BIOENGINEERING AND MEDICAL-SURGICAL SCIENCES

DISAT - Modulation of RNA Polymerase Activity via Plasmonic Hot Spots

Funded By	Dipartimento Scienza Applicata e Tecnologia [P.iva/CF:00518460019]
Supervisor	FABRIS LAURA - laura.fabris@polito.it
Contact	
Context of the research activity	RNA viruses are a class of pathogens causing significant morbidity and mortality worldwide. Their high mutation rates lead to antigenic shift and antigenic drift, which, ultimately, can enable zoonotic spillover and lead to pandemics. At the basis of the high mutation rates, among which those of influenza A virus (IAV) are the highest, lies the RNA polymerase, an enzyme responsible for transcription and replication, that, in RNA viruses, lacks proofreading ability. Recently, it has been shown that the integration of gold nanoparticles with enzymes can enable the modulation of enzymatic activity via light absorption through the nanoparticles plasmonic modes and the ensuing photothermal heating.
	Through research activities the student will work on testing the hypothesis that the illumination of hot spots in gold nanostars can be leveraged to modulate activity and fidelity of IAV's RNA polymerase through the generation of photothermal heat and hot electrons. Through experiments and computational work, the student will gain knowledge on nanotechnology, spectroscopy, plasmonics, and sensing, and will benefit from the exposure to a highly multidisciplinary and international team, in which he/she will also become proficient in the use of technical English for writing manuscripts and delivering seminars and presentations. We will build a plasmonic platform in which gold nanostars will be grown in situ in an organized array, thus bridging the gap between top-down and bottom-up nanomanufacturing protocols, and where IAV RNA polymerase will be covalently anchored at the nanostar tips with ligands that are resistant to the reaction conditions in which RNA replication occurs. The platform will then be illuminated through near infrared radiation and the production of complementary RNA (cRNA) from viral RNA (vRNA) will be monitored. Additionally, IAV RNA polymerase fidelity will be assessed in the presence and absence of illumination and other external evolutionary pressures. Polymerase fidelity will be quantified by monitoring mutation rates in the cRNA strands produced by leveraging the direct SERS method developed in the ERC-funded grant ANFIBIO, and confirmed through both Illumina and MinION nanopore sequencing. The results obtained and the fundamental

Objectives	knowledge generated will inform the design of novel photocatalysts based on the integration of hot electron-producing plasmonic nanoparticles. The student will be responsible for: 1. Heterogeneously growing bespoke plasmonic nanoparticles onto silicon substrates onto which the enzyme molecules will be bound; 2. Understanding how to modulate the activity of RNA polymerases through illumination and the ensuing hot electrons and heat; 3. Monitoring RNA polymerase activity and fidelity via surface enhanced Raman spectroscopy and tradition sequencing approaches. In particular, the student will: 1. Design, heterogeneously synthesize, and characterize six-branched gold nanoparticles also leveraging 3D printing and microfluidics; 2. Design and optimize methods to stably and reproducibly bind the enzyme molecules to gold nanoparticles so they can be equally modulated by light and heat; 3. Implement substrate-based measurements of the SERS response of RNA produced by the polymerase in various illumination conditions with rapid collection times and high spectral resolution; 4. Analyze the obtained SERS spectra with innovative statistical methods; 5. Understand how to treat and minimize the background signal generated by the complex matrix during the SERS measurements; 6. Model computationally the plasmonic response of arrays of nanoparticles through software packages such as Comsol Multiphysics and Lumerical. 7. Coherently organize and report the data collected for presentation to the other group members, collaborators, and/or audiences at conferences.

Skills and competencies for the development of the activity	We are looking for talented and driven students with preferably a M.S. degree in chemistry, materials science, or bioengineering (broadly defined) and previous expertise in: 1. Synthesis and functionalization of gold nanoparticles; 2. Basic knowledge of Raman and surface enhanced Raman spectroscopies; 3. Design and manipulation of nucleic acid probes; 4. Basic knowledge of nucleic acid amplification techniques (e.g., RT-PCR, LAMP); 5. Knowledge on 3D printing and microfluidics; 6. Basic knowledge of electric field modelling tools.