Pseudo 4D β-Carbon Edited NMR Experiments for Simultaneous Amino Acid Type Identification and Sequential Assignment of Proteins.

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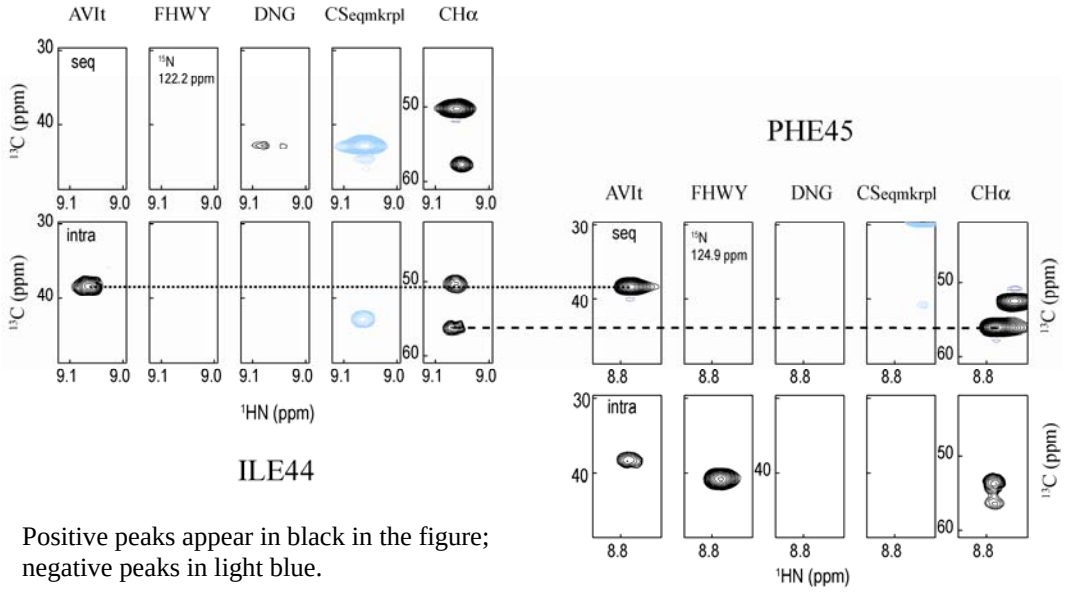
For the sequential backbone assignment of ^{13}C, ^{15}N labeled proteins a robust methodology is available. However the method shows several drawbacks like long spectrometer times or time consuming analysis of the spectra.

Here we propose a strategy based on the standard CBCA(CO)NH and CBCANH experiments, extended with a fourth pseudo-dimension consisting in the residue type. The editing methods used for the separations of the amino acid types can be shown in the panel of J. Santoro. This methodology introduces new constraints in the connection of neighbouring residues (AMino Acid TYpe COnstraint), making this analysis step more robust and easier to perform.



Positive peaks appear in black in the figure; negative peaks in light blue.

The experiments were tested with a sample of 1.7 mM ^{13}C,^{15}N-labeled Ubiquitin (76 residues) in a Bruker AV 600 spectrometer equipped with a cryoprobe. Each pseudo 4D experiment was collected in 13.5 hours using 2048 x 50 x 48 data points in ^{1}H, ^{13}C and ^{15}N dimensions, respectively.

The figure shows ^{1}HN-^{13}C planes at two ^{15}N frequencies for the different amino acid type 3D subspectra and illustrates the use of the new constraints. Intra- and inter-residual peaks used to connect two residues, besides having the same ^{13}C chemical shift, must appear in the same subspectrum type. So, a large spectral resolution in the ^{13}C dimension is no more necessary and the acquisition times can be drastically shortened. Furthermore, the assignment is in most cases immediate. In the example, we have an AVI-type residue followed by one of type FHWY and preceded by an EQMKRPL type. This allows to assign the stretch unambiguously to the fragment Leu43-Ile44-Phe45 of the Ubiquitin sequence.

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