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Hybridization chain reaction coupled with the fluorescence quenching of gold nanoparticles for sensitive cancer protein detection

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ABSTRACT

An ultrasensitive and enzyme-free sensing platform was designed for sensitive detection of anterior gradient homolog 2 (AGR2) based on the fluorescence quenching of gold nanoparticles (AuNPs) coupled with hybridization chain reaction (HCR) amplification. To construct the sensor, two fluorophore labeled hairpin probes (HP_1 and HP_2) that contained the sticky tails were designed, and AGR2 aptamer was used as an initiator for the happen of HCR between HP_1 and HP_2 to form a long nicked dsDNA duplex. Such DNA duplex could not adsorb onto the surface of AuNPs, and a strong fluorescence signal appeared due to the label of HP_1 and HP_2 with fluorophore. In the presence of AGR2, AGR2 aptamer would specifically recognize with it and accordingly could not trigger the happen of HCR. As a result, the sticky tails of HP_1 and HP_2 would adsorb onto the surface of AuNPs to bring the fluorophore into the close proximity of the AuNPs, and lead to the quenching of the fluorescence signal. By using such method, AGR2 could be sensitively detected in the range of 5.0 pM–1.0 nM based on monitoring the decrease of the fluorescence signal. Importantly, the assay could realize the detection of AGR2 effectively in diluted human serum samples.

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1. Introduction

Clinical studies have been shown that anterior gradient homolog 2 (AGR2) is directly associated with a wide range of human cancers such as esophagus, pancreas, breast, prostate and lung [1,2]. In addition, AGR2 plays an important role in proliferation, transformation, migration and metastasis of tumor cells [3,4]. Thus, sensitive detection of AGR2 is of great importance in early diagnosis and prognosis of cancers.

Aptamer is nucleic acid-based (DNA or RNA) affinity probe that obtained in vitro by an evolution process named systematic evolution of ligands by exponential enrichment (SELEX), which can bind to a wide range of targets such as small molecules [5,6], metal ions [7,8] and protein [9–11] with high specificity and affinity. Such aptamer can be chemically synthesized at low cost and display greater stability against denaturation [12]. In the past decade, a number of aptamer-based biosensors have been developed for the detection of protein coupling with colorimetric [5,13,14], fluorescent [10,15,16] and electrochemical [17–19] methods. Thereinto, fluorescence-based detection method is one of the most widely used optical sensing strategies due to the merits of it including high sensitivity, simple operation and rapid hybridization kinetics.

Hybridization chain reaction (HCR) is one of the commonly used amplification approaches, which uses a pair of complementary and kinetically trapped hairpin oligomers to propagate a chain reaction of hybridization events in an enzyme-free way at room temperature [20]. In addition, the products of HCR are highly ordered DNA duplex and the signal molecules can be attached on these helices with precisely controlled density, which can greatly improve the efficiency of signal amplification. Because of these advantages, HCR has been widely used for the detection of metal ions [21–23], DNA [24–27], protein [28–30] and RNA [31,32].

Gold nanoparticles (AuNPs) have attracted great attention in colorimetric biosensors because of their particular features, such as optical, chemical and size-dependent physical properties. The simple colorimetric changes of AuNPs from red to blue or from blue to red [33] can provide a simple mechanism for developing colorimetric biosensors. Coupling HCR with AuNPs signal amplification, a number of biosensors have been developed [34,35]. In addition, AuNPs are found to be one type of the fluorescence quencher because of the properties of them such as large surface area to volume ratio, easy surface functionalization and strong surface plasma absorption in NIR-to-IR region [36,37]. AuNPs have superquenching efficiency over a wide range of wavelengths and can quench several fluorophores simultaneously while an organic

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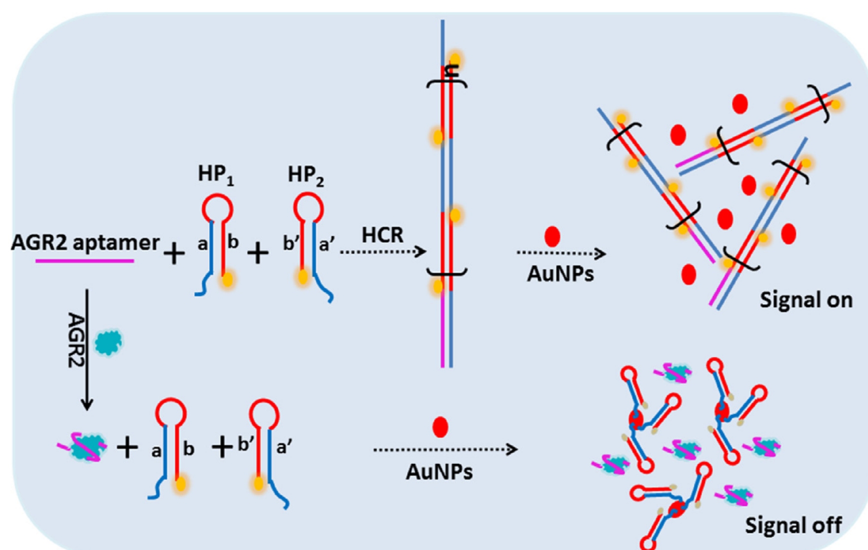

Fig. 1. The principle of AGR2 detection based on HCR amplification. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

quencher only can work well for an appropriate fluorophore [38]. By using the fluorescence quenching characteristics of AuNPs, a number of biomolecular analysis have been conducted for protein [39,40], DNA [41–43], RNA [35,44,45] and metal ions [46]. However, to the best of our knowledge, no analytical platform has yet been developed for AGR2 by using the fluorescence quenching of AuNPs coupled with HCR amplification.

Herein, by subtle combination of HCR and the fluorescence quenching of AuNPs, an ultrasensitive and enzyme-free biosensor was developed for the detection of AGR2. As shown in Fig. 1, two fluorophore labeled hairpin probes (abbreviated as HP_1 and HP_2) were firstly designed according to the literature [25]. The AGR2 aptamer (purple line) was used as an initiator for HCR event, which could hybridize with the fragment "a" (blue line) of HP_1 and opened the hairpin structure of it. The newly exposed sticky section of HP_1 (fragment "b", red color) could then hybridize with the fragment "b′" of HP_2 (red color) to open its hairpin structure and expose a sticky end (fragment "a′", blue color) for the following hybridization with the fragment "a" of the next HP_1. According to this principle, HCR happened between HP_1 and HP_2 to form a nicked DNA duplex, which could not adsorb onto the surface of AuNPs, and thus a strong fluorescence signal appeared due to the label of HP_1 and HP_2 with fluorophore. However, in the presence of AGR2, the AGR2 aptamer would specifically recognize with it and thus could not trigger the happen of HCR. Then, the sticky tails of fragment "a" in HP_1 and fragment "a′" in HP_2 would adsorb onto the surface of AuNPs, resulting in the efficient quenching of the fluorescence signal due to the close proximity of the fluorophore to AuNPs surface. Thus, the quantitative detection of AGR2 could be realized indirectly based on monitoring the decrease of the fluorescence signal.

2. Experimental section

2.1. Materials

Gold (III) chloride trihydrate ($HAuCl_4{\bullet}3H_2O$), sodium chloride (NaCl) and sodium citrate solution were purchased from Aladdin Biotech CO., Ltd (Beijing, China). AuNPs used here were synthetized according to our previous method [47], and the concentration of them calculated from the absorption spectrum by using an extinction coefficient of 2.6×10^8 M^{-1} cm^{-1} at 520 nm for approximately 13 nm particles was 10.6 nM. Anterior gradient homolog 2 (AGR2) was purchased from Shanghai Genechem Co., Ltd (Shanghai, China). Bovine serum albumin (BSA), thrombin, immunoglobulin G (IgG), trypsin, tumor necrosis factor alpha (TNF-α) and human serum were purchased from Sigma Aldrich (St. Louis, MO, USA). 20 mM of Tris-buffer (pH 7.0) was used for the dissolution of DNA strands. Ultra-pure water used in the experiments was obtained from Heal Force Smart-Nultra-pure water system. All other chemicals were analytical grade and used without further purification. All oligonucleotides were synthesized from Sangon Biotech (Shanghai, China) and the sequences of them were shown in Table 1. The DNA marker (50 bp–1500 bp) was purchased from Takara Biotech (Dalian, China) CO., LTD.

2.2. Apparatus

All fluorescent measurements were carried out on a RF-5301PC spectrofluorimeter (Shimadzu, Japan). Gel electrophoresis was performed on a GT Mini-Gel system (Bio-Rad Laboratories, Inc., Italy). The size distribution of AuNPs was obtained from transmission electron microscope (TEM, JEM-2010HR, Japan). The hydrodynamic diameter and the zeta potential of AuNPs were constructed by using particle size analyzer (MS2000, England).

2.3. Gel electrophoresis

HP_1 and HP_2 samples were heated to 95 °C for 5 min and then allowed to cool to room temperature for 1 h before using. Then, 1.0 wt% agarose gel was prepared and 4.0 μL of different DNA samples (1.0 μM) were loaded into the lanes. After that, the gel electrophoresis was performed by using 1.0 × TAE as running buffer (contained Tris base, acetic acid, and EDTA, and the pH of TAE buffer was adjusted by using NaOH) at a constant potential of 78 V for 40

Table 1
Sequences of different DNA strands.

Name	Sequence (5′–3′)
AGR2 aptamer	$CG_3TG_3AGT_2GTG_9TG_3AG_3T_2$
HP_1	$FAM\text{-}GTG_9TG_3AG_3T_2GCTAGCA_2C_3TC_3AC_9ACA_2CTC_3AC$
HP_2	$FAM\text{-}GCTAGCA_2C_3TC_3AC_9ACGTG_3AGT_2GTG_9TG_3AG_3T_2$

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