Alternative Labeling with Pyruvate: Backbone Resonance Assignment of Large Proteins from a Single Experiment

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Backbone resonance assignment of proteins is a crucial first step when determining structures, probing intermolecular interactions or investigating dynamics. These resonances are determined from a standard set of triple resonance experiments that require long coherence transfer steps during which signals can be lost. One approach is to focus on the TROSY-HNCA as it has minimal transfer steps while providing sequential connections between amino acids and it does not experience the strong CSA relaxation at ^{13}C'. Deuterated ^{13}Ca relaxes slowly and hence narrow line widths can be achieved. Usually the HNCA is not sufficient to complete assignment as the ^{13}Ca shifts are poorly dispersed and the resolution is limited to ~35 Hz due to a $^{1}J_{CaCb}$ coupling, which splits the peak and reduces its intensity (Figure 1A). This creates a significant degeneracy when matching internal and sequential resonances in the HNCA and prohibits complete assignment.

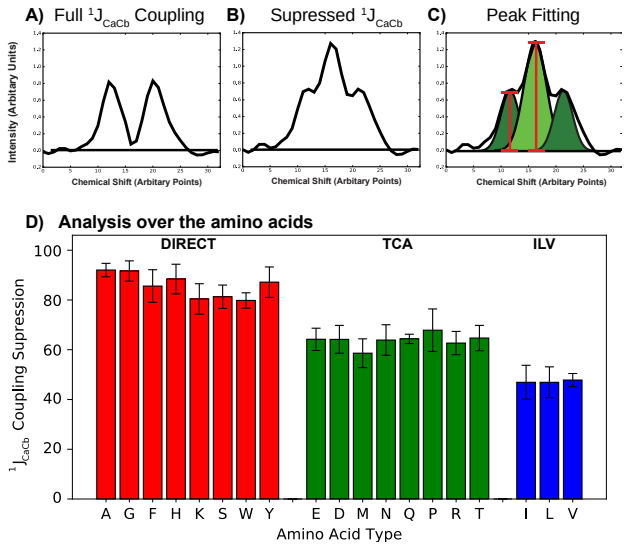

Figure 1: Suppression of $^{1}J_{CaCb}$ couplings by pyruvate labeling. A) High resolution peak in the ^{13}C dimension of an HNCA with full $^{1}J_{CaCb}$ coupling. B) Peak shape of ILE with suppressed coupling by pyruvate labeling. C) Three Peak model of peak. D) Grouping of amino acids into metabolic pathways and their average coupling suppressions.

In this work, we overcome this problem by chemically suppressing the $^{1}J_{CaCb}$ coupling through employing a new labeling scheme using a mix of ^{13}C labeled pyruvate isotopomers during protein expression. This labeling scheme results in a predominant central, uncoupled, narrow peak (Figure 1B) for all amino acids. The level of suppression is determined by fitting each peak to a 'three-peak' model which measures coupled and uncoupled peak heights (Figure 1C). These peak heights depend on the biosynthetic pathway the amino acid is derived from (Figure 1D). Amino acids directly synthesized from pyruvate are nearly singlets while the coupling in the amino acids derived from the TCA cycle is suppress by ~65%. In the ILV amino acids the coupling is suppress by about 50% (Figure 1B-C).

This method not only provides highly resolved uncoupled peaks but also unique residue specific peak shapes which become a useful property when overlap problems persist. An example is shown in Figure 2 where two sequential peaks (green and red) match an internal peak (black) in chemical shift. Visual inspection of the sequential peaks show the correct match (red) is on the right. The best match can be determined automatically with a Pearson correlation. **We assigned ~85% of the 42 kDa protein MBP using a combination of high resolution and peak shape matching with a single TROSY-HNCA experiment collected in ~3.5 days.** Additionally, sequence specific assignments can be done and errors corrected by making sure the level of coupling suppression in a proposed sequence accurately matches the amino acid sequence.

We are now using **new ^{13}Cb decoupling pulses, which do not generate Bloch-Siegert shifts**, but can selectively distinguish between some TCA and ILV amino acids. Our analysis of the transfer pathways in our pyruvate samples show that optimal $^{1}J_{NCa}$ transfer times are longer than with standard labeling. **Data on the efficiency of N->Ca and N->Ca-1 transfer for various transfer times predicts the secondary structure of a spin system.** This pyruvate labeling scheme is being used to assign the backbone of large enzymes (35–50 kDa) in the enterobactin synthesis pathway that have poor solubility. **We forsee that backbone assignment will be largely automated by an algorithm that examines a single TROSY-HNCA spectrum.**

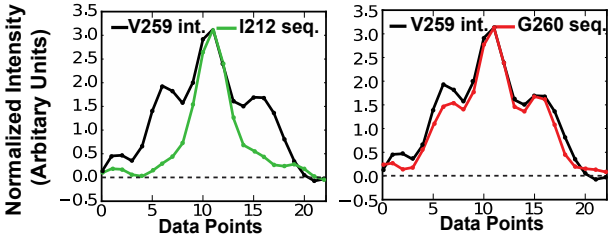

Figure 2: Solving the chemical shift redundancy problem. Both red and green (sequential peaks) from different spin systems are perfect matches for the peak in black (internal peak) by chemical shift. Their levels of $^{1}J_{CaCb}$ suppression (peak shape) makes the correct assignment clear.