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A universal lateral flow biosensor for proteins and DNAs based on the conformational change of hairpin oligonucleotide and its use for logic gate operations

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ABSTRACT

A universal lateral flow biosensor for proteins and DNAs was designed on the base of target-induced conformational changes of hairpin oligonucleotide (HO). CEA (carcinoembryonic antigen) protein and c-DNA were detected both with the naked eye and a strip reader. The scheme of detecting proteins and DNAs were based on the unique molecular recognition properties of HO to the targets to form different quantities of "active" biotin groups on the surface of gold nanoparticles (AuNPs). The output of the strip is the color of the test line, which inspired us to combine strip biosensor with logic gate. Two strip logic gates ("OR" and "INH") were designed in our paper and the combinatorial logic gates in our paper could be used to make high-throughput judgment about what targets were present in the input samples according to the output results. The biosensor facilitates a portable analysis at ambient temperature as it is simple to be conducted and no requirement of training is needed. The strip logic system is proved an excellent selection and can operate effectively as well as in human serum samples. Therefore, we indicate that such logic strips a foreseeable promise in application of intelligent point-of-care and in-field diagnostics.

1. Introduction

Molecular Boolean logic gate concept derived from electronic logic gates of conventional computer microprocessors. There is a widely accepted notion that this idea has caught many scholars' interest and inspired the generation of smart chemical and biochemical systems (Andreasson and Pischel, 2010; Chen et al., 2006; Katz and Privman, 2010) for functional materials, diagnostic and biosensing applications (Halámková et al., 2012; Vinkenborg et al., 2011; Wang et al., 2014). In the last a few years, chemical systems attained a remarkable interest from researchers and are increasingly imposed in Boolean logic operations for progress of a variety of molecule-based logic systems, such as AND, OR, XOR, NAND, NOR, INHIBIT, half-adder, and half-subtractor (Pu et al., 2011a,b; Lin et al., 2011; Liu et al., 2012; Wang et al., 2012; J. Zhang et al., 2013; L. Zhang et al., 2013; Kim et al., 2013). It could be inspiring to declare that (bio) chemical reaction systems have been in center of life reactions as same as silicon being important to computers. Cascading is supposed to perform providing the chosen substrate's output is of a same form than the input.

It is agreed by most scholars that aptamers, the artificial nucleic acid ligands, are firstly discovered by two groups in 1990 (Tuerk and Gold, 1990; Ellington and Szostak, 1990). Thereafter, aptamers have been exploited to combine with a wide range of small molecules, proteins and DNAs due to their distinct characteristics, such as the high association constant with target proteins and small molecules, easy to obtain and readily to be labelled with signal moieties, cost-effectiveness, and long-term stability (Yoshida and Yokobayashi, 2007; Pan et al., 2013). Noticeably, the combination of DNA oligonucleotides and gold nanoparticles (AuNPs) has been applied for colorimetric logic gates design (Lu et al., 2013), by taking advantage of the recognition ability of aptamers and the unique optical properties of AuNPs. It has been proved that some DNA aptamers can be split into two fragments (Liu et al., 2010; Xu et al., 2009; Zhang et al., 2008). Based on these findings, the 15-mer antithrombin aptamer and 27-mer anti-ATP

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aptamer are split into two fragments respectively in Zeng group's recent work (Chen et al., 2012). The two fragments equilibrate between the disassociated parts and the associated complex, and can reassemble to form a ligand-stabilized structure in the presence of targets. However, this method cannot be a regular approach for potential high-transmission-efficiency substrate because it has strict requirements on target. The target aptamer have to be split into two fragments and retain the recognition ability of each domain when two fragments were integrated together. Therefore, hairpin oligonucleotides (HO) caught our attention as a substitute. It embodies a unique stem–loop structure to provide high specificity, enabling the universal, simple, rapid, and sensitive detection of any proteins and DNAs (He et al., 2010, 2012; Li et al., 2007; Niu et al., 2011; Wang et al., 2008; Zhang et al., 2010).

Recently more and more effort has been made to aim at developing point-of-care (POC) diagnosis biosensors. Strip biosensors bring an optional method for this goal and have attracted great interests for their short assay time, cost-effectiveness, portable format, and stability during long-time storage (Wang et al., 2011; Liu et al., 2011; Du et al., 2012). Moreover, complex analysis procedure brought by expensive instrumentation is avoided and highly qualified personnel are not necessary. Zeng's group has successfully developed LFSB for the detection of nucleic acids (Lie et al., 2012; Liu et al., 2013), proteins (Fang et al., 2011; Ge et al., 2013), cancer cells (Liu et al., 2009), and heavy metal ions (Fang et al., 2010). The detection outcome is easy to perform by visually observing the color intensity of the red band on the test line. Furthermore, the quantitative data can be collected by recording the optical replies with a portable "strip reader". For the purpose of searching new orientations in molecular logic gates, it would be of great importance to incorporate strips with logic gates. Herein, we report a universal hairpin oligonucleotide system established for visual and sensitive detection of proteins and DNAs as well as a pair of strip logic gates that regard the red band on the test line of LFSB as outputs in response to a protein and a DNA, CEA (carcinoembryonic antigen) protein and c-DNA.

2. Experimental

2.1. Chemicals and materials

Streptavidin (SA), hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate, sucrose, Tween-20, Triton X-100, Tris(2-carboxyethyl)phosphine (TCEP), dATP (deoxyadenosine triphosphate), bovine serum albumin (BSA), CEA from human fluids ($\geq$95%, SDS-PAGE), human serum were purchased from Sigma-Aldrich (USA). Nitrocellulose membrane (HFB18004) was purchased from Millipore (Billerica, MA). Polyester fiber (VL78), laminated cards (SM31-40), HM3030 dispenser, CTD300P programmable strip cutter, DT2032 portable strip reader were purchased from Shanghai Kinbio Tech. Co., Ltd. Shanghai, China. Hairpin oligonucleotide, target DNA and control DNA probe were synthesized and purified by Sangon (Shanghai, China). The oligonucleotide sequences were as follows:

CEA-HO: 5′-HS-$(CH_2)_6$-CCA CgA TAC CAg CTT ATT CAA TTC gTg g-biotin-3′

DNA-HO: 5′-HS-$(CH_2)_6$-ACA Cgg CAg TTg ATC CTT Tgg ATA CCC Tgg CgT gT-biotin-3′

OTA-HO: 5′-HS-$(CH_2)_6$-TTT TTg ATC ggg TgT ggg Tgg CgT AAA ggg AgC ATC ggA CAA AAA A-biotin-3′

c-DNA: 5′-CCA ggg TAT CCA AAg gAT CAA CTg C-3′

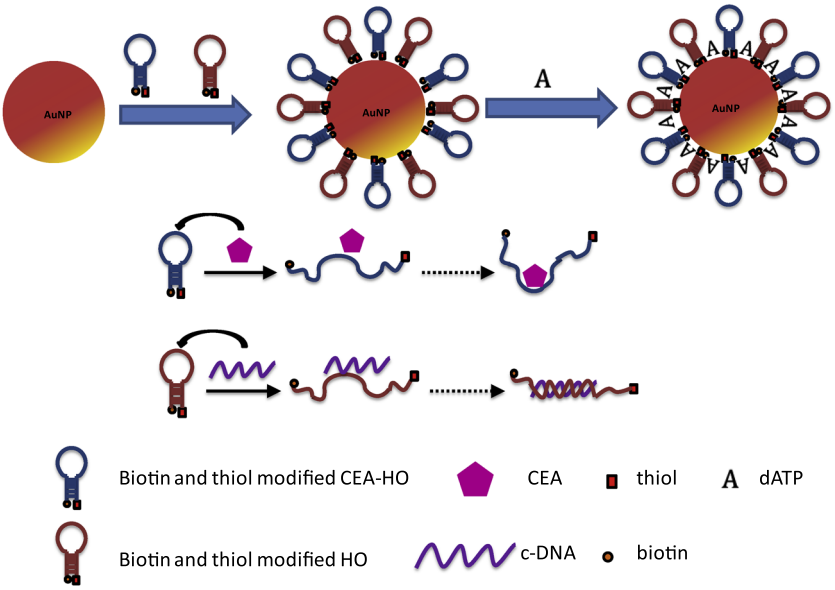
1m-DNA: 5′-CCA ggg TAT CCA ACg gAT CAA CTg C-3′

3m-DNA: 5′-CCA ggg TAT gCg ACg gAT CAA CTg C-3′

All chemicals used in this study were analytical reagent grade. All other solutions were prepared with ultrapure (18.2 M cm^{-1}) water from a Millipore Milli-Q water purification system (Billerica, MA).

2.2. Preparation of gold nanoparticles (AuNPs)

AuNPs with diameter 15 ± 3.5 nm were prepared regarding to the reported methods with slight modifications (Mao et al., 2009). All glassware applied in this preparation was completely cleaned in aqua regia (three parts HCl and one part HNO_3), rinsed in doubly distilled water, and oven-dried prior to use. In a 250 mL conical flask, 100 mL of 0.01% $HAuCl_4$ in ultrapure water were heated to boil with vigorous stirring, followed by the addition of



Scheme 1. Schematic illustration for preparation of DNA–AuNPs conjugates and conformational switch of HO.