Electronic Supplementary Information

Nanoparticle-enhanced electrical detection of Zika virus on paper microchip

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Supplementary Methods

Synthesis of PtNPs

Briefly, 36 ml of a 0.2 % solution of chloroplatinic acid hexahydrate was mixed with 464 ml of boiling deionized water. 11 ml of a solution containing 1 % sodium citrate and 0.05 % citric acid was added followed by a quick injection of 5.5 ml of a freshly prepared 0.08 % sodium borohydrate solution, containing 1 % sodium citrate and 0.05 % citric acid. The reaction continued for 10 min and the formed nanoparticles solution was gradually cooled down to room temperature.

Virus culture and isolation

Aedesalbopictus cells (C6/36) were prepared and infected with ZIKV and harvested. 4 flasks (225 cm^3) of the cells were prepared and infected with the virus. Infection was carried out by adding serum sample to the culture flask and incubating at an angle of 20 ° at 33 °C for 6 days. After incubation, the virus was harvested by treating with 23 % v/v FBS. The supernatant from the flasks was collected and centrifuged at 4000 g for 30 min. The supernatant after centrifugation was collected without disturbing the cell pellet and 500 µL aliquots were prepared and stored.

Biotinylation of Anti- Zika antibodies

Anti-Zika antibodies were biotinylated using the Biotin type A fast conjugation kit (Abcam, ab201795). 1 µL of Biotin Modifier reagent was added to every 10 µL of antibody to be labeled and was mixed gently. This was added to a vial containing lyophilized Biotin and was left for incubation at room temperature (20 ^{o}C to 25 ^{o}C) for 15 min. 1 µL of Quencher reagent was then added to every 10 µl of the antibody used. The mixture was incubated at room temperature for 4 min and stored at 4 ^{o}C.

Magnetic bead modification

200 µl of streptavidin-coated magnetic beads (Thermo Fisher Scientific- PierceTM Streptavidin magnetic beads; 88816) of 1 µm diameter was washed thrice using PBS, during which the beads were isolated using a MagnaGrIPTM (MilliPore) magnetic stand. 10 µL of the biotinylated target was added to the magnetic bead solution and left for incubation overnight on a shaker at 4 ^{o}C. Antibody conjugated beads were then washed twice using PBS and suspended in 2 mL PBS.

Supplementary Results:

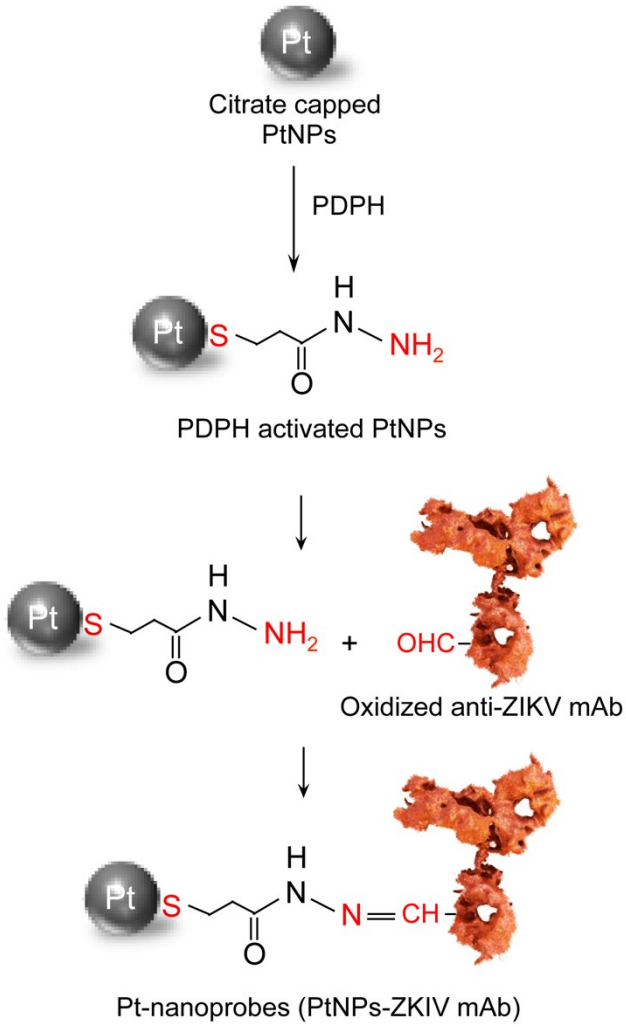

Figure S1. Detailed preparation protocol of Pt-nanoprobe. The surface of PtNPs of the prepared platinum nanoparticles (PtNPs) was modified with 3-(2-Pyridyldithio)propionyl hydrazide (PDPH) through the thiol-metal interaction. The hydrazide terminal of PDPH then allowed to couple to the free aldehyde group (CHO) in the oxidized FC region of Zika virus monoclonal antibody (anti-ZIKV mAb), forming the Pt-nanoprobes used in labeling Zika virus.

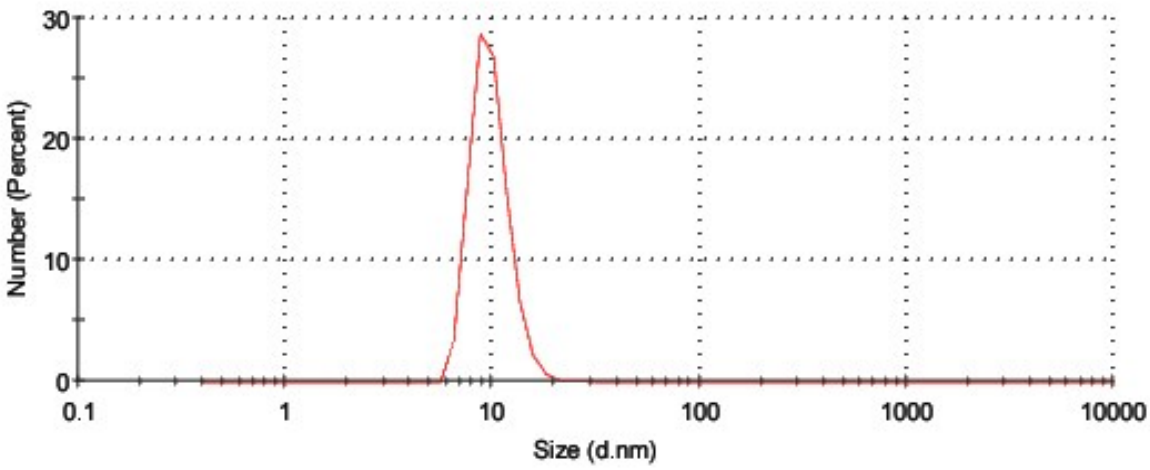

Figure S2. Dynamic light scattering (DLS) analysis of the size distribution of the prepared PtNPs used in the preparation of platinum nanoprobe (Pt-nanoprobe).

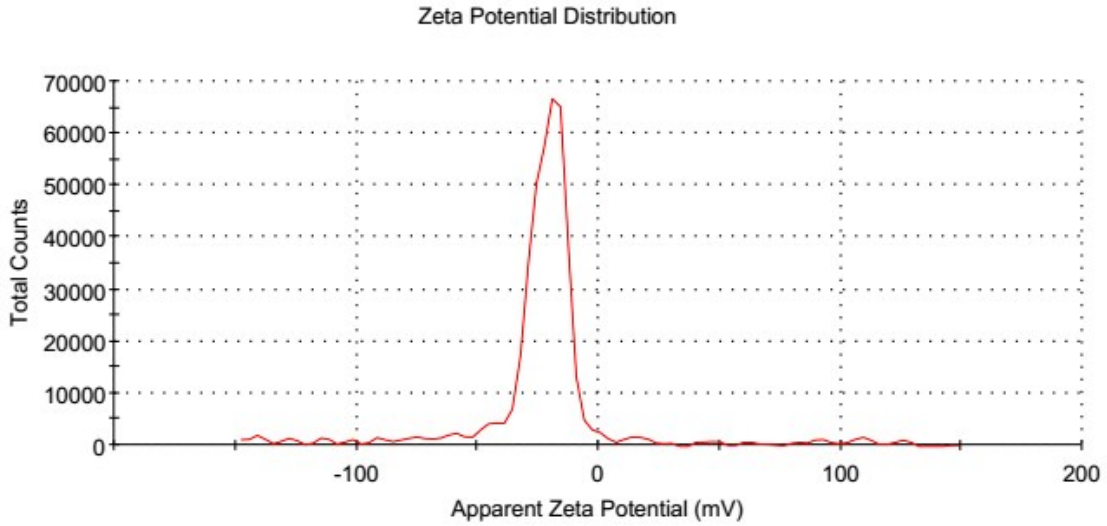

Figure S3. Zeta potential of the prepared citrate capped PtNPs used in the preparation of platinum nanoprobe (Pt-nanoprobe).

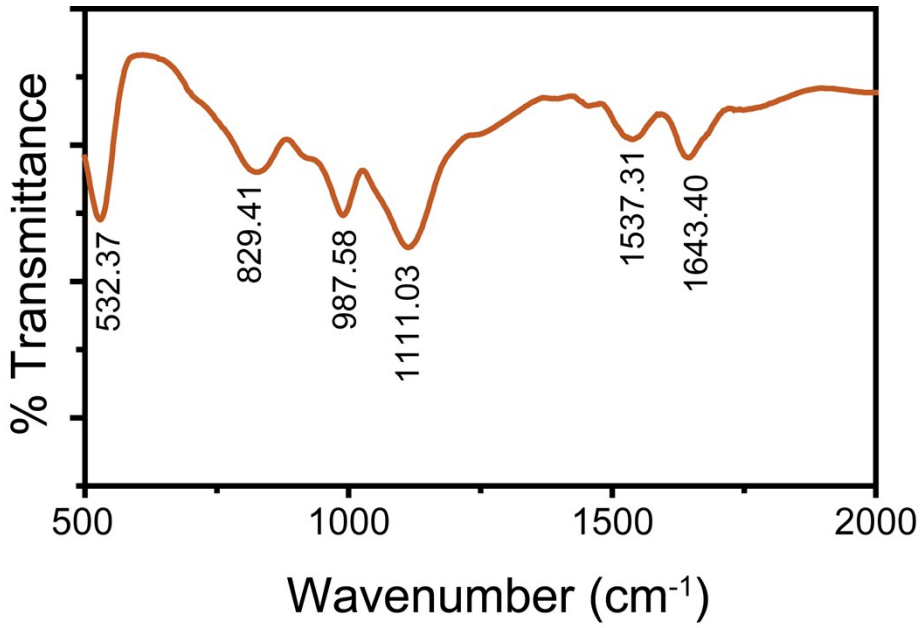

Figure S4. FTIR analysis of anti-Zika monoclonal antibody showing different peaks at 1643.40 cm^{-1}, 1537.31 cm^{-1} and 1111.03 cm^{-1} that are characteristics to the amide I and amide II bands of protein.

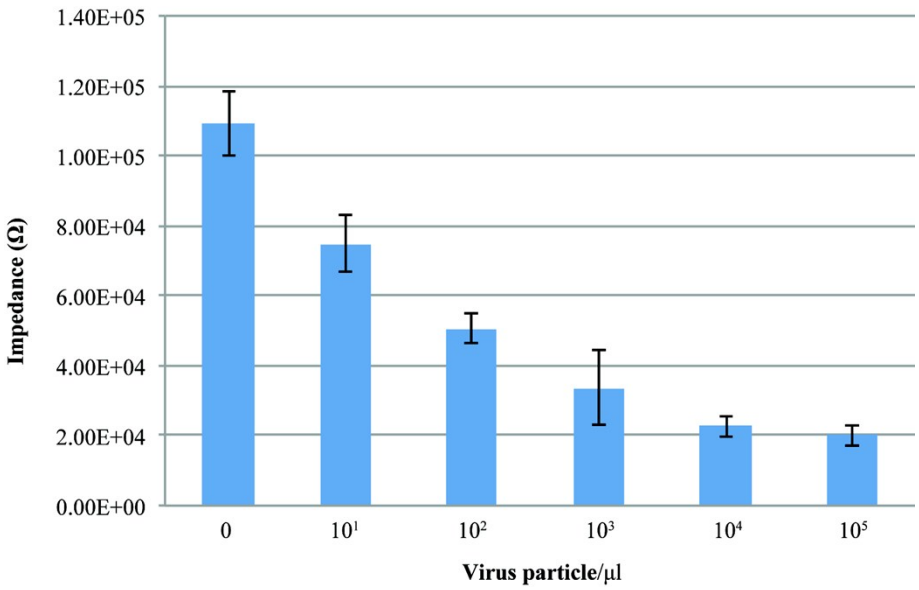

Figure S5. The detection of ZIKV on the developed paper microchip without post-capture labeling step with Pt-nanoprobes. Different concentrations of ZIKV (10^1 particle/µl to 10^5 particle/µl) were prepared in 1x PBS and captured with magnetic beads, lysed and tested on-chip. Error bars are standard deviations of mean from a total of three independent measurements.

Table S1. Summary of common types of paper-based systems recently reported for human viruses detection

Paper system[a]	Target virus[b]	Assay type	Detection Method	Detection limit	Ref.
Cellulose paper	RSV	Nucleic acid assay	Colorimetric	100 copies	[1]
	HBV	Immunoassay	Electro-chemiluminescence	34.2 pg/ml	[2]
	ZIKV	Intact virus assay	Electrical	10^2 particle/µl	Current study
Wax-printed cellulose paper	NoV	Immunoassay	Scattering	10 pg/ml	[3]
	EBOV	Nucleic acid assay	Colorimetric	10^7 copies/µl	[4]
	HIV-1	Intact virus assay	Electrical	10^7 copies/ml	[5]
LFA strip	DENV	Nucleic acid assay	Colorimetric	50 copies	[6]
	HBV			10^4 copies/ml	[7]
	HPV	Immunoassay	Colorimetric	NA	[8]
	HIV-1	Intact virus assay	Electrochemical	NA	[9]
Barcode LFA	HBV, HCV, HIV-1	Immunoassay	Colorimetric	0.003 nM	[10]
Electrochemical paper system	HHV-5	Nucleic acid assay	Electrochemical	97 copies/ml	[11]
Silica sprayed cellulose paper	IAV	Immunoassay	Electrochemical	113 PFU/ml	[12]
NC-paper system	NoV	Nucleic acid assay	Fluorometric	4.4 ng/ml, 3.3 ng/ml	[13]
Paper-Dot-ELISA chip	IAV	Immunoassay	Colorimetric	NA	[14]

[a] LFA: lateral flow assay; NC: nitrocellulose membrane; ELISA: enzyme-linked immunosorbent assay

[b] DENV: dengue virus; EBOV: Ebola virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HHV-5: herpes virus-5; HIV-1: human immunodeficiency virus-1; HPV: human papillomavirus; IAV: influenza A virus; NoV: norovirus; RSV: respiratory syndrome Virus; ZIKV: Zika virus

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