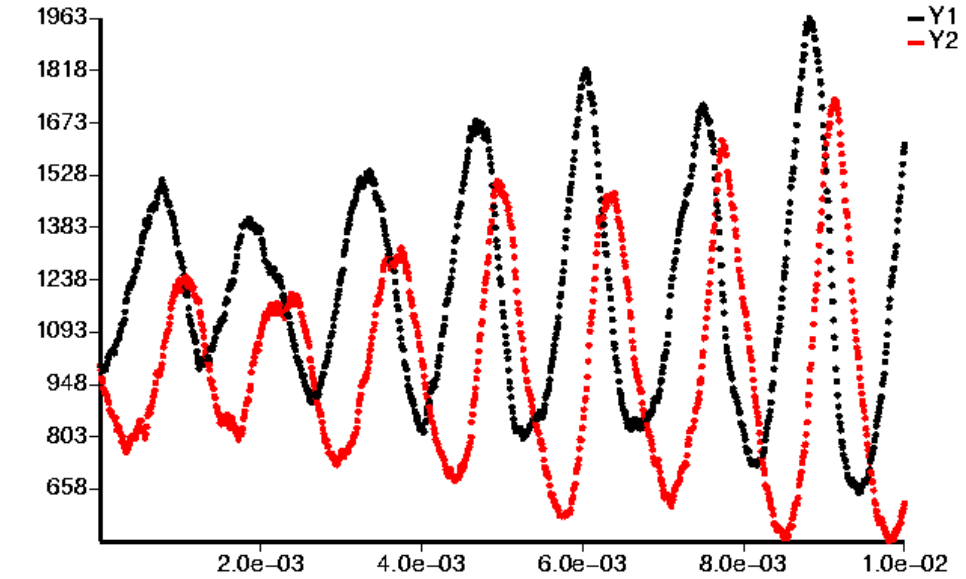


Resting number of calcium ions in a dendritic spine = 3-5 ...

On determinism and reproducibility

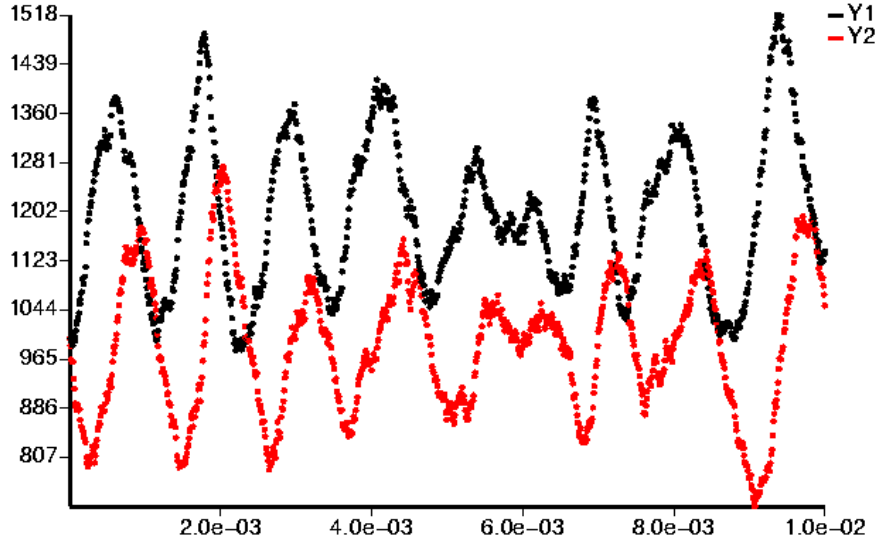


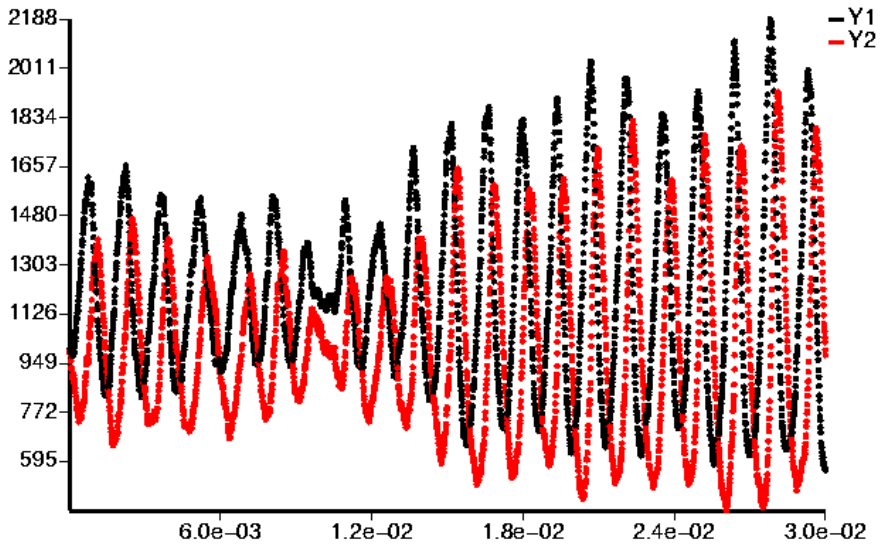
deterministic result

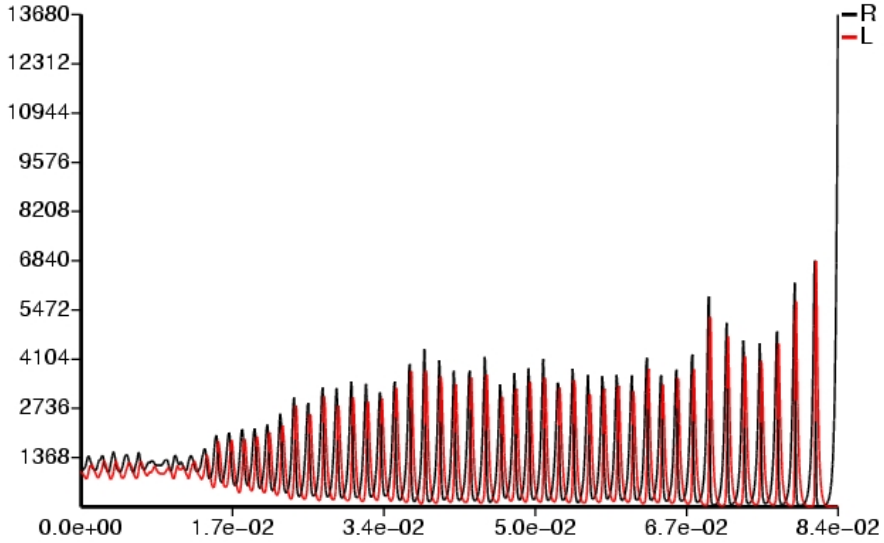


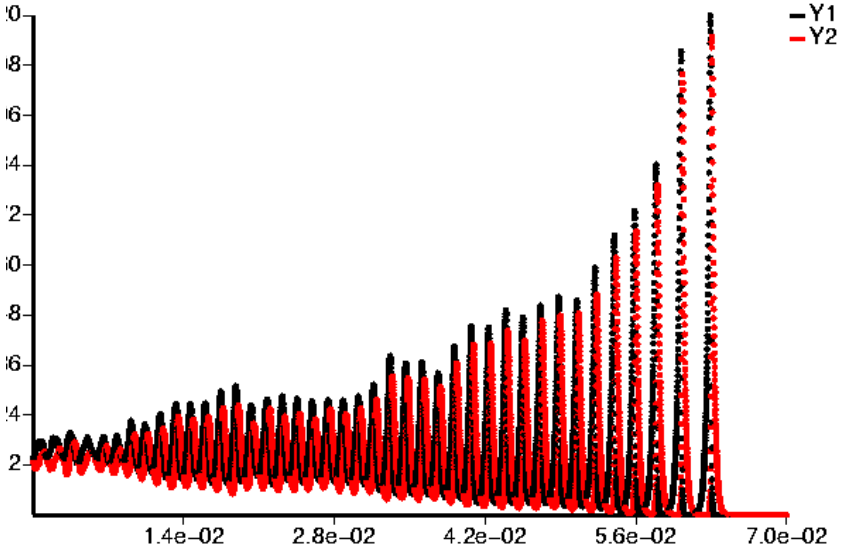
stochastic result

“Pathologic” behaviour







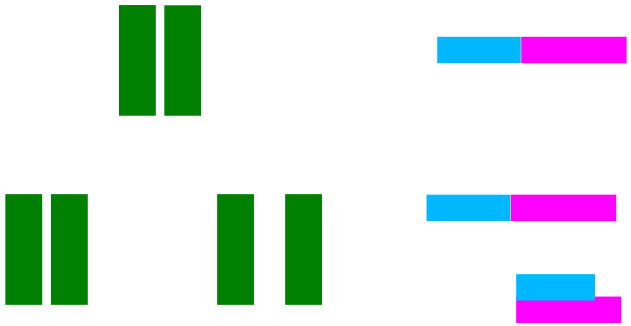



Combinatorial
Explosion

Combinatorial explosion

NMDA + CaMKII <=> NMDA-CaMKII

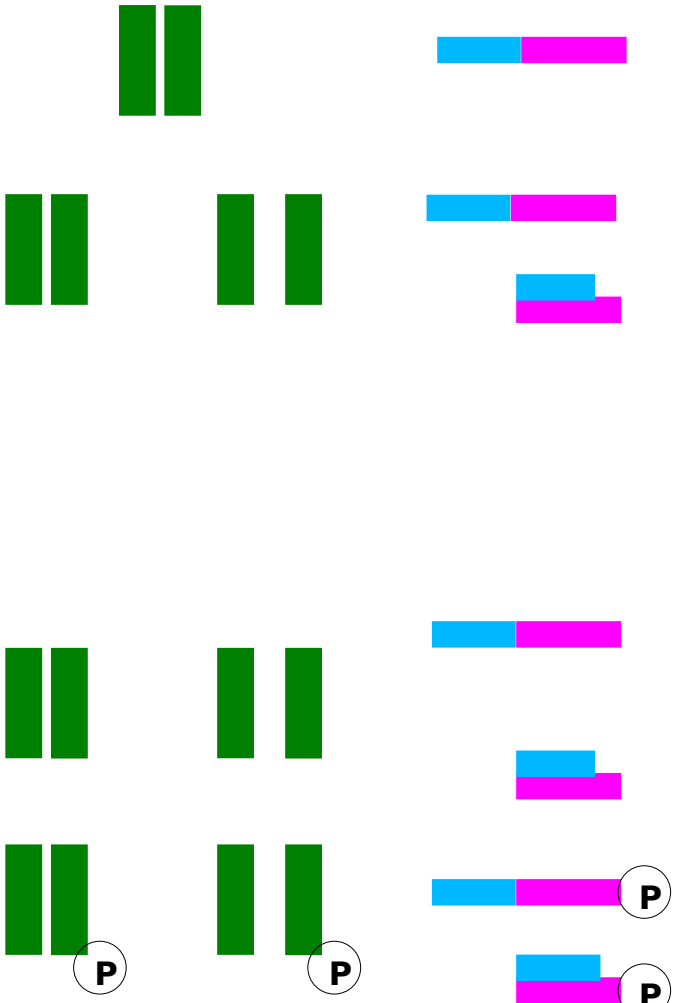
Combinatorial explosion



NMDA + CaMKII <=> NMDA-CaMKII

NMDAc + CaMKIIc <=> NMDAc-CaMKIIc
NMDAo + CaMKIIc <=> NMDAc-CaMKIIc
NMDAc + CaMKIIo <=> NMDAc-CaMKIIo
NMDAo + CaMKIIo <=> NMDAc-CaMKIIo

Combinatorial explosion



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NMDAc + CaMKIIo <=> NMDAc-CaMKIIo
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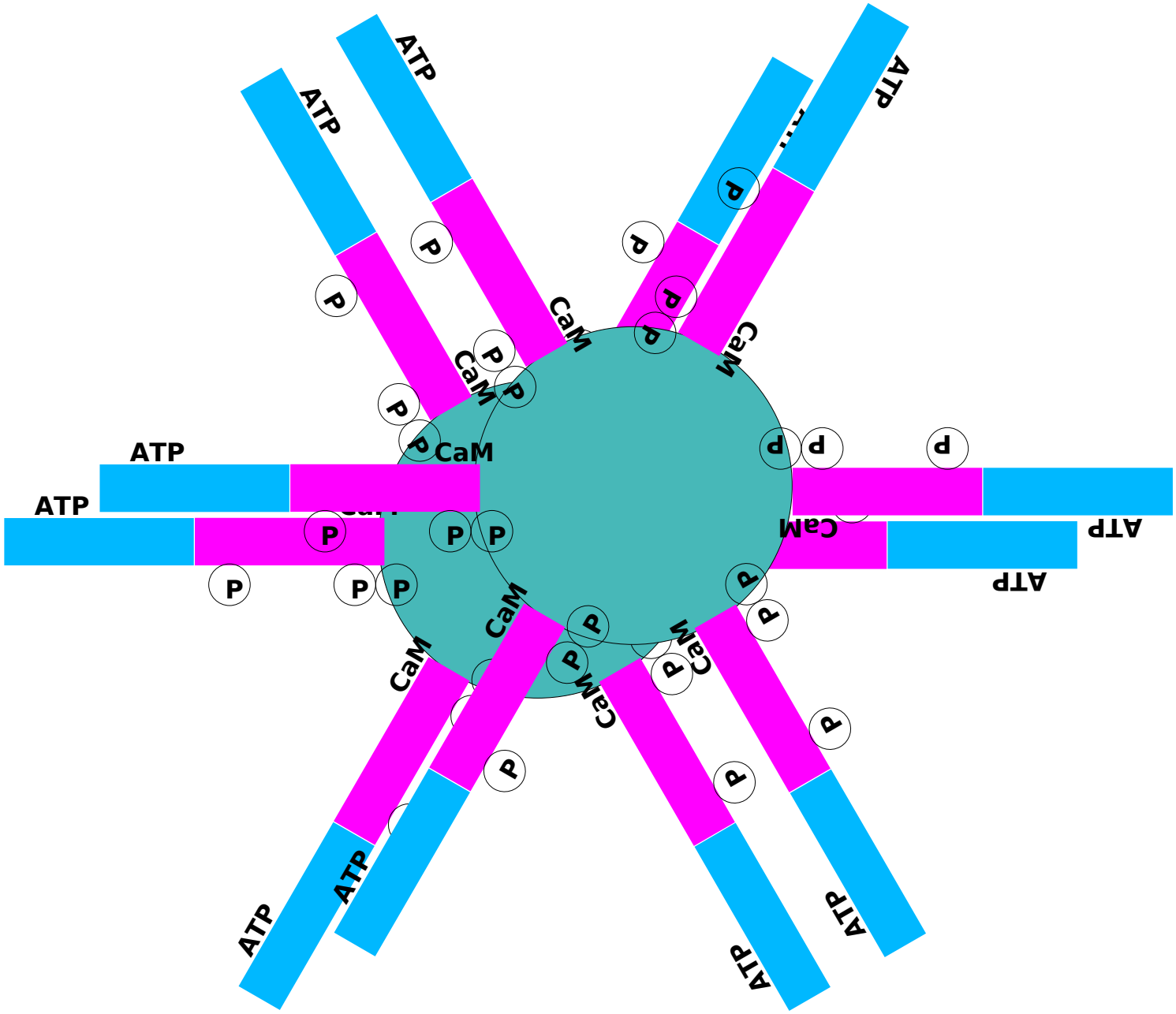
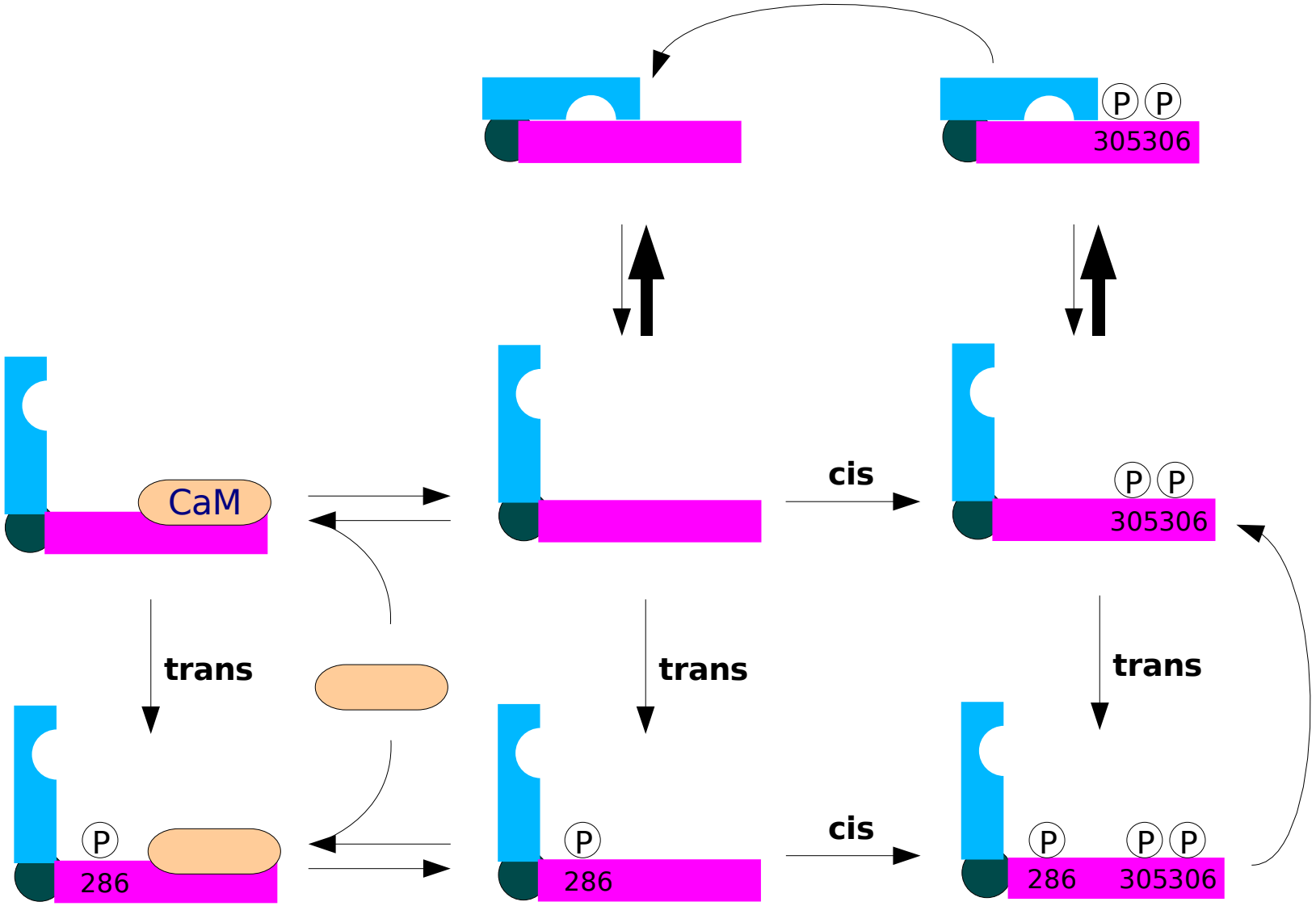
NMDAc + CaMKIIc <=> NMDAc-CaMKIIc
NMDAo + CaMKIIc <=> NMDAc-CaMKIIc
NMDAc + CaMKIIo <=> NMDAc-CaMKIIo
NMDAo + CaMKIIo <=> NMDAc-CaMKIIo
pNMDAc + CaMKIIc <=> pNMDAc-CaMKIIc
pNMDAo + CaMKIIc <=> pNMDAc-CaMKIIc
pNMDAc + CaMKIIo <=> pNMDAc-CaMKIIo
pNMDAo + CaMKIIo <=> pNMDAc-CaMKIIo
NMDAc + pCaMKIIc <=> NMDAc-pCaMKIIc
NMDAo + pCaMKIIc <=> NMDAc-pCaMKIIc
NMDAc + pCaMKIIo <=> NMDAc-pCaMKIIo
NMDAo + pCaMKIIo <=> NMDAc-pCaMKIIo
pNMDAc + pCaMKIIc <=> pNMDAc-pCaMKIIc
pNMDAo + pCaMKIIc <=> pNMDAc-pCaMKIIc
pNMDAc + pCaMKIIo <=> pNMDAc-pCaMKIIo
pNMDAo + pCaMKIIo <=> pNMDAc-pCaMKIIo

Combinatorial explosion



Additional reactions



Combinatorial explosion

- number of states = 2^n
- A molecule with 10 features = 2^{10} = 1024 states, that is 1024 pools. But most signalling molecules are present a few hundred times ...
- number of state conversions= n x 2^{n-1}
- A molecule with 10 features reacting with a molecule with 10 features =

Combinatorial explosion

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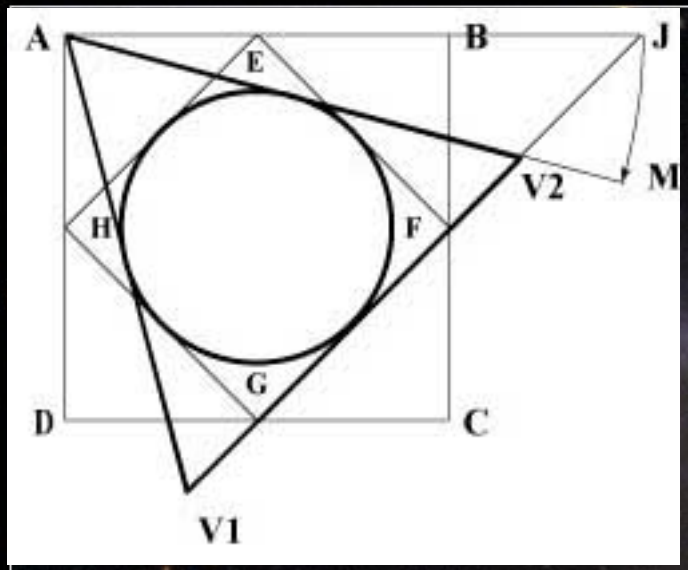
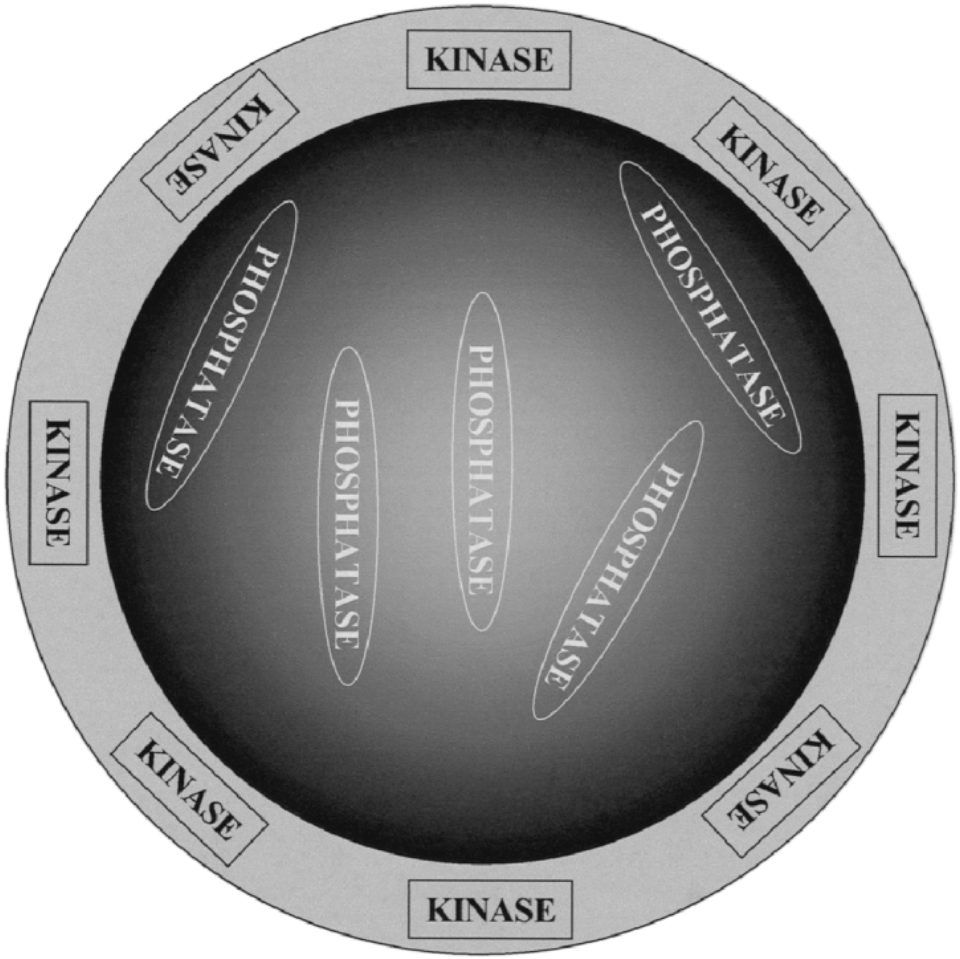
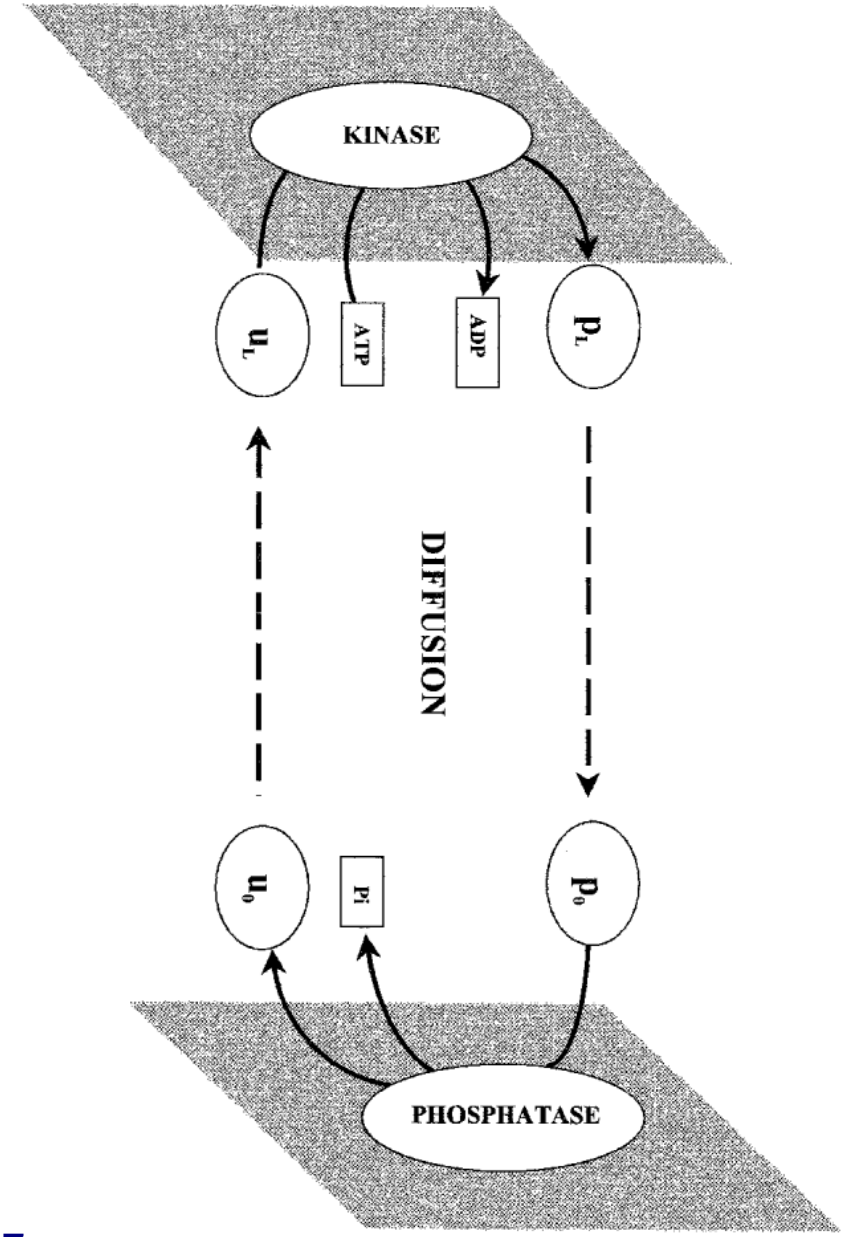
- A molecule with 10 features = 2^{10} = 1024 states, that is 1024 pools. But most signalling molecules are present a few hundred times ...
- number of state conversions= n x 2^{n-1}
- A molecule with 10 features reacting with a molecule with 10 features = 1058816 possible reactions!



Space and geometry

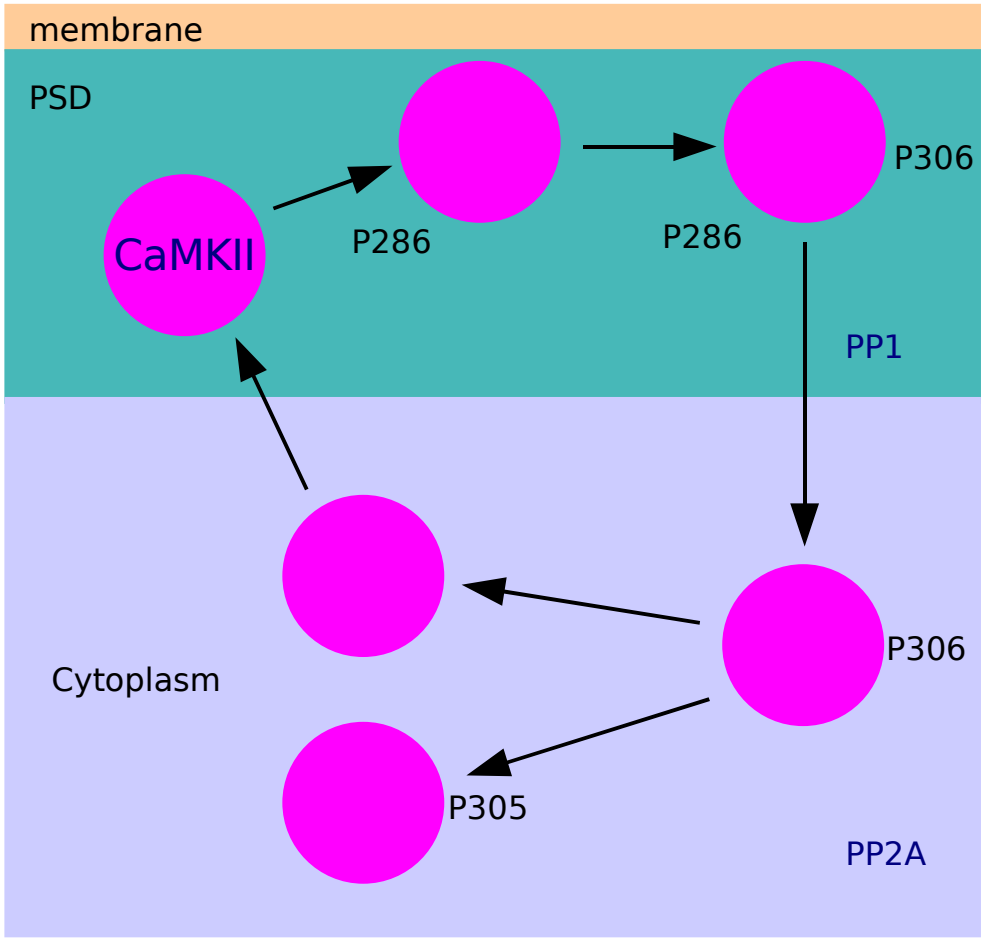
Spatial hysteresis



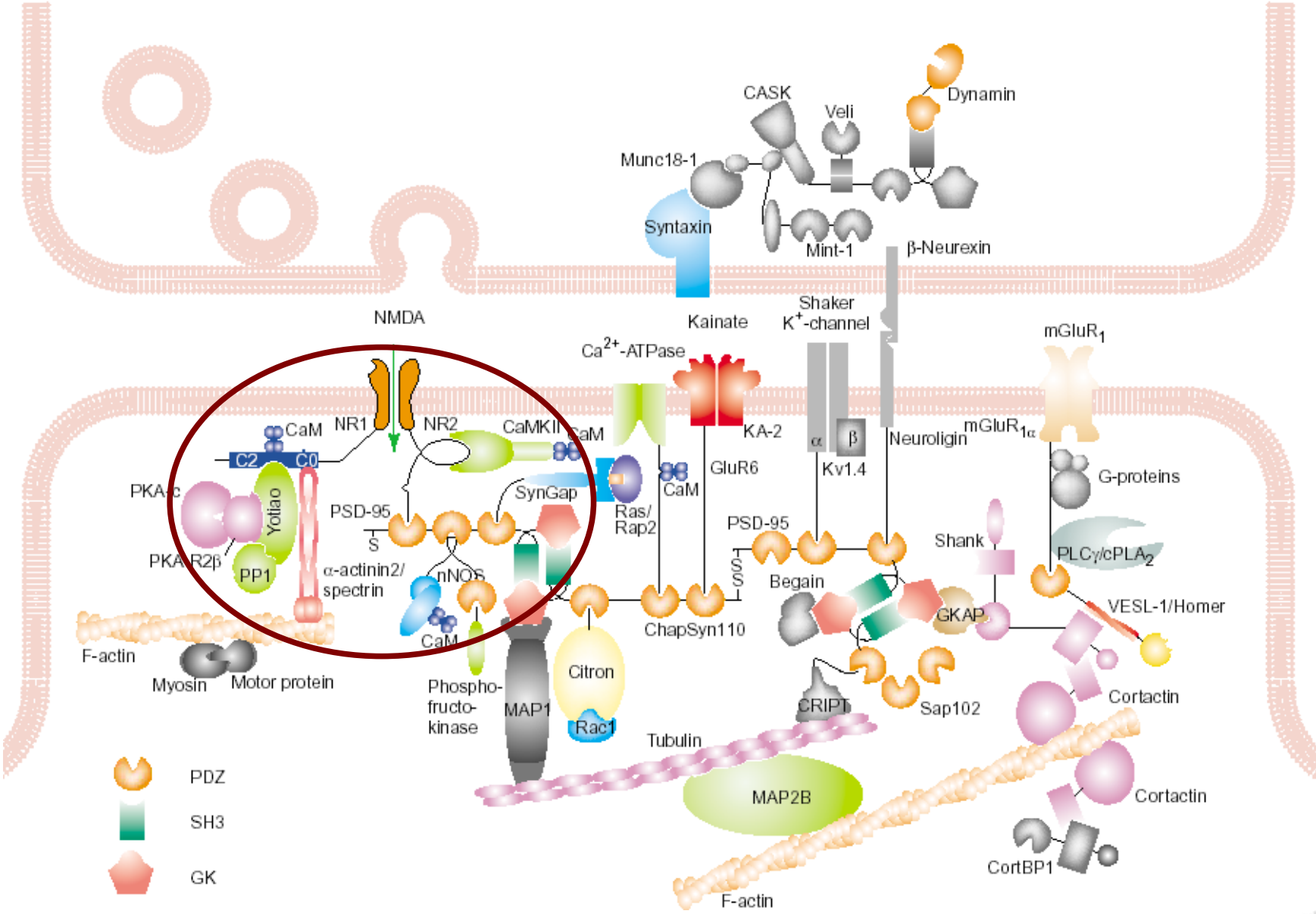


Kholodenko et al. *Biochem. J.* (2000) 350: 901–907

Spatial hysteresis



Relative positions



Husi & Grant (2001) *TINS, 24*: 259-266

Need another paradigm of simulation

Population-based simulation

- Continuous representation of populations
- Generally deterministic algorithms to simulate the evolution of populations (but not always: Gillespie)
- Generally no representation of space (but not always: finite elements)
- No movements (but not always: PDE or reaction-diffussion)
- Molecules under different states are represented by different pools

Particle-based simulation

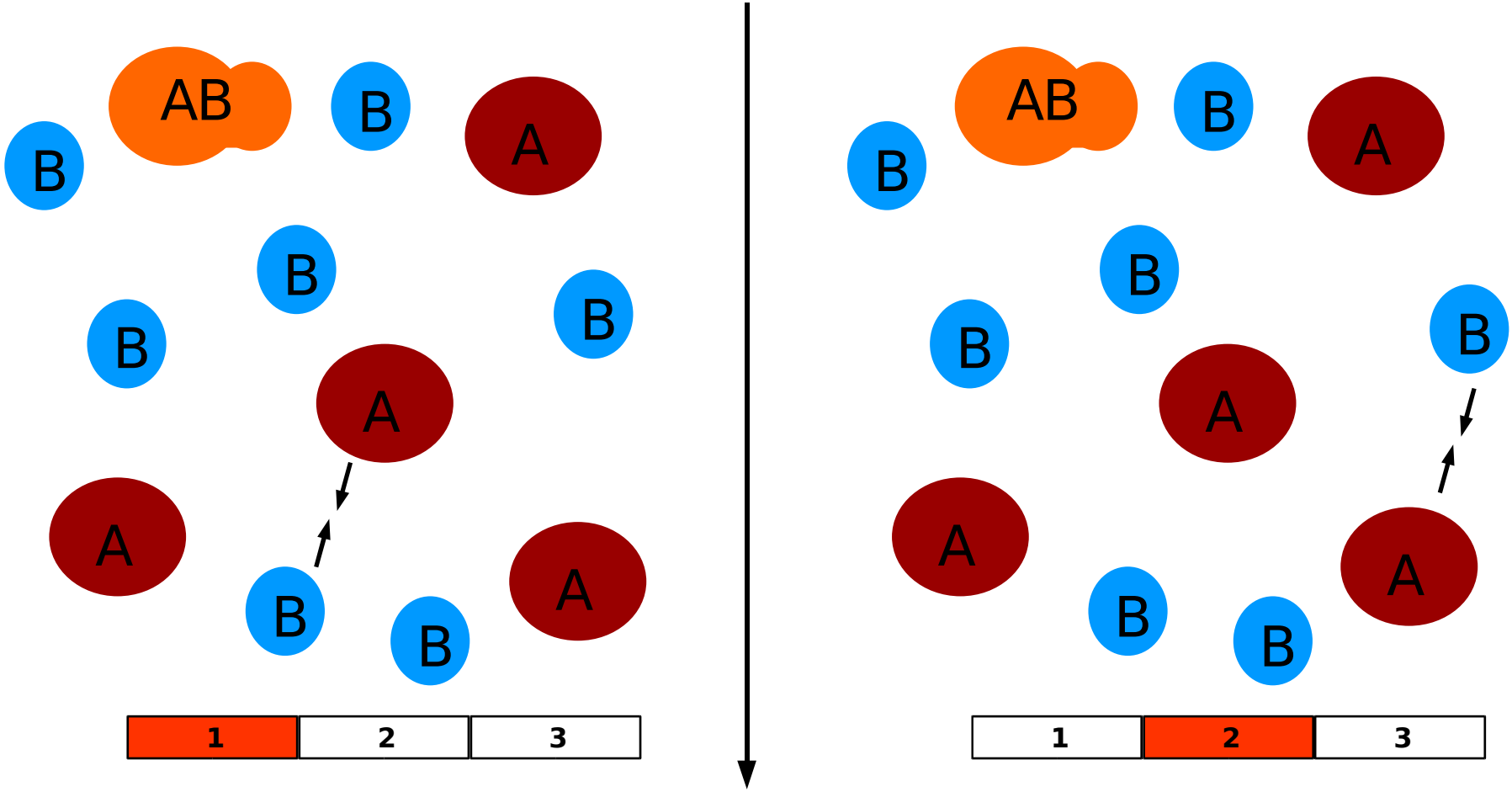
- Discrete representation of molecules
- Generally stochastic algorithms (but not always: deterministic automata)
- Generally location of molecules (but not always: StochSim v1)
- Representation of the movements of (some) molecules
- Possibility of multistates molecules

StochSim: Stochastic cellular automata

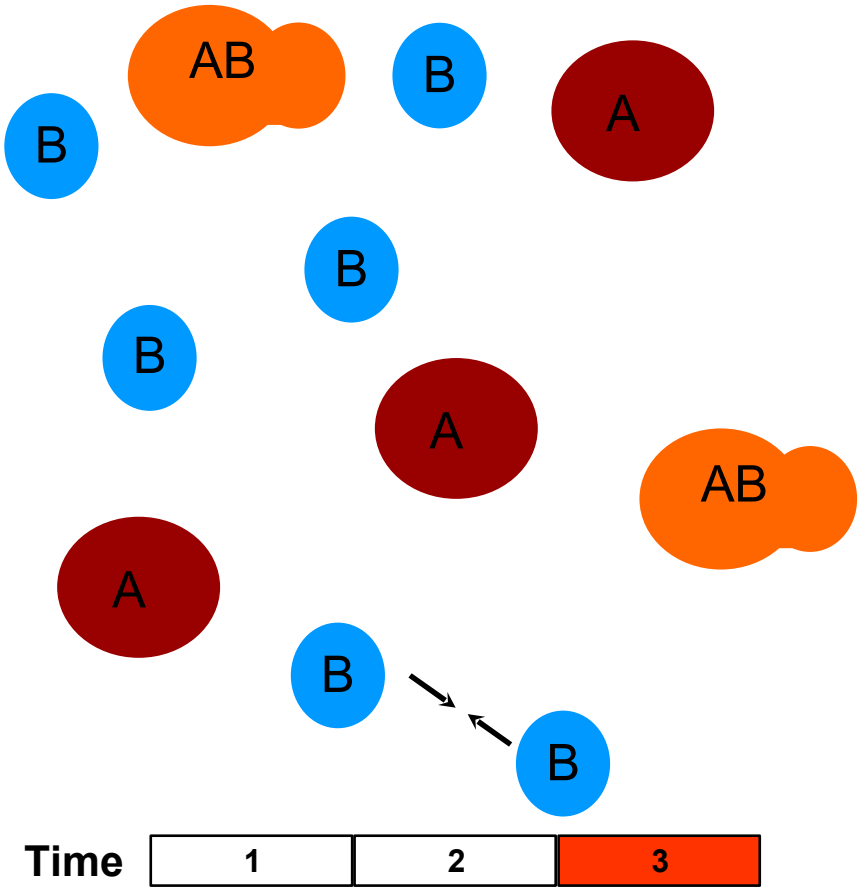
- Morton-Firth CJ, Bray D (1998) *J. Theor. Biol.* 192: 117–128.
- Le Novère N, Shimizu TS (2001) *Bioinformatics* 17: 575-576

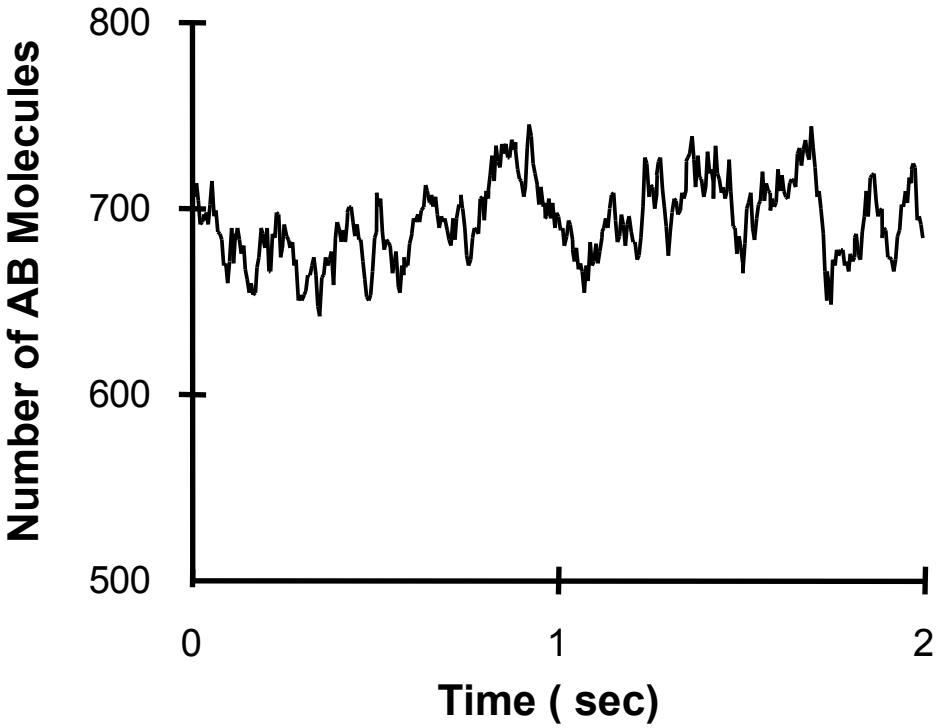
- Particle-based stochastic simulations
- Possibility of multistate complexes
- Rapid equilibria to reduce stiffness problems
- 2D lattices of various geometry

StochSim algorithm



StochSim algorithm





Kinetic constant to probability

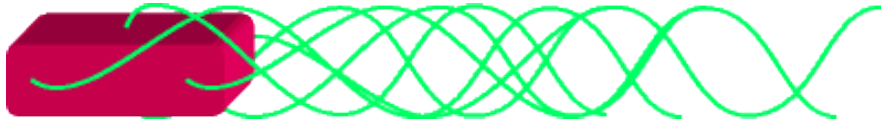
$$d[A]/dt = -k[A][B] \qquad P = \frac{k\, n(n+n_0)\Delta t}{2VN_A}$$

$$d[A]/dt = -k[A] \qquad P = \frac{k\, n(n+n_0)\Delta t}{n_0}$$

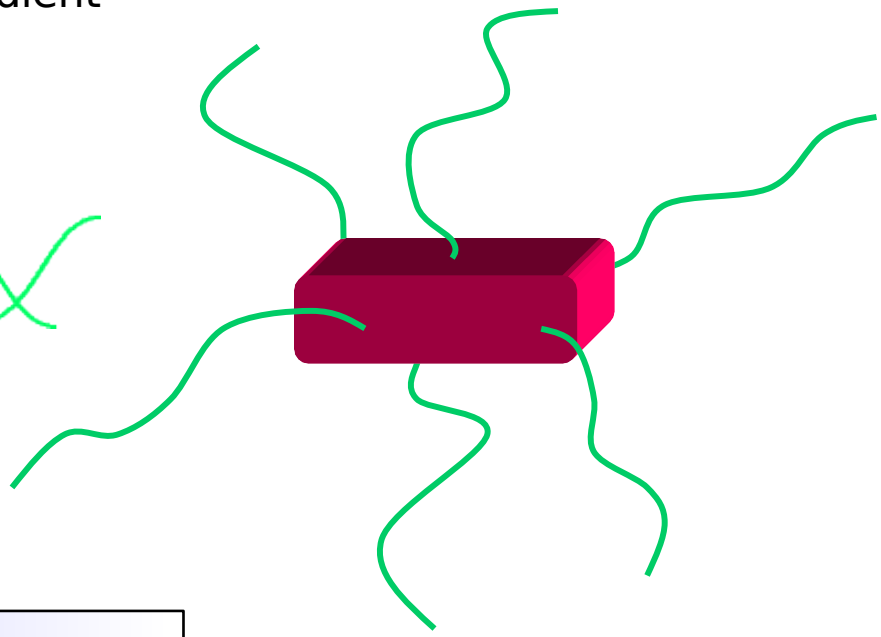
n: # molecules in the system
n_0: # pseudomolecules in the system
V: volume of the system
N_A: Avogadro constant

Mechanism of bacterial chemotaxis

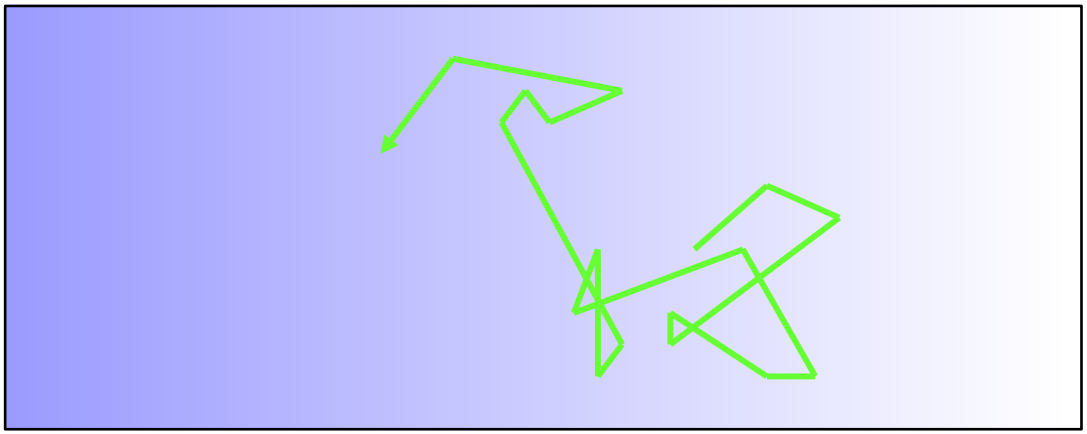
- small size ⇒ unable to read gradient
- small weight ⇒ no inertia



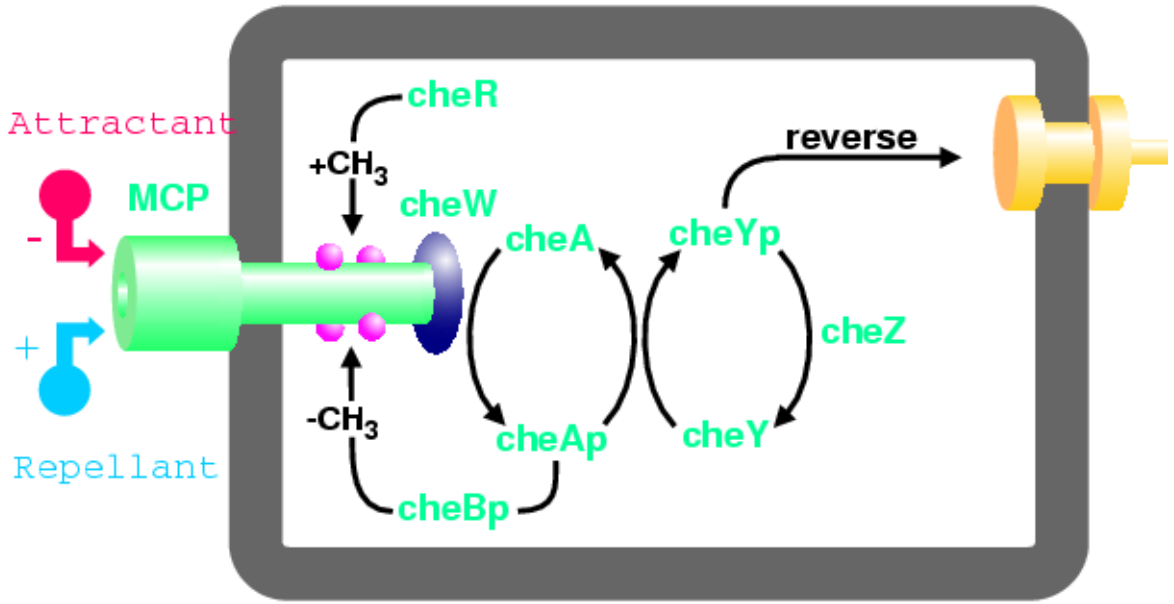
CCW = smooth



CW = tumble

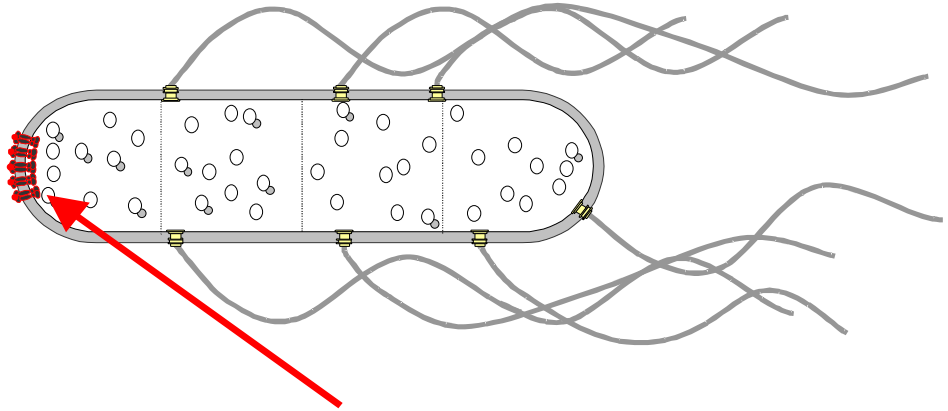


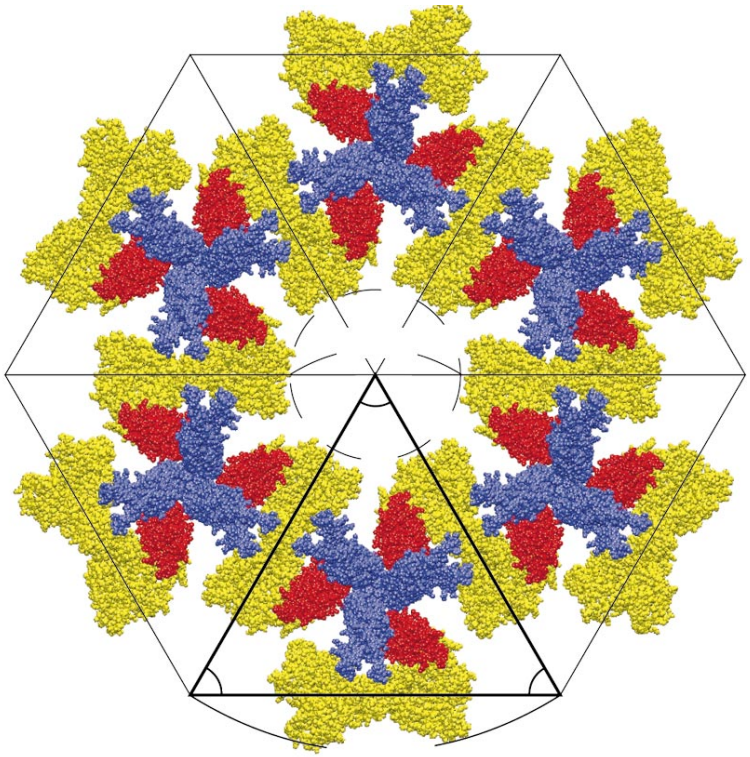
Importance of topology : the nose of the bacteria

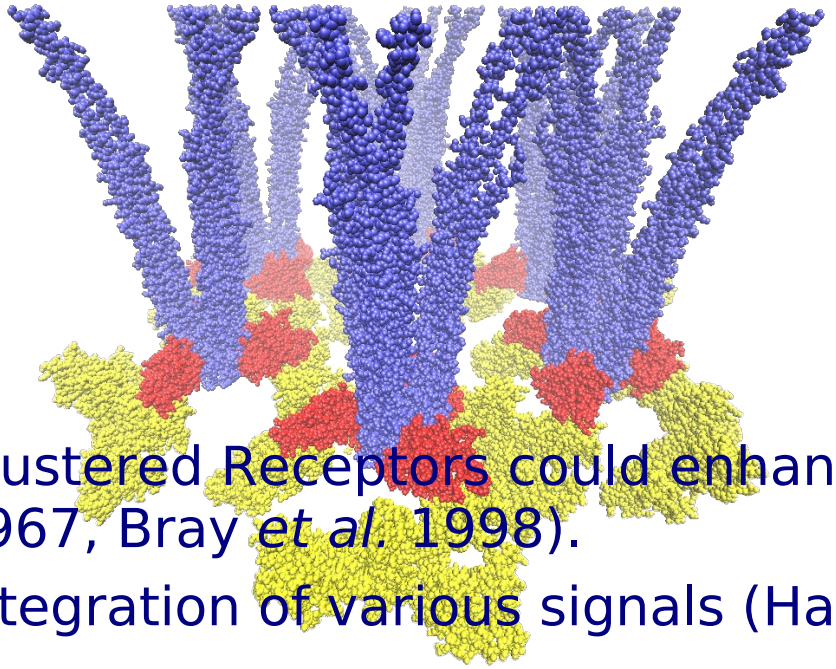


Receptor clustering and sensitivity

Chemotactic receptors form clusters at cell poles in E. coli (Shimizu, Le Novère *et al.* (2000) *Nat Cell Biol* 2: 792-796).







Clustered Receptors could enhance sensitivity (Changeux *et al.* 1967, Bray *et al.* 1998).

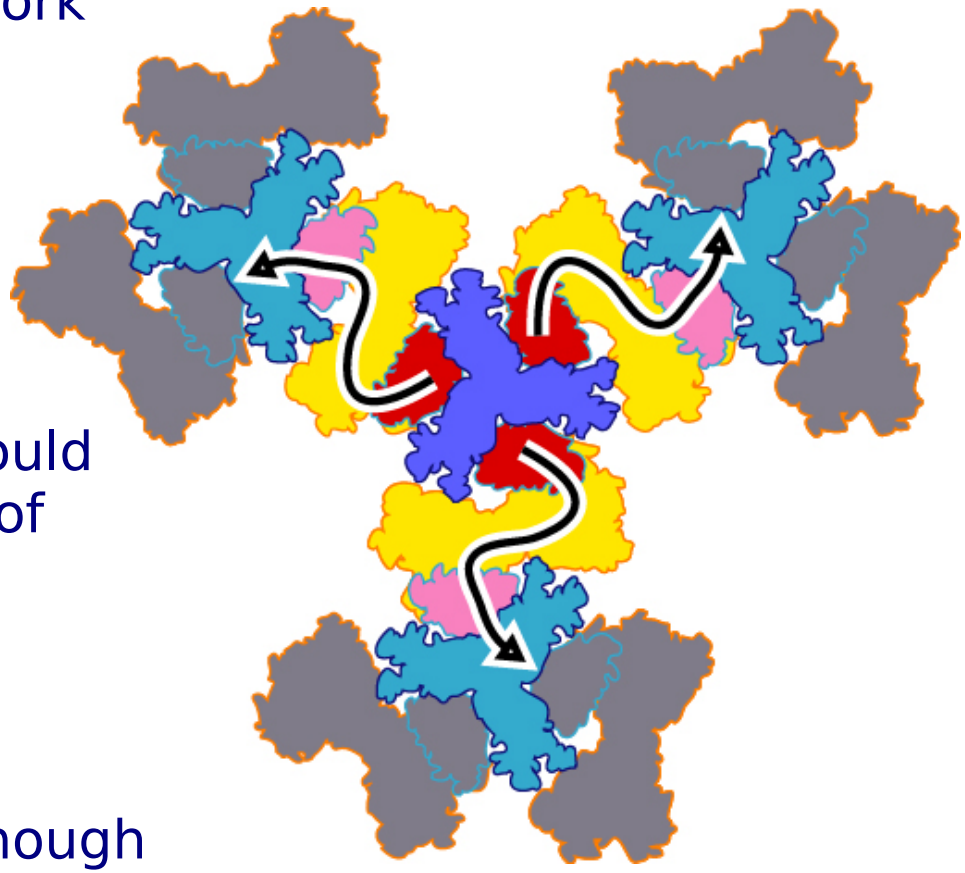
Integration of various signals (Hazelbauer et al. 1989).

Consequences for signalling

- Conformational changes could be propagated through the network via CheA/CheW
  Enhanced gain;

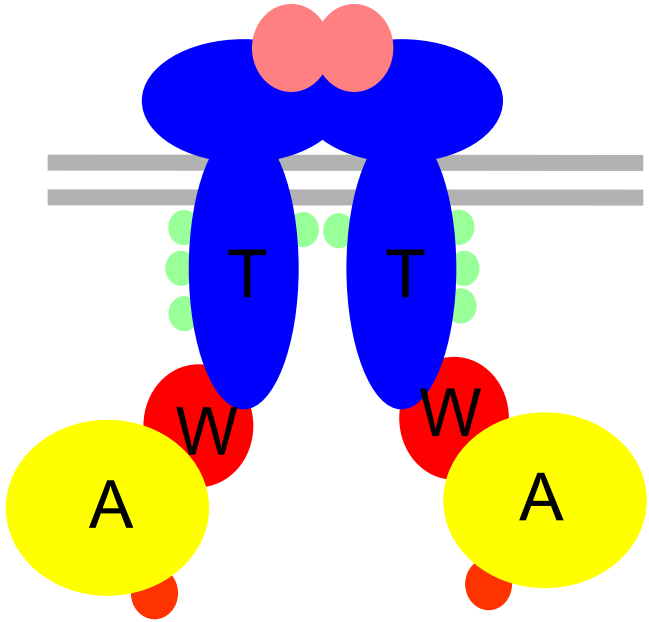
- Hybrid networks containing multiple types of receptors could integrate signals at the level of CheA activity;

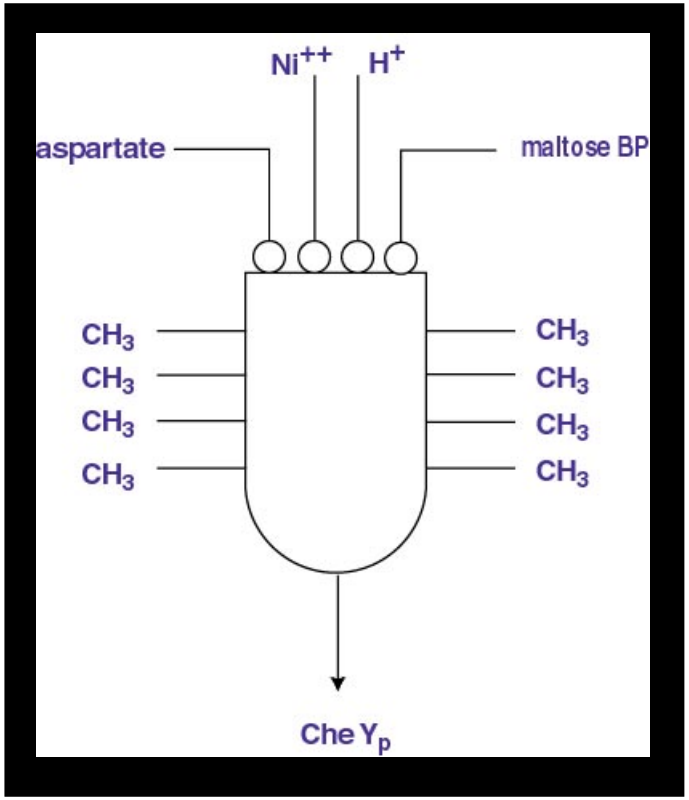
- Receptor dimers are close enough (6-10 nm) for adaptational cross-talk.



Multistate molecules

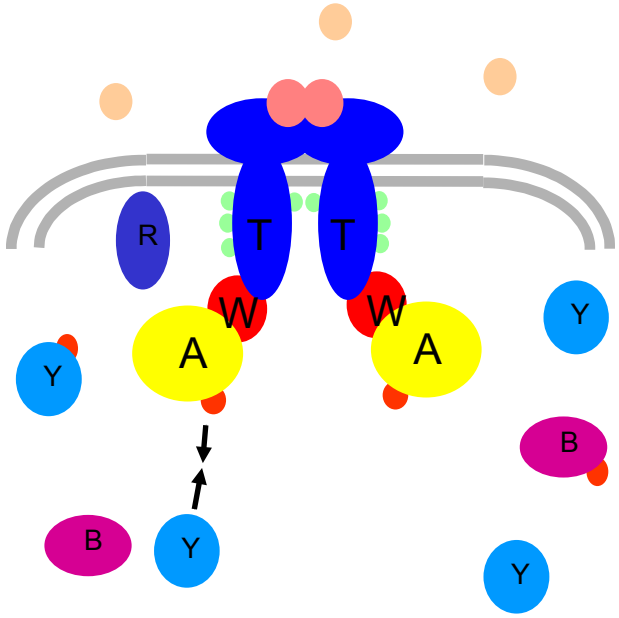
Internal features represented by binary flags. States are vectors of flags.





Multistate reactions

Reaction probabilities can be modified by the state of a participating multistate complex



$$p_{MS} = p_{base} \times p_{rel}$$

Where p_{base} is the base probability, and is p_{rel} the state-dependent relative probability.

Multistate reactions

$$(???0???) \xrightarrow{p_{base}} (???1???)$$

$$(0??0???) \xrightarrow{p_{base} \times p_{rel(0,0)}} (0??1???)$$

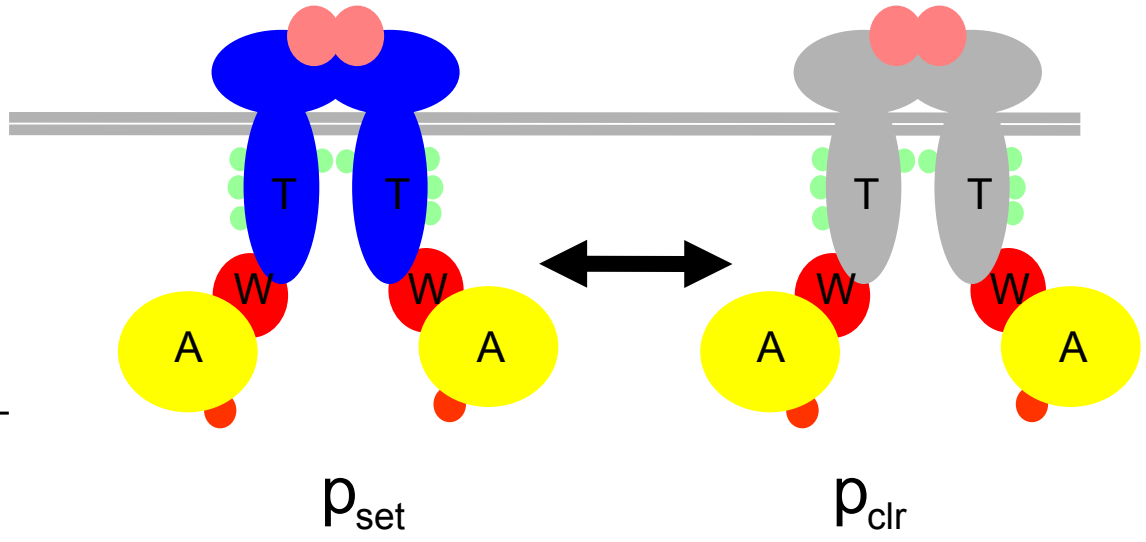
$$(1??0???) \xrightarrow{p_{base} \times p_{rel(0,1)}} (1??1???)$$

- '?' Flags do not affect the reaction
- only 4 species are needed instead of 128

Multistate rapid equilibria

- *Instantaneously* determines state of flag according to predefined probabilities.
- Probability can depend on the state of other flags.
- Primarily used to represent conformational 'flipping'.

$$\Delta G = -RT \ln \frac{p_{set}}{p_{clear}}$$



Free-energy based activation probabilities

no attractant bound			attractant bound		
Species	p	ΔG (kcal/mol)	Species	p	ΔG (kcal/mol)
	0.017	2.37		0.003	3.55
	0.125	1.18		0.017	2.37
	0.500	0.00		0.125	1.18
	0.874	1.18		0.500	0.00
	0.997	3.55		0.980	2.37

Free-energy values for coupled receptors

	no attractant bound						attractant bound					
	active neighbours	0	1	2	3	4	active neighbours	0	1	2	3	4
p		0.00	0.00	0.02	0.08	0.30		0.00	0.00	0.00	0.01	0.07
ΔG		4.47	3.49	2.50	1.51	0.53		5.55	4.56	3.58	2.59	1.61
p		0.01	0.03	0.13	0.41	0.78		0.00	0.00	0.02	0.08	0.30
ΔG		3.17	2.18	1.20	0.21	-0.77		4.47	3.49	2.50	1.51	0.53
p		0.04	0.17	0.50	0.83	0.96		0.01	0.03	0.13	0.41	0.78
ΔG		1.97	0.99	0.00	-0.99	-1.97		3.17	2.18	1.20	0.21	-0.77
p		0.22	0.58	0.87	0.97	0.99		0.04	0.17	0.50	0.83	0.96
ΔG		0.78	-0.21	-1.19	-2.18	-3.16		1.97	0.99	0.00	-0.99	-1.97
p		0.93	0.99	1.00	1.00	1.00		0.67	0.91	0.98	1.00	1.00
ΔG		-1.61	-2.59	-3.58	-4.56	-5.55		0.43	-1.41	-2.40	-3.38	-4.37

Effect of conformational spread

uncoupled

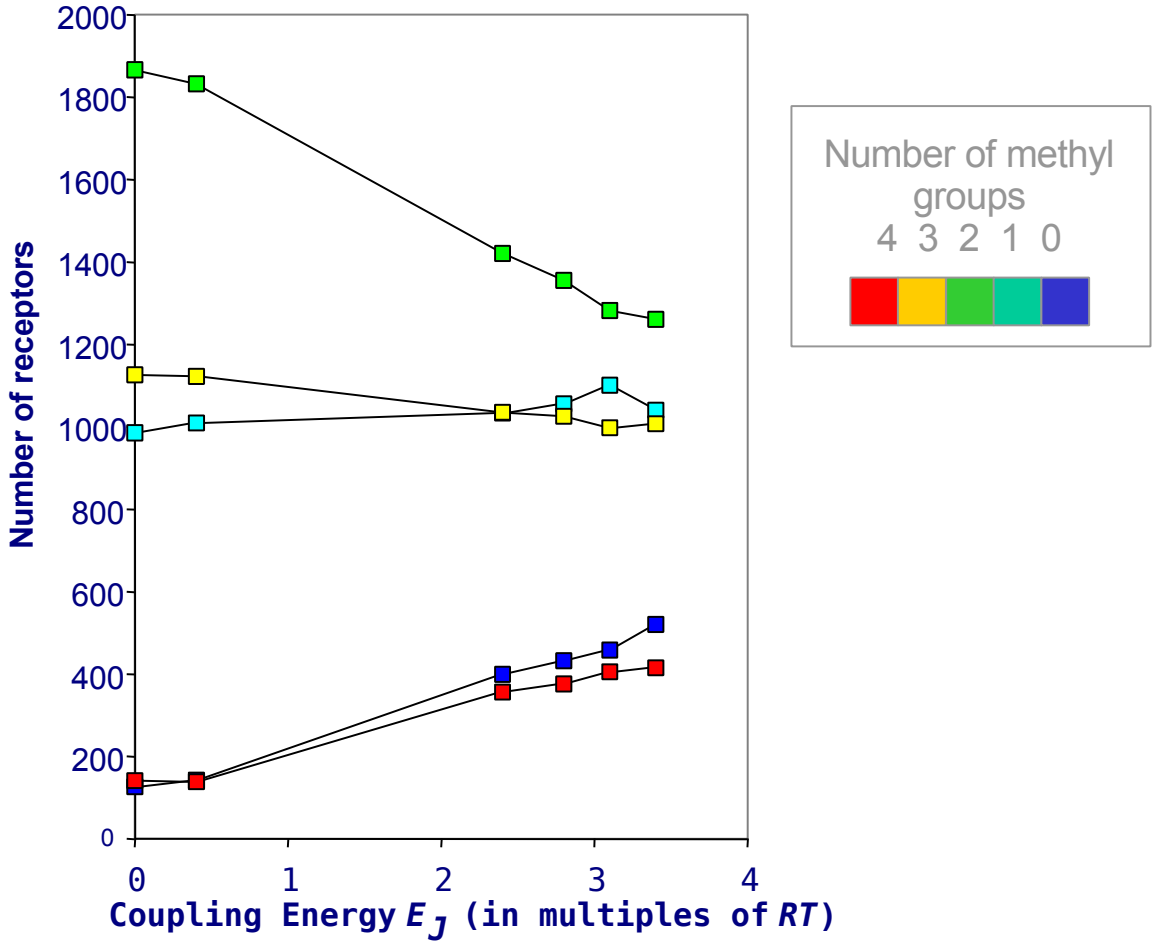
coupled

activity

methylation

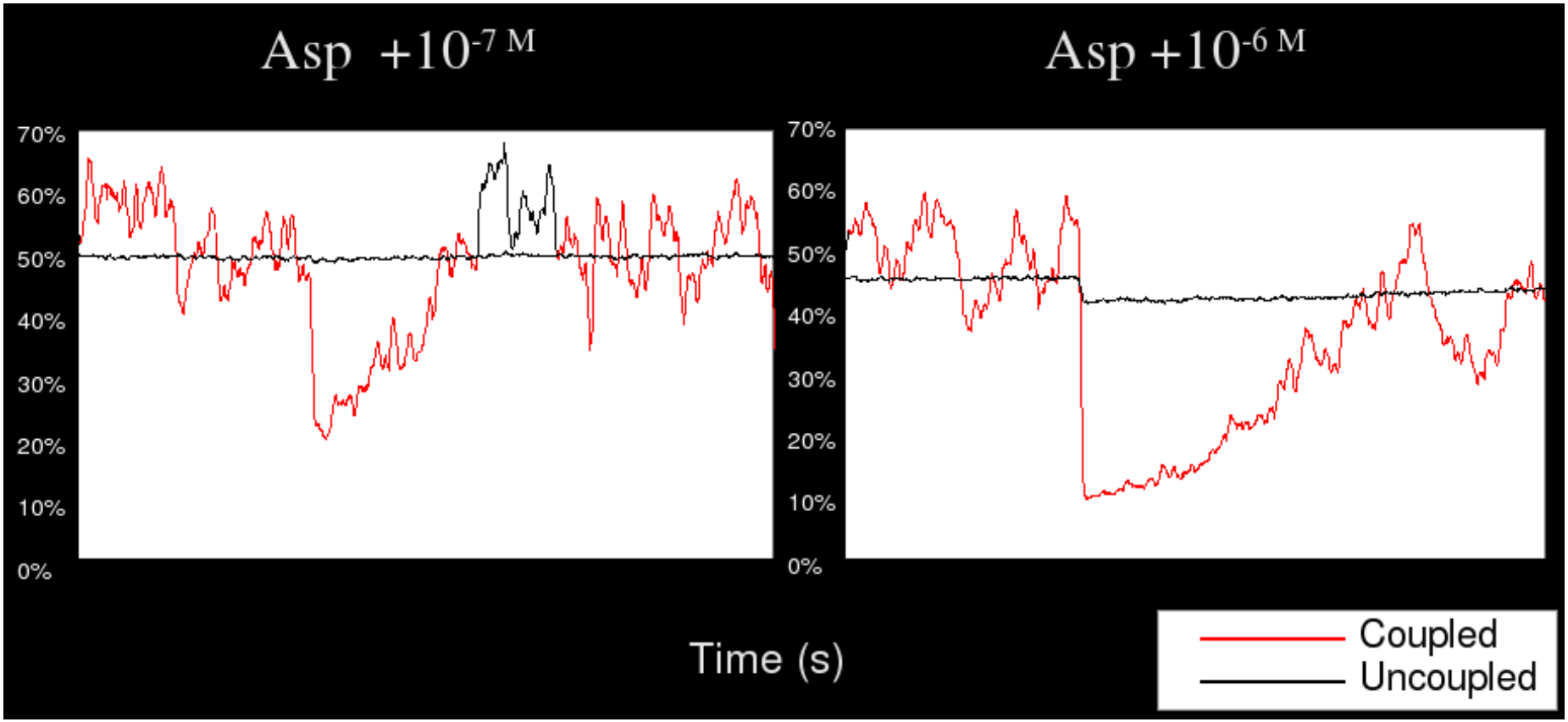
Quantitative patterns of methylation

- Steady-state population profile of receptor methylation states changes with degree of coupling



Shimizu et al. (2003) *J Mol Biol* 329:291-309.

Gain

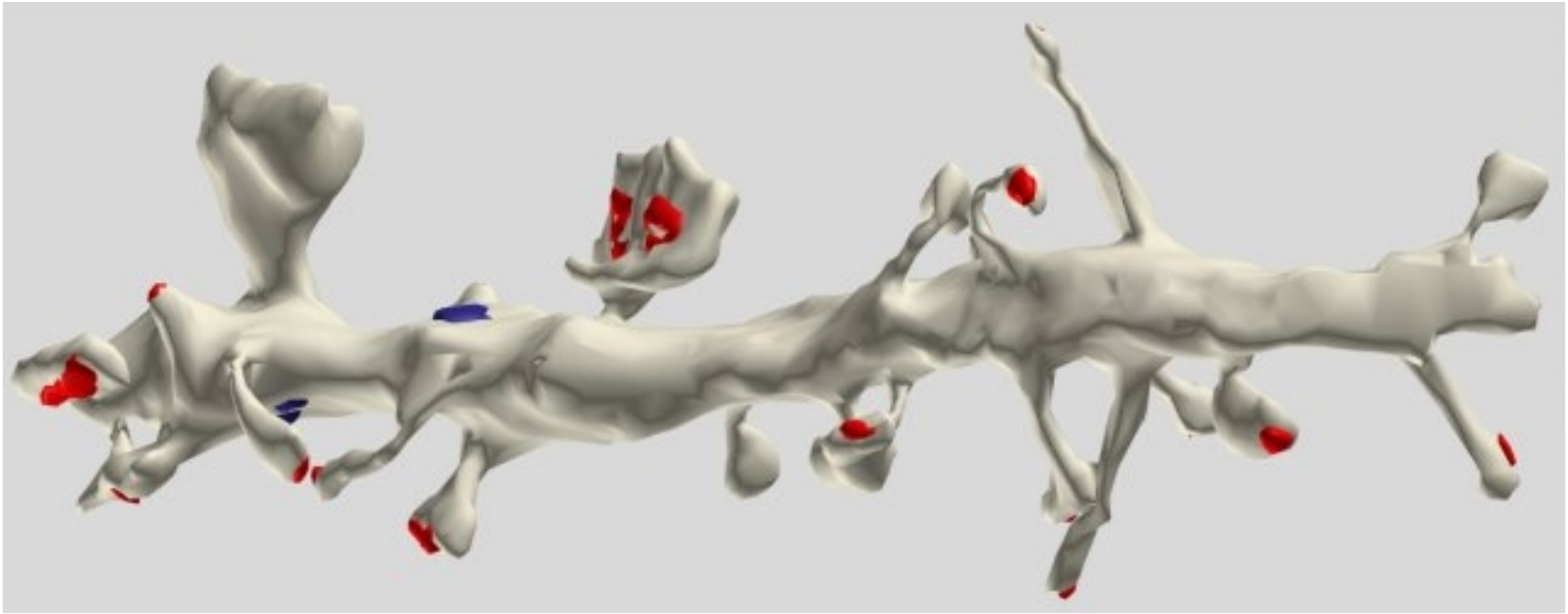


Shimizu et al. (2003) *J Mol Biol* 329:291-309.

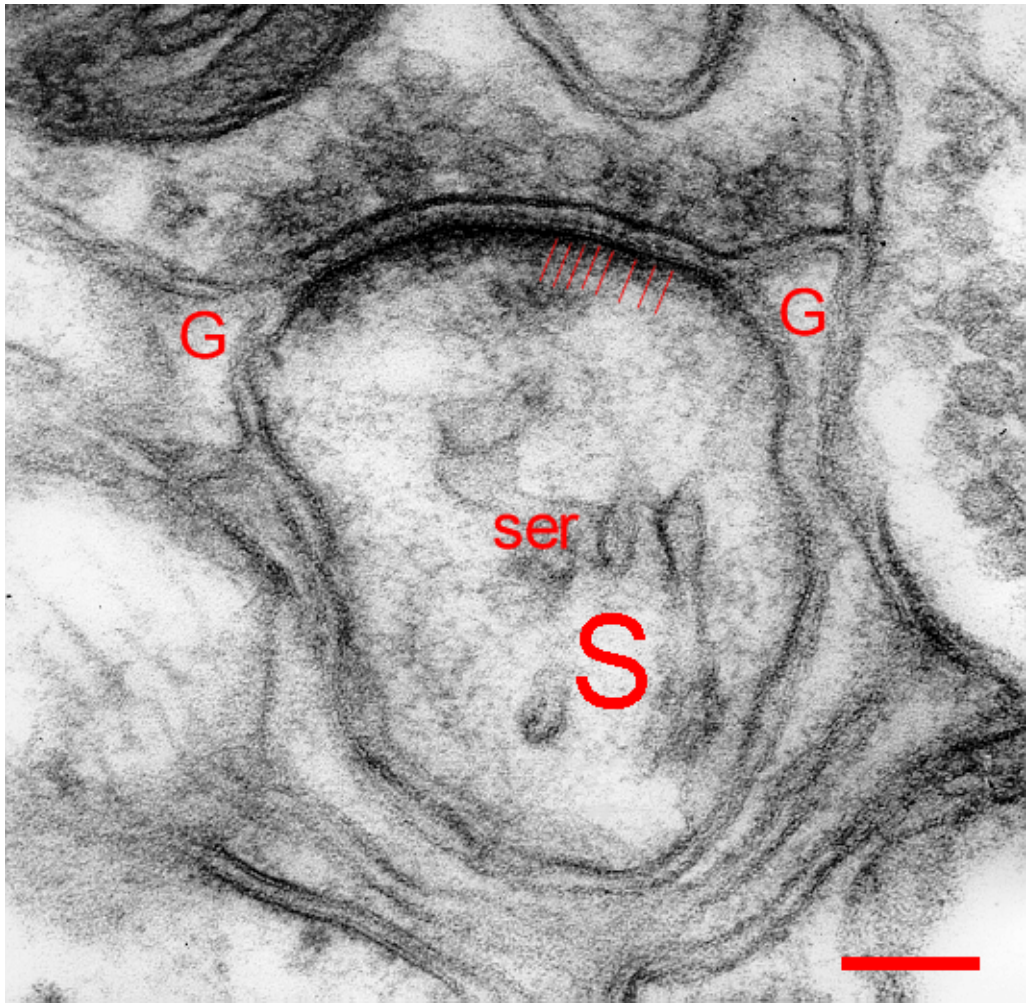
Future of StochSim

- 3D lattice
- Reactions between different multistate molecules
- New native format based on XML (extension of SBML)
- New GUI

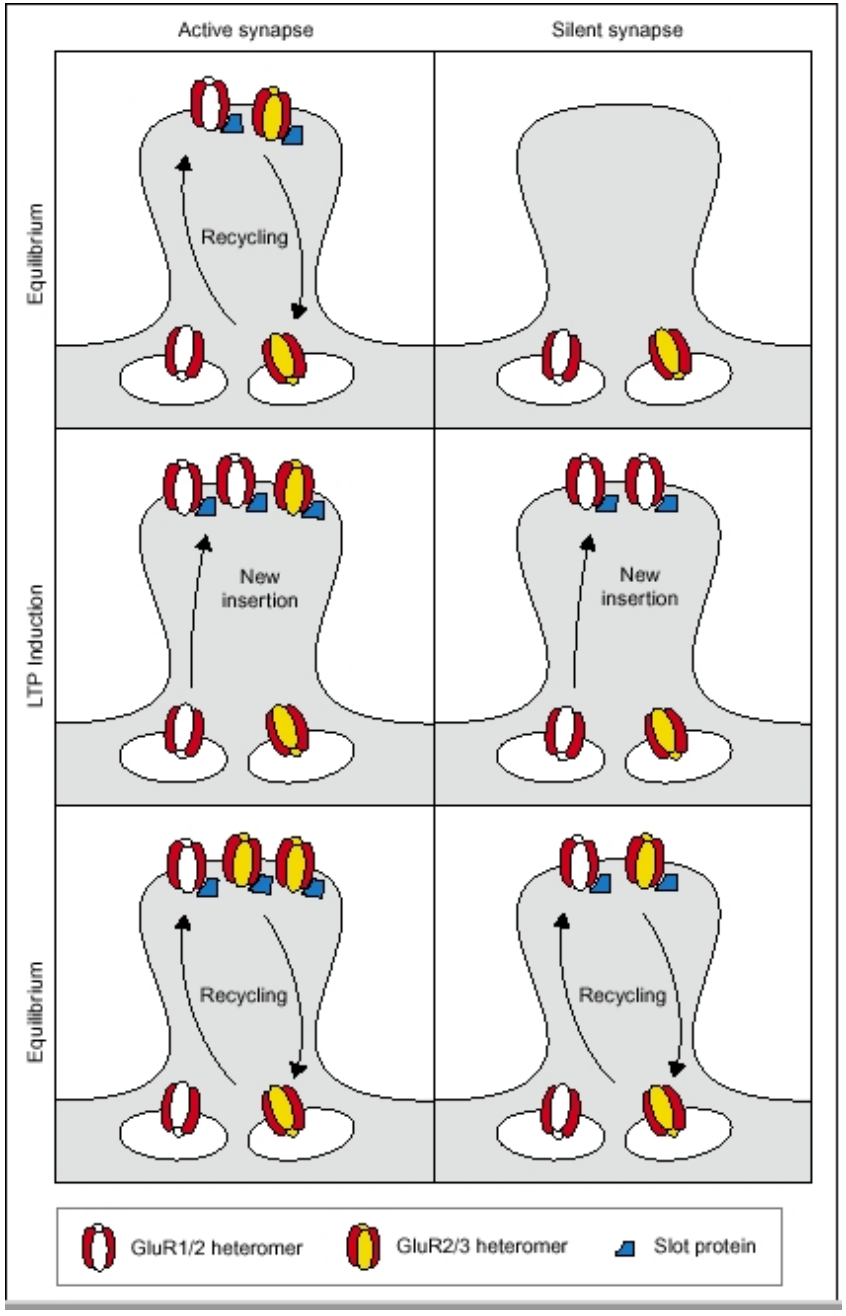
A spiny dendrite



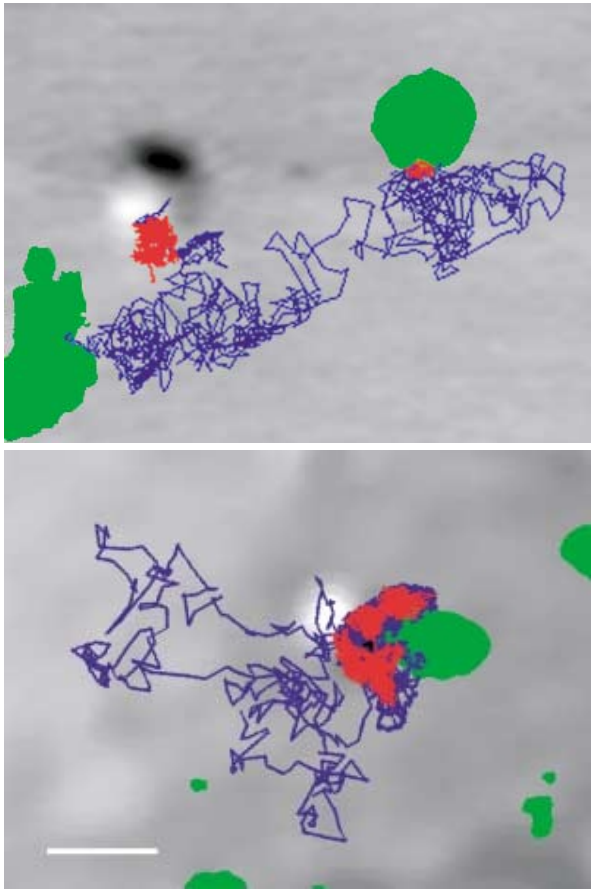
Dendritic spine



Receptors for neurotransmitters are moving



Barry and Ziff. (2002)
Curr Opin Neurobiol, 12: 279-286



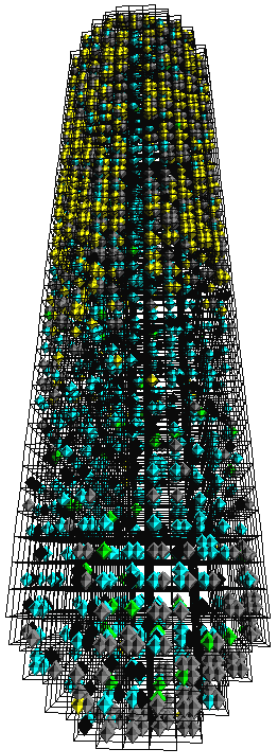
Choquet & Triller (2003)
Nat Rev Neurosci, 4: 251-265

The mesoscopic scale

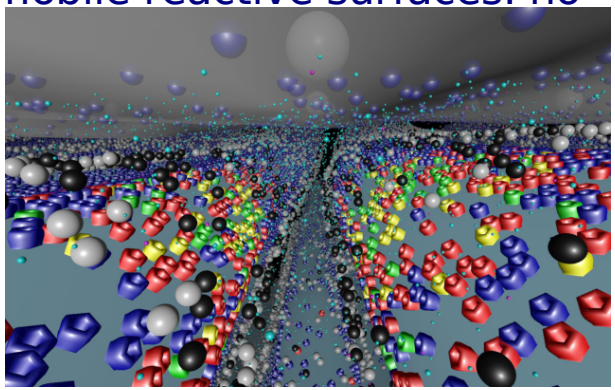
- molecule abstracted ⇒ macroscopic scale
- atomic details ⇒ microscopic scale
- Abstracted but realistic geometry ⇒ mesoscopic scale
- Relative size of object respected
- Differential location of binding sites
- realistic movements (speed and topology)

Existing software

- Population based (“spatial Gillespie”)
  - SmartCell, Mesord
  - finite elements (voxels), no individual molecules

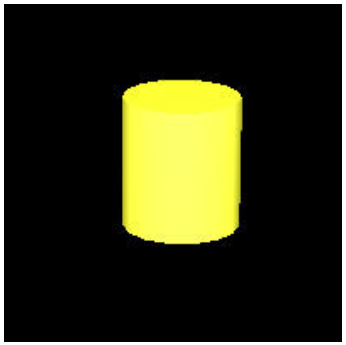


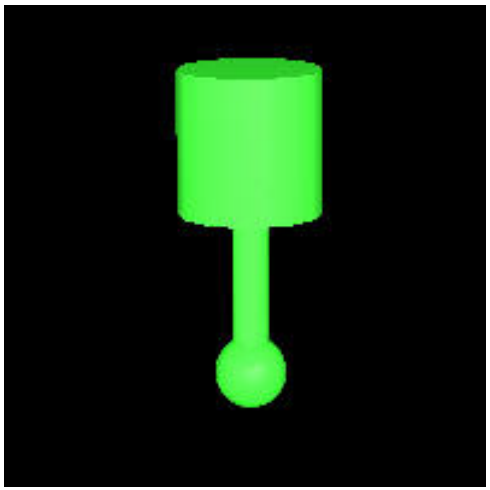
- Single-particle based
  - MCell: individual small molecules, ray-tracing. Immobile reactive surfaces. no interactions between mobile molecules

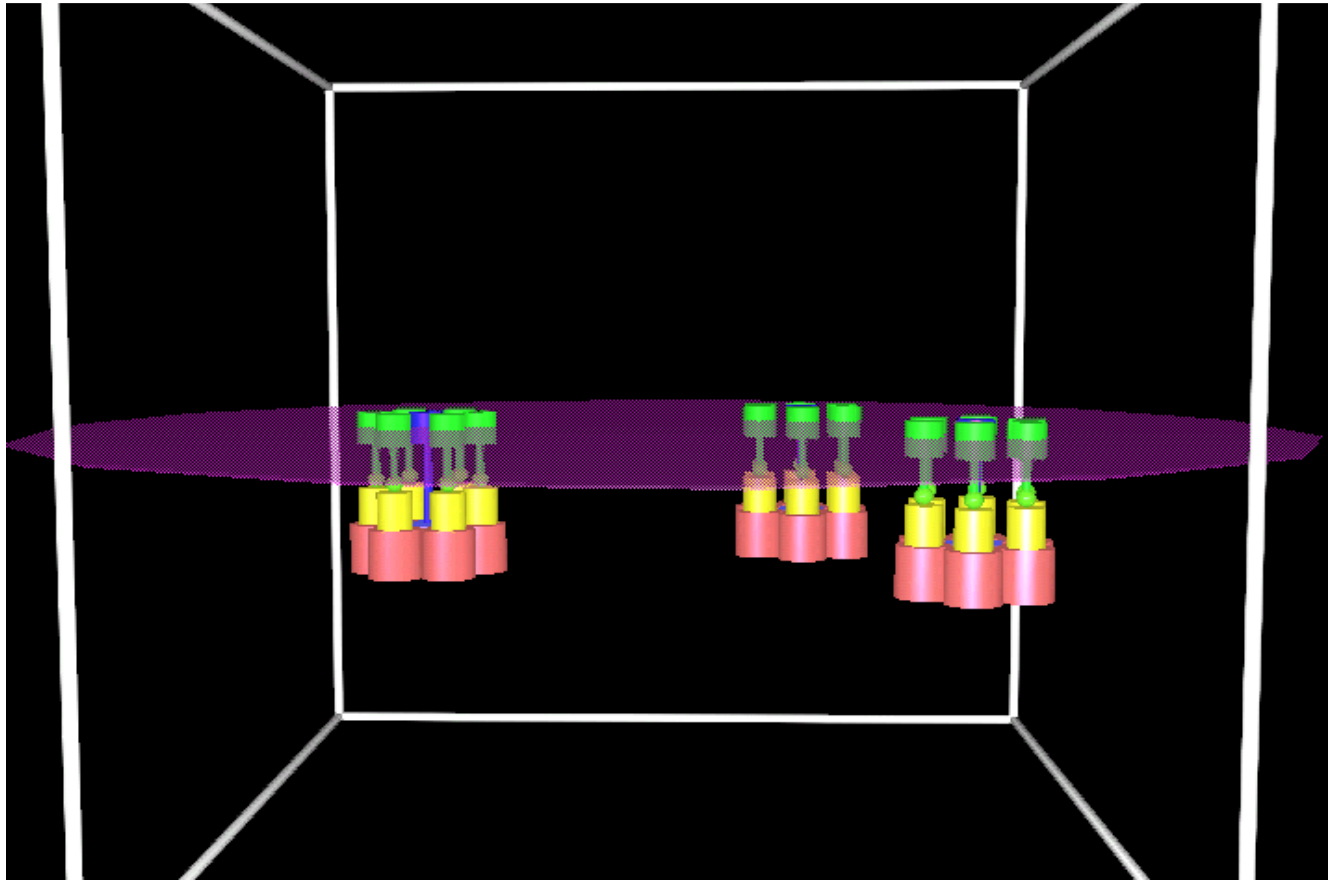


  - Smoldyn: individual small molecules, reactions between them
  - Meredys: Everything plus topology of molecules

Meredys: Particles, Objects and Clusters







Particles carry binding sites

Cluster class allows recording of Center Of Mass, radius, RMS displacement;
possibility of cluster state

Clusters are dynamically created and destroyed – transient.

Molecule diffusion

- Different diffusion spaces:
  - Static; Free diffusion; Membrane diffusion; Above membrane; Below membrane
- Two types of motion:
  - Translational $\bar{r^2} = 2\mathrm{D}_T t = 2\mathrm{kb}_T t$
  - Rotational $\bar{\theta^2} = 2 D_R t = 2\mathrm{kb}_R t$
- random walk algorithm

$$p(x,t) = \frac{1}{\sqrt{4\pi Dt}} \exp - \frac{x^2}{4\mathrm{Dt}}$$ gaussian with $\sigma^2 = 2Dt$

  - Translational $\Delta(x,y,z) = \sqrt{2\mathrm{D}_T t} \times gaussRand$
  - Rotational $\Delta\theta = \frac{\sqrt{2\mathrm{D}_R t}}{r} \times gaussRand$
- Two types of diffusion equations:
  - unrestricted brownian motion – Low Trans/Rot
  - intra-membrane diffusion (Saffman and Delbrück 1975) – High Trans/Rot

Molecule diffusion

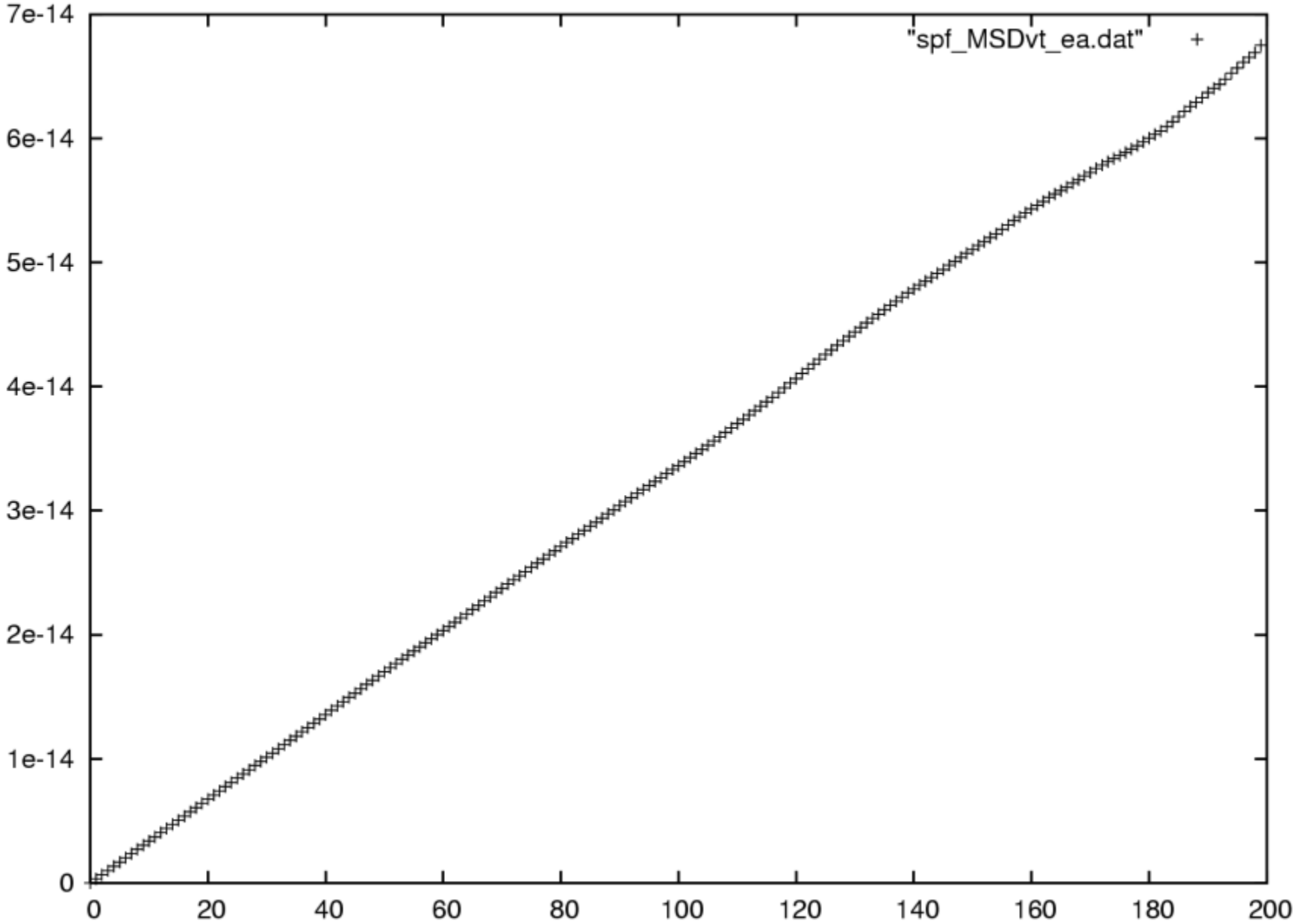
- Unrestricted brownian motion – Low Translation/Rotational

$$b_T = \frac{1}{6\pi\mu r} \qquad b_R = \frac{1}{8\pi\mu r^3} \qquad \frac{b_T}{b_R} = \frac{4}{3}r^2$$

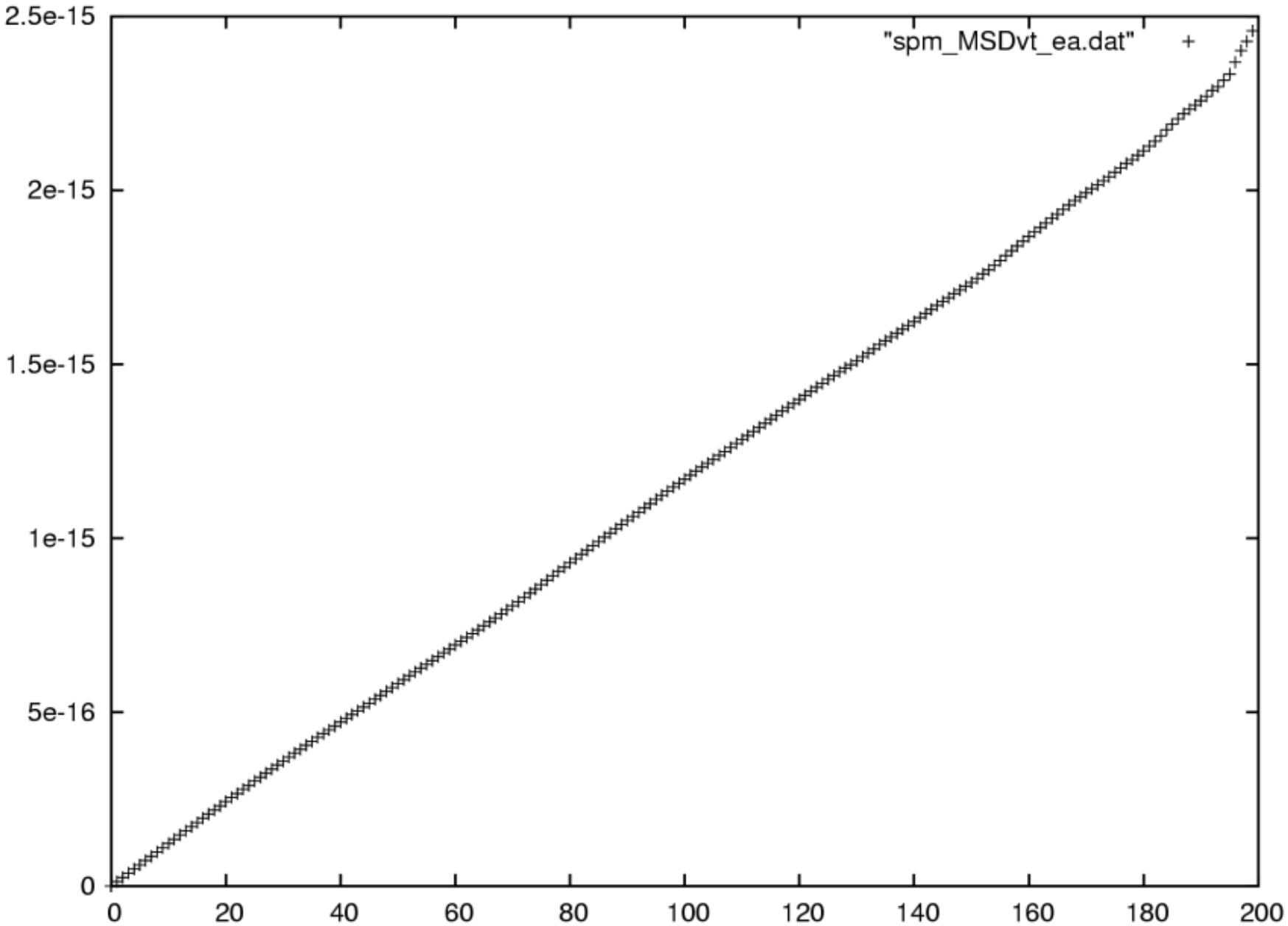
- Intra-membrane diffusion (Saffman and Delbrück 1975)
  High Translational/Rotational

$$b_T = \frac{1}{4\pi\mu h}\left(\log\left(\frac{\mu h}{\mu' r}\right) - \gamma\right) \qquad b_R = \frac{1}{4\pi\mu r^2 h} \qquad \frac{b_T}{b_R} = \left(\log\left(\frac{\mu h}{\mu r}\right) - \gamma\right) \times r^2$$

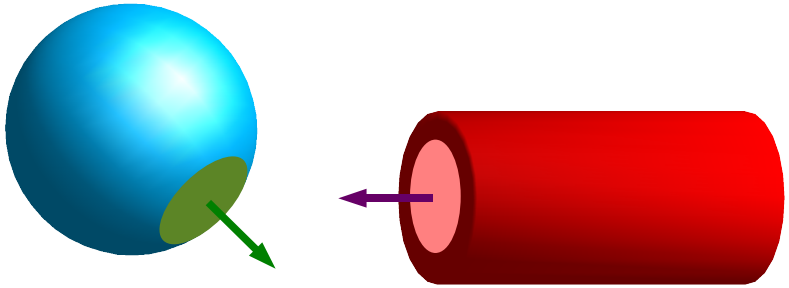
RMS displacement for free diffusion



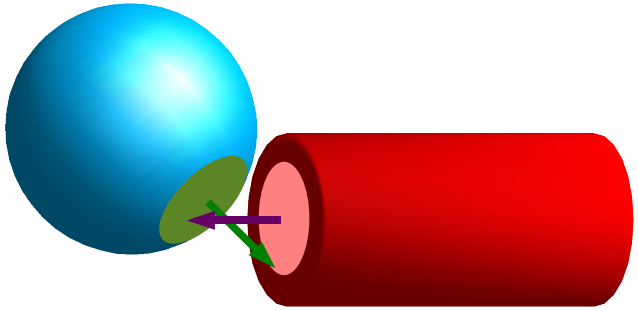
RMS displacement for membrane diffusion



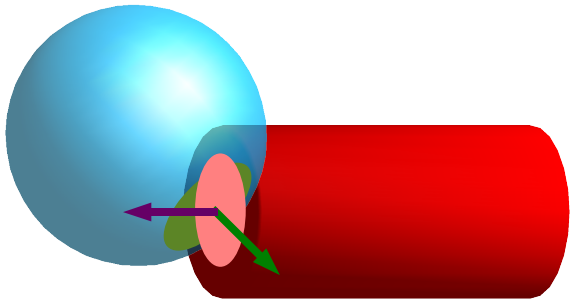
Reactions and complex formation



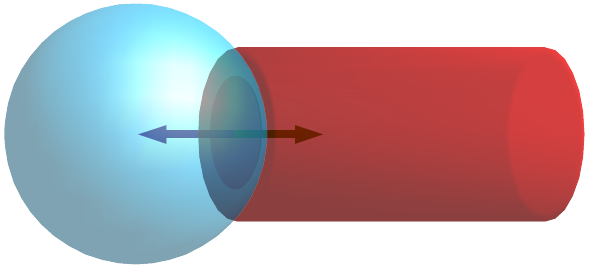
Reactions and complex formation

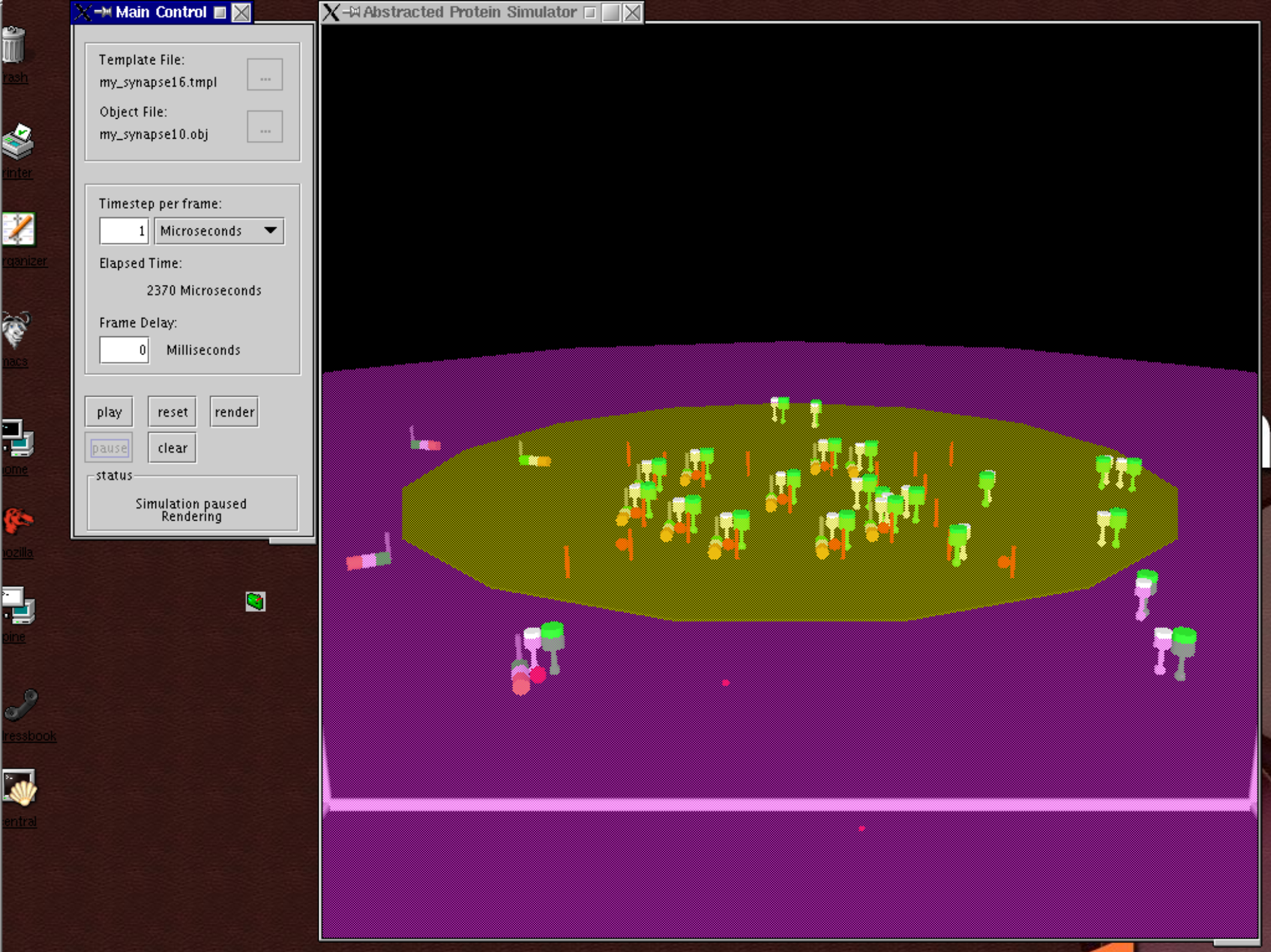


Reactions and complex formation



Reactions and complex formation

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reset
render
pause
clear
status
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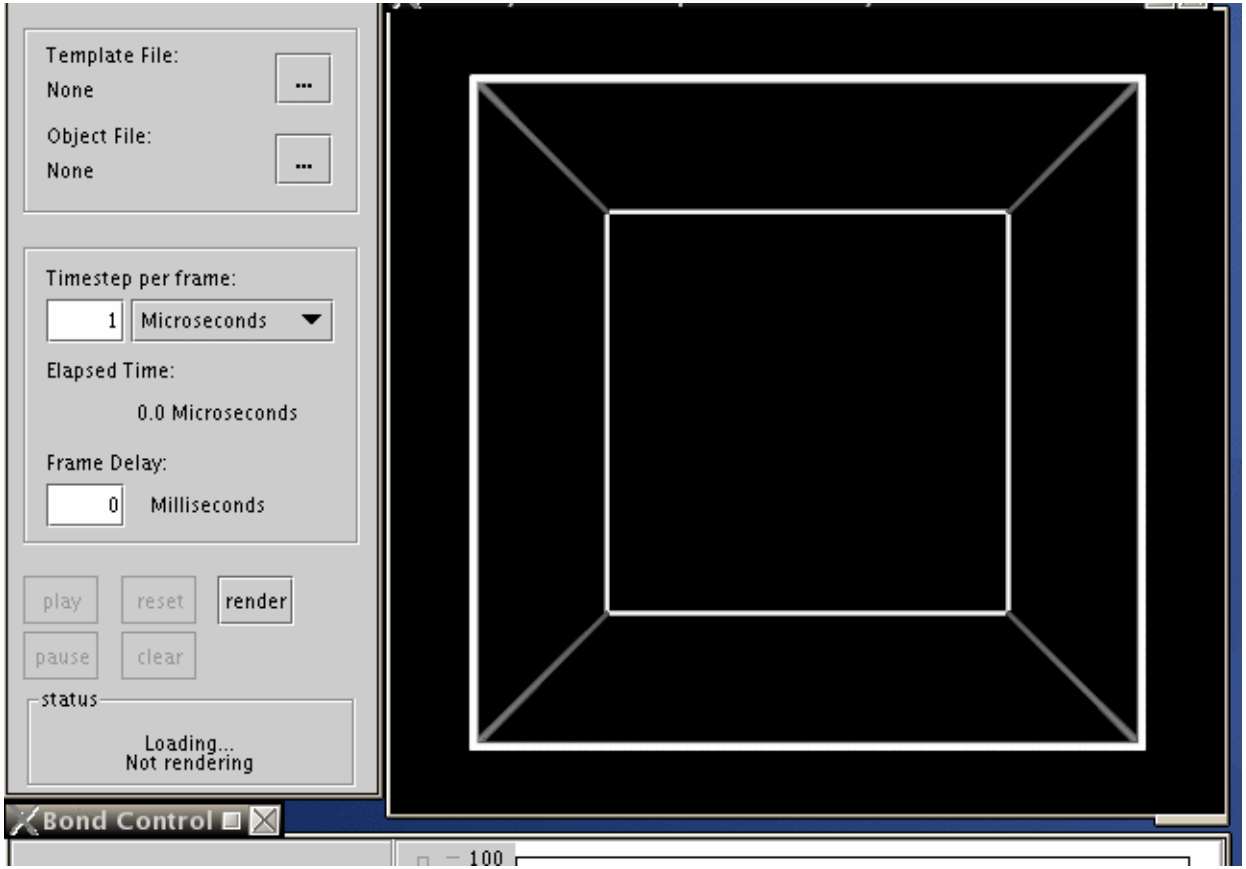
Rendering
Abstracted Protein Simulator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Milliseconds
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reset
render
pause
clear
status
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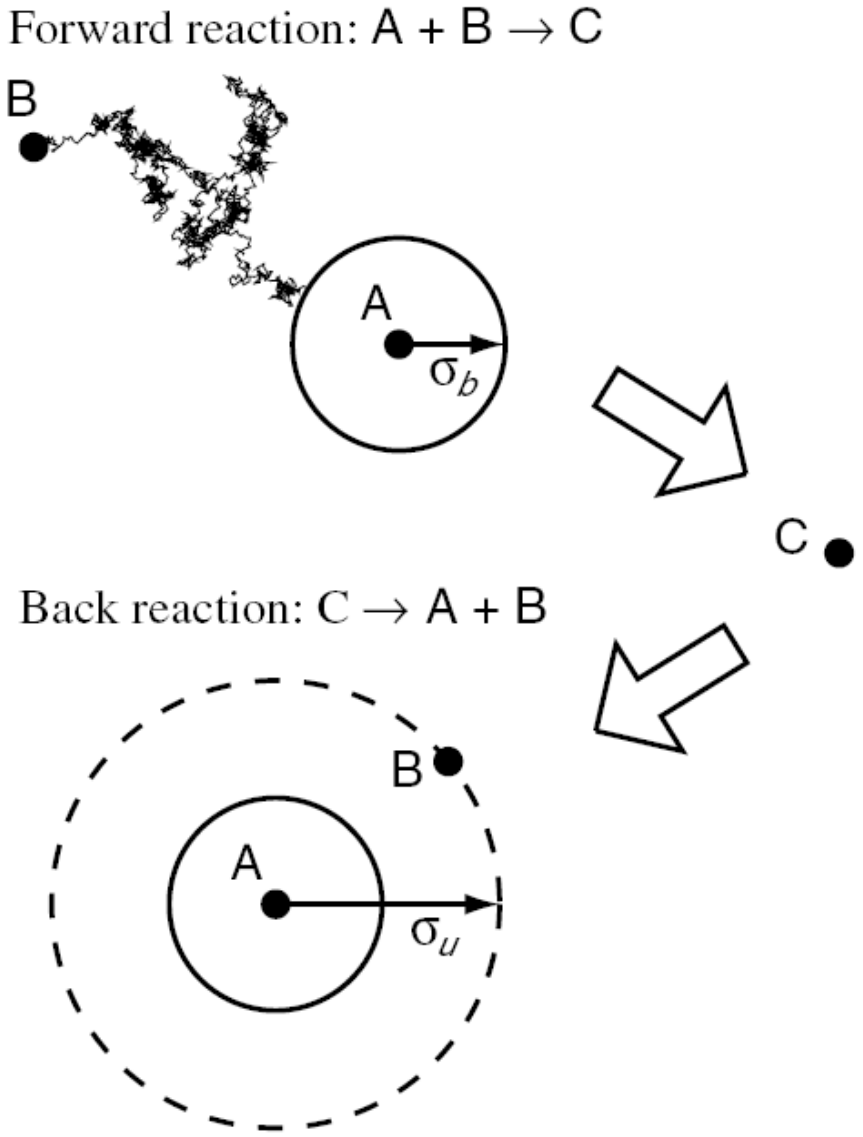
Not rendering
Bond Control
100

Remaining problems

- Probabilities of reactions are hard-coded
- Molecules can have several states, but a state does not affect the reactions
- Shape of molecules does not affect diffusion
- ...

Smoluchovski model

- Smoldyn
  (Andrews and Bray (2004) *Phys Biol* 1: 137-151)
  - + single-particle
  - + binding radius (probability of reaction) related to kinetics
  - - no volume, shape, mass,
  - - No complexes
  - - No multistate molecules



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- Lu Li
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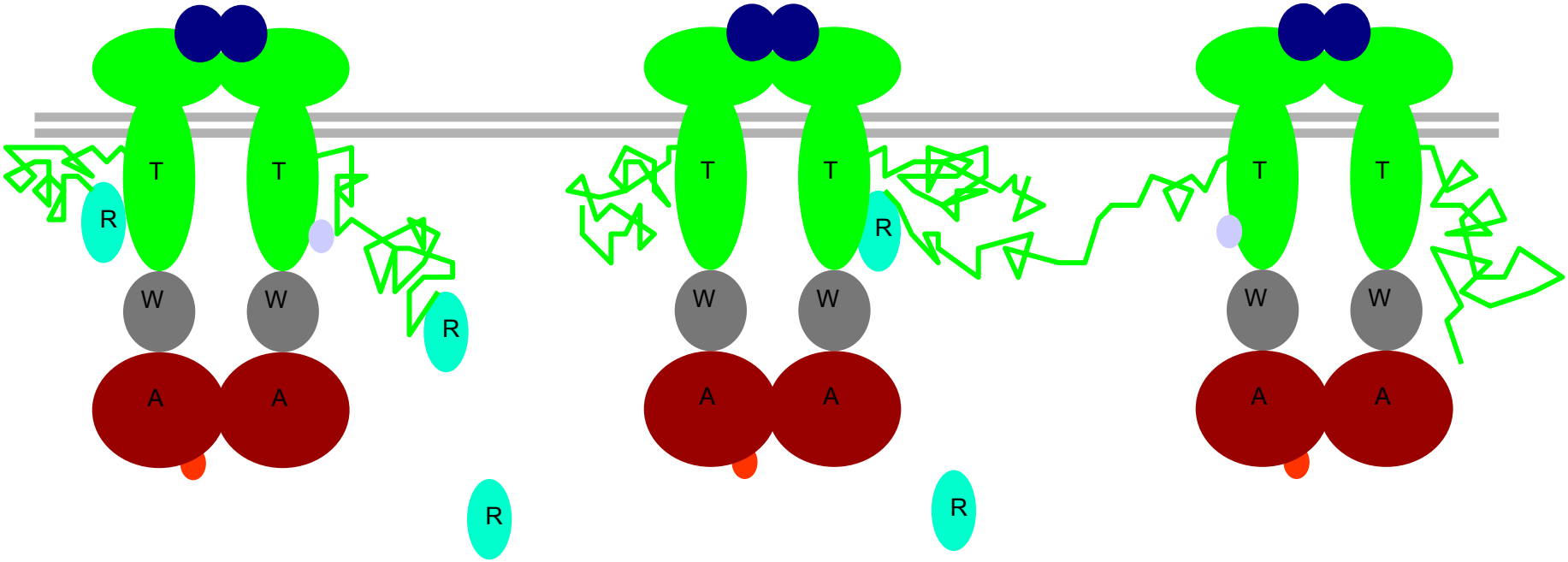
- Renaud Schiappa
- Melanie Stefan
- **Dominic Tolle**

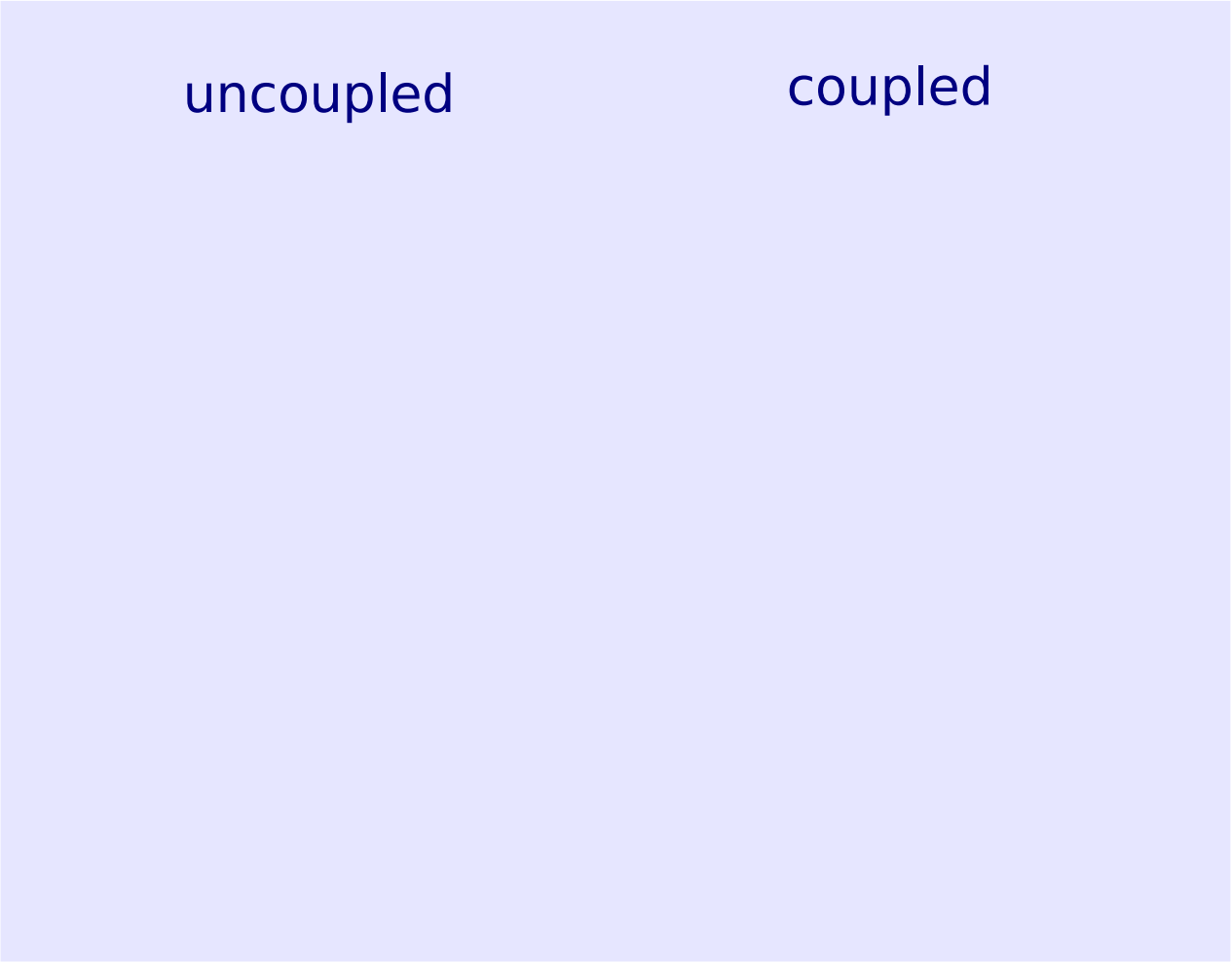
http://www.ebi.ac.uk/compneur-srv/doc/Tolle2006.pdf

Molecular brachiation

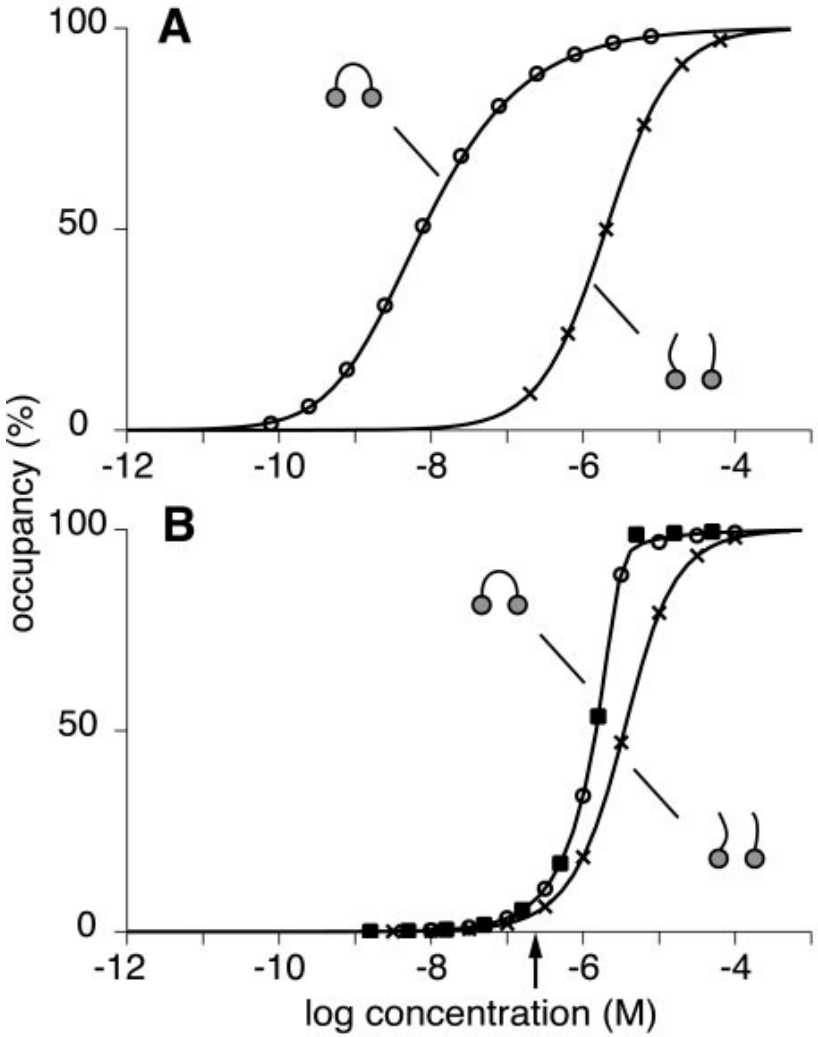
Interaction between receptors and CheR (methyltransferase)



Molecular brachiation



Effective Kd

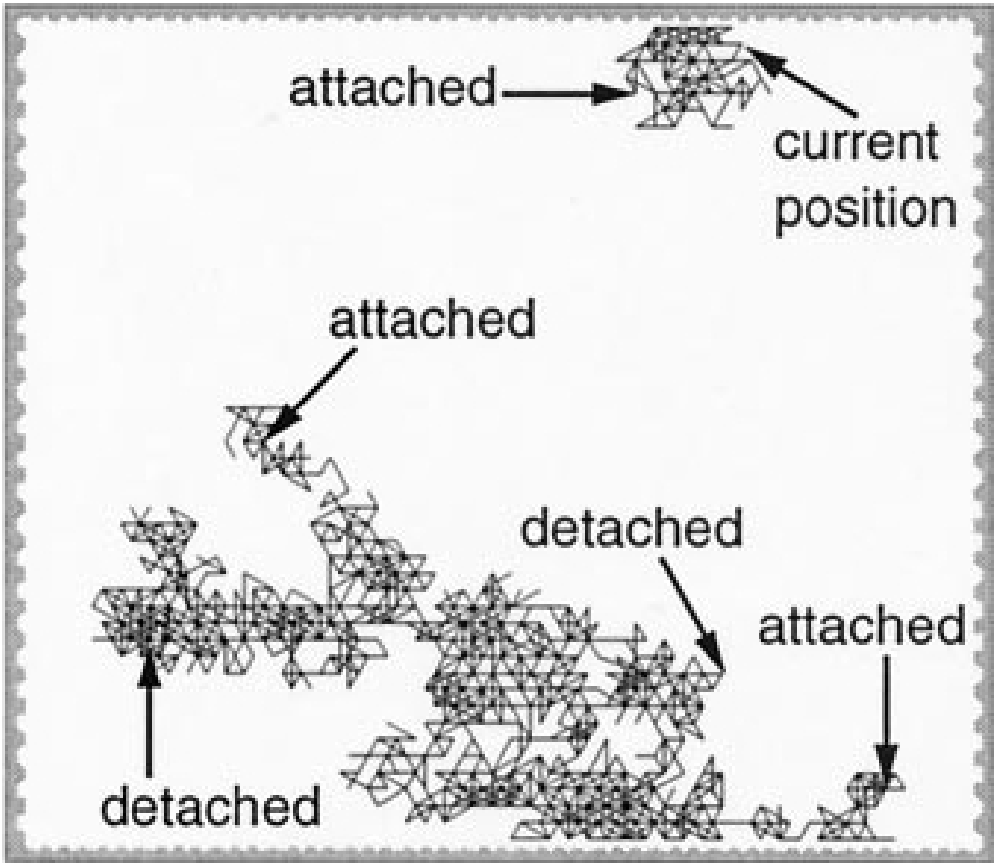


Excess brachiating molecule

Limiting brachiating molecule

Levin et al. (2002) *Biophys J* 82:1809-1817.

Segregation by affinity

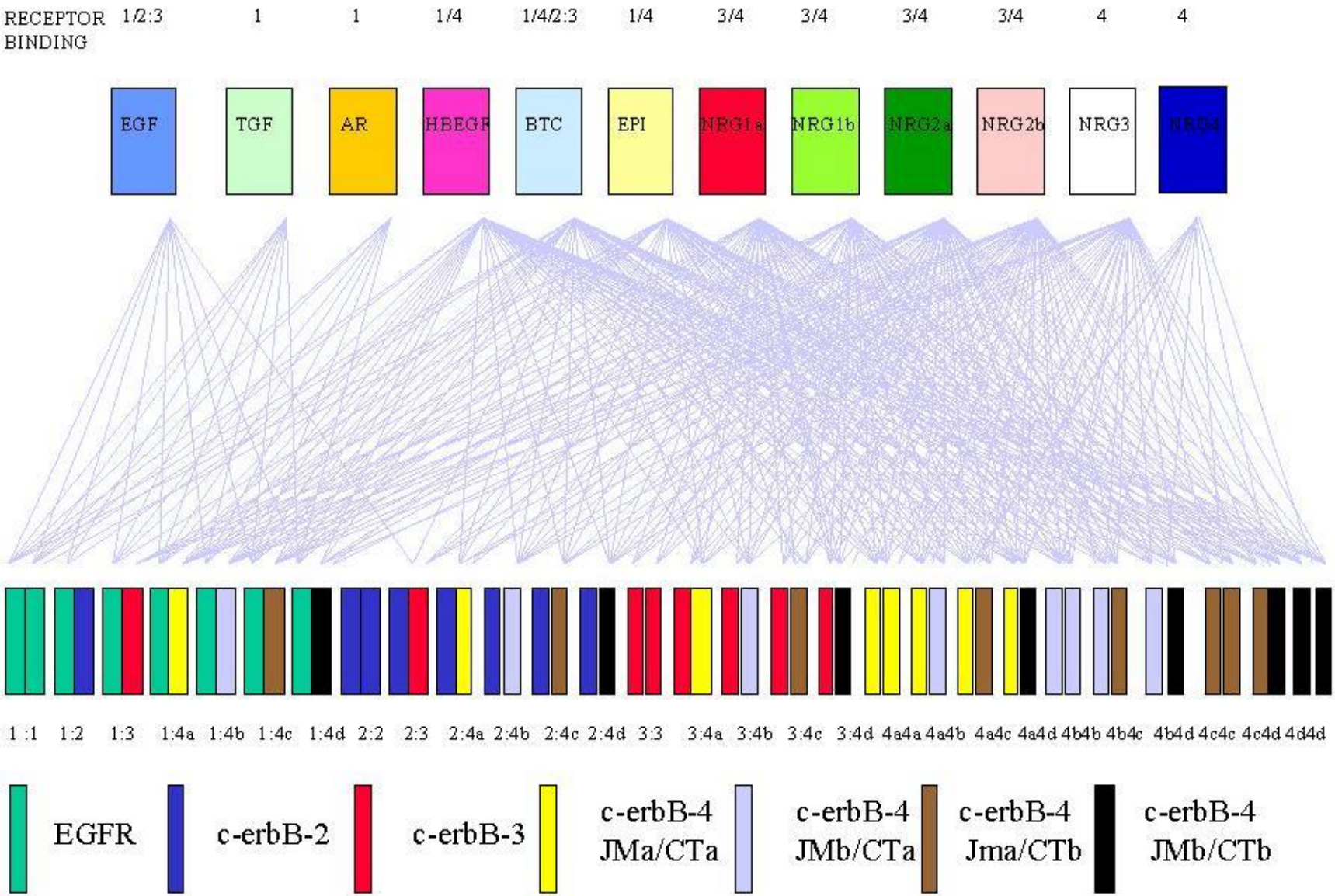


Each CheR molecule visits more receptors, some of them repetitively;

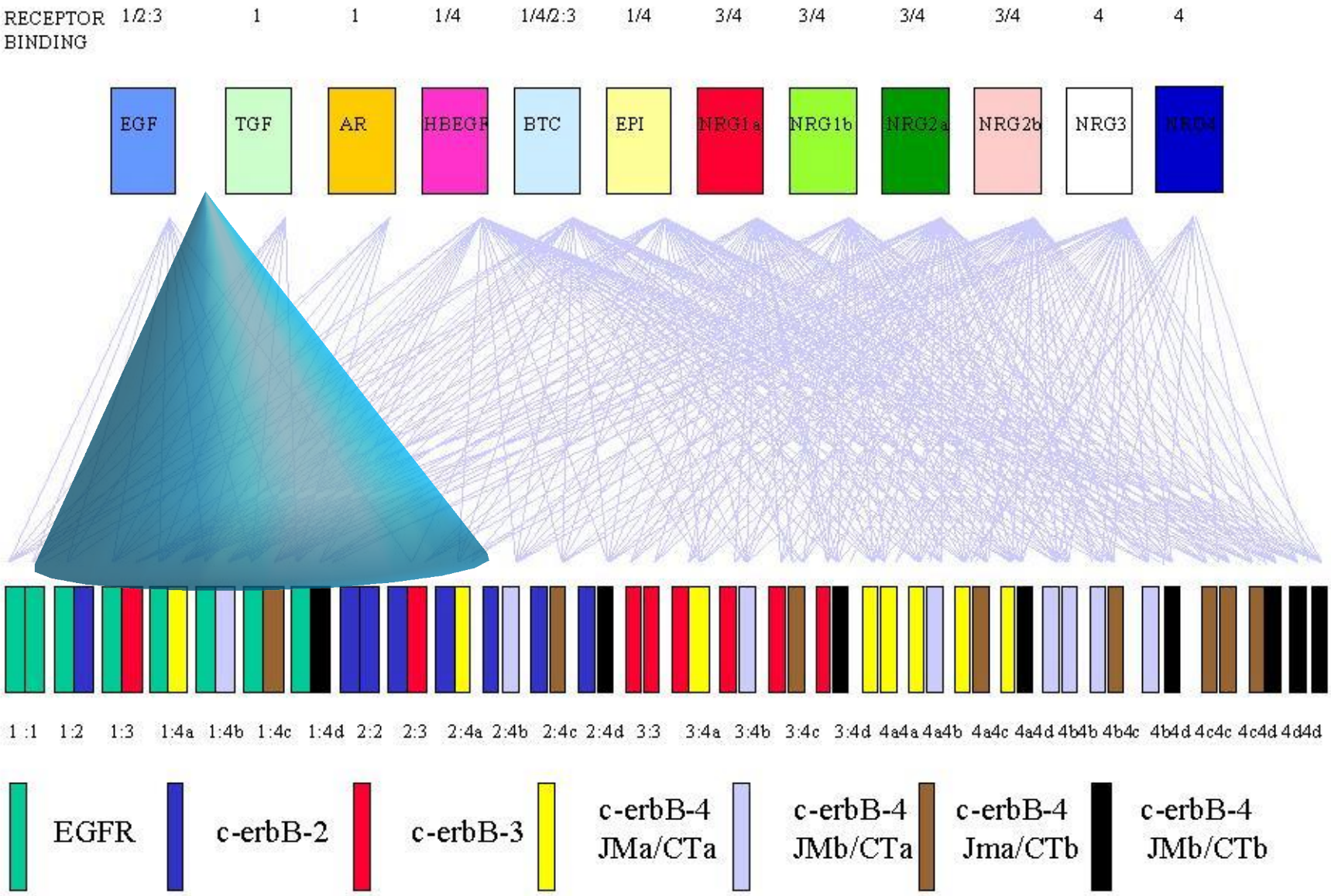
CheR molecules are trapped into the receptor lattice.

Levin et al. (2002) *Biophys J* 82:1809-1817.

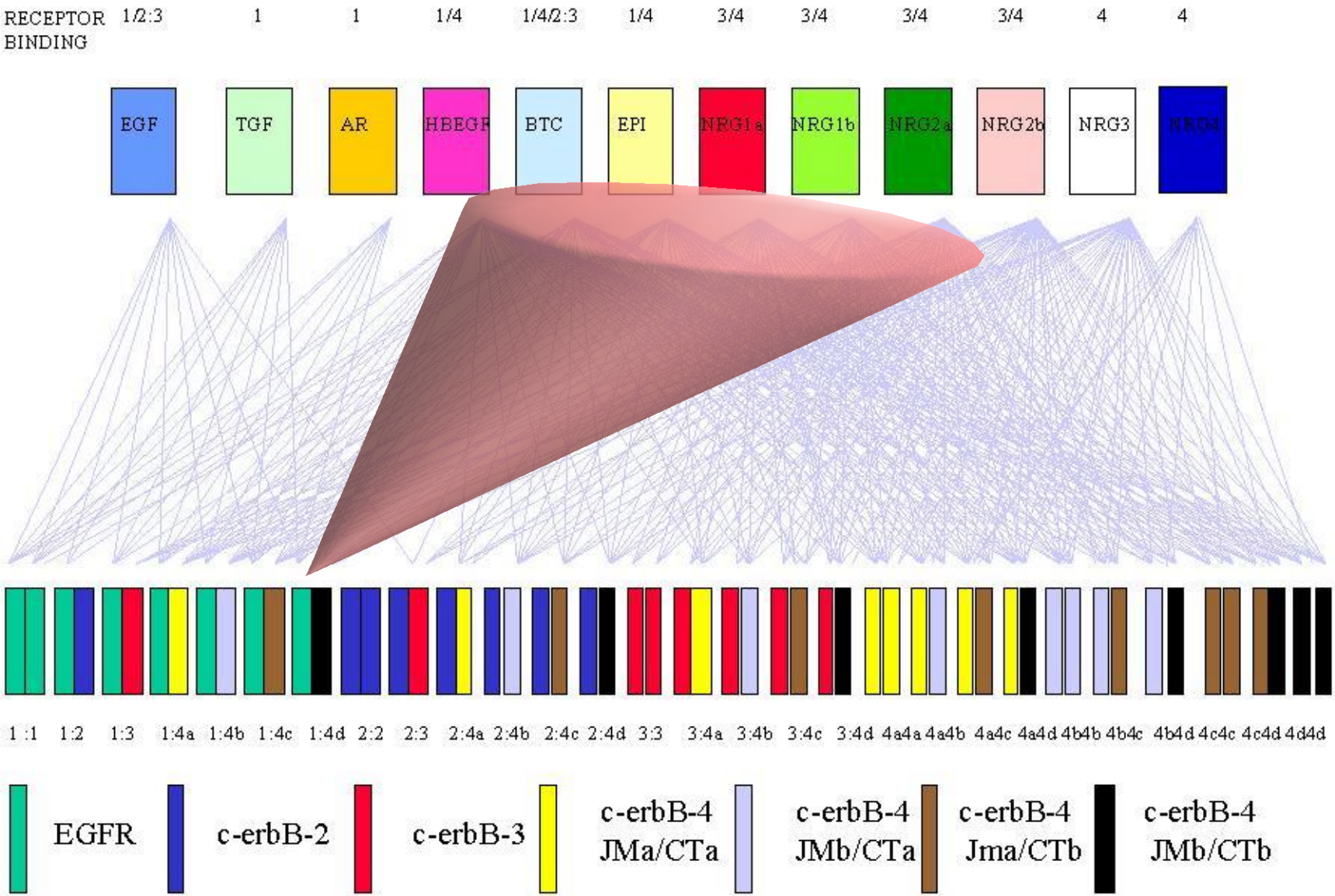
Receptor hourglass



Receptor hourglass

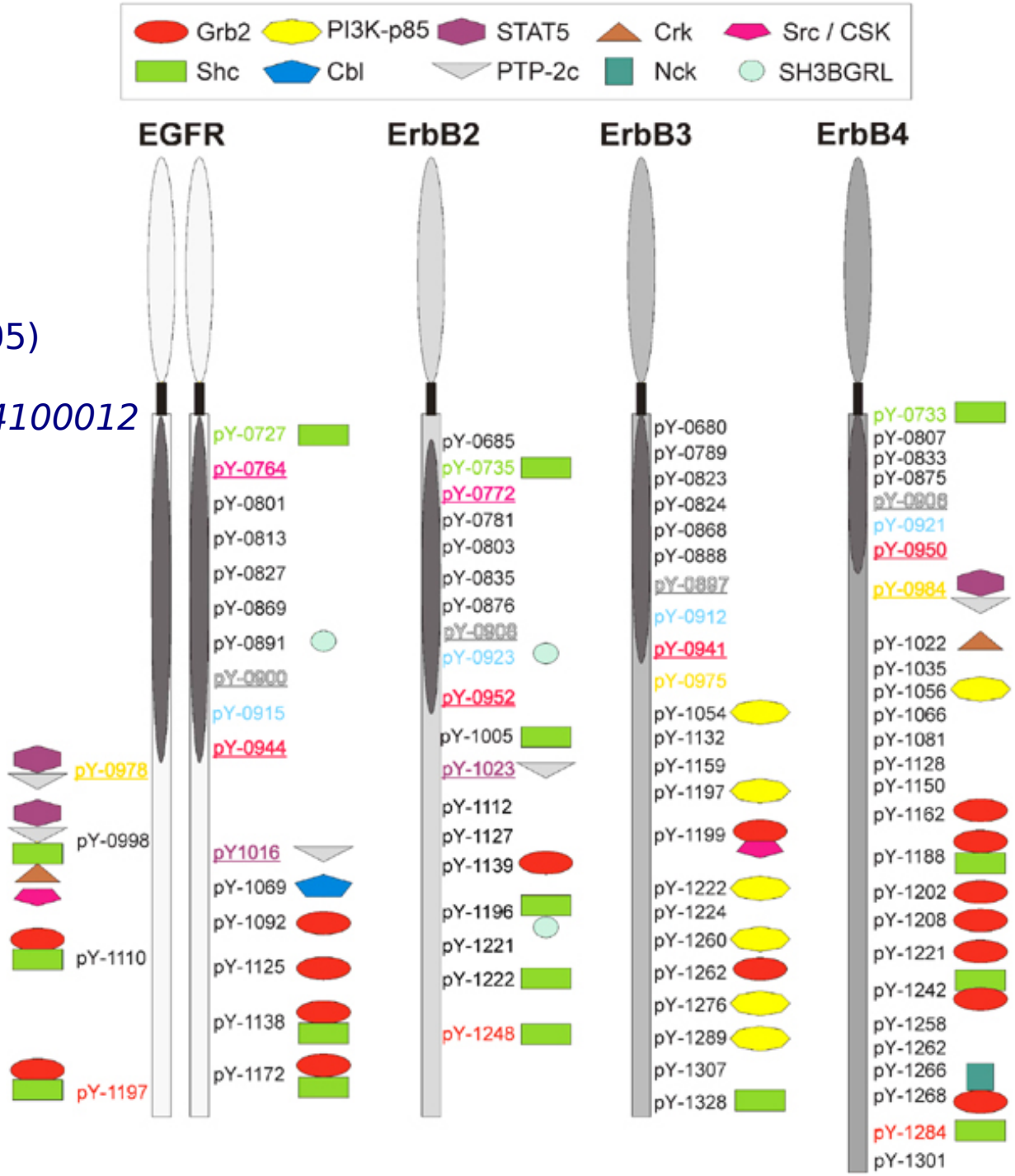


Receptor hourglass



Combinatorial explosion

Schulze *et al*. (2005)
Mol Sys Bio,
doi: 10.1038/msb4100012



Spatial cascades

Kholodenko (2003) *J Exp Biol,* 206, 2073-2082