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Target-driven self-assembly of stacking deoxyribonucleic acids for highly sensitive assay of proteins

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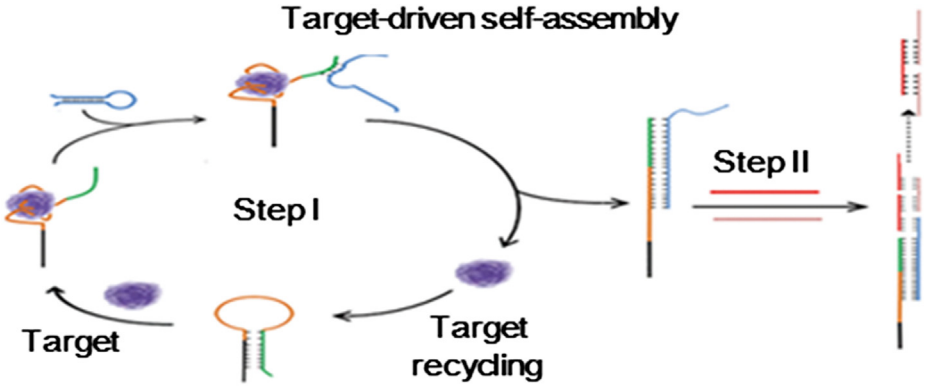
HIGHLIGHTS

- A new enzyme-free assay for highly sensitive detection of proteins is developed.
- Target-driven self-assembly of stacking DNA is adopted for signal amplification.
- The assay displays excellent performance for the detection of interferon-gamma.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, we report a new signal amplification strategy for highly sensitive and enzyme-free method to assay proteins based on the target-driven self-assembly of stacking deoxyribonucleic acids (DNA) on an electrode surface. In the sensing procedure, binding of target protein with the aptamer probe is used as a starting point for a scheduled cycle of DNA hairpin assembly, which consists of hybridization, displacement and target regeneration. Following numbers of the assembly repeats, a great deal of DNA duplexes can accordingly be formed on the electrode surface, and then switch on a succeeding propagation of self-assembled DNA concatemers that provide further signal enhancement. In this way, each target binding event can bring out two cascaded DNA self-assembly processes, namely, stacking DNA self-assembly, and therefore can be converted into remarkably intensified electrochemical signals by associating with silver nanoparticle-based readout. Consequently, highly sensitive detection of target proteins can be achieved. Using interferon-gamma as a model, the assay method displays a linear range from 1 to 500 pM with a detection limit of 0.57 pM, which is comparable or even superior to other reported amplified assays. Moreover, the proposed method eliminates the involvement of any enzymes, thereby enhancing the feasibility in clinical diagnosis.

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1. Introduction

Quantitative measurement of protein targets plays essential roles in various biomedical and biochemical researches [1]. In particular, the accurate detection of disease-associated protein biomarkers can hold great promise for understanding disease progression process and provide meaningful information for clinical diagnosis. Until now, numerous technologies, such as enzyme-linked immunosorbent assays, mass spectrometry-based proteomics and immune sensors, have been developed to meet the urgent needs of protein biomarker analysis [2–4]. Although some of these approaches have proven to be versatile in clinical practice, there remain various kinds of low-abundance protein biomarkers that cannot be detected using conventional methods because of insufficient detection sensitivity [5]. In this regard, development of more sensitive methods that enable measurement of disease-associated proteins at ultralow levels will be of great value for disease diagnosis.

As is well known, aptamer is one kind of man-made functional oligonucleotides that may bind to specific target with high affinity [6]. By incorporating with signal amplification strategies [7–9], the employment of aptamer is opening new horizons for highly sensitive detection of proteins. Amongst these strategies, enzyme-assisted nucleic acid amplification techniques, such as rolling circle amplification [10], isothermal strand displacement polymerization [11], and nuclease-aided recycling amplification [12–14] have proven powerful for achieving high sensitivity and have seen increasing use in recent years. However, the introduction of enzymes in the aptamer-based protein assays may bring additional limitations for clinical practice because enzymes are cost consuming and susceptible to contamination [15,16]. Therefore, there is a scope to develop efficient and enzyme-free amplification strategies for better clinical performance. Advances made in the field of DNA self-assembly may represent ideal options. Owing to the autonomous and programmable manner, DNA self-assembly is demonstrated to offer tremendous opportunities for producing artificial DNA nanostructures, which in turn hold out a cheerful application prospect in enzyme-free signal amplification [17–19]. DNA concatemer that arises from linear self-assembly of short DNA fragments is one of the typical DNA nanostructures, and has attracted increasing attention in the development of DNA self-assembly assisted amplified sensing methods [20,21]. For example, by incorporating DNAzyme into the concatemer structures, Wang et al. have developed an ultrasensitive electrochemical biosensor for folate receptor [20]. More recently, Liu et al. have constructed a novel dendritic DNA concetamer as an effective signal enhancer which may offer significantly improved sensitivity [21]. A further development in DNA self-assembly assisted amplification is related to toehold-mediated strand displacement, in which a branch migration process is initiated through a short single-stranded recognition sequence named as “toehold” [22]. Since the establishment by Yurke et al., such process has been widely applied to the creation of non-enzymatic DNA amplifiers [23–26], which can also be adapted to biosensing applications [27–31].

Nowadays, application of DNA self-assembly assisted amplification to protein assays shows still a scare success. To enhance the development of such strategies and further satisfy the demands for enzyme-free and sensitive detection of protein biomarkers, we here report a new strategy termed as target-driven self-assembly of stacking DNA by ingeniously combining a novel target-triggered hairpin assembly process with the above mentioned DNA concatemer propagation. In this design, the presence of target protein directly triggers special hairpin assembly, which leads to autonomous target recycles and generates initiator fragments for subsequent self-assembled formation of DNA concatemers, ultimately resulting in a drastic amplification effect. By analyzing one model biomarker, interferon-gamma (IFN-γ), which is associated with a number of infectious and autoimmune diseases [32,33], the proposed strategy can provide a detection limit of 0.57 pM, representing a promising tool for highly sensitive assay of proteins.

2. Materials and methods

2.1. Chemicals and materials

DNA oligonucleotides (HP1, HP2, S1 and S2) were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China), and their sequences are shown in Table S1 in Supplementary data. Recombinant IFN-γ was obtained from ProSpec Ltd. (Israel). Mercaptohexanol (MCH), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), cetyltrimethyl ammonium bromide (CTAB), bovine serum albumin (BSA), α-thrombin and α-fetoprotein (AFP) were ordered from Sigma–Aldrich. Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Bio Basic Inc. (Shanghai, China). Human serum was from Dingguo Biotech. Co. (Beijing, China). Other chemicals were of analytical grade and used as received.

The buffer solutions utilized in this work are listed as follows. DNA immobilization buffer: 10 mM Tris–HCl, 1 mM EDTA, 10 mM TCEP, and 0.1 M NaCl (pH 7.4). IFN-γ binding buffer: 10 mM HEPES with 150 mM NaCl (pH 7.4). DNA hybridization buffer: 10 mM phosphate buffered saline (PBS, pH 7.4) containing 1 M NaCl. Electrode washing buffer: 20 mM Tris–HCl, 5 mM $MgCl_2$, 0.1 M NaCl and 1.0% Tween-20 (pH 7.4). All the solutions were prepared with double-distilled water (18 MΩ cm), which was obtained from a Direct-8 Millipore purification system (Branstead, USA).

2.2. Preparation and characterization of positively-charged silver nanoparticles (AgNPs)

The positively-charged AgNPs were synthesized by borohydride reduction of $AgNO_3$ in the presence of CTAB [34]. Briefly, 2 mL of ethanol solution containing 1 mM CTAB was first put in 30 mL of aqueous solution of 5 mM $AgNO_3$, and the mixture was stirred for 15 min at room temperature. After that, a fresh-prepared solution of $NaBH_4$ (1 %) was added drop wise into the above mixture until the color became stable yellow green. UV–vis absorption spectroscopy and zeta potential measurements were then conducted to characterize the as-prepared AgNPs. As shown in Fig. S1 in Supplementary data, the absorption spectrum of resulting AgNPs displays a typical peak at the wavelength of 396 nm. The zeta potential of AgNPs is measured as +18.8 mV, confirming that the particles are capped with positively-charged CTAB, so that they could interact with DNA strands through electrostatic attraction.

2.3. Immobilization of HP1 on gold electrode

Before HP1 immobilization, the substrate gold electrode was carefully cleaned and pretreated. In brief, the electrode was first dipped in fresh piranha solution (concentrated H_2SO_4: 30% H_2O_2 = 3:1) for 5 min to eliminate any adsorbed material, followed by rinsing with double-distilled water. Then, it was polished with 1 μm, 0.3 μm, and 0.05 μm alumina slurry in sequence to obtain a mirror-like surface, and ultrasonically cleaned to remove the residual alumina powder. Afterward, the electrode was soaked in nitric acid (50%) for 20 min, electrochemically activated in 0.5 M H_2SO_4 using cyclic voltammetry for 30 cycles, and finally dried with nitrogen. Thereafter, thiolated (SH-) HP1 was attached to the electrode surface via gold–sulfur chemistry. Prior to the attachment, HP1 was heated to 95 °C for 5 min, followed by slowly