UNIVERSITÀ
DEGLI STUDI DI BARI
ALDO MORO

Dipartimento di Chimica

Modelling enzymatic pathways in Giant Vesicles

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COMSOL
CONFERENCE
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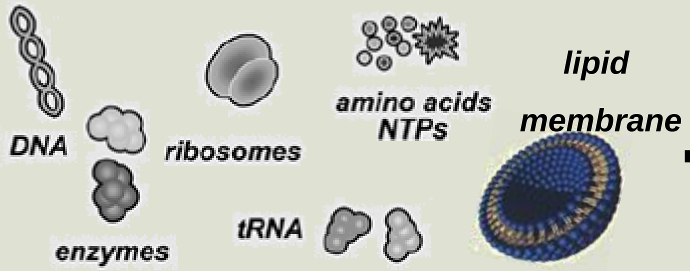
THE WESTIN GRAND MÜNCHEN

Is it possible to construct a simplified cell from separated molecules?

Origin of Life On Earth

Transition from no living to living matter



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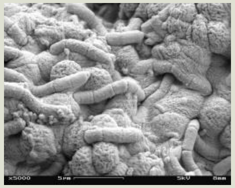


Emergence of Life in Test Tube

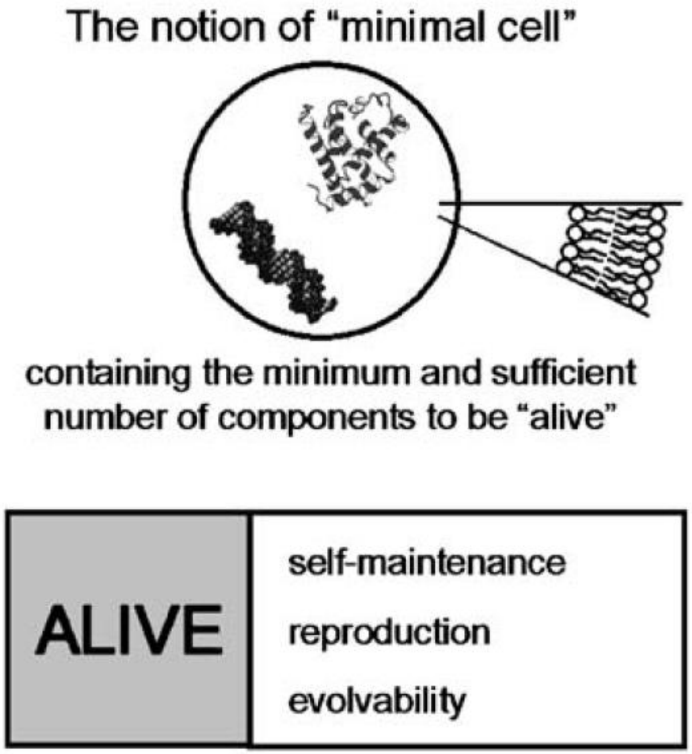
The notion of minimal cell

"…the one having the minimal and sufficient number of components to be called alive. What does "alive" mean?

Living at the cellular level means the concomitance of three properties:

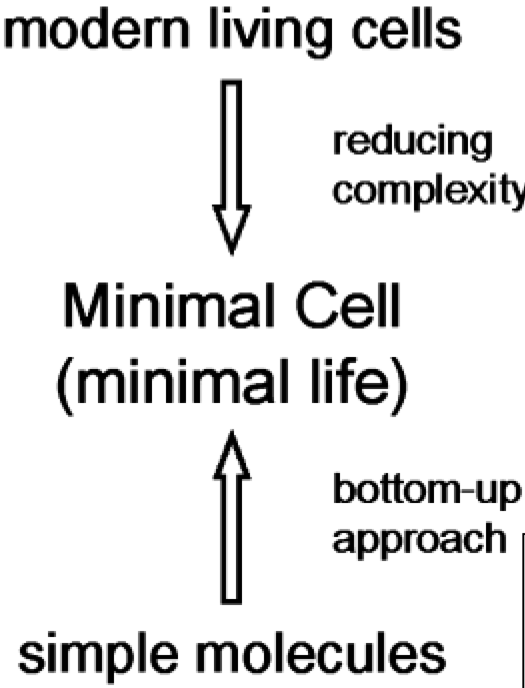
- *self-maintenance (metabolism),*
- *self-reproduction,*
- *and evolvability."*

"A living system is a system capable of self-production and self-maintenance through a regenerative network of processes which takes place within a boundary of its own making and regenerates itself through cognitive or adaptive interactions with the medium."



Damiamo and Luisi, *OLEB* (2010) 40 145

Approaches to Minimal Cells



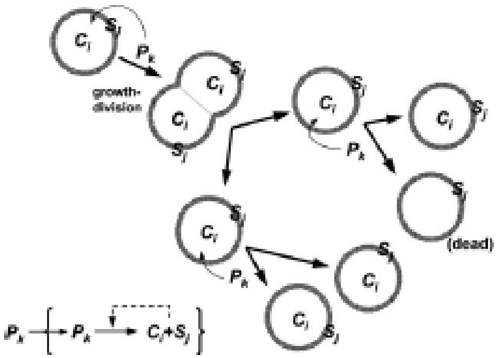
modern living cells

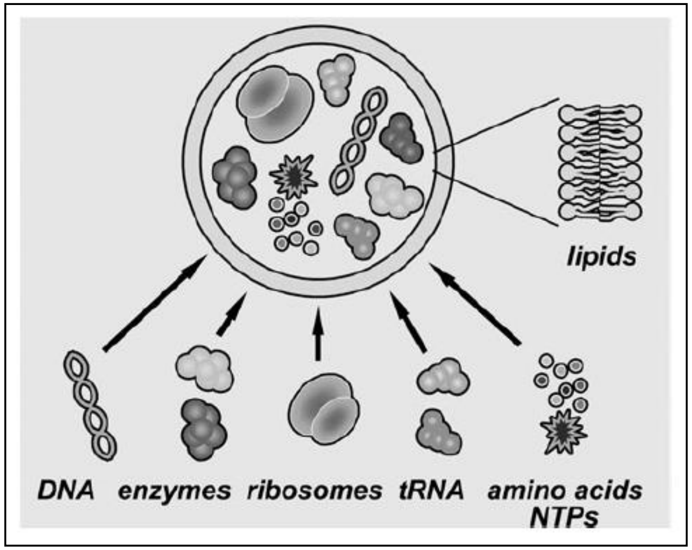
reducing complexity

Minimal Cell
(minimal life)

bottom-up approach

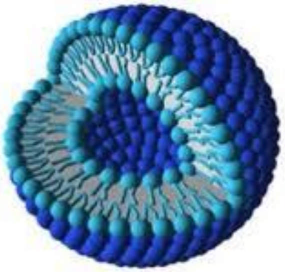
simple molecules





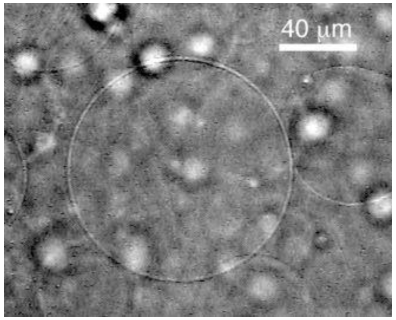
Luisi PL, *OLEB* (2006) 36 605, Stano P, *ChemComm* (2010) 46 3639

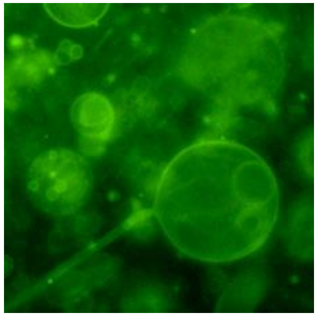
Giant lipid vesicles (GVs)



Features:

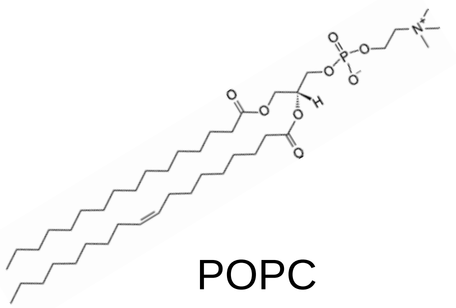
- Cell-like size (1-100μm)
- Large encapsulation volume
- Single vesicle analysis
- Direct visualization by microscopy techniques
- Use of High-throughput analysis (flow cytometry)



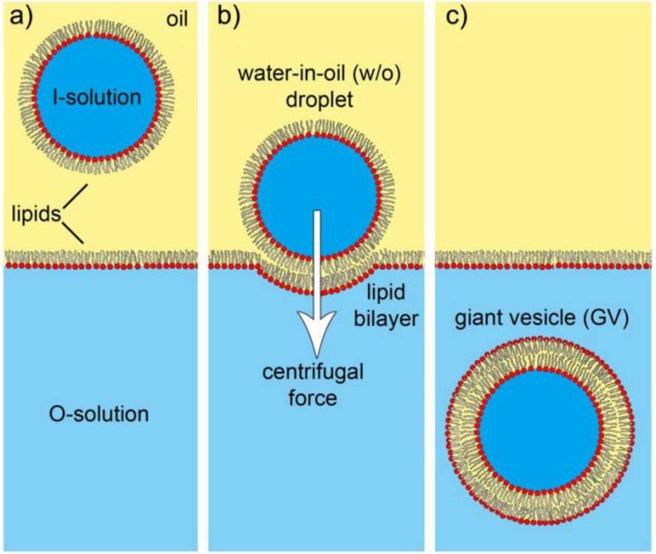


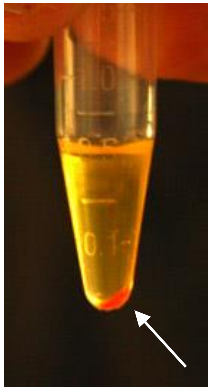
Phase Transfer Method

Pautot *et al*., *Langmuir* 2003;
PNAS 2003



POPC

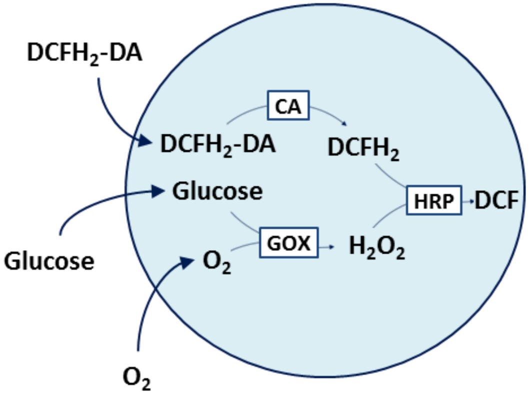




Pellet with GVs

Motivation and Aims

To develop theoretical models describing enzymatic cascade reactions taking place inside Giant Vesicles sustained by transport of substrates from the external environment.



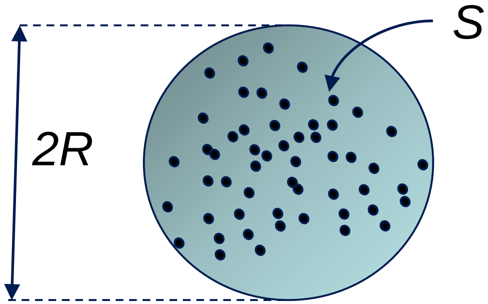
To drive the design and the chemical implementation of these system in test tube.

To achieve a better knowledge of dynamics of processes occurring in lipid compartments taking into account the poly-dispersity of these micro-sized aggregates.

To be able prepare compartmentalized chemical systems (giant lipid vesicles) designed for specific tasks (i.e. programmable) and with a determined time behavior in response to external chemical inputs (bio-computing)

General assumptions

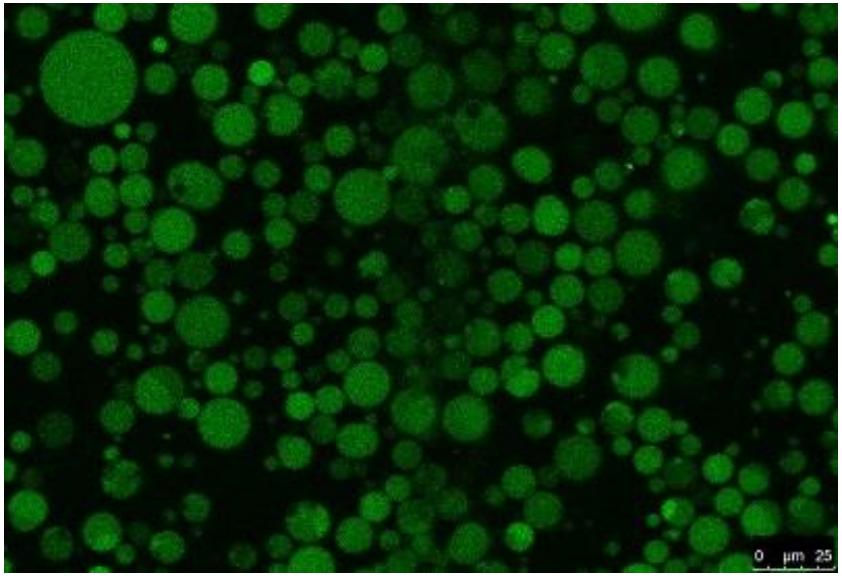
- **<u>The intrinsic stochastic effects are negligible</u>** since the volume of each reacting compartments is larger enough to encapsulate millions of reacting molecules, therefore <u>deterministic equations can be used to describe the time evolution of the system</u>.



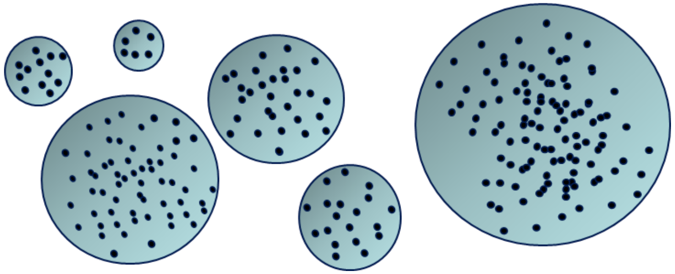
$[S]$ = 500 nM

R = 10 µm

→ $N=(4/3)\pi R^3 C N_A \approx 1.3\ 10^6$ molecules



- On the other hand, the preparation procedures of giant vesicle suspension give very poly-dispersed vesicle solution both in size of vesicles and concentration of encapsulated solutes, therefore <u>extrinsic stochastic effects must be taken into account</u>.



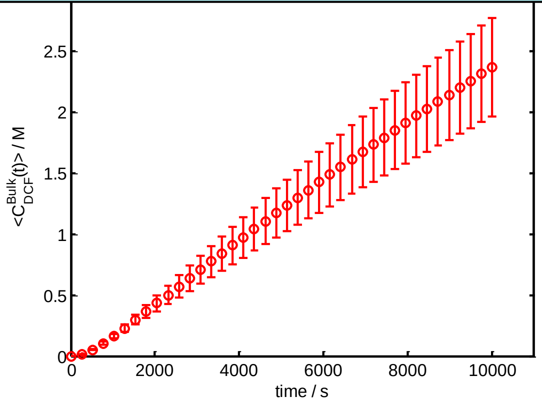
Two different approaches

0D Approach

Ordinary Differential Equation set

(ODE Set)

Evolution of of reacting species as average time course of the vesicle suspension

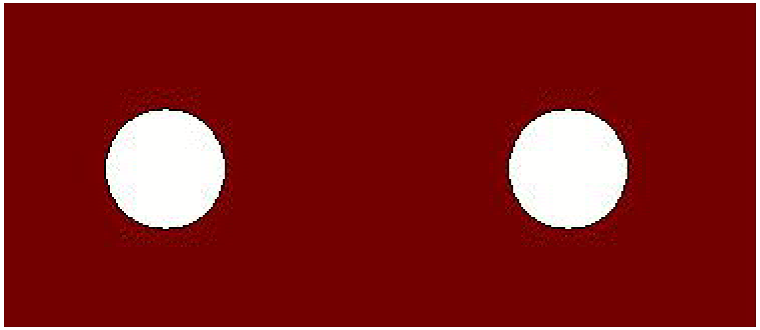


3D Approach

Partial Differential Equation set

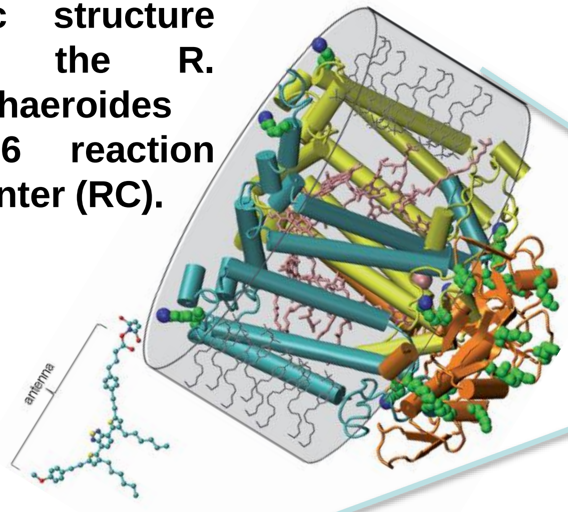
(PDE Set)

Evolution of reacting species in single vesicles both in time and space

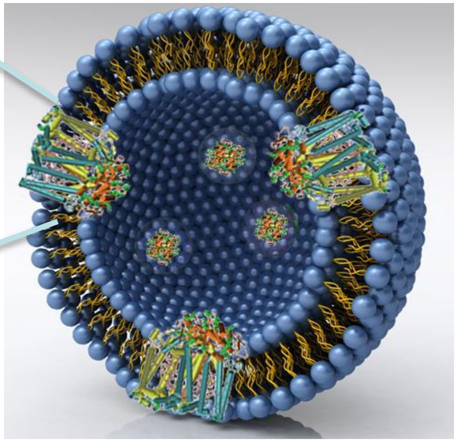


Why two approaches ?

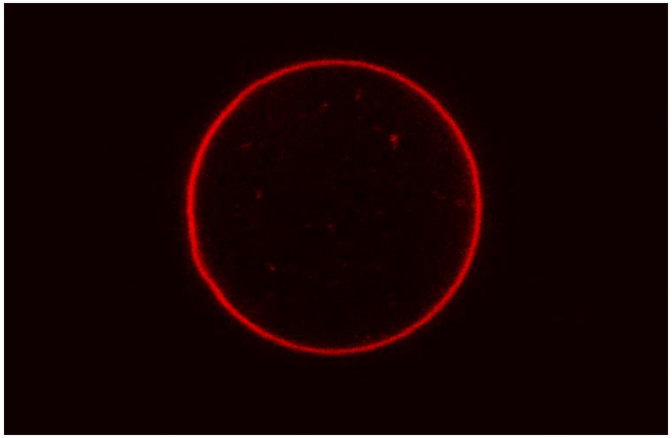
Crystallographic structure of the R. sphaeroides R26 reaction center (RC).



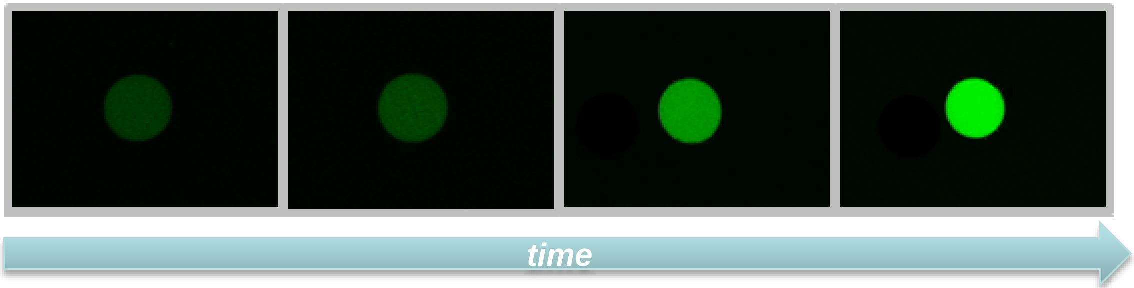
Reconstitution of the photosynthetic RC within the GV membrane



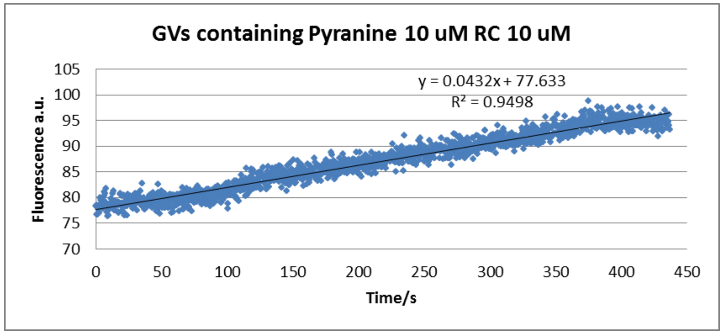
Confocal microscope image of GV made of POPC with RC reconstituted in membrane.



Increase of the pyranine internal fluorescence due to pH increase in a single vesicle followed by confocal microscopy

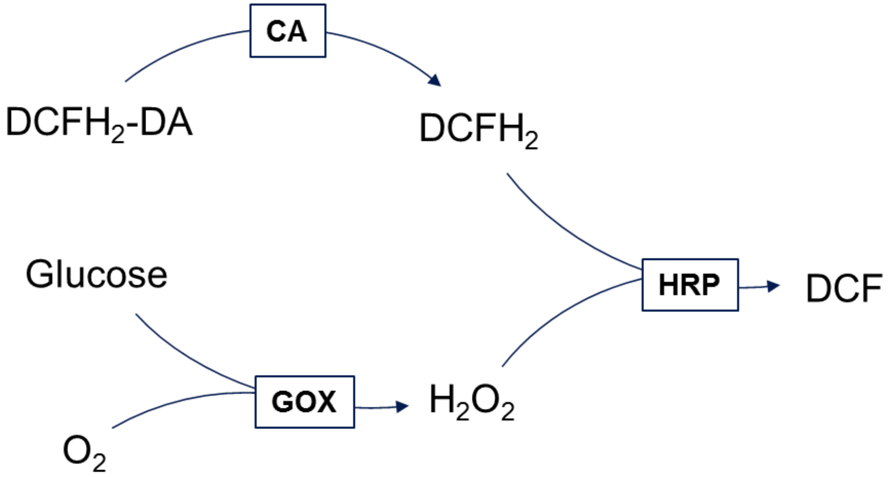


Increase of the pyranine fluoresce of a vesicle suspension followed by spectrophotometer



Submitted to PNAS

3 Enzymes Kinetic Mechanism (Y)



Symbol	Initial Concentration	Species
[CA]	1.0 μM	Carbonic Anhydrases
[HRP]	0.5 μM	Horseradish Peroxidase
[GOX]	0.06 μM	Glucose Oxidase
[DCFH$_2$DA]	20 μM	2-Clore
[DCFH$_2$]	0.0 μM	
[DCF]	0.0 μM	
[H$_2$O$_2$]	0.0 μM	Hydrogen Peroxide
[O$_2$]	200.0 μM	Oxygen
[Glu]	18.0 μM	Glucose

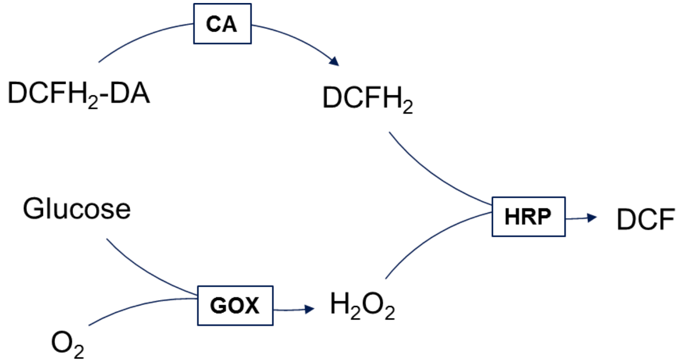
$$V_{ca} = k_c [CA] \frac{[DCFH_2DA]}{\left(K_{c,DCFH-DA} + [DCFH_2DA]\right)}$$

$$V_{gox} = k_g [GOX] \frac{[O_2]}{\left(K_{g,O_2} + [O_2]\right)} \frac{[Glu_2]}{\left(K_{g,Glu} + [Glu_2]\right)}$$

$$V_{hrp} = k_h [HRP] \frac{[DCFH_2]}{\left(K_{h,DCFH_2} + [DCFH_2]\right)} \frac{[H_2O_2]}{\left(K_{h,H_2O_2} + [H_2O_2]\right)}$$

Kinetic Constant	Value
k_g	10 s^{-1}
k_h	240 s^{-1}
k_c	4 s^{-1}
$K_{c,DCFH-DA}$	4.0e-3 M
$K_{h,H2O2}$	8.1e-5 M
$K_{h,DCFH}$	8.1e-6 M
$K_{g,O2}$	0.2e-3 M
$K_{g,Glu}$	2.0e-3 M

Model vs bulk data



CA
$DCFH_2$-DA
$DCFH_2$
Glucose
GOX
O_2
H_2O_2
HRP
DCF

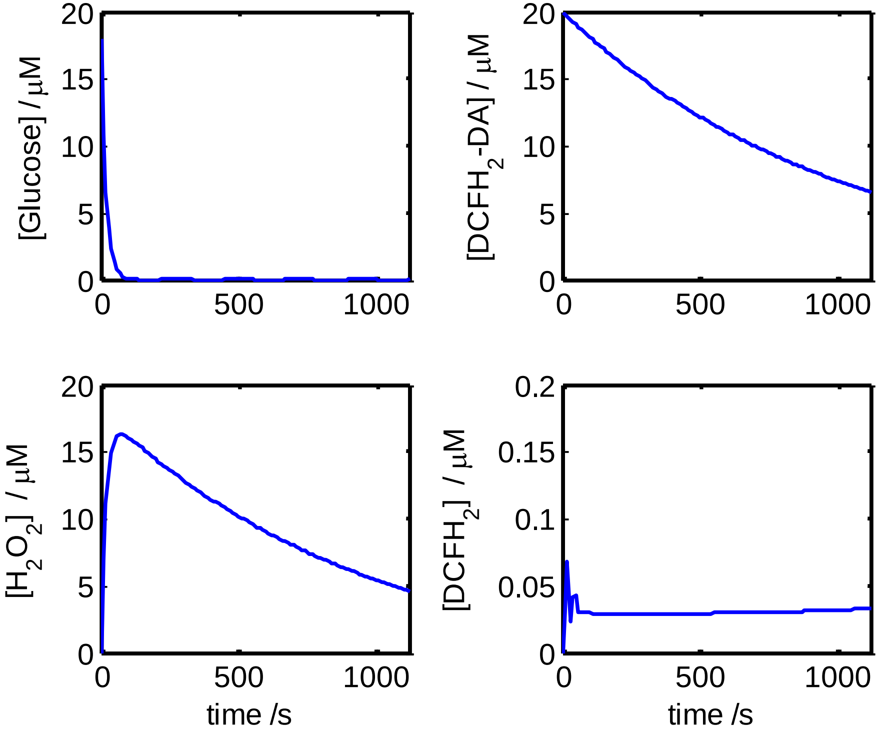
From BRENDA

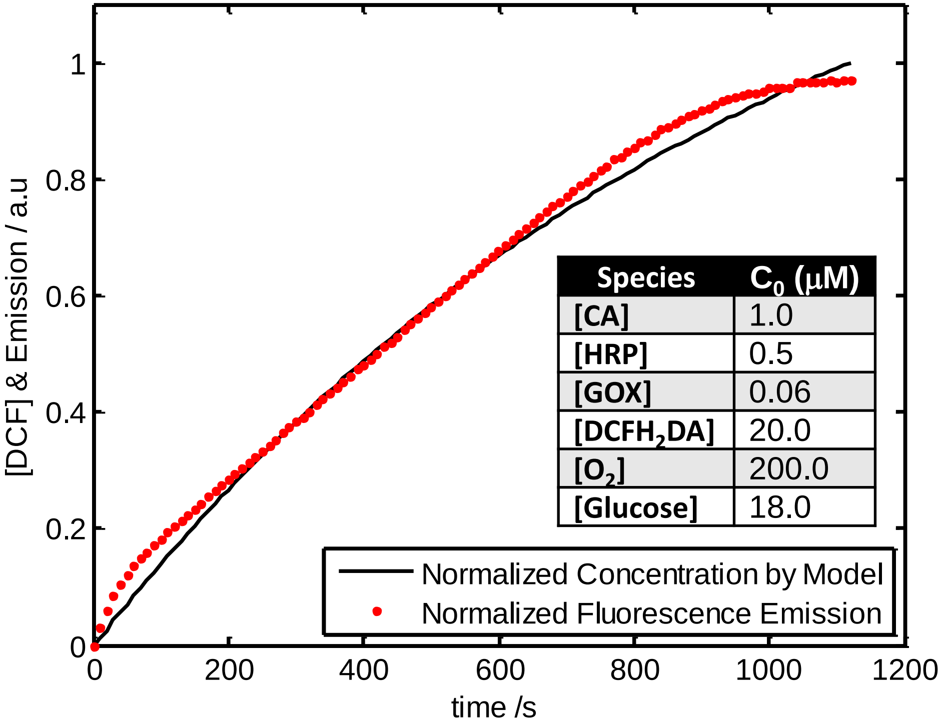
Constants	Values
k_g	10 s^{-1}
k_h	240 s^{-1}
k_c	4 s^{-1}
$K_{c,DCFH-DA}$	4.0e-3 M
$K_{h,H2O2}$	8.1e-5 M
$K_{h,DCFH}$	8.1e-6 M
$K_{g,O2}$	0.2e-3 M
$K_{g,Glu}$	2.0e-3 M

$$V_{ca} = k_c [CA] \frac{[DCFH_2DA]}{(K_{c,DCFH-DA} + [DCFH_2DA])}$$

$$V_{gox} = k_g [GOX] \frac{[O_2]}{(K_{g,O_2} + [O_2])} \frac{[Glu_2]}{(K_{g,Glu} + [Glu_2])}$$

$$V_{hrp} = k_h [HRP] \frac{[DCFH_2]}{(K_{h,DCFH_2} + [DCFH_2])} \frac{[H_2O_2]}{(K_{h,H_2O_2} + [H_2O_2])}$$

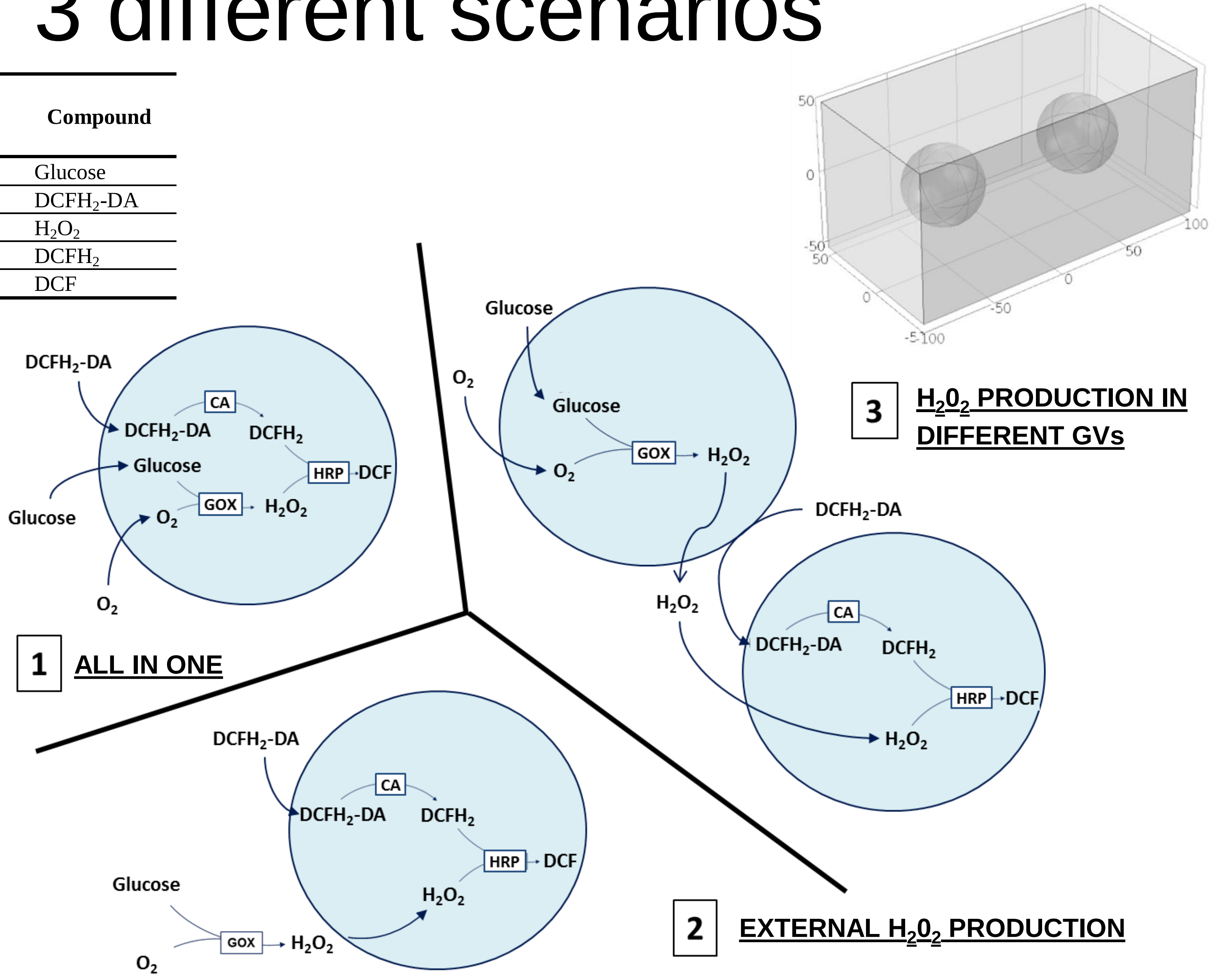




Species	C_0 (μM)
[CA]	1.0
[HRP]	0.5
[GOX]	0.06
[$DCFH_2$DA]	20.0
[O_2]	200.0
[Glucose]	18.0

3 different scenarios

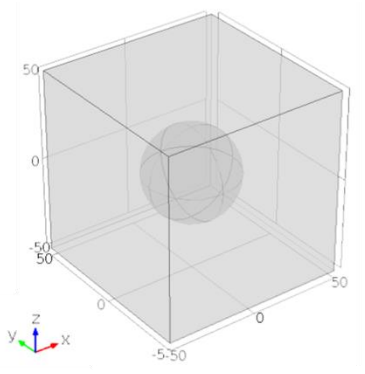
Permeability [cm/s]	Diffusion Coefficient [cm^2/s]	Compound
0.5e-7	**6.0E-06**	Glucose
1e-6	**6.0E-06**	$DCFH_2$-DA
1e-3	**1.8e-04**	H_2O_2
0.0	**6.0E-06**	$DCFH_2$
0.0	**6.0E-06**	DCF



1 ALL IN ONE

2 EXTERNAL H_20_2 PRODUCTION

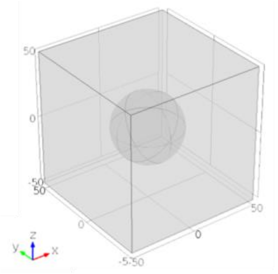
3 H_20_2 PRODUCTION IN DIFFERENT GVs



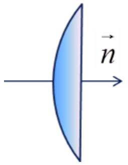
3D Model

- The global systems is decomposed in different domains (compartments)
- Free diffusion is allowed for all species in each system domains

$$\frac{\partial C_i^\delta}{\partial t} = D_i \nabla C_i^\delta$$



- Periodic Boundary Conditions are applied to the box walls
- Passive transport processes take place across the GVs' boundaries (thin lipid membrane) according to the molecular permeability ($\wp_i$)



$$\mathbf{n}\nabla C_i = \wp_i \left(C_i^{Ex} - C_i^{In}\right)$$

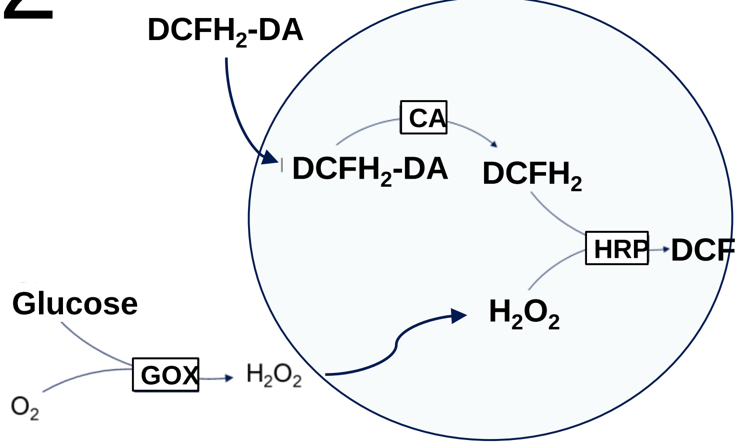
- Chemical reactions occur in system where enzymes are present according to the kinetic mechanism (R_i reaction rates)

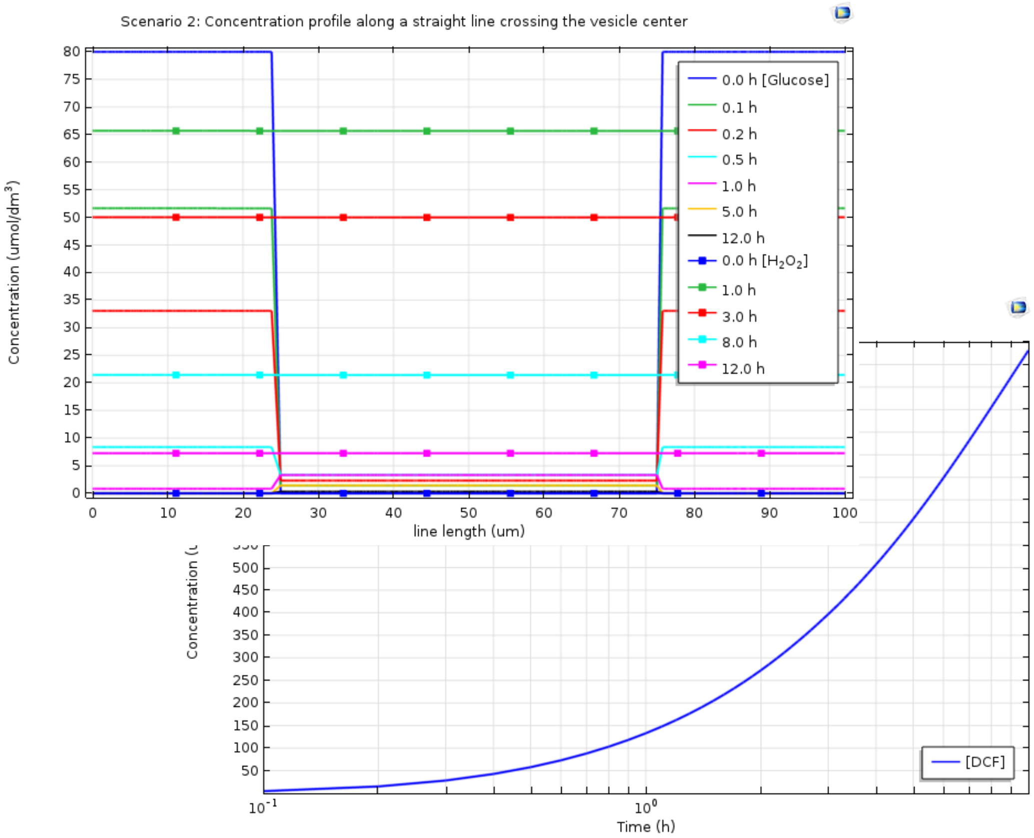
$$\frac{\partial C_i^\delta}{\partial t} = \nabla^2 C_i^\delta + \sum_{R=1}^{3} \alpha_i^R V_R$$

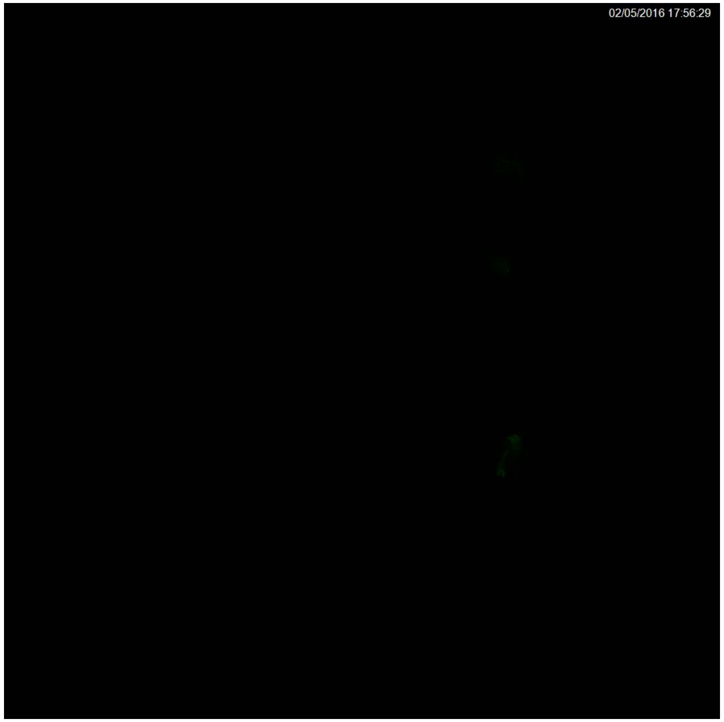
Scenario 2

Permeability [cm/s]	Diffusiion [cm²/s]	Compound
0.5e-7	6.0E-06	Glucose
1e-6	6.0E-06	$DCFH_2$-DA
1e-3	1.8e-04	H_2O_2
0.0	6.0E-06	$DCFH_2$
0.0	6.0E-06	DCF

Species	C_0 (µM)
[CA]	1.0
[HRP]	1.0
[GOX]	0.5
[$DCFH_2DA$]	100.0
[O_2]	200.0
[Glucose]	80.0

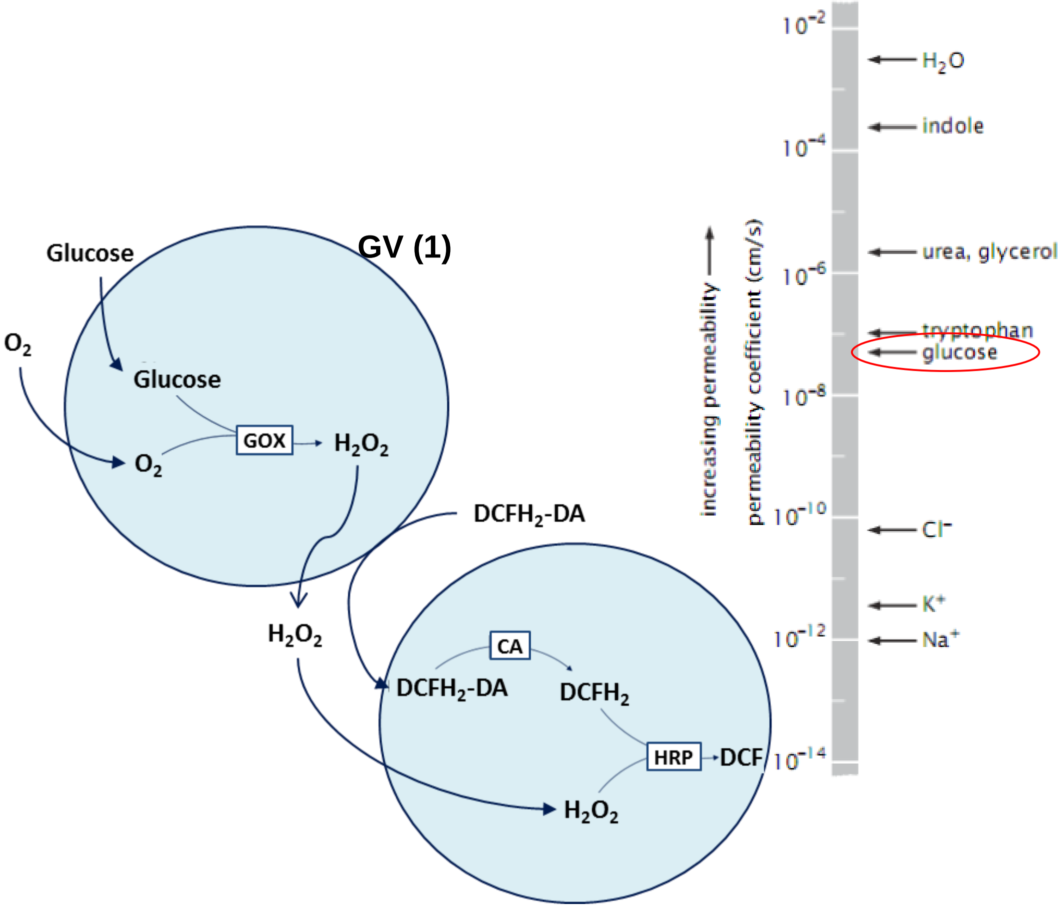




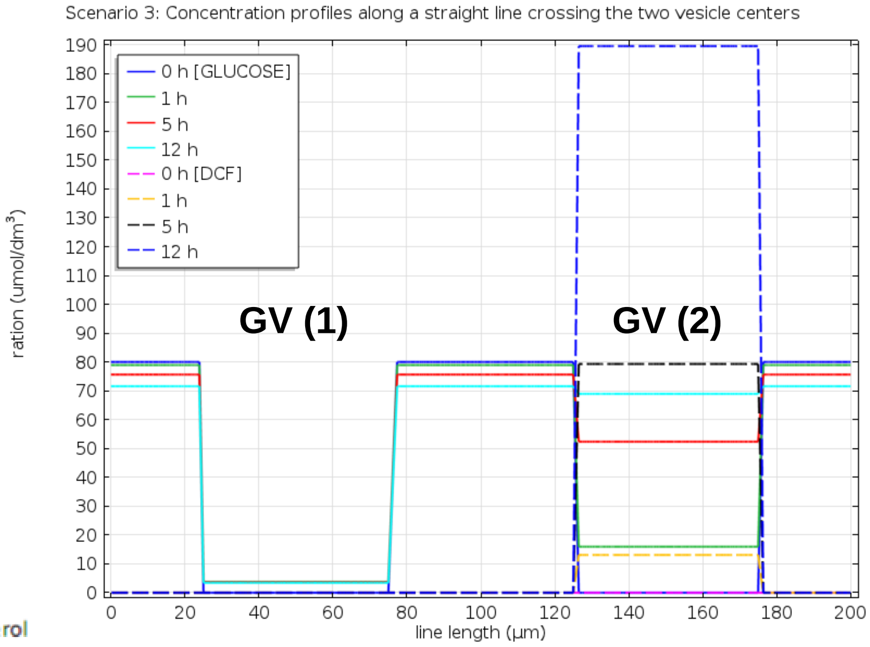


Scenario 3

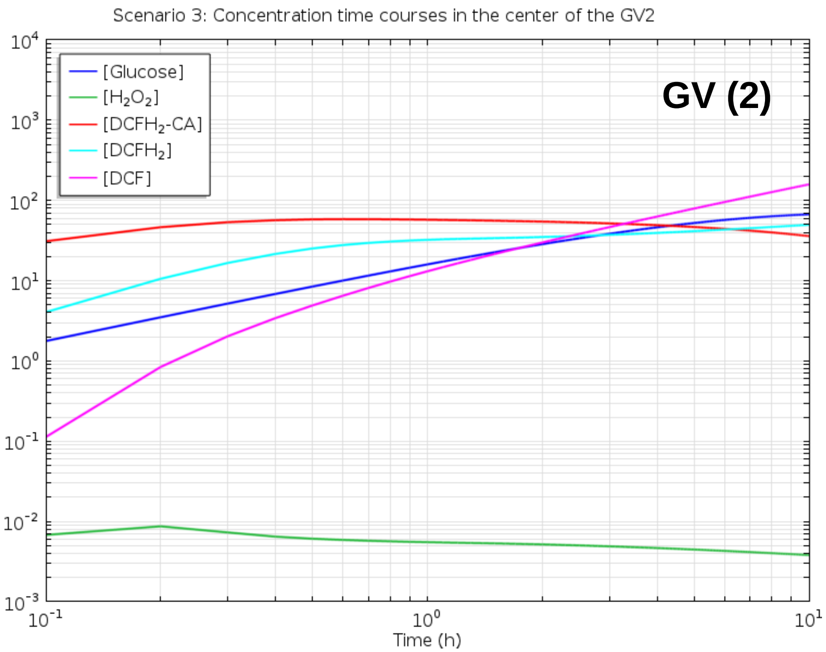
The enzymatic production of hydrogen peroxide occurs in GV (1) loaded with GOX while the fluorescent products is formed in GV (2) where HRP and CA are entrapped.



Scenario 3: Concentration profiles along a straight line crossing the two vesicle centers



Scenario 3: Concentration time courses in the center of the GV2



Conclusions

- 3D approach allows to monitor the time evolution of a single giant vesicle (or of few compartments) with more details taking into account also the diffusion of molecules in three dimensional space, although diffusion is very fast.
- POPC Giant vesicle membrane must be decorated with pore for accelerating the glucose transport.

Perspective

- To improve the comparison with experimental data.
- To build up a 3D model for light transducers giant vesicles (RC@GVs)
- To study the behaviour of GVs encapsulating magnetic NanoParticles (GV{mNP})
- To couple the GV morphology with the change of internal composition

Acknowledgments

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Thank you for your attention

"All models are wrong,

but some are useful"

George Box