A Protein-Based Three Terminal Electronic Device

ROSS RINALDI, GIUSEPPE MARUCCIO, ADRIANA BIASCO,
PAOLO VISCONTI, VALENTINA ARIMA, AND ROBERTO CINGOLANI

*National Nanotechnology Laboratory of INFM,
Dipartimento di Ingegneria dell'Innovazione, University of Lecce, Lecce, Italy*

ABSTRACT: Because of their natural functional characteristics, involving inter- and intramolecular electron transfer, metalloproteins are good candidates for biomolecular nanoelectronics. In particular, blue copper proteins, such as azurin, can bind gold via a disulfide site present on its surface and they have a natural electron transfer activity that can be exploited for the realization of molecular switches whose conduction state can be controlled by tuning their redox state through an external voltage source. We report on the implementation of a prototype of protein transistor operating in air and in the solid state, based on this class of proteins. The three terminal devices exhibit various functions depending on the relative source-drain and gate-drain voltages bias, opening a way to the implementation of a new generation of logic architectures.

KEYWORDS: nanoscale devices; molecular electronics; bioelectronics; three terminal devices

INTRODUCTION

Nanobiotechnology has been boosted by an advancement in fabrication technologies that allows construction of artificial structures in the same range of biological entities. The fascinating world of the bio-self-assembly provides new opportunities and directions for future electronics, thus opening the way to a new generation of computational systems based on biomolecules and biostructures. Recent concept-studies have demonstrated possible applications of bioelectronic devices to standard electronics (i.e., for rectification, amplification, and storage), as well as novel functionalities (i.e., for driving a biospecific assembly).¹

The blue copper protein azurin, from *Pseudomonas aeruginosa*, is involved in bacterial respiratory phosphorylation. It has a molecular mass of 14,000Da and a distorted trigonal bipyramid structure for the *copper active site*, which plays the role of an electron reservoir in the electron transfer process. The functionality of this redox protein consists of performing electron transfer between other metalloproteins (cytochrome c551 and nitrite reductase) under physiologic conditions.²

The electron transfer mechanisms of azurin have been extensively studied, both experimentally by means of scanning tunnelling microscopy (STM) based studies and theoretically.³ Electrochemical *in situ* STM of adsorbate molecules with

Address for correspondence: Ross Rinaldi, National Nanotechnology Laboratory of INFM, Dipartimento di Ingegneria dell'Innovazione, University of Lecce, Lecce, Italy.
ross.rinaldi@unile.it

low-lying redox levels strongly coupled to the environmental nuclear motion revealed that the STM process can be viewed as a coherent two-step electron transfer (ET) involving electron exchange between the local redox level and the manifolds of electronic levels in the substrate and tip. The notion of coherence implies that the intermediate electron or hole state after the first ET step does not fully relax vibrationally before the second ET step.⁴ Single-molecule behavior of redox metalloproteins, such as *P. aeruginosa* azurin, has been accurately and comprehensively investigated in liquid environment by single-molecule *in situ* STM. The properties of this class of molecules have pointed to ways of controlled orientation of well-defined monolayers of fully functional proteins, allowing a comprehensive characterization of the electronic properties of the immobilized molecules.^{5,6}

Recently Rinaldi *et al.* showed that it is possible to implement two terminal rectification devices based on an azurin monolayer.^{7,8} Taking into account that size downscaling by the usual top-down technologies is reaching its intrinsic limit, the development of new technologies for surface patterning based on self-assembly and nano-manipulation (i.e., using a bottom-up approach) becomes very important. In the case of the protein transistor we used a combination of these two approaches for the construction of the devices.

RESULTS AND DISCUSSION

The redox process involving the Cu active site of the protein in solutions is:

This reversible reaction is activated by an electron transfer mechanism that continuously reconverts the copper oxidized state (Cu^{2+}) into the reduced states (Cu^{+}). Indeed the protein can exist in these two stable configurations, and its capability to work as a one-electron carrier depends on the equilibrium between these two states. The natural electron transfer activity can be exploited for the realization of molecular switches whose conduction state can be controlled by tuning their redox state through an external voltage source (gate).

The protein transistor was fabricated on Si/SiO₂ substrates, following the method reported elsewhere.^{7,8} The geometry of the device comprises two planar Au/Cr contacts separated by a gap in the range 50–100nm, which defines the gate width. An azurin monolayer was then immobilized onto the channel by means of surface functionalization and chemisorption. The formation of extended uniform protein monolayer was controlled by means of several surface techniques, as reported elsewhere.⁹ Moreover, a controlled protein orientation was obtained by optimizing the self-chemisorption process.^{7,8} Both commercial natural azurin and synthetic and purified azurin were used to implement the devices. A silver electrode was added on the back of the Si substrate as the gate, to control the source-drain conduction.

All the fabricated devices were tested at room temperature and ambient pressure. Prior to protein deposition, a negative control on the empty devices was performed to check the effective insulation between the source (S), drain (D), and gate (G) terminals along the various current pathways. All these tests revealed I_{DS} and I_{SG} values smaller than 20pA and typical open circuit resistance larger than 100GΩ.

In FIGURE 1A we show the current voltage characteristics of a protein device for $V_{DS} > 0$ as a function of V_G in the range between 0 V and 4 V (first active region). As a general feature the I_{DS} current remains low (less than 20 pA) up to a V_{DS} bias voltage of about 2.3 V and then starts to increase, reaching 100 pA intensity at 6 V. A clear modulation effect is visible as a function of V_G . The dependence of the I_{DS} current as a function of the V_G intensity is shown in FIGURE 2A for a fixed value of V_{DS} ($V_{DS} = 4.5$ V). The current increases up to a maximum value of 200 pA at $V_G = 1.1$ V, then it decreases to the 100 pA level at $V_G = 2$ V, and finally to the open circuit value at the higher applied gate voltages.

A similar set of measurements were made under positive and negative V_{DS} bias with positive (FIG. 1A and C, respectively) and negative (FIG. 1B and D, respectively) gate bias. Four different active region resulted: $V_{DS} > 0$, $V_G > 0$ (FIG. 1A); $V_{DS} > 0$, $V_G < 0$ (FIG. 1B); $V_{DS} < 0$, $V_G > 0$ (FIG. 1C); $V_{DS} < 0$, $V_G < 0$ (FIG. 1D). In FIGURE 1B, by decreasing the gate potential, the current increases to 160 pA at $V_{DS} = 6$ V. The corresponding dependence of the I_{DS} current as a function of V_G is shown in FIGURE 2B for a V_{DS} value of 5 V. In this case we did not observe a peaked dependence, as in the case of FIGURE 2A, but a smooth increase and saturation at 115 pA in the range between -2.5 and -4 V. In the last two situations—that is, $V_{DS} < 0$, $V_G > 0$ (FIG. 2C) and $V_{DS} < 0$, $V_G < 0$ (FIG. 2D)—we observed a decrease and an increase (in absolute value) of the I_{DS} negative current with increasing gate voltage in the two cases, respectively. As a general trend, the current increases linearly for V_{DS} voltages greater than 3 V and then saturates in the range between 4.5 and 6 V. In this voltage range the operation of the protein transistor resembles that of an inorganic MOS-FET in the saturation region, with constant I_{DS} current value for each curve.

To better understand the conduction mechanism in the protein FET and the origin of the different modulation effect in the four operating modes we need to summarize the Azurin electronic properties. As mentioned above, azurin is a blue-copper electron transfer protein.¹⁰ Many important chemical and biological processes involve long-range transfer of electronic excitation energy or electric charges between donor and acceptor molecules. At large separations (several angstroms), there is no direct donor–acceptor (DA) coupling, and intervening “bridge” sites that promote the transfer are required. This mechanism has been investigated in many biological molecules, such as cytochromes, hemoglobins, DNA, and docked proteins, on time scales down to the femtosecond range. It is generally found that, in the non adiabatic regime, the rate of electron transfer decays roughly exponentially with distance between donor and acceptor sites. One of the key questions in the study of acceptor-bridge-donor complexes is the nature of the electron transfer process, which could be either a sequential (incoherent) hopping between adjacent sites or a direct quantum tunneling (superexchange) between the donor and acceptor. In the former case the bridge provides intermediate sites in which the excitation resides, whereas the only role of the bridge in the latter case is to provide virtual orbitals that determine the effective DA coupling.^{11,12} Usually, a unified description that includes sequential and tunneling processes is required to describe precisely the ET phenomena in biological media. For example, recent experiments performed on DNA to study the rate of charge transfer between two guanine bases indicate that there is a decrease in the transfer rate constant K with increasing distance only if the guanine bases are

FIGURE 1. I - V characteristics of the protein transistor in the four active regions: (A) $V_{DS} > 0$, $V_G > 0$; (B) $V_{DS} > 0$, $V_G < 0$; (C) $V_{DS} < 0$, $V_G > 0$; and (D) $V_{DS} < 0$, $V_G < 0$.

FIGURE 2. I_{DS} current intensities as a function of V_G at fixed V_{DS} values for the four active regions shown in FIGURE 1: (A) $V_{DS} = 4.5$ V; (B) $V_{DS} = -5$ V; (C) $V_{DS} = 5$ V; and (D) $V_{DS} = -5$ V.

separated by no more than three base pairs; if more bridging base pairs are present, the transfer rates exhibit only a weak distance dependence.¹³ This finding indicates that a shift from coherent superexchange charge transfer (tunneling) at short distances to a process mediated by thermally induced hopping of charges at long distance can occur in biological media, without a visible reduction of the long range electron transfer effect.

Moreover, the effect of external perturbations, such as temperature change or a dissipative external bath, must be taken into account to properly describe the process that regulates the conduction properties of the materials. In some cases the occurrence of incoherent processes arising from inelastic scattering may result in the transformation from exponential decay to ohmic behavior, with the current dominated by inelastic processes and decreasing only slowly as the inverse of the distance between the donor and acceptor sites.¹⁴ This is analogous to the situation of electron transfer through tunneling barriers in semiconductor devices. Another important parameter in the evaluation of K is the selection of the electron pathway inside the protein.¹⁵ In the case of an azurin monolayer deposited in the channel of our FET, both superexchange charge transfer inside the single azurin units and sequential hopping between adjacent proteins are involved in the conduction process. The existence of a strong internal dipole due to the surface charge distribution on the protein can affect the planar conduction in the gate channel depending whether the dipoles of the proteins in the layer are summed with a resultant in the same direction of the external source-drain field or not.^{7,8} This effect has been shown to be the origin of the rectification effects in planar two terminal devices, favoring conduction under a bias swing (positive or negative depending on the actual orientation of the proteins in the device). In the case of the characteristics of a device fabricated with commercial azurin, a similar effect is observed for positive gate voltages values (FIG. 1 A and C). In the case of the I - V measurements in FIGURE 1 C, current intensities are higher than the corresponding values in FIGURE 1 A at least by a factor 10. For negative gate voltages the measured current intensities shown in FIGURE 1 B and D are similar.

The change in the gate voltage acts in various ways depending on the cross correlation of the signs of all the external applied voltages plus the effect of the internal dipole. As a general trend the I_{DS} current decreases with increasing absolute value of the applied V_G . However, a different trend is usually observed in the case of $V_{DS} > 0$, $V_G > 0$ (FIG. 1 A) where a peaked dependence was observed in most of the realized devices. The maximum of the current is always found about $V_G = 1$ V. This is a peculiar behavior of the protein device that is not found in any inorganic counterpart and could be of interest for the realization of electronic devices with novel functionalities. In the case of devices realized with synthetic and purified azurin the current increases by a factor 10 to 100 and the V_G resonance becomes sharper. The physical origin of this trend can be found in the effect of gate voltage on the molecular levels of the protein and, in turn, on the conduction pathways inside the molecule. The protein is chemisorbed onto the SiO_2 surface by the formation of a thiolate onto the silanized surface. In this way the protein sits on the surface with the axis connecting the thiolate with the Cu ion almost perpendicular to the surface. The vertical field that propagates along this direction (gate field) modifies the electronic properties of the molecule and the oxidation state of the Cu ion, since it is strongly coupled to the thiolate by the ET pathway inside the protein. This resonance effect

FIGURE 3. STM images of Azurin molecules chemisorbed on Au(111) substrates: (A) $I_{\text{tunn}} = 0.5 \text{ nA}$, $V_{\text{gap}} = -1 \text{ V}$ and (B) $I_{\text{tunn}} = 0.5 \text{ nA}$, $V_{\text{gap}} = -0.5 \text{ V}$.

has been already observed in electrochemical STM experiments¹⁶ and in standard STM experiments performed on azurin molecules chemisorbed on Au(111) substrates at room temperature and ambient pressure. It has been demonstrated that the STM detection of the protein at the surface strongly depends on the applied bias between the tip and the gold substrate. FIGURE 3 shows two typical STM images recorded by keeping the tunneling current intensity constant to a value of 0.5 nA and changing the gap voltage from -1 V (FIG. 3A) to -0.5 V (FIG. 3B). Note that these measurements are carried out on azurin molecules immobilized *in the solid state* onto the gold surface; that is, in a condition very similar to that of the devices and totally different from that normally used in other experiments in solution. In the first image the proteins are visible on the gold surface: bright spots with the form of globular structures with dimensions of 4–6 nm in diameter are imaged by the STM. When the gap voltage is reduced to -0.5 V no more proteins are imaged, instead, at their sites on the surface, small black dips appear, due to the artifact of the piezo movement that follows the tip looking for the tunneling current. In the first case the applied potential generated a favorable level alignment between the Au substrate the molecular levels of the protein and the tip, resulting in a resonant transfer of electron through the molecular levels. An off-resonance condition is reached by shifting the gap voltage to 0.5 V, just as in the case of FIGURE 2A. For positive gap voltages it was not possible to achieve stable tunneling conditions. This means that the tunneling out of occupied states (HOMO levels) is the most favorable process.

CONCLUSIONS

We have demonstrated the first protein transistor working in air and at room temperature by using azurin metalloproteins. Various modulation effects were observed depending on the relative source–drain and gate–drain voltage bias polarity. This particular behavior was explained in terms of tuning the resonant transfer through the redox level of the molecule by means of the gate field. The fabrication of complementary devices on the same chip permits the construction of fully integrated circuits with a very small occupation area. Finally, the issues of device reproducibility, ageing, and lifetime must be addressed for molecular electronics. Among all the fabricated nanodevices, only a limited group exhibit a clear and reproducible gate effect, whereas in the others significant current fluctuations and failure occurred. Inspection by means of scanning electron microscope demonstrated that the failure was primarily due to the electrode burning and possibly metal electro-migration during operation. Electromagnetic field simulations are in progress, using finite element methods, to elucidate this important issue, to improve the device design, and, in turn, the device reproducibility.

ACKNOWLEDGMENTS

Support for this research was provided by the Italian Istituto Nazionale per la Fisica della Materia (PRA-SINPROT) and by the EU (SAMBA Project).

REFERENCES

1. BASHIR, R. 2001. DNA—mediated artificial nanobiostructures: state of the art and future directions. *Superlattices Microstructures* **29**(1): 1–16.
2. ADMAN, E.T. 1985. Structure and function of small blue copper proteins. *In* Topics in Molecular and Structural Biology, Vol. 1, Metalloproteins. P.M. Harrison, Ed: 1–42. Chemie Verlag, Weinheim.
3. KUZNETSOV, A.M. & J. ULSTRUP. 1999. *Electron Transfer in Chemistry and Biology. An Introduction to the Theory*. Wiley, Chichester.
4. FRIIS, E.P., YU.I. KHARKATS, A.M. KUZNETSOV, *et al.* 1998. *In situ* scanning tunnelling microscopy of transition metal complexes and redox metalloproteins as a vibrationally coherent electronic three-level process. *J. Phys. Chem. B* **102**: 7851–7859.
5. ZHANG, J., Q. CHI, A.M. KUZNETSOV, *et al.* 2002. Electronic properties of functional biomolecules at metal/aqueous solution interfaces. *J. Phys. Chem. B* **106**(6): 1131–1152.
6. CONTERA, S.A. & H. IWASAKI. 2002. Imaging the proteins pseudoazurin and apo-pseudoazurin on gold by STM in air: effect of the bias voltage. *Ultramicroscopy* **91**: 231–243.
7. RINALDI, R., A. BIASCO, G. MARUCCIO, *et al.* 2002. Solid-state molecular rectifier based on self-organized metalloproteins. *Advanced Materials*. **20**: 1453–1457.
8. RINALDI, R., A. BIASCO, G. MARUCCIO, *et al.* 2003. Electronic rectification in protein devices. *Appl. Phys. Lett.* **82**: 472–474.
9. SCHNYDER, B., R. KOTZ, D. ALLIATA & P. FACCI. 2002. Comparison of the self-chemisorption of azurin on gold and oxygen terminated surfaces. *Surface Interface Anal.* **34**: 40.
10. BROO, A. & S. LARSSON. 1991. Electron-transfer in azurin and the role of aromatic side groups of the protein. *J. Phys. Chem.* **95**: 4925–4928.
11. KUHN, O., V. RUPASOV & S. MUKAMEL. 1996. Effective bridge spectral density for long-range biological energy and charge transfer. *J. Phys. Chem.* **104**: 5821–5833.
12. DAVIS, W.B., M.R. WASIELEWSKI & M.A. RATNER. 1997. Electron transfer rates in bridged molecular systems: a phenomenological approach to relaxation. *J. Phys. Chem. A* **101**: 6158–6164.
13. GIESE, B., J. AMAUDRUT, A.K. KÖHLER, *et al.* 2001. Direct observation of hole transfer through DNA by hopping between adenine bases and by tunnelling. *Nature* **412**: 318.
14. NEOFIOTIS, G., R. LAKE & S. DATTA. 1992. The non-equilibrium Green's function method applied to double barrier resonant tunneling diodes. *Phys. Rev. B* **43**: 2442.
15. KAWATSU, T., T. KAKITANI & T. YAMATO. 2000. A novel method for determining the electron tunneling pathway in protein. *Inorgan. Chim. Acta* **300–302**: 862–868.
16. FACCI, P., D. ALLIATA & S. CANNISTRARO. 2001. Potential-induced resonant tunneling through a redox metalloprotein probed by electrochemical scanning probe microscopy. *Ultramicroscopy* **89**(4): 291–298.