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Novel probes for protein chip applications

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1. ABSTRACT

Protein microarrays (protein chips), due to their high throughput, have great potential to reduce the cycle time of drug discovery and to improve the efficiency of medical diagnostics. A great deal of effort has been made to facilitate the development of protein chips, such as the antibody microarrays. High-throughput fabrication of purified high-affinity probes is now one of the bottlenecks for protein microarrays. We discuss here three novel strategies to fabricate probes, namely, single chain antibodies displayed by phage, protein-oligonucleotide conjugates, and aptamers, which are promising in the protein chip technology.

2. INTRODUCTION

Highly demanded by the coming proteomic exploration, high-throughput drug discovery and individualized medical diagnostics, protein microarrays (or protein chips) as efficient and convenient tools for protein assay attract more and more attentions. Although most of previous achievements have been well documented in numerous reviews (1-16), it is still worthwhile to recall some exciting developments as follows. In 2000, Schreiber and coworkers discriminated one single spot of human immunophilin-rapamycin binding domain from the rest 10,799 IgG spots by advanced robotic arrayer and fluorescence scanner, demonstrating the feasibility of high-throughput protein detection (17). In 2001, it was Snyder's group who made the first proteomic chip, on which cloned 5800 open reading frames (ORF) proteins were spotted (18). Using this proteomic chip, they studied the interaction of calmodulin and phospholipids with whole proteome. Early in 1999, Niemeyer and colleagues firstly created a protein microarray through DNA-directed immobilization, providing a beautiful platform to combine the simplicity of DNA spotting with the complexity of protein assay (19). In 2000, another cheerful result was obtained by Golden and coworkers who succeeded in utilizing oligonucleotides, so-called aptamers, to imitate antibodies (20). The specific photoaptamers selected affinitatively from a library of photoaptamers were spotted on a substrate and the captured proteins were covalently bound to the aptamers by UV irradiation, resulting in photoaptamer microarrays. Recently, phage antibody chip was proposed as a novel

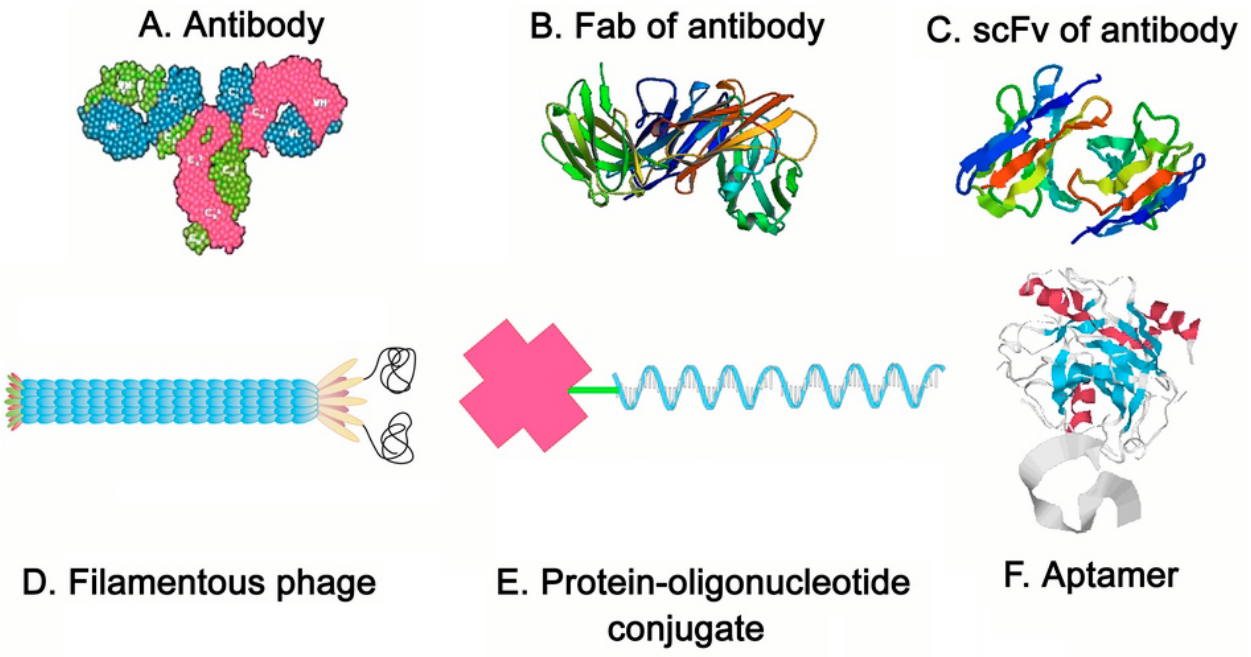

Figure 1. Schematics of six kinds of probes for protein microarrays. (A) monoclonal antibody; (B) Fab fragment of antibody; (C) scFv fragment of antibody; (D) filamentous phage displaying peptides on pIII; (E) protein-oligonucleotide conjugate; and (F) aptamer bounded with thrombin.. B, C, and F are reproduced from NCBI structure database http://www.rcsb.org/pdb/.

category of protein chips in which single chain antibody fragment (scFv) was displayed on the coat protein of filamentous phage (21). After selection and amplification, the resultant monoclonal colonies of phages were immobilized on substrates to form a phage chip, providing a hopeful way of high-throughput production of purified probes for protein chip.

Antibodies are one type of most important protein probes. They have made tremendous contribution to a wide range of applications that are based on molecular recognition. In this review, full-length antibodies, Fab fragments, and scFv (Figure 1) are referred to antibodies. Full-size antibody molecules consist of polyclonal antibodies and monoclonal antibodies (mAbs). The latter are produced in cultured cells and allow specific binding to certain antigens. As probes, full-size antibodies have some obvious advantages: 1) high diversity and specificity through natural evolution, 2) rich quantity in commercial production, and 3) good stability stemming from their ball-like structure. Fab fragments and scFv have been also proven useful as affinity bioreagents on protein chips (22, 23). Fab fragments are heterodimers consisting of the light chain and the equivalent domains of the heavy chain (V_H and C_H). These Fab fragments have essentially the same stability as the full-size antibodies do. scFv are recombinant molecules of single polypeptide containing only the variable regions of either the heavy or light chains, connected by an artificial flexible linker. Both types of the molecules retain the specificity of corresponding full-size antibodies in a context that provides new possibilities for immobilization (24), while potentially allowing for higher density of probes on surfaces. Moreover, after the recognition of targets, the sequence of the scFv can be determined, benefiting the following-up drug design and medicine development.

There are certain limitations, however, associated with antibodies. Despite of the ease of production, polyclonal antibodies usually contain multiple epitope specificities and are limited in quantity to the amount of serum that can be obtained from the immunized animal, which may impair both the specificity and density of single binding sites when used in a protein microarray. As the results, polyclonal antibodies are mostly used in sandwich assay as signaling molecules. Due to extremely high specificity mAbs are widely used. However, the identification and production of mAbs are laborious and could be very expensive in searches for rare antibodies that require screening of enormous number of colonies. Since Kohler and Milstein developed monoclonal antibody technology in 1975 (25), only a few thousands of mAbs have been produced in last three decades. On the contrary, there are almost unlimited variations of antibodies that human can produce. Thus, using existing mAbs to fulfill the task of proteomic research may be difficult. Commonly, Fab fragments are proteolytically generated from antibodies, so their production is more laborious than that of mAbs. Because of lack of the C_H domain, scFv often has lower stability, solubility, and affinity than Fab or full-size antibodies. Both antibodies and antibody fragments will encounter problems, when they are immobilized on solid surface (24). If immobilized by random non-covalent adsorption, they tend to denature due to the hydrophobic interaction with the surface. If the immobilization is through covalent bond, chemical bonding may destruct the

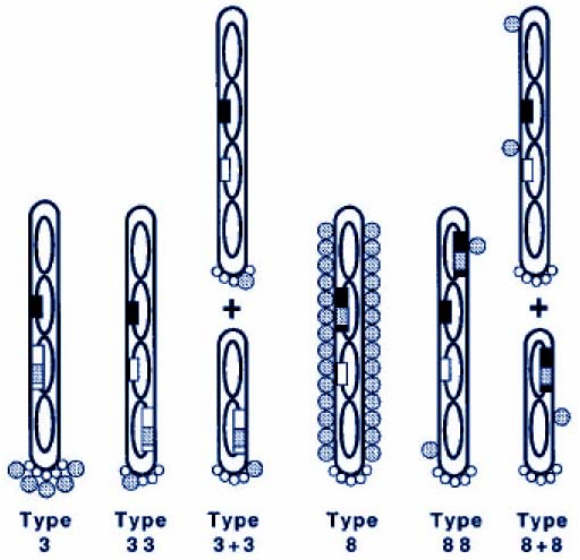

Figure 2. Types of phage display systems. The long vertical ovals represent phage virions, and the short vertical ovals represent phagemid virions. The twisted line inside each virion represents the single-stranded viral DNA. The segments encoding coat proteins pVIII and pIII are designated by black and white boxes, respectively. The hatched segments within these boxes represent foreign coding sequences spliced into a coat-protein gene, and the hatched circles on the surface of the virions represent the foreign peptides specified by these foreign coding sequences. The white circles at one tip of the virions represent the N-terminal domains of the pIII molecules. Foreign peptides displayed on pIII are either appended to the N-terminal domain (type 3 systems) or replace the N-terminal domain (type 3+3 and mostly type 33 systems). In type 8 systems, the foreign peptide is displayed on all copies of the major coat protein pVIII (2700 copies in wild-type virions), whereas in type 88 and 8+8 systems, only a minority of the pVIII copies display the foreign peptide. Reproduced with permission from Ref 26.

active sites. If the immobilization is through bridge molecules such as biotin-streptavidin and protein A or G, the immobilization density will remarkably decrease.

Considering the advantages and disadvantages of antibodies, there are at least two criteria for ideal probes based on antibodies: 1) the probe can be purified easily and form a large library of rich diversity, and 2) the probe can be arrayed efficiently and maintain high specificity. Although the progress described above is exciting, a universally applicable protein chip still faces a number of problems in many aspects, because protein chip is a complex system and requires integration for multi-disciplinary state-of-the-arts and the cutting-edge technologies. In this review, we focus on three novel probes composed of antibodies or their analogues for protein chip, which the authors' work is involved in.

3. PHAGE ANTIBODY CHIP

3.1. Phage display

Phages are viruses that infect bacterial cells and are widely used in recombinant DNA research, in which phages infect the standard recombinant DNA host, the bacterium *Escherichia coli*. Phage, an "expression vector", programs the machinery of the *E. coli* host cell to synthesize a foreign peptide whose amino acid sequence is determined by the nucleotide sequence of the insert (Figure 1D). In phage display, by splicing the foreign gene sequence into the gene for one of the coat proteins, the foreign amino acid sequence is genetically fused to the endogenous amino acids of the coat protein to make a fusion protein. The most commonly used vectors are filamentous phage strains M13, fd, and fl. Filamentous phages are flexible rods about 1 μm long and 6 nm in diameter, composed mainly of a tube of helically arranged molecules of 50-residue major coat protein pVIII; there are 2700 copies in wild-type virions, encoded by a single phage gene VIII. Inside this tube there lies the single-stranded viral DNA (ssDNA; 6407-8 nucleotides in wild-type strains). At one end of the particle there are five copies of each minor coat proteins pIII and pVI; minor coat proteins pVII and pIX are located at the other tip (26).

Among the phage-display systems, pIII and pVIII are widely used to display foreign peptides (Figure 2), including fragments of proteins (27, 28), enzymes (29), receptors (30, 31), DNA and RNA binding proteins (32), and cytokines (33). For antibody display, variety of binding ability of scFv stems from the random structures of the variable regions which are determined by the random sequences of DNA inside the phage. A number of phage antibody libraries whose sizes range from 2×10^5-6×10^{10} have been constructed (34). Those large libraries provide sufficient resources for in vitro selection of specific binding probes for each target. An overwhelming advantage of the phage antibody display is that it can deliver humanized antibodies which cannot be obtained by conventional animal-based production. To minimize the laborious handwork, remarkable progresses have been achieved in adapting phage-display selections to robotics. Novel methods for automation analysis by phage ELISA, clone picking, and sequencing are established (35, 36).

3.2. Immobilization of phage on surfaces

Immobilization of phage on surfaces is important in the fabrication of phage antibody chip. Soon after the establishment of the technique of phage display, phages began to be immobilized on substrates, for the purpose of affinity measurement (37-39) or biosensor development (40-43). Among these applications, many different methods of immobilization have been exploited, such as physical adsorption for ELISA (37-39), covalent bonding, and molecular recognition. For examples, phage has been immobilized by peptide bond between amino residue on phage and carboxyl terminal on surface (40, 43), by disulfide bond between one thiol group on phage and another on surface (40, 42), and by specific recognition between hexahistidine tag on phage and nickel coated surface (41).

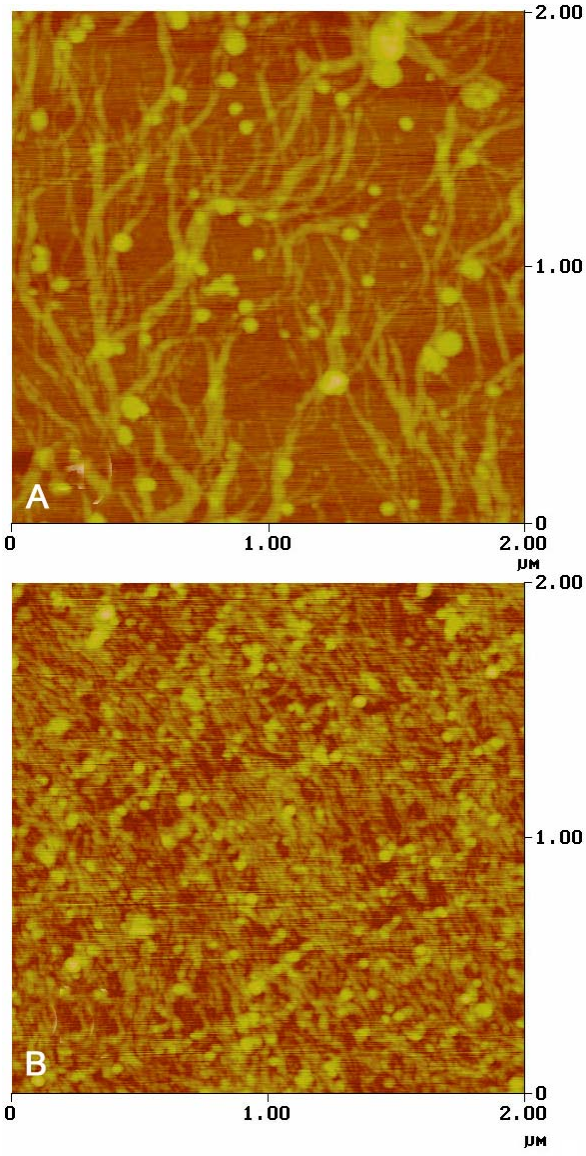

Figure 3. AFM images of phage immobilized on silicon surfaces. a) by physical adsorption and b) by chemical bonding. The images were taken in tapping mode with a single crystal silicon tip to minimize the destruction of the surfaces. Reproduced with permission from Ref 21.

The requirements for phage microarrays are even higher than phage-based biosensors. Not only should the density of the immobilized phage be optimum, but also would the orientation be kept about the same. If the immobilized phages are packed too tightly, the free space left for probe-target recognition will be not enough. Instead, if the density is too low, both the sensitivity and the specificity will certainly decrease. As to the first published phage antibody chip (21), the authors compared immobilization densities between physical adsorption and covalent bonding (44). The topologies of the surfaces treated by two methods were obtained by AFM (Figure 3). The figures illustrate that the immobilized density is medium and the orientation is ordered using covalent attachment, indicating that it is a better choice.

3.3. Application of phage antibody chip

In the first phage antibody chip, the authors made their efforts on proving the feasibility of the application in two aspects. One is preliminary diagnostics of cancer or other diseases, and another is high-throughput detection of appointed antigens (21).

As a proof-of-principle experiment of cancer diagnostics chip, five monoclonal colonies of phages, displaying respectively four scFv from mouse and one humanized scFv, were chosen for the fabrication of phage arrays (Figure 4) (21). The targets were Cy3 fluorescence labeled proteomes from normal lymphocytes and tumorous HeLa cells, respectively. After incubation, chips were thoroughly washed and scanned with microarray fluorescence scanner. From the clear images of patterned arrays, differentiated distribution of fluorescence intensities were exhibited. Although four other phages displayed almost the same fluorescence signals, the intensity of M3 phage incubated with tumorous HeLa cells was higher than that of normal cells, manifesting an overexpression of some kind of proteins in the tumorous cells. Based on this information, it is expected that phage antibody chip with sufficient amount of displayed scFv will be potentially powerful in the development of pre-test of severe diseases.

In the experiment of specific antigen detection, the ratio of the signal of positive control over that of negative control is set as the parameter for evaluation (45). For example, comparison is carried out between pIII and pVIII display systems (Figure 5). In the case of pIII display, the ratio of the positive signal to the negative is only about 2:1. This is because pIII is the minor protein of wild phage and there are only five copies of pIII on the tip of the phage. In the pIII display system, scFv is always expressed mono-valently, and most probably scFv will be either orientated parallel to or in contact with the surface which may restrict the freedom of scFv recognition. On the contrary, the result is surprisingly exciting in pVIII display system. The ratio of the positive signal to the negative increased up to 3000:1. This is attributed to the amount and status of pVIII of phage. Differed from pIII, there are about 2700 copies of pVIII which form the tube of phage. Using pVIII display system, not only the number of fused scFv will be increased, but also the orientation will be improved because there is always half of the displayed scFv stretching freely out into solution. It is the authors' view that phage antibody chip by pVIII display system will be very promising.

4. PROTEIN-OLIGONUCLEOTIDE CONJUGATES (POC)

4.1. Background

Protein and DNA, two vital components in life phenomena, have unique and complementary characters. Due to its simple but efficient base-pairing, DNA has been manipulated and designed for a lot of purposes. On the contrary, protein has more complex structure as well as

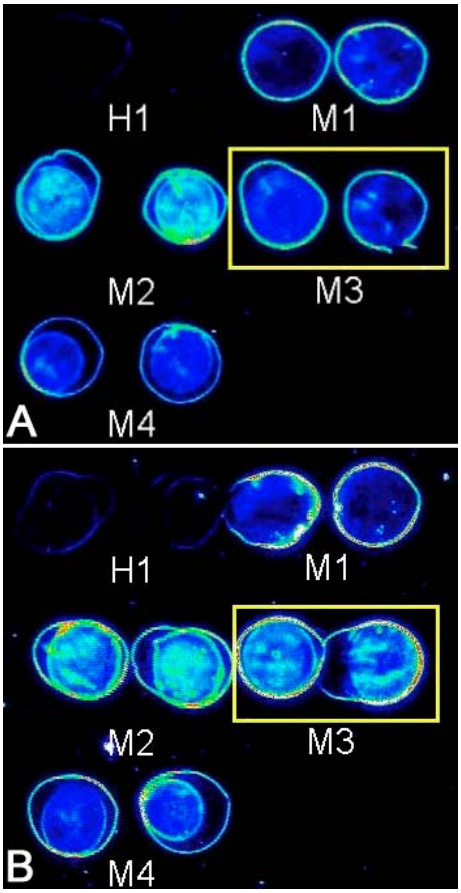

Figure 4. Recognition patterns of different proteomes by phage antibody chip. On the chip M1, M2, M3, and M4 are phages displaying monoclonal scFv of mouse-origin, and H1 are that of human-origin. The fluorescent images of the phage microarray were obtained by scanner equipped with confocal laser, and the resolution was 10 micrometers. Reproduced with permission from Ref 21.

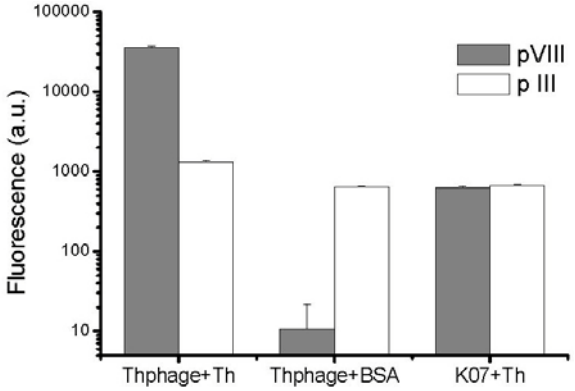

Figure 5. Comparison on the fluorescence intensities of phage antibody chips displayed by pγ and pβ. Thrombin was selected as positive target, bovine serum albumin as negative target, phage fused with thrombin-specific scFv (Thphage) as positive probe, and blank phage vector (K07) as negative probe. Reproduced with permission from Ref 45.

almost infinite functions. In 1992, POC have been utilized to develop a new technique of immuno polymerase chain reaction (IPCR) (46). Inspired by the success of DNA microarrays, POC has been adapted by Niemeyer's laboratory into microarray format, which is designated as DNA directed immobilization (DDI) (19). An obvious advantage of this methodology is that the recognition between antigens and antibodies can take place in solution, and the probe-target complex can be directed onto surfaces subsequently through duplex DNA formation. In this way, the interference of the solid surface on antigen-antibody recognition can be mostly removed. Moreover, the DNA duplex after hybridization can act as a spacer between proteins and substrates, resulting in a further reduction of protein denaturation.

Because the lateral surface structuring is carried out at the level of stable nucleic acid oligomers, the DNA microarrays can be stored almost indefinitely, while functionalization by proteins of interest via DDI can be made immediately before use. Furthermore, probes can be quickly and easily switched by hybridization, dehybridization, and rehybridization of different POCs. Recently, active control was introduced, where DNA acted as a remote controller besides as a mediator (47).

4.2. Fabrication of POC

Applications of in-line synthesis and fragment conjugation in POC fabrication have been carefully reviewed (48, 49). Specifically, the methods for making protein-oligonucleotide conjugates can be classified into three categories. The first is chemical bonding in which the covalent linkages between proteins and oligonucleotides can be disulfide, maleimide, thioether, amide bond, and ester bond (50-57). Secondly, specific molecular recognition can be used to form strong connection, such as stoichiometric binding of biotin to streptavidin (19, 58-64). At last, in vitro translation of mRNA covalently modified with a puromycin group at its 3'-end is developed recently (65-67). The peptidyl-acceptor antibiotic puromycin covalently couples the mRNA with the polypeptide chain grown at the ribosome particle, leading to a specific conjugation of the informative (mRNA) with the functional (polypeptide) moieties.

4.3. Microarrays by POC

The first DDI protein microarray was made by Niemeyer et al in 1999 (19). In that work, streptavidin modified DNA and biotinylated alkaline phosphatase, β-galactosidase, horseradish peroxidase, anti-mouse and anti-rabbit immunoglobulins were combined through biotin-streptavidin recognition. Quantitative measurements on microplates demonstrate that DDI protocol has higher immobilization efficiency than conventional immobilization techniques, such as the binding of the biotinylated proteins to streptavidin-coated surfaces or direct physisorption. In another case, Phylos Company developed a novel technique named PROfusion, which utilized peptidyl-acceptor antibiotic puromycin to connect mRNA and in vitro translated peptide (67). The feasibility of this concept of protein chip was demonstrated with

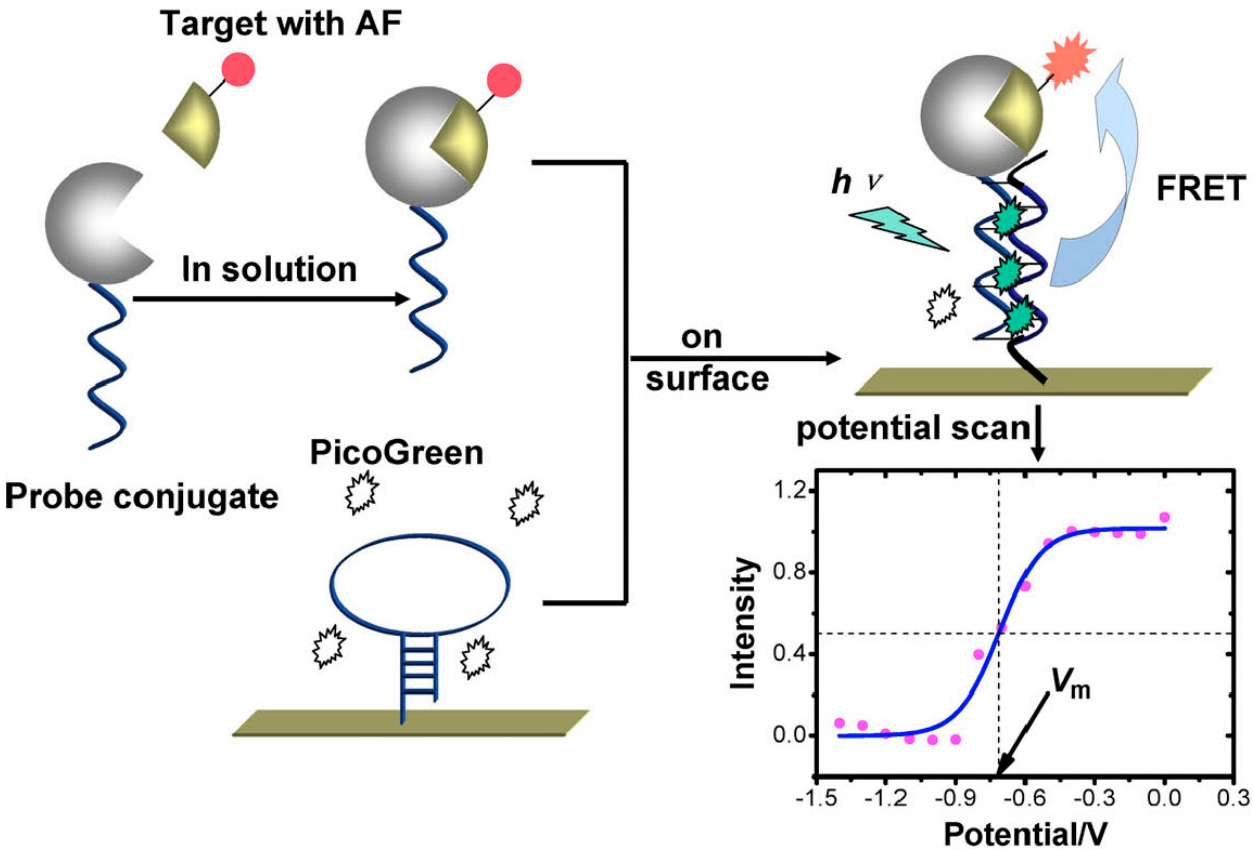

Figure 6. The schematic view of the experimental procedure of actively controlled POC chip. The probe antibody was firstly covalently connected with an ssDNA to form protein-oligonucleotide conjugate. Then the probe conjugate reacted with Alexa Fluor546 (AF)-labeled target in solution to form biomolecular complex. The complex then was hybridized with the hairpin DNA, which was immobilized on the surface. After the hybridization, PicoGreen (an intercalating dye which can act as donor to Alexa Fluro546 in FRET) was added in the solution, and a 488 nm laser was used to excite PicoGreen and the FRET signal was recorded. An SPHD curve, the normalized FRET intensity against the electric potential, was obtained by scanning the surface electric potential.

several mRNA-protein fusions, each possessing a unique peptide epitope sequence. These addressable proteins could be visualized on the microarrays by both autoradiography and highly specific monoclonal antibody binding. The anchoring of the proteins to the chip surface is proven to be robust, and the system is sensitive enough to detect sub-attomole quantities of displayed protein without signal amplification. In spite of the cheerful success of PROfusion, the technique encounters bottlenecks including displaying larger proteins and decreasing the susceptibility of conjugated mRNA.

4.4. Actively controlled chip

Wei and colleagues recently proposed a method of scanning potential hairpin denaturation (SPHD) in DNA detection, where the recognition process could be actively controlled by an electric potential (47, 68). Inspired by this approach, the negative charged DNA was used as a remote controller besides as a spacer, which was conjugated with protein probe and modulated by electric potential (69). The whole procedure is illustrated in Figure 6. Similar to the observation on hairpin DNA denaturation (47, 68), after the probe-target recognition the POC is hybridized with the hairpin DNA which is immobilized on the substrate, and an S-shape curve in the intensities of the fluorescence resonance energy transfer (FRET) is obtained by scanning the electric potential. In the SPHD method, melting potential (V_m), a critical parameter for discrimination, depends mainly on the stability of the biomolecular complex and is less sensitive to concentrations both on surface and in solution. Instead of absolute value, SPHD measures the change of signal as a function of surface electric potential, so that V_m is more stable and reproducible. Figure 7 presents such an example.

5. APTAMER

5.1. Introduction to aptamer

Aptamer is defined as a fragment of oligonucleotide, which can form 3-D structure and bind specifically to some substances. Aptamer can be used as mimics of antibodies and the identification of aptamers from very large libraries of random sequence oligonucleotides was described in 1990 (70, 71). This

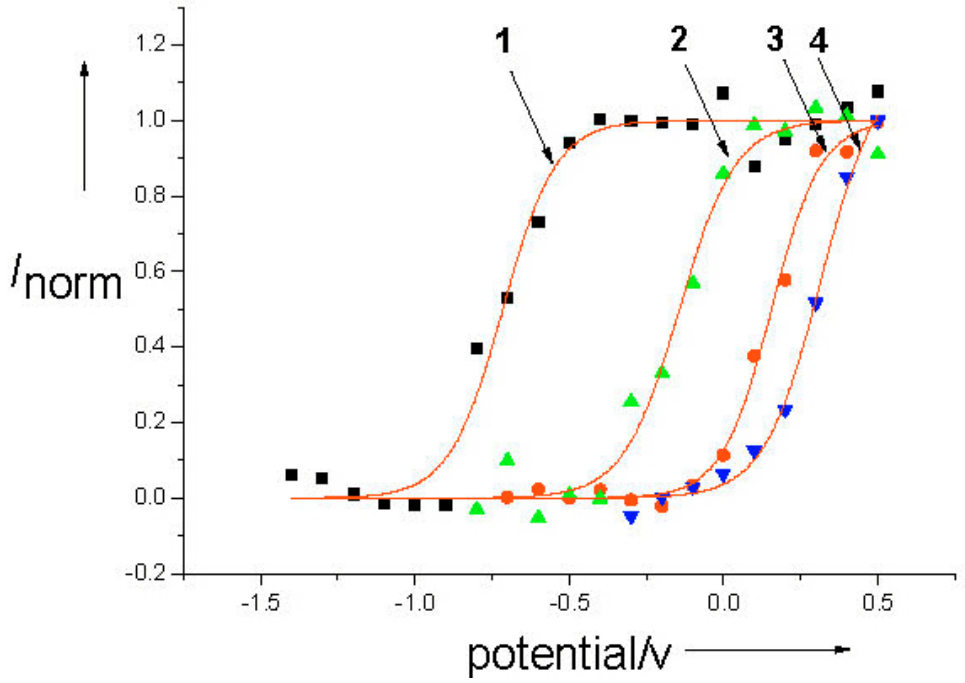

Figure 7. The SPHD curves of different systems illustrated by the normalized FRET intensity. I_{norm} is the normalized FRET intensity, and potential is the voltage reading between the working electrode (silicon) and the counter electrode (Pt wire). Curve 1, perfectly matched DNA duplex and recognized probe-target complex; Curve 2, perfectly matched DNA duplex and misrecognized probe-target complex; Curve 3, mismatched DNA duplex and recognized probe-target complex; and Curve 4, mismatched DNA duplex and misrecognized probe-target complex. The melting potentials (V_m) are: -0.72±0.06 V for curve 1, 0.01±0.10 V for curve 2, 0.16±0.09 V for curve 3, and 0.29±0.10 V for curve 4, respectively. Reproduced with permission from Ref 69.

method, entitled the SELEX process (70), is a technique for screening very large combinatorial libraries of oligonucleotides by an iterative process of in vitro selection and amplification.

Compared with antibodies, aptamers have many intrinsic advantages. 1) Ease and high reproducibility of production. Aptamers are produced automatically by DNA synthesizer with the capacity of aptamer libraries ranging from 10^{14} to 10^{15}. In addition, SELEX process becomes more and more automatical. Aptamers are purified under denaturing conditions to a very high degree of purity. Therefore, little or no variation is expected in aptamer production. 2) Wide adaptation. Because animals or cells are not involved in aptamer identification, toxins as well as molecules that do not elicit good immune responses can also be used to generate high-affinity aptamers. The 'aptamer antigens' can be small molecules including metal ions (72), organic dyes (73), drugs (74), amino acids (75), cofactors (76), aminoglycosides (77), antibiotics (78), nucleotide base analogs (79), nucleotides (80) and peptides (81), and proteins including enzymes (82), growth factors (83), antibodies (84), gene regulatory factors (85), cell adhesion molecules (86), and lectins (87). In addition, unrestricted methods are exploited in aptamer detection, such as sandwich assay (88), flow cytometry (86), electrochemisty(88, 89) and aptamer beacon (90, 91). 3) Excellent controllability and versatility. To amend the susceptibility of RNA, modifications at the 2' position of the sugar with -F or $-NH_2$ are proven to make RNA nuclease resistant (92, 93). In addition, aptamers can also be modified at the C-5 position of pyrimidines with –Br, -SH or $-NH_2$ to extend their functions (94). Unlike proteins, the denaturation of aptamers is reversible, by controlling heat, salt concentration, pH of the media, and chelating agents. It is also possible to design divalent or multivalent aptamer to improve their affinity. For instance, divalent anti-NHE (neutrophil elastase) aptamer showed a 10-fold higher affinity than the monovalent form in binding to the target either in solution or immobilized on beads (95). 4) High specificity through multiple recognitions. It would be reasonable to expect to find a series of aptamers that bind to different binding sites of a target with different affinities, which generates a unique pattern of multiple recognitions. For example, two separate groups identified that one class of aptamers bind thrombin at the fibrinogen-recognition exosite (96), whereas the other class bind at the heparin-binding exosite (97). Aptamers also exhibit a high degree of chiral recognition to target molecules and can differentiate minute differences from the targets, such as glycated proteins or isoenzymes from different tissue origin (98).

As the application in molecular detection, aptamers have been designed with stem-loop structures bred from molecular beacon, which consist of stems of a few base

pairs and fluorophores of donor and acceptor on each ends of the aptamers. This kind of aptamer designs are termed as aptamer beacon, successful in detection of thrombin in solution (90, 91). To minimize the laborious work and high costs resulting from fluorescence labeled aptamer beacon, Fang and colleagues replaced the two tagged fluorophores with intercalating dye $Ru(phen)_2(dppz)]^{2+}$ by incorporating the dye into the configured aptamers as light switch (99, 100). With measurement of the fluorescence decrease, IgE and PDGF-BB have been quantitatively determined.

5.2. Aptamer microarrays

The photoaptamer, developed by Golden et al (101, 102), is an improved form of aptamer by replacing thymidine with 5'-bromodeoxyuridine (BrdU). Photoaptamers are selected for their high affinity to target proteins using the in vitro photoSELEX protocol (20). Incorporation of photoreactive BrdU in photoaptamers provides a means to covalently cross-link the photoaptamer to the bound protein by irradiation at 308 nm UV light. The covalent bond occurs between a photoactivated BrdU within the photoaptamer and an electron rich amino acid residue of the protein that is in proximity and in the correct orientation to the BrdU (103). After this modification, even under stringent washing, captured proteins will stay with photoaptamers, resulting in high signal-to-background ratio. A 17-plex photoaptamer array has exhibited detection limit ranging from 0.006 pM to 42 pM, and k_D varied from 0.01 nM to 300 nM for several analytes including interleukin-16, vascular endothelial growth factor, and endostatin, and was able to measure proteins in 10% serum samples (103). Besides the detection limit, another study shows that with the photocross-linking, the target/non-target specificities of photoaptamer will reach 100- to $>10^6$-fold (103). Although with such successful achievement, the variation of photocross-linking efficiencies retains the range from 20% to 80%, baffling universal applications of photoaptamer microarrays.

Surprisingly, aptamer microarrays without oligonucleotide modification did not appear until recently. In 2005, two aptamer microarrays have been reported (104, 105), signaling a great advance along with the line of aptamer microarrays. Recently we were also able to immobilize aptamers of anti- and non-anti-thrombin onto silicon surfaces and observed the difference between positive and negative recognitions (106). However, our research indicated that, due to the weak force to maintain appropriate aptamer configurations and the strong aptamer-surface interaction, the specificity of recognition by aptamers on surfaces is somewhat impaired compared with that in solution. This could explain why an aptamer microarray without oligonucleotide modification did not come out in earlier days as one would hope.

6. PERSPECTIVE

With the preliminary results using phage antibody, POC, and aptamer as probes for protein microarrays, we see both the hopes and the challenges ahead. Phage antibody is a viable solution for high-throughput fabrication of purified and high-affinity antibodies, but the innateness is labor-intensive for the library of high-affinity displayed scFv. The improvement of phage antibody chip will be focused on simplifying the procedure and making it automation. The storage of the chip might be another problem, as is the case of other biochips which directly use immobilized proteins as probes. Microarrays by POC are known for its stability in storage, easiness in switching probes, and active control for recognition. However, labeling by either oligonucleotide or biotin is necessary for the formation of POC, which raises cost and complexity. Also, some fragile proteins are unsuitable for this kind of conjugation. As a promising probe, aptamer can replace antibody in most of the situations and the idea of active control can also be applied. However, the degradation of recognition power due to the aptamer-surface interaction considerably limits aptamer application. It is realized that the property of oligonucleotides immobilized on surfaces could be quite different from that in solution (47). As a result, the loss of intrinsic power of the aptamer recognition often occurs when aptamers are transferred from solution onto a solid surface. This problem has to be solved before the concept of aptamer chip is fully applicable.

7. ACKNOWLEDGMENTS

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Abbreviations: POC: protein-oligonucleotide conjugate, IgG: immunoglobulin G, scFv: single chain antibody fragment, mAb: monoclonal antibody, ORF: open reading frame, AFM: atomic force microscopy, ELISA: enzyme-linked immunoadsorbent assay, IPCR: immuno polymerase chain reaction, DDI: DNA directed immobilization, SPHD: scanning potential hairpin denaturation, FRET: fluorescence resonance energy transfer, SELEX: systematic evolution of ligands by exponential enrichment, BrdU: 5'-bromodeoxyuridine

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