

Reduced-dimensionality NMR spectroscopy for high-throughput protein resonance assignment

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A suite of reduced-dimensionality ¹³C,¹⁵N,¹H-triple-resonance NMR experiments is presented for rapid and complete protein resonance assignment. Even when using short measurement times, these experiments allow one to retain the high spectral resolution required for efficient automated analysis. “Sampling limited” and “sensitivity limited” data collection regimes are defined, respectively, depending on whether the sampling of the indirect dimensions or the sensitivity of a multidimensional NMR experiments *per se* determines the minimally required measurement time. We show that reduced-dimensionality NMR spectroscopy is a powerful approach to avoid the “sampling limited regime”—i.e., a standard set of ten experiments proposed here allows one to effectively adapt minimal measurement times to sensitivity requirements. This is of particular interest in view of the greatly increased sensitivity of NMR spectrometers equipped with cryogenic probes. As a step toward fully automated analysis, the program AUTOASSIGN has been extended to provide sequential backbone and ¹³C^β resonance assignments from these reduced-dimensionality NMR data.

Rapid resonance assignment is a prerequisite for high-throughput (HTP) structure determination and structural genomics (1). The aims of structural genomics are to (i) explore the naturally occurring “protein fold space” and (ii) contribute to the characterization of function through the assignment of atomic-resolution three-dimensional (3D) structures to proteins. The ultimate goal is to provide one or more representative 3D structures for every structural domain family in nature. It is now generally acknowledged that NMR will play an important role in this endeavor (1). The resulting demand for HTP structure determination requires fast and automated NMR data collection and analysis protocols. This impetus for the development of new methods will have broad impact in the technological infrastructure for structural biology and molecular biophysics.

Two key objectives for NMR data collection can be identified. Firstly, the measurement time should be minimized so as to lower the cost per structure and relax the constraint that NMR samples need to be stable over long time periods. Secondly, automated analysis requires recording of a redundant set of NMR spectra each affording good resolution, while it is also desirable to keep the total number of spectra small to reduce complications due to interspectral variations of chemical shifts (2). This second objective can be addressed by maximizing the dimensionality of the spectra. However, the joint realization of the first and second objective is impeded by the large lower bounds for measurement times of four (or higher) dimensional NMR spectra arising from the independent sampling of three (or more) indirect dimensions.

We distinguish “sampling limited” and “sensitivity limited” data collection regimes, depending on whether the sampling of the indirect dimensions or the sensitivity of the multidimensional NMR experiments *per se* determines the minimally achievable measurement time. Because structure determinations rely on nearly complete shift assignments routinely obtained using ¹³C,¹⁵N,¹H-triple-resonance (TR) NMR (3), the development of techniques that avoid the sampling limited regime represents an important challenge. Reduced-dimensionality (RD) TR NMR

experiments (4–7), designed for simultaneous frequency labeling of two spin types in a single indirect dimension, offer a viable strategy to circumvent sampling-limited recording of NMR spectra. RD NMR is based on a projection technique for reducing the spectral dimensionality: the chemical shifts of the projected dimension give rise to a cosine-modulation of the transfer amplitude, yielding peak doublets encoding *n* chemical shifts in an *n*-1 dimensional spectrum. Thus, for example, four-dimensional (4D) information can be obtained in a 3D experiment. This reduces the sampling requirements and the minimal measurement time by about an order of magnitude (7), which allows recording projected 4D experiments within a few hours while retaining maximal evolution times and thus a resolution routinely achieved in conventional 3D NMR spectra. Furthermore, axial coherences, arising from either incomplete polarization transfer or steady-state heteronuclear magnetization, can be observed as peaks located at the center of the doublets (6). This allows both the unambiguous assignment of multiple doublets with degenerate chemical shifts in the other dimensions and the identification of cross peak pairs by symmetrization of spectral strips about the position of the central peak. RD NMR experiments were the first designed to simultaneously recruit both ¹H and heteronuclear magnetization for signal detection (6), and RD two-spin coherence NMR spectroscopy (8) serves as a valuable radio-frequency (rf) pulse module for measurement of cross-correlated heteronuclear relaxation rates (9). Here we present a suite of nine RD TR NMR experiments (six of which are unique implementations) for complete protein resonance assignment. To integrate the RD NMR technology for rapid assignment, we have, as a step toward fully automated analysis, extended the program AUTOASSIGN (10, 11) for the use of RD NMR spectra.

Materials and Methods

NMR measurements were performed at 25°C on a Varian Inova 600 spectrometer by using a 1-mM solution of ¹³C/¹⁵N-labeled “Z-domain” of the 71-residue *Staphylococcal* protein A (12) in 90% H₂O/10% D₂O (20 mM K-PO₄; pH, 6.5). The protein’s overall rotational correlation time obtained from polypeptide backbone ¹⁵N *T*_{1ρ}/*T*₁ ratios (13) is 4.5 ns—i.e., within the range encountered for proteins from about 5 to 15 kDa.

Nine RD NMR experiments were used in conjunction with 3D HNNCACB (4–8, 14–17). Fig. 1 surveys their names and the correlated chemical shifts. In the nomenclature of the RD NMR experiments underlined letters indicate chemical shifts obtained in a common dimension. Fig. 2 displays peak patterns observed in the projected dimensions, and Fig. 6 (which is published as supporting information on the PNAS web site, www.pnas.org)

Abbreviations: HTP, high-throughput; RD, reduced-dimensionality; 2D, 3D, 4D, two-, three-, four-dimensional; COSY, correlation spectroscopy; TOCSY, total correlation spectroscopy; TR, triple-resonance; S/N, signal-to-noise.

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