Supplementary data

Structural Properties of G,T-Parallel Duplexes

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Table S1. Melting temperature (Tm) and free energies of the transition observed for the complex formed by clamp **1** and its target **2** compared with duplex **2** + **3** in magnesium buffers or in the presence of zinc.

complex	Tm (ºC)[a]	ΔG (Kcal/mol)[a]	Tm (ºC)[b]	ΔG (Kcal/mol)[b]	Tm (ºC)[c]	ΔG (Kcal/mol)[c]
1+**2**	51.9	-12.1	45.4	-10.0	46.6	-10.2
duplex **2**+**3**	50.5	-11.5	49.4	-11.0	50.1	-11.0

[a]50 mM $MgCl_2$, 10 mM sodium cacodylate pH 7.2; [b]2M NaCl pH 6.0; [c]2M NaCl, 10 mM $ZnCl_2$ pH 6.0.

Table S2. Melting temperatures (T_m) of the transitions observed for the complex formed by parallel duplexes **4**, **5**, **6**, **7** and control oligonucleotide **9** with and without their target.

Oligodeoxynucleotide	Tm (ºC)[a] with target **8**	Tm(ºC)[a] without target	Tm (ºC)[a] with RNA target **11**
clamp **4**	55.6	55.8	55.5
clamp **5**	62.2	61.7	62.0
clamp **6**	63.2	62.8	n.d.
clamp **7**	61.6	61.3	61.2
control **9**	44.0	none	50.4

[a]50 mM $MgCl_2$, 10 mM sodium cacodylate pH 7.2; n.d. not determined

Figure 1S. Circular dichroism spectra of parallel clamp **6** and the complex formed by **6** and its target **8**. Conditions 50 mM $MgCl_2$, 10 mM sodium cacodylate pH 7.2.

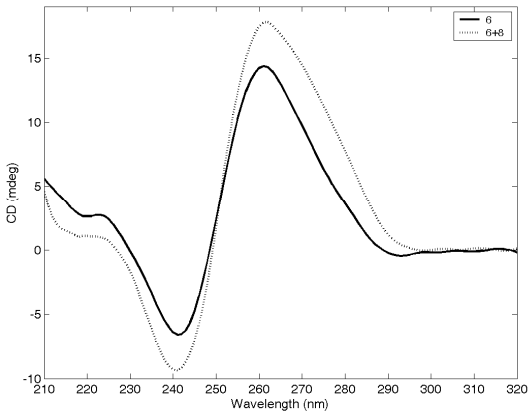
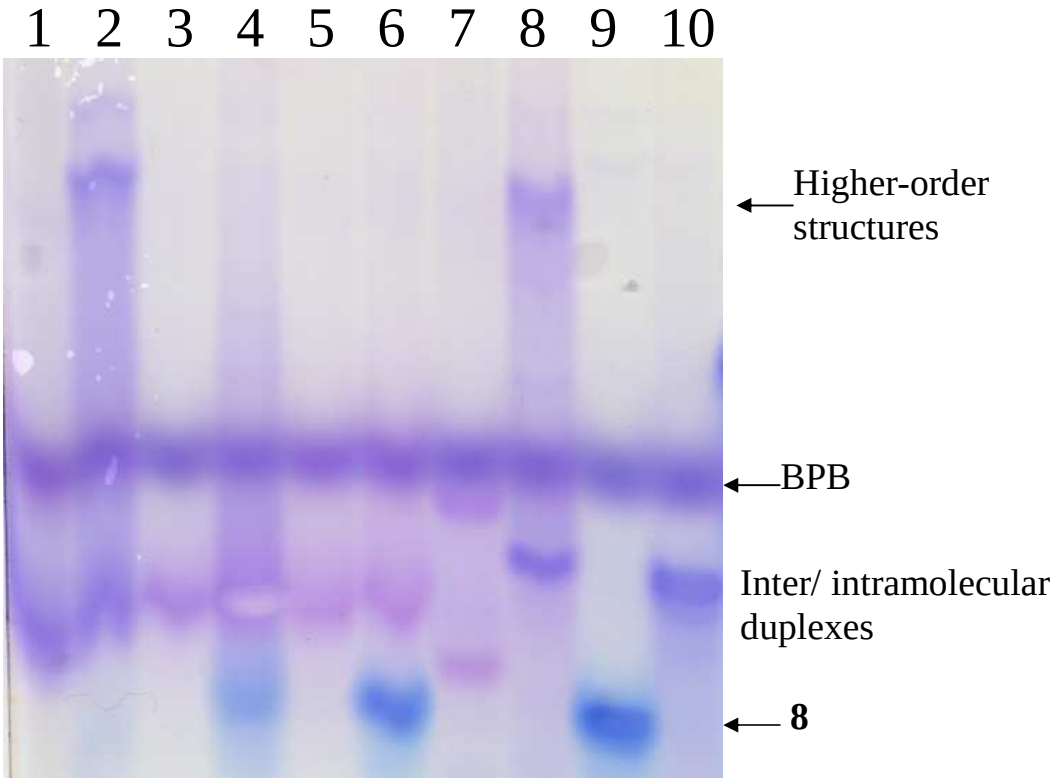


Figure 2S. Gel-shift analysis of complexes formed by parallel duplexes and its target **8**. Native 20% polyacrylamide gel electrophoresis stained with Stains-all. Lane 1: unmodified clamp **4**. Lane 2: equimolar mixture of clamp **4** and target **8**. Lane 3: clamp **5**. Lane 4: equimolar mixture of clamp **5** and target **8**. Lane 5: clamp **6**. Lane 6: equimolar mixture of clamp **6** and target **8**. Lane 7: control **9**. Lane 8: equimolar mixture of control **9** and target **8**. Lane 9: target **8**. Lane 10: equimolar mixture of **10** and target **8**. BPB: bromophenol blue dye. *Experimental Conditions.* Native polyacrylamide gel electrophoresis was carried out at 4 ºC. The 20% polyacrylamide gels [29:1 acrylamide:bis(acrylamide)] contained 90 mM Tris-borate-EDTA (TBE) pH 8.0. All DNA samples were incubated at 90 ºC for 5 min, slowly cooled, and loaded onto the gels. DNA concentration was approx. 60 micromolar (10 times higher than in melting experiments). After running overnight at 300 volts, gels were stained for 20 min in a 0.1 mg/ ml solution of Stains-all in 15% formamide in water, briefly washed with distilled water, de-stained with an IR lamp and photographed.



Non-denaturing 20% polyacrylamide gel electrophoresis was carried out at 4ºC (**Figure 2S***)*. Unmodified parallel clamp **4** alone and the duplex formed by mixing oligonucleotides **4** and **8** had similar mobility (lanes 1 and 2). The formation of the duplex is indicated by the absence of a blue spot corresponding to target **8** (lane 9). In addition to the duplex band, a smear with low mobility was observed, which may be due to higher-order structures formed by the unpaired G,T-strand (lane 2). These higher-

order structures were favored at the higher DNA concentration needed to visualize the spots with Stains-all.

Parallel duplexes carrying 8-aminoadenine **5** and **6** did not bind to target **8,** since a blue spot corresponding to target **8** was observed in both cases (lanes 4 and 6). Moreover, no higher order structures were observed in any of the samples containing duplexes **5** or **6**. This indicates that the preferred structure of these duplexes was an intramolecular hairpin form rather an intermolecular duplex. Control oligonucleotide **9** alone gave two bands of different mobility from the duplexes (lane 7), indicating that the unpaired G,T-strand was responsible for higher-order structures. Similarly, the duplex formed by **9** and **8** gave the same smear with low mobility as that observed in the duplex formed by mixing **4** and **8**. This again indicates that the unpaired G,T-strand participated in the formation of higher-order structures. Finally, the duplex formed by **10** and **8** gave a single spot in the duplex area. No higher structure band was observed because in this case there was no unpaired G,T-strand. The parallel clamp carrying 8-aminoguanine (**7**) gave the same results as parallel duplexes carrying 8-aminoadenine **5** and **6**.

Figure 3S. Scheme of the complexes observed in the gel-shift analysis.