Suspended nanoparticle crystal (S-NPC): A nanofluidics-based, electrical read-out biosensor†‡

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Here we report an approach utilizing a suspended nanoparticle crystal (S-NPC) as an electrical read-out biosensor based on a nanofluidic electrokinetics principle. As a preliminary demonstration, streptavidin-modified S-NPC with a particle diameter of 520 nm was used to detect biotin in a PBS buffer. The present result indicated that the detection range of biotin by this nanofluidics-based biosensor was about 1 nM–10 μM (in 10^{-4} × PBS) with a sensitivity of 160 nS/nM. Being easy to get established, low-cost, and having large electrical read-out signal, the present S-NPC is believed to be a promising biosensing scheme in the micro total analysis system.

Recent advances in nanofabrication expend applications of nanofluidic systems in versatile biological analysis.[1] Surface charges dominate the ionic conductance in these nanofluidic systems when the feature size of the embedded nanochannel is comparable to the Debye length ($\lambda_D \propto 1/\sqrt{c_b}$, c_b is the bulk ionic concentration).[2,3] Such unique electrokinetic property opens up the possibility to develop novel nanofluidics-based biosensors with electrical read-outs.[3–6]

No-doubt the nanochannel is the basic functional element in the nanofluidics-based biosensor. Most nanochannels reported currently are either prepared directly from natural nanostructures[7] or fabricated artificially by taking advantage of the booming microelectronics techniques.[8] So far, those nanofabrication approaches are usually complicated, expensive and time-consuming. What's more, for biological sensing applications, the inner surface of the nanochannel usually needs further modification to obtain specifically probing ability. Karnik *et al.* proposed a method of applying gate voltage to locally modify inner surface charges of a nanochannel.[9] The same group also used aminosilane chemistry to immobilize target molecules onto the inner surface of the silica nanochannel.[10] However, the present inner surface modification approaches are still limited and cannot reach requirements of the biosensing applications. Recently, Chen *et al.* found that nanoparticle crystal (NPC) self-assembled inside a microchannel exhibited typical electrokinetic properties of an individual nanochannel,[11] which gave a simple, cheap and rapid fabrication approach for a nanofluidic device. Electrokinetics of nanochannels with different characteristic sizes can be readily obtained by packing nanoparticles with corresponding diameters. More importantly, thanks to the matured nanoparticle synthesis chemistry, it is very easy to tune the surface chemical property of the nanoparticles, by which nanofluidic systems with different surface functions can be achieved.

In this communication, we studied electrokinetics of a suspended nanoparticle crystal (S-NPC) and proposed an approach using S-NPC to function as a nanofluidics-based, electrical read-out biosensor.

For a given straight nanochannel, if the diameter is larger than the Debye length, its ionic conductance (G) will be proportional to the ionic concentration of the buffer. While, if the feature size of the nanochannel is comparable to or even smaller than the Debye length, *i.e.* the electric double layers being overlapped inside the channel, the ionic conductance will be insensitive to the loaded electrolyte concentration, but be proportional to the surface charge density.[12] Similarly, in a close-packed NPC, the interstices are at nano-scale and connected with each other in series/parallel to form a nanochannel network, as show in Fig. 1a. The ionic conductance of a NPC (G_{NPC}) with a surface charge density of σ_s can be calculated as (see the ESI S1 for details†),

$$G_{NPC} \sim \left(1.16 \times 10^7(\mu_+ + \mu_-)c_b + 2.01\mu_+\frac{\sigma_s}{d}\right)\frac{S_{NPC}}{L_{NPC}} \quad (1)$$

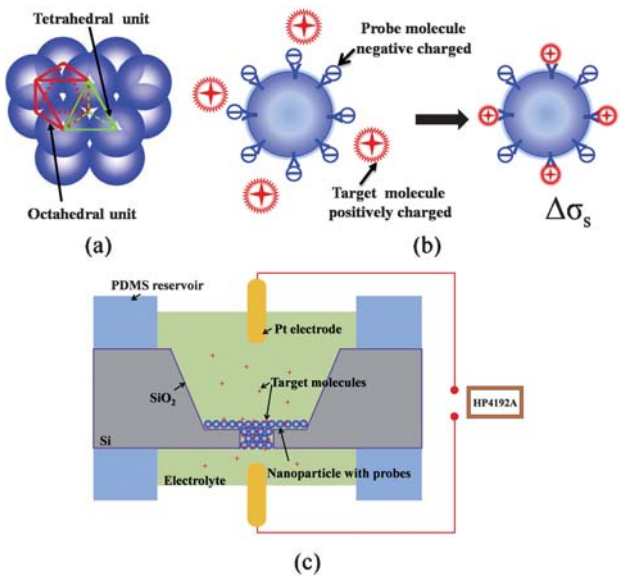

Fig. 1 (a) The crystal structure illustration of a close-packed NPC. (b) Surface charge density variation due to specific binding. (c) Schematic illustration of the present S-NPC biosensor (not to scale).

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where 1.16×10^7 and 2.01 are geometrical related constants, μ is the ion mobility, and d represents the diameter of the nanoparticle. S_{NPC} and L_{NPC} are the cross-sectional area and the thickness of the NPC, respectively. At low bulk concentration condition, *i.e.* $c_b \ll 1.73 \times 10^{-7}\mu_+\sigma_s \Big/ [(\mu_+ + \mu_-)d]$, the surface charge governs the ion conductance of the NPC, and the conductance can be simplified as,

$$G_{NPC} \sim 2.01 \cdot \frac{S_{NPC}}{L_{NPC}} \cdot \frac{\mu_+}{d} \sigma_s \quad (2)$$

When the surface charge density changes ($\Delta\sigma_s$) due to the reaction between the immobilized probes on the surface of nanoparticles and the dissociated analyte molecule as illustrated in Fig. 1b, the induced conductance variation of the NPC (ΔG_{NPC}) can be used as a trace-back signal for the analyte concentration sensing. The sensitivity of the NPC-based biosensor can be estimated as,

$$\frac{dG_{NPC}}{d\sigma_s} \sim 2.01 \cdot \frac{S_{NPC}}{L_{NPC}} \cdot \frac{\mu_+}{d} \quad (3)$$

From eqn (3), when the NPC is suspended inside a micropore (S-NPC) as shown in Fig. 1c, *i.e.* having a micrometer-sized L_{NPC}, a high sensitivity of this nanofluidics-based biosensor can be achieved.

To realize the S-NPC used in this communication, a micropore (20 μm × 20 μm) through a suspended silicon membrane (50 μm thick) was fabricated by standard silicon microfabrication process (see the ESI S2 for details†). Monodispersed nanoparticle suspension was loaded into the back chamber of the silicon micropore. After evaporation-induced self-assembling, an S-NPC was obtained inside the silicon micro-pore. The S-NPC samples were then kept in a desiccator overnight in clean room before the following experiments. To package the S-NPC for the electrical test, two PDMS replicates were reversibly bonded with the silicon substrate to function as spacious reservoirs, as shown in Fig. 2a. Platinum electrodes were used here for the electrical detection. The absolute impedance spectra of the S-NPC were measured by an impedance analyzer (HP4192A) with a bias of 0.1 V. In the present work, two kinds of nanoparticles were used. 500 nm bare silica nanoparticles (Technical Institute of Physics and Chemistry of Chinese Academic Society, China) were used to study the electrokinetics properties of the S-NPC, and 520 nm silica nanoparticles modified with streptavidin (CS01N/9387, Bangs Laboratories, Inc.) were used to construct an S-NPC biosensor for biotin detection. The SEM image in Fig. 2b shows a top view of a self-assembled streptavidin-modified S-NPC, and the confocal image in Fig. 2c gives a cross-sectional view of the close packed particles inside an S-NPC. Phosphate-buffered saline (PBS) buffers with concentrations from 10^{-5} × PBS to 1 × PBS (1 × PBS corresponds to an ionic concentration of 0.1 M) buffers were used as the electrolyte. For all the experiments in this work, 15 μl buffer was loaded into each reservoir for electrical measurements. For the biotin sensing experiments, D-biotin (B4501, Sigma) was dissolved in PBS buffers. After introducing the biotin into both reservoirs for 30 min at room temperature, the residual biotin was washed off by the PBS buffer with the same concentration. Impedances of the S-NPC before and after biotin binding were measured in the same PBS buffer. To evaluate the surface charge density of the nanoparticles, zeta potentials of the streptavidin-modified nanoparticles before and after biotin binding were measured by Zeta PALS (Brookhaven Instruments, USA). 1 ml streptavidin-modified nanoparticle suspension (400 μg/ml, diluted in 10^{-4} × PBS) was mixed without or with biotin solutions (1 ml, 5 μM, 50 μM, in 10^{-4} × PBS, respectively) for 30 min at room temperature before the measurement.

The conductance of the S-NPC constructed by 500 nm bare silica nanoparticles with the electrolyte of 10^{-5} × PBS to 1 × PBS was measured at 100 kHz (see the ESI S3 for details†). The conductance–concentration relationship of the S-NPC meets exactly the electrokinetic property of an individual nanochannel. More importantly, the conductance of the present S-NPC is much larger than single straight nanochannel, or even NPC in a microchannel. For the case of 1 mM buffer solution loaded, the conductance of the present S-NPC is around 10^{-4} S, which is much larger than those of a single straight nanochannel (10^{-9}∼10^{-10} S[2,13]) or NPC in a microchannel (∼10^{-6}S[11]). This further enlarged electrical signal can be attributed to the enormous surface -to -volume ratio, the large effective cross-sectional area, and the small thickness.

The conductance variation caused by the loaded biotin on the streptavidin modified S-NPC at different ionic concentrations is shown in Fig. 3. The ratio of conductance measured before and after

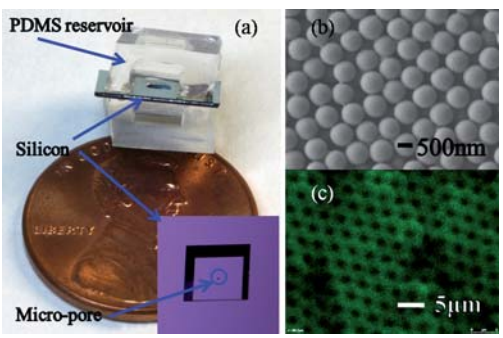

Fig. 2 (a) Photograph of a packaged S-NPC device and a silicon substrate with back-chamber upwards (compared with a penny). (b) SEM image shows a top view of an assembled streptavidin-modified S-NPC. (c) Confocal image shows the close packed particles inside an S-NPC (2.94 μm silica microbeads were used for the confocal observation) 3.6 μm deep from the top surface.

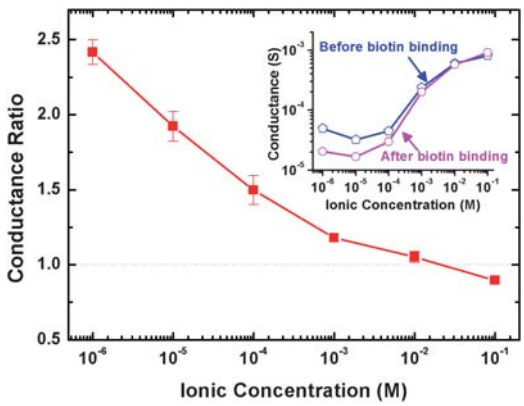

Fig. 3 Variation of the conductance ratio (the ratio of the measured conductance of the streptavidin modified S-NPC before biotin binding to that after the binding) with the buffer ionic concentrations varied from 1 μM to 0.1 M (log scale). The biotin concentration is 5 μM. Each point stands for three trials.

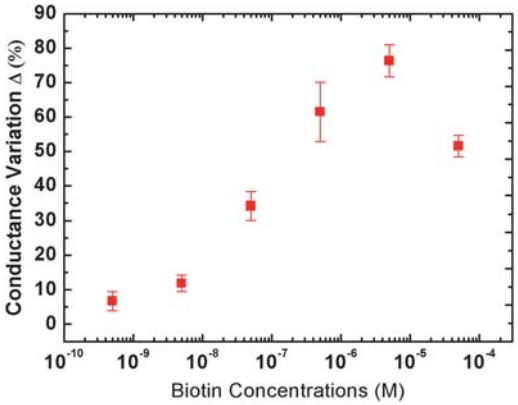

Fig. 4 Conductance variation caused by the loaded biotin on the streptavidin modified S-NPC with the biotin concentrations varied from 5 nM to 50 μM (log scale) in 10^{-4} × PBS buffer. Each point stands for three trials.

biotin binding increases from 0.90 ± 0.02 at 0.1M (ionic concentration) to 2.4 ± 0.08 at 1 μM. At high electrolyte concentrations, there is nearly no change of the conductance after the biotin binding, but at low buffer concentrations, the biotin induced conductance variation is significant. The binding of biotin to streptavidin has two effects on the conductance of the S-NPC:[3] (a) the nanochannel is partially blocked and thereby decreases the effective size of the nanochannel, (b) the surface charges of streptavidin are electrically modulated by biotin. At high bulk concentrations, only the blocking effect takes the role, but not significant. At low buffer concentrations, the electric double layers are overlapped due to the larger Debye length, and the ionic conductance of the S-NPC is proportional to the surface charge density. Zeta-potential of the streptavidin-modified nanoparticles used in the present work was −80.47 ± 0.92 mV, which meant that the surface of the S-NPC was negatively charged before biotin binding. After reacting with biotin (5 μM), the zeta-potential of the biotin–streptavidin-modified nanoparticles changed into −12.86 ± 1.12 mV, which indicated that the effective charges on the S-NPC surface were reduced by the biotin modulating, therefore the conductance of S-NPC was lower considerably after biotin binding at low bulk ionic concentrations. So the present S-NPC can be used as an electrical read-out sensor for biotin detection at a low bulk ionic concentration.

The conductance variations caused by the loaded biotin on the streptavidin modified S-NPC at different biotin concentrations in 10^{-4} × PBS (10 μM) buffer are shown in Fig. 4. The conductance variation Δ was calculated as $(G_{s-NPC} - G'_{s-NPC})/G_{s-NPC}$ where $G_{s\text{-}NPC}$ and $G'_{s\text{-}NPC}$ are conductances of the S-NPC before and after biotin binding. When the biotin concentration is 5 μM, the surface charges of the S-NPC were mostly modulated by the biotin, as aforementioned, resulting in a conductance decreasing of 76.3%. 5 nM biotin can be detected by the present S-NPC, with the conductance variation of 11.8%. When the biotin concentration was higher than 50 μM, the biotin was over-loaded compared to the binding capacity of streptavidin-nanoparticles,[14] which resulted in excessive biotin being physically absorbed on the S-NPC, turning the surface positively charged, which was confirmed by the measured zeta potential of 11.79 ± 1.39 mV. The excessive biotin absorbed on the S-NPC also reduced the feature size of the nanochannel. Therefore the conductance at this point is larger than the one when the biotin concentration was 5 μM, although the absolute charge densities on the S-NPC of these two points are almost the same. The results indicated that the present S-NPC can provide a detection range of 1 nM–10 μM (in 10^{-4} × PBS) with a sensitivity of 160 nS/nM for the biotin sensing.

In this communication, we used suspended nanoparticle crystal (S-NPC) as a nanofluidics-based, electrical read-out biosensor for the first time. The S-NPC exhibits the typical electrokinetic property of an individual nanochannel with a further enlarged electrical signal. The preliminary experiments indicated that a streptavidin modified S-NPC with a thickness of 50 μm can present a measurable conductance response to the loaded biotin with the range of 1 nM–10 μM. The present S-NPC biosensor has several advantages, such as simple, cheap and rapid fabrication, integrable with micro/nanofluidics components, and very large electrical read-out. Furthermore, nanoparticles with different sizes and various surface modifications are commercially available now, which facilitates the applications of this S-NPC biosensor in much broader areas.

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