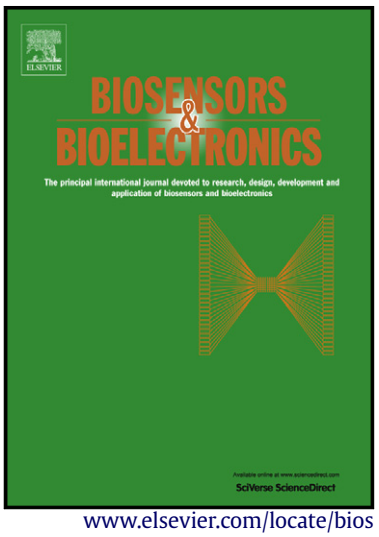


www.elsevier.com/locate/bios

Lysozyme detection on aptamer functionalized graphene-coated SPR interfaces

Palaniappan Subramanian, Adam Lesniewski, Izabela Kaminska, Alexis Vlandas, Alina Vasilescu, Joanna Niedziolka-Jonsson, Emmanuelle Pichonat, Henri Happy, Rabah Boukherroub, Sabine Szunerits

PII: S0956-5663(13)00423-5
DOI: http://dx.doi.org/10.1016/j.bios.2013.06.026
Reference: BIOS6017

To appear in: *Biosensors and Bioelectronics*

Received date: 25 April 2013
Revised date: 7 June 2013
Accepted date: 10 June 2013

Cite this article as: Palaniappan Subramanian, Adam Lesniewski, Izabela Kaminska, Alexis Vlandas, Alina Vasilescu, Joanna Niedziolka-Jonsson, Emmanuelle Pichonat, Henri Happy, Rabah Boukherroub, Sabine Szunerits, Lysozyme detection on aptamer functionalized graphene-coated SPR interfaces, *Biosensors and Bioelectronics,* http://dx.doi.org/10.1016/j.bios.2013.06.026

Lysozyme detection on aptamer functionalized graphene-coated SPR interfaces

Palaniappan Subramanian,[1] Adam Lesniewski,[2] Izabela Kaminska,[1,2] Alexis Vlandas,[3] Alina Vasilescu,[4] Joanna Niedziolka-Jonsson,[2] Emmanuelle Pichonat,[3] Henri Happy,[3] Rabah Boukherroub[1] and Sabine Szunerits[1]*

[1] *Institut de Recherche Interdisciplinaire (IRI), CNRS USR 3078, Université Lille1, Parc de la Haute Borne, 50 avenue de Halley, BP 70478, 59658 Villeneuve d'Ascq, France.*

[2] *Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224*

[3] *Institute of Electronics, Microelectronics and Nanotechnology (IEMN),UMR-CNRS 8520, Université Lille 1, Cité Scientifique, 59655 Villeneuve d'Ascq, France,*

[4] *International Center of Biodynamics, 1B Intrarea Portocalelor, sector 6, 060101, Bucharest, Romania*

Abstract

The paper reports on a surface plasmon resonance (SPR)-based approach for the sensitive and selective detection of lysozyme. The SPR sensor consists of a 50 nm gold film coated with a thin film of reduced graphene oxide (rGO) functionalized with anti-lysozyme DNA aptamer. The SPR chip coating with rGO matrix was achieved through electrophoretic deposition of graphene oxide (GO) at 150 V. Electrophoretic deposition resulted in partial reduction of GO to rGO with a thickness depending on the deposition time. For very short time pulses of 20 s, the resulting rGO film had a thickness of several nanometers and was appropriate for SPR sensing. The utility of the graphene-based SPR sensor for the selective and sensitive detection of proteins was demonstrated using lysozyme as model protein. Functionalization of rGO matrix with anti-lysozyme DNA aptamer through π-stacking interactions allowed selective SPR detection of lysozyme. The graphene-based SPR biosensor provides a means for the label-free, concentration-dependent and selective detection of lysozymes with a detection limit of 0.5 nM.

Keywords: reduced graphene oxide, electrophoretic deposition, surface plasmon resonance, aptamer, lysozyme

1. Introduction

Biosensors based on the detection principle of surface plasmon resonance (SPR) have become routine analytical tools (Wijaya, E. *et al.*, 2011). The promising potential of SPR sensors is their high sensitivity to changes in the refractive index of the sensing medium, occurring upon binding of target molecules (analytes) in solution to their affinity partners (ligands) immobilized on the SPR interface. The quality of the SPR sensor in terms of sensitivity and selectivity is defined by the optical and chemical properties of the SPR device (Touahir, L. *et al.*, 2010).

Most recently, graphene coated SPR interfaces have been theoretically (Choi, S. H. *et al.*, 2010; Wu, L. *et al.*, 2010) and experimentally (Mao, Y. *et al.*, 2011; Salihoglu, O. *et al.*, 2011; Wang, L. *et al.*, 2011; Sing, V. V. *et al.*, 2012; Szunerits, S. *et al.*, 2013) investigated as alternative SPR surfaces. The advantages of graphene-based SPR surfaces are the high surface-to-volume ratio which has proven to be beneficial for efficient adsorption of biomolecules when compared to gold (Salihoglu, O. *et al.*, 2011; Szunerits, S. *et al.*, 2013) and possible π-stacking interactions between the carbon-based ring structures of organic and biological molecules and the hexagonal cells of graphene. In the case of biotinylated BSA, its surface coverage on gold films was 230 ng cm^{-2} whereas on graphene-coated Au SPR chip it was five times more, 1205 ng cm^{-2}(Szunerits, S. et al., 2013)(Szunerits, S. et al., 2013), with a dissociation constant K_D=4.6×10^{-13} mol l^{-1}, approaching the K_D≈10^{-15} mol l^{-1} of biotin-avidin (Szunerits, S. *et al.*, 2013). This strong interaction of biomolecules is, however, a major limitation of graphene-based SPR sensors due to the lack of specificity of these interfaces. Due to the high affinity and specificity of aptamers to a wide range of target molecules, the integration of aptamers as molecular recognition elements onto graphene by simple π-stacking interactions is an easy and promising approach to make graphene-based SPR interfaces selective while keeping the advantage of high protein loading. There is currently only one real sensing application of graphene-based SPR interface relying on the capacity of α-thromin to interact with its specific aptamer in a sensitive and selective manner (Wu, M. *et al.*, 2011).

Our approach described here exploits the direct electrophoretic deposition of GO flakes onto gold SPR interfaces. Electrophoretic deposition (EPD) is a well developed and cost-effective technique (Van der Biest, O. O. *et al.*, 1999) having a number of advantages such as high deposition rate, thickness controllability, good uniformity and simplicity of scale up for the preparation of thin films from charged colloidal suspensions. This approach has been lately widely used for the deposition of micrometer to millimeter thick graphene oxide films onto different interfaces (Wu, Z.-S. *et al.*, 2009; An, S. J. *et al.*, 2010; Koh, A. T. T. *et al.*, 2012). We show here that EPD is also adapted for the deposition of very thin films of reduced graphene oxide (rGO) from GO suitable for graphene-based SPR chips (Szunerits, S. et

al., 2013). Furthermore, the resulting SPR chip can be used for selective and sensitive detection of lysozyme, an enzyme that hydrolyses the polysaccharide walls of bacteria (Vocadlo, D. J. *et al.*, 2001). Increased concentrations of lysozyme in urine and serum are associated with leukemia (Levinson, S. S. *et al.*, 2002) and renal diseases (Harisson, J. F. *et al.*, 1968). The exact determination of lysozyme concentrations is thus of clinically importance. In this paper, we show that nanomolar detection limit can be achieved using graphene-based SPR interface where the specificity is insured by the functionalization of rGO matrix with a lysozyme specific aptamer by π-stacking interaction (Kirby, R. *et al.*, 2004; Rodriguez, M. *et al.*, 2005; Cheng, A. K. H. *et al.*, 2007; Deng, C. *et al.*, 2009; Sassolas, A. *et al.*, 2011).

2. Experimental part

2.1. Materials

Graphite powder (<20 microns), hydrogen peroxide (H_2O_2), sulfuric acid (H_2SO_4), dimethylsulfoxide (DMSO) were purchased from Aldrich and used as received.

The lysozyme aptamer was purchased from Integrated DNA Technologies and had the following sequence: 5'–ATCAGGGCTAAAGAGTGCAGAGTTACTTAG-3' (Cox, C. J. et al, 2011).

2.2. Electrophoretic deposition of rGO onto gold-based SPR interfaces

Gold-based SPR interfaces were formed by depositing 5 nm of titanium and 50 nm of gold successively onto cleaned glass slides by thermal evaporation.

Graphene oxide (GO) was synthesized from graphite powder by a modified Hummers' method (Hummers, W. S. *et al.*, 1958) and the detailed experimental conditions are reported in (Das, M. R. et al., 2011). GO was exfoliated in Milli-Q water (0.5 mg/mL) by ultrasonication for 3 h until the solution became clear with no visible particulate matter.

The electrophoretic deposition was carried out using a two-electrode cell containing the GO aqueous dispersion (0.5 mg/mL) by applying DC voltage (150 V) for 20 s. Platinum (Pt) foil (1 × 2 cm^2) acts as the cathode and the SPR interface [glass/Ti (5 nm)/Au (50 nm)] substrate as the anode. The two electrodes are separated by 1 cm and are placed parallel to each other in the GO dispersion. After deposition, the interface was washed with deionized water (three times) followed by blow drying with nitrogen.

2.3. Functionalization of the SPR interface with lysozyme aptamer

A 1 μM solution of the aptamer solution in PBS was added to the graphene-modified SPR interface in the SPR cell and left incubating for 4 h at room temperature and then washed with PBS buffer.

2.4. Instrumentation

X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) experiments were performed in a PHl 5000 VersaProbe as reported previously (Kaminska, I. et al., 2012).

Raman

Micro-Raman spectroscopy measurements were performed on a Horiba Jobin Yvon LabRam HR Micro-Raman system combined with a 473-nm laser diode as excitation source. Visible light is focused by a 100x objective. The scattered light is collected by the same objective in backscattering configuration, dispersed by a 1800 mm focal length monochromator and detected by a CCD.

SPR Instrumentation

The surface plasmon resonance instrument used was an Autolab ESPRIT instrument (Eco Chemie, Utrecht, The Netherlands) working at 670 nm. The configuration of this equipment is described elsewhere (Kooyman, R. P. H. *et al.*, 1991; Wink, T. *et al.*, 1998). The instrument is equipped with a cuvette of about 100 μL. The prism used had a refractive index of n = 1.52. WinSpall 2.0 software (Max-Planck-Institute for Polymer Research, Mainz) was used to calculate the SPR curves and approximate the experimental dependences with an optical model within the framework of Maxwell macroscopic approach.

A solution of 10 mg/mL lysozyme in PBS was used as stock solution. Dissociation of lysozyme from its complex with the aptamer was achieved by first washing with PBS and then with SDS (10 %) for 20 min.

The data recorded in this manuscript are collected from five different experiments.

3. Results and discussion

3.1. Preparation and characterization of graphene-based SPR interfaces

The graphene-based SPR sensor developed in this work consists of a glass substrate onto which 5 nm titanium adhesion layer and 50 nm gold thin film are deposited, as in conventional SPR, coated with an addional layer of rGO. The rGO top layer is incorporated by electrophoretic deposition of GO, synthesized by chemical exfoliation of flake graphite through a modified Hummers' method. The rGO was deposited by EPD on gold-based SPR interfaces at an applied direct current voltage of 150 V with deposition times varing between 20-40 s (**Figure 1A**). The GO platelets with a zeta-potential of – 35 mV migrate towards the positive electrode, SPR interface, when a DC voltage is applied. Homogeneous films were deposited on the gold interface for depositon times of about 20 s (**Figure 1B**). The thickness of the deposited film was determined by comparing the SPR curves before and after electrophoretic depositon of GO for 20 s using a prism with n_{prisme} =1.52 and a wavalength of λ=670 nm (**Figure 2A**). From the SPR angle shift, a thickness of approximately 2 nm can be deduced corresponding to about 5-6 graphene layers (Szunerits, S. et al., 2013).

Figure 2B shows the Raman spectra of GO and that of the SPR chip coated with rGO (20 s deposition). The spectra display the main features of graphene-based materials with a D-band at 1351 cm^{-1} and a G-band at 1595 cm^{-1} (Wang, H. L. *et al.*, 2009) with a 0.71 D-to-G intensity ratio for GO and I_D/I_G = 0.83 for electrophoretically deposited GO. In the case of the deposited GO, the assymetic 2D band centered at ≈2700 cm^{-1} is observed, indicating graphene or graphite like structured films (Lahav, A. et al., 2009). X-ray photoelectron spectroscopy (XPS) analysis was used to gain further information on the chemical composition of the deposited graphene matrix. The C1s core level XPS spectrum of GO nanosheets is displayed in **Figure 2C** and can be deconvoluted into four components with binding energies at about 283.8, 284.7, 286.7 and 287.9 eV assigned to sp^2-hybridized carbon, C-H/C-C, C-O and C=O species, respectively. The C/O ratio of GO is 1.73 comparable to reported data in the literature (Fellahi, O. *et al.*, 2011; Lia, K.-H. *et al.*, 2011). The electrophoretically deposited GO shows a significant changes in the C1s core level spectrum (**Figure 2C**) with three dominant bands at 283.8, 285.5 and 287.3 eV. The band at 283.8 eV, due to sp^2-hybridized carbon, became predominant, suggesting the reconstitution of the graphitic network under the applied DC voltage. The bands at 285.5 and 287.3 eV are attributed to C-OH and C=O, respectively (Srivastava, S. *et al.*, 2013). The C/O ratio increased to 3.3.

3.2. Sensing of lysozyme

As already mentioned, graphene serves as a good support for biomolecules because of its large surface area (theoretical limit: 2630 m^2 g^{-1}) and rich π-conjugation structure. The efficiency of protein adsorption onto gold and gold/graphene is shown in **Figure 3A** by measuring the SPR responses upon injection of 200 nM solution of cytochrome C (MW=12 kDa), lysozyme (MW=14.4 kDa) or BSA (MW=66.5 kDa)

(**Table 1**). As expected, increasing the molecular weight of the protein while keeping the concentration constant results in increased SPR response. The change of the SPR signal is 7.7 times higher on rGO/Au than on Au SPR chips. Assuming that 1 RU (response unit) change corresponds to 1 ng cm^{-2} mass change at the sensor interface (Wang, J. L. *et al.*, 2008), the coverage is approximately 58 ng cm^{-2} on Au and as high as 405 ng cm^{-2} on rGO/Au chips for lysozyme. The resuls also shows that graphene has no preferential adsorption to any protein.

To obtain an SPR interface specific for lysozyme, the rGO/Au sensor interface was modified with a lysozyme specific aptamer through π-stacking interactions. The final SPR angle shift, resulting form the binding of the aptamer (MW=4038.6 Da), is about 228±30 RU which correlates to a surface coverage of the aptamer of about 228±30 ng cm^{-2}. The value is comparbale to the 188 ng cm^{-2} reported by Dong and co-workers for the adsorption of α-thrombin aptamer onto graphene (Wang, L. *et al.*, 2011).

The sensitivity of the SPR sensor to lysozyme was investigated upon injection of increasing concentrations of lysozyme to the SPR cell (**Figure 3B**). Five replicate measurements were performed for each experiment. In contrast to Dong *et al.* results, the binding between the aptamer and the target molecule, lysozyme, did not greatly disturb the interaction between the aptamer and the rGO matrix (Wang, L. *et al.*, 2011). The aptamer is not released from the graphene-based SPR interface and the SPR signal increased upon addition of lysozyme. A linear relationship between SPR response and lysozyme concentration in the range of 0.5-200 nM was obtained (correlation coefficient of r=0.998 according to RU= 8.1+4.49[lysozyme]) with a slope of 4.5 RU/nM. The detection limit was determined to be 0.5 nM from five blank noise signals (95 % confidence level). The reproducibility of the electrodes is expressed in terms of the relative standard deviation which is found to be 4.3 % at a lyzozyme concentration of 50 nM.

This sensitivity is comparable with an aptamer-based electrochemical lysozyme sensor (LOD=0.7 nM) (Deng, C. *et al.*, 2009) where DNA-functionalized gold nanoaprticles were used to enhance the sensitivity of the aptasensor. It is an order of magnitude lower than the reported colorimetric approach reported by Tseng *et al.* (Chen, Y.-M. *et al.*, 2008). It is also more sensitive than the label-free impedimetric aptasensor based on carbon nanotubes-modified screen printed electrodes with a LOD=862 nM (Rohrbach, F. *et al.*, 2012).

Control experiments were performed to determine the selectivity of the present SPR sensor towards lyzozyme (**Figure 3C**). After immersion in 50 nM cytochrome C, myoglobin and hemoglobin, the changes in the SPR responses were smaller when compared to that recorded with lysozyme but above 5% of the lyzosyme value. Cytochrome C showed the highest level of cross-reactivty. This might be due the

fact that this protein, with a slightly lower molecular weight, displays a comparable isoelecric point as lysozyme (**Table 1**). At neutral pH, cytochrome C has thus a positive charge and the interaction with the negatively charged anti-lysozyme DNA aptamer is favored. However, as shown in **Figure 3A**, the absence of anti-lysozyme DNA aptamer (non functionalized rGO) yields no selectivity for the detection of lyzosome and the interaction with the rGO matrix is simply governed by the size of the protein. This is also the case for gold SPR sensor, where the change of SPR signal is decreased 7.7 times when compared to gold/rGO. The cross-reactivity of (cytochrom C) is ≈25 %, while only between 7-10 % for myglobin and haemoglobin. Thus suggests limited specificity from the gold/rGO/adptamer binding surface.

.

4. Conclusion

The present study demonstrates that electrophoretical deposition of GO onto gold-based SPR interfaces is an interesting alternative for the construction of rGO coated SPR sensors. The newly formed interface allows monitoring the specific binding of lysozyme with an aptamer-modified rGO/Au surface. The sensing interface is easily fabricated by assembling the aptamer onto the rGO coated SPR interface through π-stacking interactions. This approach avoids the labeling of the DNA aptamer with functional groups such as thiol or biotin generally employed for the covalent linking of the aptamer to the sensing interface (Au chip). Indeed, regeneration of the surface did not alter the SPR characteristics. The binding event between the aptamer and lysozyme can be detected as an increase in the SPR response and the magnitude of the signal can be used as a quantitative measure of the concentration of lysozyme in the analyte sample. A detection limit of 0.5 nM was observed with a linear range up to 200 nM. This approach can be employed for the sensing of other proteins and molecules having an identified aptamer as molecular recognition partner. Furthermore, the fabrication process of the rGO-based SPR interface is highly cost-effective using GO. The electrophoretic approach is thus a competitive alternative to the formation of graphene-based SPR interfaces by mechanical transfer of high quality CVD graphene onto the SPR chip.

Acknowledgements

IK thanks the International PhD Projects Programme of the Foundation for Polish Science, co-financed from European Regional Development Fund within Innovative Economy Operational Programme ‘‘Grants for Innovation’’. R.B. and S.S. gratefully acknowledge financial support from the Centre

National de Recherche Scientifique (CNRS), the University Lille 1, Nord Pas de Calais region and the European Regional Development Fund (INTERREG IVa FW1.1.9 "Plasmobio" project). S.S thanks the Institut Universitaire de France (IUF) for financial support. AV gratefully acknowledges financial support from Marie Curie International Reintegration Grant FP7 PEOPLE-2010-RG-277126. PS acknowledges the financial support by the European Commission in RTN-project MATCON (contract no. 238201)

References:

An, S. J., Zhu, Y., Lee, S. H., Stoller, M. D., Emilsson, T., Park, S., Velamakanni, A., An, J. and Ruoff, R. S., 2010. J. Phys. Chem. Lett. 1, 1259.
Chen, Y.-M., Yu, C.-J., Cheng, T.-J. and Tseng, W.-L., 2008. Langmuir 24, 3654.
Cheng, A. K. H., Ge, B. and Yu, N.-Z., 2007. Anal. Chem. 79, 5158.
Choi, S. H., Kim, Y. L. and Byun, K. M., 2010. Opt. Expr. 19, 458.
Das, M. R., Sarma, R. K., Saikia, R., Kale, V. S., Shelke, M. V. and Sengupta, P., 2011. Colloid Surf. B: Biointerfaces 83, 16.
Deng, C., Chen, J., Nie, L., Nie, Z. and Yao, S. Z., 2009. Anal. Chem. 81, 9972.
Fellahi, O., Das, M. R., Coffinier, Y., Szunerits, S., Hadjersi, T., Maamache, M. and Boukherroub, R., 2011. Nanoscale 3, 4662.
Harisson, J. F., Lunt, G. S., Scott, P. and Blainey, J. D., 1968. Lancet 1, 371.
Hummers, W. S. and Offerman, J. R. E., 1958. J. Am. Chem. Soc. 80, 1339.
Kaminska, I., Das, M. R., Coffinier, Y., Niedziolka-Jonsson, J., Woisel, P., Opallo, M., Szunerits, S. and Boukherroub, R., 2012. Chem. Commun. 48, 1221.
Kirby, R., Cho, E. J., Gehrke, B., Bayer, T., PArk, Y. S., Neikirk, D. P., McDevitt, J. T. and Ellington, A. D., 2004. Anal. Chem. 76, 4006.
Koh, A. T. T., Foong, Y. M., Pan, L., Sun, Z. and Chua, D. H. C., 2012. App. Phys. Lett. 101, 183107.
Kooyman, R. P. H., Lenferink, A. T. M., Eenik, R. G. and Greve, J., 1991. Anal. Chem. 63, 83.
Lahav, A., Shalabaney, A. and Abdulhalim, I., 2009. Journal of Nanophotonics 3, 031501
Levinson, S. S., Elin, R. J. and Yam, L., 2002. Clin Chem 48, 1131.
Lia, K.-H., Mittal, A., Bose, S., Leighton, C., Myhoyan, K. A. and Macosko, C. W., 2011. ACS Nano 5, 1253.
Mao, Y., Bao, Y., Wang, W., Li, Z., Li, F. and Niu, L., 2011. Talanta 85, 2106.
Rodriguez, M., Kawde, A. N. and Wang, J., 2005. Chem. Commun. 4267.
Rohrbach, F., Karadeniz, H., erdem, A., Famulok, M. and Mayer, G., 2012. Anal. Biochem. 421, 454.
Salihoglu, O., Balci, O. and Kocabas, C., 2011. App. Phys. Lett. 100, 213110.
Sassolas, A., Blum, L. J. and Leca-Bouvier, B. D., 2011. Biosens. and Bioelect. 26, 3725.
Sing, V. V., Gupta, G., Batra, A., Nigam, A. K., Boopathi, M., Gutch, P. K., Tripathi, B. K., Srivastava, A., Samuel, M., Agarwal, G. S., Sing, B. and Vijayaraghavan, R., 2012. Adv. Funct. Mater. 22, 2352.
Srivastava, S., Kumar, V., Ali, M. A., Solanki, P. R., Srivastava, A., Sumana, G., Saxena, P. S., Joshi, A. G. and Malhotra, B. D., 2013. Nanoscale 5, 3043.

Szunerits, S., Maalouli, N., Wijaya, E., Vilcot, J. P. and Boukherroub, R., 2013. Anal. Bioanal. Chem. 405, 1435.

Touahir, L., Niedziółka-Jönsson, J., Galopin, E., Boukherroub, R., Gouget-Laemmel, A. C., Solomon, I., Petukhov, M., Chazalviel, J.-N., Ozanam, F. and Szunerits, S., 2010. Langmuir 26, 6058.

Van der Biest, O. O. and Vandeperre, L. J., 1999. Annu. Rev. Mater. Sci 29, 327.

Vocadlo, D. J., Davies, G. J., Laine, R. and Withers, S. G., 2001. Nature 412, 835.

Wang, H. L., Robinson, J. T., Li, X. L. and Dai, H. J., 2009. J. Am. Chem. Soc. 131, 9910.

Wang, J. L. and Zhou, H. S., 2008. Anal. Chem. 80, 7174.

Wang, L., Zhu, C., Han, L., Jin, L., Zhou, M. and Dong, S., 2011. Chem. Commun. 47, 7794.

Wijaya, E., Lenaerts, C., Maricot, S., Hastanin, J., Habraken, S., Vilcot, J. P., Boukherroub and Szunerits, S., 2011. Current Opinion in Solid State Mater. Sci. 15, 208-222.

Wink, T., Van Zuilen, S. J., Bult, A. and Van Bennekom, W. P., 1998. Anal. Chem. 70, 827.

Wu, L., Chu, H.-S., Koh, W. S. and Li, E. P., 2010. Opt. Expr. 18, 14395.

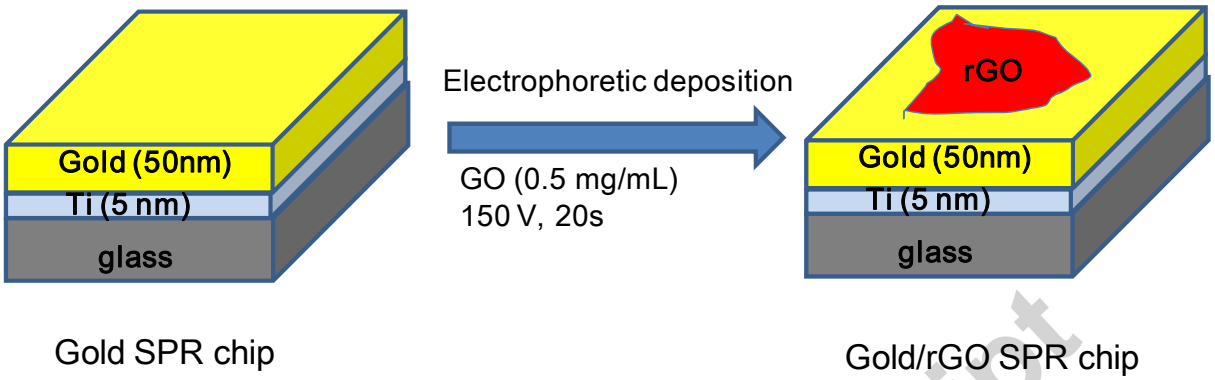
Wu, M., Kempaiah, R., Jimmy Hunag, P.-J., Maheshwari, V. and Liu, J., 2011. Langmuir 27, 2731.

Wu, Z.-S., Pei, S., Ren, W., Tang, D., Gao, L., Liu, B., Li, F., Liu, C. and Cheng, H.-M., 2009. Adv. Mater. 21, 1756.

Table 1: Molecular weight and isoelectric point of the different proteins investigated in this work.

Nr.	**protein**	**Molecular weigth / kDa**	**pI**
1	lysozyme	14.7	11.0
2	cytochrome C	12.4	10.2
3	myoglobin	17.5	6.8
4	hemoglobin	64.5	6.8

(A)



(B)

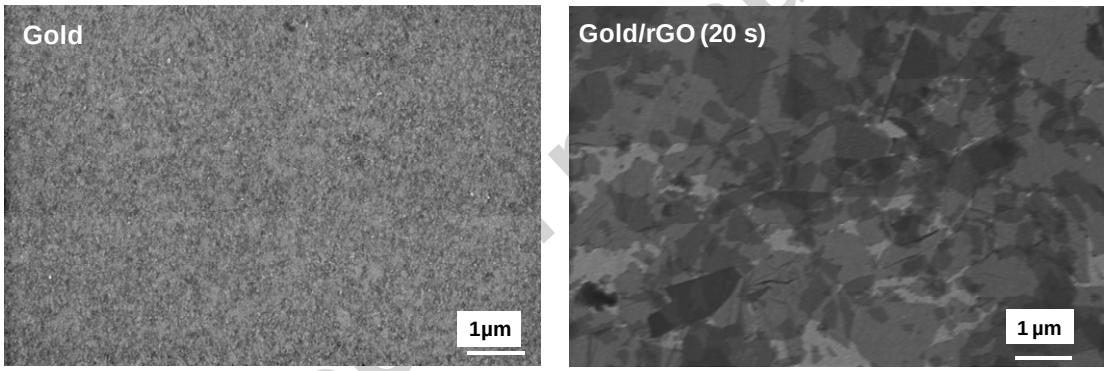
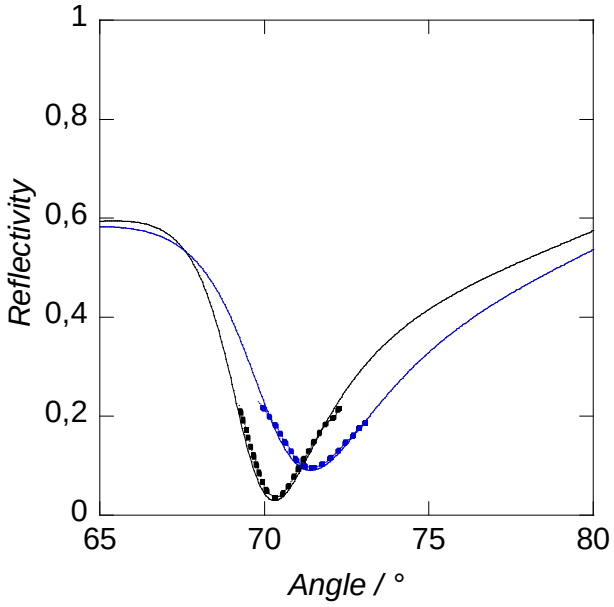
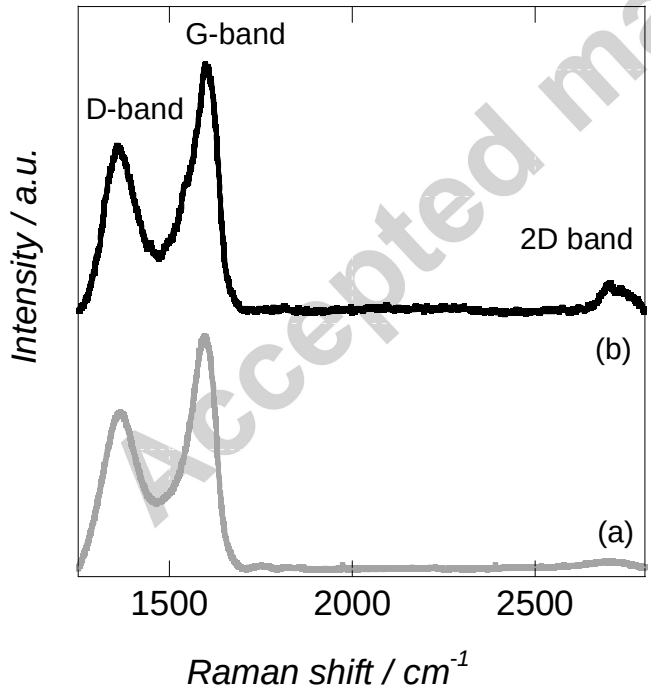


Figure 1: (A) Schematic diagram of the formation of glass/Ti/Au/rGO SPR interfaces through electrophoretic deposition of GO; (B) SEM images of glass/Ti/Au SPR chip before (left) and after deposition of rGO for 20 s (right).

(A)



(B)



(C)

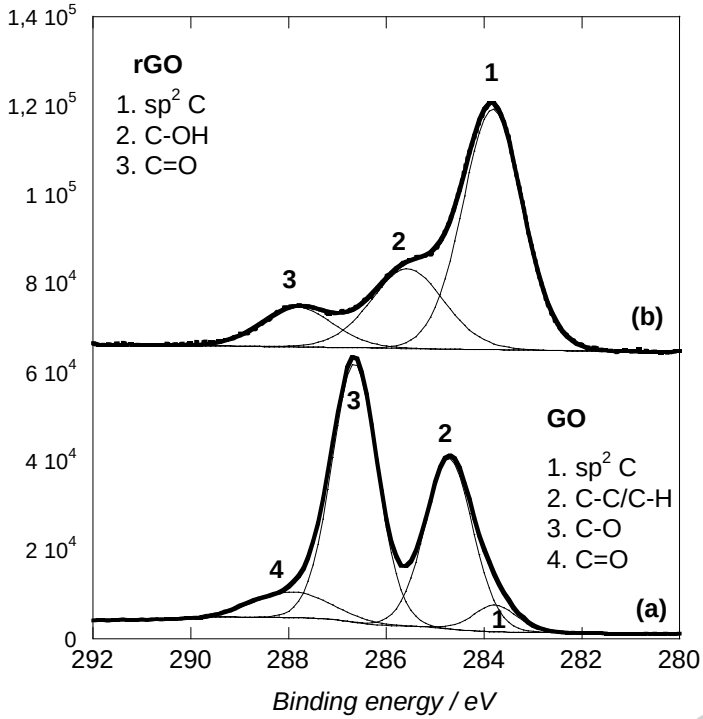


Figure 2: (A) SPR curve of the SPR sensor before (black) and after electrophoretic modification with rGO (blue): deposition time: 20s, DC voltage: 150 V. The dotted lines are the experimental data and the solid lines are SPR fits: [n_{prism}= 1.52, n_{Ti} = 2.36+i3.47 (d=5 nm); n_{Au} = 0.197+i3.67 (d=50 nm); $n_{graphene}$ = 3.0+i1.216; λ=670 nm]; (B) Raman spectra of GO before (grey) and after deposition onto the SPR chip (black); (C) C1s core-level XPS spectra of GO before (a) and after electrophoretic deposition onto the SPR interface (b).

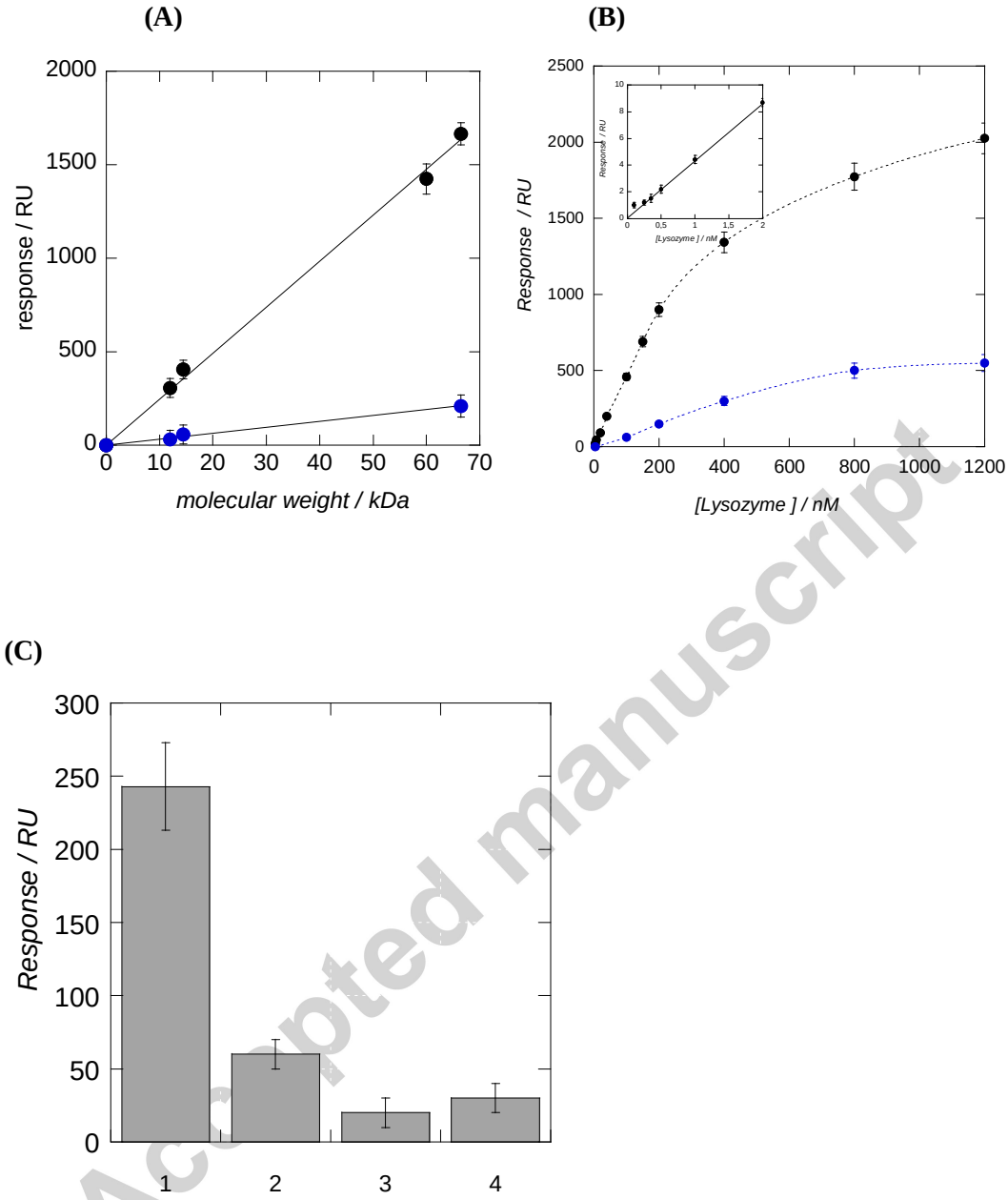


Figure 3. (A) Change of SPR response upon addition of 100 nM solution of different molecular weight proteins (cytochrome C, lysozyme, BSA) onto gold (blue) and gold/rGO SPR chip ; (B) Calibration curve for different concentrations of lysozyme on gold (blue) and gold/rGO (black) inset: standard curve as lower concentraitons; (C) Control experiments with other proteins (50 nM) after interaction for 30 min with graphene-based SPR interface followed by rinsing with PBS: 1. lysozyme, 2: cytochrome C; 3: myoglobin; 4: hemoglobin (See **Table 1**). Five replicate measurements were performed for each experiment.

* The thickness of deposited reduced graphene oxide (rGO) nanosheets can be controlled using an electrophoretic approach

* Pulses of 20 s resulted in rGO films with a thickness of several nanometers approprtiate forSPR senisng

* Functionaliztaion with anti-lysozyme DNA aptamer was achieved through p-stacking interactions with rGO

* Selective and sensitive detection of lysozyme with a detection limit of 0.5 nM was achieved