



## Polysaccharide-coated beads platform for biomolecule analysis: Evolution of SiO<sub>2</sub>-based suspension arrays

Rong Cao<sup>a,b,d,1</sup>, Demin Duan<sup>a,c,d,1</sup>, Li Jiang<sup>a</sup>, Zhuoxuan Lu<sup>a</sup>, Fang Bao<sup>a</sup>, Kexiao Zheng<sup>a</sup>, Jiong Li<sup>a,\*</sup>

<sup>a</sup> Suzhou Institute of Nano-tech and Nano-bionics, Chinese Academy of Sciences, 215123 Suzhou, PR China

<sup>b</sup> Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, 100190 Beijing, PR China

<sup>c</sup> Institute of Biophysics, Chinese Academy of Sciences, 100101 Beijing, PR China

<sup>d</sup> Graduate University of Chinese Academy of Sciences, 100049 Beijing, PR China

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### ABSTRACT

Graphically encoded SiO<sub>2</sub>-based suspension arrays are often manufactured with the 2D matrixes by popular aldehyde–aminosilane chemistry for the attachment of a variety of biomolecules, including peptides, proteins, nucleic acid probes, etc. In view of the relatively low immobilization efficiency, we have developed a novel method to prepare polysaccharide-coated silica beads, which can increase the binding capacity of biomolecules on silica beads and improve the performance of the stability of biomolecules under various denaturing conditions. In this study, two kinds of representative polysaccharides, chitosan and sodium alginate (NaAlg), were used for the surface coating. And two procedures for the immobilization of protein on NaAlg-coated beads were also studied. Under the optimized experimental conditions, NaAlg-coated beads had excellent biomolecular binding capacities which increased the fluorescence signal by almost 80%. Attending to the results obtained, we believe that the proposed methods may have significant potential for novel SiO<sub>2</sub>-based suspension array technology.

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### 1. Introduction

Biosensors and planar microarrays offer important detection tools for biomolecule analysis (Nolan & Sklar, 2002; Park, Kim, Adanyi, Varadi, & Kim, 2004; Sharma, Sehgal, & Kumar, 2003). To increase the biomolecule immobilization capacity for these technologies, which could result in a relatively high sensitivity of the assays, several methods have been used to increase the immobilization amount of biomolecules. Some examples include sensor surface modification by dextran and planar microarrays with an agarose film (Afanassiev, Hanemann, & Wölfel, 2000; Löfås, 1995). These polysaccharides were introduced not only to multiply the coupling sites but also to improve the functional properties of immobilized biomolecules, because they are long and flexible polymers with multiple active groups per molecule that could provide 3D matrixes on the surfaces (Arenkov et al., 2000; Fuentes et al., 2004; Gómez, Ramírez, & Villalonga, 2001).

To enable highly multiplexed analysis of complex biological samples, suspension microarrays have been created. In comparison with planar microarrays, suspension microarrays have several advantages, such as solution kinetics, higher sample through-

put and better quality control. However, conventional suspension arrays with fluorescence-encoded beads by using the flow cytometry also have some disadvantages, like the limited barcodes achievable and relatively high fluorescence background from polymer beads (LaFratta & Walt, 2008; Pregibon, Toner, & Doyle, 2007). Therefore, some novel suspension arrays with graphics-encoded particles have been developed, which could promise a much higher degree of multiplexing. Graphics-encoded particles can be identified by their size, shape, or composition. Some cases include shape-coded silica nanotubes, encoded sub-micron metallic nanorods, and cylindrical silica microbeads (He, Son, & Lee, 2007; Quinn, Gratalo, Haden, & Moon, 2008; Sha et al., 2006). Among them, silica particles (SiO<sub>2</sub>) with low intrinsic fluorescence, a broad range of available surface chemistries, and feasibility of micropatterns by photolithography and etching, are preferred for novel suspension array fabrication (Chiu et al., 2003; He et al., 2007; Steinberg, Stromborg, Thomas, Barker, & Zhao, 2004).

To the best of our knowledge, there is little relevant literature which has focused on polysaccharides as modifiers for graphics-encoded SiO<sub>2</sub>-based suspension arrays. Considering polysaccharides' successful application in sensors and planar microarrays, herein we used silica beads as the model system to demonstrate the conjunctive use of silica particles and polysaccharides in suspension arrays. To combine the advantage of SiO<sub>2</sub> and polysaccharides, we chose two common polysaccharides (chitosan and NaAlg) and developed a simple procedure for the preparation of

\* Corresponding author. Fax: +86 512 62872598.

E-mail address: [jjl2006@sinano.ac.cn](mailto:jjl2006@sinano.ac.cn) (J. Li).

<sup>1</sup> Joint first authors, contributed equally to this work.

NaAlg-coated silica beads, which could enhance the efficiency of binding biomolecules on the silica surfaces. As a proof-of-concept example, IgG was used in combination with NaAlg-coated beads in our study. Furthermore, we also investigated the stabilization of polysaccharide-coated beads under various denaturing conditions. We hope that the technology could be widely applied into graphically encoded SiO<sub>2</sub>-based suspension arrays for biological analysis, such as multiplex SNP genotyping analysis, genetic disease screening and multiplexed immunoassays.

## 2. Experimental

### 2.1. Materials

Silica beads (~10 μm) were obtained from BaseLine ChromTech Research Center (Tianjin, China). Chitosan (the degree of deacetylation of ~90%) and sodium alginate (NaAlg) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 3-(2-Aminoethylamino)propyl-dimethoxymethylsilane and glutaraldehyde were from Sigma–Aldrich. *N*-Hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), phosphate-buffered saline (pH 7.4, PBS) and bovine serum albumin (BSA) were purchased from Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). DyLight™ 488-conjugated goat anti-rabbit IgG (DL488 anti-rabbit IgG) was purchased from Jackson ImmunoResearch Labs. Rabbit IgG, human IgG, and goat IgG were supplied by ZhongShan Golden Bridge Biotechnology Co., Ltd (Beijing, China). Goat anti-rabbit IgG was supplied by Millipore. All reagents used in this study were of analytical grade and Milli-Q grade water was used.

### 2.2. Apparatus

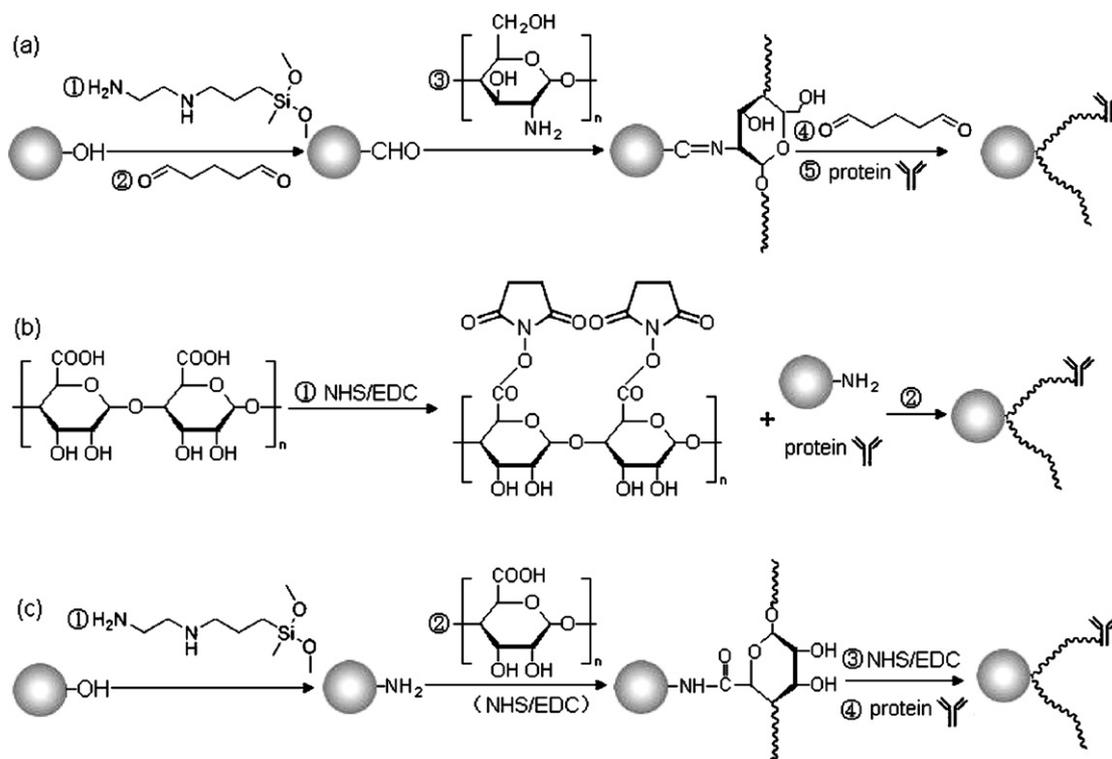
Reactions containing beads were performed in microcentrifuge tubes at room temperature with gentle shaking by the Vortex-

Genie mixer. The beads were washed by centrifugation to clear the supernatant in a Thermo Scientific Sorvall Legend Micro 17 centrifuge at 9000 rpm. The images were taken by a Nikon inverted research microscope (Eclipse Ti). Green fluorophore signals from fluorescence-labeled protein (DL488 anti-rabbit IgG) were collected by using the FITC filter (using a 20× objective). For samples in related measurement, all optical parameters, including exposure time and gain value, were kept consistent. A volume of 1 μl of silica beads dispersed in PBST buffer (pH 7.4, containing 0.1% Tween20) was dropped onto a hydrophobic glass slide. Then we performed the microscope measurement before the droplet was dried out. Bead fluorescence intensities were achieved by calculating the mean intensity of 20 random beads using the ImageJ software.

### 2.3. Preparation of chitosan-coated beads

Silica beads were modified as described previously with the following changes (Steinberg et al., 2004). The beads (~10<sup>6</sup>) were treated with 500 μl of 3% 3-(2-aminoethylamino)propyl-dimethoxymethylsilane ethanol–water (v/v, 95:5) solution for 30 min. After silanization, amino-silanized beads were rinsed thoroughly with ethanol followed by PBS buffer solution. The beads were then mixed with 500 μl of 3% glutaraldehyde/PBS solution for 2 h. Finally, washed aldehyde–silane beads were mixed with a solution of chitosan solution (from 0.1% to 0.4%, w/v) in aqueous acetic acid for 2 h to get the chitosan-coated beads.

In order to evaluate the performance of the chitosan-coated beads, DL488 anti-rabbit IgG was directly immobilized on them. The chitosan-coated beads were mixed with 500 μl of 3% glutaraldehyde/PBS solution for 2 h, then washed and resuspended in PBS buffer to react with DL488 anti-rabbit IgG (each 10<sup>5</sup> beads for 50 μl 7.5 μg/ml protein solution) for 4 h, as shown in Fig. 1a. As the same, aldehyde–silane beads, which reacted with DL488 anti-rabbit IgG as described above, were used as the control.



**Fig. 1.** Scheme of polysaccharide coatings of amino-silanized beads: (a) chitosan-coated beads; (b) NaAlg-coated beads by the one-step coating method; (c) NaAlg-coated beads by the two-step coating method.

## 2.4. Preparation of NaAlg-coated beads

### 2.4.1. One-step coating method

A one-step coating reaction (Fig. 1b) was performed by mixing activated NaAlg, the amino-silanized beads (prepared as described above) and DL488 anti-rabbit IgG. In a typical experiment, 0.2% (w/v) NaAlg solution (containing 0.15 M NaCl) was incubated with EDC/NHS (25 mg/ml in EDC and 12.5 mg/ml in NHS) for 30 min. Afterwards, excess EDC was removed by extensive dialysis against acetate buffer (10 mM, pH 5.0). Then, the mixed solution of activated NaAlg and DL488 anti-rabbit IgG (final concentration of 7.5  $\mu$ g/ml) were added to the amino beads (each  $10^5$  beads for 50  $\mu$ l mixed solution). The suspension was incubated with continuous mixing for 4 h. Finally, the beads were washed with PBST for signal detection.

### 2.4.2. Two-step coating method

A two-step coating reaction was performed through NaAlg coating followed by the protein immobilization (Fig. 1c). Typically, 0.2% (w/v) NaAlg solution (containing 0.15 M NaCl) was added to the amino beads with gentle shaking and EDC was added to a final concentration of 5 mg/ml, with NHS to a final concentration of 2.5 mg/ml. The suspension was incubated with continuous mixing for 2 h. Afterwards, NaAlg-coated beads were washed with MES buffer (0.1 M, pH 5.0, containing 0.15 M NaCl) and activated with the mixed solution (40 mg/ml in EDC and 20 mg/ml in NHS) for 30 min. Then activated NaAlg-coated beads were washed and resuspended in acetate buffer (10 mM, pH 5.0) to react with DL488 anti-rabbit IgG (final concentration of 7.5  $\mu$ g/ml) for 4 h (each  $10^5$  beads for 50  $\mu$ l mixed solution). Finally, the beads were washed with PBST for signal detection.

In these experiments where the concentration of NaAlg solution was varied, the measured fluorescence intensities were collected and studied.

## 2.5. Protein suspension arrays

Here, we performed two types of experiments using IgG and anti-IgG as models for protein (antigen/antibody) suspension arrays.

### 2.5.1. Rabbit IgG binding with DL488 anti-rabbit IgG

For the one-step coating method, activated NaAlg solution (a final concentration of 0.01%, w/v) and rabbit IgG (final concentrations of 2–50  $\mu$ g/ml) were added to the amino beads (each  $10^5$  beads for 50  $\mu$ l mixed solution) with continuous mixing for 4 h. Then, the beads were washed, resuspended in excess quenching solution (40 mM hydroxylamine in PBS with 1% BSA) and mixed well for 30 min. Control beads, modified with goat IgG, human IgG and BSA, respectively, in place of rabbit IgG, were used to determine the non-specific interaction. After washed and resuspended, rabbit IgG-modified beads and control beads were, respectively, incubated with DL488 anti-rabbit IgG for 2 h (each  $10^5$  beads for 50  $\mu$ l 7.5  $\mu$ g/ml protein solution) and then washed with PBST for signal detection.

For the two-step coating method, activated NaAlg (0.2%, w/v)-coated beads were mixed with rabbit IgG (final concentrations of 2–50  $\mu$ g/ml) for 4 h. The rest of the operation was the same as above.

### 2.5.2. Sandwich immune fluorescence assay

According to the two-step coating method, we immobilized goat anti-rabbit IgG (50  $\mu$ g/ml) on NaAlg (0.2%, w/v)-coated beads. Control beads (BSA with the corresponding concentration in place of goat anti-rabbit IgG) were used to determine the fluorescence background. Each 5000 goat anti-rabbit IgG-modified beads and the equivalent control beads were mixed together, and incubated with

100  $\mu$ l of rabbit IgG with different concentrations for 2 h. Then, the beads were rinsed by PBST buffer and mixed with 50  $\mu$ l of DL488 anti-rabbit IgG at 7.5  $\mu$ g/ml for 1 h. Finally, the beads were washed with PBST for signal detection.

## 2.6. Bead stability assays

The NaAlg-coated beads, modified with rabbit IgG, by the two-step coating method (0.2% NaAlg for beads coating, the protein concentration of 50  $\mu$ g/ml for immobilization), were incubated with 6 M guanidine hydrochloride (GuHCl), 8 M urea, or 10% SDS for 4 h. Afterwards, the beads were washed by water and PBS buffer. The residual specificity ability of the immobilized rabbit IgG was evaluated by incubation with DL488 anti-rabbit IgG for 2 h as mentioned above.

## 3. Results and discussion

### 3.1. Optimization of polysaccharide coatings of amino-silanized beads

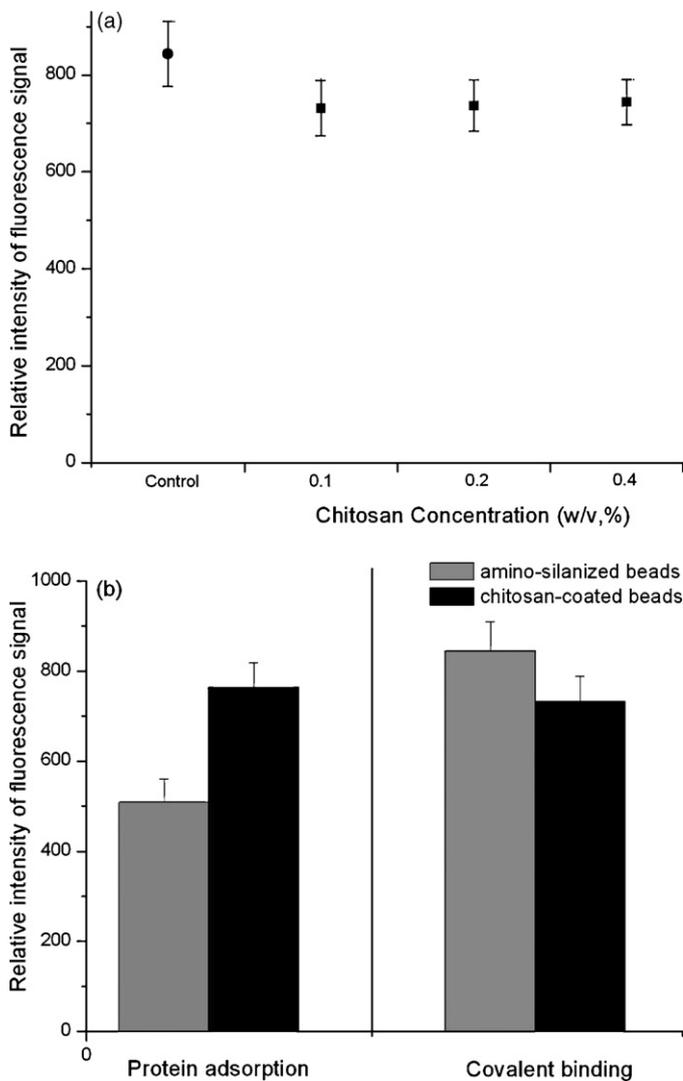
Due to polysaccharides' desirable biocompatible, biodegradable, hydrophilic and protective properties, they have received increased attention (Sæther, Holme, Maurstald, Smidsrod, & Stokke, 2008). Here, we selected two kinds of representative polysaccharides: chitosan, with amino groups, as a natural cationic polysaccharide; NaAlg, with carboxyl groups, as a natural anionic polysaccharide. The performance of the polysaccharide coatings was determined by measuring fluorescence intensity from directly immobilized DL488 anti-rabbit IgG on polysaccharide-coated beads.

#### 3.1.1. Chitosan-coated beads

Considering its porous structure, low toxicity, and the presence of free amino groups, chitosan has a great potential in a wide variety of biological applications, such as drug delivery and wastewater treatment (Schmuhl, Krieg, & Keizer, 2001; Jayakumar, Prabakaran, Reis, & Mano, 2005). The reaction of glutaraldehyde with primary amino groups of chitosan produces the aldehyde groups for protein immobilization through a Schiff's reaction. Fig. 2a shows that the amount of immobilized protein on chitosan-coated beads was smaller than that on aldehyde-silane beads. It might be explained as follows: in weak alkaline solution (PBS), there was a certain degree of chitosan phase inversion that could form the interpenetrating polymer network and introduced glutaraldehyde may lead to the formation of crosslinked chitosan. Because of amino groups partly blocked by reaction with glutaraldehyde, the efficient amino groups on the surface were decreased. Consequently, chitosan without enough freedom could not give additional reactive groups for protein immobilization. As shown in Fig. 2b, the results indicated that non-activated chitosan beads had high protein adsorption capacity. The non-specific protein adsorption may be attributed to the residual aldehyde groups on the beads and the electrostatic interactions between the amino groups in chitosan and the protein.

#### 3.1.2. NaAlg-coated beads

Like chitosan, NaAlg is also considered to be biocompatible, non-toxic, non-immunogenic and biodegradable (Yang, Chen, & Fang, 2009). However, NaAlg, comprised of mannuronic acid (M block) and guluronic acid (G block) units, has the distribution of negative charges along the polymer backbone in aqueous solution, which can provide a strong electrostatic interaction with positively charged biopolymers. Especially for protein arrays, an electrostatic interaction between the negatively charged NaAlg matrix and positively charged protein can highly concentrate the protein within

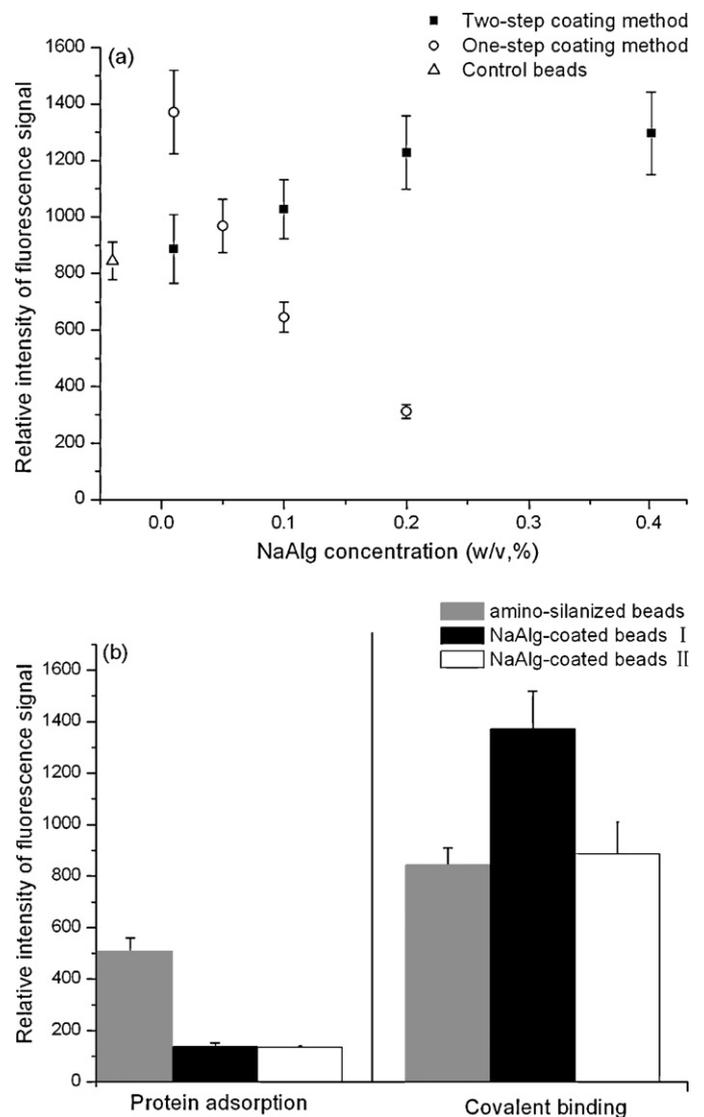


**Fig. 2.** Detection of immobilized DL488 anti-rabbit IgG on chitosan-coated beads. (a) Fluorescence signal as a function of chitosan solution concentration for DL488 anti-rabbit IgG immobilization: (circles) aldehyde–silane beads; (squares) chitosan-coated beads. (b) Adsorption or binding of DL488 anti-rabbit IgG on beads. Non-activated beads (left) and activated beads by glutaraldehyde (right). Chitosan concentration: 0.1%.

the NaAlg matrix to drive the coupling reaction. This would minimize expensive protein consumption. In addition, unlike activated chitosan by glutaraldehyde, activated NaAlg by EDC/NHS would not cause a self-crosslinking network. Thus, we could use two strategies to perform the NaAlg coating.

**3.1.2.1. One-step coating method.** Fig. 3a shows the influence of the concentration of NaAlg on protein immobilization by one-step coating method. When the concentration of NaAlg was raised from 0.01% to 0.2%, the fluorescence signal decreased. In fact, activated NaAlg solution covalently bound DL488 anti-rabbit IgG and amino beads at the same time. Thus an increased NaAlg concentration should lead to the decreased immobilized amount of protein on each polysaccharide chain. Meanwhile, multiple attachment sites on the NaAlg with very low concentration are likely to cause crosslinking between the beads (data not shown). To get the maximum binding capacity, 0.01% NaAlg was chosen for the following experiments.

**3.1.2.2. Two-step coating method.** The fluorescence signal increased with the increasing amount of NaAlg by the two-



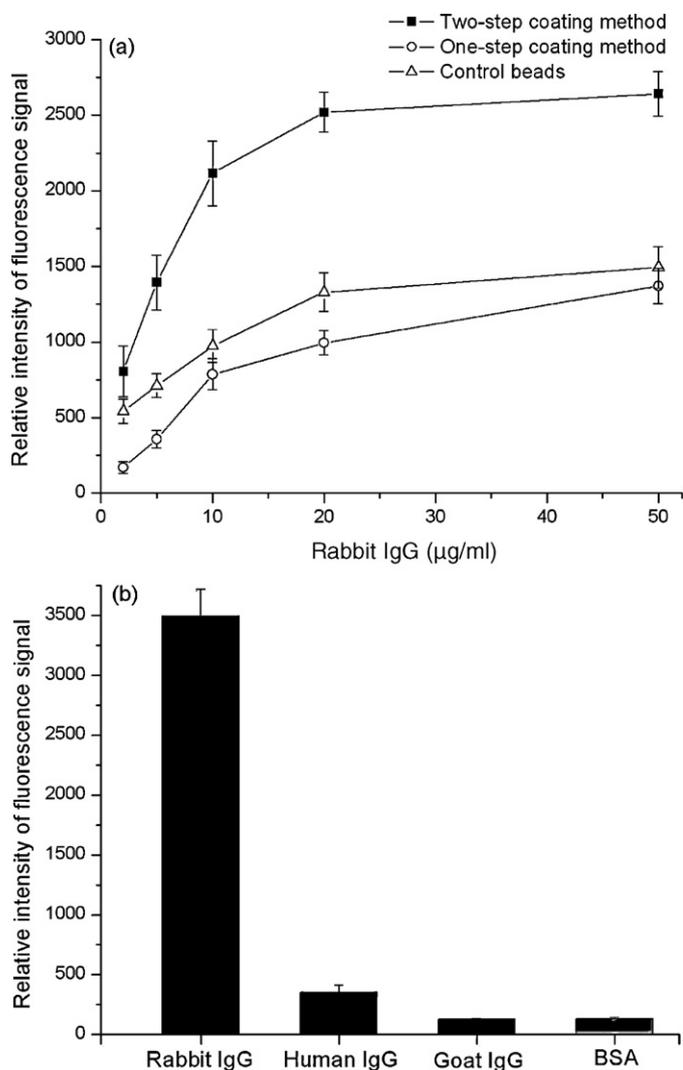
**Fig. 3.** Detection of immobilized DL488 anti-rabbit IgG on NaAlg-coated beads. (a) Fluorescence signal as a function of NaAlg solution concentration for DL488 anti-rabbit IgG immobilization: (triangles) aldehyde–silane beads; (circles) NaAlg-coated beads by one-step coating method (I); (squares) NaAlg-coated beads by two-step coating method (II). (b) Adsorption or binding of DL488 anti-rabbit IgG on beads. NaAlg concentration: 0.01%.

step coating method (Fig. 3a). Compared with aldehyde–silane beads, NaAlg-coated beads had higher immobilization capacity. With NaAlg solution concentration above 0.2%, the immobilized amount of protein showed a low dependence on the NaAlg concentration. Considering the viscosity of NaAlg solution and the ease of handling, 0.2% NaAlg was selected for this study. Besides, compared with amino-silanzed beads, the ability of the NaAlg coating to inhibit protein adsorption was also depicted (Fig. 3b).

The above results indicate that polysaccharide coatings with different chemistries had different performance for protein immobilization. The NaAlg-coated beads were better suited for covalent immobilization of biomolecules.

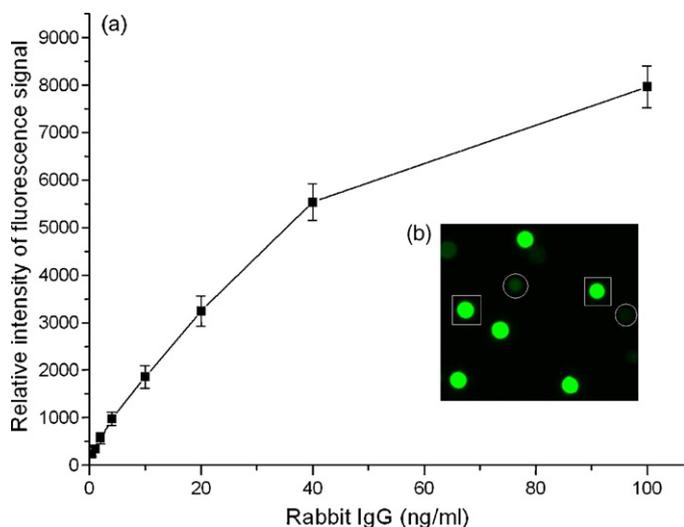
### 3.2. Characterization of rabbit IgG binding with DL488 anti-rabbit IgG

A comparison of the results from the two different methods showed the entirely different specific binding activity of the immobilized rabbit IgG (Fig. 4a). Among them, NaAlg-coated beads by



**Fig. 4.** Detection of immobilized rabbit IgG with DL488 anti-rabbit IgG. (a) Fluorescence signal as a function of rabbit IgG concentration by different methods. The aldehyde–aminosilane beads were used as the control. (b) Specific and non-specific binding discrimination. The beads, with immobilized rabbit IgG, human IgG, goat IgG or BSA (50 µg/ml) via the two-step coating method, were, respectively, incubated with DL488 anti-rabbit IgG.

the two-step coating method yielded excellent biomolecular binding capacities, which increased the fluorescence signal by almost 80%, compared with the aldehyde–silane beads. On the contrary, NaAlg-coated beads by the one-step coating method gave weak fluorescence signal. The difference in behavior of the rabbit IgG could be explained by the amount of links between the protein and NaAlg. By the two-step coating method, protein was immobilized on NaAlg-coated beads with a limited quantity of links. On the other hand, the NaAlg polymer backbone supplied a large number of links for protein immobilization via one-step coating method. Thus, the binding of the NaAlg to the surface of protein probably reduced the accessibility of DL488 anti-rabbit IgG to the active site of rabbit IgG. Kobayashi, Yanagihara, and Ichishima (1989) indicated that the molecular weight of the carboxymethyl dextran (CM-Dex) played a critical role in the final activity of CM-Dex modified enzyme. Given the similarity between CM-Dex and NaAlg, the size of the NaAlg may also affect the specific binding activity of the immobilized rabbit IgG. Namely, the molecular weight of NaAlg that we used may be too large to represent the rabbit IgG activity by the one-step coating method. However, it still seems very promising and will be possible for the use of the simple one-



**Fig. 5.** Quantitative sandwich assays: (a) diagram of the fluorescence intensity as a function of rabbit IgG concentration in the sandwich immune fluorescence assay; (b) fluorescence microscope images of the mixed NaAlg-coated beads (goat anti-rabbit IgG-immobilized beads are identified by squares; BSA-immobilized beads are circled). Only goat anti-rabbit IgG-bound beads could be observed in the strong fluorescence signal.

step coating method. For example, we can choose lower molecular weight of NaAlg or the immobilization of other biomolecules such as oligonucleotide probes. Herein, the two-step coating method was used for the following experiments. Fig. 4b shows that only rabbit IgG-bound beads interacted with corresponding DL488 anti-rabbit IgG to give the strong fluorescence signal, because of the specific binding between IgG and its corresponding antibody. There was little non-specific interaction between DL488 anti-rabbit IgG and goat IgG-bound beads/BSA-bound beads. Human IgG-bound beads might cross-react with DL488 anti-rabbit IgG, so they gave the weak fluorescence signal. Thus, BSA-bound beads were chosen as the control for the sandwich immune fluorescence assay.

### 3.3. Assessment of the sandwich immune fluorescence assay

Sandwich immunoassay was performed to detect rabbit IgG in the concentration range of 0.4–100 ng/ml (Fig. 5a). Considering the fluorescence microscope system with a 12-bit limited dynamic range, we used three different sensitivity settings to accommodate the wide range in fluorescent signal. Control beads were used to determine the background intensity. The normalized result clearly showed that the fluorescence signal intensity was linear in a broad concentration range. And as low as 0.4 ng/ml of rabbit IgG could be clearly detected. The sensitivity of suspension array is affected by the detectors (confocal microscope or normal fluorescence microscope). In this study, we had got excellent experimental results by the normal fluorescence microscope. Thus, the proposed NaAlg coating method could be applied for suspension arrays.

### 3.4. Assessment of the stability of immobilized protein

Fig. 6 shows that the stability of rabbit IgG under various denaturing conditions was improved when rabbit IgG was immobilized on the NaAlg-coated beads. This fact could be explained by the contribution of NaAlg to the maintenance of the active conformation of the protein. For example, there are some interactions between the anionic polyelectrolyte NaAlg and the anionic surfactant (SDS), like hydrophobic and electrostatic interactions, which probably reduce the accessibility of denaturant to the protein.

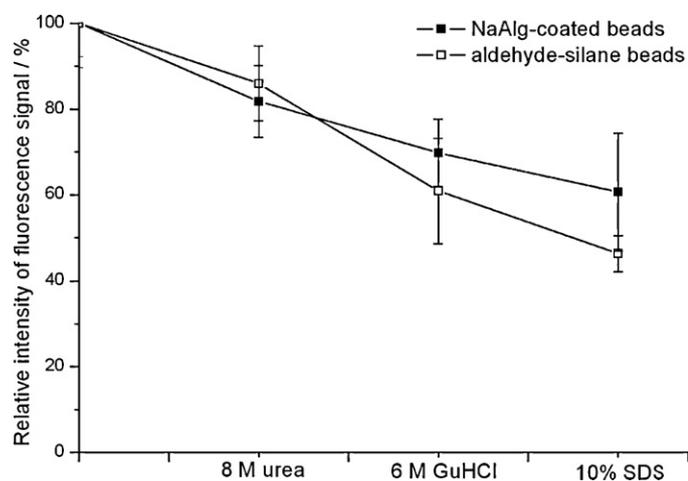


Fig. 6. Fluorescence signal of beads with varying denaturing conditions.

#### 4. Conclusions

We have developed a novel NaAlg-coated bead-based suspension array technology for protein detection. The proposed methods have taken advantage of the low fluorescence background from silica and the improved stability of protein by NaAlg. Our results clearly indicated that, with the NaAlg coating, higher fluorescence signals were obtained. Although the one-step coating method could not be used here, it still offers the great potential for other applications in suspension arrays. Therefore, future efforts will focus on the optimization of different molecular weight of NaAlg, other kinds of polysaccharides and biomolecules to make the one-step coating method as a viable tool for some biomedical applications. In addition, by silicon micromachining suspension arrays of polysaccharide-coated silica beads can also be extended to multifunctional encoded particles for high-throughput biological assays.

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#### References

- Afanassiev, V., Hanemann, V., & Wöfl, S. (2000). Preparation of DNA and protein micro arrays on glass slides coated with an agarose film. *Nucleic Acids Research*, 28, e66.
- Arenkov, P., Kukhtin, A., Gemell, A., Voloshchuk, S., Chupeeva, V., & Mirzabekov, A. (2000). Protein microchips: Use for immunoassay and enzymatic reactions. *Analytical Biochemistry*, 278, 123–131.
- Chiu, S. K., Hsu, M., Ku, W. C., Tu, C. Y., Tseng, Y. T., Lau, W. K., et al. (2003). Synergistic effects of epoxy- and amine-silanes on microarray DNA immobilization and hybridization. *Biochemical Journal*, 374, 625–632.
- Fuentes, M., Mateo, C., Garcia, L., Tercero, J. C., Guisán, J. M., & Fernández-Lafuente, R. (2004). Directed covalent immobilization of aminated DNA probes on aminated plates. *Biomacromolecules*, 5, 883–888.
- Gómez, L., Ramírez, H. L., & Villalonga, R. (2001). Modification of  $\alpha$ -amylase by sodium alginate. *Acta Biotechnologica*, 21, 265–273.
- He, B., Son, S. J., & Lee, S. B. (2007). Suspension array with shape-coded silica nanotubes for multiplexed immunoassays. *Analytical Chemistry*, 79, 5257–5263.
- Jayakumar, R., Prabakaran, M., Reis, R. L., & Mano, J. F. (2005). Graft copolymerized chitosan—Present and status applications. *Carbohydrate Polymers*, 62, 142–158.
- Kobayashi, M., Yanagihara, S., & Ichishima, E. (1989). Preparation and characterization of Taka-amylase A attached to carboxymethyl dextran by water-soluble carbodiimide. *Agricultural and Biological Chemistry*, 53, 3133–3138.
- LaFratta, C. N., & Walt, D. R. (2008). Very high density sensing arrays. *Chemical Reviews*, 108, 614–637.
- Löfås, S. (1995). Dextran modified self-assembled monolayer surfaces for use in biointeraction analysis with surface plasmon resonance. *Pure and Applied Chemistry*, 67, 829–834.
- Nolan, J. P., & Sklar, L. A. (2002). Suspension array technology: Evolution of the flat-array paradigm. *Trends in Biotechnology*, 20, 9–12.
- Park, I. S., Kim, D. K., Adanyi, N., Varadi, M., & Kim, N. (2004). Development of a direct-binding chloramphenicol sensor based on thiol or sulfide mediated self-assembled antibody monolayers. *Biosensors and Bioelectronics*, 19, 667–674.
- Pregibon, D. C., Toner, M., & Doyle, P. S. (2007). Multifunctional encoded particles for high-throughput biomolecule analysis. *Science*, 315, 1393–1396.
- Quinn, J., Gratalo, D., Haden, K., & Moon, J. (2008). *Accurate multiplex cytokine assay developed with VeraCode® technology*. <http://veracodemdx.com/downloads/VeraCodeCytokineCarboxyl.WhitePaper.pdf>
- Schmuhl, R., Krieg, H. M., & Keizer, K. (2001). Adsorption of Cu (II) and Cr (VI) ions by chitosan: Kinetics and equilibrium studies. *Water SA*, 27, 1–7.
- Sha, M. Y., Walton, I. D., Norton, S. M., Taylor, M., Yamanaka, M., Natan, M. J., et al. (2006). Multiplexed SNP genotyping using nanobarcode particle technology. *Analytical and Bioanalytical Chemistry*, 384, 658–666.
- Sharma, S. K., Sehgal, N., & Kumar, A. (2003). Biomolecules for development of biosensors and their applications. *Current Applied Physics*, 3, 307–316.
- Steinberg, G., Stromborg, K., Thomas, L., Barker, D., & Zhao, C. (2004). Strategies for covalent attachment of DNA to beads. *Biopolymers*, 73, 597–605.
- Sæther, H. V., Holme, H. K., Maurstald, G., Smidsrod, O., & Stokke, B. T. (2008). Polyelectrolyte complex formation using alginate and chitosan. *Carbohydrate Polymers*, 74, 813–821.
- Yang, J., Chen, S., & Fang, Y. (2009). Viscosity study of interactions between sodium alginate and CTAB in dilute solutions at different pH values. *Carbohydrate Polymers*, 75, 333–337.