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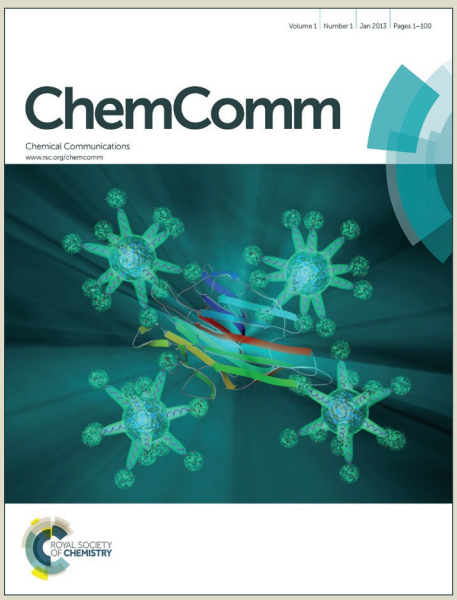

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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Hybridization chain reaction engineered dsDNA for Cu metallization: An enzyme-free platform for amplified detection of cancer cells and microRNAs

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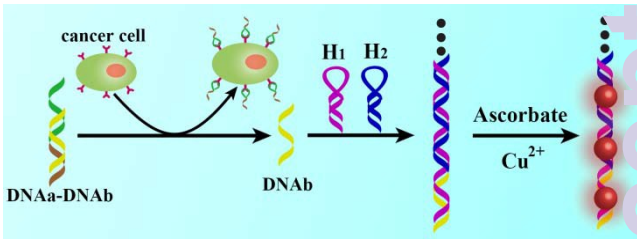
Received (in XXX, XXX) XthXXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A novel enzyme-free platform for amplified detection of cancer cells and miRNAs was constructed with high sensitivity by fluorescent Cu metallization on HCR engineered dsDNA templates.

Sensitive and accurate detection of cancer cells is crucial not only for effective diagnosis of cancer but also for monitoring and halting cancer's progress.[1] Typically, the detection methods can be divided into two major categories. One is mainly based on recognizing the biomarkers such as proteins on cancer cell membranes with optical probes, which can be used directly to analysis the whole cancer cells.[2] In this regard, various techniques, such as colorimetric assay, electrochemiluminescence, surface-enhanced Raman scattering method and flow cytometry, have been proposed.[2b-f] The other is based on the detection of the biomarkers such as microRNAs (miRNAs) inside cancer cells, where these biomolecules are expressed abnormally.[3] Up to now, diverse analytical methods (*e.g.* polymerase chain reaction assay, northern blot technique and microarray) have been developed for sensing miRNAs.[4] Although promising, these cancer cell detection methods are compromised by the requirement of cumbersome laboratory procedures, sophisticated instrument and high cost, *etc.*[5] Hence, it is in ever increasing demand to develop an easier operating method which requires simple instrumentation yet still provides high sensitivity and accuracy.

Owing to the high stability, remarkable molecular recognition property and easy modification, deoxyribonucleic acid (DNA) has been immensely employed to build smart devices for applications in biomedicine, sensing and material science.[6] For instance, a dye-labeled DNA "Nano-Claw" was proposed by Tan et al. for cancer cell diagnosis in programmable way.[7] In another work, with the help of endonuclease, fluorescent DNA probe immobilized gold nanoparticles were used to detect miRNAs.[8] However, these methods needed the participation of enzymes or dye-labeled oligonucleotides, which were expensive and complicated to operate. On the other hand, as being rich of amino groups, phosphate groups and heterocyclic nitrogen atoms, DNA offers multiple binding sites for several metal ions which could be reduced to form metallic nanoparticles that follow the profile of the DNA template.[9] To the end, the emergence of DNA metallization has offered encouraging approaches for imaging and detection, as outlined in the works reported by Mokhir's, our and other's groups.[10] Specially, fluorescent copper nanoparticle (CuNPs) have been synthesized by using double-strand DNA (dsDNA) as efficient template, which exhibited excell photophysical properties with ultrafine size.[10a] These unique characters make them particular promising for biosensing vari targets as the DNA template can be flexibly designed.[11] Although dsDNA-templated CuNPs are facile to synthesis, label-free and low-cost, the further application of CuNPs for cancer ce detection, especially in an amplified way, has not been reported yet.



Scheme 1 Schematic illustration of cancer cell detection based on th target-triggered hybridization chain reaction (HCR) and in situ formatio of fluorescent CuNPs.

Herein, for the first time, by using the dsDNA polym formed by hybridization chain reaction (HCR) as templates for copper metallization, we reported an enzyme-free approach for amplified detection of cancer cells and miRNAs. HCR ar recently emerging as a novel amplification method via triggere cascade of DNA polymerization by initiators or target molecules Two hairpin DNA strands can stably coexist in solution in the absence of the target DNA. However, the introduction of the target DNA will trigger a cascade of hybridization, which yield long dsDNA polymers with numerous repeats.[12] Our strategy is shown in Scheme 1. DNA containing the cancer cell targetin aptamer sequence (DNAa) is hybridized with the messenger DNA (DNAb) to form a partially complementary duplex (DN a-DNAb). In the presence of cancer cells, the binding of aptam rs to their targets changes their conformation and disrupts the hybridized duplex, causing the release of DNAb. Subsequently the released DNAb triggers the HCR process between tw hairpins (H_1 and H_2) to form dsDNA polymers. Afterwards, th formed dsDNA polymers are used as the template for in situ synthesis of CuNPs with strong fluorescence, which can be used

as the readout signal. With such enzyme-free amplified process, high sensitivity towards target cancer cells with a detection limit of 50 cells can be easily achieved. More importantly, the protocol exhibits excellent selectivity over normal cells, which can be readily seen by the naked eye under UV transilluminator. Therefore, a facile fluorescence amplification strategy for sensitive cancer cell detection could be realized by HCR engineered Cu metallization.

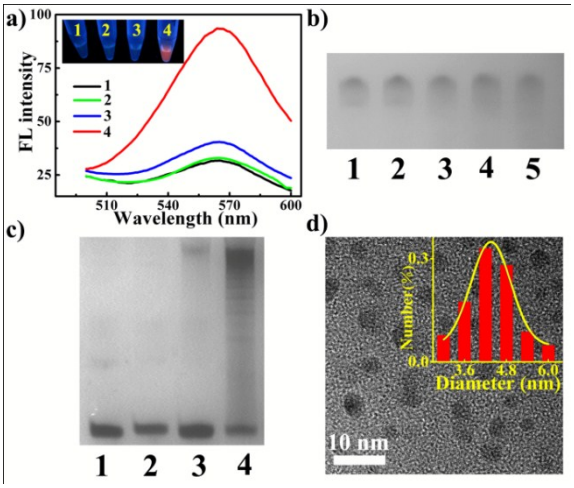


Fig. 1 (a) Fluorescence spectra of CuNPs metalized on DNA template: 1, H_1; 2, H_2; 3, H_1 + H_2; 4, H_1 + H_2 + 50 nM DNAb. Inset: photograph of DNA-templated CuNPs solutions exhibiting fluorescence under UV light. (b) Native gel electrophoretic analysis (15% PAGE) of products in the absence or presence of MCF-7 cancer cells: lane 1, DNAa-DNAb; lanes 2−5, four different numbers of MCF-7 (5×10^2, 5×10^3, 5×10^4 and 2.5×10^5) incubated with DNAa-DNAb. (c) Native gel electrophoretic analysis (15% PAGE) of the HCR process in the presence of 0 nM or 50 nM DNAb: lane 1, H_1; lane 2, H_2; lane 3, H_1 + H_2; lane 4, H_1 + H_2+ 50 nM DNAb. (d) TEM image of CuNPs metalized on the DNA template which formed via the HCR process in the presence of 100 nM DNAb. Inset: size distribution analysis of DNA-templated CuNPs.

To check the utility of this approach, a human breast cancer cell line MCF-7 was used as the target cell and aptamer sequence targeting the surface marker Mucin 1 (MUC1)[13] was employed in this study. The design of each DNA sequence was described in Table S1. DNA UV melting studies were firstly conducted to show the melting temperature (T_m) of DNAa-DNAb, H_1 and H_2. As shown in Fig. S1, at the temperature of 25 °C, DNAa-DNAb was mainly in the form of duplex while H_1 and H_2 were stable in the form of hairpins, thus no HCR process would take place in the absence of the targets. Hence, 25 °C was set as the operating temperature. Before cancer cell detection, the DNAb was used to examine the triggered HCR process and in situ formation of CuNPs. As shown in Fig. 1a, in the presence of 50 nM DNAb, the resulted CuNPs displayed a strong fluorescence signal, which had an emission maximum of 568 nm upon excitation at 345 nm. While in the absence of DNAb, the two hairpin probes were in closed form at room temperature, therefore there were no dsDNA formed and the resulted CuNPs had weak fluorescence in solution. Meanwhile, neither H_1 nor H_2 alone could result in fluorescence signal. Since the concentration of Cu^{2+} and the reaction time of HCR were important factors influencing the performance of the detection, the assay conditions were optimized as follows: 500 nM H_1, 500 nM H_2, 2 mM sodium ascorbate, 100 μM Cu^{2+} and 2 h incubation time of HCR process (Fig. S2). After that, electrophoresis experiments were conducted. As shown in Fig. 1b, in the absence of cancer cells, the band of DNAa-DNAb was present. However, litter DNAa-DNAb was observed in the presence of cancer cells. It confirmed the recognition of DNAa-DNAb towards cancer cells. Then, the DNA polymer formulated by DNAb triggered HCR process was verified. As shown in Fig. 1c, in the absence of DNAb, a nearly well-defined band (lane 3) was observed, whereas in the presence of DNAb, a long dispersed band was observed (lane 4), confirming that high molecular weight DNA structures were formed. The signal variation in circular dichroism (CD) spectra also demonstrated the formation of DNA polymers triggered by DNAb (Fig. S3). The metallization of CuNPs on the obtained dsDNA polymer templates was studied by the transmission electron microscopy (TEM) image (Fig. 1d). The average size of formed CuNPs was about 4 nm. All above experiments indicate that the approach has the potential to achieve a good performance.

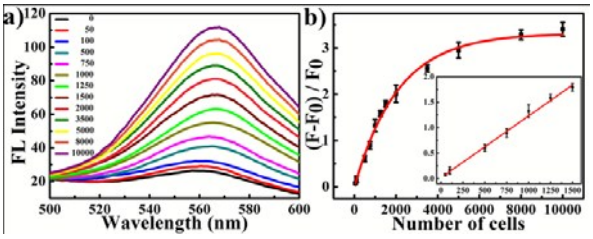


Fig. 2 (a) Fluorescence emission spectra corresponds to the analysis of different number of MCF-7 cells. (b) Plot of the fluorescence intensity measured at 568 nm as a function of the number of cells. Inset: the linear plot.

Under the optimized conditions, we then evaluated the sensitivity of this system for quantitative detection of MCF-7 cells. The detection results were shown in Fig. 2, the fluorescent intensity signals of the system enhanced along with the increase of MCF-7 cell numbers. A good linear ship was obtained over 50-1500 MCF-7 cells with a detection limit of 50 cells, which was better than or at least comparable to those of the reported methods.[2e,2f] The high sensitivity was contributed to tw amplification efforts: firstly, one cancer cell could induce the release of several DNAb. Secondly, the released DNAb triggered the amplified HCR process between H_1 and H_2. It was worthy to know that the amplification efforts did not need the participation of enzyme. More importantly, through metallization of CuNPs on resulted dsDNA templates, our approach avoided the use of dye-labeled DNA, which offered the benefits of cost efficiency and operating convenience.

We further evaluated the specificity of this platform by examining the fluorescence responses toward different kind of cells. In our experiments, five cell lines were used: MCF-7, A549, K562, HepG2 and NIH-3T3. As the normal cells, NIH-3T3 did not overexpress MUC1 receptors. As a contrast, MCF-7, A549 K562 and HepG2 were employed as cancer cell lines with overexpressed MUC1 receptors. As shown in Fig. 3, MCF-7 cells showed the strongest fluorescence while other cells including cancer cells and normal cells only induced negligible fluorescence changes, which was consistent with the reported results.[14] As MUC1 is abundantly overexpressed on epithelial cancer cells[13a], this method might be general for epithelial cancer

cells detection, especially for MCF-7 cells. On the other hand, due to the complex environment of clinical cancer sample, like epithelial cancer tissue, further efforts are needed on optimizing the current method before utilizing for practical applications.

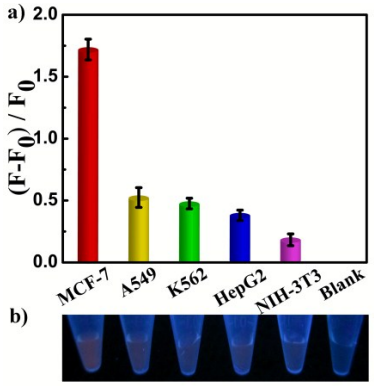


Fig. 3 The specificity for MCF-7 cells detection. (a) The fluorescence intensity of solutions containing MCF-7, A549, K562, HepG2 and NIH-3T3 cells, respectively. (b) The corresponding photograph taken under a UV transilluminator.

Being another kind of cancer cell detection method, the biomarker miRNA was further analysed by the DNA polymer-templated Cu metallization platform. For the proof-of-concept experiment, miR-21 was chosen as the target.[15] As shown in Scheme S1, upon presenting, miR-21 triggers the HCR between H_1 and H_2, resulting in the formation of large molecular dsDNA. In this way, the CuNPs are formed after introducing reducing reagent and Cu^{2+}, which results in strong fluorescence signal. As shown in Fig. S4, the enhancement of fluorescence intensity was proportional to the concentration of miR-21, and a good linear relationship between the fluorescence intensity and miR-21 concentration over the range of 0.25 nM - 25 nM was obtained. The detection limit was 0.25 nM, which was superior to some nanoparticle-based fluorometric sensor[16]. Moreover, the platform showed high specificity towards miR-21 (Fig. S5), whereas only weak fluorescent response was observed for single-base mismatched RNA (RNA1), two-base mismatched RNA (RNA2) and random RNA (RNAr). Taken together, all these results demonstrated that our strategy provided a novel, simple but sensitive approach for miRNAs detection.

In summary, for the first time, by rationally combining HCR and CuNPs, we have successfully obtained a novel enzyme-free amplified platform for selective and quantitative detection of cancer cells and miRNAs. The protocol relies on the targets triggered formation of dsDNA polymers and in situ formation of CuNPs with strong fluorescence intensity. The method used here brings about several unprecedented advantages. First of all, the method is simple and economic. The DNA-templated CuNPs are easy to synthesize and do not need the use of enzyme or dye-modified oligonucleotide. Secondly, the protocol relies on the amplified fluorescence turn-on sensing mode, which offered more sensitivity and reduced the likelihood of a false positive signal. Thirdly, the discrimination of cancer cells with normal cells can be readily seen by the naked eye under UV transilluminator. Furthermore, in cooperation with other materials, the sensor can be easily extended to other analytes. Therefore, we envision that the engineered DNA-templated CuNPs will hold great promises in a wide range of potential applications, such as fluorescence imaging, clinical diagnostics and biosensors.

Financial support was provided by the National Basic Research Program of China (Grants 2012CB720602 and 2011CB936004) and the National Natural Science Foundation of China (Grants21210002, 91213302, 21431007, 91413111).

Notes and references

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

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