Electronic Supporting Information

Graphene oxide-based electrochemical label-free detection of glycoproteins down to aM level using a lectin biosensor

L. Klukova,[a] J. Filip,[b] S. Belicky,[a] A. Vikartovska, [a] J. Tkac[a,*]

Department of Glycobiotechnology, Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 845 38 Bratislava, Slovakia
Centre for Advanced Materials, Qatar University, P.O. Box 2713, Doha, Qatar
* Corresponding author: Jan Tkac, e-mail: Jan.Tkac@savba.sk

Experimental

Chemicals

Potassium hexacyanoferrate(III), potassium hexacyanoferrate(II) trihydrate, potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), sodium acetate (CH_3COONa), acetic acid (CH_3COOH) N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), sodium hydroxide (NaOH), Concanavalin A (Con A, recognising mannose and glucose) and invertase from baker´s yeasts (INV; high content of mannose) were purchased from Sigma Aldrich (USA). Sodium periodate ($NaIO_4$), ethyleneglycol, hydrochloric acid (HCl) and ethanol for UV/VIS spectroscopy (ultra-pure) were purchased from Slavus (Slovakia). Graphene oxide aqueous dispersion solution (GO) was purchased from Graphene Laboratories Inc. (USA) and a carbo-free blocking solution was purchased from Vector Laboratories Inc. (USA). All solutions were prepared using ultrapure deionized water (DW, 0.033 μS).

Preparation of base-washed graphene oxide solution

Base-washed GO solution (c = 500 mg l^{-1}) was prepared according to Coluci *et al.*[1] with minor changes. First the GO solution was sonicated for 15 min, after that, NaOH was added (final concentration of 100 mM) and the mixture was refluxed for 2 h. The resulting product was evidently less homogeneous than the original GO. It consisted of supernatant (containing OD) and black pelletised sediment, which was collected by centrifugation and subsequently reprotonated in 1 M HCl. Such prepared product was washed with DW and lyophilised. The yielded black powder was redispersed in DW (stock solution c = 500 mg l^{-1}) prior further use and the solution is denoted as base-washed GO (GObw).

Electrode pre-treatment, biosensor preparation and EIS assays

Glassy carbon electrodes (GCE, d = 3 mm) were mechanically polished using a polishing pad and alumina slurry (particle size 1 and 0.3 μm, each for 5 min), washed by DW, treated by sonication in DW for 5 min and dried in a dustless environment. Such pretreated electrodes were modified by GO or GObw dispersion (6.5 μL; c = 80 μg ml^{-1}) *via* a drop-casting method and dried under the inert atmosphere (N_2) at room temperature (RT). After that, the electrode surface enriched with carboxylic groups was activated *via* amine coupling (1:1 mixture of 0.2 M EDC and 0.05 M NHS aqueous solutions for 15 min). Con A was subsequently immobilised onto the electrode surface (40 μL; c = 1 mg ml^{-1}) and incubated for 1 h. The last step was blocking the surface with a carbo-free solution (40 μL, 10x diluted stock solution) for 1 h (**Scheme 1**).

Prepared biosensor was incubated with 40 μL of either a solution containing INV or oxINV (glycan part of INV was oxidised using a standard oxidative protocol[2]). Each concentration of the analyte was measured in triplicate with an independent biosensor device and results are provided with a standard deviation (±SD) calculated in Excel. All measurements were carried

out on a laboratory potentiostat PGSTAT 128N (Ecochemie, Netherlands) in a three electrode cell system (auxiliary Pt, reference Ag/AgCl and a working modified glassy carbon electrode) in a phosphate buffer (PB, pH = 7.4, c = 10 mM) containing 5 mM potassium hexacyanoferrate(III) and 5 mM potassium hexacyanoferrate(II). The analysis was run at 50 different frequencies (ranging from 0.1 Hz up to 100 kHz) using Nova Software 1.10 (Ecochemie, Netherlands).

The acquired data were evaluated by the same software using a fitting of the obtained Nyquist plots according to a model Randles-Erschler equivalent circuit R(Q[RW]). Change in a charge transfer resistance (R_{CT}) relative to a reference surface (the biosensor surface after being blocked by a carbo-free blocking solution) expressed in % was used as a signal from measurements. EIS values obtained in presence of proteins were subtracted from values obtained in a plain electrolyte. Proteins were diluted in PB and all stock solutions (Con A, INV, oxINV, EDC and NHS) were stored at -20°C in aliquots.

Serum samples

From a pool of human sera samples available in our laboratory, 2 samples were selected for this study - a healthy female (born in 1973) and RA female patient (born in 1943). The RA patient met the 2010 ACR-EULAR classification criteria for RA.[3] The patients were recruited from the National Institute of Rheumatic Diseases in Piestany, Slovak Republic. Control subjects were recruited from the Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic. All subjects gave their informed written consent, and the study was approved by the Ethics Committee of the National Institute for Rheumatic Diseases, Piestany, Slovak Republic, in accordance with the ethical guidelines of the Helsinki Declaration as revised in 2000. DAS28 coefficient for RA patient mirroring the disease progress was calculated to be 6.1 (0 for healthy individual). Blood samples were collected into polyethylene tubes with

a clotting activator (S-Monovette, Sarstedt AG & Co., Germany). After the centrifugation, the serum aliquots were stored at -80 °C until they were subsequently used for the IgG isolation. For the IgG purification, the MelonGel IgG Spin Purification Kit (Thermo Scientific, U.S.) was used, offering results comparable to those using protein A or G. After the isolation procedure, IgG concentration was measured using DeNovix DS-11+ spectrophotometer at 280 nm (E1% for IgG was 13.7), all samples were diluted to a same protein concentration and were stored at -80 °C until they were analysed.

Characterisation of GO and GObw

Chemical properties of GO and GObw were investigated by Fourier-transform infrared spectroscopy (FTIR; Nicolet 6700 spectrometer, Thermo Fisher Scientific, USA). The spectrum was collected at a resolution of 4 cm^{-1} with 128 scans acquired. Diamond Smart Orbit ATR accessory was applied for measurement in a solid state.

The microstructure was characterized *via* Raman spectroscopy (DXR Raman Microscope, Thermo Fisher Scientific, USA) using 532 nm wavelength of a laser (power: 10.0 mW; exposure time: 4.0 s; number of exposures: 10).

UV/VIS spectra were performed with a spectrophotometer (Jenway 7315, UK) in an aqueous solution.

The Atomic Force Microscopy was used for visualisation of individual biosensor preparation steps – bare surface (Si/SiO_2 wafer), GO-modified surface, the interface after the activation and immobilisation of Con A, the surface blocked by a carbo-free blocking solution and the biosensor interface after binding of an analyte. Measurements were made with a peak force tapping mode in air by a Bioscope Catalyst instrument and Olympus IX71 microscope in conjunction with NanoScope 8.15 software at a scan rate of 0.5 line s^{-1} with the tip set to 200 pN

(Scan Asyst, Bruker, USA). The SCANASYST-AIR silicon tip on nitride lever (Bruker, USA, with f0=50-90 kHz and k=0.4 N m^{-1}) sharpened to have a tip radius of 2 nm was used for AFM visualisation.

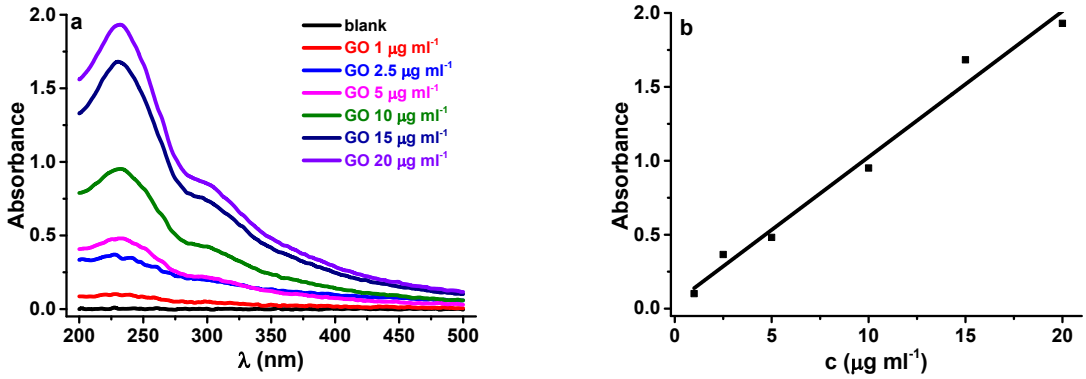

Figure S1: (a) UV-Vis characterisation of GO solutions with various GO concentrations and (b) a dependence of absorbance measured at 230 nm on GO concentration.

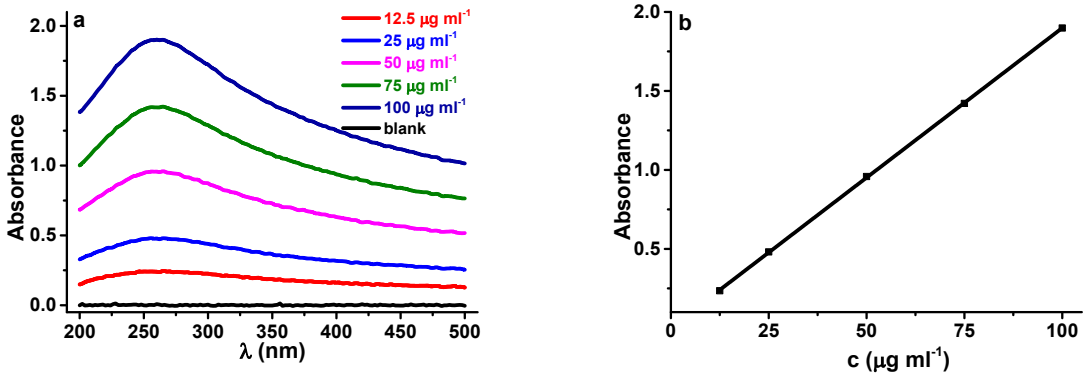

Figure S2: (a) UV-Vis characterisation of GObw solutions with various GObw concentrations and (b) a dependence of absorbance measured at 256 nm on GObw concentration.

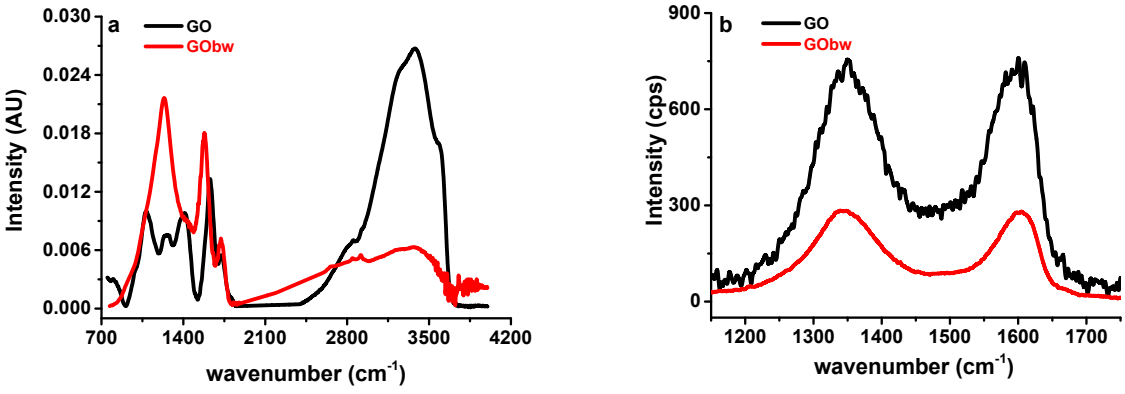

Figure S3: (a) FTIR spectra for GO and GObw and (b) Raman spectra for GO and GObw.

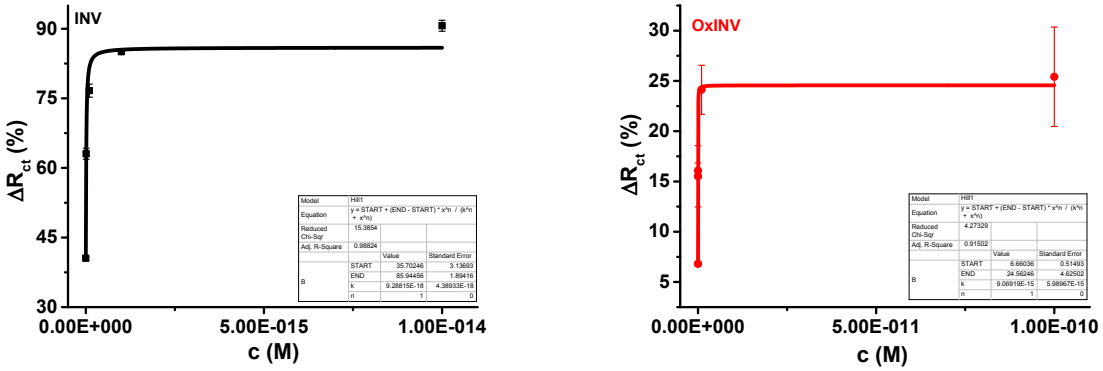

Figure S4: Fitting of calibration curves of the Con A biosensor for its analyte invertase (INV) and a non-specific binding probe oxidised invertase (oxINV) using a non-linear regression with a Hill1 model. In the inset of both figures results of the regression analysis is shown including the equilibrium dissociation constants K_{Dsurf}, which is (9.3 ± 4.4) aM with R^2=0.988 for INV and (9.1 ± 6.0) fM with R^2=0.916 for oxINV.

References

1. V. R. Coluci, D. S. T. Martinez, J. G. Honório, A. F. de Faria, D. A. Morales, M. S. Skaf, O. L. Alves and G. A. Umbuzeiro, *J. Phys. Chem. C*, 2014, **118**, 2187.
2. M. Marek, O. Valentova and J. Káš, *Biotechnol. Bioeng.*, 1984, **26**, 1223.
3. D. Aletaha, T. Neogi, A. J. Silman, J. Funovits, D. T. Felson, C. O. Bingham, N. S. Birnbaum, G. R. Burmester, V. P. Bykerk, M. D. Cohen, B. Combe, K. H. Costenbader, M. Dougados, P. Emery, G. Ferraccioli, J. M. W. Hazes, K. Hobbs, T. W. J. Huizinga, A. Kavanaugh, J. Kay, T. K. Kvien, T. Laing, P. Mease, H. A. Ménard, L. W. Moreland, R. L. Naden, T. Pincus, J. S. Smolen, E. Stanislawska-Biernat, D. Symmons, P. P. Tak, K. S. Upchurch, J. Vencovský, F. Wolfe and G. Hawker, *Arthritis Rheum.*, 2010, **62**, 2569.