

Crysta'Days | November 2023

Single crystal formation in core-shell capsules



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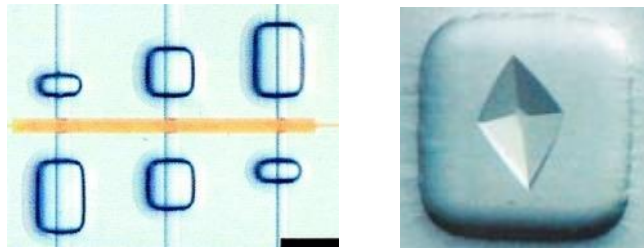


Single crystal generation

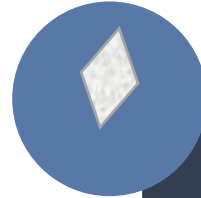
Microfluidic based methods : control at the nanoliter scale



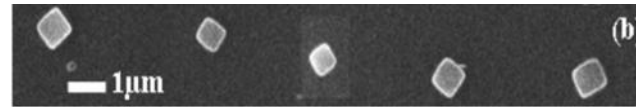
Free interface diffusion in crystallization chambers



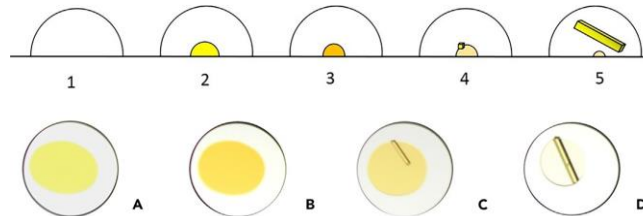
Hansen et al., *PNAS*, 2002, 99, 26, 16531-16536



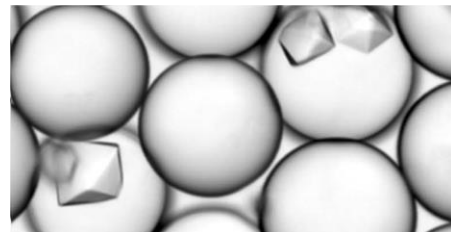
Diffusion in liquid droplets



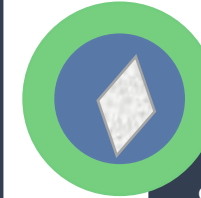
Grossier et al., *APPLIED PHYSICS LETTERS* 98, 1 2011



Tyler et al., *Chem* 6, 2020, 1755-1765



Babnigg et al., *Acta Cryst.* 2022, D89, 997-1009

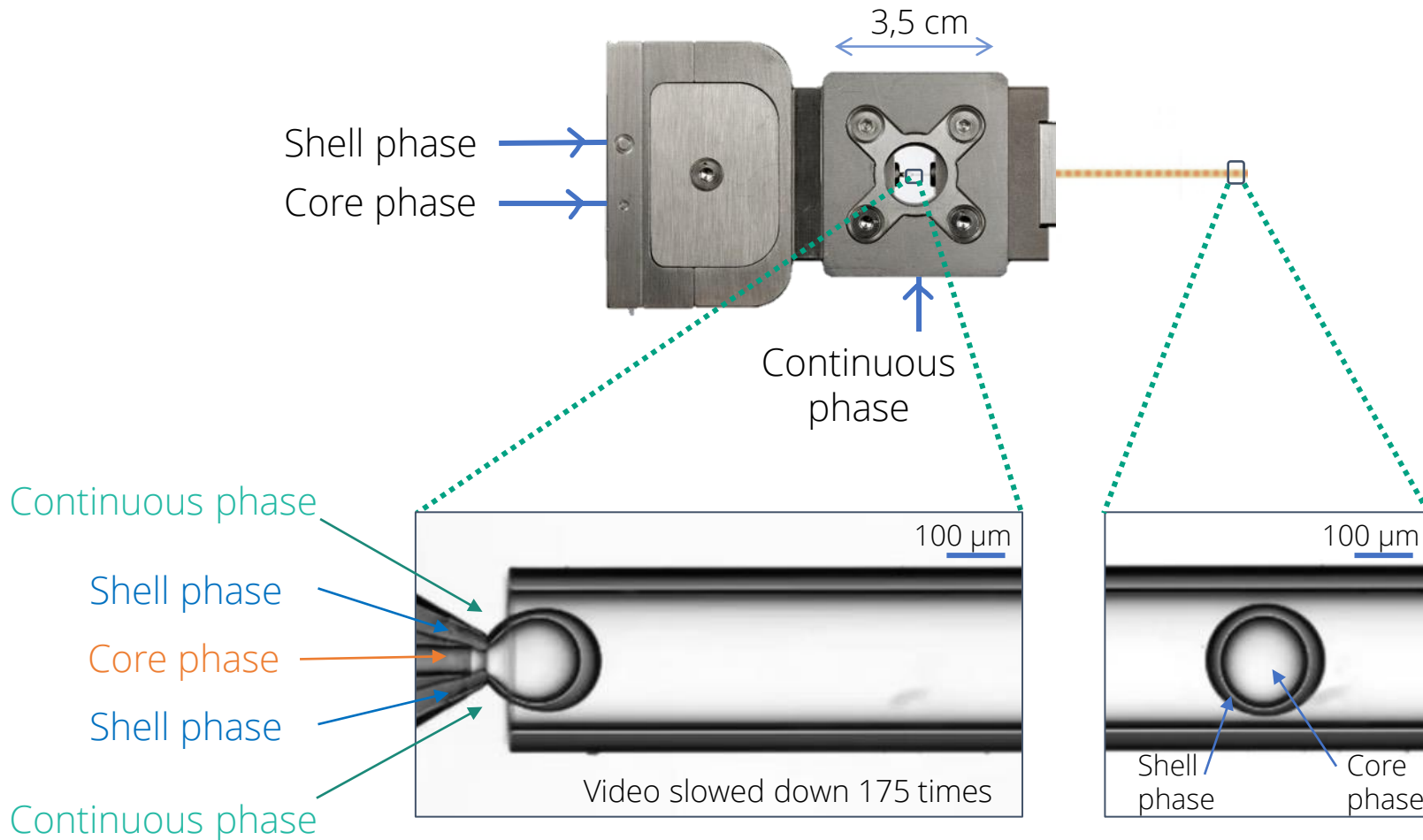


Solid microcapsules with aqueous core

- Stability of the emulsion
- Large choice of solvents

Presentation of the system

An emulsification generator for double emulsions production

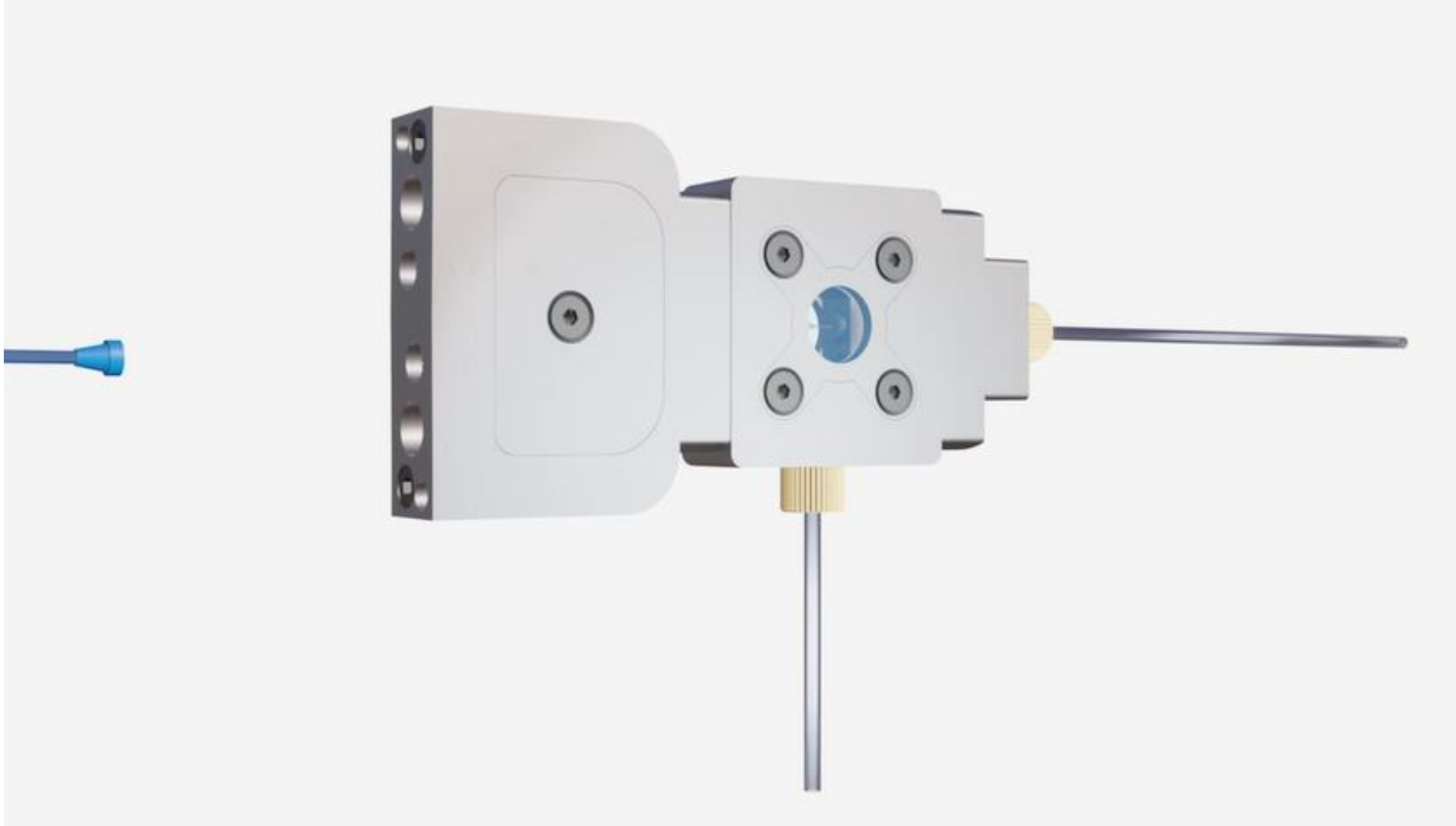


- Single emulsions
- Double emulsions
- No surface treatment needed
- Water/oil/water
- Oil/water/oil
- Highly monodispersed droplets
- Frequency > 100 Hz
- Size range: 40 to 400 μm

In this work, the diameter size of capsules is between 237 and 309 μm.

Presentation of the system

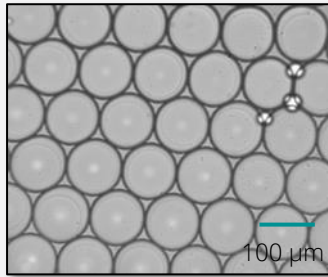
The Raydrop microfluidic droplet generator



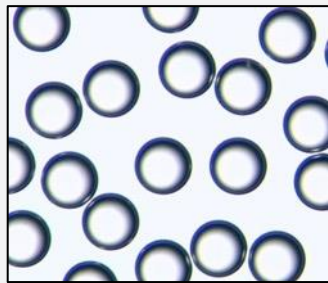
<https://youtu.be/vpbdwMb5mM0?si=s0OYyj8ap7uBxfW1>

Variety of emulsions

Simple emulsions

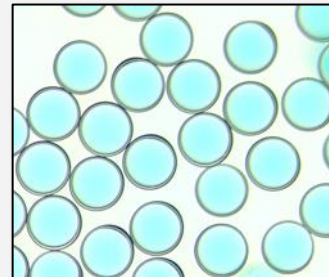


Alginate

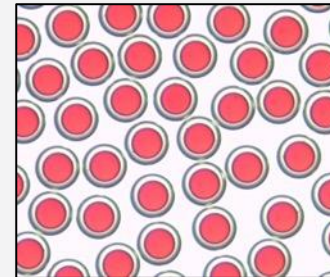


Eudragit™

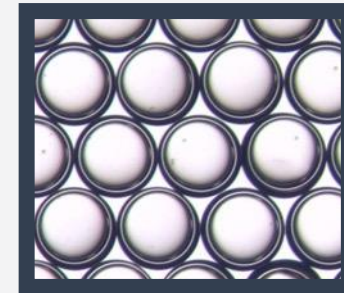
Double emulsions and microcapsules



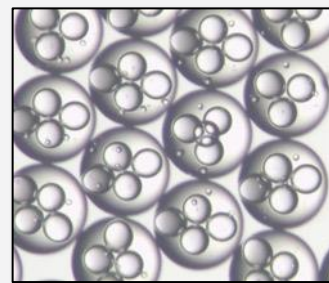
PLGA



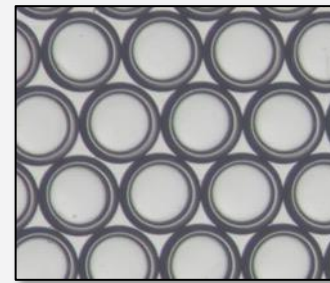
Chitosan



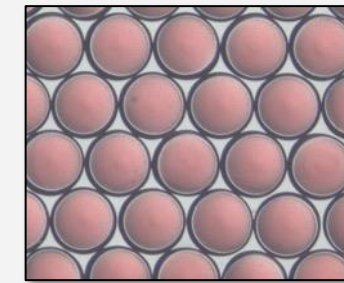
Polymethacrylate
(aqueous core)



Multiple emulsion



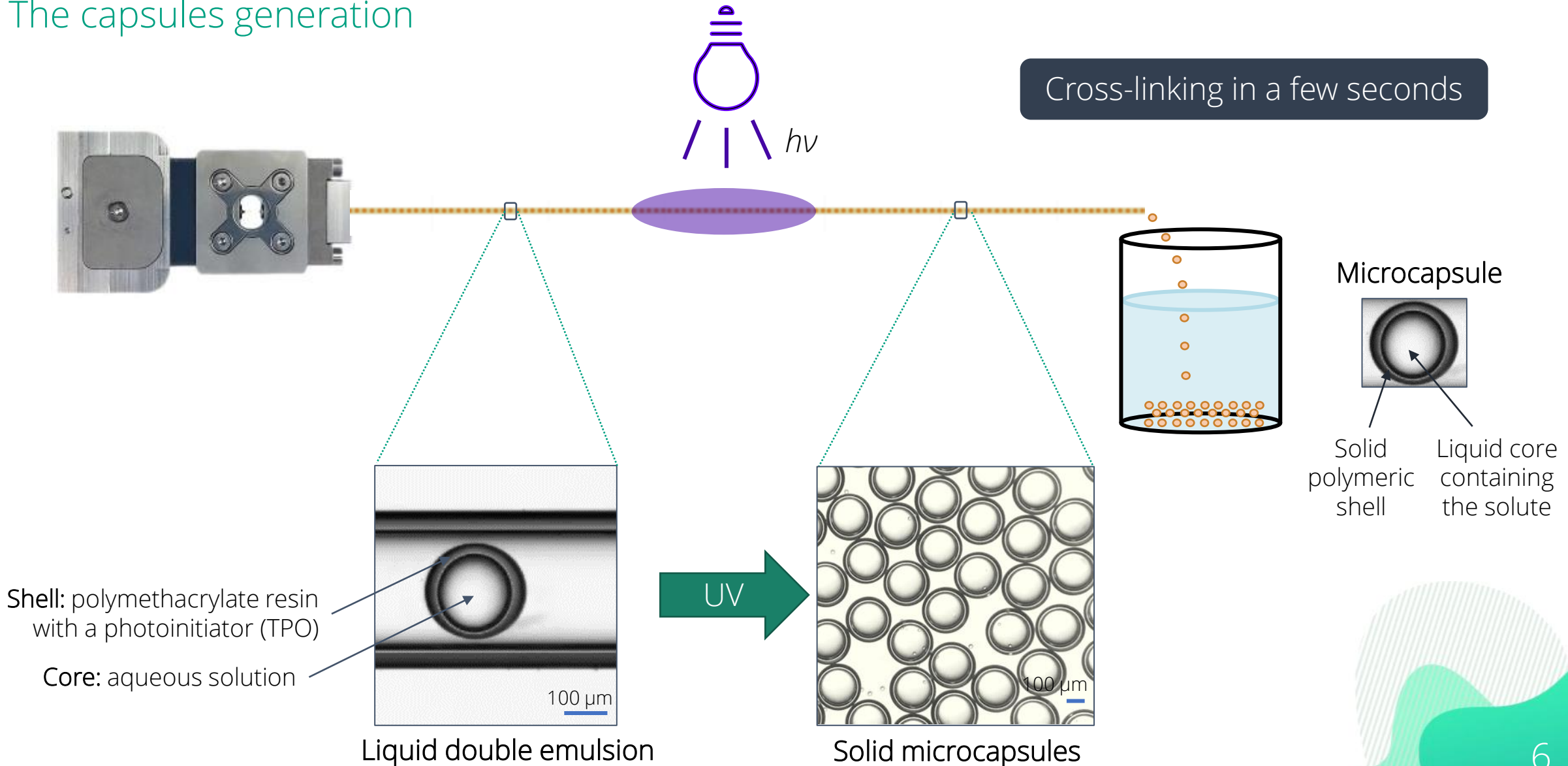
PEGDA



Polymethacrylate
(oily core)

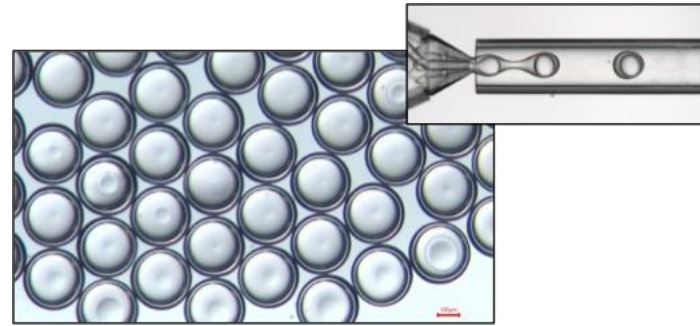
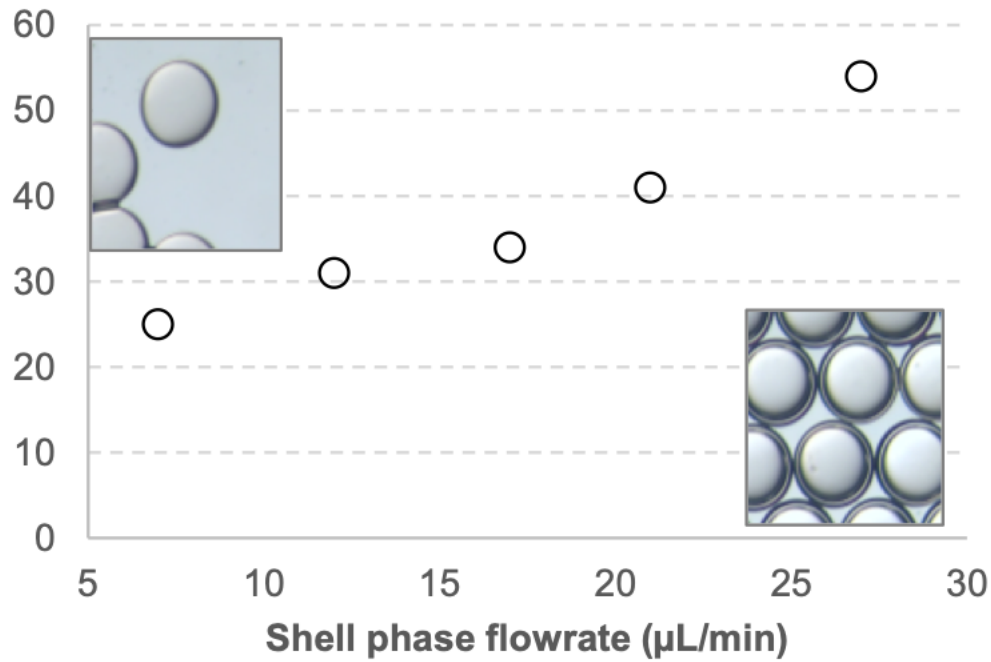
How to create UV-cured capsules?

The capsules generation

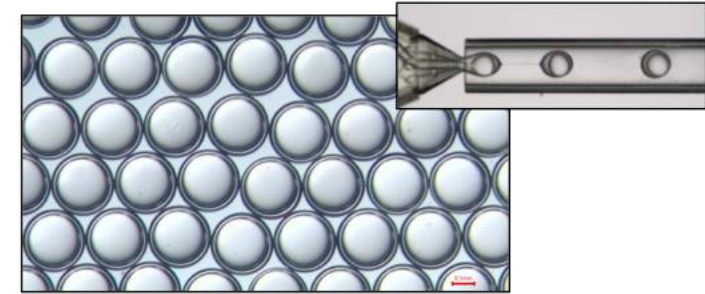


Adjustment of the shell thickness

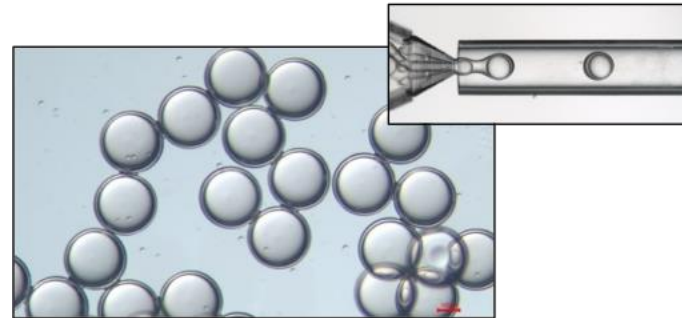
Various shell thickness



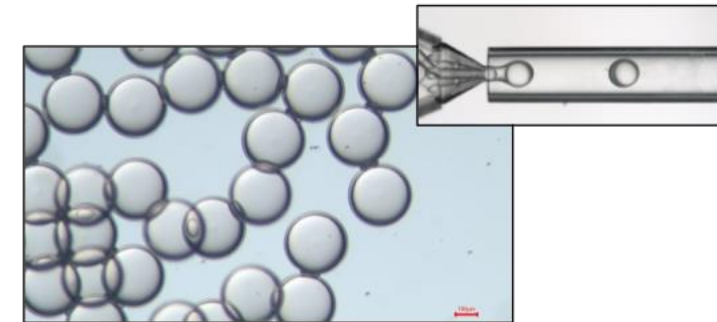
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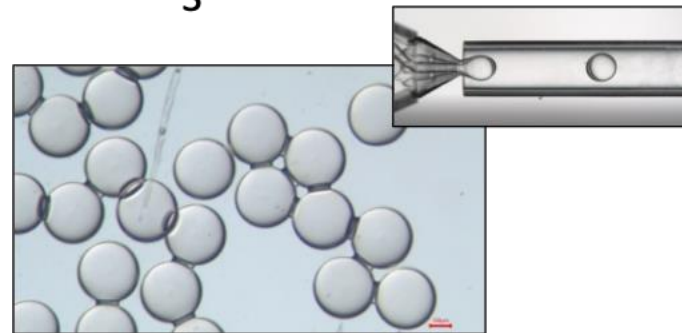
2



3



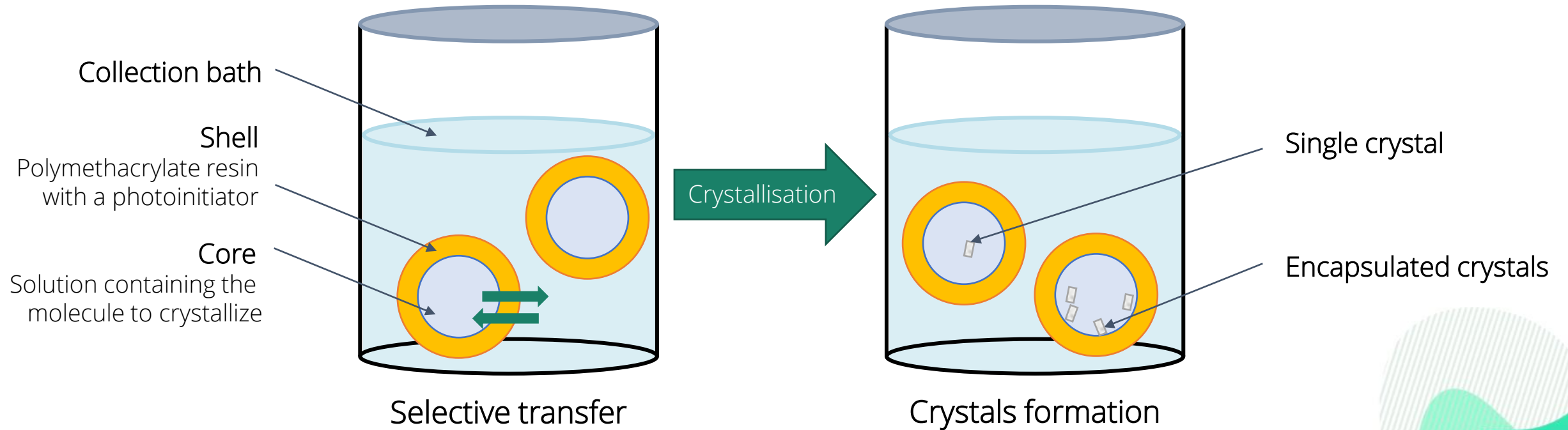
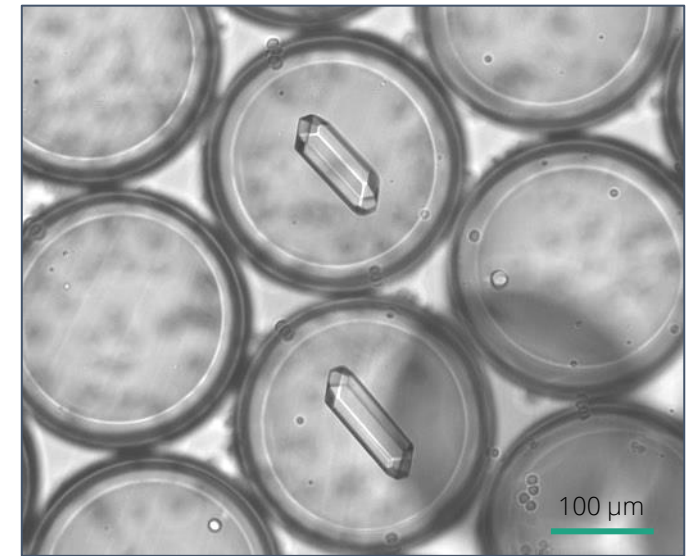
4



5

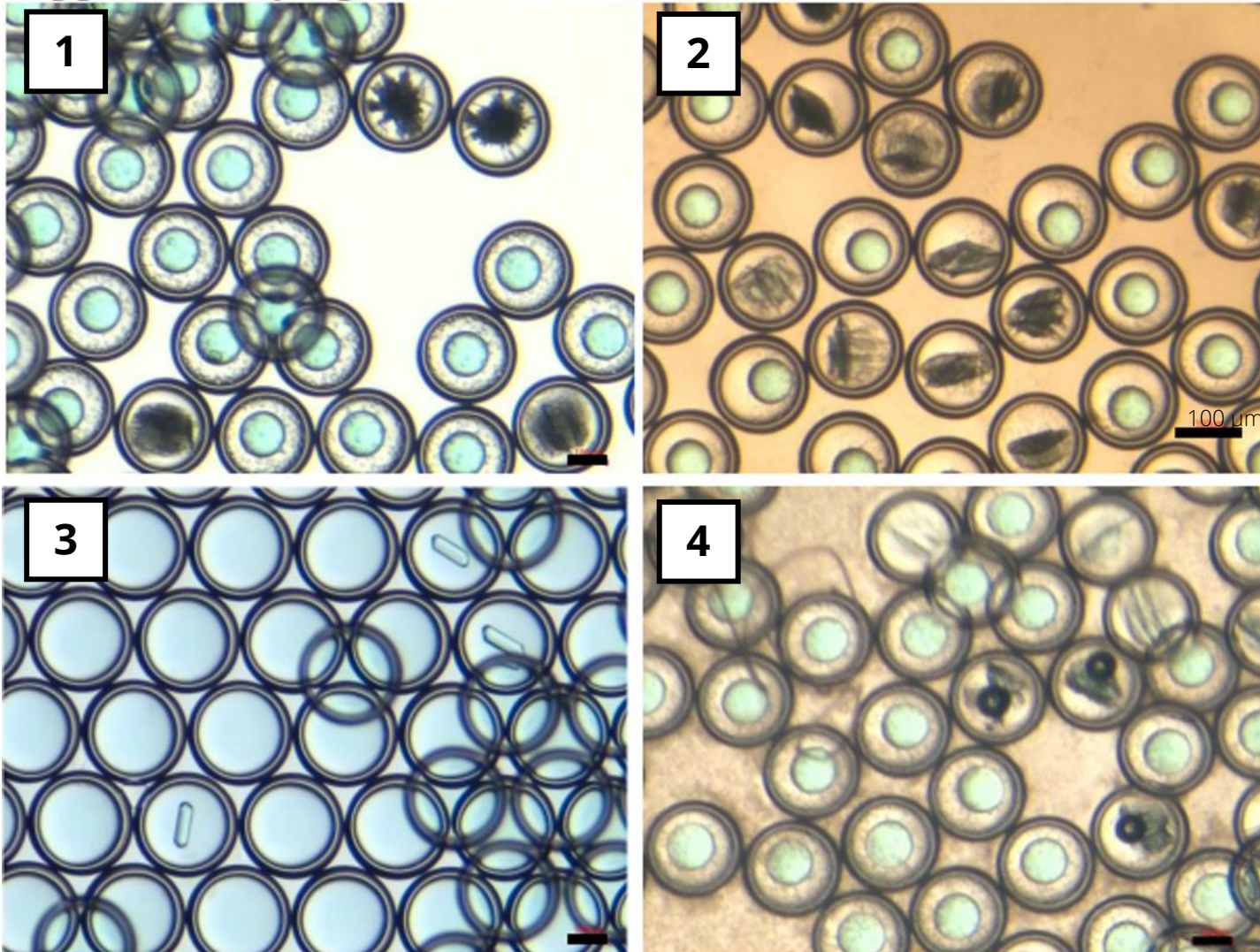
Presentation of the system

Crystal formation



Results - crystallized molecules

Small molecules crystals - Copper (II) sulfate at 200 g/L in water

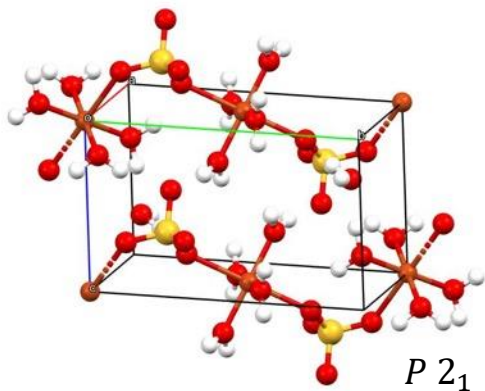
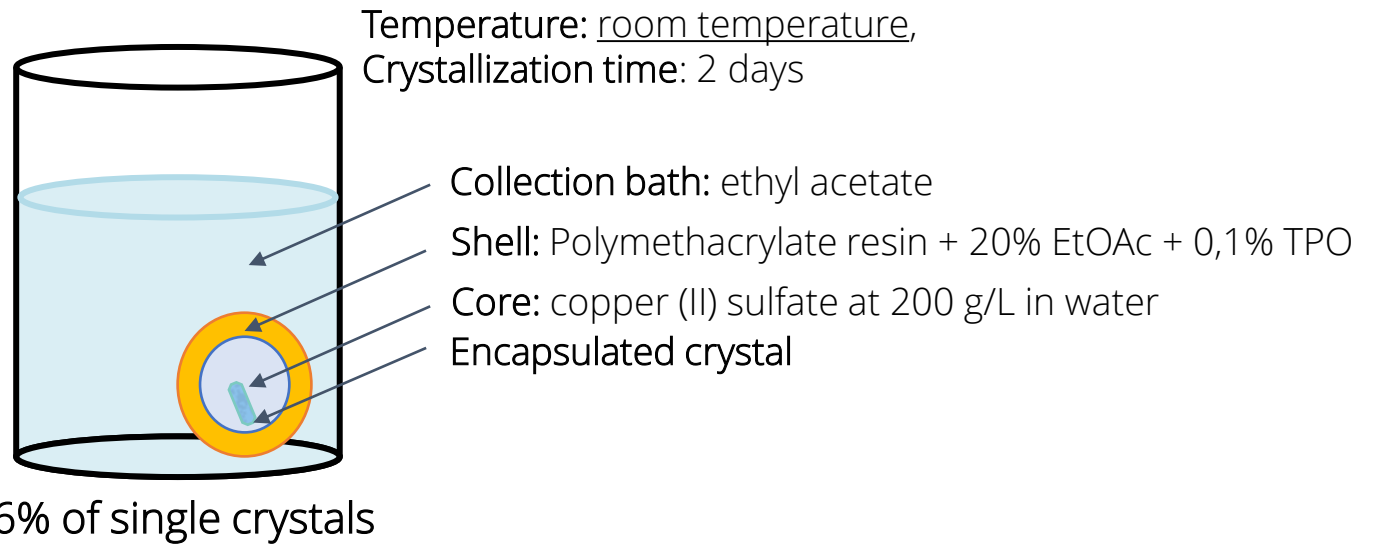
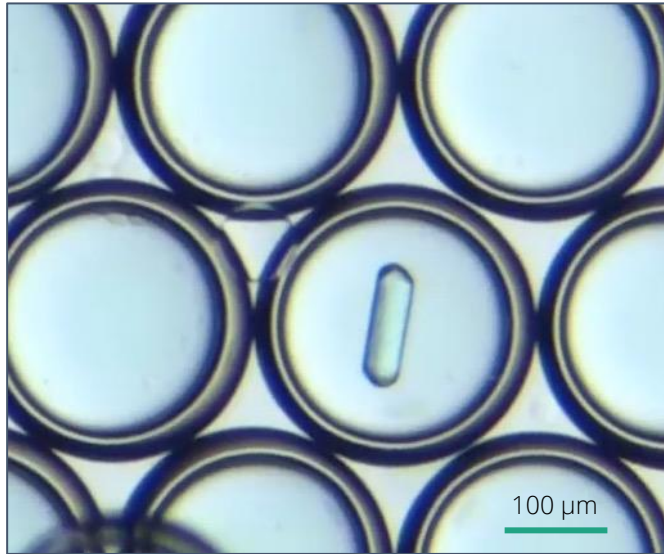


Screening parameters:

Collection bath	Storage			Picture
Air	22 °C	4.5 °C	-18 °C	
Water	22 °C	4.5 °C	-18 °C	
Acetone	22 °C	4.5 °C	-18 °C	2
Methanol	22 °C	4.5 °C	-18 °C	
Ethanol	22 °C	4.5 °C	-18 °C	1
2-propanol	22 °C	4.5 °C	-18 °C	4
Ethyl acetate	22 °C	4.5 °C	-18 °C	3

Results - crystallized molecules

Small molecules crystals - Copper (II) sulfate



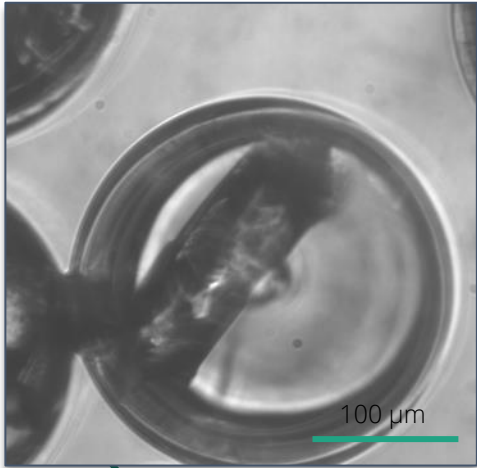
Crystal structure of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

Crystallization by antisolvent diffusion

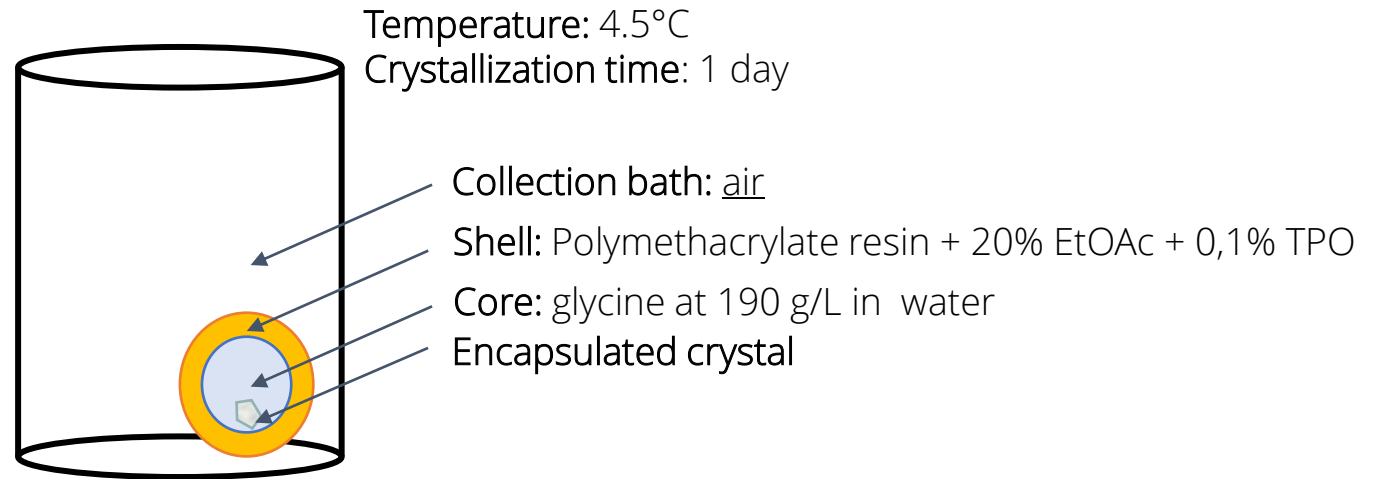
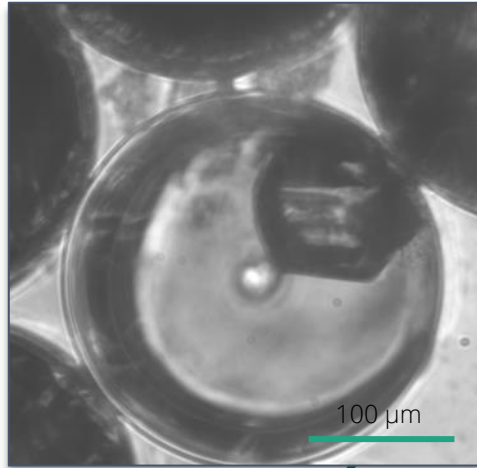
Results - crystallized molecules

Polymorphism - Glycine

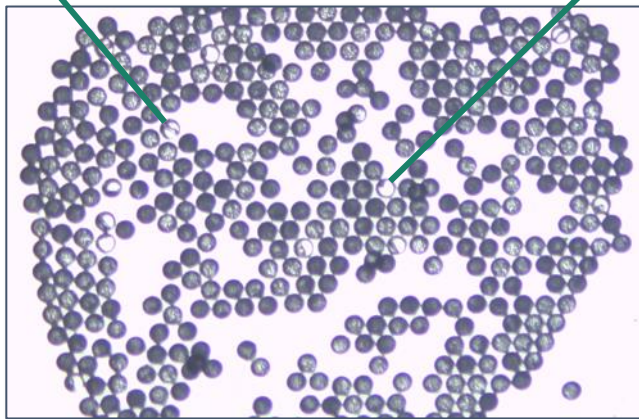
Alpha



Gamma



2.5% of single crystals



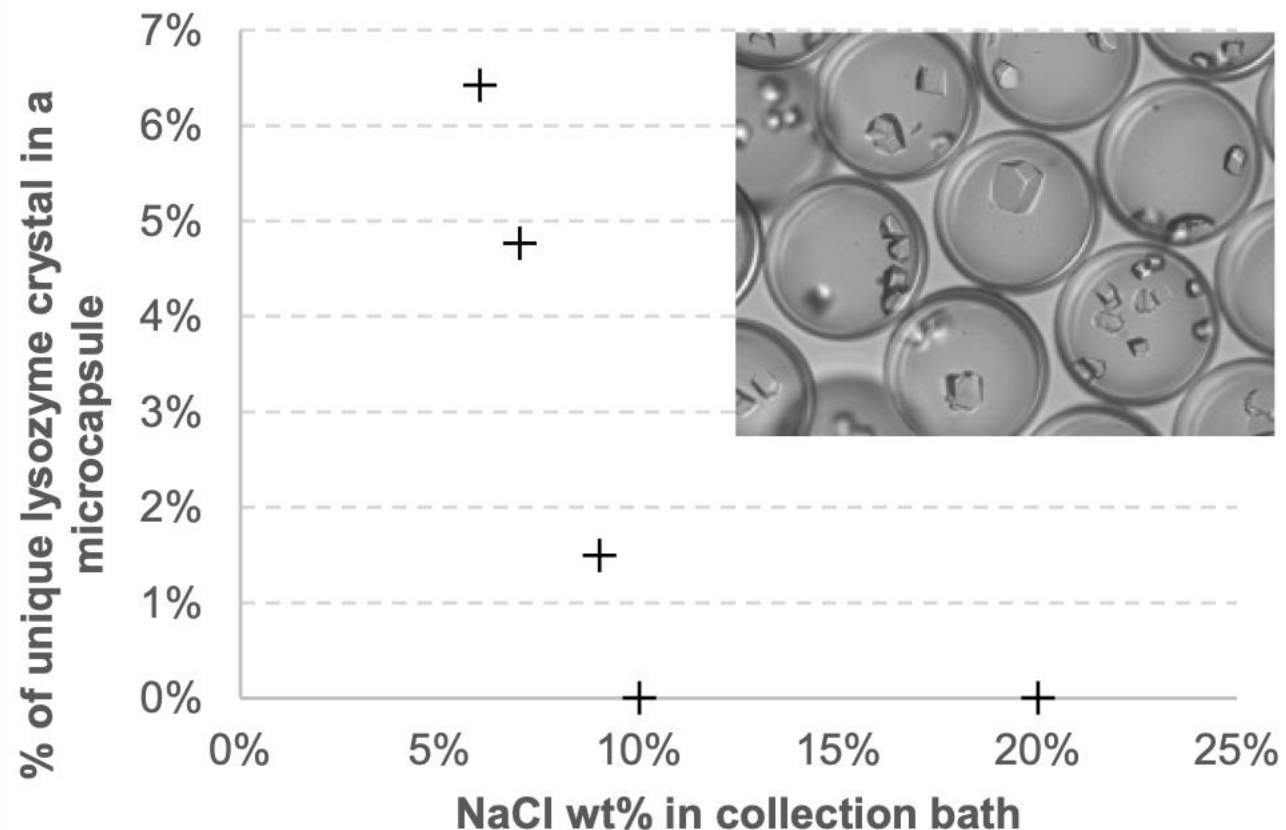
Sample of 500 capsules

← 10 seconds of production

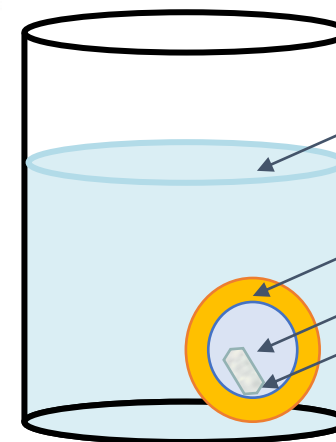
Screening of polymorphes of glycine by producing hundreds of capsules

Results - crystallized molecules

Protein crystals - Lysozyme



Temperature: 4.5°C,
Crystallization time: 3 days



Collection bath: 6% NaCl solution

Shell: Polymethacrylate resin + 20% EtOAc + 0,1% TPO

Core: 5% NaCl solution at pH 5.0 containing
Encapsulated 20 mg/mL of lysozyme
crystal

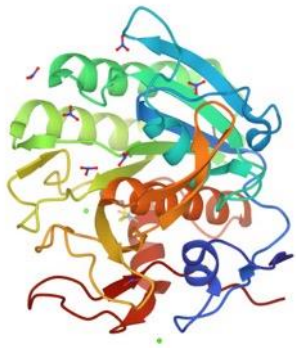
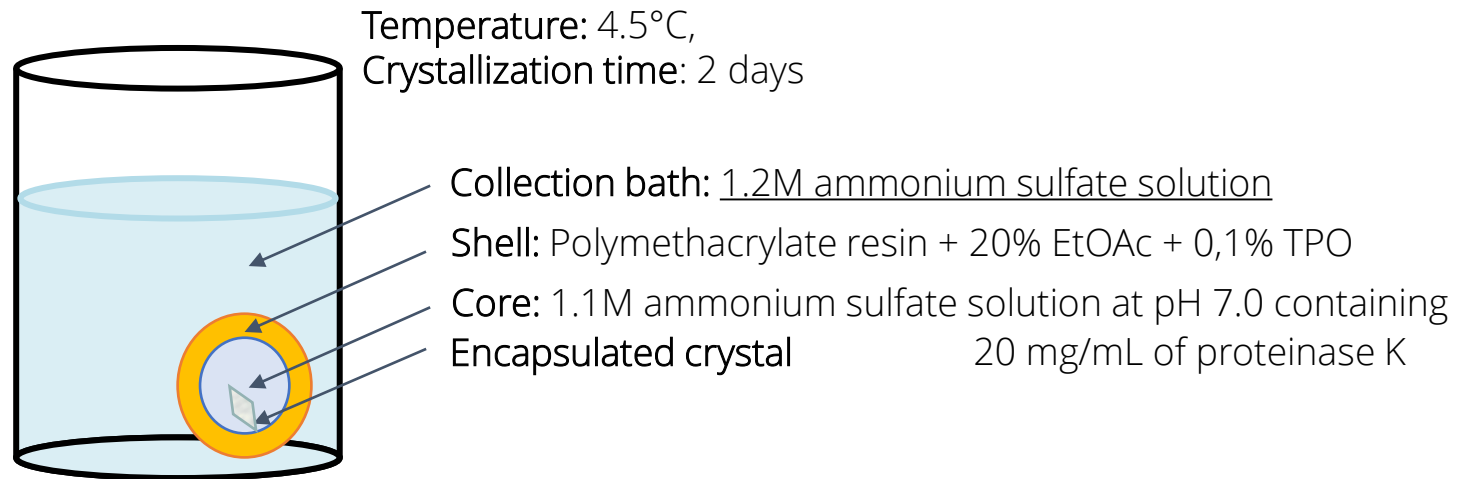
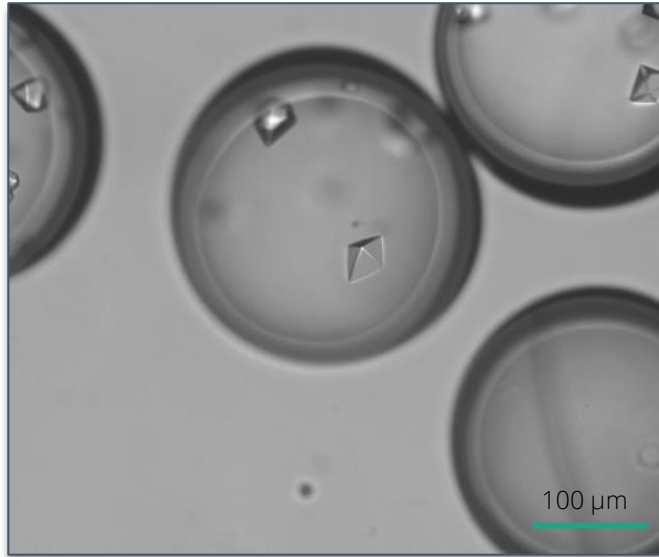


Crystal structure of lysozyme
16 kDa

Crystallization
by water
diffusion

Results - crystallized molecules

Protein crystals - Proteinase K

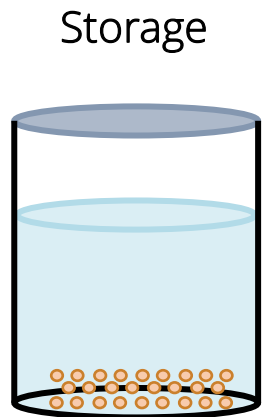


Crystal structure of proteinase K
29 kDa

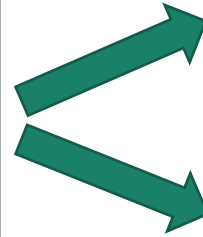
Crystallization by water diffusion

Direct crystallography

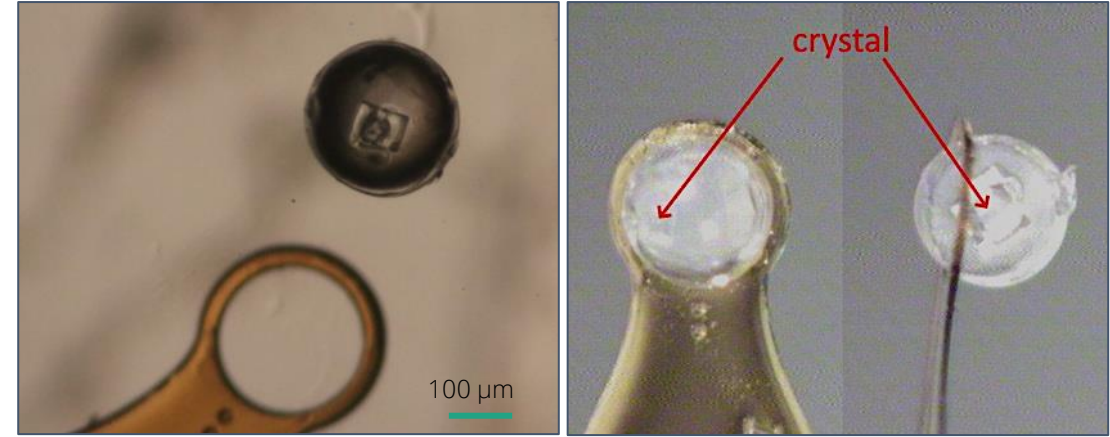
Collect of XRD data



XRD analysis

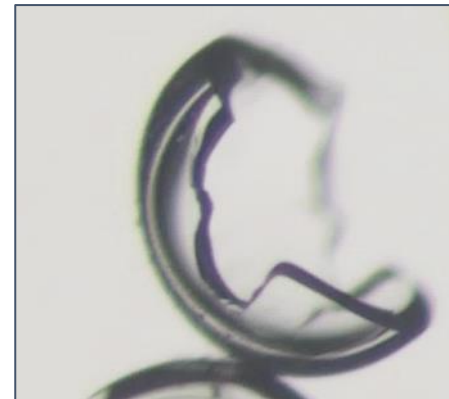


In situ



Mounting loop: 300 µm aperture

Ex situ

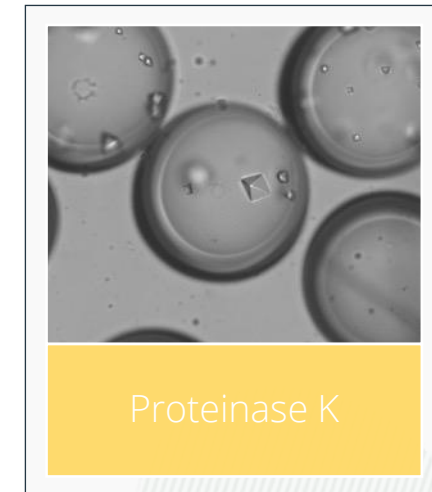
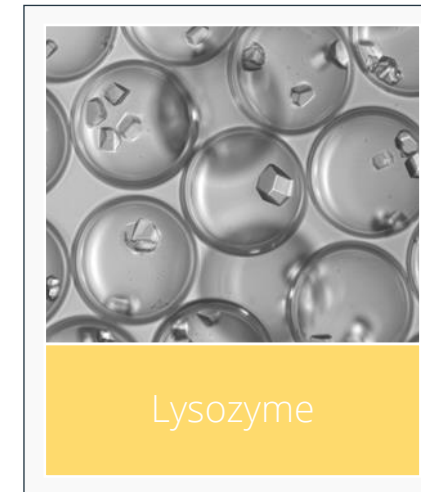
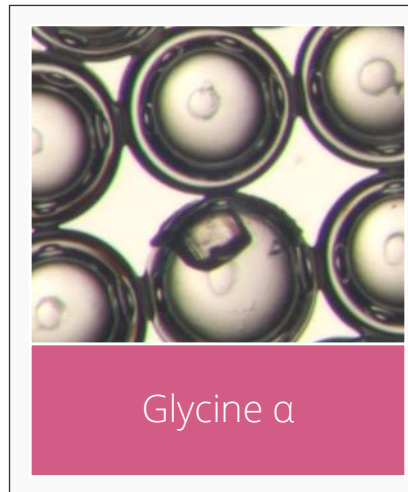
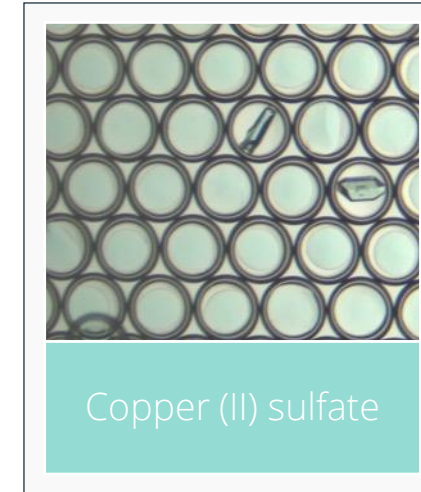


Capsules can accept :

- Room temperature
- Low temperature (up to -196°C with liquid nitrogen) without degradation of the shell.

Summary

- **High throughput**
≈2500 independent capsules/minute
- **Not resource consuming**
Down to 50 ng solute/capsule,
50 μg of solute needed for one experiment
- **Easy screening**
Fast parameter tuning
- **Crystals encapsulated in a solid shell**
Crystals protection, compatible with N₂ freezing
- **Transparent shell**
Allows direct analysis



Publication of results

ChemComm



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Single crystal formation in core–shell capsules†

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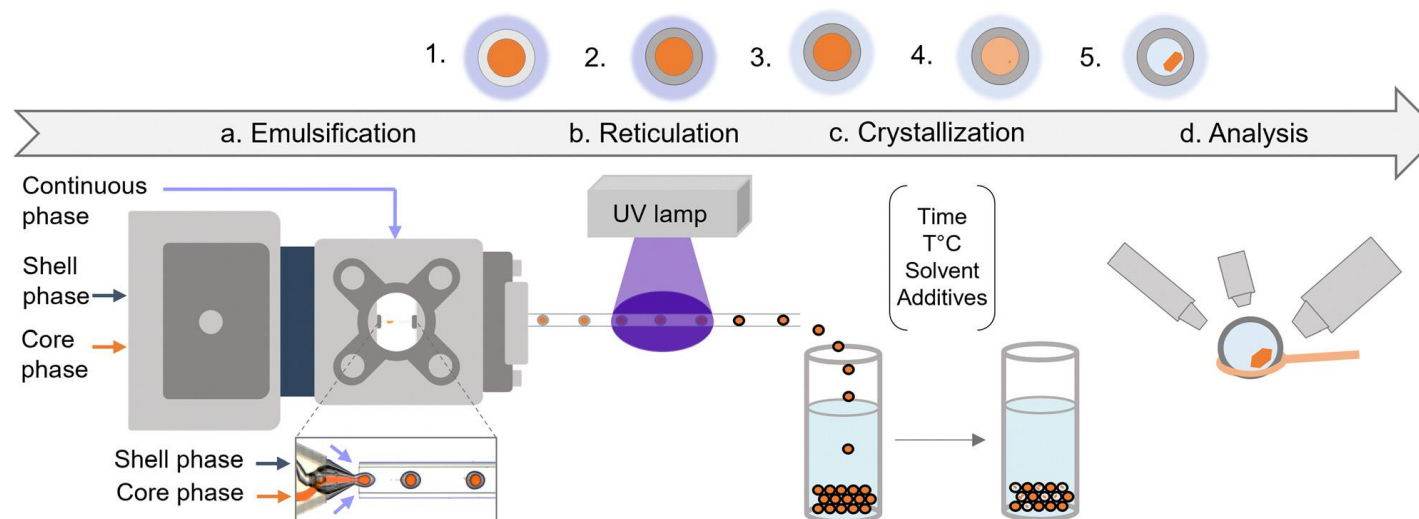
Marie Mettler,^a Adrien Dewandre,^a Nikolay Tumanov,^{id}^b Johan Wouters^b and Jean Septavaux^{id}^{*a}

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rsc.li/chemcomm

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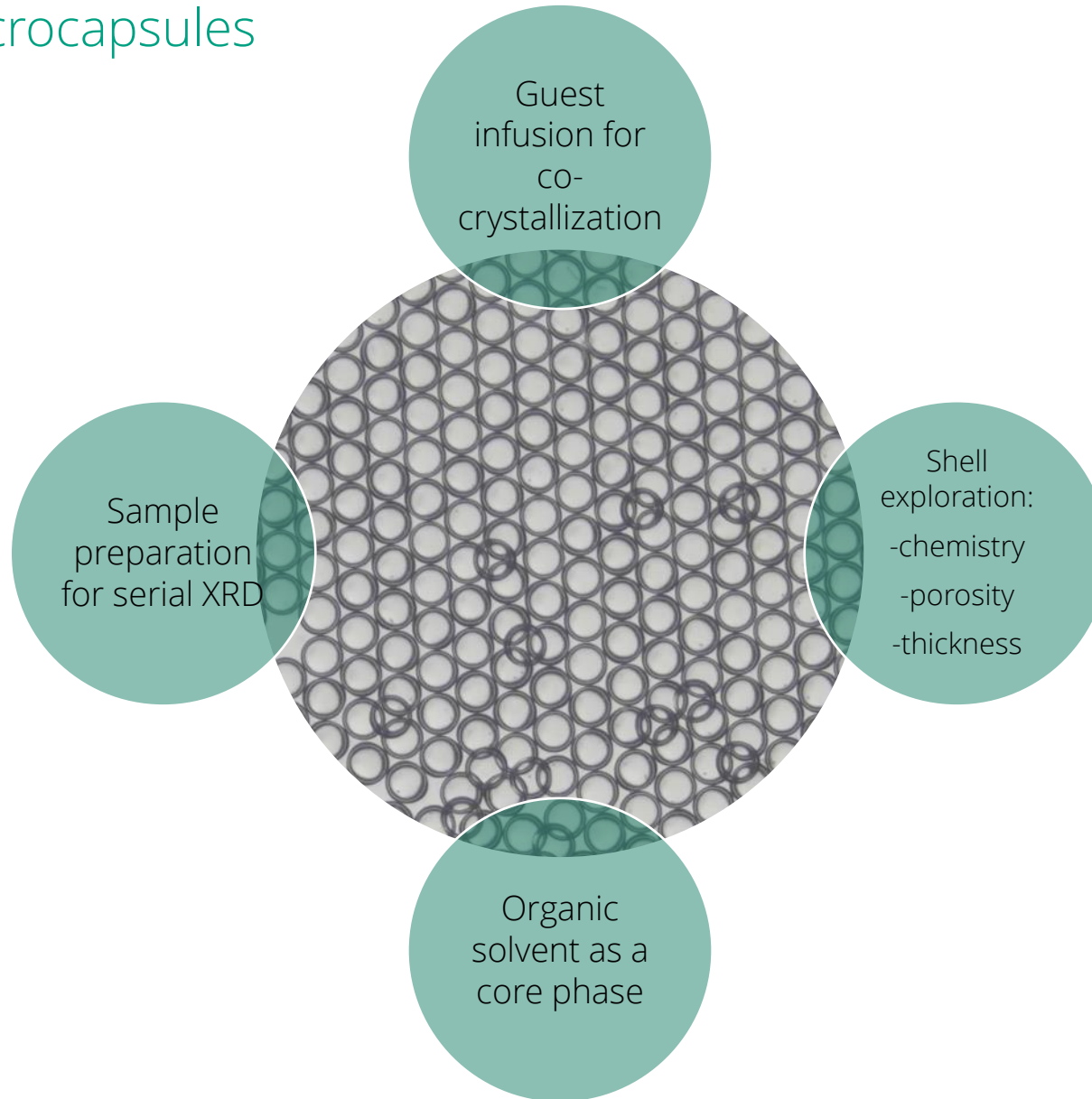
Representation of the crystallization process

Mettler *et al.*, *Chem. Commun.*, 2023, 59, 12739-12742

<https://pubs.rsc.org/en/content/articlelanding/2023/cc/d3cc03727d>

Perspectives

Single crystals in microcapsules



Aknowledgments

The Secoya team

Adrien Dewandre
Dr. Jean Septavaux
Emy Lennerts
Dr. Bart Rimez
Prof. Dr. Benoit Scheid
Bertrand Lories

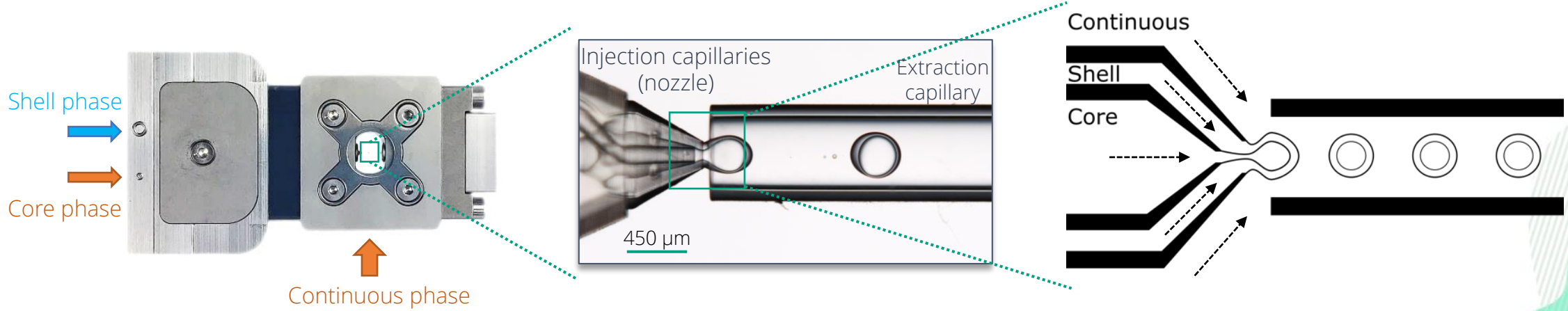
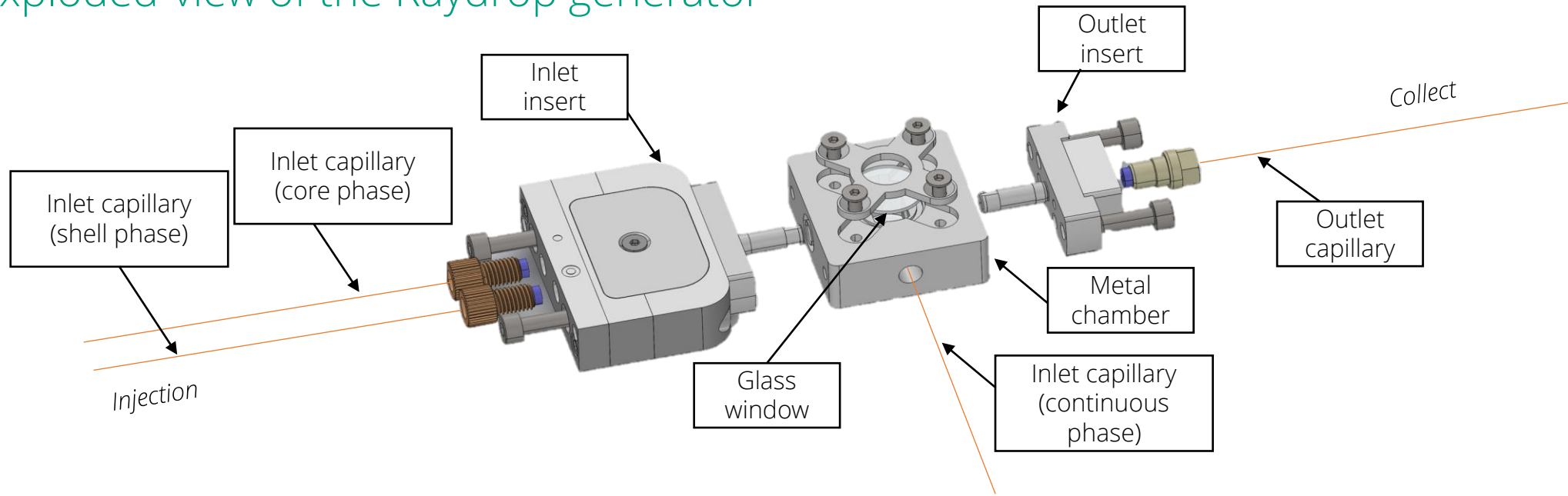
University of Namur

Dr. Nikolay Tumanov
Prof. Dr. Johan Wouter



Annexe - Presentation of the system

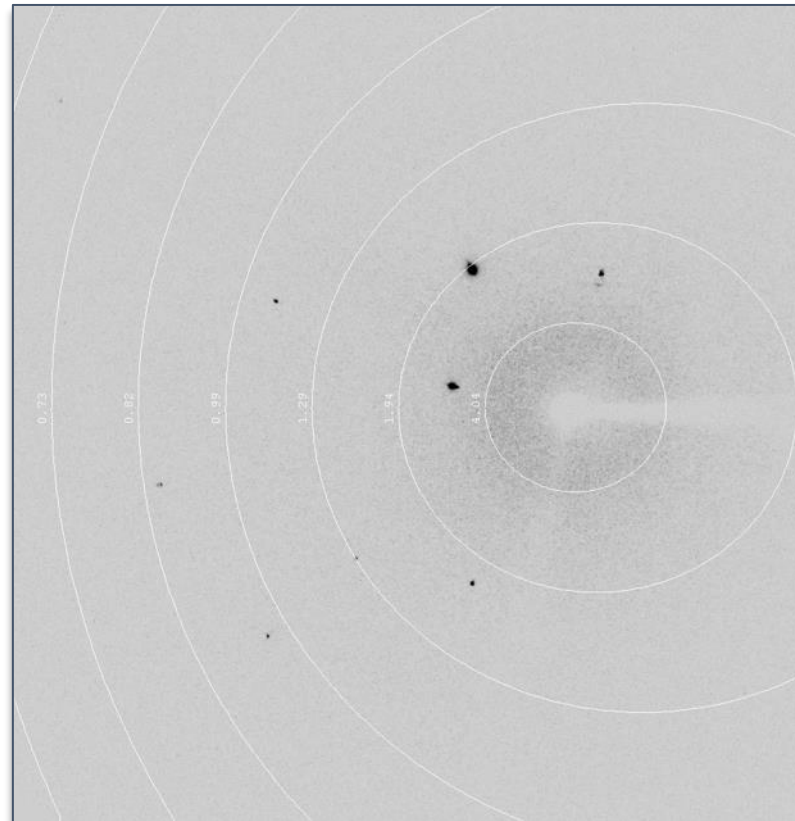
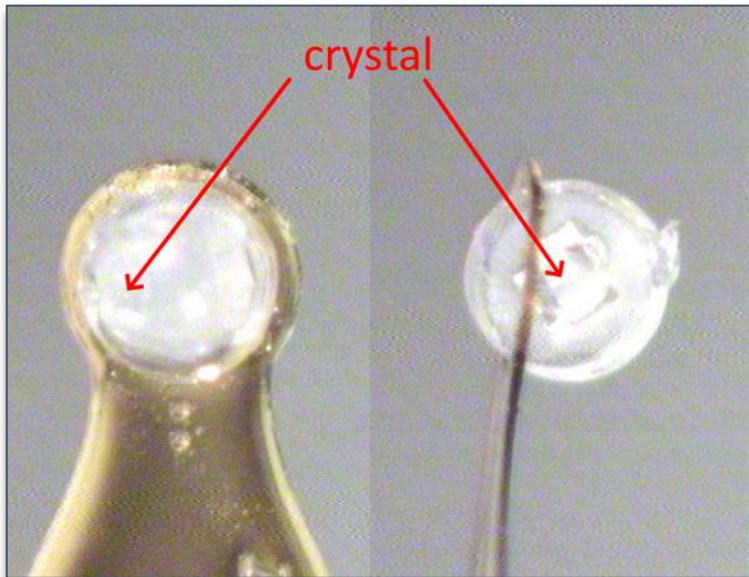
Exploded-view of the Raydrop generator



Annexe - Diffractometer

X-ray diffraction study

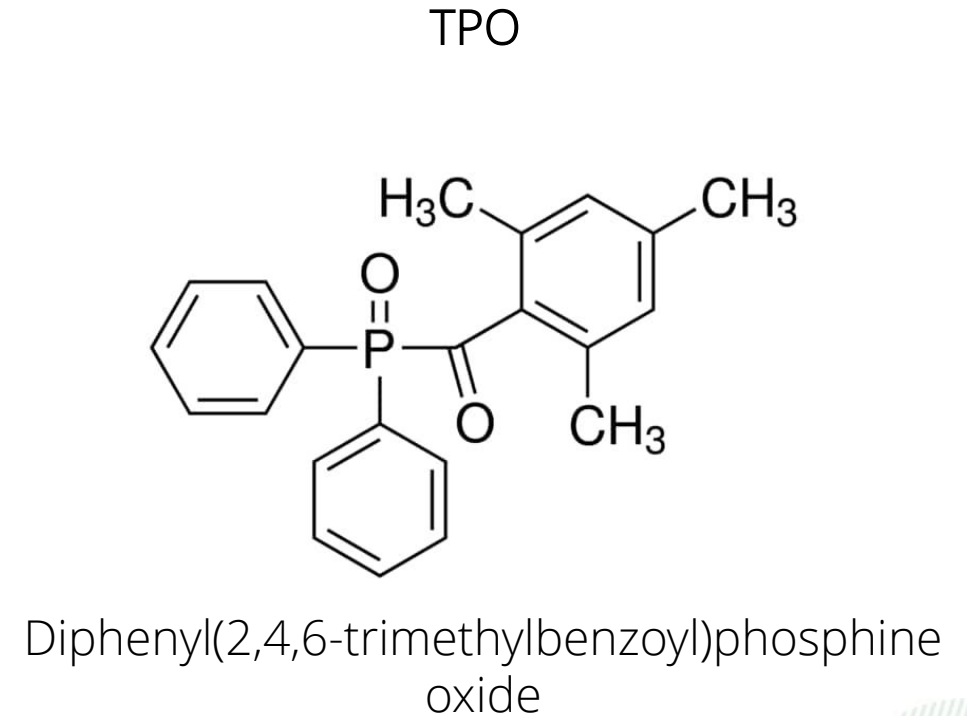
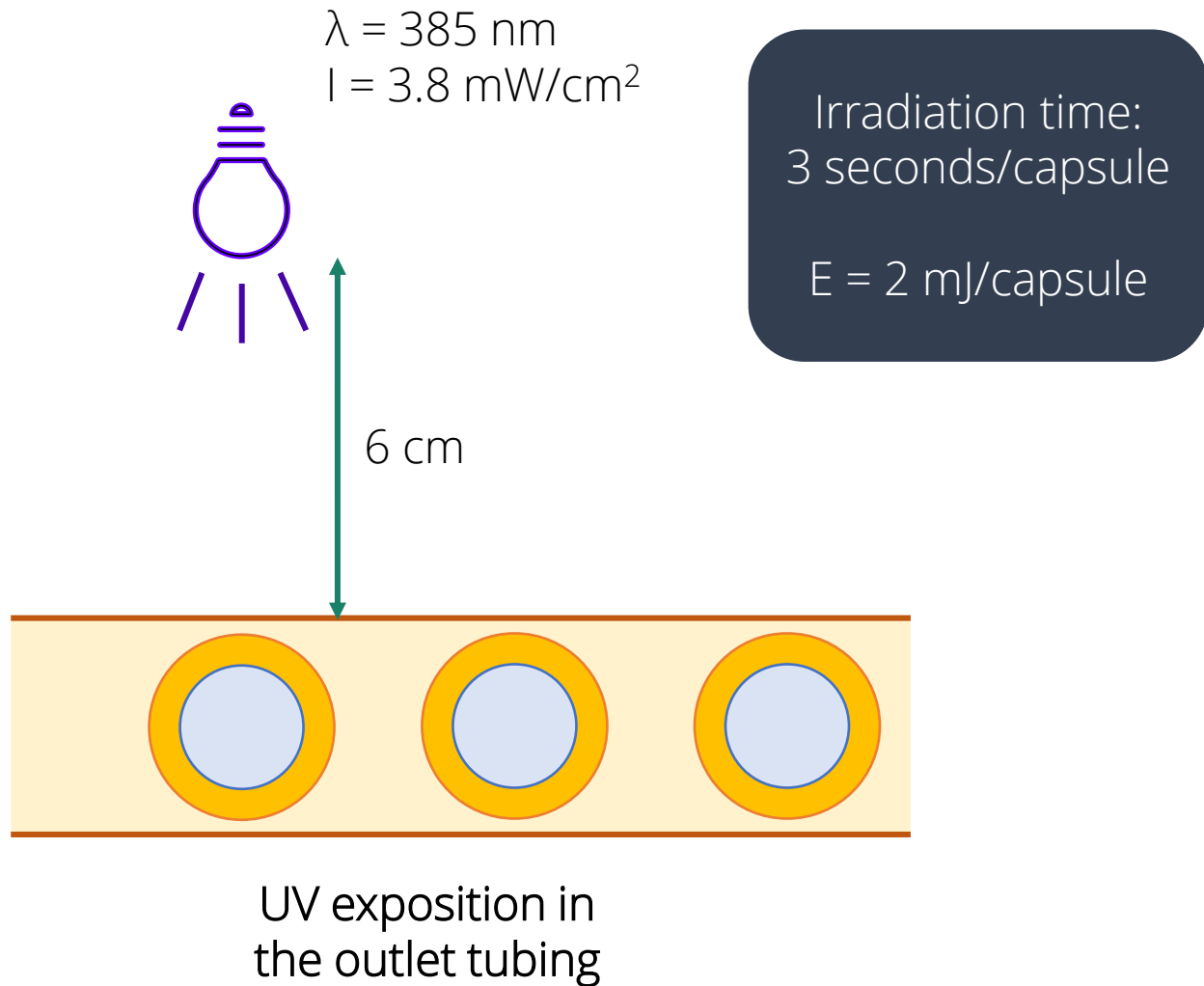
Diffractometer



Parameter	Details for NaCl
Crystal system	cubic
Space group	Fm-3m
a/Å	5.6357(3)
b/Å	5.6357(3)
c/Å	5.6357(3)
$\alpha/^\circ$	90
$\beta/^\circ$	90
$\gamma/^\circ$	90
Volume/Å ³	179.00(3)
Crystal size/mm ³	0.059 × 0.13 × 0.15

Annexe - Solidification of the shell

Resin cross-linking process with a photoinitiator



Free-radical photoinitiator