Time-Resolved Resonance Raman and Time-Resolved Step-Scan FTIR Studies of Nitric Oxide Reductase from *Paracoccus denitrificans*: Comparison of the Heme b_3-Fe_B Site to that of the Heme-Cu_B in Oxidases

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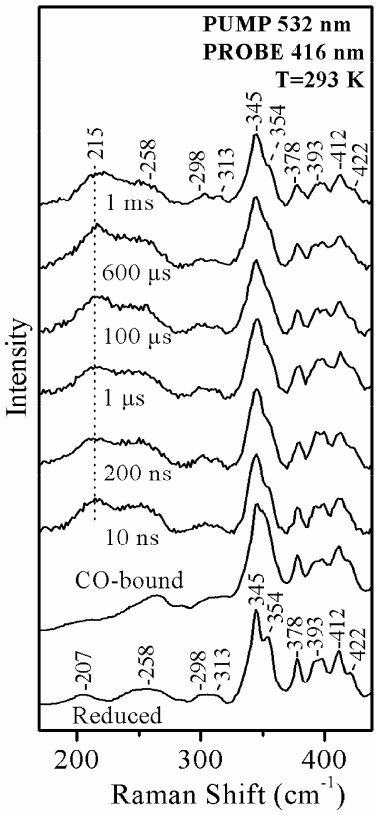
Time-resolved resonance Raman (TR^3) and time-resolved step-scan (TRS^2) FTIR spectroscopies have been used to probe the structural dynamics at the heme b_3 proximal and distal sites subsequent to carbon monoxide photolysis from fully reduced CO-bound nitric oxide reductase (NOR) and heme-copper oxidases (CcO). The TR^3 spectra of NOR exhibit structural differences relative to the equilibrium geometry of heme b_3. The most significant of these is a shift of 8 cm^{-1} to higher frequency of the 207 cm^{-1} mode that originates from ν(Fe-His), and a shift of 7 cm^{-1} to lower frequency of the ν_4 mode. The low-frequency TR^3 spectra of fully reduced NOR at the indicated times subsequent to CO photolysis are shown in the figure. We suggest that relaxation along the tilt angle of the proximal histidine with respect to the heme plane and the out-of-plane displacement of the Fe (q) are coupled, and ligand binding and dissociation are accompanied by significant changes in the angular orientation of the His ligand. The results are compared to those obtained for heme-copper oxidases. The TR^3 and TRS^2 FTIR data demonstrate significant alterations in the nature of the heme-protein dynamics between NOR and CcO resulting from specific structural differences in their respective hemepockets.



References:

1. Pinakoulaki, E. and Varotsis C. *Biochemistry* 2003, *42*, 14856-14861.