Electronic Supplementary Information

Black phosphorus nanosheets for rapid microRNA detection

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Experimental section

Chemicals

All chemicals were of analytical grade. BP crystal was obtained from Nanjing MKNANO Tech. Co.,Ltd. (http://www.mukenano.com ,China). N-methyl pyrrolidone (NMP) was purchased from Macklin Biochemical Co., Ltd (Shanghai, China). Diethyl pyrocarbonate (DEPC) was purchased from Amresco (USA). Phosphate buffered saline (PBS, 0.01 M, pH 7.2, 137mM NaCl, 2.7mM KCl) powder was obtained from Solarbio Science & Technology Co., Ltd. (Beijing, China). PBS solution was firstly dissolved using deionized Milli-Q water, and then treated with 0.1% DEPC overnight. After that, the solution was autoclaved for 15-20 minutes to remove DEPC, and then used for all fluorescence spectroscopy experiments. DNA and miRNA sequences were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). All DNA and miRNA solution were prepared using DEPC treated PBS solution.

Equipment

The topography and thickness analysis of BPNSs were performed with a BioScope Catalyst atomic force microscope (AFM, Bruker Corporation, USA) in the ScanAsyst mode in air using a PPPNCHR probe (Nanosensors, Switzerland) with a silicon tip. The size and morphology of BPNSs were characterized using a JEM-1230 Transmission Electron Microscopy (TEM, NIPPON TEKNO, Japan). The high resolution TEM (HRTEM) was performed with a JEM-2100 Transmission Electron Microscopy (NIPPON TEKNO, Japan). Samples were dispersed onto holey carbon grids with the evaporation of excess solvent. The hydrodynamic size distribution was determined on a Zetasizer Nano ZS (Malvern Instruments Ltd., UK) at 25℃. The absorbance spectrum of BPNSs was recorded at room temperature by Cary 60 UV-Vis spectrophotometer (Agilent Technologies, USA). Raman scattering spectrum of BPNSs was performed on a home-build high-resolution confocal Raman microscope equipped with a 532-nm laser as the excitation source at room temperature and a XYZ motorized sample stage controlled by PI Piezo controller E-545. A 60× objective lens with a NA of 0.90 was used thereby making the spot size at about 200 nm. The spectrum was collected by Shamrock 500i spectrograph (Andor, England) with Newton DU970P EMCCD (Andor, England). The X-ray diffraction (XRD) pattern of BPNSs was obtained on a X′ pertpro powder X-ray diffractometer (Philips, Netherland). P element content of BPNSs was determined via inductively coupled plasma atomic emission spectroscopy (ICP-AES) on OPTIMA2100DV (PerkinElmer, USA). All fluorescence measurements were performed on a Multimode microplate reader Variokan Flash (Thermo Scientific, USA) at 30℃.

Synthesis of BPNSs

The large-area BPNSs were exfoliated using our reported liquid exfoliation technique with small modifications (Figure 1A)[1]. Bulk black phosphorus (30 mg) was added to the pure NMP solution (30 mL). The mixture was put in a sonicator (SCIENTZ SDL-22DT ultrasonicator with a 40 kHz transducer and a power of 400 W, Ningbo Scientz Biotechnology Co.,LTD., China) to conduct the liquid exfoliation of the bulk BP. The temperature of the sonication bath was maintained blew 15℃. After exfoliation, the solution was centrifuged at 3000 rpm for 20 minutes to remove any nonexfoliated bulk BP. For characterization of BPNSs, the supernatant was centrifuged at 13000 rpm for 20 minutes to separate the BPNSs from the NMP. The precipitate was repeatedly water rinsing and dispersed in deionized Milli-Q water. Further, for miRNA detection, the BPNSs were concentrated to desired concentration by centrifugation.

Fluorescent miRNA assays

The fluorescent measurements were carried out in black 96-well plates at 30℃. Firstly, 100 μL of pDNA solution in the concentration of 25 nM was added into wells of plate. Then, 10 μL of BPNSs at 200 μg/mL was added to the pDNA solution. After 10 minutes of incubation at room temperature, 100 μL of target miRNA in the concentration ranging from 10 nM to 1000 nM were added to the mixture and incubated for 30 minutes. For control experiment, 100 μL of PBS was added to the mixture. For verifying the selectivity of biosensor, 100 μL of three-base mismatched miRNA (M3, M31 and M32) or non-complementary miRNA (NC) in the concentration of 250 nM was added to the mixture. After each step, the fluorescent emission spectra (excitation at 490 nm) of each well were recorded across the range of 510 nm-640 nm. During the measuring process, the fluorescent emission intensity at λ=520 nm of each well was measured every 5 minutes.

Molecule dynamic stimulation

Single-layer black phosphorus nanosheet (SL-BPNS, 7.282 nm ×7.884 nm × 0.628nm) with 1584 P atoms and an FAM-labeled ssDNA (with the sequence of pDNA) were immersed in a periodic water box with dimensions of 7.282 nm × 7.884 nm × 7.000 nm. Simultaneously, the BPNS and pDNA/miRNA duplex were immersed in a periodic water box with same parameters. In the box, there were approximately 40753 water molecules and 23 sodium ions that neutralized the ssDNA and duplex. The phosphorus atoms were modeled as uncharged Lennard–Jones particles[2]. The conformation and the parameters of the FAM molecule and BP crystal were obtained from ACPYPE[3] and COD(Crystallography Open Database), respectively. While the parameters for ssDNA and duplex were directly obtained from the AMBER ff14SB forcefield[4]. The TIP3P water model was employed in our simulation. All simulations were carried out at a constant pressure 1 bar with a constant temperature (300 K) via Gromacs 5.1.4[5] and with the Particle Mesh Ewald (PME)[6] method for full electrostatics with a cut-off of 1.2 nm. A time step of 2 fs was employed and data were collected every 1 ps. First, we performed MD simulation for 500 ps for the system of the ssDNA or duplex on the BPNS to obtain the initial conformation. Then a 30 ns MD simulation were done for the whole system. BPNS was fixed throughout the simulation. GROMACS routine utility scripts and PLUMED 2.4[7] were exploited to analyze the simulation trajectories. All structure models and snapshots were visualized using VMD[8]. The videos, which illustrated the interaction processes between BPNS and ssDNA or duplex, were created by VMD using 600 snapshots for each.

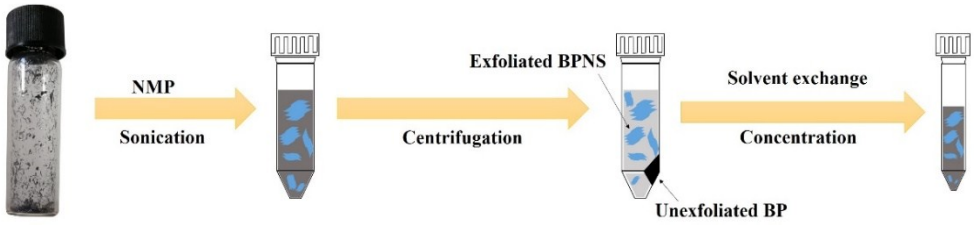

Fig.S1 Schematic illustration of BPNSs preparation procedures

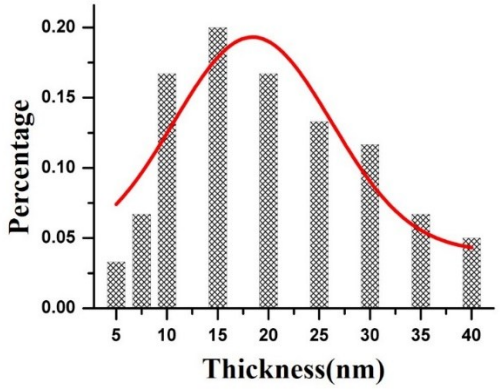

Fig. S2 Statistical analysis of the heights of 60 BPNSs determined by AFM. The average thickness of BPNSs is 18.4±1.5 nm.

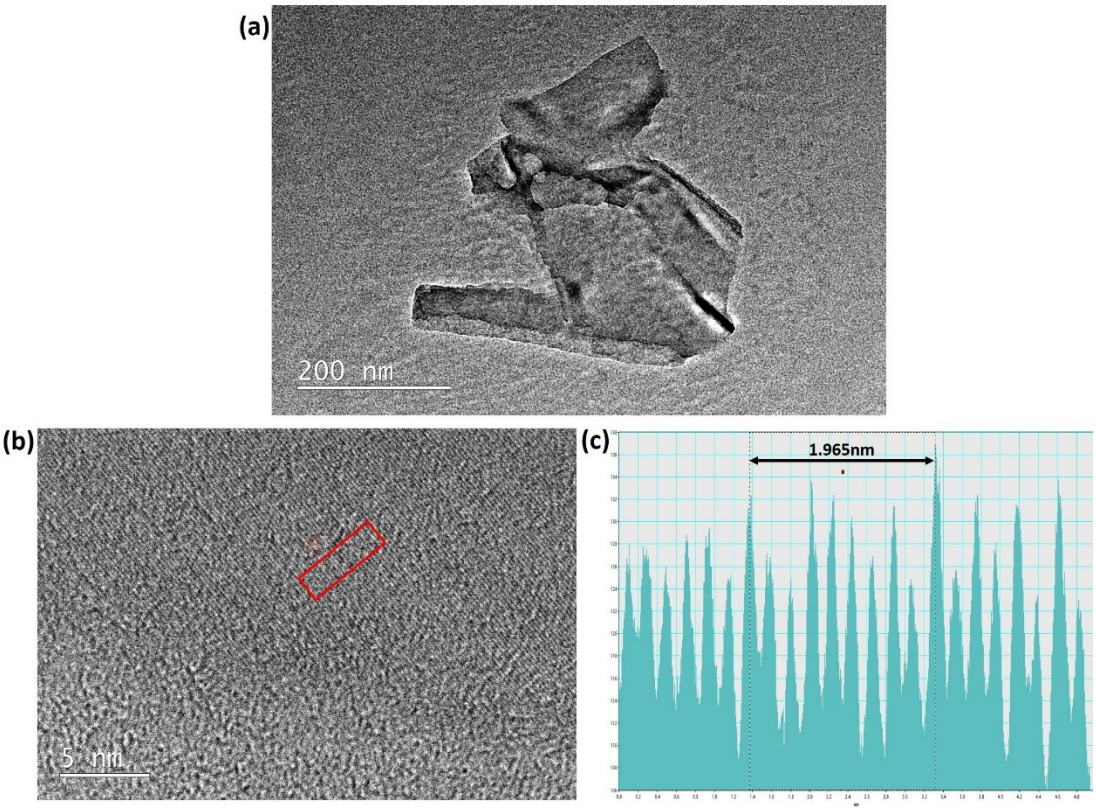

Fig. S3 The HRTEM images of BPNSs displays lattice fringes of 0.217 nm, which can be ascribed to the (002) planes of black phosphorous

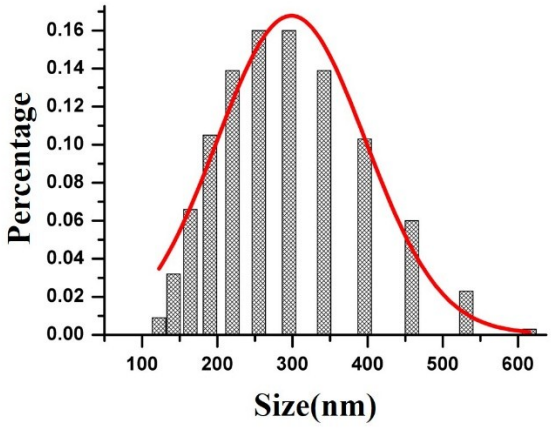

Fig. S4 The hydrodynamic size distribution of BPNSs. The average diameter of BPNSs is 298.4±6.7 nm.

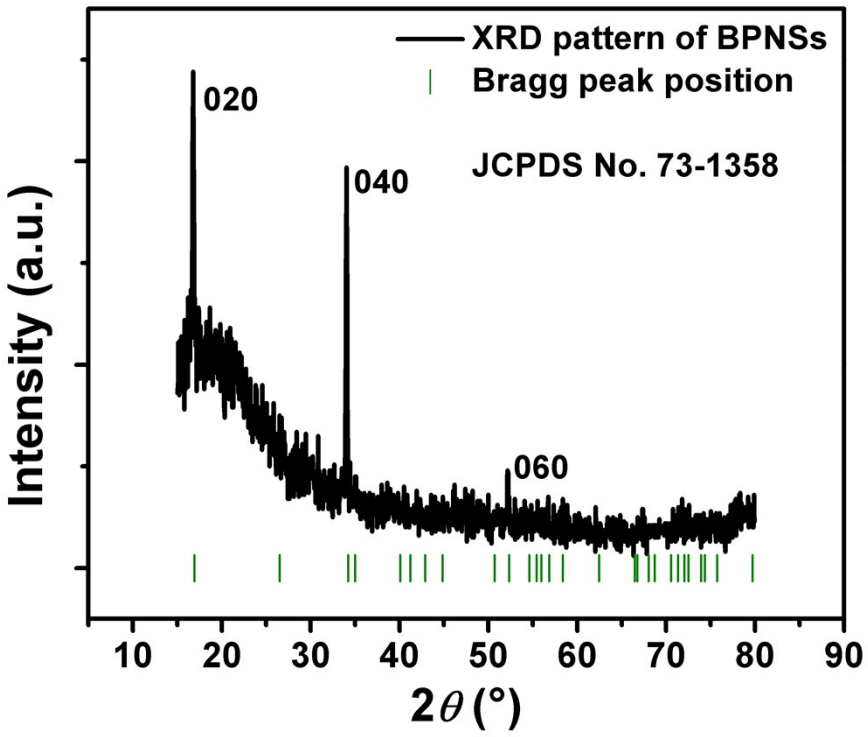

Fig. S5 The XRD pattern of BPNSs. The reflective patterned peaks are located at 2θ= 16.78°, 34.03° and 52.18°, which are attributed to the (020), (040), (060) diffractions, respectively, and is consistent with that of standard orthorhombic black phosphorus (JCPDS No.73-1358).

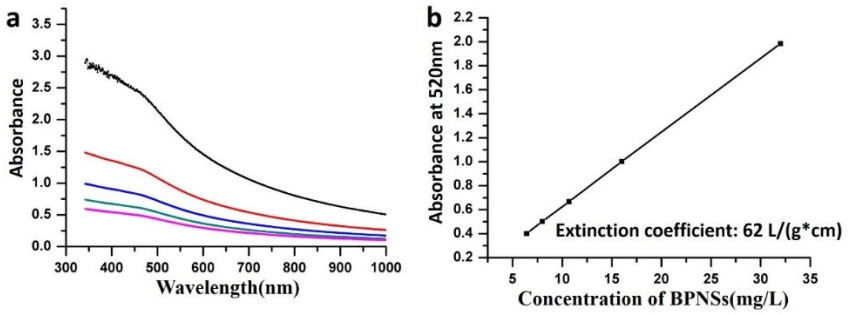

Fig.S6 (a) The absorption spectra of prepared BPNSs solution and its dilutions in the range of 340 nm-1000 nm. (b) The absorbance at wavelength λ=520 nm verse the concentration of BPNSs. The concentration of prepared BPNSs solution was 34μg/ml, which was determined by ICP-AES.

Optimization of analytical conditions

The concentration of pDNA and BPNSs were optimized to improve the performance of the biosensor. The tested pDNA concentration varied from 25 nM to 100 nM, and three concentrations of BPNSs (500μg/ml, 200μg/ml and 80μg/ml) were compared. Fig.S5a shows the fluorescence spectra of pDNA in the presence of BPNSs at different concentrations (500μg/ml, 200μg/ml and 80μg/ml), illustrating that the quenching efficiency of BPNSs for pDNA become higher as the concentration of BPNSs increased. It possibly be attributed to the increased amount of pDNA absorption on BPNSs. The results for miRNA detection were presented in Fig.S5b-d. When the concentration of BPNSs was 500μg/ml, there was no obvious linear interval for miRNA detection. Fig.S5c shows that there is a linear relationship between $\triangle F$ and C_{miRNA} in the concentration range of 10 nM to 1000 nM for 25 nM pDNA, 125 nM to 1000 nM for 50 nM pDNA and 250 nM to 1000 nM for 100 nM pDNA when the concentration of BPNSs was 200 μg/ml. While, when the concentration of BPNSs was 80μg/ml, the detection range for miRNA narrowed down to 250nM to 1000 nM for 50 nM and 125 nM to 1000 nM for 25 nM pDNA (Fig.S5d). In order to get the widest detection range, 25 nM of pDNA and 200 μg/ml of BPNSs were employed for miRNA detection.

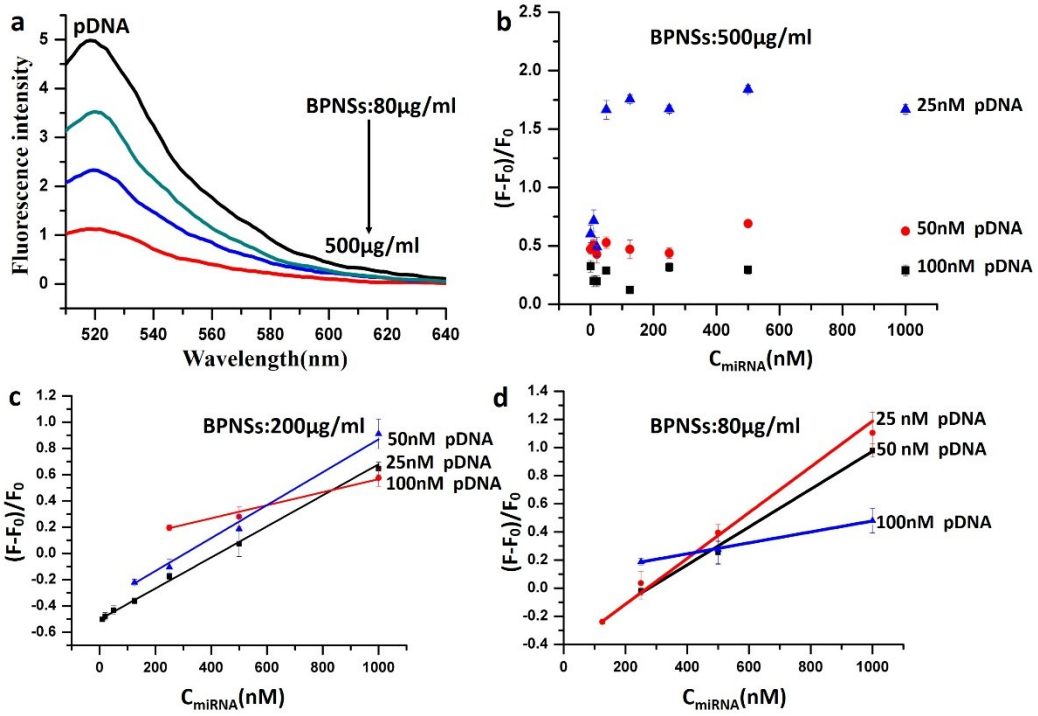

Fig. S7 (a) The fluorescence spectra of pDNA in the presence of BPNSs at different concentrations (500μg/ml, 200μg/ml and 80μg/ml). (b) The fluorescence intensity changes △F versus the concentration of target miRNA (C_{miRNA}) when the BPNSs concentration was 500μg/ml. (c) The calibration curve of △F versus C_{miRNA} when the BPNSs concentration was 200μg/ml. (d) The calibration curve of △F versus C_{miRNA} when the BPNSs concentration was 80μg/ml. The curves represent the average values of three wells with error bars showing the standard deviation of three wells at each condition.

Additional specificity test

We also tested the responses of biosensor to the "target mixed with M3" and "target mixed with NC". The results are depicted in table 1. In our experiments, the detected concentrations of "250 nM target", "250 nM target mixed with 100 nM M3" and "250 nM target mixed with 100 nM NC" are calculated by the linear equation of biosensor. It can be seen that the accuracies are in the range of 90% to 110%, which demonstrated that the signal of target miRNA remains almost the same in the presence of other miRNAs. The results further verify the specificity of the biosensor.

Table 1 The responses of biosensor to mixed samples

samples	detected concentration	accuracy
250 nM target	267.18 nM	106%
250 nM target+100 nM NC	241.52 nM	97%
250 nM target+100 nM M3	230.77 nM	92.30%

Reference

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