An Enzymatic Electrochemical Biosensing Interface Developed by The Laser-Induced Graphene Electrode

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Laser-inducing provides a cost-effective, easily-manufacturable, and environment-friendly approach to directly transfer carbon-rich polymers into graphene materials, which attracts attention from various fields, such as sensors, electrocatalysts, micro-supercapacitors, etc. Laser-induced graphene (LIG) benefits from the intrinsic properties of graphene, for example, high conductivity, high electroactivity, and high specific area. In this work the potential of laser-induced graphene in constructing an enzymatic electrochemical biosensing interface is evaluated. Here, a laminar-structured laser-induced graphene material is fabricated by laser engraving with polyimide. After deposition of the electron mediator ferrocene, a conjugated enzyme complex of bovine serum albumin-glucose oxidase (BSA-GOx) is modified on the laser-induced graphene by cross-linking. The fabricated glucose oxidase/ferrocene/LIG (GOx/Fc/LIG) biosensor achieves high sensitivity of 11.3 μA mM⁻¹ cm⁻², wide linear range of 0–11 mM, and low detection of limit of 0.04 μM. The LIG electrodes exhibit high flexibility with bending angle as high as 60° without observed conductivity change. The repeatability and robustness of the developed LIG biosensor in detection of real serum samples empower it with great potential in clinical implementation in the future.

1. Introduction
The increased global incidence of chronic metabolic diseases has become a serious health threat. Take the commonly seen metabolic disease diabetes mellitus as example, it is estimated that 537 million people have diabetes and over 6.7 million death attributes to diabetes-related causes in 2021 according to the IDF Diabetes Atlas 10th edition.[1] In treating and managing metabolic diseases, monitoring metabolite levels in body fluids is critical.[2,3] Enzymatic electrochemical biosensors, which have advantages in their timeliness, accuracy, and ease of miniaturization, are regarded as a promising approach to determine metabolite levels in vivo or in vitro.

Among common electrochemical analysis methods like cyclic voltammetry, square wave voltammetry or electrochemical impedance spectroscopy, the amperometric i-t test benefits from the ease of operation, fast response, and simplified peripheral circuits.[4,5] Therefore, the amperometric testing method has been widely adapted in constructing metabolite biosensors. However, those biosensors are still limited in low sensitivity and narrow detection range.[5] To improve the performance of enzymatic biosensors, high quality biosensing interfaces have been investigated for years. Materials with high surface-to-volume ratio and high conductivity are considered suitable for constructing biosensing interfaces.[6] Nanostructured-conductive materials, like conductive polymer nanoparticles, metal and metal oxide nanoparticles, were used...
to improve the performance of enzymatic biosensors.\textsuperscript{[7,8]} Materials with a high specific surface area can offer more active sites for enzyme immobilization. Also, materials with higher conductive and catalytic activities could help accelerate the charge transfer from bioactive molecules such as ascorbic acid, uric acid, dopamine,\textsuperscript{[14,15]} nucleic acids\textsuperscript{[16]} and small molecules.\textsuperscript{[17]} Laser-induced graphene meets the demands of high conductivity, high electroactivity, and high specific area to construct biosensing platforms. However, in typical laser-inducing processes of previous works, due to the significant difference in thermal expansion coefficients between the upper layer LIG and the lower layer PI, excessive reaction intensity was generated between LIG and PI substrate under focused laser conditions, leading to separation and easy peeling-off of the as-formed LIG. Besides, current laser-induced graphene suffers from incomplete graphitization, low conductivity, interface delamination, and instability. Overcoming these limitations and obtaining a highly sensitive, cost-effective, and reproducible electrochemical sensing interface through simplified and efficient processing methods and surface modification techniques, represent a technical bottleneck to address for wide application in wearable and point-of-care (POC) metabolite monitoring devices.

In this work, we have constructed a LIG-based enzymatic electrochemical biosensing interface and evaluated its performance in glucose sensing. A combination strategy of ultraviolet laser pre-treatment and infrared laser defocused processing was employed to obtain graphene with high conductivity and rich 3D porous structures. The constructed biosensor showed advantages in both easy and low-cost fabrication and high-performance sensing. The modified GOx/Fc/LIG biosensor exhibited wide linear range of 0–11 mM and high sensitivity of 11.3 μA mM⁻¹ cm⁻² in glucose sensing with a low detection of limit of 0.04 μM. The flexibility and robustness of developed LIG biosensor empowered it with great potential in the development of flexible bioelectronics and wearable medical devices in the future.

### 2. Results

#### 2.1. Design and Fabrication of GOx/Fc/LIG

To obtain high sensitivity and robust enzymatic electrochemical sensing interface with low-cost fabrication, we devised a LIG-based biosensor with conductive organic network for immobilization of bioactive enzymes and electron mediators. The structure and fabrication procedure of the GOx/Fc/LIG electrode is illustrated in Figure 1. First, we employed a combination strategy of ultraviolet laser pre-treatment and infrared laser defocused processing to fabricate highly conductive and porous graphene.
The ultraviolet laser pretreatment of PI film was introduced to increase its surface haze and roughness, thereby promoting its absorption of infrared laser. By employing defocused processing with adjustable focal length of the infrared laser, a more uniform distribution of laser energy was generated to allow LIG to have sufficient time for the “induction” effect to form a porous conductive morphology.

Second, the LIG material was modified with glucose oxidase and electron mediator ferrocene to form functional enzymatic sensing interface. Following the fabrication of bare LIG material with special intrinsic properties, a custom surface modification method was devised to obtain highly sensitive sensing interface with conductive organic network and conjugated enzymes and electron mediators. After washing, a ferrocene layer was assembled on bare LIG by immersing LIG in saturated ferrocene acetone solution. Then the protein complex (GOx-BSA) was drop-cast on the material and dried to form the GOx/Fc/LIG electrode. This enzyme immobilization method on LIG enables uniform and stable modification of electron mediators and enzymes on the laser-induced 3D porous graphene material, rather than uncontrollable deposition of noble metal nanoparticles as commonly used in LIG modification. Due to its hydrophobicity, ferrocene is incompatible with viscous liquids commonly used in enzyme modification like chitosan or Alginate. As a result, the protein-enzyme conjugate network for ferrocene modification adopted in our work is more efficient than using adhesive with poor ferrocene solubility. In our work, BSA and GOx were crosslinked on the rough material surface as a complex. The involvement of glutaraldehyde altered the properties of BSA-GOx solution and made it adherable to Fe/LIG surface. Those proteins with two-end hydrophilicity and hydrophobicity worked as surfactants and adhered to Fe/LIG surface. This surface modification method maximizes the intrinsic advantages of LIG material and helps with the following biosensing performance.

2.2. Morphology and Crystalline Structure of GOx/Fc/LIG

Figure 2a gives the TEM image of the induced graphene with previously described fabrication method. Unlike amorphous polymers, graphite materials have plane-to-plane polycrystalline structure and can be directly imaged by TEM. Clear lattice fringes with a distance of 0.33 nm can be seen in the TEM image, which matches the (002) plane-to-plane distance of graphite material of 3.35 Å.[18] The fabricated LIG material was composed of randomly shaped and oriented single crystalline graphene patches separated by grain boundaries. The ring-shaped diffraction patterns in Figure 2b also confirmed this phenomenon. The electron diffraction pattern of single crystalline graphene was a set of 6-fold-symmetric spots due to its lattice structure.[19] For the induced material which contained a large number of distinct graphene crystals, many sets of 6-fold-symmetric spots overlay together and thereby formed the ring-shaped patterns. Figure 2c gives a complete view of the PI film after laser treatment. The SEM image from Figure 2d reveals the micro-morphology of induced graphene. As can be seen in Figure 2d, e, the graphene sheets are closely stacked on each other. From the cross-section SEM image of LIG sheet, the thickness of laser-induced graphene layer was calculated as 75 μm. The square resistance of developed LIG was about 4.2 Ω sq⁻¹, which was lower than LIG developed in other works (about 15.7 Ω sq⁻¹).[20] This may be attributed to the condensed stackings and large thickness of graphene sheets. The morphology and measured conductivity indicated that the material may have high electrocatalytic activity, which made it suitable for biosensing interface development. Figure 2f shows LIG with enzymes and proteins immobilized on the surface. As illustrated, a thin layer of modification substances was deposited on the surface of LIG, and the morphology of the gully and hollows were maintained after modification. The conservation of morphology suggested the intrinsic properties of LIG of large specific areas may not be influenced by the surface modification.

It can be seen from Figure 3a that the typical D peak of LIG appeared at 1350 cm⁻¹. This peak was caused by the symmetric stretching vibration of sp2 carbon atoms and was related to the defects and disorder in the material structure. The G peak and 2D peak appeared at 1580 cm⁻¹ and 2700 cm⁻¹, respectively, which were related to the in-plane stretching vibration of sp2 carbon atoms and the stacking of graphene layers.[21] The ratio of D peak to G peak (ID/IG) was 0.67, which further indicated the high degree of graphitization in the LIG prepared in this study.[13,22]

The graphene structure was also substantiated by X-ray diffraction (XRD) analyses. As Figure 3b showed, in the XRD pattern, a typical C (0 0 2) peak could be observed at 2θ = 22°. The shape of the peak was blunter than those of graphite materials, which indicated a great extent of crystalline disorder. The XRD pattern demonstrated the discrete spatial structure of the graphene sheet induced by laser, which also proved the abundant defects of the material. The XRD patterns of LIG sheets after modification were also characterized, and the result showed that the introduction of ferrocene or protein materials didn’t influence the underlying LIG morphology.

X-ray electron-phonon spectra (XPS) were adapted to examine the elementary composition and chemical states after ferrocene modification (Figure 3c–e). It can be seen from Figure 3d that the high-resolution XPS spectrum of Cls from LIG (blue line) has been deconvoluted into four smaller peaks (yellow lines), which are attributed to the C-C peak (sp2 bonded carbon, corresponding to the binding energy of ≈283.96 eV), C-O peak (epoxy/hydroxyl, ≈288.02 eV), C = O peak (carbonyl, ≈287.64 eV), and O-C = O peak (carbonyl, ≈291.38 eV), suggesting hydrophilic functional groups on LIG surface. From the narrow scan of Fe 2p shown in Figure 3e, one can easily recognize the sharp Fe(II) 2p₃/₂ feature at 708.4 eV, with its corresponding 2p₁/₂ partner ≈721 eV.[24,25] The XPS spectra illustrated the presence of ferrocene in GOx/Fc/LIG. XPS results indicated that among the surface element components of the GOx/Fc/LIG electrode, the content of each component of Fe is 0.87%, O is 17.07%, C is 82.06%, respectively. In our modified biosensor, ferrocene molecules consisting of two cyclopentadienyl rings and a central iron atom anchored on the surface graphene sheet by conjugation effect between delocalized π electrons of graphene and the phenyl group of ferrocene molecules.

2.3. Wettability and Flexibility Test

In the process of surface modification of LIG, the contact angle of the material was tested in each step to evaluate the
Figure 2. a) The TEM image of LIG, the red arrow points to the edge of the lattice; b) The electron diffraction pattern of LIG; c) The appearance of fabricated LIG; d) SEM image of 1000x magnification, the inset is 5000x magnification; e) SEM image of LIG cross-section; f) SEM image of enzyme-modified LIG electrode with 1000x magnification.

As a derivative from flexible PI substrate, LIG also exhibited excellent flexibility, which made it suitable for various applications, like flexible electronics, wearable devices, etc. We measured the change in conductivity of LIG under bending conditions. As shown in Figure 4b,c, after multiple times of folding, the conductivity of the developed LIG pieces remained at high levels. Maintaining high conductivity indicated that the LIG structure experienced little destruction in the folding process. Also, the result demonstrated that the LIG electrodes can withstand bending angle as high as 60°, and no significant conductivity reduction was observed.

Figure 4c,d show the scanning electron microscopic and photographic images of the LIG material that underwent convex and concave bending conditions and remained intact. The insets illustrate the corresponding bending conditions of LIG in each figure. The morphology of LIG through this bending test demonstrates drastically different profile compared to that of LIG subjected to rigorous folding in half as shown in Figure 4f where cracks were observed with bare eyes. The densely stacked laser-induced graphene sheets remained tightly adhered on the PI substrate after the bending test, indicating excellent robustness.
of the material with mechanical disturbance. The robustness in both morphology and conductivity of the presented LIG material highlights its potential for integration in wearable devices that may endure complex natural motions.

2.4. Electrochemical Performance

The electrochemical performance of the GOx/Fc/LIG electrode has been investigated under different tests.

In our experiment, amperometric test was performed in glucose solutions at different concentrations until steady-state current was reached. The current signals were plotted in Figure 5a. In blank PBS solution, the steady-state current was 5.581 μA and increased to 9.8 μA in 1 mM glucose solution. Further, the steady-state current corresponding to different concentrations of glucose solutions increased steadily from 1 to 15 mM. At concentrations higher than 15 mM, the plateau current reached saturation. As shown in Figure 5b, the linear range of the calibration curve was from 0 mM to 11 mM (correlation coefficient $R^2 = 0.9956$). The sensitivity of the biosensor was 11.3 μA mM$^{-1}$ cm$^{-2}$, which was higher than that of other flexible carbon biosensors with ferrocene as mediator, such as the previously developed carbon paste screen-printed biosensor with modified hexacyanoferrate mediator (1.72 μA mM$^{-1}$ cm$^{-2}$). [26]

Figure 5c shows the amperometric response of the modified GOx/Fc/LIG electrode on successive addition of glucose (from 0 mM to 8 mM) into continuously stirred PBS (pH = 7.4) at an applied potential of +0.1 V. The modified electrode achieved 95% steady-state current within 15 s. This indicates good electrocatalytic activity and fast electron exchange behavior of the GOx/Fc/LIG electrode. The test current densities against analyte concentrations were plotted in the inset with a sensitivity of 29.8 μA mM$^{-1}$ cm$^{-2}$). The result also shows that the flexible GOx/Fc/LIG electrode holds potential in dynamic testing scenarios, for example interstitial fluid glucose and sweat glucose.
monitoring, etc. The selectivity of the glucose sensor was studied by measuring the current response in the presence of 2 mM lactic acid (LA) and 0.1 mM ascorbic acid (AA). Interference stock solution was added into the cell while the amperometric test was performed. As shown in Figure 5d, negligible response was observed when adding these interferences into the testing solution while a significant current increase was observed with added glucose stock solution. Given that at resting state the physiological concentration level of LA is 1–2 mM and AA about 0.1 mM [27], the existence of interfering materials at the physiological levels could not hinder normal operation of the fabricated glucose sensor. These results well demonstrated the selectivity of GOx/Fc/LIG electrode towards glucose detection in a complex environment.

The repeatability of the GOx/Fc/LIG biosensor was thereby evaluated by testing with glucose solutions using electrodes fabricated in different batches. As Figure 5e shows, the current response of the three electrodes from different batches were similar at each of the three different concentrations of glucose. The standard deviation of measured response current was <13%, indicating good repeatability of the presented biosensor. The low batch-to-batch variation suggests the production and modification of such biosensor is readily scalable and holds great promise in biomedical and clinical applications.

### 3. Discussion

In this work, a novel enzymatic electrochemical biosensing interface based on laser-induced graphene was successfully developed. Graphene was successfully induced by laser from the carbon-rich polyimide film. A relatively low power laser was used to induce graphene, and high conductivity of fabricated LIG was obtained at 4.2 Ω sq⁻¹. The TEM result showed that the fabricated LIG had polycrystalline structure. The morphology of the LIG was characterized by scanning microscopy, and the crystalline structure was characterized by Raman spectrum and X-ray Diffractometer. The SEM result showed that the fabricated graphene sheet was laminar-structured and stacked. The laser-induced graphitization process of carbon-rich polymers was evaluated by the Raman spectrum where I_D/I_G was 0.67.

The disordered crystalline structure was also demonstrated by the blunt peak at 20 = 22° in XRD patterns. The morphology and crystalline characterization result showed that the induced graphene was thin-layered and has large specific area. These properties made it suitable for constructing the electrochemical biosensing interface. Surface analysis showed that the
Figure 5. a) Amperometric i–t curve results of the LIG electrode in 0.01 M PBS (pH = 7.4) containing glucose with concentrations from 0 – 15 mM with an increment of 1 mM; b) Steady state currents plotted as the function of glucose concentrations. Inset is linear curve fitting between currents and glucose concentrations, \( R^2 = 0.9956 \) (\( N = 3 \)); c) Amperometric i–t curve with successive adding glucose stock solution in PBS solution (pH = 7.4). Arrows indicate times when 10 \( \mu \)l of 1 M glucose stock solution was added into the reaction system. Each addition contributes to increasement of glucose concentration of \( \approx 1 \) mM; d) Interference tests of the LIG electrode upon addition of glucose (Glu), lactic acid (LA) and ascorbic acid (AA); e) Batch-to-batch variation of the GOx/Fc/LIG biosensor; f) Amperometric responses of GOx/Fc/LIG in the serum sample with different concentrations of glucose.
electron mediator ferrocene was successfully anchored on the surface of graphene sheet. Further, we used glucose oxidase as the model enzyme to fabricate enzymatic biosensing electrodes with the developed material and the electrochemical performance was evaluated. It was observed that the biosensor exhibited rapid and sensitive response to the change of glucose concentrations. The electrode had a sensitivity of 11.3 μA mM⁻¹ cm⁻² and linear range of 0–11 mM. By adapting different kinds of enzymes, enzymatic electrochemical biosensors targeting at different kinds of metabolites can be developed with this LIG substrate. Therefore, laser-induced graphene can be regarded as potential electroactive substrate for enzymatic metabolite biosensing. Since the LIG was induced from flexible PI film, it also holds great potential in development of biosensors for flexible electronics and wearable devices in the future.

4. Experimental Section

Materials and Equipment: Glucose Oxidase was purchased from Sigma Aldrich (St. Louis, Missouri). Phosphate buffered saline was purchased from Sangon (Shanghai, China). Glutaraldehyde (50% in water) was purchased from TCI (Shanghai, China). Double distilled water was used throughout the experiments. Acetone was purchased from Sinopharm (Beijing, China). Polyimide (PI) films (thickness: 125 μm) were obtained from Shenzhen Changdasheng Electronics Co., Ltd (Shenzhen, China). L-aspartic acid and lactic acid were purchased from Aladdin (Shanghai, China). All electrochemical measurements were performed on the CHI760E electrochemical workstation (Chenhua, Shanghai, China). The laser source used to perform material graphitization was from Shengxiong Laser Advanced Equipment Co., Ltd (Dongguan, China).

GOx/Fc/LIG Electrode Fabrication: The LIG electrode fabrication process was performed by laser engraving PI films at the power of 4.2 W, step size of 1064 nm, speed of 50 mm s⁻¹, spot diameter of 14.17 μm, and defocus distance of 10 mm. The induced LIG sheet was cut into 1 cm × 2 cm pieces to perform further modification and electrochemical characterization.

The fabrication process was illustrated in Figure 1. The LIG sheet was immersed in ferrocene acetonitrile solution (0.1 M, 0.1% Nafton) for 1 min, and dried in the fume hood at room temperature. The protein-enzyme conjugate solution was prepared by dissolving 4 mg GOx and 7 mg BSA in 200 μL PBS, and added 20 μL 2.5% glutaraldehyde solution to trigger the cross-linking reaction using a vortex mixer. Afterwards, 10 μL of the mixed solution was immediately transferred on to the Fc/LIG material and dried at 4 °C for 24 h. Finally, the modified electrode was rinsed with double-distilled water to remove weakly bound molecules and stored at 4 °C before use.

Electrode Materials Characterization: The morphology of LIG was characterized by a field emission scanning electron microscopy (ZEISS SUPRA 55, ZEISS, Oberkochen, Germany). The crystallographic information of LIG was characterized by the X-ray diffractometer (D8 advance, Bruker, Karlsruhe, Germany) using Cu Kα radiation at λ = 1.5418 Å and scan rate of 5° min⁻¹ with the diffraction angle ranging from 10° to 80°. The Raman spectrometer were obtained on a microscopic laser confocal Raman spectrometer (LabRAM HR800, Horiba, Kyoto, Japan). The transmission electron microscopy (TEM) images were recorded using JEOL-2100plus (JEOL Ltd., Tokyo, Japan). The Auger electron spectroscopy (AES) was obtained by PHI-700 (ULVAC-PHI, Chigasaki, Japan). The X-ray photoelectron spectroscopy (XPS) was conducted by ESCALAB X-i+ (Thermo Fisher Scientific, Waltham, Massachusetts). The contact angles of different electrode materials were measured by a contact angle meter (DSA30, KRUSSE, Hamburg, Germany) where droplets of water were placed on the surface of bare LIG, Fc/LIG and GOx/Fc/LIG electrodes. The flexibility test was performed with a bending machine which can adjust the bending angle in the range of 0°–90°. A piece of fabricated LIG material with 3 cm in length and 1 cm in width was stick on the bending platform by double-sided conductive cop-er foil tape. The test piece was bent at 10°, 20°, 30°, 40°, 50°, and 60°, respectively, and recovered. At each angle, the test pieces were repeatedly bent for three times, and each time the resistance at the corresponding bending condition was measured. Following this examination, the bending degree was set to 45° and bent for 200 times to evaluate the durability. After bending for 10 times, 20 times, 50 times, 100 times, and 200 times, respectively, the resistance was measured and recorded. Furthermore, the robustness of the LIG material was tested by bending at concave and convex conditions, where the surface morphology of the LIG material was evaluated with scanning electron microscopy to examine possible cracks and exfoliation.

Electrochemical Performance Evaluation of the GOx/Fc/LIG Electrode: Electrochemical studies of the GOx/Fc/LIG electrode were performed in a three-electrode cell. The prepared GOx/Fc/LIG material was fixed on a platinum electric clip and worked as the working electrode (WE). An Ag/AgCl electrode was used as the reference electrode (RE), while a Pt coil electrode was used as the counter electrode (CE). All the potentials were referred to the standard Ag/AgCl reference electrode. All the tests were performed with amperometric I-t method in a total volume of 10 mL PBS with a bias potential of 0.1 V applied. Testing stock solutions such as glucose, lactic acid and ascorbic acid were prepared at 1 M, 2 M, and 100 mM concentrations, respectively. A three-electrode unit containing GOx/Fc/LIG as the working electrode was immersed in PBS containing glucose concentrations ranging from 1 mM to 15 mM with 1 mM interval to collect amperometric current response. The test was performed until the response current reached steady-state, and the current response at 100 s was plotted. To validate the anti-interference ability, interfering substances were added into the cell during the test. Specifically, 10 μL of glucose stock solution, 10 μL of lactic acid stock solution, and 10 μL of ascorbic acid stock solution were added into the cell at the time of 60 s, 180 s, and 280 s after the test begun, respectively, reaching final concentrations of 1 mM, 1 mM, and 100 μM, respectively. The electrochemical test result from 0 s to 440 s were recorded. To validate the dynamic monitoring capability of the GOx/Fc/LIG electrode, 10 μL of glucose stock solution was added into the cell every 100 s after test begun to make the glucose concentration increase in a gradient manner. In this test the amperometric current was also measured with stirring.

The repeatability of fabricated GOx/Fc/LIG electrode was carried out by exposing electrodes from three different batches (#1, #2, and #3) to glucose concentration gradients and measured the current response. Glucose solutions with three different concentrations (0 mM, 5 mM, and 100 mM) were used in this test. The exposed area of electrodes to the testing solution was carefully controlled. The GOx/Fc/LIG electrodes fixed on a platinum clip as the working electrode had a total area of 0.5 cm × 0.4 cm immersed into the solution in each test.

Real Serum Sample Analysis: Amperometric test of the GOx/Fc/LIG electrode in serum samples was performed in a similar way with simulated sample test. The experiment was performed in one of a 12-well culture plate instead of previously used electrochemical cell with a total sample volume of 3 mL. The electrochemical testing setup, including the working electrode configured with the platinum electric clip holding the GOx/Fc/LIG electrode, the Ag/AgCl reference electrode and the Pt coil counter electrode were immersed in the serum sample. In this experiment, the LIG material of the working electrode was modified with GOx and Fc as previously described and a total area of 0.5 cm × 0.5 cm was immersed in the analyte solution. Stepwise increase of glucose concentration was achieved by adding 6 μL of glucose stock solution (1 M) into the sample and the concentration increased gradually at 2 mM interval. The bias potential of the amperometric I-t test was set at 0.2 V, and the test results of 100 s was recorded. Experiments involving human serum samples were performed according to protocols and guidelines approved by the Ethics Review Committee of Tsinghua University (Beijing, China) and written consent was obtained from all participants.

Statistical Analysis: The limit of detection (LOD) was calculated by the following equation:

\[
\text{LOD} = \frac{3\sigma}{S}\]

where \(\sigma\) is the standard deviation of the blank signal and \(S\) is the slope of the calibration curve.
where $\delta$ stands for the standard deviation of blank signal, $S$ denotes the sensitivity in the calibration plots. Statistical data in this manuscript was presented in the manner of mean $\pm$ SD. All the calibration plots and figures were processed with Origin 2019b. Peak fitting of XPS spectra was performed with the Thermo Scientific Avantage Data System.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Author Contributions**

Conceptualization was done by M.L., G.Z., and H.W.; methodology was done by M.L., G.Z., and C.Y.; validation was done by M.L.; writing—original draft preparation was done by M.L.; writing—review and editing was done by H.W., M.L., and J.C.; visualization was done by M.L.; supervision was done by H.W. and J.C.; project administration was done by H.W. and J.C.; funding acquisition was done by H.W. All authors have read and agreed to the published version of the manuscript.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Keywords**

electrochemical biosensors, enzymatic biosensors, laser-induced graphene, sensing interface