PROTEINS APPLICATION IN ELECTRONICS

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Abstract: Molecular bioelectronics is well recognized in exploiting organic and biologic materials for electronic and optical-electronic devices. In this paper, a number of biological processes that could be used in molecular bioelectronic devices, optical memories and energy conversion are described. The objects of the research are three proteins: Photoactive Yellow Protein, Green Fluorescence Protein, Bacteriorhodopsin and their characteristic and applications.

Keywords: bioelectronics, proteins, PYP, GFP, bacteriorhodopsin

1. Introduction

Molecular bioelectronics is a relatively new field assosiated with the development of organic and biological materials within electronic and optoelectronic devices. The first molecular materials used in electronics originate from material science and are related to the development of electronic and optoelectronic devices that utilize the unique features of organic compounds macroscopic. The most successful commercial product so far are the liquid crystal displays (LCDs). However, after many years of research, the organic light-emitting devices based on dyes and polymers, synthetic electronic circuits, chemical and biochemical sensors finally have drawn attention.

Now the most challenging task in electronics are the molecular scale devices. Efforts are focused on single molecule behavior or behavior of group of molecules and precise 3D position control over single atoms and molecules. The main application of molecular scale electronics is in switches, DNA and molecular devices that could be implemented in standard silicon electronics [1]. In particular, there are molecule structures that could become a real competitor to state-of-the-art silicon microelectronics devices.

The two approaches in molecular bioelectronics, to a certain extent, coincide with the "top-down" and "bottom-up" approaches used in microelectronics. Top-down approach refers to creation of nanoscale structures, through machining for example, while the bottom-up method or nanotechnology molecular construction refers to the organic and inorganic architectures that are built atom by atom or molecule by molecule. Top-down manufacture methods are related to the part construction through cutting methods or etching processes assigned to photolithography patterns. Microelectronics industry progress is an excellent example of the top-down method. On the other hand, bottom-up manufacturing process provides single molecule product construction held together by covalent forces, much stronger than the forces that held together macro scaled components.

The long-term goal of molecular electronics is to mimic complex process behavior in nature that would allow for manufacturing molecular bioelectronics devices. In this paper, we

describe a number of biological processes which could be used in molecular bioelectronics device manufacturing, especially for sensors, optical memories and energy conversion. The objects of the research are three proteins: Photoactive Yellow Protein [2], Green Fluorescence Protein [3], Bacteriorhodopsin [4], their features, characteristic and applications in electronics.

Protein is a complex organic compound with high molecular mass consisting of amino acids bonded by peptide bonds. These molecules are the most common type in cells and represent 50% of their dry weight. Proteins are various cellular components, and they might serve as enzymes, building materials and hormonal activity.

Protein can consist of a single polypeptide chain, or may consist of several chains that are connected to each other through weak molecular bonds. Using only 20 different amino acids, a single cell builds up thousands of different proteins, each of which has a highly specialized function.

Informally, proteins can be divided into three main classes, which correlate with typical tertiary structures: globular proteins, fibrous proteins, and membrane proteins. Almost all globular proteins are soluble and many of them are enzymes. Often, fibrous proteins are structural, such as collagen – the main component of connective tissue, or keratin – the hair and nails protein component. Membrane proteins serve frequently as receptors or provide channels of polar or charged molecules to pass through the cell membrane.

2. Materials

2.1 Photoactive Yellow Protein – PYP

Ectothiorodospira halophila – small saltwater bacteria that avoids overexposure to harmful UV radiation captures blue light and moves in the opposite direction of the gradient [2]. In the cytoplasm of Ectothiorodospira halophila is a small soluble protein called photoactive yellow protein (Figure 1).

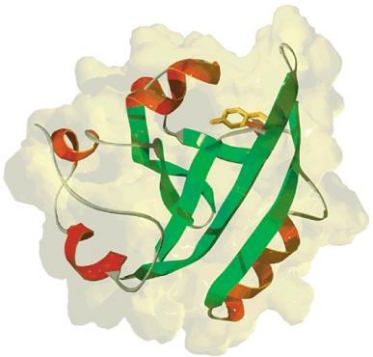

Figure 1. Photoactive yellow protein general structure. The secondary structure is represented as a bar (alpha-helices in red and beta-sheets in green) and molecular surface of PYP is in the background. Chromophore of 4-hydroxy-cinnamic acid is shown in orange.

Photoactive yellow protein contains a chromophore 4-Hydroxy-Cinnamic acid covalently bonded by γ-sulfur Cys69 (Figure 2) [5]. Chromophore is completely hidden in the hydrophobic pocket without exposure to solvents. Nonpolar environment reduces the pKa of the chromophore phenolic group to such levels that this group deprotonates at pH 7.0. Effective negative charge on the phenolic oxygen atom is stabilized by a network of hydrogen

bonds formed between Tyr42, Glu46 and Thr50 (Figure 2. (B)) [6]. Due to interactions with adjacent amino acids residues, the bound chromophore absorbs blue light photons (λ_{max} = 446 nm), and afterwards appears the yellow color that is typical for the protein.

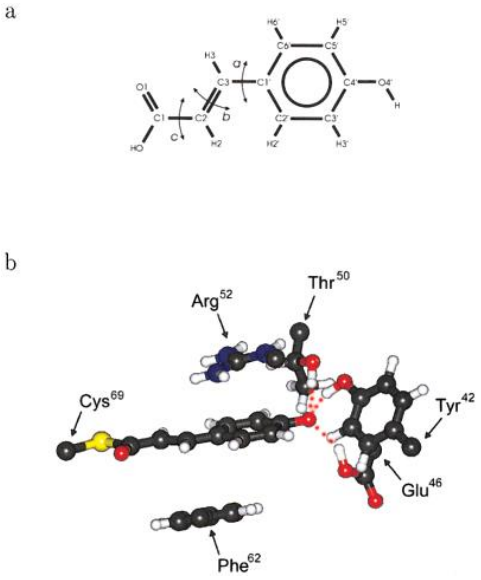

Figure 2. Chromophore of 4-hydroxy-cinnamic acid: (a) free and (b) relates to PYP.

Photoactive yellow protein also serves as a structural prototype of the widespread Per-Arnt-Sim (PAS) of the supergroup, and is a member of it as well. PAS areas are found in the signal transmitting and sensitive protein and are often involved in intermediate protein-protein interactions. In bacteria, they can serve as light sensors, redox potential or oxygen concentration sensor kinesis of histadin of two-component systems for signal transmission. PAS region is extremely flexible. PAS areas can transmit signals arising in these chemically diverse ligands in different classes of executive areas, like histidine or serine/threonine kinesis.

Due to these photoreaction studies, this protein can be used for light sensor.

2.2 Green Fluorescent Protein – GFP

Green fluorescent protein (GFP) is a protein released by the jellyfish Aequorea victoria, and when illuminated with blue light attains characteristic fluorescence in the green range. Nowadays, protein gene is widely used as a light marker in cellular and molecular biology for inquire into the expression of cellular proteins. Protein modifications for biosensors applications are developed. Even entirelly glowing animals (eg pigs) are created, where the fluorescent protein GFP is introduced into the genome and is inherited [3].

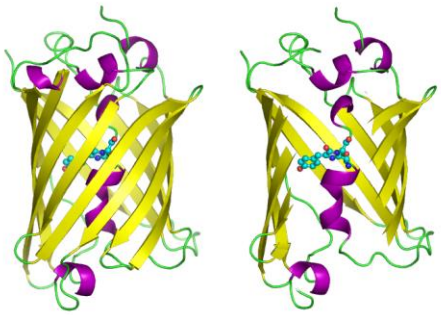

Figure 3. GFP molecules. A whole one and a cutted part of the beta barrels, so that chromophores to be clearly seen.

GFP has typical beta barel structure (Figure 3), consisting of one beta sheet with alpha helices containing one passing through the center chromophore. The internal side chains of the barrel induce specific reaction cycling peptide Ser65-Tyr66-Gly67, which leads to the formation of chromophore. This post-translational modification process is called maturation. The hydrogen bonds network and electronic interactions with the side chains affect the color of wtGFP and its derivatives. The barels' compact structure excludes the influence of the solvent molecules, keeping the chomophore fluorescence from extinguishing with water.

2.3 Bacteriorhodopsin

Bacteriorhodopsin is a light-driven proton pump in the purple membrane (PM) of Halobacterium halobium that creates a proton gradient used for the manufacture of ATP. Bacteriorhodopsin contains a "proton channel" that is essential to its function. The proton moves through two partial channels separated by a Schiff base. The proton pathway in bacteriorhodopsin is one of the first explicitly proposed to comprise HBC elements. The entire photocycle can be dissected into at least eight intermediate states. Energy is introduced by absorption of a photon, and as the cycle proceeds, the result is the translocation of one proton from the cytoplasm to the extracellular solution.

The evolution principle managed to optimize Bacteriorhodopsin to pump as much proteins as possible even if there is low light. This has led to a quantum yield of 64% for its main photoreaction and lack of refractory period. When using biological materials in technical applications very often the human planned biomolecules task is quite similar, but not identical. A small adjustment of the molecular structure may lead to forming a biomolecule, which meets the requirements much better. Such structural adjustment can be made by deliberate human intervention or a random mutation. Based on its modified functionality, molecule mutant or variant can be selected for a particular application. There are several versions of Bacteriorhodopsin [4].

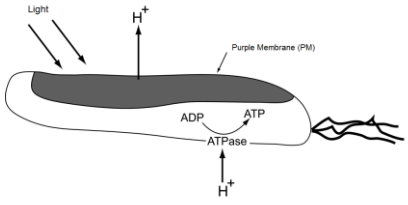

Figure 4. Schematic HS photosynthetic system presentation. BR molecules act as a light-driven proton pump. Membranne connect ATPase enzyme uses this proton gradient to generate ATP from ADP.

Figure 5 shows the Bacteriorhodopsin type within the PM. It can be seen that Bacteriorhodopsin is located in clusters of three molecules called trimmers. Trimmers form a hexagonal shape. Within the PM bacteriorhodopsin molecules are equally oriented with the same edge on the inside of the cell. This is an important requirement for granting a proton

gradient illumination. The PM size is of the order of several μm. The size of one Bacteriorhodopsin molecule is approximately 5 nm.

The protein part of the Bacteriorhodopsin complex consists of 248 amino acids arranged in seven spirals. These spirals effectively form cells in which the retinal group is located. As illustrated in figure 6 retinal group is tilted regarding the surrounding helices. Retinal chain makes an angle of about 70 ° with the normal to the PM. Retinal (vitamin A derivative) and protein absorb in the ultraviolet region (UV), but the complex with the molecule absorbs in the visible region [7].

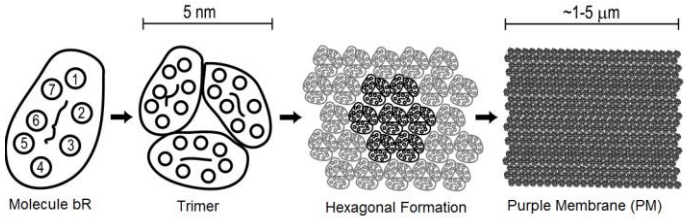

Figure 5. From left to right: Each molecule of bR is composed of seven spirals protein, which forming cell in which it has retinal group. Three bR molecules are grouped to form a trimer. These trimers form a hexagonal formation. As a result, two-dimensional grid of bR molecules formed PM.

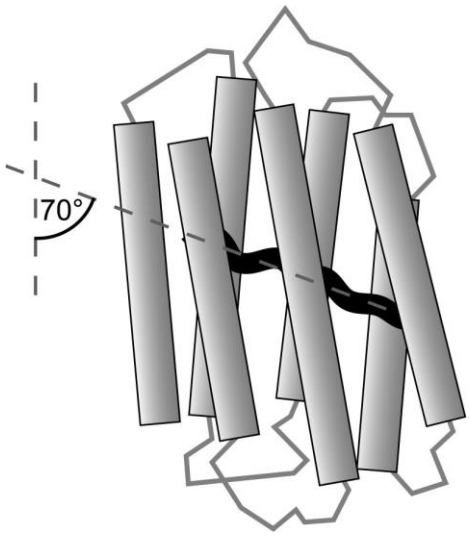

Figure 6. Structure of bR showing the retinal group (black) and nearby spirals bR-protein (gray). Retinal group forms an angle of about 70 ° with the normal to the PM.

3. Examples of successful application

3.1 GFP photo-biodiode

In this study, a molecular heterojunction consisting of molecular layers sandwiched between metal electrodes has been developed. The proposed junction was investigated for possible use as a photoswitcher and bio-photodiode. For construction of the bio-photodiode, a metal/insulator/metal MIM structured electronic device was fabricated with GFP/viologen/N-docosilquinolinium tetracyano dimethan TCNQ heterofilms used as the sensitizer, an electron relay and an electron acceptor, respectively, between the aluminum and indium tin oxide ITO glass. To investigate the photoinduced current flow of the proposed device, the

photoswitching function and rectifying characteristic of the device were measured. The transient photocurrents of the GFP homo-, GFP/viologen hetero, and GFP/viologen/TCNQ heterostructured MIM devices were measured in order to investigate the dynamic process of charge transfer of the proposed bio-photodiode [8].

Figure 7 shows the electron transfer mechanism of the molecular array consisting of the GFP/viologen/TCNQ structured bio-photodiode.

Figure 8 shows a schematic of the experimental system for the transient photocurrent measurements.

Photo switching behavior of organic photodiodes is shown in Figure 9 (a). When you submit a positive voltage, photo current is generated. By repeating the step of backlighting, repeating photocurrent is generated. This result indicates that the switching property of organic photodiodes is achieved. Photo induced unidirectional electron flow in organic photodiodes can be achieved due to the redox potential differences and e-connectivity between functional molecules as it is shown in Figure 7 (a). It may be seen how intensity of photo current depends on external pressure. Larger photo currents are generated by increasing the external voltage.

Figure 9 (b) presents the correctional characteristics as they are observed in voltamps measurements. When organic photodiodes are highlighted then photocurrent with adjacent voltage is generated. On the other hand less current is generated in dark conditions (no backlight) even when higher voltage is applied [8].

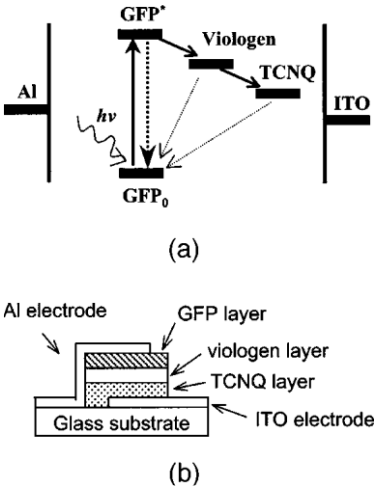

Figure 7. (a) Mechanism of photo-induced electron transfer in organic photodiodes, (b) schematic structure of organic photodiodes.

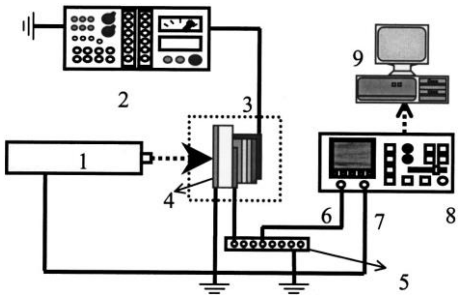

Figure 8. Setup for measuring the photocurrent: 1. Laser 2. Supply Box 3. Protective cartridge 4. Copper 5. Amplifier 6. Signal 7. Trigger 8. Oscilloscope 9. Data recording.

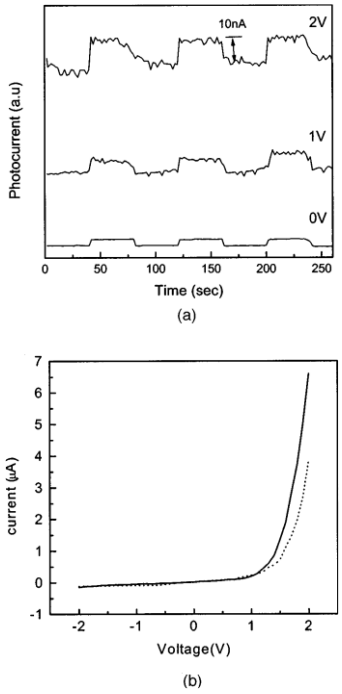

Figure 9. Photoelectric response of the bio-photodiode, a) photoswitching function; bias voltage is 2, 1.5, and 0 V. b) Rectifying property; solid line is photostate and dot line is dark state.

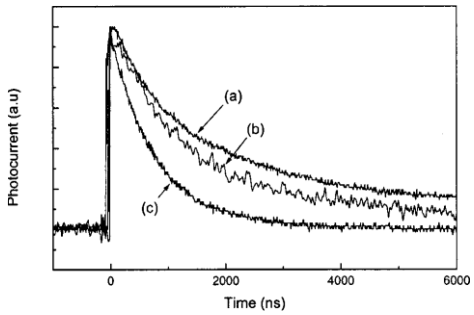

Figure 10. Transient photocurrent measurement, (a) GFP homojunction, (b) GFP/viologen heterojunction, (c) GFP/viologen/TCNQ heterojunction.

3.2 Volumetric optical memory based on bacteriorhodopsin

Optical data storage principle is to assign each lasting two states of the protein binary value – '0 'and '1' in order to keep the necessary information. For example, the B and Q state of the molecule can remain stable for many years [9].

Prospects for the 3D data storage seem more promising than the 2D storage. There are three different types of data storage that are being examined. The first is holographic storage, the second one is based on branching fotocycle and the third method uses two photons by excitation of separated points to attain storage in the material volume.

The last one is performed by the intersection of two laser beams. Each beam brings photons that have only half the energy needed to switch bR from state B to M or vice versa. During data reading, the same procedure is applied.

A different approach uses two single photons transition in bR (Figure 11) [10]. Data can be stored in proteins using a combination of red and green laser sources diodes. These sources are mounted perpendicularly to each other. Green source light reaches the protein through spatial light modulator, switching certain areas of the protein at stage O. Two milliseconds later, the red laser light, which also passes through a spatial light modulator, illuminates protein aiming to form its P condition which then passes into Q. After this two-step process a part of the protein is considered to be "1" when the protein is in the P and Q states and “0” when corresponds at O and B states. Data reading is performed in exactly the same way, except that the red source has reduced intensity. Reading green light is absorbed only by proteins in O condition, and low intensity red light represents P and Q states on the device for the injection of charge, instead of converting each O protein. Encrypted data is erased with blue light. P and Q states absorb blue light and return to their initial B state. Individual data can be deleted with blue laser and a total deletion might be done through an incoherent blue light source. Information erasement process is highly effective and memory should be protected. This procedure might allow creation of a storage system data.

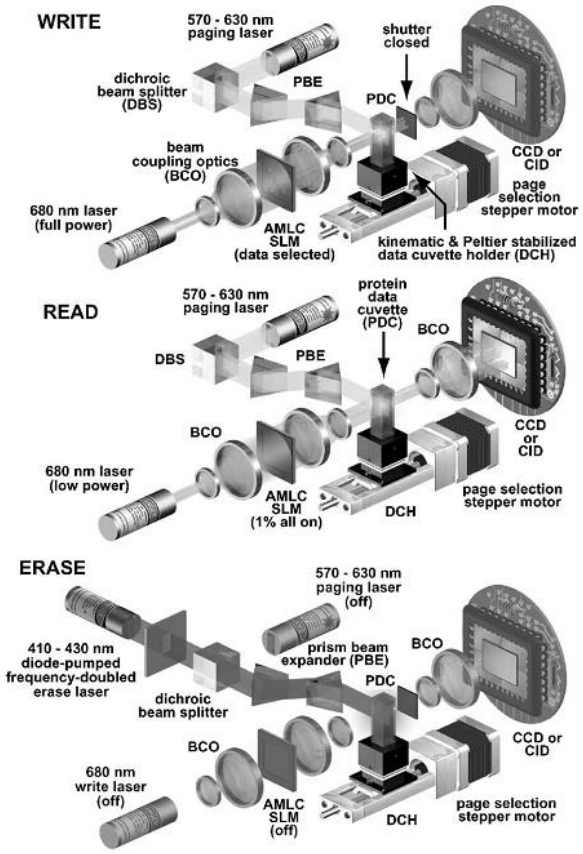

Figure 11. Setup by recording, reading and erasing on Bacteriorhodopsin. SLM - spatial light modulator; CID - a device for injecting charge; BCOs - ray optics; DBS - two-color divider rays; PBE - prisms to expand the beam; PDC - container containing protein.

The write, read and erase operations of the bacteriorhodopsin-based branched-photocycle memory. The write and read operations are both initiated by using a paging beam to activate the photocycle in a thin region within the memory medium (green light). On formation of the Ostate in this page, a write beam is activated and the data imposed on the beam using a spatial light modulator (SLM). The read process is similar, but in this case the modulator is turned off so that just enough light gets through to image the page onto the charge couple device (CCD) [or charge injection device (CID)] array. Because the O state is the only species that absorbs the write beam, the read and write processes, only involve interaction of the light beam with the O state in the paged regions. A blue laser erases an entire page. Abbreviations: beam coupling optics (BCOs); DBS, dichroic bean splitter; PBE, prism beam expander; PDC, protein data cuvette [10].

The storage capacity of this memory type is very high (10 Gbyte). Volume limits that may arise are related to the protein quality and the lens system responsible for recording and reading information. Yet, it is not known if this memory type can compete with hard drives or solid-state memory, because there are still some problems with its production. For instance, Q state in its natural form does not absorb red light well, therefore reading process requires high intensity light. On the other hand, it is possible to improve the absorbtion through genetic engineering. If such issues are resolved, the benefits of memory will be great: low cost, high

operating temperature range and there is no loss of information in the absence of power.

4. Conclusions

In the present paper, proteins for electronic applications are discussed and analyzed. All proteins reviewes conduct proton current or transfer protons and have the potential to be implemented in microelectronic systems or operate as separate components. Various attempts for the application in electronics of different protein types were discussed.

From the foregoing examples it can be concluded that these natural objects can transfer and process signals similarly to microelectronic objects. The analogy between natural objects and microelectronic circuits and devices was presented. This was clearly pointed out for bacteriorhodopsin, which has features similar to memories.

In conclusion we might say that Green Fluororescent Protein, Bacteriorhodopsin, Photoactive Yellow Protein could be used in electronics in the future. Photoactive Yellow Protein, Green Fluororescent Protein could be used for bio sensors and markers.

5. Acknowledgment

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