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Raman Spectroscopy with Nanoparticles for Investigation of Protein Tyrosine Oxidation

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Introduction

Reactive oxygen and nitrogen species play important physiological and pathological roles [1,2]. They are signaling molecules and are used by the immune system as a defense against pathogens [3,4]. If they appear in high concentrations and cannot be quenched by antioxidants, oxidative stress occurs [5,6]. Oxidative stress is implicated, for example, in Diabetes mellitus type 2, or in many neurodegenerative diseases [2,5,6].

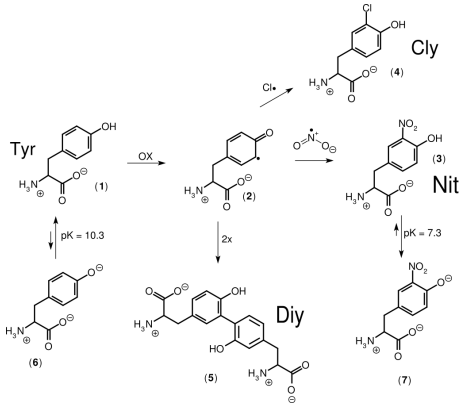


Fig. 1. Oxidized tyrosines [3,4].

For example, nitration, halogenation, and crosslinking of tyrosines are important post-translational modifications (Figure 1) and can influence protein structure [4]. The nitration is an early-stage marker of oxidative stress and neurodegeneration [7]. To understand the effect of oxidation on biophysical functions, we selectively synthesized a series of oxidized peptides and proteins. We have described the synthesis of selectively nitrated synuclein segments using Fmoc-Nit(Bn)-OH [8]. In addition to that other oxidized tyrosines can be inserted into peptides and proteins [8-10]. We have shown that double nitration significantly affected the vibrational circular dichroism spectra of synuclein segments, whereas chlorination did not cause any significant structural changes [9,10]. We used various nanoparticles to visualize protein tyrosine oxidation by surface-enhanced Raman spectroscopy (SERS), and identified markers for nitrated proteins [7,10]. We also used the SERS for studies of a neuroregenerative drug [11].

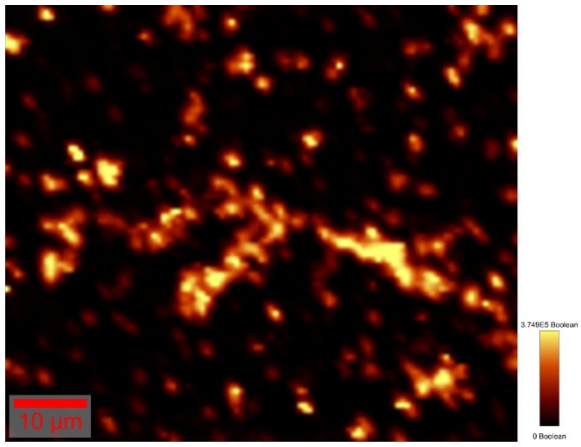
Results and Discussion

We have photochemically prepared nanosilver as a SERS substrate [7]. By adjusting the irradiation light, we could tune both plasmonic bands of pink-silver. The band yielded from the green light scattering was visible by naked-eye and was important for enhanced Raman scattering.

Protein tyrosine nitrations were monitored using surface enhanced Raman spectroscopy (SERS) with green or red laser excitation [7,10]. Two modes of measurement were used: native and induced ones. In both modes, the analyzed protein sample was mixed with silver colloid. In the induced mode, the hydrochloric acid was added to lower pH and initiate aggregation of the colloid. The induced mode is more universal for measurement of tyrosine nitration i.e., it works with many types of silver colloids such as chemical brown silver [10] and photochemical pink silver [7].

For induced mode, the characteristic pattern observed in Raman spectrum is a change of tyrosine fingerprint vibrations in region between 810 and 890 cm^{-1} [7,10]. This change is caused by strong domination of δ_{NO2} enhanced by silver surface at ca 825 cm^{-1}. The induce mode can serve for nitration detection down to 500 nM [10].

For native mode with pink-silver [7], the most dominant became the asymmetric vibration of nitro group at (1523-1527 cm^{-1}, $\nu_{NO2,as}$). This vibration in native mode can serve for nitration detection down to 114 nM [7]. Symmetric nitro band (1338-1343 cm^{-1}, $\nu_{NO2,symm}$) was visible marker of YYACAYY peptide nitration [7]; however, when the bigger synuclein models were used, the region was dominated by other vibrations of amino acids and could not serve for reliable detection of nitration [10].



We have also investigated the possibility to measure the SERS using Raman microscope. For that purpose, we have covalently anchored the silver or gold nanoparticles on glass surface using known-techniques [12-14]. The measurement in solid state brought some difficulties such as sample burning at higher powers of laser. We have achieved optical and Raman co-localization of aggregated microparticles on the surfaces. These microparticles can interact for instance with nitrobenzene – a model of nitrated species (Figure 2).

Fig. 2. Raman intensity of nitrobenzene sample with pink-silver anchored on glass surface.

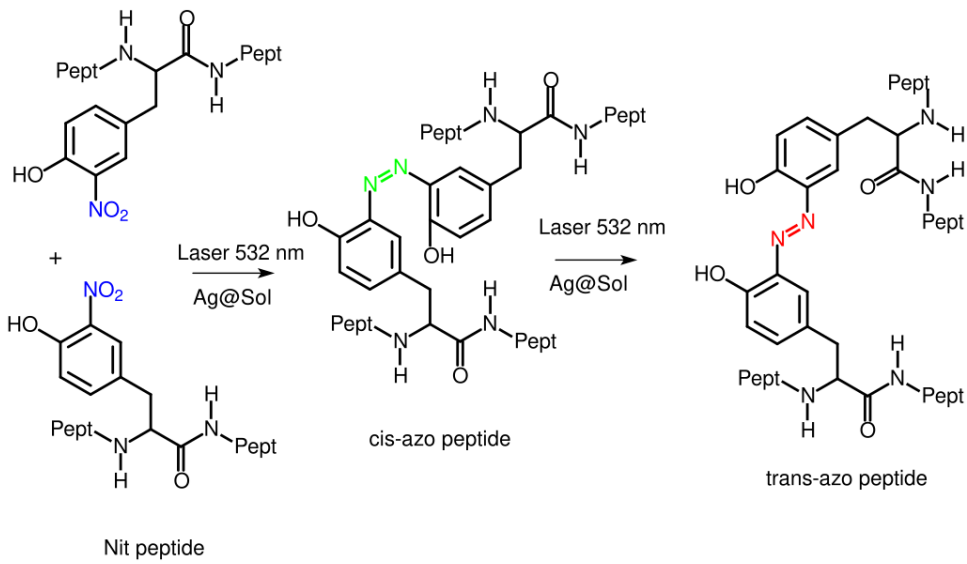


Fig. 3. Photochemical formation of azo-peptides [10].

In physics, it is well known – Heisenberg's uncertainty principle – saying that you cannot study the system without influencing it. The same is also valid for SERS studies: when the peptide is anchored on silver surface and irradiated with light it may undergo various chemical transformations.

On brown silver with [Nit^1]enkephalin, we have observed a conversion of nitro group to azo bond, which was accompanied with the crosslinking of two peptide chains together [10] (Figure 3). According to the observed Raman signals, the cis-azo bond was formed at the beginning, and later it was followed by isomerization to trans-azo peptide.

The pink-silver provided access to Cys(tBu) deprotection through the anchoring to the silver surface (Figure 4). The reaction was confirmed by an observation of S-Ag vibration and Cys vibrations

in Raman spectra [7]. This strategy is advantageous for syntheses of peptides bearing Cys residue. Protection group prevents unwanted dimerization of peptides via disulfide bridge formation. When the peptide reached the proximity of silver surface, tBu is removed and peptide stays anchored to the surface for measurement.

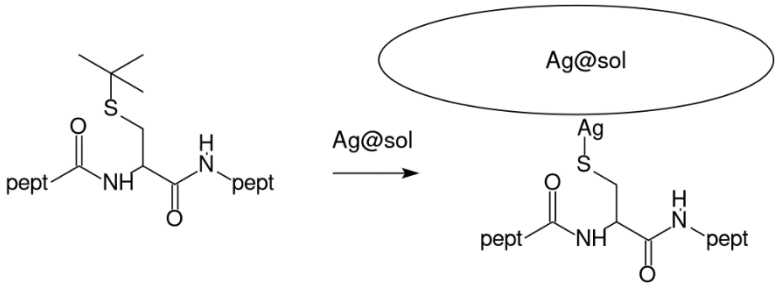


Fig. 4. Removal of tBu group by direct anchoring on silver surface [7].

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