



# ELECTROCHEMICAL BIOSENSORS FOR SENSITIVE MOLECULAR DIAGNOSTICS

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**Abstract** — Biosensors are self-contained analytical devices in which a bioreceptor is integrated with a transducer. The interaction between the bioreceptor and a target analyte generates a signal suitable for analytical purposes. In electrochemical biosensors, a change in the redox state of the biorecognition/analyte system generates a change in an electrochemical quantity which can be monitored by electroanalytical techniques. Electrochemical sensors can also be miniaturized using ultramicroelectrodes and nanoelectrodes and their arrays as transducers. These devices are characterized by high specificity and sensitivity and improved detection limits. Within the frame of the TRANS2CARE project, the Laboratory of Electrochemical Sensors (<http://lsegroup.wix.com/website-lse-group>) of the University Ca' Foscari of Venice is collaborating with some of the project partners in order to develop bioelectrochemical sensors/nanosensors for clinical/molecular diagnostics.

**Index Terms** — TRANS2CARE, electrochemistry, biosensors, affinity, proteins, electrodes, array

## 1 BACKGROUND

An electrochemical biosensor is defined as an analytical device composed by two main elements, in close proximity or even integrated one on each other: a biological recognition element and a transducer (electrode) which converts the recognition event into a measurable signal. Affinity electrochemical biosensors exploit the interaction between the recognition molecule and its specific target, to produce a detectable signal. The recognition event is strongly influenced by the characteristics (e.g., shape and size) of the interacting biomolecules and high specificity and affinity are required to ensure a strong and stable interaction. Different kind of interactions can be exploited to this aim, such as antibody-antigen complexes or the formation of the DNA double helix by hybridization between a probe and its complementary sequence. Electrochemical biosensors offer several advantages with respect to

other assays, because they are portable, cheap and the measurements are fast and easy to perform. Electrochemical sensors are furthermore provided with high sensitivity, high signal/noise ratios and can be miniaturized down to nm-size.

## 2 OBJECTIVES

Main objective of our research is the detection of DNA, proteins and other molecules of clinical interest by electrochemical detection strategies, employing nanostructured surfaces, nanoparticles, polymers with ionic properties, etc.

## 3 APPROACH & METHODS

1. Direct detection of molecules in biological samples (Fig. 1a) without functionalization of the electrode surface.
2. Modification of the electrode surface with a biological recognition layer (Fig. 1b), able to react specifically with a suitable target (= molecule of interest).

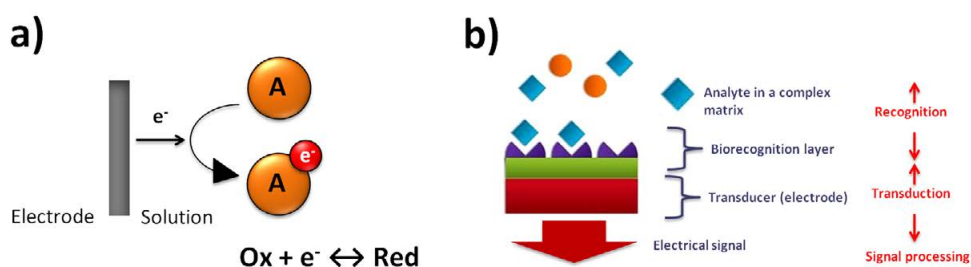


Figure 1: Schematic representation of the approaches described above.

## 4 RESULTS

The main results concern the study and development of affinity biosensors suitable for the detection of DNA hybridization or antigen/antibody interactions. For the first case, ensembles of nanoelectrodes (NEEs) [1] are used as platforms where either, the template membrane that surrounds the nanoelectrodes [2, 3] or the electrodes themselves [4], are exploited for the immobilization of the biorecognition macromolecules. These two detection schemes are summarized in Fig. 2, where the detection is performed by cyclic voltammetry (CV).

Antigen/antibody immunoreactions are exploited to analyze the antibody tissue anti-transglutaminase (anti-tTG) involved in the coeliac disease, a widespread condition characterized by an inflammatory intestinal disease associated with malabsorption. The recognition and detection principles are summarized in Fig. 3, where the detection is performed by electrochemical impedance spectroscopy (EIS).

Moreover, the use of electrochemical sensors for the electroanalytical detection of the bile pigment bilirubin (BR) and its oxidation and reduction products has been studied. BR has been chosen as target molecule because of its clinical importance in several diseases [5]. Indeed, BR can be toxic under certain conditions, especially in neonates. However a mild hyperbilirubinaemia may have a protective

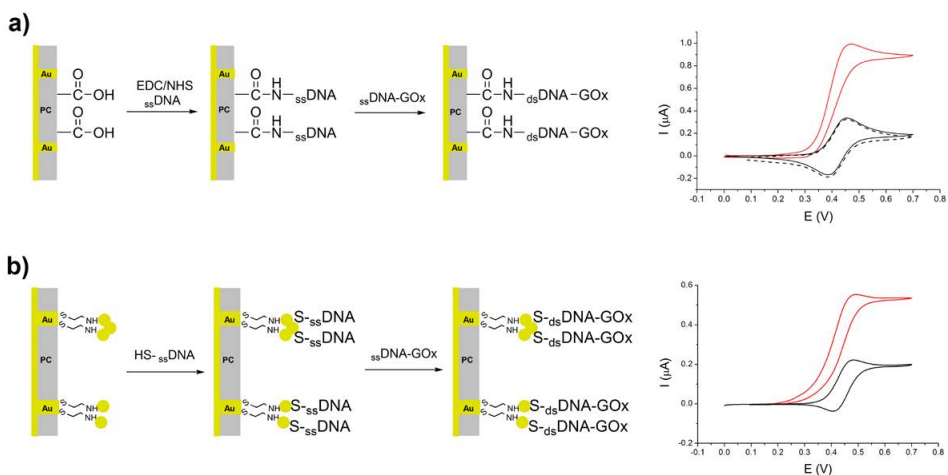


Figure 2: Functionalization schemes of NEEs where either the polycarbonate membrane (a) or the gold electrode surface (b) are exploited for the immobilization of the oligonucleotides. Cyclic voltammograms reported on the right are recorded with functionalized NEEs in a 10 mM PBS solution (pH 7.4) containing  $\text{FA}^+\text{PF}_6^-$  as redox mediator, before (full black line) and after (full red line) the addition of 50 mM glucose. Dotted line CV shows the control signal in which an unmodified NEE is used. Scan rate  $5 \text{ mV s}^{-1}$ .

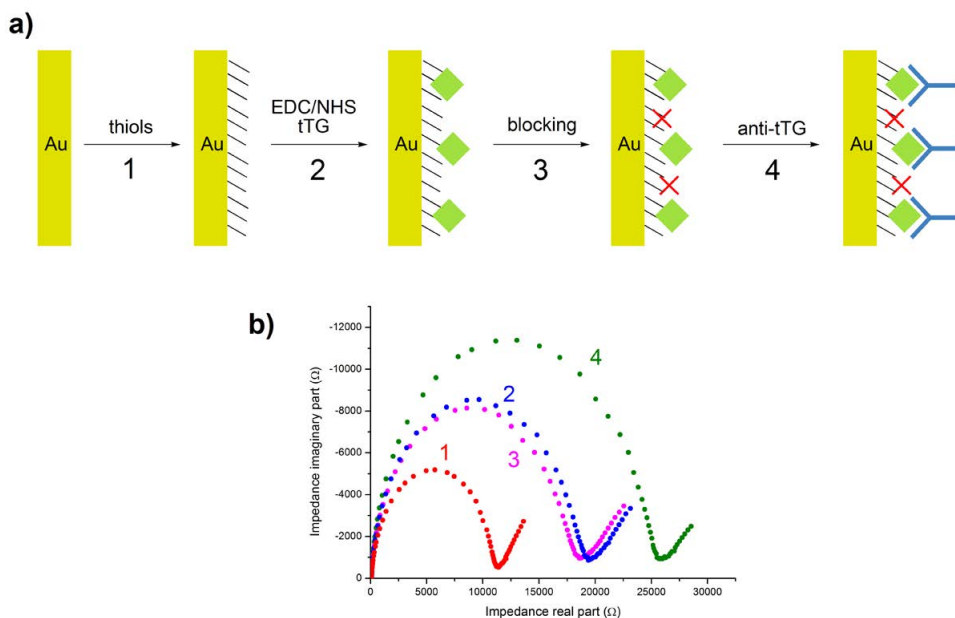


Figure 3: a) Functionalization scheme of the gold electrode surface with the antigen/antibody complex; b) Faradaic impedance spectra recorded at a gold electrode after treatment with thiols (red curve), functionalization with tTG (blue curve), blocking (magenta curve) and incubation with anti-tTG (green curve). Measurements were performed in 1X PBS buffer in the presence of  $25 \text{ mM } [\text{Fe}(\text{CN})_6]^{3-/4-}$  at a potential of  $0.22 \text{ V}$  in a frequency range from  $0.1$  to  $100 \text{ kHz}$  with an amplitude of  $0.01 \text{ V}$ .

effect against ischemic cardiovascular diseases and tumour development [6]. For this purpose, a novel kind of carbon electrodes (namely, Pyrolyzed Photoresist Carbon Electrodes - PPCEs [7]) are applied to the electrochemical characterization of BR in aprotic solvent, namely dimethylsulfoxide (DMSO). PPCE presents good electrochemical performances comparable with those of a conventional glassy carbon electrode (GCE), allowing to detect multiple electro-oxidation and electro-reduction mechanisms of BR (Fig. 4), thanks to the wide potential window accessible in DMSO. Moreover, PPCEs can be photopatterned as miniaturized arrays.

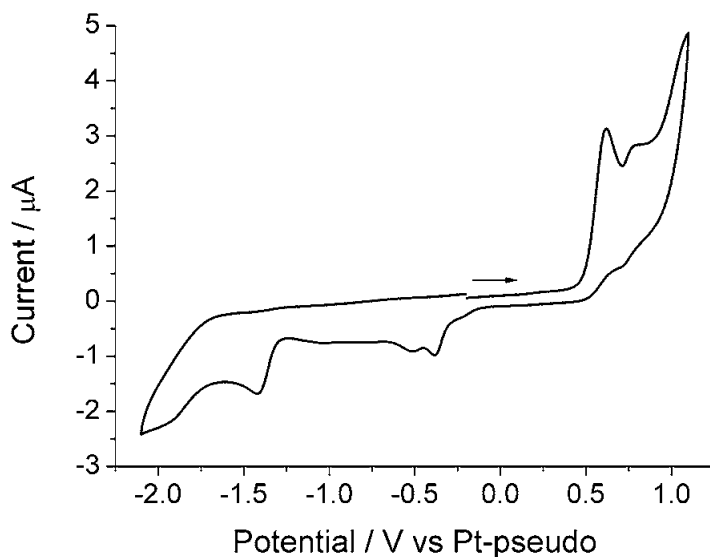


Figure 4. Cyclic voltammogram of 1 mM BR recorded with PPCE in 0.1 M TBABF<sub>4</sub>, DMSO solution. Scan rate 10 mV s<sup>-1</sup>.

Finally, the possibility to use PPCEs for the electrogeneration of the superoxide anion (O<sub>2</sub><sup>-•</sup>) by simple electrochemical reduction of dissolved oxygen has been evaluated in detail. The additional study of the reactivity of O<sub>2</sub><sup>-•</sup> with BR allows us to evaluate and confirm the radical scavenging properties of BR.

## 5 POTENTIAL NEW PRODUCTS & SERVICES

Products: sensing devices for the detection of DNA sequences; novel biosensors with high sensitivity and specificity for both the label or label-free detection of anti-tissue transglutaminase (anti-tTG) for the diagnosis of the celiac disease ; electrochemical methods and devices for the fast analysis of total and conjugated bilirubin.

Service: development of rapid, sensitive, cost-effective and easy-to-use analytical devices suitable for point of care application. Such devices will allow to use simplified detection strategies, for analysis in small volume samples (few μL).

## 6 CURRENT COLLABORATIONS

### 6.1 With other researchers:

- Luigina De Leo (TRANS2CARE, PP9)\*
- Mitja Martelanc (TRANS2CARE, PP3)
- Lovro Žibera (TRANS2CARE, LP)
- Andrea Mardegan (Veneto Nanotech S.C.p.A., Marghera-Venice)
- Ljiljana Fruk (Karlsruhe Institute of Technology, Karlsruhe, Germany)

### 6.2 With companies:

- AB ANALITICA s.r.l. of Padova

### 6.3 With hospitals:

- Institute of Child Health IRCCS "Burlo Garofolo" of Trieste\*

## 7 CONTACTS OR COLLABORATIONS NEEDED

For extending the fields of application we are looking for:

Antigen/antibody systems to be bound on the electrodes.

Redox enzymes for the development of electrocatalytic sensors.

Proposals for the application of electrochemical methods to the study of radicals and other species involved in oxidative stress and related processes.

For these reasons, collaborations with hospitals and companies are required. In addition, smart inputs from end-users can help in finding useful applications.

Target laboratories and companies:

1. Research and diagnostic laboratories
2. Companies specialized in the development and production of kits and diagnostic systems
3. Food companies
4. Companies working in the biomedical field

## 8 COMMUNICATION TOOLS

The high level of expertise in the field of electroanalytical chemistry as well as the high level of applicability, quality and performances of the presented methods is disseminated through the high quality scientific publications (see References 2-4, 7).

These topics have been presented at the following events:

## Oral presentations:

1. M. Silvestrini, P. Ugo, L. De Leo, T. Not, L. Žiberna, S. Passamonti, M. Martelanc, M. Franko, SENSITIVE MOLECULAR DIAGNOSTICS WITH ELECTROCHEMICAL BIOSENSORS AND ENSEMBLES OF NANOELECTRODES, workshop: Medicine for Nanotechnologies - Nanotechnologies for Medicine, NanotechItaly 2013, Venezia Mestre - Italy, November 29, 2013. Abstract in conference proceedings ISBN 978-88-6140-152-5.
2. M. Silvestrini, MEASURING BILIRUBIN IN SERUM USING ELECTROCHEMISTRY APPROACH, TRANS2CARE workshop - Bilirubin in Translational Medicine, Ferrara - Italy, June 6, 2013.
3. M. Silvestrini, MEASURING BILIRUBIN IN SERUM USING ELECTROCHEMISTRY APPROACH, Trans2Care workshop - Bilirubin in Translational Medicine, Izola - Slovenia, March 14 2013.

## Posters:

1. M. Silvestrini, P. Ugo, ELECTROCHEMICAL BIOSENSORS FOR SENSITIVE MOLECULAR DIAGNOSTICS, Conference: Cross-Border Italy-Slovenia Biomedical Research: Are We Ready for Horizon 2020?, Trieste - Italy, February 27, 2014.
2. M. Silvestrini, A. Mardegan, M. Cettolin, L.M. Moretto, P. Scopece, P. Ugo, ELECTROANALYTICAL APPLICATIONS OF SENSORS BASED ON PYROLIZED PHOTORESIST CARBON ELECTRODES, 2nd National Conference of Sensors, Rome – Italy, February 19 – 21, 2014.
3. A. Petelin, Z. Jenko-Pražnikar, M. Jurdana, M. Čemažar, M. Martelanc, M. Franko, M. Silvestrini, P. Ugo, L. Žiberna, S. Passamonti, SERUM BILIRUBIN AS A BIOMARKER OF CARDIOVASCULAR PRE-DISEASE STATES: FROM ANALYTICAL APPROACHES TO CLINICAL VALUE, 6th Central European Conference “Chemistry towards Biology”, Trieste - Italy, September 10 - 13, 2013.
4. M. Silvestrini, P. Ugo, L. Žiberna, S. Passamonti, L. De Leo, T. Not, M. Martelanc, M. Franko, SENSITIVE MOLECULAR DIAGNOSTICS WITH ELECTROCHEMICAL BIOSENSORS AND ENSEMBLES OF NANOELECTRODES, 6th Central European Conference “Chemistry towards Biology”, Trieste - Italy, September 10 - 13, 2013.
5. M. Silvestrini, P. Ugo and Trans2Care network, NANOTECHNOLOGY IN HEALTH CONTROL: THE EXPERIENCE WITHIN THE TRANS2CARE NETWORK, NanotechItaly 2012, Venezia Mestre - Italy, November 1, 2012. Abstract in conference proceedings ISBN 978-886140-130-3.

These topics have been the subject of two projects written for the event Trieste Next:

1. Project for the event Nordest Technology Transfer - Trieste Next 2012. Title: Electroanalytic platform for the detection of molecules of environmental/biological interest. This project received the ENTERPRISE CITY PRIZE 2013 at the event that opened the sixth edition of the Festival Enterprise City at the Municipal Theatre of Vicenza on May 8, 2013.
2. Project for the event Nordest Technology Transfer - Trieste Next 2013. Title: Electrochemical biosensors and ensembles of nanoelectrodes for molecular diagnostics.

## 9 FUNDS NEEDED

**9.1 For basic research (investigation of biological mechanisms): 50.000 €**

**9.2 For applied research (solutions for real-world problems): 20.000 €**

**9.3 For pilot & demonstrator activities (to develop a prototype): 50.000 €**

## 10 CONCLUSIONS

The know-how in the field of analytical application of electrochemical nano-biosensors acquired by the group at the University Ca' Foscari thanks to the present project can be extended and find practical application to the determination of clinically relevant molecules. Our participation in the TRANS2CARE project together with the other specialized partners, each bringing different know-how ranging from biochemistry to clinical practice, constitutes an important occasion and a challenge for new developments in the field of molecular diagnostics. Electrochemical biosensors can indeed be very helpful for obtaining quick and reliable analytical information thanks to their sensitivity, relative low cost, possibility of decentralized use and simple applicability.

## ACKNOWLEDGEMENT

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