SPOT Peptide Arrays to Study Biological Interfaces at the Molecular Level

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Abstract: A detailed knowledge on how proteins interact with other proteins or nucleic acids is essential for basic research as well as for the development of drugs and therapeutic leads. In this context, peptide arrays represent a powerful tool to analyze individual interactions within complex protein networks. Moreover, peptide arrays allow for incorporation of artificial building blocks during synthesis and secondary modification of amino acid side chains after synthesis, which makes them even more versatile. Within this review, we summarize the spectrum of applications of solid-supported peptide arrays generated by the SPOT method and provide an introduction into the entire volume dedicated to the topic.

Keywords: Epitope mapping, peptide synthesis, protein-protein interaction, protein-RNA interaction, proteomics, SPOT.

Proteins form homo- or heteromeric (macro)molecular complexes and intricate networks by interacting with small molecules, peptides, nucleic acids or other proteins. These assemblies are indispensable for maintenance of the structural and functional integrity of a cell and are critical for intra- and intercellular signaling. The protein interactions can be long-lasting or highly transient with equilibrium binding constants (K_D) ranging from picomolar to millimolar. On average, five interaction partners for any given cellular protein have been estimated, illustrating the complexity of the formed 'interactomes' and the impact of their investigation.

In order to characterize and understand these interactomes, the most basic demand is to identify the individual partner(s) participating in complex formation. Therefore, many different *in vitro* and *in vivo* techniques have been employed such as yeast-two-hybrid screens, protein-fragment complementation, a variety of co-purification strategies, atomic force microscopy (AFM), and fluorescence resonance energy transfer (FRET) (reviewed in [1]). In order to understand the molecular details of complex formation and its functional regulation, once the interaction partners have been identified, a crucial step is the determination of the binding interface between the complex components on the protein domain and amino acid residue level. Moreover, many protein-protein interactions are mediated by hot-spots, which comprise only a small part of the binding interface but account for 80% of the binding energy [2].

In this respect, peptide arrays on solid supports provide a convenient and efficient way to determine binding sites on proteins interacting with small molecules, peptides, nucleic acids or other proteins. The most frequently used way to generate these peptide arrays relies on the SPOT method, which allows for fast parallel manual or automated Fmoc-synthesis of individual peptide sequences on functionalized cellulose membranes [3-5]. Beside high density peptide arrays, currently up to 2000 individual peptide spots on a membrane of about 100 cm^2 can be synthesized routinely [6, 7].

The types of peptides, which can be synthesized, are manifold. Individual peptide sequences of up to 40 amino acids in length have been described [8], however, peptides with up to 20 amino acids provide a good compromise between the size of the offered binding site and the total amount of full-length peptide, which is limited by non-quantitative coupling of the amino acid building blocks. In order to generate small amounts of soluble peptides, these peptides can be cleaved chemically from specialized membranes or proteolytically, in case an appropriate protease cleavage site was synthesized at the basis of the growing peptide. For systematic screening of binding regions, the entire amino acid sequence of a target protein can be synthesized as an array of overlapping peptides with a typical off-set of five (rough scan) or one amino acid (fine scan). In order to determine the precise contact sites, systematic N- and C-terminal truncation variants of the peptides can be generated. Moreover, alanine-walks and consecutive exchange of each individual amino acid within a peptide by all other 19 natural amino acids allow for identification of those amino acids, which are critical for the interaction. A great advantage of the SPOT technique is the opportunity to synthesize peptides comprised of unusual amino acid building blocks such as D-amino acids, amino acids with secondary modification (e.g. phosphorylation), and amino acids with protected alpha-carboxy groups instead of the usual side chain protection group, which e. g. in the case of aspartic acid and glutamic acid allows for non-standard peptide bound formation (compare glutathione). Moreover, peptides can form inter- and intramolecular disulfide-bonds on aerial oxygen and thus become oriented and cyclised, respectively. The use of these building blocks might lead to identification of peptides with improved binding affinity or increased stability, properties which are beneficial for technical and clinical applications.

Principally there are two types of interaction sites: i.) linear binding sites of individual sequence stretches with a length of 5-30 amino acids, and ii.) composite binding sites, which represent a three-dimensional assembly of several linear binding sites distantly located on primary sequence (see Fig. **1**).

A well known example for the latter is represented by an antibody paratope, which is made up of three short complementarity determining regions (CDRs) interspersed by frame work regions. Notably, two interaction partners might make contact via all possible pairings of linear and composite binding sites. In the most complex situation, two composite binding domains interact with each other (e. g. the interaction between an antibody paratope and a composite epitope).

Peptides of roughly 20 amino acids in length are most likely unstructured. They can, however, form alpha-helices once they "shape" at the binding ligand [9].

Peptide arrays have been used successfully to determine a broad spectrum of different protein interaction sites in diverse biological context (for review see [4, 10-12], Fig. **2**). For some applications, examples of our own results are referenced, for a detailed insight into the different aspects of peptide arrays please go to the individual articles of this issue. Examples are: i.) mapping of linear and composite antibody epitopes (monoclonal hybridoma-derived antibodies as well as polyclonal sera from immunized animals or patients) [13], ii.) interaction of proteins with peptides, which on their own have important functions in the brain, in host defense as part of the innate immune response, and as potent inhibitors of enzymes,

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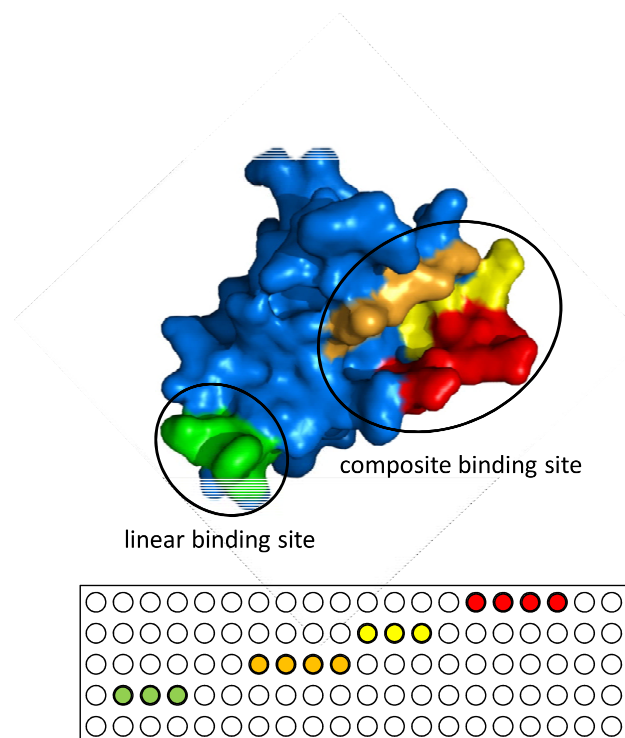

Fig. (1). Different types of binding sites. A schematic representation of a systematic peptide array with overlapping peptides of a target protein is shown. Reactive peptide spots after incubation with a soluble ligand are coloured. The corresponding amino acid sequence stretches were projected onto the three-dimensional structure of the target protein to analyze surface exposure. In this example, a linear and a tripartite composite binding site were identified.

iii.) interaction of proteins via confined protein interaction domains (e. g. SH2, SH3, WW or PDZ domains), which recognize distinct linear sequence patterns, iv.) determination of the protein binding interface independent of specialized binding domains (linear as well as composite binding sites) [14-20], v.) determination of protein kinase, phosphatase, and protease targeting sites, vi.) interaction of proteins with nucleic acids (DNA as well as RNA [21]), vii.) investigation of cell-cell interaction requirements by cellular interactions with integrin-derived peptides, viii.) screening for antimicrobial peptides based on metabolic modulation of bacteria upon binding to particular peptide species on the array, ix.) binding of proteins to secondarily modified amino acids in a defined sequence context [22], and x.) identification of sequence stretches within peptides and proteins, which specifically bind to small ligands such as metal ions.

The availability of a three dimensional structure of at least one of the binding partners is beneficial to verify the accessibility of putative interaction sites. Notably, the interaction interface between two proteins might undergo maturation due to e. g. induced fit mechanisms and therefore, the interacting faces might not be surface exposed as a whole in the monomers. Moreover, different conformers of the isolated interaction partners might exist; therefore, a high resolution structure of a single conformation of the protein might not provide all relevant information for validation of an interaction site.

A large number of the interactions between proteins are enabled or modulated by secondary modification (e.g. phosphorylation, sulfurylation, glycosylation, methylation, ubiquitylation, sumoylation) of the binding partners. Often, these modifications are recognized within a defined linear sequence context. Since secondary modification of a target protein is reversible in many cases, the interaction can be regulated in time and space. The elucidation of such targeting sites for secondary modification and the determination of related binding sites are of great interest, since intracellular signalling pathways depend on secondary modification. In this context, the SPOT method allows for the use of corresponding amino acid derivatives instead of the common ones as well as peptide modification during and after synthesis (see above). Among many others, the synthesis of phosphorylated peptides for interaction studies and the enzymatic phosphorylation and dephosporylation of non-modified peptides after synthesis are widely used since differential protein phosphorylation plays a major role in cellular signalling.

Peptide arrays are prone to identify interaction sites between a ligand protein and a particular peptide sequence, which are not part of an interaction interface in an *in vivo* situation. These accidentally identified interaction sites are not necessarily binding artefacts in a molecular sense and might thus represent specific interaction sites. However, in an *in vivo* situation the biological system might be able to suppress these non-physiological interactions by compartmentalization (e.g. proteins are located in different cellular compartments or a ligand can access its corresponding receptor only from one site of the membrane) or by burying sequence stretches within the fold of a protein thus making it inaccessible (at least in this

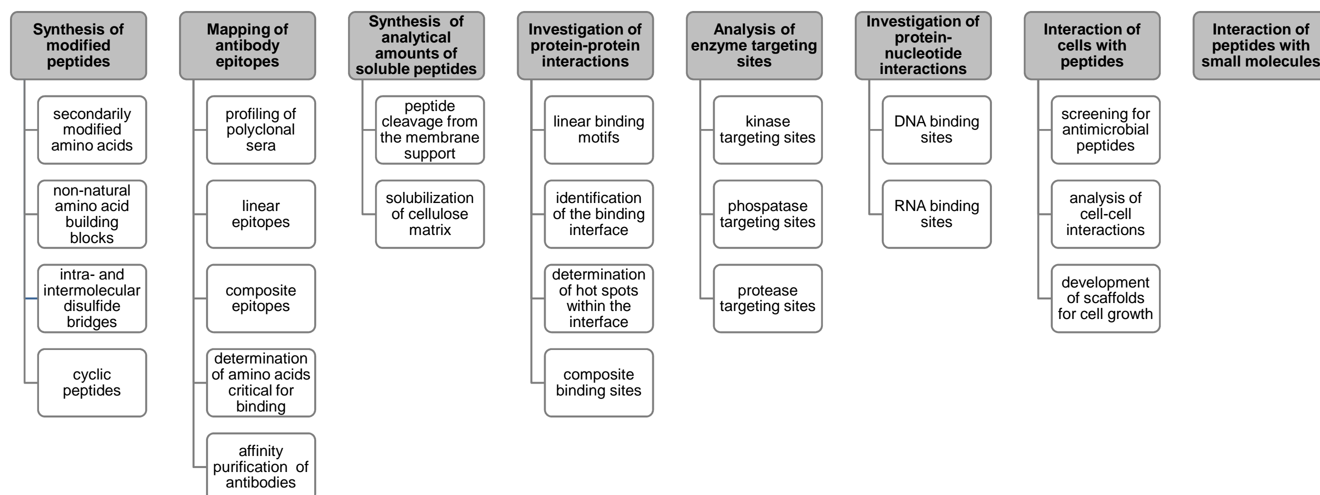

Fig. (2). A selection of applications of peptide arrays.

particular conformation of the protein) for an interaction partner inspecting the surface of a putative target protein. Therefore, binding sites determined on peptide arrays need further discrimination by *in vitro* and *in vivo* techniques with mutated variants of the parental protein. A major advantage is of course that the candidate domains have now been preselected, strongly accelerating the identification of those binding sites with *in vivo* relevance.

Within the current issue of *Mini Reviews in Organic Chemistry* we provide a detailed review of the generation and use of peptide arrays on solid supports. Beside the great potential of the method and its many successful applications we also discuss limitations and pitfalls of the technique and their circumvention. All of the articles were written by leading experts in the field whose work is gratefully acknowledged. Moreover, I like to thank the Editorial Board of *Mini Reviews in Organic Chemistry* for their trust and the opportunity to serve as a guest-editor for this issue.

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REFERENCES

[1] Piehler, J. New methodologies for measuring protein interactions *in vivo* and *in vitro*. *Curr. Opin. Struct. Biol.,* **2005**, *15*, 4-14.

[2] DeLano, W.L. Unraveling hot spots in binding interfaces: progress and challenges. *Curr. Opin. Struct. Biol.,* **2002**, *12*, 14-20.

[3] Frank, R. Spot-synthesis: an easy technique for the positionally addressable, parallel chemical synthesis on a membrane support. *Tetrahedron,* **1992**, *48*, 9217-9232.

[4] Koch, J.; Mahler, M. *Peptide Arrays on Membrane Supports*. Springer-Verlag: Berlin, Heidelberg, New York, **2002**.

[5] Merrifield, R.B. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.,* **1963**, *85*, 2149-2154.

[6] Hilpert, K.; Winkler, D.F.; Hancock, R.E. Peptide arrays on cellulose support: SPOT synthesis, a time and cost efficient method for synthesis of large numbers of peptides in a parallel and addressable fashion. *Nat. Protoc.,* **2007**, *2*, 1333-1349.

[7] Zander, N. New planar substrates for the *in situ* synthesis of peptide arrays. *Mol. Divers.,* **2004**, *8*, 189-195.

[8] Toepert, F.; Knaute, T.; Guffler, S.; Pires, J.R.; Matzdorf, T.; Oschkinat, H.; Schneider-Mergener, J. Combining SPOT synthesis and native peptide ligation to create large arrays of WW protein domains. *Angew. Chem. Int. Ed. Engl.,* **2003**, *42*, 1136-1140.

[9] Liu, Z.; Song, D.; Kramer, A.; Martin, A.C.; Dandekar, T.; Schneider-Mergener, J.; Bautz, E.K.; Dübel, S. Fine mapping of the antigen-antibody interaction of scFv215, a recombinant antibody inhibiting RNA polymerase II from Drosophila melanogaster. *J. Mol. Recognit.,* **1999**, *12*, 103-111.

[10] Brandt, O.; Dietrich, U.; Koch, J. Solid-supported peptides arrays in the investigation of protein-protein and protein-nucleic acid interactions. *Curr. Chem. Biol.,* **2009**, *3*, 171-179.

[11] Hilpert, K.; Winkler, D.F.H.; Hancock, R.E.W. Cellulose-bound peptide arrays: Preparation and applications. *Biotechnol. Genet. Eng. Rev.,* **2007**, *24*, 31-106.

[12] Schutkowski, M.; Thiele, A.; Koch, J. Peptide Arrays on Solid Supports: A Tool for the Identification of Peptide Ligands. In: *Peptides as Drugs*, Groner, B., Ed. Wiley-VCH: Weinheim, **2009**; pp. 187-217.

[13] Plewnia, G.; Schulze, K.; Hunte, C.; Tampé, R.; Koch, J. Modulation of the antigenic peptide transporter TAP by recombinant antibodies binding to the last five residues of TAP1. *J. Mol. Biol.,* **2007**, *369*, 95-107.

[14] Falk, S.; Ravaud, S.; Koch, J.; Sinning, I. The C terminus of the Alb3 membrane insertase recruits cpSRP43 to the thylakoid membrane. *J. Biol. Chem.,* **2010**, *285*, 5954-5962.

[15] Groves, M.R.; Mant, A.; Kuhn, A.; Koch, J.; Dubel, S.; Robinson, C.; Sinning, I. Functional characterization of recombinant chloroplast signal recognition particle. *J. Biol. Chem.,* **2001**, *276*, 27778-27786.

[16] Ott, V.; Koch, J.; Spate, K.; Morbach, S.; Kramer, R. Regulatory properties and interaction of the C- and N-terminal domains of BetP, an osmoregulated betaine transporter from Corynebacterium glutamicum. *Biochemistry,* **2008**, *47*, 12208-12218.

[17] Radzimanowski, J.; Ravaud, S.; Schlesinger, S.; Koch, J.; Beyreuther, K.; Sinning, I.; Wild, K. Crystal structure of the human Fe65-PTB1 domain. *J. Biol. Chem.,* **2008**, *283*, 23113-23120.

[18] Usener, D.; Schadendorf, D.; Koch, J.; Dubel, S.; Eichmuller, S. cTAGE: a cutaneous T cell lymphoma associated antigen family with tumor-specific splicing. *J. Invest. Dermatol.,* **2003**, *121*, 198-206.

[19] van der Sluis, E.O.; Nouwen, N.; Koch, J.; de Keyzer, J.; van der Does, C.; Tampé, R.; Driessen, A.J. Identification of two interaction sites in SecY that are important for the functional interaction with SecA. *J. Mol. Biol.* **2006**, *361*, 839-849.

[20] Yip, Y.L.; Smith, G.; Koch, J.; Dubel, S.; Ward, R.L. Identification of epitope regions recognized by tumor inhibitory and stimulatory anti-ErbB-2 monoclonal antibodies: implications for vaccine design. *J. Immunol.,* **2001**, *166*, 5271-5278.

[21] Dietz, J.; Koch, J.; Kaur, A.; Raja, C.; Stein, S.; Grez, M.; Pustowka, A.; Mensch, S.; Ferner, J.; Möller, L.; Bannert, N.; Tampé, R.; Divita, G.; Mely, Y.; Schwalbe, H.; Dietrich, U. Inhibition of HIV-1 by a peptide ligand of the genomic RNA packaging signal Psi. *ChemMedChem.,* **2008**, *3*, 749-55.

[22] Costagliola, S.; Panneels, V.; Bonomi, M.; Koch, J.; Many, M.C.; Smits, G.; Vassart, G. Tyrosine sulfation is required for agonist recognition by glycoprotein hormone receptors. *EMBO J.,* **2002**, *21*, 504-513.

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