Application of Automated Projection Spectroscopy (APSY) at low fields

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Rubredoxin is a small non-heme iron-sulphur protein that has been isolated from the cytoplasm of different bacteria. In sulphate reducing bacteria, its physiologic role has been proposed to be the electron donor to different enzymes involved in detoxification of reactive oxygen species, such as superoxide reductase, rubredoxin oxygen-oxidoreductase.

The gene coding for *D. gigas* rubredoxin was heterologously expressed in *E. coli*, using minimal medium which enabled the labelling of the protein with both ^{13}C and ^{15}N, and also incorporation of Zn(II) in the metal binding site. This protein sample has been used in our NMR facility to test and optimize pulse sequences and acquisition parameters of heteronuclear NMR experiments.

In previous studies[1], the ^{1}H and ^{15}N have been assigned. In this work the assignment has been completed and extended to ^{13}C, using the Automated Projection Spectroscopy (APSY)[2] approach. The spectra were acquired at a 400 MHz Bruker AvanceIII NMR spectrometer. Two examples of APSY, 4D HNCOCA and 6D HNCOCANH, are presented, analysed, and compared with the standard 3D heteronuclear NMR experiments used for sequential assignment (HNCA/HN(CO)CA and HNCO/HN(CA)CO).

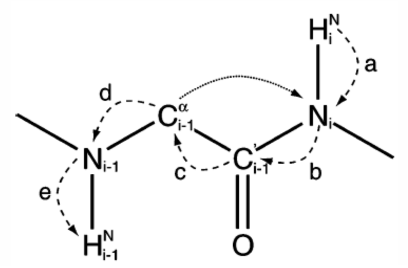

Figure 1: Magnetization transfer in the 6D APSY-seq-HNCOCANH

[1] Lamosa, P. *et al.*, *Extremophiles*, **2001**, *5*, 303-311.
[2] Hiller, S. *et al. PNAS*, **2005**, *102*, 10876-10881.

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