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Fabrication of Antibody Arrays Using Thermally Responsive Elastin Fusion Proteins

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The use of high-throughput antibody arrays for simultaneous detection of multiple analytes has gained considerable interest in the area of medical and environmental diagnostics.[1] Since detection occurs in a dedicated microregion on the array surface, the ability to spatially pattern antibodies on a defined region is essential. A variety of soft lithography, photolithography, ink-jet printing, and array printing techniques are currently available for patterning an array surface.[2] In most cases, antibody immobilization involves either covalent coupling or interaction with an immobilized ligand.[3] A principal problem with these methods is a loss of the functionality due to covalent modifications of the binding site and the random orientation which results in steric blockage of the binding sites.[3] To overcome these drawbacks, an intervening layer of protein A, G, or L (ProA, ProG, or ProL),[4] which selectively binds different types of immunoglobulin (Ig) with high affinity, has been used to immobilize antibodies with significantly improved antigen-binding capacity, sensitivity, and stability compared with covalently coupling.[5] However, creating a properly oriented film of these antibody-binding proteins remains a major challenge.

Self-assembly of biological molecules onto a patterned surface could be achieved by triggered control of hydrophobicity that enables the spatially modulated presentation of biomolecules onto an array surface.[6] This approach takes advantage of smart biomolecules with thermally tunable hydrophobic properties that can be used for selective and reversible adhesion onto a target microregion of an array surface. Elastin (ELP)-based biopolymers are stimuli-responsive polypeptides composed of repeating VPGVG that undergo a reversible phase transition from water-soluble forms into hydrophobic aggregates over a wide range of temperature and pH.[7] By creating a spatially patterned hydrophobic surface template, selective adhesion of the ELP biopolymers can be achieved above its phase transition temperature. Nath et al. have demonstrated this feasibility for protein array fabrication by direct capturing of ELP fusion proteins from solution onto surface-grafted ELPs based on triggered hydrophobic interactions.[8] Although a wide range of ELP fusion proteins can be captured using this strategy, not all antibodies or their truncated fragments are currently available for fusion construction. We report here a universal platform for antibody array fabrication by creating functional ELP fusions with ProA, ProG, or ProL for the immobilization of antibodies onto hydrophobic surfaces. Antibodies are first conjugated to the ProA, ProG, or ProL domain of the fusion proteins, followed by immobilization of the complex via temperature-triggered hydrophobic interaction between the ELP domain and the hydrophobic surface. This method provides a universal platform to immobilize antibodies in a functionally active orientation without covalent modification to the receptor. As a demonstration, we have patterned antibodies from different mammalian species by directly printing the complexes (ELP−ProA, ProG, or ProL−antibody) onto a hydrophobic glass slide using a DNA microarrayer. Functional antibody arrays for the detection of tumor markers involved in cancer diagnosis have been fabricated using this method.

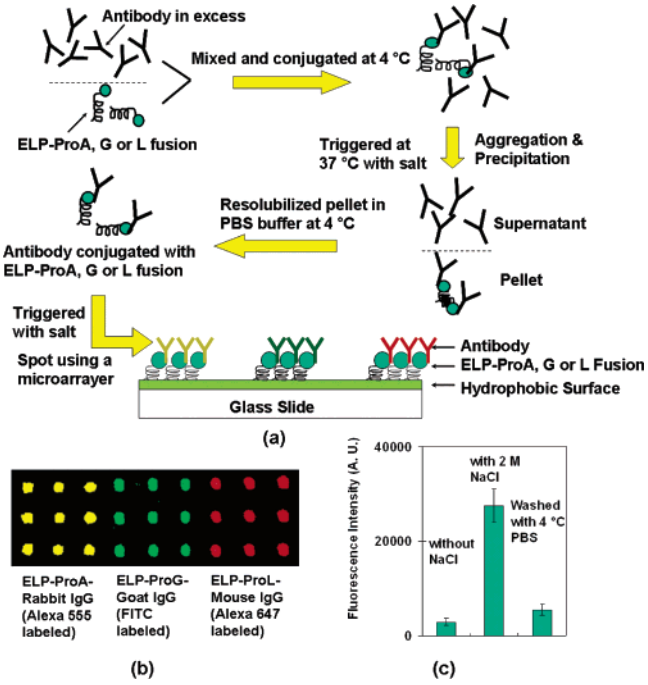

Figure 1. (a) Schematic procedure to form and purify ELP−protein fusion−antibody complexes that are spotted onto the glass slide to fabricate antibody microarrays. (b) Fluorescence image of microarrays fabricated by immobilizing antibodies from rabbit, goat, and mouse, in complexes formed with ELP−ProA, ELP−ProG, and ELP−ProL fusions, respectively. The antibodies from rabbit, goat, and mouse are labeled with fluorophores Alexa 555, fluorescein isothiocyanate (FITC), and Alexa 647, respectively. (c) Comparison of the relative fluorescence intensities detected from the complexes immobilized on hydrophobic glass surfaces at the following conditions: (i) the ELP domain is hydrophilic at room temperature (no NaCl is added); (ii) the ELP domain is triggered to its hydrophobic phase (with 2 M NaCl), and the glass surface is washed with warm PBS after spotting; (iii) same as (ii), except that the glass surface is washed with cold PBS before the complexes are completely dried.

Three different ELP fusion proteins were genetically constructed by fusing an ELP domain consisting of 78 VPGVG repeats to either ProA, ProG, or ProL. All fusion proteins were expressed to high yield (~300 mg/L) in *E. coli* and easily purified by two cycles of temperature-triggered precipitation and resolubilization (inverse temperature transition).[9] The purified ELP fusion proteins were shown to retain the reversible hydrophobic−hydrophilic transition property of the ELP as well as the antibody-binding capabilities of ProA, ProG, and ProL.[9] By fine-tuning the ionic strength of the solution, phase transition can be isothermally triggered even at room temperature.[9]

Figure 1a schematically shows the procedure to form and purify the ELP−ProA, ProG, or ProL−antibody complexes that were

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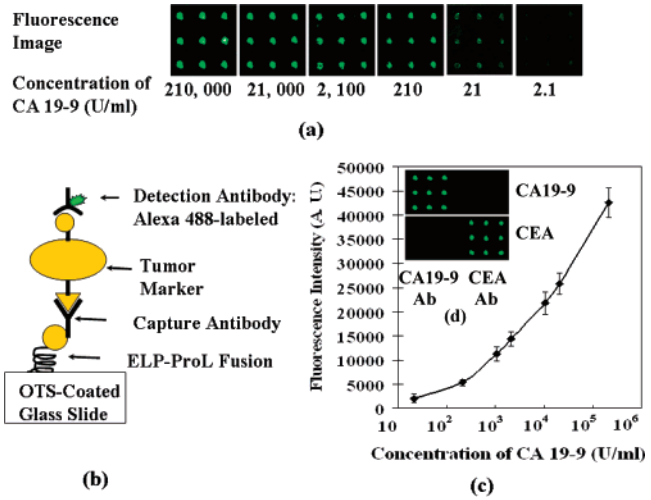

Figure 2. (a) Representative fluorescence images of the microarray when samples with different concentrations of CA 19-9 antigen are analyzed. (b) Schematic illustration of the sandwich immunoassay configuration used to construct the sensor. (c) Fluorescence intensity as a function of the CA 19-9 concentration in the samples. (d) Specificity of the antibody array. Antibodies for CA 19-9 and CEA were spotted onto the array, and samples containing either CA 19-9 or CEA were added.

spotted onto the modified glass slide. Excessive amount of antibody was mixed with one type of ELP fusion proteins (either ProA, ProG, or ProL depending on the antibody host) and conjugated with either the ProA, ProG, or ProL domain to form a complex at 4 °C when the ELP domain was hydrophilic and water-soluble. The conjugated antibody was easily separated from the unconjugated antibody by two cycles of inverse temperature transition. At 37 °C, with 1 M NaCl in the solution, the ELP domain became hydrophobic and induced aggregation of the complex. The aggregated complex was separated by centrifugation and subsequently redissolved in 4 °C phosphate buffered saline (PBS) solution. The precipitation and resolubilization cycle was repeated once before spotting the complex onto a glass slide. The surface of the glass slide was modified with a self-assembled monolayer coating formed from the precursor octadecyltrichlorosilane (OTS, $CH_3(CH_2)_{17}SiCl_3$).[10] The water contact angle of the OTS-coated glass slide was measured to be ~112°. For immobilization, the ELP domain was triggered to its hydrophobic phase by adding 1.5 M NaCl to the complex solution at room temperature. The complex was then loaded into a 96-well plate and spotted by a DNA microarrayer. As a demonstration of the multiplexing capability, this strategy was used to immobilize antibodies (labeled with different fluorophores) from three different mammalian species: rabbit, goat, and mouse, by forming complexes with ELP−ProA, ELP−ProG, and ELP−ProL fusions, respectively. Figure 1b shows the fluorescence image of the immobilized antibody array. Strong fluorescent signals were detected only on regions spotted with the corresponding labeled antibodies with each spot at a uniform size of about 40 μm and 200 μm in spacing. To verify that the immobilization of the antibodies was based on the reversible hydrophobic interaction between the ELP domain and the hydrophobic glass slide, the immobilized complexes were washed with 4 °C PBS before drying in air. Compared with the amount of complexes immobilized when washed with PBS buffer at 37 °C, more than 80% of immobilized complexes were washed away upon triggering the ELP domain to its hydrophilic phase by 4 °C PBS (Figure 1c). Additionally, only ~11% of the complexes (compared with the case when NaCl was added to trigger the ELP to its hydrophobic phase) were absorbed on the surface due to non-specific absorption when the complexes were spotted without triggering the transition of ELP to its hydrophobic phase.

A functional antibody array for the detection of tumor markers was fabricated to demonstrate utility of the immobilization method. Cancer antigen 19-9 (CA 19-9) was chosen as a representative tumor marker, which is involved in the diagnosis of liver cancer.[11] The sensor was constructed according to a sandwich immunoassay configuration (Figure 2b). Briefly, a capture antibody for CA 19-9 was immobilized onto the glass slide in the form of an ELP−ProL−antibody complex. The rest of the glass surface was blocked by bovine serum albumin to reduce the non-specific absorption of other proteins to the surface. After samples with different concentrations of antigen CA 19-9 were loaded, a second antibody (the detection antibody labeled with a fluorophore) against a different epitope of CA 19-9 was applied. The amount of CA 19-9 in the sample was quantified by relating the fluorescence signal detected using a microarray scanner (Figure 2a). Figure 2c shows the corresponding calibration plot of the fluorescence intensity as a function of the CA 19-9 concentration. The assay has a wide dynamic range covering more than 4-order of magnitude with a lower detection limit of 21 U/mL. The specificity of the array was further tested by spotting antibodies for both CA 19-9 and CEA (a second tumor marker) onto the slide surface. As shown in Figure 2d, detection was very specific with no cross-reactivity detected between the two antigens and antibodies, demonstrating the selectively of the antibody array.

In summary, we have developed a general method for antibody immobilization using ELP fusion proteins based on triggered hydrophobic interactions. Antibodies conjugated to the ELP fusion proteins can be directly printed on a hydrophobic glass slide in a functionally active orientation using a DNA microarrayer. A functional antibody array sensor for detection of tumor marker CA 19-9 has been fabricated by this method. We expect that the method presented here could be a simple and universal platform to immobilize antibodies for fabrication of a variety of microarray sensors.

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Supporting Information Available: Experimental procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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