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In situ formed copper nanoparticles templated by TdT-mediated DNA for enhanced SPR sensor-based DNA assay

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ABSTRACT

For the efficient surface plasmon resonance (SPR)-based DNA assay researching, signal amplification tactics were absolutely necessary. In this work, a sensitive SPR-DNA sensor was developed by employing in situ synthesis of copper nanoparticles (CuNPs) templated by poly-T sequences DNA from terminal deoxynucleotidyl transferase (TdT)-mediated extension, and synergistically with nano-effect deposition as the mass relay. The objective of this strategy was manifold: firstly, *t*DNA hybridized with the optimal designed probes to active the TdT-mediated DNA extension onto the surface of SPR chip, resulted a long poly-T sequences ssDNA chain in *ds*DNA terminal onto surface of gold chip and characterized by SPR signal amplitudes. Secondly, copper ion (Cu^{2+}) adsorbed into the skeleton of poly-T sequences DNA, with the aid of ascorbic acid (VC) to achieve the Cu^{2+} reduction, copper nanostructures (CuNPs) was synchronously generated onto the single nucleotide chain anchoring in *ds*DNA derivatives and the formation was featured by transmission electron micrographs (TEM) and electrochemistry. Lastly, *ds*DNA-complexed CuNPs (CuNPs@*ds*DNA) triggered the final signal amplification via real-time conversion of the additive catechol violet (CV) into oligomer or chelation precipitation by CuNPs-tagged reporters. With the proposed setups, a precise and replicable DNA sensing platform for specific target oligo was obtained with a detection limit down to 3.21 femtomolar, demonstrating a beneficial overlapping exploitation of nanomaterials and biochemical reaction as unique SPR infrastructure. Such triple-amplification strategic setups, the possibility of various methods abutment and biocompatibility weight reactor was amassed and adapted to more biological detection field.

1. Introduction

The extensive interest for applications of ultrasensitive DNA biosensors has gained in different areas, among those, DNA biosensor based on surface plasmon resonance (SPR) was of great deal for the study of biomolecular interactions and transduction of biological recognition events (Cibulskis et al., 2013; Fang et al., 2015; Stern et al., 2014). As known, the sensitivity of this sensor strongly depended on the thickness and dielectric constant (Wijaya et al., 2011; Guo, 2012), and substantial efforts had been exploited to manipulate it (Shewell et al., 2014; Wang et al., 2010). For those methods either focused on employing external labels with high refractive index such as nanoparticles including AuNPs (Ding et al., 2014; Krishnan et al., 2011), SiO_2 NPs (Cabanas-Danés et al., 2014) and Fe_3O_4 NPs (Durand et al., 2014), or on internal labels with optical dielectric property medium to enhance the mass of surface-immobilized ligands, for example, hybridization chain reaction (Yuan et al., 2015) (HCR), polymerase chain reaction (Abadian et al., 2014) (PCR), rolling circle amplification (Yan et al., 2016) (RCA) and polymerization (Wassel et al., 2016). Unfortunately, each of those techniques needed incompatible special architectural design, instrument and equipment, and relative isolation method devising, which was insufficient for the responding sensitization of bioanalyses and restrained the extensive application (Cai et al., 2015). So, it is fatal important to integrate various signal enlargement reaction for the real time and label-free detection of interactions amongst nucleic acids, with low sample consumption.

For those amplification projects, DNA with the rigidity structure and flexible sequence designing, plays a key role for serial potential methods and has accomplished a seamless integration of versatile interactions across different species (Chen et al., 2015; Ma et al., 2013; Jiang et al., 2013). By virtue of unique structure, molecule recognition properties, and high affinity for some metal ions, DNA could be as template for metallic nanostructures through the binding of metal ions

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on the DNA and subsequent chemical reduction of the DNA complexed metal ions (Becerril et al., 2009; Houlton et al., 2009). Especially, single-stranded DNA (*ss*DNA), maintaining its linear state without hybridization, was an excellent programmable building block for the integrated DNA-templated electronics (Qing et al., 2014). More important, the sequences and length of *ss*DNA construct could be in line handled by terminal deoxynucleotidyl transferase (TdT)-mediated extension (Spiga et al., 2014; Gao et al., 2013). As an unique DNA polymerase, TdT catalyzes the sequential addition of dNTPs at the 3′-OH group of an oligonucleotide primer to produce special sequence like poly-T ssDNA (Chen et al., 2014), which is the excellent programmable building block for the exact spatial positioning of metal irons, such as DNA-templated nanostructures of silver (Geng et al., 2011), palladium (Seidel et al., 2004), platinum (Monson et al., 2003), copper (Schreiber et al., 2011), and gold (Rotaru et al., 2010). Among them, *ds*DNA-supported copper nanoparticles (CuNPs@*ds*DNA) was possible to generate huge mass factor and broad application prospects, for Cu^{2+}-catalyzed activity on the basis of phenols click reaction (Ye et al., 2014), Undoubtedly, through site-specific hybridization of immobilized probe DNA with signal DNA to form *ds*DNA triggering TdT-based extension, nanoparticles technique for site-specific synthesis of CuNPs on a triangular DNA origami scaffold was achieved, which provide efficient nano-catalytican reports and classical paradigm of “up-middle-down” cascading amplification.

Catechol violet (CV) was able to converge into oligomer or chelated with metal ion, has been extensively studied in many fields of biosensors (Sun et al., 2013; Ata et al., 2014). To simplify the assay process with the purpose of manufacturing portable and affordable devices, CV in line coalescented with CuNPs@*ds*DNA or formed into oligomer, causing an notable mass augment and as an effective candidate to boost SPR response (Deng et al., 2015). As a result, a novel SPR-DNA sensor was developed by employing in situ the synthesis of CuNPs templated by poly-T sequences *ds*DNA and synergistically with nano-effect deposition (Scheme 1). Among this, the *ds*DNA platforms was formed by terminal deoxynucleotidyl transferase (TdT)-mediated extension. The process of this strategy was showed: at beginning, as bridge molecule *t*DNA initiated the TdT-mediated DNA extension to fabricate long poly-T sequences ssDNA chain in *ds*DNA terminal onto surface of gold chip and a huge SPR response, from the hybridization with the optimized probe designs. Then, CuNPs synchronous generated onto the single nucleotide chain anchoring in *ds*DNA derivatives, resulting an evidently SPR signal enlargement. At last, CuNPs@*ds*DNA triggered the final signal amplification via real-time conversion of the additive catechol violet (CV) and H_2O_2 precipitation by CuNPs-tagged reporters. Thus, this extraordinary constructs exemplify a freshly train of thought about mass-sensing modes, which reveal great promise in bioanalytical application.

2. Experimental section

2.1. Materials and reagents

3-Mercaptopropionic acid (MPA), *N*-hydroxysuccinamide (NHS),1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), ethanolamine, copper sulfate ($CuSO_4$), sodium ascorbate (VC), 3-(N-morpholino) propanesulfonic acid (MOPS), sodium chloride (NaCl), terminal deoxynucleotidyl transferase (TdT), TdT buffer (5X, 1 mol/L potassium cacodylate, 5 mmol/L $CoCl_2$, 0.05% (v/v) Triton X-100 and 0.125 mol/L Tris, pH 7.2), potassium chloride (KCl), tri(2-carboxyethyl) phosphine hydrochloride (TCEP), catechol violet (CV) and hydrogen peroxide (H_2O_2) were procured from Sigma-Aldrich Chemical Co., Ltd. All oligonucleotides used in this work were synthesized by Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai China), and purified by high-performance liquid chromatography. The stock solutions of DNA were obtained by dissolving DNA in sterile deionized water. The Cu nanoparticles reaction buffer was contained by 10 mmol/L MOPS, 150 mmol/L NaCl, pH 7.6. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was implemented throughout all assays. 0.1 mol/L pH 7.0 of phosphate buffer solutions (PBS) was prepared by mixing the stock solutions of 0.1 mol/L KH_2PO_4 and 0.1 mol/L K_2HPO_4 with 0.25 mol/L NaCl as the supporting electrolyte for DNA dilution and hybridization, respectively. Also, 0.1 mol/L pH 3.0 of phosphate buffer solutions (PBS) was compounded without 0.25 mol/L NaCl in the same method to realize the CV with H_2O_2 precipitation. All electrochemical measurements were operated in 0.5 mol/L KCl solution, preserved its N_2-saturated pressure. To facilitate better understanding this paper, an abbreviation list was needed and showed in Table 1.

All oligonucleotides sequences of assay setup were listed as follows:

Capture DNA-1 (*c*DNA-1): 5′–TAC CCT TCT CAC TGT CAG GAA CGG TGA AAG ACC AAC CA AAA AAA–NH_2–3′.

Capture DNA-2 (*c*DNA-2): 5′–TAC CCT TCT CAC TGT CAG GAA CGG TGA AAG ACC AAC CA–NH_2–3′.

Capture DNA-3 (*c*DNA-3): 5′–TAC CCT TCT CAC TGT CAG GAA CGG TGA AAG ACC AAC CA–SH–3′.

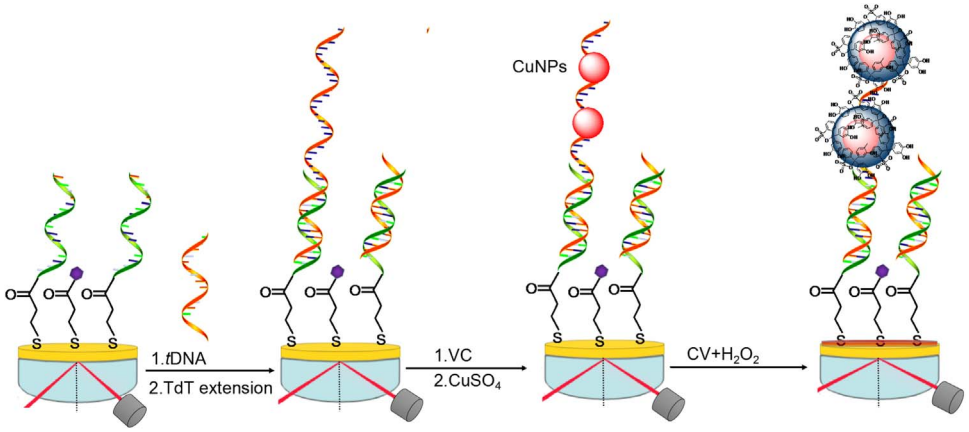
Capture DNA-4 (*c*DNA-4): 5′–TAC CCT TCT CAC TGT CAG GAA CGG TGA AAG ACC AAC CA AAA AAA–SH–3′.

Target DNA (*t*DNA): 5′–TGG TTG GTC TTT CAC CGT TCC TGA CAG TGA GAA GGG TA–3′.

One-base mismatch (T1): 5′–TGG T**C**G GTC TTT CAC CGT TCC TGA CAG TGA GAA GGG TA–3′.

Two-base mismatch (T2): 5′–TGG T**C**G GTC T**G**T CAC CGT TCC TGA CAG TGA GAA GGG TA–3′.

Three-base mismatch (T3): 5′–TGG T**C**G GTC T**G**T CAC CGT TCC TGA CAG TGA GAA **A**GG TA–3′.



Scheme 1. Schematic illustration of the synthesis CuNPs templated by TdT-mediated DNA for SPR-DNA assay.