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Core-shell assay based aptasensor for sensitive and selective thrombin detection using dark-field microscopy

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ABSTRACT

In this work, we developed a robust and ultrasensitive bio-sensor based on the target-aptamer recognition strategy and microscopic enumeration of gold nanoparticles (AuNPs) using dark field microscopy (DFM). The aptasensor with a core-shell structure consisting of a magnetic bead (MB), aptamer and AuNPs was fabricated by complementary hybridization of the DNA probe on the AuNPs surface to the aptamer coupled to the MB. Upon addition of the target molecule, the strong interaction between the aptamer and the target molecule, thrombin, results in the release of the AuNPs from the MB. The quantities of thrombin is therefore linearly correlated to the number of the released AuNPs, which can be digitally counted using DFM. To demonstrate the feasible use of the aptasensor for target detection, thrombin was evaluated as the model target. The limit of detection was determined to be 2.54 fM with dynamic range of 6 fM–100 fM. When the concentration of thrombin exceeded 100 fM, the counted number of AuNPs didn't correlate linearly to molecules of thrombin anymore, as the nanoparticles aggregated partly due to high concentration. However, the color of the solution changes to purple and the concentration of free AuNPs can be conveniently quantified by UV–Vis spectroscopy for up to 100 nM. It is noteworthy that our aptasensor is very easy to operate and requires neither complex isolation and amplification processes nor expensive instruments and consumables. Furthermore, this strategy can be easily generalized to other targets by replacing the corresponding aptamers and show great potential for the detection of biomarkers in clinical samples.

1. Introduction

The development of biosensors that recognize biological targets or processes has attracted increasing attention. Proteins, large biomolecules or macromolecules, perform various functions in organisms, including accelerating metabolic reactions, responding to stimuli, replicating DNA, and transporting molecules. The significant changes in proteins can reveal the physiological and pathological processes of an organism, which are regarded as biomarkers, indicating the biological state of a disease. These proteins are of great significance in basic research of life science [1,2], disease diagnosis [3–5], drug research [6,7], human health analysis [8,9] and biological safety monitoring [10,11]. Protein recognition based on the specificity of antigen and antibody of immune response is a widely used technique [12–14]. However, some drawbacks such as the time-consuming, costly and difficult in chemical modification and immunogenic preparation of antibody produced by the organism, restrict the use of protein analysis. Therefore, investigations aimed at the development of more convenient and efficient protein recognition are highly significant in biological systems.

Aptamers generated by the Systematic Evolution of Ligands by Exponential enrichment (SELEX) technique [15–19], are sequences of oligonucleotides that can specifically bind to a target with a stable structure, such as G-quadruplex [20], hairpin [21], and pseudoknot [22]. In contrast to antibodies, aptamers can be easily synthesized and chemically modified with little immunogenicity. The target molecules of aptamer are widely distributed, including metal ions, organic small molecules, proteins, viruses, cells and others, which allowed a range of applications of aptamer for sensitive detection of different species. Aptamers have been considered an excellent molecular recognition element due to their high affinity and good specificity for the target through complementary pairing, complementary shape, van der Waals forces, electrostatic interaction and hydrogen bonds.

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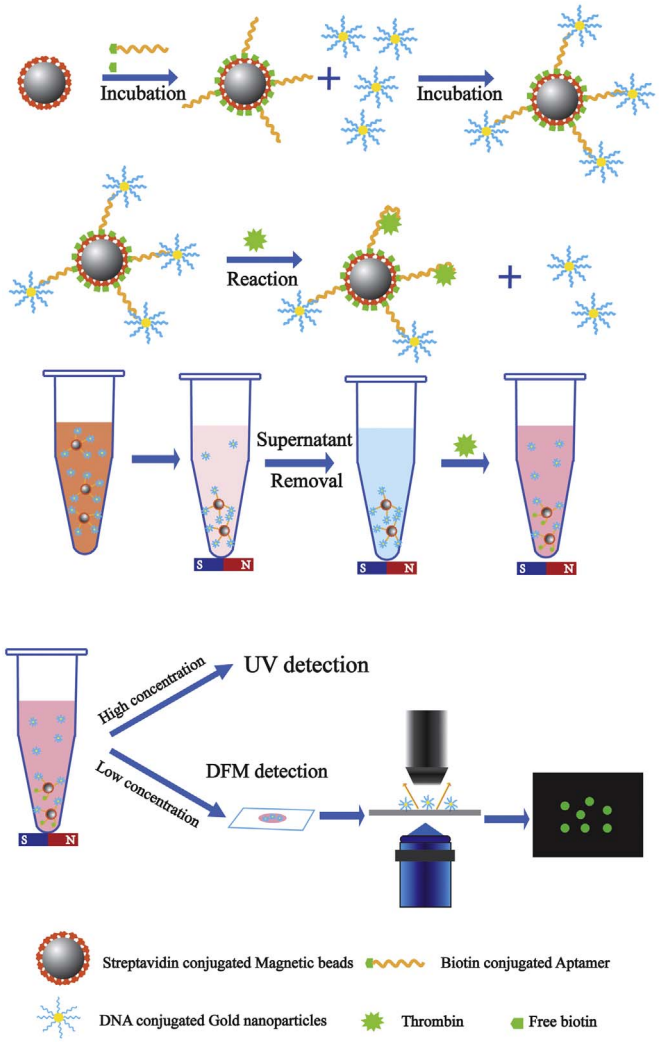
Gold nanoparticles (AuNPs) have been extensively studied for biological applications due to their inherent characteristics such as easy surface modification and good biocompatibility. Gold nanoparticle and aptamer composites have no significant systemic toxicity was observed in rats (1 mg/kg of body weight) [23]. AuNPs can be facilely prepared with well-defined shapes and sizes under controllable conditions that directly affect the optical properties of AuNPs [24] and can be utilized in a variety of fields. Several methods have been developed to assay target-aptamer recognition using AuNPs, including colorimetry [25,26], fluorescence [27,28], chemiluminescence [29], electrochemistry [30], surface-enhanced Raman [31] and others. However, the sensitivity based on the measurement of the collective signals of large number of nanoparticles is generally limited, which lead to the detection of limits achieved in the range of nanomolar to picomolar in concentration. Actually, owning to the excellent optical properties of AuNPs, especially the localized surface plasmon resonance (LSPR) effect, each particle can be detected digitally with excellent signal to noise ratio. Using a DFM, AuNPs can be unambiguously identified and quantified [32,33] which provides an excellent methodology for ultrasensitive detection for target molecules. Herein, we developed a simple and cost-effective aptasensor, which combined 1 to 1 substitution of target molecule for AuNPs through aptamer-target interaction and subsequent spectroscopic quantification/microscopic identification and enumeration of replaced AuNPs, with a detection limit as low as the fM level and an extremely wide dynamic range. The strategy is depicted schematically in Scheme 1. First, biotin-conjugated aptamers are immobilized on the surface of the streptavidin-conjugated magnetic beads (MBs) through a biotin-streptavidin interaction. AuNPs modified with DNA probes with a sequence complementary to the aptamer were added in excess of the aptamer [34,35]. An aptasensor with the core-shell structure of MB/aptamers/AuNPs has thus been fabricated. In the presence of a target, the aptamer recognizes and forms a secondary structure with the target, interrupting the complementary pairing and leading to the release of AuNPs from the MBs. The dissociated AuNPs are then quantified by ultraviolet spectroscopy or DFM digitally due to their excellent optical properties. As proof of concept, thrombin as a serine protease, playing an important role in molecular biology, is used as the model target in the present work.



Scheme 1. Schematic illustration for detection of the target. Thrombin is used as the model target.

2. Materials and methods

2.1. Reagents

DNA sequences were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). The aptamer sequence was 5′-biotin- TTTTTTTTTT TTTTTTTTTTGGTTGGTGTGGTTGG-3′. The complementary sequence (cDNA) was 5′-SH-TTTTTTTTTTTTTTTTTTTTTCCAAC-3′. Tris (2-carboxyethyl) phosphine (TCEP), hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4{\cdot}3H_2O$), thrombin (Thr), Cytochrome (Cyt), lysozyme (Lys), IgG from rabbit serum, bovine serum albumin (BSA), and Trypsin (Try) were obtained from Sigma-Aldrich (USA). Dynabeads M-280 Streptavidin was purchased from Thermo Fisher. (3-Aminopropyl) triethoxysilane (APTES) was purchased from Nanjing Chen Gong Co., Ltd. The water used in the experiment was ultrapure water from the Millipore water purification system.

2.2. Instrumentation

Scanning electron microscope (SEM) images were obtained using a ZEISS SUPRA®55 instrument (Carl Zeiss, Germany). Size measurement and distributions were measured by dynamic light scattering (DLS) on a Malvern Zetasizer Nano-ZS90 system (Malvern, Britain). Absorbance measurements were performed using a microplate reader (Tecan infinite M1000 Pro reader, Switzerland). Dark-field microscopic images were recorded using an optical BX53 microscope equipped with a color CCD (Olympus, Japan).

2.3. Synthesis of different sizes of AuNPs

The seed of the gold granule was produced by the sodium citrate reduction method. Then, using hydroxylamine hydrochloride to reduce the chloroauric acid with the addition of obtained gold granule as seeds, the gold particles with different particle sizes were produced. The detailed procedure was as follows [36]: 99 mL of ultrapure water was added to 1 mL of $HAuCl_4$ solution (24 mM), and 10 mL of sodium citrate (14.55 mM) was subsequently added. The mixture was heated under 110 ℃ for 20 min, followed by cooling in ice water for 10 min. The dark red reactive liquid was filtered through a 0.22 μm filter. The liquid was subjected to density gradient centrifugation, carried out by centrifuging at 8600 rpm for 10 min, resulting in the removal of the supernatant after centrifuging at 4000 rpm for another 10 min. The light pink supernatant was used as the seed solution. Next, different sizes of gold particles were prepared by adding 1 mL of 40 mM hydroxylamine hydrochloride solution and 1 mL of $HAuCl_4$ solution (3.52 mM) into the 1–6 mL seed solution samples. The 10 mL of gold solution was condensed to 1 mL by centrifugal washing.

2.4. Functional modification of gold nanoparticles

After incubating TCEP with cDNA for 1 h, the obtained gold particles were mixed with cDNA solution with the molar concentration ratio of cDNA: AuNPs = 10000:1 and incubated overnight. Then, the salt