Microfluidics – „Lab on a Chip“

Outline

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

Lab on a Chip Concept

pre-treatment

incubation

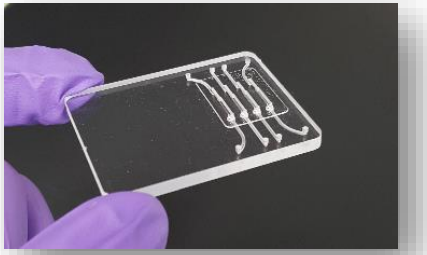
analysis

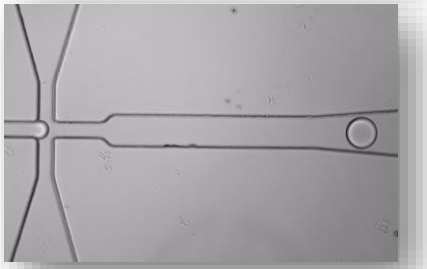
preparation

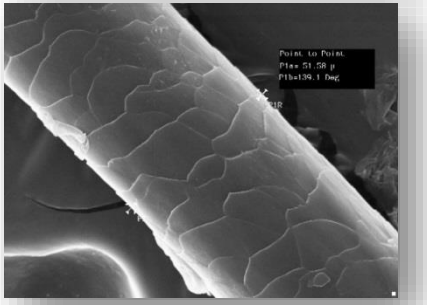
collection

Microfluidics

- „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



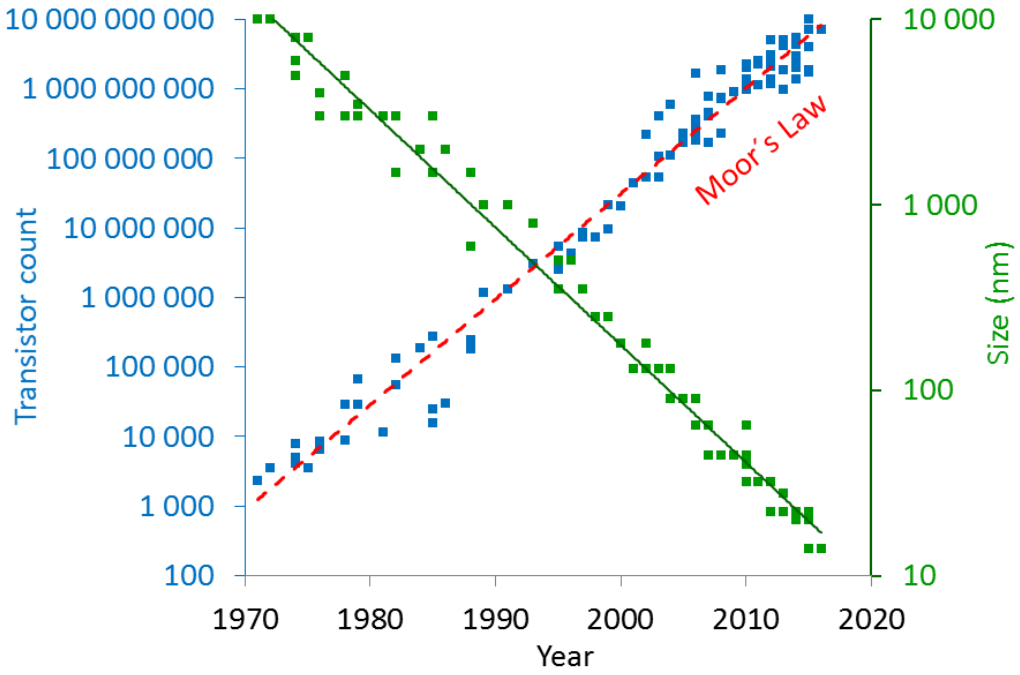




Nature 507, 181 (2014)

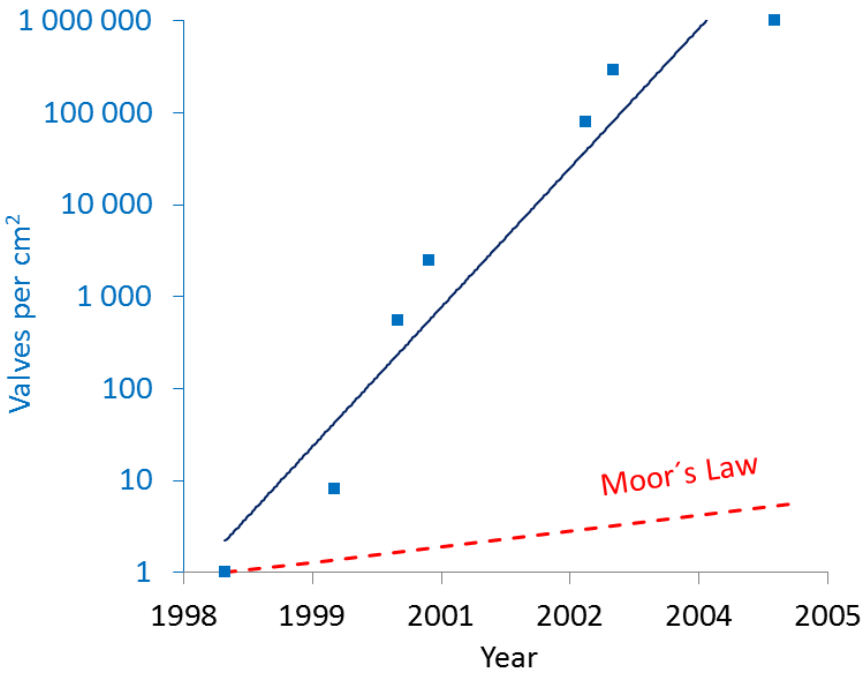
Revolution in Electronics

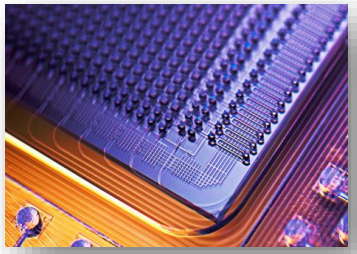
	Size (nm)	Price (USD)
Vacuum tube	100	10

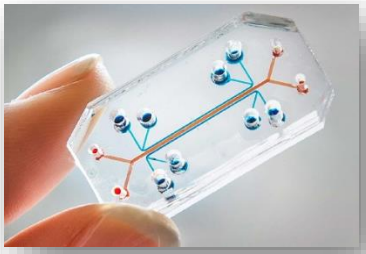


Revolution in Science?

	Volume (µL)	Throughput (assays/day)
Test tube	1 000	10



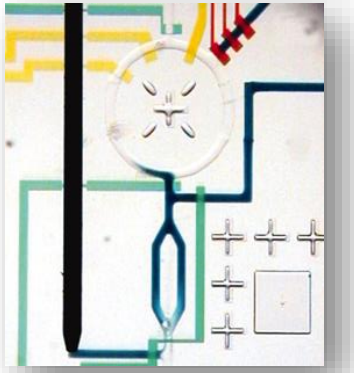




Concepts in microfluidics

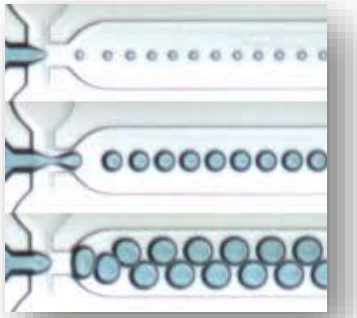
- **continuous-flow microfluidics**

  manipulation of continuous liquid flow through micro-fabricated channels



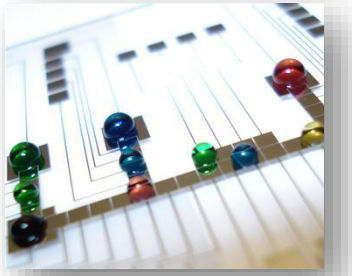
- **droplet-based microfluidics**

  manipulating discrete volumes of fluids in immiscible phases



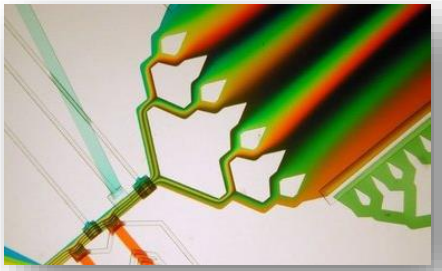
- **digital microfluidics**

  droplets manipulated on a substrate using electro-wetting

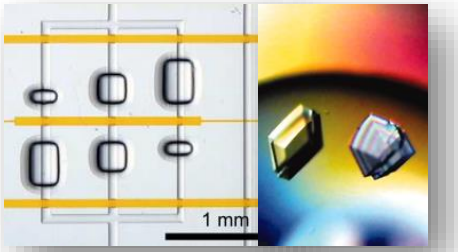


Novel Physics of Micro-Scale

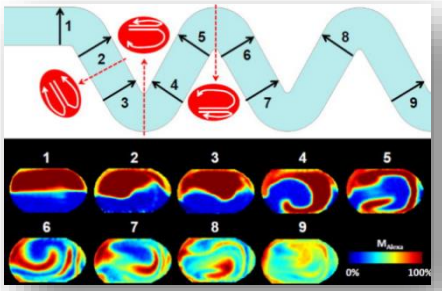
- viscosity, surface tension and capillary forces dominate
  - **lack of turbulent phenomena**



  - **absence of density-driven convection**

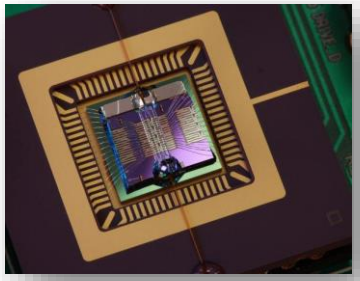
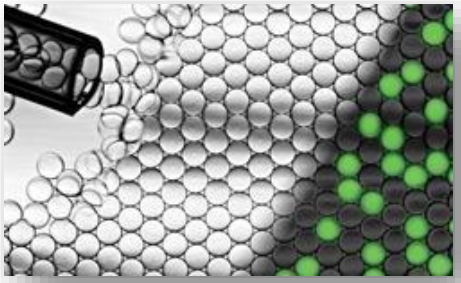


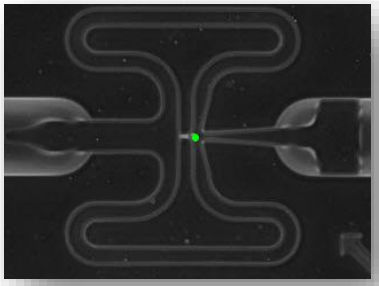
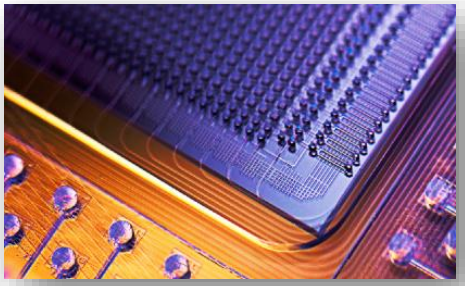
  - **strong shearing forces**



Lab on a Chip applications

- analytics and chemistry
- PCR and sequencing
- point of care diagnostics
- pharmacology
- clinical studies
- single cell biology
- protein science

Polymerase chain reaction

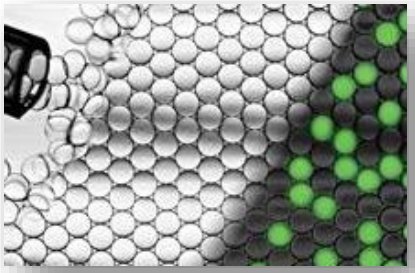
- **classical PCR**
  - slow heating/cooling cycles
  - PCR tubes (strips), 96-well MTP
  - volume 50 to 500 µL

Kary Mullis

Nobel Prize in 1993

Digital polymerase chain reaction

- **digital PCR**
  - 1 nanoliter droplets
  - 20 000 droplets per run

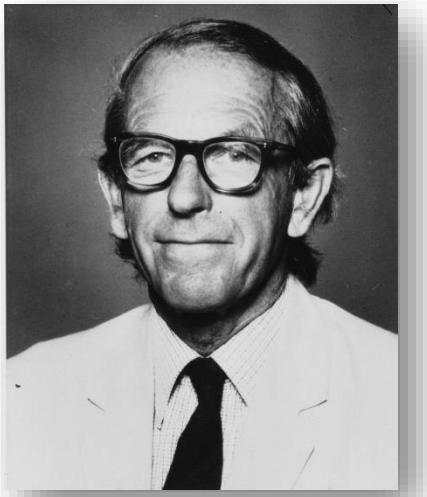


Next-generation sequencing

- **parallelization** of **single molecule** pyrosequencing
- **454 Pyrosequencing** (Roche)

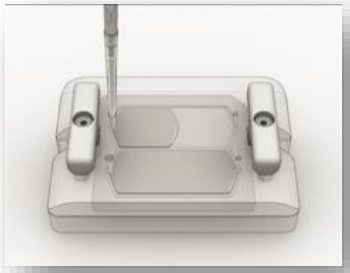
  water in oil droplets 1 picoliter (10^{-12} liters)

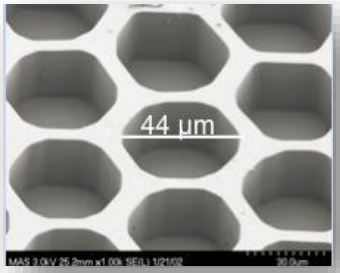
  1 mil. reads/run

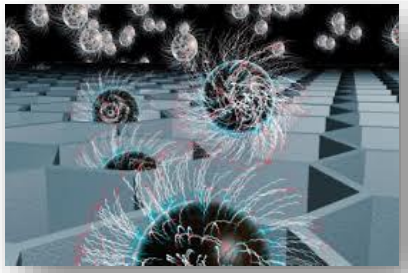


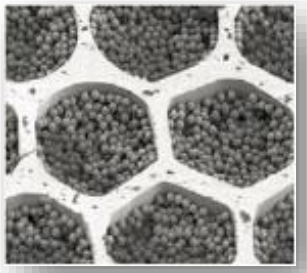
Frederick Sanger

Nobel Prize in 1980

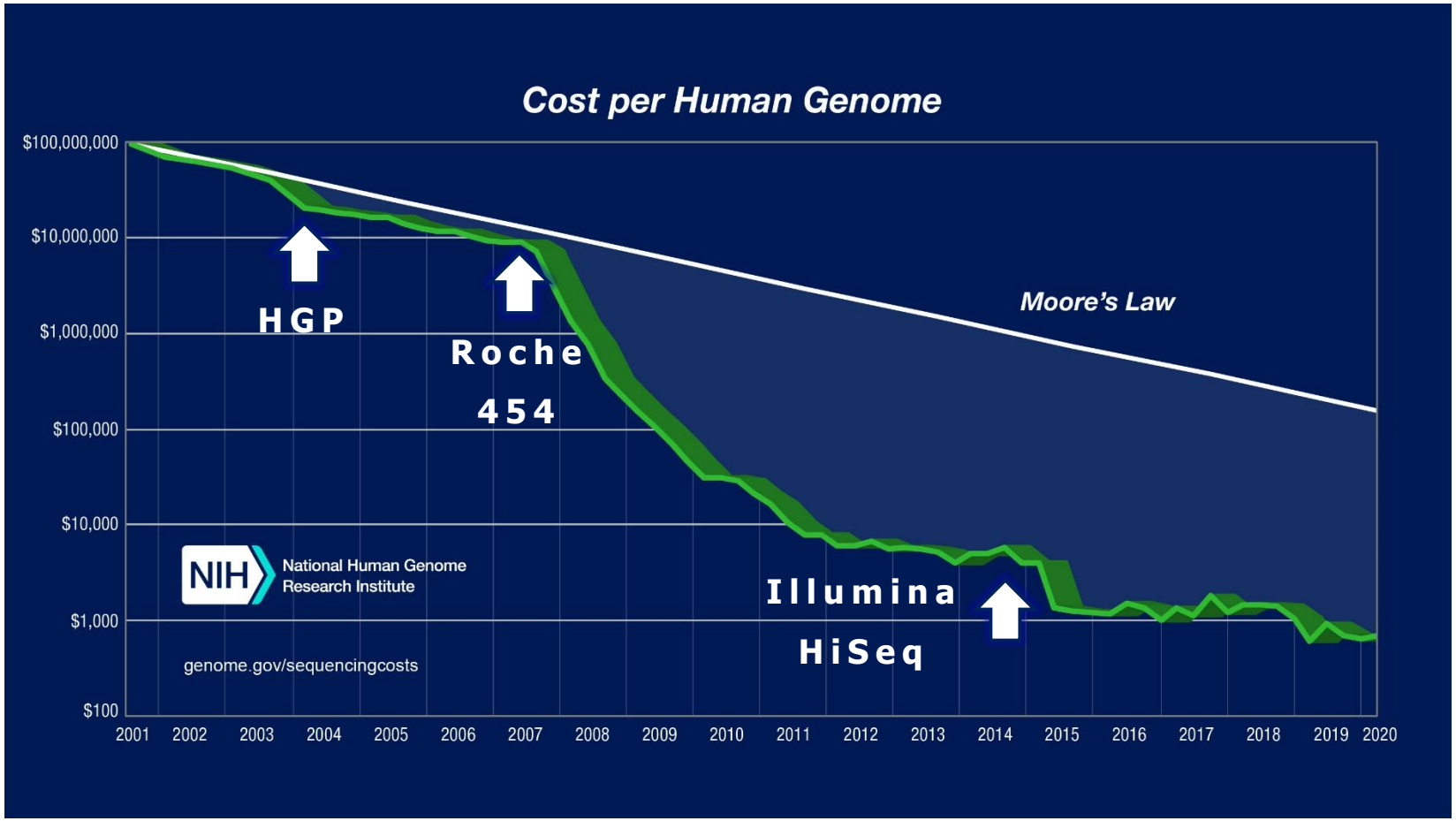








Revolution in Science?

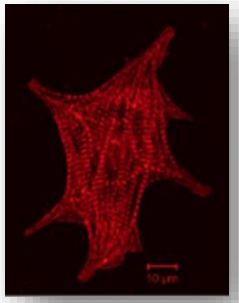


- 2003: 13 years, 3 billion USD
- 2018: days, < 1,000 USD

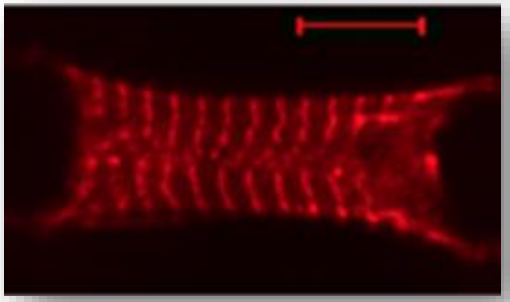
Organ(oid)s on chip

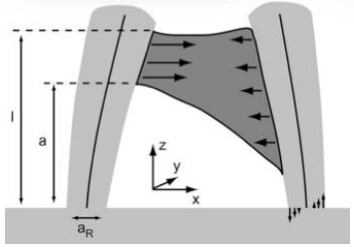
- 3D chips **mimicking human's physiological responses** (e.g., pathological, pharmacokinetic, toxicological)
- realistic *in vitro* model **closer to *in vivo* cell environment** (e.g., mechanical strain, patterning, fluid shear stresses)
- **replacing** expensive and controversial **animal testing**

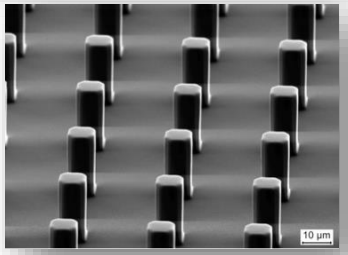
flat surface

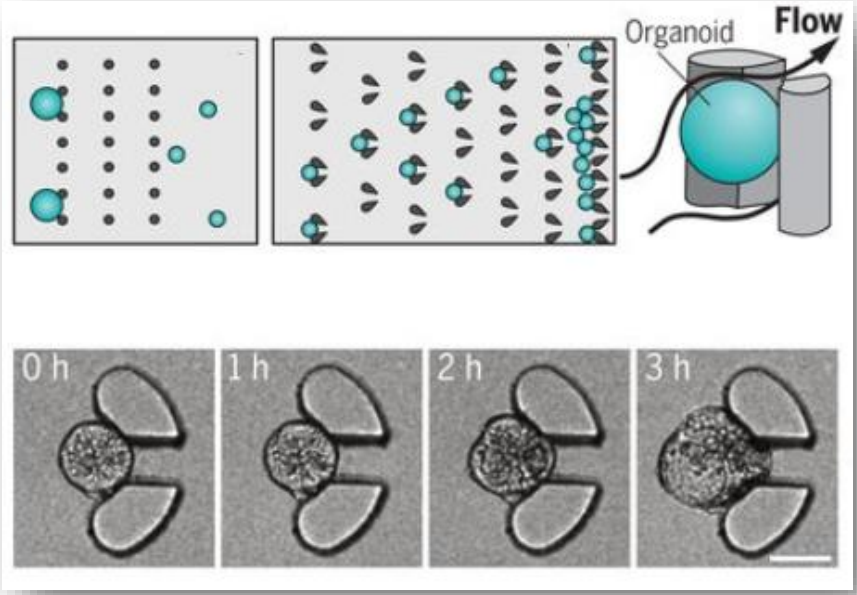


micropillar





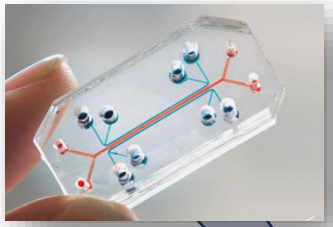




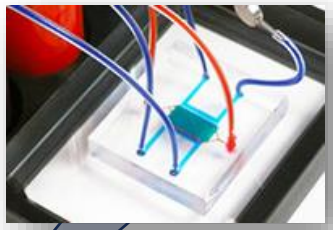
Organs on chip

Organs on chip

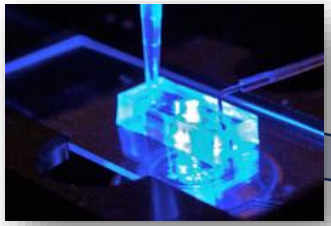
Lung

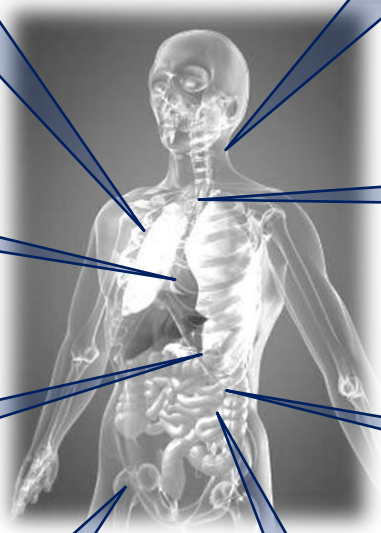


Neurovascular



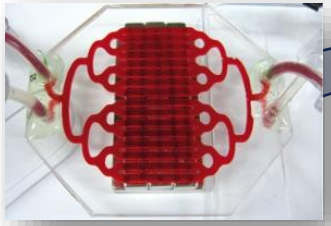
Heart





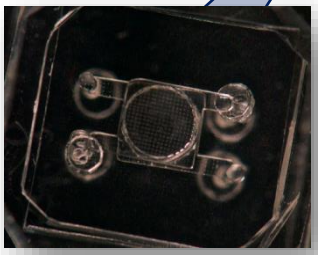
Artery

Spleen

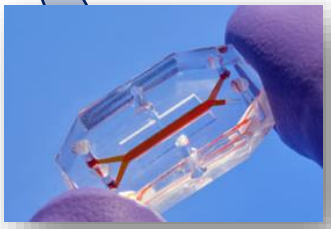


Kidney

Bone



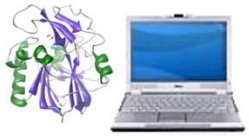
Intestine



Protein Engineering

RATIONAL DESIGN

1. Computer aided design



2. Site-directed mutagenesis

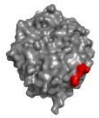
Individual mutated gene

3. Transformation

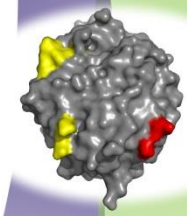
4. Protein expression

5. Protein purification

6. *not applied*



Constructed mutant enzyme



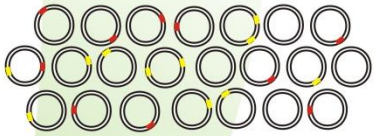
IMPROVED ENZYME

7. Biochemical testing

DIRECTED EVOLUTION

1. *not applied*

2. Random mutagenesis



Library of mutated genes
(>10,000 clones)

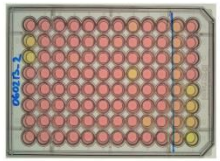
3. Transformation

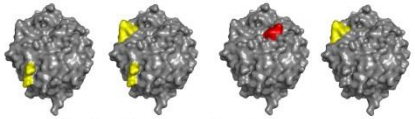
4. Protein expression

5. *not applied*

6. Screening and selection

- stability
- selectivity
- affinity
- activity

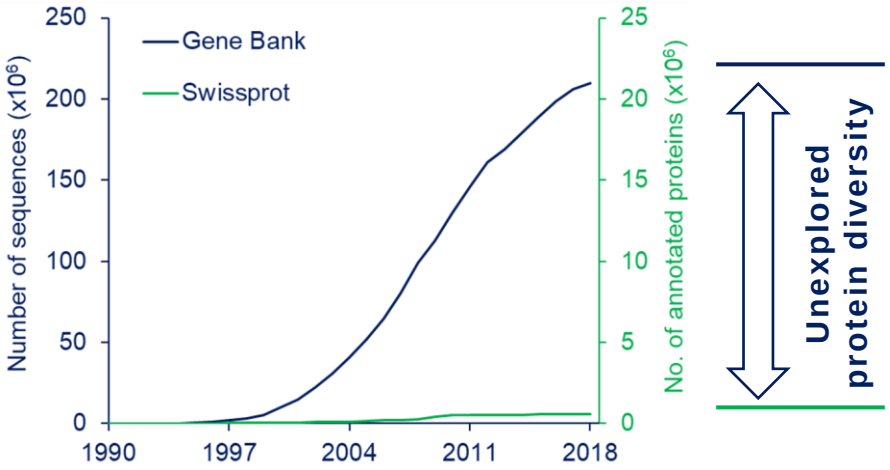




Selected mutant enzymes

Sequence diversity

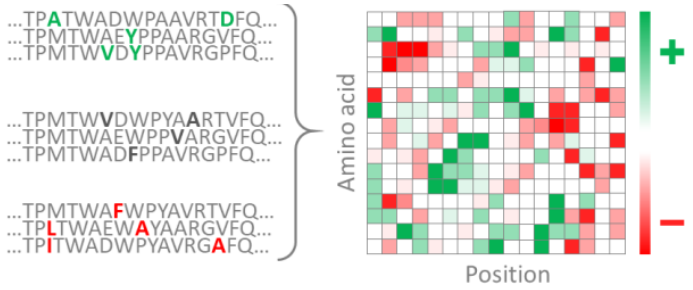
Genomic databases



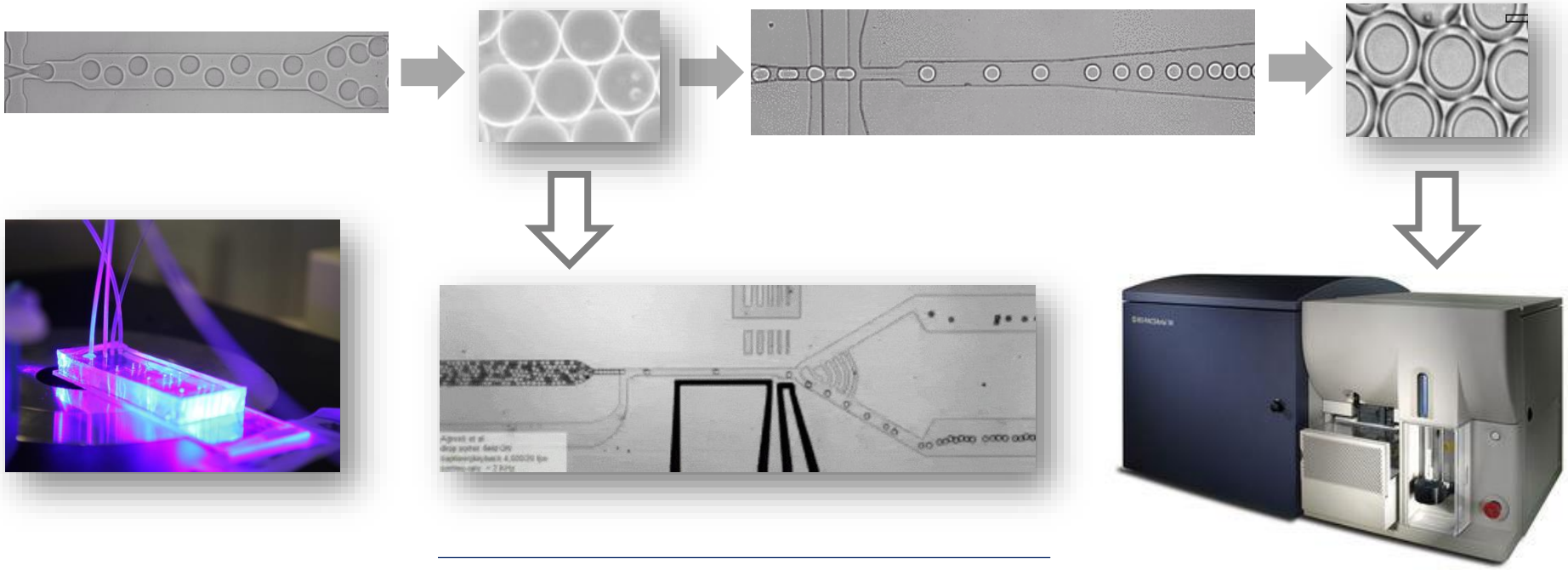
Directed evolution

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

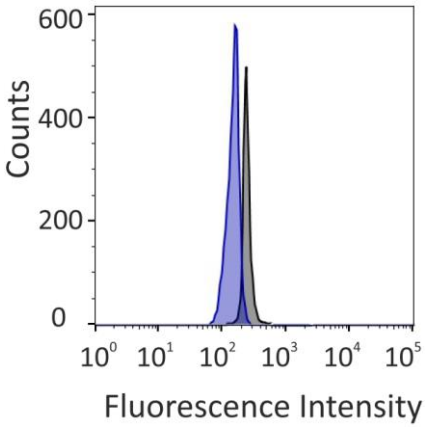
Sequence-function relationship



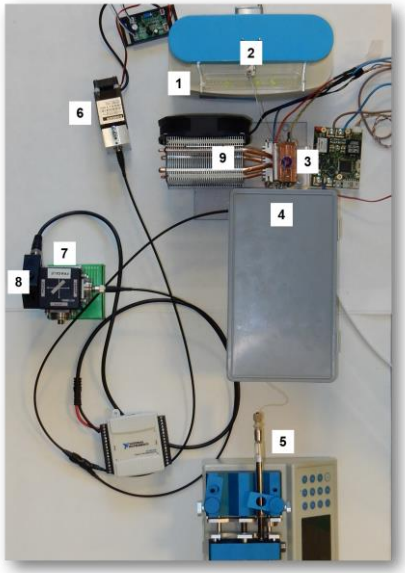
(Ultra)High-throughput screening

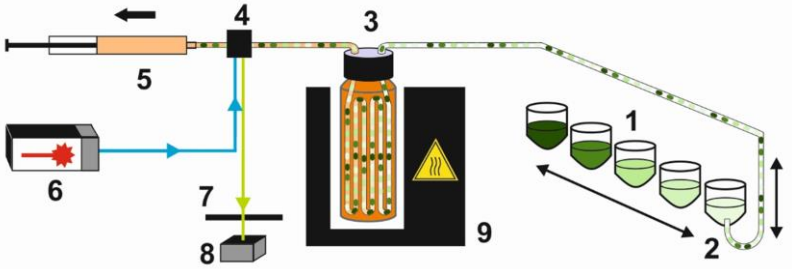


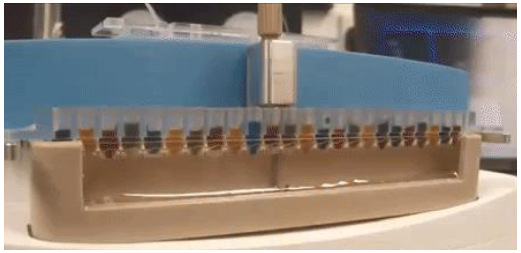
	Robotic	µFluidic
Reaction volume	100 µL	**5 pL**
Reactions / day	50 000	**$1 . 10^8$**
Total time	5 years	**3 days**
Total volume	5 000 L	**150 mL**
No. of plates / devices	250 000	**2.0**
No. of tips	28 000 000	**10**

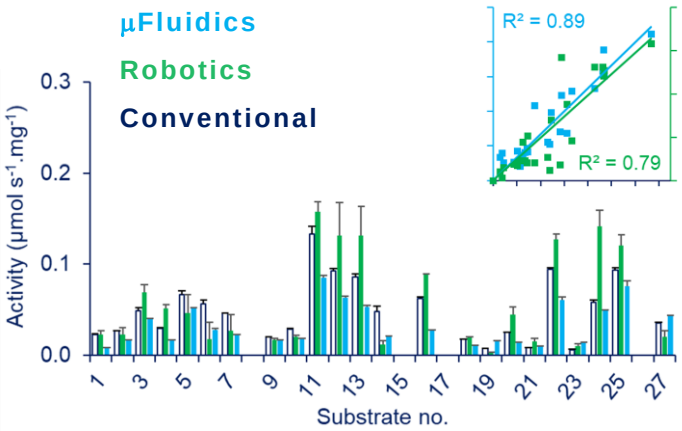


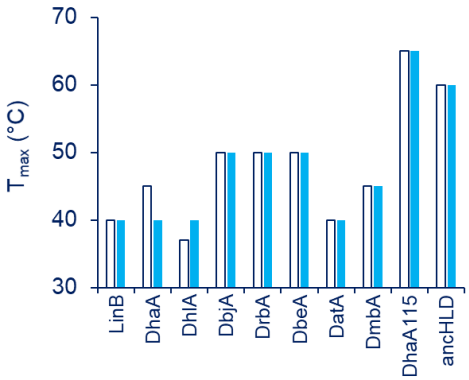
Enzyme specificity profiling





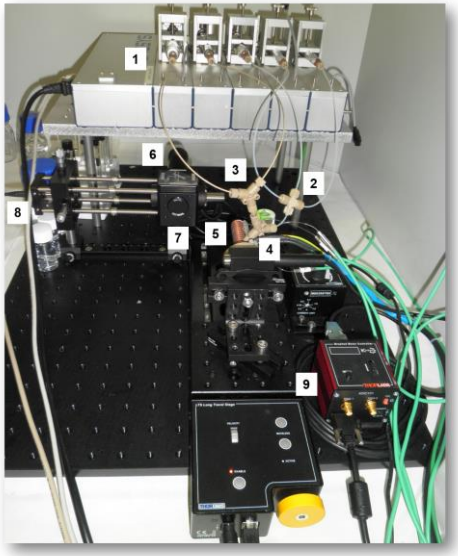


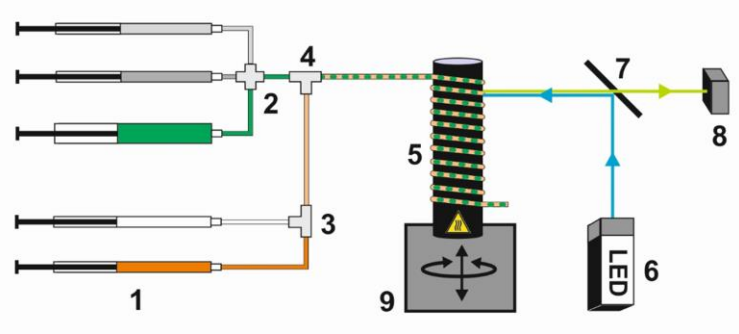




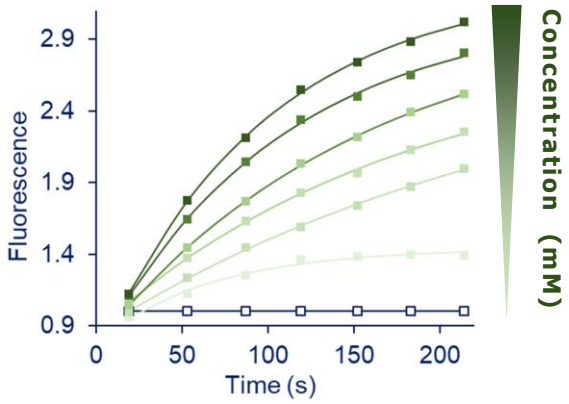
	Conventional	Robotic	µFluidic
Reaction volume (mL)	10	1	0.00015
Total enzyme (mg)	540	54	0.5
Total time (days)	100	30	5

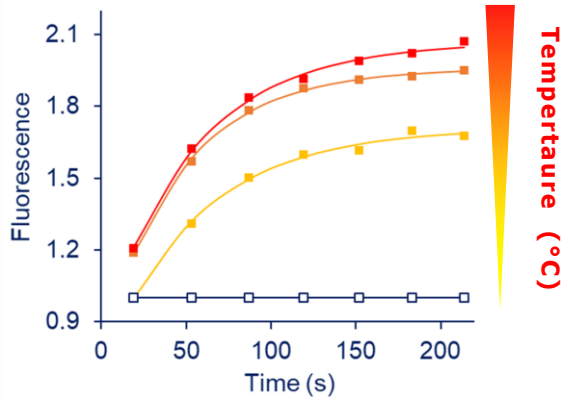
Steady-state kinetics

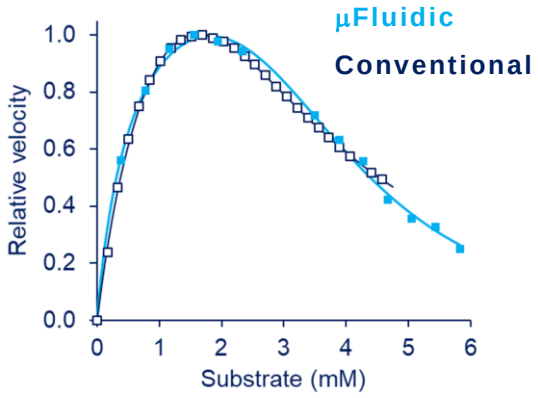




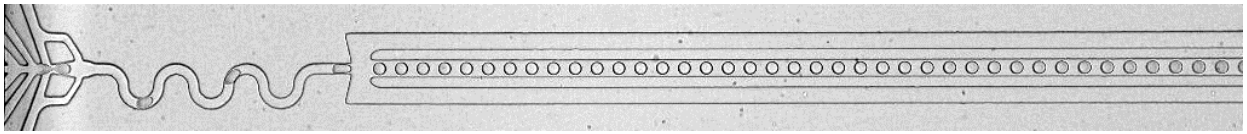
	Conventional	µFluidic
Reaction volume (mL)	2	0.00010
Total enzyme (mg)	1	0.01
Throughput per hour	5	10 000

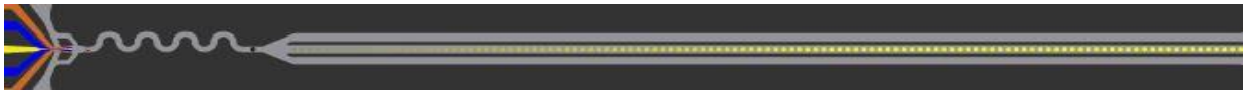


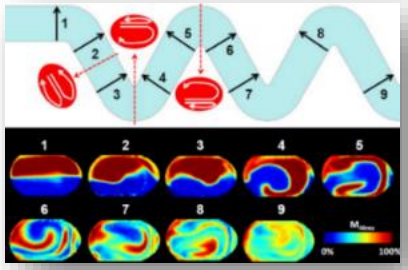


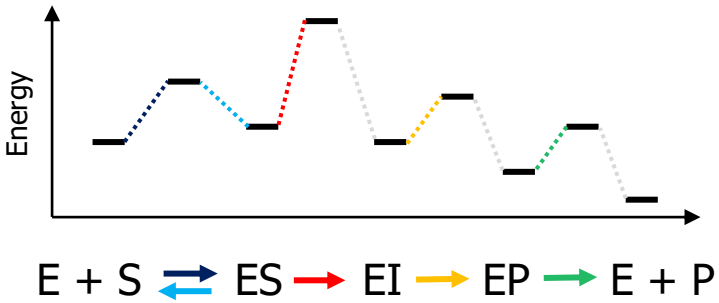


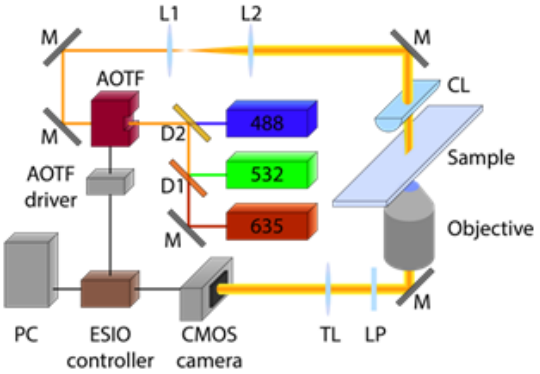
Mechanism of enzyme catalysis

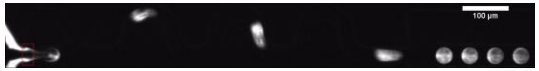




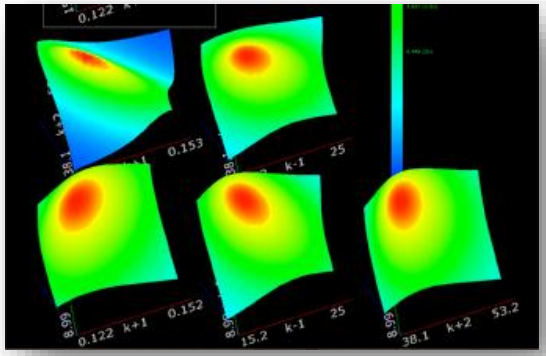




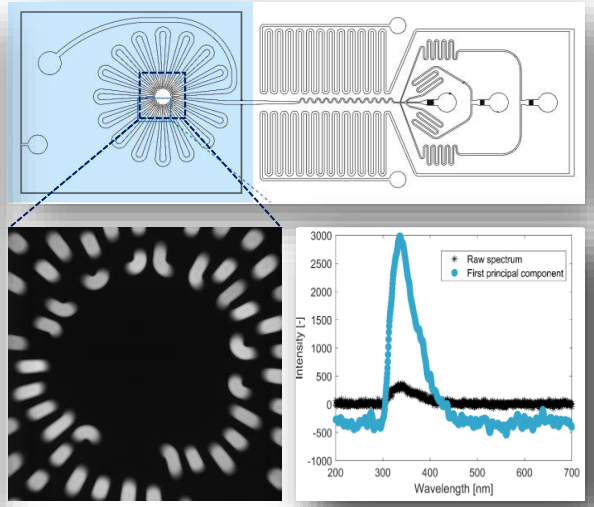


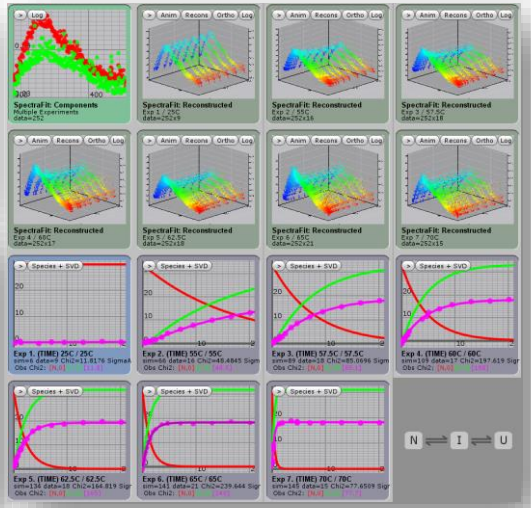


	Stopped-flow	µFluidic
Dead time	0.3 ms	**0.7 ms**
Reaction volume	100 µL	**10 pL**
Temp. equilibration	10 min	**50 ms**
Signal integration	0.5 ms	**no limit**

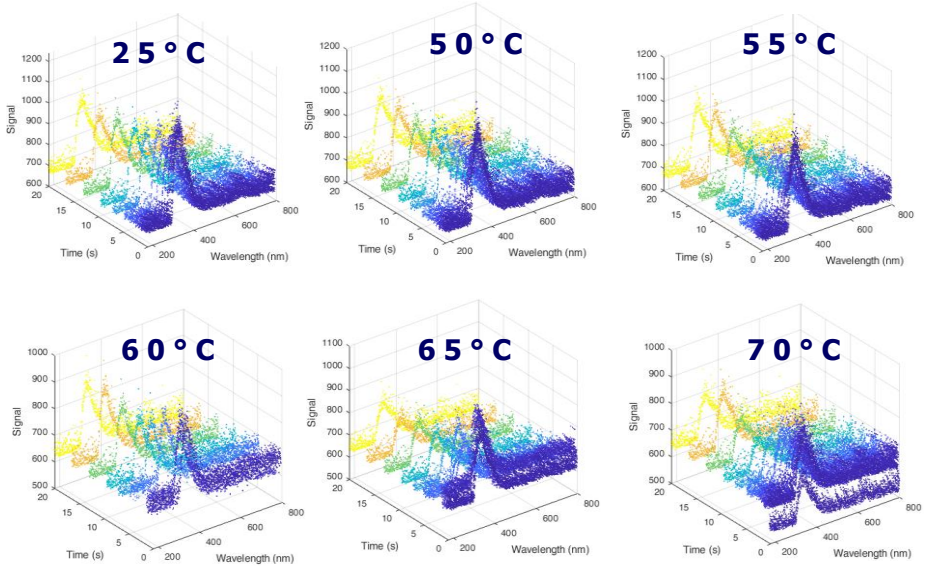


Mechanism of protein unfolding

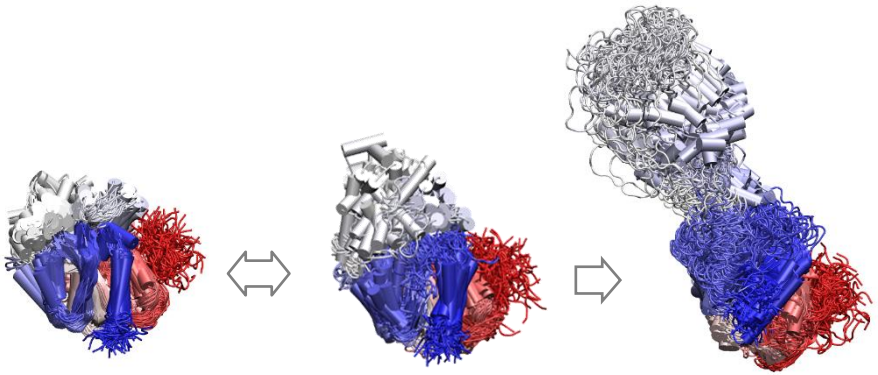




3M raw data points, 30 000 after PCA



Thermodynamic understanding

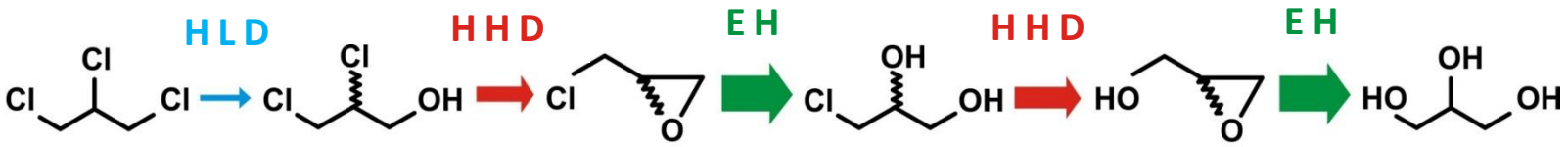


Native ⇔ Intermediate → Unfolded

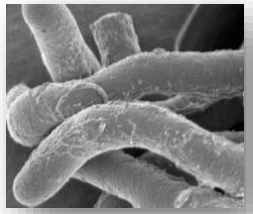
E_{a1} = 280 kJ.mol^{-1}

E_{a2} = 120 kJ.mol^{-1}

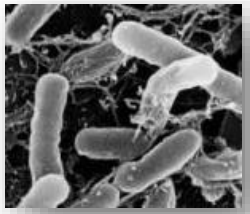
Metabolic engineering



Rhodococcus



Agrobacterium



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

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

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

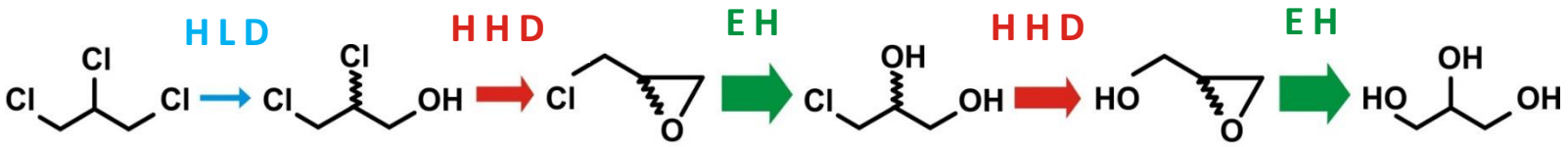
$$\frac{dc_{ECH}}{dt} = \frac{k_{cat,(R)-DCP} \times c_{HheC} \times c_{(R)-DCP}}{c_{(R)-DCP} + K_{m,(R)-DCP}} + \frac{k_{cat,(S)-DCP} \times c_{HheC} \times c_{(S)-DCP}}{c_{(S)-DCP} + K_{m,(S)-DCP}} - \frac{k_{cat,ECH} \times c_{EchA} \times c_{ECH}}{c_{ECH} + K_{m,ECH}}$$

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

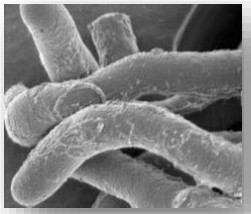
$$\frac{dc_{GDL}}{dt} = \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}} - \frac{k_{cat,GDL} \times c_{EchA} \times c_{GDL}}{c_{GDL} + K_{m,GDL} \times \left(1 + \frac{c_{GLY}}{K_i} + \frac{c_{TCP}}{K_c}\right)}$$

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

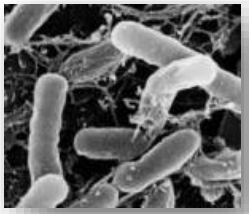
Metabolic engineering



Rhodococcus



Agrobacterium



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

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

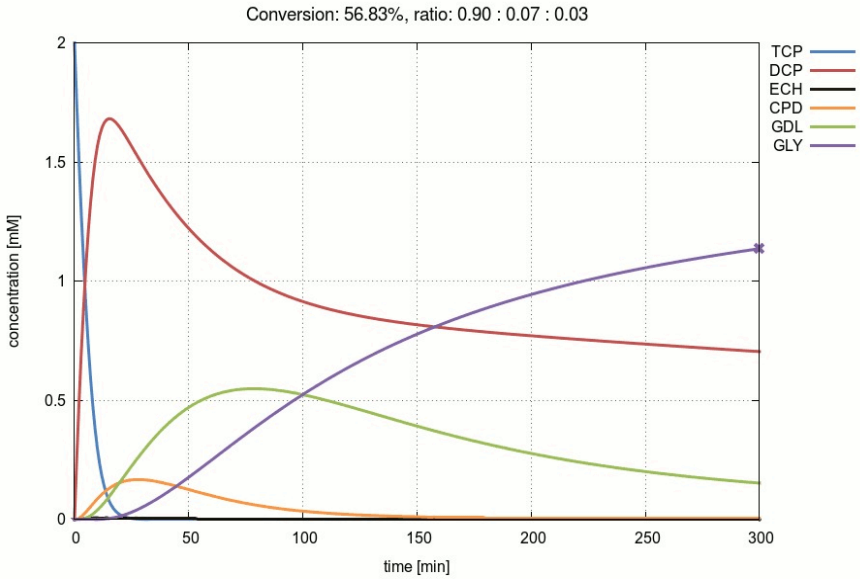
$$\frac{dc_{(S)-DCP}}{dt} = \frac{k_{cat,TCP,(S)-DCP} \times c_{DhaA} \times c_{TCP}}{c_{TCP} + K_{m,TCP}} - \frac{k_{cat,(S)-DCP} \times c_{HheC} \times c_{(S)-DCP}}{c_{(S)-DCP} + K_{m,(S)-DCP}}$$

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

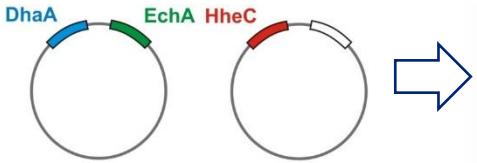
$$\frac{dc_{CPD}}{dt} = \frac{k_{cat,ECH} \times c_{EchA} \times c_{ECH}}{c_{ECH} + K_{m,ECH}} - \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}}$$

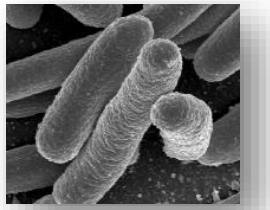
$$\frac{dc_{GDL}}{dt} = \frac{k_{cat,CPD} \times c_{HheC} \times c_{CPD}}{c_{CPD} + K_{m,CPD}} - \frac{k_{cat,GDL} \times c_{EchA} \times c_{GDL}}{c_{GDL} + K_{m,GDL} \times \left(1 + \frac{c_{GLY}}{K_i} + \frac{c_{TCP}}{K_c}\right)}$$

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

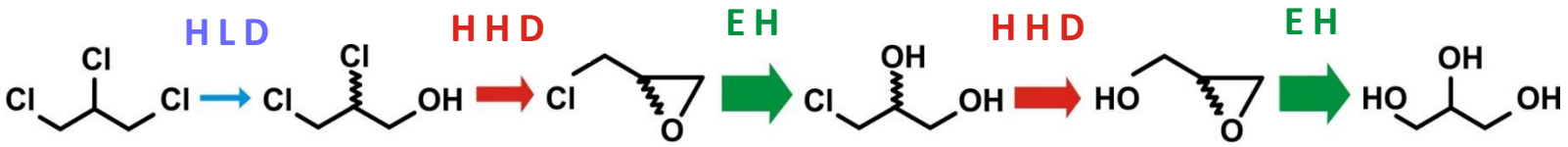


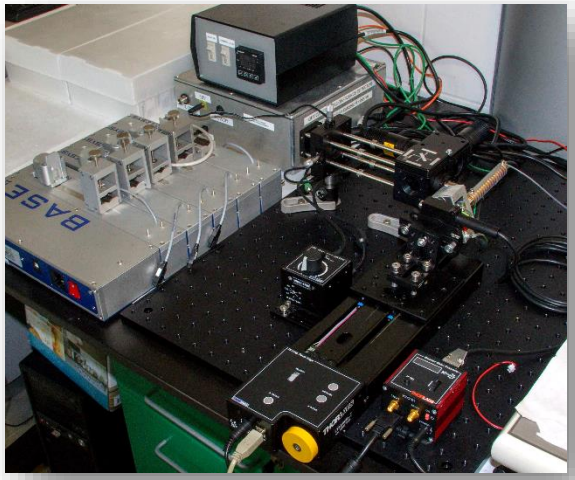
Escherichia

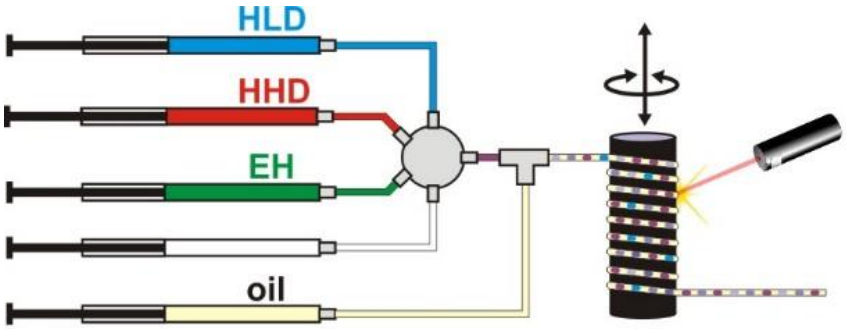




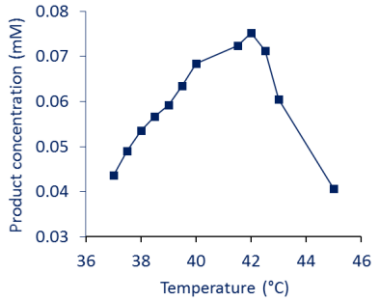
Metabolic engineering

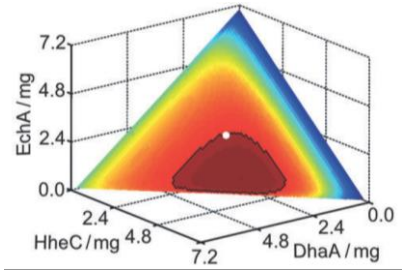






Artificial Intelligence



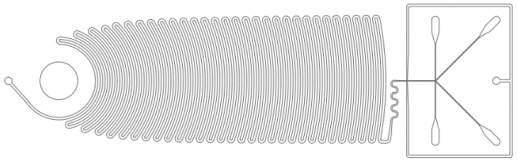


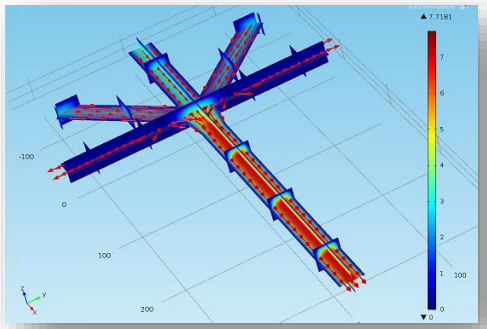
- 1 nL droplet volume
- 10 000 assays/hour

Design and fabrication

- **soft lithography** originates from semiconductor industry

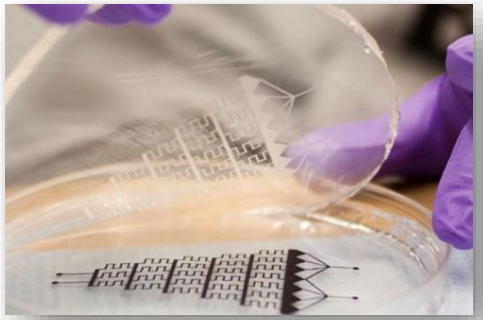
DESIGN / MODELING





MASK / MOLD

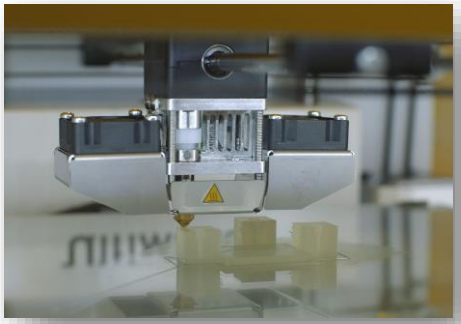
CASTING / BONDING

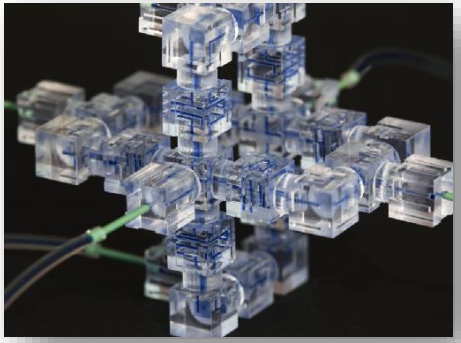


Design and fabrication

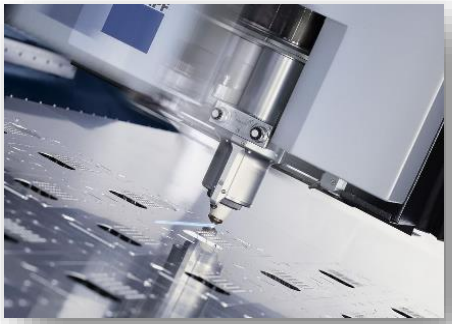
- **direct fabrication methods**

3D PRINTING

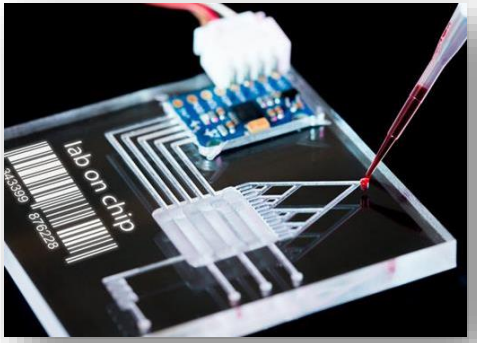




LASER CUTTING



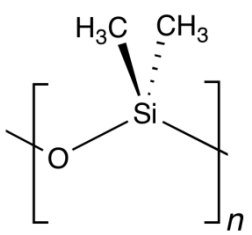
CNC μ-MILLING

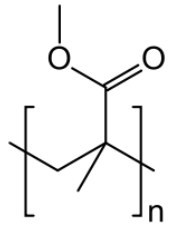


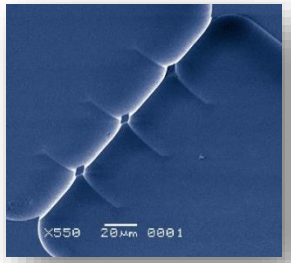
Design and fabrication

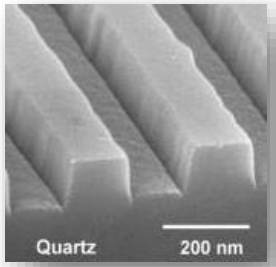
❑ materials

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



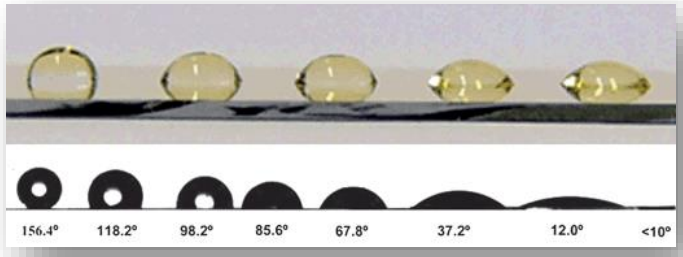


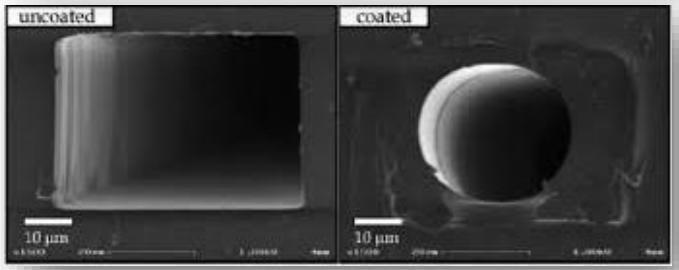




❑ surface modification

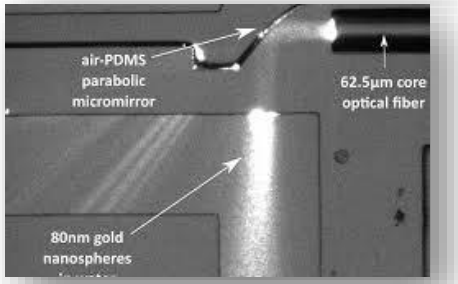
- plasma treatment
- silanization
- sol–gel coating

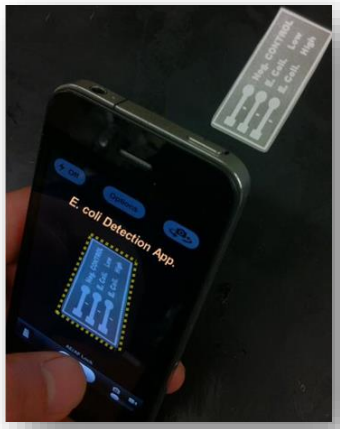




Sensing and detection

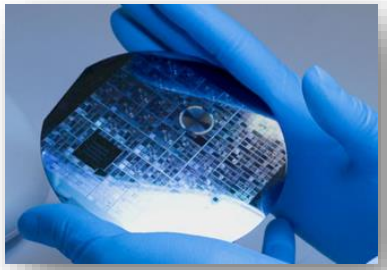
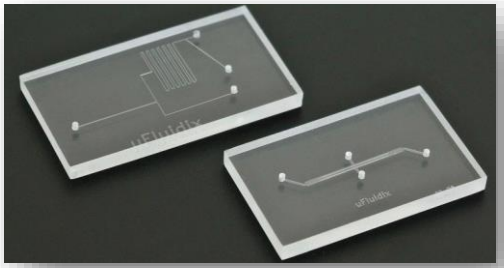
- processing of **small reagent volumes**
- **analytical timescale** and performance
- **on chip detection**
  - fluorescence (LSM, FCS, FLIM)
  - UV/VIS absorbance
  - IR spectroscopy
  - Raman scattering
  - (chemo/electro) luminescence
  - thermal conductivity
  - RI variation
- **off chip detection**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





Commercial Solutions

- 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

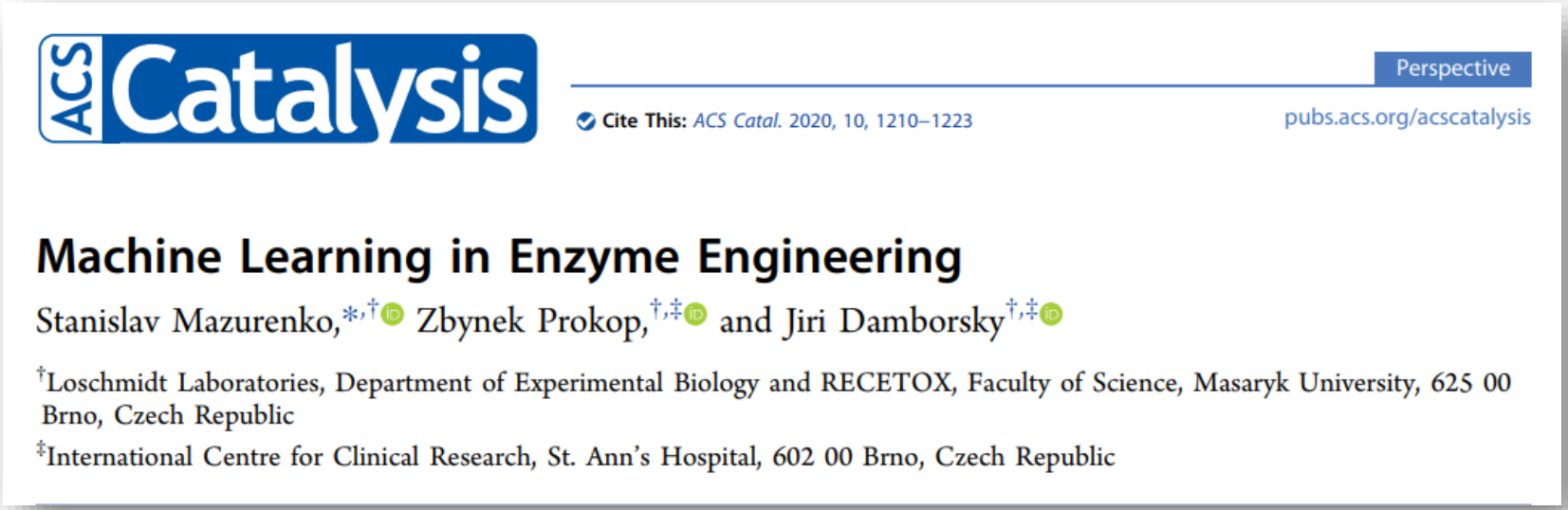
Questions

Reading

- 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)



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Perspective

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Machine Learning in Enzyme Engineering

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