X-Filtered HOESY Experiment for Detecting Intermolecular Contact between Identical Sites

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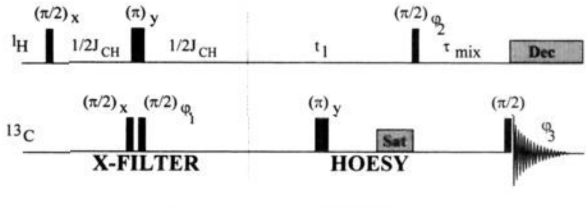
NMR[1] cross-relaxation experiments are widely used for detecting proximity in molecular systems.[2] While the proton−proton NOESY experiment is an indispensable tool for investigating protein structure in solution, its heteronuclear counterpart, the HOESY experiment,[3] is employed less frequently. Two reasons for this are the low sensitivity of the X-nucleus (typically ^{13}C) and a cross-relaxation rate diminished by the square of the respective gyromagnetic ratios. On the other hand, HOESY experiments offer advantages as well. As a first example, proton−proton spin diffusion influences heteronuclear cross-relaxation only through indirect pathways. Secondly, in systems that have slow molecular tumbling and involve long alkyl chains the spectral resolution in the ^{1}H spectrum invariably prevents the observation of separate lines while, due to larger chemical shift dispersion and lower relaxation rates, the resolution is sufficient in the heteronuclear spectrum.

Recently, these features of HOESY spectroscopy have been utilized in investigating a micellar solution exhibiting a partially unresolved ^{1}H spectrum.[4] Since micelles are aggregates of identical molecules, the observed cross peaks between various ^{1}H and ^{13}C nuclei may have both intra- and intermolecular contributions. A third advantageous feature of HOESY spectroscopy, i.e., the very low isotope labeling implied by natural abundance, can be used for separating these two contributions as demonstrated below. It can be appreciated that detection of intermolecular contacts is not so common and can afford important physicochemical information.

1D and 2D NOE methods based on isotope enrichment and editing by means of heteronuclear *J*-couplings have been in use for detecting intermolecular contacts primarily between proteins or between proteins and smaller bound molecules. The most frequent editing scheme is based on a so-called X-half-filter denoting the use of an HMQC (or HSQC) preparation prior to the proton evolution and/or detection periods.[5−11] These experiments require isotope enrichment not only for sensitivity reasons but also for minimizing the residue of the signal from uncoupled protons, relatively to protons bound to ^{13}C. These residues have no particular meaning but reflect random instabilities of the spectrometer, and as a major inconvenience, they can overlap with signals of interest. Since carbon-13 is observed, this problem simply disappears in the experiments presented here.



φ₁	φ₂	φ₃	φ₃
x	x	+	+
-x	x	-	+
x	-x	-	-
-x	-x	+	-
		Version I	Version II

Figure 1. Pulse scheme for the X-filtered HOESY experiment. Cycling φ_2 suppresses ^{13}C longitudinal relaxation during the mixing period.[4] This basic cycle is supplemented by cycling the phase of the π pulses and with Cyclops for the ^{13}C read pulse and receiver phases.

A similar design fitted for 1D and 2D HOE experiments has the obvious advantage of not requiring isotope enrichment. Moreover, as shown below, the experiment benefits from the low abundance of heteronuclei like ^{13}C by discriminating, in a straightforward way, between the inter- and intramolecular character of the detected *J*-filtered and *J*-selected contacts belonging to the same molecular unit (like methylene groups at a given position within an alkyl chain). The pulse scheme of the proposed experiments is shown in Figure 1. The HMQC preparation may, depending on the receiver phase cycle, select signals originating from protons directly bound or remote to, say, ^{13}C nuclei. First, proton chemical shift effects are refocused by the proton π pulse at time $1/J_{CH}$. Remote protons are not influenced by the rf pulses at ^{13}C frequency; therefore, alternating the sign of the acquisition (as in version I of the experiment) removes their spectral contribution, which, on the other hand, is retained if the sign of acquisition is unchanged (as in version II). For ^{13}C-coupled protons the effect of the heteronuclear *J*-coupling is refocused if the two ^{13}C $\pi/2$ pulses in the HMQC filter are of opposite phase while it is retained if those phases coincide. Thus, magnetizations of coupled protons in these two cases point toward the $+y$ and the $-y$ axes at time $1/J_{CH}$, which leads to cancellation if the two signals are added (as in version II). Since the ^{13}C signal is detected, the suppression of uncoupled protons (as would be required in a corresponding NOE experiment) is achieved without demanding extreme spectrometer stability.

The results of these 2D experiments as well as the results of a conventional 2D HOESY experiment[4] obtained on a lyotropic cubic liquid crystal are shown in Figure 2. Bicontinuous cubic phases[12] of aqueous surfactant solutions, like the one[13] investigated here, are built up of curved surfactant layers, and (since due to the symmetry of the crystal structure the static NMR couplings are averaged to zero) they provide liquid-like NMR spectra. The conventional HOESY experiment shows strong

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[1] Abbreviations and symbols used: NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; HOE, heteronuclear NOE; NOESY, two-dimensional NOE spectroscopy; HOESY, two-dimensional HOE spectroscopy; HMQC, heteronuclear multiple-quantum coherence.

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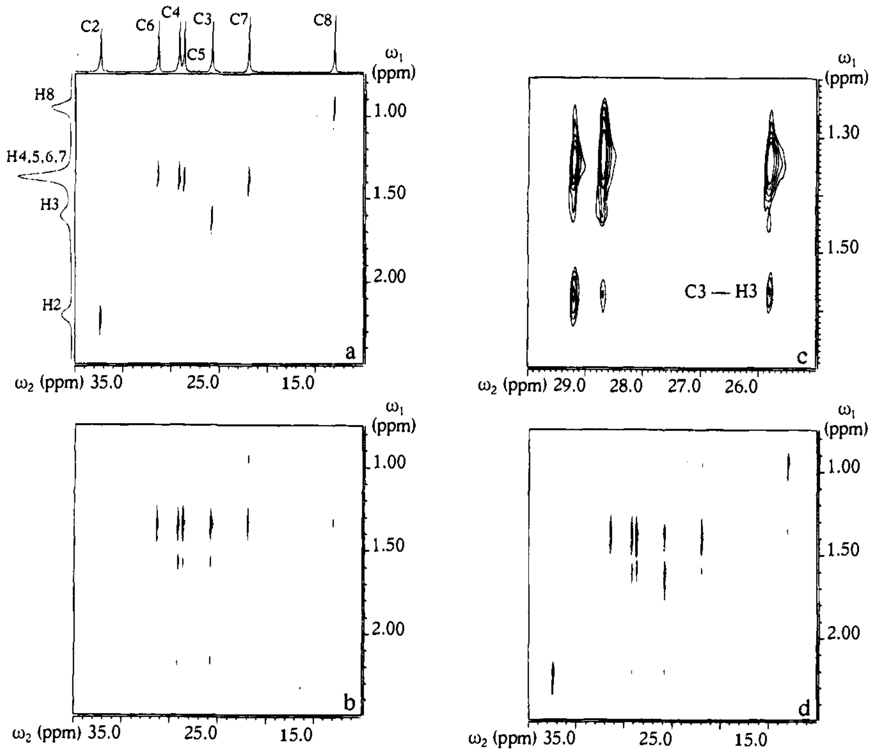

Figure 2. Pure absorption 2D spectra obtained by version I (a) and version II (b) of the X-filtered $^{13}C-^{1}H$ HOESY experiment. The expansion of a region of frame b is given in frame c while the result of a conventional HOESY experiment[4] is given in frame d. The sample is a lyotropic cubic liquid crystal, made up of a mixture of heavy water and potassium octanoate.[13] The atoms belonging to the six CH_2 groups and one terminal CH_3 group are numbered consecutively from the polar head as C2, C3, ..., C8 (C1 denoting the carboxylic carbon, not shown) and H2, H3, ..., H8. The experiments were performed at 75 MHz with 500 ms mixing time, and with 64 scans (128 for c and d) for each 256 t_1 values (incremented by 500 μs).

cross-relaxation between protons and carbons belonging to the same CH_2 units; owing to the short carbon–proton distance this finding is not unexpected. On the other hand, the 2D map shows cross peaks between protons and carbons belonging to different CH_2 units as well. More such peaks are observed in the present cubic phase than in the closely related micellar phase,[4] and this difference can be ascribed to the longer motional correlation time in the cubic phase as well as to changes in molecular conformations. All cross peaks, intra- and intermethylenes alike, may have intra- and intermolecular contributions. While these cannot be distinguished for the intermethylene peaks, the two versions of the experiment with the X-filter provide the required separation for the intramethylene peaks. The argument is as follows. The probability of a ^{13}C atom being close to protons bound to another ^{13}C atom is negligibly small because of the low natural abundance of ^{13}C. Therefore, if one selects protons which are *J*-coupled to a ^{13}C and lets them cross-relax with a ^{13}C spin, the detected cross-relaxation is overwhelmingly intramolecular (since the selection and the cross-relaxation involve the same ^{13}C spin) and, as an additional advantage, may yield the true NOE factor referred conventionally to the sole protons bound to the considered ^{13}C. If, on the other hand, one selects protons which are not *J*-coupled to a ^{13}C and they cross-relax with a ^{13}C spin belonging to a methylene unit at the same position, the cross-relaxation is dominantly intermolecular (since protons belonging to the same molecule as the ^{13}C are suppressed in this experiment).

The success of the respective experiments depends on the quality of suppressing the unwanted signal. It should be noted that the suppression of the remote protons in version I of the experiment depends neither on the magnitude of the *J*-coupling nor on the quality of the rf pulses. The suppression of the directly bound protons in version II is insensitive (due to the flatness of the cosine function around 180°) to small misset of τ and/or to small differences in J_{CH} (which was measured to be within 125 ± 3 Hz for all carbons). The goodness of the suppression is illustrated by Figure 2a–c. The intermethylene peaks are totally absent in the remote-filtered (version I) spectrum while two intramethylene peaks, the C2–H2 and the C8–H8, which are strong in the conventional spectrum, are absent in the direct-filtered (version II) spectrum. Due to ^{1}H spectral crowding the C4,5,6,7–H4,5,6,7 peaks cannot be analyzed in terms of intra- and intermolecular contributions. The C3–H3 peak in Figure 2b,c, however, is clearly of intermolecular origin. The large intensity of this in-phase peak (Figure 2c) and the absence of the C2–H2 and C8–H8 peaks exclude the origin of an incomplete suppression of cross-relaxation within the same CH_2 group. The sum of the direct- and remote-filtered spectra approximately provides the conventional HOESY spectrum in Figure 2d; the peak intensities in the composite spectrum are lower due to ^{1}H relaxation during the HMQC filter and to imperfect pulses.

This new observation opens the intriguing possibility of investigating intermolecular contacts among small- and medium-sized molecules in homomolecular systems. In particular, chain statistics for surfactant assemblies obtained by molecular dynamics simulation[14] could be tested against a new and highly distance-sensitive experimental parameter and hopefully explain (because of opposite mobility properties) the absence of contact at both chain extremities in the presently investigated system. In this context, we currently cannot discriminate between the two possible cross-relaxation pathways, i.e., the direct one between protons and ^{13}C and the relayed one via protons belonging to the same carbon position on different surfactant molecules; due to the r^{-6} dependence of the cross-relaxation rates, the second possibility is more likely. Work is in progress to clarify this point.

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