

Theory of covariance nuclear magnetic resonance spectroscopy

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Covariance nuclear magnetic resonance (NMR) spectroscopy provides an effective way for establishing nuclear spin connectivities in molecular systems. The method, which identifies correlated spin dynamics in terms of covariances between 1D spectra, benefits from a high spectral resolution along the indirect dimension without requiring apodization and Fourier transformation along this dimension. The theoretical treatment of covariance NMR spectroscopy is given for NOESY and TOCSY experiments. It is shown that for a large class of 2D NMR experiments the covariance spectrum and the 2D Fourier transform spectrum can be related to each other by means of Parseval's theorem. A general procedure is presented for the construction of a symmetric spectrum with improved resolution along the indirect frequency domain as compared to the 2D FT spectrum. © 2004 American Institute of Physics. [DOI: 10.1063/1.1755652]

I. INTRODUCTION

Over the past decades two-dimensional Fourier transform nuclear magnetic resonance (NMR) spectroscopy (2D FT NMR) has become a widely used experimental method for the structural and dynamic characterization of molecules in solution and in the solid state.¹⁻⁵ Due to its versatility the 2D FT method has found application also in other spectroscopies.^{6,7}

2D FT NMR follows the general scheme⁸

Preparation—Evolution (t_1)—Mixing (τ_m)—Detection (t_2)

(Scheme 1)

which yields a time-domain signal $s(t_1, \tau_m, t_2)$ that, after 2D Fourier transformation, provides a 2D frequency matrix $S(\omega_1, \tau_m, \omega_2) = \int_0^\infty \int_0^\infty dt_1 dt_2 \exp(-i\omega_1 t_1) \exp(-i\omega_2 t_2) s(t_1, \tau_m, t_2)$, which contains quantitative information about connectivities between individual resonances. In practice, a discretized form of $s(t_1, \tau_m, t_2)$ is recorded with a total number N_1 of t_1 values during the evolution period with a constant time increment Δt_1 and a total number N_2 of t_2 values with a time increment Δt_2 during the free induction decay (FID). The spectral resolution, defined as a measure for the smallest frequency separation for which two lines appear as clearly distinct, is along dimension ω_i ($i=1,2$) given by $\Delta \nu_i = 1/(N_i \cdot \Delta t_i)$ independent of zero-filling. Because the experimental time is proportional to N_1 , in practice N_1 is typically significantly smaller than N_2 . For $\Delta t_1 = \Delta t_2$, this leads to lower resolution along the indirect dimension ω_1 as compared to the detection dimension ω_2 . In addition, apodization parameters along t_1 must be chosen carefully in order to minimize truncation artifacts.

The recently introduced covariance NMR spectroscopy method⁹ is an alternative to 2D FT NMR spectroscopy. Instead of applying a second Fourier transformation along the t_1 evolution dimension, it constructs the covariance matrix

from a set of 1D spectra. The covariance method neither requires apodization nor phase or baseline correction along the t_1 dimension and the resolution obtained along the indirect ω_1 dimension is identical to the one along ω_2 .

In previous work an experimental demonstration and comparison between covariance NMR and 2D FT NMR was provided.⁹ In the present paper the relationship between the two methods is discussed from a theoretical perspective. The paper is organized as follows: In Sec. II the covariance method is described. In Sec. III the method is applied to the NOESY experiment¹⁰⁻¹² of the 2-spin 1/2 system as well as the general N -spin 1/2 case and compared with 2D FT. In Sec. IV, the TOCSY experiment¹³ is treated both for the 2-spin 1/2 case and the general N -spin 1/2 case and compared with 2D FT. In Sec. V, the method is formulated in terms of Parseval's theorem, which is followed by a conclusion in Sec. VI.

II. COVARIANCE SPECTROSCOPY

In covariance NMR spectroscopy the multivariate time-domain signal $s(t_1, \tau_m, t_2)$ can be obtained by using Scheme 1 and is, like in 2D FT NMR, first Fourier transformed along t_2 . This yields the real function $S(t_1, \tau_m, \omega_2)$ describing 1D absorption spectra as a function of evolution and mixing times t_1 and τ_m , respectively,

$$S(t_1, \tau_m, \omega_2) = \text{Re} \int_0^{t_{2,\text{max}}} dt_2 \exp(-i\omega_2 t_2) s(t_1, \tau_m, t_2), \quad (1)$$

where $t_{2,\text{max}}$ is the total acquisition time. In a second step, cross-covariances are computed according to⁹

$$\begin{aligned} C_{\omega_2 \omega_2'}(\tau_m) &= C_{\omega_2' \omega_2}(\tau_m) \\ &= \langle S(t_1, \tau_m, \omega_2) S(t_1, \tau_m, \omega_2') \rangle \\ &\quad - \langle S(t_1, \tau_m, \omega_2) \rangle \langle S(t_1, \tau_m, \omega_2') \rangle, \end{aligned} \quad (2)$$

where the angular brackets indicate averaging over t_1 from 0 to the maximal evolution time $t_{1,\text{max}}$, for example,

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$\langle S(t_1, \tau_m, \omega_2) \rangle = t_{1,\max}^{-1} \int_0^{t_{1,\max}} dt_1 S(t_1, \tau_m, \omega_2)$. Due to the discrete nature of data acquisition and discrete Fourier transformation, in practice $S(t_1, \tau_m, \omega_2)$ is defined at finite numbers of discrete frequencies $\{\omega_{2,j}\} (j=1, \dots, N_2)$ and discrete t_1 values. The integral over t_1 is then replaced by a sum over the t_1 increments and $C_{\omega_2 \omega_2'}$ can then be represented as a real symmetric $N_2 \times N_2$ covariance matrix \mathbf{C} .⁹

III. NOESY

The NOESY experiment monitors dipolar auto- and cross-relaxation in the laboratory frame.¹⁰⁻¹² It follows the general Scheme 1 containing three 90° pulses separated by delays t_1 and τ ,¹⁰

$$\begin{aligned} &90^\circ_{\varphi_1} - \text{evolution } (t_1) - 90^\circ_{\varphi_2} - \text{mixing } (\tau) - 90^\circ_{\varphi_3} \\ &\quad - \text{detection } (t_2). \end{aligned} \quad (\text{Scheme 2})$$

It is assumed that time-proportional phase incrementation (TPPI) (Ref. 14) is applied to the φ_1 phase to shift the carrier frequency in the indirect dimension outside the spectrum. At the beginning of the experiment the magnetizations of both spins of a homonuclear IS two-spin 1/2 system are at equilibrium, which is proportional to $(m_{I,\text{eq}}, m_{S,\text{eq}}) = (1, 1)$. For simplicity, magnetizations are treated in the following as unit-free quantities that can take values between -1 and 1 . The magnetization along the z -axis is after the second pulse (with phase $\varphi_2 = -\varphi_1$), neglecting relaxation and J -coupling evolution, given by $(m_I(t_1), m_S(t_1)) = (\cos \omega_I t_1, \cos \omega_S t_1)$, where ω_I, ω_S are the resonance frequencies of the two spins relative to the carrier frequency. During the subsequent mixing time τ , the spin evolution is governed by the Solomon equations¹⁵

$$\frac{d}{d\tau} \begin{pmatrix} m_I(t_1, \tau) \\ m_S(t_1, \tau) \end{pmatrix} = -\mathbf{R} \begin{pmatrix} m_I(t_1, \tau) - m_{I,\text{eq}} \\ m_S(t_1, \tau) - m_{S,\text{eq}} \end{pmatrix}, \quad (3)$$

where

$$\mathbf{R} = \begin{bmatrix} \rho & \sigma \\ \sigma & \rho \end{bmatrix}$$

is the relaxation matrix with $\rho = 1/T_1$ and σ is the cross-relaxation rate that is proportional to the minus sixth power of the internuclear distance r_{IS} , $\sigma = K/r_{\text{IS}}^6$. The solution to the initial condition $(m_I(t_1), m_S(t_1))$, found by standard linear algebraic methods, is

$$\begin{aligned} \begin{pmatrix} m_I(t_1, \tau) \\ m_S(t_1, \tau) \end{pmatrix} &= e^{-\rho\tau} \begin{bmatrix} \cosh(\sigma\tau) & -\sinh(\sigma\tau) \\ -\sinh(\sigma\tau) & \cosh(\sigma\tau) \end{bmatrix} \\ &\quad \times \begin{pmatrix} \cos(\omega_I t_1) - 1 \\ \cos(\omega_S t_1) - 1 \end{pmatrix} + \begin{pmatrix} 1 \\ 1 \end{pmatrix}. \end{aligned} \quad (4)$$

The final 90°_y pulse converts the longitudinal magnetization into detectable transverse magnetization that evolves with the corresponding resonance frequencies ω_I and ω_S ,

$$\begin{aligned} &(m_I(t_1, \tau, t_2), m_S(t_1, \tau, t_2)) \\ &= (m_I(t_1, \tau) e^{i\omega_I t_2}, m_S(t_1, \tau) e^{i\omega_S t_2}). \end{aligned} \quad (5)$$

Complex Fourier transformation with respect to t_2 yields according to Eq. (1) 1D absorptive spectra that depend on t_1 and τ ,

$$\begin{aligned} &S(t_1, \tau, \omega_2) \\ &= \text{Re} \int_0^\infty dt_2 e^{-i\omega_2 t_2} \{m_I(t_1, \tau) e^{i\omega_I t_2} + m_S(t_1, \tau) e^{i\omega_S t_2}\} \\ &= \pi \delta(\omega_2 - \omega_I) m_I(t_1, \tau) + \pi \delta(\omega_2 - \omega_S) m_S(t_1, \tau). \end{aligned} \quad (6)$$

In 2D FT NMR a second Fourier transformation is performed with respect to t_1 leading to a 2D spectrum with diagonal peaks at frequencies (ω_I, ω_I) and (ω_S, ω_S) with amplitudes $a_{I,I} = a_{S,S} = e^{-\rho\tau} \cosh(\sigma\tau)$ and cross peaks at frequencies (ω_I, ω_S) and (ω_S, ω_I) with relative amplitudes $a_{I,S} = a_{S,I} = -e^{-\rho\tau} \sinh(\sigma\tau)$. Additional peaks, known as axial peaks, appear at frequencies $(\omega_I, \omega_2) = (0, \omega_I)$ and $(0, \omega_S)$ that are typically suppressed by phase cycling methods.² The 2D spectrum can then be represented for the 2-spin case in a compressed form by a 2×2 matrix containing as its elements the amplitudes of diagonal and cross peaks of the 2D spectrum¹¹

$$\mathbf{S} = e^{-\rho\tau} \begin{bmatrix} \cosh(\sigma\tau) & -\sinh(\sigma\tau) \\ -\sinh(\sigma\tau) & \cosh(\sigma\tau) \end{bmatrix} = e^{-\mathbf{R}\tau}. \quad (7)$$

In the *covariance method* the variances and covariances of the spectral amplitudes are evaluated according to Eq. (2). We focus on the spectral amplitudes at the two frequencies ω_I and ω_S . The variance of $m_I(t_1, \tau)$ is $\text{Var}(I) = \langle m_I(t_1, \tau)^2 \rangle - \langle m_I(t_1, \tau) \rangle^2$. When taking into account $\langle \cos \omega_I t_1 \rangle = \langle \cos \omega_S t_1 \rangle = \langle \cos \omega_I t_1 \cos \omega_S t_1 \rangle = 0$ and $\langle \cos^2 \omega_I t_1 \rangle = \langle \cos^2 \omega_S t_1 \rangle = 1/2$, one obtains

$$\text{Var}(I) = \frac{1}{2} e^{-2\rho\tau} \{ \cosh^2(\sigma\tau) + \sinh^2(\sigma\tau) \}. \quad (8)$$

Analogous calculations yield $\text{Var}(S) = \text{Var}(I)$ and

$$\begin{aligned} \text{Cov}(I, S) &= \text{Cov}(S, I) \\ &= \langle m_I(t_1, \tau) m_S(t_1, \tau) \rangle - \langle m_I(t_1, \tau) \rangle \langle m_S(t_1, \tau) \rangle \\ &= -e^{-2\rho\tau} \cosh(\sigma\tau) \sinh(\sigma\tau). \end{aligned} \quad (9)$$

The corresponding 2×2 covariance matrix is

$$\mathbf{C} = \begin{bmatrix} \text{Var}(I) & \text{Cov}(I, S) \\ \text{Cov}(S, I) & \text{Var}(S) \end{bmatrix} = \frac{1}{2} e^{-2\rho\tau} \begin{bmatrix} \cosh^2(\sigma\tau) + \sinh^2(\sigma\tau) & -2 \cosh(\sigma\tau) \sinh(\sigma\tau) \\ -2 \cosh(\sigma\tau) \sinh(\sigma\tau) & \cosh^2(\sigma\tau) + \sinh^2(\sigma\tau) \end{bmatrix}. \quad (10)$$

Comparison with the 2D FT spectrum \mathbf{S} of Eq. (7) shows that

$$\mathbf{C} = \frac{1}{2}\mathbf{S}^2 = \frac{1}{2}\mathbf{S}^T\mathbf{S} = \frac{1}{2}e^{-2\mathbf{R}\tau}. \quad (11)$$

Hence, the covariance matrix \mathbf{C} is proportional to the 2D FT NMR spectrum recorded at twice the mixing time 2τ . Axial peaks are suppressed even without explicit phase-cycling based axial peak suppression.

It is now shown that the relationship of Eq. (11) holds for spin systems with $N > 2$ spins. It is noted that for the N -spin 2D FT NOESY spectrum the generalization of Eq. (7) holds where the peak amplitudes are given¹¹

$$S_{ij} = [e^{-\mathbf{R}\tau}]_{ij} \quad \text{or} \quad \mathbf{S} = e^{-\mathbf{R}\tau}. \quad (12)$$

For a rigid and isotropically tumbling molecule the off-diagonal elements of \mathbf{R} are $R_{ij} = K/r_{ij}^6$, where K is a prefactor that depends on the tumbling correlation time and r_{ij} is the distance between the nuclei of spins i and j . The diagonal elements are $R_{ii} = 1/T_{1,i}$, where $T_{1,i}$ is the longitudinal relaxation time of spin i .

The covariance matrix, on the other hand, is determined by

$$\mathbf{C} = \langle \mathbf{m}(t_1, \tau) \cdot \mathbf{m}(t_1, \tau)^T \rangle - \langle \mathbf{m}(t_1, \tau) \rangle \cdot \langle \mathbf{m}(t_1, \tau) \rangle^T, \quad (13)$$

where $\mathbf{m}(t_1, \tau)$ is an N -dimensional column vector containing the magnetizations of the individual spins as its components, which is the solution of the generalized Solomon equations in analogy to Eq. (4),

$$\mathbf{m}(t_1, \tau) = e^{-\mathbf{R}\tau}(\mathbf{c}(t_1) - \mathbf{1}) + \mathbf{1}, \quad (14)$$

where $\mathbf{c}(t_1) = (\cos(\omega_1 t_1), \cos(\omega_2 t_1), \dots, \cos(\omega_N t_1))^T$ and $\mathbf{1} = (1, 1, \dots, 1)^T$. The first term on the right-hand side of Eq. (13) is, after taking into account $\langle \mathbf{c}(t_1) \rangle = \langle \mathbf{c}(t_1) \rangle^T = 0$,

$$\begin{aligned} & \langle \mathbf{m}(t_1, \tau) \cdot \mathbf{m}(t_1, \tau)^T \rangle \\ &= e^{-\mathbf{R}\tau} \langle (\mathbf{c}(t_1) - \mathbf{1})(\mathbf{c}(t_1)^T - \mathbf{1}^T) \rangle e^{-\mathbf{R}\tau} - e^{-\mathbf{R}\tau} \mathbf{1} \cdot \mathbf{1}^T \\ & \quad - \mathbf{1} \cdot \mathbf{1}^T e^{-\mathbf{R}\tau} + \mathbf{1} \cdot \mathbf{1}^T, \end{aligned} \quad (15)$$

whereas the second term is

$$\begin{aligned} & \langle \mathbf{m}(t_1, \tau) \rangle \cdot \langle \mathbf{m}(t_1, \tau) \rangle^T = e^{-\mathbf{R}\tau} \mathbf{1} \cdot \mathbf{1}^T e^{-\mathbf{R}\tau} - e^{-\mathbf{R}\tau} \mathbf{1} \cdot \mathbf{1}^T \\ & \quad - \mathbf{1} \cdot \mathbf{1}^T e^{-\mathbf{R}\tau} + \mathbf{1} \cdot \mathbf{1}^T. \end{aligned} \quad (16)$$

It follows for their difference $\mathbf{C} = e^{-\mathbf{R}\tau} \langle \mathbf{c}(t_1) \cdot \mathbf{c}(t_1)^T \rangle e^{-\mathbf{R}\tau}$. In the absence of spectral degeneracies $\langle \cos(\omega_i t_1) \cos(\omega_j t_1) \rangle = \frac{1}{2} \delta_{ij}$ and therefore

$$\mathbf{C} = e^{-\mathbf{R}\tau} \frac{1}{2} e^{-\mathbf{R}\tau} = \frac{1}{2} e^{-2\mathbf{R}\tau} = \frac{1}{2} \mathbf{S}^2 = \frac{1}{2} \mathbf{S}^T \mathbf{S}, \quad (17)$$

which shows that Eq. (11) applies to spin systems of arbitrary size. As for the 2-spin case [Eq. (10)] axial peaks are automatically suppressed. Equation (17) shows that the covariance spectrum corresponds to a NOESY spectrum obtained at twice the experimental mixing time, 2τ . This explains the previous observation of significant spin diffusion effects in an experimental covariance NOESY spectrum of ubiquitin.⁹

The r_{ij}^{-6} distance dependence of the off-diagonal elements of \mathbf{R} , which determines the cross-peak amplitudes at short mixing times τ , forms the basis of the internuclear distance information obtainable from NOESY spectra.^{11,12} Be-

cause \mathbf{C} is by definition a symmetric and positive semidefinite matrix, it can be diagonalized, $\mathbf{C}\mathbf{v}_j = \lambda_j \mathbf{v}_j$, with $\lambda_j \geq 0$ and $\mathbf{C} = \mathbf{U}\mathbf{D}\mathbf{U}^T$, where \mathbf{D} is a diagonal matrix containing the eigenvalues λ_j as its diagonal elements and \mathbf{U} is an orthogonal matrix with the corresponding eigenvectors \mathbf{v}_j as its columns. It follows for spectrum \mathbf{S} and relaxation matrix \mathbf{R} ,

$$\mathbf{S} = 2^{1/2} \mathbf{C}^{1/2} = 2^{1/2} \mathbf{U}\mathbf{D}^{1/2} \mathbf{U}^T, \quad (18)$$

$$\mathbf{R} = -\frac{1}{2\tau} \ln(2\mathbf{C}) = -\frac{1}{2\tau} \mathbf{U} \ln(2\mathbf{D}) \mathbf{U}^T. \quad (19)$$

As mentioned above, if the number of t_1 increments N_1 is smaller than the number of t_2 increments N_2 , in 2D FT NMR the spectral resolution along ω_1 is lower than along ω_2 . By contrast, in covariance NMR the spectral resolutions along ω_1 and ω_2 are identical for both spectrum \mathbf{S} and relaxation matrix \mathbf{R} calculated from the eigenvectors and eigenvalues of covariance matrix \mathbf{C} using Eqs. (18) and (19). Equations (17) and (18) provide a straightforward method to get a symmetric spectrum \mathbf{S}_{sym} from a nonsymmetric 2D FT spectrum \mathbf{S} via

$$\mathbf{S}_{\text{sym}} = 2^{1/2} \mathbf{C}^{1/2} = (\mathbf{S}^T \mathbf{S})^{1/2}. \quad (20)$$

IV. TOCSY

The covariance method is applied to the TOCSY experiment¹³ for a homonuclear IS 2-spin 1/2 system. The spin Hamiltonian is given by

$$\mathcal{H} = \omega_I I_z + \omega_S S_z + 2\pi J \{I_x S_x + I_y S_y + I_z S_z\}, \quad (21)$$

where ω_I , ω_S are the Larmor frequencies relative to the carrier frequency and J is the isotropic scalar J -coupling constant (in units of Hz). Starting with the equilibrium density operator $\sigma_0 = I_z + S_z$, the relevant part of the density operator after the t_1 evolution period, neglecting spin relaxation and antiphase terms (rotating-frame zero quantum terms), is

$$\sigma(t_1) = \cos(\pi J t_1) \{ \cos(\omega_I t_1) I_x + \cos(\omega_S t_1) S_x \}. \quad (22)$$

$\sigma(t_1)$ evolves during isotropic mixing of duration τ_m under the effective Hamiltonian $\mathcal{H}_{\text{iso}} = 2\pi J \{I_x S_x + I_y S_y + I_z S_z\}$ according to¹³

$$\begin{aligned} \sigma(t_1, \tau_m) &= \cos(\pi J t_1) [\cos(\omega_I t_1) a_+(\tau_m) \\ & \quad + \cos(\omega_S t_1) a_-(\tau_m)] I_x \\ & \quad + \cos(\pi J t_1) [\cos(\omega_I t_1) a_-(\tau_m) \\ & \quad + \cos(\omega_S t_1) a_+(\tau_m)] S_x, \end{aligned} \quad (23)$$

where $a_{\pm}(\tau_m) = \{1 \pm \cos(2\pi J \tau_m)\}/2$ and only detectable terms of the density operator have been retained. Phase-sensitive detection during the period t_2 yields the complex time-domain signal

$$\begin{aligned} s(t_1, \tau_m, t_2) &= \cos(\pi J t_1) [\cos(\omega_I t_1) a_+(\tau_m) \\ & \quad + \cos(\omega_S t_1) a_-(\tau_m)] \cos(\pi J t_2) \exp(i\omega_I t_2) \\ & \quad + \cos(\pi J t_1) [\cos(\omega_I t_1) a_-(\tau_m) \\ & \quad + \cos(\omega_S t_1) a_+(\tau_m)] \cos(\pi J t_2) \exp(i\omega_S t_2). \end{aligned} \quad (24)$$

After complex Fourier transformation with respect to t_2 , 1D absorption spectra are obtained with four multiplet components whose amplitudes depend on t_1 and τ_m ,

$$\begin{aligned}
 S(t_1, \tau_m, \omega_2) &= \cos(\pi J t_1) \{ \cos(\omega_I t_1) a_+(\tau_m) \\
 &+ \cos(\omega_S t_1) a_-(\tau_m) \} \frac{\pi}{2} [\delta(\omega_2 - (\omega_I + \pi J)) \\
 &+ \delta(\omega_2 - (\omega_I - \pi J))] \\
 &+ \cos(\pi J t_1) \{ \cos(\omega_I t_1) a_-(\tau_m) \\
 &+ \cos(\omega_S t_1) a_+(\tau_m) \} \frac{\pi}{2} [\delta(\omega_2 - (\omega_S + \pi J)) \\
 &+ \delta(\omega_2 - (\omega_S - \pi J))]. \quad (25)
 \end{aligned}$$

In 2D FT NMR spectroscopy Fourier transformation along t_1 yields a spectrum with relative diagonal and cross-peak amplitudes

$$\mathbf{S} = \begin{bmatrix} a_+ & a_- \\ a_- & a_+ \end{bmatrix} \otimes \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix}, \quad (26)$$

where the first and second rows correspond to the two I spin multiplet components and the third and fourth rows correspond to the two S spin multiplet components. Zero-amplitude cross peaks occur if the scalar J -coupling is zero or if condition $\tau_m = n/J$ ($n=0,1,2,\dots$) holds and the cross-peak maxima occur at times $\tau_m = (2n+1)/(2J)$ ($n=0,1,2,\dots$).

The covariance spectrum, on the other hand, is calculated according to Eq. (2),⁹

$$\begin{aligned}
 \text{Var}(I) &= \text{Var}(S) \\
 &= \langle (\cos(\pi J t_1) \{ \cos(\omega_I t_1) a_+(\tau_m) \\
 &+ \cos(\omega_S t_1) a_-(\tau_m) \})^2 \rangle = d(a_+^2 + a_-^2),
 \end{aligned}$$

where $d = \langle \cos^2(\pi J t_1) (\cos^2(\omega_I t_1) + \cos^2(\omega_S t_1)) \rangle$ and the associated covariances are $\text{Cov}(I, S) = \text{Cov}(S, I) = 2da_+a_-$. Hence, the covariance matrix is

$$\begin{aligned}
 \mathbf{C} &= d \begin{bmatrix} a_+^2 + a_-^2 & 2a_+a_- \\ 2a_+a_- & a_+^2 + a_-^2 \end{bmatrix} \otimes \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix} \\
 &= \frac{d}{2} \begin{bmatrix} 1 + \cos^2(2\pi J \tau_m) & 1 - \cos^2(2\pi J \tau_m) \\ 1 - \cos^2(2\pi J \tau_m) & 1 + \cos^2(2\pi J \tau_m) \end{bmatrix} \otimes \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix}. \quad (27)
 \end{aligned}$$

The cross peaks have zero amplitude under the same conditions as the cross peaks of the 2D FT spectrum [Eq. (26)]. Comparison of Eqs. (26) and (27) reveals

$$\mathbf{C} = \frac{d}{2} \mathbf{S}^2 = \frac{d}{2} \mathbf{S}^T \mathbf{S}, \quad (28)$$

which has, with the exception of the prefactor d , the same form as for the NOESY case [Eqs. (11) and (17)].

Equation (28) can be generalized for spin systems with $N > 2$ spins. The isotropic Hamiltonian during the mixing period is then

$$\mathcal{H}_{\text{iso}} = \sum_{i < j}^N 2\pi J_{ij} \{ I_{ix} I_{jx} + I_{iy} I_{jy} + I_{iz} I_{jz} \}, \quad (29)$$

where J_{ij} is the scalar J -coupling constant between spins i and j . In the 2D FT TOCSY spectrum \mathbf{S} the amplitudes of the cross and diagonal peaks are given by

$$S_{ij}(\tau) = q \cdot \text{Tr} \{ I_{jz} \sigma_i(\tau) \} = q \cdot \text{Tr} \{ I_{jz} e^{-i\mathcal{H}_{\text{iso}}\tau} I_{iz} e^{i\mathcal{H}_{\text{iso}}\tau} \}, \quad (30)$$

where $q = (\text{Tr} \{ I_{jz}^2 \})^{-1}$ is a normalization factor. The matrices representing the operators \mathcal{H}_{iso} , I_{iz} , and I_{jz} are real and therefore the 2D FT TOCSY spectrum is symmetric $\mathbf{S} = \mathbf{S}^T$ because

$$\begin{aligned}
 S_{ij}(\tau) &= S_{ij}(\tau)^* \\
 &= q \cdot \text{Tr} \{ I_{jz} e^{i\mathcal{H}_{\text{iso}}\tau} I_{iz} e^{-i\mathcal{H}_{\text{iso}}\tau} \} \\
 &= q \cdot \text{Tr} \{ I_{iz} e^{-i\mathcal{H}_{\text{iso}}\tau} I_{jz} e^{i\mathcal{H}_{\text{iso}}\tau} \} = S_{ji}(\tau), \quad (31)
 \end{aligned}$$

where the invariance of the trace operation under cyclic permutation of its operator arguments was used.

For the *covariance method* the spin density operator after t_1 evolution is $\sigma(t_1) = \sum_{i=1}^N c_i(t_1) I_{iz}$, where $c_i(t_1) = \cos \omega_i t_1$. After isotropic mixing, the amplitude of the resonance of spin k is

$$\begin{aligned}
 S(t_1, \tau, k) &= q \cdot \text{Tr} \{ I_{kz} e^{-i\mathcal{H}_{\text{iso}}\tau} \sigma(t_1) e^{i\mathcal{H}_{\text{iso}}\tau} \} \\
 &= \sum_{i=1}^N c_i(t_1) q \text{Tr} \{ I_{kz} e^{-i\mathcal{H}_{\text{iso}}\tau} I_{iz} e^{i\mathcal{H}_{\text{iso}}\tau} \} \\
 &= \sum_{i=1}^N c_i(t_1) S_{ik}(\tau). \quad (32)
 \end{aligned}$$

The elements of covariance matrix \mathbf{C} are determined by

$$C_{kl} = \langle S(t_1, \tau, k) S(t_1, \tau, l) \rangle - \langle S(t_1, \tau, k) \rangle \langle S(t_1, \tau, l) \rangle. \quad (33)$$

Using $\langle S(t_1, \tau, k) \rangle = \langle S(t_1, \tau, l) \rangle = 0$ and $\langle c_i(t_1) c_j(t_1) \rangle = \frac{1}{2} \delta_{ij}$, one obtains

$$\begin{aligned}
 C_{kl} &= \sum_{i,j=1}^N \langle c_i(t_1) c_j(t_1) \rangle S_{ik}(\tau) S_{jl}(\tau) \\
 &= \frac{1}{2} \sum_{i=1}^N S_{ik}(\tau) S_{il}(\tau) = \frac{1}{2} [\mathbf{S}^T \mathbf{S}]_{kl} \quad (34)
 \end{aligned}$$

or $\mathbf{C} = \frac{1}{2} \mathbf{S}^T \mathbf{S}$ which is the same as Eq. (17). It differs from Eq. (28) by the prefactor d since, in contrast to Eq. (34), in Eq. (28) evolution under the scalar J -coupling during t_1 has been taken into account.

It is worth noting that the general form of Eq. (34) does not depend on the explicit form of \mathcal{H}_{iso} . It merely uses the fact that the propagation under the mixing Hamiltonian is unitary. Thus, Eq. (34) holds also for other experiments with different unitary mixing sequences. As in the NOESY case, a symmetric spectrum \mathbf{S} can be obtained using Eq. (20).

V. PARSEVAL'S THEOREM AND COVARIANCE NMR

The relationship $\mathbf{C} = \frac{1}{2}\mathbf{S}^T\mathbf{S}$ can be rationalized using the well-known Parseval's theorem,

$$\int_{-\infty}^{\infty} f(t)g^*(t)dt = \frac{1}{2\pi} \int_{-\infty}^{\infty} F(\omega)G^*(\omega)d\omega, \quad (35)$$

where $F(\omega)$ and $G(\omega)$ are the complex Fourier transforms of the functions $f(t)$ and $g(t)$. An analogous expression holds also for discrete time signals and spectra.

In the TOCSY experiment $\langle S(t_1, \tau_m, \omega_2) \rangle \cong 0$, because $S(t_1, \tau_m, \omega_2)$ is either close to zero in case ω_2 is far away from all resonance frequencies or $S(t_1, \tau_m, \omega_2)$ is a rapidly oscillating function in t_1 with zero average if the carrier frequency is sufficiently far away from all resonances. The latter can be achieved by setting the carrier outside the spectrum or by time-proportional phase incrementation (TPPI).¹⁴ From $\langle S(t_1, \tau_m, \omega_2) \rangle \cong 0$ follows

$$C_{\omega_2\omega_2'}(\tau_m) = C_{\omega_2'\omega_2}(\tau_m) \cong \langle S(t_1, \tau_m, \omega_2)S(t_1, \tau_m, \omega_2') \rangle. \quad (36)$$

Since $S(t_1, \tau_m, \omega_2)$ is real and $S(t_1, \tau_m, \omega_2) = 0$ for $t_1 < 0$ it can be shown using the cosine Fourier transform $S(\omega_1, \tau_m, \omega_2) = \int_0^{\infty} S(t_1, \tau_m, \omega_2) \cos(\omega_1 t_1) dt_1$,

$$\begin{aligned} C_{\omega_2\omega_2'}(\tau_m) &\propto \int_0^{\infty} dt_1 S(t_1, \tau_m, \omega_2) S(t_1, \tau_m, \omega_2') \\ &= \frac{1}{\pi} \int_{-\infty}^{\infty} d\omega_1 S(\omega_1, \tau_m, \omega_2) S(\omega_1, \tau_m, \omega_2'). \end{aligned} \quad (37)$$

In other words, the scalar product of two columns of the 2D spectrum $S(\omega_1, \tau_m, \omega_2)$ is proportional to the covariance of the corresponding time-domain signals $S(t_1, \tau_m, \omega_2)$, which coincides with the mathematical meaning of $\mathbf{C} \propto \mathbf{S}^T\mathbf{S}$ [see Eqs. (17) and (34)]. Thus, a symmetrized 2D-FT like spectrum \mathbf{S}_{sym} can be obtained using $\mathbf{S}_{\text{sym}} = 2^{1/2}\mathbf{C}^{1/2} = (\mathbf{S}^T\mathbf{S})^{1/2}$ [see Eq. (20)].

Parseval's theorem is not directly applicable to the NOESY experiment because according to Eq. (16) $\langle S(t_1, \tau_m, \omega_2) \rangle \neq 0$. However, axial peak suppression can be employed by subtracting for each FID obtained with an excitation pulse with phase φ_1 a FID with phase $\varphi_1 + 180^\circ$ creating the initial condition $-\mathbf{c}(t_1)$ at the beginning of the mixing time.² Using Eq. (14) this yields

$$\begin{aligned} \Delta\mathbf{m}(t_1, \tau) &= \{e^{-\mathbf{R}\tau}(\mathbf{c}(t_1) - \mathbf{1}) + \mathbf{1}\} - \{e^{-\mathbf{R}\tau}(-\mathbf{c}(t_1) - \mathbf{1}) + \mathbf{1}\} \\ &= 2e^{-\mathbf{R}\tau}\mathbf{c}(t_1) \end{aligned} \quad (38)$$

which has the desired property $\langle \Delta\mathbf{m}(t_1, \tau) \rangle \cong 0$ allowing the application of Parseval's theorem in analogy to Eq. (37). Alternatively, axial peaks can be suppressed by calculating

the covariance matrix $\mathbf{C}' = \frac{1}{2}\mathbf{S}'^T\mathbf{S}'$ from a subregion \mathbf{S}' of the full 2D FT spectrum \mathbf{S} that is free of axial peaks.

In this work, the covariance formalism was applied to mixed time-frequency domain data of the type $S(t_1, \tau_m, \omega_2)$ corresponding to 1D spectra along ω_2 with different evolution times t_1 . For symmetry reasons, it is possible to apply the covariance formalism to mixed frequency-time domain data of the type $S(\omega_1, \tau_m, t_2)$, which is obtained by Fourier transformation of $S(t_1, \tau_m, t_2)$ along t_1 . However, in this case the spectral resolution is determined by the sampling along the indirect t_1 dimension, which is generally lower than the one available along ω_2 .

VI. CONCLUSION

Covariance NMR spectroscopy is an attractive alternative to 2D FT NMR for the identification and analysis of nuclear spin correlations in molecular systems. The theoretical analysis presented here shows that for a large class of homonuclear 2D NMR experiments the covariance NMR spectra are mathematically related via Parseval's theorem to the corresponding 2D FT NMR spectra. Application of the matrix square-root operation to the covariance matrix yields a spectrum that has increased resolution along ω_1 and otherwise is very similar to the 2D FT spectrum. In the absence of *a priori* information about the spectrum, evolution in constant t_1 increments is well suited for the covariance approach. If, on the other hand, the chemical shift distribution is already known, alternative excitation and evolution schemes are conceivable using frequency-selective pulses providing spin correlation information that can be unraveled by means of the covariance formalism. Since covariance NMR makes fewer assumptions and has fewer restrictions, it can be considered for the types of experiments discussed here as a generalization of the 2D FT NMR method. From a data analysis perspective, the formulation of a 2D spectrum in terms of a covariance matrix has distinct advantages lending itself to diagonalization techniques, such as principal component analysis and other multivariate statistical analysis methods,¹⁶ for the downstream interpretation of the spectra in terms of spin-network topology and molecular structure.¹⁷

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