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

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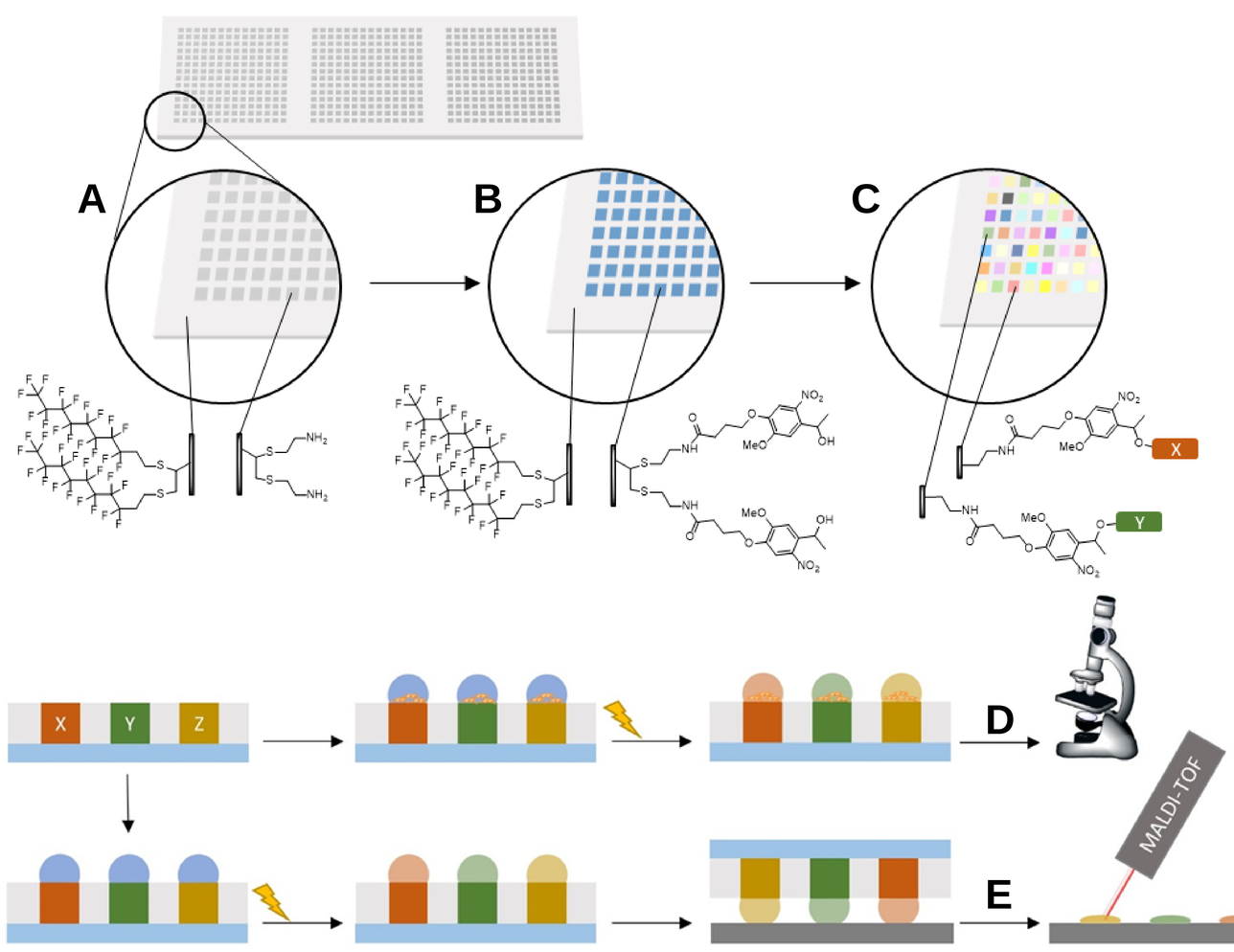
Introduction

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 several nanoliters. These droplets are then used to synthesize highly miniaturized molecule libraries which can be screened directly afterwards in cell-based assays for biological activity.



Overview

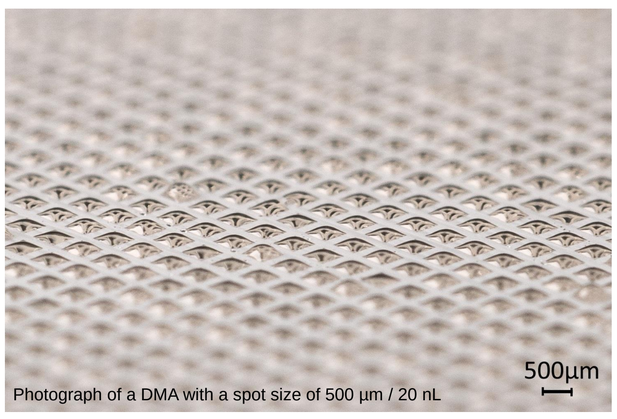
A) Hydrophilic spots offer free amine groups on porous surface, divided by superhydrophobic fluorinated borders

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

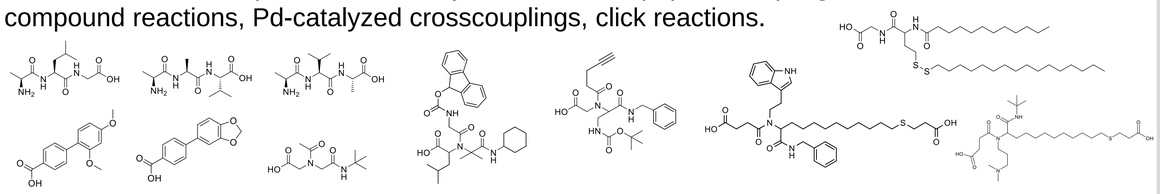
E) Alternatively to cell screenings, the array can be copied to a MALDI substrate by simple sandwiching and analysed by mass spectrometry

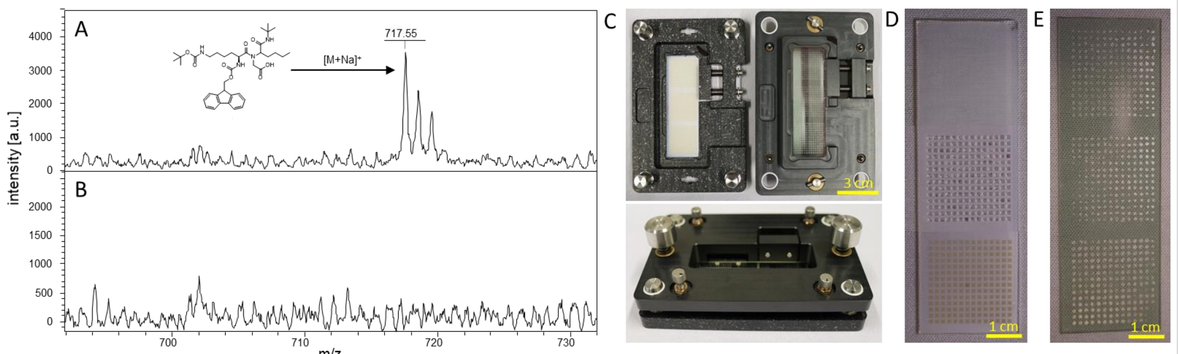


Photograph of a DMA with a spot size of 500 µm / 20 nL

Solid-Phase Synthesis of Compounds and MALDI-TOF

Various reactions are possible for library formation, like peptide coupling, three and four compound reactions, Pd-catalyzed crosscouplings, click reactions.

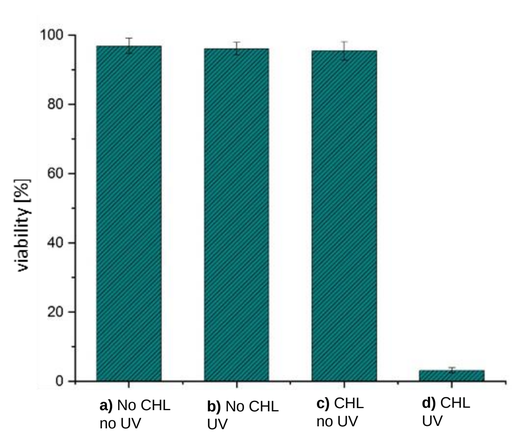


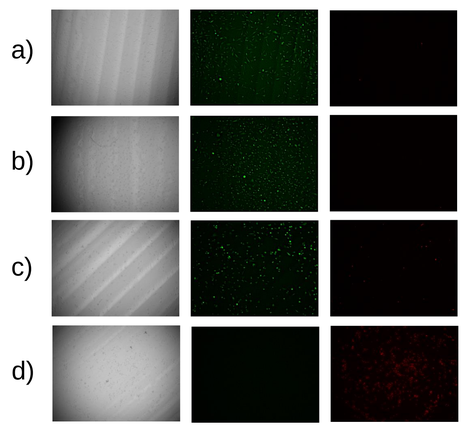


After photorelease into the droplets and addition of matrix, the array (**D**) can be copied to a MALDI substrate (ITO slide, **E**) by simple sandwiching (**C**) for mass spectrometry (detected product **A** and background **B**)

Proof-of-Concept Cell Experiments

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.





Fluorescence microscopy pictures: Brightfield \ CalceinAM \ PI

Technical Details

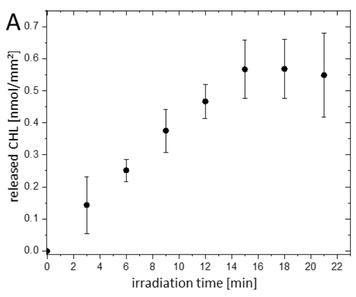
- Glass slide size: 76 mm x 25 mm x 1 mm
- Polymer thickness: 10 – 15 µm
- Pore size: 100 – 500 nm
- Functional group density: up to 1 nmol/mm²
- Accessible concentration in droplet: 1 mM
- Various sizes and shapes for spots possible

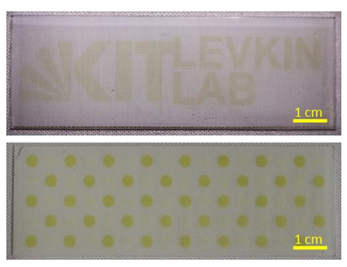
Spot size	Volume	Spots per slide
3 mm, round	5 – 10 µL	80
1 mm, square	100 nL	588
500 µM, square	20 nL	2187
350 µM, square	3 nL	4563

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**

Controlled Cleavage from Surface





UV-triggered release of the compounds can be quantitatively and spatially controlled:

A release of chlorambucil by irradiation with UV light at 365 nm at different timepoints

B spatial release of the compound by irradiation through photomasks (top: 2688 square spots per slide, bottom: 80 round spots per slide)

References

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