CHEMISTRY
A EUROPEAN JOURNAL

COMMUNICATION

DOI: 10.1002/chem.201200481

Polychromatic Decoupling of a Manifold of Homonuclear Scalar Interactions in Solution-State NMR

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Dedicated to Professor Hans Primas

Simultaneous decoupling of a manifold of homonuclear scalar interactions in high-resolution NMR spectra can be achieved by applying short phase-shifted radiofrequency (rf) pulses during the observation of the free induction decays. Simultaneous decoupling of multiple resonances allows the measurement of remaining *J*-couplings normally hidden by overlapping signals.

Decoupling is a well-known tool to simplify NMR spectra, gain resolution, and identify chemical shifts and *J*-couplings in solution. Although heteronuclear decoupling (e.g., irradiating ^{1}H while observing ^{13}C) is a routine operation, homonuclear decoupling (e.g., acquiring a ^{1}H decoupled ^{1}H spectrum) remains a challenge, because the Larmor frequencies of coupled spins can be very close. First attempts to achieve homonuclear decoupling are older than Fourier transform (FT) NMR spectroscopy and employ continuous-wave (CW) double resonance.[1] An alternative method is *J*-resolved two-dimensional (2D) spectroscopy, in which a skew projection generates a broadband homonuclear decoupled spectrum.[2] The disadvantage of most methods that achieve broadband decoupling in the indirect dimension of 2D spectra is low sensitivity and intensity distortions.[3] Homonuclear decoupling within a defined bandwidth can be achieved by using shaped inversion pulses.[4] Multiple regions can be irradiated simultaneously with several interleaved sequences in the manner of delays alternating with nutation for tailored excitation (DANTE).[5] It is possible to acquire a signal in real time while one or several homonuclear scalar interactions are removed selectively. Our method is related to an early technique known as homonuclear decoupling (HD) that is still in use, and in which the dwell time is shared between gated CW irradiation and the recording of the signal.[6] A modification uses a string of shaped inversion pulses.[4,7] In these methods, the irradiated signal disappears, and all couplings to the spin that is saturated by the rf irradiation are cancelled. The method proposed by Jesson et al.[6] uses an initial excitation pulse followed by bursts of CW irradiation, all with the same phase. In our experiment, as shown in Figure 1, only brief rf pulses with a flip angle $\beta \leq 1°$ are applied in each dwell time. By setting the phases to be initially perpendicular, analogous to the modification of Meiboom and Gill for spin–echo trains,[8] the on-resonance spin is in effect selectively spin locked and hence decoupled, and a narrow singlet is observed at the irradiation frequency. The decoupling effect can be rationalized in terms of average Hamiltonian theory (AHT).[9] By setting to zero the differences between the frequencies of the two parallel single-quantum (SQ) transitions associated with each spin, the following decoupling condition can be found:

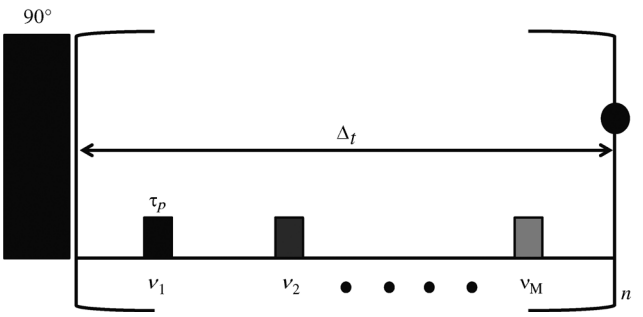

Figure 1. Scheme for polychromatic decoupling. The tall rectangle represents the initial $90°_y$ excitation pulse, whereas the small rectangles represent decoupling pulses of duration τ_p applied initially along the x axis at effective frequencies ν_m, which can be shifted at will by incrementing the rf phase from one pulse to the next within each comb $m = 1, 2, \ldots, M$. The black dot represents the acquisition of a single data-point averaged over the time interval during which the receiver is activated. The signal $s(t)$ is built up by acquiring n data points through an n-fold repetition of the loop.

$$\langle \omega_1 \rangle = \omega_1 \tau_p / \Delta t = \pi |J_{AX}| \quad (1)$$

By choosing the average rf field strength $\langle \omega_1 \rangle$ to match the coupling constant J_{AX}, the splitting vanishes. The obser-

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201200481.

vation is stroboscopic and occurs at multiples of the dwell time Δt. We propose to keep the duty cycle $\tau_p/\Delta t$ as small as possible, so that several pulses $m=1, 2, \ldots, M$ can be accommodated in each dwell time Δt with different effective frequencies ν_m. The decoupling effect can be described as a manifestation of stabilization by interconversion within a triad of coherences under multiple refocusing (SITCOM).[10] In a two-spin system without irradiation, the J-coupling normally converts an initial in-phase term I_x into an antiphase term $2I_yS_z$ during free evolution. But if decoupling pulses are inserted, a third operator, the longitudinal two-spin order $2I_zS_z$, comes into play to complete the triad. The partial conversion of $2I_yS_z$ into $2I_zS_z$ hinders the build-up of the former and thus stabilizes the initial I_x term.

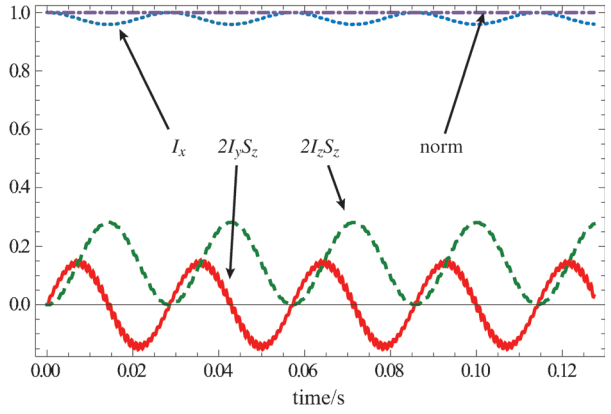

Figure 2. Simulation of three product operators belonging to the subspace $\{I_x, 2I_yS_z, 2I_zS_z\}$ (blue/dotted, red/continuous, and green/dashed lines, respectively) starting with the initial operator I_x+S_x in a two-spin system subjected to the experiment of Figure 1 with $\Omega_I=0$, $\Omega_S/(2\pi)=1$ kHz, $J_{IS}=10$ Hz, $\omega_1/(2\pi)=3.5$ kHz, $\tau_p=1$ μs, $\Delta t=100$ μs, that is, a duty cycle of 1%. The norm of the three operators $N=(\langle I_x\rangle^2+\langle 2I_yS_z\rangle^2+\langle 2I_zS_z\rangle^2)^{1/2}$ (purple/dashed–dotted line) is conserved. Upon Fourier transformation, the shallow amplitude modulation of the expectation value $\langle I_x\rangle$ gives rise to weak sidebands. The simulations were performed with mPackages.[14]

Figure 2 shows a simulation of the time dependence of these three product operators and of their norm. Because the norm is constant, coherence transfer must be confined to the subspace spanned by the triad of noncommuting operators. In the original SITCOM effect, a Carr–Purcell–Meiboom–Gill (CPMG)[8,11] sequence with moderate rf amplitudes generated $2I_yS_y$ as a third term of the triad, leading to the stabilization of I_x. Decoupling experiments with one pulse per dwell time at a single effective frequency ν_1 were applied to the $A_2M_2X_3$ system of propan-1-ol ($HOCH_2CH_2CH_3$). The conventional spectrum and three decoupled spectra with carrier frequencies set on one of the three multiplets are shown in Figure 3. The decoupling pulse length was $\tau_p=1$ μs for a dwell time $\Delta t=100$ μs. The peak-field strength was $\omega_1/(2\pi)\approx 1.6$ kHz in all cases, corresponding to a flip angle of $\beta=\omega_1\tau_p\approx 0.6°$. If the carrier frequency is set on the chemical shift $\Omega(A_2)$, the multiplicity of the coupling partner M_2 is simplified by decoupling $^3J_{AM}$, where-

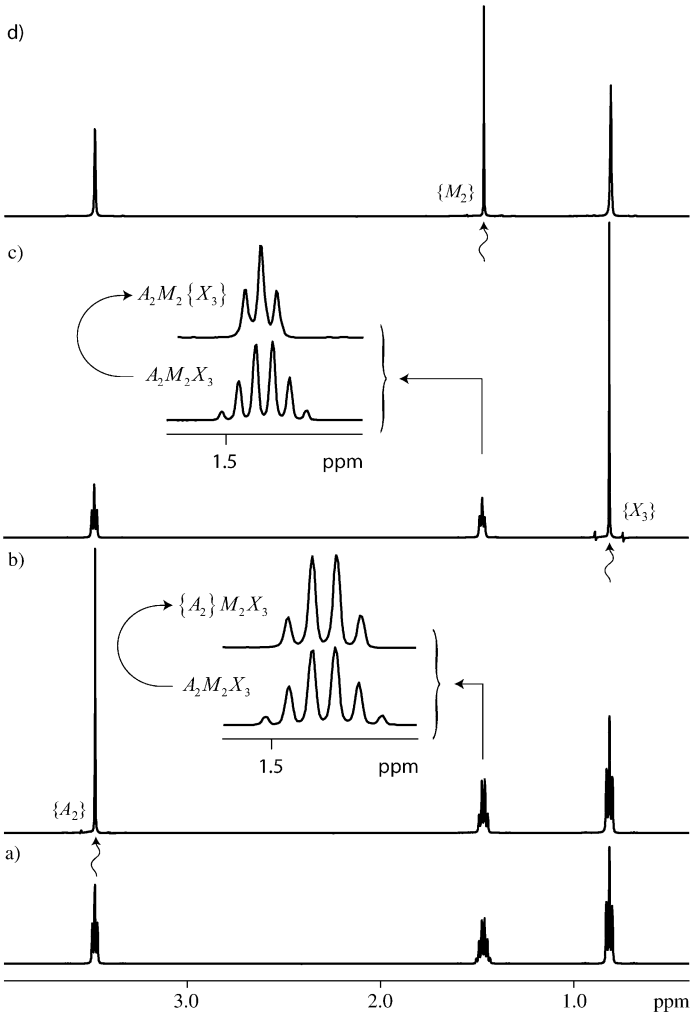

Figure 3. a) Conventional NMR spectrum of the $A_2M_2X_3$ system of propan-1-ol in D_2O at 500 MHz. b) Monochromatically decoupled spectrum obtained by setting the carrier (wavy arrow) on the A_2 triplet. The inset shows the simplification of the triplet of quadruplets of the coupling partner M_2, which collapses to a simple quadruplet. c) Partially decoupled spectrum with the carrier set on the X_3 triplet. The inset shows how the triplet of quadruplets of the coupling partner M_2 now appears as a simple triplet. d) Fully decoupled spectrum with the carrier set on the central multiplet of M_2, leaving three fully decoupled singlets. The decoupling pulse length was $\tau_p=1$ μs for a dwell time $\Delta t=100$ μs (duty cycle 1%). The peak rf field strength was $\omega_1/(2\pi)\approx 1.6$ kHz in all cases. This corresponds to an average rf field strength of $\langle\omega_1\rangle/(2\pi)\approx 16$ Hz. All spectra were processed with 1 Hz line broadening except for the expansions shown in the insets, for which no line broadening was used.

as the fine structure due to $^3J_{MX}$ is not affected, as shown in the inset of Figure 3. If the carrier frequency is set on $\Omega(X_3)$, the multiplicity of the coupling partner M_2 is simplified by decoupling $^3J_{XM}$, but the triplet due to $^3J_{AM}$ remains (inset Figure 3c.) Clearly, our sequence can decouple all interactions between the irradiated spin and its J-coupled partners, but does not affect couplings between spins that are not irradiated. In Figure 3d, the carrier frequency was set on the central M_2 resonance of the $A_2M_2X_3$ system. Because the M_2 spins are coupled to both A_2 and X_3 when

$^3J_{AX}=0$, all three resonances are decoupled in this case. Our decoupling scheme also works for strongly coupled systems, although sidebands become more prominent. This is shown by simulations and experimental data in the Supporting Information.

It is known that the effective frequency ν_m of a comb C_m of pulses can be shifted at will from the common carrier frequency ν_{rf} by a frequency shift $\Delta\nu_m=\nu_m-\nu_{rf}=\Delta\phi_m/(2\pi\Delta t)$ by shifting the phase of the k^{th} pulse of comb C_m through $k\Delta\phi_m$.[4–5,12] Because the position within the dwell time Δt of the pulses belonging to any one comb C_m is immaterial, one can readily interleave several combs with $m=1, 2, \ldots, M$. This allows in effect to irradiate simultaneously at a manifold of frequencies ν_m with $m=1, 2, \ldots, M$. Polychromatic decoupling can be a valuable tool to identify and measure J-couplings that are hidden by other signals. An example is given by the ^{1}H spectra of the cyclic peptide cyclosporine A in

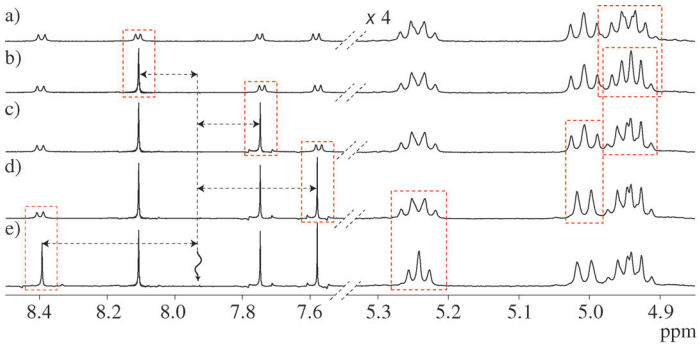

Figure 4. a) Part of a conventional ^{1}H spectrum of the cyclic peptide cyclosporine A in C_6D_6 at 500 MHz. On the left-hand side, the H^N region is expanded, whereas the H^α region is shown on the right-hand side, with a fourfold amplification of the vertical scale. b)–e) Spectra with polychromatic decoupling obtained with up to $M=4$ interleaved combs of decoupling pulses, so as to irradiate and decouple up to four H^N resonances simultaneously. A black wavy arrow indicates the common carrier frequency. The different effective irradiation frequencies are $\Delta\nu_{m=1}=-89.7$ Hz, $\Delta\nu_{m=2}=+89.7$ Hz, $\Delta\nu_{m=3}=+174.3$ Hz, and $\Delta\nu_{m=4}=-232.3$ Hz with respect to the carrier. Because the dwell time was $\Delta t=100$ µs, the phase increments of the four interleaved combs of decoupling pulses were chosen to be $\Delta\phi_{m=1}=-1.6°$, $\Delta\phi_{m=2}=+1.6°$, $\Delta\phi_{m=3}=+3.1°$, and $\Delta\phi_{m=4}=-4.2°$. The effect of decoupling $J(H^N,H^\alpha)$ is highlighted in the H^α region by dashed red rectangles. The remaining multiplet structures can be ascribed to $J(H^\alpha, H^\beta)$ couplings, which would be hard to determine in (a). The group of signals at about 4.95 ppm corresponds to two overlapping multiplets. The decoupling pulse lengths were $\tau_p=1$ µs, with a total duty cycle of 8% for the sum of the four combs. The decoupling field strengths were optimized empirically ($0.9\le\omega_1/(2\pi)\le2.3$ kHz).

Figure 4. From a) to e), up to four H^N multiplets are decoupled simultaneously by means of $M=0$, 1, 2, 3, or 4 interleaved combs of decoupling pulses, the phases of which are incremented to match the desired offsets (dashed arrows). On the right-hand side of Figure 4, the coupling partners H^α show multiplicities that are simplified, because up to four $J(H^\alpha,H^N)$ interactions are decoupled simultaneously. The stepwise simplifications are highlighted by dashed red rectangles. The residual multiplets that are caused by $J(H^\alpha,H^\beta)$ couplings can be easily determined, thus, revealing previously hidden information. The number M of effective frequencies is limited only by the total duty cycle. Our technique can be implemented in the detection or evolution periods of any multidimensional experiment to selectively decouple multiple interactions. Trains of pulses with moderate rf strength can be combined with spin–echo sequences to measure transverse relaxation rates $R_2=1/T_2$ in homonuclear coupled spin systems.[13]

In summary, we have presented a new polychromatic homonuclear decoupling sequence that is compatible with periodic signal acquisition as in normal Fourier transform spectroscopy. By inserting a manifold of polychromatic pulses in each dwell time Δt, several subsystems can be decoupled simultaneously. A considerable gain in resolution and spectral simplification can thus be obtained without distortion of signal integrals. We believe that this can help to characterize complex systems, including biological macromolecules. We would like to refer to our method as "window-acquired spin-tailoring experiment" (WASTE).

Experimental Section

All spectra were recorded with a Bruker Avance I spectrometer equipped with an 11.4 T magnet (500 MHz for protons) and a triple-resonance cryoprobe. All measurements were performed at 300 K. Typical 90° pulse lengths were about 7 µs, corresponding to an rf field strength of $\omega_1/(2\pi)\approx36$ kHz. The decoupling pulses usually had a duration $\tau_p=1$ µs, and rf field strengths $0.2<\omega_1/(2\pi)<1.6$ kHz, corresponding to flip angles smaller than 1° in all cases. The spectral widths were typically 10 kHz, so that the dwell times were $\Delta t=100$ µs, and the duty cycles 1% for monochromatic and 4% for the tetrachromatic experiments of Figure 4. Two scans were acquired for each experiment with a two-step phase alternation of the initial pulse, in concert with the receiver phase. Typically, 32 k points were acquired for each spectrum. All spectra were processed with a line broadening of 1 Hz, except when specified.

Acknowledgements

The authors thank Dr. Simone Ulzega for theoretical insight and Dr. Stefan Steuernagel and Martial Rey for valuable technical assistance. This work was supported by the Swiss National Science Foundation, the Ecole Polytechnique Fédérale de Lausanne (EPFL), the Swiss Commission for Technology and Innovation (CTI), and the French CNRS. This communication is dedicated to Hans Primas, Professor Emeritus, ETH Zürich. H. Primas was one of the Ph.D. supervisors of R. R. Ernst, and first asked the question if one could decouple all homonuclear interactions in a high-resolution spectrum, so as to observe only chemical shifts. He also taught basic quantum mechanics to one of the authors (G.B.), and often made the point that asking good questions is a far greater challenge for scientists than providing answers.

Keywords: Fourier transform spectroscopy · homonuclear decoupling · J-coupling · NMR spectroscopy

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Received: February 14, 2012
Published online: August 2, 2012

