Supplemental Section

Materials: 2-Pyrrolyl trichloromethyl ketone (2-(trichloroacetyl)pyrrole), 2-(trimethylsilyl)ethanol, sodium, 10% Pd/C, di-*t*-butyl dicarbonate (Boc-anhydride), N-(3-bromopropyl)phthalimide, tetrabutylammonium iodide, and tetrabutylammonium fluoride were purchased from Aldrich. Ethyl acetate (EtOAc), acetone, tetrahydrofuran (THF), and acetic anhydride (Ac_2O) were reagent grade from EM. All reagents were used without further purification. ^1H NMR and ^{13}C NMR spectra were recorded using a Varian Mercury instrument operating at 300 MHz. Chemical shifts are reported in parts per million relative to residual solvent signal. IR spectra were recorded on a Perkin-Elmer FTIR spectrometer. High-resolution EI mass spectra were recorded at the Mass Spectrometry Laboratory at the UCLA Mass Spectrometry Facility. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} plates. Reagent grade chemicals were used unless otherwise indicated.

Conjugate Syntheses. (Supplemental Figure S1) A Boc-protected pyrrole amino acid bearing an N-(phthalimidopropyl) moiety at the N1 position was incorporated at a unique position within each polyamide prepared by solid phase synthesis[21]. Aminolysis of resin bound polyamide **9** (10 hours/60 °C) and subsequent purification yields a polyamide-amine suitable for modification. One equivalent of L-Boc(Trt)-Cys-OH activated with DCC/HOBt was coupled to the free amine, deprotected with trifluoroacetic acid and purified via HPLC to yield

10. Polyamide thiol **10** was allowed to react with TMR-maleimide fluorophore in dimethyl formamide to afford the polyamide-TMR conjugate **3**.

Monomer Synthesis. (Supplemental Figure S2)

4-Nitro-2-(trichloroacetyl)pyrrole (11). To a cooled (-15 °C) solution of 2-(trichloroacetyl)pyrrole (250 g, 1.18 mol) in acetic anhydride (1 L) in a 4 L flask equipped with a mechanical stirrer was added fuming nitric acid (135 mL) over a period of 1 hour. The reaction mixture was then slowly allowed to warm to ambient temperature and was allowed to stir for an additional 6 hours. The mixture was then poured onto ice water (5 L) and the resulting suspension was allowed to stir vigorously, then filtered to yield a white solid. The solid was azeotroped from toluene and recrystalized from 95:5 (v:v) $CHCl_3$/EtOH to yield **10** as a tan solid (168.5 g, 0.654 mol, 55% yield): TLC (5:2 hexanes/ethyl acetate; silica) R_f 0.5; ^{1}H NMR (DMSO-d_6) δ 8.34 (d, 1 H, J = 1.5 Hz), 7.67 (d, 1 H, J = 1.5 Hz); ^{13}C NMR (DMSO-d_6) δ 173.5, 137.7, 128.5, 122.0, 115.0, 94.5; EI-MS m/e 255.9215 (M^+ 255.9209 calculated for $C_6H_3Cl_3N_2O_3$); IR (ν_{max} cm^{-1}; $CHCl_3$) 3317, 1676, 1551, 1519, 1405, 1379, 1316, 1149, 814, 742, 730.

(2-Trimethylsilyl)-ethyl 4-nitropyrrole-2-carboxylate (12). Na^0 (0.897 g, 0.039 mol) was dissolved in a solution of 2-(trimethylsilyl)ethanol (50 g, 0.423 mol) in THF (0.2 L). The resulting mixture was added to a stirred solution of **11** in THF (0.3 L). After 6 hours, the reaction was quenched by addition of concentrated H_2SO_4 (2.2 mL, 0.039 mol). The mixture was then concentrated *in vacuo* to yield a slightly yellow solid. The solid was suspended in cold (0 °C) dichloromethane

(0.1 L) and petroleum ether (0.3 L) was added. The resulting suspension was then filtered to yield **11** (91.5 g, 0.356 mol, 92% yield) as a white solid: TLC (3:2 hexanes : ethyl acetate; silica) R_f 0.5; ^{1}H NMR (DMSO-d_6) δ 8.06 (d, 1 H, J = 1.5 Hz), 7.19 (d, 1 H, J = 1.5 Hz), 4.32 (t, 2 H, J = 8.3 Hz), 1.04 (t, 2 H, J = 8.3 Hz), 0.04 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 160.1, 137.2, 124.9, 123.8, 109.8, 63.5, 17.7, -0.7; EI-MS m/e 256.0879 (M^+ 256.0879 calculated for $C_{10}H_{16}N_2O_4Si$); IR (ν_{max} cm^{-1}; $CHCl_3$) 3260, 1690, 1512, 1372, 1320, 1202, 836, 752.

(2-Trimethylsilyl)ethyl 4-[(*t*-butoxycarbonyl)amino]pyrrole-2-carboxylate (13). 10% Palladium on activated carbon (6 g) was added to a stirred solution of **12** (30.8 g, 0.120 mol) in EtOAc (0.5 L) and EtOH (2.5 mL). The resulting suspension was allowed to stir under H_2 (500 psi) for 8 hrs. The reaction mixture was then filtered over celite and the supernatant was concentrated *in vacuo* to yield a yellow oil. The oil was resuspended in EtOAc (0.24 L) and Boc-anhydride (39.3 g, 0.180 mol) was added, followed by 1M $NaHCO_3$ (0.24 L). The biphasic mixture was allowed to stir for 4 hrs. and the layers were separated. The aqueous layer was washed with EtOAc (3 x 0.25 L) and the organic layers were combined, suspended over sodium sulfate, filtered and concentrated to yield a yellow oil. The oil was dissolved in dichloromethane (0.1 L) and petroleum ether (0.4 L) and cooled at –20 °C for 1 hr. The resulting suspension was filtered to yield **13** as a finely divided white solid (29.8 g, 0.0913 mol, 76% yield): TLC (3:2 hexanes/ethyl acetate; silica) R_f 0.8; ^{1}H NMR (DMSO-d_6) δ 11.5 (s, 1 H), 9.1 (s, 1 H), 6.94 (s, 1 H), 6.58 (s, 1 H), 4.24 (t, 2 H, J = 8.4 Hz), 1.42 (s, 9 H), 1.01 (t, 2 H, J = 8.4 Hz), 0.03 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 161.0, 153.3, 125.6, 120.1, 113.2, 105.9,

79.0, 62.1, 29.0, 17.9, -0.6; EI-MS m/e 326.1656 (M^+ 326.1662 calculated for $C_{15}H_{26}N_2O_4Si$); IR (ν_{max} cm^{-1}; $CHCl_3$) 3313, 2955, 2899, 1693, 1682, 1590, 1558, 1403, 1368, 1250, 1218, 1167, 1110, 1058, 969, 860, 838, 763.

(2-Trimethylsilyl)ethyl 4-[(*t*-butoxycarbonyl)amino]-1-(phthalimidopropyl)pyrrole-2-carboxylate (14). To a solution of **13** (21.4 g, 0.042 mol) in acetone (85 mL) was added N-(3-bromopropyl)phthalimide (22.5 g, 0.084 mol), tetrabutylammonium iodide (3.1 g, 0.008 mol), and potassium carbonate (8.71 g, 0.063 mol). The resulting suspension was allowed to stir at reflux for 24 hrs. The reaction mixture was then concentrated *in vacuo* to yield a yellow solid. The solid was suspended in chloroform (0.5 L) and washed with water (4 x 0.5 L). The chloroform layer was isolated, dried (Na_2SO_4) filtered and reconcentrated to yield a yellow oil. The oil was purified by flash chromatography on silica gel using 98:2 (v:v) dichloromethane/diethyl ether as the eluent to yield **13** as a yellow solid (13.9 g, 0.027 mol, 65% yield): TLC (95:5 dichloromethane/ethyl ether; silica) R_f 0.4; ^1H NMR (DMSO-d_6) δ 9.12 (s, 1 H), 7.82 (m, 4 H), 7.21 (d, 1 H, J = 0.9 Hz), 6.60 (d, 1 H, J = 1.8 Hz), 4.25 (t, 2 H, J = 7.2 Hz), 4.13 (t, 2 H, J = 8.4 Hz), 3.55 (t, 2 H, J = 6.6 Hz), 1.95 (p, 2 H, J = 7.2 Hz), 1.42 (s, 9 H), 0.95 (t, 2 H, J = 8.1 Hz), 0.02 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 168.5, 160.7, 153.2, 134.8, 132.4, 123.9, 123.5, 118.8, 108.4, 79.1, 62.0, 46.5, 35.8, 31.1, 29.0, 17.7, -0.6; EI-MS m/e 513.2286 (M^+ 513.2295 calculated for $C_{26}H_{35}N_3O_6Si$); IR (ν_{max} cm^{-1}; $CHCl_3$) 3351, 2954, 1772, 1719, 1587, 1551, 1398, 1367, 1248, 1168, 1088, 838, 720.

4-[(*t*-butoxycarbonyl)amino]-1-(phthalimidopropyl)pyrrole-2-carboxylic acid (15). To a cooled (0 °C) solution of **14** (10.0 g, 0.020 mol) in anhydrous THF (0.1 L) was added 1M tetrabutylammonium fluoride in THF (23.4 mL, 0.023 mol) via syringe under a positive pressure of Ar. The mixture was allowed to stir at 0 °C, under Ar, for 1 hr. and slowly allowed to warm to ambient temperature. The reaction was allowed to stir an additional 8 hrs. and was quenched with 0.1 M citric acid (0.1 L). The resulting mixture was washed with EtOAc (3 x 0.3 L) and the organic portions were combined, dried (Na_2SO_4) filtered over a short silica plug, and concentrated *in vacuo* to yield **7** as a white solid (7.6 g, 0.018 mol, 92% yield): TLC (3:2 hexanes/ethyl acetate; silica) R_f 0.1; ^{1}H NMR (DMSO-d_6) δ 12.06 (s, 1H), 9.08 (s, 1 H), 7.81 (m, 4 H), 7.18 (s, 1 H), 6.56 (s, 1 H), 4.25 (t, 2 H, J = 7.2 Hz), 3.55 (t, 2 H, J = 6.6 Hz), 1.94 (p, 2 H, J = 6.9 Hz), 1.42 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 168.5, 162.2, 153.2, 134.8, 132.4, 123.6, 123.5, 119.3, 118.6, 108.6, 79.1, 46.5, 35.8, 31.2, 29.0; EI-MS m/e 413.1587 (M^+ 413.1587 calculated for $C_{21}H_{23}N_3O_6$); IR (ν_{max} cm^{-1}; $CHCl_3$) 3339, 2978, 1771, 1710, 1588, 1550, 1467, 1397, 1366, 1244, 1160, 759, 720.

Quantitative DNase I footprinting and oligonucleotide construction. All quantitative DNase I footprinting experiments and data fits were performed as described. Construction of plasmids pJT8 (containing match sites for **2** and **3**) and pVRfl with match sites for **1**, **4**, **5**, **6** was constructed as described previously.[15] Single stranded oligos PVRfl1 5′-GATCCGTCCCTTAGGCCTATGGTCCACGTTAGTGTTATGGTCCACGTTAGG TATATGGTCCACGTTAGCGCTATGGCCCA-3′ and pVRfl2 5′-

AGCTTGGGCCTATGCGCTAACGTGGACCATATACCTAACGTGGACCATA ACACTTACGTGGACCATAGGCCTAAGGGACG-3′ were ligated into Bam HI and Hind III restriction sites in pUC 19. The resulting plasmid was transformed and amplified in competent *E. coli* cells. The short DNA duplexes for the 96-well plate experiment were constructed by annealing the appropriate sequence containing single stranded oligonucleotides. The match oligonucleotides were constructed by annealing single stranded sequences 5'-GGGCTAGGCCTTGGCCC-3' and 5'-GGGCCAAGGCCTAGCCC-3' (for 1); 5'-GGG CTAGTATTTGGCCC-3' and 5'- GGGCCAAATACTAGCCC -3' (for **2**); 5'-GGG CTAGTACTTGGCCC-3' and 5'- GGGCCAAGTACTAGCCC -3' (for **3** and **7**); 5'-GGG CTAGTGTTTGGCCC-3' and 5'- GGGCCAAACACTAGCCC -3' (for **4**); 5'-GGG CTAGGTATTGGCCC-3' and 5'- GGGCCAATACCTAGCCC -3' (for **5**); 5'-GGG CTAGCGCTTGGCCC-3' and 5'- GGGCCAAGCGCTAGCCC -3' (for **6**). Protocols for annealing single stranded DNA were followed as described. Fresh oligo stocks were stored at 4 °C as 40 μM solutions in TE buffer and diluted and used as needed.

Supplemental Figures

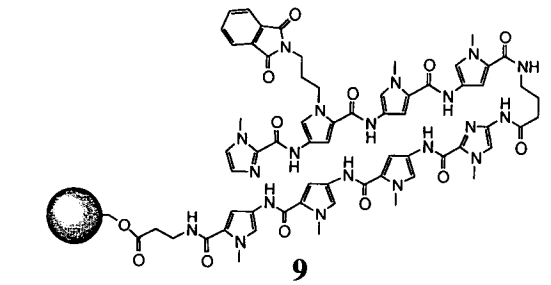
Figure S1. Synthetic scheme for conjugate **3**. i) 3-(dimethylamino)-propylamine for 10 hr at 60°C, ii) L-Boc(Trt)-Cys-OH, DCC/HOBt, iii) TFA, iv) TMR-5-maleimide.

Figure S2. Synthetic scheme for monomer 4-[(*t*-butoxycarbonyl)amino]-1-(phthalimidopropyl) pyrrole-2-carboxylic acid **14**.

Figure S3. (a) Emission profiles for 1 μM **1-6** in presence of 1 μM match 17-mer. (b) Absolute enhancement in presence of match DNA.

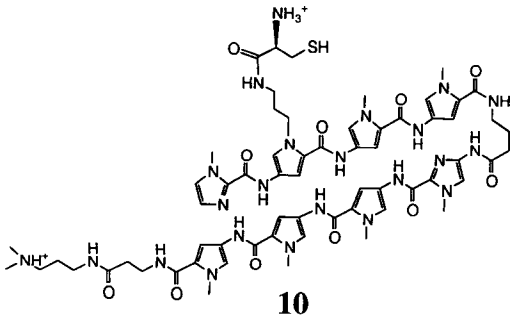
Figure S4. Absolute fluorescence values at 580 nm as reported as normalized values in Figure 5B, Table 1.

Figure S5. Representative footprinting gels. These are **3** and **2** on plasmid JT8.



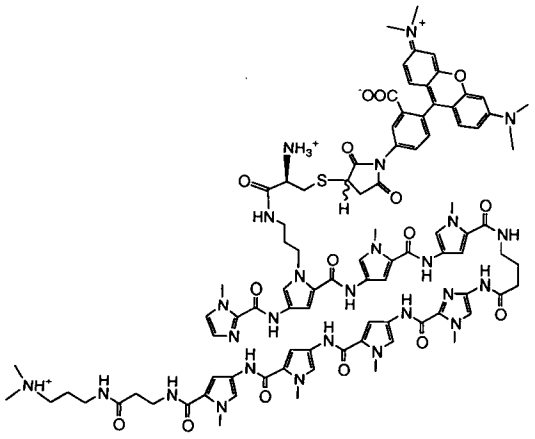
9

i, ii, iii



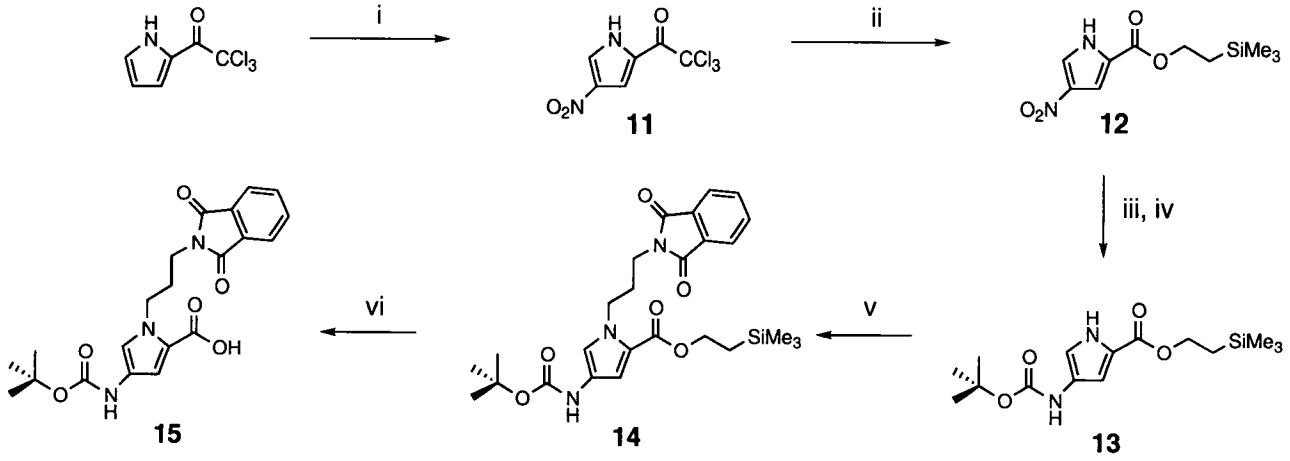
10

iv

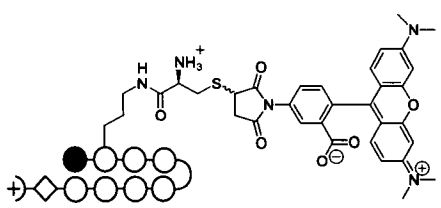


3

Supplemental Figure S1



Supplemental Figure S2



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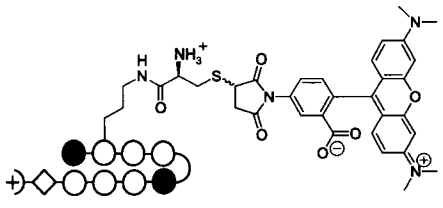

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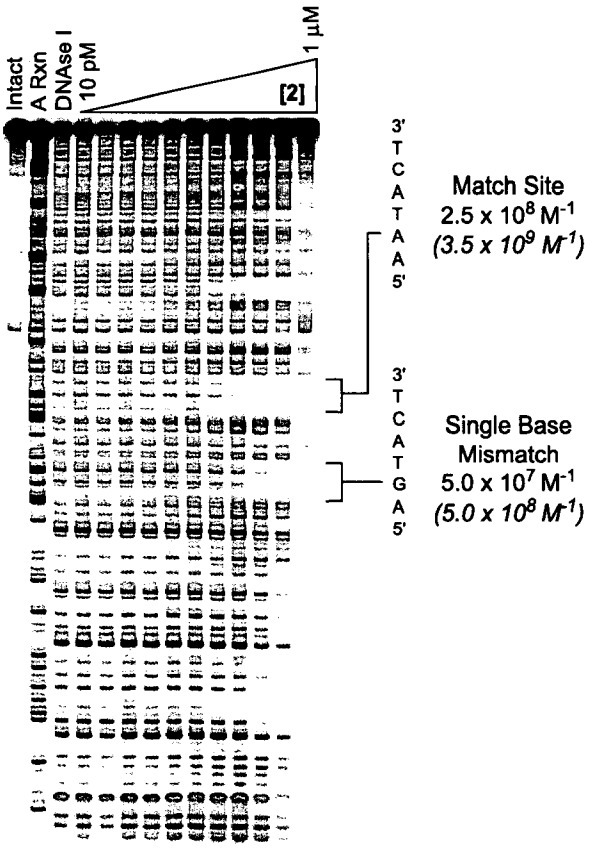

Intact
A Rxn
DNAse I
10 pM
1 µM
[2]
3' T C A T A A 5'
Match Site
2.5 x 10^8 M^-1
(3.5 x 10^9 M^-1)
3' T C A T G A 5'
Single Base
Mismatch
5.0 x 10^7 M^-1
(5.0 x 10^8 M^-1)

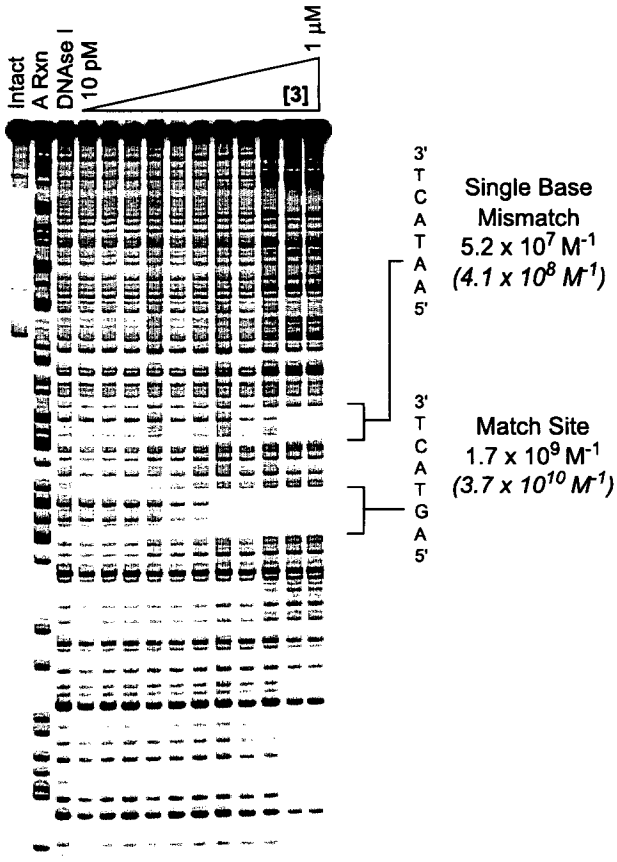
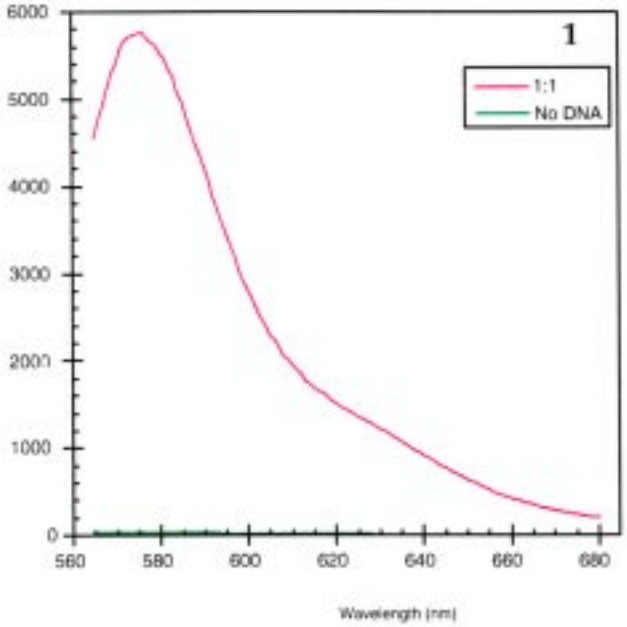
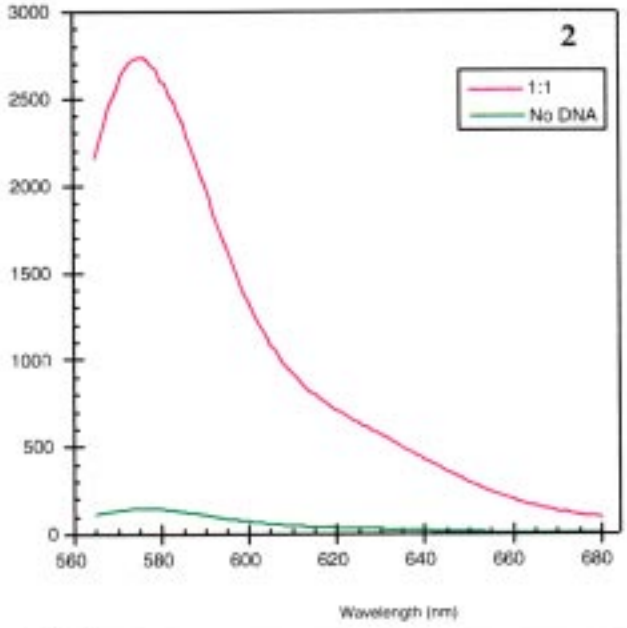
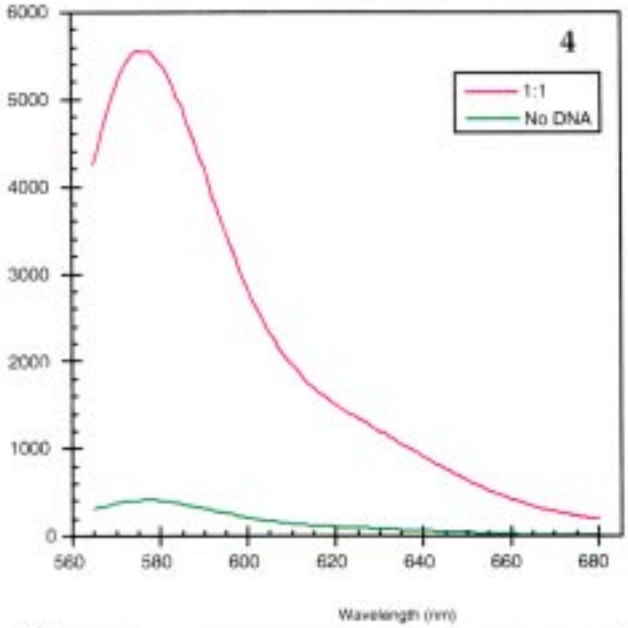

Intact
A Rxn
DNAse I
10 pM
1 µM
[3]
3' T C A T A A 5'
Single Base
Mismatch
5.2 x 10^7 M^-1
(4.1 x 10^8 M^-1)
3' T C A T G A 5'
Match Site
1.7 x 10^9 M^-1
(3.7 x 10^10 M^-1)

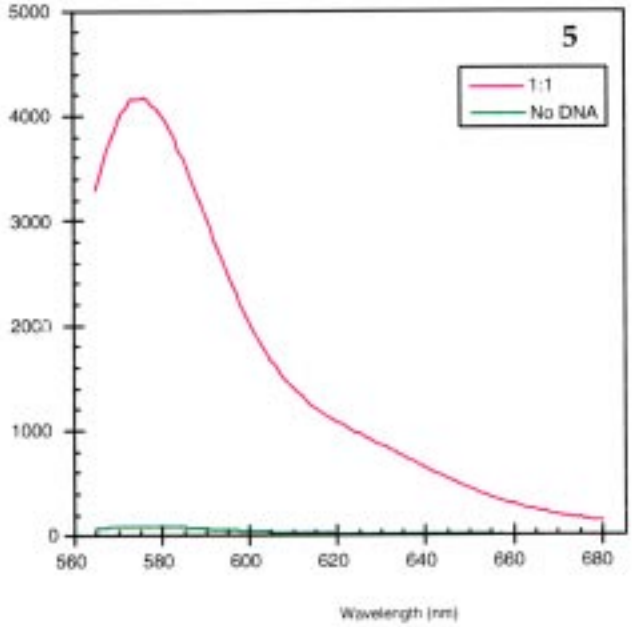
Figure S3

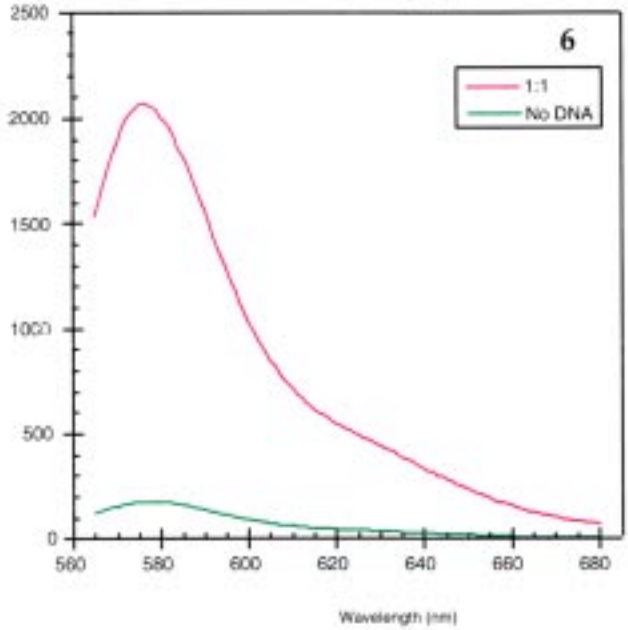
(a)











(b)

Conjugate	Enhancement
1	127
2	18
3	57
4	13
5	45
6	12

All conjugates excited at 545 nm

Supplemental Figure S4

Polyamide **1**	5'-GGCC-3'	5'-GTAT-3'	5'-GTAC-3'	5'-GTGT-3'	5'-GGTA-3'	5'-GCGC-3'
1 μM	12939191.1	1131348.81	899737.51	1364064.92	1450048.25	1072296.35
0.75 μM	11481029.8	876038.42	727249.78	1077703.97	1149271.07	918164.82
0.5 μM	9215355.53	680914.4	572976.09	818592.16	837575.82	692952.15
0.2 μM	3803710.27	435627.83	415171.57	534034.1	500861.42	434307.98
0.1 μM	1591403.79	401576.53	366217.19	401233.33	370115.41	335371.53
0.075 μM	1168461.19	355974.51	343583.79	362045.95	336901.1	310574.15
0.05 μM	765423.45	342346.15	325490.67	320122.83	308012.42	298015.34
0.02 μM	448071.42	331325.38	347730.43	302609.52	302183.63	290798.02

Polyamide **2**	5'-GGCC-3'	5'-GTAT-3'	5'-GTAC-3'	5'-GTGT-3'	5'-GGTA-3'	5'-GCGC-3'
1 μM	570016.65	4022845.57	1303296.38	1595586.12	1012941.33	530152.11
0.75 μM	554241.58	3052913.06	1093866.12	1322409.19	876011.92	474728.13
0.5 μM	561601.87	2747968.91	922928.67	1184254.45	779798.11	495840.33
0.2 μM	499241.08	1592086.77	684719.58	788705.99	497054.63	455854.65
0.1 μM	535656.61	1013989.54	527884.82	725433.36	480781.48	468551.96
0.075 μM	513549.53	809755.95	520479.85	557538.8	477624.34	448803.28
0.05 μM	546835.37	637778.47	488634.81	491011.84	441394.28	428907.53
0.02 μM	533634.24	483787.65	494710.25	484549.06	457768.74	421224.44

Polyamide **3**	5'-GGCC-3'	5'-GTAT-3'	5'-GTAC-3'	5'-GTGT-3'	5'-GGTA-3'	5'-GCGC-3'
1 μM	520860.14	1442923.3	11179701.2	555081.12	491771.09	1151309.6
0.75 μM	495892.61	1220333.52	9564491.54	496728.79	446932.73	904836.76
0.5 μM	485015.85	939979.46	7853151.63	487884.8	424936.98	721993.41
0.2 μM	441859.56	584317.88	3588983.62	462175.24	385822.47	481072.29
0.1 μM	407690.41	475004.85	1232815.79	436224.81	387416.32	415759.2
0.075 μM	437518.02	475485.18	856020.86	399725.8	411394.43	398664.19
0.05 μM	438060.88	456826.27	669845.79	408893.32	390248.93	382950.43
0.02 μM	419070.19	425536.66	452342.33	414155.82	400516.37	364587.96

Polyamide **4**	5'-GGCC-3'	5'-GTAT-3'	5'-GTAC-3'	5'-GTGT-3'	5'-GGTA-3'	5'-GCGC-3'
1 μM	1359267.96	4741949.44	2669296.69	13076416.4	2240363.03	1950368.59
0.75 μM	1257466.48	3817012.56	2321783.87	11608136.3	1583957.61	1739116.1
0.5 μM	1202947.81	3251850.25	1890508.56	9523311.14	1573015.39	1579814.01
0.2 μM	1264010.78	1880286.87	1429616.42	5435171.54	1360165.37	1262171.1
0.1 μM	1208185.66	1452348.3	1243604.42	2816231.2	1115900.25	1068140.01
0.075 μM	1209131.33	1389811.92	1338423.79	2173451.37	1088148.5	1142224.3
0.05 μM	1305072.17	1410900.85	1215336.91	1633061.35	1117834.2	1084234.21
0.02 μM	1228154.14	1184604.27	1238753.87	1242243.09	1179119.02	1015649.43

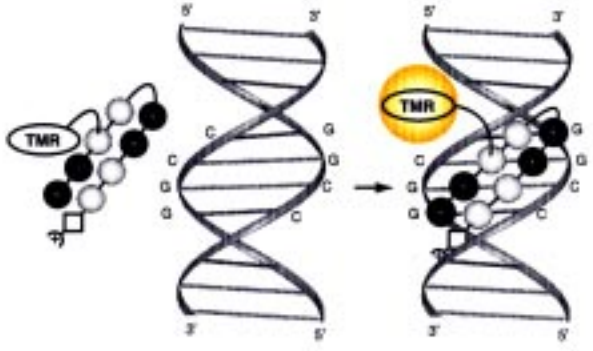
Supplemental Table S5

Polyamide **5**	5'-GGCC-3'	5'-GTAT-3'	5'-GTAC-3'	5'-GTGT-3'	5'-GGTA-3'	5'-GCGC-3'
1 μM	616551.46	659396.28	500261.61	842473.62	6546574.59	576815.88
0.75 μM	557528.74	579220.75	445159.88	704988.99	5761520.13	501653.32
0.5 μM	499942.18	502689.97	406100.03	577535.76	4803473.89	433574.96
0.2 μM	433085.62	409108.67	371452.96	427891.19	2508469.74	354385.03
0.1 μM	413019.42	384168.48	343471.78	377669.81	1129624.89	328312.48
0.075 μM	395706.51	395759.05	347227.72	362064.26	776678.22	316428.77
0.05 μM	414959.33	381877.12	338151.61	347243.51	525822.2	314347.28
0.02 μM	386801.51	370614.03	340996.82	346216.44	336123.23	302184.64

Polyamide **6**	5'-GGCC-3'	5'-GTAT-3'	5'-GTAC-3'	5'-GTGT-3'	5'-GGTA-3'	5'-GCGC-3'
1 μM	3235079.36	3937969.35	8101898	6385328.02	3586093.92	24202266
0.75 μM	2997426.86	3751273.27	6865469.7	5440221.36	3235803.99	20087280.4
0.5 μM	2587388	3448882.38	4922030.91	4320036.69	2698081.6	16263687
0.2 μM	2317478.91	2410477.63	2956643.17	2809893.37	2172081.79	7535007.66
0.1 μM	1965884.86	1907530.67	2009738.87	2068622.34	1814340.18	3543694.45
0.075 μM	1976289.08	1963873.85	1867797.37	1738081.89	1842645.74	2709840.01
0.05 μM	1935686.52	1736215.4	1784897.81	1683123.45	1775706.36	2122962.45
0.02 μM	1868595.68	1919303.05	1774404.68	1802330.58	1758540.88	1772585

Background	Polyamide **1**	Polyamide **2**	Polyamide **3**	Polyamide **4**	Polyamide **5**	Polyamide **6**
	290796.12	421219.49	364576.05	1015653.43	302187.64	1758537.81

Supplemental Table S5



Graphical Abstract