Supporting Information

Ultrasensitive Detection of Aromatic Water Pollutant Through Protein Immobilization Driven Organic Electrochemical Transistors

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Supplementary Text

Reduced GO (rGO) as an Alternative MopR Immobilization Platform

The immobilization of MopR protein on reduced graphene oxide (rGO) surface was also tested for a better assessment of the immobilization driven sensor performance. GO nanosheets are chemically reduced to get rGO by the L-ascorbic acid soft reduction method[1] and the characteristic XRD peak is found at 25° of 2θ value (Fig. S14A). The TEM analysis confirms the formation of thin layered rGO flakes (Fig. S14B). The ATPase activity of MopR for phenol detection, when immobilized on the rGO strips of equivalent concentration, is lesser by around 10% margin (Fig. S15A). Though some of the previous literature has demonstrated that protein immobilization and loading were improved upon increasing the reduction extent of GO,[2] however, the observation in our case seems to differ. Protein anchoring on GO nanosheets through physical adsorption involves a combination of hydrophobic and electrostatic interaction and the activity of the biomolecule is influenced by various factors such as protein shape, surface charge distribution, location of activity pocket, etc.[2,3] In the case of MopR, the protein favours the GO surface and increase in the hydrophobic nature of the nano-structures (like in case of rGO) intervenes with the conformation of the protein, thus lowering the activity towards phenol detection. This was also validated through the CD spectra where it was noticed that the loss of the structure for rGO was higher than GO as understood from the reduction in the ellipticity at 210 and 222 nm (Fig. S15B). When the rGO was coated on the gate and protein immobilization was done, the sensitivity of the OECT was reduced by 8-10% in the 0.02-1.25 µM phenol concentration region (Fig. S15C) and even higher (~40% reduction) in the higher analyte range.

Supplementary Methods

Protein Purification

MopR protein purification is carried out by isolating and cloning the genomic DNA of *Acinetobacter calcoaceticus NCIB8250* strain. The gene coding for the $MopR^{AC}$ construct (500 amino acid) was cloned into a modified pET vector and transformed into *E. coli* BL21(DE3) pLysS cells (Sigma-Aldrich, Cat. No. 69451-M) for purification purpose. Big volume culture of the bacteria (5 □ 1 liter) in presence of suitable antibiotics (Kanamycin and Chloramphenicol [MP Biomedicals], concentrations 35µg mL^{-1} and 30µg mL^{-1} respectively) are grown at 37 °C till the OD_{600nm} reached 0.6 at constant shaking (200 RMP). For induction purpose the growing temperature was reduced to 16 °C and 0.8 mM of IPTG (isopropyl-β-D- thiogalactopyranoside [MP Biomedicals, Cat. No. 02102101-CF]) and 5 mM $MgCl_2$ were added and the cultures are further grown for 12 hours (overnight). The protein purification was performed using Ni-NTA (Qiagen, Cat. No. 30210) affinity purification protocol. The buffer composition for the lysis, wash, and elution are following; lysis buffer: 50 mM Tris-HCl (pH 7.5), 5 mM Imidazole, 350 mM NaCl; wash buffer: 50 mM Tris-HCl (pH 7.5), 20mM Imidazole, 200 mM NaCl; elution buffer: 50 mM Tris-HCl (pH 7.5), 50-500 mM Imidazole, 150 mM NaCl. Desalting of the protein was done through EconoPac 10DG (Bio-Rad, CA, USA) column by equilibrating in desalting buffer (25 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2% glycerol). The desalted protein fractions were flash-frozen in liquid nitrogen

and stored at −80 °C. The purity of the protein was verified by running a 10% SDS-PAGE followed by Coomassie Blue (HiMedia, India) staining.

Colorimetric ATPase Assay for Phenol Detection

The detection of phenol in water samples is done by previously optimized ATPase assay.[4] Briefly, as per the biochemical reaction of MopR, phenol binding/recognition in sensor domain activates it and induces ATP hydrolysis activity (schematic at Fig. 1E). The phosphate arising from the hydrolysis activity is detected by the addition of malachite green dye in presence of ammonium molybdate. Finally, the absorbance of the solution is monitored at 630 nm and the value with respect to the blank (sample containing no phenol) is regarded as the biosensor activity. 10 mM of phenol prepared in MiliQ water, 10X ATPase buffer (200 mM Tris, 1M NaCl, 50 mM $MgCl_2$), 0.5 M EDTA, 20 µM protein ($MopR^{AC}$) and 20 mM ATP (Sigma-Aldrich, Cat. No. A2383) is used as stock for the assay. The malachite green dye (Himedia) solution was prepared in the following way; 13 mL of concentrated H_2SO_4 solution was diluted to a total volume of 100 mL and 0.14 gm of malachite green dye was added to it; the solution was then stirred at RT at 100 RPM, filter sterilized and stored. Then phosphate reaction dye (PDR) was prepared on the day of the assay by adding 2.5 mL of 7.5% ammonium molybdate (Himedia, Cat. No. GRM1018), 0.2 mL of 11% Tween 20 to 10 mL of dye solution.

After preparation of the protein coated GO strips, water samples containing the different desired concentration of phenol (2 µL volume) is added on it along with 1 mM of ATP and 1X ATPase buffer to make a total reaction volume of 20 µL. The strips were then kept at 37°C for 20 mins for the reaction to undergo. After incubation, 2 µL 0.5 M EDTA (Himedia) was added to quench the ATPase reaction. The strips were then transferred to 1.5 mL micro-centrifuge tube, 778 µL of water and 200 µL of PDR were added to make up the volume till 1 mL. The colour change was noticed instantaneously and the absorbance values were recorded at 630 nm with respect to the blank (no phenol). The absorbance values are converted to % by considering the highest value as 100% for a set of reactions and represented as 'Percentage (%) of ATPase Activity'. The comparison of the ATPase activity of the MopR coated GO strips and the pure protein activity are compared for efficiency testing. The activity of the solution version protein for phenol detection is set at 100% and the rest are compared accordingly. For the simulated wastewater samples (SWW) a set of different pollutants are mixed along with phenol and the interference was probed. SWW1 – 1 µM phenol + 1 µM [catechol, 2,3-DMP and hydroquinone]; SWW2 – 1 µM phenol + 5 µM [catechol, 2,3-DMP and hydroquinone]; SWW3 – 1 µM phenol + 10 µM [catechol, 2,3-DMP and hydroquinone].

Synthesis of Graphene Oxide (GO)

The graphene oxide is synthesized by modified Hummers' method. For that, 0.25 g of graphite (Sigma-Aldrich, Cat. No. 282863) and 0.4 g of $NaNO_3$ were taken in 12.5 mL of concentrated H_2SO_4 and stirred in an ice bath for 15 min. Then 4.0 g of $KMnO_4$ was added in small portions to obtain a greenish purple suspension followed by stirring for 6 h at 35 °C. The dark brown colour paste was diluted up to 100 mL with the slow addition of deionized water (DI) and sonicated for 20 min. 2 mL of 30% H_2O_2 was added dropwise to quench the solution. Then the golden-brown solution was centrifuged and washed with lukewarm DI water several times to adjust the pH to ∼6. Finally, the product was air-dried at 40 °C for 24 h. The product obtained was dispersed in water by 30 min sonication followed by centrifugation at 3000 rpm for 15 min. The supernatant obtained was collected as GO.

Electrical Characterization and Device Operation

Electrical measurements of the OECT are done by using a Keithley 4200A Parameter Analyzer. A Polydimethylsiloxane (PDMS) (Sigma-Aldrich, Cat. No. PHR-1518) well mould is used as a reaction medium container covering the area of the gate and channel (as shown in Fig. 3A). 1X ATPase buffer of the protein is used as the electrolyte for the biosensing reaction. The drain current (I_D) is monitored continuously in a chronoamperometric titration fashion at constant V_D (-0.7V) and V_G (+0.4V) while synthetically prepared phenol solutions are added in the medium in the increasing concentration

order. The concentration dependent I_D response vs. time plot is converted to a sensitivity plot to decipher the efficiency and the dynamic range of the device. Herein, sensitivity = $(I-I_n)/I = \Delta I/I$; I – saturated base current value (I_D) at the starting of the device, I_n – current value (I_D) at the n^{th} concentration added to the device. Similar embodiments are carried out in the absence and presence of ATP in order to demonstrate the sensitivity and detection operation of the phenol biosensing OECT. According to the design principle in absence of the ATP, the biochemical reaction will not occur keeping the effective gate voltage same; thus, the I_D will not get affected significantly. Selectivity of the OECT in case of the aromatic pollutant detection is an important figure of merit as many of them share similar structures. A series of different aromatic pollutants like – 2,3-dimethylphenol (2,3-DMP), catechol, hydroquinone, 2,4-dicholorophenol (2,4-DCP), and benzene is prepared in a similar concentration range and tested on the device. For the mixture of pollutants experiment, 2,3-DMP, catechol and phenol are mixed in equal proportion such that the concentration of each compound is same as that of the tested pure phenol sample (like 0.02 µM, 0.15 µM, 1.25 µM, and so on). The mixture sample is added to OECT biosensor and the sensitivity is compared with the pure phenol samples. For testing of the real environmental samples, water sample from Vihar Lake, Mumbai, India is collected and sterilized by a 0.22 µm filter (Millipore) to remove large debris particles. The water sample is pH adjusted to 7 and then spiked with phenol accordingly for analysis.

Synthesis of Reduced GO (rGO)

The reduction of GO was typically done by adding L-Ascorbic acid (Sigma-Aldrich, Cat. No. A92902) to 200 mL of aqueous dispersion of ultrasonicated GO at room temperature with stirring speed at 500 rpm and the stirring was continued for 8 h. Then the black solid was separated by centrifugation at 12000 rpm for 15 min and the product was washed several times with water followed by vacuum drying at 40 ^oC.

Characterization Techniques

XRD patterns of synthesized GO and rGO flakes were obtained using Rigaku SmartLab high-resolution X-ray diffractometer (XRD). TEM images of the synthesized GO and rGO were acquired on Thermo Scientific, Themis 300 G3 operated at 300 kV. The specimens of TEM were prepared by placing the aqueous suspension of GO or rGO on the carbon-coated copper grids and blotted after 30 s. Atomic Force Microscopic images of GO nanosheets were taken on Bruker (Previously Veeco), Multimode Nanoscope-IV. The samples for AFM were prepared by dropping an aqueous suspension of GO on a freshly cleaved mica surface and dried under a vacuum at 40^oC. FESEM images were taken on JEOL JSM-7600F. FTIR study of GO nanosheets were performed by Perkin Elmer Spectrum ONE. Raman spectra of GO were taken on WItec Raman Alpha 300 with an excitation laser beam wavelength of 532 nm. XPS spectra were obtained using Kratos Analytical, AXIS Supra equipped with a monochromatic Al Kα X-ray source (1468.6 eV). The Gaussian method was used for the deconvolution of the spectra. The contact angle of GO on the cleaned glass slide was measured by Holmarc's Contact Angle Meter (Model No: HO-ED-M-01). CD spectra of MopR protein and MopR in presence of GO and rGO were collected by JASCO, J-815 CD spectrometer.

Circular Dichroism (CD) Experiment

The CD spectra of the MopR protein was collected in a Jasco J-815 CD spectrometer. The protein concentration used was 0.2 mg mL^{-1}. The protein was incubated with GO/rGO of 0.2 mg mL^{-1} concentration and then CD spectra was collected. Phosphate buffer (25 mM sodium phosphate pH 7.5, 80 mM NaCl) was used for preparation of the MopR. The scanning of the spectra was done by using following configuration: temperature: 20^oC, path length: 0.1 cm using quartz cuvette, differential integration time: 8 sec, scan rate: 50 nm sec^{-1}.

Supplementary Figures

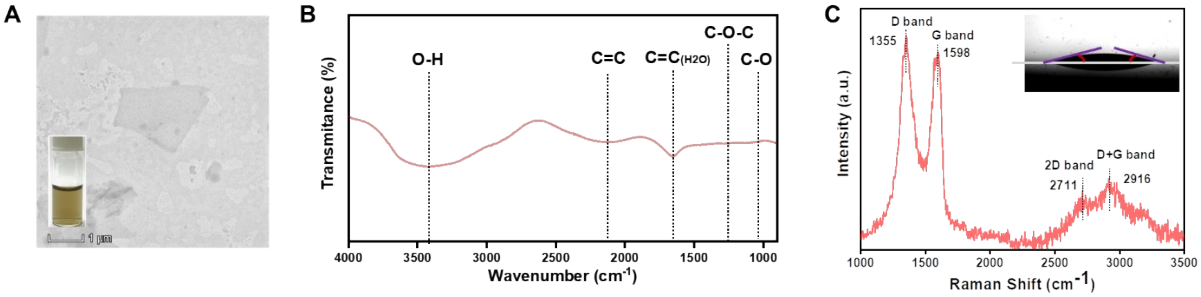


Figure S1. (A) TEM image of the GO nanosheet. Inset: aqueous dispersion of the GO. (B) FTIR characterization of the GO. (C) Raman spectra of the GO show characteristics D and G band at 1355 and 1598 cm^{-1}, respectively; Inset: The contact angle profile of GO illustrating the hydrophilic nature.

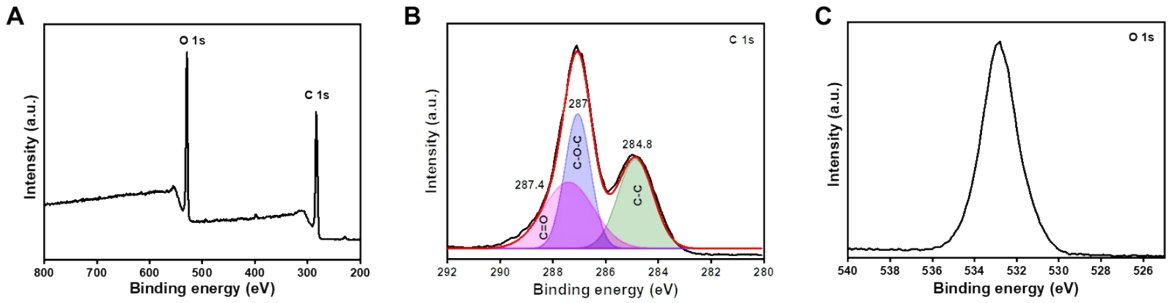


Figure S2. XPS characterization of the synthesized GO sheets. The (A) full survey scan, (B) core spectra of C 1s and (C) O 1s are shown.

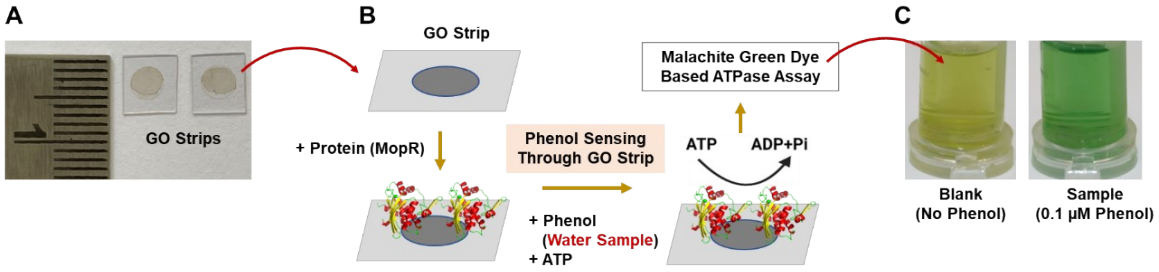


Figure S3. (A) GO-D3 coated strips for protein immobilization. (B) Schematic illustration of the MopR immobilization on the GO-D3 strips and ATPase assay. (C) Real-time colour development of the biosensor achieved through malachite green dye-based assay for phenol detection.

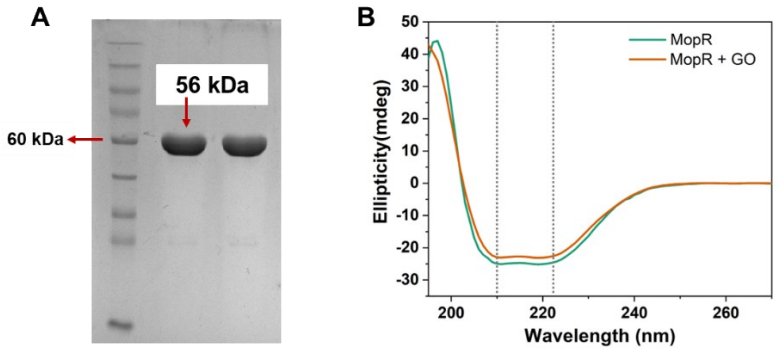


Figure S4. (A) SDS-PAGE gel of MopR protein (MopR^{AC}) of 500 amino acid length. Pure protein was observed at ~56 kDa. (B) Circular dichroism (CD) spectra of the MopR protein and MopR in presence of GO-D3.

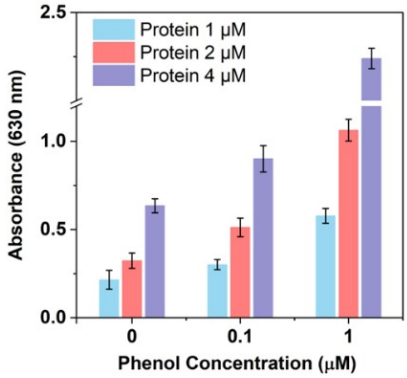


Figure S5. Phenol sensing activity of GO-D3 strips with different concentration of MopR protein immobilized.

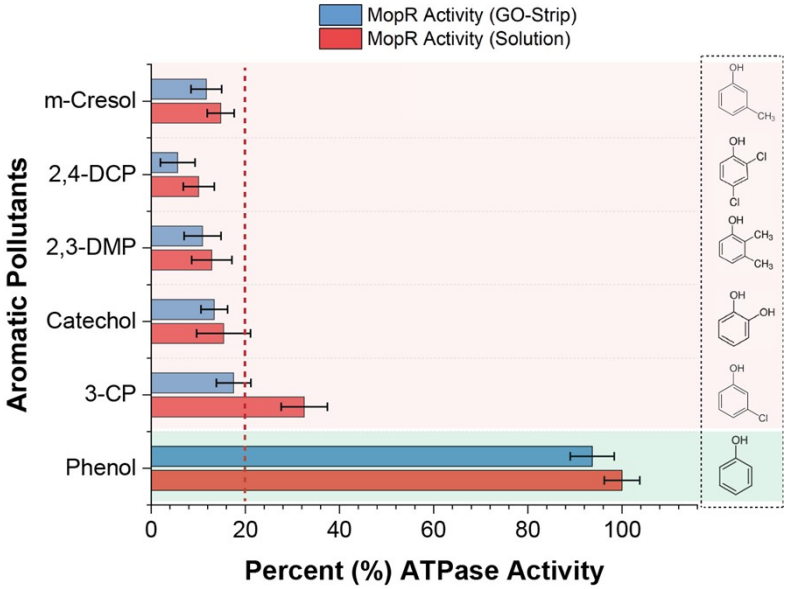
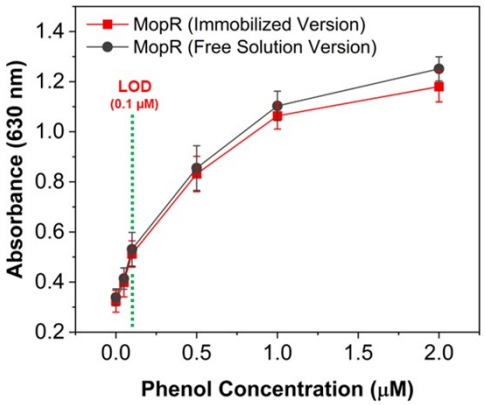


Figure S6. Comparison of the selectivity profile of the MopR immobilized GO-D3 strips and the pure protein in its *in-vitro* version. All the isostructural phenolic compounds have <20% activity (red dashed line) inferring highly selective performance of the MopR-GO-D3 nano-bio module.

A



B

Added Phenol (μM)	Detected Phenol (MopR Solution Biosensor) (μM)	Detected Phenol (MopR Immobilized Biosensor) (μM)	% Error (MopR Solution Biosensor)	% Error (MopR Immobilized Biosensor)
0.4	0.39	0.415	2.5 %	3.75 %
0.8	0.788	0.816	1.5 %	2 %

Figure S7. (A) Comparison of the MopR's activity in the 'immobilized version' (on GO-D3) along with the free 'solution version'. The protein exhibits a congruent phenol sensing response in its GO immobilized strip version. (B) Table showcasing the detected error comparison of the 'solution version' and GO 'immobilized biosensor' wherein the protein immobilized strips has very similar error in comparison to the free protein.

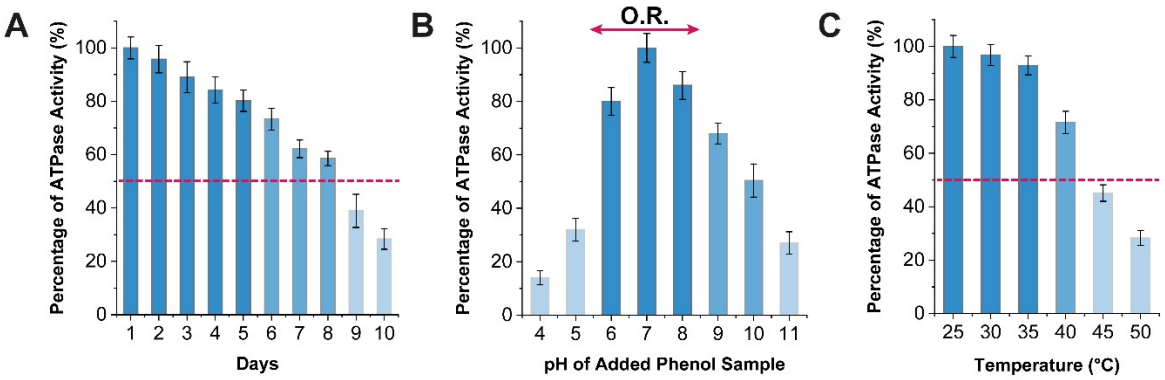


Figure S8. Stability of the MopR-GO biosensing platform. (A) Shelf-life MopR-GO strips. Around 60% of the activity is retained for 8 days (50% activity: red dashed line). (B) Phenol sensing response profile of the sensing strips for water samples having different pH. The recommended operating regime (O.R.) is mentioned. (C) Thermostability of the protein-coated strips.

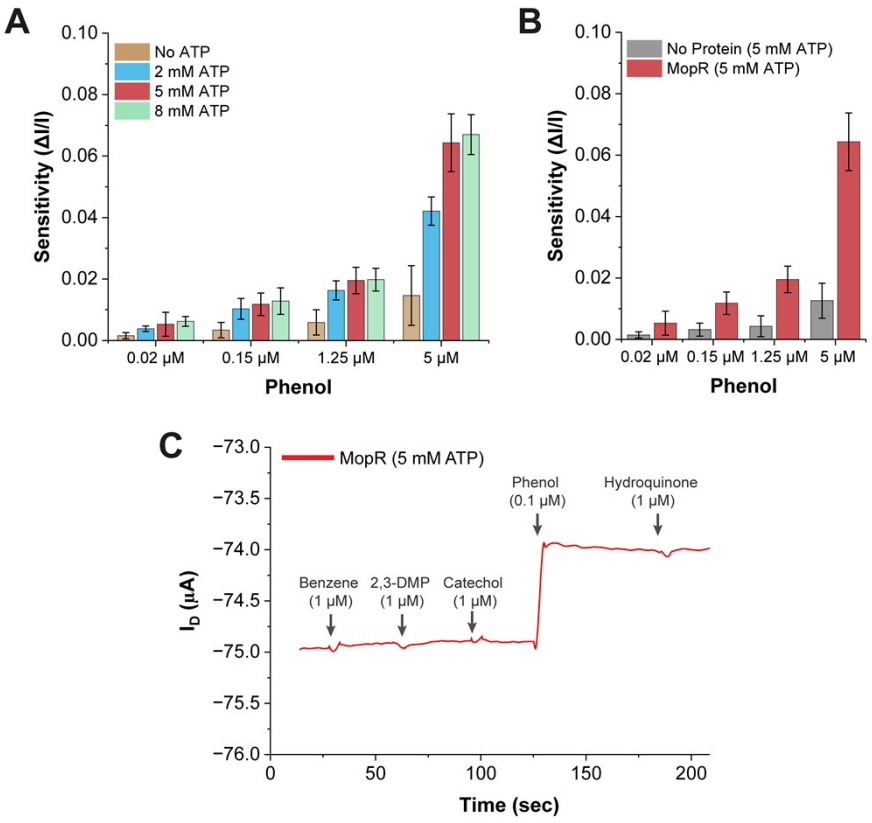


Figure S9. (A) Phenol sensitivity of the OECT device at different concentrations of ATP. (B) Sensitivity of the phenol detection response of the 'no protein' and 'protein (MopR) immobilized' device. (C) Time-channel current (I_D) response of the device for different isostructural compounds exhibiting selectivity for phenol.

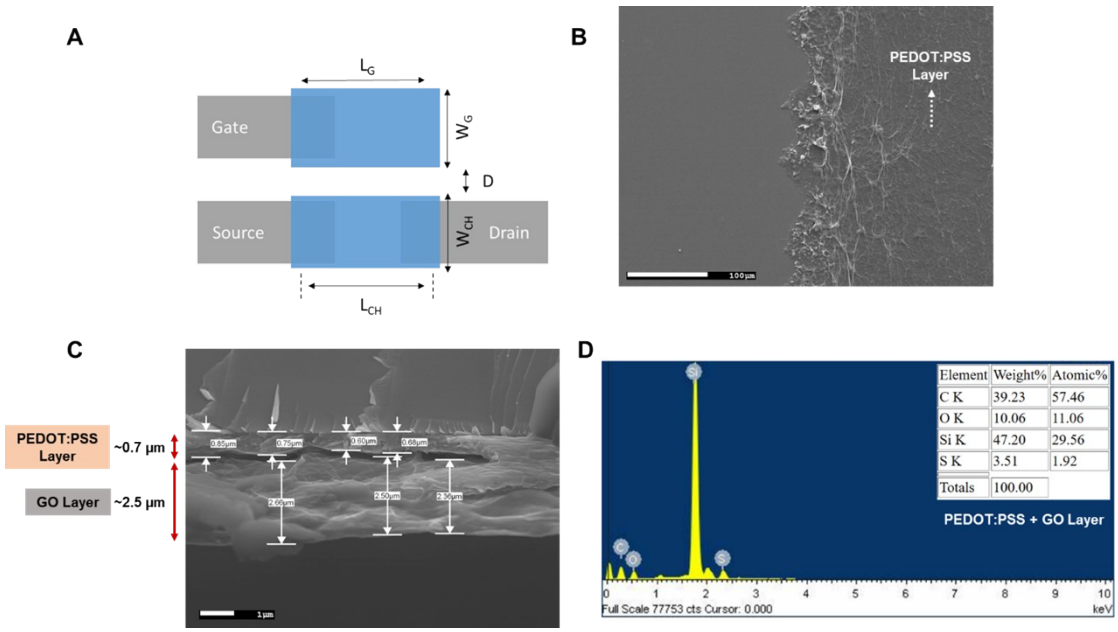


Figure S10. (A) The channel and gate geometry of the OECT. The channel: L_{CH} □ W_{CH} =8mm □ 2mm; the gate: L_G □ W_G =8 mm □ 2 mm; lateral separation: D =2 mm. (B) Top view of the PEDOT:PSS layer coated on a silicon substrate. (C) Cross-section of the GO coated PEDOT:PSS layer observed under SEM. The thickness of the PEDOT:PSS and GO layer are 0.7 μm and 2.5 μm respectively. (D) EDS spectra of the GO coated PEDOT:PSS channel layer along with the atomic composition confirming the presence of different expected elements.

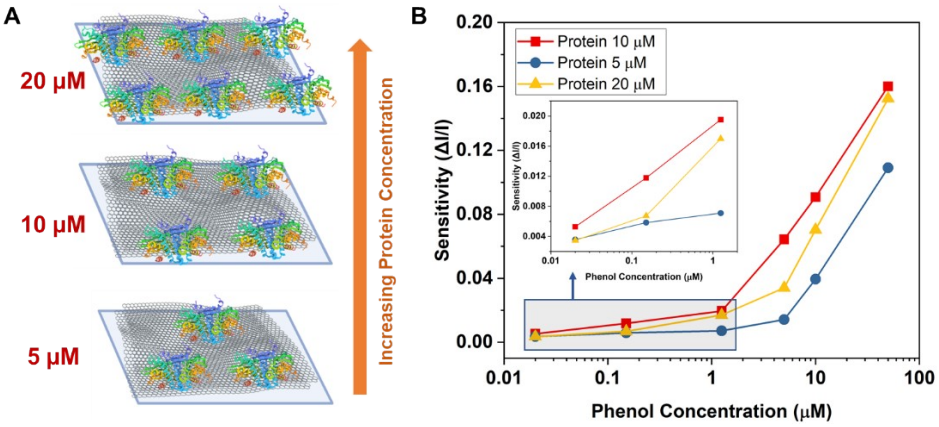


Figure S11. Optimization of the biosensing protein (MopR) concentration on the gate electrode. (A) Schematic representation of the different protein concentrations showing increasing crowding. (B) The sensitivity response of the device for different MopR concertation.

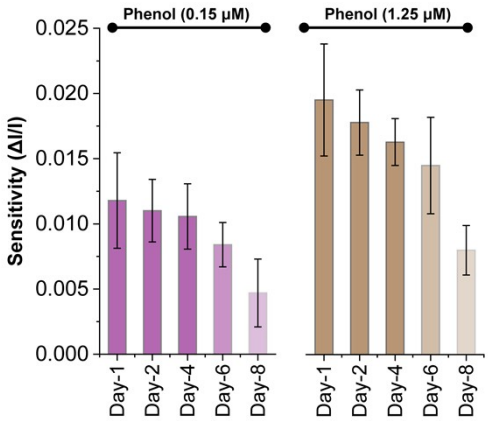


Figure S12. The phenol sensitivity response of the OECT devices at different time intervals. The protein-coated electrodes are stable for around a week.

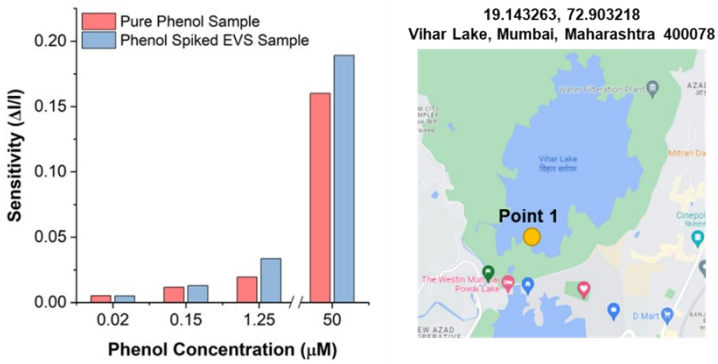


Figure S13. Real sample test on the OECT device. The sensitivity of the device for the lake water sample (referred as 'EVS') spiked with phenol and the pure water sample containing an equivalent amount of phenol is compared. The map location of the collected water sample (Vihar Lake, Mumbai, Point-1) is shown in right.

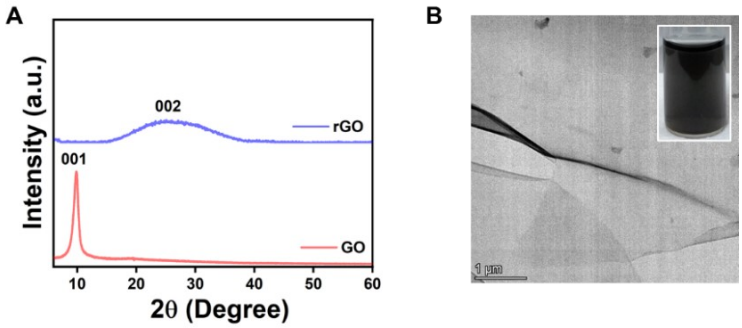


Figure S14. (A) XRD of reduced graphene oxide (rGO) is shown in comparison with the GO. The rGO samples exhibit characteristics 002 peak at 25° 2θ. (B) TEM image of the rGO. Inset: aqueous dispersion of the rGO.

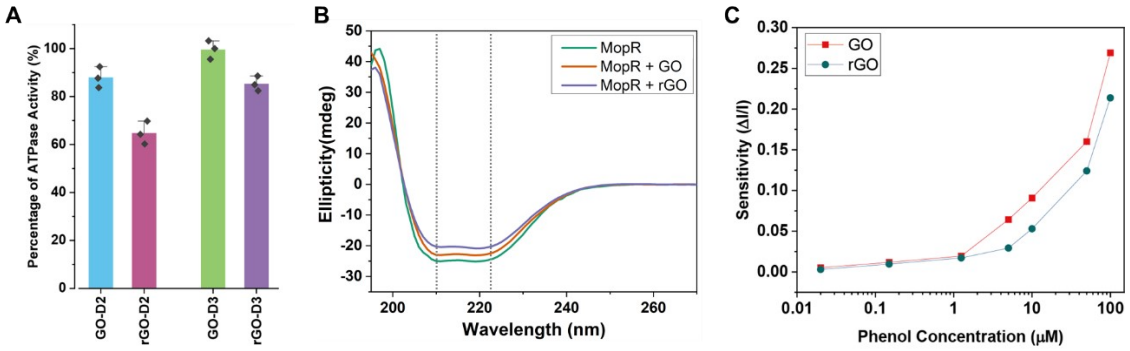


Figure S15. The ATPase activity of the MopR-rGO strips of equivalent dilution is shown in comparison to MopR-GO. The MopR-rGO biosensor strips exhibit around 15-20% lesser activity. (B) Comparative CD spectra of the MopR biosensor in presence of GO and rGO. The conformational loss of the protein was relatively higher in the case of the rGO. (C) Comparison of the sensitivity of the OECT device for phenol detection when rGO was used as a protein immobilization matrix at the gate with respect to GO.

References

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