Report - 1^{st} PhD year

Giulia Pinto

Supervisor: Prof.ssa Ornella Cavalleri

Research activity

My research focusses on the functionalization of gold substrates with DNA to develop sensitive and selective biosensors: we propose a combined approach which couples scanning probe nanolithography (AFM nanoshaving) and optical spectroscopy (spectroscopic ellipsometry) to investigate the structural and optical properties of DNA films. Complementary X-ray photoelectron spectroscopy (XPS) measurements are employed to investigate the effect of the molecular deposition protocols on the DNA adsorption.
The project is organized in three main steps. The first step deals with the study of single-strand DNA (ssDNA) self-assembled monolayers (SAMs) with the aim of optimizing the ssDNA deposition protocol. The second steps investigates the hybridization of immobilized ssDNA with the complementary DNA strands. The third step will focus on the deposition of a sensing protein exploiting the hybridization of complementary DNA conjugated with the sensing molecule.
During the first year I investigated the ssDNA deposition process. I studied the formation of both pure ssDNA SAMs and mixed SAMs formed by ssDNA and a spacer molecule, mercaptohexanol (MCH). The aim was to evaluate the influence of different parameters, like the buffer ionic strength, the adsorption time or the use of spacer molecules, on the ssDNA SAM formation.
The ionic strength of the buffer solution has been found to have a significant influence on the ssDNA adsorption. The amount of DNA deposited on the surface using a buffer with a high salt concentration (1 M) is higher than the amount deposited using a low salt concentration (1 mM). This behaviour can be explained taking into account the screening of the DNA negative charge at high ionic strength.
The formation of mixed SAMs is strongly affected by the ssDNA adsorption time as well as by the concentration of the MCH solution. SE and XPS analyses showed that increasing the adsorption time (from 3 to 24 hours) increases the ssDNA coverage. At the same time a compact ssDNA SAM hinders the following adsorption of the spacer molecule which is expected to favour the DNA hybridization. As concerns the concentration of the MCH solution, mixed layers formed using low MCH concentrations (5 uM) show slower deposition kinetics but higher sample reproducibility than samples prepared using higher MCH concentration (5 mM). Based on the results we identified short ssDNA adsorption times and low MCH concentrations as the conditions that lead to the formation of reproducible mixed SAMs well suited for hybridization experiments.
Finally I carried out preliminary experiments on the hybridization of ssDNA/MCH films with complementary strands (cDNA): successful hybridization could be inferred from the increase of the SAM thickness upon incubation in cDNA.
The molecular deposition at the different steps of the layer formation (ssDNA, mixed layer, hybridization) could be followed in situ in real time by dynamic SE measurements. The study of nanometer thick layers is a challenging task especially for the SE analysis. For this reason, from the experimental point of view the experimental set-up for in situ SE measurements was optimized, to improve system stability and data reproducibility.
As concerns data analysis, difference SE spectra have been considered in order to put in evidence the contribution of the organic layer. For a quantitative analysis of ellipsometric data, I compared ssDNA data with simulations based on suitable optical models. A simple model of transparent film well reproduces the experimental curves in the near-IR region, but it can not account for the spectral feature related to the DNA absorption (around 260 nm). I therefore tested a model of absorbing film which better reproduces experimental data in the UV region.

Exams

- "Fisica applicata alla biomedicina e ai biomateriali" - Dott. C. Canale
- Report on Linz Workshop "Linz Winter School 2019 - Advances in Single-Molecule Research for Biology and Nanoscience" - Prof. R. Ferrando
- "Structural Biology" - Prof. G. Damonte
- "Metodi ottici e spettroscopici per lo studio dei materiali" - Prof. M. Canepa

All exams have been passed.

Schools

- Linz Winter School 2019 - Advances in Single-Molecule Research for Biology and Nanoscience, January 29-31, 2019. Linz, Austria.
  https://www.jku.at/institut-fuer-biophysik/veranstaltungen/linz-winterworkshop/

Publications

- **Giulia Pinto**, Pietro Parisse, Ilaria Solano, Paolo Canepa, Maurizio Canepa, Loredana Casalis, Ornella Cavalleri.
  Functionalizing gold with single strand DNA: novel insight into optical properties via combined spectroscopic ellipsometry and nanolithography measurements.
  Soft Matter, 2019, 15, 2463.
  doi: 10.1039/C8SM02589D
- Paolo Canepa, Grazia Gonella, **Giulia Pinto**, Vladimir Grachev, Maurizio Canepa, Ornella Cavalleri.
  Anchoring of Aminophosphonates on Titanium Oxide for Biomolecular Coupling.
  Journal of Physical Chemisty C, 2019, 123, 16843.
  doi: 10.1021/acs.jpcc.9b04077

Conferences

- XXI. Annual Linz Winter Workshop - Advances in Single-Molecule Research for Biology and Nanoscience, February 1-4, 2019. Linz, Austria.
  G. Pinto, P. Parisse, I. Solano, P. Canepa, O. Cavalleri, L. Casalis, M. Canepa.
  Functionalizing gold with single strand DNA: novel insight into optical properties via combined AFM nanolithography and spectroscopic ellipsometry measurements. **(oral communication)**
  https://www.jku.at/institut-fuer-biophysik/veranstaltungen/linz-winterworkshop/
- 12^{th} European Biophysics Congress (EBSA), July 20-24, 2019. Madrid, Spain.
  Awarded an EBSA bursary to attend the conference.
  G. Pinto, P. Parisse, I. Solano, P. Canepa, M. Canepa, L. Casalis, O. Cavalleri.
  DNA SAMs on flat gold: a combined Atomic Force Microscopy and Spectroscopic Ellipsometry approach. **(poster)**
  http://www.ebsa2019.com/index.php
- Protein misfolding and amyloidosis XIII, May 23-24, 2019. Genova, Italy.
  https://unige.it/eventi/eventi.php?id=1815
- International Meeting on Nanoalloys 2019, June 4-7, 2019. Genova, Italy.
  http://nanoalloys-irn.cnrs.fr/nanoalloys-meeting/

Conference Communications - GP Coauthor

- ECOF 16, July 8-12, 2019. Paris, France.
  G. Pinto, P. Parisse, I. Solano, M. Canepa, L. Casalis, O. Cavalleri.
  DNA SAMs on flat gold: a combined Atomic Force Microscopy and Spectroscopic Ellipsometry approach.
  https://ecof16.sciencesconf.org/