

Reconstructing Photosynthetic Systems

with microfluidics

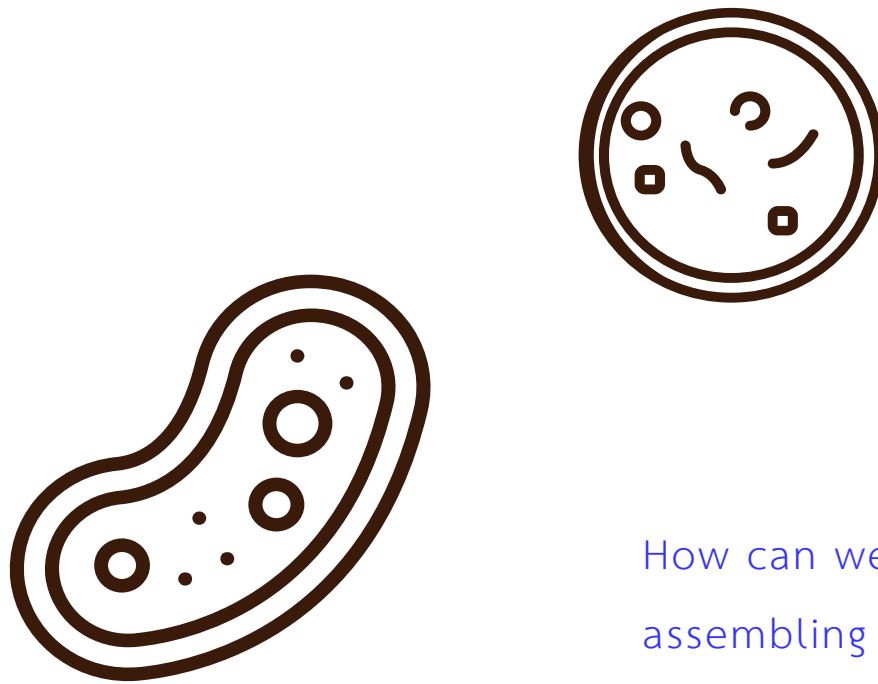
Prof. Jean-Christophe Baret

University of Bordeaux, Institut Universitaire de France

CNRS, CRPP, Soft Micro Systems

‘Only cells make cells’

Rudolf Virchow
(XIXth century)

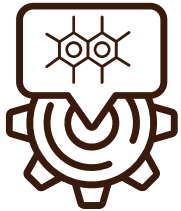


How can we engineer biological matter by
assembling biomimetic structures involving
controlled & characterized components ?

Schwille, *J. Cell. Biol.* (2011)
Schwille et al. *Angew. Chem. Int. Ed.* (2018)



... define new strategies to better control and regulate living systems making use of their flaws

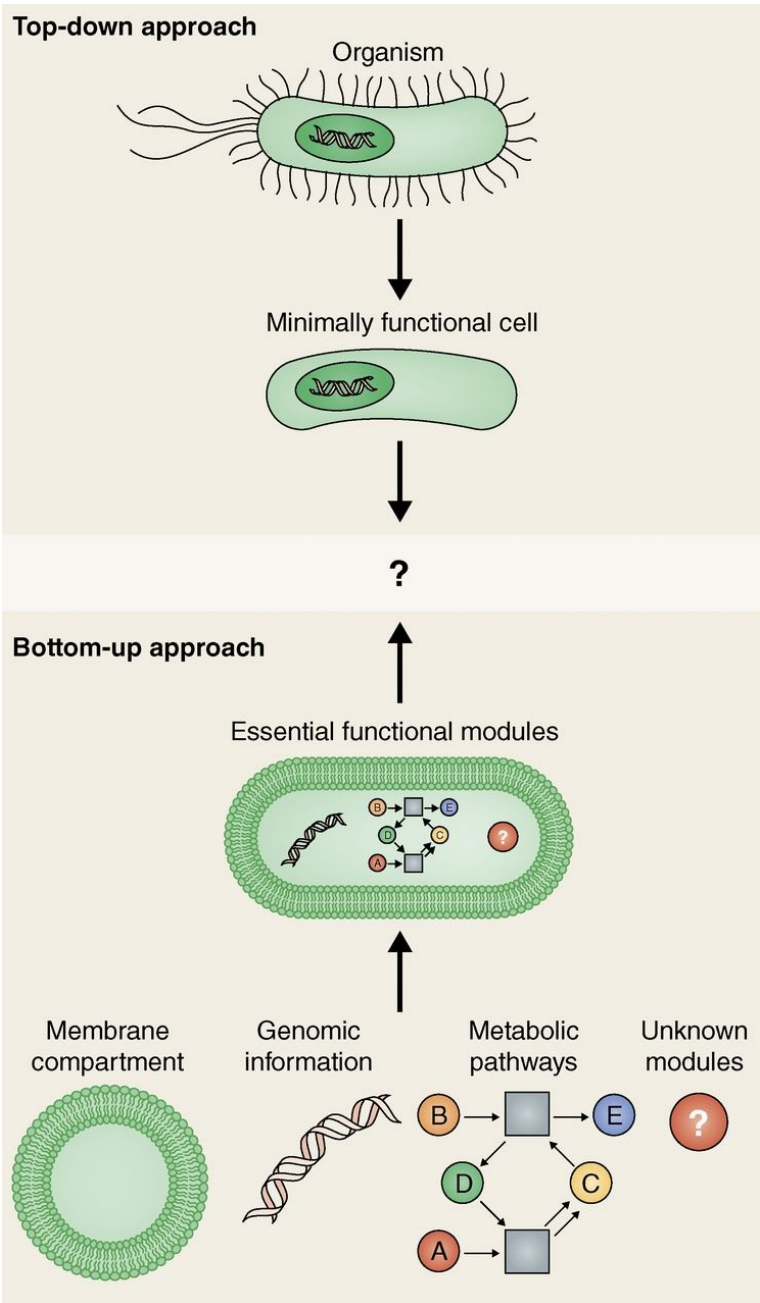


... use artificial cells in applications where living cells are not efficient or not suitable



... repair defective tissues and propose new therapeutic solutions to tissue deficiencies

Schwille, *J. Cell. Biol.* (2011)
Schwille et al. *Angew. Chem. Int. Ed.* (2018)



Essential features of living cells

Compartmentalization

Metabolism

Protein expression

Energy conversion

Growth

Division

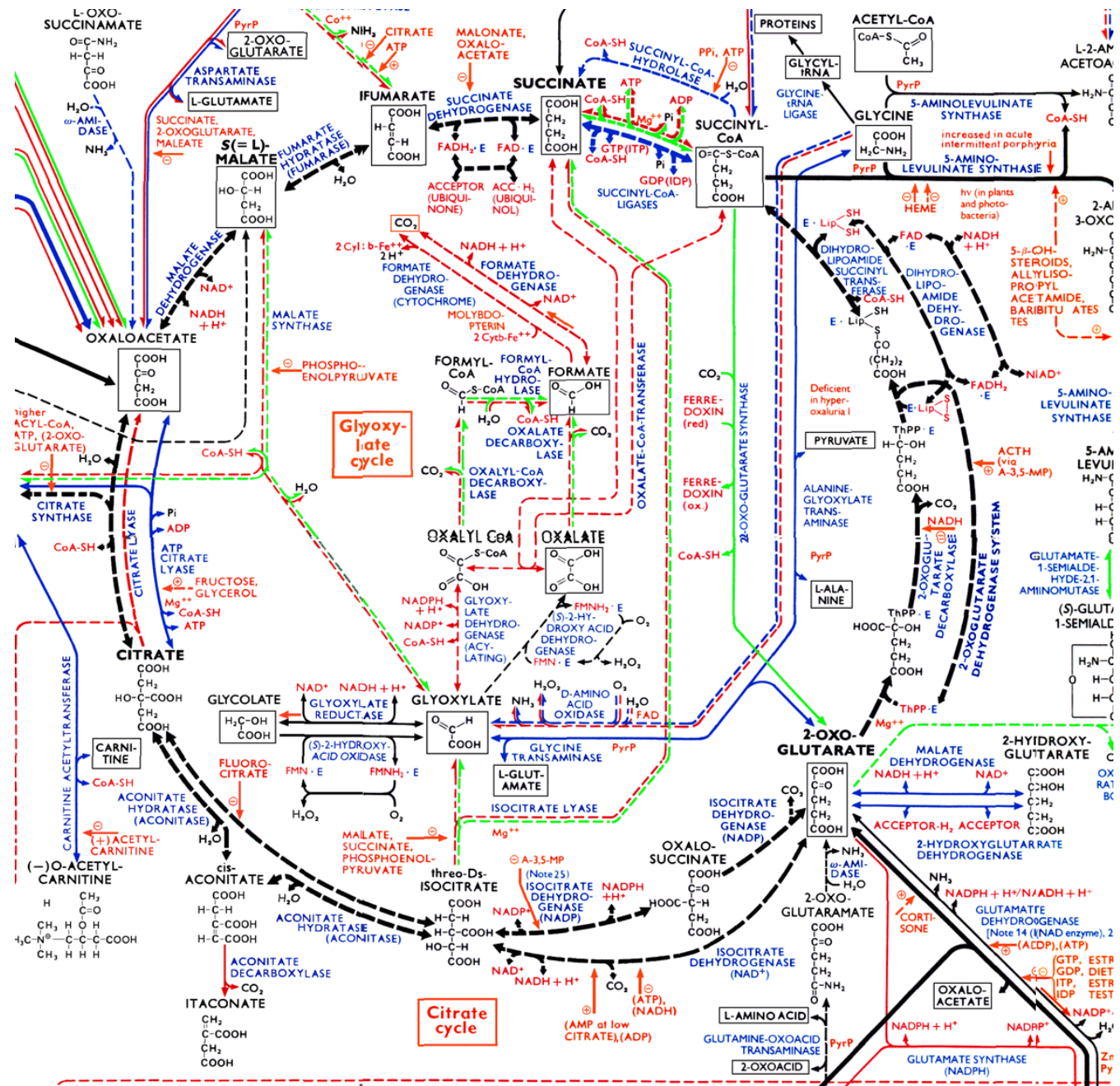
Signaling and motility

Schwille, *J. Cell. Biol.* (2011)
Schwille et al. *Angew. Chem. Int. Ed.* (2018)

The problem : biochemistry

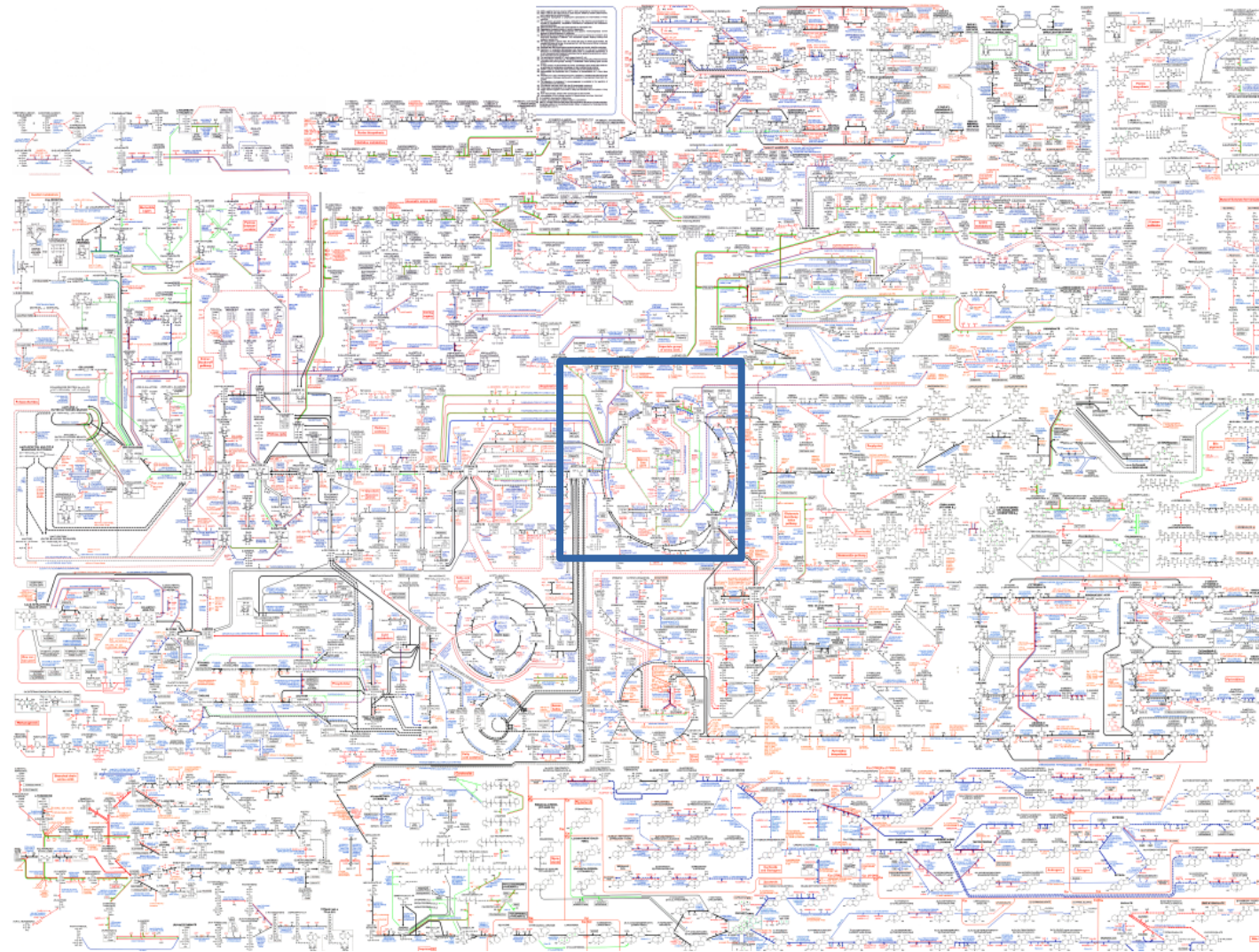
At least mine...

https://www.cc.gatech.edu/~turk/bio_sim/articles/metabolic_pathways.png



The (even bigger) problem

https://www.cc.gatech.edu/~turk/bio_sim/articles/metabolic_pathways.png



The biochemical description involves highly coupled network of reactions

Complexity arises from

- the need to perform several tasks at the same time
- the need to account for fluctuating external conditions
- adaptation & evolution

J.K. Weng, The evolutionary path towards complexity: a metabolic perspective, *New*

Phylogenetic Analysis (2014)

“How does the living organisms avoid decay ?

*The obvious answer is: by eating, drinking,
breathing and assimilating. The technical term
is metabolism.”*

E. Schroedinger,

What is life ? The physical aspect of the living cell.

Cambridge Univ. Press (1944)

How to maintain a system out-of-equilibrium ?

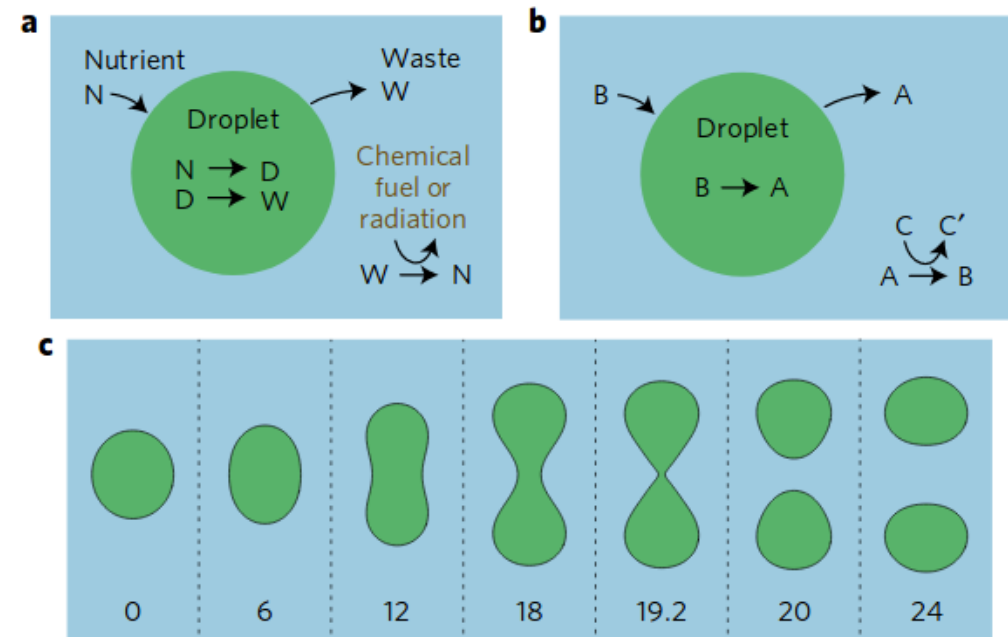
& How to make it Minimal, Simple and Integrable ?

In a bottom up synthetic biology context, there is a need to build-up a minimal version of metabolic pathways capable of maintaining the compartment out-of-equilibrium

1 reaction (a few) might be enough !

Minimal metabolism is relevant in recent models of cell division

NATURE PHYSICS DOI: 10.1038/NPHYS3984



Growth and division of active droplets provides a model for protocells, Zwicker *et al.*, *Nature Physics* (2016)

Technology for μ -compartments

Droplet-based microfluidics

Chemically Out-of-Eq. μ -compartments

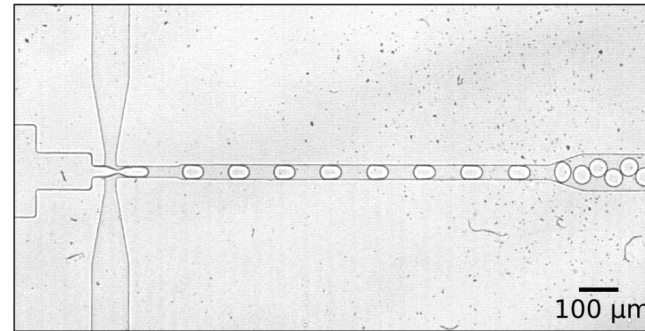
Non eq. steady states

Light controlled μ -compartments

Energy harvesting

Droplet Production

Anna et al. APL (2003)



Droplet
storage

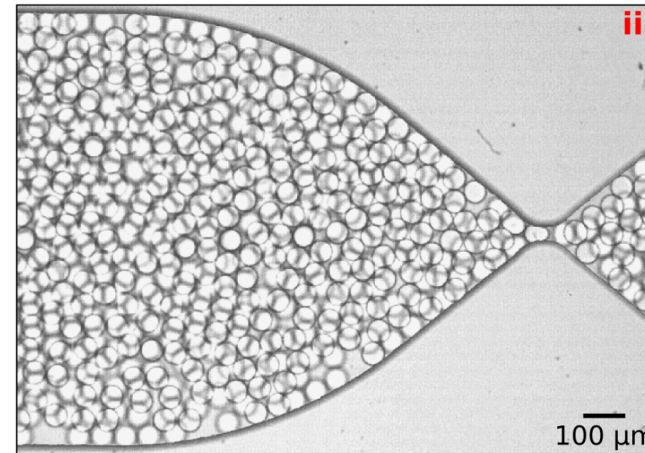


> 10 000
droplets
per second

Droplet Incubation

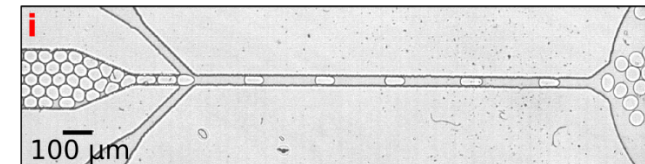
Ahn et al. APL (2006)

Frenz et al. Lab Chip (2009)



Droplet Reinjection

Holtze et al. Lab Chip (2008)



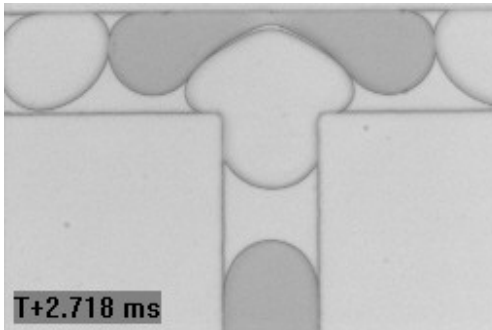
Surfactants have a crucial role in these processes

Baret, *Lab Chip* (2012)
Gruner et al., *Soft Matter* (2012)
Brosseau et al. *Soft Matter* (2014)
Gruner et al. *Current Op. Colloids Interf. Sci* (2015)
Gruner et al. *Nature Communications* (2016)
Riechers et al. *PNAS* (2016)

emulseo
biofluidic technologies

Droplet Division

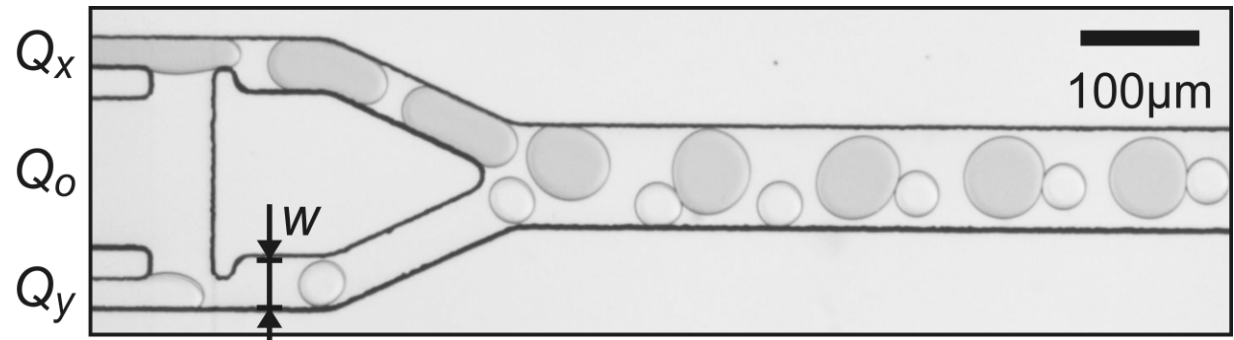
Link *et al. PRL* (2004)



Droplet Pairing

Frenz *et al. Langmuir* (2008)

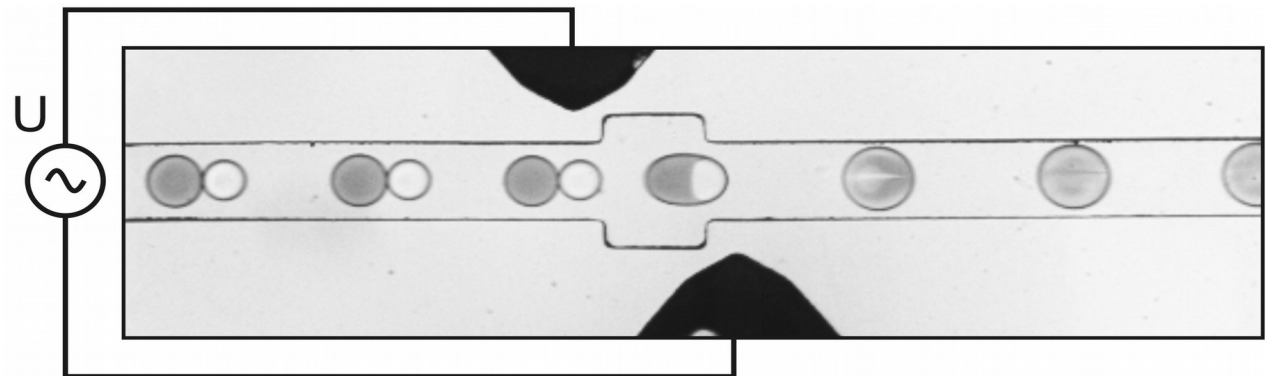
Frenz *et al. Angew. Chemie* (2008)



Droplet Fusion

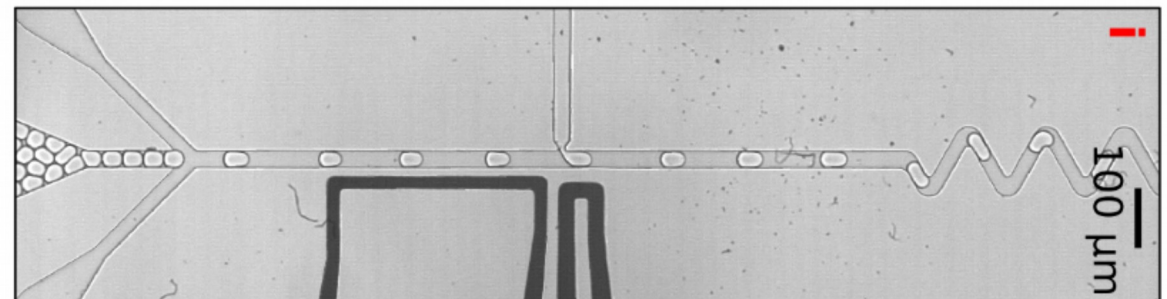
Ahn *et al. APL* (2006)

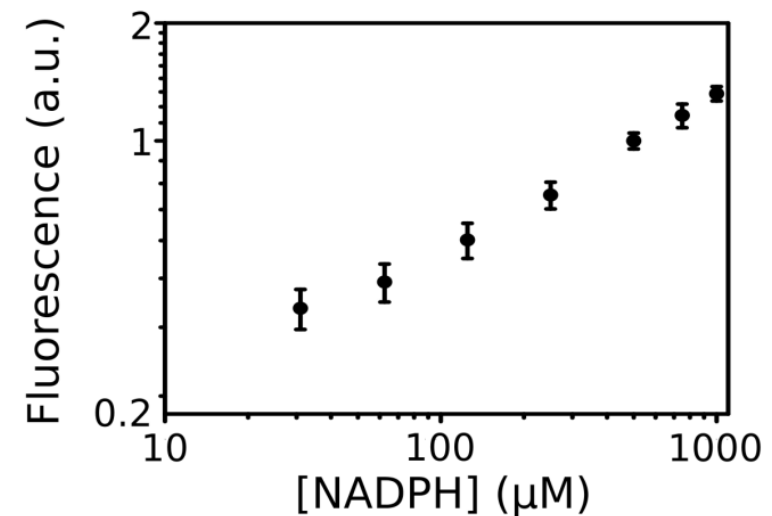
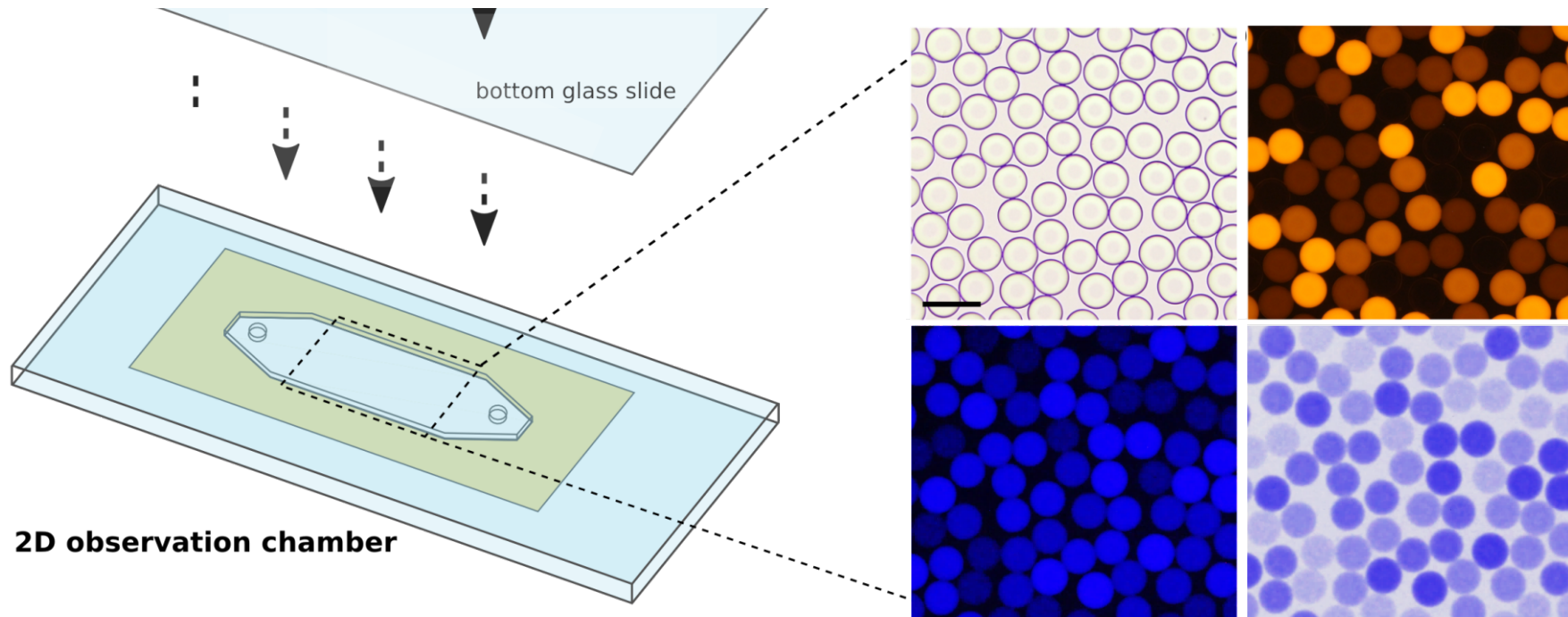
Frenz *et al. Angew. Chemie* (2008)

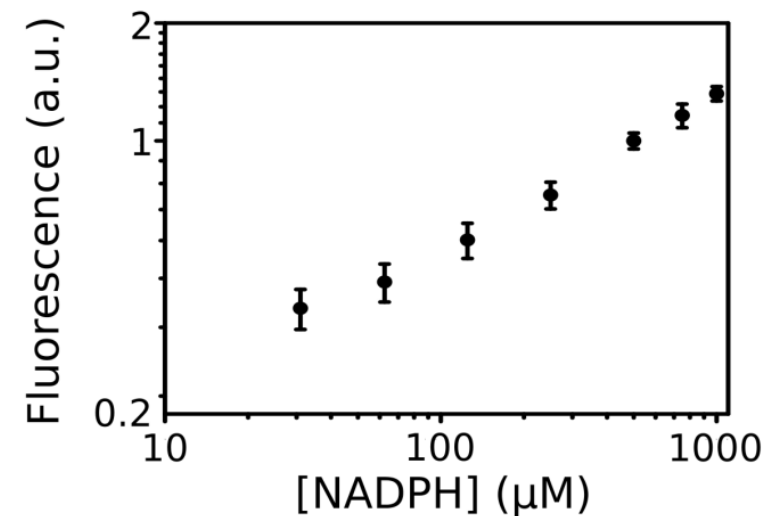
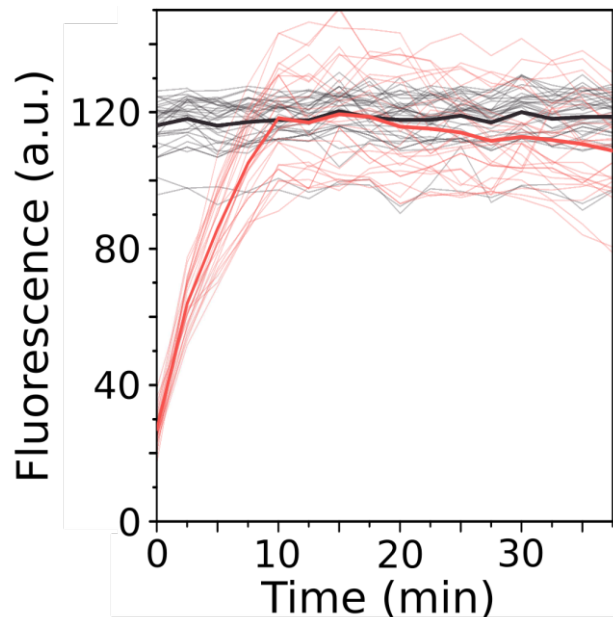
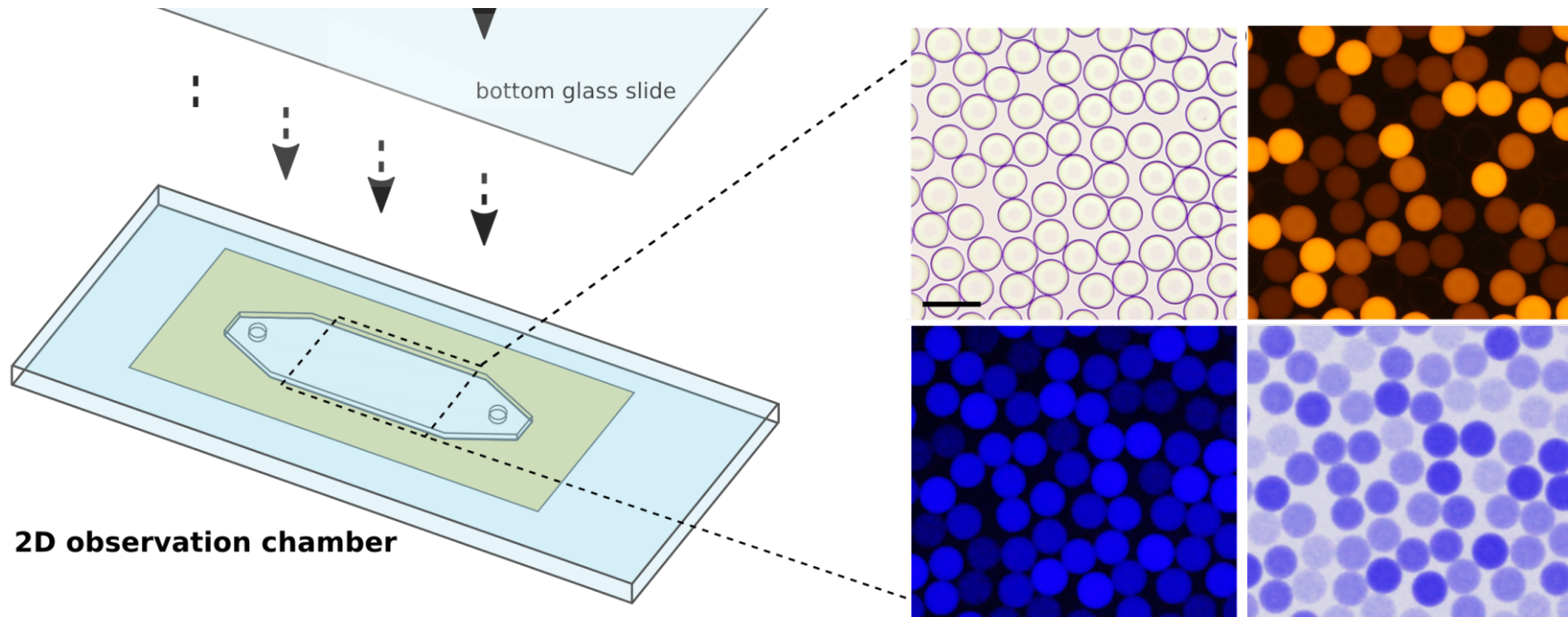


‘Pico-injection’

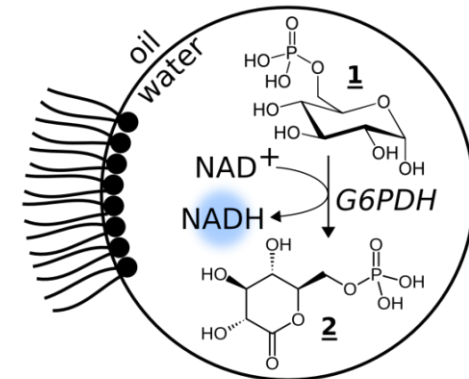
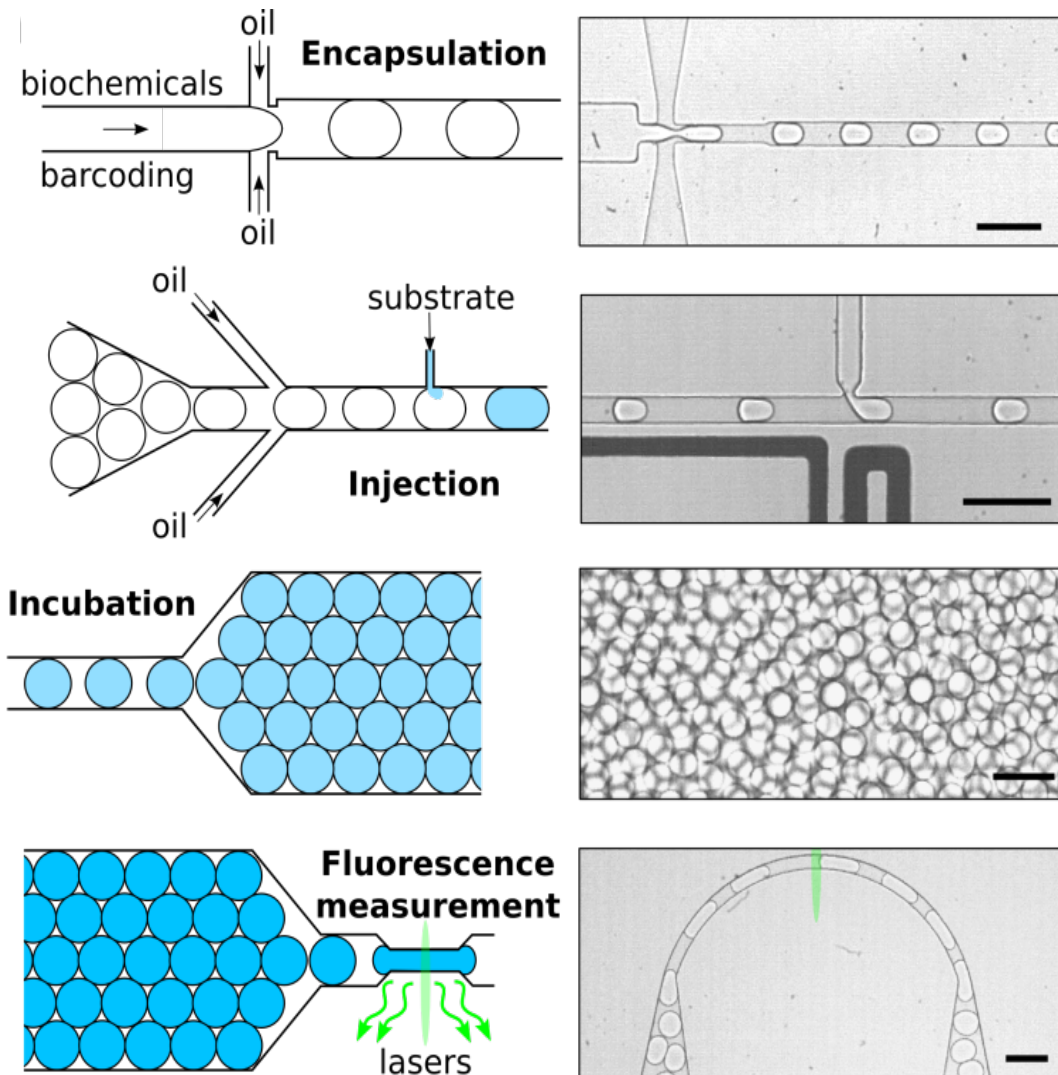
Abate *et al. PNAS* (2011)



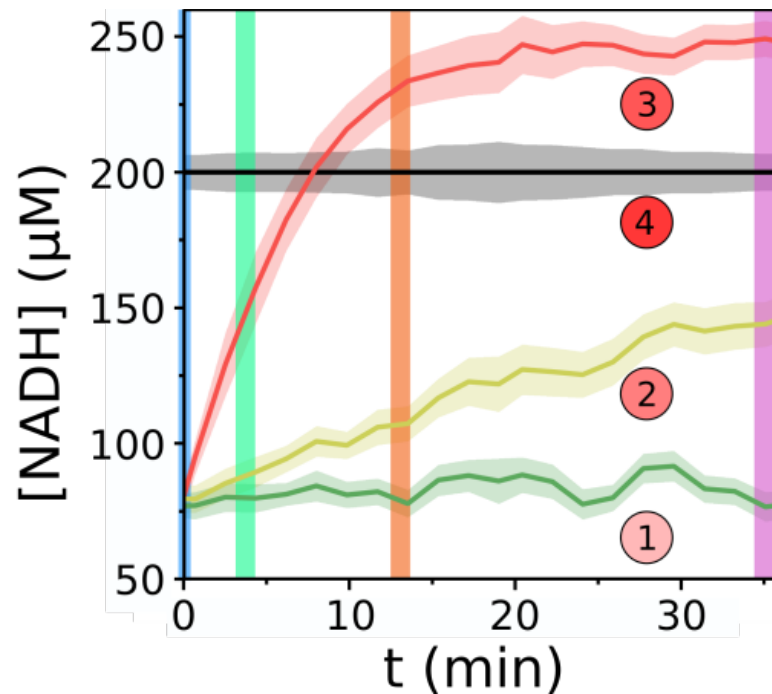
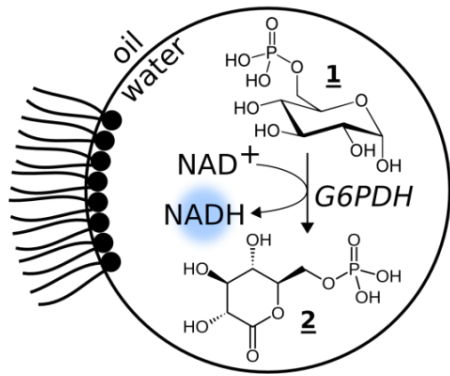




($N \approx 10\,000$)



[Beneyton et al., *Nat. Commun.*, **2018**, 9, 2931]



System runs spontaneously

$$\Delta rG < 0$$

Stops when equilibrium is reached
($\sim \text{NAD}^+$ or substrate is consumed)

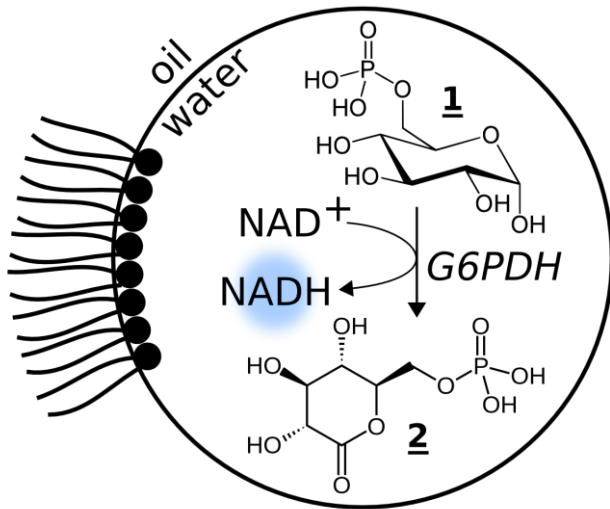
Encapsulation &
miniaturization

Quantitative
analysis

Beneyton *et al.* *Nature Comm.* (2018)

System runs ($\Delta rG < 0$)

Stops when NAD^+ cofactors are consumed

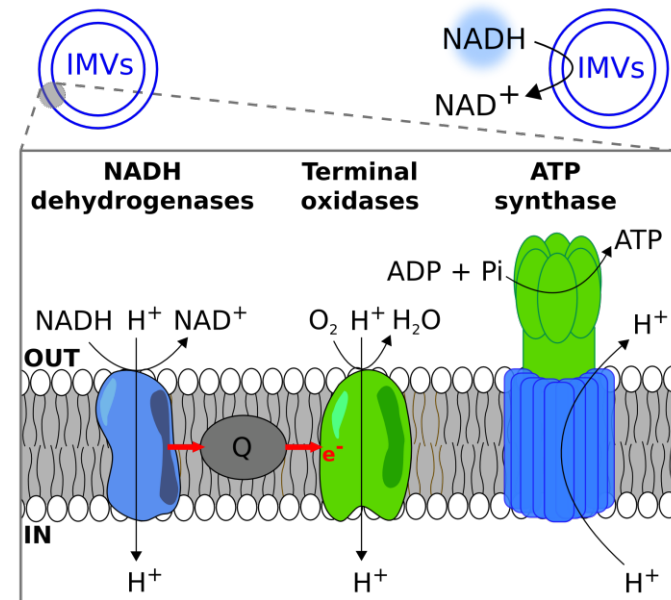


Catabolism

(NAD^+ consumption)

In-situ regeneration of NAD^+ cofactor

Inverted Membrane Vesicles from *E. coli*

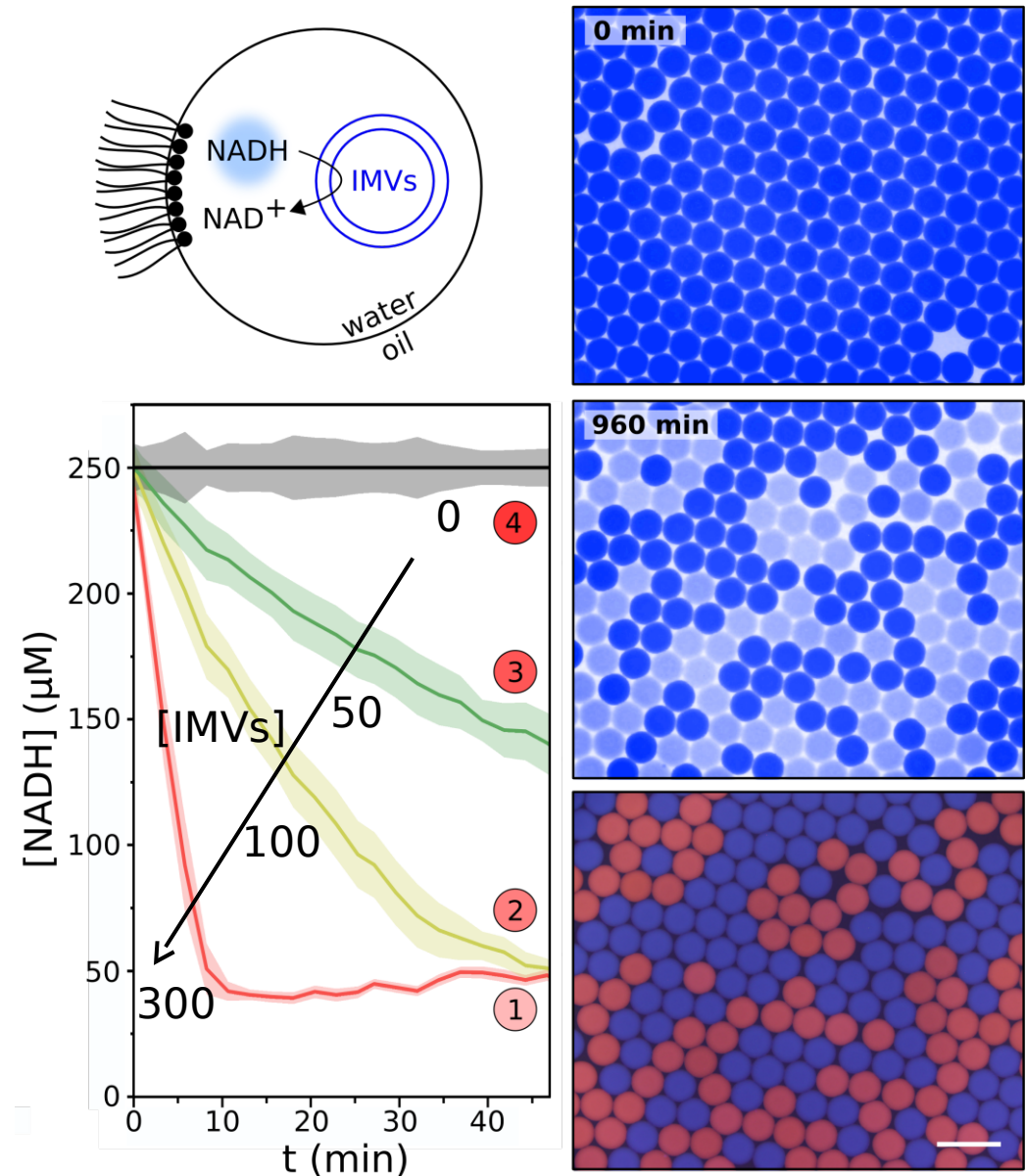


Anabolism

(NAD^+ regeneration)

Active process

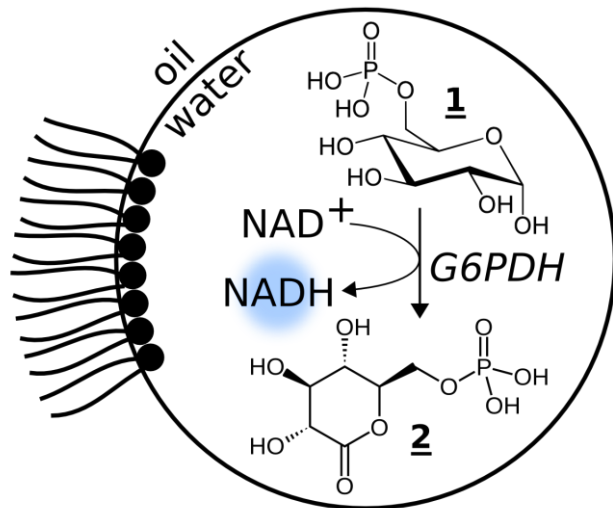
IMVs in 30 pL w/o droplets



Eq. vs Out-of-Eq.

System runs ($\Delta rG < 0$)

Stops when NAD^+ cofactors are consumed



Catabolism

(NAD^+ consumption)

+

Anabolism

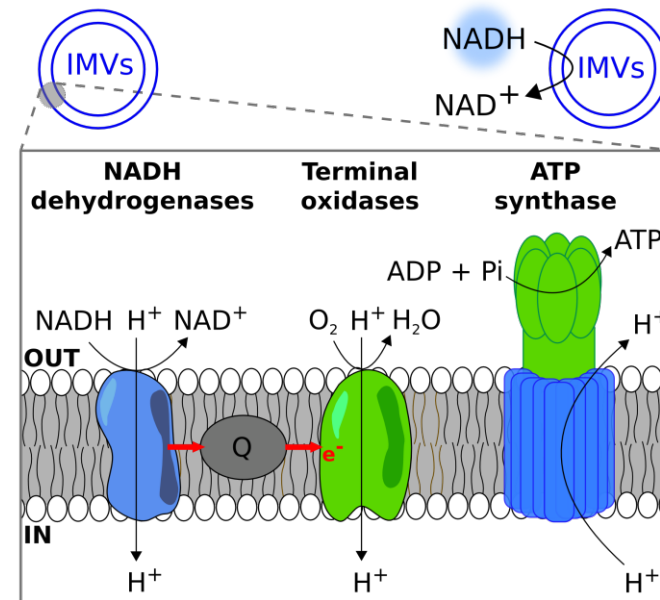
(NAD^+ regeneration)

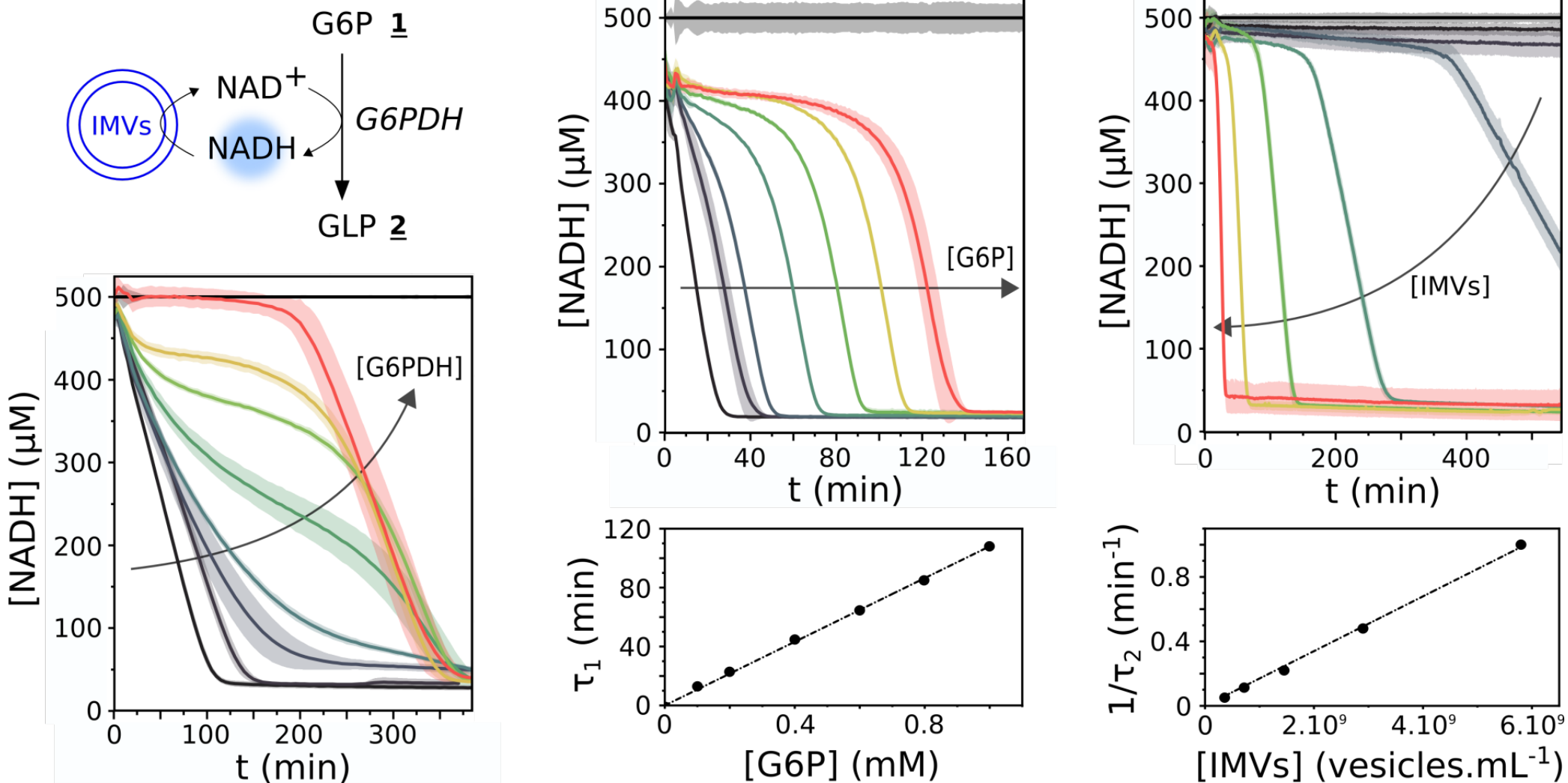
Active process

= Metabolism

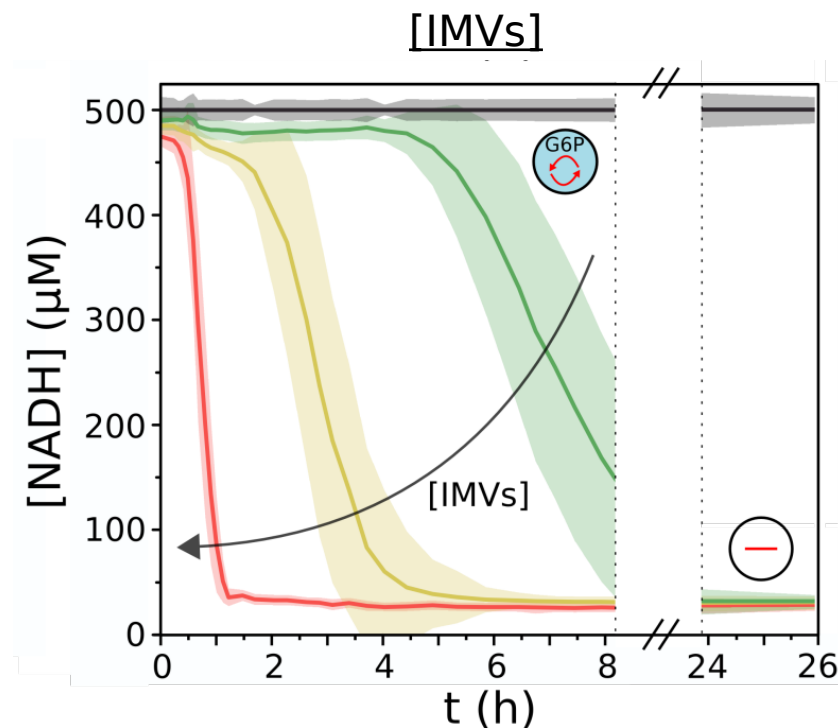
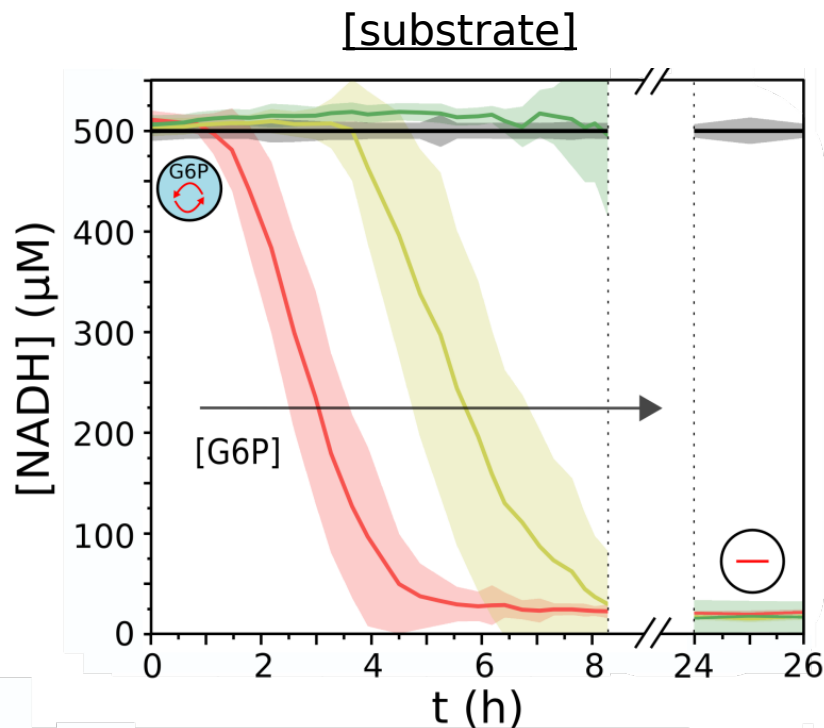
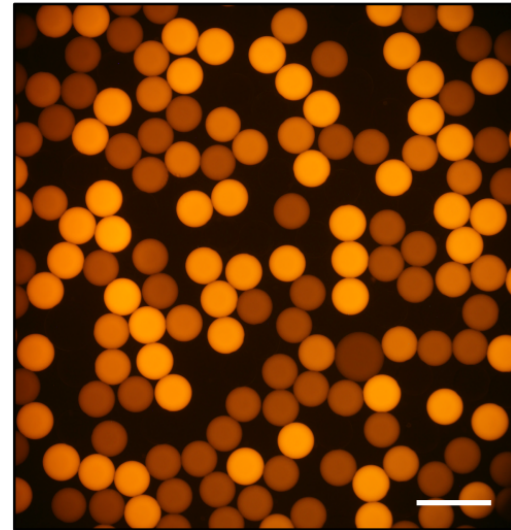
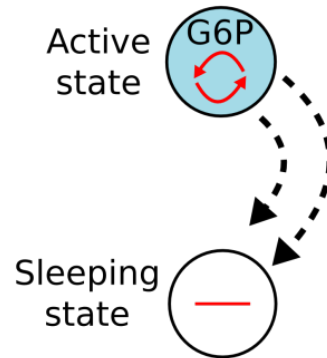
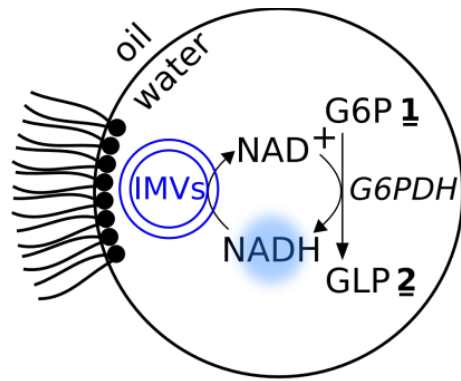
In-situ regeneration of NAD^+ cofactor

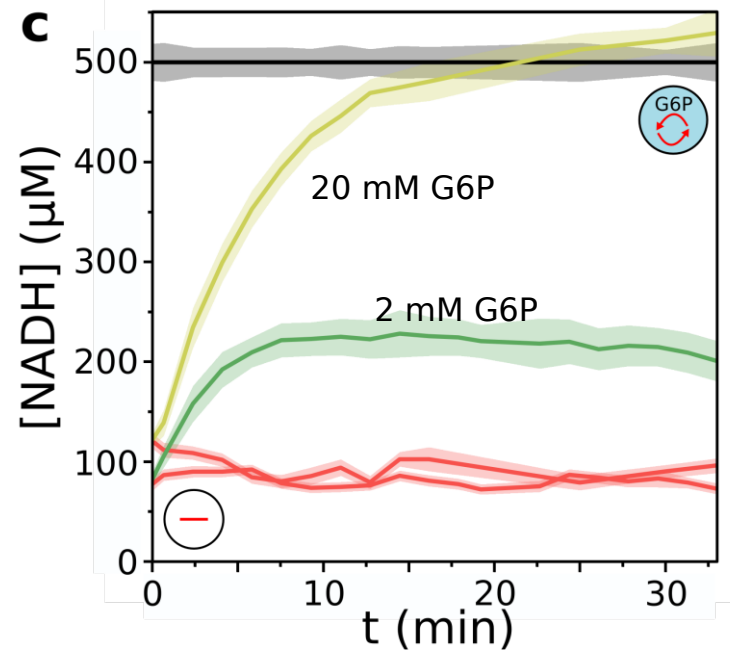
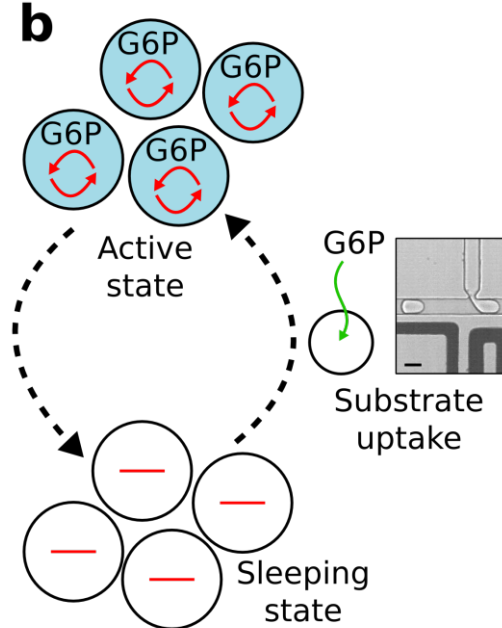
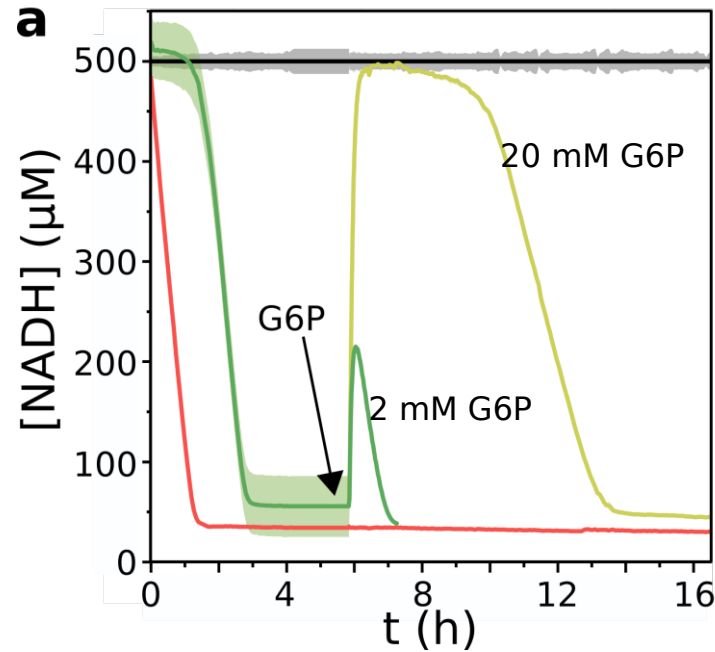
Inverted Membrane Vesicles from *E. coli*





[Beneyton et al., *Nat. Commun.*, **2018**, 9, 2931]



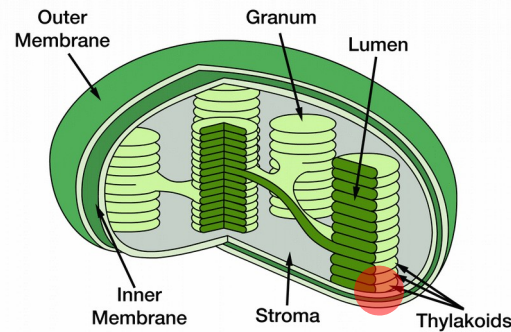


- Control of the metabolic state of thousands of independent monodisperse compartments
- Own reservoir of chemical fuel or their own fitness
- Reassembled natural parts for the regeneration of cofactors

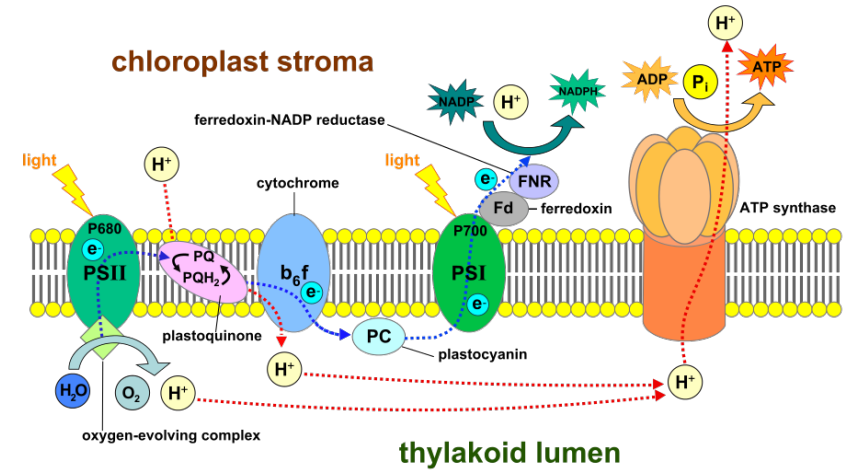
Chloroplast & Thylakoids



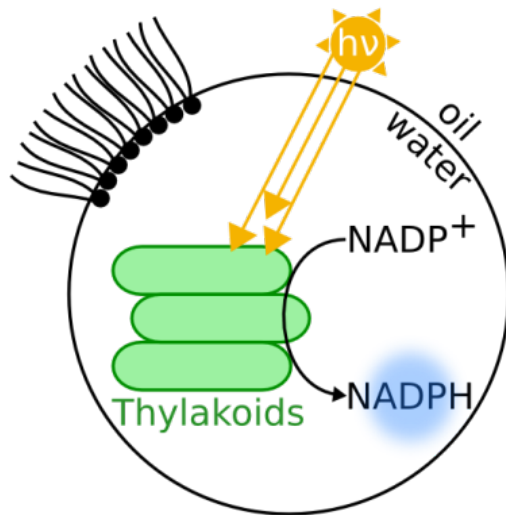
Spinach leaves



Chloroplast



Thylakoids - NADPH / ATP Regeneration



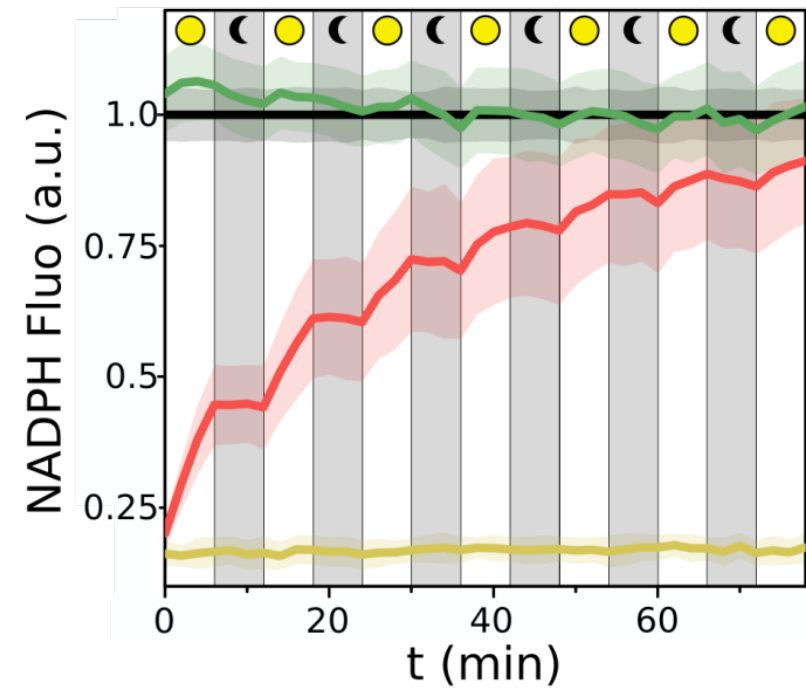
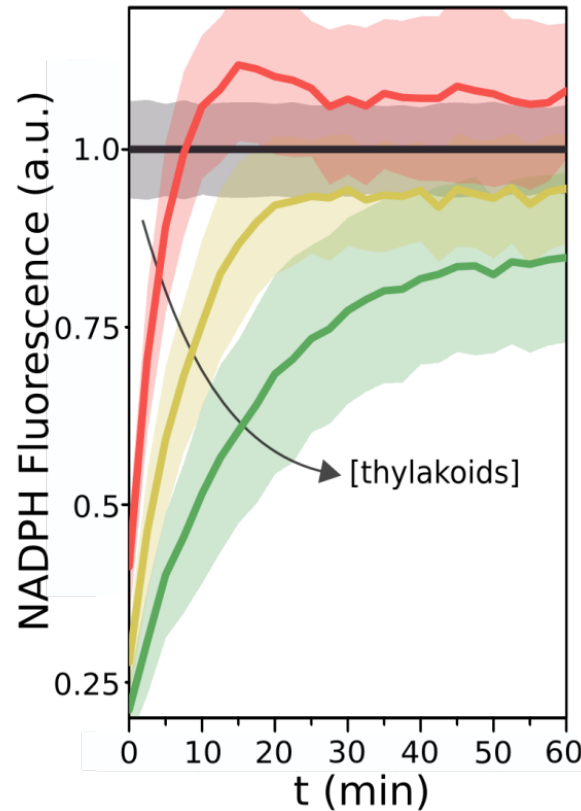
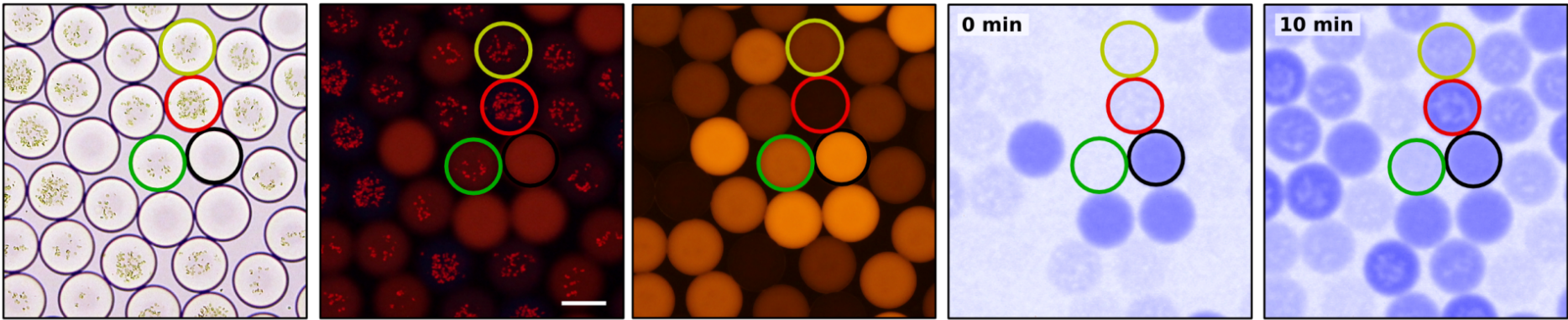
Re-engineer / mimicking chloroplasts

Harvesting light energy : Anabolism module

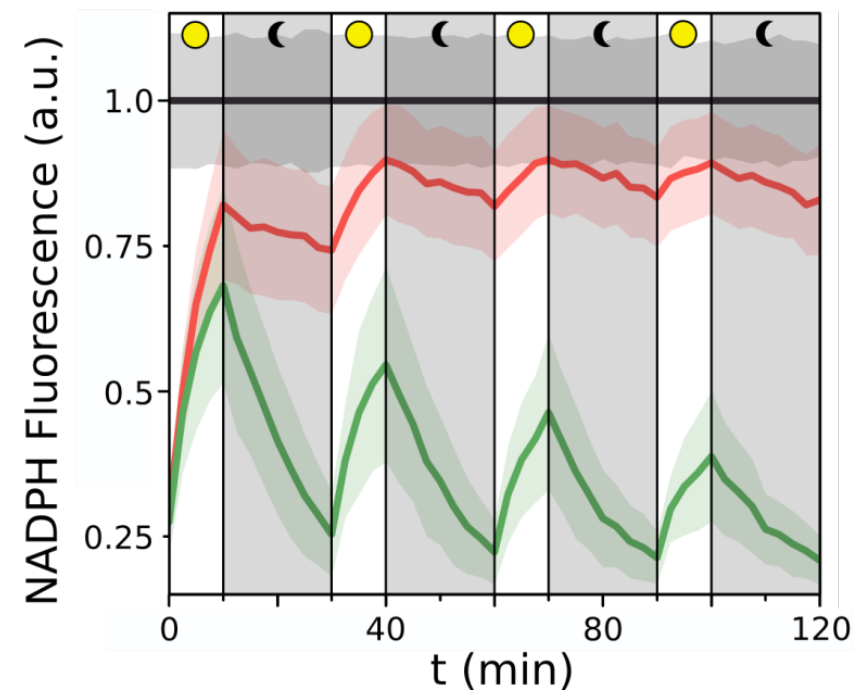
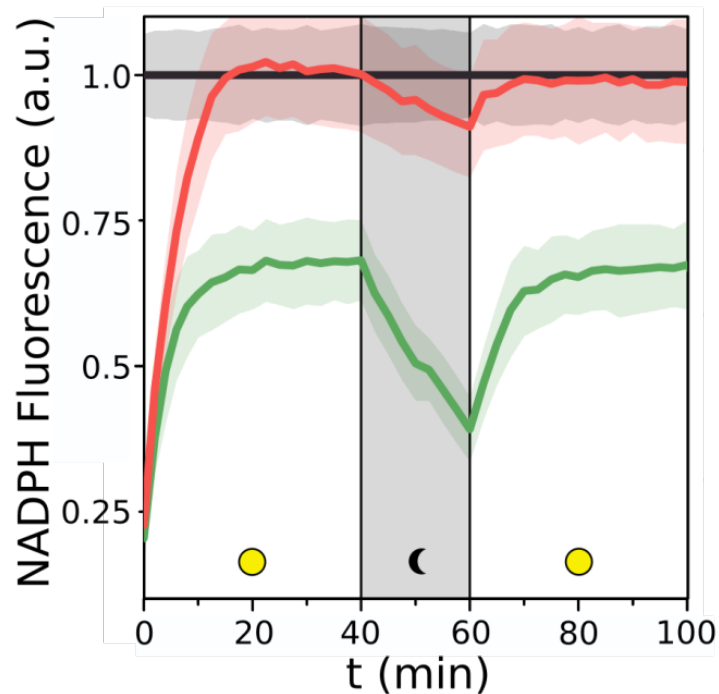
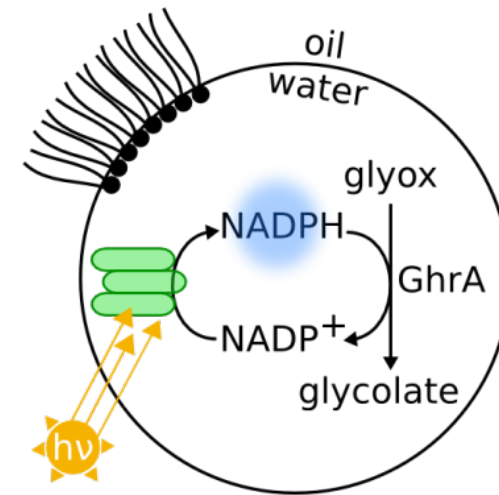
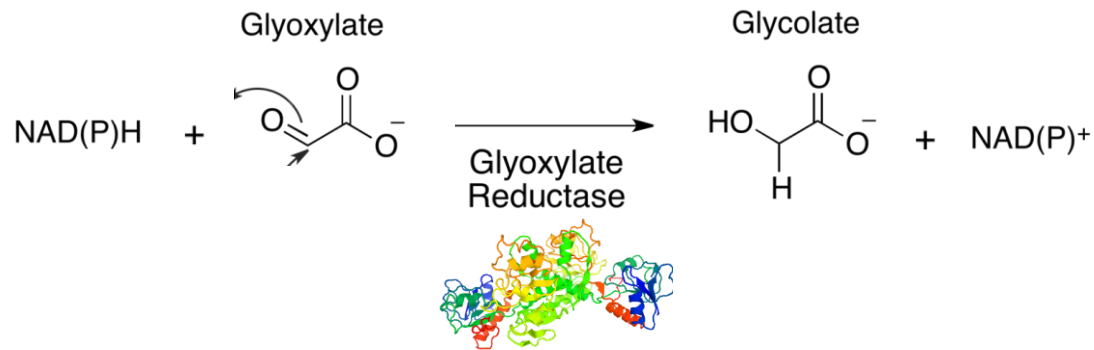
Light-triggered production of NADPH / ATP

Run out-of-equilibrium systems

Thylakoids in drops



Powering a metabolic reaction

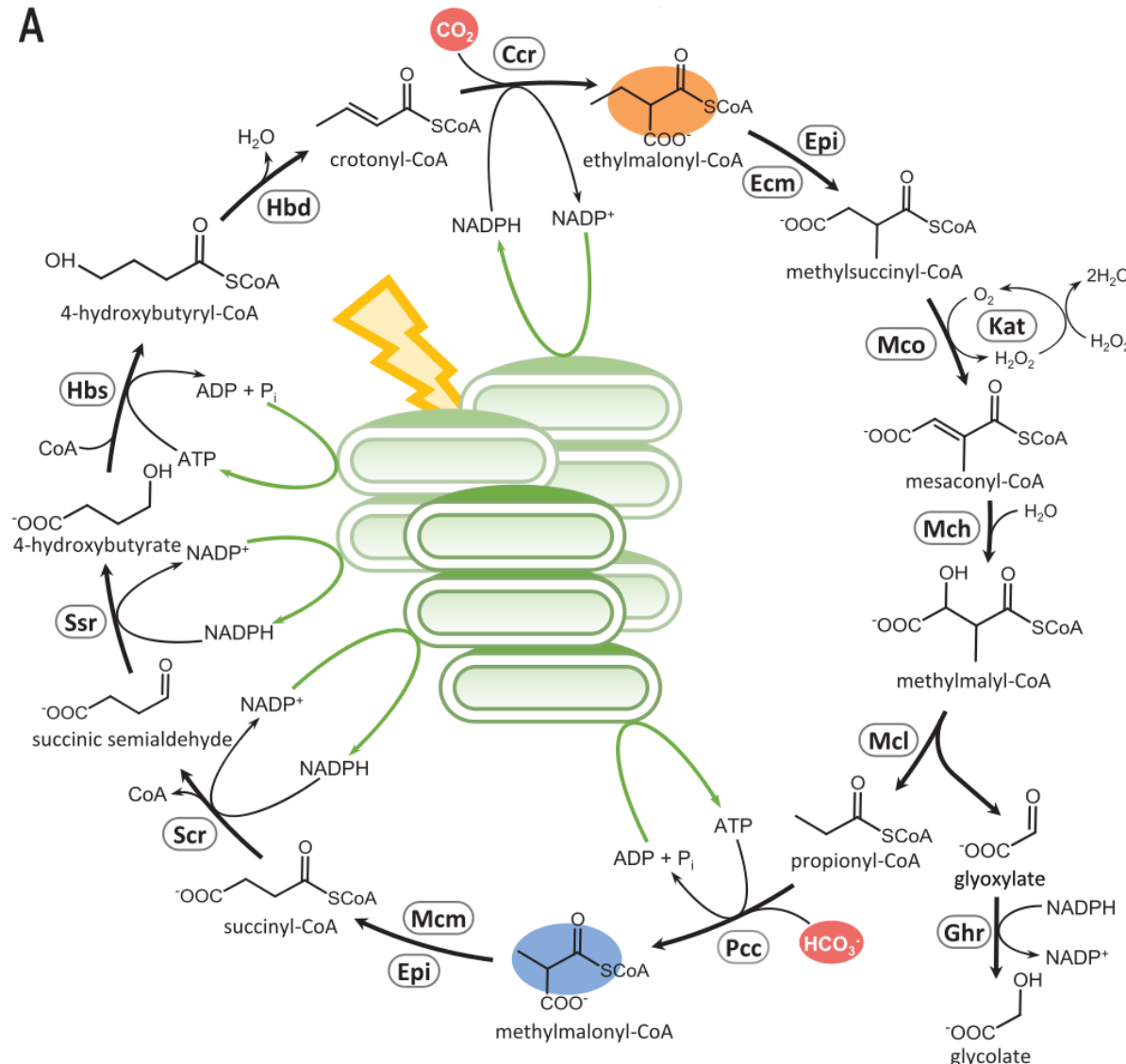


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J.-C. Baret – Novembre 2022 [[AAF - Paris]]

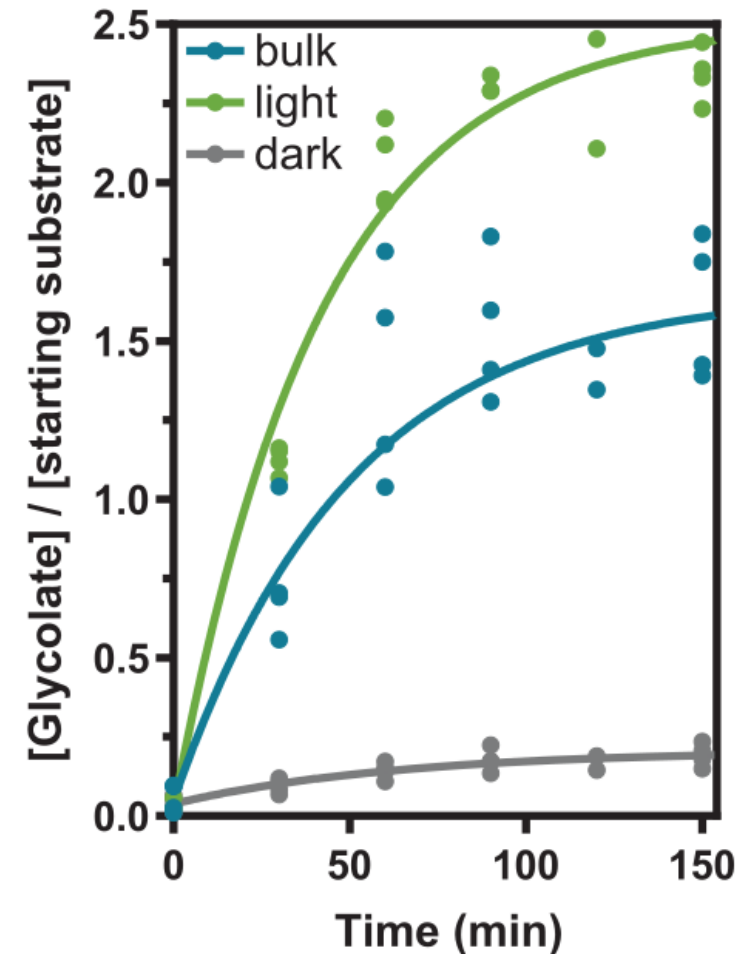
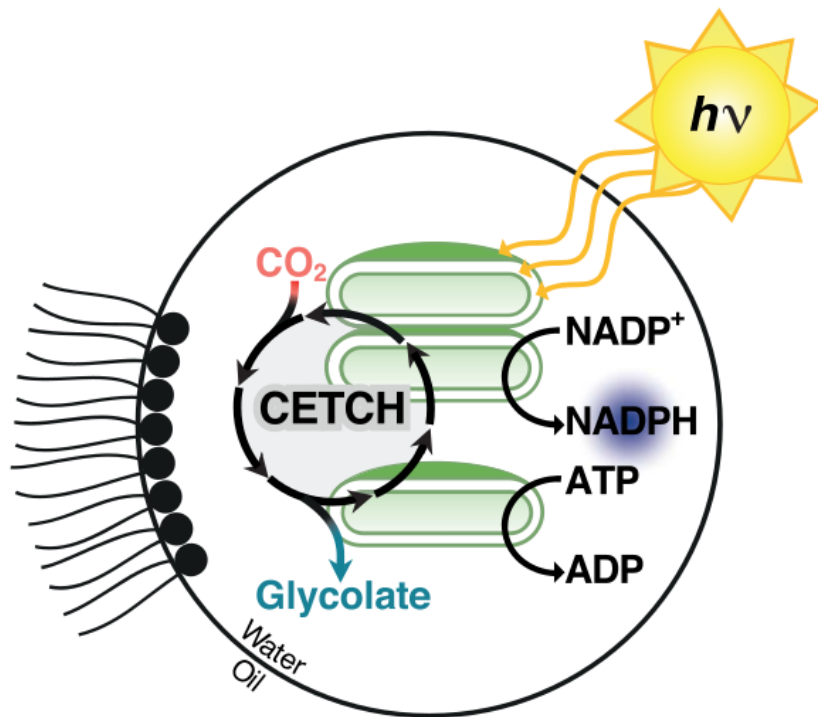
CO₂ fixation in a
microcompartment using
light

26/29



Artificial chloroplast

Integrable with more complex processes : artificial metabolic pathway



Miller, Beneyton *et al.* Science (2020)

Can we construct new / artificial cells ?

Potential for applications

New life-like systems

Benchmark for our fundamental understanding of life

We show how to construct step by step **integrable metabolic modules**.

Microfluidics will be an essential technology in the process

We need to start from a minimal approach to tackle complexity

SynBio in the Group

T. Beneyton

M. Morvan

M. Girault

T. Chotel, *et al.*

MPI Marburg

T. Erb

T. Miller, *et al.*

MPI Magdeburg

K. Sundmacher

I. Ivanov

T. Vidakovic-Koch *et al.*

maxsynbio
MAX PLANCK RESEARCH NETWORK
IN SYNTHETIC BIOLOGY



Frontiers of Life
Bordeaux

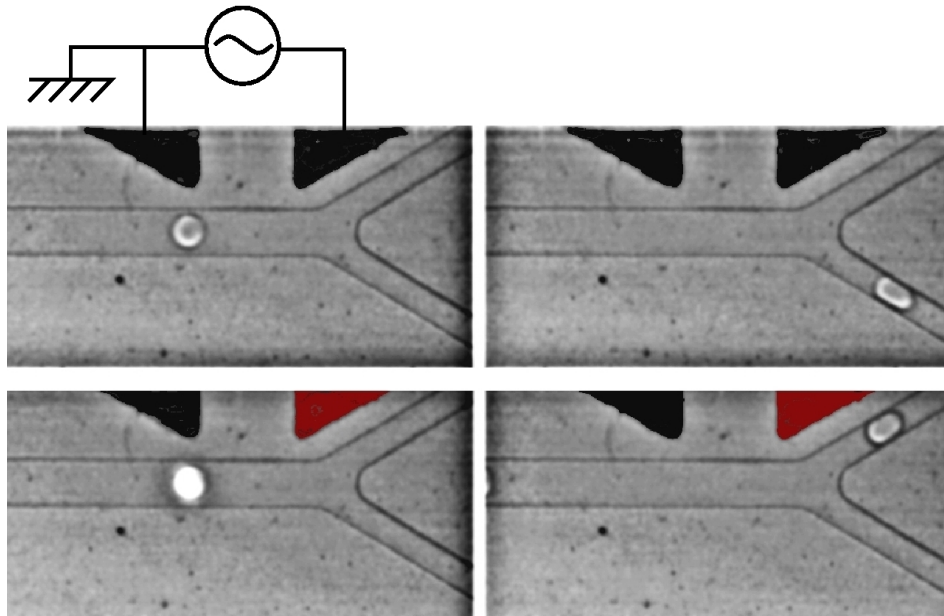


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de France



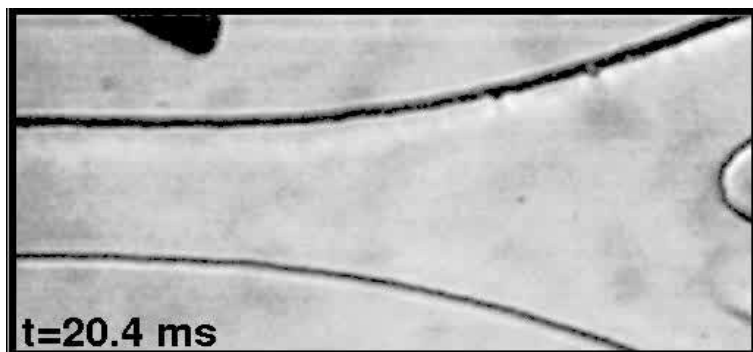
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<http://www.sms.crpp-bordeaux.cnrs.fr>

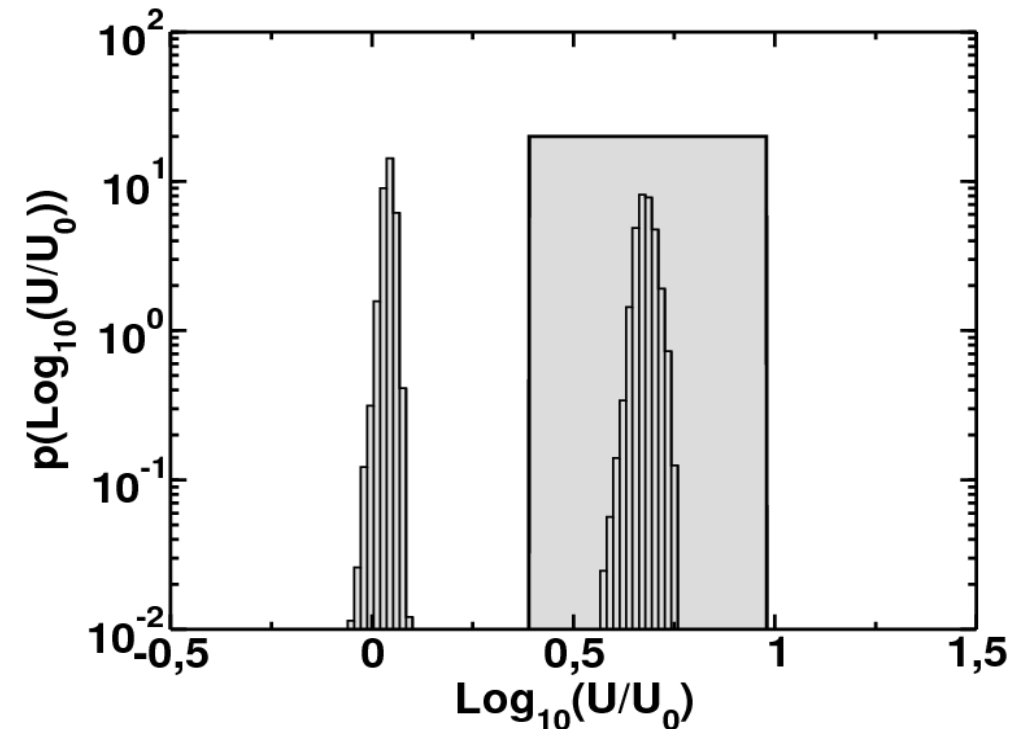


Fluorescent Activated Droplet Sorting: FADS

Possibility to sort droplets at 2 kHz



JC Baret, OJ Miller *et al. Lab Chip* (2009)



Sorting droplets at 30 kHz

Sciambi, Abate *et al. Lab Chip* (2015)