

Article

Design of tail-clamp peptide nucleic acid tethered with azobenzene linker for sequence-specific detection of homopurine DNA

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Estimated multiply charged ion peaks of PNA/DNA complexes

Table S1-5 S18–22

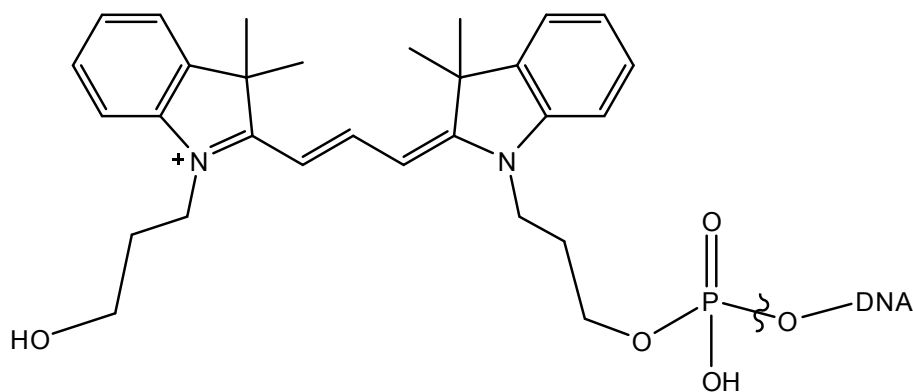
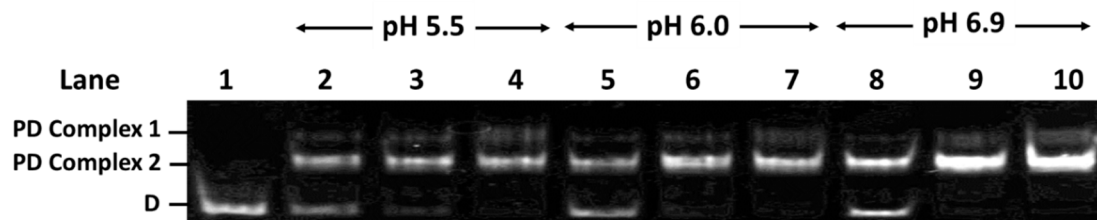


Figure S1. Chemical structure of Cy3 modified at 5' termini of DNA1 and DNA3.

A)



B)

PNA	1	2	3	4	5	6	7	8	9	10
DNA	+	+	+	+	+	+	+	+	+	+
% ratio of PD complex 1 band	0.00	13.37	20.07	29.11	7.75	13.60	21.43	7.28	13.63	19.42
% ratio of PD complex 2 band	0.00	62.67	72.99	69.63	63.37	82.77	78.57	70.23	83.65	78.52
% ratio of remaining DNA band	100.00	23.96	6.94	1.25	28.88	3.64	0.00	22.50	2.72	2.05

Figure S2. Effect of pH on complex formation of PNA11 and DNA1 under different pH conditions.

A) Each lane contains 100 nM of DNA1. Lane 1 contains no PNA. Lanes 2, 5, and 8 contain 1 equivalent of PNA11. Lanes 3, 6, and 9 contain 2 equivalents of PNA11. Lanes 4, 7, and 10 contain 3 equivalents of PNA11. Each DNA and PNA were incubated in 10 mM sodium phosphate buffer (pH6.0) at 25°C for 10 min. Gel mobility shift assays of PNA/DNA complexes were performed using 15% non-denaturing polyacrylamide gels. B) Relative band intensities of PNA/DNA complex 1 (PD complex 1), PNA/DNA complex 2 (PD complex 2), and DNA1 in a gel mobility shift assay at different PNA molar ratios and pH conditions. B) Quantitative band-intensity analysis of DNA and DNA/PNA complexes in Fig. S2A.

A)

PNA 15 [eq.] **0** **1.0** **2.0** **3.0** **5.0**



B)

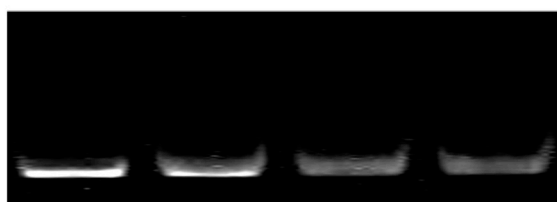
PNA 15 (eq.)	0	1	2	3	5
DNA 1	+	+	+	+	+
% ratio of shifted band	0.00	39.83	76.48	80.44	75.21
% ratio of remaining DNA band	100.00	60.17	23.52	19.56	24.79

Figure S3. Gel mobility shift analysis of complex formation between DNA1 and PNA15. All lanes contain Cy3-labelled single strand DNA 1 (100 nM) and varying amounts of *trans*-AZO PNA15. The configuration of azobenzene was thermally isomerized to the *trans*-form by incubating at 95°C for 10 min prior to the experiment. PNA and ssDNA were incubated in 10 mM sodium phosphate buffer (pH6.0) at 25°C for 10 min. The gel mobility shift assay of PNA/ssDNA complex formation was performed using a 15% non-denaturing polyacrylamide gel. B) Quantitative band-intensity analysis of DNA and DNA/PNA complexes in Fig. S3A.

A)

PNA 15 [eq.] **0** **1.0** **2.0** **3.0**

DNA 3 **+** **+** **+** **+**

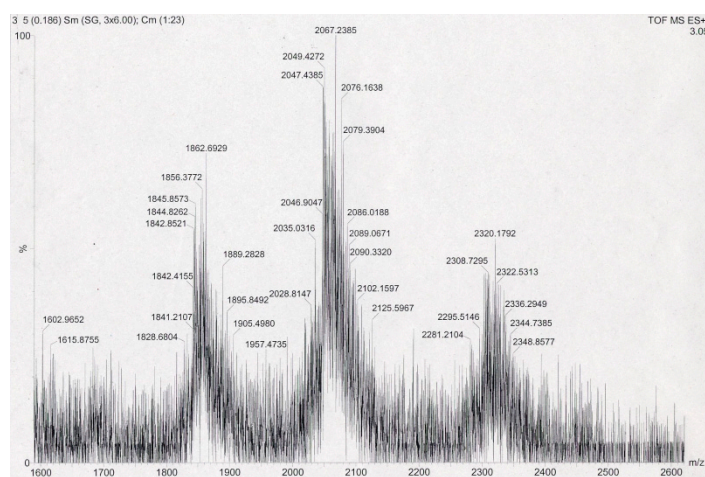


B)

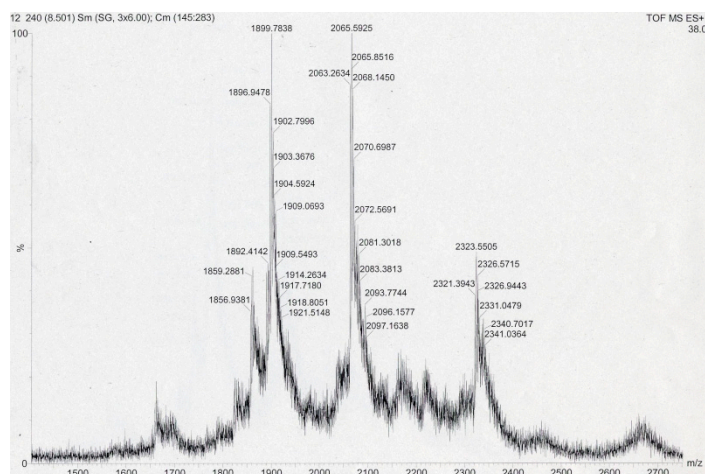
PNA 15 (eq.)	0	1	2	3
% ratio of remaining DNA band	45.651	43.893	34.13	29.48

Figure S4. Mismatch base recognition of PNA15 on the target DNA3. A) Excess amounts of PNA15 were added to each ssDNA at various concentrations, and complex formation was monitored using gel mobility shift assays. The configuration of azobenzene was thermally isomerized to the *trans*-form by incubating it at 95°C for 10 min prior to the experiment. PNA15 and ssDNA were incubated in 10 mM sodium phosphate buffer (pH6.0) at 25°C for 10 min. The gel mobility shift assay of PNA/ssDNA complex formation was performed using a 15% non-denaturing polyacrylamide gel. B) Quantitative band-intensity analysis of DNA3 in Fig. S4A.

A)



B)



C)

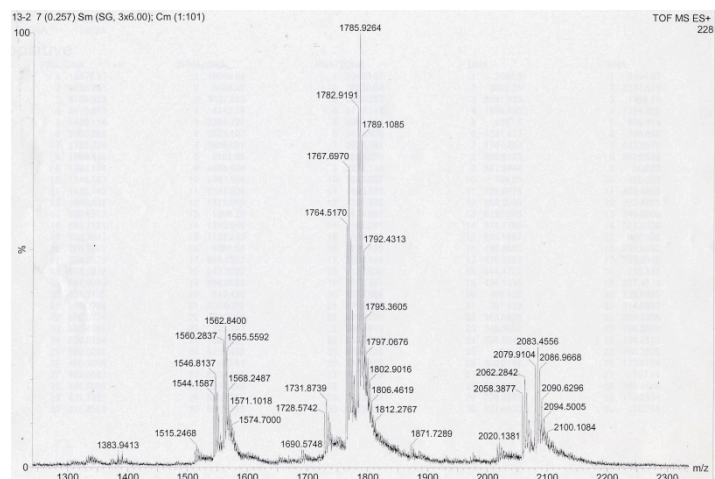
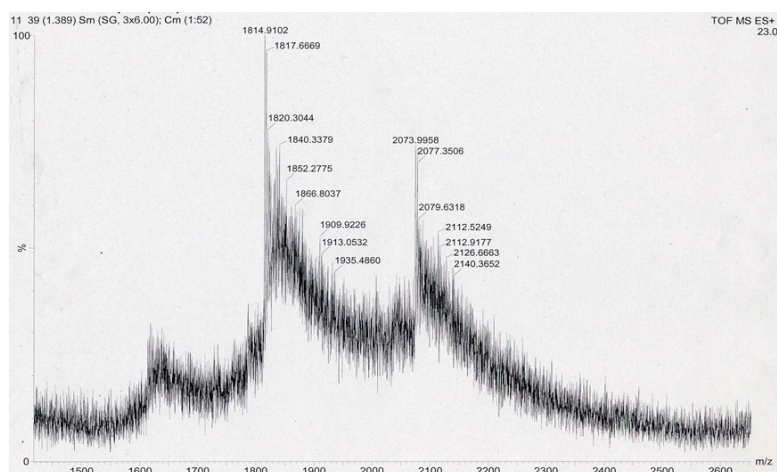


Figure S5. Stoichiometry analysis of ssDNA and TC-PNA complexes by Nano ESI-mass spectrometry. A) PNA6 and DNA1 were incubated at a 2:1 molar ratio (200 nM, 100 nM). B) PNA8 and DNA1 were incubated at a 2:1 molar ratio (200 nM, 100 nM). C) PNA15 and DNA1 were incubated at a 2:1 molar ratio (200 nM, 100 nM).

A)



B)

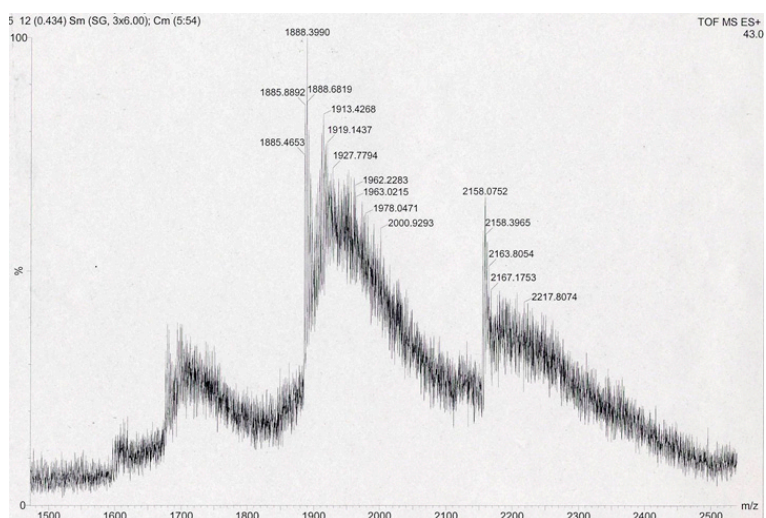


Figure S6. Stoichiometry analysis of ssDNA and PNA complexes by Nano ESI-mass spectrometry. A) PNA1 and DNA1 were incubated at a 2:1 molar ratio (200 nM, 100 nM). B) PNA2 and DNA1 were incubated at a 2:1 molar ratio (200 nM, 100 nM).

MALDI-TOF-MS analysis of PNAs

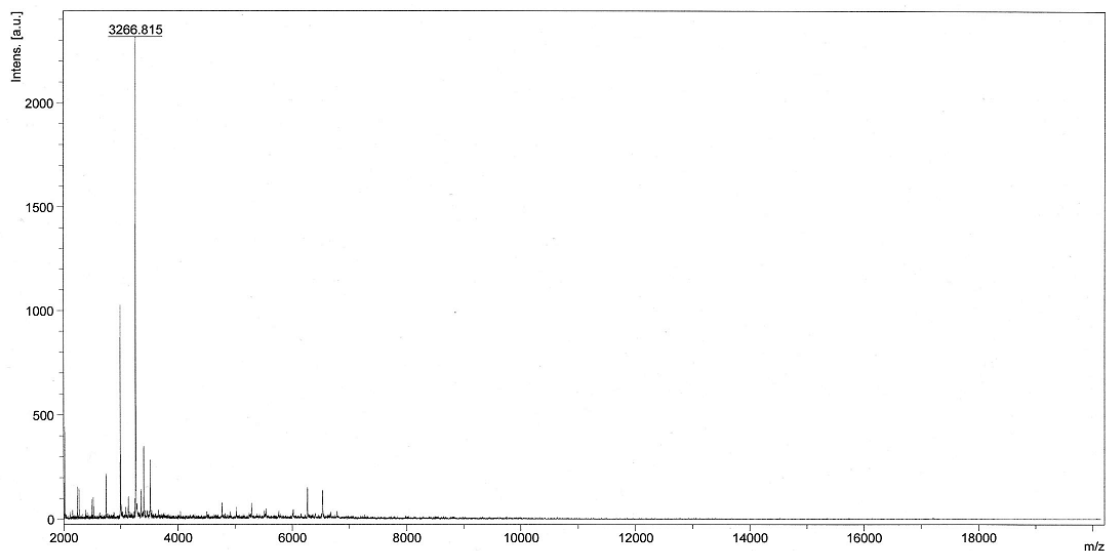


Figure S7. PNA1:TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 3266.44, observed 3266.815 [M(average)]⁺.

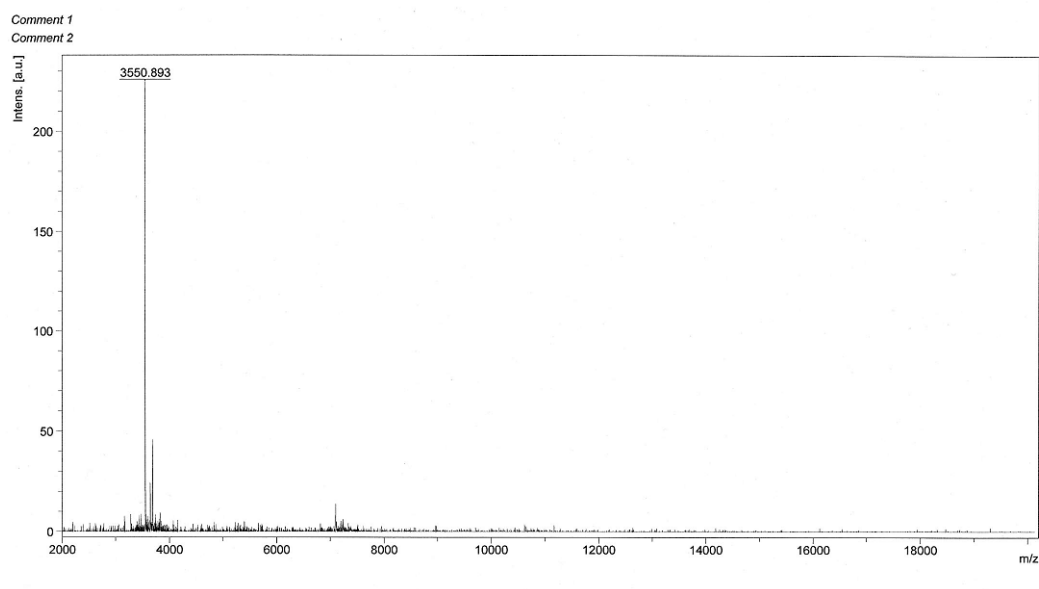


Figure S8. PNA2: AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 3549.80, observed 3550.89 [M(average)]⁺.

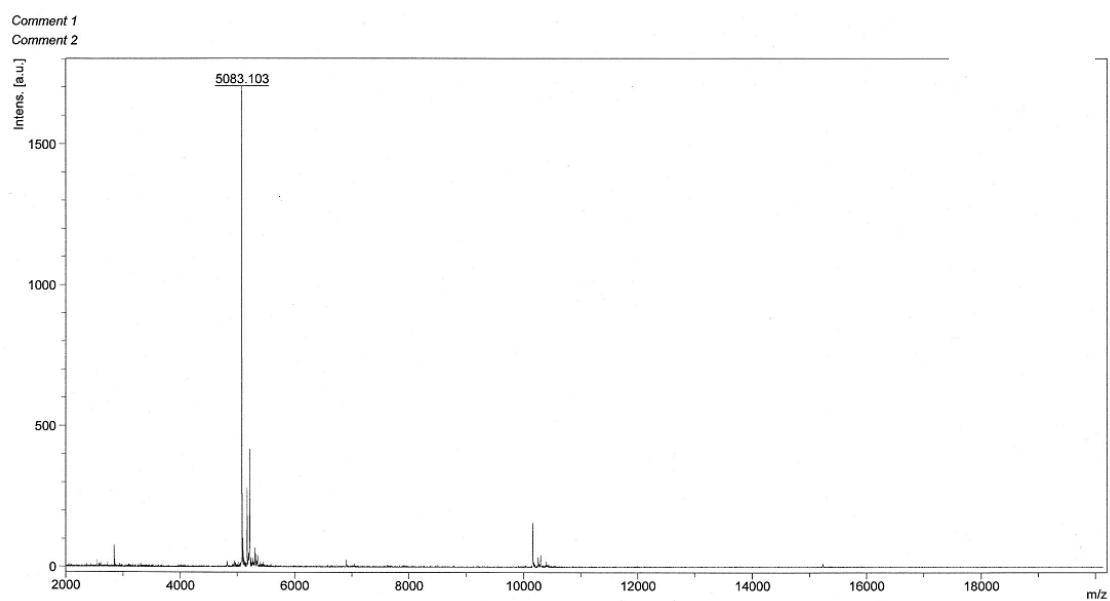


Figure S9. PNA3: K₃-CCTCT-AEEA-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5082.51, observed 5083.10 [M(average)]⁺.

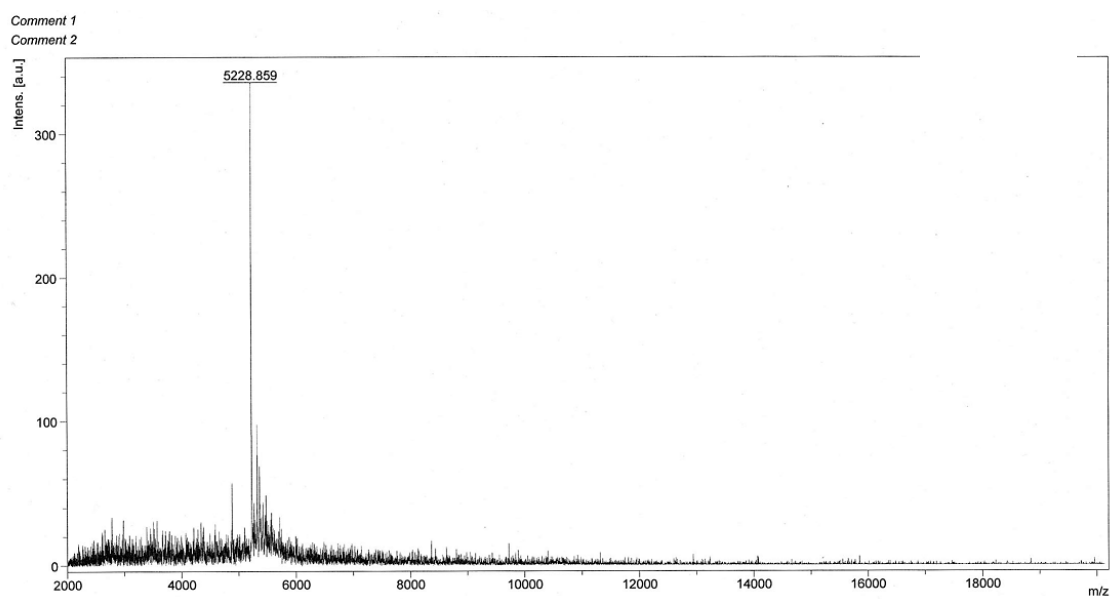


Figure S10. PNA4: K₃-CCTCT-(AEEA)₂-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5227.7, observed 5228.86 [M(average)]⁺.

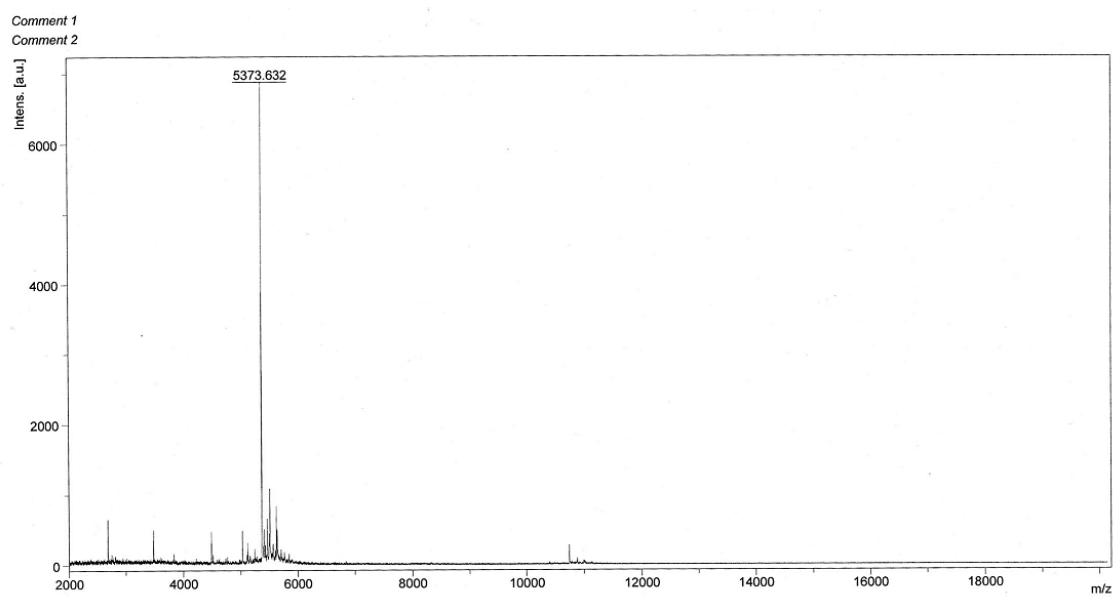


Figure S11. PNA5: K₃-CCTCT-(AEEA)₃-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5372.8, observed 5373.63 [M(average)]⁺.

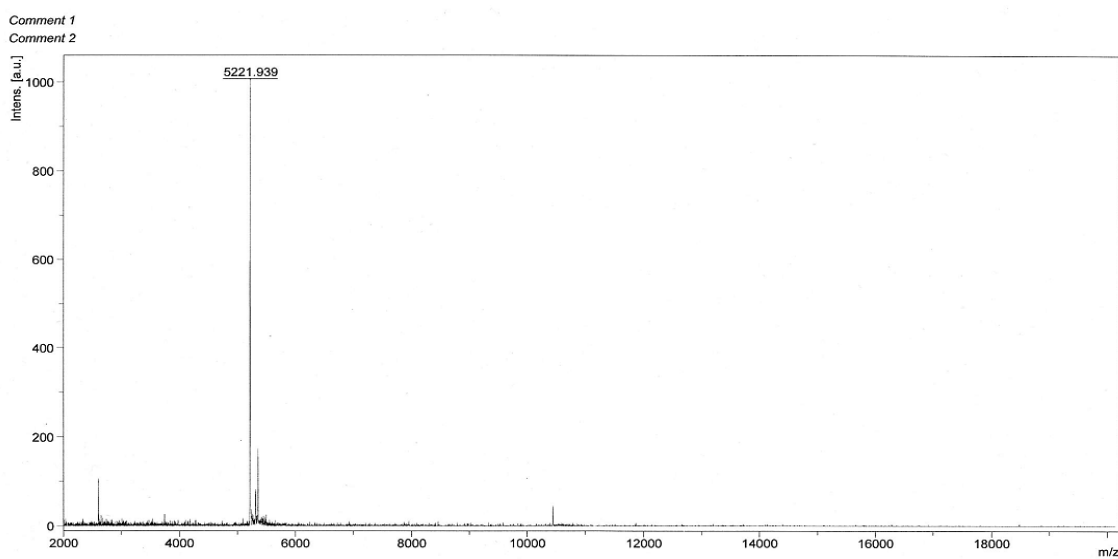


Figure S12. PNA6: K₃-CCTCT-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5220.74, observed 5221.94 [M(average)]⁺.

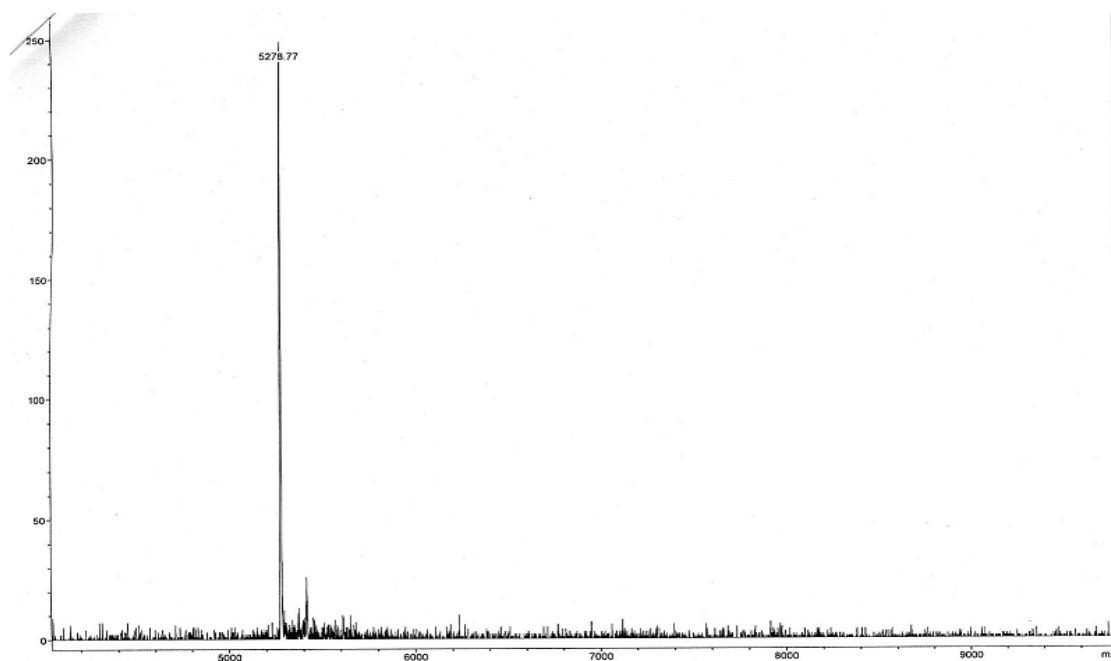


Figure S13. PNA7: K₃-CCTCT-C3-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5277.81, observed 5278.77 [M(average)]⁺.

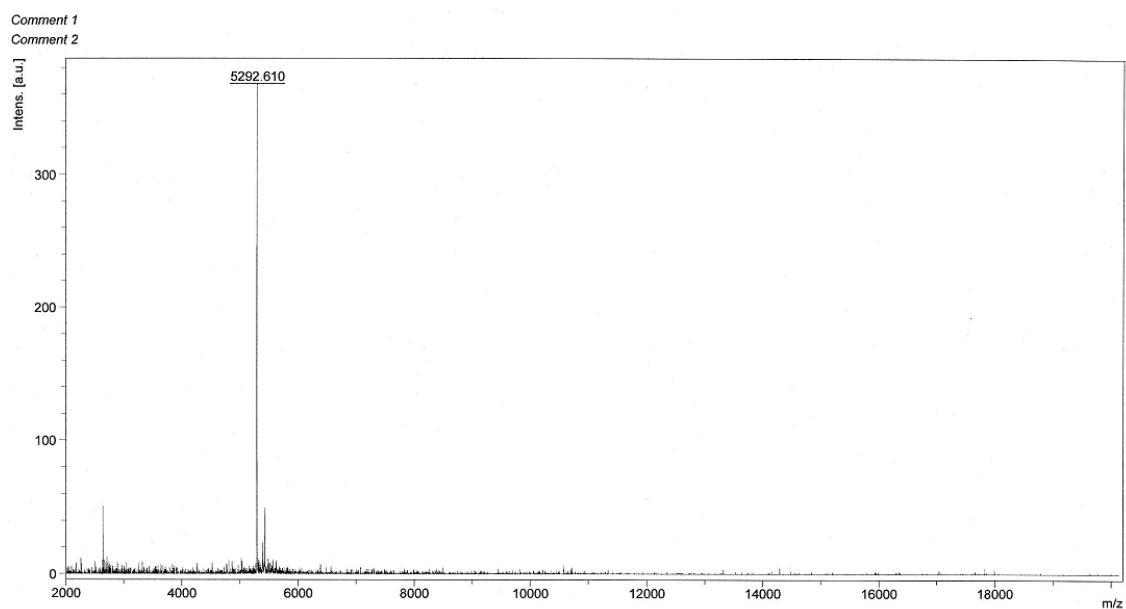


Figure S14. PNA8: K₃-CCTCT-C4-AZO-TCTCCCTTCTTT-K

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5291.82, found 5292.61 [M(average)]⁺.

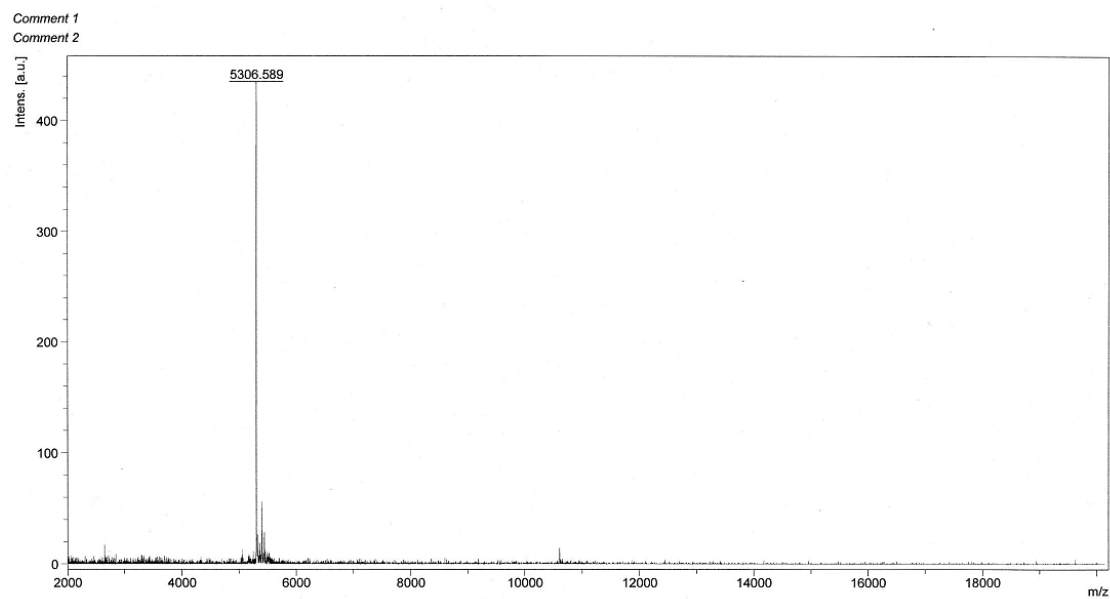


Figure S15. PNA9: K₃-CCTCT-C5-AZO-TCTCCCTTCTTT-K

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5305.86, observed 5306.59 [M(average)]⁺.

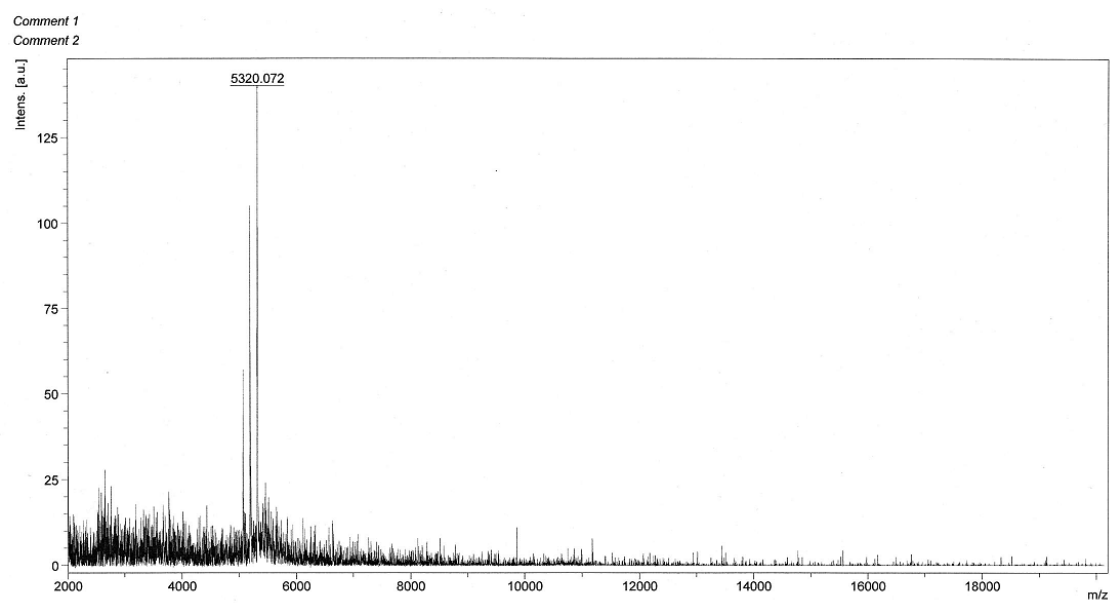


Figure S16. PNA10: K₃-CCTCT-C6-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5319.89, observed 5320.01 [M(average)]⁺.

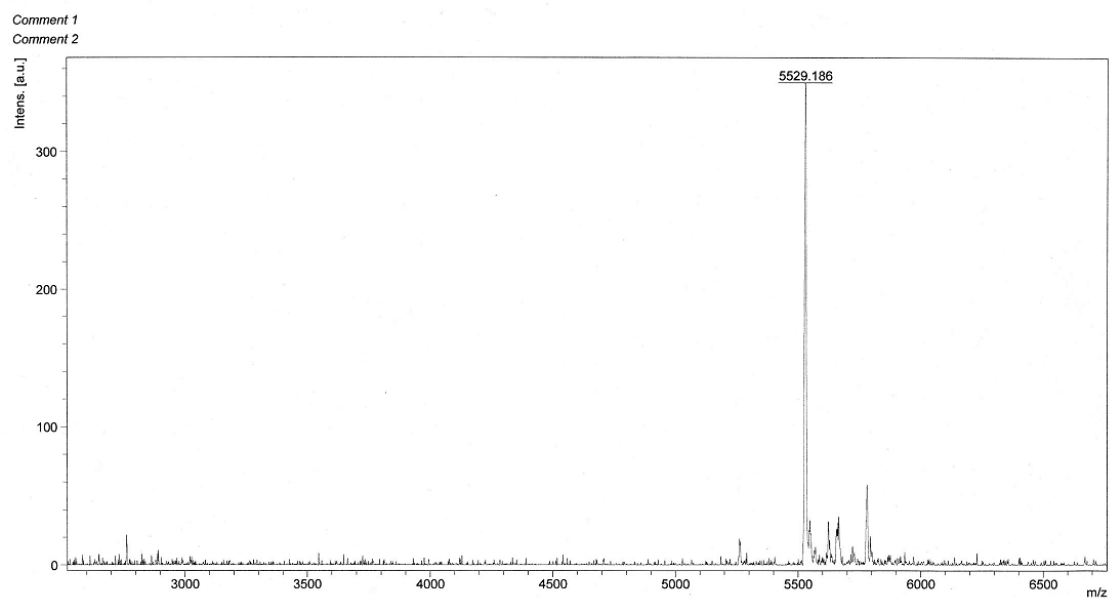


Figure S17. PNA11: K₃-CTTCCCTCT-C4-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5528.17, observed 5529.19 [M(average)]⁺.

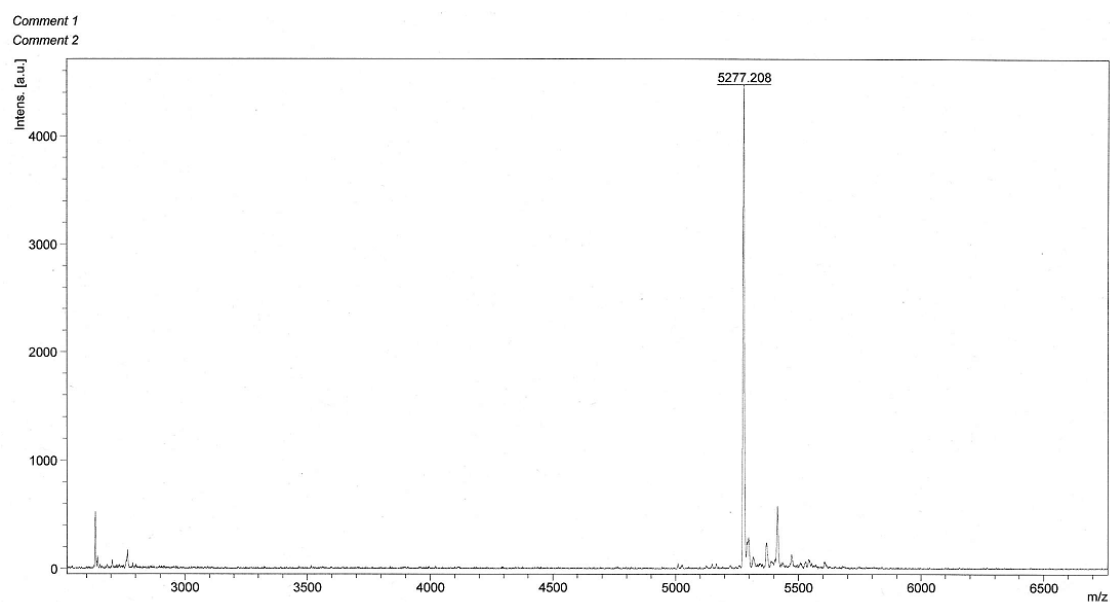


Figure S18. PNA12: K₃-TTCCCTCT-C4-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5276.87, observed 5277.21 [M(average)]⁺.

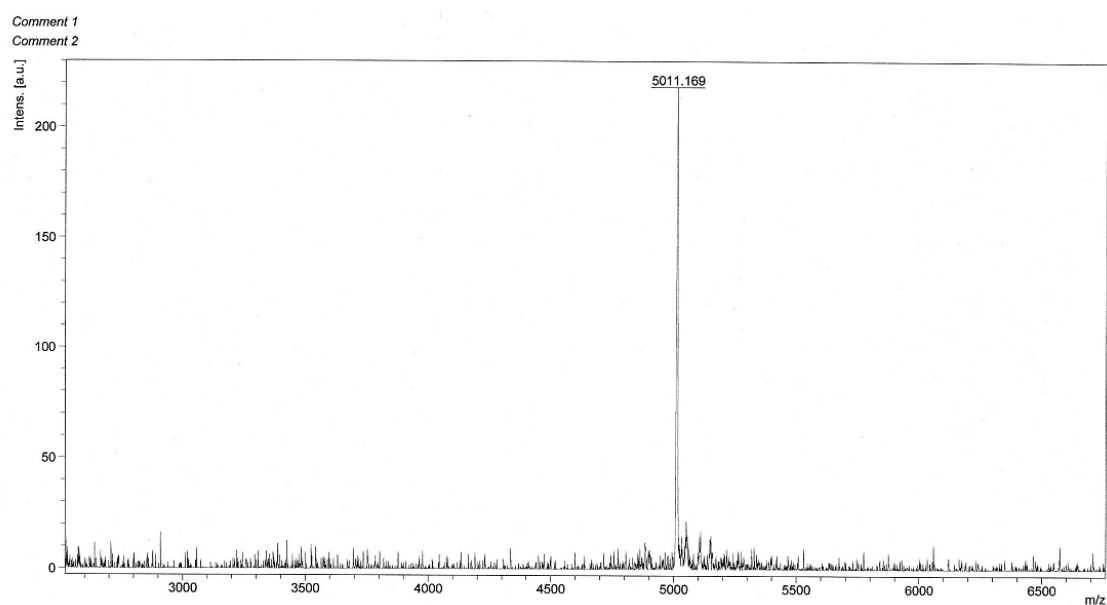


Figure S19. PNA13: K₃-TCCCTCT-C4-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 5010.62, observed 5011.17 [M(average)]⁺.

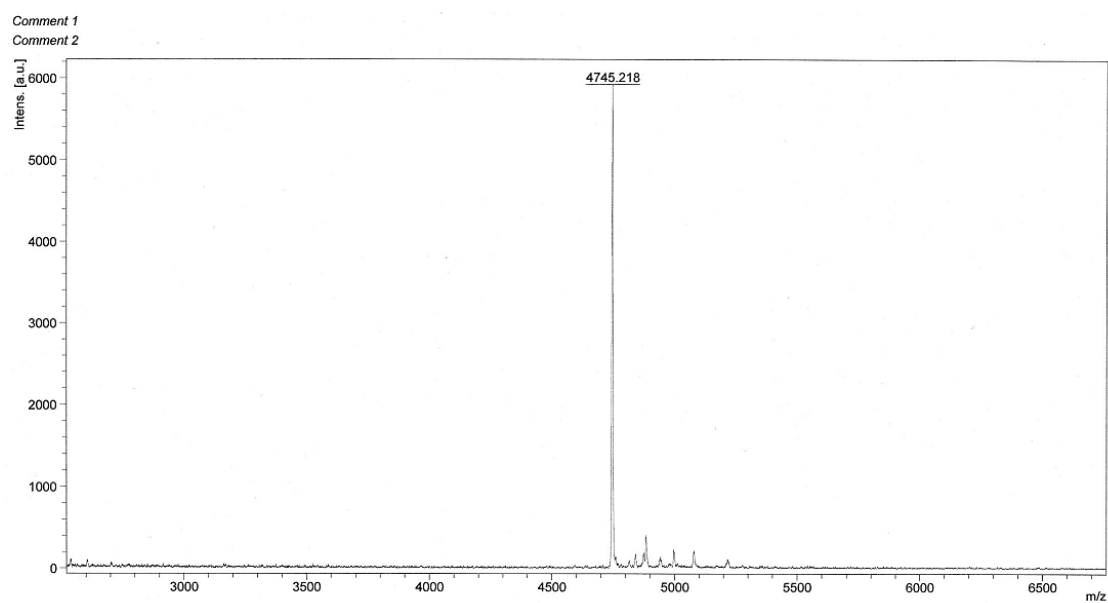


Figure S20. PNA14: K₃-CCCTCT-C4-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 4744.37, observed 4745.22 [M(average)]⁺.

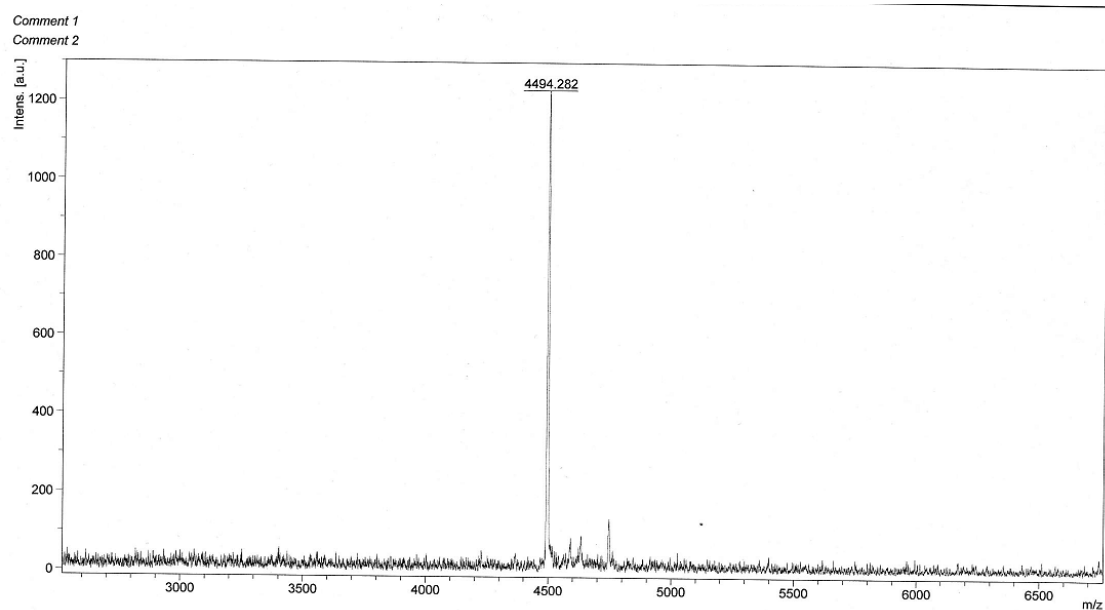


Figure S21. PNA15: K₃-CCTCT-C4-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 4493.07, observed 4494.28 [M(average)]⁺.

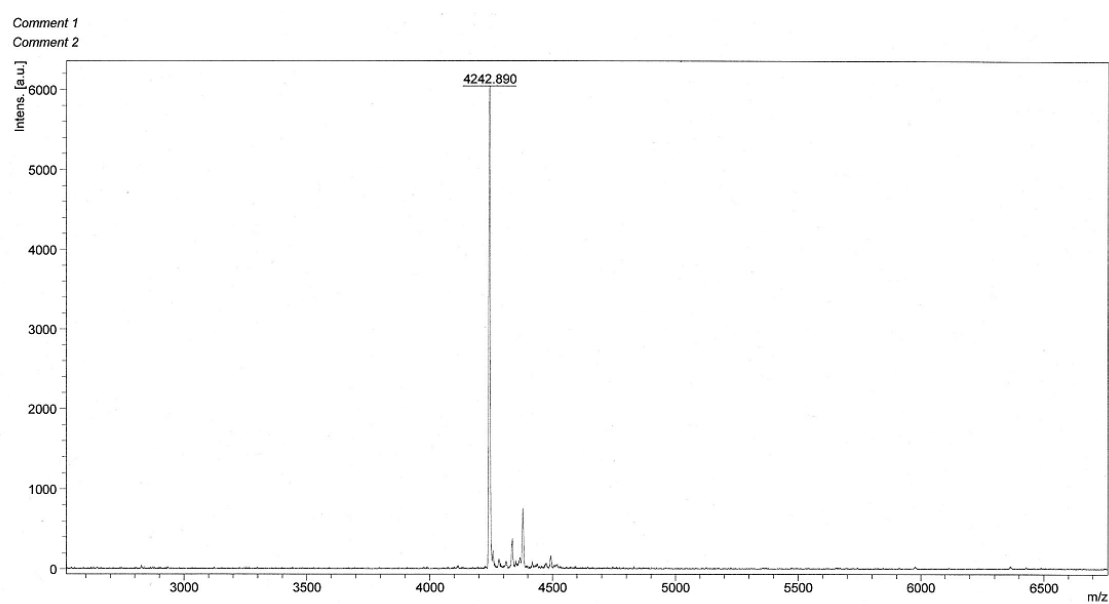


Figure S22. PNA16: K₃-CTCT-C4-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 4241.77, observed 4242.89 [M(average)]⁺.

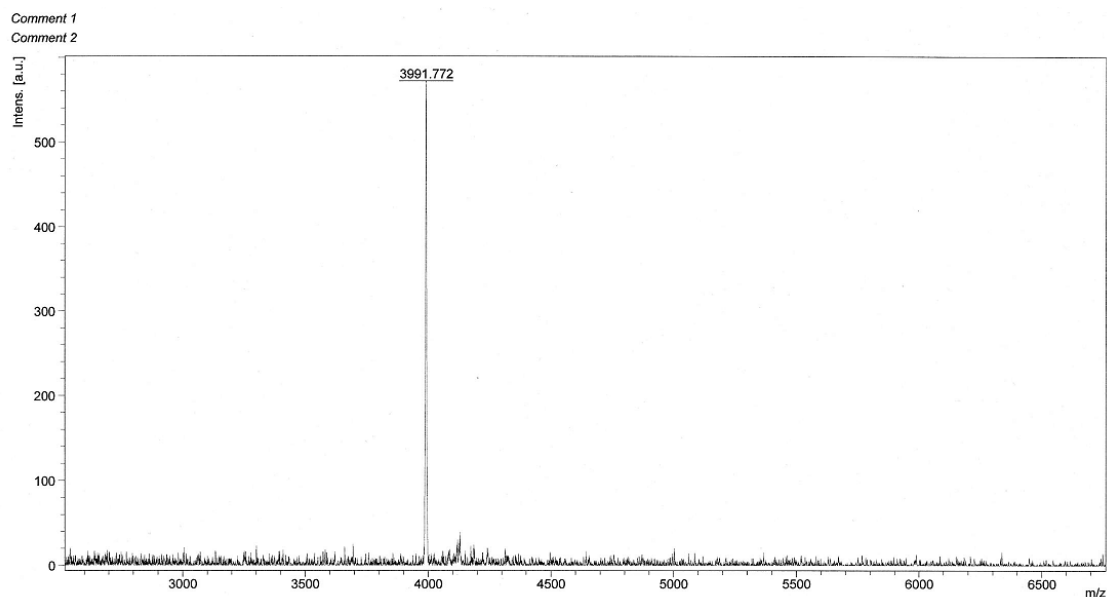


Figure S23. PNA17: K₃TCT-C4-AZO-TCTCCCTTCTTT-K.

MALDI-TOF-MS (sinapinic acid): m/z calcd. 3990.47, observed 3991.77 [M(average)]⁺.

Table S1. Estimated multiply charged ion peaks of PNA6, DNA1, and their complexes. The highlighted peaks were identified in Figure S5A.

		Molecular weight							
		PNA 6	5220.74						
		DNA 1	7982.50						
		Na ⁺	23.00						
PNA/DNA/Na ⁺		PNA/PNA/DNA/Na ⁺		PNA/DNA/DNA/Na ⁺		DNA/Na ⁺		PNA/Na ⁺	
1	13227.24	1	18447.98	1	13250.24	1	8006.50	1	5244.74
2	6614.12	2	9224.49	2	6625.62	2	4003.75	2	2622.87
3	4409.75	3	6149.99	3	4417.41	3	2669.50	3	1748.91
4	3307.56	4	4612.75	4	3313.31	4	2002.38	4	1311.94
5	2646.25	5	3690.40	5	2650.85	5	1602.10	5	1049.75
6	2205.37	6	3075.50	6	2209.21	6	1335.25	6	874.96
7	1890.46	7	2636.28	7	1893.75	7	1144.64	7	750.11
8	1654.28	8	2306.87	8	1657.16	8	1001.69	8	656.47
9	1470.58	9	2050.66	9	1473.14	9	890.50	9	583.64
10	1323.62	10	1845.70	10	1325.92	10	801.55	10	525.37
11	1203.39	11	1678.00	11	1205.48	11	728.77	11	477.70
12	1103.19	12	1538.25	12	1105.10	12	668.13	12	437.98
13	1018.40	13	1420.00	13	1020.17	13	616.81	13	404.36
14	945.73	14	1318.64	14	947.37	14	572.82	14	375.55
15	882.75	15	1230.80	15	884.28	15	534.70	15	350.58
16	827.64	16	1153.94	16	829.08	16	501.34	16	328.73
17	779.01	17	1086.12	17	780.37	17	471.91	17	309.46
18	735.79	18	1025.83	18	737.07	18	445.75	18	292.32
19	697.12	19	971.89	19	698.33	19	422.34	19	276.99
20	662.31	20	923.35	20	663.46	20	401.28	20	263.19

Table S2. Estimated multiply charged ion peaks of PNA8, DNA1, and their complexes. The highlighted peaks were identified in Figure S5B.

		Molecular weight							
PNA 8		5291.82							
DNA 1		7982.50							
Na ⁺		23.00							
PNA/DNA/Na ⁺		PNA/PNA/DNA/Na ⁺		PNA/DNA/DNA/Na ⁺		DNA/Na ⁺		PNA/Na ⁺	
1	13298.32	1	18590.14	1	21280.82	1	8006.50	1	5315.82
2	6649.66	2	9295.57	2	10640.91	2	4003.75	2	2658.41
3	4433.44	3	6197.38	3	7094.27	3	2669.50	3	1772.61
4	3325.33	4	4648.29	4	5320.96	4	2002.38	4	1329.71
5	2660.46	5	3718.83	5	4256.96	5	1602.10	5	1063.96
6	2217.22	6	3099.19	6	3547.64	6	1335.25	6	886.80
7	1900.62	7	2656.59	7	3040.97	7	1144.64	7	760.26
8	1663.17	8	2324.64	8	2660.98	8	1001.69	8	665.35
9	1478.48	9	2066.46	9	2365.42	9	890.50	9	591.54
10	1330.73	10	1859.91	10	2128.98	10	801.55	10	532.48
11	1209.85	11	1690.92	11	1935.53	11	728.77	11	484.17
12	1109.11	12	1550.10	12	1774.32	12	668.13	12	443.90
13	1023.87	13	1430.93	13	1637.91	13	616.81	13	409.83
14	950.81	14	1328.80	14	1520.99	14	572.82	14	380.63
15	887.49	15	1240.28	15	1419.65	15	534.70	15	355.32
16	832.08	16	1162.82	16	1330.99	16	501.34	16	333.18
17	783.20	17	1094.48	17	1252.75	17	471.91	17	313.64
18	739.74	18	1033.73	18	1183.21	18	445.75	18	296.27
19	700.86	19	979.38	19	1120.99	19	422.34	19	280.73
20	665.87	20	930.46	20	1064.99	20	401.28	20	266.74

Table S3. Estimated multiply charged ion peaks of PNA15, DNA1, and their complexes. The highlighted peaks were identified in Figure S5C.

		Molecular weight							
PNA 15		4507.11							
DNA 1		7982.50							
Na ⁺		23.00							
PNA/DNA/Na ⁺		PNA/PNA/DNA/Na ⁺		PNA/DNA/DNA/Na ⁺		DNA/Na ⁺		PNA/Na ⁺	
1	12513.61	1	17020.72	1	20496.11	1	8006.50	1	4531.11
2	6257.31	2	8510.86	2	10248.56	2	4003.75	2	2266.06
3	4171.87	3	5674.24	3	6832.70	3	2669.50	3	1511.04
4	3129.15	4	4255.93	4	5124.78	4	2002.38	4	1133.53
5	2503.52	5	3404.94	5	4100.02	5	1602.10	5	907.02
6	2086.44	6	2837.62	6	3416.85	6	1335.25	6	756.02
7	1788.52	7	2432.39	7	2928.87	7	1144.64	7	648.16
8	1565.08	8	2128.47	8	2562.89	8	1001.69	8	567.26
9	1391.29	9	1892.08	9	2278.23	9	890.50	9	504.35
10	1252.26	10	1702.97	10	2050.51	10	801.55	10	454.01
11	1138.51	11	1548.25	11	1864.19	11	728.77	11	412.83
12	1043.72	12	1419.31	12	1708.93	12	668.13	12	378.51
13	963.51	13	1310.21	13	1577.55	13	616.81	13	349.47
14	894.76	14	1216.69	14	1464.94	14	572.82	14	324.58
15	835.17	15	1135.65	15	1367.34	15	534.70	15	303.01
16	783.04	16	1064.73	16	1281.94	16	501.34	16	284.13
17	737.04	17	1002.16	17	1206.59	17	471.91	17	267.48
18	696.15	18	946.54	18	1139.62	18	445.75	18	252.67
19	659.56	19	896.77	19	1079.69	19	422.34	19	239.43
20	626.63	20	851.99	20	1025.76	20	401.28	20	227.51

Table S4. Estimated multiply charged ion peaks of PNA1, DNA1, and their complexes. The highlighted peaks were identified in Figure S6A.

		Molecular weight							
		PNA 1	3266.44						
		DNA 1	7982.50						
		Na ⁺	23.00						
	PNA/DNA/Na ⁺	PNA/PNA/DNA/Na ⁺	PNA/DNA/DNA/Na ⁺		DNA/Na ⁺		PNA/Na ⁺		
1	11272.94	1	14539.38	1	19255.44	1	8006.50	1	3290.44
2	5636.97	2	7270.19	2	9628.22	2	4003.75	2	1645.72
3	3758.31	3	4847.13	3	6419.15	3	2669.50	3	1097.48
4	2818.99	4	3635.60	4	4814.61	4	2002.38	4	823.36
5	2255.39	5	2908.68	5	3851.89	5	1602.10	5	658.89
6	1879.66	6	2424.06	6	3210.07	6	1335.25	6	549.24
7	1611.28	7	2077.91	7	2751.63	7	1144.64	7	470.92
8	1409.99	8	1818.30	8	2407.81	8	1001.69	8	412.18
9	1253.44	9	1616.38	9	2140.38	9	890.50	9	366.49
10	1128.19	10	1454.84	10	1926.44	10	801.55	10	329.94
11	1025.72	11	1322.67	11	1751.40	11	728.77	11	300.04
12	940.33	12	1212.53	12	1605.54	12	668.13	12	275.12
13	868.07	13	1119.34	13	1482.11	13	616.81	13	254.03
14	806.14	14	1039.46	14	1376.32	14	572.82	14	235.96
15	752.46	15	970.23	15	1284.63	15	534.70	15	220.30
16	705.50	16	909.65	16	1204.40	16	501.34	16	206.59
17	664.06	17	856.20	17	1133.61	17	471.91	17	194.50
18	627.22	18	808.69	18	1070.69	18	445.75	18	183.75
19	594.26	19	766.18	19	1014.39	19	422.34	19	174.13
20	564.60	20	727.92	20	963.72	20	401.28	20	165.47

Table S5. Estimated multiply charged ion peaks of PNA2, DNA1, and their complexes. The highlighted peaks were identified in Figure S6B.

		Molecular weight							
		PNA 2	3549.80						
		DNA 1	7982.50						
		Na ⁺	23.00						
	PNA/DNA/Na ⁺	PNA/PNA/DNA/Na ⁺	PNA/DNA/DNA/Na ⁺		DNA/Na ⁺		PNA/Na ⁺		
1	11556.30	1	15106.10	1	19538.80	1	8006.50	1	3573.80
2	5778.65	2	7553.55	2	9769.90	2	4003.75	2	1787.40
3	3852.77	3	5036.03	3	6513.60	3	2669.50	3	1191.93
4	2889.83	4	3777.28	4	4885.45	4	2002.38	4	894.20
5	2312.06	5	3022.02	5	3908.56	5	1602.10	5	715.56
6	1926.88	6	2518.52	6	3257.30	6	1335.25	6	596.47
7	1651.76	7	2158.87	7	2792.11	7	1144.64	7	511.40
8	1445.41	8	1889.14	8	2443.23	8	1001.69	8	447.60
9	1284.92	9	1679.34	9	2171.87	9	890.50	9	397.98
10	1156.53	10	1511.51	10	1954.78	10	801.55	10	358.28
11	1051.48	11	1374.19	11	1777.16	11	728.77	11	325.80
12	963.94	12	1259.76	12	1629.15	12	668.13	12	298.73
13	889.87	13	1162.93	13	1503.91	13	616.81	13	275.83
14	826.38	14	1079.94	14	1396.56	14	572.82	14	256.20
15	771.35	15	1008.01	15	1303.52	15	534.70	15	239.19
16	723.21	16	945.07	16	1222.11	16	501.34	16	224.30
17	680.72	17	889.54	17	1150.28	17	471.91	17	211.16
18	642.96	18	840.17	18	1086.43	18	445.75	18	199.49
19	609.17	19	796.01	19	1029.31	19	422.34	19	189.04
20	578.77	20	756.26	20	977.89	20	401.28	20	179.64