Supporting Information

Photoenhanced intrinsic peroxidase-like activity of a metal-free biocompatible borophene photonanozyme for colorimetric sensor assay of dopamine biomolecule

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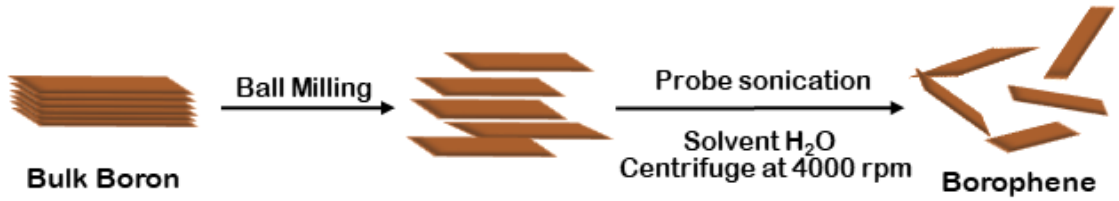
1. EXPERIMENTAL SECTION

Reagents and chemicals

3,3′,5,5′-Tetramethylbenzidine (≥ 99%), dopamine hydrochloride, uric acid (≥ 99%), D-(+) glucose (≥ 99.50%), glutathione (GSH), p-benzoquinone (p-BQ) (≥ 98%), thiourea (TH) (≥ 99%) were purchased from Sigma-Aldrich Chemical Co. Hydrogen peroxide 30% AR and L-ascorbic acid (≥ 99%) were obtained from GLR Innovations Limited, India. Sodium acetate anhydrous (≥ 99%) was obtained from HiMedia Laboratories Pvt. Ltd. whereas acetic acid glacial (≥ 99.50%) was purchased from Finar Chemicals. Human embryonic kidney (HEK293) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Methyl thiazolyl tetrazolium (MTT), dimethyl sulfoxide (DMSO), Dulbecco's Modified Eagle's medium (DMEM), phosphate buffer saline (PBS), and streptomycin were purchased from Sigma Aldrich. Fetal bovine serum (FBS) was purchased from Himedia. All the experiments were done using deionized water obtained from a Milli-Q system (Milli Pak 0.22 μm). The above-mentioned chemicals and reagents are of analytical grade and were used without further purification.

Synthesis process of borophene nanosheets

For the preparation of BNSs, we used ball mill assisted ultrasonication technique as shown in scheme S1. The bulk boron powder was put into a stainless-steel container (50 mL) along with stainless steel balls. The boron powder was ball milled for 1 h. To 100 mg of the resulting ball milled powder, 100 mL of Milli-Q water was added and the resulting mixture was subjected to probe sonication (20 KHz) for 12 h. The mixture was then centrifuged and the supernatant (BNSs) was collected for further investigations.



Scheme S1: Synthesis of BNSs nanozyme

Characterization Techniques

For the evaluation of peroxidase-like activity of borophene nanosheets (BNSs) and detection of dopamine, UV-Visible spectrophotometer (SPECORD 250 PLUS, Analytik Jena,

Germany) was used to record the absorption spectra. The synthesized BNSs was studied by several characterization techniques. For the crystalline structures, powder X-ray diffraction (PXRD) technique was adopted using a Rigaku X-ray diffractometer (ULTIMA IV, Rigaku, Japan) operating at a generator voltage of 40 kV and current 40 mA. The PXRD patterns were recorded by using CuKα (λ=1.54056 Å) as the X-ray source at a scanning rate of 3° min^{-1} in the 2θ range from 10 to 80. A field-emission scanning electron microscope (FE-SEM, Carl Zeiss: IGMA, UK), operating at an accelerated voltage of 5 kV and equipped with an energy-dispersive X-ray spectrometer (EDS) was used to examine the morphological and textural characteristics. Prior to SEM observations, powder samples were attached to an adhesive carbon tape and subjected to Au sputtering. High-resolution transmission electron microscopy (HR-TEM) was used for the microstructural study of the synthesized BNSs using a JEM-F200 (URP) equipment (JEOL Co., Ltd., Japan) run at an accelerated voltage of 200 kV. The X-ray photoelectron spectroscopy (XPS) was used to evaluate the chemical composition of the surface. Using a monochromatic Al-Kα X-ray source (1486.6 eV), XPS spectra were captured using a Thermo-Scientific ESCALAB Xi+ spectrometer (UK). With a Flurolog-3 spectrofluorometer (Horiba, Japan), fluorescence spectroscopy was used to study the production of reactive oxygen species (ROS) by BNSs.

Cytotoxicity study

Cell lines and maintenance

Human embryonic kidney (HEK293) cells were obtained from National Centre for Cell Sciences (NCCS), Pune, India. Cells were then cultured in sterilized Dulbecco's Modified Eagle's medium (DMEM) that contain 10% (v/v) Fetal bovine serum (FBS), 1% L-glutamine, and 1% streptomycin. Standard culture conditions (37 °C temperature, 5% CO_2) were maintained throughout the cell culture process.

MTT Assay (Quantitative Analysis)

Methyl thiazolyl tetrazolium (MTT) assay was performed to determine the cytotoxicity of various concentrations of BNSs on the HEK293 cells. The HEK293 cells were cultured in T25 flasks which contained DMEM medium and 10% FBS, (culture condition: 37 °C temperature, 5% CO_2 and 95% humidity). After obtaining 80% to 85% confluency the cells were trypsinized and shifted in a sterilized 96-well cell culture plate at a density of 5×10^3 cells/well. The cells were treated with different concentrations of BNSs (6.25 µg/mL –100 µg/mL) and incubated for 24 h. Then media were discarded and cleaned properly with fresh phosphate buffer saline

(PBS) solution. After that 100 µL of fresh DMEM media and 20 µL of MTT solution (5 mg/mL) were added. Again, the cells were incubated for 3 h at 37 °C. In this process formazan crystals were formed. Finally, the media was discarded, and formazan crystals were dissolved with 100 µL of dimethyl sulfoxide (DMSO) per well. In final step the absorbance values were taken at 570 nm using a micro plate reader and percentage of cell viability was calculated. The cells treated without BNSs were used as a control.

The peroxidase mimetic activity study of borophene nanozyme

To a 2.8 mL solution of sodium acetate buffer (HAc-NaAc, pH 3), TMB (30 µL, 0.5 mM) and H_2O_2 (30 µL, 3 mM) were added. To the mixture, an aqueous dispersion of BNSs (110 µL, 15 mgL^{-1}) was added and the solution was irradiated with light (440 nm, 19V, 40W max, Made in Taiwan) for 10 minutes. Then the mixture was scanned with UV-Visible spectrophotometer to monitor the formation of oxidized TMB product with a characteristic absorption peak at 652 nm.

The effect of light on the catalytic reaction was observed by varying the wavelength of light source during the course of reaction. For the study of effect of pH, the reaction was carried out by varying pH of the buffer from 2 to 8. The effect of reaction time and catalyst loading was studied by varying reaction time (from 5 to 25 min) and catalyst concentration (from 5 to 25 mgL^{-1}), respectively.

The enzyme kinetics of BNSs was studied using steady-state kinetics assay, where BNSs catalyzed TMB oxidation reactions were conducted keeping the concentration of one substrate (either H_2O_2 or TMB) constant while varying the concentration of the other. The obtained absorbance values at 652 nm ($A_{652\ nm}$), were converted to product concentrations using Beer-lambert's law and then to reaction velocities. Typical Michaelis-Menten curves were obtained by plotting these reaction velocities against corresponding substrate concentrations using formula (1). Then, corresponding Lineweaver-Burk double reciprocal plots were derived using formula (1), which provide kinetic parameters K_m and V_{max} representing the affinity of the nanozyme towards the substrate and the maximum speed of the reaction at a saturated substrate concentration, respectively.

$$v = \left(\frac{A}{t}\right) \times \varepsilon \times l \tag{1}$$

$$\frac{1}{v} = \left(\frac{K_m}{V_m}\right)\left(\frac{1}{[S]}\right) + \left(\frac{1}{V_m}\right) \tag{2}$$

Here [S] is the substrate concentration, v is the initial velocity, A is the absorbance value, t is the reaction time, l is the length of the path through which light travels in cm and ε is the molar extinction coefficient of oxidized TMB (39000 $M^{-1}cm^{-1}$).

Detection of hydroxyl radical by Fluorescence technique

For the detection of ˙OH radical, terephthalic acid (TA) was used as a fluorescent probe as it is highly selective and with ˙OH radical it results in 2-hydroxy terephthalic acid which has unique fluorescence peak around 450 nm. For the test, to a 2.8 mL solution of sodium acetate buffer (HAc-NaAc, pH 3) solution, TMB (30 µL, 0.5 mM), H_2O_2 (30 µL, 3 mM), TA solution (30 µL, 0.5 mM) and BNSs dispersion (110 µL, 15 mgL^{-1}) were added. The mixture was then irradiated with 440 nm visible light for 10 minutes. Finally, the fluorescence spectra of the samples were acquired using fluorescence spectroscopy.

Detection of ROS using scavengers

For the detection of ˙OH, $O_2^{\cdot -}$ and h^+ (photogenerated hole) in the reaction system; thiourea (TH), p-benzoquinone (p-BQ) and ethylene diamine tetraacetate (EDTA) were used, respectively. To a 2.8 mL solution of sodium acetate buffer (HAc-NaAc, pH 3) solution, TMB (30 µL, 0.5 mM), scavenging reagent (30 µL, 0.5 mM) and H_2O_2 (30 µL, 3 mM) were added followed by the addition of BNSs (110 µL, 15 mgL^{-1}). After 10 minutes of reaction, the absorbance of the mixtures were taken. For comparison, the experiment was carried out in presence and absence of the specific light source.

Detection of Dopamine

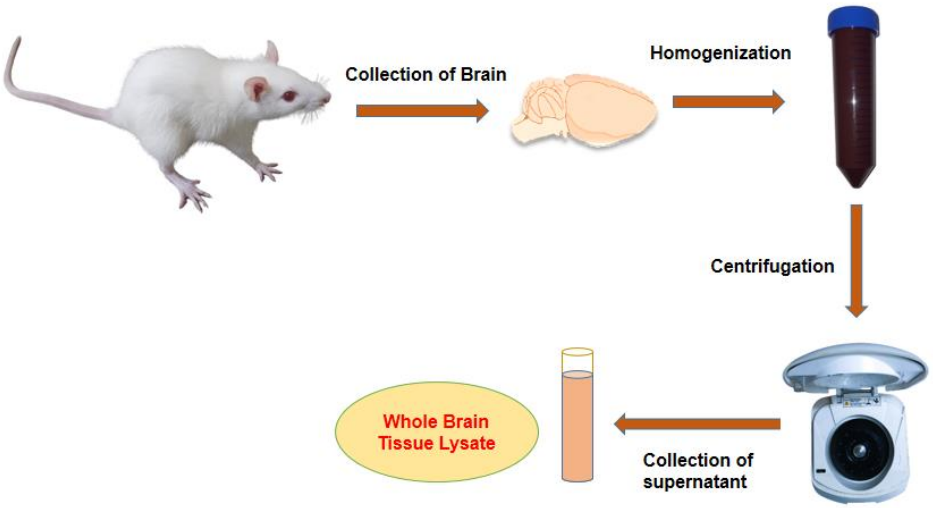
To a 2.8 mL solution of sodium acetate buffer (HAc-NaAc, pH 3) solution, TMB (30 µL, 0.5 mM), dopamine hydrochloride solution (50 µL, 50 µM), H_2O_2 (30 µL, 3 mM) and BNSs dispersion (110 µL, 15 mgL^{-1}) were added. After 10 min of irradiation with 440 nm visible light, the UV-Visible spectra of the mixture were recorded. For the standard calibration curve, the UV-Visible spectra of the mixtures with different concentrations of dopamine hydrochloride were recorded keeping the final concentrations of TMB (0.5 mM), H_2O_2 (3 mM) and BNSs (15 mgL^{-1}) in all the mixtures same. From the obtained curve, LoD (limit of detection) for dopamine was calculated.

Selectivity experiment

The selectivity of the BNSs photonanozyme towards the colorimetric detection of DA was investigated using biomolecules including glutathione, cysteine, ascorbic acid, uric acid and glucose as potential interfering substances. The experiment was performed by adding TMB (30 µL, 0.5 mM), interfering substances (50 µM), H_2O_2 (30 µL, 3 mM) and BNSs dispersion (110 µL, 15 mgL^{-1}) to 2.8 mL of sodium acetate buffer (HAc-NaAc, pH 3) solution. After irradiation with 440 nm visible light for 10 min, the UV-Visible spectra of each of the mixture were recorded.

Method for preparation of whole brain tissue lysate

The rat whole brain tissue lysate was prepared following the method described by Baruah, et al. An adult Sprague Dawley (SD) rat of 260 g body weight (BW) was euthanized using interperitoneal overdose administration of sodium pentobarbital (thiosol TM) at the dose of 150 mg/Kg BW. Once death of the animal was confirmed, necropsy was performed immediately and the whole brain was excised off carefully without losing any part. The collected brain was immediately place in cold phosphate-buffered saline (PBS, pH-7.4 solution) for proper cleaning and to prevent any tissue damage. The whole brain was weighed and cut into small pieces of about 1 mm thickness keeping in chilled PBS solution. RIPA buffer (50 µL of for each mg of tissue) was added to the lysed tissue sample to rupture the cell membrane. Homogenization of the brain tissue sample was performed at 40 Hz and kept for 1 hour in ice-cold conditions. Sample was then centrifuged at 16000 rpm for 15 min at 4 °C. Supernatant was collected and stored at -20 °C for further analysis. During this entire process, the temperature was constantly maintained at 0-4 °C. The method of brain lysate preparation is depicted in Scheme S2. The detection of dopamine in real sample studies involving rat brain tissue samples were performed using leftover tissue parts of the control group of another study approved by the Institutional Animal Ethics Committee (IAEC), CSIR-NEIST, Jorhat (IAEC Approval Reference Number: CSIR/NEISST/IAEC/01/23/007 dated 02-05-2023).



Scheme S2: Schematic representation of preparation of whole brain tissue lysate

Detection of dopamine in real samples

Two real samples- FBS and whole brain tissue lysate, were selected for DA detection using as-developed BNSs-based colorimetric assay. FBS and whole brain tissue lysate were first diluted 100 and 50 times, respectively. Then, different concentrations of standard DA solution were spiked into the diluted samples such that the final concentration of DA in the solution became 7, 10, 15, 20, 25, and 30 µM. In a typical procedure, to a solution of 2.8 mL sodium acetate buffer (pH 3), TMB (0.5 mM), H_2O_2 (3 mM), and DA spiked sample (variable concentrations) were added and irradiated with 440 nm light. After 10 minutes, UV-Vis spectra of the resulting solution was recorded and based on their $A_{652\ nm}$ value, concentration of DA present in the spiked samples were calulated. From the obtained data, recovery percentage as well as RSD for DA detection by BNSs photonanozyme were calculated and tabulated in Table S4.

2. ADDITIONAL FIGURES AND TABLES

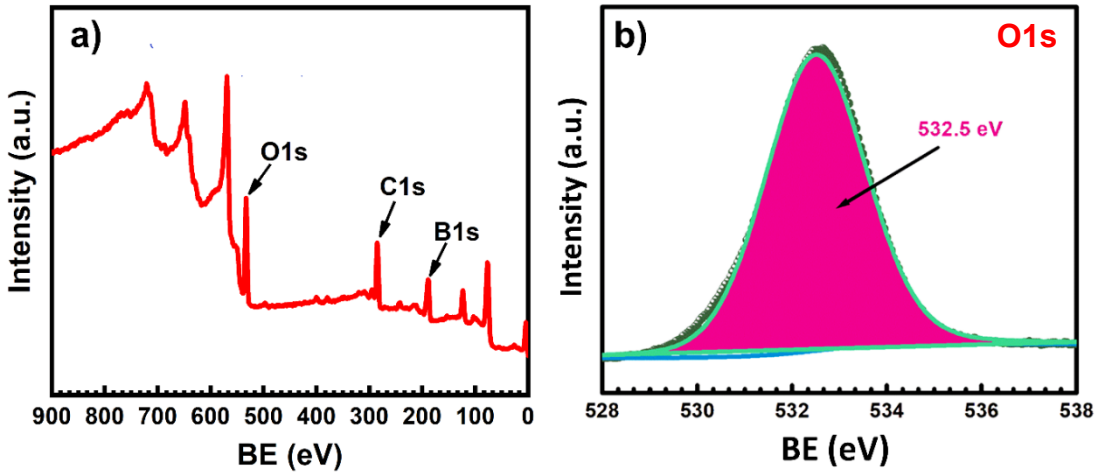


Fig. S1. a) Full scan XPS spectra of BNSs, b) High resolution deconvulated XPS spectra of O1s.

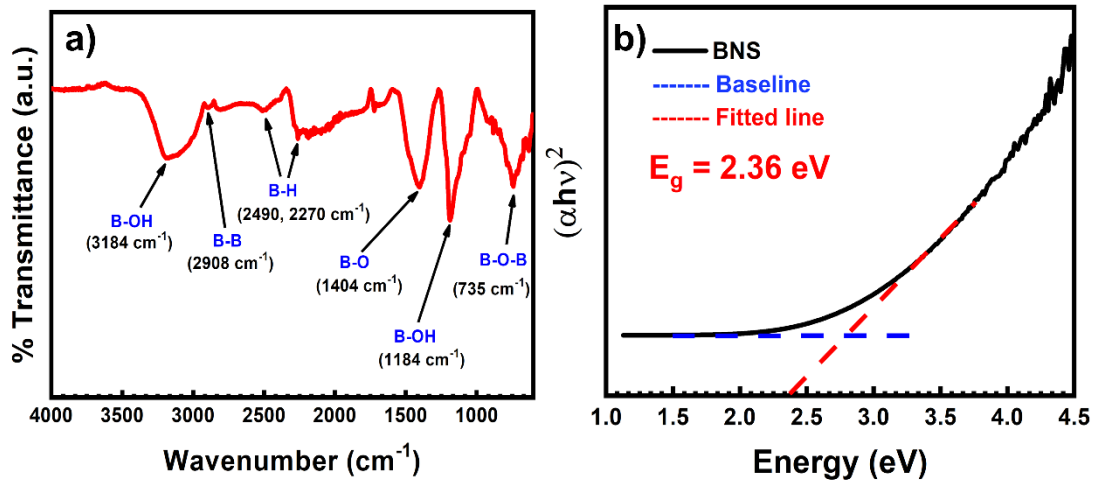


Fig. S2. a) FTIR spectra of BNSs, b) Tauc plot for BNSs.

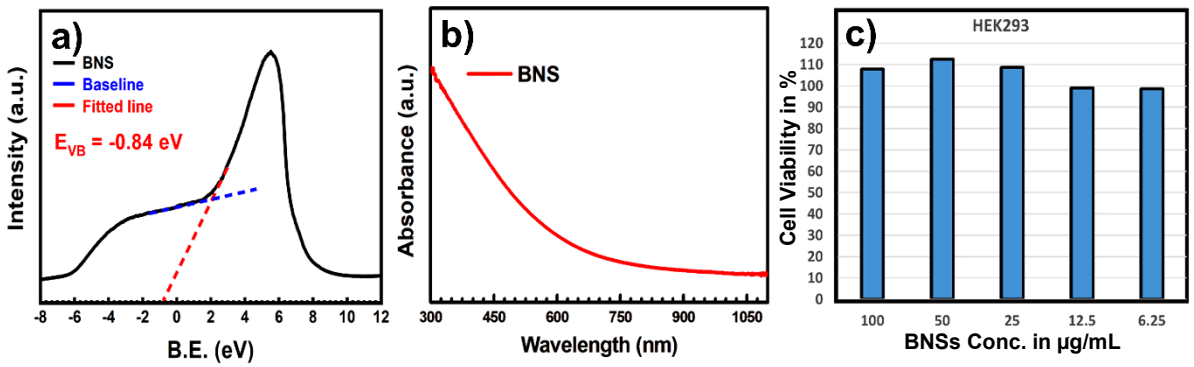


Fig. S3. a) UPS spectra and its corresponding valence band of the BNSs, b) UV-Visible spectra of BNSs and c) cytotoxicity study of BNSs.

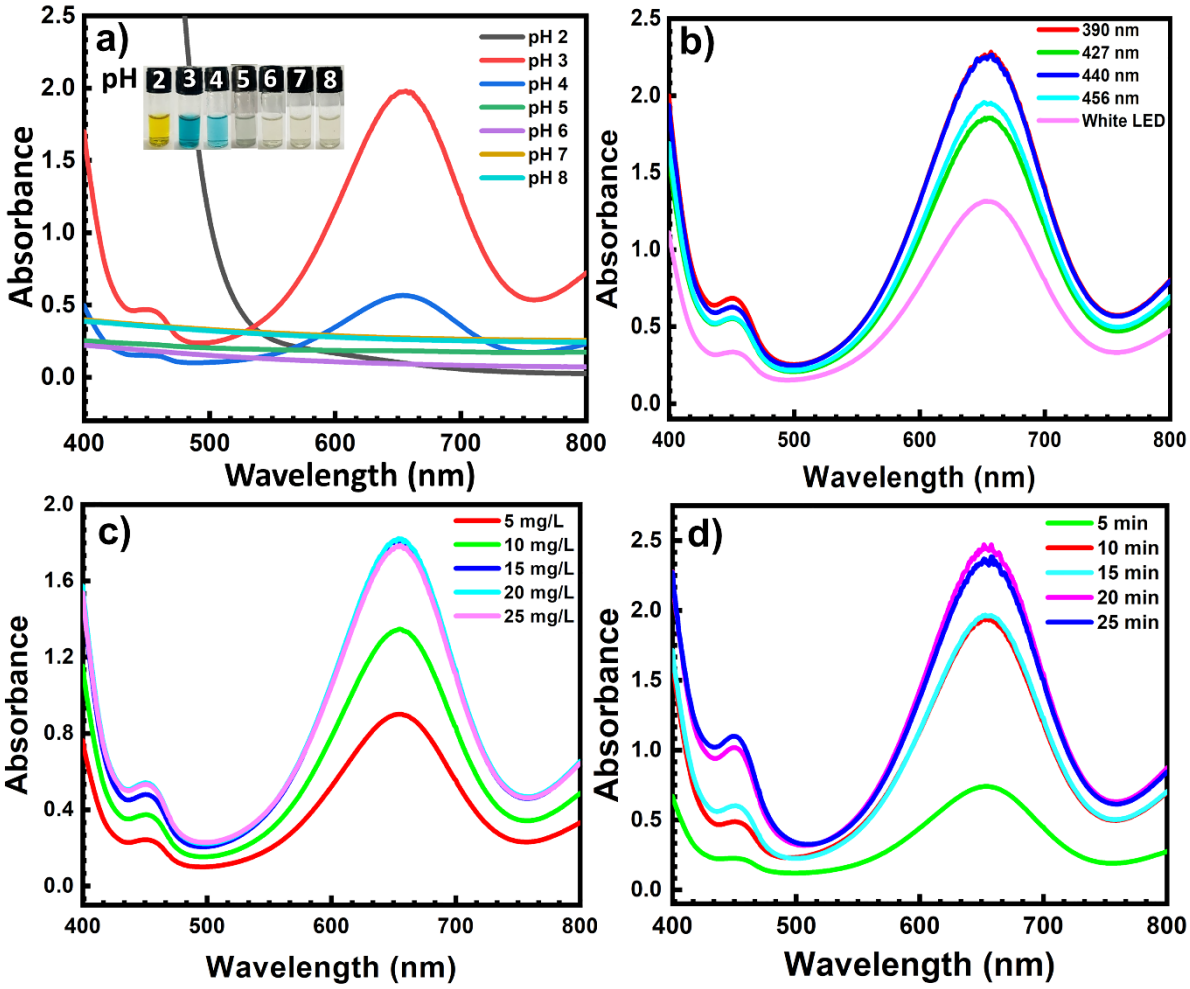


Fig. S4. UV-Visible spectra of the oxidation of the TMB at different experimental conditions (a) variation of pH in the reaction mixture from pH 2 to 8 (photographs show the oxidized TMB product from left (pH 2) to right (pH 8), (b) variable wavelength of light source, (c) variation of BNSs nanozyme catalyst concentration (5 mg/mL to 25 mg/mL), and (d) catalytic activity of BNSs towards the oxidation of the TMB at different time interval.

The peroxidase-like activity of a nanozyme depends on various factors like pH, wavelength of the light source, catalyst loading and time of the catalytic reaction**.** As shown in Fig. S3a, the catalytic activity reaches maximum at pH 3 and then decreases with increase in pH. Therefore, HAc-NaAc buffer with pH 3 was chosen. This decrease in activity is due to the fact that the solubility of TMB, which is a diamine, decreases with increase in pH value; indicating pH dependency of catalytic activity was largely correlated with the substrate rather than the nanozyme. From the study of variation of wavelength of the light source (Fig. S2b), it was found that for the 390 nm and 440 nm light source the activity comes out to be maximum. But, 390 nm falls under near UV

range, so we performed the experiments using the 440 nm light source. The experimental result of catalyst loading variation showed that with catalyst concentration of 15 mgL^{-1}, the saturation is attained (Fig. S2c). Studying the effect of time variation, we found that after 10 minutes the activity increases slowly (Fig. S2d). So, to minimize the time of catalytic reaction, we chose 10 minutes as the effective duration for the reaction. Thus, the optimal catalytic activity after 10 min of irradiation was obtained with the following experimental conditions: pH 3, 440 nm light source and 15 mgL^{-1}.

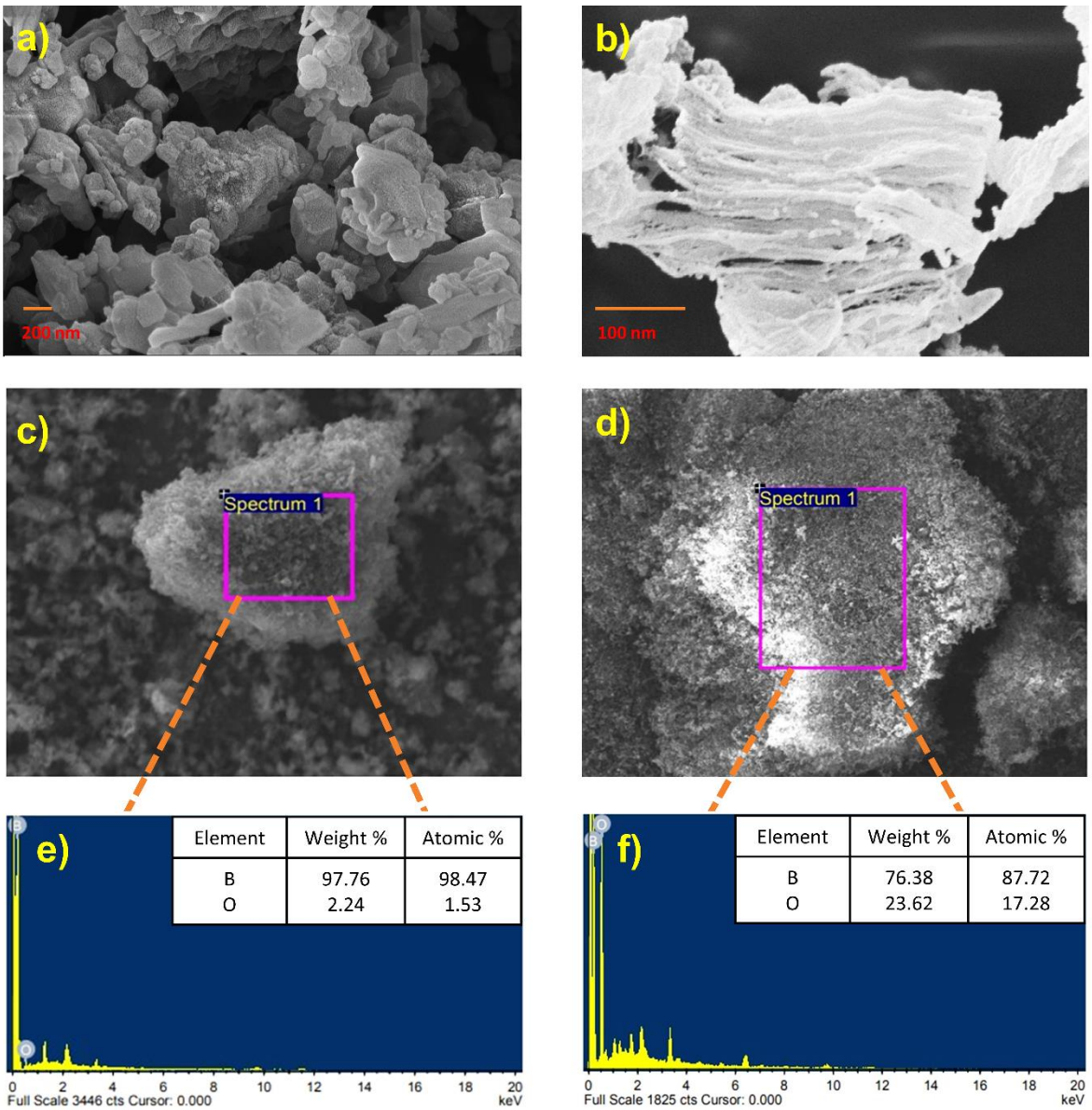


Fig. S5. a) and c) SEM images of bulk boron, b) and d) are SEM images of BNSs, e) and f) are the corresponding EDS spectrum of bulk boron and BNSs, respectively. The inset table shows the atomic % and weight % obtained from the EDS spectrum.

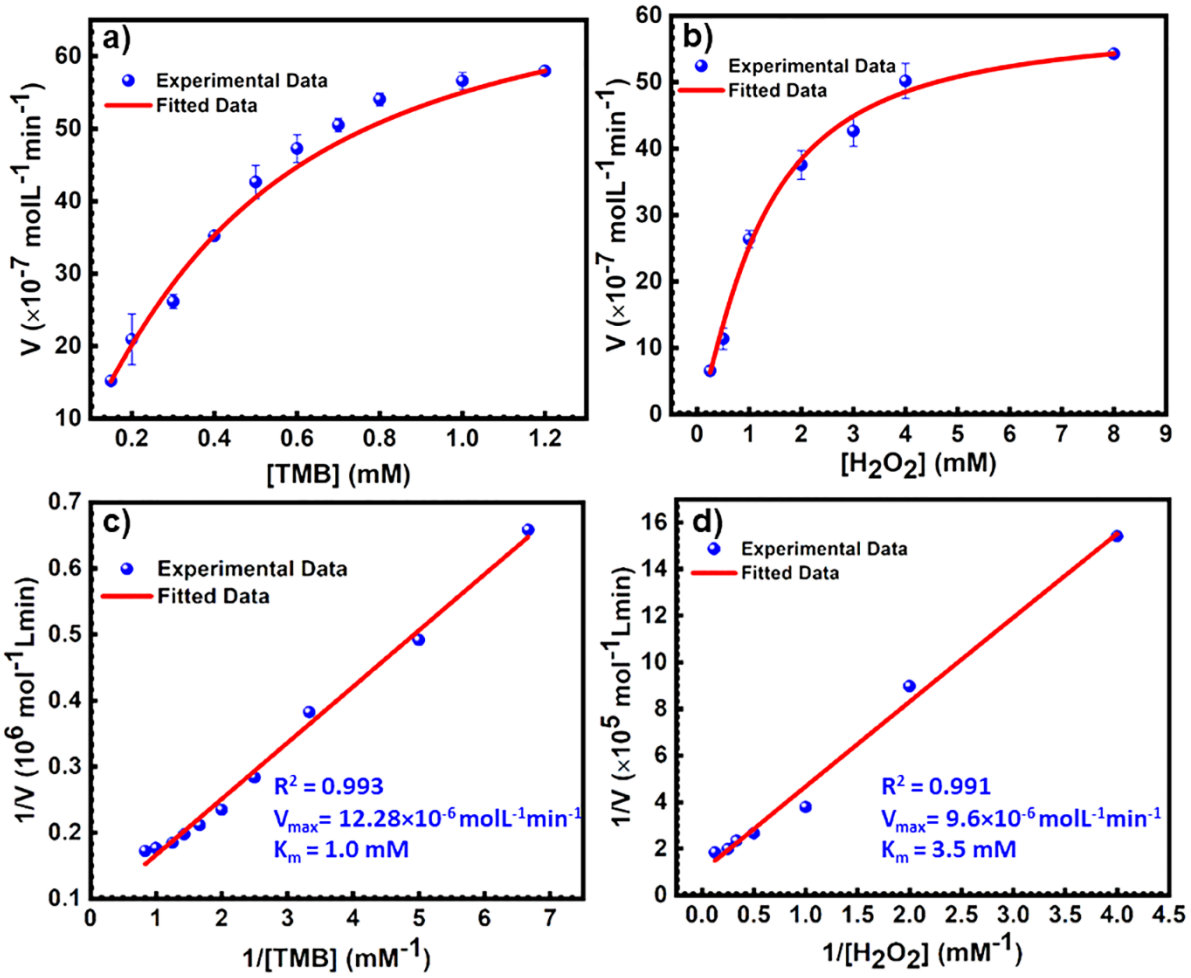


Fig. S6. Steady-state kinetic assay for BNSs photonanozyme a) variation of TMB concentration from 0.15 to 1.2 mM at a fixed H_2O_2 concentration (3 mM), b) variation of H_2O_2 concentration from 0.25 to 8.0 mM at a fixed TMB concentration (0.5 mM), c) and d) are the corresponding Lineweaver-Burk double reciprocal plot (the error bars indicating the standard deviation values for three independent analysis) (for BNSs)

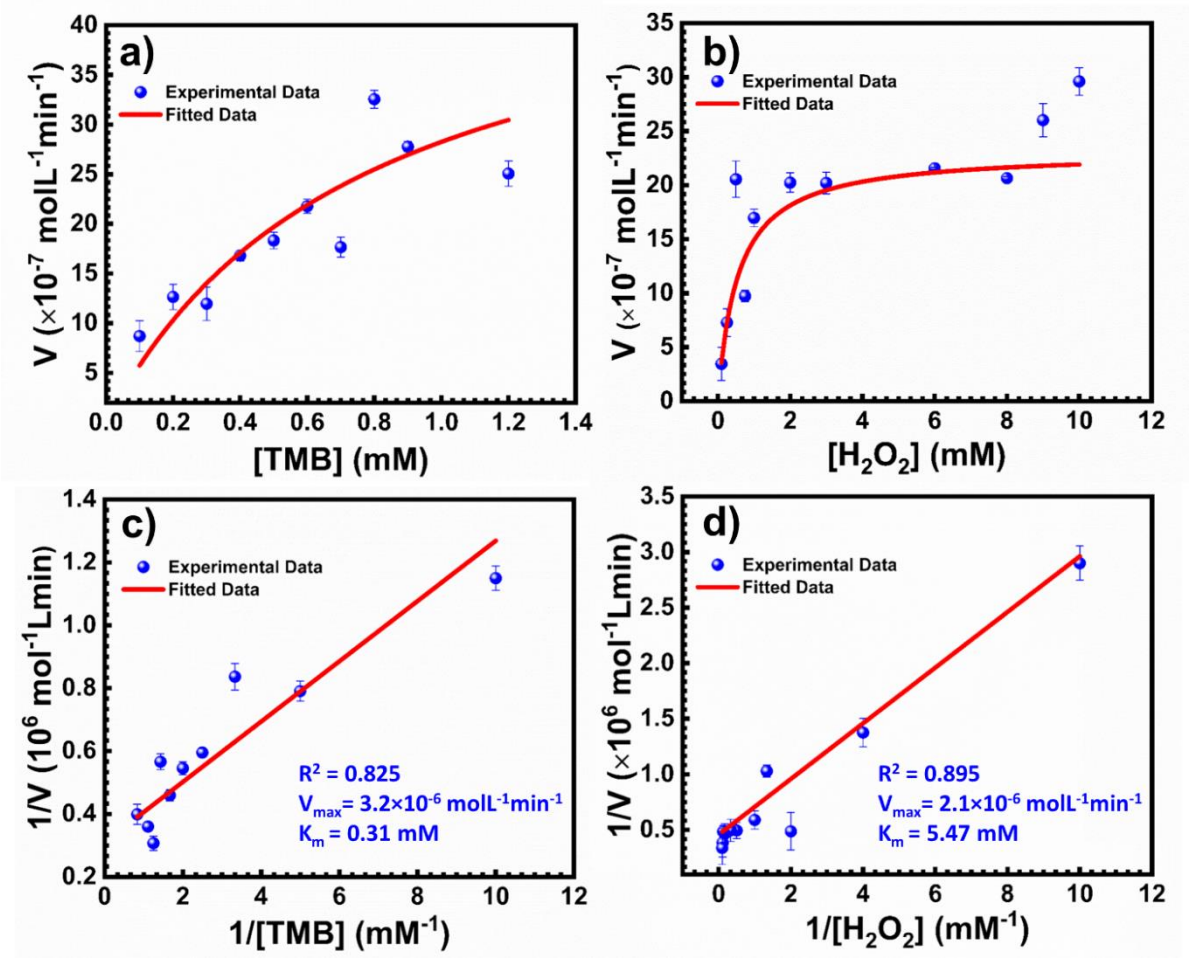


Fig. S7. Steady-state kinetic assay for bulk born a) variation of TMB concentration from 0.10 to 1.2 mM at a fixed H_2O_2 concentration (3 mM), b) variation of H_2O_2 concentration from 0.05 to 10.0 mM at a fixed TMB concentration (0.5 mM), c) and d) are the corresponding Lineweaver-Burk double reciprocal plot (the error bars indicating the standard deviation values for three independent analysis) (for bulk boron)

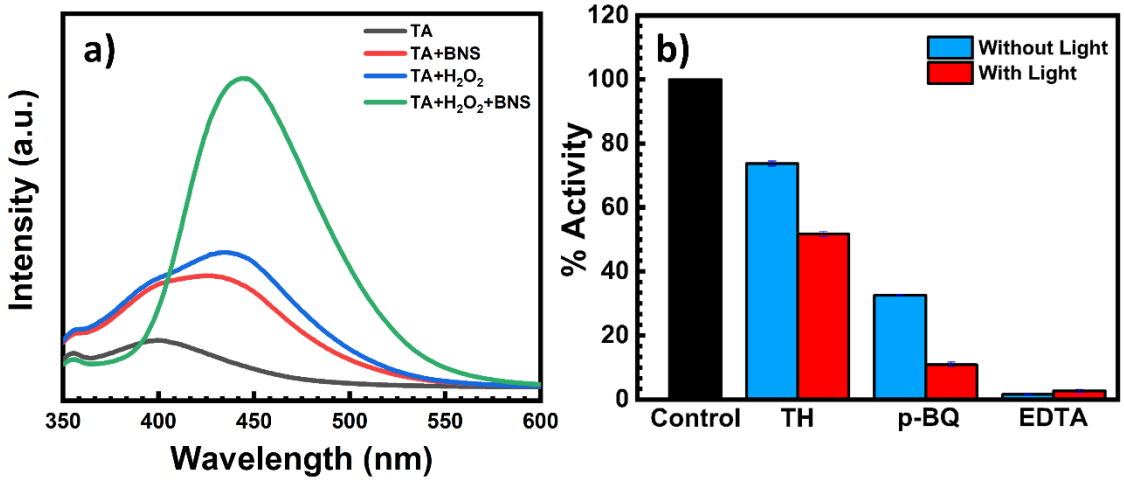


Fig. S8. a) Fluorescent spectra of BNSs using TA as a fluorescent probe, b) a comparative study of the scavenging experiments done in absence and presence of light.

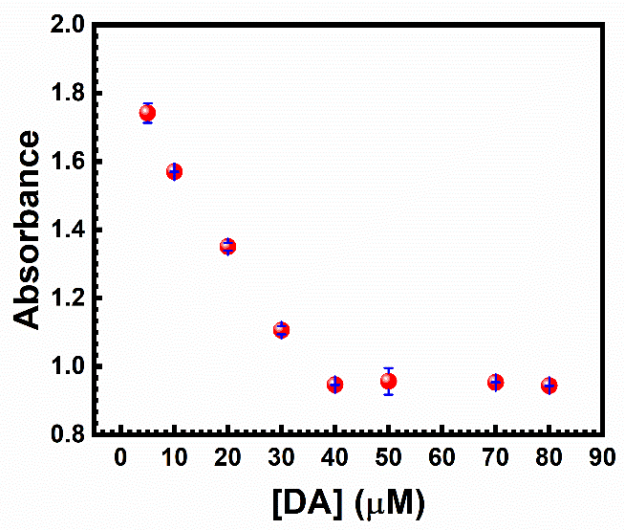
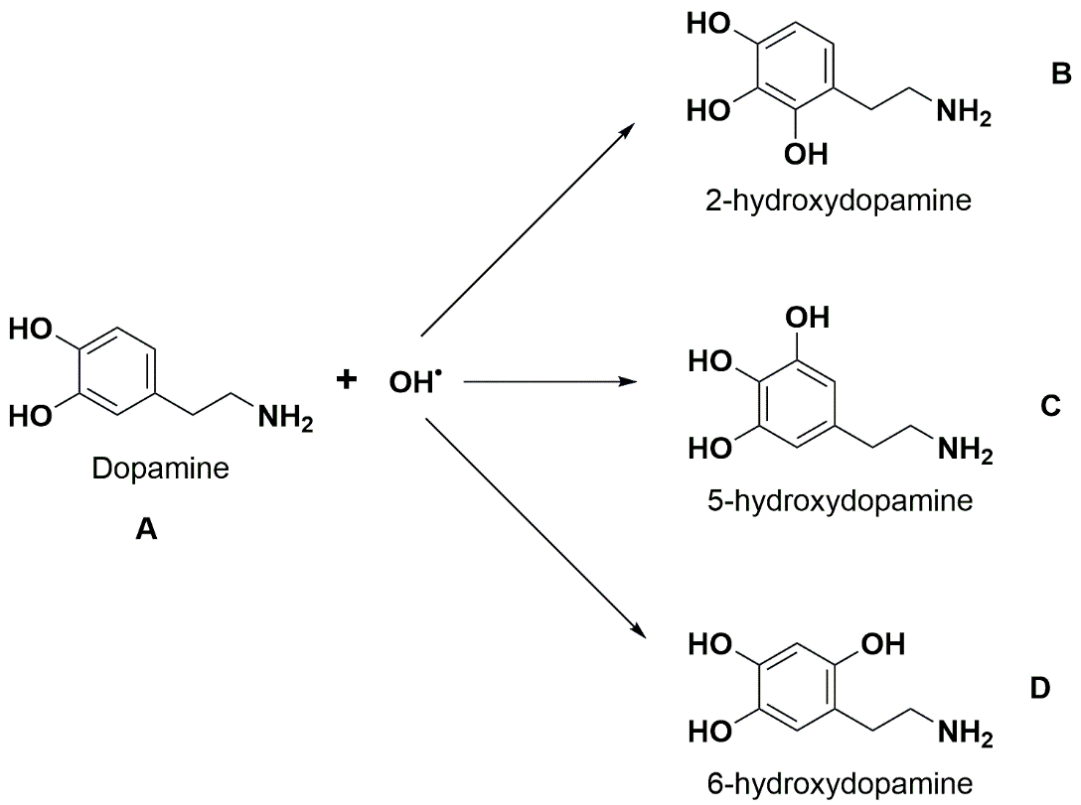


Fig. S9. Variation of the absorbance of TMB oxidation process with varying concentrations of DA.

The mechanism of inhibition of TMB oxidation by Dopamine

The selectivity experiment clearly demonstrated the greater propensity of dopamine in an acidic environment to bind with ROS like ˙OH compared to the other interfering substances and thus prevent the oxidation of TMB molecules. The unique chemical structure of dopamine contributes to its greater quenching power. The aromatic ring of dopamine contains several H-atoms which increase the electron density, mobility and the ability to bind ˙OH radicals. Dopamine forms a number of derivatives during reaction with ROS like ˙OH radicals which are illustrated in Scheme S3 of the revised supporting information. In addition, the ethyl amine group present on the side chain of phenolic ring of dopamine also effects the inhibition of TMB oxidation. As a good electron donor group, the amine group aids in accelerating electron transfer to improve the interaction of H-atoms on the aromatic ring of dopamine with ˙OH radicals.



Scheme S3: Illustration of interaction of dopamine with ROS (˙OH)

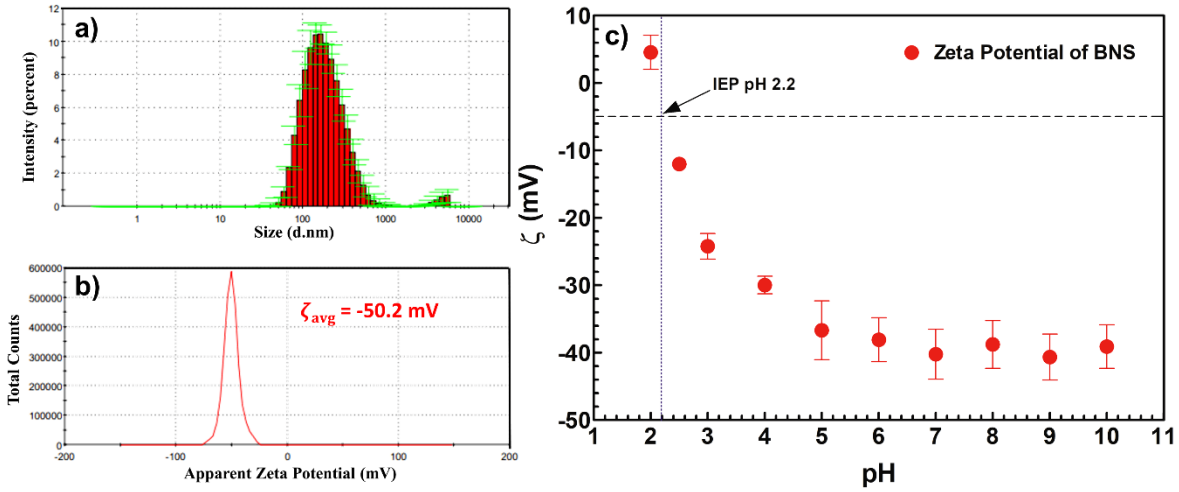


Fig. S10. a) DLS size distribution, b) Zeta potential of BNSs and c) Variation of zeta potential with pH range from 2 to 10.

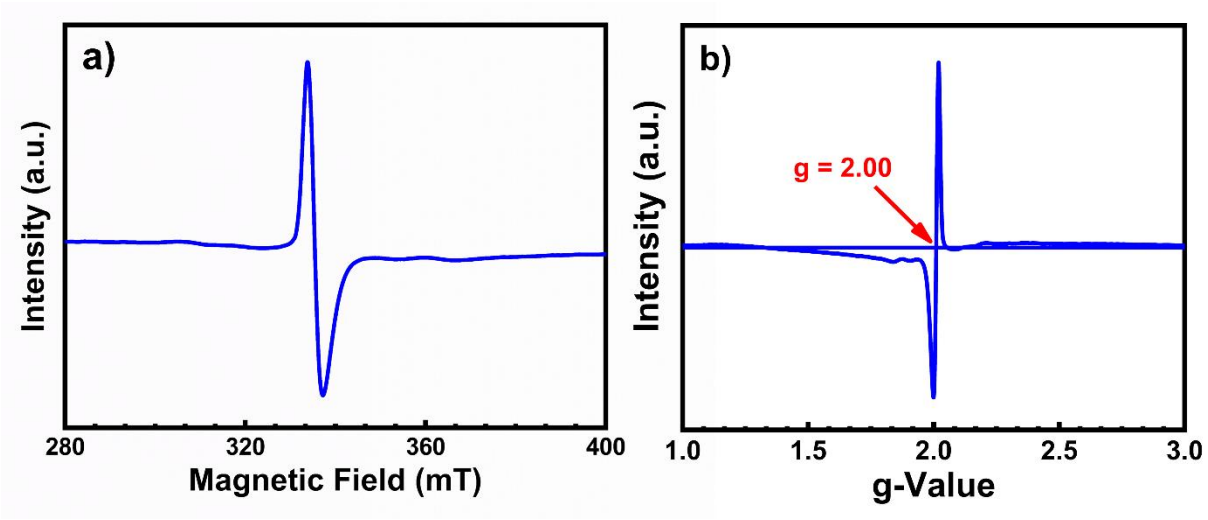


Fig. S11. a) EPR spectra of BNSs+H_2O_2 without DMPO, b) g-value for BNSs+ H_2O_2.

Table S1. Atomic percentage of the element present in the BNSs nanozyme calculated from XPS analysis

Name of the element	Peak BE (eV)	Atomic %
B1s	188.46	50.23
O1s	532.74	21.7
C1s	284.87	28.07

Table S2: Comparison of peroxidase-like activity of different nanomaterials

Catalyst	K_m (mM)		V_{max} (10^{-8} Ms^{-1})		Ref.
	TMB	H_2O_2	TMB	H_2O_2	
BNSs	1.0	3.5	20.4	16.0	This work
HRP	0.434	3.7	10	8.7	(6)
c-BN	0.157	10.88	5.98	3.45	(7)
Fe_3O_4	0.098	154	3.44	9.78	(8)
WS_2/rGO	22.406	10.001	0.9606	0.9332	(9)
Pt/hBNNSs-5	0.21	9.2	_	_	(4)

Table S3: A comparison of colorimetric/optical dopamine sensors**.**

Catalyst	Technique	Linear range	LoD	Ref.
BNSs	colorimetric	5-40 µM	6.6 µM	This Work
Ag-rGO	electrochemical	10-800 µM	5.4 µM	(1)
DOX−GO	fluorometric	1.44-11.8 µM	1.5 µM	(2)
Au-rGO	fluorometric	5-200 µM	3 µM	(3)
Pt/hBNNSs-5	colorimetric	2-55 µM	0.76 µM	(4)

Table S4a: Determination of DA in FBS using BNSs as nanozyme

FBS	Original Content	DA added (µM)	DA found (µM)	Recovery (%)	RSD (%, n=3)
1	ND	7	7.32	104.6	1.5
2	ND	15	15.04	100.3	1.4
3	ND	20	19.59	98.0	1.9
4	ND	25	23.49	93.9	3.2
5	ND	30	29.49	98.3	5.3

Table S4b: Determination of DA in Brain Tissue Lysate using BNSs as nanozyme

Brain Tissue Lysate	Original Content	DA added (µM)	DA found (µM)	Recovery (%)	RSD (%, n=3)
1	ND	10	10.42	104.2	1.4
2	ND	15	14.52	96.8	0.9
3	ND	20	20.33	101.7	1.0
4	ND	25	24.29	97.2	0.8
5	ND	30	29.80	99.3	3.8

*ND : Not determined

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