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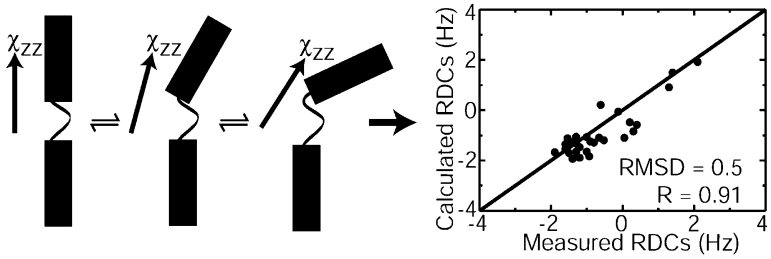

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Probing Motions between Equivalent RNA Domains Using Magnetic Field Induced Residual Dipolar Couplings: Accounting for Correlations between Motions and Alignment

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The measurement of NMR residual dipolar couplings (RDCs)[1,2] has opened a new avenue for probing motions in biomolecules over a wide range of time scales ($<10^{-3}$s).[3] Thus far, approaches for extracting structural and dynamical information from RDCs[4−6]have rested on the assumption that motions do not affect total molecular alignment. However, solute alignment in ordered media can be conformation dependent,[7]and slowly interconverting conformations can exhibit distinct alignment properties. Neglecting these effects, especially in flexible multidomain systems, can in certain cases lead to aberrations in the structural and dynamical interpretation of RDCs and diminish the ability to probe motions between domains having similar alignment propensities. Here, we introduce a new approach based on the measurement of magnetic field induced residual dipolar couplings (f(RDCs)) in nucleic acids which can take into account these correlations and demonstrate measurements of motions between two "magnetically equivalent" domains in the transactivation response element (TAR) RNA (Figure 1A).

Our approach relies on comparing magnetic susceptibility tensors (χ) measured using f(RDCs) (χ_{exp}) with values calculated (χ_{calc}) for an average conformation using tensor summation of base group susceptibilities (χ_{base}).[8,9] In Figure 1B, we depict χ-tensor frames determined independently for DI and DII in TAR (χ_{exp}) using an order tensor analysis[10,11] of $^1D_{CH}$ f(RDCs) derived from measurements of splittings at 500, 600, and 800 MHz (Figure S1) and an input TAR conformation (p(TAR)) determined previously using phage-induced RDCs (p(RDC)).[11,12] Results are depicted in the principal axis system (PAS) of χ_{calc} determined using tensor summation of base susceptibilities and the p(TAR) conformation. The overlap between the PAS of DI (in red) and DII (in blue) indicate that f(RDCs) are in excellent agreement with the global p(TAR) conformation. The overlap point also coincides with the poles of the map (DI + DII, in black), indicating excellent agreement with the calculated PAS (χ_{calc}). As shown in Table 1, the asymmetry parameter derived from this analysis (η_{exp}) is also in agreement with the total calculated value ($\eta_{calc(DI+DII)}$). In stark contrast, the magnetic susceptibility anisotropy ($\Delta\chi_{exp}$) is 51−67% smaller than the total calculated value ($\Delta\chi_{calc(DI+DII)}$) (Table 1). As shown in Figure 1C, measured RDCs (−1.9 ⇔ 2.1 Hz) are indeed uniformly attenuated relative to values predicted using χ_{calc} and the p(TAR) conformation (−3.4 ⇔ 3.4 Hz). Hence, while there is good correlation (R = 0.87), the slope of the best-fit line departs from unity (1.54), and the root-mean-square deviation (rmsd) of 1.5 Hz exceeds the uncertainty in RDCs (<0.5 Hz, Figure S1).

The results of Figure 1B argue that measured f(RDCs) are in agreement with the average orientation of DI and DII in p(TAR) (average inter-helical angle ∼49°). Inaccuracies in inter-domain

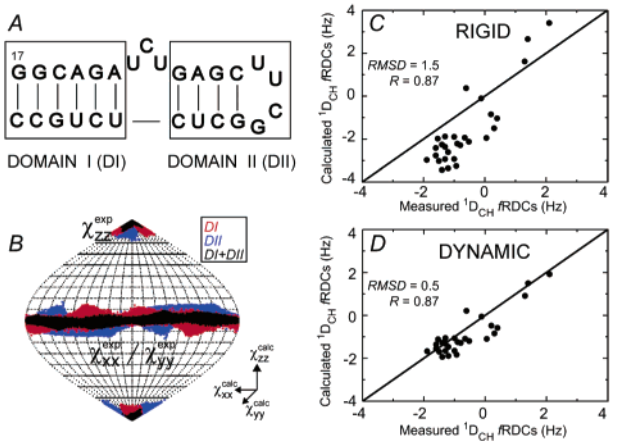

Figure 1. (A) Secondary structure of the TAR analogue used in this study (UUCG tetraloop replaces wild-type loop). (B) Sauson flaumsteed map depicting principal orientational solutions for DI (in red), DII (in blue), and DI+DII (in black) relative to a PAS calculated (χ_{calc}) using tensor summation[9] of base group susceptibilities ($\Delta\chi_{base}$)[8] and the p(TAR) conformation.[11,12] The p(TAR) conformation is composed of idealized A-form geometries for helical regions in the two stems.[11,12] The three bulge residues, which are known to be flexible in free TAR,[11] were excluded in calculating χ_{calc}. (C) Correlation plot between measured $^1D_{CH}$ f(RDCs) and values calculated using χ_{calc} and the p(TAR) conformation. (D) Correlation plot between measured $^1D_{CH}$ f(RDCs) and values calculated using χ_{calc} corrected for the effects of cone motions using eq 1. R is the correlation coefficient. The TAR NMR sample was prepared as previously described[11] and contained ∼1 mM $^{13}C/^{15}N$ labeled TAR, 15 mM sodium phosphate (pH = 6.0−6.2), 25 mM sodium sulfate and 0.1 mM EDTA. Splittings between C−H nuclei and f(RDCs) were measured in the ^{13}C dimension using spin state selective excitation/detection techniques[13] as previously described[1,11] at field strengths of 500, 600, and 800 MHz. RDCs were only considered for analysis if splittings were well resolved at all three magnetic field strengths. Random errors in RDCs estimated from the residuals in the quadratic field dependence were <0.5 Hz.

alignment of p(TAR) is therefore unlikely to be the source of the $\Delta\chi$ discrepancy. Indeed, f(RDCs) agree less with 20 previous NMR structures of TAR (1ANR)[14]wherein the inter-helical angle ranged between 36°−137° (Figure S2). While there could be local deviations from the assumed A-form geometry for helices in p(TAR), the rmsd between measured f(RDCs) and values calculated using best-fit χ-tensors are smaller than the uncertainty in RDC measurements (<0.5 Hz) and correlation coefficients are close to ideal (Table 1). The attenuations are also observed when using 20 NMR structures of TAR[14] as coordinates for helical regions in DI and DII (but keeping the global p(TAR) conformation) in determining both $\Delta\chi_{exp}$ and $\Delta\chi_{calc}$ (mean values and standard deviation over 20 structures in units of $\times 10^{-34}$ m^3/molecule: $\Delta\chi_{exp(DI)} = -147 \pm 25$; $\Delta\chi_{exp(DII)} = -144 \pm 11$; $\Delta\chi_{exp(DI+DII)} = -136 \pm 12$; $\Delta\chi_{calc(DI+DII)} = -270 \pm 19$). The $\Delta\chi_{base}$ values used in calculating

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Table 1. Experimental and Calculated χ-Tensor Parameters in TAR[a]

	rdc[b]	rmsd (Hz)[c]	R[d]	CN[e]	$\Delta\chi_{exp}$[f]	$\Delta\chi_{calc}$[g]	η_{exp}[h]	η_{calc}[i]	F_{cone}[j]	ψ_{cone} (deg)[k]
DI	13	0.4	0.93	4.7	-170 ± 20	-165	0.24 ± 0.09	0.06	0.59 ± 0.07	92 ± 10
DII	15	0.3	0.95	4.4	-176 ± 16	-160	0.21 ± 0.12	0.07	0.63 ± 0.07	86 ± 10
DI + DII	28	0.4	0.92	2.6	-156 ± 9	-286	0.15 ± 0.05	0.15	0.55 ± 0.03	97 ± 4

[a] In all cases, the experimental χ-tensor values (exp) refer to the *total* TAR χ-tensor determined using order matrix analysis of *f*(RDCs)[10] measured in DI, DII, and DI + DII[11,12] and input bond vector coordinates from the *p*(TAR) structure. Bond lengths used were C2H2 = C6H6 = C5H5 = C1′H1′ = 1.09 Å and C8H8 = 1.08 Å.[15] The calculated values (calc) were determined using tensor summation[9] of base group susceptibilities[8] and the *p*(TAR) structure.[11,12] Values for DI and DII correspond to the calculated values for the *isolated* domains while values for DI + DII correspond to the *total* calculated TAR χ-tensor. $\Delta\chi_{base}$ values were in units of $\times 10^{-34}$ (m^3/molecule): $A = -19.1$; $G = -18.3$; $C = -11.8$; and $U = -11.8$.[8] [b] Number of *f*(RDCs) used in the order matrix analysis. [c,d] Root-mean-square deviation (rmsd) and correlation coefficient (*R*) between measured *f*(RDCs) and values back calculated using the best fit χ-tensor parameters. [e] Condition numbers (CN) defined as the ratio of the largest to smallest singular value in the singular value decomposition in the χ-tensor calculations. [f] The experimental. [g] Calculated magnetic susceptibility anisotropy ($\Delta\chi = \chi_{zz} - (\chi_{xx} + \chi_{yy})/2$) in units ($\times 10^{-34}$ m^3/molecule). To account for librational motions, $\Delta\chi_{exp}$ are scaled by $1/(S_{CH}) = 1.155$, were $S^2_{CH} = 0.75$[16] is the Lipari-Szabo spin relaxation order parameter. [h] The experimental. [i] Calculated asymmetry parameter ($\eta = |(\chi_{yy} - \chi_{xx})/\chi_{zz}|$). [j,k] Values are computed using eq 1.

$\Delta\chi_{calc}$ may be in error. However, these values have successfully been used to quantitatively measure stoichiometry in multimeric DNAs[17]and in reproducing the experimental $\Delta\chi$ values in an RNA aptamer-peptide complex[18]and DNA quadruplex.[19]

Another possibility is that the assumption of a single *static* TAR structure in computing χ_{calc} is in error. We have previously reported evidence for inter-domain motions in TAR using *p*(RDCs).[11] Unlike the *p*(RDC) study, where DII appeared more ordered than DI,[11] $\Delta\chi_{exp(DI)}$ and $\Delta\chi_{exp(DII)}$ are identical within the experimental uncertainty (Table 1). This is expected regardless of inter-domain motions, as DI and DII have almost identical $\Delta\chi_{calc}$ values (Table 1). While this illustrates the difficulties in probing motions between equivalent domains, inter-domain motions can lead to the observed attenuations in $\Delta\chi_{exp}$ relative to $\Delta\chi_{calc}$. In fact, the $\Delta\chi_{exp}$ values are in better agreement with values predicted for individual domains ($\Delta\chi_{calc(DI)}$ and $\Delta\chi_{calc(DII)}$ Table 1). This suggests that χ-tensors for the two domains are nonadditive, reflecting loose coupling between the domains.

In the previous study,[11] *p*(RDCs) were interpreted as inter-domain "cone" motions,[3] assuming no correlations between motions and alignment. Here, a given domain is allowed to sample, with equal probability, all orientations relative to the mean position that are within the surface of a sphere defined by the cone radius angle ψ_{cone}. In the case of field alignment, and because the relationship between conformation and χ-tensors is known, correlations between cone motions and alignment can be explicitly taken into account. For two domains having identical anisotropies ($\Delta\chi_{DI} = \Delta\chi_{DII}$) and axially symmetric χ-tensors ($\chi_{xx} = \chi_{yy}$), it can be shown that cone motions will only lead to attenuations in $\Delta\chi_{exp}$ relative to $\Delta\chi_{calc}$ and not affect other χ-tensor parameters (Supporting Information):

$$\Delta\chi(\text{exp}) = F_{cone}\Delta\chi_{calc} \qquad (1)$$

where $F_{cone} = 0.5\cos(0.5\psi_{cone})(1 + \cos(0.5\psi_{cone}))$.[3] As shown in Figure 1D, far better agreement between measured and calculated *f*(RDCs) is obtained when scaling $\Delta\chi_{calc}$ by a factor of $F_{cone(DI+DII)} = 0.55$ (Table 1). The rmsd is reduced to 0.5 Hz, and the slope of the best-fit line now approaches unity (0.94).

The Ψ_{cone} values determined for DI, DII, and DI + DII using *f*(RDCs) range between 76° and 102° (Table 1). These motional amplitudes are larger than values previously reported using *p*(RDCs) (40−52°).[11] A number of factors can contribute to this difference. In the order tensor approach,[4] the sensitivity of the internal generalized degree of order ($\vartheta_{int} = \vartheta_{DI}/\vartheta_{DII}$) to motions is limited by the degree to which one domain is preferentially aligned, and this can lead to underestimation of motional amplitudes.[11] Considering ratios of ϑ values may also cancel the effects of local motions in both domains. In contrast, local motions would lead to underestimation of $\Delta\chi_{exp}$, overestimation of $\Delta\chi_{calc}$ and hence overestimation of ψ_{cone}. Finally, the cone model is simplistic, and other modes of motion such as twisting about individual helix axes can also contribute to the observed F_{cone} value. Nevertheless, our results together with the previous *p*RDC study[11] provide strong support for the presence of substantial directionally unbiased inter-domain motions in TAR about an average bent conformation.

In conclusion, *f*(RDCs) can be used to probe inter-domains in a manner that accounts for correlations between motions and alignment. This can allow measurements of motions between equivalent domains, as would occur in oligomeric systems, and assessment of RNA ensembles generated using NMR, molecular dynamics simulations and other techniques.

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Supporting Information Available: Field dependence of CH splittings and measured *f*(RDCs), plots of measured *f*(RDC) and values calculated using NOE structures of TAR, and derivation of eq 1. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Tolman, J. R.; Flanagan, J. M.; Kennedy, M. A.; Prestegard, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9279−9283.
(2) Tjandra, N.; Bax, A. *Science* **1997**, *278*, 1111−1114.
(3) Tolman, J. R.; Flanagan, J. M.; Kennedy, M. A.; Prestegard, J. H. *Nat. Struct. Biol.* **1997**, *4*, 292−297.
(4) Tolman, J. R.; Al-Hashimi, H. M.; Kay, L. E.; Prestegard, J. H. *J. Am. Chem. Soc.* **2001**, *123*, 1416−1424.
(5) Peti, W.; Meiler, J.; Bruschweiler, R.; Griesinger, C. *J. Am. Chem. Soc.* **2002**, *124*, 5822−5833.
(6) Tolman, J. R. *J. Am. Chem. Soc.* **2002**, *124*, 12020−12030.
(7) Zweckstetter, M.; Bax, A. *J. Am. Chem. Soc.* **2000**, *122*, 3791−3792.
(8) Bastiaan, E. W.; MacLean, C. *NMR−Basic Princ. Prog.* **1990**, *25*, 17−43.
(9) Tjandra, N.; Omichinski, J. G.; Gronenborn, A. M.; Clore, G. M.; Bax, A. *Nat. Struct. Biol.* **1997**, *4*, 732−738.
(10) Losonczi, J. A.; Andrec, M.; Fischer, M. W. F.; Prestegard, J. H. *J. Magn. Reson.* **1999**, *138*, 334−342.
(11) Al-Hashimi, H. M.; Gosser, Y.; Gorin, A.; Hu, W.; Majumdar, A.; Patel, D. J. *J. Mol. Biol.* **2002**, *315*, 95−102.
(12) Al-Hashimi, H. M.; Gorin, A.; Majumdar, A.; Gosser, Y.; Patel, D. J. *J. Mol. Biol.* **2002**, *318*, 637−649.
(13) Meissner, A.; Sorensen, O. W. *J. Magn. Reson.* **1999**, *139*, 439−442.
(14) Aboul-ela, F.; Karn, J.; Varani, G. *Nucleic Acids Res.* **1996**, *24*, 3974−3981.
(15) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 2309−2309.
(16) Fiala, R.; Czernek, J.; Sklenar, V. *J. Biomol. NMR* **2000**, *16*, 291−302.
(17) Al-Hashimi, H. M.; Tolman, J. R.; Majumdar, A.; Gorin, A.; Patel, D. J. *J. Am. Chem. Soc.* **2001**, *123*, 5806−5807.
(18) Al-Hashimi, H. M.; Gorin, A.; Majumdar, A.; Patel, D. J. *J. Am. Chem. Soc.* **2001**, *123*, 3179−3180.
(19) Al-Hashimi, H. M.; Majumdar, A.; Gorin, A.; Kettani, A.; Skripkin, E.; Patel, D. J. *J. Am. Chem. Soc.* **2001**, *123*, 633−640.

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