Electronic Supporting Information

Towards the understanding of the effect of oxygen on the electrocatalytic activity of microbial biofilms: a spectroelectro-chemical study

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Oxygen-driven oxidation of a ferrous cytochrome

The formal reduction potentials of molecular oxygen (O_2) and the outer membrane cytochromes (OMCs) are +0.16 and -0.36 V vs. SCE, respectively.[Millo et al., *Angew. Chemie - Int. Ed.*, **2011**, 50, 2625–2627] Therefore, the oxidation of a ferrous (Fe^{2+}) OMC by O_2 is a thermodynamically favoured process. To prove whether O_2 can effectively oxidize a ferrous cytochrome, we performed the following experiment. Cytochrome b5 (cyt b5), hereby chosen because it has the same His-Fe-His axial ligation as the OMCs studied in this work [Sezer et al., *Phys. Chem. Chem. Phys.* **2010**, 12, 7894-7903], was chemically reduced to the ferrous state and then exposed to O_2. Changes in oxidation state were monitored by resonance Raman spectroscopy.

A cyt b5 domain of human sulphite oxidase was used as test protein. Purification and preparation of the protein described elsewhere. [Sivanesanat al. *J. Phys. Chem. B* **2013**, 117, 11866−11872] The protein as received was in the ferric (Fe^{3+}) state, as reflected by the ν_4, ν_3, and ν_{10} bands at 1374, 1507, and 1640 cm^{-1} (Figure ESI 1, bottom spectrum). Adding sodium dithionite (Sigma Aldrich, > 85 %, molar ratio 10 : 1) to the solution reduced the protein as shown by the downshift of the ν_4 and ν_3 bands to 1361 and 1494 cm^{-1}, respectively, and the absence of the ν_{10} band, which is a distinctive trademark of His-Fe^{2+}-His cytochromes (Figure ESI 1, middle spectrum). Bubbling the solution containing the cyt b5 with O_2 (Linde Gas, grade 5) led to the immediate (i.e. within 10 s) oxidation of the protein, as can be seen in the top spectrum of Figure ESI 1, which is essentially identical to the bottom one. This experiment proves that O_2 can oxidize a ferrous cytochrome having the His-Fe^{2+}-His axial ligation, thus reinforcing our conclusions on the direct O_2-driven oxidation of the OMCs observed in our work.

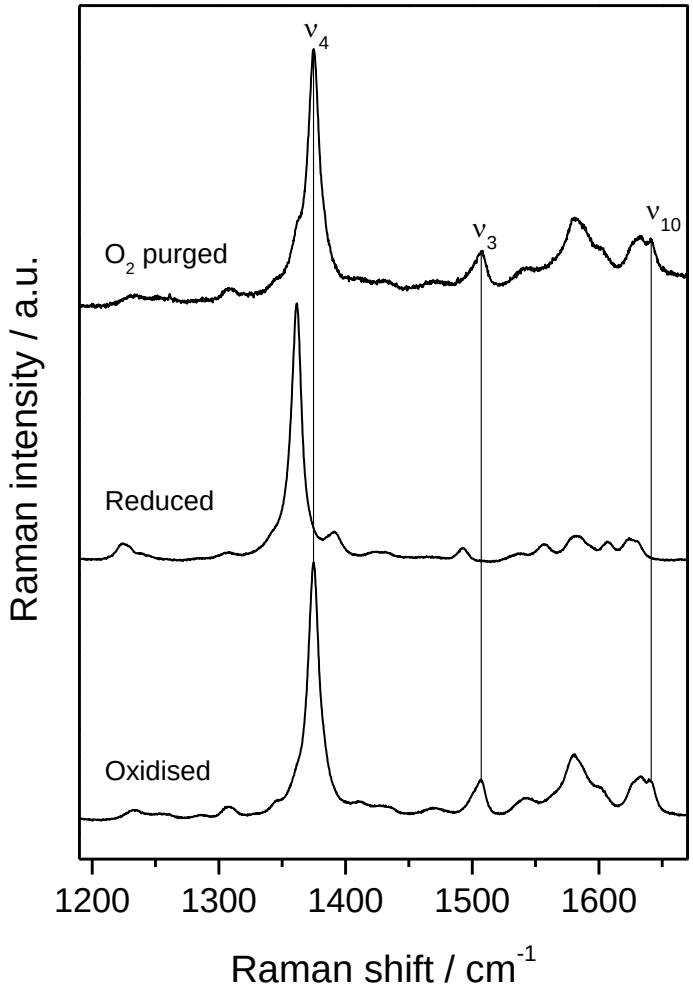

Figure ESI 1: Resonance Raman spectra of 50 μM cyt b5 in 10 mM potassium phosphate buffer solution, pH 7.0. Spectra were obtained with a 413 nm laser excitation using the apparatus described elsewhere.[Wackerbarth et al., *Appl. Spectrosc.* **1999**, 53 (3), 283−291; Ly et al., *J. Electroanal. Chem.* **2010**, 660(2), 367-376] Acquisition time was 60 s and laser power on the sample was 2 mW. To help the eyes, spectra were normalized with respect to the intensity of the ν_4 band.