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Quantification of Electron Transfer through Noncovalent Interaction between Single Molecules

（単分子間における非共有結合を介した電子移動の定量評価）

Phuc Tan Bui

July 2013

Doctoral Thesis at Osaka Prefecture University
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Table of Contents

1. Introduction 7

1.1. Scanning Tunneling Microscopy 7

1.1.1. Overview 7

1.1.2. The theory of STM 7

1.1.3. The STM experiment 10

1.2. Molecular tips 11

1.2.1. Preparation of molecular tips 12

1.2.2. Interactions between the molecular tip and the surface in chemically selective imaging 12

1.2.2.1. Charge-transfer interaction 12

1.2.2.2. Hydrogen bond interaction 15

1.2.2.3. Metal coordination bond interaction 16

1.3. Quantitative measurement of single molecule conductance by STM 17

1.3.1. Background of the single molecule conductance 17

1.3.2. Methodology overview 17

1.3.3. Outline of the thesis 21

2. Direct measurement of electron transfer through a hydrogen bond interaction between two single molecules. 24

2.1. Introduction 24

2.2. Experimental section 25
2.2.1. Schematic illustrations of the experiment 25
2.2.2. Reagents 26
2.2.3. Tip preparation 26
2.2.4. Sample preparation 27
2.2.5. $I-t$ and $I-z$ measurements 27
2.2.6. Control experiments using a clean Au substrate or tip 28
2.2.7. Statistic analysis procedure for $I-t$ and $I-z$ data. 28
2.2.8. First-principle calculations. 29

2.3. Results and discussion 30

2.4. Conclusions 36

3. Electron transfer through coordination bond interaction between single molecules: conductance switching by metal ion 37

3.1. Introduction 37

3.2. Experimental section 38

3.2.1. Reagents 39

3.2.2. Tip preparation 39

3.2.3. Sample preparation 40

3.2.4. Current measurements 40

3.2.5. Data analyses 40

3.3. Results and discussion 41
3.4. Conclusions

4. Quantitative measurement of electron transfer through a charge-transfer interaction in a single noncovalent supramolecular assembly.

4.1. Introduction

4.2. Experimental section

4.2.1. Reagents

4.2.2. Tip preparation

4.2.3. Sample preparation

4.2.4. Current measurements

4.2.5. Data analyses

4.3. Results and discussion

4.4. Conclusions

5. Electron transfer through intramolecular interactions of biomolecules: application in single-molecule DNA detection based on hybridization.

5.1. Introduction

5.2. Experimental section

5.2.1. Reagents

5.2.2. Tip preparation

5.2.3. Sample preparation

5.2.4. Current measurements
5.2.5. Data analyses 66

5.2.6. UV melting studies 67

5.3. Results and discussion 67

5.3.1. Single-molecule detection of fully matched and mismatched DNA. 67

5.3.2. Single-molecule detection of methylated DNA 75

5.4. Conclusions 78

6. Conclusions 79

References 83
1. Introduction

1.1. Scanning Tunneling Microscopy (STM)

1.1.1. Overview

“There’s Plenty of Room at the Bottom”, Feynman said at Annual Meeting of the American Physical Society in 1959. He acknowledged the limited resolution imposed by the wavelength of the probe involved such as optical microscopy with a maximum resolution of ~500 nm, infrared microscopy of ~10 µm and so on. In fact, a way to solve the challenge had been suggested by using 10 nm aperture to gain information on a molecular scale and piezoelectric scanning to control the microscope. While Ash and Nicholls provided the first demonstration of a scanning microscope by using microwave and a sub-wavelength aperture, Binnig and Rohrer [1] caught the general interest about the STM by recording the first atomically resolved image of the Si(111) (7x7) reconstruction. Since then, STM has been common in surface science because of the atomic resolution, the ability for observations in real space and operational ability in a variety of environments. The inventors of STM, Binnig and Rohrer, were awarded the Nobel Prize in 1987.

1.1.2. The theory of STM

Fig. 1.1. Illustration of the quantum mechanical tunneling effect between tip and sample [2].
Chapter 1

The basic of STM, schematically illustrated in Fig. 1.2, lies in the ability of the electron penetrate a potential barrier that would be forbidden in classical viewpoint. The electronic wave function decays exponentially and because of this extension, electrons can jump into unoccupied states of a second conductor (STM tip) if it approaches the surface sufficiently close. When a voltage bias between two conductors is applied, a tunneling current flows from the conductor to the other one with a magnitude of nanoamperes. The tunneling current, \( I \), is related to the probability of electrons crossing the barrier and decays exponentially with the tip-sample separation \( z \):

\[
I \propto e^{-2\kappa z}
\]

where

\[
\kappa^2 = \frac{2m(V_B - E)}{\hbar^2}
\]

\( E \) is the energy of the electron, \( V_B \) is the vacuum energy, \( m \) is the mass of the electron and \( \hbar \) is the Planck’s constant divided by \( 2\pi \). \( (V_B - E) \) is the local potential barrier height. The exponential dependence of current on the tip-sample distance allows STM to achieve the high resolution.

To interpret the STM images, the processes that control the flow of electrons between the tip and the surface need to be understood. As illustrated in the Fig. 1.2, the Fermi energies of the left-hand and right-hand conductor are \( E_F^L \) and \( E_F^R \), respectively. When two metal conductors are brought close to, but not electrical contact with one another (Fig. 1.2.a), the offset in the Fermi energies is equal to the difference in work functions of the two metals \( \Phi_c = E_F^L - E_F^R \). When the two metals are connected electrically (Fig. 1.2.b), the Fermi energies of the two metal line up. The vacuum level shifts and there is an electric field in the vacuum between two metal, but no current can flow from occupied states to unoccupied states between two metals because of the equally aligned Fermi levels of two metals. Because the electrons always flow from occupied states to unoccupied states, we can not only turn on the current flow but also control the direction of the current flow by adjusting the bias and the polarity of the bias between two metals (Fig. 1.2.c and 1.2.d). When the bias is applied and
two metals are sufficiently close, the wave functions of the electrons in the two metals overlap, the tunneling of electron can occur as the law of quantum mechanics.

Fig. 1.2. Illustrations of the Fermi and vacuum level positions for two metals separated by distance $d$. (a) Isolated metals. (b) Electrical contact without bias. (c) Biasing shifts the relative positions of the Fermi levels and thus creates energy band $V_B$ of unoccupied states for electron tunneling to. (d) The direction of tunneling is reversed by reversing the applied bias in case (c) [3].

STM images stand for a convolution of the density of states of occupied and unoccupied electronic states of the tip and the sample surface. In other word, STM does not image atoms, it images electronic states. Because the density of states is different in different atoms, STM images can provide the information of atom positions.
1.1.3. The STM experiment

A sharp tip is positioned within a few angstroms above the surface by using the piezoelectric crystal drive, as shown in Fig. 1.3. A bias between the tip and the sample is applied and the image is obtained by raster the tip across the sample. The tip scans over the sample while keeping either the tip-sample distance or the tunneling current constant, called as constant height mode and constant current mode, respectively. In the constant current mode, the retraction and extension of the piezocrystal actuator that are measured from a feedback circuit results in a slower scanning speed, however it reduces the risk of the tip crashing into the surface steps. In general, the constant current mode is preferred to use in the case of low magnifications and large scanned area with multiple step edges whereas scanning a flat terrace at atomic resolution the constant height mode will be better.

The scanner, piezocrystal actuator, controls the x, y and z axis movement of the tip relative to the sample. For acquiring high resolution images, the scanner must meet the severe requirements. For example, to obtain images of the surface with atomic resolution, the tip must be controlled within an accuracy of less than 1 Å in the surface’s plane and better than 0.05 Å normal to the surface. The
scanner is also required to have a high resonant frequency to reduce noise and to allow efficient feedback between recorded signal and the scanner.

1.2. Molecular tips

It has been shown that As and Ga atoms can be selectively observed in STM images with sample-negative and sample-positive biases, respectively [4]. However, STM is generally unable to discriminate different atoms or molecules. The lack of chemical selectivity in STM hinders the ability to identify chemical species. To image clearly the surfaces covered by such chemical species, techniques based on molecular tips have been developed.

![Molecular tips](image)

**Fig. 1.4.** Molecular tips facilitate the electron tunneling through chemical interactions for selective recognition of functional groups or chemical species [6].

The molecular tips are prepared by chemical modification of conventional metal tips via self-assembly processes of expected molecules typically based on thiol linkers. The chemisorbed molecule at the tip apex probes electron tunneling to or from a molecule absorbed on the sample surface. The tunneling current increases when the tip molecule forms a chemical interaction with a sample molecule, as illustrated in Fig. 1.4, because this interaction, which can be a hydrogen bonding, metal-coordination bonding or charge-transfer interaction, provides the overlap of electronic wave functions of tip and sample molecules. Therefore, the current increase is ascribed to the facilitated tunneling through the overlapped wave functions. This phenomenon is utilized to improve the poor chemical...
selectivity in conventional STM. STM with the specific molecular tip can observe specific chemical species on the sample surface, as in the previous studies of our laboratory.

1.2.1. Preparation of molecular tips [6]

The preparation of molecular tips plays a central role in determining the success of chemically selective recognition. The most simple and convenient way to prepare molecular tips is using self-assembly processes. Molecules terminated by thiols at one end are typically used for the formation of the self assembly monolayer (SAM) covering the tip. Molecularly close-packed SAMs on underlying gold STM tips are obtained by simply immersing the gold tip into dilute solutions of these molecules. The thiol group forms a covalent S–Au bond with the surface of the Au tip to anchor the molecule to the tip. The left end of the molecule, outwards free end, is the functional group that is brought closest proximity to a sample surface in STM observations. Therefore, functional group of the molecular tip plays the most important role for chemical recognition.

1.2.2. Interactions between the molecular tip and the surface in chemically selective imaging [7]

1.2.2.1. Charge-transfer interaction

The facilitation of electron tunneling through the charge-transfer interactions was utilized to image the molecular monolayer absorbed on the sample surface. Porphyrins are one of the most employed electron donors, and fullerenes possess facile electron acceptability. These two chemical species have been known to form a charge-transfer interaction between them. A fullerene molecular tip was used to observe porphyrin molecules adsorbed on the surface of highly oriented pyrolytic graphite (HOPG), as shown in Fig. 1.5.
Fig. 1.5. STM images of CoPor adlayer on HOPG. (a) Observed by using a gold tip, $V_{\text{Bias}} = -1.30$ V, $I_{\text{set-point}} = 0.30$ nA. (b) Observed by using a fullerene tip, $V_{\text{Bias}} = -1.25$ V, $I_{\text{set-point}} = 0.30$ nA. Arrows indicate the positions of the Co(II) ions. The height profiles along the lines are shown under the STM images [8].

Fig. 1.5.a presents an STM image of a cobalt(II) porphyrin (CoPor) monolayer that was observed by using a gold tip. We can observe the aligned arrays of porphyrin rings that have central protrusions as indicated by arrows in Fig. 1.5.a when gold tips were used. The protrusions are ascribed to be the central cobalt(II) ions of porphyrin rings. The facilitated electron tunneling through cobalt(II) ions has been proposed as a result of d orbital-mediated electron tunneling in a resonant way, where the unoccupied states of the half-filled $d_z$ orbitals coherently couple to the states of HOPG. We scanned the electron tunneling between single fullerene molecule and individual porphyrin molecules absorbed on HOPG by using the fullerene tips. The STM images were shown in Fig. 1.5.b. In contrast to Fig. 1.5.a, cobalt(II) ions indicated by arrows were seen as depressions, but four pyrrole groups around the cobalt ions were observed as protrusions, which reveals that the large electron tunneling occurs at the pyrrole groups. The inversion in image contrast between center cobalt(II) ions and surrounding pyrrole groups by using fullerene tips instead of gold tips was ascribed to the charge-transfer
interaction concomitant with the overlapped $\pi$ orbitals between a fullerene moiety of the molecular tip and a pyrrole moiety of the CoPor molecule.

![ STM images of SWNTs on Au(111) ](image)

This facilitation was further utilized to recognize the defect of carbon nanotubes at the atomic scale. The electron-donating molecular tips were used to selectively observe defects in single-walled carbon nanotubes (SWNTs). The molecular tips were constructed with 4-aminothiophenol (4AT) by self-assembly of 4AT onto Au STM tips. The sample SWNTs were oxidized in $\text{H}_2\text{SO}_4/\text{HNO}_3$ to attack not only existing active defect sites but also perfect sites in SWNT sidewalls and damage them to induce additional defect sites. The oxidized SWNTs were immobilized on a Au(111) surface and observed with STM in the constant current mode in the air, as shown in Fig. 1.6. By using conventional gold tips, STM image obtained in Fig. 1.6.a shows clearly the atomic structures of SWNTs but hardly detects the defects therein. In Fig. 1.6.b, when 4AT tip was used for observation, the bright protrusion (black arrow) was observed in the STM image of SWNT. Charge-transfer interaction between the electron-donating molecular tip and defect site in nanotubes facilitated
electron tunneling. Therefore, the bright protrusion that corresponds to facile electron tunneling was a defect site of SWNT.

1.2.2.2. Hydrogen bond interaction

The experimental and theoretical studies have indicated that electron tunneling through hydrogen bond interaction plays important roles particularly in biological electron transfer processes. Molecular tips allow STM measurements to recognize selectively a variety of functional groups based on hydrogen bond interaction between these functional groups and the tip molecules. The hydrogen bond interaction increases tunneling current at the functional groups on the surface, and as a result, the functional groups appear as bright protrusions. The difference in strength of hydrogen bonds was also recognized in STM images, as shown in Fig. 1.7.

Fig. 1.7. STM images of C\textsubscript{16}OC\textsubscript{10}OC\textsubscript{16} on HOPG. (a) Using unmodified gold tips. (b) Using 4MBA tips. Schematic illustration of hydrogen bond interactions between carboxyl groups of 4MBA and ether oxygen atoms in (c) unfavorable and (d) favorable orientations [7].
The orientation of \( \text{CH}_3(\text{CH}_2)_{15}\text{O}(\text{CH}_2)_{10}\text{O}(\text{CH}_2)_{15}\text{CH}_3 \) diether (abbreviated as \( \text{C}_{16}\text{OC}_{10}\text{OC}_{16} \)) was discriminated by using 4-mercaptobenzoic acid (4MBA) tips. The metal tips failed to recognize the oxygen atoms positions. As in Fig. 1.7.a, metal tips were blink to diether. Conversely, 4MBA tips were able to not only locate oxygen atoms on the surface but also recognize downward (Fig. 1.7.c) and upward (Fig. 1.7.d) oxygen atoms. Oxygen atoms with favorable orientation for hydrogen bond formation, indicated as oxygen atoms with lone-pair electrons pointing upwards in Fig. 1.7.d, can form much stronger hydrogen bonds with 4BMA tips than the downward oxygen atoms (Fig. 1.7.c). Hydrogen bond acceptors in the favorable orientation were observed as brighter lines in Fig. 1.7.b. The narrow lines next to wide lines in Fig. 1.7.b were ascribed to downward oxygen atoms (Fig. 1.7.c).

1.2.2.3. Metal coordination bond interaction

![STM images](image)

Fig. 1.8. STM images of mixed monolayer of ZnPor and NiPor with ZnPor/NiPor molar ratios (a) 1.0/0.3 (b) 1.0/0.2 and (c) 1.0/5.0 [7].

The electron tunneling facilitated by coordination bond interaction was observed in STM images in Fig. 1.8 by using 4-mercaptopyridine (4MP) tips. Fig. 1.8.a is the typical STM image of a mixed monolayer of ZnPor and NiPor with mole ratio 3:1. The central metal ions of porphyrins appear as very bright spots and moderately bright spot in the protrusion lines. These protrusions were ascribed
to facilitated electron tunneling through coordination bonds that were formed by axial ligation of the pyridyl group of tip molecule with the central metal ion of porphyrin on the surface. The ZnPor content of mixture ZnPor/NiPor solution was increased and decreased to 1.0/0.2 and 1.0/5.0 for STM images in Fig. 1.8.b and Fig. 1.8.c, respectively. The increase/decrease of the very bright spots in Fig. 1.8.b/c, appeared concomitant with the increase/decrease ZnPor content of the mixture ZnPor/NiPor, allowed authors to conclude that very bright spots and moderately bright spots was ZnPor centers and NiPor centers, respectively.

1. 3. Quantitative measurement of single molecule conductance by STM

1.3.1. Background of single molecule conductance

In molecular electronics, the requirements for measuring and controlling the electric current through a molecule are increasing essential for feasibility of molecular electronic devices. So far, single-molecule conductance has been estimated by piezo controlled electrode systems, break junction techniques and so on. However, these measurements have faced to challenges as determination of well-defined contact geometry, reducibility of the measured conductance. In addition, the electron transport at single-molecule level is an important phenomenon occurring in many chemical and biological processes. The ability to determine the single-molecule conductance offers us an opportunity to understand charge transport through single molecule. Meanwhile, the electron transfer from a single molecule to an adjacent molecule can only be understood when the conductance of single-molecule interactions is quantitatively measured. Besides, the ability in determining single-molecule conductance allows us to recognize chemical structures of a single molecule. The acquirement of chemical information from measured conductance in single molecules opens the door to build the molecular devices for chemical recognition or biosensor.

1.3.2. Methodology overview [10]

To determine the conductance of a single molecule, one must first bring it into contact with two metal electrodes. There are several requirements: (1) the measured conductance must be proven a
result of not only the sample molecules, but also a single sample molecule; (2) the molecule of interest is firmly attached to the two conductor electrodes with the specific contact geometry; (3) the measurements are performed in well-defined clean environment. In STM measurements, instead of scanning the STM tip to image individual molecules adsorbed on a conductive substrate with atomic resolution, the STM tip can be stationary positioned over a molecule to measure the tunneling current through this sample molecule. In this method, a molecule adsorbs onto a substrate to form a well-defined molecule-substrate contact, but the contact geometry of tip and molecule is difficult to determine. Therefore, measured conductance of molecules is largely scattered.

Fig. 1.9. The SAM of insulating 1-pentanethiol matrix and embedded conductor 4,4’-biphenyldithiol on Au (111).

The first typical STM measurement of single molecular wire conductance was performed by L. A. Bumm et al. [11]. The molecules of interest were inserted into a self-assembly monolayer (SAM) of less conductive molecules on Au(111) surface. When the STM tip caught the molecules of interest during scanning the mixture SAM on gold surface, the tunneling current increased and the conductance of these molecules was determined. The demonstration of this approach was shown in Fig. 1.9, in which the current through a molecular wire of 4,4’-biphenyldithiol inserted into the insulating alkanethiol monolayer was observed to have much higher conductance than that through the surrounding alkanes.
Another STM approach to measure the conductance of molecules is to attach one end of a molecule onto a substrate and the other end to an Au nanoparticle covalently. Then the STM tip is placed on the top of the nanoparticle to expectably form the electrical connection with molecules, as shown in Fig. 1.10.

Fig. 1.10. 1,5-Pentanedithiol SAM on an Au(111) substrate tethers an Au nanoparticle. The tunneling current through dithiol molecules is indirectly determined by the model of equivalent circuit on the right (a pair of tunnel capacitors $R_1,C_1$; $R_2,C_2$ in series) [12].

However, Au Ohmic contact cannot be established between the tip and the nanoparticle, but rather the tip-nanoparticle interface becomes a tunneling junction because the gold particle surface is unavoidably coated by the organic contaminant. Consequently, the nanoparticle behaves similar to a Coulomb island, so the electron transport through the molecules between the STM tip and sample is affected by the Coulomb blockade effect. The conductance of sample molecule cannot be determined directly from the measured results but from indirectly extraction by fitting the measured $I-V$ curves with the Coulomb blockade model.
A milestone in development of STM approach, which brings much larger accuracy of molecular conductance measurements, was performed by Xu & Tao [13]. A simple and clear measurement of single-molecule conductance was achieved by repeatedly creating thousands of gold-molecule-gold junctions in which molecules are directly connected to the two gold electrodes of tip and sample. The molecules of interest were terminated by two linker groups, which can anchor to the tip and sample surface, at two ends. The relative movement of the tip with the sample has three main stages of interest: (1) the tip was moved towards the sample until forming a gold-wire connection. Beside the ordinary connection of gold wire, there were also several extra connections through several molecules. (2) The tip was retracted from the sample until the gold wire was broken, but extra connections through molecules still existed. The conductance of gold wire was determined based on plateaus just before abrupt decays in current-distance curves. The gold-wire conductance was indicated as quantum steps near multiple of $G_0$ ($G_0 = 2e^2/h$). (3) After gold wire was broken, the tip continued to be pulled away from the substrate. Multiple molecular connections, which conducted electrons much less than gold-wire connection did, were broken in succession until only one molecular connection through one single molecule still existed. In the (3) stage, current-distance traces indicated plateaus just before abrupt decays or steps. The smallest step was equivalent to the electron transport through single molecular connection. The values of multiple steps correspond to the electron transport through multiple molecular connections. During the tip movement, the time at which only single molecular connection still existed in stage (3) provided the single-molecule conductance of interest. In summary, tip-sample displacement presented in this STM break junction technique allowed us to determined not only single-molecule conductance of interest but also unwanted gold-wire conductance. To avoid obtaining unnecessary gold-wire conductance, a small modification of approaching the tip to the sample was proposed. The STM tip is controlled to be proximity to, but never contact with the sample surface. This method was called the molecular junction technique. The STM molecule-junction measurements were utilized throughout the thesis.
1.3.3. Outline of the thesis

Understanding the electron transport at the single-molecule level is primarily important in utilizing a single molecule as an electronic component. Recently, there have been significant advances in the measurement of electron transport through a single molecule, and a large variety of molecules with unique electronic functions, e.g., wires, diodes, switches, and transistors, has been reported. Functional nanoelectronic devices can be realized by the controlled organization via self-assembly processes, where individual molecules are organized through noncovalent interactions between each other. These chemical interactions should allow not only the structural robustness of the molecular assemblies but also a suitable electrical connection between the constituent molecules. Thus, the next challenge lies in measuring electron transfer between single molecules interacting with each other noncovalently. However, rather little is currently known about the electron transfer properties through the noncovalent interactions at the single-molecule interface, and even the macroscopic scale.

In the researches for my thesis, molecular tips of scanning tunneling microscopy (STM) were utilized to develop novel methodologies for quantification of electron transfer through noncovalent interaction between single molecules. The molecular tips have been used to overcome poor chemical selectivity of conventional STM. In addition to this advance, these functionalized tips are also expected to provide a unique means of detecting intermolecular electron transfer given the fact that the electron tunneling takes place between a single molecule on the tip and another adjacent single molecule on a sample surface. The present doctoral thesis consists of six chapters.

Chapter 1 is Introduction, which describes the background of the present research and related previous studies. The purpose and contents of the thesis are also provided.

Chapter 2 describes quantification of electron transfer through a hydrogen bond between single molecules. In this work, STM tips were modified with self-assembled monolayers of w-carboxyl alkanethiol, and the molecular tip was brought into proximity with this sample surface. The electron transfer between the tip and sample molecules were measured while keeping the tip-sample distance constant by freezing the feedback loop of STM. The resulting current-time plot showed current jumps
Chapter 1

and consecutive plateaus. In the present measurement, the tip and sample molecules have carboxyl groups at their free ends, and the current jumps were attributed to hydrogen bond formation between these terminal carboxyl groups. The current increase brought on by the current jump, therefore, reflects electron transfer through the hydrogen bond interaction between the two single molecules. Surprisingly, the conductance of the hydrogen-bonded single molecules was found to be larger than that of the single molecule fully connected with covalent $\sigma$ bonds. The first-principle calculations were performed to qualitatively examine the origin of the superior conductivity of the hydrogen-bonded molecular junction. A prominent peak in the density of states projected onto the central region was found near the Fermi level for the hydrogen-bonded molecular junction while such a peak was absent for the covalently connected molecular junction. Because valence band structures affect the tunneling current through molecular junctions, the higher conductivity of the hydrogen-bonded junctions was attributed to the prominent peak in the projected density of states.

Chapter 3 concerns electron transfer through coordination bond interaction between single molecules. The $\omega$-carboxyl alkanethiol was again used as tip and sample molecules to explore the effect of the metal coordination on the single-molecule conductance. Without a metal ion in the measurement solution, the molecular tip formed a hydrogen bond with the sample molecule through the carboxyl groups, and facile electron transfer took place as demonstrated in Chapter 2. In the presence of the metal ion, two kinds of electron transfer were detected. One of them was through the hydrogen bond between the carboxyl groups. Another electron transfer was attributed to electron transfer through a sandwich coordination molecular junction where the molecule on the tip and substrate coordinate with the metal ion. In Chapter 2, it was demonstrated that a hydrogen bond between single molecules can conduct electrons better than a covalent $\sigma$ bond does. This chapter demonstrates that further facilitation of electron transfer occurs through metal-coordination bond interaction. These observations deepen our understanding of electron transfer between single molecules. Furthermore, the electron transfer induced by metal coordination was exploited in the construction of potential molecular switches activated by a single metal ion.
Chapter 4 deals with quantitative exploration of electron transfer from a single electron donor to a neighboring single electron acceptor. Specifically, electron transfer in a single noncovalent porphyrin-fullerene dyad was measured by utilizing an STM tip functionalized with fullerene, prepared by chemical modification of the Au tip with a fullerene derivative. In the single-molecule conductance measurements, linking groups coupling the sample with the electrodes, such as thiols, are mandatory and currently the subject of extensive research in the field of molecular electronics. Against this situation, this study introduced a novel method for electrical connection for a sample molecule to a gold substrate, i.e., ligation-mediated coupling. This protocol facilitates the detection of electron transfer without the use of a linking group and, thus, without chemical modification of the sample porphyrins. Combination of the fullerene molecular tip and ligation-mediated coupling enable quantitative characterization of the conductance of the single fullerene-porphyrin assembly. It has been demonstrated that a porphyrin-fullerene pair exhibits a rectification property; this characteristic renders the molecular assembly particularly attractive in molecular electronics. Herein, the rectification was successfully quantified. The present study opens up a way for quantitatively exploring rich electronic properties of a supramolecule at the single-molecule level.

Chapter 5 demonstrates the application of the detection of intermolecular electron transfer to single-molecule sensing. Single-stranded DNA was utilized as a probe tip for single-molecule DNA detection. Hybridization occurred between the DNA tip and the target DNA adsorbed on the Au substrate during the conductance measurements. Furthermore, it was found that the presence of a single-base mismatch or methylated nucleobase in the DNA duplex results in significant decrease in the DNA conductance. These results demonstrate that the DNA tips offer a single-molecule sensitive, rapid, and low cost methodology for single-molecule genetic diagnosis through direct electrical detection.

Chapter 6 is Conclusion. This chapter summarizes the whole results in the doctoral thesis and provides their prospects.
2. Direct measurement of electron transfer through a hydrogen bond interaction between two single molecules.

Understanding electron transfer (ET) from a single molecule to another single molecule holds essential importance to realize bottom-up molecular devices in which constituent molecules are self-assembled via noncovalent interactions between each other. However, rather little is currently known about the ET properties at the single-molecule interface. Here we employ molecular tips to quantify the ET through a hydrogen bond (H-bond) between two single molecules. We found that a hydrogen bond conducts electrons better than a covalent $\sigma$ bond does at short-range. Its conductance, however, decays steeply as the chain length of the H-bonded molecules increases. First-principle calculations were performed to reveal the electronic origin of the facile ET through the H-bond. Our results demonstrate that H-bonding in a molecular junction significantly affects its transport property.

2.1. Introduction

Understanding electron transport at the single molecule level is of primary importance in utilizing a single molecule as an electronic component [17, 18]. Recently, there have been significant advances in the measurement of electron transport through a single molecule, and a large variety of molecules with unique electronic functions, e.g., wires [19, 20], diodes [8, 21, 22], switches [23, 24] and transistors [25, 26], have been reported [27]. Functional nanoelectronic devices can be realized by controlled organization via self-assembly processes, where individual molecules are organized through noncovalent interactions between each other [28]. These chemical interactions should allow not only the structural robustness of the molecular assemblies but also a suitable electrical connection between the constituent molecules. Thus, the next challenge lies in measuring ET between single molecules interacting with each other noncovalently. Among the possible noncovalent interactions, H-bonding is ubiquitous in molecular interactions in both artificial and biological systems. However, rather little is currently known about the ET properties through the noncovalent interactions, including H-bonding, at the single-molecule interface [29-33], and even at the macroscopic scale [34-36].
We have developed molecular tips for scanning tunneling microscopy (STM). The molecular tips are prepared by chemical modification, typically via chemisorption of organosulfur compounds, of conventional metal STM tips. We demonstrated that such functionalization enables recognition of chemical identities of a single molecule [7]. Moreover, the molecular tips offer a unique means to detect the ET between single molecules. We previously proved rectified ET within a single electron donor-acceptor complex with a fullerene molecular tip [8]. Recently, molecular tips were further used to quantify electron transport induced by covalent bond formation between two single molecules [16]. In the present study, we show quantification of the ET through a hydrogen bond between single molecules. Although ET though a hydrogen bond in a single DNA base pair has been reported [29-31], we employed a simpler carboxylated alkanethiol, which allows a more direct investigation of the effect of the H-bond. We found that a hydrogen bond conducts electrons better than a covalent $\sigma$ bond does at short range. Its conductance, however, decays steeply as the ET pathway becomes longer. First-principle calculations were performed to reveal the electronic origin of the facile ET through the H-bond. Our results demonstrate that H-bonding in a molecular junction significantly affects its transport property.

2.2. Experimental section

2.2.1. Schematic illustrations of the experiment

In order to measure the electron transfer through a H-bond interaction by STM, the conventional gold tips were modified with self-assembled monolayers of $\omega$-carboxyl alkanethiols (HS-(CH$_2$)$_n$COOH, denoted shortly as C$_n$COOH) to prepare the molecular tips. A gold substrate was modified with C$_n$COOH, and the molecular tip was brought into proximity, but never contact with this sample surface. The current flowing between the tip and surface was measured in 1,2,4-trichlorobenzene. Carboxylic acids readily undergo H-bond interactions in such nonprotic solvents. During the measurements, the tip position and tip–sample distance were held constant by freezing the feedback loop of STM. This procedure was called as $I$–$t$, demonstrated in Fig. 2.1.a. In contrast, the
tip crept up and down in the direction perpendicular to the sample surface during the measurement in the $I$–$z$ mode, as schemed in Fig. 2.1.b.

![Fig. 2.1](image)

Fig. 2.1. Schematic demonstration of tunneling current measurements. The electron transfer through a H-bond was measured via temporary formation of a chemical interaction between the tip and sample molecules. (a). $I$–$t$ mode with C$_2$COOH tip over C$_2$COOH-covered sample. (b) $I$–$z$ mode with C$_2$COOH tip over C$_1$COOH-covered sample.

### 2.2.2. Reagents

The $\omega$-carboxyl alkanethiols (HS-(CH$_2$)$_n$COOH, $n = 1, 2, 3$), octanedithiol (C$_8$DT) and 1,2,4-trichlorobenzene (C$_6$H$_3$Cl$_3$) were purchased from a chemical company in Tokyo, Japan. The reagents were of the highest grade available. De-ionized water purified with a Milli-Q water purification system (Japan Millipore, Tokyo, Japan) was used for all the experiments.

### 2.2.3. Tip preparation

Small pieces of gold wire (0.25 mm diameter, 99.95%) were electrochemically etched in 3 M NaCl at AC 10 V. They were then washed by sonication in pure water, dipping in “piranha solution” (7:3 concentrated H$_2$SO$_4$/H$_2$O$_2$. Caution: piranha solution reacts violently with organic compounds and should not be stored in closed containers), and finally, thoroughly washed with pure water. For the preparation of molecular tips using the $\omega$-carboxyl alkanethiols, the gold tips were immersed into the 7.5 mM ethanolic solution of C$_6$COOH for 12 h. Full, or at least highly dense, monolayer
formation is expected on the tips based on the concentration and immersion time [37]. The modified tips were thoroughly washed with ethanol and water prior to use.

2.2.4. Sample preparation

Ultraflat gold films epitaxially grown on mica were used as Au(111) substrates [38]. The gold substrate was immersed in a 50 μM ethanolic solution of CₙCOOH or octanedithiol (C₈DT) for 30 s and 1 h for the current–time (I–t) and current–distance (I–z) measurements, respectively. Based on the selected concentration and immersion time, formation of adlayer during the thiol adsorption process should occur in the initial stages [39]. Thus, the concentration of sample molecules on the substrate surface needs to be low in order to reduce the possibility of multiple junction formation. The substrate was washed with pure ethanol and blown dry with pure nitrogen. Then, the sample cell was mounted on the substrate that was put on the STM sample holder. The cell was filled with pure 1,2,4-trichlorobenzene to prevent unwanted contamination during the measurements.

2.2.5. I–t and I–z measurements

Before performing the I–t and I–z measurements, the STM was equilibrated with the room temperature for 30 minutes to reduce the thermal drift. The piezo scanner of the STM was allowed to settle under feedback control without being driven during the equilibration. This procedure suppresses unwanted hysteresis and the creep of the piezo scanner. In the I–t measurements using the C₂COOH tips and C₂COOH-covered surfaces, the bias voltage and set-point current were +0.2 V and 0.5 nA, respectively. Successful acquisition of I–t data is contingent on careful control of these two parameters given that the tip–sample distance depends on them. The set-point current was optimized by using a constant bias voltage (0.2 V) and varying the set-point current in the range of 0.1 to 1.5 nA with acquired optimal value of 0.5 nA. Set-point values higher than 0.5 nA corresponding to closer separation of tip and sample resulted in broadening of the current histograms due to formation of multiple molecular junctions. On the other hand, with lower set-point currents, the probability of observing the plateaus (as shown in Fig. 2.2.a) decreased, plausibly because the separation was too long for junction formation. In the case of the I–t measurements using the unmodified gold tips and
C₈DT-covered surface, the bias voltage and set-point current were +0.2 V and 1 nA, respectively. In the \( I-t \) measurements, the feedback loop was disabled for 1 second, and the current flowing between the molecular tip and the sample surface was recorded at a 20 kHz sampling frequency using a data acquisition unit (SL1000, Yokogawa Electric Corporation). In the \( I-z \) measurements, the C₂COOH tip was first brought into close proximity with the sample surface using high set-point currents (7.5 nA for the C₁COOH and C₂COOH samples and 0.75 nA for the C₃COOH sample) at a bias voltage of +0.2 V. The molecular tip was then raised at a tip velocity of 5 nm/s, and the current was recorded at a 20 kHz sampling frequency using the data acquisition unit. The current measurements were repeatedly performed on different days, and each time the sample surface was freshly prepared.

2.2.6. Control experiments using a clean Au substrate or tip

No plateaus in \( I-z \) curves were observed when the C₂COOH tips were used for the measurements with clean Au substrates or when unmodified Au tips were used with the C₂COOH-covered surface. Although the carboxyl group was shown to be an effective contact group for the single molecule junction [40, 41], the above experiments exclude the possibility that the C₂COOH tip formed a molecular junction directly on the Au surface without involving the sample molecule on the substrate. We ascribe this absence of the contact between the carboxyl group and substrate surface to the small set-point current in the present study. On the basis of the reported conductance values [40, 41], HOOC(CH₂)₂COOH and HS(CH₂)₂SH molecular junctions, comprising the same number of methylene groups and contact groups as C₂COOH, exhibit the current larger than 10 nA under the bias voltage in the this study (0.2 V). The initial set-point currents in the present measurements (7.5 or 0.75 nA) are smaller than this expected current. As a result, the C₂COOH molecule on the tip cannot contact with the gold surface.

2.2.7. Statistic analysis procedure for \( I-t \) and \( I-z \) data

The current histograms were constructed from the \( I-t \) traces that exhibited the current plateaus, as shown in Fig. 2.2.a, or from the \( I-z \) traces with current steps, as shown in Fig. 2.3.b. Approximately 10% of the measured \( I-t \) and \( I-z \) traces exhibited these plateaus or increasing steps. This ratio is
comparable to that observed when alkanedithiol molecular tips were used to measure the conductance change induced by the formation of a single covalent bond with a sample alkanedithiol [16]. The bin size of the current histogram is 0.5 pA. At least 200 $I$–$t$ or $I$–$z$ traces were used to construct each histogram. For each sample molecule, multiple current histograms were constructed from several data sets (at least three) obtained using independently prepared sample surfaces. The standard deviations depicted as error bars in Fig. 2.3.c were determined by the peak values of these histograms.

2.2.8. First-principle calculations.

First-principle density functional theory calculations were performed by using the VASP code [42] that utilizes the generalized gradient approximation of Perdew, Burke and Ernzerhof (GGA-PBE) [43]. A test calculation was performed for projected density of states (PDOS) onto a (CH$_2$)$_6$ chain and a COOH end group of a HOOC(CH$_2$)$_6$COOH molecule. The result (Fig. 2.4) agrees well with literature (Fig. 4 in [44]), demonstrating the robustness of the calculation. The molecular junctions were constructed by sandwiching either the C$_2$COOH or the C$_8$DT molecule, both of which were geometrically optimized in a gas phase, between Au electrodes. A pyramidal cluster composed of 10 Au atoms was used as one of the electrodes to represent the STM tip. The other electrode was a Au(111) slab of three layers with a size of 14.42 $\times$ 17.54 Å$^2$, and repeated slab geometry was used. The molecule was placed at the hollow site at the center of the slab [45]; it was connected to the pyramidal cluster at the a-top site of the apex atom. The calculations were performed without full relaxation of the atomic positions, which is justified by the fact that the molecular junctions cannot reach their equilibrium due to continuous movement of the tip during measurements. The S-Au bond length was 2.853 Å in both cases. Single point calculations were performed with a supercell of dimensions 17.3024 $\times$ 19.1466 $\times$ 50 Å$^3$. The convergence criterion in the self-consistent iterative calculations was chosen as $10^{-5}$ eV. The plane-wave cutoff energy was 200 eV. We also employed the cutoff energy of 400 eV under the same convergence criterion ($10^{-5}$ eV) in calculations presented in Fig. 2.5. The results obtained at 200 eV and 400 eV cutoff energies are consistent with each other, demonstrating that the cutoff energy of 200 eV is sufficient. The cutoff energies of calculations were
presented in each figure caption. The Brillouin zone was sampled with special \( k \)-points on a \( 3 \times 3 \times 1 \) grid for the \( C_2COOH \) dimer and a \( 4 \times 4 \times 1 \) grid for the \( C_8DT \).

### 2.3. Results and discussion

Fig. 2.2. ET between a single H-bonded \( C_2COOH \) dimer. (a) Representative \( I-t \) plots showing jump-plateau signals measured using \( C_2COOH \) tips over \( C_2COOH \)-covered surfaces. Each plot is vertically shifted for clarity. Bias voltage: 0.2 V; set-point current: 0.5 nA. (b) Current histograms constructed from \( I-t \) plots. Pink: measurements made using \( C_2COOH \) tips over \( C_2COOH \)-covered surfaces. fwhm = 0.23. Blue: measurements made using Au tips and \( C_8DT \)-covered surfaces. fwhm = 0.26.

Fig. 2.2a shows representative current–time (\( I-t \)) plots measured using \( C_2COOH \) molecular tips over \( C_2COOH \)-covered Au(111) substrates. In addition to the background current, which is equal to the set-point value, current jumps and consecutive plateaus were observed in the \( I-t \) plots. We previously reported such jump–plateau signals, which result from the formation of a single covalent linkage between thiol groups of the molecular tip and the sample molecules [16]. In the present case, the tip and sample molecules have carboxyl groups at their free ends, and we attribute the current jumps to H-bond formation between these terminal carboxyl groups (Fig. 2.1.a, right). The current increase brought on by the current jump, therefore, reflects ET through the H-bond interaction between two single molecules. A histogram of the current increase caused by the H-bond formation was constructed from hundreds of individual current jumps (Fig. 2.2.b, pink). For comparison, we performed \( I-t \) experiments for the electron transport through a single octanedithiol (\( C_8DT \)) molecular junction, which contains 8 carbon atoms (Fig. 2.2.b, blue) [46]. The length of the H-bonded \( C_2COOH \)
dimer, composed of 6 carbon atoms in total (see Fig. 2.1.a, right), is almost the same (1.21 nm between terminal S-atoms) as that of C₈DT (1.19 nm), allowing a direct comparison. Each histogram in Fig. 2.2.b exhibits a single distinct peak; the conductance of the molecular junction was calculated using the peak current value. Despite the fact that the Au tip and substrate were connected by covalent σ bonds in the case of C₈DT, the conductance of the H-bonded C₂COOH dimer (1.5 nS) was larger than that of the C₈DT (0.99 nS).

Fig. 2.3. Distance dependence of ET through H-bond. (a) Representative I–z plots measured using C₂COOH tips over C₂COOH-covered surfaces. Each plot is horizontally shifted for clarity. Bias voltage: 0.2 V; set-point current: 7.5 nA. (b) Current histograms constructed from I–z plots measured using C₂COOH tips over Au substrates modified with C₁COOH (blue), C₂COOH (yellow), and C₃COOH (orange). A highly sensitive preamplifier (0.1 nA/V) was used for the measurements of the C₃COOH-covered surface (Inset). Larger currents cannot be measured in this case. (c) Distance dependence of conductance of molecular junction consisting of H-bonded CₙCOOH dimer (pink) and alkanedithiolate (blue). Number of C-atoms (nC) in the molecular junction, including those in carboxyl groups, is indicated for each data point.
The conductance of the H-bonded molecular junctions was further investigated by current measurements as a function of tip displacement from the substrate. After being brought into close proximity to the C$_2$COOH-covered Au(111) substrate in 1,2,4-trichlorobenzene, the molecular tip was raised. Fig. 2.3.a illustrates representative current–displacement (I–z) curves measured with the C$_2$COOH tip and C$_2$COOH-covered surface. Besides smooth exponential decay due to the electron tunneling, the I–z curves showed clear steps or plateaus. These steps are ascribed to the H-bond interaction between the C$_2$COOH molecules on the tip and sample surface. A current histogram was constructed using the current value of each data point in the I–z curves (Fig. 2.3.b, yellow). A single pronounced peak is found in the histogram; the conductance of the H-bonded C$_2$COOH dimer was calculated to be 1.4 nS, which is in agreement with the conductance determined using the I–t measurements. Generally, multiple peaks are seen in the histogram obtained from the I–z measurements, such as STM break-junction experiments. These peaks are interpreted as resulting from the formation of multiple, not single, molecular junctions between the tip and the substrate [13, 20] or different contact geometries between the anchor groups of the molecule, such as thiol, and the gold surface [46, 47]. Conversely, only one single peak appeared in the histogram in Figure 2.3.b, which demonstrates that electrons are transferred through the single H-bonded molecular junction. Although the carboxyl group was shown to be an effective contact group for the single molecule junction [40, 41], the control experiments using either a clean Au substrate or unmodified Au tip exclude the possibility that the C$_2$COOH tip formed a molecular junction directly on the Au surface without involving the sample molecule on the substrate (see section 2.2.6). The molecular junction in the present study was created by spontaneous association between the molecule freely diffusing on the substrate and the tip molecule. We, therefore, expect that the intermolecular geometry was optimized so as to form a favorable H-bond in-between. The most probable structure of the H-bond interaction between the tip and substrate is the well-known cyclic structure in which the two carboxyl groups form two very strong H-bonds (Fig. 2.1.a, right) since such a structure is shown to be most stable in both the gas phase and weakly polar to nonpolar solutions [48]. The observation of only a single peak in Fig. 2.2.b (pink) indicates that no H-bond configuration other than the cyclic structure was detected in the present experiments. Although the tilt angles of the sample and tip molecules were not
controlled in the present experiments, it has been demonstrated that the tilt angle has little, if any, effects on the junction conductance unless particularly strong electronic coupling exists between the molecule in the junction and the substrate [49, 50].

![PDOS](image)

Fig. 2.4. PDOS onto a (CH\(_2\))\(_6\) chain (blue line) and a COOH end group (red bars) of a HOOC(CH\(_2\))\(_6\)COOH molecule. Cutoff energy: 200 eV.

Next, the distance dependence of the ET was investigated for \(\omega\)-carboxyl alkanethiols with different molecular lengths for the tip and sample molecules. \(I-z\) measurements were performed, and current histograms were constructed using the C\(_2\)COOH tip over the Au(111) substrates modified with C\(_1\)COOH (Fig. 2.1.b and Fig. 2.3.b, blue), C\(_2\)COOH (Fig. 2.3.b, yellow), and C\(_3\)COOH (Fig. 2.3.b, orange). In all cases, current steps appeared in the \(I-z\) curves, and each histogram exhibited a single distinct maximum (arrow in Fig. 2.3.b). The preamplifier used for the measurement of the C\(_3\)COOH-covered surface has higher sensitivity (0.1 nA/V) but a narrower measurable current range than the preamplifier for the other measurements. The conductance values for the single H-bonded molecular pair were determined by the peak currents of the histograms. Fig. 2.3.c shows the conductance as a function of the length of the molecular pair (pink); the plot reveals that the conductance value depends exponentially upon the length. The slope of this logarithmic plot is referred to as the \(\beta\) decay constant and found to be 10 nm\(^{-1}\). The conductance values for single alkanedithiol molecular junctions (hexanedithiol, C\(_3\)DT, nonanedithiol) are also shown in Fig. 2.3.c (blue); the conductance exhibits an exponential dependence upon the molecular length. The \(\beta\) value in this case was 4.2 nm\(^{-1}\), being consistent with reported values [46, 51]. The higher \(\beta\) values for the same alkanedithiol junctions (approximately 8.5 nm\(^{-1}\)) were also reported [47, 52], and these results are attributed to the differences in the surface concentrations of the sample molecules on the substrate [47] or in the degree of surface flatness of the substrate at the atomic scale [53]. As shown in the \(I-t\) measurements,
the conductance of the molecular pair interacting with each other via the H-bond interaction was larger than that of the alkanedithiol molecular junction (where the Au tip and substrate were fully connected by σ covalent bonds) for the shorter cases. However, conductance of the H-bonded junction decay more quickly than that of the covalent junction does as the molecular length increases because of the larger β value of the H-bonded junction. The H-bonded molecular pair consequently becomes less conductive than its covalent counterpart for the larger case. A similarly large β value has previously been reported for a H-bonded molecular pair [30].

Fig. 2.5. PDOS of molecular junctions. (a) Schematic illustration of molecular junctions consisting of H-bonded C$_2$COOH dimer (left) and C$_8$DT (right). (b) PDOS of C$_2$COOH dimer (pink) and C$_8$DT (blue). Cutoff energy: 400eV. The central regions include two carboxyl and four methylene groups in the case of the C$_2$COOH dimer and the C$_8$DT, respectively. The PDOS values of the symmetrically equivalent atoms or groups (S, first CH$_2$, and second CH$_2$) were added up together.

We performed control experiments to confirm that the H-bond between the tip and sample molecules mediates the ET. The $I$–$z$ measurements were performed in the presence of urea in the solvent. Urea possesses excellent H-bond capabilities [54] and, thus, can disrupt H-bonding between the C$_2$COOH molecules on the tip and sample. With the urea addition, the plateaus as shown in Fig. 2.3.a were observed on only 0.1% of the $I$–$z$ curves out of 14,000 measurements. In an additional experiment, we employed a methyl ester of C$_2$COOH, methyl 3-mercaptopropionate, as a molecular
tip, and again, essentially no plateaus were observed in the $I-z$ curves. These results demonstrate that H-bond interaction between the tip and sample molecules plays an essential role in ET.

Fig. 2.6. PDOS of the molecular junction consisting of hydrogen-bonded C$_2$COOH dimer. (a) Schematic demonstration of the junction structure. (b) PDOS on each S (S-1 and S-2), CH$_2$ (CH2-1 to CH2-4), and COOH group (COOH-1 and COOH-2). Cutoff energy: 400eV. It should be noted that the PDOS peaks of the two sulfur atoms at different binding sites (S-1 and S-2) appeared at nearly the same position though the peak shapes are different.

After the first-principle calculations on a (CH$_2$)$_6$ chain and a COOH end group of the HOOC(CH$_2$)$_6$COOH molecule was tested successfully in Fig. 2.4, these procedures were applied to carry out to qualitatively examine the origin of the superior conductivity of the short H-bonded junctions. We investigated the electronic structures of the molecular junctions by PDOS. Transmission spectra based on non equilibrium Green’s function in conjunction with density functional theory, rather than PDOS, are often utilized to investigate molecular conductivity. It was demonstrated that PDOS peaks correlates well with transmission peaks [55]. Moreover, PDOS analyses allow for evaluating contributions of each atom or moiety of the molecular junction to the current [44, 56]. We considered either the C$_2$COOH dimer or the single C$_8$DT molecule connected to the Au electrodes at their terminals (Fig. 2.5.a). Fig. 2.5.b shows the PDOS onto terminal S-atoms, methylene groups, and the central parts of the molecular junctions of the C$_2$COOH dimer (pink) and
C₈DT (blue). The PDOS on the S-atoms and the first two methylene groups nearest to the electrodes exhibited similar peaks for these two junctions (Fig. 2.6). However, a significant difference was found in the PDOS in the central regions. There are prominent peaks centered at approximately −2.1 eV in the PDOS on the two carboxyl groups interacting with each other via the H-bond in the case of the C₂COOH dimer junction (pink in Fig. 2.5.b). In contrast, these peaks are totally absent in the PDOS on the four central methylene groups of the C₈DT junction (blue in Fig. 2.5.b). Because valence band structures affect the tunneling current through molecular junctions [44], we attribute higher conductivity of the short H-bonded junctions to the occupied states at −2.1 eV. Development of a theoretical framework that extends based on the currently available ones is required before one can make quantitative arguments about the facilitated ET.

### 2.4. Conclusions

In summary, it is shown here that a H-bond interaction facilitates electron transfer between single molecules. We revealed the superior conductivity of the H-bonded molecular junction compared to the junctions connected by the covalent σ bonds. The property of the electron transfer through the H-bond exhibited pronounced dependence on the junction length. These results indicate that the electrical connection between single molecules can be deliberately controlled by adjusting the length of H-bond linkers between each other. Furthermore, we anticipate the facilitated electron transfer can be switched on and off by changing the solution’s pH, since (de)protonation of a functional group can cause the formation or destruction of a H-bond interaction. Our results suggest that H-bonding can be utilized not only merely for structural connection but also for implementing electronic functions, including electrical connections and switching, in molecular nanoelectronic devices.
3. Electron transfer through coordination bond interaction between single molecules: conductance switching by metal ion

Molecular tips were used to investigate electron transfer through metal-coordination bonds between single molecules. Coordination of a single metal ion to two carboxylate-terminated thiolate molecules formed a sandwich-type single molecular junction. It was found that a favorable charge transfer is induced through such molecular junctions. The electron transfer facilitated by metal coordination was utilized to implement conductance switching in a molecular junction of a head-to-head pyridine dimer. The present research offers a method to control electronic functions required in the construction of functional electronic molecular devices.

3.1. Introduction

Measurements of single-molecule conductance have provided significant advances in understanding transport properties at the single-molecule level. The commonly employed techniques for such measurements include mechanically break junction and a related method based on STM. The rapidly growing progress brought by these techniques in the field of molecular electronics has revealed the influence of chemical and electronic structure on electron transmission in molecular electronic junctions [17, 18]. In light of such development, deliberate control of the conductance of a single-molecule junction has attracted much attention [47, 57, 58]. For example, switching behavior between high- and low-conductance states has been studied using electrochemical gating. In this procedure, both the reference and counter electrodes act as gate electrodes [59, 60]. Controllable electronic functions such as conductance switching effects are prerequisites for realizing functional electronic molecular devices.

The construction of such molecular devices also requires controlled organization via self-assembly processes, wherein individual molecules are organized through non-covalent interactions [28]. Therefore, understanding electron transfer between single molecules interacting with each other non-covalently is of great importance, although only little knowledge in this context is currently
available [29, 30, 33]. We have developed molecular tips for STM, which were prepared by the chemical modification of conventional metal STM tips, typically via the chemisorption of organosulfur compounds [7-9]. Very recently, we utilized ω-carboxyl alkanethiol (HS(CH$_2$)$_n$COOH, C$_n$COOH; n = 1-3) molecular tips to quantify electron transfer through a hydrogen bond between single molecules (Fig. 3.1.a), and demonstrated that a hydrogen bond conducts electrons better than a covalent σ bond does [61]. In the present study, molecular tips were utilized to examine electron transfer through a metal-coordination bond (Fig. 3.1.b). It was revealed that the coordination bond further facilitates electron transfer compared to the transfer via hydrogen bond. Moreover, this phenomenon was shown to offer a novel means to implement a switching behavior in a molecular junction.

![Fig. 3.1. Schematic illustration of a molecular junction created using a molecular tip via (a) hydrogen bond and (b) metal-coordination bond interactions.](image)

**3.2. Experimental section**

Briefly, ω-carboxy alkanethiol was employed as a ligand to explore the effect of the metal coordination on the single-molecule conductance. Metal-carboxylate coordination is widely exploited, especially in metal-organic frameworks [62-64]. STM Au tips were modified with the self-assembled monolayers of C$_1$COOH to prepare the molecular tips. A sample Au(111) surface was modified with C$_2$COOH at low coverage, which is important for the quantification of electron transfer from a single molecule to its adjacent molecule. The electron transfer was directly measured by current–distance ($I$–$z$) traces. The molecular tip was first brought in close proximity to the C$_2$COOH-modified Au
substrate in pure 1,2,4-trichlorobenzene solvent. This was achieved by applying a relatively large set-point current (7.5 nA) under the STM feedback control. The molecular tip was then raised with the feedback disabled, and the current flowing between the tip and substrate was recorded as a function of the tip–sample distance.

3.2.1. Reagents

The reagents were of the highest grade available. De-ionized water purified through a Milli-Q water purification system (Japan Millipore, Tokyo, Japan) was used in all experiments. The \( \omega \)-carboxyl alkanethiols (HS-(CH\(_2\))\(_n\)COOH, \( n = 1-3 \)), 4-mercaptobenzoic acid (SHC\(_6\)H\(_4\)COOH, abbreviated to 4MBA), 4-mercaptopyridine (SHC\(_6\)H\(_5\)N, abbreviated to 4MP), ethylenediaminetetraacetic acid ((CH\(_2\)N(CH\(_2\)COOH)\(_2\)), abbreviated to EDTA), Polydimethylsiloxane ((C\(_2\)H\(_6\)OSi)\(_n\)), abbreviated to PDMS), bromide salts (MBr\(_n\), \( n = 1, 2 \), M = metal) and 1,2,4-trichlorobenzene (C\(_6\)H\(_3\)Cl\(_3\)) were purchased from Wako Pure Chemical Industries, Ltd in Tokyo, Japan.

3.2.2. Tip preparation

Small pieces of gold wire (0.25 mm diameter, 99.95%) were electrochemically etched in 3 M NaCl at AC 10 V. They were then washed by sonication in pure water and dipping in “piranha solution” (7:3 concentrated H\(_2\)SO\(_4\)/H\(_2\)O\(_2\). (Caution: piranha solution reacts violently with organic compounds and should not be stored in closed containers.) Finally, they were thoroughly washed with pure water. To prepare molecular tips, either aliphatic (C\(_n\)COOH) or aromatic (4MBA or 4MP) thiol was dissolved in ethanol typically at 7.5 mM, and the gold tips sharpened by the electrochemical etching were immersed in the thiol solution overnight. The modified tips were then rinsed with ethanol and water prior to use. For the current measurements in aqueous solutions, the gold tips were insulated with PDMS except for their apexes to reduce ionic and polarization currents [65]. The tips were then modified by using the above thiol molecule.
3.2.3. Sample preparation

Ultraflat gold films epitaxially grown on mica were used as Au(111) substrates [38]. The gold substrate was immersed in a 50 \( \mu \)M ethanolic solution of thiol (C\(_n\)COOH, 4MBA or 4MP) for 1 h. After washing with a pure ethanolic solution, the substrate was placed on the STM sample plate and was overlapped by a sample cell. The cell was filled with either 1,2,4-trichlorobenzene or 0.1 M NaClO\(_4\) aqueous solution for the current measurements. Alternatively, ZnBr\(_2\), MnBr\(_2\), CoBr\(_2\), or NaBr was dissolved in each solution at a concentration of 10 mM. Control experiments were performed using 0.1 M NaClO\(_4\) solution containing 10 mM ZnBr\(_2\) and 10 mM EDTA.

3.2.4. Current measurements

The tunneling current measurements were performed on an SPM 5100 with a 1 nA/V pre-amplifier (Agilent Technologies, Santa Clara, CA, USA). Before each measurement, the STM was equilibrated with the room temperature for 30 minutes to reduce the thermal drift. The piezocrystal scanner of the STM was allowed to settle under feedback control without being driven during the equilibration. This procedure suppresses unwanted hysteresis and creep of the piezocrystal scanner. The molecular tip was then brought in close proximity to, but not in contact with, the modified Au surface. This procedure was achieved by applying a large set-point current (7.5 nA) under the STM feedback control. A bias voltage of 0.2 V was employed for all the measurements. After a short delay time of 100 ms, the molecular tip was pulled up at a velocity of 20 nm/s with the feedback loop disabled, and \( I-z \) traces were recorded at a 20 kHz sampling frequency using a data acquisition unit (NR-500; Keyence, Osaka, Japan). This measurement was repeated several thousand times.

3.2.5. Data analyses

The current histograms were constructed from the \( I-z \) traces that exhibited the plateaus. Plateaus were observed in \(~10\%\) of the obtained \( I-z \) traces. Other traces exhibited either simple exponential or noisier decays. This ratio is comparable to that observed in our previous work, in which the electron transfer through a hydrogen bond interaction between single molecules was measured [61].
3.3. Results and discussion.

Fig. 3.2. (a) Representative $I$–$z$ plots obtained using C$_2$COOH tip and C$_1$COOH-modified substrate in the absence (blue) and presence (pink) of Zn$^{2+}$. Bias voltage: 0.2 V; initial set-point current: 7.5 nA. (b) Current histograms constructed from $I$–$z$ plots obtained in the absence (blue) and presence (pink) of Zn$^{2+}$. Bin size: 10 pA.

We first obtained $I$–$z$ curves in the absence of metal species (Fig. 3.2.a, blue). Without a metal ion, the molecular tip formed a hydrogen bond with the sample molecule through the carboxyl groups (Fig. 3.1.a), and facile electron transfer took place as demonstrated in our previous work [61]. This effect is represented in Fig. 3.2.a (blue) as plateaus at which current maintained constant despite the increasing separation. The current value at the plateau positions was statistically assessed by a current histogram constructed using the current value of each data point in thousands of $I$–$z$ curves (Fig. 3.2.b, blue). Consequently, a single distinct peak appeared in the histogram. On the basis of the peak current of 1.1 nA, the conductance of the molecular junction formed from the hydrogen-bonded C$_1$COOH–C$_2$COOH dimer was determined to be 5.6 nS (or $7.2 \times 10^{-5} G_0$, where $G_0$ is the fundamental conductance quantum), agreeing well with our previous report [61].

Similar measurements were performed in the presence of Zn$^{2+}$ ion by using 10 mM ZnBr$_2$ solution in 1,2,4-trichlorobenzene. The resulting $I$–$z$ curves exhibited plateaus (Fig. 3.2.a, pink), and the current histogram constructed from the curves exhibited two peaks (Fig. 3.2.b, pink). Generally, multiple peaks can be observed in a current histogram obtained from $I$–$z$ curves for measuring single-molecule conductance. These peaks are interpreted as resulting from the formation of not single, but
multiple, molecular junctions between the tip and the substrate [13, 20]. These peaks may also result from different contact geometries between the anchoring groups of the molecule (such as thiol) and the gold surface [47, 46]. However, in the present work, similar measurements performed without the metal ion resulted in the histogram showing a single peak (Fig. 3.2.b, blue). These results exclude the aforementioned interpretation (multiple junctions or different contact geometries); rather, they suggest that the multiple peaks in Fig. 3.2.b (pink) arose from the effect of the metal ion on the single noncovalent molecular junction. By peak fitting, the current (conductance) values for the two peaks at high and low currents (labeled “H” and “L”, respectively) were found to be 0.99 nA (4.9 nS or $6.4 \times 10^{-5} G_0$) and 1.6 nA (7.9 nS or $1.0 \times 10^{-4} G_0$), respectively. The L peak agrees with the peak observed in Fig. 3.2.b (blue); therefore, the L peak originated from a hydrogen-bonded molecular junction that involved no metal ion (Fig. 3.1.a). Concerning the H peak, we attribute this higher peak to a sandwich coordination molecular junction where the molecules on the tip and substrate coordinate with the Zn ion (Fig. 3.1.b). The electron transport through single metal complexes has been previously reported [66-69]. Unlike in these studies, the coordination bond was formed between single molecules in situ during current measurements in the present study. Nevertheless, the present results concur with the findings of these previous studies. For example, the conductance of a single oligopeptide has been found to increase upon metal-ion binding [66]. The increase in conductance observed in the present as well as in previous works is ascribed to the enhanced electron transport via redox levels of the metal ions; this interpretation has been confirmed by first-principle calculations [67]. In our previous work, it was demonstrated that a hydrogen bond between single molecules can conduct electrons better than a covalent σ bond can. The present study demonstrates the occurrence of further facile electron transfer through metal-coordination bond interaction. These observations deepen our understanding of electron transfer between single molecules.

It is usually difficult to gain structural information about the molecular junction [47]. We anticipate here that the coordination bond is formed by the deprotonated carboxylate groups of the sample and tip molecules (Fig. 3.1.b), because such deprotonation proceeds during the substrate and
tip modification in ethanolic solutions [70]. Although chelate-type coordination is illustrated in Fig. 3.1.b, other coordination modes such as mono- and bidentate coordination are also possible [71].

![Fig. 3.3. Current histograms from $I$–$z$ curves obtained by measurements with C$_2$COOH tip and C$_1$COOH-modified substrate in the presence of (a) Co$^{2+}$ and (b) Mn$^{2+}$ ions. Bias voltage: 0.2 V; initial set-point current: 7.5 nA; bin size: 10 pA.]

The current measurements were performed in the presence of other bromide salts, i.e., CoBr$_2$ and MnBr$_2$. Again, plateaus appeared in the resulting $I$–$z$ curves, and the current histograms built from them exhibited two peaks (Fig. 3.3). In each case, the peak at lower current agreed well with the one in Fig. 3.1.b (Table 3.1), and was thus assigned to the electron transfer through the H-bonded molecular junction without the metal ion. The peaks at higher current (1.5 nA for both Co$^{2+}$ and Mn$^{2+}$, Table 3.1) were attributed to the metal-ion bridged molecular junctions (Fig. 3.1.b). We carried out the $I$–$z$ measurements with 10 mM NaBr in solution. In this case, the same results as shown in Fig. 3.2.a (blue) and 3.2.b (blue) were obtained: the current histogram showed a single peak whose position agrees well with the peak position in Fig. 3.2.b (blue; Table 3.1). These results demonstrate that a positive charge alone is not sufficient to increase the molecular conductance and that the coordination bonding between the molecules on the tip and substrate and the metal ion plays an essential role. While previous studies on electron transport through single metal complexes reported moderate dependence of their conductance on the type of transition metal ion, no such dependence was noticed in the present measurements. Electron transport through a molecule primarily depends on the gap between the highest occupied and lowest unoccupied molecular orbitals of the interested
molecule and on the alignment of these orbitals with respect to the Fermi energy of the metal electrodes. Detailed systematic theoretical calculations in these aspects might shed light on the metal dependence or independence of the junction conductance.

Table 3.1. Peak current values in the current histograms\[a\]

<table>
<thead>
<tr>
<th>Added ion</th>
<th>Peak current (nA)</th>
<th>$L$ peak</th>
<th>$H$ peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.1</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>0.99</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>0.92</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>0.95</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Na$^+$</td>
<td>1.1</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

\[a\] Measurements were performed using the C$_2$COOH tip and C$_1$COOH-modified substrate.

Fig. 3.4. Current histograms constructed from $I$–$z$ curves obtained by measurement with 4MBA tips over 4MBA-modified substrates in the (a) absence and (b) presence of Zn$^{2+}$. Bias voltage: 0.2 V; initial set-point current: 75 nA; bin size 100 pA.

The conductance increase induced by metal coordination was further investigated by using aromatic molecules. 4MBA was employed as the molecular tip, and $I$–$z$ measurements were
performed by using 4MBA-modified Au substrate in 0.1 M NaClO₄ aqueous solution. Plateaus were observed both in the absence and presence of Zn²⁺ in the measurement solution, and the current histograms resulting from the $I-z$ traces exhibited a single peak in each case (Fig. 3.4). However, the variation of current values at the peak positions depends on the presence of the metal ion. In its absence, the peak was located at 3.6 nA (Fig. 3.4.a), and the conductance value was calculated to be 18 nS (or $2.3 \times 10^{-4} G_0$). On the basis of the previous work [61], this peak is attributed to electron transfer through a hydrogen bond interaction between the carboxyl groups of the two 4MBA molecules on the tip and substrate. When Zn²⁺ ion was present in the solution during the $I-z$ measurements, the peak appeared at higher current; the peak current and conductance values were 5.7 nA and 29 nS (or $3.7 \times 10^{-4} G_0$), respectively (Fig. 3.4.b). This result is in line with the observation for the aliphatic molecules (Fig. 3.2.b). The conductance increase is attributed to metal coordination bond formation, i.e., insertion of the metal ion between 4MBA molecules on the tip and the substrate. To further substantiate this interpretation, similar measurements were carried out using the 4MBA tip and the sample in 0.1 M NaClO₄ solution containing 10 mM ZnBr₂ and EDTA. EDTA is a chelating agent capable of masking Zn²⁺ ions. The resulting current histogram is characterized by a single peak located at 3.7 nA (Fig. 3.5).

![Current histogram](image)

Fig. 3.5. Current histogram from $I-z$ curves obtained by measurements with 4MBA tips and 4MBA-modified substrate in 0.1 M NaClO₄ solution containing 10 mM ZnBr₂ and 10 mM EDTA. Bias voltage: 0.2 V; initial set-point current 75 nA; bin size: 100 pA.
This peak coincides well with the one in Fig. 3.4.a, demonstrating again the crucial role of the metal ion in the facilitation of the electron transfer. These results lead to the conclusion that coordination bond interaction within molecular junctions facilitates electron transfer between single molecules.

When the current measurements were conducted using the C₂COOH tip and C₁COOH-modified substrate, the current histogram exhibited the peak originating from a hydrogen-bonded C₁COOH-C₂COOH dimer (Fig. 3.1.a), even in the presence of Zn²⁺ ion (Fig. 3.2.b, pink). Conversely, in the case of the measurements using 4MBA tip and sample, no peak for the hydrogen-bonded 4MBA dimer appeared when Zn²⁺ was present in the solution (Fig. 3.4.b). These results were most probably caused by the difference in the solution: organic solvent (1,2,4-trichlorobenzene) was used for the former aliphatic case, whereas aqueous solution (0.1 M NaClO₄ aqueous solution) was used for the latter aromatic case. The aqueous solution facilitates deprotonation of the carboxyl groups of the 4MBA molecules when compared to the organic solvent. Consequently, these molecules easily undergo coordination bond interaction with Zn²⁺ ion; this interaction in turn suppresses the formation of the H-bonded molecular junction. Another difference between the aliphatic and aromatic systems was noticed in their conductance values. The 4MBA dimer is longer than the C₁COOH-C₂COOH dimer: the distance between the terminal sulfur atoms was 10.5 Å (10.7 Å) and 16.0 Å (16.8 Å) for the H-bonded (coordination bonded) C₁COOH-C₂COOH dimer and 4MBA dimer, respectively, based on DFT calculations using the B3LYP functional and the 6-31G(d) basis. Nevertheless, the 4MBA dimer exhibited larger conductance values both in the hydrogen- and coordination-bonded states. These observations agree with the results of previous measurements for single-molecule conductance. Aromatic molecules generally show higher conductance compared to their aliphatic counterparts because of the smaller gap between their highest occupied and lowest unoccupied molecular orbitals [17, 18].

Current measurements were also performed using a 4MP molecular tip over a 4MP-modified Au surface in a 0.1 M NaClO₄ solution. When no metal ion was added to the measurement solution, I–z curves exhibited no plateau and consequently simple exponential background current dominated the resulting current histogram (Fig. 3.6.a, blue). Because the 4MP molecules do not carry protons under
the present experimental condition, these molecules on the tip and substrate do not have chemical interactions such as hydrogen bonding. As a result, no molecular junction was formed, leading to the lack of plateaus and peaks in the $I$–$z$ curves and the current histogram, respectively. In stark contrast, the presence of Zn$^{2+}$ ions in the solution caused plateaus in the $I$–$z$ curves. On the basis of the peak that appeared in the current histogram (Fig. 3.6.b, pink), the current (conductance) value was determined to be 0.88 nA (4.4 nS or $5.7 \times 10^{-5} G_0$). By drawing analogy to the observations described above, we ascribe the peak in Fig. 3.6.b (pink) to a molecular junction in which 4MP molecules on the tip and substrate are connected to each other through the central Zn$^{2+}$ ion via coordination. In the present case, the metal ion induces the formation of the molecular junction and subsequent electron transfer; these processes are totally suppressed in the absence of the metal species. Therefore, the 4MP system constitutes a potential bistate molecular switch operated by a single metal ion.

![Fig. 3.6.](image)

3.4. Conclusions

We investigated the effect of a metal ion on the transport property between single molecules. We showed that a metal ion induces the formation of a molecular junction with carboxyl-terminated molecules via a metal coordination bond. This sandwich coordination molecular junction significantly facilitates electron transfer between single molecules. These results provide essential knowledge for
the construction of future bottom-up molecular devices. Moreover, the electron transfer induced by metal coordination was exploited in the construction of potential molecular switches activated by a single metal ion, using pyridine molecules. The next challenge in this context is reversible switching operation using various agents such as chelators.
4. Quantitative measurement of electron transfer through a charge-transfer interaction in a single noncovalent supramolecular assembly.

Electron transfer through a noncovalent interaction bears essential relevance to the functions of bottom-up supramolecular assembly. However, rather little knowledge regarding such phenomena at the single-molecule level is currently available. Herein we report the direct quantification of electron-transfer processes for a single noncovalently linked porphyrin–fullerene dyad. Facilitated electron transfer via a charge-transfer interaction in-between was successfully measured by utilizing a fullerene molecular tip. The rectification property of the supramolecular assembly was determined and quantitatively assessed. The present study opens up a way to explore quantitatively the rich electronic properties of supramolecules at the single-molecule level.

4.1. Introduction

Supramolecular donor-acceptor dyads constitute a versatile class of molecular assemblies with a variety of attractive electronic properties for molecular electronics, solar energy conversion, and catalytic and sensing applications [72, 73]. Porphyrin–fullerene dyads are among the most extensively studied systems because of their rich photo- and redox chemistry. Electronic interactions and electron transfer between the porphyrin and fullerene in solution have been well explored and documented through ensemble measurements [74-76]. Recently, there have been significant advances in the measurement of electron transport through single molecules [10]. Break-junction and similar methodologies based on scanning tunneling microscopy (STM) have been successfully applied to electron-transport measurements [17, 18]. These studies have deepened the understanding of electronic functions of molecules, including porphyrins [77-79] and fullerenes [80, 81], at the single-molecule level, which is vital for rational construction of molecular entities and nanostructures with sophisticated functionality. For example, the single-molecule conductance of a covalently bound porphyrin–fullerene dyad was measured under light illumination, allowing the observation of a long-lived charge-separated state [82]. However, knowledge concerning electron transfer between the single molecules within an assembly of single molecules interacting noncovalently, including
supramolecular donor-acceptor dyads, remains severely limited. To date, measurements of electron transfer between single molecules via hydrogen bonding [29, 30, 61] and aromatic π-π coupling [33] have been reported. Understanding charge transfer within a single supramolecular assembly is critical for building functional electronic devices, especially by a self-assembly or bottom-up process, where spontaneous formation of the supramolecular assembly plays a central role in device construction as well as conferring useful electronic functionalities [28].

Herein we demonstrate the successful quantification of electron transfer in a single noncovalent porphyrin–fullerene dyad using a fullerene–functionalized STM tip prepared by chemical modification of the Au tip with a fullerene derivative, N-methyl-2-(2-propyldithiophenyl)-fulleropyrrolidine. The sample dyad desirably demonstrates current rectification, which was quantitatively assessed herein at the single-molecule level. A novel method for electrical connection of a sample molecule to a gold substrate for molecular conductance measurements, namely, ligation-mediated coupling, is also introduced. Linking groups (e.g., thiols) that couple the sample with the electrodes are mandatory for conventional conductance measurements and are currently the subject of extensive research in the field of molecular electronics [15, 40]. In contrast to the conventional approach, the ligation-mediated coupling protocol facilitates the detection of electron transfer without the use of a linking group and thus without chemical modification of the sample porphyrin.

4.2. Experimental section

4.2.1. Reagents

The reagents were of the highest grade available. De-ionized water purified with a Milli-Q water purification system (Japan Millipore, Tokyo, Japan) was used for all of the experiments. The fullerene derivative, N-methyl 2-(2-propyldithiophenyl)fulleropyrrolidine, used for tip functionalization, was synthesized according to the procedure described in our previous report [8].
4.2.2. Tip preparation

Small pieces of gold wire (0.25 mm diameter, 99.95%) were electrochemically etched in 3 M NaCl under 10 V of AC. They were then washed by sonication in pure water, followed by dipping in “piranha solution” (7:3 concentrated H2SO4/H2O2. Caution: Piranha solution reacts violently with organic compounds, and it should not be stored in closed containers), and finally, thoroughly washed with pure water. Fullerene molecular tips (C60 tips) were prepared by dissolving the fullerene derivative in toluene at the typical concentration of 20 μM, and the gold tips were immersed in the solution overnight. The modified tips were successively rinsed with toluene and water prior to use.

4.2.3. Sample preparation

A 150-nm-thick gold film epitaxially grown on mica was used as a Au(111) substrate. The gold substrate was immersed in a 50 µM ethanolic solution of 4-aminothiophenol (4ATP) for 1 h for the chemisorption. After washing with pure ethanol, the modified substrate was immersed in a 1 mM dichloromethane solution of either 5,10,15,20-tetraphenylporphyrinatocobalt (CoTPP) or 5,10,15,20-tetraphenylporphyrinatozinc (ZnTPP) for 1 h. The substrate was mounted on a sample cell of the STM. The cell was filled with pure 1,2,4-trichlorobenzene to prevent unwanted contamination during the measurements.

4.2.4. Current measurements

The tunneling current measurements were performed on an SPM 5100 with a 1 nA/V pre-amplifier (Agilent Technologies, Santa Clara, CA). Before each measurement, the STM was equilibrated to room temperature for 30 minutes to reduce the thermal drift. The piezo scanner of the STM was allowed to settle under feedback control without being driven during the equilibration. This procedure suppresses unwanted hysteresis and creep of the piezo scanner. The C60 tip was then brought in close proximity to, but not in contact with, the modified Au(111) surface. This procedure was achieved by applying a large set-point current (7.5 nA) under the STM feedback control. A bias voltage of 0.5 V or 0.6 V was employed for main measurements, and a bias voltage range scanning from 0.3 to 0.8 V was used in extended measurements. After a short delay time of 100 ms, the C60 tip
was pulled up at a velocity of 20 nm/s with the feedback loop disabled, and \( I-z \) traces were recorded at a sampling frequency of 20 kHz using a data acquisition unit (NR-500; Keyence, Osaka, Japan). This measurement was repeated several thousand times.

### 4.2.5. Data analyses

Current histograms were constructed from the \( I-z \) traces that exhibited plateaus. Plateaus were observed for approximately 10\% of the measured \( I-z \) traces. Other traces were simple exponential or noisier decays. This ratio is comparable to that observed in our previous work in which the electron transfer through a hydrogen bond interaction between single molecules was measured [61]. For the construction of the current histograms, \( I-z \) curves that showed plateaus longer than 0.02 nm were selected, and at least 1500 selected curves were included. Each histogram was normalized with respect to the peak count of its high current signal, except for Fig. 4.4.a. The histogram in Fig. 4.4.a was normalized with respect to its single peak. The current histograms were fitted with exponential decay and Gaussian functions, which were used to fit the exponential background of the tunneling current and the peak arising from the plateaus, respectively. The fitting was performed without constrains, and the goodness of fit was evaluated by weighted sums of squared errors (WSSR). The WSSR values were in the range of 1.4-2.1.

### 4.3. Results and discussion

Initially, electron transfer between the fullerene and porphyrin was measured using the \( C_{60} \) tip and CoTPP directly adsorbed on a Au(111) surface (Fig. 4.1.a). The \( C_{60} \) tip was brought into close proximity to, but not into contact with, the CoTPP modified surface. This procedure was achieved by applying a large set-point current (7.5 nA) in the STM feedback control. The \( C_{60} \) tip was then pulled up with the feedback disabled, and current-distance (\( I-z \)) traces were recorded. These conditions prevented direct contact between the tip and substrate, unlike in break-junction techniques [17, 18], but it has been shown that molecular junctions can be formed at set-point currents of several nA [46, 83]. Nonetheless, in the measurements of the present research, the \( I-z \) traces exhibited no plateaus or constant-current regions, indicating that no molecular junctions were formed.
Fig. 4.1. (a–c) Schematic illustration of molecular junctions created using a C$_{60}$ tip. Sample surfaces: (a) MTPP directly adsorbed on Au; (b) 4ATP chemisorbed on Au; (c) MTPP axially ligated with 4ATP chemisorbed on Au. MTPP stands for a metallotetraphenylporphyrin, and M is either Co or Zn. (d–f) Representative $I$–$z$ traces for the molecular junctions presented in (a–c), respectively. Each plot has been horizontally shifted for clarity. Arrowheads indicate plateaus. Bias voltage = $-0.5$ V (sample negative); initial set-point current = 7.5 nA.

In addition, the $I$–$z$ curves were significantly noisy compared with those measured using the C$_{60}$ tip and an unmodified Au substrate (Fig. 4.2), which may be indicative of supramolecular fullerene–porphyrin association. The absence of plateaus may be ascribed to insufficient electronic coupling between CoTPP and the Au(111) surface. Terminal linking groups in the sample molecule are generally required for electronic coupling with the electrodes in single-molecule conductance measurements. However, CoTPP bears no linking group, and thus, the requisite electronic coupling with the Au substrate could not be established.

Fig. 4.2. Representative $I$–$z$ curves measured using C$_{60}$ tip. Sample surface: (a) CoTPP directly adsorbed on Au, and (b) unmodified Au substrate. Bias voltage = $-0.5$ V; initial set-point current = 7.5 nA.
Rather than introducing linking groups into the sample porphyrin, an alternative protocol for electrical connection of the molecule with the electrode was developed for the single-molecule conductance measurements: ligation-mediated coupling. The gold surface was initially modified with 4ATP via S-Au chemisorption. The 4ATP-modified substrate was washed and then immersed in a solution of CoTPP. This resulted in immobilization of CoTPP on the Au(111) surface by 4ATP through axial ligation [84-86], with consequent formation of the molecular assembly depicted in Fig. 4.1.c. Adsorption of CoTPP on the Au surface was confirmed via transmission UV/Vis spectroscopy (Fig. 4.3).

Fig. 4.3. UV/Vis spectra of CoTPP (cyan) and ZnTPP (magenta) axially ligated with 4ATP on Au.

In detail, Fig. 4.3 shows transmission UV/Vis spectra of ZnTPP and cobalt CoTPP axially ligated with 4ATP chemisorbed on a gold substrate. Ultrathin gold substrates having a nominal thickness of 25 Å were used in these spectroscopic measurements. Peaks corresponding to the Soret band were apparent at 430 nm, and were particularly pronounced for ZnTPP. The difference in the peak intensities for the ZnTPP and CoTPP species arises from the difference in the association constants for the ligation of the porphyrins by 4ATP [87]. The Soret band of the porphyrin was reported to occur at 411 nm in a CH$_3$Cl solution [84], indicating a shift of this peak in Fig. 4.3. This shift is attributed to the axial coordination by 4ATP and is consistent with the literature [84]. Instead of the relatively weak Q band, typically observed at ca. 530 nm in solution [88], the spectra are
characterized by an intense absorption in the range of 550-650 nm. This band is assigned to an enhancement of the gold surface plasmon absorption induced by chemisorbed 4ATP [84]. These peak positions are slightly different from the reported value [84], which is most probably due to the difference in the processes used to prepare the gold substrates. Further characterization of ZnTPP and CoTPP ligated with 4ATP on gold substrates was attempted with STM. In spite of the spectroscopic results described above, the porphyrin molecules could not be imaged by STM. This result agrees with the literature [86] and attributed to the conformational freedom of the porphyrins on 4ATP [86] and surface mobility of the porphyrin/4ATP molecular assemblies.

Returning to Fig. 4.1, we first investigated the electron transfer between the C_{60} tip and 4ATP without CoTPP ligation. The current measurements depicted in Fig. 4.1.b gave rise to I–z curves characterized by plateaus (Fig. 4.1.e). These plateaus indicate the formation of a molecular junction consisting of fullerene on the tip and 4ATP on the substrate via noncovalent interaction and successive electron transfer. For statistical analysis, a current histogram was constructed from the I values for the data points in the I–z curves (Fig. 4.4.a). In this procedure, I–z curves that showed plateaus longer than 0.02 nm were selected, and at least 1500 selected curves were included in constructing the histogram.

![Image](image.jpg)

Fig. 4.4. Current histograms constructed from the I–z traces measured using a C_{60} tip and (a) 4ATP chemisorbed on Au or (b) CoTPP axially ligated with 4ATP chemisorbed on Au. Bin size = 10 pA. Total fitting curves (dashed lines) and Gaussian fitting components (solid lines) are also shown. Bias voltage = −0.5 V; initial set-point current = 7.5 nA.
A single pronounced peak on an exponentially decaying background was evident, and the position of the peak corresponded to the statistical current value at which the plateaus were observed in the $I$–$z$ curves (Fig. 4.1.e). On the basis of the peak current of 0.99 nA determined by fitting the histogram, the conductance of the fullerene–4ATP molecular junction was 2.0 nS (or $2.6 \times 10^{-5}G_0$, where $G_0$ is the fundamental conductance quantum). The formation of the molecular junction is ascribed to a charge-transfer interaction between the two constituent molecules. Small association constants [89] and very weak intermolecular charge-transfer interactions [90] were previously reported for fullerene–aniline noncovalent assemblies. However, the close proximity between the $C_{60}$ moiety and 4ATP achieved herein under STM mechanical control facilitated the weak charge-transfer interaction. Although an amine group is a suitable linking group for single-molecule conductance measurements [15, 40], control experiments excluded the possibility of direct contact between 4ATP and the Au tip under the fullerene adlayer (Fig. 4.5).

Fig. 4.5. Current histogram constructed from the $I$–$z$ traces measured using unmodified Au tip and 4ATP chemisorbed on Au. Bias voltage: $-0.5$ V; initial set-point current: 7.5 nA. Bin size: 10 pA.

Fig. 4.5 is the current histogram of control experiments that were performed by employing unmodified Au tips in lieu of the $C_{60}$ tips for the current measurement with the 4ATP-modified Au(111) surfaces. Plateaus such as seen in Fig. 4.1.e were totally absent in the resulting $I$–$z$ traces. As a result, the current histogram constructed from these $I$–$z$ traces exhibited simple exponential decay (Fig. 4.5). This result is ascribed to the relatively small initial set-point current (7.5 nA), which is indicative of a large tip-sample distance to be bridged by 4ATP. For example, 1,4-benzenediamine
and 1,4-benzenedithiol molecules are expected both to conduct a current larger than 100 nA under the bias voltage of 0.2 V on the basis of the reported single-molecule conductance values [15]. These results exclude the possibility that 4ATP by itself forms the molecular junction without involving fullerene on the tip. We thus conclude that electron transfer takes place between the C₆₀ tip and 4ATP during the I–z measurements.

We next turned to quantifying the electron transfer for the single-molecular assembly comprising the fullerene and porphyrin. I–z measurements were performed using the C₆₀ tip and a sample surface on which CoTPP was adsorbed via axial ligation to chemisorbed 4ATP (Fig. 4.1.c). Steplike plateaus were apparent in the I–z curves (Fig. 4.1.f), and the current histogram compiled from the I–z curves clearly exhibited two peaks (Fig. 4.4.b). Thus, two Gaussian functions along with an exponential decay having a constant offset were used to fit the histogram. These functions fit the data very well, and the current (conductance) values for the peaks at higher and lower current in Fig. 4.4.b (labeled “Hₗ” and “Lₗ”, respectively) were 2.4 nA (4.8 nS, 6.2 × 10⁻⁵ G₀) and 0.91 nA (1.8 nS, 2.4 × 10⁻⁵ G₀), respectively. The Lₗ peak agrees with the peak in Fig. 4.4.a and is therefore attributed to electron transfer through a molecular junction containing the fullerene and 4ATP devoid of CoTPP ligation. In the I–z curves, the plateau at the Lₗ peak current always appeared without the plateau at the Hₗ peak current. This observation supports the assignment of the Lₗ peak (Fig. 4.6).

![Fig. 4.6. I–z curves measured using C₆₀ tip and CoTPP axially ligated with 4ATP chemisorbed on Au. Bias voltage: −0.5 V; initial set-point current: 7.5 nA. The Hₗ peak current (2.4 nA) is indicated by the dashed line.](image)
In contrast, the $H_{Co}$ peak appeared only when CoTPP was adsorbed on 4ATP by axial ligation and should thus be associated with CoTPP. No plateau was observed in the $I$–$z$ traces of control experiments carried out for the same sample composition (CoTPP on chemisorbed 4ATP) using unmodified Au tips. Taken together, these results indicate that electron transfer between the C$_{60}$ tip and CoTPP gave rise to the $H_{Co}$ peak. Thus, the conductance of the molecular junction consisting of the fullerene and porphyrin was successfully determined by the use of the C$_{60}$ tip and ligation-mediated coupling of the porphyrin. It has been suggested that the molecular plane of cobalt porphyrin ligated by 4ATP on Au(111) is tilted with respect to the substrate surface [86]. In the present measurements, the fullerene and the tilted porphyrin were mechanically forced into a face-to-face arrangement, which is favorable for the charge-transfer interaction, as has been demonstrated for elegantly designed fullerene–porphyrin dyads [91-93]. Since the porphyrin possesses conformational freedom induced by ligation via 4ATP [86], the CoTPP plane gradually becomes untilted as the molecular junction stretches out during the pulling-up movement of the tip in the $I$–$z$ measurements, resulting in the junction structure shown in Fig. 4.1.c. The conductance of the molecular junction was larger with CoTPP ligation than without such ligation (Fig. 4.4; also see Fig. 4.9), despite the fact that the junction length was increased by ligation. This is notable because single-molecule conductance generally decreases exponentially with increasing molecular length [10, 17, 18]. The favorable charge-transfer interaction between the fullerene and porphyrin compared with the weak interaction between the fullerene and 4ATP is the most probable cause of this anomalous behavior.

![Representative $I$–$z$ curves measured using C$_{60}$ tip and ZnTPP axially ligated with 4ATP chemisorbed on Au. Bias voltage: (a) $-0.6$ V (sample negative) and (b) $+0.6$ V (sample positive); initial set-point current: 7.5 nA.](image)
The detection of electron transfer for a single porphyrin–fullerene dyad was further substantiated by using ZnTPP axially ligated to 4ATP adsorbed on the Au substrate instead of CoTPP, which also gave rise to plateaus in the \( I-z \) curves (Fig. 4.7); the current histogram constructed from these data is presented in Fig. 4.8.

![Figure 4.8](image_url)

**Fig. 4.8.** Current histogram constructed from the \( I-z \) traces measured using a \( \text{C}_{60} \) tip and ZnTPP axially ligated with 4ATP chemisorbed on Au. Bias voltages: (a) \(-0.6\) V (sample negative); (b) \(+0.6\) V (sample positive). Initial set-point current = 7.5 nA; bin size = 10 pA.

Under a sample-negative bias voltage as used in Fig. 4.4, two clear peaks were observed (Fig. 4.8.a). By the fitting procedure used for analysis of the histogram in Fig. 4.4.b, the current (conductance) values for the peaks at higher and lower current (labeled “\( H_{\text{Zn,}^-} \)” and “\( L_{\text{Zn,}^-} \)”, respectively) were found to be 2.1 nA (3.5 nS, \( 4.5 \times 10^{-5} G_0 \)) and 1.0 nA (1.7 nS, \( 2.2 \times 10^{-5} G_0 \)), respectively. The \( L_{\text{Zn,}^-} \) conductance value agrees well with that from Fig. 4.4.a and is ascribed to the molecular junction consisting of fullerene and 4ATP without ZnTPP. The \( H_{\text{Zn,}^-} \) peak is assigned to charge transfer between the fullerene and ZnTPP adsorbed on 4ATP, analogous to the CoTPP congener. Comparison of Fig. 4.4.b and Fig. 4.8.a reveals that the conductance of the molecular junction of the fullerene-CoTPP ligated to 4ATP is higher than that of the ZnTPP counterpart (Fig. 4.9). The higher conductance suggests that the \( \text{C}_{60} \) moiety of the molecular tip experiences a stronger electronic interaction with CoTPP than with ZnTPP. The favorable interaction in the Co porphyrin–fullerene conjugate compared to the Zn porphyrin–fullerene congener has indeed been confirmed in prior electrochemical studies [94]. In the case of the Co porphyrin, significant interactions between the \( d \) orbital of the central Co atom and the \( p_z \) orbital of the fullerene carbon atom, in addition to the charge-
transfer interactions commonly observed between the metalloporphyrin and the fullerene, enhance the total porphyrin–fullerene interaction [95, 96].

![Bar graph of current and conductance values at the peak position in the histograms.](image)

*Fig. 4.9. Bar graph of current and conductance values at the peak position in the histograms. Each bar is labeled with the molecular species present on the Au substrate. Slashes represent the axial ligation. The $L_{Co}$, $L_{Zn}^-$, and $L_{Zn}^+$ peaks are the same as the peak observed for 4ATP and thus have been omitted.*

We previously demonstrated the rectification property of a porphyrin–fullerene pair [8]; this characteristic renders the molecular assembly particularly attractive in molecular electronics. Here the rectification was assessed quantitatively. The experimental system was the same as used for the measurements in Fig. 4.8.a, but an opposite (sample-positive) bias voltage was applied. Plateaus were observed in these measurements (Fig. 4.7), and two peaks were evident in the current histogram constructed from these $I$–$z$ curves (Fig. 4.8.b). Fitting analogous to that in Fig. 4.4.b and 4.8.a gave current (conductance) values of 1.4 nA (2.3 nS, $3.0 \times 10^{-5} G_0$) and 0.93 nA (1.6 nS, $1.6 \times 10^{-5} G_0$) for the peaks at higher and lower current ("$H_{Zn}^+$" and "$L_{Zn}^+$", respectively). The $H_{Zn}^+$ and $L_{Zn}^+$ peaks were again assigned to the molecular junctions containing fullerene and 4ATP with and without ZnTPP ligation, respectively. The $L_{Zn}^+$ peak position is rather consistent with that in Fig. 4.4.a. The conductance value corresponding to the current at the $H_{Zn}^+$ peak is considerably lower than that at the $H_{Zn}^-$ peak (Fig. 4.9). Both peaks arise from electron transfer between the fullerene and ZnTPP on 4ATP. Under the sample-positive bias voltage, electrons are transferred from the electron-deficient fullerene to the electron-rich porphyrin. This direction is unfavorable for the molecular diode by the acceptor–donor configuration [8], leading to the decreased conductance.
Fig. 4.10. Current histograms constructed from $I-z$ curves measured using C$_{60}$ tip and ZnTPP axially ligated with 4ATP chemisorbed on Au. Left: sample negative; right: sample positive bias. The bias voltage was indicated in each histogram. Initial set-point current: 7.5 nA. Bin size: 10 pA. The histograms at the bias voltages of $-0.6$ and $+0.6$ V are presented in Fig. 4.8 but reproduced here for comparison.

The asymmetric current flow in Fig. 4.8 was quantitatively estimated by an asymmetry ratio ($R$), defined as $R = |I(-V)|/|I(V)|$ at a given voltage V. The $I-z$ measurements were conducted at bias voltages ranging from $-0.3$ to $-0.8$ and $+0.3$ to $+0.8$ V to investigate the $R$ values. The peak currents in the resulting current histograms (Fig. 4.10) depended linearly on the bias voltage over the investigated range (Fig. 4.11.a, b), indicating that the tunneling is in the off-resonant regime. Nevertheless, $R = 1.5-1.6$ over the voltage range (Fig. 4.11.c), demonstrating the rectifying behavior. Previously, $ab$ $initio$ calculations were performed for fullerene and organic donor complexes. The calculated $I-V$ curves exhibited rectification due to polarity-dependent modification of the electron
densities of these molecular assemblies. It was also found that the rectification occurred over a broad voltage range, even at small bias voltages [97]. Those results are consistent with the rectifying properties observed here (Fig. 4.11.c). In contrast, rectification was not observed \((R = 1.0)\) when the sample surface was modified with non-ZnTPP-ligated 4ATP (Fig. 4.12). Previously, we observed such rectification by scanning tunneling spectroscopy (STS) [8]. The present \(R\) value agrees with \(R\) value of 1.6 over the same bias voltage range obtained by STS. It should be noted that in STS there existed tunneling gap of an unknown width between the porphyrin and fullerene. In contrast, this ambiguity has been eliminated in the present measurements because the measured conductance values arise from the porphyrin–fullerene assembly at the most favorable configuration [61].

![Image](image_url)  

Fig. 4.11. (a) Current and (b) conductance values at the peak positions in the current histograms in Fig. 4.10. Circles (triangles): higher (lower) current peak; Black (red): sample negative (positive) bias.  
(c) \(R\) factor calculated by the higher current peaks.
Fig. 4.12 shows current histograms obtained with C$_{60}$ tip and 4ATP-modified substrate under negative and positive bias voltages. The peak was located at 0.99 and 0.95 nA under negative and positive bias voltages, respectively. Based on these current values, $R$ factor was found to be 1.0, demonstrating that the C$_{60}$–4ATP junction exhibit no rectifying behavior.

![Current histograms](image)

Fig. 4.12. Current histograms constructed from the $I$–$z$ traces measured using C$_{60}$ tip and 4ATP chemisorbed on Au. Bias voltage: (left) $-0.5$ V and (right) $+0.5$ V; initial set-point current 7.5 nA. Bin size: 10 pA. The left histogram is presented in Fig. 4.4.a but reproduced here for comparison.

4.4. Conclusions

In summary, a molecular tip was used to quantify electron transfer in a single fullerene–porphyrin assembly linked by noncovalent interactions. The novel ligation-mediated coupling technique was developed to connect a porphyrin to a metal substrate without introducing a linking group. The combination of the molecular tip and ligation-mediated coupling enabled quantitative characterization of the conductance and rectification properties of the single fullerene–porphyrin assembly. The present study opens up a way to explore quantitatively the rich electronic properties of supramolecules at the single-molecule level. The present technique can be extended to the detection of photoinduced electron transfer between a porphyrin and fullerene, thereby furnishing useful information at the single-molecule level for the development of organic photovoltaics. Moreover, ligation with a ligand molecule affects the electronic properties of a porphyrin, similar to introducing linking groups into the sample molecule, and therefore, further systematic and theoretical investigations of this effect are required.
5. Electron transfer through intramolecular interactions of biomolecules: application in single-molecule DNA detection based on hybridization.

Single-stranded DNA (ssDNA) was utilized as a probe tip for single-molecule DNA detection. Hybridization of the DNA tip and target DNA induces electron tunneling through the resulting DNA duplex. It is demonstrated that the DNA tip allows not only genetic detection but also discovery of single-nucleotide polymorphisms at the single-molecule level.

5.1. Introduction

Sequence-specific DNA detection has been a topic of increasing interest because of its wide applications in disease identification and pathogen detection. Recent technological advances, e.g., development of a DNA microarray [98], offer opportunity for the genetic analysis. However, demands for further improvement of the sensitivity and selectivity of the DNA detection still exist. The ultimate goal of a sensing application from a technological and scientific perspective is single-molecule detection. On the one hand, the single-molecule detection enables considerable improvements in the throughput of the DNA analysis toward realization of clinical and point-of-care DNA diagnostics. On the other hand, single-molecule studies have revealed valuable knowledge regarding conformational changes and intramolecular interactions of biomolecules that cannot be determined using ensemble-based measurements [99]. Recently, there has been significant progress in single-molecule measurements. For example, much effort has been devoted to understanding electron transport through a single molecule. Break junction and similar methodologies based on STM have been successfully applied for the electron transport measurements [17, 18, 27]. The single-molecule conductance of the DNA duplex has been successfully determined by using the STM-based techniques [100-103] as well as metal nanoelectrodes [104] and nanotube electrodes [105, 106]. It is well known that a single molecule exhibits a conductance value intrinsic to its chemical structure and local environment. Measuring such conductance, therefore, allows us to identify the chemical identity of a single molecule electronically. In this study, we utilized the transport property of a single DNA molecule as a fingerprint to achieve single-molecule detection of DNA.
5.2. Experimental section

5.2.1. Reagents

The reagents were at the highest grade available on the market. De-ionized water purified with a Milli-Q water purification system (Japan Millipore, Tokyo, Japan) was used for all the experiments. All nucleotides, modified with -(CH$_2$)$_3$SH linkers at their 3’ termini and purified by HPLC, were purchased from Tsukuba Oligo Service (Ibaraki, Japan). Upon receipt of the oligonucleotides, the DNAs were dissolved in a 10 mM phosphate-buffered saline (PBS) solution to prepare 100 μM DNA solutions. The solutions were divided into aliquots and stored at –20°C. Before the experiments, each aliquot was allowed to warm to room temperature and diluted to 1 μM with 10 mM PBS. For the conductance measurements of the DNA duplex (dsDNA) using unmodified Au tips, the dsDNA was prepared by combining 1 μM T$_8$ and S$_8$ ssDNA solutions. The combined solution was then heated to 70°C for 30 minutes and allowed to cool to room temperature for several hours [101].

5.2.2. Tip preparation

Small pieces of gold wire (0.25 mm diameter, 99.95%) were electrochemically etched in 3 M NaCl at AC 10 V. Then, they were washed by sonication in distilled water, dipped in “piranha solution” (content H$_2$SO$_4$/H$_2$O$_2$ is 7/3. Caution: piranha solution reacts violently with organic compounds and should not be stored in closed container), and finally, washed thoroughly with pure water. The metal tips were insulated by covering PDMS, except for the tip apexes, in order to reduce the ionic and polarization currents [65]. To prepare molecular tips, the insulated metal tips were immersed overnight in 1 μM DNA solution at room temperature. The modified tips were washed with 10 mM PBS solution prior to use.

5.2.3. Sample preparation

Ultraflat gold films epitaxially grown on mica were used as Au(111) substrates [38]. The gold substrate was immersed in the 1 μM DNA solution for 1 h. After washing with 10 mM PBS solution,
the substrate was placed on an STM sample plate and heaped up with a sample cell. The cell was filled with 0.1 M NaClO₄ aqueous solution for the current measurements.

### 5.2.4. Current measurements

The tunneling current measurements were performed on an SPM 5100 with a 1 or 10 nA/V pre-amplifier (Agilent Technologies, Santa Clara, CA). Platinum wires were used as reference and counter electrodes. The DNA tip was first brought in close proximity to, but not in contact with, the DNA-modified Au(111) surface. This procedure was achieved by applying a high set-point current (75 nA for the 8-mer DNA tip, and 7.5 nA for the 10-mer and 12-mer DNA tips) under the STM feedback control. A bias voltage of 0.2 V was employed for all the measurements. After a short delay time of 100 ms, the DNA tip was pulled up at a velocity of 20 nm/s with the feedback loop disabled, and I–z traces were recorded at a 20 kHz sampling frequency using a data acquisition unit (SL1000; Yokogawa Electric Corporation, Tokyo, Japan, or NR-500; Keyence, Osaka, Japan). This measurement was repeated approximately 3400 times for each tip. Since the direct touch of the DNA tip with the gold substrate was carefully avoided during the whole measurement procedure, we observed no phenomenon that could be associated with loss of the probe DNA from the underlying Au tips (such as disappearance of the plateaus in the I–z curves).

In the I–t mode, after approaching the tip into the proximity of the sample surfaces, the feedback loop was disabled for 1 second for recording the current flowing between the tip and the sample surface. In the I–t measurements, the initial set-point current should be careful controlled for optimizing the formation of single-molecule interactions between tip and sample molecules. When using ssDNA tip to probe the sample covered by complementary ssDNA and methylated DNA, the set-point current was optimized at 20 nA and 10 nA respectively.

### 5.2.5. Data analyses

The current histograms were constructed from the I–z traces that exhibited the plateaus, as shown in Fig. 5.1.b. Plateaus were observed in approximately 10% of the measured I–z traces. Other traces were either simple exponential or noisier decays. The dsDNA length was estimated by assuming 3.4
Å / base-pair [107]. The length of -(CH₂)₃SH was evaluated on the basis of the optimized geometry of propanethiol calculated at the DFT level with the B3LYP functional.

5.2.6. UV melting studies

The UV melting profile for the S₈-T₈ duplex in a 10 mM PBS solution was obtained from absorbance at 250 nm. The measurements were performed with a temperature gradient of 1.0°C/min. The first derivative of the profile was used to estimate the melting temperature.

5.3. Results and discussion

5.3.1. Single-molecule detection of fully matched and mismatched DNA.

We have studied STM measurements utilizing functionalized molecular tips [7, 8, 108]. The molecular tips are constructed by chemical modification of conventional metal tips, and the foremost adsorbate molecule at the tip apex detects tunneling electrons to or from the sample surface. We
recently demonstrated that electron transfer from one single molecule to an adjacent single molecule can be quantified by using the molecular tip [16]. In the present study, gold STM tips were functionalized with ssDNA molecules by S-Au chemisorptions of mercaptopropyl linkers introduced at the 3’-terminus. Gold substrates were similarly modified with sample ssDNA. The single ssDNA molecule at the tip apex was utilized as a DNA tip to probe electron tunneling with the ssDNA-modified substrate (Fig. 5.1.a). The DNA tip was brought in close proximity to, but not contact with the sample surface. This procedure was achieved by applying a large set-point current (75 nA) under the STM feedback control. After a short dwell time of 100 ms, the DNA tip was then pulled up and current–distance (I–z) traces were recorded. All the measurements were taken in aqueous solutions.

Table 5.1. Sequences of oligonucleotide used as sample and tip molecules.

<table>
<thead>
<tr>
<th>Oligo name</th>
<th>Sequence[a]</th>
<th>Length (nm)[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>T8</td>
<td>5’-CAA CAA GC-3’</td>
<td>3.14</td>
</tr>
<tr>
<td>T8-T</td>
<td>5’-CAA TAA GC-3’</td>
<td>3.14</td>
</tr>
<tr>
<td>T8-A</td>
<td>5’-CAA AAA GC-3’</td>
<td>3.14</td>
</tr>
<tr>
<td>T8-G</td>
<td>5’-CAA GAA GC-3’</td>
<td>3.14</td>
</tr>
<tr>
<td>T8-nc1</td>
<td>5’-AGT TCT AT-3’</td>
<td>3.14</td>
</tr>
<tr>
<td>T8-nc2</td>
<td>5’-TGC ACT TA-3’</td>
<td>3.14</td>
</tr>
<tr>
<td>S8</td>
<td>5’-GCT TGT TG-3’</td>
<td>3.14</td>
</tr>
<tr>
<td>S8-T</td>
<td>5’-GCT TTT TG-3’</td>
<td>3.14</td>
</tr>
<tr>
<td>T10</td>
<td>5’-ACC AAC AAG C-3’</td>
<td>3.82</td>
</tr>
<tr>
<td>S10</td>
<td>5’-GCT TGT TGG T-3’</td>
<td>3.82</td>
</tr>
<tr>
<td>T12</td>
<td>5’-TGA CCA ACA AGC-3’</td>
<td>4.50</td>
</tr>
<tr>
<td>S12</td>
<td>5’-GCT TGT TGG TCA-3’</td>
<td>4.50</td>
</tr>
</tbody>
</table>

[a] -(CH2)3SH linkers were introduced at the 3’ terminus. [b] The linker length was included.
Fig. 5.1.b shows such traces measured using an 8-mer DNA tip, referred to as T₈ (for DNA sequences in Table 5.1), over a gold substrate modified with an 8-mer sample DNA, referred to as S₈ (Table 5.1), which was complementary to the tip molecule. When the tip and substrate surfaces are separated from each other without forming metallic wires or molecular junctions, the tunneling current should exponentially decrease as the distance increases. However, the $I$–$z$ curves (Fig. 5.1.b) exhibited plateaus for which the current remained constant despite increased separation; these plateaus indicate bridge formation between the tip and the substrate (Fig. 5.1.a). For statistical analysis, a current histogram was constructed from current values of data points in the $I$–$z$ traces (Fig. 5.1.c, top). The current histogram exhibited a single peak at 9.5 nA, which corresponds to a statistical current value where plateaus appeared in the $I$–$z$ traces. No other peak was observed in the histogram. To examine the origin of the plateaus, we performed similar measurements using unmodified Au tips and fully matched dsDNA. The dsDNA was prepared with T₈ and S₈ beforehand and adsorbed on the Au substrate for current measurements using Au tips. The plateaus were observed on $I$–$z$ traces obtained by these measurements (Fig. 5.2).

![Graph showing I–z measurements of dsDNA](image)

Fig. 5.2. $I$–$z$ measurements of dsDNA. Measurements were performed using the unmodified Au tips and the Au substrate modified with the dsDNA composed of T₈ and complementary S₈. The plots are horizontally offset for clarity. Bias voltage: 0.2 V, initial set-point current: 75 nA.

Because the dsDNA formed from S₈ and T₈ possessed thiol groups at both termini, this duplex could bridge the unmodified Au tip and substrate. We therefore attributed the current plateaus in Fig. 5.2 to electron transport through the single dsDNA [100,101] composed of S₈ and T₈. The current histogram constructed from the $I$–$z$ traces as shown in Fig. 5.2 exhibited a single pronounced peak that appeared at 9.1 nA (Fig. 5.1.c, bottom). The conductance of the sample dsDNA was successfully determined to
be 46 nS (or $5.9 \times 10^{-4} G_0$, where $G_0$ is the fundamental conductance quantum) based on this peak current. Importantly, the peak positions in the two histograms of Fig. 5.1.c agree well with each other. These results demonstrate that the plateaus in the $I$–$z$ traces measured by using the DNA tip (Fig. 5.1.c) result from the electron transport through the dsDNA. Hybridization occurred between the DNA tip and the ssDNA on the Au substrate during the $I$–$z$ measurements so that the resulting dsDNA bridges the gap between the Au electrodes (Fig. 5.1.a). In addition, the conductance showed no bias dependence up to at least 0.25 V (Fig. 5.3), as would be expected for electron transport through a molecular junction.

![Fig. 5.3. Bias dependence of the peak current in the histogram (blue) and conductance (red). A T₈ DNA tip and S₈-modified sample surface were used for the measurement.](image)

Generally, multiple peaks can be observed in the histogram constructed from $I$–$z$ measurements using break junction or related techniques. These peaks are interpreted as resulting from the formation of not single, but multiple molecular junctions [13, 20] or different contact geometries between anchoring groups of the molecule and the gold surface [46, 47]. In contrast, only one single peak appeared on the histogram of Fig. 5.1.c which demonstrates that electrons were transferred through the single DNA molecular junction. Control experiments were performed by using an 8-mer DNA tip (T₈-nc1 and T₈-nc2, Table 5.1) that was non-complementary to the S₈ target DNA on the Au substrate. The resulting $I$–$z$ traces exhibited no plateaus, indicating that no DNA molecular junctions were formed. We thus conclude that a DNA tip enables single-molecule detection of ssDNA based on electron transport induced by the in situ hybridization during the current measurements. The melting
temperature ($T_m$) of the $S_8$–$T_8$ duplex was found to be around room temperature (22.3°C) under the present experimental conditions (Fig. 5.4).

![UV melting profile](image)

Fig. 5.4. UV melting profile (at 260 nm) of $S_8$–$T_8$ duplex. Concentration of the duplex: 1 µM.

This situation is suitable for this study because single-molecule conductance was utilized for DNA detection. The $I$–$z$ measurements near the $T_m$ suppressed excessive hybridization of the target and tip DNA. Consequently, formation of multiple DNA junctions could be avoided, which facilitates the measurements of DNA single-molecule conductance as shown in the current histograms (e.g., Fig. 5.1.c). The DNA tip spent rather short time (dwell time $= 100$ ms) near the substrate on which the target ssDNA was present. However, this time duration is sufficient for the hybridization, given that the time required for the helix formation of dsDNA is approximately $10^{-6}$ to $10^{-5}$s per base pair [109, 110].

![Current histograms and conductance dependence](image)

Fig. 5.5. (a) Current histograms of the $I$–$z$ curves measured using the $T_{10}$ DNA tip and the complementary $S_{10}$ target DNA (red), and using the $T_{12}$ DNA tip and the complementary $S_{12}$ target DNA (green). 1722 and 2540 $I$–$z$ curves were used to construct the histograms for the $T_{10}$–$S_{10}$ and $T_{12}$–$S_{12}$ cases, respectively. Bias voltage: 0.2 V, initial set-point current: 7.5 nA. The Gaussian functions fitted to the histograms for the current peaks are shown. (b) Dependence of the natural
logarithm of conductance for the molecular junctions on the length of DNA (orange). The conductance values for the alkanedithiol molecular junction are also shown (blue).

In addition to the 8-mer DNA tip described above, 10- and 12-mer DNA tips (T\textsubscript{10} and T\textsubscript{12}, respectively; Table 5.1) were used to measure electron transport for complementary ssDNA of the same length as those of the DNA tips (S\textsubscript{10} and S\textsubscript{12}, respectively; Table 5.1). The $I$–$z$ curves obtained by these measurements exhibited plateaus, and their current histograms revealed single well-defined peaks at 1.5 and 0.48 nA for measurements performed using T\textsubscript{10} and T\textsubscript{12} DNA tips, respectively (Fig. 5.5.a). Conductance of each molecular junction formed by the T\textsubscript{10} and T\textsubscript{12} DNA tips was determined to be 7.6 nS (9.8 × 10\textsuperscript{-5} $G_0$) and 2.4 nS (3.1 × 10\textsuperscript{-5} $G_0$), respectively, based on peak current, and the conductance values were plotted as a function of the length of DNA tip (Fig. 5.5.b). The conductance showed an exponential dependence on the DNA length, suggesting that a tunneling or super exchange process is responsible for the charge transfer through stacked base pairs in the DNA duplex [111]. In dsDNA containing abundant G bases, charge hopping through these bases, which have a relatively low-lying highest occupied molecular orbital, is the dominant mechanism for the charge transport [112]. In contrast, the tunneling process is the most probable mechanism in the present experiments since the DNA sequences are randomly designed. Conductance values for the alkanedithiol molecular junction are also shown in Fig. 5.5.b [46]. Comparing the results shown in Fig. 5.5.b, we found that the conductance was larger for the DNA than for the alkanedithiol molecular junction. The tunneling decay constant, $\beta$ value, of the DNA was found to be 2.6 nm\textsuperscript{-1}. This value is smaller than that of the alkanedithiol (4.2 nm\textsuperscript{-1}) [17], being consistent with those reported for the electron transport through a single dsDNA molecule [100, 101]. The conductance values determined in the present study are not exactly the same as those reported previously for DNA of the same length [100, 101]. This is attributed to the differences in the DNA sequence, which significantly affects conductance [100, 101].
We next investigated the detection of a single-base mismatch in order to realize the discovery of the single-nucleotide polymorphisms (SNPs) at the single-molecule level. Electron transport induced by \textit{in situ} hybridization was measured using four kinds of 8-mer DNA tips over the Au substrate on which the common target ssDNA, S\textsubscript{8}, was adsorbed. One DNA tip, T\textsubscript{8}, was fully complementary to the target and exhibited a conductance of 48 nS upon the \textit{in situ} hybridization with S\textsubscript{8} (see above). The remaining 3 sequences (T\textsubscript{8}-T, T\textsubscript{8}-A, and T\textsubscript{8}-G) contained mismatched bases (T, A, and G, respectively) at the fourth position from their 5\textquotesingle -ends. \textit{I–z} curves measured using the mismatch-containing DNA tips with the target S\textsubscript{8} revealed plateaus. These observations suggest that formation of DNA duplexes between DNA tips and target ssDNA occurred even in the presence of the mismatched base. The T\textsubscript{8}-T tip exhibited the largest conductance (9.0 nS) among the mutated DNA tips (Fig. 5.6.a), yet this value was smaller by a factor of 5.4 compared with that of the fully matched T\textsubscript{8} tip (Fig. 5.6.b). Even larger decreases in conductance were observed for the remaining mismatched tips (3.2 and 2.6 nS for T\textsubscript{8}-A and T\textsubscript{8}-G tips, respectively; Fig. 5.6.b, also see Fig. 5.7).
Fig. 5.7. Current histograms obtained using mismatch-containing DNA tips. (a) Measured using the T₈-A DNA tip and S₈ target DNA. 1693 I–z curves were used to construct the histogram. (b) Measured using the T₈-G DNA tip and S₈ target DNA. 2203 I–z curves were used to construct the histogram. The I–z measurements were performed with a bias voltage of 0.2 V and initial set-point current of 7.5 nA.

By a control experiment using another complementary DNA, it was confirmed that the significant decrease in conductance was caused by not mere base substitutions but base mismatches (Fig. 5.8). The observed conductance decrease by the mismatched base agrees with previous reports on the electron transfer through dsDNA [101]. The presence of a mismatched base in the DNA tip or target ssDNA on the substrate causes instability in the DNA duplex structure and perturbation in the base pairing. The decreased conductance in the case of the mismatched DNA tips can be attributed to these effects [113]. The large differences in conductance values allow for a clear discrimination between the fully matched and mutated DNA using the DNA tips. For the T₈-G tip, the resulting duplex formed with the target ssDNA on the substrate contains a G:T ‘‘wobble’’ mismatch, which is thermodynamically stable [114]. Consequently, the DNA duplex was less perturbed and exhibited relatively higher conductance. Detecting this particular mismatch was not trivial, especially, by fluorescent-based assays [115]. In contrast, electron transfer induced by in situ hybridization utilizing DNA tips is sufficiently sensitive to discriminate between fully matched and mismatched dsDNA at a single-molecule level, including G:T base pairing.
Fig. 5.8. Current histogram for another complementary DNA duplex. T₈-A and S₈-T were used as the tip and target DNA, respectively. 2364 I–z curves were used to construct the histogram. The I–z measurements were performed with a bias voltage of 0.2 V and initial set-point current of 75 nA.

At the position where the mismatched bases were introduced in the case of T₈-T, T₈-A, and T₈-G, the T₈-S₈ DNA duplex contains a complementary C:G base pair. I–z measurements were performed for the duplex in which the C:G pair was replaced with another complementary A:T pair in order to investigate the effect of base substitution. T₈-A and S₈-T were used as the tip and target DNA, respectively. The current histogram constructed from the I–z measurements is characterized by a prominent peak (Fig. 5.7). On the basis of the peak current of 0.94 nA, conductance was determined to be 4.7 nS (or $6.1 \times 10^{-5} G_0$). This value is nearly identical to conductance determined for the T₈-S₈ duplex. These results demonstrate that base substitution without mismatch causes little effect on conductance values. It was reported that replacing C:G with a A:T base pair decreases the conductance of DNA [101]. In this study, no notable decrease by the base substitution was found. We attributed this to the shorter DNA length in the present work, which could make the effect of the A:T base less pronounced.

5.3.2. Single-molecule detection of methylated DNA

In the previous section, I–z measurements using the DNA tips were utilized to achieve single-molecule DNA detection and discovery of SNPs. In contrast, in the present section, we applied the current measurements with the DNA tips against time (I–t measurements) to the single-molecule DNA detection. Specifically, the ssDNA tip was bought to the proximity Au(111) substrate covered
with sample DNA, and then current between the DNA tip and sample surface was monitored with the position of the tip kept constant (Fig. 5.9.a). Such current measurements in the time domain involve no tip movement unlike the $I-z$ measurements in the previous section. As a result, this detection scheme excludes the need for actuating components, which is significantly desirable from the viewpoint of practical application of the present methodology to clinical diagnosis.

![Fig. 5.9.](image)

Fig. 5.9. (a) Schematic illustration of the current-time measurement using the DNA tip. (b) Representative $I-t$ plots measured using the $S_8$ DNA tip and the complementary $T_8$ target DNA. (c) Current histogram for measured $I-t$ plots. The Gaussian function (solid line) for the current-jump peaks are also shown. Bias voltage 0.2 V, initial set-point current: 20 nA.

Fig. 5.9.b showed the representative $I-t$ plots of the measurements using the $S_8$ DNA tip and Au substrate modified with $T_8$ DNA sample. In addition to the background tunneling current, which was equal to set-point current, sudden current jumps and plateaus higher than background were observed in measured $I-t$ data. The current jumps were ascribed to the hybridization of the DNA tip and sample DNA on the substrate. For showing the statistical value of the jump current, a histogram of current jumps was constructed from data points higher than background. The current jump histogram
exhibited a single peak at 8.5 nA (42.5 nS or $5.6 \times 10^{-4} G_0$, where $G_0$ is the fundamental conductance quantum) (Fig. 5.9.c), which corresponds to the statistical height of current jumps and consecutive plateaus. The current and conductance values determined by the $I-t$ measurements (Fig. 5.9) agree with those found in the $I-z$ method described in the Section 5.3.1. We, therefore, conclude that the $I-t$ measurements together with the use of DNA tips enable single-molecule DNA detection.

Fig. 5.10. Current histograms of $I-t$ plots measured using $S_8$ DNA tips and the $T_8$ DNA sample containing: no methylated (orange), methylated A (red) and methylated C (blue) base. The Gaussian functions fitted to the histograms for the current jump peaks are shown as black curves. Bias voltage: 0.2 V. Set-point current: 20 nA (orange), 10 nA (red & blue).

Next, we applied the present method to detection of methylated bases. The $I-t$ measurements were performed with $S_8$ DNA tips. The Au substrates in these measurements were modified with $T_8$ sample DNA that is complementary to the DNA tip but, in this case, contains either methylated A or methylated C base. $I-t$ plots acquired by these measurements exhibited current jumps and consecutive plateaus, demonstrating that hybridization takes place even in the case where nucleobases in sample DNAs was methylated. However, conductance values were found to be severely modulated by the methylation. Current histograms constructed by $I-t$ plots demonstrate the current (conductance)
values of 8.5 nA (42.5 nS or \(5.6 \times 10^{-4} G_0\)), 3.9 nA (19.5 nS or \(2.6 \times 10^{-4} G_0\)) and 2.9 nA (14.5 nS or \(1.9 \times 10^{-4} G_0\)) for sample DNAs that contains no methylated base, methylated A, and methylated C bases, respectively (Fig. 5.10). The large difference in these current or conductance values allows for discovery of DNA methylation at the single-molecule level.

5.4. Conclusions.

ssDNA was immobilized onto STM tips to construct DNA tips for measuring electron transport with the target ssDNA on a substrate. Hybridization between the ssDNA on the tip and that on the substrate occurred during the current measurement, and the resulting DNA duplex bridging the gap between the tip and substrate induced electron transport. The DNA tips thereby enable detection of target DNA at the single-molecule level. In addition and more importantly, it was found that DNA modifications, i.e., mutation (single-base mismatch) and epigenetic modification (methylation), cause a significant decrease in the conductance during \textit{in situ} hybridization, allowing for detection of SNPs. The DNA tips, therefore, offer a single-molecule sensitive, rapid, and low cost methodology for direct electrical detection, which is of paramount importance in DNA detection technology for genetic diagnosis.
6. Conclusions

Based on the discovery that the tunneling current flowing from the molecular tip to the sample surface is enhanced as the single-molecule interactions are formed to bridge the STM tip and STM sample in the previous researches of our laboratory about the chemical recognition, I continue to utilize the molecular tips to quantify the electron transfer through noncovalent interactions between a single tip molecule and a single sample molecule. In addition to quantification of electron transfer through hydrogen bond, charge transfer and coordination bond interactions, I have applied this electron transfer quantification to detect DNAs at single-molecule level such as fully matched, mismatched and methylated DNA.

Chapter 1 introduces two series of researches, which I based on to perform my thesis studies. The first series describe briefly the previous achievements of our laboratory in STM imaging, which indicated that protrusions in the images correspond to the facile tunneling current due to formation of noncovalent interactions between the tip and sample molecules. The second series review the development of STM-based methodology to measure the single-molecule conductance. Based on this methodology, I have developed to quantify the electron transfer through noncovalent interactions between two single molecules, one from tip and one from sample.

Chapter 2 describes in detail the quantitative measurement of electron transfer through a hydrogen bond between two ω-carboxyl alkanethiol molecules in which one is anchored to sample surface and the other is anchored to the tip apex, both via thiol groups. For measurement, the molecular tip was brought to the proximity, but never contact with the sample surface, the electron transfer between the tip and sample molecules were recorded in two conditions. The first condition was keeping the tip–sample distance constant by freezing the STM feedback loop, so the acquired data were current–time (I–t) plots. The second condition was displacing the tip–sample distance by moving the tip up and down to the sample, so the collected data were current–distance curves. The I–t plots showed the current jumps and consecutive plateaus. In this case, the tip and sample molecules have carboxyl groups at their free ends, and we attribute the current jumps to hydrogen bond
formation between these terminal carboxyl groups. The current increase brought on by the current jump, therefore, reflects electron transfer through the hydrogen bond interaction between two single molecules. The \( I-z \) curves, besides smooth exponential decay due to the electron tunneling, also showed clear steps or plateaus that were ascribed to the hydrogen bond interaction between single tip and sample molecules. Comparing with previous results at the short range, the conductance of the molecular pair interacting with each other via the hydrogen bond interaction was found to be larger than that of corresponding single molecule fully connected by covalent \( \sigma \) bonds. The conductance of the hydrogen-bonded junction, however, decays more quickly than that of the covalent junction as the molecular length increases. The first-principle calculations were carried out to qualitatively examine the origin of the superior conductivity of the short H-bonded junctions. A prominent peak in the density of states projected onto the central region was found near the Fermi level for the hydrogen-bonded molecular junction while such a peak was absent for the covalently connected molecular junction. Because valence band structures affect the tunneling current through molecular junctions, the higher conductivity of the hydrogen-bonded junctions was attributed to the prominent peak in the projected density of states. The property of the electron transfer through the hydrogen bond exhibited pronounced dependence on the length of single-molecule pair. These results indicate that the electrical connection between single molecules can be deliberately controlled by adjusting the length of hydrogen bond linkers between each other.

Chapter 3 mentions to investigations of the electron transfer from one \( \omega \)-carboxyl alkanethiol molecule to another \( \omega \)-carboxyl alkanethiol molecule in the present of a metal ion. There were two circumstances probably occurring in this experiment. One is the presence of metal ion insight the gap between two single molecules of \( \omega \)-carboxyl alkanethiol and therefore the coordination bond interactions of molecule-ion-molecule were probably formed. Two is the absence of metal ion insight the gap and thus only the hydrogen bonds of molecule-molecule were probably formed, as demonstrated in chapter 2. We used molecular tips of \( \omega \)-carboxyl alkanethiol and samples covered by \( \omega \)-carboxyl alkanethiol molecules for the experiments. With metal ions in the experiment solution, the measurements detected two values of electron transfer between the tip and the sample. Comparing
with our previous results in chapter 2, one value of electron transfer was ascribed to the through the hydrogen bond between two carboxyl groups. The left one was attributed to the electron transfer through coordination bonds of a metal ion with two carboxylate-terminated thiolate molecules. In other word, a metal ion induces the formation of a molecular junction with carboxy-terminated molecules via a metal coordination bond. This coordination bond formed a sandwich-type single molecular junction and was demonstrated to further facilitate the electron transfer than the hydrogen bonds do. These results provide essential knowledge for the construction of future bottom-up molecular devices and are utilized to implement conductance switching in a molecular junction of a head-to-head pyridine dimer, in which switching state is activated by a single metal ion.

Chapter 4 presents STM measurements of electron transfer from a single electron donor to an adjacent single electron acceptor. A molecular tip of fullerene and a sample covered by porphyrin molecules were used for investigations. With this setup, the electron transfer in a single noncovalent porphyrin–fullerene dyad was quantified. Instead of using linking groups, such as thiols, to couple the sample molecules to the electrode as in current subject of molecular electronics researches, we developed the novel ligation-mediated coupling technique to connect a porphyrin to a metal substrate without using a linking group. The combination of the molecular tip and ligation-mediated coupling enabled quantitative characterization of the conductance of a noncovalent porphyrin–fullerene dyad without any chemical modification of porphyrin molecules in the dyad. The measured results not only quantify the conductance but also exhibit the rectification property of the porphyrin–fullerene pair. The rectification behavior of porphyrin–fullerene dyad was also quantified in a voltage range of 0.3-0.8 V. This study opens up a way to explore quantitatively the rich electronic properties of supramolecules at the single-molecule level. In the STM measurements, the ligation-mediated coupling technique can be combined with laser illumination to detect the photoinduced electron transfer between a porphyrin molecule and a fullerene molecule, thereby furnishing useful information at the single-molecule level for the development of organic photovoltaics.
mismatched and methylated DNA was demonstrated in this chapter. Single-stranded DNA was utilized as a probe tip for single-molecule DNA detection. Hybridization between the tip ssDNA and the target DNA adsorbed on the sample surface occurred during the current measurement, and the resulting DNA duplex bridging the gap between the tip and sample induced electron transport. The DNA tips thereby enable detection of target DNA at the single-molecule level. The molecular tips of ssDNA were used to probe the complement ssDNA containing a mismatched and methylated base adsorbed on the sample surface in the measurements for single-molecule detection of mismatched and methylated DNA, respectively. While the \textit{in situ} hybridization between a ssDNA from tip and a complement ssDNA adsorbed on the sample surface during the measurement enables us to detect fully matched duplex DNA, the large difference in conductance between fully matched DNA duplex and mismatched or methylated DNA duplex allows us to discriminate the fully matched, mismatched and methylated DNA at the single-molecule level. It was found that the single-base mismatch or methylation causes a significant decrease in the conductance during \textit{in situ} hybridization, allowing for detection of single-nucleotide poly-morphisms at the single-molecule level. The DNA tips, therefore, offer a single-molecule sensitive, rapid, and low cost methodology for genetic diagnosis on the basis of direct electrical detections.

Chapter 6 summarizes the whole results and describes the prospects of the thesis.
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