Supporting Information

Dopamine-Based Paper Analytical Device for Truly Equipment-Free and Naked-Eye Biosensing Based on the Target-Initiated Catalyzed Oxidation

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EXPERIMENTAL SECTION

Chemical and Materials. All DNAs were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China), with their sequences exhibited in Table S1. Dam MTase, M.Sss I MTases, M. CviP I MTases, MsP I MTases, Dpn I, S-adenosylmethionine (SAM), Klenow fragment (KF) polymerase and Nb.BbvCI NEase were purchased from New England Biolabs, Ltd. (Beijing, China).The deoxynucleotide triphosphates (dNTPs), hemin, 5-fluorouracil, benzylpenicillin, gentamycin, dopamine hydrochloride (DA) and other chemicals with analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All solutions were prepared with ultrapure water (Millipore, Milford, MA).

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

Detection of G-quadruplex DNA in Solution. The G-quadruplex DNA assay was carried out in 85 μL of 10 mM Tris-HCI reaction solution (50 mM NaCl ， 50 mM $MgCI_2$, 100 mM KCl, pH 7.4) containing 2.35 μM hemin and target G-quadruplex DNA with different concentrations for 45 min to generate hemin/G-quadruplexes. Subsequently, 15 μL solution containing 6.0 mM H_2O_2 and 20 mM dopamine was added into the above solution. Finally, the UV-vis spectra in the range of 400-700 nm were recorded

Detection of Dam MTase activity in Solution. The Dam MTase-initiated methylation reaction was performed in 20 μL of reaction solution 10 mM Tris-HCl (50 mM NaCI, 10 mM $MgCl_2$, pH 7.5) containing 1.0 μM H1, 1.0 mM DTT, 80 μM SAM, 80 U/mLDpnI, and target Dam MTase with different concentrations for 2.0 h. After that, 50 μL of 10 mM Tris-HCl solution (50 mM NaCl, 50 mM $MgCl_2$, 100 mM KCl, 1 mM DTT, pH 7.5) comprising of 800 nM P1, 0.1 U/μL KF polymerase, 0.6 U/μL Nb.BbvCI NEase, and 350 μM dNTP was added and reacted for 1.5 h to complete the EXPAR. The mixture was then heated to 90 ℃ and maintained at 90 ℃ for 10 min to eliminate the influence of DTT. Then, 15 μL of 13.3 μM hemin was incubated with it for 45 min to generate hemin/G-quadruplexes. Another 15 μL solution containing 6.0 mM H_2O_2 and 20 mM dopamine was added into the above solution. Finally, the UV-vis spectra in the range of 400-700 nm were recorded

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Table S1. Sequences of the oligonucleotides used in the experiments

Probe Name	Sequence (5′-3′)
H1	ATATAAGAGATGGATCAAGTAACCAATGTGCAGACTTGATCC ATCTCTTATAT
A1	AAGTAAGTAGTGCGAGTTAGTGATGAAACACACACACACTTC ATCACTAACTCGCACTACTTACTT
B1	ATAGGGTTAGGGTTAGGGTTAGGGAACCAAATGTCAGCCCTA ACCCTAACCCTAT
C1	CTATTAGGGTTAGGGTTAGGGAACCAAATGTCAGCCCTAACC CTAAGAT
P1	CCCTAACCCTAACCCTAACCCCCTCAGCTCAAGTCTGCACAT TGGTTACTTGACCTCAGCTCAAGTCTGCACATTGGTTACTTGA TACTTGAACATTGGTTACTTGA
T1	GGGTTAGGGTTAGGGTTAGGG
T2	TGAGGGTGGGTAGGGTGGGTAA
T3	AGGGAGGGCGCTGGGAGGAGGG
T4	TTACGACTTTCCAACCAAATGTCAG
T5	TGACATCTGTGGTTGGAGGCATATTA

Table S2. Dam MTase assay performance of our strategy and other methods.

Method	Linear Range	Detection Limit	Ref.
Fluorescence	0.05 ~ 40 U/mL	0.003 U/mL	1
Chemiluminescence	0.025~400 U/mL	0.000129 U/mL	2
Fluorescence	0~20 U/mL	0.25 U/mL	3
Fluorescence	0~50.0 U/mL	0.01 U/mL	4
Electrogenerated chemiluminescence	0.1~100 U/mL	0.03 U/mL	5
Fluorescence	1~8 U/mL	0.014 U/mL	6
Colorimetric	6~100 U/mL	6.0 U/mL	7
Colorimetric	1~10 U/mL	0.3 U/mL	8
Colorimetric	4.0~120 U/mL (solid) 0.05~10.0 U/mL (solution)	1.46 U/mL 0.023 U/mL	This work

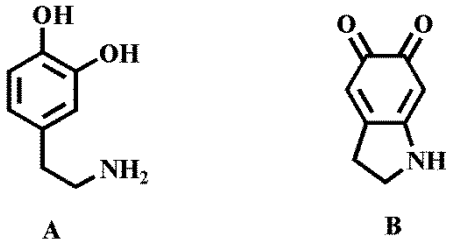

Figure S1. (A) The structure of dopamine. (B) The structure of dopachrome.

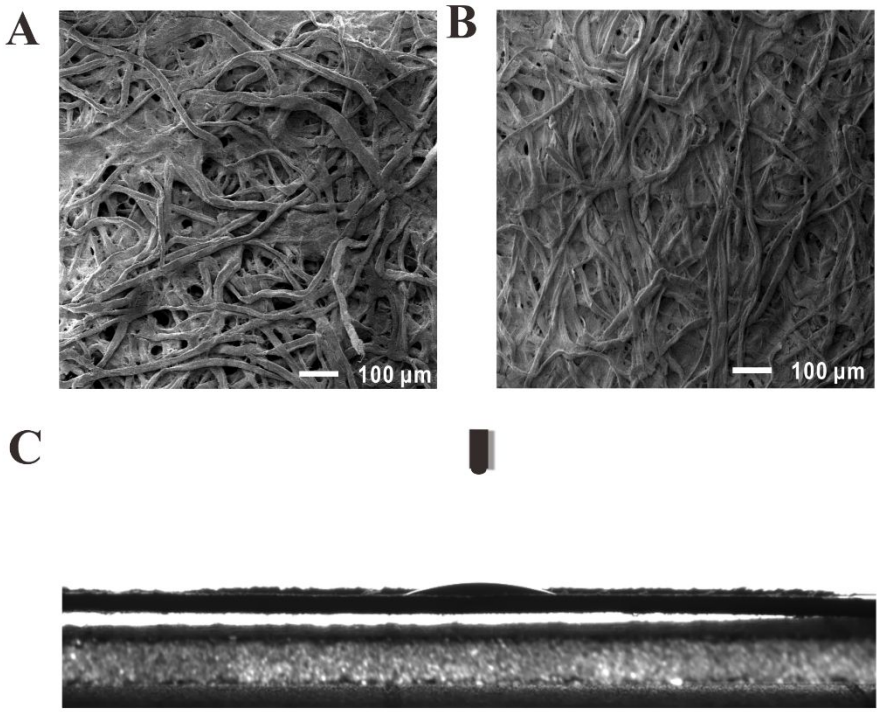

Figure S2. (A) SEM image of cellulose paper. (B) SEM image of DPAD. (C) Contact angle of cellulose paper

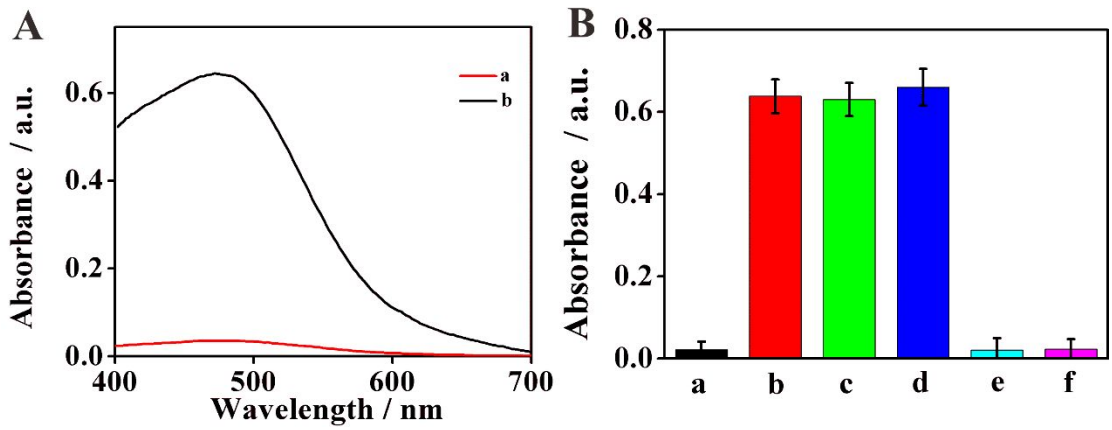

Figure S3. (A) UV-vis spectra of the solution-phase biosensor under different conditions, (a) in the presence of T1 (400 nM), and (b) in the absence of T1. (B) The absorbance of the solution-phase biosensor under different conditions: (a) blank sample, (b) T1, (c) T2, (d) T3, (e) T4, and (f) T5. The error bars represent the standard deviation of three measurements.

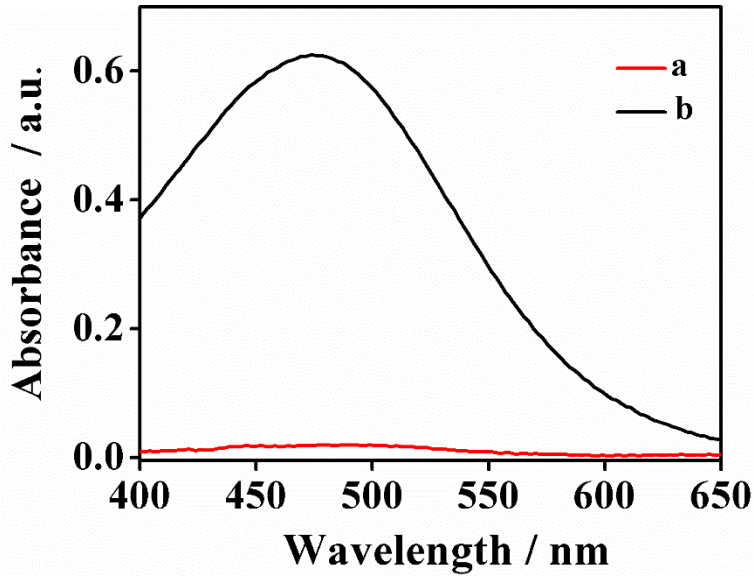

Figure S4. UV-vis spectra of the solution-phase biosensor under different conditions: (a) in the absence of target Dam MTase, and (b) in the presence of Dam MTase (10 U/mL).

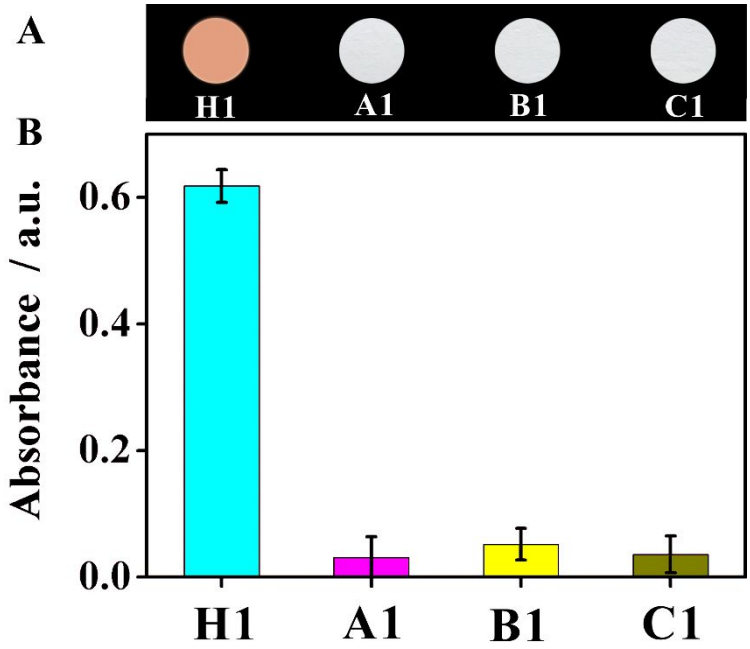

Figure S5. (A) Images of DPAD in the presence of 120 U/mL under different conditions. (B) Absorbance at 475 nm of the sensing system in the presence of 10 U/mL Dam MTase under different conditions.

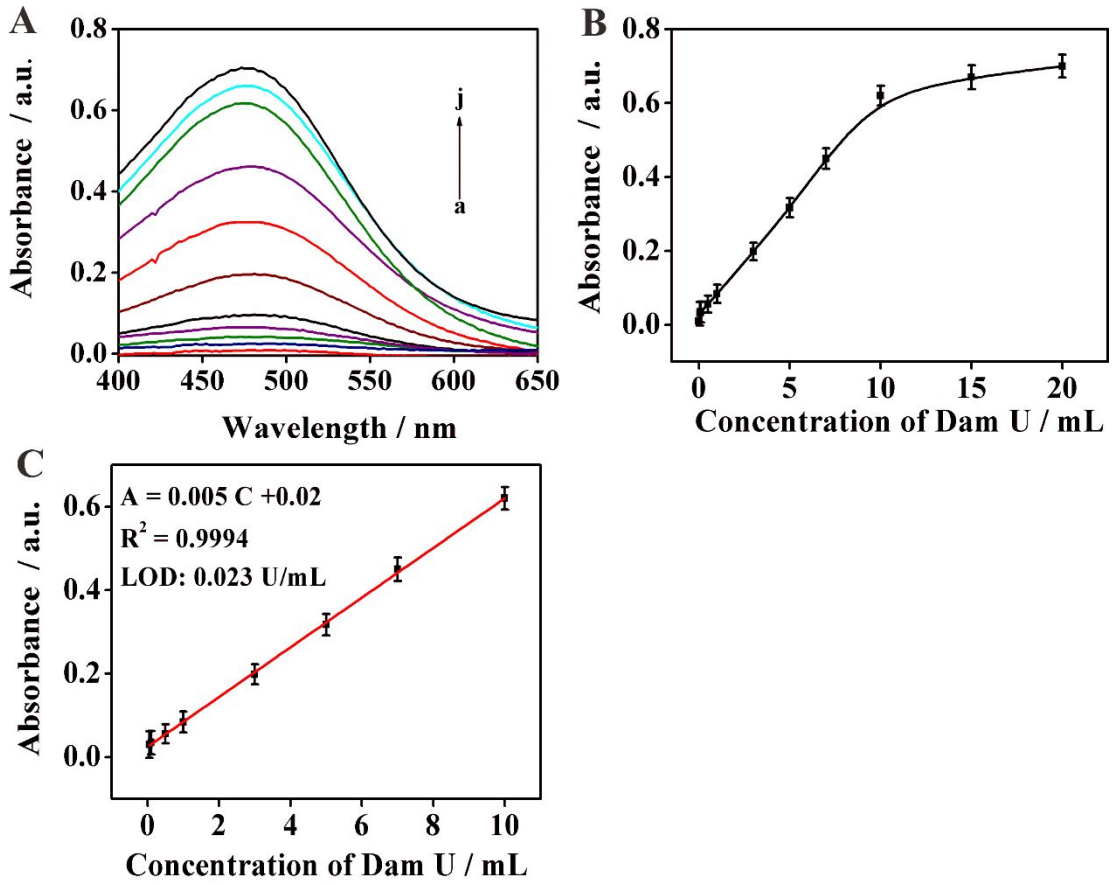

Figure S6. (A) UV-vis responses of the solution-phase biosensor in the presence of Dam MTase with different concentrations: 0, 0.05, 0.1, 0.5, 3, 5, 7, 10, 15, and 20 U/mL. (B) Absorbance at 475 nm of the sensing system versus Dam MTase with different concentrations. (C) The linear plot of absorbance versus Dam MTase concentration. The error bars represent the standard deviation of three measurements.

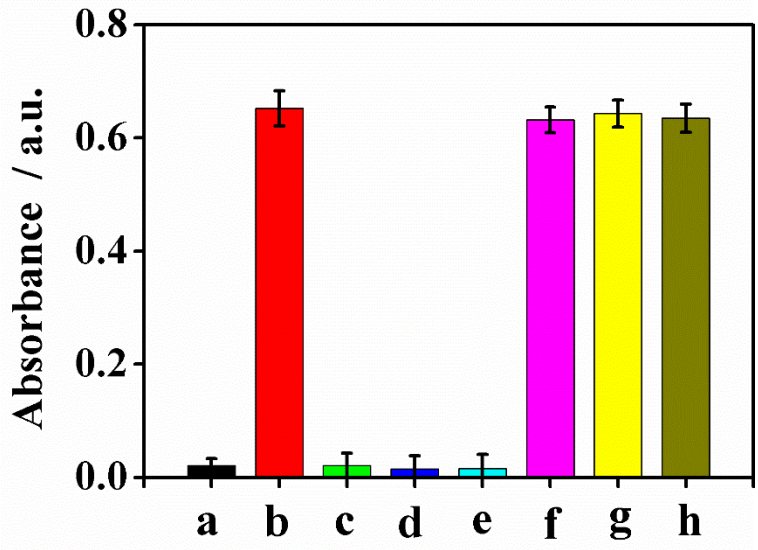

Figure S7. The absorbance of the solution-phase biosensor under different conditions: (a) blank sample; (b) Dam MTase; (c) M.Sss I; (d) M. CviP I; (e) MsP I; (f) Dam + M. Sss I; (g) Dam + M. CviP I; (h) Dam + MsP I. The error bars represent the standard deviation of three measurements.

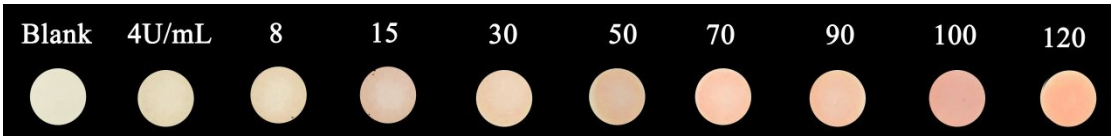

Figure S8. Stability experiment of DPAD after the storage at 4 °C for 14.0 days subject to different concentrations of Dam MTase.

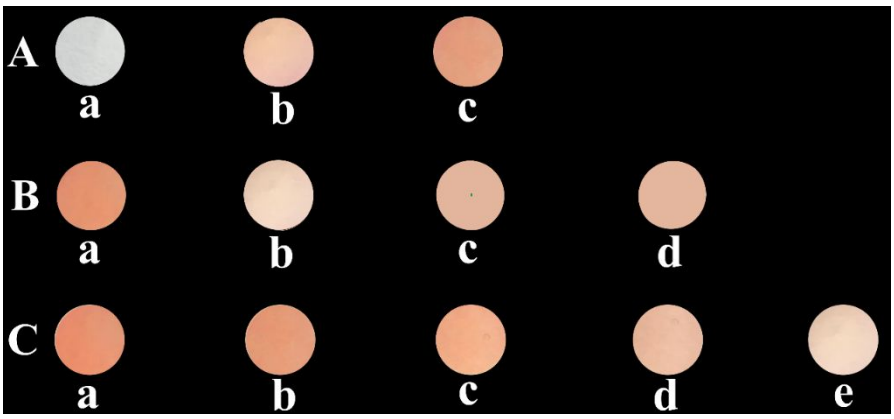

Figure S9. (A) The images of DPAD correspond to different amount of Dam MTase in human serum samples: (a) blank sample, (b) 40 U/mL, and (c) 120 U/mL. (B) The images of DPAD correspond to different inhibitors: (a) blank sample, (b) 5-fluorouracil, (c) benzylpenicillin, and (d) benzylpenicillin. The concentrations of them are 60 μM. (C) The images of DPAD correspond to 5-fluorouracil with different concentrations: (a) 0 μM, (b) 2.5 μM, (c) 5.0 μM, (d) 50 μM, and (e) 60 μM.

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