SUPPORTING INFORMATION

Bioactive Surface Design Based on Functional Composite Electrospun Nanofibers for Biomolecule Immobilization and Biosensor Applications

Sema Demirci Uzun[a], Fatma Kayaci[bc], Tamer Uyar[bc], Suna Timur[d], Levent Toppare[aefg*]*

Corresponding Authors

*Tamer Uyar, tamer@unam.bilkent.edu.tr (e-mail), +90 3122903571 (tel.), +90 3122664365 (fax)

*Levent Toppare, toppare@metu.edu.tr

a Department of Polymer Science and Technology, Middle East Technical University, 06800, Ankara, Turkey

b Institute of Materials Science and Nanotechnology, Bilkent University, 06800, Ankara, Turkey

c UNAM-National Nanotechnology Research Center, Bilkent University, Ankara, 06800, Turkey

d Faculty of Science, Biochemistry Department, Ege University Bornova, 35100, İzmir, Turkey

e Department of Chemistry, Middle East Technical University, 06800, Ankara, Turkey

[f] Department of Biotechnology, Middle East Technical University, 06800, Ankara, Turkey

g The Center for Solar Energy Research and Application (GÜNAM), Middle East Technical University, 06800, Ankara, Turkey

Materials

All chemicals used in monomer synthesis (hydrobromic acid, bromine, dichloromethane (DCM)) were purchased from Sigma except for thiophene which was purchased from Acros Organics (Geel, Belgium). All chemicals used for electropolymerization ($LiClO_4$, $NaClO_4$, acetonitrile (ACN), dichloromethane (DCM)) were purchased from Aldrich. Multi-walled carbon nanotubes (O.D. x L 6–9 nm x 5 μm, >95% carbon), nylon 6,6 pellets (relative viscosity: 230.000-280.000), formic acid (98-100 %) were purchased from Sigma-Aldrich. Glucose oxidase (GOx, β-D-glucose: oxygen 1-oxidoreductase, EC 1.1.3.4, 39800 units/g) from *Aspergillus niger*, D-glucose, sodiumborohydride were purchased from Sigma (St. Louis, USA). Acetonitrile, hydrochloric acid, sodium hydroxide were purchased from Merck (Darmstadt, Germany). Tetrahydrofuran (THF) was purchased from Fisher and purified over benzophenone and sodium. Beverages were of commercial types.

Apparatus

Cyclic voltammetry and amperometric measurements were carried out using Ivium Compact Stat potentiostat (The Netherlands) in a cell equipped with a conventional three electrode configuration. As the working electrode, graphite electrode (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) was used. Ag/AgCl (3 M KCl saturated with AgCl) and a Pt electrode (Metrohm, Switzerland, www.metrohm.com) were used as the reference and counter electrodes, respectively.

In electrospinning studies, KD Scientific (KDS 101) syringe pump is utilized to control feed rate of polymer solution. The applied high voltage for electrospinning was obtained by using high voltage power supply (Spellman, SL Series).

The conductivity measurement of the solutions used for electrospinning was performed by using a bench top conductivity meter (Mettler Toledo, FE-30 FiveEasy) at room temperature.

Contact angle measurements were performed for each polymer and biosensor based surface using the sessile drop method with a KSV CAM 200 contact angle meter (KSV Instruments, Finland). During the experiments, a drop of distilled water was dripped into each surface. The drop profile was recorded with a CCD camera allowed monitoring the changes in contact angle for each surface. All reported data were given as the average with standard deviation of fifteen measurements.

Scanning electron microscope (SEM, FEI – Quanta 200 FEG) was used to investigate the morphology of the nanofibers and estimate the average fiber diameter (AFD) by measuring around 100 fiber diameters. Prior to SEM imaging, a nominal ~5.0 nm Au/Pd alloy was sputtered on the samples. The detailed morphological investigation of the nylon 6,6/4MWCNT nanofibers was performed by transmission electron microscope (TEM, FEI – Tecnai G2 F30). For TEM imaging, the nanofibers dispersed in ethanol was drop-casted onto holey carbon coated TEM grid, and allowing them to dry under an IR lamp for a few minutes.

Synthesis of 4-(4,7-di(thiophen-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde (BIBA) Monomer

Firstly, 2,1,3- benzothiadiazole was brominated in the presence of HBr and Br_2. Subsequently, 4,7-dibromobenzo[c][1,2,5]thiadiazole was reduced with $NaBH_4$ in ethanol. To obtain the acceptor unit, 3,6-dibromobenzene-1,2-diamine (4.0 mmol, 1.01 g) was reacted with terephthalaldehyde (16 mmol, 2.15 g) in addition of $ZrCl_4$ in acetonitrile (ACN) under reflux conditions for 24 hours. After purifying product 4-(4,7-dibromo-*1H*-benzo[*d*]imidazol-2-yl)benzaldehyde (0.8 mmol, 0.33 g) was dissolved in anhydrous THF, tributyl(thiophen-2-yl)stannane was drop wise added to the solution under argon atmosphere. In reflux condition,

dichlorobis(triphenylphosphine)- palladium(II) (0.02 mmol, 0.02 g,) was added as a catalyst to proceed the Stille Coupling process. The reaction was ended with TLC monitoring. After purification with the column chromatography, the product was yielded as a yellow solid (45 %, 0.24 g) (eluent: DCM).

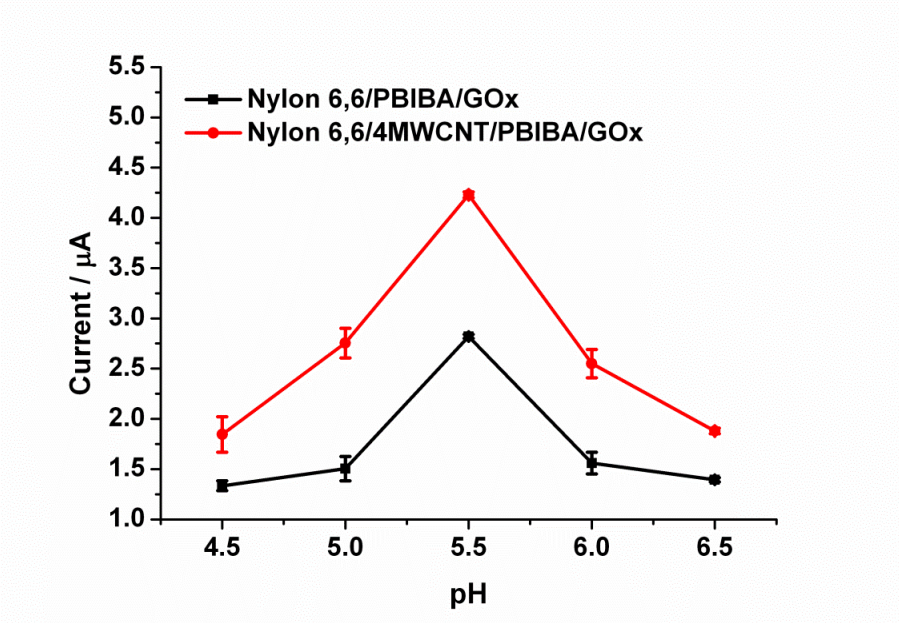

Figure S1 The effect of pH on the biosensor responses, 25 ºC, -0.7 V. The measurements were performed with 0.40 mM glucose. Error bars show the standard deviation (SD) of three measurements.

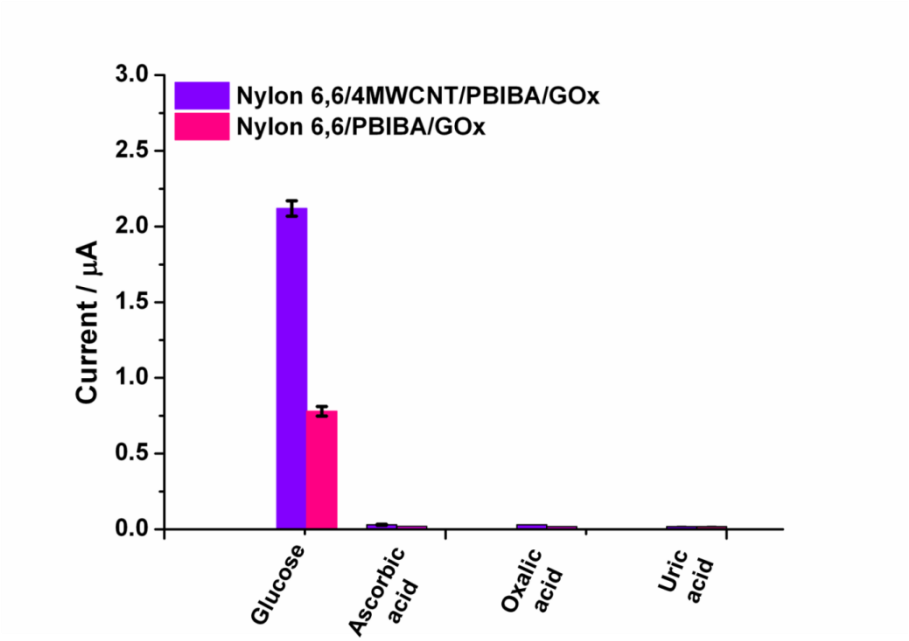

Figure S2 Comparison of the modified biosensors response to glucose (0.1 mM), ascorbic acid (0.1 mM), oxalic acid (0.1 mM) and uric acid (0.1 mM) in NaOAc buffer (pH 5.5) at -0.7 V.

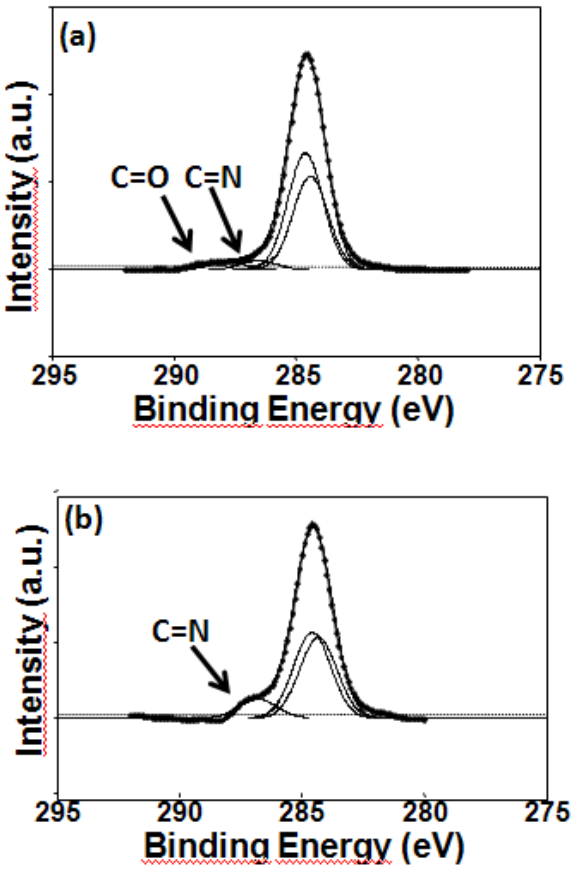

Figure S3 C1s XPS spectra of a) before b) after GOx immobilization on 4MWCNT/Nylon 6,6/PBIBA nanocomposite surface.