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Hyperdimensional NMR Spectroscopy

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We propose a new approach to high-dimensional NMR based on a limited set of low-dimensional measurements. An ideal *N*-dimensional (ND) NMR spectrum would provide all possible correlations between *N* nuclei. By exploiting the projection−reconstruction (PR) technique,[1−5] we show how to obtain all the $N(N-1)/2$ pairwise correlation spectra for the case that $N = 10$. This would be quite impractical if performed by the conventional method, owing to excessive signal loss in the successive coherence transfer stages and the need to explore all evolution dimensions independently. A true ten-dimensional experiment could take as long as a million years to complete. Despite recent improvements in speed achieved by spatially encoded single-scan NMR,[6,7] Hadamard spectroscopy,[8,9] GFT-NMR,[10,11] and *PR*-NMR,[1−5] it is generally accepted that the extension of multidimensional NMR spectroscopy beyond $N = 4$ is essentially impractical.[12]

We show here that limited radial sampling of evolution space, followed by projection−reconstruction,[1−3] achieves the same result and reduces the measurement time by many orders of magnitude. The first point to appreciate is that two separate coherence transfer experiments A → B and A → C can provide the B−C correlation spectrum *indirectly*.[4] This method is related to the "covariance spectroscopy" of Zhang and Brüschweiler[13] who derived $^{13}C-^{13}C$ correlations from 2D $^{1}H-^{1}H$ TOCSY and $^{13}C-^{1}H$ HSQC experiments. Note, however, that the covariance algorithm operates only on homonuclear spin systems and breaks down when there are overlapping chemical shifts.

The main reason for invoking high-dimensional experiments is ambiguity in the assignment of the correlation peaks, caused by overlap of chemical shifts. Consider a hypothetical 3D spectrum, S(F1,F2,F3). Two separate 2D experiments represented by the orthogonal S(F1,F3) and S(F2,F3) planes do not provide sufficient information to define the full S(F1,F2,F3) matrix if there are degenerate chemical shifts in one of the dimensions. This ambiguity is resolved by forcing the reconstruction of S(F1,F2,F3) to be compatible with a new spectrum recorded in a plane tilted about the F3 axis through an angle $0° < \alpha < 90°$. This tilted spectrum is obtained by Fourier transformation of data acquired by linking t_1 and t_2 together in a suitable ratio.[1−4] This *valid* reconstruction of S(F1,F2,F3) is then projected onto the S(F1,F2) plane to give the required correlation spectrum. (See the Supporting Information for a detailed illustration.) This is the essence of the method—even when chemical shifts overlap, the ambiguity can be resolved by the "TILT" technique.[14,15] Analogous conclusions apply to higher-dimensional experiments.

Hyperdimensional NMR can be defined as a technique that derives all possible direct and indirect correlation spectra from a limited set of low-dimensional measurements, as opposed to the conventional method where all correlations are measured directly. As an illustration, 800 MHz spectra are shown for a 0.3 mM aqueous solution of the 4 kDa protein agitoxin,[16] isotopically enriched in ^{13}C and ^{15}N. Owing to the relative simplicity of the spectra, only a few tilted projections were needed for these reconstructions. For spectra with more overlap, it would be useful to link three evolution times together, generating doubly tilted projections.[4]

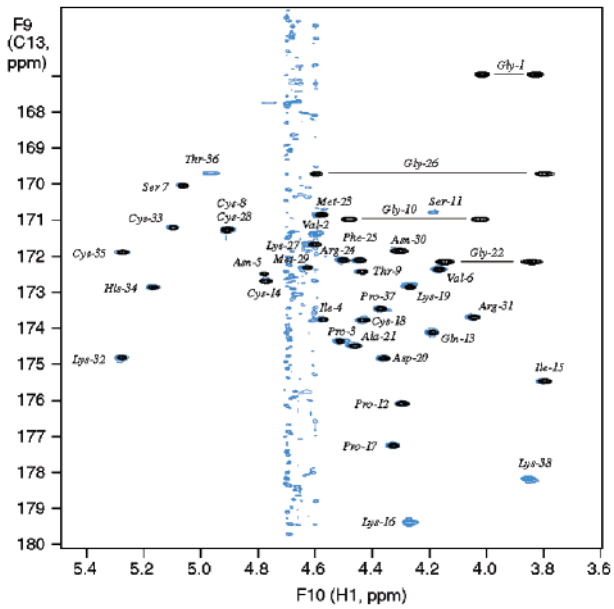
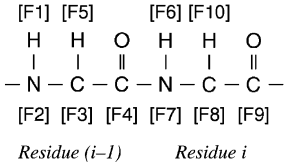


Figure 1. The F9F10 projection of the 10D spectrum of agitoxin comparing correlations between CO(*i*) and HA(*i*) obtained by indirect reconstruction (black contours) with the F1F3 projection of the conventional 3D HCACO spectrum (blue contours).

Consider two adjacent amino acid residues:



If all possible correlations were to be recorded in a single measurement, this would be *ten-dimensional* NMR. The proposed technique derives direct and indirect correlation spectra from a limited set of simpler experiments (Table 1).

To test the method, we compare the indirect reconstruction of the F9F10 projection of the hypothetical 10D agitoxin spectrum with the conventional 3D HCACO spectrum projected onto the F1F3 plane (Figure 1). Both spectra show correlations between CO(*i*) and HA(*i*). On the whole, there is a good fit between the two spectra, and the few differences are easily explained. The reconstructed spectrum lacks responses from the residues adjacent to prolines (which have no NH protons, thus precluding indirect measurements). The conventional spectrum fails to detect Gly residues since this region overlaps the CB responses and was not excited; this spectrum is also marred by a band of artifacts from

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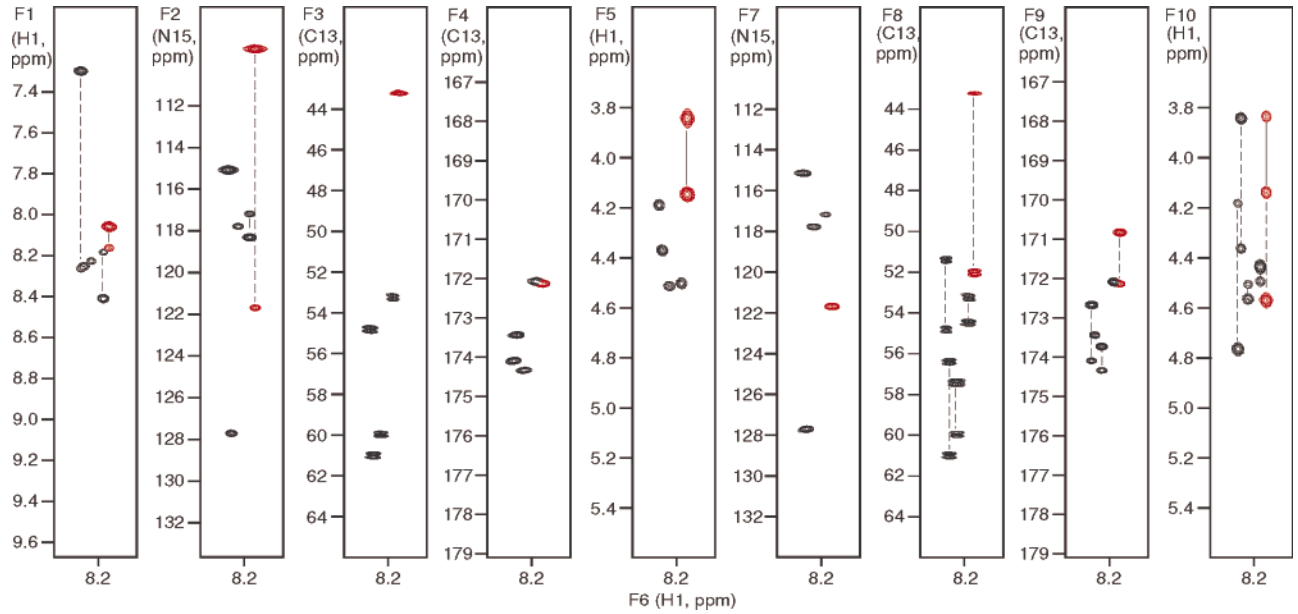

Figure 2. Strip plots illustrating indirect reconstructions of correlations between HN(*i*) [F6] and the remaining nine sites in two adjacent amino acid residues. The 0.18 ppm range centered at 8.2 ppm encompasses five different proton shifts, one of which is highlighted in red, representing intra-residue correlations to Met-23 or inter-residue correlations to Gly-22.

Table 1. Pulse Sequences Used to Observe the Direct and Indirect Correlations to the ^{1}HN spin [F6] Shown in Figure 2 as Strip Plots of a Hypothetical Ten-Dimensional Spectrum of Agitoxin

Direct Correlations

plane	correlation	pulse sequence
F6F7	HN(*i*)−N(*i*)	2D ^{15}N HSQC
F6F4	HN(*i*)−CO(*i*−1)	3D *PR*-HNCO
F6F8	HN(*i*)−CA(*i*)	3D *PR*-HNCA
F6F3	HN(*i*)−CA(*i*−1)	3D *PR*-HN(CO)CA

Indirect Correlations

plane	correlation	pulse sequence combination
F6F10	HN(*i*)−HA(*i*)	3D *PR*-HNCA and 2D ^{13}C HSQC
F6F5	HN(*i*)−HA(*i*−1)	3D *PR*-HN(CO)CA and 2D ^{13}C HSQC
F6F2	HN(*i*)−N(*i*−1)	3D *PR*-HN(CO)CA and 3D *PR*-HNCA
F6F1	HN(*i*)−HN(*i*−1)	3D *PR*-HN(CO)CA and 3D *PR*-HNCA
F6F9	HN(*i*)−CO(*i*)	4D *PR*-HNCOCA[a] and 3D *PR*-HNCA

[a] Starting with magnetization on HN(*i*+1).

imperfectly suppressed water signals, absent in the indirect reconstruction.

While inflation of a full 10D data matrix would be impractical, any desired 2D plane or projection can be extracted from the experimental data set. Of the 45 possible orthogonal projections, we show the correlations between HN(*i*) [F6] and all nine remaining sites in the form of strip plots encompassing five different proton shifts (Figure 2). These spectra correlate HN(*i*) with HN(*i*−1), N(*i*−1), CA(*i*−1), CO(*i*−1), HA(*i*−1), N(*i*), CA(*i*), CO(*i*), and HA(*i*). Although demonstrated for the case of a small protein, these results suggest a new protocol for structural studies of molecules of any type.

This work may stimulate thinking about *N*-dimensional NMR in a rather different way. The conventional approach would explore a sequence of *N*−1 direct correlations, eventually building up a mesh that connects all the coupled chemical sites. In contrast, the proposed indirect correlation measurements tie together the same mesh in an alternative manner. Instead of having to *deduce* certain indirect correlations, the spectroscopist can *reconstruct* the corresponding correlation spectra in the form of projections of a hypothetical *N*-dimensional data matrix, allowing him to evaluate the reliability of the experimental data. Projection−reconstruction experiments are also completed more rapidly and can provide significantly improved resolution (see the example in the Supporting Information).

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Supporting Information Available: Experimental details; comparison of reconstructed and conventional 3D HN(CA)CO spectra; resolving ambiguities due to overlap, and step-by-step reconstruction of indirect correlation spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Spectra were recorded on a Varian 800 MHz spectrometer at 30 °C using standard pulse sequences from the "BioPack" library; further experimental details are set out in the Supporting Information.

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