Selective $^{15}N \leftrightarrow {}^{13}C$ Polarization Transfer for Triple-Resonance NMR Experiments of Uniformly Labeled Aligned Protein Samples

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Introduction

The study of aligned membrane protein samples by solid-state NMR provides a wealth of information that is difficult to obtain by other methods, such as X-ray crystallography and solution NMR spectroscopy. In particular, extensive information regarding the proteins' orientation and dynamics in the membrane environment can often be gathered from straightforward two-dimensional (2D) $^{1}H/^{15}N$ double-resonance NMR experiments.[1] However, for larger protein systems, signal overlap becomes a significant problem. To overcome this, various 2D and 3D $^{1}H/^{13}C/^{15}N$ triple-resonance experiments have been developed allowing for sequential assignment along the peptide chain and spreading signals over the additional ^{13}C dimension.[2] Uniformly ^{13}C labeling often makes these spectra ambiguous because the polarization transfer steps employed are unselective. Here, a selective $^{15}N \leftrightarrow {}^{13}C$ polarization transfer method is demonstrated using a model crystal sample which should be useful for obtaining unambiguous $^{13}C/^{15}N$ correlation spectra of aligned protein samples.

Experimental

Experiments were performed using a wide bore Bruker Avance 600 spectrometer equipped with a home-built low-E triple-resonance probe for aligned samples.

Results and Discussion

Figure 1a shows the 1D ^{15}N HSQC NMR signal of a single crystal [U-13C,15N]NAVL sample as a function of the mixing time τ (as shown in the HSQC pulse sequence of **Figure 1b**). The red and grey traces were obtained by using soft and hard ^{13}C π-pulses, respectively. The rf field for the red soft pulses is optimized following a commonly used trick where the pulse rotation for the aliphatic carbons is approximately 360°. Such a scheme decouples the homonuclear interaction from aliphatic carbons and transfer ^{15}N coherence selectively only to the directly bound CO. The selective transfer is evident from the sinusoidal $\sin^2(\pi D \cdot \tau)$ behavior of the red trace and provides a more efficient transfer than when using hard π-pulses. The coherence transfer oscillation also allows the measurement of ^{15}N -^{13}C dipolar coupling without the interference from interactions with other carbons and ^{13}C homonuclear interactions. The grey trace displays a complicated behavior because the coherence transfer involves multiple carbon spins.

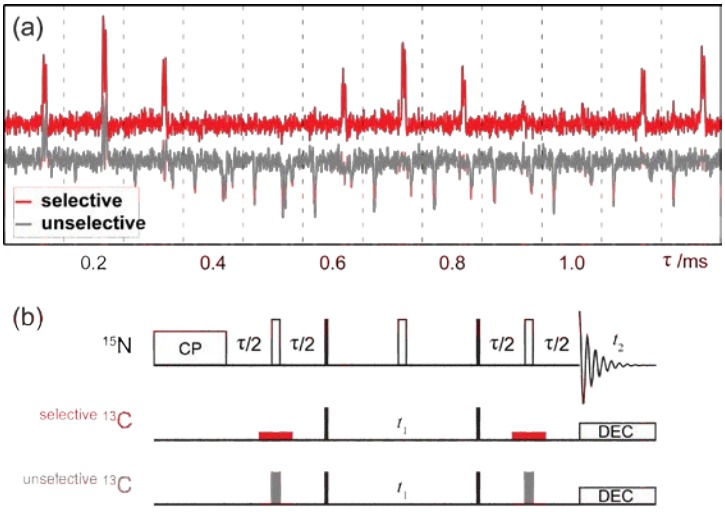

Figure 1 (a) Build up curves for $^{15}N \rightarrow {}^{13}C \rightarrow {}^{15}N$ HSQC magnetization transfer using selective (red trace) and unselective (gray trace) π-pulses during the mixing time τ. (b) HSQC pulse sequence (black bars ≡ $\pi/2$-pulses, open bars ≡ π-pulses, red bars ≡ soft π-pulses, grey bars ≡ hard π-pulses).

Conclusions

Unambiguous N/CO correlation can be obtained for aligned protein samples by using soft ^{13}C π-pulses that transfer coherence efficiently and selectively to CO carbons during HSQC mixing. The presented method should improve the resolution and greatly simplify acquisition of NC correlation spectra.

Acknowledgements

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References

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