Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Antibody-powered DNA switches to initiate hybridization chain reaction for amplified fluorescence immunoassay

Jia-Yang He, Ze-Hui Chen, Hui-Lin Deng, Ruo Yuan* and Wen-Ju Xu*

Key Laboratory of Luminescence Analysis and Molecular Sensing (Southwest University), Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China.

Table S1. Base sequences of oligonucleotides used in this work

Names	Base sequence (5'→3')
Recognition strand (RS)	CAGTGGCGTTTTATTCTTGT-Dig
Report hairpin 1 (RH1)	AGAATAAAACGCCACTGCCG*AAGCGATCGCCGCGCGG*-Cy3
Report hairpin 2 (RH2)	AGAATAAAACGCCACTGCCGCG*AAGCGATCGCCGCGCGC*GCGG-Cy3
Report hairpin 3 (RH3)	AGAATAAAACGCCACTGCCGCGCG*AAGCGATCGCCGCGC*GCGCGCGCGG-Cy3
Help strand 1 (HS1)	AGAATAAAACGCCACTG**ATTTTTTTTTTTTTTTTCTT***CGGATCGCTT*
Help strand 2 (HS2)	AGAATAAAACGCCACTG**ATTTTTTTTTTTTTTTTCTT***CGGCGATCGCTT*
Help strand 3 (HS3)	AGAATAAAACGCCACTG**ATTTTTTTTTTTTTTTTCTT***CGCGGCGATCGCTT*
Quench hairpin 1 (QH1)	TGTCGGCGATCGCTTCCGCG-BQH-2
Quench hairpin 2 (QH2)	TGTCGCGGCGATCGCTTCCGCGCG-BQH-2
Quench hairpin 3 (QH3)	TGTCGCGCGGCGATCGCTTCCGCGCGCG-BQH-2

Characterizations

Firstly, the hybridization reaction of different oligonucleotide samples was characterized using PAGE. From Fig. S1A, the individual RS, QH, RH and HS manifested their own characteristic mobility, separately corresponding to lane 1, 2, 3 and 4. While their mixture showed lower electrophoretic movement (lane 5), indicating the successful hybridization reaction between them. However, the addition of target anti-Dig in the above resultant solution caused several bands of different moving mobility (lane 6), where the slowest one standing in the uppermost

place might attribute to the HCR product by RH and QH base pairing in this assay system. Obviously, the production of HCR was much higher than DNA marker (lane 7) demonstrating that a DNA nanowire with a very high molecular weight was generated successfully.

Correspondingly, we also measured the UV-Vis absorption spectra of the above samples. Obviously from Fig. S1B, the weak absorptions of QH, RS, RH and HS were observed (curve 1-4). But for their mixture, a strong absorption peak was emerged (curve 5), and a similar UV-Vis response was also obtained after adding anti-Dig (curve 6), which might prove the efficient formation of more DNA double strands assisted by HCR.

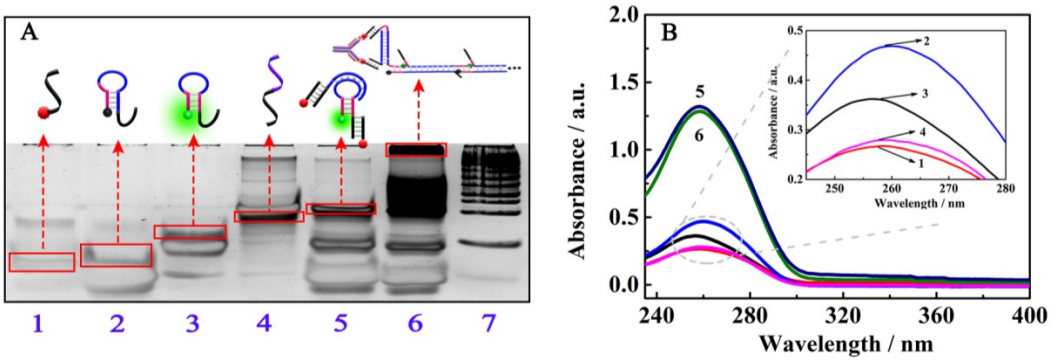

Fig. S1 (A) PAGE image of RS (lane 1), QH (lane 2), RH (lane 3), HS (lane 4), RS+QH+RH+HS (lane 5), RS+QH+RH+HS+anti-Dig (lane 6) and DNA marker (25, 50, 75, 100, 150, 200, 300, 400, 500 bp) (7). All the samples (each with 2.0 μM) were incubated at 37 ℃ for 2 h. (B) UV-Vis absorption spectra of QH (1), RS (2), RH (3), HS (4), QH+RS+RH+HS (5), QH+RS+RH+HS+anti-Dig (6)

Optimization of reaction time

We investigated the effect of different reaction time in our detection system on the final BHQ-2-quenched fluorescence signal, mainly involving in the formation of the metastable DNA structure and the conjugation reaction of anti-Dig with two Dig

haptens. Apparently from Fig. S2A, when mixing RS, HS and RH, the measured fluorescence (FL) intensity of Cy3 was gradually decreased with the increasing of reaction time from 0 to 2.5 h, and leveled off at about 2 h. Upon treated with anti-Dig in the resultant solution in 2.5 h, the fluorescence emission of Cy3 was efficiently quenched by BHQ-2, and the biggest extinction was firstly appearted at about 1.0 h (Fig. S2B), suggesting the optimal time of 1 h for anti-Dig coujgation reation.

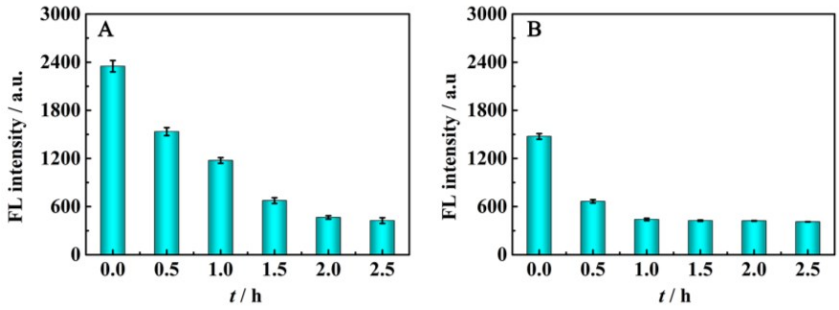

Fig. S2 The fluorescence intensity of the detection system in different reaction time: (A) RS+HS+RH and (B) RS/HS/RH+anti-Dig. (The concentration of anti-Dig was 1 μM. Error bars, *s*, *n*=5).

Table S2 Detection strategies and analytical performance of different immunoassay methodologies.

Methods	Strategy	Targets	Linear range	LOD	Ref.
CV and EIS	Paper-based electrochemical biosensor	Anti-SARS-CoV-2	6.7 pM to 6.7 nM	6.4 nM	[1]
DPASV	Electrochemical immunoassay	Anti-Dengue	0 to 2.6 nM	0.68 nM	[2]
ELISA	Microfluidic-integrated multicolor immunosensor	Anti-HIV-1 p24	0 to 47 pM	3.3 pM	[3]
BL	Thread-based bioluminescent sensor	Anti-HIV1-p17	0 to 250 nM	7.7 nM	[4]

FL	Antibody-mediated small molecule detection	Anti-DNP	30 to 200 nM	20 nM	[5]
FL	Antibody-powered DNA switches integrated HCR	anti-Dig	10 pM to 1.0 μM	8.1 pM	Our work

CV: cyclic voltammetry; EIS: electrochemical impedance spectroscopy; DPASV: differential pulse anodic stripping voltammetry; ELISA: enzyme linked immunosorbent assay; BL: bioluminescence.

Table S3 Recovery of anti-Dig in human serum samples with our developed method (*n*=5).

Samples	Spiked (nM)	Measured (nM)	Recovery (%)	RSD (%)
1	0.10	0.103	103	3.0
2	1.0	1.01	101	3.1
3	10	9.88	98.8	3.5
4	100	96.7	96.7	4.5

References

1 A. Yakoh, P. Umaporn, S. Rengpipat, N. Hirankarn, O. Chailapakul, S. Chaiyo, *Biosens. Bioelectron*. 2021, **176**, 112912.

2 G. A. Ortega, J. C. Zuaznabar-Gardona and E. Reguera, *Biosens. Bioelectron*. 2018, **116**, 30-36.

3 D. Liu, Y. Q. Zhang, M. Y. Zhu, Z. Z. Yu, X. M. Ma, Y. L. Song, S. F. Zhou and C. Y. Yang, *Anal. Chem*. 2020, **92**, 11826-11833.

4 K. omimuro, K. Tenda, Y. Ni, Y. Hiruta M. Merkx, and D. Citterio, *ACS Sens*. 2020, **5**, 1786-1794.

5 M. Rossetti, R. Ippodrino, B. Marini, G. Palleschi and A. Porchetta, *Anal. Chem*. 2018, **90**, 8196-

8201.