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RELAZIONE ATTIVITA' ANNUALE DEI PERFEZIONANDI/DOTTORANDI – SECONDO ANNO
REPORT ON THE PHD ACTIVITY – SECOND 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**
Corso 1602: Ciclo di seminari – Biophysical Sciences	Da sostenere	45

CORSI FREQUENTATI SENZA SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (ATTENDANCE ONLY)	**NUMERO DI ORE HOURS**
Corso 1425: Fluorescent biosensors II	24
English for writing and presenting research papers course (Adrian Wallwork)	16
English for CVs and job interviews course (Adrian Wallwork)	4

ALTRE ATTIVITÀ FORMATIVE (SEMINARI, WORKSHOP, SCUOLE ESTIVE, ECC.) – DESCRIZIONE OTHER PHD ORIENTED ACTIVITIES (SEMINARS, WORKSHOPS, SUMMER SCHOOLS, ETC) – DESCRIPTION	**NUMERO DI ORE HOURS**
31/01/2019 Workshop: Advanced theranostic nanomedicine in oncology. IIT	9
28/03/2019 Seminar: In vitro to real: a novel integrative strategy to prevent colorectal cancer within the diet-host-microbiota triangle. Dott. Josep Rubert	1
30/05/2019 Seminar: Investigating Brain Disease: from Patients to Model Organisms and Model Organisms and Beyond. Prof. Angeliki Louvi	1
10-11/06/2019 Workshop: "Highlights in nanoscience" . NEST	16
13/06/2019 Seminar: Present and future of Forensic Biology. Dott. Andrea Berti	1
26/06/2019 Seminar: Exploitation of nanoparticle–protein corona for emerging	1

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therapeutic and diagnostic applications. Prof. Giulio carracciolo	
29/06/2019 – 06/07/2019 Summer School: ISSON2019 (International SummerSchool on Nanoscience and Nanotechnology	64
12/09/2019 Seminar: The King and the Crown: Impact of the Nanoparticle-Protein Corona in Nanomedicine. Prof. Giulio Caracciolo	1
3/10/2019 Seminar: Laboratory of cellular therapy, program of cell therapy and immuno-oncology. Prof. Dominici Massimo-UNIMORE	1
04/10/2019 Seminar: Biomimetic materials: opportunities in biomedicine. Prof. Ennio Tasciotti	1

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

Second year research activity was focused on two projects:

1) Development of renal clearable nanoparticle-based biosensors for early cancer diagnosis (main research)
2) Biomarker discovery from glioblastoma multiforme cancer exosomes (internal collaboration with FPS research groups)

1. Development of renal clearable nanoparticle-based biosensors for early cancer diagnosis:

In vivo nanoparticle-based biosensors would have the potential to improve current diagnostic capabilities. Indeed, the outcome of most pathologies such as cancer is strictly related with the ability to perform early diagnosis of the disease. This research aims at developing triggerable metal-based nanoarchitectures, composed by several small clearable nanoparticles, able to selectively recognise an analyte in vivo and provide a measureable feedback on the presence and concentration of the biomarker even at ultra-low concentrations.

Gold nanoparticle (13 nm diameter) biosensor design:

We have assembled a biosensor composed by 13 nm diameter gold nanoparticles.
These particles are not renal clearable but show good aggregation-dependent optical properties. Particles were decorated with selected and complementary DNA oligonucleotides. These sequences lead to the formation of the aggregates and are responsive to the presence of the analyte. The working principle of the sensor is shown in figure 1A.
Two main structures of oligos were designed, named dimeric and trimeric, where two or three sequences are respectively involved. For each structure we used “S” and “antiPSMA” group of sequences (fig.1B). In particular, antiPSMA sequences include an aptameric sequence against PSMA, a biomarker for prostate cancer.
Design and *in silico* characterisation for these sequences were performed with mFold. Sequences annealing was further confirmed by High Performance Liquid Chromatography (HPLC).

Physical characterisation of the biosensor:

Aggregates were obtained incubating nanoparticles that expose complementary sequences on their surface. Aggregated particles show plasmonic shift and increased hydrodynamic radius.
Melting curve of the aggregates was measured following the size change of the aggregates with DLS at different temperatures. We observed that aggregates disassemble upon reaching their melting temperature, with a steeper curve shown by trimeric assemblies.

In cuvette evaluation of the biosensor:

Biosensing ability of aggregates was tested toward oligonucleotides and PSMA protein.

Oligonucleotide biosensor: The aggregate structure responsive to oligonucleotides (Figure 1B: S1:S3:S4 trimer) recognizes sequences complementary to S1. Under the tested conditions, limit of detection is lower than 100 nM.

Protein biosensor: The dimeric version of the aggregate responsive to PSMA (Figure 1B) shows sensitivity in the low pM range, and it is based on the partial dehybridization from the complementary B sequence of the sensing aptamer sequence, that occurs upon binding to the protein biomarker. This in turn leads to destabilization, and hence disassembly, of the entire nanostructure. The trimeric antiPSMA aggregate working principle is essentially the same; its sensitivity level must be further investigated.

Development of ultrasmall based nanoparticle biosensor:

We are developing of a second generation of biosensors, compatible with clearance requirement, translating the system on ultrasmall quantum dots and ultrasmall gold nanoparticles.

2 nm gold nanoparticles were synthetized and decorated with our sequences. As for the previous biosensor, we prepared the aggregates and measured their melting curves.

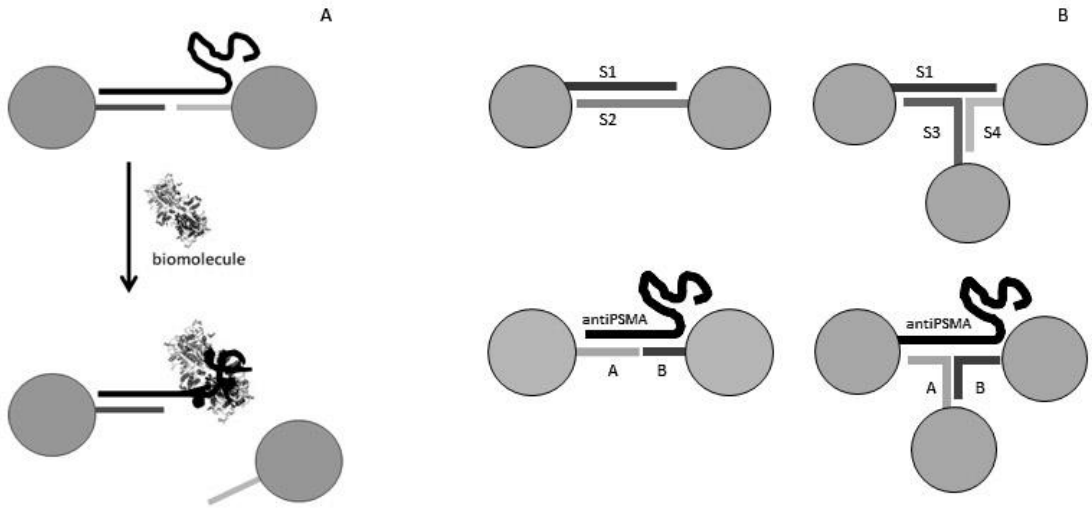

Fig. 1. (**A**) Sensing principle of the aggregate (**B**) Schematic of the aggregates structures. From up left to bottom right: S1:S2 dimer; S1:S3:S4 trimer; antiPSMA dimer and antiPSMA trimer.

2. Biomarker discovery from glioblastoma multiforme cancer exosomes

As parallel research line, we have started a collaboration for the discovery of novel circulating biomarkers. In particular, we are interested in profiling membrane proteins from glioblastoma multiforme (GBM) exosomes. Exosomes are extracellular vesicles that play a role in long distance communication between cells and are relevant in cancer development. Exosomes are considered a

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good source of biomarkers, for these reasons we are interested in profiling membrane proteins from exosomes isolated from GBM primary cells. The identification of unique membrane biomarkers on their surface would help their recovery and quantification in peripheral blood of patients, with important impact on screening and early diagnosis procedures.

Exosomes were isolated from GBM primary cells. Cells were obtained thanks to Azienda Ospedalierio Universitaria Pisana.
Exosomes were concentrated with three different methods (ultracentrifugation, ultrafiltration coupled to dialysis and size exclusion chromatography columns) in order to establish the better isolation method for our experiments. Proteomic analyses are being carried out.

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

Dr. Giovanni Signore
Dr. Stefano Luin (relatore interno)

DATA **DATE**	04/10/2019	**FIRMA** **SIGNATURE**