Electronic Supplementary Information (ESI)

Human serum albumin templated MnO_2 nanosheets as an efficient biomimetic oxidase for biomolecule sensing

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Materials and apparatus

Glucose, glucose oxidase, trypsin, lysozyme, tyrosinase, and acetyl cholinesterase (AchE), acid phosphatase (ACP), human serum albumin (HSA), manganous chloride ($MnCl_2$), 3,3′,5,5′-tetramethylbenzidine (TMB), and sodium hydroxide were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ascorbic acid 2-phosphate (AAP) was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). All reagents were used as received without further purification. All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance >18.2 MΩ.

Transmission electron microscope (TEM) images were obtained from JEM-2100 (JEOL, Japan) with a 200 kV accelerating voltage. X-ray photoelectron spectroscopy (XPS) measurements were recorded on an ESCALAB 250Xi (Thermo Scientific, America). UV-vis absorption spectrum was recorded at room temperature on a UV-2550 spectrophotometer (Shimadzu, Japan). Absorbance of the oxidized TMB (oxTMB) at 652 nm was monitored for quantitative analysis.

Kinetic analysis and catalytic mechanism study

The Michaelis-Menten behavior of the catalytic reactions was investigated by monitoring the absorbance of TMB at 652 nm. The experiments were carried out at room temperature with different concentrations of TMB in NaAc buffer (20 mM, pH 4.0) in presence of MnO_2 NS (20 μg/mL). Lineweaver-Burk plots, $1/v = (K_m/V_{max})(1/[S]) + 1/V_{max}$, was used to calculate the Michaelis-Menten constant, where v represents the initial velocity, V_{max} stands for the maximal reaction velocity, [S] is the concentration of substrate and K_m is the Michaelis constant.

Application to real samples

In order to prove the viability of our proposed sensors for practical applications, human serum was adopted as a model matrix. The serum from volunteers was collected by the First Affiliated Hospital of Zhengzhou University, and informed consent was

obtained for the use of human serum. All the blood samples were obtained through venipuncture and centrifuged at 10,000 rpm for 10 min after standing for 2 h at room temperature. Acetonitrile was introduced into serum samples with the fixed volume ratio1:1 (acetonitrile/serum). After vigorously shaking for 15 min, the mixture solution was centrifuged at 10,000 rpm for 10 min. Then, a certain concentration of ACP was spiked to the 1% diluted human serum samples to check the percentage recovery. The detection procedure was the same as those described in the aforementioned experiment for ACP detection in buffer. All experiments were performed in compliance with the relevant laws and institutional guidelines and approved by Life-Science Ethics Review Committee of Zhengzhou University.

Samples analysis-determination of ACP in cell extracts

PC-3 human prostate carcinoma cell lines were cultured in RPMI 1640 medium supplemented with 12% fetal calf serum, 100 μg mL^{-1} streptomycin, and 100 units mL^{-1} penicillin. The collected cells were resuspended in 20 μL of 20 mM NaAc buffer (pH 4.5) containing 150 mM NaCl. With the addition of 20 μL lysis buffer (20 mM NaAc buffer, 150 mM NaCl, 1% Triton X-100, 0.4 mM phenylmethylsulfonyl fluoride, pH 4.5), the mixture was incubated for 1.5 h at 4 °C with occasional shake. Cell debris was removed by centrifugation at 10000 rpm for 10 min, and the supernatant was recovered. The detection procedure was the same as those described in the aforementioned experiment for ACP detection in buffer. Specific activity is defined as the enzyme units per mg protein. Protein content of the extracts was determined using the method of Bradford, with bovine serum albumin as standard.[1] Meanwhile, all of the samples were also analyzed by a commercial kit (Acid Phosphatase Colorimetric Assay Kit, Cayman Chemical Company, Michigan, USA).

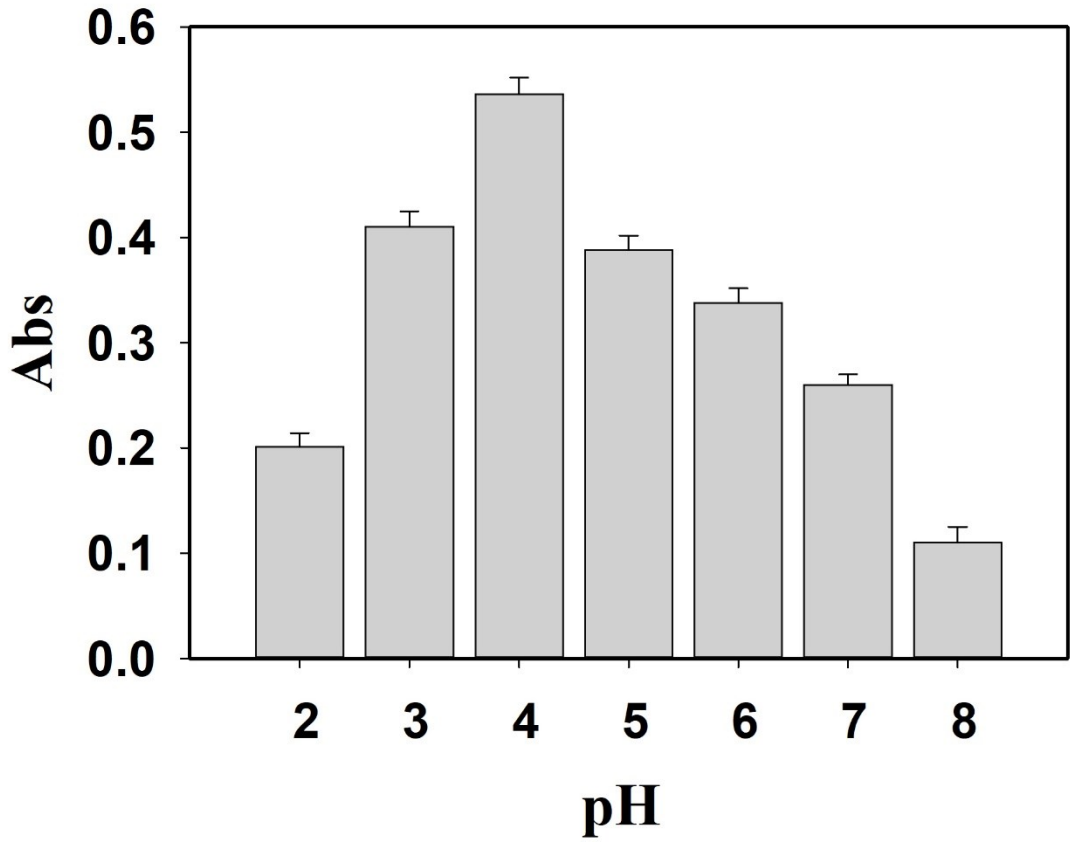

Fig. S1. Dependence of the oxidase-like activity on pH. Error bars are standard deviation of three repetitive experiments.

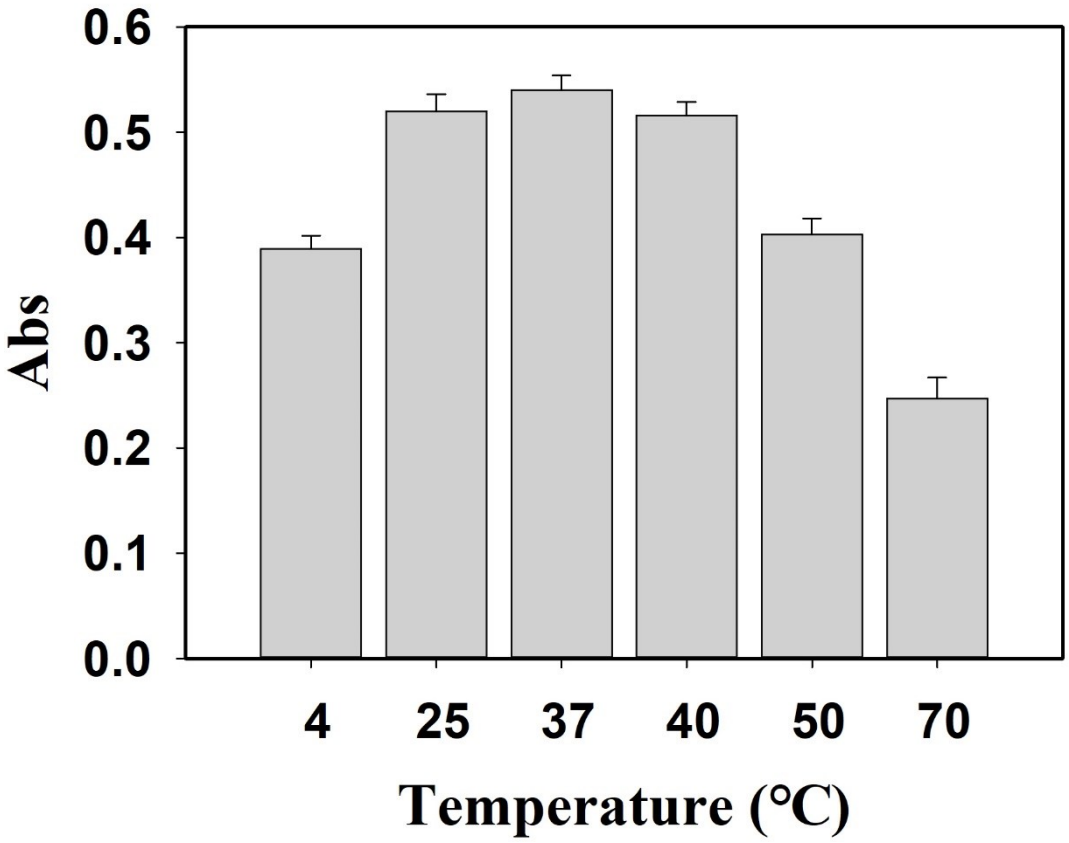

Fig. S2. Dependence of the oxidase-like activity on temperature. Error bars are standard deviation of three repetitive experiments.

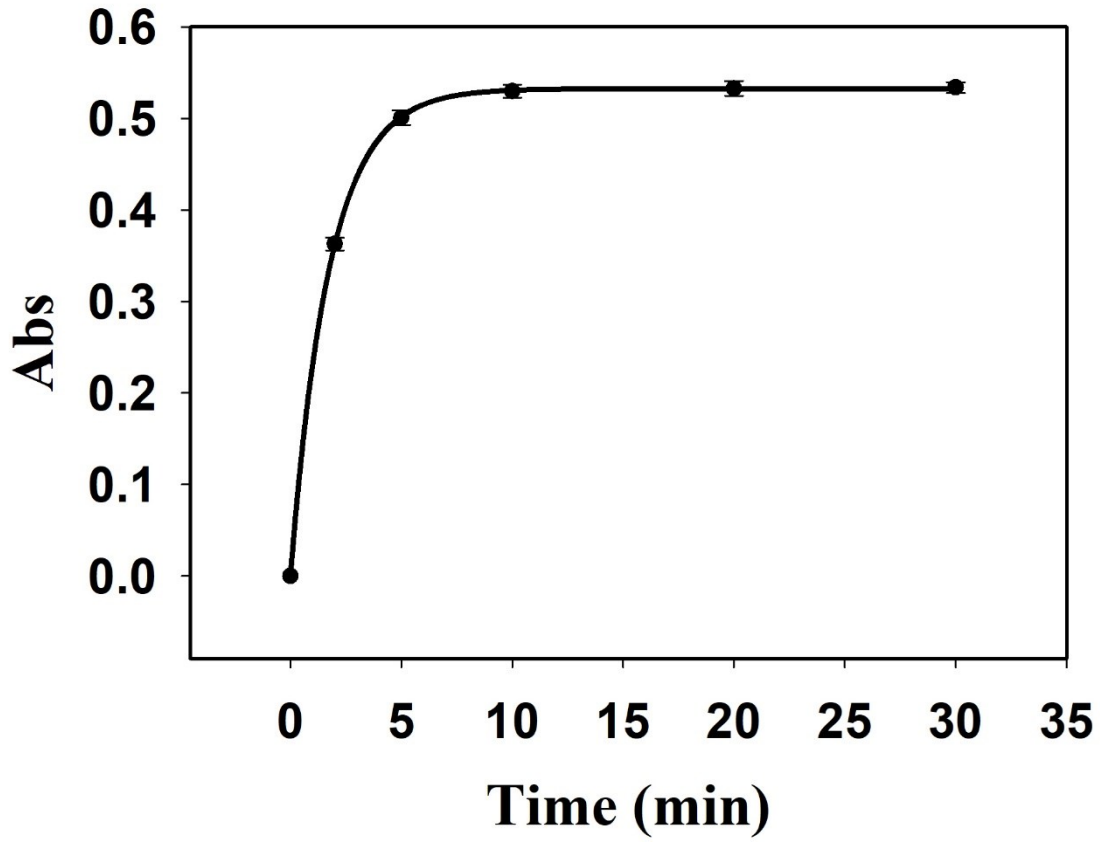

Fig. S3. The effect of reaction time in this assay. The results were the average of three repetitive experiments with error bars indicating the standard deviation.

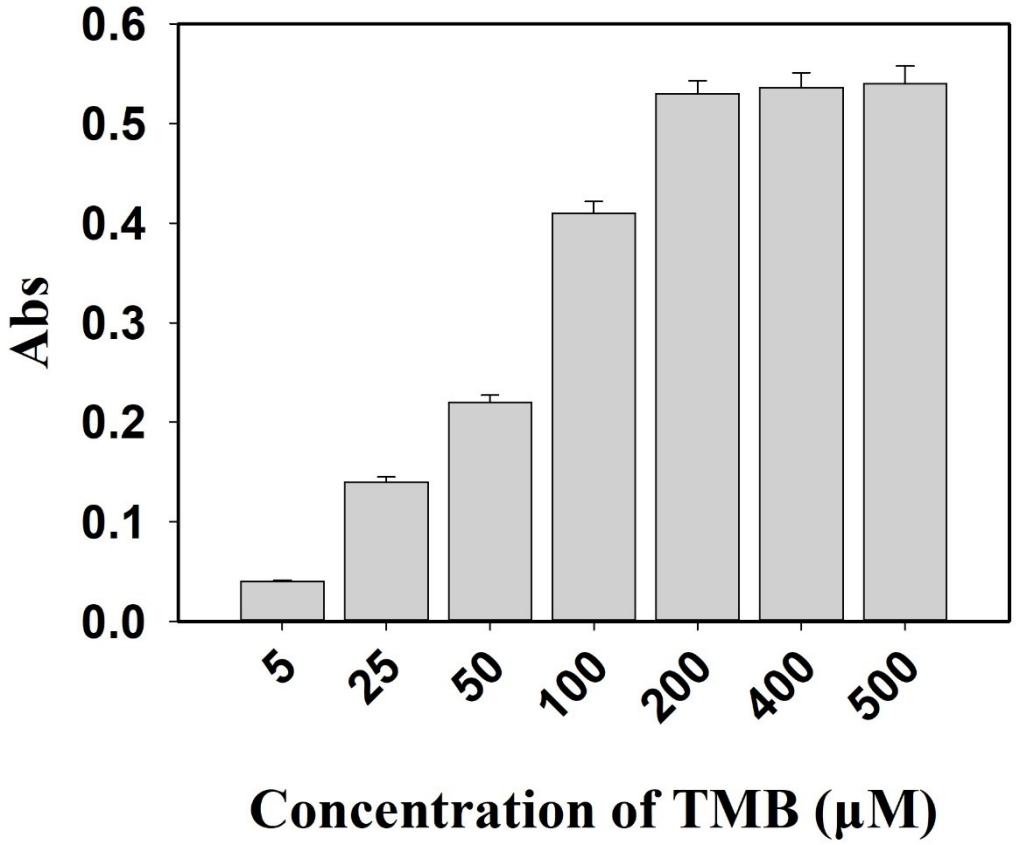

Fig. S4. Optimization the concentration of TMB. Error bars are standard deviation of three repetitive experiments.

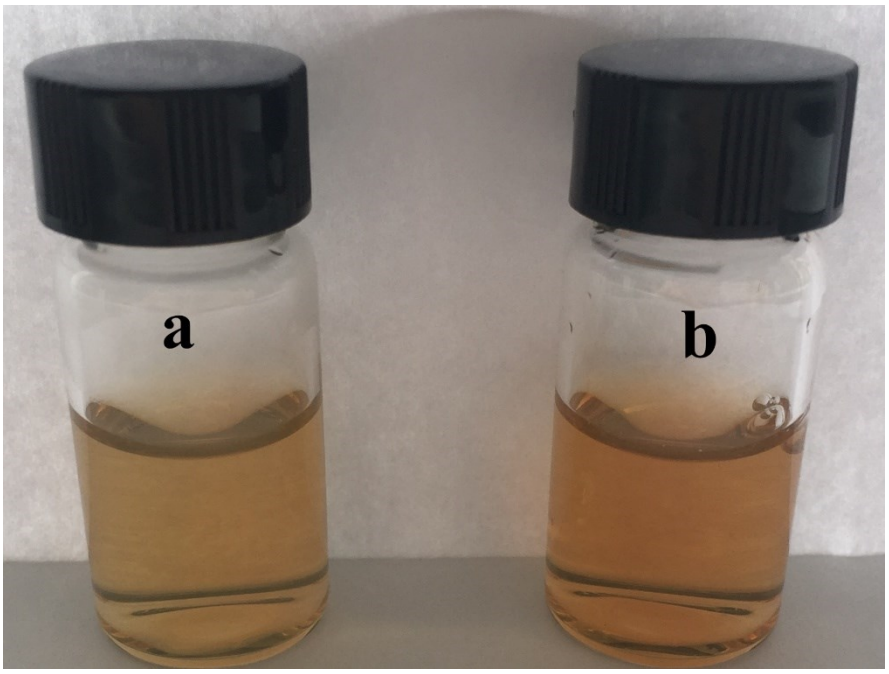

Fig. S5. Appearance of MnO_2 NS dispersed in (a) PBS and (b) human serum.

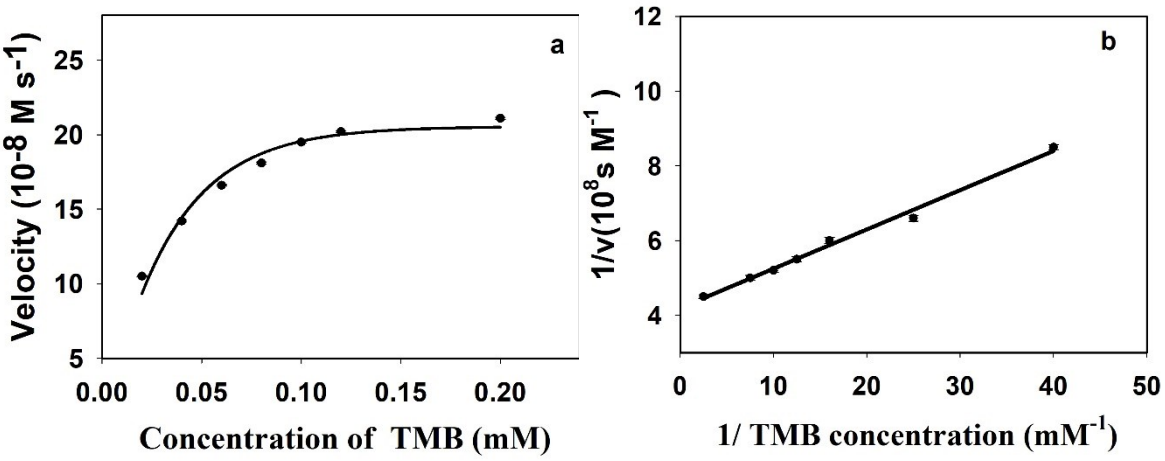

Fig. S6. (a) Michaelis−Menten curve of MnO_2 NS with TMB; (b) Double reciprocal plots of the Michaelis-Menten equation.

Table S1. Michaelis–Menten constant (K_m) and maximum velocity (v_{max}) obtained from the double reciprocal plots which have been compared with the natural enzyme HRP and other artificial enzyme mimetic nanomaterial for TMB oxidation.

Oxidase mimics	K_m (mM)	$V_{max}\times10^{-8}$ ($M\cdot s^{-1}$)	Reference
MnO_2 NS	0.042	21.2	Our work
HRP	0.434	10.0	2
Cu NCs	0.648	5.96	3
N-GQDs	11.9	0.38	4
Pt NPs	0.6	16.2	5
Co_3O_4 NPs	0.037	6.27	6
C-Dots	0.039	3.61	7

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