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Mass effect of redox reactions: A novel mode for surface plasmon resonance-based bioanalysis

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ABSTRACT

The pursuit of more specific and sensitive response is a perpetual goal for modern bioassays. This work proposed a novel label-free strategy about redox-related mass effect based on the surface plasmon resonance (SPR) technique for ultrasensitive determination of DNA. The protocol starts with the modification of SPR gilded disk with the capture DNA (*c*DNA). After the conjugation of immobilized *c*DNA with the target DNA (*t*DNA), the hybridization chain reaction was triggered by the introduction of mutual partial complementary primers to elongate the terminal into a nanoscale duplex. As it is reported that porphyrin could intercalate into the grooves of the double-stranded DNA (*ds*DNA) scaffold, multiple positive-charged Fe^{III} *meso-tetra*(*N*-methyl-4-pyridyl) porphine (FeTMPyP) with symmetric structure were uptaken for in situ formation of porphyrin-*ds*DNA complex. Given FeTMPyP a highly efficient catalysis for the peroxide reduction, its presence as a biomimetic cofactor was validated via circular dichroism and UV–vis spectroscopy, demonstrating a tight binding as well as high catalytic activity and stability. Using 4-chloro-1-naphthol as a proton donor, the catalytic reduction of H_2O_2 would oxidize it into insoluble benzo-4-chloro-hexadienone, which simultaneously deposited on the heterogeneous interface, leading to a significant amplification in both SPR response and topological height profile. The signal increment was proportional to the concentration of *t*DNA, thus an ultrasensitive SPR-based DNA assay was developed with a linear range over four orders of magnitudes and a *sub*-femtomolar detection limit of 0.73 fM. The developed methodology exemplifies a different way of thinking about mass-sensing modes, extending conventional SPR-based DNA analysis to relevant biomedical applications.

1. Introduction

Surface plasmon resonance (SPR) has become a leading technique for in situ bioaffinity assay of diverse targets (Stern et al., 2014; Tang et al., 2014). A key edge of SPR lays at its capability for on-line measurement of biomolecules without the prior fluorescent or enzymatic labeling (Fang et al., 2015; Singh et al., 2015). However, the detection limit of such label-free strategy normally remains at nanomolar level, while nonspecific adsorption in the physiological media further restrained the biomedical applications of conventional SPR. To improve the sensitivity, innovative paradigms have been devised, including resonance energy transfer between surface plasmon of gold nanoparticles and biomolecule-gated fluorescent probe (Stobiecka and Chałupa, 2015; Stobiecka, 2014); and modulated plasmonic fields via fine-tuning the dielectric function of sensory interface that behaved specifically as variation of SPR reflectance, fluorescence quenching in gated resonance energy transfer, and resonance elastic light scattering (Stobiecka and Hepel, 2011). Meanwhile, nanomaterials like silica (Cabanas-Danés et al., 2014) and iron oxide (Durand et al., 2014; Shewell et al., 2014) particle-based tracing tags were also employed for the signal amplification, which could give rise to great mass accumulation and consequent changes in the refractive index near the bioconjugated interface. Remarkably, an in vitro determination of prostate specific antigen down to 300 aM was achieved by the probe of superparamagnetic microbeads (Yao et al., 2013). Nevertheless, so far only special microscopic shapes consisting of certain elements are qualified for the responding sensitization of SPR-based bioanalyses (Pelossof et al., 2012); more than that, it is apparent to be a standard protocol for surface biofunctionalization of applied materials. Both take negative impact on the flexibility and the cost-effectiveness of SPR platform.

The development of DNA-centered biotechnologies has accomplished a seamless integration of versatile interactions across different species (Chen et al., 2015a; Ma et al., 2013; Jiang et al.,

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2013). By programming and manipulating the nucleotide sequences, structural complex could be fabricated spontaneously and templated specifically for signal transduction (Prieto-Simó and Samitie, 2014; Sugiyama et al., 2014). One unique polymeric architecture comes out of the hybridization chain reaction (HCR), a facile and efficient adjustment of the length of double-stranded DNA (*ds*DNA) upon an initiator. The periodical long duplex could incorporate abundant intercalators such as methylene blue (Huang et al., 2015), pyrene (Häner et al., 2010; Mayer-Enthart et al., 2010), and hemin (Zang et al., 2014) into a super-sandwich framework and worked altogether as a reporter unit in biosensors without deliberate labeling. Especially, when adapted in SPR analysis, the elongated backbones shall alternate the permittivity next to the substrate (Zagorodko et al., 2014). Furthermore, to trigger a synergistic weight increment along with the terminal extension of *ds*DNA, one may dynamically introduce insolubles from some groove-bound catalytic reactions following a complete biorecognition event. A large volume of precipitation then gradually overweighs the previous HCR scaffolds. An optional catalyst here is considered to be iron(III) *meso*-tetra(*N*-methyl-4-pyridyl) porphine or simply, FeTMPyP.

As a kind of metalloporphyrins, FeTMPyP plays a prominent role in the peroxidase-like catalysis (He et al., 2012), whose activity prevails over other iron, manganese and cobalt derivatives and even competes against those of natural enzymes (Tang et al., 2013; Xue et al., 2012). Unfortunately, covalent bonding with FeTMPyP to the antibody would reduce to terribly slow catalytic rate due to its easy dimerization (Houseman et al., 2002). By making full use of highly symmetric stereochemistry, *ds*DNA has been discovered accommodating individual FeTMPyP automatically and tightly within its intrinsic groove regardless of sequence (Li et al., 2014), while it happens to be no interaction between porphyrin and the single-stranded DNA (*ss*DNA) (Hou et al., 2014; Jang et al., 2014). On one hand, the catalytic efficiency of FeTMPyP as a mimicking cofactor could be preserved at its best. On the other hand, the amount of captured FeTMPyP is able to increase in cascade by the implementation of HCR. In this work, an HCR-assisted biomimetic catalysis-induced precipitation was tailored to SPR-based DNA assay (Scheme 1), in which a water-soluble proton donor named 4-chloro-1-naphthol (ClN) was selected as the substrate. Multiple FeTMPyP–*ds*DNA composite could oxidize ClN rapidly into its sparingly soluble benzo-4-chlorohexadienone (BCl) counterpart in the presence of H_2O_2. Based on the mass effect of product accumulation at the sensing interface, a novel method of unlabeled redox reactions was proposed here for the promotion of SPR output, resulting in an ultrasensitive determination of functional DNA with high specificity. This construct thus exemplify a different way of thinking about mass-sensing modes, showing great promise in bioanalytical applications.

2. Experimental section

2.1. Materials

4-Chloro-1-naphthol (ClN), *N*-hydroxysuccinamide (NHS), mercaptopropionic acid (MPA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and ethanolamine were purchased from Sigma-Aldrich (Shanghai, China). Iron(III) *meso*-tetrakis(4-*N*-methylpyridyl)porphyrin (FeTMPyP) was ordered from J&K Scientific Inc. (Shanghai, China). Ethidium bromide stock solution, bromophenol blue, tris(hydroxymethyl)aminomethane/borate/ethylenediamine tetraacetic acid (EDTA) or TBE buffer (5 ×), and Agarose H were procured from Sangon BioTech Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and used as received. Phosphate-buffered saline (PBS, pH 7.4, containing 0.25 M NaCl and 1 M K_2HPO_4/KH_2PO_4) was prepared to enhance the DNA-hybridization yield, which was also employed as the running buffer in instruments. Ultrapure water obtained from a Millipore water purification system ($\geq$ 18 MΩ, Milli-Q, Millipore) was implemented throughout all assays. Human serum samples were kindly provided by Nanjing Medical University Cancer Hospital and Jiangsu Cancer Hospital (Nanjing, China), which was diluted with the above hybridization buffer [1:1 (v/v)] before the recovery testing. All oligonucleotides were customized at Sangon BioTech Co., Ltd. (Shanghai, China) and purified via high-performance liquid chromatography, the sequences of which were listed as follows:

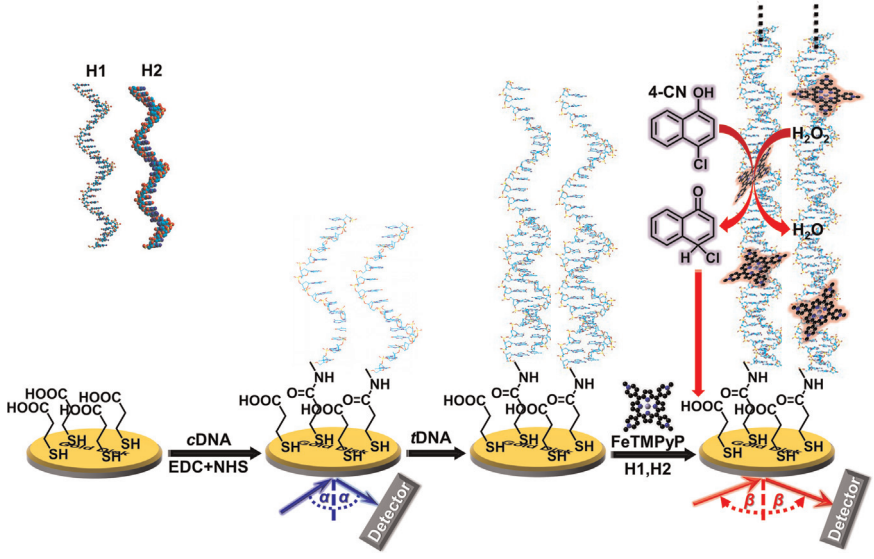
Capture probe (*c*DNA): 5′-NH_2-TATTAACTTTACTCC-3′

Target DNA (*t*DNA): 5′-TCAGCGGGGAGGAAGGGAGTAAAGTTAATA-3′

Hairpin probe 1 (H1): 5′-CTTCCT***CCCCGCTGA***CAAAGT***TCAGCGGGG***-3′

Hairpin probe 2 (H2): 5′-***TCAGCGGGG***AGGAAG***CCCCGCTGA***ACTTTG-3′

1-Base mismatch: 5′-TCAGCGGGGAGGAAGGGAGTAAA**A**TTAATA-3′



Scheme 1. Schematic illustration of the SPR-based assay protocol.