

## **Resonance Raman vibrations of the covalent heme-protein linkage in cytochromes $c_3$ are fingerprints for the protein structure**

Roberto E. Di Paolo, Patrícia M. Pereira, Inês Gomes, Filipa M.A. Valente, Inês A.C. Pereira,  
Ricardo Franco

*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal*

Resonance Raman (RR) spectroscopy was used to investigate conformational differences in the hemes of Cytochromes  $c_3$ , a family of low-redox potential multi-heme cytochromes found in the cytoplasm and/or membranes of sulfate reducing bacteria of the *Desulfovibrio* and *Desulfomicrobium* genera. The most studied member of the family is the tetraheme cytochrome  $c_3$  which has a globular structure where all four hemes are almost perpendicular to each other. The other members of this family display this characteristic heme arrangement and an overall similar structure, although some of them are larger proteins with a higher number of hemes, like the nine-heme or the sixteen-heme cytochromes. Some species contain also ortologs of the tetraheme cytochrome  $c_3$  which have distinct physiological functions but are not easily distinguished by biochemical methods.

Comparative analysis of the low frequency region RR spectra revealed important shifts for the two lines assigned to vibrations of the covalent heme-protein linkage [ $\nu(\text{C}_a\text{S})$ ]. The value of the frequency separation between those two lines allowed clustering the analyzed proteins into distinct groups, which correlate with differences in the protein structure. These observations indicate that the RR line pattern for [ $\nu(\text{C}_a\text{S})$ ] vibrations of the covalent heme-protein linkage for the hemes of cytochromes of the  $c_3$  family is very sensitive to the overall protein structure, and thus RR is a useful tool for a positive structural identification of cytochromes within this family.