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Electronic Supplementary Information

In-situ amplified colorimetric immunoassay for quantitative detection of dengue virus coupling with dual-enzyme-functionalized UiO-66(Ce) framework

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Chemical and Reagents. Dengue Virus NS1 Type 2 standards and related ELISA kits were purchased from Newbioscience Biotech. Inc (mEA-H12114, KA&M-BIO, https://www.biomart.cn). Dengue Virus Type 2 NS1 Polyclonal Antibody (PA5-32207) were supplied by thermofisher Co. (Cambridge, MA). Glucose oxidase (GOx) from *Aspergillus niger* were purchased from Dingguo Biotechnol. Inc. (Beijing, China) (Type X-S, lyophilized powder, 100,000 – 250,000 units g^{-1} solid). Horseradish peroxidase was purchased from Aladdin. Acrylic resin F127 (probenecid tri-block F127), acetic acid, sodium perchlorate, ceric ammonium nitrate, triethylbenzene, and some of the solvents used in the synthesis were purchased from Aladdin (Shanghai, China). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 3,3',5,5'-tetramethylbenzidine (TMB), thomas brilliant blue G-250 dye, hydrogen peroxide (H_2O_2) were purchased from local suppliers. All high-binding polystyrene 96-well microplates were acquired from Greiner Bio-One (Frickenhausen,705071, Germany). All reagents used were of A.R. grade. Ultrapure water obtained from a Millipore water purification system (18.2 MΩ cm^{-1}, Milli-Q, Millipore) was used throughout the experiment.

Experimental Characterization. X-ray Diffraction (XRD) characterization was obtained by a D8 ADVANCE (Bruker, Germany). The nanoparticles were characterized by using transmission electron microscopy (TEM, FEI Talos F200s). UV-vis absorption spectra were recorded by the Infinite M200 Pro NanoQuant, (Tecan, Switzerland). Specific surface area was determined by gas adsorption apparatus (BSD 660, BEISHIDE instrument tec. Co LTD).

BJH Method for Microporous Analysis. The pore size distribution curve of UiO-66(Ce), determined by the Barrett-Joyner-Halenda (BJH) method, is based on the following equation1:

$$r = -\frac{2\sigma V cos\theta}{RTln(p/p0)} \quad \textbf{(1)}$$

Where σ (N cm^{-1}) represents the surface tension of adsorbent liquid; T is the test temperature; P/Po represents the relative pressure; V (mL mol^{-1}) represents the molar volume of the liquid phase of the adsorbent; R represents the ideal gas constant; θ is contact angle between the curved moon face and the solid wall.

Determination of Enzyme Catalytic Activity. First, UiO-66(Ce)@GOx/HRP was dispersed in a PBS buffer solution for prepared a suitable solution (1 mg mL^{-1}). Next, these enzyme solutions were co-incubated with substrate solutions (e.g., glucose solution for GOx and a mixture of TMB and H_2O_2 for HRP) at 37°C to initiate the catalytic reaction. After a set time interval, the change in absorbance of the reaction system at a specific wavelength (e.g., for TMB oxidation reaction use 652 nm wavelength) and the change in blood glucose meter oscillogram were measured using a spectrophotometer to monitor the consumption of substrate or the generation of product. By analyzing the rate of change in absorbance, the catalytic activity of the enzyme can be calculated. The enzymatic activity of glucose oxidase is defined by the following equation 2

$$Ea = \frac{(C1 - Co) * V}{t * m} \quad \textbf{(2)}$$

where C1 and C0 represent the glucose concentration (μM) in the final and initial states, respectively, V represents the reaction volume (L), t represents the catalytic time (min), and m represents the catalyst mass (mg). The catalytic activity of peroxidase was determined by previous reports.[1, 2]

Evaluation of Enzyme Catalytic Stability. The stability of the synthesized multi-enzyme reactor was assessed prior to performing an ELISA-like DENV assay. In our experiments, we assessed the stability of the synthesized biomineralization probes by determining their chromogenic signals per unit time for the glucose-TMB system. Specifically, we assessed the catalytic efficiency of biomineralization probes stored under room temperature laboratory conditions over a period of 60 days by using a glucose-TMB hybrid system. The colorimetric value of the substrate (A0) was recorded on day 0, and the absorbance of the TMB mix (652 nm) for each test was A. Residual enzyme activity was defined as A/A0. In this reaction, the glucose concentration was set at 50 mM, and the TMB concentration was set at 100 μL in a total volume of 1 mL. The catalytic reaction time was set to 10 min.

Preparation of mAb_1-Coated Microplate. Monoclonal antibodies to DENV2-NS1 were prepared and bonded in microtiter plates as follows. The purchased mAb_1 (50 μL per well, 10 μg mL^{-1}) was added into a high-binding 96-well microplate containing sodium carbonate buffer (0.05 M, pH 9.6), and then incubated 24 h at 4 °C. A plastic film is wrapped over the microplate to prevent evaporation of the liquid. The completed incubated microplates were removed and washed three times with PBA (pH 7.4) buffer containing 0.05% Tween 20 (v/v). This was followed by incubation for 1 h by adding

closure buffer (300 uL of PBS solution per well (10 mM, pH 7.4) including 1.0 wt % BSA). The aforementioned prepared microtiter plates were prepared for the further assay.

Preparation of dAb_2 (UiO-66(Ce)@GOx/HRP-pAb_2). To covalently attach the secondary antibody to UiO-66(Ce), we first dispersed UiO-66(Ce) in PBS buffer (5 mL, 1 mg mL^{-1}) and then added EDC (10 mg mL^{-1}, 1 mL) and NHS (5 mg mL^{-1}, 1 mL) to activate the carboxyl groups on the surface of UiO-66(Ce). After a period of incubation at room temperature (25 ℃), the carbonyl intermediate was formed, followed by the addition of secondary antibody solution. Finally, conjugates were obtained by centrifugation at 4 ℃ (10 min, 13 000 g), and dispersed in 1.0 mL of 2 mM sodium carbonate solution containing 1.0 wt % BSA and 0.1 wt % sodium azide, pH 7.4, and stored at 4 ℃ for further use.

Enzyme-Linked Immunosorbent Assay (ELISA) for DENV2-NS1. When using the Human Dengue Virus Type 2 NS1 Protein ELISA Kit, the standard was first diluted, and then the enzyme plate was spiked with samples, including the standard and diluted samples to be tested, followed by warming at 37°C for 30 minutes. After that, the plate was washed 5 times with diluted washing solution, followed by addition of enzyme labeling reagents to each well (except for blank wells) and warming again. After warming, the washing step was repeated. For color development, color developer A was added first, followed by color developer B. The color development was carried out at 37°C for 10 minutes, protected from light. After the color development was completed, the reaction was terminated by adding termination solution to each well, and the blue color changed to yellow. Finally, the absorbance (OD) of each well was measured at 450 nm, and the actual concentration of the sample was calculated according to the standard curve. Some tests of actual samples beyond the linear range were obtained by multiple equal dilution assays.

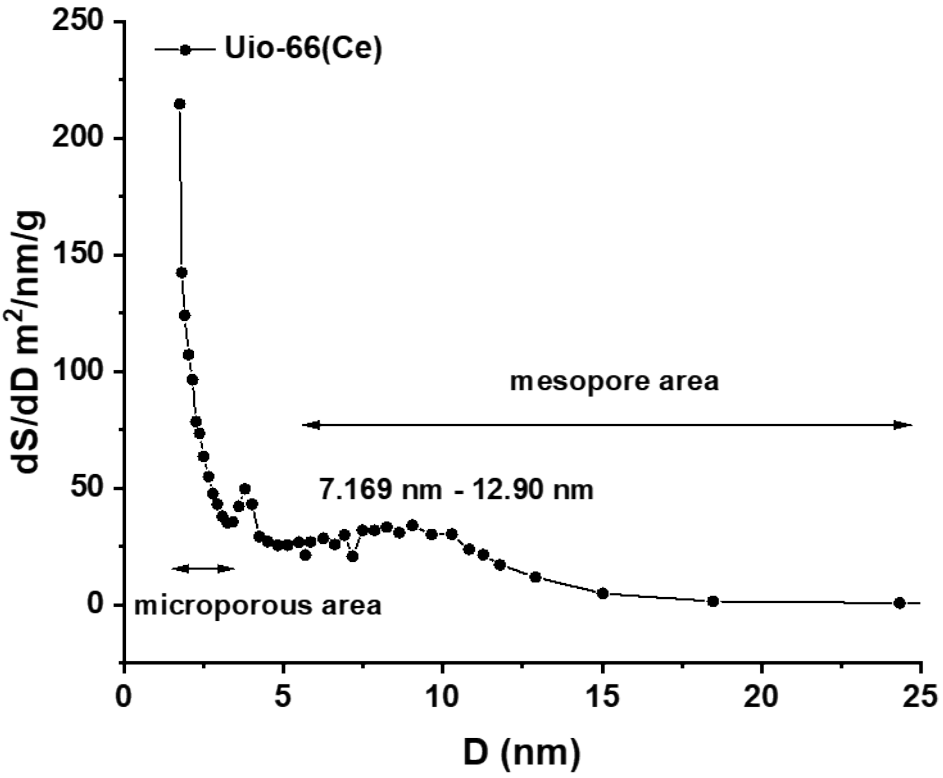

Fig. S1 Pore size distribution curves of UiO-66(Ce) determined based on the BJH approach.

Table S1. Comparison table of the working scope of the testing method for DENV2-NS1.

No.	Methods	Detection of limit	Dynamic Response Range	Re.
1	Colorimetric	1.56 ng mL^{-1}	1.0 – 400 ng mL^{-1}	3
2	Electrochemical	None	100 pM - 100 μM	4
3	Surface plasmon resonance	0.06 μg mL^{-1}	0.2 – 2.0 μg mL^{-1}	5
4	Fluorescent	0.15 ng mL^{-1}	0.01 – 1000 ng mL^{-1}	6
5	Electrochemical	0.015 μg mL^{-1}	0.04 – 0.6 μg mL^{-1}	7
6	Electrochemical	1.36 pg mL^{-1}	1 – 100 pg mL^{-1}	8
7	Colorimetric	2.6 pg mL^{-1}	10 – 100 pg mL^{-1}	9
8	Colorimetric	39.7 pg mL^{-1}	0.05 – 100 ng mL^{-1}	This work
	ELISA kit*	None	0.078 – 5 ng mL^{-1}	Used in this work

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