Printed-Circuit-Board-Based Two-Electrode System for Electronic Characterization of Proteins

Sara Talebi, Souhad M. A. Daraghma, Ramesh T. Subramaniam,* Subha Bhassu, Georgepeter Gnana Kumar, and Vengadesh Periasamy*

ABSTRACT: Proteins have been increasingly suggested as suitable candidates for the fabrication of biological computers and other biomolecular-based electronic devices mainly due to their interesting structure-related intrinsic electrical properties. These natural biopolymers are environmentally friendly substitutes for conventional inorganic materials and find numerous applications in bioelectronics. Effective manipulation of protein biomolecules allows for accurate fabrication of nanoscaled device dimensions for miniaturized electronics. The prerequisite, however, demands an interrogation of its various electronic properties prior to understanding the complex charge transfer mechanisms in protein molecules, the knowledge of which will be crucial toward development of such nanodevices. One significantly preferred method in recent times involves the utilization of solid-state sensors where interactions of proteins could be investigated upon contact with metals such as gold. Therefore, in this work, proteins (hemoglobin and collagen) were integrated within a two-electrode system, and the resulting electronic profiles were investigated. Interestingly, structure-related electronic profiles representing semiconductor-like behaviors were observed. These characteristic electronic profiles arise from the metal (Au)—semiconductor (protein) junction, clearly demonstrating the formation of a Schottky junction. Further interpretation of the electronic behavior of proteins was done by the calculation of selected solid-state parameters. For example, the turn-on voltage of hemoglobin was measured to occur at a lower turn-on voltage, indicating the possible influence of the hem group present as a cofactor in each subunit of this tetrameric protein.

INTRODUCTION

With the world advancing toward miniaturization, the exciting prospect of fabrication of nanodevices may only be practically possible through molecular electronics.7 Current conventional evaporation and deposition methods involving inorganic silicon-based electronics do not allow progression beyond the microscale owing to the natural physical limitations.2 In this context, biomolecules such as nucleic acids and proteins are of interest due to their unique functional and complementary properties such as molecular recognition, self-assembly and folding, and the ability to mediate electric charges.3,4 These nanoscale polymers offer high potential for structural manipulation that could be utilized in nanoconstructions for various applications.5,6 The key advantage of the molecular approach is the ability to design and fabricate devices using a cost-effective and environmentally friendly “bottom-up” approach.7

More recently, researchers have been exploring the possibility of using proteins8 to exploit built-in advantages for tasks such as electron transport, photochemical conversion, and molecular recognition.9 Proteins, also known as polypeptides, are the most structurally complex molecules that can be found in an organism. Formed by a combination of amino acids through peptide bonds, proteins form three-dimensional (3D) structures that directly reflect their functions. The fundamental properties of amino acids, mainly their hydrophobicity, steric attributes, and the electronic properties of their side chains (R-group), determine the 3D structure of each protein.10 Amino acids as building blocks of proteins play an important role in defining a protein structure by the formation of α helixes and β sheets. Interactions between amino acid side chains influence folding preferences. Proteins carry electric charges which arise from conformational forces between side chains and domains. Electron flow through a protein molecule involves charge transport within the molecule and electron exchange with the environment.11 This process has been investigated at different levels including short-range and long-range conductance of single molecules and supramolecular structures.12–14 The tools and techniques used for elucidation of electrical characterization of proteins in this case mainly involve scanning probe microscopy (SPM) with an...
effort to minimize structural changes or damage during the process.\textsuperscript{15}

The process of directed electron movement, or electron flow, through protein structures is called electron transport (ETp) or electron transfer (ET), highly influenced by its localized environment. The process in which the electron movement occurs while all or part of the protein is in direct contact with an ionically conducting electrolyte, which can function as an electron sink or source through a redox process is called ET. ET in proteins has been studied in a number of model proteins such as cytochrome \textit{c},\textsuperscript{16} plastocyanin,\textsuperscript{17} and azurin\textsuperscript{18–20} and involves indirect measurement through electrochemical techniques. On the other hand, in the absence of an electrolyte or presence of a nonconducting solution, the electron movement is called ETp.\textsuperscript{21,22} ETp studies mainly involve current (I)–voltage (V) characterization of protein monolayers in the solid-state using techniques such as atomic force microscopy (AFM) and scanning tunneling microscopy (STM).\textsuperscript{23} Unlike ET, the principles involved in ETp in proteins are less understood, mainly due to the lack of ionic charges surrounding the protein in solid-state monolayers. Therefore, the ET mechanisms, such as hopping and tunneling, have been utilized as models to describe and interpret ETp in proteins.

Recently, printed circuit board (PCB) technology has been introduced as a cost-efficient and reliable method for the fabrication of biosensing platforms.\textsuperscript{24,25} The term Lab-on-PCB introduced recently\textsuperscript{26} is an indication of growing interest in this aspect. Commercially fabricated PCB provides industrial-grade electrode quality while maintaining standardized design. In an effort to investigate the electronic profiles due to ETp in selected proteins, the current study uses a PCB-based electrode configuration that allows \textit{I}–\textit{V} characterization of metal–protein–metal (MPM) structures. This cost-effective PCB-based method was proved to be able to generate highly reproducible electronic profiles and may be developed into a potentially simple and accurate protein characterization tool. Hemoglobin (Hb), a globular tetramer with a molecular weight of about 64 kDa, and collagen (Coll) type I, consisting of triple helices of elongated fibrils weighing around 300 kDa, were selected as two structurally different proteins for this study.

\section*{RESULTS AND DISCUSSION}

Figure 1 shows the \textit{I}–\textit{V} characteristics of Hb (a) and Coll (b) in the positive region. Nonlinear profiles are observed for both proteins in the positive bias region, representing junction properties akin to a Schottky junction similar to the RNA- and DNA-specific junctions reported previously.\textsuperscript{27–30} This could imply that DNA, RNA, and proteins (in this case Hb and Coll) demonstrate a semiconductor-like behavior when “sandwiched” between two metal electrodes. It can be observed that Coll conducts higher current compared to Hb (Figure 1c).

To interpret the electronic behavior of the proteins in the two-electrode system proposed in this work, it is important to consider the factors affecting conductivity in proteins. The studies on ETp in proteins are limited to certain model proteins such as cytochromes, azurin, and bacteriorhodopsin\textsuperscript{23} thus, the results could not be generalized to all proteins. Recent studies, however, show that conductance in proteins depends on the protein structures. A study on Hb and superoxide dismutase (SOD) as \textalpha{}- and \textbeta{}-domain proteins respectively showed that the current signal of Hb is stronger than that of SOD; therefore, the \textalpha{}-domain is more conductive.\textsuperscript{31} Both proteins used in this work are \textalpha{}-domains; therefore, some other structural differences might be the reason behind this. The electronic properties of the amino acid side chains and their conformation influence the folding preferences along the peptide backbone, thereby determining the 3D structure of proteins.\textsuperscript{10}

In a study on electron coupling in model peptides with \textalpha{}-helix and \textbeta{}-strand motifs including all natural amino acids, it was proven that backbone-mediated electron coupling is significantly influenced by the type of amino acids and the side chain conformations.\textsuperscript{32} Using electronic structure calculation, it was predicted that electronic coupling through the bridges containing proline is remarkably higher than those containing all other side chains, concluding proline as a superbridge. This finding was also in agreement with previous experiments demonstrating proline to facilitate ET.\textsuperscript{33,34} Proline is the second most abundant amino acid in Coll, and its presence might be related to the higher current in this protein compared to Hb.
As can be seen from Figure 1, the deviation from mean is relatively higher in Coll above voltage 2 V. Molecular damage or structural changes occur when biomolecules are exposed to a high electric field. Especially in proteins, exposure to an electric field affects their conformational properties causing structural transitions from helices to random coils, and the unfolding significantly influences their electronic properties. However, the degree of molecular changes is reversely proportional to structural stability. Unlike Coll, the average $I$–$V$ graph plotted for Hb exhibits smaller standard deviations indicating the higher reproducibility of the profiles even at higher voltages. Hb is a well-known example of a highly stable protein as a result of the multi-subunit (tetramer) quaternary structure. In general, previous studies on the electronic properties of RNA and DNA molecules, and the current protein study, demonstrated the highest instability of conductance profiles above 2 V in RNA. This may result from the lower degree of molecular stability owing to the single-stranded polynucleotide structure as compared to the double helix of DNA or the highly complex 3D structure of proteins.

In the negative region, nonlinear $I$–$V$ profiles with an insulating region followed by a significant current conductance above a knee voltage were observed, where both proteins showed similar $I$–$V$ characteristics (Figure 2). The different conductance patterns as compared to the positive region could be attributed to the fact that the current flow across a semiconductive barrier in the positive and negative regions is governed by different mechanisms. The electronic behavior of biomolecules upon application of a negative bias is poorly understood. Therefore, there is a need for further research to better elucidate the negative biased $I$–$V$ characteristics of proteins.

The sweep profiles of Hb and Coll were obtained by combining the $I$–$V$ profiles of each sample in the positive and negative regions to avoid molecular damage if exposed to a drastic change of voltage (−3 to +3 V or vice versa), commonly practiced in inorganic $I$–$V$ characterization. As demonstrated in Figure 3a, the sweep profile of each sample generally exhibits similarity to what is observed for a conventional semiconductor junction. The semi-log $I$–$V$ characteristics of protein-specific Schottky structures are shown in Figure 3b. The effect of series resistance ($R_s$), arising from the contact wires or bulk resistance of the protein molecule itself, is noticeable when the current curve of the In $I$–$V$ plot is dominated by $R_s$ at higher voltages. Lower series resistance, in general, demonstrates the higher efficiency of the junctions, indicating lower internal resistance.

A number of solid-state parameters were extracted from the $I$–$V$ characteristics using two different methods. Resistance profiles ($R$–$V$) of the protein samples calculated based on the conventional method while neglecting the effect of series resistance is shown in Figure 3c. The value of shunt resistance ($R_{sh}$) was obtained as the highest peak value of the $R$–$V$ profiles at 133.067 and 53.944 MΩ for Hb and Coll, respectively. Higher shunt resistance suppresses the recombination of charge carriers at the junction interface and decreases current leakage.

According to the conventional method, while ignoring the effect of series resistance, the $I$–$V$ characteristics of the forward bias are defined by

\[
I = I_0 \exp \left( \frac{qV}{nkT} \right)
\]  

(1)

where $V$ is the voltage drop across the barrier, $n$ is the ideality factor, $I_0$ is the reverse saturation current, $k$ is the Boltzmann constant, $T$ is the temperature, and $q$ is the electron charge. The value of ideality factor ($n$) can be derived using the gradient of the ln $I$–$V$ graphs in the positive region using eq 1 rewritten as

\[
n = \frac{q}{kT} \frac{dV}{d(\ln I)}
\]  

(2)

Idiety factor measures the conformity of a diode to an ideal diode with 1 being the scale. Using the above formula $n$ was calculated as 17.91 and 19.80 for Hb and Coll, respectively. Reverse saturation current ($I_0$) and knee voltage, as negative bias solid-state parameters, have also been shown in Table 1.

Figure 2. $I$–$V$ characteristics of (a) Hb and (b) Coll and (c) comparison of both in the negative bias region of 0 to −3 V. Experiments were carried out in five replicates to show the reproducibility of the profiles.
lead to the eventual electrical breakdown of the junction (not observed within the voltage range applied in this work), was obtained from the negative bias $I−V$ plots for each sample. However, Cheung and Cheung proposed that the effect of $R_s$ should not be neglected; therefore, the forward bias characteristics of the $ln I−V$ should be calculated using

$$I = I_0 \exp \left[ \frac{q(V - IR_s)}{nkT} \right]$$

(3)

where $IR_s$ is the voltage drop across the series resistance of the device. Therefore, the value of $R_s$ can be determined from

$$\frac{dV}{d(ln I)} = \frac{nkT}{q} + IR_s$$

(4)

By plotting $\frac{dV}{d(ln I)}$ versus $I$ (Figure 4), the value of $R_s$ can be determined from the gradient of the linear part of the graph and the ideality factor from the term $\frac{nkT}{q}$, which is the y-axis intercept. $R_s$ values obtained from this method were calculated as 397.60 and 277.50 kΩ for Hb and Coll, respectively. The ideality factor of the protein–metal diodes according to Cheung and Cheung's method showed significant divergence from the values calculated conventionally, which was in agreement with the literature reported previously. The $n$ values were reduced to 6.68 and 12.76 for Hb and Coll, respectively. The larger ideality factor values generally indicate a deviation from ideal diode-like behavior. The ideality factor value of 1 basically represents an ideal diode, where rectification purely occurs as a result of charge emission due to thermionic processes. In the Hb and Coll samples studied, the higher than unity values calculated demonstrate the existence of secondary charge transfer mechanisms contributing to the charge injection process.

**Table 1. Selected Electronic Solid-State Parameters Calculated from the Positive and Negative Biased $I−V$ Profiles**

| sample | turn-on voltage (V) | series resistance, $R_s$ (kΩ) | shunt resistance, $R_{sh}$ (MΩ) | ideality factor, $n$ | reverse saturation current, $I_0$ (nA) | knee voltage (V) |
|--------|---------------------|-------------------------------|-------------------------------|-------------------|-------------------------------|--------------|
| Hb     | 1.10                | 397.60                        | 133.067                       | 17.91             | 6.89                          | −1.30        |
| Coll   | 1.25                | 277.50                        | 53.944                        | 19.80             | 21.01                         | −1.10        |

Figure 3. Sweep profiles of Hb and Coll in the range of −3 to 3 V (a); semilog current–voltage characteristics of the Au/protein/Au Schottky structures (b); and resistance profiles of protein samples showing distinctive peaks of shunt resistance values for each sample tested (c). Characteristic secondary resistive peaks can also be observed for each type of protein, indicative of secondary current pathways at higher voltages.
Table 1 lists some of the solid-state parameters calculated according to thermionic emission (TE) theory using conventional and Cheung and Cheung methods. Based on the $I-V$ profiles, the turn-on voltages for Hb and Coll were determined as 1.10 and 1.25 V, respectively. Although Coll appears to conduct higher current, Hb exhibited a lower turn-on voltage due to the existence of a hem group as a cofactor in each subunit of the Hb tetramer. In previous studies involving immobilized dry-state proteins, cofactors bound to proteins were observed to affect the ETp in solid-state measurements. Cofactors in proteins create relatively efficient current transport paths and facilitate electronic charge migration and protein–electrode electronic coupling. As such, Hb required a lower turn-on compared to Coll to switch-on the "bio-diode".

The samples for this work were used in solution form. Therefore, it is important to understand the origin of the electric signals observed. Deionized water and a very low concentration of acetic acid were used to solubilize Hb and Coll to preserve the native conformation of the proteins. Although both solvents are known to have no electrolytic activities, CV measurements of the protein solutions as well as the solvents alone were carried out to conclude the purely electronic nature of the signals observed in this work. No redox activity within the potential window of $-2$ to 2 V was observed for both samples and therefore ETp could be concluded as the only phenomenon involved in current flow at the metal–protein junction.

## CONCLUSIONS

Understanding the electronic properties of biomaterials when incorporated into conventional or known electrical structures could provide useful information for future applications in nanoelectronics. In this work, a PCB-based two-electrode system was proposed as a simple and cost-effective method of elucidating the electronic properties of selected proteins when incorporated into a Schottky junction. The selected MPM structures in this work showed distinct electronic profiles similar to conventional metal–semiconductor junctions. Coll conducted higher current than Hb, which might be as a result of high proline content, previously proven to facilitate ET in proteins. However, Hb appeared to require a lower turn-on voltage to start conducting, which could possibly be due to the presence of a hem group in its subunits as a cofactor. In conclusion, the quantitative solid-state electronic parameters extracted from the $I-V$ characteristics could be used to guide researchers for selecting the most efficient metal–protein combination for the fabrication of bio-devices. In addition, further experiments using this method could potentially lead to the development of protein (or biomolecule) characterization platforms based on $I-V$ characteristics.

## MATERIALS AND METHODS

Protein samples, namely hemoglobin porcine and collagen Type I from rat tail, were purchased in lyophilized form (Sigma-Aldrich). Samples were prepared by solubilizing Hb and Coll in ultrapure deionized water and 0.01 mM acetic acid, respectively, at known concentrations.

To form the Au/protein/Au junction structure, a two-electrode system was designed to enable maximum surface contact, which was then fabricated on a PCB (Asia Printed
Circuit Sdn. Bhd (Perak, Malaysia). Mass fabrication of the designed PCB was sourced out and accomplished by Asia Printed Circuit Sdn. Bhd (Perak, Malaysia). Using CAD/CAM Software, patterns including circuit film and solder mask layers were converted to Gerber format and plotted out according to the design. A single-sided 1.6 mm fiberglass-resin laminate (FR4) with a copper thickness of 36 μm was panelized for the imaging process. The panel was laminated with a UV light-sensitive dry polymer film. The plotted-out circuit film was laid and aligned on top of the laminated panel, followed by exposure to UV to bind it to the panel. Following this, the panel went through a chemical photo-developing process to wash away the unexposed dry polymer film areas underneath the circuit lines and pads. A 4–5 μm layer of Nickel (Ni) was electrolytically plated on top of the circuit lines and pads followed by electrolytic plating of Au (0.049–0.052 μm or 2 μm). After stripping and etching processes to remove the unwanted dry polymer and copper areas, the panel was printed with epoxy solder mask ink to cover the circuit lines leaving only the electrode and connecting pads exposed.

Two electrode pads with a gold layer of 2 μm thickness were electrochemically fabricated on top of the FR4 board as illustrated in Figure 5. Prior to measurements, the sensors were submerged in acetone for 1 min and rinsed with deionized water. Subsequently, the sensors were rinsed with isopropyl alcohol (IPA) for 1 min followed by rinsing with deionized water and dried under a nitrogen stream.

For electronic characterization, 10 μL of each protein solution was transferred to the sample loading site of the immersion process. The panel was laminated with a UV light-sensitive dry polymer film area underneath. A source measure unit (SMU) (236 SMU, Keithley) was utilized to apply a voltage of 0 to −3 V in the positive and negative regions, respectively. The corresponding current measurements were recorded, and I–V profiles for each of the proteins were generated prior to the calculation of the selected solid-state parameters. Governed by thermionic emission theory,16 we employed the conventional17,48 and Cheung and Cheung’s49 methods.

**AUTHOR INFORMATION**

**Corresponding Authors**

Ramesh T. Subramaniam — Centre for Ionics University of Malaya, Department of Physics, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia; Email: Ramesh@um.edu.my

Vengadesh Periasamy — Low Dimensional Materials Research Centre (LDMRC), Department of Physics, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia; orcid.org/0000-0002-0435-4944; Email: vengadesshp@um.edu.my

**Authors**

Sara Talebi — Low Dimensional Materials Research Centre (LDMRC), Department of Physics, Faculty of Science, Centre for Ionics University of Malaya, Department of Physics, Faculty of Science, and Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Souhad M. A. Daragha — Low Dimensional Materials Research Centre (LDMRC), Department of Physics, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Subha Bhassu — Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Georgeper Gnana Kumar — Department of Physical Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai 625021, Tamil Nadu, India; orcid.org/0000-0001-7011-3498

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b03831

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors would like to acknowledge the financial support from FRGS (FP038-2017A) and PRGS (PR003-2019A) grants.

**REFERENCES**

(1) Birge, R. R.; Gillespie, N. B.; Izaguirre, E. W.; Kusnetzow, A.; Lawrence, A. F.; Singh, D.; Wise, K. J. Biomolecular Electronics: Protein-Based Associative Processors and Volumetric Memories. *J. Phys. Chem. B* 1999, 103, 10746–10766.

(2) Nawrocki, W. Physical limits for scaling of integrated circuits. *J. Phys.: Conf. Ser.* 2010, No. 012059.

(3) Alfinito, E.; Pouset, J.; Reggiani, L. PROTEOTRONICS: The emerging science of protein-based electronic devices. *J. Phys.: Conf. Ser.* 2015, No. 012002.

(4) Saen-Oon, S.; Lucas, M. F.; Guallar, V. Electron transfer in proteins: theory, applications and future perspectives. *Phys. Chem. Chem. Phys.* 2013, 15, 15271.

(5) Chow, D. C.; Johannes, M. S.; Lee, W.-K.; Clark, R. L.; Zauscher, S.; Chilkoti, A. Nanofabrication with biomolecules. *Mater. Today* 2005, 8, 30–39.

(6) Abu-Salah, K. M.; Ansari, A. A.; Alrokayan, S. A. DNA-Based Applications in Nanobiotechnology. *J. Biomed. Biotechnol.* 2010, 2010, 1–15.

(7) Lee, C. W.; Kim, O. Y.; Lee, J. Y. Organic materials for organic electronic devices. *J. Ind. Eng. Chem.* 2014, 20, 1198–1208.

(8) Maruccio, G.; Bramanti, A. Nano electronic Devices Based on Proteins. In *Nanostructure Science and Technology*; Offenhauser, A.; Rinaldi, R., Eds.; Springer, 2009; pp 139–189.

(9) Davis, J. J.; Morgan, D. A.; Wraithmell, C. L.; Axford, D. N.; Zhao, J.; Wang, N. Molecular bioelectronics. *J. Mater. Chem.* 2005, 15, 2160.

(10) Dwyer, D. S. Electronic Properties of the Amino Acid Side Chains Contribute to the Structural Preferences in Protein Folding. *J. Biomol. Struct. Dyn.* 2001, 18, 881–892.

(11) Bianco, P. The Origin of Bioelectrochemistry: An Overview. In *Encyclopedia of Electrochemistry*; Wiley-VCH Verlag GmbH & Co: KGaA, 2007.

(12) Zhang, B.; Song, W.; Pang, P.; Lai, H.; Chen, Q.; Zhang, P.; Lindsay, S. Role of contacts in long-range protein conductance. *Proc. Natl. Acad. Sci. U.S.A.* 2019, 116, 5886–5891.

(13) Ing, N. L.; El-Naggar, M. Y.; Hochbaum, A. I. Going the Distance: Long-Range Conductivity in Protein and Peptide Bioelectronic Materials. *J. Phys. Chem. B* 2018, 122, 10403–10423.

(14) Ron, I.; Pecht, I.; Sheves, M.; Cahen, D. Proteins as Solid-State Electronic Conductors. *Acc. Chem. Res.* 2010, 43, 945–953.

(15) Ratner, M. A brief history of molecular electronics. *Nat. Nanotechnol.* 2013, 8, 378–381.

(16) Andolfi, L.; Cannistraro, S. Conductive atomic force microscopy study of plastocyanin molecules adsorbed on gold electrode. *Surf. Sci.* 2005, 598, 68–77.

(17) Chi, Q.; Zhang, J.; Friis, E. P.; E.T. Andersen, J.; Ulstrup, J. Electrochemistry of self-assembled monolayers of the blue copper protein Pseudomas aeruginosa azurin on Au(111). *Electrochem. Commun.* 1999, 1, 91–96.

(18) Li, W.; Sepunaru, L.; Amdursky, N.; Cohen, S. R.; Pecht, I.; Sheves, M.; Cahen, D. Temperature and Force Dependence of...
Nanoscale Electron Transport via the Cu Protein Azurin. *ACS Nano* 2012, 6, 10816–10824.
(19) Farver, O.; Pecht, I. Long range intramolecular electron transfer in azurins. *J. Am. Chem. Soc.* 1992, 114, 5764–5767.
(20) Facci, P. *Biomolecular Electronics: Bioelectronics and the Electrical Control of Biological Systems and Reactions*, 1st ed.; William Andrew, 2014.
(21) Claudio, N. *Molecular Bioelectronics: The 19 Years of Progress*, 2nd ed.; World Scientific, 2016.
(22) Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism and function. *Cell* 2004, 116, 281–297.
(23) Ron, I.; Sepunaru, L.; Izhakov, S.; Belsenkova, T.; Friedman, N.; Pecht, I.; Sheves, M.; Cahen, D. Proteins as Electronic Materials: Electron Transport through Solid-State Protein Monolayer Junctions. *J. Am. Chem. Soc.* 2010, 132, 4131–4140.
(24) Dutta, G.; Regoutz, A.; Moschou, D. Commercially Fabricated Printed Circuit Board Sensing Electrodes for Biomarker Electrochemical Detection: The Importance of Electrode Surface Characteristics in Sensor Performance. *Proceedings* 2018, No. 741.
(25) Salvo, P.; Henry, O. Y.; Dhaenens, K.; Acero Sanchez, J. L.; Gielen, A.; Weire Solnestam, B.; Lundeberg, J.; O’Sullivan, C. K.; Vanfleteren, J. Fabrication and functionalization of PCB gold electrodes suitable for DNA-based electrochemical sensing. *Bio-Med. Mater. Eng.* 2014, 24, 1705–1714.
(26) Moschou, D.; Tserepi, A. The lab-on-PCB approach: tackling the µTAS commercial upscaling bottleneck. *Lab Chip* 2017, 17, 1388–1405.
(27) Talebi, S.; Daragham, S.; Subramaniam, S. R. T.; Bhassu, S.; Periasamy, V. Exploring the Electronic Properties of Ribonuclear Acids Integrated Within a Schottky-Like Junction. *J. Electron. Mater.* 2019, 48, 7114–7122.
(28) Periasamy, V.; Rizan, N.; Al-Ta’i, H. M. J.; Tan, Y. S.; Tajuddin, H. A.; Iwamoto, M. Measuring the Electronic Properties of DNA-Specific Schottky Diodes Towards Detecting and Identifying Basidiomycetes DNA. *Sci. Rep.* 2016, 6, No. 29879.
(29) Rizan, N.; Yew, C. Y.; Nkiam, M. R.; Krishnasamy, J.; Bhassu, S.; Hong, G. Z.; Periasamy, V.; et al. Electronic Properties of Synthetic Shrimp Pathogens-derived DNA Schottky Diodes. *Sci. Rep.* 2018, 8, No. 896.
(30) Azmi, S. Z.; Vello, V.; Rizan, N.; Krishnasamy, J.; Talebi, S.; Gunaseelam, P.; Periasamy, V.; et al. Electronic profiling of alga-derived DNA using DNA-specific Schottky diode. *Appl. Phys. A: Mater. Sci. Process.* 2018, 124, No. 559.
(31) Zhang, X. Y.; Shao, J.; Jiang, S. X.; Wang, B.; Zheng, Y. Structure-dependent electrical conductivity of protein: its differences between alpha-domain and beta-domain structures. *Nanotechnology* 2015, 26, No. 125702.
(32) Berstis, L.; Beckham, G. T.; Crowley, M. F. Electronic coupling through natural amino acids. *J. Chem. Phys.* 2015, 143, No. 225102.
(33) Cordes, M.; Jacques, O.; Kötgen, A.; Jasper, C.; Boudebsou, H.; Giese, B. Development of a Model System for the Study of Long Distance Electron Transfer in Peptides. *Adv. Synth. Catal.* 2008, 350, 1053–1062.
(34) Shah, A.; Adhikari, B.; Martic, S.; Munin, A.; Shahzad, S.; Ahmad, K.; Kraatz, H.-B. Electron transfer in peptides. *Chem. Soc. Rev.* 2015, 44, 1015–1027.
(35) Jiang, Z.; You, L.; Dou, W.; Sun, T.; Xu, P. Effects of an Electric Field on the Conformational Transition of the Protein: A Molecular Dynamics Simulation Study. *Polymers* 2019, 11, No. 282.
(36) Bostick, C. D.; Mukhopadhyay, S.; Pecht, I.; Sheves, M.; Cahen, D.; Lederman, D. Protein bioelectronics: a review of what we do and do not know. *Rep. Prog. Phys.* 2018, 81, No. 026601.
(37) Leck, H. J. *Theory of Semiconductor Junction Devices*, 1st ed.; Pergamon Press: New York, 1976.
(38) Kiuru, T.; Dahlberg, K.; Mallat, J.; Raisanen, A. V.; Narhi, T. In *Comparison of Low-Frequency and Microwave Frequency Capacitance Determination Techniques for mm-Wave Schottky Diodes*, Proceedings of the 6th European Microwave Integrated Circuits Conference; IEEE: Manchester, UK, 2011; pp 10–11.