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Enzyme-guided plasmonic biosensor based on dual-functional nanohybrid for sensitive detection of thrombin

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ABSTRACT

Rapid and sensitive methodologies for the detection of protein are in urgent requirement for clinic diagnostics. Localized surface plasmon resonance (LSPR) of metal nanostructures has the potential to circumvent this problem due to its sensitive optical properties and strong electromagnetic near-field enhancements. In this work, an enzyme mediated plasmonic biosensor on the basis of a dual-functional nanohybrid was developed for the detection of thrombin. By utilizing LSPR-responsive nanohybrid and anaptamer-enzyme conjugated reporting probe, the sensing platform brings enhanced signal, stability as well as simplicity. Enzymatic reaction catalyzed the reduction of Au^{3+} to Au° *in situ*, further leading to the rapid crystal growth of gold nanoparticles (AuNPs). The LSPR absorbance band and color changed company with the nanoparticle generation, which can be real-time monitoring by UV–visible spectrophotometer and naked eye. Nanohybrid constructed by gold and magnetic nanoparticles acts as a dual functional plasmonic unit, which not only plays the role of signal production, but also endows the sensor with the function of magnetic separation. Simultaneously, the introduction of enzyme effectively regulates the programming crystal growth of AuNPs. In addition, enzyme also serves as signal amplifier owing to its high catalysis efficiency. The response of the plasmonic sensor varies linearly with the logarithmic thrombin concentration up to 10 nM with a limit of detection of 200 pM. The as-proposed strategy shows good analytical performance for thrombin determination. This simple, disposable method is promising in developing universal platforms for protein monitoring, drug discovery and point-of-care diagnostics.

1. Introduction

Development of sensitive and rapid methods for detection of proteins is in urgent requirement for medical diagnostics and therapeutics. (Jans and Huo, 2012; Song et al., 2010; Whitcombe et al., 2011; Deng et al., 2014; Wang et al., 2014) Various strategies, including electrochemical analyses, (Nie et al., 2009) enzyme-linked immunosorbent assay (ELISA), (Fredriksson et al., 2002) PAGE, (Vallejo-Illarramendi et al., 2013) have been demonstrated for detecting different kinds of proteins. Although these well established methods have been broadly used, they are time-consuming, complex, and usually rely on expensive and sophisticated instrument. Therefore, it is still of great challenge to develop protein sensors for rapid and simple point-of-care (POC) diagnostics that can be used by untrained personnel. (Liu et al., 2013; Luppa et al., 2011)

Localized surface plasmonresonance (LSPR) has emerged as an increasingly popular tool for protein analysis with high sensitivity due to its optical properties and strong electromagnetic near-field enhancements.(Haes et al., 2004; McFarland and Van Duyne, 2003; Zhao et al., 2009) LSPR of gold nanoparticle comes from light excited collective surface plasmon oscillations on the surface of metallic nanoparticles. It demonstrates the characteristics of sharp absorption peak in the visible wavelengths range at the macro level. The LSPR properties greatly affect by size and interparticle distance of the metal nanoparticles, which is reflected in color change and absorption peak shift. (Mayer and Hafner, 2011) Pioneer works have shown its advantages in constructing diverse biosensor for disease diagnostics. (Sepúlveda et al., 2009) However, these assays are usually lack of efficient separation method, which is a big limitation for practical POC diagnostic in homogeneous solution. Fe_3O_4 magnetic nanoparticle has been widely applied in isolation area owing to its excellent superaramagnatic ability. Additionally, high surface area and good biocompability make it feasible to hybrid with other nanomaterials and biomolecules. (Boterashvili et al., 2012; Chemla et al., 2000; Goon et al., 2009; Jiang et al., 2008; Perez et al., 2003; Rogstad et al., 2013; Tong et al., 2012) Nanocomposites made of gold and Fe_3O_4

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nanoparticlenot only provide aneffective solution to the sophisticated operation of traditional plasmonic biosensors, but also extend its application in point-of-care diagnostics.

As we all know, enzyme acts as an efficient catalyst to tailor the shape and size of nanostructure. It guides programming crystal growth of AuNPs and tunes its chemicophysieal property consequently. (de La Rica and Stevens, 2012; Liz-Marzan, 2006; Rodríguez-Lorenzo et al., 2012; Willner et al., 2006) Thus, enzyme-AuNPs conjugates can provide biocatalytic systems that serve as amplifying units in practical applications. Pioneer works which take advantage of these properties for biosynthsis have been well established. (Ahmad et al., 2002; Rangnekar et al., 2007) Combining the high efficient catalysis property of enzyme with the sensitive LSPR-response character of AuNPs will offer a sensitive sensing strategy. However, the development of biodetection derived from this concept is still on the early stage.

Here we constructed a dual functional AuNPs/Fe_3O_4 nanohybrid, upon which a novelplasmonic sensor for protein detection was built for the first time. On this platform, the signal probe modified with glucose oxidase can *in situ* generate H_2O_2 to initiate the crystal growth and produce a colorimetric readout signal. Aptameris chosen as the recognition element owing to its high binding affinity and chemical stability compared with its antibody counterparts. (Farokhzad et al., 2006; Huizenga and Szostak, 1995; Macaya et al., 1993; Pavlov et al., 2004) Aptamer I was used to capture target analyte specifically, which also allows easy removal of excess protein and other unbound objects with magnetic separation. Aptamer II, which is connected to glucose oxidase (Aptamer II/GOx), binds to the captured protein. Subsequently, nanoparticle growth reaction is performed where the product of H_2O_2 can reduce the Au^{3+} ions in the solution to Au°. The depositon of Au° on the AuNPs surface can enlarge the size of the nanoparticle, which significantly alters the LSPR property. As a result, a significant plasmonic absorption peak variation emerged, along with the color change of the mixture, which can be recognized by naked eye and measured through spectrophotometer. Here, we used thrombin as a model target, as it is essential in regulation of tumor growth, angiogenesis and metastasis. (Chang et al., 2010; Cho et al., 2008; He et al., 2007; Pavlov et al., 2004) Taking advantage of enzyme assisted AuNPs crystal growth and magnetic separation, simple, sensitive and selective sensing platform can be successfully realized in homogeneous solution.

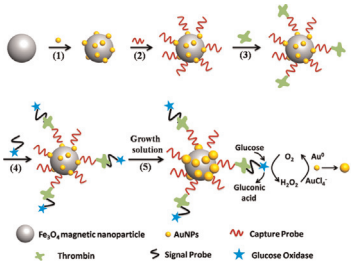
2. Experimental section

2.1. Reagents

Thrombin (from human plasma) was purchased from Sigma. The DNA sequences were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. DNA sequence of thrombin aptamer was listed as follows: Aptamer I (P1): 5′-SH-(CH_2)-TTT AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3′; Aptamer II (P2), 5′-NH_2-(CH_2)-GGT TGG TGT GGT TGG-3′. Glucose oxidase, bovine serum albumin (BSA), immunoglobulin G (IgG) and hemoglobin were bought from Beijing DingGuo Biotech. Co., Ltd. $HAuCl_4$ was bought from Alfa Aesar (Tianjing, China). Other regents of analytical grade were purchased from Beijing Chemical Co. (Beijing, China). Deionized water was used for all solutions preparation.

2.2. Synthesis of AuNPs/Fe_3O_4 nanohybrid

The gold colloid and the Fe_3O_4 nanoparticles were synthesized according to previous reports. (Brown et al., 2000; Hui et al., 2008; Jana et al., 2001) As for the preparation of the nanohybrid, 100 mL of 0.6 mg/mL Fe_3O_4 nanoparticle solution was first sonicated for 15 min, following by slow addition of 2.5 mL of 2.5 mg/mL (polyethylenimine) PEI solution (pH 10.0). Magnetic separation method was used to remove redundant PEI for three times. Afterwards, 45 mL gold colloid solution was added to the PEI coated Fe_3O_4 solution. The resulting solution was washed with deionized water for three times to remove the redundant gold colloids and stored in 10 mg/mL BSA at room temperature for future use.



Scheme 1.
Schematic Illustration of AuNPs/Fe_3O_4 Nanohybrid Based Plasmonic Sensor and the Mechanism of Thrombin Detection. (1) AuNPs are loaded onto Fe_3O_4 magnetic nanoparticles surface to obtain the dual functional composite nanomaterial; (2) Capture probe (thrombin aptamer I) is bounded to the nanohybrid via covalent bond; Owing to the double aptamer recognition mechanism, in the presence of thrombin (3) and aptamer II/glucose oxidase(4), a sandwiched structure was formed and (5) with the addition of "gold nanoparticle growth solution" ($HAuCl_4$ and glucose), crystal growth is initiated, resulting in size enlargement, absorption peak shift and color change.

2.3. DNA functionalization of AuNPs/Fe_3O_4 nanohybrid

Thiol-modified thrombin aptamer was attached onto AuNPs/Fe_3O_4 nanohybridin accordance with previous reported protocols. (Zhang et al., 2012) Briefly, 10 uL of 100 uM thiol-modified thrombin aptamerI stock solution was mixed with 1 mL above prepared AuNPs/Fe_3O_4 nanohybrid solution and incubated for 2 min. Afterwards, the sample was adjusted to acidity with 300 mM citrate · HCl buffer (pH 3.0). Concentrated NaCl was gradually added into the solution to increase the NaCl concentration to 300 mM with gentle shake. Extra AuNPs were removed through PBS washing and magnetic separation. The obtained DNA-functioned AuNPs/Fe_3O_4 nanohybrid was redispersed in 10 mg/mL (PBS) solution for future use.

2.4. Preparation of glucose oxidase modified thrombin aptamerII.

The glucose oxidase modified signal probe was prepared with the aid of glutaraldehyde, which isfrequently used as an amine-reactive cross linking reagent. (Hao et al., 1998; Thompson et al., 1991; Williams and Tjian, 1991) In general, 35 uL of 200 uM amine group-modified thrombin aptamer II stock solution was mixed with 35 uL of 400 uM glucose oxidase solution and stirred for 10 min. Freshly prepared glutaraldehyde solution (70 uL, 2 wt%) was put into the mixture and shaked on an oribital shaker for 1 min, followed by 30 min incubation at room temperature. After that, Tris–HCl buffer (10 uL, 50 mM, pH 7.0) was used to terminate the reaction. Unreacted glucose oxidase was removed by centrifugal ultrafiltration (Millipore, Amicon Ultra-2, molecular weight cutoff: 30 kDa) and redissolved in PBS solution.

2.5. Assembly of the plasmonic sensor

For the detection of the target, 100 uL thrombin aptamerI modified AuNPs/Fe_3O_4 nanohybrid prepared above was mixed with 100 uL PBS solution (10 mM PBS, 100 mM NaCl, pH 7.40) containing a certain amount of thrombin and incubated for 30 min. After careful wash and separation, the nanohybrid was