Supporting Information

TABLE S1. Comparison of base proton chemical shifts predicted by SHIFTS 4.1. for the AGGG duplex with the observed proton shifts.

Proton	Predicted order		Predicted shift (ppm)	Observed order		Observed Shift (ppm)
CH3	THY	5	1.21	THY	5	1.218
	THY	11	1.26	THY	11	1.314
	THY	26	1.52	THY	26	1.533
	THY	6	1.55	THY	6	1.606
	THY	22	1.57	THY	22	1.633
	THY	17	1.58	THY	17	1.675
AH2						
	ADE	23	6.26	ADE	23	6.945
	ADE	7	6.48	ADE	7	7.29
	ADE	12	6.87	ADE	12	7.43
	ADE	18	7.39	ADE	18	7.43
	ADE	3	7.42	ADE	24	7.545
	ADE	24	7.94	ADE	3	7.724
H6/H8						
	THY	5	7.22	THY	5	7.143
	THY	11	7.24	THY	11	7.221
	THY	22	7.29	THY	22	7.341
	THY	6	7.33	THY	6	7.341
	THY	26	7.33	THY	17	7.4
	THY	17	7.36	THY	26	7.448
	GUA	8	7.58	GU	10	7.472
	GUA	9	7.6	GU	9	7.507
	GUA	4	7.64	GU	4	7.545
	GUA	10	7.65	GU	8	7.55
	GUA	13	7.7	GU	13	7.679
	GUA	28	7.87	GU	2	7.938
	GUA	2	7.88	GU	28	7.957
	GUA	15	7.94	GU	15	7.986
	CYT	25	7.51	CYT	25	7.24
	CYT	19	7.52	CYT	19	7.29
	CYT	14	7.58	CYT	20	7.454
	CYT	20	7.6	CYT	27	7.472
	CYT	16	7.63	CYT	21	7.496
	CYT	21	7.63	CYT	16	7.529
	CYT	1	7.72	CYT	14	7.54
	CYT	27	7.72	CYT	1	7.6
	ADE	24	8.19	ADE	24	8.117
	ADE	3	8.3	ADE	3	8.147
	ADE	7	8.38	ADE	7	8.171
	ADE	23	8.38	ADE	12	8.187
	ADE	18	8.43	ADE	23	8.243
	ADE	12	8.44	ADE	18	8.302

FIGURE S1. Fe^{2+}-dependent shifting of aromatic proton resonances within the AGGG-containing duplex. The duplexed sample (0.65 mM) was in 90% H_2O/10% D_2O, 130 mM NaCl. Arrows indicate resonances that undergo significant chemical shift changes.

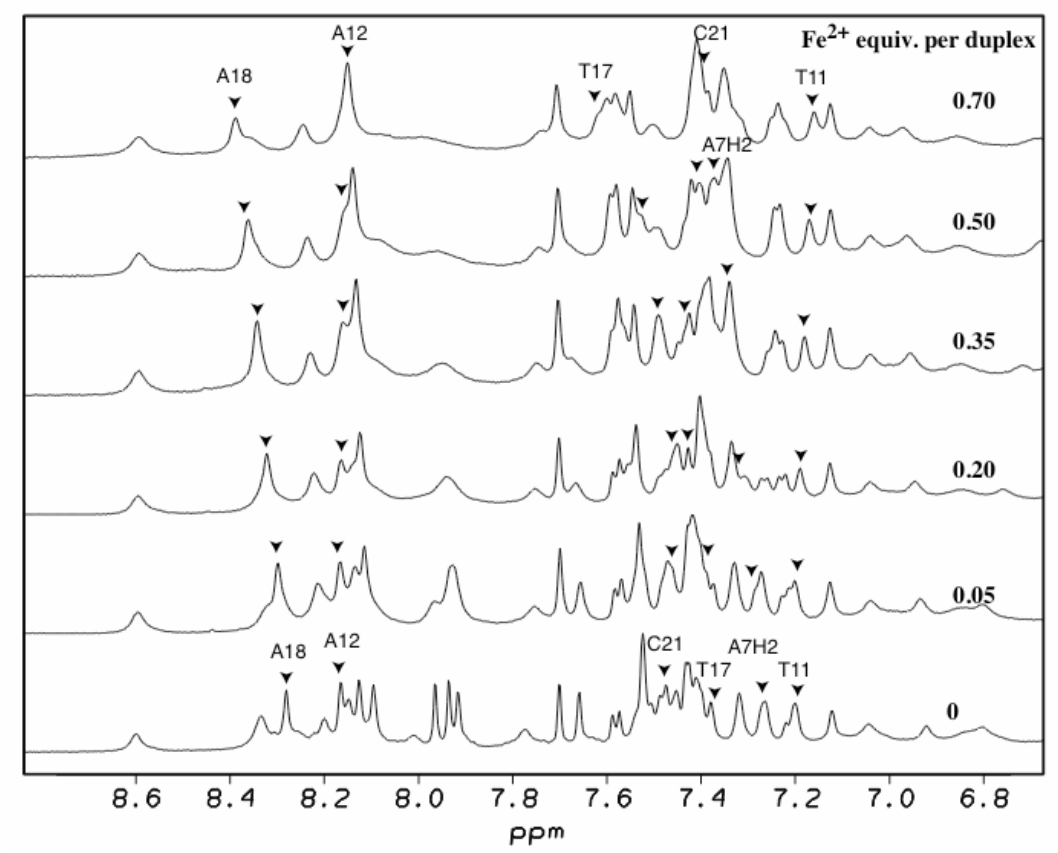
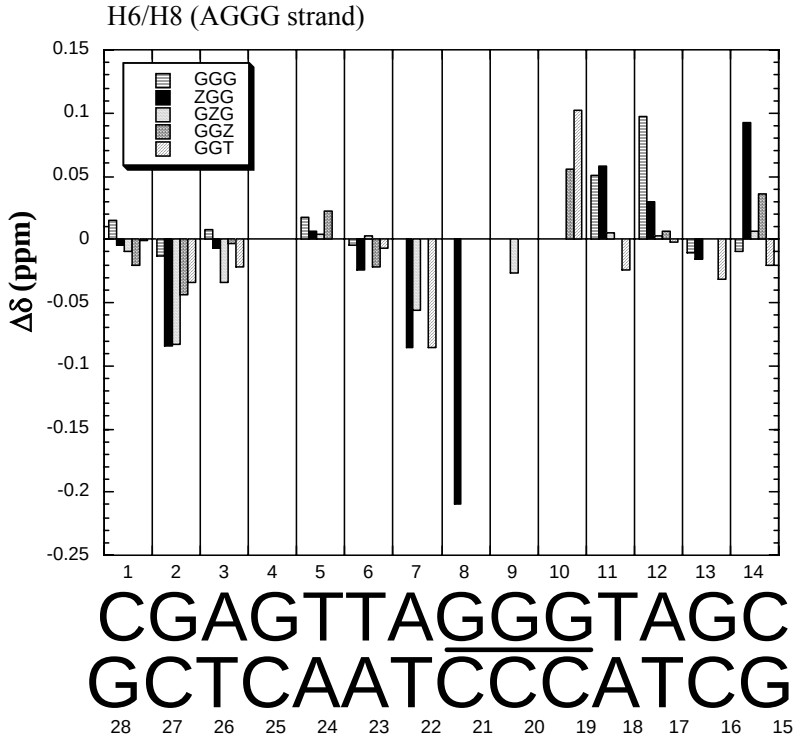


Figure S2. Magnitude and direction of chemical shift changes for the aromatic H6/H8 protons of the various duplexes with 0.5 equivalents of Fe^{2+} per duplex.



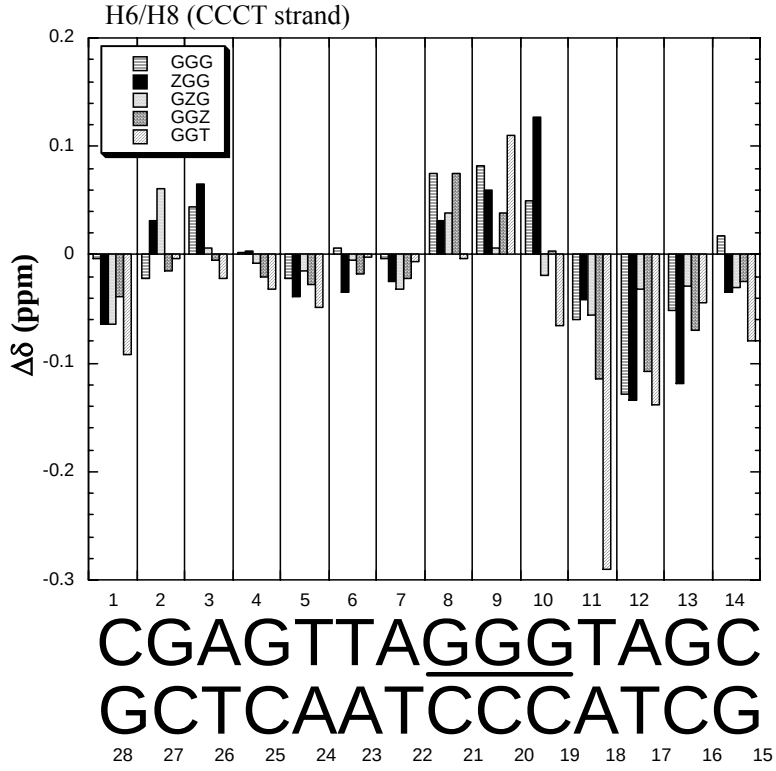


FIGURE S3. Solvent exchangeability of imino protons in the AGGG-containing duplex as a function of Fe^{2+} concentration. Imino proton exchangeability was measured on a 1 mM sample in 90% H_2O/10% D_2O, 130 mM NaCl, using the solvent saturation transfer experiment described in Materials and Methods. Peak intensities of imino protons were measured using the cursor in FELIX 97.0 after each addition of Fe^{2+}.

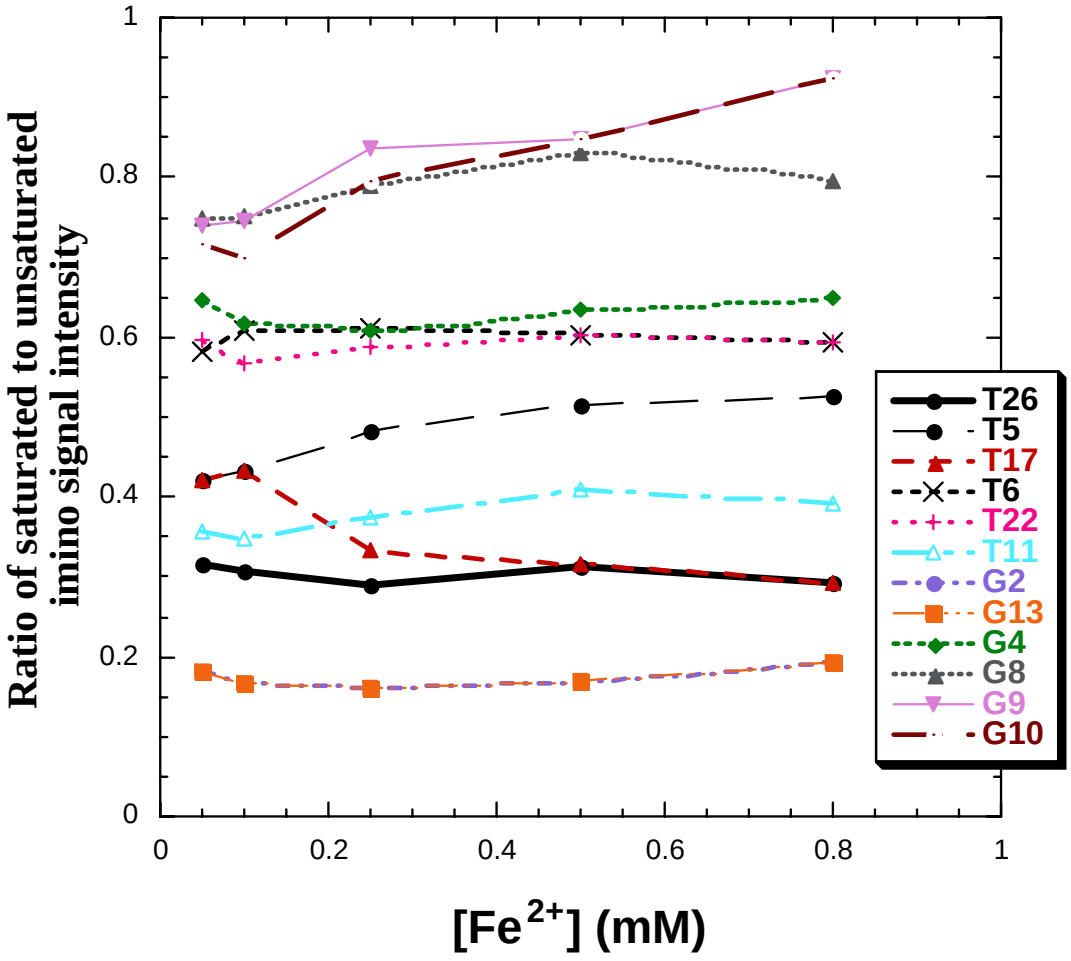


FIGURE S4. Estimation of the dissociation constant K_d for Fe^{2+}-binding at the AGGG sequence. The K_d was calculated from Fe^{2+}-dependent changes in the G9 NH resonance. The data were fitted to the K_d equation using Kaleidagraph 3.0. The observed changes in chemical shift ($\Delta\delta_{obs}$) during the course of an Fe^{2+} titration were measured by subtracting the peak position (in ppm) of the G9 imino proton resonance from its position in the absence of Fe^{2+} (δ_{free}). These changes were then plotted as a function of total added Fe^{2+} concentration, $[Fe^{2+}]$. $\Delta\delta_{obs}$ can be defined as:

$\Delta\delta_{obs} = 1/DNA_{total}[\Delta\delta_{bound}(Fe^{2+}{}_{bound}) + \Delta\delta_{free}(DNA_{total} - Fe^{2+}{}_{bound})]$,

where:

$Fe^{2+}{}_{bound}/DNA_{total}$ is the fractional occupancy, F_{bound},

$\Delta\delta_{bound}$ is the chemical shift change when $F_{bound} = 1$,

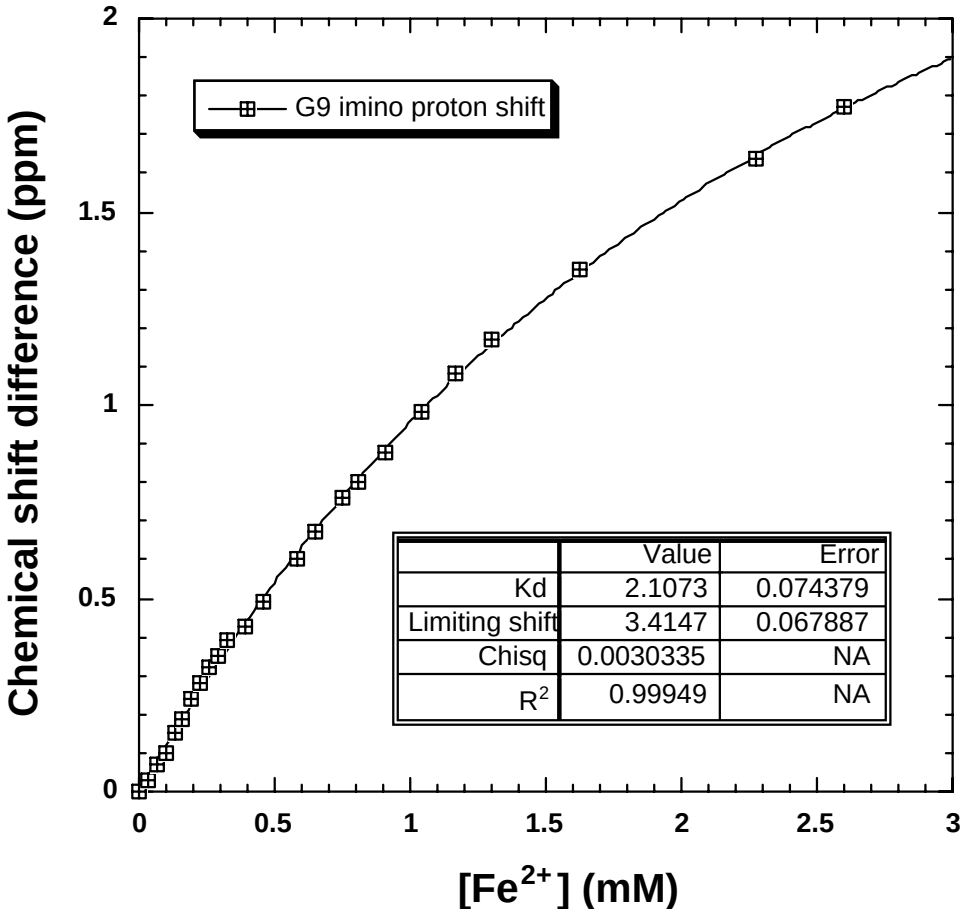
$\Delta\delta_{free}$ is the chemical shift change when $F_{bound} = 0$ (by definition, $\Delta\delta_{free} = 0$), and

$1 - (Fe^{2+}{}_{bound}/DNA_{total}) = 1 - F_{bound}$.

Using the standard equations involving K_d and F_{bound}, $\Delta\delta_{obs}$ can be expressed as a function of $[Fe^{2+}]$ as follows:

$\Delta\delta_{obs} = \Delta\delta/(2DNA_{total})[(DNA_{total}+[Fe^{2+}]+K_d)-\{(DNA_{total}+[Fe^{2+}]+K_d)^2-4(DNA_{total})[Fe^{2+}]\}^{1/2}]$

where $\Delta\delta$ is the magnitude of $\Delta\delta_{bound}$. The data were fitted to the above equation with $[Fe^{2+}]$ and $\Delta\delta_{obs}$ as variables to obtain values for K_d and limiting shift, $\Delta\delta$.


G9 imino proton shift
Chemical shift difference (ppm)
[Fe2+] (mM)
0
0.5
1
1.5
2
2.5
3
Value
Error
Kd
2.1073
0.074379
Limiting shift
3.4147
0.067887
Chisq
0.0030335
NA
R2
0.99949
NA