LASER-SCRIBED GRAPHENE ELECTRODES AS AN ELECTROCHEMICAL IMMUNOSENSING PLATFORM FOR CANCER BIOMARKER ‘EIF3D’

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Abstract
eIF3d is a protein biomarker which has a potential for the diagnosis of various cancers. Herein, a bio-platform was constructed for eIF3d sensing by using LSG and surface functionalization with anti eIF3d antibody via EDC/NHS chemistry. Following the surface modifications, XPS and several electrochemical methods were used. Difference in the signals were related to biomarker amounts between 75-500 ng/mL. LOD was calculated as 50.4 ng/mL. Selectivity of biosensor was tested by using of various interference molecules. eIF3d was also successfully detected in synthetic biological samples. Thus, to the best of our knowledge, this study is one of the rare studies on use of LSGs in immunosensor studies.

Keywords: Laser-scribed graphene electrodes, Cancer, Biosensors, Affinity-based sensors, Biomarkers, EIF3D

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1. Introduction

As one of the core subunits of eukaryotic translation initiation factor 3 (eIF3) complex, subunit D (eIF3d) is reported to have an important oncogenic role in many different cancer types, such as prostate \(^1\), colon \(^2\), lung \(^3\), breast \(^4\) and bladder cancer \(^5\). eIF3d is known to specifically recognize and bind to the 7-methylguanosine cap of a subset of mRNAs and these interactions are considered essential in cell proliferation regulation \(^6\). Down-regulation of eIF3d is discovered to be associated with inhibition of proliferation of cancer cells, indicating its prognostic value in cancer diagnostics \(^3\,\,^4\,\,^7\). To date, eIF3d expression has been detected in either tissue extracts \(^1\,\,^5\,\,^7\) or cell lines \(^1\,\,^3\) using conventional immunohistochemistry \(^1\,\,^7\) or western blot analyses,\(^1\,\,^3\) respectively. Being a newly discovered potential clinical biomarker for various cancer types, detection of eIF3d protein holds great promise in the field of cancer diagnostics. While the methods used in detection of eIF3d expression are sensitive and reliable, they also suffer from being time consuming, complicated, expensive and lacking practicality \(^8\). The need for a reliable, simple and real-time monitoring in the clinical practice drives the motivation in developing biosensors. A biosensor is often defined as an analytical device which can convert a biological event to a measurable and processable signal using a transducer \(^9\). Several different qualitative and/or quantitative measurement techniques such as optical (colorimetric \(^10\), fluorescence-based \(^11\), surface-enhanced resonance spectroscopy \(^12\) etc.) and electrochemical (amperometric \(^13\), potentiometric \(^14\), impedimetric \(^15\) etc.) methods have been applied to biosensors using antibodies \(^16\), aptamers \(^17\) and enzymes \(^18\) as recognition agents. Electrochemical biosensing platforms satisfy the need of a rapid, simple, sensitive, selective, low-cost and efficient detection method of proteins \(^19\).

Considering the diversity of carbon-based materials used in electrochemical sensing, it is clearly seen that graphene has attracted significant attention due to its superior structural, physicochemical and morphological properties \(^20\). Studies have been revealed that graphene has high thermal conductivity, mechanical strength, high optical transparency and good potential for energy storage, nanoelectronics, catalysis, sensing and biomedicine \(^21\). There are several methods employed for graphene
production in the literature such as conventional CVD, Hummers’ method, other chemical and thermal reduction methods \[22\]. However, these methods are weak in terms of scalability and requires expensive instrumentation, long synthesis processes \[23\]. Therefore, it is important to use a strategy for graphene production includes simultaneous reduction, cheap and mask-less patterning on relevant substrates with good resolution for many potential applications \[24\]. Laser scribed graphene (LSG) technology has grown attention with being a promising, reliable, and mask-free patterning method simultaneously derived from polyimide substrate by molecular rearrangement on the surface upon laser exposure \[25\]. The first study presenting the super capacitor fabrication of laser irradiated graphite oxide films was performed by Gao et al. \[26\]. This work followed by Strong et al. \[27\] demonstrating thermal reduction of graphite oxide, generation of graphene and exfoliation of the layers by laser. Recently, chemical and biosensing applications have been realized by researchers proving that LSG electrodes offers a promising alternative sensing platform with high surface area, multilayer structure, high porosity, stability and conductivity \[25b, 28\].

As innovative platforms, few numbers of LSG based bio-sensors and limit of detection (LOD) values for these bio-sensors were available in the literature and these are summarized in Table 1. In this work, LSG electrodes were used for one-step biomolecule immobilization after a practical surface functionalization. In this study, eIF3d protein was selected as a potential biomarker in cancer diagnosis to investigate antibody/receptor interaction by using LSG platforms. LSG-based immunosensor platform was previously designed for the detection of biological materials, \[29\] however, to the best of our knowledge, this is the first study for the detection of the eIF3d biomarker. Therefore, this study is one of the critical models of the use of LSG electrodes as the immunosensor platform which provides an effective surface for the biomolecule immobilization. For the future, LSG electrodes can be easily adapted for the multiple detection of other biomarkers as well as critical analytes in diagnosis of various diseases.

Table 1. Application of LSGs as biosensing platform in the literature.

<table>
<thead>
<tr>
<th>Bio-/Sensor Type</th>
<th>Target Analyte</th>
<th>LOD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAAMI based electrochemical sensor</td>
<td>IgG</td>
<td>6.0 pg/mL</td>
<td>[29]</td>
</tr>
<tr>
<td>PEDOT based electrochemical sensor</td>
<td>Dopamine</td>
<td>0.3 μM</td>
<td>[30]</td>
</tr>
<tr>
<td>Aptamer based electrochemical sensor</td>
<td>Thrombin</td>
<td>5.0 pM</td>
<td>[25a]</td>
</tr>
<tr>
<td>Copper nanoparticles based electrochemical sensor</td>
<td>Biogenic amines</td>
<td>11.6 μM</td>
<td>[31]</td>
</tr>
<tr>
<td>Non-enzymatic electrochemical sensor</td>
<td>Glucose</td>
<td>0.35 μM</td>
<td>[24]</td>
</tr>
</tbody>
</table>

**2. Material and Methods**

**2.1. Chemical and Reagents**

1-Pyrenebutyric acid (PBA), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), eIF3d antibody (Prestige Antibodies), eIF3d, alfa fetoprotein (AFP), human epidermal growth factor receptor 2 (Her2) antigen (PrEST Antigens), bovine serum albumin (BSA) (lyophilized powder, ≥96% (agarose gel electrophoresis)), potassium hexacyanoferrate (III) (HCF) which used as redox probe, potassium chloride, dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). In addition, disodium hydrogen phosphate, sodium phosphate monobasic, sodium chloride and potassium chloride were supplied from Sigma Chemical Company (St. Louis, MO, U.S.A.) for buffer solutions. Sodium phosphate buffer (50 mM, (pH 7.4)) was used as a working buffer.

**2.2. Apparatus**

Conductive graphene layers were patterned on a commercial polyimide (PI) sheet (Kapton Width:12”, Utech Products, USA) by CO2 laser irradiation (Universal Laser Systems® PLS6.75) directly. Laser spot diameter and wavelength parameters were selected as ~150 μm and 10.6 μm respectively. PalmSens4 potentiostant (Palm Instruments, Houten, Netherlands) was used for electrochemical measurements such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and
electroanalytical impedance spectrometry (EIS) measurements. For the electrochemical measurements, LSG as a working electrode, platinum (Pt) as counter electrode and Ag/AgCl (3.0 M KCl, Metrohm, Switzerland) as reference electrode were used [25a, 32]. All electrochemical experiments were carried out in electrochemical reaction cell (10 mL), at ambient conditions, in the presence of 5.0 mM Fe(CN)$_6^{3-/4-}$ as redox probe. DPV and CV measurements were conducted in a potential range from -0.4 V to 0.8 V. EIS measurements were obtained in the frequency range of 0.02 Hz to 10 Hz at 0.18 V. X-Ray photoelectron spectrometer (XPS) (Thermo Scientific, U.S.A.) and scanning electron microscopy (SEM, Thermo Fischer Scientific Apreo S LoVac model, Oregon, USA) were used for monitoring of surface modification. Bruker D8 ADVANCE (Karlsruhe, Germany) X-ray diffractometer (XRD) with Cu Kα radiation (1.5406 Angstrom) in a 2θ range of 20°–80° and Horiba Scientific ARAMIS Raman spectrometer with a 473 nm cobalt laser source were used to characterize the graphene crystallinity and the structure.

2.3. Electrode Fabrication

The LSG electrodes were designed with 3 mm diameter, 1.2 mm width and 2.2 cm height using Tanner software. The mask-free fabrication of the electrodes was performed by using CO2 laser irradiation following our previous work [28a]. A flexible polyimide sheet was cleaned before the laser irradiation. The optimization of laser parameters was performed to achieve the LSG production with the highest resistivity value, given in Table S1 and S2. The laser power, laser speed, pulse and z distance were chosen as 3.2 W, 2.8, 1000 pulses per inch, and 2.5 mm respectively to produce the best quality of the multilayer graphene with a high surface area and homogeneity. The resistivity value of the graphene was measured as 58 Ω/square. The area between the working area and connections was passivated before conducting the measurements to achieve a good isolation and keep the working area stable. Schematic illustration of LSG electrode fabrication was shown in Scheme 1.

2.4. Surface Functionalization/Modification

Optimization of PBA concentration was performed and the optimum concentration for PBA was determined as 25 mM (Fig S1). LSG electrodes were initially functionalized with PBA (25 mM) in DMSO for 1 h at ambient conditions. Then, the electrode was immersed in mixture of EDC (50 mM) and NHS (50 mM) solutions (in 1X, pH 6.0, PBS) for 90 min [25a]. Activated surfaces was washed with PBS (1X, pH 6.0) and then, 10 µg/mL eIF3d antibody (in 1X, pH 7.4 PBS) [25a] was dropped on the modified LSG surface and allowed to incubate 2 h. Afterward, the electrode surface was washed with PBS (1X, pH 7.4) for removing the unbound protein moieties. After immobilization step, BSA (1.0 mg/mL) was used to block the surface for 30 min to avoid nonspecific binding which are especially important in the sensor performances in real samples as well in the presence of interference molecules. Finally, eIF3d as target analyte in different concentrations (750; 500; 250; 150; 100; 75 ng/mL in 1X, pH 7.4, PBS) were dropped on the modified LSGs, respectively and incubated for 30 min for the selective capturing by the biofunctional surfaces through the antibody/analyte recognition reaction. In addition, incubation time optimization for antibody/analyte recognition reaction was conducted and the optimum incubation time was determined as 30 min (Fig S2). All modification steps were followed via electrochemical methods such as CV, DPV and EIS. In addition, SEM and XPS analysis were used to prove surface functionalization as well as binding reactions. Step-by-step surface modification and detection principles of the biosensing platform were summarized in Scheme 1.
Furthermore, some analytical parameters of the biosensor platform such as LOD, coefficient of variation (%), repeatability (±S.E.) were determined. LOD value was calculated by using the \(\frac{3 \times \text{Standard Error}}{m}\) value formula, where the coefficient of variation (%) value was obtained with \(\frac{\text{Standard error}}{\text{Average}} \times 100\) formula and repeatability (± S.E.) value was found from 10 consecutive measurements.

In addition to analytical features, reusability was investigated for the proposed platform. Briefly, eIF3d analyte (250 ng) was added to the LSG biosensor and the signal response was registered. Then, it was washed with 100 mM KCl for removing the bound eIF3d from the surface by distorting the affinity based biomolecular interactions (in between anti-eIF3d and eIF3d) using highly salted medium. And then, the same LSG biosensor was used again for the following measurements by repeating the same conditions in terms of eIF3d amount (250 ng). This procedure was repeated three times and the difference in current signals of each measurement were evaluated.

2.5. Effects of Interfering Substances and Sample Application

Selectivity is an important parameter in terms of sensor performance and was tested by using some possible interferences such as AFP, HER2 as model protein biomarkers, and BSA due to the presence in a biological samples especially in serum. These substances were prepared in 250 ng/mL concentrations (in 1X, pH 7.4 PBS) and applied on LSGs modified as LSG/PBA/Antibody, instead of target biomarker (eIF3d). Data were compared with the signal response of eIF3d (250 ng/mL) and relative signal responses were calculated.

To investigate, the effect of sample matrix and the applicability of proposed system, synthetic serum and urine samples (preparation procedures were given in Table S3), were used and eIF3d (250 and 150 ng/mL) were added in these synthetic samples. Afterwards, spiked solutions were applied on modified LSGs instead of eIF3d standards and then, signal responses were registered.
the final step, biomarker amount in the samples were calculated via linear graph.

3. Results and Discussion

3.1. Surface Characterization

CV, EIS, XPS, XRD, Raman and SEM analyses were performed to verify the presence of graphene layers surface modifications as well as analyte binding. Each surface modification steps were monitored with CV and EIS data, (Fig 1). Initially, PBA was added to bare LSGs to create additional carboxyl groups on the graphene surface for the efficient antibody binding [25a]. Afterwards, PBA modified LSGs were treated with EDC/NHS and then, eIF3d antibody was dropped on the surface. Prior to the analyte addition for the antibody/analyte binding reaction, the surface was blocked with BSA to avoid non-specific interactions which could reduce the sensing performances in complex matrices such as tissue homogenates as well as serum and urine.

Each modification steps besides the analyte binding were created massive structures as a result of integration of additional molecules to the surface. These interactions prevent the transfer of the redox probe to the electroactive area that caused a drop and difference in CV and DPV peaks, while an increase in EIS data.

In addition to CV results, EIS measurements were performed for surface characterization and summarized in Fig 1B. EIS results were defined with Nyquist circulation diagram in Fig 1B that was formed from Warburg impedance (W), resistances (Rs and Rct) and capacitance (C1). Solution resistance on the electrochemical cell was shown as Rs; whereas electron transfers resistance was shown as Rct (C1) was symbolized double layer capacitance. Nyquist diagram is electrical semicircle and were evaluated and fitted with Randle’s equivalent circuits. EIS data are well agreed with the CV results. As PBA, antibody and eIF3d analytes were immobilized on the bare LSG surface, resistant values (Rct) were increased. This is an expected result as an indication of bulky structures as a result of successful binding to the electrode surface. Furthermore, data for anodic and cathodic CV peaks as well as Rct values were summarized as Table S4 in SI (Supporting information)

![Fig 1](image-url)  
**Fig 1.** A) CV for bare LSG; LSG/PBA; LSG/PBA/Anti-eIF3d; LSG/PBA/Anti-eIF3d/BSA and LSG/PBA/anti-eIF3d/BSA/eIF3d (75 ng/mL). [Measurements were obtained in the potential range between -0.4 V and 0.8 V at scan rate of 50 mVs, 5.0 mM Fe(CN)₆³⁻/⁴⁻]. B) EIS measurements for bare LSG; LSG/PBA; LSG/PBA/Anti-eIF3d; LSG/PBA/Anti-eIF3d/BSA and LSG/PBA/anti-eIF3d/BSA/eIF3d.
In addition to electrochemical measurements, XRD and Raman spectroscopies were performed to confirm the presence and quality of graphene and results were summarized in SI (Fig S3).

XPS measurements were also performed to prove the binding and recognition reactions, respectively. Critical bonds in these surface modifications were summarized in Fig 2. Moreover, to confirm PBA interactions and forming of carboxyl groups on LSGs, C 1s and O 1s spectra were examined. 6 Peaks were observed in C 1s spectra. Peak at 284.17 eV was shown pure graphitic sites, while peak at 284.78 eV was shown C-C or C=C binding. Peak at 285.3 eV was indicated the presence of hydrocarbons in PBA. Peak at 285.95 eV was also reported C-N or C=N binding, this peak is thought to result from nitrocellulose in the material used for the surface isolation prior to the using of LSGs. Peaks at 288.21 eV and 289.1 eV were pointed out C=O and ester carbon of PBA. Furthermore, O 1s spectra results were examined for modified surface with PBA. Two peaks were obtained at 532.67 eV and 533.78 eV and these peaks were proved that carboxyl groups formed at the surface as functional groups. Since, the peak at 532.67 eV indicated O=C while another peak at 533.78 eV could be interpreted O-H.

In the next modification step, C 1s spectra was recorded for proving of the bond between carbon end of PBA and -NH₂ terminal of antibody. The presence of C-C, C=C or C-H bonds in antibody structure, the peaks were occurred at 284.21 eV and 284.99 eV. Also, availability of 3 peaks at 285.58 eV, 286.39 eV and 288.4 eV, were proved the successful antibody binding via EDC/NHS chemistry. As predicted, carbonyl (C=O) peak was seen at 285.58 eV. In addition, the peak at 286.39 eV represented the C-N bond, while the peak at 288.41 showed C-N=O. XPS measurement was not used to prove receptor and biomarker interaction which is mainly based on the physical interaction. It is only used to verify step by step surface modification.

Furthermore, electrode surface was imaged via SEM (with 25 000X magnification). As shown in Fig 2, PBA treatment didn’t affect the surface morphology. On the other hand, it can be clearly seen that eIF3d antibody molecules were successfully immobilized in 3D structure of LSG surface especially inner part of the cavities. The most important advantage is that this immobilization strategy doesn’t prevent the antibody/antigen interaction that provides selective capturing of the target analyte.
3.2. Analytical Features

Initially, step-by-step modification of LSGs was monitored via DPV technique (Fig 1C). DPV results were in consistence with the CV and EIS results as expected. It was seen that DPV peaks were decreased with as a result of surface covering by PBA, antibody binding as well as blocking with BSA.

A calibration graph was obtained between eIF3d amount (ng/mL) and current (ΔµA). The drops in DPV peak currents are related with analyte amounts (Fig 1D). Linear range of the platform was determined between 75 and 500 ng/mL eIF3d. Equation for linearity and correlation coefficient were obtained as $I$ (µA)=0.014 [eIF3d] (µA/ng/mL) + 0.313 and $R^2$=0.996, respectively. A deviation from the linearity was obtained after 500 ng/mL. DPV curves for different eIF3d concentrations were shown in Fig S4. Also, analytical features for the biosensor platform were determined and LOD value was calculated as 50.43 ng/mL. All obtained analytical features for LSG were summarized in Table 2A. According to the literature researches, eIF3d biomarker is usually extracted from tissues and LOD value for the biomarker could not be found. As our best knowledge, a biosensor system for the eIF3d has not been reported yet. However, a commercially ELISA kit is available. However, a commercial ELISA kit is available in the market [48]. Related studies with the biomarker were summarized in Table 2B.

Table 2. (A) Some analytical features of the biosensor platform for eIF3d biomarker [Standard error values were obtained by three consecutive measurements]. (B) Methods in literature for determination of eIF3d.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample Type</th>
<th>Linear Range</th>
<th>LOD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistochemistry</td>
<td>Tissue</td>
<td>-</td>
<td>-</td>
<td>[49]</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>Tissue</td>
<td>-</td>
<td>-</td>
<td>[7b]</td>
</tr>
<tr>
<td>Chromatography and immunohistochemistry</td>
<td>Tissue</td>
<td>-</td>
<td>-</td>
<td>[50]</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>Tissue</td>
<td>-</td>
<td>-</td>
<td>[51]</td>
</tr>
<tr>
<td>ELISA</td>
<td>Blood, tissue, serum etc.</td>
<td>0.25-8 ng/mL</td>
<td>0.1 ng/mL</td>
<td>[48]</td>
</tr>
<tr>
<td>Biosensor</td>
<td>Serum and urine</td>
<td>75-500 ng/mL</td>
<td>50.43 ng/mL</td>
<td>This work</td>
</tr>
</tbody>
</table>

Electrode-to-electrode reproducibility which is one of crucial parameters effecting to obtain reliable data, was also examined. The variation of three individual LSG based biosensor is calculated as 3.67% of the mean signal change for 500 ng/mL eIF3d.

Moreover, reusability of the biosensors was tested and given in Fig S5. Prior to reuse of biosensors, highly salted medium was applied to the working electrodes to remove biomolecular affinity-based interactions on the surface. In fact, LSGs are considered as disposable systems due to cost-effective features besides numerous advantageous such as their easy adaptable and innovative characteristics but reusability of the biosensor is not directly related to the substrate or electrode materials, it is mainly depending on the surface chemistry used in biomolecule immobilization and the basis of molecular interactions used for the measurement of the sensing responses. In our case, an affinity-based interaction was used and while removing the bound analyte via salty medium, leaking or partly denaturation of the recognition molecule might be occurred. This fact could cause variation in current signals in subsequent measurements. Hence, freshly prepared LSG biosensors was used in each trial.
The alteration in response signal of the biosensing system was related with the selective capturing of the target biomarker by the immobilized antibodies and the resulted limitation of transfer of the redox probe due to the changes in surface properties as a result of binding reactions. On the other side, nonspecific binding of interfering substances having the protein structures such as other protein biomarkers could also affect the diffusion features, therefore cause to a drop in the peak currents similar with the real target molecule (eIF3d). To examine this parameter effecting the sensing performance, interference experiments were performed by the addition of selected molecules such as AFP, HER2, BSA and mixtures of these molecules with eIF3d instead of the target protein biomarker. Obtained signal responses were then compared with the eIF3d data. The relative responses for these molecules were under 30% (28.9%, 26.4% and 20.2% for HER2, BSA, AFP, respectively (Fig 3A) \(^{[35, 52]}\). In addition, the relative responses mixtures of these molecules with eIF3d were found 104.95% for HER2+eIF3d, 105.17% for AFP+ eIF3d and 111.88% for BSA+eIF3d (Fig 3B). Hence, it can be said that designed bio-platform selectively recognizes eIF3d biomarker in compared to the other protein biomarkers.

For the sample application, the synthetic serum and urine samples were spiked with eIF3d solutions in two different concentrations (150 and 250 ng/mL). Then, these solutions were directly added to LSG/PBA/Antibody/BSA surface.

After measurement of the signal responses at previous operation conditions mentioned, signal values were compared the data belonging to the standard eIF3d solutions (150 and 250 ng/mL). Recovery and relative standard deviation (RSD) values were calculated and results were summarized in Table 3.
4. Conclusion

In conclusion, we have investigated the use of LSG electrodes as promising tool serving as a sensing platform. The fast LSG production by CO2 laser provides a straightforward and mask-free mass production with high reproducibility and active surface area. Easy surface functionalization provides on-step biomolecule immobilization for the biosensor fabrications in different surface size and geometry. Various biorecognition molecules from aptamers to specific peptide fractions can be easily immobilized via this approach and custom-tailored biosensors for different applications might be fabricated. In this study, the use of eIF3d biomarker, which is the harbinger of many types of cancer, and the use of LSG electrode on cancer detection were investigated. An immunosensor has been designed with the use of the eIF3d biomarker with the LSG electrode, which is rarely found in the literature. A fast, user-friendly, cost-effective immunosensor has been designed that can be used successfully for cancer detection. In the future in the light of this study, LSG electrodes, which have just started to be fabricated, may be used in the immunosensor design today, can guide different immunosensor studies for single or multi-detection of different cancer types with desired one-step patterning.

5. Acknowledgements

The authors would like to express their acknowledgments to the financial support of funding from King Abdullah University of Science and Technology (KAUST), Saudi Arabia. Biosensing experiments as well as SEM and XPS analysis were conducted in Ege University, Central Research Test and Analysis Laboratory Application and Research Center (EGE-MATAL).

6. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

7. Data Availability

The data used to support the findings of this study are included in the article.

List of Figure Legends

Scheme 1. Schematic illustration of LSG electrode fabrication, step-by-step surface modification of LSG electrode and detection principles of the eIF3d sensing platform.

- Fig 3. A) CV for bare LSG; LSG/PBA; LSG/PBA/Anti-eIF3d; LSG/PBA/Anti-eIF3d/BSA and LSG/PBA/anti-eIF3d/BSA/eIF3d (75 ng/mL), [Measurements were obtained in the potential range between -0.4 V and 0.8 V at scan rate of 50 mV/s, 5.0 mM Fe(CN)3/4-]. B) EIS measurements for bare LSG; LSG/PBA; LSG/PBA/Anti-eIF3d; LSG/PBA/Anti-eIF3d/BSA and LSG/PBA/Anti-eIF3d/BSA/eIF3d (75 ng/mL). [EIS measurements for bare LSG; LSG/PBA; LSG/PBA/Anti-eIF3d were shown in the inset (i); Measurements were obtained at 0.02 Hz to 10 kHz and at 0.18 V]. C) DPV data for bare LSG, LSG/PBA; LSG/PBA/Anti-eIF3d, LSG/PBA/Anti-eIF3d/BSA and LSG/PBA/Anti-eIF3d/BSA/eIF3d (75 ng/mL); [Measurement were carried out in potential range from -0.4 V to 0.8 V at the scan rate of 50 mV/s, 5.0 mM Fe(CN)3/4-]. D) Calibration curve for eIF3d biomarker; [Error bars were obtained by 3 or 4 measurements].

- Fig 4. SEM images of bare LSG electrode (A) modified electrode-LSG/PBA- (B) and LSG/PBA/Antibody (C). (Scale value: HV: 5.00 kV; 25 000X; WD: 10.7). XPS High resolution spectra of D) C 1s peaks for LSG/PBA E) O 1s peak for LSG/PBA F) C 1s peaks for LSG/PBA/Antibody.

- Fig 3. Biosensor response for the various cancer biomarkers interfering molecules (A), and mixtures of various cancer biomarkers with eIF3 (B). [All biomarker solutions and mixture solutions were prepared 250 ng/mL concentrations. The same conditions with Fig 2 were used for the measurements and the error bars were obtained by 3 consecutive measurements].

8. References

GRAPHICAL ABSTRACT