Combining Self-Assembled Monolayers and Mass Spectrometry for Applications in Biochips

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Annu. Rev. Anal. Chem. 2008. 1:767–800

First published online as a Review in Advance on March 28, 2008

The *Annual Review of Analytical Chemistry* is online at anchem.annualreviews.org

This article's doi:
10.1146/annurev.anchem.1.031207.112903

1936-1327/08/0719-0767$20.00

Key Words

high-throughput assays, protein arrays, SAMDI, surface chemistry, systems biology

Abstract

Biochip arrays have enabled the massively parallel analysis of genomic DNA and hold great promise for application to the analysis of proteins, carbohydrates, and small molecules. Surface chemistry plays an intrinsic role in the preparation and analysis of biochips by providing functional groups for immobilization of ligands, providing an environment that maintains activity of the immobilized molecules, controlling nonspecific interactions of analytes with the surface, and enabling detection methods. This review describes recent advances in surface chemistry that enable quantitative assays of a broad range of biochemical activities. The discussion emphasizes the use of self-assembled monolayers of alkanethiolates on gold as a structurally well-defined and synthetically flexible platform for controlling the immobilization and activity of molecules in an array. The review also surveys recent methods of performing label-free assays, and emphasizes the use of matrix-assisted laser desorption/ionization mass spectrometry to directly observe molecules attached to the self-assembled monolayers.

1. PERSPECTIVES AND OVERVIEW

Assays of biochemical activities are fundamental to biological research, drug discovery, clinical diagnostics, food and environmental safety, biological warfare and other areas (1–5). The development and application of bioanalytical methods therefore continue to be dominant themes in analytical chemistry. Current research is motivated by several goals, including the analysis of small sample volumes (such as lysates from individual cells and other complex samples), massively parallel assays of large families of activities, and label-free detection formats. The development of biochips—or patterned arrays of immobilized molecules—addresses these themes and represents a significant achievement in genomic analysis. Such development also offers promising opportunities in proteomics, glycomics, and related topics.

This review provides a chemical perspective on the status of biochip arrays. We begin with an overview of the preparation and use of biochips, discuss current work with self-assembled monolayers (SAMs) to tailor the interfacial layer, and emphasize current efforts to apply label-free approaches to the biochip arrays.

1.1. Enter Biochip Microarrays: Oligonucleotides

The first high-density oligonucleotide microarray was reported by Fodor and colleagues in 1991 (6). The oligonucleotides were directly synthesized on a glass slide and lithographic masks were used to photochemically activate designated spots on the substrate for coupling each nucleotide. The first arrays had densities of 100,000 spots/cm^2 (6) and enabled the broad-scale identification of RNA transcripts in cellular samples. The sample DNA was fluorescently tagged, and hybridization of DNA to the immobilized oligonucleotides was detected with a fluorescence scanner and quantitated to reveal the pattern of gene expression associated with a cellular activity (7). Within ten years, these DNA arrays were a common tool in life sciences laboratories, providing unprecedented information on the global patterns of activities in cells (8–10). The technology is now mature, as arrays that comprise several million oligonucleotides (11) are now commercially available.

1.2. Extension to Protein and Small-Molecule Arrays

The rapid impact of DNA chips on the field of biology provided a strong motivation to develop arrays based on other classes of molecules, including peptides, proteins, carbohydrates, and small molecules. Yet, the development of these seemingly analogous arrays has proven much more difficult and still awaits broader commercialization. These difficulties with development begin with the tendency of proteins to adsorb nonspecifically to essentially all man-made materials (12–15). Unwanted adsorption is the source of background signals in many assays of biological samples (16). It is not an issue with most gene chip experiments because the isolation and amplification of DNA in samples exclude significant levels of protein. For protein arrays, this nonspecific adsorption is often accompanied by denaturation and therefore contributes to a loss of activity of the immobilized proteins (17). The immobilization of proteins or

small molecules also presents challenges that DNA arrays do not. With regard to the latter, the uniform structure of oligonucleotides allows the development of straightforward immobilization strategies that apply equally well to all members of the array. The varied structures of proteins—which differ in molecular weight, charge, stability, and aggregation state—substantially complicate the development of universal strategies for immobilization. The methods that are now used, and which are discussed in this review, are limited in that they do not provide for a uniform activity of the proteins in the array. Solutions for these challenges in developing methods to prepare and apply biochip arrays outside the genomics arena are found in surface engineering, and are a central topic of this review.

1.3. Applications of Protein and Small-Molecule Arrays

Notwithstanding the challenges identified above, early examples of protein and small-molecule arrays presage the value that these tools will bring to the life sciences. Snyder and colleagues prepared the first protein arrays for proteome-wide surveys of biochemical interactions (**Figure 1**) (18). This group cloned a collection of 5800 yeast open-reading frames into a yeast high-copy expression vector to express each protein as its glutathione-S-transferase (GST) fusion. These proteins were spotted on glass slides presenting glutathione ligands and were then assayed to identify those sets of proteins that were binding partners for calmodulin and phosphoinositide. The arrays identified several activities, both known and previously unknown. However, these early examples were encumbered by significant levels of false negative and false positive findings (19). Because the proteins were not individually purified and characterized, several of the intended proteins were not present in the array and others

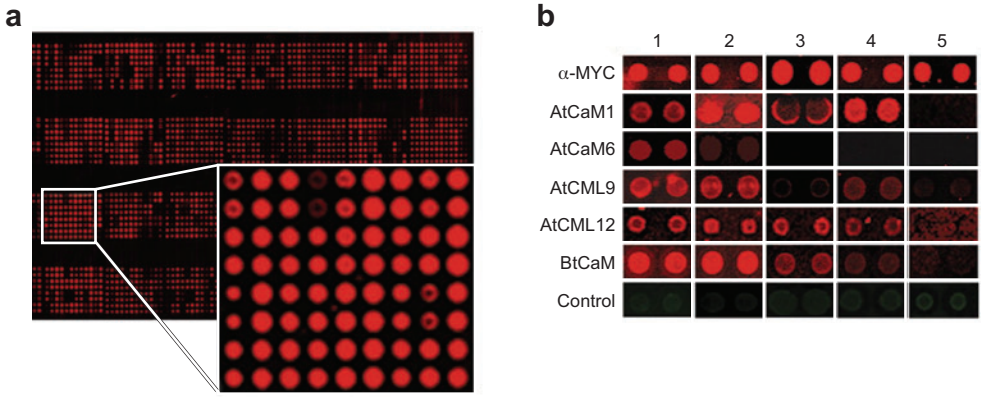


Figure 1

(*a*) Example of a high-density protein microarray presenting 1133 recombinant proteins on a nitrocellulose-coated glass slide. The array has been stained with a fluorescently labeled antibody that recognizes the MYC affinity tag. (*b*) The array was probed with fluorescently labeled calmodulin (CaM) and calmodulin-like proteins (CML) to identify the binding partners of these proteins.

were likely present in altered forms due to denaturation, proteolysis, or association with other partners, all of which can compromise specific interactions.

MacBeath and colleagues reported arrays having approximately 100 proteins, which represented the set of SH2 adaptor proteins from the human proteome (20), and addressed the ambiguities associated with protein expression by individually cloning, sequencing, and purifying each protein. These proteins were immobilized to aldehyde-coated glass slides and probed with a set of fluorescently labeled peptides to map out the consensus sequences and specificities of each adaptor domain (21). The significance of this work is that it permitted determination of the relative binding affinities of the peptides for each protein target and revealed specificity profiles of the receptors that may be involved in cancer. By probing the arrays with short peptide ligands, the authors avoided the complications that arise with nonspecific adsorption of macromolecules. Another common approach, based on antibody arrays, has been demonstrated for profiling cytokine levels in breast cancer cells (22) and blood (23) as well as for profiling human prostate cancer sera (24). Ligler and colleagues, for example, have developed a multianalyte-array biosensor that detects and identifies multiple analytes simultaneously from real-world samples (25).

The development of small-molecule arrays has also been slow due to the difficulty in arraying thousands of molecules with different functionalities and controlling nonspecific interactions with the surface (5). Shair et al. created reaction microarrays in which they determined the enantiomeric excess of tens of thousands of compounds simultaneously by comparing the relative affinities of two chiral fluorescent tags for each compound (26). Schreiber and colleagues prepared a library of 3780 molecules using the "one bead–one stock solution" approach, then created a small-molecule array by immobilizing this collection to a glass slide using a quill-pin contact printing robot (27). The arrays were treated with a fluorescently labeled protein with the aim of identifying those spots to which the protein localized, and therefore the small molecules with selective affinity for the target protein. To control the nonspecific adsorption of the protein, the array was first treated with the protein bovine serum albumin (BSA)—a common strategy used to passivate sites for nonspecific adsorption (28)—yet this experiment was still plagued by substantial levels of nonspecific adsorption.

2. BIOCHIP ARRAYS: COMPONENTS

In this section, we present a primer on the component technologies that are necessary for preparing and applying biochips. For each area, we describe the methods that are currently the most commonly used and identify the benefits and limitations specific to each. This section is followed by a detailed survey of SAMs and their combination with mass spectrometry (MS) detection methods, the topic that is the focus of this review.

2.1. Surface Chemistries

The surface of the biochip is not a passive element in the assay; rather, it optimally fulfills several roles. These roles include binding of the molecules that make up the

array; providing an environment that optimizes the activities of immobilized molecules; reducing nonspecific interactions of molecules in the sample with the chip; and providing substrates that are compatible with specific detection methods. A broad range of surface chemistries have been used and can be categorized as follows: (1) surface chemistries that employ polymeric materials, (2) those that use hydrogels, and (3) those that use SAMs as supports for the array. The first group includes the use of polystyrene, polycarbonate, and poly(ethylenimine) to immobilize molecules (29). The properties of these surfaces can be modified through reactive processes, including aqueous oxidation, plasma treatment, and mechanical roughening. In general, however, the resulting surfaces are complex in that they comprise several chemical functional groups and therefore require an empirical procedure for optimizing their use. Hydrogels are lightly cross-linked polymers that undergo enormous swelling in water—typically with more than 100-fold increases in volume—and therefore provide a three-dimensional environment that more closely approximates that present in solution (30). These materials, which include polyacrylamide, dextran, and agarose, are typically grafted onto a polymeric substrate and offer the benefits of being relatively effective at preventing nonspecific protein adsorption and of providing a higher loading capacity of immobilized species. The use of a hydrogel in assays, however, can be complicated by mass transport effects of soluble proteins through the gel and therefore necessitates caution when used in quantitative assays of biochemical activities. SAMs allow the most stringent control over surface structure and therefore provide a well-defined environment around immobilized molecules. These substrates are prepared by the self-assembly of precursor molecules, either alkanethiols or alkylsilanes, onto gold or hydroxylated substrates, respectively, and are discussed further below (31–34).

2.2. Immobilization Strategies

An important concern with chip-based assays is that immobilization alters the activity of the molecule, sometimes resulting in an almost complete loss of activity. This consequence for activity is of most concern with proteins, although it applies to small molecules as well. There are several reasons for this. First, on immobilization, part of a protein is oriented towards the substrate and is not available for interactions with soluble partners. Second, for surfaces that promote nonspecific adsorption—as is the case for the overwhelming majority of all materials—immobilized proteins will undergo denaturation. The presence of the surface can also inhibit large-scale conformational changes of proteins that can be required for their biochemical activity. We discuss below the four classes of immobilization strategies that are used, and group them according to whether the immobilization relies on specific or nonspecific interactions and whether it results in a covalent or noncovalent linkage of the molecule to the substrate.

2.2.1. Nonspecific and noncovalent immobilization. The oldest and experimentally most straightforward methods are those that rely on physical adsorption of proteins or molecules to surfaces. As a general rule, essentially all proteins will adsorb to essentially all surfaces. This is true for surfaces that are hydrophobic or are

positively or negatively charged; those that present hydrogen bond donors or acceptors; or even those that are modified with Teflon-like chemistries (12–15). Adsorption is usually fast—sometimes near the diffusion limit—and often yields a single layer of protein (13). The resulting layer is typically heterogeneous in structure, both in the orientation and denaturation state of the presented protein, and is dependent upon the conditions employed in the adsorption (35). For example, the use of solutions having higher concentrations of protein results in less denaturation because the proteins have less time to unfold (with an accompanying larger footprint) before the neighboring sites become occupied with adjacent proteins (36). Hence, these methods require substantial tuning to optimize the activities of protein. Whereas these methods can be valuable for assays of single proteins, the demand to optimize many immobilization events makes them impractical for the preparation of arrays. A final concern with proteins that are immobilized noncovalently is that they can exchange with soluble proteins during an assay. This concern, known as the Vroman effect, has been characterized extensively for materials that contact blood and remains a concern in bioassays (37).

2.2.2. Nonspecific and covalent immobilization. Surfaces presenting functional groups that can be used to covalently link the molecule to the chip avoid the exchange of immobilized proteins, and can be applied to low-molecular-weight molecules (including peptides) that would otherwise have poor affinity for the surface. Indeed, glass slides modified with N-hydroxysuccinimide (NHS) esters or with aldehydes are available commercially and are frequently used in the preparation of DNA and protein arrays, respectively (38–41). When used to immobilize arrays of proteins, these slides result in a heterogeneous display of the immobilized species, as each protein on average has several side chains that carry reactive functional groups, and require that the molecules be purified prior to arraying (42).

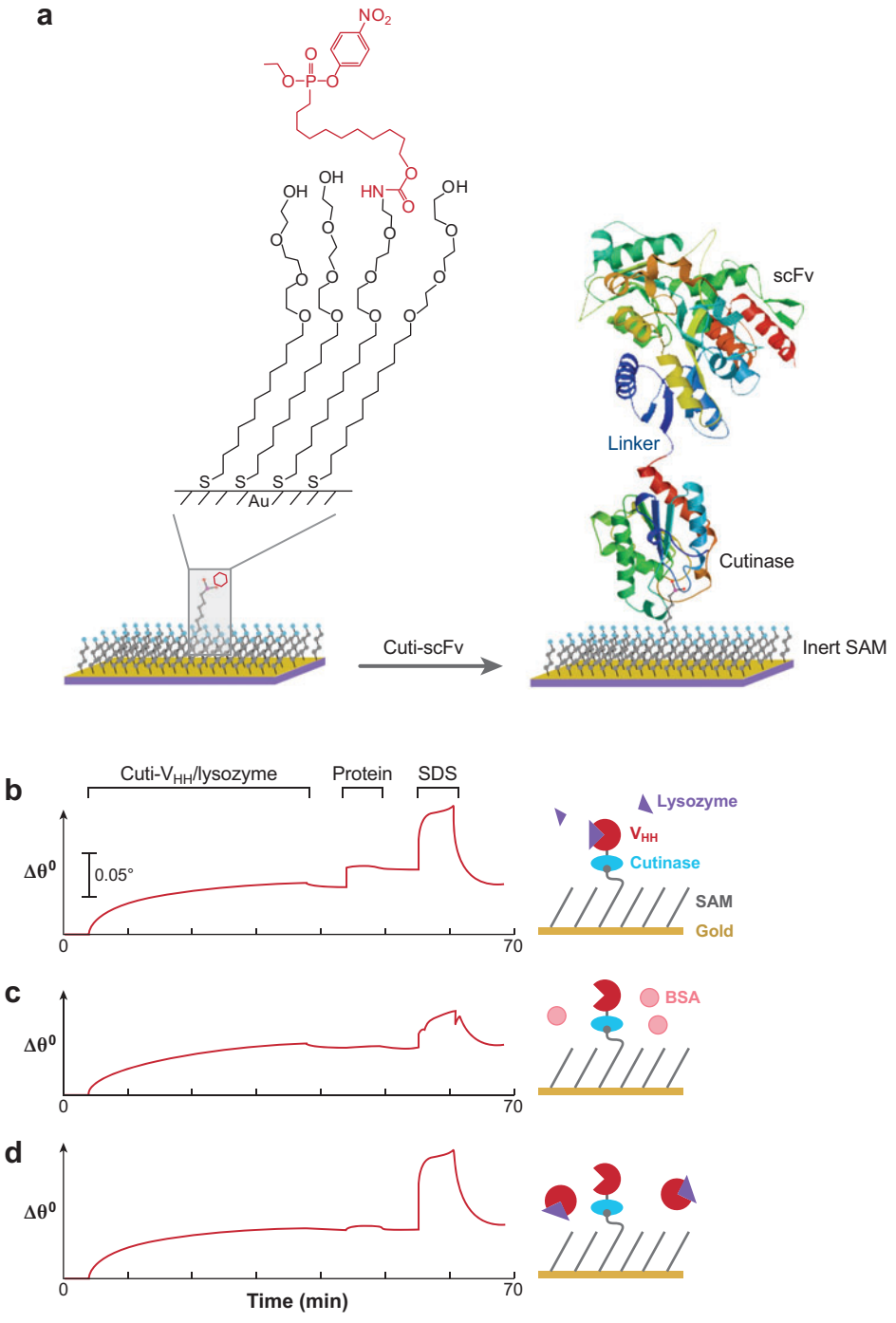
2.2.3. Specific and noncovalent immobilization. The use of surfaces that present molecular groups that can selectively interact with a tag on the protein provides for more control over the immobilization process and eases the requirement to purify the molecules prior to arraying. Attachment of biotin-tagged ligands to substrates that are modified with a layer of streptavidin is the most common example of a specific but noncovalent strategy for immobilizing molecules (38). The many reagents available to biotinylate ligands together with the specificity and high affinity of the ligand-protein interaction make this technique effective for biochip applications (43). For proteins, it is now possible to express recombinant forms with a tag that can be biotinylated with the enzyme BirA from *Escherichia coli* (44). The expression of proteins that have a hexahistidine tag allows direct immobilization to surfaces presenting a chelated Ni(II) ion (45, 46). The his-Ni(II) interaction has high affinity (45, 47) and is reasonably stable on the time scale of most biochip assays, making it the most important strategy used in protein arrays (48). The binding of GST fusions to glutathione-modified substrates has also been a common approach to protein immobilization, but is limited by the weak affinity of this ligand-protein complex. With a dissociation rate constant of about 0.1 s^{-1} (49), the immobilized proteins would be expected to dissociate from

the substrate during an experiment. The apparent stability of chips prepared using GST fusions is likely dependent on nonspecific interactions between the protein and surface and on the possible dimerization of GST with an increased affinity for the substrate. Jiang and coworkers have described a strategy to use proteins tagged with an oligonucleotide for immobilization to an oligonucleotide array (50). The specificity inherent in hybridization of oligonucleotides allows the array to be prepared from a mixture of all protein-DNA conjugates.

2.2.4. Specific and covalent immobilization. The above-described strategies that make use of immobilization domains provide the best control in positioning proteins at surfaces and reduce the demand for purification by allowing proteins to be selectively immobilized from mixtures, although they may not always provide sufficient stability for biochemical assays, particularly when stringent wash conditions are employed. To address this last limitation, we investigated a strategy wherein the immobilization domain of a fusion protein was made to interact with an irreversible inhibitor, leading to a selective and covalent attachment of the protein to the substrate. We demonstrated this method with the selective binding of the serine esterase cutinase to a class of phosphonate ligands to give a covalent adduct between the ligand and an active site serine residue (**Figure 2**) (51). The ability to prepare protein reagents using recombinant methods and to selectively immobilize target proteins without rigorous purification also prevents the loss of activity that often accompanies the manipulations used in purifying proteins. Johnsson and coworkers reported a similar approach using the human DNA repair protein O^6-alkylguanine–DNA alkyltransferase as the immobilization domain (52, 53). Another study used the substrate binding domain of poly(hydroxyalkanoate) depolymerase (54). Finally, Camarero and coworkers developed a method based on a *trans*-slicing process. The protein of interest was fused to an N-intein that was complementary to a C-intein on the monolayer. Upon association of the two domains, a splicing reaction resulted in release of the intein and a covalent attachment of the protein to the monolayer (55).

2.3. Content

A challenge in assembling biochip arrays that is often skirted by investigators concerns the preparation of the library of reagents to be arrayed. Whereas oligonucleotides are commercially available in high volume and are inexpensive (approximately $2 for 1 μg of a 20-mer), peptides, proteins, carbohydrates, and other small molecules are not generally available commercially. When they are, however, the cost for large libraries can be prohibitive. Solid-phase synthetic methods are important for generating large pools of small molecules and have been applied successfully to peptides and, more recently, to certain classes of oligosaccharides and small molecules (56, 57). Proteins, with typical masses ranging from 10 to 100 kD but occasionally as high as 1000 kD, must be prepared using biological methods (58). The preparation of large pools of proteins using parallel automated methods are complicated by the relatively poor yields inherent to cloning and expression (even in the *E. coli* host with good expression vectors, approximately one-third of the expected proteins are produced), the



improper folding of proteins and resulting insoluble particles, and the purification of proteins. Further, larger proteins and those requiring posttranslational modifications often require mammalian host expression systems (58). Early studies of protein arrays used massively redundant arrays (40), and the sizes of arrays are still almost always limited by the available molecular content. Clearly, the generation of large numbers of functional reagents remains a bottleneck in the biochip field.

2.4. Arraying

Commercial oligonucleotide arrays are now available with 1-μm feature sizes, whereas the still-limited content available for other classes of molecules eases the requirement to spot sizes of 50–100 μm. The current methods can be categorized as those that apply solutions of reagents to discrete regions of a slide, those that use light to activate regions of a slide for immobilization of reagents, and those that rely on self-assembly of the array. The first group of methods has benefited from the development of a variety of mechanical devices for delivering nanoliter-scale reagent droplets to defined regions of a surface (59); these methods include pin arrayers and ink-jet printing tools (60, 61). These robotic arrayers can reliably deliver molecules to arrays with feature sizes approaching 100 μm, but these arrays often have poor uniformity with regard to size and composition of the spots. Spots often have a bright ring of immobilized molecules around the perimeter, likely derived from the rapid evaporation of the droplet after it is delivered to the substrate, and can compromise quantitative comparison of spots across the array (62). Microfluidic devices prevent the evaporation of drops and therefore represent an exciting alternative method for the controlled delivery of biomolecules to surfaces (63–65). Delamarche and coworkers reported a microfluidic capillary system that autonomously transports submicroliter volumes capable of fabricating cellular microarrays that can measure cellular secretions after exposure to stimuli using the authors' "micromosaic immunoassay" (66, 67). Finally, contact methods that rely on applying a stamp or other applicator to a substrate can be effective. Crooks and coworkers described an interesting example wherein a stamp was used to pick up an array of oligonucleotides—which was initially hybridized to an array of oligonucleotides—and then to deliver the molecules to a target plate, where they were immobilized in the geometry of the array (68).

The combination of photolithography, which can irradiate a substrate with a pattern of light at micrometer-scale resolution, with photochemical protecting groups

Figure 2

(*a*) Illustration of a scheme to use self-assembled monolayers (SAMs) presenting an irreversible phosphonate inhibitor of the serine esterase cutinase to immobilize a single-chain antibody. Surface plasmon resonance spectroscopy shows the immobilization of the fusion protein and subsequent association of antigen (*b*). Control experiments show that the surfaces prevent the nonspecific adsorption of bovine serum albumin (BSA) (*c*) and that soluble inhibitors can block specific interactions at the surface (*d*). In each case, treatment of the monolayers with detergent (SDS) resulted in the removal of the antigen, but not the covalently immobilized fusion protein.

can be applied to the synthesis of an array of molecules directly on the biochip surface. This strategy was used to prepare the first DNA chips and is still important for preparation of the highest density DNA chips. Gulari and coworkers prepared peptide arrays by using photogenerated acids to spatially direct the deprotection of amino groups during peptide synthesis (69). For smaller-sized arrays, a series of droplets can sequentially be applied and removed from regions of the surface. Frank developed this SPOT method for preparing arrays of peptides on nitrocellulose resins (70), and Chang and coworkers have used this method to prepare arrays of small molecules (71). These methods are exciting because of the efficiency with which complex arrays can be prepared, but they are challenging to develop. Because the final molecules are attached to the substrate and cannot be purified, the syntheses must proceed in high yield. The small number of molecules present on the surface, however, makes it extremely challenging to assess the quality of the synthesis and to optimize reaction yields. Below, we describe the development of MS methods that address this limitation.

Monk and Walt have described a clever approach to preparing arrays by self-assembly, wherein they allowed oligonucleotide-modified beads to attach to the ends of a bundled optical fiber (72). Using split-pool synthesis, a large library of beads was created such that each bead presented a unique sequence of oligonucleotides. Assembly of the beads on the fiberoptic bundle yielded an array, but with the curious problem that the address of each oligonucleotide was unknown. A training procedure, whereby known mixtures of fluorescently tagged oligonucleotides were hybridized with the array, was then used to map the locations of oligonucleotides within the array. This application is commercially viable and shows that the expense associated with the training procedure can be lower than that associated with the deterministic synthesis or placement of high-density arrays.

2.5. Detection Methods

Biochip arrays are used to identify binding interactions between soluble molecules and their partners on the array and to identify those molecules on the array that are substrates for an enzyme. Most experiments rely on detection of a fluorescent signal to identify these interactions. In protein binding experiments, for example, the target protein is labeled and then applied to the biochip array, after which analysis with a flatbed scanner identifies those spots that have retained the soluble protein (40, 73). Similarly, the identification of molecules in an array that are substrates for an enzyme can be performed using fluorescently labeled antibodies that bind the product (74, 75). Fluorescence detection methods offer the benefits of being fast and sensitive and of using commercially available scanners. The use of a fluorescent chromophore, however, can alter or block the function of the labeled protein, can lead to increased nonspecific interactions of the protein with the substrate, and precludes the simultaneous analysis of several target proteins. A larger concern with this and other label-dependent methods is that unanticipated biochemical activities cannot be identified because the choice of labeling strategy requires knowledge of the activity to

be assayed. We note that radioisotopes can be employed in similar ways, but because of safety concerns this approach is less common (48, 76).

The limitations inherent to the use of labels have motivated the development of label-free detection methods that can monitor interactions of unmodified analytes. The most common approaches are based on optical strategies to monitor changes in the refractive index of the medium adjacent to the biosensor, which increases as proteins bind to the surface and displace solvent (77–80). Surface plasmon resonance spectroscopy (SPR) now represents a standard method for monitoring the interactions of a soluble and an immobilized binding partner and has the benefit of providing real-time, and therefore kinetic, measurements on interactions (81–83). Corn et al. have developed imaging SPR instruments to monitor the hybridization of oligonucleotides (84). To improve sensitivity, the authors harnessed an enzymatic amplification technique (85) to achieve a 10^6-fold improvement over nonamplified formats (86). Georgiadis and coworkers have applied imaging SPR to obtain kinetic and thermodynamic measurements on the specific binding of drugs to DNA arrays and were able to discriminate between different binding sites on the same DNA biosensor (87).

Below, we discuss the development of mass spectrometric methods for label-free detection of biochips. In addition, we note that several methods are now in early stages of development and may offer alternative label-free strategies for analyzing biochips. These approaches are based on electrochemical detection (88, 89), piezo resonance sensors (90), and calorimetric methods (91, 92).

3. SELF-ASSEMBLED MONOLAYERS FOR BIOCHIPS

We and others have made extensive use of SAMs in biochip applications because of the unmatched control over surface structure and properties that these films provide (93). In this section, we describe the characteristics that make SAMs well suited for use in preparing biochips, including the chemical flexibility to tailor the interface and the availability of inert surface chemistries. We review examples that demonstrate monolayers in quantitative assays of biochemical activities. The subsequent section continues this discussion with an overview of MS as a label-free method for analyzing monolayers and with a review of applications to biochip assays.

SAMs of alkanethiolates of gold were first described nearly 25 years ago and have remained the most important strategy for preparing structurally well-defined and complex organic surfaces (94, 95). The monolayers assemble from a solution of terminally substituted long-chain alkanethiols, and the assembly completes in several hours. Extensive work has shown that the alkanethiolates anchor to the (1,1,1) surface of gold in a hexagonal lattice to give a well-packed array of alkyl chains in the *trans*-extended conformation (31, 96–98). This arrangement positions the terminal functional group of the alkanethiolates at the surface and therefore provides a straightforward strategy for engineering the chemistry of a surface. Moreover, monolayers may be prepared from a mixture of two or more alkanethiols to introduce multiple functional groups onto the surface and to control the density of the active functional

group (99–101). The monolayers are stable under the conditions employed in biochip applications, but they desorb at temperatures of 80°C or with irradiation of UV light (102, 103).

3.1. Inert Surfaces

The most significant finding concerning the monolayers that are well suited to bioanalytical applications were the reports by Prime and Whitesides that monolayers presenting short oligomers of the ethylene glycol group prevented the nonspecific adsorption of protein (104, 105). The mechanisms that underlie this property are not fully elucidated—it is not yet possible, for example, to design a new inert surface—but they are believed to be related to the conformational entropy of the glycol oligomers and the structure of solvent in proximity to the surface (106, 107). Empirical methods have been used to identify additional examples of functional groups that render monolayers inert (108). These include the mannitol group, which can maintain inertness for several weeks (109); however, the glycol groups remain the most important for biochip applications.

3.2. Immobilization Chemistries

Several chemistries have been developed for immobilizing ligands to monolayers (**Figure 3**). Surfaces that present the immobilization group at 1–2% density among the oligo(ethylene glycol) chains are generally effective in optimizing the amount of immobilized molecule (and therefore signal in an assay), providing for a uniform environment of molecule (by avoiding significant crowding), and reducing nonspecific interactions with the surface (110). The use of monolayers that present a maleimide group against a background of tri(ethylene glycol) groups is particularly convenient for the immobilization of peptides (111). An important benefit with this approach is that the density of ligand is determined by the density of the reactive group on the monolayer—not by the kinetics of the coupling reaction, which depend on the concentration of the reagent—and therefore arrays that present a multitude of molecules do so at uniform density. This property allows direct comparison of activities across the array. We have also used the Diels-Alder reaction for immobilization of diene-conjugated peptides to monolayers that present the benzoquinone group (76). Other immobilization methods have used the cycloaddition of azide and terminal alkyne groups (112) and the reaction of amino-substituted ligands with monolayers presenting anhydride groups (113).

Numerous methods have been described for immobilizing proteins, including the use of monolayers presenting a NTA-Ni(II) ligand for the immobilization of His-tagged proteins (46). Another approach has used proteins engineered to present a single cysteine residue on their surface that can mediate immobilization to monolayers that present maleimide groups (114). Abbott and coworkers prepared a variant of RNase A wherein a cysteine residue was activated as a mixed disulfide with 2-amino-5-thiobenzoic acid and which then reacted with a thiol on the monolayer to immobilize the protein with a disulfide tether (115).

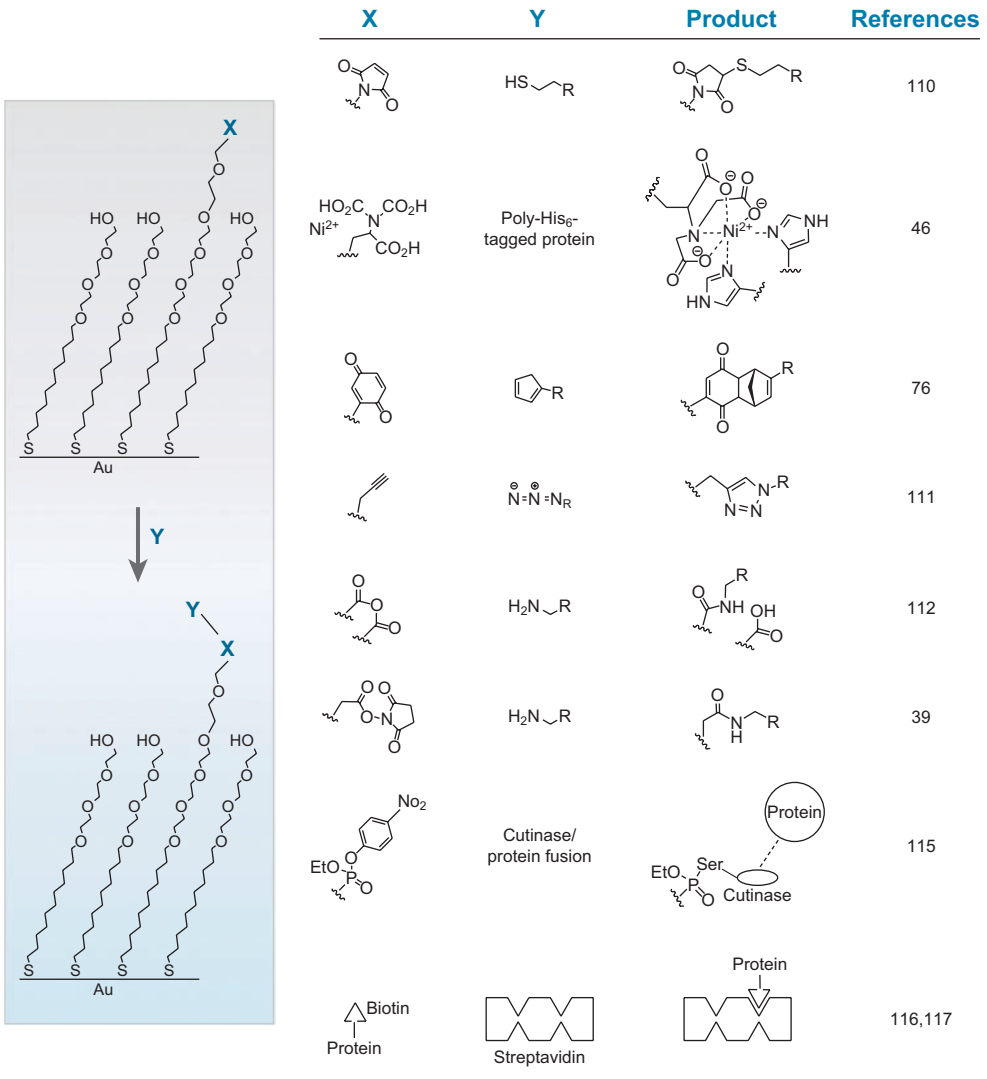


Figure 3

A chart summarizing several chemistries used to immobilize ligands to self-assembled monolayers. In each case, monolayers presenting the group X are used to immobilize ligands conjugated to group Y.

We have applied the cutinase fusion protein strategy described earlier to prepare a small antibody array (116). By uniformly orienting the antibodies on the surface, each antibody had a specific activity greater than 90% and the density could be controlled to optimize the activities of the antibodies. For example, we found that the fraction of antibodies that bound antigen was constant for antibodies that bound small antigens, but this fraction decreased with increasing densities of an antibody that bound

large antigens. This example reflects the lateral crowding at the surface when the antigen has a larger footprint than the immobilized antibody. Taken together, these methods provide controlled chemistries for optimizing the activities of immobilized proteins.

3.3. Molecular Recognition on Monolayers

Early examples of monolayers that were designed to selectively interact with proteins demonstrated the association of carbonic anhydrase with an immobilized benzenesulfonamide ligand and the binding of streptavidin to an immobilized biotin group (117, 118). For example, one study used SPR to show that the amount of bound protein increased with the density of ligand; the authors found that the rate constants for association and dissociation of the protein were similar to those for the homogeneous phase interaction. Indeed, several additional examples, including the binding of proteins to clustered carbohydrates (119), established the importance of immobilizing ligands to inert surfaces so as to minimize the nonspecific interactions that contribute to background signal, as well as to optimize the activity of the immobilized molecules. These examples served as the starting point for the development of monolayers for the preparation of biochip arrays and for a wide range of biochemical assays.

3.4. Quantitative Assays with Monolayers

In the first report of a carbohydrate array, we demonstrated quantitative assays of protein binding and enzyme activity using a monolayer presenting ten monosaccharides. The carbohydrates were prepared with a cyclopentadiene group that was used to immobilize the sugars to a monolayer presenting benzoquinone groups (120). The uniform density that this strategy ensures is particularly important for assays of carbohydrate-binding proteins, as many of these interactions are oligovalent and the association constants can vary with the density of the ligand. We probed the array with a panel of fluorescently labeled lectins and in each case we clearly identified the binding specificity of each protein (**Figure 4**). Further, when these experiments were performed in the presence of a soluble carbohydrate, we measured a dose-dependent inhibition of the binding to immobilized ligands; these data permitted a quantitative analysis of binding affinities. We also used the arrays to profile the specificity of a galactosyltransferase enzyme. By profiling the array with a panel of lectins both before and after the enzyme reaction, we inferred the carbohydrates that were modified by the enzyme. As part of this work, we demonstrated that the yield for the reaction was constant for densities of ligand up to 70%, but that the yield decreased at higher densities (121). This finding again reflects the crowding of ligands that renders some of them inaccessible to the enzyme.

In another experiment, we demonstrated an assay of the src kinase (76). We again used the Diels-Alder reaction to immobilize a peptide substrate for the kinase and monitored the phosphorylation reaction using a ^{32}P-labeled phosphate group. Application of a sample containing src and ATP resulted in phosphorylation of the peptide,

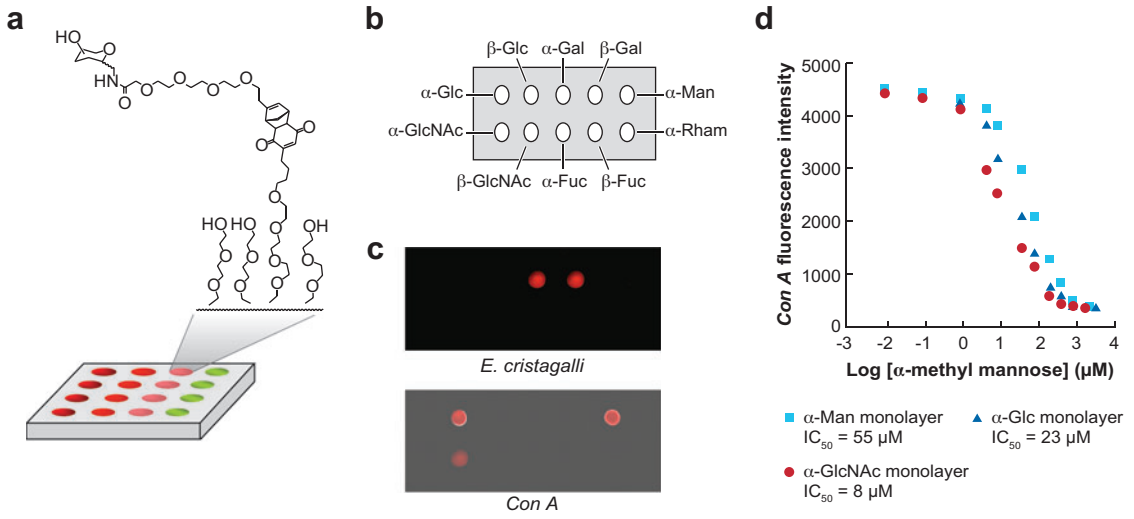


Figure 4

(*a*) Carbohydrate array prepared by immobilizing diene-conjugated carbohydrates to monolayers presenting a benzoquinone group. (*b*) The resulting arrays were treated with *Erythrina cristagalli* and *Concanavlin A* (*Con A*), two fluorescently labeled lectin proteins, to reveal (*c*) the binding specificities of the proteins. The addition of soluble carbohydrates gives a dose-dependent inhibition of the binding of lectin to the monolayer and (*d*) provides quantitative information on the binding affinities.

whereas a control peptide whose active tyrosine was substituted with a phenylalanine residue was inactive. Significantly, we observed levels of phosphorylation that were 75-fold greater than the background count, which again reflects the effectiveness of the glycol groups in preventing nonspecific interactions. By applying an array of microliter droplets, each of which contained the kinase and varying concentrations of a known inhibitor, we obtained titration curves that revealed the dissociation constant of the inhibitor (76).

These examples demonstrate the high performance of monolayers that combine immobilized ligands with oligo(ethylene glycol) layers in biochemical assays of protein binding and enzyme activity. The control over presentation of the ligand, which includes the density and orientation of the immobilized molecules, and the effectiveness of the ethylene glycol layer in preventing unwanted interactions at the surface and in maintaining the activities of immobilized molecules are very well suited to the construction of biochip arrays for a broad range of applications and represent a significant advance over many of the current—and commercially available—surface chemistries now in use. The preceding examples have all used label-dependent methods to determine biochemical activities on the chip and therefore carry the same limitations discussed earlier. In the next section we describe the development of a mass spectrometric technique to characterize monolayers and describe several classes of assays that can be performed using this label-free method.

4. ASSAYS WITH SELF-ASSEMBLED MONOLAYERS FOR MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

Monolayers serve as efficient substrates for matrix-assisted laser desorption/ionization (MALDI) MS. Upon irradiation with a laser, the alkanethiolates are released from the gold surface (through a loss of the sulfur-gold bond) and give rise to peaks whose masses correspond to the terminally substituted alkanethiolates. Early work by the Wilkins and Hanley groups investigated laser desorption of alkanethiolates and showed that both the monomers and dimers of the alkanethiolates were observed, along with fragments of these species (122, 123). We find that the use of matrix substantially reduces fragmentation of the intact alkanethiolates, thereby producing clean and easily interpretable spectra. Most significantly, the use of monolayers that are functionalized with appropriate chemical and biological functionality permits a broad range of assays. This section gives several examples of these assays using the technique termed self-assembled monolayers for matrix-assisted laser desorption/ionization (SAMDI) MS.

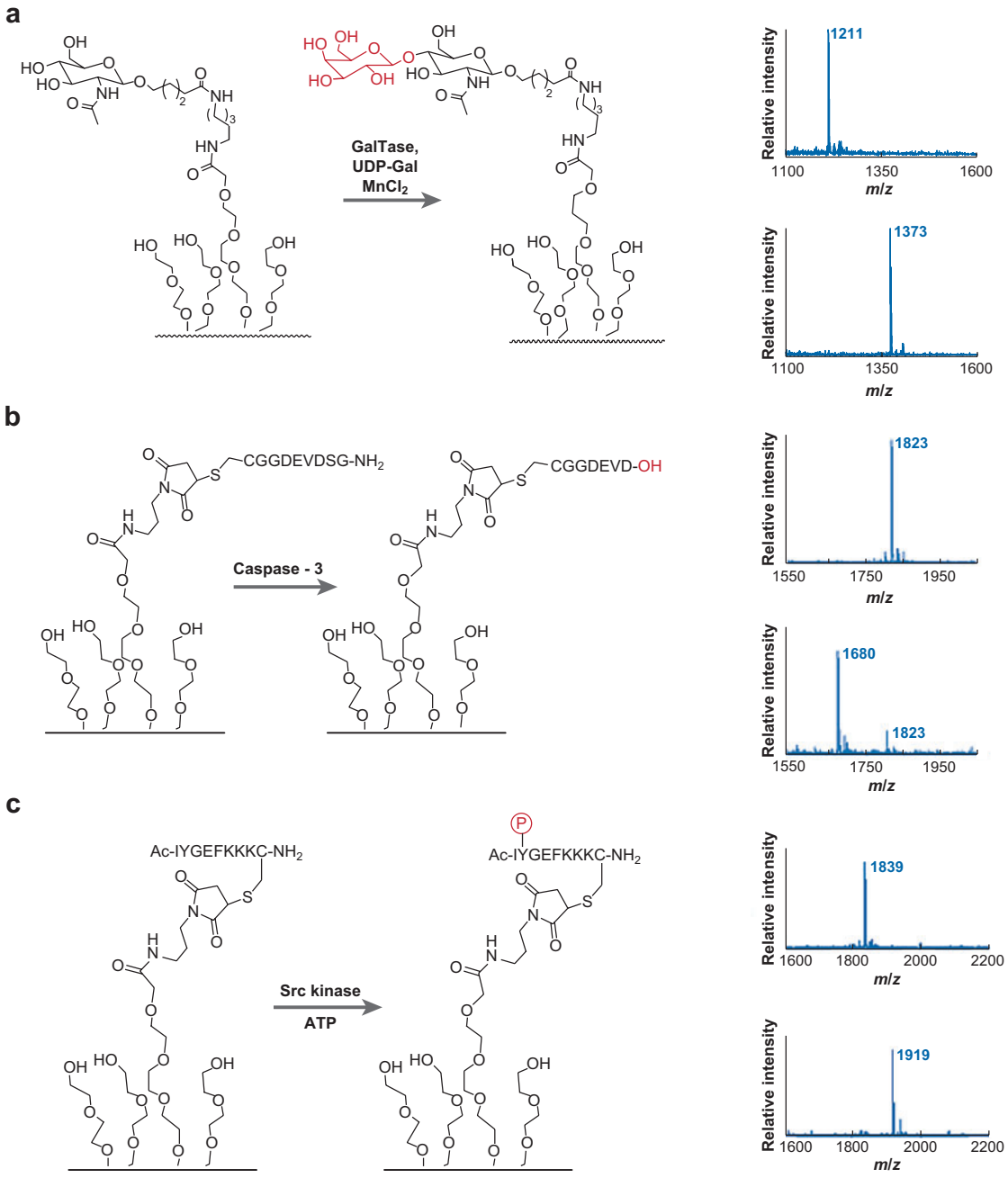
4.1. Enzyme Activity Assays

Most enzymes act on substrates and generate products whose masses are distinct from the substrate. For example, kinases add phosphate groups to hydroxyl-bearing side chains of proteins, proteases cleave proteins by hydrolyzing an amide bond, and acetylases convert the amino groups of lysine residues to the corresponding acetamides (124). SAMDI offers the ability to perform label-free assays of enzyme activities, starting with the immobilization of the relevant enzyme substrate to monolayers that are otherwise inert. Treatment of the monolayers with the enzyme and any required cofactors leads to the product, which can then be observed in the mass spectrum. **Figure 5** shows several examples of enzyme activity assays.

In a kinase activity assay, we immobilized a cysteine-terminated peptide to a monolayer presenting the maleimide group (125). A SAMDI spectrum showed clear peaks corresponding to the immobilized peptide and to the background tri(ethylene glycol)–terminated alkanethiolates, and also showed a lack of peaks for the initial maleimide-terminated alkanethiolate; this demonstrated that the immobilization reaction was complete. After the surface was treated with src kinase, a SAMDI spectrum revealed

Figure 5

Examples of enzyme activity assays performed with self-assembled monolayers for matrix-assisted laser desorption/ionization mass spectrometry (SAMDI MS), wherein monolayers presenting carbohydrate or peptide ligands were treated with an enzyme. In each case, mass spectra revealed peaks corresponding to the masses of the substituted alkanethiolates before and after modification of the enzyme. Examples are shown for the galactosylation of an immobilized carbohydrate by (*a*) β(1,4)-galactosyl transferase and (*b*) proteolysis of a peptide by caspase-3, as well as (*c*) phosphorylation of a peptide by src kinase.



that the peaks representing peptide-alkanethiol conjugates shifted by 80 Da, as expected for the increase in mass following phosphorylation. In this and most other examples, multiple peaks were observed for the anticipated analyte that represented the alkanethiolates in their monomeric and disulfide forms. We do not know whether the disulfides formed upon desorption of the monolayer from the gold surface, which is well precedented in thermal reactions (126), or whether the disulfides formed in the ion cloud following desorption. We also observed adducts of the alkanethiols with multiple counter ions, including proton, sodium, and potassium. In any event, we did not see significant fragmentation of the functionalized alkanethiolates and consequently obtain spectra that are straightforward to interpret. Other examples have characterized the methylation of arginine by protein arginine methyltransferase 1 (127), the protease activity of caspase-3 (128), and the modification of carbohydrates by glycosyltransferase (120).

These examples illustrate several benefits inherent to the SAMDI method. First, the use of MS provides a label-free method for assaying a broad range of biochemical activities. The lack of labels eliminates several steps in the assay and avoids the need for developing antibodies to label an intended analyte, thereby significantly reducing development time and decreasing the risk that introduction of the label will interfere with the biological activity being assayed. MS also permits simultaneous assay of distinct enzyme activities—which would normally be incompatible because of different labeling strategies—to be performed on the same chip and thus with a single sample.

MS also provides instructive information about the analyte. Whereas SPR and related optical methods provide information on the amount of protein that interacts with an immobilized ligand, they do not depend on the composition of those proteins. The ability of SAMDI to identify each species at the surface according to its molecular weight allows a straightforward discrimination between signals due to specific analytes and to background. This ability also allows observation of both the products and substrates of an enzyme and therefore can verify that the immobilized molecule was indeed on the chip (thereby identifying possible false negatives); further, SAMDI can provide a better assessment of the yield of the enzymatic reaction. Moreover, the mass resolution of this method also permits multianalyte assays to be performed. In one example, we immobilized a mixture of four peptides (**Figure 6**), each of which was clearly resolved in the SAMDI spectrum. Treatment of the monolayer with mixtures of kinases resulted in selective phosphorylation reactions, which could be analyzed by SAMDI to determine which kinases were present in the sample (125).

4.2. Solution-Phase Assays

A common concern with chip-based assays is that immobilization of a molecule may compromise its biochemical activity. For example, immobilization of a protein in an improper orientation can prevent its association with a binding partner, and immobilization of a substrate using a short tether can prevent its access to a buried active site of an enzyme. We performed an assay of a methyl transferase enzyme using a monolayer that presented a peptide substrate; we found that the peptide was fully inactive (127). We then repeated the reaction in solution using a cysteine-terminated

a

Kinase	Substrate peptide	Molecular weight	Molecular weight on surface (H^+)
PKA	P2: Ac-LRRASLGC-NH_2	915.5	1839
CaMKII	P4: Ac-KRQQSFDLFBC-NH_2	1368.7	2293
CKI	P5: Ac-CKRRALpSVASLPGL-NH_2	1590.8	2515
Erk	P7: Ac-TGPLSPGPFGC-NH_2	1072.5	1996

b

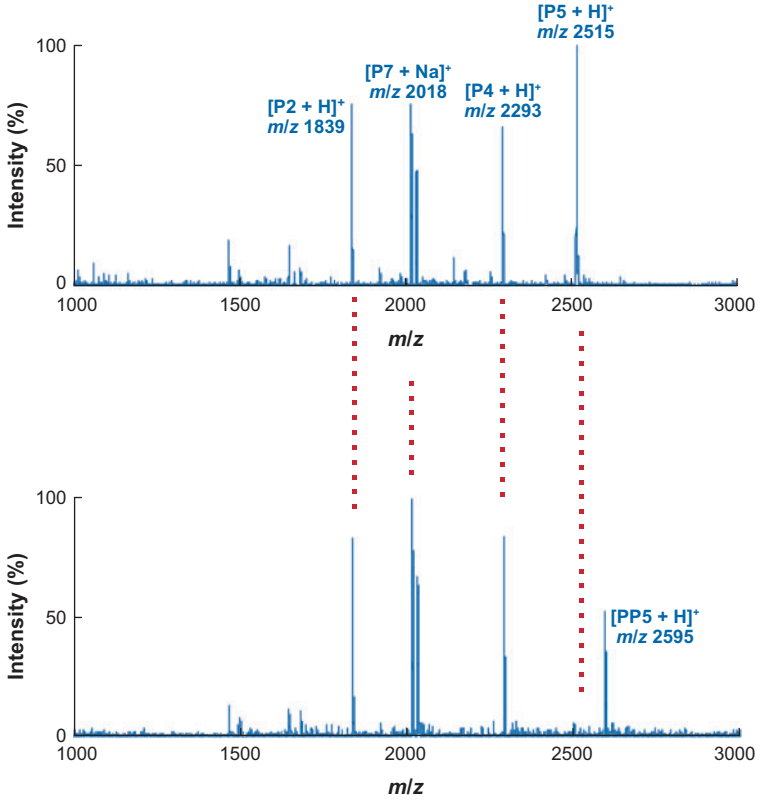


Figure 6

An example of multianalyte assays enabled by self-assembled monolayers for matrix-assisted laser desorption/ionization mass spectrometry (SAMDI MS). A mixture comprising four cysteine-terminated peptides, each of which was a selective substrate for one of four kinases (*a*) was immobilized to a monolayer presenting maleimide groups. The four peptides had distinct masses and each gave a distinct peak in the mass spectrum. Treatment of the monolayer with CKI kinase (*b*) resulted in a monolayer where only P5 had shifted by 80 Da, revealing the selective phosphorylation of this peptide.

peptide. At the end of the reaction, we applied the reaction mixture directly to the monolayer. Because there were no other thiols in the assay mixture, the peptide selectively immobilized to a monolayer presenting a maleimide group, after which the monolayer was rinsed and the peptide was analyzed by SAMDI.

Indeed, in this format the peptide was efficiently methylated and a time course of the reaction—obtained by spotting microliter aliquots from a single reaction mixture to a monolayer at different times—revealed a kinetic profile that agreed with that obtained using conventional assays based on high-pressure liquid chromatography. This approach should be applicable to other assays provided that the substrate contains an immobilization tag that permits selective reaction with a functional group on the monolayer. Significantly, this method combines the advantages offered by homogeneous phase reactions for maintaining biochemical activity and liquid handling in microtiter plates with those offered by immobilized format assays for simplifying sample preparation and analysis.

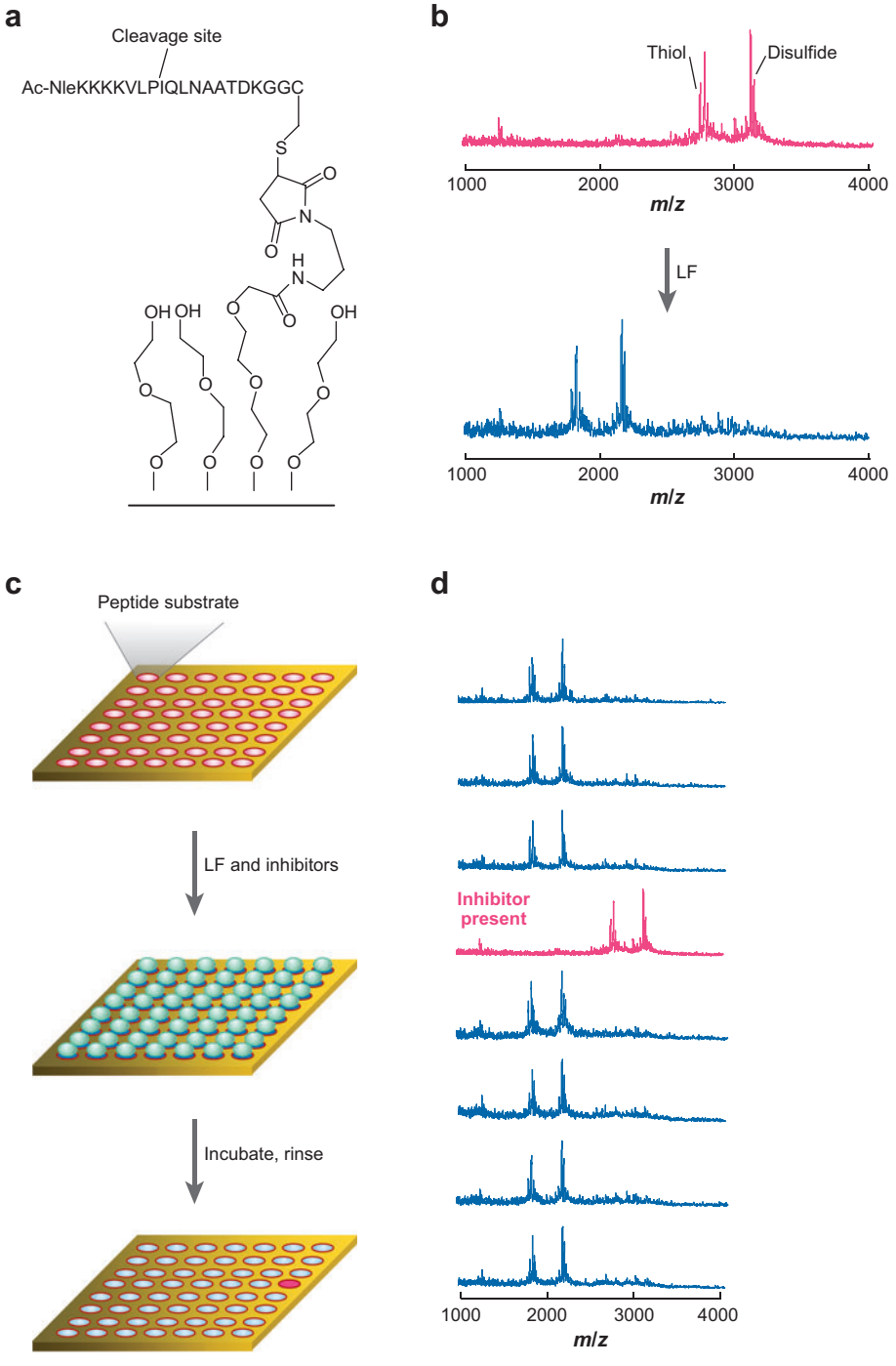
4.3. High-Throughput Screening

MS methods have not been applied to the discovery of inhibitors of enzymes. MS has significant benefits, but they are limited by the need to enrich the sample with the intended analyte and remove salt. This makes sample preparation impractical for screening applications that require many tens of thousands of independent experiments.

Because the SAMDI method uses substrates wherein the intended analyte is covalently attached, sample preparation requires only that the substrate be rinsed and then spotted with matrix, both of which are compatible with high-throughput manipulations. We demonstrated this approach by screening a library of 10,000 compounds to identify selective inhibitors against the anthrax lethal factor toxin, a protease that cleaves MAP kinase proteins in the host cell (**Figure 7**) (129). A peptide substrate for lethal factor was immobilized to a monolayer modified with a 10 × 10 array of circular islands. Droplets containing the enzyme and a pool of eight compounds from the library were applied to each island, allowed to stand for 1 h, and then rinsed. Mass spectra acquired from islands present on a dozen plates showed complete cleavage of the immobilized peptide on the majority of spots, whereas 1% of the spots showed partial or absent cleavage, revealing that those pools contained an inhibitor of the protease. Compounds from the active pools were then assayed individually, and a

Figure 7

Self-assembled monolayers for matrix-assisted laser desorption/ionization (SAMDI) used to perform a screen of 10,000 small molecules to identify inhibitors of the anthrax lethal factor (LF) protease. (*a*) A peptide substrate for LF was immobilized to a monolayer presenting maleimide groups. (*b*) Treatment of the monolayer with recombinant protease resulted in cleavage of the peptide, which was then analyzed by SAMDI mass spectrometry (MS). (*c*) Chemical screens were performed by arraying 100 droplets containing the protease and eight compounds from the library, followed by analysis of the spots with MS. (*d*) The analysis clearly identified the spots that had an inhibitor in the reaction mixture.



single candidate with 1 μM dissociation constant was identified and found to be active in cell culture assays.

This example highlights the benefits of performing high-throughput screens with mass spectrometric methods. Most importantly, the approach avoids the high rate of false positive results that are common to fluorescence methods (because compounds in the chemical library are fluorescent at the wavelengths used in the assay) and minimizes the time required to develop and format a new assay. The SAMDI method, however, has a significantly lower throughput than fluorescent methods, requiring approximately 1 h to analyze a single plate. We expect advances in the sensitivity and automation of instruments to accelerate the throughput, but not to the levels now common in fluorescent approaches.

4.4. Chemical Reaction Screening

The task of reaction discovery in synthetic chemistry is also rooted (in part) in numerous trial-and-error experiments to identify reagents and conditions that promote a desired reaction. Yet, the same chip-based tools that are under intense development for biological applications have not, with one notable exception, been applied to reaction discovery.

Liu and coworkers reported a strategy that uses oligonucleotides that are functionalized with common functional groups (130). They used a panel of reagents that permitted each pair of functional groups to be brought into proximity via the hybridization of complementary oligonucleotides. When treated with reagents that promoted a reaction resulting in the joining of the functional groups, the resulting cross-linked oligonucleotides could be isolated and applied to an oligonucleotide array to identify the sequences of DNA (and, therefore, the identity of the functional groups that had reacted). This work is significant because it can identify unanticipated reactions. Most current methods in chemistry instead begin with a known transformation and a product that can be detected, then screen for reagents that efficiently promote the reaction (131, 132).

The SAMDI method complements that reported by Liu et al. in that both methods are able to identify unanticipated reactions; however, the MS method can identify those reactions (1) that do not result in coupling of the two reagents, (2) that require a stoichiometry of the reagents other than 1:1, and (3) that use reagents and solvents that are otherwise incompatible with DNA templates. Indeed, a recent report showed the utility of SAMDI for developing reactions of immobilized molecules with soluble reagents (133). Whereas current methods require a combination of several analytical methods to characterize the products of interfacial reactions (with a substantial effort required for each reaction), SAMDI rapidly provides information for the products formed as well as the approximate yields. We recently reported 15 new reactions that were developed with the aid of the SAMDI method, including palladium-mediated cross-coupling of aromatic halides. In each case, we were able to rapidly identify conditions that gave a high-yielding conversion. For example, treatment of a monolayer presenting terminal alkyne groups with sodium methoxide in deuterated water promoted the exchange of the terminal hydrogen with a deuterium atom. The mass

spectrum clearly showed the mass increase associated with the addition of a single neutron to the alkyne. To investigate the application of this method to the identification of unanticipated reactions, we prepared monolayers that presented a single functional group at low density against a nonreactive background, and treated the monolayers with an array of common reagents. The spots were then analyzed by SAMDI to identify those that gave a high-yielding conversion to a new product whose structure was not readily deduced from the mass. This screen identified a novel reaction, wherein a primary amine reacted with three equivalents of an aldehyde under mild conditions to provide an N-alkylpyridinium product (133).

4.5. Assays of Cellular Activities

Many applications seek to measure enzyme or protein binding activities in complex samples, including extracts prepared from cell culture and bodily fluids taken for clinical analysis, and are often complicated by significant levels of nonspecific signal. In these cases, the MS approaches are especially valuable in that specific and nonspecific analytes can be distinguished based on their masses. In one instance, we used a SAMDI assay to monitor the activities of caspase proteases that initiate the apoptotic pathways in cells (several caspase enzymes undergo sequential activation when the cell death machinery is activated). The assay now widely used in biology relies on the collection of cell lysate and the addition of fluorescently tagged tetrapeptides that are substrates for the caspases. The substrates incorporate the fluorogenic reporter at the amide bond that undergoes hydrolysis and therefore forces a large nonnatural residue into the enzyme active site. In consequence, the peptide substrates have poor specificity for the enzymes, leading to cleavage by several of the caspase family members. Using the SAMDI assay, we immobilized longer peptide sequences that spanned both sides of the cleavage site (128). Treatment of the monolayers with lysates from cells that had been stimulated to activate the apoptotic pathway resulted in cleavage of the peptides and quantitation of caspase activity in the cells. A direct comparison of the SAMDI and fluorogenic assays revealed that the use of longer peptides in SAMDI gave improved enzyme-type specificity and also showed that the SAMDI assay can measure endogenous enzyme activities in complex cell lysates. Another study reported on the measurement of kinase activities in cell lysates (134).

4.6. Clinical Immunoassays

MS has been an important method in clinical diagnostic laboratories. Significant early work performed by Nelson and colleagues used MS to perform immunoassays on SAMs (135–139). The development of surface-enhanced laser desorption/ionization (SELDI) MS (140), which is based on the partially selective enrichment of proteins to substrates with chemistries that are electrostatic or hydrophobic to varying degrees, has prompted many efforts to apply MS for identifying and analyzing biomarkers (141). However, the presence of mixtures on the SELDI plates still complicates analysis of the spectra. Furthermore, these techniques are limited by reliance on either physical adsorption or the covalent attachment of random amino acids, which can

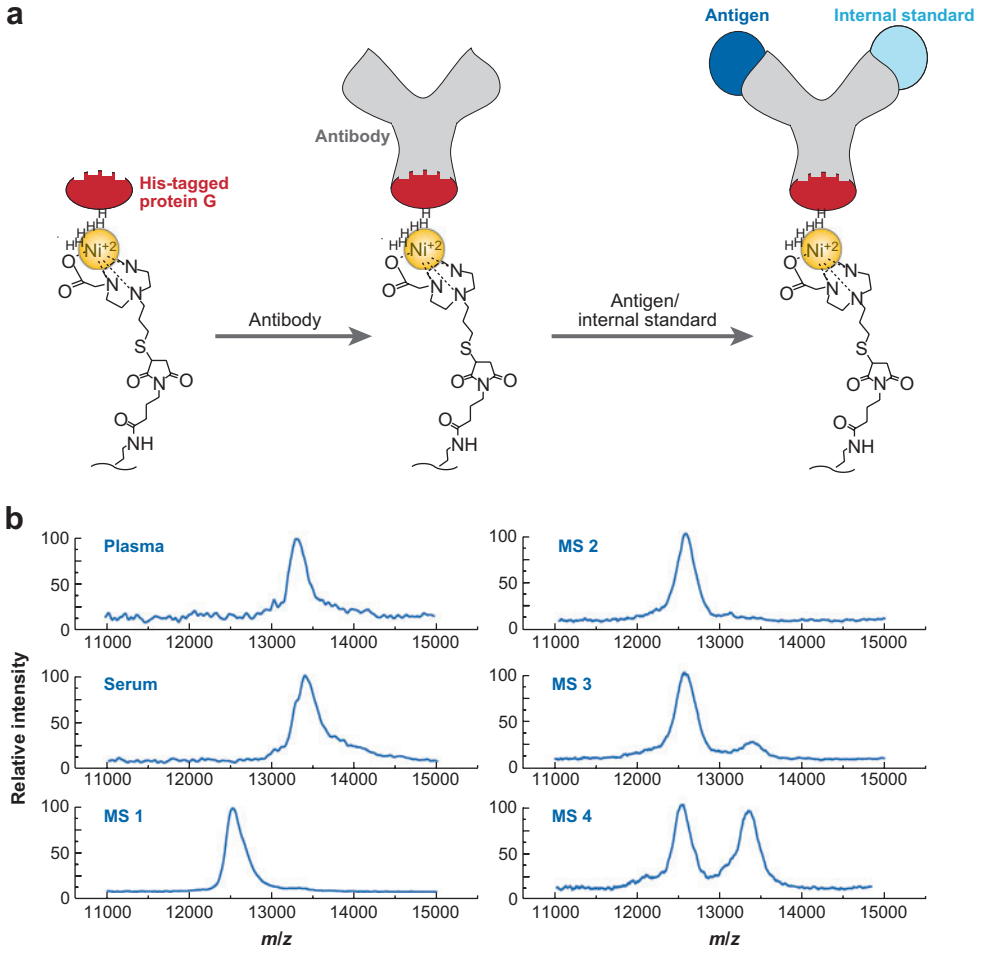


Figure 8

A mass spectrometry (MS) assay used to perform an immunoassay of cystatin C in patient clinical samples. (*a*) A His-tagged protein G is immobilized to a monolayer and used to immobilize an antibody against cystatin. (*b*) Treatment of the immunosensor with plasma, serum, or cerebral spinal fluid from patients with multiple sclerosis reveals the proteolytic form of the antigen associated with multiple sclerosis.

lead to nonspecific adsorption and a lack of control over orientation and density. The use of the surface chemistries described above can repair these limitations and may accelerate the use of MS methods in clinical immunoassays. We applied this approach to the analysis of cystatin (an inhibitor of cysteine proteases found in several tissues and a candidate biomarker for multiple sclerosis) in cerebral spinal fluids taken from healthy patients and from patients diagnosed with multiple sclerosis (142). The protein was present in a truncated form in the latter population, and is therefore a good candidate for analysis by MS (**Figure 8**). We prepared a monolayer that presented an

antibody with equal affinity for both the full and truncated forms of cystatin. Application of 1 μL of sample to the monolayer resulted in capture of the protein antigen, and analysis by SAMDI clearly discriminated between the two forms of protein and identified the samples taken from the patients with multiple sclerosis.

5. FUTURE PERSPECTIVES

In this review, we have addressed the status of biochip arrays from a surface chemistry perspective, emphasized the use of SAMs to control the immobilization of molecules and their activities, and discussed the ability of MS to perform a broad range of biochemical assays. The use of well-defined surface chemistries allows the activities of immobilized molecules to be optimized and used for quantitative measurement of binding affinities and enzyme activity. Analyzing the biochips with MS substantially simplifies the formatting of new assays and permits distinct biochemical activities to be profiled on the same chip. These benefits will be particularly important for applications in higher-density biochips, where current methods still suffer from high rates of false positive and negative information and where applications are often constrained by the strategies available for labeling biochemical activities.

Current efforts emphasize the preparation and analysis of monolayers that have hundreds to thousands of molecules in the array and thus approach the common densities of current protein and small-molecule arrays. Monolayers are compatible with many of the arraying tools—with the exception of those wherein the pin contacts the substrate and can damage the monolayer—that are important for preparation of biochips on glass slides. A difficulty with the monolayers stems from the lower throughput inherent to mass spectrometric methods as compared to fluorescence-imaging methods, which use flatbed scanners. Entry-level instruments can acquire data from the monolayers at a rate of approximately 250 spots/h. Modern instruments with higher sensitivity are expected to accelerate this rate by a factor of five; the incorporation of multiple assays in each spot may lead to a further tenfold throughput, allowing most protein and small-molecule arrays to be analyzed in one day. As with current MALDI MS, better methods for uniformly depositing matrix on the substrates will be important.

The early work in biochip arrays for applications outside of nucleic acids has been exciting and points to the many important applications that these tools will enable. Advances in surface chemistry will be vital to realizing these opportunities and to expanding the applications that may be targeted. This review provides an early status report on the surface chemistries that are most relevant to biochips and which will be important to furthering this important technology in bioanalytical chemistry.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Liotta LA, Espina V, Mehta AI, Calvert V, Rosenblatt K, et al. 2003. Protein microarrays: meeting analytical challenges for clinical applications. *Cancer Cell* 3:317–25
2. Lueking A, Cahill DJ, Mullner S. 2005. Protein biochips: a new and versatile platform technology for molecular medicine. *Drug Discov. Today* 10:789–94
3. Petrik J. 2006. Diagnostic applications of microarrays. *Transfus. Med.* 16:233–47
4. Robinson WH. 2006. Antigen arrays for antibody profiling. *Curr. Opin. Chem. Biol.* 10:67–72
5. Ma HC, Horiuchi KY. 2006. Chemical microarray: a new tool for drug screening and discovery. *Drug Discov. Today* 11:661–68
6. Fodor SPA, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D. 1991. Light-directed, spatially addressable parallel chemical synthesis. *Science* 251:767–73
7. Schena M, Shalon D, Davis RW, Brown PO. 1995. Quantitative monitoring of gene-expression patterns with a complementary-DNA microarray. *Science* 270:467–70
8. Brown PO, Botstein D. 1999. Exploring the new world of the genome with DNA microarrays. *Nat. Genet.* 21:33–37
9. Chittur SV. 2004. DNA microarrays: tools for the 21st century. *Comb. Chem. High T. Scr.* 7:531–37
10. Lockhart DJ, Winzeler EA. 2000. Genomics, gene expression and DNA arrays. *Nature* 405:827–36
11. Haushalter RC. 2004. Method for in situ, on-chip chemical synthesis. *U.S. Patent No.* 20040175710
12. Mrksich M, Sigal GB, Whitesides GM. 1995. Surface-plasmon resonance permits in-situ measurement of protein adsorption on self-assembled monolayers of alkanethiolates on gold. *Langmuir* 11:4383–85
13. Tengvall P, Lundstrom I, Liedberg B. 1998. Protein adsorption studies on model organic surfaces: an ellipsometric and infrared spectroscopic approach. *Biomaterials* 19:407–22
14. Elwing H. 1998. Protein absorption and ellipsometry in biomaterial research. *Biomaterials* 19:397–406
15. Margel S, Vogler EA, Firment L, Watt T, Haynie S, Sogah DY. 1993. Peptide, protein, and cellular interactions with self-assembled monolayer model surfaces. *J. Biomed. Mater. Res.* 27:1463–76
16. Haab BB, Dunham MJ, Brown PO. 2001. Protein microarrays for highly parallel detection and quantification of specific proteins and antibodies in complex solutions. *Gen. Biol.* 2:0004.1–04.13
17. Kusnezow W, Hoheisel JD. 2003. Solid supports for microarray immunoassays. *J. Mol. Recognit.* 16:165–76
18. Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, et al. 2001. Global analysis of protein activities using proteome chips. *Science* 293:2101–5
19. Michaud GA, Salcius M, Zhou F, Bangham R, Bonin J, et al. 2003. Analyzing antibody specificity with whole proteome microarrays. *Nat. Biotechnol.* 21:1509–12

20. Machida K, Thompson CM, Dierck K, Jablonowski K, Karkkainen S, et al. 2007. High-throughput phosphotyrosine profiling using SH2 domains. *Mol. Cell* 26:899–915
21. Jones RB, Gordus A, Krall JA, MacBeath G. 2006. A quantitative protein interaction network for the ErbB receptors using protein microarrays. *Nature* 439:168–74
22. Lin Y, Huang R, Chen L, Li S, Shi Q, et al. 2004. Identification of interleukin-8 as estrogen receptor regulated factor involved in breast cancer invasion and angiogenesis by protein arrays. *Int. J. Cancer* 109:570–15
23. Brooks RA, Wimhurst JA, Rushton N. 2002. Endotoxin contamination of particles producing misleading inflammatory cytokine responses from macrophages in vitro. *J. Bone Joint Surg.* 84:295–99
24. Miller JC, Zhou HP, Kwekel J, Cavallo R, Burke J, et al. 2003. Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. *Proteomics* 3:56–63
25. Taitt CR, Golden JP, Shubin YS, Shriver-Lake LC, Sapsford KE, et al. 2004. A portable array biosensor for detecting multiple analytes in complex samples. *Microbial Ecol.* 47:175–85
26. Korbel GA, Lalic G, Shair MD. 2001. Reaction microarrays: a method for rapidly determining the enantiomeric excess of thousands of samples. *J. Am. Chem. Soc.* 123:361–62
27. Kuruvilla FG, Shamji AF, Sternson SM, Hergenrother PJ, Schreiber SL. 2002. Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays. *Nature* 416:653–57
28. Peterfi Z, Kocsis B. 2000. Comparison of blocking agents for an ELISA for LPS. *J. Immunoassay* 21:341–54
29. Oh SJ, Hong BJ, Choi KY, Park JW. 2006. Surface modification for DNA and protein microarrays. *OMICS: J. Interg. Biol.* 10:327–43
30. Rubina AY, Dementieva EI, Stomakhin AA, Darii EL, Pan'kov SV, et al. 2003. Hydrogel-based protein microchips: manufacturing, properties, and applications. *Biotechniques* 34:1008–22
31. Vericat C, Vela ME, Salvarezza RC. 2005. Self-assembled monolayers of alkanethiols on Au(111): surface structures, defects and dynamics. *Phys. Chem. Chem. Phys.* 7:3258–68
32. Schaeferling M, Schiller S, Paul H, Kruschina M, Pavlickova P, et al. 2002. Application of self-assembly techniques in the design of biocompatible protein microarray surfaces. *Electrophoresis* 23:3097–105
33. Wink T, van Zuilen SJ, Bult A, van Bennekom WP. 1997. Self-assembled monolayers for biosensors. *Analyst* 122:R43–R50
34. Love JC, Estroff LA, Kriebel JK, Nuzzo RG, Whitesides GM. 2005. Self-assembled monolayers of thiolates on metals as a form of nanotechnology. *Chem. Rev.* 105:1103–69
35. Johnson RD, Arnold FH. 1995. The Temkin isotherm describes heterogeneous protein absorption. *Biochim. Biophys. Acta* 1247:293–97

36. Seigel RR, Harder P, Dahint R, Grunze M, Josse F, et al. 1997. On-line detection of nonspecific protein adsorption at artificial surfaces. *Anal. Chem.* 69:3321–28
37. Turbill P, Beugeling T, Poot AA. 1996. Proteins involved in the Vroman effect during exposure of human blood plasma to glass and polyethylene. *Biomaterials* 17:1279–87
38. Yam CM, Deluge M, Tang D, Kumar A, Cai CZ. 2006. Preparation, characterization, resistance to protein adsorption, and specific avidin-biotin binding of poly (amidoamine) dendrimers functionalized with oligo(ethylene glycol) on gold. *J. Colloid. Interf. Sci.* 296:118–30
39. Patel N, Davies MC, Hartshorne M, Heaton RJ, Roberts CJ, et al. 1997. Immobilization of protein molecules onto homogeneous and mixed carboxylate-terminated self-assembled monolayers. *Langmuir* 13:6485–90
40. MacBeath G, Schreiber SL. 2000. Printing proteins as microarrays for high-throughput function determination. *Science* 289:1760–63
41. Tessier PM, Lindquist S. 2007. Prion recognition elements govern nucleation, strain specificity and species barriers. *Nature* 447:556–61
42. Rao SV, Anderson KW, Bachas LG. 1998. Oriented immobilization of proteins. *Mikrochim. Acta* 128:127–43
43. Smith CL, Milea JS, Nguyen GH. 2006. Immoblization of nucleic acids using biotin-strept(avidin) systems. *Top. Curr. Chem.* 261:63–90
44. Viens A, Mechold U, Lehrmann H, Harel-Bellan A, Ogryzko V. 2004. Use of protein biotinylation in vivo for chromatin immunoprecipitation. *Anal. Biochem.* 325:68–76
45. Nieba L, Nieba-Axmann SE, Persson A, Hamalainen M, Edebratt F, et al. 1997. BIACORE analysis of histidine-tagged proteins using a chelating NTA sensor chip. *Anal. Biochem.* 252:217–28
46. Sigal GB, Bamdad C, Barberis A, Strominger J, Whitesides GM. 1996. A self-assembled monolayer for the binding and study of histidine tagged proteins by surface plasmon resonance. *Anal. Chem.* 68:490–97
47. Lata S, Reichel A, Brock R, Tampe R, Piehler J. 2005. High-affinity adaptors for switchable recognition of histidine-tagged proteins. *J. Am. Chem. Soc.* 127:10205–15
48. Zhu H, Klemic JF, Chang S, Bertone P, Casamayor A, et al. 2000. Analysis of yeast protein kinases using protein chips. *Nat. Genet.* 26:283–89
49. Armstrong RN. 1991. Glutathione S-transferases: reaction-mechanism, structure, and function. *Chem. Res. Toxicol.* 4:131–40
50. Boozer C, Ladd J, Chen S, Yu QM, Homola J, Jiang S. 2004. DNA direction protein immobilization of mixed ssDNA/oligo(ethylene glycol) self-assembled monolayers for sensitive biosensors. *Anal. Chem.* 76:6967–72
51. Hodneland CD, Lee YS, Min DH, Mrksich M. 2002. Selective immobilization of proteins to self-assembled monolayers presenting active site-directed capture ligands. *Proc. Natl. Acad. Sci. USA* 99:5048–52
52. Kindermann M, George N, Johnsson N, Johnsson K. 2003. Covalent and selective immobilization of fusion proteins. *J. Am. Chem. Soc.* 125:7810–11

53. Sielaff I, Arnold A, Godin G, Tugulu S, Klok HA, Johnsson K. 2006. Protein function microarrays based on self-immobilizing and self-labeling fusion proteins. *ChemBioChem* 7:194–202
54. Lee SJ, Park JP, Park TJ, Lee SY, Lee S, Park JK. 2005. Selective immobilization of fusion proteins on poly(hydroxyalkanoate) microbeads. *Anal. Chem.* 77:5755–59
55. Kwon Y, Coleman MA, Camarero JA. 2006. Selective immobilization of proteins onto solid supports through split-intein-mediated protein trans-splicing. *Angew. Chem. Int. Edit.* 45:1726–29
56. de Paz JL, Noti C, Seeberger PH. 2006. Microarrays of synthetic heparin oligosaccharides. *J. Am. Chem. Soc.* 128:2766–67
57. Duffner JL, Clemons PA, Koehler AN. 2007. A pipeline for ligand discovery using small-molecule microarrays. *Curr. Opin. Chem. Biol.* 11:74–82
58. Hunt I. 2005. From gene to protein: a review of new and enabling technologies for multi-parallel protein expression. *Protein Expres. Purif.* 40:1–22
59. Haab BB. 2001. Advances in protein microarray technology for protein expression and interaction profiling. *Curr. Opin. Drug Disc.* 4:116–23
60. Roda A, Guardigli M, Russo C, Pasini P, Baraldini M. 2000. Protein microdeposition using a conventional ink-jet printer. *Biotechniques* 28:492–96
61. Barbulovic-Nad I, Lucente M, Sun Y, Zhang MJ, Wheeler AR, Bussmann M. 2006. Bio-microarray fabrication techniques: a review. *Crit. Rev. Biotechnol.* 26:237–59
62. Deng Y, Zhu XY, Kienlen T, Guo A. 2006. Transport at the air/water interface is the reason for rings in protein microarrays. *J. Am. Chem. Soc.* 128:2768–69
63. Wang PC, DeVoe DL, Lee CS. 2001. Integration of polymeric membranes with microfluidic networks for bioanalytical applications. *Electrophoresis* 22:3857–67
64. Hansen C, Quake SR. 2003. Microfluidics in structural biology: smaller, faster. . . better. *Curr. Opin. Struc. Biol.* 13:538–44
65. Weibel DB, Whitesides GM. 2006. Applications of microfluidics in chemical biology. *Curr. Opin. Chem. Biol.* 10:584–91
66. Lovchik R, von Arx C, Viviani A, Delamarche E. 2007. Cellular microarrays for use with capillary-driven microfluidics. *Anal. Bioanal. Chem.* 390(3):801–8
67. Bernard A, Michel B, Delamarche E. 2001. Micromosaic immunoassays. *Anal. Chem.* 73:8–12
68. Kim J, Crooks RM. 2007. Replication of DNA microarrays prepared by in situ oligonucleotide polymerization and mechanical transfer. *Anal. Chem.* 79:7267–74
69. Gao XL, Zhou XC, Gulari E. 2003. Light directed massively parallel on-chip synthesis of peptide arrays with t-Boc chemistry. *Proteomics* 3:2135–41
70. Frank R. 1992. Spot-synthesis: an easy technique for the positionally addressable, parallel chemical synthesis on a membrane support. *Tetrahedron* 48:9217–32
71. Walsh DP, Chang YT. 2004. Recent advances in small molecule microarrays: applications and technology. *Comb. Chem. High T. Scr.* 7:557–64
72. Monk DJ, Walt DR. 2004. Optical fiber-based biosensors. *Anal. Bioanal. Chem.* 379:931–45

73. Lynch JL, deSilva CJS, Peeva VK, Swanson NR. 2006. Comparison of commercial probe labeling kits for microarray: towards quality assurance and consistency of reactions. *Anal. Biochem.* 355:224–31
74. Sapsford KE, Charles PT, Patterson CH, Ligler FS. 2002. Demonstration of four immunoassay formats using the array biosensor. *Anal. Chem.* 74:1061–68
75. Arenkov P, Kukhtin A, Gemmell A, Voloshchuk S, Chupeeva V, Mirzabekov A. 2000. Protein microchips: use for immunoassay and enzymatic reactions. *Anal. Biochem.* 278:123–31
76. Houseman BT, Huh JH, Kron SJ, Mrksich M. 2002. Peptide chips for the quantitative evaluation of protein kinase activity. *Nat. Biotechnol.* 20:270–74
77. Cooper MA. 2003. Label-free scanning of bio-molecular interactions. *Anal. Bioanal. Chem.* 377:834–42
78. Haake HM, Schutz A, Gauglitz G. 2000. Label-free detection of biomolecular interaction by optical sensors. *Fresen. J. Anal. Chem.* 366:576–85
79. Schaferling M, Nagl S. 2006. Optical technologies for the read out and quality control of DNA and protein microarrays. *Anal. Bioanal. Chem.* 385:500–17
80. Fang Y. 2006. Label-free cell-based assays with optical biosensors in drug discovery. *Assay Drug. Dev. Techn.* 4:583–95
81. Homola J, Vaisocherova H, Dostalek J, Piliarik M. 2005. Multi-analyte surface plasmon resonance biosensing. *Methods* 37:26–36
82. Fujiwara K, Watarai H, Itoh H, Nakahama E, Ogawa N. 2006. Measurement of antibody binding to protein immobilized on gold nanoparticles by localized surface plasmon spectroscopy. *Anal. Bioanal. Chem.* 386:639–44
83. Boozer C, Kim G, Cong SX, Guan HW, Londergan T. 2006. Looking towards label-free biomolecular interaction analysis in a high-throughput format: a review of new surface plasmon resonance technologies. *Curr. Opin. Biotech.* 17:400–5
84. Goodrich TT, Lee HJ, Corn RM. 2004. Direct detection of genomic DNA by enzymatically amplified SPR imaging measurements of RNA microarrays. *J. Am. Chem. Soc.* 126:4086–87
85. Goodrich TT, Lee HJ, Corn RM. 2004. Enzymatically amplified surface plasmon resonance imaging method using RNase H and RNA microarrays for the ultrasensitive detection of nucleic acids. *Anal. Chem.* 76:6173–78
86. Lee HJ, Li Y, Wark AW, Corn RM. 2005. Enzymatically amplified surface plasmon resonance imaging detection of DNA by exonuclease III digestion of DNA microarrays. *Anal. Chem.* 77:5096–100
87. Wolf LK, Gao Y, Georgiadis RM. 2007. Kinetic discrimination of sequence-specific DNA-drug binding measured by surface plasmon resonance imaging and comparison to solution phase measurements. *J. Am. Chem. Soc.* 129:10503–11
88. Yang LJ, Li YB, Erf GF. 2004. Interdigitated array microelectrode-based electrochemical impedance immunosensor for detection of *Escherichia coli* O157:H7. *Anal. Chem.* 76:1107–13
89. Xu DK, Xu DW, Yu XB, Liu ZH, He W, Ma ZQ. 2005. Label-free electrochemical detection for aptamer-based array electrodes. *Anal. Chem.* 77:5107–13

90. Rabe U, Kopycinska M, Hirsekorn S, Arnold W. 2002. Evaluation of the contact resonance frequencies in atomic force microscopy as a method for surface characterisation (invited). *Ultrasonics* 40:49–54
91. Jelesarov I, Bosshard HR. 1999. Isothermal titration calorimetry and differential scanning calorimetry as complementary tools to investigate the energetics of biomolecular recognition. *J. Mol. Recognit.* 12:3–18
92. Clas SD, Dalton CR, Hancock BC. 1999. Differential scanning calorimetry: applications in drug development. *Pharm. Sci. Technol.* 2:311–20
93. Whitesides GM, Kriebel JK, Love JC. 2005. Molecular engineering of surfaces using self-assembled monolayers. *Sci. Prog.* 88:17–48
94. Nuzzo RG, Allara DL. 1983. Adsorption of bifunctional organic disulfides on gold surfaces. *J. Am. Chem. Soc.* 105:4481–83
95. Finklea HO, Avery S, Lynch M, Furtsch T. 1987. Blocking oriented monolayers of alkyl mercaptans on gold electrodes. *Langmuir* 3:409–13
96. Porter MD, Bright TB, Allara DL, Chidsey CED. 1987. Spontaneously organized molecular assemblies. 4. Structural characterization of normal-alkyl thiol monolayers on gold by optical ellipsometry, infrared spectroscopy, and electrochemistry. *J. Am. Chem. Soc.* 109:3559–68
97. Strong L, Whitesides GM. 1988. Structures of self-assembled monolayer films of organosulfur compounds adsorbed on gold single crystals: electron-diffraction studies. *Langmuir* 4:546–58
98. Poirier GE, Pylant ED. 1996. The self-assembly mechanism of alkanethiols on Au(111). *Science* 272:1145–48
99. Bain CD, Whitesides GM. 1988. Formation of 2-component surfaces by the spontaneous assembly of monolayers on gold from solutions containing mixtures of organic thiols. *J. Am. Chem. Soc.* 110:6560–61
100. Biebuyck HA, Bian CD, Whitesides GM. 1994. Comparison of organic monolayers on polycrystalline gold spontaneously assembled from solutions containing dialkyl disulfides or alkenethiols. *Langmuir* 10:1825–31
101. Yan L, Marzolin C, Terfort A, Whitesides GM. 1997. Formation and reaction of interchain carboxylic anhydride groups on self-assembled monolayers on gold. *Langmuir* 13:6704–12
102. Ryan D, Parviz BA, Linder V, Semetey V, Sia SK, et al. 2004. Patterning multiple aligned self-assembled monolayers using light. *Langmuir* 20:9080–88
103. Bensebaa F, Ellis TH, Badia A, Lennox RB. 1998. Thermal treatment of n-alkanethiolate monolayers on gold, as observed by infrared spectroscopy. *Langmuir* 14:2361–67
104. Prime KL, Whitesides GM. 1991. Self-assembled organic monolayers: model systems for studying adsorption of proteins at surfaces. *Science* 252:1164–67
105. Prime KL, Whitesides GM. 1993. Adsorption of proteins onto surfaces containing end-attached oligo(ethylene oxide): a model system using self-assembled monolayers. *J. Am. Chem. Soc.* 115:10714–21
106. Herrwerth S, Eck W, Reinhardt S, Grunze M. 2003. Factors that determine the protein resistance of oligoether self-assembled monolayers: internal hydrophilicity, terminal hydrophilicity, and lateral packing density. *J. Am. Chem. Soc.* 125:9359–66

107. Welle A, Grunze M, Tur D. 1998. Plasma protein adsorption and platelet adhesion on poly[bis(trifluoroethoxy)phosphazene] and reference material surfaces. *J. Colloid. Interf. Sci.* 197:263–74
108. Ostuni E, Chapman RG, Holmlin RE, Takayama S, Whitesides GM. 2001. A survey of structure-property relationships of surfaces that resist the adsorption of protein. *Langmuir* 17:5605–20
109. Luk YY, Kato M, Mrksich M. 2000. Self-assembled monolayers of alkanethiolates presenting mannitol groups are inert to protein adsorption and cell attachment. *Langmuir* 16:9604–08
110. Houseman BT, Mrksich M. 2001. The microenvironment of immobilized Arg-Gly-Asp peptides is an important determinant of cell adhesion. *Biomaterials* 22:943–55
111. Houseman BT, Gawalt ES, Mrksich M. 2003. Maleimide-functionalized self-assembled monolayers for the preparation of peptide and carbohydrate biochips. *Langmuir* 19:1522–31
112. Devaraj NK, Miller GP, Ebina W, Kakaradov B, Collman JP, et al. 2005. Chemoselective covalent coupling of oligonucleotide probes to self-assembled monolayers. *J. Am. Chem. Soc.* 127:8600–01
113. Chapman RG, Ostuni E, Yan L, Whitesides GM. 2000. Preparation of mixed self-assembled monolayers (SAMs) that resist adsorption of proteins using the reaction of amines with a SAM that presents interchain carboxylic anhydride groups. *Langmuir* 16:6927–36
114. Hong HG, Jiang M, Sligar SG, Bohn PW. 1994. Cysteine-specific surface tethering of genetically engineered cytochromes for fabrication of metalloprotein nanostructures. *Langmuir* 10:153–58
115. Luk YY, Tingey ML, Dickson KA, Raines RT, Abbott NL. 2004. Imaging the binding ability of proteins immobilized on surfaces with different orientations by using liquid crystals. *J. Am. Chem. Soc.* 126:9024–32
116. Kwon Y, Han ZZ, Karatan E, Mrksich M, Kay BK. 2004. Antibody arrays prepared by cutinase-mediated immobilization on self-assembled monolayers. *Anal. Chem.* 76:5713–20
117. Mrksich M, Grunwell JR, Whitesides GM. 1995. Biospecific adsorption of carbonic-anhydrase to self-assembled monolayers of alkanethiolates that present benzenesulfonamide groups on gold. *J. Am. Chem. Soc.* 117:12009–10
118. Haussling L, Ringsdorf H, Schmitt FJ, Knoll W. 1991. Biotin-functionalized self-assembled monolayers on gold: surface-plasmon optical studies of specific recognition reactions. *Langmuir* 7:1837–40
119. Horan N, Yan L, Isobe H, Whitesides GM, Kahne D. 1999. Nonstatistical binding of a protein to clustered carbohydrates. *Proc. Natl. Acad. Sci. USA* 96:11782–86
120. Houseman BT, Mrksich M. 2002. Carbohydrate arrays for the evaluation of protein binding and enzymatic modification. *Chem. Biol.* 9:443–54
121. Houseman BT, Mrksich M. 1999. The role of ligand density in the enzymatic glycosylation of carbohydrates presented on self-assembled monolayers of alkanethiolates on gold. *Angew. Chem. Int. Edit.* 38:782–85

122. Trevor JL, Lykke KR, Pellin MJ, Hanley L. 1998. Two-laser mass spectrometry of thiolate, disulfide, and sulfide self-assembled monolayers. *Langmuir* 14:1664–73
123. Gong WH, Elitzin VI, Janardhanam S, Wilkins CL, Fritsch I. 2001. Effect of laser fluence on laser desorption mass spectra of organothiol self-assembled monolayers on gold. *J. Am. Chem. Soc.* 123:769–70
124. Walsh CT. 2006. *Posttranslational Modifications of Proteins: Expanding Nature's Inventory*. Greenwood Village, Colo.: Roberts
125. Min DH, Su J, Mrksich M. 2004. Profiling kinase activities by using a peptide chip and mass spectrometry. *Angew. Chem. Int. Edit.* 43:5973–77
126. Di Valentin C, Scagnelli A, Pacchioni G. 2005. Theory of nanoscale atomic lithography: an ab initio study of the interaction of "cold" Cs atoms with organthiols self-assembled monolayers on Au(111). *J. Phys. Chem. B* 109:1815–21
127. Min DH, Yeo WS, Mrksich M. 2004. A method for connecting solution-phase enzyme activity assays with immobilized format analysis by mass spectrometry. *Anal. Chem.* 76:3923–29
128. Su J, Rajapaksha TW, Peter ME, Mrksich M. 2006. Assays of endogenous caspase activities: a comparison of mass spectrometry and fluorescence formats. *Anal. Chem.* 78:4945–51
129. Min DH, Tang WJ, Mrksich M. 2004. Chemical screening by mass spectrometry to identify inhibitors of anthrax lethal factor. *Nat. Biotechnol.* 22:717–23
130. Kanan MW, Rozenman MM, Sakurai K, Snyder TM, Liu DR. 2004. Reaction discovery enabled by DNA-templated synthesis and in vitro selection. *Nature* 431:545–49
131. Copeland GT, Miller SJ. 2001. Selection of enantioselective acyl transfer catalysts from a pooled peptide library through a fluorescence-based activity assay: an approach to kinetic resolution of secondary alcohols of broad structural scope. *J. Am. Chem. Soc.* 123:6496–502
132. Petra DGI, Reek JNH, Kamer PCJ, Schoemaker HE, van Leeuwen PWNM. 2000. IR spectroscopy as a high-throughput screening technique for enantioselective hydrogen-transfer catalysts. *Chem. Commun.* 683–84
133. Li J, Thiara PS, Mrksich M. 2007. Rapid evaluation and screening of interfacial reactions on self-assembled monolayers. *Langmuir* 23:11826–35
134. Su J, Bringer MR, Ismagilov RF, Mrksich M. 2005. Combining microfluidic networks and peptide arrays for multi-enzyme assays. *J. Am. Chem. Soc.* 127:7280–81
135. Nedelkov D. 2006. Mass spectrometry-based immunoassays for the next phase of clinical applications. *Exp. Rev. Prot.* 3:631–40
136. Nedelkov D, Nelson RW. 2001. Analysis of native proteins from biological fluids by biomolecular interaction analysis mass spectrometry (BIA/MS): exploring the limit of detection, identification of nonspecific binding and detection of multi-protein complexes. *Biosens. Bioelectron.* 16:1071–78
137. Nelson RW, Krone JR, Bieber AL, Williams P. 1995. Mass-spectrometric immunoassay. *Anal. Chem.* 67:1153–58

138. Nedelkov D, Kiernan UA, Niederkofler EE, Tubbs KA, Nelson RW. 2006. Population proteomics: the concept, attributes, and potential for cancer biomarker research. *Mol. Cell Proteomics* 5:1811–18
139. Nedelkov D, Kiernan UA, Niederkofler EE, Tubbs KA, Nelson RW. 2005. Investigating diversity in human plasma proteins. *Proc. Natl. Acad. Sci. USA* 102:10852–57
140. Engwegen JYMN, Gast MCW, Schellens JHM, Beijnen JH. 2006. Clinical proteomics: searching for better tumour markers with SELDI-TOF mass spectrometry. *Trends Pharmacol. Sci.* 27:251–59
141. Albrethsen J, Bogebo R, Olsen J, Raskov H, Gammeltoft S. 2006. Preanalytical and analytical variation of surface-enhanced laser desorption-ionization time-of-flight mass spectrometry of human serum. *Clin. Chem. Lab. Med.* 44:1243–52
142. Patrie SM, Mrksich M. 2007. Self-assembed monolayers for MALDI-TOF mass spectrometry for immunoassays of human protein antigens. *Anal. Chem.* 79:5878–87