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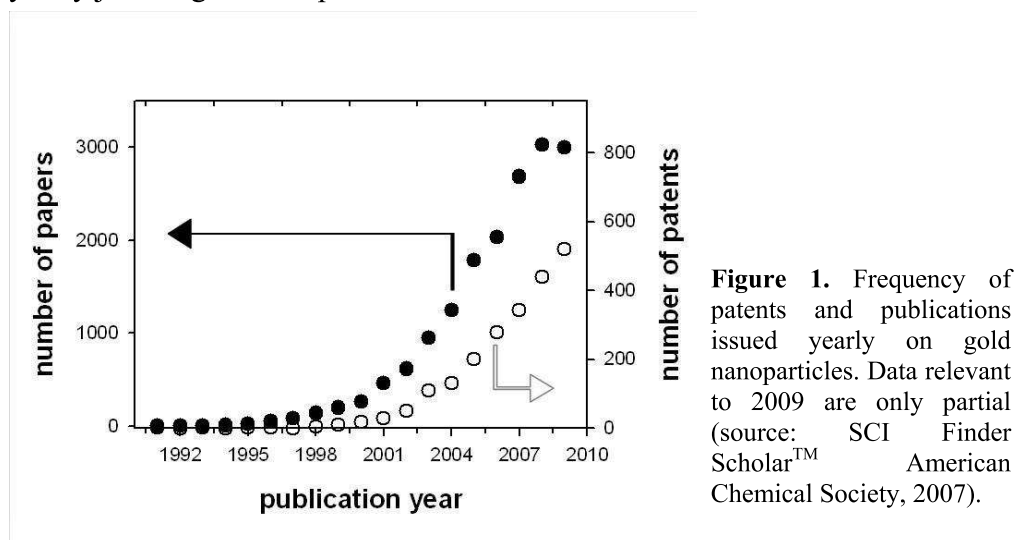
## **GS – SCI**

## GOLD NANOSTRUCTURES FOR SENSING APPLICATIONS

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### Abstract

Metal nanoparticles (NP) are the subject of an exponentially growing scientific interest. Figure 1 provides a bird view of the literature statistics, showing the number of papers and patents issued yearly just on gold nanoparticles.



**Figure 1.** Frequency of patents and publications issued yearly on gold nanoparticles. Data relevant to 2009 are only partial (source: SCI Finder Scholar™ American Chemical Society, 2007).

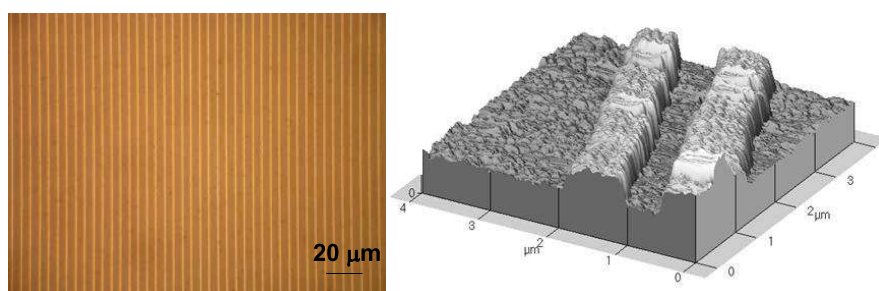
In the present study, electrochemical techniques have been used to prepare gold-based nanomaterials with a controlled morphology and chemical composition. Colloidal gold@surfactant core@shell nanoparticles have been obtained via the so-called sacrificial anode technique [1-2]. As synthesised Au-NPs had an average core diameter of  $5.0 \pm 1.1$  nm and their surface spectroscopic characterization showed the presence of two chemical statuses, namely nanostructured Au<sup>(0)</sup> (its abundance being higher than 90%) and Au<sup>(I)</sup>. The nanomaterial was then deposited on the top of a capacitive Field Effect Transistor sensor and subjected to a mild thermal annealing aiming at removing the excess of stabilizing surfactant molecules. The morphology of annealed Au-NP layers is shown in Figure 2, where a typical Scanning Electron Microscopy image is reported.



**Figure 2.** SEM micrograph of gold nanoparticles deposited on the top of the FET capacitive sensor and annealed at 200°C for 1 hour.

Measurements on the gas sensitivity of the Au-NP sensors towards a number of gases found in automotive gas exhausts were performed. The device employing annealed Au-NPs as sensing layer showed the largest response towards  $\text{NO}_x$ , and much smaller –if any- responses towards  $\text{NH}_3$ ,  $\text{H}_2$ , CO, hydrocarbons and other interferent species.

A controlled electrodeposition technique was also used to grow gold nanowires (Au-NWs) on silica slides [3]. Arrays of hundreds Au-NWs with a rectangular cross section were obtained, their height, width and length being respectively equal to 80 nm, 300nm, and 1 cm. Due to their peculiar properties, elongated structures such as gold nanorods and NWs appear particularly promising for sensing and biosensing applications, and straightforward examples have been already given in recent literature [4-5].



**Figure 3.** Optical and Atomic Force Microscopy images of arrays of gold nanowires deposited on silica slides by means of the so-called Lithographically Patterned Nanowire Electrodeposition (LPNE) technique [3].

Both Au-NWs and layers composed of thermally annealed AuNPs were employed as non-conventional promoters for the Laser Desorption Ionization – Mass Spectrometry (LDI-MS) detection of low-molecular weight analytes, such as amino acids and peptides. LDI-MS responses showed that both Au-nanomaterials lead to a highly efficient and preferential analyte desorption/ionization, as a limited number of low intensity interferent peaks can be detected. Noteworthy, MS spectra were always dominated by analyte signals [6].

Finally, Covalent Virus Layer (CVL)-modified micro-gaps between gold pads were studied as model device architecture for the impedimetric detection of specific antigens under liquid-flow conditions.

Electronic transduction of bio-recognition events can also provide new perspectives for the development of Organic Field Effect Transistor (OFET) sensors. Recently, a new bilayer OTFT architecture was proposed by Torsi et al. for the enantio-selective recognition of chiral molecules [7]. In this contest, a totally innovative OFET device has been realized, by fully integrating in the electronic device a protein recognition element, through novel protein supramolecular architectures [8] that will be reported and discussed, as well.

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## BIOLUMINESCENT WHOLE-CELL BIOSENSORS FOR ON FIELD ANALYSIS

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### Abstract

Bioluminescence (BL) has revealed an extremely useful analytical tool enabling ultrasensitive detection in biotechnological applications. The typical bioanalytical applications of BL proteins include the investigation of protein–protein interactions, protein conformational changes, protein phosphorylation, second-messengers expression and, in general, the study of gene expression and gene regulation. Since BL proteins can be detected down to very low levels, they allow ultrasensitive detection of the target analytes and monitoring of the physiological phenomena under investigation [1,2]. These BL features, associated to instrumental and technical advancements in miniaturization, enable the analysis of small-volume samples, which leads to the development of miniaturized and high-throughput assays. Genetically engineered cells (bacteria, yeasts, or mammalian cells) able to produce a BL signal in response to a target analyte represent powerful analytical tools for environmental, medical, and food analysis, and are characterized by low cost and high rapidity and sensitivity.

Nevertheless some important issues have still to be addressed to make these biosensing systems true analytical biosensors, such as portability, i.e., using BL whole-cell biosensors for on field monitoring and point-of-care analysis, and reliability. Indeed, their main pitfall is the high variability of the BL signal produced by the engineered cell, in fact the emitted light changes according to the metabolic state of the cell. To solve this problem we introduced in the cell a vitality internal control to correct the analytical signal. The recent availability of new reporter genes with improved BL properties, together with technical improvements, prompted the development of multiplexed cell-based assays using more BL proteins (e.g., green- and red-emitting luciferases, secreted luciferases, aequorin...) under the regulation of constitutive and inducible promoters in the same cell. Triple- and four-color bioassays, which combine spectral unmixing of green- and red-emitting luciferases with gaussia luciferase and aequorin, were developed thus allowing to measure three separate targets with high sensitivity and rapidity.

In addition, a new polymeric matrix was developed to encapsulate and keep cells alive for long periods of time [3] in order to obtain ready-to-use portable devices. Different recombinant yeast and bacterial biosensors were immobilized in a customized well microplate to obtain a bioluminescent cell array that can be stored for up to 2 months at 4°C without losing cell vitality. A device was constructed with the cell array in contact, through an optical taper, with an imaging light sensor, a portable charge-coupled device (CCD) camera able to localize and quantify the luminescent signal. "Contact" light detection, in which the signal is produced on (or very close to) the detection surface, allows to achieve much higher optical efficiency than that of conventional camera-based imaging systems. The applicability of this multiplexed biosensing platform containing whole-cell biosensors to measure hormonal activity in clinical and environmental samples is reported.

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## ELECTROCHEMICALLY SYNTHESIZED MOLECULARLY IMPRINTED POLYMERS FOR SENSING APPLICATIONS

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### Abstract

Considerable scientific research is nowadays devoted to the development of tailor-made receptors capable of recognizing and binding the molecular target with high affinity and selectivity. Molecular imprinting revealed to be an attractive tool for designing selective materials. During imprinting process polymeric matrices with specific binding sites are generated by template-induced prearrangement of complementary interactive functional groups [1]. A schematic representation of molecular imprinting principle is given in Figure 1. Pre-assembly of functional monomers around the template is driven by their molecular interactions. The polymerization “freezes” binding groups within the imprinted cavity. Removal of the template affords binding sites complementary in size and in chemical functionality to the original template [1]. A molecular memory is thus introduced into the polymer that is able to recognize the template and to selectively interact with it during the rebinding step [1].

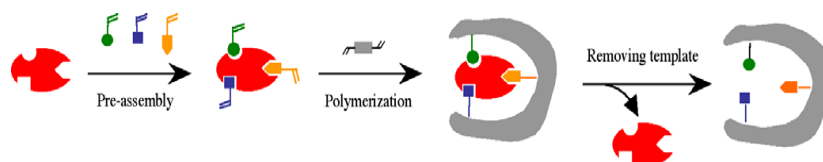


Figure 1 – Schematic representation of imprinting process (from [1]).

Among different analytical applications for which Molecularly Imprinted Polymers (MIPs) have been successfully used [2], MIP-based sensors attract increasing attention on account of their specificity, stability, robustness and low cost [3]. A particularly important aspect in the design of MIPs sensors is MIP integration with the transducer and different strategies have been employed to address this key issue. Among these, MIP electropolymerization has emerged as an effective way of synthesizing and anchoring MIP to the transducer surface [4]. By this approach the film grows on the electrode surface, so good adherence is guaranteed. Moreover, film preparation is simple and quick and its thickness is controlled by varying the amount of circulated charge. During the last decade, several electrosynthesized MIPs have been proposed as recognition element, particularly in electrochemical sensors [see e.g. 5-6].

The present communication reviews applications of electrosynthesized MIPs proposed in our research group since the first original proposal on the topic [4] to now, with a particular emphasis on the choice of functional monomers, on the proposed characterizations and on the explored sensing applications in the detection of biologically and environmentally relevant analytes.

The development of an electrosynthesized poly(3-thiophene acetic acid) imprinted on atrazine will be described [7]. After NMR studies of the binding affinity between monomer and template, X-ray

Photoelectron Spectroscopy (XPS) characterization was used for the evaluation of polymer-template interaction thus evidencing a remarkable imprinting effect.

A novel imprinting scheme, combining for the first time electropolymerization with metal-ion coordination, has been proposed [8] and collected results will be illustrated. A MIP for a pesticide (4-(2,4-dichlorophenoxy)butyric acid (2,4-DB)) has been prepared from a Co-porphyrin (Co(III)tetrakis(o-aminophenyl) porphyrin) as functional monomer. Such an approach aims to combine advantages of electropolymerization with ones related to the use of metal complexes in imprinting procedures [9]. After verification of template entrapment and subsequent removal by Fourier-Transform Infrared and XPS spectroscopies, the imprinting effect was verified by comparing electrochemical responses of MIP and not-imprinted polymer (NIP) tested by Cyclic Voltammetry and by Chronoamperometry. In both cases MIP revealed an enhanced electrocatalytic activity towards 2,4-DB reduction. Moreover, MIP based electrodes evidenced a good selectivity against both pesticides and structurally related compounds.

Another novel fascinating approach for MIP-transducer integration based on electropolymerization will be presented. A MIP for the alkaloid ephedrine (MIPE) has been immobilized in an electrosynthesised polypyrrole (PPY) film for the development of a voltammetric sensor for ephedrine [10]. This strategy offers advantages of electropolymerization and, at the same time, the decoupling of imprinting and deposition steps allows the separate optimization of parameters of each process. The electrode was prepared using an unconventional “upside-down” geometry for the three-electrode cell. As a consequence, a high MIP loading was obtained, as revealed by SEM characterization. The sensor exhibited a satisfactory performance in ephedrine detection and in the rejection of tested potential interferences.

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