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Metalloprotein based scalable field effect transistor with enhanced switching behaviour

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ABSTRACT

Molecular devices based on organic and bio-molecules have been sought as an alternative to silicon-based devices to overcome their limitations for the development of biosensors and bioelectric devices. Metalloproteins are an integral part of several biochemical reactions and are shown to work as transistors and memory devices. In this work, we have reconstituted variants of recombinant azurin (redox active copper protein from *Pseudomonas aeruginosa*) by modifying the metal ion. Azurin variants were prudently immobilized on three different devices which acted as a channel (200 nm) between the source and the drain electrodes and the devices realized in highly scalable and reproducible manner to act as a field effect transistor (FET). Electrical measurements illustrated p-type FET behavior for all three variants. The devices possessed low subthreshold swing and the high on-off current ratio of the order of 10^5 for Cu-azurin, 3.4×10^2 for Fe-azurin and 2.3×10^2 for Ni-azurin. The FET device showed consistent behavior in the highly stable mode as measured for a span of 8 weeks. These FETs having high throughput with long-term stability and their robustness is likely to escalate the tremendous stride in the development of hybrid molecular devices.

1. Introduction

Over the last few decades, we have progressed from silicon transistors to micro-chips, which has been used to develop micro-processors and other nano-devices fuelling the utmost requirement of future electronics. But several limitations, such as, problems in doping and tunnelling after a certain level of miniaturization have been shown by silicon-based devices. Since Aviram and Ratner have shown that molecules can behave as semiconductors [1], a lot of work has been conducted showing biomolecules working as transistors [2], diodes [3], capacitors [4] which can be realized into biosensors [5,6], memory devices [7,8], and logic gates [9,10]. Bio-nanodevices possess several advantages over silicon-based devices like integration into much smaller areas, multifunctioning and faster response time [11]. Nowadays, these devices have attracted considerable interest as they are operationally simple, cheap and compatible for real-time detection. Though these devices can replace silicon-based devices to overcome their limitations, there are issues related to stability and robustness of the attached protein on the device.

Generally, metalloproteins have been employed in bio-nanodevices as they have unique characteristics such as electron-transfer capability, the possibility of gating redox activity and nanometric size, which make them appealing for bioelectronics applications at the nanoscale [12,13]. Since the activity of redox metalloproteins can be tuned by an external potential, hence, they are good candidates for creating hybrid sub-micrometer-sized electronic components [14,15].

Pseudomonas aeruginosa azurin (shown in Fig. 1(a)) is one of the simplest and smallest (14 kDa) copper containing oxidoreductase which is required for respiratory phosphorylation of the bacteria [16]. Azurin is also known as blue protein due to the presence of copper ion at the redox active centre of the protein [17]. Copper ion present in the active site provides the blue colour (λ_{max} = 620 nm), and excellent stability to this protein (T_m = 86 °C) [18]. In order to use protein as an element of the electronic device, it is highly desirable to preserve the stability and functionality of protein using appropriate film fabrication technology [19]. Since most of the proteins from mesophilic organisms get denatured at just 40 °C [20], high stability and small size of azurin make this protein a favorable

Abbreviations: AFM, atomic force microscopy; FET, field effect transistor; SS, subthreshold swing; TC, transfer characteristics; T_m, melting temperature.

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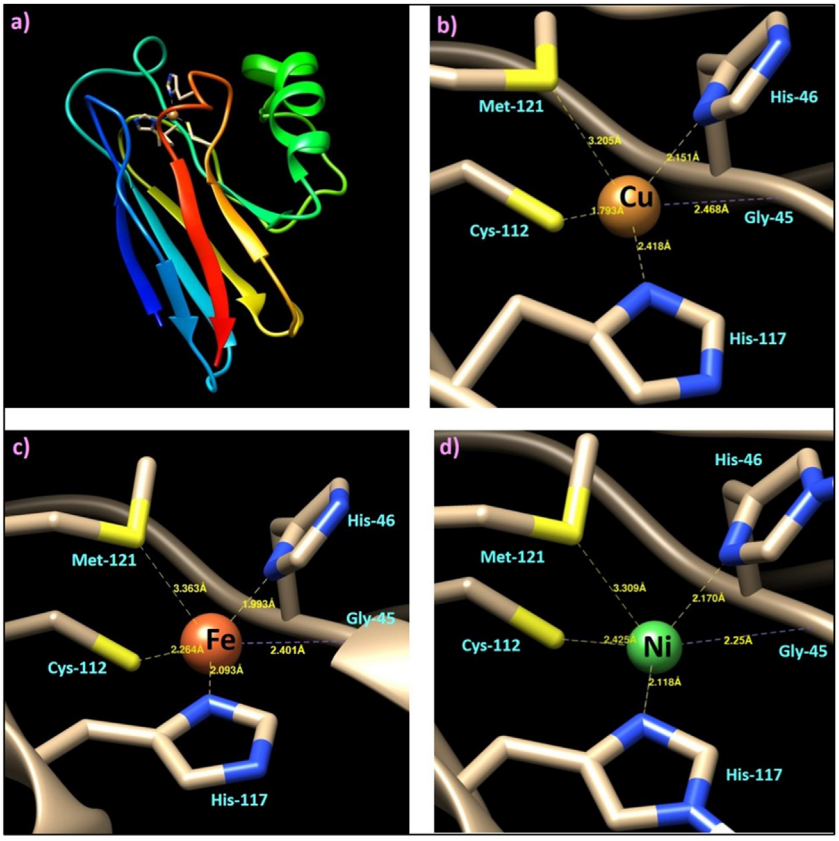

e)	Cu	Fe	Ni
Gly-45	2.47Å (Trigonal bipyramid)	2.40Å (Trigonal bipyramid)	2.30Å (Trigonal bipyramid)
His-46	2.15Å (Tetrahedral)	1.99Å (NA)	2.00Å (NA)
Cys-112	1.79Å (NA)	2.26Å (Trigonal bipyramid)	2.55Å (Trigonal bipyramid)
His-117	2.42Å (Trigonal bipyramid)	2.09Å (Tetrahedral)	2.17Å (Trigonal bipyramid)
Met-121	3.21Å (Trigonal bipyramid)	3.36Å (Trigonal bipyramid)	3.32Å (Trigonal bipyramid)

Fig. 1. (a) Crystal structure (generated by UCSF Chimera, https://www.cgl.ucsf.edu/chimera) of native *Pseudomonas aeruginosa* azurin. Structural depiction of the metal site in (b) native azurin (PDB ID: 1AZU) and its (c) Fe derivative (PDB ID: 4HZ1) and (d) Ni derivative (PDB ID: 1NZR). e) Table showing the distance of amino acid residues with metal ions and their best fitted geometry.

molecule for the development of bio-nanodevices. Its robust and ordered self-assembly on the gold surface (via thiol linkage) has resulted in a number of hybrid molecular nanodevices [21,22].

Azurin has been shown to behave like a transistor and its current transfer properties have been studied [23]. It has been demonstrated that secondary coordination sphere interaction, hydrophobicity, and hydrogen bonding can tune the redox potential of azurin [24]. Thus, it may be plausible to modulate the charge transfer properties of proteins and thus accomplish a metalloprotein field-effect transistor (FET) by manipulating their secondary structure or by altering the redox site. In this work, we have purified recombinant azurin protein using histidine tag and have altered the redox centre of protein by incorporating Fe and Ni ions to the apo-azurin (without metal ion) for studying its electron transfer behavior. The metal ions were bound to Gly-45, His-46, Cys-112, His-117 and Met-121 at redox active site of metalloprotein azurin as shown in Fig. 1.

2. Experimental

2.1. Azurin preparation

Azurin gene (450 bp) from *Pseudomonas aeruginosa* was cloned in fusion with 6x histidine tag and expressed in *Escherichia coli*. Recombinant apo-azurin (14 kDa) was purified in large quantity by Ni-NTA affinity chromatography. The metal ion of redox cen-