Exploring Protein-Protein Interactions with Synthetic Peptide Arrays

Rudolf Volkmer* and Victor Tapia

Institute für Medizinische Immunologie, Charité-Universitätsmedizin Berlin, Hessische Str. 3-4, 10115 Berlin, Germany

Abstract: Development of array technologies started in the late 1980s, and today this technique has become a powerful tool for high throughput approaches in biology and chemistry. Progress was mainly driven by the human genome project and associated with the development of several new technologies, leading to the birth of additional ''omic'' topics such as proteomics, glycomics, or antibodyomics. In this review we focus strictly on peptide arrays applied to investigate protein-protein interactions, starting from single events up to complete interaction networks (interactomes). The specificities and interaction networks of protein interaction domains (PIDs) are our central theme. Picking practical examples from the literature, we review some general concepts about peptide arrays on planar supports for mapping protein-protein interactions.

Keywords: Peptide arrays, SPOT technology, protein interaction domains, peptide microarrays.

1. INTRODUCTION

The organization of living systems depends on complex networks of molecular interactions whereby proteins are a central component and it is tempting to suggest the existence of a molecular recognition code [1]. In fact, structural modules and motifs may have isolated functional "meaning" like words in human language [2, 3]. To pursue this analogy, we can think of cellular wiring as a masterpiece of evolutionary tinkering, with structural elements used many times in different whole protein contexts, where by trial and error some rules of interconnectivity have achieved a favorable feature or message [4, 5]. Moreover, as in human language, one can interpret an analogous hierarchical organization: linear sequence of polypeptide chains, fold elements, compound structural motifs, protein complexes, and protein "machines" [6-8] and indeed protein architecture is modular. A protein is composed of single domains (modules) separated on discrete sequence patterns, which in turn comprise folding motifs [9]. In general, isolated protein modules have the same globular folding as in the context of the whole protein, and therefore a reductionist approach could be applied in practice [10-14].

Structural analysis of functional protein complexes suggests at least two classes of protein-protein interactions. In the first class, the complementary surfaces of the interacting partners are both extensive. This means that the residues involved in each interacting surface only come together upon protein folding. The second class comprises asymmetric interactions, where a protein interaction domain (PID) may dock a short linear sequence motive on the partner protein. While interactions over extensive surfaces cannot be inferred, the binding determinants of a PID may be mapped to short linear motifs [15-18].

Since the early 1990s biological library techniques such as phage display [19, 20], yeast two-hybrid [21] and pull-down assays (affinity chromatography) in combination with mass spectrometry [22-24] have been predominately used to reveal cellular protein-protein interaction networks. In particular, phage display has become one of the major techniques for applying highly diverse combinatorial peptide libraries, e.g. to discover PID interaction networks. Additionally, bioinformatics and computational tools were developed to find modular domains and their cognate ligands [25]. Nowadays, several databases are freely available, such as MINT (http://mint.bio.uniroma2.it/mint) and the SMART database [26]. Array technologies, especially protein arrays, arrived late in the field of protein-protein interactions [27-29]. Here, critical factors such as native folding stability or functionality proved to be an enormous challenge in the production of protein arrays. Peptides, in contrast, are easier to handle and retain partial features of protein function. Thus, peptide arrays [30, 31] are suitable to support proteomic research, particularly in the case of PID recognition.

2. PROTEIN INTERACTION DOMAINS

The era of extensive genome sequencing has revealed many PIDs. The interaction partners, and therefore the functions of such proteins can be determined by identifying the critical binding sites for one family member through evolutionary tracing [4], or functional protein arrays [8], and then mapping the relevance of single site mutations. Many of the PIDs in proteins can be grouped into families that show clear evidence of their evolution from a common ancestor. The PID families recognize specific sequence or structural motifs. Table **1** summarizes the structural sequence motifs recognized by the most common protein interaction domains. Within each family, variations in the chemical characteristics of the domain-binding pocket modulate finer peptide recognition specificity, and as a consequence, determine the selection of functional protein partners *in vivo* [15]. In general, one can conclude that PID recognition is ruled by (1) posttranslational modifications such as phosphorylation of the amino acids Ser, Thr, and Tyr, and methylation or acetylation of lysine or arginine [32-38] and/or by (2) short linear sequence motifs [39, 40] as shown in Table **1**.

One difficulty in deriving pathways or networks of PID-mediated protein-protein interactions is that the difference in affinities between 'specific' and 'non-specific' interactions is small and reported to be less than two orders of magnitude in the case of SH2 domains [16, 41] and its peptide ligands [42]. Even when the recognition specificity of intact proteins is greater than that for the corresponding isolated peptide domain, affinity is not raised above one order of magnitude, as shown for SH3 domains [43, 44]. Moreover, the fact that metazoan SH3 domains can replace the yeast Sho1-SH3 and rescue the cellular response to environmental high osmolarity emphasizes that the specificity of SH3-mediated interactions is not great [45]. In summary, the described facts lead to a picture of large and promiscuous SH3-mediated interaction networks.

Since it is possible to generate mutant SH3 domains that have up to 40-fold higher affinity than their wild-types [46], one must consider the potential of these domains as research tools and a source of lead compounds for pharmaceutical development. Furthermore, one question cannot be ignored: what is the functional advantage of maintaining relative low affinity and selectivity for interactions? Moreover, how can SH3-dependent interaction pathways achieve such precise cellular responses?

Nowadays, the picture of linear functional pathways is being revolutionized by a more probabilistic view of dynamic equilibrium

*Address correspondence to this author at the Institut für Medizinische Immunologie, AG Molekulare Bibliotheken, Charité - Universitätsmedizin Berlin, Hessische Str. 3-4, 10115 Berlin, Germany; Tel: +49-(0)30-450 524267; Fax: +49-(0)30-450 524942; E-mail: rve@charite.de

1570-193X/11 $58.00+.00

Table 1. Protein Interaction Domain Binding Specificity. Upper part: Linear Peptide Sequences (Consensus Motifs) Specifically Recognized by a Domain Family. Lower part: Kind of Posttranslational Modification Recognized by the Corresponding Domain Family

Domain Family	**Consensus Motifs**[a]
SH2	*po*Y
SH3	*Class I:* ([+]xxPxxP); *class II:* (PxxPx[+]); *atypical class I:* RKxxYxxY, PxxRxxKP; *atypical class II:* PxxDY, Px(P/A)xxR, ΦxRPx
WW	*Class I:* PPxY; *class II:* PPLP; *class III:* RPPP(R); *class IV: po*(S/T)P
EVH1/WH1	*Class I:* FPPPP; *class II:* TPPxxF; plus DLPPPEPYVQT, LEVAQTTALPD
PDZ	*Class I:* x(S/T)xΦ$_{COOH}$; *class II:* xΦxΦ$_{COOH}$
GYF	PPGΦ (until now)
PTB	NPx*po*Y; NPxY
FHA	*po*Txxx
WD40	LP*po*TP; D*po*SGxx*po*S
PH	Phosphoinositide (Phospholipids)
Posttranslational modifications	**Recognized by the domain families**
Phosphorylation at Tyr	SH2, PTB
Phosphorylation at Ser/Thr	FHA, WD40 MH2, Polo Box, BRCT, FF, WW and 14-3-3 proteins
Acetylation of Lys	Bromodomains
Methylation of Lys	SET
Methylation of Arg	Chromodomains
Hydroxlation of Pro	VHL
Monoubiquitination	UIM, CUE, UBA

[a]Φ: hydrophobic amino acids (VILFWYM); [+]: positively charged (KR); *po*: phosphorylated

between multiple interactions, where "the central organizing principle is a vast and ever-shifting web of interactions, from which output is gauged by global changes in complex binding equilibria" [47, 48].

3. PROTEIN INTERACTION MAPS – SCANNING THE INTERACTOME

Elucidation of functional signal transduction pathways, biochemical functions or gene regulation, is initially addressed in proteomics by deriving interaction networks ideally depicting all interactions in the cell. Several attempts have been made for the yeast interactome. While yeast is easier to culture than mammal tissues, and its proteome is simpler than other eukaryotes, it seems that most of the protein interactions in yeast find orthologues in 'higher' eukaryotes, including humans [23].

Landmarks in systematic efforts to analyze the yeast interactome have used automated yeast two-hybrid approaches [49, 50], phage display [51], and affinity chromatography [23, 24]. A comparison of datasets derived by individual methods demonstrates that different approaches have different potential. For example, affinity chromatography is biased towards tight interactions such as those involving extensive complementary surfaces, while interactions where one of the two partners contains at least one PID are more frequently found in the two-hybrid database. The higher sensitivity of so-called synthetic approaches (yeast two-hybrid, phage display and SPOT synthesis) make them better suited for detecting PID-mediated interactions, since their peptide affinity in terms of *Kd* are in the range of 10 – 100 μM or even higher. However, this advantage is counterbalanced by low specificity, especially with the yeast two-hybrid approach.

To correct this deficiency, it is recommended to double check the information fed into interaction databases. This can be achieved by deriving two interaction networks through orthogonal synthetic methods and then considering only the intersection between the two datasets. The strength of this combined approach for delivering physiologically relevant interactions has been proven for phage display/yeast two-hybrid [51], phage display/SPOT technology [52, 53] and phage display/yeast two-hybrid/SPOT technology intersected datasets [54]. A notable conclusion here is that the intersected dataset of proteins able to interact with a given PID is larger than expected if cellular events are viewed as precise wiring of proteins in the cell. Although a set of these potential binders may have no physiological relevance due to different temporal or tissue expression, or structures disrupted *in vitro*, etc., the paradox of promiscuous recognition versus mutually exclusive responses seems to be inherent to PID-mediated interactions. Recent work of Landgraf *et al.* [52] and Tonikian *et al.* [54] supports the observation that many natural peptides with biochemical potential to bind any given SH3 domain (found in the proteomic study) are indeed used *in vivo* to mediate the formation of complexes.

4. SYSTEMATIC SETS OF PEPTIDES TO MAP PROTEIN BINDING

The design principles of peptide arrays useful for mapping protein-protein interactions can be classified into protein sequence-based, and *de novo* approaches [55, 56]. In general, the design principles are the same as for B-cell epitope mapping [57, 58]. However, some specifics such as peptide length, choice of the carrier material, and a diverse range of affinity have to be taken into account due to the broad spectrum of protein-protein interactions.

Scans of Overlapping Peptides and Cognate Approaches

Protein sequence-derived peptide arrays provide the basic tools to elucidate interactions between proteins. Scans of overlapping peptides [59, 60] are often applied as an initial approach to mapping the binding site of interacting proteins. This involves synthesizing the entire sequence of a protein as short, linear, overlapping peptides, for example arrayed on a planar surface, and subsequently tested for binding of the partner protein. In general, 6- to 15-mer overlapping peptides were used for B-cell epitope mapping protocols [30, 55, 59]. This length is sufficient for antibody-protein interactions, since linear epitopes do not exceed this range [58]. In contrast, pepscans comprising overlapping peptides of different lengths (up to 34-mer) were synthesized to elucidate the binding sites of Pex proteins [61-63], Tat proteins [64, 65], and protein components of the maltose importer [66, 67]. The pepscan approach works excellently if a binding site is formed by a linear sequence motif recognized by the interacting partner protein. This is the case for the Pex 19p/Pex 13p interaction [61]. A cellulose membrane containing an array of 20-mer peptides designed to represent the entire Pex13p sequence in an overlapping arrangement was probed for binding a Pex19p recombinant GST fusion protein. Immunological detection of bound Pex19p-GST by a monoclonal anti-GST antibody resulted in staining of serial spots covering amino acids 191 to 222 of Pex13p.

However, the outcome of binding experiments are often not so clear, and therefore we strongly recommend applying sets of overlapping peptides with variable length. Variable peptide lengths were used to map the binding motif within the protein $EIIA^{Glc}$ recognized by MalK [67]. Here, cellulose membrane-bound peptide arrays representing the complete $EIIA^{Glc}$ sequence were screened for MalK binding. The peptide arrays consisted of 13-mers, 16-mers, or 31-mers overlapping with adjacent peptides by 12, 15, and 30 amino acids, respectively. Comparison of these three peptide arrays helped to localize the MalK binding sites of $EIIA^{Glc}$ more precisely. It is also an advantageous practice to perform binding experiments in both directions. This was performed when mapping subunit-subunit interactions between MalF, MalG and MalK [66].

Besides peptide length, the number of overlapping amino acids between the consecutive peptides defines a peptide scan. In general, peptides are shifted by one to three positions along the linear sequence. Simple peptide scans are sufficient for revealing linear binding motifs, as shown for the Pex 19p/Pex 13p interaction. Mapping of discontinuous binding motifs may be performed successfully applying pepscans of variable peptide length, as demonstrated for the maltose importer machinery [66, 67]. However, mapping of discontinuous binding sites is still a great challenge and further useful approaches were developed [68, 69].

Amino Acid Substitution Scans and Cognate Approaches

After elucidating a binding motif, details can be elaborated by amino acid substitution scans and substitution analyses. Key residues of a binding motif are those amino acids that are effectively in contact with the binding partner. Critical amino acids are those residues that facilitate adoption of a certain binding conformation prior to, or upon binding. These residues define the specificity and binding free energy. The concept of alanine scanning [70] was developed to identify these residues and was successfully applied to cellulose membrane-bound peptide arrays [71]. Residues that cannot be exchanged by alanine without loss of binding are regarded as key residues for the interaction. Further scans such as glycine scans, tyrosine scans and proline scans have also been reported [72-75]. All these scans reveal effects that depend on the amino acid side chains, with the exception of proline, which influences peptide conformation. There are also reports about further modifications of amino acid substitution scans [76-78].

Applying all genetically encoded amino acids for an amino acid substitution scan results in a complete amino acid substitution scan or (complete) substitution analysis. Here, each amino acid of the original sequence is replaced by all other 19 genetically encoded amino acids. This approach has been used quite often in peptide array technology since the beginning (for more references see [31, 79]). Substitution analyses are well-suited for SPOT technology to map the structure-activity relationship of protein binding motifs, or even of a complete protein domain [80-82].

In general, protein-protein interactions of physiological relevance depend on a defined position of the binding site and are mediated by specific non-covalent interactions of amino acid side chains. In consequence, this results in a distinct substitution analysis pattern representing the significance and contribution of each amino acid for the protein-protein interaction [80].

Further sequence-derived peptide arrays useful for exploring protein-protein interactions are truncation and deletion libraries. These types of assays are carried out to determine the minimal length of a binding motif [55, 59, 79].

Inverted Peptide Arrays with Free C-termini

PDZ domains [83-85] anchor transmembrane proteins to the cytoskeleton and hold signaling complexes together [86, 87]. In general, PDZ domains recognize the C-terminal four to seven residues of their protein binding partner and they require a free C-terminus for ligand recognition (Table **1**). In other words, PDZ domains are PIDs known to recognize short linear peptides containing a free C-terminus. Unfortunately, SPOT-synthesized peptides lack free C-termini due to their C-terminal fixation to the cellulose support. The first reliable and robust SPOT synthesis concept for synthesizing inverted peptide arrays with free C-termini was published in 2004 and the approach was recently improved [88, 89]. Fortunately, the 3-brompropyesters [90] used in the first approach is replaced by the commercially available HMPA linker allowing for a more convenient procedure. The inverted peptide array approach has been applied to map the specificity of several PDZ domains [88, 89, 91-94].

5. INFLUENCING THE RELIABILITY AND QUALITY OF PEPTIDE ARRAYS APPLIED FOR MAPPING PROTEIN BINDING

Generally, combined approaches should be used for mapping the binding sites of protein complexes [51-54]. The strength of those combined approaches to deliver physiologically relevant interactions has been proven for the combination of a yeast two-hybrid with SPOT technology approach, here demonstrated by the work of Pires *et al.* [95]. Yeast two-hybrid and bioinformatics suggest that the SH3-domain of Pex 13p provides binding sites for two proteins, the PTS1 receptor Pex5p and the putative docking protein Pex14p. As shown in Table **1**, SH3-binding peptides are characterized by the presence of a general PXXP consensus sequence, and indeed such a motif can be found in the sequence of Pex 14p. To analyze the binding sites of the two SH3-ligands Pex5p and Pex14p in more detail, Pex5p-derived overlapping 26-mer synthetic peptides covering the putative binding region, and Pex14p-derived 12-mer synthetic peptides covering the entire Pex14p protein sequence were tested for their interaction with the SH3-domain of Pex13p. Indeed a proline-rich motif of Pex14p representing a typical type II SH3-ligand motif was identified as the SH3-binding site of Pex14p. In contrast, the SH3-binding site in Pex5p was unambiguously mapped to non-PXXP sequences containing the sequence QPWTDQFEKLEKEV, which represents amino acid residues 202–215 of Pex5p. Both peptides can bind simultaneously to the SH3 domain, suggesting that Pex 13p acts as an adapter protein.

The quality of protein-protein-interaction studies using synthetic peptide arrays prepared by SPOT synthesis is influenced by

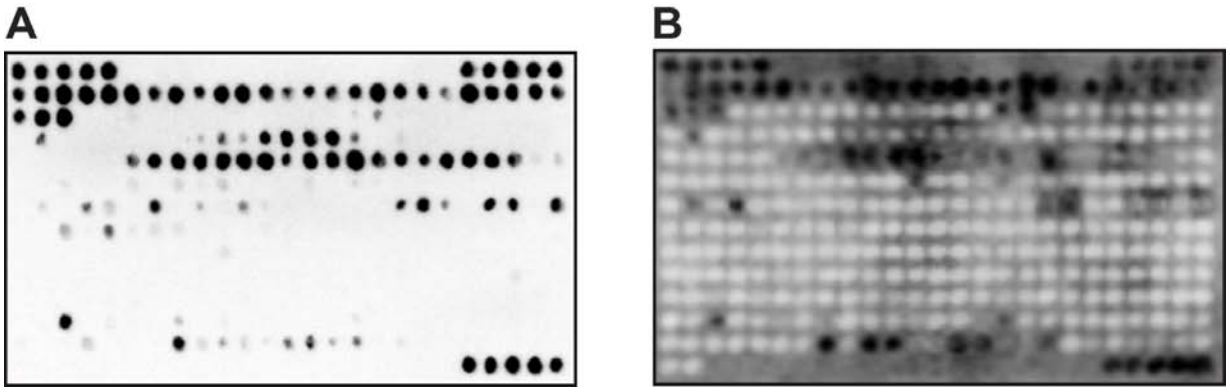

Fig. (1). The influence of membrane type upon read out quality.
Two identical peptide arrays (295 potential yeast SH3 binding peptides, 15-mers [28]) were synthesized alternatively on an ether-type membrane (Fig. **1A**) or on a standard ester-type membrane (Fig. **1B**). Both arrays were probed for binding the yeast Myo 5 SH3 domain. Even although identical synthetic, incubation, and visualization protocols were used, the read-out quality was dramatically different. The best results were obtained using an ether-type membrane.

technological aspects. As shown in Fig. (**1**), the read-out quality is strongly influenced by the kind of membranes used in the binding experiment. When two identical peptide arrays were synthesized alternatively on an ester-type membrane or an ether-type membrane, even though identical synthesis, incubation, and visualization protocols were used, the read-out quality was drastically different. The best results were obtained using an ether-type membrane [52, 96]. Therefore, CAPE membrane proves to be first class, especially for SH3 domain interaction studies, but also for several other kinds of biological tests [52, 54, 61, 95, 97, 98]. Unfortunately, CAPE membranes cannot be used in a fully automated SPOT synthesis process [99] due to their mechanical brittleness. It is worth testing other membranes for their signal-to-noise ratio during on-support assays, e.g. the di-amino-membrane from the Blackwell lab [52, 100], or commercially available membranes from AIMS [101, 102].

6. PEPTIDE ARRAYS FOR PROTEOMIC APPROACHES

The strength of SPOT peptide assays is its unbiased, comprehensive and systematic approach to evaluating a given protein for binding linear peptide sequences, which may also be additionally modified. The clear advantage of the array format can be fully exploited to study protein interactions where one of the partners participates in complex formation by docking through a relatively short peptide within a receptor protein. In fact, a fairly large set of protein-protein interactions are mediated by families of domains, such as SH2, SH3, EVH1, GYF, PDZ or WW domains (Table **1**). These domains are referred to as protein interaction domains (PID), acting as receptors to accommodate short peptides in their binding pockets [39, 40, 103].

In an ideal scenario, unique peptides representing the entire proteome of an organism would be synthesized on an array and assayed individually for interactions with a PID of interest. However, this straightforward approach is not technically feasible since the number of short peptides, even in a proteome as simple as that of baker's yeast, is in the order of 10^7. This figure is far beyond the limits of current technologies for peptide array synthesis, even for approaches such as peptide microarrays [104, 105], photolithography on a glass surface [106] or peptide laser printer technology [107, 108]. In practice, a filtering step is required to generate an array of manageable size.

One approach described by Landgraf and co-workers [52] applies a strategy called WISE (Whole Interactome Scanning Experiment). It combines phage display techniques together with SPOT technology and bioinformatics. The consensus sequences of yeast SH3 domains for example, are deduced from screening random peptide repertoires, such as phage display libraries, which generally results in strict consensus sequences [109]. For the next step the authors relaxed the strict consensus sequences obtained; for example the strict consensus sequence of the yeast SH3 domain Rvs167 defined as RxFPRxP was relaxed to R/KxxPxxP. Subsequently, all sequences within the yeast proteome matching a relaxed consensus of a given SH3 domain were identified by computational methods. This approach was repeated for eight yeast SH3 domains. For each domain, approximately 1,500 peptides matching the relaxed patterns were selected for synthesis. The peptides were generated on cellulose membranes, and the membranes were probed with the corresponding SH3 domain fused to glutathione S-transferase (GST). Finally, the bound domains were detected using an anti-GST antibody. Fortunately, the intensity of each binding-spot could be measured quantitatively. The average number of peptides in the yeast proteome that have the potential to bind SH3 domains with an affinity that may have physiological relevance was found to be surprisingly high, ranging from a few peptides, in the case of the Abp1 and Boi2 SH3 domains, to several tenths, in the case of the Yfr024w SH3 domain. Given the hypothesis that all these peptides are equally expressed inside the cell and exposed to the solvent in the folded protein structure, these findings raise the question of whether the observed binding promiscuity has any physiological implication. Many proteins are organized in discrete complexes [23, 24], however, many physiologically relevant protein interactions do not lead to the formation of stable complexes, and the WISE approach suggest that SH3-mediated interactions may belong to this latter class. The authors consider a new scenario in which proteins, even when not forming stable complexes, are seldom isolated in solution, but navigate in the cell by moving from one weak partner to another. The semiquantitative data provided by the WISE approach, complemented with the results of large scale expression and localization studies, may eventually allow one to model these different settings. Nevertheless, the identified target peptide can be used as a lead to develop tighter binding molecules in order to interfere with complex formation *in vivo*.

Very recently, the WISE approach was expanded to the complete SH3 domain interactome of yeast [54]. A consortium comprising the Boone, Cesareni, Drubin, Kim, Sidhu and Volkmer labs applied a combined approach of orthogonal experimental proteomic tools, such as phage display, yeast two-hybrid and SPOT technology, which were combined with sophisticated computational and mathematical tools. The results from the three complementary experimental techniques were integrated using a Bayesian algorithm to generate a high-confidence yeast SH3 domain interaction map. We found that the interaction map was enriched for proteins involved in endocytosis. Additionally, experiments demonstrated that parts of the constructed SH3 domain interactome are actually used *in vivo* to mediate formation of several endocytic complexes, especially the SH3 domains of Lsb3p and Lsb4p.

Compared to the model organism yeast, there are considerably more PIDs, in the human proteome, which harbors approximately 300 SH3 domains. Nevertheless, Wu and co-workers have started a systematic study to identify SH3 domain-mediated human protein-protein interactions by synthetic peptide array target screening [110]. A peptide array of 1536 potential peptide ligands, derived from the SWISS-PROT database, was used to probe a group of 12 human SH3 domains. However, mapping peptide arrays derived from the total human proteome to revealing a complete human SH3 domain interactome is yet not technically feasible for the SPOT technology approach. Even applying sophisticated filtering steps, the number of peptides is still too high and unmanageable.

Screening human PDZ domains for interaction partners was performed with an array of 6223 human C-termini (11-mers) derived from the SWISS-PROT database [88]. These peptides were synthesized on a cellulose membrane using the method of inverted peptide arrays with free C-termini. Mapping was performed with a tagged PDZ domain, using several tags [88, 89, 91-94]. In analogy to the human SH3 domain dilemma, elucidation of the complete human PDZ interactome is also not feasible by standard SPOT technology. Recently, the labs of Sidhu, Bader, and Boone published impressive work where they scanned billions of random peptides with phage display to accurately map the binding specificity for approximately half of the over 330 PDZ domains in human and *Caenorhabditis elegans* proteomes [111].

7. INTERFERING WITH PROTEIN-PROTEIN INTERACTIONS FOR DRUG DISCOVERY APPLICATIONS

The majority of currently used drugs are directed towards enzymes, G-coupled receptors, carriers, nuclear hormone receptors and ion channels [112, 113]. One explanation for this preference is that those classic proteins for which small-molecule drugs have been designed bind naturally small molecules or linear peptide sequences. Unfortunately the majority of protein-protein interactions do not have natural small-molecule partners and the discovery of drugs binding those protein-protein interfaces could not start from a small natural substrate [114]. An exception that proves the rule, protein interaction domains (see chapter 2) offers the luxury to recognize natural short linear sequences [38] and, fortunately, those small size sequences are attractive leads for drug discovery [115]. Examples include inhibitors that bind different SH2 domains [116, 117] or small molecules which interfere with 14-3-3 molecules [118].

The standard-size contact surfaces of protein-protein interactions are large (1200 – 2000 Å) [119] and involve amino-acid residues that are not contiguous in the primary sequence of the protein [120]. Due to the fact that the vast majority of protein-protein interactions do not have natural small-molecule partners, high-throughput screening does not automatically identify promising interfering compounds.

The finding of the so called “hotspots” by Wells and coworkers in 1995 [121] is a remarkable breakthrough in finding small molecules or peptides that target protein-protein interfaces [122, 123]. Mutation analysis studies show that a small subset of residues belonging to the large protein-protein binding interface contributes most of the free energy of the binding. Such hotspots constitute less than half of the contact surface of a protein involved in the protein-protein interaction and are usually found at the centre of the contact interface [114]. Interestingly, systematic analysis of hotspots revealed a distinct amino acid composition with tryptophan, arginine, and tyrosine as the fundamental ones [123]. Furthermore a protein involved in a protein-protein interaction not seldom show promiscuous binding to several other targets using the same hotspot region [124]. These findings paved the way for interfering protein-protein interactions with small molecules and impressive examples are given in the review of Wells *et al.* [114] and Gerrad *et al.* [125].

CONCLUSION

Hopefully, this review illustrates that peptide arrays are a well-established screening tool for biologically active peptides. The technology has established itself as a highly flexible, robust, and reliable research method. The equipment for the SPOT technology is commercially available, does not require special conditions, and can be implemented in nearly every biochemical laboratory. However, the capability to prepare high-quality peptide arrays efficiently and economically, as well as implementing cost-effective and rapid analytical techniques to generate and process data from peptide arrays are milestones for the future development.

ACKNOWLEDGEMENTS

The author thanks the Deutsche Forschungsgemeinschaft (SFB 449, SFB/TR 19, SFB 618, VO 885/1-2, VO 885/3-2, FOR 806) and the BMBF (01EZ0737) for financial support.

REFERENCES

[1] Sudol, M. From Src Homology domains to other signaling modules: proposal of the protein recognition code. *Oncogene*, **1998**, *17*, 1469-1474.
[2] Smith, J. M. Natural selection and the concept of a protein space. *Nature*, **1970**, *225*, 563-564.
[3] Jacob, F. *The Possible and the Actual (Jessie and John Danz Lectures).* University of Washington Press: Washington, **1994**.
[4] Lichtarge, O.; Bourne, H. R.; Cohen F. E. An evolutionary trace method defines binding surfaces common to protein families. *J. Mol. Biol.*, **1996**, *257*, 342-358.
[5] Gimona, M. Protein linguistics - a grammar for modular protein assembly? *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 68-73.
[6] Searls, D. B. Linguistic approaches to biological sequences. *Comput. Appl. Biosci.*, **1997**, *13*, 333-344.
[7] Searls, D. B. The language of genes. *Nature*, **2002**, *420*, 211-217.
[8] Phizicky, E.; Bastiaens, P. I. H.; Zhu, H.; Snyder, M.; Fields, S. Protein analysis on a proteomic scale. *Nature*, **2003**, *422*, 208-215.
[9] Creighton, T. E. *Proteins Structure and Molecular Properties.* W. H. Freeman and Company: New York, **1996**.
[10] Holm, L.; Sander, C. Dictionary of recurrent domains in protein structures. *Proteins*, **1998**, *33*, 88-96.
[11] Das, S.; Smith, T.F. Identifying nature's protein Lego set. *Adv. Protein Chem.*, **2000**, *54*, 159-183.
[12] Morais Cabral, J. H.; Petosa, C.; Sutcliffe, M. J.; Raza, S.; Byron, O.; Poy, F.; Marfatia, S. M.; Chishti, A. H.; Liddington, R. C. Crystal structure of a PDZ domain. *Nature*, **1996**, *382*, 649-652.
[13] Musacchio, A.; Noble, M.; Pauptit, R.; Wierenga, R.; Saraste, M. Crystal structure of a Src-homology 3 (SH3) domain. *Nature*, **1992**, *359*, 851-855.
[14] Mayer, B. J.; Saksela, K. SH3 Domains. In: *Modular Protein Domains*, Cesareni, G.; Gimona, M.; Sudol, M.; Yaffe, M. Eds. Wiley-VCH: Weinheim, **2005**; pp. 37-58.
[15] Cesareni, G.; Panni, S.; Nardelli, G.; Castagnoli, L. Can we infer peptide recognition specificity mediated by SH3 domains? *FEBS Lett.*, **2002**, *513*, 38-44.
[16] Sadowski, I.; Stone, J. C.; Pawson, T. A noncatalytic domain conserved among cytoplasmic protein-tyrosine kinases modifies the kinase function and transforming activity of Fujinami sarcoma virus P130gag-fps. *Mol. Cell. Biol.*, **1986**, *6*, 4396-4408.
[17] Mayer, B. J.; Ren, R.; Clark, K. L.; Baltimore, D. S. A putative modular domain present in diverse signaling proteins. *Cell*, **1993**, *73*, 629-630.
[18] Ren, R.; Mayer, B. J.; Cicchetti, P.; Baltimore, D. Identification of a ten-amino acid proline-rich SH3 binding site. *Science*, **1993**, *259*, 1157-1161.
[19] Smith, G. P. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science*, **1985**, *228*, 1315-1317.
[20] Scott, J. K.; Smith, G. P. Searching for peptide ligands with an epitope library. *Science*, **1990**, 249, 386-390.
[21] Fields, S.; Song, O. A novel genetic system to detect protein-protein interactions. *Nature*, **1989**, *340*, 245-246.
[22] Lu, P. J.; Zhou, X. Z.; Shen, M.; Lu, K. P. Function of WW domains as phosphoserine- or phosphothreonine-binding modules. *Science*, **1999**, *283*, 1325-1328.
[23] Gavin, A. C.; Bösche, M.; Krause, R.; Grandi, P.; Marzioch, M.; Bauer, A.; Schultz, J.; Rick, J. M.; Michon, A. M.; Cruciat, C. M.; Remor, M.; Höfert, C.; Schelder, M.; Brajenovic, M.; Ruffner, H.; Merino, A.; Klein, K.; Hudak, M.; Dickson, D.; Rudi, T.; Gnau, V.; Bauch, A.; Bastuck, S.; Huhse, B.; Leutwein, C.; Heurtier, M. A.; Copley, R. R.; Edelmann, A.; Querfurth, E.; Rybin, V.; Drewes, G.; Raida, M.; Bouwmeester, T.; Bork, P.; Seraphin, B.; Kuster, B.; Neubauer, G.; Superti-Furga, G. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature*, **2002**, *415*, 141-147.

[24] Ho, Y.; Gruhler, A.; Heilbut, A.; Bader, G. D.; Moore, L.; Adams, S. L.; Millar, A.; Taylor, P.; Bennett, K.; Boutilier, K.; Yang, L.; Wolting, C.; Donaldson, I.; Schandorff, S.; Shewnarane, J.; Vo, M.; Taggart, J.; Goudreault, M.; Muskat, B.; Alfarano, C.; Dewar, D.; Lin, Z.; Michalickova, K.; Willems, A. R.; Sassi, H.; Nielsen, P. A.; Rasmussen, K. J.; Andersen, J.R.; Johansen, L. E.; Hansen, L. H.; Jespersen, H.; Podtelejnikov, A.; Nielsen, E.; Crawford, J.; Poulsen, V.; Sørensen, B. D.; Matthiesen, J.; Hendrickson, R. C.; Gleeson, F.; Pawson, T.; Moran, M. F.; Durocher, D.; Mann, M.; Hogue. C. W.; Figeys, D.; Tyers, M. Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry. *Nature*, **2002**, *415*, 180-183.

[25] Linding, R.; Letunic, I.; Gibson, T. J.; Bork, P. Computational Analysis of Modular Protein Architectures. In: *Modular Protein Domains*, Cesareni, G.; Gimona, M.; Sudol, M.; Yaffe, M. Eds. Wiley-VCH: Weinheim, **2005**; pp. 439-476.

[26] http://smart.embl-heidelberg.de

[27] MacBeath, G.; Schreiber, S. L. Printing proteins as microarrays for high-throughput function determination. *Science*, **2000**, *289*, 1760-1763.

[28] Zhu, H.; Bilgin, M.; Bangham, R.; Hall, D.; Casamayor, A.; Bertone, P.; Lan, N.; Jansen, R.; Bidlingmaier, S.; Houfek, T.; Mitchell, T.; Miller, P.; Dean, R. A.; Gerstein, M.; Snyder, M. Global analysis of protein activities using proteome chips. *Science*, **2001**, *293*, 2101-2105.

[29] Zhu, H.; Klemic, J. F.; Chang, S.; Bertone, P.; Casamayor, A.; Klemic, K. G.; Smith, D.; Gerstein, M.; Reed, M. A.; Snyder, M. Analysis of yeast protein kinases using protein chips. *Nat. Genet.*, **2000**, *26*, 283-289.

[30] Frank, R. SPOT-synthesis: An easy technique for the positionally addressable, parallel chemical synthesis on a membrane support. *Tetrahedron*, **1992**, *48*, 9217.

[31] Volkmer, R. Synthesis and application of peptide arrays: Quo vadis SPOT technology. *ChemBioChem.*, **2009**, *10*, 1431-1442.

[32] Pawson, T.; Scott, J. D. Protein phosphorylation in signaling--50 years and counting. *Trends Biochem. Sci.*, **2005**, *30*, 286-290.

[33] Linding, R.; Jensen, L. J.; Ostheimer, G. J.; van Vugt, M. A.; Jørgensen, C.; Miron, I. M.; Diella, F.; Colwill, K.; Taylor, L.; Elder, K.; Metalnikov, P.; Nguyen, V.; Pasculescu, A.; Jin, J.; Park, J.G.; Samson, L. D.; Woodgett, J. R.; Russell, R. B.; Bork, P.; Yaffe, M. B.; Pawson, T. Systematic discovery of in vivo phosphorylation networks. *Cell*, **2007**, *129*, 1415-1426.

[34] Garcia, B. A.; Shabanowitz, J.; Hunt, D. F. Characterization of histones and their post-translational modifications by mass spectrometry. *Curr. Opin. Chem. Biol*., **2007**, *11*, 66-73.

[35] Trelle, M. B.; Jensen, O. N. Functional proteomics in histone research and epigenetics. *Expert. Rev. Proteomics*, **2007**, *4*, 491-503.

[36] Jensen, O. N. Interpreting the protein language using proteomics. *Nat. Rev. Mol. Cell. Biol.*, **2006**, *7*, 391-403.

[37] Larsen, M. R.; Trelle, M. B.; Thingholm, T. E.; Jensen, O. N. Analysis of posttranslational modifications of proteins by tandem mass spectrometry. *Biotechniques*, **2006**, *40*, 790-798.

[38] Seet, B. T.; Dikic, I.; Zhou, M. M.; Pawson, T. Reading protein modifications with interaction domains. *Nat. Rev. Mol. Cell. Biol.*, **2006**, *7*, 473-483.

[39] Pawson, T.; Nash, P. Assembly of cell regulatory systems through protein interaction domains. *Science*, **2003**, *300*, 445-452.

[40] Pawson, T. Protein modules and signalling networks. *Nature*, **1995**, *373*, 573-580.

[41] Ladbury, J. E.; Arold, S. Searching for specificity in SH domains. *Chem. Biol*., **2000**, *7*, R3-R8.

[42] Songyang, Z.; Shoelson, S. E.; Chaudhuri, M.; Gish, G.; Pawson, T.; Haser, W. G.; King, F.; Roberts, T.; Ratnofsky, S.; Lechleider, R. J. SH2 domains recognize specific phosphopeptide sequences. *Cell*, **1993**, *72*, 767-778.

[43] Lee, C. H.; Leung, B.; Lemmon, M. A.; Zheng, J.; Cowburn, D.; Kuriyan, J.; Saksela, K. A single amino acid in the SH3 domain of Hck determines its high affinity and specificity in binding to HIV-1 Nef protein. *EMBO J.*, **1995**, *14*, 5006-5015.

[44] Arold, S.; O'Brien, R.; Franken, P.; Strub, M. P.; Hoh, F.; Dumas, C.; Ladbury, J. E. RT loop flexibility enhances the specificity of Src family SH3 domains for HIV-1 Nef. *Biochemistry*, **1998**, *37*, 14683-14691.

[45] Zarrinpar, A.; Park, S.-H.; Lim, W. A. Optimization of specificity in a cellular protein interaction network by negative selection. *Nature* **2003**, *426*, 676-680.

[46] Hiipakka, M.; Poikonen, K.; Saksela, K.-D. SH3 domains with high affinity and engineered ligand specificities targeted to HIV-1 Nef. *J. Mol. Biol.*, **1999**, *293*, 1097-1106.

[47] Mayer, B. J. SH3 domains: complexity in moderation. *J. Cell. Sci.*, **2001**, *114*, 1253-1263.

[48] Jørgensen, C.; Linding, R. Simplistic pathways or complex networks? *Curr. Opin. Genet. Dev*., **2010**, *20*, 15-22.

[49] Ito, T.; Chiba, T.; Ozawa, R.; Yoshida, M.; Hattori, M.; Sakaki, Y. A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc. Natl. Acad. Sci. U.S.A.*, **2001**, *98*, 4569-4574.

[50] Uetz, P.; Giot, L.; Cagney, G.; Mansfield, T. A.; Judson, R. S.; Knight, J. R.; Lockshon, D.; Narayan, V.; Srinivasan, M.; Pochart, P.; Qureshi-Emili, A.; Li, Y.; Godwin, B.; Conover, D.; Kalbfleisch, T.; Vijayadamodar, G.; Yang, M.; Johnston, M.; Fields, S.; Rothberg, J. M. A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. *Nature*, **2000**, *403*, 623-627.

[51] Tong, A. H. Y.; Drees, B.; Nardelli, G.; Bader, G. D.; Brannetti, B.; Castagnoli, L.; Evangelista, M.; Ferracuti, S.; Nelson, B.; Paoluzi, S.; Quondam, M.; Zucconi, A.; Hogue, C. W. V.; Fields, S.; Boone, C.; Cesareni, G. A combined experimental and computational strategy to define protein interaction networks for peptide recognition modules. *Science*, **2002**, *295*, 321-324.

[52] Landgraf, C.; Panni, S.; Montecchi-Palazzi, L.; Castagnoli, L.; Schneider-Mergener, J.; Volkmer-Engert, R. Cesareni, G. Protein interaction networks by proteome Peptide scanning. *PLoS Biol.*, **2004**, *2*, E14.

[53] Castagnoli, L.; Costantini, A.; Dall'Armi, C.; Gonfloni, S.; Montecchi-Palazzi, L.; Panni, S.; Paoluzi, S.; Santonico, E.; Cesareni, G. Selectivity and promiscuity in the interaction network mediated by protein recognition modules. *FEBS Lett.*, **2004**, *567*, 74-79.

[54] Tonikian, R.; Xin, X.; Toret, C. P.; Gfeller, D.; Landgraf, C.; Panni, S.; Paoluzi, S.; Castagnoli, L.; Currell, B.; Seshagiri, S.; Yu, H.; Winsor, B.; Vidal, M.; Gerstein, M. B.; Bader, G. D. Volkmer, R.; Cesareni, G.; Drubin, D. G.; Kim, P. M.; Sidhu, S. S.; Boone, C. Bayesian modeling of the yeast SH3 domain interactome predicts spatiotemporal dynamics of endocytosis proteins. *PLoS Biol*., **2009**, *7*, e1000218.

[55] Wenschuh, H.; Volkmer-Engert, R.; Schmidt, M.; Schulz, M.; Schneider-Mergener, J.; Reineke, U. Coherent membrane supports for parallel microsynthesis and screening of bioactive peptides. *Biopolymers*, **2000**, *55*, 188-206.

[56] Reineke, U.; Volkmer-Engert, R.; Schneider-Mergener, J. Applications of peptide arrays prepared by the SPOT-technology. *Curr. Opin. Biotechnol.* **2001**, *12*, 59-64.

[57] Reineke, U. Antibody epitope mapping using de novo generated synthetic peptide libraries. *Methods Mol. Biol.*, **2009**, *524*, 203-211.

[58] van Regenmortel, M. H. V. In: *Immunochemistry*, van Oss, C. J.; van Regenmortel, M. H. V. Eds. Marcel Dekker, Inc.: New York, **1994**.

[59] Frank, R.; Overwin, H. SPOT synthesis. Epitope analysis with arrays of synthetic peptides prepared on cellulose membranes. *Methods Mol. Biol.*, **1996**, *66*, 149-169.

[60] Geysen, H. M.; Meloen, R. H.; Barteling, S. J. Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proc. Natl. Acad. Sci. USA*, **1984**, *81*, 3998-4002.

[61] Rottensteiner, H.; Kramer, A.; Lorenzen, S.; Stein, K.; Landgraf, C.; Volkmer-Engert, R.; Erdmann, R. Peroxisomal membrane proteins contain common Pex19p-binding sites that are an integral part of their targeting signals. *Mol. Biol. Cell.*, **2004**, *15*, 3406-3417.

[62] Schell-Steven, A.; Stein, K.; Amoros, M.; Landgraf, C.; Volkmer-Engert, R.; Erdmann, R.; Rottensteiner, H. Identification of a novel, intraperoxisomal pex14-binding site in pex13: association of pex13 with the docking complex is essential for peroxisomal matrix protein import. *Mol. Cell Biol.*, **2005**, *25*, 3007-3018.

[63] Saveria, T.; Halbach, A.; Erdmann, R.; Volkmer-Engert, R.; Landgraf, C.; Rottensteiner, H.; Parsons, M. Conservation of PEX19-binding motifs required for protein targeting to mammalian peroxisomal and trypanosome glycosomal membranes. *Eukaryot. Cell*, **2007**, *6*, 1439-1449.

[64] Schreiber, S.; Stengel, R.; Westermann, M.; Volkmer-Engert, R.; Pop, O. I.; Müller, J.P. Affinity of TatCd for TatAd elucidates its receptor function in the *Bacillus subtilis* twin arginine translocation (Tat) translocase system. *J. Biol. Chem.*, **2006**, *281*, 19977-19984.

[65] Pop, O. I.; Westermann, M.; Volkmer-Engert, R.; Schulz, D.; Lemke, C.; Schreiber, S.; Gerlach, R.; Wetzker, R.; Muller, J. P. Sequence-specific binding of prePhoD to soluble TatAd indicates protein-mediated targeting of the Tat export in Bacillus subtilis. *J. Biol. Chem.*, **2003**, *278*, 38428-38436.

[66] Blüschke, B.; Eckey, V.; Kunert, B.; Berendt, S.; Landmesser, H.; Portwich, M.; Volkmer, R.; Schneider, E. Mapping putative contact sites between subunits in a bacterial ATP-binding cassette (ABC) transporter by synthetic peptide libraries. *J. Mol. Biol.*, **2007**, *369*, 386-399.

[67] Blüschke, B.; Volkmer-Engert, R.; Schneider, E. Topography of the surface of the signal transducing protein EIIA that interacts with the MALK subunit of the maltose ATP-binding casette transporter ($MALFGK_2$) of salmonella typhimurium. *J. Biol. Chem.*, **2006**, *281*, 12833-12840.

[68] Reineke, U.; Hoffmüller, U.; Schneider-Mergener J. Patent DE 198 31 429 A1, 2000.

[69] Meloen, R. H.; Langeveld, J. P.; Schaaper, W. M.; Slootsta, J. W. Synthetic peptide vaccines: unexpected fulfillment of discarded hope. *Biologicals*, **2001**, *29*, 233-236.

[70] Cunningham, B. C.; Wells, J. A. High-resolution epitope mapping of hGH-receptor interactions by alanine-scanning mutagenesis. *Science*, **1989**, *244*, 1081-1085.

[71] Bolger, G. B.; Baillie, G. S.; Li, X.; Lynch, M. J.; Herzyk, P.; Mohamed, A.; Mitchell, L. H.; McCahill, A.; Hundsrucker, C.; Klussmann, E.; Adam, D. R.; Houslay, M. D. Scanning peptide array analyses identify overlapping binding sites for the signalling scaffold proteins, beta-arrestin and RACK1, in cAMP-specific phosphodiesterase PDE4D5. *Biochem. J*., **2006**, *398*, 23-36.

[72] Liang, M.; Mahler, M.; Koch, J.; Ji, Y.; Li, D.; Schmaljohn, C.; Bautz, E. K. F. Generation of an HFRS patient-derived neutralizing recombinant antibody to Hantaan virus G1 protein and definition of the neutralizing domain. *J. Med. Virol.*, **2003**, *69*, 99-107.

[73] Blüthner, M.; Koch, J.; Mahler, M. Mutational analysis and structure predictions. In: *Peptide Arrays on Membrane Supports – Synthesis and Applica-*

tions, Koch, J.; Mahler, M. Eds. Springer Verlag: Heidelberg, **2002**; pp. 123-139.

[74] Wälchli, S.; Espanel, X.; Harrenga, A.; Rossi, M.; Cesareni, G.; van Huijsduijnen, R. H. Probing protein-tyrosine phosphatase substrate specificity using a phosphotyrosine-containing phage library. *J. Biol. Chem.*, **2004**, *279*, 311-318.

[75] Williams, A. D.; Portelius, E.; Kheterpal, I.; Guo, J. T.; Cook, K. D.; Xu, Y.; Wetzel, R. Mapping abeta amyloid fibril secondary structure using scanning proline mutagenesis. *J. Mol. Biol*., **2004**, *335*, 833-842.

[76] Podolnikova, N. P.; Gorkun, O. V.; Loreth, R. M.; Yee, V. C.; Lord, S. T.; Ugarova, T. P. A cluster of basic amino acid residues in the gamma370-381 sequence of fibrinogen comprises a binding site for platelet integrin alpha(IIb)beta3 (glycoprotein IIb/IIIa). *Biochemistry*, **2005**, *44*, 16920-16930.

[77] Espanel, X.; Sudol, M. Yes-associated protein and p53-binding protein-2 interact through their WW and SH3 domains. *J. Biol. Chem.*, **2001**, *276*, 14514-14523.

[78] Espanel, X.; Huguenin-Reggiani, M.; van Huijsduijnen, R. H. The SPOT technique as a tool for studying protein tyrosine phosphatase substrate specificities. *Protein Sci.*, **2002**, *11*, 2326-2334.

[79] Reineke, U.; Schneider-Mergener, J.; Schutkowski, M. Peptide arrays in proteomics and drug discovery. In: *BioMEMS and Biomedical Nanotechnology, Vol.II Micro and Nano-technologies for Genomics and Proteomics*, Ozkan, M.; Heller, M. J. Eds. Springer: New York, **2005**, pp. 161-282.

[80] Przezdziak, J.; Tremmel, S.; Kretzschmar, I.; Beyermann, M.; Bienert, M.; Volkmer, R. Probing the ligand binding specificity and analyzing the folding state of SPOT synthesized FBP28 WW domain variants. *ChemBioChem.*, **2006**, *7*, 780-788.

[81] Portwich, M.; Keller, S.; Strauss, H. M.; Mahrenholz, C. C.; Kretzschmar, I.; Kramer, A.; Volkmer, R. A network of coiled coil associations derived from synthetic GCN4 leucine zipper arrays. *Angew. Chem. Int. Ed*., **2007**, *46*, 1654-1657.

[82] Toepert, F.; Pirés, J. R.; Landgraf, C.; Oschkinat, H.; Schneider-Mergener, J. Synthesis of an array comprising 837 variants of the hYAP WW protein domain. *Angew. Chem. Int. Ed*., **2001**, *40*, 897-900.

[83] Jemth, P.; Gianni, S. PDZ domains: folding and binding. *Biochemistry*, **2007**, *46*, 8701-8708.

[84] Hung, A.Y.; Sheng, M. PDZ domains: structural modules for protein complex assembly. *J. Biol. Chem.*, **2002**, *277*, 5699-5702.

[85] Nourry, C.; Grant, S. G.; Borg, J. P. PDZ domain proteins: plug and play! *Sci. STKE* **2003**, *2003*, RE7.

[86] Sheng, M.; Sala, C. PDZ domains and the organization of supramolecular complexes. *Annu. Rev. Neurosci*., **2001**, *24*, 1-29.

[87] van Ham, M.; Hendriks, W. PDZ domains-glue and guide. *Mol. Biol. Rep*., **2003**, *30*, 69-82.

[88] Boisguerin, P.; Leben, R.; Ay, B.; Radziwill, G.; Moelling, K.; Volkmer-Engert, R. An improved method for the synthesis of cellulose membrane-bound peptides with free C termini is useful for PDZ domain binding studies. *Chem. Biol.*, **2004**, *11*, 449-459.

[89] Boisguerin, P.; Ay, B.; Radziwill, G.; Fritz, R. D.; Moelling, K.; Volkmer, R. Characterization of a putative phosphorylation switch: adaptation of SPOT synthesis to analyze PDZ domain regulation mechanisms. *ChemBioChem.*, **2007**, *8*, 2302-2307.

[90] Licha, K.; Bhargava, S.; Rheinländer, C.; Becker, A.; Schneider-Mergener, J.; Volkmer-Engert, R. Highly parallel nano-synthesis of cleavable peptide-dye conjugates on cellulose membranes. *Tetrahedon Lett*., **2000**, *41*, 1711-1715.

[91] Wiedemann, U.; Boisguerin, P.; Leben, R.; Leitner, D.; Krause, G.; Moelling, K.; Volkmer-Engert, R.; Oschkinat, H. Quantification of PDZ domain specificity, prediction of ligand affinity and rational design of super-binding peptides. *J. Mol. Biol*., **2004**, *343*, 703-718.

[92] Joshi, M.; Vargas, C.; Boisguerin, P.; Diehl, A.; Krause, G.; Schmieder, P.; Moelling, K.; Hagen, V.; Schade, M.; Oschkinat, H. Discovery of low-molecular-weight ligands for the AF6 PDZ domain. *Angew. Chem. Int. Ed*., **2006**, *45*, 3790-3795.

[93] Radziwill, G.; Weiss, A.; Heinrich, J.; Baumgartner, M.; Boisguerin, P.; Owada, K.; Moelling, K. Regulation of c-Src by binding to the PDZ domain of AF-6. *EMBO J.*, **2007**, *26*, 2633-2644.

[94] Cushing, P. R.; Fellows, A.; Villone, D.; Boisguérin, P.; Madden, D. R. The relative binding affinities of PDZ partners for CFTR: a biochemical basis for efficient endocytic recycling. *Biochemistry*, **2008**, *47*, 10084-10098.

[95] Pires, J. R.; Hong, X.; Brockmann, C.; Volkmer-Engert, R.; Schneider-Mergener, J.; Oschkinat, H.; Erdmann, R. The ScPex13p SH3 domain exposes two distinct binding sites for Pex5p and Pex14p. *J. Mol. Biol.*, **2003**, *326*, 1427-1435.

[96] Volkmer-Engert, R.; Hoffman, B.; Schneider-Mergener, J. Stable attachment of the HMB-linker to continuous cellulose membranes for parallel solid phase spot synthesis. *Tetrahedron Lett*., **1997**, *38*, 1029-1032.

[97] Halbach, A.; Lorenzen, S.; Landgraf, C.; Volkmer-Engert, R.; Erdmann, R.; Rottensteiner, H. Function of the PEX19-binding site of human adrenoleukodystrophy protein as targeting motif in man and yeast. PMP targeting is evolutionarily conserved. *J. Biol. Chem*., **2005**, *280*, 21176-21182.

[98] Fest, S.; Huebener, N.; Weixler, S.; Bleeke, M.; Zeng, Y.; Strandsby, A.; Volkmer-Engert, R.; Landgraf, C.; Gaedicke, G.; Riemer, A. B.; Michalsky, E.; Jaeger, I. S.; Preissner, R.; Forster-Wald, E.; Jensen-Jarolim, E.; Lode, H. N. Characterization of GD2 peptide mimotope DNA vaccines effective against spontaneous neuroblastoma metastases. *Cancer Res*., **2006**, *66*, 10567-10575.

[99] The MultiPep SPOT is the first commercial available fully automated SPOT robot system (Intavis Bioanalytical Instruments AG, Köln, Germany).

[100] Bowman, M. D.; Jeske, R. C.; Blackwell, H. E. Microwave-accelerated SPOT-synthesis on cellulose supports. *Org. Lett.*, **2004**, *6*, 2019-2022.

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

[102] AIMS Scientific Products, Braunschweig, Germany, http://www.aims-scientific-products.de

[103] Pawson; T.; Raina, M.; Nash, P. Interaction domains: from simple binding events to complex cellular behaviour. *FEBS Lett.*, **2002**, *513*, 2-10.

[104] Reimer, U.; Reineke, U. Schneider-Mergener, J. Peptide arrays: from macro to micro. *Curr. Opin. Biotechnol*., **2002**, *13*, 315-320.

[105] Volkmer, R. Peptide Chips. In: *Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine, Volume 2*. Ganten, D.; Ruckpaul, K. Eds. Springer: Berlin, Heidelberg, New York **2006**, 2, pp. 1372-1377.

[106] Fodor, S. P.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. Light-directed, spatially addressable parallel chemical synthesis. *Science*, **1991**, *251*, 767-773.

[107] Beyer, M.; Nesterov, A.; Block, I.; König, K.; Felgenhauer, T.; Fernandez, S.; Leibe, K.; Torralba, G.; Hausmann, M.; Trunk, U.: Lindenstruth, V.; Bischoff, F. R.; Stadler, V.; Breitling, F. Combinatorial synthesis of peptide arrays onto a microchip. *Science*, **2007**, *318*, 1888.

[108] Stadler, V.; Felgenhauer, T.; Beyer, M.; Fernandez, S.; Leibe, K.; Güttler, S.; Gröning, M.; König, K.; Torralba, G.; Hausmann, M.; Lindenstruth, V.; Nesterov, A.; Block, I.; Pipkorn, R.; Oustka, A.; Bischoff, F. R.; Breitling, F. Combinatorial synthesis of peptide arrays with a laser printer. *Angew. Chem. Int. Ed.*, **2008**, *47*, 7132-7135.

[109] Tonikian, R.; Zhang, Y.; Boone, C.; Sidhu, S. S. Identifying specificity profiles for peptide recognition modules from phage-displayed peptide libraries. *Nat. Protoc*., **2007**, *2*, 1368-1386.

[110] Wu, C.; Ma, M. H.; Brown, K. R.; Geisler, M.; Li, L.; Tzeng, E.; Jia, C. Y. H.; Jurisica, I.; Li, S. S.-C. Systematic identification of SH3 domain-mediated human protein-protein interactions by peptide array target screening. *Proteomics*, **2007**, *7*, 1775-1785.

[111] Tonikian, R.; Zhang, Y.; Sazinsky, S. L.; Currell, B.; Yeh, J. H.; Reva, B.; Held, H. A.; Appleton, B. A.; Evangelista, M.; Wu, Y.; Xin, X.; Chan, A. C.; Seshagiri, S.; Lasky, L. A.; Sander, C.; Boone, C.; Bader, G. D.; Sidhu, S. S. A specificity map for the PDZ domain family. *PLoS Biol*., **2008**, *6*, e239.

[112] Hopkins, A. L.; Groom, C. R. The druggable genome. *Nat. Rev. Drug Discov.*, **2002**, *1*, 727-730.

[113] Betz, U. A.; Farquhar, R.; Ziegelbauer K. Genomics: success or failure to deliver drug targets? *Curr. Opin. Chem. Biol.*, **2005**, *9*, 387-391.

[114] Wells, J.A.; McClendon, C. L. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature*, **2007**, *450*, 1001-1009.

[115] Wilson, A. J. Inhibition of protein-protein interactions using designed molecules. *Chem. Soc. Rev.*, **2009**, *38*, 3289-3300.

[116] Shakespeare, W. C. SH2 domain inhibition: a problem solved? *Curr. Opin. Chem. Biol.*, **2001**, *5*, 409-415.

[117] Toogood, P. L. Inhibition of protein-protein association by small molecules: approaches and progress. *J. Med. Chem.*, **2002**, *45*, 1543-1558.

[118] Rose, R.; Erdmann, S.; Bovens S.; Wolf, A.; Rose, M.; Henning S.; Waldmann, H.; Ottmann, C. Identification and structure of small-molecule stabilizers of 14-3-3 protein-protein interactions. *Angew. Chem. Int. Ed*., **2010**, *49*, 4129-4132.

[119] Horton, N.; Lewis, M. Calculation of the free energy of association for protein complexes. *Protein Sci.*, **1992**, *1*, 169-181.

[120] Conte, L. L.; Chothia, C.; Janin, J. The atomic structure of protein-protein recognition sites. *J. Mol. Biol.*, **1999**, *285*, 2177-2198.

[121] Clackson, T.; Wells, J. A. A hot spot of binding energy in a hormone-receptor interface. *Science*, **1995**, *267*, 383-386.

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

[123] Moreira, I. S.; Fernandes, P. A.; Ramos, M. J. Hot spots – A review of the protein-protein interface determinant amino-acid residues. *Proteins*, **2007**, *68*, 803-812.

[124] DeLano, W. L.; Ultsch, M. H.; de Vos, A. M.; Wells, J. A. Convergent solutions to binding at a protein-protein interface. *Science*, **2000**, *287*, 1279-1283.

[125] Gerrard, J. A.; Hutton, C. A.; Perugini, M. A. Inhibiting protein-protein interactions as an emerging paradigm for drug discovery. *Mini Rev. Med. Chem.*, **2007**, *7*, 151-157.

Received: April 06, 2010 Revised: July 07, 2010 Accepted: July 12, 2010