# Catalytic reactions in nano- micro-compartments: How to build a bio-synthetic lego?

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*Basel 2019*

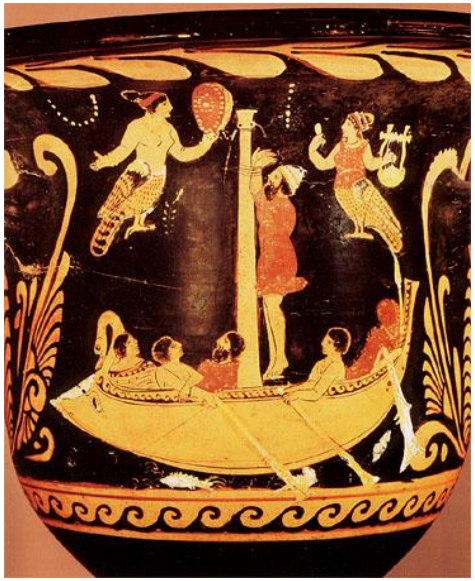
# Challenges for today's medicine

To produce very sensitive compounds for diagnostic (contrast agents, fluorescent dyes, magnetic probes)

To produce new biological active compounds/systems with an improved activity to decrease doses.

- To find global solutions for an increased population
- Optimum treatment conditions for each individual

# Hybrid systems?



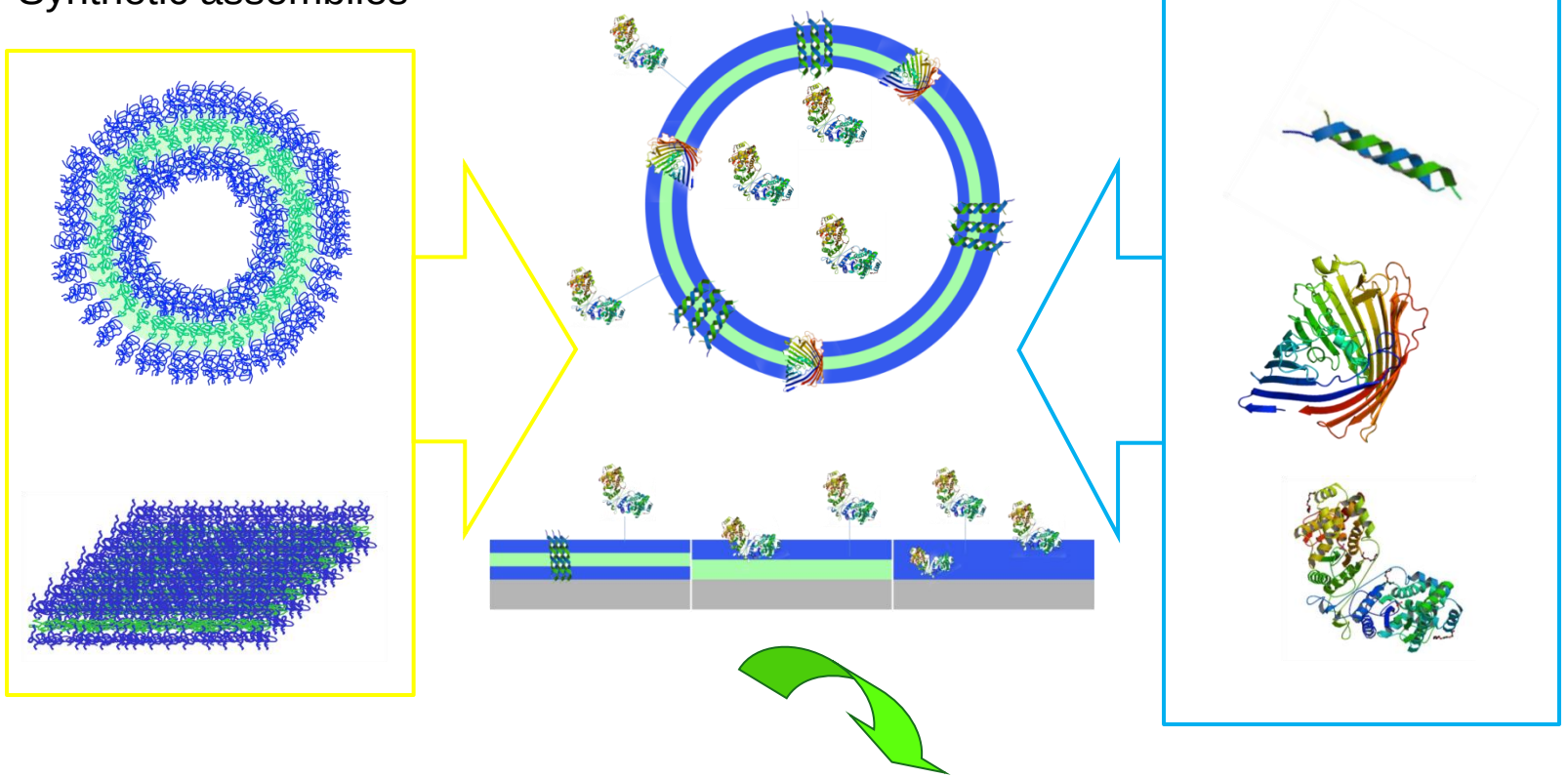
Art objects-
Greek period


Hybrid cars

# Hybrid systems

Synthetic assemblies

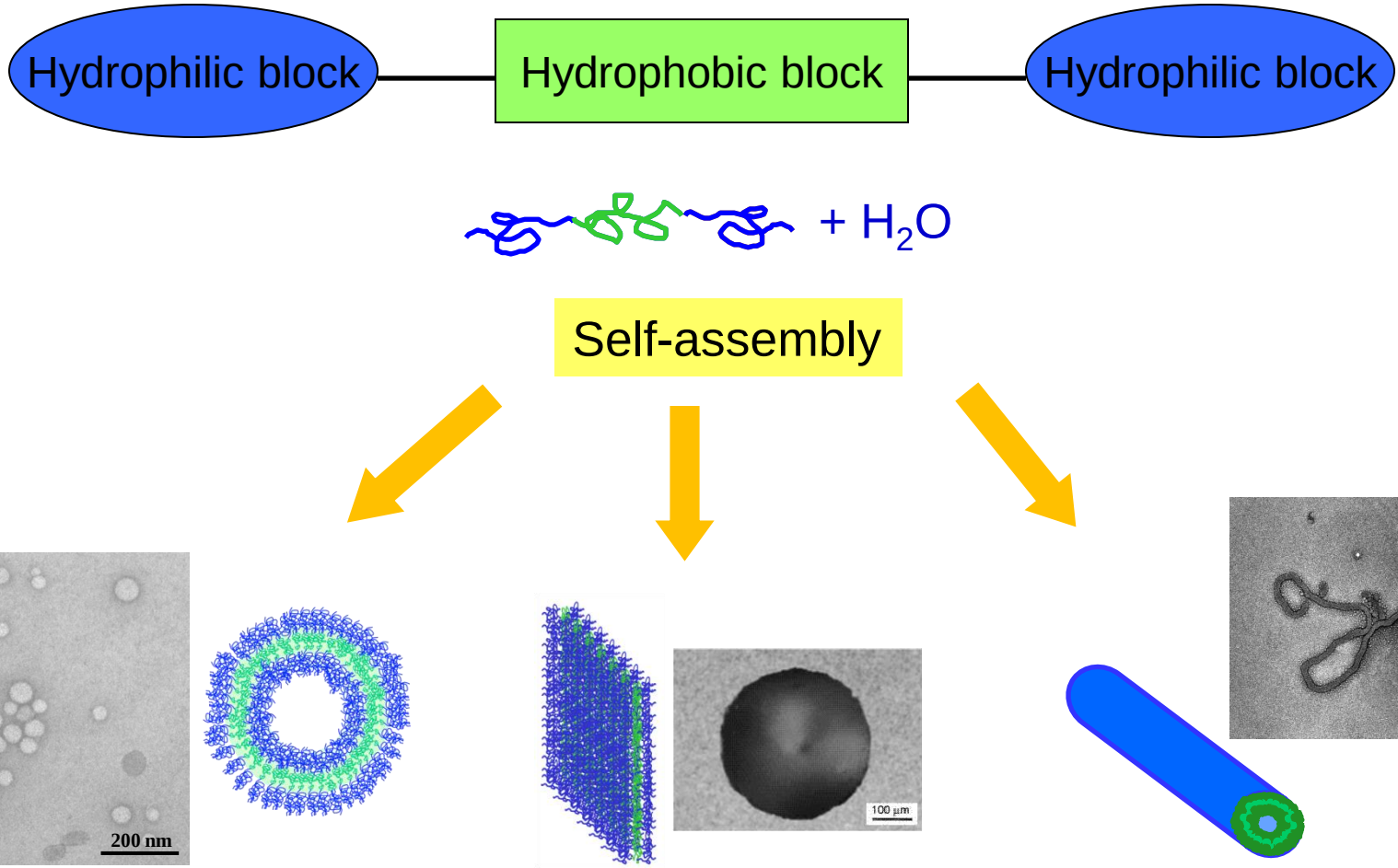
Biomolecules



➢ Catalytic compartments, active surfaces, mimics of cells, biosensors, etc

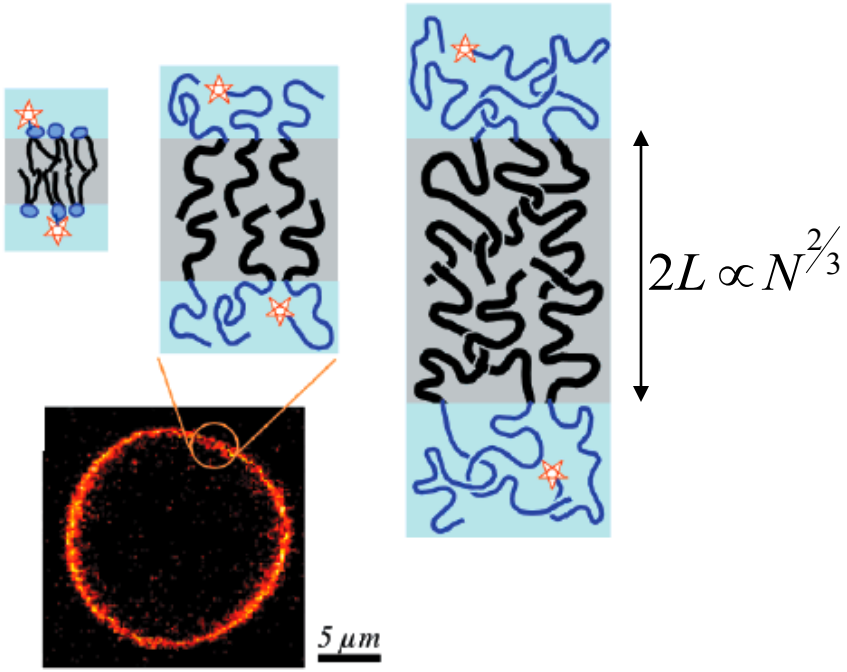
M. Garni, S. Thamboo, C. Schenenberger, C.G. Palivan, *BBA- Biomembranes*, 2017
S. Rigo, C. Chun, L. Maurizzi, X. Zhang, J. Xu, C.G. Palivan, *Advanced Science*, 2018

# Self-assembly of amphiphilic copolymers

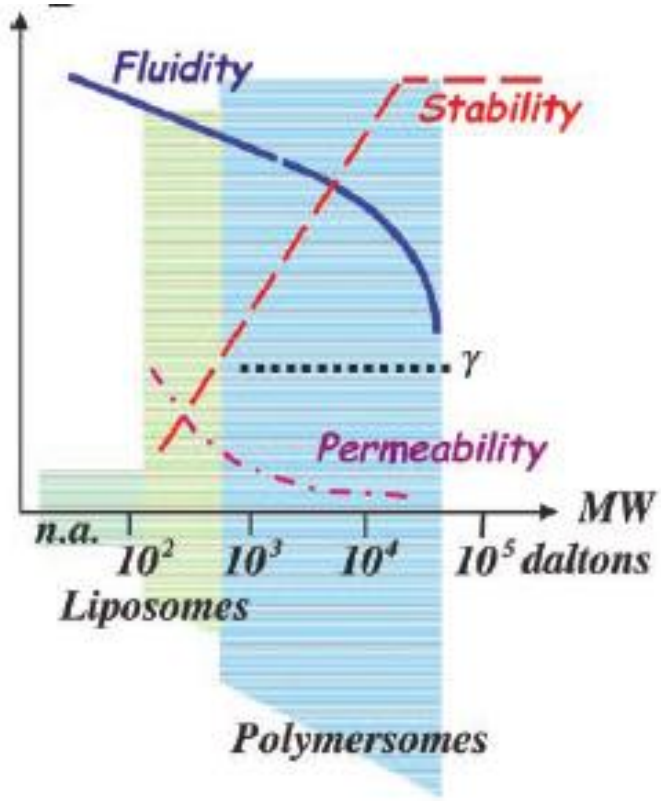


# Thickness vs properties of polymer membrane

Polymer vesicles (polymersomes):

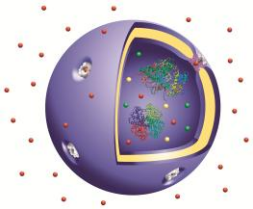



Discher D.E. & Eisenberg A. Science, 2002, 297, 967

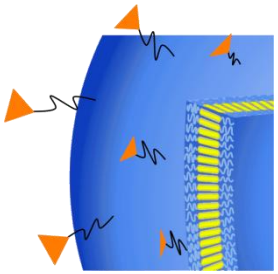



Lee J.C.M. et al., Macromolecules 2002, 35, 323

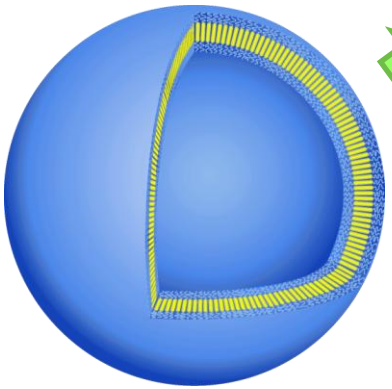
# Bio-Synthetic assemblies

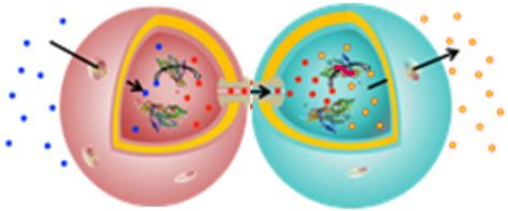


Nanoreactors, Artificial Organelles

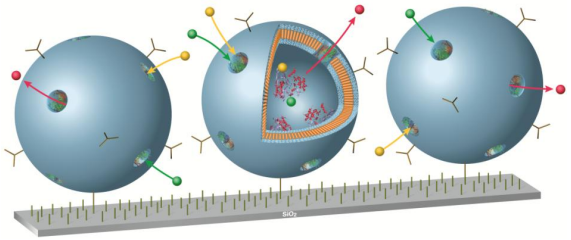


Functionalized carriers

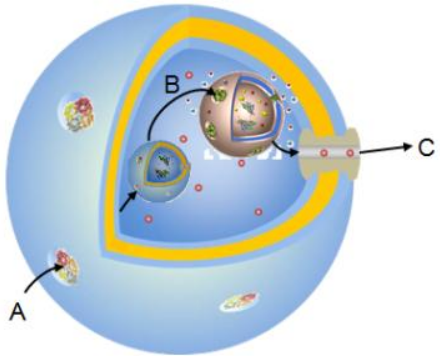




Interconnected Organelles



„Active“ surfaces

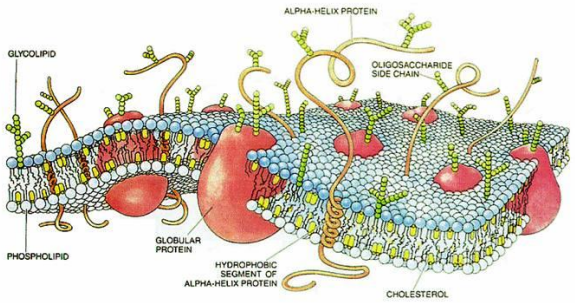



Compartments-in-compartment,
artificial cells

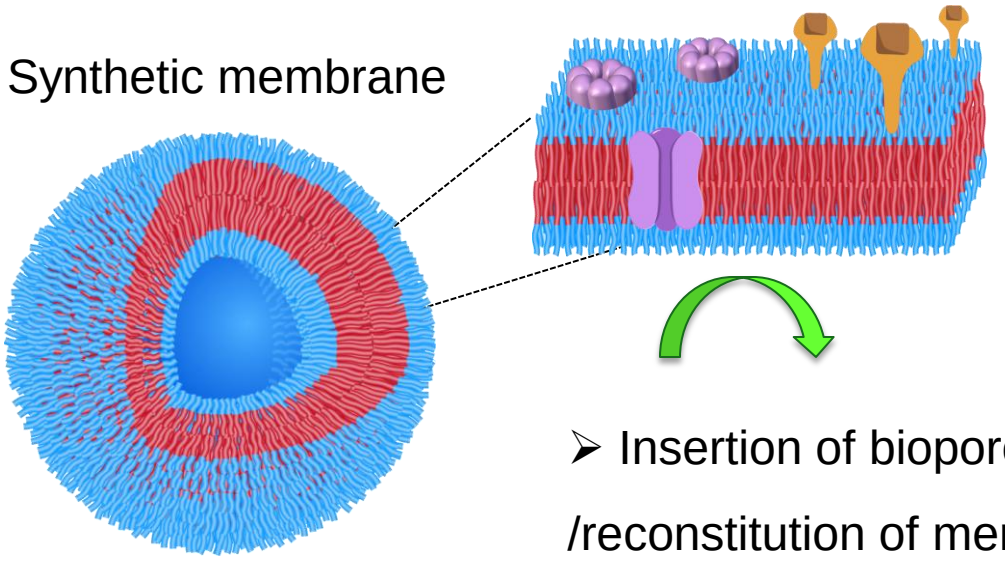
- Catalytic nanocompartments
- Catalytic nanocompartments in tandem
- Artificial organelles
- Artificial organelles wih triggered activity

# Polymer membranes: mimic of biomembranes

Cell membrane




Synthetic membrane



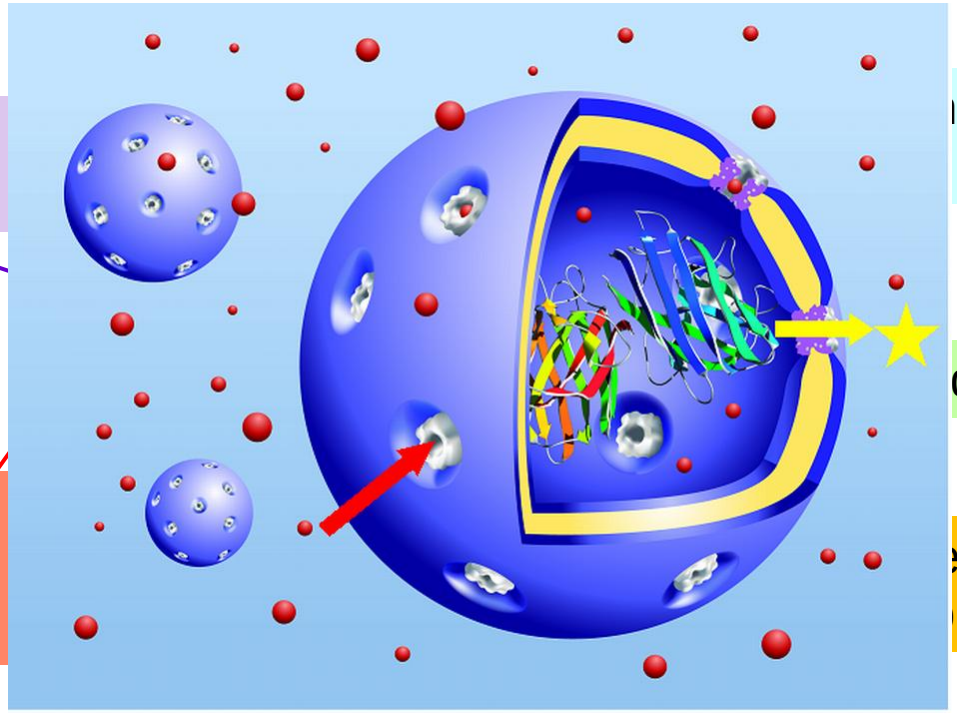
- Self-organisation
- Targeting
- Immobilisation on solid support

- Insertion of biopores /reconstitution of membrane proteins
- Generation of hybrid membranes

Palivan et al. *Chem. Soc. Rev. 2016.*

# Catalytic nanocompartments: Concept

Polymer compartment



ızymes,

ducts

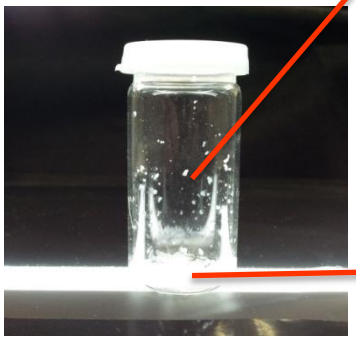
iopores

Harmful molecules, substrates ,

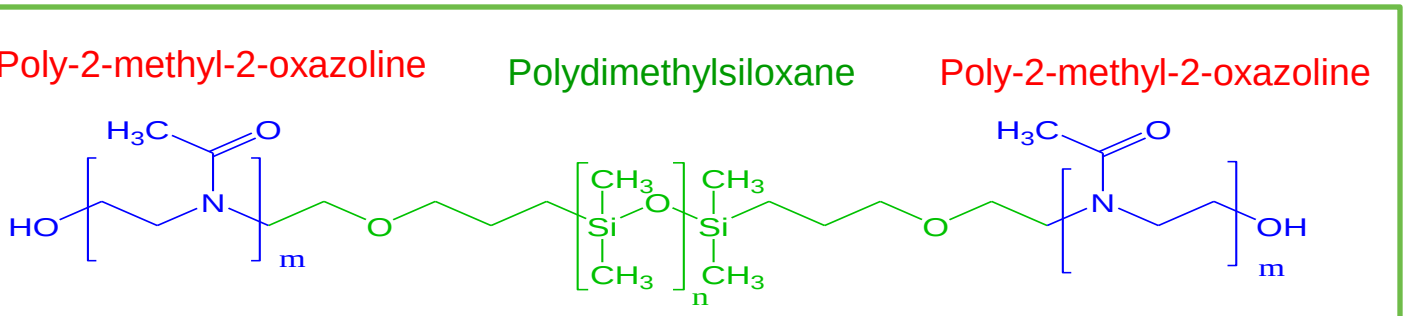
- ✓ full activity of encapsulated enzyme/mimics
- ✓ protection against hostile outside environment
- ✓ activation / deactivation on demand

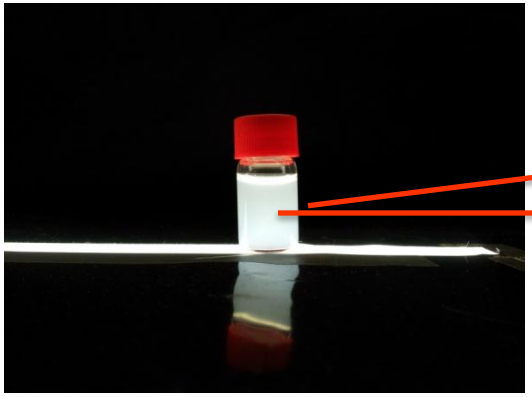

P. Tanner, S. Egli, V. Balasubramanian, O. Onaca, C. G. Palivan, W. Meier, FEBS Letter, **2011**
M. Lomora, G. Gunkel-Grabole, S. Mantri, C. G. Palivan, *Chem. Comm.*, **2017**
A. Belluati, I. Craciun, J. Liu, C.G. Palivan, *Biomacromolecules*, **2018**.

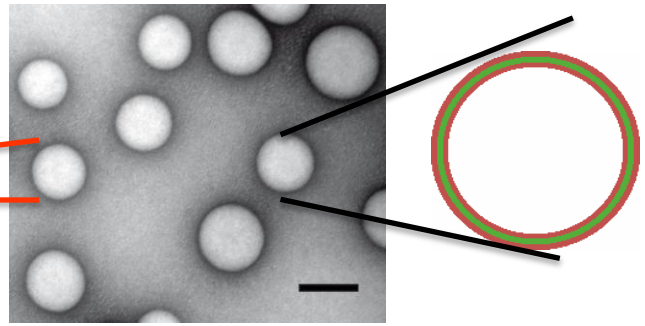
# Raw material: amphiphilic block copolymer



Poly-2-methyl-2-oxazoline Polydimethylsiloxane Poly-2-methyl-2-oxazoline



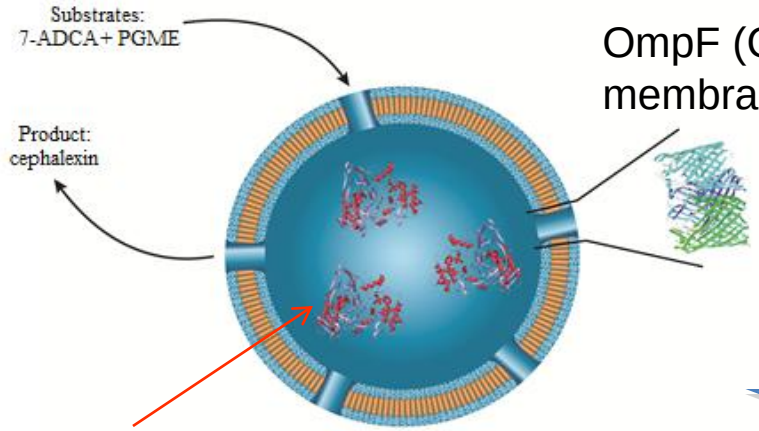


scale bar = 100 nm)

Hollow spheres: polymersomes/GUVs
( 50 nm – 1 µm)

O. Onaca, D.W. Hughes, V. Balasubramanian, M. Grzelakowski, W. Meier, C. G. Palivan**,** Macromol. Biosci, **2010**.

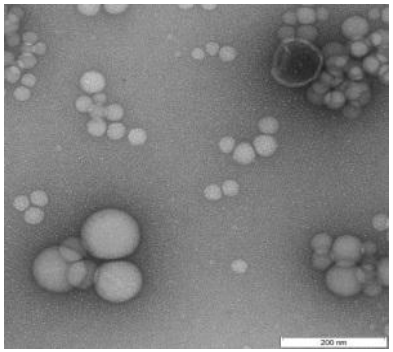
# Catalytic nanocompartments: antibiotics production



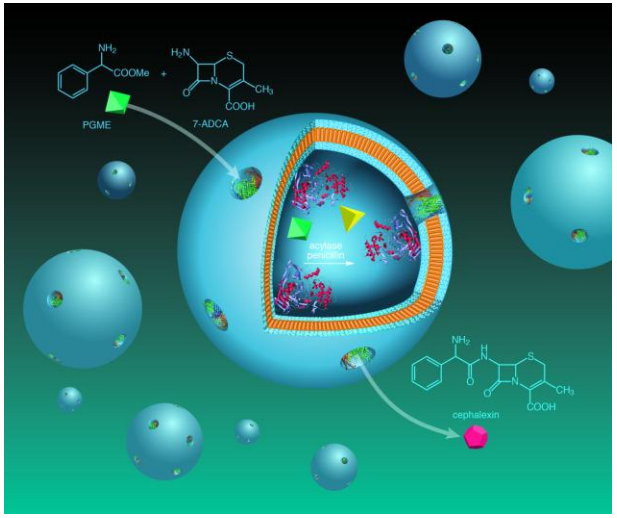

OmpF (Outer membrane protein F)

Penicillin acylase

TEM



scale bar 200 nm

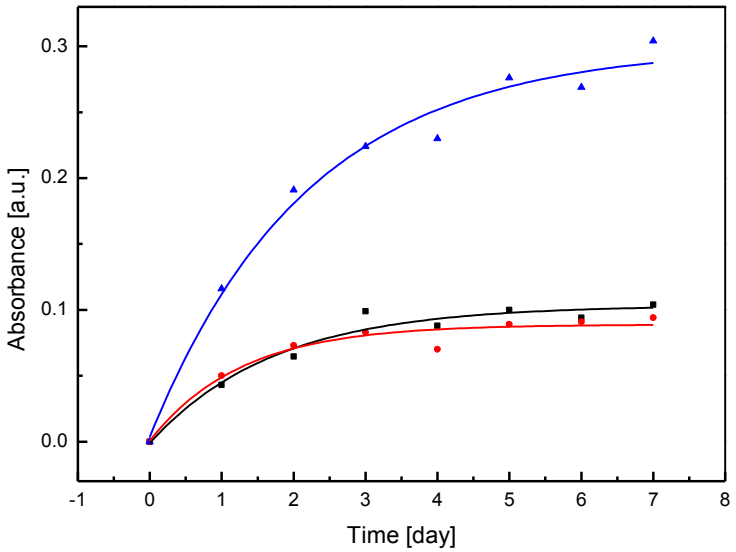



K. Langowska, C.G. Palivan, W. Meier, *Chem. Comm.*, 2013.
K. Langowska, J. Kowal, C. G. Palivan, W. Meier *J. Mater. Chem. B*, 2014.

# *In situ* production of antibiotics

Reaction progress curves for substrates solution with:

a. Denatured free enzyme solution (black),
b. Non - permeabilized catalytic nanocompartments (red)
c. Catalytic nanocompartments (blue).



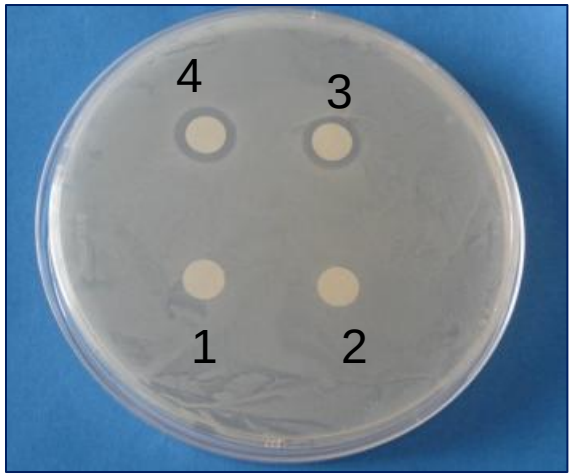

| Sample | Yield [%] | Encapsulation efficiency [%] | Cephalexin concentration after 7 days [mM] |
|---|---|---|---|
| Substrates (PGME + 7-ADCA) | 7.0 | - | 3.1 |
| Polymersomes without OmpF | 5.3 | 10.0 | 2.4 |
| Nanoreactors prepared at room temperature | 21.0 | 11.0 | 9.7 |
| Nanoreactors prepared at 7 C. | 25.5 | 9.4 | 11.8 |

✓ The progress of the cepahlexin formation reaction: continuous production during 7 days.

K. Langowska, C.G. Palivan, W. Meier, *Chem. Comm.*, **2013**.

# Antimicrobial activity:

Bacterial growth in the presence of nanoreactors adsorbed on a surface (MHB agar plate inoculated with bacteria).




1: Control (empty polymersomes)

2: Substrate solution

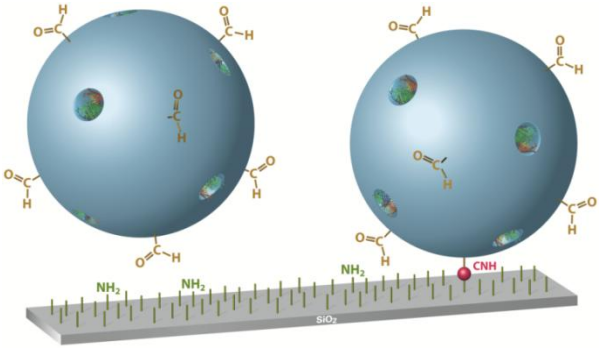
3: Catalytic nanocompartments prepared at RT; after incubation for 24 h at 37 °C.

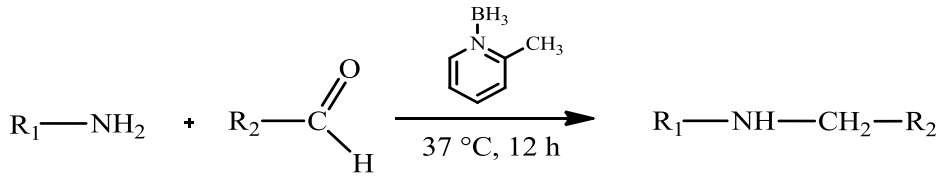
4: Catalytic nanocompartments prepared at 8 °C; after incubation for 24 h at 37 °C.

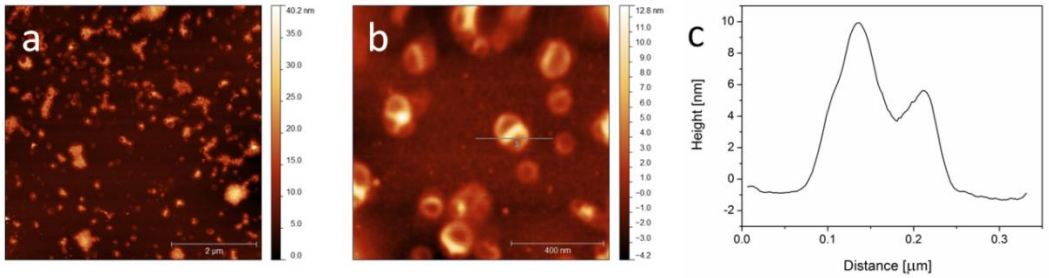
✓ Zones of inhibition were observed only around catalytic nanocompartments encapsulating enzymes.

K. Langowska, C.G. Palivan, W. Meier, *Chem. Comm.*, **2013**, 49, 128

# Active surfaces for antibiotics production



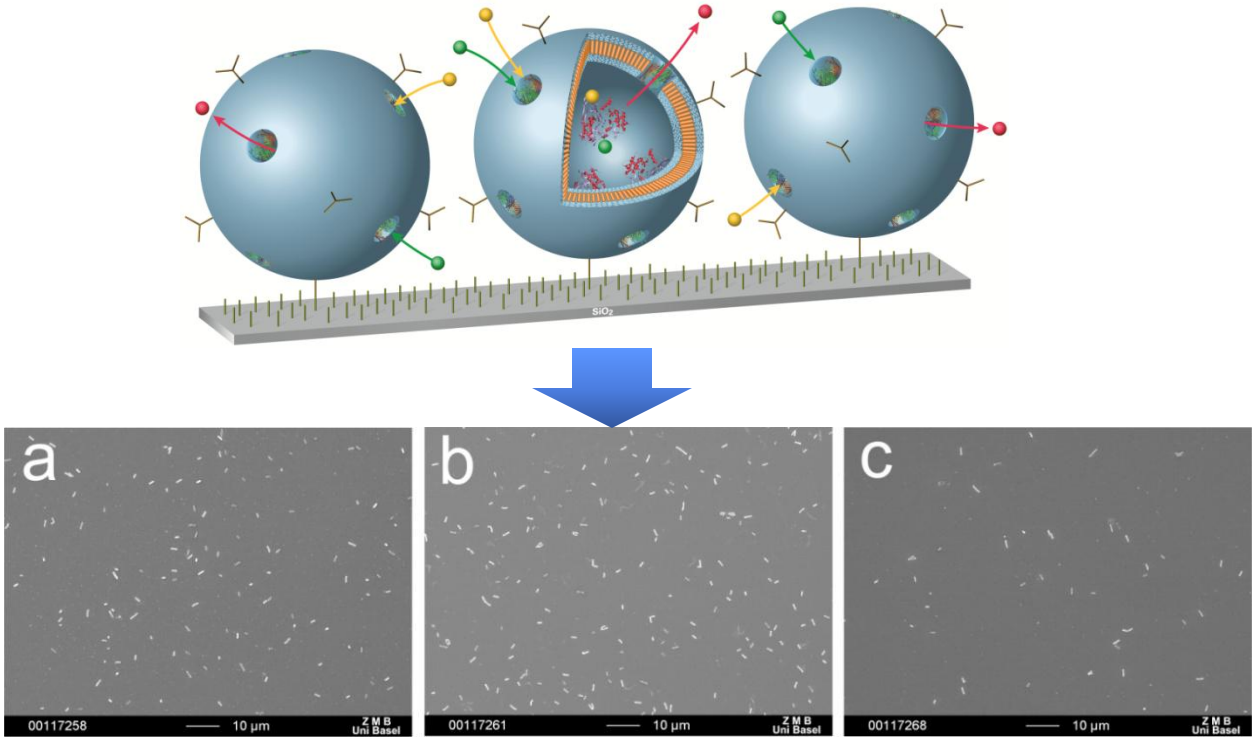




AFM height image of polymer catalytic compartments immobilized on an aminated silicon surface. Scale bar (a) 2 µm; scale bar (b) 400 nm.; (c) cross section profiles of (b).

✓ Successful immobilisation of nanoreactors on solid support (morphology preserved).

# Antimicrobial activity of active surfaces



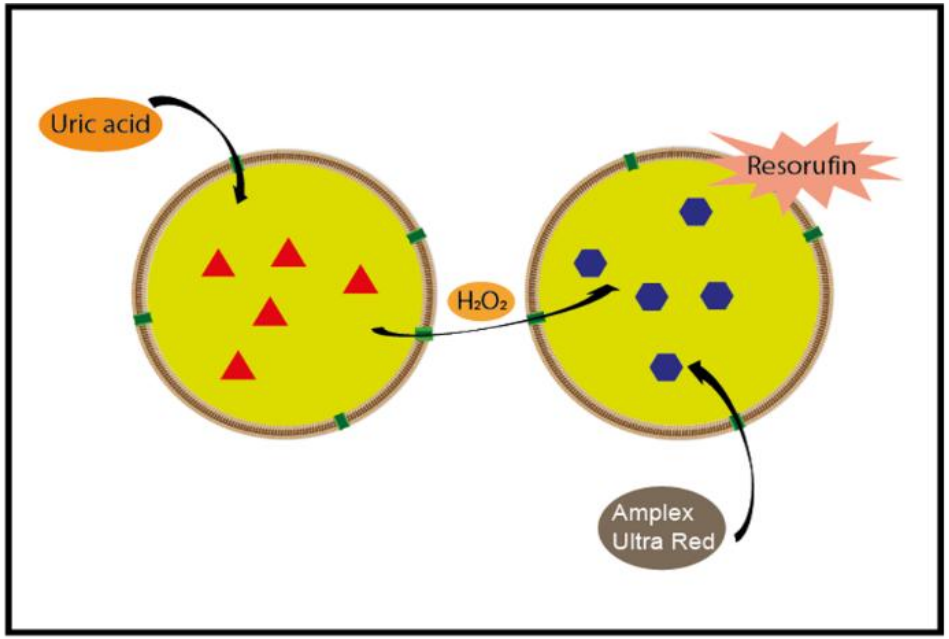

SEM images of *E. coli* attached to: a) silanized silicon surface; b) surface with immobilized non-permeable polymersomes encapsulating enzyme; c) surface with immobilized catalytic compartments. Scale bar: 10 µm.

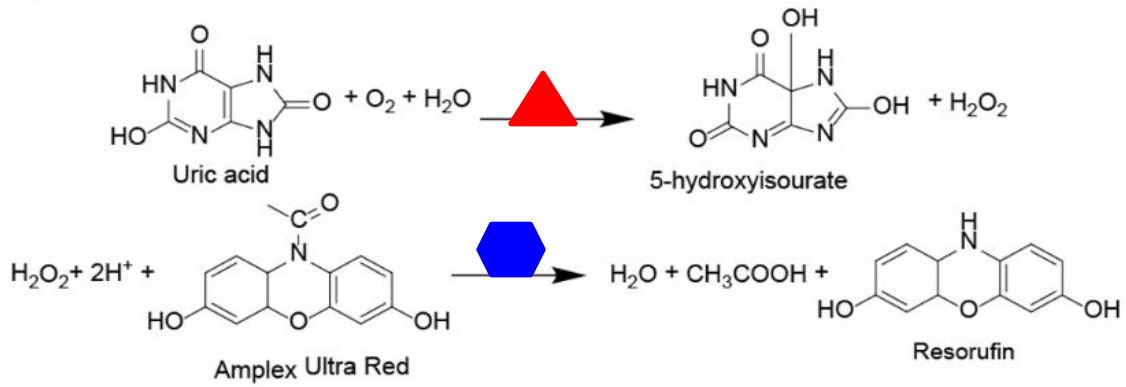
✓ Active surfaces efficiency: Inhibition of bacterial growth up to 7 days.

K. Langowska, J. Kowal, C.G. Palivan, W. Meier, J. Chem. Mat. B., **2014**
S. Rigo, C. Chun, L. Maurizzi, X. Zhang, J. Xu, C.G. Palivan, *Advanced Science*, **2018**.
S. Rigo, G. Gunkel-Grabole, W. Meier, C. G. Palivan, *Langmuir*, **2018**.

- Catalytic nanocompartments
- Catalytic nanocompartments in tandem
- Artificial organelles
- Artificial organelles wih triggered activity

# Catalytic nanocompartments in tandem



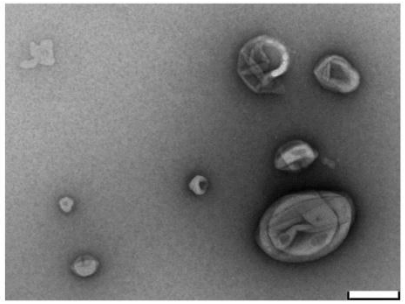

A. Belluati, I. Craciun, J. Liu, C.G. Palivan, *Biomacromolecules*, 2018.

# Catalytic nanocompartments: UOX & HRP

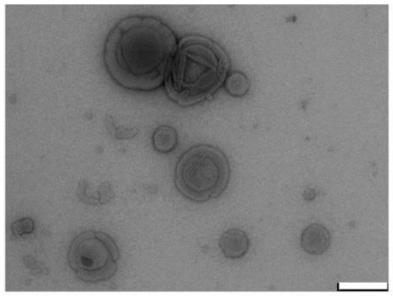
Uricase (UOX)

Horseradish peroxidase (HRP)

TEM

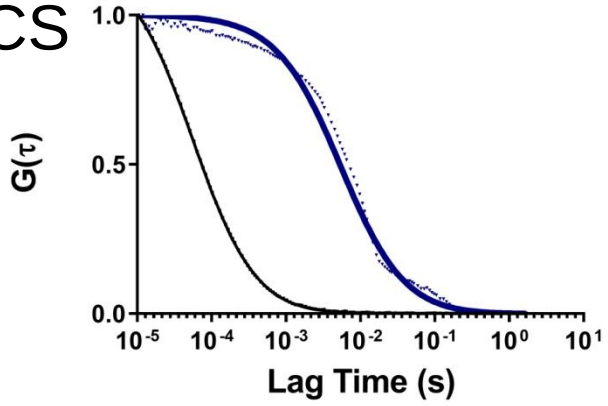


TEM micrograph of UOX-nanocompartments (scale bar: 200 nm)

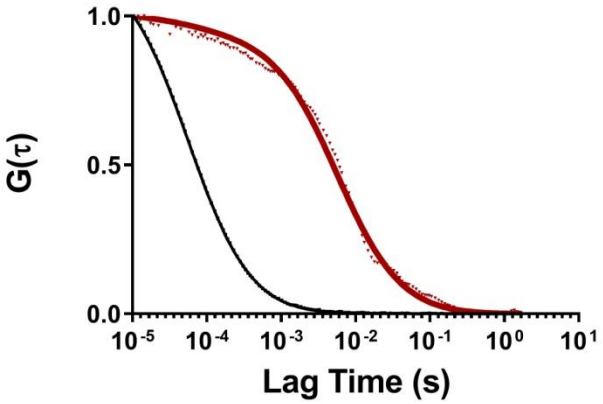


TEM micrograph of HRP-nanocompartments (scale bar: 200 nm)

FCS

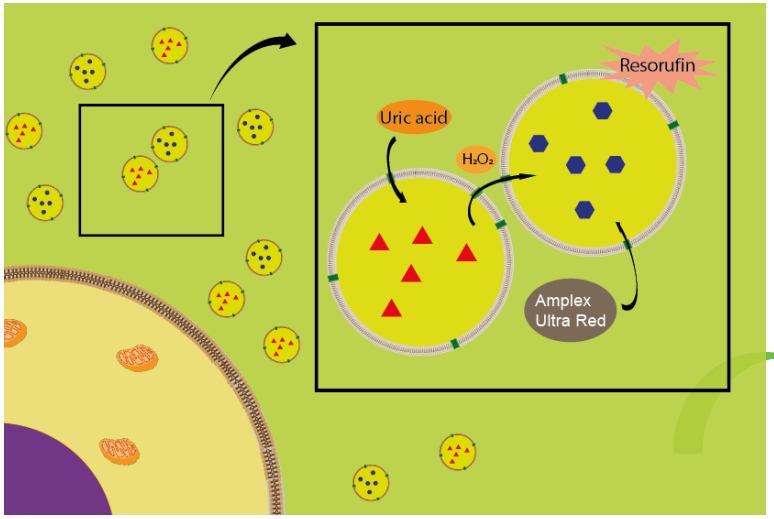


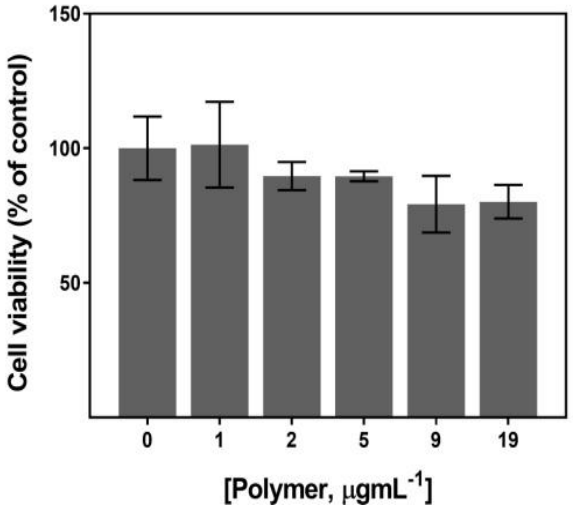

Normalized FCS autocorrelation curve of the dye labeled UOX-loaded nanocompartments and of the free dye.




Normalized FCS autocorrelation curve of the dye labeled HRP-loaded nanocompartments and of the free dye

# Efficiency of the cascade reaction *in vitro*

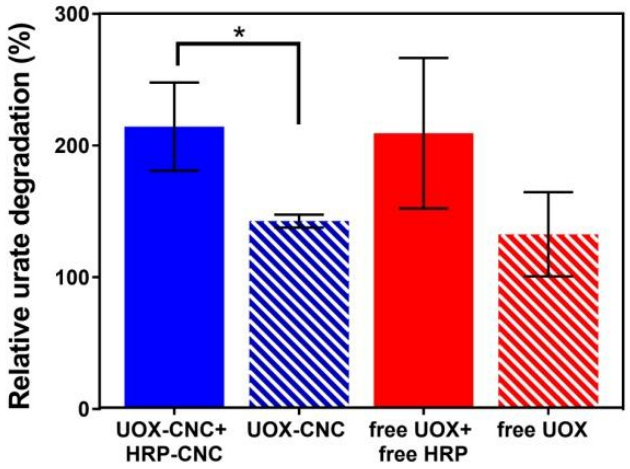


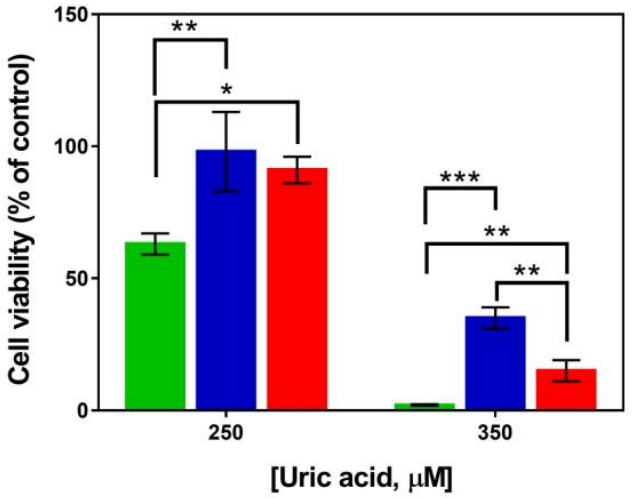

*Cell viability of HEK293T cells incubated with catalytic nanocompartments in tandem.*

# Efficiency of the cascade reaction *in vitro*

blood serum




HEK293T



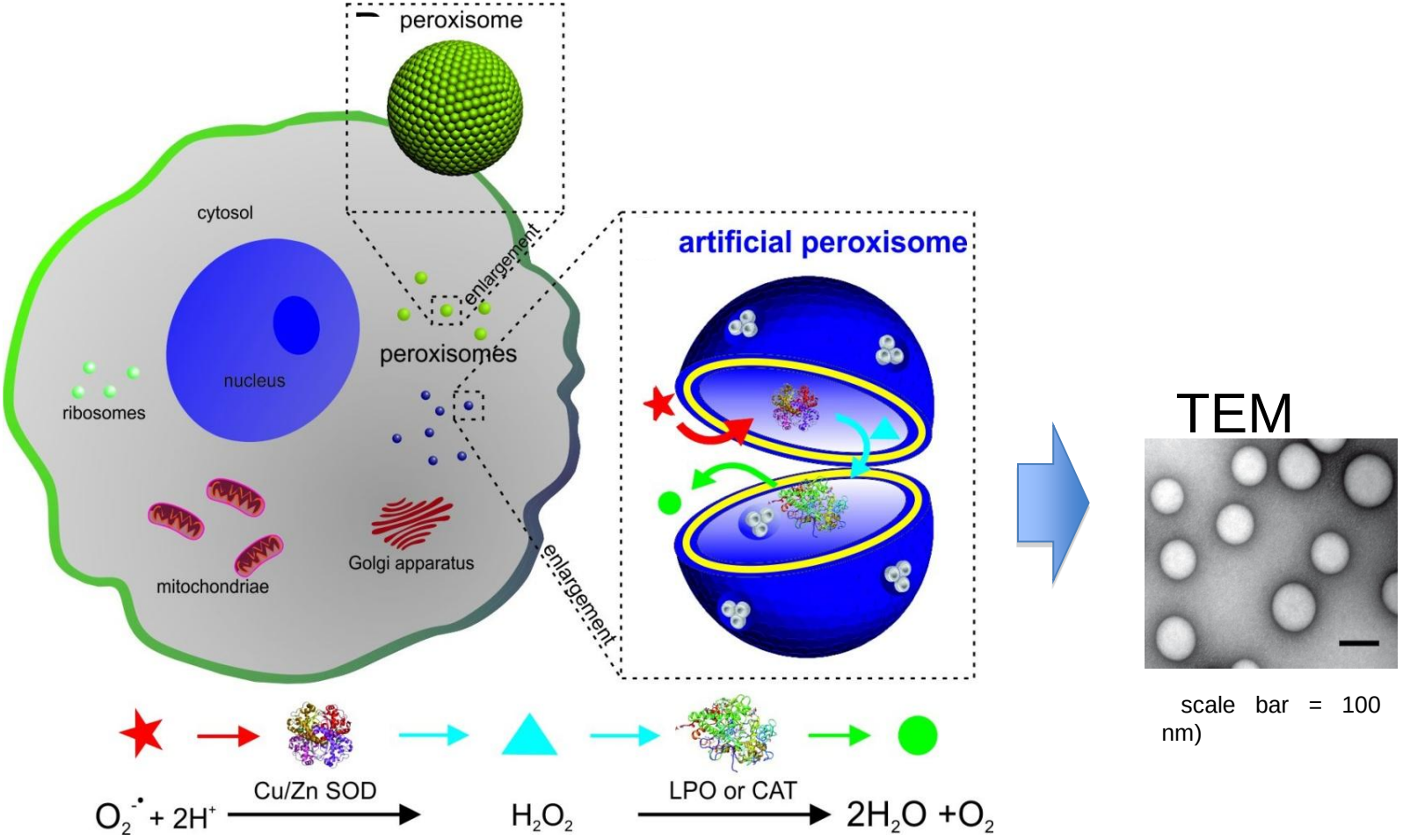

✓ Clearance of uric acid by catalytic nanocompartments in tandem in blood serum.

✓ Detoxifying activity of the cascade reaction inside catalytic nanocompartments in tandem on HEK293T cells (hyperuricemia).

A. Belluati, I. Craciun, J. Liu, C.G. Palivan, *Biomacromolecules*, 2018.

- Catalytic nanocompartments
- Catalytic nanocompartments in tandem
- Artificial organelles
- Artificial organelles wih triggered activity

# Artificial peroxisomes - Concept

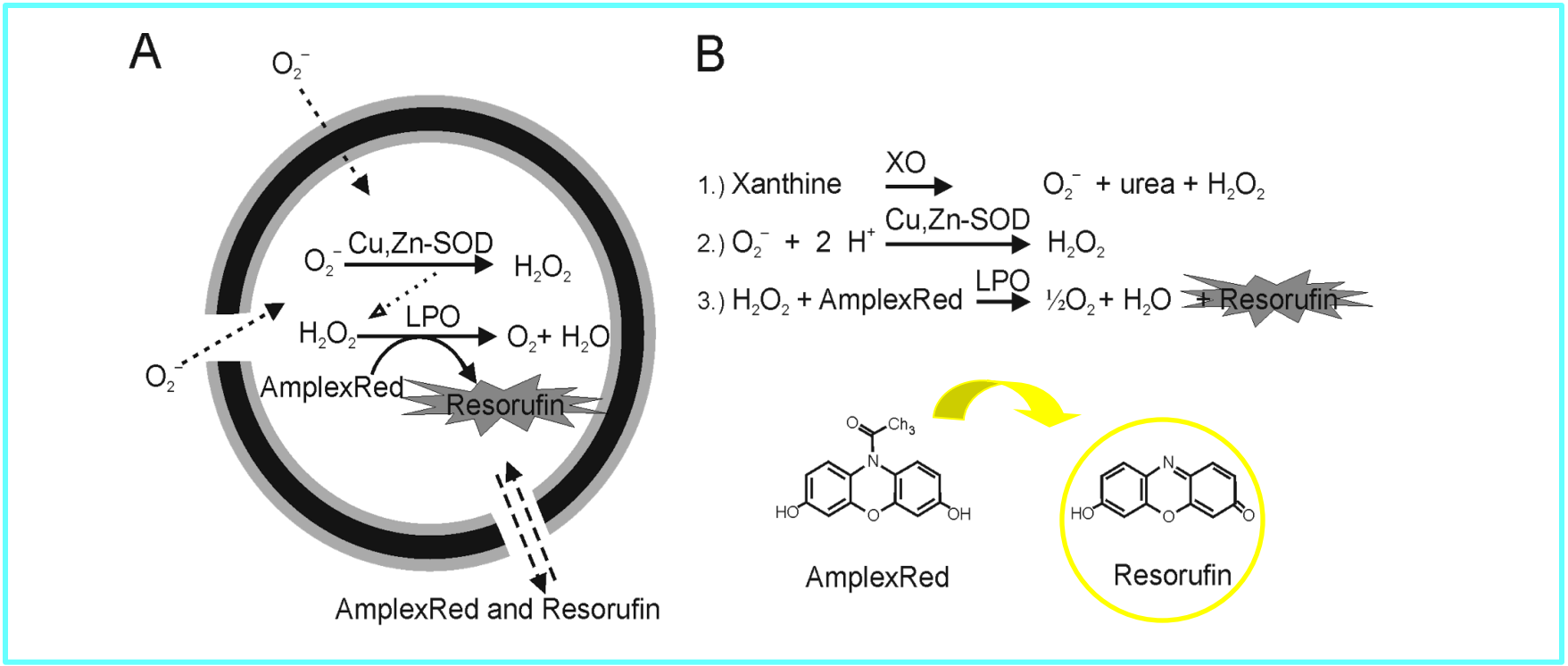




P. Tanner, O. Onaca, V. Balasubramanian, W. Meier, C. G. Palivan**,** *Chem. Eur. J*, **2011**
P. Tanner, P. Baumann, R. Enea, O. Onaca, C. Palivan, W. Meier, *Accounts of Chemical Research*, **2011**
C.G. Palivan, R. Goers, A. Najer, X. Zhang, W. Meier, *Chem. Soc. Rev*, **2016**.

# Enzymatic cascade reaction

**Function**: detection and detoxification of reactive oxygen species

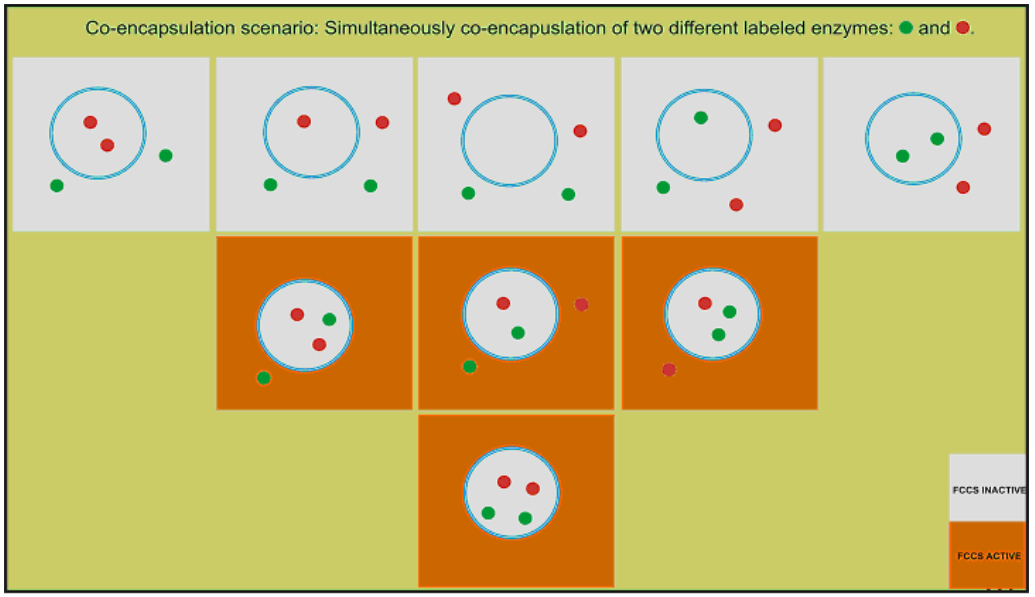



**Tandem**: SOD and Lactoperoxidase/Catalase

P. Tanner, V. Balasubramanian, C.G. Palivan, Nano Letters, **2013**.

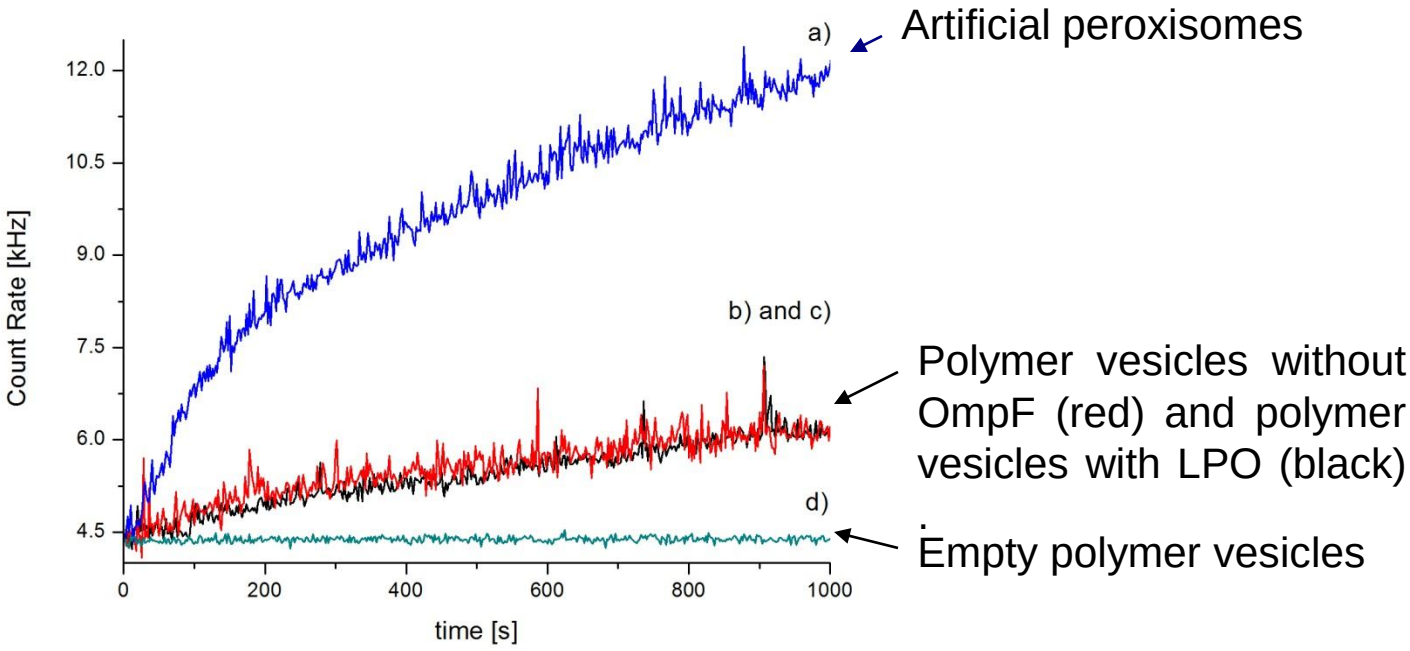
# Co-encapsulation: A real challenge

Scenario for a co-existence of nanocompartments populations containing various encapsulated enzymes :




✓ Catalytic nanocompartments with co-encapsulated enzymes: 10-20% of vesicles contain both enzymes inside their cavity.

# Artificial peroxisomes : activity assay

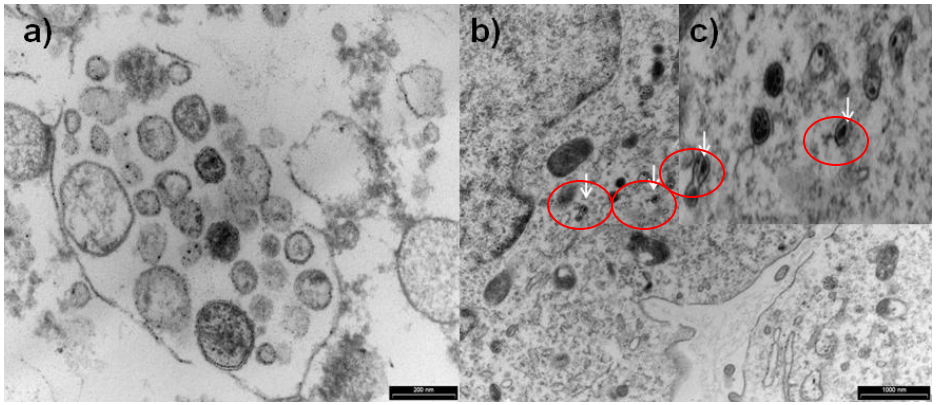



✓ Artificial peroxisomes: tandem of enzymes detoxify *in situ* both superoxide radicals and $H_2O_2$.

P. Tanner, V. Balasubramanian, C.G. Palivan, Nano Letters, 2013

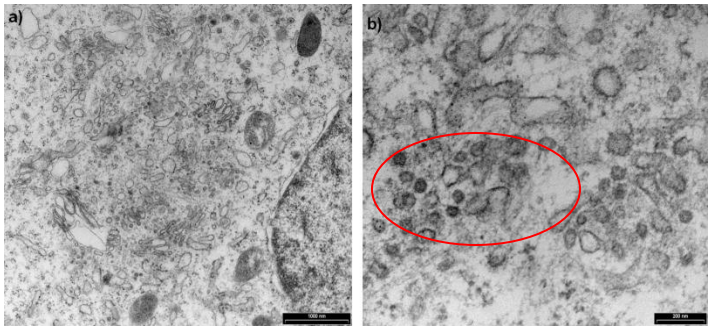
# Artificial peroxisomes in HeLa cells

TEM images of HeLa cells incubated for 24 h with SOD-LPO containing peroxisomes:




a) Intracellular area, as an endosome-like compartment; b) Cytoplasm area containing shrunken polymeric vesicles (indicated by arrows); c) Zoom of shrunken polymeric vesicles in the cytoplasm (indicated by arrows).

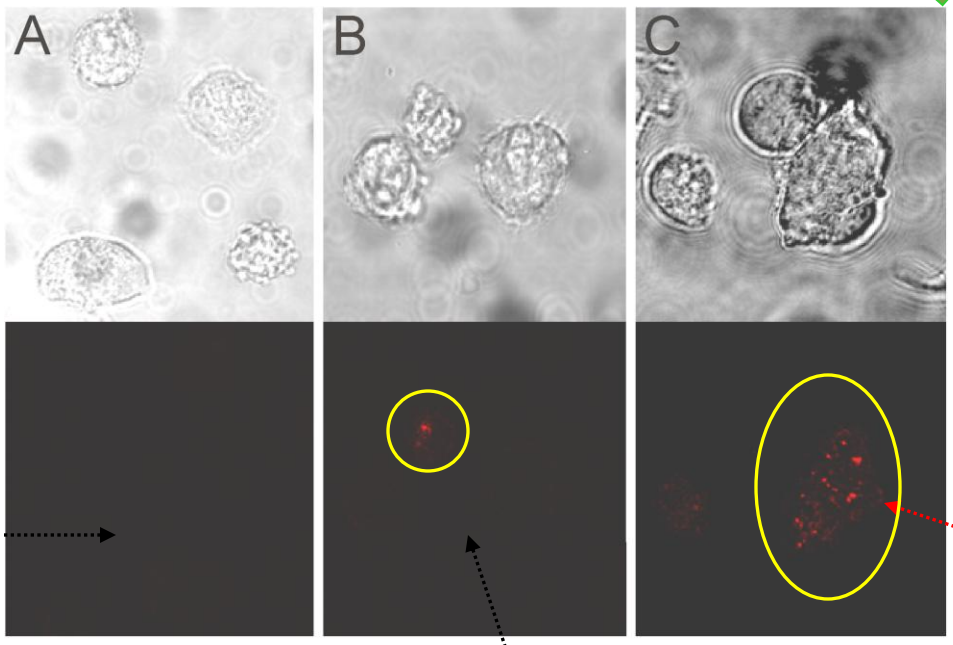
Localisation after 48 hours:




a) Cytoplasm area containing polymeric vesicles; b) Zoom of polymeric vesicles in the cytoplasm.

# Artificial peroxisomes

*In vitro* uptake and activity assay (HeLa cells):




Normal cells treated with empty polymersomes

Normal cells treated with AOs

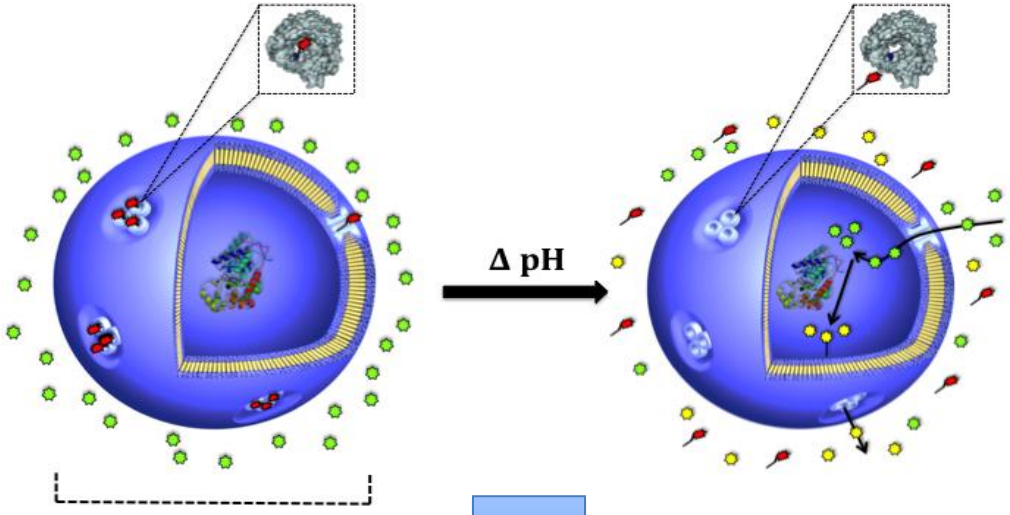
Cells exposed to **oxidative stress** and treated with AOs

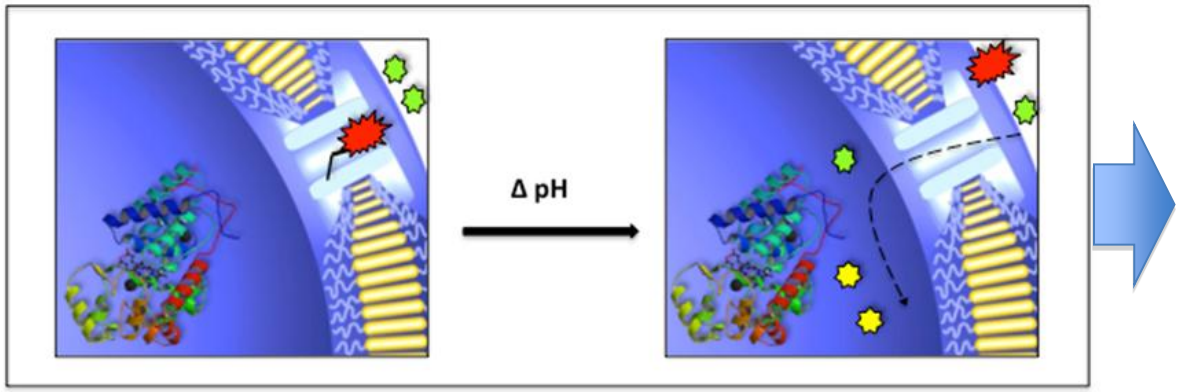
✓ Artificial organelles significantly degrade intracellular harmful radicals inside cells.

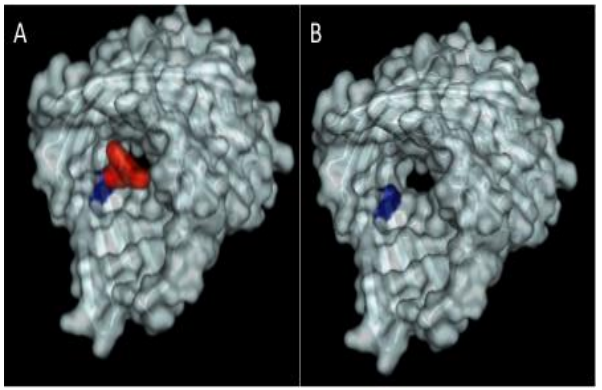

P. Tanner, V. Balasubramanian, C. Palivan, Nano Letters, **2013**.
C.G. Palivan, R. Goers, A. Najer, X. Zhang, W. Meier, *Chem. Soc. Rev.*, **2016**
A. Beluatti, I. Craciun, C. E. Meyer, S. Rigo, C.G. Palivan, Current Opinion in Biotechnology, **2019**.

- Catalytic nanocompartments
- Catalytic nanocompartments in tandem
- Artificial organelles
- Artificial organelles wih triggered activity

# Artificial organelles with triggered activity

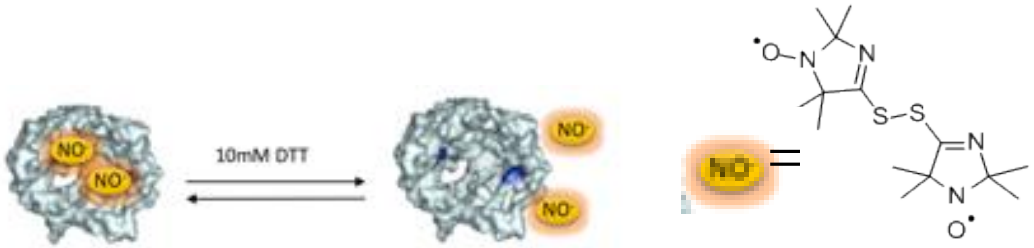


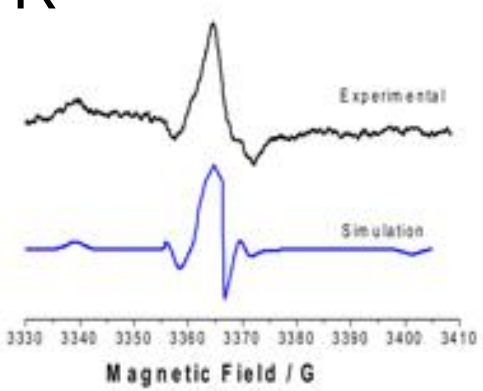



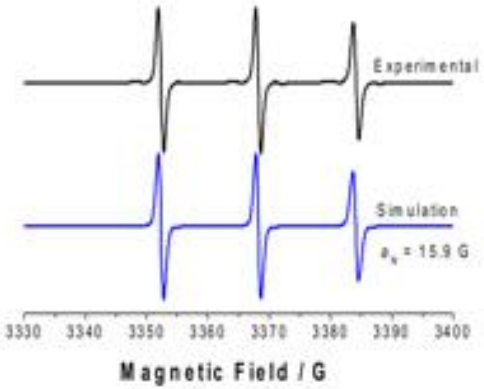

T. Einfalt, R. Goers, A. I.Dinu, A. Najer, M. Spulber, O. Onaca-Fischer, C. G. Palivan , *Nano Letters*., **2015**.
C. Edlinger, T. Einfalt, M. Spulber, A. Car, W. Meier, C. G. Palivan, *Nano Letters*, **2017**.

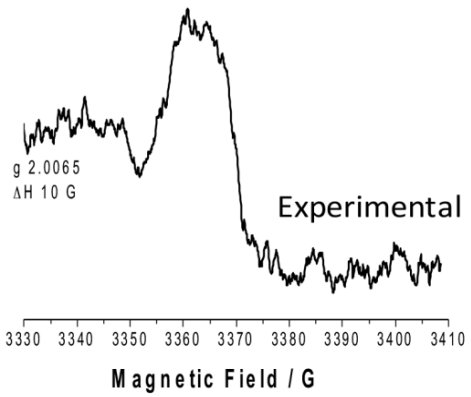
# Engineering a «protein gate»

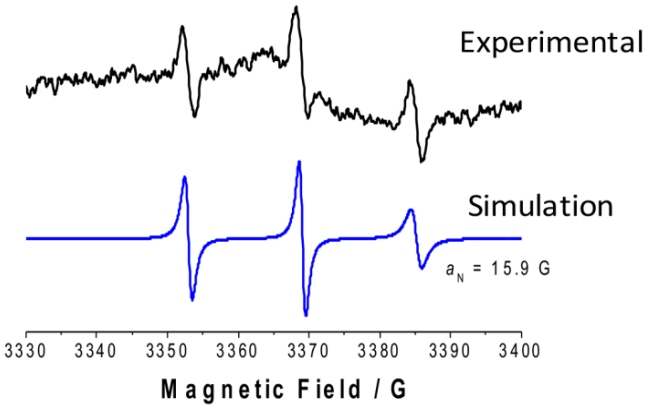



EPR

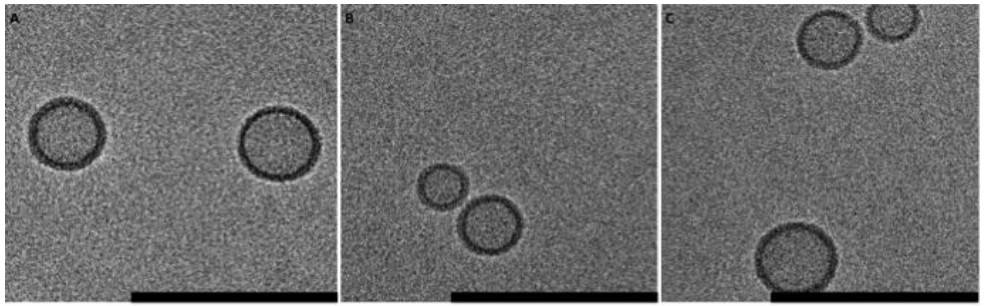


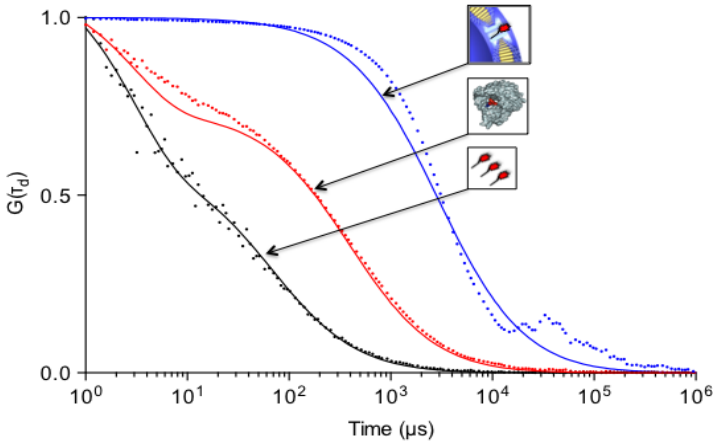



# Artificial organelles with protein „gate“




Cryo-TEM: **A**. Catalytic Nanocompartments without OmpF, **B**. Catalytic Nanocompartments with reconstituted OmpF-WT, and **C**. Catalytic Nanocompartments with reconstituted OmpF-CA-Cy5. Scale bar = 200 nm.

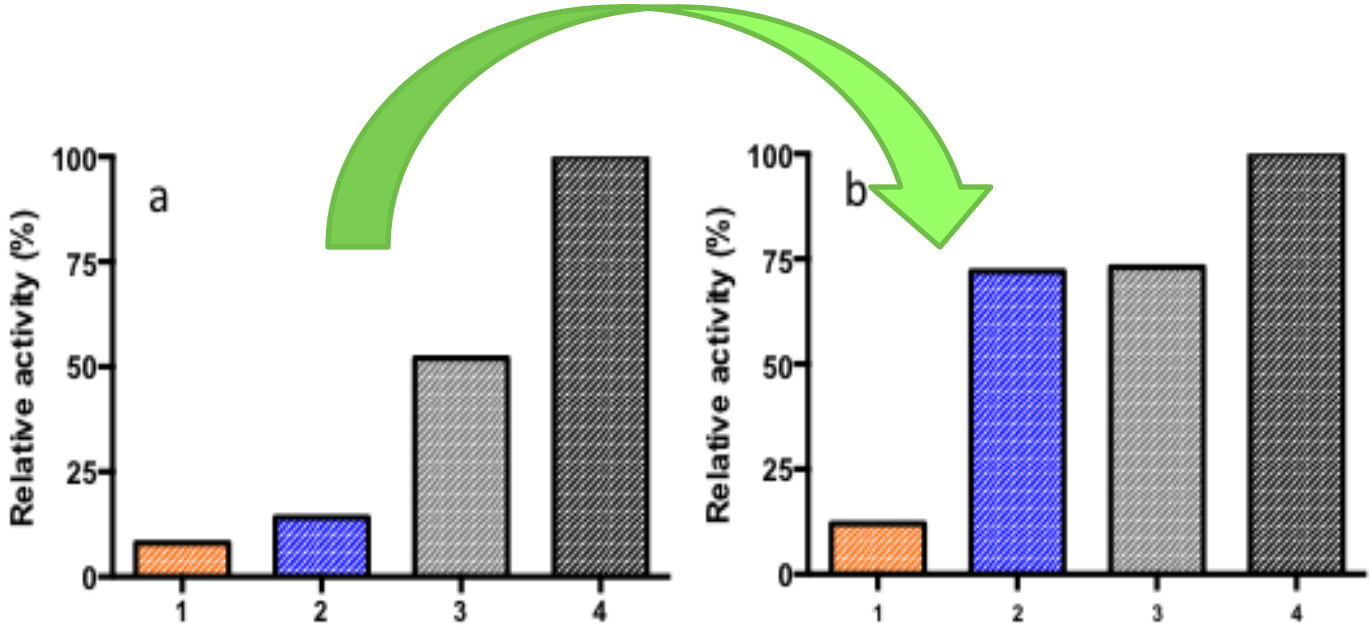



***FCS autocorrelation curves of*** **:**

- Cy5-hydrazide (Black),
- OmpF-CA-Cy5 (Red)
- OmpF-CA-Cy5 in the membrane of nanocompartments (Blue).

T. Einfalt, R. Goers, A. I.Dinu, A. Najer, M. Spulber, O. Onaca-Fischer, C. G. Palivan , *Nano Letters*., **2015**.
C. Edlinger, T. Einfalt, M. Spulber, A. Car, W. Meier, C. G. Palivan, *Nano Letters*, **2017**.

# Triggered nanoreactors




***Amplex red conversion kinetics*** :at pH 5.5, at time 0 (a)

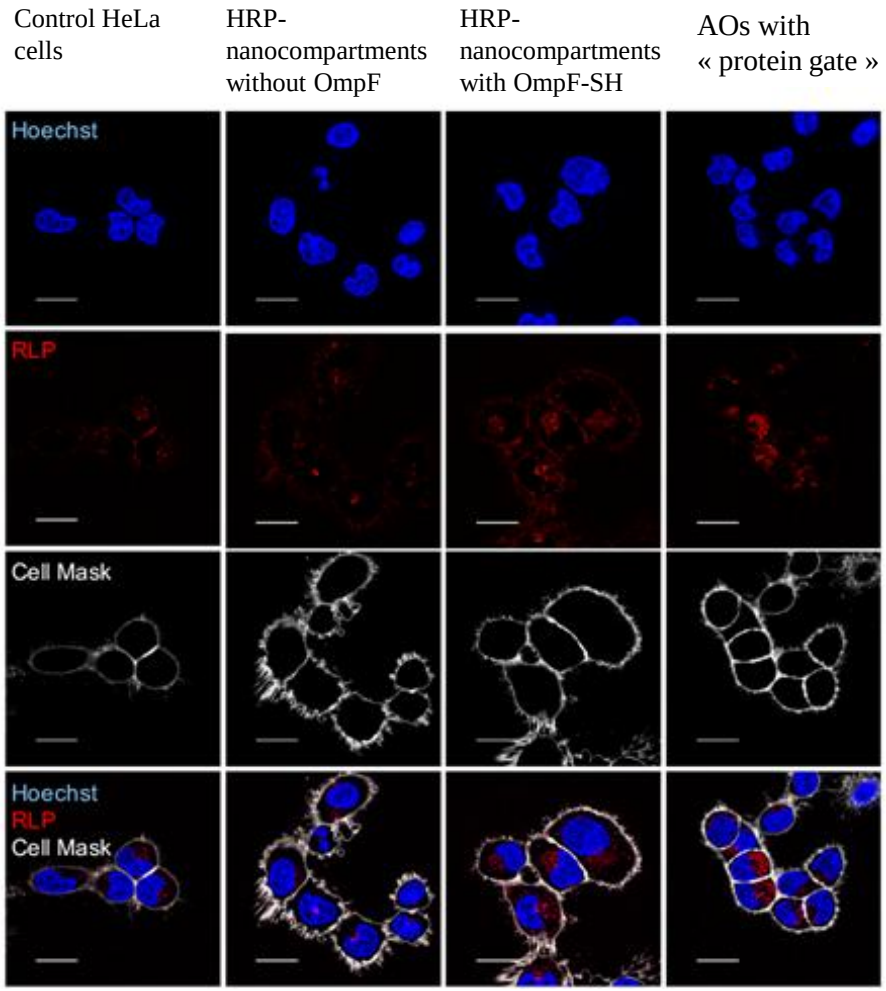
(1) unpermeabilised nanoreactors (orange)
(2) OmpF-CA-Cy5 (blue)
(3) OmpF-CA (grey),
(4) Ompf-WT (black)

***Amplex red conversion kinetics*** :at pH 5.5, After 1 hour (B)

(1) unpermeabilised nanoreactors (orange)
(2) OmpF-CA-Cy5 (blue)
(3) OmpF-CA (grey),
(4) Ompf-WT (black)

# Triggered artificial organelles *in vitro*

*HeLa cells*




Blue: Hoechst 33342 nucleus stain

Cyan: CellMask Deep Red Plasma membrane stain

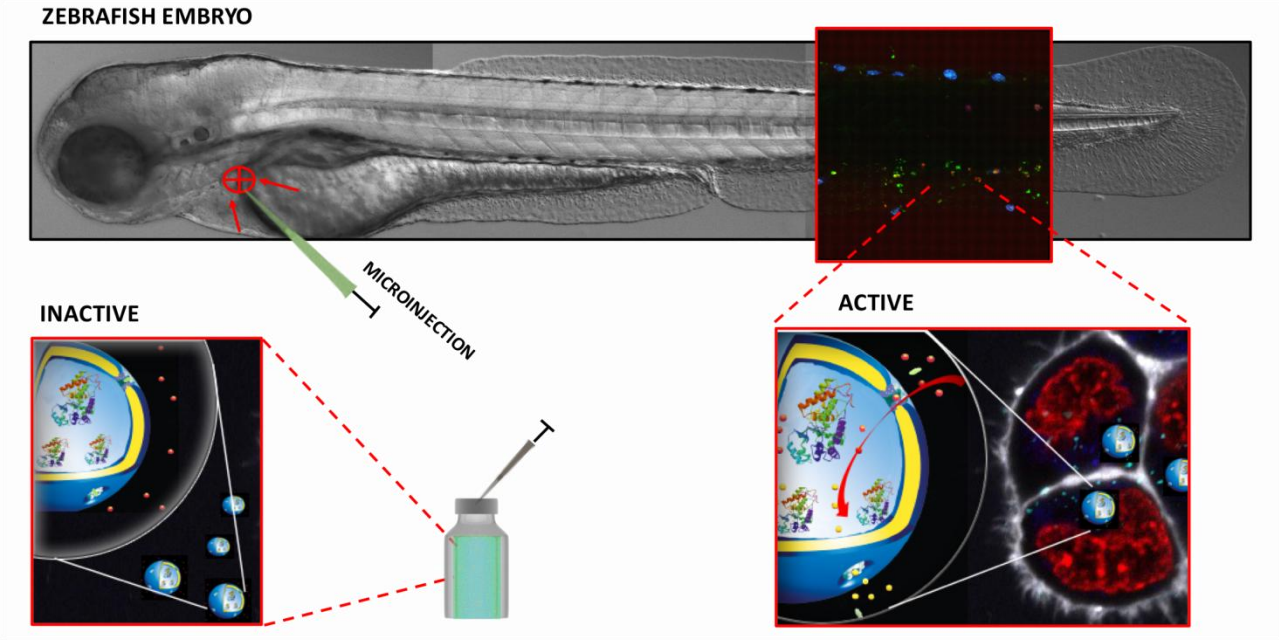
Red: converted Amplex UltraRed product.

Scale bar: 20µm

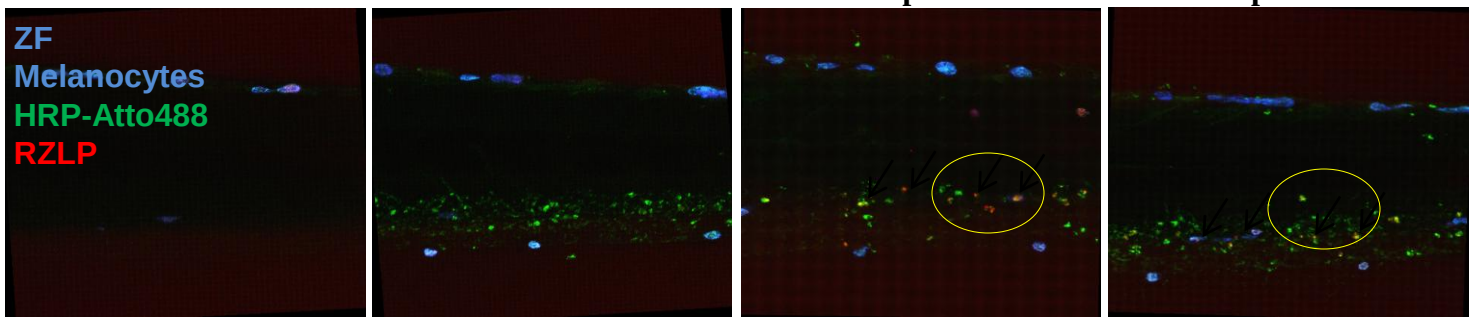
✓ Artificial organelles are activated *in vitro* by reductive changes.

T. Einfalt, D. Witzigmann, C. Edlinger, S. Sieber, R. Goers, A. Najer, M. Spulber, O. Ozana-Fischer, J. Huwyler, C. G. Palivan, *Nature Comm.*, **2018**.

# Artificial Organelles *in vivo*



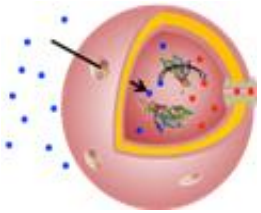

| Control ZF embryo | HRP-Atto488 nanocompartments | HRP-Atto488-nanocompartments with OmpF-SH | HRP-Atto488 nanocompartments with OmpF-S-S-CF |
|---|---|---|---|

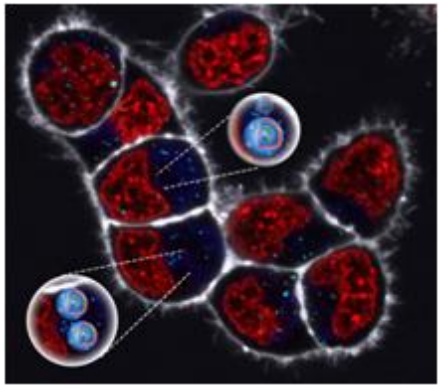


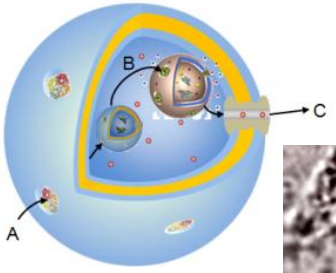

✓Artificial organelles are non-toxic, biocompatible and functional *in vivo* !

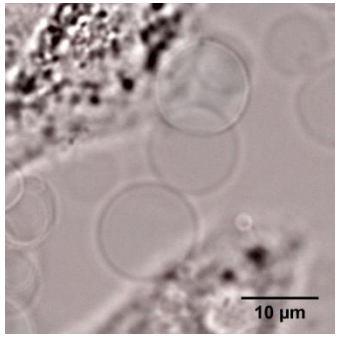
T. Einfalt, D. Witzigmann, C. Edlinger, S. Sieber, R. Goers, A. Najer, M. Spulber, O. Ozana-Fischer, J. Huwyler, C. G. Palivan, *Nature Comm.*, **2018**.

# Conclusion and outlook








- Cellular implants
- Active surfaces or membranes with complex functionality
- Molecular factories ...

# Acknowledgements

***Collaborators:***

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Prof. Sabine Van Doorslaer (Antwerp University)

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Prof. Dirk Schneider (Mainz University)

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# Thank you for your attention