Cellulose-bound Peptide Mini- and Microarrays for Large Scale Peptide Screening Applications

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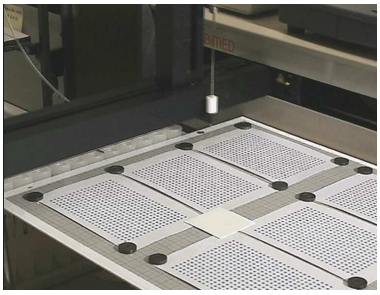
INTRODUCTION

The array format has become a well advanced and widely applied platform for parallel high throughput screening in life science research. Analyses which involve large numbers of probe molecules arrayed on a planar substrate and simultaneous interrogation of these with extensive series of samples are becoming a major approach in global functional genomic experiments. Since its introduction in 1990 as a convenient method to synthesize *in situ* arrays of peptides for rapid epitope mapping, SPOT synthesis [1] has found numerous applications in studying protein-protein interactions through systematic peptide fragment analysis [2].

We developed the SC^2 process to overcome the current limitations of cellulose-bound macroarrays produced by SPOT-synthesis while maintaining the advantageous features of the cellulose support [3]. The process is very flexible concerning the type of array substrate, format and spot densities and allows massive miniaturization and parallelisation. The benefits of this new format of cellulose-bound peptide arrays will be exemplified with studies on peptide recognition by protein domains (SH3) and immunodiagnostics for human viral and bacterial infections.

Conventional SPOT-Analysis

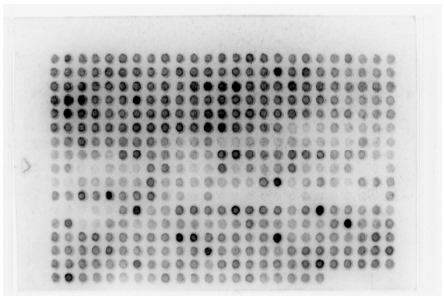


Parallel SPOT-synthesis on a cellulose membrane

The format produced with conventional SPOT synthesis robots can be miniaturized down to 1 mm sized spots at a distance of 2 mm on commercially available optimized cellulose membranes when delivering only nanoliter volumes of reagents. This allows the production in parallel of up to 20,000 peptides in one run on the full table of the instrument (28x28 cm).

These types of cellulose-bound macroarrays have been very successfully applied to a large variety of biological tests and are compatible with most standard read-out systems. The particular special features of cellulose-bound probe molecules which support this success are a) the perfect biocompatibility of the cellulose support with very low background affinity and b) the high local concentration of the synthetic probe (about 10mM) allowing the detection of low affinity binders with a K_d almost to 1mM.
As long as the biological assaying of these macroarrays does not irreversibly transform the probe molecules, they can be reused many times upon stripping off all biologicals from the assay experiment. Depending on the biological assay, however, this stripping can be quite insufficient and thus one array may only be usable once. Furthermore, one synthesized array can be processed only serially through a set of experiments, which requires several identical synthetic arrays to proceed in parallel. Additionally, a conventional SPOT-type macroarray requires considerably large volumes for the assay and, thus, the amount of available sample can become limiting.

Protein Binding Assay

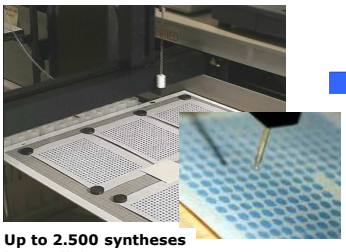


Size of the cellulose membrane is 8 x 12 cm.
Array of spots (2mm in diameter) is 25 x 17 = 425

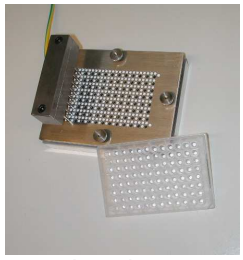
SH3-Domains are involved in many cellular signalling processes. They bind to specific proline-rich regions in target proteins. Nck1 – an adaptor protein consisting of three SH3 and one SH2 domain – is known to bind to the N-WASP protein involved in actin polymerization and recruit it to sites of tyrosine phosphorylation. A systematic single replacement set (each position in the sequence replaced by all other coding amino acids of the 21mer peptide sequence –PPPHSSGPPPPPARGRGAPPY- from the proline-rich region of N-WASP was prepared and probed with a SH3-GST fusion protein.
In the example, this membrane bound peptide array was incubated with the second SH3-domain of Nck1 to determine its preferred recognition motifs. Bound SH3-domains were detected using peroxidase-coupled α-GST antibodies and chemiluminescence exposure of x-ray film.

Process - Peptide Miniarrays on Glass Slides

Synthetic peptide libraries prepared by SPOT-Syntheses



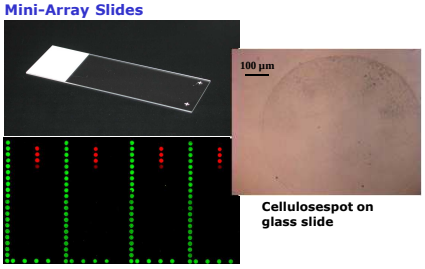
Up to 2.500 syntheses in parallel



Separation and Solubilisation

Printing on specially prepared[4] glass slides with 500 µm pin

Mini-Array Slides

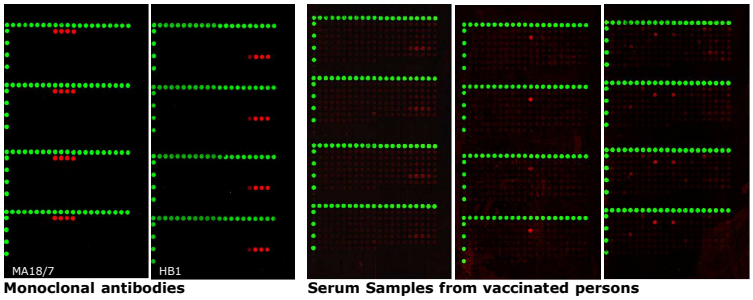


Cellulosespot on glass slide

Up to 800 compounds per slide.
1 synthesis gives up to 100.000 slide copies.

Applications

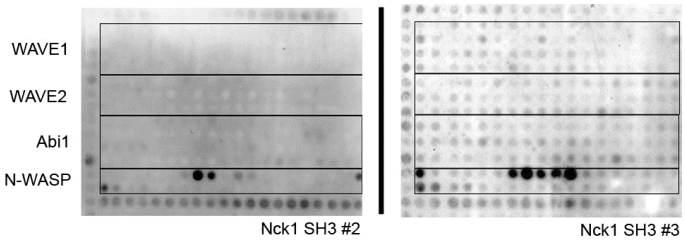
Epitope Mapping of αHBVsag1-Antibodies



Monoclonal antibodies **Serum Samples from vaccinated persons**

Probing with monoclonal antibodies Ma18/7 and HB1 reveal the epitopes known from literature.

Peptide Recognition by SH3-Protein-Domain



Cellulose-bound peptides on glass slides perform equally well in protein interaction experiments.
In the examples, slides presenting proline-rich peptide ligands of SH3-domains were incubated with the second and third SH3-domains of Nck1 fused to GST. Bound SH3-domains were detected using peroxidase-coupled α-GST antibodies and chemiluminescence exposure of x-ray film.

CONCLUSIONS

The SC^2-process presented here for manufacturing mini-arrays of synthetic probe compounds exploits the beneficial features of cellulose-bound compound arrays made by SPOT synthesis. However, it adds more experimental steps to the whole manufacturing process and, thus, requires considerable additional effort. Therefore, this is only reasonable if indeed a great number of copies of the same array are required for a larger series of experiments like profiling serum collections with antigen peptide maps or profiling recombinant protein extracts with generic compound libraries.
The new chemical micro-arrays perform as reliably as the original, successful macro-SPOTs system on cellulose membranes. Single or only a few serial experiments still should be preferably performed with this macro-array format, except a limiting amount of sample makes miniaturisation a strict prerequisite as with samples from e.g. small model organism like mouse or worm, tissue from patients or a few sorted cells; in this case, the surplus of effort is certainly justified.

PERSPECTIVES

Central facilities or commercial suppliers should benefit from this process as arrays made of generic types of chemical libraries can be produced at much lower costs per array. With 0.5 ml of SC^2-spotting solution obtained from one single cellulose-compound disc segment, at least 10^5 mini-array copies can be printed. In this respect, it is also of interest that we successfully printed and assayed SC^2-spotting solutions on many other types of surfaces including plastic sheets made of PVC, PP and other polymer sheets. This could be exploited to adopt SC^2-arrays to numerous alternative customized formats other than the conventional microscope slide.
SC^2-arrays are available as CelluSpots™ from Intavis Bioanalytical Instruments[5].

References:

1. Frank, R. *Tetrahedron* **1992**, *48*, 9217-9232
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3. Dikmans, A. et.al., *QSAR Comb. Sci. 25*, **2006**, No.11, 1069-1080
4. Available from AIMS Scientific Products GmbH, Braunschweig, Germany
5. Intavis Bioanalytical Instruments AG, Köln, Germany

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