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Electron tunnelling through single azurin molecules can be on/off switched by voltage pulses

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Redox metalloproteins are emerging as promising candidates for future bio-optoelectronic and nano-biomemory devices, and the control of their electron transfer properties through external signals is still a crucial task. Here, we show that a reversible on/off switching of the electron current tunnelling through a single protein can be achieved in azurin protein molecules adsorbed on gold surfaces, by applying appropriate voltage pulses through a scanning tunnelling microscope tip. The observed changes in the hybrid system tunnelling properties are discussed in terms of long-sustained charging of the protein milieu. © *2015 AIP Publishing LLC*.
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Redox metalloproteins play a key role in a wide variety of biological processes, such as photosynthesis and respiration, due to their capability to efficiently shuttle electrons among active sites of partner proteins.[1] In the last years, their electron transfer (ET) properties have been extensively studied in view of potential bioelectronics and biosensor applications.[2–7] In particular, much effort has been devoted to control their ET processes at single molecule level, by means of external signal inputs; this deserving challenging perspectives also in the applicative field of biomemories.[8–11]

Among redox metalloproteins, most attention has been focused on the blue copper protein Azurin (Az) for several reasons, among which: (i) the protein displays a remarkable robustness combined with an efficient electron conjugation ability with metal electrodes, giving rise to stable hybrid nanostructures;[11–22] (ii) it is characterized by a very efficient and fast intramolecular charge transfer, whose molecular pathway is quite well established;[23] and (iii) it maintains its biorecognition capability towards natural partners and biomolecules of clinical interest, even immobilized onto inorganic substrates.[24,25]

The Az active site, which is crucially involved in the ET process, contains a copper (Cu) ion which is able to switch between two stable oxidation states (Cu^{1+}/Cu^{2+}).[26] Within the active site, the Cu ion is liganded to five Az aminoacidic residues, according to a peculiar distorted tetrahedral symmetry that gives rise to the characteristic ligand field energy levels (d-d optical transitions), which, in turn, endows Az with an intense ligand-to-metal charge transfer (LMCT) absorption band.[26] This symmetry, which moreover determines the protein redox potential, can be finely tuned by the vibrational "quake" induced by the docking of the partner, resulting in a facilitated physiological ET.[1]

By means of femtosecond pump–probe and resonant Raman spectroscopy experiments, combined to theoretical-computational approaches, it has been shown that the excitation of the LMCT optical band could induce the activation of the Az active site phonons and, to a minor extent, of the protein collective modes of some biological relevance.[27–29]

Thus, an intriguing interplay among ET, optical, and vibrational properties seems to characterize the physiological functioning of Az. This would make the proteins suitable candidates for applications in bio-optoelectronic devices, provided that the cross interaction among electronic, optical, and vibrational behaviour could be appropriately controlled.

Within this contest, we have recently demonstrated that electron tunnelling through single Az molecules onto a semiconducting surface can be significantly enhanced by optical excitation of the LMCT band at 632 nm;[10] on the other hand, it has been shown that the ET properties of Az molecules bound to gold electrodes can be controlled by tuning the electrochemical potential[11–15] or by stress-induced structure modification.[17,30]

In this paper, we show that electron tunnelling through Az molecules assembled on a gold (Au) electrode can be reversibly on/off switched by appropriate voltage pulses applied by a scanning tunnelling microscopy (STM) tip placed on the top of a single Az protein molecule.

Controlled Az/Au hybrid systems were prepared by incubating the Au substrates (which have been previously annealed with a butane flame, to obtain re-crystallized atomically flat (111) terraces over hundreds of nanometers) with an Az solution (100 μM in 50 mM ammonium acetate, pH 4.6) for about 12 h at 4 °C. Then, they were rinsed with ultrapure water and accurately dried with pure nitrogen. STM measurements have been performed by using an Agilent PicoLE 5100 microscope, with final preamplifier sensitivity of 1 nA/V and mechanically cut Au tip.

The experimental conditions are schematically outlined in Fig. 1(a). The Az molecule is sketched as being bound to the Au surface through its disulphide bridge, with the active site oriented towards the STM tip; such a binding having been reported in several experiments.[13–21]

Fig. 1(b) shows a typical STM image obtained from our hybrid Az/Au system. The globular objects observed are remarkably stable in time, against repetitive scanning, as witnessed by the image in Fig. 1(c), which has been acquired

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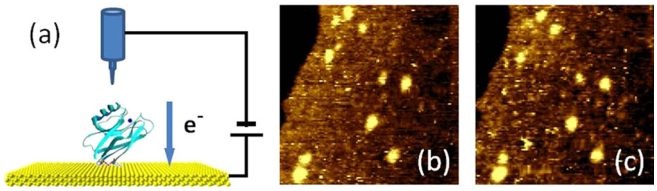

FIG. 1. (a) Schematic representation of the working conditions, at positive sample bias. (b) and (c) STM images of an Az/Au sample acquired at $V_{bias} = +0.5$ V, I = 0.06 nA, scanning rate = 4.1 Hz, scan size = 50 × 50 nm, and z scale = 0.250 nm: (b) after approaching and (c) after 20 min of continuous scanning.

after continuous scanning for 20 min. The average lateral size of the bright spots is 3.9 ± 0.6 nm, in good agreement with the crystallographic dimensions of Az (4.4 ± 0.9 nm (Ref. 31)); while their apparent height results to be much smaller (0.22 ± 0.04 nm), as it is generally remarked for STM images of Az proteins on metal surfaces.[13,14,16]

For homogeneous conductive materials imaged by STM at constant current value, the brightness of the observed topographic features is directly correlated to the real vertical dimension of the imaged structures. On the contrary, when almost non-conductive objects (such as biomolecules) are assembled on conductive surfaces, their STM image contrast cannot be solely attributed to the relief of the structures under investigation. Indeed, the corresponding piezo vertical displacement (which is much smaller than the real topographic dimensions) results from an increase in the tunnelling current, likely due to non-negligible conductive properties whose origin is still quite controversial. Biomolecule conductance has been tentatively attributed to the hydration water molecules,[32] to the presence of dissolved ions,[33] or to a subtle balance among non-resonant and resonant tunnelling, and hopping processes occurring within the biomolecular bonds.[34] In particular, it has been suggested that the brightness of the STM features associated to the Az protein (i.e., its capability of sustaining an electron tunnelling current, that we will call "tunnelling conductivity," from here on) could be due to a two-step resonant tunnelling.[12–15] Such a process occurs when a metalloprotein is entangled between two metal electrodes (STM tip and surface, in our case) and, under the application of a suitable bias, it results having an electronic state energetically aligned with the Fermi level of an electrode. This molecular electronic state is then exploited by the tunnelling electrons, in order to reduce the tunnelling distance, also improving the tunnelling rate. Generally, the electronic states involved in such a process are the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO) that correspond to the oxidized and reduced state of the Az redox centre, respectively. Thus, the brightness of the STM image of an Az molecule adsorbed on an Au surface should be somewhat dependent on the accessibility of such electronic states, that should be properly aligned with the tip and surface Fermi levels.[12–15]

After having imaged single Az proteins, we have placed the STM tip on top of individual molecules, with subnanometer accuracy (consistently with the piezo drift value), and we have submitted them to 0.05 s long voltage pulses in the 1.0–3.5 V range (either positive or negative), after having disengaged the feedback.

Surprisingly enough, we found that voltage pulses of suitable intensity were able to reversibly switch off and on the Az spot brightness (Fig. 2), i.e., its tunnelling conductivity. Actually, the spot marked by the white arrow in Fig. 2(a) has completely disappeared in Fig. 2(b), after the application of a positive pulse of 3.0 V on top of it, according to the voltage *vs* time scheme shown in Fig. 2(d).

Further, the spot brightness is completely recovered when a negative pulse of the same intensity is applied at the same tip location, as it is clearly visible in Fig. 2(c). This off/on switching of the Az feature brightness in the STM images (i.e., of the protein tunnelling conductivity) was successfully repeated for several positive/negative voltage pulse cycles, upon different molecules, without registering any image displacement and for bias voltages in the 2.0–3.5 V range. No change in the protein tunnelling conductivity was, instead, observed when bright spots were first submitted to negative voltage pulses.

Such tunnelling conductivity off/on switching as a function of local voltage pulses, as applied by means of a nanosized tip, has never been observed before in redox biomolecules, and might deserve a strong interest for future development of biomaterial-based memories and bio-optoelectronic devices with biosensing properties. Indeed, the occurrence of a similar phenomenon has been registered in isolated atoms,[35] inorganic nanocrystals,[36,37] small organic molecules,[38,39] and peptides,[40] and it has been interpreted as being due to a modification of the tip-system-substrate energy level alignment, as induced by conformational changes or charging effects.

Charging effects have been invoked for those nanosystems characterized by a relatively high electrical capacity and asymmetric conduction characteristics; resulting in long-sustained modification of the conduction state on the time scale of STM measurements (tens of minutes). In particular, these are inorganic, semiconductor nanocrystals with asymmetric electrode contact resistances, also embedded in a polymeric matrix.[36,37]

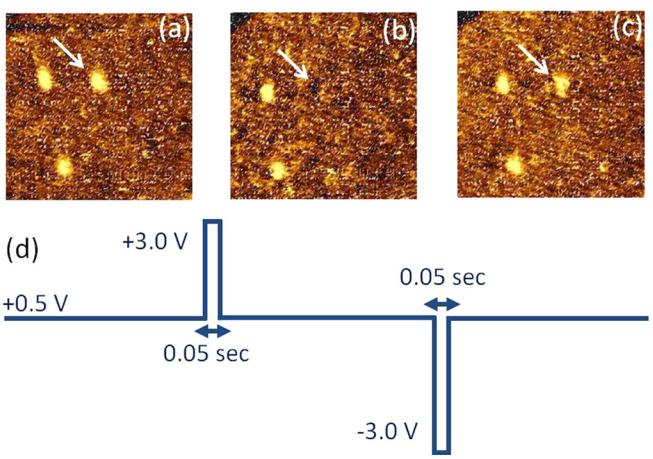

FIG. 2. STM images of an Az/Au sample (scan size = 50 × 50 nm; z scale = 300 pm; $V_{bias} = +0.5$ V; I = 0.06 nA; and scan rate = 4.1 Hz): (a) after approaching the surface; (b) after the application of a positive bias pulse (+3.0 V; 0.05 s); and (c) after the application of a negative bias pulse (−3.0 V; 0.05 s). The white arrows indicate the place where voltage pulses have been applied, according to the voltage *vs* time plot (not in scale) shown in panel (d).

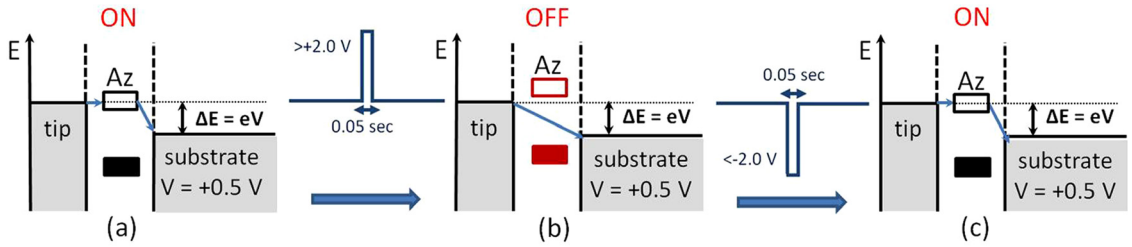

FIG. 3. Sketches of the energy level diagrams of the tip-Az-Au system at a moderate positive sample bias (a), after a positive bias pulse more intense than +2.0 V (b), and after a further negative bias pulse of the same intensity (c). Unoccupied (LUMO) and occupied (HOMO) electronic molecular levels are symbolized by empty and full rectangles, respectively, according to Ref. 14.

Due to its nanosized dimensions and dielectric nature, Az protein is known to be endowed with a relatively high electrical capacity, when entangled within hybrid nanostructures;[21,22] the corresponding capability of trapping charges having been exploited to build up biomemories.[11] Also, some asymmetry in the conduction characteristics of the Az/Au system has been previously put into evidence, as mainly due to the different strength of the electronic coupling that the protein establishes with the substrate (*via* the S-Au bonding) and the tip.[15–18] Moreover, the "off" conduction state of the protein, as induced by the voltage pulses, is stable upon several consecutive image scans.

According with this line of evidences, we could attribute the observed off/on switching of the Az/Au system tunnelling conductivity to a charging of protein milieu. In particular, we suggest that charges could be trapped at the Az-Au interface, with the Az-Au contact acting as a conductance "bottle-neck" (the effect is observed only when injecting electrons from the tip toward the surface, through the protein, by setting positive sample bias as shown in Fig. 1(a)).

The phenomenological model we are proposing to explain the overall process is resumed in Fig. 3. The energy level diagram of the tip-Az-Au system, under the application of a moderate positive bias to the sample for STM imaging, is shown in Fig. 3(a). The Fermi level of the Au surface is situated at a lower energy with respect to that of the tip, due to the applied voltage (+0.5 V in the sketched case). According to Marcus' theory, the molecular electronic states are represented with a broadening of 0.3 eV (Ref. 14) and with an energy gap between HOMO and LUMO that is twice the activation energy for charge exchange, also called reorganization energy λ.[18] By assuming that λ is similar in STM and in electrochemical ET,[12] its value can be estimated to be about 0.5 eV,[18] with a resulting HOMO-LUMO gap of about 1.0 eV. Moreover, the Az electronic states are pinned at the substrate Fermi level, as due to the Az-Au coupling.[18] Under these hypothesis, the LUMO is aligned with the tip Fermi level (see Fig. 3(a)), and an efficient two-step resonant tunnelling is allowed.[12–15]

When a short, positive voltage pulse (above the 2.0 V threshold) is applied to the Az/Au system, the induced electron flux may overcome the protein conduction capabilities and an amount of charges may be trapped within the protein milieu. This is likely due to: (i) the relatively high electrical capacitance displayed by Az when entangled in hybrid nanostructures;[21,22] (ii) the limited conductance of the hybrid system, mainly controlled by the S-Au bond which constitutes a sort of "bottle-neck" for the conduction at the Az-Au interface (the overall Az/Au conductance has been measured to be about $G \approx 10^{-5}\ G_0$, where $G_0 = 2e^2/h \approx 77.4\ \mu S$ is the quantum conductance).[15]

The potential created by trapped charges affects the energy level alignment of the tip-Az-Au system,[36,37] inducing, in particular, a shift of the protein energy levels towards higher energies, as shown in Fig. 3(b). If such a shift is intense enough (higher than 0.15 eV, in the sketched case), the Az LUMO moves out from the energy range suitable for allowing resonant tunnelling, as shown in Fig. 3(b), and only the less efficient direct non-resonant tunnelling between tip and substrate is then allowed. Within this picture, trapped charges and limited conduction would result in a long-sustained protein low tunnelling state.[36] Upon the application of a successive, reversed voltage pulse, the trapped charges are removed and, in turn, the hybrid system energy levels are shifted back with a concomitant restoring of the more efficient resonant tunnelling (Fig. 3(c)).

In summary, we have observed that the capability of single Az molecules adsorbed on an Au surface to sustain an electron tunnelling current (i.e., their tunnelling conductivity) can be controlled through voltage pulses applied by means of an STM tip. In particular, electron trapping within the protein can be induced or removed, and this may tune the energy level alignment of the hybrid system; likely allowing, over a charging threshold, a reversible switching of the tunnelling regime from an Az-mediated resonant tunnelling to a direct, non-resonant tip-to-surface tunnelling. Such a technique to control off/on switching of the transport capabilities of a nanosized biological object assembled on an Au electrode could be particularly interesting in view of future application, also in the field of biomemories and biooptoelectronic devices.

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[1]A. R. Bizzarri and S. Cannistraro, in *Encyclopedia of Condensed Matter Physics*, edited by G. Bassani, G. Liedl, and P. Wyder (Elsevier, 2005), pp. 361–369.
[2]S. Shleev, J. Tkac, A. Christenson, T. Ruzgas, A. I. Yaropolov, J. W. Whittaker, and L. Gorton, Biosens. Bioelectron. **20**, 2517 (2005).
[3]B. Willner, E. Katz, and I. Willner, Curr. Opin. Biotechnol. **17**, 589 (2006).
[4]I. Ron, L. Sepunaru, S. Itzhakov, T. Belenkova, N. Friedman, I. Pecht, M. Sheves, and D. Cahen, J. Am. Chem. Soc. **132**, 4131 (2010).
[5]J. M. Artés, M. López-Martínez, I. Díez-Pérez, F. Sanz, and P. Gorostiza, Electrochim. Acta **140**, 83 (2014).

[6]I. Delfino, B. Bonanni, L. Andolfi, C. Baldacchini, A. R. Bizzarri, and S. Cannistraro, J. Phys.: Condens. Matter **19**, 225009 (2007).
[7]C. Baldacchini, M. A. Herrero Chamorro, M. Prato, and S. Cannistraro, Adv. Funct. Mater. **21**, 153 (2011).
[8]J. J. Davis, B. Peters, and W. Xi, J. Phys.: Condens. Matter **20**, 374123 (2008).
[9]D. Li, P. M. Gannett, and D. Lederman, Nanotechnology **23**, 395705 (2012).
[10]C. Baldacchini, A. R. Bizzarri, and S. Cannistraro, Appl. Phys. Lett. **104**, 093702 (2014).
[11]J.-W. Choi, B.-K. Oh, and Y. J. Kim, Appl. Phys. Lett. **91**, 263902 (2007).
[12]Q. Chi, O. Farver, and J. Ulstrup, Proc. Natl. Am. Soc. **102**, 16203 (2005).
[13]E. P. Friis, J. E. T. Andersen, Yu. I. Kharkats, A. M. Kuznetsov, R. J. Nichols, J.-D. Zhang, and J. Ulstrup, Proc. Natl. Acad. Sci. U. S. A. **96**, 1379 (1999).
[14]P. Facci, D. Alliata, and S. Cannistraro, Ultramicroscopy **89**, 291 (2001).
[15]J. M. Artés, I. Díez-Pérez, and P. Gorostiza, Nano Lett. **12**, 2679 (2012).
[16]L. Andolfi, A. R. Bizzarri, and S. Cannistraro, Thin Solid Films **515**, 212 (2006).
[17]W. Li, L. Sepunaru, N. Amdursky, S. R. Cohen, I. Pecht, M. Sheves, and D. Cahen, ACS Nano **6**, 10816 (2012).
[18]J. M. Artés, M. Lopez-Martinez, A. Giraudet, I. Díez-Pérez, F. Sanz, and P. Gorostiza, J. Am. Chem. Soc. **134**, 20218 (2012).
[19]S. Raccosta, C. Baldacchini, A. R. Bizzarri, and S. Cannistraro, Appl. Phys. Lett. **102**, 203704 (2013).
[20]A. K. Yagati, T. Lee, J. Min, and J.-W. Choi, Bioelectrochemistry **83**, 8 (2012).
[21]Q. Chi, J. Zhang, J. E. T. Andersen, and J. Ulstrup, J. Phys. Chem. B **105**, 4669 (2001).
[22]P. R. Bueno, F. Fabregat-Santiago, and J. J. Davis, Anal. Chem. **85**, 411 (2013).
[23]O. Farver and I. Pecht, J. Am. Chem. Soc. **114**, 5764 (1992).
[24]S. Santini, A. R. Bizzarri, T. Yamada, C. W. Beattie, and S. Cannistraro, J. Mol. Recognit. **27**, 124 (2014).
[25]F. Domenici, A. R. Bizzarri, and S. Cannistraro, Int. J. Nanomed. **6**, 2033 (2011).
[26]E. I. Solomon, D. E. Heppner, E. M. Johnston, J. W. Ginsbach, J. Cirera, M. Qayyum, M. T. Kieber-Emmons, C. H. Kjaergaard, R. G. Hadt, and L. Tian, Chem. Rev. **114**, 3659 (2014).
[27]C. Arcangeli, A. R. Bizzarri, and S. Cannistraro, Biophys. Chem. **78**, 247 (1999).
[28]T. Cimei, A. R. Bizzarri, S. Cannistraro, G. Cerullo, and S. De Silvestri, Chem. Phys. Lett. **362**, 497 (2002).
[29]A. R. Bizzarri, L. Andolfi, M. Taranta, and S. Cannistraro, Biosens. Bioelectron. **24**, 204 (2008).
[30]J. J. Davis, N. Wang, A. Morgan, T. Zhang, and J. Zhao, Faraday Discuss. **131**, 167 (2006).
[31]H. Nar, A. Messerschmitd, R. Huber, M. van de Kamp, and G. W. Canters, J. Mol. Biol. **221**, 765 (1991).
[32]M. Amrein, H. Gross, and R. Guckenberger, "STM of proteins and membranes," in *STM and SFM in Biology*, edited by O. Marti and M. Amrein (Academic Press Inc., San Diego, USA, 1993), pp. 127–175.
[33]J. C. Genereux and J. K. Barton, Chem. Rev. **110**, 1642 (2010).
[34]S. Lindsay, J. Chem. Educ. **82**, 727 (2005).
[35]J. Repp, G. Meyer, F. E. Olsson, and M. Persson, Science **305**, 493 (2004).
[36]E. Nahum, Y. Ebenstein, A. Aharoni, T. Mokari, U. Banin, N. Shimoni, and O. Millo, Nano Lett. **4**, 103 (2004).
[37]A. Gambardella, M. Prezioso, and M. Cavallini, Sci. Rep. **4**, 4196 (2014).
[38]Z. J. Donhauser, B. A. Mantooth, K. F. Kelly, L. A. Bumm, J. D. Monnell, J. J. Stapleton, D. W. Price, Jr., A. M. Rawlett, D. L. Allara, J. M. Tour, and P. S. Weiss, Science **292**, 2303 (2001).
[39]M. Alemani, M. V. Peters, S. Hecht, K.-H. Rieder, F. Moresco, and L. Grill, J. Am. Chem. Soc. **128**, 14446 (2006).
[40]K. Kitagawa, T. Morita, and S. Kimura, Angew. Chem. **117**, 6488 (2005).