# High Throughput On-Chip Synthesis and Screening of Miniaturized Compound Libraries

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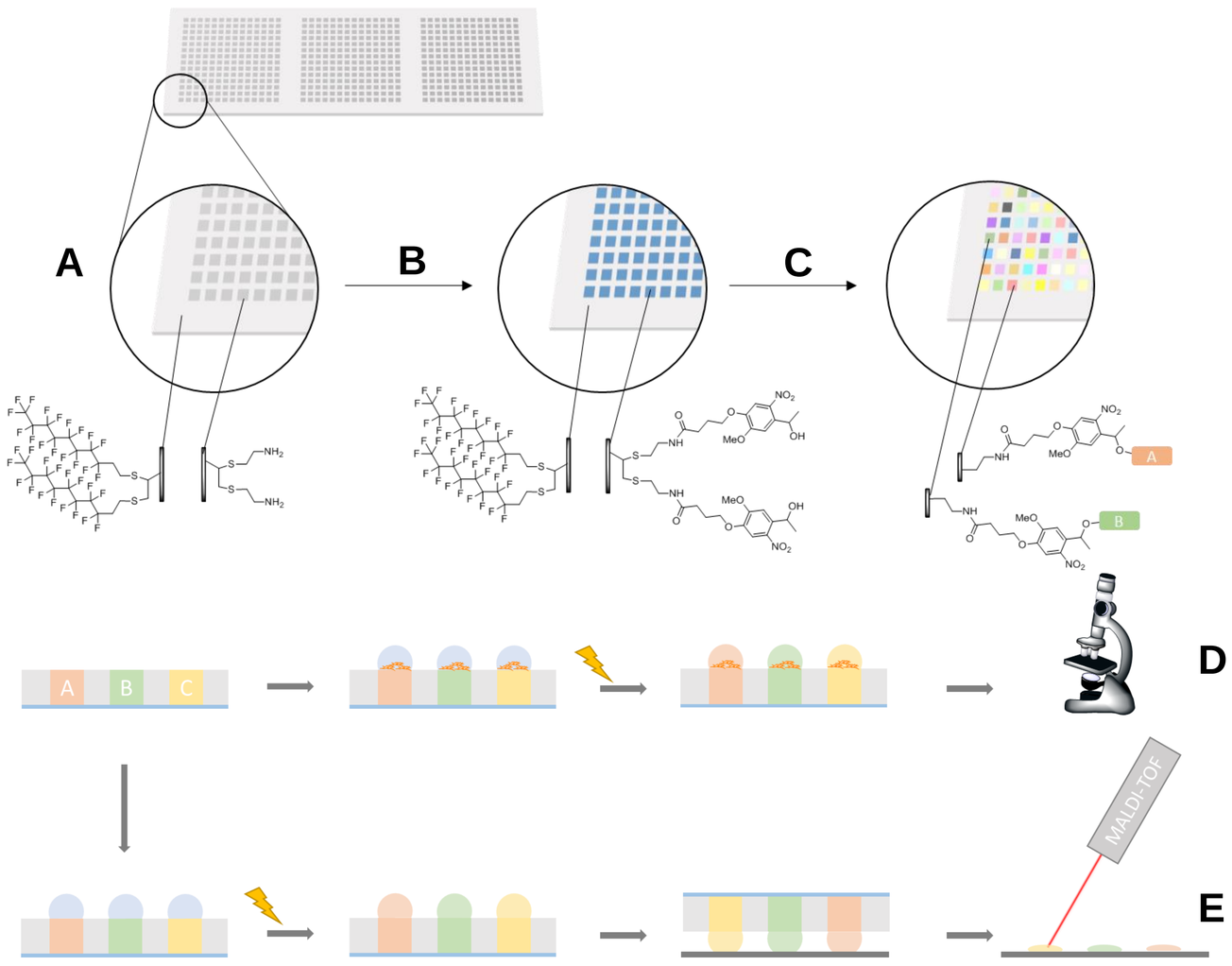
**Introduction**

A microscopic glass slide is coated with a nanoporous polymer and spatially treated via UV-click chemistry to yield an array of distinct, self-forming droplets (droplet microarray, DMA) with volumes down to 20 nL. These droplets are then used to synthesize highly miniaturized, solid phase supported molecule libraries which can be screened directly afterwards in cell-based assays for biological activity.




## Overview

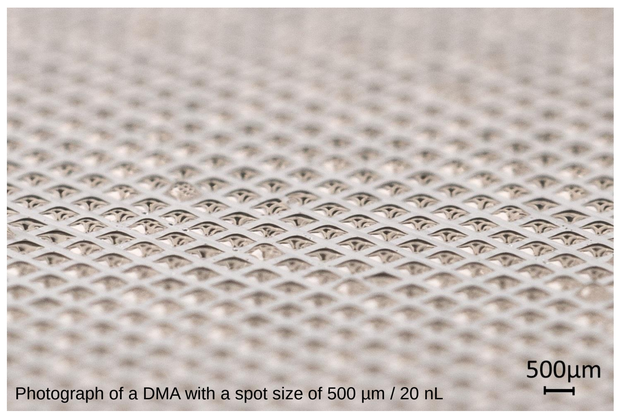
**A)** Hydrophilic spots offer free amine groups on porous surface, divided by superhydrophobic fluorinated borders

**B)** Photocleavable linker is attached in the distinct spots as anchor point for synthesis

**C)** Various reactions can be performed on solid phase, yielding a microarray of compounds

**D)** Spreading of cell suspension spontaneously forms separated nanoliter-sized droplets into which the compound is released by irradiation with UV light at 365 nm to start the screening

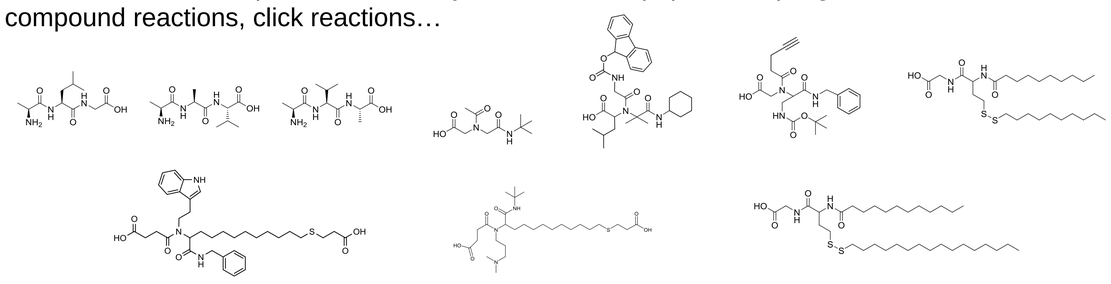
**E)** Applying solvents like DMF or DMSO allow photorelease of the compounds for high throughput MALDI-TOF analysis after sandwiching to a steel substrate with preloaded matrix

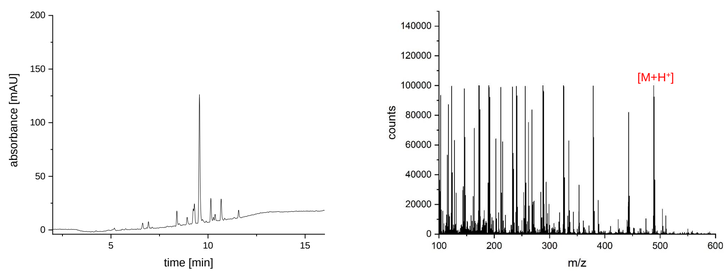


Photograph of a DMA with a spot size of 500 µm / 20 nL

## Solid-Phase Synthesis of Compounds

Various reactions are possible for library formation, like peptide coupling, three and four compound reactions, click reactions…

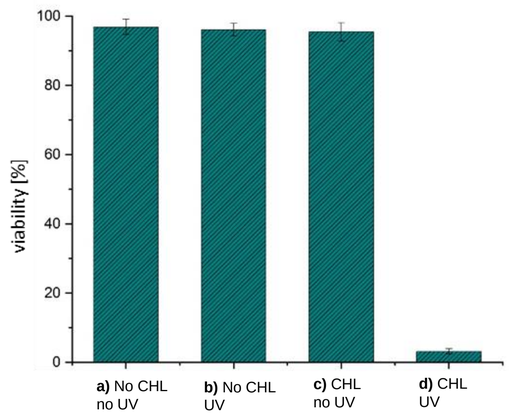


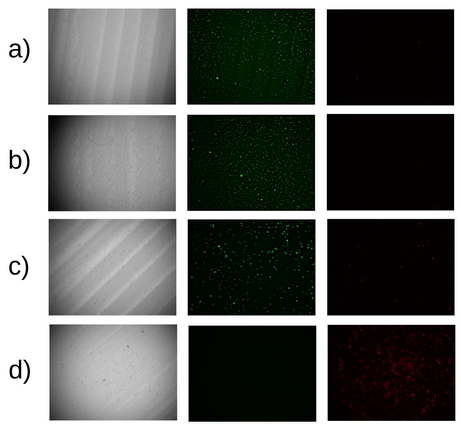



Analysis via LC-MS (left) or MALDI-TOF (right) after cleavage from the surface

## Proof-of-Concept

Chlorambucil (CHL) sensitive CHO-K1 cells were seeded into the spots (with / without CHL) and half of the spots were irradiated with UV light at 365 nm. Only the combination of UV light and linker-bound CHL resulted in a drop of viability.



Microscopic pictures: Brightfield \ CalceinAM \ PI

## Technical Details

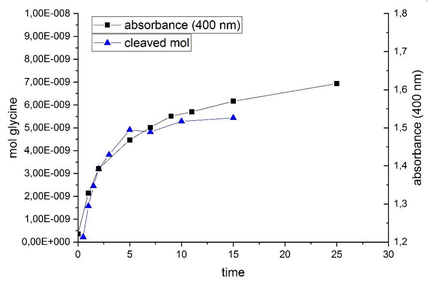
- Glass slide size: 76 mm x 25 mm x 1 mm
- Polymer thickness: 10 – 15 µm
- Pore size: 100 – 500 nm
- Functional group density: up to 1 nmol/mm²
- Accessible concentration in droplet: 10 mM
- Various sizes and shapes for spots possible

| Spot size | Volume | Spots per slide |
|---|---|---|
| 3 mm, round | 5 – 10 µL | 80 |
| 1 mm, square | 100 nL | 588 |
| 500 µM, square | 20 nL | 2187 |
| 350 µM, square | 3 nL | 4563 |

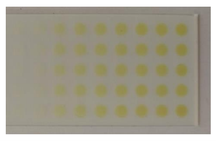
## Controlled Cleavage from Surface

Variation of compound concentration by different irradiation times

top: The change of absorbance at 400 nm of the polymer coating during cleavage, quantified and compared to amount of yielded sample molecule (glycine)




bottom: DMA with round spots (d = 3 mm) after UV-irradiation. Different irradiation times (prolonged from left to right) led to graded intensity of the yellow color.



## Summary

- **Highly miniaturized synthesis of compound libraries**
- **Simple parallelization of reactions**
- **Straightforward connection to on-chip biological and cellular screening**
- **Allows spatial, temporal and quantitative control over screening conditions**
- **High-Throughput, short innovation cycles**

**References**

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