First Year Report

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Summary of research activity:

My activity regards the design and characterization of a DNA-based biosensing platform capable of fast, specific, and sensible recognition of oligonucleotide sequences. This is implemented through a 3-step process. Initially, a gold substrate is functionalized with thiolated single strands of DNA, complementary to the sequence we want to detect (probe DNA, pDNA). Then, a molecular spacer, 6-Mercapto-1-Exanol (MCH), is introduced to improve the molecular organization of the pDNA film. The system is lastly exposed to the target sequence (tDNA) for detection, with particular interest for the RdRp/Helicase sequence of Sars-Cov-2.

I investigated the self-assembly of thiolated DNA strands on gold substrates and the detection of the tDNA with a multi-technique approach, coupling spectroscopic and microscopic methods. With atomic force microscopy, I performed nano-shaving measurements to monitor the morphological evolution of the surface and obtain information about the thickness of the DNA film at each step of deposition. Optical analysis of the system was performed by Spectroscopic Ellipsometry, an in-situ, non-perturbative method using two different detection configurations: conventional external reflection mode (hereafter referred to as SE) and Total Internal Reflection Ellipsometry (TIRE). Complementary X-ray photoelectron spectroscopy (XPS) measurements were carried out to investigate the molecular deposition of DNA on the gold surface and support the optical data.

Through SE it has been possible to monitor real-time changes in the optical thickness and observe the DNA absorption at 260 nm, detected at the single monolayer level. For the data analysis, SE difference spectra were analyzed to enhance the contribution of the organic film. For the quantitative analysis of the SE data, I employed a 5-layer optical model that accounts for the DNA UV absorption and the strong interaction between the thiol group and the gold surface.

Analysis of SE data provided an indication of hypochromism in DNA SAMs. Hypochromism, well known for DNA in solution, consists of a decrease in the UV absorbance of double-stranded DNA compared to its single-stranded counterpart. While this effect has been detected in many experiments dealing with DNA molecular solutions, it has not been reported for DNA films on surfaces. I verified hypochromism with the selected viral sequences by spectrophotometry measurements in solution and then observed hypochromism in SE experiments. In fact, no increase in absorbance is detected upon incubation and hybridization with tDNA, i.e. an increase of DNA strands. As far as we know, it is the first time that hypochromism is observed for DNA monolayers immobilized on a surface. Therefore, our experiments could add information on this attracting phenomenon in a situation different from usual experiments in solution.

As a further step, from a more applicative perspective, I investigated the selectivity of the sensing platform by introducing non-target DNA. Results demonstrate that the system can discriminate between the human coronavirus HKU1 and the SARS-CoV-2. Furthermore, the system was found to preserve the same recognition capability in a molecularly crowded environment, in which both HKU1 and Sars-Cov-2 are present. Decreasing the Sars-CoV-2 concentration while keeping constant an excess HKU concentration does not affect detection.

Finally, I used a modified set-up of the spectroscopic ellipsometer to perform TIRE experiments, using the Kretschmann configuration. This method combines SE with surface plasmon resonance (SPR), leading to an increase in system sensibility. While the LoD (Limit of Detection) of SE is 25nM

of the target sequence, TIRE can detect down to 1nM. A part of my future activity will focus on methods to reduce the minimum measurable concentration. One of the strategies will be the use of nanostructured gold films as support for TIRE measurements.

List of courses:

Biologia Molecolare: exam done
Spettroscopie e Materiali per la fotonica: attended
Biosensors: attending
AFM BioMed Summer School: attended

List of publications:

G. Pinto, S. Dante, **S.M.C. Rotondi**, P. Canepa, O. Cavalleri, M. Canepa. *Spectroscopic Ellipsometry Investigation of a Sensing Functional Interface: DNA SAMs Hybridization.* Adv. Mater. Interfaces 2022.

List of conferences and presentations:

S.M.C. Rotondi, S. Dante, G. Pinto, A. Lagomarsino, P. Canepa, M. Canepa, O. Cavalleri. *Towards a label-free biosensor: studying DNA SAMs with a multi-technique investigation.* **Poster** presented at 1st MOSBRI Conference with bursary, June 20-22 2022, Paris, France.

AFM BiomMed School, July 11-16 2022, Marseille, France.

S.M.C. Rotondi, S. Dante, G. Pinto, A. Lagomarsino, P. Canepa, M. Canepa, O. Cavalleri. *Label-free detection of diseases-related oligonucleotide sequences.* **Oral communication** at XXVI National Congress SIBPA with bursary, September 11-14 2022, San Miniato, Italy.

S.M.C. Rotondi, S. Dante, G. Pinto, P. Canepa, M. Canepa, O. Cavalleri. *Optical detection of viral sequences through spectroscopic ellipsometry.* **Poster** presented at 17th European Conference on Organized Films, September 14-16 2022, Cordoba, Spain.