Electronic Supplementary Information

Diagnosis of *Mycobacterium Tuberculosis* Using Palladium-platinum Bimetallic Nanoparticles on Paper-Based Analytical Devices

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EXPERIMENTAL SECTION

Design, manufacturing, and surface modification of the dnPAD. The dnPAD used in this study consists of two 3D printed plastic cartridges and four layers of Whatman® cellulose chromatography paper, including grade 1 Chr for the first layer (referred to as the detection pad) and grade 3MM Chr for the second to fourth layers (referred to as the absorbent pads). The process for preparing the wax-printed paper is shown in **Fig. S1**. The first layer was the detection pad, whose main function was the area for DNA hybridization. We used hydrophobic wax printing to define circular wells (6.0 mm in diameter) as detection wells on the hydrophilic paper. After printing, the first layer of paper was dried in an oven at 110 °C for ten minutes to form a hydrophobic barrier. The second layer of paper was also printed with wax to define circular wells (7.5 mm in diameter) and then dried in an oven at 150 °C for twenty minutes.[1] The following two absorbent pads were printed with wax by the same process to form four square-shaped patterns (12 mm × 12 mm) per layer and also dried in an oven at 150°C for twenty minutes. Their main function was absorbing the washing buffer in order to wash the detection wells. Following these steps, the detection and absorbent pads were cut into a size of 34 mm × 28.5 mm.

In the detection pad, we used CMC to non-covalently modify the cellulose chromatography paper, which could increase the platform's hydrophilicity, and then used EDC/NHS to improve the binding affinity.[2] First, we dissolved 0.5 mg mL^{-1} CMC into 25 mM $CaCl_2$ solution, then CMC solution (100 μL) was added to the detection wells, which reacted with the cellulose paper at room temperature for ten minutes, followed by drying at 40 °C for five minutes. Afterwards, NaAc buffer (100 μL) containing EDC (0.1 M) and NHS (0.4 M) was added to the CMC-modified detection wells and allowed to react for thirty minutes, followed by drying at 40 °C for five minutes. Finally, capture DNA (3 μL), NaCl (3 μL), and blocking buffer (various %

BSA in PBS with 0.1% Tween-20) (3 μL) were added to the detection wells sequentially and incubated at room temperature for ten minutes. The surface-modified detection pad was then dried at room temperature and kept at 4 °C before testing.

Preparation of the Pd@Pt NPs conjugated with reporter DNA. Pd@Pt NPs (20 μL), reporter DNA (40 nM, 180 μL), and deionized water were evenly mixed and incubated at 37 °C for ten minutes. Then, the mixture was centrifuged at 790 ×g for five minutes (23 °C) to remove excess reporter DNA. Finally, the reporter DNA-Pd@Pt NPs (detector) were resuspended in PBS solution and stored at 4 °C for further use.

The effect of solution pH on the reporter DNA-Pd@Pt NPs. First, the reporter DNA-Pd@Pt NPs (0.01 mg mL^{-1}) were added into a NaAc-HAc buffer solution (0.2 M, pH = 3.0-8.0). Subsequently, TMB (0.41 mM) and H_2O_2 (0.25 M) were added, and the mixture was allowed to react for 60 seconds. Following the reaction, the absorbance peak intensity at 652 nm was measured to assess the catalytic activity of the reporter DNA-Pd@Pt NPs at various pH values.

Optimization of target DNA detection on the dnPAD. We divided the detection wells into control (C) and test (T) wells used for blank and sample groups. Firstly, target DNA (3 μL) was added to the T well, and the same volume of PBS was added to the C well. Then, the reaction was incubated at 65 °C for two minutes, followed by a thirty-second cooling period. Next, the reporter DNA-Pd@Pt NPs (3 μL) was added to all wells, followed by incubation at 65 °C for two minutes and a thirty-second cooling period.

After the reaction was completed, washing buffer (5% BSA in PBS with 0.1% Tween-20) (100 μL) was added to remove any non-specific binding substances (e.g., capture DNA, BSA, target DNA, and reporter DNA-Pd@Pt NPs). Finally, TMB substrate (3 μL) was added to all wells, and the resulting color change was produced by the catalytic reaction between TMB and reporter DNA-Pd@Pt NPs. After the five-minute reaction, we used a smartphone to capture the images of the color change results on the detection pad (shutter speed (S) = 1/125, ISO = 50, exposure value (EV) = 0, white balance (WB) = 5250 K). The B/R value of the images was analyzed using ImageJ (National Institutes of Health, Bethesda, MD) based algorithm to obtain the concentration of target DNA. In addition, we optimized various parameters in the detection process of target DNA, including the reaction temperature (58, 65, and 68 ℃), reaction time (1, 3, 5, 7, and 9 minutes), capture DNA (0.3, 3.0, and 5.0 μM), NaCl (0.01, 0.10, and 1.0 M), BSA (1, 5, and 10%), reporter DNA (0.5, 1.0, and 2.5 μM), and reporter DNA-Pd@Pt NPs (0.05, 0.10, and 0.51 mg mL^{-1}). The optimal conditions were confirmed through experiments, which were the reaction temperature of 65 ℃, the reaction time of five minutes, 3 μM of the capture DNA, 0.1 M of NaCl, 5% of BSA, 1.0 M of the reporter DNA, and 0.1 mg mL^{-1} of the reporter DNA-Pd@Pt NPs.

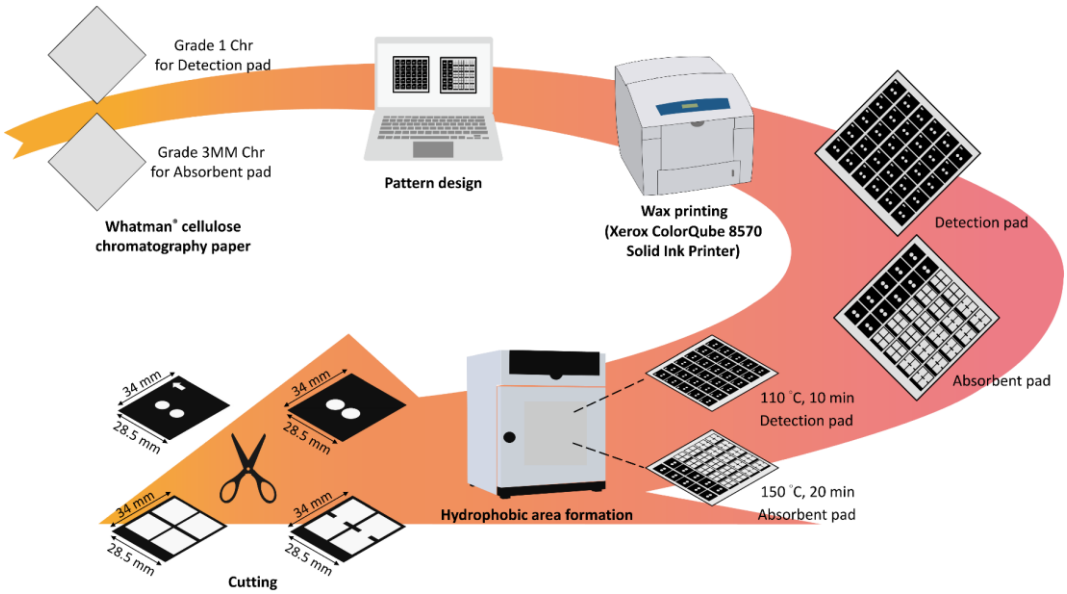

Fig. S1. Schematic diagram of the pattern design, wax printing, hydrophobic area formation, and cutting procedures for the Whatman® cellulose chromatography paper sheets.

Table S1. DNA sequence information required for DNA hybridization.

Sequence name	Genome position	Sequence information (5′→3′)	Tm (℃)
Capture DNA	960-972	NH_2-$(CH_2)_6$-GTCCAGCTCATGC	42
Target DNA	960-985	CAGGTCGAGTACGCCTTCTTGTTGGC	63
Reporter DNA	973-985	GGAAGAACAACCG-$(CH_2)_6$-NH_2	40

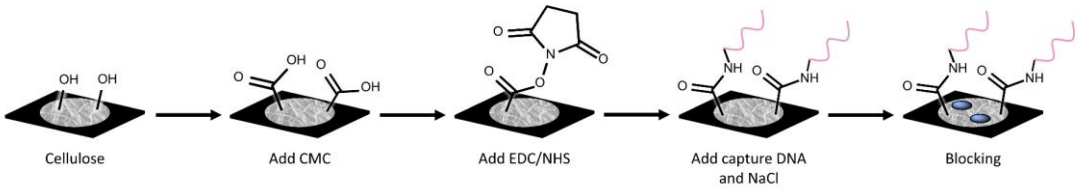

Fig. S2. Schematic diagram of the pretreatment procedures of the un-treated wax areas, including the CMC-EDC/NHS surface modification, adding of the capture DNA and NaCl, and BSA blocking.

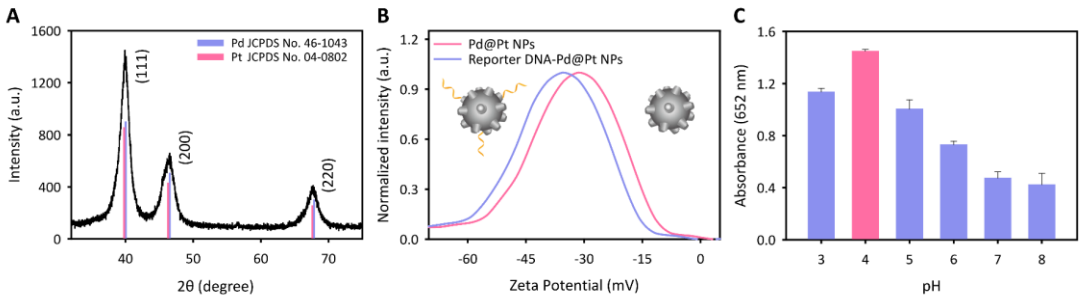

Fig. S3. (A) XRD images of the Pd@Pt NPs. (B) Zeta-potential analysis of the Pd@Pt and reporter DNA-Pd@Pt NPs. (C) The effect of pH value in 0.2 M NaAc-HAc buffer on the absorbance value of the reporter DNA-Pd@Pt NPs.

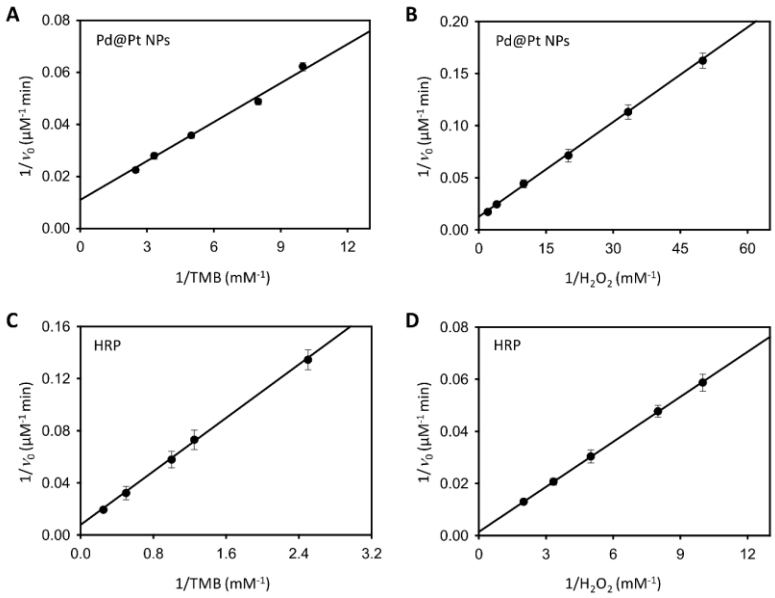

Fig. S4. Catalytic kinetics of Pd@Pt NPs and HRP. Double reciprocal plots of catalytic kinetics of Pd@Pt NPs reacting with (A) TMB, (B) H_2O_2. Double-reciprocal plots of HRP reacting with (C) TMB, (D) H_2O_2.

Table S2. Comparison of the catalytic kinetic parameters (K_m, v_{max}, k_{cat} and k_{cat} / K_m) of the Pd@Pt NPs and HRP.

Enzyme	Substrate	K_m (mM)	v_{max} (M s^{-1})	k_{cat} (s^{-1})	K_{cat} / K_m (M^{-1} s^{-1})
Pd@Pt NPs	TMB	0.46	1.49×10^{-8}	1.23×10^{6}	2.65×10^{9}
	H_2O_2	0.24	1.25×10^{-8}	5.12×10^{5}	2.14×10^{9}
HRP	TMB	6.58	2.11×10^{-6}	8.49×10^{2}	1.29×10^{5}
	H_2O_2	4.12	1.23×10^{-5}	4.59×10^{3}	1.12×10^{6}

Table S3. The comparison of catalytic efficiency (k_{cat} / K_m) of different enzymes.

Enzyme	Substrate	K_{cat} / K_m (M^{-1} s^{-1})	Ref.
Pd NPs	TMB	5.1×10^{-1}	3
PdCu Nanocorals	TMB	8.1×10^{-1}	3
Pd cubes	TMB	2.5	4
Dendritic Pt nanoparticles	TMB	5.9	5
Pt-Pd NPs	TMB	8.0	6
Pd@Pt core–frame nanodendrities	TMB	1.7×10^{1}	4
Pt hollow nanodendrites	TMB	2.1×10^{1}	4
Au NRs	TMB	1.7×10^{2}	7
Pd@AuNR	TMB	3.5×10^{2}	8
FeS_2/SiO_2	TMB	2.3×10^{3}	9
Au@Pt NPs	TMB	6.1×10^{8}	10
Pd@Pt-HRP NPs	TMB	9.7×10^{8}	11
Ni@Pt NPs	TMB	1.3×10^{9}	12

Pd@Pt NPs	TMB	2.7×10^{9}	This work

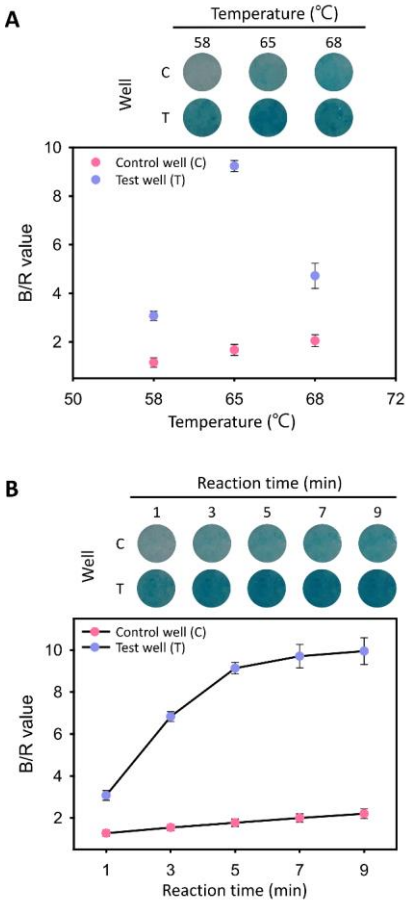

Fig. S5. The effect of temperature and reaction time on the dnPAD sensing system. (A) Colorimetric results and the corresponding B/R value comparison chart for different temperatures, including 58, 65, and 68 ℃. (B) Colorimetric results and the corresponding B/R value comparison chart for different reaction times, including 1, 3, 5, 7, and 9 min.

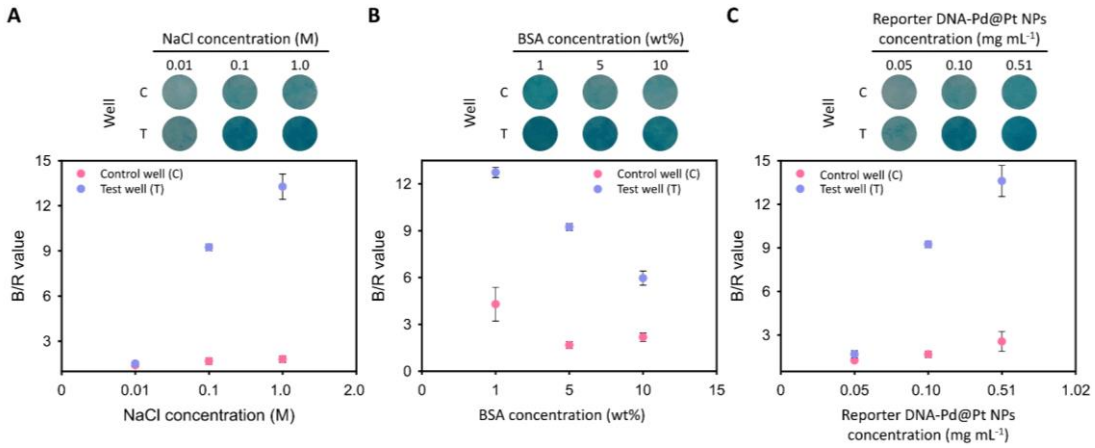

Fig. S6. The effect of concentration of NaCl, BSA, and reporter DNA-Pd@Pt NPs on the dnPAD sensing system. (A) Colorimetric results and the corresponding B/R value comparison chart for different concentrations of the NaCl, including 0.01, 0.1, and 1 M, (B) Colorimetric results and the corresponding B/R value comparison chart for different concentrations of the BSA, including 1, 5, and 10%, (C) Colorimetric results and the corresponding B/R value comparison chart for different concentrations of the reporter DNA-Pd@Pt NPs, including 0.05, 0.10, and 0.51 mg mL^{-1}.

Table S4. The component and cost of the dnPAD.

Component cost	Supplier	Cost/Device (dollars)
Cartridge (ABS)	GO HOT TEK	$ 0.310
Cellulose papers	Whatman	$ 0.004
Capture DNA	Protech Technology Enterprise	$ 0.006
Reporter DNA	Protech Technology Enterprise	$ 0.001
Pd@Pt NPs	-	$ 0.032
TMB substrate	Cloud-Clone	$ 0.008
CMC, EDC/NHS, BSA, PBST, H_2O	Various	$ 0.008
	Total (USD)	$ 0.369

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