LOSCHMIDT LABORATORIES

Microfluidics – „Lab on a Chip"

Outline

- 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

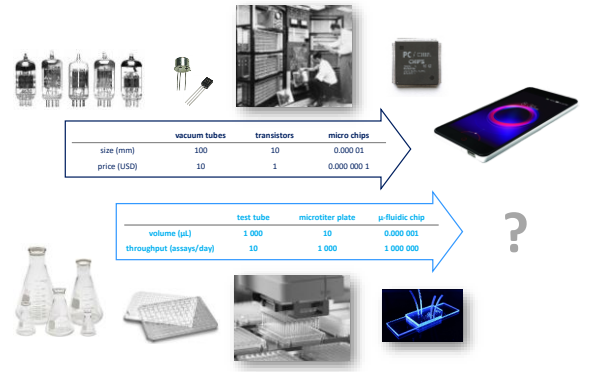
Lab on a Chip Concept

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)

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 (µL)	1 000	10	0.000 001
throughput (assays/day)	10	1 000	1 000 000

?

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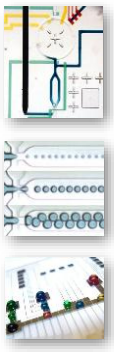
Concepts in microfluidics

- **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



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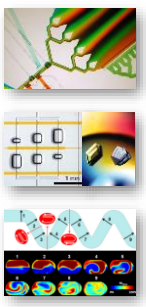
Novel Physics of Micro-Scale

- 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



Nature Biotechnol. 20, 826 (2002) *Appl. Phys. Lett. 83, 4664 (2003)* *PNAS 99, 16531 (2002)*

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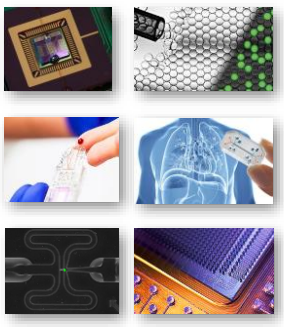
Lab on a Chip applications

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



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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




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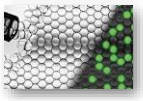
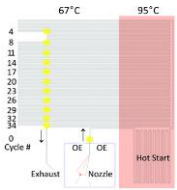
Digital polymerase chain reaction

- **digital PCR (single molecule)**
  - 1 nanoliter droplets
  - 20 000 droplets per run
  - fast heat transfer

Analytical Chemistry, 2008, 80, 8975

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Next-generation sequencing

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

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

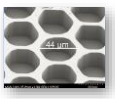
  1 mil. reads/run, 10 USD/Mbase

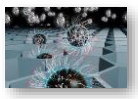
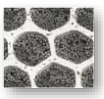
Frederick Sanger

Nobel Prize in 1980





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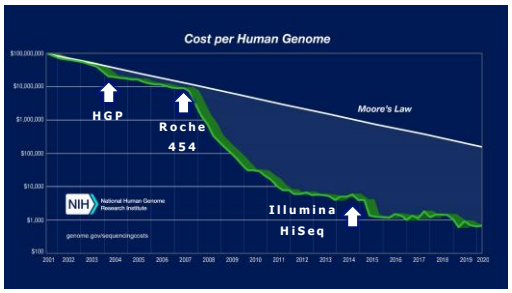
Revolution in DNA analysis



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

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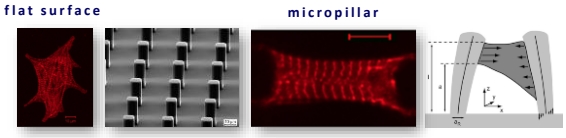
Organ(oid)s on chip

- 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)



Nature 471, 661–665 (2011) *Biophysical Journal 94(5) 1854–1866*

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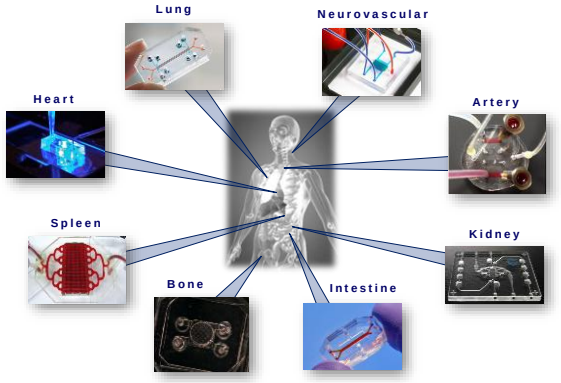
Organs on chip



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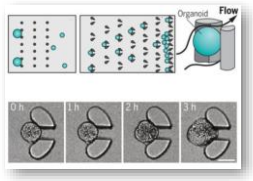

- **can replace** expensive and controversial **animal testing**

Nature 471, 661–665 (2011) *Science 364, 960-965 (2019)*

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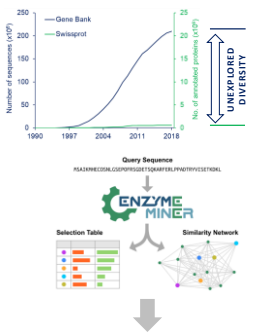
Protein Discovery and Engineering



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

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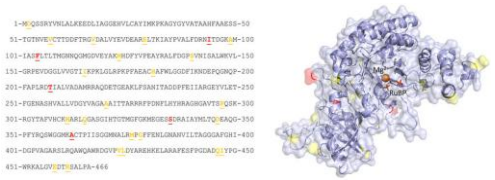
Protein Discovery and Engineering



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

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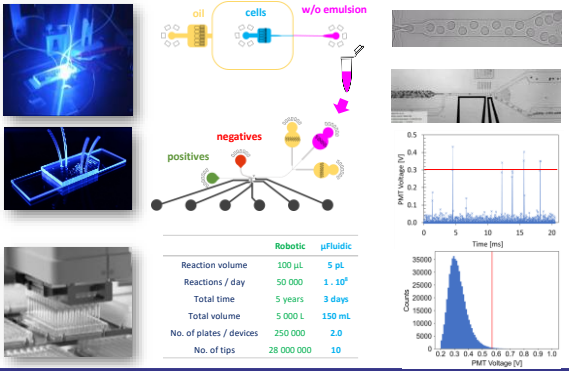
High-throughput on chip sorting (FADS)



	Robotic	µFluidic
Reaction volume	100 µL	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

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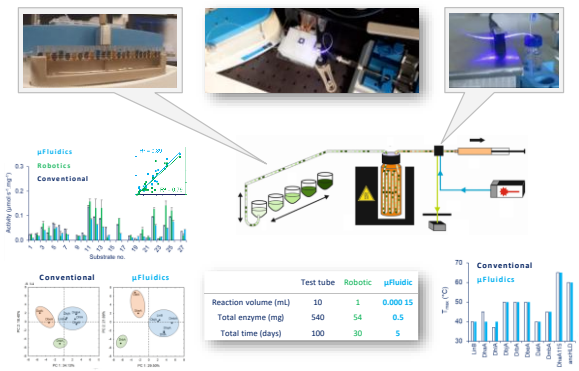
Activity and specificity in µ-droplets



	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

Anal. Chem. 2019, 91: 10008-10015

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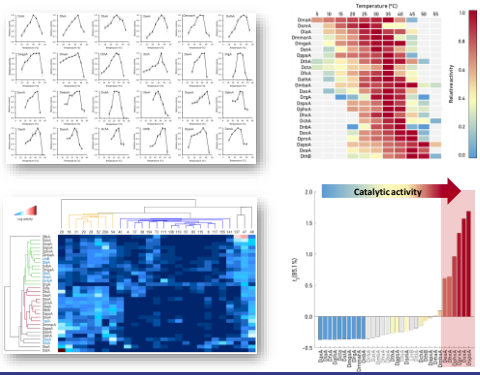
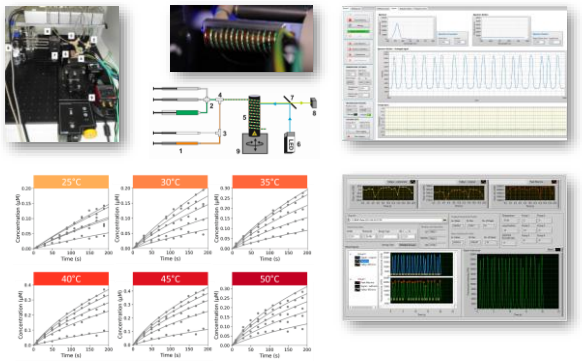
Activity and specificity in µ-droplets



Vasina et al., Chem Catalysis 2: 2704

20

Kinetics and thermodynamics in µ-droplets



Vasina et al., Chem Catalysis 2: 2704

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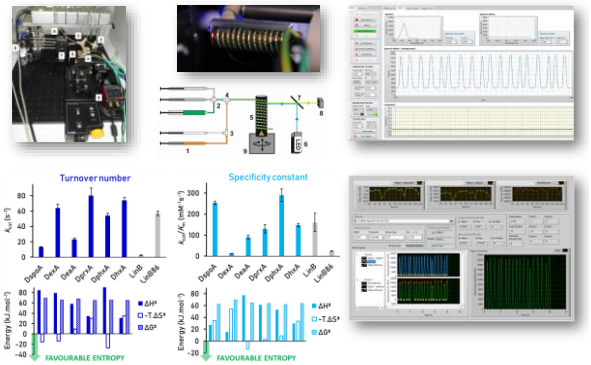
Kinetics and thermodynamics in µ-droplets



Vasina et al., Chem Catalysis 2: 2704

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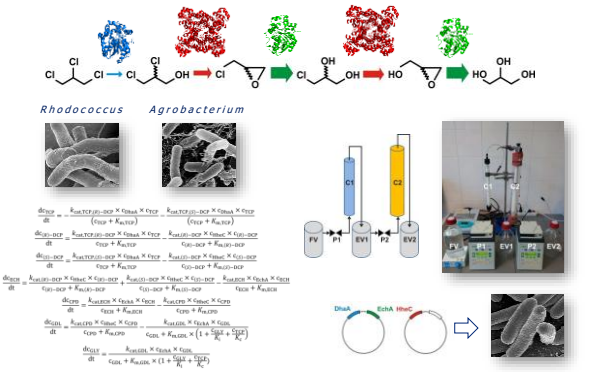
Synthetic biology



ChemBioChem 15: 1891 (2014) *ACS Synth. Biol. 3: 172 (2014)*

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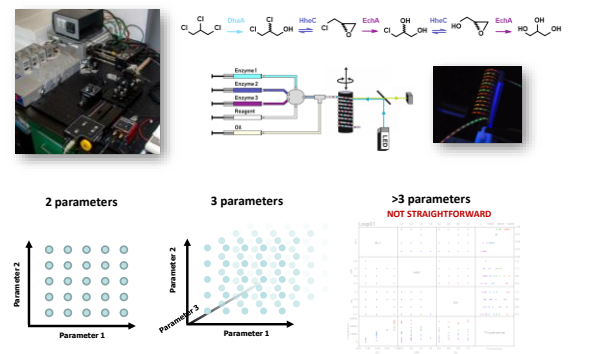
Enzyme cascades in µ-droplets



Small, 11: 4009 (2015)

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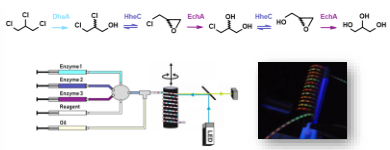
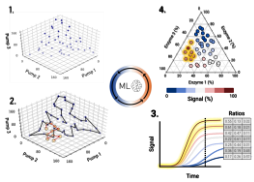
Enzyme cascades in µ-droplets



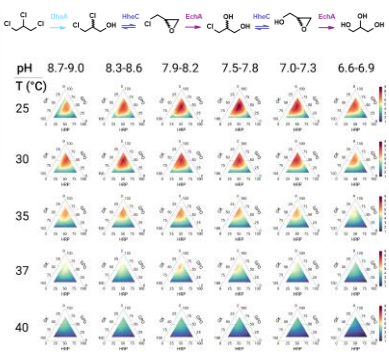
1. Covering of combinatorial space
2. Measurement order definition
3. Signal acquisition
4. Data processing and evaluation
5. Feedback loop (ML-based)



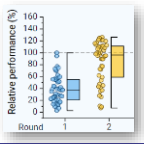
Small, 11: 4009 (2015)

25

Enzyme cascades in µ-droplets



ML-based tracking



Small, 11: 4009 (2015)

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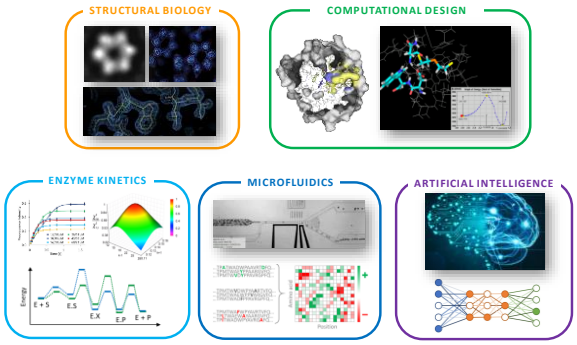
Multidisciplinary modern biotechnology



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Design and fabrication

- **soft lithography** originates from semiconductor industry

DESIGN / MODELING | MASK / MOLD | CASTING / BONDING

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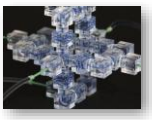
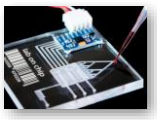
Design and fabrication

- **direct fabrication methods**

3D PRINTING | LASER CUTTING | CNC μ-MILLING





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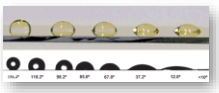
Design and fabrication

- **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





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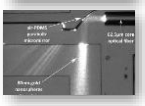
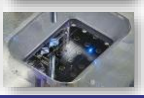
Sensing and detection

- 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

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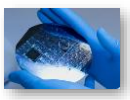
Commercial instruments and services

- customized design and fabrication



- entire technologies


Nature Meth. 10, 1003 (2013) *Nature 499, 505 (2013)*

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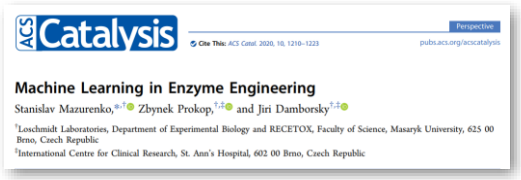
Conclusions

- 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

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**)


ACS Catalysis

Perspective

Cite This: ACS Catal. 2020, 10, 1210−1223

pubs.acs.org/acscatalysis

Machine Learning in Enzyme Engineering

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