Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Supporting Information

Equipment-Free and Visualized Biosensor for Transcription Factor Rapid Assay Based on Dopamine-Functionalized Cellulose Paper

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Experimental Section

Chemical and Materials. Exonuclease III (Exo III) were purchased from New England Biolabs Inc. (Beverly, MA, USA). Hemin, dopamine hydrochloride (DA·HCl), tris(hydroxymethy)aminomethane (Tris), $MgCl_2$, KCl, and other chemicals with analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). NF-κB p50 was purchased from from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA), C-Jun and insulin were purchased from Solarbio (Beijing, China).The Milli-Q water (Millipore Corp., Bedford, MA, U.S.A.) was used in all experiments. Whatman cellulose chromatography paper were purchased from Sigma-Aldrich Company (St. Louis, USA). All oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) with their sequences listed in Table S1.

Apparatus. Fourier transform infrared spectra (FT-IR) were recorded on a NICOLET 380 FT-IR spectrometer (Nicolet Thermo, USA). Scanning Electron Microscope (SEM) and energy dispersive spectroscopy (EDS) images were conducted on a HITACHI S4800 SEM (Hitachi, Japan). The gel electrophoresis characterization was conducted on a Gel Doc XR + Imaging System (BIO-RAD, America) by using SYBR Gold as the dye. Contact angle was recorded on an OCA25 Contact angle measuring instrument (Dataphysics, Germany). UV-vis spectra were performed on a U-4100 Ultraviolet spectrometer (Hitachi, Japan). All measurements were carried out at room temperature.

Fabrication of DA-PBs. DA·HCl (0.0038 g, 0.02 mmol) was dissolved into 1.0 mL ultrapure water to prepare the stock solution (20 mM). Then 5.0 μL DA solution was dropped into cellulose paper surface. After that, the DA-PBs was vacuum dried overnight at room temperature.

Detection of NF-κB p50 in solution. Prior to analysis, P1-P2 duplex was prepared through the heating treatment (the solution comprising of P1 and P2 was heated to 95 ^{o}C, and maintained at 95 ^{o}C for 5 min. After that, the solution was slowly cooled down to room temperature.), and P3 was treated following the same procedure to form the desired hairpin structure. The target-initiated binding experiment was carried out in 30 μL Tris-HCl solution (10 mM, 100 mM KCl, 20 mM $MgCl_2$, 0.25 mM DTT, 0.1 mM EDTA, 0.1 mg/mL yeast tRNA, 10% glycerol, pH 7.5) containing 2.0 μM P1-P2 duplex and NF-κB p50 with different concentrations for 20 min to complete the recognizable experiment. Subsequently, 30 μL Tris-HCl solution (10 mM, 100 mM KCl, 20 mM $MgCl_2$, 0.25 mM DTT, 0.1 mM EDTA, pH 7.5) comprising of 100 U/mL Exonuclease III (Exo III) and 30.0 μM P3 was added and the mixing solution was permitted to react for 30 min. The mixture was then heated to 90 ^{o}C and maintained at 90 ^{o}C for 10 min. After that, 20 μL of 30 μM hemin was incubated with the above mixing solution for 45 min to generate hemin/G-quadruplexes. Another 20 μL solution containing 5 mM H_2O_2 and 15 mM DA was added into the above solution. Finally, the UV-vis spectra of the reaction system in the range of 400-700 nm were recorded.

Detection of NF-κB p50 Using DA-PBs. The target-initiated binding experiment was carried out in 30 μL Tris-HCl solution (10 mM, 100 mM KCl, 20 mM $MgCl_2$, 0.25 mM DTT, 0.1 mM EDTA, 0.1 mg/mL yeast tRNA, 10% glycerol, pH 7.5) containing 20 μM P1-P2 duplex and NF-κB p50 with different concentrations for 20 min. Subsequently, 30 μL Tris-HCl solution (10 mM, 100 mM KCl, 20 mM MgCl2, 0.25 mM DTT, 0.1 mM EDTA, pH 7.5) comprising of 600 U/mL Exonuclease III (Exo III) and 300 μM P3 was added and the solution was permitted to react for 30 min. The mixture was then heated to 90 ^{o}C and maintained at 90 ^{o}C for 10 min. Subsequently, 20 μL of 300 μM hemin was incubated with it for 45 min to generate hemin/G-quadruplexes. Another

20 μL solution containing 50 mM H_2O_2 was added into the above solution. Finally, 3 μL of the resulting solution was dropped on the surface of DA-PBs and reacted for 20 s. Then, the images of DA-PBs were obtained from the mobile phone, and the signal intensity was read directly by the naked eyes for equipment-free detection of NF-κB p50.

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Table S1. Sequences of the oligonucleotides used in the experiments

Probe Name	Sequence (5′-3′)
P1	GGGACTTTCCAACCAAATGTCAGCCCTAACCCTAACCCTATA TATAT
P2	ATATATATAGGGTTAGGGTTAGGGCTGACATTTGGTTGGAAA GTCCC
P3	ATAGGGTTAGGGTTAGGGTTAGGGAACCAAATGTCAGCCCTA ACCCTAACCCTATATATAT
P1'	ACTTATCAGCTTAAGGATCTTCTCGACAGCTCTAGTAG
P2'	GCTACTAGAGCTGTCGAGAAGATCCTTAAGCTGATAAGT
P1''	TTAGCGACTCGTTAGCAAGGCTGAGGTCTTCAGCAAGCTGT
P2''	ACAGCTTGCTGAAGACCTCAGCCTTGCTAACGAGTCGCTA
P1'''	TGAGGTCAAGTCTGCACATTGGTTAGCTGAGGTCAAGTCTGC ACATTGGTTA
P2'''	TAACCAATGTGCAGACTTGACCTCAGCTAACCAATGTGCAGA CTTGACCTCA

Table S2. NF-kB P50 assay performance of our strategy and other methods.

Method	Linear Range	Detection Limit	Ref.
Colorimetric	5 pM~2 nM	3.8pM	1
Chemiluminescence	10 fM~1 nM	6.03×10^{-15} M,	2
Electrochemical	10 pM ~ 5000 pM	10pM	3
Fluorescence	30 pM~ 1.5 nM.	10pM	4
Electrochemical	1 pM~ 5 nM	0.23 pM	5
Fluorescence	0.5 nM~50 nM	0.2nM	6
Fluorescence	0 ~1000 pM	2.2pM	7
Colorimetric	40 nM ~1500 nM (solid)	40 nM	This work
	0.8 nM ~30 nM (solution)	0.26 nM	

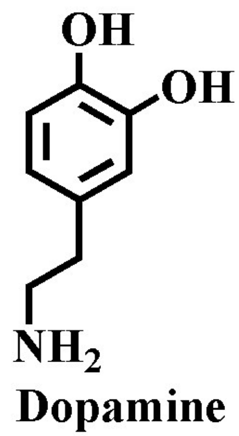
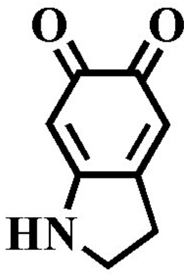


Fig. S1 Molecular structure of dopamine



Aminochrome

Fig. S2 Molecular structure of Aminochrome

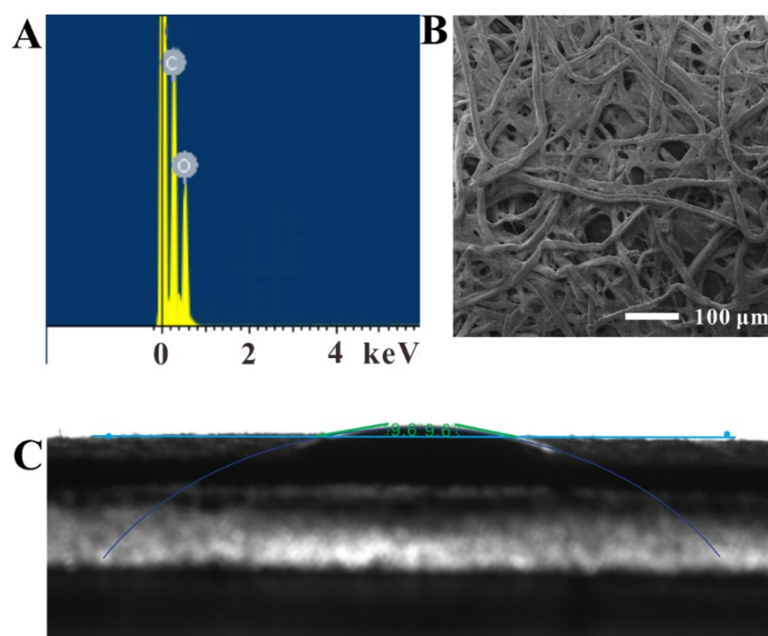


Fig. S3 (A) Energy dispersive spectroscopy of cellulose paper. (B) SEM image of cellulose paper. (C) Contact angle of cellulose paper

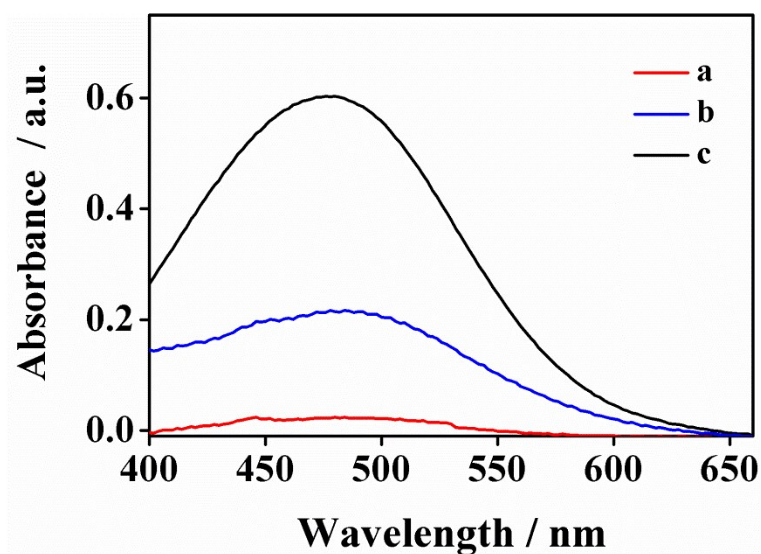


Fig. S4 UV-vis spectra of the solution-phase biosensor under different conditions: (a) in the absence of target NF-κB p50, (b) in the presence of 10 nM NF-κB p50, and (c) in the presence of 30 nM NF-κB p50.

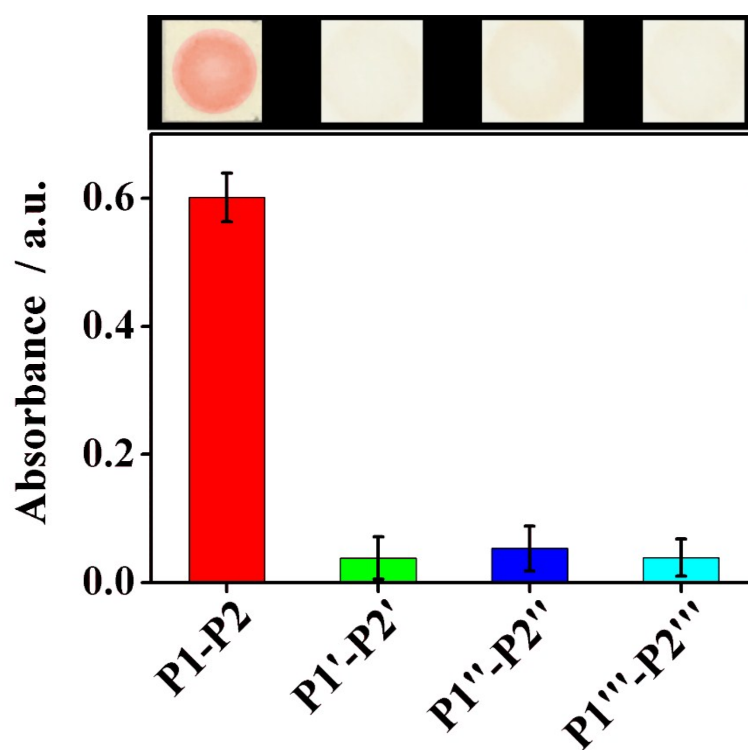


Fig. S5 The output signals of the developed solid sensor and solution-phase sensor by different DNA correspond to target analyte.

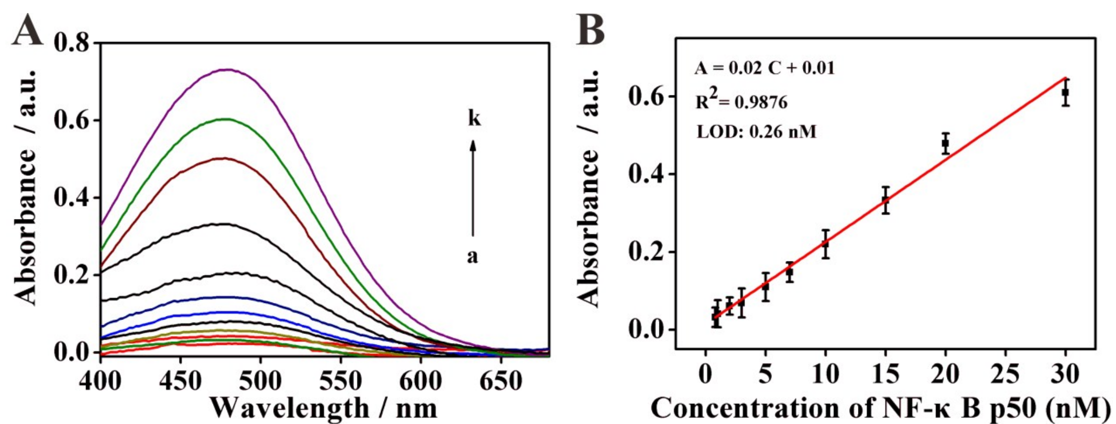


Fig S6. (A) UV-vis responses of the solution-phase biosensor in the presence of NF-κB p50 with different concentrations: 0, 0.8, 1, 2, 3, 5, 7, 10, 15, 20, 30, and 40 nM. (B) The linear plot of absorbance versus Dam MTase concentration. The error bars represent the standard deviation of three measurements.

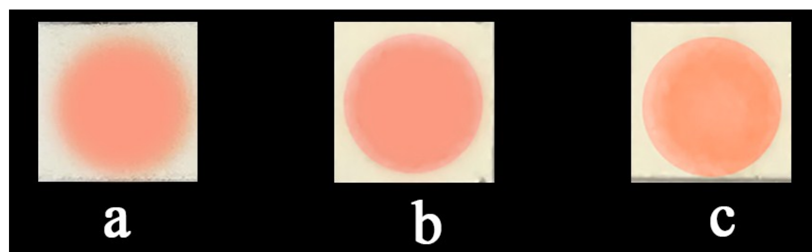


Fig. S7 Color signals of three differently fabricated DA-PBs for 1500 nM NF-κB p50 assay.

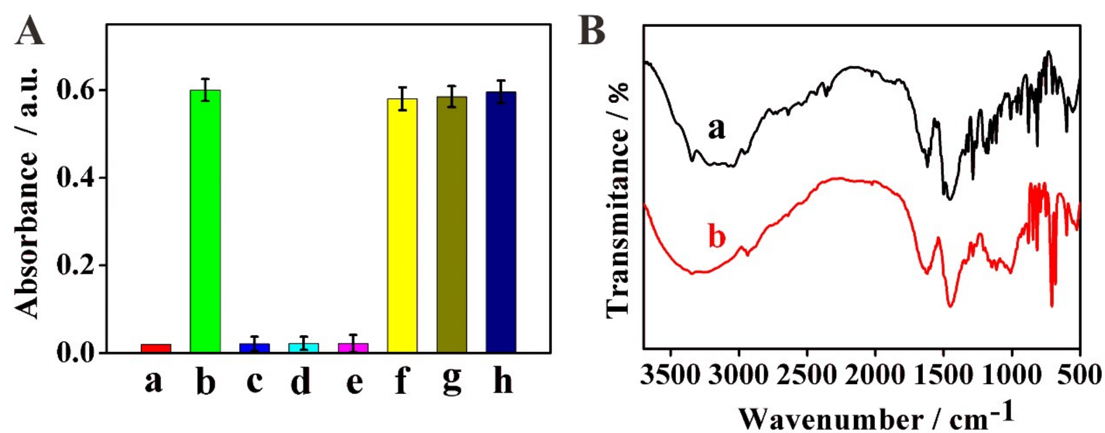


Fig. S8 (A) The absorbance of the solution-phase biosensor under different conditions: (a) control sample; (b) NF-κB p50; (c) Insulin; (d) C-Jun; (e) BSA; (f) NF-κB p50 + Insulin; (g) NF-κB p50 + C-Jun; (h) NF-κB p50 + BSA. The error bars represent the standard deviation of three measurements. (B) FT-IR spectra of different samples: (a) dopamine, (b) the treated DA-PBs after storing for 7.0 days at 4 ^{o}C.

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