

BIOENGINEERING AND MEDICAL-SURGICAL SCIENCES

DISAT - Modulation of RNA Polymerase Activity via Plasmonic Hot Spots

Funded By	Dipartimento DISAT
------------------	--------------------

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 can enable zoonotic spillover and lead to pandemics. At the basis of the high mutation rates, lies the RNA polymerase, an enzyme responsible for transcription and replication. Recently, it has been shown that the integration of gold nanoparticles with enzymes can enable the modulation of enzymatic activity via light absorption. 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. Bando FARE - progetto PHROGS 1216/2022 Modulazione Plasmonica Mediata da Hot Spot dell'Attività dell'RNA Polimerasi Virale Indagata Tramite Surface Enhanced Raman Spectroscopy – CUP E13C22002520005
---	--

	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. The results obtained and the fundamental 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;
--	--

Objectives	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. Basic knowledge on synthesis and functionalization of gold nanoparticles; 2. Basic knowledge of optical spectroscopy; 3. Basic knowledge of nucleic acid probes; 4. Basic knowledge of nucleic acid amplification techniques (e.g., RT-PCR, LAMP); 5. Basic knowledge on 3D printing and microfluidics.