ChemistryOpen
Minireviews
doi.org/10.1002/open.202000025
Chemistry Europe
European Chemical Societies Publishing

Protein Assays on Organic Electronics: Rational Device and Material Designs for Organic Transistor-Based Sensors

Tsukuru Minamiki, Riku Kubota, Yui Sasaki, Koichiro Asano, and Tsuyoshi Minami*[a]

Artificial receptor-based protein assays have various attractive features such as a long-term stability, a low-cost production process, and the ease of tuning the target specificity. However, such protein sensors are still immature compared with conventional immunoassays. To enhance the application potential of synthetic sensing materials, organic field-effect transistors (OFETs) are some of the suitable platforms for protein assays because of their solution processability, durability, and compact integration. Importantly, OFETs enable the electrical readout of the protein recognition phenomena of artificial receptors on sensing electrodes. Thus, we believe that OFETs functionalized with artificial protein receptors will be a powerful tool for the on-site analyses of target proteins. In this Minireview, we summarize the recent progress of the OFET-based protein assays including the rational design strategies for devices and sensing materials.

1. Introduction

1.1. Current Status of Protein Assays and Their Issues

The development of protein assays is of enormous benefit to various fields; from basic research in biology to medical diagnosis[1a] and drug discovery.[1b] This is because proteins play important roles in the composition of the human body and the administration of biological functions.[2] Some of the most general methods for protein analyses are immunoassays including enzyme-linked immunosorbent assays (ELISAs),[3] immunochromatography,[4] and western blotting (WB) combined with gel electrophoresis techniques.[3] The immunoassay can sensitively and selectively detect analytes because of the specificity of antibodies for target proteins.[1] Although the conventional assays have significantly contributed to protein analyses in basic research, medicine, and industry, their applications om the on-site quantitative detection and real-time monitoring of proteins are practically limited. This limitation originates from the characteristics of the measurement apparatuses and antibody materials in the immunoassay. For instance, the long-term storage and utilization of antibodies for continuous measurements of protein levels are challenging because of the instability of most antibodies against chemical or thermal stimulation.[5] Furthermore, large-sized equipment (ex., spectrometers, microplate readers, etc.) are required for the quantitative analyses of analytes. Hence, the possible place to accurate detection of target proteins has been limited to large-scale facilities (i.e., major hospitals and institutes) with well-trained operators. In this regard, significant attention is being devoted to the development of compact transducers for immunoassays such as surface plasmon resonance (SPR)[6] and quartz crystal microbalance (QCM).[7] These transduction platforms can accomplish the on-site determination of biomarker proteins. However, they have not gained extensive usage owing to their high costs and low mechanical robustness. Thus, more suitable sensing materials and transducers are desired for the achievement of point-of-care testing and daily management of health conditions through the quantitative information of proteins.

[a] Dr. T. Minamiki, Dr. R. Kubota, Y. Sasaki, K. Asano, Prof. T. Minami
Institute of Industrial Science
The University of Tokyo
4-6-1 Komaba, Meguro-ku
Tokyo 153-8505 (Japan)
E-mail: tminami@iis.u-tokyo.ac.jp

1.2. Toward Next-Generation Protein Assays: Combination of Organic Electronic Devices with Artificial Receptors

As already mentioned, artificial receptors (synthetic sensing materials) are among the most attractive materials for the development of easy-to-use protein assays for various situations. Despite the recent advances in organic synthesis chemistry and supramolecular chemistry, protein recognition by artificial receptors is still challenging topics for use in practical applications.[8] Compared to the naturally-derived sensing materials for proteins (i.e,. antibodies), artificial receptors are superior in terms of chemical and physical stabilities, production costs, and fine-tuning of the sensing ability in line with the required targets. While artificial receptors possess such excellent properties, their applications are still restricted to the purification and extraction of proteins (ex., protein purification columns). This limitation stems from the following reasons. First, the specificity of the artificial receptors for target proteins is insufficient for sensing applications. In immunoassays, the selective detection of proteins can be achieved by an assembly of a highly-ordered interactive portion (=paratope) of the antibody for the residual part of the target proteins (=epitope).[2–4] Unfortunately, it is difficult to imitate such antibody structures as the effective protein-recognition field by only using organic synthetic approaches. Moreover, conventional artificial receptors convert the sensing information of proteins into optical signals. In other words, the large-sized apparatus is also required for the quantitative analysis of the target proteins.

For the realization of on-site protein analyses by utilizing artificial receptors, we believe that organic field-effect transistors (OFETs) are among the best candidate devices as transducers for artificial receptors (Figure 1). OFETs have several attractive features including lightweight, mechanical flexibility and durability, compact integration, and low-cost processability.[9] Thes suggest that OFETs are suitable devices for the preparation of on-site sensing systems. Furthermore, OFET-based devices can directly read the protein recognition behavior of artificial receptors as electrical signals, implying that protein analyses could be performed on a single chip. More importantly, the artificial receptors integrate as self-assembled monolayers (SAMs) on the sensing electrode (=the solid/liquid

interface) of OFETs, which enables the improvement of their sensing ability for proteins.

Thus far, several types of protein assays utilizing OFETs have been reported. In this Minireview, we provide a comprehensive overview of the design strategy for the construction of devices and materials of OFET-based protein sensors. Furthermore, the sensing ability of OFETs with concrete demonstrations in protein detection is discussed in chapters 3 and 4.

2. Device Design of OFETs for the Accurate and Reproducible Detection of Proteins

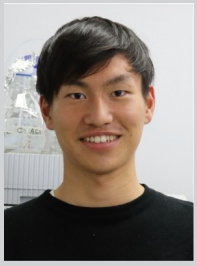
Protein sensing should generally be performed in water; however, the electrical performance of organic semiconductor materials is generally deteriorated when exposed to aqueous media.[10] Therefore, to achieve protein detection with OFET devices, a specific device design is required for exclusive use in protein sensing with practical accuracy and reproducibility. Toward this end, low-voltage operatable OFETs (=transducer) with extended-gate electrodes (=sensing portion) have been proposed (Figure 2). OFETs are commonly composed of three-terminal electrodes (=the source, drain, and gate), a dielectric layer, and an organic semiconductor layer. In the extended-gate structure, the drive unit including the organic semiconductor layer and the detection unit (=extended-gate) is separated, implying that the device can stably detect targets in the water.

The basic operation principle of the extended-gate OFET device is as follows. The accumulation of electric charges at the interface between the semiconductor and dielectric layers occurs due to the voltage impression at the gate terminal (V_{GS}) (i.e., the formation of the semiconductor channel). Then, electric currents will flow through the channel region by applying the

Tsukuru Minamiki was born in 1990 in Hokkaido, Japan. He received his Ph.D. from Yamagata University under the direction of Professor Shizuo Tokito in 2016. During his Ph.D. research, he was appointed as a JSPS Research Fellow (DC) at the same university. He started his academic carrier as a JSPS Postdoctoral Research Fellow (PD) at the University of Tokyo, working with Associate Professor Tsuyoshi Minami. He has been a Research Scientist at National Institute of Advanced Industrial Science and Technology (AIST) since 2018. He is also a Research Collaborator at Institute of Industrial Science, the University of Tokyo. His research interests focus on organic electronics, functional polymers and molecular assemblies for sensing applications, and microchip-based bioassay systems.

Riku Kubota was born in 1988 in Tokyo, Japan. He received his Ph.D. degree in Engineering from Tokyo Metropolitan University in 2016 under the supervision of Professor. Hiroyoshi Kawakami. Between 2016 and 2019, he worked with the same group engaging in the development of metalloporphyrins for biomedical applications, and supramolecular systems for redox catalyses. Currently, he is a Project Research Associate at the University of Tokyo, working with Associate Professor Tsuyoshi Minami. His current researches focus on supramolecular materials for organic field-effect transistors and optical chemosensor arrays.

Yui Sasaki was born in Aomori, Japan, in 1992. She is currently a doctoral course student under the supervision of Associate Professor Tsuyoshi Minami at the University of Tokyo. She is also a JSPS Research Fellow for Young Scientists (DC1) at the same univerisity. During her Ph.D. course, she worked with Professor Yang Tian of East China Normal University (China) in 2019, and Professor Karsten Haupt of Compiègne University of Technology (France) in 2020 on collaborative projects. Her research interests are molecular self-assemled systems for chemical sensors.



Koichiro Asano was born in 1995 in Osaka, Japan. He obtained his B.Sc. at the University of Tokyo in 2019. He is currently a master's course student under the supervision of Associate Professor Tsuyoshi Minami at the University of Tokyo. His research fields are supramolecular chemistry and chemical sensors.

Tsuyoshi Minami was born in Saitama, Japan, in 1983. He obtained his Ph.D. degree from Tokyo Metropolitan University, under the supervision of Prof. Yuji Kubo, in 2011. During his Ph.D. research, he worked with Professor Tony D. James of University of Bath on collaborative researches. Between 2011 and 2013, he was a Postdoctoral Research Associate at Bowling Green State University, working with Associate Professor Pavel Anzenbacher, Jr. In 2013, he was appointed as a Research Assistant Professor at the same university. Thereafter, he proceed to Research Center for Organic Electronics of Yamagata University as an Assistant Professor in 2014. He was appointed as a Lecturer at the University of Tokyo in 2016, and then he has been an Associate Professor since 2019 at the same university. Currently, he is also Visiting Associate Professor at Tokyo Metropolitan University and Yamagata University. His research interests are supramolecular analytical chemistry, self-assembled materials, gold nanoparticles and organic transistors for sensing applications.

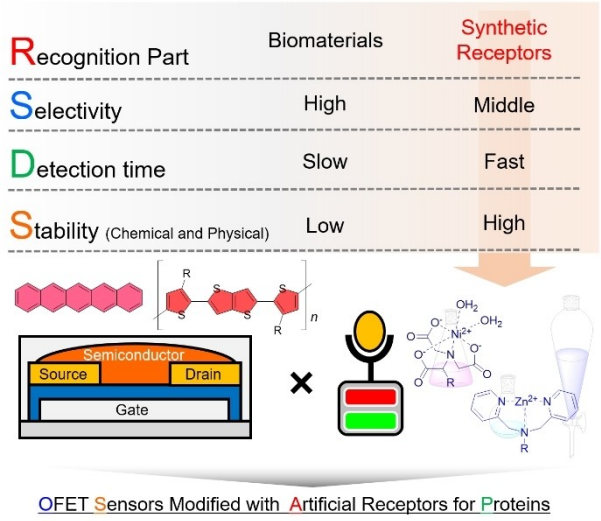


Figure 1. Comparison of the sensing properties of biomaterials and synthetic receptors for protein.

drain voltage (V_{DS}). Thus, the OFET devices behave as a switch in electronic circuits.[11] Here, the required gate voltage for exchanging the operation phase between the electrical "on" and "off" states in the OFETs is called the threshold voltage (V_{TH}). The relationship between the currents and voltages is defined as the following equation (Eqn. (1)):

$$I_{DS} = \frac{W}{2L}\mu C(V_{GS} - V_{TH})^2 \quad (1)$$

where W and L are the width and length of the channel, μ is the field-effect mobility, and C is the capacitance of the dielectric layer at a channel area, respectively.

When target proteins with an electrical charge (Q) are captured at the surface of the extended-gate electrode, V_{TH} changes with increasing protein concentration as shown in the following equation (Eqn. (2)).[12]

$$\Delta V_{TH} = \frac{\Delta Q}{C} \quad (2)$$

The proposed equations indicate that the extended-gate OFET can electrically respond to the protein recognition on the sensing electrode. Since proteins are positively or negatively charged depending on pH conditions, the modification of the gate electrode by protein recognition materials including artificial receptors can allows the electrical detection of proteins by OFETs.

To avoid the water-induced degradation of the OFET during the protein assay, a low-voltage operation of the electrical device is crucial because the unintentional electrochemical phenomena might be induced by applying a high voltage to the aqueous media. According to Eqn (1) and the following Eqn (3), the operation voltage of OFETs depends on the dielectricity and thickness of the dielectric layer.

$$C = \frac{\varepsilon_0 \varepsilon_r}{d} \quad (3)$$

Here, ε_0 is the dielectric constant of the vacuum, ε_r is the relative permittivity of the dielectric material, and d is the thickness of the dielectric layer. These indicate that the low-voltage operation of OFETs for protein sensors can be achieved by utilizing high-capacitive dielectric elements. For instance, we have constructed the extended-gate type OFETs for protein sensing by employing a hybrid-type ultra-thin dielectric film which consists of an oxide film and a self-assembled monolayer (SAM) material.[13] More details of the fabrication process and applied materials for the extended-gate type OFETs are summarized in a recent review article.[14]

Since the first report of a protein assay utilizing an extended-gate type OFET,[13] various types of devices have been reported by many research groups. The representative results of protein detection by OFETs are summarized for each material from the next chapter.

3. Protein Detection Based on Organic Transistors Modified with Biomaterials

3.1. OFET-Based Immunoassays for Proteins

For the comparison of the protein sensing abilities of the extended-gate type OFETs with the general protein assays (i.e.,

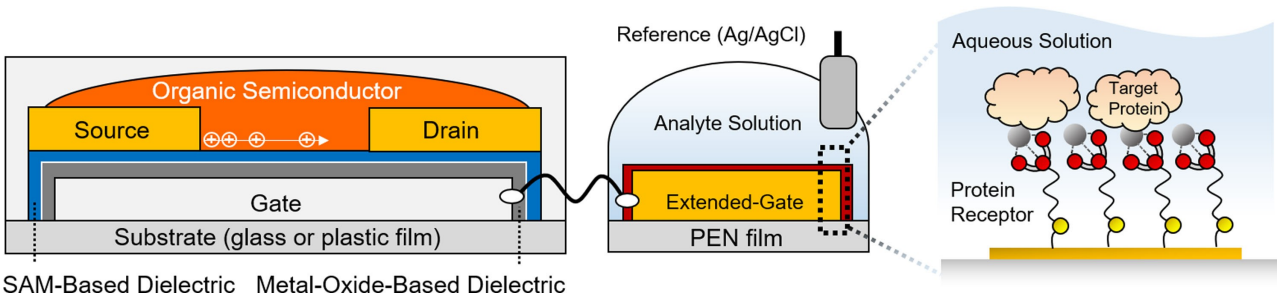


Figure 2. Schematic illustration of the typical device structure of extended-gate type OFETs.

immunoassays), antibodies were the most benefitical materials for the preparation of the OFET-based sensor devices.[15] Herein, we introduce our previous report on the chromogranin A (CgA) detection by using the OFET modified with an anti-CgA antibody (Figure 3).[16] CgA is an acidic glycoprotein that exists in the secretory granules of many endocrine and neuroendocrine cells, and acts on the production and transport of hormone granules.[17] Tumors derived from neuroendocrine cells increase the CgA concentration in the serum and plasma. Thus, CgA can be utilized as a biomarker for neuroendocrine tumors such as carcinoid tumors, pheochromocytoma, neuroblastoma, and small cell lung cancer.[18] Moreover, mental stress might be managed by monitoring CgA in real-time because CgA is associated with mental disorders such as depression.[19] The antibody-modified OFET for the detection of CgA was prepared by the following multi-step procedure. First, the sensing electrode surface was covered with a carboxy-terminated SAM, and streptavidin protein was then allowed to react with the SAM through an amide coupling reaction. Streptavidin is widely employed as a reaction scaffold for proteins due to its extremely high binding-constant with biotin (K_d ~10^{-15} M).[20] Thereafter, a biotinylated anti-CgA antibody was immobilized with the streptavidin-coated sensing electrode (Figure 3a).

As shown in Figure 3b, the positive shifts in the OFET characteristics were observed by increasing the CgA concentration. The positive shifts indicate the accumulation of anionic molecules on the surface of the extended-gate electrode. CgA possesses a negative charge at a neutral pH, meaning that the

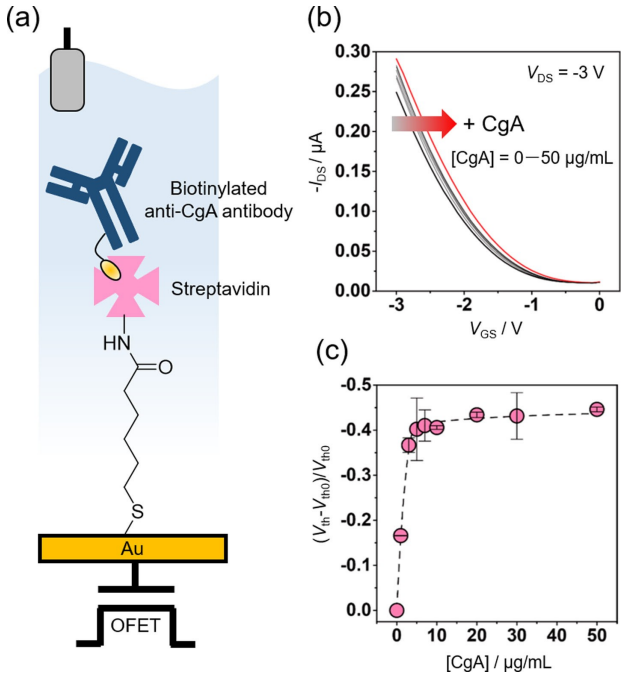


Figure 3. (a) The OFET-based immunosensor for the detection of chromogranin A (CgA). (b) Transfer characteristics of the OFET-based immunosensor with increasing CgA concentration in a PBS solution containing 0.1 wt% HSA. (c) Changes in the threshold voltage of the OFET due to each concentration of CgA in the PBS solution. Reproduced with permission from Ref. 16. Licensed by Creative Commons Attributions 4.0 International (CC BY 4.0).

obtained shift of the output signal could be derived from the capture of CgA. In the fabricated OFET, the limit of detection (LOD) for CgA was estimated to be 0.31 μg/mL (ca. 6 nM) (Figure 3c). Notably, the required time for the OFET-based detection of CgA was 0.5 hours, which was much shorter than that of the typical ELISA (~2.5 hours).[21] The reduced detection time resulted from the unnecessary pre-treatment of the target proteins. Therefore, the OFET could be applied as the device platforms for the on-site detection of proteins without any pre-treatment requirement. However, the calculated LOD value in the demonstrated OFET modified with the antibody was outside the practical concentration range of CgA in real samples. (ca. 0.3 nM).[22] This might be derived from the long gap between the protein recognition portion and the electrode surface. In extended-gate devices, the available region for the electrical read-out of the charge of the targets is generally limited to several nanometers from the electrode surface (=the Debye shielding effect).[23] Therefore, large-sized materials for protein sensing such as the antibody could affect the sensitivity of OFET-based sensors. The relationship between the Debye shielding effect and the sensitivity of the OFET sensor was previously investigated by Bonfiglio et al.[24] Thus, the molecular size of sensing materials and the interfacial design for the extended-gate electrode surface are crucial to improving the sensitivity of the OFET-based protein assays.

To investigate the benefit of the interfacial design to the sensing properties of the OFET-based protein sensors, Song et al. reported a strategy for the surface of the sensing electrode to improve the sensitivity of the OFET-based immunosensor.[25] To detect glial fibrillary acidic protein (GFAP) which is known as a biomarker for traumatic brain injuries,[26] the extended-gate surface was treated with an anti-GFAP antibody along with polyethylene glycol (PEG) layers (Figure 4a). The PEG modification of the extended-gate electrode induces changes in the dielectric properties of the interface between the electrode/aqueous solution. Thus, the electrical responses on the electrode (=the inner region of the calculated Debye length) can be enhanced in accordance with molecular weight of PEGs. The sensing capabilities of the PEG-modified OFET was investigated by employing PEGs with different molecular weights. As shown in Figure 4b, the PEGs with high molecular weights (=PEG 4000, 6000, and 8000) resulted in significant changes in the drain current, while the changes in the drain current were not observed in the OFETs modified with the low-molecular-weight PEGs (=PEG 200, 400, and 1000). The obtained value for the lowest LOD in the fabricated OFETs was determined to be 1.0 ng/mL. These investigations support the fact that the consideration of the Debye shielding effect was crucial to achieving high-sensitive protein assays based on OFET devices.

3.2. Aptamer-Functionalized OFETs for Protein Detection

As mentioned above, the improvement of the OFET-based immunoassays with antibodies is limited by their large sizes. To achieve more sensitive detections of proteins employing OFETs,

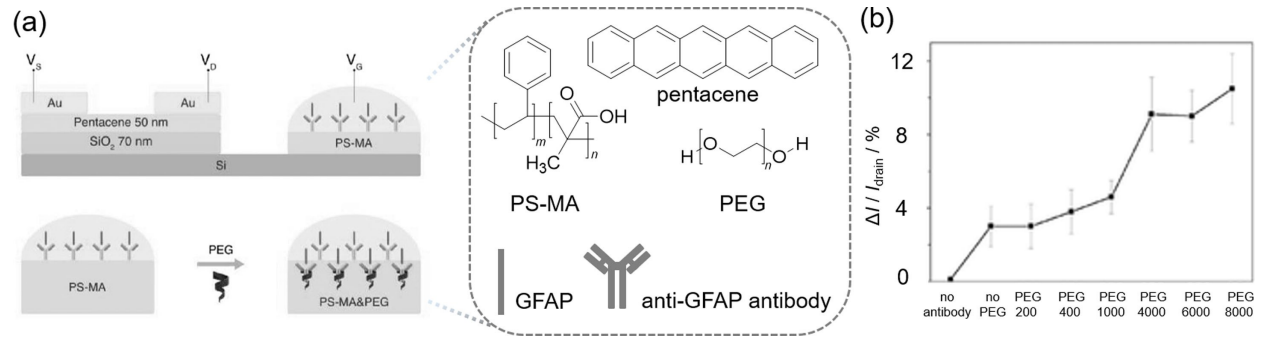


Figure 4. (a) Device architecture of the OFET-based immunosensor for the detection of the glial fibrillary acidic protein (GFAP), and the chemical structures of the materials in the device. The applied anti-GFAP antibody was labeled with a fluorescent probe [i.e., fluorescein isothiocyanate (FITC)] for the confirmation of the immobilization of the antibody on the electrode. (b) Changes in the drain current of the OFET devices modified with each polyethylene glycol (PEG) by adding 100 ng/mL GFAP in the PBS solution. Reproduced and adapted with permission from Ref. 25. Copyright 2017 John Wiley & Sons.

the utilization of small-sized materials for protein recognition is required. With the described limitations in immunoassays, aptamers might be the alternative effective biomaterials for the construction of OFET-based protein assays because of their small size and high-affinity for targets. Aptamers can be readly produced by molecular biology-based methods including systematic evolution of ligands by exponential enrichment (SELEX).[27] Importantly, the aptamer materials demonstrate higher chemical and physical durability than those of antibodies, meaning that various fabrication processes can be employed for the introduction of aptamers into OFETs. For example, Bortolotti et al. produced an aptamer-functionalized OFET for the detection of the interleukin-6 (IL-6) protein.[28] Since IL-6 is presently regarded as a target protein to inhibit its variety of pathological processes, the IL-6 levels can be utilized as biomarkers for the determination of infectious diseases, cancers, and inflammations.[29] To compare the sensing ability of the OFET modified with both antibody and aptamer, the anti-IL-6 antibody or the aptamer which possesses specificity for IL-6 were immobilized on the gate electrode of the OFET. Although the affinities of both sensing materials for IL-6 were almost the same, the electrical responses in the aptamer-modified OFET were slightly more sensitive than those in the OFET-based immunosensor (Figure 5). This suggests that the aptamer-based OFET can be applied for the sensitive detection of the target proteins.

Another example of the aptamer-based OFET was demonstrated by Dorfman et al.[30] The OFET functionalized with the aptamer was designed for the detection of a toxic protein (= ricin).[31] The fabricated device was electrically responsive to increasing the ricin levels (> 1 μg/mL) (Figure 6). In addition, the comparative experiment supported the fact that the electrical changes in the OFET characteristics stemmed from the specific binding of the aptamer to ricin. While the detection of ricin was achieved in real samples such as milk and orange juice, the LOD values obtained in the real samples (ca. 1 ng/mL) were 10 times higher than those obtained in the buffer solution. This is because a large amount of electrical charges in aptamers induces the electrostatic shielding effect to protein recognition in aqueous media exhibiting a strong ionic strength. Moreover, although aptamers are much smaller than antibodies, the electrical responses of OFETs are still restricted by the Debye shielding effect.

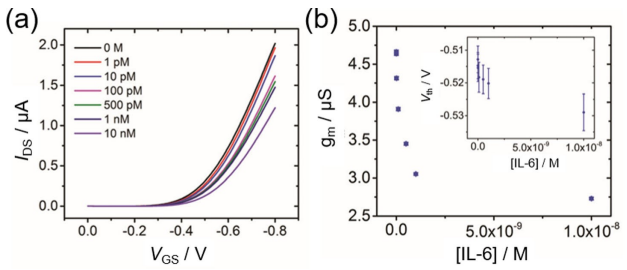


Figure 5. (a) Transfer characteristics of the OFET modified with an aptamer for each concentration of interlekin 6 (IL-6). (b) Changes in the electrical parameter (channel conductance; g_m) of the OFET with increasing CgA concentration. Reproduced with permission from Ref. 28. Licensed by Creative Commons Attributions 4.0 International.

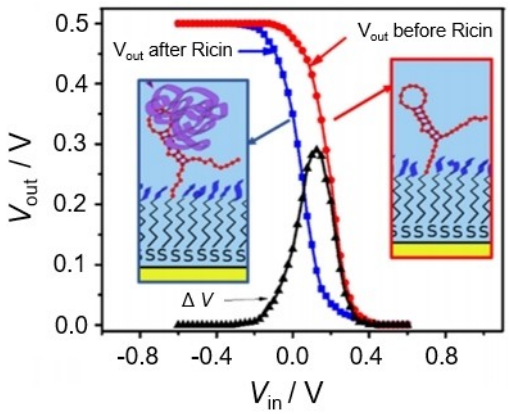


Figure 6. Output signals of the OFET-based aptamer sensor before and after the addition of ricin. Insets illustrate the aptamer-modified extended-gate electrodes. Reproduced and adapted with permission from Ref. 30. Copyright 2016 The American Chemical Society.

4. More Effective Materials for OFET-based Protein Assays: Coordination-Bonding Driven Artificial Receptors

Aptamers have proven to be useful materials for the preparation of OFET-based protein sensors. However, the screening of usable aptamer materials for the detection of target proteins should be performed at each time. Considering the protein sensing results obtained from the biomaterial-based OFETs, we decided to apply artificial receptor materials in the fabrication of OFET-based protein sensors. Coordination bonds are effective as the driving forces for protein recognition based on artificial receptors because they can selectively interact with target residues in proteins.[32] Importantly, metal-complex-based artificial protein receptors can be readily prepared by simple synthesis techniques. Furthermore, their advantage is not only related to their relatively smaller sizes (compared to antibodies and aptamers); it is also related to their high stability against chemical- and physical stimulations. Thus, these types of protein sensing materials can contribute to the practical on-site protein assays based on OFETs.

To compensate for the limitations of the artificial receptors in terms of affinity for target proteins, the artificial receptors were integrated into the form of SAMs onto the electrodes.[33] The selective protein recognition by OFETs can be expected through the cooperative interactions of the integrated artificial receptors with the target proteins.[34] This chapter summarizes our demonstrative results with the OFET-based protein assays integrated with SAMs of artificial receptors.

4.1. Artificial Receptor for the Recognition of the Protein Residue: Nickel(II)-Nitrilotriacetic Acid Monolayer

Since serum albumin is known as a biomarker protein for hepatic failure and nephrotic syndrome,[35,36] the development of an easy-to-use albumin sensor is crucial for medical diagnoses. Hence, we decided to employ a SAM of nickel(II)-nitrilotriacetic acid (Ni^{II}-nta complex) as the sensing portion in the device (Figure 7a).[37] The Ni^{II}-nta complex exhibits a high binding affinity for consecutive histidine residues in proteins, meaning that it can be utilized as the sensing portion of the OFET for the detection of histidine-rich proteins including albumin.[38] For the functionalization of the OFET by the Ni^{II}-nta complex, the nta ligand containing a thiol terminal group was immobilized on the Au extended-gate electrode as the SAM. Thereafter, the nta-modified electrode was immersed in the aqueous buffer containing Ni^{II} to form the Ni^{II}-nta complex on the electrode. As a titration result, the electrical response of the OFET changed with increasing albumin concentration (Figure 7b). The determined LOD value was 40 pg/mL (ca. 0.6 pM). In addition, the device selectively responded to albumin (Figure 7c). The obtained sensitivity in our fabricated device was higher than those of the previous reports using fluorescent probes and immunoassays for albumin.[39–41] This increased sensitivity could be attributed to the following reasons: 1) A long hydrophobic alkyl chain can improve the accessibility of the Ni^{II}-nta terminal to the histidine residues in albumin. 2) The molecular size of the artificial receptor in the SAM might be appropriate for capturing the target proteins at the electrode/liquid interface (*vide supra*).

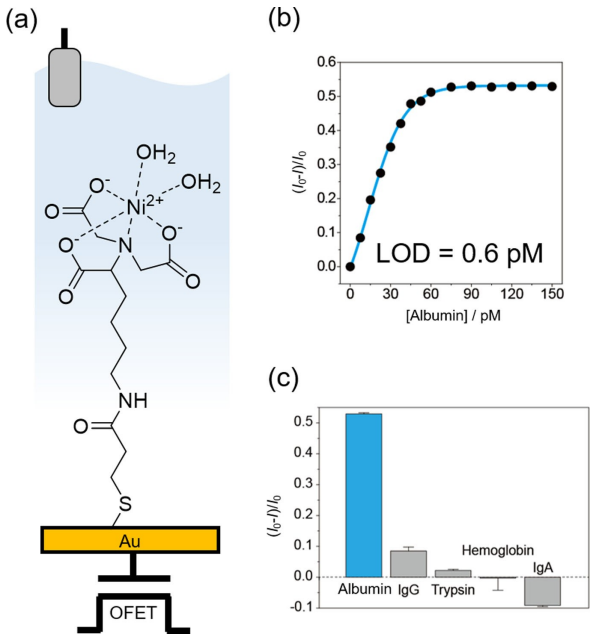


Figure 7. (a) The OFET functionalized with the nickel(II)-nitriloacetic acid (Ni^{II}-nta) SAM for the detection of albumin. (b) Changes in the drain current of the OFET with increasing albumin levels. (c) Changes in the drain current of the OTFT by adding each protein. Reproduced with permission from Ref. 37. Licensed by Creative Commons Attributions 4.0 International.

4.2. Artificial Receptor for the Protein Residue with Chemical Modifications: Zinc(II)-Dipicolylamine Monolayer

To expand the application fields of the OFET-based protein assays, we carried out the electrical detection of a phosphorylated protein by using the artificial receptor-based OFET. The detection of protein phosphorylation is crucial because it is one of the key post-translational modifications (PTMs) for proteins such as metabolic regulation,[42] signal transduction,[43] and carcinogenesis.[44] To demonstrate the detectability of PTMs by OFETs, a zinc(II)-dipicolylamine (Zn^{II}-dpa) complex was employed as an artificial receptor for a phosphorylated protein (= α-casein) (Figure 8a).[45] Because the ingestion of α-casein (as a component of the cow milk) induces food allergies, the detection of α-casein is significant for the quality control of the milk-containing products in food industries.[46] The Zn^{II}-dpa complex can selectively form coordination bonds with the phosphorylated sites of proteins including α-casein.[47,48] To modify the extended-gate electrode with the Zn^{II}-dpa SAM, we firstly synthesized the dpa ligand with the thiol moiety. Thereafter, the thiol-terminated dpa was immobilized on the surface of the extended-gate. Finally, the dpa on the electrode was treated with Zn^{II} to form the SAM of the Zn^{II}-dpa complex. As a

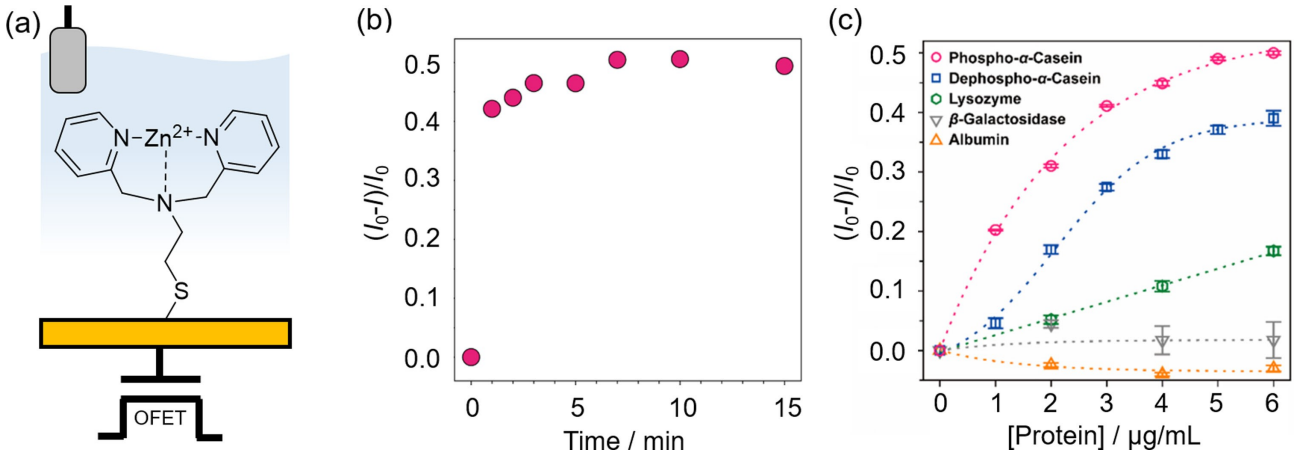


Figure 8. (a) The extended-gate type OFET modified with the artificial phosphoprotein receptor (= the Zn^{II}-dpa SAM). (b) Time-dependency of the electrical response of the OFET to α-casein (6 μg/mL) in a HEPES buffer solution (10 mM) with NaCl (100 mM) at pH 7.4. (c) Changes in the drain currents of the OFET due to the addition of each protein. Reproduced and adapted from Ref. 45. Copyright 2016 The American Chemical Society.

result, we obtained the electrical responses of the OFET to the phosphorylated α-casein by using the Zn^{II}-dpa SAM. On the other hand, no change was observed in the OFET when the dpa monolayer was utilized without Zn^{II}. These results suggest that the electrical responses stemmed from the coordination bonding between α-casein and the Zn^{II}-dpa complex on the extended-gate electrode. Notably, the electrical response was almost saturated in the first few minutes (Figure 8b), indicating that the rapid detection of α-casein by the OFET could be achieved compared with conventional protein assays including ELISAs. Importantly, the fabricated OFET sensor demonstrated a strong response to the phosphorylated α-casein, whereas its response to the partially dephosphorylated one was relatively low (Figure 8c). Furthermore, there was almost no response to the non-phosphorylated proteins. Thus, the OFET sensor with the Zn^{II}-dpa complex as a SAM has the potential to detect the phosphorylation of proteins quantitatively. Overall, these results will lead to the development of OFET-based protein assays for the rapid and quantitative determination of PTMs.

5. Summary and Outlook

In this Minireview, we summarized the recent progress of the development of the OFET-based protein sensors. While OFET-based immunoassays are the simplest approach to achieve the selective detection of target proteins, further improvements in their sensitivity have been deemed difficult due to the Debye shielding effect that occurs at the sensing electrodes; this is because of the large molecular size of antibodies. Hence, the utilization of small-sized materials for protein recognition could contribute to the enhancement of the sensitivity of the OFET-based sensors to target proteins. From this perspective, the aptamers obtained by molecular evolution engineering have been applied as alternative sensing materials for antibodies to prepare the OFET-based protein sensors with high sensitivity. Although the detection of the target protein in real samples was achieved by the aptamer-modified OFET sensor, the protein detection with the sensitivity of a pM level was successfully demonstrated by the artificial receptor-functionalized OFET. The employment of the artificial receptors for the construction of the OFET-based protein sensors was effective in improving the LOD value for analytes and also the electrical detection of the chemical information in proteins such as PTMs (ex., protein phosphorylation). Thus, we believe that OFET devices combined with artificial receptors are powerful platforms for the development of on-site quantitative assays for proteins including disposable sensors for the high-throughput analyses of proteins and wearable sensors for the monitoring of biomarker levels.

While the development of portable protein assays with high-sensitivity, quantitativity, and accuracy is still at its beginning stage, the design of the protein assays from both the "materials" and "devices" viewpoints paved an avenue for the achievement of novel analytical technologies (since there are many types of biomarker proteins and various required situations). In contrast, the accurate discrimination of each target in a crude analyte solution utilizing the artificial receptor-based protein assays is still a challenging topic in the achievement of its practical applications such as in healthcare and medical diagnosis. Thus, the concept of chemosensor arrays affords a hint in the realization of the simultaneous detection of target proteins without any requirement for pre-treatment. In fact, we have proposed high-throughput detection methods of various targets utilizing cross-reactive optical chemosensor systems (i.e., chemosensor arrays) combined with the statistical analysis techniques.[49] Hence, the third viewpoint ("analyses") might further enhance the value of the OFET-based protein sensors with artificial receptors. Consequently, we believe that OFET-based integrated circuits combined with cross-reactive artificial receptors as an OFET array will lead to the development of high-throughput protein sensors that behave as next-generation electronic devices in the near future.

Acknowledgements

This work was financially supported by the Japan Society for the Promotion of Science (JSPS, Grant-in-Aid for Scientific Research, 17H04882) and the Noguchi Institute. YS also thanks JSPS, Research Fellow for Young Scientists (DC1), No. 18 J21190.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: organic transistors · protein assays · self-assembled monolayers · artificial receptors · host-guest chemistry

[1] a) S. Hanash, *Nature* **2003**, *422*, 226–232; b) L. Cohen, D. R. Walt, *Chem. Rev.* **2019**, *119*, 293–321.
[2] J. M. Berg, J. L. Tymoczko, G. L. Gatto Jr., L. Stryer, *Biochemistry, 8th ed.*; Freeman: New York, **2015**.
[3] J. R. Crowther in *Molecular Biomethods Handbook* (Eds.: R. Rapley, J. M. Walker), Humana Press, New York, NY, **1998**, pp. 595–617.
[4] J. B. G. Kwapinski in *Methodology of Immunochemical and Immunological Research*, Wiley-Interscience, John Wiley & Sons Inc., New York, NY, **1973**.
[5] a) A. W. P. Vermeer, W. Norde, *Biophys. J.* **2000**, *78*, 394–404; b) R. M. Daniel, M. Dines, H. H. Petach, *Biochem. J.* **1996**, *317*, 1–11.
[6] S. Zeng, D. Baillargeat, H.-P. Ho, K.-T. Yong, *Chem. Soc. Rev.* **2014**, *43*, 3426–3452.
[7] C. I. Cheng, Y.-P. Chang, Y.-H. Chu, *Chem. Soc. Rev.* **2012**, *41*, 1947–1971.
[8] R. Kubota, I. Hamachi, *Chem. Soc. Rev.* **2015**, *44*, 4454–4471.
[9] H. Sirringhaus, *Adv. Mater.* **2014**, *26*, 1319–1335.
[10] D. Kumaki, T. Umeda, S. Tokito, *Appl. Phys. Lett.* **2008**, *92*, 093309.
[11] G. Horowitz, *Adv. Mater.* **1998**, *10*, 365–377.
[12] T. Sakata, R. Fukuda, *Anal. Chem.* **2013**, *85*, 5796–5800.
[13] T. Minamiki, T. Minami, R. Kurita, O. Niwa, S. Wakida, K. Fukuda, D. Kumaki, S. Tokito, *Appl. Phys. Lett.* **2014**, *104*, 243703.
[14] R. Kubota, Y. Sasaki, T. Minamiki, T. Minami, *ACS Sens.* **2019**, *4*, 2571–2587.
[15] T. Minamiki, Y. Sasaki, S. Su, T. Minami, *Polym. J.* **2019**, *51*, 1–9.
[16] T. Minamiki, T. Minami, Y. Sasaki, S. Wakida, R. Kurita, O. Niwa, S. Tokito, *Sensors* **2016**, *16*, 2033.
[17] B. H. Fasciotto, J. C. Denny, G. H. Greeley Jr, D. V. Cohn, *Peptides* **2000**, *21*, 1389–1401.
[18] J. M. Conlon, *Regul. Pept.* **2010**, *165*, 5–11.
[19] J. E. Allgrove, E. Gomes, J. Hough, M. Gleeson, *J. Sports Sci.* **2008**, *26*, 653–661.
[20] P. S. Stayton, S. Freitag, L. A. Klumb, A. Chilkoti, V. Chu, J. E. Penzotti, R. To, D. Hyre, I. Le Trong, T. P. Lybrand, R. E. Stenkamp, *Biomol. Eng.* **1999**, *16*, 39–44.
[21] M. Stridsberg, B. Eriksson, K. Öberg, E. T. Janson, *J. Endocrinol.* **2003**, *177*, 337–341.
[22] Y. Nishikawa, J. Li, Y. Futai, N. Yanaihara, K. Iguchi, T. Mochizuki, M. Hoshino, C. Yanaihara, *Biomed. Res.* **1998**, *19*, 245–251.
[23] T. Goda, Y. Miyahara, *Langmuir* **2012**, *28*, 14730–14738.
[24] S. Lai, M. Barbaro, A. Bonfiglio, *Appl. Phys. Lett.* **2015**, *107*, 103301.
[25] J. Song, J. Dailey, H. Li, H.-J. Jang, P. Zhang, J. T.-H. Wang, A. D. Everett, H. E. Katz, *Adv. Funct. Mater.* **2017**, *27*, 1606506.
[26] F. K. Korley, R. Diaz-Arrastia, A. Wu, J. K. Yue, G. T. Manley, H. I. Sair, J. V. Eyk, A. D. Everett, *J. Neurotrauma* **2016**, *33*, 215–225.
[27] E. J. Cho, J.-W. Lee, A. D. Ellington, *Annu. Rev. Anal. Chem.* **2009**, *2*, 241–264.
[28] C. Diacci, M. Berto, M. Di Lauro, E. Bianchini, M. Pinti, D. T. Simon, F. Biscarini, C. A. Bortolotti, *Biointerphases* **2017**, *12*, 05F401.
[29] C. A. Hunter, S. A. Jones, *Nat. Immunol.* **2015**, *16*, 448–457.
[30] S. P. White, S. Sreevatsan, C. D. Frisbie, K. D. Dorfman, *ACS Sens.* **2016**, *1*, 1213–1216.
[31] European Food Safety Authority (EFSA), *EFSA J.* **2008**, *6*, 1–38.
[32] K. L. Haas, K. J. Franz, *Chem. Rev.* **2009**, *109*, 4921–4960.
[33] J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo, G. M. Whitesides, *Chem. Rev.* **2005**, *105*, 1103–1170.
[34] Z. Xu, S. Jia, W. Wang, Z. Yuan, B. J. Ravoo, D.-S. Guo, *Nat. Chem.* **2019**, *11*, 86–93.
[35] R. Spinella, R. Sawhney, R. Jalan, *Hepatology* **2016**, *10*, 124–132.
[36] R. P. Hull, D. J. Goldsmith, *BMJ.* **2008**, *336*, 1185–1189.
[37] T. Minamiki, Y. Sasaki, S. Tokito, T. Minami, *ChemistryOpen* **2017**, *6*, 472–475.
[38] G. B. Sigal, C. Bamdad, A. Barberis, J. Strominger, G. M. Whitesides, *Anal. Chem.* **1996**, *68*, 490–497.
[39] P. Xue, K. Zhang, Z. Zhang, Y. Li, F. Liu, Y. Sun, X. Zhang, C. Song, A. Fu, B. Jin, K. Yang, *Appl. Biochem. Biotechnol.* **2012**, *166*, 1604–1614.
[40] K. Zhang, C. Song, Q. Li, Y. Li, K. Yang, B. Jin, *Hum. Vaccines* **2010**, *6*, 652–658.
[41] D.-L. Ma, W.-L. Wong, W.-H. Chung, F.-Y. Chan, P.-K. So, T.-S. Lai, Z.-Y. Zhou, Y.-C. Leung, K.-Y. Wong, *Angew. Chem. Int. Ed.* **2008**, *47*, 3735–3739.
[42] O. Pagel, S. Loroch, A. Sickmann, R. P. Zahedi, *Expert Rev. Proteomics* **2015**, *12*, 235–253.
[43] L. N. Johnson, R. J. Lewis, *Chem. Rev.* **2001**, *101*, 2209–2242.
[44] M. Ashcroft, M. H. G. Kubbutat, K. H. Vousden, *Mol. Cell. Biol.* **1999**, *19*, 1751–1758.
[45] T. Minamiki, T. Minami, P. Koutnik, P. Anzenbacher Jr, S. Tokito, *Anal. Chem.* **2016**, *88*, 1092–1095.
[46] P. Spuergin, M. Walter, E. Schiltz, K. Deichmann, J. Forster, H. Mueller, *Allergy* **1997**, *52*, 293–298.
[47] A. Ojida, T. Kohira, I. Hamachi, *Chem. Lett.* **2004**, *33*, 1024–1025.
[48] T. Minami, T. Minamiki, S. Tokito, *Chem. Lett.* **2016**, *45*, 371–373.
[49] V. Hamedpour, Y. Sasaki, Z. Zhang, R. Kubota, T. Minami, *Anal. Chem.* **2019**, *91*, 13627–13632.

Manuscript received: January 30, 2020
Revised manuscript received: April 22, 2020