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Peptide arrays: towards routine implementation

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Peptide arrays have attracted wide interest as tools for discovering biochemical interactions. Because many protein binding and enzyme activities are directed towards peptides, the preparation of arrays having hundreds to thousands of immobilized peptides offers an unprecedented opportunity for identifying interactions of proteins. This short opinion reviews recent progress in the preparation of peptide arrays and their use in the characterization of biomolecular interactions and the discovery of new reagents for biological researches. This body of work establishes the feasibility of this technology and suggests that it will find much wider use in research groups.

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Current Opinion in Chemical Biology 2004, **8**:554–558

This review comes from a themed section on
Analytical techniques
Edited by Renato Zenobi and Fred Regnier

Available online 23rd August 2004

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2004.08.007

Introduction

Proteins play key roles in essentially all cellular processes. These roles include the cell-surface receptors that mediate cell adhesion, the molecular recognition events by which adaptor proteins localize biochemical activities, and the enzymatic activities that underlie metabolic function and signal transduction. In many cases, the biological activities of the proteins can be recapitulated by shorter peptides that are taken from the primary sequences (although often with partial loss of activity). Hence, arrays of peptides can serve as valuable tools for identifying biologically active motifs and for profiling cellular activities. In this review, we provide an overview of important work over the past three years that has contributed to the development and use of peptide arrays (Figure 1).

The excitement for peptide microarrays — together with protein, carbohydrate and small-molecule arrays — stems from the impact that oligonucleotide arrays have had in basic and applied research. A significant characteristic of the arrays is that they allow biochemical interactions to be identified from thousands of possibilities and have in turn motivated many researchers to pursue discovery-driven, rather than hypothesis-driven, research. An important difference between peptide arrays and the oligonucleotide arrays is found in the range of applications they can be used to address. DNA arrays are used almost entirely to profile mRNA populations in cells and rely exclusively on hybridization of sample DNA with the immobilized DNA. Peptide arrays, by contrast, can be used to screen diverse interactions, including the binding of proteins, the action of enzymes, the adhesion of cells, the binding of metals, and many others.

The diverse range of applications of peptide arrays — and the different protocols that are specific to each application — has in part slowed the implementation of these tools. Further issues that have made peptide arrays more challenging to implement include the expense of preparing large numbers of peptides, the development of chemistries and supports that give good control in presenting immobilized peptides, the prevention of non-specific interactions at the surface, and the development of analytical methods to detect activities on the chips. This opinion reviews recent work that has made significant progress in each of these themes and suggests areas that will benefit from further development.

Preparation of peptide arrays

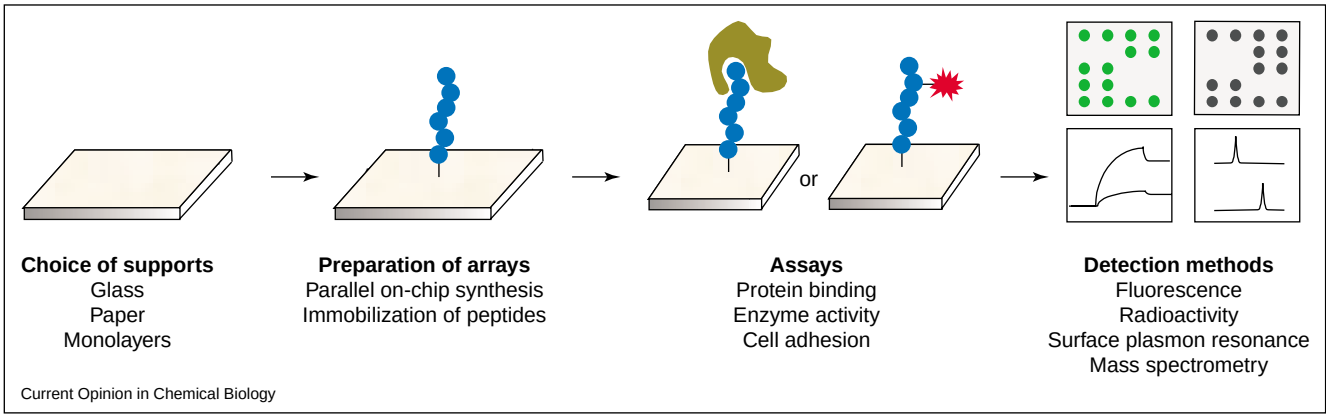
Several methods have been reported for the preparation of peptide arrays. These methods can be classified by the assembly of peptides on the surface — *in situ* peptide synthesis and immobilization of functionalized peptides onto chips — and by the properties of the support. We next describe recent progress in these developments and outline the considerations that dictate which approaches are best-suited for a particular application.

Parallel on-chip synthesis

The *in situ* synthetic approaches have the primary benefit that they avoid conventional synthesis of each of the peptide sequences that are presented in the array. The expense and time required to prepare hundreds of peptides — including the purification of those peptides before immobilization — prohibit routine applications. These considerations have motivated the development of the *in situ* approaches, which benefit from using small amounts of reagents and avoiding purification and immobilization of peptides.

The two general approaches for *in situ* peptide synthesis are photolithographic synthesis and SPOT synthesis. The

Figure 1



Aspects of peptide arrays described in this review.

photolithographic approach was first reported by Fodor *et al.* in 1991 [1]. This group translated solid phase methods for peptide synthesis directly to the array support by developing photolabile protecting groups that would allow synthesis to proceed only at regions of the surface that are illuminated. This group demonstrated the preparation of an array containing 1024 peptides by using photolithographic masks to repeatedly deprotect selected regions of the array for addition of amino acids. Since this first report, several improved photolithographic synthesis methods have been reported, including the use of photoresist films reported by McGall *et al.* in 1996 [2] and development of maskless array synthesis by Singh-Gasson *et al.* in 1999 [3]. This latter development was exciting because the use of a micro-mirror array served as a virtual mask for the photolithographic exposures, leading to lower expense, faster throughput and higher densities in array preparation. While these two reports were directed at the preparation of oligonucleotide arrays, there is a clear route to their application for peptide arrays. More recently, Pellois *et al.* [4••] reported a strategy that could use conventional amino acid building blocks — based on the acid-labile tert-butoxycarbonyl group — for *in situ* peptide synthesis. This strategy relied on the use of a photoacid that gives chemical deprotection of the peptide in regions of illumination. Taken together, these reports advanced the photolithographic preparation of peptide arrays. The requirement for light sources, masks and optics, however, can still discourage many researchers from accessing these methods.

The second technique used for *in situ* peptide synthesis is the SPOT method, first reported by Frank in 1992 [5,6]. In the SPOT synthesis, peptides are synthesized by sequential spotting of small (typically microliter) volumes of activated amino acids to a porous membrane. A benefit of the SPOT method is that it is based on the same chemistry used in conventional solid-phase synthesis. Further, the arrays may be assembled either by manual or robotic distribution of reagents, making this method accessible to researchers in the biological sciences. The SPOT synthesis and applications have recently been reviewed by Frank [7••]. In a recent report in 2003, Toepert *et al.* [8] described a strategy that combines SPOT synthesis and chemical ligation to generate large peptide arrays which present 38-mer peptides.

Immobilization of peptides

The alternative method to *in situ* peptide synthesis relies on immobilization of peptides that have been previously synthesized by chemical or recombinant methods. This strategy is important when small numbers of peptides are needed in the array or when the peptides will be used to prepare large numbers of identical arrays, situations that can justify the expense of peptide synthesis and purification.

There are many strategies available for immobilizing peptides. For arrays, it is important that the method not require tedious protocols to chemically modify peptides with tags for reaction with a support. The use of terminal cysteine residues provides an effective route and can be used in several schemes. In one example, Falsey *et al.* [9] reported the immobilization of a cysteine-terminated peptide to glyoxylyl glass surfaces, by way of a thiazolidine ring adduct. The reaction of cysteine residues with surfaces presenting maleimide groups [10], bromomethylketone groups [11], and disulfides [12•] also provide reliable methods for immobilization. Non-natural side chain functionalities can also be used, and are best when they can be incorporated during the solid-phase synthesis of the peptide. Salisbury *et al.*, for example, reported the use of an alkoxyamine-terminated peptide for immobilization to aldehyde derivatized surfaces [13•] and Lesaicherre *et al.* reported the use of biotinylated peptides for immobilization to avidin-coated glass slides

[14]. This latter group has also applied the native chemical ligation approach to immobilize cysteine-terminated peptides to a glass surface presenting a thioester [15]. Strategies that require synthetic modification of the peptide are less useful for routine preparation of peptide arrays, but may be important in applications that require high selectivity in the immobilization reaction [16,17••].

Choice of support

The choice of the material that serves as the support for the peptide array has received relatively little attention. Yet, the properties of the support can be critical to the performance of the arrays in diverse applications. Supports that are inert toward the non-specific adsorption of protein, for example, would both reduce the level of false-positive interactions in assays and ensure that a higher fraction of the immobilized peptides are available for interactions with soluble probes. A common strategy to prevent these false-positive results is to treat the array with a blocking protein that will adsorb and prevent further unwanted adsorption in an assay. The consequence of this approach is that the proteins can also block the interactions of immobilized peptides. Self-assembled monolayers of alkanethiolates on gold that present oligo(ethylene glycol) groups have emerged as highly effective inert surfaces that avoid the need for customary blocking procedures [18].

Peptide arrays are most important in experiments that aim to identify new interactions from many possibilities. In practice, the arrays are used qualitatively and must be followed with conventional (often solution-phase experiments) to quantitatively characterize the interactions. We have demonstrated that the use of surface chemistries that present the immobilized peptides in a uniform environment on an otherwise inert background enables quantitative analysis of interactions directly on the surface [17••]. This example illustrated the measurement of inhibition constants of src kinase by small molecules. We expect that the use of this and analogous strategies will be important to both simplifying the use of peptide arrays and improving the quality of data obtained with the assays. A related issue in peptide arrays is control over the density of peptide ligands in each region. Arrays that guarantee a uniform density of peptides in each spot would enable direct comparisons of relative activities across the array. In practice, the densities can vary by an order of magnitude, making these comparisons difficult. Further, in assays of polyvalent interactions — i.e. identifying ligands for cell adhesion — the activity of the peptides is highly dependent on the density [19]. Control over density is clearly an important goal in further development of peptide arrays.

The choice of support also determines the analytical methods that can be used to analyze the array after an experiment. The use of radioactive and fluorescent labels has been the most important, but recent work has introduced 'label-free' methods that offer wider utility. These latter methods have the principal advantages that the assays are more straightforward — because they avoid the steps associated with applying and processing the label — and they can identify unanticipated activities. One technique under development is an imaging form of surface plasmon resonance (SPR) spectroscopy. The Corn group has quantitatively characterized binding of anti-FLAG antibodies to FLAG peptides using SPR imaging [12•]. A second approach is based on mass spectrometry methods, which are applicable to identifying proteins that bind to an array and enzymatic activities that modify the peptides. We have shown that MALDI-TOF MS is particularly well-suited to the characterization of enzyme activities [20••,21,22] (Figure 2). Nelson *et al.* [23] reviewed a method combining SPR and MALDI-TOF MS to analyze biomolecular interactions on the chips. In another example of label-free detection, Kroger *et al.* [24] utilized reflectometric interference spectroscopy to read changes in reflectance pattern when the optical thickness changes from the binding of biomolecules to the surface.

Applications

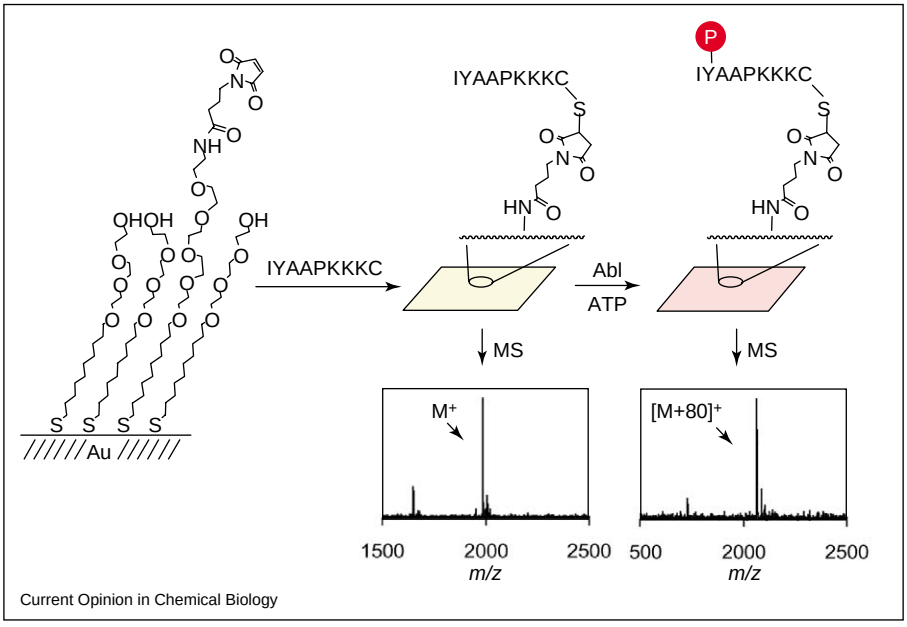
Identification of binding interactions

One of the important biological applications of peptide arrays aims to characterize binding events of proteins with peptide ligands. In one example, Usui *et al.* characterized binding of α-amylase, trypsin, calmodulin and myosin using arrays presenting 250 different peptides [25]. This group demonstrated that lysozyme, glucosidase, galactosidase, BSA, α-amylase and trypsin each displayed unique fingerprint patterns that could be used to correlate the identity of the bound proteins. Peptide arrays have also been used in diagnostic applications on clinical samples. In one example, Duburcq *et al.* [26] characterized the binding of antibodies to an array with an immunofluorescence assay and went on to apply the method to serodetection of antibodies directed against hepatitis B/C viruses, human immunodeficiency virus, Epstein-Barr virus and syphilis antigens. In another example, Boisguerin *et al.* [27•] developed a method to construct cellulose-bound peptide arrays to study recognition of PDZ domains. This latter study accomplished the immobilization of peptides at the N-termini, which was necessary to preserve the C-terminal motifs that are bound by the PDS domains.

Development of reagents

The discovery of inhibitors for enzymes and the identification of active substrates for enzymes are very important in basic biology and drug discovery. Huang *et al.* [28] reported the use of peptide arrays to optimize peptide inhibitors acting against β-lactamases and identified a hexa-peptide that inhibits broad classes of β-lactamases with activities in the 100 μM range. In an example that used peptide arrays to identify enzyme substrates,

Figure 2



Design of peptide arrays for characterizing enzyme activities by MALDI-TOF MS. A peptide substrate for Abl kinase was immobilized to a maleimide-presented self-assembled monolayer (SAM). The monolayer was treated with Abl kinase and phosphorylation was characterized by MALDI-TOF MS.

Salisbury *et al.* [13•] developed peptide arrays for the identification of peptide substrates of several proteases, including trypsin, granzyme B and thrombin. The authors designed fluorogenic peptide substrates that result in an increased fluorescence response upon protease-mediated cleavage. Lesaicherre *et al.* [29] performed activity assays of kinases — including p60 tyrosine kinase and PKA — with peptide arrays, and characterized the phosphorylation reactions with fluorescently labeled antibodies.

Other applications

Peptide arrays have also been used to identify ligands that are active for mediating cell adhesion. Falsey *et al.* [9] used a peptide array to study the binding specificity of a peptide for various cell lines and characterized signaling processes in adherent cells using immunofluorescence techniques. In another interesting application, Gao *et al.* [30] used peptide arrays to identify ligands that selectively bind to Pb(II) salts. This example reveals a strategy for applying peptide arrays to develop metal sensors for monitoring toxic metals.

Outlook

Peptide arrays have been a topic of interest for many years but have seen significant research and development activity only in the past five years. Recent work has established a series of approaches for preparing the peptide arrays and, most significantly, has demonstrated the utility of these tools in a diverse set of applications. The next five year period will see a significant growth in the use of peptide arrays as research tools in chemistry and biology laboratories.

Acknowledgement

We are grateful to the National Institutes of Health.

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