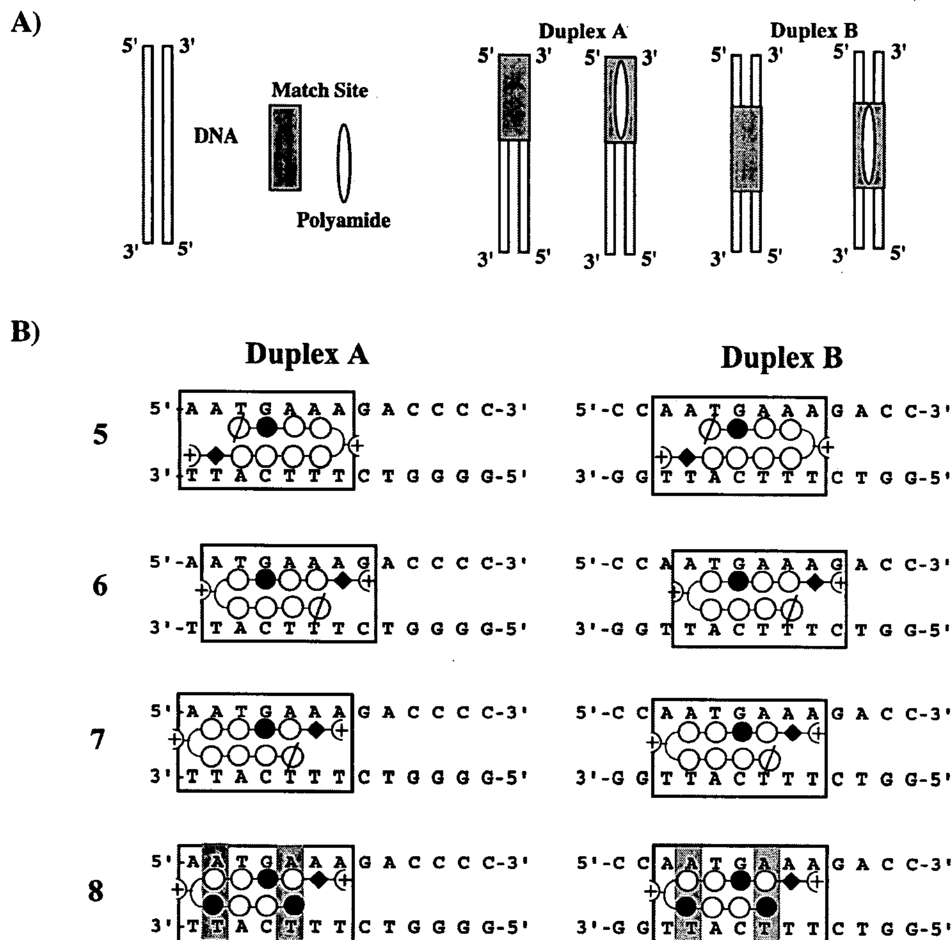
Supporting information World Wide Web Edition

Figure S1



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Supporting Information, Table S1

Table S1. DNA melting with internal and terminal polyamide binding sites.[a]

	Ka (DNase I)[b] (M^{-1})	Tm Duplex **B**[c] (C)	Tm Duplex **A**[d] (C)	ΔTm **B**[c] (C)	ΔTm **A**[d] (C)	ΔΔTm[e] (C)	% ΔΔTm/ ΔTm **B**
---	---	45.9	46.9	---	---	---	---
5	$4.0x10^{10}$	69.9	68.0	24.0	21.1	2.9	12%
6	$1.7x10^{10}$	67.5	66.4	21.6	19.5	2.1	10%
7	$3.0x10^{9}$	63.0	62.5	17.1	15.6	1.5	9%
8	$\leq 5x10^{6}$	54.8	53.9	8.9	7.0	1.9	21%

Supporting Information, Figure Legends

Figure S1 A) Cartoon of the DNA melting experiments. The same binding site is placed at the end and middle of two short DNA duplexes. **B)** Sequences of the duplexes **A** and **B** with ball-and-stick models for the polyamides showing the binding site in a box and mismatches in gray. Duplex **A** represents the terminal 13 bp of the M-MuLV LTR, 5'-AATGAAAGACCCC-3'. Duplex **B** has the same nucleotide composition, with two base pairs moved from the 3' to the 5' end of the duplex, which shifts the binding site of the polyamides away from the end of the duplex. Polyamide **5** places Dp against position 1 of duplex **A** and position 3 of duplex **B**. Polyamide **6** places the turn ($(R)^{H_2N}\gamma$) against position 2 of duplex **A** and position 4 of duplex **B**. Polyamide **7** places the turn ($(R)^{H_2N}\gamma$) against position 1 of duplex **A** and position 3 of duplex **B**. Polyamide **8** is a double base pair mismatch for both duplexes.

Table 2.2 [a]Melting curves were obtained by standard UV-vis spectroscopy (80° C—15° C, 1° C/minute, scan at 260 nM every 30 seconds) for both duplexes (2 μM) in the absence and presence of 2 μM polyamide (**5-8**). These experiments were preformed using buffered conditions (10 mM sodium cacodylate, 10 mM KCl, 10 mM $MgCl_2$, 5 mM $CaCl_2$) designed by Pilch *et al.* to be similar to those used for DNase I footprinting titrations (*47*). [b]See Table 1 and Experimental Procedures. [c]ΔTm **B** is defined as the difference in melting temperature of duplex **B** with the addition of polyamide. [d]ΔTm **A** is defined as the difference in melting temperature of duplex **A** with the addition of polyamide. [e]ΔΔTm is defined as ΔTm **B** - ΔTm **A**. All polyamides tested increased the stability of both duplexes as measured by an increase in the melting temperature (Tm) of the duplex in the presence of the polyamide. For duplex **B** with the internal match site, the ΔTm for match polyamides was significantly higher than the mismatch polyamide and the order of increasing ΔTm matched the order of increasing Ka as measured by DNase I footprinting (**8**<<**7**<**6**<**5**). The same order was observed for duplex **A**, and the ΔTm of the match polyamides were all significantly higher than the mismatch. This indicates that polyamides can bind specifically to a match site at the absolute end of a DNA fragment with high affinity, although in each case ΔTm **A** was slightly lower than ΔTm **B**. For each of the match polyamides, the ΔΔTm was approximately 10% of ΔTm **B**. A rough calculation using the relationship $\Delta Tm \propto \ln Ka$ suggests that polyamides **5-7** have a 3-fold lower affinity for the terminal match site as compared to an internal match site.