Rapid and ultra-sensitive detection of AMP using a fluorescent and magnetic nano-silica sandwich complex†

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We report here a novel AMP biosensor based on the aptamer-induced disassembly of fluorescent and magnetic nano-silica sandwich complexes with a direct detection limit of 0.1 μM.

Nucleic acids aptamers are isolated from random-sequence nucleic acid libraries by "*in vitro* selection".[1,2] Like monoclonal antibodies, aptamers can bind to their targets with high affinity and specificity. A number of aptamers have been generated against a wide variety of targets, including metal ions, small organic compounds, metabolites, and proteins.[3,4] In comparison with antibodies and enzymes, aptamers are relatively easy to synthesize, more stable to biodegradation, and less vulnerable to denaturation.[5,6] These advantages make aptamers particularly useful for biosensing applications in environmental monitoring and medical diagnostics. Several methods have been developed for transformation of the aptamer-binding events into physically detectable signal, most of which are based on fluorescent dye-modified DNA detection.[7–12] However, two major elements limit the sensor sensitivity and reproducibility. The first is the relatively low signal amplification. Because one DNA probe can only be labeled with one or a few fluorophores, the fluorescence signal becomes too weak to be detected when the target concentration is low. The second is the poor photostability of the fluorophore. Most organic dye molecules suffer from photobleaching, which can cause irreproducible results.[13]

To achieve the desired signal amplification and reproducibility for ultra-sensitive detection, several types of nanomaterials, such as quantum dots, silica, and metal nanoparticles, have been explored.[11,13–15] Among them, gold nanoparticles (AuNP) have been successfully used for detection of adenosine based on their unique size and distance-dependent optical properties.[11] The disassembly of gold nanoparticle aggregates can be detected directly by observing the color change of the solution from blue to red or monitored by UV-Vis absorption spectroscopy. However, the main limitation[11] of this approach is low sensitivity (0.3 mM). Under certain circumstances, such as in environmental pollution prevention and clinical diagnosis, higher sensitivity is desirable. Fluorescent silica nanoparticles (FNP) which can entrap a large amount of fluorophores have been developed for this purpose.[13,15–19] There are three major advantages for FNP as a fluorescence probe for ultra-sensitive detection. First, a large number of fluorophores can be encapsulated inside a single silica nanoparticle, which can emit strong fluorescence when excited. Therefore, compared with fluorophore-labeled DNA, the fluorescence signal is greatly amplified making it possible for ultra-sensitive detection. Second, FNP is highly photostable provided by the silica matrix shielding effect. The fluorophores are well protected from environmental oxygen when doped inside the silica network and can emit a constant fluorescent signal that is sufficient for detection of trace amounts of the target molecule. Third, the silica surface has been considered as a universally biocompatible and versatile substrate for the immobilization of biomolecules. Biomolecular immobilization has been developed for various applications using well-developed immobilization protocols based on the silica surface.[13,16,17]

In this report, we synthesized amino-modified fluorescent silica nanoparticles (FNPs) and magnetic silica microspheres (MSMPs) 70 nm and 200 nm in diameter, respectively (Fig. 1(a) and (c)), Particle size distributions and fluorescence spectra are shown in Fig. S1 and S2, respectively.† Glutaraldehyde (GA) was used for immobilization of amino-modified oligonucleotides on the silica surface as a linker molecule. Bovine serum albumin (BSA) was used to block the nonspecific adsorption and improve the efficiency of hybridization.[17] The structure-switching signaling aptamer was introduced to assemble FNPs and MSMPs into sandwich complexes.[10,12] After hybridization, the unattached FNPs were removed by repeatedly washing and magnetic separation.

The adenosine monophosphate (AMP) sensor is constructed as shown in Scheme 1A. The sensor is composed of three components: MSMPs functionalized with 3′-amino-modified DNA (MSMP probe), FNPs functionalized with 5′-amino-modified DNA (FNP probe), and a linker DNA (Detection probe). The detection probe can be divided into three segments. The first segment (**Segment a**) can hybridize with the ***MSMP probe***. The second segment (**Segment b**) can hybridize with the last five bases of the ***FNP probe***. The third

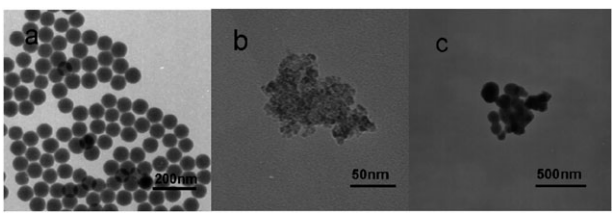

Fig. 1 TEM images of (a) fluorescent silica nanoparticles (FNPs); (b) Fe_3O_4 nanoparticles, (c) magnetic silica microsphere (MSMPs).

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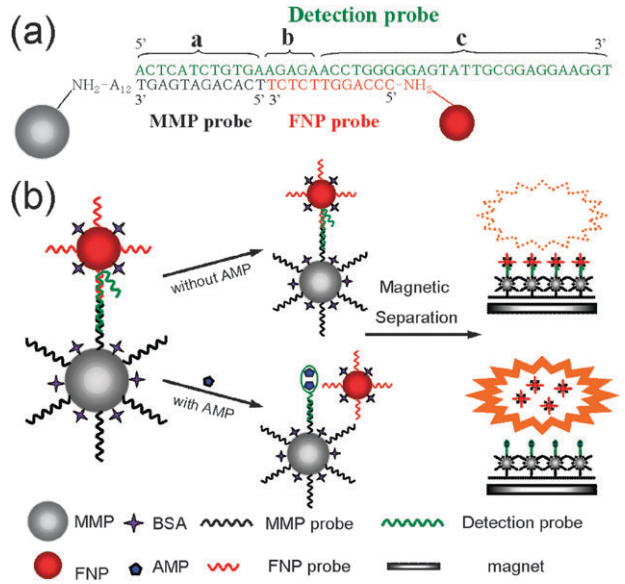

Scheme 1 (a) DNA sequences in the complex: Detection probe (green), MSMP probe (black), FNP probe (red). (b) Schematic representation of the process for AMP detection based on FNPs.

segment (**Segment c**) is the ***AMP aptamer sequence*** which can hybridize with the first seven bases of the ***FNP probe***. In the presence of AMP, the aptamer would change its structure to bind AMP and does not hybridize with the first seven bases of the ***FNP probe*** (Scheme 1). Therefore, only five bases of the ***Detection probe*** are left to hybridize with the ***FNP probe***, this makes the hybridization unstable at room temperature.[11] As a result, FNPs will dissociate from MSMPs, resulting in disassembly of the sandwich complex. Upon magnetic separation, the dissociated FNPs can be collected for fluorescence measurement. The process of AMP detection is shown in Scheme 1B. Without AMP, FNPs will be separated together with MSMPs away from the aqueous medium by magnetic separation, which makes the aqueous medium exhibit weak fluorescence. However, with the addition of AMP, the dissociated FNPs would still remain in the aqueous medium and the aqueous medium exhibits strong fluorescence. The design of the AMP-induced disassembly was adapted from a recent report.[11a] Lu and coworkers employed an adenosine-induced disassembly of gold nanoparticles for fast colorimetric sensing of adenosine with a detection limit of 0.3 mM.[11a] However, when fluorescent silica nanoparticles were used, a 3000-fold greater sensitivity could be achieved. The direct detection limit can be as low as 0.1 μM, 500-fold lower than using quantum dots.[11b]

Quantitative analysis was performed[20–22] by monitoring the fluorescence emission intensity at 586 nm (λ_{ex} = 458 nm) of the aqueous medium after magnetic separation.[15] Along with the increase of the AMP concentration, the fluorescence intensity at 586 nm gradually increased (Fig. 2A). Using this method, we can directly detect as low as 0.1 μM AMP (Fig. 2B). In addition, AMP analogues, such as uridine monophosphate (UMP), cytidine monophosphate (CMP) and guanosine monophosphate (GMP), do not dramatically increase the fluorescence (Fig. 3), indicating that the sensor has

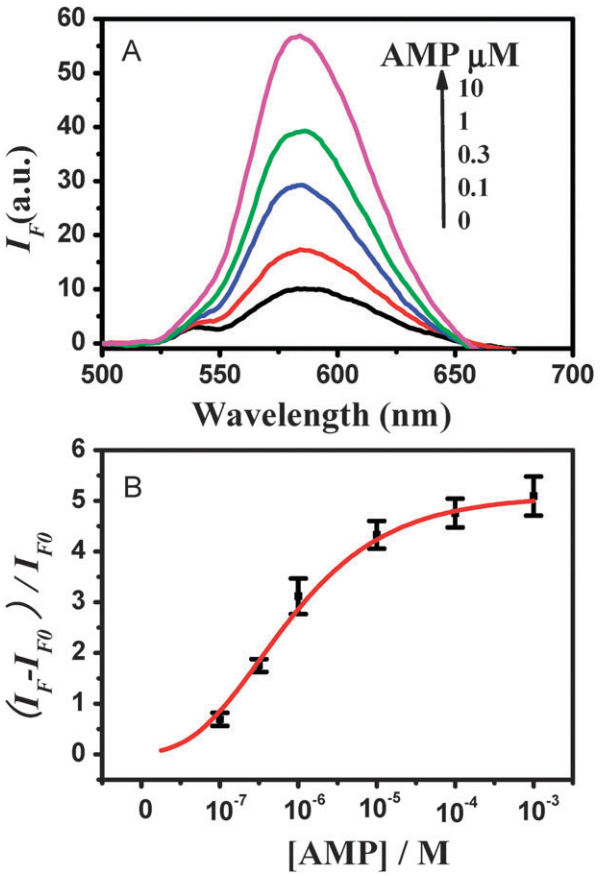

Fig. 2 (a) Fluorescence emission spectra of the aqueous medium after magnetic separation: 0 μM AMP (black line), 0.1 μM AMP (red line), 0.3 μM AMP (blue line), 1 μM AMP (green line), 10 μM AMP (magenta line) in SSC1 buffer (25 mM sodium citrate, 150 mM NaCl, pH 7.4); (b) Quantification of AMP concentration by monitoring the relative fluorescence intensity ($(IF - I_{F0})/I_{F0}$, where I_F and I_{F0} are fluorescence emission intensity at 586 nm of the aqueous medium with or without AMP). Excitation wavelength was 458 nm. Data were obtained from three independent measurements.

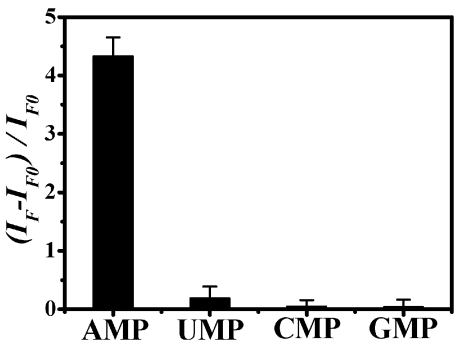

Fig. 3 Sensor AMP selectivity against AMP analogues, UMP, CMP and GMP in SSC1 buffer (25 mM sodium citrate, 150 mM NaCl, pH 7.4). AMP, UMP, CMP and GMP were each 5 μM.

high selectivity for AMP. The high AMP selectivity can be attributed to the AMP aptamer binding specificity.[23,24]

Furthermore, the effects of pH and ionic strength on FNPs fluorescence in the absence or presence of AMP were investigated under our experimental conditions. In the absence of

AMP, as shown in the ESI Fig. S3 A and B,† FNPs fluorescence hardly changed in our studied pH range or salt concentration. In the presence of AMP, FNPs fluorescence did not change in the pH range from 5.4 to 9.4 (Fig. S4 A†), suggesting that the aptamer fragment binding to AMP is not influenced by pH over a wide range of pH. With increasing concentration of NaCl, in the presence of AMP, FNPs fluorescence decreased (Fig. S4 B†). It is well known that increasing the salt concentration can enhance hybrid DNA duplex stability. This can decrease the AMP binding affinity and make it more difficult for the disassembly of silica nanoparticles induced by the aptamer structural switching upon adenosine binding. The effects of pH and ionic strength on FNPs-MSMPs complex are consistent with the previous reports on AuNP aggregates.[25]

In summary, we have demonstrated a new AMP biosensor based on the aptamer-induced disassembly of a nano-magnetic and fluorescent silica sandwich complex. This sensor exhibits high selectivity, a low detection limit, and high photostability. Compared with using gold nanoparticles, 3000-fold greater sensitivity can be achieved. The direct AMP detection limit can be as low as 0.1 μM. Since the surface of the silica nanoparticle serves as a biocompatible and versatile substrate for the immobilization of biomolecules, and aptamers bind to their targets with high affinity and specificity, as pointed out previously,[11] this aptamer-based design can be useful for medical diagnostics and environmental monitoring.

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