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When graphene meets lectin for sensitive glycan analysis

The importance of glycans (chains of carbohydrates) in human body is relatively well known. They have a number of essential roles not only in physiology, but also in origin of various diseases. The majority of proteins and lipids have a saccharidic component attached, which structure often reflects the physiological state of the organism and its changes correlate to the development of the disease. In fact, many of the currently approved biomarkers are glycoproteins, which means by focusing on the glycan part of the glycoprotein we will be able to develop more reliable diagnostic (also prognostic or predictive) methods. Unfortunately, to detect these minute alterations, a robust and expensive instrumentation is necessary (usually mass spectrometry and chromatographic methods are employed). Electrochemical methods, like the one we used in our work, are a promising solution addressing this issue, as they allow fast, simple and economical way of analysis. Moreover, thanks to their ability to be miniaturized, electrochemical approach provide a perspective to develop a point-of-care device, more accessible to clinicians and patients.

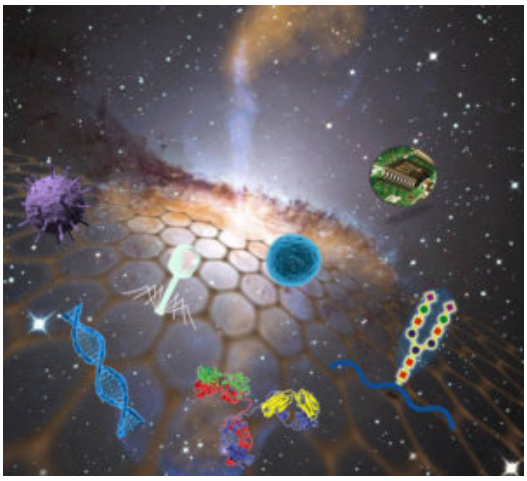

Fig. 1.

Here, we present a biosensor that combines two key elements. Graphene as a revolutionary material, discovered only in 2004 by Andre Geim and Konstantin Novoselov, for which they were awarded the 2010 Nobel Prize in Physics. Graphene is a two dimensional material (one atom thick), composed only from carbon atoms, organized in honeycomb lattice structure. Thanks to its exceptional properties such as mechanical strength, heat and electric conductivity or transparency, graphene found various applications. Especially electric conductivity is an essential characteristic for employment of graphene in biosensor construction. Second necessary element is a lectin. Lectins are group of proteins that specifically bind carbohydrate moieties, either free or as a part of glycoconjugates. Lectins can be isolated from various sources (plants, animals) and different lectins have a different specificity. For example lectin Concanavalin A used in this work, isolated from jack-bean (*Canavalia ensiformis)*, binds α-D-glucose and α-D-mannose. There are already biosensors and bioassays reported that combine lectins and graphene-derived materials, however lectin is usually attached to the surface *via* a linker or a cross-linking polymer and is used for immobilization of antibodies, enzymes or even whole cells, not for the analysis of glycans.

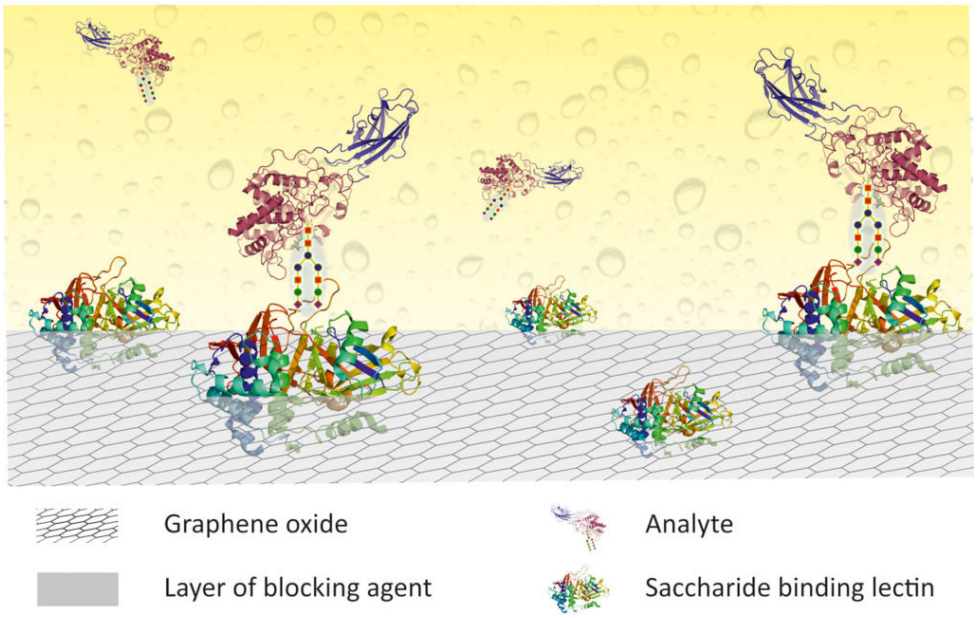

Fig. 2. The key components of the graphene-based lectin biosensor for detection of glycoproteins.

An important aspect in biosensor development is tuning its surface and also electrical properties. Graphene itself is a conductive and hydrophobic material. This may negatively affect the structure of immobilized lectin ConA when in a direct contact with the surface. Contrary to that, graphene oxide (GO), prepared by Hummers method, contains more oxygen groups and oxidation debris (mixture of highly oxidized polyaromatic carboxylated fragments) that are responsible for decreased conductivity, but increased hydrophilicity. When the lectin was immobilized to the surface of graphene oxide, the lectin retains its native structure unlike with hydrophobic graphene surface where the adsorption of the lectin may cause the biointerface to be disfunctional. We also inspected the importance of oxidation debris for the sensitivity of prepared biosensor. Commercially available GO was base-washed (GObw) in order to remove oxidation debris. After evaluation of biosensor prepared from GObw it was observed, that its sensitivity compared to the one prepared on GO was significantly lower. We prepared the first ever published biosensor where lectin for glycan/glycoprotein analysis was directly immobilized on the GO surface, but we also demonstrated that presence of the oxidation debris is essential for biosensor development.

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