# Electronic Supplementary Information (ESI)

# for

# Sensitive colorimetric assay of T4 DNA ligase by integrating the oxidase nanozyme of LaMnO$_{3.26}$ coupled with hyperbranched amplification reaction

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**Materials and reagents.**

La(NO$_3$)$_3$·6H$_2$O, Mn(CH$_3$COO)$_2$·4H$_2$O, ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, ammonium hydroxide aqueous solution (NH$_4$OH), adenosine triphosphate (ATP), sodium pyrophosphate decahydrate (PPi), sodium phosphate (Pi), p-benzoquinone, t-butanol, histidine, triethylenediamine, and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 3,3',5,5'-Tetramethylbenzidine (TMB), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid ammonium salt) (ABTS), 2,2,6,6-tetramethylpiperidine (TEMP), and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were purchased from Inokai Technology Co., Ltd. (Beijing, China). T4 DNA ligase, dATP, ammonium persulfate (APS), N,N,N',N'-tetramethylethylenediamine, and Acryl/Bis 30% solution (29:1) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Terminal transferase (TdT) and its 10×TdT buffer (50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate, pH 7.9@25°C), and endonuclease IV (Endo IV) were bought from New England Biolabs (Ipswich, USA). Bovine serum albumin (BSA), polymerase, T4 polynucleotide kinase (T4 PNK), glucose oxidase (GOx), lysozyme, trypsin, thrombin, E. coli ligase, and superoxide dismutase (SOD) were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). All solutions were prepared with ultrapure water (18 MΩ/cm) provided from a Milli-Q purification system.

All oligonucleotides were HPLC-purified and synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the oligonucleotides were listed as follows:

Hairpin DNA: 5'–PO$_4$–TAT AGC TCA ATC GCA CTA GCT ATA TAT AGC TAA CCA CTA ACA GCT ATA–3';

Assistant probe: 5'–PO$_4$–TTT TTT TTT TTT TTT TTT TTX TTT TT–NH$_2$–3' (The X in the assistant probe indicates the abasic site mimic).

**Instruments**

The X-ray diffraction (XRD) pattern was obtained with a D8 Advance X-ray diffractometer using the Cu Kα radiation (Brook AXS, Germany). Scanning electron microscopy (SEM) images were determined by a Hitachi S-4800 high resolution scanning electron microscope (Hitachi, Japan). Transmission electron microscope (TEM) images were performed by a JEM-2100 plus transmission electron microscope (Hitachi, Japan). Fourier transform infrared (FT-IR) spectra were recorded on a FALA-2000104 spectrometer (Boman, Canada). The Raman spectra were received on an In Via Raman microscope spectrometer (Renishaw, UK) with the laser excitation wavelength fixed at 785 nm. The X-ray photoelectron spectra (XPS) were acquired with an Axis supra spectrometer (Kratos, UK). UV–vis absorption spectroscopic measurements for solutions were carried out using a TU–1901 spectrophotometer (Puxi, China). The absorption experiments for the detection of T4 DNA ligase were carried out on 96–well plates with a SpectraMax M5 microplate reader (Molecular Devices, USA).
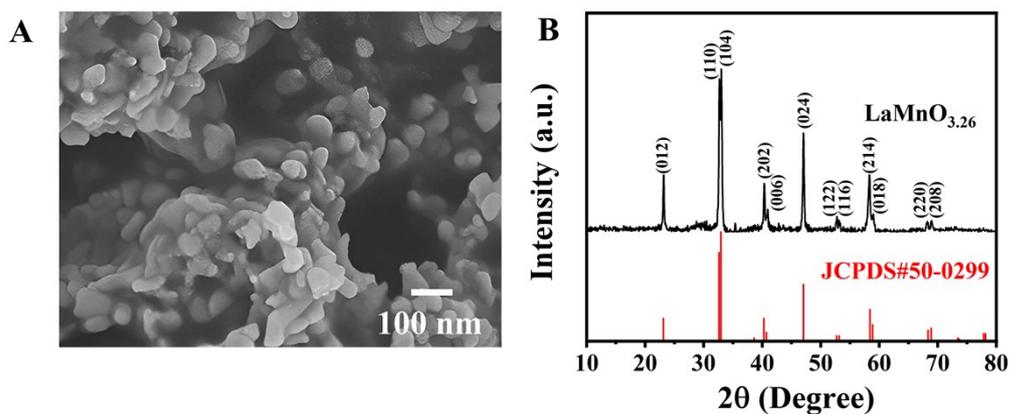
**Fig. S1** (A) SEM image and (B) XRD pattern of the synthesized LaMnO$_{3.26}$.
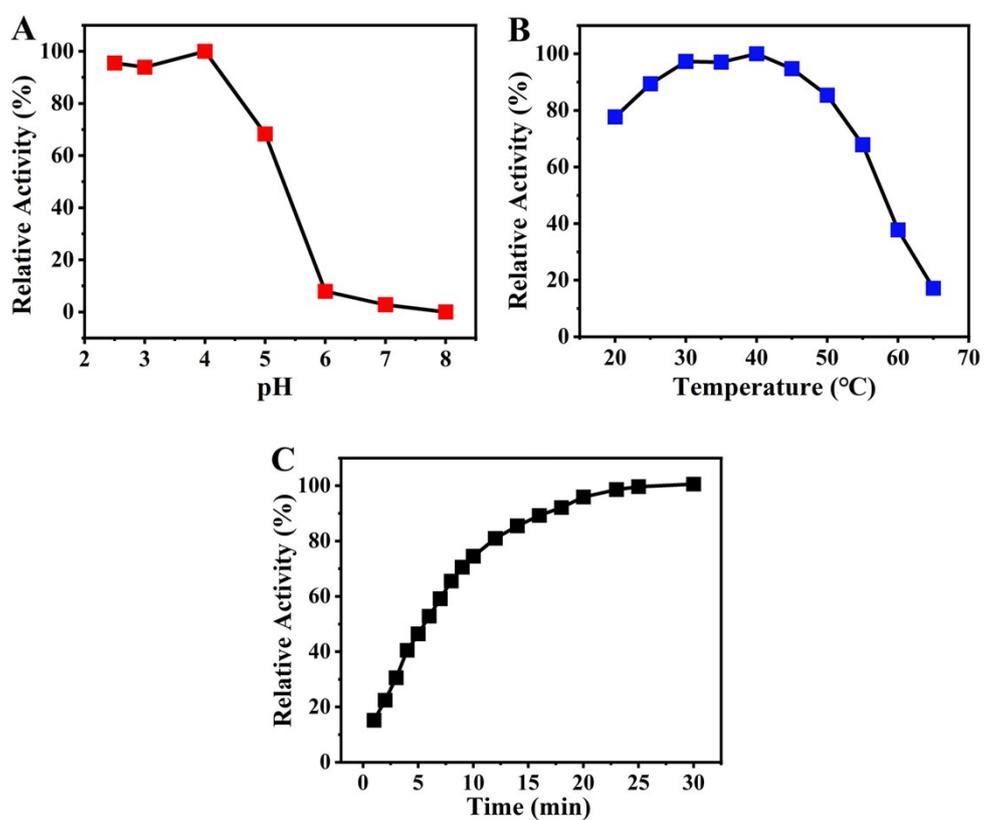


**Fig. S2** Relative catalytic activity of LaMnO$_{3.26}$ mimetic oxidase at (A) different pH of reaction solutions, (B) different temperatures, and (C) different reaction times.
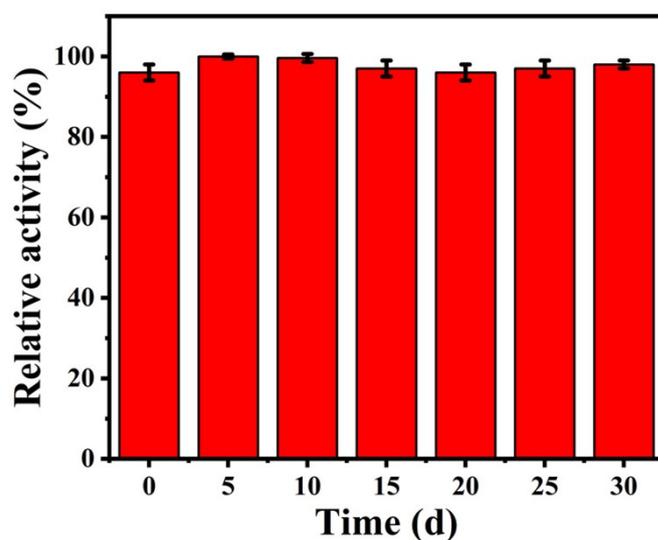
**Fig. S3** Catalytic activity variations of LaMnO$_{3.26}$ nanozyme in 30 days. Error bars represent the standard deviation (SD) for three measurements.

**Table S1**. Comparison of the kinetic parameters between LaMnO$_{3.26}$ and other reported nanomaterials

| Materials | $K_m$ (mM) | $V_{max}$ ($10^{-8}$M•S$^{-1}$) | Reference |
|---|---|---|---|
| NiPd hNPs | 0.110 | 1.52 | 1 |
| CoMo hybrids | 0.177 | 19.5 | 2 |
| NiCo$_2$O$_4$ | 0.127 | 0.127 | 3 |
| 3-CoV MMOs | 0.444 | 9.5 | 4 |
| Au-PtNCs | 0.362 | 13.6 | 5 |
| Nanoceria | 0.42 | 10.0 | 6 |
| LaMnO$_{3.26}$ | 0.071 | 21.9 | This work |
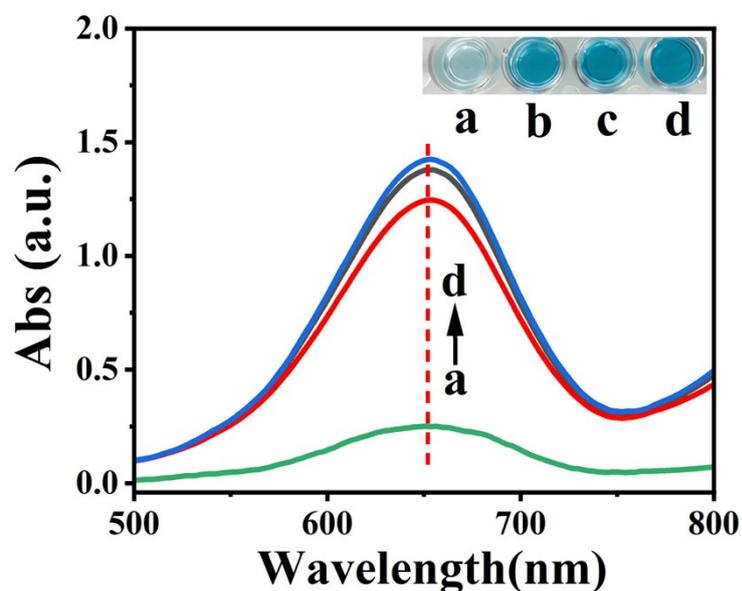
**Fig. S4** Absorption diagram of the nanozyme mediated catalytic system when in the presence of different substances: (a) PPi, (b) ATP, (c) Pi, and (d) control. The inset shows the corresponding colors of the reaction solutions. Reaction conditions: the concentration of PPi is 10 mM and the concentrations of ATP and Pi are 100 mM.
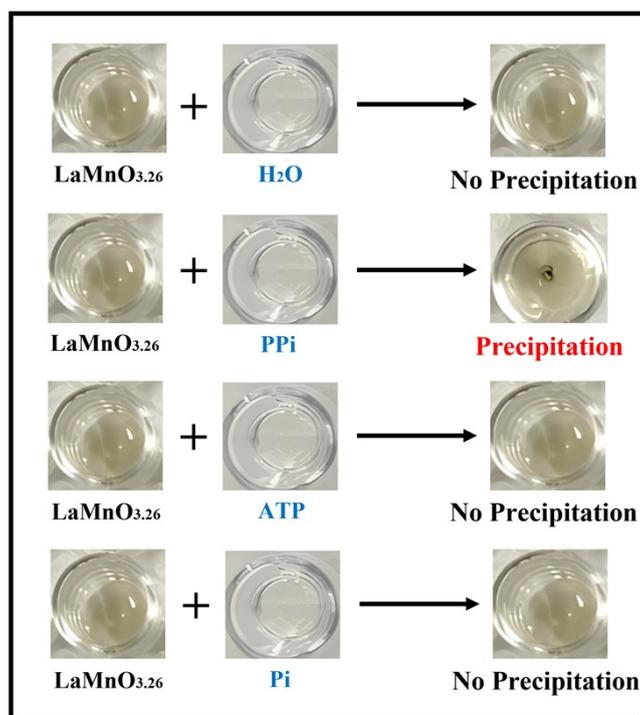


**Fig. S5** Graphs of the changes of dispersion state of the $LaMnO_{3.26}$ nanozyme after mixing with different substances.
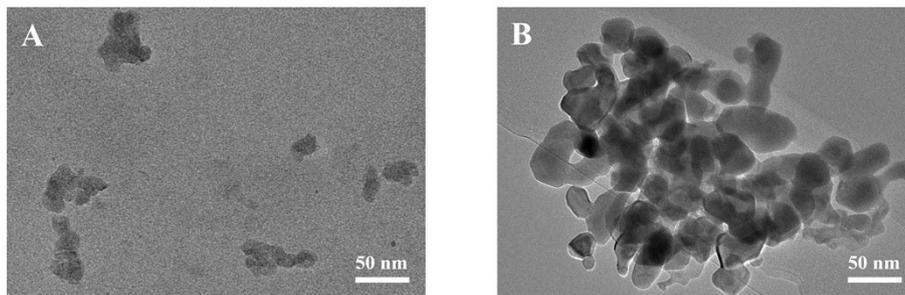
**Fig. S6** TEM images of the LaMnO$_{3.26}$ nanomaterials (A) before and (B) after mixed with PPi.
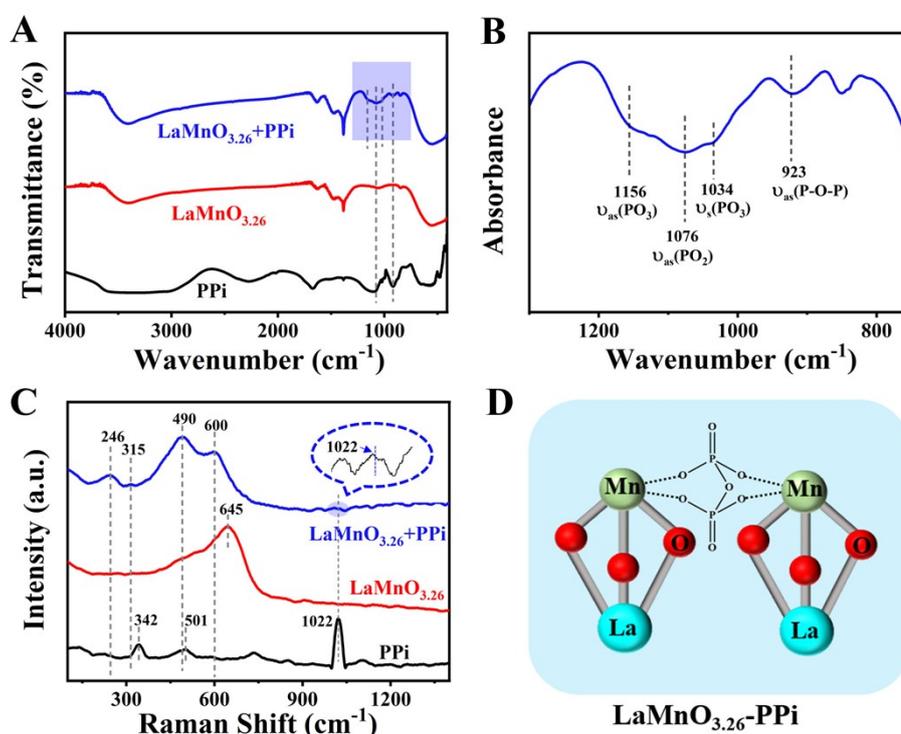


**Fig. S7** (A) FT-IR spectra of different samples, (B) detailed IR spectra of the purple shaded part in (A), (C) Raman spectra of different samples, (D) Schematic diagram of the structure of PPi complexed with LaMnO$_{3.26}$.
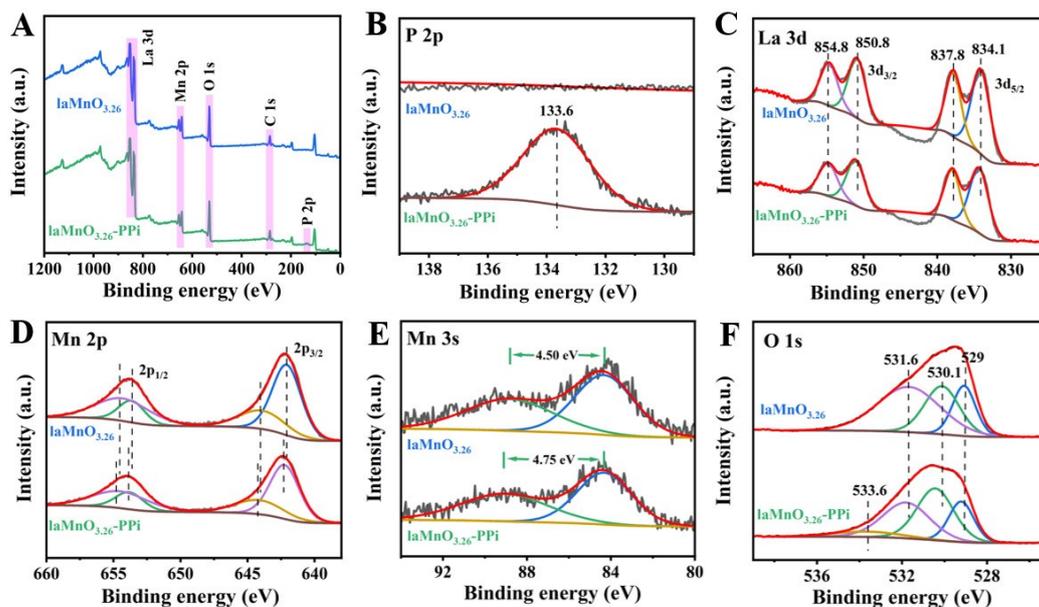
**Fig. S8** (A) XPS survey spectra of LaMnO$_{3.26}$ and PPi treated LaMnO$_{3.26}$. High-resolution XPS spectra of (B) P 2p, (C) La 3d, (D) Mn 2p, (E) Mn 3s, and (F) O 1s of LaMnO$_{3.26}$ and PPi treated LaMnO$_{3.26}$.
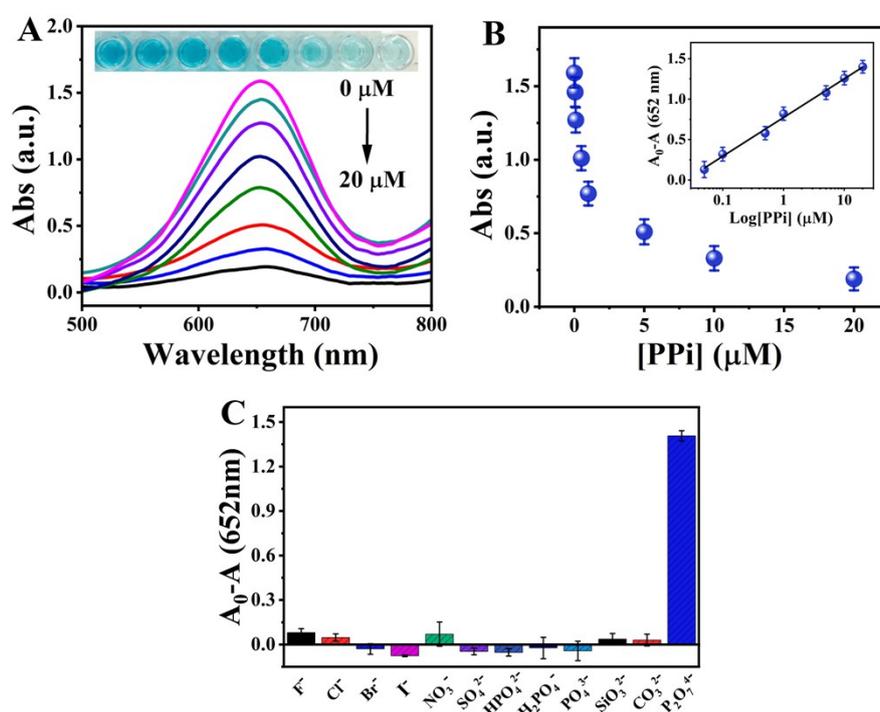


**Fig. S9** (A) Effect of PPi concentration on the oxidation of TMB by LaMnO$_{3.26}$ (PPi concentrations of 0, 0.05, 0.1, 0.5, 1, 5, 10 and 20 μM), (B) Linearity responses for PPi,

(C) Effects of various interfering anions on the catalytic oxidation of TMB by LaMnO$_{3.26}$. Error bars indicate the standard deviation of the three replicate measurements. A and A$_0$ are the absorbance intensities of the detection system in the presence and absence of PPi, respectively.
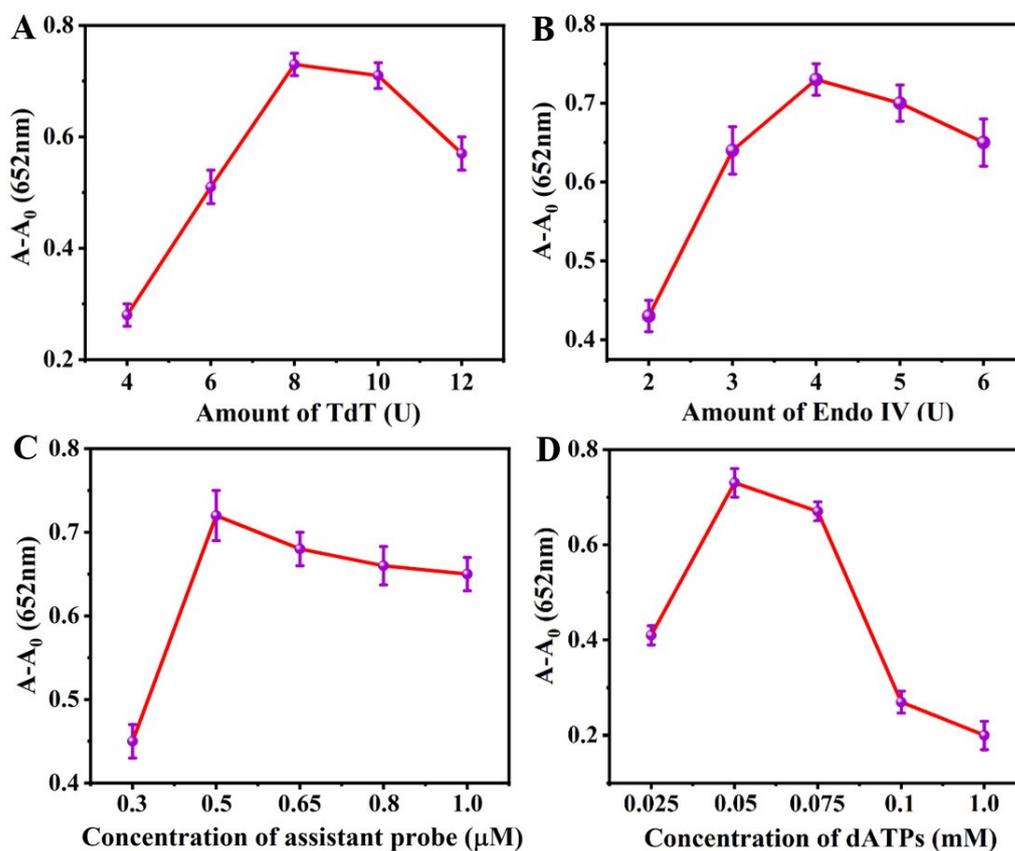


**Fig. S10** Absorption increment (A-A$_0$) values versus the concentration of (A) TdT, or (B) Endo IV, or (C) assistant probe, or (D) dATPs. A and A$_0$ are the absorbance intensities of the detection system in the presence and absence of T4 DNA ligase, respectively. The T4 DNA ligase concentration is 10 U/mL. Error bars indicate the standard deviation of three independent experiments.
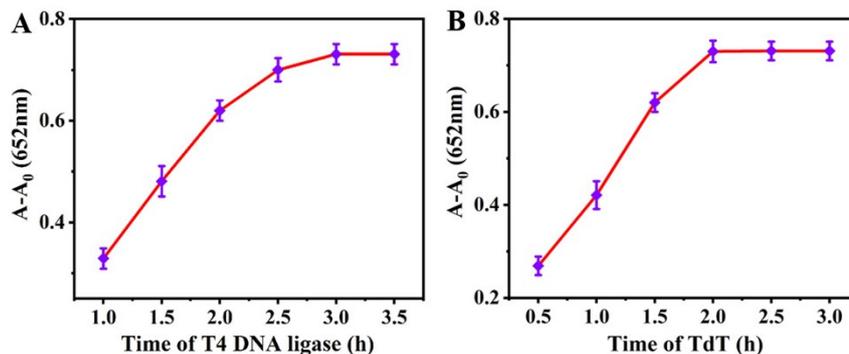
**Fig. S11** Influence of (A) ligation time of T4 DNA ligase, and (B) amplification time of TdT on the response of the detection system. A and $A_0$ are the absorbance intensities in the presence and absence of T4 DNA ligase, respectively. Error bars indicate the standard deviation of three independent experiments.

**Table S2.** Comparison with other reported methods for the detection of T4 DNA ligase.

| Method | Linear Range (U/mL) | LOD (U/mL) | Reference |
|---|---|---|---|
| Fluorescence | 0~2.5 | $1.2 \times 10^{-2}$ | 7 |
| Electrochemical | $5.0 \times 10^{-3}$~5 | $2.5 \times 10^{-3}$ | 8 |
| Colorimetry | 0.2~5 | 0.2 | 9 |
| Colorimetry | $4.8 \times 10^{-3}$~6 | $1.6 \times 10^{-3}$ | This work |

**Table S3.** Recovery of T4 DNA ligase in the diluted human serum samples (n=4).

| Samples | Spiked amount (U/mL) | Detected amount (U/mL) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| 1 | 0.03 | 0.03±0.001 | 103.3 | 3.3 |
| 2 | 0.15 | 0.15±0.003 | 102.0 | 2.0 |
| 3 | 0.60 | 0.65±0.041 | 108.3 | 6.3 |
| 4 | 1.00 | 0.98±0.025 | 98.0 | 2.5 |

# References

1 Q. Wang, L. Zhang, C. Shang, Z. Zhang, S. Dong, *Chem. Commun.*, 2016, **52**, 5410-5413.

2 Y. Ding, G. Wang, F. Sun, Y. Lin, *ACS Appl. Mater. Interfaces*, 2018, **10**, 32567–32578.

3 L. Su, W. Dong, C. Wu, Y. Gong, Y. Zhang, L. Li, G. Mao, S. Feng, *Anal. Chim. Acta*, 2017, **951**, 124−132.

4 Y. Wang, C. Chen, D. Zhang, J. Wang, *Appl. Catal. B*, 2020, **261**, 118256.

5 J. Feng, P. Huang, F. Wu, *Analyst*, 2017, **142**, 4106.

6 H. Cheng, S. Lin, F. Muhammad, Y. W. Lin, H. Wei, *ACS Sens.*, 2016, **1**, 1336–1343.

7 Y. Guo, Q. Wang, Z. Wang, X. Chen, L. Xu, J. Hu, R. Pei, *Sens. Actuators B Chem.*, 2015, **214**, 50–55.

8 X. Y. Wang, P. Dong, W. Yun, Y. Xu, P. G. He, Y. Z. Fang, *Talanta*, 2010, **80**, 1643-1647.

9 K. Y. He, W. Li, Z. Nie, Y. Huang, Z. L. Liu, L. H. Nie, S. Z. Yao, *Chem. Eur. J.*, 2012, **18**, 3992-3999.