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Published on Web 01/10/2003

GFT NMR, a New Approach To Rapidly Obtain Precise High-Dimensional NMR Spectral Information

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Abstract: Widely used higher-dimensional Fourier transform (FT) NMR spectroscopy suffers from two major drawbacks: (i) The minimal measurement time of an *N*-dimensional FT NMR experiment, which is constrained by the need to sample $N - 1$ indirect dimensions, may exceed by far the measurement time required to achieve sufficient signal-to-noise ratios. (ii) The low resolution in the indirect dimensions severely limits the precision of the indirect chemical shift measurements. To relax on constraints arising from these drawbacks, we present here an acquisition scheme which is based on the phase-sensitive joint sampling of the indirect dimensions spanning a subspace of a conventional NMR experiment. This allows one to very rapidly obtain high-dimensional NMR spectral information. Because the phase-sensitive joint sampling yields subspectra containing "chemical shift multiplets", alternative data processing is required for editing the components of the multiplets. The subspectra are linearly combined using a so-called "*G*-matrix" and subsequently Fourier-transformed. The chemical shifts are multiply encoded in the resonance lines constituting the shift multiplets. This corresponds to performing statistically independent multiple measurements, and the chemical shifts can thus be obtained with high precision. To indicate that a combined *G*-matrix and FT is employed, we named the new approach "GFT NMR spectroscopy". GFT NMR opens new avenues to establish high-throughput protein structure determination, to investigate systems with a higher degree of chemical shift degeneracy, and to study dynamic phenomena such as slow folding of biological macromolecules in greater detail.

Introduction

NMR[1] spectra need to exhibit (i) signal-to-noise (S/N) ratios warranting reliable data interpretation, (ii) digital resolutions ensuring adequate precision for the measurement of NMR parameters such as chemical shifts, and (iii) a dimensionality at which a sufficient number of NMR parameters is correlated.[2,3] Above a minimal "target dimensionality" N_t at which most of the peaks are resolved, increased dimensionality does not aim at resolving peak overlap but at increasing the number of correlations obtained in a single data set. This eliminates ambiguities when several multidimensional NMR spectra are combined for resonance assignment, for example, when using ^{1}H,^{13}C,^{15}N triple-resonance NMR to assign protein resonances.[3] An increase in dimensionality is, however, limited by the need to independently sample the indirect dimensions because this leads to longer measurement times. Although the measurement time can be somewhat reduced by aliasing signals[3] or accepting a lower digital resolution in the indirect dimensions, high dimensionality often prevents one from tuning the measurement time to a value that ensures one will obtain sufficient, but not unnecessarily large, S/N ratios.

In view of these considerations, "sampling" and "sensitivity limited" data collection regimes are defined[4] depending on whether the sampling of the indirect dimensions or the sensitivity of the FT NMR experiment determines the minimal measurement time. In the sensitivity limited regime, long measurement times are required to achieve sufficient S/N ratios, so that the sampling of indirect dimensions is not necessarily constraining the adjustment of the measurement time. In the sampling limited regime, some or even most of the instrument time is invested for sampling, which yields excessively large S/N ratios.[5] In view of the ever increasing sensitivity of NMR instrumentation, new methodology to avoid the sampling limited regime is needed.[4]

In general, phase-sensitive acquisition of an *N*D FT NMR experiment[2,3] requires sampling of $N - 1$ indirect dimensions with $n_1 \times n_2 \ldots n_{N-1}$ complex points representing $n_{\mathrm{FID}} = 2^{N-1} \cdot \prod_{j=1}^{N-1} n_j$ FIDs. The resulting steep increase of the minimal measurement time, T_{m}, with dimensionality prevents one from recording five- or higher-dimensional FT NMR spectra: acquir-

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(1) Abbreviations used: NMR, nuclear magnetic resonance; 1D, 2D, 3D, 4D, 5D, one-, two-, three-, four-, five-dimensional; FID, free induction decay; FT, Fourier transformation; GFT, combined *G*-matrix and Fourier transformation; NOE, nuclear Overhauser effect; COSY, correlation spectroscopy; NOESY, nuclear Overhauser enhancement spectroscopy; rf, radio frequency; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate.

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