

Supplementary Figure 1. Stoichiometric mixing study indicates that poly A Zic1 DNA 47mer anneals exclusively into a unimolecular G4-DNA structure. 1 nM 5'-[³²P]-labeled Poly A Zic1 37mer and 47mer (sequences as shown) were annealed under conditions that induce G4-DNA formation either alone (lanes 1-2), with equimolar amounts of either unlabeled oligonucleotide (lanes 3-6), or together (lane 7). Annealed products were determined by non-denaturing polyacrylamide gel electrophoresis.

Supplementary Figure 2. Titration of a fixed amount of G4R1/RHAU with increasing amounts of G4-DNA indicates 70% active protein. (A.) Non-denaturing electropherogram of titration of a fixed concentration of G4R1/RHAU (250 pM) with increasing concentrations of Poly A Zic1 47mer G4-DNA (lanes 1-10 ; control lane 11 had no G4R1/RHAU and 10 pM 5'-[³²P]-labeled Poly A Zic1 47mer). The amount of radioactivity per sample was kept constant by mixing 10 pM 5'-[³²P]-labeled Poly A Zic1 47mer with sufficient unlabeled G4-DNA to achieve the appropriate Poly A Zic1 47mer G4-DNA concentration. (B) The percent bound and unbound Poly A Zic1 47mer G4-DNA derived from the electropherogram shown in (A) is displayed graphically. The experimental data fit well to a quadratic equilibrium binding equation, assuming 70% active G4R1/RHAU with a K_d of the binding interaction of 5pM; the predicted percent binding of 250 pM and 300 pM G4-DNA was 66% and 56 %, respectively, with experimentally determined binding percentages of 67% and 57%, respectively.

Supplementary Figure 3. GMSA of dimethyl sulfate-treated Poly A Zic1 47mer incubated with G4R1/RHAU. 1 nM dimethyl sulfate-treated 5'-[³²P]-labeled Poly A Zic1 47mer was annealed under conditions that induce G4-DNA formation in non-dimethyl sulfate treated oligonucleotides, then incubated with increasing concentrations of purified recombinant G4R1/RHAU (lane 1, 0 pM; lane 2, 2.5 pM; lane 3, 5 pM; lane 4, 7.5 pM; lane 5, 10 pM; lane 6, 40 pM; lane 7, 80 pM). Lanes 8 and 9 show non-dimethyl sulfate-treated Poly A Zic1 47mer annealed into a G4-DNA structure incubated in the absence (lane 8) or presence of 80 pM G4R1/RHAU (lane 9). Shown is a representative (of 3 repetitions) non-denaturing electropherogram.

Supplementary Figure 4. Nondenaturing gel electrophoresis determines the conditions and characteristics for producing pure Band 2 and Band 3 from 5-[³²P]-labeled Poly A Zic1 DNA 47mer incubated with a complementary PNA. (A) Nondenaturing gel electropherogram of a titration of increasing concentrations of an 11mer PNA incubated with 1 nM 5'-[³²P]-Poly A Zic1 47mer DNA in RES buffer containing 50mM KCl at 98°C for 10 min, followed by cooling to room temperature (lane 1, 0 nM; lane 2, 0.3 nM; lane 3, 0.6 nM; lane 4, 1.25 nM; lane 5, 2.5 nM; lane 6, 5 nM; lane 7, 7.5 nM; lane 8, 10 nM; lane 9, 500 nM). Pure Band 2 was observed in lanes 4-8 (PNA: DNA molar ratios of 1.25-10:1); Band 3 was observed in lane 9 (PNA: DNA molar ratio of 500:1). (B) nondenaturing gel electropherogram of 1 nM 5'-[³²P]-Poly A Zic1 47mer DNA incubated with 2 nM PNA at 50 °C for 18 hours in RES buffer plus increasing concentrations of KCl. (lane 1, 6.25 mM, lane 2, 12.5 mM, lane 3, 25mM, lane 4, 50mM, lane 5, 75mM.). G4-DNA structures (Bands 1 and 2), but not the PNA: DNA hybrid (Band 3), are destabilized in the presence of low concentrations of KCl.

Supplementary Figure 5. CD spectra of Bands 1, 2, and 3 at different temperatures in no salt, LiCl or KCl indicates that Band 3 is a PNA: DNA hybrid. (A) CD spectra of pure Band 1 produced by forming Poly A Zic1 DNA 47mer into G4-DNA were obtained in three different salt conditions: upper spectra, no salt; middle spectra, 50 mM LiCl; lower spectra, 50 mM KCl. Spectra were recorded at different temperatures as follows: 25 °C (dark blue), 37 °C (pink), 70 °C (light blue) and 98 °C (green).-(B) CD spectra of pure Band 2 or (C) pure Band 3 formed under

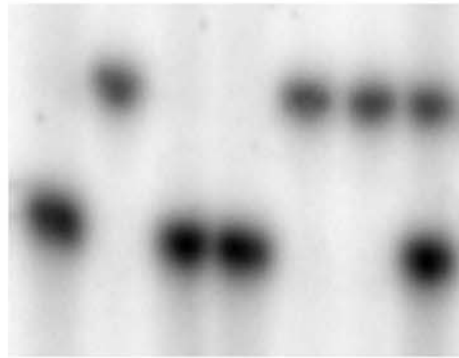
conditions discussed in Supplementary Figure 4 by incubation of Poly A Zic1 DNA 47mer with a complementary PNA were obtained in three different salt conditions: upper spectra, no salt; middle spectra, 50 mM LiCl; and lower spectra, 50 mM KCl. Spectra were recorded at different temperatures as follows: 25 °C (dark blue), 37 °C (pink), 75 °C (brown), and 98 °C (green)°C. The PNA alone produced no peaks of molar ellipticity in its CD spectrum (data not shown).

Supplementary Figure 6. GMSA indicates that G4R1/RHAU does not tightly bind PNA: DNA duplex formed without significant excess PNA. Lanes 1-3 of a non-denaturing gel electropherogram show a titration of labeled Poly A Zic1 47mer G4-DNA incubated with increasing molar ratios of a PNA with Watson-Crick complementarity to the G4-DNA-forming sequence, but without G4R1/RHAU. Lanes 4-12 show PNA:DNA duplex created by annealing equimolar PNA and 1nM 5'-[³²P]-labeled Poly A Zic1 47mer DNA incubated in the presence of increasing concentrations of G4R1/RHAU ranging from 1 pM (lane 4) to 300 pM (lane 12). Lanes 13-15 show 1nM 5'-[³²P]-labeled Poly A Zic1 47mer G4-DNA incubated without PNA in the absence (lane 13) or presence (lanes 14 and 15) of 30 pM G4R1/RHAU. In lane 15, a 10-fold molar excess of PNA was added concomitant with the addition of G4R1/RHAU.

Supplementary Figure 7. A G4R1/RHAU resolution assay of Poly A Zic1 DNA 47mer G4-DNA in the presence of a complementary PNA shows that G4R1/RHAU-catalyzed unwinding of G4-DNA requires ATP hydrolysis. Shown is a phosphorimage of a non-denaturing gel electropherogram of 1 nM 5'-[³²P]-labeled Poly A Zic1 G4-DNA 47mer incubated with 10 nM of a PNA with Watson-Crick complementarity to the G4-DNA-forming sequence in the presence of increasing concentrations of G4R1/RHAU without ATP (lanes 1-6), with 10 mM AMP-PNP (lanes 7-12), with 10 mM ATP (lanes 13-18). Lane 19 shows 5'-[³²P]-labeled Poly A Zic1 G4-DNA 47mer incubated without PNA or G4R1/RHAU. Lanes 20-22 show a titration of 5'-[³²P]-labeled Poly A Zic1 G4-DNA 47mer incubated with increasing concentrations of PNA, but without G4R1/RHAU.

Supplementary Figure 1

³² P -47mer	-	+	-	-	+	+	+
Unlabeled 47mer	-	-	+	-	+	-	-
³² P -37mer	+	-	+	+	-	-	+
Unlabeled 37mer	-	-	-	+	-	+	-



Lane 1 2 3 4 5 6 7

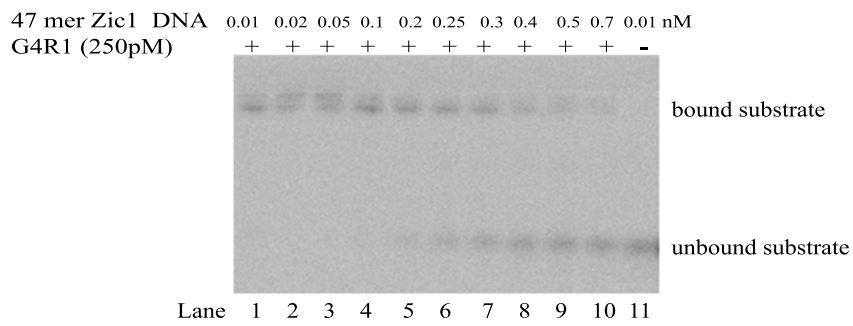
47mer: 5'-AAA AAA AAA AGG GTG GGG GGG GCG GGG GAG GCC GGG GAA AAA AAA AA-3'

37mer: 5'-AAA AAA AAA AGG GTG GGG GGG GCG GGG GAG GCC GGG G-3'

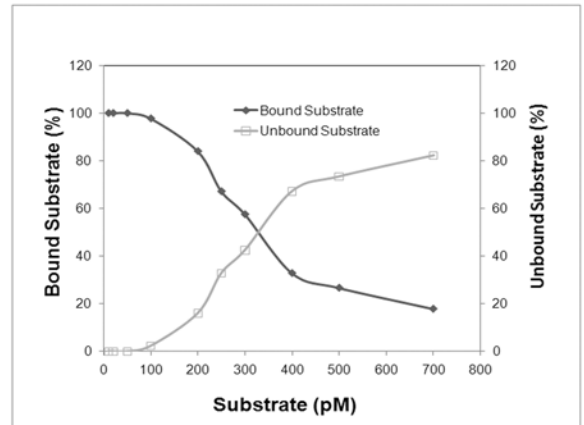
Bold G's involved in G4 formation

Supplementary Figure 2

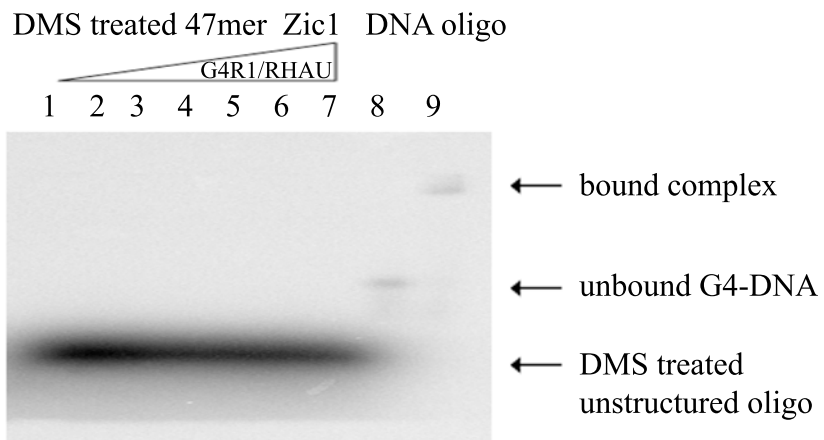
A



B



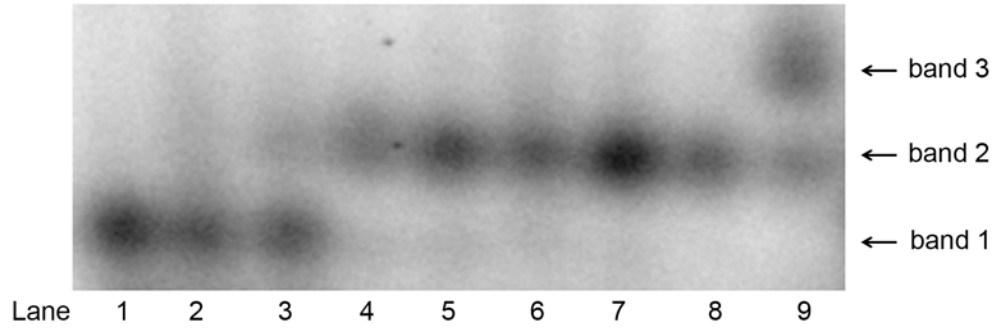
Supplementary Figure 3



Supplementary Figure 4

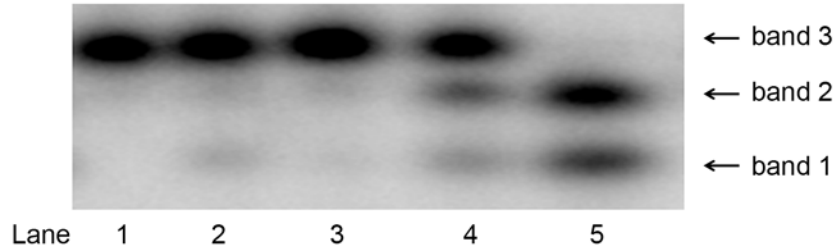
A

Zic1 47 mer DNA (nM)	1	1	1	1	1	1	1	1	1
PNA (nM)	0	0.3	0.6	1.25	2.5	5.0	7.5	10	500
KCl (mM)	50	50	50	50	50	50	50	50	50



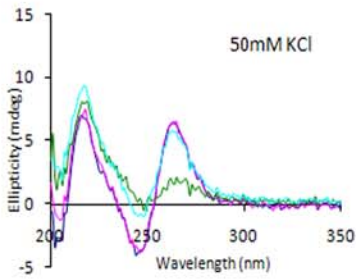
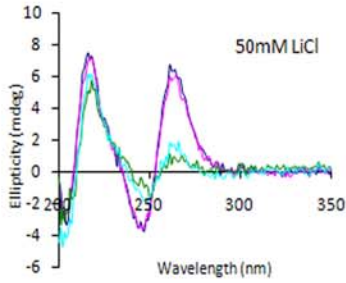
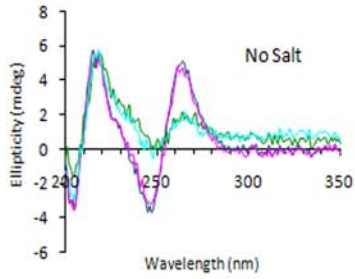
B

Zic1 47 mer DNA (nM)	1	1	1	1	1
PNA (nM)	2	2	2	2	2
KCl (mM)	6.25	12.5	25	50	75

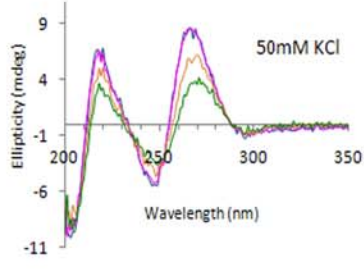
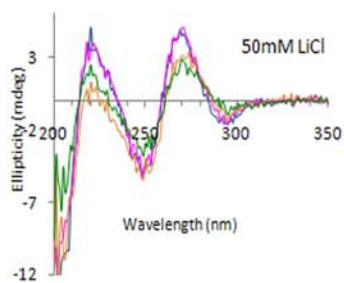
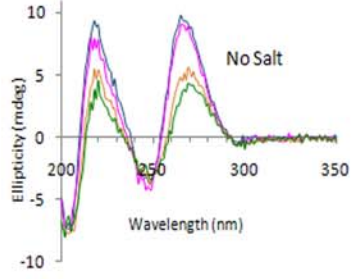


Supplementary Figure 5

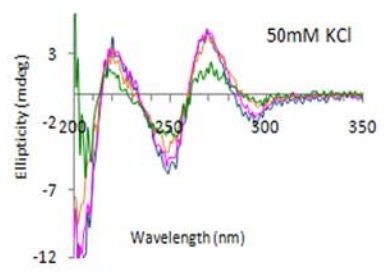
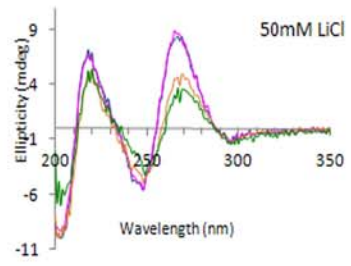
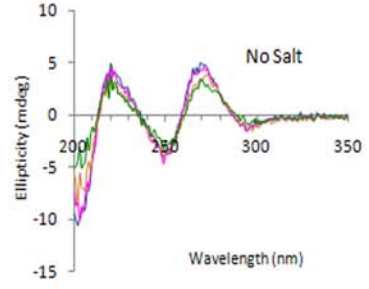
A) Band 1



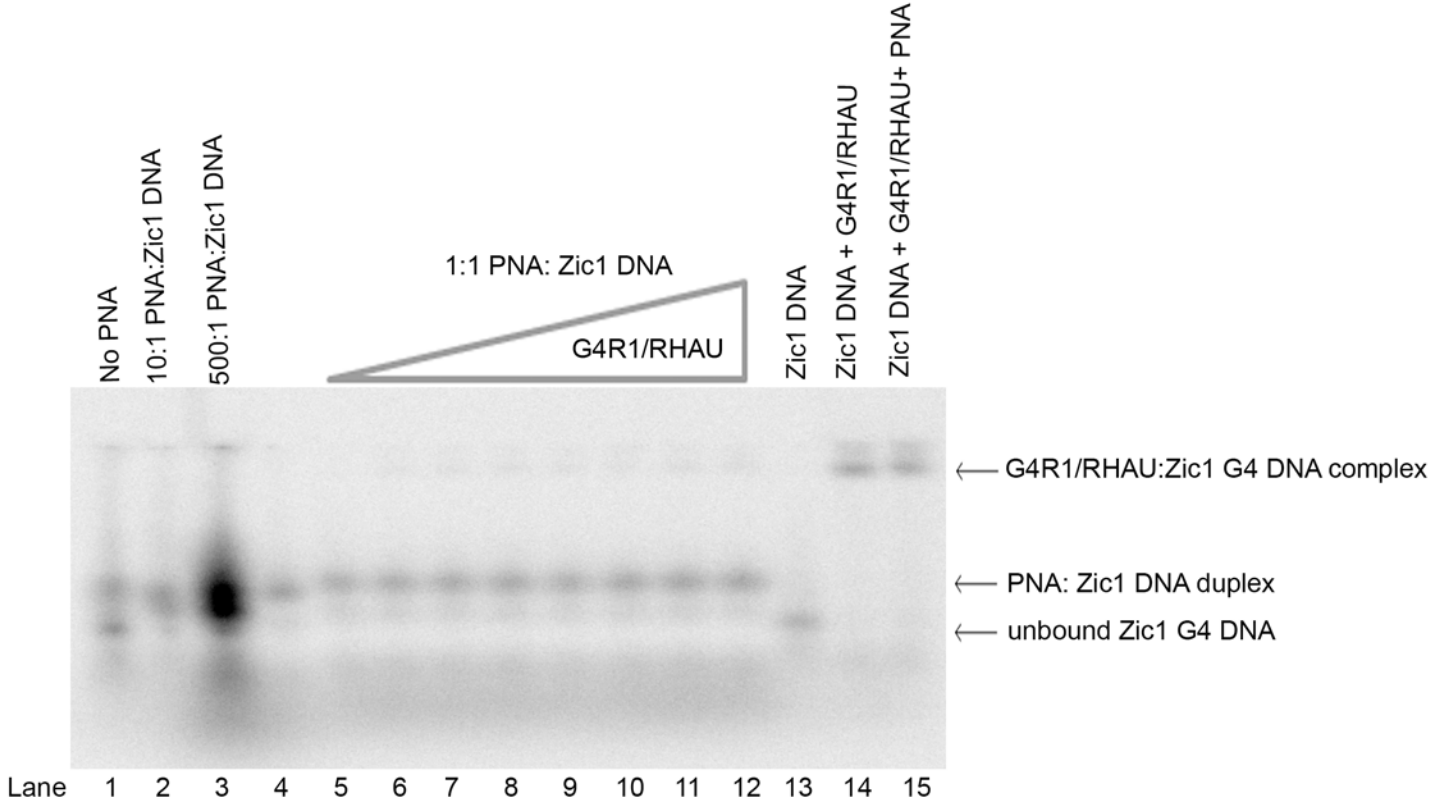
B) Band 2



C) Band 3



Supplementary Figure 6



Supplementary Figure 7

