PROTEOTRONICS: The emerging science of protein-based electronic devices

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Abstract. Protein-mediated charge transport is of relevant importance in the design of protein based electronics and in attaining an adequate level of understanding of protein functioning. This is particularly true for the case of transmembrane proteins, like those pertaining to the G protein coupled receptors (GPCRs). These proteins are involved in a broad range of biological processes like catalysis, substance transport, etc., thus being the target of a large number of clinically used drugs. This paper briefly reviews a variety of experiments devoted to investigate charge transport in proteins and present a unified theoretical model able to relate macroscopic experimental results with the conformations of the amino acids backbone of the single protein.

1. Introduction
Proteotronics was introduced as a new science by the Authors in 2014 [1]. The word originates from the combination of proteomics, introduced in 1996 by Marc Wilkins to indicate the large-scale study of proteins structures and functions, and electronics, introduced by Thomas Edison in 1883, to indicate the development of devices manipulating electrical current to perform useful tasks. The main objective of proteotronics is to propose and develop innovative electronic devices, based on the selective action of specific proteins. Here, we briefly survey the pillars of this emerging science by reporting a series of experiments appropriate to investigate the electrical properties of a given protein and a theoretical approach based on an impedance network protein analogue (INPA) able to provide a unifying microscopic interpretation of the experimental data. For a comprehensive review on the subject readers are sent to Ref. [2]

2. Physical system of interest
Proteins appear like linear chains of lego-like building blocks (the amino acids) able to swiftly (few microseconds) organize in space so as encoded in their sequence. In other words, proteins are polymers constituted by twenty different types of amino acids (the alphabet of proteins) with a sequential length containing $20 \div 1000$ amino acids. Figure 1 shows a segment structure of a typical transmembrane protein, bovine rhodopsin, sensitive to light. Here we are interested in sensing proteins that under an external stimulus (e.g., light, odours, drugs, etc) change their tertiary structure going from a native to an activated state. The conformational change is crucial
Figure 1. Segment structure of bovine rhodopsin.

Figure 2. OR-7D4 in its ribbon representation: native state in purple and activated state in green.

Figure 3. Cartoon of a protein embedded into the analogous impedance network.

Figure 4. Overlap between amino acids and equivalent-circuit element. $C_{\alpha i}$ identifies the center of the sphere corresponding to the $\alpha$-carbon atom of the $i$-th amino acid.

for the external/internal communication of a living cell. Figure 2 reports the ribbon structure of an olfactory receptor (OR), the chimpanzee OR-7D4, in its native (purple) and activated (green) state. In this respect, a sensing protein is the ultimate miniaturization of a sensor. In
nature, the information associated with the conformational change moves to the brain through a biological chain. This ability to detect the change of an electrical property of the single protein correlated to the conformational change is crucial for the development of a nano-bio-sensor, a significant example of proteotronics [2].

**Figure 5.** Schematic of a three electrodes electrochemical-cell. WE, RE and CE refer, respectively, to working, reference, and control electrodes.

3. Theoretical model

The theoretical model implements an impedance protein network analogue (INPA) where the 3D backbone representation of a single protein is associated with a topological network (graph), consisting of nodes and links, as depicted in Fig. 3. By connecting couples of nodes with the rule that they must be closer than an assigned interaction radius, $R_a$, the graph turns into an impedance network when an elemental impedance is associated with each link. The elemental impedance is taken as that of an RC parallel circuit, as shown in Fig. 4,

$$Z_{i,j} = \frac{l_{i,j}}{A_{i,j}} \left( \frac{1}{\rho^{-1} + i \epsilon_{i,j} \epsilon_0 \omega} \right)$$

where $A_{i,j} = \pi (R_a^2 - l_{i,j}^2/4)$, is the cross-sectional area between the spheres of radius $R_a$ centered on the $i,j$ nodes, respectively, $l_{i,j}$ is the distance between these centers, $\rho = 10^{10}$ $\Omega$m the resistivity of each amino acid, with the indicative value following the fit with experiments, (the microscopic mechanism responsible of charge transfer is taken to be of tunneling type), $i = \sqrt{-1}$ is the imaginary unit, $\epsilon_0$ is the vacuum permittivity and $\omega$ is the circular frequency of the applied harmonic voltage. The relative dielectric constant pertaining to the couple of $i,j$ amino acids, $\epsilon_{i,j}$, is expressed in terms of the intrinsic polarizability of the $i,j$ amino acids.

By positioning the input and output electrical contacts on the first and last node, respectively, for a given applied bias the network is solved within a linear Kirchhoff scheme and its global impedance spectrum, $Z(\omega)$, is calculated in the frequency range $10^{-3}$ $\div$ $10^5$ Hz which is standard.
for experiments. By construction, besides the small-signal dynamic response, this network produces a parameter dependent static current-voltage (I-V) characteristic determined as:

\[ V = Z(0)I \]  

(2)

To this purpose, we assume that the 3D structures of the protein in its native (without ligand) and activated (with ligand) states are known from the protein data bank available in the literature or from an homology modeling. The role of the medium (lipids) surrounding the protein is here neglected. Indeed, the medium is not involved in the conformational change and so it does not imply a variation of the global impedance.

4. Results and discussions

This section briefly outlines the main experimental techniques and the associated experimental data available from the literature together with the microscopic modeling carried out with the INPA technique.

Figure 5 shows the schematic of the electrochemical impedance spectroscopy (EIS) carried out on a self assembled monolayer of a given protein with a macroscopic area of a few cm\(^2\) at a small applied voltage in the mV range and in the frequency range \(10^{-3} \div 10^5\) Hz. Figure 6 reports the impedance of the rat OR-I7 at different odorant concentrations and represented as a Nyquist plot.

Figure 7 reports the set-up for an I-V characteristic carried out on thin-films of proteorhodopsin (pR) in the voltage range 0 \(\div\) 100 V corresponding to average electric fields in the range 1 \(\div\) 100 kV/cm and in the presence or absence of a visible light. Figure 8 reports the photocurrent of two proteins, bacterio-rhodopsin (bR) and pR belonging to the family of opsins, that are sensitive to visible light [5]. The experiments are successfully described by the INPA model or by a simple linear fit inspired by the theoretical model.

Figure 9 reports the set-up for I-V measurements on monolayers of single protein with a macroscopic surface area of a few cm\(^2\). The contacts are designed to permit the illumination of the sample by visible light. Figure 10 reports a set of I-V characteristics in the range \(-1 \div 1\) V for the case of bR together with the theoretical interpretation obtained by the INPA model.
Figure 11 is a schematic of the atomic force microscope (AFM) set-up used for the I-V measurements on protein monolayers properly assembled on a conductive substrate in the range $-10 \div +10$ V. Figure 12 reports a set of results carried out on bacterio-rhodopsin samples for three indentations of the tip, 1.2, 2.8 and 4.6 nm from the bottom contact, with the theoretical interpretation performed using the INPA model.

![Schematic of the metal-protein-metal nanostructure for I-V measurements in the presence of light.](image1)

![I-V characteristics of bR in the native (2NTU) and activated (2NTW) states. Data were calculated by using a Gaussian distribution of barrier heights between amino acids. The continuous and dashed lines report the experimental data in the native and activated state, respectively.](image2)

5. Conclusions and perspectives
Proteotronics is an emerging science aiming at investigating the coupling between the protein world (proteomics) and electronics. In particular, major results can be summarized as follows. (i) The correlation between the 3D structure of proteins and their sensing properties is confirmed from an experimental and a theoretical point of view. (ii) The electrical properties of a single protein is found to be similar to that of a medium-gap semiconductor with Ohmic resistivities estimated in the range of $10^5 \Omega \cdot cm$ at room temperature. (iii) The current-voltage characteristics are found to exhibit a superlinear behavior at increasing applied voltages, which is associated with a sequential tunneling mechanism of charge transfer between neighbouring amino acids. Typical barrier heights are in the range $50 \div 300$ meV and barrier width lower than 1 nm. (iv) The electrical change associated with the conformational change following a sensing action of the protein is found to be in the range from a few per cent up to about a factor of two in magnitude. This variation can be positive or negative and, from a theoretical interpretation, is consistent with the change in the spatial location of the amino acids of the single protein. In a conformational change, the variation in the average volume of a protein is estimated to be of a few per cent. (v) A microscopic modeling based on an impedance network protein analogue (INPA) is found to provide a unified interpretation of experiments available from literature. Once the tertiary structure of a protein is given, INPA is useful to visualize the amino acids interconnection through the construction of the contact maps and to predict the electrical properties in a wide range of applied bias. INPA is actually a numerical code based
on a Monte Carlo technique and suitable for further implementation to include advances in the knowledge and characterization of proteins. Yet, the scarcity of data and the simplifications in the model reflect the discrepancies between numerical and experimental findings which, for the first step of an emerging science, is a plausible result.

![Figure 11. Schematic of the AFM set-up including the detection.](image)

Figure 11. Schematic of the AFM set-up including the detection.

Figure 12. I-V characteristics obtained by simulations with extended contacts at different indentations in nanometers including a leakage current. The thick continuous lines refer to experimental data [6] and the symbols to INPA numerical simulations.

(vi) A first attempt to develop a new generation of nano-bio-sensors using the sensing property, manifested by a conformational change, into an electrical response is reported. A proof of concept on the viability of such an attempt was successfully demonstrated for several sensing proteins (olfactory receptors: rat OR I7, human OR 17-40, light receptors: bacteriorhodopsin, proteorhodopsin, etc.). As a final comment, we stress that proteotronics can be considered as a branch of pantatronics, a new term announced here to underline the historical development and the overwhelming trend of electronics as applied to a multitude of fields of scientific/technological interest (atoms, chemistry, mechanics, molecules, solids, spin, etc.).

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