# $^{1}H$, $^{15}N$ and $^{13}C$ Assignment and NMR Solution Structure of Rubredoxin

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Rubredoxin is a small (6 kDa) non-heme iron sulphur protein that has been isolated from sulphate-reducing bacteria and archaea. It has been proposed to be an electron donor of various enzymes, such as superoxide reductase, and rubredoxin oxygen-oxidoreductase. The gene coding for *D. gigas* rubredoxin was heterologously expressed in *E. coli*, using minimal medium which enabled the uniform labelling of the protein with $^{13}C$ and $^{15}N$, and also the incorporation of Zn(II) in the metal center.

This protein sample was selected as a standard sample for testing purposes, in our Departmental NMR facility. It was used as a case study to set up protocols for acquisition, processing, and structure calculation of proteins, using triple resonance NMR experiments. The following series of spectra were acquired and analysed in a 400 MHz and 600 MHz Bruker AvanceIII spectrometers: $^{1}H$-$^{15}N$ HSQC, $^{1}H$-$^{13}C$ HSQC, HNCO/HN(CA)CO, HNCA/HN(CO)CA, HNCACB/HN(CO)CACB, (H)CCH-TOCSY, $^{1}H$-$^{15}N$ NOESY-HSQC, $^{1}H$-$^{13}C$ NOESY-HSQC, $^{1}H$-$^{1}H$ NOESY. In addition, $^{13}C$-direct detection experiments were also performed (CON, CACO, CBCACO, C-COSY).

With the above methods, a complete $^{1}H$, $^{15}N$ and $^{13}C$ assignment was attained. The availability of the $^{13}C$ sample and the use of the aforementioned experiments yielded a more complete assignment than the previously published work that used an only $^{15}N$-labelled sample[1]. The current solution structure, with the new assignments (S22) is still highly similar to the previously reported one.

[1] Lamosa, P. *et al.*, *Extremophiles*, **2001**, *5*, 303-311.

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