When Solid State Physics Meets Proteins; A Current - Voltage - Temperature approach to "BIO-NANO"

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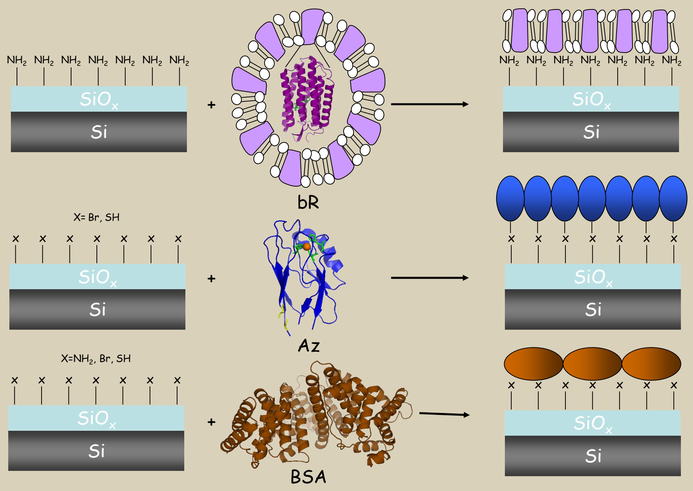
Electron transfer (ET) through proteins is a fundamental process in biology. It has been and is studied intensively in solution. *Solid state electron transport (**ETp**) across proteins, sandwiched between two solid electrodes*, an evolution of molecular electronics, aims at understanding the extent to which protein features/functions are expressed (and used) in this new configuration. Most studies to date were conducted with one or just a few molecules in the junction.

We show that one can prepare and electrically characterize high quality, large area monolayer junctions with three different families of proteins: *Bacteriorhodopsin* (bR), a membrane protein-chromophore complex with proton pumping function, *Azurin* (Az), a blue-copper ET metallo-protein, and *Bovine Serum Albumin* (BSA) and that we can gain information about their **ETp** mechanism by applying solid state physics methods, such as current-voltage temperature (I-V-T) measurements to these proteins. We find dramatic changes in the proteins' **ETp** activation energies and mechanisms, for bR and Az, respectively. Our results shed new light on **ETp** properties in proteins *(mostly they resemble molecular wires, more than insulators)* and lead us a step further towards utilizing the functional characteristics of these and related biocomplexes as actual electronically conductive components.

Making monolayers

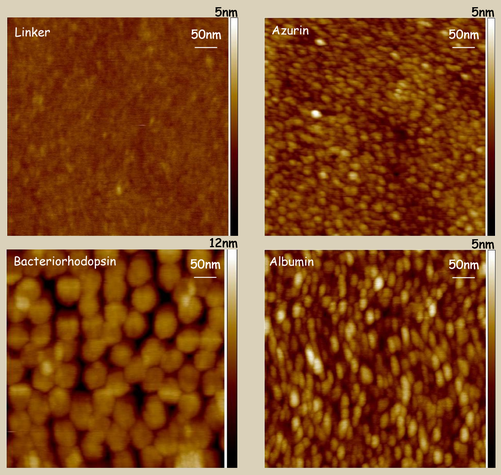
Large area protein monolayers are prepared by self-assembly of the protein molecules on chemically modified surfaces of SiOx on highly doped Si, with well-defined electrical behavior.

Protein assembly on the substrate is via electrostatic binding followed by vesicle fusion (bR), covalent attachment (Az) and physisorption (BSA).



Monolayer characterization

Each preparation step is characterized by Ellipsometry and AFM topography. The thin SiOx on high doped Si guarantees ohmic behavior of the Si/SiOx surface. The short linker layer is both dense and smooth, allowing high surface coverage (80-90%) by the proteins. The discrepancies between ellipsometry values and AFM protein heights are due to the presence of voids in the cases of Az and BSA and to vesicle curvature in the case of bR.

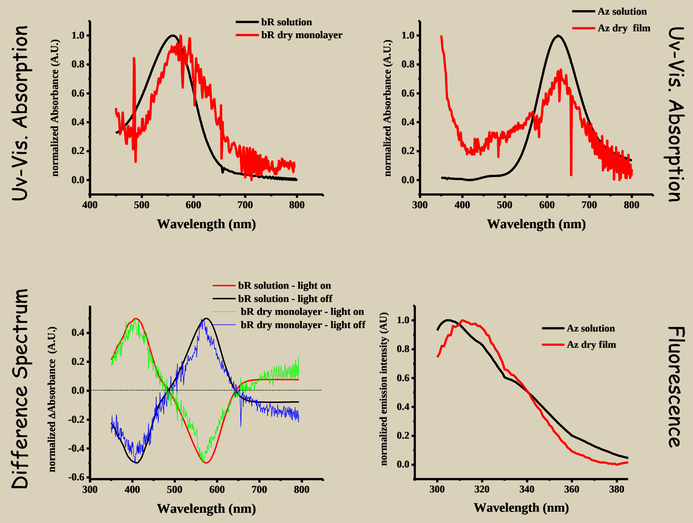


AFM height images of protein monolayers (500nm x 500nm)

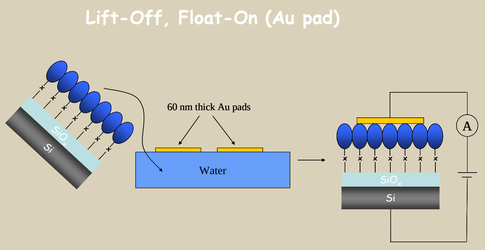
	Ellipsometry Value (Å)	Length (Å) (normal to surface)
Oxide	11-12	
Linkers	6-7	7
Azurin	15-18	36
Bacterio-rhodopsin	75-80	50
Albumin	14-18	40

Do proteins keep their conformation?

Optical absorption and fluorescence of bR and Az are very sensitive to changes in protein conformations. bR pigment absorption and photo-activity (formation of M_{412} intermediate of the bR photo-cycle) are observed on dry monolayers (top and bottom left). The absorption band and unique short wavelength fluorescence emission of Az are also retained in dry Az films (top and bottom right).

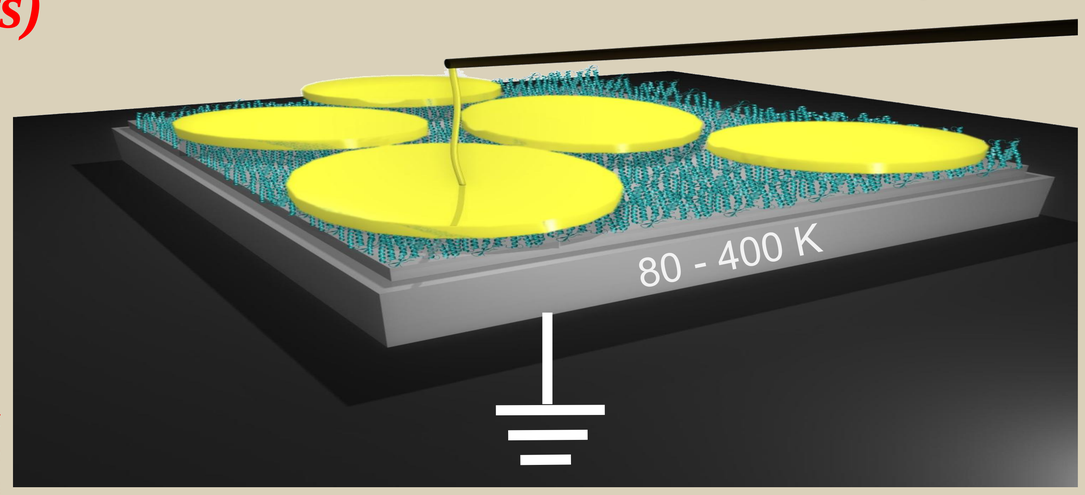


Closing the circuit (making top contacts)



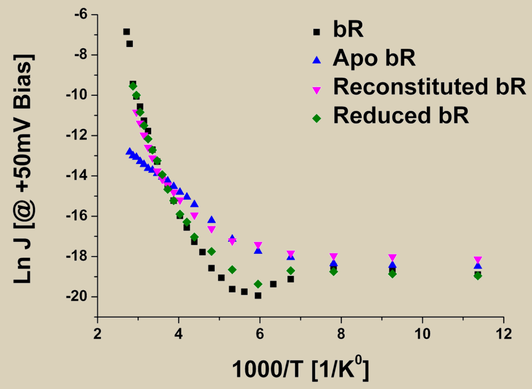
LoFo is a methods for top contact deposition. The method, is known to give non-damaging contact to soft monolayers and to allow highly reproducible measurements: < 5% of the measurements gave short circuits and the standard error in the rest of the measurements was < 10%.

Solid state physics applied to Biology Determining ETp Mechanism

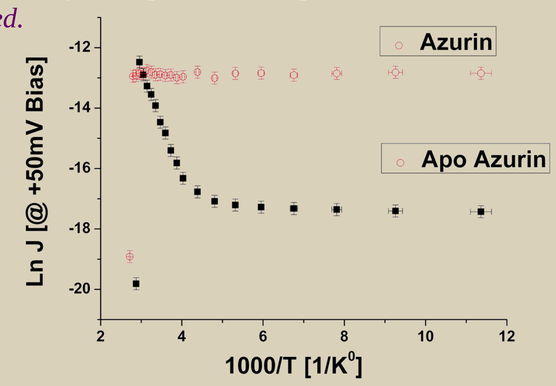


Current vs. Voltage as a function of Temperature (I-V-T) curves of monolayers of the proteins. The ability to measure the current under temperature variation allowed the possibility of determining ETp mechanism, with out any perturbation from the surroundings (solvents) as in the classical case.

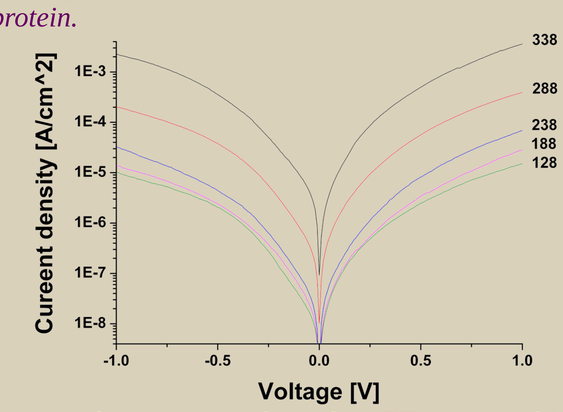
Results with modified proteins (biomolecular signature)



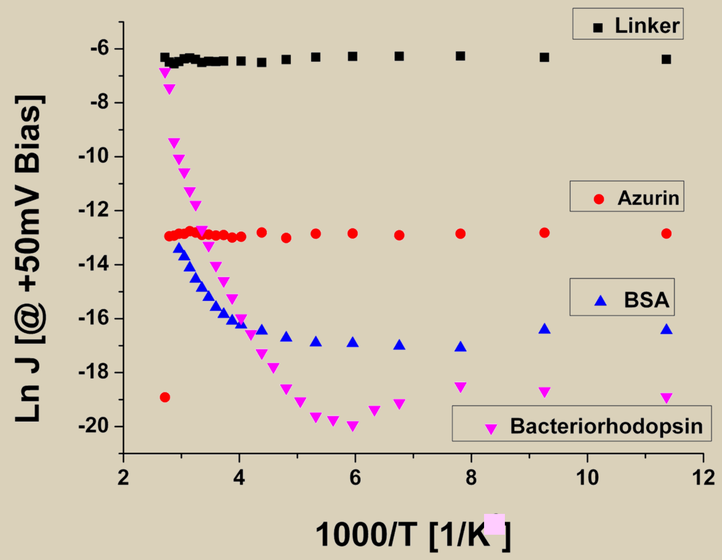
The reduction of the charge in the retinal chromophore (Reduced bR) of bR, does not change the temperature dependence of ETp in comparison to WT bR. However, removing the retinal from its binding site (Apo bR) seems to "open" a new pathway for ETp. After reconstitution of the Apo bR, a competition between the two pathways is observed.



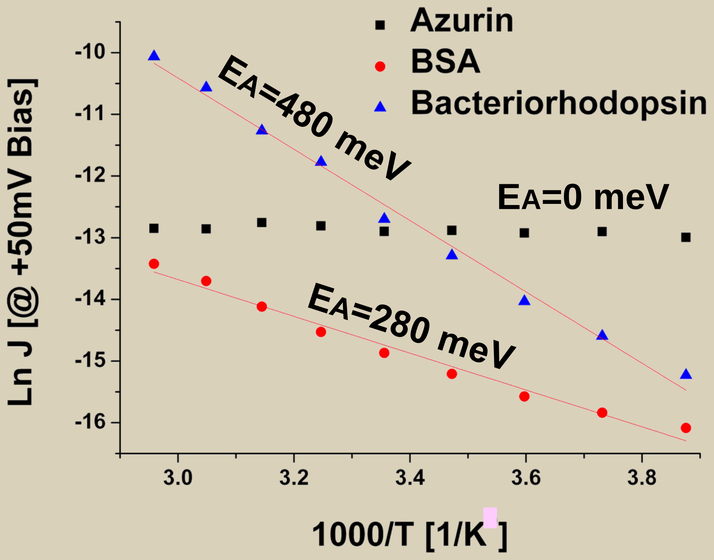
Dramatic change in ETp mechanism from temperature independent to temperature dependent is detected due to removal of single Cu ion from the protein.



The occurrence of temperature dependent ETp even at high biases (1 V) excludes the possibility that the activation energy barrier is due to the contacts.



Comparison of temperature dependence of three proteins. The control experiment of the system with just the linker shows temperature independent ETp. Surprisingly, Azurin produced temperature independent ETp as well, except at high temperature, where an irreversible sharp drop in the current corresponds to the protein's denaturation. Remarkably, at high temperatures, bacteriorhodopsin, a natural proton pump protein, yielded higher currents than the redox protein Azurin.



Activation energies (E_A), extracted from Arrhenius equation of the different proteins.

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