Appendix A. Electronic Supplementary Information

Self-Assembled Chromogen-loaded Polymeric Cocoon for Respiratory Virus Detection

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Materials and methods

Biological agents

Anti-IV/A/H1, H2, and H3 (Anti-HA) and anti-IV/A/H3N2 antibodies (anti-IV/A/H3N2) were purchased from Sino Biological Inc. (Beijing, China). Clinically isolated IV/A/Yokohama/110/2009/H3N2 was kindly provided by Dr. C. Kawakami of the Yokohama City Institute of Health, Japan. The recombinant baculovirus expressed-Hepatitis E virus-like particles (HEV-LPs) were provided by Dr. Tian Chen-Li from National Institute of Infectious Disease Japan. Zika virus and white spot syndrome virus (WSSV) were provided by Prof. K. Morita of Institute of Tropical Medicine, Nagasaki University, and Dr. J. Satoh of National Research Institute of Aquaculture, Japan Fisheries Research, and Education Agency respectively. Norovirus (NoV) was kindly provided by Mr. F. Abe of Shizuoka Institute of Environmental and Hygiene, Shizuoka, Japan. Norovirus-like particle (NoV-LP) was expressed using a baculovirus expression system [1]. All experiments were conducted using deionized (DI) water.

Preparation of CuNFs.

Inspired by the protein inorganic hybrid nanoflowers, CuNFs, were prepared by a one-pot bio-mineralization process with some modifications[2, 3]. Typically, 100 μL of BSA (5 mg/mL) was dissolved into 10 mL of 1 × PBS (pH 7.4) for incubation of 0.5 h under slow stirring. Then, 400 μL of $CuSO_4 \cdot 5H_2O$ solution (120 mM) and 50 μL of 10% KCl solution were added to the BSA solution and further incubated for an additional 48 h. The obtained solution was centrifuged (6500 g, 15 min) at 7°C and washed three

times. Thus, obtained CuNFs were freeze-dried and stored at −20°C for further usage.

Catalytic activity of CuNFs.

CuNFs were analyzed for its peroxidase-like activity in the catalytic oxidation of TMB by H_2O_2 according to previously report[4] with some modification. The steady-state kinetic assay was carried out at room temperature on the CuNFs in the working solution, containing 40 mM TMB reagents and 500 mM H_2O_2 (1:4, v/v in DI water). The assays were performed in series of concentration of co-substrate to a fixed concentration of the substrate in the corresponding working solution. The absorbance of the developed color was measured at 653 nm using UV-Vis spectroscopy.

Preparation of Anti-IV/A/HA antibody-conjugated TMB-NPS@PLGA.

The conjugation of anti-HA antibody (Ab) or Anti-IV/A/H3N22 Ab to TMB-NPs@PLGA was done by EDC/NHS conjugation chemistry as described in our previous work [5].

Calculation of the Limit of Detection (LOD)

Based on the calibration curve of the developed assay, the LOD of the developed assay on the Influenza virus A/H1N1 and A/H3N2, and spike protein of SARS-CoV-2 were determined based on the y-intercept (c) and the gradient slope (S) of the calibration line and the standard deviation (σ) of the lowest detectable signal (blank), respectively.[6]

As the calibration curve is represented in semi-log graph, the corresponding LOD is defined as [LOD] = $e^{3.3\sigma/S}$.

$$Y = S \cdot \log(X) + c \quad (1)$$

$$Y_{LOD} = S \cdot \log(X_{LOD}) + c \quad (2)$$

Where Y_{LOD} denotes the absorbance value obtained from the lowest concentration of the virus sample by the developed assay. X_{LOD} indicates the lowest concentration of virus-containing sample which can be detectable by the developed assay.

As $Y_{LOD} \leq \Delta A_{Blank} + 3.3\sigma$ (3)

The $\Delta A_{\mathrm{Blank}}$ is 0 as ΔAbs is represented with $\mathrm{A}-\mathrm{A}_{\mathrm{Blank}}$.

From Eq. (2) and (3)

$$S \cdot \log(X_{LOD}) + c = 3.3\sigma \quad (4)$$

$$S \cdot \log(X_{LOD}) = 3.3\sigma - c \quad (5)$$

From Eq. (5), the X_{LOD} was defined as follow,

$$X_{LOD} \leq e^{\frac{3.3\,\sigma - c}{S}} \quad (6)$$

The illustration of the LOD calculation is shown in Fig. S5.

Table S1. The Kinetic Parameter of CuNFs in comparison to natural peroxidase

Catalyst	Substrate	K_m (mM)	v_{max} (μM/s)
CuNFs	TMB	0.07	0.12
CuNFs	H_2O_2	6.50	0.02
HRP	TMB[7]	0.43	0.10
HRP	H_2O_2[7]	3.70	0.09

Table S2. Comparison of Previous Work Related to IV/A Detection

Detection method	Strain of IV	LOD	Reference
LSPR-induced immunofluorescence	A/New Caledonia/20/99	0.09 pg/mL	8
Metal enhanced fluorescence	A/New Caledonia/20/99	1 ng/mL	9
Peroxidase-mimicking nanozyme	A/New Caledonia/20/99	10 pg/mL	10
LSPR fiber-optic	A/Vietnam/1203/ 2004	13.9 pg/mL	11
Electrochemical immunosensor	A/Poland/08/2006	2.2 pg/mL	12
Immunochromatography assay	A/California/12/2009	76.7 ng/mL	13
Nanovesicle based immunoassay	A/New Caledonia/20/99	0.05 pg/mL	(This work)

Table S3. Clinically Isolated IV/A/H3N2 Detection

	Virus Concentration (pfu/mL)						
Detection Method	5000	1000	100	50	25	10	1
Commercial IV detection kit	+	-	-	-	-	-	-
HRP-based ELISA[6, 8]	+	+	-	-	-	-	-
AuNPs-based Immunoassay[8]	+	+	+	+	-	-	-
This study	+	+	+	+	+	+	-

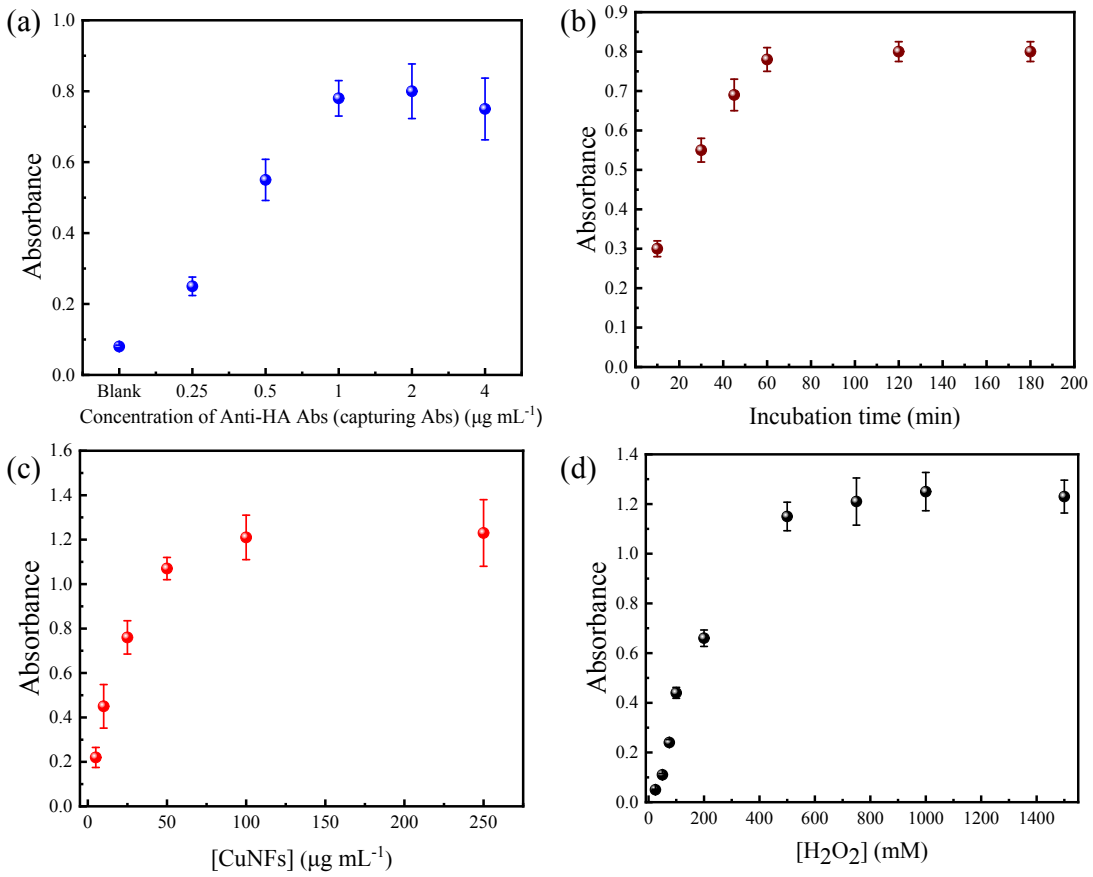

Fig. S1. The optimization of (a) the concentration of capturing antibody, (b) the incubation time of the virus-antibody binding, (c) concentration of the CuNFs and (d) hydrogen peroxide in the developed TMB-NPs@PLGA based method for virus sensing. The concentration of the Influenza virus A/H1N1 was 100 pg mL^{-1}.

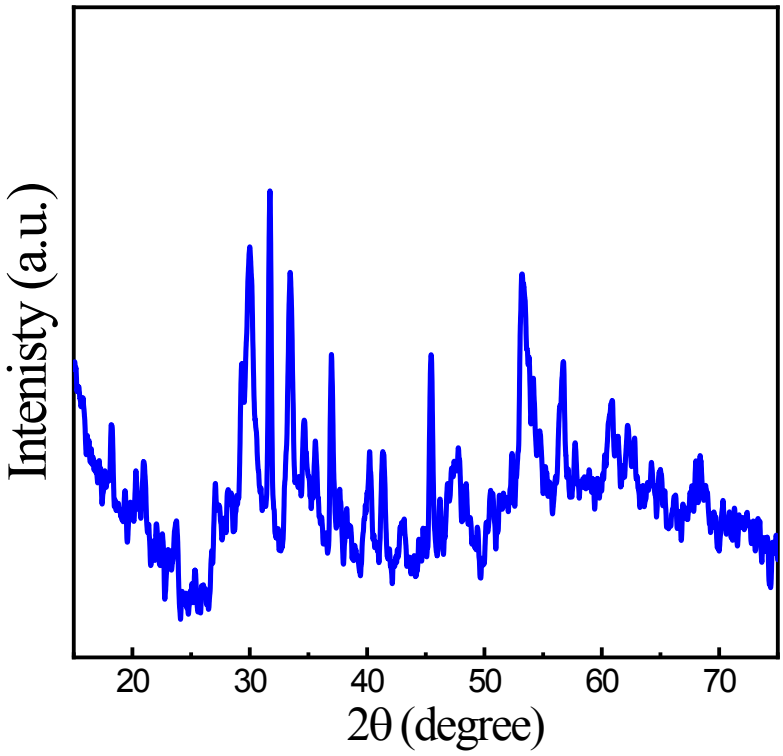

Fig. S2. XRD pattern of CuNFs

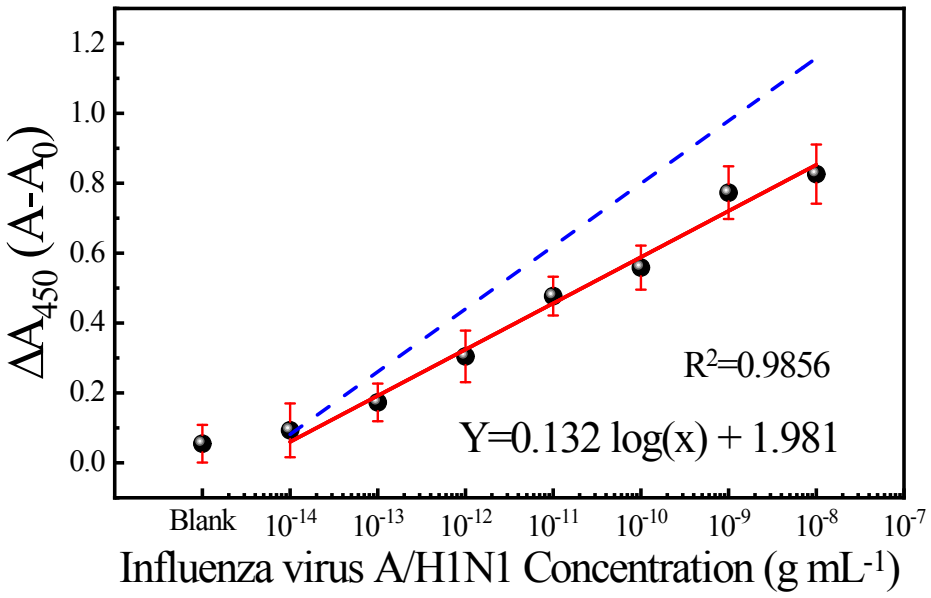

Fig. S3. The calibration line of the IV/A/H1N1 detection by TMB-NPs@PLGA based colorimetric detection. The error bar represents the SD of the triple measurements.

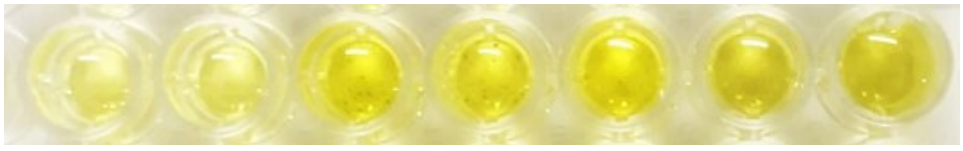

Fig. S4. Digital illustration of the developed color in the Influenza virus A detection using the developed immunoassay

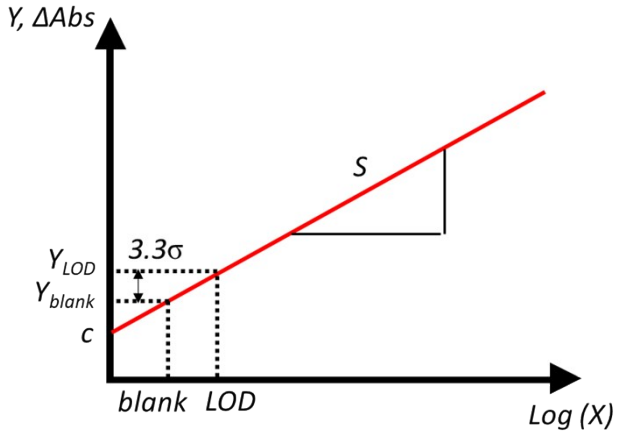

Fig. S5. The illustration of the LOD calculation

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