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Multilayer hemin/G-quadruplex wrapped gold nanoparticles as tag for ultrasensitive multiplex immunoassay by chemiluminescence imaging

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ABSTRACT

A multilayer hemin/G-quadruplex DNAzyme wrapped gold nanoparticle (M-DNAzyme/AuNP) tag was designed for ultrasensitive chemiluminescence (CL) imaging. By combining with a disposable protein array, an ultrasensitive and high-throughput multiplex CL immunoassay method was proposed for simultaneous detection of four cancer biomarkers. The M-DNAzyme/AuNP tag was prepared by assembling high ratio of alkylthiol-capped signal DNA containing multiple G-quadruplex sequences to biotinylated DNA on AuNPs and then reacting with hemin to form multilayer hemin/G-quadruplex DNAzyme units. It could be bound to the biotinylated secondary antibody of sandwich immunocomplex by biotin–streptavidin conjugation to catalyze a CL reaction on a protein array, which produced strong CL emission. Under optimal conditions, the CL signals could be simultaneously collected by a charge-coupled device for ultrasensitive multiplex CL imaging of cancer biomarkers. Using α-fetoprotein, human chorionic gonadotrophin-β, carcinoma antigen 125, and carcinoembryonic antigen as model analytes, the proposed immunoassay method showed high sensitivities and wide linear ranges in a simple, cheap and high throughput way. The M-DNAzyme/AuNP as a universal signal tag as well as the protein chip could be suitable for mass production for economical, portable and multianalyte assay, showing a promising potential in application to clinic and other relative fields.

1. Introduction

Accurate and high throughput detection of biomarkers in serum or tissue samples plays an essential role in early screening and diagnosis of diseases (Stoeva et al., 2006; Yu et al., 2006; Wu et al., 2007a, 2007b). Tumor markers exist in blood at trace levels in the absence of a tumor and their levels rise upon the formation of tumor. The extremely low concentrations of most biomarkers during the early stage of diseases and the limited specificity of single marker in cancer diagnosis (Wu et al., 2007a, 2007b) lead to urgent need of the ultrasensitive multiplex immunoassay methods for simultaneously detecting a panel of tumor markers with easy operation, low cost, and small sample consumption.

Various protein arrays or immunosensor arrays have been designed for electrochemical (Kojima et al., 2003; Wilson and Nie, 2006a; Wu et al., 2007a, 2007b) and optical (Knecht et al., 2004; Deiss et al., 2009; Hu et al., 2010; Kwon et al., 2011) readout of multiplex analytes. Chemiluminescence (CL) immunosensor array benefiting from the advantages of simple sensor set-up and without the need of external light source or optics is one of the most developed technologies. In order to improve the sensitivity of CL immunosensing, numerous signal amplification strategies based on various bionanocomposite probes have been designed by loading a large amount of natural enzymes such as horseradish peroxidase (HRP) on nano-carriers (Bi et al., 2009a, 2009b; Zhen et al., 2010). However, the limited species and amount of natural enzymes assembled on the nano-carriers have become a bottleneck. Thus low molecular weight DNAzyme formed by binding a G-quadruplex DNA strand with a hemin molecule has been attempted to be used as a substitute in CL analysis (Niazov et al., 2004; Willner et al., 2008; Wang et al., 2011). As a promising HRP-mimicking enzyme, DNAzyme can catalyze the oxidation of luminol by H_2O_2 to generate CL emission (Xiao et al., 2004). Comparing with the natural enzymes, DNAzyme can be easily produced and possesses better stability and robustness. To further enhance the sensitivity, this work assembled a multilayer structure containing multiple DNAzyme units on the nano-carriers to achieve the signal amplification of CL transduction.

The multilayer DNAzyme structure can generally be prepared using enzyme-assisted DNA replication strategies, such as rolling

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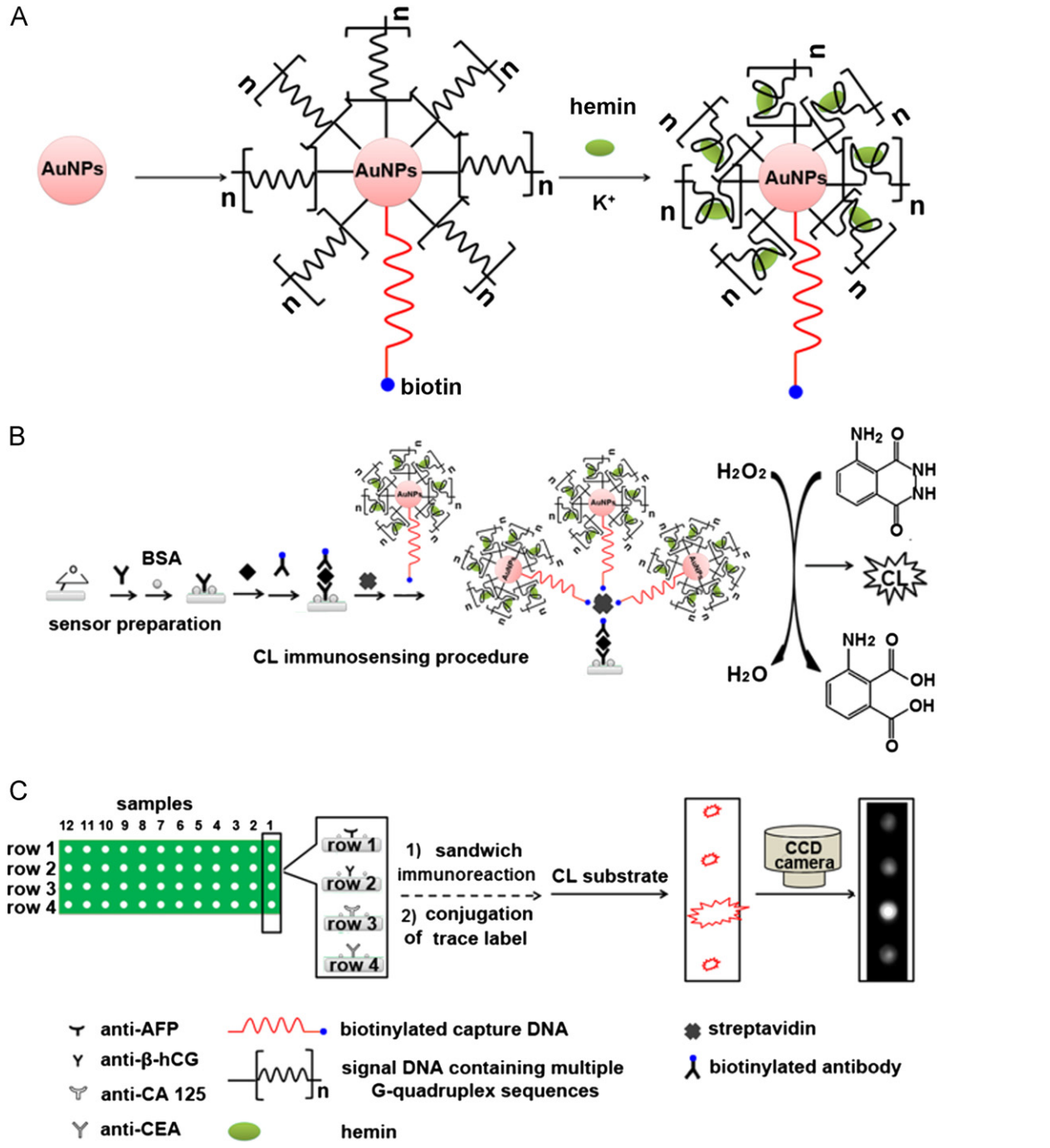

Scheme 1. Schematic diagrams of (A) preparation of M-DNAzyme/AuNP with the optimal *n* value of 3, (B) sensor preparation and CL immunosensing procedure, and (C) multiplex CL imaging immunoassay of four tumor markers using an immunosensor array.

circle amplification (Cheglakov et al., 2007; Bi et al., 2010), hybridization chain reaction (Shimron et al., 2012) and polymerase chain reaction (Cheglakov et al., 2006). These processes which demand the assistance of primers, polymerase or nicking enzymes are high-cost and complex. Herein, the multilayer structure was prepared by directly assembling high ratio of multiple G-quadruplex sequences to biotinylated DNA on gold nanoparticles (AuNPs), followed by the binding of hemin to the sequences to form multilayer DNAzyme units (Scheme 1A). AuNP can enhance the CL emission of luminol–H_2O_2 system (Zhang et al., 2005) and is one of the most used nano-carriers in CL bioassay (Niazov et al., 2004; Bi et al., 2009a, 2009b). The assembly and formation processes of multilayer DNAzyme wrapped AuNPs (M-DNAzyme/AuNP) were very simple, and the probe produced a limit of detection down to fM level, which was much lower than the previously reported multilayer DNAzyme strands for CL detection of protein (6.6 pM) (Bi et al., 2010). The presence of biotinylated DNA enabled the multilayer units to be a universal signal tag for CL immunoassay by the specific recognition of biotin to avidin.

By combining the universal tag with a disposable protein immunosensing array and a sensitive charge-coupled device (CCD) detector, an ultrasensitive and high-throughput CL imaging method was proposed for simultaneous immunoassay of multiple biomarkers. Compared to the limit of detection of 4.1 pg mL^{-1} for CL immunoassay of carcinoembryonic antigen (CEA) using