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Aptamer induced multicoloured Au NCs-MoS$_2$ “switch on” fluorescence resonance energy transfer biosensor for dual color simultaneous detection of multiple tumor markers by single wavelength excitation

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HIGHLIGHTS

- Dual colored Au NCs were used as energy donors of the FRET-based aptasensor for the first time.
- Multiple tumor markers could be simultaneously detected with a single excitation.
- Serums from normal and hepatoma patients could be visually discriminated by this FRET-based aptasensor.

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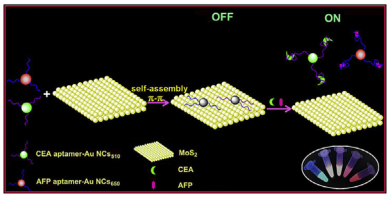
GRAPHICAL ABSTRACT



ABSTRACT

An aptamer induced “switch on” fluorescence resonance energy transfer (FRET) biosensor for the simultaneous detection of multiple tumor markers (e.g., AFP and CEA) combining molybdenum disulfide (MoS$_2$) nanosheets with multicolored Au NCs by a single excitation was developed for the first time. Here, AFP aptamer functionalized green colored Au NCs (510 nm) and CEA aptamer functionalized red colored Au NCs (650 nm) are used as energy donors, while MoS$_2$ is used as energy receptor. On the basis of recording the change of the recovered fluorescence intensity at 510 nm and 650 nm upon the addition of targets CEA and AFP, these two tumor markers can be simultaneously quantitatively detected, with detection limits of 0.16 and 0.21 ng mL^{-1} (3σ) for AFP and CEA, respectively. In addition, it is noteworthy that the developed biosensor can not only realize accurate quantitative determination of multiple tumor markers by fluorescent intensity, but also be applied in semi-quantitative determination through photo visualization. More importantly, confocal microscope experiments prove that serums from normal and hepatoma patients can also be visually and qualitatively discriminated by this FRET-based biosensor with a single excitation wavelength, indicating promising potential of this assay for clinical diagnosis.

1. Introduction

Highly sensitive and high-throughput sensing technologies for the detection of tumor markers are of great significance due to their versatile potential applications in early diagnosis and treatment for

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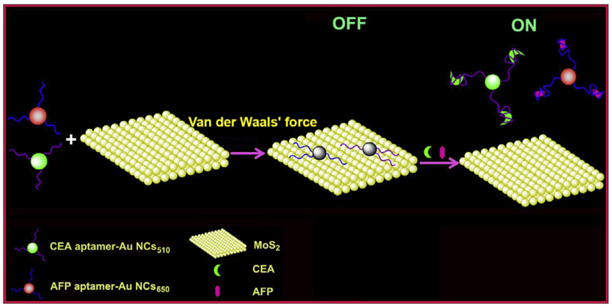
cancer [1]. Notably, many cancers are associated with multiple tumor markers, and the detection of a single tumor marker may cause false diagnosis which restricts diagnostic value in clinical analysis [2]. Simultaneous detection of multiple tumor markers, with the advantage of enhancing detection throughput, shortening analytical time and detection cost, can effectively improve the accuracy of early cancer diagnosis over the single marker assay. Currently, owing to the unique superiority of high sensitivity, convenience and low cost, a series of fluorescent techniques for multiple tumor marker analysis have been established [3–5]. In most cases, multiple lasers are needed to excite each fluorophore to collect the emissive fluorescence when multiple tumor marker are detected simultaneously. Thus, the background noise originating from multiple excitation wavelengths are difficult to be excluded, which inevitably restrict their clinical applications [6]. Therefore, simultaneous fluorescent detection of multiple tumor markers with a single excitation wavelength is highly desirable but remains a challenge.

Fluorescence resonance energy transfer (FRET), based on the nonradiative energy transfer from the fluorescent donor to the acceptor within short proximity (<10 nm), makes it possible for simultaneous detection of multiple tumor markers by recording emission intensities at different wavelengths with single excitation [7]. Until now, a series of FRET nanosensors have been developed to accomplish separate detection of diverse tumor markers with organic dyes or semiconductor quantum dots (QDs) as energy donors [8,9]. However, their practical application is limited owing to the non-negligible shortcomings, such as easy photo bleaching and poor photo stability for organic dyes as well as high toxicity for QDs. Compared with organic dyes or QDs, gold nanoclusters (Au NCs) have been widely utilized in biosensing and bio-imaging due to their excellent biocompatibility, stable fluorescent emitting and good photo stability in the past few decades [10–14]. In addition, their size-dependent emission spectra make them good candidates for simultaneous excitation at a single wavelength [15]. Therefore, it is expected that FRET biosensors for the detection of multiple tumor markers may be developed using different colored Au NCs as energy donors, and more interestingly, these multicolored Au NCs may possibly be excited by a single wavelength. In addition, owing to the strong fluorescence quenching ability and high affinity to single-stranded DNA [16], molybdenum disulfide (MoS_2) nanosheets are considered to be promising fluorescent quencher candidates for the preparation of FRET-based aptasensors platforms. In 2013, Zhang's group firstly revealed the high fluorescence quenching ability of MoS_2 nanosheet for fluorescent dye labelled ssDNA [17]. After that, MoS_2 nanosheet based FRET biosensors for tumor markers detection have been developed, including epithelial cell adhesion molecule [18], DNA [19], DNA methyltransferase (MTase) activity [20] and thrombin [21] et al. Nevertheless, although MoS_2 have great potentials in FRET-based aptasensors, their integration with multicolored Au NCs for the development of aptasensor for simultaneous detection of multiple targets has not been explored.

In this work, we report a novel aptamer induced “switch on” FRET biosensor for simultaneous detection of multiple tumor markers using dual colored Au NCs as energy donors with a single excitation wavelength. As shown in Scheme 1, simultaneous detection of multiple tumor markers (e.g., AFP and CEA) are performed in the following procedure. Firstly, AFP aptamer functionalized green colored Au NCs (510 nm) and CEA aptamer functionalized red colored Au NCs (650 nm) are simultaneously assembled onto the surface of MoS_2 nanosheets through van der Waals force. Thus, the fluorescence of the dual colored Au NCs was simultaneously quenched through static quenching due to the strong fluorescence quenching ability of MoS2 nanosheets. Secondly, upon the addition of the target tumor markers (AFP and CEA), green and red fluorescence will be simultaneously recovered along with the releasing of the two aptamer functionalized Au NCs, because of the higher affinity between aptamer and target tumor markers [22,23]. Therefore, simultaneous detection of multiple tumor markers can be achieved by monitoring the change of the recovered fluorescence intensity at 510 nm and 650 nm with a single excitation. Compared with the traditional methods for simultaneous detection, several obvious advantages of this sensor array make it particularly attractive: (1) the biggest advantage of our present method is that multiple tumor markers could be simultaneously detected by a single excitation wavelength. (2) dual colored Au NCs with lower toxicity were firstly used as energy donors instead of semiconductor quantum dots, showing better biocompatibility. More importantly, the determination of multiple tumor markers can be achieved not only by recording fluorescence recovery intensity but also by photo visualization. Furthermore, serums from normal and hepatoma patients can also be visually discriminated by this FRET-based biosensor using single excitation wavelength, showing feasible potential for diagnostic applications.



Scheme 1. Schematic illustration of the dual color FRET biosensor for simultaneous detection of multiple tumor markers.

2. Materials and methods

2.1. Materials

$HAuCl_4 \cdot 3H_2O$ (Hydrogen tetrachloroaurate trihydrate), carcinoembryonic antigen (CEA), α-fetoprotein (AFP), 11-mercaptoundecanoic acid (MUA), MoS_2 nanosheets and tetrakis (hydroxymethyl) phosphonium chloride (THPC), Glutathione (GSH), bovine serum albumin (BSA),1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and N-hydroxylsuccinimide sodium salt (NHS) were purchased from Sigma-Aldrich (USA). All solutions were prepared using ultrapure water from a Millipore Simplicity 185 water purification system (Millipore, USA). DNA aptamers wwere purchased from Shanghai Sangon Biotechnology (Shanghai, China), and their sequences are as follows: AFP aptamer, 5′-GGCAGGAAGACAAACAAGCTTGGCGGCGGGAAGGTGTTTAAATTCCCGGGTCTGCGTGGTCTGTGGTGCTGT-NH_2-3’; CEA aptamer, 5′-ATACCAGCTTATTCAATT-NH_2-3’. Serum samples were obtained from the Eighth Peoples' Hospital of Qingdao, and they were collected with informed consent from the human subject. In addition, the sample collection was approved by Institutional Review Board of Eighth Peoples' Hospital of Qingdao. All reagents were of analytical reagent grade.

2.2. Instruments

The morphologies of all samples were characterized by a JEM-2100 transmission electron microscope (TEM) with an