# Carbon dots enhanced peroxidase-like activity of platinum nanozyme

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## Experimental section

***Synthesis of CDs:*** 1.6 g of carbon fibre powder was refluxed in 120 mL of $HNO_3$ and $H_2SO_4$ ($V_{HNO_3}$:$V_{H_2SO_4}$=2:1) for 4 h. Then, the resulting solution was collected and neutralized with $NaHCO_3$ to the pH~3. The resultant solution containing CDs was first filtered by 0.22 μm BIOSHARP membrane filters and further dialyzed in a 3500-Da dialysis bag for 7 days.

***Preparation of artificial urine sample:*** The artificial urine sample was prepared according to the previously report.[1] In short, L-lactic acid (1.1 mM), citric acid (2.0 mM), urea (170 mM), $NH_4Cl$ (25 mM), $CaCl_2 \cdot 2H_2O$ (2.5 mM), NaCl (10 mM), $NaHCO_3$ (25 mM), $K_2HPO_4$ (7.0 mM), $KH_2PO_4$ (7.0 mM), $MgSO_4$(2.0 mM), and $Na_2SO_4$ (10 mM) were mixed in DI water.

***Effect of metal ions on the catalytic activity of Pt@CDs:*** 50 μL of $H_2O_2$ (2 mM in 0.33 M sodium acetate and 0.0166 M citric acid (buffer A for short)), 50 μL of TMB (2 mM in 1 mM ethylenediaminetetraacetic acid disodium salt and 9.8 mM citric acid (buffer B for short) ), 1 μL of each kind of metal ion solution (1 mM) and 2.5 μL of Pt@CDs were mixed and incubated at 40 ℃ for 10 min. The mixture was measured by the absorbance at 652 nm on a plate reader.

***Detection of $H_2O_2$ and dopamine (DA) in real samples***: The same protocol for $H_2O_2$ detection was applied in two commercial drinks, fresh orange juice and milk. A series of $H_2O_2$ solution with varying concentrations were added to a 1000-fold diluted orange juice or milk before analysis. The content of $H_2O_2$ were determined by the standard addition method. Similarly, the same protocol for DA detection was applied in a 1000-fold diluted serum. The contents of DA were determined by the standard addition method.

***Mask ascorbic acid (AA) in dopamine detection***: Briefly, AA solutions with different concentrations (30, 150, 300 μM) were incubated with $Cu^{2+}$ and EDTA. The concentrations of $Cu^{2+}$ and EDTA were twice that of AA. The DA solution was treated

in the same way. Then, the same protocol for DA detection was applied. The absorbance at 652 nm was recorded. The absorbance variation ($\Delta A = A_{blank} - A$) of each group was calculated. $A_{blank}$ and A represent the absorbance intensity of Pt@CDs + TMB + $H_2O_2$ system in the absence and presence of DA or interfering AA masked by $Cu^{2+}$, respectively.

***Morphological Characterization of Bacteria***: Typically, PBS, Pt@CDs, $H_2O_2$ (100, μM), Pt@CDs+ $H_2O_2$ (100 μM) were added into 500 μL of $10^7$ cfu/mL *E. coli*, then incubated for 3 h at 37 ℃ under 180-rpm shaking. The obtained samples were harvested by centrifugation at 3000 rpm for 10 min at 4 ℃, and then washed with PBS. 2.5% glutaraldehyde solution was added to fix *E. coli* and the mixture was incubated at 4 ℃ for 4 h. Subsequently, different concentration of ethanol solution (30, 50, 70, 90, 100 %) were used for bacterial dehydration. The final samples were dipped onto clean silicon wafer and dried overnight. After sputtering deposition of Au, the samples were imaged by SEM (ZsrissSigma 300).

## Supporting Figures

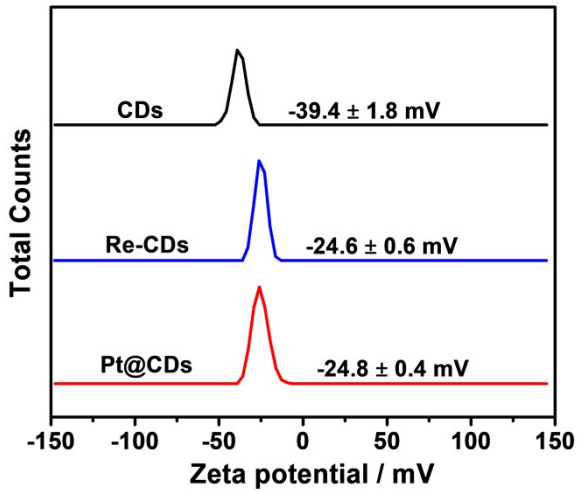


**Fig. S1** The zeta potential of CDs, Re-CDs, and Pt@CDs.

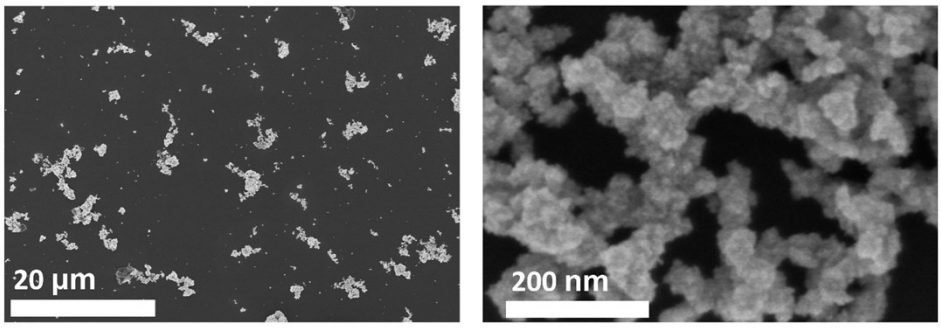


**Fig. S2** SEM images of PtNPs prepared in absence of CDs.

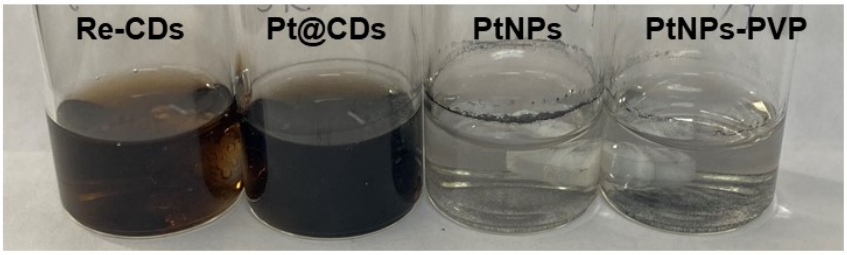


**Fig. S3** Photograph of Re-CDs, Pt@CDs, PtNPs prepared in absence of CDs, and PtNPs prepared in the presence of PVP (PtNPs-PVP).

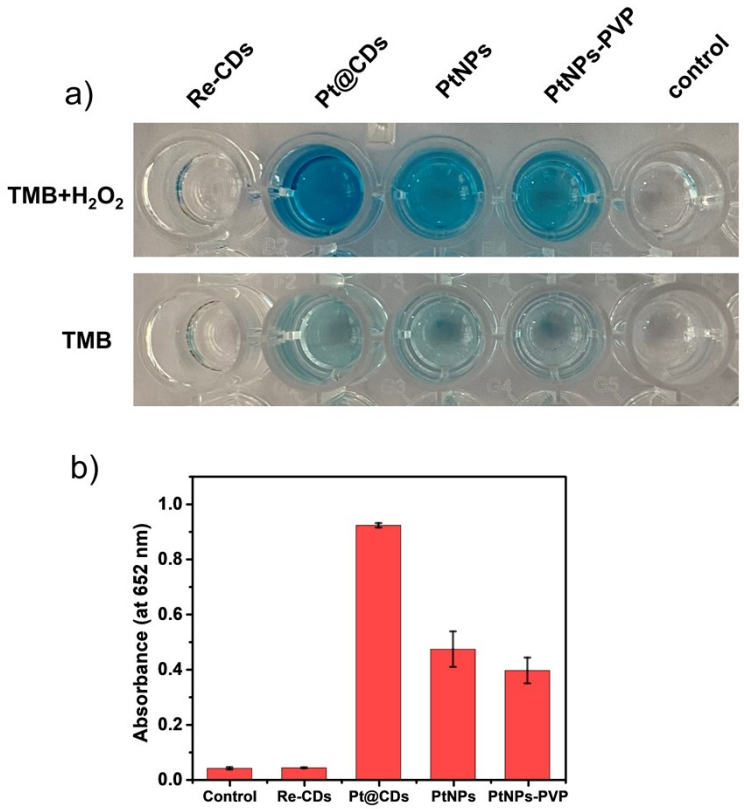


**Fig. S4** Comparison of catalytic activities for TMB-$H_2O_2$ among Re-CDs, Pt@CDs, PtNPs, and PtNPs-PVP.

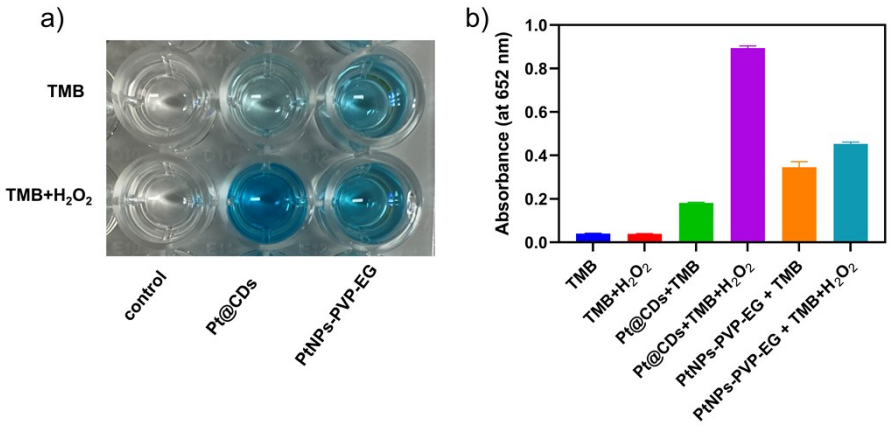


**Fig. S5** Comparison of catalytic activities for TMB-$H_2O_2$ between Pt@CDs and PtNPs-PVP-EG.

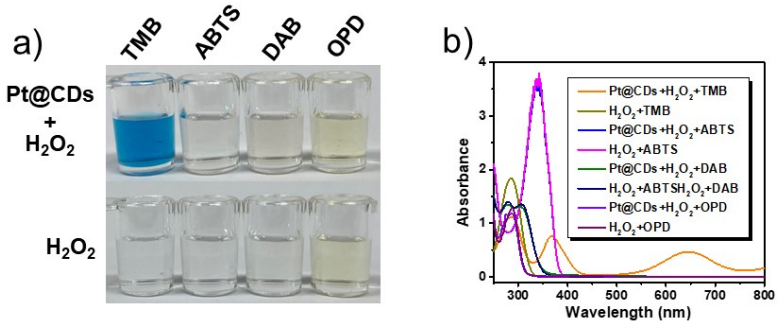


**Fig. S6** The comparison of Pt@CDs-catalyzed $H_2O_2$ oxidization among various organic peroxidase substrates of TMB, ABTS, DAB, and OPD.

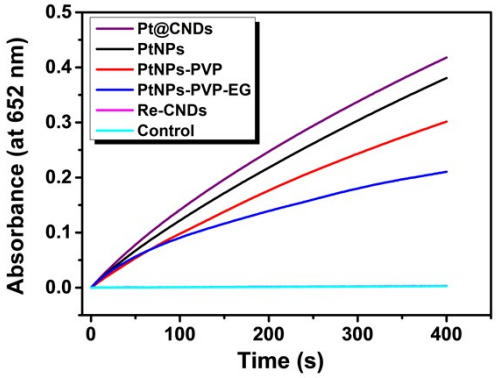


**Fig. S7** Reaction–time curves of TMB oxidation reactions catalyzed by Pt@CDs, PtNPs, PtNPs-PVP, PtNPs-PVP-EG, and Re-CDs as indicated.

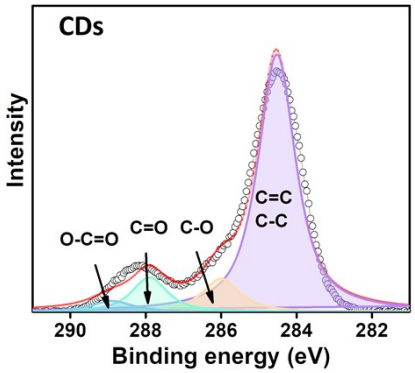


**Fig. S8** The C 1s high-resolution XPS with identification of peaks by curve fitting of CDs

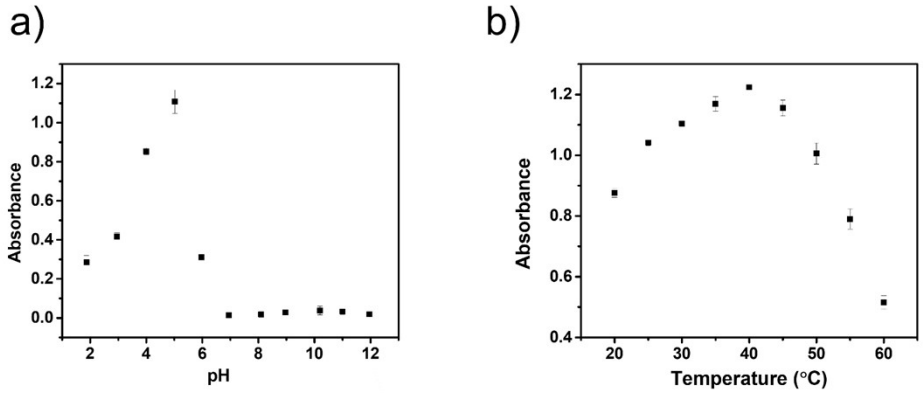


**Fig. S9** Effects of pH (a) and temperature (b) on the catalytic performance of Pt@CDs.

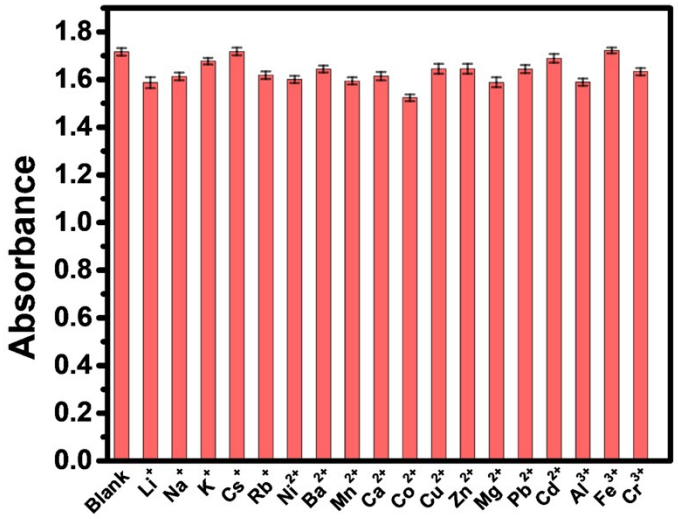


**Fig. S10** Effects of various metal ions (10 μM) on the Pt@CDs-TMB-$H_2O_2$ reaction.

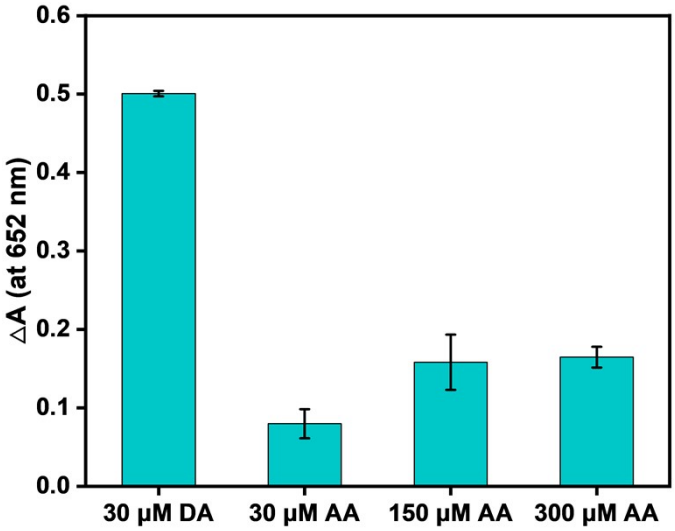


**Fig. S11** Response of the Pt@CDs-TMB-$H_2O_2$ reaction toward DA and AA in the present of $Cu^{2+}$ with twice the concentration of AA.

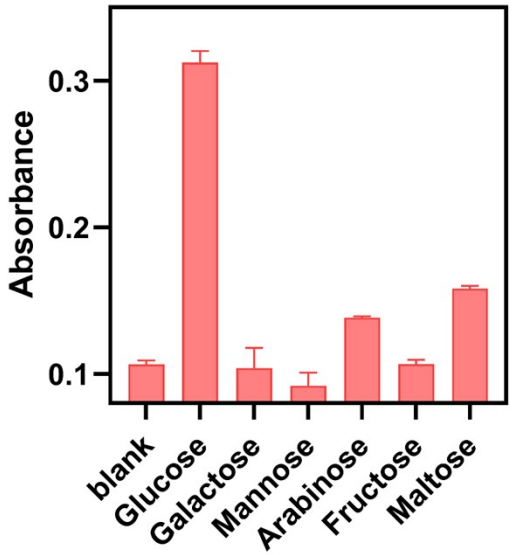


**Fig. S12** Selectivity of the proposed assay for glucose. The concentrations of galactose, mannose, arabinose, fructose were 10 mM and the concentration of maltose was 5 mM, whereas the concentration of glucose was 1 mM.

**Table S1**. Comparison of the $K_m$ and $V_{max}$ values of different Pt-based nanozymes.

| Enzyme | Substrate | $K_m$ (mM) | $V_{max}$ ($10^{-8}$M/s) | Ref. |
|---|---|---|---|---|
| **HRP** | TMB | 0.434 | 10.0 | 2 |
| | $H_2O_2$ | 3.7 | 8.71 | |
| **PVP-PtNCs** | TMB | 0.031 | 11.53 | 3 |
| | $H_2O_2$ | 78.31 | 23.79 | |
| **PVP/PtRu NZs** | TMB | 0.76 | 11.53 | 4 |
| | $H_2O_2$ | 36.0 | 23.79 | |
| **BSA-PtNPs** | TMB | 0.217 | 15.4 | 5 |
| | $H_2O_2$ | 68.4 | 28.7 | |
| **BSA/Pt-NPs** | TMB | 0.119 | 21.0 | 6 |
| | $H_2O_2$ | 41.8 | 16.7 | |
| **Apoferritin-PtNPs** | TMB | 0.22 | 55.8 | 7 |
| | $H_2O_2$ | 187.25 | $3.2\times10^4$ | |
| **NAC–Pt NCs** | TMB | 0.132 | 48.3 | 8 |
| | $H_2O_2$ | 35.00 | 31.7 | |
| **Citric acid-RhPt** | TMB | 0.129 | 68.15 | 9 |
| | $H_2O_2$ | 6.18 | 92.70 | |
| **Citrate-PtNPs** | TMB | 0.055 | 0.58 | 10 |
| | $H_2O_2$ | 63 | 0.53 | |
| **DNA-Pt2.9** | TMB | 0.056 | 58.2 | 11 |
| | $H_2O_2$ | 48.0 | 56.8 | |
| **DNA-Pt2.1** | TMB | 0.0329 | 11.9 | 11 |
| | $H_2O_2$ | 74.4 | 30.5 | |
| **DNA-Pt1.8** | TMB | 0.0162 | 1.93 | 11 |
| | $H_2O_2$ | 117.2 | 5.19 | |
| **$Pt_{600}$-GLP NCs** | TMB | 0.17 | 5.04 | 12 |
| | $H_2O_2$ | 2.06 | 7.51 | |
| **Pt@CDs** | TMB | 0.061 | 11.8 | This |
| | $H_2O_2$ | 26.5 | $9.1\times10^4$ | work |

**Table S2.** Relative content of different oxygen-containing functional groups (calculated by integrating fitting curve area of C 1s XPS).

| | C=C | C-O | C=O | -COO |
|---|---|---|---|---|
| CDs | 76.9% | 9.9% | 9.7% | 3.5% |
| Re-CDs | 77.1% | 13.1% | 5.8% | 4.0% |
| Pt@CDs | 78.2% | 10.9% | 7.7% | 3.2% |

**Table S3.** Determination of $H_2O_2$ in orange juice (n=3)

| Sample | Added (μM) | Found (μM) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| 1 | 400.00 | 389.01 | 97.25 | 5.14 |
| 2 | 500.00 | 516.00 | 103.20 | 3.45 |
| 3 | 800.00 | 790.44 | 98.81 | 4.97 |

**Table S4.** Determination of $H_2O_2$ in milk (*n*=3)

| Sample | Added (μM) | Found (μM) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| 1 | 200.00 | 194.89 | 97.45 | 6.68 |
| 2 | 400.00 | 407.11 | 101.78 | 6.37 |
| 3 | 1000.00 | 1009.33 | 100.93 | 4.58 |

**Table S5.** Determination of DA in serum (*n*=3)

| Sample | Added (μM) | Found (μM) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| 1 | 125.00 | 125.69 | 100.55 | 4.97 |
| 2 | 150.00 | 152.61 | 101.74 | 3.96 |
| 3 | 250.00 | 256.46 | 102.58 | 3.84 |

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