

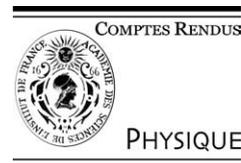


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Highly polarized nuclear spin systems and dipolar interactions in NMR/Systèmes de spins nucléaires fortement polarisés et interactions dipolaires en RMN

## Cross-correlated relaxation in NMR of macromolecules in the presence of fast and slow internal dynamics

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### Abstract

In this paper we present an analysis of correlation and spectral density functions involved in autorelaxation and cross-correlated relaxation in the magnetic resonance of macromolecules. Internal dynamics of the macromolecule are described in terms of two distinct fluctuation processes with different, slow and fast, correlation times. The approach developed in this work takes into account the possible coupling between both fluctuating internal processes. **To cite this article:** *L. Vugmeyster et al., C. R. Physique 5 (2004).*

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### Résumé

**Relaxation par corrélation croisée en RMN de macromolécules en présence de dynamiques internes lente et rapide.**

Dans cet article, nous présentons une analyse des fonctions de corrélation et de densité spectrale impliquées dans l'auto-relaxation et la relaxation par corrélation croisée en résonance magnétique des macromolécules. La dynamique interne d'une macromolécule est décrite par deux processus distincts de fluctuations internes respectivement lent et rapide, associés à des temps de corrélation différents. L'approche développée dans ce travail prend en compte un possible couplage entre ces deux types de mouvements internes. **Pour citer cet article :** *L. Vugmeyster et al., C. R. Physique 5 (2004).*

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**Keywords:** NMR; Relaxation; Order parameter; Lipari–Szabo; Internal mobility

**Mots-clés :** RMN ; Relaxation ; Paramètres d'ordre Lipari–Szabo ; Mouvements internes

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### 1. Introduction

Internal dynamics of biological macromolecules such as proteins or nucleic acids span a wide range of time scales. A proper description of internal motions is essential for a better understanding of their biological function. However, the measurement of NMR relaxation rates does not provide information on internal dynamics alone, since the rates are determined by a combination of overall tumbling and internal motions. One therefore has to resort to theoretical models to separate contributions from both types of motions. If rotational diffusion can be assumed to be isotropic, the overall tumbling of a macromolecule can

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## Nomenclature

$C_{uv}(t)$	total cross-correlation function
$C_{uv}^{\text{glob}}(t)$	correlation function for global motion
$C_{uu}^{\text{int}}(t), C_{uv}^{\text{int}}(t)$	correlation functions for internal motion
$C_{uu}(t)$	autocorrelation function
$C_{uv}^{\text{int},q}(t)$	cross-correlation function associated with the dynamics of the local environment
$D_{mk}(\Psi) \equiv D_{mk}^{(2)}(\Psi)$	second-rank Wigner matrix elements
$G(\Omega_u, \Omega_v, t; \Omega_{u0}, \Omega_{v0}, 0)$	joint probability density
$G(\Omega_u, \Omega_v, t   \Omega_{u0}, \Omega_{v0}, 0)$	conditional transition probability density
$J_{uu}(\omega)$	spectral density of autocorrelation, Fourier transform of $C_{uu}(t)$
$J_{uv}(\omega)$	spectral density of cross-correlation, Fourier transform of $C_{uv}(t)$
$\Omega_u$	solid angle describing the position of $\mathbf{u}$ in the molecular frame
$\Omega_u^L(t)$	solid angle describing the position of $\mathbf{u}$ in the laboratory frame
$\tilde{\Omega}_u$	solid angle describing the position of $\mathbf{u}$ with respect to the local environment reference frame
$S_{uv}$	cross-correlation order parameter, in general encompassing both fast and slow motions
$S_{uu}$	autocorrelation order parameter, in general encompassing both fast and slow motions
$S_{uu}^f$	autocorrelation order parameter for fast motion
$\tilde{S}_{uu}^f$	autocorrelation order parameter for fast motion as defined by Clore et al.
$S_{uu}^s$	autocorrelation order parameter for slow motion
$S_{uv}^s$	cross-correlation order parameter for slow motion
superscript $q$	quasi-equilibrium value
superscript eq	true equilibrium value
$\langle \theta_{uv} \rangle$	average angle between vectors $\mathbf{u}^q$ and $\mathbf{v}^q$ , which is fixed in a rigid fragment
$\tau_c$	isotropic global correlation time
$\tau_u^f$	time constant for fast internal motions of $\mathbf{u}$
$\tau_u^s$	time constant for slow internal motions of $\mathbf{u}$
$Y_{2m}$	second order spherical harmonics function
$\Psi$	represents the three Euler angles relating the molecular frame to the laboratory frame
$\Psi_u$	represents the three Euler angles relating the local environment reference frame of $\mathbf{u}$ to the molecular reference frame

be described by a single correlation time  $\tau_c$ . If the tumbling motion is anisotropic, however, its description requires three distinct correlation times  $\tau_m$  ( $m = 0, 1, 2$ ). Internal dynamics are often described by invoking one or two local correlation times which may vary along the molecule. Conventional NMR methods provide information on auto-relaxation rates ( $R_1$  and  $R_2$ ) and on cross-relaxation rates (Overhauser effects) involving various nuclei (essentially protons, nitrogen-15, carbon-13 and phosphorus-31 in biomolecules). The most commonly used technique to characterize local internal dynamics consists in determining the longitudinal and transverse relaxation rates  $R_1$  and  $R_2$  of nitrogen-15 and the steady-state Overhauser enhancement factor  $\eta$ . These experiments provide information on the rigidity of an N–H<sup>N</sup> bond vector.

In recent years, an increasing number of NMR methods have been described [1,2] that allow one to determine a wide range of cross-correlated relaxation rates that are due to various dipole–dipole and anisotropic chemical shift interactions. In particular, cross-correlated relaxation rates of multiple-quantum coherences provide insight into correlated dynamics of various interactions, such as cross-correlation between two dipole–dipole interactions (DD/DD), between two chemical shift anisotropy interactions (CSA/CSA), or combinations such as DD/CSA [2–9]. The measurement of such rates appears especially promising since they allow one to estimate the degree of correlation between the motions of two CSA tensors or dipole–dipole interactions, and therefore of the motions of atoms and chemical bonds. In recent work by Peluquessy et al. [6] it has been shown that internal dynamics on a time scale that is slower than overall tumbling contributes to cross correlated relaxation of zero- and double-quantum coherences.

The fluctuating orientation of a dipole–dipole interaction (DD) or of a principal component of a CSA tensor can be represented by a vector  $\mathbf{u}$ . The dynamics of such a vector  $\mathbf{u}$  are usually described in terms of a model-free [10] order parameter  $S_u^2$  [11–15]. The model-free formalism was originally designed to take account of internal motions characterized by a single time constant, assumed to be much shorter than the correlation time  $\tau_c$ , so that the internal and overall motions may be considered to be statistically independent. Violations of the assumption of the statistical independence between internal and overall motions

has been discussed recently [16,17]. The Lipari–Szabo formalism was extended by Clore et al. [18] to encompass two distinct internal time scales, i.e., a very fast internal motion and a much slower one, both of which are still assumed to be statistically independent of overall tumbling. In order to extract information about correlated dynamics from cross-correlated relaxation rates, it is necessary to have a proper theoretical framework which relates these rates to a set of correlation times that characterize both the internal and overall dynamics of the molecule. A first step in this direction has been proposed by Daragan and Mayo [19] who made an analysis of the relevant correlation functions for various rotational models. Note that the model-free approach has also been applied to anisotropic overall motion [20,21], thus extending previous work [22]. The present paper represents an attempt to extend the current theory to the case of cross-correlated relaxation in the presence of two types of internal dynamic processes. The dynamics of each interaction  $\mathbf{u}$  will be described by three parameters, i.e., two local correlation times  $\tau_u^f$  and  $\tau_u^s$  corresponding to fast and slow internal processes, in addition to the isotropic global correlation time  $\tau_c$ . The environment fluctuates on a much slower timescale so that the bond vectors  $\mathbf{u}$  and  $\mathbf{v}$  undergo rapid fluctuations about their respective quasi-equilibrium positions. In addition, we shall assume that the fluctuations of  $\mathbf{u}$  and  $\mathbf{v}$  are statistically independent on a timescale which is short compared to the characteristic time of the environment. In practical applications, e.g., if we consider relaxation of two-spin coherences involving neighboring N–H<sup>N</sup> bonds, each vector N<sub>u</sub>–H<sub>u</sub><sup>N</sup> is associated with both a short time constant  $\tau_u^f$  and a long time constant  $\tau_u^s$ .

The description of internal dynamics that is presented in this paper is only valid for dipole–dipole interactions between nuclei that are at a fixed distance from each other, and for chemical shift anisotropy interactions described by second rank tensors with components which are independent of internal motions. However, for simplicity, the theory is specialized in this paper to the case of axially symmetric tensors. If the interactions of interest are the dipoles N<sub>u</sub>–H<sub>u</sub><sup>N</sup> and N<sub>v</sub>–H<sub>v</sub><sup>N</sup>, the cross-correlation function corresponds to the correlation between the motions of the bond vectors  $\mathbf{u}$  and  $\mathbf{v}$ . Therefore, the dynamics of spin interactions may provide direct insight into the dynamics of bond vectors. For the sake of simplicity, we shall speak of ‘bond vector dynamics’, keeping in mind that the same formalism is also applicable to long-range DD interactions such as C’H<sup>N</sup> (i.e., between a carbonyl carbon and an amide hydrogen) and to various correlations such as CSA/CSA, CSA/DD, etc., although the principal axes of the relevant tensors are not necessarily parallel to any bond vectors.

We shall analyze the effects on cross-correlated spectral density functions of the simple two-step model for the internal dynamics presented above and we shall provide guidance on how to extract the characteristics of correlated fluctuations from experimental relaxation rates.

## 2. Model and basic equations

Considering two bond vectors  $\mathbf{u}$  and  $\mathbf{v}$  that are normalized to unit length, the cross-correlation function  $C_{uv}(t)$  can be written as

$$C_{uv}(t) = \frac{1}{5} \langle P_2(\mathbf{u}(t) \cdot \mathbf{v}(0)) \rangle, \quad (1)$$

where  $\langle \cdot \rangle$  indicates the ensemble average over tumbling and internal motions, and  $P_2(x) = \frac{1}{2}(3x^2 - 1)$ . Using the addition theorem for modified spherical harmonics, we obtain:

$$C_{uv}(t) = \frac{1}{5} \frac{4}{5} \sum_{m=-2}^2 \langle Y_{2m}(\Omega_u^L(t)) Y_{2m}^*(\Omega_v^L(0)) \rangle, \quad (2)$$

where  $\Omega_u^L(t)$  and  $\Omega_v^L(0)$  are the solid angles describing the positions of the two bond vectors  $\mathbf{u}$  and  $\mathbf{v}$  with respect to the laboratory reference frame. Contributions of overall tumbling and internal motions to the cross-correlation function (Eq. (2)) can be separated provided that they are statistically independent [10]. By introducing the solid angles  $\Omega_u$  and  $\Omega_v$  to describe the orientations of the vectors  $\mathbf{u}$  and  $\mathbf{v}$  with respect to a molecular frame

$$Y_{2m}(\Omega_u^L) = \sum_k D_{mk}(\Psi) Y_{2k}(\Omega_u), \quad (3)$$

where  $D_{mk}(\Psi) \equiv D_{mk}^{(2)}(\Psi)$  are second-rank Wigner matrix elements and  $\Psi$  represents the three Euler angles relating the molecular frame to the laboratory frame [23]. For isotropic global tumbling, Eqs. (2), (3) lead to:

$$C_{uv}(t) = C^{\text{glob}}(t) C_{uv}^{\text{int}}(t), \quad (4)$$

where  $C^{\text{glob}}(t)$  is the autocorrelation function of global tumbling:

$$C^{\text{glob}}(t) = \frac{1}{5} \sum_k \langle D_{km}(\Psi(t)) D_{km}^*(\Psi(0)) \rangle, \quad (5)$$

and where  $C_{uv}^{\text{int}}(t)$  is the cross-correlation function in the molecular frame:

$$C_{uv}^{\text{int}}(t) = \frac{4\pi}{5} \sum_m \langle Y_{2m}(\Omega_u(t)) Y_{2m}^*(\Omega_v(0)) \rangle. \quad (6)$$

For isotropic overall tumbling, one has:

$$C^{\text{glob}}(t) = \frac{1}{5} \exp\left(-\frac{t}{\tau_c}\right). \quad (7)$$

The calculation of the correlation functions  $\langle Y_{2m}(\Omega_u(t)) Y_{2m}^*(\Omega_v(0)) \rangle$  can be reduced to the average values  $\langle Y_{2m}(\Omega_u(t)) \rangle$  under given initial conditions. We can write the average  $\langle Y_{2m}(\Omega_u(t)) Y_{2m}^*(\Omega_v(0)) \rangle$  in terms of the probability density function  $G(\Omega_u, \Omega_v, t; \Omega_{u0}, \Omega_{v0}, 0)$  which characterizes the joint stochastic process of the motions of the vectors  $\mathbf{u}(t)$  and  $\mathbf{v}(t)$ :

$$\langle Y_{2m}(\Omega_u(t)) Y_{2m}^*(\Omega_v(0)) \rangle = \int d\Omega_u(t) d\Omega_v(t) d\Omega_u(0) d\Omega_v(0) G(\Omega_u, \Omega_v, t; \Omega_{u0}, \Omega_{v0}, 0) Y_{2m}(\Omega_u) Y_{2m}^*(\Omega_{v0}), \quad (8)$$

where  $G(\Omega_u, \Omega_v, t; \Omega_{u0}, \Omega_{v0}, 0)$  is the joint probability density of the solid angles  $\Omega_u$  and  $\Omega_v$  associated with the vectors  $\mathbf{u}$  and  $\mathbf{v}$ . In terms of the conditional transition probability  $G(\Omega_u, \Omega_v, t | \Omega_{u0}, \Omega_{v0}, 0)$  this can be written:

$$G(\Omega_u, \Omega_v, t; \Omega_{u0}, \Omega_{v0}, 0) = G(\Omega_u, \Omega_v, t | \Omega_{u0}, \Omega_{v0}, 0) G(\Omega_{u0}, \Omega_{v0}), \quad (9)$$

where  $G(\Omega_{u0}, \Omega_{v0})$  is the probability density of the initial orientations. Introducing the marginal conditional transition probability characterizing the stochastic function  $\Omega_u(t)$  alone:

$$\tilde{G}(\Omega_u | \Omega_{u0}, \Omega_{v0}) = \int d\Omega_v G(\Omega_u, \Omega_v | \Omega_{u0}, \Omega_{v0}), \quad (10)$$

one obtains:

$$\langle Y_{2m}(\Omega_u(t)) Y_{2m}^*(\Omega_v(0)) \rangle = \int d\Omega_u d\Omega_{u0} d\Omega_{v0} \tilde{G}(\Omega_u | \Omega_{u0}, \Omega_{v0}) G(\Omega_{u0}, \Omega_{v0}) Y_{2m}(\Omega_u) Y_{2m}^*(\Omega_{v0}) \quad (11)$$

which implies that:

$$\langle Y_{2m}(\Omega_u(t)) Y_{2m}^*(\Omega_v(0)) \rangle = \langle Y_{2m}(\Omega_u(t)) \rangle_0 Y_{2m}^*(\Omega_{v0}), \quad (12)$$

where:

$$\langle Y_{2m}(\Omega_u(t)) \rangle_0 = \langle Y_{2m}(\Omega_u(t)) \rangle_{\{\Omega_{u0}, \Omega_{v0}\}} = \int d\Omega_u \tilde{G}(\Omega_u | \Omega_{u0}, \Omega_{v0}) Y_{2m}(\Omega_u). \quad (13)$$

Recall that the dynamics of the vectors is modelled by a two-step process with fast motions about a quasi-equilibrium vector, and slow fluctuations of the latter about the true equilibrium. We define  $\Psi_u$  as the set of Euler angles that relate the local environment frame of the vector  $\mathbf{u}$  to the molecular reference frame. The position of  $\mathbf{u}$  with respect to the former frame is denoted by the Euler angles  $\tilde{\Omega}_u$ :

$$Y_{2m}(\Omega_u(t)) = \sum_k D_{mk}(\Psi_u(t)) Y_{2k}(\tilde{\Omega}_u(t)). \quad (14)$$

The motion of the vector  $\mathbf{u}$  about its quasi-equilibrium position is much faster than the motion of the local environment about the average conformation, so that it may be assumed that  $\Psi_u$  is essentially constant during the time  $t \approx \tau_u^f \ll \tau_u^s$  during which  $\tilde{\Omega}_u$  fluctuates about its quasi-equilibrium value. At longer times,  $\tau_u^f \ll t \approx \tau_u^s$ ,  $\Psi_u$  moves about its equilibrium value. Therefore, the transition probability  $P(\Omega_u, t | \Omega_{u0}, 0) = P(\Psi_u; \tilde{\Omega}_u, t | \Psi_{u0}; \tilde{\Omega}_{u0}, 0)$  may be factorized approximately as:

$$P(\Psi_u, \tilde{\Omega}_u, t | \Psi_{u0}, \tilde{\Omega}_{u0}, 0) \approx P(\Psi_u, t | \Psi_{u0}, 0) P_{\Psi_u}(\tilde{\Omega}_u, t | \tilde{\Omega}_{u0}, 0). \quad (15)$$

Note that in Eq. (15) the transition probability from  $\tilde{\Omega}_u(0)$  to  $\tilde{\Omega}_u(t)$  depends on the instantaneous orientation  $\Psi_u(t)$  of the environment. Therefore, the average  $\langle Y_{2m}(\Omega_u(t)) \rangle$  is:

$$\langle Y_{2m}(\Omega_u(t)) \rangle = \sum_k \int d\Psi_{u0} d\Psi_u d\tilde{\Omega}_{u0} d\tilde{\Omega}_u D_{mk}(\Psi_u) Y_{2k}(\tilde{\Omega}_u) P(\Psi_u, t | \Psi_{u0}, 0) P_{\Psi_u}(\tilde{\Omega}_u, t | \tilde{\Omega}_{u0}, 0) P(\Psi_{u0}; \tilde{\Omega}_{u0}). \quad (16)$$

Assuming that the initial orientations are independent:

$$P(\Psi_{u0}, \tilde{\Omega}_{u0}) = P(\Psi_{u0}) P(\tilde{\Omega}_{u0}) \quad (17)$$

the average in Eq. (16) is:

$$\langle Y_{2m}(\Omega_u(t)) \rangle = \sum_k \int d\Psi_{u_0} d\Psi_u D_{mk}(\Psi_u) P(\Psi_u, t | \Psi_{u_0}, 0) P(\Psi_{u_0}) \langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(t)} \quad (18)$$

with:

$$\langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(t)} = \int d\tilde{\Omega}_u d\tilde{\Omega}_{u_0} Y_{2k}(\tilde{\Omega}_u) P_{\Psi_u}(\tilde{\Omega}_u, t | \tilde{\Omega}_{u_0}, 0) P(\tilde{\Omega}_{u_0}). \quad (19)$$

In order to obtain analytical results, one may assume that both processes can be described in terms of a strong collision model. Thus, the transition probability  $P_{\Psi_u}(\tilde{\Omega}_u, t | \tilde{\Omega}_{u_0}, 0)$  is given by:

$$P_{\Psi_u(t)}(\tilde{\Omega}_u, t | \tilde{\Omega}_{u_0}, 0) = P_{\Psi_u(t)}^{\text{eq}}(\tilde{\Omega}_u) + (\delta(\tilde{\Omega}_u - \tilde{\Omega}_{u_0}) - P_{\Psi_u(t)}^{\text{eq}}(\tilde{\Omega}_u)) e^{-t/\tau_u^f}. \quad (20)$$

Thus at short times, i.e., for  $t \ll \tau_u^f$ , one has approximately:

$$\langle Y_{2m}(\Omega_u(t)) \rangle \approx \int \sum_k d\Psi_{u_0} D_{mk}(\Psi_u(0)) P(\Psi_{u_0}) [\langle Y_{2k}(\tilde{\Omega}_u(0)) \rangle e^{-t/\tau_u^f} + \langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(0)}^{\text{eq}} (1 - e^{-t/\tau_u^f})] \quad (21)$$

or:

$$\langle Y_{2m}(\Omega_u(t)) \rangle \approx \sum_k \langle D_{mk}(\Psi_u(0)) \rangle \langle Y_{2k}(\tilde{\Omega}_u(0)) \rangle e^{-t/\tau_u^f} + \sum_k \langle D_{mk}(\Psi_u(0)) \rangle \langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(0)}^{\text{eq}} (1 - e^{-t/\tau_u^f}). \quad (22)$$

Defining the quasi-equilibrium average value as:

$$\langle Y_{2m}(\Omega_u(t)) \rangle^q = \sum_k \langle D_{mk}(\Psi_u(t)) \rangle \langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(t)}^{\text{eq}}. \quad (23)$$

For  $t \ll \tau_u^f$  one has:

$$\langle Y_{2m}(\Omega_u(t)) \rangle^q \approx \langle D_{mk}(\Psi_u(0)) \rangle \langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(0)}^{\text{eq}} = \langle Y_{2m}(\Omega_u(0)) \rangle^q \quad (24)$$

which is approximately constant. Therefore:

$$\langle Y_{2m}(\Omega_u(t)) \rangle \approx \langle Y_{2m}(\Omega_u(0)) \rangle e^{-t/\tau_u^f} + \langle Y_{2m}(\Omega_u(0)) \rangle^q (1 - e^{-t/\tau_u^f}). \quad (25)$$

The average  $\langle Y_{2m}(\Omega_u(t)) \rangle$  obeys the following differential equation:

$$\frac{d}{dt} \langle Y_{2m}(\Omega_u(t)) \rangle = -\frac{1}{\tau_u^f} (\langle Y_{2m}(\Omega_u(t)) \rangle - \langle Y_{2m}(\Omega_u(t)) \rangle^q). \quad (26)$$

Similarly, for  $\tau_u^f \ll t$ , the fast process has reached the quasi-equilibrium state  $\langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(t)}^{\text{eq}}$ , so that one only needs to consider the slow motions of the environment:

$$\langle Y_{2m}(\Omega_u(t)) \rangle = \sum_k \int d\Psi_{u_0} d\Psi_u D_{mk}(\Psi_u(t)) P(\Psi_u | \Psi_{u_0}) P(\Psi_{u_0}) \langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(t)}^{\text{eq}}. \quad (27)$$

If the slow fluctuations of  $\Psi_u(t)$  also obey a strong collision model, Eq. (18) takes the form:

$$\langle Y_{2m}(\Omega_u(t)) \rangle = \sum_k \int d\Psi_{u_0} d\Psi_u \langle Y_{2k}(\tilde{\Omega}_u) \rangle_{\Psi_u(t)}^{\text{eq}} D_{mk}(\Psi_u) P(\Psi_{u_0}) [P^{\text{eq}}(\Psi_u) + (\delta(\Psi_u - \Psi_{u_0}) - P^{\text{eq}}(\Psi_u)) e^{-t/\tau_u^s}] \quad (28)$$

which gives:

$$\begin{aligned} \langle Y_{2m}(\Omega_u(t)) \rangle &= \sum_k \int d\Psi_{u_0} d\Psi_u P^{\text{eq}}(\Psi_u) D_{mk}(\Psi_u) P(\Psi_{u_0}) \langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(t)}^{\text{eq}} (1 - e^{-t/\tau_u^s}) \\ &\quad + \sum_k \int d\Psi_{u_0} d\Psi_u \delta(\Psi_u - \Psi_{u_0}) D_{mk}(\Psi_u) P(\Psi_{u_0}) \langle Y_{2k}(\tilde{\Omega}_u(t)) \rangle_{\Psi_u(t)}^{\text{eq}} e^{-t/\tau_u^s}. \end{aligned} \quad (29)$$

Using the fact that at long times  $\langle Y_{2m}(\Omega_u(t)) \rangle \approx \langle Y_{2m}(\Omega_u(t)) \rangle^q$ , one finally gets:

$$\langle Y_{2m}(\Omega_u(t)) \rangle^q = \langle Y_{2m}(\Omega_u(t)) \rangle^{\text{eq}} (1 - e^{-t/\tau_u^s}) + \langle Y_{2m}(\Omega_u(0)) \rangle^q e^{-t/\tau_u^s}, \quad (30)$$

where  $\langle Y_{2m}(\Omega_u) \rangle^{\text{eq}}$  is the average value of  $Y_{2m}(\Omega_u)$  in the true equilibrium. Thus  $\langle Y_{2m}(\Omega_u(t)) \rangle^q$  obeys the differential equation:

$$\frac{d}{dt} \langle Y_{2m}(\Omega_u(t)) \rangle^q = -\frac{1}{\tau_u^f} (\langle Y_{2m}(\Omega_u(t)) \rangle^q - \langle Y_{2m}(\Omega_u(t)) \rangle^{\text{eq}}). \quad (31)$$

Using Eqs. (12), (26) and (31) one obtains the following equations for the correlation functions:

$$\frac{dC_{uv}^{\text{int}}(t)}{dt} = -\frac{1}{\tau_u^f} (C_{uv}^{\text{int}}(t) - C_{uv}^{\text{int},q}(t)) \quad (32)$$

$$\frac{dC_{uv}^{\text{int},q}(t)}{dt} = -\frac{1}{\tau_u^s} (C_{uv}^{\text{int},q}(t) - C_{uv}^{\text{int,eq}}), \quad (33)$$

where  $C_{uv}^{\text{int},q}(t)$  is the correlation function associated with the dynamics of the local environment, and  $C_{uv}^{\text{int,eq}} = S_{uv}^2$  is the cross-correlation order parameter.

$$C_{uv}^{\text{int},q}(t) = \frac{4\pi}{5} \sum_m \sum_{kk'} \langle D_{mk}(\Psi_u^s(t)) D_{mk'}^*(\Psi_v^s(0)) \rangle \langle Y_{2k}(\tilde{\Omega}_u) \rangle^{\text{eq}} \langle Y_{2k'}^*(\tilde{\Omega}_v) \rangle^{\text{eq}}, \quad (34)$$

$$S_{uv}^2 = \frac{4\pi}{5} \sum_m \sum_{kk'} \langle D_{mk}(\Psi_u^s) \rangle^{\text{eq}} \langle D_{mk'}^*(\Psi_v^s) \rangle^{\text{eq}} \langle Y_{2k}(\tilde{\Omega}_u) \rangle^{\text{eq}} \langle Y_{2k'}^*(\tilde{\Omega}_v) \rangle^{\text{eq}}. \quad (35)$$

The cross-correlation order parameter of Eq. (35) obeys the usual definition:

$$S_{uv}^2 = \lim_{t \rightarrow \infty} \frac{4\pi}{5} \sum_m \sum_{kk'} \langle D_{mk}(\Psi_u^s(t)) D_{mk'}^*(\Psi_v^s(0)) Y_{2k}(\tilde{\Omega}_u(t)) Y_{2k'}^*(\tilde{\Omega}_v(0)) \rangle. \quad (36)$$

Solving Eqs. (32), and (33), we obtain the final result for the cross-correlation function, Eq. (4), in the presence of global, fast and slow internal dynamics:

$$C_{uv}(t) = \frac{1}{5} e^{-t/\tau_c} \{ \langle P_2(\mathbf{u}(0) \cdot \mathbf{v}(0)) \rangle e^{-t/\tau_u^f} + (C_{uv}^{\text{int},q}(0) - S_{uv}^2) \frac{\tau_u^s}{\tau_u^s - \tau_u^f} (e^{-t/\tau_u^s} - e^{-t/\tau_u^f}) + S_{uv}^2 (1 - e^{-t/\tau_u^f}) \}. \quad (37)$$

We define an order parameter  $S_{uv}^{2(f)}$  for the fast motions as:

$$S_{uv}^{2(f)} = \sum_k \langle Y_{2k}(\tilde{\Omega}_u) \rangle^{\text{eq}} \langle Y_{2k'}^*(\tilde{\Omega}_v) \rangle^{\text{eq}}, \quad (38)$$

where normalization of the Wigner functions  $\sum_m D_{mk}(\Psi^s) D_{mk'}^*(\Psi^s) = \delta_{kk'}$  has been used. The cross-correlation rates that can be observed in NMR are proportional to the spectral densities  $J_{uv}(\omega)$ , which are defined as Fourier transforms of  $C_{uv}(t)$ :

$$J_{uv}(\omega) = \frac{2\pi}{5} \left\{ \frac{S_{uv}^2 \tau_c}{1 + \omega^2 \tau_c^2} + \frac{\tau_u^{f'}}{1 + \omega^2 (\tau_u^{f'})^2} \left[ \langle P_2(\mathbf{u}(0) \cdot \mathbf{v}(0)) \rangle - S_{uv}^2 - (C_{uv}^{\text{int},q}(0) - S_{uv}^2) \frac{\tau_u^s}{\tau_u^s - \tau_u^f} \right] + \frac{\tau_u^{s'}}{1 + \omega^2 (\tau_u^{s'})^2} (C_{uv}^{\text{int},q}(0) - S_{uv}^2) \frac{\tau_u^s}{\tau_u^s - \tau_u^f} \right\} \quad (39)$$

with

$$(\tau_u^{f'})^{-1} = (\tau_u^f)^{-1} + (\tau_c)^{-1}, \quad (\tau_u^{s'})^{-1} = (\tau_u^s)^{-1} + (\tau_c)^{-1}. \quad (40)$$

### 3. Autocorrelation spectral density

The expression for the order parameter  $S_{uu}^2$  follows from Eq. (36) by replacing  $\tilde{\Omega}_v(0)$  by  $\tilde{\Omega}_u(0)$  and  $\Psi_v^s = \Psi_u^s$ :

$$S_{uu}^2 = \lim_{t \rightarrow \infty} \frac{4\pi}{5} \sum_m \sum_{kk'} \langle D_{mk}(\Psi_u^s(t)) D_{mk'}^*(\Psi_u^s(0)) Y_{2k}(\tilde{\Omega}_u(t)) Y_{2k'}^*(\tilde{\Omega}_u(0)) \rangle. \quad (41)$$

In this case the initial value of the correlation function for the local environment is equal to the order parameter for the fast motions (see Eqs. (34) and (38)):

$$(S_{uu}^f)^2 \equiv C_{uu}^{\text{int},q}(0) = \sum_k \langle Y_{2k}(\tilde{S}_u) \rangle^{\text{eq}} \langle Y_{2k'}^*(\tilde{S}_u) \rangle^{\text{eq}}. \tag{42}$$

The autocorrelation spectral density  $J_{uu}(\omega)$  in the presence of global, fast and slow internal motions is thus:

$$J_{uu}(\omega) = \frac{2\pi}{5} \left\{ \frac{S_{uu}^2 \tau_c}{1 + \omega^2 \tau_c^2} + \frac{\tau_u^{f'}}{1 + \omega^2 (\tau_u^{f'})^2} \left[ 1 - S_{uu}^2 - (S_{uu}^{2(f)} - S_{uu}^2) \frac{\tau_u^s}{\tau_u^s - \tau_u^f} \right] + \frac{\tau_u^{s'}}{1 + \omega^2 (\tau_u^{s'})^2} (S_{uu}^{2(f)} - S_{uu}^2) \frac{\tau_u^s}{\tau_u^s - \tau_u^f} \right\}. \tag{43}$$

Eq. (43) can be written in a form identical to the one obtained by Clore et al. [18]:

$$J_{uu}(\omega) = \frac{2\pi}{5} \left\{ \frac{S_{uu}^2 \tau_c}{1 + \omega^2 \tau_c^2} + \frac{(1 - (\tilde{S}_{uu}^f)^2) \tau_u^{f'}}{1 + \omega^2 (\tau_u^{f'})^2} + \frac{((\tilde{S}_{uu}^f)^2 - S_{uu}^2) \tau_u^{s'}}{1 + (\omega \tau_u^{s'})^2} \right\}, \tag{44}$$

where the extended Lipari–Szabo order parameter  $(\tilde{S}^f)^2$  describing the fast dynamics of the vector  $\mathbf{u}$  (which we shall call  $(\tilde{S}_{uu}^f)^2$  for consistency of notation) relates to our definitions in the following manner:

$$(\tilde{S}_{uu}^f)^2 = \alpha S_{uu}^{2(f)} + (1 - \alpha) S_{uu}^2; \tag{45}$$

with:

$$\alpha = \frac{\tau_u^s}{\tau_u^s - \tau_u^f}. \tag{46}$$

Since  $\alpha > 1$  and  $S_{uu}^f > S_{uu}$ , it follows that  $\tilde{S}_{uu}^f > S_{uu}^f$ . Thus, the approach of Clore et al. [18] gives a higher estimate of the order parameter corresponding to fast fluctuations than our theory. The cause of such a discrepancy is that Clore et al. considered slow and fast motions as two independent processes. By contrast, Eq. (43) has been derived from a two-step model of the dynamics. As mentioned above, at short times on the order of  $\tau_u^f$ , the autocorrelation function  $C_{uu}^{\text{int}}(t)$  approaches a quasi-equilibrium value  $C_{uu}^{\text{int},q}(t)$  for some instantaneous orientation of the local environment. Then, on a slower time-scale of  $\tau_u^s$ , the function  $C_{uu}^{\text{int},q}(t)$  approaches its limit  $S_{uu}^2$ . In the limit of very small  $\tau_u^f \rightarrow 0$ ,  $\alpha \rightarrow 1$  and the order parameters for the fast motion in both the extended MF approach and the present theory become equal ( $\tilde{S}_{uu}^f \rightarrow S_{uu}^f$ ). This corresponds to the situation where fast motions are averaged at each time on the characteristic timescale of the slow motion evolution. The present theory takes into account the fact that at larger values of  $\tau_u^f$  this decoupling approximation breaks down. Note that the autocorrelation spectral density does not provide any information about slow dynamics if  $\tau_u^s \gg \tau_c$ . In this limit, according to Eq. (40),  $\tau_u^{s'} \approx \tau_c$  and Eq. (44) reduces to

$$J_{uu}(\omega) = \frac{2\pi}{5} \left\{ \frac{(\tilde{S}_{uu}^f)^2 \tau_c}{1 + \omega^2 \tau_c^2} + \frac{(1 - (\tilde{S}_{uu}^f)^2) \tau_u^{f'}}{1 + \omega^2 (\tau_u^{f'})^2} \right\}. \tag{47}$$

#### 4. Limit of vanishing correlations

In the absence of correlations between the vectors  $\mathbf{u}$  and  $\mathbf{v}$  we have, with the use of Eq. (6) and the addition theorem for spherical harmonics:

$$\langle P_2(\mathbf{u}(0) \cdot \mathbf{v}(\mathbf{0})) \rangle = C_{uv}^{\text{int},q}(0) = S_{uv}^2, \tag{48}$$

so that Eq. (39) reduces to

$$J_{uv}(\omega) = \frac{2\pi}{5} \frac{S_{uv}^2 \tau_c}{1 + \omega^2 \tau_c^2}, \tag{49}$$

independently on the relative magnitudes of  $\tau_c$ ,  $\tau_u^f$  and  $\tau_u^s$ .

If the fast fluctuations are axially symmetrical with respect to the local environment, we have  $Y_{2m}(\tilde{\Omega}_u) = Y_{20}(\tilde{\Omega}_u)\delta_{m0}$ , and, therefore, only the components  $D_{m0}(\Psi_u^s) = Y_{2m}(\Psi_u^s)$  contribute to Eq. (36). Since  $D_{m0}$  does not depend on the third Euler angle, the latter could be set to zero and the first two Euler angles represent the solid angle  $\Omega_u^s$  describing the orientation of the bond vector  $\mathbf{u}$  with respect to the local axis of the slowly fluctuating environment.

Hence, for fast axially symmetric fluctuations the cross-correlated order parameter can be written, according to Eq. (36):

$$S_{uv}^2 = S_{uu}^f S_{vv}^f (S_{uv}^s)^2, \quad (50)$$

where  $(S_{uv}^s)^2$  is given by

$$(S_{uv}^s)^2 = \frac{4\pi}{5} \sum_m (Y_{2m}(\tilde{\Omega}_u^s))^{\text{eq}} (Y_{2m}^*(\tilde{\Omega}_v^s))^{\text{eq}}. \quad (51)$$

If the slow fluctuations of the local environment are also axially symmetric with respect to its equilibrium orientation, we can apply the Wigner transformation to Eq. (51) in the same manner as above, again using the addition theorem for spherical harmonics. This will result in:

$$S_{uv}^2 = S_{uu}^f S_{vv}^f S_{uu}^s S_{vv}^s P_2(\mathbf{u}^{\text{eq}} \cdot \mathbf{v}^{\text{eq}}) \quad (52)$$

and therefore

$$J_{uv}(\omega) = \frac{2\pi}{5} \frac{S_{uu}^f S_{vv}^f S_{uu}^s S_{vv}^s P_2(\mathbf{u}^{\text{eq}} \cdot \mathbf{v}^{\text{eq}}) \tau_c}{1 + \omega^2 \tau_c^2}, \quad (53)$$

where  $\mathbf{u}^{\text{eq}}$  and  $\mathbf{v}^{\text{eq}}$  are unit vectors that define the average orientations. Here  $P_2(\mathbf{u}^{\text{eq}} \cdot \mathbf{v}^{\text{eq}})$  is a ‘structural factor’ which can be determined from independent measurements, e.g., from X-ray [24] or from NMR [25], and  $S_{uu}^f$ ,  $S_{vv}^f$ ,  $S_{uu}^s$  and  $S_{vv}^s$  describe the dynamics on different time scales. Thus if the order parameters  $S_{uu}^f$ ,  $S_{vv}^f$  have been determined from independent measurements (e.g., from autocorrelation spectral densities), the measurement of cross-correlation rates provides information on the order parameter of the slowly fluctuating environment, provided that there is no correlation between the vectors.

## 5. Cross-correlation spectral density for very slow motions of the local environment

For  $\tau_u^s \gg \tau_c$  Eq. (37) reduces to:

$$C_{uv}(t) = \frac{1}{5} e^{-t/\tau_c} \{ e^{-t/\tau_u^f} [(P_2(\mathbf{u}(0) \cdot \mathbf{v}(0))) - C_{uv}^{\text{int},q}(0)] + C_{uv}^{\text{int},q}(0) \}. \quad (54)$$

The first term in Eq. (54) describes the decay of correlations between the vectors  $\mathbf{u}$  and  $\mathbf{v}$  due to fast fluctuations for  $t \approx \tau_u^f$ . At  $t \gg \tau_u^f$  the correlation between the vectors is proportional to  $C_{uv}^{\text{int},q}(0)$ , and the decay of  $C_{uv}(t)$  is due to the global motion of the molecule. The spectral density corresponding to Eq. (54) is equal to

$$J_{uv}(\omega) = \frac{2\pi}{5} \left\{ [(P_2(\mathbf{u}(0) \cdot \mathbf{v}(0))) - C_{uv}^{\text{int},q}(0)] \frac{\tau_u^{f'}}{1 + \omega^2 (\tau_u^{f'})^2} + \frac{C_{uv}^{\text{int},q}(0) \tau_c}{1 + \omega^2 \tau_c^2} \right\}, \quad (55)$$

which for  $\tau_u^f \ll \tau_c$  reduces to

$$J_{uv}(\omega) = \frac{2\pi}{5} \frac{C_{uv}^{\text{int},q}(0) \tau_c}{1 + \omega^2 \tau_c^2}. \quad (56)$$

In this regime the spectral density depends on the quasi-static distribution of local orientations and on their correlations, given by  $C_{uv}^{\text{int},q}(0)$ . This can easily be shown for the case of axially symmetric fast fluctuations for which

$$C_{uv}^{\text{int},q}(0) = S_{uu}^f S_{vv}^f (P_2(\mathbf{u}^q \cdot \mathbf{v}^q)), \quad (57)$$

where  $\mathbf{u}^q$ ,  $\mathbf{v}^q$  are the unit vectors indicating the quasi-equilibrium orientations of the vectors  $\mathbf{u}$  and  $\mathbf{v}$ . Define  $\theta_{uv}$  as the angle between  $\mathbf{u}^q$  and  $\mathbf{v}^q$ . Then, in the ‘rigid fragment’ limit, i.e., when  $\theta_{uv}$  is time-independent, one has  $\langle P_2(\theta_{uv}) \rangle = P_2(\langle \theta_{uv} \rangle)$ , so that the corresponding spectral density can be written as:

$$J_{uv}^{\text{rigid}}(\omega) = \frac{2\pi}{5} \frac{S_{uu}^f S_{vv}^f P_2(\cos(\theta_{uv})) \tau_c}{1 + \omega^2 \tau_c^2}. \quad (58)$$

Eq. (58) can be compared with Eq. (53), which is valid in the absence of correlations. A remarkable feature of the spectral density in these two distinct regimes is the proportionality of  $J_{uv}(\omega)$  to either  $P_2(\cos(\theta_{uv}))$  for rigid fragments or to  $P_2(\mathbf{u}^{\text{eq}} \cdot \mathbf{v}^{\text{eq}})$  in the absence of correlations. Neither of these proportionalities will however survive in the case of partial correlations. Note that in general the average angle between two vectors given by  $\langle\theta_{uv}\rangle$  is not equal to the angle between the average positions of the two vectors  $\mathbf{u}^{\text{eq}}$  and  $\mathbf{v}^{\text{eq}}$ .

Consider, by way of illustration, the thermal probability distribution  $G(\mathbf{u}^q, \mathbf{v}^q)$  in the form

$$G(\mathbf{u}^q, \mathbf{v}^q) \propto \exp\{-U/kT\} \quad (59)$$

with the energy  $U$  given by:

$$U = U_u[1 - P_2(\mathbf{u}^q \cdot \mathbf{u}^{\text{eq}})] + U_v[1 - P_2(\mathbf{v}^q \cdot \mathbf{v}^{\text{eq}})] + U_{uv}[1 - P_2(\cos(\theta_{uv} - \theta_0))]. \quad (60)$$

Here the first and the second terms are responsible for the alignment of  $\mathbf{u}^q$  and  $\mathbf{v}^q$  along their average directions  $\mathbf{u}^{\text{eq}}$  and  $\mathbf{v}^{\text{eq}}$ . The last term represents the coupling potential which induces correlations between  $\mathbf{u}^q$  and  $\mathbf{v}^q$ . It tends to restore the angle between  $\mathbf{u}^q$  and  $\mathbf{v}^q$  to a fixed value  $\theta_0$ , regardless of the directions of  $\mathbf{u}^{\text{eq}}$  and  $\mathbf{v}^{\text{eq}}$ . Therefore, the various terms in Eq. (60) give rise to a complicated dependence of  $J_{uv}(\omega)$  on  $\theta_{uv}$ , which in general cannot be related to  $\langle\theta_{uv}\rangle$  in a simple manner.

The limit of vanishing correlations arises if the last term in Eq. (60) is neglected. In this case,  $J_{uv}(\omega)$  is proportional to  $P_2(\mathbf{u}^{\text{eq}} \cdot \mathbf{v}^{\text{eq}})$ . On the other hand, recall that in the rigid fragment limit,  $J_{uv}(\omega)$  is proportional to  $\langle P_2(\mathbf{u}^q \cdot \mathbf{v}^q) \rangle = \langle P_2(\cos \theta_{uv}) \rangle = P_2(\cos \langle\theta_{uv}\rangle)$ . It is apparent that these simple relationships are lost if all three terms in Eq. (60) are taken into account. To illustrate this point we consider the special case where  $U_{uv} \gg U_u, U_v$ , near the regime of rigid fluctuations, where  $\theta_0$  equals  $\langle\theta_{uv}\rangle$ . The distribution function  $G$  simplifies to  $G(\theta_{uv}) \propto \exp\{-U[1 - P_2(\cos(\theta_{uv} - \langle\theta_{uv}\rangle))]/kT\}$ . Thus, we have

$$\langle P_2(\mathbf{u}^q(0) \cdot \mathbf{v}^q(0)) \rangle \equiv \langle P_2(\cos \theta_{uv}) \rangle = \int \frac{1}{2} (3 \cos^2 \theta_{uv} - 1) G(\theta_{uv}) d\theta_{uv}. \quad (61)$$

If the two vectors are rigidly held with respect to each other, i.e., if the slow motions are totally correlated:

$$G(\theta_{uv}) = \delta(\theta_{uv} - \langle\theta_{uv}\rangle), \quad (62)$$

where  $\delta(\theta_{uv} - \langle\theta_{uv}\rangle)$  is a Dirac delta function. For small fluctuations near  $\langle\theta_{uv}\rangle$ ,  $G(\theta_{uv})$  is a symmetrical function with respect to  $\langle\theta_{uv}\rangle$ , which gives:

$$\langle P_2(\cos \theta_{uv}) \rangle = (2(\cos^2(\theta_{uv} - \langle\theta_{uv}\rangle)) - 1) P_2(\cos \langle\theta_{uv}\rangle) + \frac{1}{2} (1 - \langle \cos^2(\theta_{uv} - \langle\theta_{uv}\rangle) \rangle). \quad (63)$$

This illustrates the fact that, even in a simple case,  $\langle P_2(\cos \theta_{uv}) \rangle$  is not proportional to  $P_2(\cos \langle\theta_{uv}\rangle)$ .

A more quantitative comparison of NMR cross-correlated relaxation rates with the function  $J_{uv}(\omega)$  requires specific microscopic models. However, if slow fluctuations can be assumed to be axially symmetric, the deviations of the spectral density for a given pair of vectors  $\mathbf{u}$  and  $\mathbf{v}$  from proportionalities to  $P_2(\cos \langle\theta_{uv}\rangle)$  or  $P_2(\mathbf{u}^{\text{eq}} \cdot \mathbf{v}^{\text{eq}})$  may be ascribed to partial correlation of bond vector dynamics.

## 6. Conclusion

We have proposed a framework for the analysis of the dynamics of macromolecules in the presence of internal motions, that occur on two different time scales and global isotropic tumbling. The internal dynamics were introduced as a two-step process with fast motions with respect to a quasi-equilibrium conformation of a local environment and slow fluctuations of the latter. The autocorrelated spectral density is compared to that given by a well-known extension of the model-free treatment by Clore et al. [18]. In the present work, it is found that the order parameter of the extended model-free approach, as defined by Clore et al., is overestimated. We have analyzed the cross-correlated spectral densities under the assumption of independence of the fast time scale fluctuations. Moreover, slow internal motions on a timescale longer than overall tumbling have no effect, neither on the autocorrelated spectral density (as expected) nor on the cross-correlated spectral density in the rigid fragment case. Hence, slow motions on a timescale that is slower than overall tumbling can be observed in NMR cross-correlated relaxation experiments only if it is partially or totally uncorrelated. This work may provide a way to extract information about slow motions from experimental NMR data.

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