Supporting Information

Dual Stimuli-Responsive Supramolecular Boron Nitride with Tunable Physical Properties for Controlled Drug Delivery

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Experimental sections

Materials

Hexagonal boron nitride (BN), poly(propylene glycol) diacrylate (PPG diacrylate, average molecular weight of 800 g/mol, ca. 14 repeat units), adenine, potassium tert-butoxide and phosphate buffered saline (PBS) of the highest purity grades were obtained from Sigma-Aldrich (St. Louis, MO, USA). All solvents and PBS were of analytical grade and purchased from TEDIA (Fairfield, OH, USA). Dimethylformamide was stirred over calcium hydride overnight and distilled under reduced pressure before use. Doxorubicin hydrochloride (DOX) was purchased from Cayman Chemical Co., Ltd., Ann Arbor, MI, USA. Cellulose dialysis membranes with a molecular weight cutoff (MWCO) of 1000 and 6000−8000 Da were acquired from CelluSept T1 (Braine-l'Alleud, Belgium).

Characterization

RigakuUltima IV wide-angle X-ray diffraction (WXRD; Rigaku, Tokyo, Japan) patterns were analyzed using a Bruker phase diffractometer (Cu Kα, λ = 1.54 Å with a nickel monochromatic at 40 kV). Raman spectra were obtained from 200 to 1600 cm^{-1} at room temperature with a laser Raman spectrometer (30 mW at 532 nm; Model CRM 2000; WITec Inc., Ulm, Germany). Aqueous samples were spin-coated onto silicon surfaces, then the water was evaporated slowly at room temperature for 1 day. Ultraviolet-visible (UV-Vis) spectroscopy data were recorded using a UV-Vis spectrophotometer (Jasco V-550 type, Tokyo, Japan). Fluorescence spectra were achieved using a 1 cm-path quartz cell on a Jasco FP-8300 spectrophotometer equipped with Zenon lamp at a slit width of 10 nm. An excitation wavelength of 400 nm was used; fluorescence emission was monitored from 420 to 900 nm. A field emission scanning electron microscope (FE-SEM; JSM-6500F, JEOL, Japan) and transmission electron microscope (TEM, H-7000; Hitachi, Tokyo, Japan) were used to analyze sample morphology and microstructure. The thicknesses and morphology of exfoliated BN nanosheets were determined using an Atomic Force Microscope (NX10, AFM Park Systems, Suwon, South Korea). Sample preparations for FE-SEM and AFM have followed the same procedure as for Raman. For the process of TEM specimen preparation, samples were suspended in water and dropped onto the surface of 200 mesh carbon-coated copper grid with a carbon film, then the

water was removed slowly at room temperature for 1 day. Dynamic light scattering (DLS, Nano Brook Zeta PALS, Brookhaven Instruments Corporation, Holtsville, NY, USA) was performed to measure zeta potential and determine the average hydrodynamic size of samples.

Exfoliation of bulk-BN into BN nanosheets with A-PPG

Bulk BN (25 mg) and A-PPG (25 mg) were added to water (10 mL) and ultrasonicated (DELTA, Ultrasonic Cleaner D150H, Taiwan; operating at 43 KHz and 150 W) for 3 h at 25 °C. The supernatant suspension containing well-dispersed BN nanosheets was collected by centrifugation at 8000 rpm for 3 min. The dispersed solution was freeze dried and dispersed into aqueous solution or PBS for further experiments.

Cytotoxicity of BN/A-PPG nanosheets

The *in vitro* cytotoxicity of blank A-PPG and 50/50 BN/A-PPG nanosheets against a murine macrophage cell line (RAW 264.7 cells) and human breast adenocarcinoma cell line (MCF-7 cells) was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) viability assay. Cells were seeded in 96 well-plates in 100 μL of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 5% penicillin and cultured at 37 °C in 5% CO_2. After 24 h, the culture medium was removed and replaced with 100 μL of fresh medium containing different concentrations of blank A-PPG or 50/50 BN/A-PPG (0.1-100 μg/mL). Cells were incubated for 2 h, rinsed twice with PBS, 100 μL of growth media was added to each well, incubated for 48 h, then 10 mL of MTT solution (5 mg/mL) was added to each well, incubated for 24 h, and the blue formazan crystals generated by live cells were dissolved in 100 μL DMSO. The absorbance of each well was measured at 570 nm using a microplate reader. Untreated cells were used a control. Relative cell viability (%) was determined by comparing the absorbance values of treated cells with control wells. Each experiment was repeated independently a minimum of three times with six wells per sample in each experiment. Average cell viability (%) was calculated as:

$$\text{Cell viability (\%)} = A_{sample}/A_{control} \times 100$$

Where A_{sample} is the absorbance of the polymer-treated cells and $A_{control}$ is the absorbance

of control untreated cells.

Lower critical solution temperature (LCST) determination

The LCSTs of the 50/50 BN/A-PPG composite at concentrations ranging from 2 mg/mL to 5 mg/mL were measured using a UV–Vis spectrophotometer at a heating rate of 0.5 °C/min. Temperature was increased from 10 to 60 °C. Transmittance of the composite solutions was assessed at 500 nm after equilibration at each temperature. LCST was defined as the temperature at which the percent transmittance of an aqueous solution decreased to 50%.

Preparation of DOX-loaded BN/A-PPG nanosheets

Drug encapsulation was conducted using a similar process as the previous literature.[11] Briefly, the 50/50 BN/A-PPG composite (1.2 mg) was added to 3 mL of PBS solution containing 0.05, 0.1, or 0.2, 0.4 or 0.8 mg/mL DOX. The solutions were ultrasonicated at 25 °C for 20 min to disperse the BN nanosheets, then the mixture was stirred vigorously and incubated at 25 °C. Residual free DOX was eliminated from the composite mixture by dialysis for 24 h. The DOX-loaded content was measured by reading absorbance (λ = 488 nm) using UV-Vis spectroscopy, with reference to a calibration plot prepared using serially diluted free DOX. The drug loading content (DLC) and encapsulation efficiency (EE) of DOX were calculated using:

$$\text{DLC (\%)} = \frac{Amount\ DOX\ loaded\ on\ BN/A\text{ - }PPG}{Amount\ of\ DOX/BN/A\text{ - }PPG\ Complex} \times 100$$

$$\text{EE (\%)} = \frac{Amount\ DOX\ loaded\ on\ BN/A\text{ - }PPG}{Amount\ of\ DOX\ input} \times 100$$

***In vitro* DOX release assay**

Temperature- and pH-dependent drug deliveries from DOX-loaded BN/A-PPG complexes were investigated using a dialysis method as a function of time**.** To evaluate DOX release, DOX-loaded BN/A-PPG complex in PBS solution (1 mL) was placed in a dialysis bag with a MWCO of 1000 Da, and dialyzed against discharging buffer solutions of different pH values (pH = 7.4, 5.5 at 37 °C) and at different temperatures (35 or 40 °C

at pH 7.4) under reservoir-sink conditions. At each predetermined time interval, samples of the release medium were taken from the outer phase buffer medium and the concentration of DOX was determined by UV-Vis spectroscopy at λ = 486 nm. After each time-point, the outer phase PBS medium was replaced with an equal volume of fresh PBS to maintain a fixed volume. Cumulative percentage release was calculated using:[S1]

$$\text{Concentration of drug } (\mu g/mL) = (\text{slope} \times \text{absorbance}) \pm \text{intercept}$$

$$\text{Amount of drug } (mg/mL) = \text{concentration} \times \text{bath volume}$$

$$\text{Cumulative percentage release } (\%) = \frac{Volume\ of\ sample\ withdrawn\ (mL)}{Bath\ volume\ (v)} \times P\ (t-1) + Pt$$

Where Pt = percentage release at time t

Where $P\ (t-1)$ = Percentage release previous to 't'

Statistical Analysis

All experiments were repeated at least three times with similar results, and the data are presented as mean ± standard deviation. Statistical significance was established using the Student's *t*-test.

Table S1: FWHM values (from Fig. 1d) for the (002) diffraction peaks of pristine BN and composites at 25 °C.

Samples	FWHM (°)
BN	0.2543
50/50 BN/A-PPG	0.5665
30/70 BN/A-PPG	0.6157
10/90 BN/A-PPG	0.7949

Table S2: Summary of preparing drug-loading nanomedicines with various functional materials as nanocarriers.

Functional nanocarriers	Drug	Drug-loading content (wt %)	References
Block copolymer	DOX·HCl	18.5	52 (a)
Liposome	DOX·HCl	30	52 (b)
Chitosan	5-Fluorouracil	20.1	52 (c)
Multiwalled carbon Nanotube	DOX	1.5	52 (d)
Graphene oxide	Irinotecan	2.7	52 (e)
Dendrimer	DOX	16.9	52 (f)
Lipid Nanoemulsions	DOX	7.4	52 (g)

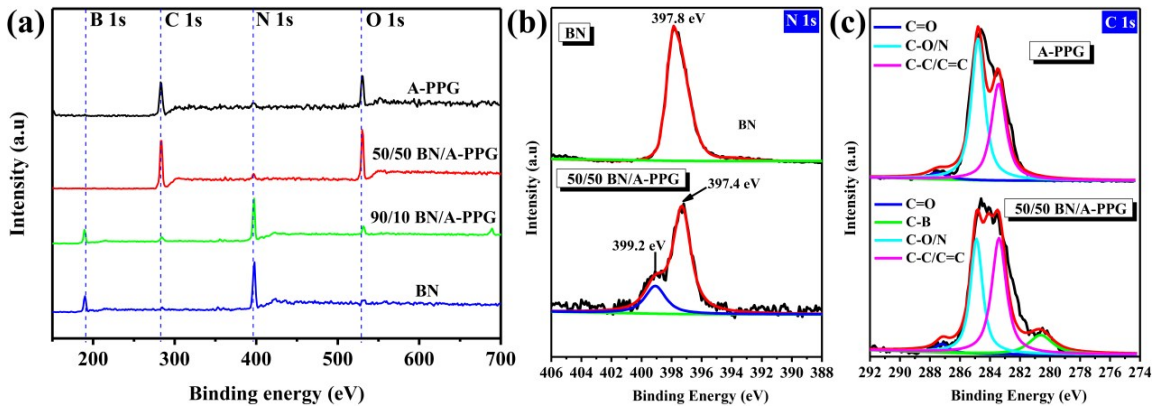

Fig. S1: (a) Wide-scan XPS spectra of pristine BN, A-PPG and BN/A-PPG composites, high-resolution XPS spectra of **(b)** N 1s for pristine BN and 50/50 BN/A-PPG composite, **(c)** C 1s for pure A-PPG and 50/50 BN/A-PPG composite.

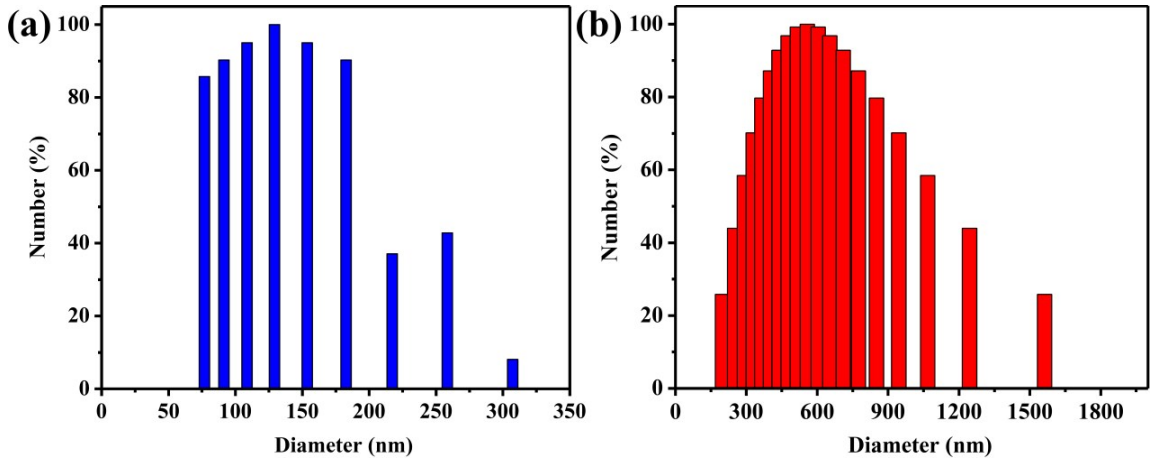

Fig. S2: Particle size distributions measured at 90° for **(a)** pure A-PPG and **(b)** 50/50 BN/A-PPG composite.

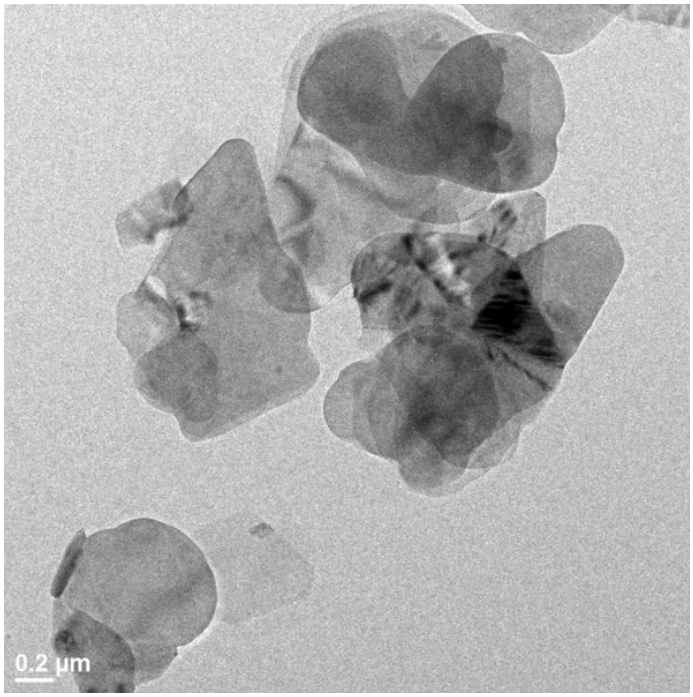

Fig. S3: Low-magnification TEM image of the 50/50 BN/A-PPG composite.

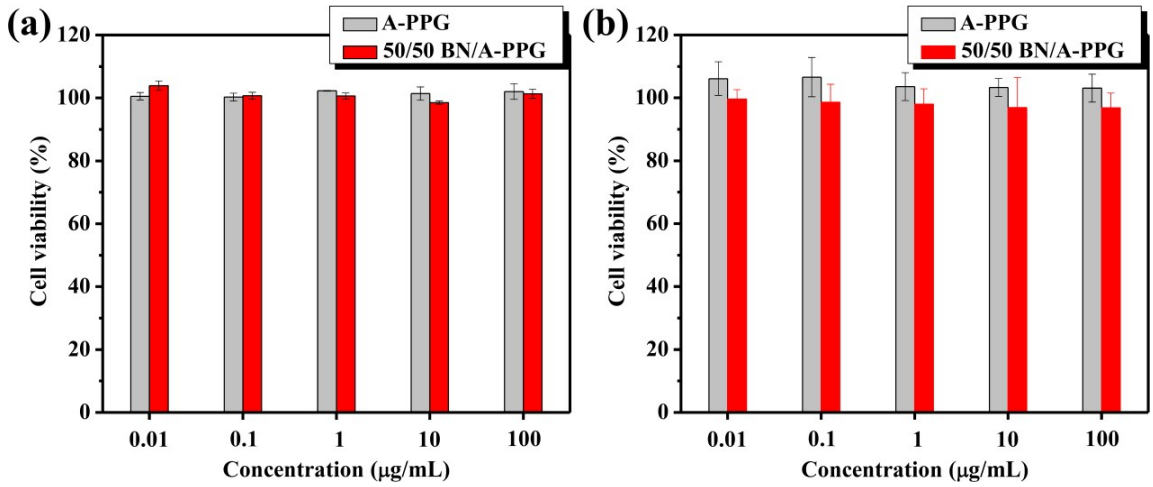

Fig. S4: Viabilities of **(a)** RAW 264.7 and **(b)** MCF-7 cells after incubation with different concentrations of pristine A-PPG or 50/50 BN/A-PPG composite for 48 h at 37 °C.

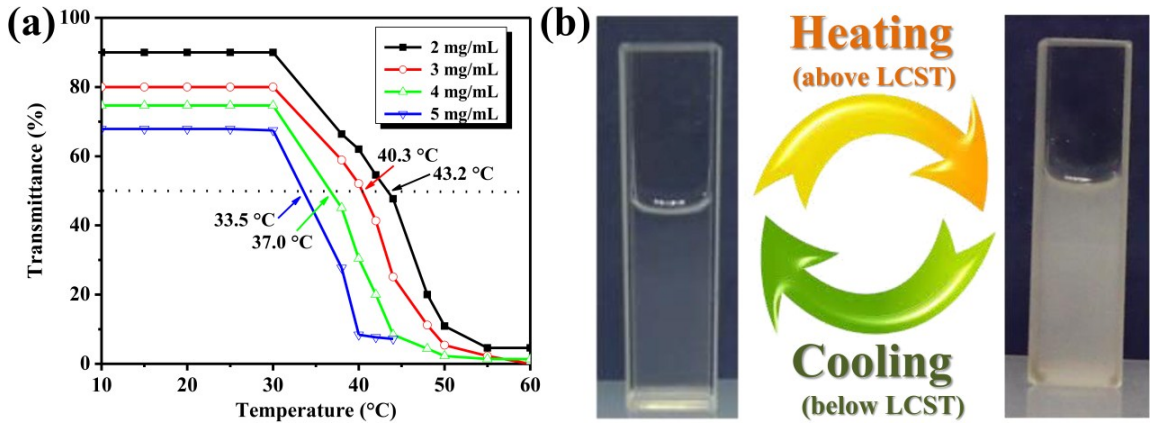

Fig. S5: (a) Transmittance as a function of temperature and composite concentration for 50/50 BN/A-PPG nanosheets in aqueous solution. **(b)** Thermoreversible LCST-type phase behavior: photographs show an aqueous solution of BN/A-PPG composite below (left) and above the LCST (right).

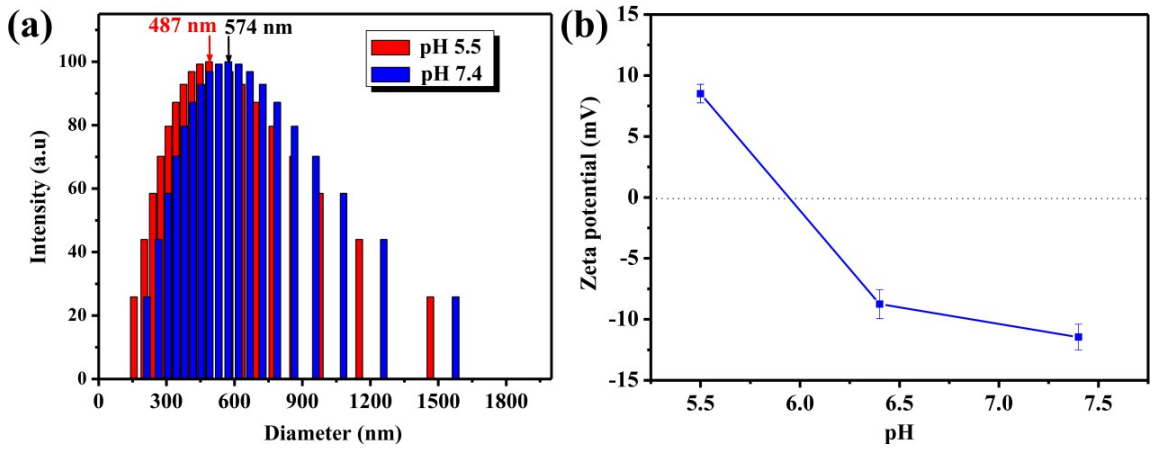

Fig. S6: (a) Particle size and (b) Zeta potential of the 50/50 BN/A-PPG composite in aqueous medium at different pH values.

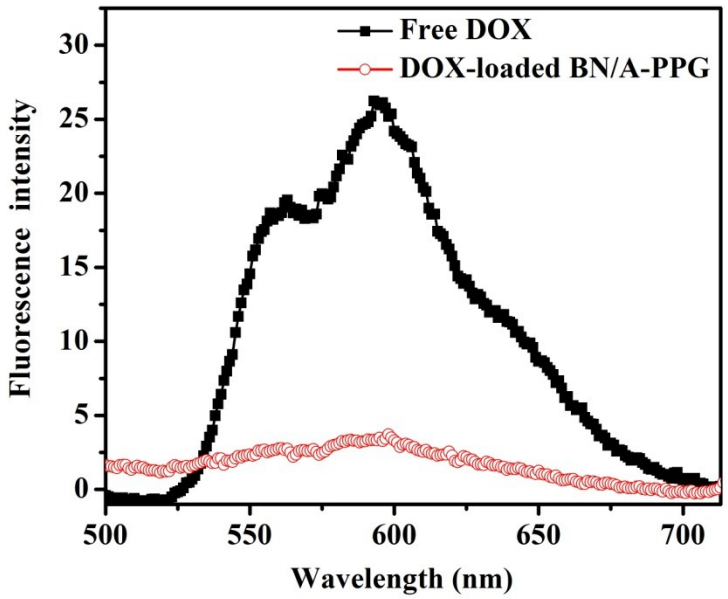

Fig. S7: PL spectra of pristine DOX and the BN/A-PPG composite in PBS solution at the same DOX concentration (λ_{ex} = 488 nm).

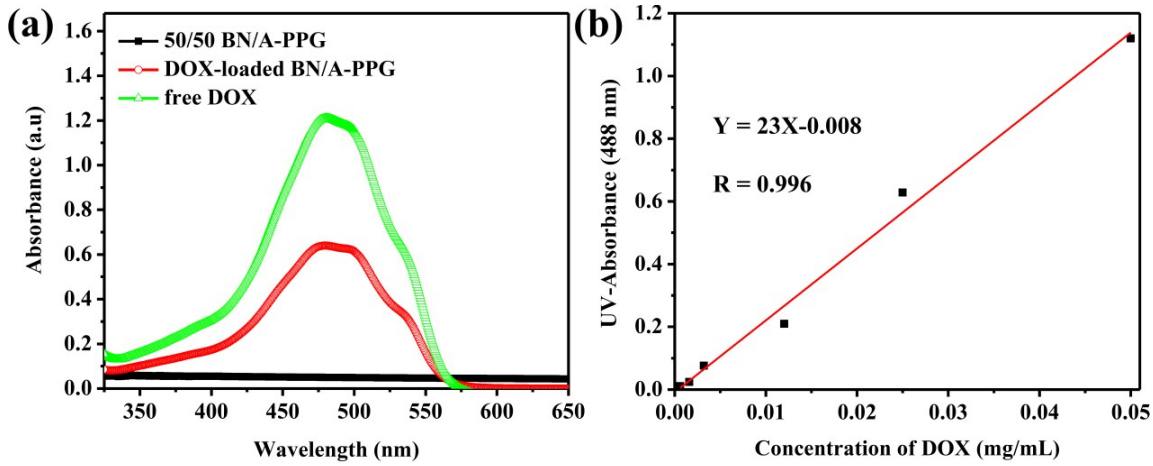

Fig. S8: (a) UV-Vis spectra for pristine DOX, BN/A-PPG and DOX-loaded BN/A-PPG composites in PBS solution at the same DOX concentration. **(b)** Calibration curve of free DOX in PBS (pH = 7.4), obtained by measuring the absorption of different concentrations free of DOX via UV-Vis spectrophotometry at 488 nm.

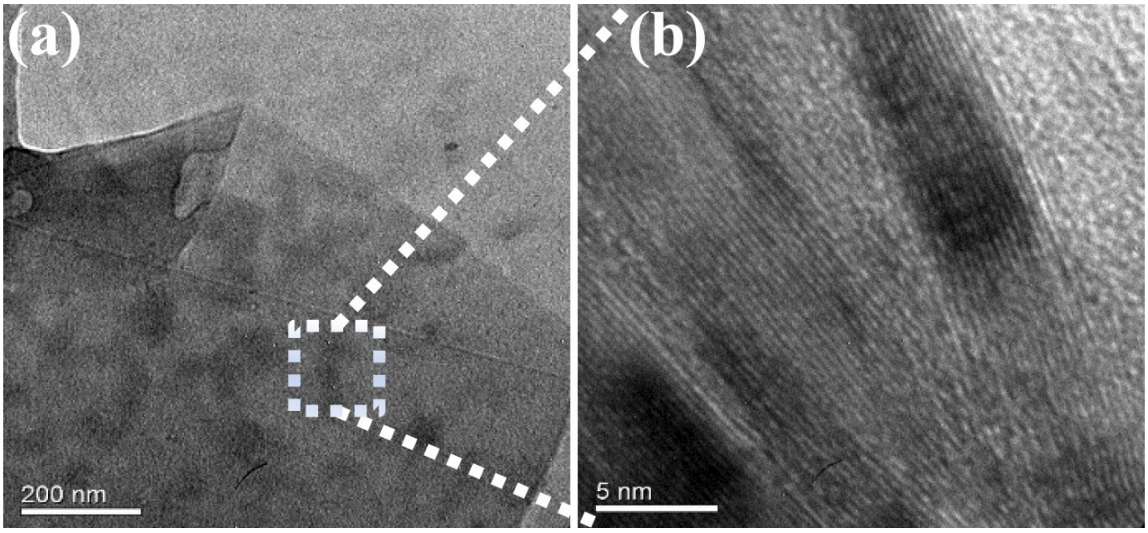

Fig. S9: TEM images of **(a)** DOX-loaded BN/A-PPG composite and **(b)** enlarged view of (a) DOX loaded on the lamellar structure of BN nanosheets.

References:

S1. A. R. Chandrasekaran, C. Y. Jia, C. S. Theng, T. Muniandy, S. Muralidharan and S. A. Dhanaraj, *J. Appl. Pharm. Sci.*, 2011, **1**, 214.