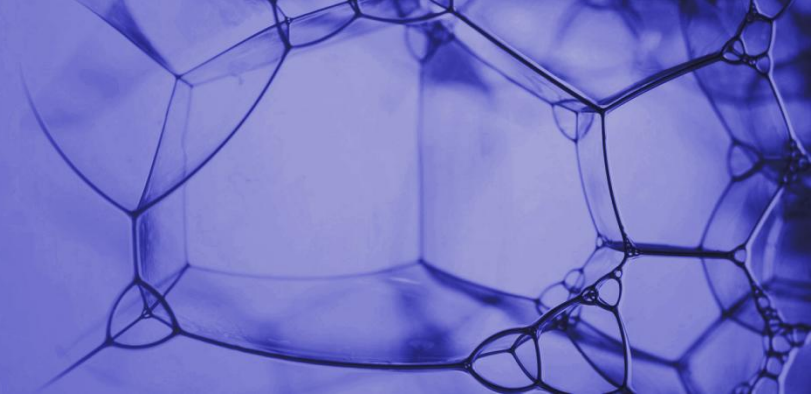


**LOSCHMIDT
LABORATORIES**



Microfluidics – „Lab on a Chip“

Outline

A decorative image in the top right corner showing a close-up of a microfluidic chip's internal channels, which are small, interconnected glass or plastic structures used for precise fluid control at the micro-scale.

- ❑ introduction to microfluidics
- ❑ physics of micro-scale
- ❑ lab on a chip applications
 - life and medical science
 - discovery of novel proteins
 - protein and metabolic engineering
- ❑ design and fabrication
- ❑ sensing and detection

Lab on a Chip Concept

incubation



pre-treatment



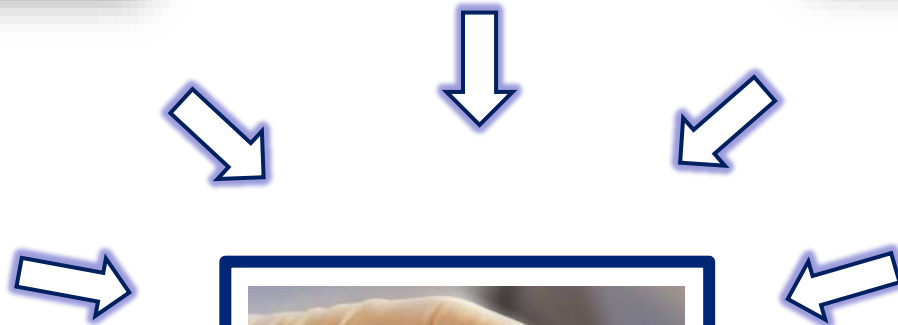
analysis



preparation

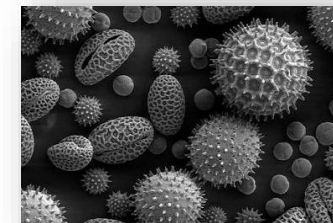
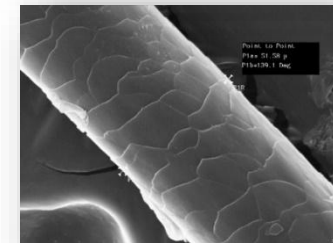
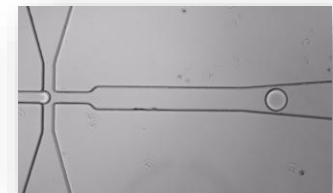


collection

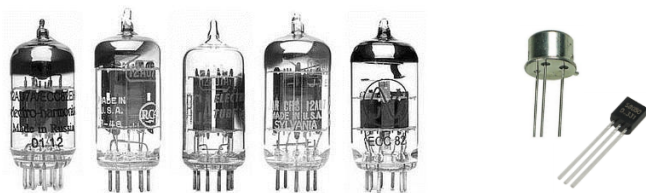


□ „behavior, control and manipulation of fluids geometrically constrained to a small dimensions“

- dimensions (1'-100' μm)
- volumes (nL, pL, fL)
- unrivalled precision of control
- (ultra)high analytical throughput
- reduced sample and power consumption
- facile process integration and automation

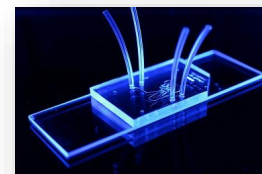
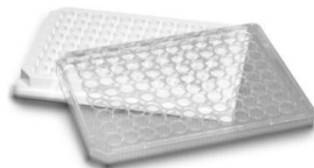


Miniaturization & integration



	vacuum tubes	transistors	micro chips
size (mm)	100	10	0.000 01
price (USD)	10	1	0.000 000 1

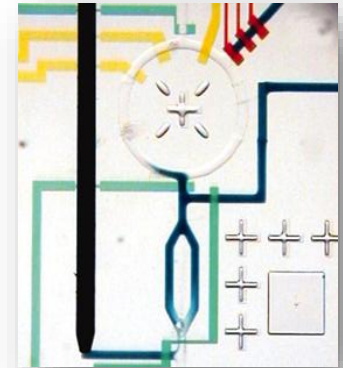
	test tube	microtiter plate	μ -fluidic chip
volume (μ L)	1 000	10	0.000 001
throughput (assays/day)	10	1 000	1 000 000



Concepts in microfluidics

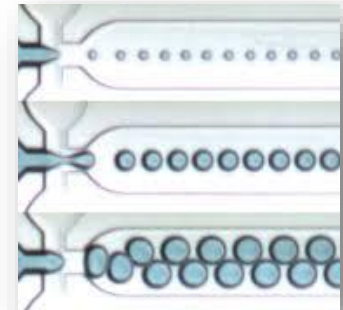
- ❑ **continuous-flow microfluidics**

manipulation of continuous liquid flow
through micro-fabricated channels



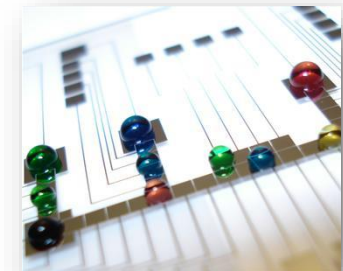
- ❑ **droplet-based microfluidics**

manipulating discrete volumes of fluids
in immiscible phases



- ❑ **digital microfluidics**

droplets manipulated on a substrate
using electro-wetting



Novel Physics of Micro-Scale

□ viscosity, surface tension and capillary forces dominate

▪ **lack of turbulent phenomena**

+ nontrivial chemical gradients

to study chemotaxis

▪ **absence of density-driven convection**

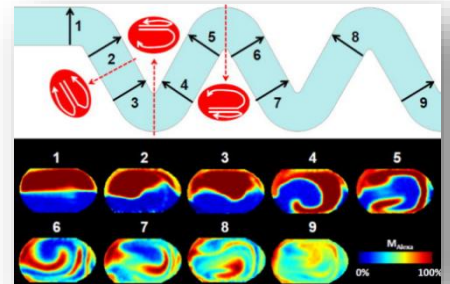
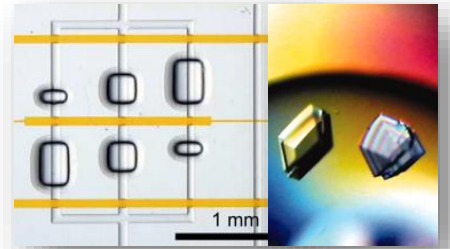
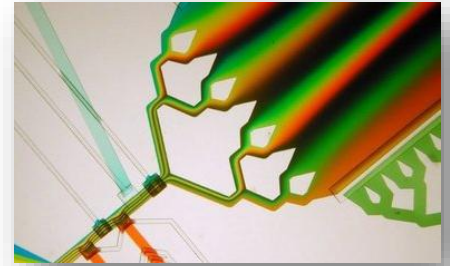
+ free interface diffusion, efficient

protein crystallization kinetics

▪ **strong shearing forces**

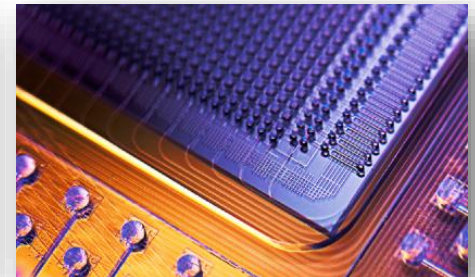
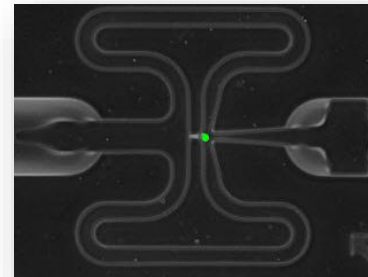
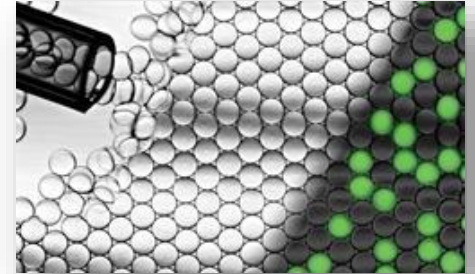
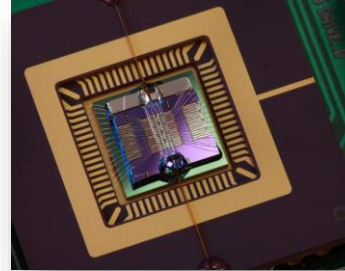
+ fast mixing kinetics of protein

folding and/or catalysis



Lab on a Chip applications

- ❑ analytics and chemistry
- ❑ PCR and sequencing
- ❑ point of care diagnostics
- ❑ pharmacology
- ❑ clinical studies
- ❑ single cell biology
- ❑ high throughput biology



Polymerase chain reaction

❑ classical PCR

- slow heating/cooling cycles
- PCR tubes (strips), 96-well MTP
- volume 50 to 500 μL



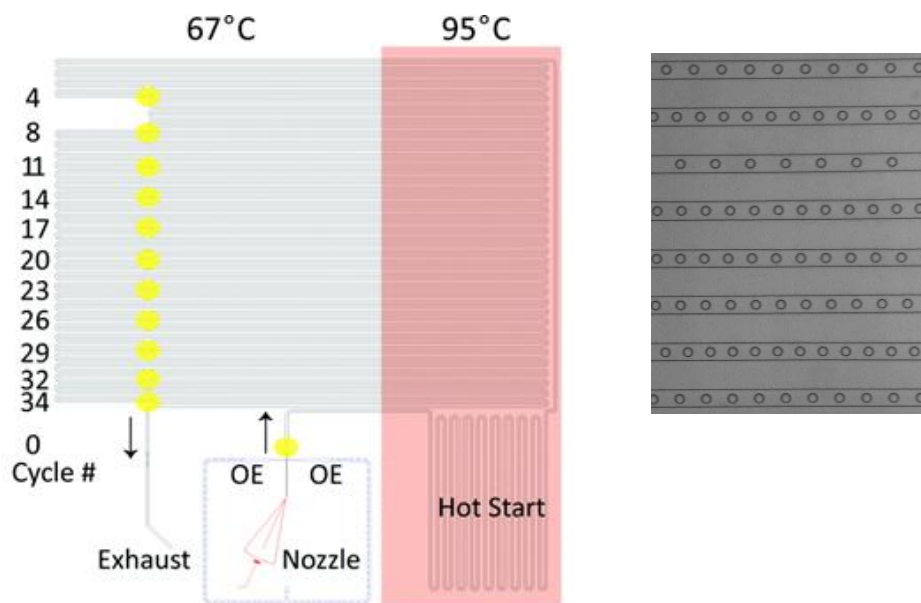
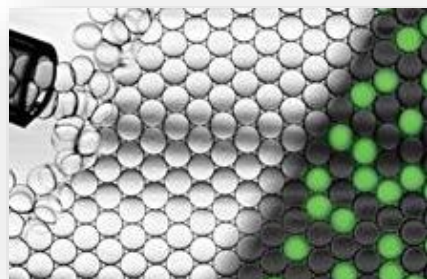
Kary Mullis

Nobel Prize in 1993

Digital polymerase chain reaction

□ digital PCR (single molecule)

- 1 nanoliter droplets
- 20 000 droplets per run
- fast heat transfer



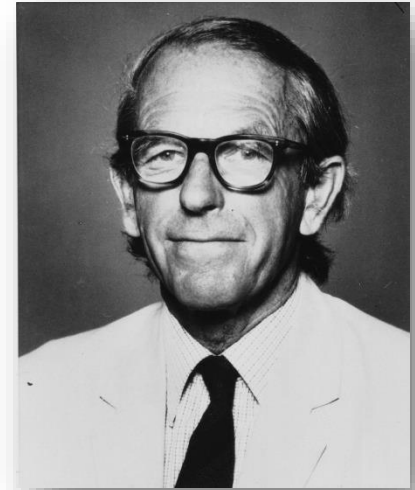
Next-generation sequencing

- parallelization of single molecule pyrosequencing

- 454 Pyrosequencing (Roche)

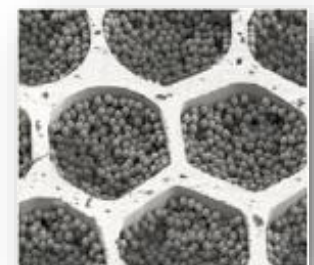
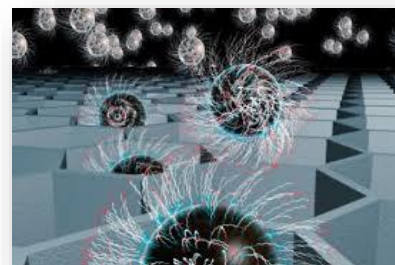
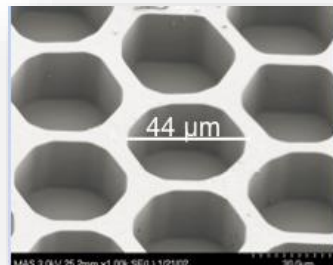
water in oil droplets 1 picoliter (10^{-12} liters)

1 mil. reads/run, 10 USD/Mbase

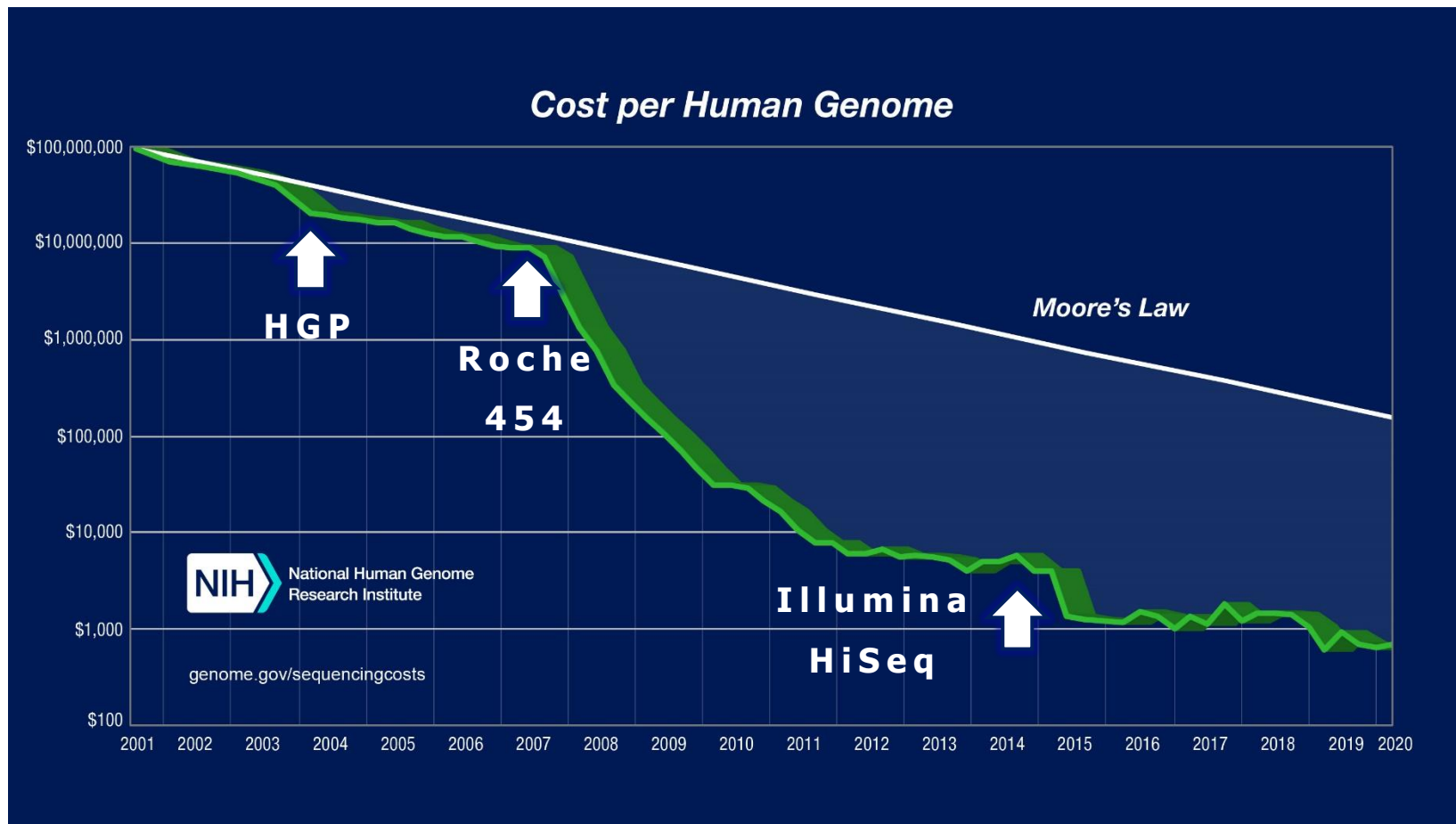


Frederick Sanger

Nobel Prize in 1980



Revolution in DNA analysis

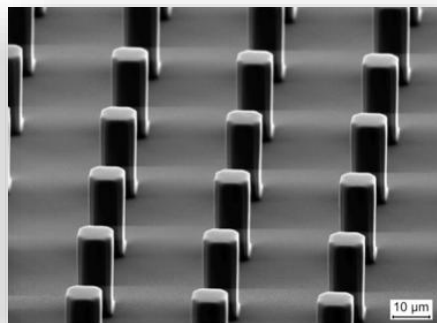
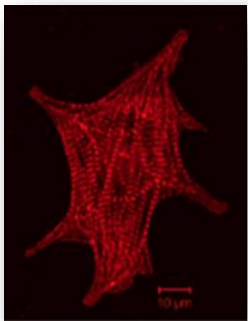


- ❑ 2003: 13 years, 3 billion USD
- ❑ 2018: days, < 1,000 USD

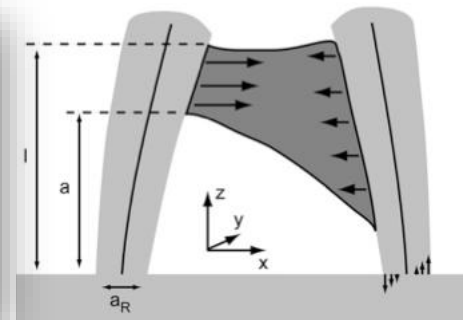
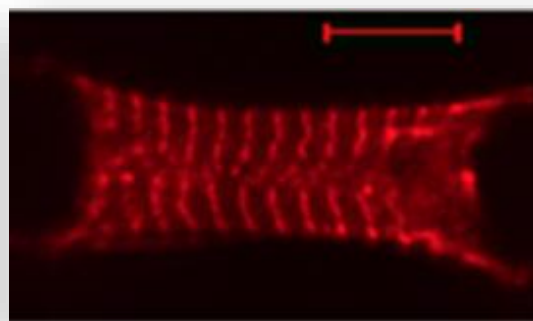
Organ(oid)s on chip

- ❑ 3D chips mimicking human's physiological responses
(e.g., pathological, pharmacokinetic, toxicological)
- ❑ realistic *in vitro* model closer to *in vivo* cell environment
(e.g., mechanical strain, patterning, fluid shear stresses)

flat surface

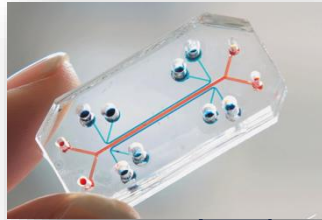


micropillar

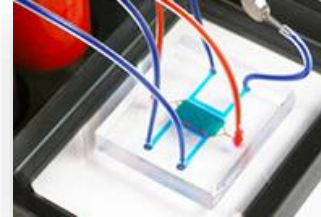


Organs on chip

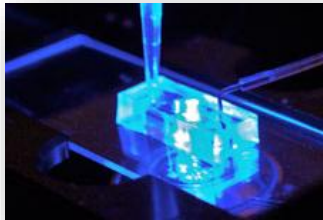
Lung



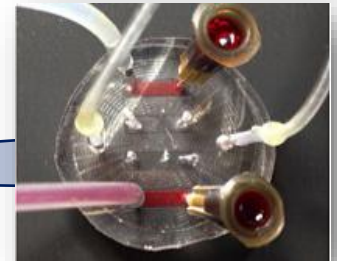
Neurovascular



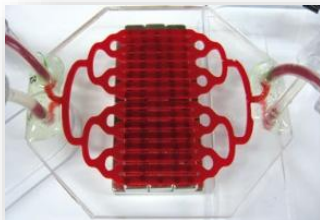
Heart



Artery



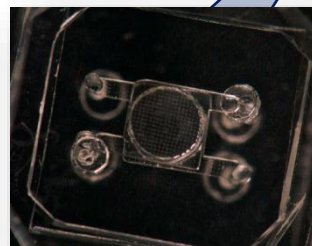
Spleen



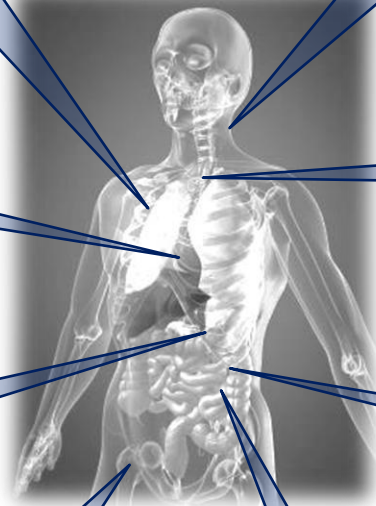
Kidney



Bone

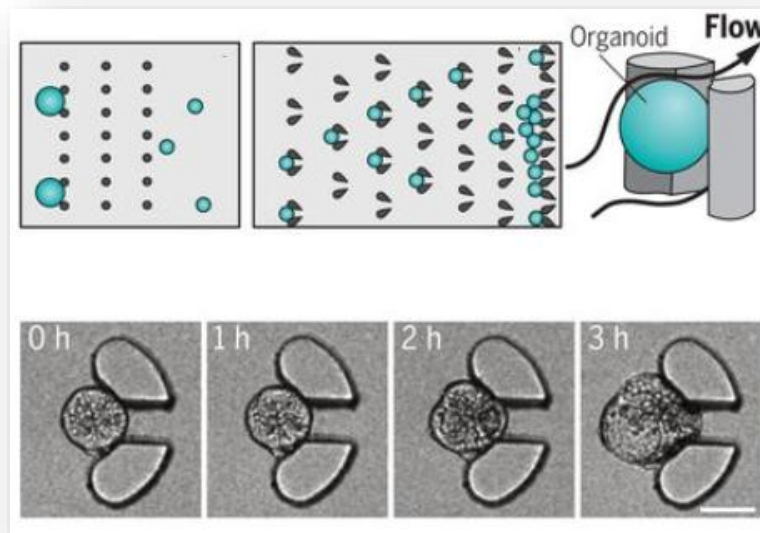


Intestine



Organ(oid)s on chip

- ❑ **3D chips mimicking human's physiological responses**
(e.g., pathological, pharmacokinetic, toxicological)
- ❑ **realistic *in vitro* model closer to *in vivo* cell environment**
(e.g., mechanical strain, patterning, fluid shear stresses)



- ❑ **can replace expensive and controversial animal testing**

Protein Discovery and Engineering

ENZYME MINER v1.0 Automated mining of soluble enzymes with diverse structures, catalytic properties and stabilities

Submit new job Help Example Acknowledgements

ABOUT

EnzymeMiner identifies putative members of enzyme families and facilitates the selection of promising targets for experiments. The server mines sequences that are likely to show the desired catalytic activity. Key selection criteria are: (i) predicted soluble expression in *Escherichia coli*, (ii) sequence identity, and (iii) deposit date. The search query can be a sequence from the Swiss-Prot database or a custom sequence with specified essential residues. The output is an interactive selection table and a sequence similarity network.

User guide | Example results Hide

JOB INPUT

Swiss-Prot sequences Custom sequences

1.1.1.1 - Alcohol dehydrogenase. (240) Load example

Select sequences from table (max. 40) Select sequences from similarity network (max. 40)

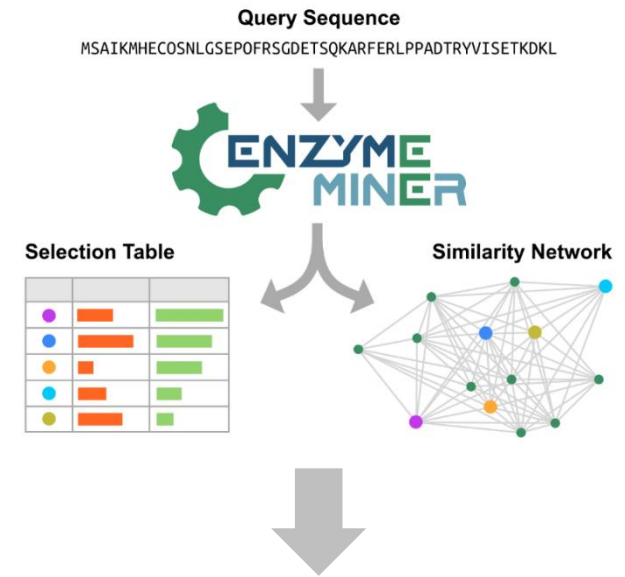
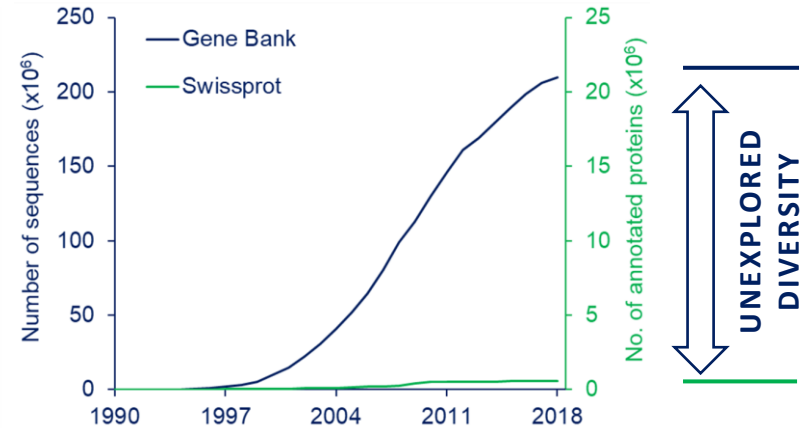
Accession	ER	Length	Sequence plot
<input type="checkbox"/> A0A075TMP0	9	340	
<input type="checkbox"/> A1A835	7	369	
<input type="checkbox"/> A1CFL1	7	388	
<input type="checkbox"/> A1L4Y2	14	394	
<input type="checkbox"/> A2XAZ3	14	381	
<input type="checkbox"/> A5IYX5	1	309	
<input type="checkbox"/> A6ZTT5	0	382	
<input type="checkbox"/> A7ZIA4	7	369	
<input type="checkbox"/> A7ZX04	7	369	
<input type="checkbox"/> B1J085	7	369	
<input type="checkbox"/> B1LIP1	7	369	
<input type="checkbox"/> B4M8Y0	4	254	
<input type="checkbox"/> E1ACQ9	8	339	
<input type="checkbox"/> F2Z678	10	351	
<input type="checkbox"/> F8DVL8	0	383	

Selected 0 of 240 (max. 40) 1 to 15 of 240 Page 1 of 16

Filter sequences by Pfam domains...

Select all Deselect all Show selected only

Advanced options

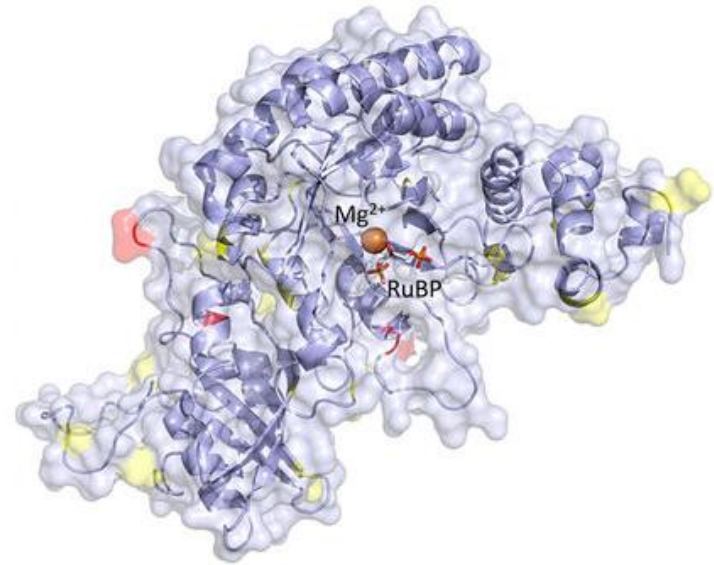


Nucleic Acids Res. 48, W104–W109 (2020)

FUNCTIONAL CHARACTERISATION

Protein Discovery and Engineering

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51-TGTNVEVCTTDDFTRGV^DDALVYEVDEARE^ELTKIAYPVALFDRN^ITDGK^AM-100
101-IAS^FLT^LTMGNNQGMGDVEYAK^MHDFYVPEAYRALFDG^PSVNISALWKVL-150
151-GRPEVDGGLVVGT^IIKPKLGLRPKPF^AEACH^HAFWLGGDFIKNDEPQGNQP-200
201-FAPLRD^TIALVADAMRRAQDETGEAKLFSANITADDPFEI IARGEYVLET-250
251-FGENASHVALLVDGYVAGAA^AAITTARRRFPDNFLHYHRAGHGAVTS^FQSK-300
301-RGYTAFVHCK^MARL^QGASGIHTGTMGFGKMEGES^SDRAIAYMLT^QDEAQG-350
351-PFYRQSWGGMK^ACTPIISGGMNALR^MPC^GFFENLGNANVILTAGGGAFGHI-400
401-DGPVAGARSLRQAWQAWRDGVP^VL^DYAREHKELARAFESFPGDAD^QI^IYPG-450
451-WRKALGV^EDT^RSALPA-466



No.	Coverage (95%)
1	94
2	3 066
3	98 163
4	3 141 251
5	100 520 093

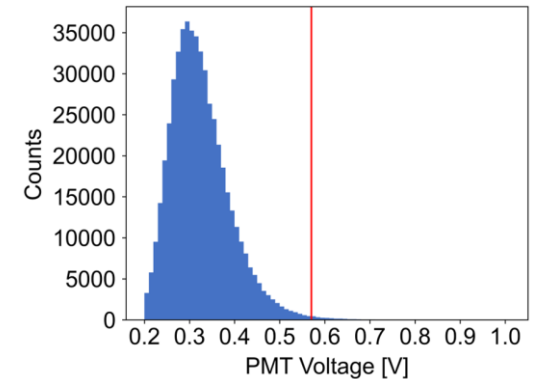
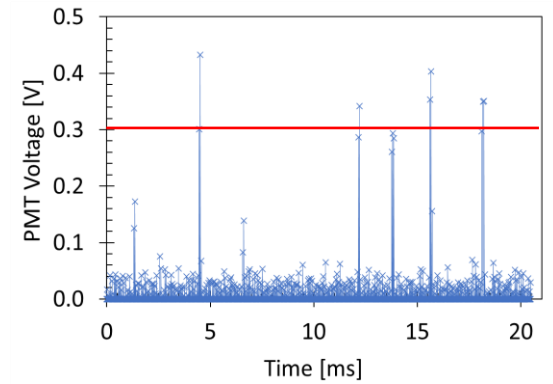
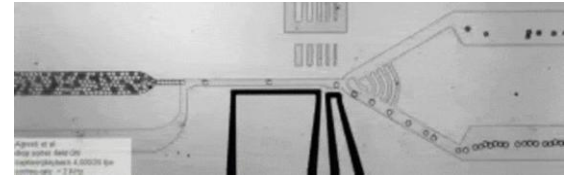
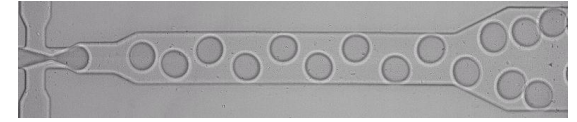
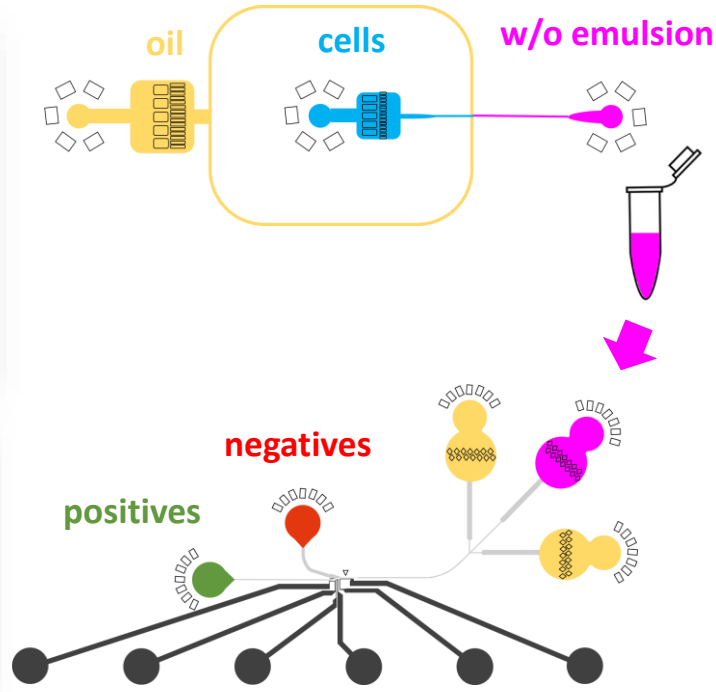
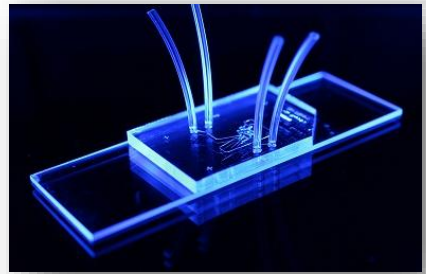
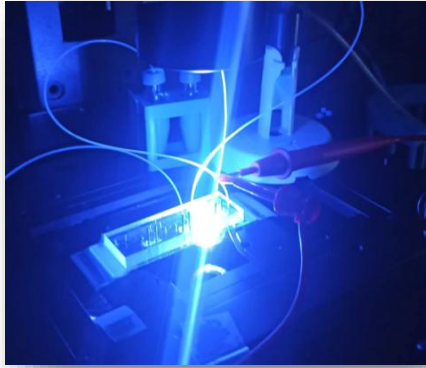


SCREENING AND IDENTIFICATION
OF POSITIVE HITS



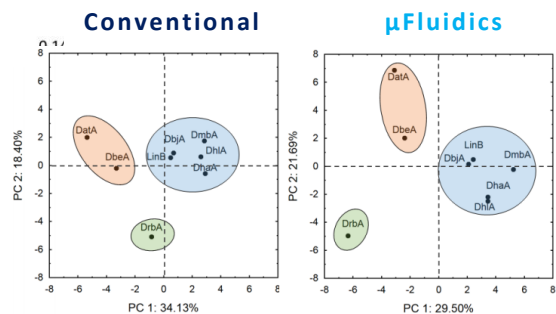
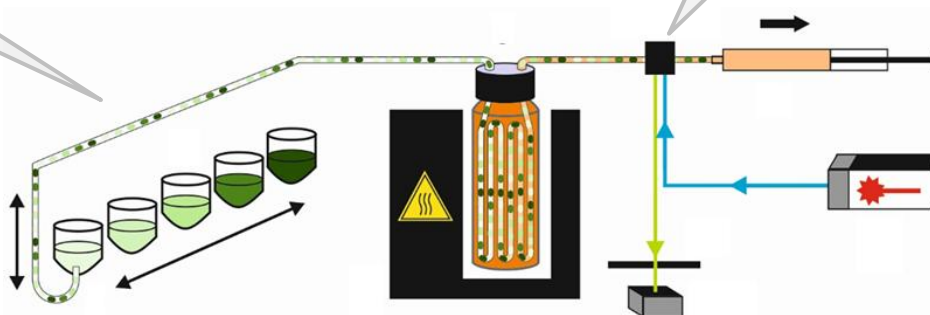
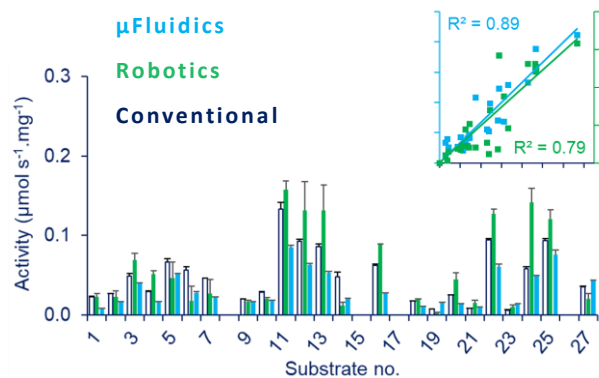
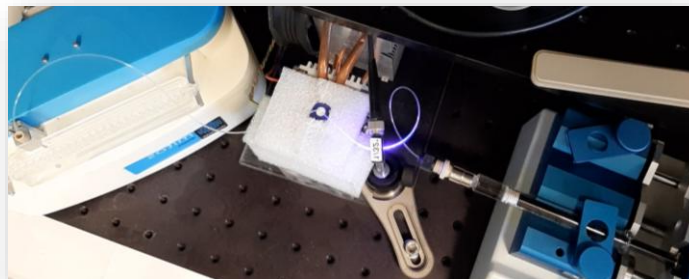
FUNCTIONAL CHARACTERISATION

High-throughput on chip sorting (FADS)

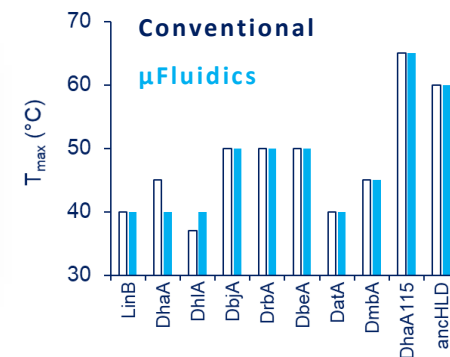


	Robotic	μ Fluidic
Reaction volume	100 μ L	5 pL
Reactions / day	50 000	$1 \cdot 10^8$
Total time	5 years	3 days
Total volume	5 000 L	150 mL
No. of plates / devices	250 000	2.0
No. of tips	28 000 000	10

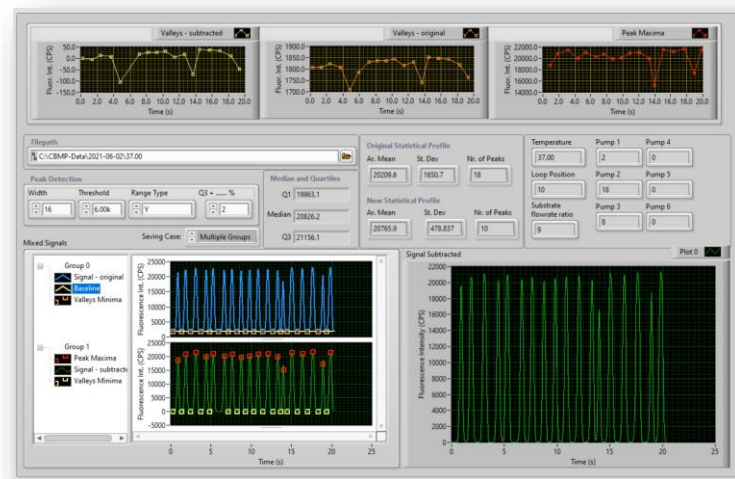
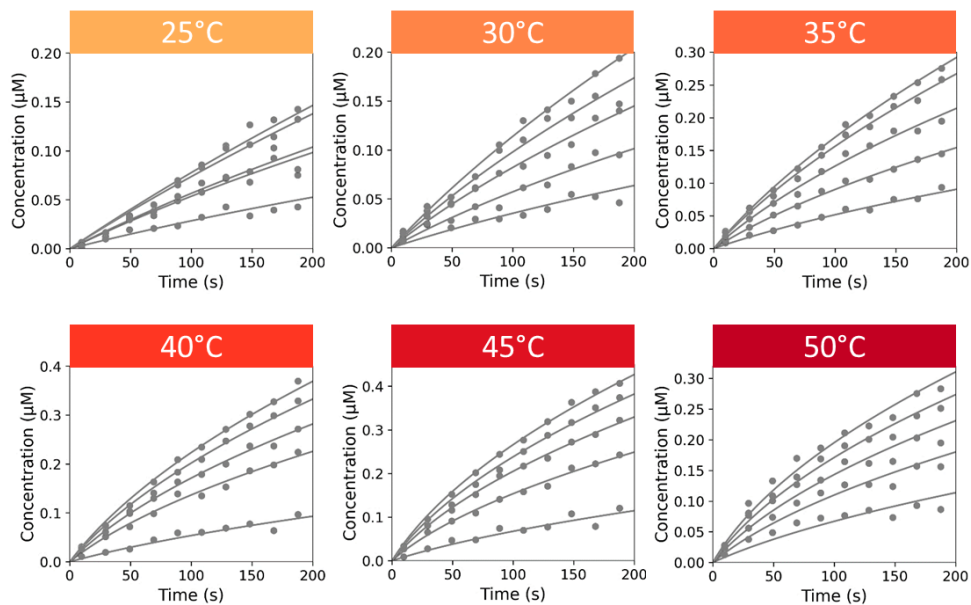
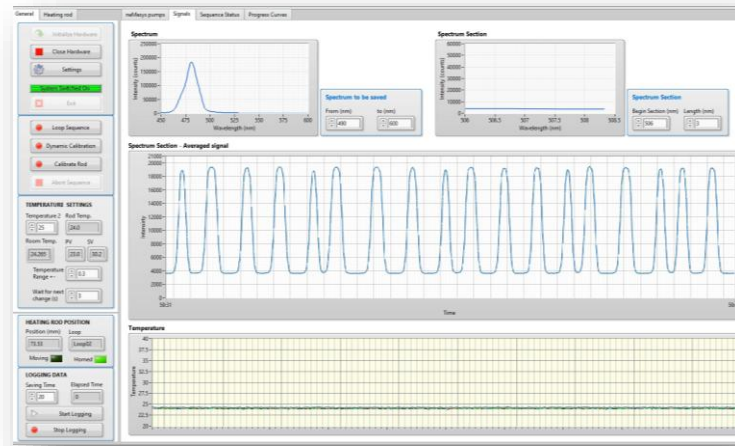
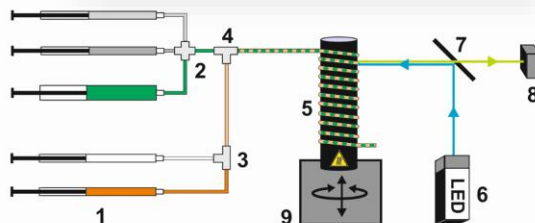
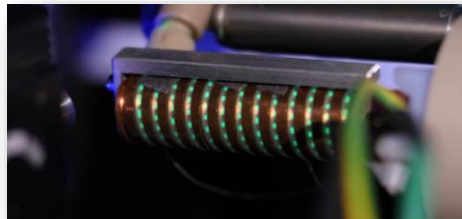
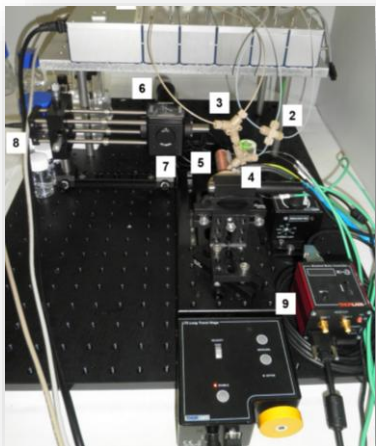
Activity and specificity in μ -droplets



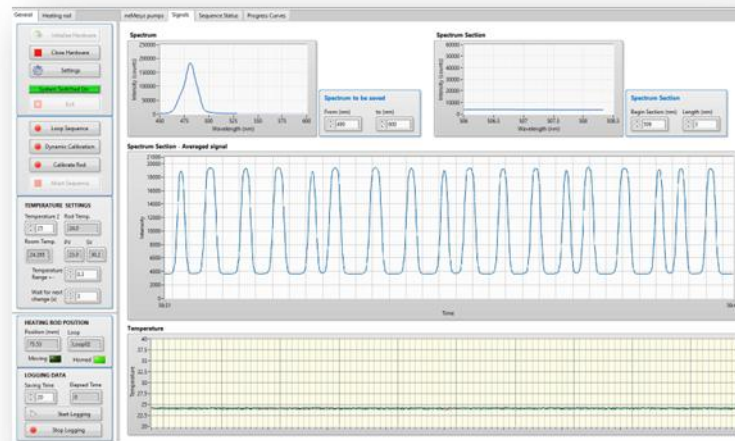
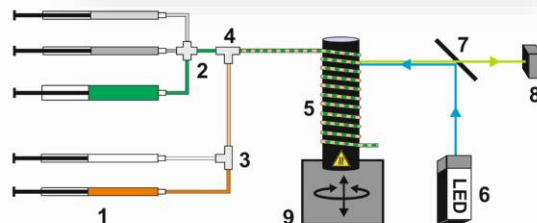
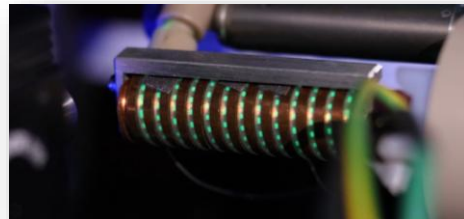
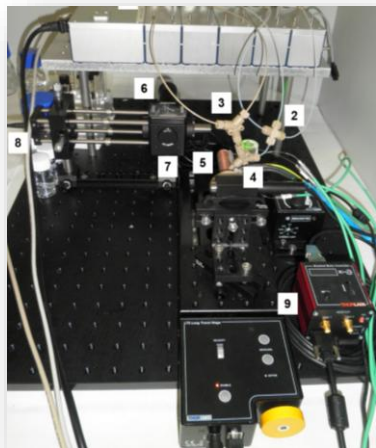
	Test tube	Robotic	μFluidic
Reaction volume (mL)	10	1	0.000 15
Total enzyme (mg)	540	54	0.5
Total time (days)	100	30	5



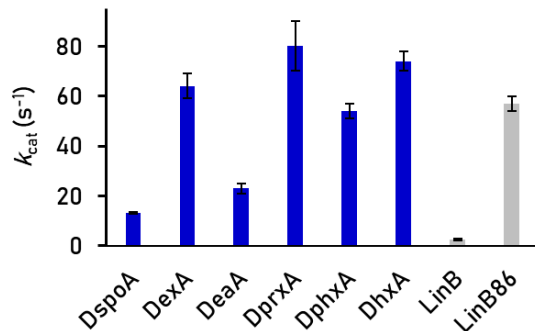
Kinetics and thermodynamics in μ -droplets



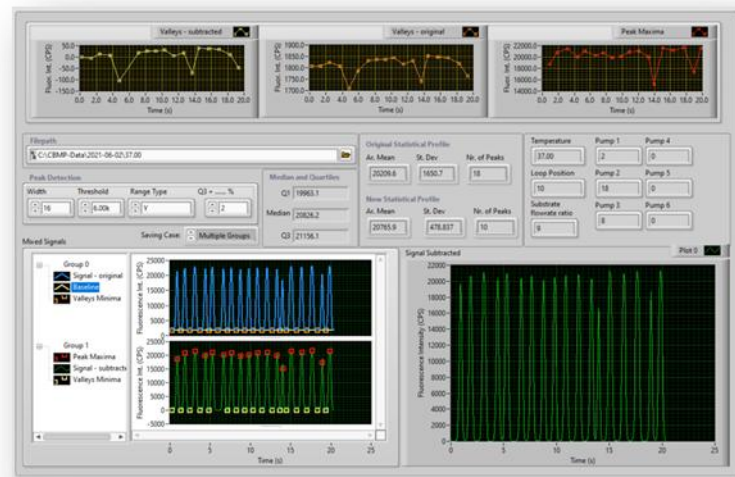
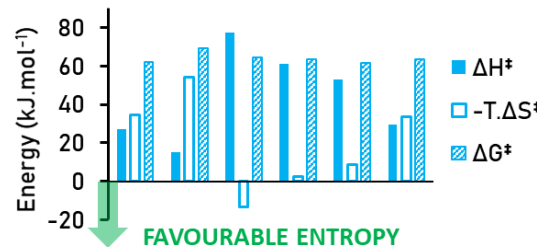
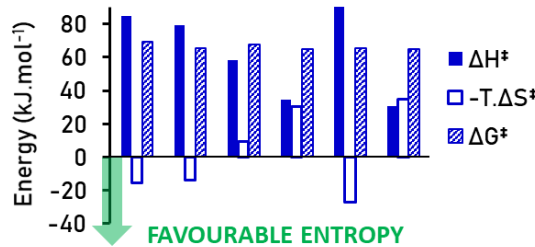
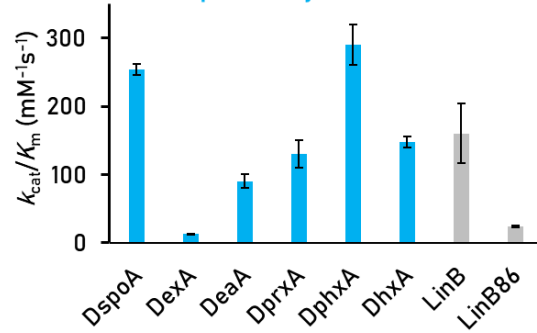
Kinetics and thermodynamics in μ -droplets

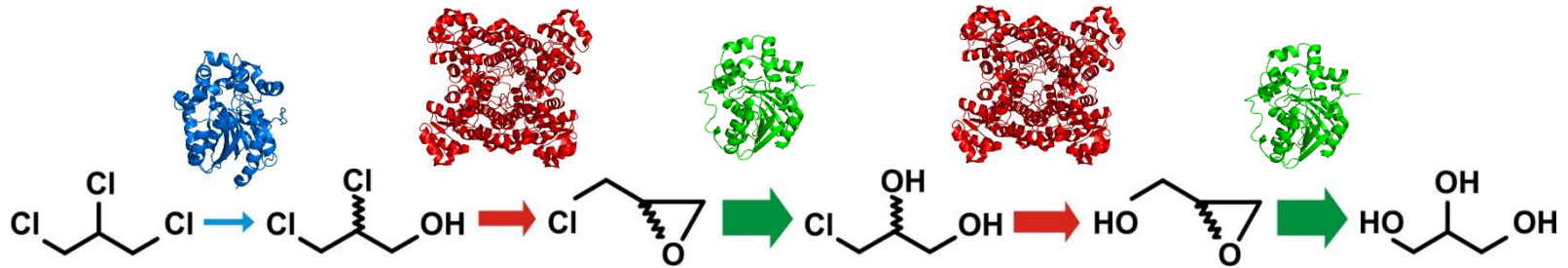


Turnover number

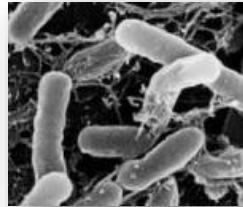
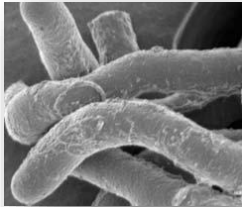


Specificity constant





Rhodococcus *Agrobacterium*



$$\frac{dc_{TCP}}{dt} = \frac{k_{cat,TCP,(R)-DCP} \times c_{DhaA} \times c_{TCP}}{(c_{TCP} + K_{m,TCP})} - \frac{k_{cat,TCP,(S)-DCP} \times c_{DhaA} \times c_{TCP}}{(c_{TCP} + K_{m,TCP})}$$

$$\frac{dc_{(R)-DCP}}{dt} = \frac{k_{cat,TCP,(R)-DCP} \times c_{DhaA} \times c_{TCP}}{c_{TCP} + K_{m,TCP}} - \frac{k_{cat,(R)-DCP} \times c_{HheC} \times c_{(R)-DCP}}{c_{(R)-DCP} + K_{m,(R)-DCP}}$$

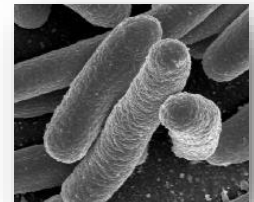
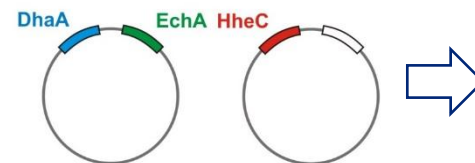
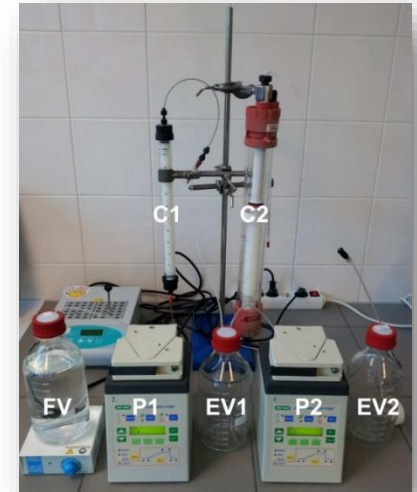
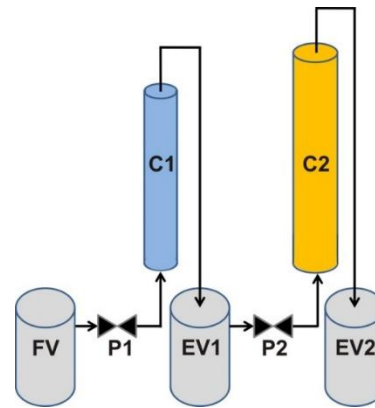
$$\frac{dc_{(S)-DCP}}{dt} = \frac{k_{cat,TCP,(S)-DCP} \times c_{DhaA} \times c_{TCP}}{c_{TCP} + K_{m,TCP}} - \frac{k_{cat,(S)-DCP} \times c_{HheC} \times c_{(S)-DCP}}{c_{(S)-DCP} + K_{m,(S)-DCP}}$$

$$\frac{dc_{ECH}}{dt} = \frac{k_{cat,(R)-DCP} \times c_{HheC} \times c_{(R)-DCP}}{c_{(R)-DCP} + K_{m,(R)-DCP}} + \frac{k_{cat,(S)-DCP} \times c_{HheC} \times c_{(S)-DCP}}{c_{(S)-DCP} + K_{m,(S)-DCP}} - \frac{k_{cat,ECH} \times c_{EchA} \times c_{ECH}}{c_{ECH} + K_{m,ECH}}$$

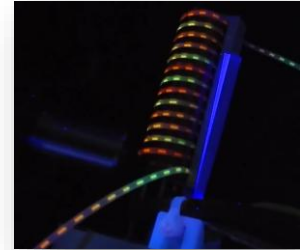
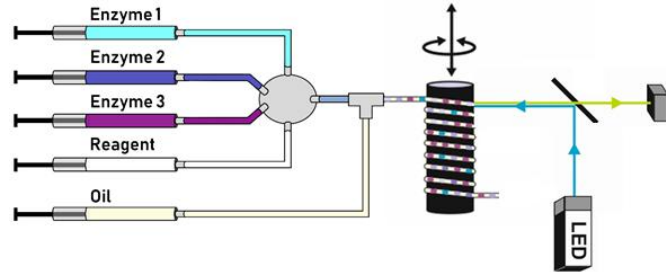
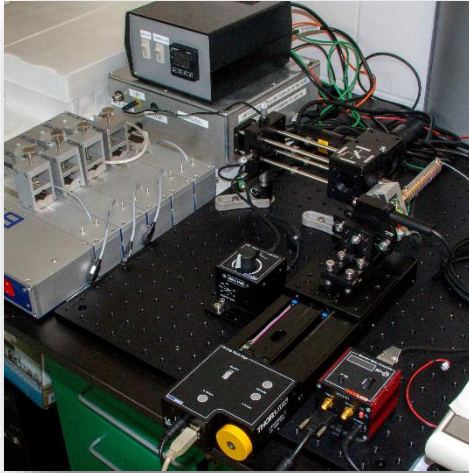
$$\frac{dc_{CPD}}{dt} = \frac{k_{cat,ECH} \times c_{EchA} \times c_{ECH}}{c_{ECH} + K_{m,ECH}} - \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}}$$

$$\frac{dc_{GDL}}{dt} = \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}} - \frac{k_{cat,GDL} \times c_{EchA} \times c_{GDL}}{c_{GDL} + K_{m,GDL} \times \left(1 + \frac{c_{GLY}}{K_i} + \frac{c_{TCP}}{K_c}\right)}$$

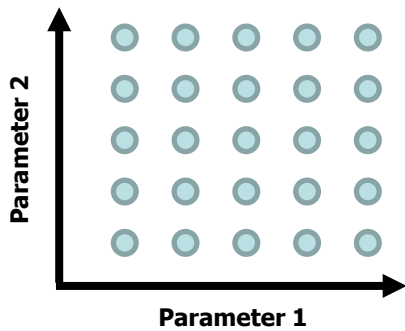
$$\frac{dc_{GLY}}{dt} = \frac{k_{cat,GDL} \times c_{EchA} \times c_{GDL}}{c_{GDL} + K_{m,GDL} \times \left(1 + \frac{c_{GLY}}{K_i} + \frac{c_{TCP}}{K_c}\right)}$$



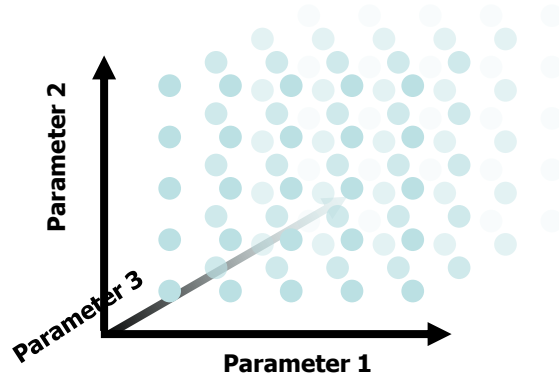
Enzyme cascades in μ -droplets



2 parameters

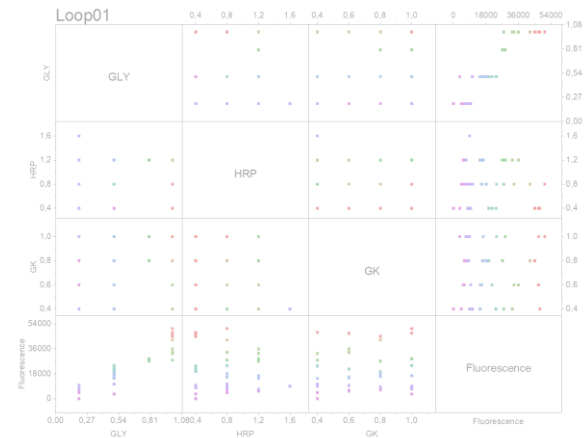


3 parameters

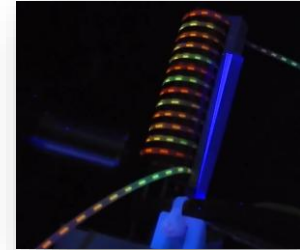
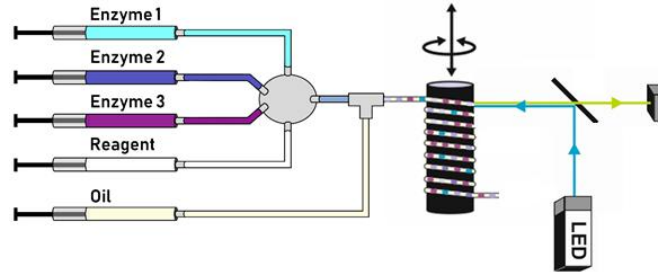
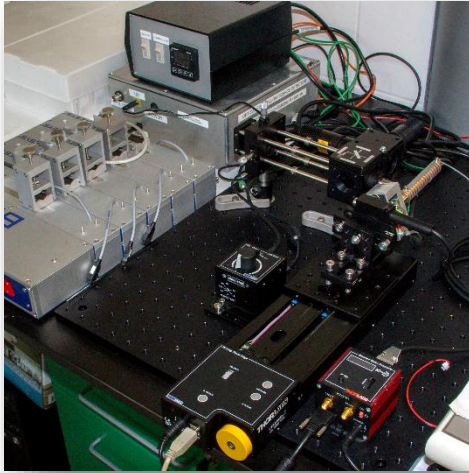


>3 parameters

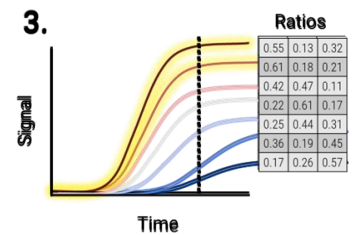
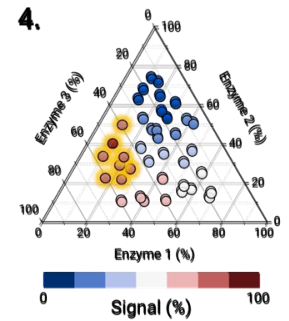
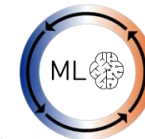
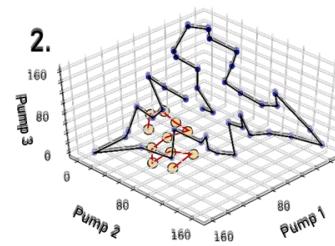
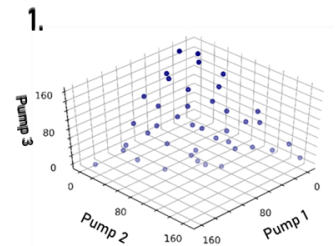
NOT STRAIGHTFORWARD



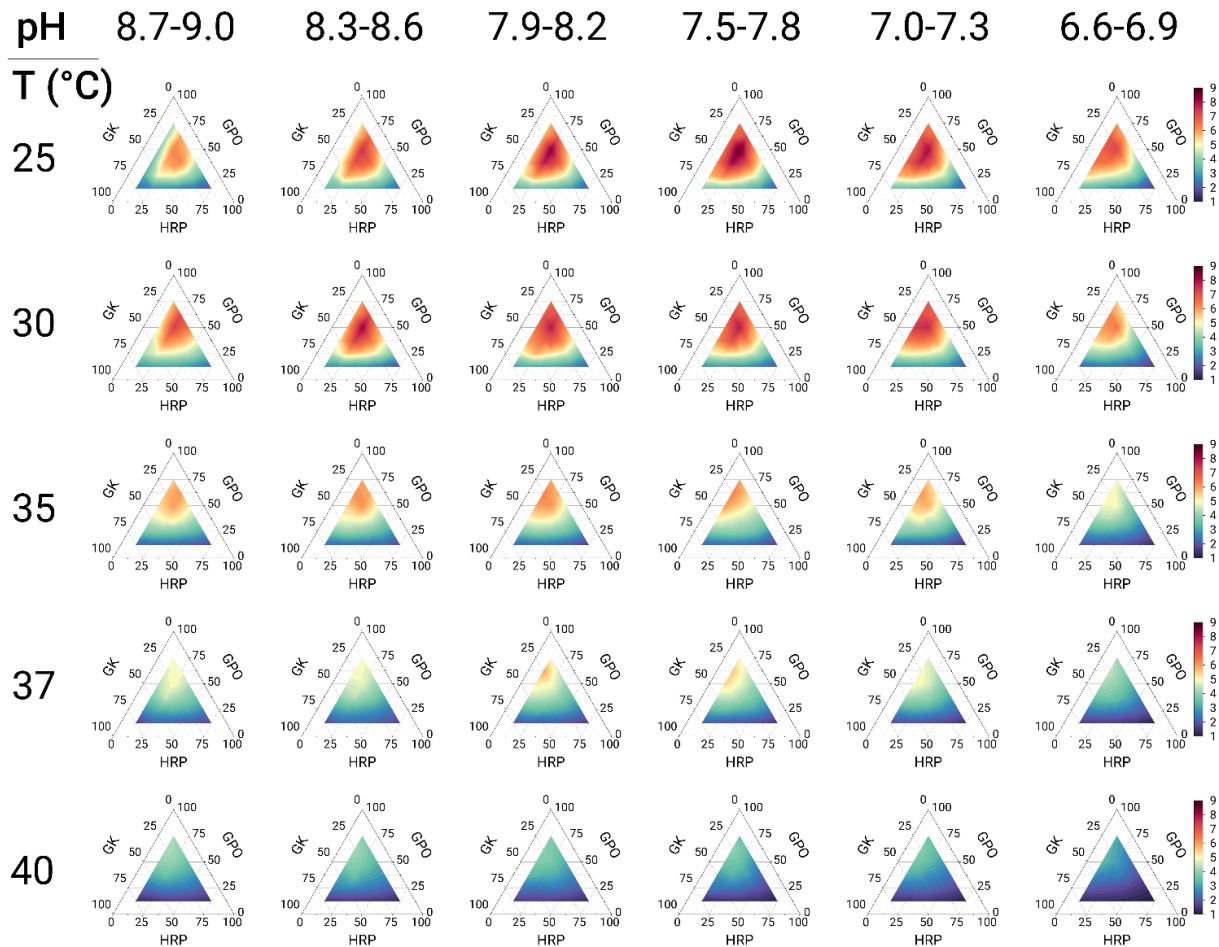
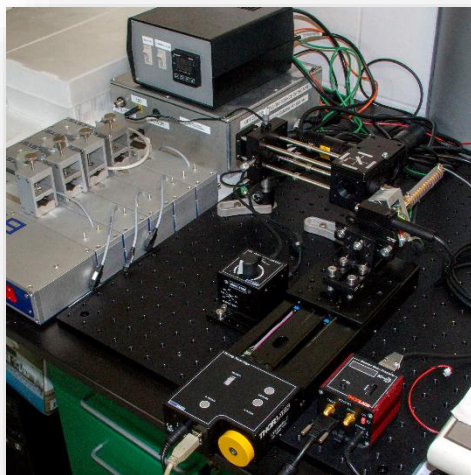
Enzyme cascades in μ -droplets



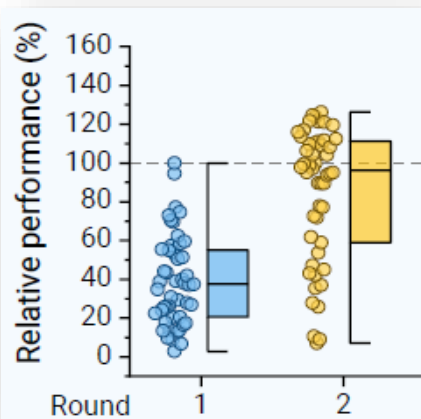
1. Covering of combinatorial space
2. Measurement order definition
3. Signal acquisition
4. Data processing and evaluation
5. Feedback loop (ML-based 🟡)



Enzyme cascades in μ -droplets

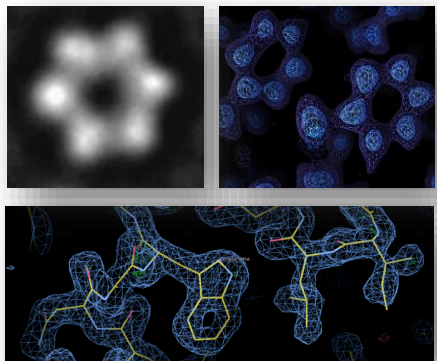


ML-based tracking

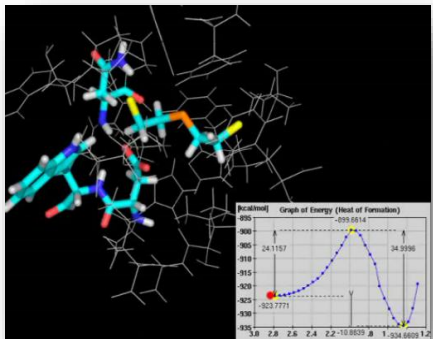
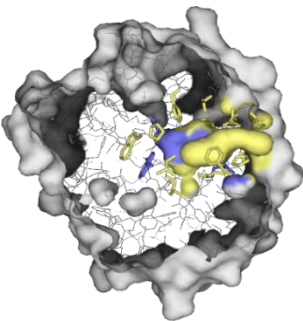


Multidisciplinary modern biotechnology

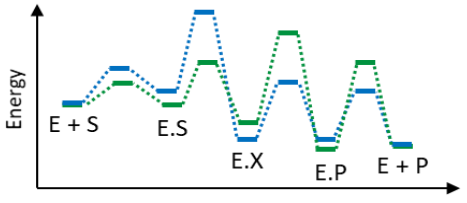
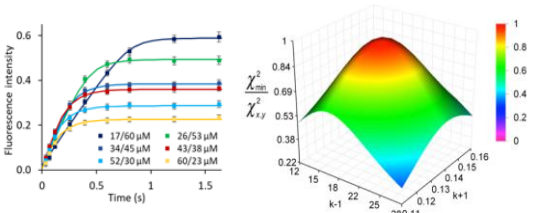
STRUCTURAL BIOLOGY



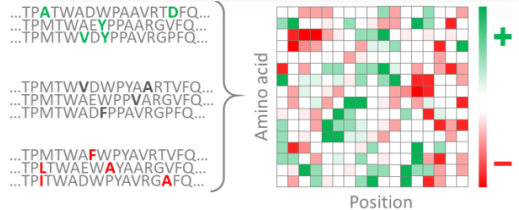
COMPUTATIONAL DESIGN



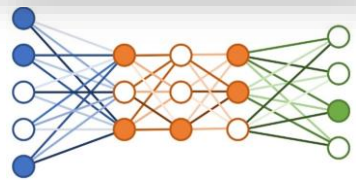
ENZYME KINETICS



MICROFLUIDICS

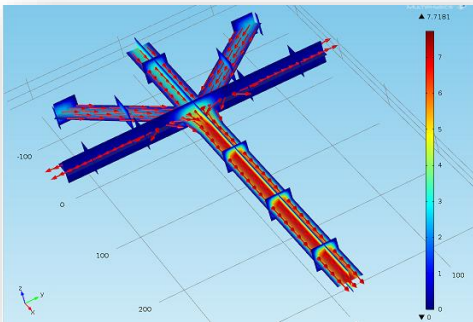
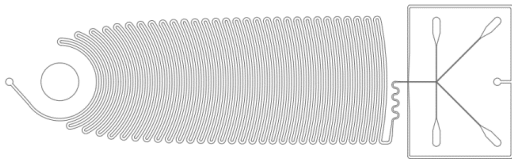


ARTIFICIAL INTELLIGENCE



- **soft lithography** originates from semiconductor industry

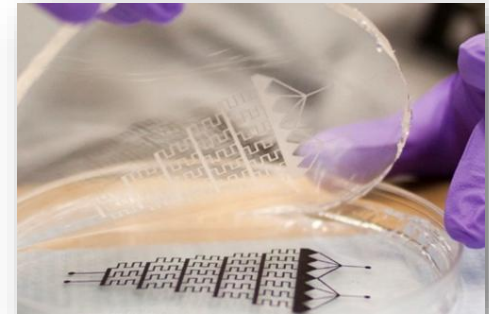
DESIGN / MODELING



MASK / MOLD

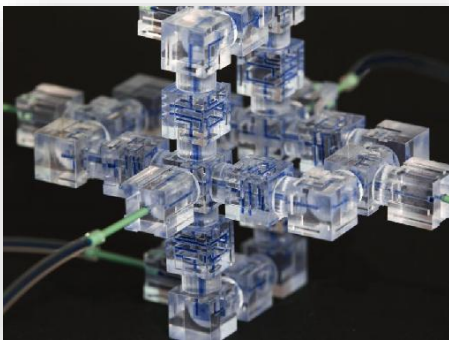
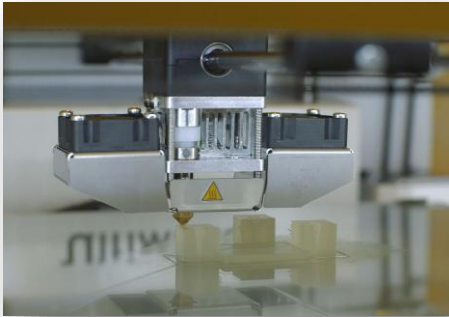


CASTING / BONDING



□ direct fabrication methods

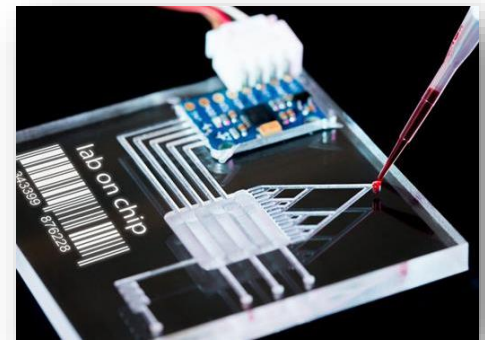
3D PRINTING



LASER CUTTING



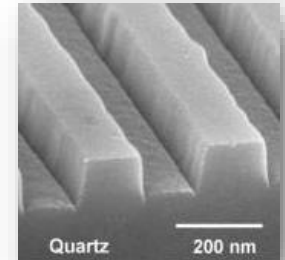
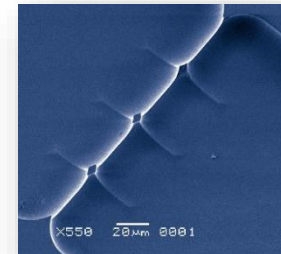
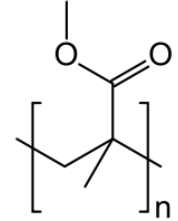
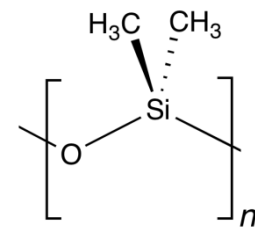
CNC μ -MILLING



Design and fabrication

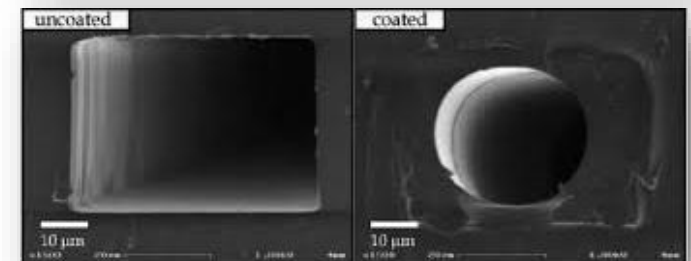
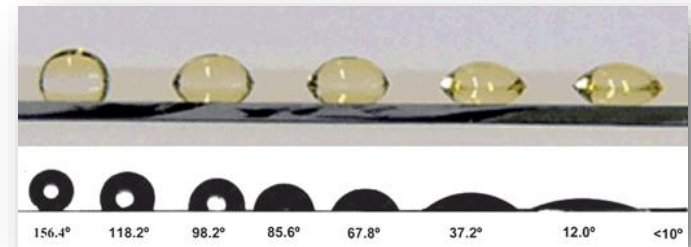
□ materials

- inert and transparent
- PDMS - poly(dimethyl siloxane)
- PMMA - poly(methyl methacrylate)
- fused silica, quartz and glass



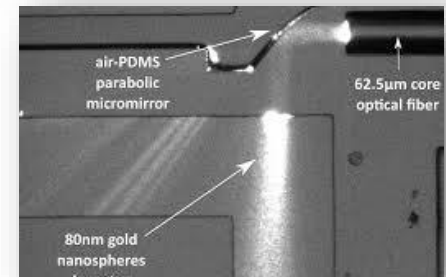
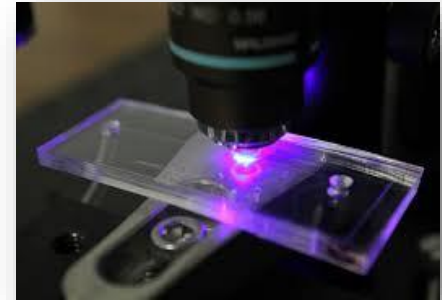
□ surface modification

- plasma treatment
- silanization
- sol-gel coating

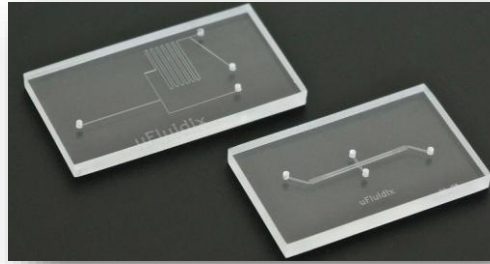
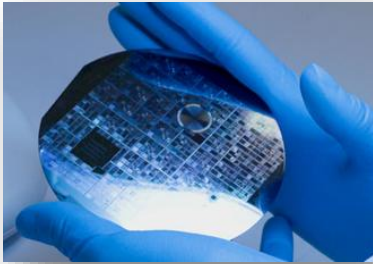


Sensing and detection

- ❑ processing of **small reagent volumes**
- ❑ **analytical timescale** and performance
- ❑ **on chip detection**
 - fluorescence (LSM, FCS, FLIM)
 - UV/VIS absorbance
 - IR spectroscopy
 - Raman scattering
 - (chemo/electro) luminescence
 - thermal conductivity
 - RI variation
- ❑ **off chip detection**
 - GC, HPLC, MS
 - NMR, X-ray



- customized design and fabrication



- entire technologies



Conclusions

- ❑ reduced sample/reagent/power consumption
- ❑ superior performance and novel physics
- ❑ applications in life and medical sciences
- ❑ in-house as well as commercial technologies

microfluidics revolutionize science & technology

- ❑ Mazurenko, S., 2020: **Machine Learning in Enzyme Engineering**. *ACS Catalysis*, 10, 1210–1223
- ❑ 3. DATABASES RELEVANT TO ENZYME ENGINEERING
3.3. *Emerging Methods for High-Throughput Data Collection*
(page 1213 - 1216)



✓ Cite This: *ACS Catal.* 2020, 10, 1210–1223

Perspective

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Machine Learning in Enzyme Engineering

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[‡]International Centre for Clinical Research, St. Ann's Hospital, 602 00 Brno, Czech Republic