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Author of the doctoral dissertation: **mgr inż. Patryk Pyrcz**

Supervisor: **Prof. Dr Hab. Jacek Waluk**

Auxiliary supervisor: **Dr Sylwester Gawinkowski**

Title of the doctoral dissertation: *Towards Stable Single-Molecule SERS Measurements with High Temporal Resolution.*

Abstract

Surface-enhanced Raman scattering based techniques provide unique insights into a wide range of processes with unprecedented sensitivity, reaching the level of single-molecule detection. Achieving such a level of sensitivity is possible through the use of so-called plasmonic nanocavities – regions characterized by anomalously high electromagnetic field enhancement factors arising from the excitation of localized plasmon resonances. The objective of this dissertation was to investigate chemical and physical processes involving molecules (including single molecules) occurring within plasmonic nanocavities using surface-enhanced Raman scattering (SERS) and surface-enhanced femtosecond stimulated Raman scattering (SE-FSRS) techniques.

The first part of the dissertation focuses on elucidating the temporal evolution of single-molecule SERS (SM-SERS) spectra using aggregated gold nanorods as the plasmonic substrate and porphycene (Pc) as the Raman reporter. The temporal evolution of SM-SERS spectra was characterized at two extreme temperatures, namely 298 K and 83 K. Initially, the influence of temperature on the lifetimes of individual Pc molecules under SERS conditions was investigated, demonstrating that at 83 K the lifetime is statistically fifteen times longer than at 298 K. Subsequently, time traces of Pc SM-SERS spectra were analyzed, and fluctuations in Raman band amplitudes as well as spectral fluctuations were examined by investigating the relationships between selected Raman bands of Pc. The performed analysis of the Spearman correlation coefficient revealed that, regardless of temperature, amplitude fluctuations of Raman bands exhibit significantly higher correlation coefficients compared to fluctuations in band positions. As the temporal resolution of single-spectrum acquisition improved – from 500 milliseconds to a few milliseconds – the distributions of Spearman correlation coefficient values for both amplitude and positional fluctuations of Raman bands shifted toward weaker correlations. In the case of fluctuations in the amplitude of the Raman signal, the degree of shift was greater compared to the fluctuations in the positions of the Raman bands.

Analysis of the Spearman correlation coefficient revealed that amplitude fluctuations are independent of spectral fluctuations of the Raman bands, suggesting the presence of two distinct

mechanisms responsible for these fluctuations in SM-SERS spectra. In the case of amplitude fluctuations in the SM-SERS spectra of Pc and the observation of relative changes in Raman bands corresponding to the $2A_g$ and $4A_g$ vibrational modes, the most probable mechanism is their selective enhancement by atomically confined near-field cavities. In contrast, for spectral fluctuations – characterized by abrupt, rapid shifts in Raman band positions – the proposed mechanism involves the formation of so-called picocavities. These structures arise from spontaneous interaction formed between individual gold adatoms on the nanoparticle surface and the Pc molecule.

In the following part of the work, the focus was placed on reducing fluctuations in SM-SERS spectra. An approach was employed in which the investigated molecule, thionine (Th), was incorporated into a supramolecular complex with cucurbit[7]uril (CB[7]). The study examined the effect of encapsulation in CB[7] on: (i) the SERS spectrum in both aqueous and dry environments, (ii) photostability under plasmonic conditions, and (iii) the detection probability and temporal evolution of SM-SERS spectra. The performed investigations, supported by quantum-chemical calculations (DFT), revealed that Th encapsulated in CB[7] adopts a perpendicular orientation, which provides a favorable alignment of the transition dipole moment of Th with respect to the electromagnetic field vector inside the nanocavity. Consequently, a more dynamic decay of the SERS signal was observed for Th–CB[7] complexes compared to Th freely oriented on the metal surface. Single-molecule measurements further demonstrated an increased detection probability and a reduced coefficient of variation for SM-SERS spectral amplitude fluctuations in the case of Th–CB[7]. The more stable fluctuation profile was interpreted as resulting from restricted mobility of Th within CB[7], caused by the suppression of translational motions in the nanocavity after encapsulation.

The final part of the dissertation was devoted to the development of a methodology based on SE-FSRS as a potential tool for studying single molecules on ultrafast timescales. Optimization studies were carried out using silica-coated *trans*-1,2-bis(4-pyridyl)ethylene-functionalized spherical gold oligomers, during which the influence of key experimental parameters on the acquisition of SE-FSRS spectra at a repetition rate of 1 kHz was investigated. These parameters included the energy and wavelength of the Raman pump pulse, as well as the time delay between the Raman pump and the probe pulse. In the subsequent part, attention was focused on elucidating the origin of the broad background that accompanies SE-FSRS signals. Using monodisperse colloids of silica-coated gold nanobipyramids, it was demonstrated that the background observed in SE-FSRS spectra is strongly correlated with the plasmon resonance spectrum, while its spectral position remains constant and independent of the Raman pump excitation wavelength. Based on the experimental results and supporting calculations, it was suggested that the appearance of this background is associated with the photothermal effect, accompanied by local temperature changes in plasmonic nanoparticles.