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Supplemental Material for "Recognition of Single-stranded Nucleic Acids..."

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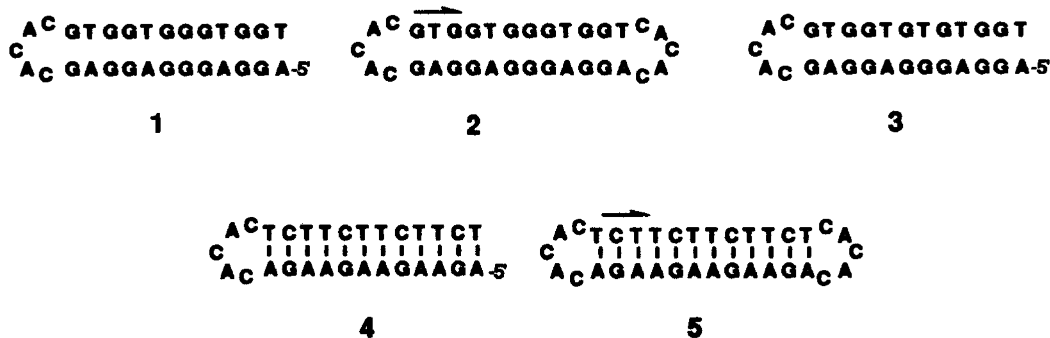
Synthetic methods for linear and circular oligonucleotides. DNA oligomers were synthesized on an Applied Biosystems 392 automated synthesizer using the standard phosphoramidite method.[1] RNA oligonucleotides were prepared using t-butyl-dimethylsilyl-protected phosphoramidites (Applied Biosystems), and following the oligoribonucleotide synthesis procedure of Usman.[2] Tetrabutylammonium fluoride in THF (Aldrich) was dried over molecular sieves prior to use in the desilylation step.[3] For synthesis of circular DNAs the linear precursors were 5'-phosphorylated on the synthesizer using a commercially available reagent purchased from Cruachem.[4] Oligonucleotides were purified by preparative 20% denaturing polyacrylamide gel electrophoresis and quantitated by absorbance at 260 nm. Extinction coefficients for the oligomers were calculated by the nearest neighbor method.[5]



Note that compounds **4** and **5** are not described in the main text; these were synthesized to test whether connected Watson-Crick duplexes could bind pyrimidine-rich targets by Hoogsteen complexation. The cyclizations of the 5'-phosphorylated precursors of the circular ligands **2** and **5** were carried out essentially as described earlier.[6,7] Both precursors were reacted at 50 μM concentration; the cyclization of **2** was aided by the template dCTCCTCCCTCCT (55 μM), while the cyclization of **5** was self-templating. The ligations were carried out in a buffer containing imidazole•HCl (200 mM, from a 0.5 M pH 7.0 stock), and $NiCl_2$ (100 mM). Solid BrCN was added with vortex mixing to give a final calculated concentration of 125 mM, and the reactions were allowed to proceed at 25°C for 12 hr. As the reaction proceeds a light tan precipitate is observed, and previous studies have shown that this precipitate contains the

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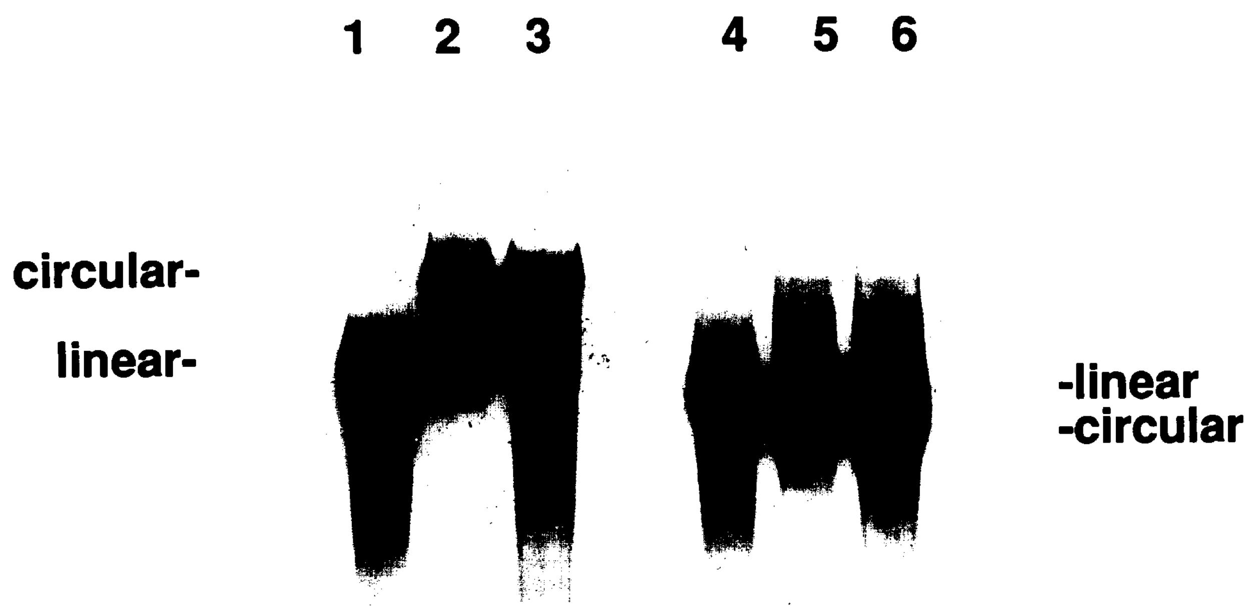

Fig. 1. Stained denaturing 20% PAGE gel with characterization of circular compounds **2** (lanes 1-3) and **5** (lanes 4-6). Lanes 1,4: Linear precursor prior to cyclization. Lanes 2,5: Isolated circular products. Lanes 3,6: Circular compounds partially digested with S1 nuclease, showing initial product of cleavage having the same mobility as the linear 34mers.

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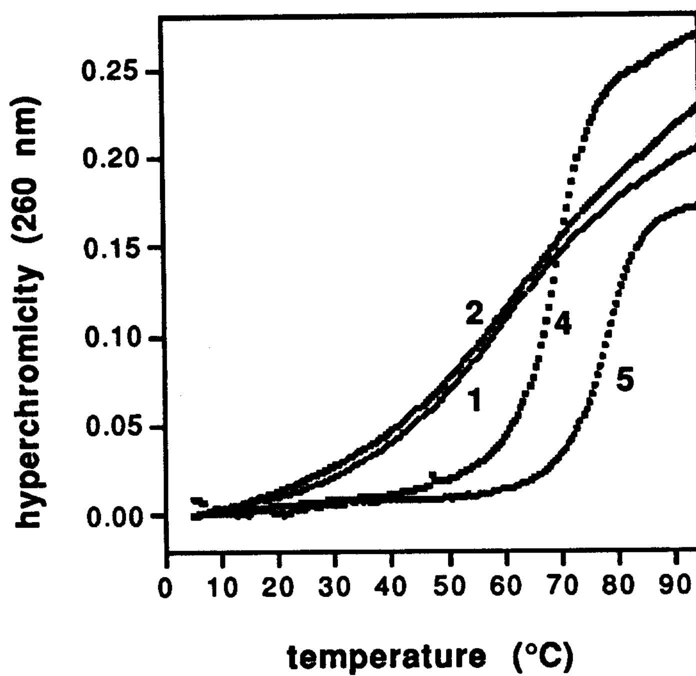

Fig. 2. Thermal denaturation curves measured at pH 7.0 for the ligands **1,2,4,5** alone. Note that the transitions for **4** and **5** are present in the melting plots for their complexes (see Fig. 3).

majority of the nucleic acids. The solutions (including solids) were dialyzed against water and lyophilized. The resulting solid was loaded onto a preparative 20% denaturing PAGE gel for separation. The circular products were isolated by excision from the gel after visualization by UV shadowing; the circle **2** migrated at a rate ~0.9 times that of the linear 34 nt precursor, while the circle **5** migrated at ~1.05 the rate of its 34mer precursor (see Fig. 1, below).

The circularity of **2** and **5** was confirmed by partial digestion by S1 nuclease. The reactions were carried out using 1 nmol DNA in 5.1 µL of a buffer containing 50 mM NaOAc, 50 mM NaCl, and 5 mM $ZnCl_2$. Then 0.4 units nuclease S1 (0.9 µL, Pharmacia) was added, and the mixtures incubated at 37°C for 10 min. Reactions were stopped by addition of 6 µL of an 8 M urea, 30 mM EDTA solution, and the mixtures were loaded onto a 20% denaturing analytical PAGE gel. Products were visualized for photography (see Fig. 1) with Stains-all dye (Sigma). Both compounds show a single initial product which migrates with the mobility of the 34mer precursor.

Thermal denaturation experiments. Solutions for the thermal denaturation studies contained a 1:1 molar ratio of oligonucleotide ligand (**1-5**) and complementary 12-nt pyrimidine oligomer (1 µM each). Solutions were buffered with 10 mM Na•PIPES (1,4-piperazine-bis(ethanesulfonate), Sigma) at pH 7.0. Some experiments were also carried out at pH 5.5, as described in the text. The buffer pH is that of a 500 mM stock solution at 25°C; after dilution the final solution pH was shown to be within 0.1 unit of the buffer stock. Also present in the denaturation solutions were 100 mM NaCl and 10 mM $MgCl_2$. After the solutions were prepared they were heated to 90°C and allowed to cool slowly to room temperature prior to the melting experiments.

The melting studies (see Figs. 2,3 for plots) were carried out in teflon-stoppered 1 cm pathlength quartz cells under nitrogen atmosphere on a Varian Cary 1 UV-vis spectophotometer equipped with thermoprogrammer. Absorbance (260 nm) was monitored while temperature was raised from 5.0 to 95°C at a rate of 0.5°C/min. Melting temperatures (T_m) are determined by computer fit of the first derivative of absorbance with respect to 1/T. Uncertainty in T_m is estimated at ± 0.5°C based on repetitions of experiments.

Native gel experiments. (See fig. 4) pH 7.0 solutions of ratio 2:1, 1:1 and 1:2 circle : substrate oligomer (0.25 or 0.5 nmol each) were prepared at 4°C in 5 µL of a buffer containing 70 mM Tris•borate, 10 mM $MgCl_2$, and 6% glycerol and incubated for 4 hr prior to loading on a 20% nondenaturing PAGE gel. The gel was electrophoresced at 2.5 mW at 4°C using the same buffer as the electrophoresis buffer, and the resulting bands were visualized with Stains-all dye.

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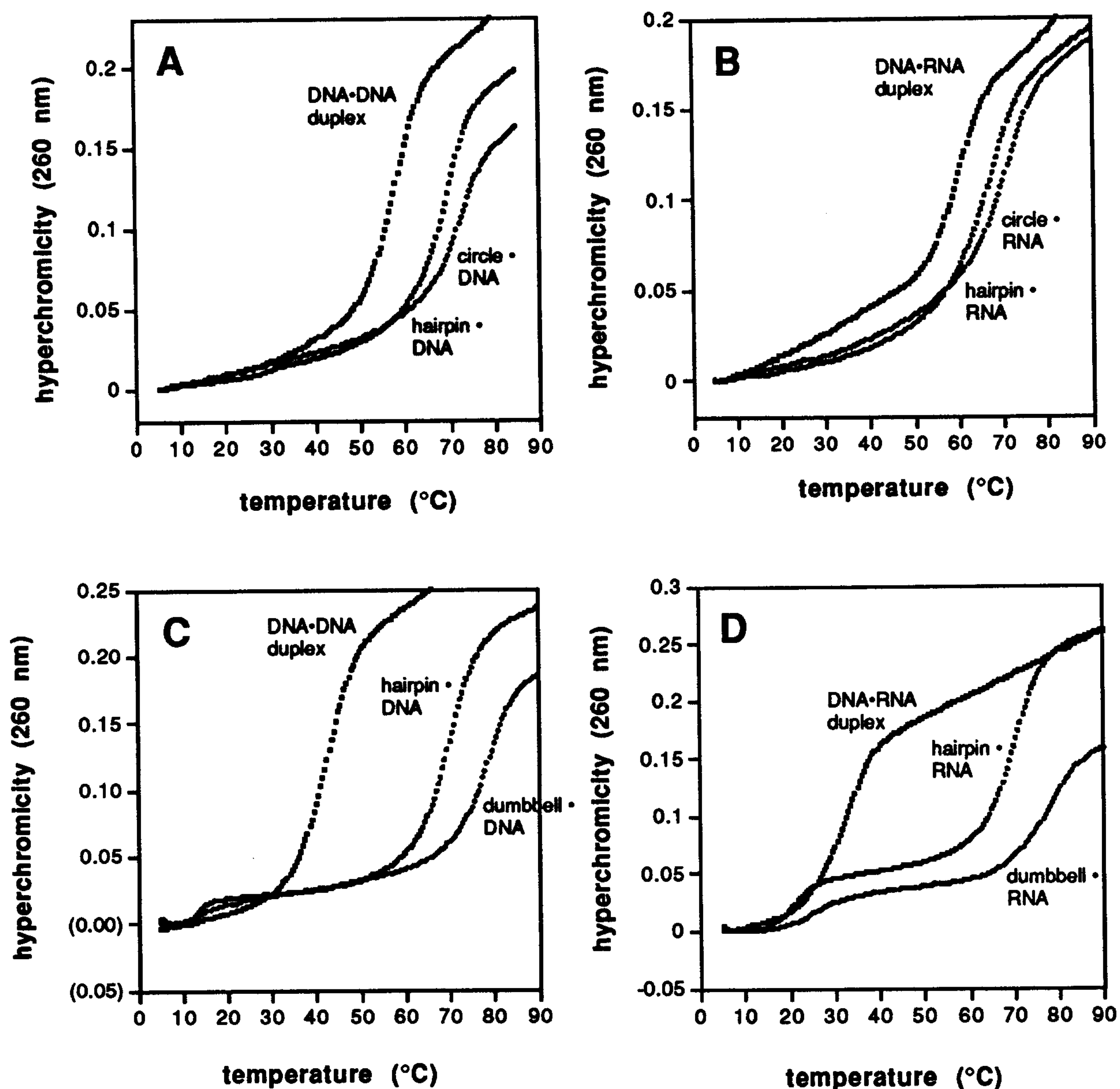

Fig. 3. Thermal denaturation curves measured at pH 7.0 for the complexes in this study (see Table I in text for structures). **A.** Purine-rich ligands binding a pyrimidine DNA complement. **B.** Purine-rich ligands binding a pyrimidine RNA complement. **C.** Hairpin and dumbbell ligands binding a pyrimidine DNA complement (note triplex to duplex transitions at 10-20°C). **D.** Hairpin and dumbbell ligands binding a pyrimidine RNA complement (note triplex to duplex transitions at 20-30°C). See experimental section for details.

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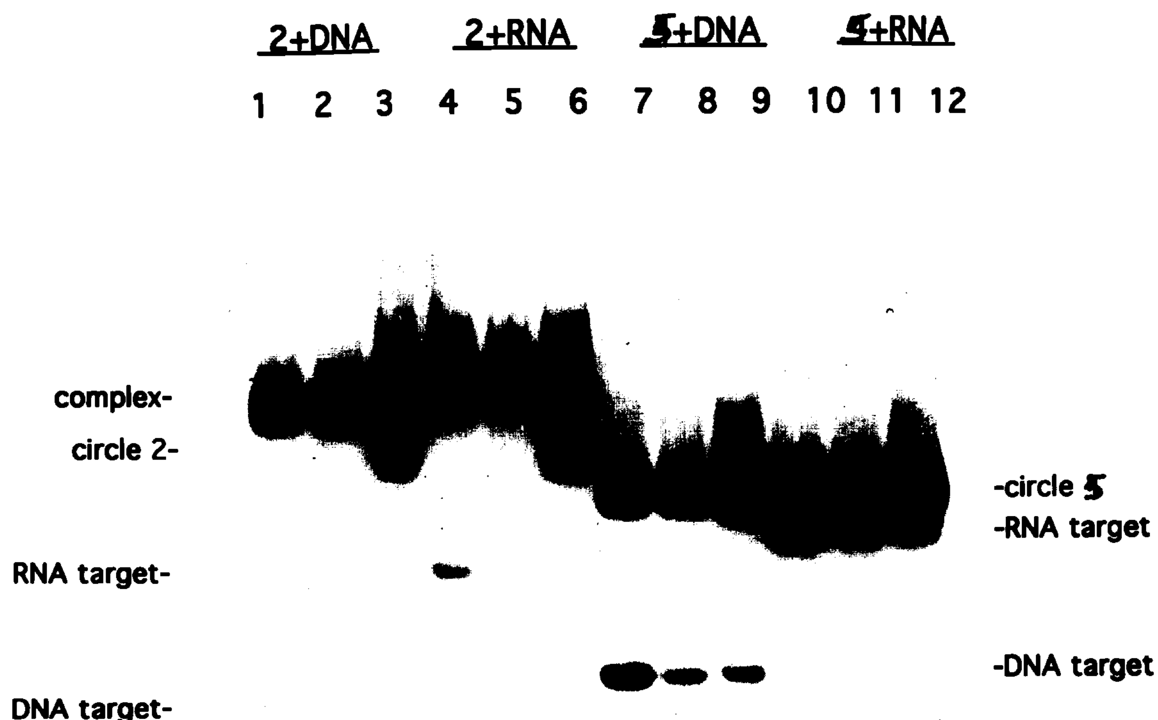

Fig. 4. Stained 20% native polyacrylamide gel of mixtures of circle **2** (lanes 1-6) and circle **5** (lanes 7-12) with DNA and RNA complements at pH 7.0. Lanes 1-3: 2:1, 1:1, 1:2 DNA complement with circle **2**. Lanes 4-6: 2:1, 1:1, 1:2 RNA complement with circle **2**. Lanes 7-9: 2:1, 1:1, 1:2 DNA complement with circle **5**. Lanes 10-12: 2:1, 1:1, 1:2 DNA complement with circle **5**. Note 1:1 stoichiometry for the complexes of 2; also note that under these conditions the weak ligand **5** apparently does not bind the complements. Oligonucleotide strands are 50 μM each, and mixtures were incubated at pH 7.0, 4°C as described under "Native gel experiments."

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