Determining Stoichiometry in Homomultimeric Nucleic Acid Complexes Using Magnetic Field Induced Residual Dipolar Couplings

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Nucleic acid oligomerization is an important step for driving formation of structural elements that are involved in a variety of recognition events. Higher-order DNA architectures are implicated as recognition motifs in elements of telomeric, centromeric, and triplet disease sequences,[1] while homodimerization of retroviral RNA into "duplex" and "kissing" complexes plays an important role in various stages of viral replication and genome packaging.[2] Despite the importance of oligomerization, direct characterization of nucleic acid multimeric states by nuclear magnetic resonance (NMR) spectroscopy[3] has traditionally been difficult owing to the chemical shift degeneracy that arises in symmetric homomultimers. In addition to depriving insight into thermodynamic factors that govern nucleic acid oligomerization in solution, this degeneracy can hinder determination of multimeric stoichiometry,[4] which is of critical importance for accurate interpretation of NMR distance constraints during high-resolution structure determination.[5,6] Here, we introduce a new approach for probing *intermolecular* interactions in nucleic acids that relies on the measurement of magnetic field induced residual dipolar couplings (*fi*RDCs),[7] and demonstrate an application to the direct determination of *multimeric stoichiometry* in higher-order DNA architectures.

Under high magnetic fields (B), nucleic acid molecules assume a sufficient level of molecular alignment to allow measurement of *fi*RDCs as contributions to normally observed scalar couplings.[7] The magnitudes of observed *fi*RDCs depend quadratically on the magnetic field strength, and on the principal values of the magnetic susceptibility tensor (χ_{ii} $i = \{x,y,z\}$), which for nucleic acids are dominated by the diamagnetic susceptibilities of aromatic base groups ($\chi_{ii}^{(\text{base})}$).[7e] Because stacking interactions favor coplanar arrangement of base planes in nucleic acids, their corresponding χ-tensors tend to be close to axially symmetric ($\chi_{yy} \approx \chi_{xx}$),[6,8] with principal values (χ_{ii}) that increase approximately linearly with the total number of bases due to constructive addition of base susceptibilities (Figure 1). Thus, comparisons between experimental χ_{ii} values determined for a *multimer* ($\chi^{(m-\text{mer})}$) with corresponding values expected for a *monomer* ($\chi^{(1-\text{mer})}$) can provide a new route for the determination of multimeric stoichiometry. In what follows, we develop a framework for this determination that is independent of a priori structural information.

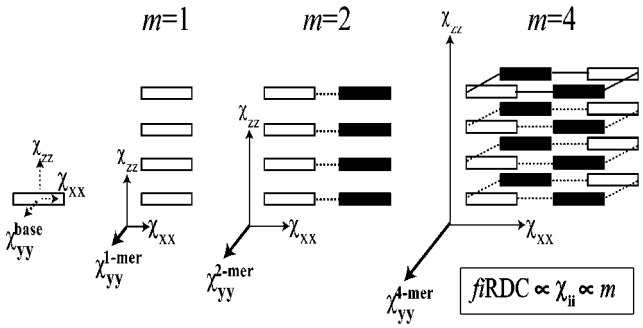

Figure 1. Sensitivity of *fi*RDC to nucleic acid multimeric stoichiometry m. Base planes are depicted as black rectangles. Black filled rectangles are used to distinguish identical monomeric units in a multimer.

In general, a principal value, $\chi_{ii}^{(m-\text{mer})}$, can be derived from the RDC value, D_{ii}, measured for an interaction vector oriented along the corresponding ith principal direction.[9] For nucleic acids, χ_{yy} is a good target for experimental determination. First, because base interaction vectors are perpendicular to their own principal anisotropy ($\chi_{zz}^{(\text{base})}$), they will preferentially be positioned within the $\chi_{yy}-\chi_{xx}$ plane of the total $\chi^{(m-\text{mer})}$ principal axis system (Figure 1). Second, as nucleic acids tend to have close to axially symmetric χ-tensors,[6,8] many RDC values measured for interaction vectors in the $\chi_{yy}-\chi_{xx}$ plane will provide a good estimate for the value of D_{yy}. Moreover, due to the negative susceptibility anisotropy expected from extended diamagnetic nucleic acids ($\chi_{zz} < 0$), the value of D_{yy} will correspond to the largest RDC value (D^+) (+ve for $^1D_{\text{NH}}$ and -ve for $^1D_{\text{CH}}$) from a given set of measurements. The observed value for D^+ hence provides an estimate for the value of D_{yy}, which in turn has a direct correspondence with the desired *total multimeric* $\chi_{yy}^{(m-\text{mer})}$ value,

$$\chi_{yy,\text{exp}}^{(m-\text{mer})} = \frac{D^+}{D_{\text{IS}}^{\text{max}}} \quad \text{and} \quad D_{\text{IS}}^{\text{max}} = \left(\frac{\mu_0}{4\pi}\right)\frac{\gamma_1\gamma_2 h}{2\pi^2 r_{\text{IS}}^3} \qquad (1)$$

where all symbols have their usual meaning. While in principle any interaction vector can be used to determine D^+, we will focus on *fi*RDCs between directly bonded imino nitrogen/proton (*fi*$^1D_{\text{NH}}$) in ^{15}N-labeled molecules.

Provided knowledge of the monomer structure, the χ_{yy} value for a monomer ($\chi_{yy}^{(1-\text{mer})}$) can be calculated from *explicit tensor summation* of individual base χ-tensors($\chi_{yy}^{(\text{base})}$).[7e] However, to enable this determination in the absence of a priori structural information, we first assume that base groups are *perfectly coplanar*. The value of $\chi_{yy}^{(1-\text{mer})}$ for a monomer composed of N residues can then be calculated from a simple scalar addition of $\chi_{yy}^{(\text{base})}$ values. This leads to an expression for $\chi_{yy,\text{exp}}^{(m-\text{mer})}$ as a function of $\chi_{yy,\text{calc}}^{(1-\text{mer})}$, and the multimeric stoichiometry m,

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(1) Patel, D. J.; Bouaziz, S.; Kettani, A.; Wang, W. *Structures of guanine-rich and cytosine-rich quadruplexes formed in vitro by telomeric,centromeric and triplet repeat disease sequences*; Oxford University Press: Oxford, 1999.
(2) Patton, J. T.; Spencer, E. *Virology* **2000**, *277*, 217−225.
(3) Mollova, E. T.; Pardi, A. *Curr. Opin. Struct. Biol.* **2000**, *10*, 298−302.
(4) (a) Gehring, K.; Leroy, J. L.; Gueron, M. *Nature* **1993**, *363*, 561−565. (b) Dardel, F.; Marquet, R.; Ehresmann, C.; Ehresmann, B.; Blanquet, S. *Nucl. Acids Res.* **1998**, 26, 3567−3571. (c) Aboulela, F.; Nikonowicz, E. P.; Pardi, A. *FEBS Lett.* **1994**, *347*, 261−264. (d) Lapham, J.; Rife, J. P.; Moore, P. B.; Crothers, D. M. *J. Biomol. NMR* **1997**, *10*, 255−262.
(5) O'Donoghue, S.; Nilges, M. *Calculation of Symmetric Oligomer Structures from NMR Data*; Plenum: New York, 1999; Vol. 17.
(6) Al-Hashimi, H. M.; Majumdar, A.; Gorin, A.; Kettani, A.; Skripkin, E.; Patel, D. J. *J. Am. Chem. Soc.* **2001**, *123*, 633−640.
(7) (a) Bothner-By, A. A. In *Encyclopedia of Nuclear Magnetic Resonance*; Grant, D. M., Harris, R. K., Eds.; Wiley: Chichester, 1995; pp 2932−2938. (b) Prestegard, J. H.; Tolman, J. R.; Al-Hashimi, H. M.; Andrec, M. In *Biological Magnetic Resonance*; Krishna, N. R., Berliner, L. J., Eds.; Plenum: New York, 1999; Vol. 17, pp 311−355. (c) Tolman, J. R.; Flanagan, J. M.; Kennedy, M. A.; Prestegard, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9279−9283. (d) Kung, H. C.; Wang, K. Y.; Goljer, I.; Bolton, P. H. J. Magn. Reson., Ser. B **1995**, 109, 323−325. (e) Bastiaan, E. W.; MacLean, C. *NMR* **1990**, *25*, 17−43.
(8) Tjandra, N.; Omichinski, J. G.; Gronenborn, A. M.; Clore, G. M.; Bax, A. *Nat. Struct. Biol.* **1997**, *4*, 732−738.
(9) Clore, G. M.; Gronenborn, A. M.; Bar, A. *J. Magn. Reson.* **1998**, *133*, 216−221.

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$$\chi_{yy,\mathrm{exp}}^{(m-\mathrm{mer})} = m \times \chi_{yy,\mathrm{calc}}^{(1-\mathrm{mer})} = m \times [\sum_{n=1}^{N} \chi_{yy,n}^{(\mathrm{base})}] \qquad (2)$$

To account for deviations from base coplanarity, we consider two limiting cases. Deviations in base planes that result from rotations about a *common* axis that is fixed in orientation relative to the molecular frame (planar disorder) will *not* affect the value χ_{yy} calculated using eq 2, but can have an impact on our ability to obtain D_{yy} from the observed value of D^+ because of departures from axial symmetry that accompany this case. Nevertheless, it is expected that such deviations will be quite small, based on calculations of asymmetry parameters ($\eta = |(\chi_{xx} - \chi_{yy})/\chi_{zz}|$) corresponding to available multimeric structures ($\eta < 0.23$, average $\eta = 0.1$, Figure S1). On the other hand, the limiting case of *random* deviations from base coplanarity will simply *quench* the true value of $\chi_{yy}^{(1-\mathrm{mer})}$ relative to the value determined using eq 2. To account for this reduction, we introduce a correction factor, Γ, which can be estimated from the observed dispersion of *fi*RDCs,

$$\Gamma = 1 - \left(\sqrt{2}\,\frac{\sigma_D}{D^+}\right) \quad \text{and} \quad \sigma_D = \sqrt{\langle D_i^2\rangle - \langle D_i\rangle^2} \qquad (3)$$

where the D_i refer to the set of dipolar coupling measurements. Consolidating eqs 1 and 2, and introducing the factor Γ, leads to a final formula for the multimeric stoichiometry, m,

$$m = \frac{10\mu_0 kT}{B^2 D_{\mathrm{IS}}^{\mathrm{max}}(r=1.0)}\,\frac{D^+ r_{\mathrm{IS}}^3}{\chi_{yy,\mathrm{calc}}^{(1-\mathrm{mer})}\Gamma S_{\mathrm{IS}}} \qquad (4)$$

where the Lipari Szabo spin relaxation order parameter, S, has also been included to account for fast librational motions.[7b] Assuming a moderately flexible nucleic acid, a value of $S^2_{\mathrm{NH}} = 0.75$ is appropriate.[10] By assuming isotropic deviations, stoichiometry values determined using eq 3 for Γ will correspond to an *upper* bound value (m^+), while a *lower* bound value (m^-) can obtained by setting $\Gamma = 1$.

Using the above procedure and experimental *fi*RDC data, stoichiometry values were determined for two DNA multimers (a) a previously characterized homodimeric DNA quadruplex (*d*DQ),[6,11] and (b) a tetrameric DNA quadruplex (*d*TQ) for which structure determination has recently been completed in our laboratory.[12] In Table 1, we show the distribution of measured fi^1D_{NH} values, and in Table 2, we report individual DNA sequences, and $\chi_{yy,\mathrm{exp}}^{(\mathrm{mol})}$, $\chi_{yy,\mathrm{calc}}^{(1-\mathrm{mer})}$, Γ and m^+/m^- values calculated using eqs 1, 2, 3, and 4, respectively. As shown in Table 2, the determined stoichiometry for *d*DQ of (1.8−2.5, $\langle m\rangle = 2.1$) argues that *d*DQ is a *homodimer*, in agreement with previous stiochometric studies and high-resolution NMR structure.[11] On the other hand the determined stoichiometry for TQ of (3.3−4.3, $\langle m\rangle = 3.8$), argues that TQ is a *homotetramer*, in agreement with the structure of this DNA molecule determined using both NOE and experimentally derived hydrogen-bonding constraints.[12] To test the robustness of our procedure, we determined the stoichiometry of the highly irregular "arrowhead" DNA structure (DA)[13] which displayed some of the largest deviations from axial symmetry ($\eta = 0.21$, Figure S1), as well as for a dimeric RNA "kissing" complex having A from helical stems (*r*DK),[14] this time using simulated RDC data (Table 1). As shown in Table 2, the determined stoichiometries are also in agreement with the dimeric architectures of these nucleic acid molecules.

Table 1. *fi*RDC s between Imino Nitrogen/Proton (fi^1D_{NH}) in Units Hz; D^+ Values (eq 1) Are Shown in Bold

*d*DQ[a]	*d*TQ[a]	*d*DA[b]	*r*DK[b]	
1.1 ± 0.2	2.0 ± 0.2	0.54	1.77	1.77
1.0 ± 0.2	1.6 ± 0.2	0.71	1.78	0.34
1.0 ± 0.2	1.3 ± 0.2	0.29	1.73	1.49
1.0 ± 0.2	2.1 ± 0.2	0.68	1.86	
0.5 ± 0.2	1.4 ± 0.2			

[a] $^1D_{\mathrm{NH}}$ values were *experimentally* measured for *d*DQ (dimeric quadruplex)[11] as previously described and reported[6] and for *d*TQ (tetrameric quadruplex)[12] using the same procedure at 18.72 T. [b] $^1D_{\mathrm{NH}}$ values were *calculated* for guanine resides in *d*DA (DNA dimeric "arrowhead")[13] and *r*DK (dimeric RNA "kissing" complex)[14] using a χ-tensor determined using *explicit* tensor summation of $\chi^{(\mathrm{base})}$,[7e] coordinates from high-resolution structures (PDB 1b3p and 1f5u, respectively), and using $r_{\mathrm{NH}} = 1.047$ Å, $S_{\mathrm{NH}} = 0.866$ and $B = 18.72$ T.

Table 2. Nucleic Acid Stoichiometry from *fi*RDC

oligonucleotide sequence	$\chi_{yy,\mathrm{exp}}^{(\mathrm{mol})}$ [a]	$\chi_{yy,\mathrm{calc}}^{(1-\mathrm{mer})}$ [b]	Γ[c]	m^+/m^- [d]	$\langle m\rangle$[e]
$dG_3T_2CAG_2$ (*d*DQ)	77 ± 14	48.7	0.73	2.4 ± 0.4/ 1.8 ± 0.3	2.1 ± 0.3
$dGCG_2AG_2AT$ (*d*DQ)	147 ± 14	51.1	0.78	4.3 ± 0.4/ 3.3 ± 0.3	3.8 ± 0.3
dG_2AG_2AT (*d*DQ)	43.3	41.1	0.68	2.1/1.4	1.8
$rG_2UG_3AGACGUC_3AC_2$ (*r*DK)	127.4	93.3	0.61	2.6/1.6	2.1

[a] Experimental values of $\chi_{yy}^{(m-\mathrm{mer})}$ (in units m^3/molecule × 10^{34}) determined using eq 1 and D^+ values from Table 1. [b] Calculated values of $\chi_{yy}^{(1-\mathrm{mer})}$ (in units m^3/molecule × 10^{34}) using eq 1 and $\chi_{yy}^{(\mathrm{base})}$ values from the literature.[7e] [c] Correction factor calculated from the distribution of $^1D_{\mathrm{NH}}$ coupling in Table 1 using eq 3. [d] Upper (m^+) and lower (m^-) values for multimeric stoichiometry calculated using eq 4, $r_{\mathrm{NH}} = 1.047$ Å and $S_{\mathrm{NH}} = 0.866$. [e] Mean stoichiometry ($(m^+ + m^-)/2$).

In conclusion, a small number of *fi*RDCs measured at high precision, can be used to accurately determine nucleic acid multimeric stoichiometry without the need for any a priori structural information or even resonance assignments. Inclusion of normalized fi^1D_{CH}[9] would enhance the accuracy of our approach, especially in reporting deviations in base planarity not accounted for by fi^1D_{NH} couplings. The measurement of *fi*RDC is therefore a general and powerful probe of nucleic acid intermolecular interaction and oligomerization.

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Supporting Information Available: Bar graph of calculated η values for 23 DNA structures, and derivation of Γ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Fiala, R.; Czernek, J.; Sklenar, V. *J. Biomol. NMR* **2000**, *16*, 291−302.
(11) Kettani, A.; Basu, G.; Gorin, A.; Majumdar, A.; Skripkin, E.; Patel, D. J. *J. Mol. Biol.* **2000**, *301*, 129−146.
(12) da Silva, M. W.; Kettani, A.; Al-Hashimi, H. M.; Majumdar, A.; Gorin, A.; Chernichenko, N.; Skripkin, E.; Patel, D. J. In preparation.
(13) Kettani, A.; Bouaziz, S.; Skripkin, E.; Majumdar, A.; Wang, W.; Jones, R. A.; Patel, D. J. *Struct. Folding Des.* **1999**, *7*, 803−815.
(14) Kim, C. H.; Tinoco, I. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9396−9401.