

MINIATURIZED ON-CHIP SYNTHESIS FOR HIGH-THROUGHPUT CELL SCREENING

Marius Brehm¹, Alisa Rosenfeld¹, Alexander Welle², Vanessa Trouillet³, Stefan Heissler², Pavel A. Levkin¹

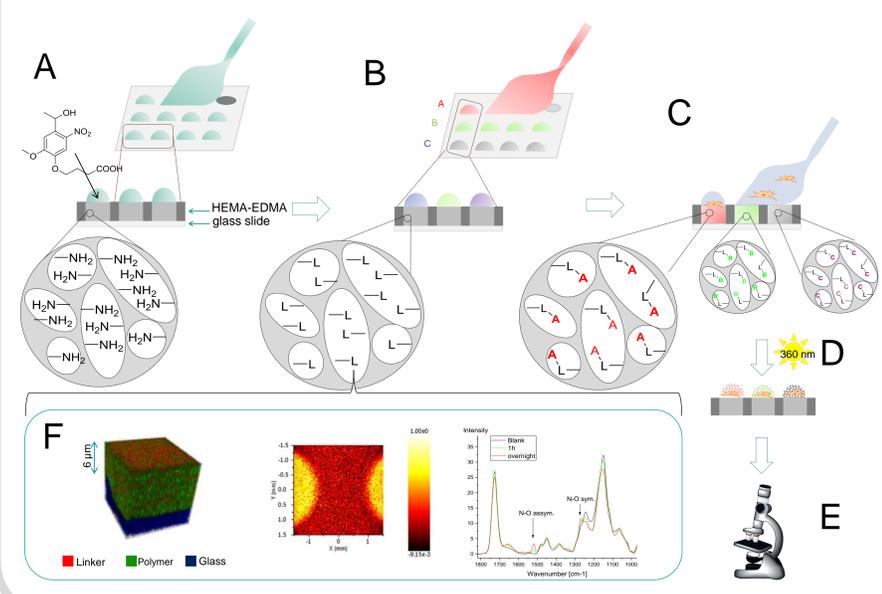
¹ Karlsruhe Institute of Technology (KIT), Institute of Toxicology and Genetics (ITG), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

² Karlsruhe Institute of Technology (KIT), Institute of Functional Interfaces (IFG), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

³ Karlsruhe Institute of Technology (KIT), Institute for Applied Materials (IAM), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

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

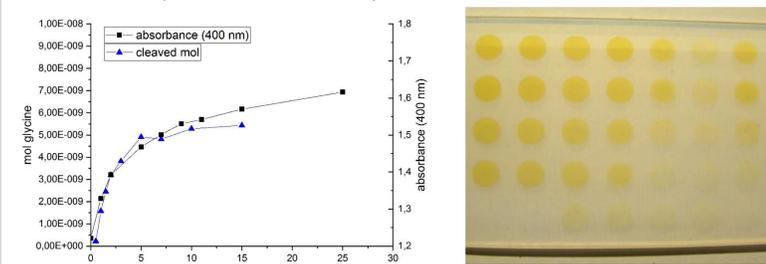
A) Attachment of photolinker to the amino groups grafted exclusively in hydrophilic spots
B) Reactants are added into the distinct droplets to create a combinatorial library of substances, attached to the linker
C) After the synthesis is done, cells are seeded by applying cell suspension via rolling droplet onto DMA
D) starting the screening on demand by irradiation with UV light of 360 nm
E) Read-out
F) Analysis of the substrate:

- TOF-SIMS depth profiling shows equal distribution of linker
- XPS image of superhydrophobic border assigned to amide and nitro group
- ATR-IR to monitor reaction progress

G) Close-up photograph of DMA spot size: 500 μm / 20 nL

Controlled cleavage from surface

Variation of compound concentration by different irradiation times



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

Left: change of absorbance during cleavage, quantified and compared to concentration of yielded sample molecule (glycine)

Technical Details

- Glass slide size: 76 mm x 25 mm
- Polymer thickness: 10 – 15 μm
- Pore size: 100 – 500 nm
- Functional group density: up to 2 nmol / mm²
- Maximum accessible concentration: 50 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

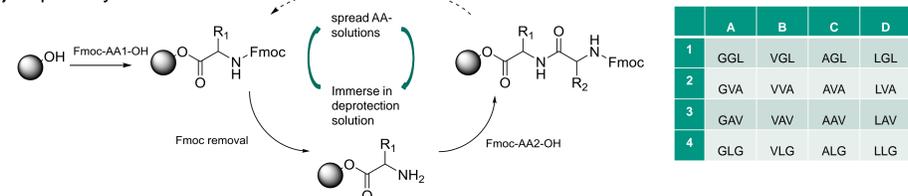
- Highly miniaturized synthesis of compound libraries
- Simple parallelization of reactions
- Straightforward connection to on-chip biological and cellular screening
- Allows spatial, temporal and quantitative control over screening conditions
- High-Throughput, short innovation cycles

References

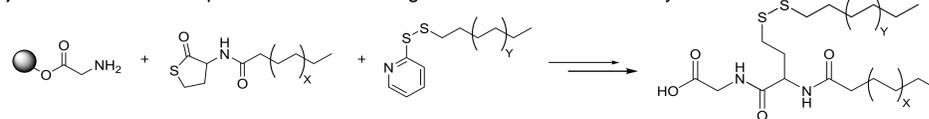
- Feng, W., Ueda, E., Levkin, P.A. *Adv. Mater.*, **2018**, *30* (20), 1706111.
Molla, M.R., Böser, A., Rana, A., Schwarz, K., Levkin, P.A. *Bioconjugate Chem.*, **2018**, *29* (4), 992–999.
Geyer, F.L., Ueda, E., Liebel, U., Grau, N., Levkin, P.A. *Angew. Chem.*, **2011**, *50* (36), 8424–8427.
Molla, M.R., Böser, A. and Levkin, P.A., *Organische Moleküle zur Verwendung als Transfektionsmittel*, Patent application no.: 102018104783.5. München: Deutsches Patent- und Markenamt.

Solid-phase synthesis of compounds

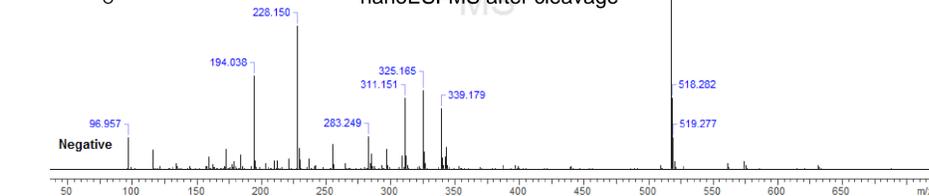
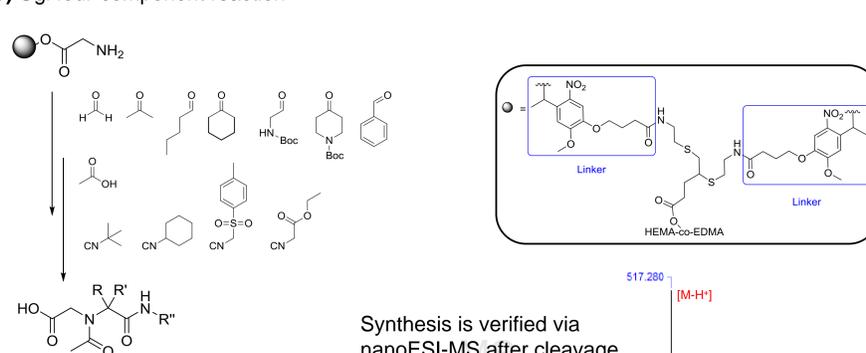
A) Peptide synthesis



B) Direct formation of liposomes after cleavage via Thiolactone chemistry



C) Ugi four-component reaction



Benz, M., Levkin, P.A., Molla, M.R., Rosenfeld, A., Brehm, M. Karlsruhe Institut für Technologie. Verfahren zur Behandlung von mindestens einer Zelle mit einem chemischen Syntheseprodukt in einem Microarray. Patent application no.: 102018002880.2. München: Deutsches Patent- und Markenamt.

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