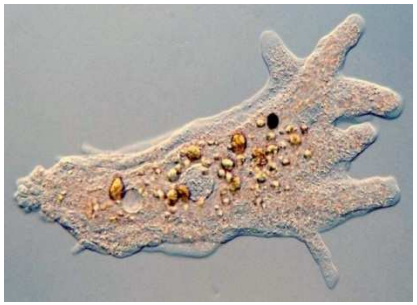
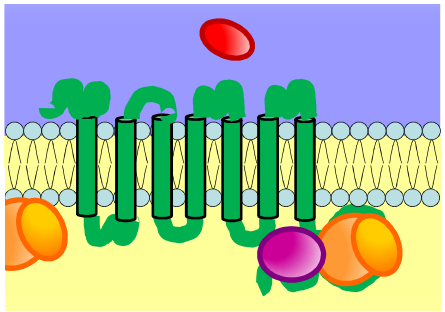
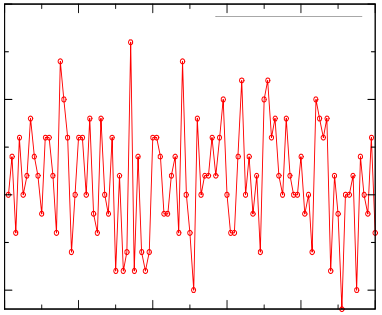
Universiteit Leiden

Reverse engineering of the gradient sensing system in *Dictyostelium* requires stochastic modeling







NDNS+ workshop

Eurandom, Eindhoven University of Technology

dr. Sander Hille

Friday, 16 April 2010

Ongoing joint work …

Experimental:

Biophysics group: Single Molecule Microscopy – SMM (University Leiden)

Thomas Schmidt

Freek van Hemert *(finished PhD end December 2009)*

Ewa Snaar-Jagalska (Leiden Institute of Biology)

Leiden Institute of Physics

Modeling, analysis & simulation:

Johan Dubbeldam (Mathematical Physics, TU Delft)

Technische Universiteit Delft

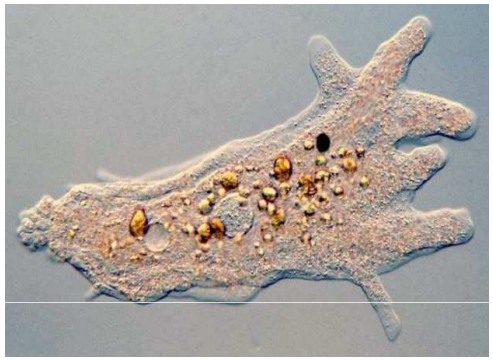
Eidrees Ghariq *(BSc student in Physics & Math, UL)*

SH (Mathematical Institute, University Leiden)

Mathematisch Instituut
Universiteit Leiden

Dictyostelium discoideum

An introduction:
A so-called ‘slime mould’



~20 µm

An amoeba

Living in the soil, e.g. in decomposing wood

A macroscopic colony

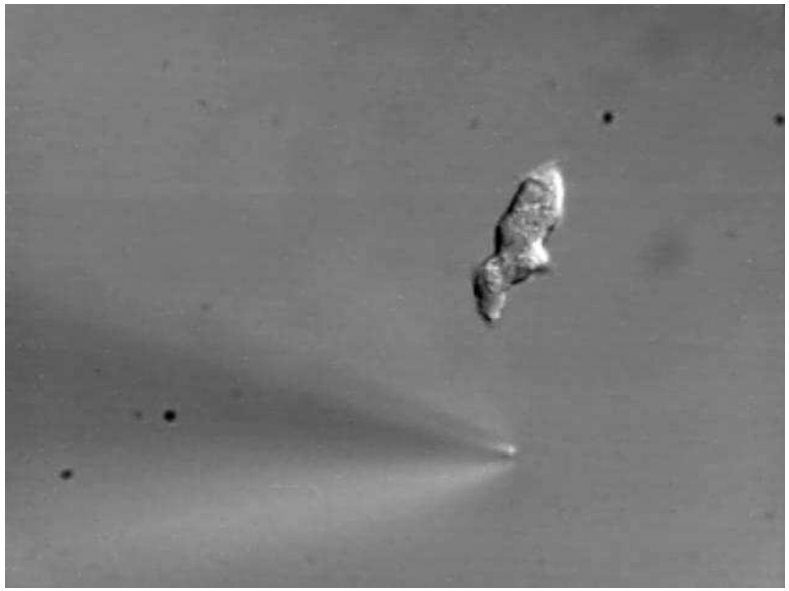
Chemotactic movement

Single cell movement:

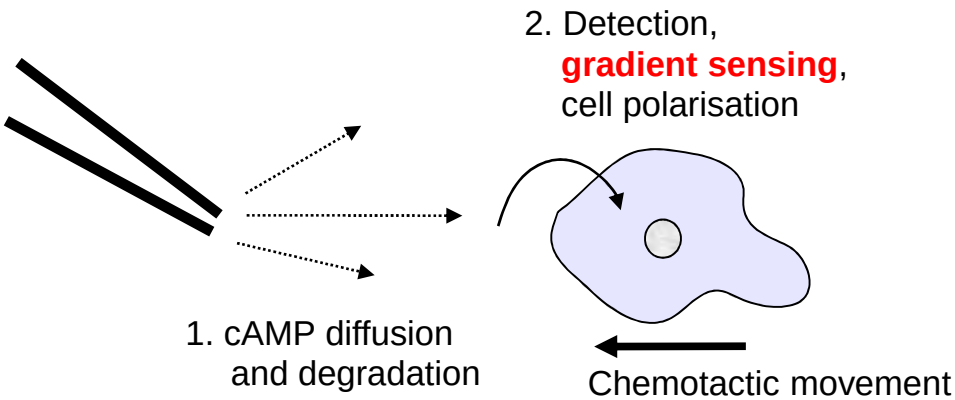
In response to external signal of a chemotactic compound, here **cAMP**

*(**c**yclic **A**denosine **M**ono-**P**hosphate)*

Micropipette with cAMP →

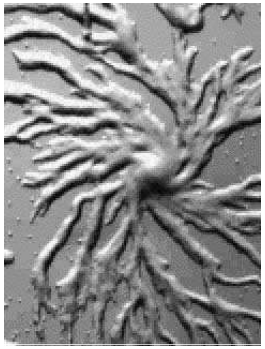


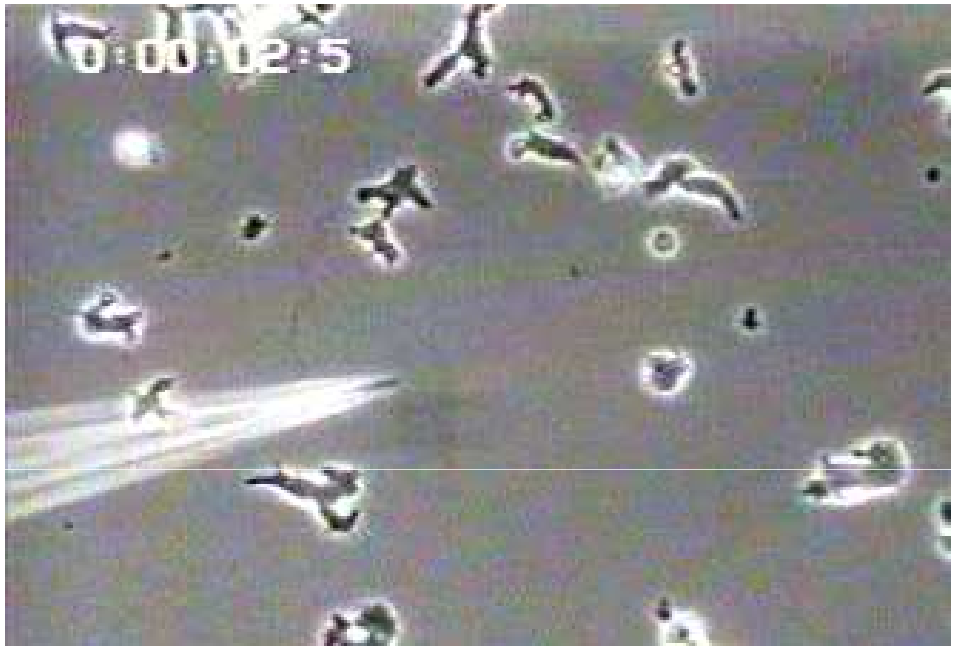
Source: Dictyostelium cinema (Firtel lab)



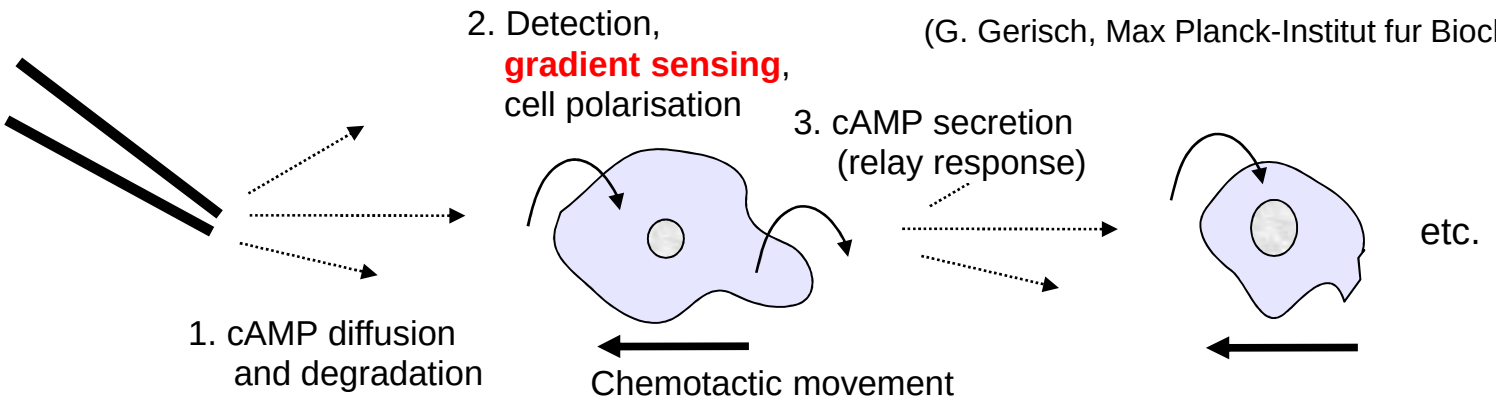
Chemotactic movement and gradient sensing

Multi-cell movement:



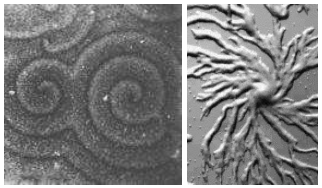


(G. Gerisch, Max Planck-Institut fur Biochemie)



Modeling phenomena in chemotaxis

Macroscopic level

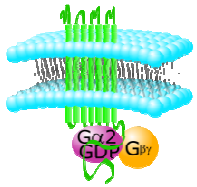


Aggregation

Mesoscopic level

Single cell movement
Cell-cell interaction

Microscopic level



Internal cell signaling network
Molecular movement & interactions

Deterministic:

Patlak-Keller-Segel chemotaxis model

Kinetic models for chemotaxis

Hydrodynamic models for chemotaxis

Deterministic:

Local Excitation Global Inhibition
(Deverotes et al. , 2004)

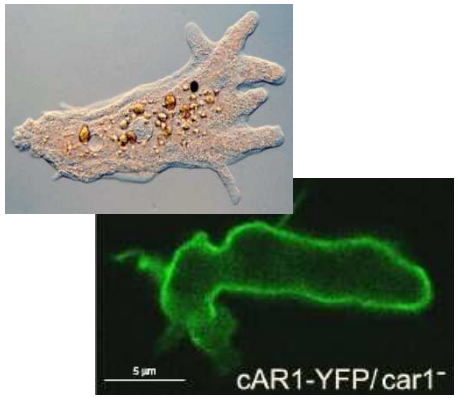
Depletion model
(Postma & Van Haastert , 2001)

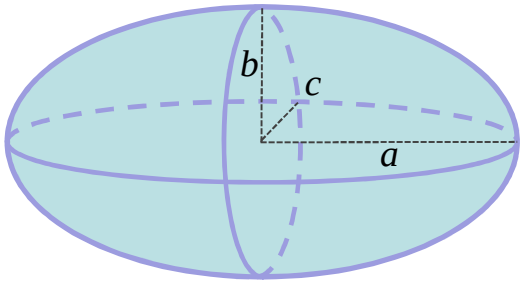
Balanced Inactivation model
(Levine, Kessler & Rappel, 2006)

Stochastic:

Stochastic Excitable System model
(Naoki, Sakamura & Ishii, 2008)

Microscopic level
-- Cell dimensions --





Ellipsoidal cell:
$2a$ x $2b$ x $2c \approx$ 20 x 10 x10 µm

Volume:

$$V = \frac{4}{3}\pi abc \approx 1.04 \times 10^3 \mu\mathrm{m}^3$$

Surface area:

$$A \approx 4\pi \left(\frac{a^p b^p + a^p c^p + b^p c^p}{3} \right)^{1/p}, \qquad p = 1.6075$$

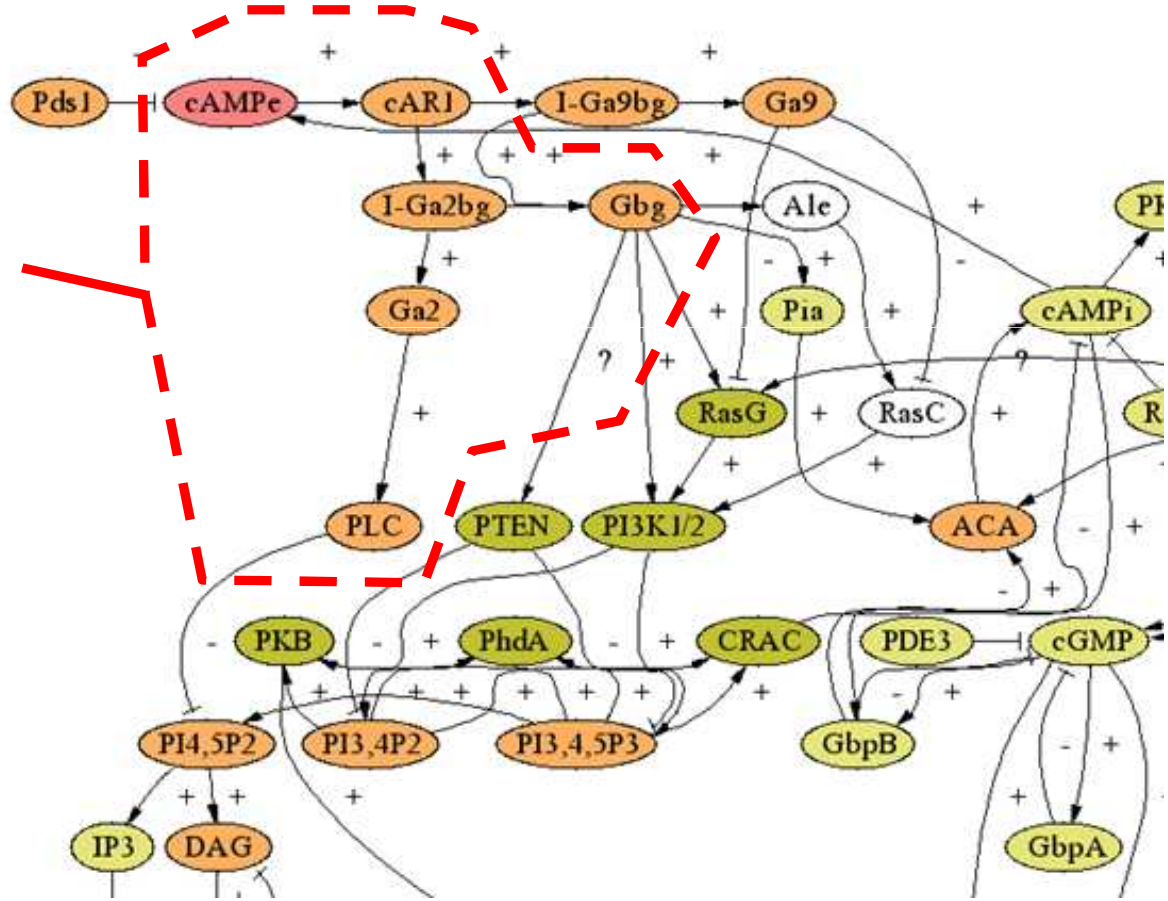
(Knud-Thompson formula)

$$\approx 5.4 \times 10^2 \mu\mathrm{m}^2$$

The molecular players

Small part of a large signaling network:

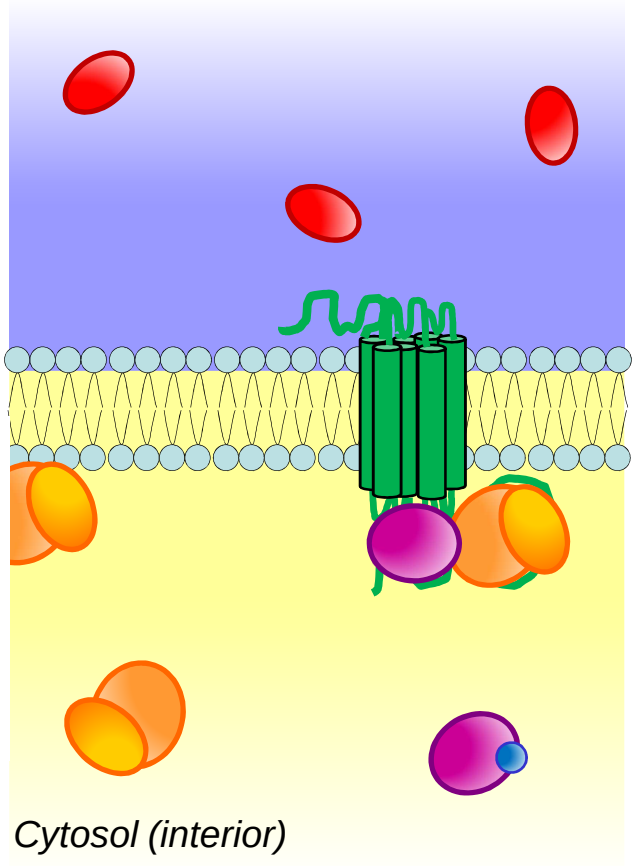
cAMP gradient sensing



Other compounds contribute to effective and efficient chemotaxis, but are **not essential** for gradient sensing

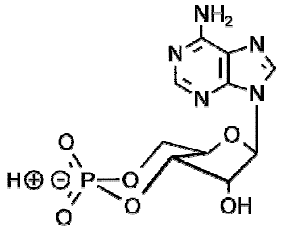
The molecular players

Environment (exterior)



Cytosol (interior)

cAMP



Plasma membrane
(lipid bilayer)

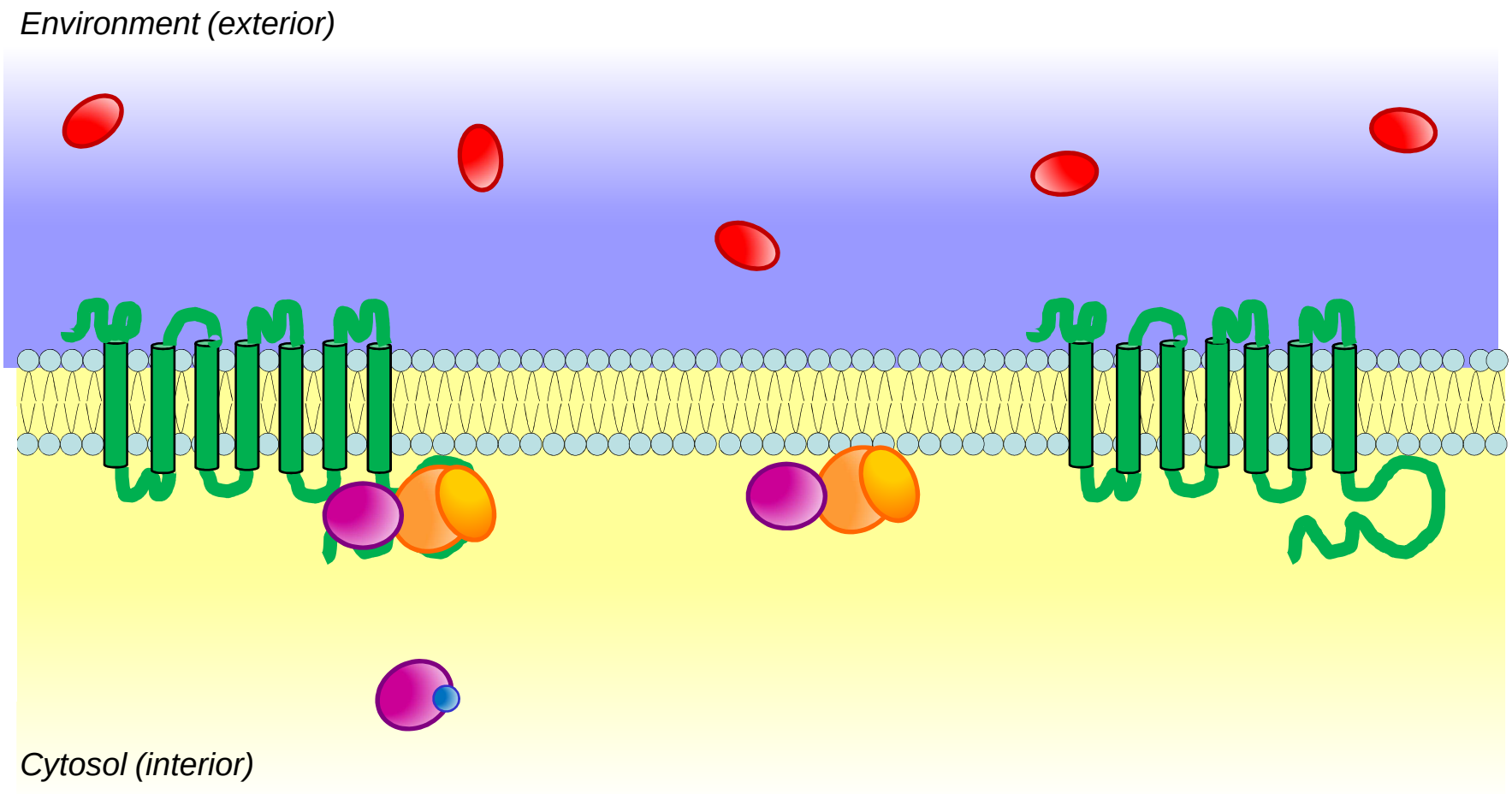
Seven-transmembrane G-protein coupled receptor (GPCR) for cAMP (**cAR1**)

G-potein complex; heterotrimer:

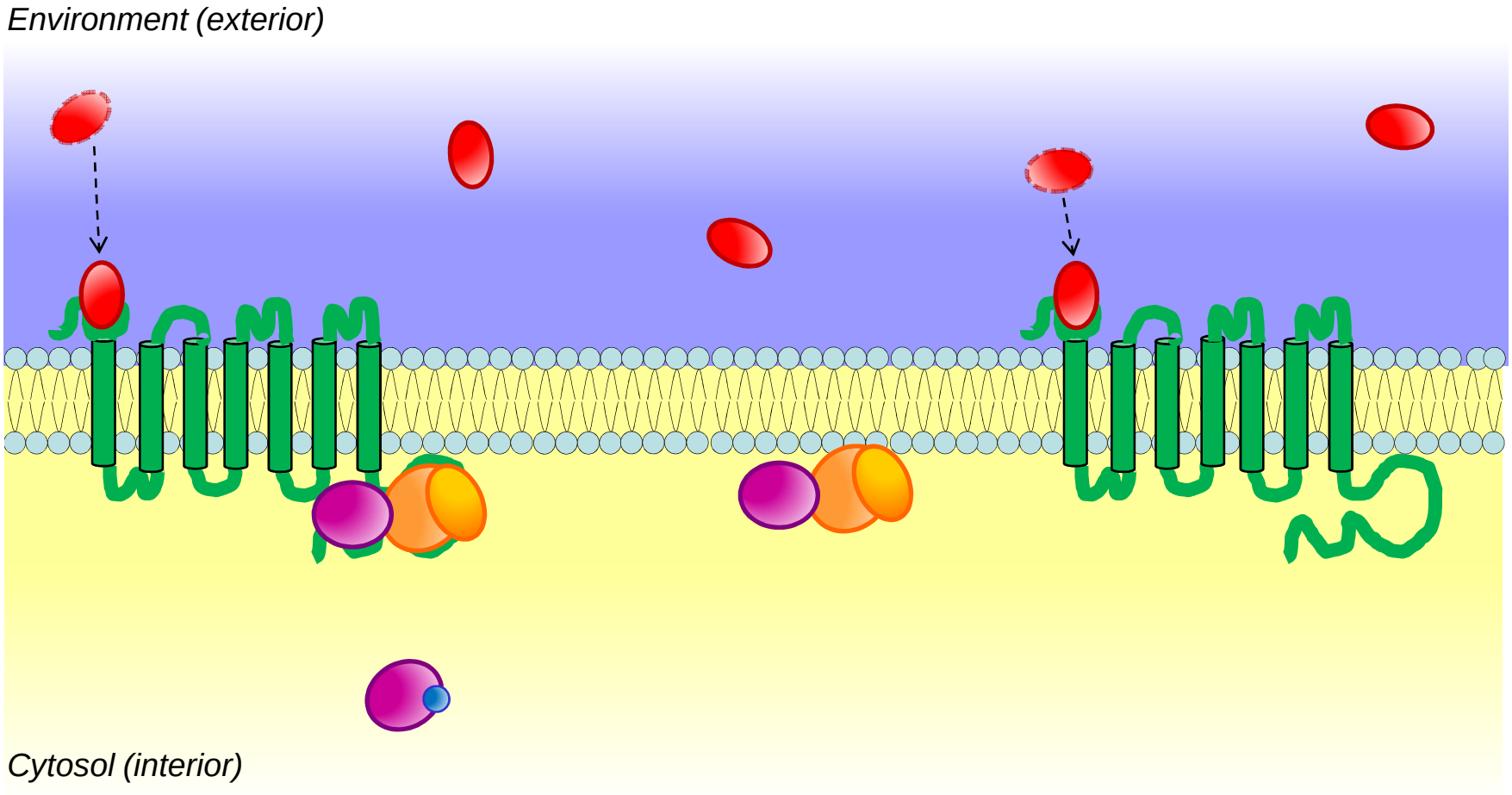
- Gα2-GDP subunit
- Gβγ subunit
  - Gβ subunit
  - Gγ subunit

Gα2-GTP subunit
(phosphorylated Gα2-GDP)

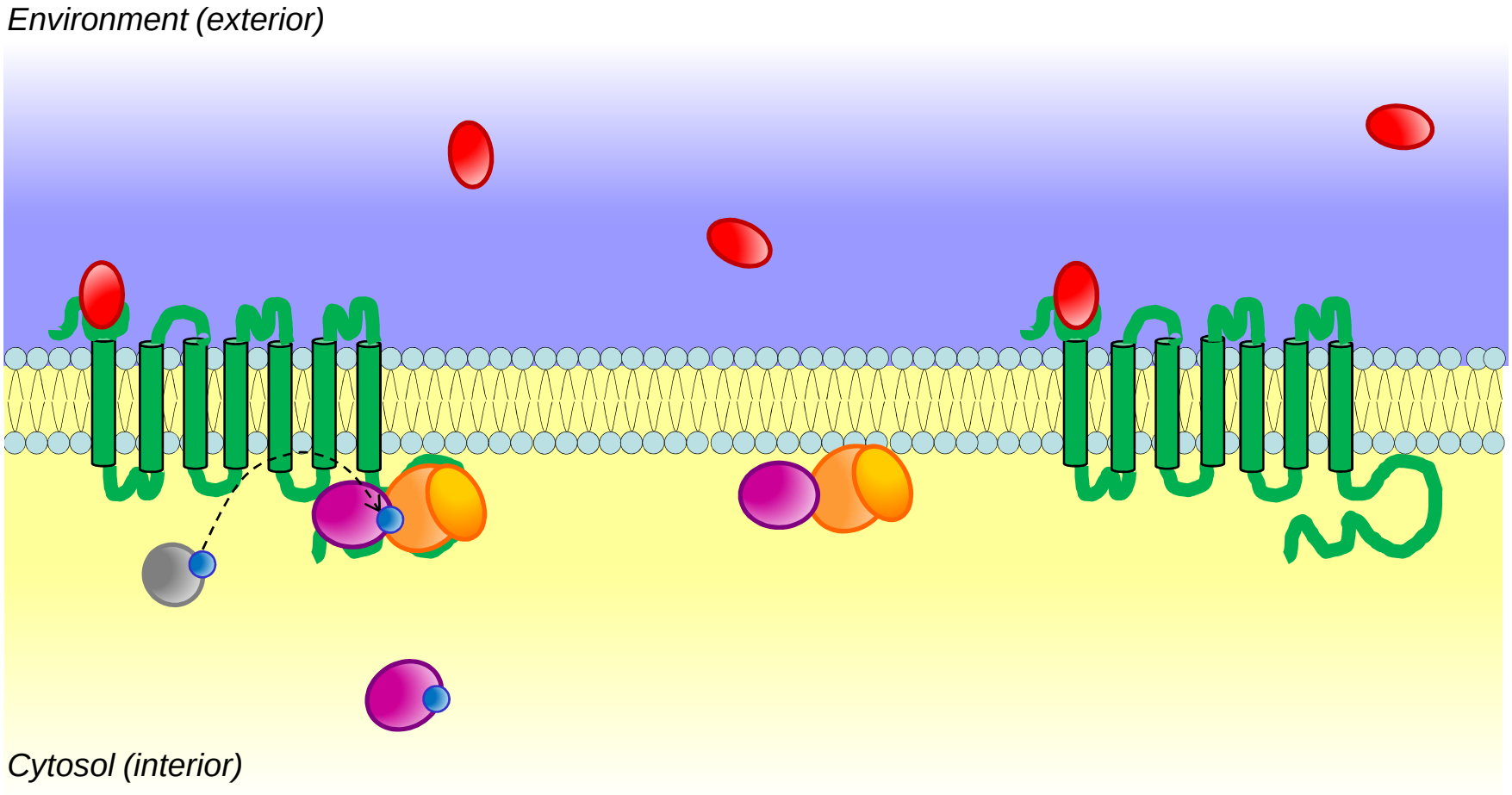
The molecular players
-- their interactions --



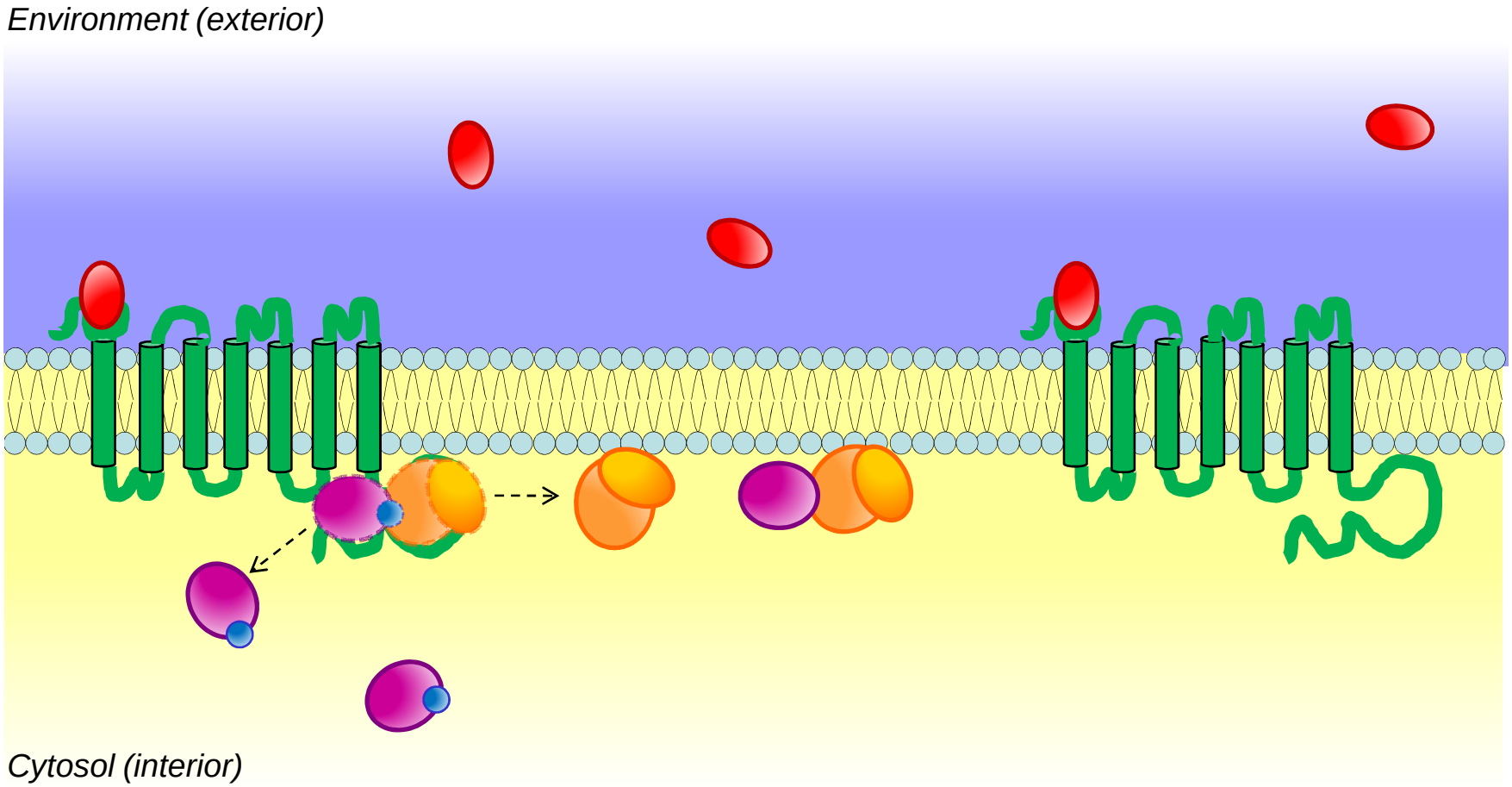
The molecular players
-- their interactions --



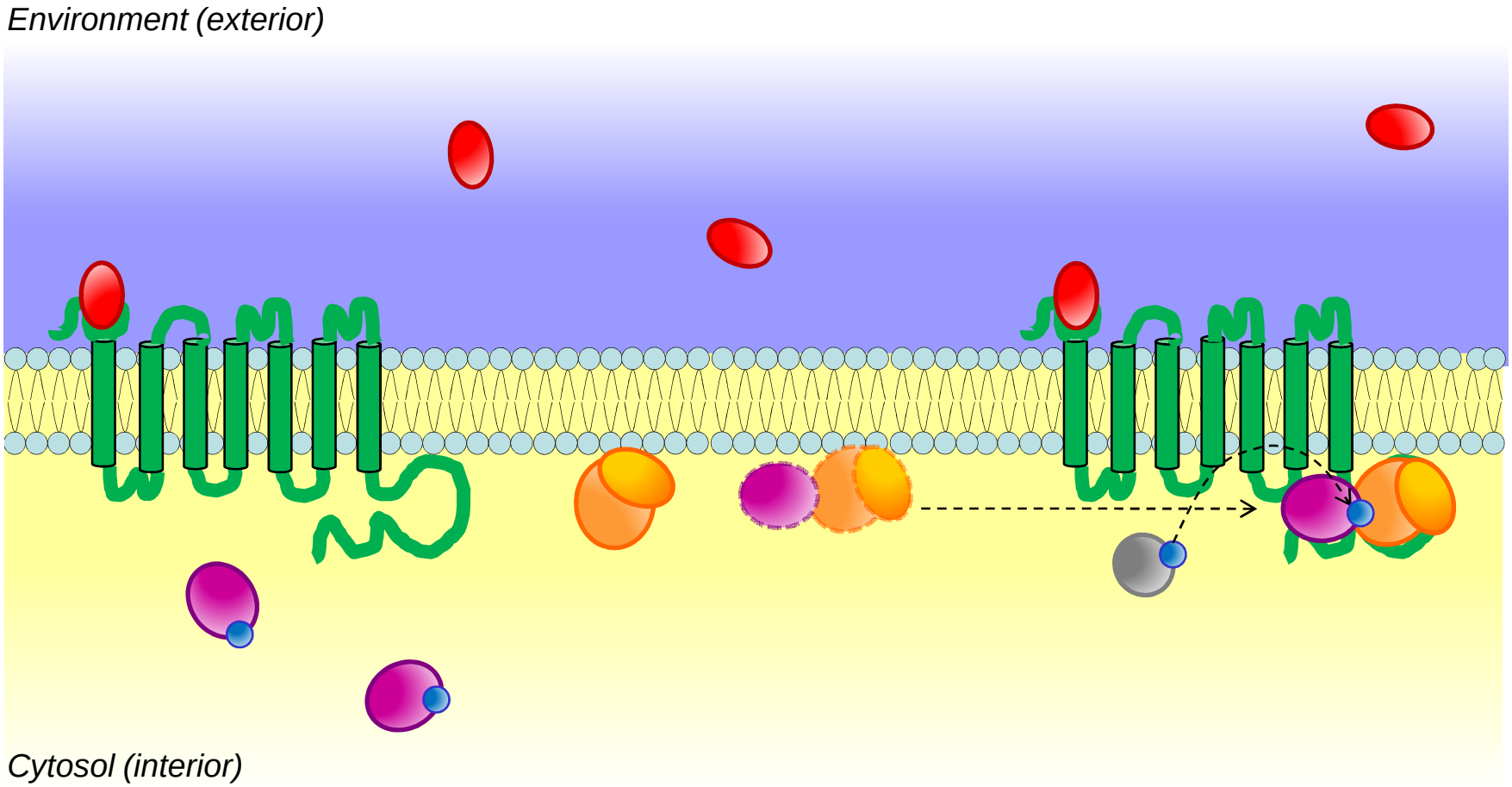
The molecular players
-- their interactions --



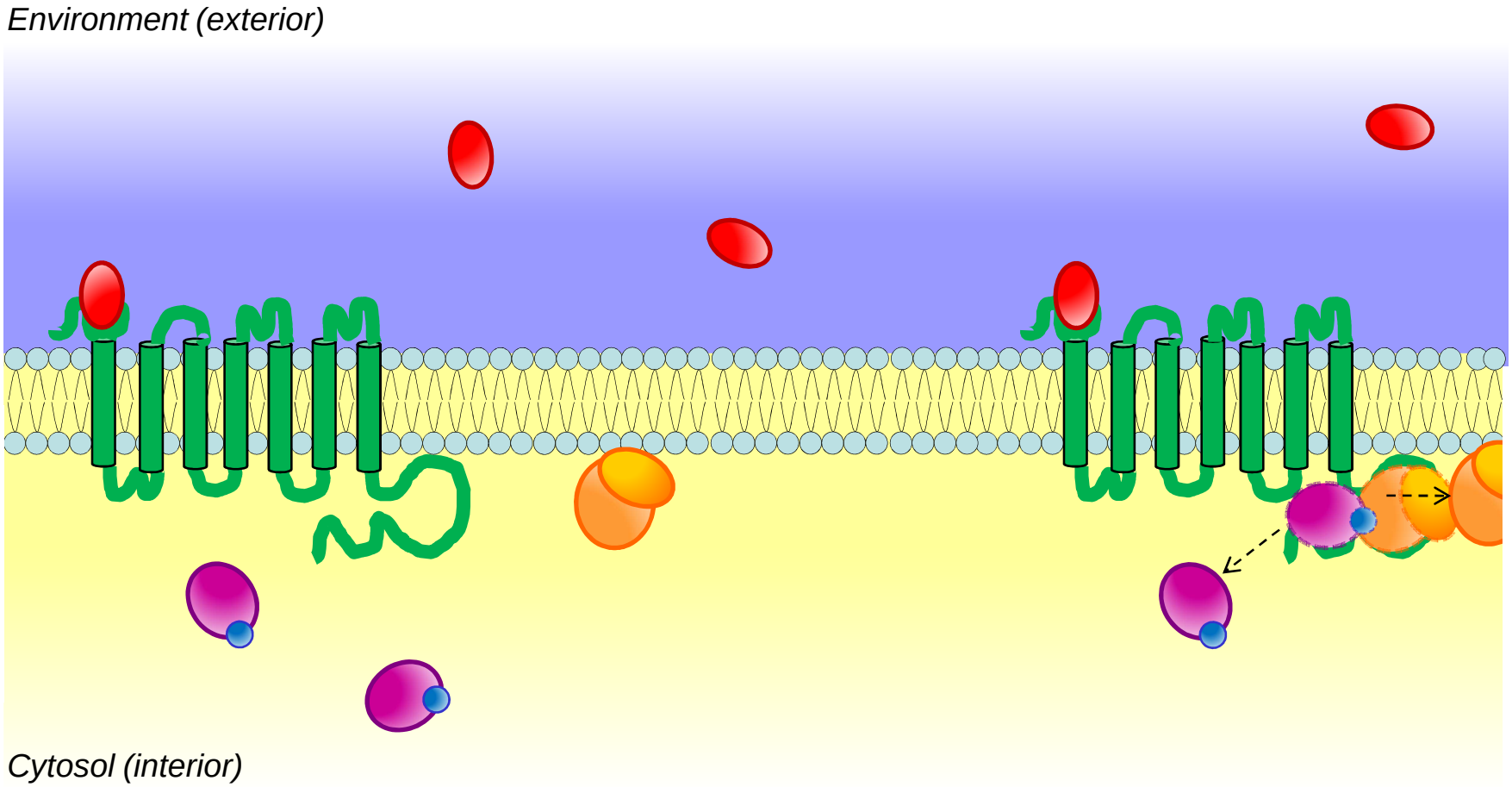
The molecular players
-- their interactions --



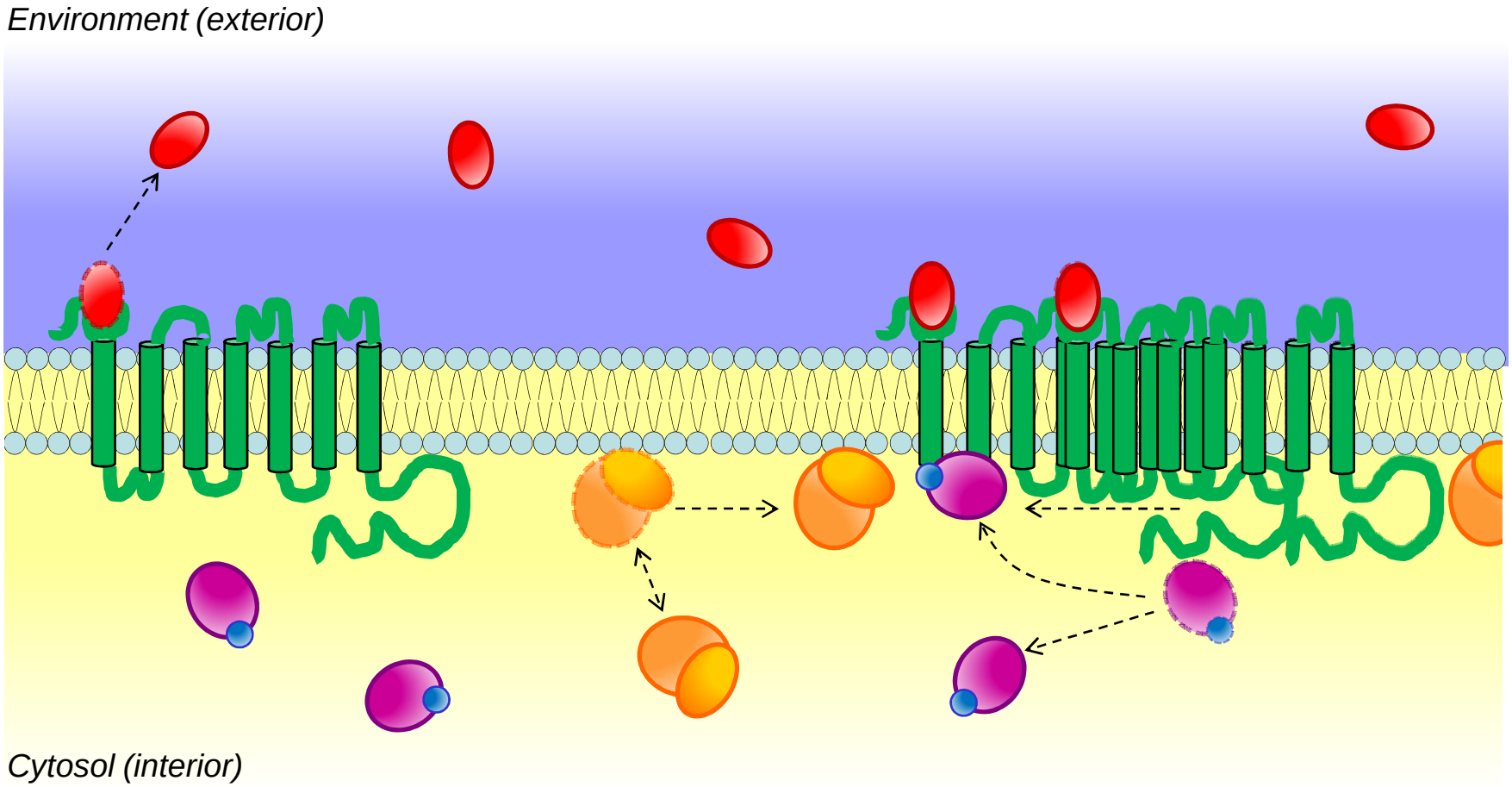
The molecular players
-- their interactions --



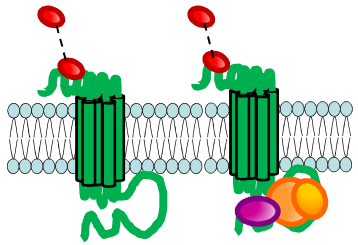
The molecular players
-- their interactions --



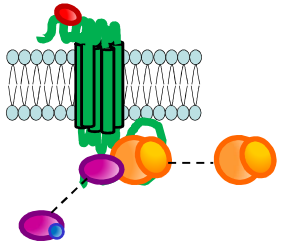
The molecular players
-- their interactions --



Put in chemical reactions…



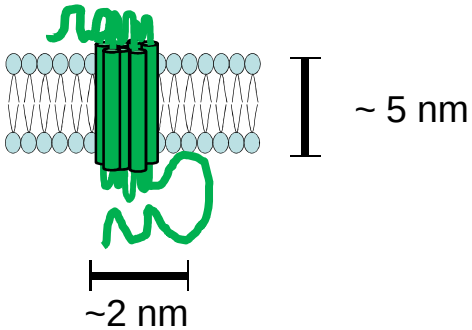
$$
\begin{array}{lrcl}
\text{(R1)} & \text{cAMP} + \text{cAR1} & \rightleftarrows & \text{cAR1}^* \\
\text{(R2)} & \text{cAMP} + \text{cAR1} \cdot \text{G}\alpha_2\beta\gamma & \rightleftarrows & \text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma \\
\text{(R3)} & \text{cAR1} + \text{G}\alpha_2\beta\gamma & \rightleftarrows & \text{cAR1} \cdot \text{G}\alpha_2\beta\gamma \\
\text{(R4)} & \text{cAR1}^* + \text{G}\alpha_2\beta\gamma & \rightleftarrows & \text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma \\
\text{(R5)} & \text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma & \rightarrow & \text{cAR1}^* + \text{G}\alpha_2\text{P} + \text{G}\beta\gamma \\
\text{(R6)} & \text{G}\alpha_2\text{P} & \rightarrow & \text{G}\alpha_2 \\
\text{(R7)} & \text{G}\alpha_2 + \text{G}\beta\gamma & \rightleftarrows & \text{G}\alpha_2\beta\gamma
\end{array}
$$



The molecular players
-- some facts and figures --

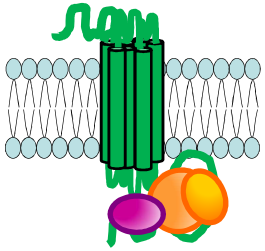
(Resulting from the literature and experiments by Van Hemert, Schmidt and coworkes)

cAMP diffusivity: ~ 100 $\mu m^2/s$



cAR1: First cAMP receptors that become expressed upon starvation; most sensitive

~ 54 x 10^3 per cell *(from fluorescence measurements)*



Van Hemert *et al.:*
single-molecule epifluorescence microscopy results suggest

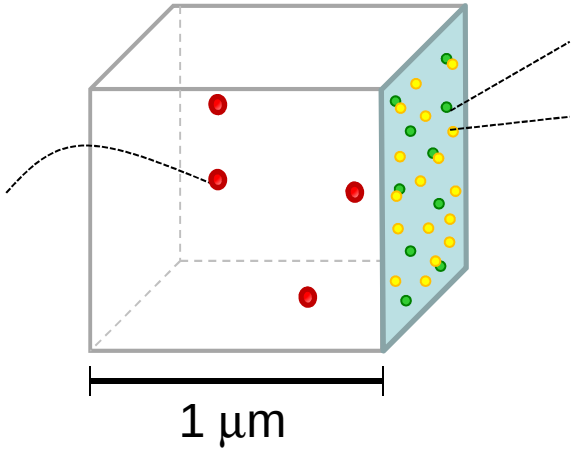
~ 30 % of $G\alpha 2\beta\gamma$ pre-coupled to cAR1 initially

~ 50 % of cAR1 is pre-coupled to $G\alpha 2\beta\gamma$

→ ~ 70 – 90 x 10^3 molecules of $G\alpha 2\beta\gamma$ per cell

The molecular players
-- some facts and figures --

Sparsely distributed molecules:



cAMP:
66 nM
~ 40 / μm^3

cAR1: ~ 100 / μm^2

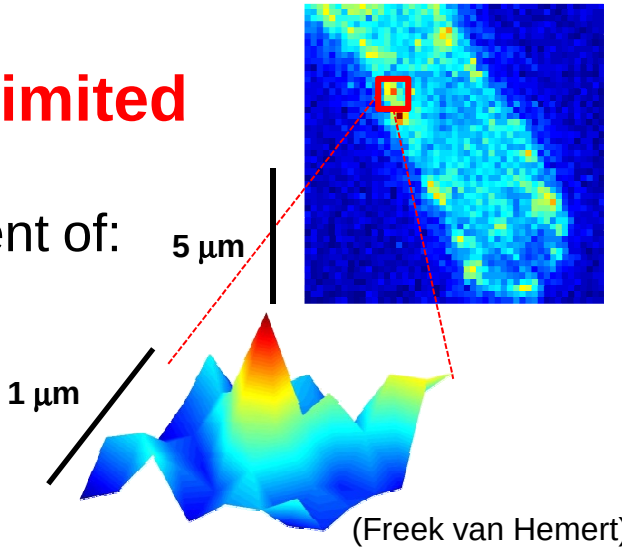
Ga2bg: ~ 130 – 170 / μm^2

Binding area ● for cAMP ≈
100 x 3 nm^2 = 300 nm^2
= 0.03 % of membrane area

→ **Many reaction rates are diffusion-limited**

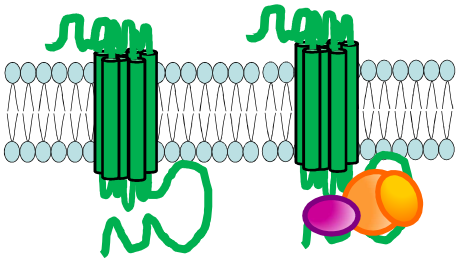
Single Molecule Microscopy enables measurement of:

-- Diffusivities
-- Fractions with different diffusivity
-- Off-rates



(Freek van Hemert)

The molecular players
-- some facts and figures --



Van Hemert *et al.:* (PhD thesis, p.60)

Fast diffusing fraction: $D_1 = 0.015 \pm 0.002 \mu\mathrm{m}^2/s$

Slow diffusing fraction: $D_2 = 0.007 \pm 0.001 \mu\mathrm{m}^2/s$

Gβγ subunit:

Van Hemert *et al.:* (PhD thesis, p.35)

Fast diffusing fraction: $D = 0.15 \pm 0.01 \mu\mathrm{m}^2/s$

Slow diffusing fraction: $D = 0.012 \pm 0.001 \mu\mathrm{m}^2/s$

Gα2 subunit:

One of 12 types; e.g. Gα4 involved in chemotaxis towards bacteria (= food)

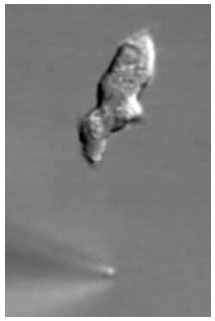
Van Hemert *et al.:* (PhD thesis)

Fast diffusing fraction: $D = 0.14 \pm 0.01 \mu\mathrm{m}^2/s$

Slow diffusing fraction: $D = 0.015 \pm 0.001 \mu\mathrm{m}^2/s$

Cell response
-- some facts and figures --

Cells can quickly detect and react to very shallow gradients:



Within 13 minutes; gradient of ~ 0.4 nM/μm (midpoint level ~ 40/μm^3)

(0.4 nM $\approx$ 0.25 / μm^3)

In various experiments: 40/μm^3 at anterior, 35/μm^3 at posterior (20 μm long)

Cells can cope with gradients that cover orders of magnitude in steepness and midpoint

Cells function properly in signals with noise-levels so high, that it may experience inverted gradients over time intervals
(Miyanaga et al. Biosystems (2007) 88(3), 251-260)

cAR1 at leading edge (anterior) has 2x increased cAMP off-rate and increased mobility. After activation, Gα2 has increased affinity for the membrane and cAR1

The main question: Reverse engineering

Reverse Engineering:

Reconstructing and understanding the interactions of components and the rates and the diffusivities in relation to the overall functioning of the network as observed and measured.

The experimental evidence did not fit any of the existing models for gradient sensing completely.

How is the cAMP gradient sensing system in *Dictyostelium* organised such that it functions properly,

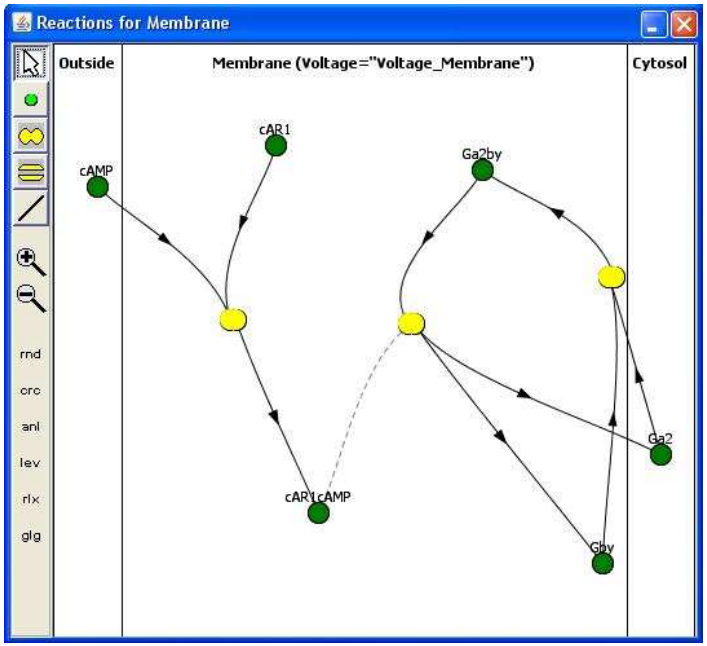
-- with these low molecule numbers,
-- over large ranges of gradients and midpoint levels,
-- in gradients that may even invert over time intervals?

Modeling & simulation
-- initial model --

v0 Freek van Hemert simulated an initial model in the **VCell** online computational environment:

$$
\begin{array}{lrcl}
\text{(R1)} & \text{cAMP} + \text{cAR1} & \rightleftarrows & \text{cAR1}^* \\
\text{(R5')} & \text{G}\alpha_2\beta\gamma & \rightarrow & \text{G}\alpha_2\text{P} + \text{G}\beta\gamma \\
\text{(R7')} & \text{G}\alpha_2 P + \text{G}\beta\gamma & \rightarrow & \text{G}\alpha_2\beta\gamma
\end{array}
$$

Catalysed by cAR1*



Diffusivities:

cAR1:	0.04 $\mu m^2/s$
cAR1*:	0.19 $\mu m^2/s$
$G\alpha 2\beta\gamma$:	0 $\mu m^2/s$
$G\alpha 2$:	24 $\mu m^2/s$
$G\beta\gamma$:	0.5 $\mu m^2/s$

Modeling & simulation
-- initial model --

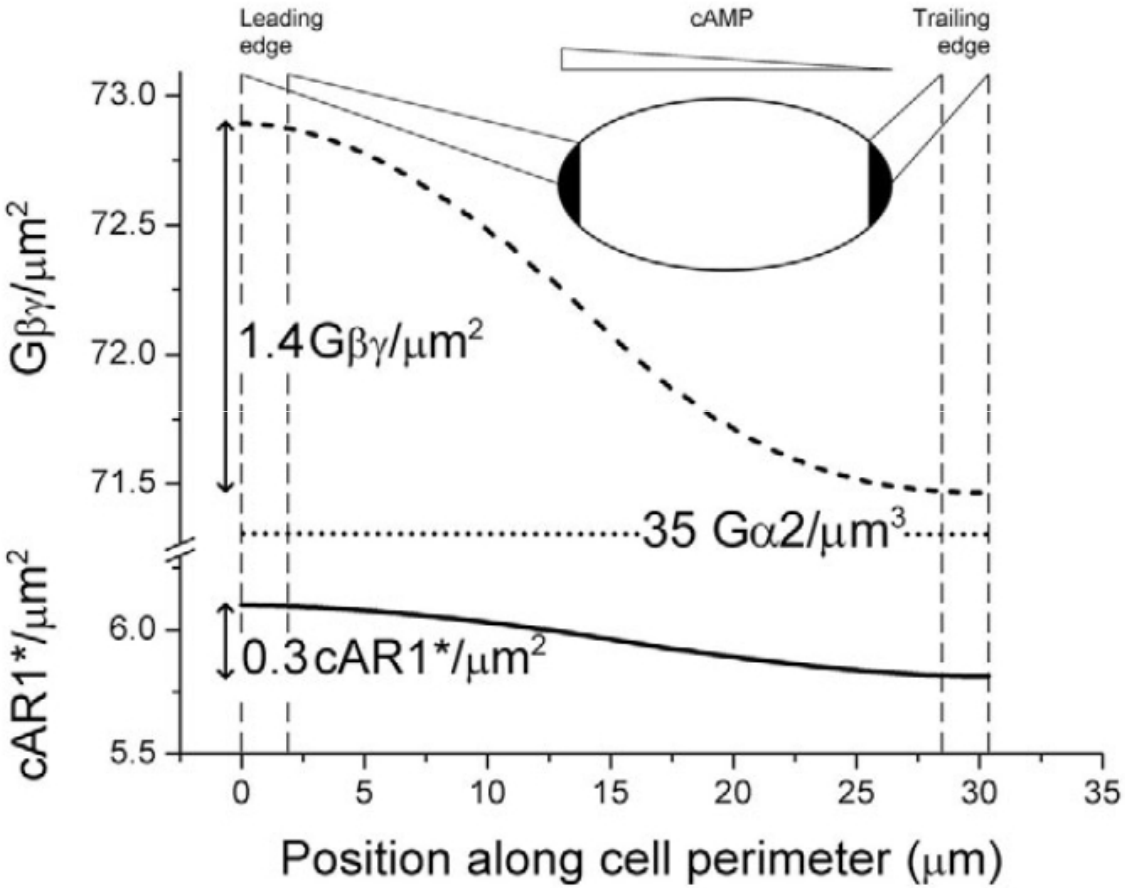
Results

cAMP concentration front:
66 nM = 40 / μm^3

cAMP concentration back:
58 nM = 35 / μm^3

Gradient: 0.4 nM / μm

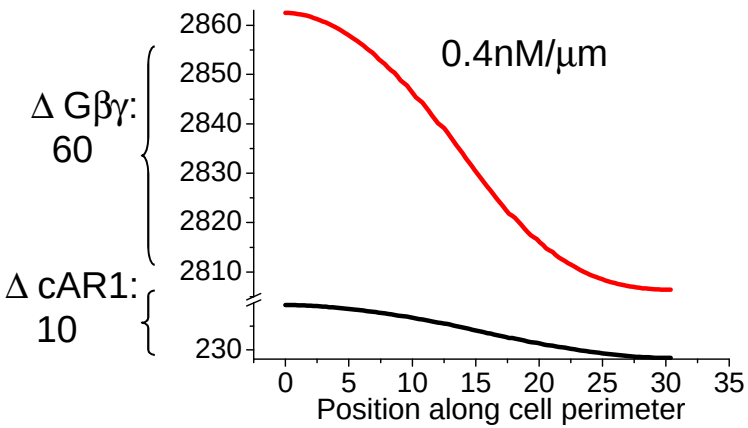
Linear amplification of cAR1* signal into Gα2$\beta\gamma$ of factor ~ 5



(1 nM = 0.6 / μm^3)

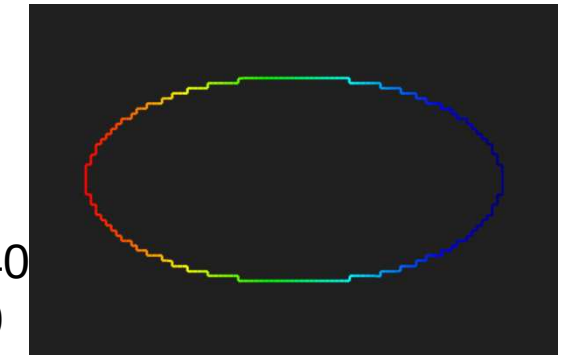
(De Keijzer *et al.* J. Cell Sci. **121**(10), 2008)

Modeling & simulation
-- type of VCell ouput --



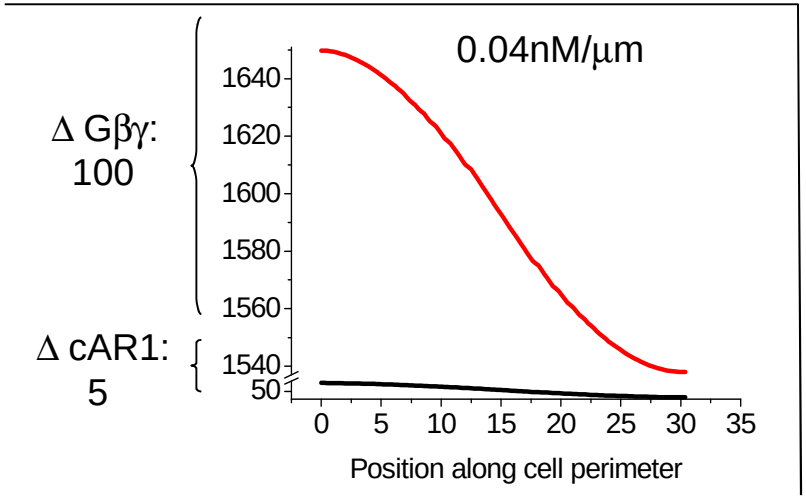
Front:

cAR1* : 240
Gβγ : 2860

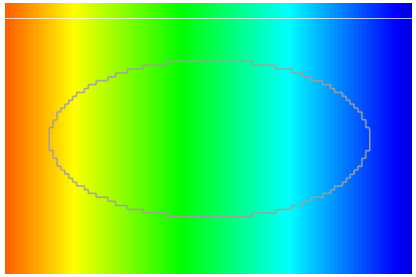


Back:

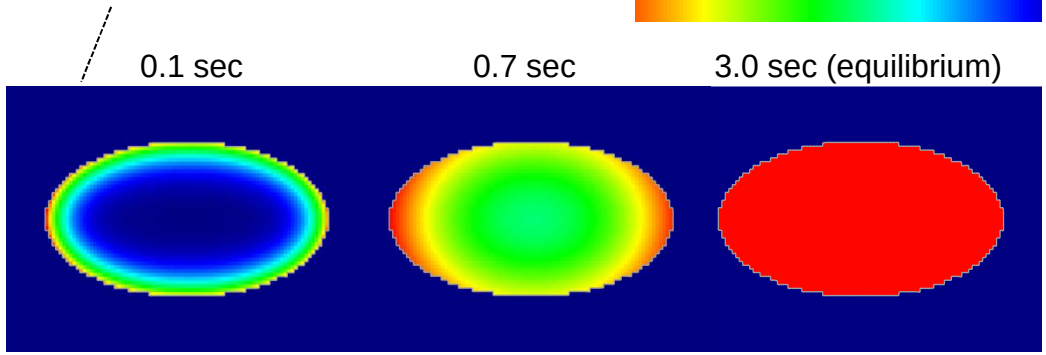
cAR1* : 230
Gβγ : 2800



cAMP gradient
(0.4 nM/µm)



Gα2: No gradient information (D=24 µm^2/s ...)



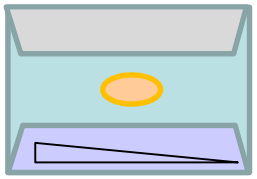
Objectives of research collaboration

The VCell results initiated the collaboration

First as a jointly supervised BSc project: *(Eidrees Ghariq, Physics & Math)*

1.) **Reproduce the VCell results in a 'better controllable' computational environment,**
   - -- with a more detailed model,
   - -- with more flexibility (towards future use in micro-fluidics experiments),
   - -- but easy-to-use for the experimental biophysicists.

('FEMLAB')



2.) **Is a deterministic model appropriate, or should one consider a stochastic model?**

3.) **Development of a deterministic or stochastic model that will explain the experimental data**

Full model specification
-- model v1 --

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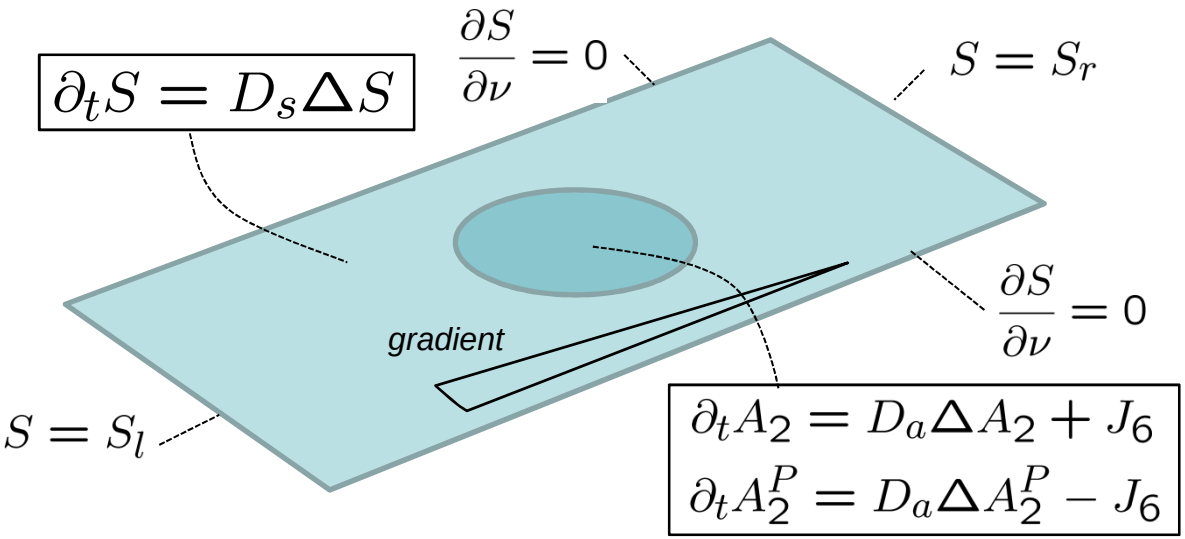
Mathematisch Instituut

In 2D subdomains & boundary conditions:



Index:

	cAMP	S
	cAR1	R
	cAR1*	R*
	Gα2βγ	G
	cAR1-Gα2βγ	R_G
	cAR1*-Gα2βγ	R^*_G
	Gβγ	B
	Gα2	A_2
	Gα2P	A_2^P

J_k : net reaction flux of (R*k*); given by Mass Action Kinetics, e.g.:

$$J_6 = k_6^+ A_2^P$$

$$
\begin{array}{lrcl}
\text{(R1)} & \text{cAMP} + \text{cAR1} & \rightleftarrows & \text{cAR1}^* \\
\text{(R2)} & \text{cAMP} + \text{cAR1} \cdot \text{G}\alpha_2\beta\gamma & \rightleftarrows & \text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma \\
\text{(R3)} & \text{cAR1} + \text{G}\alpha_2\beta\gamma & \rightleftarrows & \text{cAR1} \cdot \text{G}\alpha_2\beta\gamma \\
\text{(R4)} & \text{cAR1}^* + \text{G}\alpha_2\beta\gamma & \rightleftarrows & \text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma \\
\text{(R5)} & \text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma & \rightarrow & \text{cAR1}^* + \text{G}\alpha_2\text{P} + \text{G}\beta\gamma \\
\text{(R6)} & \text{G}\alpha_2\text{P} & \rightarrow & \text{G}\alpha_2 \\
\text{(R7)} & \text{G}\alpha_2 + \text{G}\beta\gamma & \rightleftarrows & \text{G}\alpha_2\beta\gamma
\end{array}
$$

Full model specification
-- model v1 --

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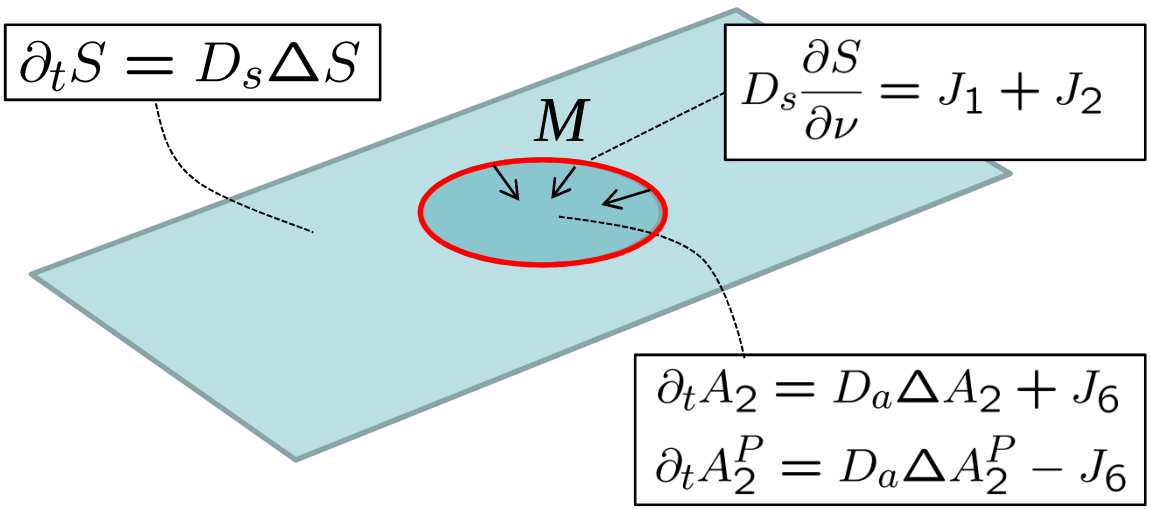
In 2D subdomains & boundary conditions:



Index:

	cAMP	S
	cAR1	R
	cAR1*	R*
	Gα2βγ	G
	cAR1-Gα2βγ	R_G
	cAR1*-Gα2βγ	R^*_G
	Gβγ	B
	Gα2	A_2
	Gα2P	A_2^P

$$J_1 = k_1^+ S|_M R - k_1^- R^*$$
$$J_2 = k_2^+ S|_M R_G - k_2^- R_G^*$$

(R1)	$\text{cAMP} + \text{cAR1}$	$\rightleftarrows$	cAR1^*
(R2)	$\text{cAMP} + \text{cAR1} \cdot \text{G}\alpha_2\beta\gamma$	$\rightleftarrows$	$\text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma$
(R3)	$\text{cAR1} + \text{G}\alpha_2\beta\gamma$	$\rightleftarrows$	$\text{cAR1} \cdot \text{G}\alpha_2\beta\gamma$
(R4)	$\text{cAR1}^* + \text{G}\alpha_2\beta\gamma$	$\rightleftarrows$	$\text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma$
(R5)	$\text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma$	$\rightarrow$	$\text{cAR1}^* + \text{G}\alpha_2\text{P} + \text{G}\beta\gamma$
(R6)	$\text{G}\alpha_2\text{P}$	$\rightarrow$	$\text{G}\alpha_2$
(R7)	$\text{G}\alpha_2 + \text{G}\beta\gamma$	$\rightleftarrows$	$\text{G}\alpha_2\beta\gamma$

Full model specification
-- model v1 --

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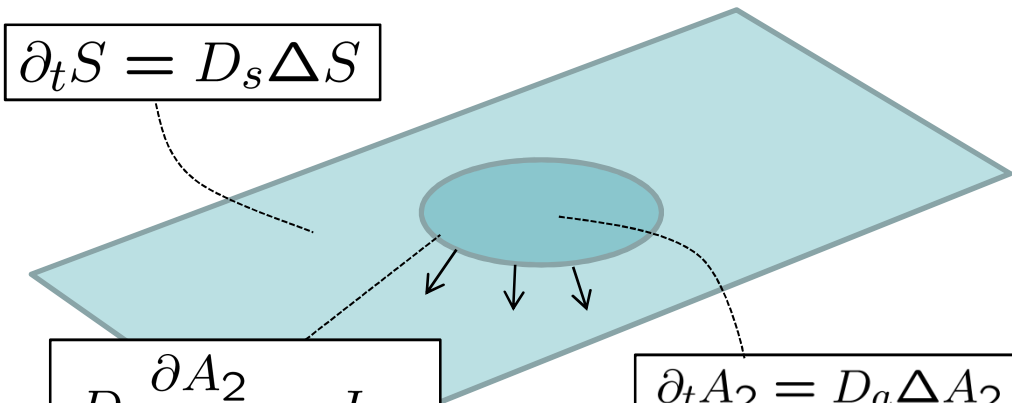
In 2D subdomains & boundary conditions:

$$\partial_t S = D_s \Delta S$$



$$D_a \frac{\partial A_2}{\partial \nu} = J_7$$
$$D_a \frac{\partial A_2^P}{\partial \nu} = -J_5$$

$$\partial_t A_2 = D_a \Delta A_2 + J_6$$
$$\partial_t A_2^P = D_a \Delta A_2^P - J_6$$

Index:

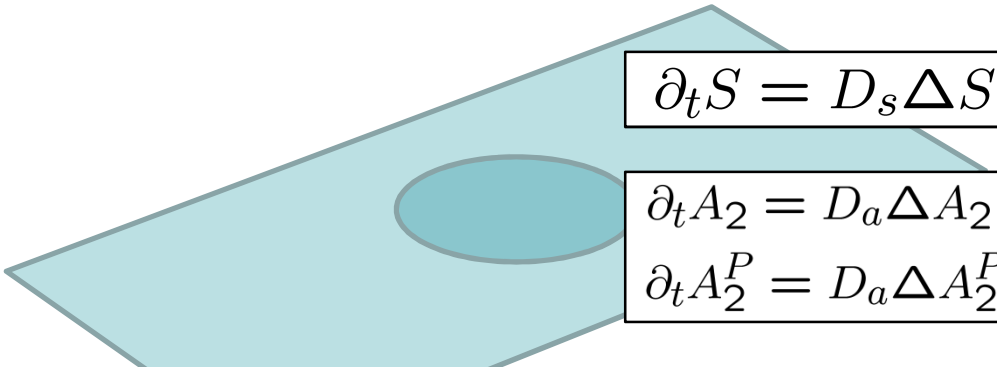
	cAMP	S
	cAR1	R
	cAR1*	R*
	Gα2βγ	G
	cAR1-Gα2βγ	R_G
	cAR1*-Gα2βγ	R^*_G
	Gβγ	B
	Gα2	A_2
	Gα2P	A_2^P

$$J_7 = k_7^+ A_2|_M B - k_7^- G$$
$$J_5 = k_5^+ R_G^*$$

(R1)	$\text{cAMP} + \text{cAR1}$	$\rightleftarrows$	cAR1^*
(R2)	$\text{cAMP} + \text{cAR1} \cdot \text{G}\alpha_2\beta\gamma$	$\rightleftarrows$	$\text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma$
(R3)	$\text{cAR1} + \text{G}\alpha_2\beta\gamma$	$\rightleftarrows$	$\text{cAR1} \cdot \text{G}\alpha_2\beta\gamma$
(R4)	$\text{cAR1}^* + \text{G}\alpha_2\beta\gamma$	$\rightleftarrows$	$\text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma$
(R5)	$\text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma$	$\rightarrow$	$\text{cAR1}^* + \text{G}\alpha_2\text{P} + \text{G}\beta\gamma$
(R6)	$\text{G}\alpha_2\text{P}$	$\rightarrow$	$\text{G}\alpha_2$
(R7)	$\text{G}\alpha_2 + \text{G}\beta\gamma$	$\rightleftarrows$	$\text{G}\alpha_2\beta\gamma$

Full model specification
-- model v1 --

Reaction-diffusion system on 2D domains…



$$\partial_t S = D_s \Delta S$$

$$\partial_t A_2 = D_a \Delta A_2 + J_6$$
$$\partial_t A_2^P = D_a \Delta A_2^P - J_6$$

… coupled to reaction-diffusion system on 1D membrane (*M*):

$$\begin{aligned}
\partial_t R &= D_R \Delta R - J_1 - J_3 \\
\partial_t R^* &= D_{R^*} \Delta R^* + J_1 - J_4 + J_5 \\
\partial_t G &= D_G \Delta G - J_3 - J_4 + J_7 \\
\partial_t R_G &= D_{R_G} \Delta R_G - J_2 + J_3 \\
\partial_t R_G^* &= \qquad\qquad J_2 + J_4 - J_5 \\
\partial_t B &= D_B \Delta B + J_5 - J_7
\end{aligned}$$

Index:

	cAMP	S
	cAR1	R
	cAR1*	R*
	Gα2βγ	G
	cAR1-Gα2βγ	R_G
	cAR1*-Gα2βγ	R^*_G
	Gβγ	B
	Gα2	A_2
	Gα2P	A_2^P

Full model v1
-- comments --

Recall that:

The diffusivities have been measured with good accuracy, *in vivo:*

$$D_s = 100\ \mu m^2/s \qquad D_B = 0.15\ \mu m^2/s$$

$$D_R = D_{R^*} = D_{R_G} = 0.012\ \mu m^2/s \qquad D_a = 0.14\ \mu m^2/s$$

Most reaction rates are diffusion-limited: hence derivable from these diffusivities

Note:

Diffusivities stated above differ substantially from those used in model v0:

(Increasing experimental precision and knowledge about the biochemistry & physics of the system)

Diffusivities v0:

cAR1:	0.04 $\mu m^2/s$
cAR1*:	0.19 $\mu m^2/s$
Gα2βγ:	0 $\mu m^2/s$
Gα2:	24 $\mu m^2/s$
Gβγ:	0.5 $\mu m^2/s$

Full model v1
-- Intermediary conclusions --

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Thus…

Comparison to VCell simulation results became irrelevant

We have now a model though in an 'easy-to-use', 'controllable' computational environment

One should focus on the Reverse Engineering objective

But …

Model seems too 'complex' for Reverse Engineering purpose

Full model can (should) be simplified in many ways though, e.g.:

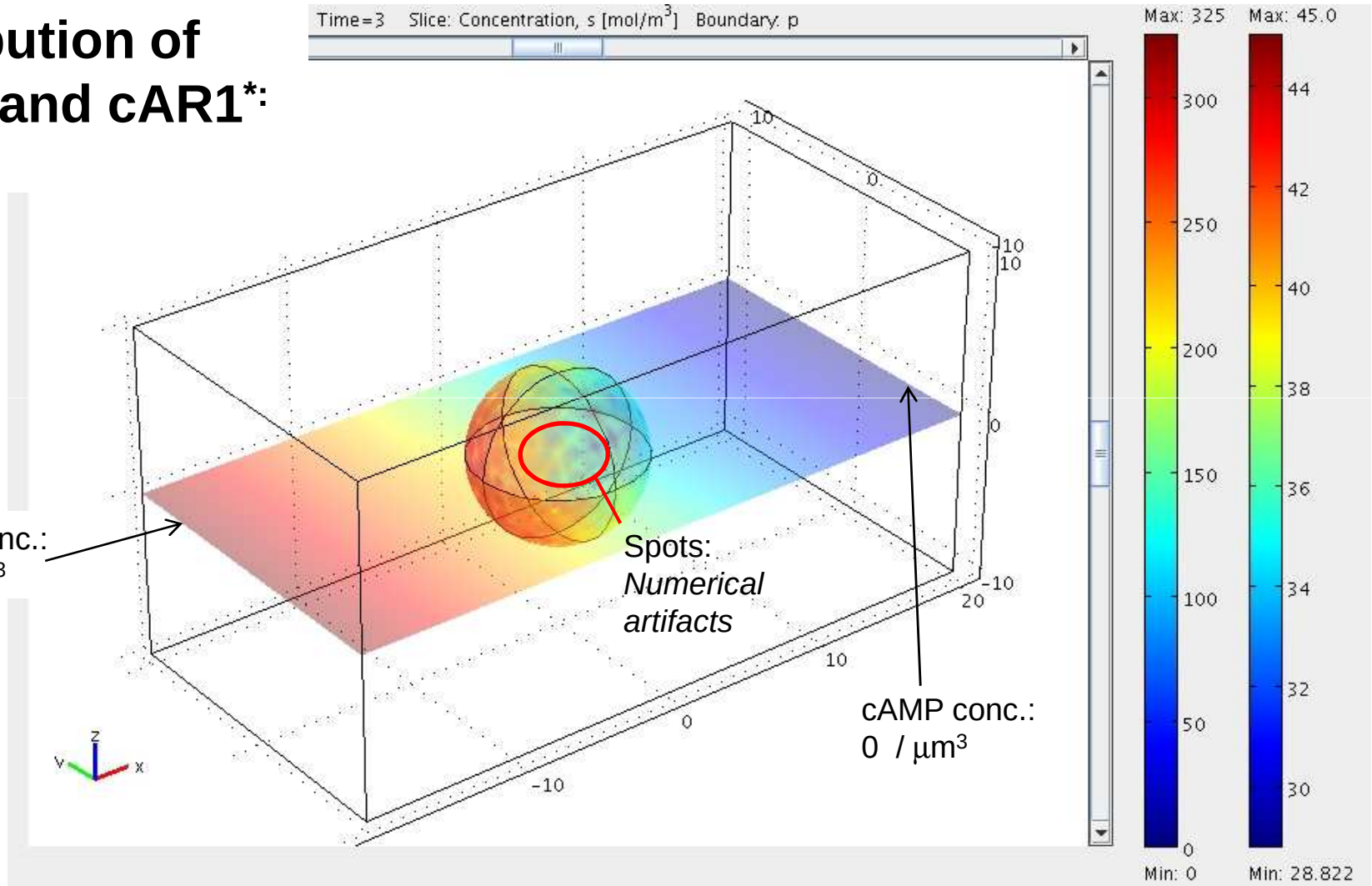
-- quasi-steady state for S

-- Michaelis-Menten kinetics for

$$
\begin{array}{lrcl}
\text{(R2)} & \text{cAMP} + \text{cAR1} \cdot \text{G}\alpha_2\beta\gamma & \rightleftarrows & \text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma \\
\text{(R4)} & \text{cAR1}^* + \text{G}\alpha_2\beta\gamma & \rightleftarrows & \text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma \\
\text{(R5)} & \text{cAR1}^* \cdot \text{G}\alpha_2\beta\gamma & \rightarrow & \text{cAR1}^* + \text{G}\alpha_2\text{P} + \text{G}\beta\gamma
\end{array}
$$

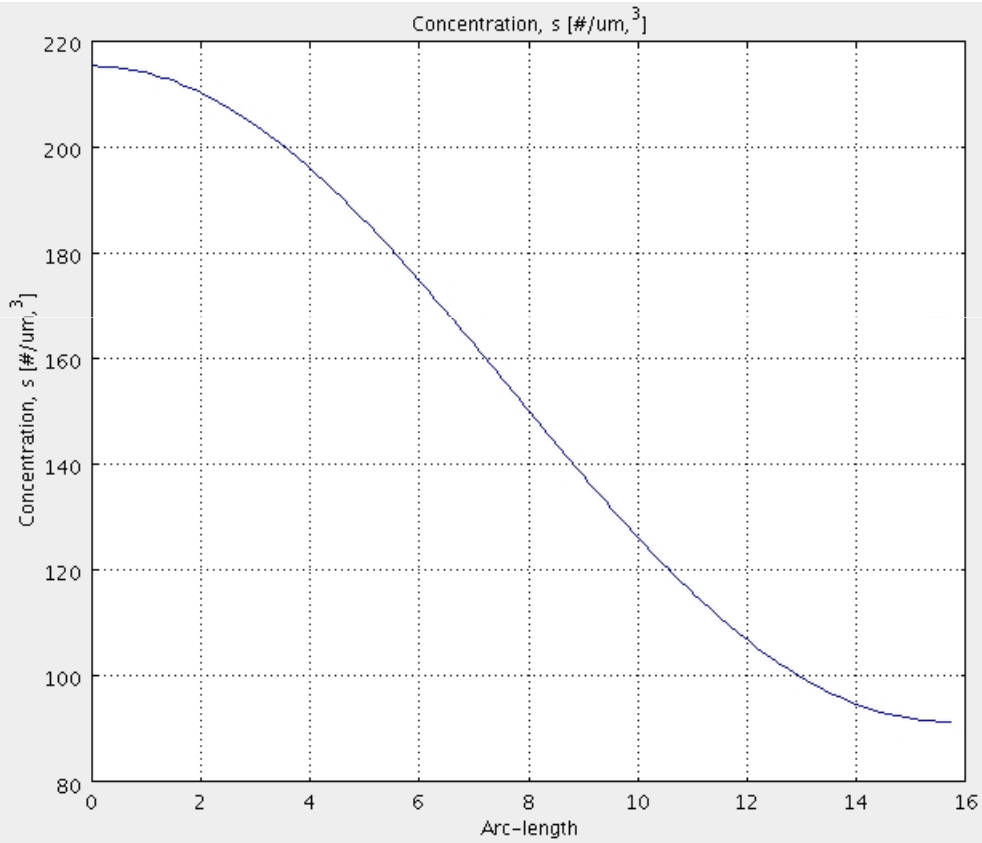
Modeling & simulation
-- some results model v1 – 3D --

Distribution of cAMP and cAR1*:
(t=3 s)

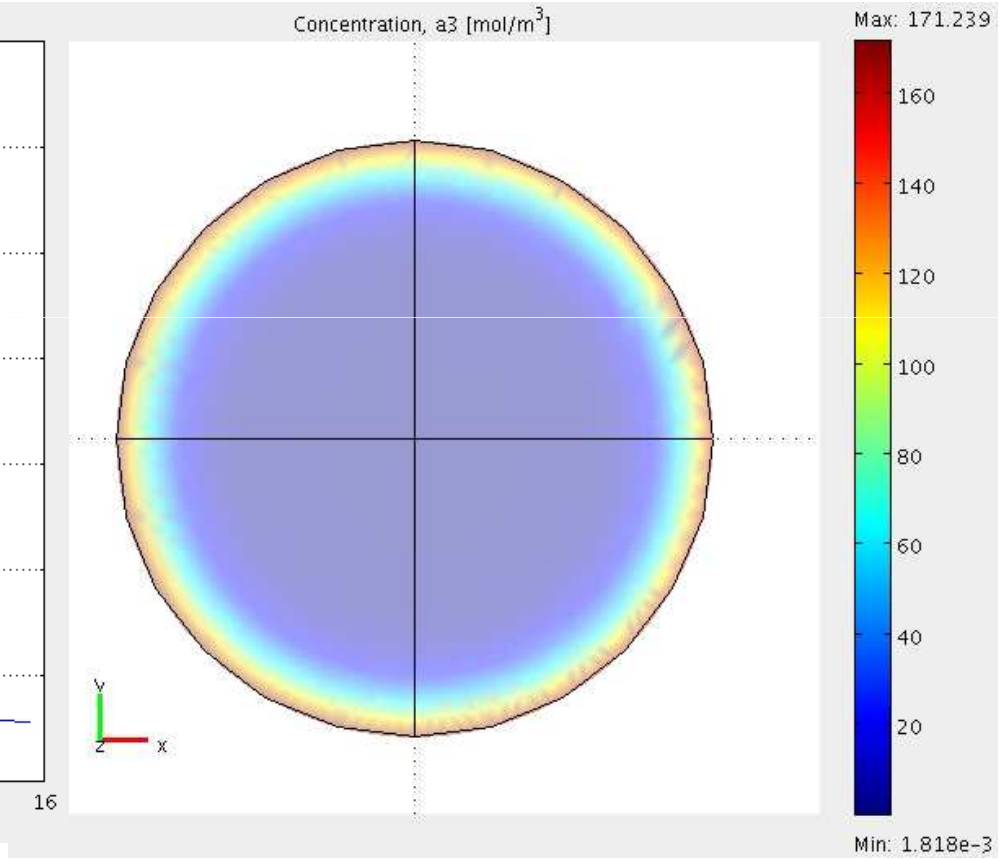


Modeling & simulation
-- some results model v1 – 3D --

cAMP concentration at membrane:

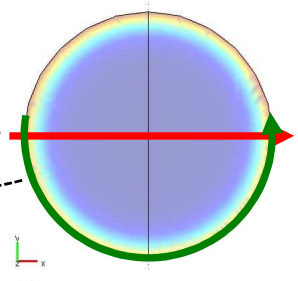


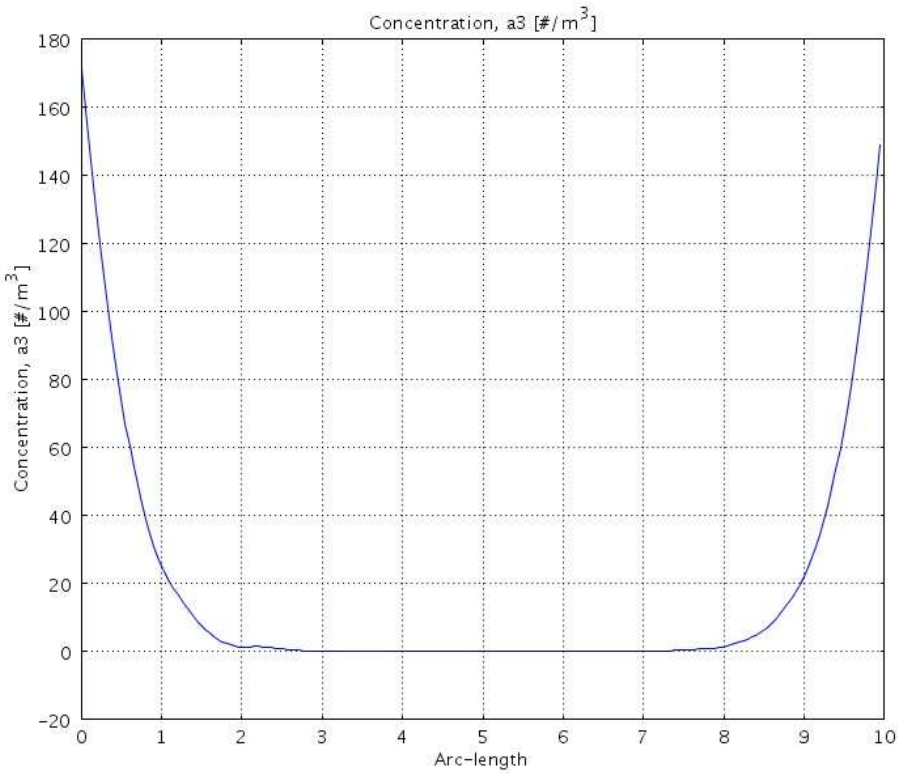
Interior distribution of Gα2P:

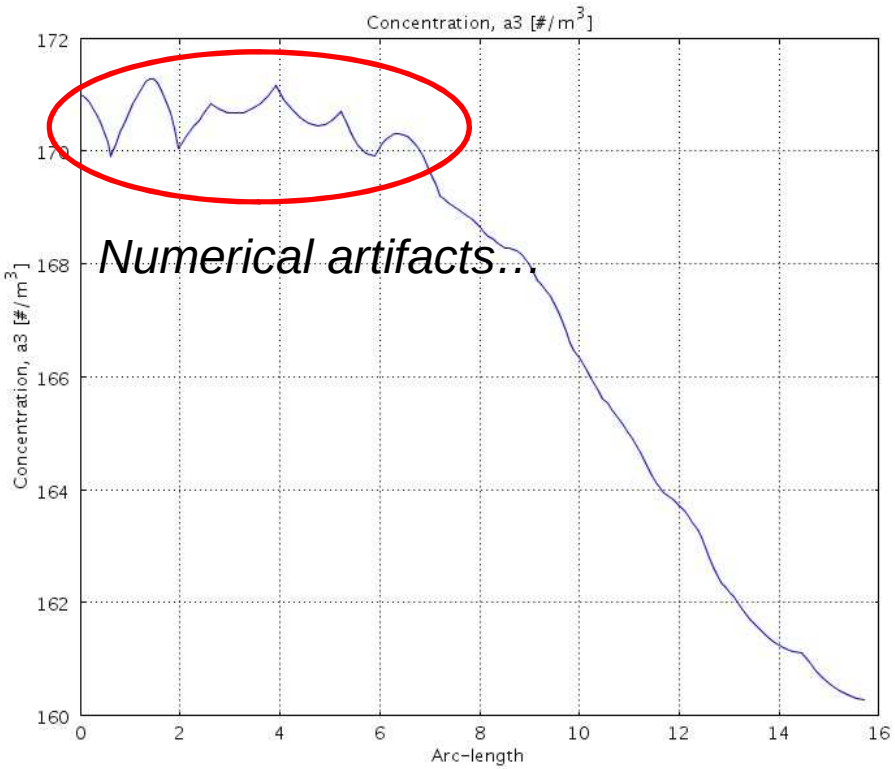


Modeling & simulation
-- some results model v1 – 3D --

Concentration profile of Gα2P along curves:







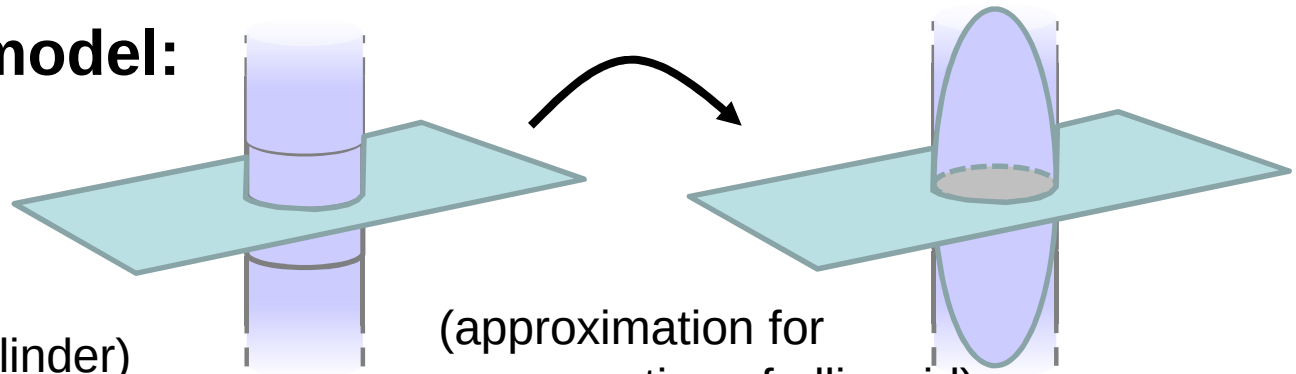
Modeling & simulation
-- the 2D model v1 --

The 2D deterministic model is computationally much less intensive. Thus it is better suited for ‘probing’ the dynamics

The computational cost for 3*D stochastic simulation* cannot be justified by our purpose of Reverse Engineering and validation of deterministic models

→ **Consider the 2D model from this point onwards**

Relation to 3D model:

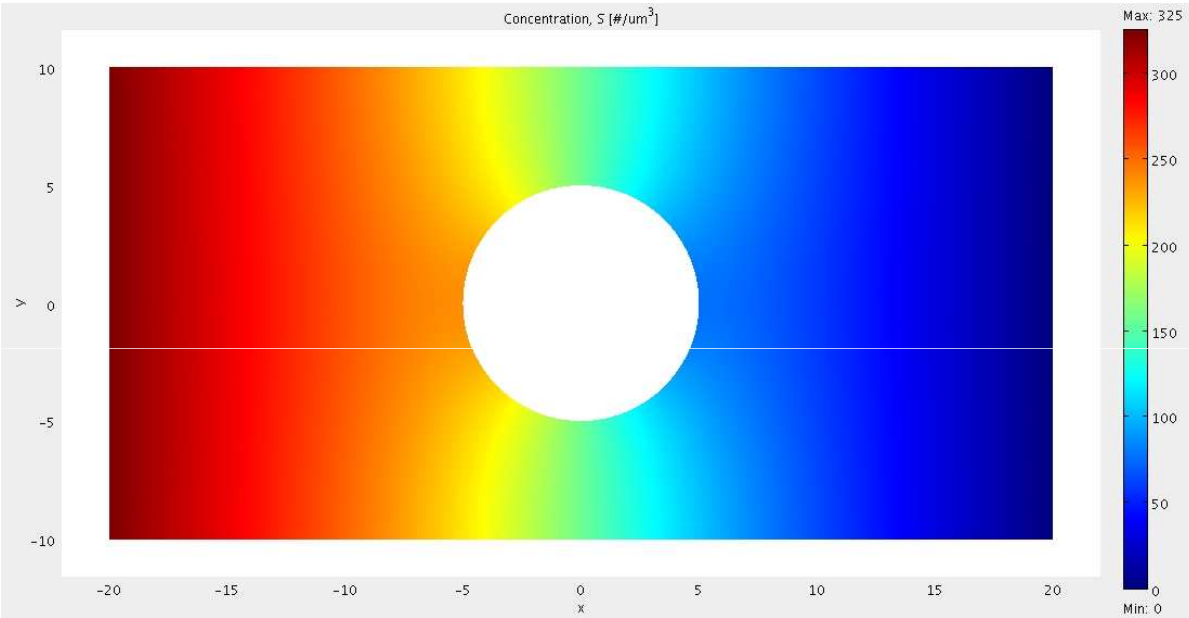


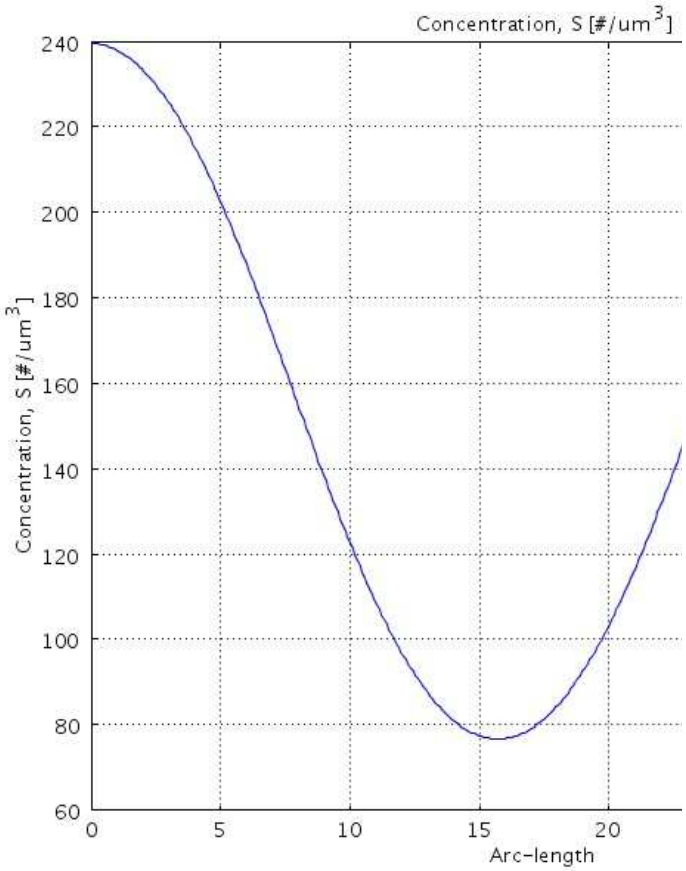
(infinitely extended cylinder)

(approximation for cross-section of ellipsoid)

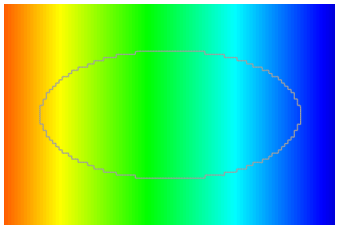
Simulation 2D model

cAMP concentration gradient: (325/µm^3 left, 0 right)





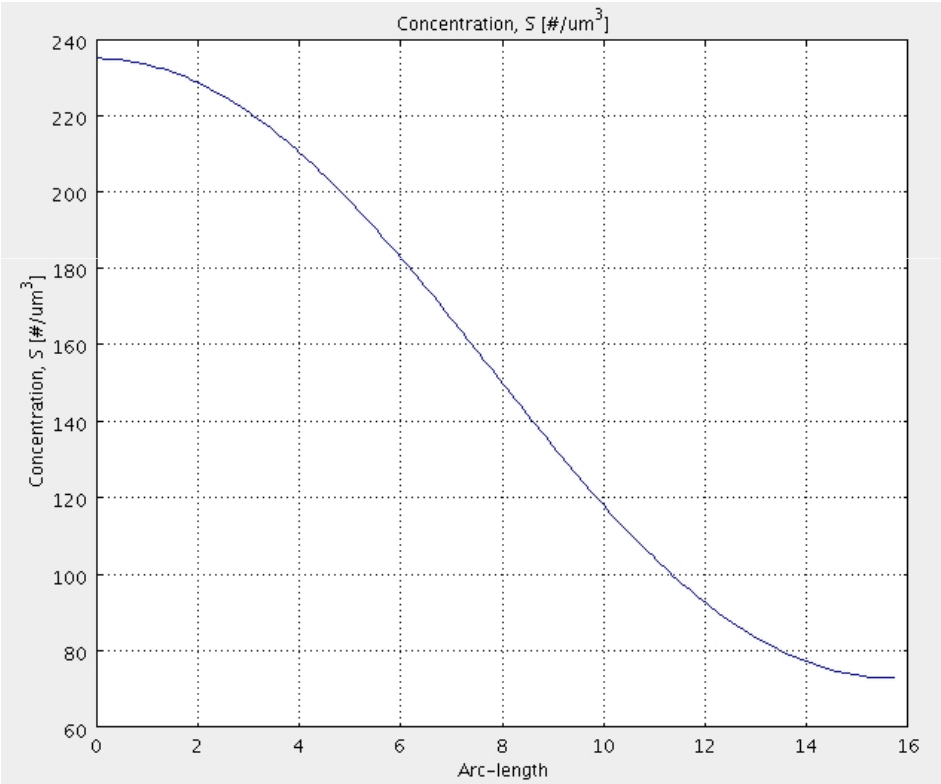
Recall:
Virtual Cell result



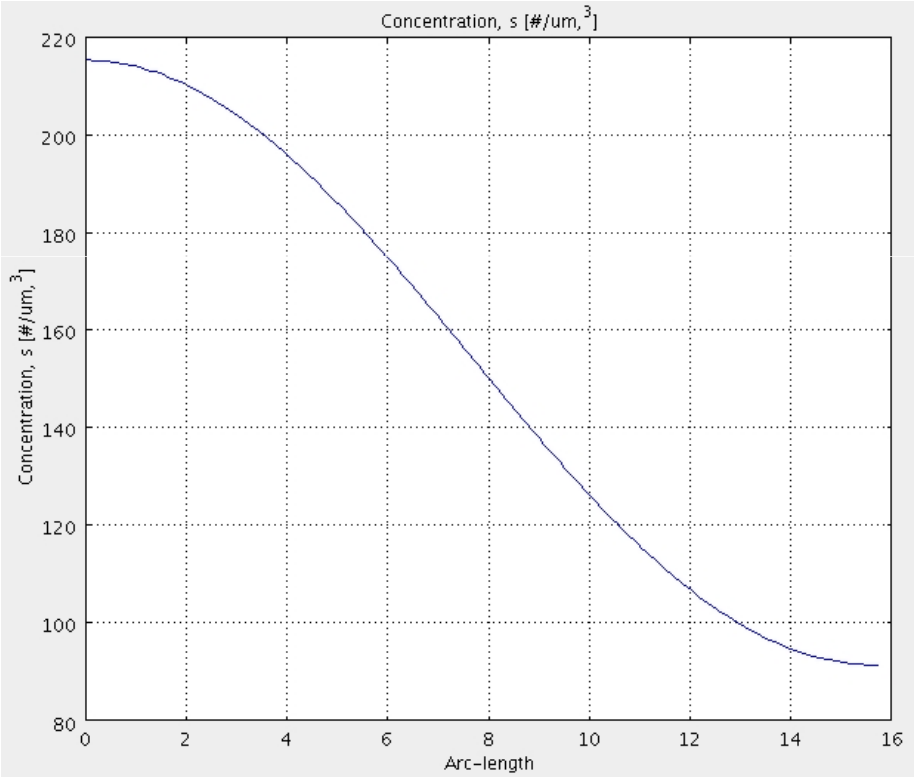
Comparison 2D – 3D simulations

cAMP concentration gradient: (325/um^3 left, 0 right)

2D



3D

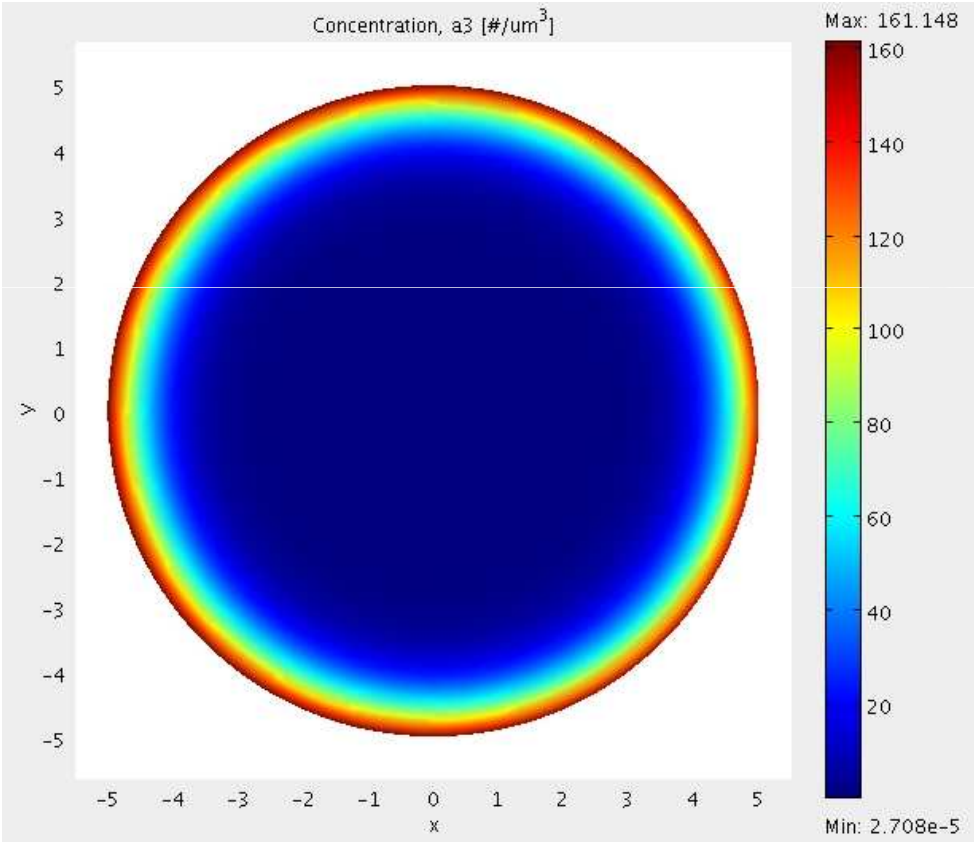


Comparison 2D – 3D simulations

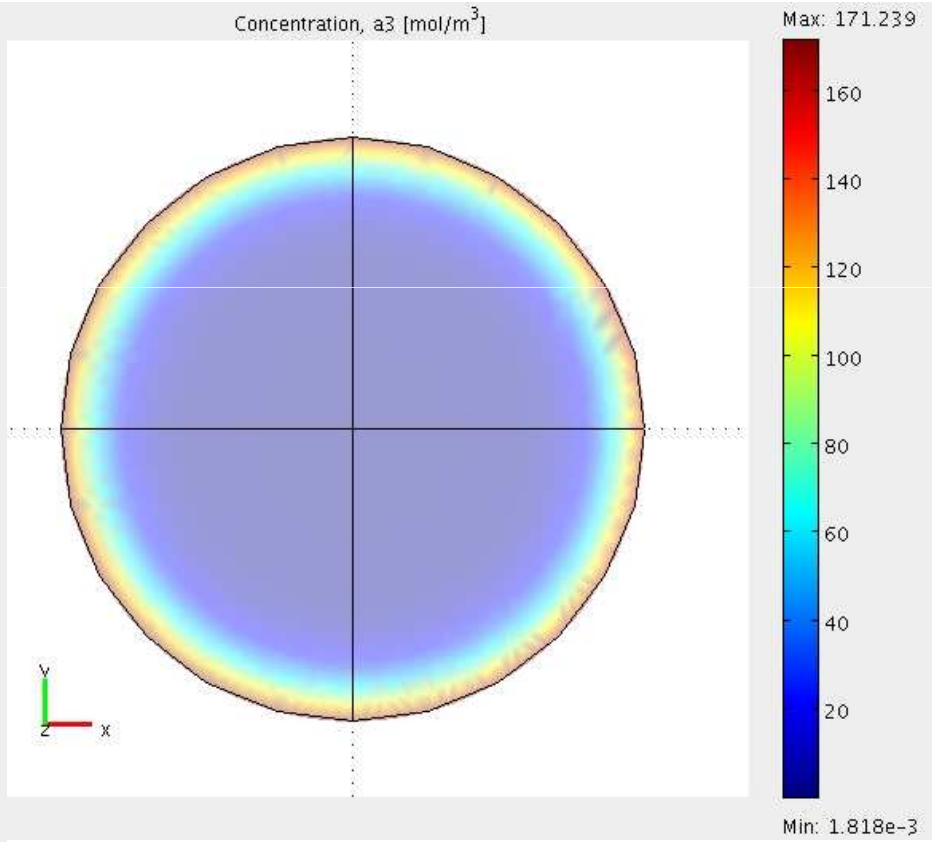
Gα2P concentration

(325/μm^3 left, 0 right)

2D



3D



How suitable are deterministic models for our problem?

The low molecule numbers are suspicious

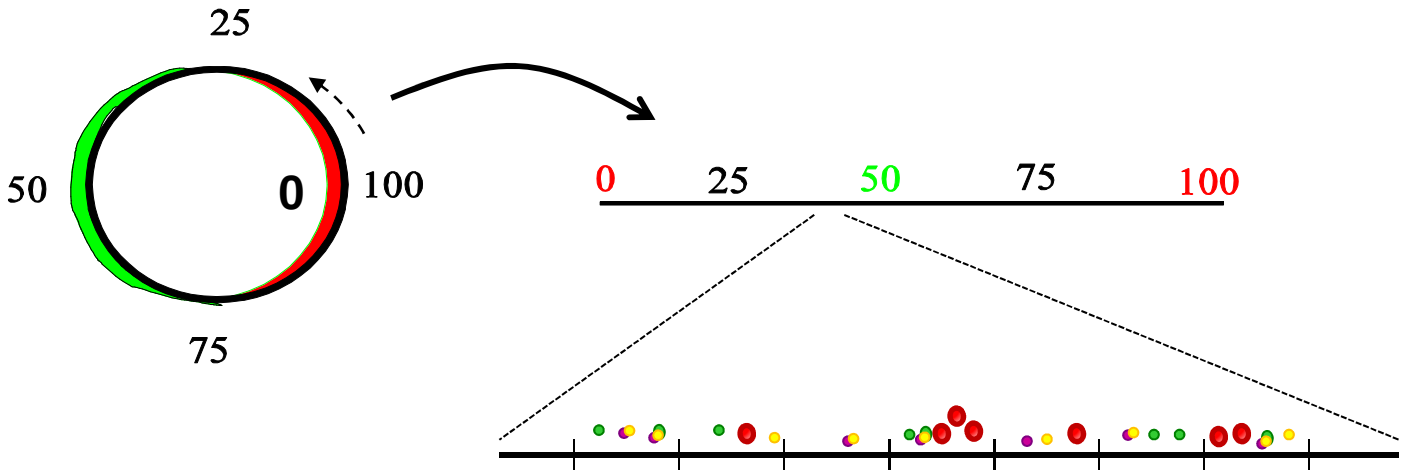
Objective: how large is the variation in the stochastic simulation?

Stochastic simulation set-up

(Simulations realised by Johan Dubbeldam (TUD))

Uses Gillespie algorithm for stochastic chemical reaction simulation, extended with diffusion

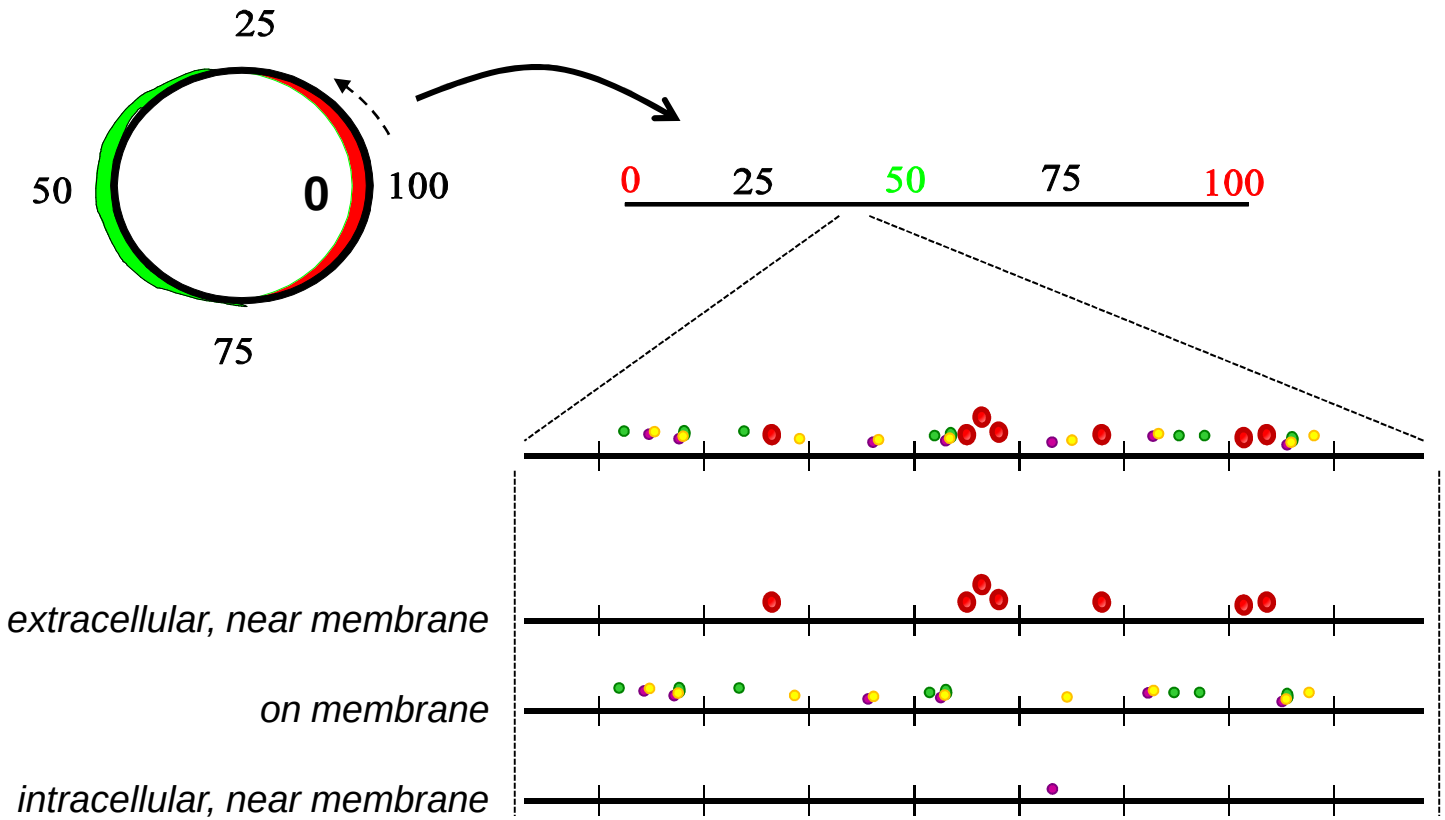
Cell circumference divided into 100 segments (each corresponds to ~ 0.31 μm)



Each segment contains molecules; multiple chemical species in a segment

E.g. initial # cAMP in segment i: $\approx N_{\text{min}} + A \cdot \frac{1}{2}\cos(2\pi(i-50)/100)$

Stochastic simulation set-up

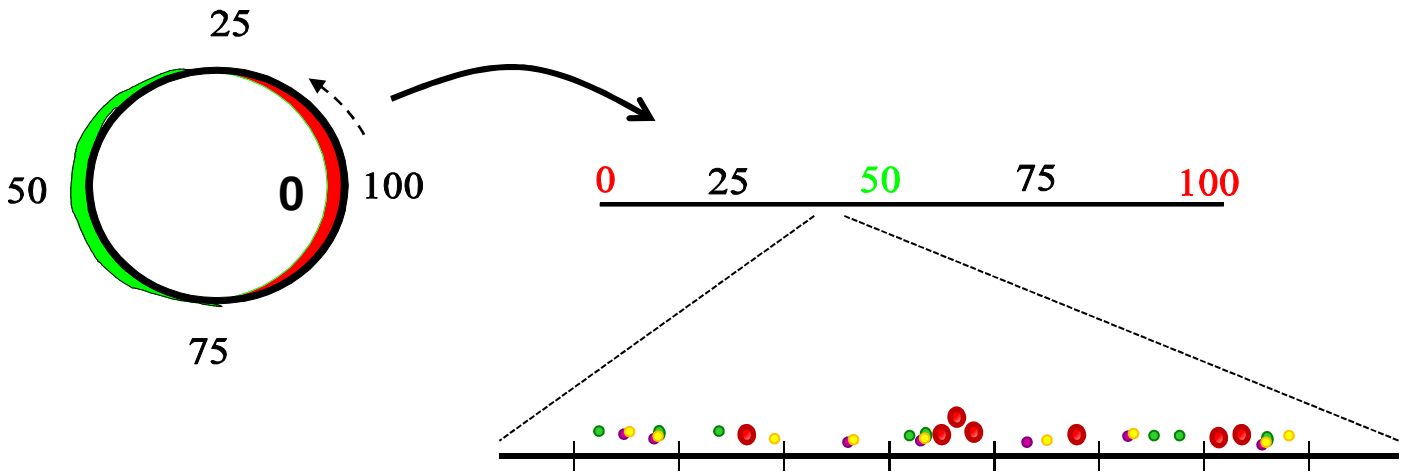


In a discrete time step (~ 1 ms physical time):

-- molecules may react within a single segment (w.r.t. reaction probabilities)

-- molecules may perform random walk (transition probabilities relate to diffusivities))

Stochastic simulation set-up



Limited set of reactions:

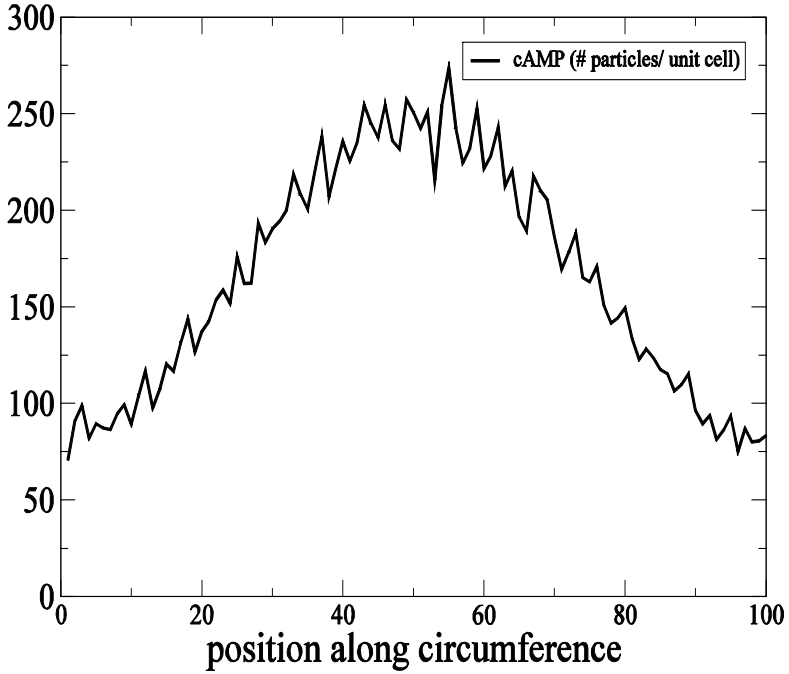
(R1)	$\mathrm{cAMP} + \mathrm{cAR1}$	$\rightleftarrows$	$\mathrm{cAR1}^*$
(R2)	$\mathrm{cAMP} + \mathrm{cAR1} \cdot \mathrm{G}\alpha_2\beta\gamma$	$\rightleftarrows$	$\mathrm{cAR1}^* \cdot \mathrm{G}\alpha_2\beta\gamma$
(R3)	$\mathrm{cAR1} + \mathrm{G}\alpha_2\beta\gamma$	$\rightleftarrows$	$\mathrm{cAR1} \cdot \mathrm{G}\alpha_2\beta\gamma$
(R4)	$\mathrm{cAR1}^* + \mathrm{G}\alpha_2\beta\gamma$	$\rightleftarrows$	$\mathrm{cAR1}^* \cdot \mathrm{G}\alpha_2\beta\gamma$
(R5)	$\mathrm{cAR1}^* \cdot \mathrm{G}\alpha_2\beta\gamma$	$\rightarrow$	$\mathrm{cAR1}^* + \mathrm{G}\alpha_2\mathrm{P} + \mathrm{G}\beta\gamma$

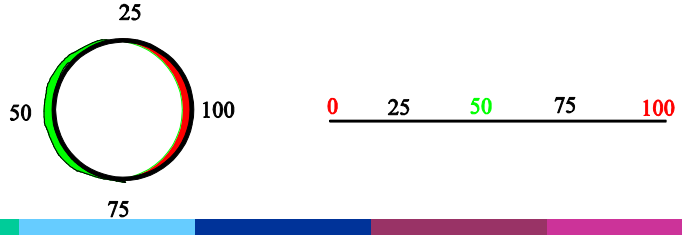
1000 receptors initially homogeneously distributed over segments

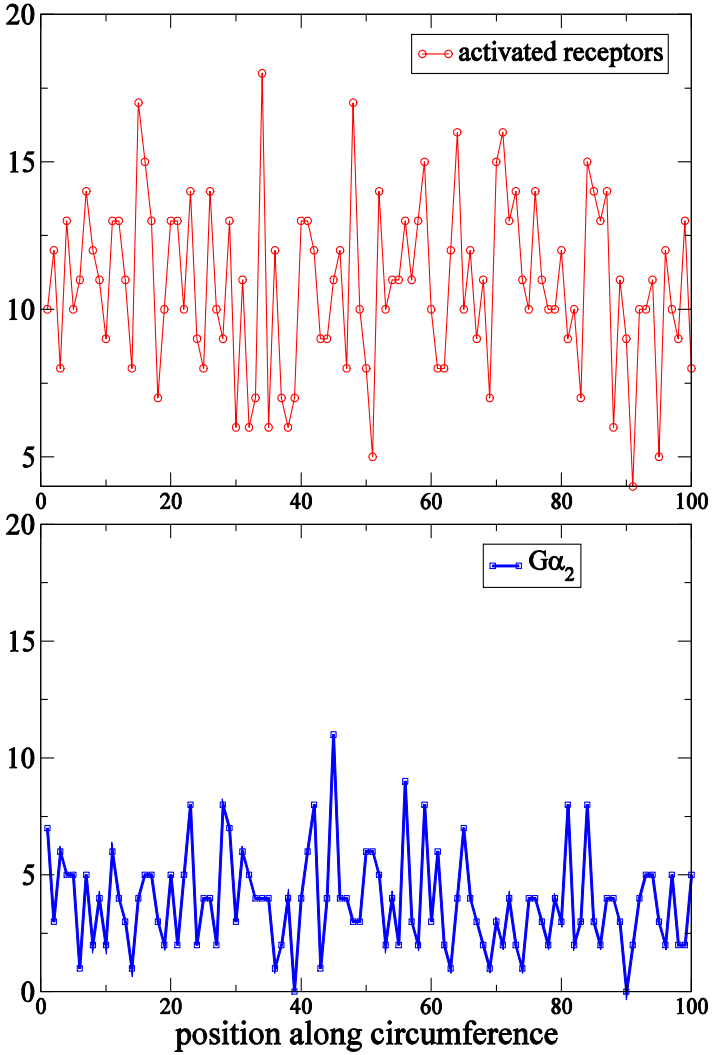
A simulation result

Distributions after 10^4 iterations (~ 10 s)

cAMP







A simulation result

Distributions after 10^4 iterations

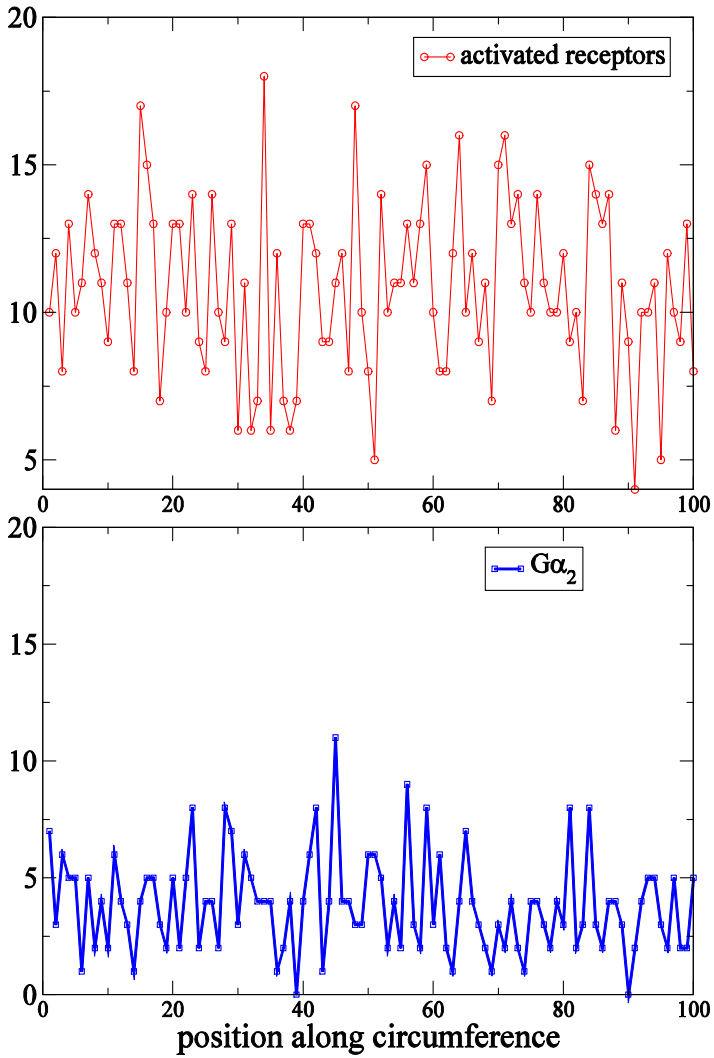
(~ 10 s)

In this parameter setting:

Large noise in cAR1* and/or Gα2P signal

A realisation seems to miss gradient information in cAR1* and/or Gα2P signal

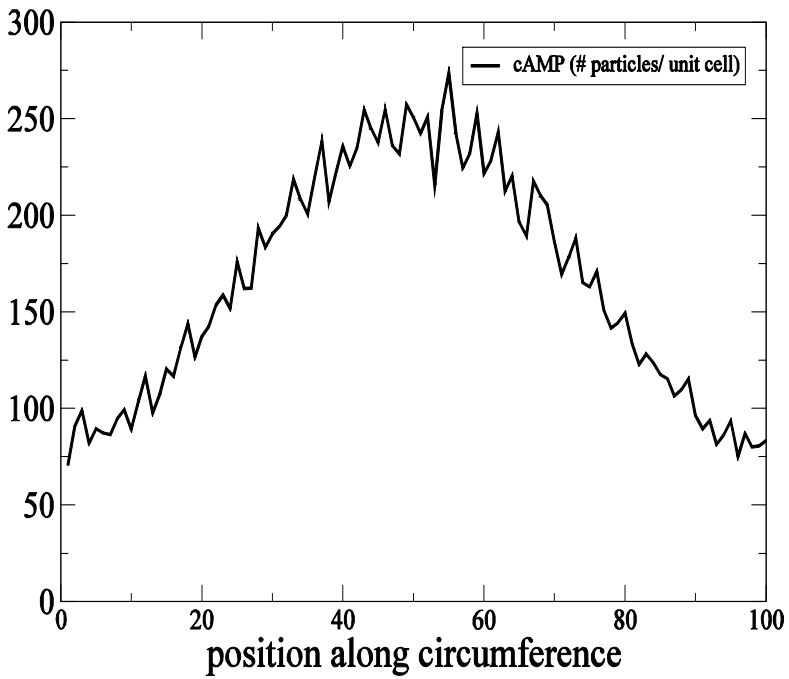
No signal amplification

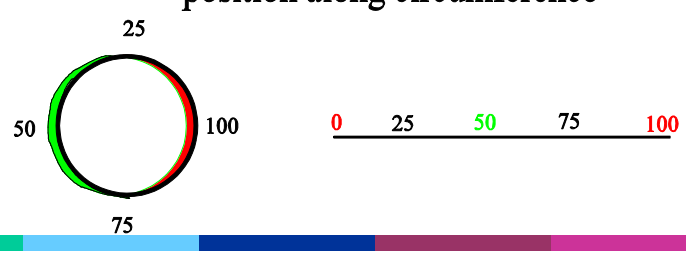


A simulation result

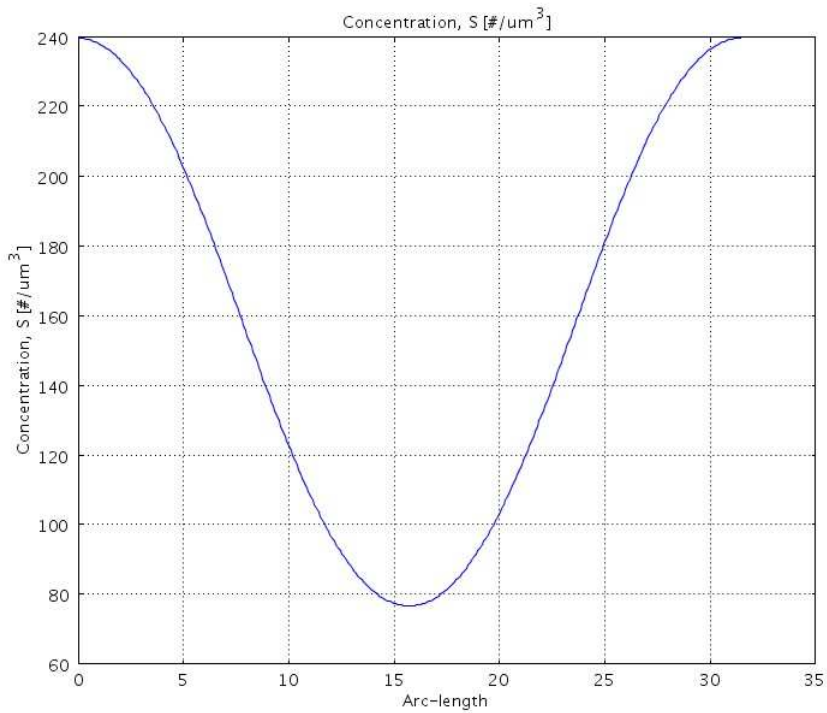
Distributions after 10^4 iterations

cAMP **(~ 10 s)**



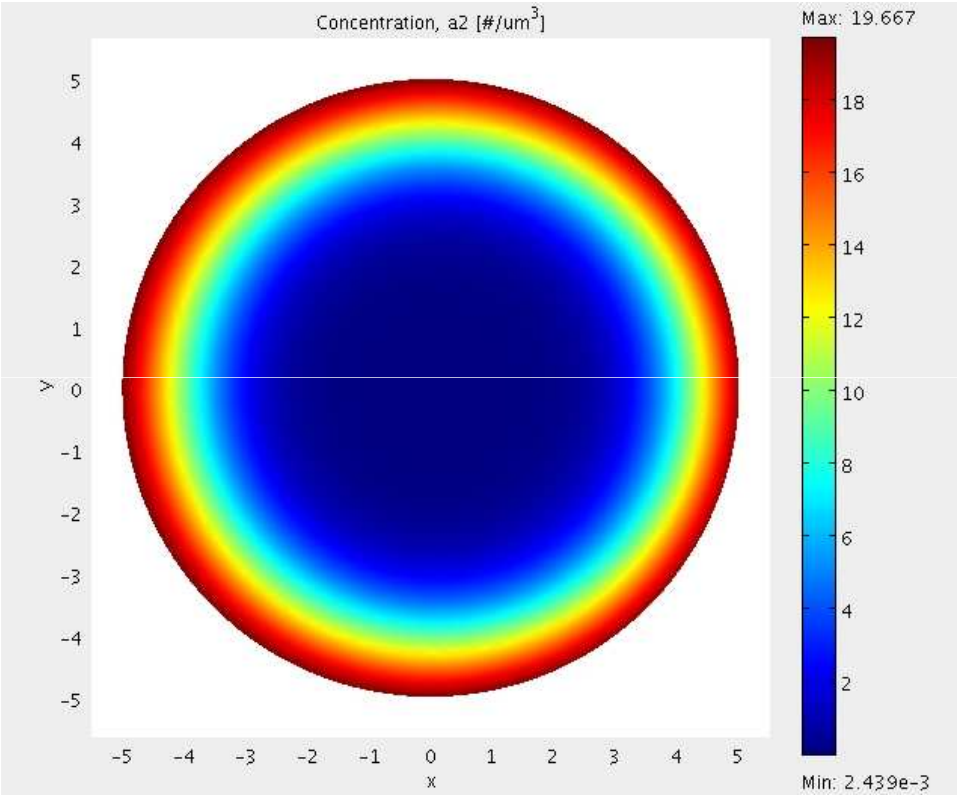


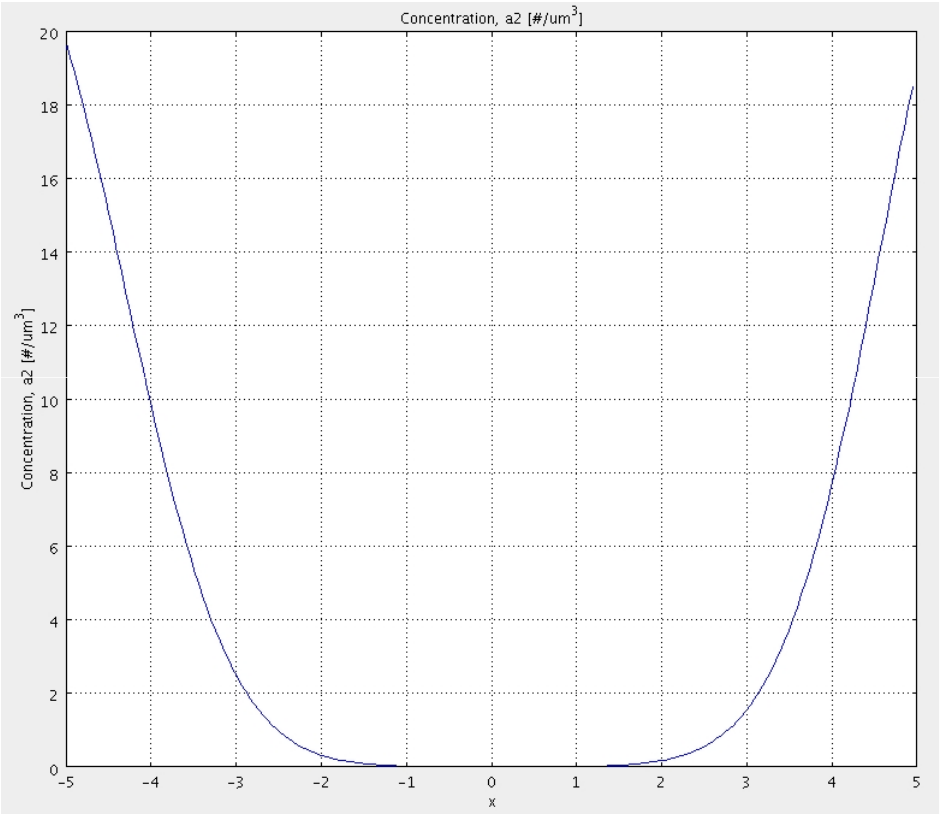
cAMP distribution according to determistic simulation



A deterministic simulation result

(different parameters) **Gα2P concentration**





Hardly any gradient information either…

Outlook

THE QUESTION remains,

How can the signaling system sense a gradient in such a **noisy environment** at low cAMP concentrations in a '**trustworthy' manner**?

-- The system seems to biochemically perform time-averaging

How can this be realised through and understood in view of the compounds, diffusivities and off-rates?

-- Currently looking in detail at stochastic properties of the cAMP-cAR1 and cAR1-Gα2$\beta\gamma$ interactions

Using simulations, analysis and experimental results