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Fast 2D NMR Ligand Screening Using Hadamard Spectroscopy

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Chemical shift perturbation NMR experiments are widely used for structure-based drug discovery.[1] The main advantage of NMR methods based on protein observation is the possibility to map the ligand interaction site, due to the single residue selectivity offered by 2D HSQC or HMBC experiments. Knowledge of the individual protein residues affected by ligand binding can be directly related to binding models derived by computer docking and used to rationally design new ligands in a structure-based design cycle.[2] The advantages of structure-based ligand development are nicely illustrated in the SAR by NMR approach of Fesik and co-workers.[3] Accurate measurement of chemical shifts using 2D spectra during ligand titrations can be used to extract quantitative binding data. Line width changes or the presence of duplicate signals provide information on the exchange regime.[4]

There is an increasing interest in new NMR methods that can reduce the time needed to record 2D correlation spectra. A recent example is the SOFAST approach, based on the optimization of longitudinal relaxation that allows a substantially higher repetition rate and, therefore, a shorter total accumulation time.[5] A different approach is based on spatial encoding of the indirect detection using selective pulses and gradients, allowing a single scan acquisition of 2D spectra.[6] Both methods are generally applicable to the acquisition of spectra without any previous knowledge of the expected signal positions. However, in ligand screening or titration experiments, the initial frequencies of the signals whose perturbation is sought are known, and in general, the expected perturbations are small. This a priori knowledge can be used to speed up spectral acquisition by measuring only the frequencies of interest using

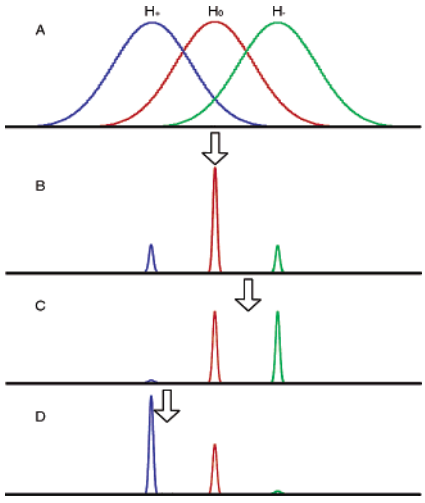

Figure 1. Intensity encoding of ligand-induced shifts. In three different Hadamard encoded experiments, the centers of the Gaussian excitation profiles for a particular site are displaced to higher and lower frequencies (A). While the positions of the cross-peaks are determined by the center of the excitation band, the observed intensities in Hadamard spectra reflect the actual frequency of the cross-peaks, indicated by the position of the arrows in B−D.

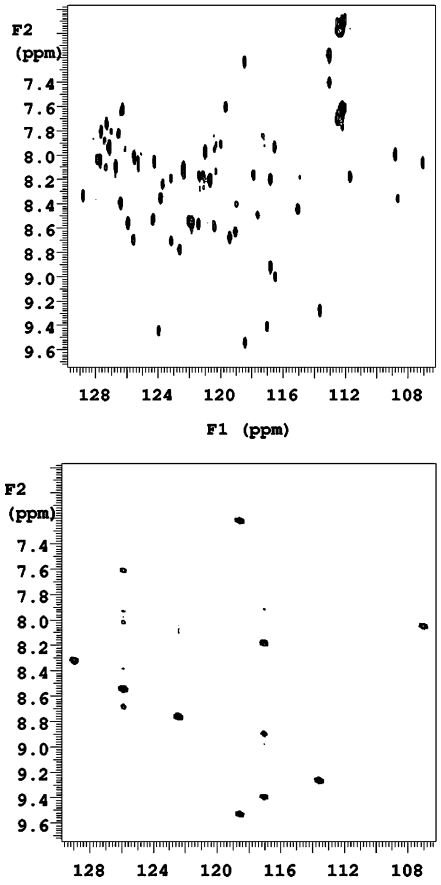

Figure 2. HSQC (top, ni = 128) and Hadamard HSQC (bottom, eight frequencies) of a WW domain of protein FBP28. Both spectra were acquired with four transients. Measurement times are 2234 and 87 s.

Hadamard encoding. In Hadamard spectroscopy, the evolution time in the indirect dimension is replaced by phase-modulated multisite selective excitation.[7] For recent reviews, see ref 8.

Recent applications of Hadamard encoded spectroscopy have made use of the increased time resolution resulting from very fast spectral acquisition, while maintaining the spectroscopic resolution associated with 2D spectra. Specific examples include measurement of proton−deuteron exchange kinetics[9] or frequency-resolved diffusion and relaxation measurements of chemically similar species in mixtures.[10] The use of Hadamard encoded filters to measure methyl−methyl cross-relaxation has also been demonstrated.[11]

Hadamard encoded HSQC spectra only contain cross-peaks with selected heteronuclear frequencies (e.g., ^{15}N). Ligand-induced ^{15}N shifts that exceed the excitation bandwidth result in the disappearance of the cross-peak. While this may be enough for screening applications, no information on the magnitude and direction of the shift is obtained.

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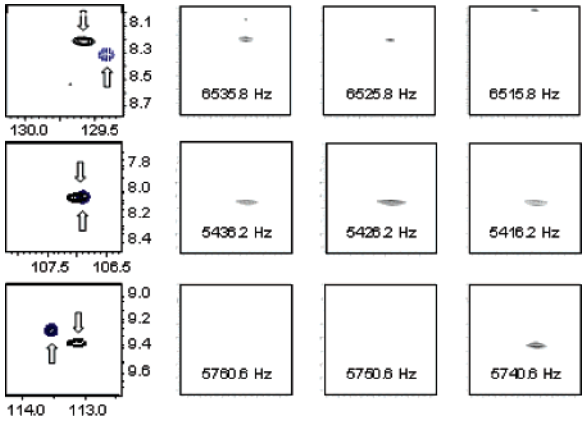

Figure 3. Ligand perturbation measured by standard HSQC and Hadamard HSQC. Each row corresponds to a different residue. The top and bottom rows are from ligand-shifted residues. The center row is from an unperturbed residue. The leftmost column is a superposition of HSQC cross-peaks of a ^{15}N-labeled WW domain in the absence (up pointing arrows) and in the presence (down pointing arrows) of its cognate peptide. The other three columns to the right are expansions of Hadamard spectra in the presence of ligand at the indicated excitation frequencies corresponding to offsets of +10, 0, and −10 Hz, respectively, from the frequency of the signal in the free protein.

Practical implementations of Hadamard spectroscopy make use of shaped pulses with a finite selectivity in order to limit the length of the excitation train and to avoid unacceptable relaxation losses. Heteronuclei with frequencies that are different from the selected ones, but fall within one of the excitation bands, give rise to a cross-peak at the nominal selected frequency but with a reduced intensity that depends on the shape of the excitation pulse (Figure 1). Typically, Gaussian shapes are used. Small frequency shifts can be estimated from intensity changes. However, since Gaussian shapes are symmetrical, the information about the sign of the frequency shift is lost. It can be easily recovered, however, by comparing the intensities of Hadamard encoded spectra with nominal excitation frequencies uniformly displaced by an amount comparable to the excitation bandwidth. We typically use a set of three spectra (a "Hadamard constellation") that, together, span a frequency range of 50−100 Hz. This is comparable to the ligand-induced shifts that are usually observed.

Figure 2 compares a HSQC and a Hadamard encoded HSQC of a WW domain of FBP28 in which seven ^{15}N frequencies were selected. Additional peaks with similar heteronucleus frequency are resolved in the proton dimension.

Figure 3 shows expansions of three Hadamard spectra of the WW domain in the presence of an unlabeled peptide ligand. The excitation frequencies are those of the unperturbed signal (center) or have an offset of plus (left) or minus (right) 10 Hz.

The leftmost column shows expansions of the same peaks in conventional HSQC spectra of the free protein and the complex.

The top (bottom) row corresponds to a protein peak that is shifted downfield (upfield) in the complex. The center row corresponds to an unperturbed signal.

A decrease of signal intensity in the Hadamard spectrum with the excitation frequencies of the free protein identifies those observed residues that are affected by ligand binding. Signals in Hadamard spectra with excitation frequency offsets provide information on the sign of the induced shifts. For small shifts, a simple quantitative estimation of the magnitude of the shift can be obtained from the relative intensities measured in two Hadamard spectra. Details are given in the Supporting Information.

Hadamard encoded heterocorrelation spectra provide obvious advantages for high-throughput 2D NMR screening. Considerable time savings are achieved both in acquisition, by measuring only the regions of interest, and in automatic data analysis, as the apparent ^{15}N frequencies are known a priori. A possible limitation comes from the use of selective pulses that would cause sensitivity losses for large proteins and the need to focus on well-resolved signals for proper quantification of ligand-induced shifts.

Hadamard encoding can be applied in combination with other fast NMR methods: the recently reported Hadamard encoded SOFAST−HMQC holds promise of reducing the acquisition time to a few seconds.[12]

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Supporting Information Available: A quantitative analysis of the conversion from intensity ratios to frequency shifts and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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