Supporting Information

Modular DNA Circuits for Point-of-Care Colorimetric Assay of Infectious Pathogens

Dan Zhu,[1] Zihao Ma,[1] Zichun Wang,[1] Qingyun Wei,[1] Xiaojian Li,[1] Jingjing Wang,[2] Shao Su,[1] Xiaolei Zuo,[3,4] Chunhai Fan,[4] Jie Chao,[1,*] Lianhui Wang[1,*]

[1] State Key Laboratory of Organic Electronics and Information Displays & Jiangsu Key Laboratory for Biosensors, Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing University of Posts and Telecommunications, 9 Wenyuan Road, Nanjing 210023, China

[2] Hebei Provincial Key Laboratory of Ophthalmology, Hebei Provincial Eye Hospital, Xingtai 054001, China

[3] Institute of Molecular Medicine, Shanghai Key Laboratory for Nucleic Acids Chemistry and Nanomedicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China

[4] School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules and National Center for Translational Medicine, Shanghai Jiao Tong University, Shanghai 200240, China

[*] To whom correspondence should be addressed: Jie Chao (iamjchao@njupt.edu.cn); Lianhui Wang (iamlhwang@njupt.edu.cn)

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Table S1. Sequences of oligonucleotides used in this work.

Name	Sequence (5′-3′)
Biotin-S1	biotin- TTTTTTTTTTTCTCACTAACTGCATA
Ebola-S2	GCCGATAGTTGAGGGAAAAGACCCACCTATGCAGTTAG TGAGA
Ebola-S3	GGTGGTGGTGGTTGTGGTGGTGGTGGGTCTTTTCCCTC AAC
Ebola-S4	CTAACTGCATAGGTGGGTCTTTTCCCTCAAC
H1N1-S2	GACCATGAGCTTGCTGTGGAGCCACCTATGCAGTTAGTG AGA
H1N1-S3	GGTGGTGGTGGTTGTGGTGGTGGTGGCTCCACAGCAAG CT
H1N1-S4	CTAACTGCATAGGTGGCTCCACAGCAAGCT
Zika-S2	CGGGATTCTTCATGATGCCAGCCACCTATGCAGTTAGTG AGA
Zika-S3	GGTGGTGGTGGTTGTGGTGGTGGTGGCTGGCATCATGA AG
Zika-S4	CTAACTGCATAGGTGGCTGGCATCATGAAG
Dengue-S2	ATAATCCCTTCTGGTGTGTTGCCACCTATGCAGTTAGTGA GA
Dengue-S3	GGTGGTGGTGGTTGTGGTGGTGGTGGCAACACACCAGA AG
Dengue-S4	CTAACTGCATAGGTGGCAACACACCAGAAG
H7N9-S2	AATTCCTGCTTGTTCTCTCTTCCACCTATGCAGTTAGTGA
H7N9-S3	GGTGGTGGTGGTTGTGGTGGTGGTGGAAGAGAGAACA AGC
H7N9-S4	CTAACTGCATAGGTGGAAGAGAGAACAAGC
Ebola DNA[a]	GTCTTTTCCCTCAACTATCGGC
Ebola RNA	GUCUUUUCCCUCAACUAUCGGC

H1N1 DNA[a]	CTCCACAGCAAGCTCATGGTC
Zika DNA[b]	CTGGCATCATGAAGAATCCCG
Dengue DNA[b]	CAACACACCAGAAGGGATTAT
H7N9 DNA[a]	AAGAGAGAACAAGCAGGAATT
SM-Ebola DNA	GTCTTTTCCC**C**CAACTATCGGC
HBV DNA[a]	AAATTCGCAGTCCCCAACCTCC
HIV DNA[a]	ACTGCTAGAGATTTTCCACAT
MAL DNA[a]	AAAATTAAGTGTTCATAACAGA
Random DNA[a]	TAGCTTATCAGACTGATGTTGA

[a] We obtained the microorganisms' genomic DNA from GenBank (Ebola DNA: Ebola virus-Mayinga, Zaire, 1976, complete genome, GenBank accession AF086833.2; H1N1 DNA: Influenza A virus (A/California/07/2009(H1N1)) segment 4 hemagglutinin (HA) gene, complete cds, GenBank accession NC_026433.1; H7N9 DNA: Influenza A virus (A/Shanghai/4664T/2013(H7N9)) segment 6 neuraminidase (NA) gene, complete cds, GenBank accession KC853231.1; HBV DNA: Hepatitis B virus DNA, complete genome, strain Fukuoka Red Cross HBV e-negative 1992, GenBank accession D28880.1; HIV DNA: Human immunodeficiency virus 1, complete genome, GenBank accession NC_001802.1; MAL DNA: P.falciparum 18S ribosomal RNA not in asexual parasites, GenBank accession M19173.1 and identified the specific regions that these PCR primers would amplify.

[b] sequences adapted from ref. (*1*).

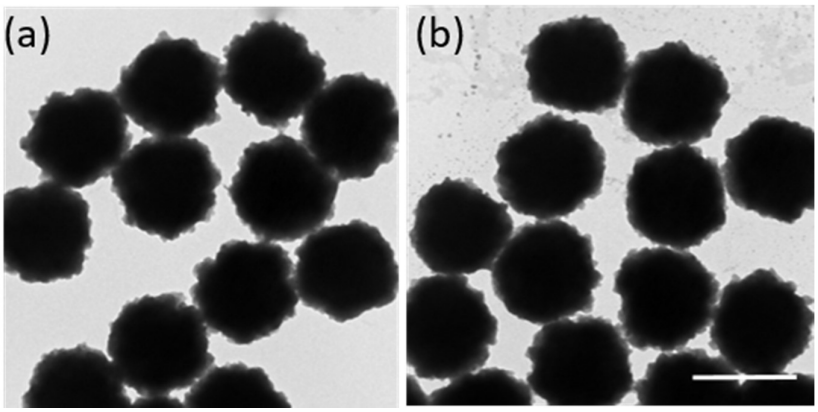

Figure S1. Transmission electron microscope (TEM) images of magnetic beads (a) before and (b) after biotin-S1 immobilization. The scale bar was 1 μm.

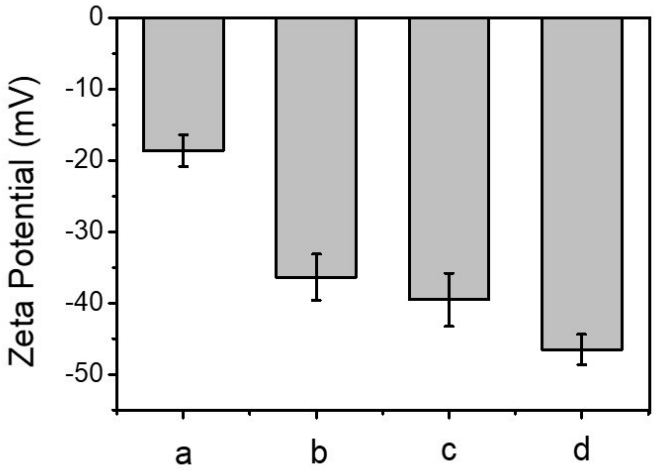

Figure S2. Zeta potential (mV) of (a) naked magnetic beads, (b) biotin-S1 modified magnetic beads, (c) biotin-S1/S2 modified magnetic beads and (d) biotin-S1/S2/S3 modified magnetic beads.

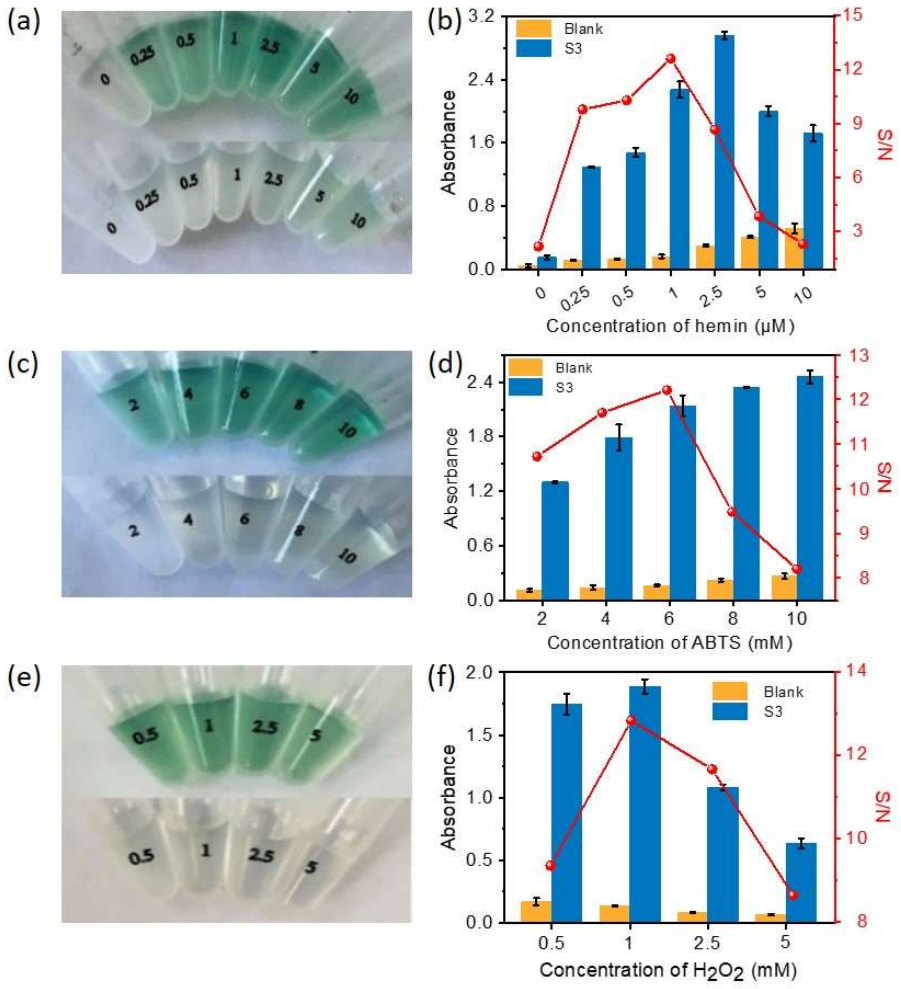

Figure S3. Optimization of the concentration of (a-b) hemin, (c-d) ABTS and (e-f) H_2O_2 in the absence and presence of 100 nM Ebola-S3 DNAzyme. (a, c, e) The photographs of DNAzyme under different concentrations. (b, d, f) The corresponding absorbance at 421 nm in a, c and e. The histogram represents the absorbance peak at 421 nm.

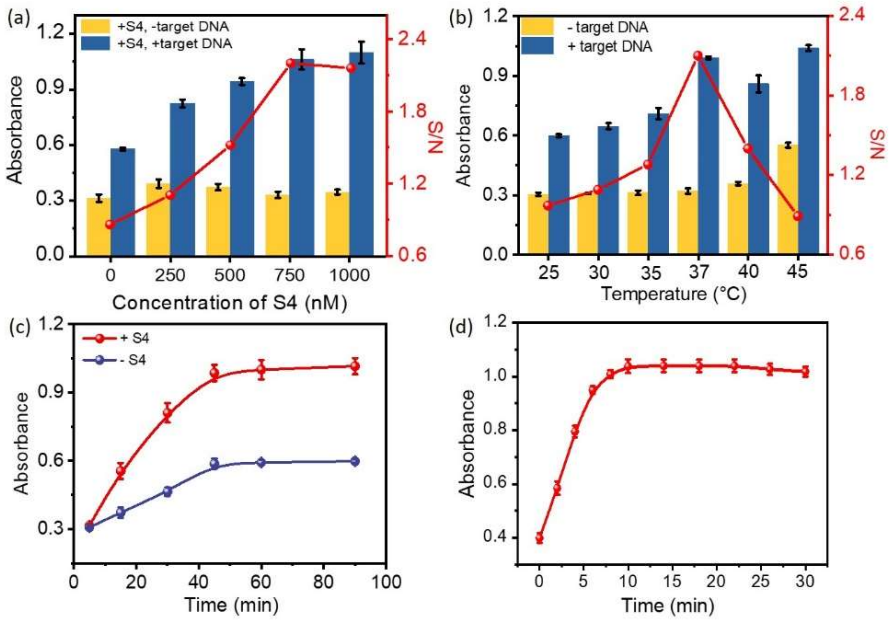

Figure S4. (a) Influence of different concentration (0, 250, 500, 750 and 1000 nM) of S4 in the presence and absence of 100 nM target DNA. (b) Influence of different temperature (25, 30, 35, 37, 40 and 45 °C) in the presence and absence of 100 nM target DNA. (c) The kinetic study of DNA circuit in the presence and absence of 750 nM S4. (d) The kinetic study of catalytic reaction of ABTS in the presence of 6 mM ABTS and 1 mM H_2O_2. The histogram in (a-b) and plots in (c-d) represents the absorbance peak at 421 nm.

Table S2. Analytical performance of different methods for detecting genetic targets of infectious pathogens.

Method	Instrument-dependent	Target	Linear Range	Detection Limit	Time	Ref.
Electro-chemistry	Yes	Single (Ebola)	10 nM-75 nM	4.7 nM	~ 2.5 h	2
Electro-chemistry	Yes	Single (H7N9)	1 pM-2.5 nM 2.5 nM -100 nM	0.75 pM	~ 1.5 h	3
Fluorescence	Yes	Single (H5N1)	500 pM-2 μM	500 pM	24 h	4
Colorimetry	No	Single (Dengue)	0-12 μM	0.12 μM	15 min	5
Colorimetry	No	Single (Ebola)	1 pM-1 nM	5.45×10^7 copies/mL	5 min	6
Colorimetry	No	Multiple (H7N9, Ebola, H1N1, Zika, Dengue)	0-125 nM (H7N9); 0-125 nM (Ebola); 0-125 nM (H1N1); 0-100 nM (Zika); 0-75 nM (Dengue)	24.0 pM (H7N9); 314.1 nM (Ebola); 875.0 pM (H1N1); 613.5 pM (Zika); 135.5 pM (Dengue)	< 2 h	This work

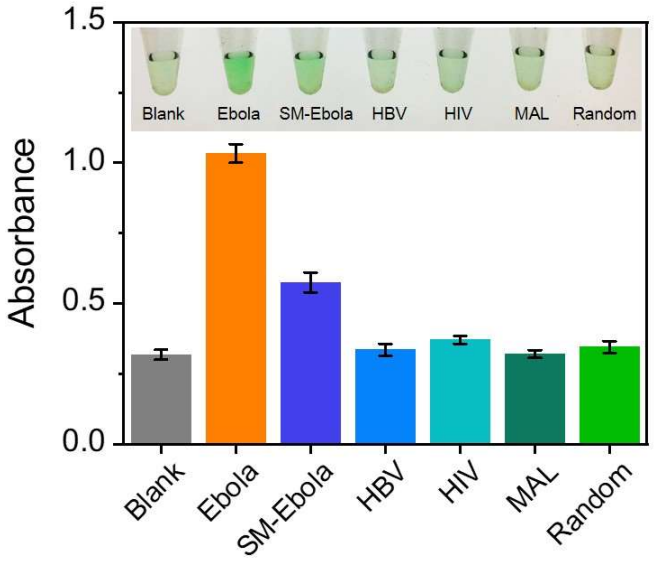

Figure S5. The absorption of the DNA circuits specific for Ebola gene in the absence (control) and presence of target Ebola DNA, single-base mismatched Ebola DNA (SM-Ebola), Hepatitis B Virus (HBV), HIV, Malaria (MAL) and a non-complementary virus gene with a random sequence (Random) under the same experimental conditions. The concentrations of all virus gene were 100 nM, respectively.

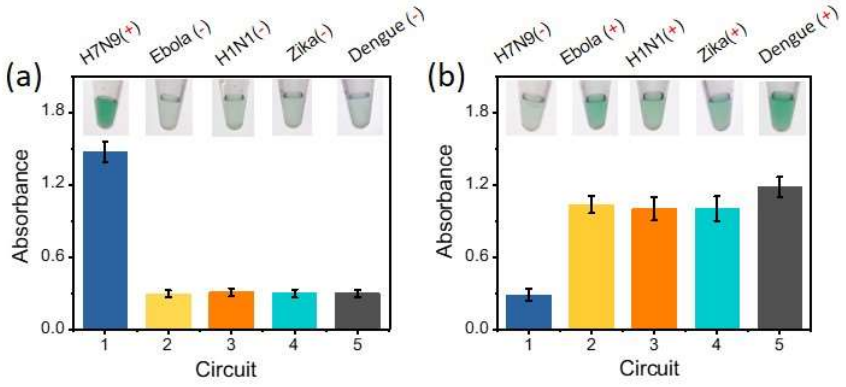

Figure S6. Parallel detection of unknown genetic targets by DNA circuits: (a) H7N9 DNA; (b) the mixture of Ebola DNA, H1N1 DNA, Zika DNA and Dengue DNA. The concentration for each target DNA was 100 nM. The histogram represents the absorbance peak at 421 nm and insets are their corresponding photographs.

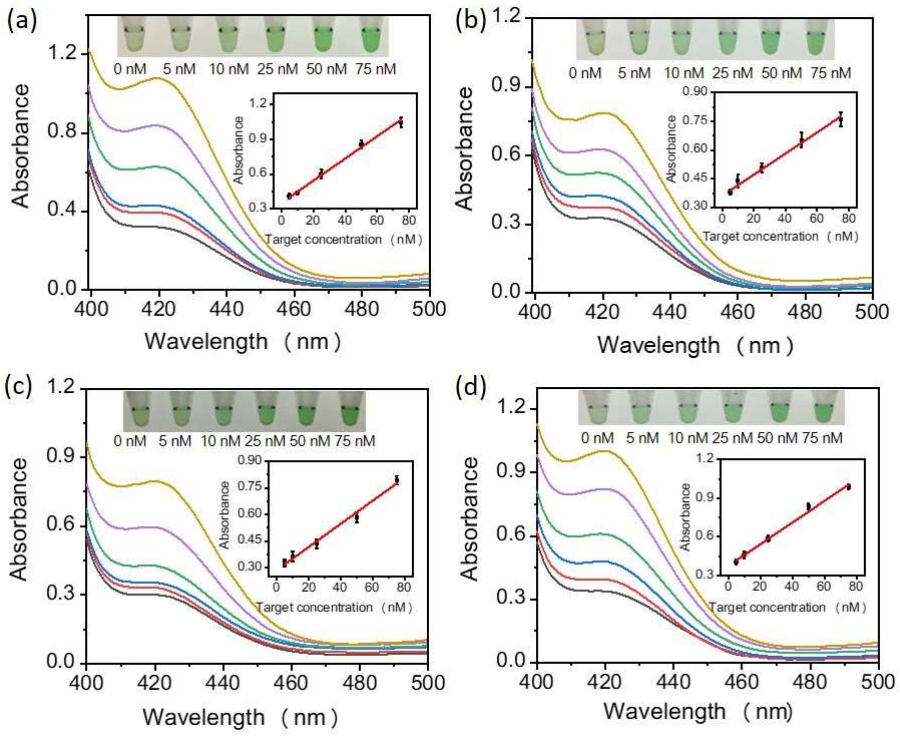

Figure S7. The UV-Vis spectra of DNA circuit for (a) H7N9 DNA, (b) H1N1 DNA, (c) Zika DNA and (d) Dengue DNA with different concentrations from 0-75 nM in the human serum samples. Insets are the corresponding photographs and calibration curves. For H7N9 DNA, the linear regression equation was $A_{421\ nm} = 0.009614\ c + 0.3497$ ($R^2 = 0.9916$); for H1N1 DNA, the linear regression equation was $A_{421\ nm} = 0.005548\ c + 0.3599$ ($R^2 = 0.9942$); for Zika DNA, the linear regression equation was $A_{421\ nm} = 0.006522\ c + 0.2872$ ($R^2 = 0.9942$); for Dengue DNA, the linear regression equation was $A_{421\ nm} = 0.008380\ c + 0.3756$ ($R^2 = 0.9876$), respectively.

Table S3. Recovery tests for H7N9 DNA in diluted human serums (n = 3).

Sample	Added (nM)	Found (nM)	Recovery (%)	RSD (%)
1	10	9.6 10.2 10.6	101.3	5.0
2	25	26.9 25.2 24.6	102.3	4.8
3	50	50.3 54.4 51.2	103.9	4.3

Table S4. Recovery tests for H1N1 DNA in diluted human serums (n = 3).

Sample	Added (nM)	Found (nM)	Recovery (%)	RSD (%)
1	10	10.2 10.5 9.7	101.3	4.0
2	25	27.4 25.6 24.7	103.6	5.5
3	50	49.7 54.1 50.3	102.7	4.8

Table S5. Recovery tests for Zika DNA in diluted human serums (n = 3).

Sample	Added (nM)	Found (nM)	Recovery (%)	RSD (%)
1	10	9.8 10.4 10.1	101.0	3.0
2	25	25.9 25.1 24.7	100.9	2.4
3	50	51.2 47.1 49.0	98.2	4.1

Table S6. Recovery tests for Dengue DNA in diluted human serums (n = 3).

Sample	Added (nM)	Found (nM)	Recovery (%)	RSD (%)
1	10	10.4 9.4 10.1	99.7	5.1
2	25	26.1 23.8 25.4	100.4	4.7
3	50	52.1 50.2 54.7	104.7	4.5

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