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Selective lectin multimodal biosensing on functionalized graphene

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Introduction

Graphene has a wide potential for applications in biotechnology such as selective biosensor due to its electronic properties and strict monolayer character. The outstanding sensitivity is hampered by null inherent selectivity, which must be introduced by secondary processing, such as chemical functionalization. Graphene can be functionalized using chemical treatment that enables surface coverage with glucosamines of different length.
Lectins are widespread group of proteins that can recognize and bind sugars with a high degree of specificity. Binding with glycans play key role as recognition determinants and modulators of numerous physiological and pathological processes [1]. This is the main motivation of our study, which is focused on biosensor for their selective detection. *N*-acetylglucosamine (GlcNAc) oligomers bind lectins specifically and reversibly. We investigate the affinity of on-surface grafted glucosamines of different length towards lectin WGA (Triticum vulgaris). In the experiments we use mono- to penta-GlcNAcs and evaluate the strength of binding by competitive series. The GlcNAc-grafted graphene can be used as a selective active layer of lectin sensors using spectroscopic, microscopic, electrical or mass detection [2].

Methods

Monolayer graphene was grown using the low-pressure chemical vapor deposition on a copper foil, transferred on silicon wafer and fluorinated using XeF_2. Reaction with gaseous XeF2 introduces C-F defects in the structure, which are smoothly exchanged for strong nucleophiles, such as propargylamine. The triple bond can be then exploited in the CuAAC reaction with azide-terminated GlcNAc oligomers to tether these carbohydrates onto the monolayer (Fig.1).

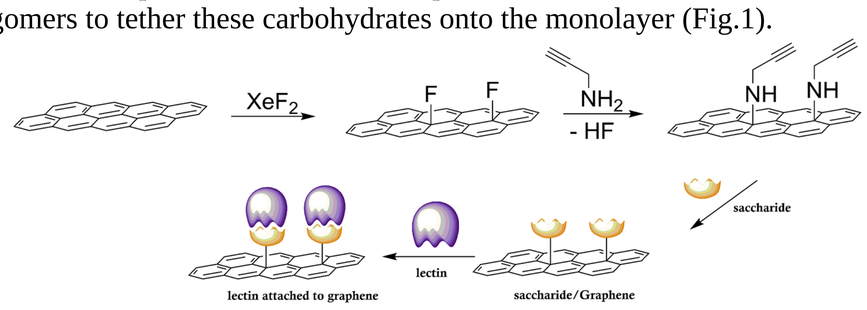

Fig. 1: Functionalization of graphene, covalent grafting of carbohydrates and selective binding of WGA.

We used a wide range of methods to confirm the specific binding between surface-grafted GlcNAcs and WGA – AFM, mass spectrometry, fluorescence microscopy, SERS (Surface-enhanced Raman spectroscopy), contact angle and electrical measurements. The results are summarized in Fig. 2 and confirm the chemo-selective binding.

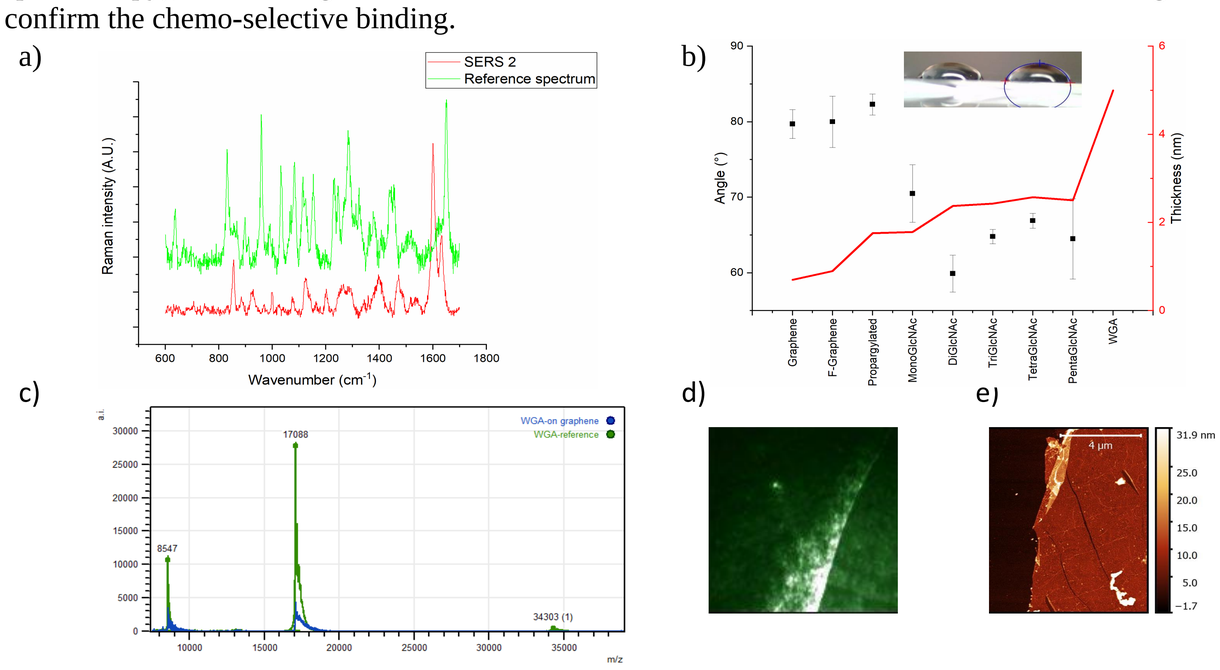

Fig. 2: Modes of detection using the GlcNAc functionalized graphene: a) SERS spectrum showing graphene modes (D 1330 cm-1, G 1600 cm-1) and characteristic vibrational bands from the reference GlcNAc spectrum. b) Results from AFM and contact angle measurement – the growing layer thickness confirms GlcNAcs binding and high about 5 nm verifies bounded lectin (red line). Surface hydrophilicity of graphene increases with hydrophilicity of bound groups. c) Mass spectrometry results confirmed the presence lectin attached to graphene. d) Fluorescence imaging – FITC-labelled WGA is selectively binding to functionalized graphene (visible contrast at the edge of graphene). e) Example of graphene AFM image. Layer thickness was evaluated from height histograms and is plotted in graph b).

Binding avidity

AFM measurements allow to evaluate changes in the height profile that corresponds to the competitive reactions and releasing of the lectin.

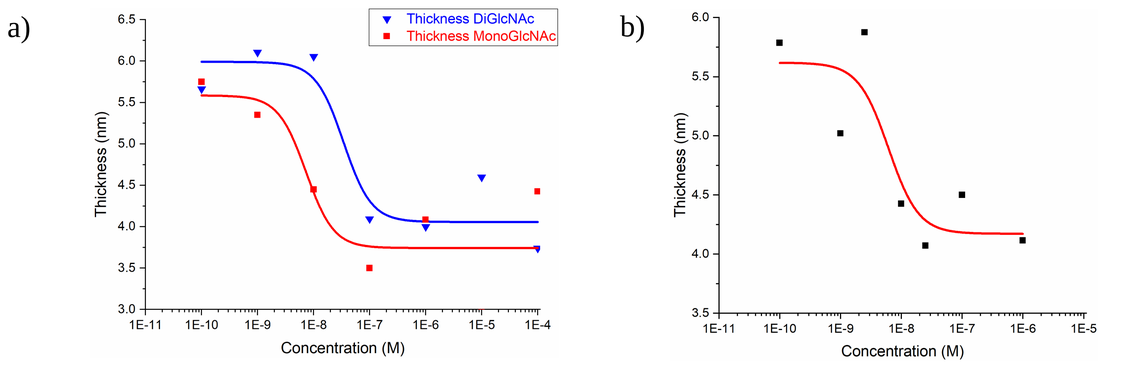

Fig. 3: Results of AFM measurements after applying series of competitive glucosamines. With increasing concentration of the competitor the bound lectin is released and the height profile decreases. a) Results for Mono- and Di-GlcNAc on graphene with MonoGlcNAc as the competitor. With longer chain of GlcNAc the avidity to lectin increase by one order of magnitude. ($k_{MonoGlcNAc}$ = 7.3E−9 , $k_{DiGlcNAc}$ = 3.4E-8). b) Results for lectin bound to PentaGlcNAc on graphene in competition with TetraGlcNAc ($k_{TetraGlcNAc}$ = 6.2E-9). All data fitted with Hill function with positively cooperative binding (Hill coefficient = 1.75 [3]).

Biosensors operating with electrical detection in graphene (resistance, GFET) are particularly attractive [4] [5]. Stern layer is established on the surface of functionalized graphene in solution. Upon lectin binding the charge/ion dynamics in Stern layer is frozen, which can be detected as a change of conductivity.

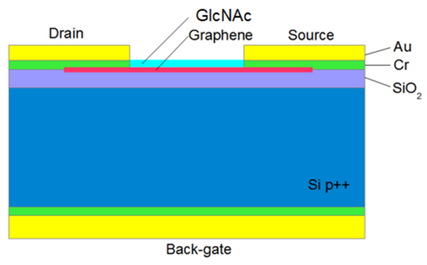

Fig. 4: GFET configuration

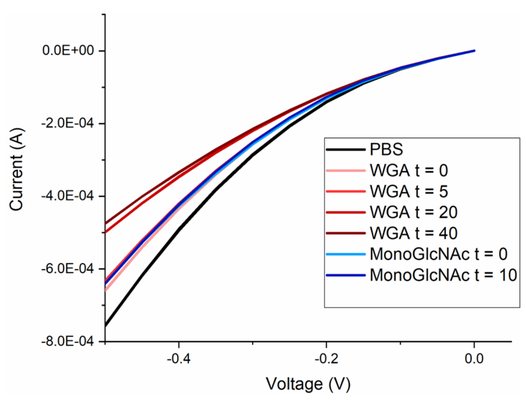

Fig. 5: I-V characteristics of functionalized graphene-based biosensor in the course of the reversible lectin binding and unbinding by MonoGlcNAc.

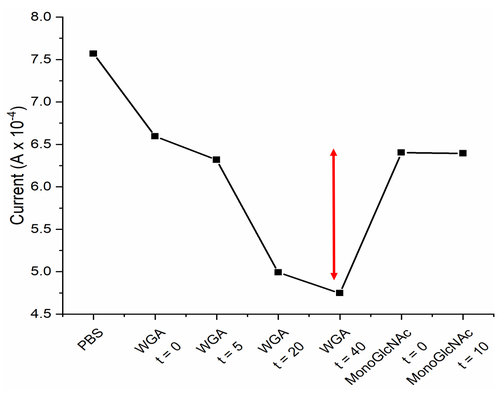

Fig. 6: Readout of the electrical signal from Fig.5 for voltage -0.5 V. Current dropped by 30 % when WGA is bound and restores after competition.

Conclusions

We have demonstrated by variety of methods (SERS, AFM, CA) that we have successfully grafted the graphene surface by GlcNAcs oligomers. Graphene surface becomes more hydrophilic and thicker with increasing length of the carbohydrate moiety and spectral fingerprint is present in SERS.
The glucosamines mioties ensure selective binding towards lectin (WGA). We verified the lectin binding on functionalized graphene by mass spectrometry, fluorescence imaging and AFM. The reversibility of protein-carbohydrate binding was confirmed by competitive essay: increasing concentration of GlcNAc competitor leads to release of the bound lectin. With increasing length of the carbohydrate oligomer on graphene, avidity for lectin increases as well.
Finally, we exploited the results to create a prototype of an electrical sensor which can detect lectin in complex ionic solutions.

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