

# Supporting Information

## Preparation of ultra-thin hexagonal boron nitride nanoplate for cancer cell imaging and neurotransmitter sensing

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## **1. Materials and Methods**

### **1.1. Materials**

Pristine boron nitride (98%), sulfuric acid (99.99 %) and hydrogen peroxide (30 wt %) were purchased from Sigma-Aldrich (St. Louis, MO). N-Butanol was purchased from Samchun pure chemicals co. ltd (Gyeonggi-do, Korea). Cell culture reagents, including fetal bovine serum (FBS), penicillin, streptomycin, trypsin/EDTA, and Dulbecco's phosphate buffer saline (PBS) were purchased from Gibco BRL (Carlsbad, CA, USA). 3-(4,5-dimethylthiazol-2-yl)-2 and 5-diphenyl tetrazolium bromide (MTT) were also obtained from Sigma-Aldrich (St. Louis, MO).

### **1.2. Exfoliation and functionalization of boron nitride**

The synthesis procedure of functionalized boron nitride is demonstrated by the schematic diagram and the flow chart shows in Figure 1 and Figure S1, respectively. Certain amount of pristine boron nitride powder were irradiated in microwave followed by dissolve in solution and bath sonication for 12 h. Distilled water, hydrogen peroxide or sulfuric acid were added and ultrasonication was continued for 10 minutes. The mixture of boron nitride was refluxed for overnight (95-100 °C) in a dark environment. Adequate amount of tert-butanol was added to the solution for extracting the water soluble fraction of boron nitride. The fraction of boron nitride which was dissolved in aqueous part was collected and dried by freeze dryer for process 1 and 3. But for process 3, the aqueous part was dialyses against water to remove byproduct of sulfuric acid before freeze drying. The dialysis process was continued for 6 h and water was replaced for 2 times with 2 h of interval. Dialysis and drying process was carried out in dark environment to protect fluorescence intensity from light.

### **1.3. Characterization**

Transmission electron microscopy (TEM) analysis were conducted on a JEOL 2100 field-emission TEM system. Atomic force microscopy (AFM) were conducted under the tapping mode on a Veeco Digital Instruments Multimode Scanning Probe Microscope with a Nanoscope III Controller. The X-ray diffraction pattern of hBN and hBN-OH were obtained using Rigaku D/MAX-2550 pc with monochromatic CuK radiation. UV-vis spectra were measured at 20°C with UV-VIS spectroscopy (Mecasys Co. Ltd., Korea) equipped with a 10-mm quartz cell (path length of 1 cm). Fluorescence spectra was recorded on a PL spectroscopy (Sinco, Korea). Raman spectroscopy was studied using a Thermo-Nicolet-Almega dispersive Raman spectrometer with 532 nm excitation. The surface charges of hBN and hBN-OH were performed on Zeta sized ELS Z (Otsuka Electronics, South Korea). Thermal gravimetric analysis (TGA) was measurement on a TA-Q50 thermo-gravimetric analyzer (TA, DE). Band gap values of fluorescence hBN-OH was measured based on absorbance and reflectance of the respective hBN-OH. The calculation methods has been elaborately discussed elsewhere.<sup>1</sup> In brief, the powder hBN-OH was dissolved in water and sprayed on a transparent plasma film. After drying the film was placed on spectrophotometer for measuring reflection spectra. In this model, the prism/grating double monochromatic system is used. The lenses which were conveniently used in a monochromatic system have all been replaced with mirrors. This is done to eliminate image deviation due to chromatic aberration. A Metler Toledo DSC822e/200 under N<sub>2</sub> atmosphere used for DSC analysis. Around 3 to 5 mg sample of each hBN materials were taken for analysis. Both samples measurements were preceded by heating to 120 °C at a 10 °C min<sup>-1</sup> heating rate to remove thermal history. After that, the samples were cooled down to – around 100 °C at a cooling rate of 10 °C min<sup>-1</sup>. At that time, heating curves obtained at heating rate of 10 °C min<sup>-1</sup>.

## 2. Quantum yield measurements

Functionalized boron nitride dissolve in water and make various concentrations of samples. Quinine sulphate used as standard materials. It's dissolved in 0.1M H<sub>2</sub>SO<sub>4</sub> to make various concentrations.

All samples and standard concentration measurements by UV with solution absorbance in the range 0.03 to 0.07. That concentration measure BT Photoluminescence Spectra with calculate integrate area of this peak to enter below equations

$$QY_x = QY_s * [A_x/A_y] * [F_s/F_x] * [n_x/n_s].^2$$

Here, “x” and “s” refer to the sample and the reference material, respectively. A and F refers to the integrated area under the emission spectrum and the fraction of exciting light absorbed at the excitation wavelength (same of both samples) and refractive index refers to ‘n’ of the solvent.<sup>2</sup>

### 3. Cell imaging and cytotoxicity study

Epithelial carcinoma (KB) cells were obtained from Korea Cell Bank (Seoul, Korea) and cultured in a 75 mm<sup>3</sup> T flask. The cell were transferred to 8-well plate at concentration of 1x10<sup>5</sup> cells/well. The cells were incubated for overnight to allow them to adhere on the bottom surface. hBN-OH was dispersed in PBS at a concentration of 0.1 mg/mL and 10 μL of the suspension was added to the well. The hBN-OH and KB cells were co-incubated for 4 h followed by washing with PBS for 3 times. The cells were observed by confocal microscope to observe accumulation of fluorescence hBN-OH.

For cytotoxicity observation, KB cells were seeded into a 96- well plate (1x10<sup>5</sup> cells/ well) and incubated for 24 h. Different concentrations of hBN and hBN-OH (1, 10, 25, 50 and 100 μg/mL) were added to the 96-well plate. RPMI-1640 medium was used as the control. The cell solutions were incubated for another 24 h under dark conditions at 37 °C. 50 μL of MTT aqueous solution was added to every well for 4 h before the 24 h incubation period ended. Upper layer of the solution discarded from plate. 150 μL of MTT solubilisation solution was added to each well to dissolve the formazan crystals and the optical absorbance was assessed using a Varioskan flash (Thermo Scientific, USA) at a wavelength of 570 nm.<sup>3</sup> Cell viability calculated using below mentioned equations

Cell Viability (%) = (absorbance of sample cells/ absorbance of control cells) x100.

#### 4. *In vitro* cells uptake study

Cellular uptake study of functional boron nitride, sample was incubated with KB cells. The cells were culture at 37 °C in a humidifier atmosphere containing 5% CO<sub>2</sub> in a RPMI-1640 medium with 10% fetal bovine serum. Cells monolayer around 5x10<sup>4</sup> cells/mL were harvested by 0.25% trypsin-0.03% EDTA solution. The cells (20 µL) in RPMI-1640 media were seeded in an 8 well plate and pre-incubated for 24 h before the assay. 50µg/mL of functional boron nitride was added to 8 well plate and the incubated for 2 h. Cells containing wells were washed by PBS to remove unable to uptake the particles. Before observing the cells by CLSM were preserved by paraformaldehyde solution to get clear cells morphology.<sup>4</sup>

#### 5. Scanning electron microscopy (SEM) of cell morphology

KB cells were cultured according to the method described in confocal microscopic imaging method. The cells were prepared for SEM imaging according to the method reported previously.<sup>5</sup> In brief, the cells were dispersed in fixing solution composed of 1% glutaraldehyde and 0.1M sodium cacodylate. The cells were then fixed and dehydrated following various steps; such as fixing in 1% tannic acid followed by dehydrate through a series of alcohol. Finally, the dehydrated cells were sputter coated with gold and observed by SEM.

#### 6. Haemolysis assay of the hBN-OH

Fresh blood was collected from SD rat for haemolysis assay of the hBN-OH. Erythrocytes of blood were collected by centrifugation and washed with PBS for three times. The erythrocytes was mixed with additional PBS and re-centrifuge. The functional hBN with various concentrations (0-200 µg/mL) was added with 1 mL erythrocytes solution ad incubated for 4 h at 37 °C. Absorbance of the supernatant was analysis at 394 nm to measure percentage of hemolysis.<sup>6</sup>

## 7. Dopamine sensing

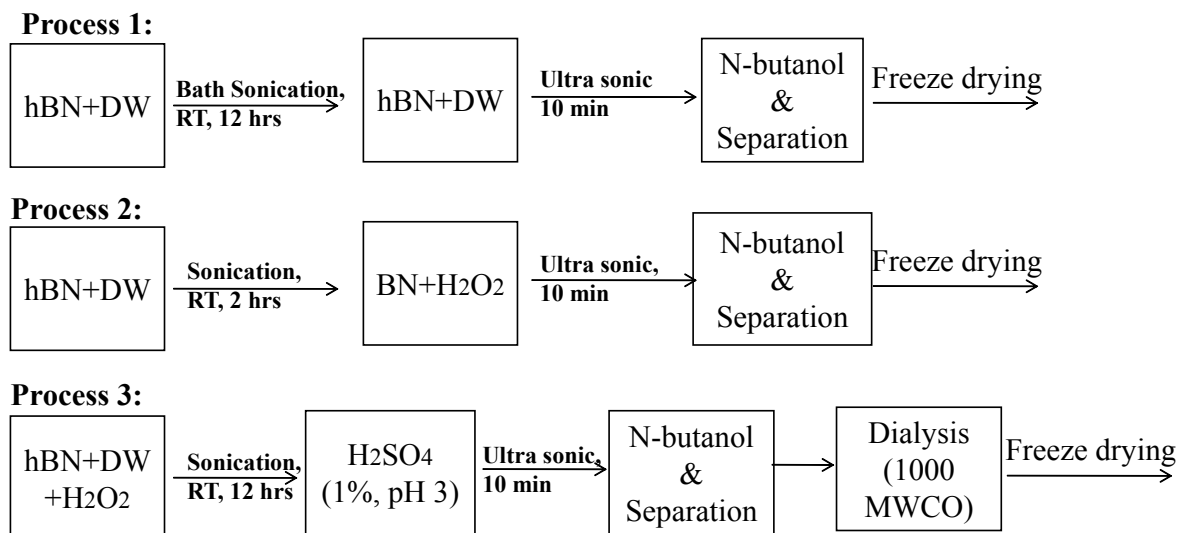
Cyclic voltammetry assay was conducted with a CHI 7031D electrochemical workstation (CHI, USA) according to the method described earlier.<sup>7</sup> In brief, glassy carbon, Ag/AgCl and Pt wire were used as a working electrode, reference electrode and counter electrode, respectively. hBN-OH was dissolved in water as a concentration of 1 mg/mL and vortex for uniform dispersion. The glassy carbon electrode was coated with the hBN-OH solution through drop cast process and dried in air. The electrode was prepared with dopamine dissolved in PBS (pH 7.4) with 1.5  $\mu$ M concentration. The parameters of the cyclic voltammeter was: potential window between -0.1~+0.5 V. In case of black, the glassy electrode was not coated with anything (bare electrode) and finally the electrolytes (dopamine) was mixed with hBN-OH and incubated for 10 min before analysis by bare glassy electrode.

## 8. Colloidal stability test

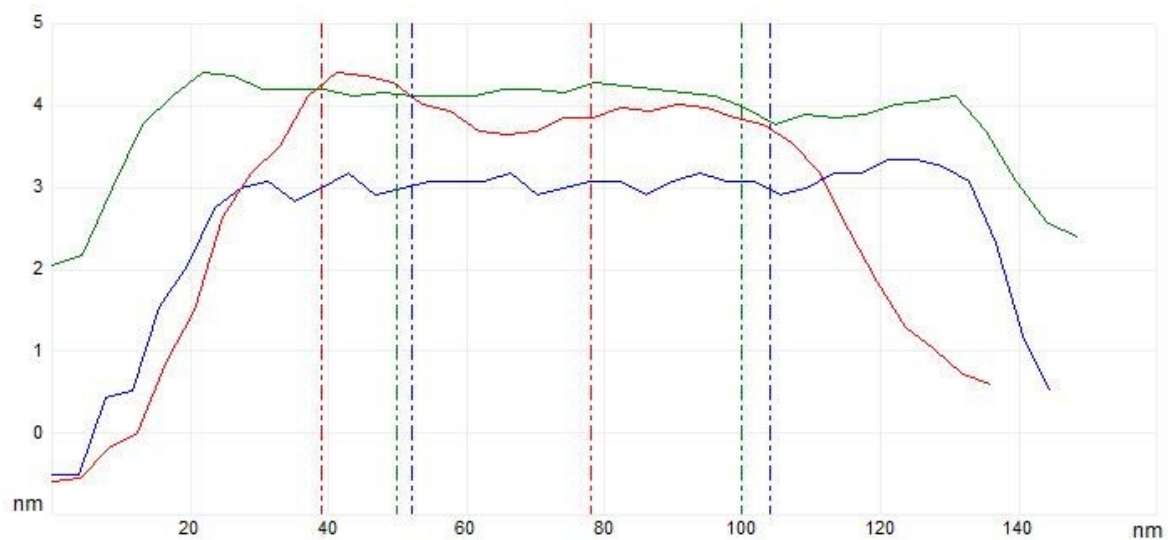
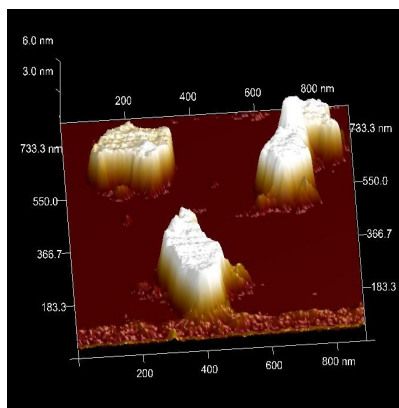
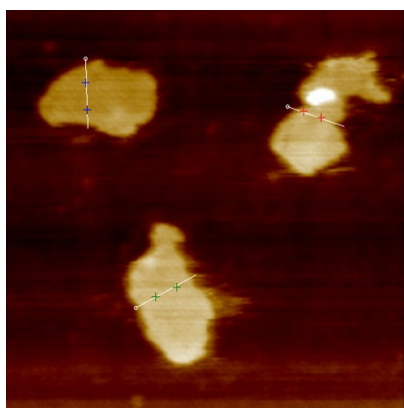
hBN-OH itself is not stable enough when dissolved in water or PBS since the surface is highly anionic in nature. Therefore, we aim to observe its stability and dispersibility in cationic polymer solutions such as PEI and bPEI solution. For experiment, the hBN-OH was dissolved in PBS (10 mg/mL) and divided into three different groups. PEI (1 K Da) and bPEI (25K Da) was added to the two hBN-OH containing PBS (1%) solution and another was kept separately without adding anything. Visual dispersibility was monitored and images were taken at 0, 6, 12 and 24 h after dispersion.

## 9. Statistical analysis

Significant differences were assessed with a one-way analysis of variance (ANOVA) (Origin Pro 8.0), and the data were presented as the means  $\pm$  S.E

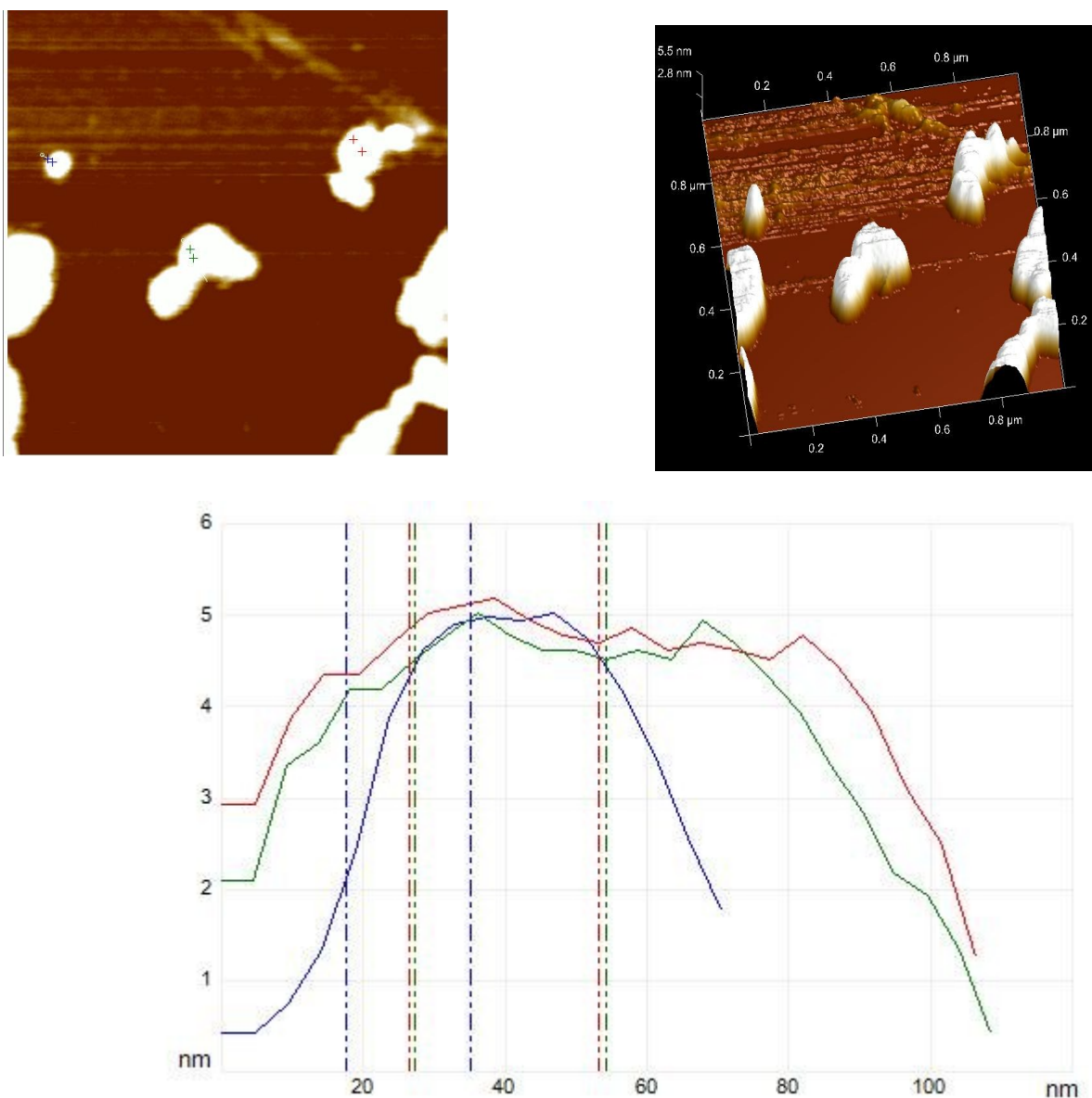


**Figure S1.** The scheme shows different process of fabrication and functionalization of hBN.

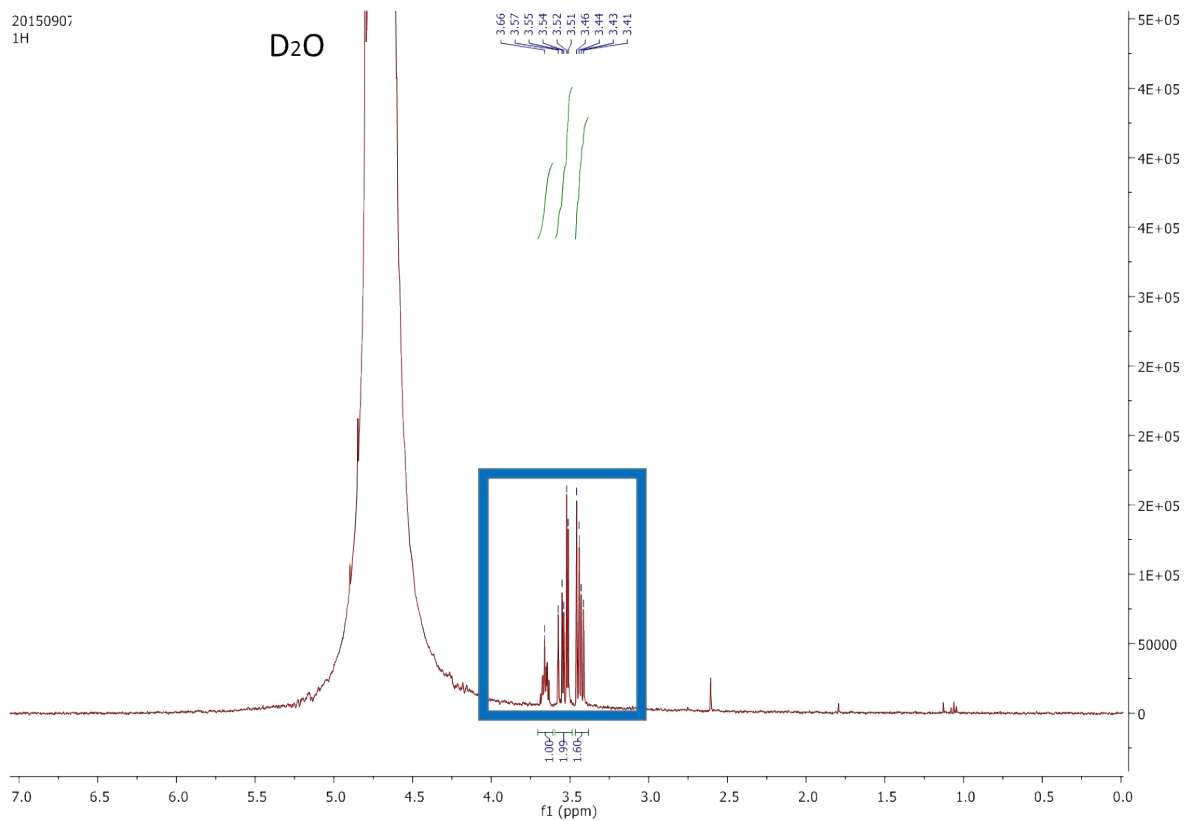


**Figure S2.** Size and height of exfoliated boron nitride according to process 1.

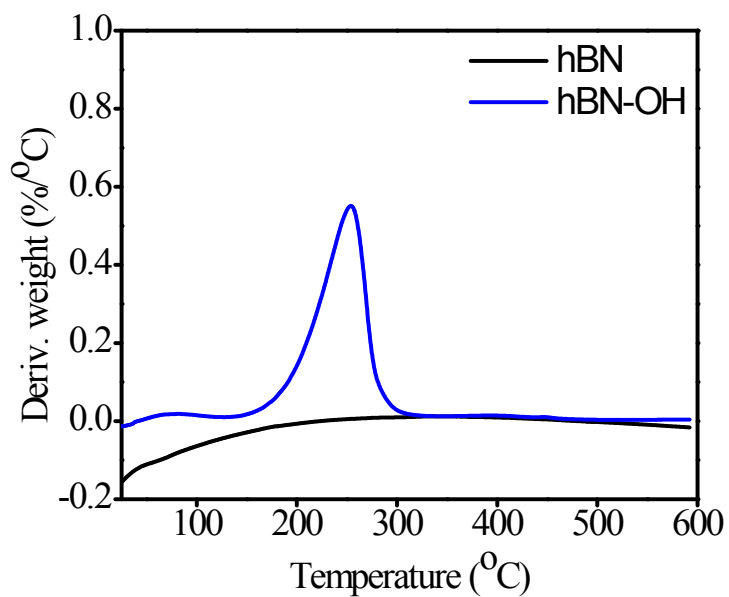




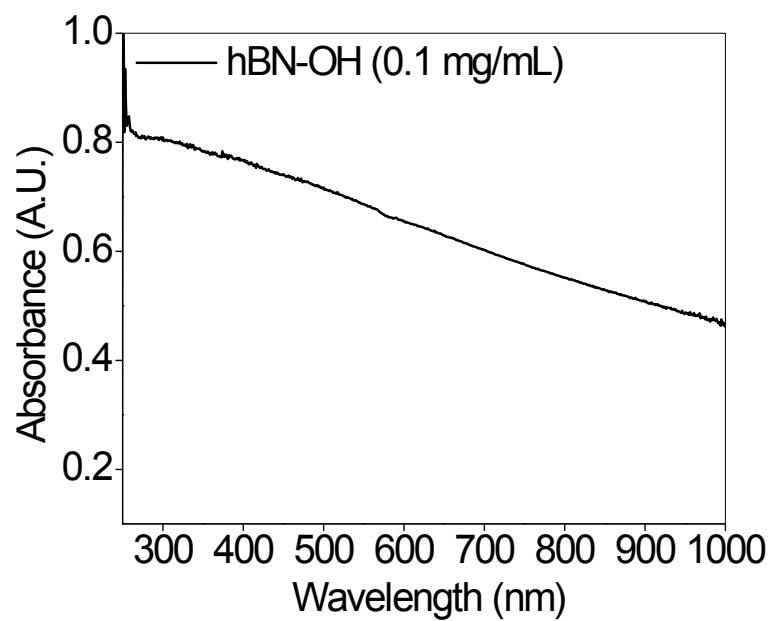
**Figure S3.** Size and height of exfoliated boron nitride according to process 2.



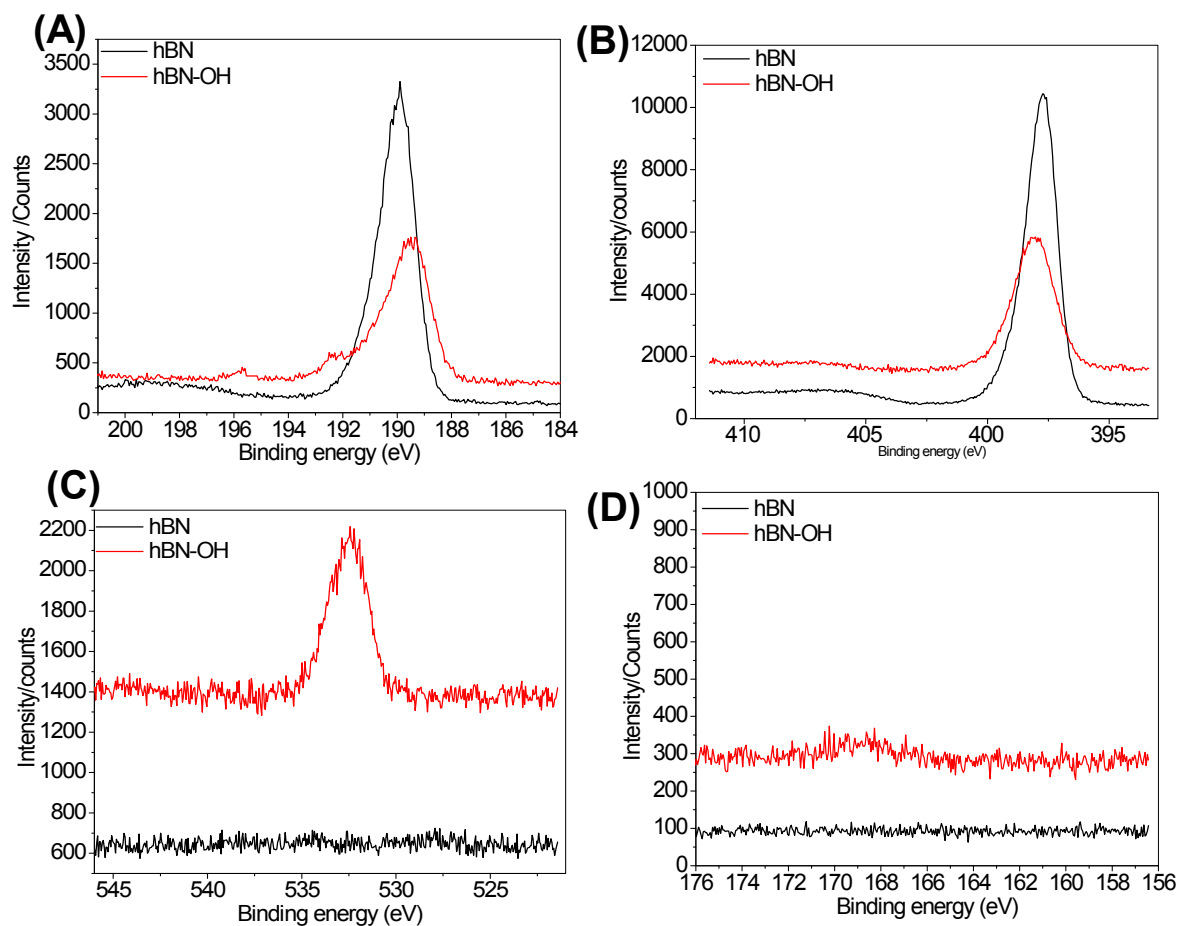
**Figure S4.** <sup>1</sup>H-NMR spectrum of hBN-OH show appearance of proton peaks.



**Figure S5.** The first derivative of the mass-change of pristine hBN and hBN-OH as a function of temperature represent  $T_{\max}$  of individual derivatives.



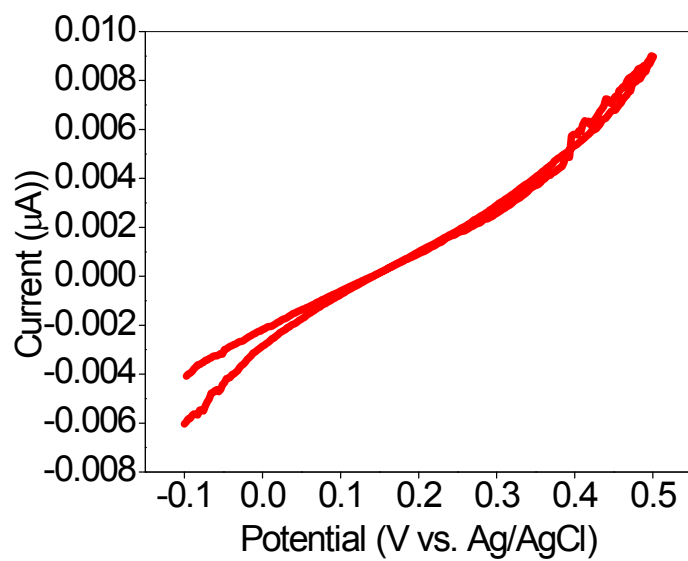
**Figure S6.** UV-vis absorbance spectrum of hBN-OH.



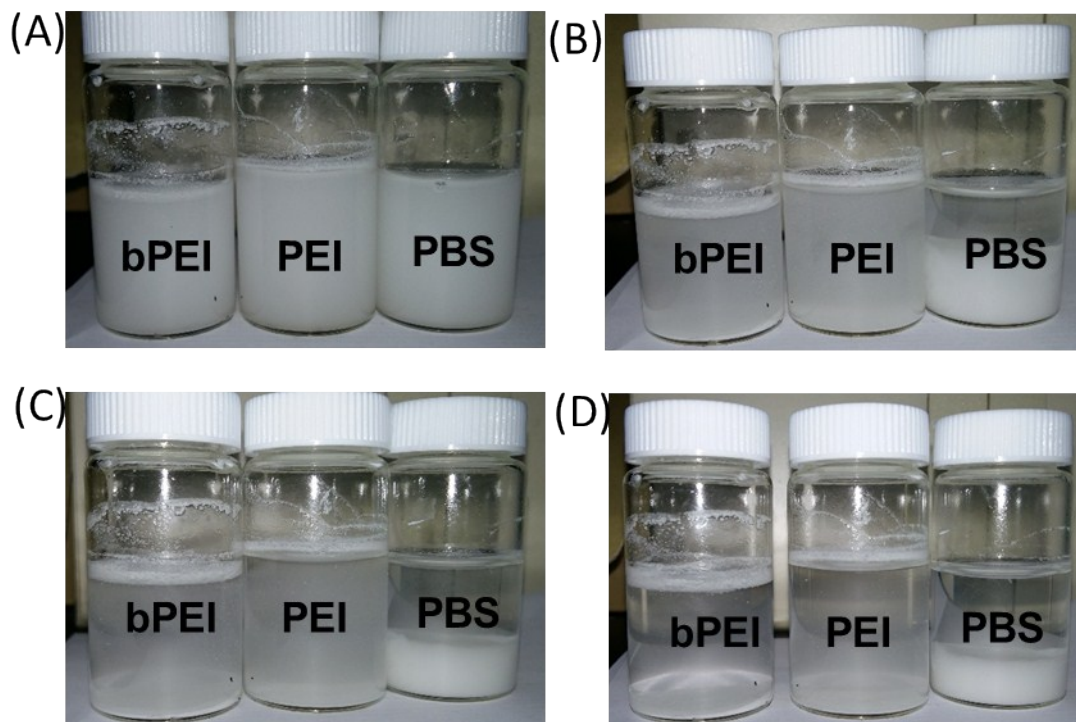
**Figure S7.** Individual peak survey of hBN and hBN-OH, (A) B1s, (B) N1s, (C) O1s, and (D) S1s.

**Table S1.** Relative atomic content of hBN and hBN-OH calculated from the XPS survey of hBN and hBN-OH. B/N ratio is 0.66 and 0.88 in hBN and hBN-OH, respectively.

Content	hBN	hBN-OH
<b>B</b>	38.21	27.72
<b>N</b>	57.89	31.48
<b>O</b>	0	38.73
<b>C</b>	1.92	0
<b>S</b>	0	0.3

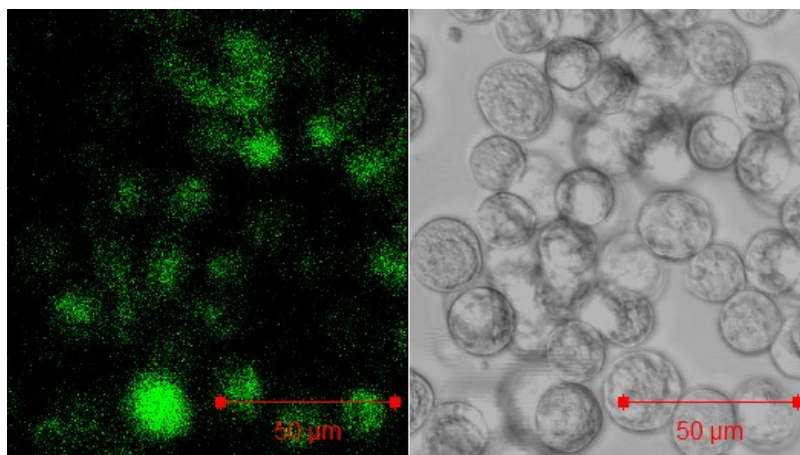


**Figure S8.** Cyclic voltammogram of un-modified glassy carbon (bare GC) electrode in a 0.1 M PBS solution (pH 7.4) containing 10  $\mu\text{M}$  dopamine (DOPA) at a scan rate of 50  $\text{mV s}^{-1}$ .



**Figure S9.** hBN-OH was dissolved in 1 M PBS (10 mg/mL), PEI (1% in PBS), bPEI (1% in PBS). Colloidal stability was observed at (a) 0 h, (b) 6 h, (c) 12 h and (d) 24 h where we have observed that complete precipitation of hBN-OH found at 4 h in PBS solution due to high concentration. However hBN-OH dispersed in PEI and bPEI solutions (1% in PBS) form partial precipitation at 4, 12 and 24 h of observation even at same concentration of hBN-OH (10 mg/mL). The hBN-OH nano-sheets go through interaction with the hydrophobic PEI and bPEI which are known as highly cationic polymers when dissolved together. Therefore, dispersion duration of hBN-OH highly increased. This results suggest that, polymeric modification of the hBN-OH could significantly increase stability that could further use for biological application such as drug/gene delivery carrier.





**Figure S10.** Confocal microscopic images of KB cells after 4 h of co-incubation with hBN-OH.

## References

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