Report - 2nd PhD year

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Research activity

PhD project: "Functionalization of gold substrates with DNA to develop sensitive and selective biosensors."

During the first year of PhD, I investigated the deposition of single-strand DNA (ssDNA) films and of mixed films formed by ssDNA and spacer molecules. I focussed on the influence of the buffer ionic strength on the DNA self-assembly and I performed preliminary measurements on the role of the ssDNA incubation time: both studies have been concluded in the first half of the second year. Then, I focussed on the study of the hybridization of mixed films (ssDNA + molecular spacer) with complementary DNA (cDNA) strands. DNA self-assembled monolayers (SAMs) have been studied through microscopic (Atomic Force Microscopy, AFM) and spectroscopic (Spectroscopic Ellipsometry, SE, and X-ray Photoelectron Spectroscopy, XPS) methods, in order to obtain information about morphology and thickness (AFM), dielectric properties and optical absorption (SE) and surface coverage (XPS). Moreover, Quartz Crystal Microbalance with Dissipation (QCM-D) measurements were performed to obtain information on molecular density too.
In particular, during the second PhD year I focused on:

- thick ssDNA films (XPS);
- effect of the buffer ionic strength (1mM vs 1M) on the mechanical properties of ssDNA SAMs (AFM);
- effect of the incubation time (3h vs 24h) on ssDNA SAMs (SE, AFM, XPS);
- hybridization of mixed SAMs with non-labelled cDNA (SE, AFM, XPS, QCM-D), labelled cDNA (SE), non-complementary DNA (ncDNA) (SE);
- evaluation of the minimal concentration of cDNA solution detectable by SE (in hybridization experiments);
- SAMs of dsDNA strands hybridized in solution prior to chemisorption (SE).

In order to check that ssDNA preserves its structure upon chemisorption, XPS measurements have been performed on thick DNA films, deposited on indium substrates, and compared with XPS data on ssDNA SAMs on gold. The same molecular-related signals were observed, with, as expected, an increase of the ratio between molecular signals and substrate signal. Moreover, it can be noted that in the case of SAMs the binding energy position of the S2p signal (162 eV) indicates the occurrence of molecular chemisorption while in the case of thick films ssDNA molecules are only physisorbed on the indium substrate (S2p signal around 164 eV).
To complete the analysis of the role of ionic strength in ssDNA films formation, performed in the first PhD year, I investigated the mechanical properties of ssDNA SAMs with AFM using the JPK Nanowizard (DIFILAB project). This microscope can operate in Quantitative Imaging mode which can provide information on the sample mechanical properties, like the Young modulus, through the acquisition of force-distance curves. It is important to note that the evaluation of the mechanical properties of ultrathin films such as DNA SAMs is a challenging task, since it is difficult to uncouple the contribution of the substrate from that of the film. From the analysis of QI data it has been shown that SAMs chemisorbed at higher ionic strength are thicker, more compact and rigid than those chemisorbed at low ionic strength. This is reasonably related to the reduction of the intermolecular repulsion due to the screening of the DNA charges at high ionic strength. These results have been included in a publication on Materials (doi: 10.3390/ma13132888).
Then, the role of the incubation time (3 vs 24 hours) was studied in order to optimize the experimental protocol. Increasing the incubation time:

- SE detected an higher optical thickness (parameter influenced by both thickness and refractive index);
- AFM nanoshaving experiments measured an increase of the film thickness (1.7 nm for 3h incubated ssDNA films, 2.9 nm for 24h incubated ssDNA films);
- XPS measurements showed an increase of the ssDNA coverage. Moreover, it has been observed that the S2p signal associated to S-Au bond (at a binding energy of 162 eV), dominant in ssDNA SAMs incubated for 24h, is accompanied, in 3h-incubated ssDNA SAMs, by a lower energy signal (at 161.4 eV), usually associated to a different sulfur-gold coordination.

Finally, I studied the hybridization of mixed SAMs with cDNA. Hybridization was monitored by different method:

- XPS detected an increase of the ratio between carbon and sulfur signal intensities upon hybridization;
- AFM nanoshaving measurements, performed in collaboration with Dr. Pietro Parisse (IOM-CNR, Trieste), showed an increase of the film thickness at each deposition step (ssDNA: 1.7 nm, ssDNA + molecular spacer: 2.4 nm, dsDNA: 4.5 nm). These results were compared with preliminary experiments performed at the Genova Physics Department with JPK AFM, which show promising results (www.difi.unige.it/it/ricerca/difilab).
- QCM-D experiments on DNA SAMs have been performed in collaboration with Dr. Silvia Dante (IIT, Genova). These measurements provided information on film density and packing. QCM-D data indicated that the molecular spacer immobilized after ssDNA deposition both fills empty sites and replaces weakly bound ssDNA. The hybridization was detected by an increase of mass/area ratio. QCM-D experiments support AFM shaving results;
- SE detected an increase of the optical thickness and a more intense optical absorption upon hybridization.

In particular, a more detailed study on hybridization was performed with SE. First, I verified that the increase of optical thickness after incubation in cDNA solution was due to hybridization using fluorescently labelled complementary strands (dye absorption was observed together the optical thickness increase) and that hybridization was selective (it did not occur for incubation in ncDNA). Second, preliminary measurements have shown that SE is able to detect hybridization at cDNA concentrations as low as 20 nM (in previous experiments, it was used a 1 μM solution). Third, SE experiments on dsDNA hybridized in solution were performed showing that strands hybridized in solution form films that present an optical thickness which is one half of the optical thickness showed by films prepared by three-step deposition (ssDNA/molecular spacer/cDNA).
In parallel to this project, I participated in a collaboration guided by Dr. Silke Krol (National Institute of Gastroenterology IRCCS "S. de Bellis" Research Hospital, Bari). This study focused on the role of polymer surface coatings in inducing spheroid formation when used as substrates for cell cultures. The study showed that small differences in the polymer composition can have a drastic impact on the cell behavior. In particular, I performed a morphological analysis of the polymer coatings through AFM in liquid.

Courses

- 2020 Fall WVASE Online Short Course - September 14 - October 5, 2020.
  https://www.jawoollam.com/resources/short-courses/2020-fall-wvase-online-short-course

Publications

- **Giulia Pinto**, Paolo Canepa, Claudio Canale, Maurizio Canepa, Ornella Cavalleri.
  Morphological and Mechanical Characterization of DNA SAMs Combining Nanolithography with AFM and Optical Methods.
  Materials, 13(13), 2888 (2020).
  doi: 10.3390/ma13132888
- Simona Serrati, Chiara Martinelli, Antonio Palazzo, Rosa Maria Iacobazzi, Mara Perrone, Roberto Santoliquido, Quy K. Ong, Zhi Luo, Ahmet Bekdemir, **Giulia Pinto**, Ornella Cavalleri, Annalisa Cutrignelli, Valentino Laquintana, Nunzio Denora, Francesco Stellacci, Silke Krol.
  Reproducibility warning: The curious case of Polyethylene glycol 6000 and spheroid cell culture
  PLoS ONE, 15(3): e0224002 (2020).
  doi: 10.1371/journal.pone.0224002

Conferences

- 106^o Congresso Nazionale Società Italiana di Fisica 2020. September 14-18, 2020 **(communication) G. Pinto**, P. Canepa, M. Canepa, O. Cavalleri.
  DNA sequence detection by coupling microscopy and spectroscopy methods.
  https://congresso2020.sif.it