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Non-enzymatic detection of glucose levels in human blood plasma by a graphene oxide-modified organic transistor sensor

An extended-gate-type organic transistor (OFET) functionalized with an artificial receptor can selectively detect target glucose as well as minimize interferent effects such as protein adsorption on the detection electrode in human blood plasma by graphene oxide.

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Non-enzymatic detection of glucose levels in human blood plasma by a graphene oxide-modified organic transistor sensor†

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We herein report an organic transistor functionalized with a phenylboronic acid derivative and graphene oxide for the quantification of plasma glucose levels, which has been achieved by the minimization of interferent effects derived from physical protein adsorption on the detection electrode.

The launch of the concept of point-of-care (POC) testing has vigorously promoted the development of sensor technology.¹ Among them, portable sensors for glucose are in high demand because patients with diabetes require self-checking of glucose levels in daily life.² In particular, diabetic complications could be caused by the fluctuation of plasma glucose levels or constant high glucose levels,³ meaning that methods for accurate glucose monitoring are essential. Toward accurate detection of glucose in human blood samples, enzymatic sensors have been widely developed to date. The biosensors based on the detection principle of catalytic reactions of enzymes (*e.g.*, glucose oxidase) possess high selectivity and sensitivity, while interferent effects on the enzyme electrodes could be induced by oxidation of electrochemically active species (*e.g.*, ascorbic acid) in human blood samples.⁴ Moreover, the instability of the biogenic recognition materials against physical and/or chemical stimuli is still a concern in the actual sensing environment.² Taking into consideration the robustness of the sensing system, artificial receptors⁵ are promising recognition materials for real-sample analysis.⁶

The representative artificial receptor for glucose is phenylboronic acid (PBA),^{5a,5c} which reacts with a *cis*-diol moiety

through a dynamic covalent bond. However, general artificial receptors confront issues to gain high selectivity against a specific analyte because of their inherent cross-reactivity.⁶ Hence, sophisticated designs considering the conformation between the *cis*-diol moiety and the boronic acid moiety are necessary for the accurate detection of glucose.⁷ Although such elaborate artificial receptors have been developed to increase the binding affinity to glucose, the influence of a large amount of interferents in human blood samples is not still ignorable. In other words, sufficient sensor designs at molecular levels to retain the favorable discrimination power in real-sample analysis are still at the frontier. To this end, we decided to change our perspective to a molecular recognition portion at a nano-scale for maximizing the selectivity of a single artificial receptor. The significant difference in nanomaterials from single artificial receptors is an extensive large surface originating from the assembled or conjugated structures.⁸ Therefore, the large surface allows a sufficient number of intermolecular interactions at the interface.

A self-assembled monolayer (SAM) functionalized with a molecular recognition moiety provides a two-dimensional detection portion. Considering the nature of SAM-based chemical sensing, their selectivity is significantly influenced by the morphology of the surface.⁹ Although the discrimination of the slight structural differences in saccharides is challenging in a single artificial receptor, the functionalized SAMs could tune the selectivity by the density of receptors. In this assay, a simple structural artificial receptor, 4-mercaptophenylboronic acid (4-MPBA)¹⁰ was selected as a building block for the SAM.

Certainly, the applications of SAMs have been expanded to real-sample analysis in analytical chemistry, and an approach using SAMs has been applied to inhibit interferent effects on metal surfaces.¹¹ In this method, the SAMs play a role in trapping the interferent proteins,¹² whereas their inhibition ability at a large amount of interferents is limited owing to their adsorption capacity. Thus, we focused on the principle of repulsion for the minimization of interferent effects. In the case

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† Electronic supplementary information (ESI) available: Preparation of GO solution, fabrication and characterization details of the OFET and the extended-gate Au electrode, investigation of the detectability of the OFET-based sensor, and spike and recovery tests. See DOI: <https://doi.org/10.1039/d2cc07009j>



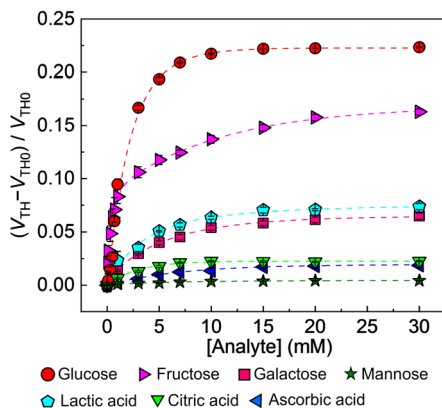


Fig. 3 Response profiles of the GO-OFET-based chemical sensor with 4-MPBA against seven analytes in 0.1 M phosphate buffer with 0.1 M NaCl at pH 7.4. Titration isotherms were obtained by collecting V_{TH} at each concentration of analytes from 0 to 30 mM. The terms V_{TH0} and V_{TH} , respectively, indicate threshold voltages before and after adding the analytes. Three repetitive evaluations were carried out for each analyte.

(Fig. S9(a), ESI \dagger),¹⁰ which might be explained by the change in the surface potential of the electrode derived from proton transfer upon forming the boronate ester.²² Moreover, a sigmoidal response to glucose was observed at a low concentration range (Fig. S7(b), ESI \dagger inset), implying a formation change from a 2:1 to 1:1 complex of 4-MPBA and glucose with an increase in glucose concentration.²³ Furthermore, the selectivity test showed a different response pattern to saccharides from a cross-reactive response in a previous report,^{10a} which might be due to the difference in the molecular densities (Fig. S3(b), ESI \dagger).^{21a}

Next, the role of GO for the minimization of the interferent effects, such as physical protein adsorption¹⁶ to the sensing portion, was investigated by an antifouling ability test against negatively charged proteins (*i.e.*, immunoglobulin G and serum albumin) contained in human blood plasma. In Fig. S10 (ESI \dagger), the OFET-based sensor without GO displayed the gradual time-dependent change in the threshold voltages in the presence of the proteins, suggesting accumulation of the negatively charged proteins on the extended-gate electrode. In contrast, a negligible response to the proteins was observed in the case of the GO-OFET. The ζ -potential of the utilized GO material represented -22.9 ± 1.4 mV at pH 7.4, whereby the suppressed response to the proteins could be explained by electrostatic repulsion between the negatively charged GO surface and the proteins. Moreover, the GO-OFET was also examined in non-diluted human blood plasma toward real-sample analysis (Fig. 4). The OFET without GO displayed much higher interferent effects stemming from the physical adsorption of the proteins to the sensing portion in human blood plasma. Meanwhile, the GO-OFET showed a moderate time-dependent response, which was derived from plasma glucose (*vide infra*) capturing on 4-MPBA as well as suppression of the protein adsorption by GO.

Finally, the GO-OFET was applied to the quantification of glucose levels in human blood plasma. The original concentration of plasma glucose (*i.e.*, 4.4 ± 0.2 mM ($n = 3$)) was

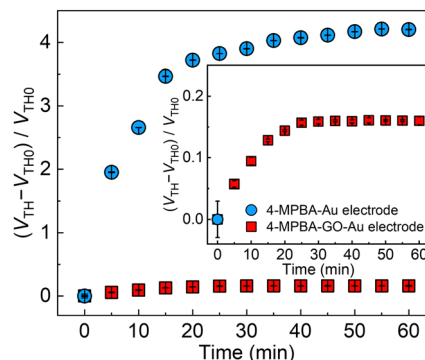


Fig. 4 Antifouling ability test for the OFET in non-diluted human blood plasma. The red squares and blue circles mean the 4-MPBA-attached extended-gate Au electrode with and without GO, respectively. Three repetitive evaluations were carried out for each electrode. Inset shows the enlarged figure for V_{TH} shifts.

determined by a commercial biochemical analyzer using a dry chemistry system. As shown in Fig. 5(a), the transfer curves of the GO-OFET stepwise shifted with an increase in glucose concentration. The obtained titration isotherm represented a non-linear saturation curve (Fig. 5(a), inset), which indicated

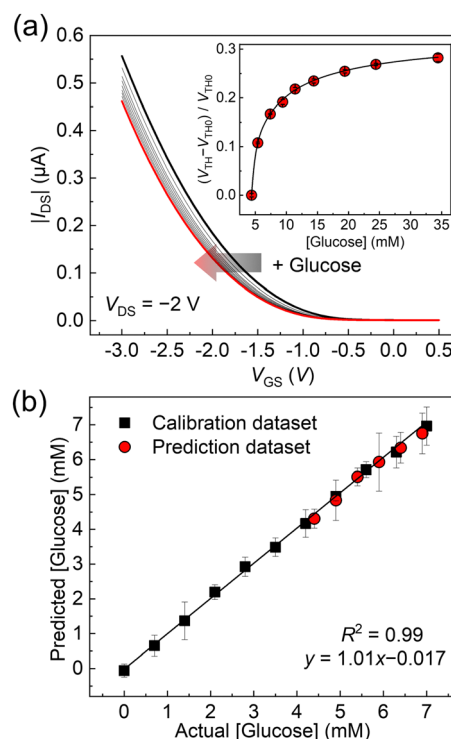


Fig. 5 (a) Transfer characteristics of the GO-OFET-based chemical sensor with 4-MPBA upon the addition of glucose in human blood plasma. The inset represents the titration isotherm obtained by collecting V_{TH} at each concentration of analytes from 4.4 to 34.4 mM with three repetitive measurements. The initial concentration means the original concentration of the plasma glucose determined by the commercial biochemical analyzer (4.4 ± 0.2 mM, ($n = 3$)). (b) Result of the spike and recovery test for glucose in human blood plasma. Three repetitive evaluations were carried out for each concentration.



the successful quantitative detection of glucose levels in human blood plasma. To the best of our knowledge, this is the first example to detect glucose levels in human blood plasma by using FETs functionalized with the PBA derivatives. As a further attempt, a spike and recovery test for glucose in human blood plasma was carried out (Fig. 5(b)). The glucose concentrations were predicted by utilizing the calibration line, and the recovery rates against glucose were determined to be 87–110% (Table S1, ESI†). Indeed, the predicted plasma glucose concentration by the OFET-based sensor (4.3 ± 0.27 mM, in Table S1, ESI†) was comparable to the result of the commercial biochemical analyzer. Therefore, the estimated recovery rates implied the feasibility of accurate analysis by the glucose sensor based on the GO-OFET in real-world scenarios.

In summary, we developed a GO-OFET functionalized with 4-MPBA for accurate detection of glucose levels in human blood plasma. The PBA derivatives have been widely utilized as artificial saccharide receptors, whereas the discrimination of saccharides in real samples containing large amounts of interferents has been challenging owing to the limitation of the receptor design. In our approach, we expected that the selected nanomaterial (*i.e.*, GO) could maximize the sensing ability of the OFET modified with 4-MPBA. Indeed, the GO-OFET successfully quantified glucose concentrations in human blood plasma, which was accomplished by the minimalization of the physical adsorption of the interferent proteins. Judging from the accurate detection of plasma glucose levels by the GO-OFET, we believe that it has potential for the establishment of a POC glucose device based on the fusion technology of organic electronics and nanocarbon chemistry.

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Conflicts of interest

There are no conflicts of interest to declare.

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