

Innovative Peptide Tools for Proteomics and Drug Discovery

a report by

Jerini Peptide Technologies

Jerini Peptide Technologies (JPT) is a provider of peptide based tools and services, ranging from custom services to the provision of highly innovative and proprietary peptide research tools (Kinase Profiling Tools, Protease Profiling Tools and ImmunoTools) for target validation, screening purposes and immunological applications in the pharmaceutical industry and academic institutes. JPT has an implemented quality management system certified according to the Deutsches Institut für Normung, European Standard and the International Organization for Standardization (DIN EN ISO) standard 9001:2000. JPT is in possession of several proprietary technologies which include a fully automated high-throughput peptide synthesis and screening approach (Spot™ technology), as well as a method for the generation of complex peptide micro-arrays (PepStar™ technology).

Peptide Array Product Description

PepSpots™

PepSpots¹ are peptides or peptide derivatives covalently linked to cellulose or polypropylene membranes via their C-terminal end. The peptides are assembled automatically according to the needs of the customer. The underlying technology is the Spot technology, a technique by which individual peptides are assembled in an ultra-fast and economical manner at unique spatial addresses (spots) on planar membranes by means of standard solid-phase synthesis chemistry. Up to 25,000 peptides can be prepared on a single membrane using this method. Following the completion of computer-controlled peptide synthesis in stages, using JPT's proprietary robotic systems, peptide side chains, are de-protected – with the result that the peptides desired by the customer are ready to screen, still attached to the membrane. This spotted membrane constitutes a so-

called peptide macro array, in essence a defined peptide library.

The quality of PepSpot peptides corresponds with those obtained from traditional Fmoc-based solid phase procedures, i.e. an average purity of approximately 70% can be achieved for peptides up to 15 amino acids in length. Smaller peptides display significantly higher purities, whereas the quality of peptides comprising more than 20 amino acids drops to the range of 50% to 60% purity.

Features

- The underlying technology is extremely fast, accurate and economical.
- No other technique can provide as many peptides in a comparable timeframe.
- Cellulose represents a very hydrophilic solid support that is well suited for incubation with proteins because of its inherently low, non-specific binding characteristics.
- The extremely high peptide loading of the PepSpots (up to one nanomole per square millimetre) enables the reliable detection of low affinity interactions.

Applications

The key application for PepSpots is the biological screening of protein/protein interactions. One special subset application in this area is the systematic identification of epitopes for the characterisation of antigen/antibody interactions or of protein/protein interactions using PepSpots representing overlapping peptide scans through the primary sequence of the target protein.² The ability

1. U Reineke, A Kramer and J Schneider-Mergener, *Curr. Top. Microbiol. Immunol.*, 243 (1999) 23; U Reineke, R Volkmer-Engert and J Schneider-Mergener, *Curr. Opin. Biotech.*, 12 (2001) 59; U Reimer, U Reineke and J Schneider-Mergener, *Curr. Opin. Biotech.*, 13 (2002) 315.
2. U Reineke, R Sabat, H-D Volk and J Schneider-Mergener, *Protein Sci.*, 7 (1998) 951; U Reineke, A Kramer and J Schneider-Mergener, *Antibody Engineering* (Springer Lab Manual), ed. R Kontermann and S Dübel (Berlin, Heidelberg: Springer-Verlag, 2001, p. 443.



to detect weak interactions when employing this method enables the reliable and efficient identification and mapping of discontinuous epitopes. In addition, PepSpots are frequently used as an economical tool to systematically optimise the performance of already characterised, biologically active peptides.

ProteaseSpots™

ProteaseSpots are peptide derivatives covalently linked to cellulose membranes via their C-terminal end. The peptides are modified by the attachment of a fluorescent probe like fluoresceine or anthranilic acid at the N-terminal end or at the side chain of the N-terminal amino acid. The underlying technology is the Spot™ synthesis technology. Up to 2,000 ProteaseSpot peptides are prepared on a single membrane. Membrane disks surrounding the ProteaseSpots containing the bound peptides are automatically punch-pressed into 96-well microtiter plates. The result is a defined library of protease peptides that are ready to screen, still attached to the cellulose membrane via the C-terminal amino acid.

Features

- No other technique can provide as many fluorescently labelled peptides in a comparable timeframe.
- Cellulose represents a very hydrophilic solid support that is well suited for incubation with proteases with their potential substrate sequences.
- Fluorescently labelled peptide fragments released during protease-mediated cleavage of recognised peptides are readily detected using standard fluorescence microtiter plate reader equipment, subsequent to the transfer of aliquots of the reaction solution into 'daughter' microtiter plates.
- Identification of cleavage sites is straightforward via the mass-spec analysis of released peptide fragments in daughter microtiter plates.
- ProteaseSpots enable the estimation of kinetic constants for protease-mediated substrate cleavage in a highly parallel manner, via timed incubations with samples.

Applications

The key application for ProteaseSpots is the detection and identification of protease cleavage sites. One very special application is the *de novo* identification of substrates for orphan proteases, holding the promise of significant acceleration of

high-throughput screening assays of such targets. In addition, ProteaseSpots are frequently used as an economical alternative to improve previously characterised protease substrates, which provides the added benefit of potential systematic optimisation of substrate performance.

PepStar™ Peptide Microarrays

PepStar microarrays comprise peptide derivatives equipped with a free C-terminal carboxylic function that are covalently linked to glass surfaces via their N-terminal end. The density of peptides is approximately 15 femtomoles per square millimetre. The peptides are automatically assembled either as fixed sets or according to the specific needs of individual customers. These peptide microarrays are produced via Spot synthesis technology. Generally, an N-terminal reactivity tag (with optimised functionality, allowing for chemoselective re-immobilisation to appropriately modified surfaces) is separated from the glass surface by a long, hydrophilic linker molecule. Membrane-bound peptide Spots are punch-pressed into 96-well microtiter plates, where peptides are then released chemically and filtered into daughter plates, resulting in ready-to-print peptide stocks.

After a reformatting step into 384-well microtiter plates, aliquots (0.1–1 nanolitres (nl)) of the peptide solutions are deposited onto aldehyde-modified glass surfaces (standard glass microscope slide format) using a non-contact printing device. The N-terminal reactivity tag allows a chemoselective reaction with aldehyde moieties on the glass surface, resulting in directed covalent attachment of the peptides. The amount of peptide released from one cellulose Spot is sufficient to print up to 250 identical PepStar microarrays, underlining the cost-efficiency of the PepStar production process.

The chemoselective re-immobilisation in combination with capping steps employed during peptide assembly results in a reactivity purification, leading to extremely high levels of purity in the printed peptides. The average purity of PepStar peptides is approximately 90%.

PepStar microarrays are categorised by the number of peptides deposited: low-density microarrays contain up to 384 peptides in three identical subarrays, a total of 1,152 spots per slide, while high-density peptide microarrays carry of 2,304 peptides in three identical subarrays, a total of 6,912 spots per slide.

The high-density microarrays are exclusive to the full service component of the JPT business model. For

this service, the customer has only to provide JPT with appropriate biological material and JPT does all the experimental work. The bioinformatics group then analyses the data and generates a report, sent to the customer as a PDF document.

The low-density microarrays are delivered to the customer together with a protocol. There are several pre-made microarrays with a fixed content available (PhosphoSite-Detector™ microarrays, Protease Cleavage Site Collection microarrays and RandomLibrary microarrays). The content on low-density peptide microarrays could be customised, too.

Features

- Underlying technology is extremely fast, accurate and economical, especially for PepStar microarrays containing standardised libraries.
- No other technique can provide as many immobilised and purified peptides in a comparable time frame.
- The optimised glass surfaces are perfectly compatible with radioactive or fluorescence assays.
- Reagent amounts are extremely low: about 300µl per experiment or only 20nl per data point.
- Built-in quality control by virtue of triplicate subarrays.
- Broad range of chemical modifications available (fluorophore/quencher pairs, biotinylation, acetylated or methylated lysines, phosphorylated serines, threonines or tyrosines, etc.)
- PepStar peptide microarrays are fully compatible with standard equipment used for DNA microarrays, e.g. scanners, hybridisation chambers or washing stations.

The underlying benefits of these features include compression of project timelines derived from the speed with which the Spot-mediated PepStar manufacturing process can deliver microarrays for follow-up experiments. Additionally, the use of glass surfaces compatible with familiar radioactive or fluorescent reagents reduces assay development costs. The breadth of peptide modifications available also means that PepStar microarrays can be readily incorporated into a wide variety of experimental programs. Reagent costs are reduced

significantly by the much smaller volume requirements of the microarray format. Finally, the prevalence of DNA microarray equipment in biopharma means lower cost barriers to customers adopting PepStar technology.

Applications

The key application for PepStar peptide microarrays is the profiling of enzymatic activities, particularly of kinases and proteases. Only a limited set of these enzymes has been characterised with respect to substrate specificity, creating substantial need to efficiently identify substrates of orphan proteases and kinases. Additionally, most of the known protease/kinase substrates are not suitable for current high-throughput screening assays as full-length proteins due to solubility issues, expense of purification or suboptimal K_m values. PepStar peptide microarrays represent an effective tool to identify^{3,4} and optimise³ substrates for these classes of enzymes, providing sustainable reductions of assay development time. Moreover, it is well accepted that the information concerning substrate specificity is the key starting point for enzyme inhibitor development.

PepStar peptide microarrays enable the precise characterisation of substrate specificities,⁴ the identification of selective substrates within a given panel of similar enzymes (e.g. proteases or kinases) and the mapping of the pharmacophoric space of enzyme super substrates. Custom-made PepStar peptide microarrays representing overlapping peptide scans through kinase substrate proteins (PhosphoSite-Detector) allow for the reliable identification of phosphorylation sites in downstream targets of kinases, a prerequisite for the understanding of biological function in kinase-mediated cell signalling. Additionally, peptide scans overlapping through the kinase itself allow for the determination of autophosphorylation sites as well as the identification of upstream kinases regulating the function of the target kinase. PepStar peptide microarrays could also be used for profiling complex mixtures (e.g. cell lysates) regarding the activity of kinase/phosphatases or proteases. This product allows for the differentiation of various activation states of cells as well as the characterisation of the cellular effects of inhibitors directed against single members of particular classes of enzymes.

Another application of PepStar peptide microarrays is the profiling of antibody specificities. For example, one growing class of tools for proteomics research are

3. J M Lizcano, M Deak, N Morrice, A Kieloch, C J Hastie, L Dong, M Schutkowski, U Reimer and D R Alessi, *J. Biol. Chem.*, 277 (2002) 27,839.
4. L Rychlewski, M Kschischo, L Dong, M Schutkowski and U Reimer, *J. Mol. Biol.*, 336 (2004) 307.

phospho-specific antibodies, i.e. antibodies recognising a specific phosphorylated form of a particular phosphoprotein. PepStar phosphopeptide microarrays could be used to efficiently map the specificity and cross-reactivity of phospho-specific antibodies. Yet another potential application of PepStar peptide microarrays is their use in clinical diagnostics. A collection of peptides representing various pathogens could be printed onto PepStar microarrays, enabling the very efficient detection of antibodies directed against these antigens from human blood samples and allowing for the rapid detection and identification of infectious agents. ■

Contact Information

Jerini Peptide Technologies

Jerini AG

Invalidenstr. 130

10115 Berlin, Germany

Tel.: +49 30 9 78 93 0

Fax: +49 30 9 78 93 105

e-Mail: peptide@jerini.com

<http://www.jerini.com/peptide>