Chemical Robotics in Medicine: Components, Perspectives and Applications

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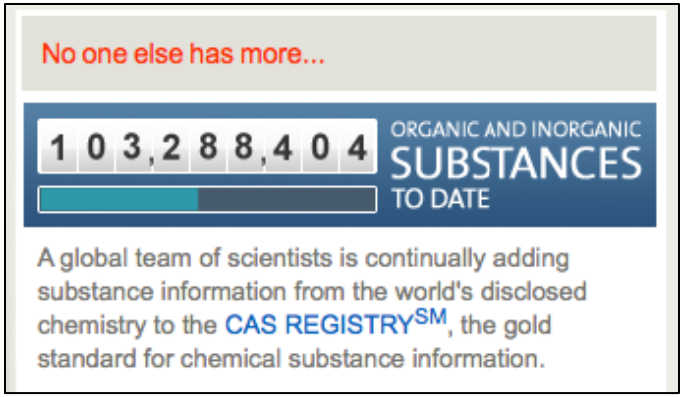
Centralizovaný rozvojový projekt MŠMT č. C29:
„Integrovaný systém vzdělávání v oblasti výskytu a eliminace reziduí léčiv v životním prostředí“

Motivation and Aims

How many molecules are known to mankind?

Source: www.cas.org



How many of them are FDA-approved drugs?

DrugBank
Open Data Drug & Drug Target Database

Statistics

Drug Statistics

Total Number of Small Molecule Drugs	7451
Total Number of Biotech Drugs	289
Total Number of Approved Drugs	1741
Total Number of Experimental Drugs	5064
Total Number of Illicit Drugs	186
Total Number of Withdrawn Drugs	163
Total Number of Drugs	7740

Total Number of Approved Drugs 1741

Motivation and Aims

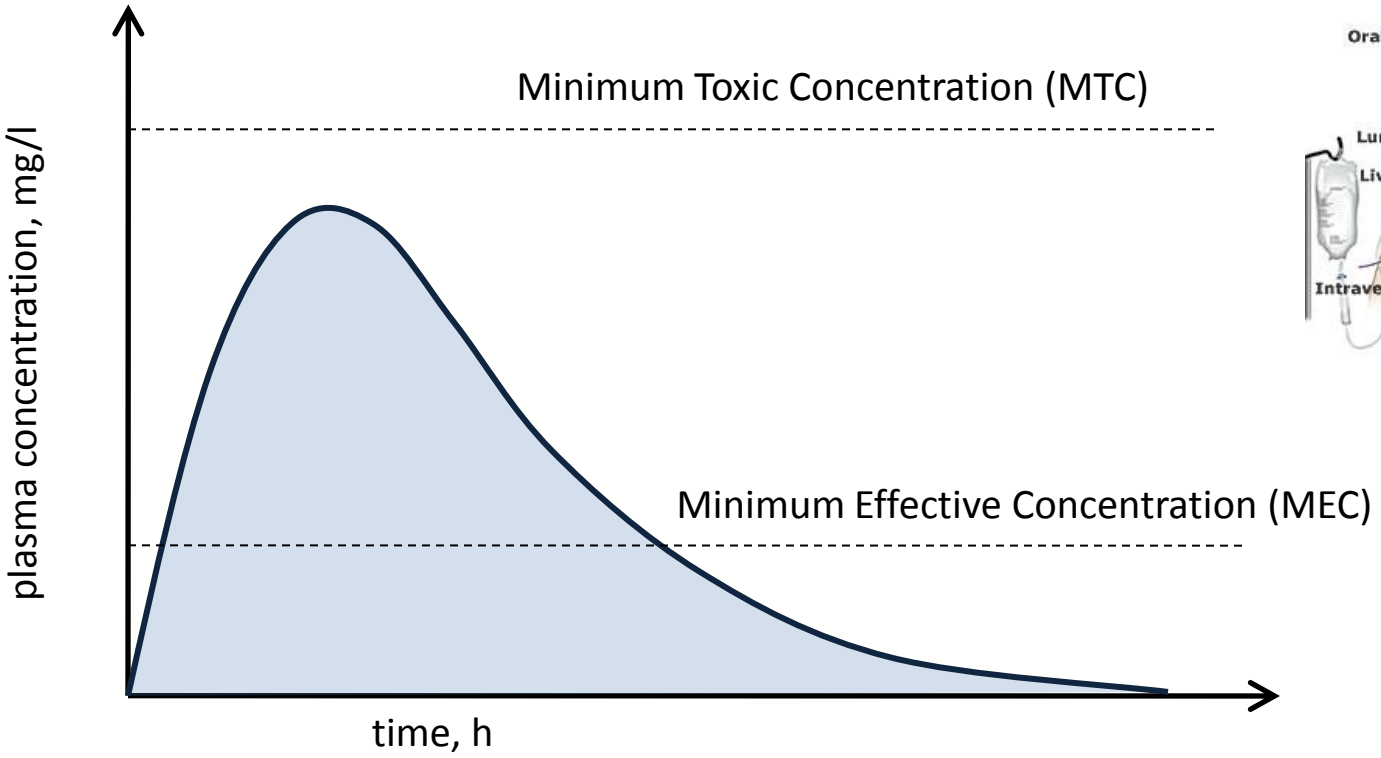
1,741 : 89,578,080 ≈ **1 : 50,000**

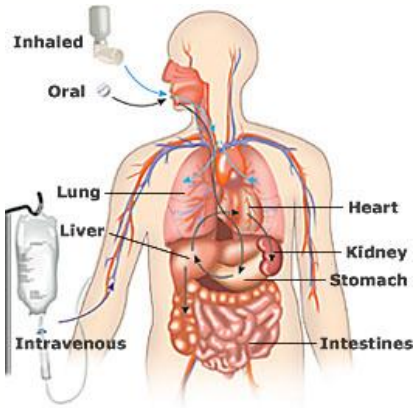
Why?

1. Low/insufficient potency
2. Too high potency/toxicity
3. Unsuitable for established manufacturing, formulation and supply chain paradigms

Problem of high potency

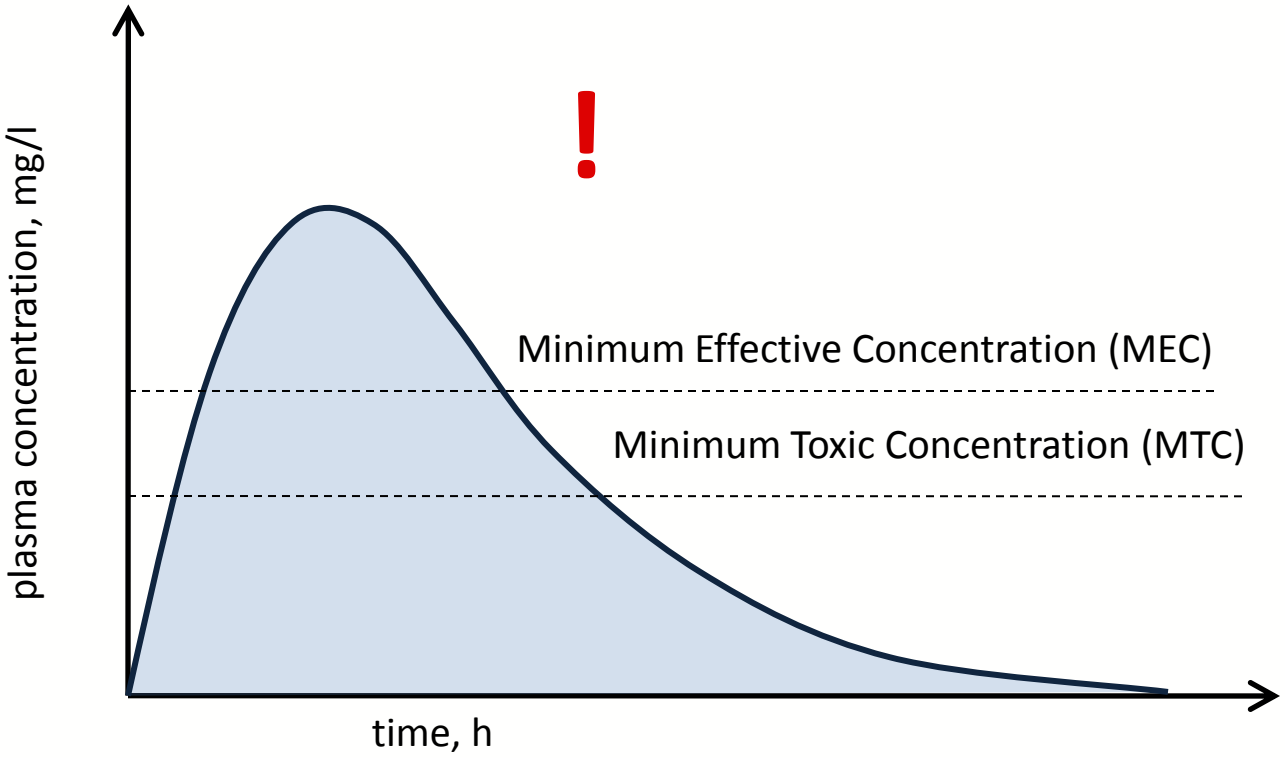
Pharmacokinetic profile – single dose response

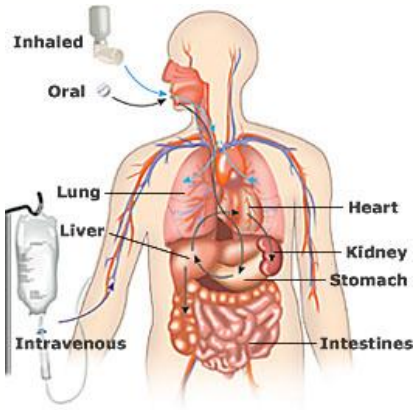




Problem of high potency

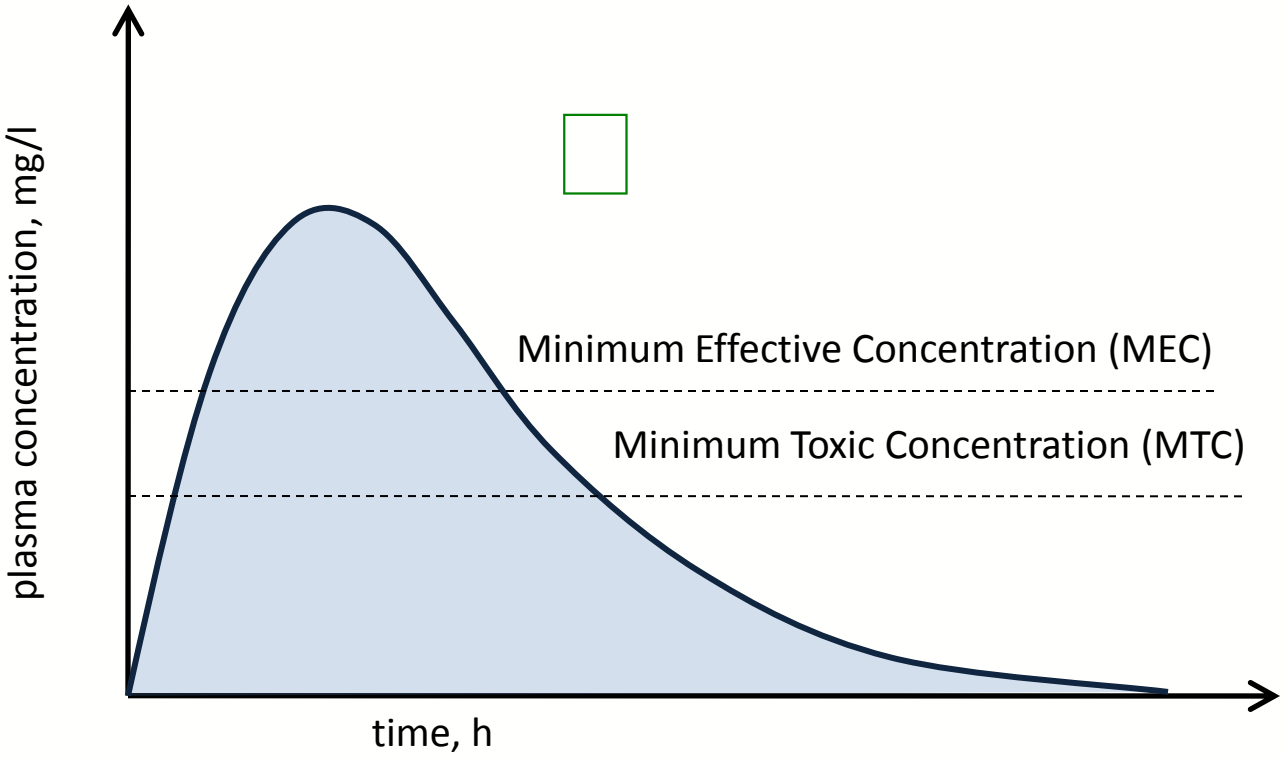
Problem: systemic application of pre-existing API

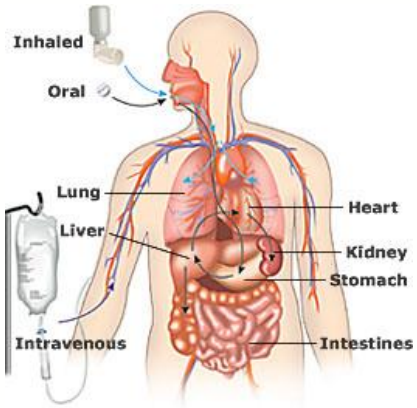




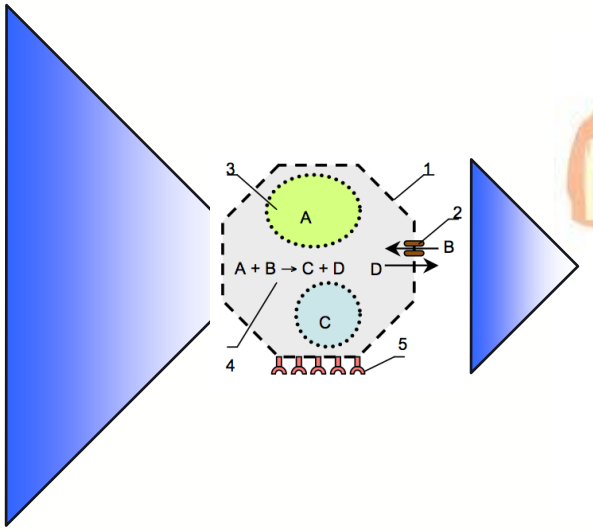
Problem of high potency

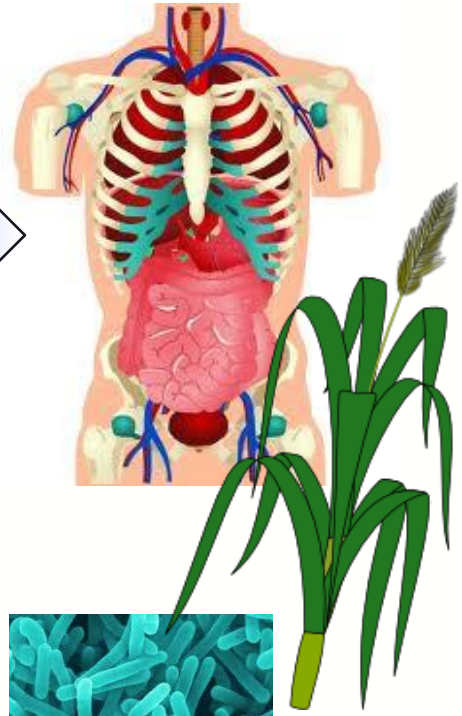
Possible approach: local, in-situ production of API





In-particle production of API



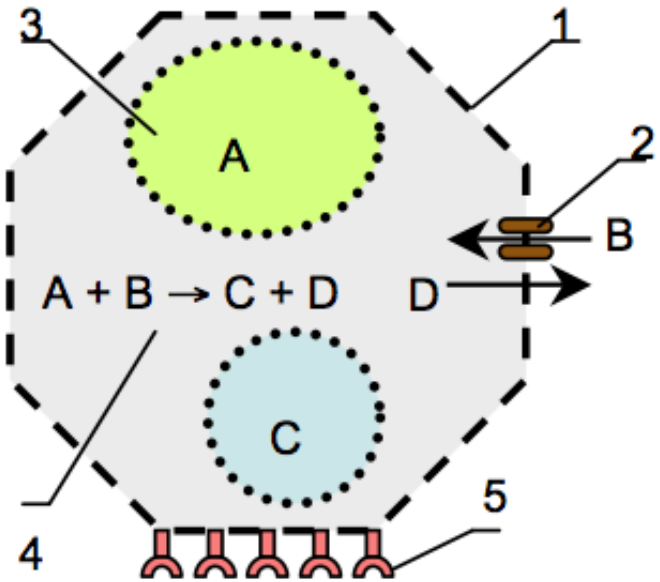


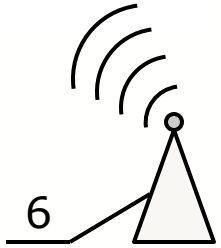
Engineering challenges

1) Design and synthesis of structured microparticles („embodiment“)
2) Control of local reaction-diffusion process
3) Control of particle transport/desposition

Design of a chemical microrobot

Chemical robot = Internally structured functional particle



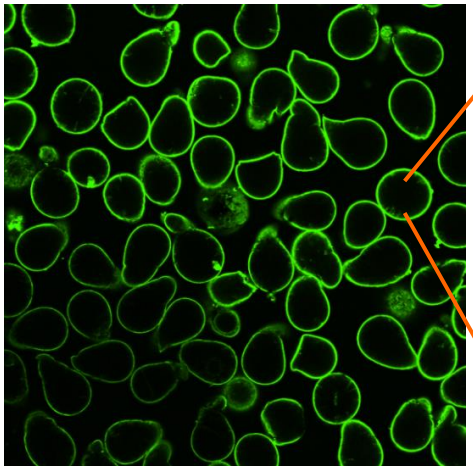


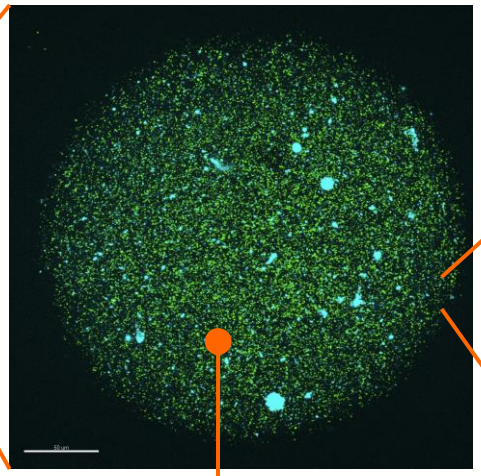
Structural and functional sub-systems:

1. Semi-permeable shell (**integrity**)
2. Variable diffusion rate (**transport**)
3. Internal reservoirs (**storage**)
4. Chemical **reaction** "on-demand"
5. Surface functionalisation (**adhesion**)
6. Remote control (**communication**)

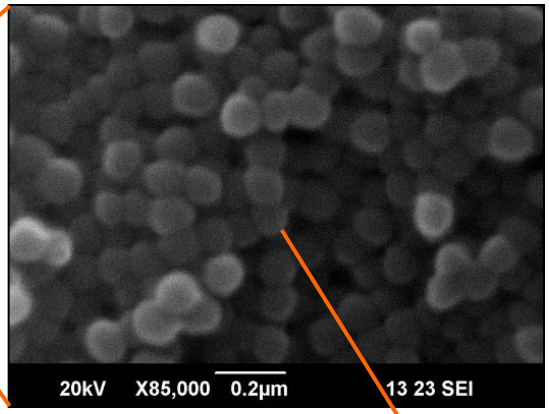
Modularity!!!

Physical implementation



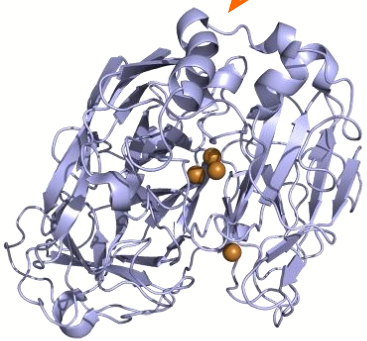


Mesoporous SiO_2 shell
(sol-gel or LbL assembly)

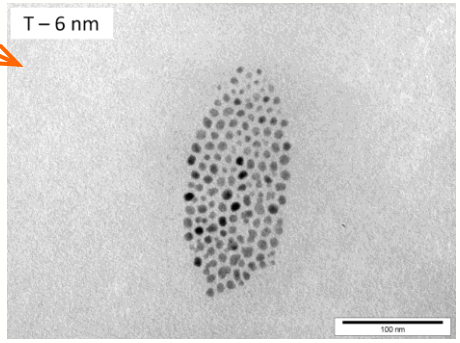


Core-shell hydrogel microparticles

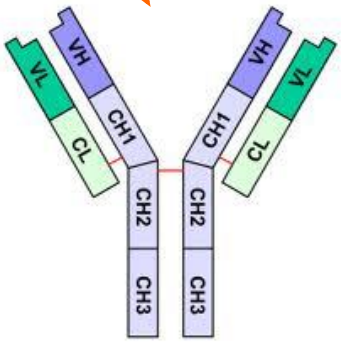
Storage vesicles (reversible, step change of difusion)



Hydrogel with immobilised enzymes

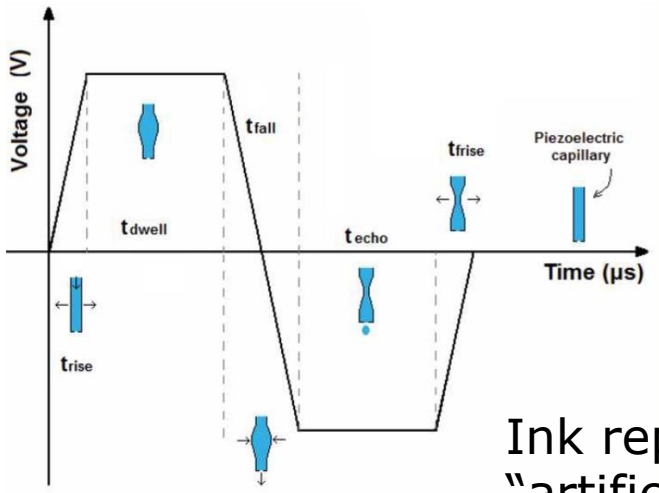


Radiofrequency responsive nanoparticles

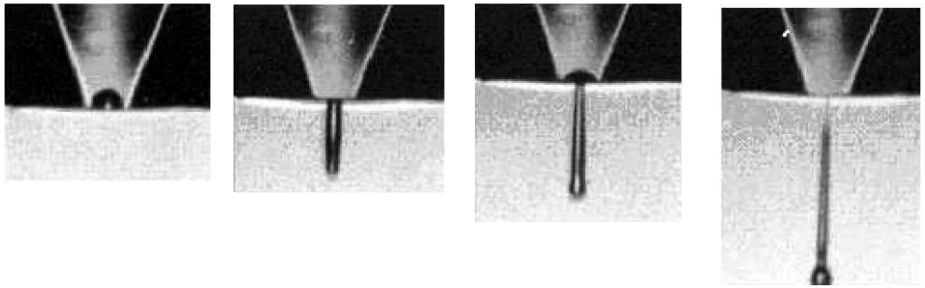


Covalently coupled antibody

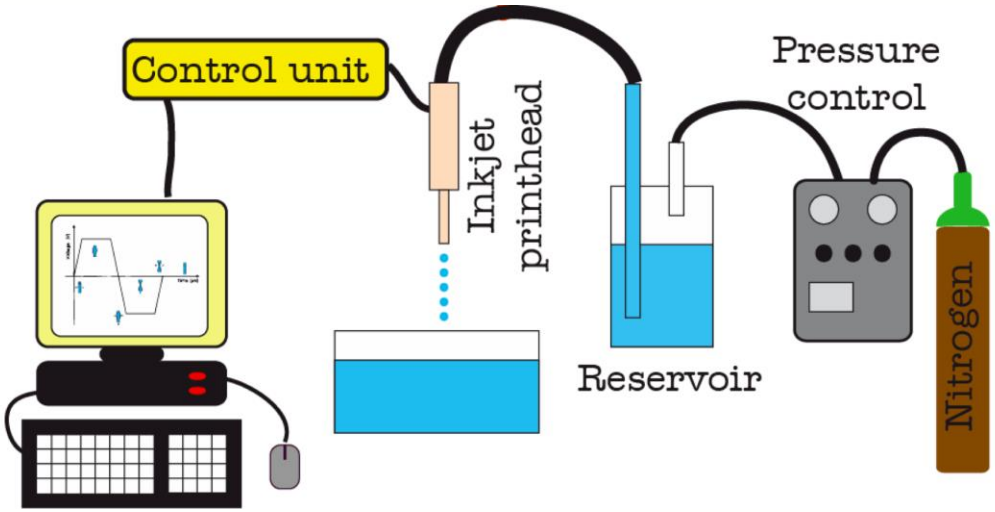
1. Embodiment

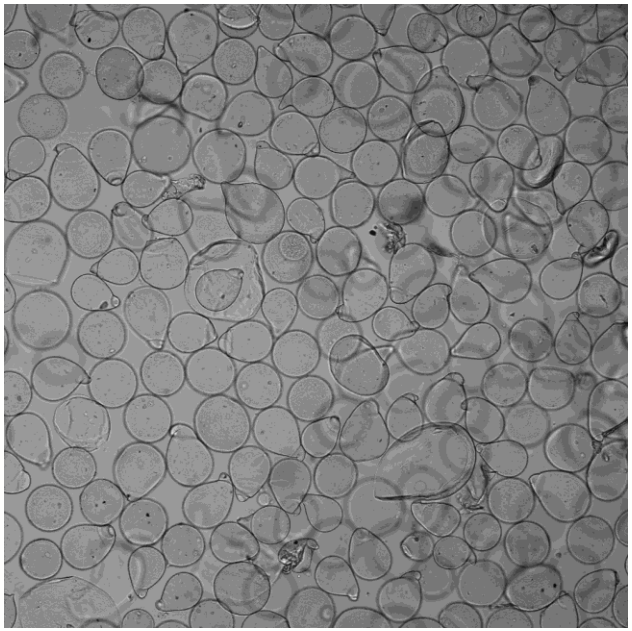


Drop-on-Demand inkjet printing (MicroFab Inc.)



Ink replaced by "artificial cytoplasm"

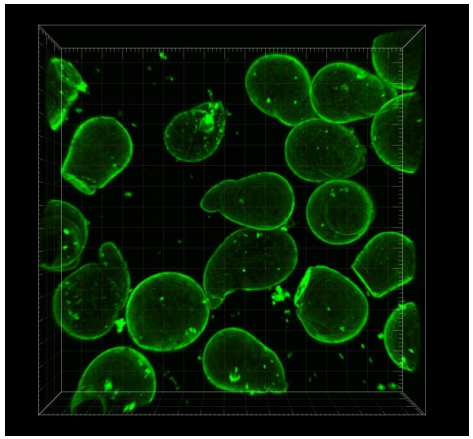


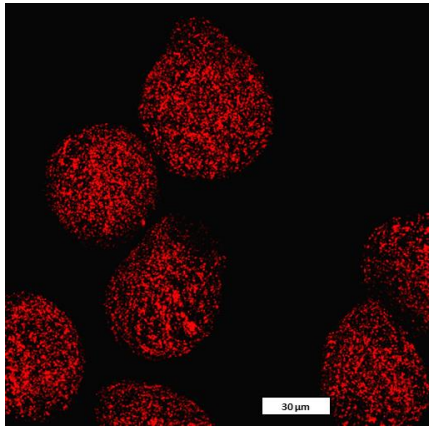


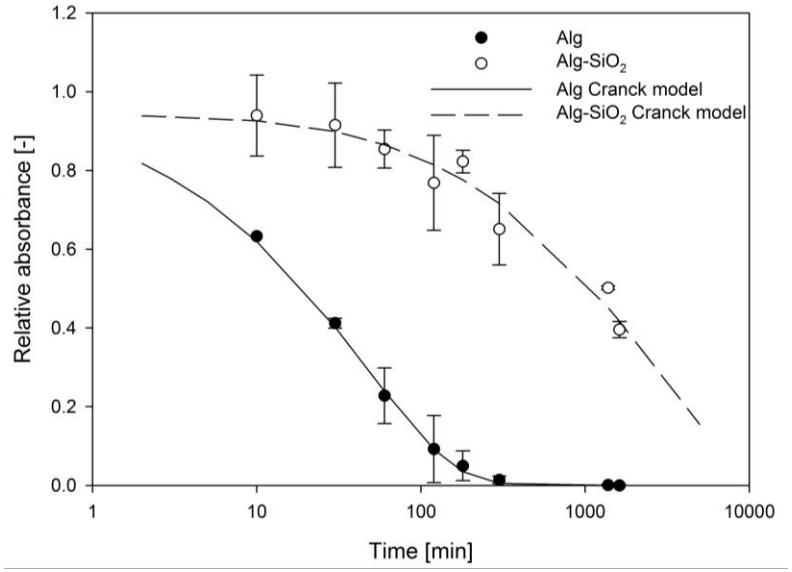
Powder Technol. 200 (2010) 254-259
Chem. Eng. Sci. 66 (2011) 3829-3835

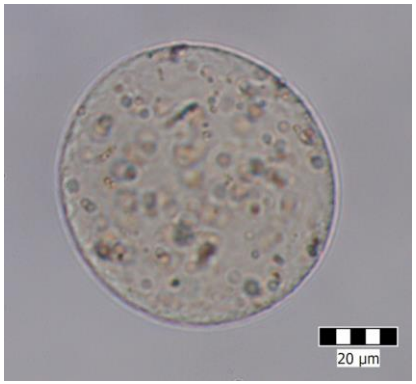
1. Embodiment

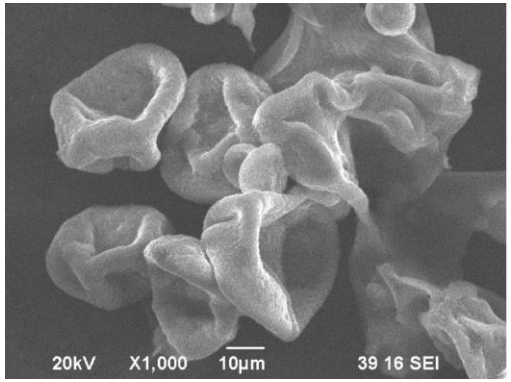
- Sol-gel or LbL (layer-by-layer) deposition process
- SiO_2 layer is flexible but improves chemical and mechanical stability
- Prevents leakage of internal components and allows surface functionalisation





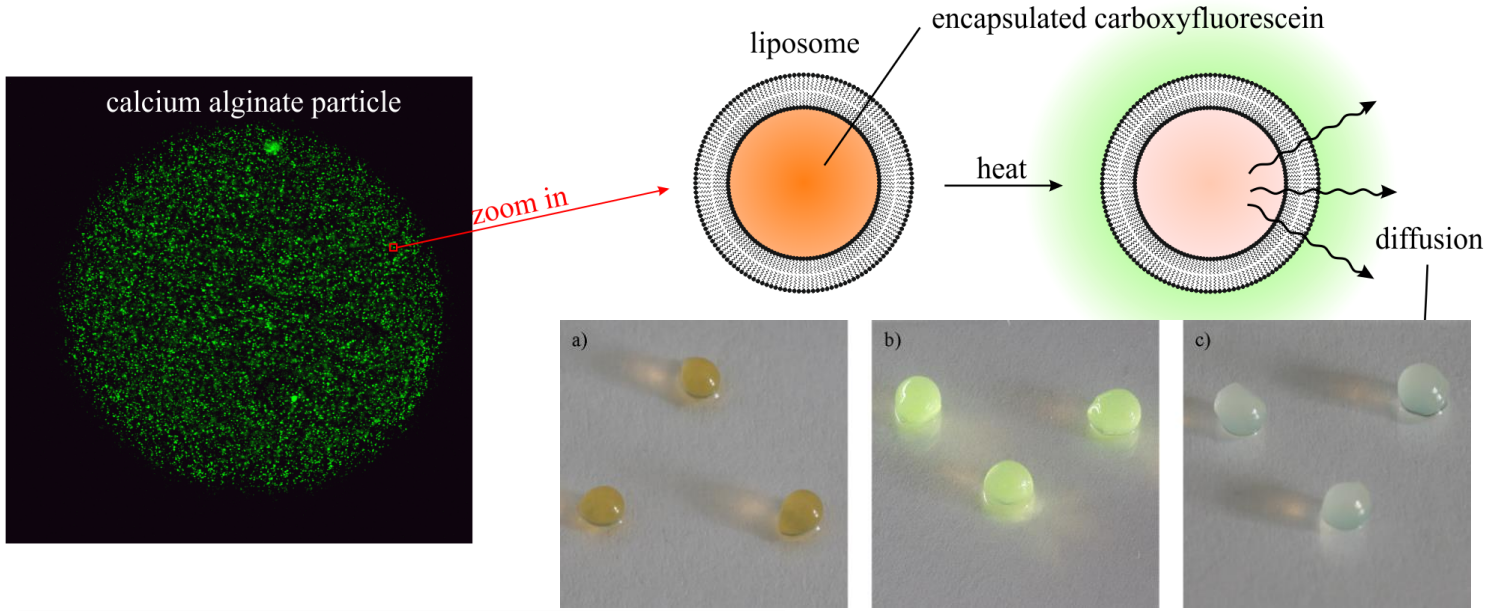


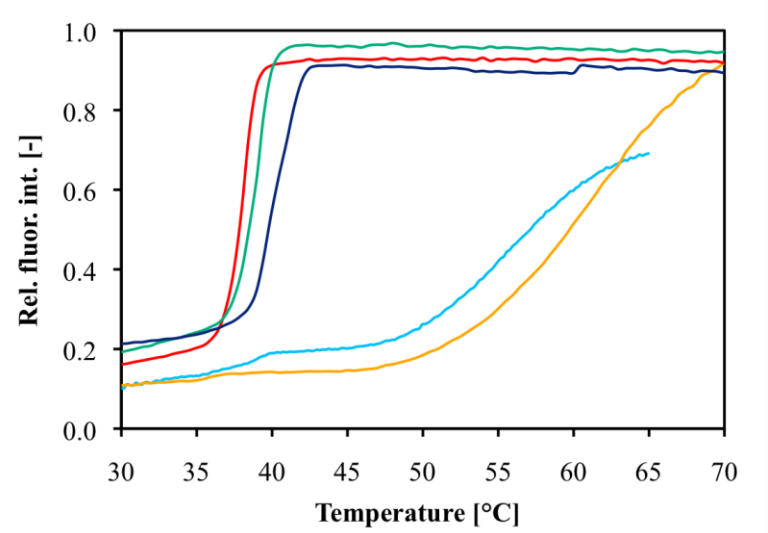


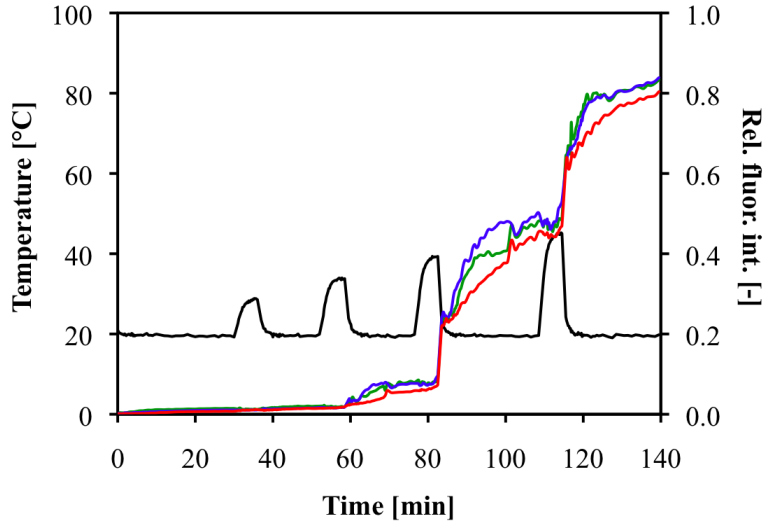


J. Coll. Interf. Sci. 357 (2011) 109-115
Coll. Surf A 410 (2012) 52-57

2. Storage and Release

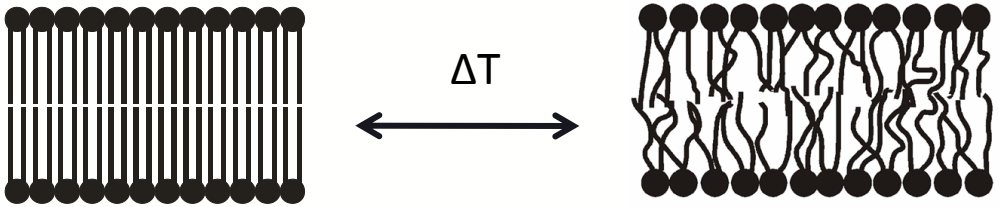


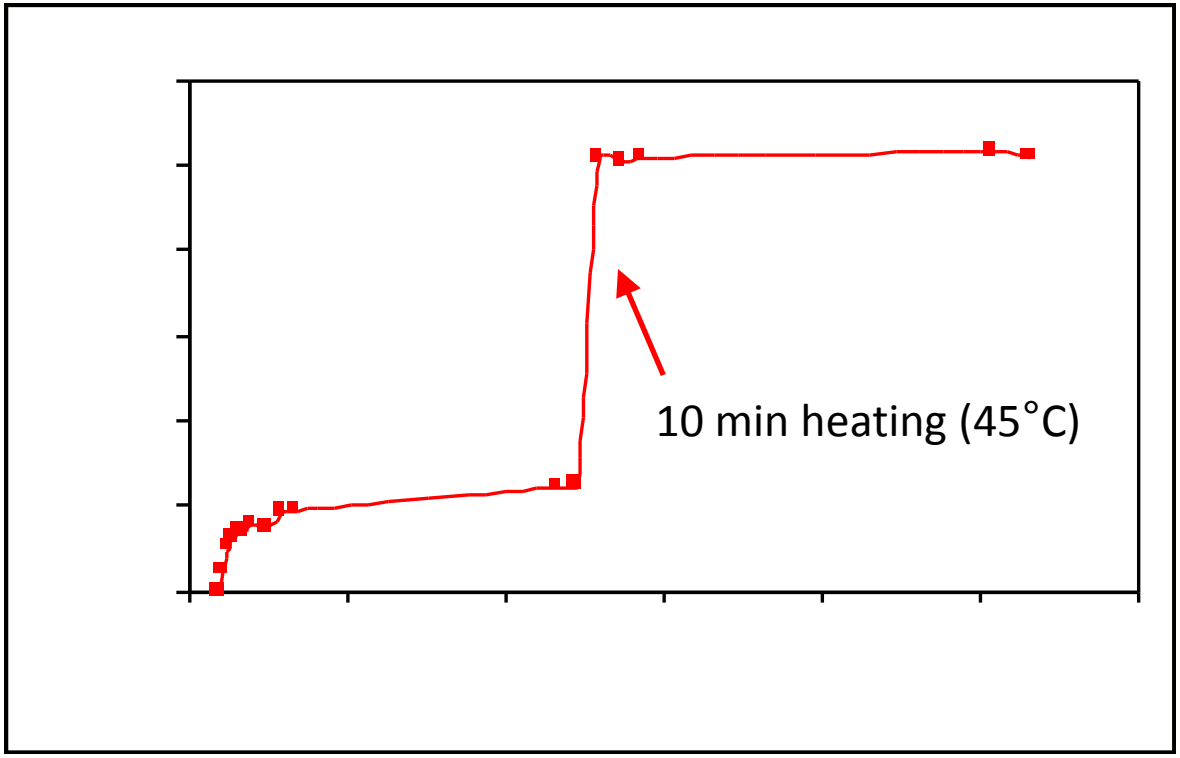




2. Storage and Release

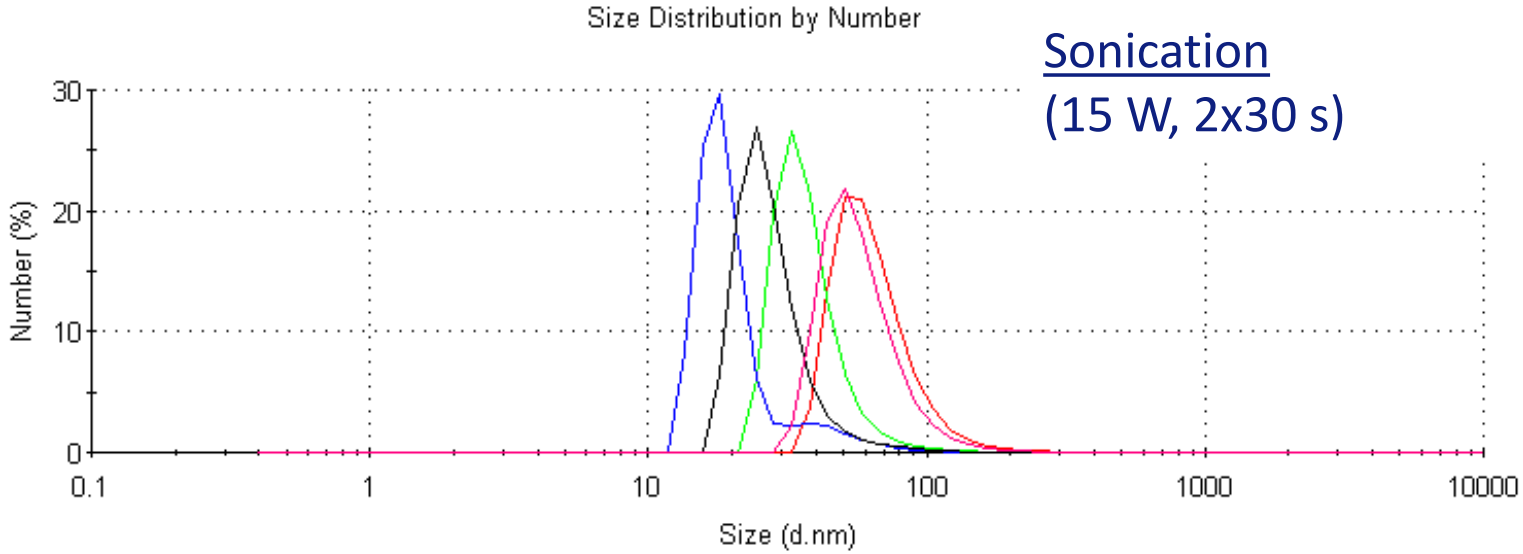
Phospholipid bi-layer



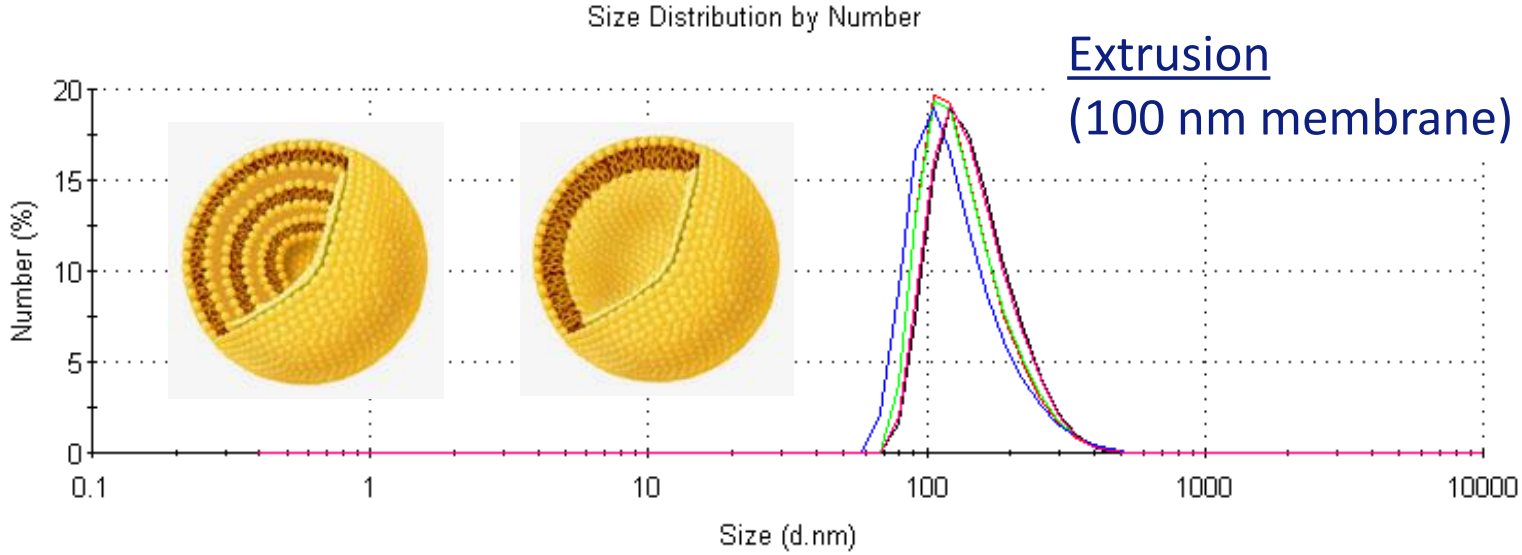


J. Coll. Interf. Sci. **394**, 380-385 (2013)

2. Storage and Release

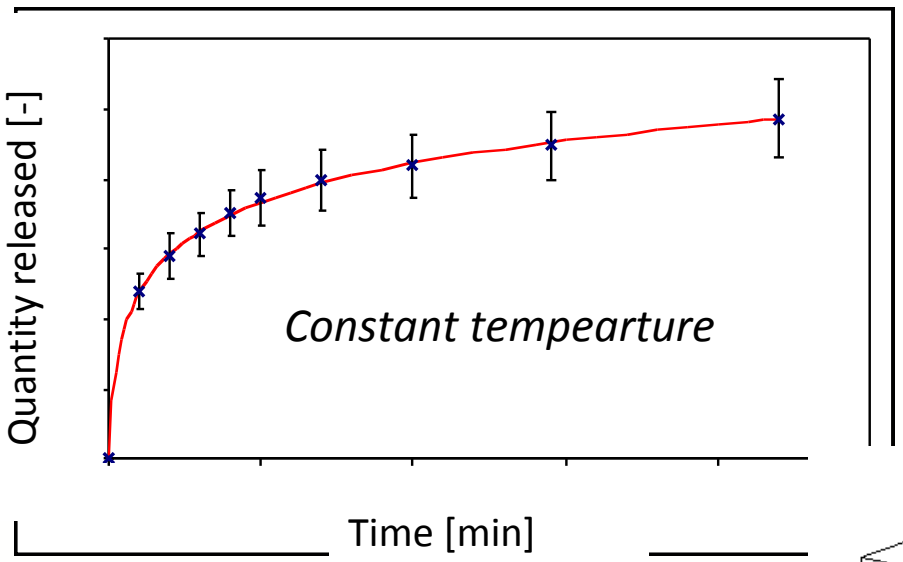


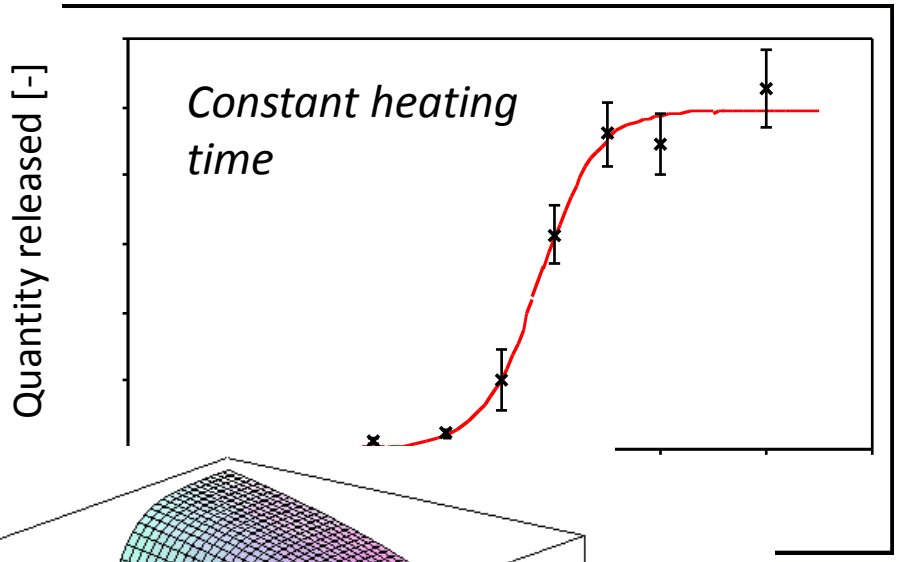
Sonication
(15 W, 2x30 s)

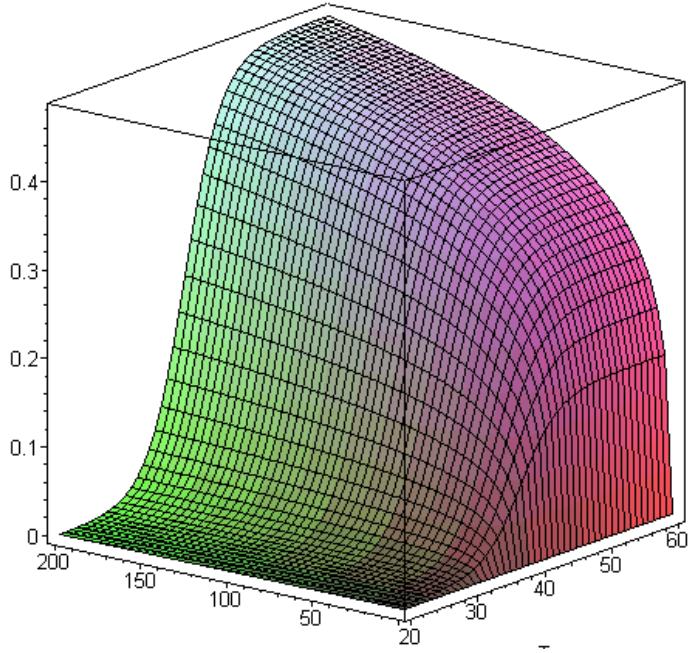


Extrusion
(100 nm membrane)

2. Storage and Release





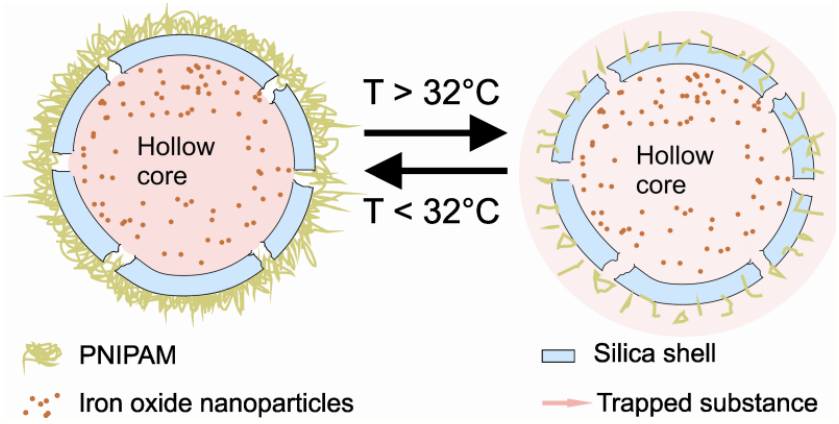


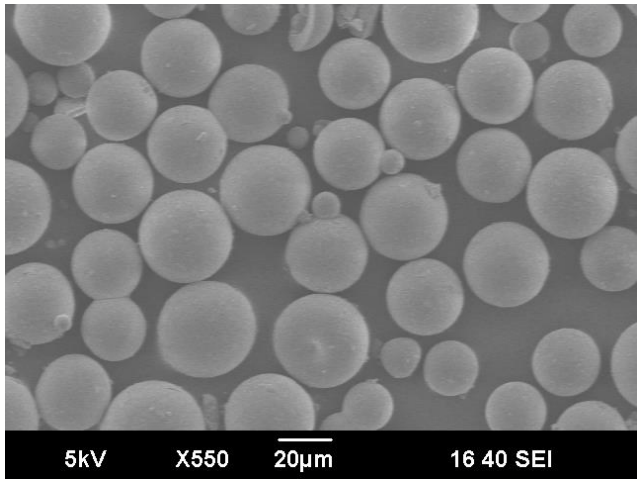
Parametric **response surface** is the basis for diffusion control

$$I_{rel}(t,T) = \frac{0{,}08129\ln(1{,}794t+1)}{1+e^{-0{,}3676(T-38{,}59)}}$$

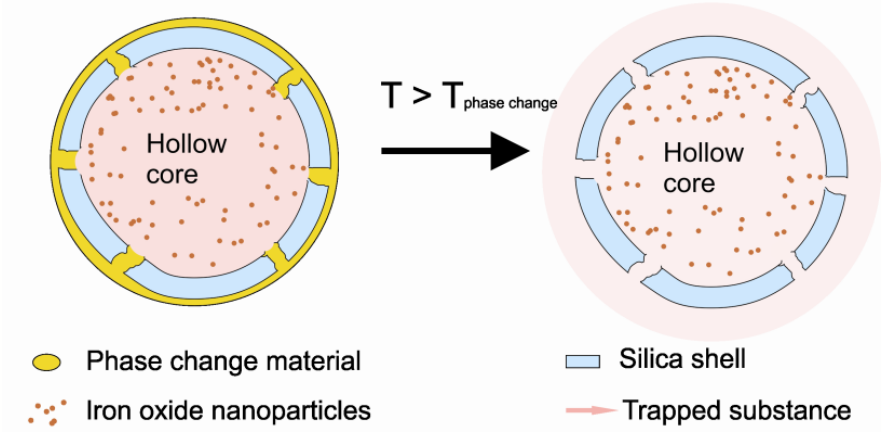
2b. Storage and Release – other mechanisms

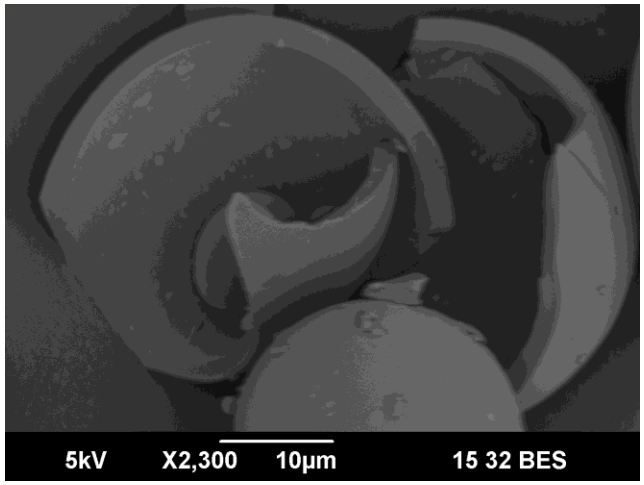
Gating effect using grafted polymer brushes



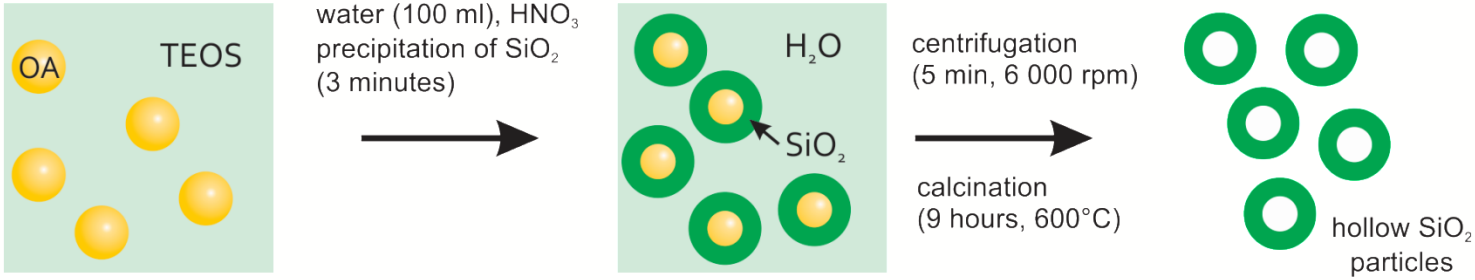


Gating effect using a phase change material

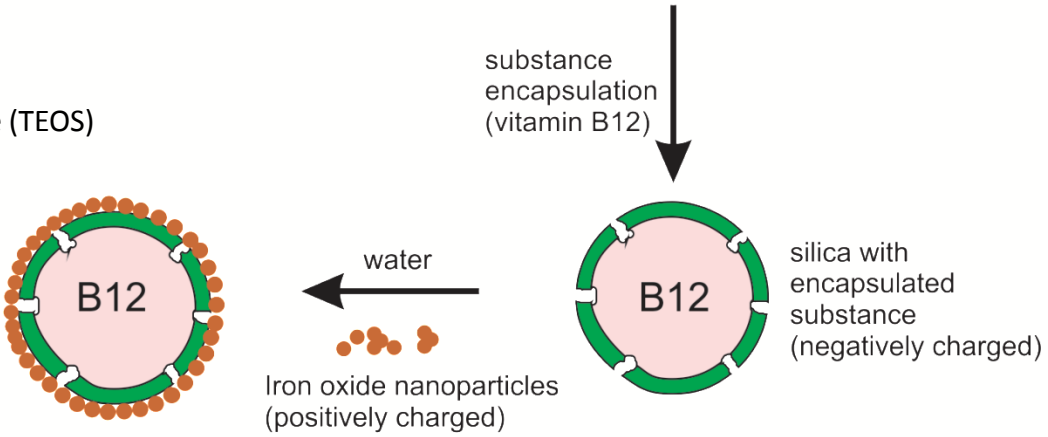


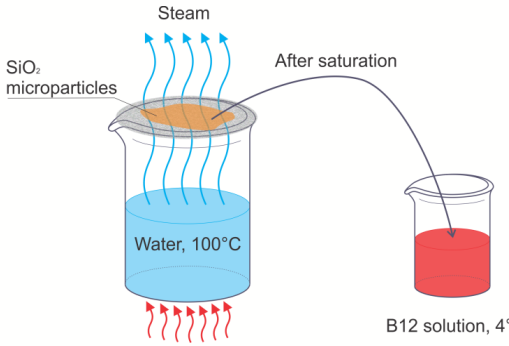


2b. Storage and Release – other mechanisms



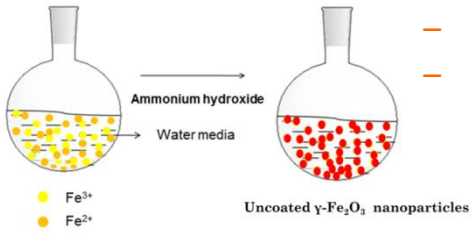
- Soft templating method
  - Silica precursor – tetraethyl orthosilicate (TEOS)
  - Liquid templates – octylamine (OA)
- Substance encapsulation
  - Using hot water vapour to displace the air contained in the holow core
  - Pressure difference across the shell



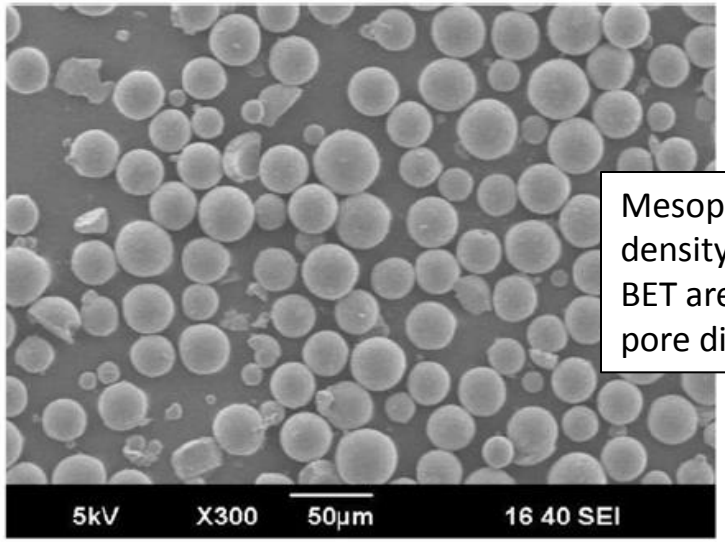


composite
iron oxide/silica
particles

- Superparamagnetic iron oxide nanoparticles
  - Co-precipitation method
  - $Fe^{2+} + 2\ Fe^{3+} + 8\ OH^- \rightarrow Fe_3O_4 + 4\ H_2O$

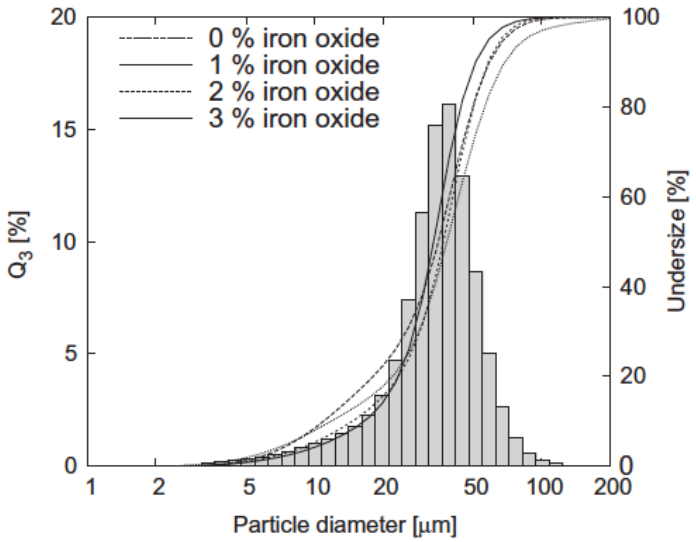


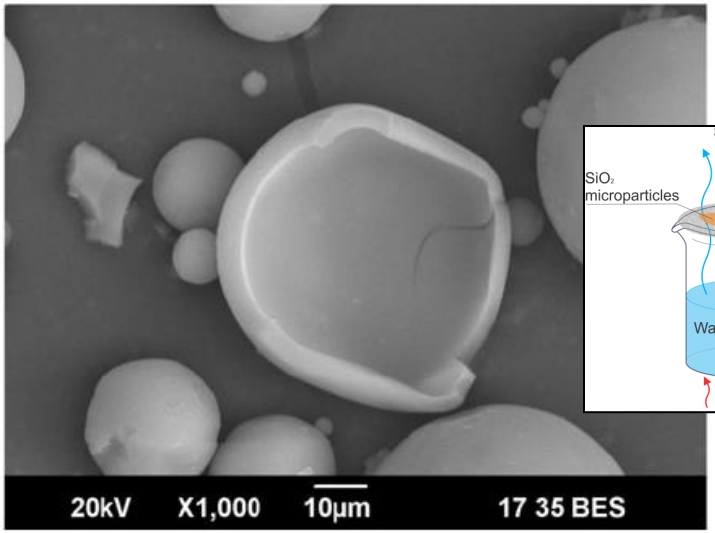
2b. Storage and Release – other mechanisms



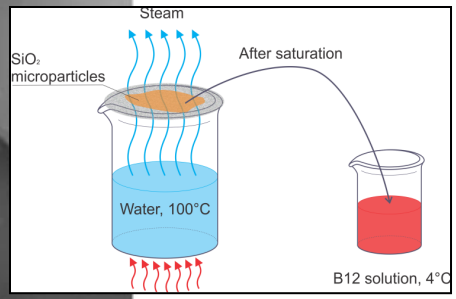
Size distribution

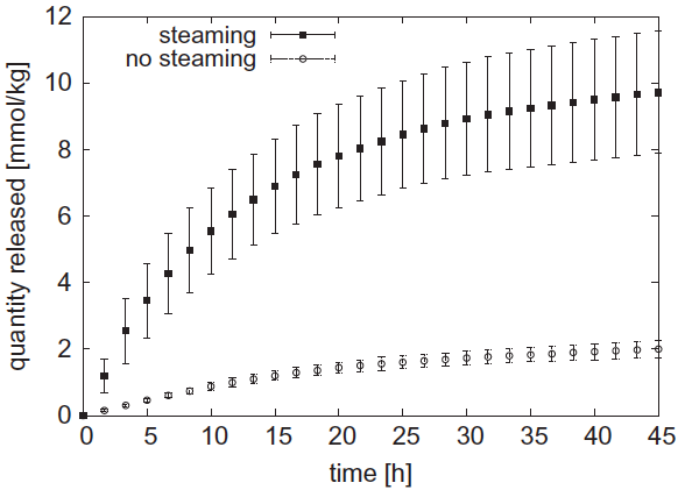
Mesoporous SiO_2 shell:
density 2.095 g/cm^3
BET area 834.6 m^2/g
pore diameter 6.03 nm





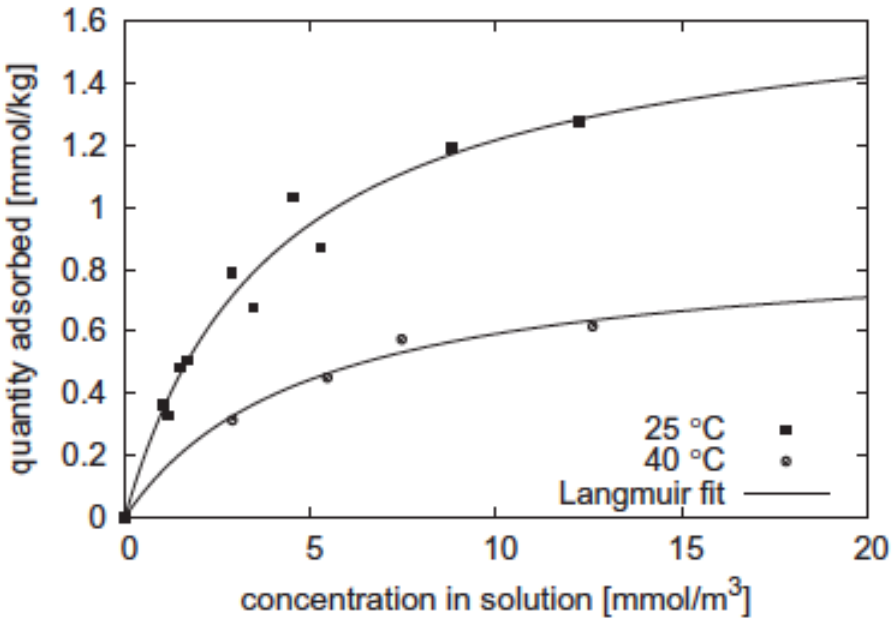
Particle loading



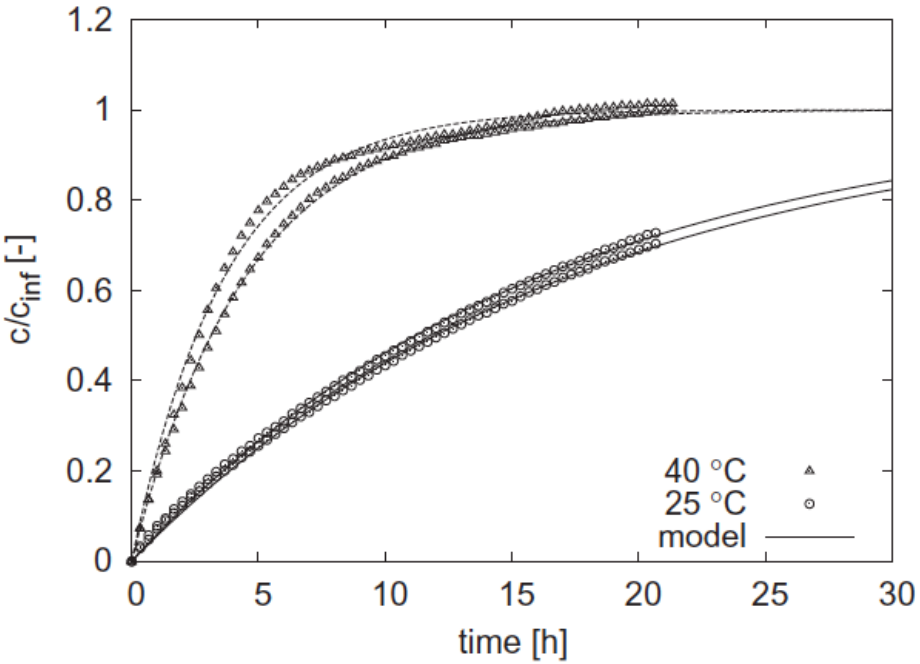


2b. Storage and Release – other mechanisms

Adsorption equilibrium $q = q_m \frac{K_a c}{1 + K_a c}$



Diffusion model $c(t) = c_\infty(1 - \exp(-\beta t))$



$$\frac{d\Delta c}{dt} = -A\frac{D_{eff}}{\delta}\Delta c\left(\frac{1}{V_p} + \frac{1}{V_{out}}\right)$$

$$V_p = V_c + \epsilon V_s + (1-\epsilon)V_s\rho_s\frac{\partial q}{\partial c}$$

D_{eff} (25 °C) = 2.58 ± 0.67 x 10^{-10} cm^2/s

D_{eff} (40 °C) = 3.69 ± 0.21 x 10^{-10} cm^2/s

Micropor. Mesopor. Mater. **159**, 119-125 (2012)
Chem. Eng. J. **232**, 591-598 (2013)

3. Communication & Control

Remotely controlled toys

Remotely controlled chemistry

3. Communication & Control

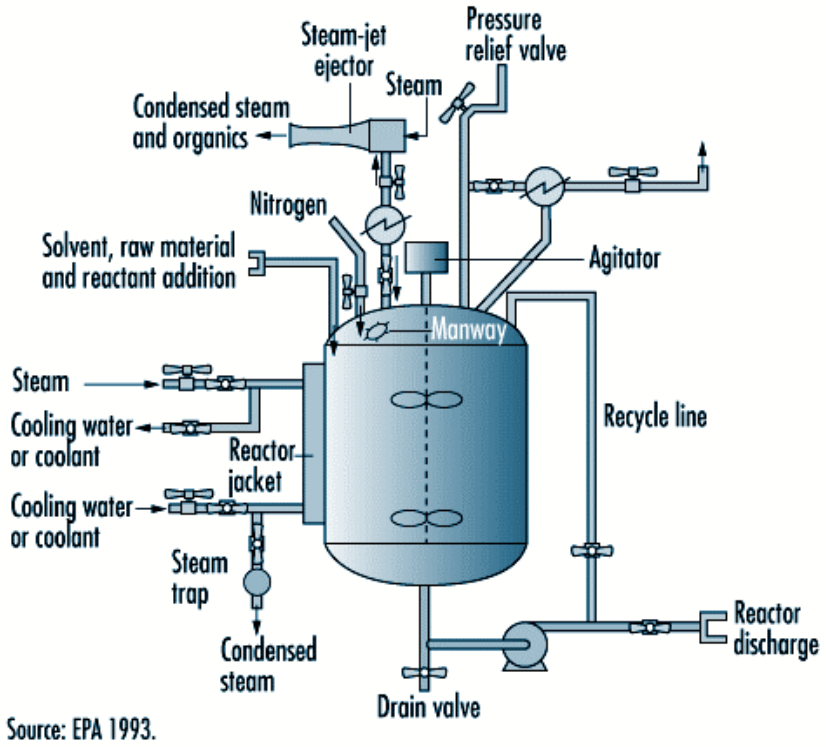
$$A \rightarrow products$$

$$r_A = -k(T)c_A = -k_\infty \exp\left(\frac{-E_a}{RT}\right)c_A$$

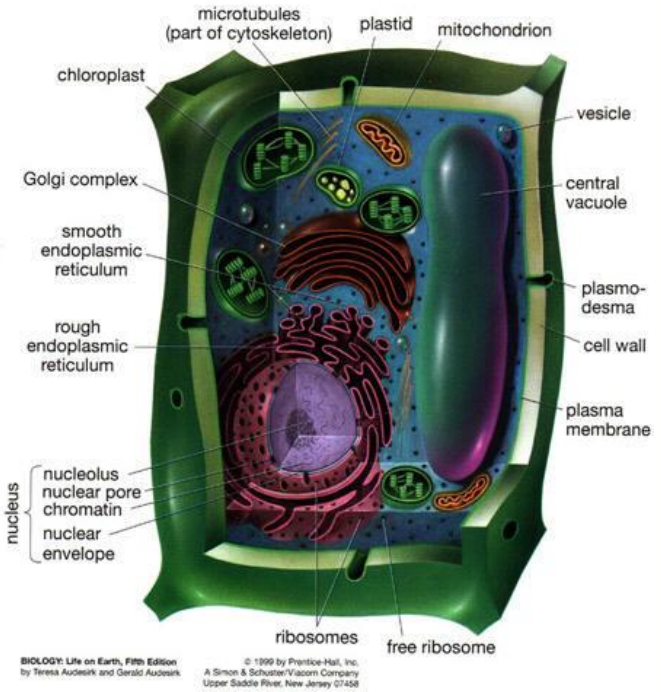
Presence of a catalyst

Reactant concentration

Temperature

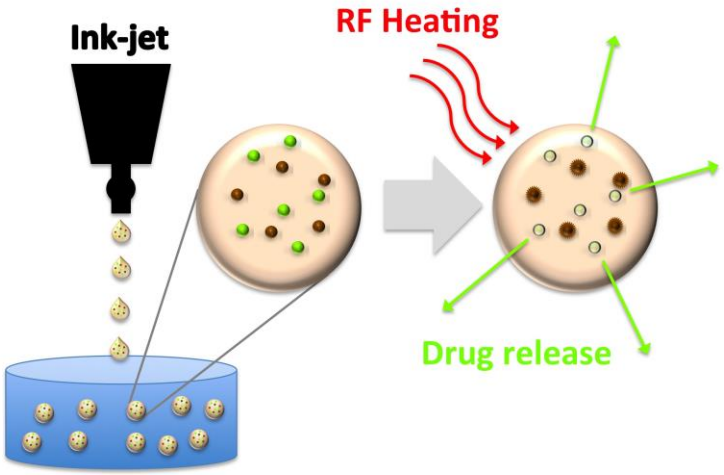


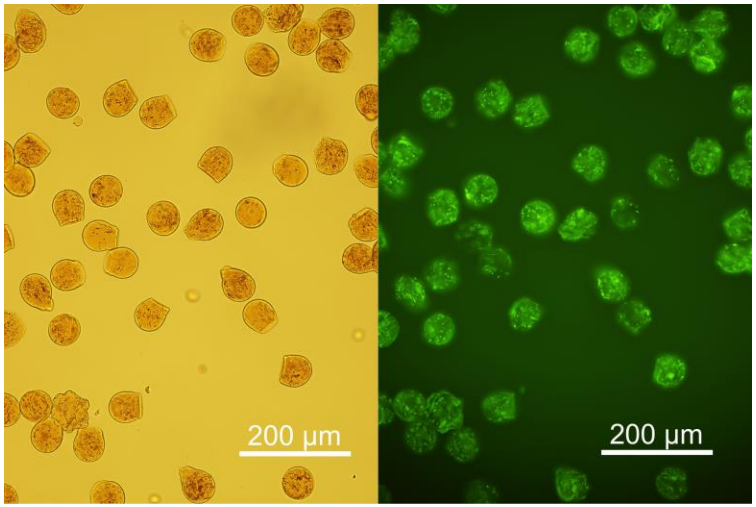
Chemical reactor

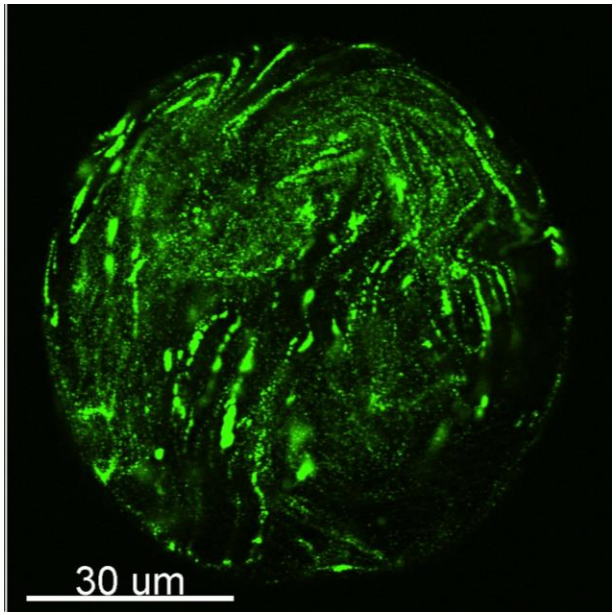


Cell

3. Communication & Control

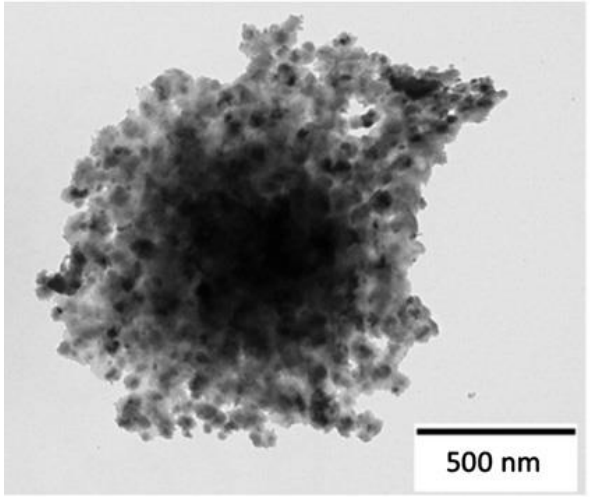




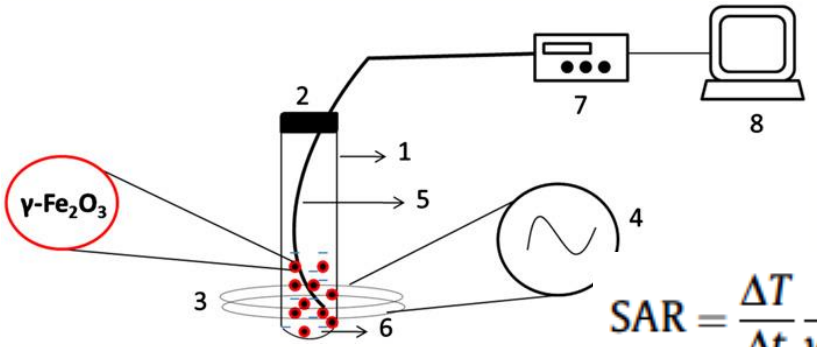


J. Coll. Interf. Sci. ***394*** *(2012) 380-385*
Coll. Surf A ***410*** *(2012) 52-57*
Langmuir ***29*** *(2013) 4381-4387*

3. Communication & Control

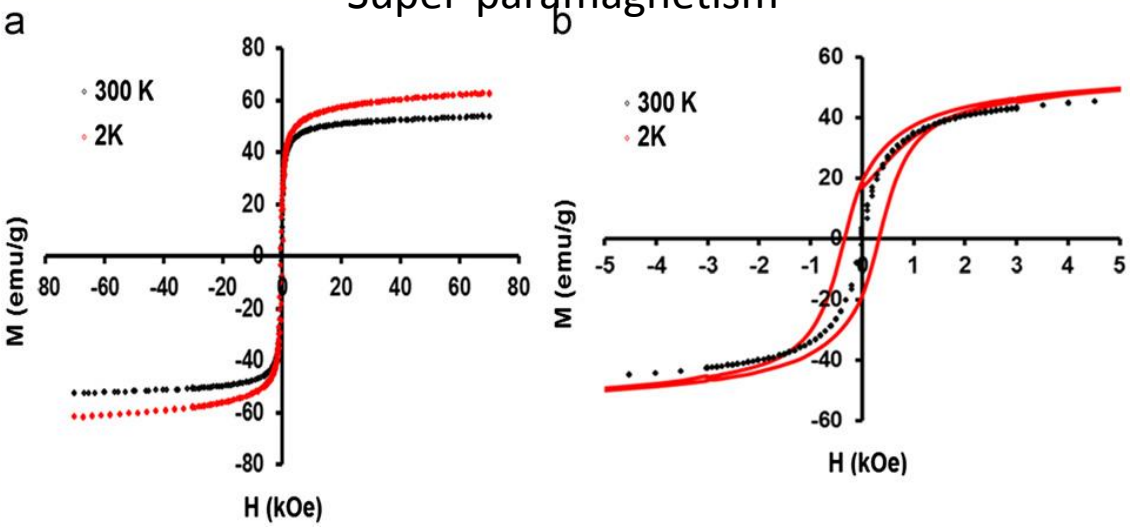


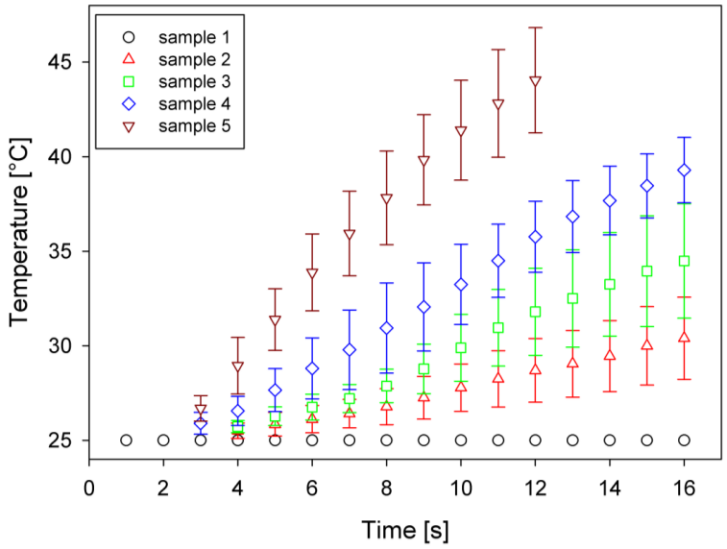
20 mT, 400 kHz, 2.5 kW RF coil
(CEIA PowerCube 32/400)



$$SAR = \frac{\Delta T}{\Delta t}\frac{c_p}{w_{Fe_3O_4}}$$

Super-paramagnetism

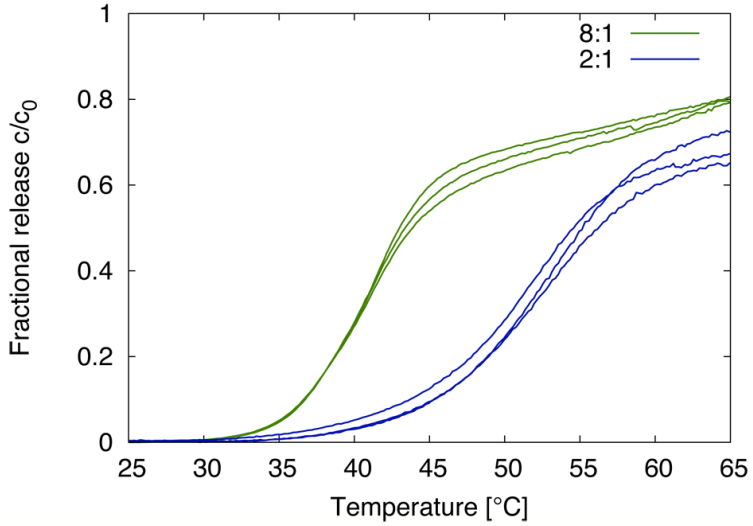




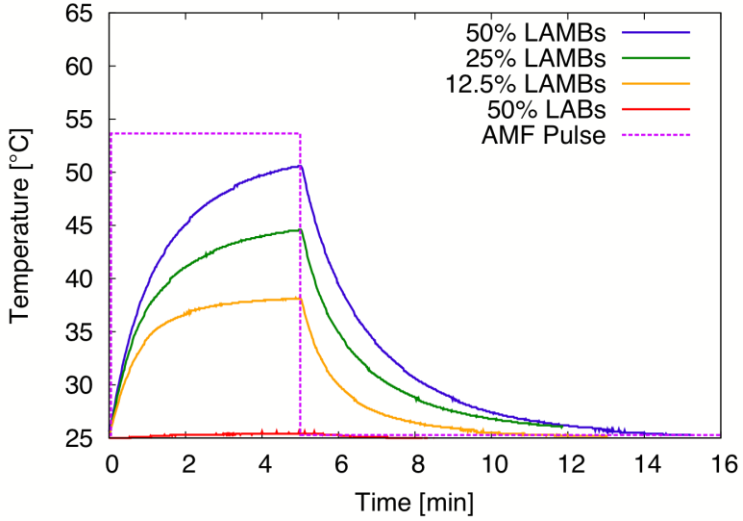
J. Solid. State Chem. **200** (2013) 150-156

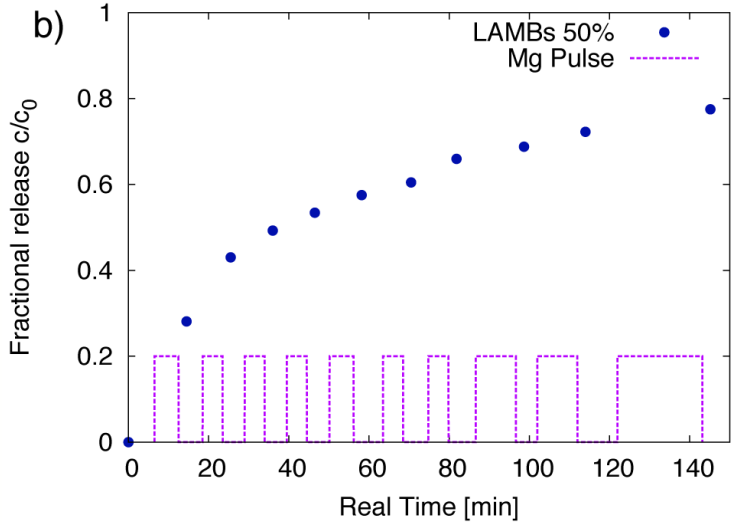
3. Communication & Control

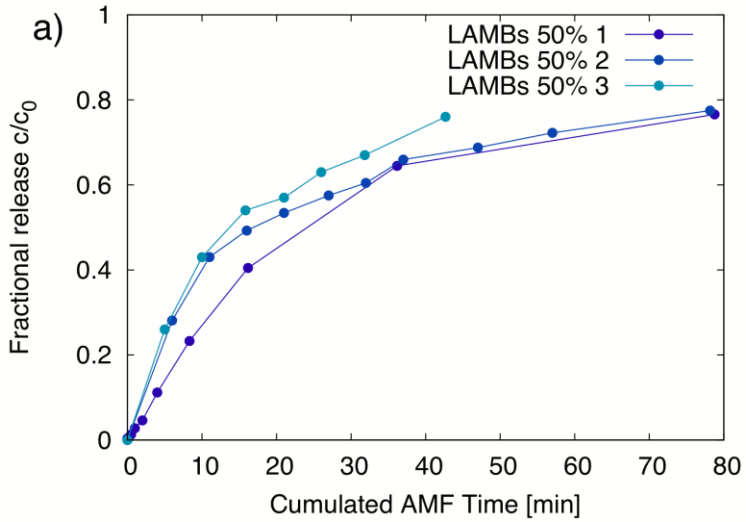
Effect of DPPC:cholesterol ratio



Effect of particle concentration

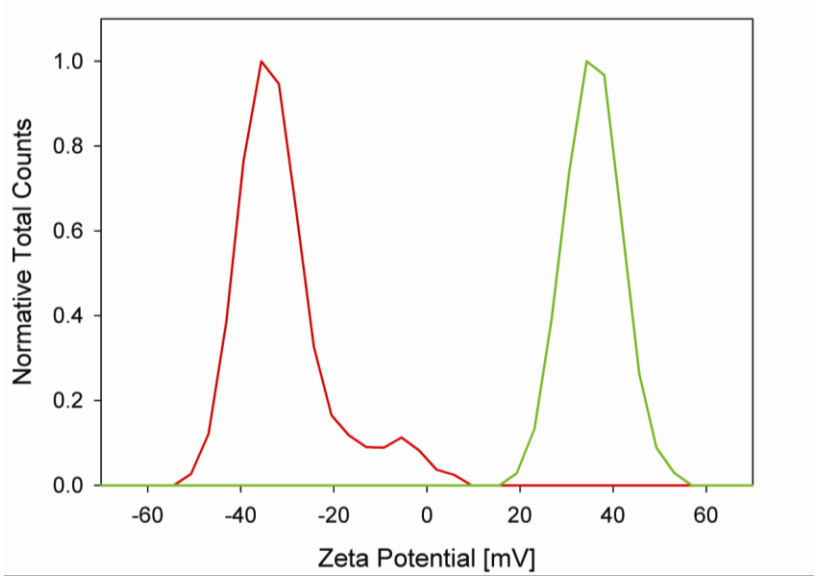


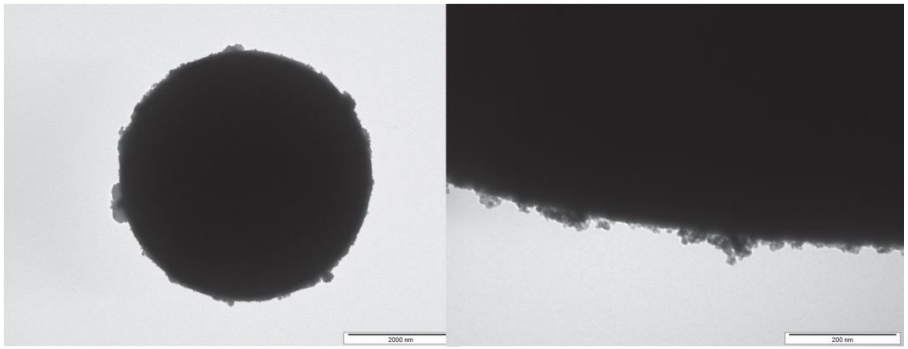




3b. Communication & Control

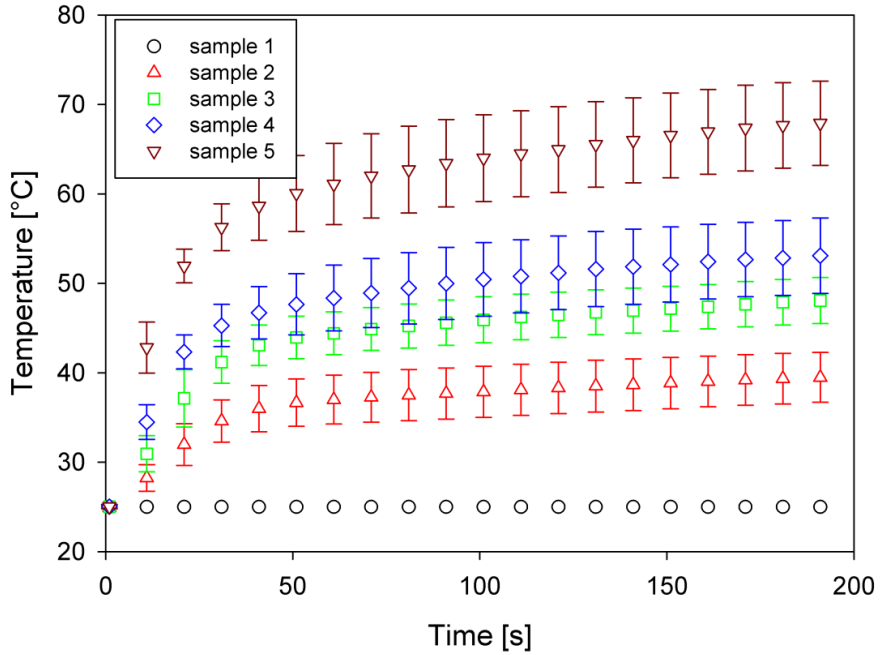
Iron oxide – silica composites





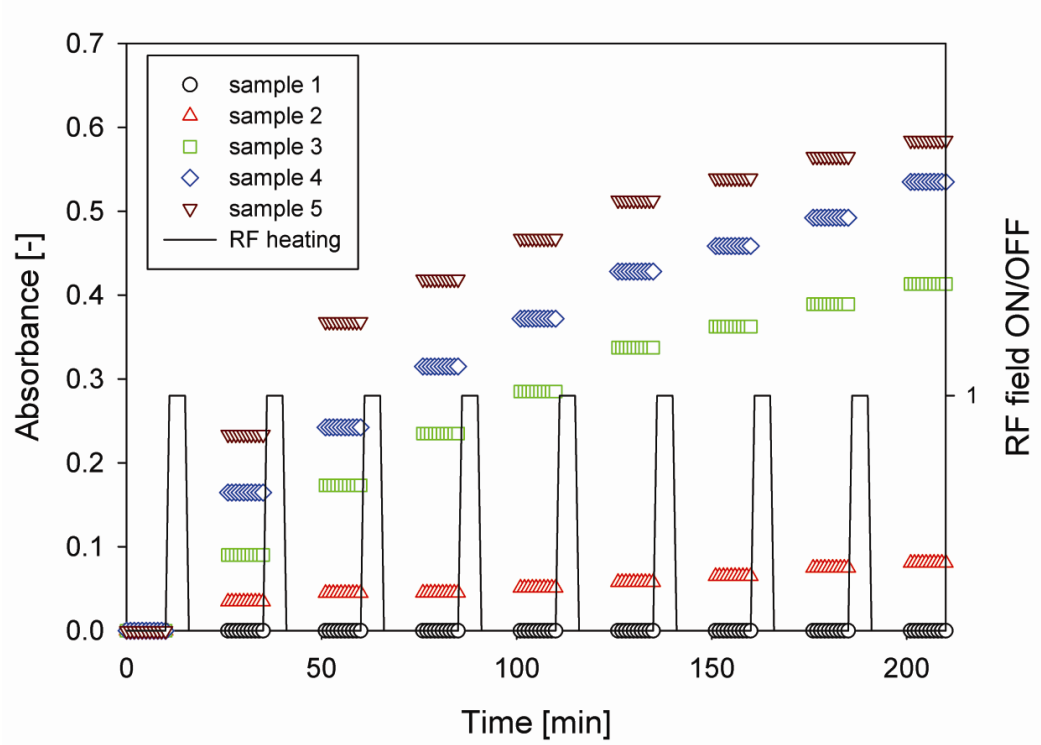
Sample no.	Silica:iron oxide ratio (w/w)
1	1 : 0.0
2	1 : 0.1
3	1 : 0.2
4	1 : 0.4
5	1 : 0.8

Radiofrequency heating



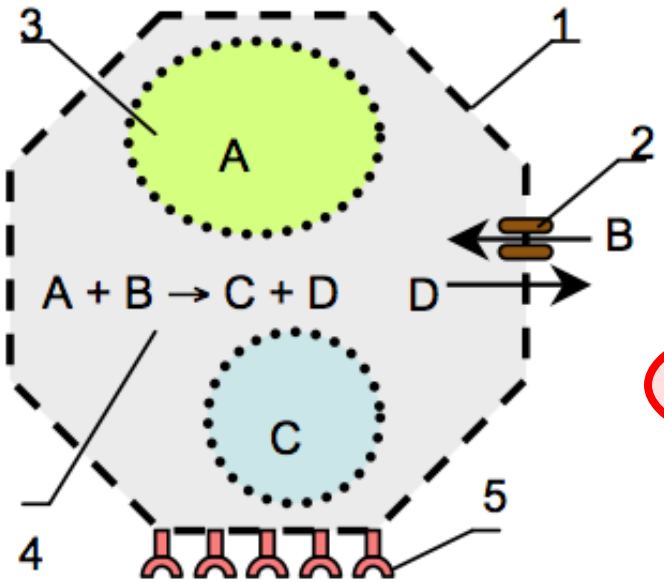
3b. Communication & Control

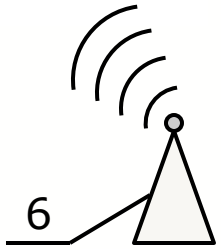
Radiofrequency controlled diffusion “on demand”



Kovacik et al., Chem. Eng. J. **232**, 591-598 (2013)

Design of a chemical microrobot





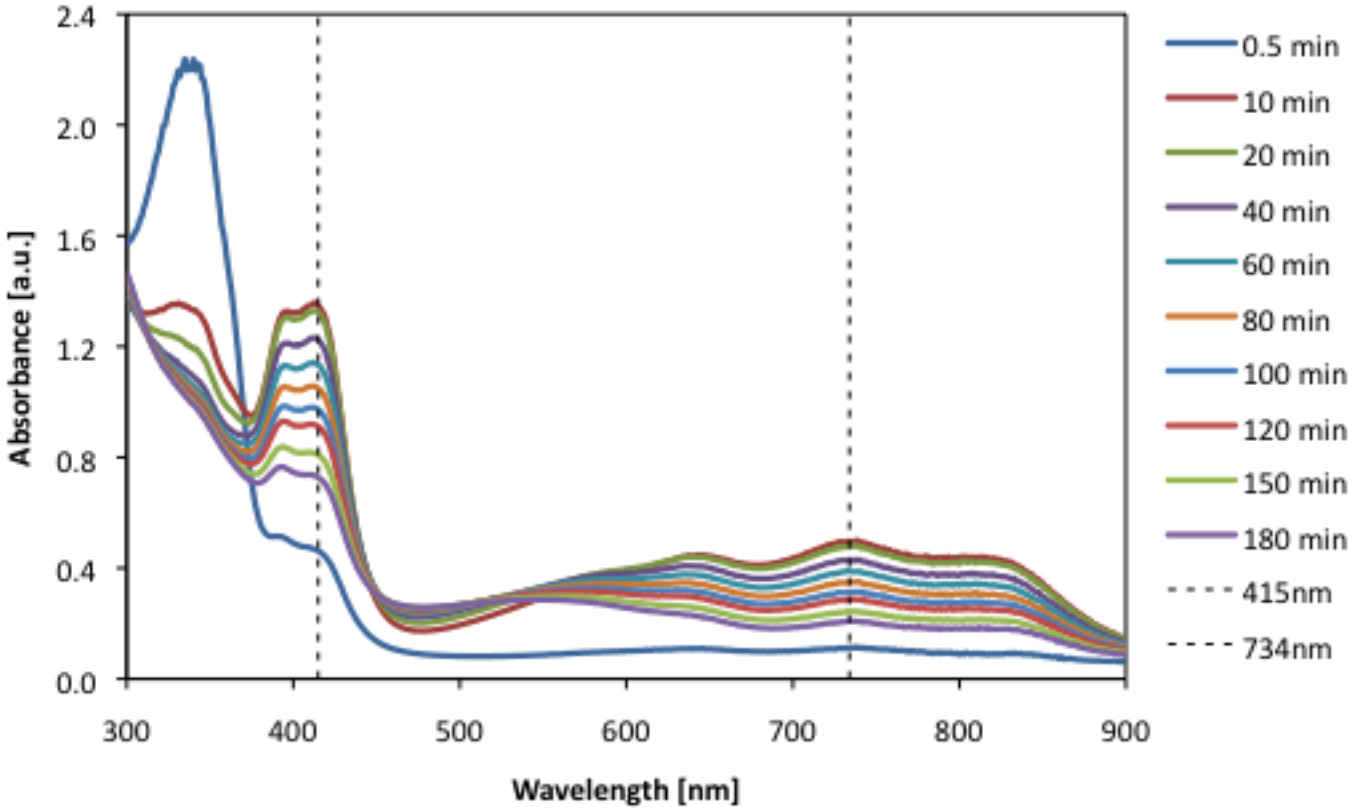
Structural and functional sub-systems:

1. Semi-permeable shell (**integrity**)
2. Variable diffusion rate (**transport**)
3. Internal reservoirs (**storage**)
4. Chemical **reaction** "on-demand"
5. Surface functionalisation (**adhesion**)
6. Remote control (**communication**)

4. Reaction kinetics

In-situ enzymatic production of an unstable active from stable precursor

$$[ABTS^{2-}]_{in} \xrightarrow{k_{rel}} [ABTS^{2-}]_{out} \xrightarrow{k_{enz}} [ABTS^{1-}]_{ox} \xrightarrow{k_2} [decayed\ product]$$



4. Reaction kinetics

$$\frac{dc_{in}}{dt} = -k_1 c_{in}$$

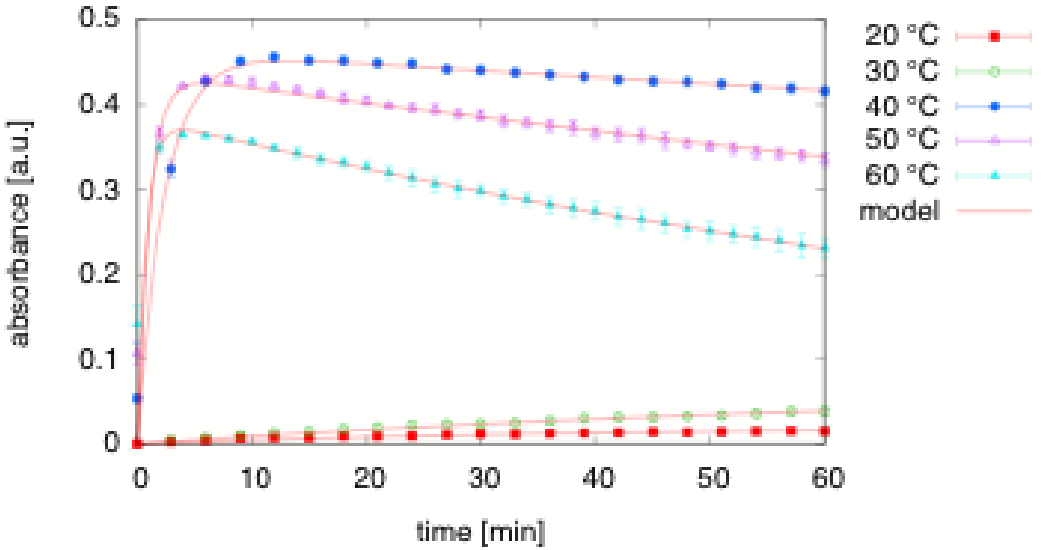
$$\frac{dc_{ox}}{dt} = -k_2 c_{ox} + k_1 c_{in}$$

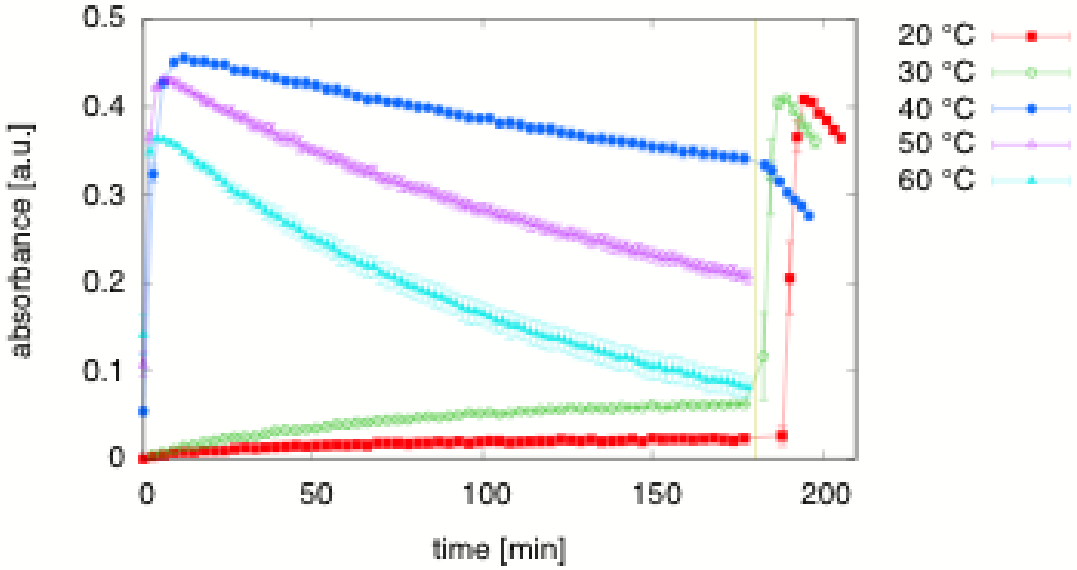
Sigmoidal dependence on T

$$k_1 = k_m \frac{1}{1 + \exp\left(-b(T - T_m)\right)}$$

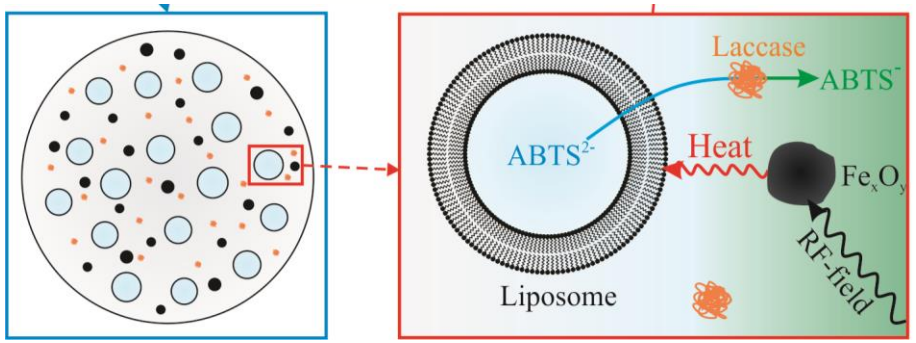
Arrhenius law

$$\ln k_2 = \frac{A}{T} + B$$

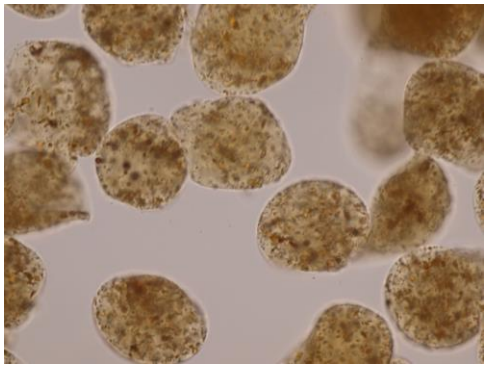


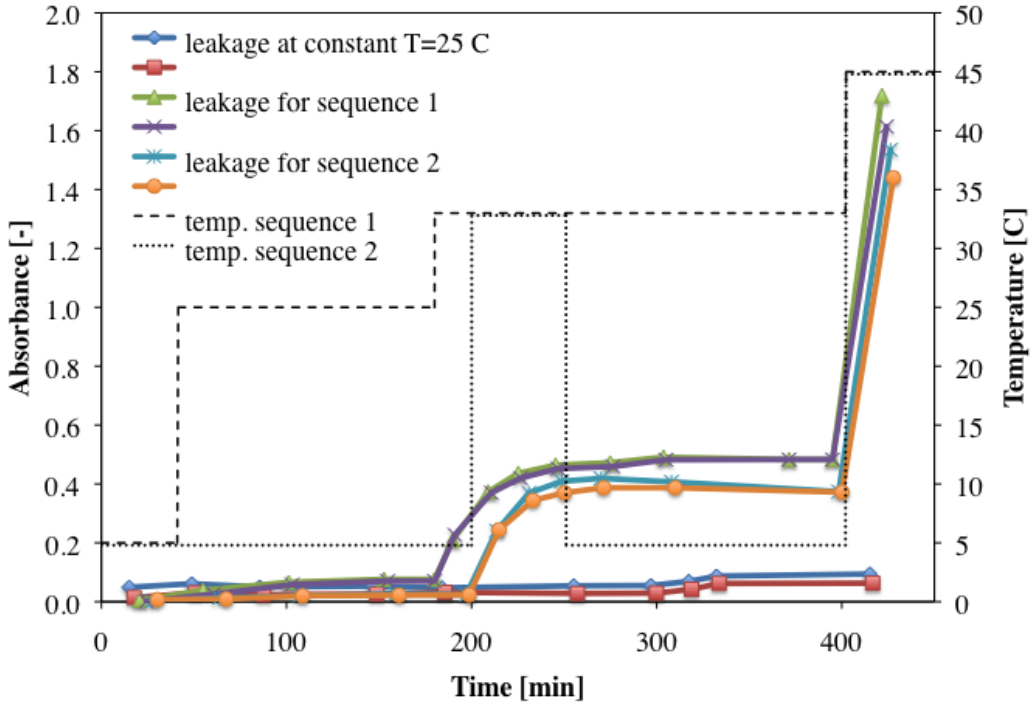


4. Reaction kinetics



Enzyme: laccase
Substrate: ABTS

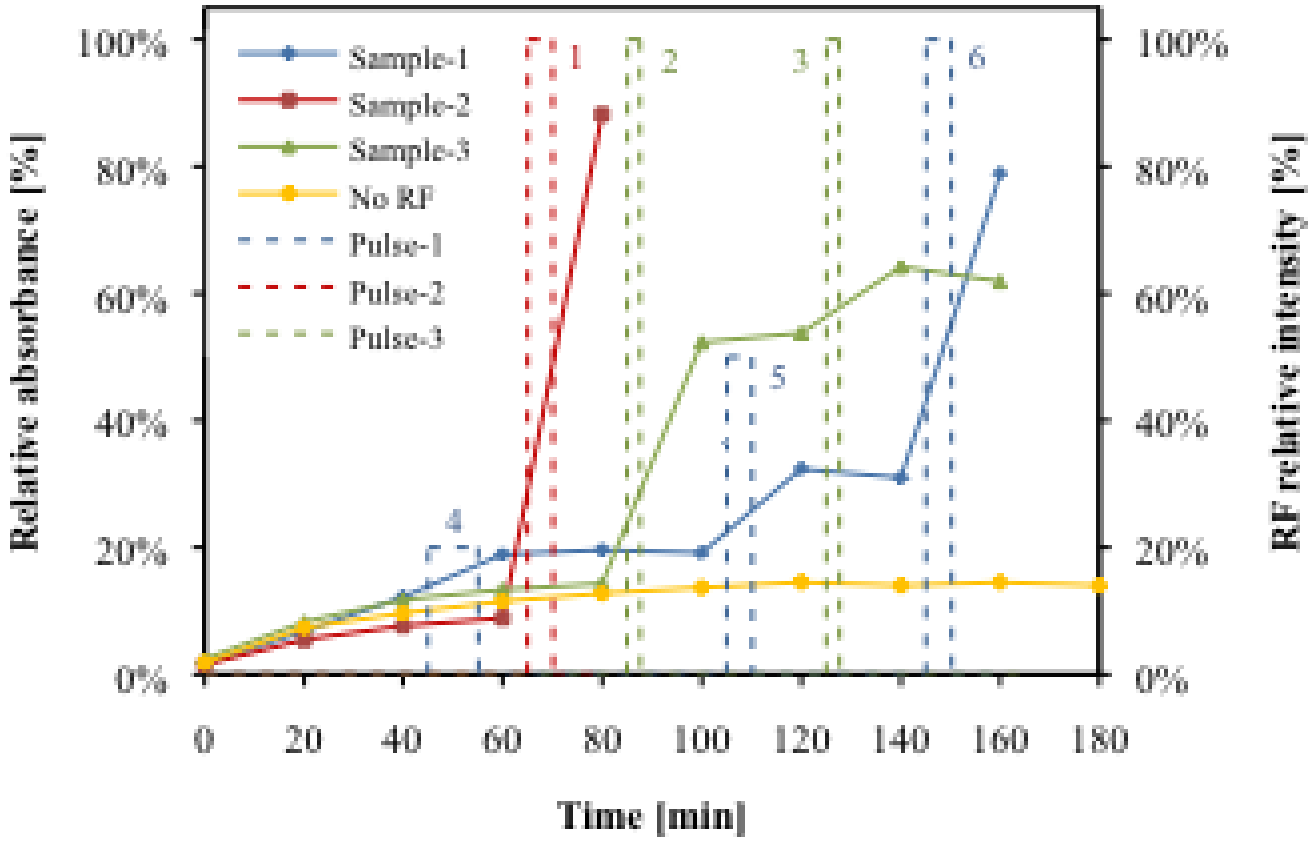




Ullrich et al., *Chem. Eng. Sci.* 2014

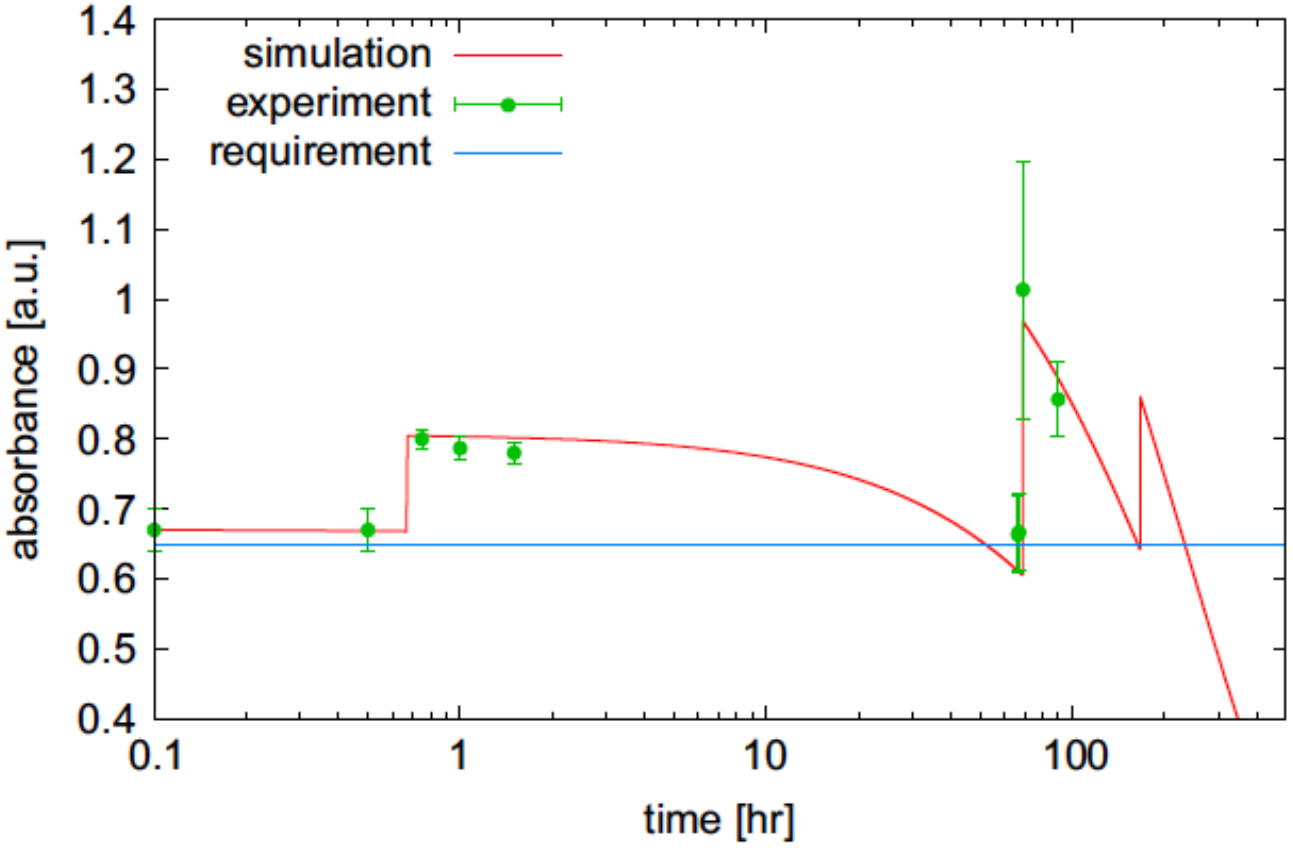
4. Reaction kinetics

Radiofrequency pulse sequences to control production rate of active



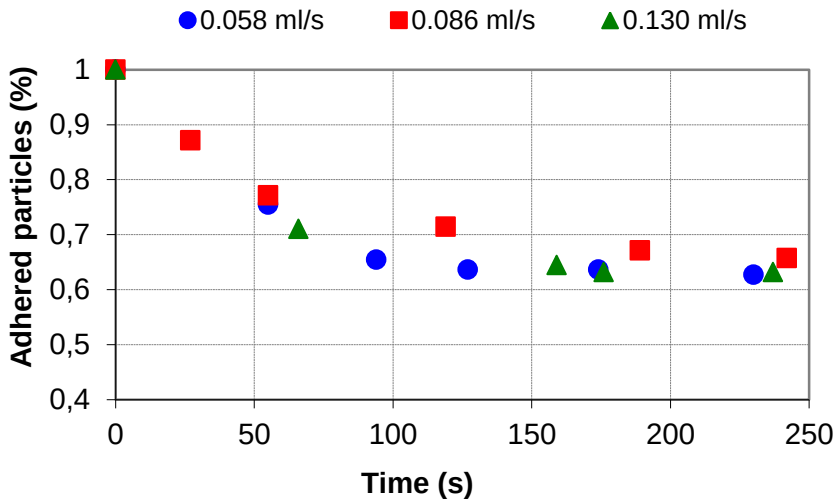
4. Reaction kinetics

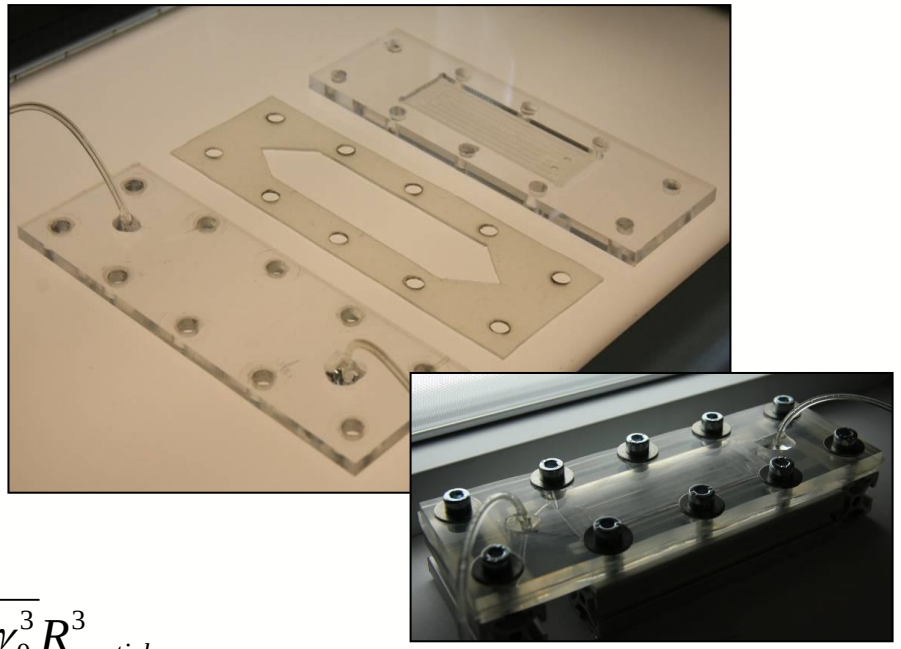
Model-predictive control to maintain active concentration above threshold

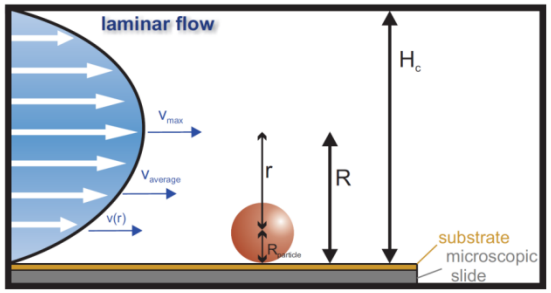


Ullrich et al., *Chem. Eng. Sci.* 2014

5. Control of particle adhesion





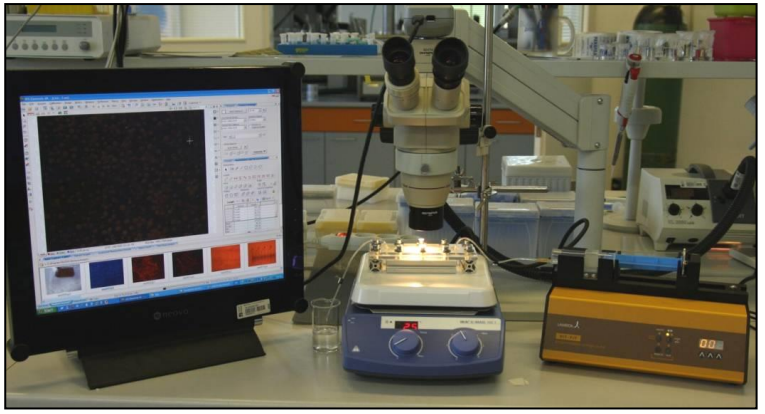


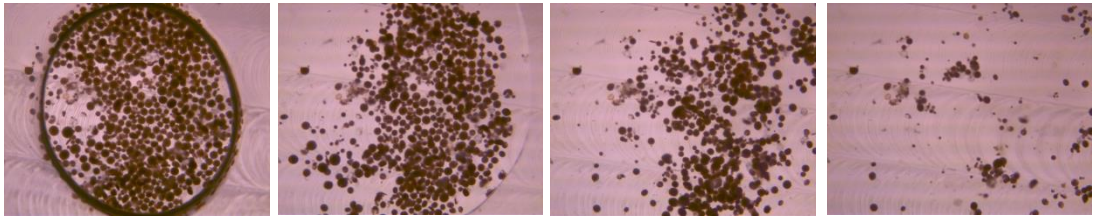
Saffman lift force:

$$F_{lift} = 81.2\sqrt{\rho_w \mu_w \gamma_0^3} R_{particle}^3$$

Stokes drag force:

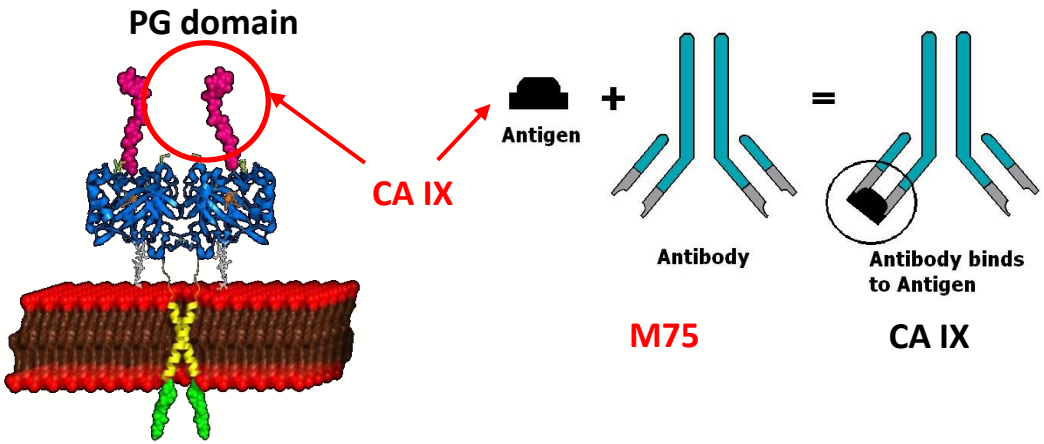
$$F_{drag} = 6\pi R_{particle} v(r)$$

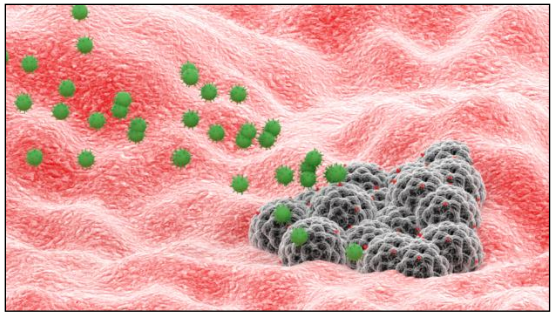




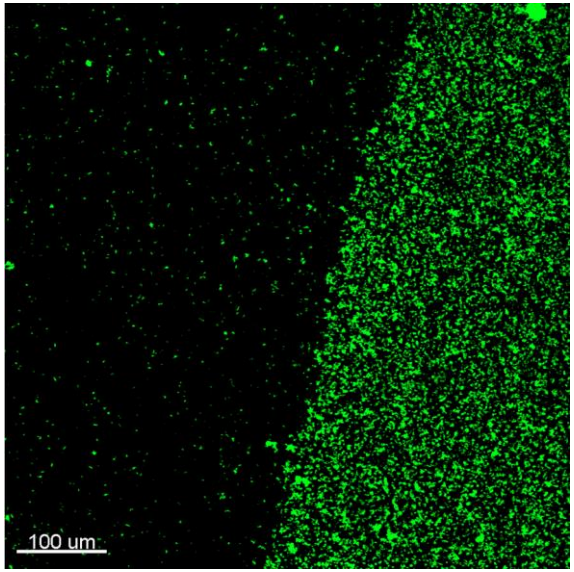
1 s 15 s 20 s 40 s

5. Control of particle adhesion





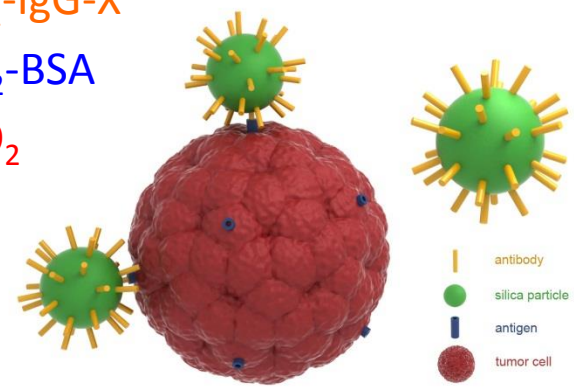
- **antigen CA IX** (carbonic anhydrase 9)
  - transmembrane protein
  - expressed in cancers (lung, colon, breast, cervix, ovaries, brain, oral cavity...)
- **antibody M75**
  - recognizes unique PG domain of CA IX

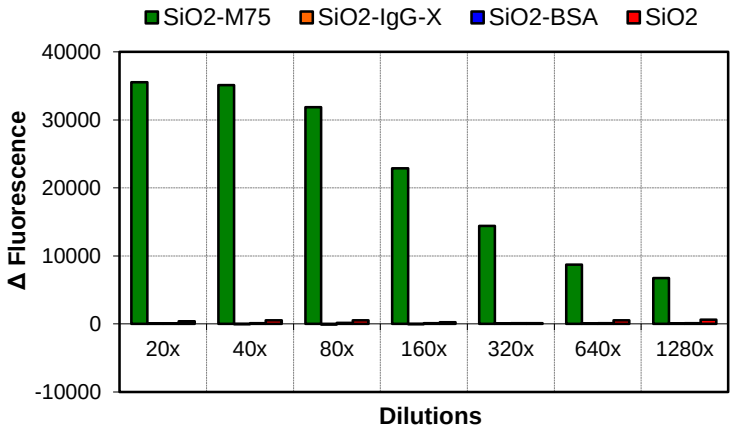


Tokarova et al., *Nanoscale* **5** (2013) 11490-11498

5. Control of particle adhesion

- 4 types of particles:
  - specific : SiO_2-M75
  - unspecific: SiO_2-IgG-X
    SiO_2-BSA
  - unmodified: SiO_2

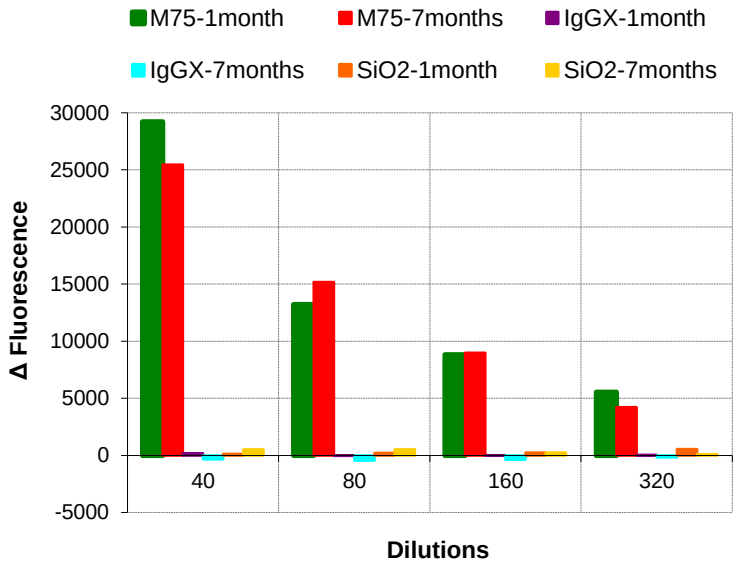




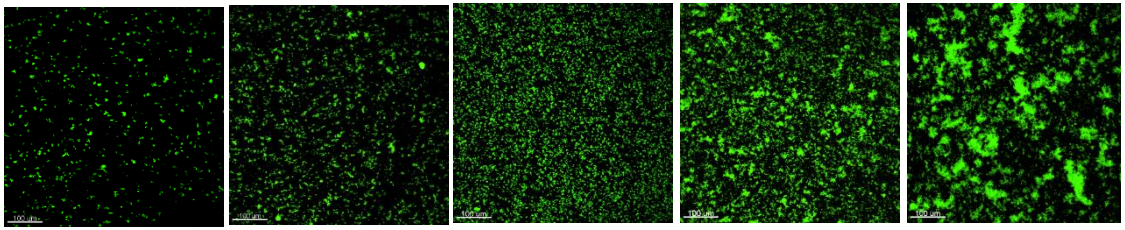
- covalent coupling of proteins and antibodies to obtain monolayer

- ELISA - *enzyme-linked immunosorbent assay* - spectrophotometric detection of specific protein (antibody)

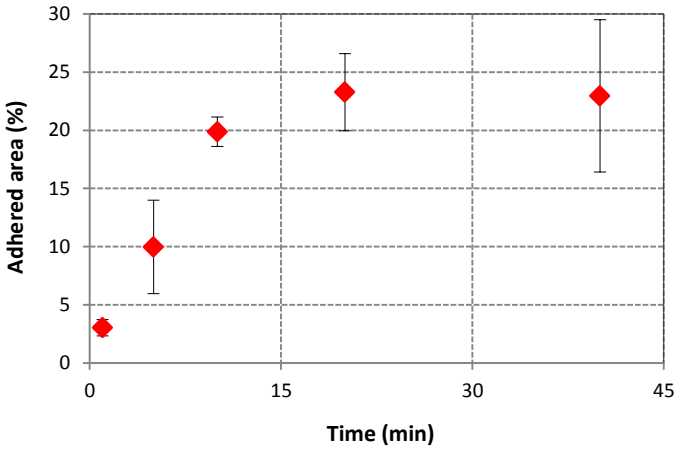
- *ELISA-like* – no need of secondary antibody for detection



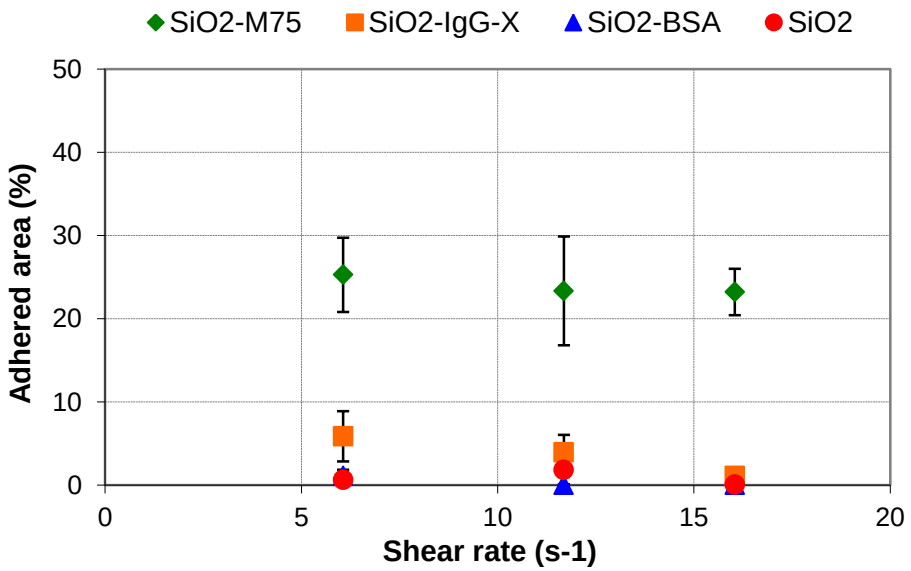
5. Control of particle adhesion

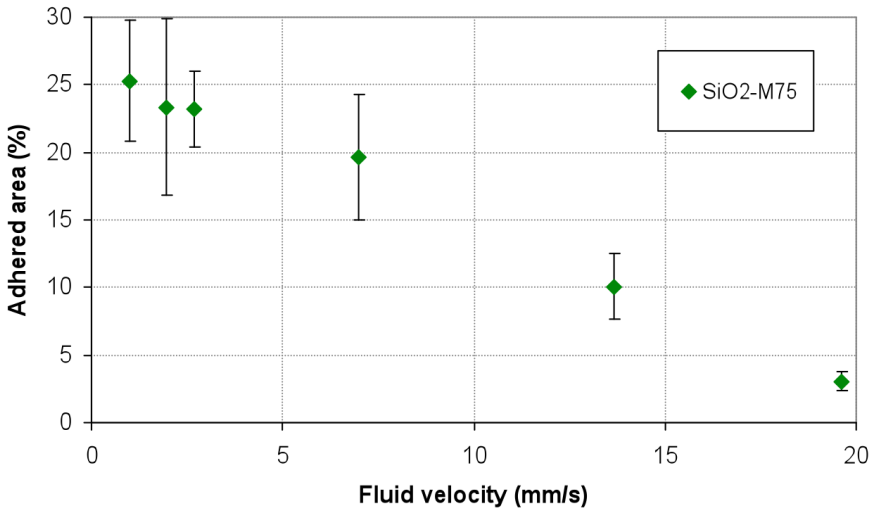


1min 5min 10min 20min 40min



- increasing exposure time of SiO_2-M75 with PG-MBP modified plastic slides



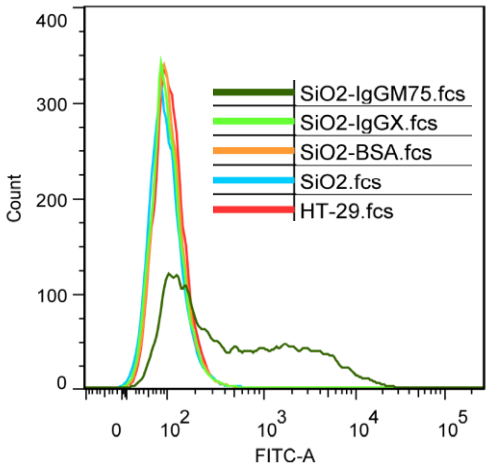


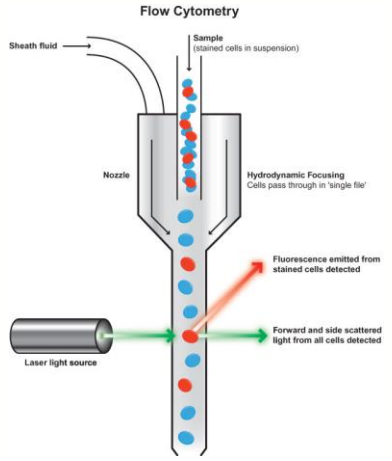
5. Control of particle adhesion

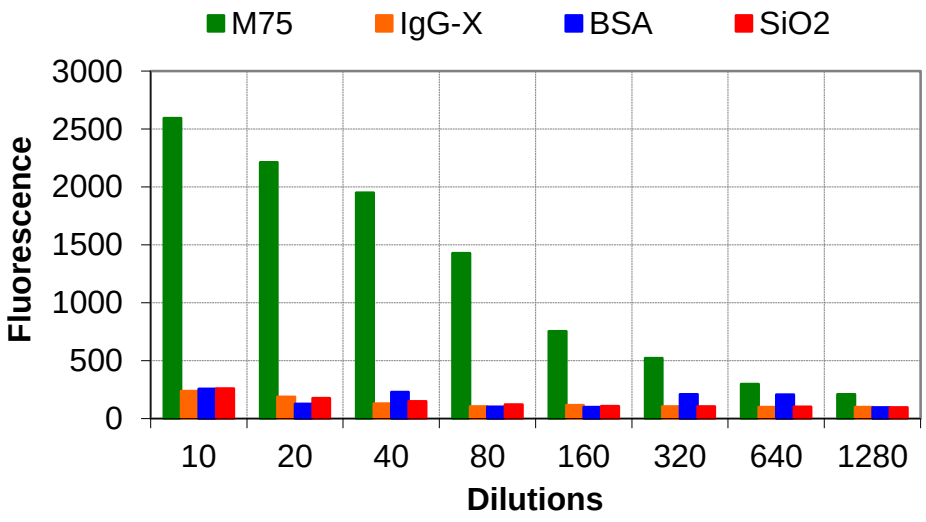
HT-29 cell line (derived from colorectal carcinoma) expressed antigen CA IX which binds with M75-modified particles

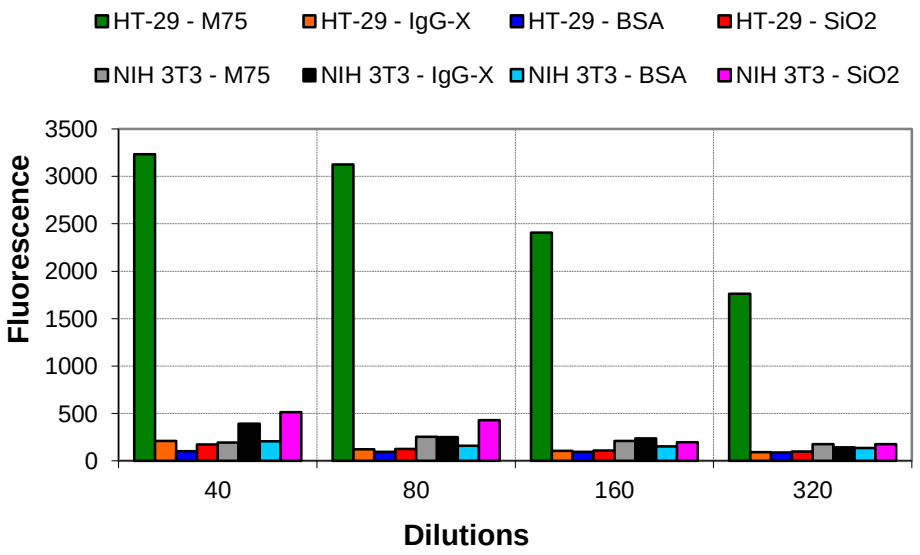
NIH 3T3 (embryonic fibroblast cell line)

- no expression of CA IX on surface
- negative control





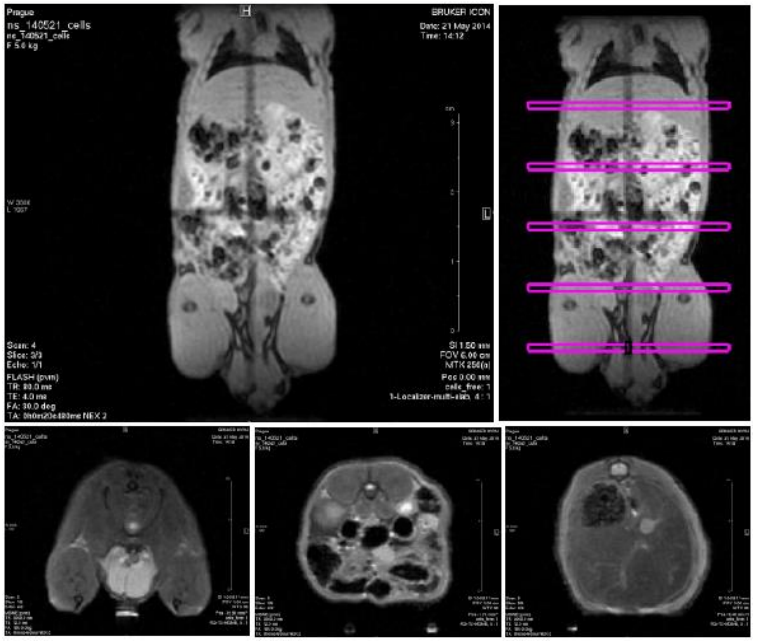


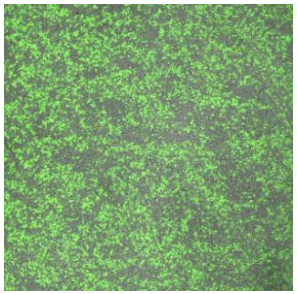


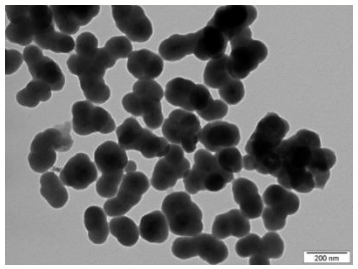
5. Control of particle adhesion

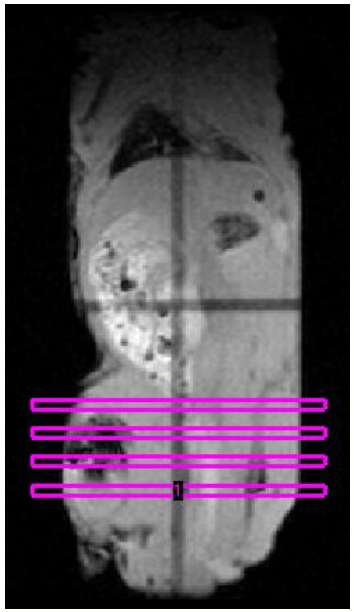
On-going work: *in vivo* studies

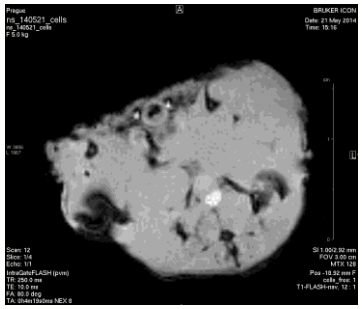
NU/NU nude mice
Human carcinoma HT-29
Multi-modal imaging (MRI & Fluorescence)

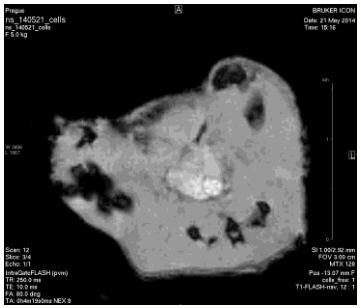




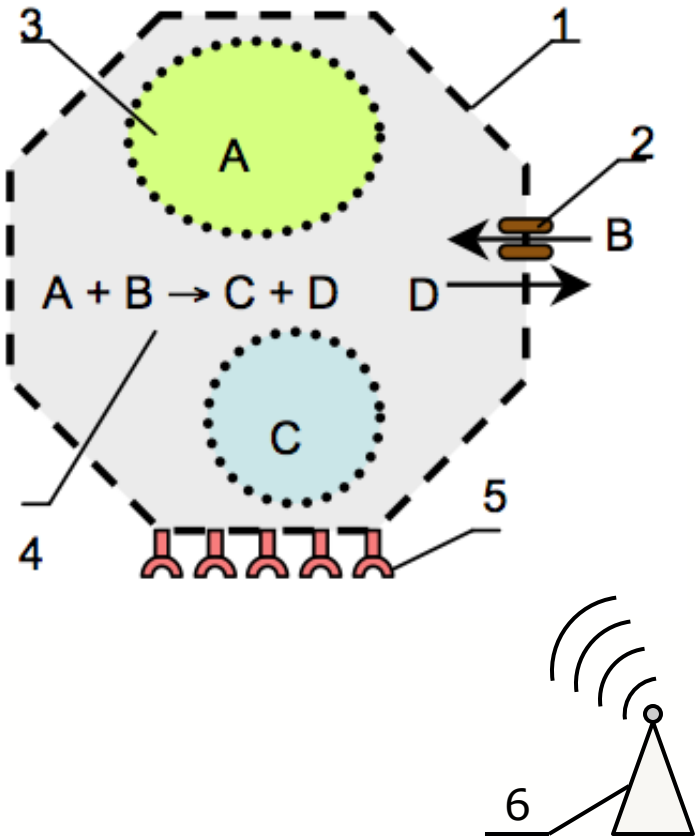








Design of a chemical microrobot



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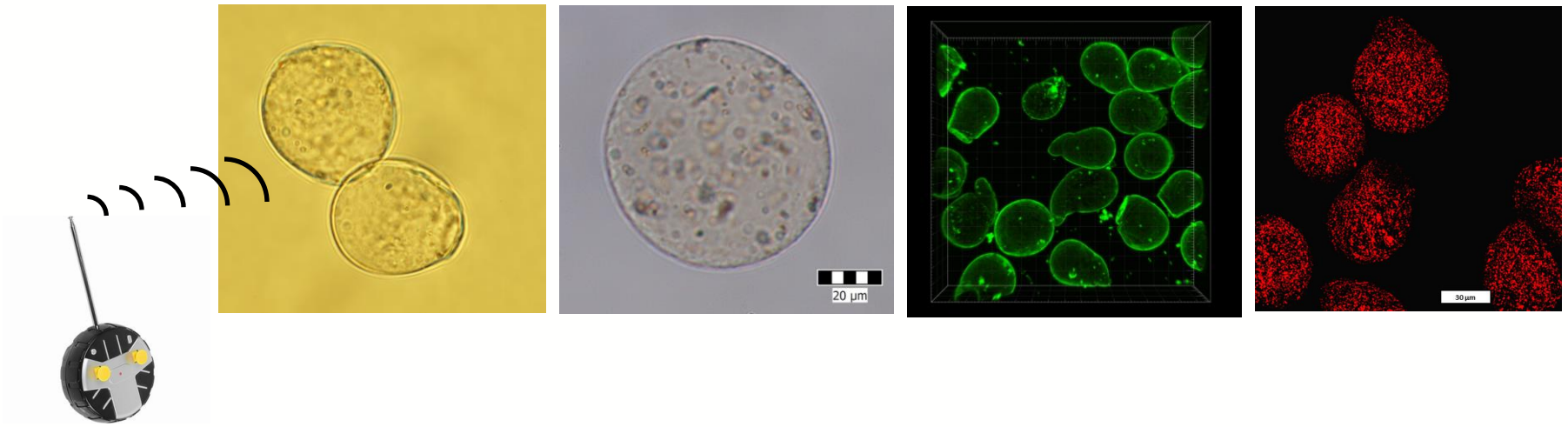
2. Variable diffusion rate (**transport**)
3. Internal reservoirs (**storage**)
4. Chemical **reaction** "on-demand"
5. Surface functionalisation (**adhesion**)
6. Remote control (**communication**)

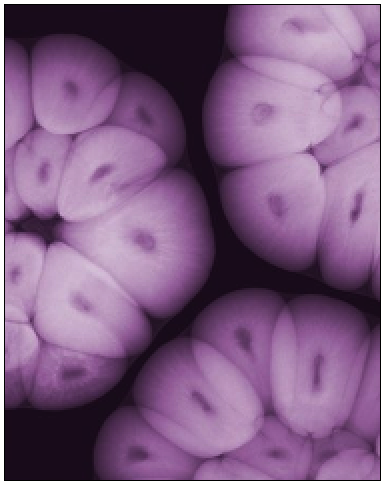
Conclusions

- Multi-compartment particles - “chemical robots”
- Remotely controlled diffusion by RF signals
- Control of enzymatic reaction
- Control of adhesion via antigen-antibody interaction

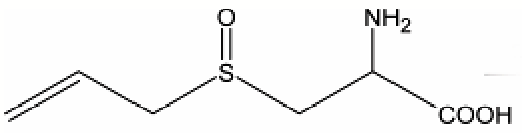


Applications

1) Cancer theragnostics (MRI imaging + RF triggered drug release)
2) Delivery of natural fungicides (crop protection)
3) Delivery of unstable natural antibiotics - allicin

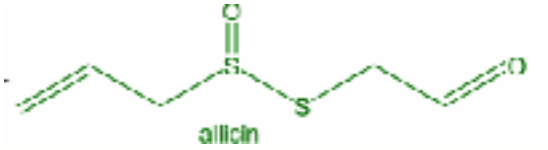


Garlic bulb
(*Allium sativum*)



Aliin (stable precursor)

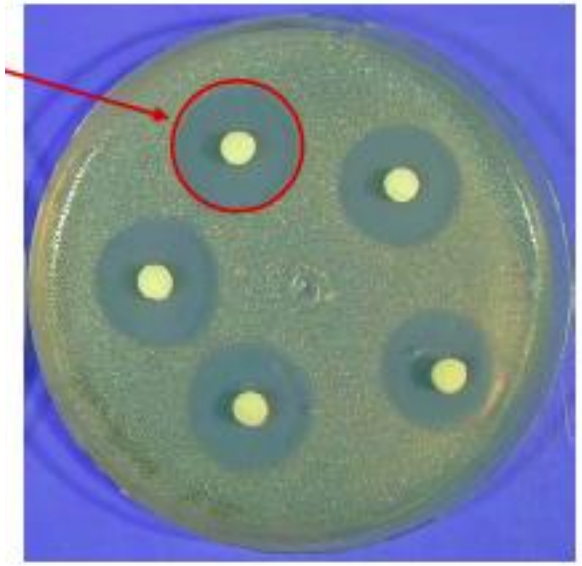
Enzyme allinase



Allicin (bactericidal but unstable)

Inhibition zones

E. coli DH5α



Thank you

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