

AZURIN PROTEIN FOR THE DEVELOPMENT OF BIONANODEVICES

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AZURIN PROTEIN FOR THE DEVELOPMENT OF BIONANODEVICES

by

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Dedicated to
My dad, Sh. Ganga Ram
and
My dear husband, Parvesh Kumar

*“The purpose of morality is to teach you, not to suffer and die,
but to enjoy yourself and live.”*

- Ayn Rand

CERTIFICATE

This is to certify that the thesis entitled “**Azurin Protein for the Development of Bionanodevices**” being submitted by **Ms. Neeti Kalyani** to the **Indian Institute of Technology Delhi** for the award of the degree of **Doctor of Philosophy** in Biochemical Engineering and Biotechnology, is a record of the authentic research work carried out by her under my supervision and guidance. She has fulfilled all the requirements for submission of this thesis, which to the best of my knowledge has reached the required standard. The results contained in this thesis have not been submitted in part or full to any other university or institute for the award of any other degree or diploma.

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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_{620}/Abs_{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 spectroscopy. 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 (Aluminium/Azurin/Indium tin oxide/Polyethylene terephthalate) structure based on

recombinant azurin was used for the flexible memory applications. Highly stable LRS (low resistance state) and HRS (high resistance 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.

सार

स्यूडोमोनास एरुजिनोसा ऐज़ूरीन, जो कि एक तांबा आयन युक्त छोटा सा अपचयोपचय धातु प्रोटीन है, को निकेल-एन. टी. ऐ. (नाइट्रिलोट्रायएसिटिक एसिड) द्वारा एकल चरण शुद्धिकरण के लिए एक एफ़िनिटी टैग (6xहिस्टिडाइन) के साथ pET28a वेक्टर में क्लोन किया गया। ऐज़ूरीन को ओवरएक्सप्रेस और शुद्ध किया गया और वाइल्ड टाइप स्यूडोमोनास एरुजिनोसा ऐज़ूरीन की तुलना में रिक्ॉम्बिनेंट ऐज़ूरीन 50 गुना ज़्यादा प्राप्त हुआ। रिक्ॉम्बिनेंट ऐज़ूरीन को पराबैंगनी-दृश्यमान स्पेक्ट्रोस्कोपी द्वारा चिन्हित किया गया और शीर्ष 620 nm पर 0.174 के शुद्धता अनुपात (Abs620 / Abs280) के साथ पाया गया। ऐज़ूरीन, फ्लोरोसेंस और सी. डी. स्पेक्ट्रोस्कोपी द्वारा भी चिन्हित किया गया। प्रोटीन के विद्युत् रासायनिक व्यवहार का अध्ययन चक्रीय वोल्तामेट्री और प्रवाहकीय परमाणु बल माइक्रोस्कोपी (कंडक्टिव एटॉमिक फोर्स माइक्रोस्कोपी) द्वारा किया गया। ऐज़ूरीन प्रोटीन में चक्रीय वोल्तामेट्री द्वारा ऑक्सीकरण और अपचयन शीर्ष क्रमशः 280 mV और 178 mV पर प्राप्त हुए। ऐज़ूरीन वेरिएंट्स (Cu-ऐज़ूरीन, Fe-ऐज़ूरीन और Ni-ऐज़ूरीन) प्रोटीन के धातु-आयन केंद्र को संशोधित करके पुनर्निर्मित किए गए। वे पराबैंगनी-दृश्यमान अवशोषण और फ्लोरोसेंस स्पेक्ट्रोस्कोपी द्वारा भी चिन्हित किये गए। सभी तीन अज़ुरिन वेरिएंट्स की अपचयोपचय क्रिया ऑक्सीकरण और अपचयन शीर्ष द्वारा देखी गई। एज़ुरिन वेरिएंट्स तीन अलग-अलग उपकरणों पर इम्मोबिलाईज़ किये गए जिन्होंने स्रोत और निकास इलेक्ट्रोड के बीच एक माध्यम (200 nm) के रूप में कार्य किया और तीनों उपकरण अत्याधिक स्केलेबल और पुनरुत्पादित फील्ड इफ़ेक्ट ट्रांजिस्टर की तरह काम करने के लिए अनुकूल पाए गए। विद्युत मापन द्वारा सभी तीन उपकरणों में p-प्रकार के फील्ड इफ़ेक्ट ट्रांजिस्टर का व्यवहार देखा गया। उपकरणों में कम सबथ्रेशोल्ड स्विंग (200 mV/डेकेड) और उच्च ऑन-ऑफ़ विद्युत प्रवाह अनुपात (10^5) मिला। फील्ड इफ़ेक्ट ट्रांजिस्टर उपकरणों के व्यवहार

को आठ सप्ताह की अवधि के लिए मापा गया और अत्याधिक स्थिर व्यवहार प्राप्त हुआ। इन फील्ड इफेक्ट ट्रांज़िस्टर्स में दीर्घकालिक स्थिरता के साथ उच्च श्रृंखला दिखाया गया और सराहनीय सुदृढ़ता प्रदर्शित हुई। लचीली मेमोरी अनुप्रयोगों के लिए रिकॉम्बिनेंट ऐज़ुरिन पर आधारित एलुमीनियम / ऐज़ुरिन / आई. टी. ओ. / पी. ई. टी. (इंडियम टिन ऑक्साइड / पोलिएथिलिन टेरिफथेलेट) संरचना का उपयोग किया गया। 500 चक्रों के लिए अत्याधिक स्थिर एल. आर. एस. (मंद प्रतिरोधी अवस्था) और एच्. आर. एस. (उच्च प्रतिरोधी अवस्था) पायी गयी। लचीले यन्त्र को कई बार मोड़ने के बाद भी स्थिर प्रवाह और दोनों स्विचिंग अवस्थाएं पायी गयी। एज़ुरिन धातु आयन वेरिण्ट्स आधारित मेमोरी उपकरणों का भी अध्ययन किया गया। 10 चक्रों के लिए सभी तीन उपकरणों में स्थिर एल. आर. एस. और एच्. आर. एस. प्राप्त हुए। Cu-एज़ुरिन आधारित यन्त्र ने बड़ी मेमोरी विंडो के साथ सबसे अच्छा मेमोरी व्यवहार दिखाया। इसके अलावा, सभी तीन उपकरणों ने अच्छा दोहराव और स्थिरता प्रदर्शित की। रिकॉम्बिनेंट एज़ुरिन द्वारा अच्छा संचालन व्यवहार दिखाया गया है और इसे विद्युतीय संवेदको के विकास के लिए इस्तेमाल किया जा सकता है। ये उपकरण प्रोटीन आधारित मेमोरी को अन्य जैव-संगत और जैव-एकीकृत विद्युतीय यन्त्र के साथ एकीकृत करने के लिए तकनीकी कमी को पूरा करते हैं, जो की बेहद वांछनीय है।

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LIST OF ABBREVIATIONS

| | |
|----------------------|--|
| °C | Degree Celsius |
| 11-MUA | 11-mercapto-undecanoic acid |
| Abs _{280nm} | Absorption at 280 nm |
| Abs _{620nm} | Absorption at 620 nm |
| Abs _{max} | Absorption maxima |
| AFM | Atomic force microscopy |
| APS | Ammonium persulfate |
| APTS | 3-aminopropyl triethoxysilane |
| Az | Azurin |
| BSA | Bovine serum albumin |
| cAFM | Conductive atomic force microscopy |
| CD | Circular dichroism |
| CHAPS | 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate |
| cm | Centimeter |
| CV | Cyclic voltammetry |
| cytC | Cytochrome c |

| | |
|-------|--|
| DNA | Deoxyribonucleic acid |
| DTSSP | 3,3-dithiobis(sulphosuccinimidyl propionate) |
| E^0 | Standard reduction potential |
| EBL | Electron beam lithography |
| ECSTM | Electrochemical scanning tunneling microscopy |
| ET | Electron transfer |
| ETp | Electron transport |
| FET | Field-effect transistor |
| FTIR | Fourier transform infrared spectroscopy |
| h | Hour |
| HEPES | 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid |
| HOMO | Highest occupied molecular orbital |
| HRS | High resistance state |
| I_D | Drain current |
| IPTG | Isopropyl- β -D-thiogalactopyranoside |
| ITO | Indium tin oxide |
| I-V | Current-voltage |

| | |
|-------------------|-------------------------------------|
| kDa | Kilodaltons |
| k_{ET} | Electron transfer rate constant |
| $kVcm^{-1}$ | Kilo volt per centimeter |
| LB | Luria Broth |
| LRS | Low resistance state |
| LUMO | Lowest unoccupied molecular orbital |
| $M^{-1}cm^{-1}$ | Per molar per centimeter |
| mg | Milligram |
| mg/mL | Milligram per milliliter |
| mg/L | Milligrams per liter |
| mm | Millimeter |
| mM | Millimolar |
| $n\Omega \cdot m$ | Nanoohm-meter |
| MPTS | 3-mercaptopropyl trimethoxysilane |
| mV | Millivolt |
| $mVsec^{-1}$ | Millivolt per second |
| MWCO | Molecular weight cut-off |

| | |
|--------|-------------------------------|
| nA | Nanoampere |
| NHE | Normal hydrogen electrode |
| Ni-NTA | Nickel-nitrilotriacetic acid |
| nm | Nanometer |
| OLED | Organic light emitting diode |
| pA | Picoampere |
| PCR | Polymerase chain reaction |
| PET | Polyethylene terephthalate |
| PMMA | Poly(methyl methacrylate) |
| POC | Point of care |
| rpm | Revolutions per minute |
| s | Second |
| SDS | Sodium dodecyl sulphate |
| SEM | Scanning electron microscopy |
| STM | Scanning tunneling microscopy |
| SS | Subthreshold swing |
| TB | Terrific Broth |

| | |
|--------------------|---------------------------------------|
| TC | Transfer characteristics |
| TEMED | N,N,N',N'-tetramethylethylene diamine |
| T _m | Melting temperature |
| Tris | Tris(hydroxymethyl)aminomethane |
| μM | Micromolar |
| UV | Ultraviolet |
| V | Volt |
| V _{DS} | Drain-source voltage |
| V _{GS} | Gate-source voltage |
| v/v | Volume by volume |
| V _{reset} | Reset voltage |
| V _{set} | Set voltage |
| V _{TH} | Threshold voltage |
| w/v | Weight by volume |

LIST OF SYMBOLS

| | |
|------------|------------------------------|
| α | Alpha |
| β | Beta |
| cm | Centimeter |
| ϵ | Molar absorption coefficient |
| g | Relative centrifugal force |
| μ | Micro |
| λ | Wavelength |