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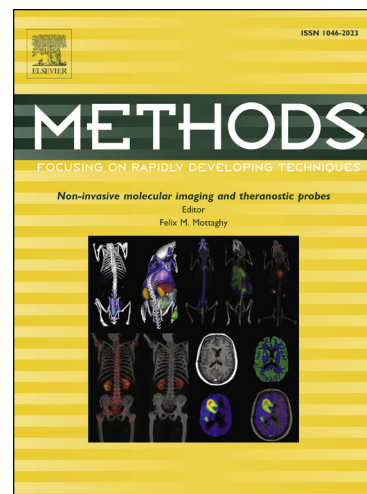
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Direct Detection of Carbon and Nitrogen Nuclei for High-Resolution Analysis of Intrinsically Disordered Proteins using NMR Spectroscopy

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Abstract

Nuclear magnetic resonance spectroscopy (NMR) is a powerful technique for characterizing the structural and dynamic properties of intrinsically disordered proteins and protein regions (IDPs & IDRs). However, the application of NMR to IDPs has been limited by poor chemical shift dispersion in two-dimensional (2D) ¹H-¹⁵N heteronuclear correlation spectra. Among the various detection schemes available for heteronuclear correlation spectroscopy, ¹³C direct-detection has become a mainstay for investigations of IDPs owing to the favorable chemical shift dispersion in 2D ¹³C-¹⁵N correlation spectra. Recent advances in cryoprobe technology have enhanced the sensitivity for direct detection of both ¹³C and ¹⁵N resonances at high magnetic field strengths, thus prompting the development of ¹⁵N direct-detect experiments to complement established ¹³C-detection experiments. However, the application of ¹⁵N-detection has not been widely explored for IDPs. Here we compare ¹H, ¹³C, and ¹⁵N detection schemes for a variety of 2D heteronuclear correlation spectra and evaluate their performance on the basis of resolution, chemical shift dispersion, and sensitivity. We performed experiments with a variety of disordered systems ranging in size and complexity; from a small IDR (99 amino acids), to a large low complexity IDR (185 amino acids), and finally a ~73 kDa folded homopentameric protein that also contains disordered regions (133 amino acids/monomer). We conclude that, while requiring high sample concentration and long acquisition times, ¹⁵N-detection often offers enhanced resolution over other detection schemes in studies of disordered protein regions with low complexity sequences.

Keywords: Intrinsically disordered proteins, IDP, NMR, ¹³C-Detection, ¹⁵N-Detection

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