Supporting Information

Next Generation Hairpin Polyamides with

(*R*)-3,4-Diaminobutyric Acid Turn Unit

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Construction of Plasmids pCDMF-1 and pCDMF-2: Oligonucleotides were purchased from Integrated DNA Technologies. The plasmids pCDMF-1 and pCDMF-2 were constructed by annealing the oligonucleotides: 5'-AGCTGCGGCTCGAGACGGCTAACCCATCGAGACGGCTAGCCCATCG AGACGGCTATCCCATCGAGACGGCTACCCCATCGAGAGGATC-3' and 5-GATCGATCCTCT CGATGGGGTAGCCGTCTCGATGGGATAGCCGTCTCGATGGGCTAGCCGTCTCGATGGGTTA GCCGTCTCGAGCCGC-3'; 5'-AGCTGCGAGACGGCTCGAGACGGCTTGAACATCGAGACGG CTCGAGACGGCTTGACCATCGAGACGGCTCGAGACGGCTC-3' and 5-GATCGAGCCGTCTCG AGCCGTCTCGATGGTCAAGCCGTCTCGAGCCGTCTCGATGTTCAAGCCGTCTCGAGCCGTC TCGC-3', respectively, followed by ligation into the BamHI/HindIII restriction fragment of pUC19 using T4 DNA ligase. The plasmid was then transformed into Escherichia coli JM109 competent cells. Ampicillin-resistant white colonies were selected from 25 mL Luria–Bertani (LB) agar plates containing 50 mg/mL ampicillin treated with XGAL and isopropyl-β-D-thiogalactopyranoside (IPTG) solutions and grown overnight at 37 °C. Cells were harvested the following day and purification of the plasmid was performed with a Wizard Plus Midiprep DNA purification kit (Promega). DNA sequencing of the plasmid insert was performed by the sequence analysis facility at the California Institute of Technology.

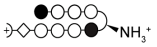
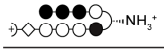
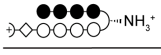
DNase I Footprinting Titrations: Polyamide equilibrations and DNase I footprint titrations were conducted on the 5′ end-labeled PCR product of pCDMF-1 and pCDMF-2 according to standard protocols.[12] DNA was incubated with polyamides or water (control) for 12 h at room temperature prior to reaction with DNase I.

Table S1. Melting temperatures of DNA/polyamide complexes for all four base pair variations at the turn position of hairpin polyamides.[a]

	A•T		T•A		C•G		G•C	
	5′-CGA **TGGTC<u>A</u>** AGC-3′		5′-CGA **TGGTC<u>T</u>** AGC-3′		5′-CGA **TGGTC<u>C</u>** AGC-3′		5′-CGA **TGGTC<u>G</u>** AGC-3′	
Polyamides	T_m / °C	ΔT_m / °C	T_m / °C	ΔT_m / °C	T_m / °C	ΔT_m / °C	T_m / °C	ΔT_m / °C
—	57.2 (±0.1)	—	55.8 (±0.1)	—	59.7 (±0.3)	—	60.4 (±0.2)	—
 (**5**)	70.6 (±0.2)	13.4	69.0 (±0.3)	13.2	65.9 (±0.3)	6.2	64.3 (±0.1)	3.9
 (**6**)	74.1 (±0.3)	16.9	72.9 (±0.2)	17.1	67.3 (±0.2)	7.6	64.7 (±0.2)	4.3
 (**7**)	76.1 (±0.2)	18.9	73.2 (±0.1)	17.4	69.7 (±0.3)	10.0	66.1 (±0.2)	5.7
 (**8**)	77.5 (±0.3)	20.3	74.2 (±0.1)	18.4	70.1 (±0.1)	10.4	66.8 (±0.2)	6.4

[a]All values reported are derived from at least three melting temperature experiments with standard deviations indicated in parentheses. ΔT_m-values are given as $T_m^{(DNA/polyamide)}$ - $T_m^{(DNA)}$.

Table S2. Equilibrium association constants for hairpin polyamides determined by quantitative DNase I footprint titrations.[a]

Polyamides		A•T 5′-CGA **TGTTCA** AGC-3′ K_a / M^{-1}	T•A 5′-CGA **TGTTCT** AGC-3′ K_a / M^{-1}
	(**1**)	3.0 (±0.8) x 10^{10} [b]	n. d.
	(**2**)	2.6 (±0.6) x 10^{10}	n. d.
	(**3**)	2.1 (±0.1) x 10^{10}	n. d.
	(**4**)	2.7 (±0.3) x 10^{10}	n. d.
		5′-CGA **TGGTCA** AGC-3′	5′-CGA **TGGTCT** AGC-3′
	(**5**)	1.3 (±0.7) x 10^{10} [b]	n. d.
	(**6**)	3.1 (±0.5) x 10^{10}	n. d.
	(**7**)	2.4 (±0.3) x 10^{10}	n. d.
	(**8**)	2.3 (±0.3) x 10^{10}	n. d.
		5′-CGA **TGGGCA** AGC-3′	5′-CGA **TGGGCT** AGC-3′
	(**9**)	n. d.	n. d.
	(**10**)	n. d.	1.5 (±0.2) x 10^{10}
	(**11**)	n. d.	3.0 (±0.4) x 10^{9}
	(**12**)	n. d.	5.9 (±0.9) x 10^{9}
		5′-CGA **TGGGGA** AGC-3′	5′-CGA **TGGGGT** AGC-3′
	(**13**)	2.8 (±0.2) x 10^{7} [b]	n. d.
	(**14**)	n. d.	6.6 (±1.8) x 10^{9}
	(**15**)	n. d.	9.4 (±3.0) x 10^{7}
	(**16**)	n. d.	2.1 (±0.6) x 10^{8}

[a]Equilibrium association constants reported are mean values from at least three quantitative DNase I footprint titration experiments. Standard deviations are shown in parentheses. [b]Equilibrium association constants have been reported previously [11] (n. d. = not determined).

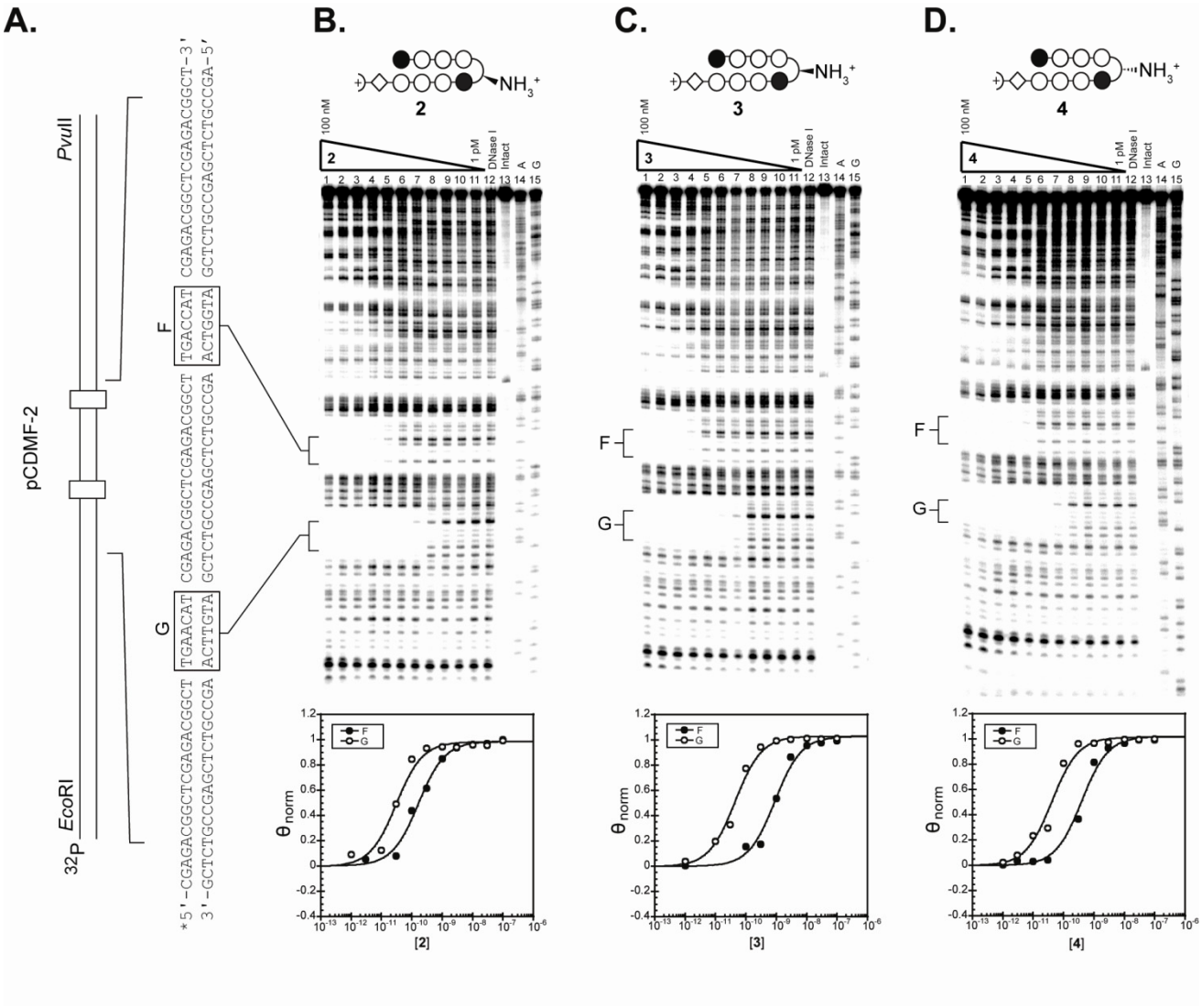


Figure S1. Quantitative DNase I footprint titration experiments for polyamides **2**, **3**, and **4** on the 285 base pair, 5′ end-labeled PCR product of plasmid pCDMF-2: lanes 1-11, 100 nM, 30 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, 10 pM, 3 pM, and 1 pM polyamide, respectively; lane 12, DNase I standard; lane 13, intact DNA; lane 14, A reaction; lane 15, G reaction.

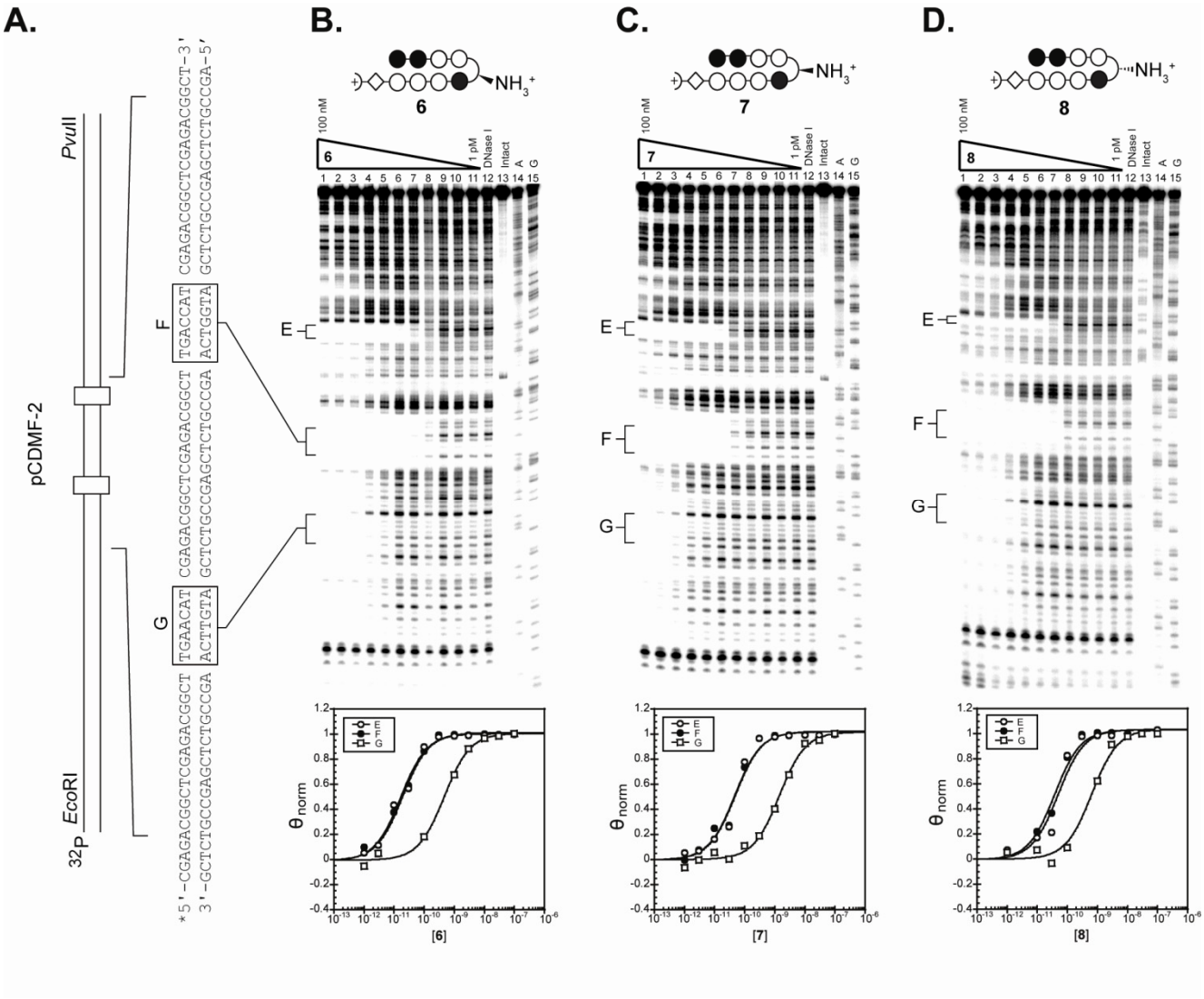


Figure S2. Quantitative DNase I footprint titration experiments for polyamides **6**, **7**, and **8** on the 285 base pair, 5′ end-labeled PCR product of plasmid pCDMF-2: lanes 1-11, 100 nM, 30 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, 10 pM, 3 pM, and 1 pM polyamide, respectively; lane 12, DNase I standard; lane 13, intact DNA; lane 14, A reaction; lane 15, G reaction.

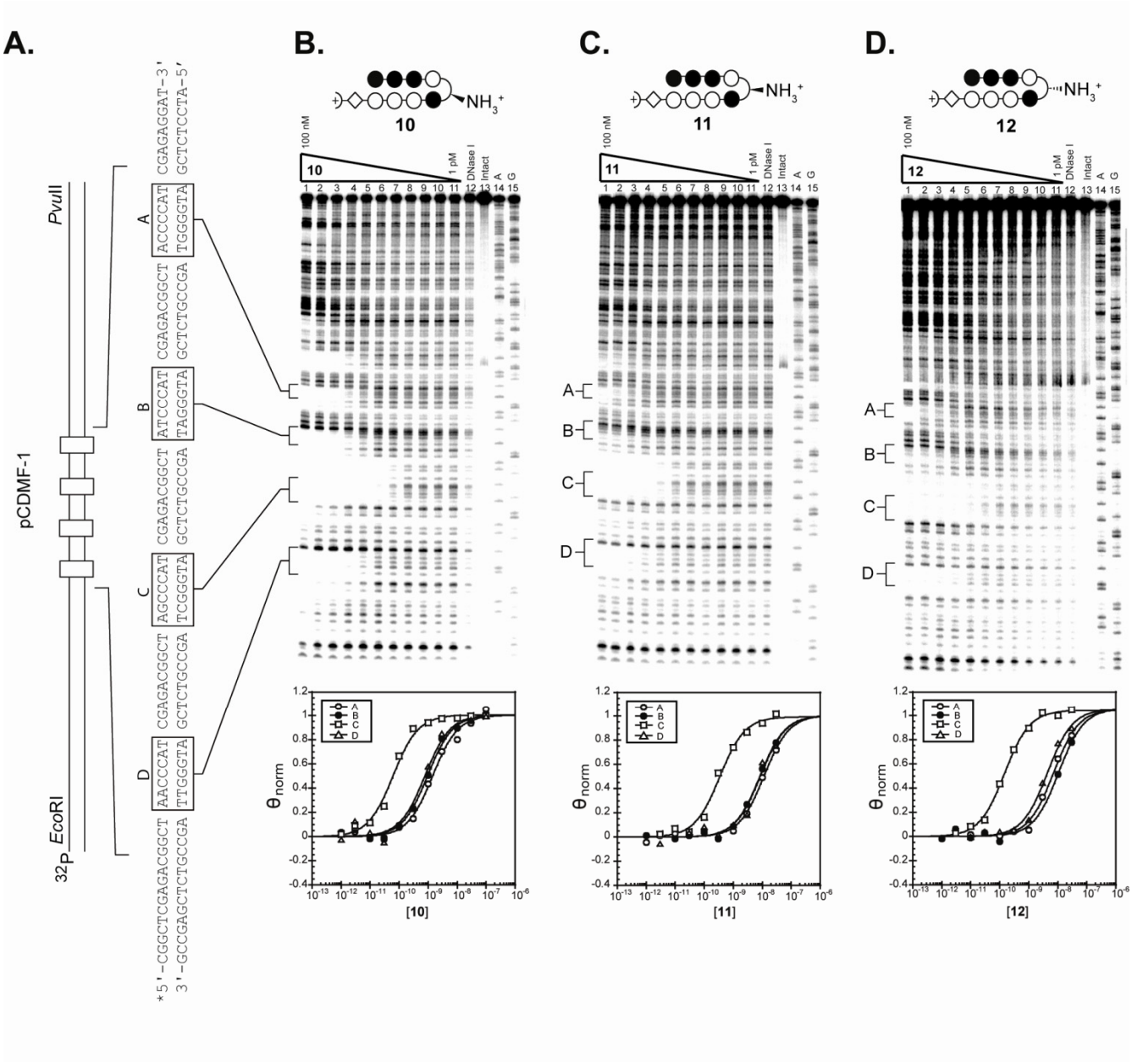


Figure S3. Quantitative DNase I footprint titration experiments for polyamides **10**, **11**, and **12** on the 293 base pair, 5′ end-labeled PCR product of plasmid pCDMF-1: lanes 1-11, 100 nM, 30 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, 10 pM, 3 pM, and 1 pM polyamide, respectively; lane 12, DNase I standard; lane 13, intact DNA; lane 14, A reaction; lane 15, G reaction.

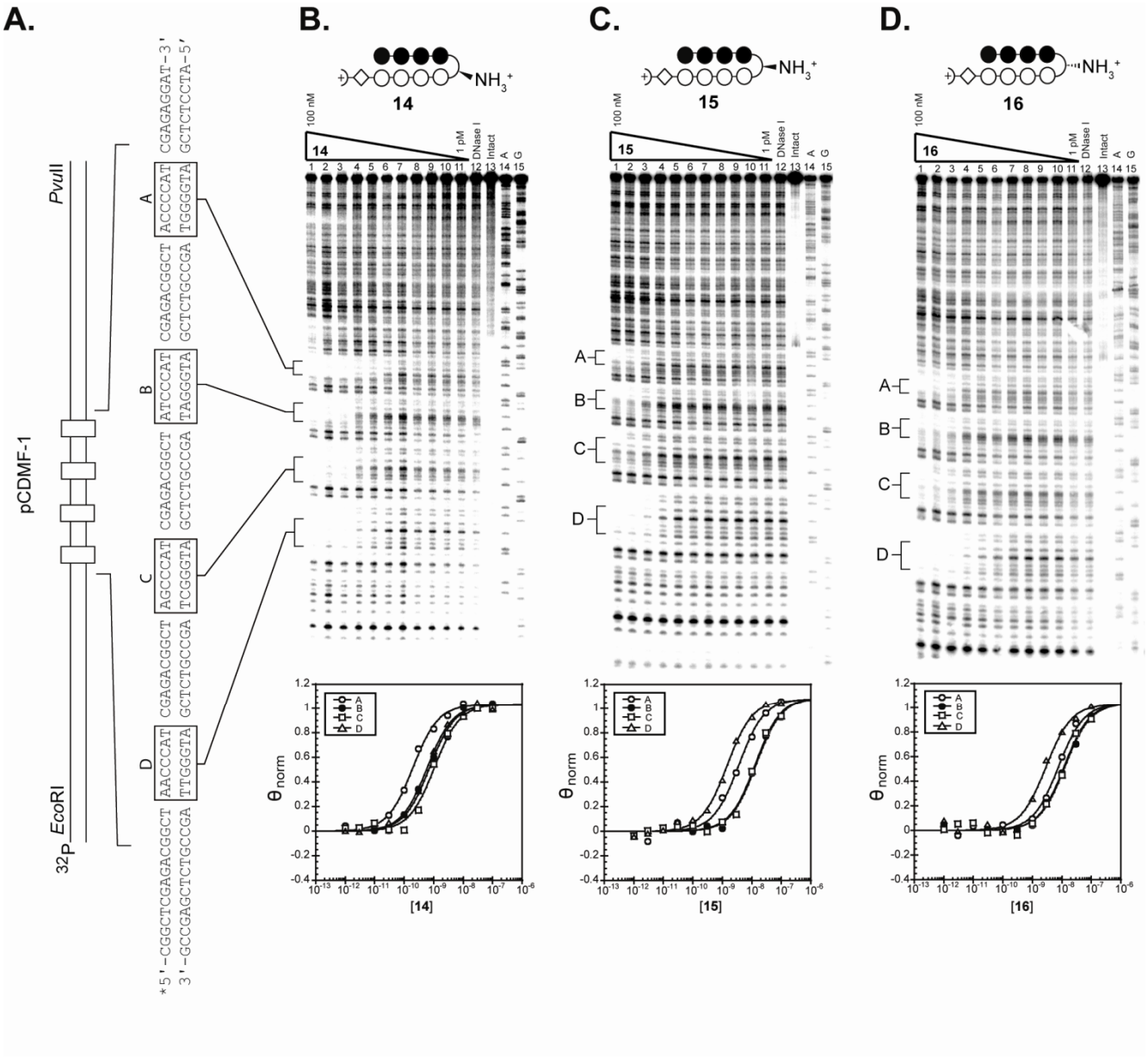


Figure S4. Quantitative DNase I footprint titration experiments for polyamides **14**, **15**, and **16** on the 293 base pair, 5′ end-labeled PCR product of plasmid pCDMF-1: lanes 1-11, 100 nM, 30 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, 10 pM, 3 pM, and 1 pM polyamide, respectively; lane 12, DNase I standard; lane 13, intact DNA; lane 14, A reaction; lane 15, G reaction.

Table S3. Melting temperatures of polyamides targeted to DNA-sequence 5′-AGAACA-3′ in complex with DNA.[a]

DNA sequence = 5′-TTGC **AGAACA** GCAA-3′			
Polyamides		T_m / °C	ΔT_m / °C
–		60.1 (±0.2)	–
	(**20**)	74.4 (±0.2)	14.3
	(**21**)	76.3 (±0.2)	16.2
	(**22**)	64.6 (±0.1)	4.5
	(**23**)	66.9 (±0.1)	6.8

[a]All values reported are derived from at least three melting temperature experiments with standard deviations indicated in parentheses. ΔT_m-values are given as $T_m^{(DNA/polyamide)}$ - $T_m^{(DNA)}$.

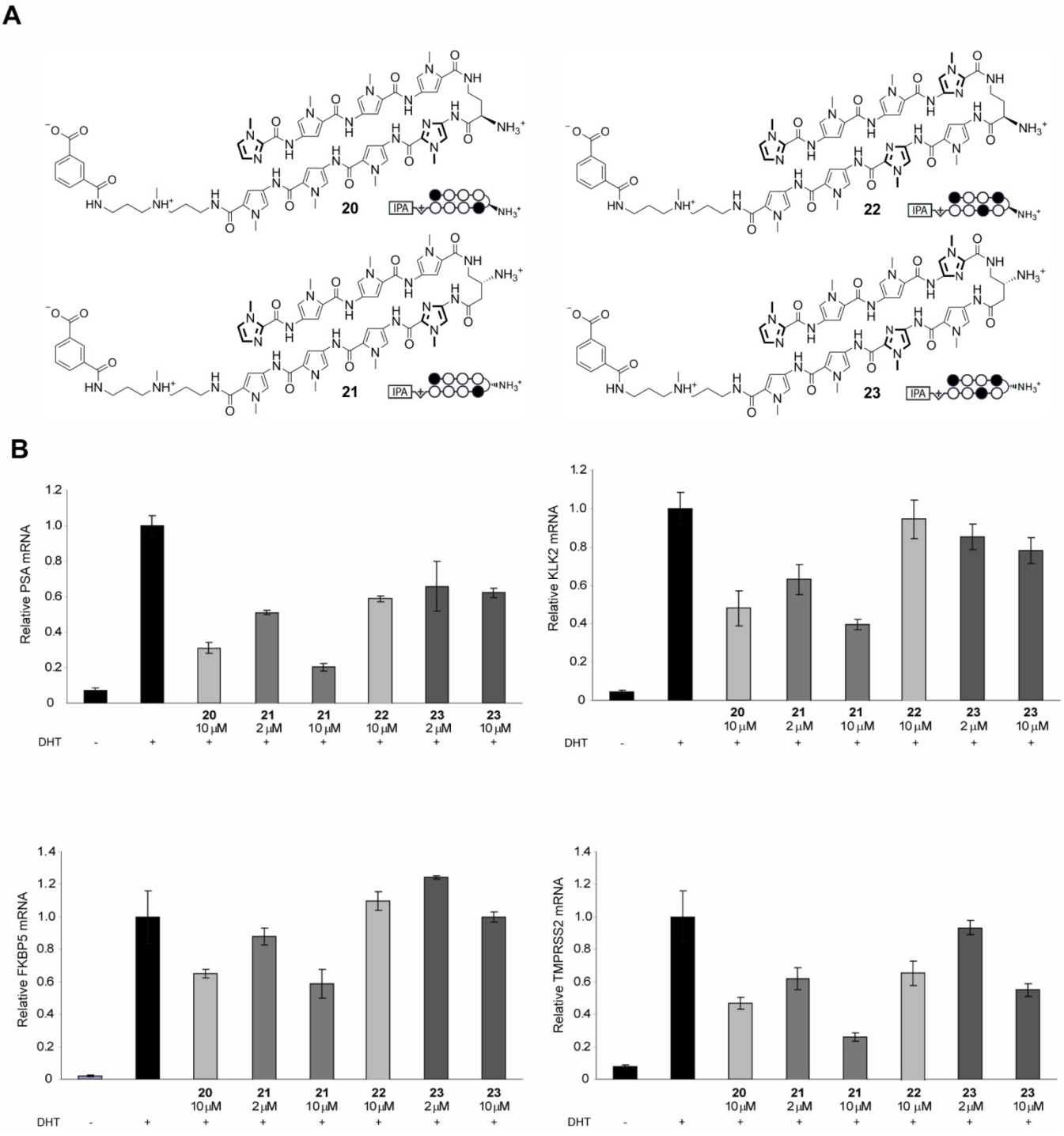


Figure S5. A) Chemical structures and ball-and-stick models of matched and mismatched polyamides **20**-**23**, respectively, targeted to 5′-AGAACA-3′. B) Inhibition of DHT-induced PSA, KLK2, FKBP5, and TMPRSS2 expression by **20**-**23** measured by quantitative real-time RT-PCR.