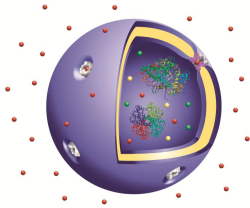


Bio-synthetic compartments as mimics of natural organelles and cells

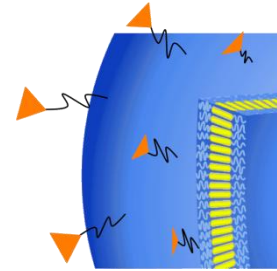
Cornelia Gabriela Palivan
University of Basel

Philadelphia, 2020

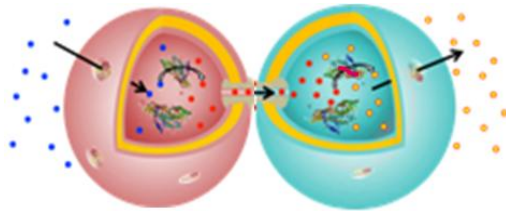
Bio-Synthetic assemblies



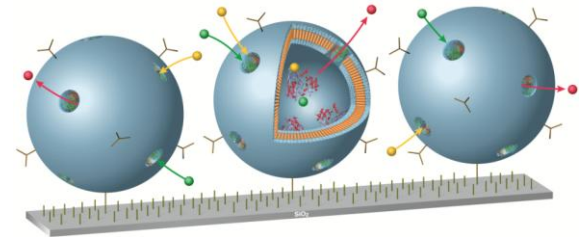
Nanoreactors, Artificial Organelles



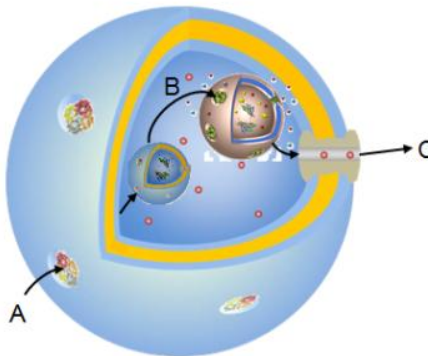
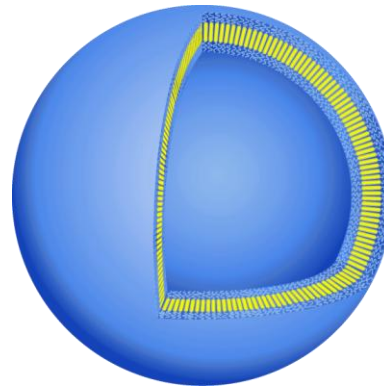
Functionalized carriers



Interconnected Organelles



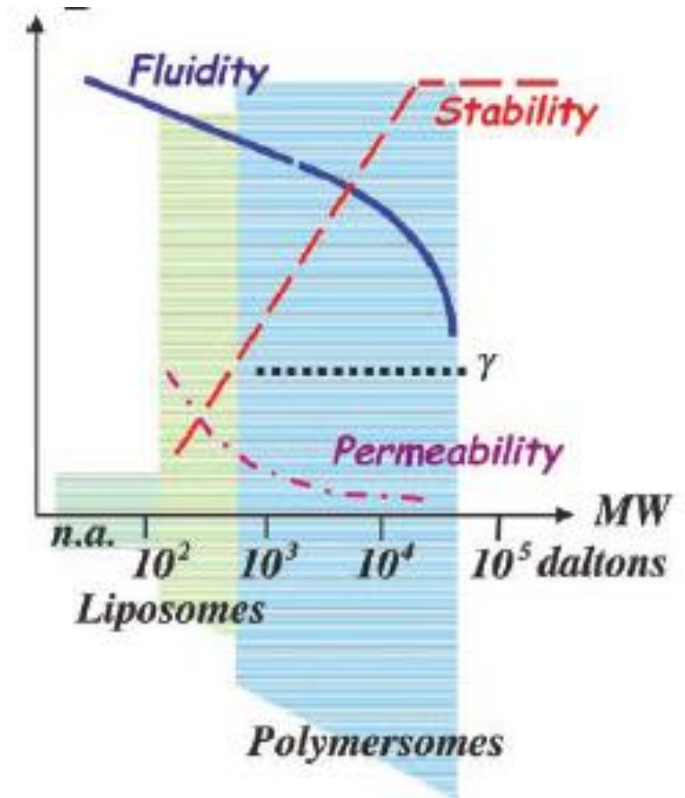
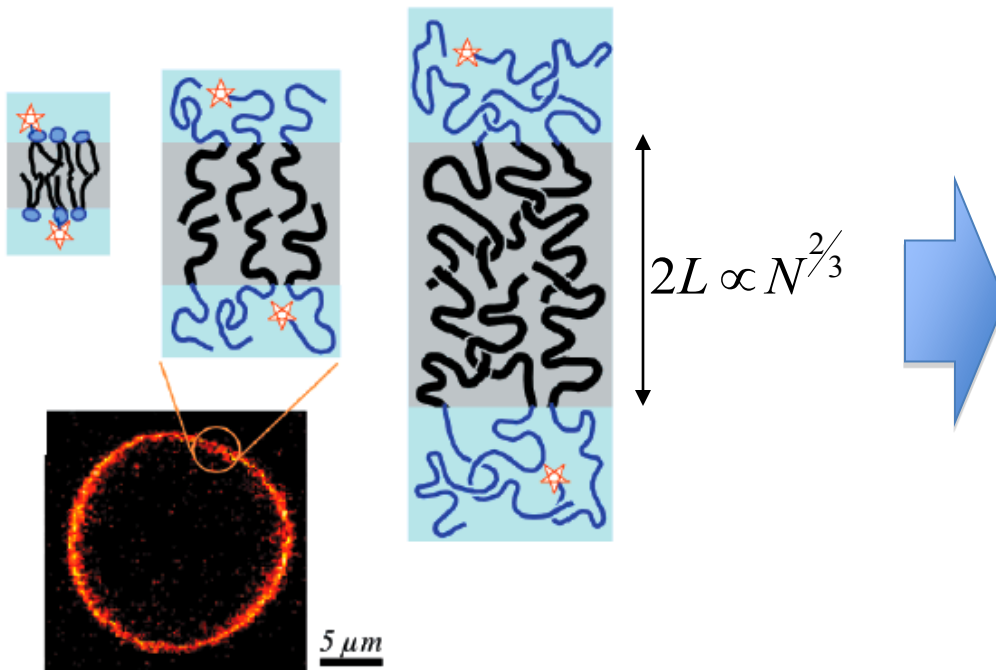
„Active“ surfaces



Compartments-in-compartment,
artificial cells

Thickness vs properties of polymer membrane

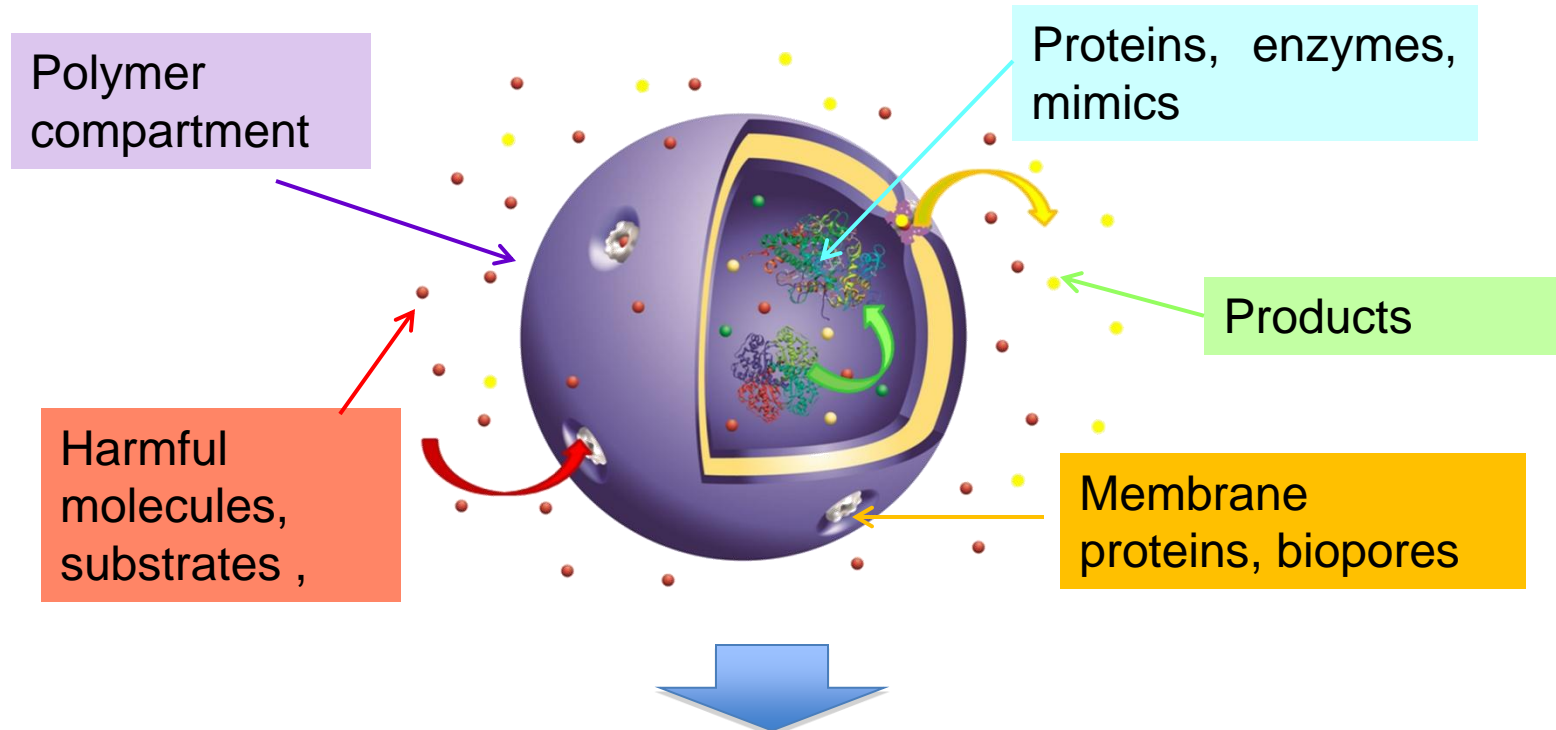
Polymer vesicles (polymersomes):



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

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

Catalytic nanocompartments: Concept



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

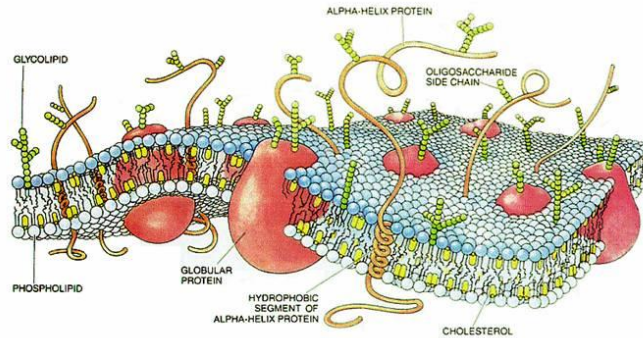
P. Tanner, S. Egli, V. Balasubramanian, O. Onaca, C. G. Palivan, W. Meier, *FEBS Letter*, **2011**, 585, 1699.

M. Lomora, G. Gunkel-Grabole, S. Mantri, C. G. Palivan, *Chem. Comm.*, **2017**, 53, 10148.

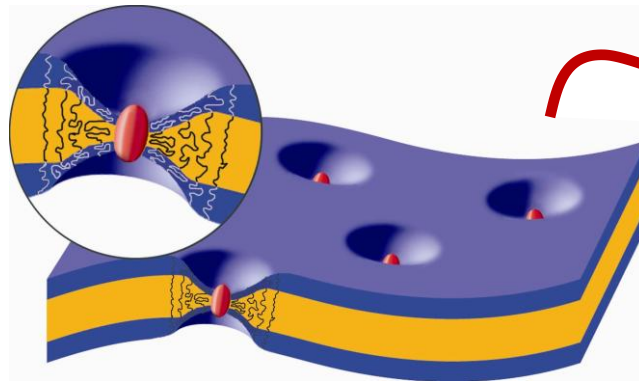
A. Belluati, I. Craciun, J. Liu, C.G. Palivan, *Biomacromolecules*, **2018**, DOI: 10.1021/acs.biomac.8b01019.

Polymer membrane: mimic of biomembranes

Cell membrane



Polymer membrane



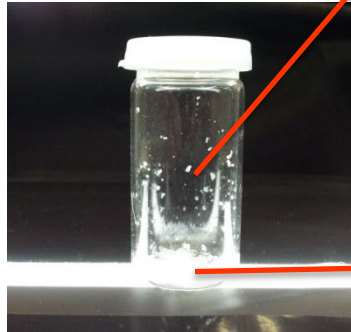
OmpF (Outer membrane protein F)

Nardin et al. *Chem. Commun.*, **2000**, 1433.

M. Garni, S. Thamboo, C. Schenenberger, C.G. Palivan, *BBA- Biomembranes*, **2017**, 1859, 619.

- Protein decorated membranes
- Artificial organelles
- Mimics of cells

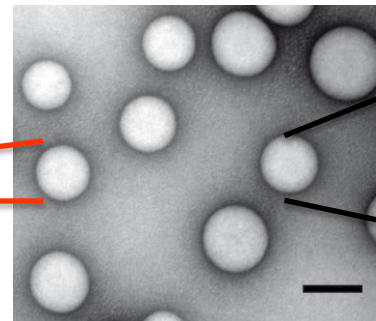
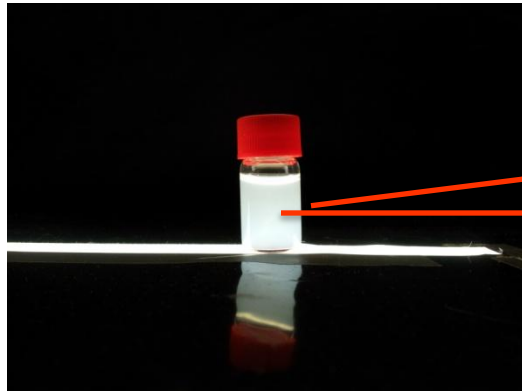
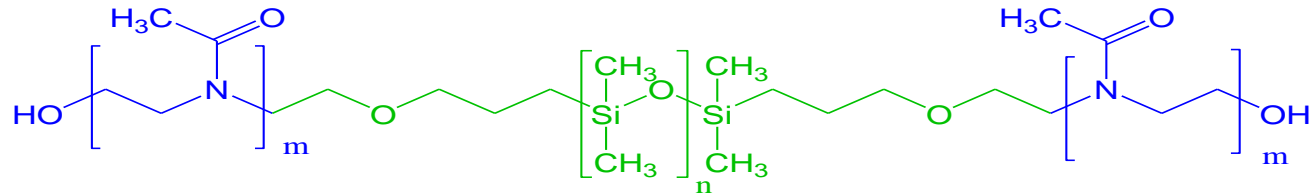
Raw material: amphiphilic block copolymer



Poly-2-methyl-2-oxazoline

Polydimethylsiloxane

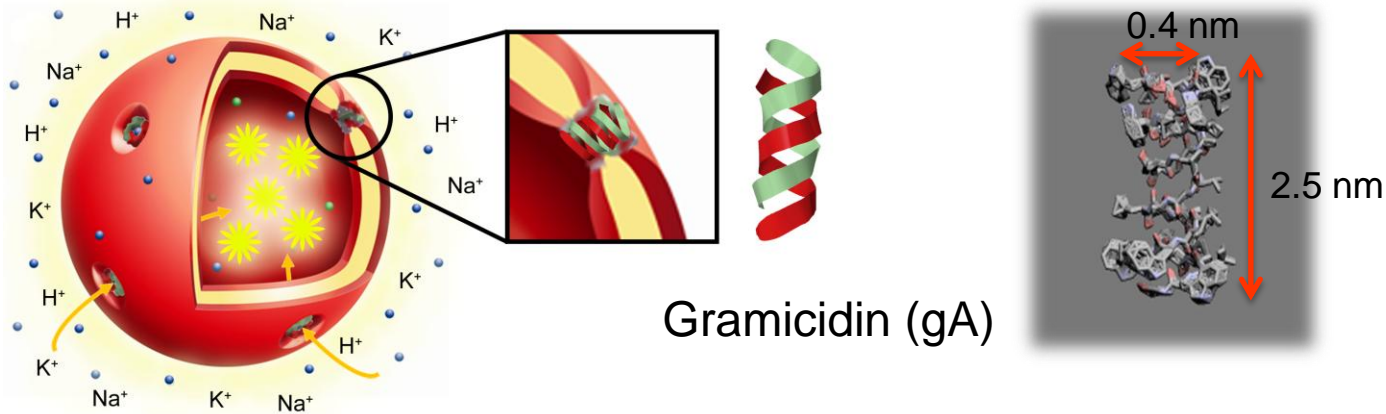
Poly-2-methyl-2-oxazoline



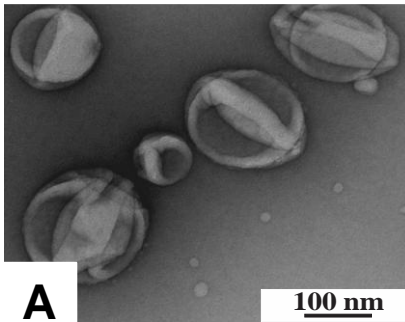
scale bar = 100 nm)

Hollow spheres: vesicles
(50 nm – 1 μ m)

Polymersomes engineered for ion selective permeability

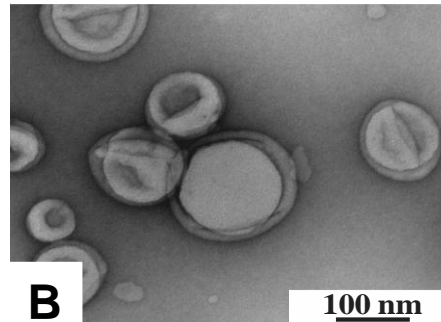


without gA, high pH

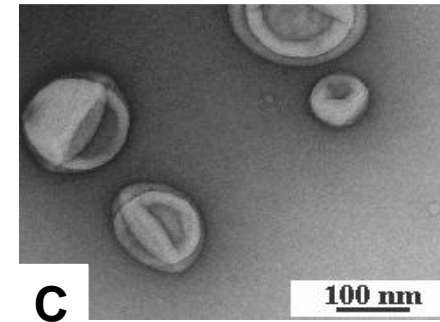


Rh = 111 nm
Rg = 104 nm
 $\rho = Rg/Rh = 0.91$

with gA, high pH



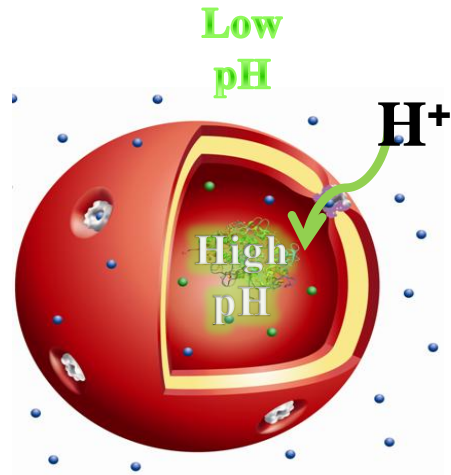
with gA, low pH



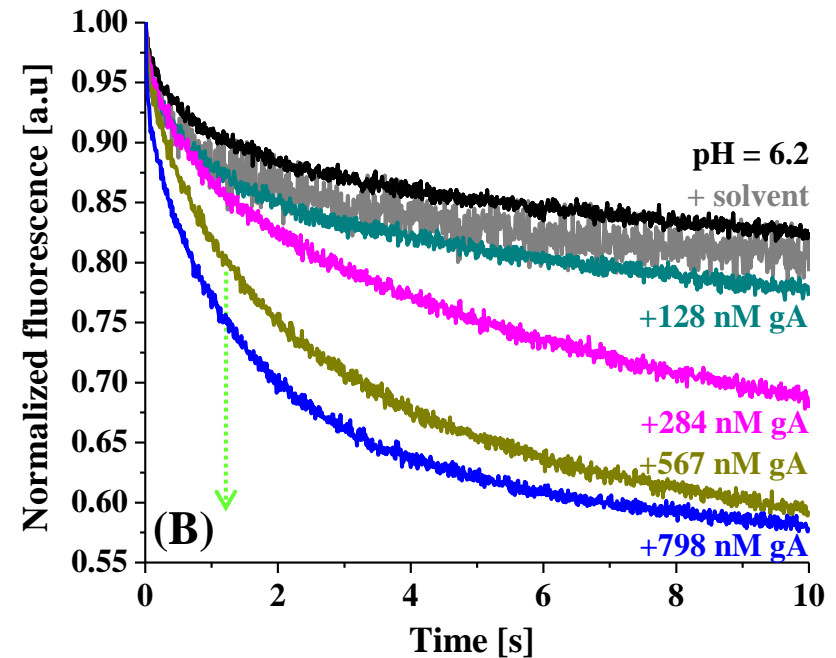
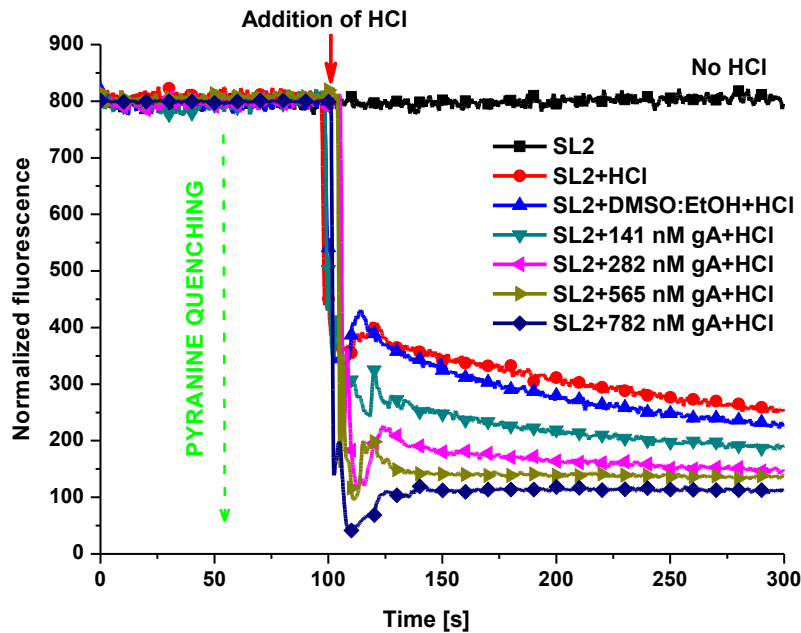
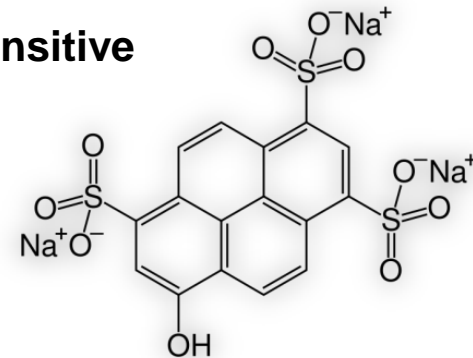
Rh = 111 nm
Rg = 102 nm
 $\rho = Rg/Rh = 0.91$



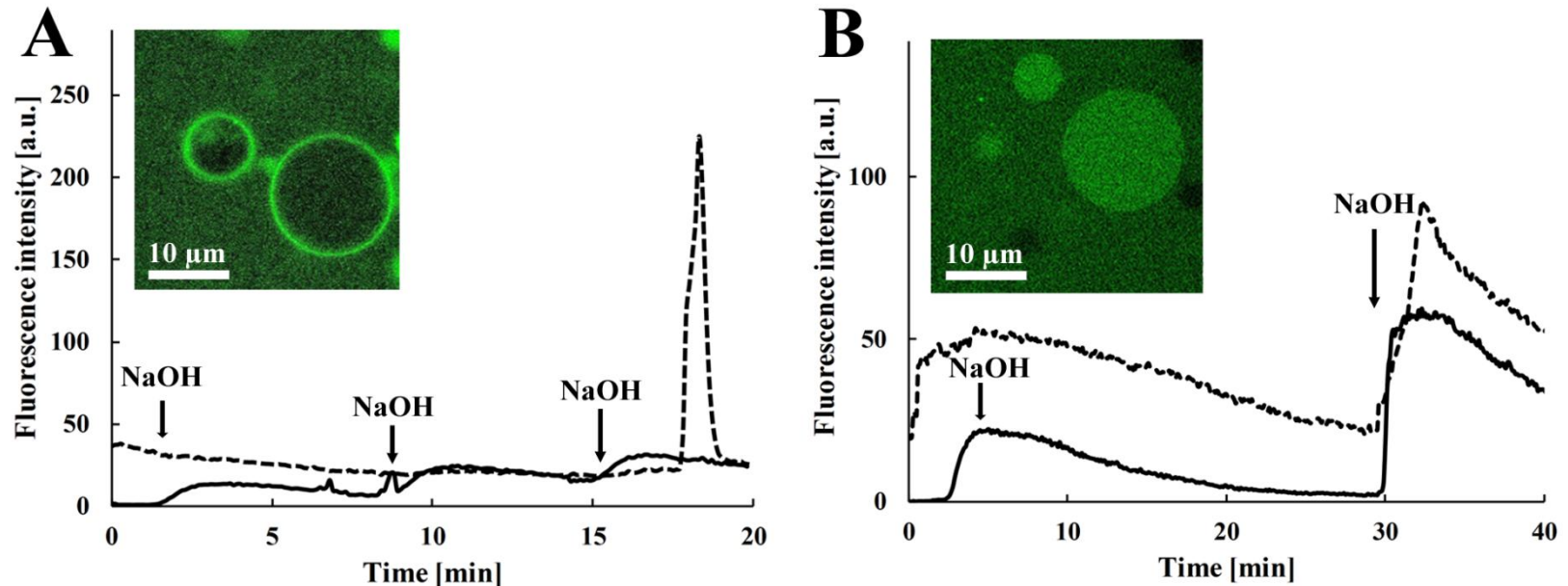
Polymersomes with functional gA biopores



Pyranine: pH-sensitive



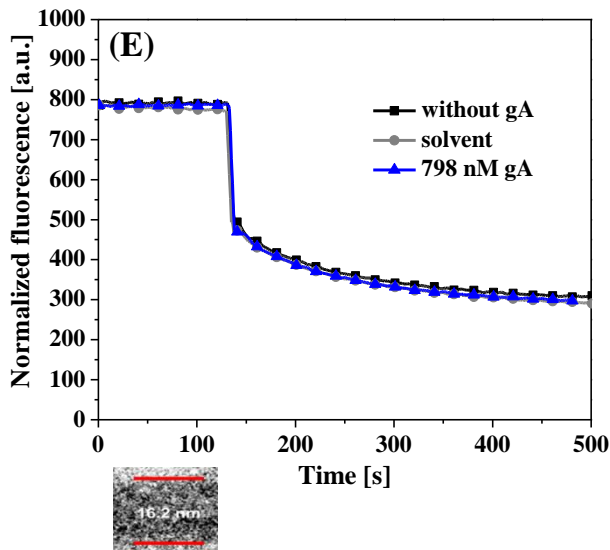
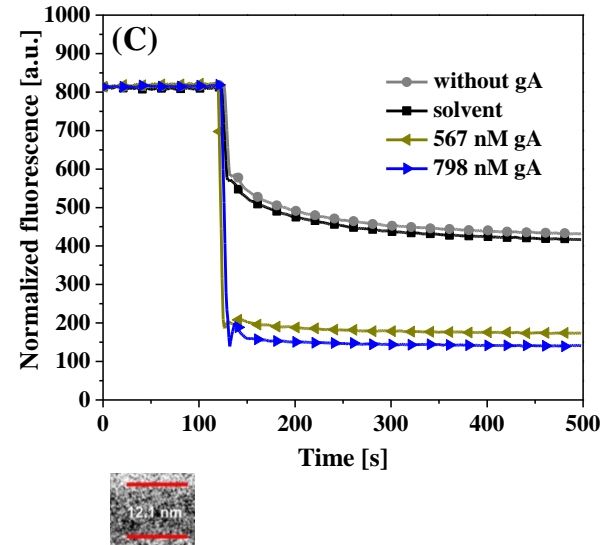
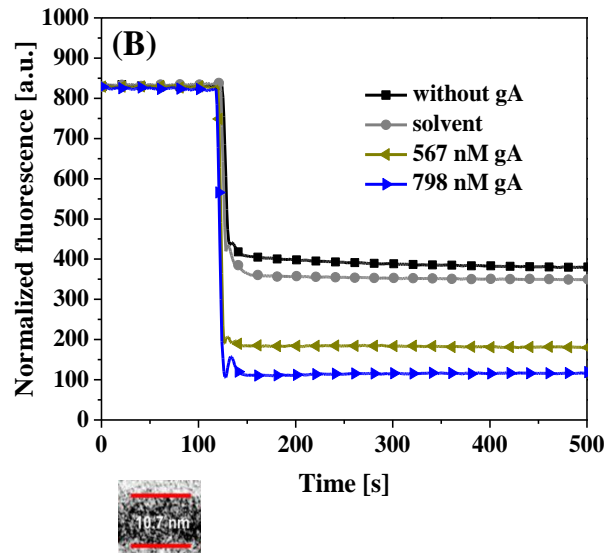
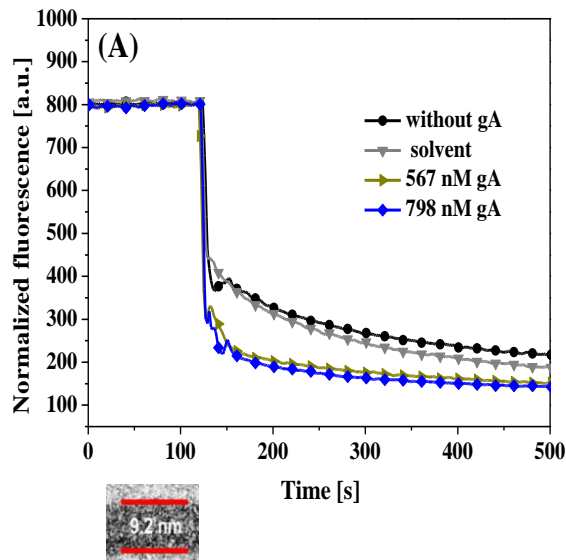
Polymersomes with engineered ion selective permeability (H^+ , Na^+ , K^+)



Fluorescent intensity change over time of 5(6)-carboxyfluorescein (CF) inside and outside of polymer GUVs in absence (A), and in upon insertion of gA (B). Insets: representative LSM images showing the principle of blocking the membrane to protons (A) and permeability of the membrane to protons by insertion of gA (B). Fluorescence intensity inside GUVs (dotted line), and outside GUVs (solid line).

✓ Both polymersomes and giant vesicles with engineered ion selective permeability of the membrane (membrane thickness 9.2 nm).

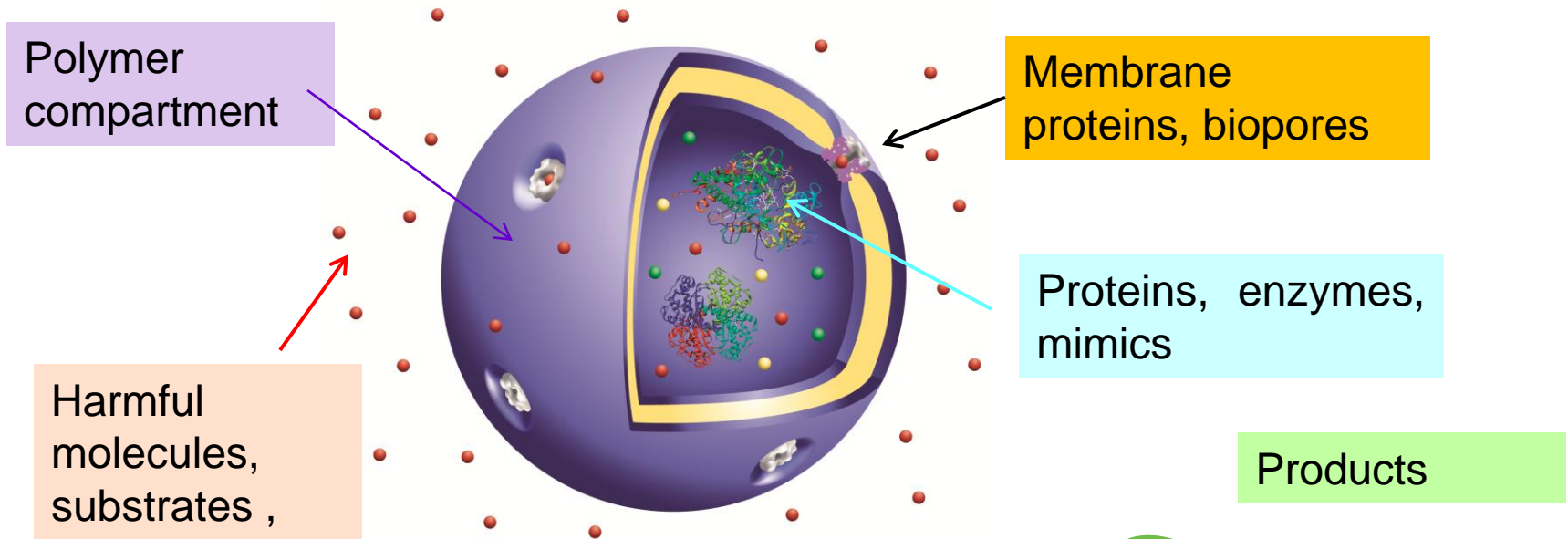
Membrane thickness: gA insertion ?



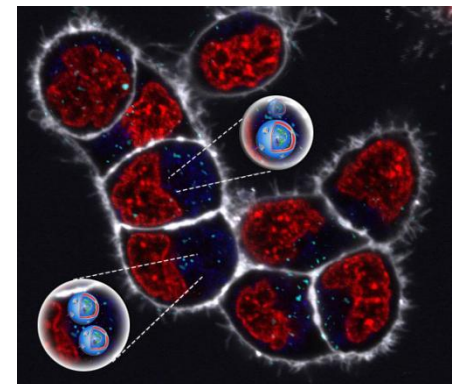
gA successful insertion and functionality in synthetic membranes with a thickness up to 13.2 nm (4 times thicker than gA size) !

- Protein decorated membranes
- Artificial organelles
- Mimics of cells

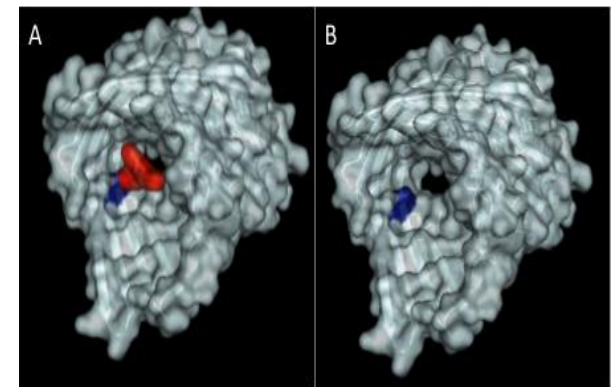
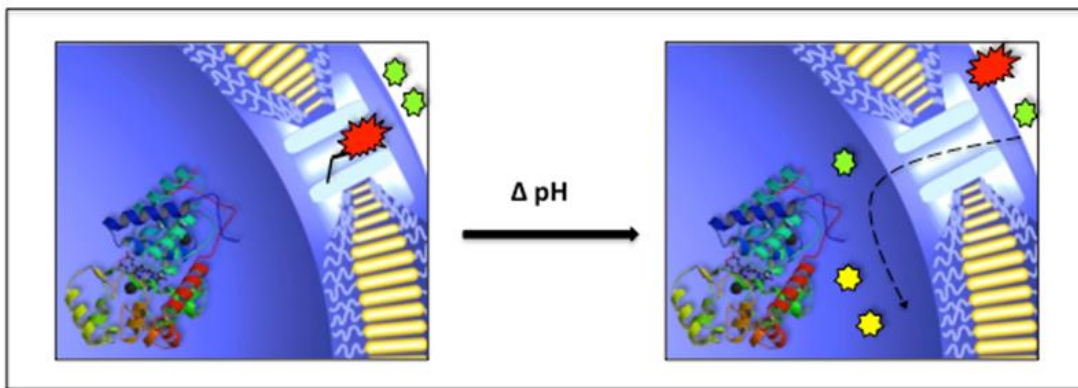
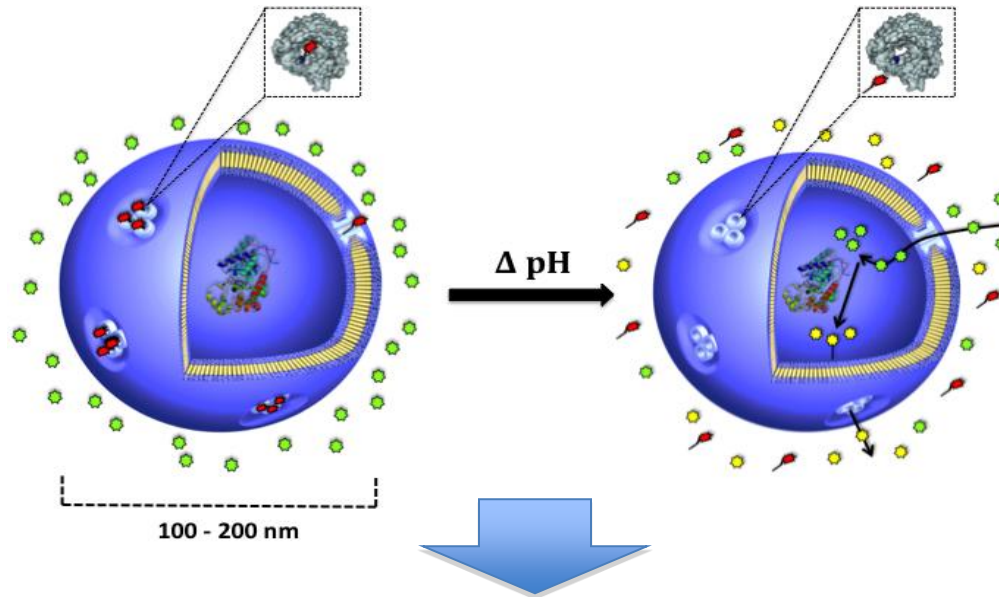
Artificial organelles: Concept



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



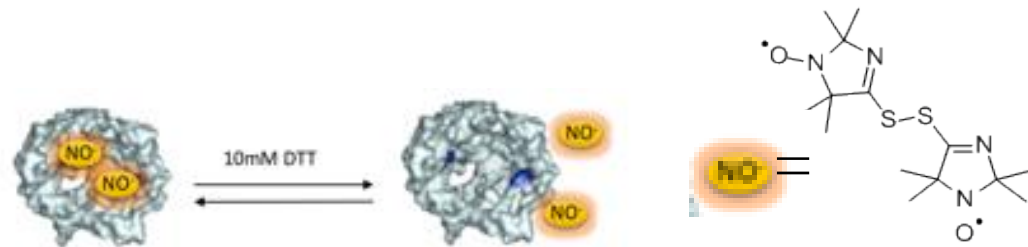
Artificial organelles with triggered activity



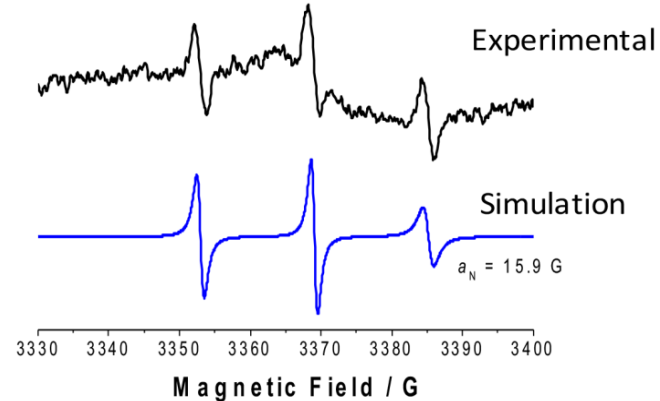
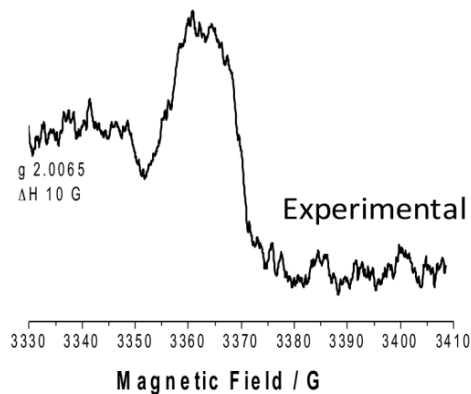
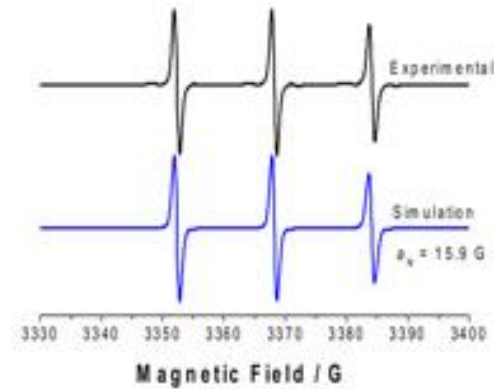
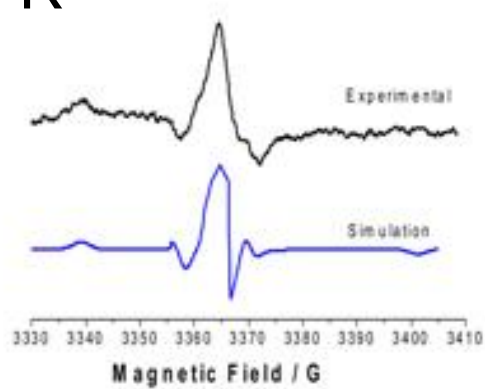
T. Einfalt, R. Goers, A. I.Dinu, A. Najer, M. Spulber, O. Onaca-Fischer, C. G. Palivan, *Nano Letters.*, **2015**.

C. Edlinger, T. Einfalt, M. Spulber, A. Car, W. Meier, C. G. Palivan, *Nano Letters*, **2017**.

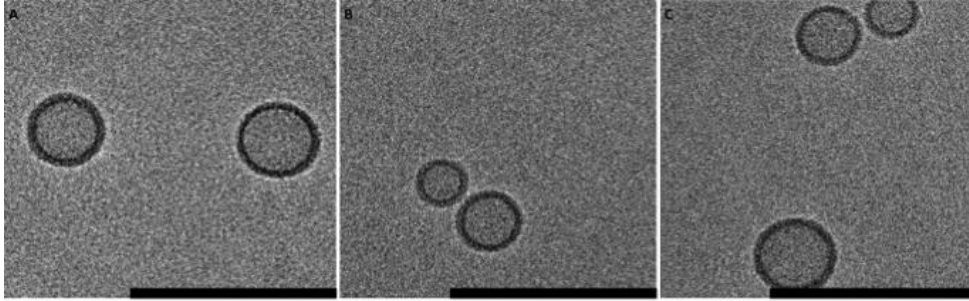
Engineering a «protein gate»



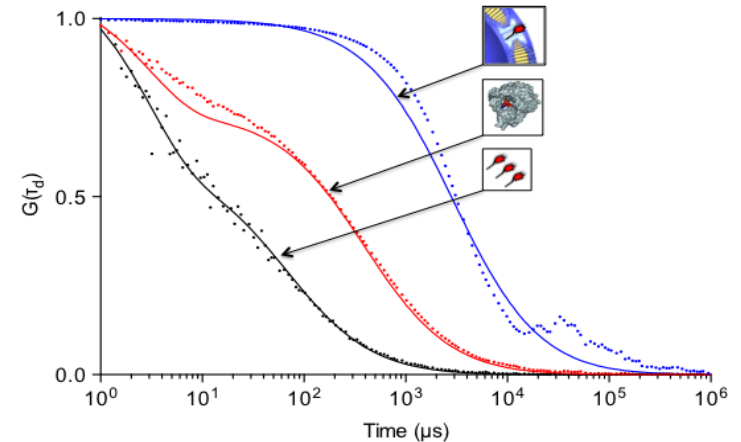
EPR



Artificial organelles with protein „gate“



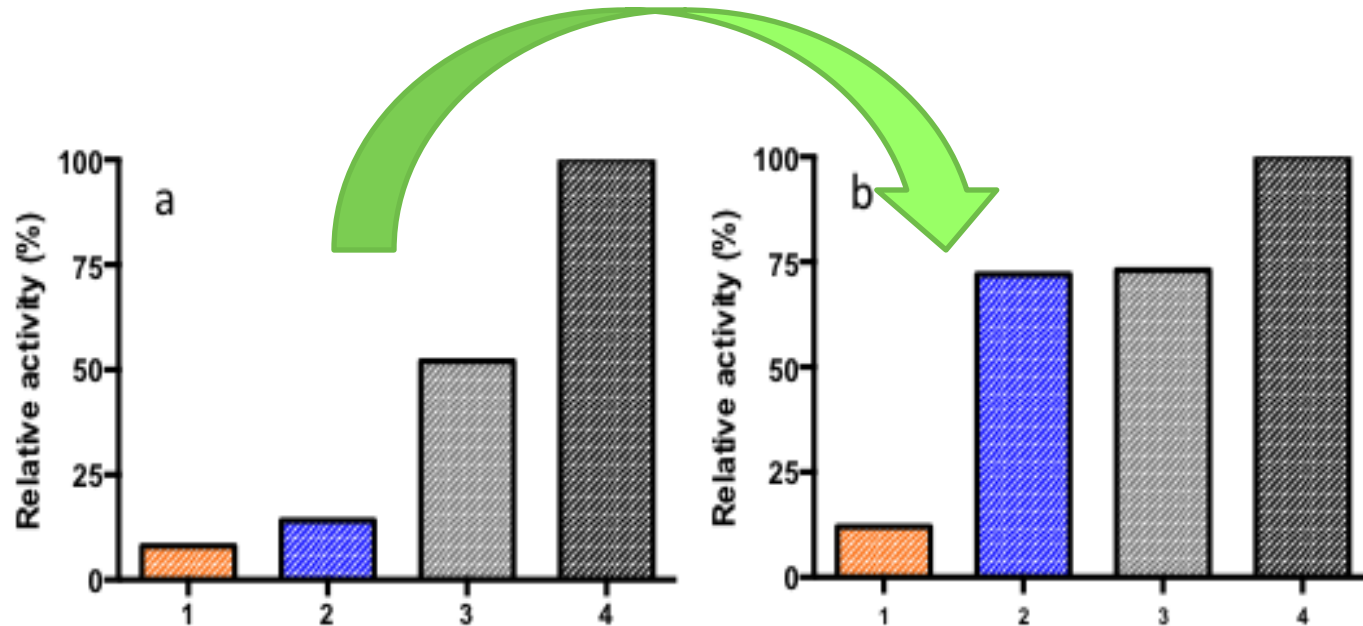
Cryo-TEM: **A.** Aos without OmpF, **B.** Aos with reconstituted OmpF-WT, and **C.** Aos with reconstituted OmpF-CA-Cy5. Scale bar = 200 nm.



FCS autocorrelation curves of :

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

Artificial organelles with triggered activity



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

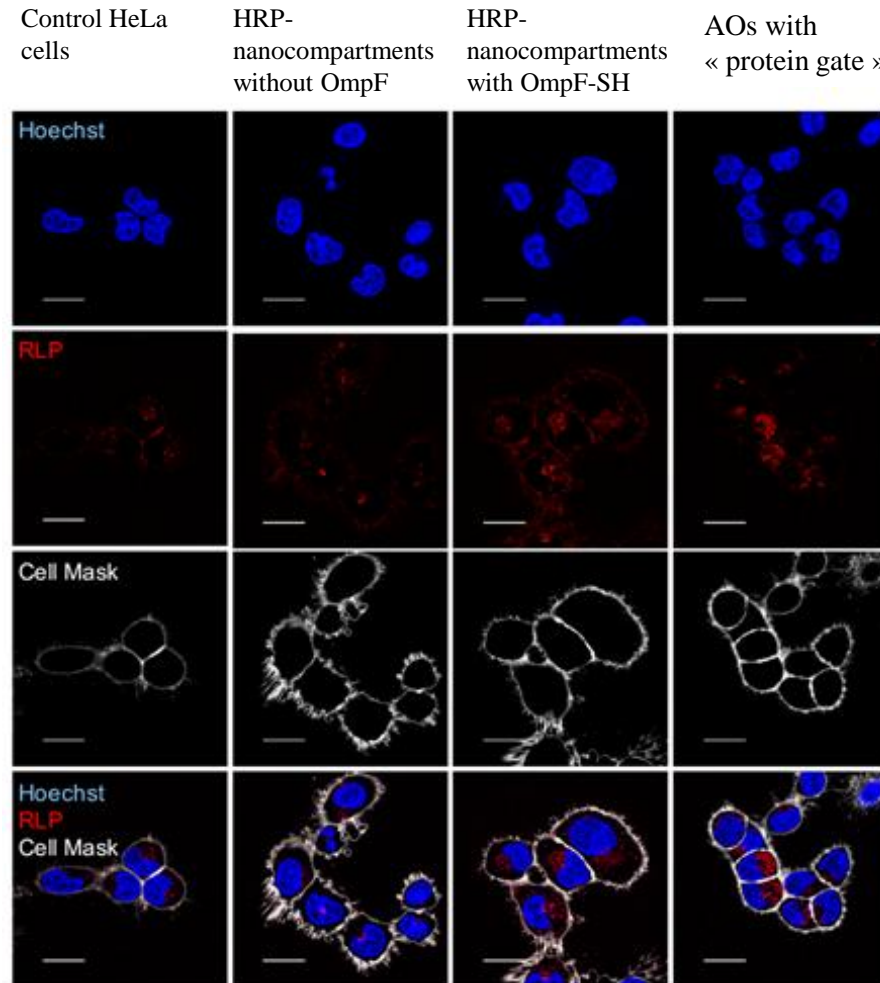
- (1) unpermeabilised nanoreactors (orange)
- (2) OmpF-CA-Cy5 (blue)
- (3) OmpF-CA (grey),
- (4) OmpF-WT (black)

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

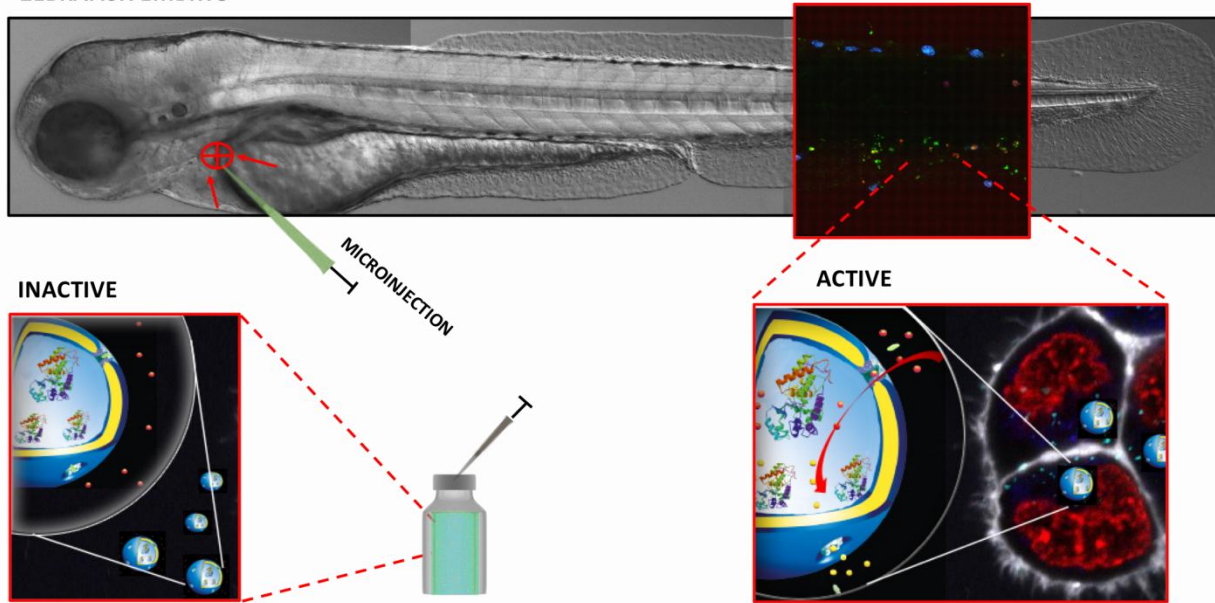
UltraRed product.

Scale bar:
20µm

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

Artificial Organelles *in vivo*

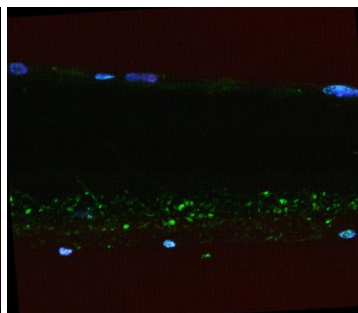
ZEBRAFISH EMBRYO



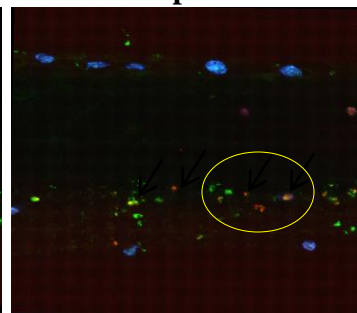
Control ZF embryo



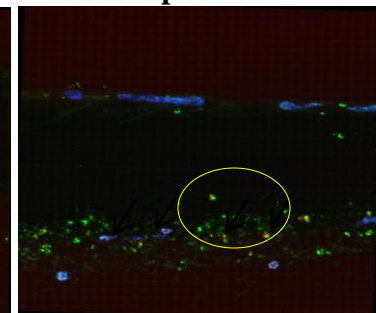
HRP-Atto488
nanocompartments



HRP-Atto488-
nanocompartments with
OmpF-SH



HRP-Atto488
nanocompartments with
OmpF-S-S-CF



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

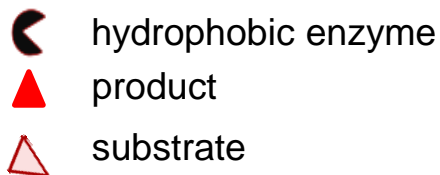
- Protein decorated membranes
- Artificial organelles
- **Mimics of cells**

Mimics of cellular processes

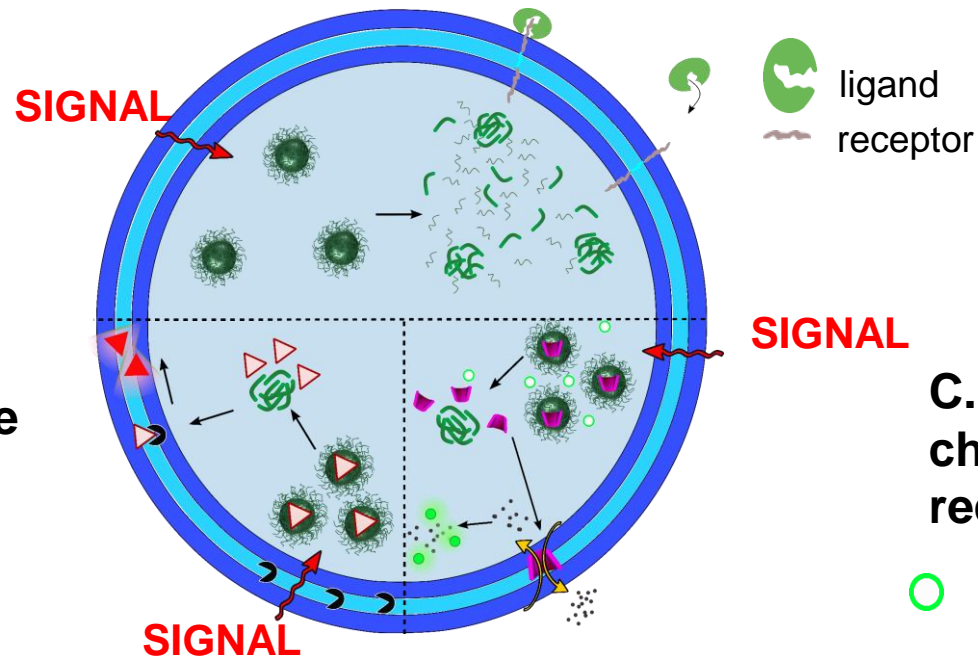
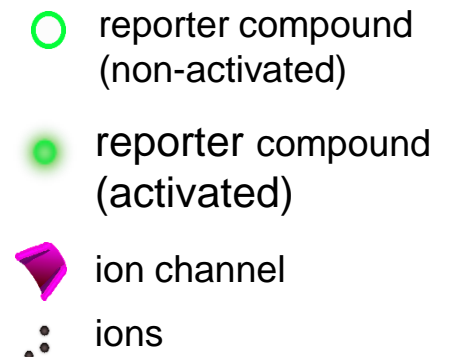
A. Stimuli-responsiveness



B. Triggered enzyme action by recombination with substrate

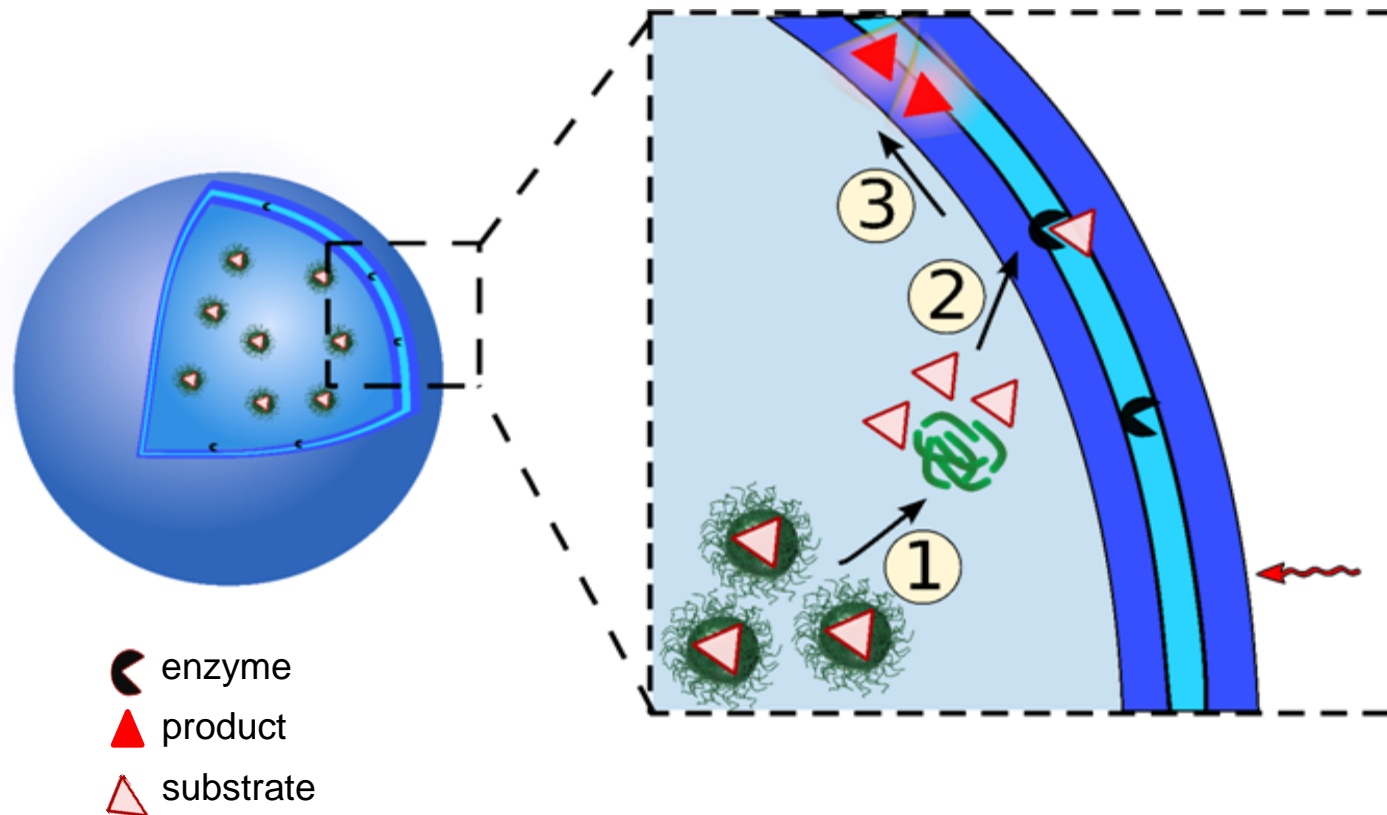


C. Triggered ion channel recruitment

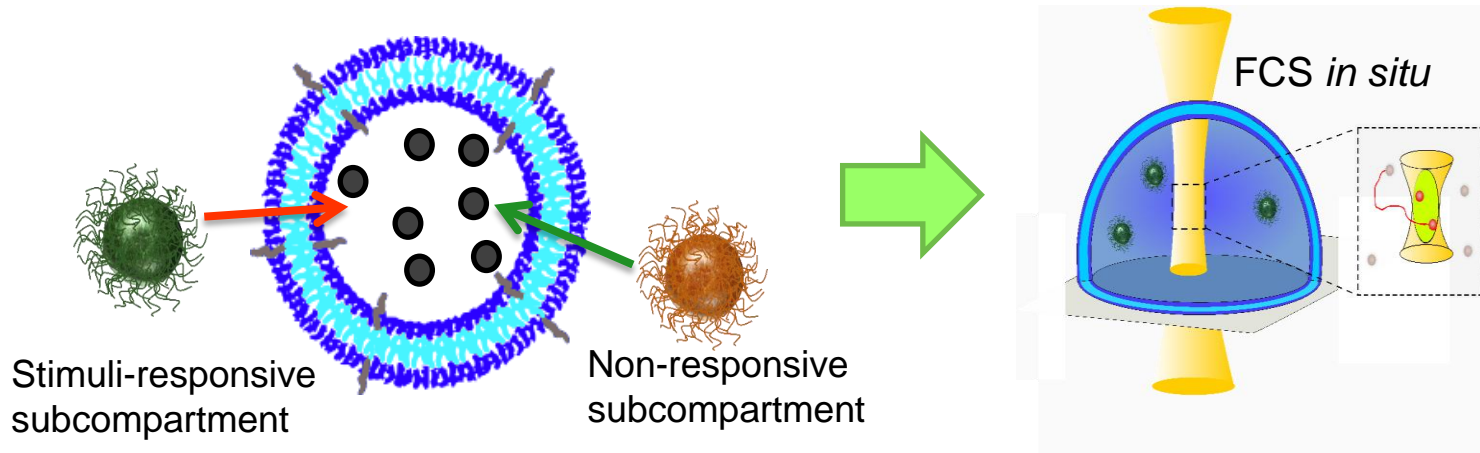


Artificial cells- enzymatic reactions

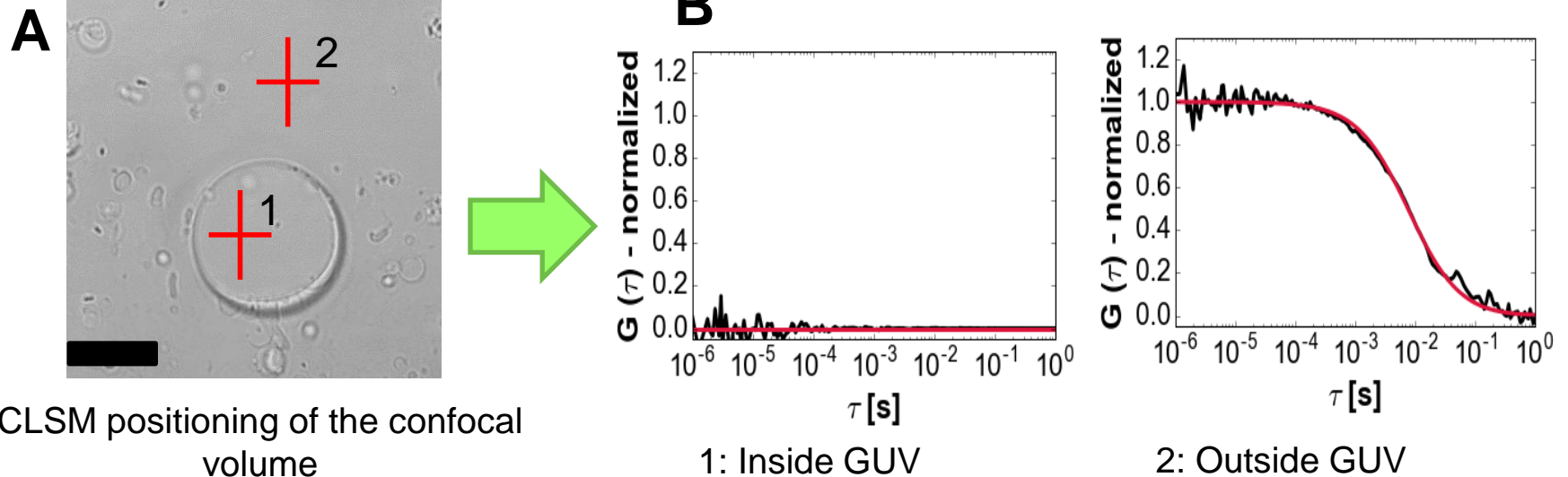
Concept: Triggered activity of enzymes inside artificial cells by stimuli-responsive recombination of sequestered substrates with the enzymes



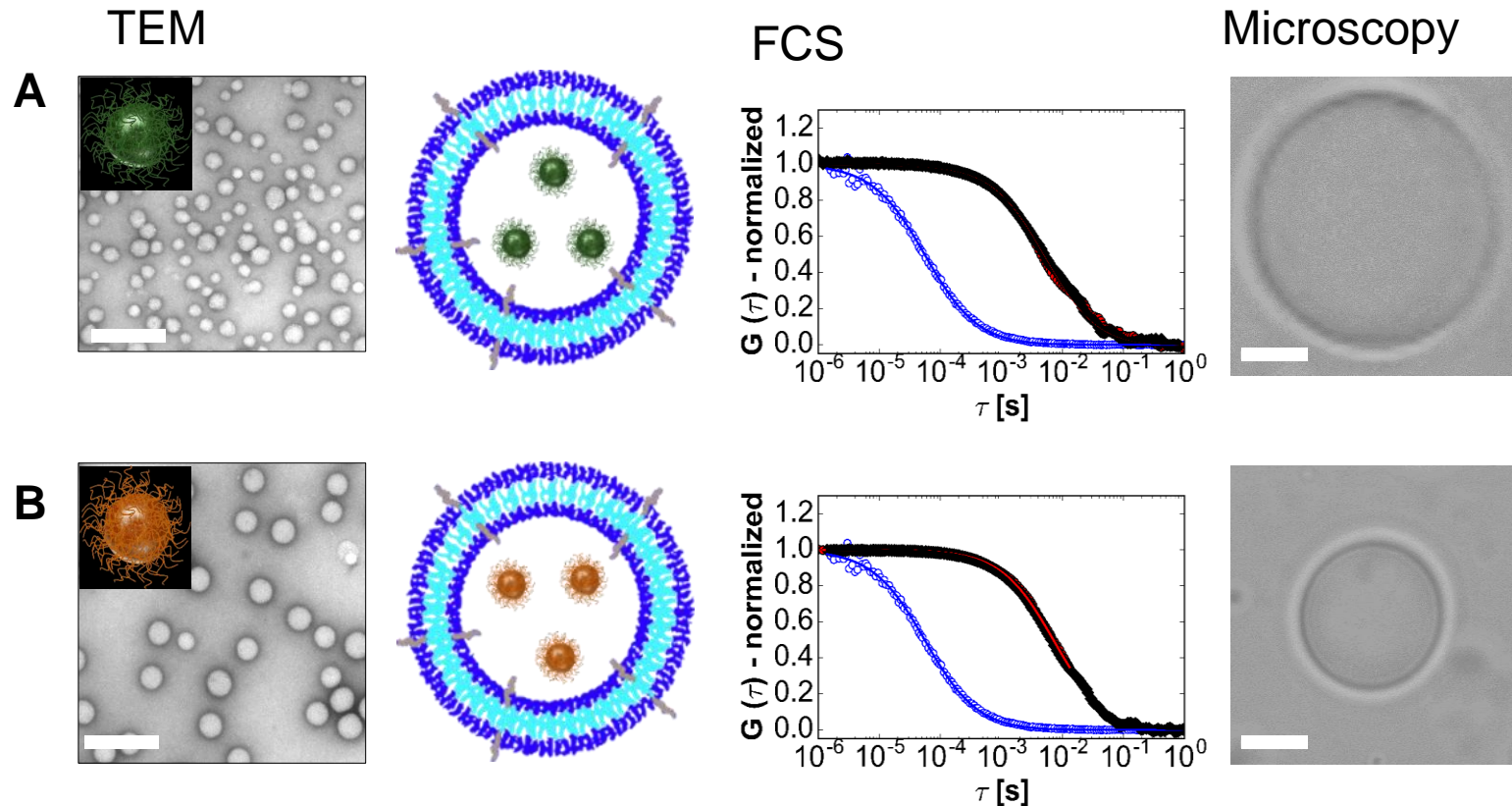
Artificial cells- *in situ* elements



FCS autocorrelation curve

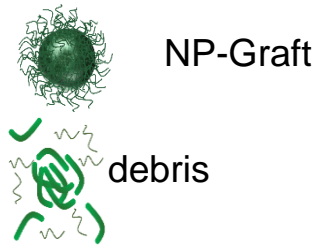
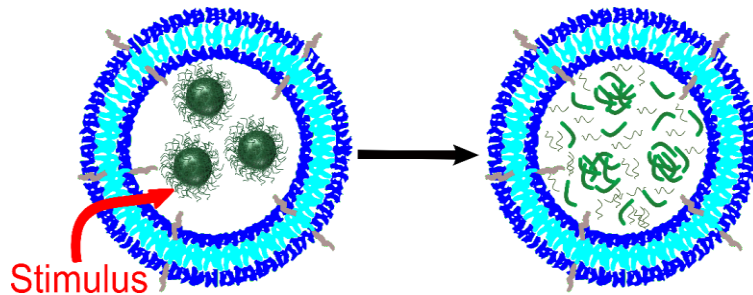


Encapsulation of stimuli-responsive nanoparticles

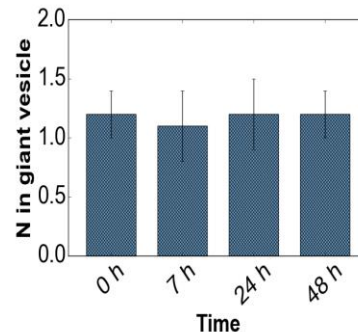
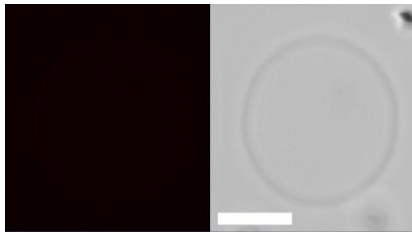
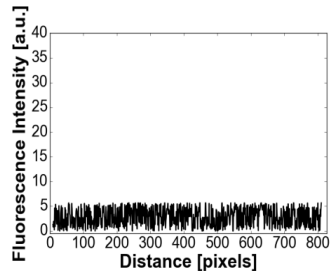


✓ Successful encapsulation of nanoparticles (stimuli-responsive/inert).

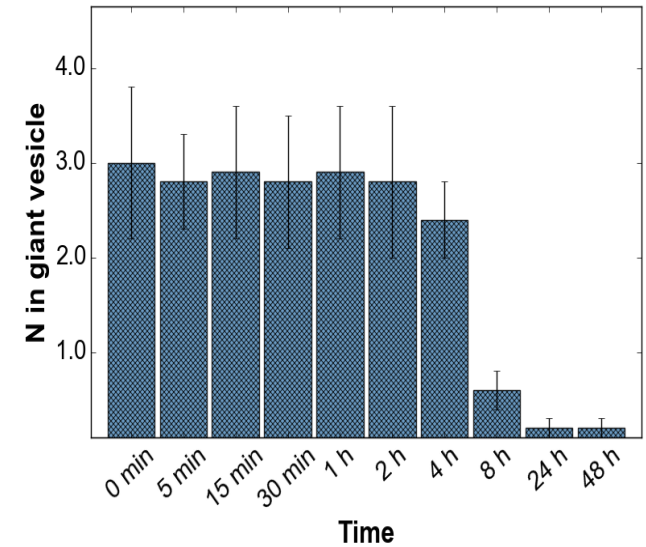
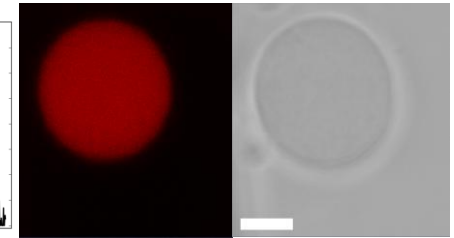
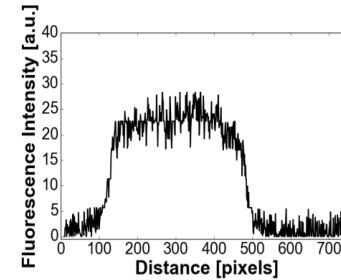
In situ stimuli-responsiveness of the nanoparticles



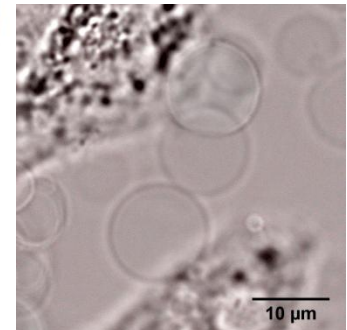
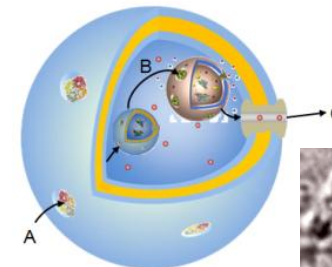
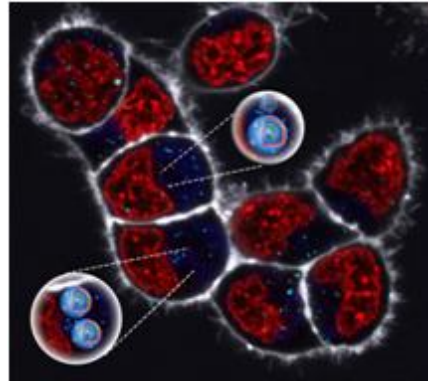
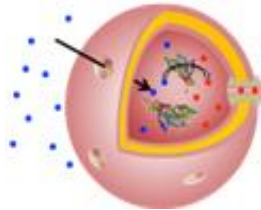
No stimulus:



In the presence of stimulus:



Conclusion and outlook



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

Acknowledgements



Collaborators:

Prof. Wolfgang Meier (University of Basel)
Prof. Sabine Van Doorslaer (Antwerp University)
Prof. Joerg Huwyler (University of Basel)
Prof. Abhay Pandit (NUI Gallaway)
Prof. Dirk Schneider (Mainz University)

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University of Basel