

Correction to "Theory of Nonrigid Rotational Motion Applied to NMR Relaxation in RNA"

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Here we aim to provide corrections to the previously published expressions. The original manuscript (OM) sought to provide a bridge between solid-state NMR-derived motional models and solution experimental results by deriving an expression for the transition probability of the orientation of an atomic bond attached to a molecule that is undergoing both (a) overall rotational diffusion and (b) an exchange between two or more different conformational states. The procedure involved finding an eigenfunction expansion of the transition probability for a single conformer, and then generalizing the time dependence of this expansion to incorporate an exchange between conformers with different rotational diffusion tensors (under the assumption of collinear diffusion tensors at the moment of exchange; for more on this, see the Discussion).

The proposed corrections apply to the latter step of finding the generalized time dependent coefficients. The OM expressions do not explicitly show the dependence of the chosen single conformer eigenfunctions on the diffusion tensor of the given conformer. An oversight on the part of the authors in this regard resulted in incorrect expressions for (a) the time evolution equation for these time-dependent coefficients (eq 18) and (b) the diffusion tensor dependences in the correlation function (eq 21). In the following sections, we remedy these equations and illustrate the results of utilizing the new expressions in our simulations of the solution relaxation times T_1 and T_2 . It will be shown that differences between the new simulation results and those in the OM are much smaller than the experimental errors in the relaxation times, implying that the discussions of the physical implications of the results presented in the OM remain entirely valid.

The first section provides a detailed rederivation of the equations in question to clarify the points of difference between the OM and the new expressions. The next section presents the new simulation results, together with graphs of "residuals" between the OM relaxation times and the relaxation times calculated using the updated expressions.

THEORY

The analysis remains the same as in the original manuscript up to eq 16. Having established an expression (eq 15 from the OM) for the transition probability of a single conformer, we need to solve the time evolution equation for the case of exchange between members of a discrete set of conformers (eq 16

from the OM):

$$\begin{aligned} \frac{\partial}{\partial t} P(\vec{\Omega}, \beta, t | \vec{\Omega}_0, \alpha) = & - \sum_{i,j=1}^3 \hat{L}_i D_{ij}^{\beta} \hat{L}_j P(\vec{\Omega}, \beta, t | \vec{\Omega}_0, \alpha) \\ & + \sum_{\gamma=1}^{N_{\text{conformers}}} R_{\beta\gamma} P(\vec{\Omega}, \gamma, t | \vec{\Omega}_0, \alpha) \end{aligned} \quad (\text{C.1})$$

In eq C.1, the diffusion tensors of any pair of exchanging conformers are assumed to be momentarily collinear during the near-instantaneous jump (see Discussion for more on this assumption).

Choosing the eigenfunctions of one conformer (labeled by V) out of the entire set as the primary basis, the transition probability for any conformer β at time t given an initial conformer α

$$\begin{aligned} P(\vec{\Omega}, \beta, t | \vec{\Omega}_0, \alpha) \\ = \sum_{l=0}^{\infty} \sum_{m=-l}^l \left(\sum_{p,p'=1}^{2l+1} c_{lm}^{pp',\beta\alpha}(t) A_{mp}^{(l)*}(\vec{\Omega}, V) A_{mp'}^{(l)}(\vec{\Omega}_0, V) \right) \end{aligned} \quad (\text{C.2})$$

where the initial condition is given by

$$P(\vec{\Omega}, \beta, 0 | \vec{\Omega}_0, \alpha) = \delta^{\beta\alpha} \delta(\vec{\Omega} - \vec{\Omega}_0) \quad (\text{C.3})$$

Note that, in formulating expression C.2, we used the fact that the operators in the time evolution equation (C.1) commute with the operators \hat{L}^2 and the lab frame \hat{L}_z , and so the most general expansion in terms of $\vec{\Omega}$ and $\vec{\Omega}_0$ is given by eq C.2 (i.e., the expansions over an additional set of l and m indices are contracted out by delta functions). Equation C.2 replaces eq 17 from the OM.

Applying the initial condition to expression C.2 leads to the following constraint on the coefficients $c_{lm}^{pp',\beta\alpha}(t)$:

$$c_{lm}^{pp',\beta\alpha}(0) = \delta^{pp'} \delta^{\beta\alpha} \quad (\text{C.4})$$

To utilize the expansion C.2 in eq C.1, we need to find the impact of the rotational diffusion operator for an arbitrary conformer β on the common eigenfunction basis. For the sake of clarity we shall use the following representation for functions of $\vec{\Omega}$:

$$A_{mp}^{(l)*}(\vec{\Omega}, V) \equiv |A_p(V)\rangle$$

The l and m indices are left implicit due to the fact that an inner product with the eigenstate of any other conformer will force

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the l and m indices to be the same, i.e.

$$\begin{aligned}\langle A_p(\beta) | A_p(V) \rangle &= \int A_{MP}^{(L)}(\vec{\Omega}, \beta) A_{mp}^{(l)*}(\vec{\Omega}, V) d\vec{\Omega} \\ &= \delta_{Ll} \delta_{Mm} \sum_{k=-L}^L f_{L,k}^{(p)}(\beta) f_{L,k}^{(p)}(V)\end{aligned}\quad (\text{C.5a})$$

having utilized the expansions of the respective $A_{mp}^{l*}(\vec{\Omega}, V)$'s in terms of Wigner matrices (see eqs 12 and 13a from the OM).

If $\beta = V$, i.e., for the same conformer, expression C.5a reduces to a product of delta functions over all indices (see eq 13b from the OM),

$$\langle A_p(\beta) | A_p(\beta) \rangle = \delta_{Ll} \delta_{Mm} \delta_{p,p} \quad (\text{C.5b})$$

Plugging expression C.2 into eq C.1,

$$\begin{aligned}\sum_{l,m,p,p'} A_{mp'}^l(\vec{\Omega}_0, V) \frac{dc_{lm}^{pp',\beta\alpha}(t)}{dt} | A_p(V) \rangle \\ = - \sum_{l,m,p,p'} A_{mp'}^l(\vec{\Omega}_0, V) c_{lm}^{pp',\beta\alpha}(t) \left(\sum_{ij=1}^3 \hat{L}_i D_{ij}^\beta \hat{L}_j \right) | A_p(V) \rangle \\ + \sum_{\gamma=1}^{N_{\text{Conformers}}} R_{\beta\gamma} \sum_{l,m,p,p'} A_{mp'}^l(\vec{\Omega}_0, V) | A_p(V) \rangle c_{lm}^{pp',\gamma\alpha}(t)\end{aligned}\quad (\text{C.6})$$

Next, we can multiply both sides by $A_{MP'}^{L*}(\vec{\Omega}_0, V)$ and integrate over all values of $\vec{\Omega}_0$, leading to

$$\begin{aligned}\sum_p \frac{dc_{LM}^{pP',\beta\alpha}(t)}{dt} | A_p(V) \rangle \\ = - \sum_p c_{LM}^{pP',\beta\alpha}(t) \left(\sum_{ij=1}^3 \hat{L}_i D_{ij}^\beta \hat{L}_j \right) | A_p(V) \rangle \\ + \sum_{\gamma=1}^{N_{\text{Conformers}}} R_{\beta\gamma} \sum_p | A_p(V) \rangle c_{LM}^{pP',\gamma\alpha}(t)\end{aligned}\quad (\text{C.7})$$

In the LHS term and the second term on the RHS, expression C.5b was used. We now left-multiply both sides of eq C.7 with $\langle A_p(V) |$ to yield

$$\begin{aligned}\sum_p \frac{dc_{LM}^{pP',\beta\alpha}(t)}{dt} \langle A_p(V) | A_p(V) \rangle \\ = - \sum_p c_{LM}^{pP',\beta\alpha}(t) \langle A_p(V) | \left(\sum_{ij=1}^3 \hat{L}_i D_{ij}^\beta \hat{L}_j \right) | A_p(V) \rangle \\ + \sum_{\gamma=1}^{N_{\text{Conformers}}} R_{\beta\gamma} \sum_p \langle A_p(V) | A_p(V) \rangle c_{LM}^{pP',\gamma\alpha}(t)\end{aligned}\quad (\text{C.8})$$

The LHS and the second term on the RHS yield delta functions over the subscripts of the eigenfunctions,

$$\begin{aligned}\frac{dc_{LM}^{pP',\beta\alpha}(t)}{dt} = - \sum_p c_{LM}^{pP',\beta\alpha}(t) \langle A_p(V) | \left(\sum_{ij=1}^3 \hat{L}_i D_{ij}^\beta \hat{L}_j \right) | A_p(V) \rangle \\ + \sum_{\gamma=1}^{N_{\text{Conformers}}} R_{\beta\gamma} c_{LM}^{pP',\gamma\alpha}(t)\end{aligned}\quad (\text{C.9})$$

The final procedure is to find an expression for the first term on the RHS of eq C.9. The eigenfunctions of any single conformer form a complete basis with respect to Euler angle space, and so we can insert the identity operator

$$\hat{I} = \sum_{L',M',Q} | A_Q(\beta) \rangle \langle A_Q(\beta) | \quad (\text{C.10})$$

on either side of the rotational diffusion operator in eq C.9:

$$\langle A_p(V) | \left(\sum_{ij=1}^3 \hat{L}_i D_{ij}^\beta \hat{L}_j \right) | A_p(V) \rangle = \sum_Q \sum_R \langle A_p(V) | A_Q(\beta) \rangle \langle A_Q(\beta) | \left(\sum_{ij=1}^3 \hat{L}_i D_{ij}^\beta \hat{L}_j \right) | A_R(\beta) \rangle \langle A_R(\beta) | A_p(V) \rangle \quad (\text{C.11})$$

Once again, the L' and M' indices are forced to be equal to L and M , respectively, by eq C.5a.

Given that the operator $(\sum_{ij=1}^3 \hat{L}_i D_{ij}^\beta \hat{L}_j)$ is diagonal in the eigenfunction basis for conformer β , we obtain

$$\langle A_p(V) | \left(\sum_{ij=1}^3 \hat{L}_i D_{ij}^\beta \hat{L}_j \right) | A_p(V) \rangle = \sum_Q \langle A_p(V) | A_Q(\beta) \rangle \lambda_Q(\beta) \langle A_Q(\beta) | A_p(V) \rangle \quad (\text{C.12})$$

We can use (C.5a) to simplify this expression in terms of known coefficients:

$$\langle A_P(V) | (\sum_{i,j=1}^3 \hat{L}_i D_{ij}^\beta \hat{L}_j) | A_P(V) \rangle = \sum_Q (\sum_{k_1=-L}^L f_{L,k_1}^{(P)}(V) f_{L,k_1}^{(Q)}(\beta)) \lambda_Q(\beta) (\sum_{k_2=-L}^L f_{L,k_2}^{(Q)}(\beta) f_{L,k_2}^{(P)}(V)) \quad (\text{C.13})$$

Thus, the equations for the time dependent coefficients are

$$\frac{dc_{LM}^{PP',\beta\alpha}(t)}{dt} = - \sum_P \sum_Q (\sum_{k_1=-L}^L f_{L,k_1}^{(P)}(V) f_{L,k_1}^{(Q)}(\beta)) \lambda_Q(\beta) (\sum_{k_2=-L}^L f_{L,k_2}^{(Q)}(\beta) f_{L,k_2}^{(P)}(V)) c_{LM}^{PP',\beta\alpha}(t) + \sum_{\gamma=1}^{N_{\text{conformers}}} R_{\beta\gamma} c_{LM}^{PP',\gamma\alpha}(t) \quad (\text{C.14})$$

In general, this results in a $(2L+1)^2 N_{\text{conformers}} \times (2L+1)^2 N_{\text{conformers}}$ matrix for the time-dependent coefficients. However, if we confine our attention to the $L = 2$ case, we notice from Table 1 in the OM that the $f_{2,k}^{(P)}$'s are diffusion tensor-dependent only for two of the diffusion eigenvalues, $P = 2$ and $P = 3$. Moreover, the subspace of coefficients for $P = 1, 4$, and 5 is orthogonal to that for $P = 2$ and 3 . This implies that

$$\sum_{k=-2}^2 f_{2,k}^{(P)}(\beta) f_{2,k}^{(Q)}(V) = \delta_{PQ} \quad \text{for } P, Q = 1, 4, 5 \quad (\text{C.15})$$

and so for $P, P' = 1, 4, 5$

$$\frac{dc_{2M}^{PP',\beta\alpha}(t)}{dt} = -\lambda_P(\beta) c_{2M}^{PP',\beta\alpha}(t) + \sum_{\gamma=1}^{N_{\text{conformers}}} R_{\beta\gamma} c_{2M}^{PP',\gamma\alpha}(t) \quad (\text{C.16a})$$

In addition, as the initial condition C.4 forces the time-dependent coefficients to be diagonal in the indices P and P' at time $t = 0$, the form of eq C.16a implies that these coefficients will remain diagonal for all time:

$$\frac{dc_{2M}^{PP,\beta\alpha}(t)}{dt} = -\lambda_P(\beta) c_{2M}^{PP,\beta\alpha}(t) + \sum_{\gamma=1}^{N_{\text{conformers}}} R_{\beta\gamma} c_{2M}^{PP,\gamma\alpha}(t) \quad \text{for } P = 1, 4, 5 \quad (\text{C.16b})$$

The equations block-diagonalize into $N_{\text{conformers}} \times N_{\text{conformers}}$ blocks for each of these three values of P .

The remaining $4N_{\text{conformers}} \times 4N_{\text{conformers}}$ subspace can be diagonalized separately (using eq C.14):

$$\frac{dc_{LM}^{PP',\beta\alpha}(t)}{dt} = - \sum_{P=2,3} \sum_{Q=2,3} (\sum_{k_1=-L}^L f_{L,k_1}^{(P)}(V) f_{L,k_1}^{(Q)}(\beta)) \lambda_Q(\beta) (\sum_{k_2=-L}^L f_{L,k_2}^{(Q)}(\beta) f_{L,k_2}^{(P)}(V)) c_{LM}^{PP',\beta\alpha}(t) + \sum_{\gamma=1}^{N_{\text{conformers}}} R_{\beta\gamma} c_{LM}^{PP',\gamma\alpha}(t) \quad \text{for } P, P' = 2, 3 \quad (\text{C.17})$$

These represent $4N_{\text{conformers}}$ equations for the coefficients $c_{2m}^{22,\beta\alpha}(t)$, $c_{2m}^{23,\beta\alpha}(t)$, $c_{2m}^{32,\beta\alpha}(t)$, and $c_{2m}^{33,\beta\alpha}(t)$. A further simplification is possible considering that in eq C.17 only those coefficients are coupled that share the same P' index. So it is possible to separately diagonalize the equations with $P' = 2$ and those with $P' = 3$ (i.e., the equations for $\{c_{2m}^{22,\beta\alpha}(t), c_{2m}^{32,\beta\alpha}(t)\}$ are separate from those for $\{c_{2m}^{23,\beta\alpha}(t), c_{2m}^{33,\beta\alpha}(t)\}$). This yields two separate $2N_{\text{conformers}} \times 2N_{\text{conformers}}$ matrix equations.

In conclusion, the $(2L+1)^2 N_{\text{conformers}} \times (2L+1)^2 N_{\text{conformers}}$ -dimensional rate equations represented by eq C.14 simplify into three $N_{\text{conformers}} \times N_{\text{conformers}}$ -dimensional matrix equations (eqs C.16b) and two $2N_{\text{conformers}} \times 2N_{\text{conformers}}$ -dimensional matrix equations (eqs C.17).

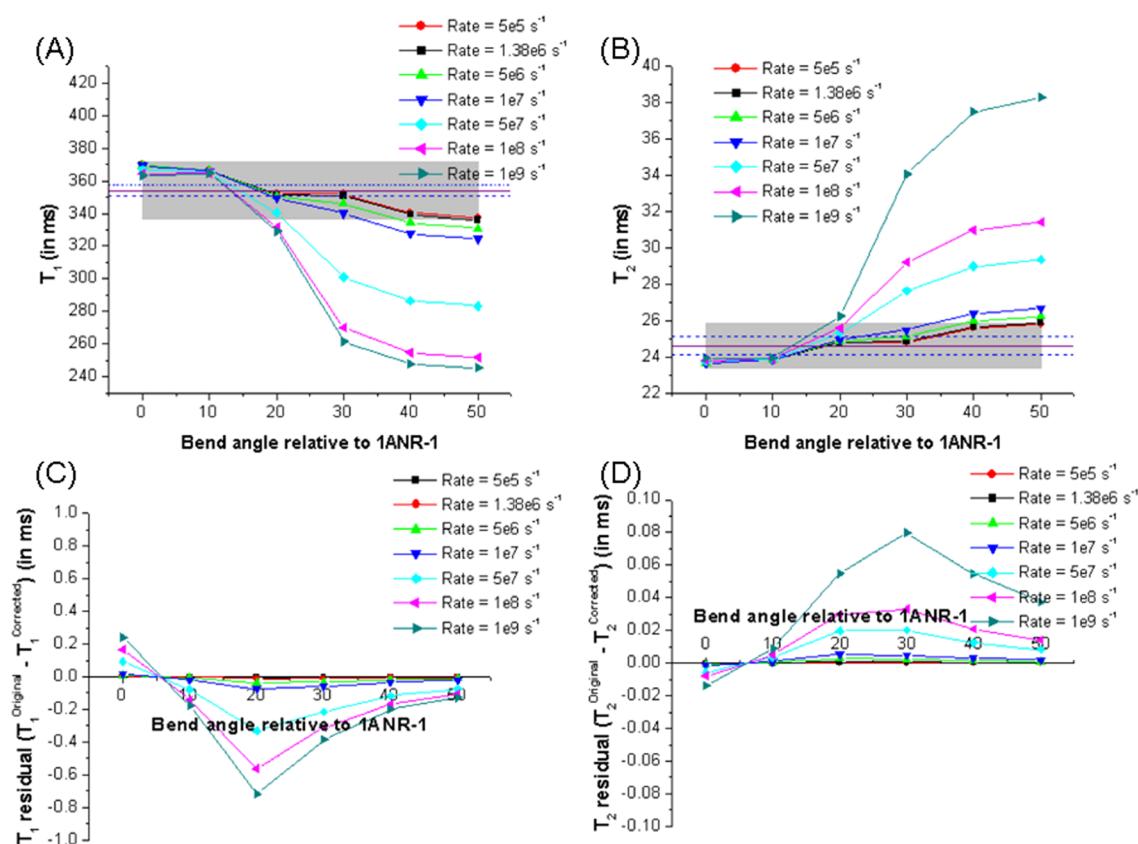


Figure 1. Relaxation times vs bend angle for structures within the Tw15 series considering various exchange rates from 5×10^5 to 1×10^9 s⁻¹: (A) T_1 times; (B) T_2 times. Experimental values are marked by solid purple lines, and the error bars are indicated by dashed blue lines. The experimental relaxation times are $T_1^{\text{expt}} = 354 \pm 3$ ms and $T_2^{\text{expt}} = 24.6 \pm 0.5$ ms. Also, shown in gray is the $\pm 5\%$ error region. The two lower panels show the differences between the relaxation times calculated using the original theory and those calculated using the corrected expressions: (C) $T_1^{\text{original}} - T_1^{\text{correction}}$ and (D) $T_2^{\text{original}} - T_2^{\text{correction}}$.

Returning to the correlation function, and to the general l case, we evaluate the following expression using expansion C.2:

$$\begin{aligned}
 \langle D_{ma}^{(l)*}(\vec{\Omega}_0) D_{ma}^{(l)}(\vec{\Omega}_t) \rangle_{\alpha\beta} &= \int d\vec{\Omega}_0 \int d\vec{\Omega} P(\vec{\Omega}_0) P(\vec{\Omega}, \beta, t | \vec{\Omega}_0, \alpha) D_{ma}^{(l)*}(\vec{\Omega}_0) D_{ma}^{(l)}(\vec{\Omega}_t) \\
 &= \int d\vec{\Omega}_0 \int d\vec{\Omega}_t P(\vec{\Omega}_0) D_{ma}^{(l)*}(\vec{\Omega}_0) D_{ma}^{(l)}(\vec{\Omega}_t) \sum_{L=0}^{\infty} \sum_{M=-L}^L \sum_{P,P'=1}^{2L+1} c_{LM}^{PP',\beta\alpha}(t) A_{MP'}^{(L)}(\vec{\Omega}_0, V) A_{MP}^{(L)*}(\vec{\Omega}_t, V) \\
 &= \sum_{L=0}^{\infty} \sum_{M=-L}^L \sum_{P,P'=1}^{2L+1} c_{LM}^{PP',\beta\alpha}(t) \left(\frac{1}{2L+1} \right) \sum_{K,K'=-L}^L f_{L,K}^{(P)}(V) f_{L,K'}^{(P')}(V) \delta_{L,K} \delta_{K',a} \delta_{Ka} \delta_{Mm} \\
 &= \left(\frac{1}{2L+1} \right) \sum_{P,P'=1}^{2L+1} c_{lm}^{PP',\beta\alpha}(t) f_{l,a}^{(P)}(V) f_{l,a'}^{(P')}(V)
 \end{aligned} \tag{C.18}$$

Equation C.18 is the updated version of eq 19 from the OM.

This leads to the final expression for the correlation function (eq 4 from the OM):

$$\langle P_i(\hat{n}(0) \cdot \hat{n}(t)) \rangle = \frac{4\pi}{2l+1} \sum_{a,a'=-l}^l \sum_{\alpha,\beta=1}^{N_{\text{conformers}}} \sum_{P,P'=1}^{2l+1} c_{lm}^{PP',\beta\alpha}(t) f_{l,a}^{(P)}(V) f_{l,a'}^{(P')}(V) Y_{la}^*(\theta_\beta, \phi_\beta) Y_{la'}(\theta_\alpha, \phi_\alpha) P_{\text{eq}}(\alpha) \tag{C.19}$$

Equation C.19 is the updated version of eq 20 from the OM.

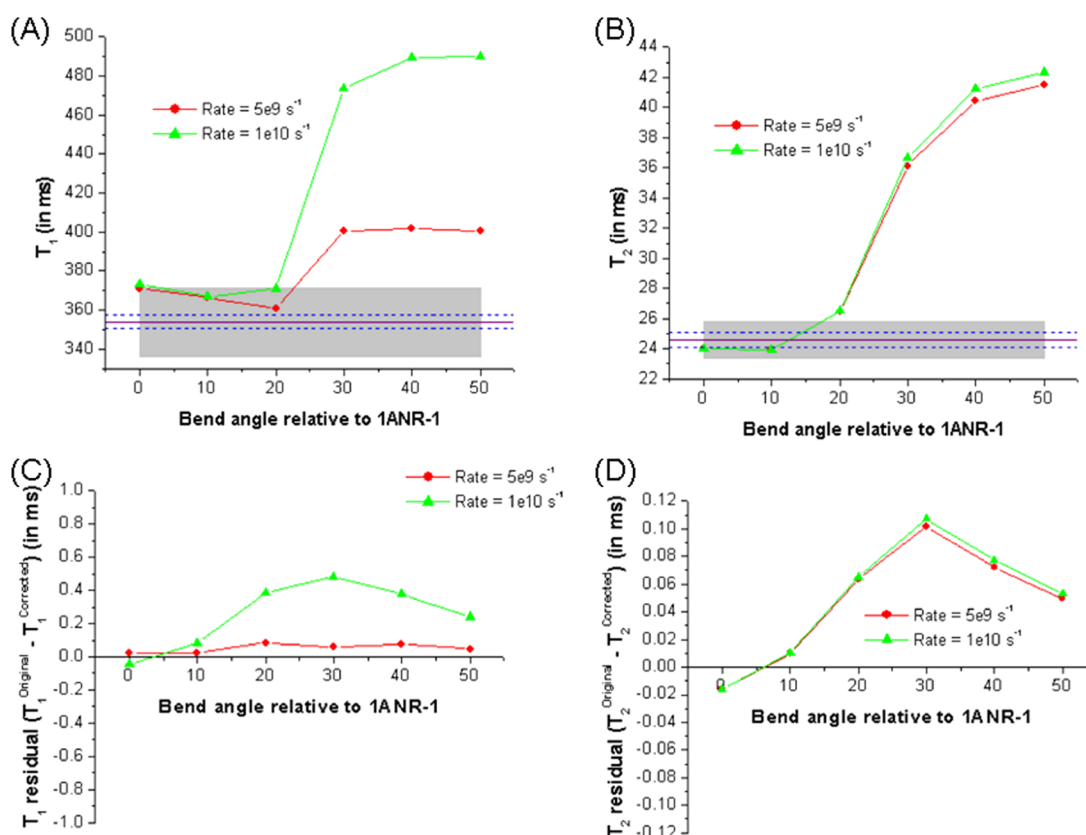


Figure 2. Relaxation times vs bend angle for structures within the Tw15 series considering the two exchange rates of 5×10^9 and $1 \times 10^{10} \text{ s}^{-1}$: (A) T_1 times; (B) T_2 times. Experimental values are marked by solid purple lines, and the error bars are indicated by dashed blue lines. The experimental relaxation times are $T_1^{\text{expt}} = 354 \pm 3 \text{ ms}$ and $T_2^{\text{expt}} = 24.6 \pm 0.5 \text{ ms}$. Also, shown in gray is the $\pm 5\%$ error region. The two lower panels show the differences between the relaxation times calculated using the original theory and those calculated using the corrected expressions: (C) $T_1^{\text{original}} - T_1^{\text{correction}}$ and (D) $T_2^{\text{original}} - T_2^{\text{correction}}$.

Again, specializing to the $l = 2$ case relevant for NMR,

$$\begin{aligned}
 C(t) &= \langle P_2(\hat{n}(0) \cdot \hat{n}(t)) \rangle \\
 &= \sum_{\alpha, \beta=1}^{N_{\text{conformers}}} \left(c_{2M}^{11, \beta \alpha}(t) \left\{ \frac{3}{4} \sin^2 \theta_\beta \sin^2 \theta_\alpha \sin(2\phi_\beta) \sin(2\phi_\alpha) \right\} + \frac{3}{2} \{ c_{2M}^{22, \beta \alpha}(t) f_{2,2}^{(2)}(V) f_{2,2}^{(2)}(V) + c_{2M}^{32, \beta \alpha}(t) f_{2,2}^{(3)}(V) f_{2,2}^{(2)}(V) \right. \right. \\
 &\quad + c_{2M}^{23, \beta \alpha}(t) f_{2,2}^{(2)}(V) f_{2,2}^{(3)}(V) + c_{2M}^{33, \beta \alpha}(t) f_{2,2}^{(3)}(V) f_{2,2}^{(3)}(V) \} \sin^2 \theta_\beta \sin^2 \theta_\alpha \cos(2\phi_\beta) \cos(2\phi_\alpha) \\
 &\quad + \frac{\sqrt{3}}{2\sqrt{2}} [\{ c_{2M}^{22, \beta \alpha}(t) f_{2,2}^{(2)}(V) f_{2,0}^{(2)}(V) + c_{2M}^{32, \beta \alpha}(t) f_{2,2}^{(3)}(V) f_{2,0}^{(2)}(V) + c_{2M}^{23, \beta \alpha}(t) f_{2,2}^{(2)}(V) f_{2,0}^{(3)}(V) \\
 &\quad + c_{2M}^{33, \beta \alpha}(t) f_{2,2}^{(3)}(V) f_{2,0}^{(3)}(V) \} \sin^2 \theta_\beta \cos(2\phi_\beta) (3 \cos^2 \theta_\alpha - 1) + \{ c_{2M}^{22, \beta \alpha}(t) f_{2,0}^{(2)}(V) f_{2,2}^{(2)}(V) + c_{2M}^{32, \beta \alpha}(t) f_{2,0}^{(3)}(V) f_{2,2}^{(2)}(V) \\
 &\quad + c_{2M}^{23, \beta \alpha}(t) f_{2,0}^{(2)}(V) f_{2,2}^{(3)}(V) + c_{2M}^{33, \beta \alpha}(t) f_{2,0}^{(3)}(V) f_{2,2}^{(3)}(V) \} \sin^2 \theta_\alpha \cos(2\phi_\alpha) (3 \cos^2 \theta_\beta - 1)] \\
 &\quad + \frac{1}{4} \{ c_{2M}^{22, \beta \alpha}(t) f_{2,0}^{(2)}(V) f_{2,0}^{(2)}(V) + c_{2M}^{32, \beta \alpha}(t) f_{2,0}^{(3)}(V) f_{2,0}^{(2)}(V) + c_{2M}^{23, \beta \alpha}(t) f_{2,0}^{(2)}(V) f_{2,0}^{(3)}(V) + c_{2M}^{33, \beta \alpha}(t) f_{2,0}^{(3)}(V) f_{2,0}^{(3)}(V) \} \\
 &\quad \cdot (3 \cos^2 \theta_\beta - 1)(3 \cos^2 \theta_\alpha - 1) + c_{2M}^{44, \beta \alpha}(t) \left\{ \frac{3}{4} \sin(2\theta_\beta) \sin(2\theta_\alpha) \sin(\phi_\beta) \sin(\phi_\alpha) \right\} \\
 &\quad + c_{2M}^{55, \beta \alpha}(t) \left\{ \frac{3}{4} \sin(2\theta_\beta) \sin(2\theta_\alpha) \cos(\phi_\beta) \cos(\phi_\alpha) \right\} \Big) P_{\text{eq}}(\alpha)
 \end{aligned} \tag{C.20}$$

This is the final update to expressions in the OM, where eq C.20 replaces eq 21 in the OM. Note that this expression differs from that in the original manuscript only in the $P = 2, 3$ cases and includes the cross-terms $c_{2M}^{23, \beta \alpha}(t)$ and $c_{2M}^{32, \beta \alpha}(t)$.

It is worth briefly mentioning the numerical procedures we used for solving the equations for the time-dependent coefficients. For eqs C.16b, we set up the $N_{\text{conformers}} \times N_{\text{conformers}}$ evolution matrices on the RHS for the coefficients

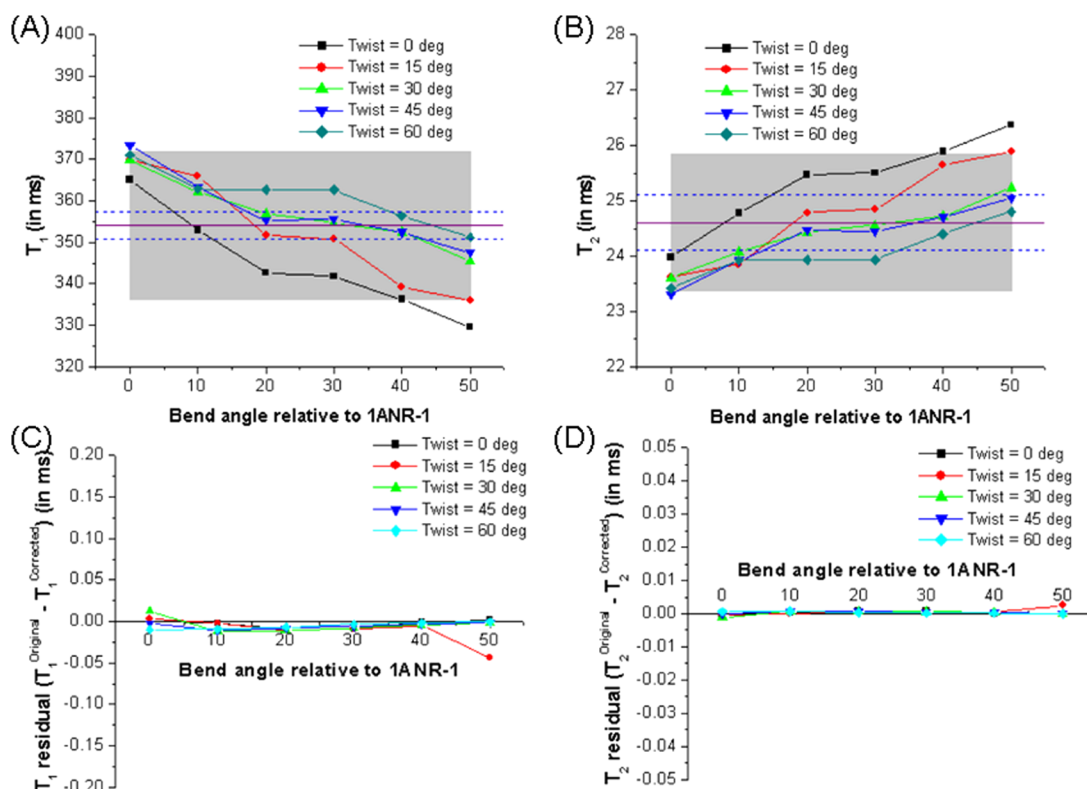


Figure 3. Relaxation times vs bend angle for structures with various degrees of positive twist about the upper helix, ranging from 0° to $+60^\circ$: (A) T_1 times; (B) T_2 times. Experimental values are marked by solid purple lines, and the error bars are indicated by dashed blue lines. The experimental relaxation times are $T_1^{\text{expt}} = 354 \pm 3$ ms and $T_2^{\text{expt}} = 24.6 \pm 0.5$ ms. Also, shown in gray is the $\pm 5\%$ error region. The two lower panels show the differences between the relaxation times calculated using the original theory and those calculated using the corrected expressions: (C) $T_1^{\text{original}} - T_1^{\text{correction}}$ and (D) $T_2^{\text{original}} - T_2^{\text{correction}}$.

(for each value of P), and then solved for the eigenvalues and eigenvectors. For the initial condition C.4, it can easily be shown that

$$c_{2M}^{PP,\beta\alpha}(t) = (\tilde{T} e^{-\tilde{\Lambda}t} \tilde{T}^{-1})_{\beta\alpha} \quad (\text{C.21})$$

where \tilde{T} is the matrix with the eigenvectors of the evolution matrix as its columns, and $\tilde{\Lambda}$ is the diagonal matrix of eigenvalues. A similar procedure holds for the cases where $P, P' = 2, 3$. Here, we set up vectors of the form $\tilde{V}_{2,\alpha}(t) = \{c_{2m}^{22,1\alpha}(t), c_{2m}^{32,1\alpha}(t), c_{2m}^{22,2\alpha}(t), c_{2m}^{32,2\alpha}(t), \dots, c_{2m}^{22,N_{\text{conformers}}\alpha}(t), c_{2m}^{32,N_{\text{conformers}}\alpha}(t)\}^T$ for each value of α and solved the coupled equations (just as for the $P, P' = 1, 4, 5$ cases) by setting up the evolution matrix on the RHS of eq C.17 and finding its eigenvalues and eigenvectors. However, applying the initial condition to obtain an expression similar to eq C.21 requires an additional step, as the initial conditions force all the $P \neq P'$, i.e., all the even-numbered coefficients of the vector above, to be 0 at time $t = 0$. Now, if you consider the case with $P' = 3$, i.e., the solution for the vector $\tilde{V}_{3,\alpha}(t) = \{c_{2m}^{23,1\alpha}(t), c_{2m}^{33,1\alpha}(t), c_{2m}^{23,2\alpha}(t), c_{2m}^{33,2\alpha}(t), \dots, c_{2m}^{23,N_{\text{conformers}}\alpha}(t), c_{2m}^{33,N_{\text{conformers}}\alpha}(t)\}^T$, the initial condition requires all the odd-numbered coefficients to be 0. Next, we noticed that the evolution matrices for both $P' = 2$ and $P' = 3$ are the same, due to the lack of any explicit P' dependence in the matrix elements. In light of these facts, it is possible to find the combined solutions to both sets of coefficients as

$$\begin{aligned} c_{2M}^{22,\beta\alpha}(t) &= (\tilde{T} e^{-\tilde{\Lambda}t} \tilde{T}^{-1})_{2\beta-1,2\alpha-1} & c_{2M}^{32,\beta\alpha}(t) &= (\tilde{T} e^{-\tilde{\Lambda}t} \tilde{T}^{-1})_{2\beta,2\alpha-1} \\ c_{2M}^{23,\beta\alpha}(t) &= (\tilde{T} e^{-\tilde{\Lambda}t} \tilde{T}^{-1})_{2\beta-1,2\alpha} & c_{2M}^{33,\beta\alpha}(t) &= (\tilde{T} e^{-\tilde{\Lambda}t} \tilde{T}^{-1})_{2\beta,2\alpha} \end{aligned} \quad (\text{C.22})$$

$\alpha, \beta = 1, \dots, N_{\text{conformers}}$

\tilde{T} is a $2N_{\text{conformers}} \times 2N_{\text{conformers}}$ -dimensional matrix with the eigenvectors of the evolution matrix as its columns, and $\tilde{\Lambda}$ is the diagonal $2N_{\text{conformers}} \times 2N_{\text{conformers}}$ -dimensional matrix of eigenvalues.

RESULTS

We have repeated several of the simulations from the OM for TAR-RNA (Figure 1 of the OM) using the corrected equations for the time-dependent coefficients (eqs C.16b and C.17) and the correlation function (eq C.20) and present these results in the following. The relaxation times are calculated in each case for an exchange between a structure modified in one or more parameters from the lowest energy model of TAR-RNA (PDB code 1ANR),¹ labeled 1ANR-1, and 1ANR-1 itself. For comparison, the experimental relaxation times are $T_1 = 354 \pm 3$ ms and $T_2 = 24.6 \pm 0.5$ ms.²

In Figure 1, we have recalculated the solution relaxation times T_1 (Figure 1A) and T_2 (Figure 1B), for the set of structures termed as the “Tw15 series” (structures shown in Figure 2A of the OM), as a function of the conformational exchange rate. The rate is varied from 5×10^5 to 1×10^9 s⁻¹. Shown in the lower two panels are the “residuals”, i.e., the differences between the relaxation times published in the OM and the new simulation results, $T_1^{\text{original}} - T_1^{\text{correction}}$ (Figure 1C) and $T_2^{\text{original}} - T_2^{\text{correction}}$ (Figure 1D). As can be seen, the difference in results is less than 0.8 ms in magnitude for the T_1 times and less than 0.08 ms in magnitude for the T_2 times, significantly smaller than the respective experimental errors. (Compare to Figure 6 of the OM.)

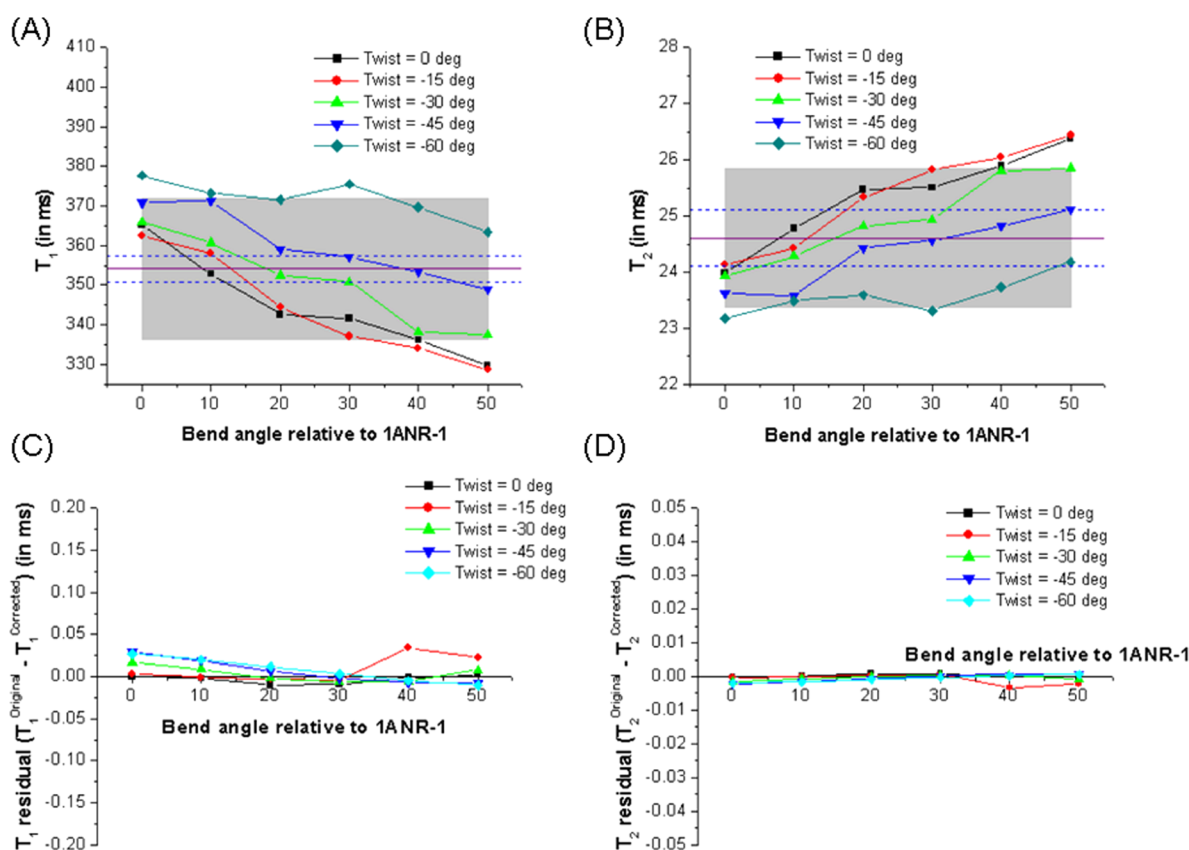


Figure 4. Relaxation times vs bend angle for structures with various degrees of negative twist about the upper helix, ranging from -60° to 0° : (A) T_1 times; (B) T_2 times. Experimental values are marked by solid purple lines, and the error bars are indicated by dashed blue lines. The experimental relaxation times are $T_1^{\text{expt}} = 354 \pm 3$ ms and $T_2^{\text{expt}} = 24.6 \pm 0.5$ ms. Also, shown in gray is the $\pm 5\%$ error region. The two lower panels show the differences between the relaxation times calculated using the original theory and those calculated using the corrected expressions: (C) $T_1^{\text{original}} - T_1^{\text{corrected}}$ and (D) $T_2^{\text{original}} - T_2^{\text{corrected}}$.

Figure 2 shows the relaxation times calculated for the Tw15 series with exchange rates of 5×10^9 and $1 \times 10^{10} \text{ s}^{-1}$. These have been shown separately, as in the OM, to show clearly the turnover in the T_1 trend as the rates increase. Whereas the T_1 values for rates considered in Figure 1 steadily decrease with increases in the rate for the same bend angle, there is a reversal of this trend at a rate between 1×10^9 and $5 \times 10^9 \text{ s}^{-1}$, as can be seen from the increasing T_1 values for the rates in Figure 2A. The T_2 values (Figure 2B) do not show this turnover. Also shown are the T_1 residuals (Figure 2C) and T_2 residuals (Figure 2D). The magnitude of the residuals is once again seen to be smaller than the experimental errors (at most ~ 0.5 ms for T_1 and ~ 0.1 ms for T_2). This is further confirmation of the earlier statement that the qualitative discussion in the OM still remains valid. (Compare to Figures 8 and 9 of the OM.)

Figures 3 and 4 show the T_1 (Figures 3A and Figure 4A) and T_2 (Figures 3B and Figure 4B) values for structures whose upper helices have been modified from 1ANR-1 to include a specified twist about the upper helical axis, in addition to the bend angles indicated on the x -axes. The upper helical axis is defined using the program 3DNA.³ Figure 3 shows the relaxation times for positive twists (in a right-hand rule sense), along with the T_1 residuals (Figure 3C) and the T_2 residuals (Figure 3D). The panels in Figure 4 show analogous results for negative twists applied to the upper helix. The residuals are well below the experimental errors in all cases shown, being at most ~ 0.03 ms for T_1 and ~ 0.003 ms for T_2 . (Compare to Figures 10 and 11 of the OM.)

DISCUSSION

Although we have not redone the simulations for all the various cases considered in the OM, we believe that the range of parameters considered here do show sufficiently that numerically the original theory and the corrected expressions do not vary significantly enough to alter the qualitative arguments presented in the OM. However, it is important to note that such may not be the case for a set of structures that may deviate substantially from cylindricity, or for an exchange between structures that may have very different diffusion tensors. Therefore, we present the corrected version of the theory in this correction to remedy the situation.

It is worth restating the assumption, mentioned in the OM, that the exchange process considered here is such that there is no change in the orientation of the molecules during the instantaneous jump between conformers; i.e., the diffusion tensors of any pair of conformers are momentarily collinear during the jump. This assumption allows for the derivation of a relatively simple closed-form expression for the correlation function and is valid as long as the different exchanging conformers do not vary significantly. This condition of validity can be achieved in applications by considering a larger number of conformers along the trajectory between two very disparate conformers, thereby better approximating a continuum transition. For a derivation of spectral densities for the general case of noncollinear diffusion tensors at the moment of exchange, the reader may refer to the work of Ryabov et al.⁴

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