# Nanometerscale modification of biological membranes by field emission scanning tunneling microscopy

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# Nanometer-scale modification of biological membranes by field emission scanning tunneling microscopy

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Manipulation and modification at atomic and nanometer scales of some semiconductor and metallic samples has been made possible by scanning tunneling microscopy (STM). This has generated novel approaches for designing new devices at nanometer scale. The poor electronic conductivity of biological molecules has prevented the extension of those methods to them. Here, it is described how a low current STM operated in the field emission regime allows, reproducible imaging and selective modification of biological membranes. A method is presented (i) to visualize at high-resolution hydrated purple membrane sheets, (ii) to produce nanometer-scale marks on them, and (iii) to image the altered membranes.

Nanometer- and atomic-scale modification of some semiconductor[1–3] and metallic[4,5] surfaces has been demonstrated by using one or a combination of the interactions present in scanning tunneling microscopy (STM): contact forces, Van der Waals forces, electric fields, and electron beam induced processes. This has stimulated a variety of methods for fabrication of nanometer-scale structures and devices.[6,7] Submicrometer lithography has also been performed by exposing some polymer layers to the STM electron current.[8,9] However, the extension of those results to other biological and technological relevant materials as biological membranes has been hindered by the difficulties of the STM for imaging poor conducting materials.[10]

In this letter, it is demonstrated that an STM operated in the field emission regime and at very low currents ($I \leqslant 2$ pA) can generate high-resolution images of hydrated biological membranes and subsequently modify them by increasing the current. Marks which lateral dimensions between 15 and 100 nm can be made on purple membrane sheets.

Purple membrane (PM) is a natural membrane crystal made of a single protein species (bacteriorhodospin). It appears in oval sheets of about 1 $\mu$m in diameter and ~4.8 nm in thickness.[11] The proteins are packed in an hexagonal symmetry with a lattice constant of 6.3 nm. The protein acts as a light driven proton pump. Due to its remarkable optical properties, PM is drawing technological interest for its potential applications as a high performance component of optical computers.[12]

Imaging has been performed in the constant current mode. The approach of Guckenberger *et al.* of using low currents and high voltages is followed.[13] Typical parameters are $I \sim 0.2$ pA and $V \sim -7$ V (sample negative). The modifications are performed by approaching the tip, from the imaging distance until mechanical contact.

There are two physical parameters that control the tip-membrane distance: (i) the applied voltage and (ii) the set current. The imaging is only possible for currents below $2 \pm 0.5$ pA and applied voltages above $5.5 \pm 0.3$ V (Ref. 14). The dispersion with respect to mean values is tip dependent. When those conditions are not fulfilled, the tip dives into the membrane and by scanning the tip a pattern can be written. The modifications are visualized by retracting the tip to imaging values and scanning the sample.

A 10-$\mu\ell$ drop of an aqueous suspension containing 0.1 mg/ml of PM is sprayed onto a piece (5 mm×5 mm) of highly oriented pyrolitic graphite (HOPG). With no further preparation the sample is introduced in a STM chamber that allows control relative humidity. Prior to the deposition, freshly cleaved graphite has been glow discharged for 5 s at $10^{-4}$ mbar. All measurements have been performed in air at 35% relative humidity. The low current STM used here has been described elsewhere.[10] A relevant factor for imaging PM at high resolution is the sharpness of the tips. Tips with curvature radius smaller than 30 nm are needed.[15] All images are raw data with no other processing than the subtraction of the background plane.

A topographic image (0.16 pA and −7 V) of a membrane is shown in Fig. 1. The membrane is clamped between two terraces separated by a step of carbon atoms. The thick-

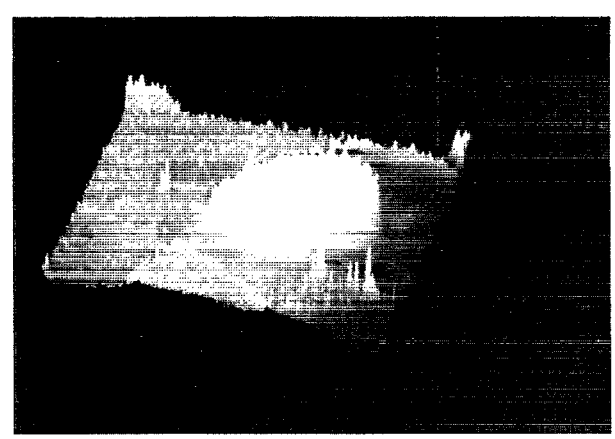

FIG. 1. Topographic image, viewed in perspective, of a purple membrane patch (2 $\mu$m×2 $\mu$m). Some small fragments of PM (about 20 nm) are also seen in the lower left corner. There is a good agreement between the thickness of the membrane (4.6 nm from the image) and its known value (4.8 nm). The granular aspect of the substrate has been created during the glow discharge. Image taken in air at 35% relative humidity; $I = 0.16$ pA and $V = -7$ V (sample negative).

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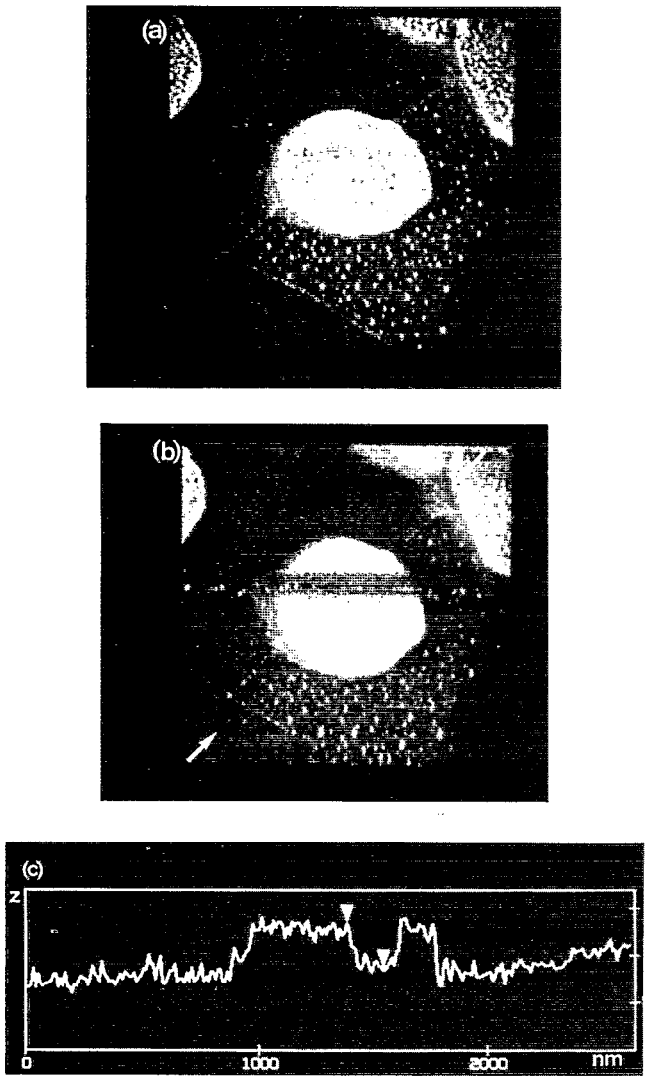


FIG. 2. Topographic images (top view) 2 $\mu$m×2 $\mu$m of PM patches. (a) A patch of PM lies between to HOPG terraces separated by a monoatomic step. Small PM fragments are also visible as bright spots. (b) The same patch after a stripe of proteins have been removed. The removal is achieved by scanning 20 lines, 5 nm apart at 4.5 pA. In the middle of the trench there is a small stripe of 15-nm width (it is hardly visible due to the scale). This was created by scanning three lines, 5 nm apart at 6 pA. (c) A cross section [it follows the direction of the arrows in (b)] shows the structure created and allows height measurements (4.5 nm between markers); $z$ axis, 16.7 nm. Images taken at $I=0.16$ pA and $V=-7$ V.

ness of the membrane as measured from the image is 4.6 ±0.5 nm, i.e., in good agreement with the value measured by other techniques[11] (~4.8 nm). This figure illustrates the ability to faithfully image biological molecules with this method. The presence of a monoatomic step (0.34 nm) on the substrate is noticeable in the forefront. Tiny PM fragments are seen in the lower right corner. The smaller structure detected has a diameter of about 20 nm. This corresponds to a fragment of about ten proteins.

Figure 2 shows a membrane before and after a section of it has been removed. The modification, a trench of membrane's diameter ×110 nm×3.8 nm has been made by scanning the selected region at 4.5 pA and at −7 V. The depth of the cut is controlled by the set of the current. Higher currents

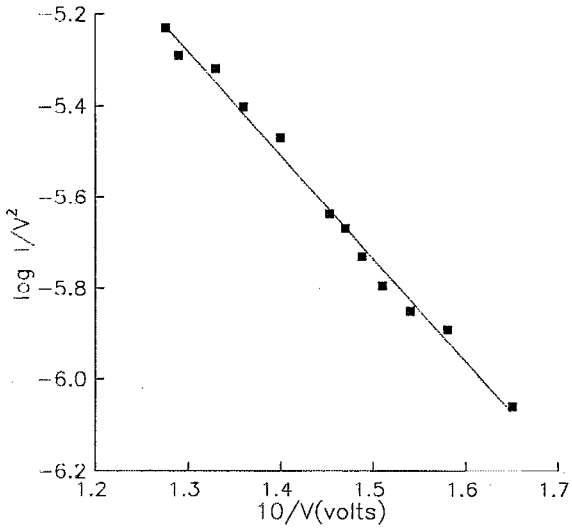


FIG. 3. Current vs voltage characteristics. The linear dependence follows the Fowler–Nordheim equation for a field emission process. The $I$-$V$ curves have been taken on a HOPG surface close to the PM.

imply deeper cuts. At 4.5 pA approximately $\frac{3}{4}$ of the protein backbone are removed or smashed by the tip [see Fig. 2(c)]. In this example, the trench is made by scanning 20 consecutive lines 5 nm apart.

The degree of precision and potential of this method for nanometer-scale modification is shown in Fig. 2(b). In the middle of the trench, there is a 15-nm stripe of clean graphite. All the proteins have been removed. This has been accomplished by scanning three lines at 6 pA. Alternatively, marks can be made by scanning the PM at very low currents (less than 2 pA) and decreasing the voltage below 5.5 V. Both methods produce similar results.

Topographic images are obtained at −7 V and 0.16 pA. At those values, measured tip-sample separation ($S$) are about 5–6 nm. Those values are incompatible with a tunneling mechanism between tip and sample $\{I \propto V \exp[-10.25\Phi(\mathrm{eV})^{1/2}S(\mathrm{nm})]\}$, where $\Phi$ is the effective work function. In order to identify the mechanism of electron transport, $I$-$V$ characteristics have been measured. These plots have been taken by interrupting the constant current mode. The tip is held at a defined position over the sample, then the voltage is ramped and simultaneously the current is recorded.

A typical plot of $\log(I/V^2)$ vs $1/V$ (Fig. 3) shows a linear dependence. This is distinctive of a field emission process described by the Fowler–Nordheim equation for planar emitters[16]

$$I = AV^2 \exp\left(-\frac{6.85\Phi^{3/2}S}{V}\right),$$

where A is a factor that depends on the emitting area. There are several significant differences between this emission process and the one measured by field emission microscopy (FEM).[16] In these experiments, electrons are emitted from relatively smooth surfaces while in FEM they leave sharp tips of radius of curvature of about 20 nm. In FEM, the separation between electrodes are in the range of mm and

extraction voltages go from hundreds to thousands of volts. Here, distances are smaller than 10 nm and applied voltages around −7 V. Nevertheless, the electrical fields present here (~1 V/nm) are of the same order than those of FEM.

This field emission mode is fully compatible with the electronics associated with STM. Several authors have operated the STM at high voltages,[8,13] but as far as the author knows, no field emission characteristics were determined.

Imaging of biological membranes is possible at both polarities, however, negative polarities are preferred. When electrons are emitted from the tip the resolution is worse. Tip-sample separations are larger by a factor of 2–3 when the sample is biased positive, this in turn decreases the resolution.

There is an active controversy surrounding STM experiments on biomolecules, both theoretical and experimental. Conduction enhancement by energy relaxation in disordered systems,[17] pressure induced resonant tunneling,[18] and ion assisted conduction[19] mechanisms have been proposed. For these and the results of Guckenberger *et al.*,[13,20] a process where electrons are field emitted from the molecules is more likely. In addition to plots as those of Fig. 3, a field emission mechanism is also supported by the threshold observed in the applied voltage (5.5 V) required for imaging the membranes, i.e., values above the work function of the substrate (4.7 eV). However, the specific details of how electrons are emitted through proteins remains a challenging problem.

There is an increasing tendency to incorporate biomolecules into physical devices; biosensors, new photonic materials, or artificial biological neural networks. To exploit all the benefits derived from using biomolecules as components of devices a new generation of tools are needed to characterize and to manipulate them in environmental conditions. The operation of a low current STM in the field emission regime produces high resolution and reproducible images of hydrated purple membranes. The transition from field emission to tunneling regimes turns the tip into a modifying tool. Marks of 15 nm of lateral dimensions have been generated on the membrane.

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