Supporting Information

3D Branched Crystal Carbon Nitride with Enhanced Intrinsic Peroxidase-Like Activity: a Hypersensitive Platform for Colorimetric Detection

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EXPERIMENTAL SECTION

Materials

All regents (Melamine, LiCl, KCl, CH_3COONa, CH_3COOH, H_2O_2, TMB, ABTS, $MgCl_2$, KCl) used were analytic degree in this paper without any purification in use. DNA oligomers were purchased from Sangon Biotech (Shanghai, China). Ultrapure water was obtained through a Millipore water purification system (Laikie Instrument Co., Ltd., Shanghai, China).

Instruments

Scanning electron microscopy (SEM) images were obtained through field emission SEM (Hitachi S4800). Transmission electron microscopy (TEM) images and energy-dispersive X-ray spectroscopy (EDS) elemental mapping were gained from high-magnification TEM (FEI Tecnai G2 F30 S-Twin). X-ray diffraction (XRD, D8 ADVANCE Powder) equipped with a Cu-Kα radiation was used for crystal phase structures of all the as-prepared samples. The chemical structures of as-prepared samples were obtained through Fourier Transform Infrared (FTIR, Bruker VERTEX 70 FTIR). Jasco V-550 spectrometer was employed for ultraviolet-visible (UV-vis) absorption spectra. Electron paramagnetic resonance (EPR, Bruker 300E spectrometer, Germany) was employed for ·OH detection. The water contact angle (WCA) was determined by an optical contact angle and interface tension meter (KINO SL 200KB).

3DBC-C_3N_4 preparation

10 g of melamine was placed in a crucible with a cover and heated to 550 °C for 4 h with a heating rate of 12 °C/min, and then yellow power g-C_3N_4 obtained after natural cooling to room temperature. A mixture of 1 g of obtained yellow power and the 6 g of KCl/LiCl 11:9 (weight/weight) was heated to 550 °C for 4 h under Ar with a heating rate of 12 °C/min. When the temperature of the mixtures was cooled down to room temperature by natural cooling, the as-prepared mixture was suddenly washed by ice water and dried at 60 °C for 12 h. The 3DBC-C_3N_4 with nanoneedles was fabricated.

Peroxidase-like activity assays

0.1 mg/mL as-prepared nanomaterials were mixed with 5 mM TMB and 50 mM H_2O_2 at acetate buffer (pH = 4) for 30 min in room temperature. For analyzing ssDNA on the

peroxidase-like properties of 3DBC-C_3N_4, the mixture of 3 μM ssDNA (A_{22}, T_{22}, C_{22}, G_{22}) or different cytosine length (C_5, C_{10}, C_{22}, C_{44}, C_{80}) in buffer and 0.1 mg/mL 3DBC-C_3N_4 in acetate buffer (pH = 4) was incubated at room temperature for 1 h, followed by the substrate 5 mM TMB and 50 mM H_2O_2 addition, sequence shown in Table S1. ssDNA attaching 3DBC-C_3N_4 in different concentration of saline ion was maintained at room temperature for 1 h, followed by the substrate 5 mM TMB (5 mM ABTS) addition. After being incubated for 30 min at room temperature, the absorbance of these solutions in a cell was measured with 652 nm excitation.

Steady-State Kinetic Analysis

Kinetic experiments were carried out in a 1.5 mL EP tube containing 0.1 mg/mL pre-prepared nanomaterials in acetate buffer (pH = 4) with varied concentrations of TMB (H_2O_2) and 50 mM H_2O_2 (5 mM TMB). A Line-Burk plot was provided for Michaels-Menten constant calculated: $V_0=(V_{max}+[S])/(K_m+[S])$, where V_0 is the initial velocity, V_{max} is the maximal reaction velocity, [S] correspond to the substrate concentration, and K_m is the Michaelis-Menten constant, equaling to [S] when the reaction velocity reached half of V_{max}.

EPR and Zeta

EPR was carried out for ·OH detection. After nanomaterials with or without ssDNA and H_2O_2 mixed for 30 min at room temperature. The 5, 5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was added into the mixture as the spin trapping agent for ·OH detection. Zeta potential measurement was also investigated for different treatments of surface charge effect at acetate buffer (pH = 4).

OTC detection

OTC aptamer and 3DBC-C_3N_4 were incubated room temperature for 30 min, aptamer sequence listed in Table S1. Then different concentrations of OTC were added for hybridizing with its aptamer in room temperature for 1 h. The mixture was mixed with 5 mM TMB and 50 mM H_2O_2 at room temperature for 30 min. The absorbance of the cell with solution was measured at 652 nm.

Cell viability assay and bioimaging analyze

Liver hepatocellular carcinoma ($HepG_2$) cells were cultured in Dulbecco's modified

Eagle's medium (DMEM) in a Petri dish, containing 10% fetal bovine serum. The Petri dish was incubated in a humidified atmosphere containing 5% CO_2 at 37 °C. $HepG_2$ cells were dispersed in a 96 well microtiter plate at 1.0 * 10^5 cells per well. Different concentrations of 3DBC-C_3N_4 were added into $HepG_2$ cells and then incubated for 24 h. Subsequently, the cells were washed five times with 0.1 mM PBS buffer and 100 μL of 5 mg/mL. Methyl thiazolyl tetrazolium (MTT) in PBS buffer was added to each well for 5 h. After MTT solution discarded, 200 μL of DMSO was added to solubilize the formazan crystals, and then the solution in each well was collected. The absorbance was measured at 490 nm by a RT 6000 microplate reader. The 3DBC-C_3N_4 was incubated in Hela cells for 24 h. After PBS washing, fluorescence was detected via laser scanning confocal microscopy (Leica, Wetzlar, German).

RESULTS

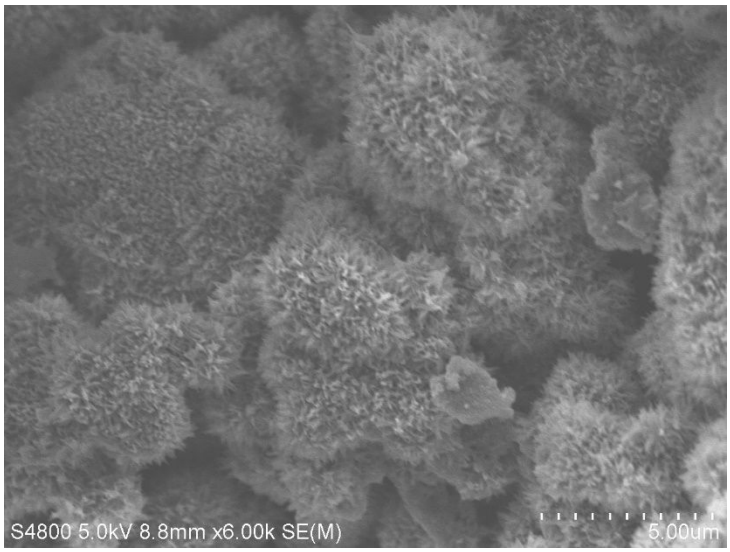

Figure S1. Low magnification SEM images of 3DBC-C_3N_4.

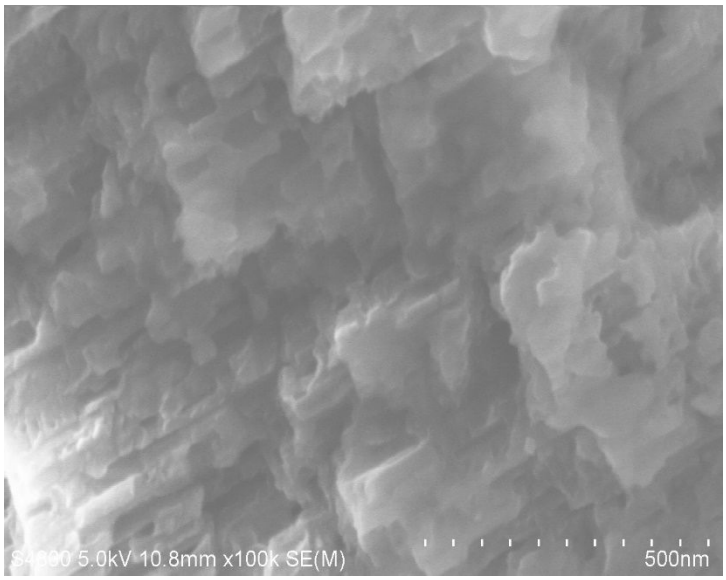

Figure S2. Low magnification SEM images of g-C_3N_4.

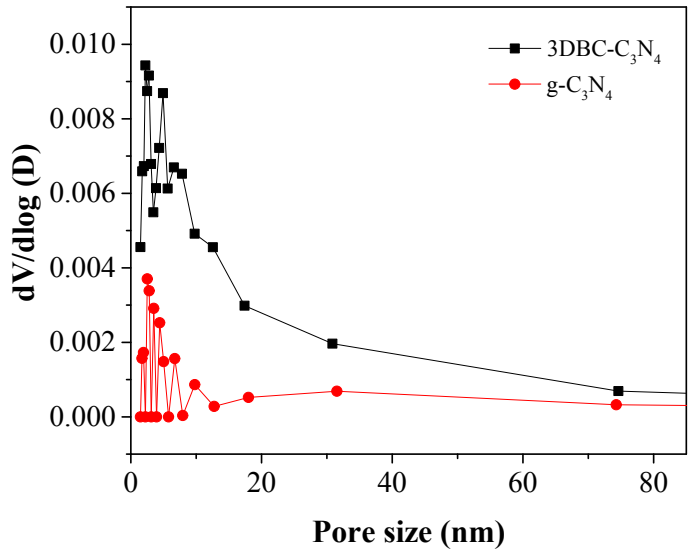

Figure S3. Pore-size distribution curves of 3DBC-C_3N_4 and g-C_3N_4

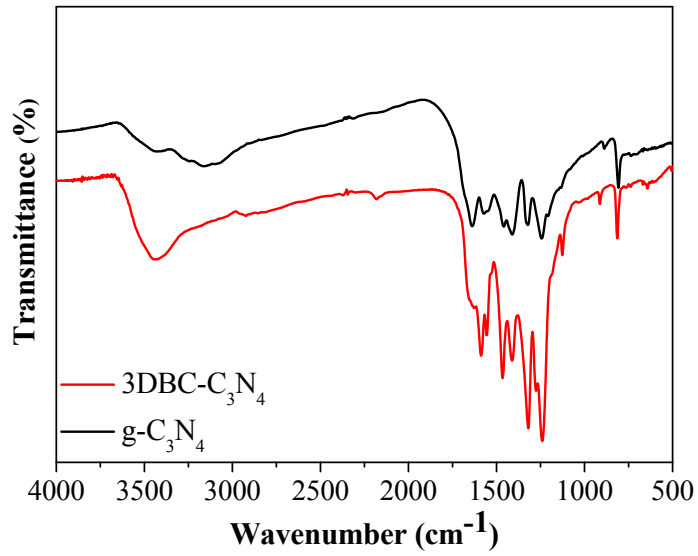

Figure S4. FT-IR of 3DBC-C_3N_4 and g-C_3N_4.

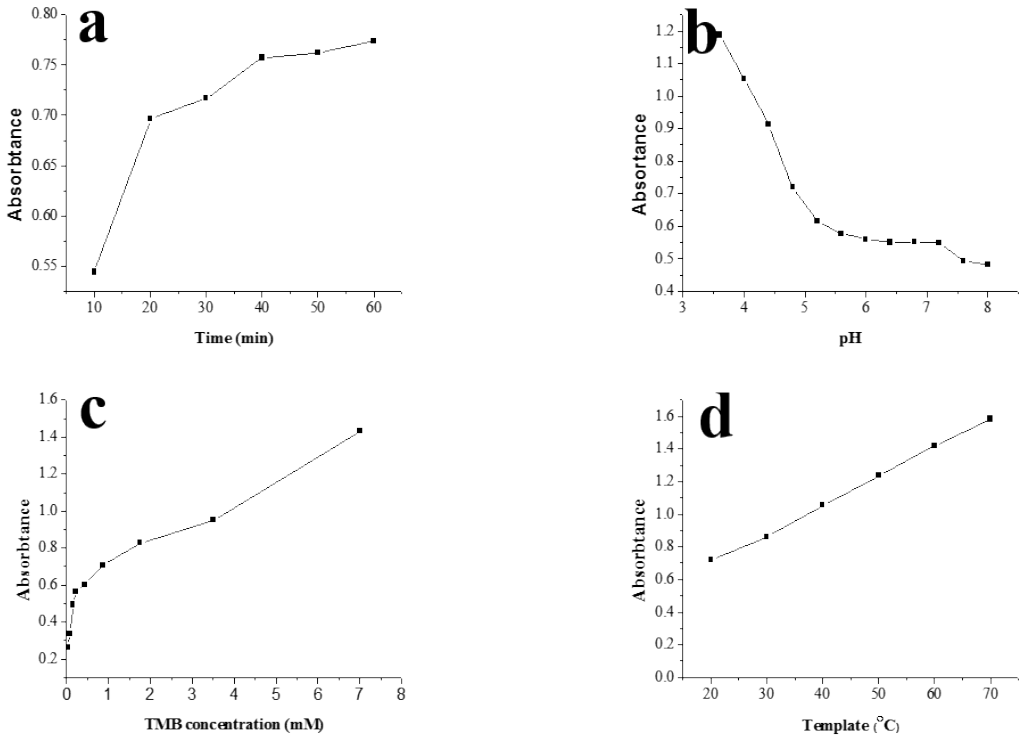

Figure S5. The effect of incubated time (a), pH (b), concentration of TMB (c) and temperature (d) on peroxidase-like of 3DBC-C_3N_4.

Table S1. Sequence of nucleic oligomers used in this paper

Nucleotide	Sequence (5'-3')
A22	AAAAAAAAAAAAAAAAAAAAAA
T22	TTTTTTTTTTTTTTTTTTTTTT
C22	CCCCCCCCCCCCCCCCCCCCCC
G22	GGGGGGGGGGGGGGGGGGGGGG
OTC aptamer	CGTACGGAATTCGCTAGCCGAGTTGAGCCGGGCGCGGTA CGGGTACTGGTATGTGTGGGGATCCGAGCTCCACGTG

Table S2. Comparison of available sensors for OTC detection

Method	Analytical method	Ranges	LODs	Reference
Light scattering agglutination assay based on aptamer-conjugated	Photon count	100-10^4 ppb	100 ppb	[1]

polystyrene latex microphores				
A microfabricated cantilever array based on aptamerself-assembled monolayer sensor	Differential deflection	1.0-100 nM	0.2 nM	[2]
Using electrochemilumiluminescence (ECL) based on (RuSiNPs)/Nafion film modified electrode	ECL intensity	0.1-100 μM L^{-1}	0.1μM L^{-1}	[3]
A colorimetric assay based on gold nanoparticles	UV-Vis absorbance	0.42-16 μg mL^{-1}	0.17μgmL- $^{-1}$	[4]
Using one-pot carbon nanoparticles sensors	Fluorescence intensity	0.06-6 μM	6.9 nM	[5]
Using a fluorescein-labeled long-chain aptamer assembled onto reduced graphene oxide	Fluorescence intensity	0.1-2 μM	10 nM	[6]
Based on 3D graphene supported bimetallic nanocomposites with aptamer	UV-Vis absorbance	0.01-0.25μM	8nM	[7]

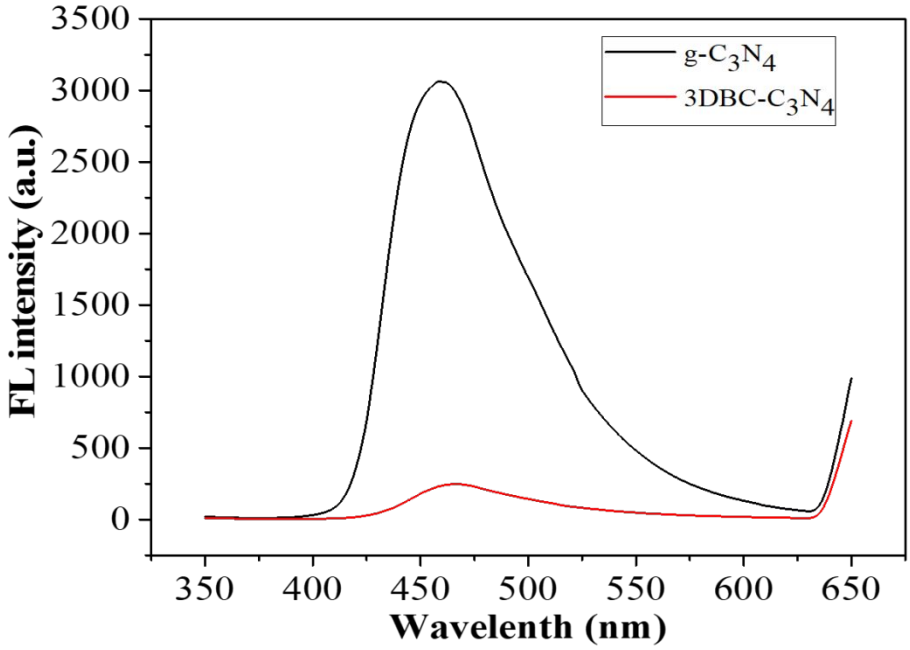

Figure S6. Fluorescence spectra of 3DBC-C_3N_4 and g-C_3N_4.

REFERENCES

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(1) Kim, K.; Gu, M. B.; Kang, D. H.; Park, J. W.; Song, I. H.; Jung, H. S.; Suh, K. Y. High-Sensitivity Detection of Oxytetracycline Using Light Scattering Agglutination Assay with Aptasensor. *Electrophoresis* **2010,** *31* (18), 3115-3120.
(2) Hou, H.; Bai, X.; Xing, C.; Gu, N.; Zhang, B.; Tang, J. Aptamer-Based Cantilever Array Sensors for Oxytetracycline Detection. *Anal. Chem.* **2013,** *85* (4), 2010-2014.
(3) Zhao, Y.; Ye, C.; Liu, W.; Chen, R.; Jiang, X. Tuning the Composition of AuPt Bimetallic Nanoparticles for Antibacterial Application. *Angew. Chem., Int. Ed.* **2014,** *53* (31), 8127-8131.
(4) Shen, L.; Chen, J.; Li, N.; He, P.; Li, Z. Rapid Colorimetric Sensing of Tetracycline Antibiotics with In Situ Growth of Gold Nanoparticles. *Anal. Chim. Acta* **2014,** *839*, 83-90.
(5) Yang, X.; Luo, Y.; Zhu, S.; Feng, Y.; Zhuo, Y.; Dou, Y. One-Pot Synthesis of High Fluorescent Carbon Nanoparticles and Their Applications as Probes for Detection of Tetracyclines. *Biosens. Bioelectron.* **2014,** *56*, 6-11.
(6) Zhao, H.; Gao, S.; Liu, M.; Chang, Y.; Fan, X.; Quan, X. Fluorescent Assay for Oxytetracycline Based on a Long-Chain Aptamer Assembled onto Reduced Graphene Oxide. *Microchim. Acta* **2013,** *180* (9-10), 829-835.
(7) Yuan, F.; Zhao, H.; Zang, H.; Ye, F.; Quan, X. Three-Dimensional Graphene Supported Bimetallic Nanocomposites with DNA Regulated-Flexibly Switchable Peroxidase-Like Activity. *ACS Appl. Mater. Interfaces* **2016,** *8* (15), 9855-9864.