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Silicon nanodot-based aptasensor for fluorescence turn-on detection of mucin 1 and targeted cancer cell imaging

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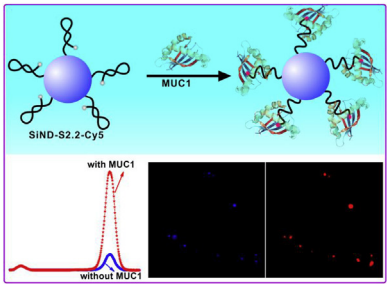
HIGHLIGHTS

- The dual-color SiND-based aptasensor was applied in homogeneous MUC1 assay.
- The SiND acted as a DNA carrier, a reference, and a fluorescence quencher.
- This biosensor exhibited high selectivity toward MUC1 against other proteins.
- This aptasensor displayed low toxicity and could be used for live cell imaging.
- This biosensor was applied to detect MUC1 in human serum samples.

GRAPHICAL ABSTRACT



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ABSTRACT

We report herein a new dual-color fluorescent aptasensor for detection of tumor marker mucin 1 (MUC1) and targeted imaging of MCF-7 cancer cells based on the specific interaction between MUC1 and its aptamer S2.2. The aptasensor was prepared by covalent attachment of the cyanine (Cy5)-tagged aptamer S2.2 to fluorescent silicon nanodot (SiND). The fluorescence of S2.2-Cy5 could be quenched by the SiND carrier in the absence of MUC1, and its fluorescence was restored in the presence of MUC1 due to structure switching of S2.2. This aptasensor exhibits specificity for MUC1-possitive MCF-7 cells rather than MUC1-negative MCF-10A cells and Vero cells. The SiND plays multiple roles in this fluorescence assay, making the method easier compared with other approaches. The limit of detection and precision of this method for MUC1 was 1.52 nM and 3.6% (10 nM, n = 7), respectively. The linear range was 3.33 −250 nM, and the recoveries in spiked human serum were in the range of 87−108%. This is a simple, selective, sensitive and reliable method, which can well achieve not only quantitative analysis of tumor marker but also dual-color visualization of single cancer cells.

1. Introduction

Cancer attracts the worldwide attention with a marked increase in incidence and mortality. Based on the global cancer statistics estimates, the number of new cancer cases and cancer deaths occurred in 2012 are about 14.1 million and 8.2 million, respectively

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[1]. The metastasis of cancer cells is responsible for most cancer deaths [2]. Alterations in many molecular expression in cancer cells accompany the metastatic process [3]. Moreover, the levels of tumor markers in serum are related to the stages of cancer [4]. Therefore, the development of novel approaches for accurate and sensitive detection of tumor markers holds enormous potential for early diagnosis and therapy of cancer [5].

Currently, the analytical techniques for detection of tumor markers include fluorescence spectroscopy [5–7], UV–vis spectroscopy [8], electrochemical method [9–11], and surface-enhanced Raman scattering (SERS) [12]. Fluorescence spectroscopy has been used extensively for analytical measurements due to the features of easy-to-operate, rapid analysis capability, cost efficiency, high sensitivity, and direct study of molecular processes without prior separation [13]. The reported nanomaterials for fluorescence detection of tumor markers include oxidized mesoporous carbon nanospheres (OMCN) [5], CdSe/ZnS quantum dots (QDs) [6], graphene oxide (GO) [7], which are all based on ligand-receptor interactions. The tumor-targeting ligands (e.g., aptamers, antibodies, and peptides) can recognize cancer specific antigens [14]. Aptamers are short single-stranded RNA or DNA molecules, which are generally selected through an in vitro process, known as systematic evolution of ligands by exponential enrichment (SELEX) [15,16]. They offer several advantages over other targeting ligands in bioapplications, such as smaller size, simpler structures, more stability in harsh biological conditions, and relatively high affinity and specificity to their receptors [17]. The aptamer based biosensors (aptasensors) have been developed for detection, imaging, and therapy of cancer [18]. In our previous work [19], an aptamer-functionalized CdTe:Zn^{2+} QDs was designed for targeted cancer imaging. Some details could be obtained from the visualized images, for example, the location of QDs in cells. However, quantitative bioanalysis was not performed. It can be expected that the combination of fluorescence spectroscopy-based quantitative analysis with fluorescence imaging will offer more information [20]. Recently, Li et al. reported an OMCN featured fluorescent aptasensor for quantitative detection of tumor marker and cancer-targeted imaging, which displayed robust potential for application in cancer diagnosis and therapy [5]. In order to achieve highly precise analysis, it is desirable to develop a self-referencing aptasensor integrating reference probe and signal probe into one structure, which can reduce the influences of analyte-independent factors and facilitate multicolor imaging of single cancer cells [10,21–23].

Mucin 1 (MUC1), a large cell-surface-associated glycoprotein, is applied as a diagnostic marker in cancer, and is expected to be used as a therapeutic target for cancer [24–26]. This transmembrane mucin contains an extracellular domain comprising of a region of nearly identical repeats of 20 amino acids per repeat, a hydrophobic membrane-spanning domain of 31 amino acids, and a cytoplasmic domain of 69 amino acids [6,27]. The overexpression of MUC1 is associated with several various human epithelial adenocarcinomas [28–30], and it is the most common tumor marker of breast cancer [4,31]. The aptamer S2.2, a targeting ligand for MUC1 antigen, includes three base pairs, three consecutive mispairs and a four base-pair stem capped by three thymine residues [32–34]. It was selected by Ferreira et al. using the SELEX methodology, and they also applied fluorescently tagged S2.2 to image breast cancer MCF-7 cells [32]. Rhinehardt et al. found that the binding of MUC1-G peptide (a 9 amino acids immunodominant epitope of the 20 amino acids variable tandem repeat) with the thymine loop of S2.2 induced structural changes in the aptamer [35]. Several methods for detection of MUC1 have been reported based on the binding event that breaks the hairpin structure of S2.2. Cheng et al. constructed a S2.2-related CdSe/ZnS QDs and used it for fluorescence "turn-off" detection of MUC1 [6]. Ma et al. immobilized methylene blue-tagged S2.2 on gold for electrochemical "turn-off" sensing of MUC1 [11]. These previous findings inspire us to design the cyanine (Cy5)-tagged S2.2-functionalized silicon nanodot (SiND-S2.2-Cy5) aptasensor for specifically targeting MUC1.

Silicon nanocrystals, an emerging new class of QDs, have been widely used in fluorescence detection and imaging [36–38]. The blue-emitting SiNDs with a diameter of 2.5 nm synthesized in our laboratory also have been applied in fluorescence imaging at the cellular level [21,39]. Fascinatingly, the SiNDs can quench the fluorescence of Cy5 in Cy5-tagged S2.2 effectively, and the fluorescence of Cy5 can be restored in the presence of MUC1. Herein, a SiND-S2.2-Cy5 aptasensor has been constructed via a facile covalent attachment with the bifunctional crosslinker (Sulfo-SMCC) for "turn-on" sensing of MUC1 because of structure switching (Fig. 1). Briefly, the amino groups on the surface of SiNDs can react with the succinimide of Sulfo-SMCC to produce SiND-Maleimide. Then, SiND-Maleimide reacts with Cy5-S2.2-SH through Michael reaction to obtain the SiND-S2.2-Cy5. Compared with the aptasensors reported in the literature [5–7,11,12], this metal-free fluorescent aptasensor integrates multiple advantages. First of all, the aptasensor is prepared by the covalent coupling method using the self-fluorescent nanomaterial as the carrier and fluorescence quencher, facilitating its application in a complex matrix and decreasing the likelihood of a false positive signal in a cellular environment. Secondly, the "turn-on" sensor is more commendable than the "turn-

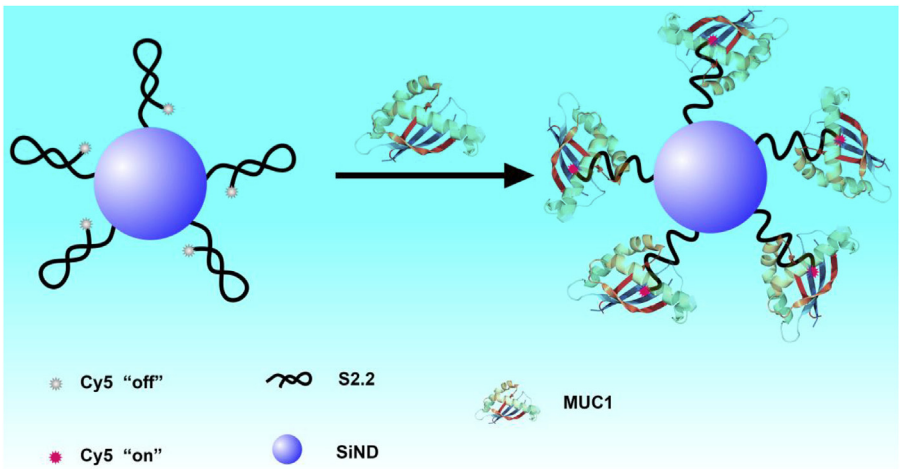

Fig. 1. Schematic presentation of the SiND-S2.2-Cy5 aptasensor for detection of MUC1.

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