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UNIVERSITÀ
DEGLI STUDI DI BARI
ALDO MORO

Dipartimento di Chimica

In vitro and *in silico* minimal cell models

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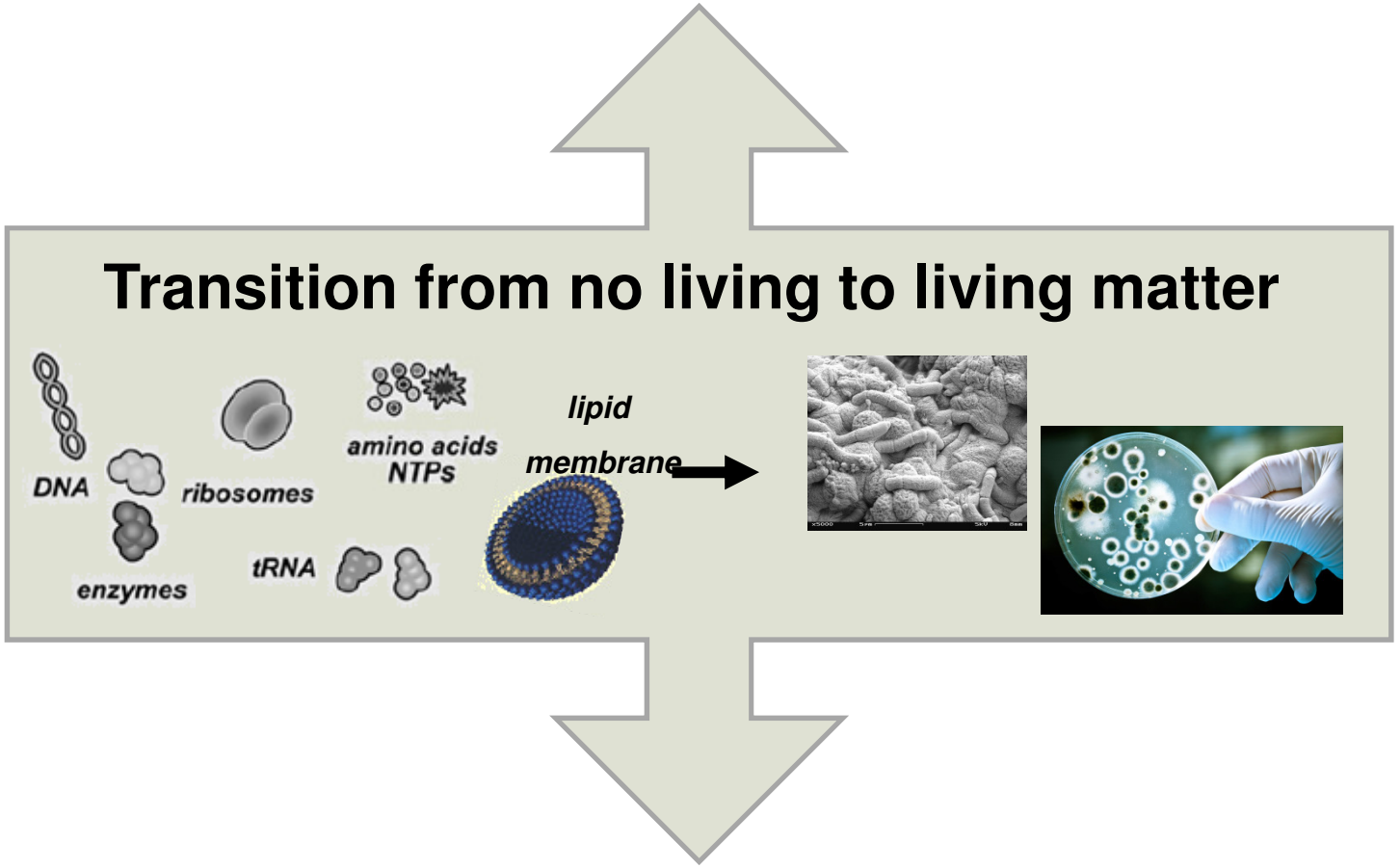
2nd International Summit on
Integrative Biology
August 04-05, 2014 Hilton-Chicago/Northbrook, Chicago, USA

Outline

- Introduction
  - Notion of minimal cell
  - Stochastic vs Deterministic models
- In vitro models
  - Polymer-Enzymes Complex for cascade reactions
  - Membrane Pore formation
  - Photosyntetic Reaction Renter membrane recostitution
- In silico models
  - Osmotic Synchronization
- Conclusions

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

Origin of Life On Earth



Emergence of Life in Test Tube

(*) Luisi's group, ETH Zurich and Roma3 Univ.

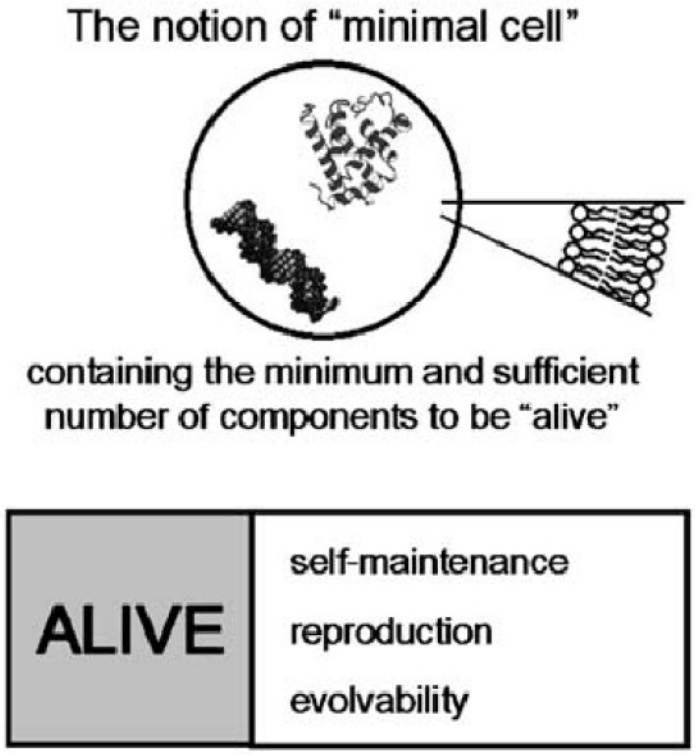
The notion of minimal cell

"…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

Semi-synthetic approach to MC

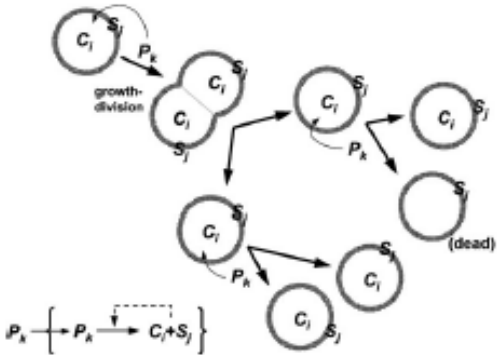
modern living cells

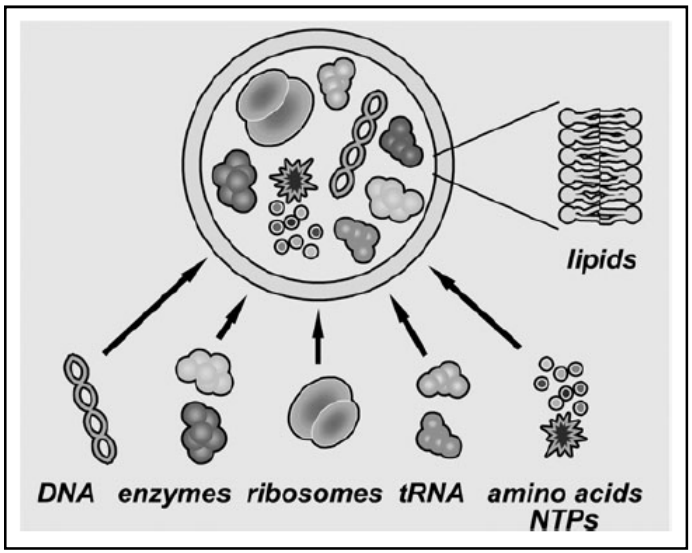
reducing complexity

Minimal Cell (minimal life)

bottom-up approach

simple molecules





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

Aim of Modeling

Bridging the gap between *in silico* and *in vitro* experiments in the bottom-up approach to the minimal artificial cell

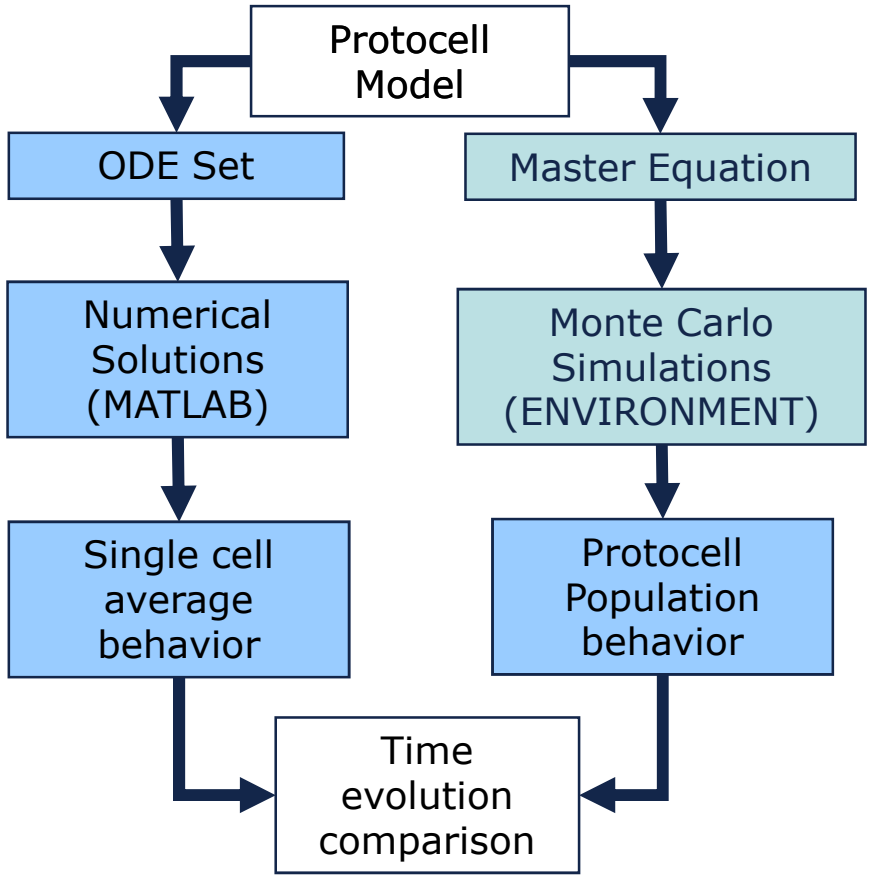
Deterministic	Stochastic
Macroscopic	Microscopic
Positive real numbers of Molecules	Integer numbers of Molecules
Reaction Rates	Propensity Probabilities
Average Behaviour	Average + Fluctuations

Theoretical approaches:

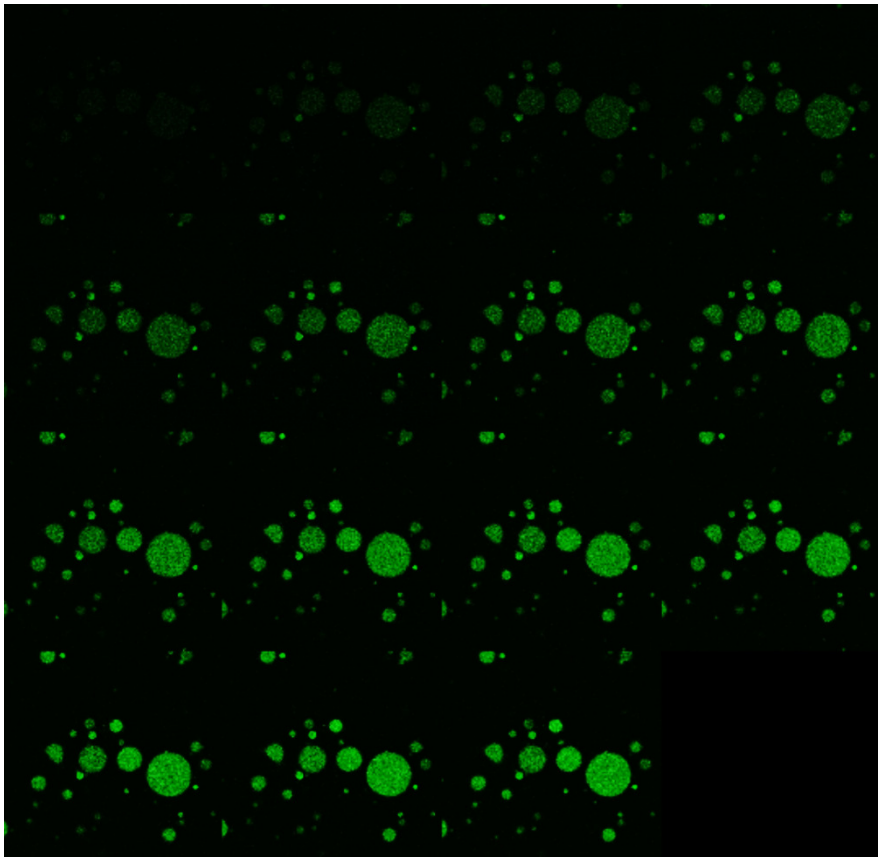
- **Deterministic**
- **Stochastic**

Theoretical models have been developed in order to analyse the feasibility of minimal cell models (*protocells*) able to exhibit:

- Self-maintenance
- Self-reproduction
- Evolution



GFP Expression in a w/o emulsion

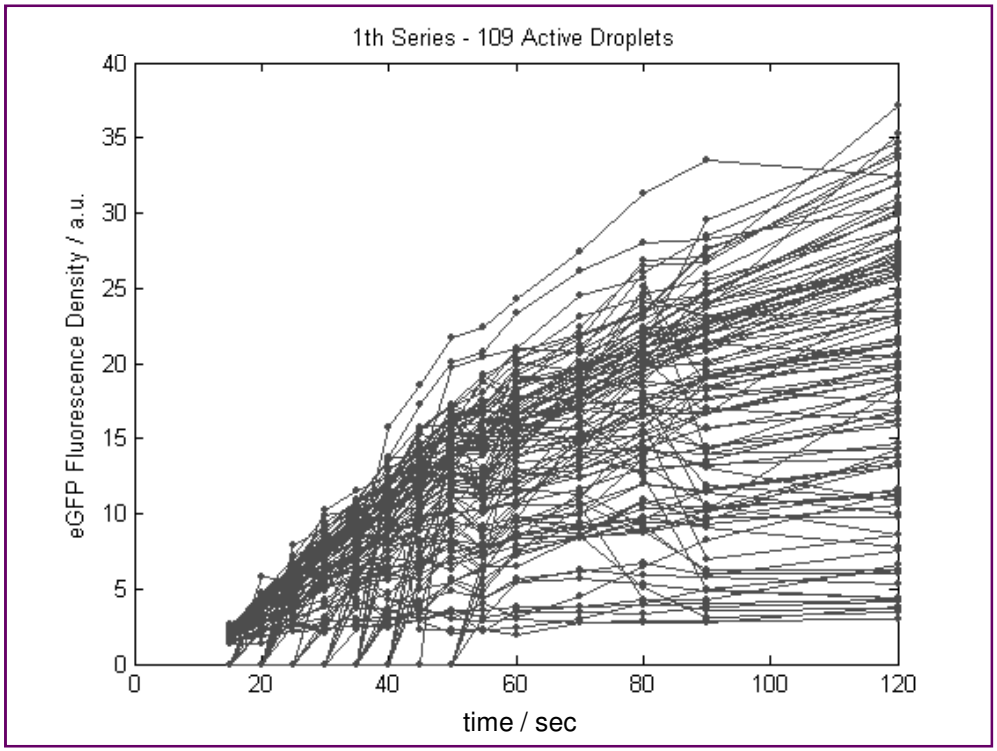


PURESYSTEM encapsulated in a water/oil emulsion

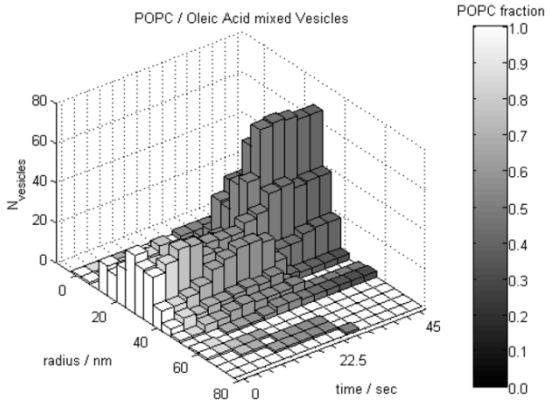
Data from Luisi's Lab experiments by P.Carrara, M.Caputo and P.Stano

The time behavior of each compartment in the population could be highly affected by random fluctuations:

- Intrinsic effects
- Extrinsic effects

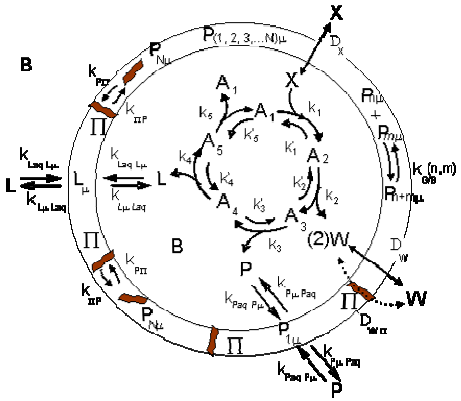


Lipid Vesicle Dynamics



Mavelli F., Ruiz-Mirazo K.,
Phys Biol 7, (2010) , 36002

Minimal Petide Cell

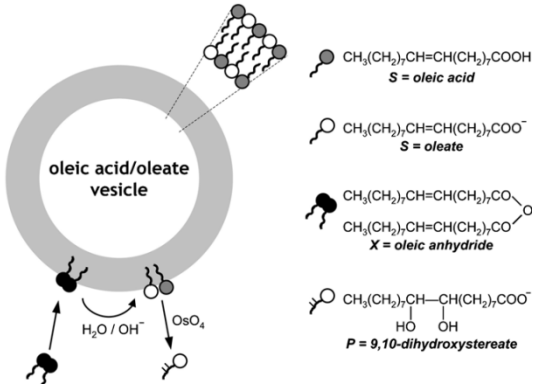


Ruiz-Mirazo K., Mavelli F.,
Biosystems 91, (2008), 374

ENVIRONMENT

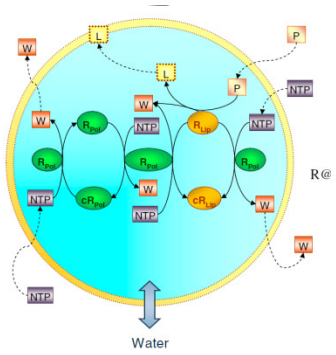
Mavelli F., Ruiz-Mirazo K.,
Phil. Trans. R. Soc. Lon. B
362, (2007) , 1789.

Autopoietic Vesicles



Mavelli F., Stano P.,
Phys Biol 7, (2010) , 16010

Ribocell Model



Mavelli F. (2012) BMC Bioinformatics
13 , supp4, S10

In vitro modeling

Giant lipid vesicles (GVs)

Features:

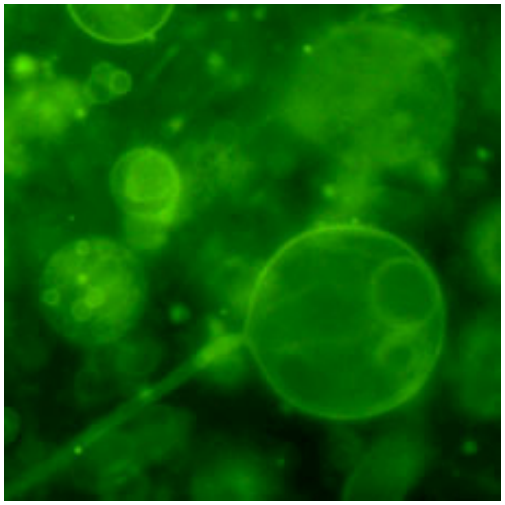
- Cell-like size
- Large encapsulation volume
- Single vesicle analysis
- Direct visualization by microscopy techniques
- Use of High-throughput analysis (flow cytometry)

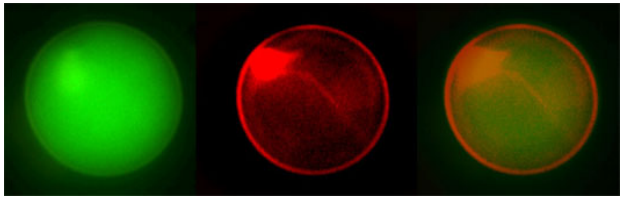
Conventional preparation methods:

- Natural swelling
- Electroformation

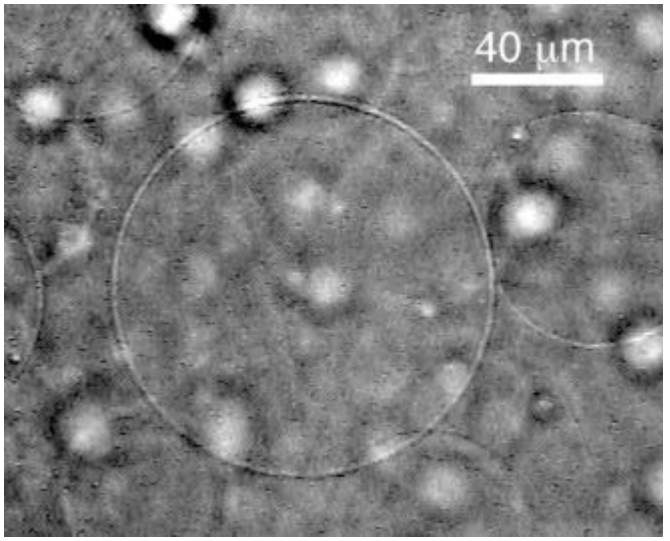
New Method

- Phase Transfer Method

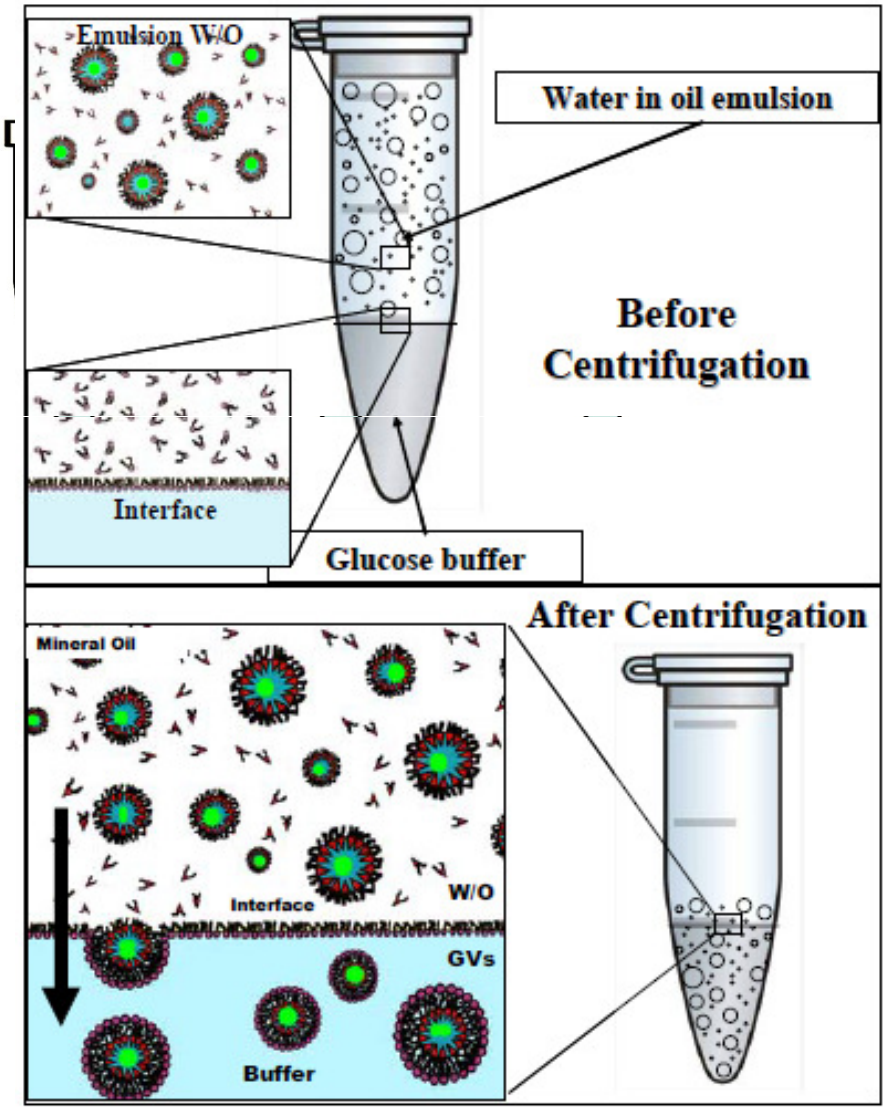




Giant vesicles (1-100µm)

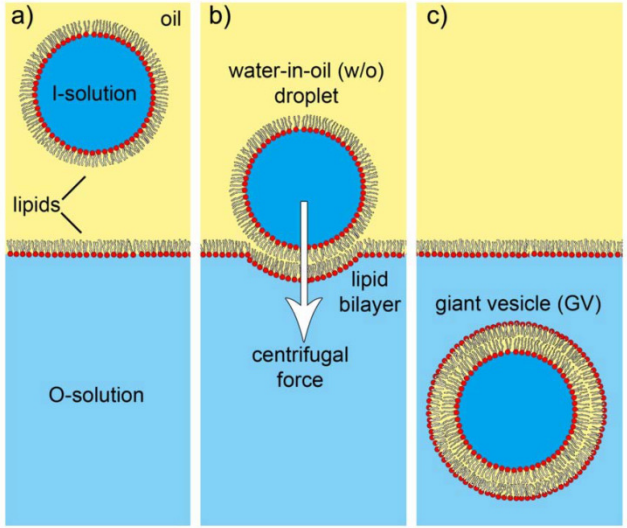


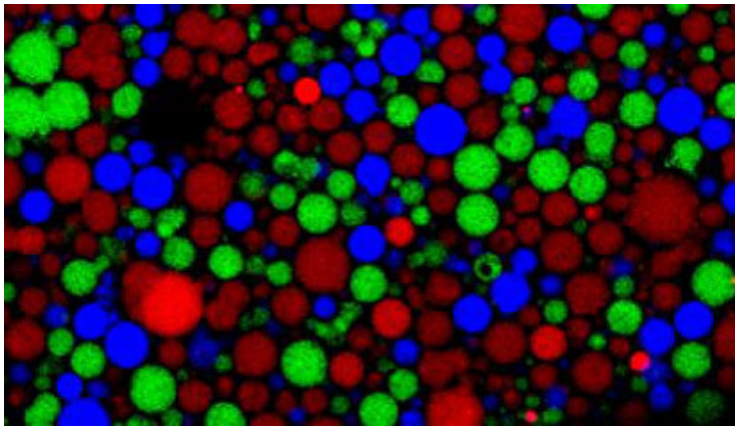
The “Droplet transfer method”



The “droplet transfer” method

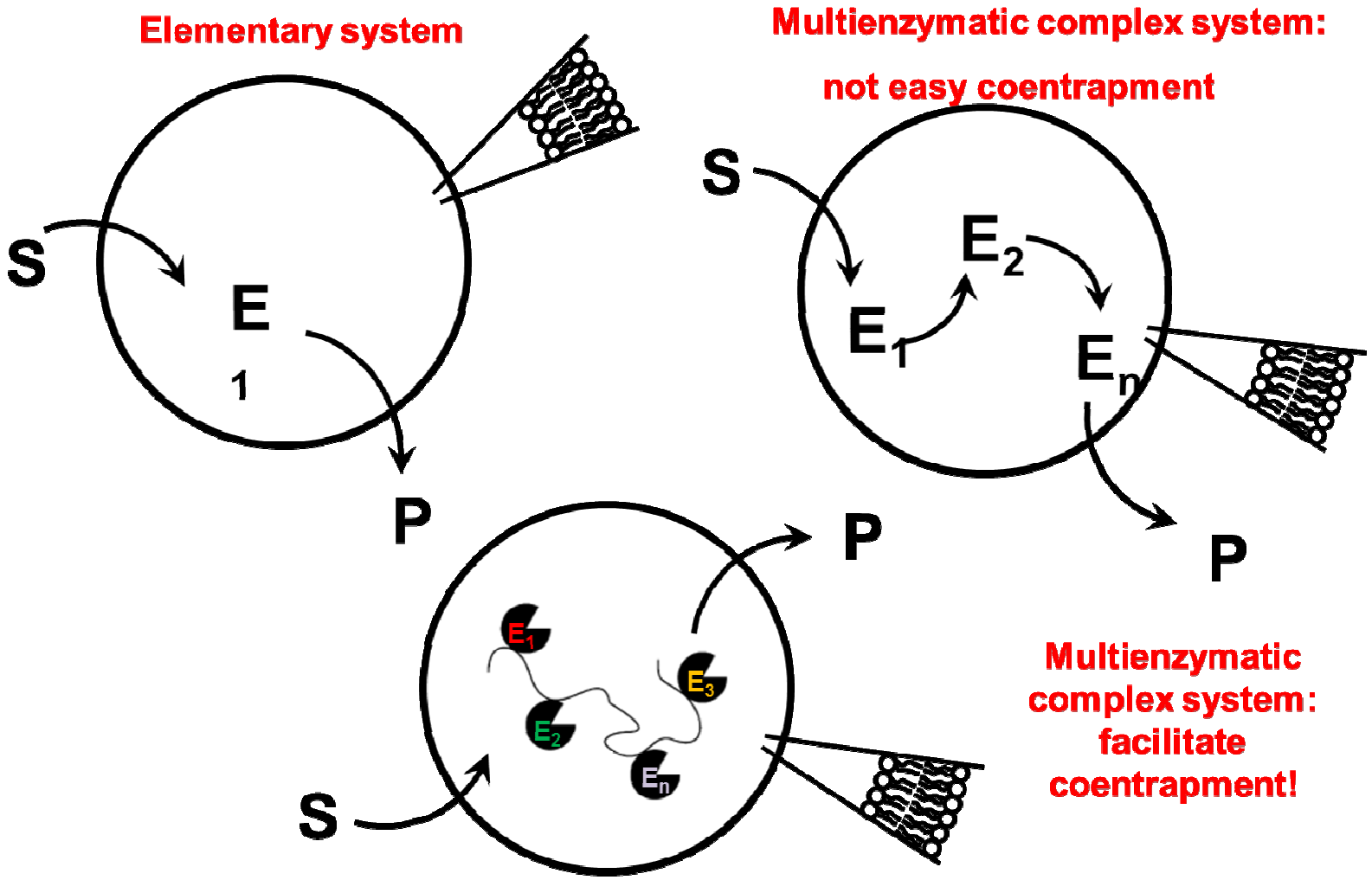
Pautot et al., *Langmuir* 2003; *PNAS* 2003



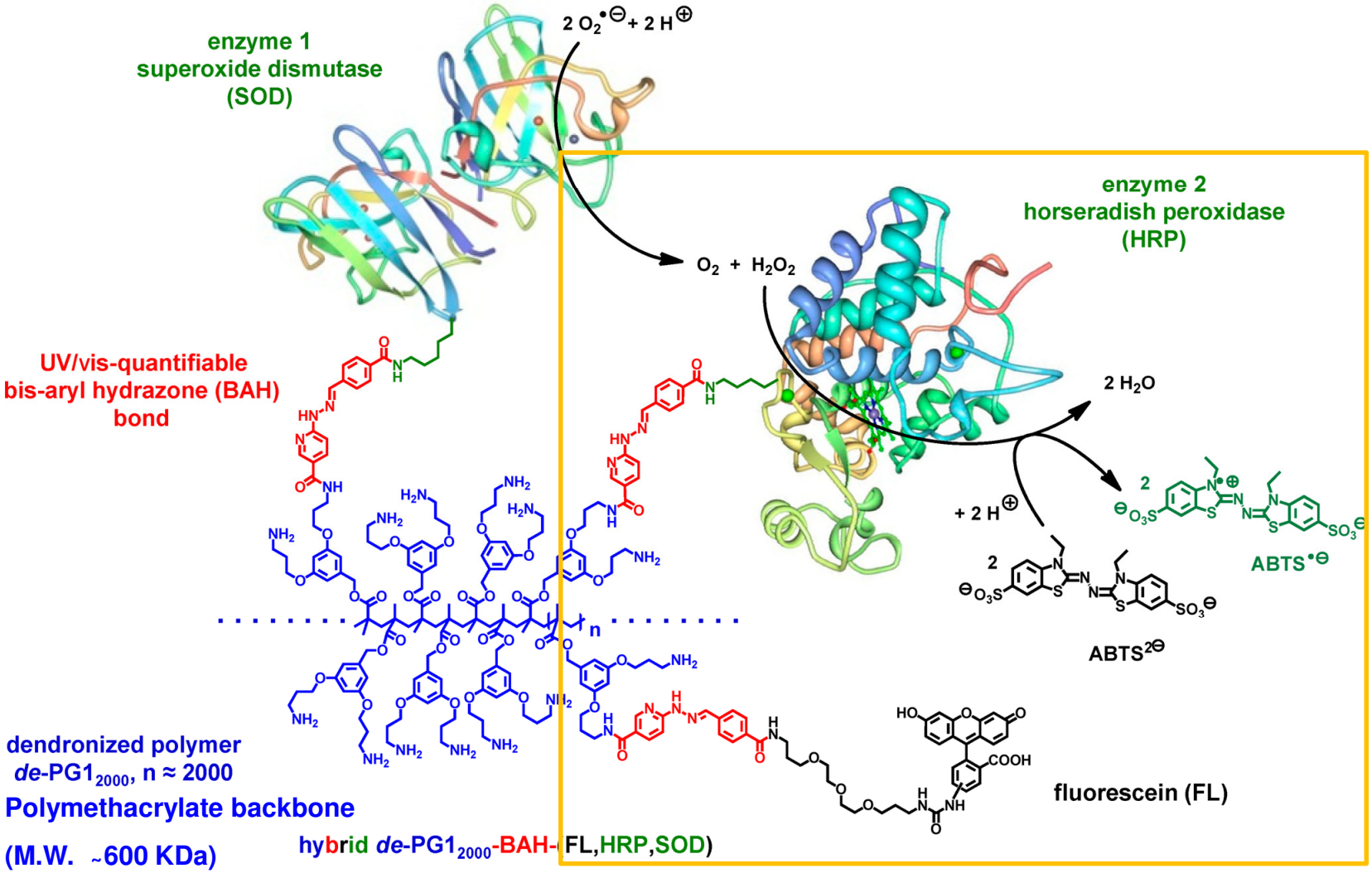


Carrara, Altamura, Stano, Luisi, *submitted*

GVs as biochemical reactors
Improving enzyme encapsulation

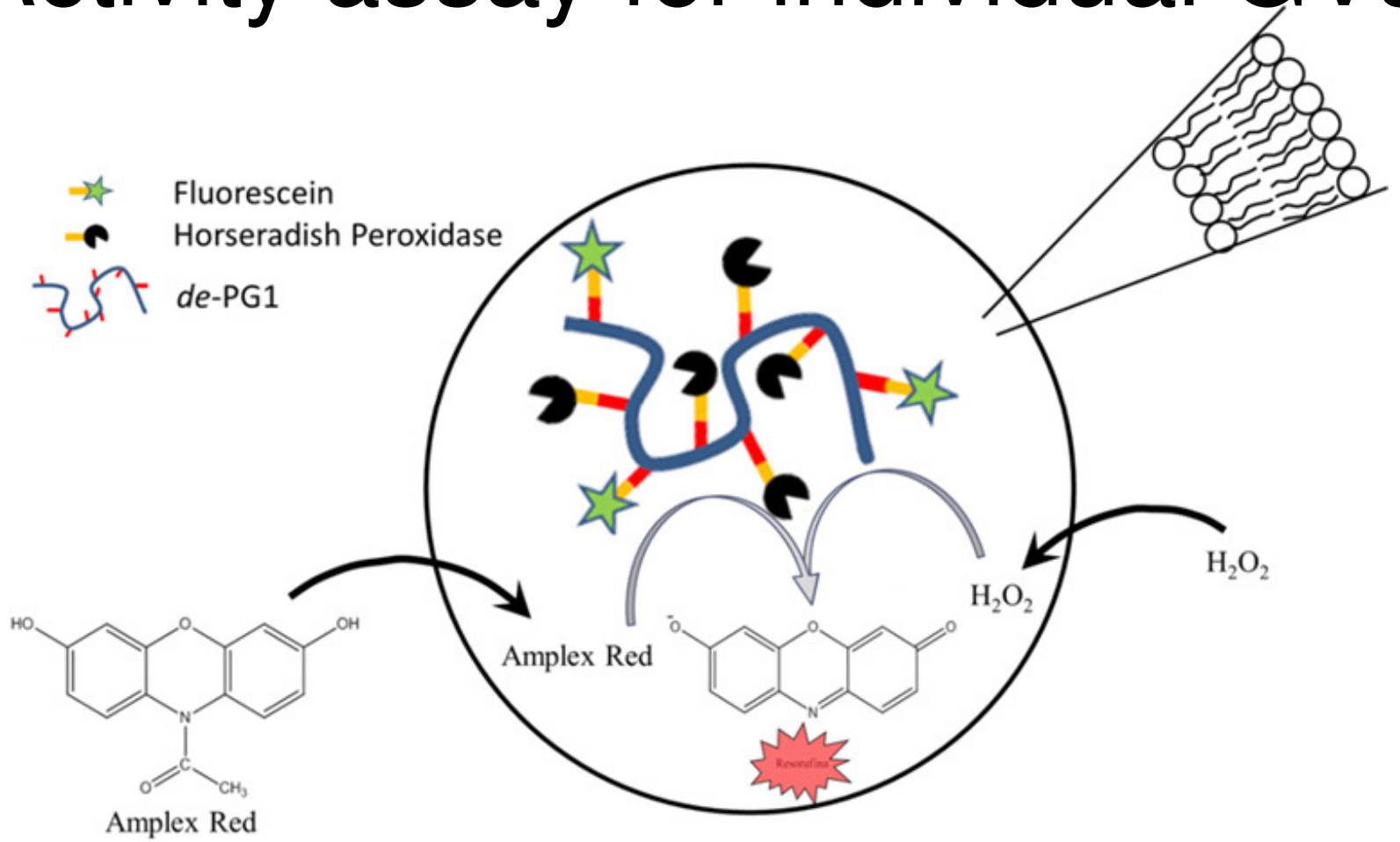


de-PG1-(BAH)-FL-HRP-SOD synthesis:



Grotzky A. et al. *JACS* 2012

Activity assay for individual GVs



Enzymatic system

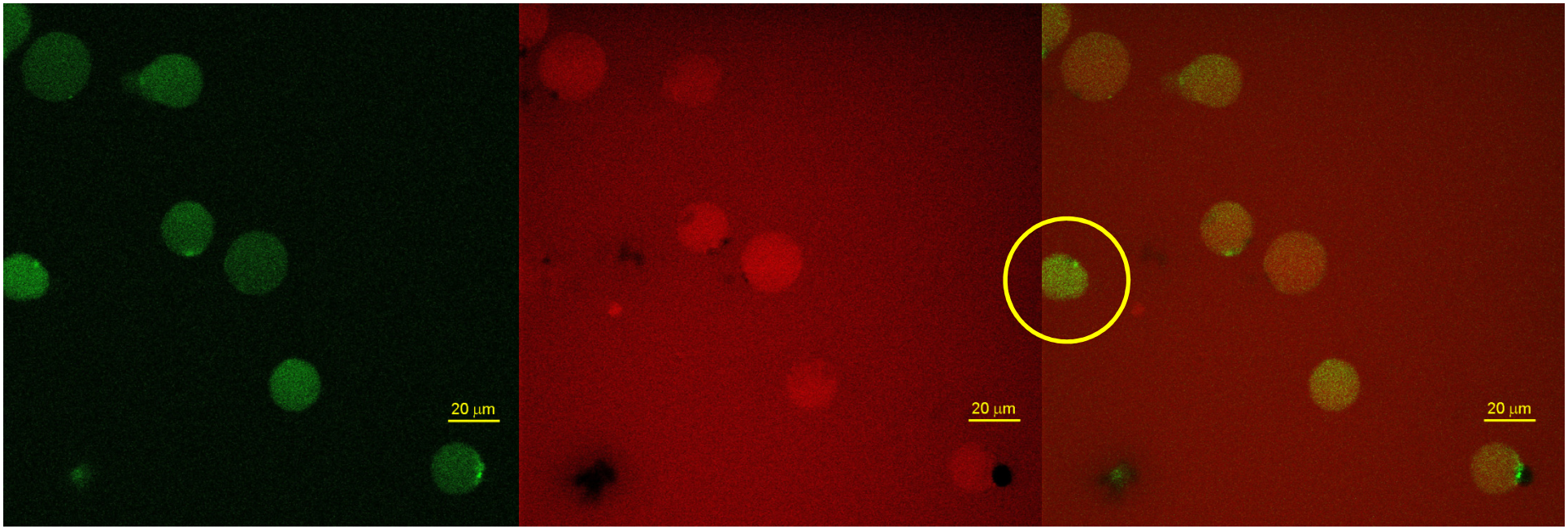
de-PG1-FI-HRP entrapment

- [HRP] = 1 μM,
- [Amplex Red] = 10 μM,
- [H_2O_2] = 10 μM

Langmuir Article pubs.acs.org/Langmuir

Structure and Enzymatic Properties of Molecular Dendronized Polymer–Enzyme Conjugates and Their Entrapment inside Giant Vesicles

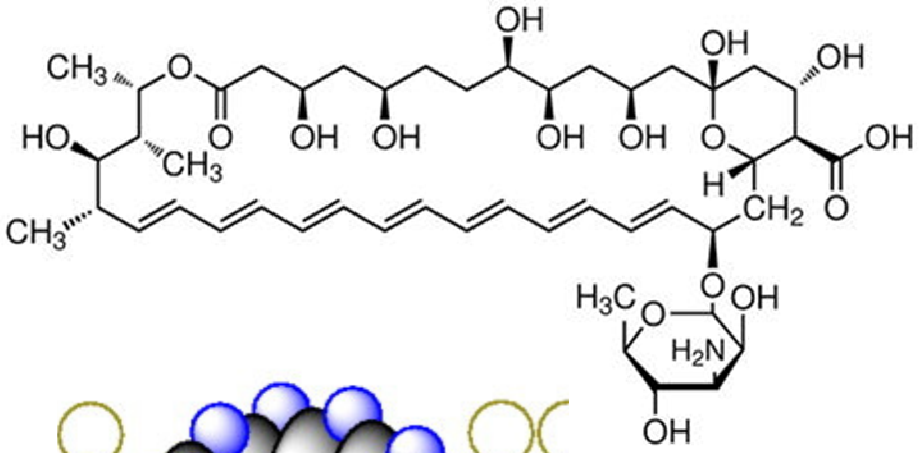
Andrea Grotzky,[†] Emiliano Altamura,[†,‡] Jozef Adamcik,[§] Paolo Carrara,[∥] Pasquale Stano,[∥] Fabio Mavelli,[‡] Thomas Nauser,[⊥] Raffaele Mezzenga,[§] A. Dieter Schlüter,[†] and Peter Walde*[,†]

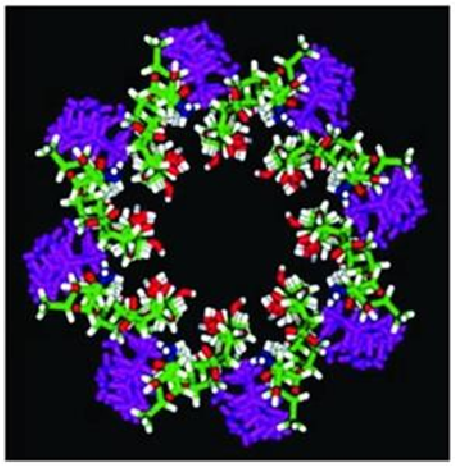


t = 200 s

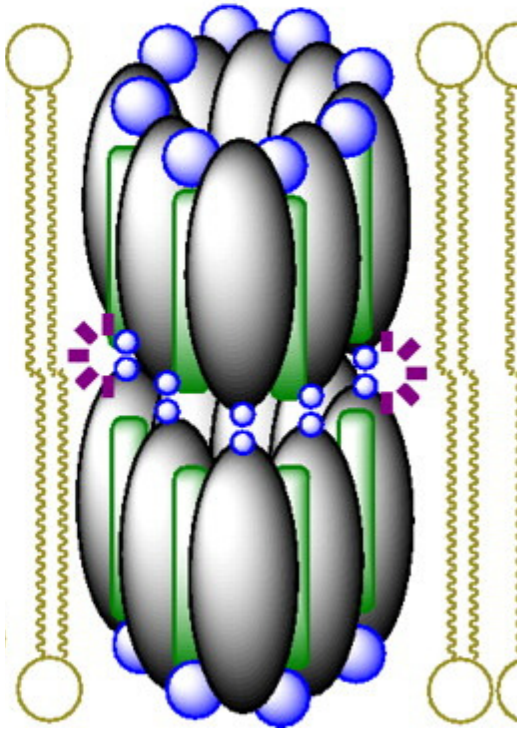
Membrane engineering

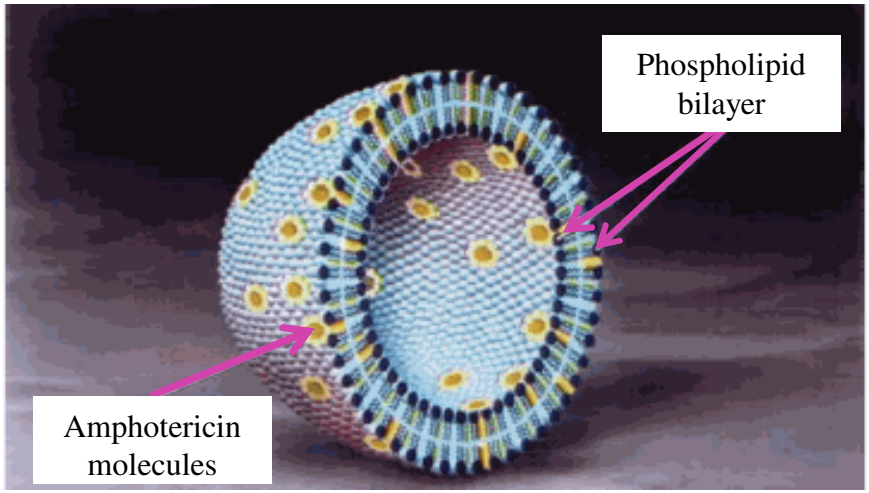
Pore formation: Amphotericin B



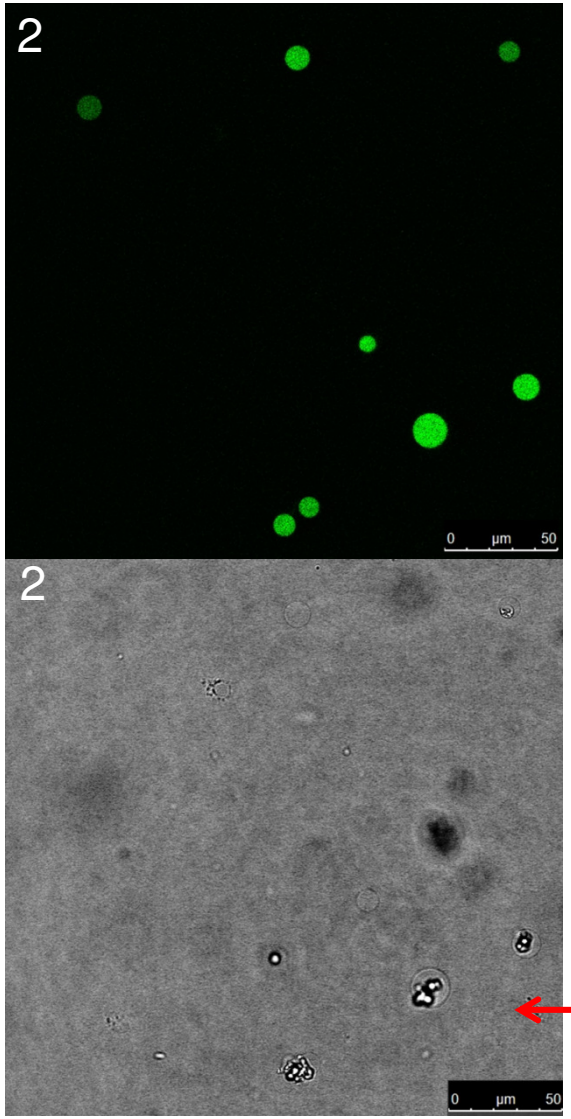


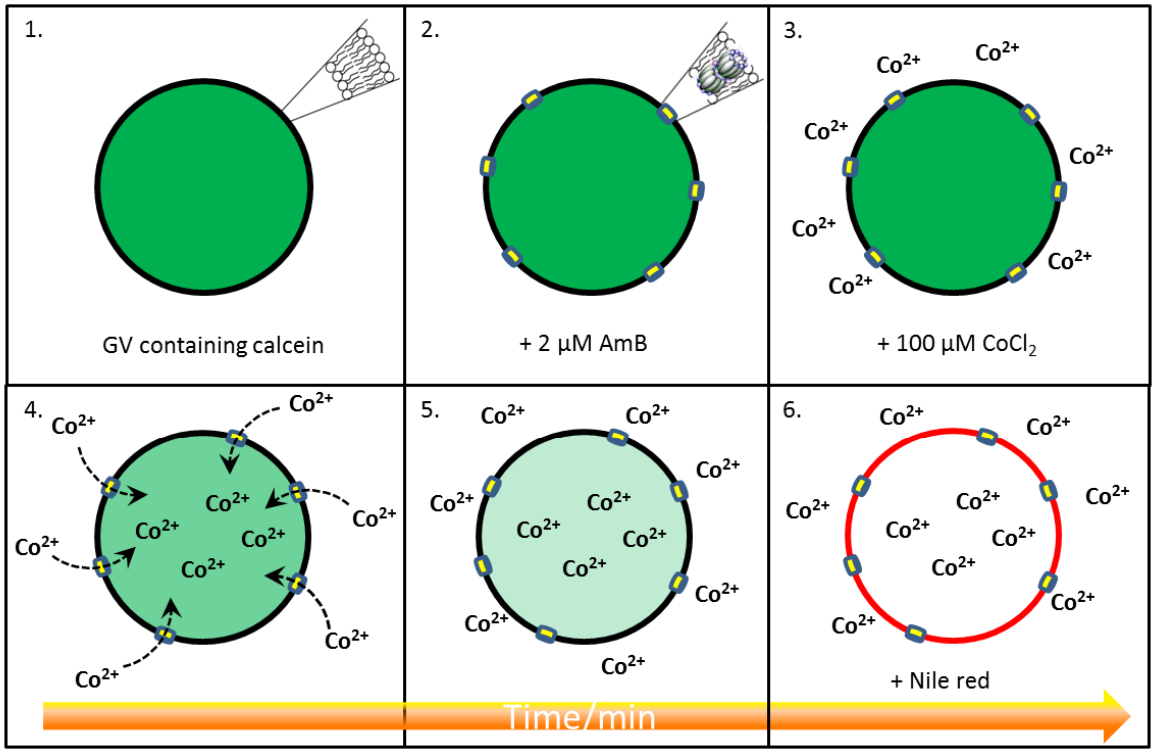
Among the variety of antifungal antibiotics Amphotericin B (AmB) is an amphiphilic molecule that forms pores in phospholipid membranes (Khutorsky 1996), allowing the diffusion of glucose, as experimentally demonstrated for nano-sized liposomes (Fujita *et al*. 2013).





The assay for the pore formation

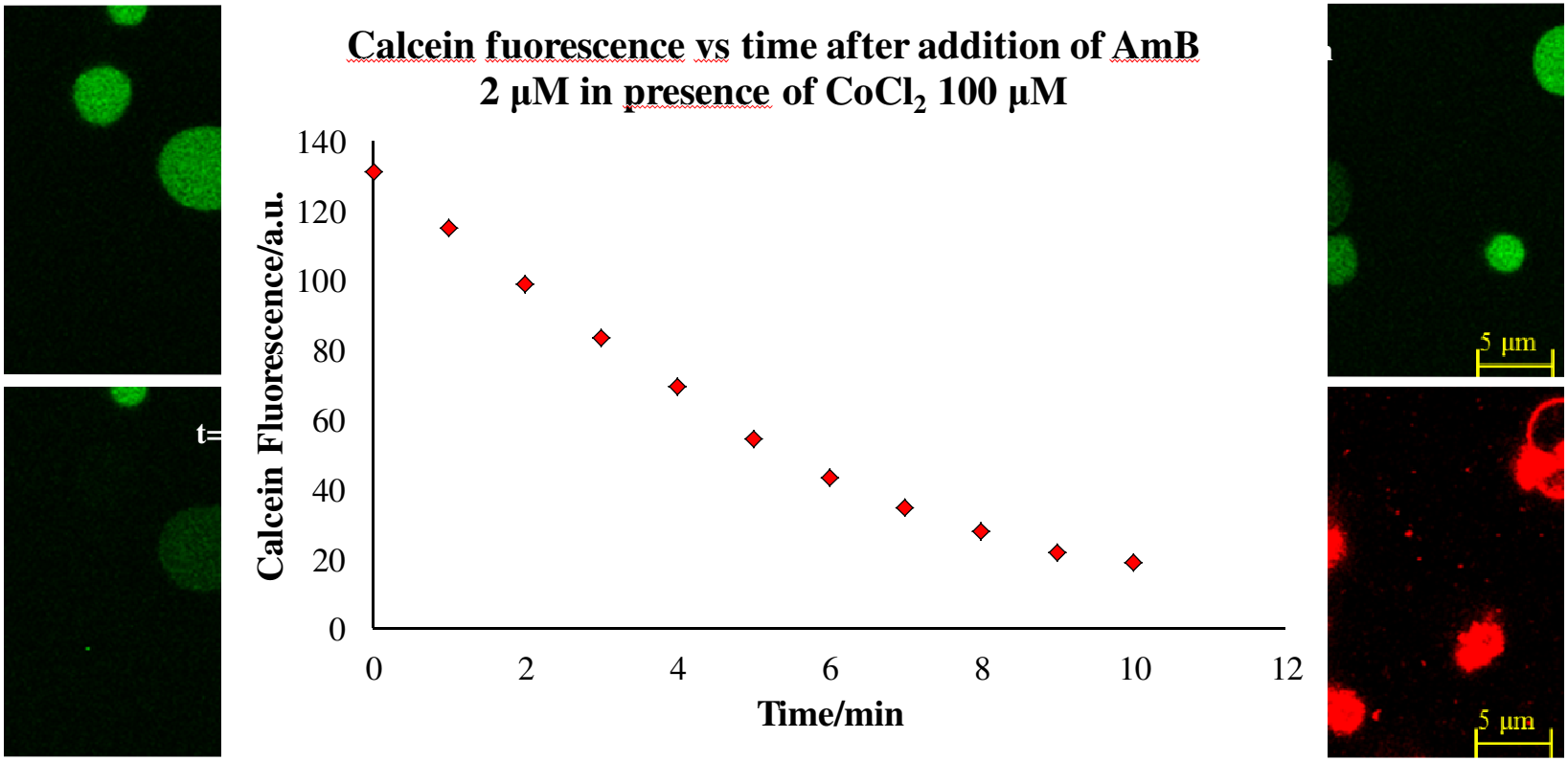




GV containing calcein and AmB pore formation, followed by internal calcein fluorescence extinction after addition of a Co^{2+} ions to the vesicle.

GVs encapsulating 10 µM calcein after the addition of AmB 2 µM

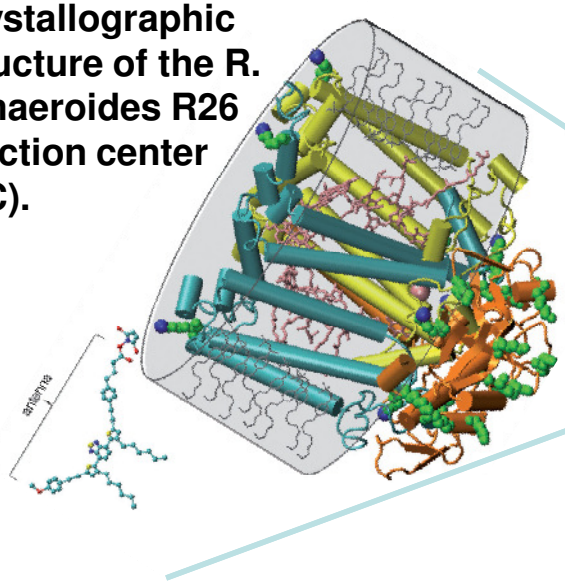
The assay for the pore formation



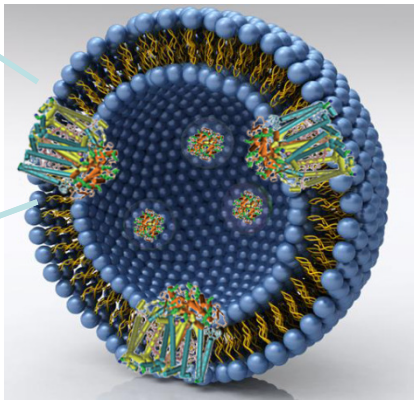
GVs containing Calcein 10 µM. In the external buffer $CoCl_2$ 100 µM is added as quencher.
Time scan recorded after the addition of Amphotericin B 2 µM, that is able to create pores through the membrane with an high dimensional selectivity.
The graph indicates the decrease of calcein green fluorescence during time due to the quenching effect of the Co^{2+} ions.

Photosynthetic Reaction Center in GVs

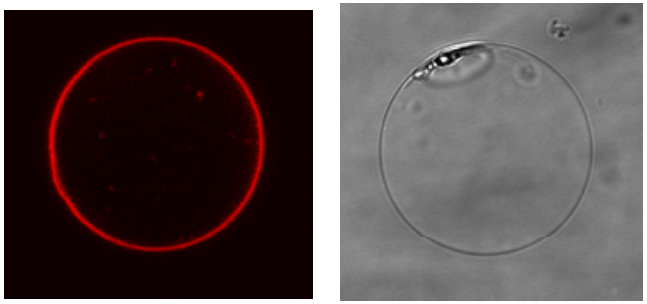
Crystallographic structure of the R. sphaeroides R26 reaction center (RC).



Reconstitution of the photosynthetic RC within the GV membrane

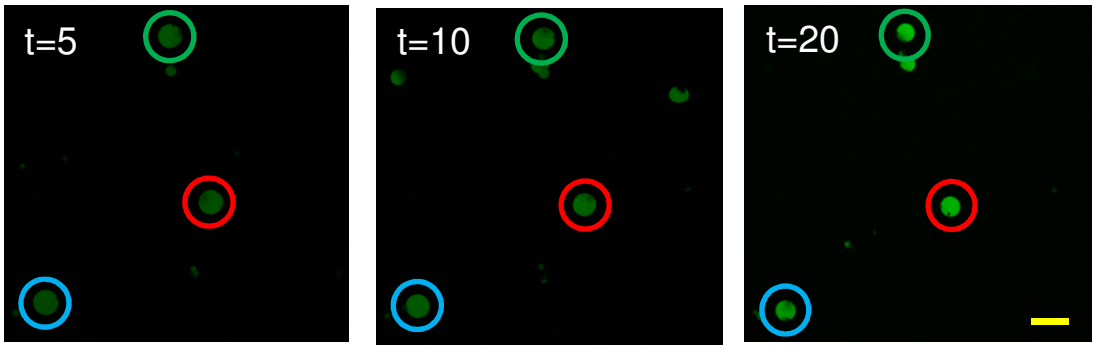


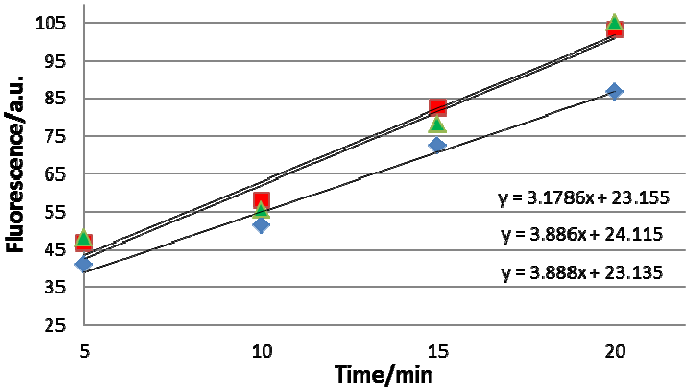
Confocal microscope images of GV made by POPC with RC reconstituted in membrane.



Manuscript in preparation

pH increase of the vesicle internal solution due to the activity of RCs monitored by the fluoresce of an entrapped dye (pyranine)

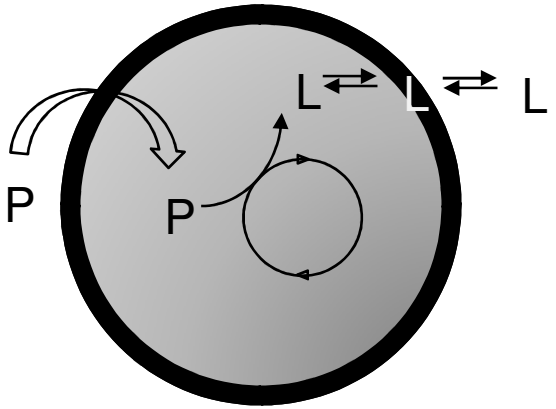


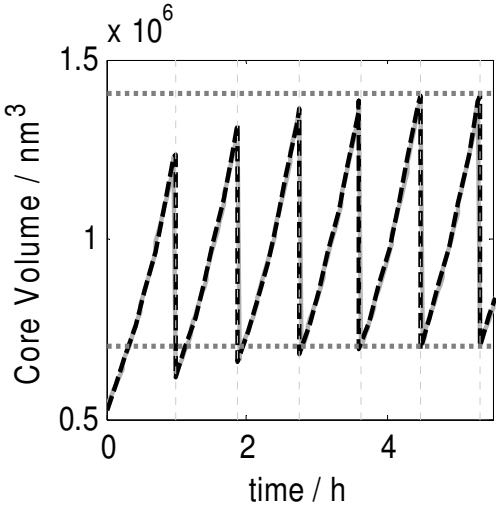


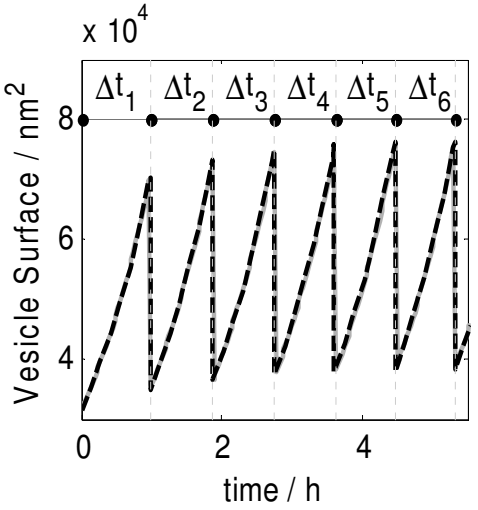
In silico modeling

Protocells stationary reproduction

We would like to determine the conditions that drive minimal self-producing vesicles to regular growth/division cycles, making possible that subsequent generations inherit size and internal chemical composition.







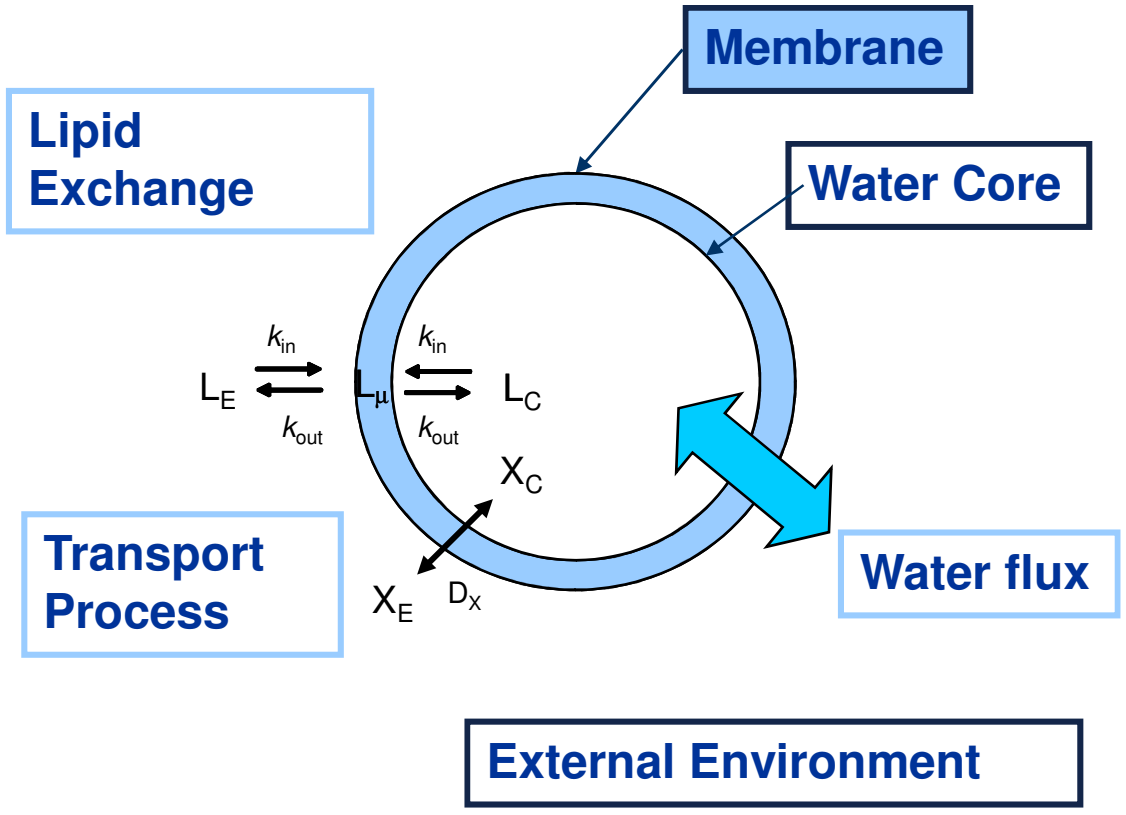
In silico Vesicles

Vesicles are described as compartmentalized reacting systems (CSTR) made of two different homogeneous domains:

- the membrane
- the water core

Lipids and molecules can be exchanged between the membrane and water core, between the membrane and the external environment.

Transport processes can also occur, exchanging molecules directly from the external environment to the internal water pool.



Mavelli F, Ruiz-Mirazo K, (2007) Phil. Trans. Royal Soc. London B 362, 1789.
Mavelli F, Ruiz-Mirazo K, (2010) Phys Biol 7, 036002

Stability of closed membrane

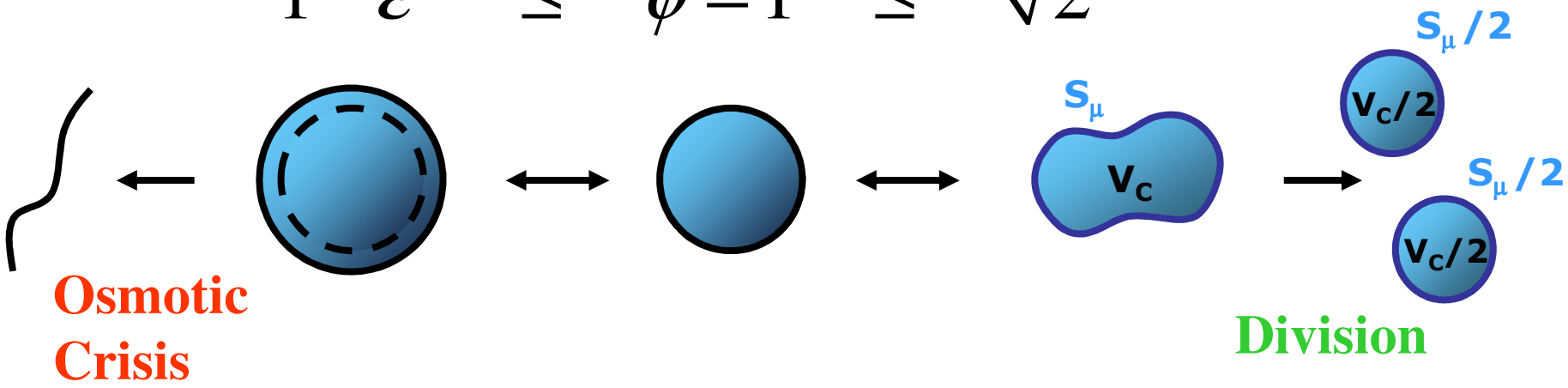
Reduced Surface

$\phi = S_\mu / \sqrt[3]{36\pi V_{core}^2}$ ratio of the actual membrane surface S_μ and the area of a sphere with the actual volume V_c of the core

Vesicle

swollen spherical deflated

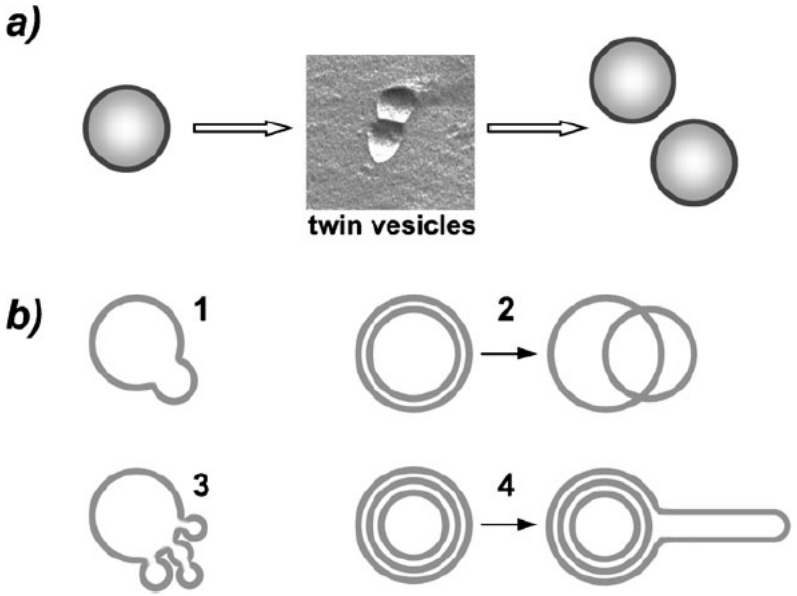
$$1-\varepsilon \leq \phi = 1 \leq \sqrt[3]{2}$$



Vesicle division

Small unilammellar vesicles

Giant uni-lammellar vesicles



Giant multi-lammellar vesicles

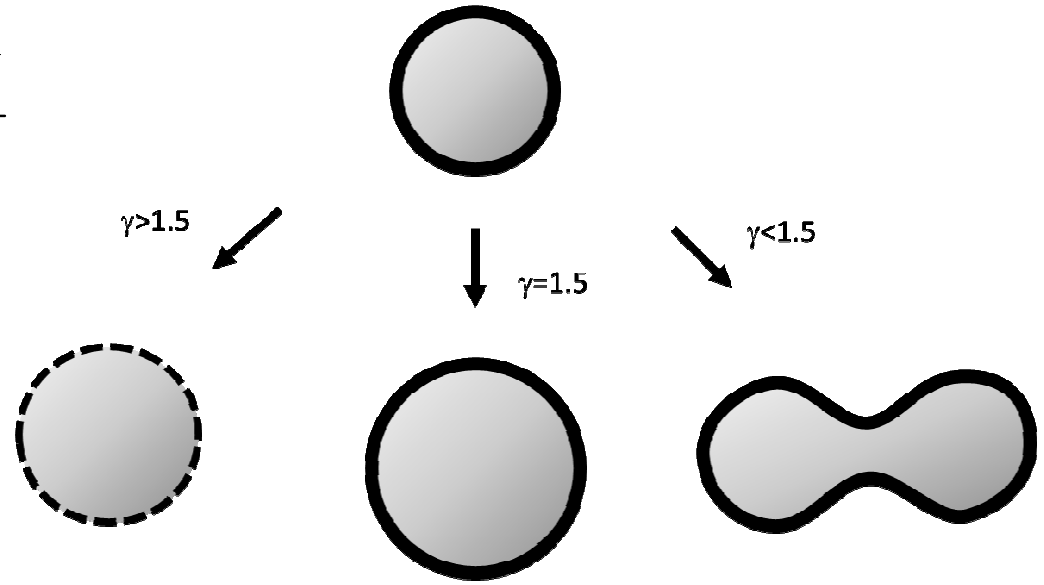
Fig. 5 Freeze–fracture electron microscopy of oleate vesicles (taken after 40 s from the addition of oleate micelles) reveals that "twin vesicles" can be the actual intermediate of the self-reproduction. Twin vesicles are not present at the end of the reaction. Adapted from Stano *et al.*[29h] Several intermediates have been observed in the case of the transformations occurring to oleate giant vesicles after the addition of oleate micelles: (1) budding mechanism;[33a,b] (2) translocation;[33a,b] (3) evagination;[36b] (4) tubular growth and division.[36a]

Stano P, *ChemComm* (2010) 46 3639

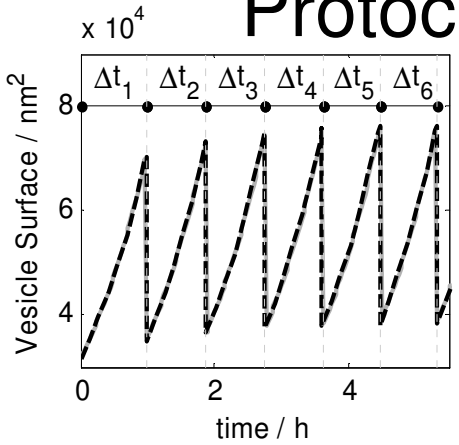
Growth control coefficient

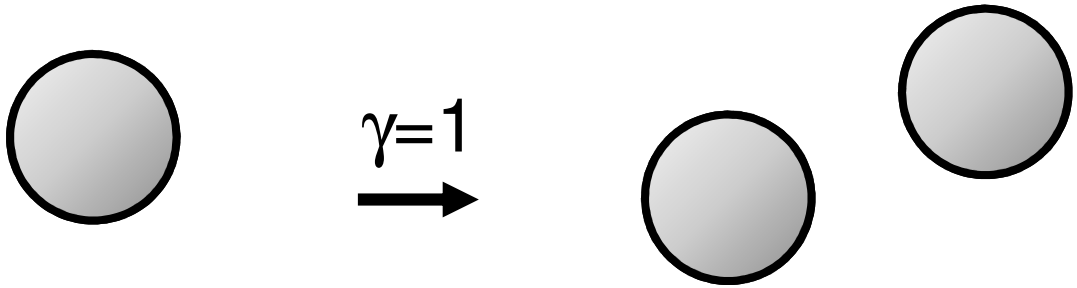
$$\gamma = \left(\frac{1}{V_g}\frac{dV}{dt}\right) \Big/ \left(\frac{1}{S_g}\frac{dS}{dt}\right) = \frac{S_g}{V_g}\frac{dV}{dS}$$

γ is a dimensionless observable is defined as the ratio between the relative velocities of variation of core volume and membrane surface of a vesicle



Protocells stationary reproduction





Mavelli F, Ruiz-Mirazo K , (2013) Integr. Biol., 5, 324-341

Osmotic synchronization

$$\gamma = 1$$

$$\sum_{\rho}^{\text{Reactions}} \Delta m_\rho r_\rho + \frac{S}{V} \sum_{i}^{\text{Species}} \wp_i \left([X_i]_{Env} - [X_i] \right) - v_L = \frac{C^T \alpha_L}{2} \frac{N_A V}{S} v_L$$

$$v_R = \sum_{\rho}^{\text{Metabolic Reactions}} \Delta m_\rho r_\rho$$

different metabolic steps occurring inside the protocell, each at a particular rate r_ρ, with a net number of molecules produced or consumed Δm_ρ

$$v_{Tp} = \frac{S}{V} \sum_{i}^{\text{Species}} \wp_i \left([X_i]_{Env} - [X_i] \right)$$

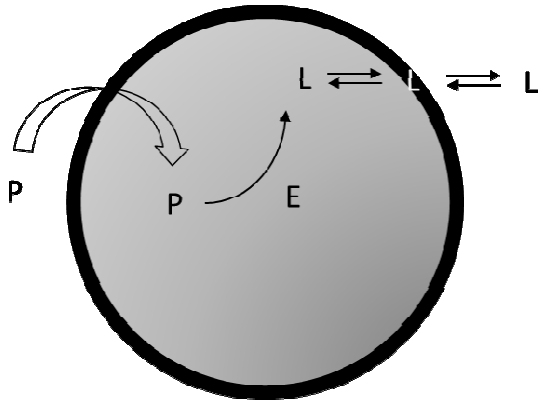
net fluxes of molecules that can come in or escape across the membrane through passive transport

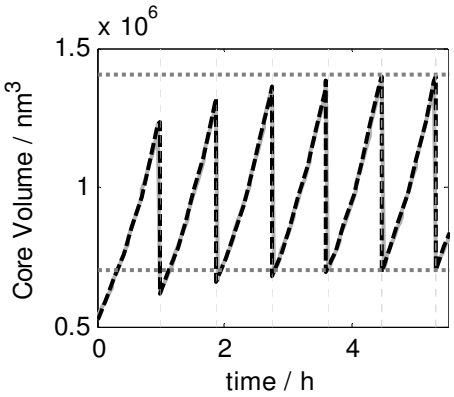
v_L

metabolic lipid production rate

Case Study

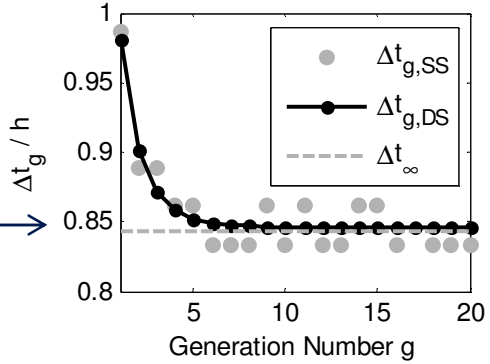
Self-producing enzymatic vesicle: a hypothetical model where the production of lipid L takes place through the chemical transformation of a precursor molecule P, assumed to occur only in the presence of an additional compound E,





$$R_\infty = \frac{6n}{\alpha_L N_A C^T}$$

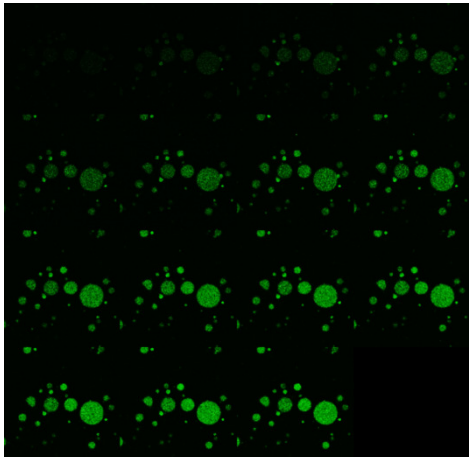
$$\Delta t_\infty = \frac{2}{\alpha_L [P]_{Ext}} \left(\frac{ln2}{N_A \wp_P} + \frac{4\pi R_\infty^2}{k} \right)$$

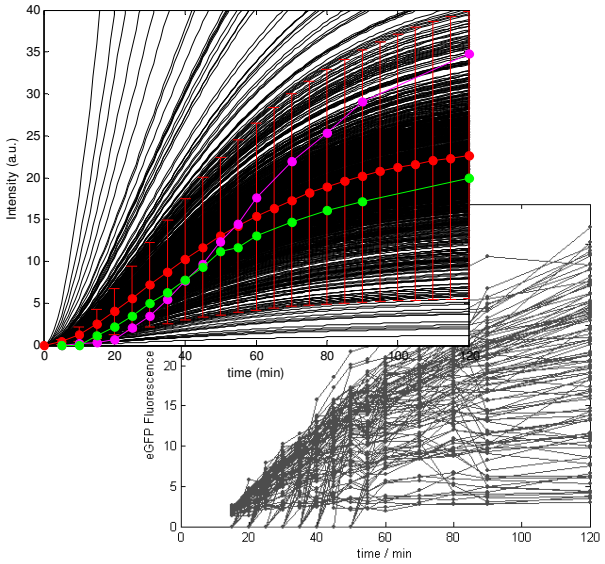


- **Stochastic simulation outcomes (in grey)**
- **the deterministic curve by numerical integration (in black).**

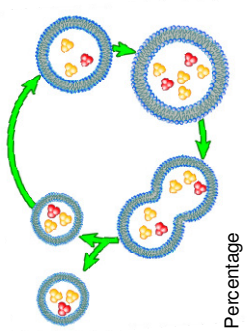
Theoretical modelling

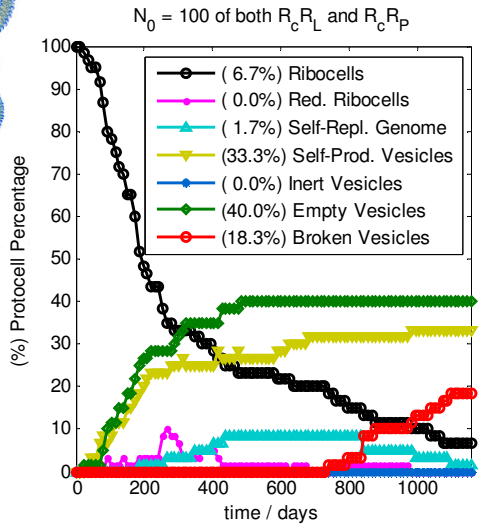
GFP Expression in a w/o emulsion

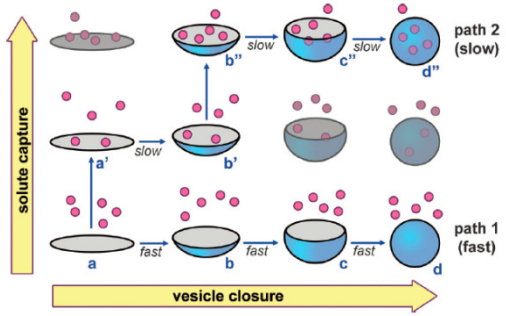




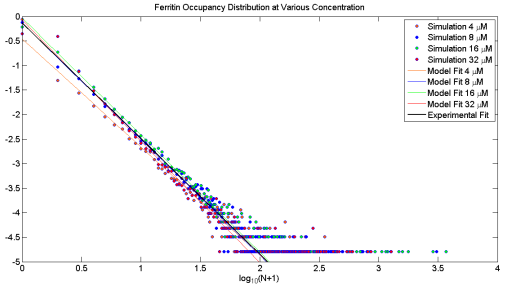
the Ribocell model



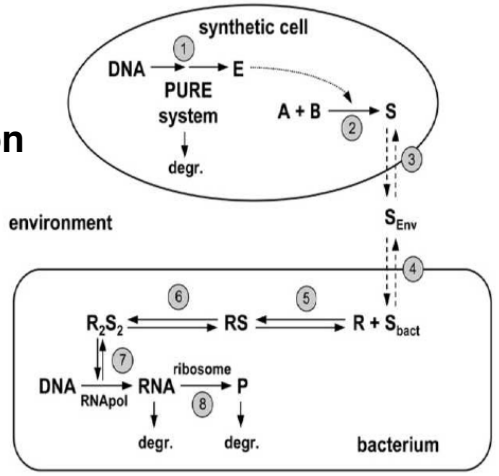




the Crowding effect



Chemical communication between artificial cells and bacteria



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Conclusions

In vitro and *in silico* minimal artificial cell models (protocells) have been present.

By the integration between numerical model and protocells implemented in the test tube it will be possible to design and synthetize a real minimal artificial cell in a no too far future.

Thank you for your attention

"All models are wrong,

but some are useful"

George Box

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