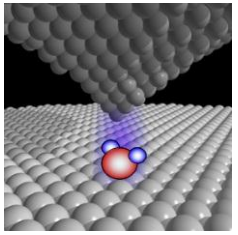


Actinides and Heavy Metals Interactions on Proteins and Their Biosensing

Center of Surface Science and Nanotechnology (CSSNT)

Molecular Recognition and Interactions Laboratory (LIRM)

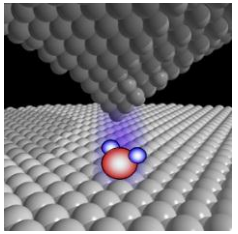
Project leaders:

Michael Odorico
Ingénieur-Chercheur

Marius Enachescu
Professor Dr.rer.nat.

PhD student: Marian Bota

Contents



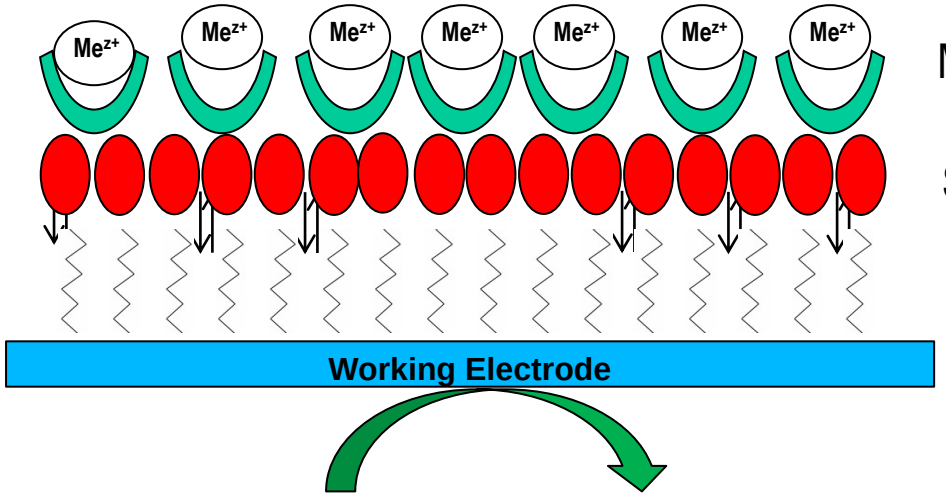
Goal of the project

The goal of this project is to develop novel techniques for assessing biosensor surfaces built with self-assembled monolayers (SAMs) as well as the design of the biosensor and its testing.

The main tasks of this project:

(i) to perform reproducible characterization of a biofunctionalized surface in order to analyze the distribution of single proteins and evaluate their capacity of binding metals of interest. Main characterization techniques involved will be scanning probe microscopy in imaging and spectroscopic modes giving information on the surface coverage and mechanical properties of the layer, its surface contact potential and dielectric constant variations, as well as confocal RAMAN microscopy for complementary characterization.

(ii) to design the biosensor and to perform its testing, mainly involving Electrochemical Impedance Spectroscopy (EIS) and related techniques.



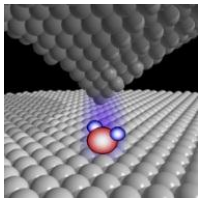
Me^{z+} (Me = UO_2^{2+}, Zn^{2+}, Pb^{2+})

Specific metal binding protein

SAM

Objectives and detailed work plan

(3 years project)



WP 1. ***Developing self-assembled monolayers (SAM) on silicium and gold***

- **Task 1.1.** Identifying monoclonal antibodies/receptors that will bind to the heavy metals — **LIRM**
- **Task 1.2.** Including the Identified antibodies/receptors on SAM on silicium and gold — **LIRM**

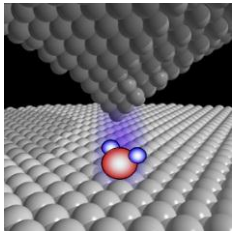
WP 2. ***Characterization of self-assembled monolayers before/after bonding with heavy metals***

- **Task 2.1.** Micro Raman characterization — **CSSNT**
- **Task 2.2.** AFM and SPFM studies, heavy metals (HM) interactions and electrical properties mapping — **LIRM, CSSNT**

WP3. ***Bioseonsor design activities: Characterization of electrochemical behavior of SAM in a solution of heavy metals***

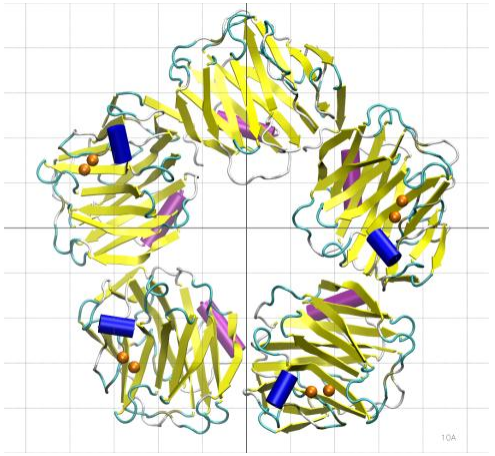
- **Task 3.1.** Cyclic voltametry studies on the SAM covered surfaces/electrode — **CSSNT**
- **Task 3.2.** Optimization of the electrochemical response of the electrode to the presence of heavy metals — **LIRM, CSSNT**

Preliminary obtained results:

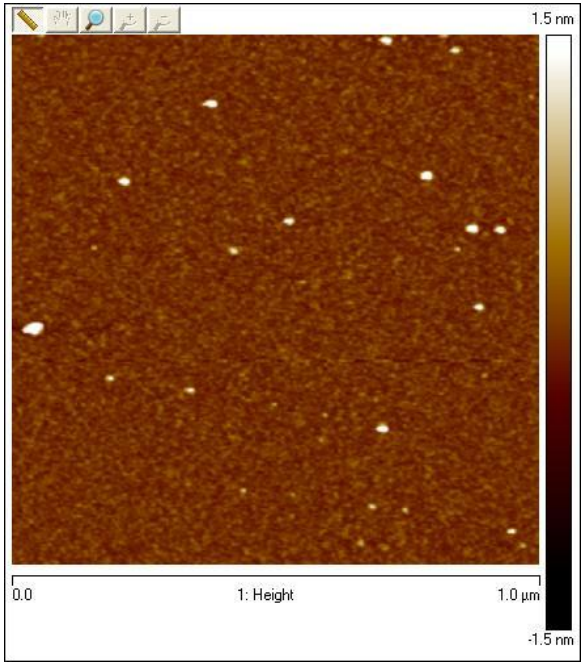


WP.1. Developing self-assembled monolayers (SAM) on silicium and gold

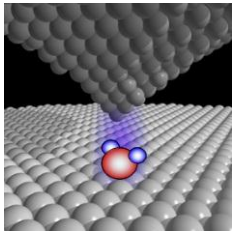
Task 1.1. Identifying monoclonal antibodies/receptors that will bind to the heavy metals



- From a list of protein that were already characterized for interacting with actinides, we selected the C-reactive protein
- The apparent affinity of UO_2^{2+} for native CRP was almost 100-fold higher than that of Ca^{2+} (Pible et al., 2010)
- Biochemical experiments confirmed the predicted binding site for UO_2^{2+} .



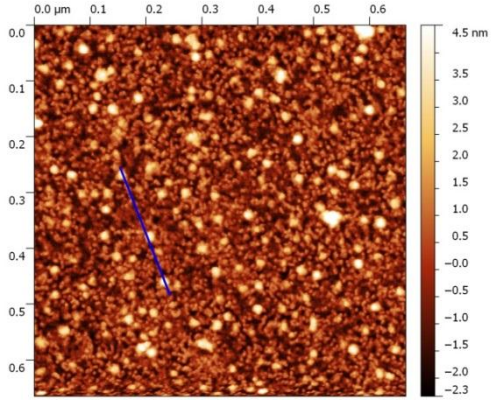
Preliminary obtained results

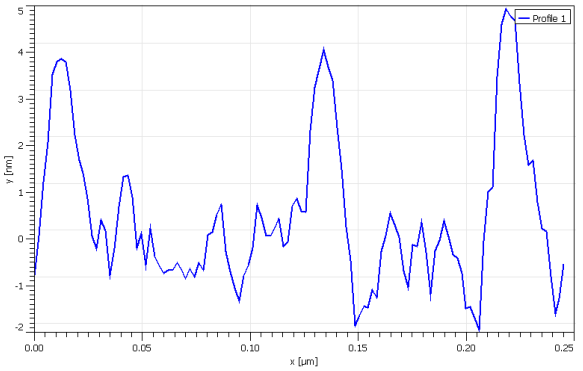


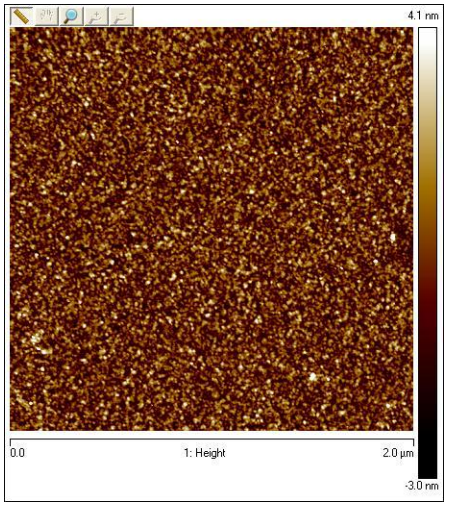
WP.1. Developing self-assembled monolayers (SAM) on silicium and gold

Task 1.2. Including the Identified antibodies/receptors on SAM on silicium and gold

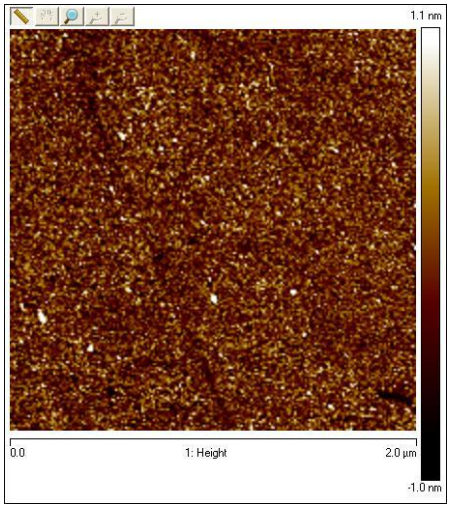
Height image of chemically fixed antibodies on mica-grafted surfaces



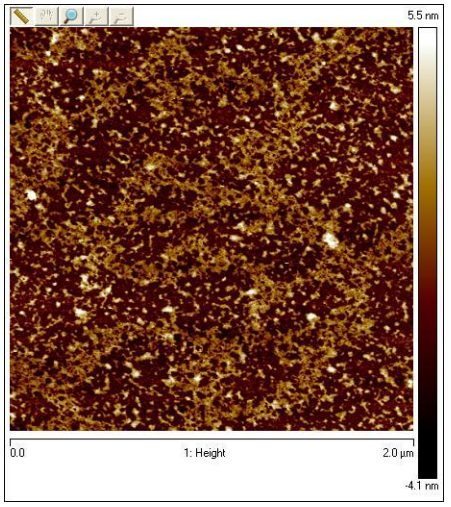




Topographic AFM image in air of a 30% COOH-SAM surface.

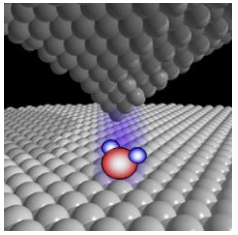


Topographic AFM image in air of a 100% COOH-SAM surface.



Topographic AFM image in air of a 100% COOH-SAM surface with antibody molecules deposited.

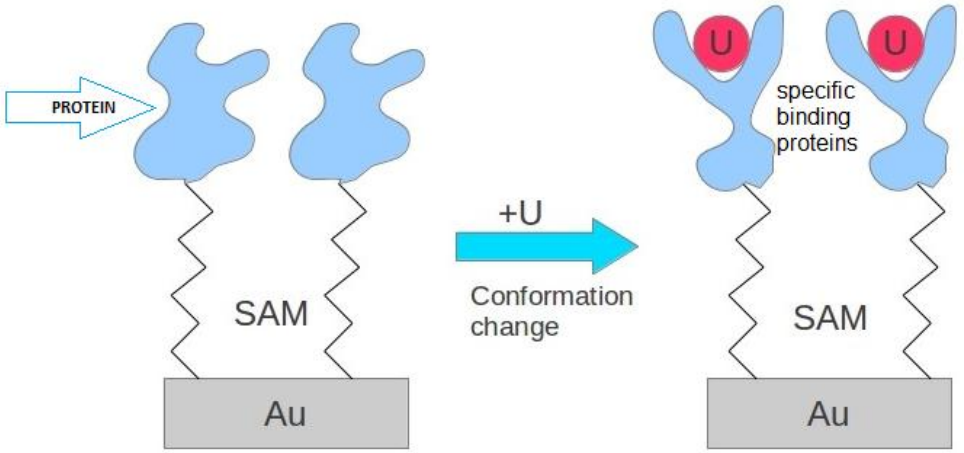
Planned characterizations in the near future:



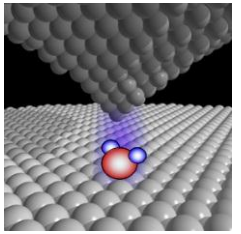
Task 2.1. Micro Raman characterization

- Characterization of the samples will be done using a Raman-Confocal microscope. This method provides “fingerprint” spectra that are unique to each specific compound and contain information about chemical composition and structure.
- Using this technique we can differentiate between bound and unbound proteins.

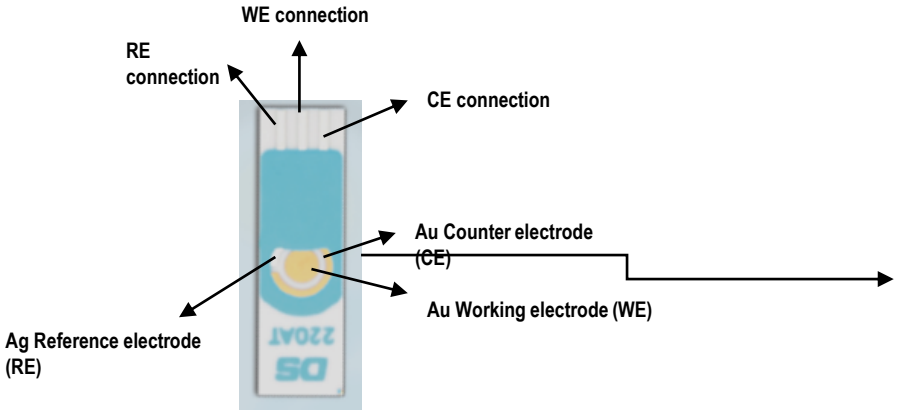
Task 2.2. AFM and SPFM studies, heavy metals (HM) interactions and electrical properties mapping

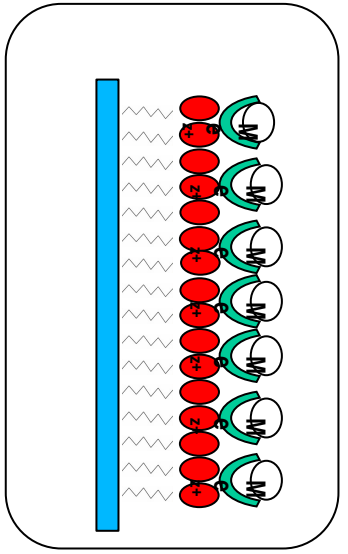


Biosensor design activities: Characterization of electrochemical behavior of SAM in a solution of heavy metals



Design of the sensing electrode

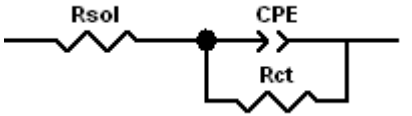




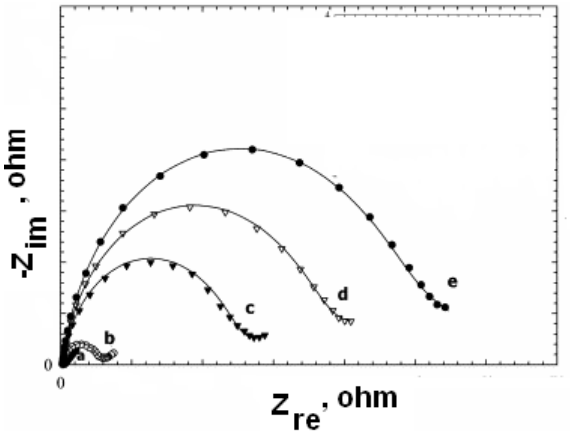
Au WE

SAM-Protein-Metal complex nanostructure

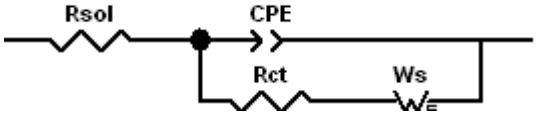
To better fit experimental results the duble layer capacitance should be estimated involving a CPE – constant phase element)



Au screen printed electrode (Au-SPE)

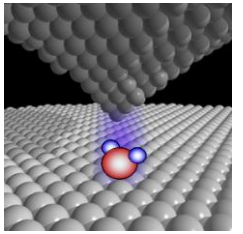


Qualitative Nyquist plots for: (a) bare Au electrode; (b) Au-SAM; (c) Au-SAM-Protein; (d, e) Au-SAM-Protein- UO_2^{2+}, different concentrations



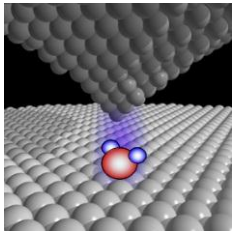
If the process becomes mass transfer limited, a Warburg element may be introduced

Foreseen impact and cooperation perspectives



- The final goal of the project is to demonstrate the possibility of using self assembled monolayers chemically coupled with target proteins to identify presence of heavy metal ions.
- The demonstration of a high selectivity by adapting nanotechnology techniques would open interesting perspectives in the whole domain of nanosciences and nanotechnology
- Possibility of developing a high sensitivity metal detection biosensor.
- This project will act as a catalyst in promoting and developing existing cooperation between UPB - CSSNT and LIRM-CEA by exploiting complementarities and creating synergies among current research fields of participating research teams, resulting in a mutual cross-fertilization of both human potential and infrastructure capabilities.

CSSNT Brief Description



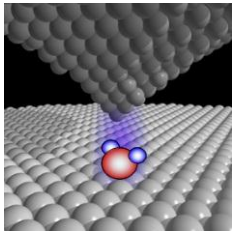
University POLITEHNICA of Bucharest - UPB
- the oldest and largest technical university from Romania
- 55 Chairs, in 13 Faculties and 5 Departments
- 40 Research Centers

Research Area: Surface Science & Nanotechnology
Equipment available:
Dedicated built UHV deposition chamber for “MBE”-PLD
Custom built chamber for CNTs and nanoparticles deposition
High power excimer laser - Compex Pro 205
Raman-Confocal microscopy
Home built Atomic Force Microscope
Phillips TEM
FEI SEM

Human resources:
6 PhD students
7 PostDoc students
4 Professors
4 Associate personnel

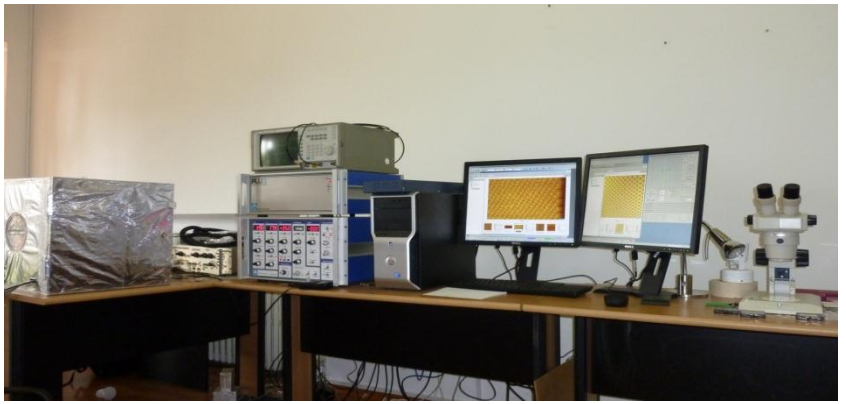
The main interest of the UPB-CSSNT is to develop the scientific knowledge for the **processing** and **characterization** of micro- or nano-materials and structures for applications in biosensors, solar cells, optics, smart clothing and optoelectronics.

CSSNT Equipment

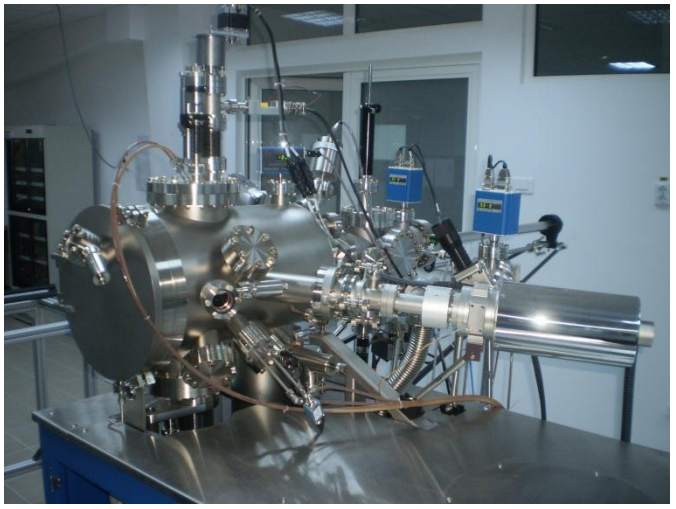


Horiba LabRam 800
- Raman-Confocal microscope-

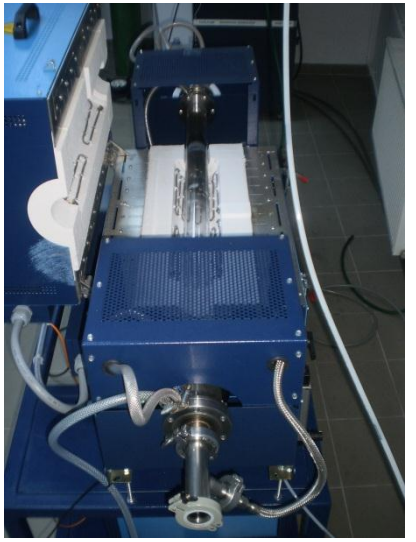
Home built AFM
- Topographic characterization with nanometric resolution-

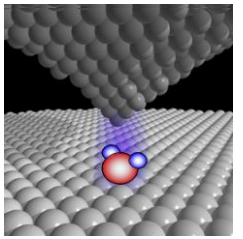


"MBE" Pulse Laser Deposition
- Facility for special ultra-thin and thin films depositions -



Facility for preparation of carbon nanoparticules and carbon nano-tubes(CNTs) by pulse laser technique.





Thank you for your attention!