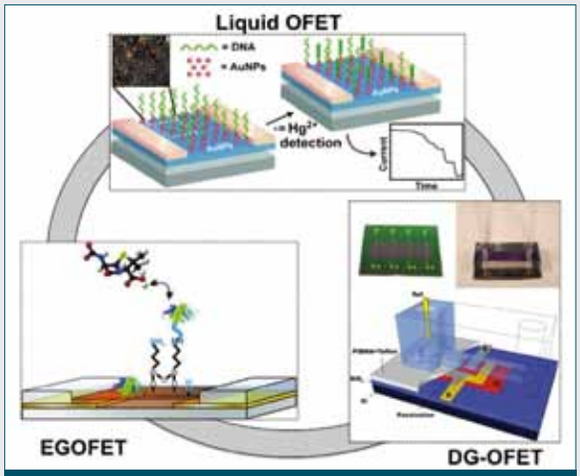

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BIO-SENSING BASED ON ELECTROCHEMICALLY-GATED ORGANIC FIELD-EFFECT TRANSISTORS

Organic field-effect transistors gated by an electrolyte in direct contact with the organic semiconductor have been recently reported as ultra-sensitive potentiometric devices. The operations and perspectives of these device architectures are discussed.

Organic bioelectronics is a promising platform for the next generation of biosensors, because of low-cost processing, high-throughput, integration on flexible/stretchable substrates, and biocompatibility. Different device architectures are being developed, with the aim to provide ultra-sensitive biosensors, cell signal transducers, implantable devices. Envisioned applications include "*real-time*" monitoring during a transient state (like an immune/inflammatory response), triggering and stimulating the regeneration of an injured site (as spinal cord injury) and the logic control of drug-eluting devices [1].

OFET sensors operate as interfacial devices. Relevant interfaces are involved during OFET operation: i) charge injection interface between electrodes and the organic semiconductor (OSC), ii) bottom gate dielectric/OSC, where the analyte modifies the charge carrier density, iii) OSC/environment which is capacitively coupled by a second (top) dielectric layer. As an example, DNA can be detected because of its negative charge that effectively couples to the OFET, when covalently tethered to electrodes [2] or physically adsorbed on the OSC surface [3]. Specific bio-recognition can be integrated by means of chemical tailoring of materials and/or interfaces. A clear example was the discrimination of the enantiomers of β-citronellol by endowing OSC with chiral pending group [4]. A biotin sensor has been demonstrated by embedding a phospholipid layer buried underneath the OSC film [5]. In all these cases, OFET is exposed to aqueous environment, but is operated in a "dry" state.

OFET operations in liquid have been demonstrated by developing architectures suited for water immersion or exposure such as single and dual gate Organic Field-Effect Transistor (OFET), Electrolyte-Gated Organic Field-Effect Transistor (EGOFET) [6] and Organic Electrochemical Transistor (OECT) [7], as shown in Fig. 1. Both OFETs and EGOFETs respond to small changes of electrostatic potential at the interface between the OSC and the aqueous solution. Differently from OFETs, OECT responds to doping/de-doping by ions diffusing into the bulk of a conductive polymer from the environment. Our contribution aims to highlight only the role of interfaces in OFETs.

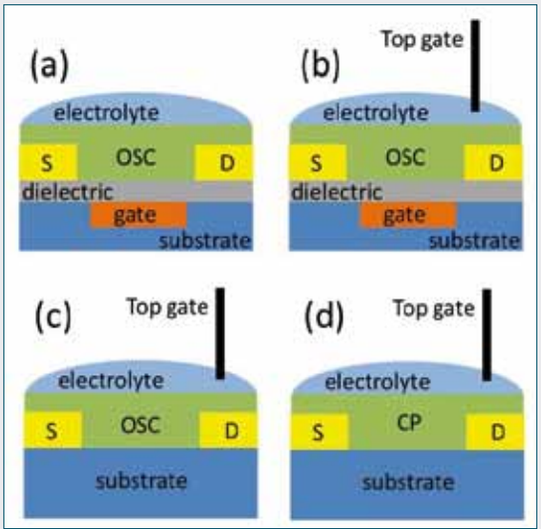

Fig. 1 - a) Liquid Organic Field-Effect Transistor; b) Dual-Gate Organic Field-Effect Transistor; c) Electrolyte-Gated Organic Field-Effect Transistor; d) Organic Electrochemical Transistor. S, D and CP stand for source, drain and conductive polymer

In OFET in contact with an aqueous solution, the change of the OSC/environment interfacial potential, Ψ_0, is amplified by the threshold voltage shift according to $\Delta V = -C_{top}/C_{bottom} \cdot \Psi_0$, where C_{top} and C_{bottom} are top and bottom gate dielectrics respectively. With a water-stable OSC, a bottom gate device can be directly exposed to the aqueous environment. Ultra-sensitive response

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is ascribed to the capacitive coupling mediated by the Debye-Helmholtz layer. Specific recognition was achieved by adsorbing/grafting the recognition probe on the channel surface. Successful examples include immunoassays with adsorbed antibodies [8], and gold nanoparticles coated with DNA aptamers designed for binding Hg ions [9]. The device works better at low ionic strength, since the probe-target size exceeds the typical screening length scale that is dependent on the ionic strength.

To extend the operating voltage range and impart further stability, a high-k dielectric can be placed between the OSC and the environment. Examples include cross-linked thin polymer layers [10], ferroelectric polymers [11], ultra-thin oligo-alkane films [12]. The interfacial potential Ψ_0 is controlled by an electrode immersed in the medium, and modulated by adsorption, change of electrochemical potential, cell bioelectricity etc. Dual gate OFETs have been used as pH-meters, similarly to ISFET, but with the clear advantage of a sensitivity exceeding the Nernst limit [13]. The capacitance of the OSC/electrolyte interface can be measured with a dual gate OFET [14], and shown to yield a capacitive coupling more than 500 times larger than a traditional silica layer. The bottom gate modulates the response. Moving from bi-distilled water to 0.1 M NaCl solution, the double layer capacitance rises from 7.8(±0.8) $\mu F/cm^2$ to 14.6(±2.0) $\mu F/cm^2$ according to the ionic strength increase. The characteristic time constant of the electrical charging/discharging is 4.6 ms, and the detection limit of potential changes in the electrolyte is 50 μV, thus enabling the extracellular detection of cell signals. EGOFET is like dual gate OFETs but operates only with a top gate immersed in the aqueous solution. Stability can be improved by blending P3HT and PMMA [15], or by the use of a phospholipidic membranes cast on OSC film [16]. The control of the Debye-Helmholtz layer through the gate electrode/electrolyte interface is prompting EGOFET as promising ultra-sensitive biosensors and transducers. Detection of DNA [17] and penicillin [18] has been achieved by the direct interaction between the target and the functionalized OSCs. Central to the sensitivity are the electrical dipoles and the local pH changes in proximity to the conductive channel. A further example is an EGOFET supported by a biotinylated phospholipidic bilayer that confers a high-grade of sensitivity and selectivity towards streptadivin [19]. In this respect, our group has recently presented two further examples: i) a dopamine biosensor [20] and ii) an extracellular transducer for neuronal signals [21]. The former is an EGOFET, whose sensing core resides at the gate electrode/electrolyte interface (Fig. 2a). This gives an unprecedented opportunity to decouple the recognition system from the electrical transducer allowing a more flexible engineering. In terms of sensitivity, the threshold voltage senses dopamine down to pM scale (Fig. 2b). The latter proves the EGOFET capability to stimulate and record the collective bioelectric signals of neurons grown on top of it (Fig. 2c and Fig. 2d). This again proves the concrete possibility to fabricate EGOFETs as interfacing and implantable supports.

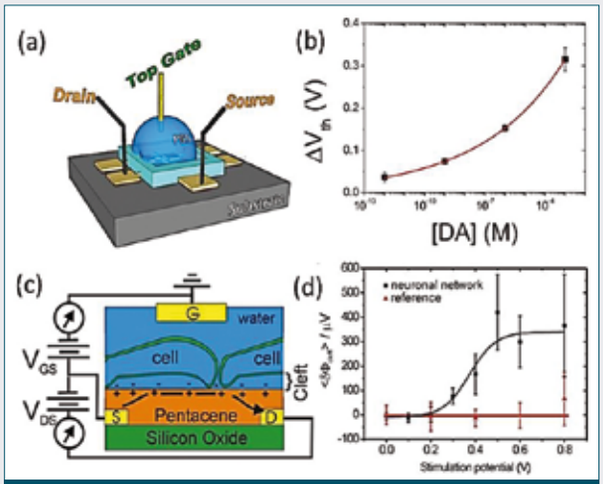

Fig. 2 - a) EGOFET biosensor architecture; b) threshold voltage sensitivity for dopamine (reprinted with permission from [19], copyrights @ 2013 Elsevier); c) extracellular transducer for neurons; d) change of the potential cleft after stimulation (reprinted with permission from [20], copyrights @ 2013 Royal Society Chemistry)

In conclusion, this brief perspective deals with the main OFET configurations capable to operate as biosensors. The great potential of these devices is their ultra-sensitivity together with the possibility to make them biocompatible, aiming at point of care tests that are appealing to several areas such as healthcare, medical diagnostics, food monitoring, e-textile etc.

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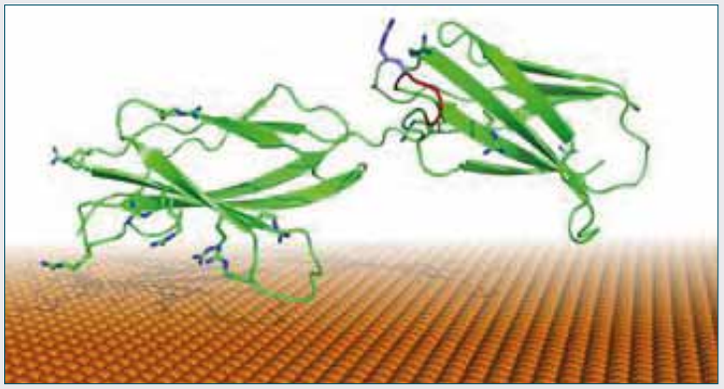

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PROTEIN-SURFACE INTERACTIONS

The relevance of the interaction between proteins and solid surfaces in organic bio-electronics is remarked, and the related open issues are discussed. In particular, the potentialities and the current limitations of molecular modelling applied to this field are critically discussed.

The interaction between proteins and solid surfaces (organic or inorganic) is a central issue in organic bio-electronics. On the one hand, specific proteins may be part of an organic bio-electronics device themselves: properly chosen proteins (e.g., enzymes, antibodies, DNA-binding proteins) are ideal to provide specificity toward organics molecules or other biomolecules, or to work as redox couples able to respond to applied potentials (electron transfer proteins). For these applications, the proteins need to be supported on a surface, and depending on the setup, may also need to exchange electrons with the surface. On the other hand, in case organics bioelectronics are used to investigate cells, the latter sense their environment also by the proteins in the extracellular matrix. Again, the interaction of such proteins with the surface, e.g., of a organic device, concurs to determine how the cell behaves, and in turn may provide information on the biological activity of the cell itself.

Concerning the first aspect, a fundamental point to be understood is how the protein functionality to be exploited is affected by the immobilization on the surface. This translates to specific questions, such as: what are the stable orientations of the adsorbed protein on the surface? Does the adsorption induce changes in the protein structure and internal dynamics and how would these changes affect functionality? How is the peculiar environment of the interface (e.g., water at interfaces, presence of charges) directly modifying the relevant protein properties?

Concerning instead the interaction of cells with surfaces, a fundamental question from the molecular point of view is: given a certain protein composition of extracellular matrix, which proteins adsorb on a given surface, as a function of time? This requires to consider not just the kinetics of the initial protein-surface encounter complex but also the subsequent processes that involve proteins orientational and structural reorganization and possibly displacement from the surface by the other proteins. To this a second question follows: how does the adsorbed proteins interact with the cell, i.e., which biological reactions do they trigger? Again, clarifying these issues requires to answer other specific questions besides those already mentioned above, such as: what is the relative diffusion rates of the proteins toward the surface, and then the probability of being adsorbed? What proteins can be displaced from the surface, and by what other proteins? Which "cryptic epitopes" [1] can be made accessible by structural reorganization upon adsorption? And which normal epitopes become instead inaccessible [2]?

As in other physical-chemistry research fields, molecular modeling (e.g., ab initio calculations, molecular mechanics, coarse grained approaches, multiscale models involving combination of them) has the potential of providing a complete microscopic picture (a chemical understanding) of protein-surface interactions. Joint with experimental work, it can be exploited to enhance the information accessible by the measurements [3], see Fig. 1, as already done in other contexts such as spectroscopies of organic molecules in gas phase and in solution. Moreover, when the modeling becomes accurate enough, it can also acts directly as a technological tool to assist the design of devices with better performance.

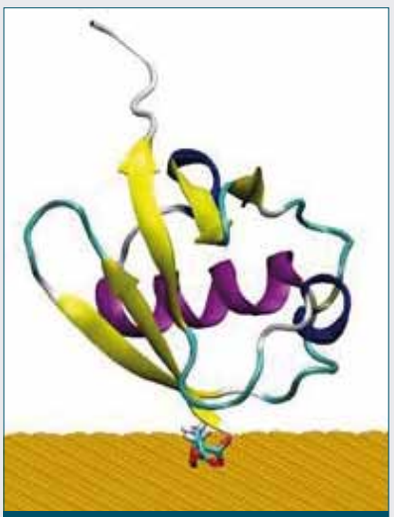

Fig. 1 - Most likely orientation of ubiquitin on Au in the presence of citrate ions as jointly determined by NMR and protein-surface docking [3]

Several issues need to be addressed for enabling accurate molecular modeling of protein-surface interactions. Some of them are common to the simulation of proteins in solution. The trade-off be-

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tween accuracy and computational feasibility makes at present classical molecular dynamics simulations the main tool to study proteins. Well-known limitations of classical MD are:

i) the accuracy of the underlying force fields (not just for the protein, but also for water and ions);
ii) the limited time scale accessible (100 ns-1 μs, ms only with a dedicated machine [4]);
iii) the incapability of directly simulating electronic phenomena (electronic spectroscopy, electron transfer rates). All these issues define areas of intense current research, such as the development of better (e.g., polarizable) force field, of effective enhanced sampling techniques, and of hybrid multiscale approaches (e.g., QM/MM/Continuum), respectively.

Then, there are issues specific of protein-surface systems [5]. To mention the most pressing:

i) we need to properly interface computational approaches developed separately for surfaces and for biomolecules. The task is less difficult for ab initio methods (smaller number of system specific assumptions) which however have limited applicability "as is" due to the prohibitive computational cost. More work is required for empirical approaches (e.g., functional forms of the force fields is often different for solids and proteins); parameterization of protein-surface force fields (either empirically or using ab initio calculations) is a very active field of research [6];
ii) liquid water on a surface is different from bulk water (from the structural, dynamical, dielectric and chemical point of view); the use of bulk models (e.g., implicit solvents) at interface requires re-validation, and often adjustments or new developments [6, 7];
iii) comparison with experiments, needed first to validate the models and then to exploit them as interpretative tools, is best done when it is possible to directly evaluate *in silico* the same quantities experimentally measurable. This requires developing models for the probing techniques used at the surfaces (either specific for them such as scanning probe approaches, or adapted from bulk techniques), as well as for the measurable outcomes in organic bio-electronics;
iv) relevant surfaces are far from being ideal. Often we do not have enough experimental information to build a reliable model, and molecular modeling should also consider strategies to tackle this uncertainty (e.g., use modeling itself to build the defected surface, consider many realizations and see which one better agrees with experiments).

All these issues represent stimulating challenges for the computational scientist community; solving them, that requires continuous interaction with experimentalists, will provide new tools useful to understand and then to improve organic bio-electronics devices.

Acknowledgements: The author thanks IIT under Seed project MOPROSURF and MIUR under FIRB project Italnanonet for funding.

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OPTICAL SENSING IN DIAGNOSTICS

Optical sensing devices are important tools for diagnostics in the biomedical field. We briefly discuss the concepts of optical techniques as well as the minimum requirements needed for optical sensing devices in order to afford systems for low-cost and easy-to-use real-life applications.

Nowadays there is an emerging need for reliable, fast, low-cost and easy-to-use sensing systems in many fields, such as security [1], environmental sciences, pharmacology, diagnostics and therapeutics. The last two areas, in particular, are at the forefront of medical research due to the great impact on welfare and society [2-4].
A sensing system consists of a probe able to identify a target through a recognition process that has to be transduced in a measurable signal. The requirements needed for applicable and marketable sensing systems are i) target specificity, ii) high sensitivity, iii) repeatability and reproducibility of the output signals, iv) fast response, v) stability of the probe, vi) easy and cost-effective detection. Optical sensors have the potential to address all these requirements, as discussed below.
The term optical sensor generally defines a probe whose interaction with a specific target is transduced in a change of optical properties. Optical sensing can be pursued via spectroscopic (colorimetric and fluorimetric) detection or by means of evanescent-field or plasmon resonance based methods.
The latter detection techniques mainly use label-free sensors and are based on variations of physical properties (refractive index or dielectric constant) of the local surface environment of the sensor upon binding with the target [5]. In this overview we will focus on label-based spectroscopic optical sensing that exhibits several peculiar advantages. The transduction signal reflects variations of the electronic properties of the probe upon target binding [6] in either the ground state (colorimetric sensor) or the excited state (luminescent sensor), as depicted in Fig. 1. Recognition can be based on the formation of a non covalent complex between the probe and the target, on a chemical modification of the probe/target molecular structure, *i.e.* with the formation of a strongly absorbing or intensely emitting unit, or on conformational changes induced by the binding event.
The majority of optical sensors developed so far for biomedical applications are based on fluorescence detection [7-9]. The chosen fluorophores are typically stable organic molecules exhibiting well defined fluorescence signatures (*i.e.* spectrum, quantum yield and lifetime) so that any variations of these features can be easily detected. The transduction mechanisms are essentially fluorescence quenching or fluorescence enhancement upon target binding, and change in emission colour or lifetime.
Examples based on fluorescence quenching are present in the literature [10], however, for optical sensing in biological systems, such strategy is not reliable due to the complexity of the local environment that molecular probes experience in living matter, which can strongly interfere with the detection.
Fluorescence enhancement detection, or "off-on switching" is considerably more reliable. Several examples have been reported, mainly for the selective sensing of ions, where the switching from "dark" to "light" is unambiguously shown [11-14].
Signalling based on fluorescence spectral variations is also promising, particularly in optical microscopy techniques; it can be achieved via monomer-excimer switched emission [15, 16] or *via* Förster resonance energy transfer (FRET) phenomena involving a donor-acceptor based sensor system [17].
Colorimetric sensors are less common but recently have gained attention in the biomedical field [18, 19]. The variation of the absorption spectrum of a chromophore upon binding with a specific analyte can be quite remarkable and specific.
Marked colour changes can be detected with very simple detection techniques or even by naked eye, making this approach one of the most technically and economically affordable. However, some highly specific dyes can exhibit low absorption cross sections and this requires further progress in signal amplification strategies to reach acceptable sensitivities [20].
In biomedical diagnosis, sensing mechanisms often exploit natural biochemical processes, *e.g.* the antibody-antigen specific binding. The design of sensory systems able to interact with target biomolecules can follow two main strategies: i) optically silent but biologically active molecules covalently labelled with optical probes (small chromophores or fluorophores can be also genetically targeted in proteins) [21] or ii) individual units containing both the biological and the signalling functionality.
The transition from fundamental studies in solution (which are needed to know the key photophysical properties of both targets and probes and the recognition mechanisms) to the construction of sensors devices that can be implemented in diagnostic tools, needs several converging competences, ranging from material chemistry to organic synthesis and from spectroscopy to biochemistry. The goal is the immobilization of the probe on a properly functionalized surface while trying to maintain both its optical and biological activity. Several parameters influencing these functions need to be carefully evaluated, such as surface composition, free volume of the functional group, probe density, and distance between the probe and the surface [22, 23].
Innovative techniques are emerging to control surface functionalization

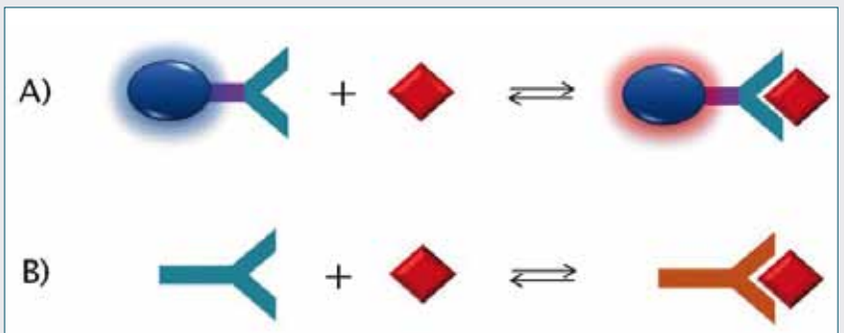

Fig. 1 - Schematic representation of A) a luminescent sensor and B) a colorimetric sensor

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Fig. 2 - Comparison of confocal fluorescence intensity image (left) and confocal fluorescence lifetime image (right); authors' unpublished data

such as platform approach, co-attachment of spacers, non-covalent piercing of probes into SAMs [24-26], with substantial room for further progress.

Detection techniques range from bulk spectroscopy (steady-state and time-resolved) to more sophisticated optical approaches for the investigation of materials deposited on solid surfaces. During the last decade, targeting detection at the single molecule level has prompted remarkable advances in temporal and spatial resolution of optical microscopies. Confocal fluorescence microscopy is now the most widely utilized technique for imaging luminescent probes in living systems. In particular, one of its applications, Fluorescence Lifetime Imaging Microscopy (FLIM), can ultimately exploit the potential of label-based sensing relative to label-free techniques [27-29].

Imaging based on the spatial distribution of the fluorescence lifetime of the probe in place of - or complementary to - fluorescence intensity can be much more informative and quantitative on the specific binding event (Fig. 2). Recently developed "super-resolution" microscopy techniques have become one of the most powerful microscopic tools available [30]. Among these, with the acronym RESOLFT (REversible Saturable OpticaL Fluorescence Transitions) are usually generalized different techniques, such as STED- (STimulated Emission Depletion) and GSD- (Ground State Depletion) microscopy, that take advantage of saturation of an optical transition to create a "dark" region around the bright fluorescent spot used for the analysis [31, 32]. Spatial resolutions of the order of tens of nanometers can be achieved, making these techniques crucial to study recognition events in constrained environments such as device surfaces. However, these super-resolution techniques, though very powerful, are far from being cost-effective and easy-to-use and often require the use of sophisticated hardly affordable fluorescent labels. A truly groundbreaking progress for optical sensing applications will entail the development of sensors devices where the detection can be achieved with user-friendly, portable and cheap techniques. The so called "point-of-care detection" is an essential requirement in resource-limited settings and recent literature shows how medical research in diagnostics is now focused on this issue, due to the great social impact that may arise [33]. In this context, optical sensors devices represent a real challenge for fast, sensitive and selective sensing.

Acknowledgments: The authors thank the MIUR-CNR project Nanomax N-CHEM for financial support.

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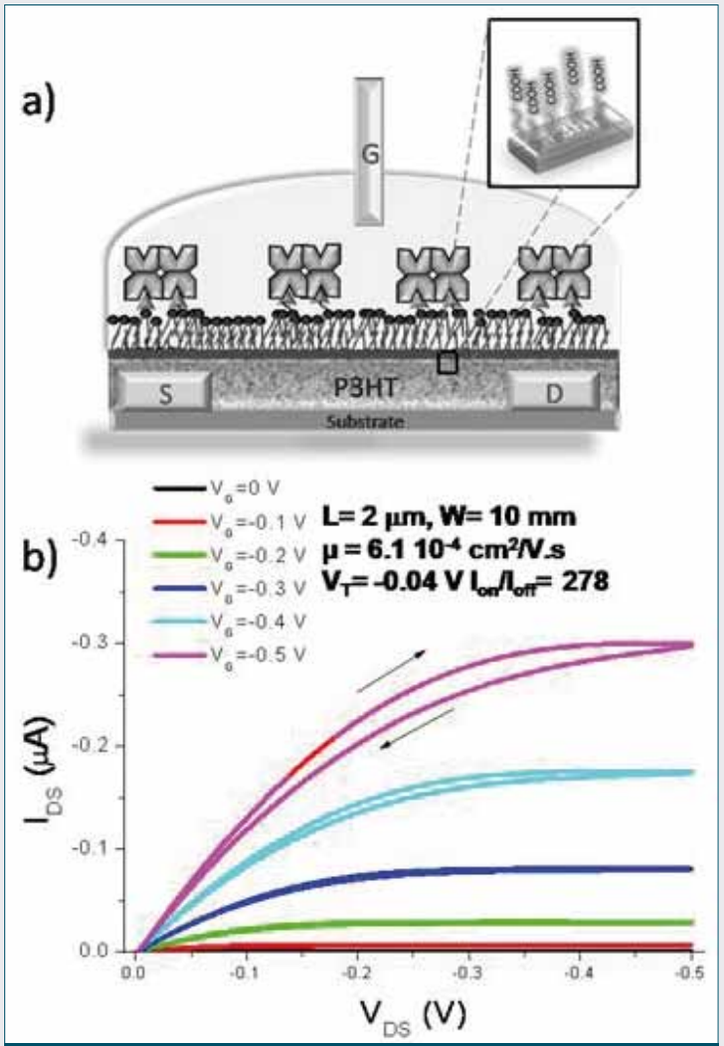

Fig. 1 - a) Structure and b) I_{DS} vs. V_{DS} curves of the biotinylated PL-EGOFET

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INTEGRATION OF BIOMOLECULES IN EGOFET DEVICES. ORGANICSEMICONDUCTOR SURFACE MODIFICATION

Plasma Enhanced Chemical Vapor Deposition (PE-CVD) process was employed to functionalize the poly(3-hexylthiophene) (P3HT) surface of an Electrolyte Gated Organic Field Effect Transistor (EGOFET). The modified surface served for oriented immobilization of biotinylatedphospholipid (PL) layer. Both the functionality of the bio-layer and the electrical proprieties of the organic semiconductor were retained. The EGOFET device was successfully used as biosensor for the detection of streptavidin.

Introduction

Organic field effect transistors (OFET) have attracted much attention the past few years for their promising applications in chemical and biological sensors [1, 2]. A transistor-based sensor combines a multi-parametric sensor and an amplifier, thus enabling the development of sensing devices with high sensitivity and low detection limit [3, 4]. Among OFET configurations, Electrolyte Gated Organic Field Effect Transistors (EGOFETs) are considered very promising for sensing applications due to their ability to operate in liquid media at low voltage (less than 1 V) [5], easy fabrication and compatibility with biological molecules [6]. These devices can be fabricated with solution processed techniques and integrated on various substrates, including flexible ones. Moreover, molecular recognition can be realized by proper surface tailoring of the structural layers of the transistor (e.g. OS and gate electrode). Therefore, EGOFETs have been successfully used for sensing DNA [7], dopamine [8], proteins [9] etc.

In previous reported configurations of EGOFET biosensors, the bio-recognition element is immobilized either on the metal (usually gold) surface of the gate electrode using self assembled monolayers or on the surface of the semiconductor. In the former case, the recognition event takes place at the interface between the electrolyte and the gate electrode and indirectly affects the charge distribution in the semiconductor channel. On the other hand, for the immobilization of biomolecules on the organic semiconductor surface, functional groups such as -OH, -COOH, $-NH_2$ able to anchor the desired biospecies are essential. Synthesis of properly tailored conjugated polymers is most commonly utilized. Main bottleneck of this approach is that the presence of functional groups located either on the backbone or on the side chains can disrupt or even destroy the electrical properties of the pristine polymers [10]. Alternative methods to immobilize bioprobes to the semiconductor's active layer surface without affecting the bulk properties of the polymer are required. Herein, an alternative method for surface functionalization of P3HT organic semiconductor (OS) and immobilization of bioprobes that ensures good semiconductive functionality is discussed.

Questo articolo è stato presentato nel corso di "Avogadro Colloquia", Bologna, 29 ottobre 2012. L'evento è stato promosso dalla SCI ed organizzato in collaborazione con l'Alma Mater Studiorum Università di Bologna, l'Università di Bari "A. Moro" e il CNR.

EGOFET configuration OS surface functionalization

In the most common architecture, the EGOFET consists of a semiconducting polymer film in contact with an electrolyte in top gate bottom contact configuration. A gate electrode is immersed in the electrolyte and source and drain electrodes provide electrical contact to the channel. Considering that biological sensing applications take place in aqueous media, water-gated organic field effect transistor can be utilized. In this case, water can act both as the electrolyte and the media responsible for carrying the analyte sample. However, the EGOFET structure suffers by two main issues:

a) the biological recognition elements must be placed on the hydrophobic surface of the OS;

b) the contact with an electrolyte solution often results in doping of the semiconductor.

In order to tune the hydrophobic surface of the P3HT layer, plasma deposited ethylene/acrylic acid (pdEthAA) nanometric coatings were deposited on the surface of OS. PE-CVD process has the advantage of lower deposition temperatures, which is crucial in many semiconductor thin film deposition processes [11].

Carboxylic -COOH groups were formed at the surface of plasma processed P3HT without affecting the bulk properties of the material and served as excellent anchor groups for binding biomolecules. A phospholipid bilayer was deposited afterwards so as to prevent the doping of the semiconductor.

The supported PL bilayer was linked to biotin thus allowing the electronic biosensing of streptavidin in solution. Fig. 1a shows the structure of water-gated OFET along with the modified P3HT and the bitotinylated phospholipids layer.

Electrical and sensing performance of the EGOFET device

The electrical performance of the EGOFET based sensor with biotinylated phosholipids is depicted in Fig. 1b. The EGOFET electrical measurements were carried out by using a 2 μL droplet of water (HPLC grade) or PBS (phosphate buffered saline 10 mM, pH=7.4) as gate dielectric. The thickness of the pdEthAA coating was optimized in order to minimize any effect on the electrical performances of the EGOFETs. A coating time of 3 seconds found to have negligible impact on the figures of merit of the device compared to pristine P3HT [11]. The EGOFET device was employed for sensing streptavidin.

The calibration curve for different concentrations of streptavidin is shown in Fig. 2 as the relative response versus the analyte's concentration. The drain current increased upon exposure of biotinylated PL-EGOFET biosensor to streptavidin. This increase has been attributed to the accumulation of more holes in the semiconductor/PL interface due to binding of streptavidin on the surface of biotinylated PL bearing a negative charge in the aforementioned pH that the sensing measurement is realized [9].

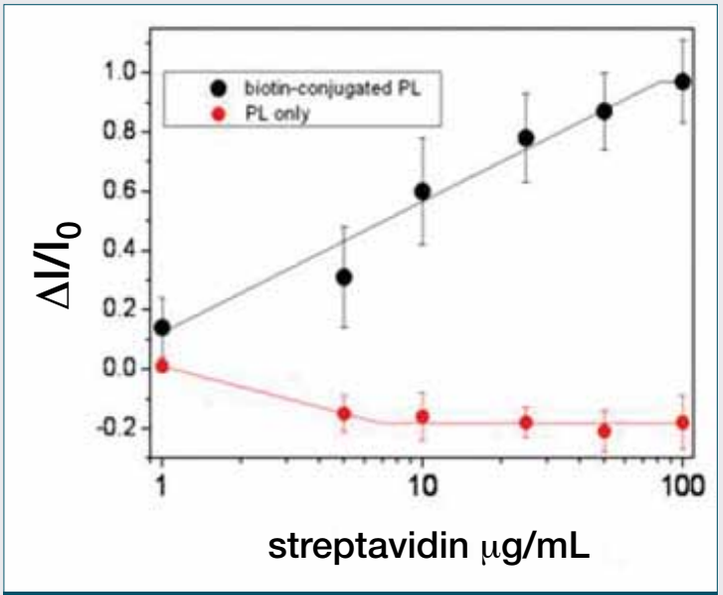

Fig. 2 - Relative response of the biotin-PL and bare PL-EGOFET transducers exposed to different concentrations of streptavidin

Conclusions

The results showed that PE-CVD is a valid approach to functionalize P3HT surfaces with carboxyl groups, leaving unaffected the EGOFET performances. Moreover, the presence of -COOH groups on the surface of the semiconductor allowed oriented deposition of the PLs layer. The PL layer served as a barrier layer holding the ions from immigrating from the electrolyte to the organic semiconductor layer. In addition, biomolecules denaturation is prevented and high sensitivity and selectivity is obtained.

Acknowledgment: B.R. Pistillo (formerly Univ. Bari) is acknowledged for useful discussion.

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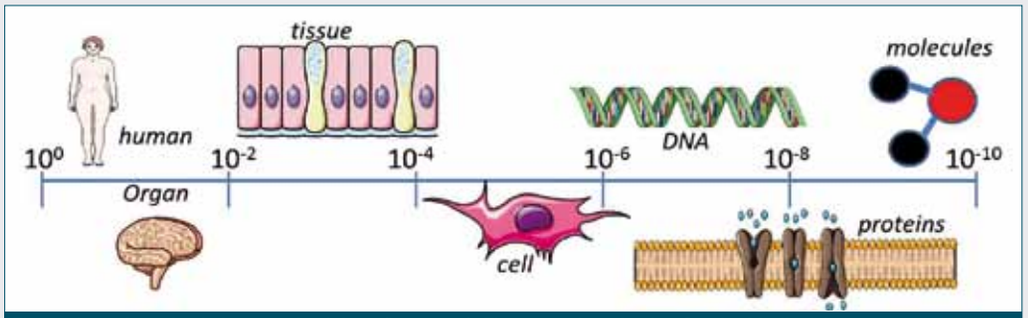

Fig. 1 - Length scales in human physiology (in meters). Figure produced using Servier Medical Art

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ORGANIC ELECTRONICS AT THE INTERFACE WITH BIOLOGY: A BIOLOGIST'S PERSPECTIVE

In this article I will set the stage for the use of organic electronics in biological applications by considering three basic questions: 1. Why do we want to interface with biological systems? 2. What are the levels of complexity in biological systems? 3. Why use organic electronics for interfacing with biological systems. I will outline the key advantages of organic materials and show examples from literature where key properties of organic electronic materials have been used to great effect to probe biological systems, or develop tools that will be beneficial to human health. I will conclude by attempting to outline future applications for organic electronics at the interface with biology.

Why do we want to interface with biological systems?

Biologists seek to understand the basic processes involved in biological systems. This is both for fundamental research, to understand the complex mechanisms involved in life but also for applications including but not limited to human health, the environment and also biotechnology. For the purpose of this article I will limit myself to the consideration of human health only, an already vast application area. Our interface with biology can be classified into three categories. The first category is a fundamental understanding of biological processes in human physiology; the second is the development of diagnostics to be able to detect disease or infection in humans; and the third is to develop treatments and preventative measures for human disease. These three categories are obviously interrelated and it is clear that knowledge of fundamental processes and systems is required to achieve both effective diagnostics and treatment. It is also clear that there are many unanswered questions which will rely on the development of new technologies to achieve answers.

What are the levels of complexity in biological systems?

A frequent difficulty encountered by physical scientists and engineers when attempting to interface with the world of biology, is finding a suitable application that will best utilize their technology. Unfortunately there is a communication gap between the two worlds, exacerbated by different vocabularies and differences in background which make exchange difficult. Fortunately efforts are afoot to improve education, journals which were hitherto the domain of engineers are becoming multidisciplinary, e.g. *Advanced Healthcare Materials* and *Journal of Materials Chemistry B.* Increasingly, faculties at universities are reaching out to start collaborations colleagues in other colleges. Also encouraging is the number of cross-disciplinary labs, departments and centers being created.

Engineers and physical scientists like to consider machines as a whole, to understand how the component parts are interconnected and to have an overall view of the working system, a so called top down view. Biology is often conversely considered from a bottom up view, starting from individual molecules moving to cells and sometimes continuing on to the whole organism, although in many cases biologists restrict themselves to a single length scale, classifying themselves as either molecular biologists, cell biologists or physiologists, rarely taking an engineer's whole system view. In this article, I will briefly describe human physiology from a top down view to paint a more appealing picture to the engineer or physical scientist.

If we consider animal physiology from a top down view we should view the organism as the product of assembly of a multilevel process. Important length scales in this multilevel process are shown in Fig. 1. The first sub level of the human organism is the organ system, examples including the nervous system, the digestive system, the reproductive system etc. These organs systems are in turn made up of a variety of different tissue types. By simplifying somewhat we can consider 4 main types - epithelial tissue that is exposed to the outside and covers all surfaces of organs in the body, connective tissue which underlies

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and surrounds other tissue types, muscle tissue which generates the force to move, and finally nervous tissue which controls the processes involved in movement and communication. Tissues are in turn made up mainly of cells, of which there are over 200 types known in animal physiology. Well known cell types include neurons, blood cells, muscle cells and epithelial cells. Continuing downwards, cells are made up of and contain a series of macromolecules including protein complexes that carry out all of the cellular functions, synthesize DNA etc., nucleotide macromolecules such as the DNA double helix that carries our genetic inheritance, lipids which form important structures and compartments surrounding and inside cells, and finally polysaccharides that have such diverse roles as components of connective tissue and markers on proteins to signal to cells. The final level of organization is at the molecular level. The building blocks of biological processes (conserved in all phyla) are the following molecules: amino acids, nucleotides, sugars and lipids. All of the macromolecules cited above are ultimately made up of these building blocks. With the help of metals ions such as calcium, sodium and potassium and a couple of other factors, such as vitamins and cofactors, nature carries out all of the billions of reactions and processes involved in life. This grossly oversimplified description of human physiology is the result of a couple of centuries of research, research that has been greatly aided by advances in technology, particularly in the last 50 years or so.

Historically, progress in both fundamental and applied areas of biological sciences has relied on new developments in technology. If we take the example of microscopy, consistent advances in optics have allowed higher and higher resolution allowing researchers to view whole animals, organs, cells and subcellular complexes. Super resolution methods have become available break through the theoretical diffraction limit (outlined by Ernst Abbe in 1873) making it possible to visualize objects of less than 250 nm. These new methods include PALM (photoactivated localization microscopy) or STORM (stochastic optical reconstruction microscopy) [1] and two photon excitation microscopy a technique that allows depth imaging in tissues even up to 1 mm [2]. Biologists (in particular clinicians) are often relatively reluctant to embrace novel technologies. This is understandable given the length of time and workload required for validation of new technologies, a burden which rests almost entirely with the end-user. However, occasionally a breakthrough occurs which undeniably benefits the community of life scientists and ultimately the human race due to improvements in medicine and healthcare such as the example cited above for microscopy. Some key examples of technology advances which have advanced medicine include PCR (polymerase chain reaction) [3], rapid DNA sequencing [4], peptide fingerprinting (MALDI-enabled peptide identification) [5], cell counting and flow cytometry [6], surface plasmon resonance (protein-protein interactions)[7] and many, many more.

At the last annual general meeting of the American Society of Cell Biology in San Francisco, an inspiring keynote address was given by US secretary for energy and Nobel laureate Steven Chu, who underlined the necessity for biologists and physical scientists and engineers to collaborate to push forward the frontiers of biology. Dr. Chu himself won the Nobel prize for his work on cooling and trapping of atoms with laser light [8, 9], in 1997, with Claude Cohen-Tannoudji and William Daniel Phillips. Long a proponent of using physical methods to understand biology, Chu has studied biofilm formation [10], protein modification in live cells [11] and RNA folding [12] using techniques such as fluorescence resonance energy transfer, atomic force microscopy, and optical tweezers. Chu is just one example of a pioneer who realized the benefits that could arise from applying fundamental knowledge in physics to applications, notably in biological sciences.

Why use organic electronics for interfacing with biological systems?

Traditionally, electronics have been an important component of technology applied to biology. Either involved in the transducer mechanism to allow an electrical readout, or simply downstream for signal processing or amplification. The field of *bioelectronics* has involved a more intimate coupling of electronics with biology, for example in electrodes for probing brain function, development of “active“ prosthetics, or biosensors for use in diagnostics. In most cases electronics have relied on inorganic conductors or semiconductors such as silicon. However, an emerging trend is the use of organic electronics, using ‘carbon based’ semiconducting polymers or small molecules [13]. Organic electronics originated in the 1960s from research on organic crystals [14] and the Nobel prize was awarded to Heeger, Mac Diarmid and Shirakawa in the late 1970s for their discovery that the semiconducting polymer polyacetylene could become highly conducting when doped [15]. The field of organic electronics saw a large growth during the 1990s and early 2000s with devices such as organic light emitting diodes (OLEDs), organic photovoltaics (OPV) and organic thin film transistors (OTFTs), but this development has since leveled off for OPVs and OTFTs [16]. Applications of organic electronics in biology, commonly termed organic bioelectronics [17] had a later start and are still showing a high level of growth in terms of publications per year. There are a variety of reasons which make organic electronic materials well suited for interfacing with biological systems. These advantages are discussed below.

Mixed conduction and ideal interfaces

The ability of organic electronic materials to conduct ions, in addition to electrons and holes, facilitates their communication with biological systems, which have tightly regulated ionic fluxes, and where ions act to transduce signals. Organic electronic materials can also form ideal interfaces with biological media, translating into extremely useful properties in devices that can increase sensitivity. In many cases the electrolyte can be considered as part of the device, the ions of the electrolyte continuously exchanged with the conducting polymer. This intimate connection between the device and the electrolyte, and by extension the biomoiety used for sensing or probing, ultimately results in a cleaner, more direct, interaction. This is in contrast to traditional silicon electronics, where oxide layers can buffer the connection

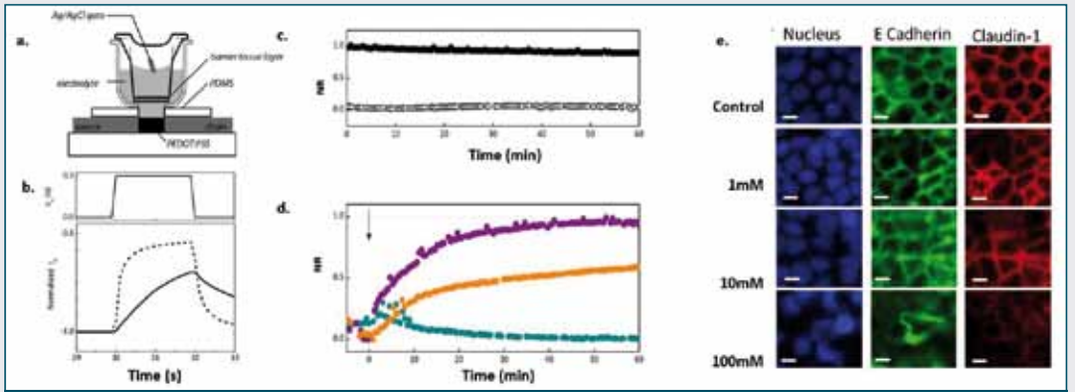

Fig. 2 - Operation of the OECT for toxin detection: a) illustration of OECT barrier tissue sensor. S, D, and G refer to the source, drain and gate electrodes; b) example of a typical OECT response with and without cells (right): the drain current (ID) response to a gate voltage pulse (top), the presence of cells is shown in black and the insert alone with a dashed line (bottom); c) control normalized response of OECT with cells (white) and without cells (black), with no exposure to ethylene glycol-bis(beta-aminoethyl ether)-*N,N,N',N'*-tetra acetic acid (EGTA); d) *in situ* monitoring of NR on addition of 1 mM (dark cyan), 10 mM (orange) and 100 mM (violet) EGTA. EGTA is added at time = 0, as indicated by the arrow; e) immunofluorescence of proteins in the apical junction upon exposure to EGTA. Monolayers were exposed to various concentrations of EGTA for 2 h and then stained with antibodies against apical junction proteins. In panel a: DAPI (blue), E-cadherin (green) and occludin (red); in panel b: DAPI (blue), E-cadherin (green) and claudin-1 (red). Cells were exposed to 0, 1, 10 and 100 mM EGTA from top to bottom. The scale bar is 10 μm

between the device and the biomoiety. This 'cleaner' interface leads to low interface impedance, which, for neural probe applications has proved invaluable as devices with lower impedance can record neural signaling with higher fidelity [18, 19]. The mixed conductivity of organic materials has been used to great effect in interfacing with biological systems. For example, the Berggren group used an organic electrochemical ion pump (OEIP) to deliver an ionic species (a neurotransmitter) to cochlear neurons in a guinea pig (*in vivo*) resulting in electrical depolarization of neurons [20]. An organic electrochemical transistor (OECT) has been used by my own lab to detect ion flow through barrier tissue *in vitro* as a means of detecting damage to these cell layers caused by pathogens and toxins resulting in an electrical readout [21-23], illustrated in Fig. 2. The OECT is uniquely sensitive to ions as the conducting polymer channel can be considered in a sense as having a 3-dimensional capacity for ion uptake due to ion penetration in the volume of the film [24]. The OECT has further been used to detect ion flow through ion channels embedded in lipid bilayers (again resulting in an electrical readout) [25a] and also to measure neuronal activity *in vivo* with unparalleled sensitivity [25b].

Freedom in chemical modification and ease of processing

The nature of polymer synthesis allows for a level of chemical modification not possible with inorganic materials. Various moieties can be covalently added to a polymer chain for the purpose of increased biological functionality. *In situ* polymerization has been explored extensively over the last two decades as a means of physical entrapment of various molecules, including large polyanions and bulky proteins [26]. This has mostly been from a tissue engineering perspective with a view to using conducting polymers as scaffold coatings for either drug [27] or cofactor [28, 29] release. Tuning chemistries is an extremely useful tool in optimizing materials for surface functionalization via vapor phase polymerization [30] and monomers have been produced that allow easy chemical linking of biological moieties to the polymer background using electropolymerisation [31]. An elegant example of the use of a modified CP was again by the Berggren group, who showed an example of a polymer system that could be used to detach cell layers in a 'smart petri dish' configuration [32]. Electrochemical oxidation was used to release the top layer of the polymer system, and thus release cells adhering to this layer. Cell detachment is normally done with an enzymatic treatment which cleaves adhesive surface proteins on the cells, rendering a significant population of the cells non-viable. This new method allows detachment of the cells without damaging the surface proteins. Although new conducting polymers and variants are coming through the pipeline, commercially available conducting polymers still constitute the majority of use in biomedical applications due to the ease of use and preparation of devices. Ink formulations are also available, opening the door to large scale printing of devices. Easily scaled-up processing techniques, such as spray coating and other roll-to-roll compatible techniques, also translate to low cost devices and it has been formally demonstrated that all-conducting polymer (PEDOT:PSS) devices are possible and have been demonstrated for use in glucose sensing [33]. In developing single use devices for point-of-care diagnostics, this remains extremely important.

Soft mechanical properties

One of the major application areas for bioelectronics has been for interfacing with the brain to either record signals or stimulate neuronal activity. Traditionally, silicon/metal electrodes or implants are used, however there is a significant mechanical mismatch that generates problems when the probes are implanted into the soft tissue of the brain, since metal and silicon probes have very high modulus of elasticities [34]. Martin and co-workers have shown how conducting polymers can be used to coat electrodes to improve biocompatibility and even result in an increased signal due to improved impedance of the CP coated electrodes [35, 36]. An added advantage of CPs are their conformability, which can allow better signal transduction, for example in neural applications [37]. Flexibility was often cited as an advantage for applications such as OLEDs that were envisioned coating surfaces of buildings and integrated in textiles, and is no less the case for development of novel sensing technology, either for wearable sensors [38], or indeed for direct incorporation into textiles [39].

Outlook

The idea behind this brief overview is not to try to trivialize the complexity of biological systems, but simply to show the community of physical scientists and engineers that biology is accessible from many different angles. Organic electronics provide a novel tool kit for interfacing with biology that is not meant to replace existing electronics, but rather to complement it. As the examples cited above show, in certain cases, the unique properties of organic electronics provide a solution to an otherwise unmet problem. The future appears to be bright for organic bioelectronics. To date organic electronics have been used to interface with a multitude of different biorecognition elements including DNA [40], bacteria [41], lipids [42], cells (neurons [43], epithelial cells [44], fibroblasts [45]…), tissue, organs (brains [36], ear [20]), and many more. Nonetheless, this is only the tip of the iceberg. Up until now a large focus of organic bioelectronics has been on neuroscience, an obvious target due to the electrical activity of neural cells and networks. However, other electrically active cells exist include cardiac cells and muscle cells. In addition to concentrating on the central nervous system, light could be shed on the peripheral nervous system or even the neuromuscular junction. As mentioned above, many different biological processes involve ion transfer or flow, including ion channels, signal transduction, and oxidative phosphorylation. This article has principally focused on human physiology, however, there are of course many other targets including microorganisms, plants etc. Perhaps biotechnology combined with organic electronics could result in new ways to harvest energy. One important aspect that should not be forgotten, and is particularly relevant to the chemistry community, is that materials development needs to keep abreast with the applications. In the future one could envision materials designed for specific applications, with functionalities including bioerodibility, biodegradability but remaining highly conducting [46]. In conclusion, this is an extremely exciting time to be involved in this area of research and it is clear that many novel and useful applications for biology will result from this interdisciplinary field.

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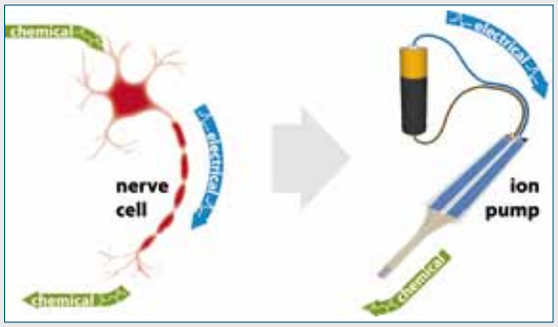

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MIMICKING NEURAL SIGNALLING

The neuron remains the most efficient interface to other neurons. To develop new brain-machine interfaces, we propose to mimic the neuron's functionality using organic bioelectronics. We detail the organic electronic ion pump and its potential to form the basis for an artificial neuron.

Nerve cells exemplify one of nature's solutions for electrochemical transducers. They take chemical and electrical inputs, process them biochemically, and deliver the appropriate signalling substances. Furthermore, neurons are highly selective to which signals they sense, can effectively transduce chemical and electrical signals, and can influence their environment by diffusive delivery of individual molecules (without requiring liquid flow). A human-made device that could operate in such a manner would provide a new, powerful method of interfacing with, modulating, and providing therapies for biological systems. Today, the predominant methods of modulating or otherwise interfacing with biological systems fall under two broad categories: pharmaceutical and electrical. Pharmaceutical techniques are appealing in their chemical selectivity. However, the basic method of systemic dosage suffers from drawbacks such as non-localized delivery, potential for significant side-effects, or toxicity in unintended regions [1, 2]. By exploiting advances in microfabrication, fluidic devices with channel dimensions on the order of microns have been developed for delivery of pharmaceuticals and bio-active substances [3]. Such devices have been demonstrated in a wide range of *in vitro* applications [4] and have been proposed for incorporation into established therapeutic implants [5]. As with more traditional methods, such as osmotic pumps, fluidic delivery has the advantage of administering any soluble compound to a well-defined region. The primary disadvantages are the dynamics - it is difficult to stop and start the flow precisely - and the fact that the flow can disrupt the fragile biochemical environment of the target [6]. Electrical methods such as deep brain stimulation (DBS) [7] show promise in their high spatial localization, but suffer from a poorly-understood mechanisms and the inability to discriminate among various cell types; all tissue in the region of the electric field is stimulated, leading to potential side effects. In addition, the majority of therapies do not take advantage of the body's own signalling system to regulate their function. An ideal system for therapeutically modulating biochemical signalling would combine the advantages of pharmaceuticals (chemical selectivity) and electrical stimulation (spatial localization), while at the same time utilizing the body's own signalling substances. Such a system appears to be functionally identical to a neuron itself; a neuron can be considered a chemical-to-electrical-to-chemical signal transduction unit. Chemical signals in the form of neurotransmitters are detected at the synapse, are then converted into an action potential and conveyed electrically down the axon, and finally are translated back into chemical release at the downstream synapse. It is important to note that the synaptic release of chemical substances via exocytosis is diffusive, avoiding both chemical and pressure-related disruption of otherwise stable biochemical gradients [6]. Recently, our group has demonstrated the conducting polymer-based organic electronic ion pump (OEIP) (Fig. 1) [8-10]. In OEIPs, electronic input is converted into the highly spatially and temporally resolved release of neurotransmitters and other biosubstances. In this way, the technology mimics the electronic-to-chemical signal transduction of a biological

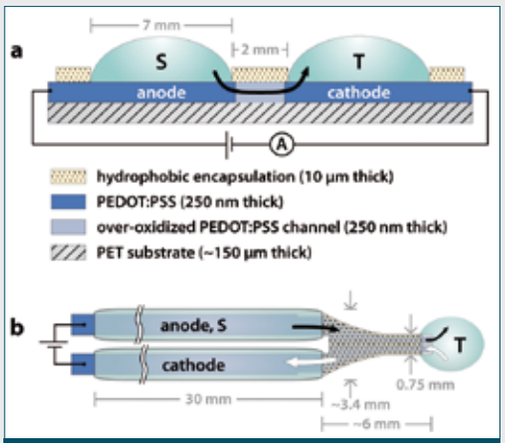

Fig. 1 - The organic electronic ion pump (OEIP): a) schematic side view showing one variation of the geometry. S and T indicate the source and target electrolytes, where cells would be cultured in the target system for *in vitro* experiments; b) schematic of the implantable encapsulated device utilized for *in vivo* applications. In this case, the target system is an arbitrary physiological electrolyte. In both figures, the black arrows indicate the flow of cations toward the target system, and the white arrow indicates the flow of (arbitrary) cations to complete the electrochemical circuit. Figure adapted from [9]

Questo articolo è stato presentato nel corso di "Avogadro Colloquia", Bologna, 29 ottobre 2012. L'evento è stato promosso dalla SCI ed organizzato in collaborazione con l'Alma Mater Studiorum Università di Bologna, l'Università di Bari "A. Moro" e il CNR

neuron. Substances are "pumped" electrophoretically based on the molecule's ionic charge, rather than delivered by liquid flow. Delivery is thus diffusive and avoids convective disruption, and thereby further mimics biological neurons in their diffusive exocytotic delivery. Due to the precise electrochemical relationships governing the OEIP, the administered dosage can be easily controlled by adjusting the electronic current. Finally, since the mechanism is electrophoretic rather than liquid flow-based, it can be stopped and restarted rapidly. OEIPs can precisely deliver positively or negatively [11] charged ions and molecules, including certain neurotransmitters. The technology has been used *in vitro* to establish pH gradients [12] and control neural cell signaling [8, 10], and *in vivo* (Fig. 1b) to modulate hearing function in a living animal by targeting a specific pathway in the cochlea [9]. OEIP technology have been used to develop chemical - rather than electronic - diodes [13] and transistors [11, 14, 15] (Fig. 2). These so-called "active" circuit elements behave in a non-linear fashion, that is, the output signal is not directly proportional to the input signal. This sort of non-linear behavior is a hallmark of neural signaling, which is threshold-based: a threshold concentration of neurotransmitters is required to trigger an action potential. With these biochemical "iontronic" active components, large-area matrix release could be realized, as in large area displays. Also, the ability to amplify biochemical "input" signals could pave the way for ionic computing and its eventual incorporation into existing biological networks. Indeed, we have already demonstrated basic logic functionality. Ionic diodes have been used to develop AND gates [13], and NOT and NAND gates have been demonstrated using ionic transistors [15]. It is important to note that since OEIP technology is based on thin films of conducting polymers and polyelectrolytes, devices can be manufactured in a variety of ways, using techniques including photolithography, *in situ* electrosynthesis, spin coating, screen printing, inkjet printing, and even high-throughput roll-to-roll printing [16, 17]. Furthermore, the organic electronic materials employed exhibit flexibility and rheological compatibility with tissue [18], making them ideal materials with which to develop devices for biological applications. Systems for micro-scale, electronically controlled substance delivery have been largely limited. Either delivery is flow-based, or it is difficult to control rapidly and dynamically, as in controlled release surfaces. Developing systems, which could translate dynamic, potentially automatic, electronic signals into accordingly dynamic (flow-free) delivery, could thus pave the way for new therapies for a variety of cell signalling disorders. Additionally, some applications would benefit from wide-area, or multiple-site functionality, whereby macroscopic settings such as wound healing, spinal cord injury, or even anti-fouling, could be addressed. Mimicking the electronic-to-chemical transduction of biological neurons with OEIPs and iontronic technology thus represents an ideal foundation for a new generation of treatments: (i) malfunctioning signaling pathways could be manipulated via the same regulating chemicals naturally used in the healthy state; (ii) automatic electronic regulation could provide therapy without requiring interaction of the patient or practitioner; and (iii) adverse effects arising from disturbance of the biological microenvironment would be minimized by diffusive, synapse-like delivery. Furthermore, the organic nature of the OEIPs and other iontronic components makes them ideal for interfacing biological systems and traditional electronics. We foresee a range tools leveraging these organic bioelectronic technologies either as a complement to existing therapies, or as the fundamental element in entirely new therapeutic systems.

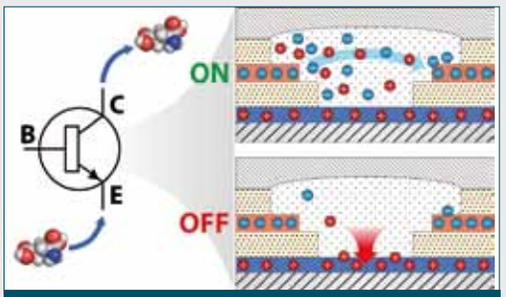

Fig. 2 - An organic electronic ion bipolar junction transistor (IBJT). A control voltage on the base, B, regulates the flow of anions from the emitter, E, to the collector, C, in the same manner as in OEIPs. In the ON state, cations in the (charge neutral) channel provide charge compensation for the transported anions. In the OFF state, the channel is depleted of cations, and anionic charge cannot be compensated. Figure adapted from [11]

Acknowledgements: This research was carried out within the OBOE Center (www.oboecenter.se) funded by the Swedish Foundation for Strategic Research (SSF) and the Swedish Innovation Office (VINNOVA). Additional funding was provided by the Swedish Research Council, the Knut and Alice Wallenberg Foundation, the Royal Swedish Academy of Sciences, the Önnesjö Foundation, and the EU project FP7-NMP-2011-280772 *Implantable Organic Nano-Electronics* (iONE-FP7).

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