Supplemental Information

General. Boc-β-alanine-(4-carboxamidomethyl)-benzyl-ester-copoly(styrene-divinylbenzene) resin (Boc-β-PAM-resin), *N,N'*-dicyclohexylcarbo-diimide (DCC), *N*-hydroxybenzotriazole (HOBt), and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexa-fluorophosphate (HBTU) were purchased from Peptides International. Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBop) was from Novabiochem. *N,N*-Diisopropylethylamine (DIEA) and *N,N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were purchased from Applied Biosystems. (*R*)-2-Fmoc-4-Boc-diaminobutyric acid was from Bachem, dichloromethane (DCM) was reagent grade from EM, and trifluoroacetic acid (TFA) was from Halocarbon. All other chemicals were obtained reagent-grade from Aldrich (unless otherwise stated) and used without further purification. ^{1}H NMR spectra were recorded on a Varian Mercury 300 instrument. Chemical shifts are reported in parts-per-million downfield from the signal for Me_4Si, with reference to the solvent residual signal. UV spectra were measured on a Hewlett-Packard model 8452A diode array spectrophotometer. Matrix-assisted, laser desorption/ionization time-of-flight mass spectrometry was carried out at the Protein and Peptide Micro-analytical Facility at the California Institute of Technology. HPLC analysis was performed on a Beckman Gold system using a RAINEN C_{18}, Microsorb MV, 5 μm, 300 × 4.6 mm reversed-phase column in 0.1% (w/v) TFA with acetonitrile as eluent and a flow rate of 1.0 mL/min, gradient elution 1.25% acetonitrile/min. Preparatory HPLC was carried out on a Beckman HPLC using a Waters DeltaPak 100 × 25 mm, 100 μm C_{18} column, 0.1% (w/v) TFA, 0.25% acetonitrile/min. 18MΩ water was obtained from a Millipore MilliQ water purification system, and all buffers were 0.2 μm filtered. DNA oligonucleotides were synthesized by the Biopolymer Synthesis Center at the California Institute of Technology and used without further purification. Plasmids were sequenced by the Sequence/Structure Analysis Facility (SAF) at the California

Institute of Technology. dNTP's (PCR nucleotide mix), and all enzymes (unless otherwise stated) were purchased from Roche Diagnostics and used with their supplied buffers. pUC19 was from New England Biolabs. Deoxyadenosine [α-^{32}P] triphosphate and deoxytyrosine [α-^{32}P] triphosphate was from NEN. Deoxyadenosine [γ-^{32}P] triphosphate was from ICN. AmpliTaq DNA polymerase for PCR (polymerase chain reaction) was from Perkin-Elmer and used with the supplied buffers. RNase-free water (used for all DNA manipulations) was from US Biochemicals. Ethanol (200 proof) was from Equistar, isopropanol from Mallinckrodt. Pre-mixed tris-borate-EDTA (Gel-Mate, used for gel running buffer) was from Gibco. Bromophenol blue and xylene cyanol FF were from Acros. All reagents were used without further purification. DNA manipulations were performed according to standard protocols.[13]

Synthesis of 2-EDTA. The Fmoc protecting group at the turn primary amine was removed by treatment with piperidine and the free amine reacted with excess succinic anhydride. Following cleavage from resin with 3,3'-diamino-*N*-methyldipropylamine, Boc-protection of the tail primary amine with Boc_2O and HPLC purification, the modified linker-appended 6-ring hairpin polyamide Im-Im-Py-*(R)*$^{HNCO(CH_2)_2CO_2H}\gamma$-Py-Py-Py-β-Dp-Boc (**5L2-Boc**) was obtained. Coupling with 6-ring hairpin polyamide **6b** as described above then gave Im-Im-Py-*(R)*[Im-Im-Py-*(R)*$^{HNCO(CH_2)_2CO}\gamma$-Py-Py-Py-β-Dp]$^{NH}\gamma$-Im-Py-Py-β-Dp-Boc (**2-Boc**), which was isolated by reversed phase HPLC. Finally, the Boc group was removed by treatment with TFA, and the resulting free amine at the tail was acylated using EDTA dianhydride, which provided pure **2-EDTA** after hydrolysis of the remaining anhydride moiety with 0.1 M NaOH and separation of the crude reaction mixture by reversed phase HPLC. The dicationic head-to-head linked hairpin polyamide dimers are soluble in aqueous solution up to 1 mM. No significant decrease in solubility was observed with increasing length of the diacid linker moiety.

Im-Im-Py-*(R)*$^{HNCO(CH_2)_2CO_2H}$γ-Py-Py-Py-β-Dp-Boc (5L2-Boc). Im-Im-Py-*(R)*^{HNFmoc}γ-Py-Py-Py-β-PAM-resin was treated with 80% (v/v) piperidine in DMF at r.t. for 2 h. The resin was washed with DMF (3 ×), dried and subsequently a solution of succinic anhydride (10.0 equiv.) and DMAP (1.0 equiv.) in DMF was added, followed by DIEA. The mixture was shaken at r.t. for 4 h, drained and then the resin washed with DMF (3 ×) and DCM (3 ×) and dried. A sample of the obtained Im-Im-Py-*(R)*^{HNCOCH_2CH_2CO_2H}γ-Py-Py-Py-β-PAM-resin (200 mg) was placed in a glass 20 mL peptide synthesis vessel and treated with neat 3,3'-diamino-*N*-methyldipropylamine (1.5 mL) at 55 °C with periodic agitation for 18 h. The reaction mixture was filtered to remove resin, the residue washed with DMF (1 mL) and the combined filtrate and washing was treated with 40 mL of cold (–20 °C) Et_2O. The precipitated crude polyamide was recovered by centrifugation, washed with Et_2O (20 mL), dried under HV and then dissolved in dry DMF (2 mL) and DIEA (0.5 mL). Boc_2O (5.0 equiv.) was added and the mixture stirred at r.t. for 2 h. Subsequently, 0.1% (wt/v) TFA was added (7.5 mL) and the resulting solution purified by reversed phase HPLC. Im-Im-Py-*(R)*$^{HNCO(CH_2)_2CO_2H}$γ-Py-Py-Py-β-Dp-Boc (**5L2-Boc**) was recovered upon lyophilization of the appropriate fractions as a yellowish powder (12.4 mg, 9% recovery): UV (H_2O) λ_{max} 260 (28000), 310 (52140); ^{1}H NMR (DMSO-d_6) δ 10.31 (s, 1 H), 9.96 (s, 1 H), 9.94 (s, 1H), 9.91 (s, 1 H), 9.75 (s, 1 H), 9.16 (bs, 1 H), 8.26 (d, 1 H, J = 8.1 Hz), 8.02 – 8.11 (m, 2 H), 8.00 (t, 1 H, J = 5.4 Hz), 7.56 (s, 1 H), 7.47 (s, 1 H), 7.24 (s, 1 H), 7.23 (s, 1 H), 7.19 (d, 1 H, J = 1.6 Hz), 7.17 (d, 1 H, J = 1.6 Hz), 7.09 (d, 1 H, J = 0.8 Hz), 7.05 (d, 1 H, J = 1.6 Hz), 6.97 (bs, 2 H), 6.93 (d, 1 H, J = 1.6 Hz), 6.87 (d, 1 H, J = 1.6 Hz), 4.41 (q, 1 H, J = 7.2 Hz), 4.00 (s, 6 H), 3.84 (s, 6 H), 3.80 (s, 6 H), 3.31 – 3.43 (m, 2 H), 2.80 – 3.31 (m, 12 H), 2.72 (d, 3 H, J = 4.8 Hz), 2.39 – 2.48 (m, 2 H), 2.35 (t, 2 H, J = 7.2 Hz), 1.65 – 2.04 (m, 6 H), 1.38 (s, 9 H); MALDI-TOF-MS calcd. for $C_{57}H_{78}N_{19}O_{13}$ (M + H): 1236.6. Found 1236.7.

Im-Im-Py-*(R)*[Im-Im-Py-*(R)*$^{HNCO(CH_2)_2CO}$γ-Py-Py-Py-β-Dp]^{NH}γ-Im-Py-Py-

β-Dp-Boc (2-Boc). To a solution of Im-Im-Py-(*R*)$^{HNCO(CH_2)_2CO_2H}$γ-Py-Py-Py-β-Dp-Boc (**5L2-Boc**) (5.0 μmol, 6.2 mg) and Im-Im-Py-(*R*)^{H_2N}γ-Im-Py-Py-β-Dp (**6b**) (4.8 μmol, 4.8 mg) in 200 μL of dry DMF was added HOBt (50.0 μmol, 8.0 mg, 10.0 equiv.) followed by PyBop (25.0 μmol, 13.0 mg, 5.0 equiv.) and DIEA (100 μL). The reaction mixture was shaken at RT for 4 h, then 0.1% (wt/v) TFA was added (6 mL) and the resulting solution purified by reversed phase HPLC. Im-Im-Py-(*R*)[Im-Im-Py-(*R*)$^{HNCO(CH_2)_2CO}$γ-Py-Py-Py-β-Dp]^{NH}γ-Im-Py-Py-β-Dp-Boc (**2-Boc**) was recovered upon lyophilization of the appropriate fractions as an off-white powder (8.3 mg, 78% isolated yield): UV (H_2O) λ_{max} 260 (56000), 310 (104280); ^{1}H NMR (DMSO-d_6) δ 10.30 (s, 2 H), 10. 29 (s, 1 H), 10.13 (s, 1 H), 10.10 (s, 1 H), 9.93 (s, 1 H), 9.92 (s, 1H), 9.91 (s, 1 H), 9.75 (s, 2 H), 9.30 (bs, 1 H), 9.19 (bs, 1 H), 8.30 (d, 1 H, J = 8.1 Hz), 8.28 (d, 1 H, J = 8.1 Hz), 7.98 – 8.11 (m, 6 H), 7.57 (s, 2 H), 7.46 (s, 2 H), 7.45 (s, 1 H), 7.25 (s, 1 H), 7.22 (s, 2 H), 7.21 (s, 1 H), 7.16 (s, 3 H), 7.08 (s, 2 H), 7.06 (s, 1 H), 6.94 – 7.02 (m, 5 H), 6.88 (s, 2 H), 4.53 (q, 1 H, J = 7.2 Hz), 4.41 (q, 1 H, J = 7.2 Hz), 4.00 (s, 6 H), 3.99 (s, 6 H), 3.95 (s, 3 H), 3.84 (s, 6 H), 3.82 (s, 3 H), 3.80 (s, 12 H), 3.30 – 3.44 (m, 4 H), 3.19 – 3.30 (m, 4 H), 2.92 – 3.16 (m, 12 H), 2.74 (d, 6 H, J = 5.1 Hz), 2.71 (d, 3 H, J = 5.1 Hz), 2.40 – 2.47 (m, 4 H), 2.35 (t, 4 H, J = 7.2 Hz), 1.64 – 2.05 (m, 10 H), 1.37 (s, 9 H); MALDI-TOF-MS calcd. for $C_{102}H_{135}N_{38}O_{20}$ (M + H): 2212.1. Found: 2212.2.

Im-Im-Py-(*R*)[Im-Im-Py-(*R*)$^{HNCO(CH_2)_2CO}$γ-Py-Py-Py-β-Dp]^{NH}γ-Im-Py-Py-β-Dp-EDTA (2-EDTA). Im-Im-Py-(*R*)[Im-Im-Py-(*R*)$^{HNCO(CH_2)_2CO}$γ-Py-Py-Py-β-Dp]^{NH}γ-Im-Py-Py-β-Dp-Boc (**2-Boc**) (1.5 μmol, 3.3 mg) was dissolved in 80% (v/v) TFA in DCM (0.5 mL) and the solution stirred at r.t. for. 30 min. Subsequently the reaction mixture was diluted with 0.1% (wt/v) TFA (1 mL) and MeCN (1 mL) and then lyophilized. The residue was taken up in dry DMSO (200 μL) and treated with a solution of EDTA dianhydride (15.0 mmol, 20 mg, 10.0 equiv.) in dry DMF/DMSO 1:1 (200 μL) and DIEA (200 μL) at 55 °C for 5 min. Then 0.1 M NaOH (1.5 mL) was added and the reaction mixture heated at 55 °C for another 15 min. After cooling to r.t., 0.1% (wt/v)

TFA was added (6 mL) and the resulting solution purified by reversed phase HPLC. Im-Im-Py-*(R)*[Im-Im-Py-*(R)*$^{HNCO(CH_2)_2CO}$γ-Py-Py-Py-β-Dp]^{NH}γ-Im-Py-Py-β-Dp-EDTA (**2-EDTA**) was recovered upon lyophilization of the appropriate fractions as a white powder (0.6 mg, 17% isolated yield): UV (H_2O) λ_{max} 260 (56000), 310 (104280); ^{1}H NMR (DMSO-d_6) δ 10.28 (s, 2 H), 10. 27 (s, 1 H), 10.10 (s, 1 H), 9.98 (s, 1 H), 9.92 (s, 1 H), 9.89 (s, 1H), 9.88 (s, 1 H), 9.71 (s, 2 H), 9.20 (bs, 2 H), 9.15 (bs, 1 H), 9.05 (bs, 1 H), 8.29 (d, 1 H, J = 7.6 Hz), 8.25 (d, 1 H, J = 7.6 Hz), 7.97 – 8.06 (m, 7 H), 7.56 (s, 2 H), 7.46 (s, 2 H), 7.45 (s, 1 H), 7.25 (d, 1 H, J = 1.5 Hz), 7.22 (d, 1 H, J = 1.5 Hz), 7.21 (d, 1 H, J = 1.5 Hz), 7.20 (d, 1 H, J = 1.5 Hz), 7.19 (s, 1 H), 7.16 (d, 1 H, J = 1.5 Hz), 7.15 (d, 1 H, J = 1.5 Hz), 7.08 (s, 1 H), 7.07 (s, 2 H), 6.99 (d, 1 H, J = 1.5 Hz), 6.98 (s, 2 H), 6.96 (d, 1 H, J = 1.5 Hz), 6.88 (d, 2 H, J = 1.5 Hz), 4.59 (q, 1 H, J = 6.8 Hz), 4.43 (q, 1 H, J = 6.8 Hz), 4.01 (s, 6 H), 4.00 (s, 6 H), 3.95 (s, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.80 (s, 9 H), 3.79 (s, 3 H), 3.52 – 3.65 (m, 8 H), 3.08 – 3.19 (m, 16 H), 2.80 – 3.04 (m, 8 H), 2.74 (d, 6 H, J = 5.0 Hz), 2.72 (d, 3 H, J = 5.0 Hz), 2.43 – 2.52 (m, 4 H), 2.32 – 2.39 (m, 4 H), 1.74 – 2.04 (m, 4 H), 1.70 – 1.84 (m, 6 H); MALDI-TOF-MS calcd. for $C_{107}H_{141}N_{40}O_{25}$ (M + H): 2386.1. Found: 2386.2.

Supplemental Figure S1. Synthesis of the affinity cleaving analog Im-Im-Py-*(R)*[Im-Im-Py-*(R)*$^{HNCO(CH_2)_2CO}$γ-Py-Py-Py-β-Dp]^{NH}γ-Im-Py-Py-β-Dp-EDTA (**2-EDTA**) starting from Im-Im-Py-*(R)*^{NHFmoc}γ-Py-Py-Py-β-PAM-resin: (i) 80% Piperidine/DMF; (ii) succinic anhydride, DMAP, DIEA, DMF, 4 h; (iii) 3,3'-diamino-*N*-methyldipropylamine, 55 °C, 18 h; (iv) Boc_2O, DIEA, DMF, 2 h; v) PyBop, HOBt, DIEA, DMF, 4 h; vi) 80% TFA/DCM, 0.4 M PhSH, 30 min; vii) EDTA dianhydride, DIEA, DMSO/DMF 3:1, 55 °C, 5 min; then 0.1 M NaOH, H_2O/DMSO/DMF 15:3:1, 55 °C, 15 min.

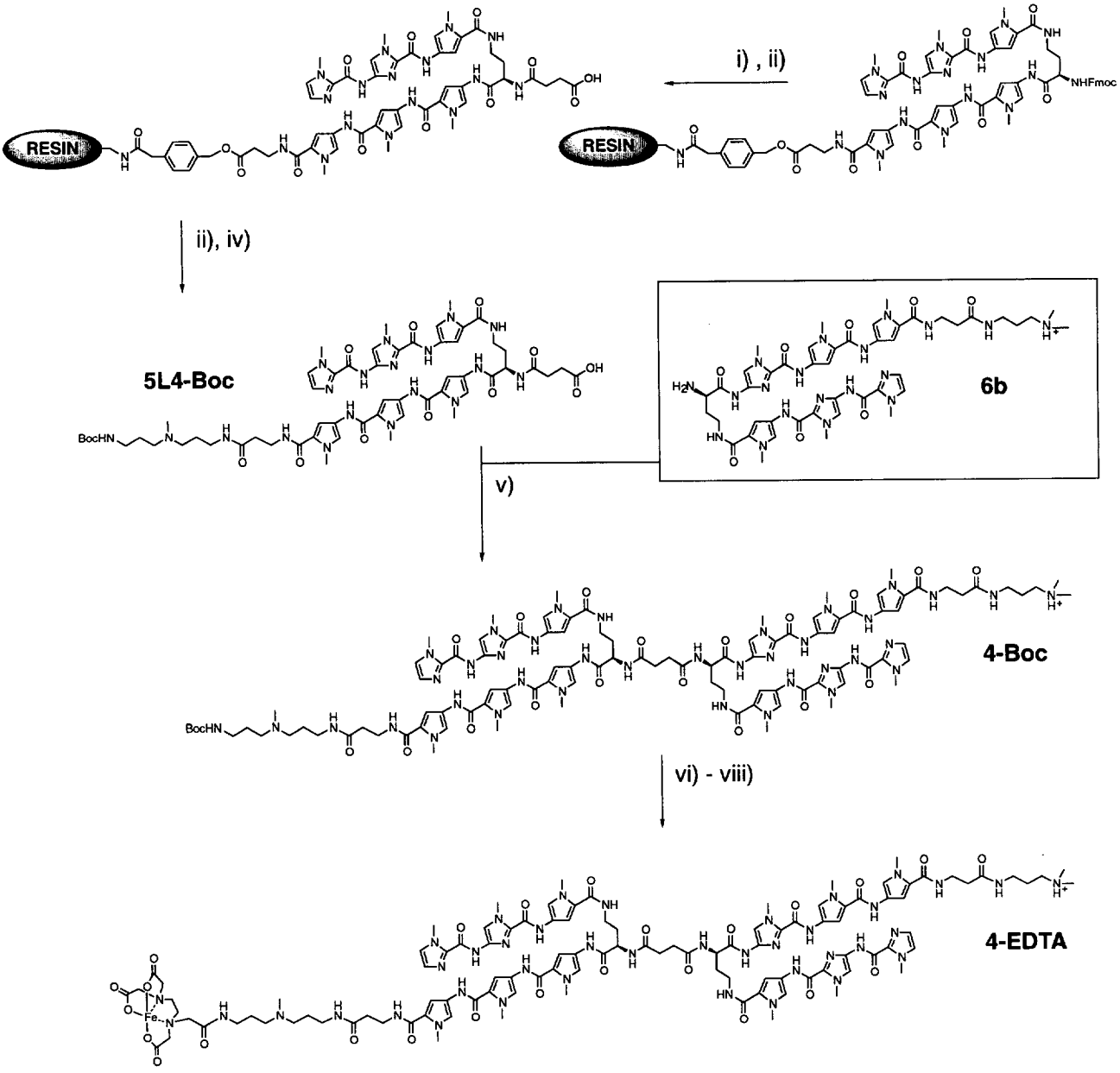
Supplemental Figure S2. Quantitative DNase I footprinting experiment with Im-Im-Py-*(R)*[Im-Im-Py-*(R)*$^{HNCO(CH_2)_2CO}$γ-Py-Py-Py-β-Dp]^{NH}γ-Im-Py-Py-β-Dp (**2**) on the 3'-^{32}P-

labeled 278-bp *Eco*RI/*Pvu*II restriction fragment derived from the plasmid pVRSS. Quantitative DNase I footprint titration experiment with **2**: lane 1, intact DNA; lane 2, A-specific reaction; lane 3, DNase I standard; lane 4 – 19, 0.5 pM, 1 pM, 2 pM, 5 pM, 10 pM, 20 pM, 50 pM, 100 pM, 200 pM, 500 pM, 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 50 nM **2**. All reactions contained 10 kcpm labeled DNA and were carried out at 22 °C at pH 7.0 in the presence of 10 mM Tris-HCl, 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$ with an equilibration time of 36 h. (b) Binding isotherms for the 10-bp, 11-bp, and 12-bp match sites from the quantitative DNase I footprint titration experiment with **2**. Θ_{norm} values were obtained according to published methods.[8] The solid lines are best fit Langmuir binding titration isotherms obtained by a non-linear least squares algorithm.

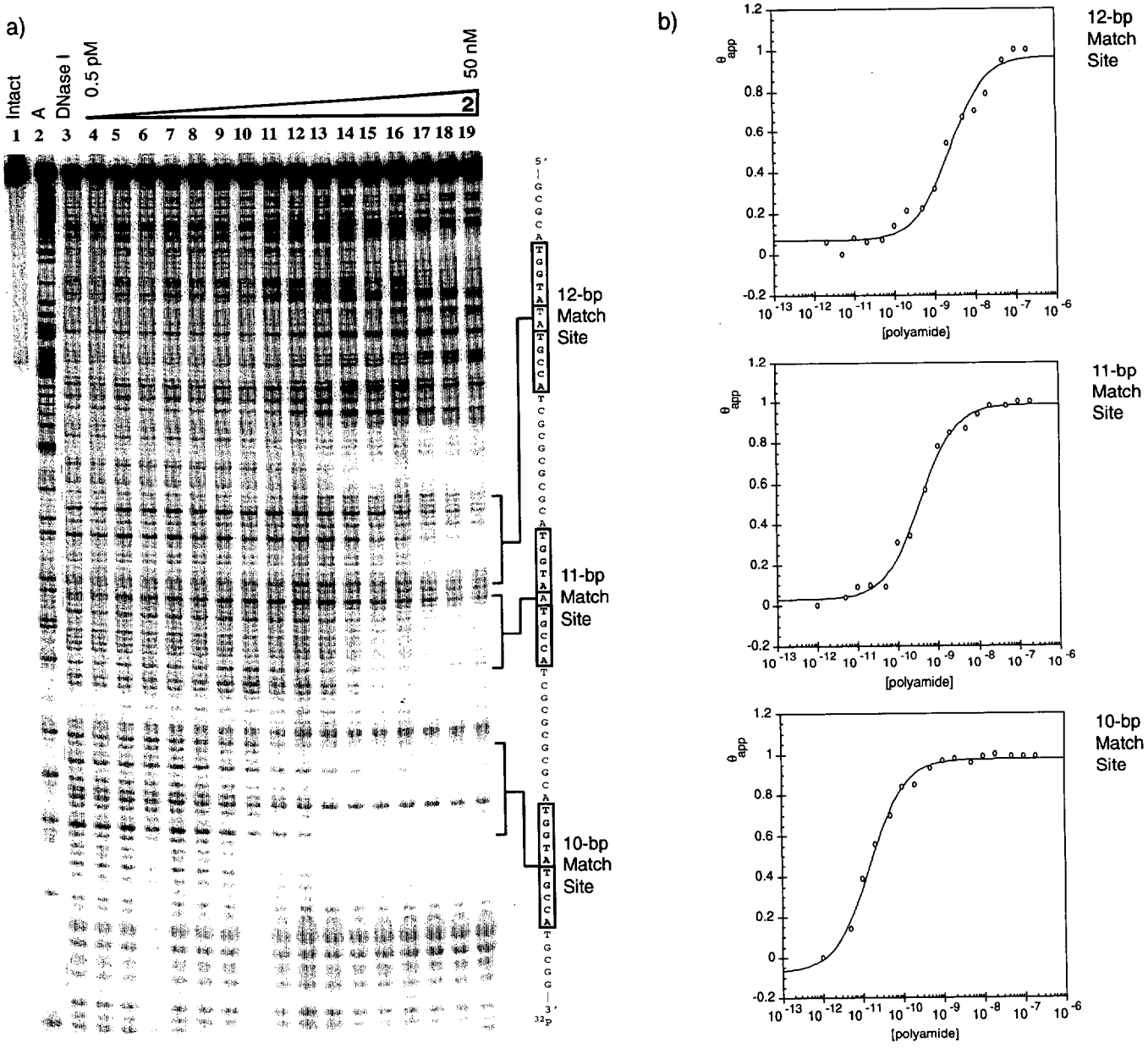
(c) Illustration of the *Eco*RI/*Pvu*II restriction fragment derived from plasmid pVRSS, containing 10-bp, 11-bp, and 12 bp match target sites. Plasmid **pVRSS** insert: 5'- G A T C C C G C A T G G C A T A C C A T G C G C G C G A T G G C A T T A C C A T G C G C G C G A T G G C A T A T A C C A T G C G C A -3' • 5'- G G C G T A C C G T A T G G T A C G C G C G C G C T A C C G T A A T G G T A C G C G C G C G C T A C C G T A T A T G G T A C G C G T T C G A -3'. We thank V. C. Rucker for the design and preparation of plasmid pVRSS.

(d) The weak footprint above the 12 bp match site is a mismatch site (5'-TGCGCAAGCTT-3') outside the insert which is inherently present on the pUC19 plasmid. From the size of the footprint, it appears to be a single hairpin binding site, but we are uncertain how to correctly assign it. The single and double mismatch sites are examined on plasmid PWLH1 (Figure 4).

(e) There is compression between the 11 bp and 12 bp sites which appear directly adjacent from the A chemical sequencing reaction. We confirmed the presence of the intervening $(CG)_4$ tract by dideoxysequencing.



Supplement Figure S1



Supplement Figure S2