2DIR spectroscopy study of oxidation – from biomarker quantification to spectral imaging of tissue sections.

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Electron-Vibration-Vibration two-dimensional infrared spectroscopy is used here to study oxidation biomarkers, such as 3-nitrotyrosine. We aim to demonstrate how the spectral information obtained through this technique could be used to generate 2DIR images able to map the localisation of biomarkers across healthy and diseased human tissue sections.

The use of Electron-Vibration-Vibration (EVV) two-dimensional infrared (2DIR) spectroscopy [1,2] for the label-free and non-destructive study of oxidation biomarkers will be discussed. This technique employs one near-IR and two mid-IR picosecond excitation beams to probe vibrational couplings in a sample, allowing for direct observation of vibrational couplings from amino acid side-chains. As a result, we are able to quantify post-translational modifications such as those occurring under oxidative stress. Here, tyrosine (Tyr) nitration is used as a model due to its importance in inflammatory diseases. In recent work [2], EVV 2DIR spectroscopy has been reported to detect 3-nitrotyrosine (nTyr) levels down to 5% in peptide mixtures (Figures 1 and 2), with an estimated detection limit of 1% nitration. Additionally, we have shown that spectral images can be constructed from FFPE tissue sections using EVV 2DIR spectroscopy [1]. We aim to further discuss these results and to demonstrate how the spectral information obtained through this technique could be used to map the localisation of biomarkers across healthy and diseased human tissue sections, allowing for differential analysis.

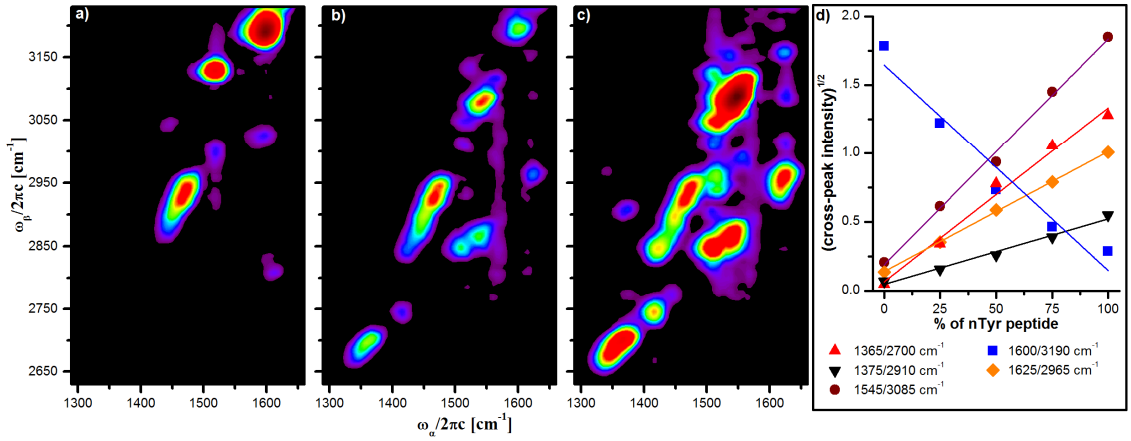

Figure 1: Experimental EVV 2DIR spectra for mixtures of the Tyr and nTyr heptamer peptides at pH 9.1 containing (a) 0, (b) 50 and (c) 100% of the deprotonated nTyr peptide. (d) Calibration curves obtained by plotting the square root of the normalized cross-peak intensity against the known percentage of nTyr peptide.

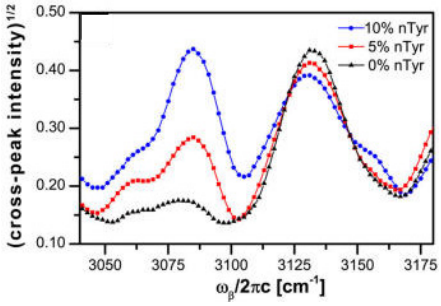

Figure 2: Line profiles measured across EVV 2DIR spectral features using a fixed $\omega_\alpha/2\pi c$ frequency value of 1545 cm^{-1} for 10, 5, and 0% nTyr samples. The normalised intensity of the 1545/3085 cm^{-1} cross-peak values for 5% and 10% mixtures were used to calculate the percentage of nTyr through the calibration curve shown in (Figure 1.d). The results were 6 and 11%, respectively, giving a reasonably good agreement with the values of 5.4 and 12.1%, which were independently measured via LC-MS.

[1] F. Fournier et al., Acc. Chem. Res. **42**(9), 1322–31 (2009).
[2] L. Rezende Valim et al., J. Phys. Chem. B **118**, 12855−12864 (2014).