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Polymers for peptide/protein arrays

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Abstract: Peptide and protein arrays have gained increasing attention due to their potential application in many areas of research, clinical diagnosis, and pharmacy. A typical array consists of a support containing immobilized peptides or proteins positioned in an addressable format. The greatest advantage of the arrays is the possibility for miniaturization, which relies on dividing the surface into miniature spots, thus allowing for hundreds/thousands of analyses to be simultaneously performed using minimal amounts of a precious biological material. The quality of assays with the use of peptide and protein arrays depends on the surface properties, *e.g.*, hydrophilicity, homogeneity, density of functional groups, surface morphology, *etc*. In recent years, it was shown that the quality of the assays might be improved by introducing polymers acting as spacers between the peptide and the solid support. This approach causes changes in the surface properties, *e.g.*, it reduces the undesirable non-specific adsorption of biomolecules, increases the density of functional groups, or can improve the biological activity of biomolecules attached to the surface. In this review, various types of polymers that are used for peptide and protein arrays and their impact on the assay quality are discussed.

Keywords: peptide arrays, protein arrays, polymeric surfaces, microarrays, polymeric linkers, poly(ethylene glycol), dendrimers.

Polimery w macierzach peptydowych/białkowych

Streszczenie: Peptydy lub białka naniesione w regularnych, uporządkowanych pozycjach na nośnik stały tworzą tzw. macierze. Układy takie wzbudzają coraz większe zainteresowanie, ponieważ można je wykorzystywać do prowadzenia analiz w biochemii, diagnostyce klinicznej czy farmacji. Największą zaletą macierzy jest możliwość miniaturyzacji. Podział powierzchni macierzy na mikroplamki (mikrospoty) pozwala na wykonywanie do kilkuset analiz jednocześnie z wykorzystaniem minimalnej ilości cennego materiału biologicznego. Jakość analiz przeprowadzanych przy użyciu macierzy peptydowych i białkowych zależy od takich właściwości powierzchni, jak: hydrofilowość, jednorodność, gęstość obsadzenia grupami funkcyjnymi, morfologia, itp. W ostatnich latach wykazano, że można poprawić jakość analiz w wyniku wprowadzenia polimerów między peptyd/białko a podłoże. Polimery zmieniają właściwości powierzchni macierzy, np. redukują niepożądaną adsorpcję biocząsteczek, zwiększają gęstość obsadzenia powierzchni grupami funkcyjnymi lub poprawiają dostępność biocząsteczek związanych

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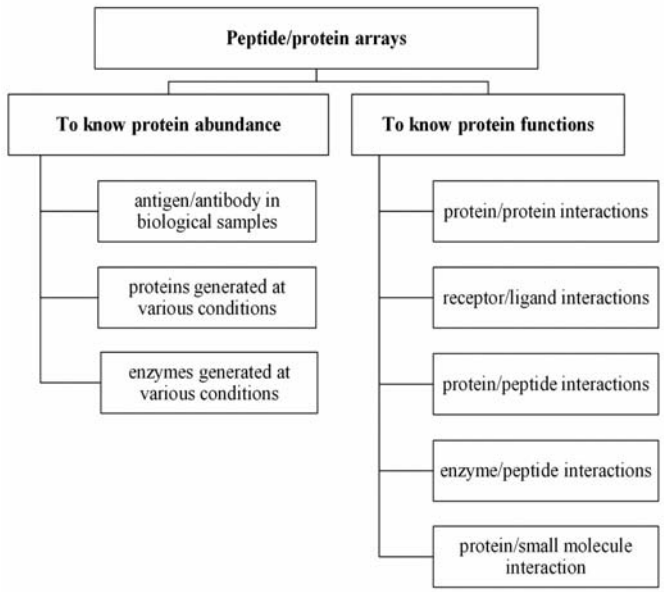
z powierzchnią. W niniejszej pracy omówiono różne typy polimerów stosowane do otrzymywania macierzy peptydowych i białkowych oraz ich wpływ na jakość przeprowadzanych analiz.

Słowa kluczowe: macierze peptydowe, macierze białkowe, powierzchnie polimerowe, mikromacierze, łączniki (linkery) polimerowe, poli(glikol etylenowy), dendrymery.

Peptide/protein arrays have received much attention due to the wide scope of their applications in biological and medical research [1—3]. They can be used for the detection of biomolecules [4], *e.g.*, enzymes, proteins, or antibodies, or for the determination of biological functions [5—8], such as enzymatic activity, protein-protein interactions, drug profiling *etc*. In Scheme A, the major areas for the application of protein/peptide arrays are listed.



Scheme A. Various areas of protein/peptide arrays applications

The greatest advantage of peptide/protein arrays is the possibility for miniaturization of the system (more than 200 spots per cm^2) [9], which allows for a large number of analyses to be performed simultaneously with a minimal amount of an expensive biological material [10]. The application of microarrays significantly reduces the time and costs of analysis compared to those for standard analytical methods. These advantages triggered the development of technologies for array production and miniaturization [11—13] and enlarged the scope of their applications for diagnostics, treatment and monitoring of therapy [14—20].

Generally, peptide/protein arrays consist of a solid support, divided into spots that contain immobilized peptides/proteins designed to specific biomolecules which target the surface (target biomolecules). During the interaction of a target biomolecule with a peptide/protein array, three basic actions may occur: (i) binding of the target molecules, (ii) chemical transformation of the peptide/protein, or (iii) release of peptide/protein fragments. These interactions result in changes of the surface's properties which are screened using various detection methods. The general scheme of an array experiment is shown in (Fig. 1).

The quality of an assay with the use of peptide/protein arrays depends on the proper choice of detection techniques for analysis of the array after the experiment. Common detection techniques rely on mass spectrometry, surface plasmon resonance, atomic force microscopy, and quartz crystal microbalances, as well as labeling me-

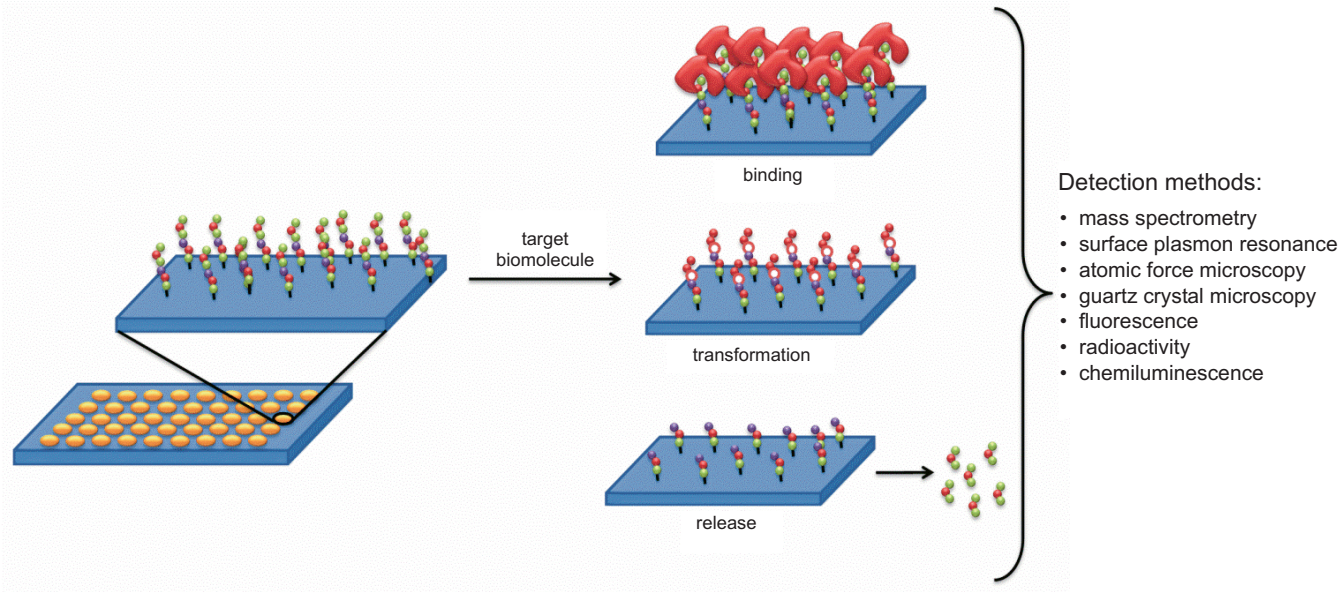


Fig. 1. The idea of the peptide-array experiments

thods, such as fluorescence, radioactivity, and chemiluminescence [21—26]. Most of the assays employ some type of labeling usually the highly sensitive technique of fluorescence. Label-free methods have the principal advantages that the assays can identify unanticipated activities and are more straightforward because they avoid steps associated with labeling. Although label-free detection methods are highly desirable, they demand experience, and their availability is still not high enough to be commonly applied for peptide/protein microarrays.

The high quality of experiments using peptide/protein arrays can be achieved by the proper choice of the support for the selected detection technique, which ensures the appropriate peptide/protein binding capacity, retains the biological activity of the immobilized peptide/protein, decreases the non-specific protein adsorption, and improves the accessibility of the target molecule to the immobilized peptide/protein. Therefore, substantial efforts have been made to prepare various types of supports for peptides/protein immobilization [27—35].

Recently, synthetic polymers have become attractive materials for peptide/protein arrays due to the vast choice of their physical and chemical properties. The introducing of polymers provides the possibility to modify the surface parameters, such as the homogeneity, roughness, philicity, density of functional groups, and spacing between the surface and the peptide/protein, which ensures the high quality and sensitivity of the assay. Polymers may be used directly as a support or can be applied to modify inorganic substrate that moderate the physical and chemical properties of the surface. Polymers in peptide/protein arrays can serve as polymeric films or polymeric linkers (linear, branched) between the peptide/protein and the support.

The aim of this work is to provide an overview of the polymers used for the preparation of peptide/protein arrays and to describe their impact on the quality of the assays. Various types of peptide/protein arrays with synthetic polymers covalently bound to the surface are discussed. The scope of this review is limited to peptide/protein arrays on planar supports.

METHODS FOR PEPTIDE/PROTEIN IMMOBILIZATION

Many efforts have been undertaken to develop the optimal method for the attachment of peptides/proteins to the solid support. The main methods of immobilizing bioactive compounds onto the array surface are adsorption, entrapment, biorecognition, and covalent attachment [27, 36—38] (Fig. 2).

The adsorption methods (Fig. 2 A), despite their simplicity, have several drawbacks, the most important of which is a risk that the adsorbed peptides will be removed during the experimental steps. Methods based on the physical entrapment of proteins in polymeric gels (Fig. 2 B) preserve the three-dimensional structure of proteins and eliminate problems with denaturation, but the gel creates a barrier for target biomolecules, and the experiments require long incubation times. The biorecognition method (Fig. 2 C) relies on a ligand-receptor pairing process that limits the area of their application, and it is mainly used for antibody/antigen immobilization. The covalent attachment of peptides/proteins onto a functionalized surface (Fig. 2 D) commonly used method. This method results in the formation of stable bonds between the compounds and the support.

Inorganic supports, especially glass, are the most popular supports for array preparation due to their availability, low price, well-characterized coupling chemistries, and favorable optical properties, which permit fluorescence imaging. There are a number of different strategies for the immobilization of biomolecules onto modified glass surfaces [39]. Commonly, the glass surface is modified with functionalized organosilanes to generate functional groups [40, 41], usually amine [42—45], epoxy [46—48], aldehyde [47, 49], thiol [50, 51], succinimidyl esters or isothiocyanate [52], able to react with peptide/protein functions. Organosilanes can directly provide the functional groups for peptide/protein immobilization or can react with a bifunctional reagent bearing the desired reactive group [38, 53]. Apart from glass, inorganic supports such as silicon [54] and gold [55] were also applied for peptide/protein arrays.

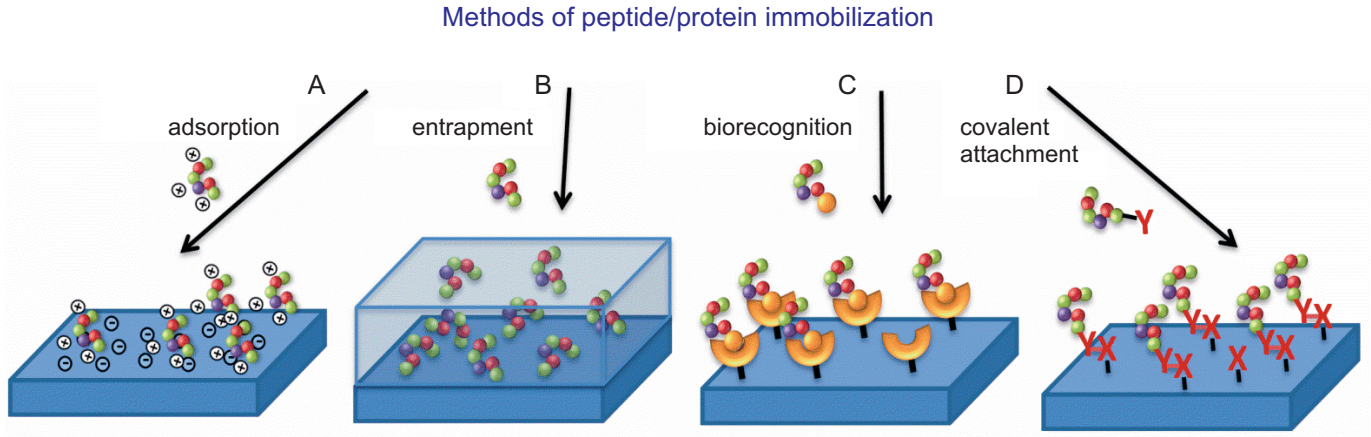


Fig. 2. Immobilization of peptides/proteins

POLYMERIC SUPPORTS FOR PEPTIDE/PROTEIN ARRAYS

Self-supporting polymeric supports

Due to a great choice of physical and chemical properties, synthetic polymers have become an attractive alternative to inorganic supports in protein/peptide arrays (Fig. 3). Self-supporting polymeric films, such as poly(methyl methacrylate) (PMMA) [56], polycarbonate (PC) [57], polydimethylsiloxane (PDMS) [58], and poly(ethyl terephthalate) [59] have been successively applied for the production of peptide/protein microarrays [60, 61].

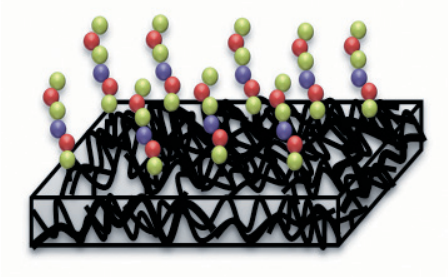


Fig. 3. Self-supporting polymeric supports for peptide arrays

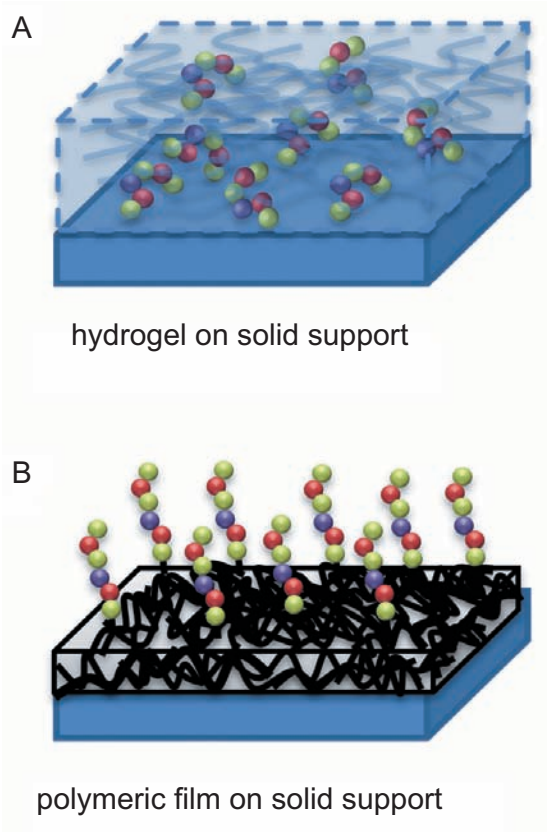


Fig. 4. A) a hydrogel, B) a polymeric film deposited onto solid supports

Various methods have been developed for the modification of polymeric surfaces, leading to functional groups for the immobilization of peptide/proteins [38]. Common techniques employed for this task involve the use of plasma oxidation [62] followed by treatment with appropriate organosilanes for the functionalization of PDMS [63], treatment of PMMA with 1,6-hexanediamine for the introduction of reactive amino groups [64] or sulfonation reactions on PC to provide sulfonated surfaces [37]. Polymers containing functional groups in their structure were also applied as self-supporting films for peptide/protein arrays. They do not require modification when used for the immobilization of a protein/peptide. For example, a self-supporting film of poly(hydroxyethyl methacrylate-co-glycidyl methacrylate) carrying reactive epoxy groups was successively applied to attach invertase, which has activity in the production of glucose and fructose from sucrose with respect to its free counterpart [65].

Polymeric films deposited on solid supports

Polymer/peptide arrays with polymer films can be divided into two major categories: surfaces coated with polymer hydrogels (Fig. 4 A) or polymeric films (Fig. 4 B) containing functional groups capable of the covalent attachment of peptides/proteins.

Polyacrylamide [66—68] and agarose [68, 69] are the most commonly applied polymer hydrogels. In this type of array, the biomolecules are physically entrapped in the hydrogel deposited on the solid support. The hydrophilic character of the hydrogel makes it possible to maintain the aqueous environment during experiments, thus preventing the denaturation of proteins and providing the proper environment for the reaction. However, the three--dimensional gel structure represents a barrier for diffusion of the target biomolecule and requires very long incubation times, especially for low-abundance proteins [66].

Covering the support by a polymeric film is a simple way to modulate the chemical and physical properties of the surface, leading to an improved quality of assays. For this purpose, many types of functional polymers have been used, *e.g.*, polylysine [70, 71], poly[*N*,*N*-dimethylacrylamide-co-*N*-acryloyloxysuccinimmide-co-3-(trimethoxysilyl) propyl methacrylate) (poly(DMA-NAS-MAPS)] [35, 72], poly(ethyleneimine) [73], poly(glycidyl methacrylate) [74], poly(aminoethyl methacrylate), and poly[(ethylene glycol) methacrylate] [75].

The immobilization of proteins/peptides on the polymeric films may increase the immobilization density or reduce the non-specific adsorption of biomolecules, depending on the applied polymer. For example, Chang Ming Li *et al*. [74] covered a PMMA support with a poly(glycidyl methacrylate) (PGMA) film using UV-induced radical polymerization. The presence of the PGMA

layer, carrying functional epoxy groups, led to an increase in the immunoglobulin G immobilization density compared to direct immobilization.

Yoon-Sik Lee *et al*. [76] coated several hydrophilic polymers containing functional groups [poly(aminoethyl methacrylate), poly(glycidyl methacrylate), poly(ethylenimine), chitosan, poly(glycidyl methacrylate)-*g*-poly(ethylenimine) and poly(glycidyl methacrylate)-*g*-PEG] on a glass support by two different methods: radical polymerization and coupling of a polymeric film to a modified glass. All of the polymer-grafted surfaces showed good repealing properties against the non-specific adsorption of proteins. They displayed very clear streptavidin-patterned surface properties making them high quality supports for protein-chip applications.

Stadler *et al*. [75] used UV-induced radical polymerization to obtain a poly[(ethylene glycol) methacrylate] (PEGMA) film on a glass support. The OH groups of the PEG side chains were modified with Rink amide linkers, which permit for peptide synthesis directly on the polymeric surface. The authors showed that PEGMA-covered glass slides are valuable supports for peptide arrays that resist non-specific protein adsorption and allow for high-quality mapping of specific interactions of monoclonal antibodies (anti-HA, anti-FLAGs) with appropriate peptide epitopes (YPYDVPDYA, DYKDDDDK-C).

POLYMERIC LINKERS IN PEPTIDE/PROTEIN ARRAYS

Linear linkers

The peptide/protein arrays with linear polymeric linkers between the peptide and the support may be synthesized using several approaches (Fig. 5).

First, the polymer chains must be attached to the support, which may be accomplished by "grafting to" or "grafting from" methods [77, 78]. The "grafting to" of a heterobifunctional polymer is mainly used due to its simplicity compared to "grafting from" approaches, which involve living ionic or controlled polymerization methods. Another method for fabricating a well-defined monolayer is the use of self-assembling processes with linker-thiols on gold supports (SAM) [79].

The peptide may be covalently attached to the polymeric linker by coupling reactions [80, 81] or can be synthesized directly on the surface [75]. Coupling methods, which involve a reaction between the functional groups of the amino acids present in the protein sequences and the functional groups of a solid support, are generally used in the case of protein immobilization [13]. Alternatively, such peptide/protein arrays may be obtained by a one-step immobilization of the entire polymer-peptide/protein conjugate [54, 55, 82].

The insertion of a linker can improve the accessibility of the peptide/protein attached to the surface and keeps the bound biomolecules away from the surface, resulting in a much faster and more efficient reaction with the target biomolecules. Corn *et al*. [83] employed a SAM of an amine-functionalized alkanethiol on the gold support for the preparation of arrays. The amine groups of the alkanethiol were transferred to thiols and reacted with the *N*-terminal cysteine of the FLAG peptide (C-DYKDDDDK). The presence of serine-glycine (SG) and serine-glycine-serine-glycine (SGSG) as linkers between the cysteine and the FLAG sequence improves the efficiency of the peptide interactions with the monoclonal antibody anti-FLAG M2 (Fig. 6). Most of the antibody adsorption was observed at the peptide with the SGSG spacer.

Poly(ethylene glycol) (PEG) is the most frequently used linker for the arrays. Trzebicka *et al*. [54] found that introducing six ethylene glycol units between the CF-GRMLG peptide and the surface improves the availability of the peptide for trypsin action. A quantitative yield for enzymatic hydrolysis was observed only in the case for which the peptide was bound through the PEG linker. Moreover, the insertion of the short PEG linker increased the density of the peptide immobilization

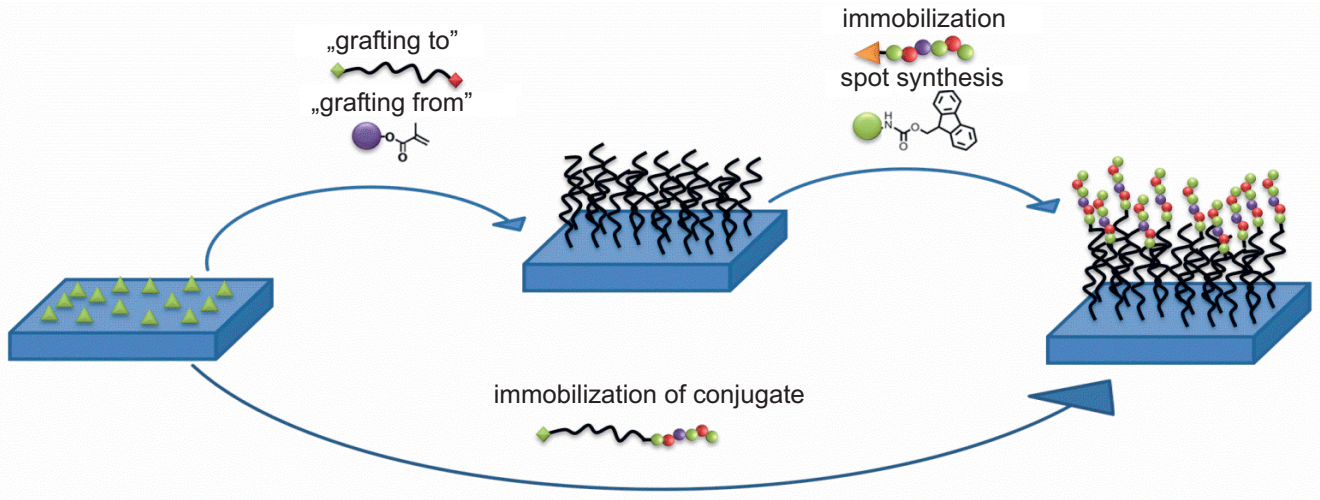


Fig. 5. Preparation methods leading to peptide arrays with polymeric linkers

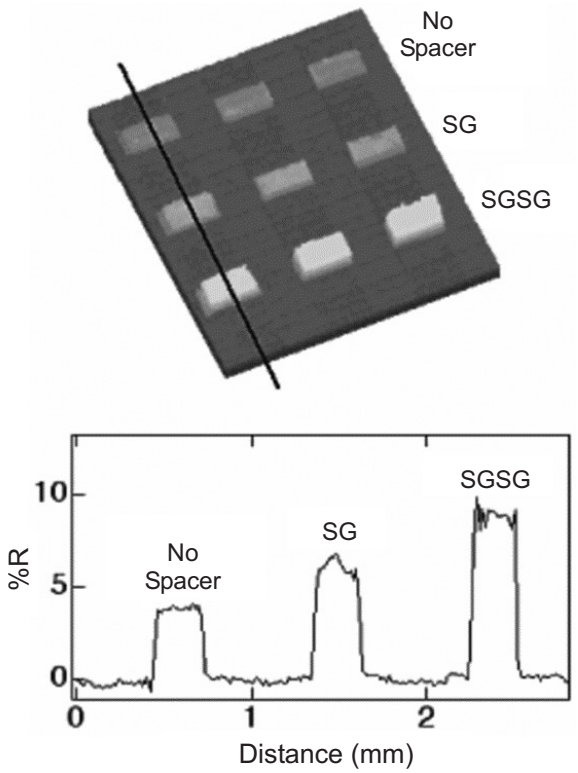


Fig. 6. The relative surface coverage (R) of the peptide arrays with an anti-FLAG M2; reprinted with permission from [83], copyright (2002) American Chemical Society

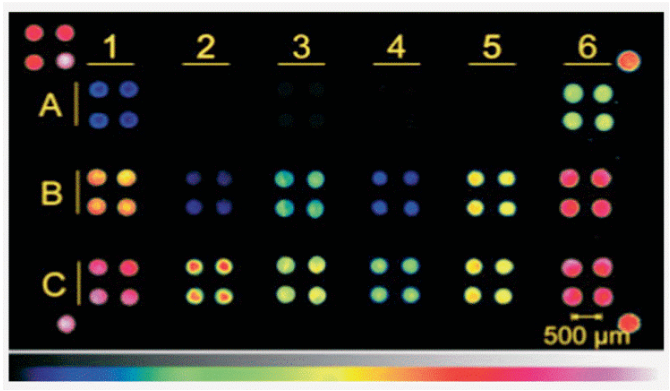


Fig. 7. Fluorescence intensities of a peptide-neutravidin conjugate printed onto amine coated glass slides with (A) biotin without linker, (B) biotin-aminohexanoic acid, (C) biotin-PEG_{12}, and probed with primary antibodies and the Cy5-labeled secondary antibodies (1) HSV-Tag, (2) T7-Tag, (3) Myc, (4) Pol, (5) Con, (6) Hel; the increased fluorescence intensities are displayed in the coloration order from black (no fluorescence), to blue, green, yellow, red, purple, and white (signal saturation); reprinted from [45], copyright (2006) with permission from Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

two-fold, thus improving the sensitivity of the enzyme assay.

Another example of improving the assay by an additional PEG spacer was shown by Andresen *et al*. [45]. They demonstrated that a PEG spacer between various peptides and the support increased the antibody binding to a peptide-neutravidin conjugate, which was physically adsorbed onto the amino-modified glass (Fig. 7).

However, in some cases, the immobilization of peptides *via* an excessively long PEG chain may cause undesirable effects. This effect was observed by Katayama *et al*. [55], who studied phosphorylation by protein kinases. The immobilization of the peptide CGIYGEFKKK-NH_2 through PEG decreased density of the peptide on the surface and the sensitivity of the kinase assay compared to the peptide attachment by sulfosuccinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxalate.

Another advantage of PEG as a linker is its resistance to the non-specific adsorption of proteins [84—86] that makes PEG the most commonly used linker for the preparation of peptide/protein arrays. The presence of hydrophilic PEG chains reduces the non-specific adsorption of biomolecules to the surface and provides a low background for measurements, resulting in improved sensitivity of the assay [44, 80, 84, 87]. For example, Lesaicherre *et al*. [80] investigated the kinase activity using peptide arrays with various peptide sequences. They showed that the intercalation of a PEG layer between the slide surface and the immobilized peptides minimized the non-specific binding of biomolecules, thus increasing the sensitivity of an assay. Additionally, the alkanethiols that are terminated by a short PEG chain were shown to effectively prevent the non-specific adsorption of proteins [88, 89].

Recently, the relevance of various lengths of PEG linkers in studying trypsin as a model protease using peptide arrays was described [90]. The authors showed that the optimal CF-GRMLG peptide immobilization density was reached for PEG with 12 ethylene glycol units, whereas the strongest anti-biofouling effect was observed for PEG with 27 units.

The peptide/protein arrays with PEG linkers are listed in Table 1.

Another class of linkers for protein/peptide arrays are polymers based on polymethacrylates [34, 43, 81, 91—93]. Klok *et al*. obtained polymer layers of poly(glycidyl methacrylate) (PGMA) and its copolymers with poly[2-(diethylamino)ethyl methacrylate] (PGMA-co-PDEAEMA) using surface-initiated atom-transfer radical polymerization (SI-ATRP) from silicon [92] and tantalum-pentoxide [81] surfaces. It was demonstrated that the copolymers of PGMA-co-PDEAEMA containing reactive epoxy functionalities were able to effectively bind proteins (lysozyme, bovine serum albumin) from aqueous solutions at room temperature. Due to the catalytic activity of the tertiary amine groups, the incorporation of the DEAEMA comonomer increases the rate of the protein immobilization reaction and leads to an enhanced protein-binding capacity. This increased the sensitivity of the biotin-streptavidin interactions and recombinant human tumor necrosis factor-α assays.

Li *et al*. [43, 94] used SI-ATRP from modified glass to obtain layers of poly[glycidyl methacrylate-co-po-

T a b l e 1. Peptide/protein arrays with PEG linkers

Support	PEG	Biomolecule	Application	Role of PEG	Ref.
glass	amino-PEG_3-succinimic acid	EIYGEFF	p60c-src protein tyrosine kinase analysis	increased accessibility	[44]
glass	NHS-PEG-NHS	YIYGSFK	kinase phosphorylation	prevents non-specific binding	[80]
glass	NH_2-PEG-NH_2	YIYGSFK	binding of FITC-labeled anti-phosphotyrosine		[80]
glass	NHS-PEG_{78}-COOH	$GGSGSGH_{10}$	binding of penta-anti-His antibody labeled with Alexa488	prevents the non-specific adsorption, increased accessibility	[87]
glass	NHS-PEG_{78}-COOH	antibody IgG_{saf32}	binding of second antibody		[87]
gold	NHS-PEG_{20}-MAL	CGIYGEFKKK-NH_2	cSrc phosphorylation		[55]
gold	NHS-PEG_{78}-MAL	CGIYGEFKKK-NH_2	cSrc phosphorylation		[55]
gold	CG(PEG_{12})IYGEFKKK-NH_2 conjugate		cSrc phosphorylation		[55]
silicon	Fmoc-NH-PEG_6-COOH	CF-GRMLG	trypsin hydrolysis	increased accessibility and immobilization density	[54]
glass	alkyene-PEG_3-cyclodiene	biotin	binding of streptavidin-FITC		[114]
glass	alkyene-PEG_3-cyclodiene	S-tag peptide	binding of FITC-labeled S-protein		[114]
glass	NHS-C(O)-PEG-C(O)-NHS	N_3-PEG_8-KETAAAKFERQHMDS conjugate	binding of S-protein and RNase S′		[115]
glass	NHS-C(O)-PEG-C(O)-NHS	K(N_3)ETAAAKFERQHMDS	binding of S-protein and RNase S′		[115]
gold	HS-$(CH_2)_{11}$-PEG_6	ferritin, immunoglobulin G, nebulin fragment (ND66 fragment), myosin	unfolding and nanomechanics of proteins	reduces nonspecific AFM tip-surface interactions	[116]
glass	biotin-PEG_{12}-MASMTGGQQMGTN biotin-PEG_{12}-TQPELAPEDPEDS biotin-PEG_{12}-EEQKLISEEDLLR biotin-PEG_{12}-DKDDAFYIVKRCI biotin-PEG_{12}-IVFTDDKLSNMRI biotin-PEG_{12}-NKTSLPTNIAFEL		antibodies interactions	increased accessibility and immobilization density	[45]
glass	NH_2-PEG_3	green fluorescent protein (GFP) glutathione S-transferase (GST)	binding of antibodies		[117]
glass	NH_2-PEG_8-N_3	green fluorescent protein (GFP) glutathione S-transferase (GST)	binding of antibodies		[117]
gold	HS-$(CH_2)_8PEG_7$ HS-$(CH_2)_8PEG_5$	AcIYGEFKKKC-NH_2	c-Src Kinase activity		[118]
glass	diene-PEG_4-biotin		binding of streptavidin		[82]
glass	diene-PEG_4-protein A		binding of FITC labeled antibody		[82]
gold	HS-(X)-PEG_5	glycopeptides			[119]
glass	NH_2-PEG-NH_2	biotin-G_6	binding of Cy3-streptavidin		[120]
glass	NH_2-PEG_{45}-NH_2	anti-HRP antibody	binding of enzyme HRP	prevents non-specific adsorption	[121]

ly(ethylene glycol) methacrylate] [P(GMA-co-PEGMA)] copolymer brushes. In the brushes, the glycidyl methacrylate units provide epoxy groups for efficient, covalent antibody attachment (Fig. 8), whereas PEG moiety can effectively prevent the nonspecific adsorption of biomolecules, thus improving the sensitivity of antibody immunoassays with different antigens.

Polymer brushes of poly[oligo(ethylene glycol)$_6$ methacrylate], poly[oligo(ethylene glycol)$_{10}$ methacrylate] and poly[(2-hydroxy ethyl) methacrylate] obtained by SI-ATRP from glass slides were investigated by Klok *et al*. [91] for the immobilization of fusion proteins. The surface hydroxyl groups were activated with *p*-nitrophenyl chloroformate. Subsequent O6-benzylguanine functionaliza-

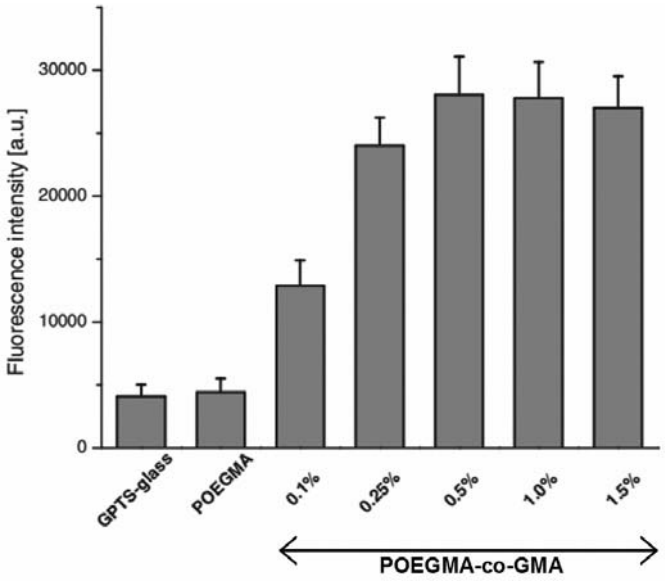


Fig. 8. Fluorescent intensities of Cy3-labeled anti-goat immunoglobin G microarrays fabricated on epoxy-modified glass, POEGMA modified glass, and various POEGMA-co-GMA-modified glasses; reprinted from [94], copyright (2010) with permission from Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

tion allowed for chemoselective immobilization of O6-alkylguanine-DNA-alkyltransferase (AGT) (Fig. 9).

All polymeric surfaces described by Klok *et al*. [91] are resistant to the non-specific adsorption of proteins, which makes them an attractive platform for the development of protein microarrays.

Recently, Yanxia Zhang *et al*. [95] applied random copolymers of poly(AMA-co-HEMA) for immobilization of the peptide Ac-HWRGWVA. It was demonstrated that this system selectively binds immunoglobulin-G molecules and is resistant to non-specific interactions when tested in complex protein solutions.

Branched linkers

Branched macromolecules have also been immobilized onto supports to link peptides/proteins. A variety of branched structures are used, including: dendrimers, dendrons, and stars (Fig. 10).

Dendrimers and dendrons, with their well-defined, monodispersed structure and high density of functional end-groups, are very attractive for peptide/protein microarray preparation [96]. The dendritic structure for the fabrication of protein/peptide arrays can be synthesized *in situ* by stepwise reactions initiated from the surface [97] or obtained by the attachment of presynthesized dendrimers or dendrons to the surface using coupling reactions [52] or SAM techniques of dendron thiols [98]. Generally, the strategy based on the attachment of prepa-

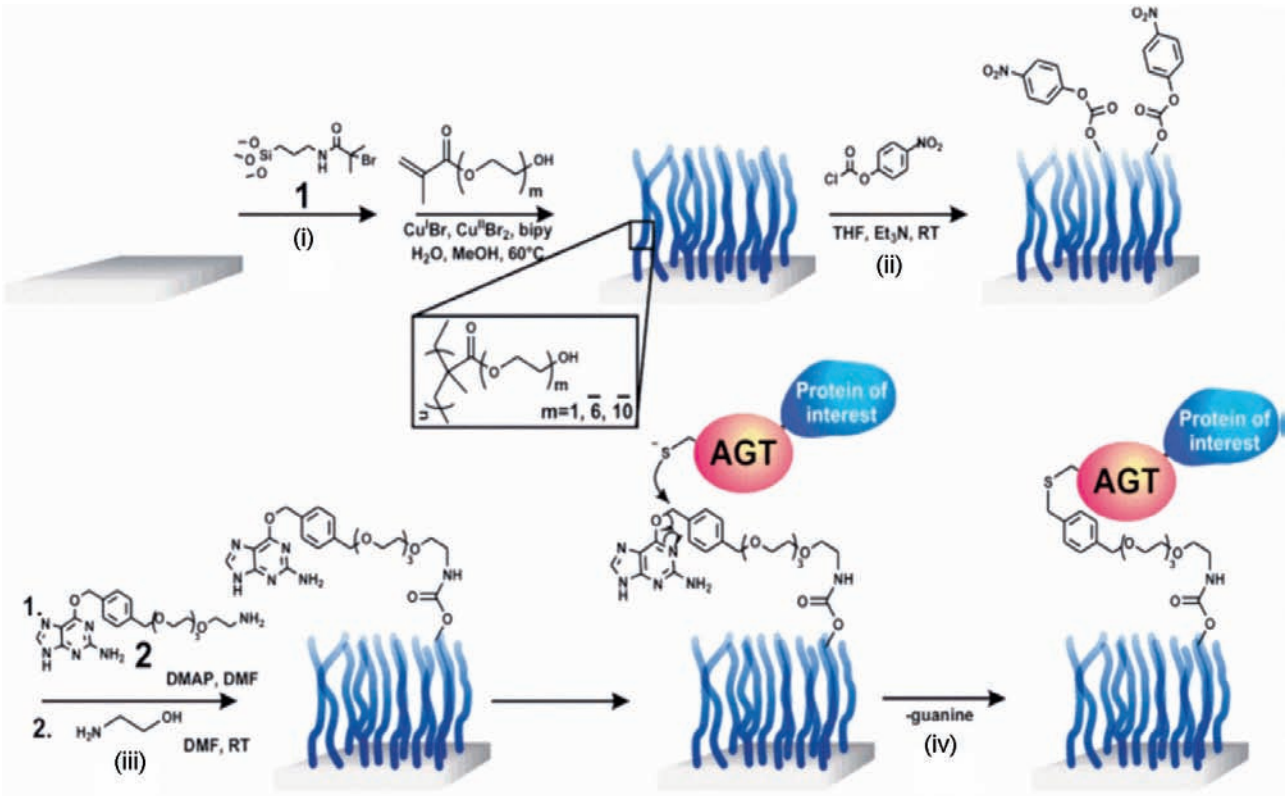


Fig. 9. Fabrication of protein-functionalized polymer brushes: (i) grafting of ATRP initiator 1 and surface-initiated ATRP; (ii) activation of hydroxyl groups with *p*-nitrophenyl chloroformate (NPC); (iii) (1) functionalization with benzylguanine derivative and (2) and quenching of residual NPC groups; and (iv) immobilization of AGT fusion proteins on benzylguanine-displaying surfaces; reprinted with permission from [98], copyright (2005) American Chemical Society

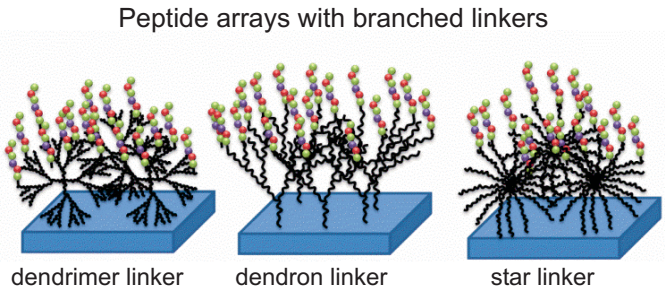


Fig. 10. Peptide arrays with branched polymeric linkers of various structures

red dendritic polymers is preferred because the reactions on the surface often suffer from lower yield. The dendrimers synthesized from the surface frequently have irregular structures and a lower number of functional end-groups than theoretically predicted, as was reported by Beier and Hoheisel [97]. They synthesized dendritic linkers from glass and polypropylene supports by several time-consuming modification steps. The structure of the linkers present on the surfaces was unclear and revealed inhomogeneous distributions of the immobilized biomolecules within individual spots.

The well-defined microarrays prepared by the attachment of presynthesized dendritic polymers were described for polyamidoamine (PAMAM) [99—103], polyphosphine [104, 105], and poly(propylene imine) [106].

The main advantage of the dendritic linker structure over a non-covered surface in peptide/proteins arrays is an increase in the biomolecule binding capacity due to its three-dimensional structure and large amount of terminal functional groups. Benters *et al*. [52] investigated PAMAM-modified glass surfaces for the fabrication of protein and DNA microarrays. The coating of the surface with polymerized dendrimers, with 64 primary amino groups in the outer sphere, led to an increase in the immobilization density of the biomolecules. Moreover, the crosslinking of the dendritic polymer layer by heterobifunctional reagents (disuccinimidylglutarate or phenylenediisothiocyanate) improved the stability of the surface against the harsh washing and regeneration conditions applied during microarray analyses.

The quality of the assays with the use of microarrays with dendritic linkers depends on the linker generation. Ajikumar *et al*. [102] described a simple, efficient fabrication method for a high-density PAMAM-COOH dendrimer-based glass support for protein microarrays. The performance of the fabricated surface for protein microarrays was evaluated in terms of the assay specificity and sensitivity, particularly with complex biological samples. A higher generation of PAMAM-COOH-coated surfaces (G4.5) has lower nonspecific protein adsorption compared to the lower generation of the dendrimer or succinimic acid surfaces. In addition, the spot homogeneity of the immobilized antibody improves with higher generations of PAMAM-COOH.

Lemcoff *et al*. [107] linked three generations of acetal-terminated benzylether dendrimers on a silica support for bovine serum albumin (BSA) immobilization. They demonstrated a significant, positive dendritic effect, showing that the use of dendrimers led to at least two times more efficient protein binding on the silica surface than the monovalent analogue. The BSA-binding capacity increased with the dendrimer generation. Pathak *et al*. [106] obtained a series of poly(propyleneimine) dendrimers of generations 1-5 on Si and glass surfaces for protein immobilization (BSA and alkaline phosphatase). They showed that the dendrimer generations past G-3 led to a higher density of immobilized BSA (Fig. 11). Moreover, the presence of higher generation (G-4 and G-5) dendrimers caused an increased activity of the immobilized alkaline phosphatase.

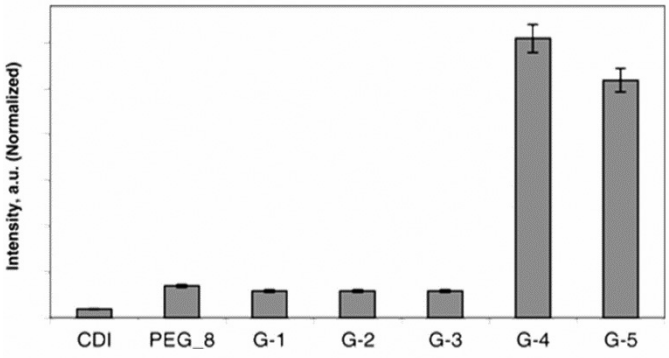


Fig. 11. Protein coverage on various generation dendrimers as well as control surfaces estimated using the integration of fluorescence intensity on identical surface areas; reprinted with permission from [106], copyright (2004) American Chemical Society

Another type of branched linker applied for protein/peptide arrays are polymeric stars. For the preparation of star surfaces for peptide/protein arrays, the PEG is generally used to form star arms. It was reported [108, 109] that, due to their anti-fouling properties, PEG-stars allowed for highly sensitive arrays able to detect even one protein molecule [110]. Mitra *et al*. [111] attached eight-armed PEG-octovinylsulfone-immobilized stars to mercaptosilanized glass and used them for the immobilization of proteins (polyclonal goat IgG, streptavidin, methionine aminopeptidase, DNA, and thrombin). The PEG coatings displayed low levels of non-specific protein adsorption and enabled highly quantitative single-molecule protein detection with the use of the total internal reflection fluorescence technique.

Groll *et al*. [112] coated six-armed PEG stars onto an amine-modified glass surface and used this system for streptavidin and His-tag enhanced green fluorescent protein. The star arms were terminated with isocyanate groups capable of reaction with the support and the proteins. The PEG-star layers prevent nonspecific protein adsorption, and thus the proteins could be selectively

bound to the surface, providing a high-quality spot pattern. Nienhaus *et al*. [113] used the same isocyanate-terminated PEG stars on an amine-modified glass surface for the immobilization of RNase H, a small enzyme that serves as a model system in protein folding. The PEG stars layer ensured the resistance to the non-specific adsorption of biomolecules.

CONCLUSIONS

The quality of assays with the use of peptide/protein arrays may be highly improved by introducing a polymer between the peptide/protein and the support. Generally, all polymeric linkers (branched and linear) and polymeric films between the peptide/protein and the support improve the mobility and accessibility of the immobilized biomolecules on the arrays, resulting in their higher activity and sensitivity toward target biomolecules, due to a much faster and more efficient reaction.

The polymer deposited onto the support may significantly increase the immobilization density of the biomolecules, thus improving the sensitivity of the assays. The density of the biomolecules strongly depends on the polymer length and architecture. The short, linear linkers expose their functional end-groups, which leads to a higher immobilization density than direct immobilization. The application of dendritic linkers results in a higher loading capacity of biomolecules than the linear analogs. The amount of biomolecules on such surfaces increases with the dendrimer/dendron generation. Additionally, the loading capacity may be increased by introducing polymeric films containing functional groups due to their three-dimensional network structure.

To improve the sensitivity of assays, polymers that possess anti-biofouling properties are often applied. Particularly valuable examples are poly(ethylene oxide) and its derivatives, making possible the detection of single protein molecules on the surface.

A very attractive solution comprises the introduction of copolymers containing functional groups that are able to bind proteins/peptides and minimize the non-specific adsorption. Such polymers may simultaneously increase the immobilization density and reduce the undesirable adsorption of biomolecules from the medium.

Despite the development of various polymeric supports for peptide/protein microarrays, it is difficult to select one optimal and universal surface. The type of an assay should be individually treated, and the surface coverage should be selected, taking into account several factors: the type of biomolecules investigated, the applied detection strategy, and the type of biological event; this indicates the necessity for further research.

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