Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

One-Step Colorimetric Detection of Antibody Based on Protein-Induced Unfolding of G-quadruplex Switch

Xiaolu Hu,[‡a] Chao Li,[‡a] Chang Feng,[a] Xiaoxia Mao,[b] Yang Xiang,[*a] Genxi Li[*ab]

[a]State Key Laboratory of Pharmaceutical Biotechnology and Collaborative Innovation Center of Chemistry for Life Sciences, Department of Biochemistry, Nanjing University, Nanjing 210093, P. R. China

[b]Center for Molecular Recognition and Biosensing, School of Life Sciences, Shanghai University, Shanghai 200444, P. R. China

Experimental section

Reagents and materials.

The DNA strand with the following sequence was synthesized and HPLC purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). G-rich DNA: 5'-DIG-TTTTGGGTAGGGCGGGTTGGGTTTT-DIG-3', fluorescence-G-rich DNA: 5'-DIG-TTTTG(HEX)GGTAGGGCGGGTTGGG(BHQ-1)TTTT-DIG-3'. The oligonucleotide was used as provided and diluted in 10 mM Tris-HCl buffer (100 mM NaCl, 1 mM EDTA, pH 7.4), to give a stock solution of 100 μM. A hemin stock solution (5 mM) was prepared in dimethyl sulfoxide (DMSO) and stored in the dark at -20 °C. All other chemicals used in this work were of analytical grade and directly used without additional purification. TMB (3, 3′, 5, 5′-tetramethyl benzidine) was purchased from Neogen (Lexington, KY) in the format of a ready-to-use reagent (enhanced K-blue substrate, H_2O_2 included). Polyclonal anti-digoxigenin antibody (DIG-Ab, Cat. Number: ab76907) was purchased from Abcam Corporation. DL-Dithiothreitol (DTT, Cat. Number: 43815), agarose (Cat. Number: A9539), Immunoglobulin G (IgG, Cat. Number: I5006), immunoglobulin E (IgE，Cat. Number:AG30P), anti-dinitrophenol antibody (DNP-Ab, Cat. Number: D9656) and bovine serum albumin (BSA, Cat. Number: 05470) were obtained from Sigma-Aldrich (Shanghai, China).

Instrumentations.

All solutions were prepared with ultrapure water (18.2 MΩcm) from a Milli-Q purification system (Bedford, MA). Optical density (OD) at 650nm of the solution was performed by UV–vis spectroscopy (UV-1800, Shimadzu, Japan). Circular dichroism (CD) spectrum was measured on a Chirascan spectropolarimeter that was purchased from Applied Photophysics Ltd. (Great Britain).

Preparation of G-quadruplex switch.

All the samples were prepared with reaction buffer (40KT buffer, pH 6.5, 100 mM Tris, 50 mM MES, 40 mM KCl, 0.05% Triton X-100, 1% DMSO). G-quadruplex DNAzyme was prepared according to previously reported literature with a minor modification. G-rich DNA was diluted to 2 μM in above reaction buffer, heated at 95 °C for 3 min, gradually cooled to room temperature. Hemin (5 mM) was then added and incubated at room temperature for 30 min to form the hemin/G-quadruplex complex.

Circular dichroism experiment.

The process of sample preparation was similar to the preparation of G-quadruplex switch as described above, but higher concentrations were used. 1 μM DIG-Ab was added into treatment tube to incubate for 1h at RT. Then different volumes of the reaction buffer were added to each tube to 200 μL. The final concentration of the oligonucleotide was 5 μM. CD measurements were carried out on a spectropolarimeter equipped with a programmable temperature control unit. The

spectra from 320 to 200 nm were obtained in 1mm path length cuvettes and averaged from three scans with the reaction buffer background subtracted.

Colorimetric assay.

After 20 μL G-rich DNA (10 μM) and 2 μL hemin (100 μM) formed G-quadruplex switch, DIG-Ab with a series of concentration was added. Then different volumes of the 40KT buffer were added to each sample solution to 100 μL. Control experiment (in the absence of DIG-Ab) was conducted at the same condition. After incubation at room temperature for 1 h, the peroxidation reaction was initiated by the addition of 100 μL of TMB substrate. The mixture was kept for 40 min, and different colors were observed with naked eye owing to different concentrations of DIG-Ab. Then UV-vis spectra were collected and the photos were taken immediately.

Notes and references

1. P. Travascio, Y. F. Li and D. Sen, *Chem. Biol.*, 1998, **5**, 505-517.
2. L. Zhu, C. Li, Z. Zhu, D. W. Liu, Y. Zou, C. M. Wang, H. Fu and C. J. Yang, *Anal. Chem.*, 2012, **84**, 8383-8390.
3. Z. Y. Wang, J. Zhao, J. C. Bao and Z. H. Dai, *ACS Appl. Mater. Interfaces*, 2016, **8**, 827-833.
4. L. Zhang, J. Zhu, T. Li and E. Wang, *Anal. Chem.*, 2011, **83**, 8871-8876.
5. S. Shimron, F. Wang, R. Orbach and I. Willner, *Anal. Chem.*, 2012, **84**, 1042-1048.

Results and discussion

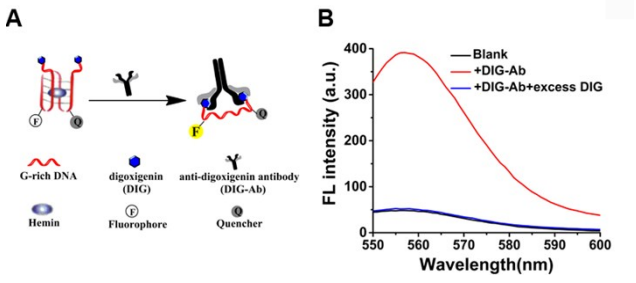

Fig. S1 Confirmation of the principle by fluorescence labled G-quadruplex switch. (A) The scheme of DIG-Ab detection by using fluorescence labled G-quadruplex switch. (B) Fluorescence signals in response to 100 nM DIG-Ab (red line) and 100 nM DIG-Ab with excess DIG molecule (blue line), the control group without DIG-Ab and DIG molecule (black line).

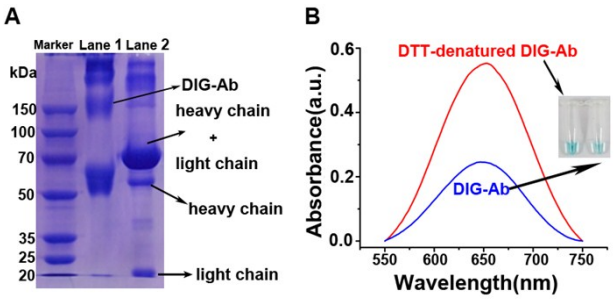

Fig. S2 The effect of DTT-denatured DIG-Ab on the G-quadruplex switch. (A) SDS-polyacrylamide gel electrophoresis results of DTT-denatured DIG-Ab and DIG-Ab. The characteristic bands of DIG-Ab fragments are observed after incubation with 20 mM DTT for 1h (lane 2: DTT-denatured DIG-Ab); in contrast, the negative control without 20 mM DTT show the band of integrated DIG-Ab at 150 kDa (lane 1: DIG-Ab). (B) UV-vis absorption spectra of TMB substrate after it reacted with 50 nM DTT-denatured DIG-Ab and 50 nM DIG-Ab. Inste shows the corresponding color changes of resolution.

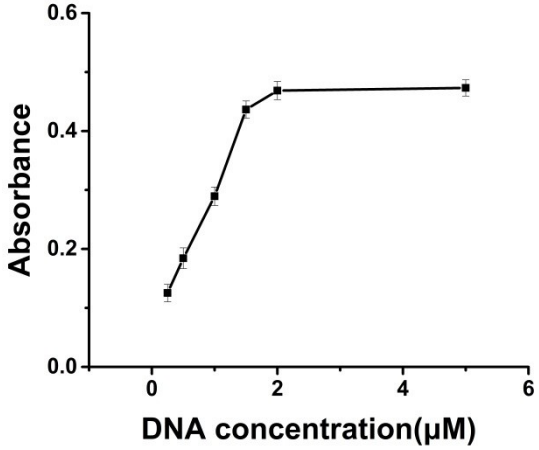

Fig. S3 Optimization of concentration of the G-quadruplex-containing DNA (0.25, 0.5, 1, 1.5, 2 and 5 μM). Error bars represent the standard deviations of three parallel measurements.

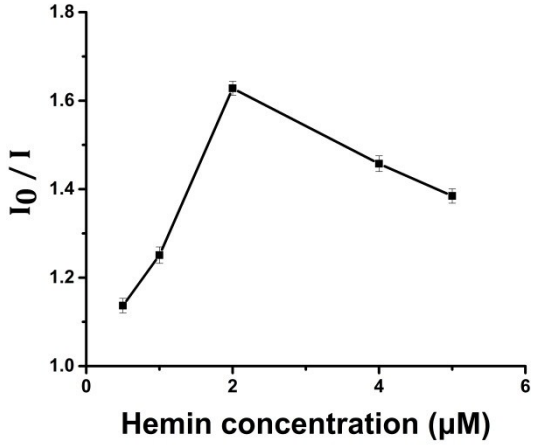

Fig. S4 Optimization of concentration of hemin (0.5, 1, 2, 4 and 5 μM). Error bars represent the standard deviations of three parallel measurements.

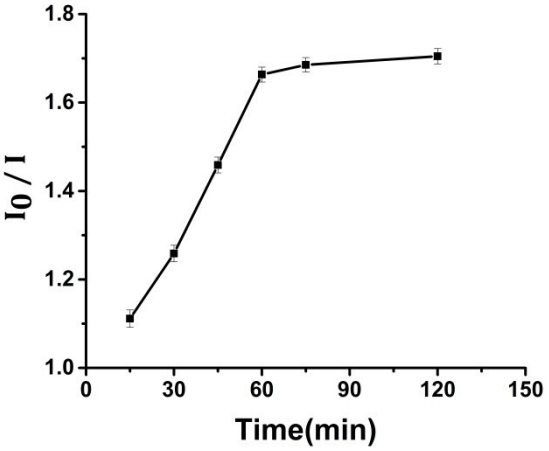

Fig. S5 Optimization of concentration of antibody incubation time (15, 30, 45, 60, 75 and 120 min). Error bars represent the standard deviations of three parallel measurements.

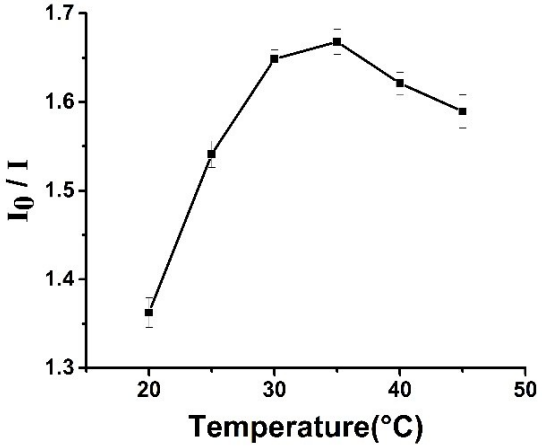

Fig. S6 Optimization of antibody incubation temperature (20, 25, 30, 35, 40 and 45

°C). Error bars represent the standard deviations of three parallel measurements.

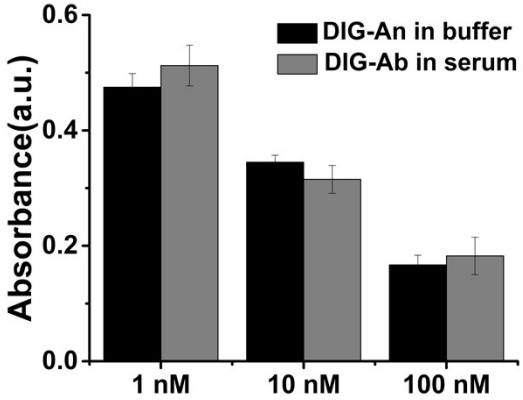

Fig. S7 Results obtained from the testing of DIG-Ab spiked in serum and buffer (DIG-Ab concentration is 1, 10, and 100 nM).