Supporting Information

Steric hindrance inhibition of strand displacement for homogeneous and signal-on fluorescent detection of human serum antibodies

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Experimental Section

Materials and reagents: [Tris(hydroxymethy-l)aminomethane] hydrochloride (Tris-HCl), dithiothreitol (DTT) and ethylenediaminetetraacetic acid (EDTA) were provided by Sangon Biotech Co., Ltd (Shanghai, China). Sulfosuccinimidyl-4-[N-maleimidomethyl]-cyclohexane-1-carboxylate (sulfo-SMCC) was provided by Thermo Scientific (Rockford, IL, USA). Human serum was received from Sigma-Aldrich (St. Louis, MO). Polyclonal anti-digoxigenin (anti-Dig) from sheep, mouse anti-dinitrophenol (anti-DNP, monoclonal), alpha-1 fetoprotein antibody (anti-AFP), streptavidin (STV) and human immunoglobulin G (IgG) were bought from Abcam Co., Ltd (Shanghai, China). The following oligonucleotides (Table S1) involved in this work were all ordered from Takara Biotechnology Co., Ltd. (Dalian, China).

Table S1 Oligonucleotides used in the experiments.

Oligonucleotide	Sequence
Molecular beacon (MB)	5'-FAM ATACGGTTTACATCGCTCACAGAAACCGTAT -Dabcyl-3'
Dig-modified probe (Dig-MP)	5'-Dig-TAGTTCATACGGTTTCTGTGAGCGATGT-3'
Thiol-modified competing probe (CP)	5'-ACATCGCTCACAGAAACCGTATGAACTA-$(CH_2)_6$-HS-3'

Preparation of anti-AFP-conjugated CP (anti-AFP-CP): The conjugation of the thiol-modified CP with anti-AFP was performed according to previous report with slight modifications.[1] In brief, anti-AFP (2.0 mg mL^{-1}) was firstly incubated with sulfo-SMCC with a 30-fold excess than anti-AFP for 120 min at 25 °C in PB buffer (55 mM phosphate, 150 mM NaCl, 20 mM EDTA, PH 7.2). In the meantime, 15 μL of 100 μM thiolated CP was reacted with 20 μL of 100 mM DTT in PB buffer for 60 min at 37 °C to reduce the disulfide bonds. This was followed by further purification of reacted CP with the GE Healthcare Illustra MicroSpin G-50 Columns (Marlborough, MA, USA). Next, the sulfo-SMCC-activated anti-AFP was incubated with the DTT-treated CP at 4 °C overnight, followed by dialysis (Slide-A-Lyzer, Thermo Scientific) against PB buffer overnight to obtain the anti-AFP-CP.

Fluorescent anti-Dig sensing procedure: The mixture of Dig-MP (1 μM) and MB (1 μM) in 50 mM Tris-HCl reaction buffer (150 mM NaCl, 10 mM $MgCl_2$, pH 7.0) was heated at 90 °C for 5 min, then was cooled down to room temperature to form Dig-MP/MB with a strong fluorescence signal due to the unfolding of the MB structure.

Subsequently, various concentrations of anti-Dig were incubated with Dig-MP/MB (100 nM) in the reaction buffer at room temperature for 30 min. After that, anti-AFP-CP was added to the mixture at the final concentration of 80 nM and further incubated for 75 min. Finally, reaction buffer was added to the mixture to a total volume of 200 μL and fluorescence measurements were carried out to obtain the data according to our previous precedure.[2] In brief, fluorescence responses of the sample solutions were recorded on a RF-5301PC spectrophotometer (Shimadzu, Tokyo, Japan) with a 150W Xenon lamp (Ushio Inc, Japan) as the excitation source at room temperature. Emission spectra of the solutions were collected from 300 nm to 800 nm with specific excitation wavelength of 490 nm. The excitation and emission slit widths were both set at 5 nm.

Table S2 Comparison of different methods for antibodies detection

Detection method	Detection mechanism	Detection limit	Ref.
Electrochemistry	Steric hindrance effects	10 nM	3
Electrochemistry	Structure switching of dsDNA	5 nM	4
Electrochemistry	Structure switching of dsDNA	10 nM	5
Fluorescence	DNA-based molecular beacon	10 nM	6
Fluorescence	DNA-mediated binding assay	1 nM	7
Fluorescence	Steric hindrance inhibition of strand displacement	5.6 nM	This work

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