Single-molecule junctions are a versatile platform to investigate electron-transport characteristics toward the realization of molecular electronics.1–5 Moreover, these junctions hold a significant importance in sensing applications.6 Examples of such analytical applications include single-molecule detection of DNA,7–10 peptides,11 and glucose.12 Single-molecule junctions can be reliably prepared based on a break-junction technique using scanning tunneling microscopy (STM).13 The formation of a single-molecule junction typically involves the chemisorption of the target molecule onto metal electrodes, i.e., the STM tip and the substrate. The molecules, therefore, need to bear functional groups that bind to the electrodes. Considerable research has been devoted to seeking suitable functional groups for robust junction formation. Consequently, thiols, amines, and pyridyl nitrogens have been commonly employed in recent studies.14,15 However, the requirement of such functional groups as linkers hampers a wide applicability of molecular junctions for the single-molecule sensing. We have recently proposed a novel methodology, ligation-mediated coupling, to tackle this problem. In this technique, both the STM tip and the substrate are modified with organic molecules that can bind to a given analyte molecule. In the previous work, we used a fullerene-functionalized STM tip (a C60 tip) and a substrate modified with 4-aminothiophenol (4ATP). A metal tetraphenylporphyrin (MTPP) served as a sample molecule.16 The charge-transfer interaction and coordination bonding of a C60 tip and 4ATP, respectively, with MTPP led to the formation of a supramolecular assembly between the tip and the substrate. The electron-transport properties can be consequently interrogated with the supramolecule, in spite of the absence of linkers in the sample MTPP: the current rectification phenomena arising from the donor-acceptor interaction between fullerene and porphyrin were found. In the present work, we further improved the ligation-mediated coupling, and applied this strategy to a complex biological molecule (Fig. 1a). We selected microperoxidase 11 (MP11, Fig. 1b), which is undecapeptide bearing a prosthetic heme group obtained by the proteolytic digestion of cytochrome c.17,18 Since MP11 preserves the peroxidase activity, this oligopeptide has been utilized as a versatile model system for heme peptides.

An Au wire was electrochemically etched to prepare each STM tip.19 The tips were chemically modified by immersion overnight in a 7.5 mM ethanolic solution of 4-mercaptopyridine (4MP). An Au(111) surface, prepared by thermal evaporation, was used as a substrate. This substrate was also modified with 4MP by immersion for 1 min in a 50 μM solution. After washing with pure ethanol, the 4MP-modified substrate was further immersed in a 1 mM toluene solution of Zn tetraphenylporphyrin (ZnTPP) or MP11. STM measurements were performed on a SPM 5100 (Agilent Technologies, CA). The UV/Vis spectra were obtained using a V-650 spectrophotometer (JASCO, Tokyo). The spectra were acquired in the transmission mode using ultrathin gold films of 5 nm (nominal thickness) on glass as substrates. In order to stabilize the supramolecular assembly, both the substrate and the STM tip were modified with 4MP instead of

![Fig. 1](image-url)
ATP used in the previous work, since the stability constants of the metal-pyridine complexes are generally higher than those of the metal-aniline complexes. We first used porphyrins, i.e., ZnTPP, which is a close analogue of the hemes in hemoprotein, including MP11. Figure 2a shows the UV/Vis spectrum for the 4MP-modified substrate after immersion in the ZnTPP solution. The peak at 430 nm is attributed to the Soret band of ZnTPP. In a benzene solution of ZnTPP, the Soret band was observed at 423 nm, and the red shift for the surface-tethered ZnTPP was attributed to charge transfer caused by the axial ligation of 4MP. Figure 2a exhibits a broad absorption in the range of 500 – 600 nm. This band can be assigned to both the Q band of ZnTPP and an enhancement of the gold surface plasmon absorption induced by chemisorbed 4MP. UV/Vis spectroscopic studies show the surface adsorption of ZnTPP via the ligation with 4MP chemisorbed on the gold surface.

Next, we measured the electron transport using the ZnTPP/4MP molecular assembly on the Au surface. In this measurement, an STM tip covered with a self-assembled monolayer of 4MP was first brought into close proximity to the sample surface modified with the ZnTPP/4MP assembly. The 4MP molecular tip was then pulled up, and the current, from which the conductance was calculated, was recorded. In the resulting conductance traces, step-like plateau regions appeared, as shown in Fig. 2b. The observation of the plateaus indicates the formation of a molecular junction involving the 4MP molecule on the tip and the ZnTPP/4MP assembly on the substrate surface. The conductance traces were statistically analyzed by constructing a conductance histogram (Fig. 2c). A clear peak was found in the histogram. In general, multiple peaks appear when multiple molecules simultaneously bridge the gap between the STM tip and the substrate. The single peak in Fig. 2c thus indicates the formation of a junction containing a single molecular specie. When unmodified Au tips, instead of the 4MP molecular tips, were used for similar measurements, no plateaus and peaks were found in the conductance traces and histogram, respectively. These results suggest that the molecular junction comprises the 4MP on the tip and ZnTPP/4MP assembly on the substrate. The junction formation is ascribed to a coordination bond interaction between the central metal ion of ZnTPP and the pyridine nitrogens of the 4MP molecules on the tip and the substrate (see Fig. 1a). On the basis of the peak position in the histogram, the conductance of the single ZnTPP-containing assembly was determined to be $5.0 \times 10^{-5} G_0$, where $G_0$ is the fundamental conductance quantum, which equals to $2e^2/h$. This value lies in the same order of magnitude as that reported in the previous work, where 4ATP and fullerene molecules were used to form the assembly. These results demonstrate that 4MP can be used to form the supramolecular assembly for electron transport measurements.

The formation of a molecular junction mediated by a coordination bond interaction was further applied to MP11. This oligopeptide was first adsorbed on a 4MP-modified Au(111) surface similar to ZnTPP. An intense Soret band appeared at 413 nm in the UV/Vis spectrum (Fig. 3a), which showed a red shift, as compared with the band observed in solution (400 nm). The comparison of Fig. 3a with Fig. 2a reveals a smaller absorbance of the MP11-modified surface than the ZnTPP-modified one. We attributed this to a decreased surface concentration of MP11 because of its bulky peptide fragment. This result shows successful immobilization of MP11 on the sample surface, probably due to the coordination bond interaction of 4MP with the heme iron. Having confirmed the presence of MP11 on the surface, we carried out a conductance measurement using the 4MP molecular tip and an Au substrate modified with the MP11/4MP assemblies. The resulting
respectively. The conductance of the FeTPP-containing junction and the peak in the conductance traces and histogram, junction of 4MP/FeTPP/4MP was apparent based on the plateaus similar to the case of ZnTPP, the formation of the molecular metal center (see Fig. 1b), was used as a sample molecule. FeTPP, which bears close similarity to the heme in terms of the functions of hemoproteins, even in the presence of peptide chains. The present methodology enables direct electrical connection to 4MP molecules on the tip and substrate. We thus conclude that molecular junction formed with the 4MP tip and the MP11/4MP assembly. Bias voltage, 0.4 V; bin size, 1.25 × 10⁻⁵ G₀.

In summary, we successfully measured the conductance of a molecular junction was determined to be 4.3 × 10⁻⁴ G₀, based on the peak position in the histogram (Fig. 3b). To investigate the identity of the molecular junction, FeTPP, which bears close similarity to the heme in terms of the conductance histogram for the 4MP/FeTPP/4MP assembly. Bias voltage, 0.4 V; bin size, 1.25 × 10⁻⁵ G₀.

Fig. 3 (a) UV/Vis spectrum of MP11 axially ligated with 4MP on the Au surface. (b) Conductance histogram obtained by a measurement with the 4MP molecular tip and the Au substrate modified with the MP11/4MP assembly. Bias voltage, 0.4 V; bin size, 1.25 × 10⁻⁵ G₀.

Conductance traces exhibited plateaus, and a single pronounced peak appeared in the histogram (Fig. 3b), demonstrating the formation of a molecular junction. Again, the existence of the single peak indicates that a single molecular entity or assembly bridges the gap between the tip and the substrate. The conductance of the molecular junction was determined to be 4.3 × 10⁻⁴ G₀, based on the peak position in the histogram (Fig. 3b). To investigate the identity of the molecular junction, FeTPP, which bears close similarity to the heme in terms of the metal center (see Fig. 1b), was used as a sample molecule. FeTPP was immobilized on the 4MP-modified Au surface, and the 4MP molecular tip was used to measure the conductance. Similar to the case of ZnTPP, the formation of the molecular junction of 4MP/FeTPP/4MP was apparent based on the plateaus and the peak in the conductance traces and histogram, respectively. The conductance of the FeTPP-containing junction was found to be 4.4 × 10⁻⁴ G₀ (Fig. S1, Supporting Information). Importantly, this conductance nicely agrees with the corresponding value determined in the conductance measurement using MP11 (see above). These results indicate that the molecular junction formed with the 4MP tip and the MP11/4MP sample was composed of the heme of MP11 and two coordinating 4MP molecules on the tip and substrate. We thus conclude that the present methodology enables direct electrical connection to prosthetic heme groups, which are mandatory for the biological functions of hemoproteins, even in the presence of peptide chains.

In summary, we successfully measured the conductance of a single molecular assembly containing the heme peptide using molecular tips and functionalized substrates. The present technique can be extended to a single-molecule detection of a biological specimen, and also open up a unique way for utilizing biological functions in molecular electronic devices.

Acknowledgements

This work is supported by JSPS KAKENHI Grants 26288070 and 16K14018.

Supporting Information

Conductance histogram for the 4MP/FeTPP/4MP assembly. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References

1. S. V. Aradhya and L. Venkataraman, Nature Nanotech., 2013, 8, 399.

2. N. J. Tao, Nature Nanotech., 2006, 1, 173.

3. F. Chen, J. Hihath, Z. F. Huang, X. L. Li, and N. J. Tao, Annu. Rev. Phys. Chem., 2007, 58, 535.

4. H. Song, M. A. Reed, and T. Lee, Adv. Mater., 2011, 23, 1583.

5. K. Moth-Poulsen and T. Bjørnholm, Nature Nanotech., 2009, 4, 551.

6. T. Nishino, Anal. Sci., 2014, 30, 81.

7. T. Nishino and P. T. Bui, Chem. Commun., 2013, 49, 3437.

8. P. T. Bui, T. Nishino, H. Shiigi, and T. Nagaoka, Chem. Commun., 2015, 51, 1666.

9. S. Chang, J. He, A. Kibel, M. Lee, O. Sankey, P. Zhang, and S. Lindsay, Nature Nanotech., 2009, 4, 297.

10. S. Huang, J. He, S. Chang, P. Zhang, F. Liang, S. Li, M. Tuchband, A. Fuhrmann, R. Ros, and S. Lindsay, Nature Nanotech., 2010, 5, 868.

11. Y. Zhao, B. Ashcroft, P. Zhang, H. Liu, S. Sen, W. Song, J. Im, B. Gyarfas, S. Manna, S. Biswas, C. Borges, and S. Lindsay, Nature Nanotech., 2014, 9, 466.

12. T. Nishino, H. Shiigi, M. Kiguchi, and T. Nagaoka, Chem. Commun., 2017, 53, 5212.

13. B. Xu and N. J. Tao, Science, 2003, 301, 1221.

14. F. Chen, X. Li, J. Hihath, Z. Huang, and N. Tao, J. Am. Chem. Soc., 2006, 128, 15874.

15. L. Venkataraman, J. E. Klare, I. W. Tam, C. Nuckolls, M. S. Hybertsen, and M. L. Steigerwald, Nano Lett., 2006, 6, 458.

16. P. T. Bui, T. Nishino, Y. Yamamoto, and H. Shiigi, J. Am. Chem. Soc., 2013, 135, 5238.

17. J. G. Kleingardner and K. L. Bren, Acc. Chem. Res., 2015, 48, 1845.

18. F. Nastri, M. Chino, O. Maglio, A. Bhagi-Damodaran, Y. Lu, and A. Lombardi, Chem. Soc. Rev., 2016, 45, 5020.

19. D. Gingery and P. Buhmann, Rev. Sci. Instrum., 2007, 78, 113703.

20. G. Kalyuzhny, A. Vaskevich, G. Ashkenasy, A. Shanzer, and I. Rubinstein, J. Phys. Chem. B, 2000, 104, 8238.

21. D. J. Quimby and F. R. Longo, J. Am. Chem. Soc., 1975, 97, 5111.

22. G. Kalyuzhny, A. Vaskevich, G. Ashkenasy, A. Shanzer, and I. Rubinstein, J. Phys. Chem. B, 2000, 104, 8238.

23. Z. Zhang, S. Hou, Z. Zhu, and Z. Liu, Langmuir, 2000, 16, 537.

24. V. Arima, E. Fabiano, R. I. R. Blyth, F. Delia Sala, F. Matino, J. Thompson, R. Cingolani, and R. Rinaldi, J. Am. Chem. Soc., 2004, 126, 16951.

25. M. S. Inkpen, M. Lemmer, N. Fitzpatrick, D. C. Milan, R. J. Nichols, N. J. Long, and T. Albrecht, J. Am. Chem. Soc., 2015, 137, 9971.

26. W. Huang, Z. Zhang, X. Han, J. Tang, Z. Peng, S. Dong, and E. Wang, Biophys. Chem., 2001, 94, 165.