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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

# Carbon Nanoparticle-Protected Aptamers for Highly Sensitive and Selective Detection of Biomolecules Based on Nuclease-Assisted Target Recycling Signal Amplification

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Received (in XXX, XXX) XthXXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXXXX 20XX

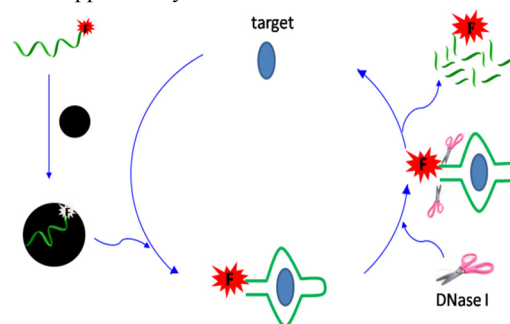
DOI: 10.1039/b000000x

Based on the protective property of carbon nanoparticles for aptamers against the digestion of nuclease, we have developed a nuclease-assisted target recycling signal amplification method for highly sensitive detection of biomolecules, such as ATP and kanamycin. The high binding specificity between aptamer and target leads to excellent selectivity of the assay.

Because of their unique structural, catalytic, and photophysical characteristics, nanomaterials have recently been combined with specific molecular recognition elements to create new tools for life science and biotechnology. Gold or silver nanoparticles, silica nanoparticles and carbon nanomaterials have emerged and found widespread applications,<sup>1</sup> especially in antisense therapy, drug delivery, and diagnostics *in vivo* and *in vitro*.<sup>2</sup> Among these, carbon nanomaterials have attracted more and more attention, with carbon nanotubes, graphene, graphene oxide, and C<sub>60</sub> being successfully employed in many biomedical and bioanalytical applications.<sup>3</sup> Specifically, the fluorescence quenching and protective property of carbon nanomaterials, such as graphene oxide, towards nucleic acid probes enable their application in intracellular monitoring and diagnostics.<sup>2c, 4</sup> The interaction between fluorescent probes and carbon nanomaterials leads to fluorescence resonance energy transfer, while the addition of targets weakens the interaction between probes and nanomaterials and hinders the fluorescence resonance energy transfer (FRET), thus increasing the fluorescence intensity.<sup>4a</sup> In our group, we have made use of the protective properties of graphene oxide to single-stranded DNA against nuclease digestion to develop a cyclic enzymatic amplification method (CEAM) for sensitive and multiplex detection of miRNA in complex biological samples.<sup>5</sup>

Recently, new carbon nanomaterials, carbon nanoparticles (CNPs), have also been demonstrated to be chemically inert and biocompatible with low cytotoxicity. Li *et al* reported that

fluorescent DNA probes can be adsorbed on CNPs by  $\pi$ - $\pi$  stacking, resulting in quenching of the probes by fluorescence resonance energy transfer, thus serving as a promising fluorescent sensing platform.<sup>6</sup> Subsequently, combined with DNA probes, a new platform for sensing of DNA, proteins, nucleases and metal ions based on CNPs has been explored.<sup>7</sup> However, up to now, there are still no reports on the protective properties of CNPs to single-stranded DNA from enzymatic cleavage. These properties may provide innovative detection strategies based on enzymatic signal amplification. In this work, we report that single-stranded fluorescent DNA probes adsorbed on CNPs can be effectively protected from enzymatic cleavage. Based on this finding, we have successfully developed a nuclease-assisted target recycling signal amplification method for the analysis of biomolecules, such as ATP and kanamycin, using single-stranded aptamers. Due to the ability of aptamers to bind to a wide range of targets with high affinity and specificity,<sup>8</sup> the proposed assay shows significant advantages in high sensitivity, excellent selectivity and general applicability.



Scheme 1. Working principle of DNase I-assisted target recycling signal amplification for target analysis based on carbon nanoparticles protection and quenching of single-stranded aptamer probes.

The working principle of the method is illustrated in Scheme 1. In the absence of targets, dye-labelled aptamer probes are in the flexible single-stranded state and adsorb on the CNPs and, as a result, the fluorescence of dyes is quenched by CNPs. Upon the addition of specific targets, the probes bind to the targets and the aptamer/target complexes desorb from the CNPs surface, with the probes becoming fluorescent. More importantly, the probe immediately becomes the substrate for DNase I digestion,

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†Electronic Supplementary Information (ESI) available: Experimental Section, Materials, and Fluorescence measurements. See DOI:

subsequently releasing the target to bind another probe on CNPs and initiate the next round of cleavage, resulting in significant fluorescent signal amplification.

To demonstrate the feasibility of the carbon nanoparticle-based target recycling signal amplification method, adenosine triphosphate (ATP) was chosen as the model target. The following ATP aptamer sequence was prepared: 5'- ACC TGG GGG AGT ATT GCG GAG GAA GGT-FAM-3'.<sup>9</sup> Native polyacrylamide gel electrophoresis was performed to demonstrate the protective property of CNPs to aptamers against DNase I digestion and the target-assisted cleavage of the aptamer by DNase I. As shown in Figure 1A, in the absence of CNPs, the ATP aptamer alone (lane 2) and aptamer interacted with ATP (lane 3) were digested by DNase I. In contrast, after incubation with CNPs for 30 min, the ATP aptamer was resistant to DNase I cleavage (lane 4), while the presence of target ATP led to digestion of ATP aptamer by DNase I (lane 5), indicating that the ATP/aptamer complex had desorbed from CNPs and the aptamer was hydrolyzed by DNase I. These results verified that CNPs can protect ATP aptamer from DNase I digestion, and after binding to the target ATP, the ATP aptamer desorbs from CNPs and is hydrolyzed by DNase I.

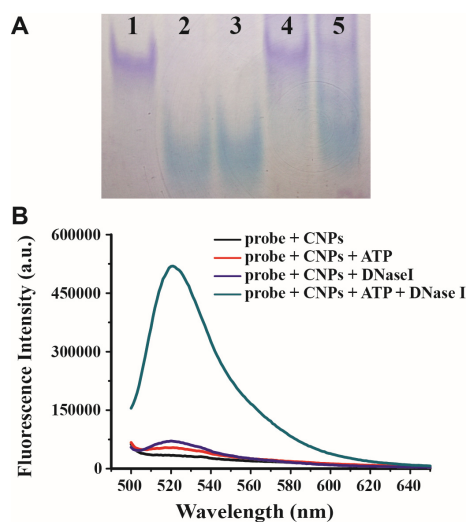


Figure 1. (A) Native PAGE analysis of ATP aptamer with CNPs and DNase I. Lane1: ATP aptamer; lane2: ATP aptamer + DNase I; lane3: ATP aptamer + ATP + DNase I; lane4: ATP aptamer + CNPs + DNase I; lane5: ATP aptamer + CNPs + ATP + DNase I. (B) Fluorescence emission spectra of ATP aptamer under different conditions.

Fluorescence measurements were conducted to confirm the DNase I-assisted target recycling process and fluorescence signal amplification. As demonstrated in Figure 1B, the ATP aptamer showed low fluorescence intensity after incubation with CNPs, verifying the efficient adsorption and quenching ability of CNPs towards fluorescent single-stranded DNA probes. After the addition of target ATP, there was a slight increase in the fluorescence intensity due to the 1:1 binding event. Without target ATP, the presence of DNase I also led to a slight fluorescent enhancement, suggesting that some of the aptamers adsorbed on CNPs were hydrolyzed by DNase I.<sup>10</sup> By contrast, in the presence of both DNase I and ATP, the aptamer bound with target ATP and desorbed from CNPs. The aptamer was then digested by DNase I, followed by the release of ATP and cyclic cleavage reaction, which gave rise to a remarkable fluorescence enhancement. The result established that DNase I-assisted digestion of ATP aptamer

led to a target recycling reaction, thus resulting in amplified fluorescence intensity.

The DNase I-assisted target recycling signal amplification leads to highly sensitive detection of ATP. The fluorescence intensity was measured with different concentrations of target ATP. As illustrated in Figure 2A, a dramatic increase in fluorescence intensity was observed as the concentration of ATP increased from 0 to 1 mM. The fluorescence intensity change exhibits a good linear positive correlation with ATP concentration as shown in Figure S1 (see ESI). The detection limit of the method was calculated to be 0.2 nM based on the  $3\sigma$  method. To confirm that it is the efficient DNase I-assisted target recycling signal amplification that contributes to the high sensitivity of the approach, control experiments were carried out with different concentrations of ATP reacting in the absence of DNase I. By contrast, the limit of detection was 10  $\mu$ M without signal amplification (Figure S2, ESI). Thus, the DNase I-assisted target recycling signal amplification method lowers the limit of detection by 4 orders of magnitude for the detection of ATP.

We further tested the selectivity of the ATP detection method. Due to the inherent specificity of the aptamer, the binding of probe and ATP was found to be highly selective. As shown in Figure 2B, ATP analogues, such as GTP, CTP, and UTP, did not induce obvious signal enhancement, consistent with the fact that they do not bind with ATP aptamer sequences strongly enough to cause the desorption of the probe from CNPs surface for nuclease digestion. These results verified the high specificity of our method for ATP detection. Considering the significance of ATP analysis in biological samples, we challenged our approach to detect ATP in DMEM cell media. As demonstrated in Figure S3, the fluorescence intensity increases with the addition of increasing concentrations of ATP and 1 nM ATP can be easily detected. The result confirmed the adaptability of our amplification approach for ATP detection in complex biological samples.

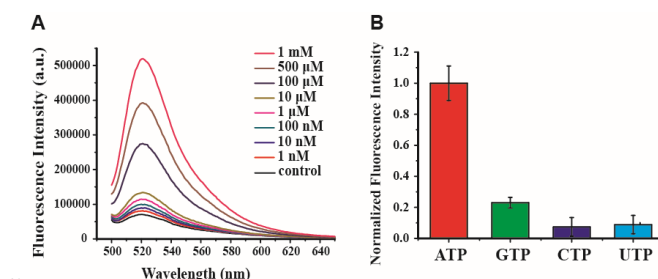


Figure 2. (A) Fluorescence emission spectra of ATP aptamer with different concentrations of ATP. (B) Selectivity of DNase I-assisted target recycling signal amplification method for ATP over GTP, CTP and UTP with concentration of 10  $\mu$ M each.

To demonstrate that our signal amplification strategy can be applied to the detection of other molecules using particular aptamers, we further designed a DNase I-assisted target recycling signal amplification method for kanamycin detection. Kanamycin is an aminoglycoside antibiotic, which interacts with the 30S subunit of prokaryotic ribosome to block translation.<sup>11</sup> It has been applied in a wide range of infection treatments. However, excessive use of kanamycin in food and medicine may cause serious side effects, such as nephrotoxicity, ototoxicity, neuromuscular blocking effects, hematopoietic system toxicity and allergic reactions.<sup>12</sup> Therefore, a rapid and convenient method for kanamycin detection with high sensitivity and selectivity is in great demand. The kanamycin aptamer sequence was prepared 5'-AGA TGG GGG TTG AGG CTA AGC CGA CCG TAA GTT GGG CCG T-FAM-3'.<sup>13</sup> Figure 3A illustrates

the fluorescence intensity upon addition of different concentrations of kanamycin. The fluorescence intensity increased with increasing kanamycin concentration and the fluorescence intensity change showed a linear relationship with the concentration of kanamycin within the range from 10 nM to 1  $\mu$ M (Figure S4, ESI). The detection limit was calculated to be 2.7 nM based on the  $3\sigma$  method, far below the maximum residual limits (MRL) in food products (e.g., 62 nM in Korea, 83 nM in Japan and 206 nM in the European Union). To evaluate the specificity of our assay for kanamycin detection, we challenged the system with several kanamycin analogues, such as gentamicin, streptomycin and tetracycline. As shown in Figure 3B, the fluorescence signal produced by the analogues was only 10%-15% of that produced by kanamycin, thus verifying the high specificity of the proposed CNPs-based target recycling signal amplification method.

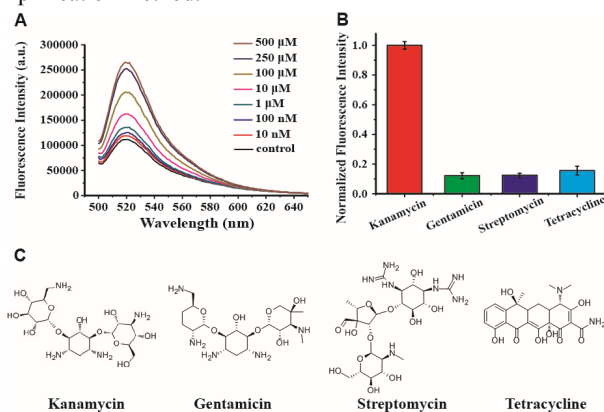


Figure 3. (A) Fluorescence emission spectra of kanamycin aptamer with different concentrations of kanamycin. (B) Selectivity of DNase I-assisted target recycling signal amplification method for kanamycin over gentamicin, streptomycin and tetracycline with concentration of 10  $\mu$ M each. (C) Structures of kanamycin and its analogues.

In conclusion, for the first time we demonstrated that single-stranded DNA probes absorbed on CNPs can be effectively protected from digestion by DNase I. Based on CNP-protected single-stranded aptamer probes, we have developed a DNase I-assisted target recycling signal amplification strategy for highly sensitive and selective analysis of biomolecules, such as ATP and kanamycin. The proposed approach shows advantages in high sensitivity, excellent specificity and simplicity. The low background fluorescence intensity, together with the cyclic DNase I-assisted signal amplification, gives rise to the high sensitivity of the assay for ATP detection, with a detection limit four orders of magnitude lower than that of non-amplified methods. Furthermore, the high binding selectivity between aptamer and target leads to high selectivity of our assay. More importantly, such high sensitivity and selectivity is achieved by simply mixing the aptamer, the CNPs, the target and DNase I, then conducting the fluorescent measurement. Therefore, the reported DNase I-assisted target recycling signal amplification strategy based on CNPs-protected single-stranded aptamer probes is expected to find wide applications for highly sensitive and selective detection of biomolecules.

We thank the National Basic Research Program of China (2010CB732402, 2013CB933703), the National Science Foundation of China (91313302, 21205100, 21275122,

21075104), the National Science Foundation for Distinguished Young Scholars of China (21325522) and Open Funding Project of the State Key Laboratory of Bioreactor Engineering for their financial support.

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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