Azurin protein for the development of bionanodevices

Abstract

*Pseudomonas aeruginosa* azurin, a small copper containing redox metalloprotein was cloned in pET28a vector in fusion with an affinity tag (6 X histidine) for single step purification using Ni-NTA (Nickel-nitrilotriacetic acid). The protein was overexpressed and purified and the yield was 50 times higher for recombinant azurin than reported values for wild type azurin from *P. aeruginosa*. Recombinant azurin was characterized by UV-visible spectroscopy and the characteristic peak at 620 nm was observed with purity ratio (Abs\textsubscript{620}/Abs\textsubscript{280}) of 0.174. Azurin was also characterized by fluorescence, and CD spectroscopy. The electrochemical behavior of protein was studied by cyclic voltammetry and conductive atomic force microscopy. In cyclic voltammetry azurin protein showed oxidation and reduction peaks at 280 mV and 178 mV, respectively. Azurin variants (Cu-azurin, Fe-azurin and Ni-azurin) were reconstituted by modifying the metal ion center of the protein. They were characterized by UV-visible absorbance and fluorescence microscopy. All the three azurin variants showed good redox active characteristics observed by the presence of oxidation and reduction peaks. Azurin variants were prudently immobilized on three different devices which acted as a channel (200 nm) between the source and 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 possess low subthreshold swing (200 mV/decade) and high on-off current ratio ($10^5$). The FET devices showed consistent behavior in highly stable mode as measured for a span of 8 weeks. These FETs having high throughput with long term stability and exhibited commendable robustness. The Al/Azurin/ITO/PET structure based on recombinant azurin was used for the flexible memory applications. Highly stable LRS (low resistive state) and HRS (high resistive state) were demonstrated for 500 cycles. The flexible device has also exhibited stable current and both switching states upon bending test for multiple cycles. Azurin metal ion variants based memory devices were also studied. Stable HRS and LRS were observed in all three devices for 10 cycles. The Cu-azurin based device showed best memory behavior with larger memory window. Also, all three devices exhibited good repeatability and endurance. Recombinant azurin has shown good conducting behavior and may be used for the development of electronic biosensors. These devices also fill the technological gap to integrate protein based memory with other biocompatible and bio-integrated electronic device, which is extremely desirable.