Supporting Information

A novel high-sensitivity electrochemical sensor for cancer-cell detection by means of phosphorene functionalized by Sialic-Acid Bio-molecules

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Synthesis of phosphorene sheets:

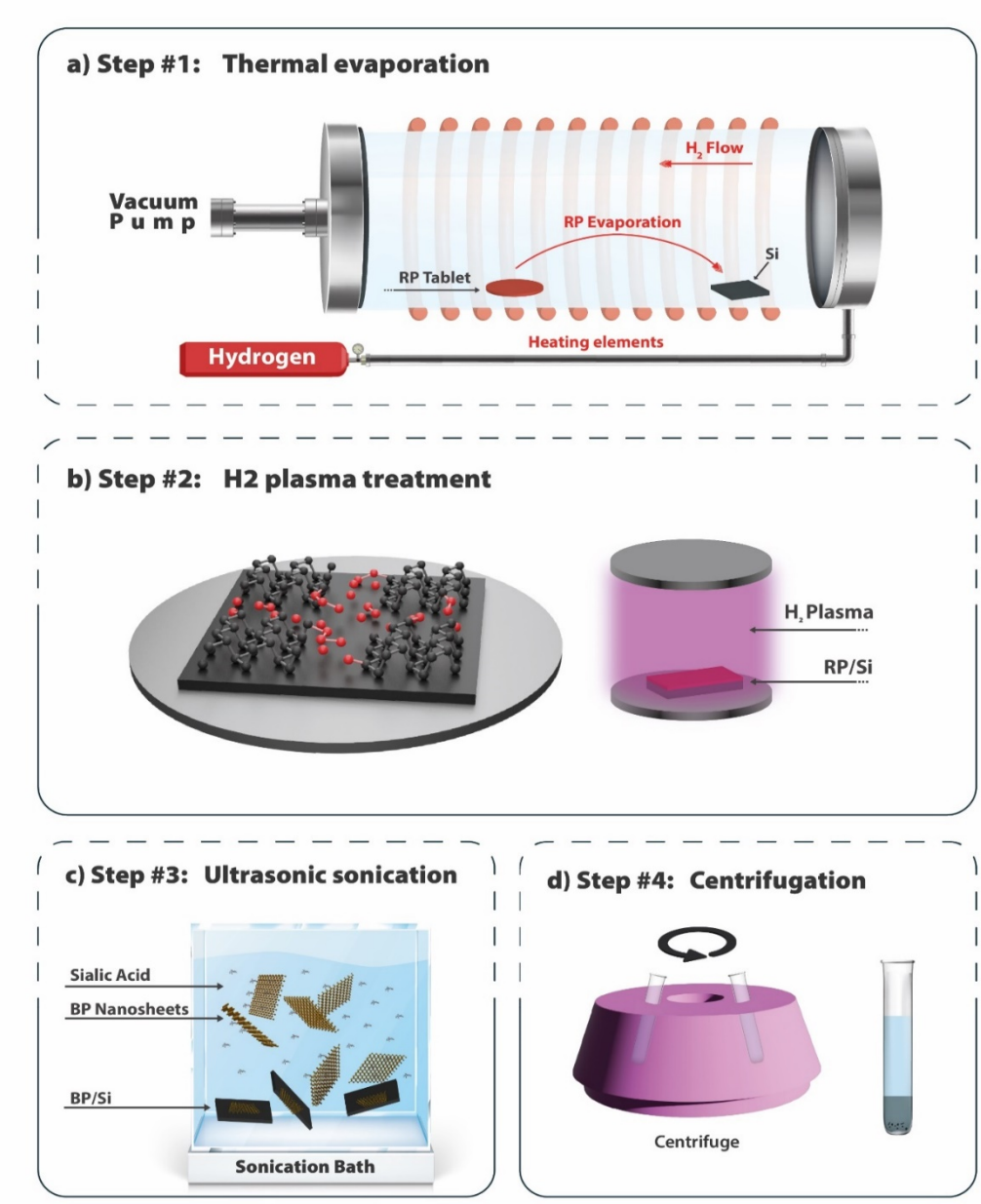

Figure S 1. Preparation and functionalization steps: The schematic drawing of (a) deposition and growth reactor, containing a hot zone where red phosphorus is evaporated and the cold zone to deposit the amorphous film on the silicon substrate. (b) The plasma treatment at an elevated temperature of 450^{o}C using hydrogen plasma, (c) immersion of phosphorene-coated Si substrates in sialic acid solution and finally, (d) centrifugation to separate the immersed sheets from thick or bulky structures[1].

To investigate the evolution of nanosheets and the effect of bio-molecule passivation on the structure of the phosphorene:

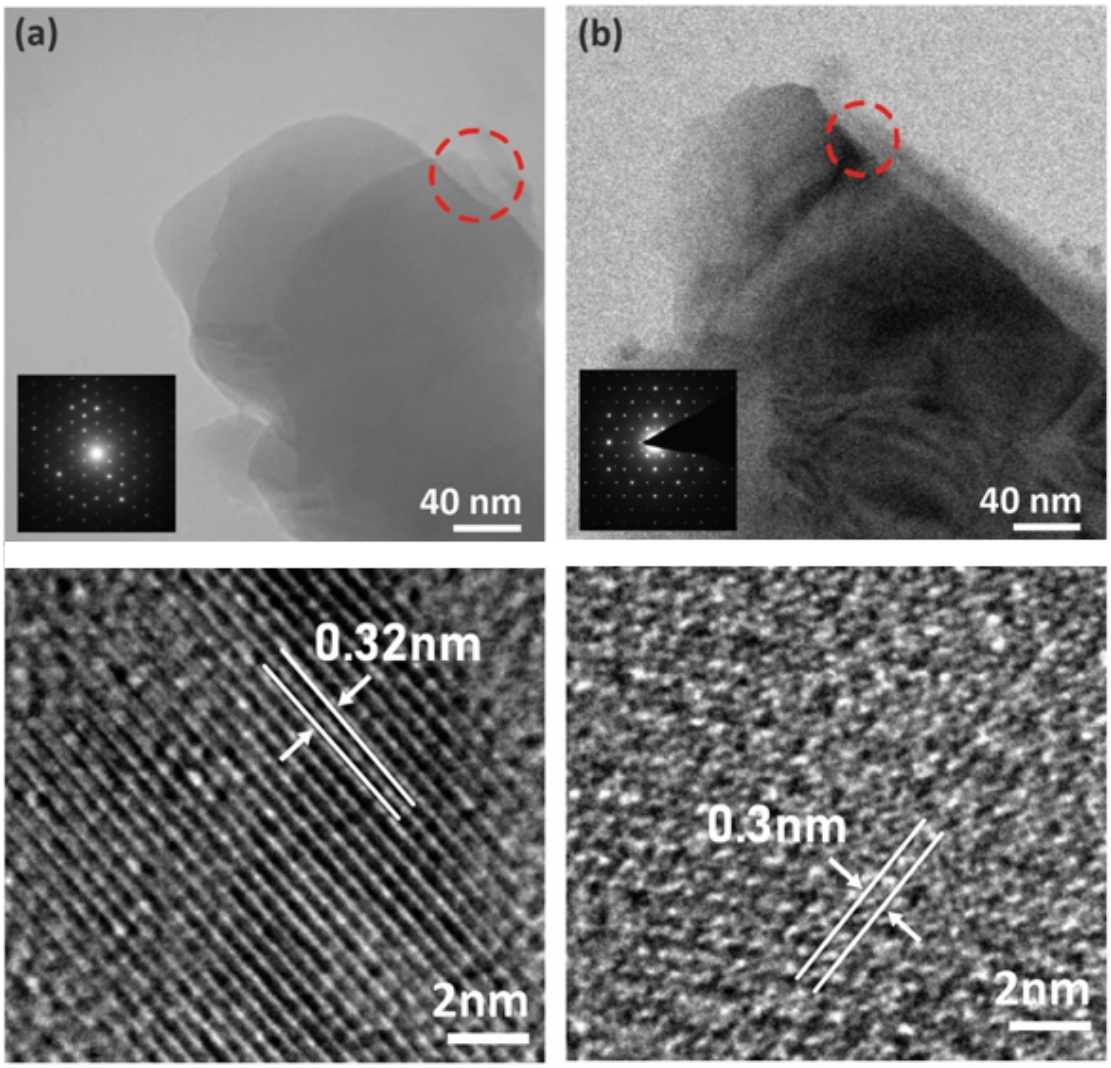

Figure S 2. Electron Microscopy analysis: TEM (a) images of pristine phosphorene structures, indicating their lattice spacing as well as layered structure. (b) The TEM images corresponding to SA-treated phosphorene sheets. The high-resolution image demonstrates the evolution of lattice fringes with a slightly smaller spacing compared to the pristine features. While the pristine (untreated) BP sheets show a spacing of 0.32 nm, the SA-functionalized sheets possess a spacing of 0.3 nm. We believe the attachment of these bio-molecules to the phosphorus atoms would induce strain in a direction to shrink the spacing. The spotty surface of the SA-treated specimen is believed to be due to the high coverage of P-atoms, functionalized with biomolecules[1].

Toxicity analysis:

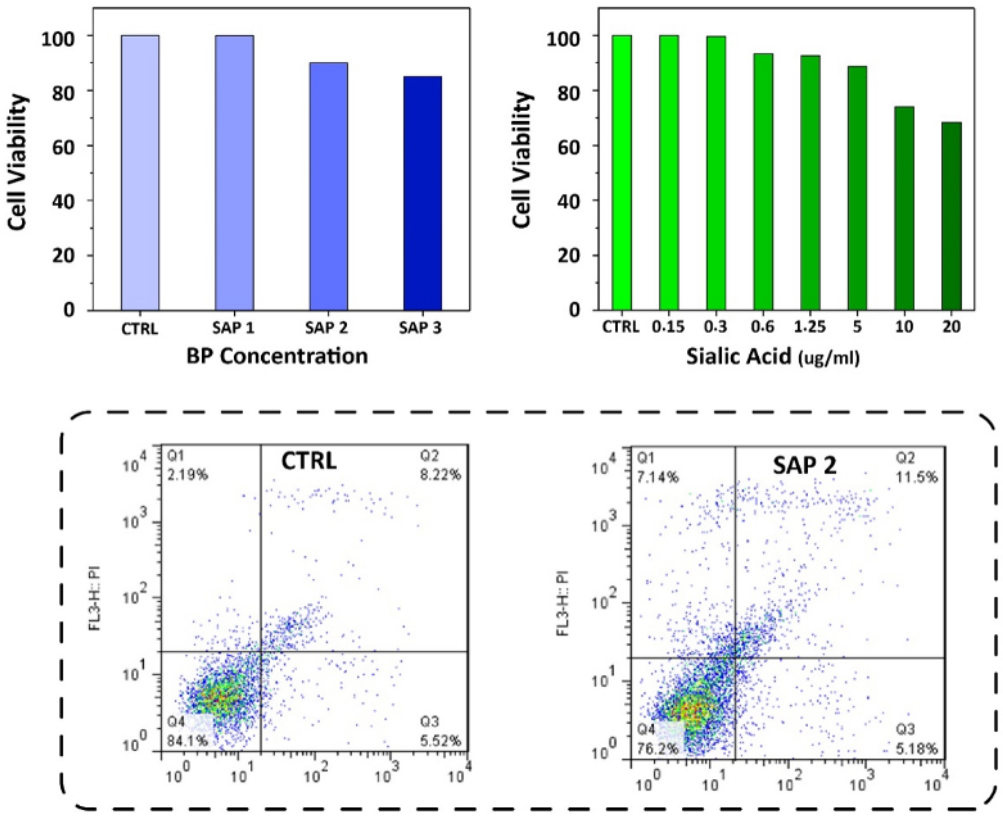

Figure S 3. Toxicity assays: cytotoxicity assays of Sia-passivated sheets on MDA-MB-231cell line. (a) the results of MTT assays on cell viability for different concentrations of SAP. By raising the concentration, one can see a drop in viability to 72%. (b) Similar investigations with varying percentages of BP concentrations indicate a reduction in viability at higher values of BP. (c) flow cytometry data showing little toxicity once comparing the control data with the Sia-passivated sheet exposure[1].

Table S 1. Nanosheet concentration[1]

SAP1	SAP2	SAP3
0.3 mg/mL	0.8 mg/mL	2 mg/mL

To determine nanosheet concentration, exfoliated BPNShs in ethanol and dried them in a vacuum.

Then BPNShs were weighted.

Table S 2. RSD

Modified electrodes	Cell Count (cell/mL)	Repeats			RSD (%)
		1	2	3	
A	50	1297.35`	1252.93	1371.46	4.64
B	100	1343.58	1364.90	1398.46	2.05
C	1000	2397.43	2480.76	2410.12	1.78
D	5000	2791.82	2879.88	2843.84	1.56
E	10,000	3105.5	3038.4	3106.4	1.27

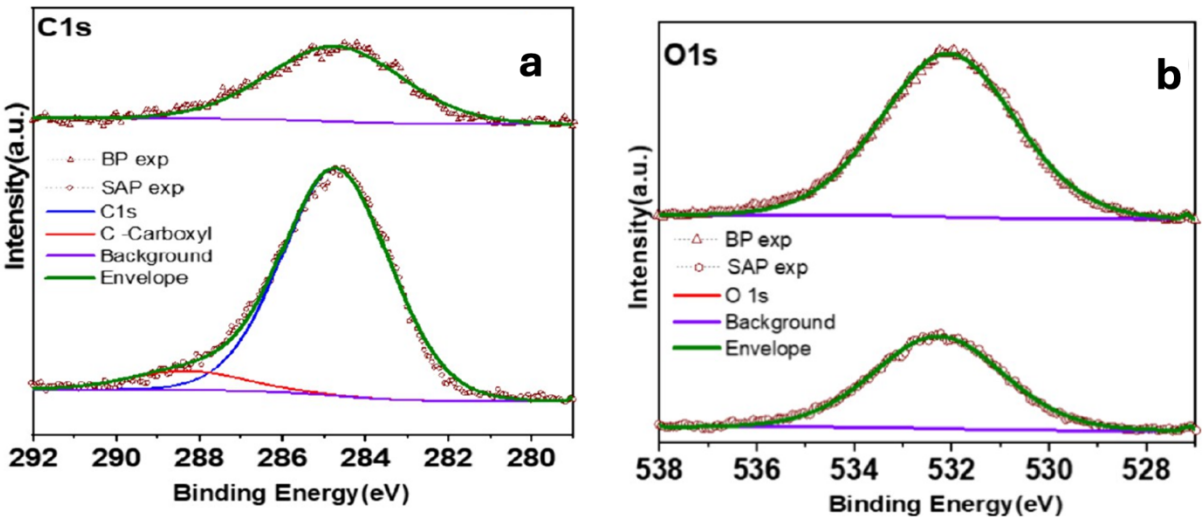

Figure S4. High-resolution spectra of (a) C1s, and (b) O1s.

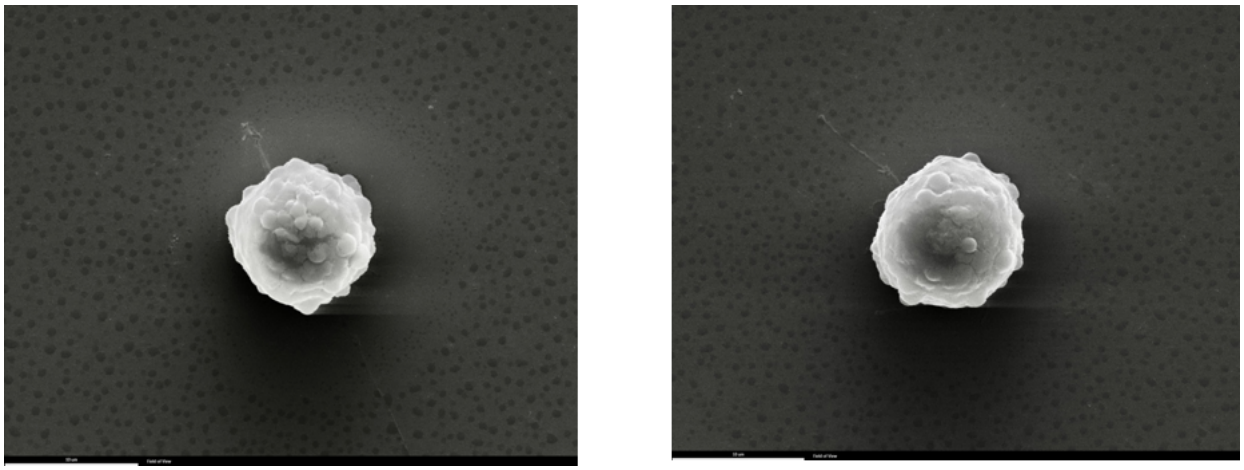

Figure S5. Cell Capturing with Bare BP

Cell Capturing with Bare BP

As a control, cell capture was tested on bare phosphorene surfaces under identical conditions. SEM images (Figure X) show negligible cell adhesion, confirming the absence of specific interactions. This is largely due to the rapid degradation of unfunctionalized BP in aqueous and ambient environments, which compromises surface stability and biorecognition. These results highlight the critical role of sialic acid functionalization in enabling both stable and selective cell attachment.

MATERIALS AND CHEMICALS

We purchased red phosphorous (RP) powder (>97%) from Merck (Hohenbrunn, Germany). SA was from the Tokyo Chemical Industry (Tokyo, Japan). MDA-MB-231, MCF-7, HT-29 (colon cancer cells), and HUVEC cell lines were from The National Cell Bank of the Iranian Pasteur Institute. These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma), 10% FBS, and 1% penicillin/streptomycin (Gibco). In this study, a 500 mM phosphate-buffered solution (PBS; pH 7.4) was used. K_3,4[$Fe(CN)_6$] was purchased from Merck (Darmstadt, F.R. Germany). Moreover, N-acetylneuraminic or SA has been obtained from Sigma-Aldrich.

APPARATUS

The materials were analyzed through morphological images obtained via transmission electron microscopy using Philips CM300 TEM equipment, scanning electron microscopy using a Hitachi S4160 FE-SEM machine equipped with energy-dispersive X-ray spectroscopy (EDX), and atomic force microscope using an NT-MDT AFM apparatus in noncontact mode with an NSG30 tip (240 kHZ resonance frequency). The optical properties of the sheets were examined with a Teksan-Opus Raman spectroscope, which uses an Nd:YAG green laser (λ = 532 nm) at a low power of approximately 7 mW. To avoid any damage to the flakes, a long work distance ×60 (LWDx60) objective lens was used.

CELL CULTURE

MDA-MB-231, MCF-7, HT-29, and HUVEC cells were cultured in Dulbecco's modified Eagle's medium containing 10% FBS and 100 μg/mL double antibiotics (penicillin-streptomycin) in a 5% CO_2 incubator at 37 °C. After culturing, the cell sediment was treated with trypsin to obtain a uniform cell suspension. The cells were then harvested and separated from the medium by centrifugation at 1000 rpm for 5 minutes and washed three times with sterile PBS. Next, the cells were dispersed in various concentrations using the cell culture media. Finally, the cell concentration was evaluated by using a Neubauer cell counting chamber.

FLUORESCENCE MICROSCOPY CHARACTERIZATION

BP-SA covered the well substrates of some 96-well plates for cell imaging. Then, 100 μL of the MDA-MB-231 and MCF-7 cell suspension (10^4 cells/mL) was put in the wells and incubated in 5% CO_2 at 37 °C for 60 min. After washing the substrates with PBS to remove the free cells, the cells captured by the nanomaterial were stained with AO. Cells bound on the nanomaterial were observed under an ultraviolet (UV) light excitation state and light field using a fluorescence microscope.

SEM images OF CELL CAPTURE

For SEM images of cells, the BP-modified substrate was immersed in SA solution. Then, the MDA-MB-231 and MCF-7 cell suspensions were dropped on the substrate, and the cells were incubated in 5% CO_2 at 37 °C for 60 min. After PBS washing of the substrates, the cells captured by the nanomaterial were fixed with %2.5 glutaraldehyde (GA) and dehydrated by ethanol. Cells bound on the nanomaterial were observed under an electron beam using SEM.

ELECTROCHEMICAL MEASUREMENT

All EC measurements were performed on an SP100 Zive potentiostat/galvanostat/impedance analyzer using an as-fabricated electrode. The modified WE was exposed to 80 μL of cell suspensions at 37 °C for 1h and then washed with PBS to remove unbound cells. EIS was used to analyze the frequency ranging from 0.1 to 100 KHz in PBS with 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1).

COMPUTATIONAL METHOD

Density functional theory (DFT) calculations were performed within the framework of the projector augmented-wave formalism [2] as implemented in the Vienna ab initio simulation package [3]. Structural relaxations and electronic properties of phosphorene were achieved by using the Perdew-Burke-Ernzerhof (PBE) of the generalized gradient approximation [4]. An energy cut-off of 500 eV was used for plane-wave basis expansion. All the structures were relaxed until the energy and force on each atom became smaller than 10^{-5} eV and 0.01 eV/Å, respectively. The Brillouin zone was sampled by 6 × 6 × 1 and 12 × 12 × 1 k-grid meshes for geometry optimization and electronic property calculations, respectively. To avoid the interaction between different imaging structures due to periodic boundary condition and electrical polarization, a vacuum larger than 20 Å were applied. To model molecular adsorption on phosphorene monolayer, a 6 × 7 supercell comprised of 168 phosphorus atoms was constructed. Because this system was dealt with periodic boundary conditions, this supercell size ensures that the minimum separation distance between neighboring periodic images is larger than 10 Å.

References:

1. Khazamipour, N., et al., *Linker-free Functionalization of Phosphorene Nanosheets by Sialic Acid Biomolecules.* Langmuir, 2024. 40(13): p. 7067-7077.
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3. Kresse, G. and J. Furthmüller, *Efficient iterative schemes for ab initio total-energy calculations using a plane-wave basis set.* Physical review B, 1996. 54(16): p. 11169.
4. Wang, Y. and J.P. Perdew, *Correlation hole of the spin-polarized electron gas, with exact small-wave-vector and high-density scaling.* Physical Review B, 1991. 44(24): p. 13298.