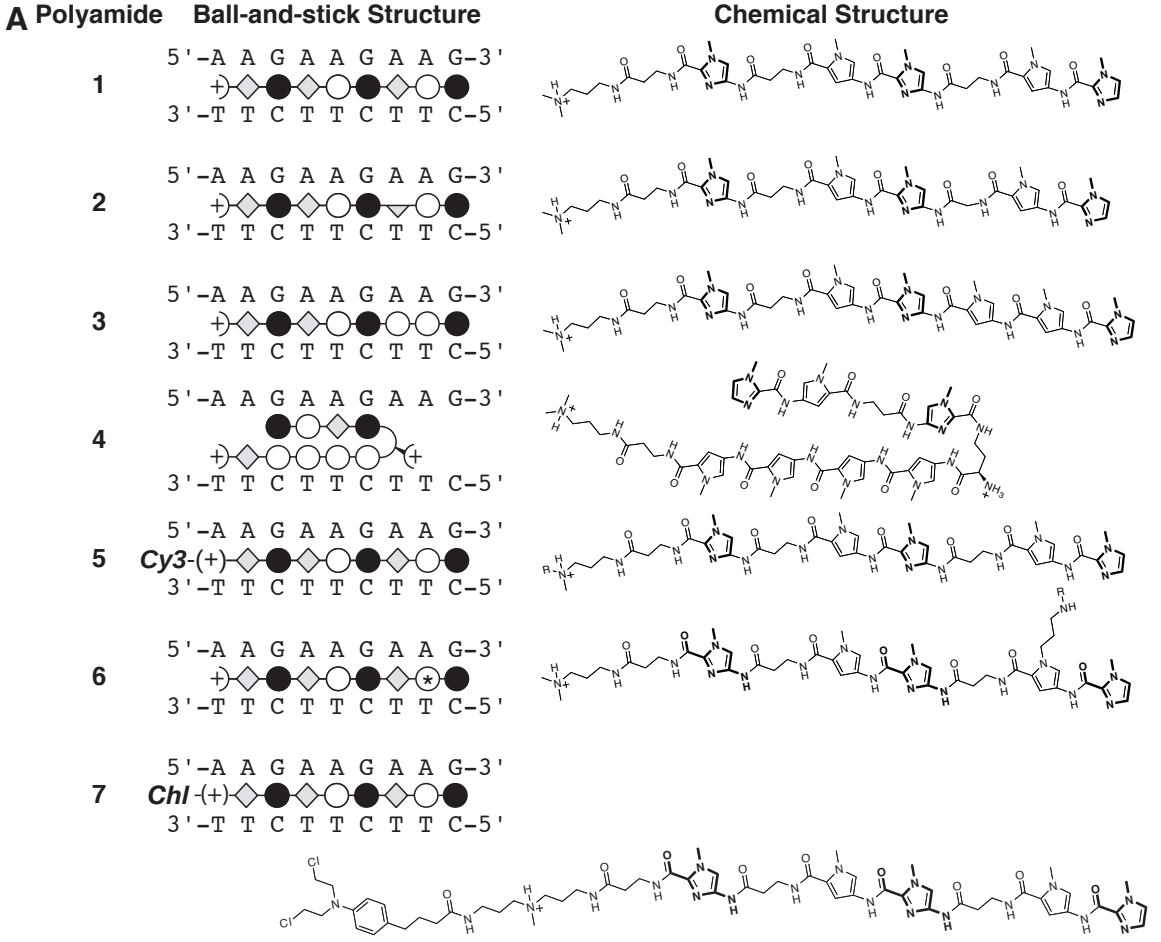
Appendix B

DNase I Footprinting and Alkylation Assays of Linear β-linked Compounds and Hairpin Polyamides Targeted to GAA Repeats

Enclosed in this appendix are several footprinting gels of linear *β*-linked polyamides and a single hairpin polyamide (overview in Figure B.1) targeted to $(AAG)_3 \cdot (CTT)_3$ (Figures B.2-B.5). A single thermal cleavage gel of chlorambucil alkylator Im-Py-*β*-Im-Py-*β*-Im-*β*-Dp-Chl (polyamide **7**) alkylating $(GAA)_{33} \cdot (TTC)_{33}$ is shown (Figure B.6). Table B.1 summarizes the findings in Figures B.2–B.5. Table B.2 summarizes MALDI-TOF Mass Spectrometric data collected for the seven compounds used in these studies.

The design of plasmid pJWP12 (Figure B.1B) was based on initial CSI motif data for polyamide **6**, in which 5'-AAGATGTTC-3' was the preferred motif, 5'-AAGAAGTTC-3' an acceptable similar site for the primary motif, and 5'-AAGAAGAAG-3' the binding site for which the polyamide was originally designed. Data from Figure B.2 shows that the affinity of **1** for the principal motif, 5'-AAGATGTTC-3' is less than that for the designed site, 5'-AAGAAGAAG-3.' Chapter 3 probes this discrepancy more in depth. The CSI microarray intensities correlate well with polyamide binding affinities but do not necessarily correlate with the sequence motif generated from the 300 highest affinity binding sites.

Polyamide alkylation studies were undertaken with polyamide **7** due to a collaboration with the Robert D. Wells laboratories at Texas A&M. Preliminary data suggested that polyamide **7** may be able to upregulate frataxin expression in GM15850 cells.



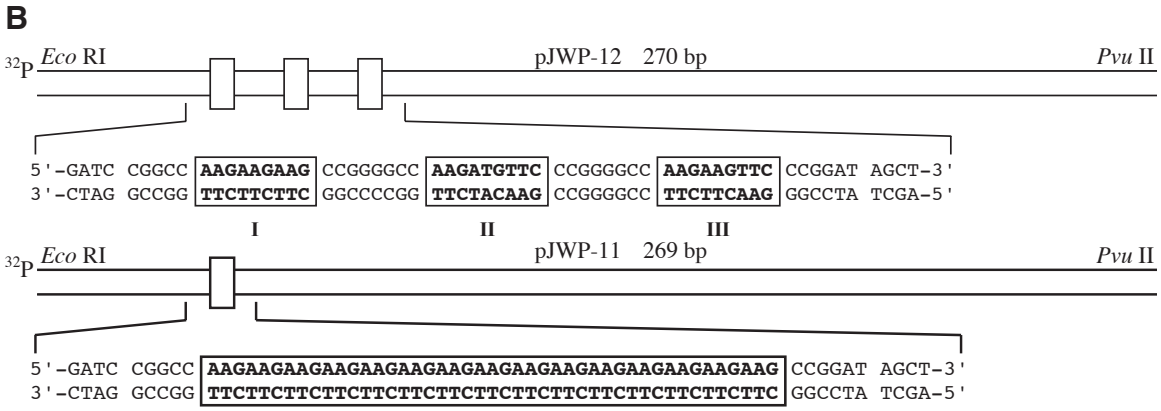


Figure B.1. Overview of Polyamides Studied on pJWP12 and pJWP11 and plasmid sequences of pJWP12 and pJWP11. a) Polyamides studied. b) Plasmid insert sequences

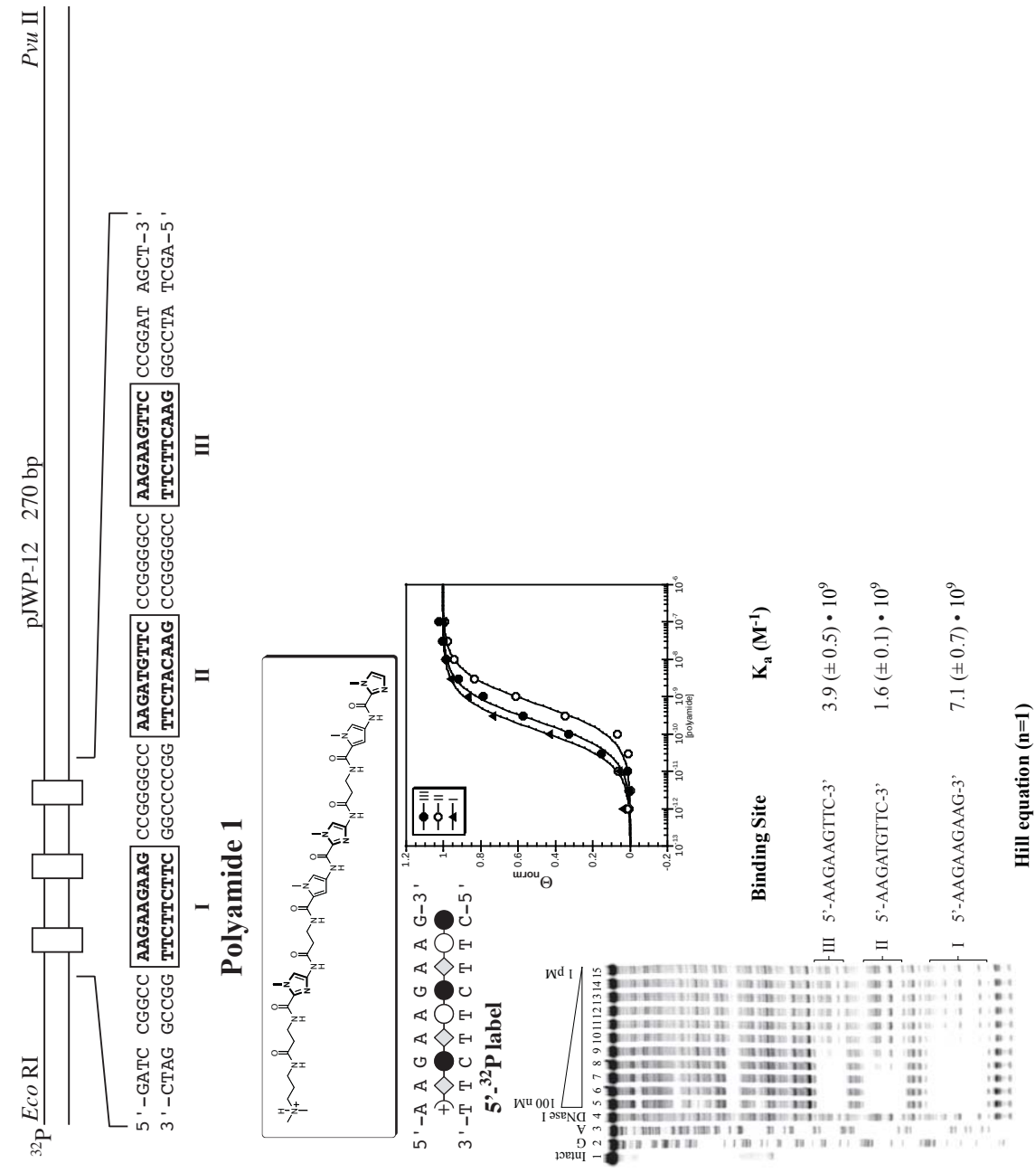


Figure B.2. Quantitative DNase I footprint of a linear *β*-linked polyamide targeting GAA repeats. CSI Motif preferred binding site **III** does not correlate with polyamide preferred binding site **I**.

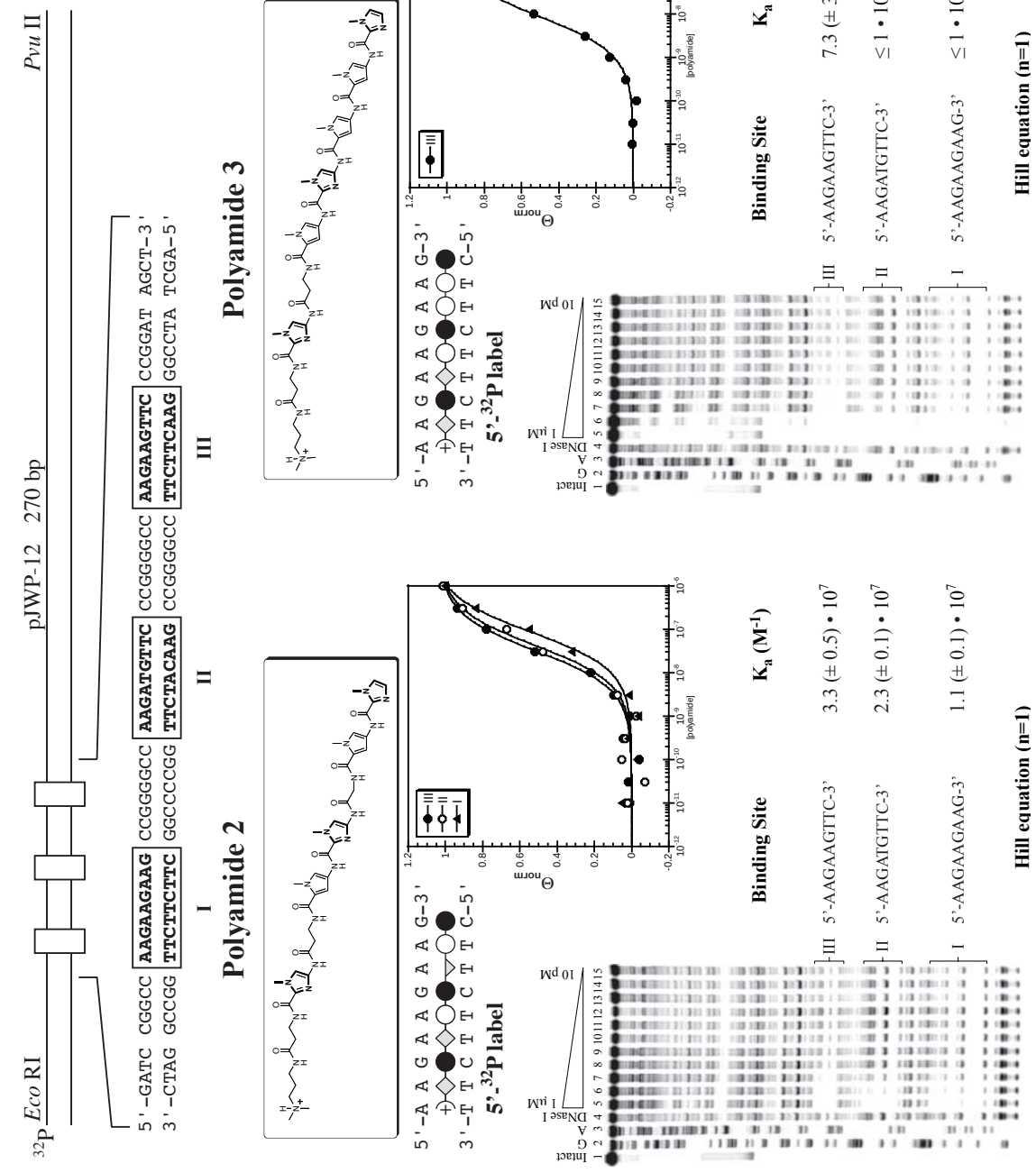


Figure B.3. Quantitative DNase I footprints for two linear *β*-linked polyamides, one containing a *β* → *Gly* point mutation and the other containing a *β* → Py point mutation

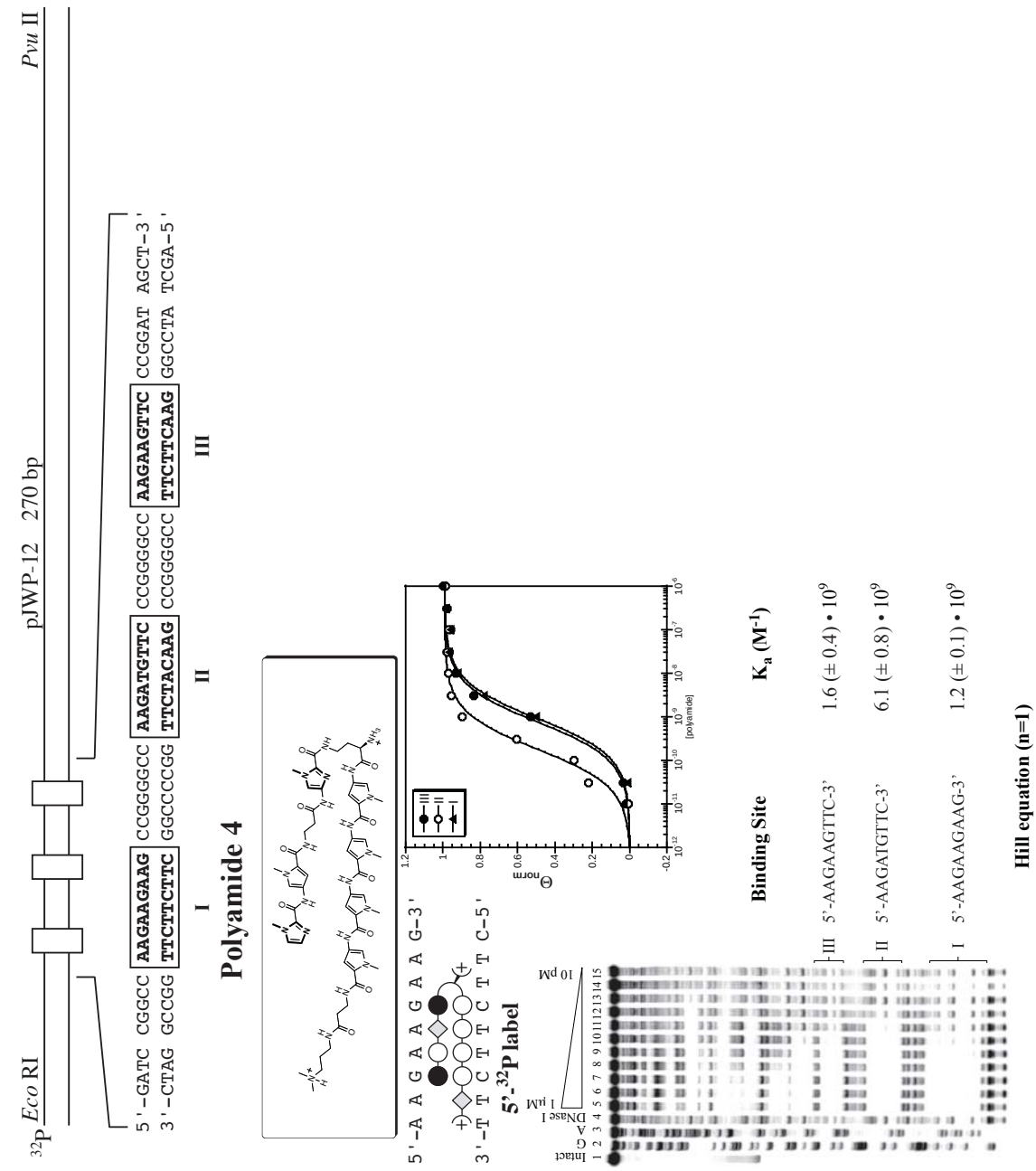


Figure B.4. Quantitative DNase I footprint of a hairpin polyamide targeting GAA repeats

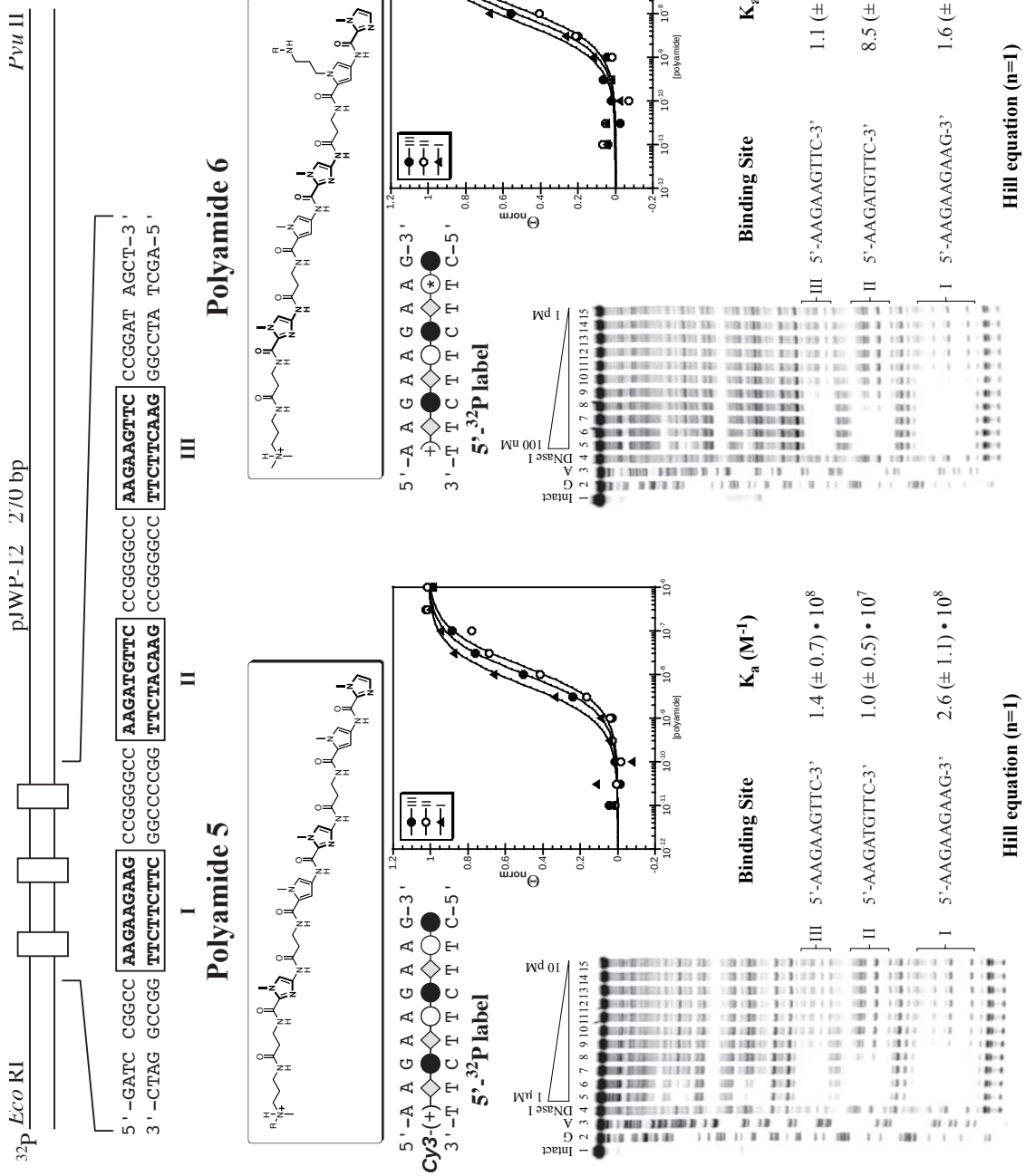


Figure B.5. Quantitative DNase I footprint of two Cy3-labeled, linear *β*-linked polyamides targeting GAA repeats

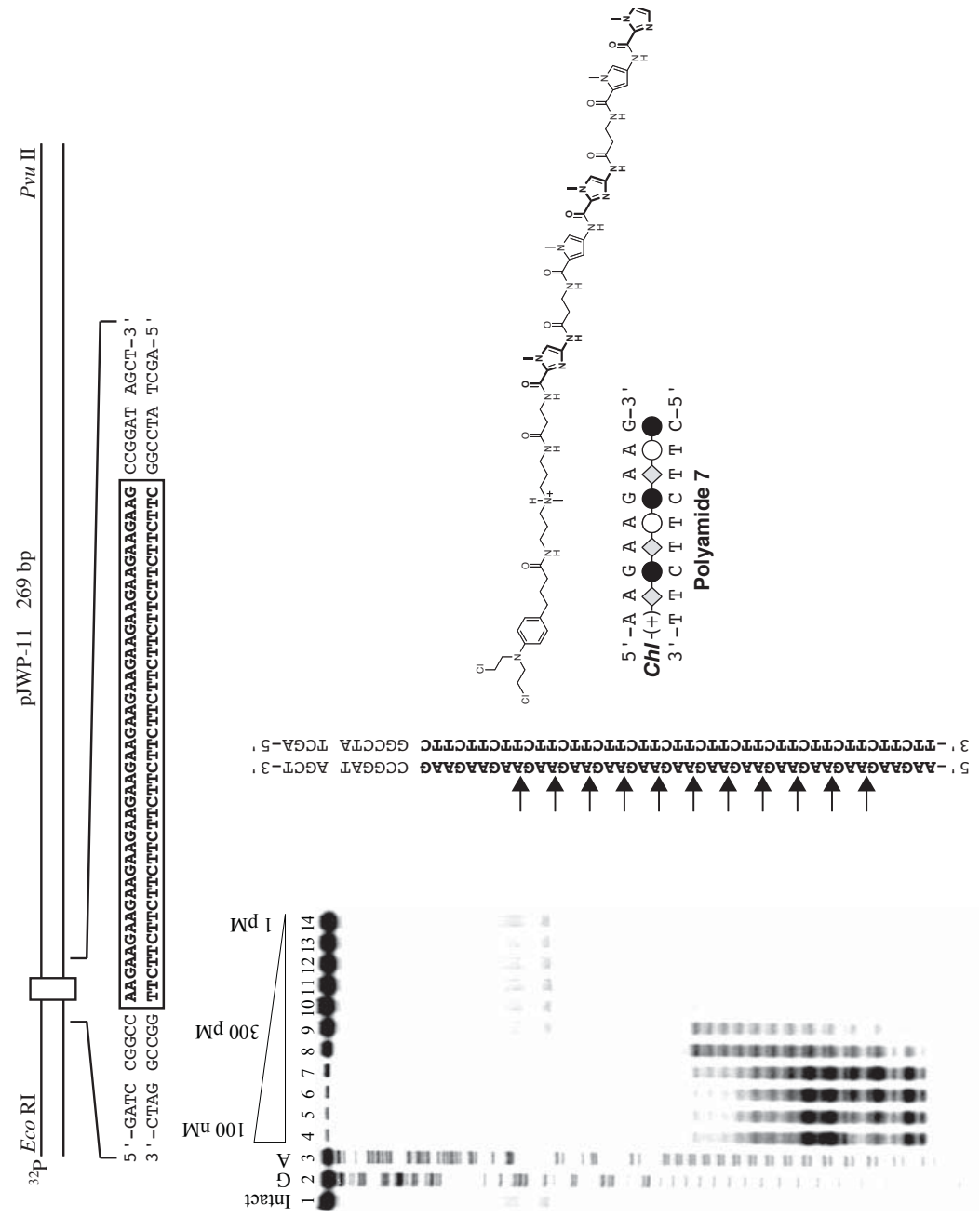


Figure B.6. A Chlorambucil-labeled GAA-targeting β-linked polyamide alkylates $(GAA)_{33}$ repeat sequences

Table B.1. Summary data from Figures B.2–B.5. All values are listed as K_a (M^{-1}) of the polyamide for the specified binding site. Bolded entries represent the highest affinity within a row

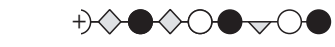
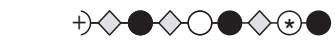
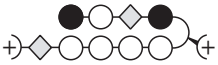
Polyamide	Ball-and-stick Structure	AAGAAGAAG	AAGATGTTC	AAGAAGTTC
1		**$7.1 (\pm 0.7) \cdot 10^9$**	$1.6 (\pm 0.1) \cdot 10^9$	$3.9 (\pm 0.5) \cdot 10^9$
2		$1.1 (\pm 0.1) \cdot 10^7$	$2.3 (\pm 0.1) \cdot 10^7$	**$3.3 (\pm 0.5) \cdot 10^7$**
3		$\leq 1 \cdot 10^7$	$\leq 1 \cdot 10^7$	**$7.3 (\pm 3.5) \cdot 10^7$**
4		$1.2 (\pm 0.1) \cdot 10^9$	$6.1 (\pm 0.8) \cdot 10^9$	**$1.6 (\pm 0.4) \cdot 10^9$**
5	***Cy3***-(+) 	**$2.6 (\pm 1.1) \cdot 10^8$**	$1.0 (\pm 0.5) \cdot 10^7$	$1.4 (\pm 0.7) \cdot 10^8$
6		**$1.6 (\pm 0.3) \cdot 10^8$**	$8.5 (\pm 1.5) \cdot 10^7$	$1.1 (\pm 0.1) \cdot 10^8$

Table B.2. MALDI-TOF Mass Spectral Data for polyamides **1–7**

Polyamide	Ball-and-stick Structure	$[M+H]^+$ calc'd	$[M+H]^+$ obsv'd
1		914.4	914.4
2		900.4	900.5
3		965.5	965.4
4		1187.6	1187.6
5	***Cy3***-(+) 	1569.7	1569.6
6		1569.7	1569.7
7	***Chl***-(+) 	1242.6	1242.5