Supporting Information for

DNA-Modulated Single-atom Nanozyme with Enhanced Enzyme-Like Activity for Ultrasensitive Detection of Dopamine

*Zhihan Wu,[a] Wendong Liu,[a] Haijun Lu, [a] Hongyan Zhang, [a] Zhe Hao, [a] Fanghua Zhang, [a] Ruizhong Zhang, [*a] Xiyan Li [*b] and Libing Zhang [*a]*

[a] Tianjin Key Laboratory of Molecular Optoelectronic Sciences, Department of Chemistry, Tianjin University, Tianjin 300072 P. R. China

E-mail: libing.zhang@tju.edu.cn (L.Z.); zhangrz2019@tju.edu.cn

[b] Institute of Photoelectronic Thin Film Devices and Technology, Solar Energy Conversion Center, Key Laboratory of Photoelectronic Thin Film Devices and Technology of Tianjin, Engineering Research Center of Thin Film Photoelectronic Technology of Ministry of Education, Nankai University, Tianjin 300350 P. R. China

*Corresponding author.

E-mail: xiyan.li@nankai.edu.cn

Materials and methods

Chemical and materials.

All oligonucleotide sequences were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China) and shown in Table S1. All sequences were rapidly denatured at 95°C for 5 min and then cooled to room temperature for the following studies. Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), Hemin (95%), 2-methylimidazole (2-MI, 98%), 3,3′,5,5′-tetramethylbenzidine (TMB), hydrogen peroxide (H_2O_2, 30%), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ascorbic acid, *L*-cysteine, *L*-phenylalanine were purchased from Aladdin (Shanghai, China). DA and *L*-tyrosine were purchased from Meryer (Shanghai, China). Glutathione, glucose, lactose, and uric acid were purchased from Energy Chemical (Shanghai, China). All reagents were used directly without further purification. Ultrapure water (≥18 MΩ, Millipore) was used in the whole experiment.

Apparatus and characterization.

Transmission electron microscopy (TEM) images and element mappings were gained from a JEM-2100F with an accelerating voltage of 200 kV. Scanning electron microscope (SEM) images were performed on a TESCAN MIRA LMS (Czech). X-ray photoelectron spectroscopy (XPS) measurement was performed on a Thermo Scientific K-Alpha with Al Ka X-ray radiation as the X-ray source for excitation. The crystal structure of samples was identified by X-ray diffractometer (XRD) using Cu Kα radiation (Rigaku SmartLab SE, Japan). Zeta potential of the materials was measured on a nano-particle size and Zeta potential analyzer (Zetasizer nano ZS90, Malvern Panalytical). Ultraviolet-visible (UV-vis) absorption spectra was collected on a UV-vis-NIR spectrophotometer (UV-3600 Plus, Shimadzu, Japan). Fluorescence analysis was carried out on a FS05 steady-state transient fluorescence spectrometer (Thermo Field, USA).

Synthesis of N-C

The synthetic route of N-C is similar to that of Fe-N-C, except that hemin is not added in the first step to obtain ZIF-8.

Kinetic measurements.

The steady-state kinetic analysis was carried out using 0.5 μg mL-1 Fe-N-C or DNA/Fe-N-C in reaction volume of acetate buffer (20 mM, pH 4.0) at room temperature with diverse concentrations of H_2O_2 (TMB) and 0.5 mM TMB (5 mM H_2O_2). According to the typical Michaelis-Menten equation: $v = V_{max}$ [C] / (K_M + [C]), where ν is the initial velocity, V_{max} is the maximal reaction velocity, [C] is the concentration of substrate (TMB or H_2O_2), and K_M is Michaelis constant.

Selectivity analyses of DNA/Fe-N-C for dopamine (DA).

To verify the selectivity of the DNA/Fe-N-C-based sensing platform for DA, various amino acids and small molecules such as cysteine, phenylalanine, tyrosine, uric acid, lactose, glucose, glutathione, ascorbic acid were similarly tested replaced with 40 μM of DA.

Detection of ·OH

Typically, Fe-N-C or DNA/Fe-N-C solution (10 μg mL^{-1}), terephthalic acid (TA) solution (0.5 mM) and H_2O_2 solution (10 mM) were mixed in acetate buffer (pH 4.0, 20 mM) until the final volume reached 1 mL. After incubating for 3 h, the solutions were used for the detection of ·OH.

Supporting tables and figures

Table S1 Sequences of synthesized DNA

Name	Sequences (5′-3′)
G_{14}	GGGGGGGGGGGGG
T_{14}	TTTTTTTTTTTTT
C_{14}	CCCCCCCCCCCCC
A_{14}	AAAAAAAAAAAAA
A_{20}	AAAAAAAAAAAAAAAAAAAA
A_{30}	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
A_{40}	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Random DNA	TACCCTGTAGAACCGAATTTGTG
Random FAM-DNA	TACCCTGTAGAACCGAATTTGTG, FAM at the 5′ terminus

Table S2 Comparison of the apparent Michaelis-Menten constant (K_M) and maximum reaction rate (V_{max}) of the catalytic reactions.

Catalyst	Substrate	K_M (mM)	V_{max} (10^{-8} M s^{-1})	References
Fe-N-C	TMB	0.15	8.20	This work
	H_2O_2	1.60	7.61	
DNA/Fe-N-C	TMB	0.08	7.75	This work
	H_2O_2	3.03	11.69	
HRP	TMB	0.43	10.00	[1]
	H_2O_2	3.70	8.70	

Table S3 Comparison of different methods for DA detection.

Methods	Materials	Linear range (μM)	LOD (nM)	References
Fluorescence	GNC-Ce^{3+}	0.1-1000	10.85	[2]
Fluorescence	BSA-CuNCs	0.50-50	280	[3]
ECL	CdSeTe/ZnS QDs	3.75-450	100	[4]
Electrochemistry	CAuNE	1-100	5830	[5]
Colorimetric	$CuFe_2O_4$/Cu_9S_8/ PPy h-CuS	2-20	1000	[6]
Colorimetric	$Bi_2Fe_4O_9$	0.15-50	50	[7]
Colorimetric	Pt/hBNNSs-5	2-55	760	[8]
Colorimetric	h-CuS NCs	2-150	1670	[9]
Colorimetric	CGNPs	1-20	1150	[10]
Colorimetric	FeN_3-PtN_4-SAzyme	1-10	109	[11]
Colorimetric	Fe-N-C	5-100	4170	This work
Colorimetric	DNA/Fe-N-C	0.01-4 5-100	9.56	This work

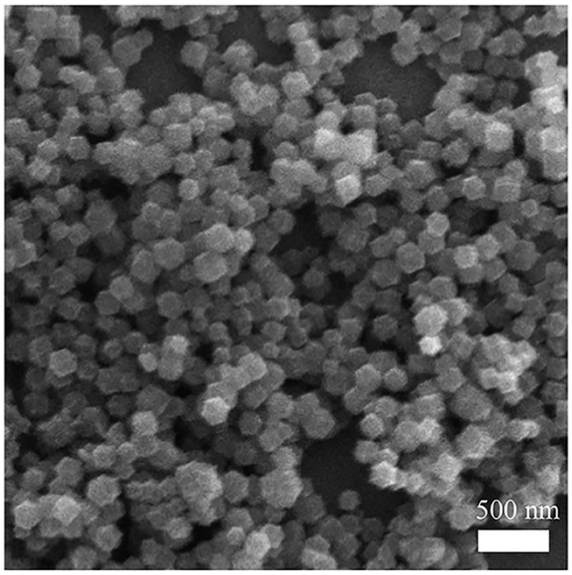

Fig. S1 SEM image of Fe-N-C.

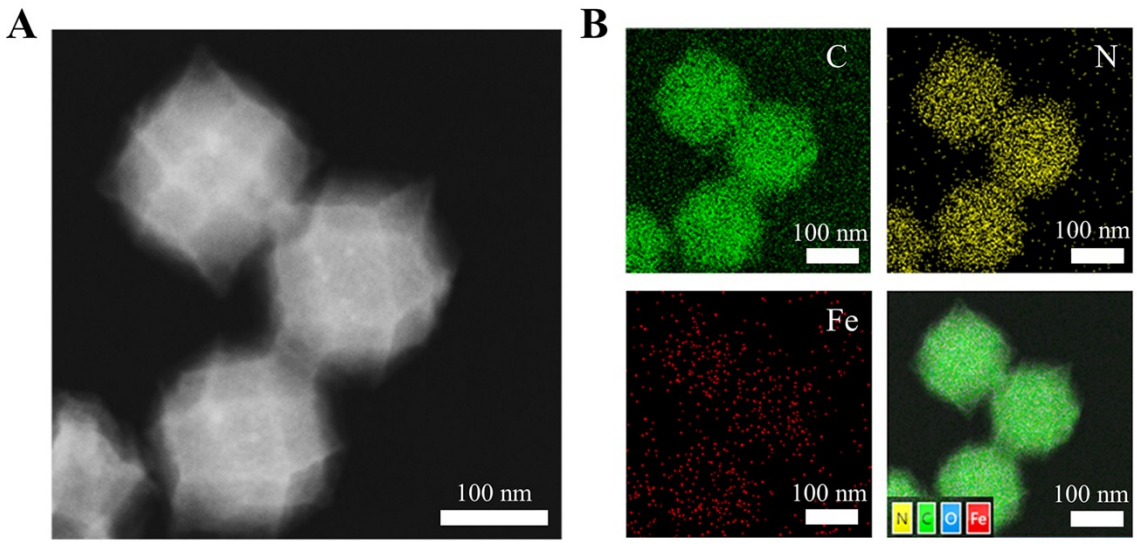

Fig. S2 (A) HAADF-STEM image of Fe-N-C. (B) the elemental mapping images of C (green), N (yellow), Fe (red) and their overlapping image.

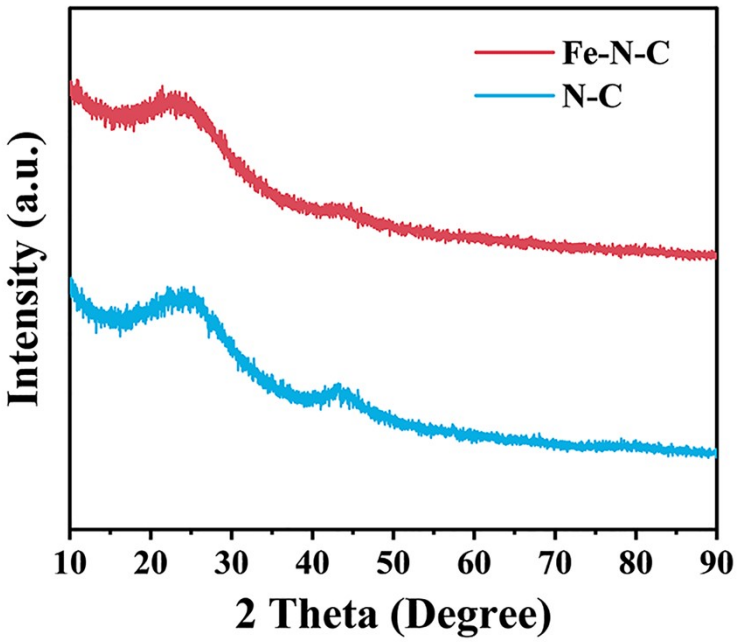

Fig. S3 XRD pattern of N-C and Fe-N-C.

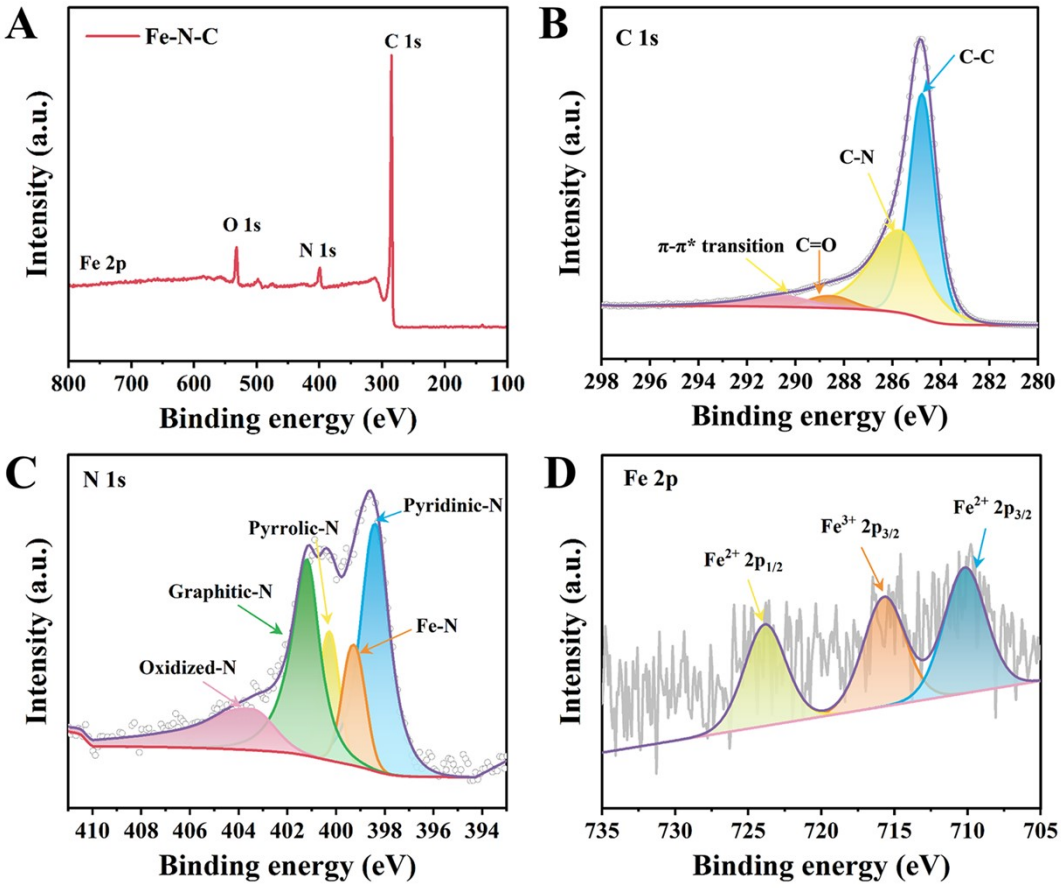

Fig. S4 (A) XPS survey spectrum of Fe-N-C. (B) High-resolution XPS spectra of C 1s, (C) N 1s, and (D) Fe 2p.

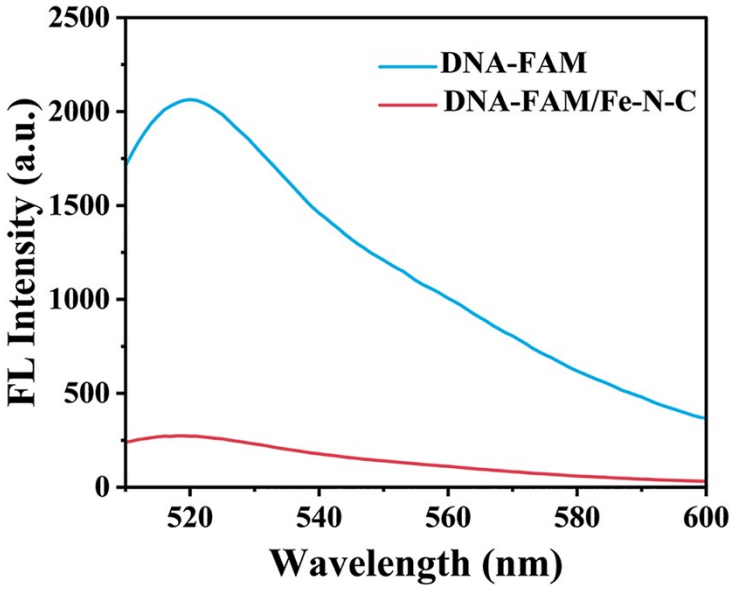

Fig. S5 Fluorescence spectra of FAM-DNA responding to 5 μg mL^{-1} Fe-N-C.

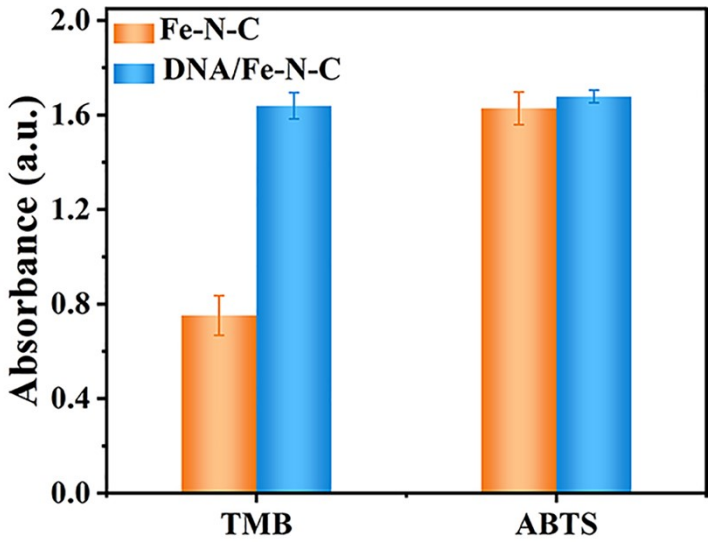

Fig. S6 POD-like activity of Fe-N-C using ABTS, and TMB as peroxidase substrates before and after adding DNA. Absorbance was determined at 652 nm. The error bars are standard deviations obtained from three independent measurements.

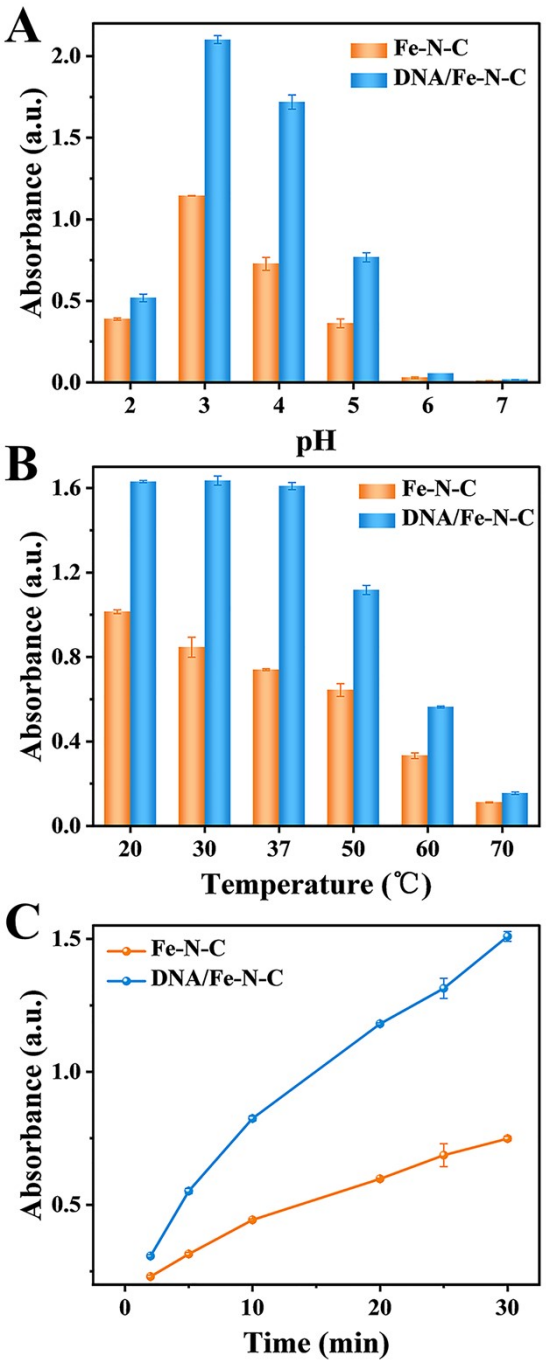

Fig. S7 The absorbance variation of Fe-N-C and DNA/Fe-N-C depending on pH (A) and temperature (B) and time (C). Absorbance was determined at 652 nm. The error bars are standard deviations obtained from three independent measurements.

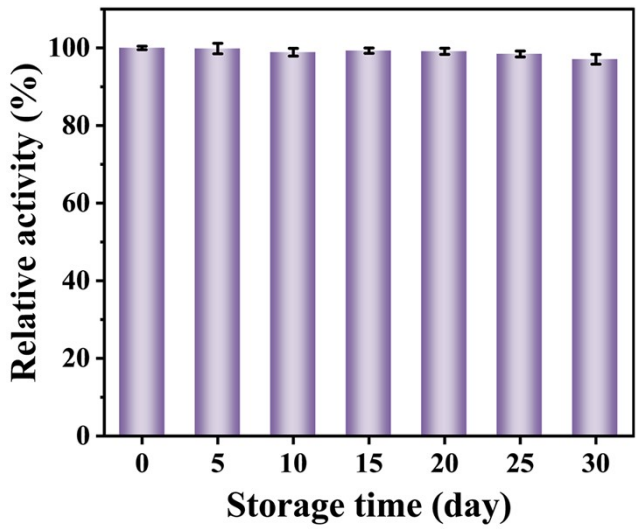

Fig. S8 Stability tests of DNA/Fe-N-C for 30 days. Condition: 0.5 μg mL^{-1} Fe-N-C, 10 nM DNA, 0.4 mM TMB, 4 mM H_2O_2, HAc-NaAc buffer (20 mM, pH 4.0), a reaction time of 20 minutes and a reaction temperature of 37°C. The error bars are standard deviations obtained from three independent measurements.

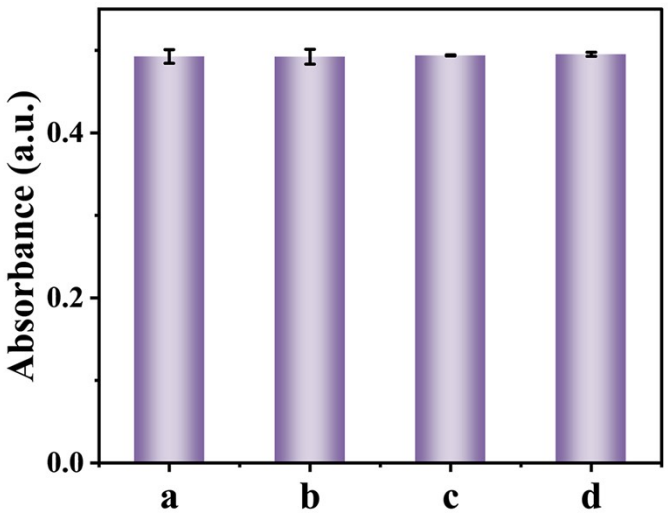

Fig. S9 The absorbance of DNA/Fe-N-C in different conditions of (a) pH=7; (b) pH=4; (c) pH=7, H_2O_2; (d) pH=4, H_2O_2. Absorbance was determined at 260 nm. The error bars are standard deviations obtained from three independent measurements.

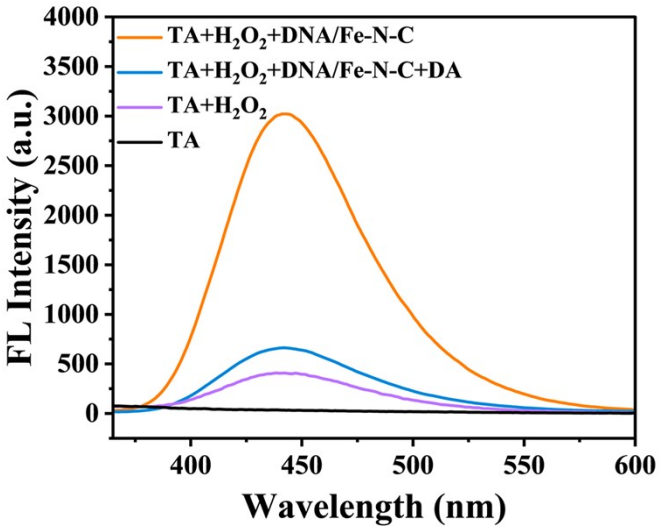

Fig. S10 Fluorescence spectra were measured under excitation at 315 nm for the interaction of TA with DNA/Fe-N-C, DNA/Fe-N-C+DA, and H_2O_2, respectively.

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