

Realization of a four-step molecular switch in scanning tunneling microscope manipulation of single chlorophyll-a molecules

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Single chlorophyll-a molecules, a vital resource for the sustenance of life on Earth, have been investigated by using scanning tunneling microscope manipulation and spectroscopy on a gold substrate at 4.6 K. Chlorophyll-a binds on Au(111) via its porphyrin unit while the phytol-chain is elevated from the surface by the support of four CH₃ groups. By injecting tunneling electrons from the scanning tunneling microscope tip, we are able to bend the phytol-chain, which enables the switching of four molecular conformations in a controlled manner. Statistical analyses and structural calculations reveal that all reversible switching mechanisms are initiated by a single tunneling-electron energy-transfer process, which induces bond rotation within the phytol-chain.

Since the beginning of 1990, the scanning tunneling microscope (STM) has been demonstrated to be a useful tool to manipulate single atoms/molecules on supporting substrates with atomic-scale precision (1–4). To date, most STM manipulation experiments are performed on single atoms or small molecules, and the applications of this technique are concentrated mainly in the materials science research area. In this article, we extend the STM manipulation procedures to biology-related research areas and investigate a relatively large plant molecule known as chlorophyll-a.

Chlorophyll-a induces green color in plant leaves and is a key ingredient in photosynthesis, one of the most important biological processes, which converts sunlight into chemical energy in plants (5–9). Chlorophyll-a is also important from the evolutionary standpoint because photosynthesis played a central role in the early development of life on Earth (6). But, just as chlorophyll has been vital in the development and sustenance of plants and life forms, it may prove to be just as essential to the advancement of “green” energy research and nanotechnology. Because of their nontoxic nature and their abundance in the natural world, plant molecules like chlorophyll-a are given special interest in the quest for green energy resources and for the development of environment-friendly nanoscale devices (10–12). Chlorophyll-a consists of two main components: a porphyrin unit as the “head” and a long carbon-chain as the “tail.” In the light-harvesting reaction centers found in plant leaves, chlorophyll-a conforms into various shapes by bending the phytol tail. Molecular conformation is a key process in many biological functions, and controlling the conformational changes of biological molecules with submolecular precision is a dream for many scientists. Here, we are not only able to resolve the structure of single chlorophyll-a molecules but also to reversibly switch four molecular conformations in a controlled manner; the detailed switching mechanisms are explained by means of both experimental analyses and theoretical calculations.

Results and Discussion

Chlorophyll-a is weakly bound to the Au(111) surface, and the molecules are easily displaced during imaging. The STM images of chlorophyll-a on Au(111) show both single molecules and regions of self-assembled molecular clusters preferentially lo-

cated at the elbows of Au(111) herringbone reconstruction (Fig. 1*a*). To understand the adsorption geometry of chlorophyll-a, we will first discuss the structure of the molecule in the clusters. Chlorophyll-a clusters grow epitaxially on Au(111) and form a close-packed structure with a unit cell length of 1.6 nm (Fig. 1*a*). Inside the clusters, the molecules position in pairs with their “heads” facing each other. In a single row along the long molecular axis direction, the molecules assemble in an alternating “head–tail–tail–head” arrangement (Fig. 1*b*). From the atomically resolved STM images, the orientation of the molecules with respect to the Au(111) surface is determined. The long molecular axis is aligned along the [211] surface directions of Au(111). Such a close-packed self-assembly of chlorophyll-a is significant, mimicking the *in vivo* packing of chlorophyll-a in the photosynthetic membrane and possibly having important applications in solar cells and medical devices (10, 12).

The calculated structure of a free-standing chlorophyll-a molecule using the parameterized method PM3 (ArgusLab software; Planaria Software, Seattle, WA) shows that the phytol-chain is folded on the porphyrin unit (Fig. 1*c*) (15). The PM3 method is based on a simplified Hartree–Fock theory using NDDO (neglect of differential diatomic overlap) integral approximation. It is a self-consistent field method that takes in account electrostatic repulsion and exchange stabilization. Chlorophyll-a adsorbs on Au(111) by maintaining a similar structure: The porphyrin unit lies flat on the surface while the phytol-chain is folded on top and elevated from the surface by the support of four CH₃ groups. In the high-resolution STM images, the phytol appears as a chain of three triangular units, which are assigned as the elevated C–H groups corresponding to the following carbon atoms: (C4, C5, C6), (C8, C9, C10), and (C12, C13, C14), respectively (Fig. 1*c* and *d*). The carbon atoms C3, C7, C11, and C15 are attached to the CH₃ groups and are located slightly lower than their neighbors in this conformation (Fig. 1*d*).

Isolated chlorophyll-a molecules on Au(111) show a similar structure as in the clusters (Fig. 2*a*) with less detailed features: Instead of three triangular units, they appear as three lobes in the STM images. This effect might be partly due to a higher mobility of the phytol-chain in isolated molecules during STM imaging as opposed to the ones in the clusters, which are locked-in by the neighboring molecules. In this adsorption geometry, the binding of phytol-chain to the surface is weak, and thus it is relatively easy to change its conformation by using an inelastic tunneling spectroscopy scheme (2).

Four conformations of chlorophyll-a, marked as 1, 2, 3, and 4 (Fig. 2), have been selectively switched by injecting tunneling

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Abbreviation: STM, scanning tunneling microscope.

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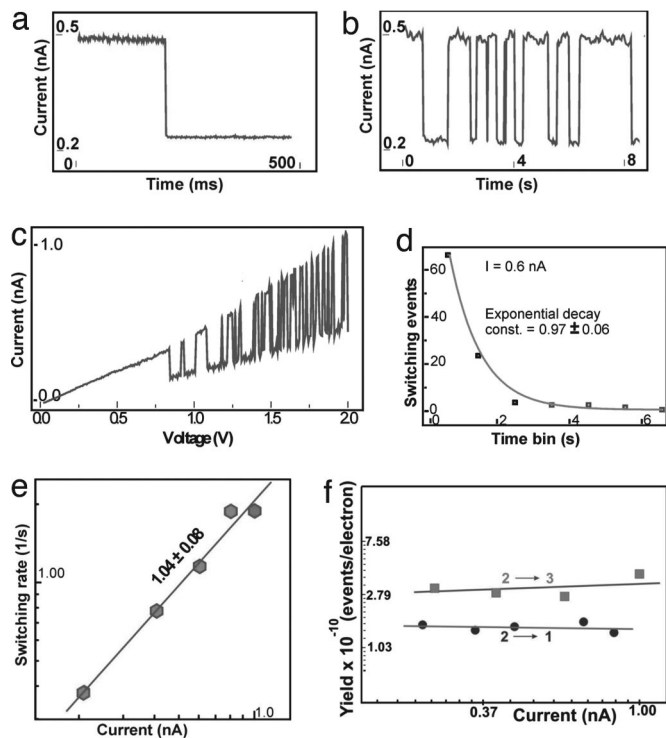


Fig. 3. Switching operation and mechanism. (a) An abrupt change in the tunneling current is associated with 1-to-2 switching. The current trace is recorded for a fixed voltage pulse of 1.5 V. (b) For a longer electron injection period, a two-step multiple-switching signal is observed. (c) An I - V curve of chlorophyll-*a* shows fluctuation of current above 0.8 V due to switching between 1 and 2. (d) A binned distribution of the switching events corresponding to 0.6 nA. (e) A logarithmic switching rate vs. current graph shows a unity slope. (f) The yield plots for 2-to-1 and 2-to-3 switching events. All of the data sets used for the plots are recorded with a 1.5-V bias.

free-standing chlorophyll-*a* structures using the parameterized method PM3 (15). The calculations reveal that the three bent molecular conformations observed here are caused by rotations of parts of the phytol-chain. The 1-to-2 switching involves bending of the phytol at C12 joint. This is caused by the rotation of the $\sigma_{C11-C12}$ bond (indicated in Fig. 2 with a red arrow in 1), which rotates the last part of the phytol (from C12 to C15) in a counterclockwise direction resulting in a 60° tail bending (Fig. 2). The 2-to-3 switching is induced by bending the phytol at C8 joint by rotating the σ_{C7-C8} bond (shown in Fig. 2 with a blue arrow in 2). This rotates a part of the phytol, from C8 to C15, in a counterclockwise direction. A clockwise rotation of the end part of the phytol at C13 in conformation 3 switches to the conformation 4 (indicated in Fig. 2 with an orange arrow in 3). Because the phytol-chain is lifted up from the surface by the support of CH_3 groups, such chain rotations can easily take place. The calculations reveal that chlorophyll-*a* can have a rich variety of conformations. We conclude that our ability to switch only four chlorophyll-*a* conformations on Au(111) is due to the limitation of the phytol tail rotation imposed by the surface. By comparing the calculated results with the experiment, we find location A (Fig. 2) as the C12 and C13 region and B as the C8 joint.

During the tip-induced switching process, the tunneling current is recorded as a function of time and the conformational changes can be recognized from the abrupt changes in current intensity (Fig. 3a). By increasing the process duration, the molecule can be switched between the two conformations back and forth, producing a two-step current signal (Fig. 3b) (16–19), which functions like a toggle switch. The activation barrier for

switching is determined by acquiring I - V spectroscopy data on isolated chlorophyll-*a* molecules, which reveal the current fluctuation above 0.8 V due to the conformational changes (Fig. 3c) (21). In general, rotational excitations of a free molecule require lower energies than electronic excitations (20). The 0.8-eV barrier ($1 \text{ eV} = 1.602 \times 10^{-19} \text{ J}$) determined here may include contribution from the surface. The I - V curve in Fig. 3c shows that when the current increases at the larger voltages, the frequency of switching also increases (21). For a fixed bias, the average switching frequency can be adjusted by varying the tunneling current only. The increase or decrease in the current induces faster or slower switching rates, respectively.

To understand the tunnelling-electron-induced switching process, we have performed statistical analyses taken over 1,200 switching events by using a 1.5-V bias and at different currents for the four conformations of the molecule. As described above, the switching can already be performed with a 0.8-V bias. 1.5 V is chosen here to ensure a single-electron tunnelling process. Fig. 3d shows the plot of conformational changes vs. time for the 1-to-2 switching with a fixed current of 0.6 nA. The time constant of this exponential decay has been determined as $0.97 \pm 0.06 \text{ s}$. The switching rates are determined from the inverse of the time constants. The linear dependence of switching rate on the tunneling current is illustrated in Fig. 3e, where each data point is determined by plotting an exponential curve shown in Fig. 3d. The rate, R , and tunneling current, I , are related as

$$R \propto I^N, \quad [1]$$

where N is the number of inelastic tunneling electrons involved in the energy-transfer process (22–24) to the molecule that induces conformational switching. From the slope of this curve, N is determined as ≈ 1 . Therefore, this switching process is initiated by a single-tunneling-electron energy-transfer process. Similar results have been found for the 2-to-3 and 3-to-4 switching processes, and thus single-tunneling-electron energy transfer causes all of the switching events described in this article. We have also determined the quantum yields for the switching events. Fig. 3f provides the yield plots for the 2-to-1 and 2-to-3 switching events. Here, the yield, Y , is related to the switching rate, R , and current, I , as

$$Y = Re/I, \quad [2]$$

where e is the electronic charge. The determined yields are 3.2×10^{-10} for switching from 2 to 3 and 1.5×10^{-10} for the switching from 2 to 1. Almost zero slopes of the yield plots further verify the single-electron energy-transfer process (25). The measured yields for 3-to-2 and 3-to-4 switching events are 3.8×10^{-10} and 3.3×10^{-10} , respectively. To demonstrate our ability of control further, we present snapshot images from a STM movie (Movie 1, which is published as supporting information on the PNAS

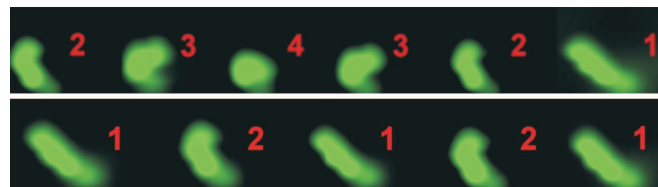


Fig. 4. Frames of STM images from a STM movie showing reversible switching of conformations 1, 2, 3, and 4. Each manipulation step involves taking the STM image of chlorophyll-*a*, inelastic tunneling spectroscopy excitation to switch the conformation, and then retaking the molecule image. Image parameters: 2.8 nm \times 3.6 nm scan, 0.5 V, and 93 pA; switching parameters: 1.5 V and 0.6 nA. The images taken after each switching event are sequentially presented.

