Abstract

Title:	Optimization of surface-enhanced Raman scattering spectroscopy for study of biologically important biomolecules and their interactions
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Abstract:

The main goal of this thesis was to optimize surface-enhanced Raman scattering (SERS) spectroscopy for study of biologically important biomolecules. For that purpose we focused on substrates based on gold colloidal nanoparticles immobilized to silanized glass plates. Stable, uniform and highly reproducible SERS-active substrates have been prepared by using aminopropyltrimethoxysilane and citrate-reduced gold nanoparticles thermally stabilized after their immobilization. Model biomolecules 5,10,15,20-tetrakis(1-methyl-4-pyridyl)porphyrin (TMPyP) and 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TSPP) were studied on these substrates by using a classical Raman spectrometer in macro-mode and a confocal Raman microspectrometer. Conditions for SERS spectroscopy of porphyrins were optimized with respect to sensitivity and reproducibility. SERS microspectroscopy showed several advantages over SERS measurements in macromode: possibility of surface spectral mapping, easier manipulation with samples, shorter collection times and absence of strong Raman signal from glass support. Both techniques show limit of detection (LOD) of porphyrins ~ 5×10^{-8} M and prove very good spectral reproducibility of substrates in both mm- and μm- scale. SERS enhancement was optimized by measuring of TMPyP SERS spectra using six excitation wavelengths (excitation profile). Results show that SERS intensity of TMPyP is correlated with the extinction spectrum of the system Au surface/TMPyP although the position of the maximum of intensity differs for particular vibrational modes depending on the molecular resonance. Maximal enhancement is obtained for 568.2 nm excitation that provides LOD of TMPyP 2×10^{-8} M.

Keywords: SERS, SERS microspectroscopy, immobilized gold nanoparticles, porphyrins, excitation profile