Supporting Information

Peroxidase Activity of the Coronene Bisimide Supramolecular Architecture and the Applications in Colorimetric Sensing of H_2O_2 and Glucose

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Experimental section

Optimization of the H_2O_2 assay

The effect of CTDI concentration

The catalytic activity of CTDI was studied. TMB, H_2O_2 and different amounts of CTDI were added to the sample buffer solution, mixed briefly and incubated in a 37 ºC water bath for 2 h. The absorption spectra were measured, and the intensity changes of the absorbance maximum of the oxidized TMB at 652 nm were followed. Final concentrations: TMB, 500 μM; H_2O_2, 100 μM; CTDI, 0, 5, 10, 15, 20, 25 and 30 μM, respectively; buffer, 50 mM HAc-NaAc, pH 5.0; total sample volume, 200 μL.

The effect of buffer pH value

TMB and H_2O_2 were added to buffer solutions of different pH value in the absence or presence of CTDI. The sample solutions were mixed briefly and incubated in a 37 ºC water bath for 2 h, and the absorption spectra were recorded. Conditions: TMB, 500 μM; H_2O_2, 100 μM; CTDI, 20 μM; buffer, 50 mM, NaAc-HAc, pH 3.5, 4.0, 4.5, 5.0, 5.5; Na_2HPO_4-NaH_2PO_4, pH 6.0, 6.5, 7.0.

The effect of buffer concentration

A HAc-NaAc buffer solution of different concentrations (Final concentrations: 5 − 60 mM, pH 5.0) was added to a sample solution containing 500 μM TMB, 100 μM H_2O_2 and 20 μM CTDI, mixed briefly and incubated in a 37 ºC water bath for 2 h, and the absorption spectra were recorded.

The effect of reaction time

H_2O_2 of various concentrations (Final concentrations: 0, 10, 100, 300, 500 μM, respectively) was added to the sample buffer solution containing 500 μM TMB and 20 μM CTDI. The sample solutions were incubated at 37 ºC and the absorption spectra were recorded at different incubation time (0 − 150 min, buffer, 50 mM HAc-NaAc, pH 5.0).

Kinetic assay

The peroxidase-like catalytic activity of the CTDI nanofibers was studied. The assay was conducted using 20 μM CTDI in buffer solution (50 mM, NaAc-HAc, pH 5.0, 37 ºC). TMB concentration was kept constant (0.5 mM) and H_2O_2 concentration was varied. Then H_2O_2 concentration was kept constant (50 mM) and TMB concentration was varied. The reactions were monitored via the UV-vis absorption changes at 652 nm. The reaction rates were calculated (molar extinction coefficient of oxidized TMB: 39,000 $M^{-1}\cdot cm^{-1}$). The Michaelis–Menten constant was calculated using the Lineweaver–Burk plot: $1/\nu = K_m/V_{max}\cdot(1/[S]+1/K_m)$, where ν is the initial reaction velocity, V_{max} is the maximal reaction velocity, and [S] is the concentration of the substrate.

Optimization of the glucose assay

The effect of GOx concentration

GOx of various concentrations (Final concentrations: 0, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2.5, 5 U/mL, respectively) was added to the sample solution containing 100 μM glucose, 500 μM TMB, and 20 μM CTDI. Samples were incubated in a 37 ºC water bath for 2 h and the absorption spectra were recorded (buffer, 50 mM HAc-NaAc, pH 5.0).

The effect of reaction time

Glucose and GOx were added to the sample solutions containing TMB and CTDI. Samples were incubated at 37 ºC and the absorption spectra were recorded at a certain period of time (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 min, respectively). Final concentrations: glucose, 25 μM; GOx, 0.5 U/mL; TMB, 500 μM; CTDI, 20 μM; buffer, 50 mM HAc-NaAc, pH 5.0.

Reuse of the nanofibers catalyst

CTDI, TMB and GOx were added to dilute human blood sample solutions. Samples were incubated at 37 ºC for 2 h and the absorption spectra were recorded. After the reaction, the sample solutions were passed through a filter (MWCO: 100 kD) via centrifugation at 10,000 rpm for 5 min. The CTDI catalyst was separated and washed two times with water, and used for another cycle of the catalytic reaction. Final concentrations: CTDI, 20 μM; GOx, 0.5 U/mL; TMB, 500 μM; buffer, 50 mM HAc-NaAc, pH 5.0.

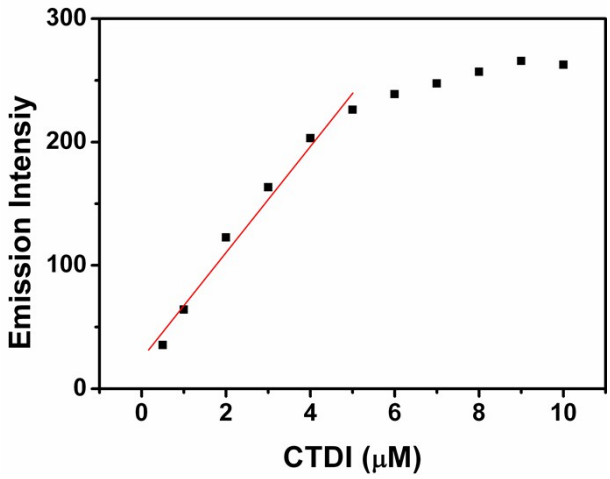

Fig. S1 Changes in emission intensity of CTDI at 492 nm in ethanol with concentration.

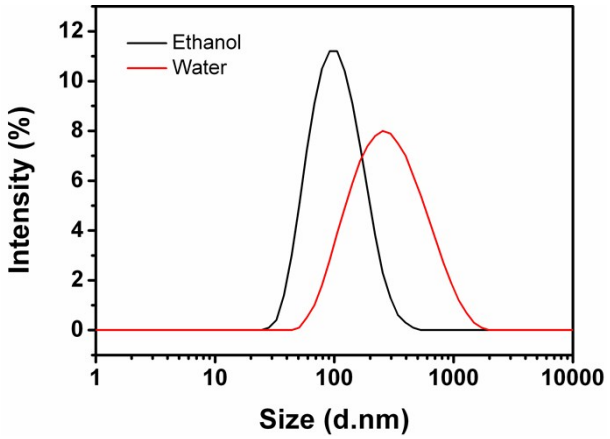

Fig. S2 DLS: size distribution of 10 μM CTDI in ethanol and water solution.

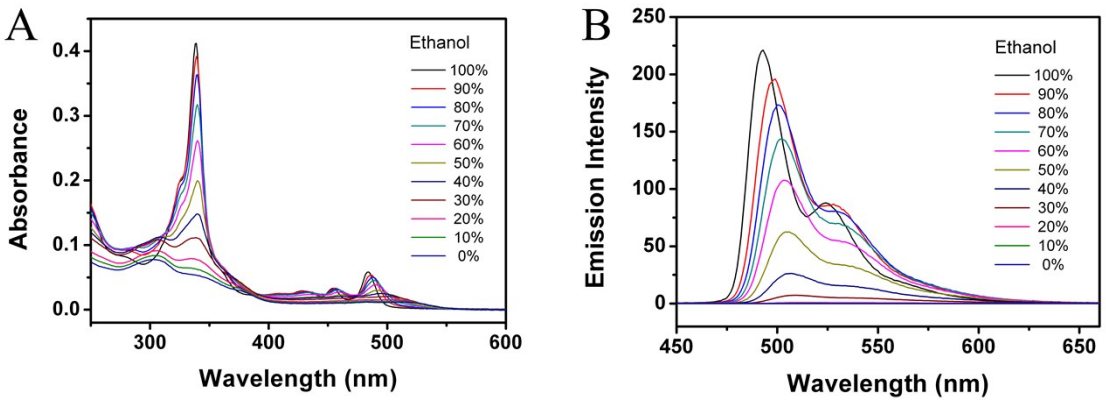

Fig. S3 Changes in UV-vis absorption (A) and emission (B) spectra of 5 μM CTDI in ethanol – water solvent mixture with ethanol concentration.

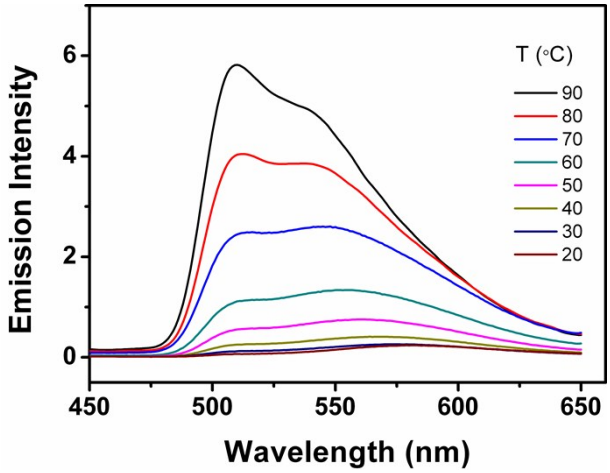

Fig. S4 Changes in emission spectrum of 5 μM CTDI in water at different temperatures. The solution temperature was increased from 20 to 90 ºC.

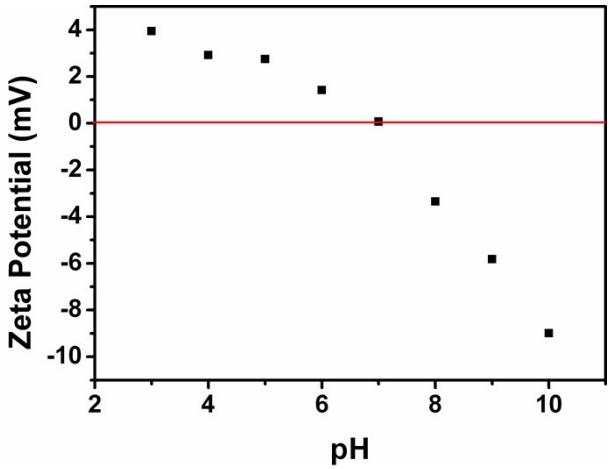

Fig. S5 Zeta potential value changes of the CTDI nanofibers in different buffer solutions. Conditions: 25 mM buffer, NaAc-HAc, pH 3.0 – 5.0; Na_2HPO_4-NaH_2PO_4, pH 6.0 – 9.0; Na_2CO_3-$NaHCO_3$, pH 10.0.

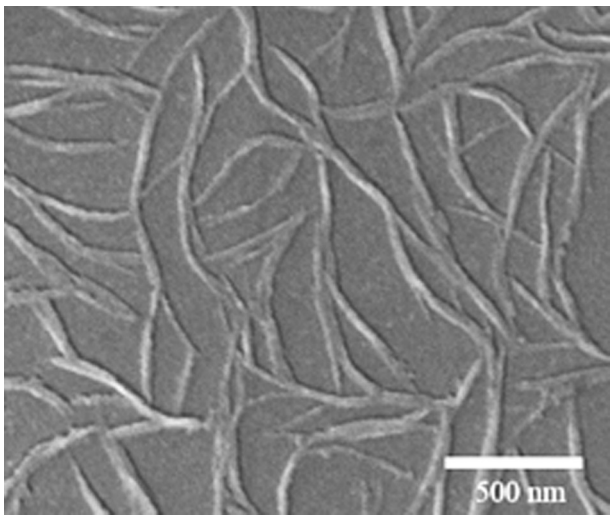

Fig. S6 SEM images of the self-assembled CTDI nanofibers in ethanol.

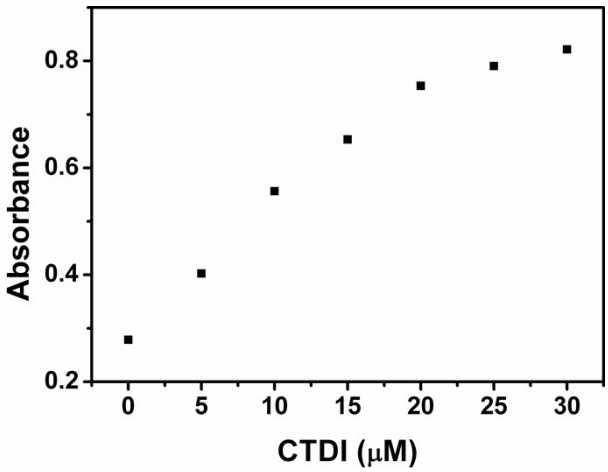

Fig. S7 Changes in maximum absorption of TMB (at 652 nm) with CTDI concentration. Sample mixture contains 500 μM TMB and 100 μM H_2O_2.

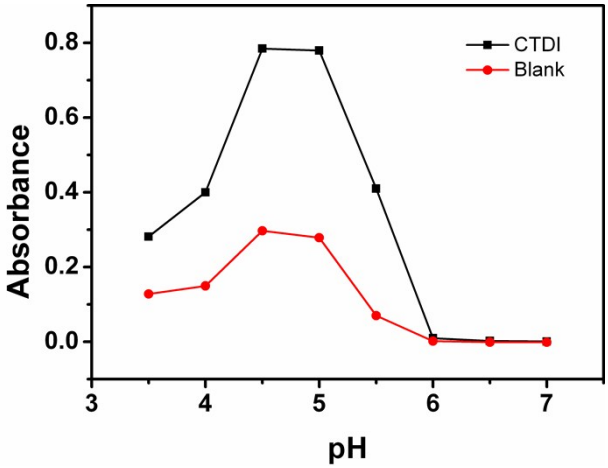

Fig. S8 Changes in maximum absorption of TMB at 652 nm with assay solution buffer pH value in the presence or absence of CTDI (blank control). Conditions: TMB, 500 μM; H_2O_2, 100 μM; CTDI, 20 μM; buffer, 50 mM, NaAc-HAc, pH 3.5 – 5.5; Na_2HPO_4-NaH_2PO_4, pH 6.0 – 7.0.

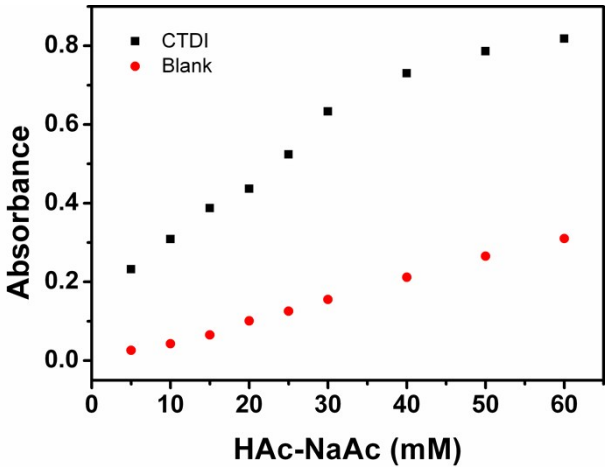

Fig. S9 Changes in maximum absorption of TMB at 652 nm with buffer concentration in the presence or absence of CTDI (blank control). Conditions: TMB, 500 μM; H_2O_2, 100 μM; CTDI, 20 μM; buffer: 5 – 60 mM NaAc-HAc, pH 5.0.

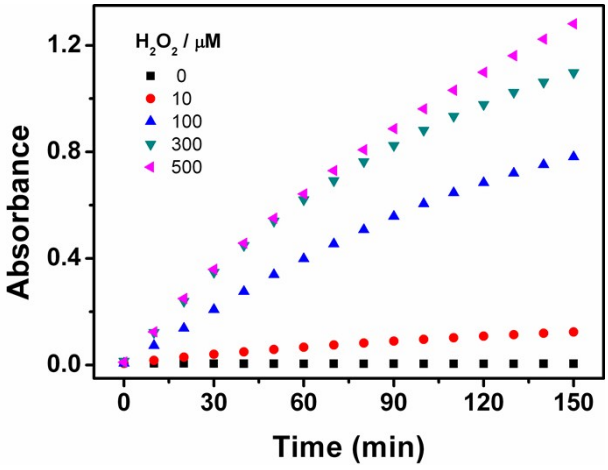

Fig. S10 Changes in TMB maximum UV-vis absorption at 652 nm with reaction time.

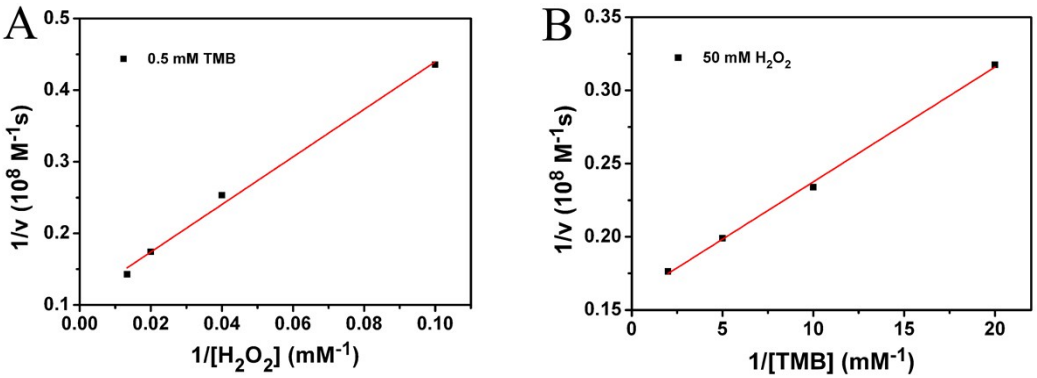

Fig. S11 Double-reciprocal plots for the CTDI nanofibers catalytic reaction. (A) TMB concentration was kept constant (0.5 mM), H_2O_2 concentration was varied; (B) H_2O_2 concentration was kept constant (50 mM), TMB concentration was varied. Conditions: CTDI, 20 μM; buffer, 50 mM, NaAc-HAc, pH 5.0.

Table S1. Comparison of the kinetic parameters (K_m and V_{max}) of the natural enzyme HRP and some carbon-based artificial peroxidase.

Catalyst	Substance	K_m (mM)	V_{max} (10^{-8} M·s^{-1})	Ref.
CTDI	TMB	0.049	6.28	This work
nanofibers	H_2O_2	30.85	9.29	
GO-COOH	TMB	0.024	3.45	19
	H_2O_2	3.99	3.85	
HRP	TMB	0.275	1.24	19
	H_2O_2	0.214	2.46	
C-Dots	TMB	0.039	3.61	22
	H_2O_2	26.77	30.61	
$C_{60}[C(COOH)_2]_2$	TMB	0.233	0.347	32
	H_2O_2	24.58	0.401	

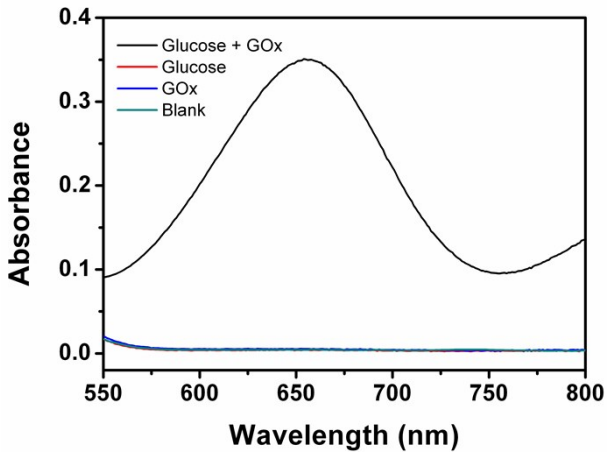

Fig. S12 Changes in UV-vis absorption spectrum of TMB (500 μM) in the presence of 500 μM glucose + 125 mU/mL GOx; 500 μM glucose; or 125 mU/mL GOx. CTDI: 20 μM. Blank curve is the UV-vis absorption spectrum of TMB (500 μM) in the presence of 20 μM CTDI.

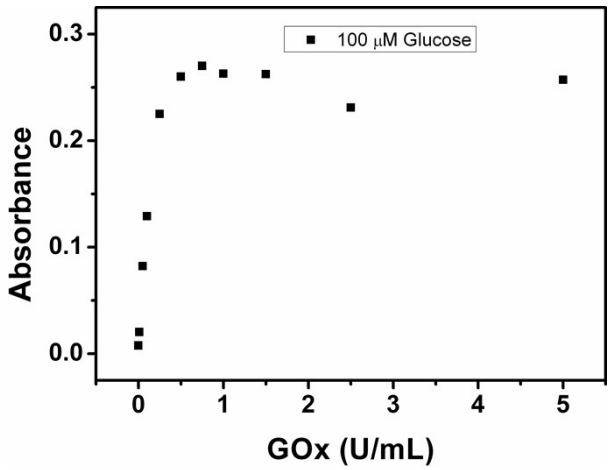

Fig. S13 The TMB absorption intensity changes at 652 nm with GOx concentration.

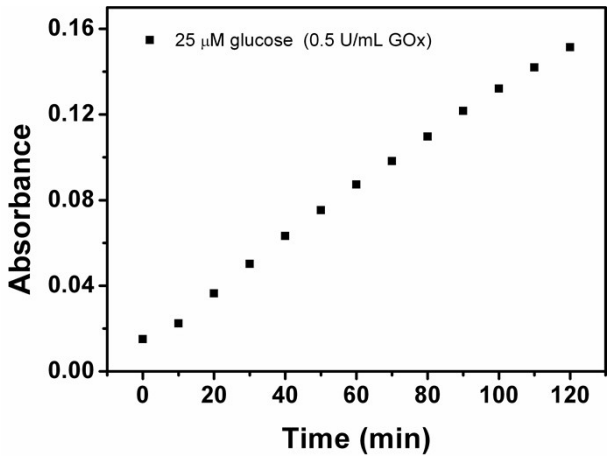

Fig. S14 Changes in TMB absorption value at 652 nm with reaction time. Conditions: 500 μM TMB, 20 μM CTDI.

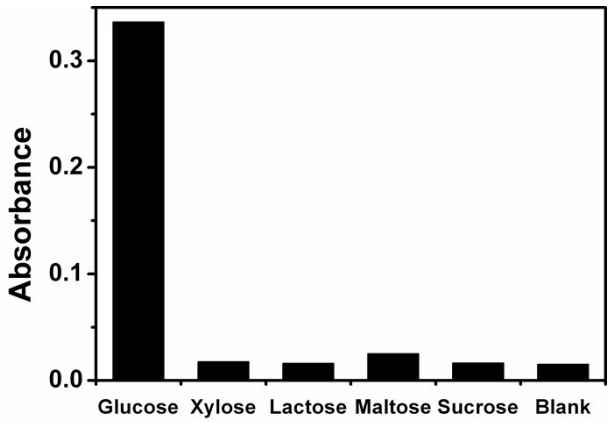

Fig. S15 Selectivity of the glucose assay. Final concentrations: TMB, 500 μM; CTDI, 20 μM; GOx, 0.5 U/mL; glucose, xylose, lactose, maltose, sucrose, 200 μM each.

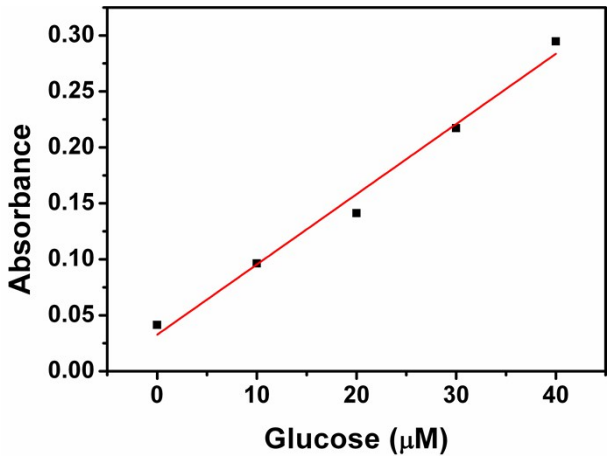

Fig. S16 Maximum TMB absorption value changes at 652 nm with glucose concentration in dilute blood sample. The spiked glucose concentration: 0, 10, 20, 30, 40 μM (A_{652} = 0.0063C + 0.033, correlation coefficient R^2 = 0.983).

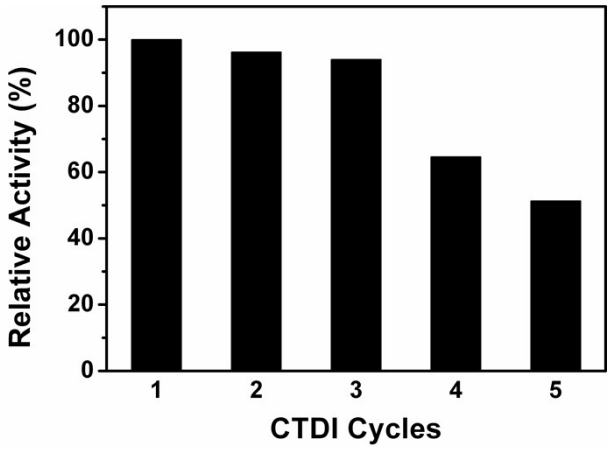

Fig. S17 Catalytic performance of the nanofibers catalyst after several cycles of reuse in dilute blood sample. After each round of reaction, the catalyst was separated via passing through a filter (MWCO: 100 kD), and used for another cycle of the catalytic reaction. Final concentrations: CTDI, 20 μM; GOx, 0.5 U/mL; TMB, 500 μM; buffer, 50 mM HAc-NaAc, pH 5.0.

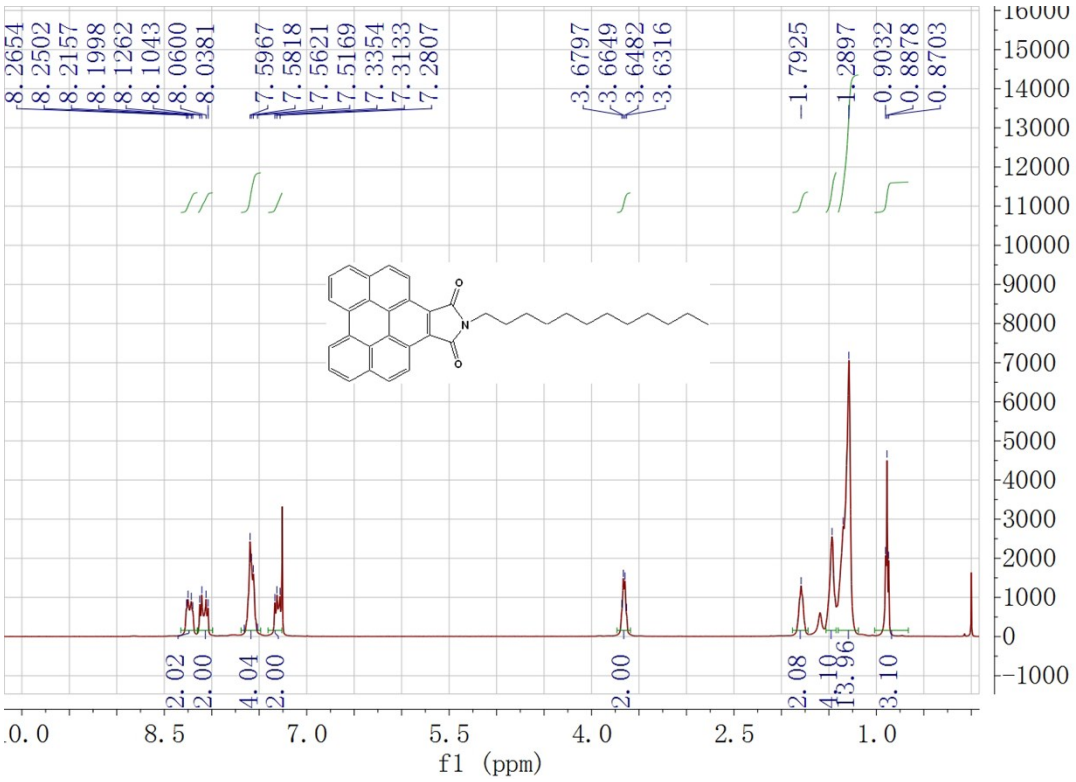

Fig. S18 ^{1}H NMR spectrum of compound **2**.

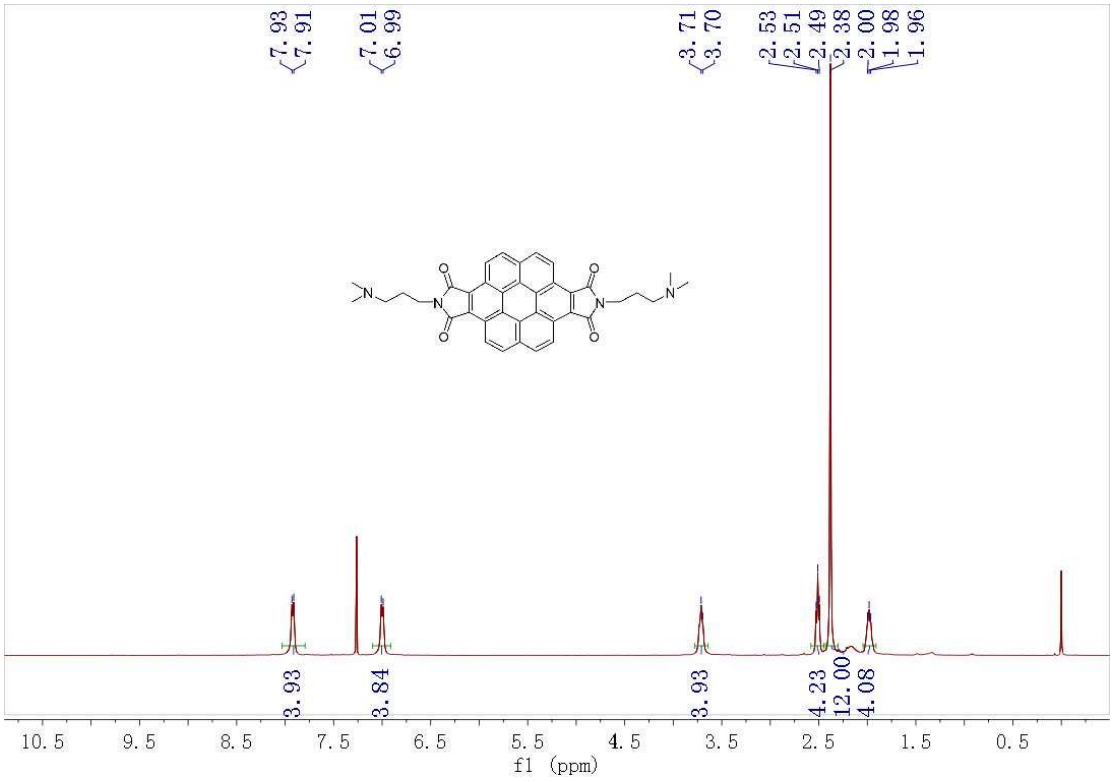

Fig. S19 ^{1}H NMR spectrum of compound **5**.

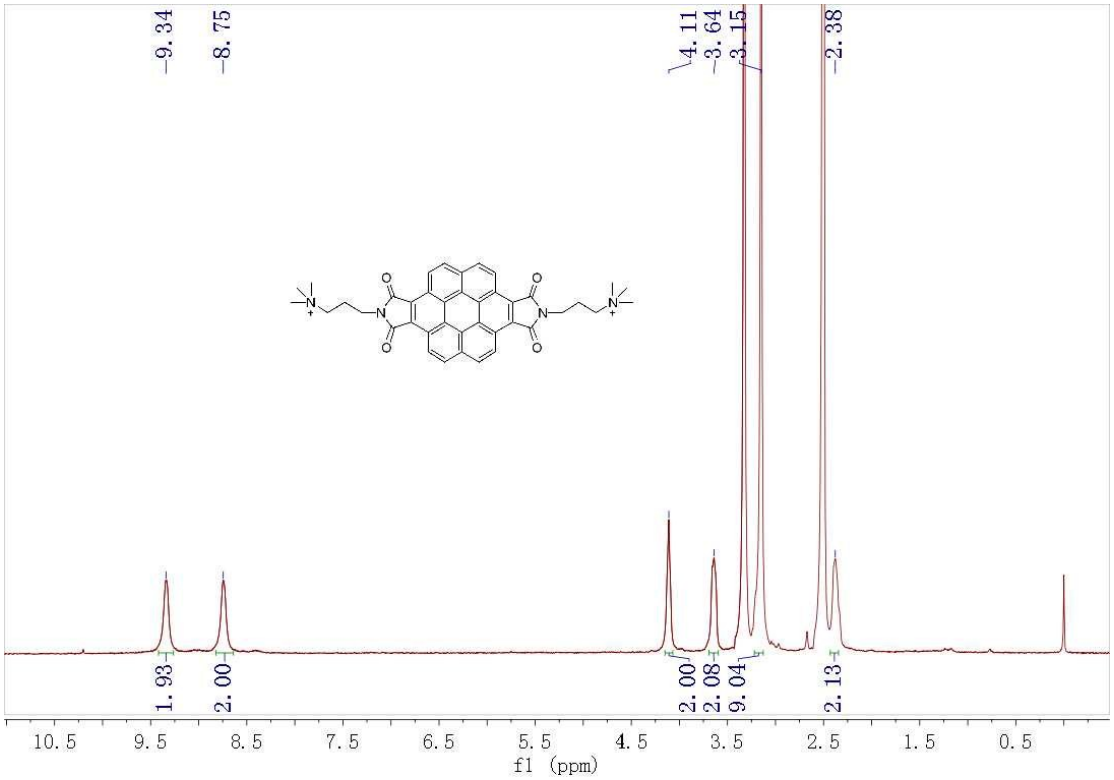

Fig. S20 ^{1}H NMR spectrum of CTDI.

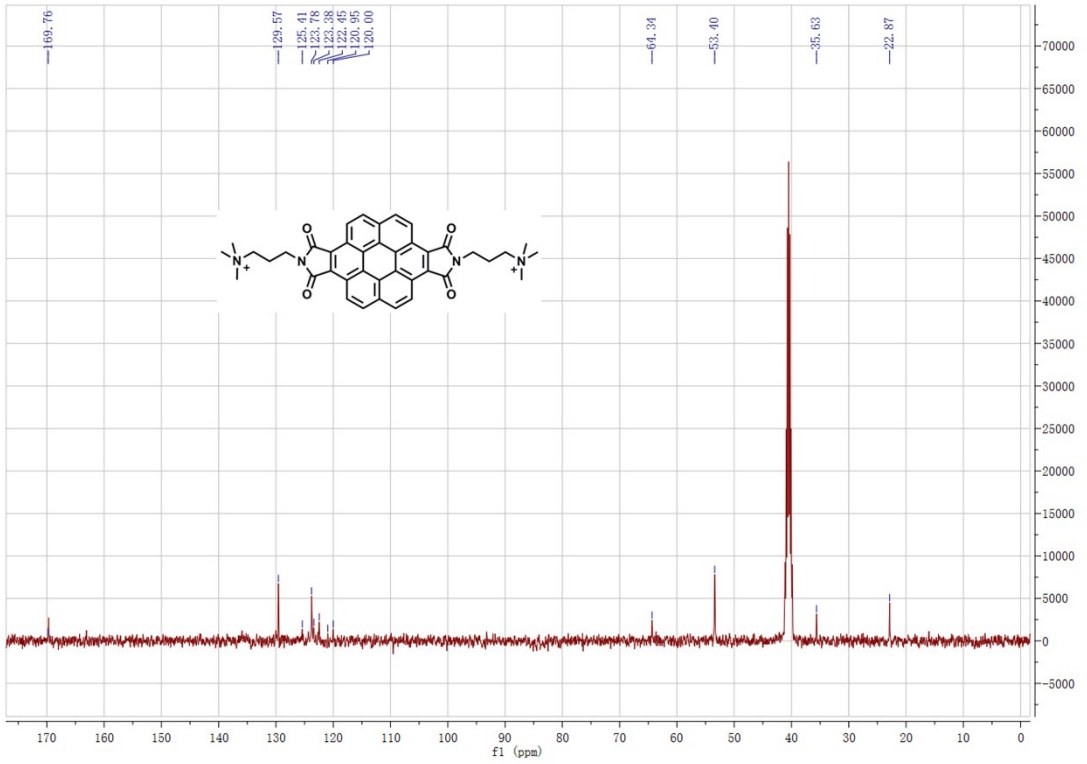

Fig. S21 ^{13}C NMR spectrum of CTDI.

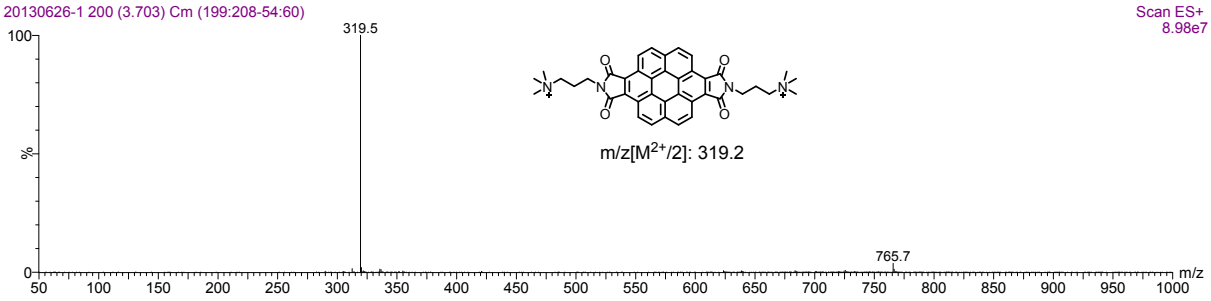

Fig. S22 Electrospray ionization mass spectrum of CTDI.