

Supporting information

Surface Enhanced Resonance Raman Detection of a Catalytic Intermediate of DyP-type Peroxidase

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Materials and Methods

The cloning and overexpression of ppDyP gene in *Escherichia coli* and protein purification followed previously optimised procedures.⁸

Silver supports for SERR experiments were electrochemically roughened and subsequently immersed into ethanolic solution of aminooctanethiol (AOT) and mercaptohexanol (MOH) in 1:3 (M/M) ratio for 16–24 h to form a self-assembled monolayer (SAM).^{9, 10} Prior to SERR experiments, the SAM coated Ag supports were immersed for 10–15 min into 200 μL of 50 mM Tris-HCl + 200mM NaCl, pH 7.6 buffer containing ca. 0.4 μM PpDyP. The SERR experiments were performed using a homemade SERR cell. A rotating cuvette (from Hellma), containing 100 μM PpDyP, was used for RR experiments.

Spectra were measured at RT with 413 nm line of krypton ion laser (Coherent Innova 302), using a confocal spectrograph (Jobin Yvon LabRam), equipped with a liquid N₂ cooled CCD camera; 5 and 1.1 mW laser power and 60 and 20 s accumulation time, were employed for the measurements of RR and SERR spectra, respectively. After polynomial baseline subtraction the spectra were subjected to component analysis as described previously, using a homemade software.^{9, 10}

Figures

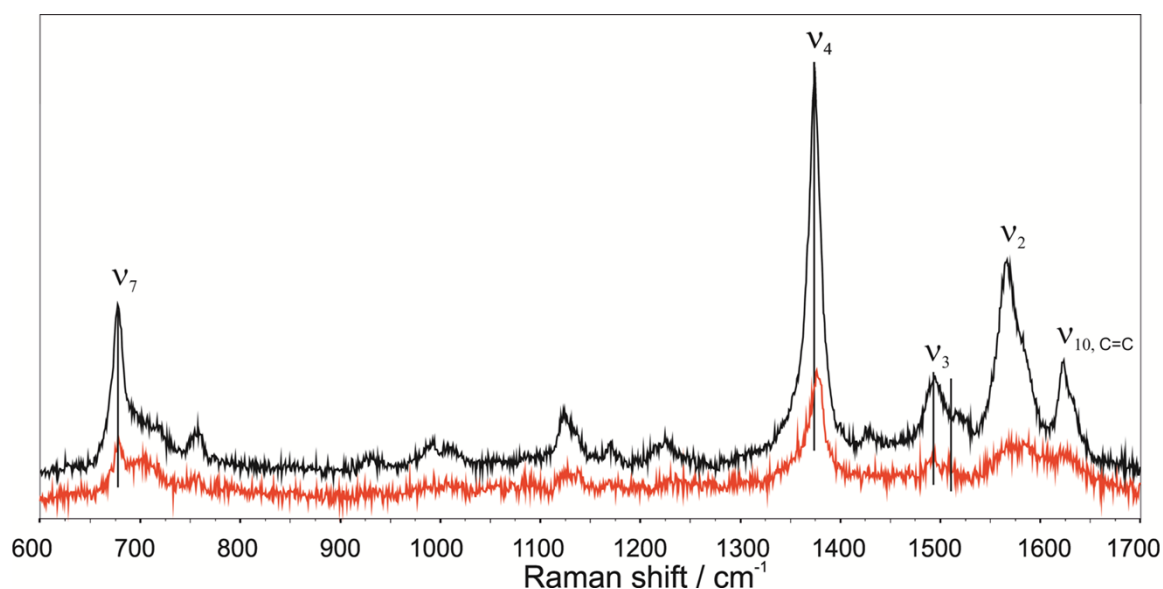


Figure SI 1. Experimental SERR spectra of ferric PpDyP immobilised on AOT/MOH SAM in the absence (black trace) and presence (red trace) of hydrogen peroxide. Spectra show unaltered frequency of ν_7 mode (which can be regarded as an internal standard) and upshifted ν_4 and ν_3 modes. Spectra were acquired from the same sample, under identical experimental conditions (laser power 1.1 mW, 20s accumulation time; co-addition of two spectra). It is noteworthy that there are no indications of Fe(IV)=O stretching mode in the low frequency region of the spectra; this mode has never been detected in HRP Cpd I, and appears at $\sim 780\text{cm}^{-1}$ in Cpd II.¹⁴