

Purification of High-Complexity Peptide Microarrays by Spatially Resolved Array Transfer to Gold-Coated Membranes

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Arrays of small molecules on functional solid supports are important tools in modern research.^[1] With them a high number of compounds can be simultaneously screened for interactions with a sample. It is an ongoing challenge in chemistry and material sciences to steadily increase the number of compounds per area of the solid support. Regarding the natural complexity of proteins and peptides, these molecules are a prime example of where high-throughput methods are needed to increase the screening efficiency.^[2] However, the synthesis of complex protein and peptide arrays is demanding because natural proteins and peptides are composed of at least 20 different amino acids implying a high combinatorial diversity. Efforts have been taken to synthesize peptide arrays by lithographic methods.^[3] However, this approach is still not marketable due to expensive equipment, nonstandard building blocks, labor-intensive protocols, and low synthesis efficiencies.^[4] In fact, the first practicable and fully combinatorial approach to produce complex arrays of synthetic peptides was the so-called SPOT synthesis.^[5] By means of this spatially resolved spotting technique, which had been state-of-the-art for more than a decade, arrays of up to 25 different peptide spots per cm² can be directly synthesized on a modified cellulose sheet.^[6] Over the last years, our group has significantly improved the feature density of complex peptide arrays introducing a new microparticle-based synthesis approach. Customized arrays with about 800 peptide spots per cm² can be routinely synthesized using a specialized laser printer and up to 40 000 spots per cm² are possible on

a custom-designed complementary metal oxide semiconductor (CMOS) microchip.^[7]

Despite of the progress in array complexity, a fundamental problem inheres in any synthesis which is performed in situ on the solid support: Due to imperfect coupling reactions or difficult amino acid sequences, the number of incomplete peptides increases with the peptide length.^[8] The efficiency of the SPOT technique has been reported to range from below 40% to more than 92% which clearly indicates fluctuations depending on the length and sequence of the respective peptides.^[9] The classic protecting group strategy prevents peptides with missing amino acids but, nonetheless, shorter peptide artefacts can lead to false-positives or false-negatives in binding assays and reduce the sensitivity of the assay. As a consequence, methods for the purification of peptide arrays have become subject to research. Peptides from arrays synthesized by the SPOT technique can, for example, be cleaved by dry aminolysis in ammonia vapor, extracted from punched-out membrane pieces and re-spotted on a solid support.^[10] This method yields arrays with purified peptides but is tedious and expensive.

Here, we present the first method which is capable of directly purifying high-complexity peptide arrays. The method is based on the transfer of the entire microarray from the synthesis support to a gold-coated membrane, whereby only the full-length array members are immobilized again (**Scheme 1**). Peptide artefacts and impurities are removed in situ (i.e., without the loss of spatial information provided by the synthesis).

To synthesize peptide arrays for the purification experiments, we used both available microparticle-based synthesis approaches: the laser printer technique and microchip-based synthesis. Thus, either standard microscopy glass or custom-designed microchips were applied as a support material. We coated both types of surfaces with 50–60 nm thick polymeric films consisting of 10 mol% poly(ethylene glycol)methacrylate (PEGMA) and 90 mol% polymethylmethacrylate (PMMA) by surface initiated atom transfer radical polymerization (siATRP) as described in the Supporting Information and in the literature.^[11] We routinely esterified the alcohols of the poly(ethylene glycol) (PEG) side-chains with three β -alanine residues to insert a short molecular spacer between the PEG brushes and the peptides.^[11a] To allow for the peptide cleavage after the array synthesis, we augmented the polymer coating with the acid-labile Rink amide (RAM) linker because of its rigidity, the reported good cleavage efficiency and the opportunity to simultaneously cleave peptides and side-chain protecting groups in a single step.^[12] The RAM linker was coupled to the amino-terminated

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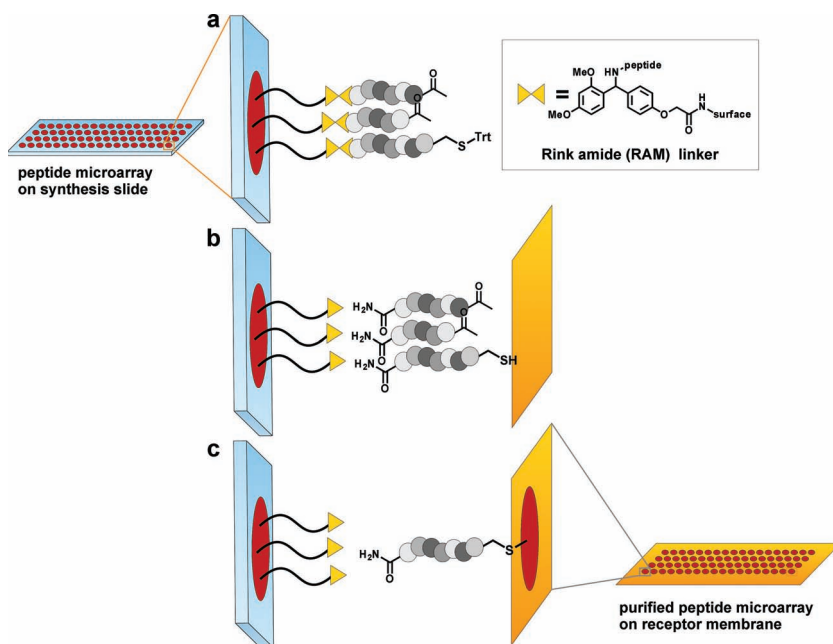
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Scheme 1. Schematic of the peptide-array purification by specific transfer to a gold-coated membrane. a) Only full-length peptides obtain an N-terminal cysteine (Trt = trityl protecting group). b) TFA/toluene treatment simultaneously cleaves the RAM linker and side-chain protecting groups. c) Only cysteine-bearing peptides (full-length array members) couple to the gold-coated membrane, whereas acylated fragments and impurities are removed.

PEG surfaces in up to 90% yield as determined by UV–vis spectrometry upon fluorenylmethoxycarbonyl (Fmoc) cleavage.^[11a] The density of amino groups on RAM linker terminated surfaces prior to the peptide synthesis was in the range of 1.5–2.0 nmol per cm².

Afterwards, we prestructured the surfaces with glycine spots (laser printer approach) or alanine spots (microchip approach) in a defined pattern. In our synthesis strategies, Fmoc protected and orthopentafluorophenyl (Opfp)-activated amino acid compounds were embedded in small polymeric microparticles. Each sort of amino acid microparticles was selectively addressed onto the synthesis support either using a custom-built laser printer or by electric field patterns generated on a CMOS microchip which is described in more detail elsewhere.^[7b,13] Currently, the highest achievable resolution in the routine peptide synthesis amounts to about 800 different peptide spots per cm² with the laser printer (about 281 000 peptide spots on a 19.1 cm × 19.1 cm synthesis area) and 10 000 per cm² on the latest (optimized) microchip generation.^[14] The coupling reaction between amino groups on the surface and amino acids from the microparticles takes place in distinct reaction spheres as soon as the solid polymer matrix is melted at temperatures around 90 °C (Supporting Information, Scheme S1). In the laser printer approach, we chose a rather simple pattern of 180 peptide spots per array.

biological “key/lock” systems such as biotin/avidin. We therefore chose the thiol moiety in cysteine as a specific head group because the adsorption of thiolated molecules to gold-coated surfaces in defined self assembled monolayers (SAMs) has been reported to be catalyzed in TFA acidic media.^[17] To equip only the full-length array members with a thiol head group, cysteine had to be introduced in the last synthesis step. In a second microparticle-based synthesis step we thus coupled

Each spot printed with the glycine microparticles was approximately 512 μm in diameter with 1024 μm center-to-center spacing (Figure 1a,b). On the current (optimized) microchip, we assembled alanine microparticles on each of the 16 384 pixel electrodes. The electrodes on the chip have a dimension of 84 μm × 84 μm pixels with 100 μm pitch (Figure 1d,e) which is equivalent to a microarray resolution of 10,000 peptides per cm².^[15] After routine coupling, washing, blocking, and deprotection steps (Supporting Information, Scheme S1), we coupled the pre-synthesized human influenza hemagglutinin A (HA) peptide Fmoc-NH-GGGYPYDVPDY-AGGG-OH to all glycine/alanine spots from solution using standard activation chemistry. The HA wild type sequence (YPYDVPDYA) which has been extensively used in protein engineering as a general protein tag^[16] was here elongated with an N- and C-terminal GGG spacer to ensure good accessibility for the specific antibody to HA in the later detection. To purify the peptide array from synthesis artefacts by transferring it to a second solid support a specific bond formation was intended. The use of TFA in organic solvents to cleave the RAM linker *a priori* excluded

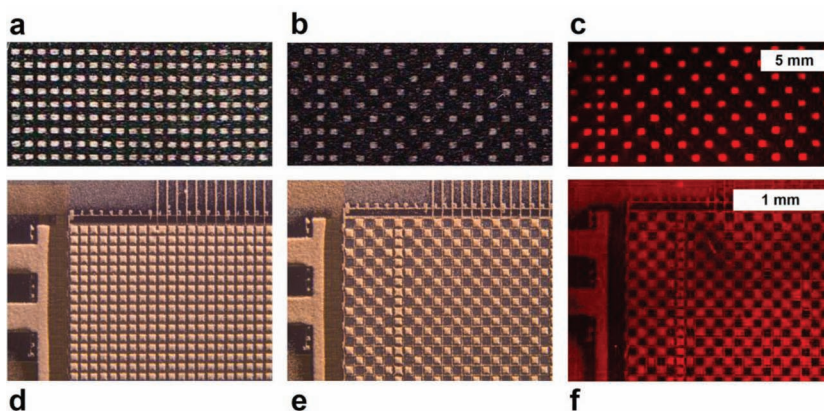


Figure 1. Peptide-array synthesis and immunostaining on the gold-coated membrane after the transfer. a) Glycine microparticles (yellowish) printed onto the synthesis support with the laser printer. b) Cysteine microparticles (yellowish) printed after HA epitope coupling (not shown). c) Immunostaining of the transferred peptide array on the gold-coated membrane with the ATTO 700-labeled monoclonal antibody to HA. d) Alanine microparticle deposition pattern on the CMOS microchip. e) Cysteine microparticle deposition pattern after HA epitope coupling to all alanine spots (not shown). f) Immunostaining of the transferred peptide array on the gold-coated membrane with the Cy5-labeled monoclonal antibody to HA.

cysteine to only select HA peptide spots in a distinct pattern. At that point, the side-chains of the amino acid residues, including the N-terminal cysteine, were still shielded with the standard acid-labile protecting groups.

Regarding the receptor surface for the array transfer we favored an application of flexible polymer membranes because we considered two rigid slides with a fluid film in between to give rise to lateral diffusion. Moreover, two slides would be hard to separate again due to capillary forces. In this context, polyvinylidene fluoride (PVDF) membranes show outstanding properties regarding their mechanical robustness, thermal stability, and chemical resistance.^[18] We found commercially available PVDF membranes with average pore sizes of 100 nm (Merck Millipore Durapore) and 450 nm (Merck Millipore Immobilon-P or Immobilon-FL) to resist the TFA acidic conditions of the peptide cleavage and side-chain deprotection. We sputter-coated these membranes with gold (Supporting Information, Figure S1) to enable specific coupling of peptides containing an N-terminal cysteine. Due to their flexibility, we expected the gold-coated membranes to be easily contacted with the array supports and separated from them again after the peptide-array transfer. Moreover, we considered the membrane texture to provide a reservoir of cleavage medium and to suppress trapping of air bubbles which would hamper the spatially resolved transfer of peptides.

In a first proof-of-principle experiment we transferred the synthesized HA peptide microarrays to a PVDF membrane coated with an approximately 20–30 nm thick gold layer. To ensure good contact between receptor membrane and synthesis slide we placed the gold-coated membrane on a filter paper soaked with the transfer medium to provide a reservoir of transfer medium and to generate a TFA atmosphere in the petri dish (Supporting Information, Figure S2). We then pressed the synthesis slide face-down onto the receptor membrane and weighted it for a transfer time of 30 min which we determined to be a reasonable time frame to transfer a considerable amount of peptide (Supporting Information, Figure S3). The cleavage solution was 50 vol% TFA in toluene. After the peptide transfer, we incubated the receptor membrane in an excess of the cleavage solution for additional 30 min to ensure complete removal of side-chain protecting groups. We then placed the receptor surface in a 2 mM solution of *O*-(2-mercaptoethyl)-*O'*-methylhexaethyleneglycol (EG₇-SH) in ethanol overnight to render the remaining parts of the gold-coated membrane protein-repelling and more hydrophilic.^[19] Afterwards, we routinely blocked the membrane in Rockland Blocking Buffer for Near Infra Red Fluorescent Western blotting (Rockland buffer, Rockland Immunochemicals Inc.) for 30 min to additionally suppress nonspecific protein adsorption, especially on the non-coated reverse side of the membrane which is not coated with the EG₇-terminated thiol film. We subsequently immunostained the transferred peptides from the laser printer

array with the ATTO 700-labeled monoclonal antibody to HA and scanned the membrane with the Odyssey Infrared Imager (Odyssey Imager, LI-COR Inc.) at 21 μm resolution. The array transfer from the microchip surface was, in contrast, immunostained with the Cy5-labeled monoclonal antibody to HA and scanned with the GenePix 4000B imager (Molecular Devices, LLC) due to the higher resolution of this scanner (5 μm). To be able to scan with the GenePix 4000B imager, we had to glue the membrane to a microscopy slide using spray adhesive. The scans showed that, in both approaches, only the set of cysteine-terminated peptide spots was transferred (Figure 1c,f). We found lateral diffusion to be negligible even if the microarray resolution accounted for 10 000 peptide spots per cm² in the microchip-based approach (Figure 1c and Figure 2). However, in the microchip approach we had to use the 100 nm pore size membrane (Merck Millipore Durapore) because otherwise the array tended to blur. In both approaches, the fluorescence detection on the gold-coated membranes featured strong signals and low background staining. The experiment showed selective transfer of cysteine-terminated HA (HA-SH) peptides, whereas the HA peptides without a thiol group (HA-acyl) were not captured on the receptor membrane. Since cysteine can be selectively attached to full-length peptides in the last step of any in situ synthesis the described findings highlight a method to purify peptides in high-complexity arrays. Regarding the receptor membrane, we found gold-coated PVDF to be a stable and biologically compatible material on which we could routinely perform immunostainings in aqueous buffer solutions, especially, when the membrane was rendered more hydrophilic upon EG₇-SH treatment.

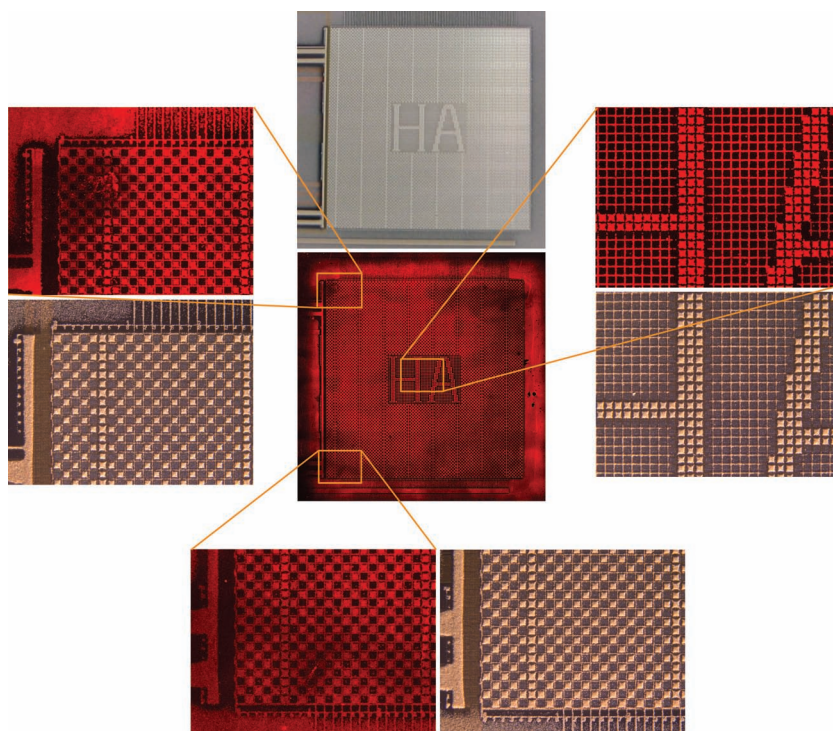


Figure 2. Detailed comparison of the cysteine microparticle deposition pattern (yellowish) on the microchip (grey) with the fluorescence pattern of HA peptides on the gold-coated PVDF membrane after the microarray transfer.

In summary, our method provides a fast and efficient way to purify high-complexity peptide arrays. Only two additional steps are required in the combinatorial synthesis following the N^α-Fmoc strategy: First, the standard solid support is reacted with the RAM linker to introduce a cleavable anchor group. Secondly, an N-terminal cysteine is added to the peptides in the last synthesis step. Due to the protecting-group strategy and routine acylation steps, only full-length peptides obtain this thiolated amino acid which is implicitly required for the purification effect in the transfer. However, library members should be devoid of cysteine except for the N-terminal head group which can be achieved by quasi-isosteric cysteine-alanine replacement or, with less microenvironmental influence, by cysteine-serine substitution.^[20] Cleavage from the synthesis support, transfer, and rebinding to the membrane is achieved in a single step which allows for a fast processing and for a simple automation of the array purification. We demonstrated specific transfer of the microarray down to a resolution of 10 000 peptide spots per cm². Even at such high complexities the array quality was not diminished by lateral diffusion suggesting that arrays of even higher resolution might be purified by this method in the future. Aside from the purity of array members, quantitative analyses using *in situ* synthesized microarrays also suffer from variations in the peptide density which is due to the sequence dependence of the peptide yield. However, if the number of binding sites on the gold-coated membranes can be adjusted to the lowest assumed yield we expect our method to be also capable of normalizing the peptide density over the entire microarray. A limitation of binding sites can simply be achieved by preincubation of the membrane in differently concentrated solutions of EG₇-SH as demonstrated in Figure S4 in the Supporting Information. High-purity peptide arrays with normalized peptide densities will pave the way for demanding peptide-protein interaction studies in high-throughput which will be of immense merit for proteomics and biomedical research. Furthermore, the method could be adapted to transfer peptide arrays to specialized sensor surfaces which could then be used for the label-free detection of binding events in high-throughput. In particular, methods such as surface plasmon resonance imaging (SPRI) require analytes to be immobilized on metal-coated surfaces.^[21]

Experimental Section

Synthesis Surface Preparation: Detailed protocols for the synthesis surface preparation can be found in the Supporting Information or in the literature.^[11b,11c]

RAM Linker Coupling: A 0.1 M solution of the Fmoc RAM linker (1059 mg) in anhydrous DMF (20 mL) was prepared in a nitrogen flask. The linker was activated by adding anhydrous *N,N'*-diisopropylcarbodiimide (DIC, 369 μL), stirring for 5 min, then adding anhydrous *N*-methylimidazole (NMI, 317 μL). The reaction mixture was directly added to the synthesis surface which was placed in a petri dish inside a desiccator. The desiccator was evacuated and flooded with argon 3 times and left over night. Each coupling step was followed by standard washing steps and routine capping in 10 vol% acetic anhydride (Ac₂O) and 20 vol% *N,N*-diisopropylethylamine (DIPEA) in DMF for 30 min, as well as Fmoc deprotection with 20 vol% piperidine in DMF for 20 min as described in the Supporting Information and in the literature.^[7a,13,22] The coupling efficiency was determined by UV-vis spectrometry upon

Fmoc deprotection as described in the Supporting Information and in the literature.^[11a]

Microparticle Deposition and Coupling Reaction: Microparticles containing the orthopentafluorophenyl (Opfp) activated and Fmoc protected amino acids were selectively printed to the linker-modified surfaces with our custom-built laser printer or addressed onto the microchip as extensively described in the literature.^[7,14] First, glycine or alanine microparticles were addressed to each spot/pixel (Figure 1a,d). After HA peptide coupling (see below) cysteine microparticles were deposited in a selected pattern (Figure 1b,e). After each deposition step, the solid support was put in an oven and heated at 90 °C for 90 min under argon atmosphere to melt the particles and initiate the coupling reaction. After cooling to room temperature, the surface was processed as described in the Supporting Information (routine synthesis cycle) and in the literature.^[7a,13,22]

HA Peptide Coupling: A 1 mM solution of Fmoc-NH-GGGYPYDVPDYACGG-OH (solid phase synthesized, fully side-chain protected, HPLC purified) in anhydrous DMF (500 μL) was prepared. The peptide was activated by adding a solution (500 μL) of 1-hydroxybenzotriazole (HOBt, 10 mM) and *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU, 10 mM), stirring for 5 min and adding DIPEA (1.7 μL). The surface was placed in a petri dish inside a desiccator, brought to argon atmosphere and covered with the prepared solution. After 20 h the surface was washed 3 times for 5 min each with DMF, 2 times for 5 min each with methanol, rinsed with acetone, then dried in a stream of argon. The coupling step was followed by routine capping with Ac₂O and Fmoc deprotection as described above. A selected cysteine microparticle pattern was subsequently deposited as described in the microparticle deposition section.

Preparation of the Gold-Coated PVDF Membrane: A piece of Immobilon-P, Immobilon-FL, or Durapore membrane was placed in the MED 020 Modular High Vacuum Coating System (Bal-Tec AG). The machine was evacuated to less than 2×10^{-4} mbar and argon pressure was set to 5×10^{-2} mbar. Gold sputter-coating was executed at 60 mA for 12–15 s which was equivalent to 20–30 nm gold thickness on plane silicon surfaces according to a previous instrument calibration.

Microarray Transfer to Gold-Coated Membrane: A piece of gold-coated PVDF was put on top of a circular filter paper inside a petri dish. Filter paper and membrane were soaked with 50 vol% TFA in toluene (500 μL). The array was immediately put on the membrane face down, weighted, and left on the membrane for 30 min. After the transfer, membrane and array were carefully separated. The membrane was washed 5 times for 5 min each with toluene, 2 times for 2 min each with ethanol, then immediately incubated in the EG₇-SH solution (see below).

Blocking Prior to the Immunoassay: A 2 mM solution of EG₇-SH in ethanol was prepared. The membrane was directly immersed in this solution and left for 24 h. After washing 5 times for 2 min each with ethanol and 2 times for 2 min each with water, the membrane was incubated in Rockland buffer for 60 min to additionally block the reverse side. The membrane was washed in phosphate buffer saline (0.15 M) with 0.05 vol% Tween-20 (PBS-T) for 5 min and directly immersed in the staining solution.

Immunostaining: The monoclonal mouse 12CA5 IgG antibody to HA was either labeled with the ATTO 700 dye or the Lightning-Link Cy5 dye using commercial protein labeling kits (amine-reactive kits from ATTO-TEC GmbH and Innova Biosciences Ltd. respectively) and the protocols as supplied by the manufacturers. A 1:1000 dilution of the respective antibody in PBS-T (5 mL) with additional 0.1 vol% Rockland buffer was freshly prepared. The surfaces were rocked in this solution for 60 min, washed five times for 5 min each with PBS-T, and two times for 2 min each with water. Before the scan the surfaces were carefully dried in a stream of compressed air. To scan membrane samples with the GenePix 4000B imager they were glued to a standard microscopy slide using spray adhesive. The readout was performed at 635 nm excitation with 33% device power, +130 μm focus offset and at 5 μm resolution. Scans with the Odyssey Imager were performed at 685 nm excitation with 21 μm resolution and a detector intensity of 6.0 while the membranes were pinned down with a 20 cm × 20 cm glass plate made of low fluorescence glass.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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- [1] a) C. N. LaFratta, D. R. Walt, *Chem. Rev.* **2008**, *108*, 614; b) M. Uttamchandani, S. Yao, in *Small Molecule Microarrays*, Vol. 669, (Eds: M. Uttamchandani, S. Q. Yao), Humana Press, New York **2010**, 1.
- [2] M. P. H. Stumpf, T. Thorne, E. De Silva, R. Stewart, J. A. Hyeong, M. Lappe, C. Wiuf, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6959.
- [3] a) S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, D. Solas, *Science* **1991**, *251*, 767; b) J. P. Pellois, X. Zhou, O. Srivannavit, T. Zhou, E. Gulari, X. Gao, *Nat. Biotechnol.* **2002**, *20*, 922.
- [4] X. Gao, J. P. Pellois, Y. Na, Y. Kim, E. Gulari, X. Zhou, *Mol. Diversity* **2004**, *8*, 177.
- [5] R. Frank, *Tetrahedron* **1992**, *48*, 9217.
- [6] F. Breitling, A. Nesterov, V. Stadler, T. Felgenhauer, F. R. Bischoff, *Mol. Biosyst.* **2009**, *5*, 224.
- [7] a) V. Stadler, T. Felgenhauer, M. Beyer, S. Fernandez, K. Leibe, S. Guettler, M. Groening, K. Koenig, G. Torralba, M. Hausmann, V. Lindenstruth, A. Nesterov, I. Block, R. Pipkorn, A. Poustka, F. R. Bischoff, F. Breitling, *Angew. Chem. Int. Ed.* **2008**, *47*, 7132; b) M. Beyer, A. Nesterov, I. Block, K. Koenig, T. Felgenhauer, S. Fernandez, K. Leibe, G. Torralba, M. Hausmann, U. Trunk, V. Lindenstruth, F. R. Bischoff, V. Stadler, F. Breitling, *Science* **2007**, *318*, 1888.
- [8] a) W. C. Chan, P. D. White, *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*, Oxford University Press, New York **2000**; b) H. Wenschuh, M. Beyermann, S. Rothemund, L. A. Carpino, M. Bienert, *Tetrahedron Lett.* **1995**, *36*, 1247.
- [9] a) F. Molina, D. Laune, C. Gougat, B. Pau, C. Granier, *Pept. Res.* **1996**, *9*, 151; b) A. Kramer, U. Reineke, L. Dong, B. Hoffmann, U. Hoffmueller, D. Winkler, R. Volkmer-Engert, J. Schneider-Mergener, *J. Pept. Res.* **1999**, *54*, 319; c) M. Takahashi, A. Ueno, H. Mihara, *Chem. Eur. J.* **2000**, *6*, 3196; d) B. Ay, M. Streitz, P. Boisguerin, A. Schlosser, C. C. Mahrenholz, S. D. Schuck, F. Kern, R. Volkmer, *Biopolymers* **2007**, *88*, 64.
- [10] a) A. M. Bray, N. J. Maeji, A. G. Jhingran, R. M. Valerio, *Tetrahedron Lett.* **1991**, *32*, 6163; b) R. Volkmer, *ChemBioChem* **2009**, *10*, 1431.
- [11] a) M. Beyer, T. Felgenhauer, F. R. Bischoff, F. Breitling, V. Stadler, *Biomaterials.* **2006**, *27*, 3505; b) V. Stadler, M. Beyer, K. Koenig, A. Nesterov, G. Torralba, V. Lindenstruth, M. Hausmann, F. R. Bischoff, F. Breitling, *J. Proteome Res.* **2007**, *6*, 3197; c) V. Stadler, R. Kirmse, M. Beyer, F. Breitling, T. Ludwig, F. R. Bischoff, *Langmuir* **2008**, *24*, 8151.
- [12] a) H. Rink, *Tetrahedron Lett.* **1987**, *28*, 3787; b) M. S. Bernatowicz, S. B. Daniels, H. Koester, *Tetrahedron Lett.* **1989**, *30*, 4645; c) F. Guillier, D. Orain, M. Bradley, *Chem. Rev.* **2000**, *100*, 2091.
- [13] F. Loeffler, C. Schirwitz, J. Wagner, K. Koenig, F. Maerkle, G. Torralba, M. Hausmann, F. R. Bischoff, A. Nesterov-Mueller, F. Breitling, *Adv. Funct. Mater.* **2012**, *22*, 2438.
- [14] F. Loeffler, J. Wagner, K. Koenig, F. Maerkle, S. Fernandez, C. Schirwitz, G. Torralba, M. Hausmann, V. Lindenstruth, F. R. Bischoff, F. Breitling, A. Nesterov, *Aerosol Sci. Technol.* **2011**, *45*, 65.
- [15] K. Koenig, I. Block, A. Nesterov, G. Torralba, S. Fernandez, T. Felgenhauer, K. Leibe, C. Schirwitz, F. Loeffler, F. Painke, J. Wagner, U. Trunk, F. R. Bischoff, F. Breitling, V. Stadler, M. Hausmann, V. Lindenstruth, *Sens. Actuators, B* **2010**, *147*, 418.
- [16] Y. T. Chen, C. Holcomb, H. P. H. Moore, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 6508.
- [17] H. J. Himmel, A. Terfort, C. Woell, *J. Am. Chem. Soc.* **1998**, *120*, 12069.
- [18] F. Liu, N. A. Hashim, Y. Liu, M. R. M. Abed, K. Li, *J. Membr. Sci.* **2011**, *375*, 1.
- [19] a) K. L. Prime, G. M. Whitesides, *JACS* **1993**, *115*, 10714; b) S. Herrwerth, W. Eck, S. Reinhardt, M. Grunze, *J. Am. Chem. Soc.* **2003**, *125*, 9359.
- [20] L. Moroder, *J. Pept. Sci.* **2005**, *11*, 187.
- [21] C. Lausted, Z. Y. Hu, L. Hood, C. T. Campbell, *Comb. Chem. High Throughput Screening* **2009**, *12*, 741.
- [22] a) C. Schirwitz, I. Block, K. Koenig, A. Nesterov, S. Fernandez, T. Felgenhauer, K. Leibe, G. Torralba, M. Hausmann, V. Lindenstruth, V. Stadler, F. Breitling, F. R. Bischoff, *Curr. Protoc. Protein Sci.* **2009**, *18.2*, 1; b) C. Schirwitz, F. F. Loeffler, T. Felgenhauer, V. Stadler, F. Breitling, F. R. Bischoff, *Biointerphases* **2012**, *7*, 47.