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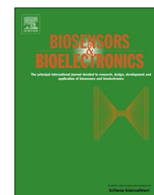

Visual multiple recognition of protein biomarkers based on an array of aptamer modified gold nanoparticles in biocomputing to strip biosensor logic operations

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ABSTRACT

We developed a strip biosensors array based on aptamer-modified gold nanoparticles as receptors and combined the protein-aptamer binding reaction with the streptavidin-biotin interaction as well as the sandwich format. We found that a series of protein receptors obtained a distinct response pattern to each target protein. Three proteins have been well distinguished with the naked eyes and a portable reader without mutual interference, accompanying with lower limit of detection and wider linear range. A complete set of four elementary logic gates (AND, OR, INH, and NAND) and eight combinative logic gates (AND–OR; AND–INH; OR–INH; INH–NAND; AND–OR–INH; AND–INH–NAND; OR–INH–NAND; AND–OR–INH–NAND) are thoroughly realized using this array, which could eventually be applicable to the keypad-lock system with enhanced complexity in the near future. Moreover, this array shows excellent linear relationships, anti-interference capability, real human serum samples applicability, long-term storage stability and reproducibility. All indicate that this design has very good prospects for development.
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1. Introduction

Biomolecules possess excellent properties as building blocks, such as naturally availability in quantities, good biocompatibility, structurally diversity, chirality, multiple possible coordination modes, and prices amenability (Pu et al., 2014; Angelos et al., 2009). In the case of biomolecular ligands, aptamers are one of the ideal bio-linkers, which are single-stranded DNA or RNA oligonucleotides selected from large combinatorial libraries by systematic evolution of ligands by exponential enrichment (SELEX) (Wilson and Szostak, 1999; Tuerk and Gold, 1990; Cox and Ellington, 2001; Berezovski, 2005). The so-called aptamers offer great flexibility in terms of structure variants to bind to a variety of targets with high affinity and specificity, and they are also promising candidates for bioanalytical applications and new computing systems (Famulok and Mayer, 2011; Liu et al., 2010). Moreover, when a single aptamer splits into two fragments, they usually can form associated sandwich-type complexes with their targets (Feng et al., 2015). So far, most examples to control protein activity using aptamer are based on the streptavidin (SA)-biotin interaction or use a limited number of protein-binding aptamers (Qin et al., 2015a,b). Herein we extend this concept of a split aptamer-based strip biosensors array by modifying with gold nanoparticles (AuNPs) for visual multiple recognition of proteins.

Their synthetic accessibility and highly predictable binding properties make aptamers ideal building blocks to construct molecular logical circuits (Janssen et al., 2015; Stojanovic et al., 2014), which are molecular-scale computers that perform Boolean operations to carry out binary computational functions (Seelig et al., 2006). Despite previous burgeoning developments, most of the molecular logic systems have the following disadvantages: (1) it is difficult to scale them up for assembling large networking systems owing to the interference between reactions in the same chemical system and the incompatibility of various chemical gates operating under different conditions (Silva and Uchiyama, 2007); (2) they usually employ a fluorescent signal as the output, which relies on advanced instruments for the readout (Uchiyama et al., 2004); and (3) they often lack the desired portability and require sophisticated synthesis processes, which adds to the complexity, cost, and overall assay time (Lin et al., 2012).

Therefore, it would be highly desirable to develop logic system that is simple in design, not limited to solution-based applications, and involves a reliable and convenient readout. Moreover, a series of independently working logic gates could be easily brought together to function as concatenated logic gates. So, we designed a strip biosensors array to be an ideal pattern to monitor logic

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operations. Upon binding to proteins, it will generate a direct and visible readout, which can be readily distinguished by the naked eye. This also offers a platform for applying to the "keypad lock" system duo to its output signals are dependent not only on the proper combination of inputs but also on the order in which these inputs are introduced. Moreover, this array shows excellent accuracy for detecting protein biomarkers, anti-interference capability, real human serum samples applicability, and long-term storage stability. All indicate that this design has very good prospects for development.

2. Materials and methods

2.1. Chemicals and materials

Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate, sucrose, trehalose, Tween-20, TritonX-100, Tris (2-carboxyethyl) phosphine (TCEP), deoxyadenosine triphosphate (DATP), $Na_3PO_4 \cdot 12H_2O$, 30% H_2O_2, phosphate buffer saline (PBS, PH 7.4, 0.01 M), bovine serum albumin (BSA), carcinoembryonic antigen (CEA) from human fluids (Z95%, SDS-PAGE), human serum, thrombin (TB) from human plasma were purchased from Sigma-Aldrich (USA). Streptavidin (SA) were purchased from Promega (USA). Goat anti Rabbit IgG were purchased from Thermo Scientific. Nitrocellulose membrane (HFB18002) was purchased from Millipore (Billerica, MA). Polyester fiber (VL78, CH27), laminated cards (SM31-40), HM3030 dispenser, CTD300P programmable strip cutter, DT2032 portable strip reader were purchased from Shanghai Kinbio Tech. Co., Ltd. Shanghai, China. Transmission electron microscopic (TEM) images were obtained on FEI Tecnai G20 (America) with an accelerating voltage of 200 kV. Hairpin oligonucleotide (HO), single-stranded DNA (ssDNA) and mucin 1 protein (MUC1) were synthesized and purified by Sangon (Shanghai, China).

The aptamer sequences were as follows:

Thrombin hairpin oligonucleotide (TB-HO): 5′-Biotin-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-(CH2)6-SH-3′

Thrombin single-stranded DNA (TB-ssDNA): 5′-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-(CH2)6-SH-3′

Thrombin test DNA (TB-tDNA): 5′-Biotin-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3′

Thrombin control DNA (TB-cDNA): 5′-Biotin-AG TCA CCC CAA CCT GCC CTA CCA CGG ACT-3′

Mucin 1 protein hairpin oligonucleotide (MUC1-HO): 5′-Biotin-G CAG TTG ATC CTT TGG ATA CCC TGG-(CH2)6 -SH-3′

Mucin 1 protein single-stranded DNA (MUC1-ssDNA): 5′-G CAG TTG ATC CTT TGG ATA CCC TGG-SH-3′

Mucin 1 protein test DNA (MUC1-tDNA): 5′-Biotin-G CAG TTG ATC CTT TGG ATA CCC TGG-3′

Mucin 1 protein control DNA (MUC1-cDNA): 5′-Biotin-CCA GGG TAT CCA AAG GAT CAA CTG C-3′

Carcinoembryonic antigen hairpin oligonucleotide (CEA-HO): 5′-Biotin-CCA CGA TAC CAG CTT ATT CAA TTC GTG G-(CH2)6-SH-3′

Carcinoembryonic antigen single-stranded DNA (CEA-ssDNA): 5′-A TAC CAG CTT ATT CAA TTC GTG G-SH-3′

Carcinoembryonic antigen test DNA (CEA-tDNA): 5′-Biotin-CCA CGA TAC CAG CTT ATT CAA T-3′

Carcinoembryonic antigen control DNA (CEA-cDNA): 5′-Biotin-CCA CGA ATT GAA TAA GCT GGT ATC GTG G-3′

All chemicals used in this study were purchased from standard commercial sources and all were analytical reagent grade. All buffer solutions were prepared with ultra-pure (18.2 $M cm^{-1}$) water from a Millipore Milli-Q water purification system (Billerica, MA).

2.2. Preparation of condensed gold nanoparticles (AuNPs) and HO

AuNPs with different average diameter were prepared using the citrate reduction method according to the reported (He et al., 2012). All glasswares used in this study were thoroughly cleaned in aqua regia (three parts HCl and one part HNO_3), rinsed in doubly distilled water, and oven dried prior to use. Different volumes of 1% trisodium citrate was put in a 250 mL, round-bottom flask, containing 100 mL of 0.01% boiled $HAuCl_4$ aqueous solution. After the color of the solution changed, boiling was pursued for an additional 10 min; then the colloid solution was stirred for another 15 min without heating any more. The resulting AuNPs solution was stored in amber laboratory bottles at 4 °C and characterized by TEM and UV. We condensed the AuNPs by centrifugation for 15 min at 12,000 rpm. After discarding the supernatant, the remanent red pellets were collected at 4 °C. Finally, the AuNPs with average diameter 15 nm $\pm$ 3.5 nm were chosen to be condensed as 5-fold AuNPs solution to prepare the HO-AuNPs-ssDNA receptors.

Hairpin oligonucleotide (HO) was modified with a biotin at its 5′ end and a thiol at its 3′ end. It experienced a centrifugal 10 min under 1200 rpm first, then dispersed in the buffer (Tris–HCl (20 mM, pH 7.4), 100 mM NaCl, and 5 mM $MgCl_2 \cdot 6H_2O$). The solution was incubated in 95 °C water for 5 min and then allowed to slowly cool to room temperature (RT) over another 30 min so that the probe HO correctly folded into a hairpin structure. Tris(2-carboxyethyl) phosphine (TCEP), a thiol deoxidizer, was used to cut the disulfide bond formed between the thiol groups. And deoxyadenosine triphosphate (DATP), a monomer unit of nucleic acid, was used as sealant to prepare the HO-AuNPs conjugates (Huang et al., 2014).

2.3. Preparation of HO-AuNPs-ssDNA conjugates

Different molar ratios of the slight modified HO and thiolated single-stranded DNA (3/1, 2/1, 1/1, 1/2, and 1/3) (Qin et al., 2015a,b) and 15 μL of 1 mM TCEP were added into 1 mL of the 5-fold concentrated AuNPs solution to prepare HO-AuNPs-ssDNA conjugates, respectively. After shaking 2 h at RT, 90 μL of 15 mM DATP were attached to the solution and shook another 1 h at RT. Then, the HO-AuNPs-ssDNA conjugates were put at 4 °C for 6 h to increase the stability of the conjugates. The excess reagents were removed by centrifugation for 15 min at 12,000 rpm. After discarding the supernatant, the red pellets were washed twice with an aqueous solution containing 20 mM $Na_3PO_4 \cdot 12H_2O$, 5% BSA, 0.25% Tween-20, and 10% sucrose, through the centrifugation time of 10 min at 12,000 rpm; finally, redispersed in 1 mL the same buffer solution and stored at 4 °C before further use. All receptors (a–f) were synthesized in this way.

2.4. Preparation of streptavidin-biotinylated test/control DNA (SA-tDNA/SA-cDNA) conjugates

The streptavidin-biotinylated test/control DNA (SA-tDNA/SA-cDNA) conjugates were obtained using the previously reported method (Ge et al., 2013; Fang et al., 2014). Briefly, five hundred microlitre of 4 mg/ml of streptavidin was mixed with 10 μM biotinylated test/control DNA. The mixture was incubated for 2 h at room temperature. After adding 400 μL PBS (pH=7, 0.01 M) into the mixture, the solution was centrifuged in dialysis tube for 10 min at 12,000 rpm under RT, then discarded the supernatant. The above step was repeated for three times. The remaining solution in filter was diluted to 600 μL with PBS.

2.5. Preparation of the lateral flow biosensor (LFB)

The biosensor consists of three components: sample pad,

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