Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

β-alanine and *N*-terminal cationic substituents affect polyamide-DNA binding

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Materials and synthesis details

DNA:

All the DNA oligomers in this study were purchased from Integrated DNA Technologies, Inc. (IDT, Coralville, IA) and the purity is checked by ESI-MS. The DNAs used are shown below:

λB DNA: 5'-Biotin-CCAAATAAAAGG**AAGTGAA**ACCAAG*CTCT*CTTGGTTTCACTTCCT TTTATTTGG-3';

SC1 DNA: 5'-Biotin-CGGCCAAGCCGG**AAGTGAG**TGCC*TCTC*GGCACTCACTTCCGGC TTGGCCG-3';

GAGA mutant DNA: 5'-Biotin- CCAAATAAAAGA**GAGTGAA**ACCAAG*CTCT*CTTGGTTT CACTCTCTTTTATTTGG-3'

Short λB DNA: 5'-GGA**AGTGA**AC*CTCT*GTTCACTTCC-3'

Mutant 1: 5'-GGA**AGAGA**AC*CTCT*GTTCACTTCC-3'

Mutant 2: 5'-GGA**AGTGT**AC*CTCT*GTTCACTTCC-3'

Mutant 3: 5'-GGA**AGTTA**AC*CTCT*GTTCACTTCC-3'

Mutant 4: 5'-GGA**ACTGA**AC*CTCT*GTTCACTTCC-3'

Mutant 5: 5'-GGA**AGTCA**AC*CTCT*GTTCACTTCC-3'

The three long DNAs with biotin at 5' were used for SPR experiments. The short λB DNA was used for Thermal melting and Circular Dichroism experiments. The five mutant sequences were used for Thermal melting.

Compound synthesis:

Boc-β-alanine-PAM resin was purchased from Peptides International. Polyamide building blocks, monomers or dimers, were purchased from A Chemtek, Inc. (Worcester, MA) and purification of polyamides was done by the Boc-PAM solid phase method as reported,[1] with some modifications. In particular, dimer building blocks were purchased from A Chemtek or were prepared in-house and used in place of sequential monomers. This was especially the case when monomer coupling would have involved the poorly-nucleophilic imidazole amino group from the growing, resin-bound polyamide reacting with an active ester in solution, as per

Dervan's original recommendations.[1] Application of dimer building blocks are indicated by parentheses in polyamide syntheses, as for FH1024: TMG-(PyIm)-β-Im-γ-(Py-β)-(PyPy)-β-PAM resin. For example, (PyPy) indicates that the pyrrole-pyrrole dimer was used. When unavailable commercially, dimer building blocks were made by standard methods as previously described.[2] The final products were purified by reverse-phase HPLC and characterized with NMR and HR mass spectrometry. The TMG substituted PAs were synthesized by virtually identical solid phase methods up to the pyrrole group (4-amino-N-methylpyrrole-2-carboxyamide). To generate the final building block where TMG is connected to the H2N-Py moiety, HATU[3] has been used[4]. The PA concentration was determined by accurate weighting on a five place analytical balance and dissolving the compound in an appropriate volume of solvent.

KA2034:

HRMS calc'd for $C_{56}H_{71}N_{21}O_{11}$ 1213.56452, found 1213.5574

^{1}H NMR (600 MHz, DMSO-d_6) δ = 10.37 (s, 1 H), 10.35 (s, 1 H), 10.05 (d, *J* = 1.2 Hz, 1 H), 9.93 (s, 1 H), 9.89 (s, 1 H), 9.89 (s, 1 H), 9.82 (s, 1 H), 9.31 (br. s., 1 H), 8.10 (d, *J* = 1.8 Hz, 1 H), 8.05 (t, *J* = 5.9 Hz, 1 H), 8.02 (t, *J* = 5.7 Hz, 1 H), 7.97 (t, *J* = 6.2 Hz, 1 H), 7.88 (t, *J* = 6.2 Hz, 1 H), 7.50 (s, 1 H), 7.40 (s, 1 H), 7.28 (d, *J* = 1.8 Hz, 1 H), 7.25 - 7.21 (m, 2 H), 7.17 (d, *J* = 1.8 Hz, 1 H), 7.15 (d, *J* = 1.8 Hz, 1 H), 7.09 - 7.05 (m, 2 H), 6.98 (d, *J* = 2.1 Hz, 1 H), 6.88 (d, *J* = 1.8 Hz, 1 H), 6.87 (d, *J* = 1.8 Hz, 1 H), 3.94 (s, 3 H), 3.91 (s, 3 H), 3.85 (s, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.52 (q, *J* = 6.7 Hz, 2 H), 3.42 - 3.35 (m, 2 H), 3.27 (q, *J* = 6.6 Hz, 2 H), 3.12 (q, *J* = 6.5 Hz, 2 H), 3.04 - 2.97 (m, 2 H), 2.75 (d, *J* = 5.0 Hz, 6 H), 2.60 (t, *J* = 6.5 Hz, 2 H), 2.35 (t, *J* = 7.2 Hz, 2 H), 2.27 (t, *J* = 7.5 Hz, 2 H), 1.83 - 1.77 (m, 2 H), 1.77 - 1.70 (m, 2 H)

^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.5, 171.0, 169.0, 168.5, 161.2, 158.6, 158.5, 158.5, 158.4, 158.0, 157.9, 157.8, 136.1, 135.8, 134.0, 133.6, 122.8, 122.7, 122.7, 122.7, 122.2, 122.2, 122.1, 122.0, 122.0, 120.7, 119.3, 118.5, 118.4, 118.1, 117.9, 114.3, 113.6, 106.9, 104.8, 104.7, 104.3, 103.9, 54.7, 42.3, 40.0, 38.1, 36.2, 36.0, 35.9, 35.6, 35.4, 34.9, 34.9, 34.7, 33.1, 25.6, 24.5

KA2035:

HRMS calc'd for $C_{59}H_{72}N_{22}O_{11}$ 1264.57542, found 1264.5696

^{1}H NMR (600 MHz, DMSO-d_6) δ = 10.31 (s, 1 H), 10.26 (s, 1 H), 10.07 (s, 1 H), 9.93 (s, 1 H), 9.90 (s, 2 H), 9.89 (s, 1 H), 9.84 (s, 1 H), 9.24 (br. s., 1 H), 8.12 (d, *J* = 1.8 Hz, 1 H), 8.07 - 7.99 (m, 4 H), 7.55 (s, 1 H), 7.50 (s, 1 H), 7.39 (d, *J* = 1.8 Hz, 1 H), 7.28 (d, *J* = 1.8 Hz, 1 H), 7.22 (d, *J* = 1.8 Hz, 1 H), 7.22 (d, *J* = 1.8 Hz, 1 H), 7.17 - 7.15 (m, 2 H), 7.15 (d, *J* = 1.8 Hz, 1 H), 7.07 (s, 2 H), 7.00 (d, *J* = 1.8 Hz, 1 H), 6.88 (d, *J* = 1.2 Hz, 2 H), 3.98 (s, 3 H), 3.94 (s, 3 H), 3.87 (s, 3 H), 3.85 (s, 3 H), 3.85 (s, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.80 (s, 3 H), 3.42 - 3.35 (m, 2 H), 3.30 (q, *J* = 6.8 Hz, 2 H), 3.12

(q, *J* = 6.5 Hz, 2 H), 3.03 - 2.97 (m, 2 H), 2.75 (d, *J* = 4.7 Hz, 6 H), 2.35 (t, *J* = 7.3 Hz, 2 H), 2.28 (t, *J* = 7.6 Hz, 2 H), 1.85 - 1.78 (m, *J* = 7.1, 7.1, 14.5 Hz, 2 H), 1.78 - 1.71 (m, 2 H)

^{13}C NMR (151 MHz, DMSO-d_6) δ = 171.0, 169.0, 161.2, 158.7, 158.6, 158.6, 158.5, 158.1, 157.9, 155.7, 136.1, 135.9, 134.1, 134.1, 134.0, 122.8, 122.7, 122.7, 122.7, 122.2, 122.2, 122.1, 122.0, 122.0, 121.2, 120.8, 119.3, 118.5, 118.1, 117.9, 114.7, 114.3, 105.5, 104.8, 104.8, 104.3, 104.0, 54.7, 42.3, 40.4, 40.0, 38.0, 36.3, 36.2, 36.1, 35.9, 35.6, 35.4, 34.9, 34.9, 33.1, 25.6, 24.5

EA calc'd for $C_{59}H_{72}N_{22}O_{11}(3CF_3CO_2H)(9H_2O)$: C, 44.12%; H, 5.30%; N, 17.41%. Found: C, 43.87; H, 4.92%; N, 17.13%.

KA2040:

HRMS: calc'd for $C_{53}H_{70}N_{20}O_{11}$ 1162.55362, found 1162.5466

^{1}H NMR (600 MHz, DMSO-d_6) δ = 10.37 (s, 1 H), 10.35 (s, 1 H), 10.05 (s, 1 H), 9.88 (s, 1 H), 9.84 (s, 1 H), 9.75 (s, 1 H), 9.25 (br. s., 1 H), 8.10 (d, *J* = 1.5 Hz, 1 H), 8.07 - 8.03 (m, 2 H), 8.02 (t, *J* = 5.7 Hz, 1 H), 7.96 (t, *J* = 6.0 Hz, 1 H), 7.89 (t, *J* = 6.0 Hz, 1 H), 7.50 (s, 1 H), 7.39 (s, 1 H), 7.28 (d, *J* = 1.8 Hz, 1 H), 7.16 (d, *J* = 1.8 Hz, 1 H), 7.15 (d, *J* = 1.8 Hz, 1 H), 7.09 (d, *J* = 1.8 Hz, 1 H), 6.98 (d, *J* = 2.1 Hz, 1 H), 6.86 (d, *J* = 1.8 Hz, 1 H), 6.85 (d, *J* = 1.8 Hz, 1 H), 6.63 (d, *J* = 1.5 Hz, 1 H), 3.94 (s, 3 H), 3.90 (s, 3 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 3.77 (s, 3 H), 3.55 - 3.49 (m, 2 H), 3.45 - 3.40 (m, 2 H), 3.40 - 3.35 (m, 2 H), 3.28 - 3.22 (m, 2 H), 3.14 - 3.08 (m, 2 H), 3.04 - 2.97 (m, 2 H), 2.74 (d, *J* = 5.0 Hz, 6 H), 2.60 (t, *J* = 6.6 Hz, 2 H), 2.52 - 2.47 (m, 2 H), 2.35 (t, *J* = 7.2 Hz, 2 H), 2.24 (t, *J* = 7.5 Hz, 2 H), 1.81 - 1.70 (m, 4 H)

^{13}C NMR (151 MHz, DMSO-d_6) δ = 171.0, 169.0, 168.5, 167.8, 162.7, 161.2, 161.2, 158.6, 158.5, 158.4, 158.4, 158.1, 157.9, 136.1, 135.8, 133.9, 133.6, 122.7, 122.7, 122.7, 122.0, 122.0, 121.9, 120.7, 119.3, 118.1, 117.9, 117.6, 117.2, 114.3, 113.5, 104.8, 104.3, 104.0, 103.4, 54.7, 42.3, 40.0, 38.1, 36.2, 36.0, 35.9, 35.9, 35.8, 35.6, 35.5, 35.4, 34.9, 34.9, 34.7, 33.1, 25.5, 24.5

EA calc'd for $C_{53}H_{70}N_{20}O_{11}(3CF_3CO_2H)(6H_2O)$: C, 43.92%; H, 5.31%. Found: C, 44.15; H, 4.95%.

KA2041:

HRMS: calc'd for $C_{53}H_{70}N_{20}O_{11}$ 1162.55362, found 1162.5466

^{1}H NMR (600 MHz, DMSO-d_6) δ = 10.31 (s, 1 H), 10.26 (s, 1 H), 10.07 (d, *J* = 0.9 Hz, 1 H), 9.90 (s, 1 H), 9.88 (s, 1 H), 9.84 (s, 1 H), 9.77 (s, 1 H), 9.26 (br. s., 1 H), 8.12 (d, *J* = 1.8 Hz, 1 H), 8.08 - 7.99 (m, 4 H), 7.55 (s, 1 H), 7.49 (s, 1 H), 7.39 (d, *J* = 1.5 Hz, 1 H), 7.28 (d, *J* = 1.8 Hz, 1 H), 7.16 (d, *J* = 1.8 Hz, 1 H), 7.15 (d, *J* = 1.8 Hz, 1 H), 7.14 (d, *J* = 1.8 Hz, 1 H), 7.10 (d, *J* = 1.8 Hz, 1 H), 7.00 (d, *J* = 1.8 Hz, 1 H), 6.86 (d, *J* = 1.8 Hz, 1 H), 6.85 (d, *J* = 2.1 Hz, 1 H), 6.64 (d, *J* = 1.8 Hz, 1 H), 3.98 (s, 3 H), 3.93 (s, 3 H), 3.87 (s, 3 H), 3.85 (s, 3 H), 3.81 (s, 3 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.45 - 3.40

(m, 2 H), 3.40 - 3.35 (m, 2 H), 3.28 (q, *J* = 6.7 Hz, 2 H), 3.11 (q, *J* = 6.5 Hz, 2 H), 3.03 - 2.98 (m, 2 H), 2.74 (d, *J* = 5.0 Hz, 6 H), 2.51 - 2.48 (m, 2 H), 2.35 (t, *J* = 7.2 Hz, 2 H), 2.25 (t, *J* = 7.5 Hz, 2 H), 1.79 (td, *J* = 7.3, 14.5 Hz, 2 H), 1.76 - 1.71 (m, 2 H)

^{13}C NMR (151 MHz, DMSO-d_6) δ = 174.1, 172.1, 170.9, 164.4, 164.3, 161.8, 161.7, 161.7, 161.5, 161.3, 161.1, 161.0, 160.9, 158.9, 139.2, 139.1, 137.2, 137.2, 125.9, 125.8, 125.3, 125.2, 125.2, 125.0, 125.0, 124.3, 124.0, 122.5, 121.3, 121.1, 120.7, 120.1, 117.9, 117.4, 108.6, 107.9, 107.4, 107.1, 106.5, 57.8, 45.4, 43.2, 41.2, 39.4, 39.4, 39.2, 39.1, 39.0, 38.7, 38.6, 38.5, 38.0, 38.0, 36.2, 28.7, 27.6

KA2114:

HRMS: calc'd for $C_{58}H_{76}N_{22}O_{11}$ 1256.6064, found 1256.6028

^{1}H NMR (600 MHz, DMSO-d_6) δ = 10.31 (s, 1 H), 10.26 (s, 1 H), 10.09 (s, 1 H), 9.90 (s, 1 H), 9.85 (s, 2 H), 9.83 (s, 1 H), 9.68 - 9.60 (m, 1 H), 8.12 (d, *J* = 1.2 Hz, 1 H), 8.10 - 8.00 (m, 5 H), 7.89 (br. s., 4 H), 7.55 (s, 1 H), 7.50 (s, 1 H), 7.39 (d, *J* = 1.2 Hz, 1 H), 7.29 (d, *J* = 1.8 Hz, 1 H), 7.17 (d, *J* = 1.2 Hz, 1 H), 7.15 (d, *J* = 1.8 Hz, 2 H), 7.10 (d, *J* = 1.8 Hz, 1 H), 7.00 (d, *J* = 1.8 Hz, 1 H), 6.85 (d, *J* = 1.2 Hz, 1 H), 6.84 (d, *J* = 1.2 Hz, 1 H), 6.67 (d, *J* = 1.8 Hz, 1 H), 3.98 (s, 3 H), 3.94 (s, 3 H), 3.87 (s, 3 H), 3.85 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.77 (s, 3 H), 3.46 - 3.39 (m, 2 H), 3.39 - 3.33 (m, 2 H), 3.29 (q, *J* = 6.7 Hz, 2 H), 3.21 - 3.14 (m, 1 H), 3.14 - 3.09 (m, 2 H), 3.09 - 2.95 (m, 3 H), 2.91 - 2.82 (m, 2 H), 2.73 (d, *J* = 4.7 Hz, 3 H), 2.53 - 2.46 (m, 2 H), 2.34 (t, *J* = 7.0 Hz, 2 H), 2.28 (t, *J* = 7.3 Hz, 2 H), 1.96 - 1.86 (m, 2 H), 1.84 - 1.72 (m, 4 H)

^{13}C NMR (151 MHz, DMSO-d_6) δ = 174.1, 172.2, 170.9, 165.8, 164.4, 164.3, 161.8, 161.7, 161.7, 161.7, 161.5, 161.4, 161.2, 161.0, 161.0, 158.9, 139.2, 139.1, 137.2, 137.2, 125.9, 125.9, 125.8, 125.3, 125.2, 125.2, 125.1, 125.0, 124.4, 124.0, 122.5, 121.2, 121.1, 120.8, 120.5, 118.6, 117.9, 117.4, 108.6, 107.9, 107.3, 107.0, 106.7, 56.4, 55.2, 43.2, 41.2, 39.4, 39.4, 39.3, 39.2, 39.1, 38.9, 38.7, 38.7, 38.6, 38.5, 38.0, 38.0, 36.3, 28.8, 27.1, 24.9

EA calc'd for $C_{58}H_{76}N_{22}O_{11}(4CF_3CO_2H)(7H_2O)$: C, 43.09%; H, 5.15%; N, 16.75%;. Found: C, 43.10; H, 4.77%; N, 16.07%

KA2115:

HRMS: Calc'd for $C_{55}H_{75}N_{21}O_{11}$ 1205.5954, found 1205.5932

^{1}H NMR (600 MHz, DMSO-d_6) δ = 10.37 (s, 1 H), 10.35 (s, 1 H), 10.06 (s, 1 H), 9.85 (s, 1 H), 9.84 (s, 1 H), 9.81 (s, 1 H), 9.71 - 9.60 (m, 1 H), 8.11 (d, *J* = 1.2 Hz, 1 H), 8.08 (t, *J* = 5.9 Hz, 1 H), 8.06 - 8.00 (m, 2 H), 7.97 (t, *J* = 5.9 Hz, 1 H), 7.93 - 7.82 (m, 5 H), 7.50 (s, 1 H), 7.40 (s, 1 H), 7.28 (d, *J* = 1.8 Hz, 1 H), 7.17 (d, *J* = 1.8 Hz, 1 H), 7.14 (d, *J* = 1.2 Hz, 1 H), 7.11 (d, *J* = 1.8 Hz, 1 H), 6.98 (d, *J* = 1.8 Hz, 1 H), 6.85 (d, *J* = 1.8 Hz, 1 H), 6.84 (d, *J* = 1.2 Hz, 1 H), 6.67 (d, *J* = 1.8 Hz, 1 H), 3.94 (s, 3

H), 3.91 (s, 3 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.78 (s, 3 H), 3.52 (q, *J* = 6.5 Hz, 2 H), 3.47 - 3.40 (m, 2 H), 3.40 - 3.32 (m, 2 H), 3.27 (q, *J* = 6.8 Hz, 2 H), 3.22 - 3.15 (m, 1 H), 3.15 - 3.09 (m, 2 H), 3.09 - 2.96 (m, 3 H), 2.92 - 2.82 (m, *J* = 5.9 Hz, 2 H), 2.73 (d, *J* = 4.7 Hz, 3 H), 2.60 (t, *J* = 6.5 Hz, 2 H), 2.53 - 2.46 (m, 2 H), 2.34 (t, *J* = 7.3 Hz, 2 H), 2.26 (t, *J* = 7.3 Hz, 2 H), 1.96 - 1.85 (m, 2 H), 1.83 - 1.73 (m, 4 H)

^{13}C NMR (151 MHz, DMSO-d_6) δ = 171.0, 169.1, 168.6, 167.8, 162.7, 161.3, 161.2, 158.7, 158.6, 158.5, 158.4, 158.4, 158.4, 158.2, 158.0, 157.9, 136.1, 135.8, 134.0, 133.6, 122.8, 122.8, 122.7, 122.1, 122.0, 122.0, 121.9, 121.1, 120.8, 119.4, 119.1, 118.8, 118.1, 117.9, 117.6, 116.9, 115.0, 114.4, 113.6, 113.0, 104.8, 104.2, 103.9, 103.6, 53.3, 52.1, 40.0, 38.1, 36.3, 36.3, 36.2, 36.0, 35.9, 35.8, 35.6, 35.6, 35.5, 35.4, 34.9, 34.9, 34.7, 33.1, 25.6, 24.0, 21.8

EA calc'd for $C_{55}H_{75}N_{21}O_{11}(5CF_3CO_2H)(6H_2O)$: C, 41.43%; H, 4.92%; N, 15.61%; F, 15.12%. Found: C, 41.51; H, 4.48%; N, 15.34%; F, 14.40%.

FH1024

^{1}H NMR (500 MHz, DMSO-d_6) δ =10.46(s, 1 H), 10.37(s, 1 H), 9.87(s, 1 H), 9.85(s, 1 H), 9.76(s, 1 H), 9.67(s, 1 H), 9.44(br. s, 1 H), 8.09~8.02(m, 3 H), 7.96(t, J=5.84Hz, 1 H), 7.84(s, 1 H), 7.81(t, J=5.85Hz, 3 H), 7.52(s, 1 H), 7.38(s, 1 H), 7.16(d, J=1.8Hz, 1 H), 7.15(d, J=1.8Hz, 1 H), 7.09(d, J=1.8Hz, 1 H), 6.90(d, J=1.8Hz, 1 H), 6.87(d, J=1.8Hz, 1 H), 6.81(d, J=1.8Hz, 1 H), 6.63(d, J=1.8Hz, 1 H), 3.94(s, 3 H), 3.9(s, 3 H), 3.87(s, 3 H), 3.81(s, 3 H), 3.79(s, 3 H), 3.77(s, 3 H), 3.53(q, J=6.01Hz, 2 H), 3.4(m, 4 H), 3.25(q, J=6.28Hz, 2 H), 3.14(m, 4 H), 3.04(m, 4 H), 2.91-2.87(m, 12 H), 2.74(d, J=5.03Hz, 4 H), 2.64(m, 1 H), 2.6(t, J=6.28Hz, 2 H), 2.36(t, J=7.2Hz, 3 H), 2.24(t, J=7.14Hz, 2 H), 1.9(m, 2 H), 1.71(m, 4 H)

FH1026

^{1}H NMR (500 MHz, DMSO-d_6) δ =10.46(s, 1 H), 10.25(s, 1 H), 9.88(s, 1 H), 9.84(s, 1 H), 9.79(s, 1 H), 9.77(s, 1 H), 9.70(s, 1 H), 9.46(br. s, 1 H), 8.09~8.0(m, 4 H), 7.83(d, 4 H), 7.57(s, 1 H), 7.49(s, 1

H), 7.37(d, J=1.53Hz, 1 H), 7.19(d, J=1.53Hz, 1 H), 7.15(d, J=1.53Hz, 1 H), 7.10(d, J=1.53Hz, 1 H), 6.92(d, J=1.68Hz, 1 H), 6.86(d, J=1.53Hz, 1 H), 6.83(d, J=1.53Hz, 1 H), 6.63(d, J=1.53Hz, 1 H), 3.99(s, 3 H), 3.96(s, 3 H), 3.93(s, 3 H), 3.89(s, 3 H), 3.82(s, 3 H), 3.80(s, 3 H), 3.78(s, 3 H), 3.44~3.26(m, 8 H), 3.173~3.00(m, 12 H), 2.99-2.87(m, 4 H), 2.74(d, J=4.9Hz, 4 H), 2.63(m, 1 H), 2.36(m, 3 H), 2.25(t, J=7.64Hz, 2 H), 1.91(m, 4 H), 1.71(m, 4 H)

FH1028

^{1}H NMR (500 MHz, DMSO-d_6) δ =10.46(s, 1 H), 10.37s, 1 H), 9.92(s, 1 H), 9.88(s, 1 H), 9.82(s, 1 H), 9.67(s, 1 H), 9.44(br. s, 1 H), 8.09 (t, J=5.76Hz, 1 H), 8.04 (t, J=5.76Hz, 1 H), 8.00 (t, J=5.76Hz, 1 H), 7.81(m, 4 H), 7.52(s, 1 H), 7.39(s, 1 H), 7.21(d, J=1.8Hz, 1 H), 7.20(d, J=1.8Hz, 1 H), 7.19(d, J=1.8Hz, 2 H), 7.09(d, J=1.8Hz, 1 H), 6.91(d, J=1.8Hz, 1 H), 6.87(d, J=1.8Hz, 1 H), 6.81(d, J=1.8Hz, 1 H), 3.93(s, 3 H), 3.91(s, 3 H), 3.87(s, 3 H), 3.86(s, 3 H), 3.85(s, 3 H), 3.83(s, 3 H), 3.81(s, 3 H), 3.53(q, J=6.32Hz, 2 H), 3.38 (q, J=6.92Hz, 2 H), 3.27(q, J=6.62Hz, 2 H), 3.15~3.01(m, 8 H), 2.99-2.87(m, 10 H), 2.74(d, J= 4.9Hz, 4 H), 2.63(m, 1 H), 2.59(t, J=6.62Hz, 2 H), 2.36(t, J=7.23Hz, 3 H), 2.25(t, J=7.53Hz, 2 H), 1.91(m, 4 H), 1.71(m, 4 H)

KJK6162

1H NMR (300 MHz, DMSO-d6) δ =10.50(s, 1 H), 10.34(s, 1 H), 9.94(m, 2H), 9.89(m, 2 H), 9.70(s, 1 H), 8.74(d, J=4.56Hz, 2 H), 8.51(d, J=8.45Hz, 2 H), 8.12(s, 1 H), 7.56(s, 1 H), 7.50(q, J=4.42Hz, 4 H), 7.38(d, J=1.38Hz, 1 H), 7.23(d, J=1.83Hz, 1 H), 7.17(d, J=6.89Hz, 2 H), 7.11(d, J=1.8Hz, 1 H), 7.04(d, J=1.83Hz, 1 H), 6.91(d, J=1.54Hz, 1 H), 6.86(d, J=1.97Hz, J=4.09Hz, 2 H), 6.80(t, J=1.97Hz, 1 H), 6.59(s, 1 H), 5.75(s, 5 H), 4.19(q, J=5.45Hz, 3H), 3.98(s, 3H), 3.93(s, 3 H), 3.87(s, 3 H), 3.86(s, 3 H), 3.84(s, 3 H), 3.82(s, 3 H),

3.81(s, 3 H), 3.79(s, 3 H), 3.67(s , 2 H), 3.60(s , 4 H), 3.17(s, 6 H), 3.15(s, 6 H), 3.08(t, J=0.73 Hz, 1 H), 2.88(t, J=1.90Hz, 1 H), 2.68(s, 2H), 2.08(s, 2H), 1.22(m, 2 H), 1.16(t, J=7.38Hz, 2 H)

Biophysical methods

Surface Plasmon Resonance (SPR)

SPR measurements were performed using Biacore T200 optical biosensor systems (GE Healthcare, Inc., Piscataway, NJ). Filtered and degassed HEPES buffer (10 mM HEPES, 400 mM NaCl, 1 mM EDTA and 0.05% v/v surfactant P20, pH 7.4) was used in SPR experiments. The measurement process is the same as described previously.[5] A biotinylated hairpin DNA (5'-biotin-CCAAATAAAAGGAAGTGAAACCAAG*CTCT*CTTGGTTTCACTTCCTTTTATTT GG, Fig.1) was immobilized on the surface of a sensor chip pre-coated with streptavidin. A reference channel was prepared without DNA immobilization for baseline correction. To form the PA-DNA complex, an increasing concentration of PA was injected onto the chip surface at a flow rate of 100 µL/min for 210 seconds until a steady state was reached. This high flow rate was used to minimize mass transfer effects. The association was followed by a pure running buffer flow at the same rate, causing the complex to dissociate. At the end of each cycle, 1 M NaCl was used as regeneration solution and injected over the surface to completely wash off the residual PA and prepare the surface for the next cycle.

The kinetic analyses were performed using standard 1:1 global fitting model with mass transport parameters incorporated as described previously.[6,7] The equilibrium constant of some compounds were determined using a steady state fit, because the kinetic parameters went beyond the detection limit and were too fast to determine. This often features the weak binding compounds.

Thermal Melting (Tm)

Thermal melting studies were conducted on a Cary 300 Bio UV/visible spectrophotometer (Varian) in filtered buffer containing 10 mM HEPES (pH 7.4), 50 mM NaCl and 1 mM EDTA. Samples were heated at a rate of 0.5 °C/min, and the corresponding absorbance at 260 nm was recorded and plotted against the temperature. The T_m of DNA was measured at 3 µM, and an equivalent amount of PA was added to obtain the T_m of PA-DNA complex. The difference between the T_m of DNA in the absence and presence of PA is thus a ΔT_m.

Circular Dichroism Spectroscopy (CD)

CD spectra were obtained using a Jasco J-810 spectrometer (Jasco Inc., Easton, MD) with a scan range from 400 nm to 230 nm at 25 °C. The spectra were averaged over four scans with a scan speed of 50 nm·min^{-1} and a buffer blank correction. A 5μM DNA solution was firstly scanned and the PAs were then titrated into the same cuvette at increasing concentration ratios. The complex was scanned under the same condition. A hairpin DNA without a biotin label (5'-GGAAGTGAAC*CTCT*GTTCACTTCC-3') was used and the experimental buffer is the same as that used in thermal melting study.

Results and Figures

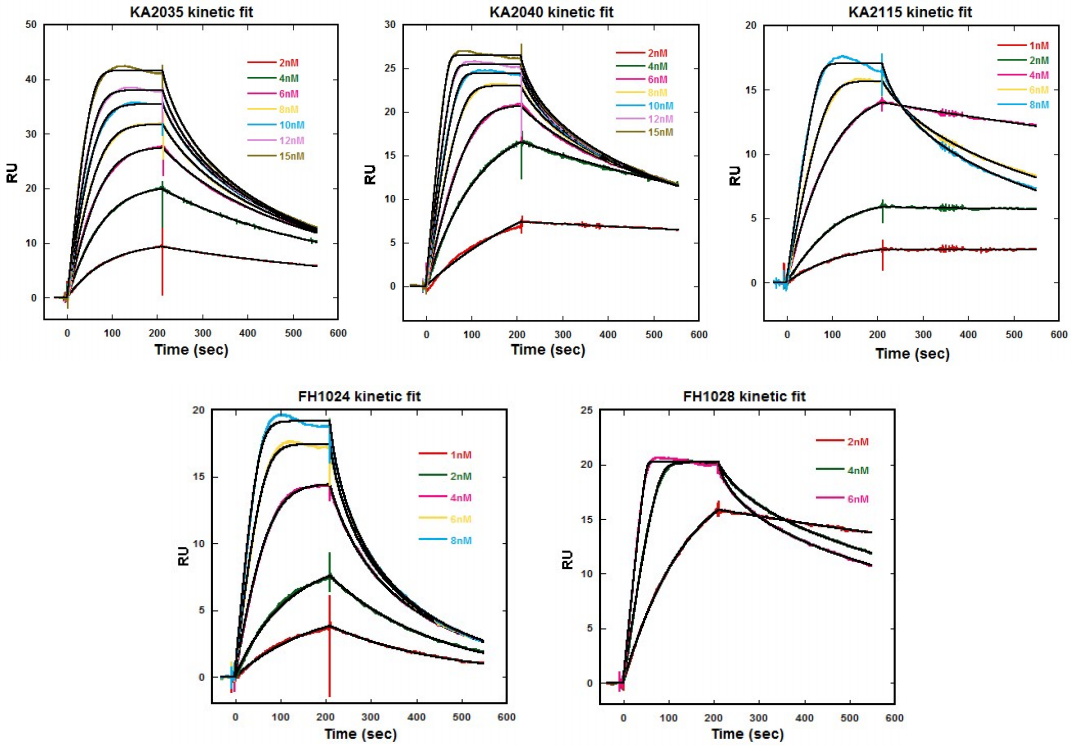
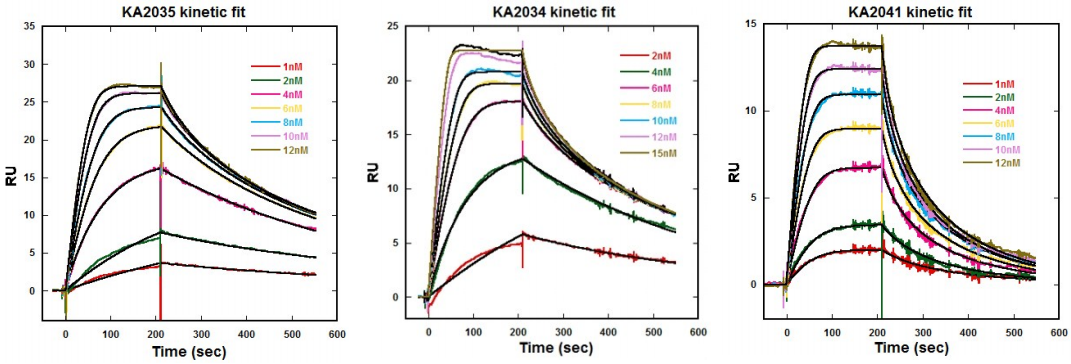
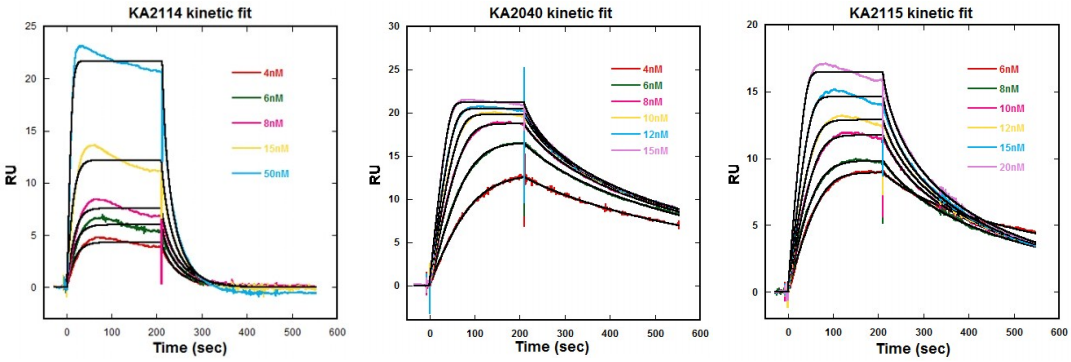


Fig. S1. SPR sensorgrams of PAs binding to λB DNA. The colored lines are experimental sensorgrams. The black overlays are 1:1 global kinetic fits.



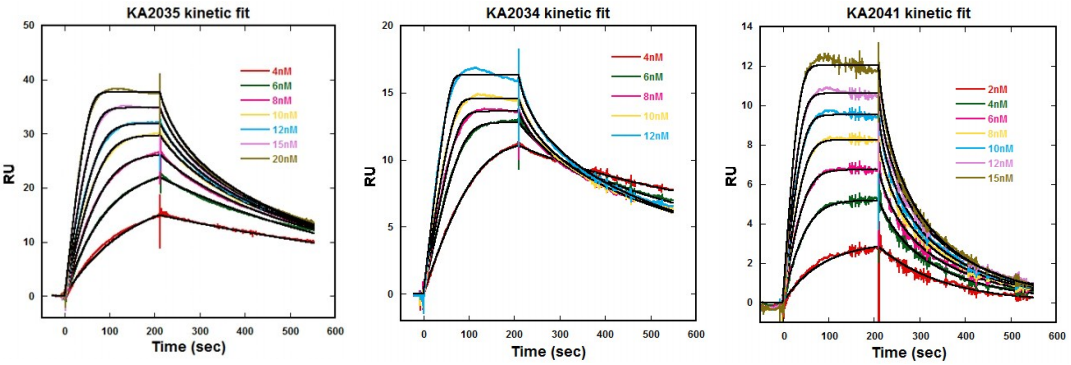


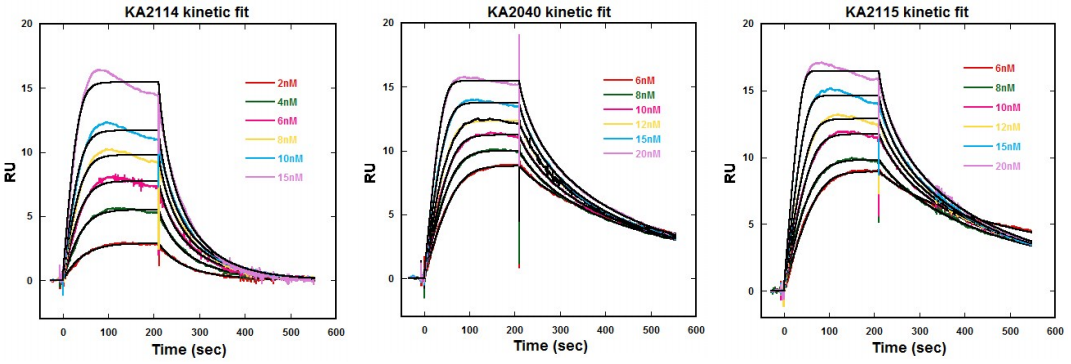
SC1 DNA PA binding site

5'-biotin-CGGCCAAGCCGGAAGTGAGTGC C T
GCCGGTTCGGCCTTCACTCACG T C

GAGA mutant PA binding site

5'-biotin-CCAAAAGAGAGTGAAAACCAAG C T
GGTTTTCTCTCACTTTGGTTC T C





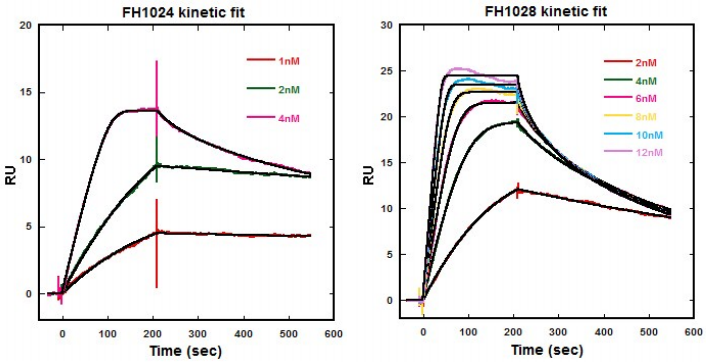


Fig. S2. SPR sensorgrams of PAs binding to SC1 (up) and GAGA mutant (down) sequences. The colored lines are experimental sensorgrams. The black overlays are 1:1 global kinetic fits.

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