Supporting Information

Electrografted Monolayer of Polyaniline as a Tuneable Platform for Glucose Biosensor

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Table of Contents

Instrumentation

Indium tin oxide (ITO) coated glass slides of 15-25 Ω sq^{-1} resistivities were purchased from Sigma Aldrich. The electrochemical investigations were carried out by PalmSens 4 or EmStat (PalmSens Instruments BV, the Netherlands) potentiostat. Measurements of the contact angle were carried out using an EasyDrop Contact Angle Measuring Instrument by KRUSS GmbH using ultrapure water as a drop source. Each contact angle value is generated and averaged from 3 measurements with droplets being positioned places on the surface. The UV-Vis spectra were measured using a spectrophotometer by Agilent Cary 60 UV-Vis (Agilent Technologies, USA) at 25 ^{o}C. The morphology of the prepared surfaces were analyzed by atomic force microscopy (Dimension Icon AFM system; Bruker, USA), operated in tapping mode in the air.

Chemicals

Potassium chloride (KCl), boric acid (H_3BO_3), potassium dihydrogen phosphate (KH_2PO_4), acetic acid (CH_3COOH), 30% ammonia aqua solution ($NH_3 \cdot H_2O$) were obtained from Carl Roth. Aniline (C_6H_7N, 99%), 3-(phenylamino)propyltrimethoxysilane ($C_{12}H_{21}NO_3Si$, 96%) were purchased from Alsa Aesar. Hydrogen peroxide 30% aqueous, 2-propanol, acetone were purchased from Reachem. Glucose oxidase (from *Aspergillus niger*), sulfuric acid (H_2SO_4), *D*-(+)-glucose ($C_6H_{12}O_6$), 4-aminothiophenol were purchased from Sigma-Aldrich. All the experiments were conducted using type II water (R > 18 MΩ) purified in a Milli-Q system. Solution potassium phosphate buffer (PPB, 50 mM) with 100 mM KCl was prepared by dissolving the KH_2PO_4 and K_2HPO_4 as well as KCl salts. pH of the PPB solution was adjusted by 1 M HCl or NaOH solutions addition and measured using a pH meter (Serie EC–31, Phoenix Instrument, Germany). Britton-Robinson (BR) buffers with pH 3 to 10 were prepared by mixing 40 mM boric acid, 40 mM phosphoric acid, and 40 mM acetic acid, and pH of the solution was adjusted by 1 M acetic acid or NaOH solutions.

Activation of ITO and Au electrodes and preparation of the initiating monolayers

Firstly, bare ITO electrodes were cleaned with isopropyl alcohol followed by acetone for 10 minutes using an ultrasonic bath. After that, the ITO electrodes were gently washed with ultrapure water and then cleaned in an ultrasonic bath for another 10 minutes. Then, electrodes were dipped in a solution of ammonia (75%) and hydrogen peroxide (15%) in 3:1 (v/v) at 80°C for 20 minutes. After this pre-treatment, the ITO electrodes were rinsed with copious amounts of

the ultrapure water. The cleaned and activated ITO electrodes were left at 120 °C to dry up. After that, the ITO/PhNHPrSi self-assembly monolayer (SAM) was prepared onto the activated ITO electrodes by covering with 50 mM of 3-(phenylamino)propyltrimethoxysilane (PhNHPrSi) solution in toluene and left overnight. Afterwards, the ITO/PhNHPrSi electrodes were cleaned in an ultrasonic bath with toluene to remove arised sediments.

The Au electrode with 0.20 cm diameter (area is 0.031 cm^2) was firstly cleaned with Micropolish alumina 0.3 μm gel for 5 min by mechanical rubbing. Afterwards, the electrodes were cleaned with distilled water and put in an ultrasonic bath for another 5 min. Then, the electrode was cleaned by CV in 50 mM KOH (from 0 V to -2.6 V vs Ag/AgCl; 40 scans; at a scan rate of 300 mV s^{-1}) and afterwards in 0.5 M H_2SO_4 solution (from -0.2 V to 1.75 V vs Ag/AgCl; 40 scans; at a scan rate of 300 mV s^{-1}). After the electrochemical cleaning, the Au electrodes were washed with distilled water and inserted in Eppendorf tubes containing 300 μL of 5 mM 4-aminothiophenol dissolved in ethanol, and stored in refrigerator at 4 °C for 12 hours. Furthermore, the electrodes were washed in an ultrasonic bath for 2 min with distilled water.

Electrochemical synthesis of the PANI monolayers

The measurements were done by three electrode cells. A titanium plate of its area of 1 cm^2 was used as a counter electrode. Ag/AgCl with saturated KCl as a reference electrode was used throughout these electrochemical measurements. The coated ITO/PhNHPrSi electrode was used as the working electrode (the area of the electrodes was 1.2 cm^2). The electrochemical synthesis of the monolayers was carried out by cyclic voltammetry (CV). Thirty cycles of CV were performed from 0 to 1 V vs Ag/AgCl with a scan rate of 50 mV s^{-1} in a solution containing 50 mM aniline in 0.1 M H_2SO_4. After the synthesis, the freshly prepared electrode was washed with ultrapure water using an ultrasonic bath for 35 minutes.

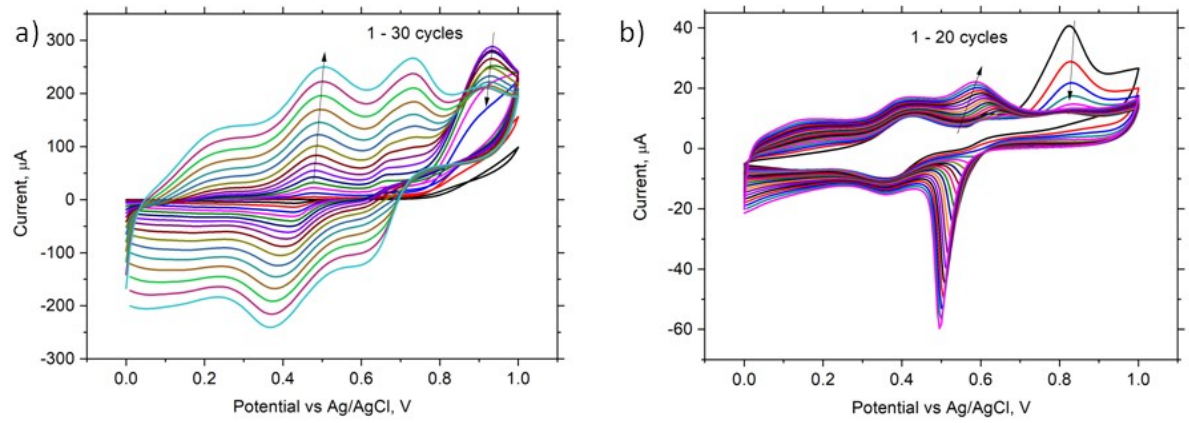

Figure S1. Electrochemical synthesis of the electrocrafted PANI monolayer onto a) the ITO and b) gold substrates

To prepare the electrocrafted PANI monolayer onto Au substrate, the same electrodes were used except for the Au working electrode. Twenty cycles of CV were performed from 0 to 1 V vs Ag/AgCl with a scan rate of 50 mV s^{-1} in a solution containing 50 mM aniline in 0.1 M H_2SO_4 (Fig. S1). After the synthesis, the freshly prepared electrode was washed with ultrapure water using an ultrasonic bath for 35 minutes.

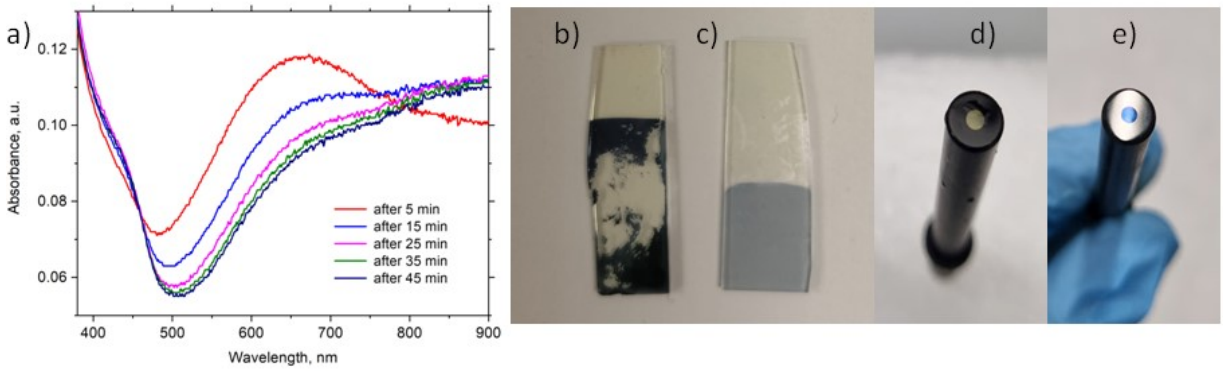

Figure S2. a) UV-vis spectra during ultrasonic washing of the ITO/PANI and ITO/PhNHPrSi/PANI, and photography of b) the ITO/PANI and c) ITO/SPhN/PANI electrodes after the ultrasonic 30 min. washing in dist. water; the Au/SPhN/PANI electrode d) after electropolymerization in 0.1 M H_2SO_4 and e) after the ultrasonic 30 min. washing in dist. water.

Spectroscopy analysis of monolayer

The ITO/PhNHPrSi/PANI electrode was placed in the spectrophotometric cuvette containing 20 mM Britton-Robinson (BR) buffer. The UV-Vis spectrum of the ITO/PhNHPrSi/PANI electrode was registered after different pH from 3 to 10 in BR buffer. The absorbance spectrum of PANI layer was evaluated with respect to a reference spectrum of the ITO/PPTMeSi electrode registered prior to the experiment under similar initial conditions.

Theoretical modelling of the electrografted PANI molecules

For optimisation of the PANI structures, density functional theory (DFT) was used. The structures were optimised by using a hybrid functional B3LYP and a 6-31G(d,p) basis set with the polarization functions on H, C and N atoms followed by calculations of their harmonic vibrational frequencies to verify their stability in the gas phase. The reduced leucoemeraldine, pernigraniline, and emeraldine forms with eight fragments of aniline and one fragment of 3-(phenylamino)propyltrimethoxysilane were used as the models of the electrografted PANI molecules. To compute the FT-IR spectra of these molecules, method based on a hybrid B3LYP functional and 6-31G(d,p) basis set was employed. Also, time depended TD-DFT was applied to compute UV-vis spectra of these forms. A hybrid exchange–correlation the Coulomb-attenuating functional CAM-B3LYP and a 6-31+G(d,p) basis set with a diffuse function (+) were used. For modelling the aquatic environment, the polarizable continuum solvation model for water (C-PCM, dielectric = 78.3) was used. Up to 40 electronic transitions of the molecules were computed. To compare the theoretical data with the spectral shapes of the experimental spectra, the theoretical UV-vis transitions as the Gaussian distribution functions with full width at half maximum of 0.25 eV were weighted. All the computations were carried out by using Spartan'18 software (Spartan'18 for Windows version 1.4.0 Wavefunction, Inc.).

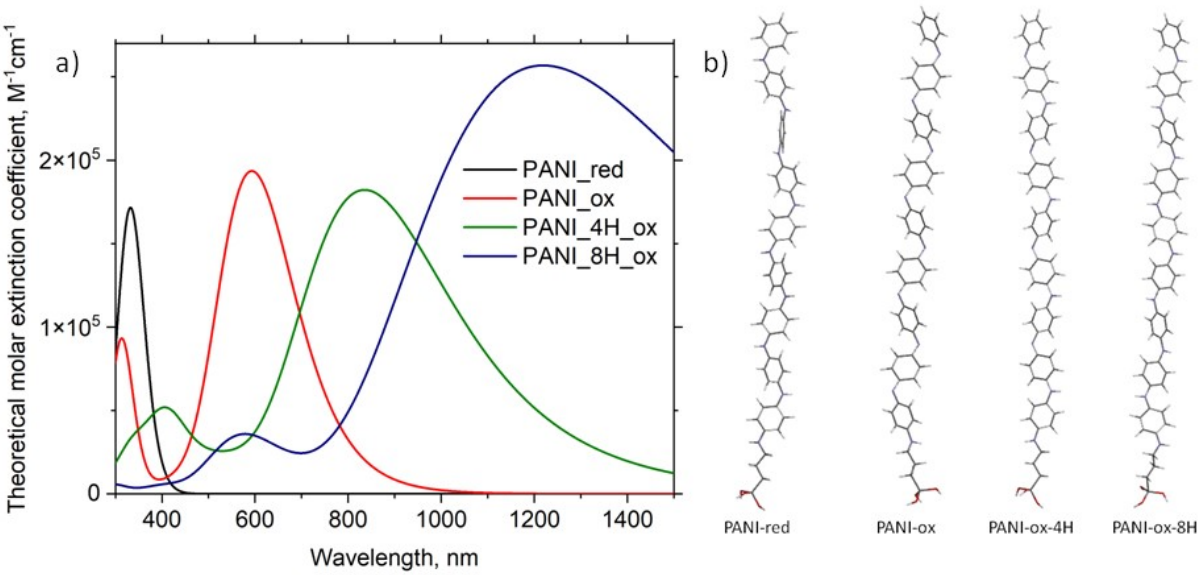

Figure S3. a) The computed UV-vis spectra of the PANI-red, PANI-ox, PANI-ox-4H, and PANI-ox-8H forms of proposed molecules, and b) the optimized structures of these forms

Preparation and investigation of the Au/SPhN/PANI/GOx bioelectrode

The drop of 3 μL of 1 mg mL^{-1} GOx solution in PPB was added onto the electrocrafted PANI monolayer, and the drop was allowed to dry. Before electrochemical investigations, permeable to the analyte (but not to the enzyme) dialysis cellulose-based membrane was added onto the bioelectrode. For electrochemical investigations of this Au/SPhN/PANI/GOx working bioelectrode, an electrochemical cell of 10 mL volume was used. Titanium plate of its area of 1 cm^2 as a counter electrode and Ag/AgCl with saturated KCl as a reference electrode was used throughout these electrochemical measurements. In 10 mL of PPB (50 mM, pH 7.0), solution of 1 M Glu was added in small amounts by mixing using a pipetman. Electrochemical response was measured by chronoamperometry at the potential of -0.4 V vs Ag/AgCl. The Cormay Serum HN sample (Lot/Seria: HN09) was used as the real sample with the declared range of Glu concentration of 4.13–5.05 mM. A sample of 200 μL of this HN serum was added in 2 mL of PPB (50 mM, pH 7.0) solution by mixing and the electrochemical response was measured by chronoamperometry at the potential of -0.4 V vs Ag/AgCl. This experiment was repeated three times (N = 3) and the results were averaged.

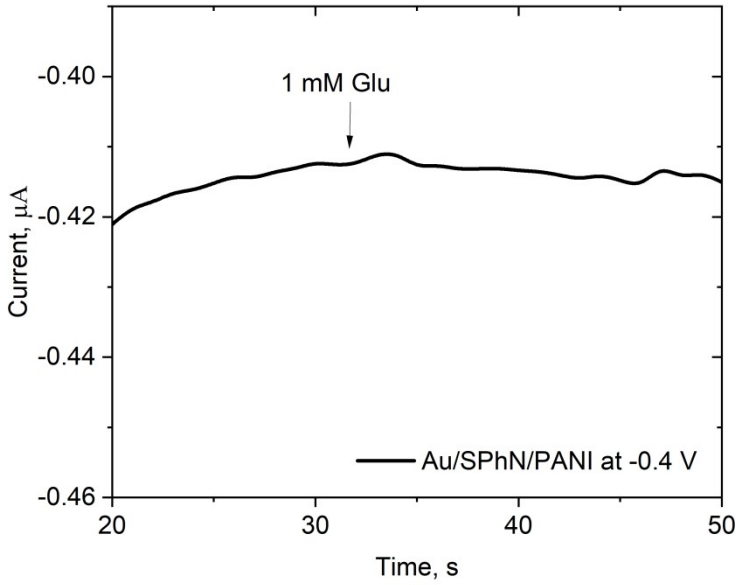

Figure S4. The current response of the Au/SPhN/PANI electrode after the addition of 1 mM Glu at -0.4 V vs Ag/AgCl and pH 7

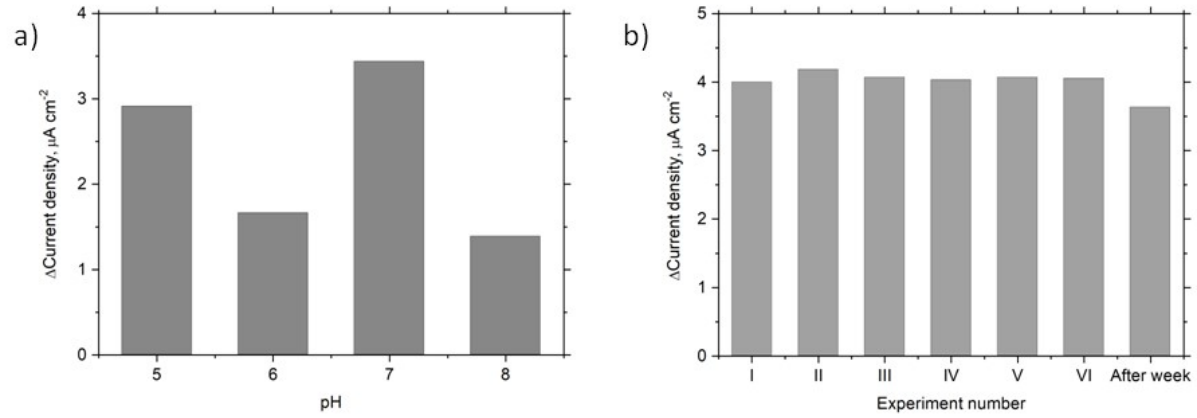

Figure S5. The current responses of this bioelectrode a) at different pHs; b) by repeating the tests six times and after storing at 4 °C in week when 1 mM of Glu and at -0.4 V vs Ag/AgCl

The limit of detection (LOD) at using the 3σ approach was calculated using the equation S1:

$$LOD = \frac{3S}{a} \qquad \text{(S1)}$$

where S represents the standard deviation of the response, a denotes the slope of the calibration curve.

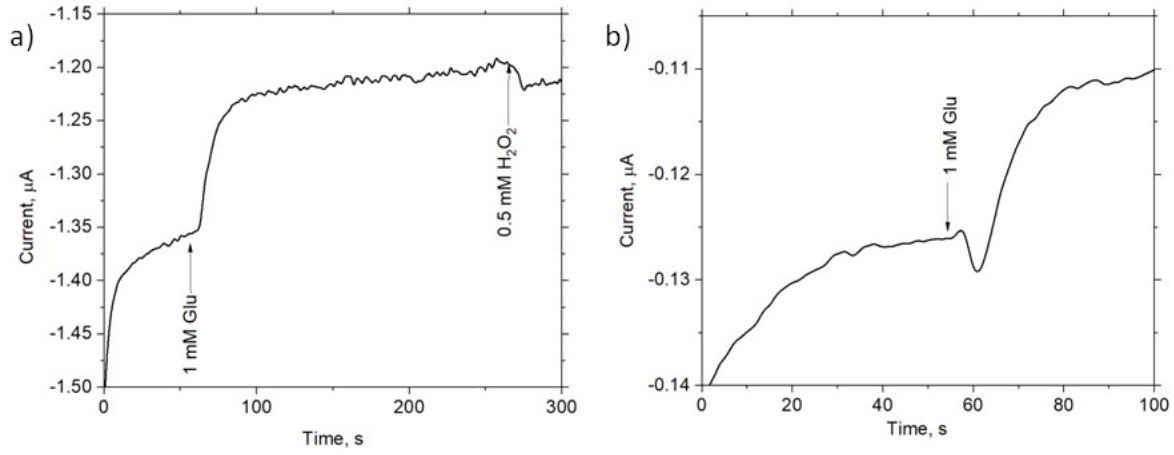

Figure S6. The current response of the Au/SPhN/PANI/GOx bioelectrode a) after the addition of 1 mM Glu and 0.5 mM H_2O_2 and b) after passing argon gas through the electrolyte and the addition of 1 mM Glu at -0.4 V vs Ag/AgCl