

Determination of RNA Sugar Pucker Mode from Cross-Correlated Relaxation in Solution NMR Spectroscopy

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Cross-correlated relaxation provides precious structural and dynamic information. Recently, cross-correlation rates were actively exploited to study protein structure and dynamics.^{1–7} Methods based on cross-correlated relaxation can also be extended to oligonucleotides and thus provide additional information to that obtained from the measurement of NOEs and ³J coupling constants.^{2,8,9} Moreover, cross-correlation rates which linearly depend on the overall rotational correlation time, τ_c , appear an attractive tool to study molecules with increasing molecular weight. We propose here a method, to accurately measure carbon–proton dipole–dipole cross-correlated relaxation rates, and to determine from them the sugar-puckering modes in nucleic acids.

We focus on the rate $\Gamma_{C_i H_i, C_j H_j}^c$ that arises from cross-correlated relaxation between the dipolar interactions of two distinct carbon–proton spin pairs ($C_i H_i$ and $C_j H_j$) and is given by

$$\Gamma_{C_i H_i, C_j H_j}^c = \frac{2}{5} \frac{\gamma_H^2 \gamma_C^2}{r_{C_i H_i}^3 r_{C_j H_j}^3} \left(\frac{\mu_0}{4\pi} \right)^2 \hbar^2 (S_{ij}^c)^2 \left(\frac{3 \cos^2 \theta_{ij} - 1}{2} \right) \tau_c \quad (1)$$

where γ_H , γ_C are the magnetogyric ratios, μ_0 is the susceptibility of the vacuum, $r_{C_i H_i}$ and $r_{C_j H_j}$ are the carbon–proton distances, \hbar is the Planck constant, S_{ij}^c is an order parameter taking internal mobility of the dipole tensors of $C_i H_i$ and $C_j H_j$ into account, θ_{ij} is the projection angle between these dipole tensors, which are oriented parallel to the respective carbon–proton bond vectors, and τ_c is the overall correlation time.

Cross-relaxation due to $\Gamma_{C_i H_i, C_j H_j}^c$ mediates coherence transfer between double and zero quantum coherence $4H_{iz}C_{ix}C_{jy}$ and $4H_{jz}C_{jx}C_{iy}$. In the proposed *quantitative* Γ -HCCH NMR experiment, (Figure 1) after frequency labeling C_i (with ω_{C_i}), double and zero quantum coherence DQ/ZQ $4H_{iz}C_{ix}C_{jy}$ is created at time point a . During the mixing time $\tau_M = n/J_{CC}$ (with n integer), the (DQ/ZQ) operator $4H_{iz}C_{ix}C_{jy}$ can only be created through cross-correlated relaxation due to $\Gamma_{C_i H_i, C_j H_j}^c$. Evolution of chemical shift and heteronuclear scalar coupling is refocused for $\Delta' = 0$. The latter operator is then picked up in a reverse manner, and it gives

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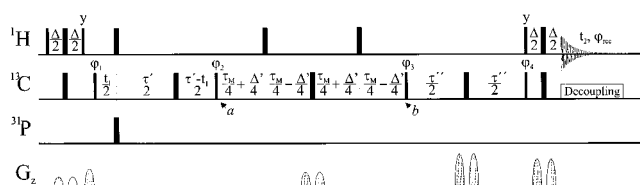


Figure 1. Pulse sequence of the 2D *quantitative* Γ -HCCH. Narrow and thick bars represent 90° and 180° pulses. The default phase for pulses is x . $\Delta' = 0$ ms for the *quantitative* Γ -HCCH-cross experiment and $\Delta' = 3.36$ ms for the *quantitative* Γ -HCCH-reference experiment. $\Delta = 3.2$ ms, $\tau' = \tau'' = 1/(4J_{CC}) = 6.25$ ms, $\tau_M = 1/(J_{CC}) = 25$ ms. ¹³C-decoupling was applied during acquisition with $\gamma B_1/2\pi = 2.5$ kHz. The relaxation delay was 1.5 s. The experiments were performed on a BRUKER DRX600 with a ¹H,¹³C,³¹P-TXI-probe with z gradients. Quadrature detection in ω_1 was achieved by States-TPPI phase incrementation of phase φ_1 . 128 scans per t_1 (34 complex points, spectral width: 6024 Hz) increment were recorded with 2K points in t_2 (spectral width: 6010 kHz). The total time for one experiment was 3.5 h. The experiment was also implemented in the 3D version by including evolution of proton chemical shift in the first INEPT step. The phase cycle employed was: $\varphi_1 = x, -x$; $\varphi_2 = x, x, x, x, -x, -x, -x, -x$; $\varphi_3 = x, x, x, x, x, x, x, -x, -x, -x, -x, -x, -x, -x, -x$; $\varphi_4 = x, x, -x, -x$; $\varphi_{rec} = x, -x, -x, x, -x, x, x, -x, -x, x, x, -x, -x, x, x$.

Table 1. Cross-Correlated Relaxation Rates $\Gamma_{C_i H_i, C_j H_j}^c$ (Hz) as Derived from the Experiment shown in Figure 1^a

	U4	U5	U6	U7
$\Gamma_{C_1 H_1, C_2 H_2}^c$	-1.8 ± 0.5	7.5 ± 0.5	5.8 ± 0.5	6.4 ± 0.3
$\Gamma_{C_3 H_3, C_4 H_4}^c$	13.9 ± 0.5	-2.0 ± 0.6	5.0 ± 0.3	-2.5 ± 0.1
$\Gamma_{C_1 H_1, C_2 H_2}^c / \Gamma_{C_3 H_3, C_4 H_4}^c$	-0.13	-3.75	+1.16	-2.56
$^3J_{H_1 H_2}$	2.6 ± 0.3	8.7 ± 0.1	6.8 ± 0.1	8.1
$^3J_{H_3 H_4}$	8.9 ± 0.2	1.6 ± 0.2	4.7 ± 0.1	3.1 ± 0.2

^a The indices i and j have been converted to the conventional sugar nomenclature. Proton–proton coupling constants $^3J_{H_1 H_2}$ and $^3J_{H_3 H_4}$ measured from *forward directed* HCC-TOCSY–CCH-E.COSY¹³ are also reported.

rise to a cross-peak at $(\omega_{H_i}, \omega_{C_j})$. Cross-correlated relaxation between any chemical shift anisotropy (CSA) and the CH dipolar interaction is refocused. Homonuclear NOE between H_i and H_j , starting from $4H_{iz}C_{ix}C_{jy}$, creates $4H_{ij}C_{xi}C_{yj}$, which does not contribute to the cross-peak. Altogether, the following transfers are achieved in the sequence:

$$4H_{iz}C_{ix}C_{jy} \rightarrow 4H_{iz}C_{ix}C_{jy} [\cosh(\Gamma_{C_i H_i, C_j H_j}^c \tau_M) \cos^2(\pi J_{CH} \Delta') - \sinh(\Gamma_{C_i H_i, C_j H_j}^c \tau_M) \sin^2(\pi J_{CH} \Delta')] - 4H_{jz}C_{jx}C_{iy} [\sinh(\Gamma_{C_i H_i, C_j H_j}^c \tau_M) \cos^2(\pi J_{CH} \Delta') + \cosh(\Gamma_{C_i H_i, C_j H_j}^c \tau_M) \sin^2(\pi J_{CH} \Delta')] \quad (2)$$

The last term gives rise to the cross-peak at $(\omega_{C_i}, \omega_{H_j})$ due to coherence transfer between $4H_{iz}C_{ix}C_{jy}$ and $4H_{jz}C_{jx}C_{iy}$. Therefore, in the experiment with $\Delta' = 0$, the intensity of the cross-peak (I^{cross}) is proportional to $\sinh(\Gamma_{C_i H_i, C_j H_j}^c \tau_M)$, whereas for $\Delta' = 1/2 J_{CH}$, the intensity of the cross-peak (I^{ref}) is proportional to $\cosh(\Gamma_{C_i H_i, C_j H_j}^c \tau_M)$. By comparing the intensity of the cross-peak measured in the two experiments one can determine

$$I^{\text{cross}} / I^{\text{ref}} = \tanh(\Gamma_{C_i H_i, C_j H_j}^c \tau_M) \quad (3)$$

The obtained relaxation rates are reported in Table 1.

The dependence of cross-correlated relaxation on the projection angle between the two dipole vectors can be exploited

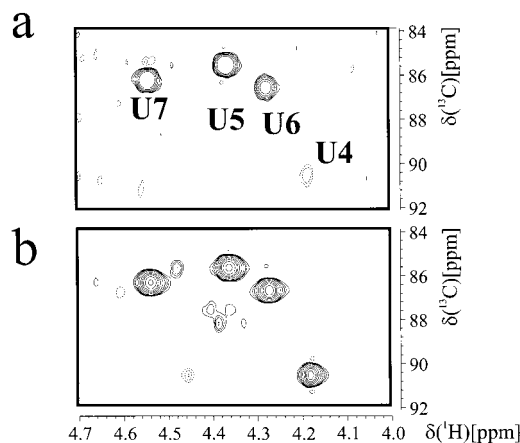


Figure 2. $H_{2'}, C_{1'}$ region of the *quantitative* Γ -HCCH cross experiment (a) and of the *quantitative* Γ -HCCH reference experiment (b). The spectra are acquired on the 5'-CGCUUUUGCG-3' hairpin, ^{13}C -labeled in the uridine residues by chemical synthesis.¹² The spectra are shown, for clarity, with different thresholds (1:4). The assignment of the cross-peaks is also shown.

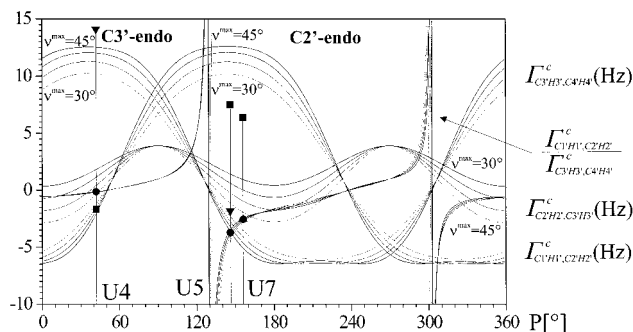


Figure 3. The calculated cross correlated relaxation rates $\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c$, $\Gamma_{C_{2'H_{2'}}, C_{3'H_{3'}}}^c$, and $\Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c$ and the ratio $\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c / \Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c$ are reported in solid lines as a function of the pseudorotation pucker P for $\tau_c = 1.5$ ns and for four values of the pucker amplitude ν^{\max} (30° , 35° , 40° , 45°). The experimental data for $\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c$ (squares), $\Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c$ (triangles) and for the ratio $\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c / \Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c$ (circles) are overlaid on the graph. The regions of the graph corresponding to $C_{2'}$ -endo and $C_{3'}$ -endo conformations,^{10,11} shown on top, are shaded in gray.

to determine local conformations in ribose rings. Figure 3 shows the cross correlation rates $\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c$, $\Gamma_{C_{2'H_{2'}}, C_{3'H_{3'}}}^c$, and $\Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c$ as a function of pseudorotation pucker (P) and amplitude (ν^{\max}).^{10,11} It can be noted that discrimination between sugar pucker modes $C_{2'}$ -endo and $C_{3'}$ -endo^{10,11} can be achieved from observation of opposite signs of the two rates $\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c$ and $\Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c$. The $\Gamma_{C_{2'H_{2'}}, C_{3'H_{3'}}}^c$ rate instead is not informative since *configuration of the two bond vectors* is *cis*. $\Gamma_{C_{2'H_{2'}}, C_{3'H_{3'}}}^c$ assumes nearly the same values in the two conformations. Therefore, analysis of the relative signs and magnitudes of the two rates $\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c$ and $\Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c$ provides a very straightforward method to distinguish between the two main sugar pucker conformations. However, as can be observed from the various plots with different ν^{\max} , the absolute values of the rates

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Table 2. Pseudorotation Pucker as Derived from Dipole–Dipole Cross-Correlated Relaxation and Coupling Constant Data^a

	U4	U5	U6	U7
P [$^\circ$]	42 $^\circ$	146 $^\circ$	31 + 13% (42 $^\circ$) 69 + 13% (146 $^\circ$)	156 $^\circ$
P [$^3J_{H,H}$]	44 $^\circ$	144 $^\circ$	32% (44 $^\circ$) 68% (144 $^\circ$)	134 $^\circ$

^a For U6, the observed averaged coupling constants and relaxation rates can be reproduced assuming a two-state conformational equilibrium between the two sugar conformations found for U4 and U5 and the relative populations are given.

are affected by variations of the sugar pucker amplitude. Figure 3 also shows the ratio

$$\frac{\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c}{\Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c} = \frac{(\mathcal{S}_{1,2}^c) 3 \cos^2 \theta_{1,2} - 1}{(\mathcal{S}_{3,4}^c) 3 \cos^2 \theta_{3,4} - 1} \quad (4)$$

which instead is less sensitive to the variations of ν^{\max} ; it does not depend on τ_c , and if the fluctuations of the respective dipole tensors are comparable, it is also less sensitive to $\mathcal{S}_{i,j}^c$. The sugar pucker conformations obtained from cross correlated relaxation through the analysis of the ratio $\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c / \Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c$ are in good (U7) to excellent (U4, U5) agreement with those derived independently from $^3J(H,H)$ coupling constant data (Table 2), which verifies the validity of the new approach. Residues U4 ($P = 42^\circ$), U5 and U7 ($P = 146^\circ, 156^\circ$) are in agreement with a single conformation. The cross-correlated relaxation rates for U6 can only be explained assuming conformational averaging as is also evident from $^3J(H,H)$ (Table 2). Therefore we propose the ratio $\Gamma_{C_{1'H_{1'}}, C_{2'H_{2'}}}^c / \Gamma_{C_{3'H_{3'}}, C_{4'H_{4'}}}^c$ as a measure of the sugar-pucker mode, while closer analysis of individual rates will reveal information on fluctuations of the sugar pucker on various time scales.

In conclusion, we have introduced a new way to determine the local geometry of ribose ring puckering which does not rely on any model-derived parametrizations. The cross-correlated relaxation rates scale linearly with the molecular size, and thus the proposed method is expected to be robust even for large RNA molecules and their complexes. This is in contrast to determination of $J(H,H)$ coupling constants in larger RNA for which peak positions are affected by cross-correlated^{14,15} relaxation rates, and only simulation of multiplet components yields correct coupling constant information.¹⁶ The measurement of dipole–dipole cross-correlated relaxation by means of this new *quantitative* Γ principle provides an unambiguous way to measure cross-correlated relaxation rates which is expected to be of general applicability in RNA, DNA, and protein NMR spectroscopy.

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Supporting Information Available: The source code to calculate cross-correlated relaxation rates as a function of correlation time, pseudorotation phase, and amplitude (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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