Recombinant azurin-CdSe/ZnS hybrid structures for nanoscale resistive random access memory device

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\textbf{ABSTRACT}

In the present study, we developed a biohybrid material composed of recombinant azurin and CdSe-ZnS quantum dot to perform as a resistive random access memory (ReRAM) device. Site specific amino acid sequences were introduced in azurin to bind with the surface of CdSe-ZnS nanoparticle allowing the formation of a hybrid and voltage-driven switching enabled to develop a resistive random access memory (ReRAM) device. The analytical measurements confirmed that the azurin and CdSe-ZnS nanoparticles were well conjugated and formed into a single hybrid. Further, reversible, bistable switching along with repeatable writing-reading-erasing processes on individual azurin/CdSe-ZnS hybrid at nanoscale was achieved on the hybrid device. The device was programmed tested for 50 cycles with an ON/OFF ratio and measured to be of three orders of magnitude. The developed device shown good stability and repeatability and operates at low voltages thus makes it promising candidate for future memory device applications.

1. Introduction

While the semiconductor industry reaches to its fundamental and technological challenges, new solutions and technologies have been sought and evaluated (Wondrak, 1999). By employing innovative architectural methods, enhancement in device functionality and density with increased switching speed can be achieved (You, 2014). Thus, with bionanoelectronics it is expected to achieve superior performance and with recent research on biomolecular processing (Lee et al., 2014) with memory operations (Yagati et al., 2013) assures the technological feasibility of this research filed. However, the main obstacle for practical realization of bionanoelectronics is the interface that integrates the biological components to the microelectronic circuitry. In order to achieve efficient and versatile biohybrid devices there should be an excellent coupling between biological and inorganic components without any denaturation and should avoid complex fabrication methods. Conventionally, the conjugation between biomolecules and inorganic molecules with chemical linkers or electrostatic interactions often produce aggregates with poor control over the number of biomolecules attached to a single nanoparticle (Dirks et al., 2009; Katz and Willner, 2004).

Semiconductor nanoparticles such as quantum dots (QDs) whose excitons are confined in all three spatial dimensions (Reed, 1993) and their electronic characteristics are closely related to the size and shape of individual QDs. Quantum confinement plays an important in the electronic properties of QDs and possess discrete energy levels (Fischbein and Drndic, 2005). CdSe QDs are often capped with organic ligands which act as tunnel barriers for charge transport between adjacent particles. Core-shell type particles passivated with inorganic shell structures are more robust in comparison with organically passivated dots and also has greater feasibility towards processing and incorporation into various structures (Wilson et al., 1993). CdSe-ZnS nanoparticles are used as memory elements by observing charge trapping/detraping events and Coulomb blockade (CB) at room temperature. It is reported that, if an electron tunneling through a very small capacitance junction encounters a charge of $-e/2 < Q < e/2$, then electrons will be blocked from tunneling until that charge has been shunted. Experiments on QDs show a zero-conductance region equal to $E_g + 2E_H$, where $E_g$ is the band gap of the material and $E_H$ is the coulomb potential of a charge on the QD, also referred to as the charging energy (Hummon et al., 2010). Therefore, QDs are used memory elements by controlling the charge trap and release events.

Azurin a well characterized redox protein from \textit{Pseudomonas aeruginosa}, with excellent electron transfer and optical features which...
make it an especially valuable candidate for bioelectronics applications (Yagati et al., 2009). Mostly, biohybrid materials composed of proteins, QDs have gained much attention because of their various applications in biosensors, drug delivery, and in development of memory devices (Portney et al., 2008; Tseng et al., 2006). Recently, resistive switching random access memory (ReRAM) devices based on proteins (Wang et al., 2015b), natural organic material (Hosseini and Lee, 2015), Pt nanoparticles combined with ferritin (Uenuma et al., 2011), natural silk fibron (Wang et al., 2015a, 2016) coupled with Au nanoparticles (Gogurla et al., 2013), memristive devices formed with egg albumen film (Chen et al., 2015) were developed. However, coupling recombinant azurin with CdSe-ZnS QDs has not yet been reported.

Thus, we demonstrate a strategy for the formation of biohybrid molecule by incorporating genetically modified specific peptide sequences in wild azurin to achieve recombinant azurin which has the ability to couple Au surface and also enables direct coupling to CdSe-ZnS on outer shell to achieve a bio-inorganic hybrid structure for the development of a ReRAM device (Fig. 1). Analytical methods were performed to examine biohybrid formation and to assess the electrical properties of hybrid molecules I-V measurements were conducted and applied for the development of a nanoscale ReRAM device.

2. Experimental details

2.1. Materials and reagents

To fabricate the resistive memory device, Au (111) coated Si (100) wafers were purchased from Inostek (Korea). CdSe-ZnS nanoparticles and Lipoprotein were purchased from Sigma-Aldrich (USA). All oligonucleotides were supplied by Bioneer (Korea). Ethanol, H₂SO₄, and 30% H₂O₂ were purchased from Daejung Chemicals and Metals Co Ltd (Korea). The water used for all experiments was distilled and deionized by Millipore [(Milli-Q) water (DDW > 18 MΩ)].
2.2. Construction of recombinant azurin gene

To construct recombinant azurin gene with two cysteine groups and NNPMHQN peptide sequences, the genomic DNA from *Pseudomonas aeruginosa* ATCC (American Type Culture Collection) 15692 was extracted and purified using QIAamp DNA mini kit (Qiagen, USA). The *azu* gene was amplified by PCR from genomic DNA of *Pseudomonas aeruginosa* ATCC 15692. The forward primer and reverse primer were designed to include the Ndel restriction enzyme site, the EcoRI restriction enzyme site, respectively (Fig. S1). The PCR product was obtained and purified, using QIAquick gel extraction kit (Qiagen, USA), and digested with Ndel and EcoRI restriction enzymes (New England Biolabs, USA) (Fig. S2 (a)). The digested DNA fragment was ligated with a pET-21a (+) vector (Novagen, USA), which was predigested with Ndel and EcoRI, using Quick ligation kit (New England Biolabs, USA) (Fig. S2 (b)). The recombinant azurin gene encoding two cysteine-modified azurin (mazu1 gene) was amplified by Qiagen Change lightening multi-site-directed mutagenesis kit (Agilent Technologies, USA), from the pET-21a (+) vector::recombinant azurin gene. The correct incorporations of two Cys sequence and NNPMHQN peptide sequence were used to insert NNPMHQN peptide sequence (Fig. S1). The PCR product A, lane 3: product B). The two DNA fragments (product A, B) were confirmed by DNA sequence analysis, respectively (Fig. S2(c), S3).

2.3. Expression and purification of recombinant azurin

The recombinant azurin (MW 14.6 kDa) with modified genes was expressed from *E. coli* BL21 DE3 by recombinant DNA technology. The plasmids, containing genes for recombinant azurin variant, were transformed into *E. coli* BL21 (DE3). The transformants were grown to an optical density (OD) of 0.600 at 35 °C in shake flasks containing 1 L of Luria-Bertani medium (0.5% yeast extract, 1.0% tryptophan, and 1.0% NaCl) with 50 mg/mL ampicillin. Expression was induced by adding isopropyl β-D-thiogalactopyranoside (IPTG) to a concentration of 0.840 mM. The transformed cells were grown for an additional 4.0 min, rinsed thoroughly with distilled water, and then dried in an N2 stream. The recombinant azurin solution was concentrated by centrifugation at 4000 rpm, 4.0 to 5.0 (50 mM sodium acetate). CuSO4 (0.5 M×20 mM) was added to the azurin solution and taken up by the recombinant apo-azurin for 4.0 to 5.5 (50 mM sodium acetate), yielding azurin-containing supernatant. Then, the recombinant apo-azurin fractions which did not contain copper were eluted (elution pH 4.6–4.8, respectively) on a CM cellulose ion exchange column with a pH gradient ranging from 4.0 to 5.0 (50 mM sodium acetate). CuSO4 (0.5 M×20 μL) was added to the azurin solution and taken up by the recombinant apo-azurin for 12 h at 4 °C with mixing. The recombinant azurin was purified and concentrated by centrifugation at 4000g for 50 min at 4 °C with MWCO 3k Amicon ultra-centrifugal filter (Millipore, USA).

2.4. Device fabrication

Au (111) substrates were kept in piranha solution (1:3 v/v, H2O2, and conc. H2SO4 for 4.0 min, rinsed thoroughly with distilled water, and then dried in an N2 stream. The recombinant azurin solution (10 μm) was then dropped onto the Au substrate for 6 h at 4 °C. The thiol groups of two cysteine residues will enable anchoring of the Au surface by covalent bonding. After washing with DI water, 20 μL of CdSe-ZnS solution (10 μm) was immobilized on azurin directly in which ZnS outer shell can be bound with NNPMHQN sequences of azurin.

2.5. Electrical and optical characterizations

STM measurements were performed with Digital Instruments Multimode STM with Nanoscope IV controller (Veeco Metrology, CA, USA). Measurements were conducted by using mechanically cut Pt/Ir tips. For I-V measurements, the STM tip was located on desired position for bare Au, CdSe-ZnS, lipoprotein, and over a azurin/CdSe-ZnS nanoparticle hybrid. No additional force was applied to the tip and the same set point of 0.5 nA with a bias voltage of 0.1 V was used for all experiments.

The UV–Vis optical absorbance of azurin, CdSe-ZnS, and azurin/CdSe-ZnS were measured by using an HP-8453 photodiode array (PDA) spectrophotometer (Agilent technologies) equipped with a pellett temperature controller.

3. Results and discussion

3.1. Production of recombinant azurin

The formation of a bio-inorganic hybrid composed of azurin coupled with CdSe-ZnS was achieved by selecting a protein binding motif with specific recognition for the ZnS outer shell (Lee et al., 2002; Sarikaya et al., 2003). The Primers were used to substitute a Cys residue at M13, K92 of azurin 128 residues using multiple site-directed mutagenesis (MSDM) and introduce Asn-Asn-Pro-Met-His-Glu-Asn (NNPMHQN) peptide sequence between L102 and K103 of azurin 128 residues using splicing by overlap extension (SOE).

The mazu1 gene (M13C, K92C) encoding two cysteine-modified azurin was performed from *E. coli* strain DH5α, which was used as the host for sub cloning by site-directed mutagenesis (SDM) in a previous study (Lee et al., 2010). The thiol groups of two cysteine residues will enable anchoring of the Au surface by covalent bonding. General DNA recombinant techniques were employed throughout this experiment (Chung et al., 2013). The mazu1 gene was amplified by MSDM, from the pET-21a (+) vector::azu gene. The NNPMHQN peptide sequence was inserted into mazu1 gene, which is confirmed to have a binding affinity to the ZnS surface. To introduce the NNPMHQN sequences to the pET-21a (+) vector::mazu1 gene, we used SOE using PCR (Reikofski and Tao, 1992), SOE by PCR, as used in the present research, involves three separate polymerase chain reactions (PCRs) (Fig. 2(a)): The QD 1F, QD 1R, QD 2F, and QD 2R primers were required for each construct of the two steps PCR reactions (Fig. S1). The QD 1F and QD 2R primers were designed to contain a Ndel restriction enzyme site, respectively and the QD 1R and QD 2F primers were designed to generate fragments of overlapping sequences. One of the first step PCRs produces a DNA fragment with Ndel restriction enzyme site (product A), and the other a DNA fragment with BamHI restriction enzyme site (product B). Fig. 2(b) shows first step products (product A, B) of SOE by PCR on agarose gel electrophoresis (lane 1: size marker, lane 2: product A, lane 3: product B). The two DNA fragments (product A, B) produced in the first step PCR reactions were mixed to form the template for the second step PCR. The final product (SOE product) was amplified by second step PCR. Fig. 2(c) shows second step products (SOE product) of SOE by PCR on agarose gel electrophoresis (lane 1: DNA size marker, lane 2: SOE product (annealing temperature: 53 °C), lane 3: SOE product (annealing temperature: 55 °C).

For the production of recombinant azurin, the mazu2 gene fragments were confirmed by gel electrophoresis on a 1.0% agarose gel (Fig. 2(d)). Lane 1 shows the DNA size marker. Lane 2 shows the pET-21a (+) vector (top band) and mazu2 gene (bottom band). Lane 3 shows the Ligates of pET-21a (+): mazu2 gene. The mazu2 gene was verified by DNA sequencing (Fig. S3). From the result, the NNPMHQN sequences were found in the mazu2 gene along with M13C and K92C. The prepared mazu2 gene was cloned from of *Pseudomonas aeruginosa* genomic DNA. This gene was inserted into the pET-21a (+) vector.
with NdeI, BamHI sites. The pET-21a (+) vector::mazu2 gene (psukim 003) was transformed into E. coli BL21 (DE3). The transformants were then expressed and purified. The purified recombinant azurin was confirmed using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fig. 2(e) depicts the SDS-PAGE result. Lane 1 shows the size marker and lane 2 shows the wild type azurin. In this case, the molecular weight is around 15.0 kDa. Lane 3 shows the band of recombinant azurin which shows slightly larger (around 16.0 kDa) than wild type azurin owing to the additional peptide insertion.

The formation of recombinant azurin with three SDM was analyzed using UV–Vis spectroscopy. It is observed that the absorption peak is in the visible region of the spectrum as shown in Fig. 2(f), the Cu (II) form of azurin is dominated by ligand to copper transitions, especially by those involving the cysteine ligand. The intense peak in the 625 nm peaks were due to the ligand-to-metal-charge transfer transition, corresponding to the $S$(Cys)$_x \rightarrow Cu(II)|p_z^3|_y$ transition which has a band gap energy of 1.98 eV. Thus, the recombinant azurin has similar characteristics compared to wild-type azurin. This indicates that recombinant azurin with three site directed mutations was successfully achieved and enabled for further experiments.

### 3.2. Formation of azurin/CdSe-ZnS hybrid molecules

Energy filtered transmission electron microscopy (EF-TEM) analysis on individual CdSe-ZnS nanoparticles and azurin/CdSe-ZnS conjugates were performed to analyse the hybrid molecules. EF-TEM images of the core-shell nanoparticles were presented which shows isolated particles over entire surface, having lattice spacing of the CdSe core of 0.22 nm. The size of the core-shell nanoparticles as estimated from the images found to be around ~5 nm Fig. 3(a). The azurin/CdSe-ZnS conjugated surface depicts that many CdSe-ZnS particles were attached to the underlying protein molecules thus confirming the formation of biohybrid material. These hybrid molecules were found over entire surface Fig. 3(b). Moreover, Energy dispersive analysis (EDA) on individual CdSe-ZnS nanoparticles and azurin/CdSe-ZnS conjugates were performed to analyse the hybrid molecules. Elemental analysis was performed to the CdSe-ZnS nanoparticles and azurin/CdSe-ZnS conjugates in Fig. 3(c) and (d). EDA spectrum for azurin/CdSe-ZnS conjugates shows the presence of S, Zn, Se, Cd, and Cu elements (Fig. 3(d)). The AFM, UV–Vis and XPS experiments were conducted to confirm the azurin/CdSe-ZnS hybrid in Supplementary materials (Fig. S4, S5).

### 3.3. Electrical characteristics of azurin/CdSe-ZnS hybrid system

The current-voltage ($I-V$) curves were measured for azurin/CdSe-ZnS and also on individual elements such as bare Au, CdSe-ZnS, and lipoprotein were measured at 295 K by positioning the STM conductive tip on the molecules without tunneling current feedback. The tunneling current was recorded while the tip-bias voltage was swept from ±3 V. Fig. 4 shows electrical $I-V$ properties of Au, CdSe-ZnS/Au, lipoprotein/Au, and azurin/CdSe-ZnS/Au electrodes respectively. In the case of bare Au, a linear $I-V$ characteristic (Ohmic behavior) was observed (Fig. 4a), obviously the bare Au substrate showed the general metallic
conductor (linear) property. Further, CdSe-ZnS/Au (Fig. 4b) exhibits the nonlinear I-V properties, which is due to semiconductor nature that has enough energy band gap resulting in a rectifying property in a two-terminal system. This forms a double-tunnel junction configuration having small resistive switching behavior; however, no hysteresis effect was found in these measurements. Also, lipoprotein/Au (Fig. 4c) adsorbed substrate which doesn’t have any metal center in the protein, showed a minute nonlinearity in I-V curves. It is expected that, lipoproteins provided the energy band gap that makes such nonlinearity. Also, this system doesn’t exhibit any peculiar behavior and makes it difficult to use in any resistive memory applications. Also, several reports on azurin/Au showed symmetrical and sigmoidal I-V characteristics without any hysteretic nature (Yagati et al., 2012). However, azurin/CdSe-ZnS/Au hybrid showed a distinct electrical bipolar switching behavior. This electrical bipolar behavior resulted from the conjugation resulted in an electronic phenomenon caused by the charge transfer between the two nanomaterials comprising hybrid.

As shown in Fig. 4d, the resistive switching with hysteretic behavior on the azurin/CdSe-ZnS hybrid was observed when trace and retrace linear voltage scans are applied from 0 to +3.0 V and –3.0 to 0 V, respectively. Initially, the I-V curve of the hybrid shows a low conductivity state, and this state was defined as “OFF”. When the potential reaches 2.0 V, an abrupt increase in conductivity occurs; this is defined as the “ON” state. This state indicates a transition of the hybrid molecule from an initial OFF state to an ON state that is equivalent to the “write” process in a digital ReRAM device. Thus, the switch-on voltage (V_{ON}) for azurin/CdSe-ZnS hybrid is 2.0 V. When a negative voltage is applied continuously, the hybrid molecule remains in the ON state until 0.3 V. Then, it returns to the low conductivity state (OFF state). This reverse voltage state is equivalent to the “erase” process in the ReRAM device.

3.4. Assessment of ReRAM function of azurin/CdSe-ZnS hybrid system

Several I-V curves were obtained on hybrids located at different positions to confirm the resistive memory property of system. Fig. 5a shows the I-V characteristics of azurin/CdSe-ZnS when a bias voltage was applied from ±3.0 V, respectively. As shown in the figure, the device starts switching to a conductive state (ON state) when the applied voltage is 2 V, with a sharp increase in the current, and remains in that state until the reverse voltage 0.9 V (OFF state) was applied. The device shows electrical hysteresis behavior which is an essential feature of bistable memory devices. State “1” and state “0” correspond to the relatively high current (ON state) and the relatively low current states (OFF state), respectively. The bistable transition from ON and OFF state is equivalent to the “write” and “erase” process in a digital memory device. This change in transition from low to high conductivity is because of the charge donor azurin transferring electrons due to the Cu (I) and Cu (II) densities of states through tunneling to the lower energy core of the CdSe-ZnS core-shell nanoparticle. It is assumed that free electrons will tunnel through the conjugate by forming a double tunnel junction, which has a distribution of many energy levels sandwiched between the two metal electrodes. Hysteresis was observed in reverse scanning from high to low bias, suggesting small amount of charge is stored in the CdSe-ZnS core. This is due to the charge transfer from azurin to CdSe-ZnS particles under the applied electric field, consequently change in the conductivity of the hybrid system. When charge site in azurin is established, a sudden jump of current accompanying the charge tunneling through the nanoparticles is observed. Hence, an electric field induced charge transfer mechanism takes place between the azurin and CdSe-ZnS core resulting the observed memory phenomena.

To understand the carrier transport mechanism in ReRAM device for the two conducting states, the experimental I-V data was fitting with theoretical model, Fig. 5. In the OFF state, the plot of \(\ln(J) vs V^{1/2}\)
in the sweeping voltage ranging from 0 to 2 V before the electrical transition can be fitted to a straight line with Eq. (1) (Lee et al., 2012), Fig. 5b.

$$J = AT^2 \exp \left[ -q \left( \frac{\Phi_T - \sqrt{qE/kT\varepsilon}}{kT} \right) \right]$$ (1)

where $J$ and $V$ are the current density and voltage, respectively; $A$, $T$, $k$, $\Phi$, $q$, $\pi$, and $\varepsilon_r$ and $\varepsilon_0$ are the Richardson constant, temperature, Boltzmann constant, Schottky energy barrier, electron charge, ratio of circumference to diameter, and $\varepsilon_r$ is the permittivity of free space, respectively. The observed linearity characteristic suggests that the carrier transport mechanism in the OFF state is dominated by the thermionic emission, and the conduction mechanism is dominated by the charge injection from electrodes.

For ON state conductive switching, the experimental data of the azurin/CdSe-ZnS hybrid device, shown in the plot of $\ln(J/V)$ vs $V^{1/2}$ in Fig. 5c. Data is well fitted with a linear dependence although there are some small deviations at low voltages. This result indicates that bulk-limited Poole-Frenkel emission is the dominant mechanism for the electrical transport of carriers through this interface. The expression of $I_{p,f}$ as a function of $V$ corresponds to (Schulman et al., 2015)

$$I = q\mu N_C E \exp \left[ -q \left( \phi_T - \sqrt{qE(kT\varepsilon_r)} \right) \right]$$ (2)

where $\mu$ is the electronic drift mobility, $N_C$ is the density of states in the conduction band, $q\phi_T$ ($=\Phi_T$) is the trap energy level, and the other notations are the same as defined before. This process indicated that hybrid nanocomposite act as the charge traps to realize the electrical switching and memory effect.

The mechanism could be explained as charge can be efficiently trapped in the molecule; i.e., a resonant state (redox state) of molecule as a conducting channel, in which the highest occupied molecular orbital (HOMO) or lowest unoccupied molecular orbital (LUMO), will trap charges injected from close Fermi levels of the contacts. Azurin active site contains a copper (Cu) ion which is able to switch between two stable states (Cu$^{1+}$/Cu$^{2+}$) and the arrangement of active site with ligands gives ligand-to-metal charge transfer (LMCT) for the two redox states (i.e., the ligand-centered HOMO and metal centered LUMO).

The molecular orbital energies of azurin can be estimated in the solution state through cyclic voltammetry (CV). CV for azurin depicted redox peaks at 0.2 and 0.35 V vs Ag/AgCl (Yagati et al., 2013), which is equivalent to 0.15 and 0.30 V vs saturated calomel electrode (SCE) respectively. Also, from the STS curves the $V_{on}$ for azurin/CdSe-ZnS was approximated as 2.0 V. (Hipps, 2006; Scudiero et al., 2002) disclosed a relation to convert the electrochemical potential referenced to SCE to a vacuum level as

$$V_{abs} (eV) = qE^{°}(SCE) + 4.7 eV$$

where $E^{°}$ is the redox potential and 4.7 eV is approximated according to vacuum level, which is suitable expression for analyzing reduction process. However, for oxidation process the equation offered by (Schmidt et al., 1994; Scudiero et al., 2002) was utilized, and the ionization energies

$$V_{i}=4.7 eV+(2.0)E^{ox}(SCE)_{1/2}$$

in which $E^{ox}(SCE)_{1/2}$ is the half-wave oxidation potential. Thus, the redox potentials of azurin can be converted to solid-state potentials in STM using these two equations, and the energy levels ligand centered oxidation and metal centered reduction are 5.3 and 4.85 respectively ($V_{i}=4.7 eV+(1.0)×0.3=5.3$ eV and $V_a=4.7 eV+0.15=4.85$ eV). The actual solid-state ionization and
electron affinity energies of HOMO and LUMO in azurin will deviate slightly from those in vacuum. Further, the HOMO/LUMO of CdSe-ZnS between electrodes and the work function of the electrodes were adopted from the literature. The existence of the electrical bistability might be attributed due to the existence of the internal electric field generated by trapped charge carriers in CdSe/ZnS core-shell QDs. The OFF state can be recovered by the application of ~1.0 V. The proposed model can be compared with double barrier tunnel junction in which the electron tunnel from azurin to tip through CdSe-ZnS.

The hybrid conjugate acts as a carrier blocking material, resulting in the blockage of electrons due to the relatively large energy barrier between the work function of the Au electrode and the HOMO level of the hybrid layer. When Au electrode acts as –ve terminal, electron from Au will pass towards Pt electrode via azurin/CdSe-ZnS hybrid molecule. Azurin is well-known for good conductor so electron can reach to protein through thermionic injection, but some amount of energy required to overcome resistance of CdSe-ZnS semiconductor material hence after $V_{ON}$ (2.0 V) the electron from azurin can reach to LUMO of CdSe-ZnS and then transfer towards the Pt electrode (Fig. 6a). When a positive applied voltage is applied to the electrode, after the injection of electrons from the Pt/Ir tip into the LUMO level occurs through the Poole-Frenkel tunneling process, the electrons existing at the LUMO level are transported among the hybrid molecules along the direction of the applied bias voltage through the tunneling process (Fig. 6a).

To determine whether the azurin/CdSe-ZnS hybrid device possessed sufficient stability for use as a resistive random access memory (ReRAM) device, its current response for write and erase cycles were tested for each operational voltage (Fig. 6b). The write and erase operations were performed at a voltage of $\pm 2.0$ V and ‘erase’ pulse of 1.0 V (both with a time width of $10^{-4}$ s), were used to turn the device ON and OFF, respectively. The device was programmed for 50 cycles with an ON/OFF ratio of around $10^3$. However, with an increasing number of cycles, the device can be rationalized from the degradation of the single hybrid interface through joule heating during the operation of the device. Thus, in terms of the performance the statistics of $V_{ON}$ and $V_{OFF}$ for the hybrid device was 2.0 and 0.5 respectively with an ON/OFF ratio $10^3$ and possessed stable $V_{ON}$ and $V_{OFF}$ over 50 switching cycles. Besides using the biohybrid as a functional material, the resistive switching memory effect can be significantly tuned by controlling the composite system with different size of QDs and various types of metalloproteins which possess different redox potentials and also mixed protein systems for multibit and multilevel storage systems.

The actual performance of the device is better than the other protein based memory (Meng et al., 2011) which possess diminished hysteresis behavior with shorter retention times and virus/QD system (Portney et al., 2008) has larger $V_{ON}$ and $V_{OFF}$ switching voltages with shorter retention time. Nevertheless, there is also some challenging tasks such as maintaining good memory performance on soft and curvilinear surfaces and also fully integrating these hybrid devices in future memory devices with full functionality is also necessary.

4. Conclusion

In conclusion, we present a strategy for the development of a
biohybrid material involving quantum dot and azurin. The binding strategy to form a hybrid is as follows; (i) the direct assembly of recombinant azurin on an Au surface without any linker by the incorporation of cysteine residues with an aim to introduce well-ordered and better functionality of the biomaterial, (ii) the incorporation of a genetically engineered peptide sequence (NNPMHQN) which is composed of specific amino acids that selectively bind to the inorganic surface, thus forming a hybrid. Further, the $I$-$V$ measurements on hybrid structure exhibited electrical bistability for ReRAM functions. The ON/OFF ratio of the device was measured to be of three orders of magnitude after repeated cycles. Also, the stability and durability of the proposed system can be improved by incorporating different size nanoparticles. Furthermore, different types of recombinant proteins may influence the dipole interaction between the CdSe-ZnS and protein. Thus, the construction of ReRAM device composed of azurin/CdSe-ZnS/Au hybrid particles are promising for developing next-generation semiconductor devices.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2016.11.037.

References