

Supplementary Information

for manuscript entitled

Effect of Preorganization on the Affinity of Synthetic DNA Binding Motifs for Nucleotide Ligands

by

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2. Selected HPLC Chromatograms of Modified Oligonucleotides

For oligonucleotides **2-6**, see Experimental Part for HPLC gradients.

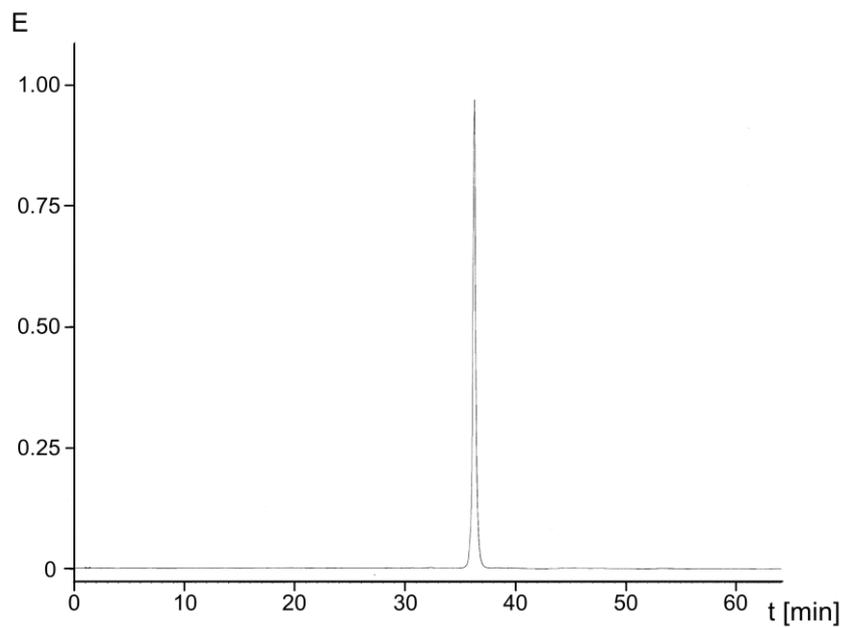


Figure S1. HPLC chromatogram of oligonucleotide **2**.

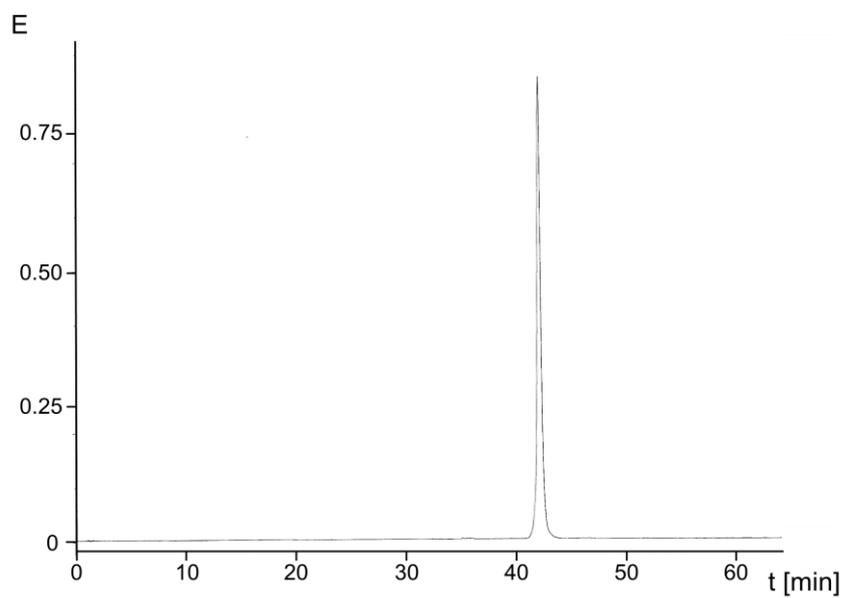


Figure S2. HPLC chromatogram of oligonucleotide **3**.

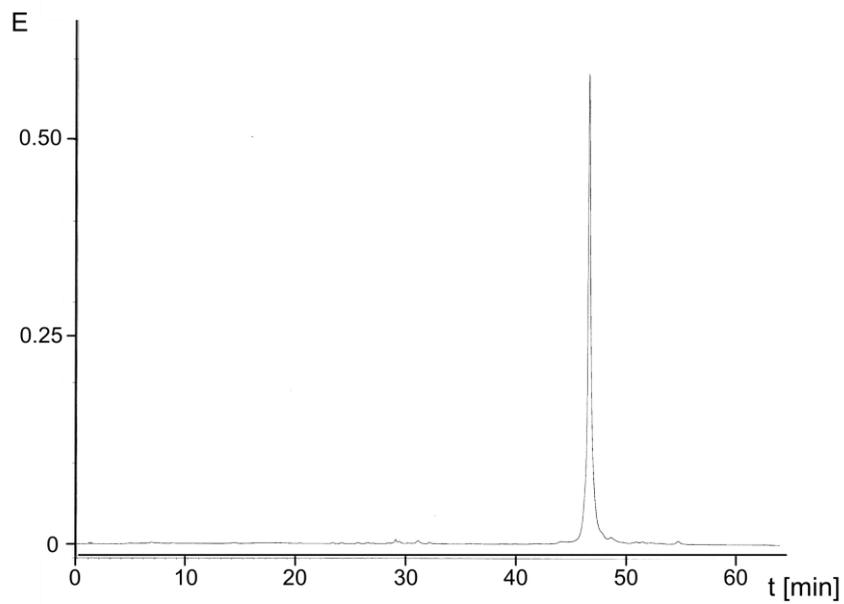


Figure S3. HPLC chromatogram of oligonucleotide **5**.

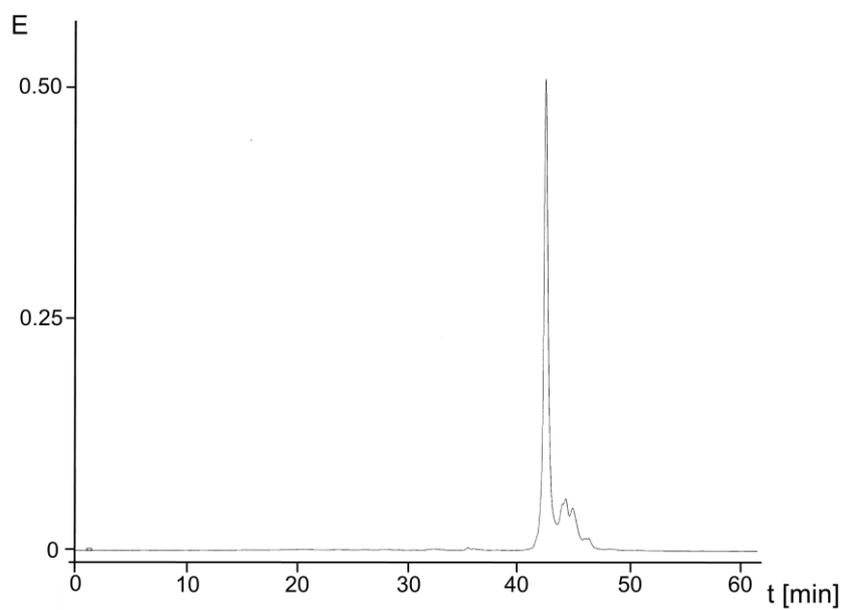


Figure S4. HPLC chromatogram of oligonucleotide **6**.

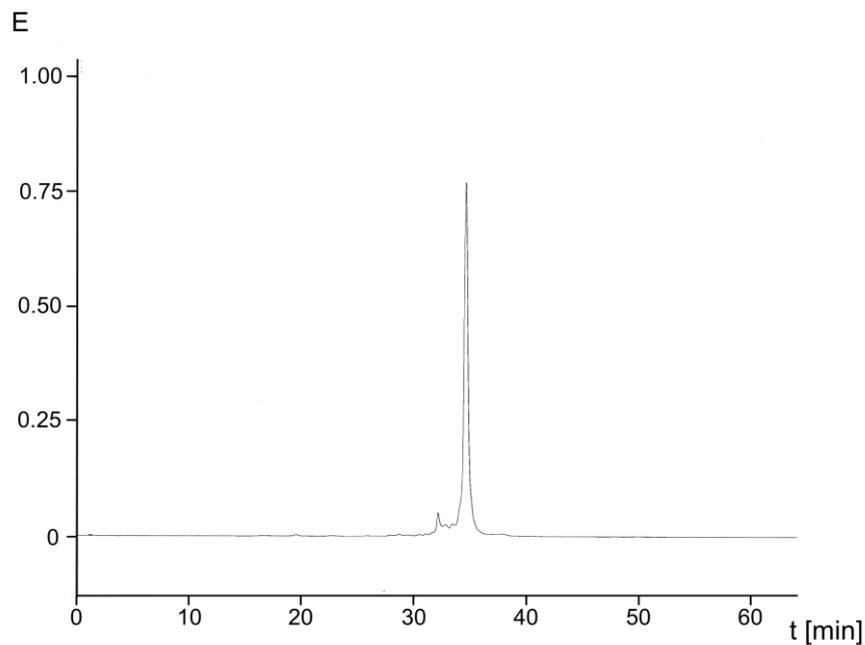


Figure S5. HPLC chromatogram of oligonucleotide **15**; gradient: 0% acetonitrile for 5 min, then of 0% to 10% acetonitrile in 20 min, 10% to 20% in 25 min, 20% to 25% in 10 min, then 25% to 80% in 5 min.

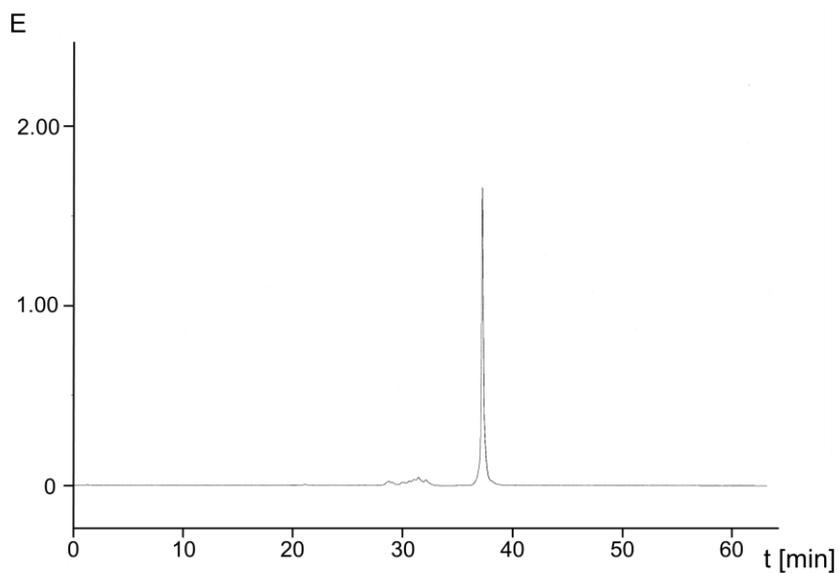


Figure S6. HPLC chromatogram of oligonucleotide **16**; gradient identical to that reported in the legend to Figure S5.

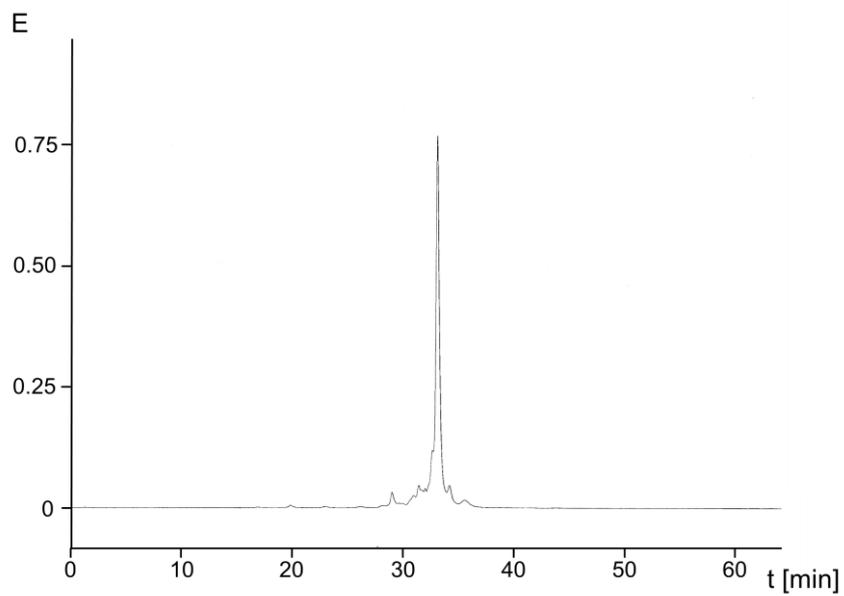


Figure S7. HPLC chromatogram of oligonucleotide **17**; gradient identical to that reported in the legend to Figure S5.

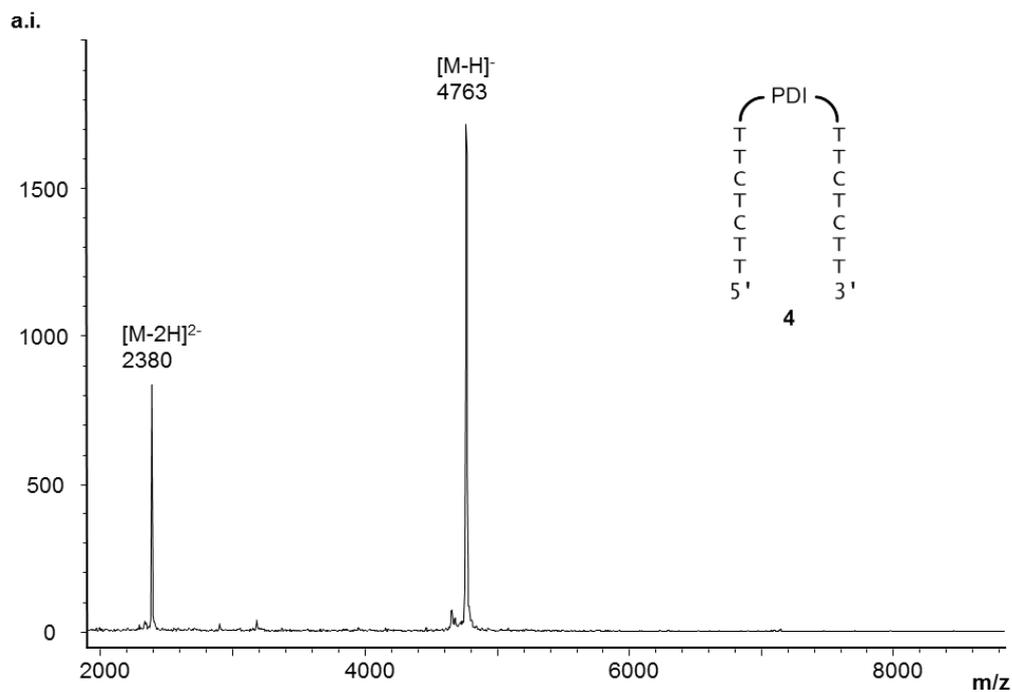


Figure S10. MALDI-TOF mass spectrum of oligonucleotide **4**, as measured in linear negative mode. An assignment of the ions is shown above the relevant peaks.

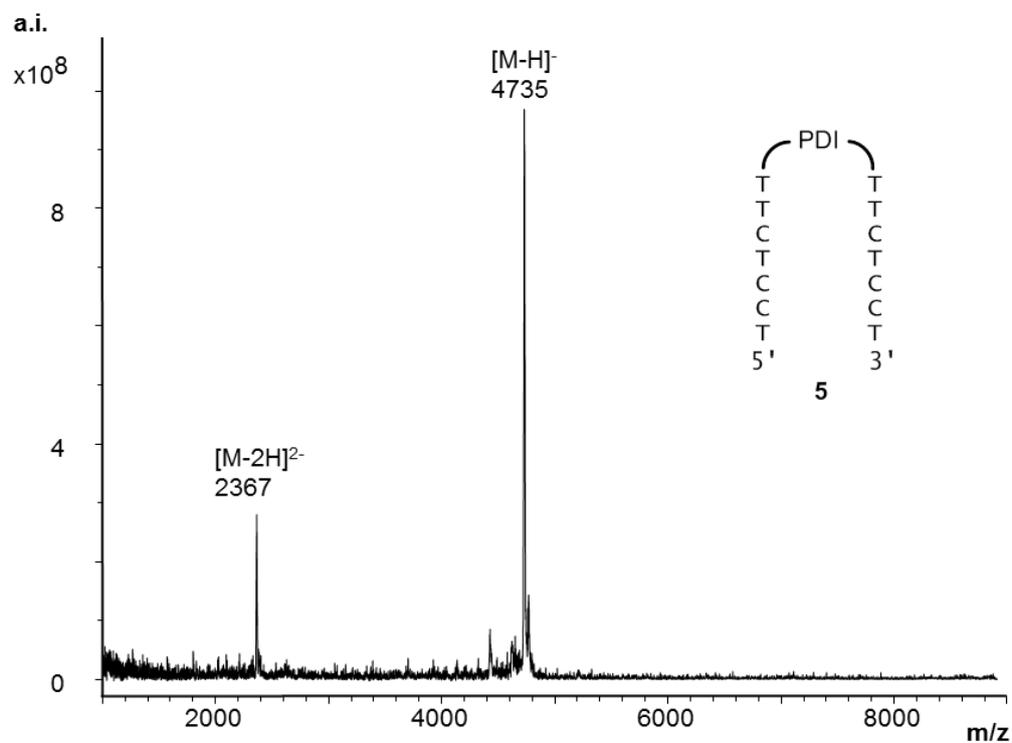


Figure S11. MALDI-TOF mass spectrum of oligonucleotide **5**, as measured in linear negative mode. An assignment of the ions is shown above the relevant peaks.

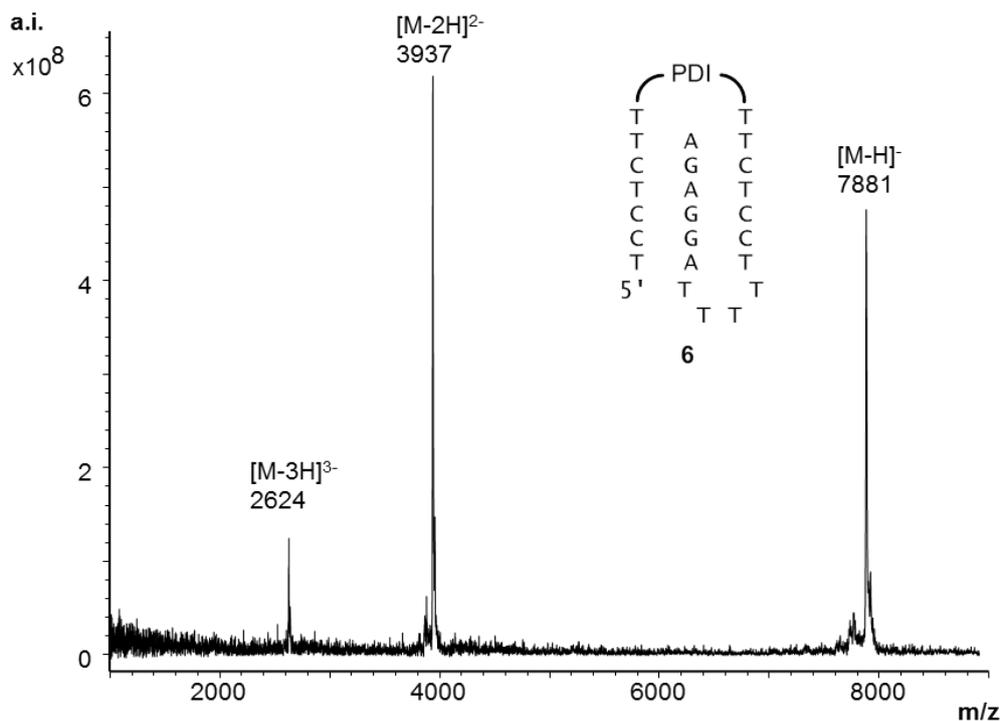


Figure S12. MALDI-TOF mass spectrum of oligonucleotide **6**, as measured in linear negative mode. An assignment of the ions is shown above the relevant peaks.

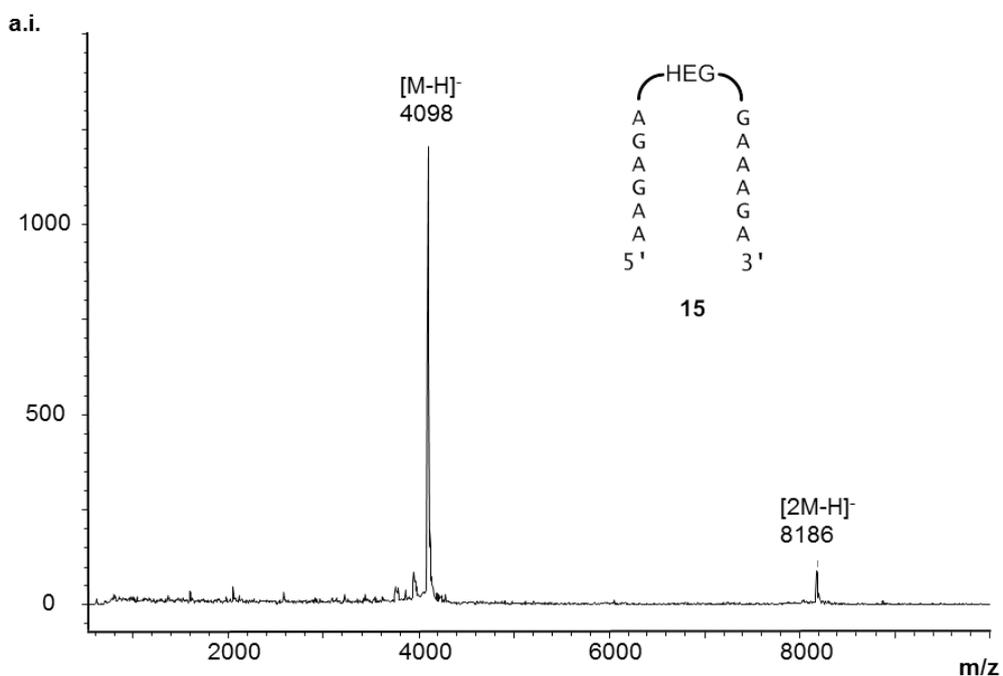


Figure S13. MALDI spectrum of motif **15**. Measured in linear negative mode with matrix / comatrix: 2,4,6-trihydroxyacetophenone / diammonium hydrogen citrate in ethanol.

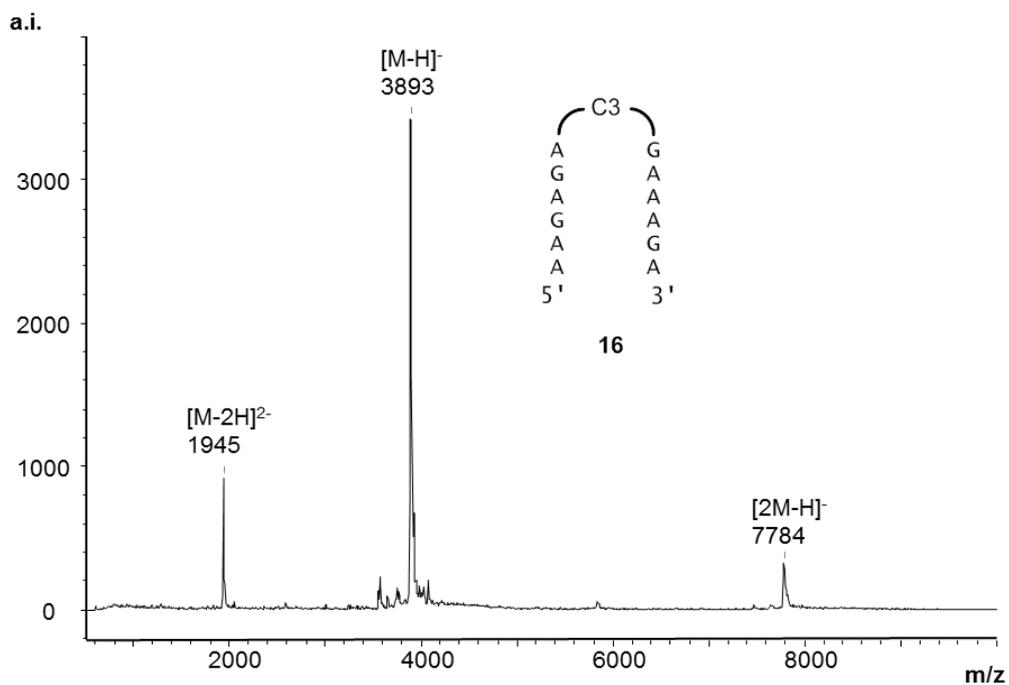


Figure S14. MALDI spectrum of motif **16**. Measured in linear negative mode with matrix / comatrix: 2,4,6-trihydroxyacetophenone / diammonium hydrogen citrate in ethanol.

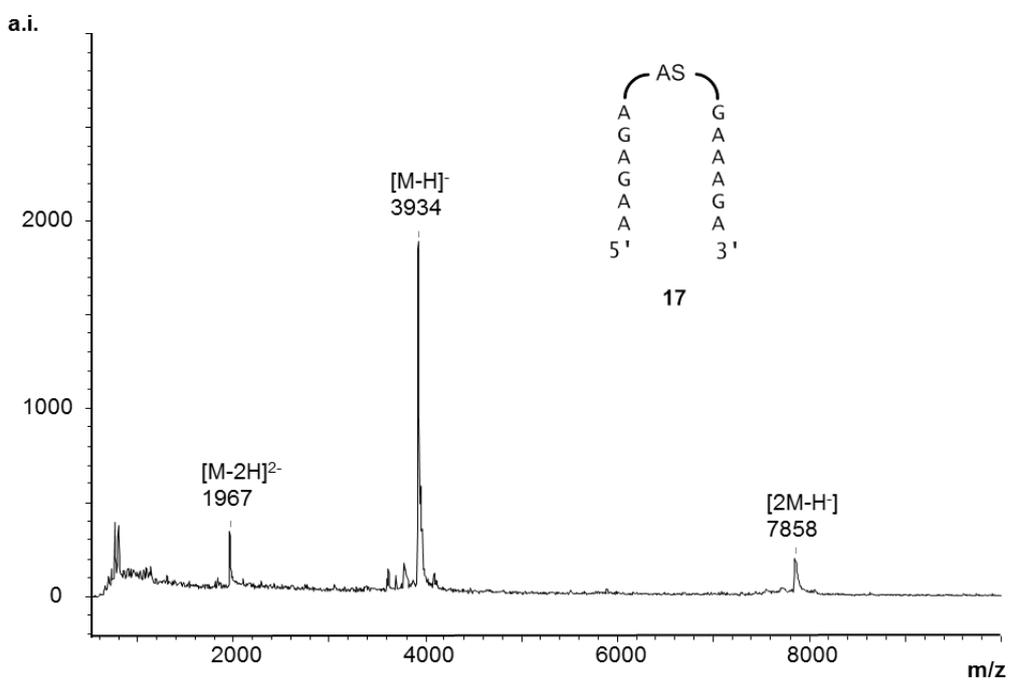


Figure S15. MALDI spectrum of motif **17**. Measured in linear negative mode with matrix / comatrix: 2,4,6-trihydroxyacetophenone / diammonium hydrogen citrate in ethanol.

3. Additional UV/VIS-Spectra from Filtration Assays

Intramolecular triplex formed by **6**

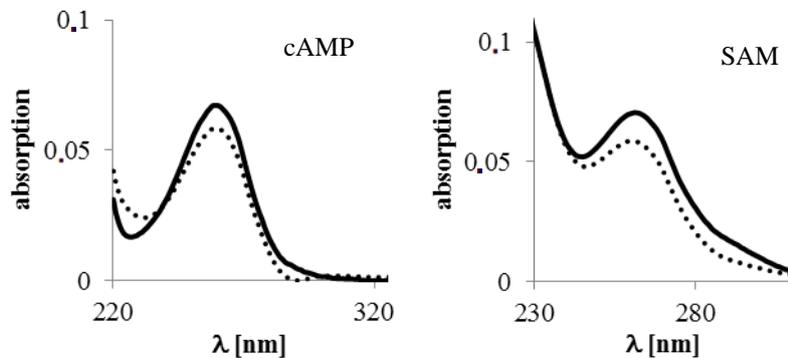
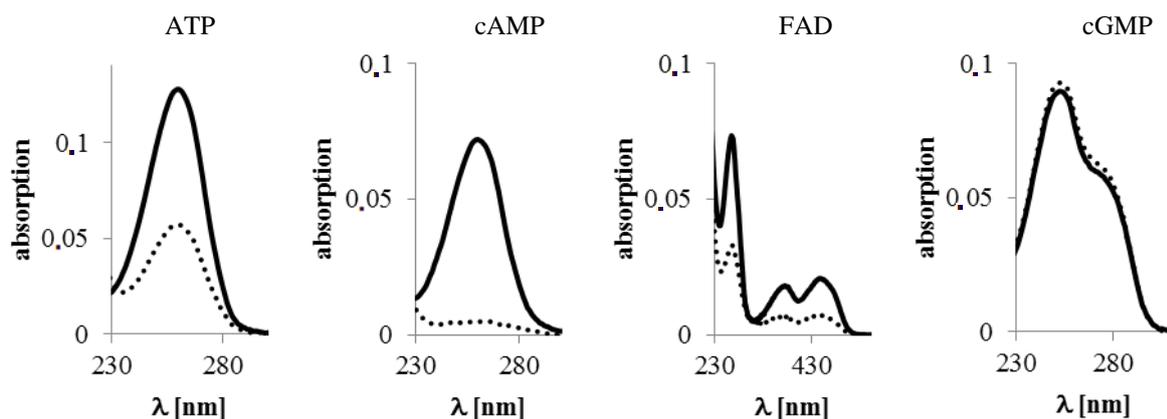
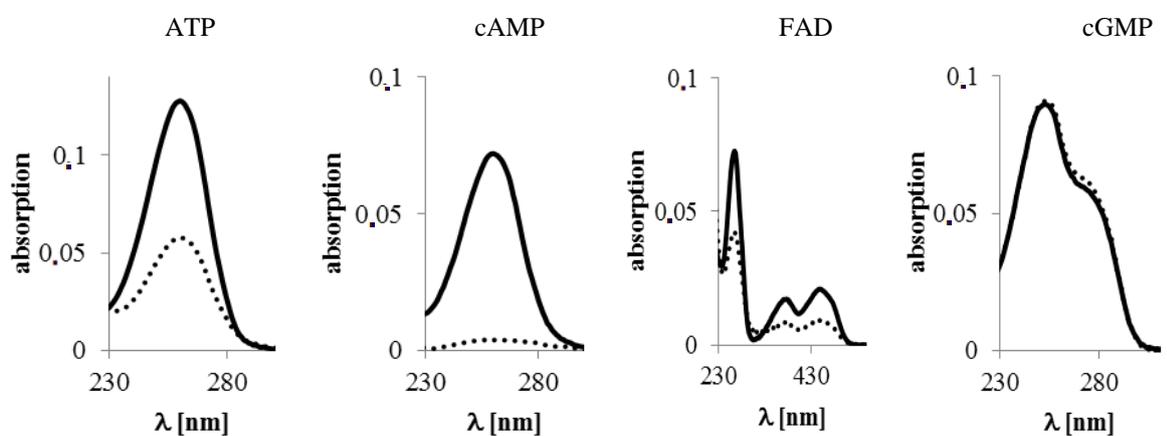


Figure S16. UV/VIS-spectra from equilibrium filtration assays with triplex **6** with cAMP or SAM as ligands. The dashed curves are spectra of filtrates, and solid curves are from a control sample containing the ligand only. Conditions: 10 μM triplex, 10 μM ligand, 10 mM phosphate buffer (pH 6.0), 1 mM NaCl.

Triplex **16:19**



Triplex **17:19**



Triplex **18:19**

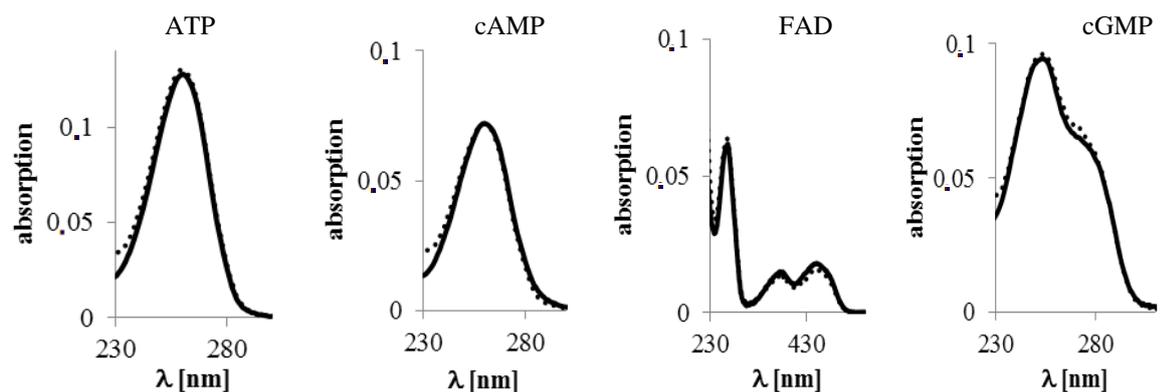


Figure S17. UV/VIS-spectra from equilibrium filtration assays with triplex **16** – **18** and **19** with ATP, cAMP, FAD or cGMP as ligands. The dashed curves are spectra of filtrates, and solid curves are from a control sample containing the ligand only. Conditions: 10 μ M triplex, 10 μ M ligand, 10 mM phosphate buffer (pH 6.0), 1 mM NaCl.

4. Additional UV-Melting Curves

Triplex **13:19** with or without cAMP, ATP, or FAD as ligands

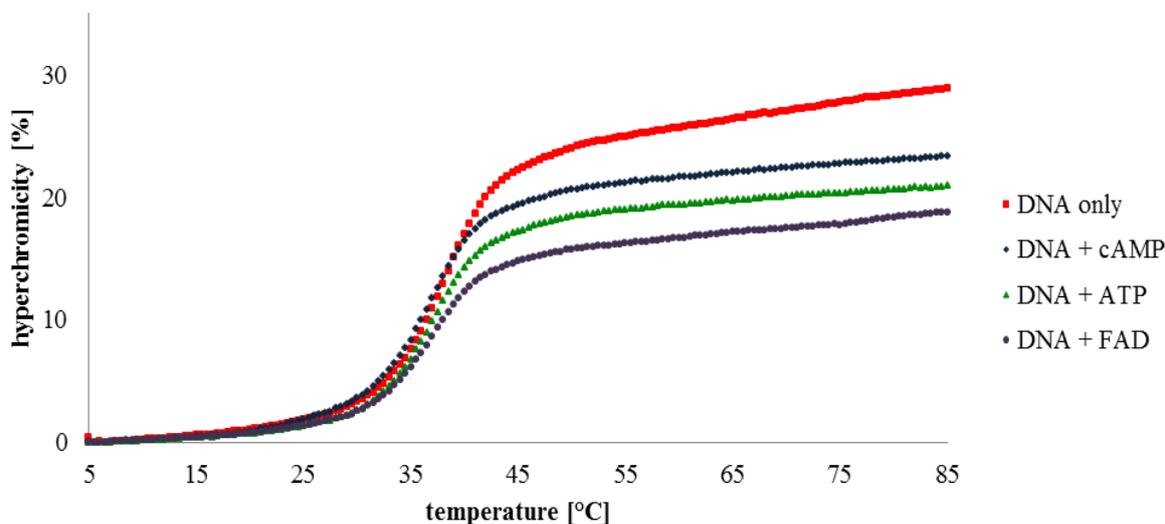


Figure S18. UV melting curves of triplex **13:19** alone and in the presence of cAMP, ATP, or FAD. Conditions: 1 μ M triplex, 5 μ M ligand, 10 mM phosphate buffer (pH=6.0), 1 mM NaCl, at 260 nm and a heating rate of 1 °C/min.

Triplex **14:19** with or without cAMP, ATP, or FAD as ligands

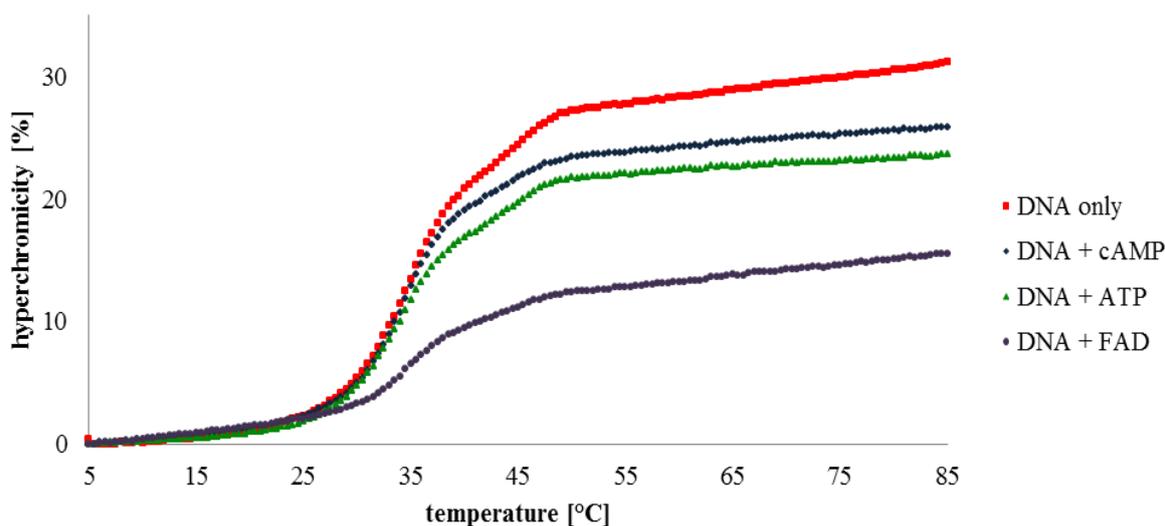


Figure S19. UV melting curves of triplex **14:19** alone and in the presence of cAMP, ATP, or FAD. Conditions: 1 μ M triplex, 5 μ M ligand, 10 mM phosphate buffer (pH=6.0), 1 mM NaCl, at 260 nm and a heating rate of 1 °C/min.

Triplex **15:19** with or without cAMP, ATP, or FAD as ligands

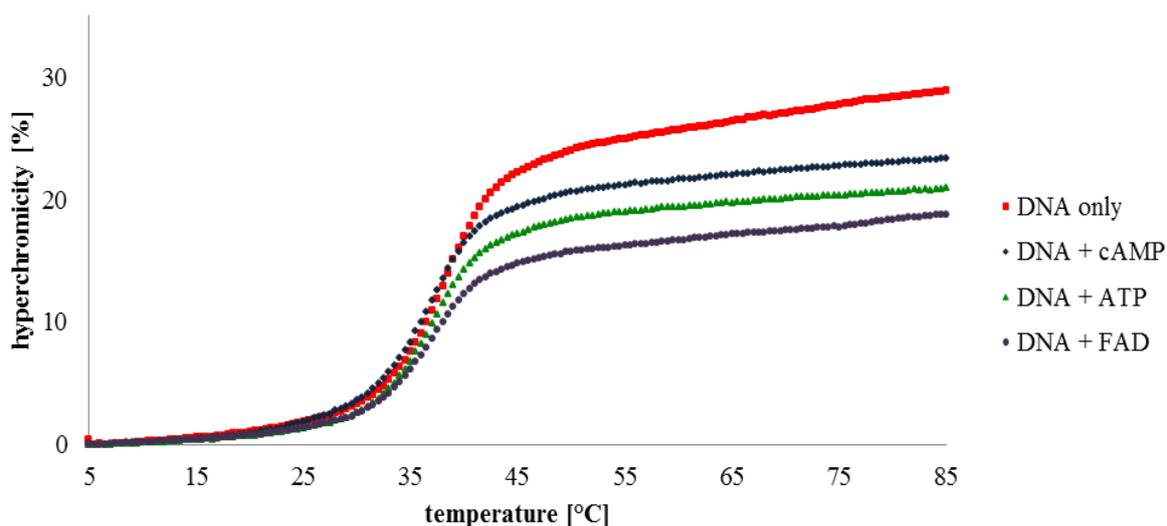


Figure S20. UV melting curves of triplex **15:19** alone and in the presence of cAMP, ATP, or FAD. Conditions: 1 μ M triplex, 5 μ M ligand, 10 mM phosphate buffer (pH=6.0), 1 mM NaCl, at 260 nm and a heating rate of 1 °C/min.

Triplex **16:19** with or without cAMP, ATP, or FAD as ligands

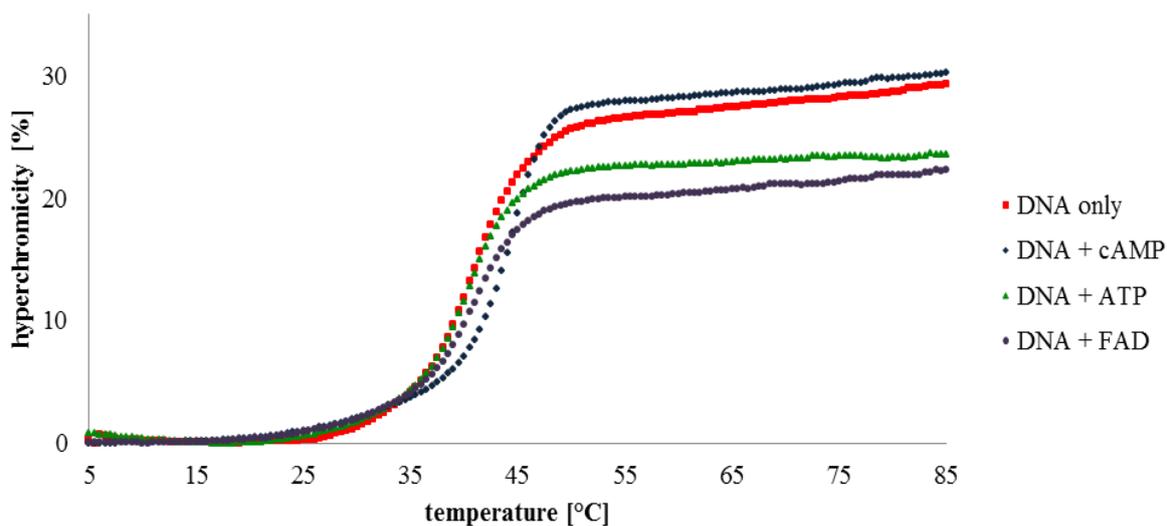


Figure S21. UV melting curves of triplex **16:19** alone and in the presence of cAMP, ATP, or FAD. Conditions: 1 μ M triplex, 5 μ M ligand, 10 mM phosphate buffer (pH=6.0), 1 mM NaCl, at 260 nm and a heating rate of 1 °C/min.

Triplex **17:19** with or without cAMP, ATP, or FAD as ligands

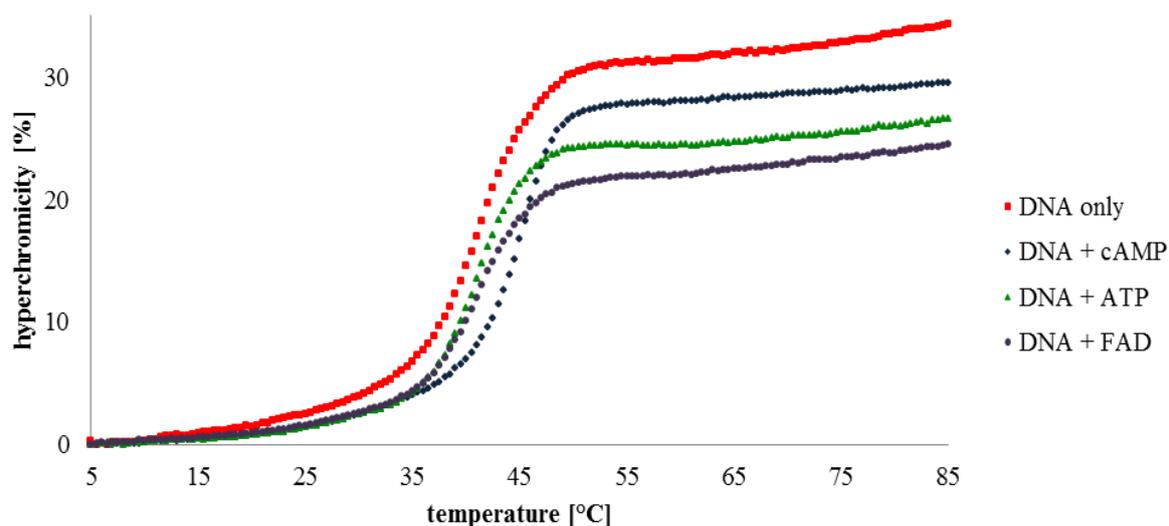


Figure S22. UV melting curves of triplex **17:19** alone and in the presence of cAMP, ATP, or FAD. Conditions: 1 μ M triplex, 5 μ M ligand, 10 mM phosphate buffer (pH=6.0), 1 mM NaCl, at 260 nm and a heating rate of 1 $^{\circ}$ C/min.

Triplex **18:19** with or without cAMP, ATP, or FAD as ligands

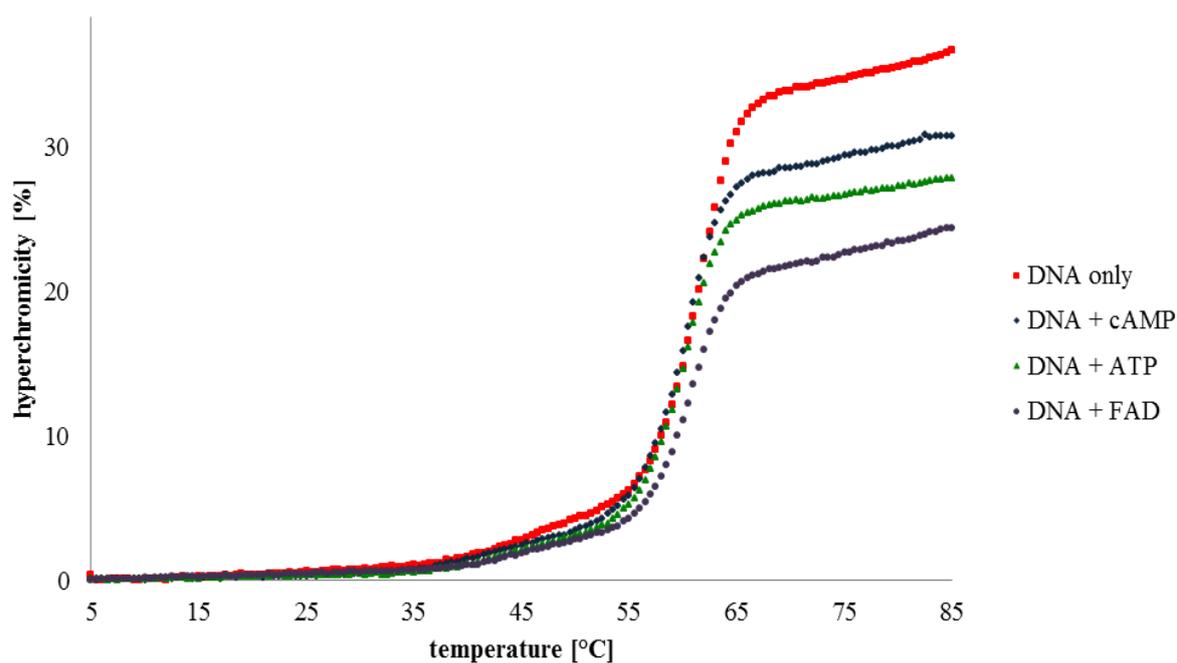


Figure S23. UV melting curves of triplex **18:19** alone and in the presence of cAMP, ATP, or FAD. Conditions: 1 μ M triplex, 5 μ M ligand, 10 mM phosphate buffer (pH=6.0), 1 mM NaCl, at 260 nm and a heating rate of 1 $^{\circ}$ C/min.

5. Effect of Magnesium Ions and pH 7.0 on UV-Melting Points

Table S1. UV-Melting points (T_m 's) of triplexes of **17** and **19** in the presence or in the absence of cAMP at pH values of 6.0 or 7.0, with or without $MgCl_2$ in the buffer.^a

Entry No.	Sequence	c($MgCl_2$) [mM]	Melting Point [°C]	
			pH = 6	pH = 7
1	17:19	0	40.6	33.1
2	17:19 , cAMP	0	44.7	38.7
3	17:19	10	41.9	34.5
4	17:19 , cAMP	10	46.0	40.0

^a Conditions: 1 μ M DNA, 5 μ M cAMP, 10 mM phosphate buffer (pH = 6.0 or pH = 7.0), 1 M NaCl, heating rate 1 °C/min. Single UV transitions, most probably encompassing the triplex-to-duplex and duplex-to-single-strand transitions were observed.

6. Confirming Binding of cAMP by RP-HPLC

Binding of cAMP by the triplex of **17** and **19** was confirmed by RP-HPLC analysis of the complex. For this, equilibrium filtration of an aqueous solution containing **17**, **19**, and cAMP was performed, as described in the Experimental Part of the main Paper under *Binding Constants via Equilibrium Filtration*. The complex retained by the filter was harvested. The resulting solution was then injected into the HPLC instrument. Prior to this, a reference solution of cAMP had been injected to establish its retention time and to serve as standard for quantitation. The HPLC conditions (55°C and increasing acetonitrile content) are denaturing, so each of the components of the ternary complex elutes separately. Figure S25 shows the resulting chromatogram.

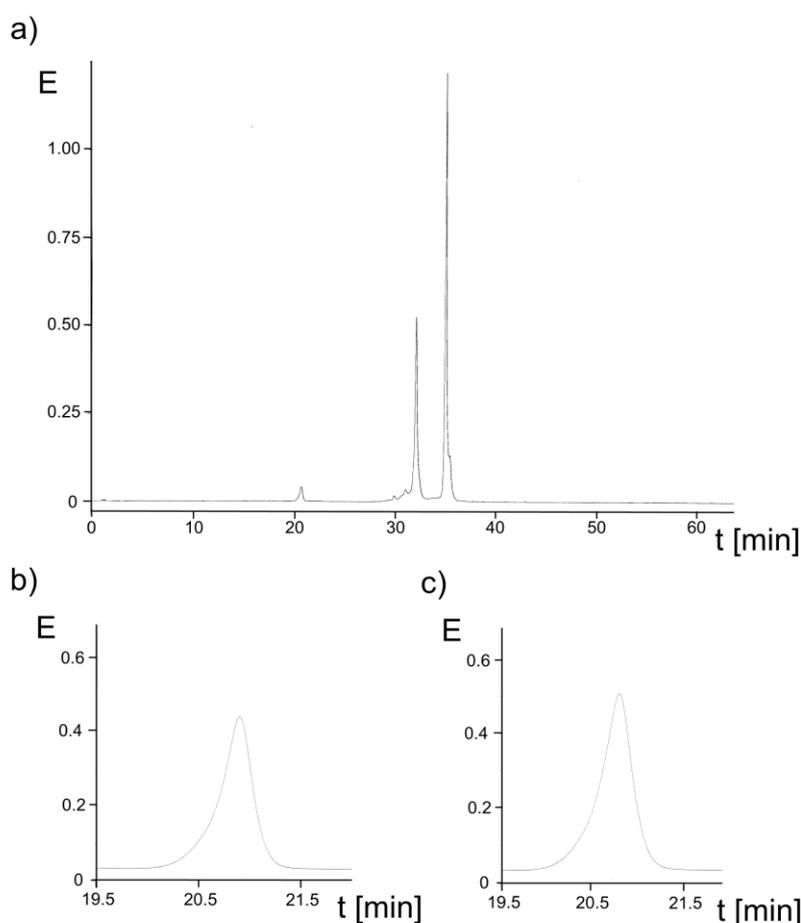


Figure S25. Confirming binding of cAMP by RP-HPLC (C4 column). a) Chromatogram of the complex obtained by equilibrium filtration (3 kDa cut-off), as measured with elution at 55°C column temperature and a gradient of 0% acetonitrile for 5 min, followed by 0% to 10% acetonitrile in 20 min, 10% to 20% acetonitrile in 25 min, 20% to 25% in 10 min, and 25% to 80% in 5 min. $t_{R, \text{cAMP}} = 21$ min. b) Expansion of the region showing the cAMP peak. c) The same region of a reference chromatogram of cAMP alone. Manual integration of the peak for cAMP gave a dissociation constant (K_d) of approx. 0.1 μM , with an estimated error of ± 0.05 μM .