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RELAZIONE ATTIVITA' ANNUALE DEI PERFEZIONANDI/DOTTORANDI – TERZO/QUARTO ANNO
REPORT ON THE PHD ACTIVITY – THIRD/FORTH YEAR

NOME E COGNOME NAME AND SURNAME	Giulia Matteoli
DISCIPLINA PHD COURSE	Nanoscience

CORSI FREQUENTATI CON SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (WITH FINAL EXAM)	**VOTAZIONE RIPORTATA MARK**	**NUMERO DI ORE HOURS**

CORSI FREQUENTATI SENZA SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (ATTENDANCE ONLY)	**NUMERO DI ORE HOURS**

ALTRE ATTIVITÀ FORMATIVE (SEMINARI, WORKSHOP, SCUOLE ESTIVE, ECC.) – DESCRIZIONE OTHER PHD ORIENTED ACTIVITIES (SEMINARS, WORKSHOPS, SUMMER SCHOOLS, ETC) – DESCRIPTION	**NUMERO DI ORE HOURS**
November 2019_Applied Nanotechnology and Nanoscience International Conference (ANNIC 2019)	30
May 2020_ WORKSHOP "ELLIS against Covid-19"	4
June 2020_WEBINAR Alfatest "Nanomedicina e l'importanza della monodispersità, ma come ottenerla"	1
June 2020_ WEBINAR Alfatest "Perchè dovrei pensare alla sintesi chimica in flusso continuo?"	1
November 2019 – March 2020 : Weekly seminars at Fondazione Pisana per la Scienza (Internal and External Speakers)	15

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ATTIVITÀ DI RICERCA SVOLTA (MAX. 8.000 CARATTERI)*
RESEARCH ACTIVITY (MAX. 8000 CHARACTERS)

(a longer report in the document attached)

My PhD research activity is focused on the development of nanoparticle-based biosensors for early detection of Prostate Cancer. During this third year of PhD I concluded the characterisation and the sensing response of a first type of biosensor, specific for Prostate Membrane Protein Antigen (PSMA), designed for *ex vivo* application and I started to assemble and characterised a second type of biosensor specific for Prostate Specific Antigen (PSA) that have features that make it suitable for *in vivo* application.

In Prostate Cancer, the second most diagnosed cancer in men, survival rate drops from nearly 100% if Prostate Cancer is diagnosed early to 30% when diagnosed in advanced state. In fact, a tumor can grow for years before it is detected, and complete eradication of the disease is strictly related to our diagnostic abilities. However, sensitivity level in diagnostic has not improved in the last decades. One of he main hurdles is the intrinsic limit of detection proper of any analytical technique. If we consider the sensitivity of ELISA, the main bioanalytical technique used in clinics for protein detection, we can detect an antibody with a dissociation constant of 10^{-9} M in standardised clinical tests. This limit of detection is dictated mainly by the attainment of chemical equilibrium between the antigen and the antibody. As a consequence, the limit of detection of chemical equilibrium based reactions, such as ELISA, strictly depends on the concentration of the biomarker and not on volume of the sample. One way to overcome this limitation consists to quantify a biomarker with an irreversible recognition step by the sensor. In these reactions sensitivity depends on the absolute number of molecules (instead of concentration) and, as a consequence, would increase with the screening of larger sample volumes. With this in mind, I have developed two triggerable metal-based nanoarchitectures able to respond to the presence of a protein biomarker (PSMA or PSA). These sensors are composed by DNA functionalised Gold nanoparticles (NPs) that interact together to form a cluster of nanoparticles. This interaction occurs because the DNA sequences are designed to anneal stably in absence of the analyte. The recognition of the sensor with the target protein occurs because these DNA sequences include an aptamer specific to the biomarker. The interaction between the aptamer to the protein makes the annealing of the sequences unstable and triggers the release of single NPs in an irreversible way. The choice of Gold NPs permits to monitor the release of single NPs from the cluster and changes in size of the cluster exploiting Gold NPs optical proprieties.

PSMA responsive sensor: The first sensor developed is responsive to Prostate Specific Membrane Antigen (PSMA) and is designed for *ex vivo* application. In fact, detection of PSMA in urine samples and in urinary exosomes discriminates between Prostate Cancer and healthy patients. PSMA responsive sensor is composed of single 13 nm diameter Gold NPs, decorated with selected nucleotide sequences. DNA sequences and their melting temperatures were firstly designed *in silico* and then confirmed with absorbance measurements at 260 nm. Then, PSMA sensor was fully characterised for its chemical-physical proprieties and thermal stability. Clusters disassemble upon reaching DNA sequences melting temperature, consistently with the dependence of assembly stability on the hybridization of the nucleotide sequences. PSMA sensing response was then assessed in two systems of increasing complexity, i.e. human recombinant PSMA and PSMA positive exosomes, which are the real biological target of the sensor. PSMA responsive sensor has a limit of detection of 50 pM to human recombinant PSMA releasing single NPs. In this reaction we observed a reduction in size of the cluster confirmed also by a plasmonic shift towards shorter wavelengths. Then, we incubated the sensor with PSMA positive exosomes. In this case we observed an increase in size of the system, probably due to the interaction of the NPs decorated with the aptameric sequence on PSMA present on the surface of the exosome. In this case we were able to detect PSMA-positive exosomes at a concentration of 10^{10} exosomes/ml.

PSA responsive sensor: The second type of sensor is specific to Prostate Specific Antigen (PSA), a biomarker whose presence in serum is crucial for early detection of Prostate Cancer recurrence after radical prostatectomy. PSA

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responsive sensor has been designed for *in vivo* application, thus for screening total blood volume (larger sample volume). In this case the cluster is composed by single 2 nm Gold NPs, whose size is compatible to renal clearance requirements and suitable for *in vivo* application. In fact, recognition of PSA by the sensor would produce the release of single renal clearable NPs that would be rapidly secret in urine. PSA responsive sensor chemical-physical characterisation is completed: melting temperatures of the sequences and of clustered nanoparticles were confirmed and indicate that the cluster is stable at body temperature. The sensor needs to be tested for it PSA sensing ability for the conclusion of this project.

*se si intende sottoporre una relazione di ricerca più estesa, utilizzare il campo per una descrizione sintetica e allegare il documento in formato .pdf

If you are going to submit a longer report, please fill the box with a synthetic abstract and attach a document in pdf format

EVENTUALI PUBBLICAZIONI **PUBLICATIONS (IF AVAILABLE)**
Oral communication at Applied Nanotechnology and Nanoscience International Conference – ANNIC 2019. Oral title: “DNA-driven Gold Nanoparticle assembly tailored to biomarker quantification”

NOME DEL RELATORE **THESIS ADVISOR**
Internal supervisor: Prof. S. Luin External supervisor: Dr. G. Signore

DATA **DATE**	16/10/2020	**FIRMA** **SIGNATURE**