Electronic Supplementary Information (ESI)

Proximity-induced hybridization chain assembly with small-molecule linked DNA for single-step amplified detection of antibodies

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Experiment section

Chemicals and Materials

The DNA probes listed in Table S1 were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). Anti-digoxigenin antibody (Dig-Ab), human immunoglobulin G (IgG), anti-PSA antibody (PSA-Ab) and anti-dinitrophenol antibody (DNP-Ab) were obtained from Abcam Co., Ltd (Shanghai, China). Streptavidin (SA), bovine serum albumin (BSA) and digoxigenin were purchased from Sigma-Aldrich (Shanghai, China). All other reagents were analytical grade and used without further purification.

Instruments

The fluorescence spectra were recorded at room temperature in a quartz cuvette on F-7000 fluorescence spectrophotometer (Hitachi, Japan). The real-time fluorescence spectra were obtained in 96-well plates on a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA).

Gel electrophoresis analysis

Gel electrophoresis was performed on 3% (w/w) agarose gel containing 0.5 μg/mL ethidium bromide and 0.5 μg/mL gold view at room temperature in 0.5 × TBE (44 mmol/L Tris-Boric Acid; 1 mmol/L EDTA). A 10 μL DNA sample was mixed with 1 μL 10 × loading buffer, then the mixture was added in the well of agarose gel. The gels were run at 100 V for 50 min. Then, the gels were visualized using a digital camera under strong UV illumination within Tanon 4200SF gel imaging system.

Anti-digoxigenin antibody detection

All DNA probes were dissolved in 1 × PBS buffer. The concentrations were determined by a UV-2450 spectrophotometer (Shimadzu, Japan). In a typical process, different concentrations of Dig-Ab were added into detection solution (40 nM I1, 40 nM I2, 100 nM H1 and 100 nM H2) respectively in 1 × PBS buffer (10 mM Phosphate, 137 mM NaCl, 2.7 mM KCl, 5 mM $MgCl_2$, pH 7.4). After incubation for 3 h at 37 °C, the fluorescence spectra were recorded from 500 to 600 nm using an excitation wavelength

of 488 nm.

For the detection of Dig-Ab in complex biological media, Dig-Ab with different final concentrations were added into detection solution containing 10% human serum. The mixture was incubated for 3 h at 37 °C, and then the fluorescence spectra were recorded from 500 to 600 nm using an excitation wavelength of 488 nm.

Selectivity analysis

The selectivity of antibody-responsive hybridization chain assembly for Dig-Ab against other proteins was evaluated as follows: 50 nM Dig-Ab or 500 nM other protein was added into the detection solution, and subsequently incubation for 3 h at 37 °C. The fluorescence spectra were recorded from 500 to 600 nm using an excitation wavelength of 488 nm.

Table S1. Sequences of synthesized DNA probes.[a]

Name	Sequence (5'-3')
I	TGCTGTGGATCGAGCGGTAGTTGTCA
I1	Dig-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCAGTCTTAGTTGTCA
I2	TGCTGTGGATCGAGCGGTGACTGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-Dig
H1	TGACAACTACCGCTCGAT(FAM)CCACAGCAATGCTTCATGCTGTGGAT(BHQ1)CGAGCGGT
H2	TGCTGTGGATCGAGCGGTAGTTGTCAACCGCTCGATCCACAGCATGAAGCAT

[a] The DNA sequences marked with purple are initiator. Underline sequences indicate complementary regions of the probes to form hairpin structure.

Scheme S1. Schematic illustration of the competitive binding assay of programmable hybridization chain assembly.

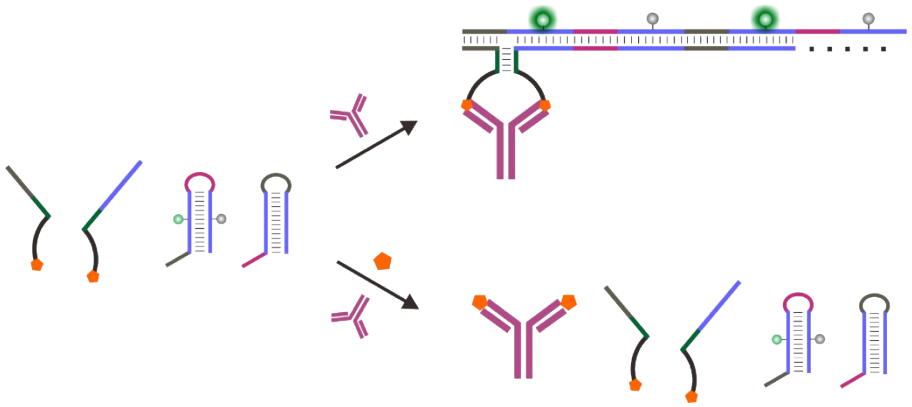

Fig. S1. Gel electrophoresis analysis of the binding of I1 and I2 with Dig-Ab. lane 1, DNA marker; lane 2, 8 μM I1; lane 3, 8 μM I2; Lane 4, 8 μM I1 and 8 μM I2; lane 5, 8 μM I1, 8 μM I2 plus 8 μM Dig-Ab.

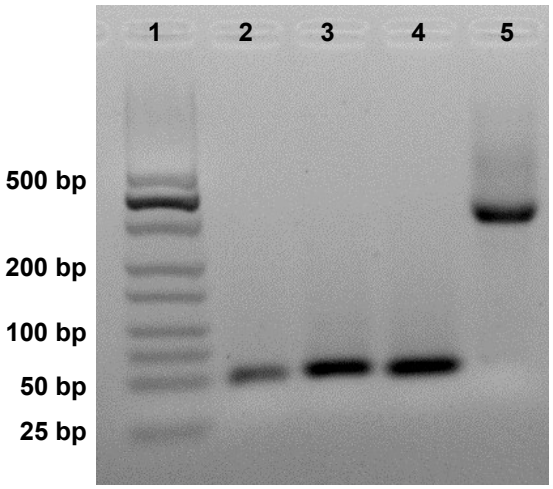

Fig. S2. Optimization of the concentration of I1 and I2 (20 nM, 30 nM, 40 nM, 50 nM, 60 nM). The concentration of Dig-Ab was 30 nM.

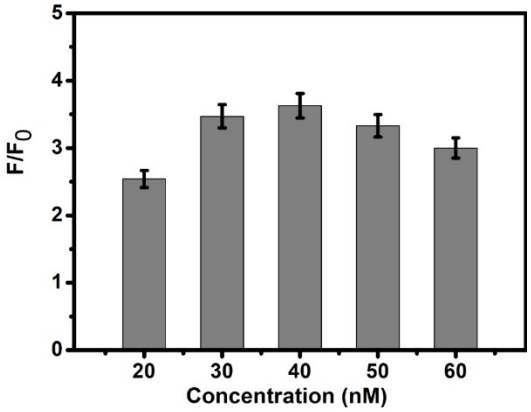

Fig. S3. Real-time fluorescence signals reading of antibody-responsive hybridization chain assembly with (red) or without (black) Dig-Ab.

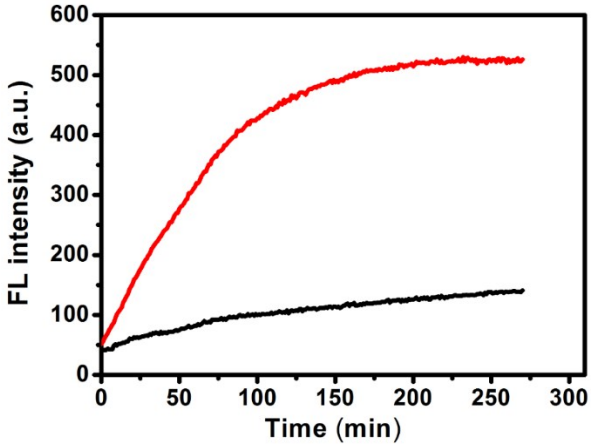

Fig. S4. (A) Fluorescence spectral response of free Dig with varying concentrations; (B) Fluorescence intensity versus the concentration of free Dig. The concentrations of Dig-Ab were 50 nM, H1 and H2 were 100 nM, I1 and I2 were 40 nM.

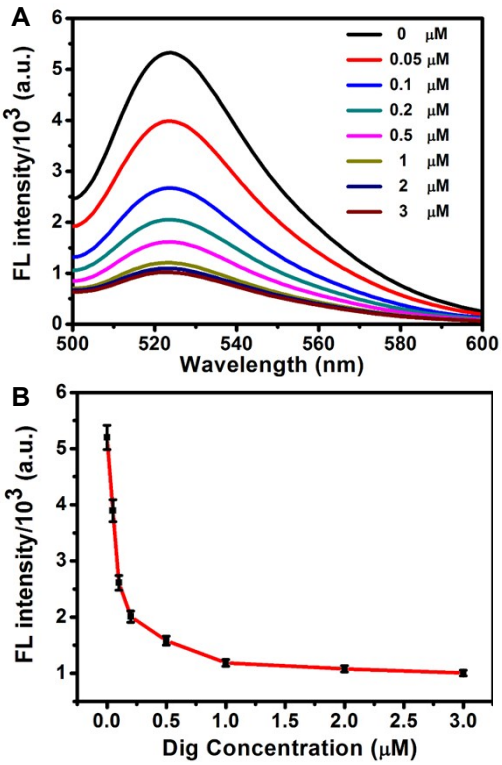

Table S2. Comparison of the detection performance toward Dig-Ab with different methods.

Technique	Detection limit	Sensing strategy	Reference
Fluorescence	5.6 nM	Steric hindrance inhibition of strand displacement	1
Fluorescence	1 nM	DNA-mediated homogeneous binding assay	2
Fluorescence	10 nM	DNA-based beacon conformation-switching	3
Fluorescence	0.33 nM	Binding-induced nanoswitch	4
Fluorescence	1 nM	Antibody-templated assembly of RNA structure	5
Electrochemistry	0.67 nM	DNA-mediated strand displacement	6
Electrochemistry	1 nM	Bioelectrochemical switches	7
Electrochemistry	5 nM	Target induced signal response	8
Electrochemistry	10 nM	Steric hindrance effects	9
Nanopore	0.5 nM	Analyte-triggered triplex molecular beacon nanoswitch	10
Fluorescence	0.032 nM	Proximity-induced hybridization chain assembly	This work

Table S3. Recovery experiments of Antibody in serum sample.

Samples	Spiked Antibody (nM)	Found Antibody (nM)	Recovery (%)	RSD (n=3, %)
1	5	4.72	94.4	2.5
2	10	10.31	103.1	3.8
3	25	24.32	97.3	2.7
4	35	33.01	94.3	4.3

Reference:

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