Report - 3^{rd} PhD year

Giulia Pinto

Supervisor: Prof.ssa Ornella Cavalleri

Research activity

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

During the first two years of PhD, I investigated the deposition of single-strand probe DNA (pDNA) films, the behaviour of mixed films formed by pDNA and spacer molecules and finally experiments on the hybridization with the target sequence (tDNA).
DNA self-assembled monolayers (SAMs) have been mainly studied through Spectroscopic Ellipsometry (SE). SE measurements were complemented by Quartz-Crystal Microbalance with Dissipation (QCM-D), X-rays Photoelectron Spectroscopy (XPS) and Atomic Force Microscopy (AFM) obtaining kinetic, compositional and thickness information.
During the third PhD year I focussed on:

- the effect of the incubation time (from 30 minutes to 3 hours) on pDNA SAMs;
- the reversibility of the hybridization process and the reusability of the sensing platform;
- the development of an optical model which helps the interpretation of SE spectra;
- QCM-D experiments on DNA SAMs;
- the influence of the nucleic acid sequence on the DNA self-assembly;
- the development of a Surface Plasmon Enhanced Ellipsometry set-up.

During the first PhD years, the role of the pDNA incubation time (3 vs 24 hours) was studied in order to optimize the experimental protocol. In the last year, the time was further reduced to 30 minutes. Some slight differences in pDNA SAMs could be detected (lower optical thickness for lower incubation time) while the efficiency of hybridization with tDNA was not affected by the pDNA incubation time. The possibility to vary the time of incubation in pDNA without changing the hybridization efficiency allows a flexible time management of the experiments. Moreover, reducing the incubation to about an hour is also convenient for the "industrial" development of the biosensor.
As concerns the hybridization of mixed SAMs with tDNA, the process was tested in the first years employing labelled target strands for the validation of the process and not-target strands for the selectivity between probe and target sequences. To complete the characterization of DNA SAMs and understand the actual possibility to design DNA-based biosensors, in this last year I analyzed the recovery of the platforms through denaturation/renaturation cycles. In fact, the reversibility of the hybridization process is one of the main advantages of DNA-based biosensors, due to the cost saving. Experiments showed a complete recovery of the SAMs surface at least for 5 times of hybridization/denaturation cycles. Moreover, an important issue in terms of biosensor development is the preservation of the selective recognition properties of the sensing platform upon storage in dry conditions. To this end, I checked and successfully verified the occurrence of hybridization and denaturation cycles on mixed SAMs upon at least one week storage in dry conditions.
Finally, an accurate and detailed optical model was built to fully characterize DNA SAMs and provide key information about UV absorption features in SE spectra. This analysis allowed to distinguish between a single-strand DNA film ("blank" platform biosensor) and a double strand DNA (biosensor which has recognized the target sequence) adsorbed on gold. Moreover, this work provided a proof of DNA hypochromism on ultrathin films of DNA (effect well known for DNA in solution, that causes a 20%-30% optical absorption reduction for double strand DNA compared to single strand DNA).
During the entire year, I performed QCM-D experiments under the supervision of Dr. Silvia Dante (IIT, Genova). These measurements provided further information on DNA films, allowing to investigate the DNA self-assembly kinetics and to evaluate the surface molecular density of single and double strand DNA films. Preliminary measurements have also shown that QCM-D is able to detect hybridization at tDNA concentrations as low as 15 nM (in previous experiments, a 1 μM solution was used).
The last part of the thesis focussed on the study of different nucleic acid sequences, such as the RdRp-Helicase sequence from a SARS-CoV-2 gene region. The detection of this target sequence is commercially attractive, given possible COVID19 pandemic-related applications. I investigated both tDNA and tRNA

strands, detecting no relevant experimental differences.
Furthermore, I worked with Dr. Francesco Bisio (CNR-SPIN, Genova) to assemble a home-made system able to perform Surface Plasmon Enhanced Ellipsometry (SPEE) experiments, method which combines surface plasmon resonance (SPR) effect and spectroscopic ellipsometry in total internal reflection condition. SPEE measurements allow to monitor chemical and physical processes at metal interfaces in situ, being very sensitive to small changes (due for example to molecular adsorption) and to perform measurements in opaque solutions. Preliminary experiments performed on DNA SAMs actually showed a higher sensitivity respect with standard SE measurements, suggesting that this set-up could help to lower the minimum detectable concentration of tDNA.
In parallel to my PhD project, I participated in a collaboration with Dr. Loredana Casalis (Elettra Sincrotrone Trieste S.C.p.A., Trieste) and Dr. Pietro Parisse (IOM-CNR, Trieste) about extracellular vesicles (EVs), an interesting intercellular communication system. In particular, I started the optical characterization through SE experiments of supported lipid bilayers and of their interaction with EVs.

Publications

Giulia Pinto, Silvia Dante, Silvia Maria Cristina Rotondi, Paolo Canepa, Ornella Cavalleri, Maurizio Canepa.
Label-free molecular investigation via spectroscopic ellipsometry: a rapid method for DNA detection.
submitted

Conferences

- **13th European Biophysics Congress (EBSA)**
  July 24-28, 2021. Vienna, Austria.
  G. Pinto, P. Canepa, C. Toccafondi, O. Cavalleri, M. Canepa.
  An untapped tool for biological samples: spectroscopic ellipsometry **(poster)**
  https://www.ebsa2021.org

- **XXV Congresso Nazionale SIBPA 2021**
  June 28-July 01, 2021. Online conference.
  Awarded a SIBPA bursary to attend the conference.
  G. Pinto, S. Dante, S.M.C. Rotondi, P. Canepa, M. Canepa, O. Cavalleri.
  Label-free molecular investigation via spectroscopic ellipsometry: optical detection of a Sars-CoV-2 gene region **(oral communication)**
  https://www.sibpa.it/CongressoNazionaleSIBPAParma/

- **65th Biophysical Society Annual Meeting**
  February 22-26, 2021. Online conference.
  G. Pinto, S. Dante, P. Parisse, P. Canepa, L. Casalis, M. Canepa, O. Cavalleri.
  Nucleic acid sequence detection by a multi-technique approach **(poster)**
  https://www.biophysics.org/2021meeting/