instrument at UMBC was purchased with support from the NIH (Grants GM42561 and AI30917 to M.F.S.).

Note Added in Proof. Additional $^1H-^{113}Cd$ scalar coupling involving the Ala(43) methyl group, which was originally attributed to incomplete cancellation in the $^1H-^{113}Cd$ HSED spectra, has been detected and confirmed in a 2D $^1H-^{113}Cd$ heteronuclear multiple quantum correlation experiment. This scalar coupling is probably due to direct orbital overlap between the methyl protons of Ala(43) and the Cys(42) sulfur (6- and 8-bond $^1H-^{113}Cd$ scalar coupling mediated by the Ala(43) NH···S hydrogen bond or by the covalent bonds, respectively, would appear implausible) and thus may have important implications for understanding intraprotein electron transfer.

Supplementary Material Available: Figures of the full 2D NOESY spectra of ^{113}Cd- and zinc-substituted *P. furiosus* rubredoxin, the downfield region of the 500-MHz 2D NOESY spectrum of ^{113}Cd(Rd), and a stacked plot of the CPMG data for ^{113}Cd(Rd) (4 pages). Ordering information is given on any current masthead page.

Minor Groove Binding of $[Ru(phen)_3]^{2+}$ to $[d(CGCGATCGCG)]_2$ Evidenced by Two-Dimensional Nuclear Magnetic Resonance Spectroscopy

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The chiral transition metal complex $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline), Figure 1, exists in two enantiomeric forms, Δ and Λ, which bind differently to DNA.[1-6] Despite extensive studies, the structural DNA-binding characteristics have remained unclear. Both minor[7-9] and major groove binding[4,8,10,11] have been suggested, and various modes of binding have been proposed, intercalation of one of the phenanthroline wings being one of them.[1,3-5,11]

Here we report the first two-dimensional NMR study on the interaction of $[Ru(phen)_3]^{2+}$ with the self-complementary decanucleotide duplex $[d(CGCGATCGCG)]_2$.[12] The results provide evidence for binding of both Δ- and Λ-$[Ru(phen)_3]^{2+}$ to the AT region in the minor groove of the oligonucleotide and strongly indicate a non-intercalative type of binding.

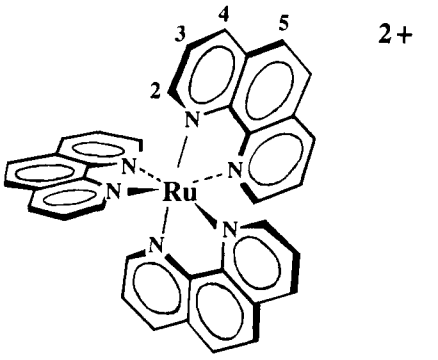

Figure 1. $[Ru(phen)_3]^{2+}$, Δ-enantiomer.

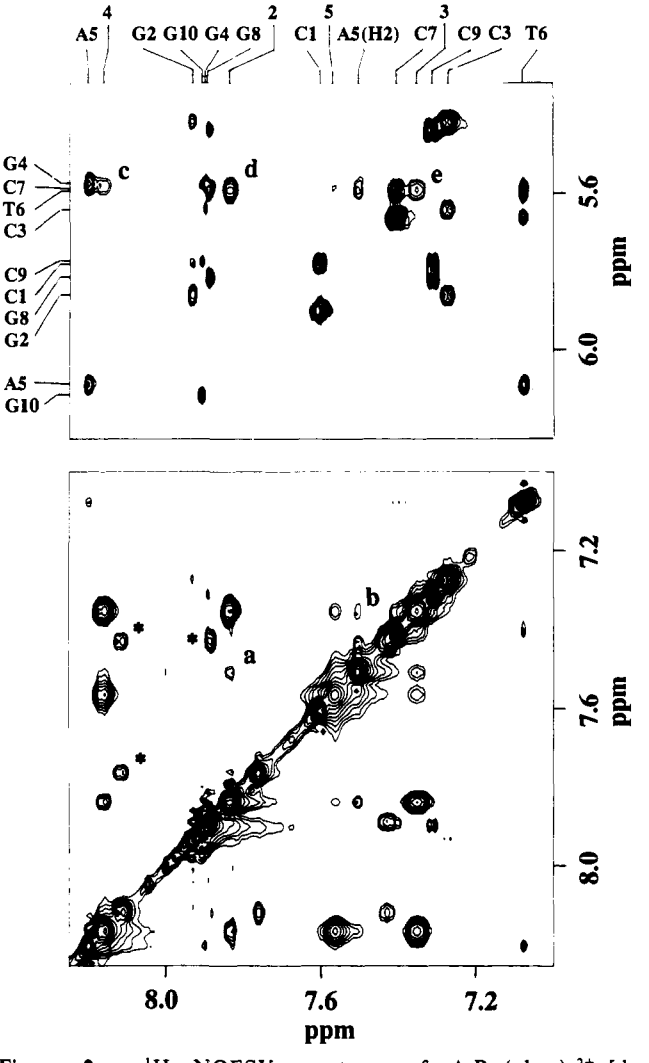

Figure 2. ^1H NOESY spectrum of Δ-$Ru(phen)_3^{2+}$-$[d(CGCGATCGCG)]_2$, molar ratio 0.3, 4 mM duplex at 41 °C, in 10 mM phosphate buffer (D_2O), uncorrected pD = 7.0. Upper panel: aromatic-H1′ region. Lower panel: aromatic-aromatic region. Cross peaks labeled as (a) 2-H2(A5), (b) H2(A5)-3, (c) 4-H1′(G4), (d) 2-H1′(G4/T6/C7), and (e) 3-H1′(T6/C7). Asterisks indicate intramolecular cross peaks from small amounts of Λ-$[Ru(phen)_3]^{2+}$ present. Assignments are indicated for the four nonequivalent protons of Δ-$[Ru(phen)_3]^{2+}$ (2, 3, 4, and 5) and for the aromatic protons H6 and H8 of the bases (horizontally) and the H1′ sugar protons (left-hand side), respectively (cf. Patel et al.[13]). The NOESY spectrum was acquired on a Bruker AM 500 spectrometer in the phase-sensitive mode with TPPI and a mixing time of 450 ms.

Selected regions of a NOESY spectrum of Δ-$[Ru(phen)_3]^{2+}$-$[d(CGCGATCGCG)]_2$ (molar ratio 0.3) are shown

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(12) $RuCl_3$ was obtained from Aldrich Chemical Company Inc. Racemic $[Ru(phen)_3]^{2+}$ was synthesized and separated into chiral isomers according to Dwyer and Gyarfas (Dwyer, F. P.; Gyarfas, E. C. *J. Proc. R. Soc. N.S.W.* **1949**, 170-173). The concentration of $[Ru(phen)_3]^{2+}$ was spectroscopically determined using a molar absorptivity of 19 000 M^{-1} cm^{-1} at 447 nm. $[d(CGCGATCGCG)]_2$ was obtained from the Department of Immunology, University of Uppsala, Sweden.
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Table I. NOESY[a] Cross Peaks between $[Ru(phen)_3]^{2+}$ and $[d(CGCGATCGCG)]_2$ at 41 °C

Δ-$[Ru(phen)_3]^{2+}$ (0.3)[b]	Λ-$[Ru(phen)_3]^{2+}$ (0.95)[b]
2-H2(A5)	2-H2(A5)
2-H1′(G4/T6/C7)[c]	2-H1′(A5)
2-H4′(C7)	2-H1′(T6)
3-H2(A5)	2-H4′(G4/T6/C7)[c]
3-H1′(T6/C7)[c]	3-H2(A5)
3-H4′(C7)	3-H1′(A5)
4-H1′(G4)	4-H1′(A5)
	4-H1′(T6)
	4-H1′(C7)
	4-H4′(G4/T6/C7)[c]
	5-H1′(T6)
	5-H1′(C7)
	5-H4′(G4/T6/C7)[c]

[a] Mixing time = 450 ms. [b] Concentration ratio (metal complex/oligonucleotide duplex). [c] Indistinguishable due to spectral overlap.

in Figure 2. The complex is in fast exchange; thus only one set of resonances is seen. A number of NOESY cross peaks between protons on the metal complex and on the oligonucleotide are observed, indicating interproton distances of less than 5 Å. Cross peaks a and b arise from dipolar interactions between the aromatic proton H2 of adenine, located at the bottom of the minor groove, and protons 2 and 3 on the phenanthroline chelates. Also the sugar protons H1′ and H4′, both facing the minor groove, exhibit NOESY cross peaks with the phenanthroline protons (cross peaks c, d, and e in the spectrum). It was found that relatively long mixing times, 450 ms, were required for the buildup of the chelate–oligonucleotide cross peaks. This is due to the exchange kinetics of the complex, which is rapid compared to the cross-relaxation rate, and to the relatively weak binding, resulting in shorter effective mixing times.

A list of observed $[Ru(phen)_3]^{2+}$–oligonucleotide cross peaks for both the Δ- and Λ-enantiomers is presented in Table I. Both enantiomers exhibit similar chelate–oligonucleotide cross-peak patterns, although more cross peaks were observed with the Λ-form, however at a higher chelate/oligonucleotide ratio. No protons located in the major groove were found to be involved in NOE interactions with either $[Ru(phen)_3]^{2+}$ enantiomer.

The sequential connectivity pattern of the oligonucleotide cross peaks is not significantly affected by the binding of $[Ru(phen)_3]^{2+}$, indicating that a B-type conformation is retained. As has previously been reported,[9] and extensive upfield shift of H2 on A5 (approximately 0.3 ppm) is observed upon addition of either Δ- or Λ-$[Ru(phen)_3]^{2+}$ to a concentration ratio of 1.0 metal complex/oligonucleotide duplex. Considerable upfield shift changes are also seen for the H1′ protons on the sugar rings of G4, A5, T6, and C7, while only slight shift changes are seen for protons in the major groove. This indicates a change in the magnetic environment in the minor groove around G4, A5, T6, and C7, most likely due to ring-current effects from closely located phenanthroline rings.

These observations demonstrate that both the Δ- and Λ-enantiomers of $[Ru(phen)_3]^{2+}$ bind in the minor groove of $[d(CGCGATCGCG)]_2$. Moreover, for both enantiomers all NOE contacts are centered around the AT step of the oligonucleotide, demonstrating that the binding preferentially occurs to this part of the sequence.

It has previously been suggested that $[Ru(phen)_3]^{2+}$ binds intercalatively to DNA, with one of the phenanthroline moieties inserted between two base pairs.[1,3,4,11] The present study supplies a number of arguments against an intercalative type of binding to $[d(CGCGATCGCG)]_2$. First, no NOESY cross peaks between either the imino protons or the aromatic protons in the major groove and the phenanthroline protons are seen. Second, no significant intensity reduction of the DNA sequential connectivity cross peaks is seen upon titration with $[Ru(phen)_3]^{2+}$, indicating that no base-pair separation occurs. Third, the binding kinetics is rapid and no line broadening is observed. This is in contrast to intercalators, which usually bind with intermediate exchange kinetics, causing line broadening of the surrounding DNA protons.

In conclusion, the NMR experiments presented show, for the first time unambiguously, minor groove binding, most likely non-intercalative, of both Δ- and Λ-$[Ru(phen)_3]^{2+}$ to $[d(CGCGATCGCG)]_2$ with the highest affinity for its AT region.

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Backbone Dynamics of Calbindin D_{9k}: Comparison of Molecular Dynamics Simulations and ^{15}N NMR Relaxation Measurements

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Recent methodological advances in NMR spectroscopy[1] have made feasible the measurement of ^{15}N and/or ^{13}C T_1 and T_2 relaxation rate constants as well as $\{^1H\}X$ NOEs for a large number of sites in macromolecules such as proteins.[2-5] For proteins in solution, both the overall molecular tumbling as well as the internal motions contribute to reorientation and therefore to NMR relaxation. Thus, spin relaxation is controlled by dynamics on the picosecond to nanosecond time scale, which makes NMR relaxation data eminently suited for testing the accuracy of molecular dynamics (MD) simulations.[6] Recently a method has been developed for generating three-dimensional solution structures from NMR data using relaxation matrix back calculations[7] that incorporate information regarding internal dynamical properties from MD simulations, rather than from experimental measurements, to improve the accuracy of the generated structures. Consequently, comparisons between MD simulations and experimental data have acquired renewed urgency.

To evaluate the capacity of protein MD simulations to reproduce dynamical behavior on the picosecond time scale, we have analyzed three MD runs of the protein calbindin D_{9k} in water that differ in force field and atom representation. Simulation U[8] uses a united atom representation for aliphatic CH_n groups, simulation A[9] uses an explicit all atom representation, and simulation N[10] uses all atoms, a reenforced hydrogen-bonding potential, and mode-separated temperature scaling.[11] The starting coordinate for all three simulations was the X-ray crystal structure of native calbindin D_{9k}.[12] The computer program MUMOD[13] performed the simula-

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