Determination of Aptamer and Apatmer-siRNA species by Surface enhanced Raman (SERS), Surface-Enhanced Infrared Absorption (SEIRA) and Nano-IR spectroscopic techniques

Abstract*:*

The research proposal is based on the ongoing activities of the Molecular Spectroscopy Group (MSG) at the Department of Industrial Chemistry "Toso Montanari". The group focuses on the experimental and computational study of the structural and spectroscopic properties of molecular systems in the condensed phases. To achieve this, experimental techniques such as micro-Raman, micro-IR, UV/visible spectroscopy, along with X-ray and AFM measurements in collaboration with other researchers, are employed.

The present proposal highlights the group's intent to employ spectroscopic methods such as Surface-Enhanced Raman (SERS), Surface-Enhanced Resonance Raman (SERRS), SEIRA (Surface-Enhanced Infrared Absorption), and Nano-IR, for the detection of aptamers and aptamer-siRNA species within a limit as low as 100 pM in a complex matrix made of a biocompatible gel containing entrapped anti-cancer drugs, aiming to advance fundamental research closely tied to practical applications.

Glioblastoma (GB) is the most aggressive primary brain tumor, with a 5-year survival rate of only 5.7%. Its resistance to chemotherapy, diffuse invasiveness, and location beyond the blood-brain barrier (BBB) limit treatment options. As a result, new drug delivery strategies are crucial. Recent evidence suggests that Hedgehog-GLI (HH) pathway inhibitors could offer therapeutic potential for GB.

The large project funding this fellowship aims to develop a novel drug delivery approach for glioblastoma (GB), using locoregional treatment with biocompatible gels containing temozolomide (TMZ) combined with various anti-cancer agents targeting GLI. These agents include newly developed small molecule inhibitors (GLIi) and innovative Aptamer-siRNA conjugates (Apt-siGLI). This strategy is designed to overcome the blood-brain barrier and drug resistance, reduce systemic toxicity, and increase the concentration of therapeutic drugs at the tumor site.

In this research, a key focus is the characterization of siRNA and the identification and quantification of Apt-siGLI within the synthesized chitosan-based thermogels (THG and HYG). In this context, Raman and Resonance Raman spectroscopies and imaging will be used to detect the sensitive vibrations of the aptamer binding sites, possibly enabling the differentiation between bound and unbound aptamers within the gels. By using Resonance Raman spectroscopy, with excitation in the 200-300 nm wavelength range, we expect the aptamer signal to be selectively enhanced by several orders of magnitude through precise modulation of the excitation wavelength. Additionally, a label-free SERRS (Surface-Enhanced Resonance Raman Scattering) protocol could be employed, where the analyte is adsorbed on metal nanoparticles and identified based on its intrinsic Raman spectrum, further enhancing the detection sensitivity of the aptamer. For SERS, we will develop an experimental protocol using metallic nanoparticles to enhance the signal, aiming to achieve aptamer detection in the pM concentration range with our in-house spectroscopic setup. For the SERRS technique, we will exploit our established collaboration with the ELETTRA synchrotron facility in Trieste. In

both approaches, because quantifying the aptamer itself is the crucial, a label-free approach is most suitable, despite potential sensitivity challenges.

Along with SERS spectroscopies, we will attempt the aptamer detection also using SEIRA (Surface-Enhanced Infrared Absorption), the spectroscopic technique that enhances the infrared absorption signals of molecules adsorbed on certain nanostructured metallic surfaces as a result of the interaction between the electromagnetic field of the IR light and the surface plasmons. In fact, SEIRA can be effectively used to detect label-free the distinct vibrational modes of the aptamers active in the infrared spectrum, such as stretching vibrations of phosphate backbones or specific nucleobases. The ultimate goal of the project would be to apply a Nano-IR technique. This would allow for the analysis of much smaller sample volumes, making it more suitable for studying low-concentration aptamer systems or very thin films of aptamers, as it can detect molecular interactions in highly localized regions, even at single-molecule levels.

Work Plan

As outlined in the project plan, the primary experimental techniques for studying the systems of interest will include Raman and FTIR microscopy, already available at the Department of Industrial Chemistry "Toso Montanari," along with Nano-IR spectroscopy, which will be operational at Navile Campus by the end of 2024. The main objective is to develop SERS and AFM-IR techniques to detect molecular analytes in complex samples at picomolar concentrations. This will involve managing, maintaining, and potentially enhancing the experimental setup throughout the research activity.

The specific goals of our proposal are:

1. Implement the existing micro-IR/Raman experimental setup at the hosting group.
2. Plan vibrational spectroscopic measurements using synchrotron-based systems.
3. Optimize SERS techniques using the in-house micro-Raman spectrometers.
4. Set up and optimize the new Nano-IR system at the hosting campus for nanometric infrared resolution.