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Microfluidic methods for enzyme engineering

Bottom-up integration in metabolic synthetic systems

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CNRS, CRPP, Soft Micro Systems

CRPP

maxsynbio
MAX PLANCK RESEARCH NETWORK IN SYNTHETIC BIOLOGY

Frontiers of Life Bordeaux

Dr. Thomas Beneyton	T. Erb and his group	Prof. S. Lecommandoux
Dr. Nicolas Martin	T. Miller	Prof. H. Kellay
Dr. Laura Alvarez	M. Scheffen	Dr. A. Innis
Dr. Yeseul Park	K. Sundmacher and his group	Dr. J. Amédée
Dr. Maude Ducrocq	I. Ivanov	Dr. C. Lartigue
Bastien Lambert	T. Vidakovic-Koch *et al.*	Dr. V. Desvergnes
Zi Lin	D. Tang and her group	Prof. Laure Beven
Rafael Jimenez	C. Love	Dr. Jean-Paul Douliez
Vivien Willems…	…	…

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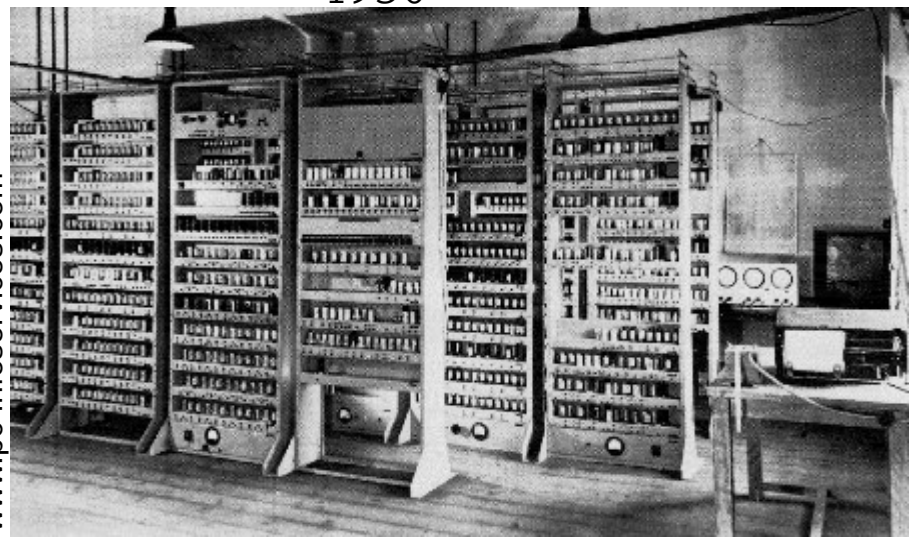
Technology background

1950

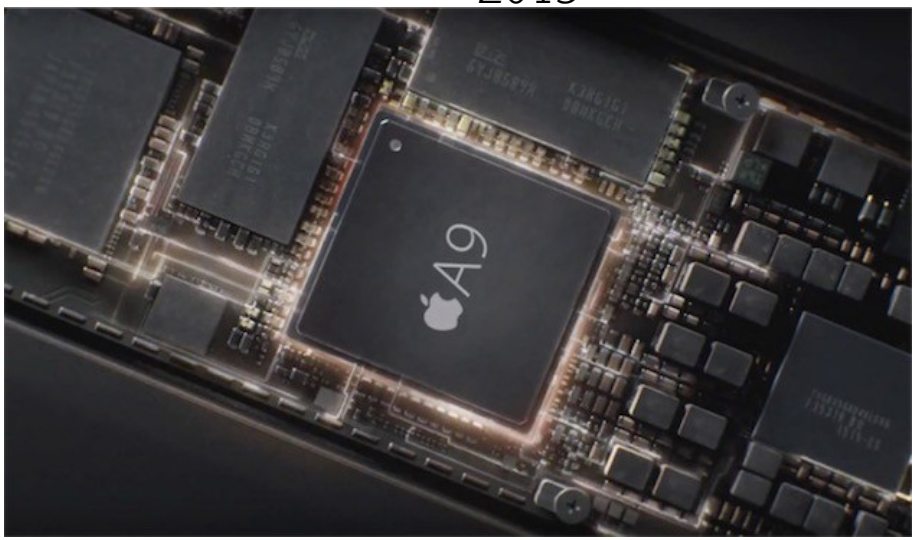


www.pc-infoservices.com

Runs at 500 kHz

512 word memory

2015



Runs at ~GHz

GB of memories

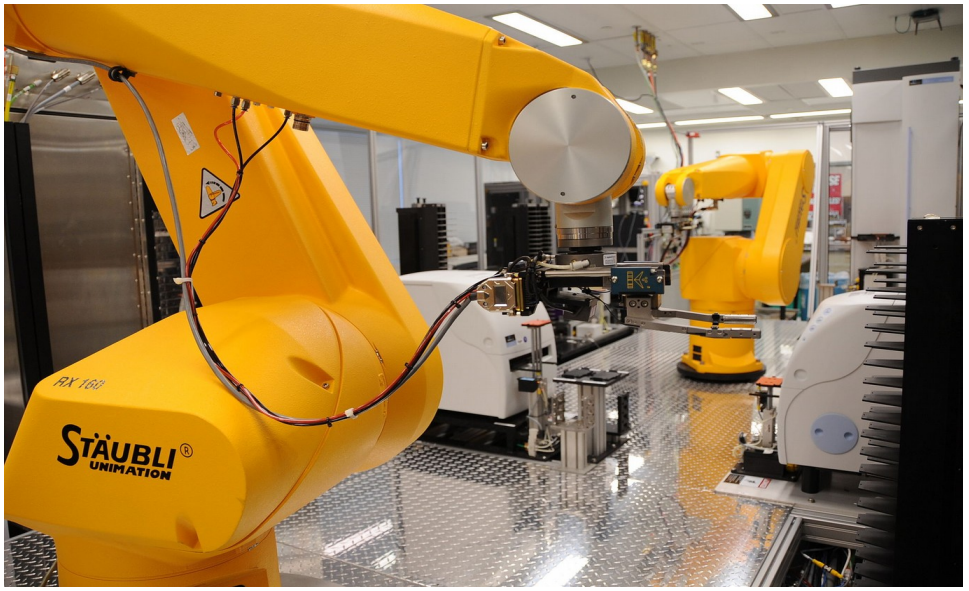
10–14 nm resolution for transistor

The most striking example of the power of :

Miniaturization | Automatization | Systems integration

Technology background : µflu

ca. 2000

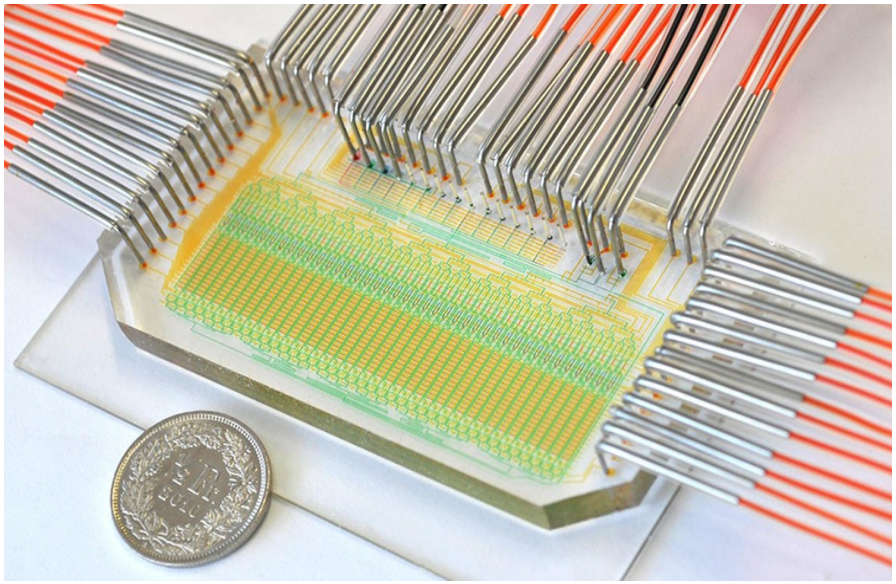


https://en.wikipedia.org/wiki/High-throughput_screening#/media/File:Chemical_Genomics_Robot.jpg

Performs 1 assay per s

µL volumes

ca. 2010+



S. Tay Lab – ETH Zurich

Performs 1 000 assays per s

pL volumes

Translating to Biology the power of

Miniaturization | Automatization | Systems integration

Droplet-Based Microfluidics

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VOLUME 86, NUMBER 18 PHYSICAL REVIEW LETTERS 30 APRIL 2001

Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device

Todd Thorsen,[1] Richard W. Roberts,[1] Frances H. Arnold,[1] and Stephen R. Quake[2]
[1]*Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125*
[2]*Department of Applied Physics, California Institute of Technology, Pasadena, California 91125*
(Received 9 January 2001)

Droplets as micron-sized microreactors, actuated in a carrier oil in microchannels

nature reviews methods primers https://doi.org/10.1038/s43586-023-00212-3

Primer Check for updates

Droplet-based microfluidics

Thomas Moragues[1], Diana Arguijo[2], Thomas Beneyton[3], Cyrus Modavi[4], Karolis Simutis[5], Adam R. Abate[4], Jean-Christophe Baret[3,6], Andrew J. deMello[1] ✉, Douglas Densmore[2] & Andrew D. Griffiths[5]

Protein engineering

Antibody screening

Molecular diagnostics

Single cell analysis

Strain selections

...

Protein Engineering

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Objective

Optimise catalysts for use in industrial processes, diagnostic systems,...

Principle

Improve the enzyme by cycles of mutations and selection (directed evolution) :

mimicking Natural Evolution

Implementation in microfluidics

(1) Generate millions of mutants of a native enzyme, expressed in a host or in vitro

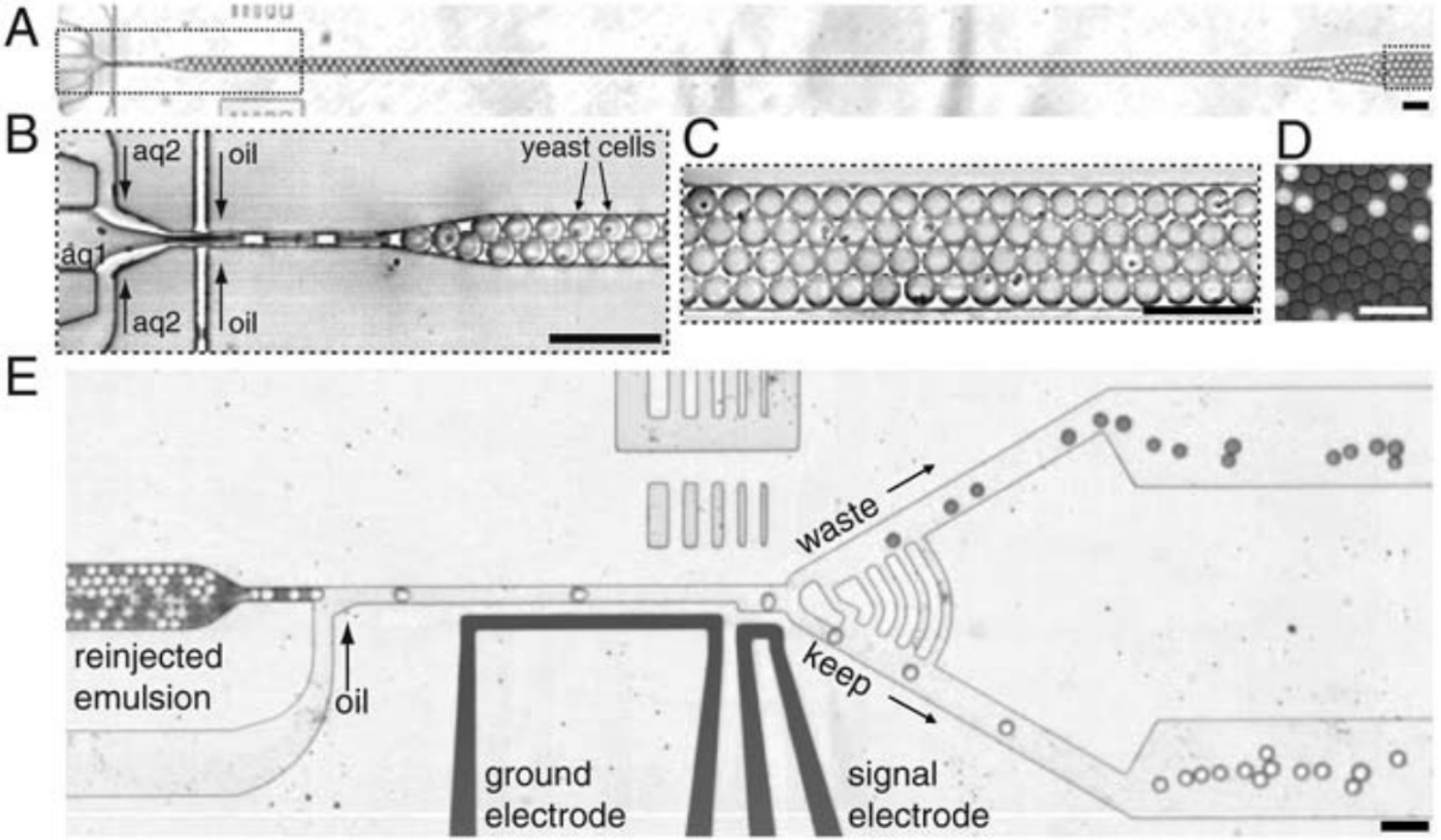
(2) Analyse each individually in droplets, select the best ones (hits – typically 1000)

(3) Recover the DNA and repeat (1) from the hits / refine the screen using standard assays

Baret et al. Lab Chip 2009

Agresti et al. PNAS 2010

Integration



pL

kHz

Millions

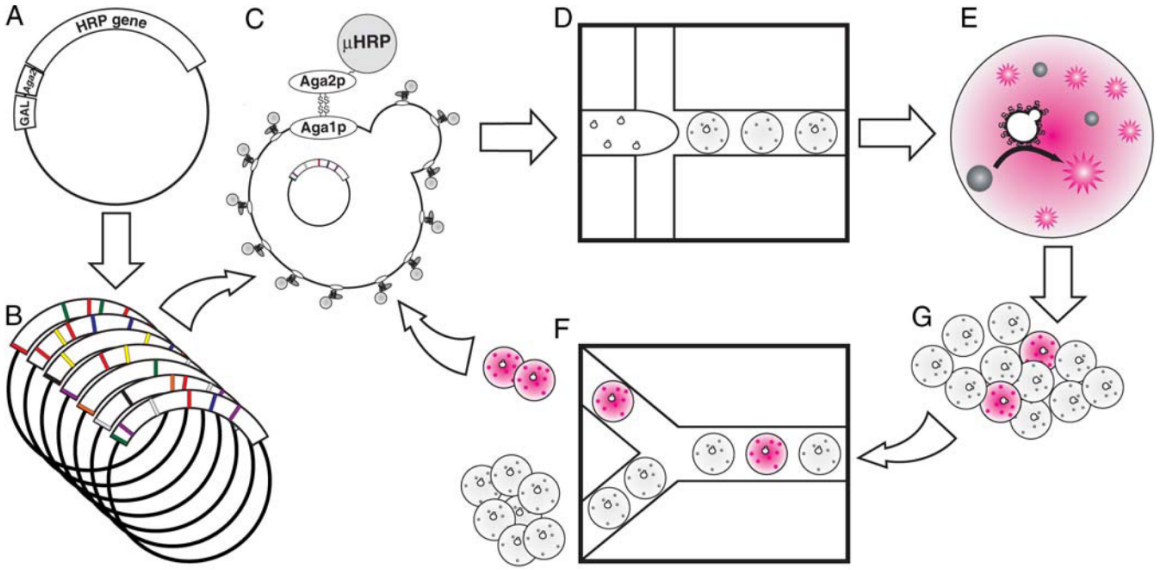
Fluorescence

Cell expression

Baret et al. Lab Chip 2009

Agresti et al. PNAS 2010

HRP Proof of concept



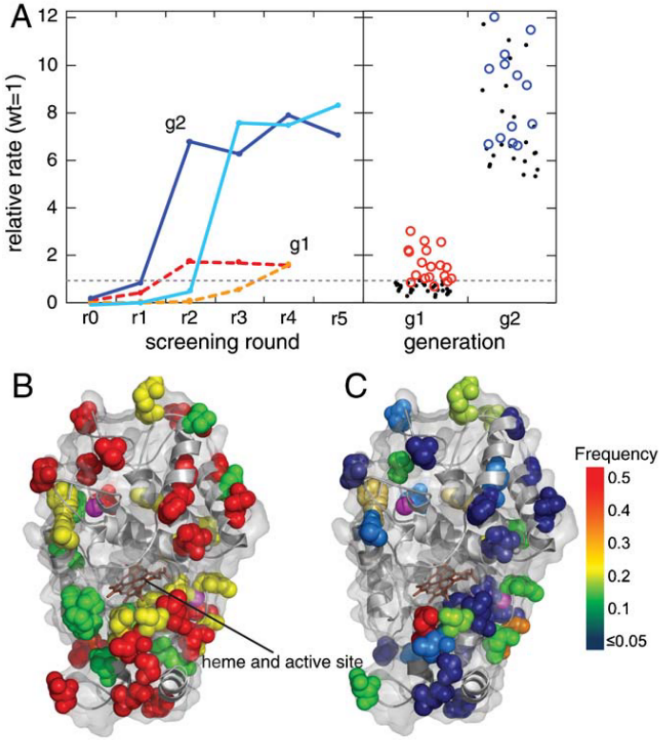


Table 1. Comparison of time and costs* for the complete screen using traditional methods and in microfluidic emulsions

	Robot	Microfluidic drops
Total reactions	5×10^7	5×10^7
Reaction volume	100 μL	6 pL
Total volume	5,000 L	150 μL
Reactions/day	73,000	1×10^8
Total time	~2 years	~7 h
Number of plates/devices	260,000	2
Cost of plates/devices	$520,000	$1.00
Cost of tips	$10 million	$0.30
Amortized cost of instruments	$280,000	$1.70
Substrate	$4.75 million	$0.25
Total cost	$15.81 million	$2.50

Agresti et al. PNAS 2010

Protein Engineering by Dir. Evo.

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Table 2. Summary of the Evolved Biomolecules Described in This Review[a]

	selection pressure	no. rounds	expression		library design	reference
			E. coli	other		
Enzymes						
phosphotriesterase	fluorescence	1	cytoplasmic		random	39
TNA polymerase	fluorescence	1	cytoplasmic		semirational	40
peroxidase	fluorescence	2		*S. cerevisiae*	semirational	41
esterase	fluorescence	5	cytoplasmic		semirational	43
dehydrogenase	absorbance	2	cytoplasmic		random	46
oxidase	fluorescence	1	cytoplasmic		semirational	74
sulfatase	fluorescence	1	display		semirational	75
aldolase	fluorescence	2	cytoplasmic		semirational	77
	fluorescence	6	cytoplasmic		random	78
Ribozymes						
X-motif (RNA)	fluorescence	9		PCR	random	46
Antibodies						
anti-tranferrin (K562)	fluorescence	1		hybridoma cells		83
anti-PD-1	fluorescence	2		*S. cerevisiae*		86
Aptamers						
iSpinach	fluorescence	5		IVTT	semirational	90
	fluorescence	5		IVTT	semirational	91
Mango III	fluorescence	9		IVTT	semirational	92
Mango III (A10U), iMango III	fluorescence	4		IVTT	rational	93
Gemini-561, o-Coral	fluorescence	4		IVTT	semirational	94

[a]A total of 8 enzymes, 5 aptamers, 1 ribozyme, and 2 antibodies have been reviewed. We have also dissected the key components underlying these directed evolution experiments.

Directed evolution in drops: molecular aspects and applications,

A. Manteca, A. Gadea, D. van Assche, P. Cossard, M. Gillard-Bocquet, T. Beneyton, A. Innis, J.-C. Baret, ACS Synthetic Biology (2021)

Evolving single enzymes of practical / industrial / therapeutic interest in microfluidics is demonstrated.

Limitations when the enzyme must be produced by organisms that can evolve, if the enzyme is toxic to the cell...

We also need to integrate functionalities of interest in metabolic pathways

Microfluidics use in evolving systems for CO2 fixation

(Collaboration with Tobias Erb, MPI Marburg)

- *Example 1 : Evolving enzymes of CO2 fixation pathways*
- *Example 2 : Reconstruction of pathways in droplets*

1 - Glycolyl-CoA Carboxylase (GCC)

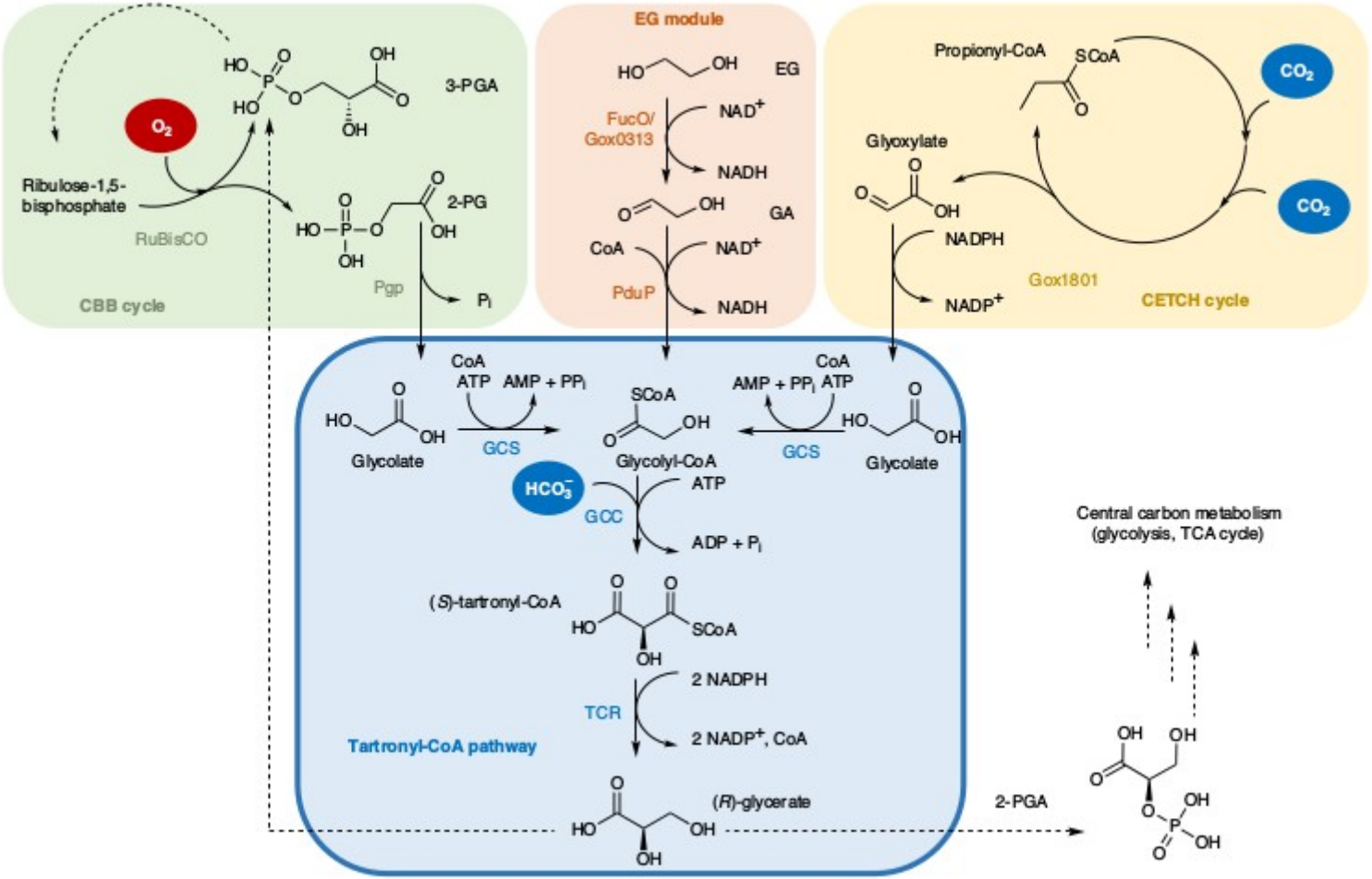
Engineering CO2 fixation strategies is of relevance in the context of high atmospheric CO2 concentrations

Tartronyl-CoA pathway :

Proposed to produce glycolate from CO2

But :

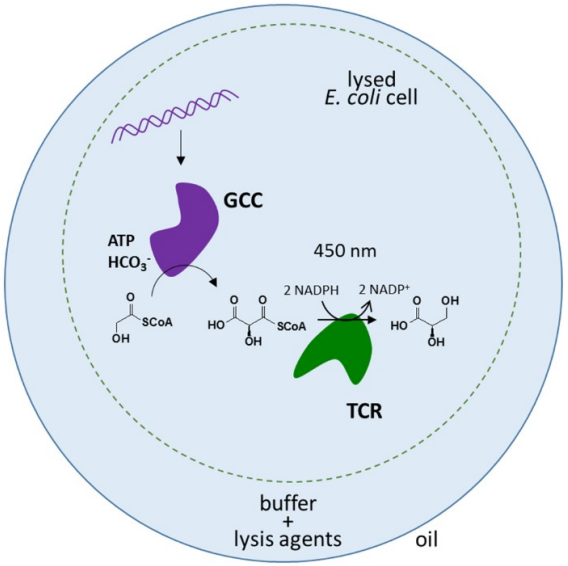
GCC is lacking

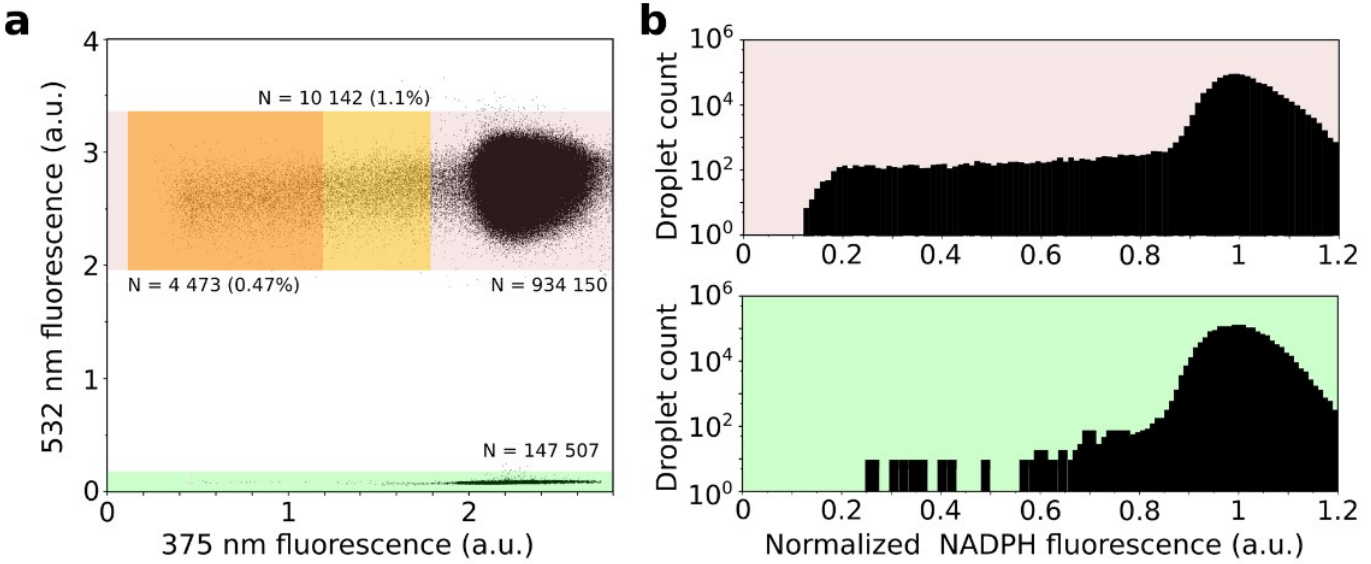


Trudeau et al., PNAS 2018

Scheffen, (...), and Erb, *Nature Catalysis* 2021

1 - Glycolyl-CoA Carboxylase (GCC)





Screening approach based on NADPH readout in droplets

Library analysis in microfluidics reveals about 5 % of active variants

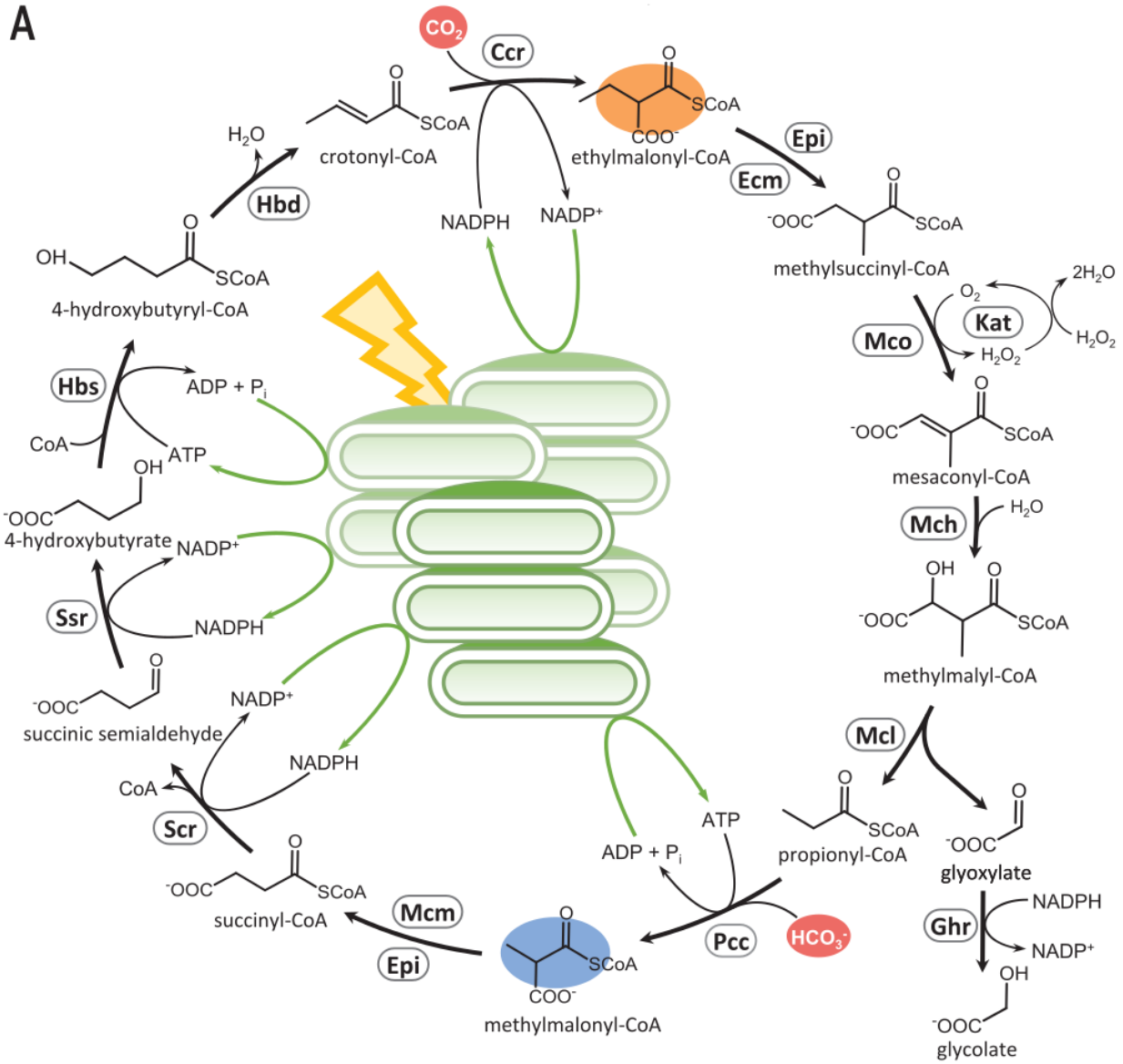
Further screen in plates allows to identify hits

A GCC is finally integrate to run the TaCo Pathway

Microfluidic workflows fully integrate in screening strategies for protein engineering : rapid readout, HTS, pre-screening,...

Scheffen et al. Nature Catalysis 2021

2 – Artificial metabolic cycles



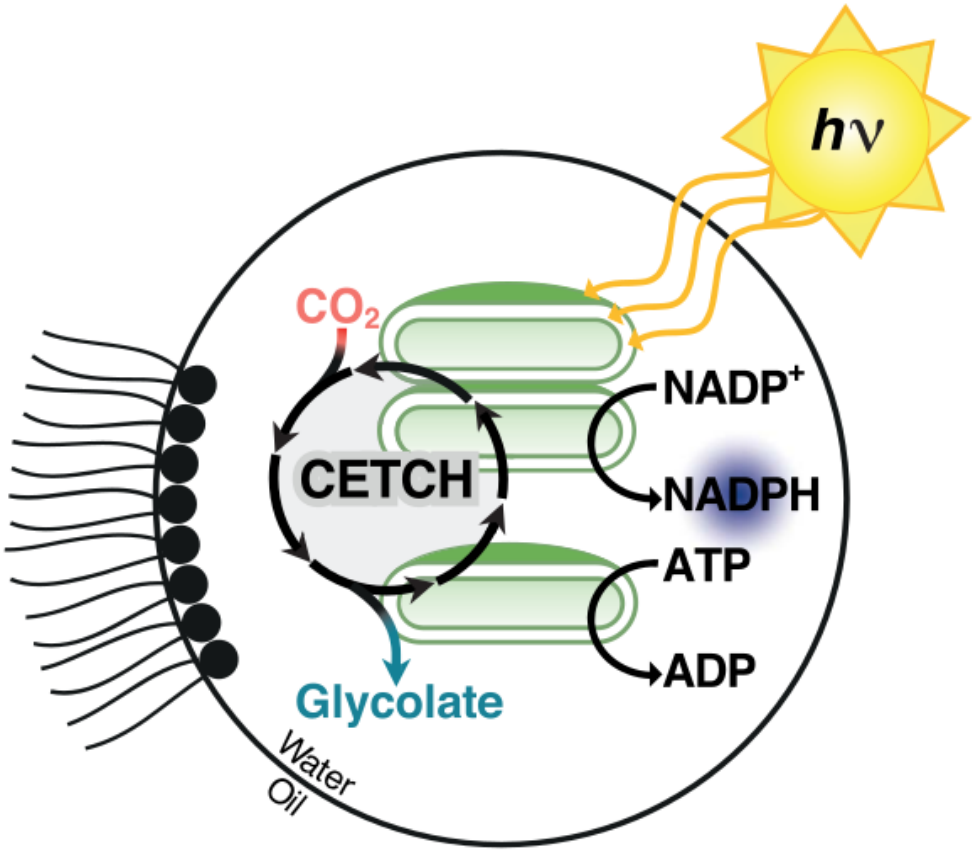
CETCH cycle (T. Erb)

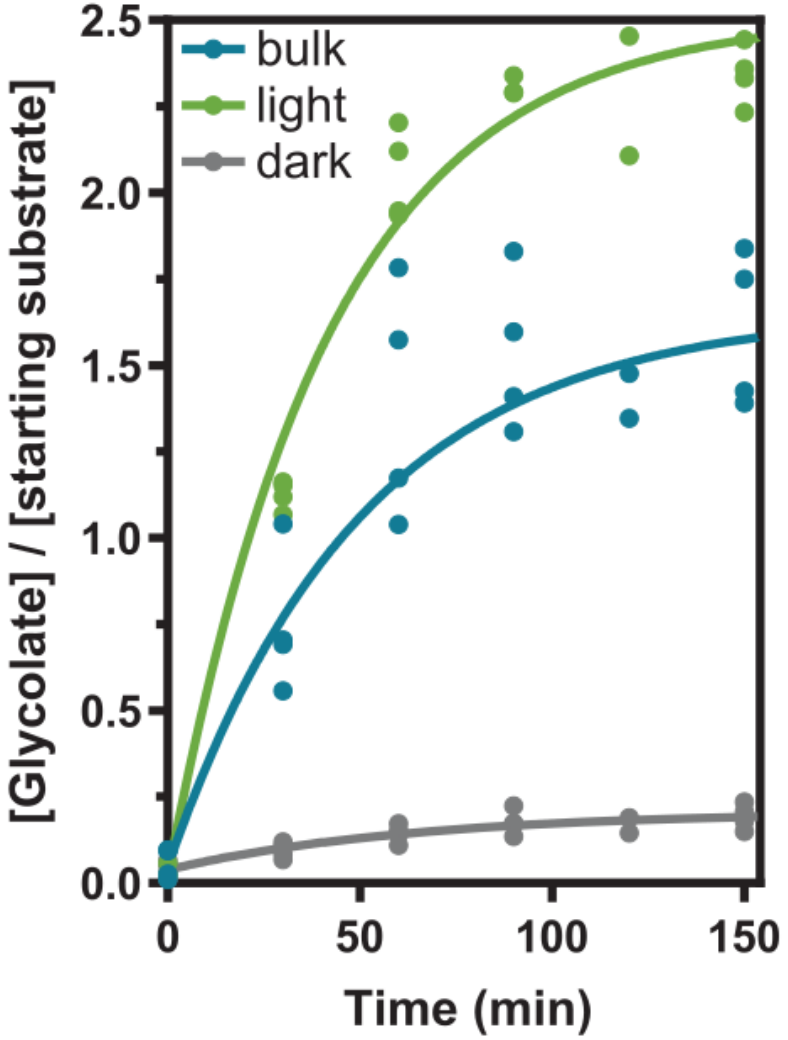
CO_2 fixation in a microcompartment using light

Schwander et al. Science (2016)

Miller, Beneyton et al. Science (2020)

2 - Artificial metabolic cycles





Efficient biomimetic synthesis of C_2 compounds from CO_2

Miller, Beneyton et al. Science (2020)

2 - Artificial metabolic cycles

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Droplets as microreactors for the integration and miniaturization of metabolic cycles (artificial)

Further use possible for screening (eg fluorescence readout)

Microfluidics usable to screen experimental conditions (compositions)

Miller, Beneyton et al. Science (2020)

Take Home Message

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Protein engineering benefits from microfluidic methods

Microfluidics for the selection of hits

Microfluidics generate data (phenotypes) at high throughput that can be linked to genotypes (DNA recovery)

Usable to improve molecules (biocatalysts)

Train IA-based models to predict sequence/function properties ?