Supplementary Material (ESI) for Chemical Communications

Supporting information:

Fluorescence-based Detection of Single Nucleotide Permutation in DNA *via* Catalytically Templated Reaction

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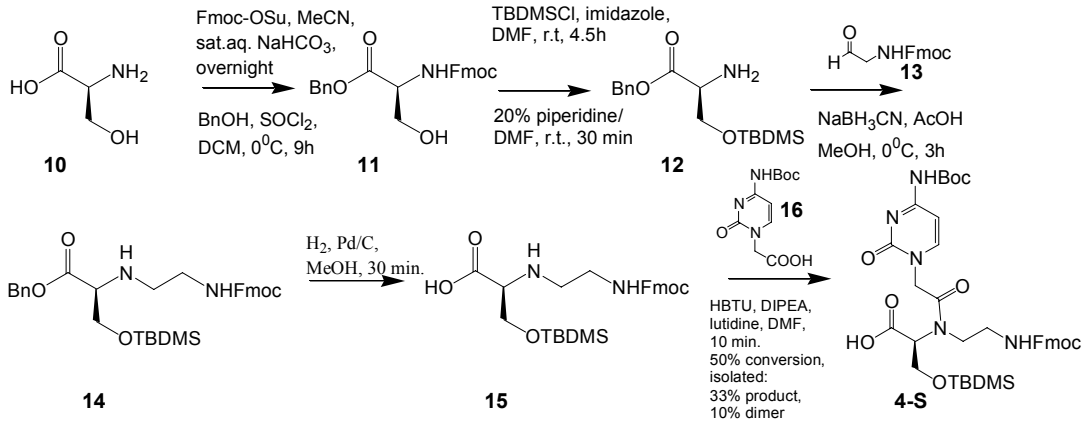
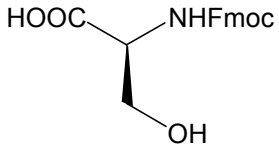
1. General experimental procedures

Reactions for the synthesis of PNAs and their monomers were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions. Anhydrous solvents were obtained by passing them through commercially available alumina column (Innovative technology, Inc., MA). PNA monomers were prepared as previously described. DNA oligomers were purchased from Sigma Proligo. Substituted polystyrene resins (100-200 mesh, 1% DVB) were purchased from Novabiochem. Deionized water was obtained by purification with a Mili-Q Ultra Pure Water system. Reversed-phase chromatography was performed using pre-packed columns Supelco Discovery DSC-18. NMR spectra were recorded on Bruker Advance-400 instrument and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. IR spectra were measured using a FT-IR Nicolet Magna 200 spectrophotometer, in reflective mode (ATR) directly from solid or liquid pure samples; wave numbers are given in cm^{-1}. The mass spectra were measured on a Surveyor MSQ spectrometers (ESI) using Hewlett Packard Series 1100 HPLC, column Interchrom C18, US0550, HS Strategy 3 RP, 50x2 (mm), particle size 3.0μm 0-1 min with water and acetonitrile containing 0.01% of TFA: 0% MeCN, 1-5 min: 0-100% MeCN, 5-6 min: 100% MeCN, 6-7 min 100%-0% MeCN, 7-8 min 0% MeCN. The MALDI spectra were measured using Brucker Daltonics AutoflexII TOF/TOF spectrometer. Automated PNA syntheses were performed on an Applied biosystems Expdite 9000. The fluorescence measurements were made using microplate fluorometer GeminiXS (Molecular Devices).

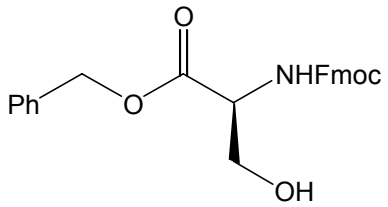
Preparation of the (O-TBS)-serine-modified PNA monomer 4-S:



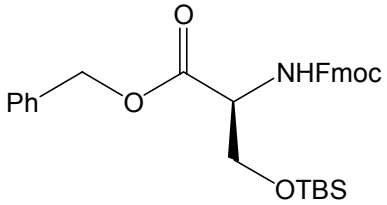
Scheme S-1. Synthesis of 2-(O-TBDMS-hydroxymethyl)-PNA monomer.



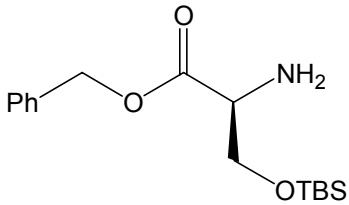
Fmoc-Ser-OH (17): (*S*)-serine **10** (1.05 g, 10.0 mmol, 1.0 equiv) was dissolved in 50 mL of sat. aq.$NaHCO_3$. Fmoc-OSu (3.37 g, 10.0 mmol, 1.0 equiv) was dissolved in acetonitrile (50 mL). The solutions were mixed and stirred for 12 h after which the acetonitrile was evaporated and the resulting mixture was extracted with EtOAc (3 x 50 mL). The organic layers were combined and washed 3x100 mL 0.5% aqueous HCl, 2x100 mL brine, dried with Na_2SO_4 and concentrated *in vacuo* to afford 3.02 g of **17** as a white solid (92% yield). **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.69 (2H, d, *J* = 7.5 Hz), 7.55 (2H, t, *J* = 7.5 Hz), 7.43 (1H, br s), 7.33 (2H, t, *J* = 7.5 Hz), 7.24 (2H, t, *J* = 7.5 Hz), 6.75 (1H, br s), 6.34 (1H, d, *J* = 7.5 Hz), 4.46 (1H, m), 4.32 (2H, m), 4.11 (1H, m), 4.10-3.80 (2H, br m). **^{13}C NMR δ(ppm):** 173.8, 156.8, 143.6, 141.2, 127.7, 127.1, 125.1, 119.9, 67.4, 62.8, 55.8, 46.9. **MS ESI $[M+H]^+$:** 328.15, RT = 5.18 min. **HRMS MALDI (m/z):** $C_{18}H_{17}NO_5Na$ $[M+Na]^+$ = calc. 350.1005, measured 350.1004. **IR ν(cm^{-1}):** 3310, 2964, 1694, 1539, 1449, 1337, 1254, 1050.



Fmoc-Ser-OBn (11): Fmoc-Ser-OBn **17** (2.75 g, 8.4 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (50 mL) and benzyl alcohol (4.35 mL, 42 mmol, 5.0 eq) was added prior to cooling the reaction mixture to 0 ºC. Thionyl chloride (0.92 mL, 12.6 mmol, 1.5 equiv) was added dropwise and the solution was stirred fo 3h at 0ºC. Another portion of $SOCl_2$ (0.92 mL, 12.6 mmol, 1.5 equiv) was then added and reaction was stirred at 23 ºC for 6 h before quenching with sat. aq. $NaHCO_3$ (100 mL). The mixture was extracted EtOAc (2 x 100 mL) and the combined organic layers were washed brine (3 x 100 mL), dried over Na_2SO_4 and evaporated. The crude product was purified with flash chromatography (hexanes:EtOAc 3:1) affording 2.55 g (73% yield) of the desired ester **11** as white solid. **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.79 (2H,d, *J* = 7.5 Hz), 7.62 (2H, d, *J* = 7.5 Hz), 7.44 (2H, t, *J* = 7.5 Hz), 7.38 (5H, s), 7.34 (2H, t, *J* = 7.5 Hz), 5.77 (1H, d, *J* = 6.4 Hz), 5.26 (2H, s), 4.52 (1H, m), 4.45 (2H, d, *J* = 3.8 Hz), 4.25 (1H, ddd, *J* = 6.4 Hz, 1.6 Hz, 1.5 Hz), 4.06 (1H, dd, *J* = 10.5 Hz, 1.6 Hz), 3.97 (1H, dd, *J* = 10.5 Hz, 1.5 Hz), 2.47 (1H, br s). **^{13}C NMR δ(ppm):** 143.6, 141.3, 135.1, 128.7, 128.6, 128.2, 127.7, 127.1, 127.0, 125.0, 120.0, 67.6, 67.3, 63.3, 56.1, 47.1 (two quarternary carbons missing) **MS ESI $[M+H]^+$:** 418.21, RT = 4.98 min. **HRMS MALDI (m/z):** $C_{25}H_{23}NO_5Na$ $[M+Na]^+$ = calc. 440.1474, measured 440.1485. **IR ν(cm^{-1}):** 3065, 2948, 1717, 1519, 1450, 1338, 1195, 1059, 737.

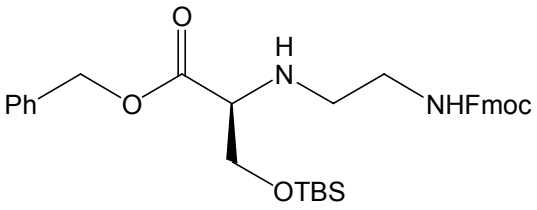


Fmoc-Ser(TBS)-OBn (18): Fmoc-Ser-OBn **(11)** (2.33 g, 5.58 mmol, 1.0 equiv) was dissolved in DMF (10 mL) together with imidazole (1.52 g, 22.32 mmol, 4.0 equiv) and *tert*-butyldimethylsilyl chloride (1.68 g, 11.16 mmol, 2.0 eq) was added. The reaction was stirred for 5 h at 23ºC then quenched with brine (100 mL) and extracted with EtOAc (3 x 50 mL). The organic layers were combined and washed brine (3 x 100 mL), dried with Na_2SO_4 and concentrated *in vacuo* affording 2.62 g of a crude product which was engaged directly in the subsequent step. **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.85 (2H, d, *J* = 7.3 Hz), 7.68 (2H, t, *J* = 6.8 Hz), 7.47-7.34 (9H, m), 5.76 (1H, d, *J* = 8.8 Hz), 5.26 (1H, d, *J* = 3.9 Hz), 5.26 (1H, d, *J* = 3.9 Hz) 4.56 (1H, dt, *J* = 2.5 Hz, 8.8 Hz), 4.46-4.41 (2H, m), 4.30 (1H, ddd, *J* = 8.8 Hz, 3.0 Hz, 2.6 Hz), 4.18 (1H, dd, *J* = 10.0 Hz, 2.6 Hz), 3.94 (1H, dd, *J* = 10.0 Hz, 3.0 Hz), 0.94 (9H, s), 0.07 (3H, s), 0.06 (3H, s). **^{13}C NMR δ(ppm):** 170.4, 156.0, 143.8, 141.3, 135.3, 128.7, 128.6, 128.4, 128.3, 127.8, 127.7, 127.2, 127.1, 125.2, 125.1, 125.0, 120.0, 67.3, 63.7, 56.1, 47.1, 25.7, 18.1, -3.5, -5.5. **MS ESI $[M+H]^+$:** 532.44, RT = 6.19 min. **HRMS MALDI (m/z):** $C_{31}H_{38}NO_5Si$ $[M+H]^+$ = calc. 532.2520, measured 532.2539. **IR ν(cm^{-1}):** 3035, 2955, 2883, 2856, 1741, 1721, 1499, 1451, 1343, 1251, 1191, 1058, 740.

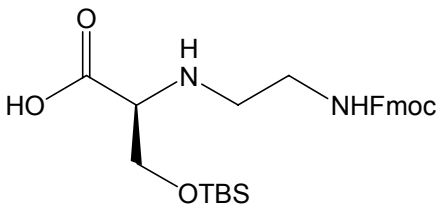


H-Ser(TBS)-OBn (12): The crude Fmoc-Ser(TBS)-OBn **18** (2.62 g) was dissolved in DMF (40 mL) and piperidine (10 mL) was added. After 30 min. at 23ºC, the solvents were evaporated under reduced pressure and the resulting crude was purified by flash chromatography (hexanes:EtOAc 2:1 to hexanes:EtOAc 1:3) to afford 1.36 g (78% after 2 steps) of the H-Ser(TBS)-OBn **12** as a white crystalline solid. **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.35 (5H), 5.18 (1H, d, *J* = 1.5 Hz), 5.18 (1H, d, *J* = 1.5 Hz), 3.98 (1H, dd, *J* = 9.8 Hz, 4.4 Hz), 3.83 (1H, dd, *J* = 9.8 Hz, 3.9 Hz), 3.57 (1H, dd, *J* = 4.4 Hz,

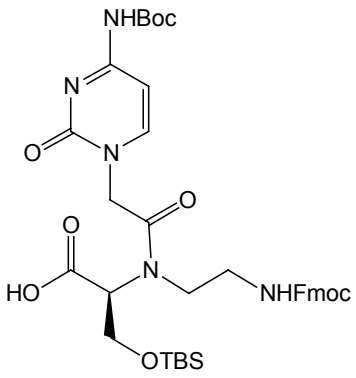
3.9 Hz), 1.76 (2H, br s), 0.87 (9H, s), 0.06 (3H, s), 0.04 (3H, s). **^{13}C NMR δ(ppm):** 174.0, 135.7, 128.5, 128.3, 128.2, 66.7, 65.3, 56.6, 25.7, 18.2, -5.5. **MS ESI [M+H]$^+$:** 310.19, RT = 4.18 min., **HRMS MALDI (m/z):** $C_{16}H_{28}NO_3Si$ $[M+H]^+$ = calc. 310.1839, measured 310.1853. **IR ν(cm^{-1}):** 3223, 2951, 2929, 2883, 2857, 1743, 1677, 1663, 1461, 1341, 1256, 1116.



N-(Fmoc-aminoethyl)-Ser(TBS)-OBn (14): H-Ser(TBS)-OBn **12** (317 mg, 1.024 mmol, 1.0 equiv) dissolved in MeOH (3 mL) at 0ºC with Fmoc glycinal **13** were stirred at that temperature for 30 min prior to the addition of sodium cyanoborohydride (35.4 mg, 0.563 mmol, 0.55 equiv) and acetic acid (32.2 μL, 0.563 mmol, 0.55 equiv). After 1 h, TLC analysis indicated complete consumption of the substrates and the solvents were evaporated. The crude product was then stirred with sat. aq. $NaHCO_3$ (50 mL) and extracted EtOAc (3 x with 50 mL). The organic layers were combined and washed 2x with sat. aq. $NaHCO_3$, once with brine, dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified with flash chromatography (hexanes:EtOAc 2:1) affording 372 mg (63% yield) of a translucid oil which crystalized upon drying *in vacuo*. **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.80 (2H, d, *J* = 7.5 Hz), 7.63 (2H, d, *J* = 7.5 Hz), 7.40 (2H, t, *J* = 7.5 Hz), 7.38 (5H), 7.34 (2H, t, *J* = 7.5 Hz), 5.38 (1H, m), 5.20 (2H, s), 4.41 (2H, d, *J* = 7.0 Hz), 4.27 (1H, t, *J* = 7.0 Hz), 3.93 (1H, dd, *J* = 4.3 Hz, 9.7 Hz), 3.86 (1H, m), 3.42 (1H, m), 3.36-3.26 (2H, m), 2.90-2.69 (2H, m), 0.90 (9H, s), 0.07 (3H, s), 0.05 (3H, s). **^{13}C NMR δ(ppm):** 173.0, 156.5, 144.0, 141.3, 135.7, 128.6(CH), 128.3(CH), 128.2(CH), 127.6(CH), 127.0(CH), 125.1(CH), 119.9(CH), 66.7(CH_2), 66.6(CH_2), 64.5(CH_2), 62.8(CH), 47.3(CH_2), 47.2(CH), 40.9(CH_2), 25.8(CH_3), 18.2, -5.5(CH_3), -5.6(CH_3). **MS ESI [M+H]$^+$:** 575.45 RT = 5.41 min. **HRMS MALDI (m/z):** $C_{33}H_{43}N_2O_5Si$ $[M+H]^+$ = calc. 575.2942, measured 575.2889. **IR ν(cm^{-1}):** 3063, 2952, 2929, 2883, 2856, 1716, 1667, 1450, 1333, 1253, 1102, 734.

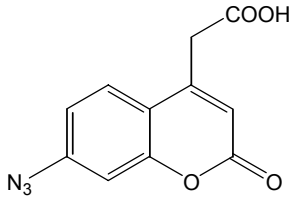


N-(Fmoc-aminoethyl)-Ser(TBS)-OH (15): Benzyl ester **14** (913 mg, 1.59 mmol, 1.0 equiv) dissolved in 35 mL of methanol was mixed with 5% Pd/C (1 wt.% Pd, 183 mg) and after deoxygenation (triple flush of the 500 mL reaction flask with N_2/vacuum) was stirred vigorously for 1h under hydrogen at atmospheric pressure. TLC analysis indicated complete consumption of the starting material (EtOAc 1:2, R_f starting material 0.6, the product on the start line) and the reaction flask was then degassed, flushed 3 times with N_2 and the suspension was filtered through a celite pad, which was subsequently washed with additional 100 mL of methanol. After evaporation and drying overnight *in vacuo*, the expected product was obtained pure (690 mg, 90% yield) as white solid. **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.76 (2H, d, *J* = 7.3 Hz), 7.63 (2H, d, *J* = 7.3 Hz), 7.49 (1H, s), 7.39 (2H, t, *J* = 7.3 Hz), 7.29 (2H, t, *J* = 7.3 Hz), 4.47 (1H, br s), 4.32 (2H, m), 4.20 (2H, m), 4.16 (1H, m), 3.65 (2H, br s), 3.60 (1H, m), 3.29 (2H, br m), 0.87 (9H, s), 0.07 (6H, s). **^{1}H NMR (DMSO) δ(ppm):** 7.90 (2H, d, *J* = 7.3 Hz), 7.68 (2H, d, *J* = 7.3 Hz), 7.48 (1H, s), 7.39 (2H, t, *J* = 7.3 Hz), 7.32 (2H, t, *J* = 7.3 Hz), 4.31 (2H, d, *J* = 6.72 Hz), 4.22 (1H, t, *J* = 6.44 Hz), 3.93-3.86 (2H, m), 3.30 (1H, s), 3.24 (2H, d, *J* = 5.36 Hz), 2.88 (2H, d, *J* = 6.2 Hz), 0.84 (9H, s), 0.03 (6H, d). **^{13}C NMR ($CDCl_3$) δ(ppm):** 170.5, 157.0, 143.9, 141.2, 127.7, 127.1, 125.3, 119.9, 67.1, 63.2, 61.0, 49.6, 47.1, 46.7, 25.7, 18.1, -5.6. **MS ESI $[M+H]^+$:** 485.41, RT = 5.05 min. **HRMS MALDI (m/z):** $C_{26}H_{37}N_2O_5Si$ $[M+H]^+$ = calc. 485.2472, measured 485.2440. **IR ν(cm^{-1}):** 3067, 2952, 2928, 2884, 2856, 1702, 1634, 1539, 1407, 1252, 1087, 838, 739.



Modified PNA-monomer 4-S: N^7-Boc-cytosine-2-acetic acid **16** (263 mg, 0.98 mmol, 1.1 eq, preparation described in [1]) was dissolved in DMF and HBTU (337 mg, 0.89 mmol, 1.0 eq) followed by Et*i*Pr_2N (191 µL, 1.16 mmol, 1.3 equiv) were added. After stirring 20 min. at 23 ºC, Et*i*Pr_2N (191 µL, 1.16 mmol, 1.3 equiv) and backbone **15** (430 mg, 0.89 mmol, 1.0 equiv) were added subsequently and the reaction was quenched after 10 min with 0.5% HCl (200 mL). The precipitate thus obtained was filtered, washed with water (50 mL), dissolved in 100 mL EtOAc, dried with anhydrous Na_2SO_4 concentrated *in vacuo*. The crude product was purified with flash chromatography (acetone 100%, then acetone:MeOH 19:1, 9:1, 3:1) to afford 220 mg (33% yield) of the pure product **4**. **^{1}H NMR (DMSO) δ(ppm):** 10.26 (1H, s), 7.87 (2H, m), 7.66 (2H+1H, m), 7.39 (2H, m), 7.30 (2H, m), 6.96 (1H, m), 4.75 (1H, m), 4.19 (2H, m), 4.12 (2H, m), 3.93 (1H, m), 3.70-3.10 (6H, m), 1.44 (9H, s), 0.83

(9H, s), 0.02 (6H, m). **^{13}C NMR (CD_3OD) δ(ppm):** 168.2, 163.9, 162.7, 157.6, 149.9, 148.9, 143.8, 141.1, 140.3, 128.8, 126.6, 123.7, 119.3, 95.5, 82.1, 66.7, 60.1, 54.4, 53.1, 46.9, 44.3, 42.1, 24.8, 24.7, 17.4, -4.9. **MS ESI $[M+H]^+$:** 736.84, **$[M+H\text{-}Boc]^+$:** 636.63. RT = 5.90 min. **HRMS MALDI (m/z):** $C_{37}H_{4\ 9}N_5O_9SiNa$ $[M+Na]^+$ = calc. 758.3198, measured 758.3292. **IR ν(cm^{-1}):** 2928, 1748, 1653, 1558, 1498, 1450, 1369, 1240, 1149.



7-azidocoumarin-4-acetic acid (3): 7-aminocoumarin-4-acetic acid[2] **(1)** (3.0 g, 13.69 mmol, 1.0 equiv) was suspended in water (65 mL) and cooled to 0ºC. Concentrated sulfuric acid (16 mL) was added slowly to maintain the reaction at 0ºC and the solution became homogeneous. A solution of sodium nitrite (1.13 g, 16.42 mmol, 1.2 equiv) in water (20 mL) was added drop wise over 30 min and the mixture was stirred 1h at 0ºC. A solution of sodium azide (1.47 g, 22.58 mmol, 1.65 equiv) in water (12 mL) was added drop wise to the reaction mixture and it was stirred. overnight while warming up to room temperature. The product precipitated as dark-yellow solid. It was filtered on glass fritted funnel, washed extensively with water and dried *in vacuo* to remove traces of water thus affording 2.66 g (79% yield) of pure azidocoumarin **2**. As chromatography resulted in partial decomposition and the product was pure by LCMS and NMR techniques, the compound was used for coupling with PNA oligomers after careful drying without further purification. **^{1}H NMR (DMSO) δ(ppm):** 7.71 (1H, d, *J* = 8.2 Hz), 7.15 (1H, s), 7.11 (1H, d, *J* = 8.8 Hz), 6.44 (1H, s), 3.90 (2H, s). **^{13}C NMR (DMSO) δ(ppm):** 171.0, 160.0, 154.6, 150.0, 143.8, 127.6, 116.5, 116.1, 115.5, 107.4, 37.6. **MS ESI $[M+H]^+$:** 246.05, RT = 4.34 min. **HRMS MALDI (m/z):** $C_{11}H_8N_3O_4$ $[M+H]^+$ = calc. 246.0515, measured 246.0508. **IR ν (cm^{-1}):** 2124, 2103, 1729, 1689, 1614, 1393, 1311, 1169.

Synthesis of the PNA oligomers:

preparation of the lysine-functionalized solid support: Rink resin (500 mg, 0.7 mmol/g, 0.35 mmol, 3.5 equiv 100-200 mesh, Novabiochem) was swollen in CH_2Cl_2 (5 mL) for 20 min. The Fmoc group was removed (20% piperidine/DMF, 20 min.) and the resin washed extensively with DMF and CH_2Cl_2. Fmoc-Lys(Boc)-OH (46.5 mg, 0.1 mmol, 1.0 equiv) was dissolved in anhydrous N-methylpyrrolidone (NMP, 5 mL) and HOBt (101 mg, 0.75 mmol, 7.5 equiv) followed by diisopropylcarbodiimide (DIC, 77.4 μL, 0.5 mmol, 5.0 equiv) were added. The mixture was stirred for 15 min prior to addition to the resin and then 3 hr with the resin. The unreacted amino groups were capped (30 min) with solution of acetic anhydride (0.92 mL) and 2,6-lutidine (1.3 mL) in DMF (20

mL). The resulting resin of loading c.a. 0.2 mmol/g was deprotected again (20% piperidine in DMF, 10 min.). Fmoc-Lys(Boc)-OH (186 mg, 0.4 mmol, 4.0 eq) was dissolved in NMP (5 mL) and HBTU (133 mg, 0.35 mmol, 3.5 equiv) followed by Et*i*Pr$_2$N (66 µL, 0.4 mmol, 4.0 eq) and 2,6-lutidine (71 µL, 0.6 mmol, 6.0 equiv) were added. The mixture agitated 15 min prior to addition to the resin and agitated 2 hr with the resin. The resin was then washed with DMF and CH_2Cl_2 and capped (30 min., acetic anhydride/2,6-lutidine, as previously), washed again with DMF and CH_2Cl_2, and dried by washing with diethyl ether and drying under reduced pressure on a filter for 30 min. The resulted Fmoc-Lys(Boc)-Lys(Boc)-Rink resin (loading c.a. 0.2 mmol/g) was stored at -20ºC for further use. Conversely, the same resin could be obtained using 0.2 mmol/g Rink resin using two HBTU couplings.

preparation of the serine-PNA-functionalized solid support: Rink resin (500 mg, 0.7 mmol/g, 0.35 mmol, 3.5 equiv 100-200 mesh, Novabiochem) was swollen 20 min. in CH_2Cl_2 (5 mL). Fmoc protecting group was removed (20% piperidine/DMF, 20 min.) and the resin washed extensively with DMF and CH_2Cl_2. Monomer **4-S** (73.6 mg, 0.1 mmol, 1.0 equiv) and HOBt (67.5 mg, 0.5 mmol, 5.0 equiv) were dissolved in anhydrous NMP (5 mL), DIC (155 µL, 1 mmol, 10 equiv) was added and the mixture was stirred for 15 min prior to addition to the resin. After 3 h, the resin was washed with DMF and CH_2Cl_2 and the unreacted amino groups were capped with solution of acetic anhydride (0.92 mL) and 2,6-lutidine (1.3 mL) in DMF (20 mL) for 30 min. The resin was subsequently washed with DMF and CH_2Cl_2 and dried by washing with diethyl ether and drying under reduced pressure on a filter for 30 min. The resulted Fmoc-2-(O-TBS)-C(Boc)PNA-Rink resin (loading c.a. 0.2 mmol/g) was stored at -20ºC for further use. Conversely, the same resin could be obtained by HBTU coupling with a 0.2 mmol/g Rink resin.

Automated synthesis of the C-azidocoumarin-terminated PNA probes 7: Fmoc-2-(O-TBS)-C(Boc)PNA-Rink resin (0.2 mmol/g, 10 mg, 2 µmol, preparation described above) was loaded to the automatic PNA-oligomer synthesizer (Expedite, Applied Biosystems), deprotected (20% piperidine/DMF), coupled with Fmoc-protected PNA monomers **4** (0.2 M soln. in NMP, 4.0 equiv) using HBTU (0.175 M soln. in NMP, 3.5 equiv) and bases (4.0 equiv Et*i*Pr$_2$N with 6.0 equiv 2,6-lutidine in NMP-0.2/0.3 M). The resin was capped after every step (acetic anhydride/2,6-lutidine) and the N-terminal Fmoc protecting group was removed (20% piperidine/DMF). When the desired sequence was obtained and characterized (Analysis of polymer bound sequences was performed by cleaving a few beads with 4:1 TFA/cresol or 19:1 TFA/thiethylsilane for 30 min then precipitate the product from 10 volumes of diethyl ether, and pellet the product by centrifugation - 5 min at 19 000 g. The pellet was redissolved in MeCN:water 1:1 and analyzed by LCMS), two subsequent couplings with lysine residues were performed using semi-automatic equipment for solid-phase synthesis. The resin was transferred into a reactor tube with a filter, swelled for 20 min. in CH_2Cl_2 and the Fmoc protecting group removed if necessary (20% piperidine/DMF, 5 min.). Fmoc-Lys(Boc)-OH (18.8 mg, 0.04 mmol, 20 equiv) was dissolved in 0.175 M soln. of HBTU in NMP (200 µL, 0.035 mmol, 17.5

equiv) and 200 µL of Et*i*Pr$_2$N /2,6-lutidine solution (0.2 M/0.3 M, 0.04 /0.06 mmol, 20/30 equiv) was added. The coupling mixture was added to the resin after a few minutes and the resin was agitated for 30 min. The resin was filtered off, washed with DMF, deprotected (20% piperidine/DMF) and the second lysine residue (BocLys(Boc)OH) was introduced using the same procedure. The silyl protecting group was removed with 1M tetrabutylammonium fluoride (TBAF) solution in DMF (1 mL, 1h, twice) and the resin was washed extensively with DMF and CH_2Cl_2. 7-azidocoumarin-4-acetic acid (9.8 mg, 0.04 mmol, 20 eq) was dissolved in 0.175 M soln. of HBTU in NMP (200 µL, 0.035 mmol, 17.5 equiv). 200 µL of Et*i*Pr$_2$N /2,6-lutidine solution (0.2 M/0.3 M, 0.04 /0.06 mmol, 20/30 equiv) was added. The coupling mixture was added after 15 min preincubation to the resin and stirred overnight. If the subsequent LC/MS analysis revealed unreacted substrate, coupling with the coumarin derivative was repeated. When coupling was complete as judged by LC/MS analysis from the cleavage of an analytical sample, the resin was filtered off, washed extensively with DMF and CH_2Cl_2, cleaved with 4:1 TFA/cresol or 19:1 TFA/thiethylsilane (0.5-1 mL) for 2 h. The resulting mixture was separated from the resin and precipitated from 10 volumes of ether. The resin was cleaved again (0.5 volume of the previous cleaving mixture, 30 min., then the filtrate precipitated from 10 vol. Et_2O). The suspensions were centrifuged (9600 rpm, 4 ºC, 20 min.), resuspended in fresh Et_2O of the same volume and centrifuged again under the same conditions. The crude precipitates were redissolved in water and purified with reverse-phase chromatography using gradient of MeCN in water (with 0.1% TFA). Purity of the fractions were analyzed with MALDI and LCMS. The pure product **7** was collected at 20-30% MeCN in water. The pure fractions were combined and lyophilized. Stored under N_2 at -20ºC as solid.

Automated synthesis of the N-phosphine-terminated PNA probes 9: Fmoc-Lys(Boc)-Lys(Boc)-Rink resin (0.2 mmol/g, 10 mg, 2 µmol) was loaded to the automatic PNA-oligomer synthesizer (Expedite, Applied Biosystems), deprotected (20% piperidine/DMF), coupled with Fmoc PNA monomers **4** (0.2 M soln. in NMP, 4.0 equiv) using HBTU (0.175 M soln. in NMP, 3.5 equiv) and bases (4.0 equiv Et*i*Pr$_2$N with 6.0 equiv 2,6-lutidine in NMP-0.2/0.3 M). The resin was capped after every step (acetic anhydride/2,6-lutidine) and the N-terminal Fmoc protecting group was removed (20% piperidine/DMF). The proper synthesis of the sequence was verified by cleavage of an analytical sample as described above. Coupling of 2-diphenylphosphinobenzoic acid was performed using semi-automatic equipment for solid-phase synthesis. The resin was transferred into a reactor tube with a filter, swelled for 20 min. with CH_2Cl_2, the Fmoc protecting group removed if necessary (20% piperidine/DMF, 5 min.) and 2-diphenylphosphinobenzoic acid (12.2 mg, 0.04 mmol 20 equiv) was dissolved in 0.175 M soln. of HBTU in NMP (200 µL, 0.035 mmol, 17.5 equiv). 200 µL of ETIPR2N/2,6-lutidine solution (0.2 M/0.3 M, 0.04 /0.06 mmol, 20/30 equiv) was added. The coupling mixture was directly mixed with the resin and stirred 30 min. The resin was filtered off, washed with DMF and the coupling was repeated for another 30 min. The resin was filtered off, washed extensively with DMF and CH_2Cl_2, cleaved with 4:1 TFA/cresol or 19:1 TFA/thiethylsilane (0.5-1 mL) as

described above. The crude precipitates were redissolved in water and purified with reverse-phase chromatography using gradient of MeCN in water (with 0.1% TFA). Purity of the fractions were analyzed with MALDI and LCMS, the pure product being eluted at 20-30% MeCN in water. The most abundant by-product (phosphine oxide) was usually well separated. The pure fractions were combined, lyophilized and stored under N_2 at -20ºC as solid.

Oligomers – MALDI analysis

C'- N_3Coum-CGTGCA-KK-NH_2 **(7)** MALDI $[M+H]^+$ calc. 2156.90, found 2156.62.

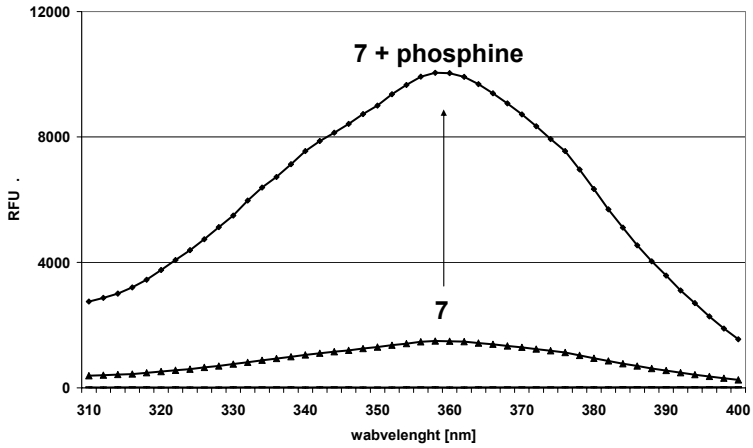
C'- KK-ATAGGC-PPh_3 **(9)** MALDI $[M+H]^+$ calc. 2211.94, found 2212.24

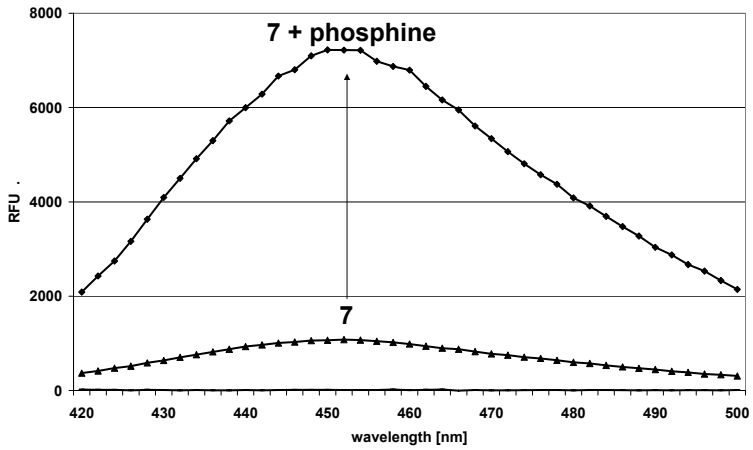
C'- N_3Coum-CGTGCAGC-KK-NH_2 **(7)** MALDI: $[M+H]^+$ calc. 2699.11, found 2699.10

C'- KK-CCATAGGC-PPh_3 **(9)** MALDI: [M+H] calc. 2714.14, found 2715.39.

Absorption/emission quenched azidocoumarin and its reduced product

Absorption (top) and emission (bottom) maxima of azidocoumarin-PNA conjugate 7 (5 μM in 50% formamide for 455 nm emission and 360 nm excitation respectively)





Hybridization reactions:

Commercially available DNA templates were purchased as desalted 100 μM solutions in deionized water (Sigma Proligo, 5 O.D.) and diluted to the concentrations of 10, 2 and 0.2 μM. The PNA probes were dissolved in deionized water to 1 mM concentration which was the basic stock solution. The probes were diluted to their final concentrations with 1 mM PBS buffer solution (pH = 7.0) or with a 50% formamide solution in 1 mM PBS buffer (pH = 7.0). The stock solutions were stored in the buffer at -20ºC.

Black 96-well plates (500 μL/well, NUNC) for fluorescent readout were used to perform the templated reactions. 240 μL of the azide-PNA solution and DNA template solution (1.2-12 μL of one of the stock solutions, depending on the desired final DNA percentage and concentration) were mixed. 240 μL of the phosphine-PNA (two fold higher concentration than the azide-PNA) was added using a multichannel pipette and the fluorescence readout was recorded immediately. Negative controls – equal volumes of the azide- and phosphine-PNA with 1mM PBS buffer of the volume equal to the DNA solutions used in the experiment. Background level for the buffer and reagents – 480 μL 1 mM PBS (buffer), 240 μL 1mM PBS + 240 μL azide-PNA solution (azide-PNA background), 240 μL 1mM PBS + 240 μL phosphine-PNA solution (phosphine-PNA background). The level of full conversion of the fluorogenic substrate to the fluorescent product was measured using 240 μL azide-PNA + 240 μL of 1mM TCEP solution. The optimal measurement wavelengths were found by scanning the ranges 300-400 nm (excitation) and 400-500 nm (emission) for the biggest difference between the fluorescence level of the starting azide and the amine product yielding 360 nm (excitation) and 455 nm (emission) as the optimal wavelength.

The fluorescence readout of the templated reactions was performed for 45-120 min. in duplicates and the average of both the results were plotted.

Linear dependence of fluorescence level on the product concentration was initially confirmed within the range of 4-0.03 μM of azide-PNA concentration (error within 5.4-6.9 %, as standard deviation) by reaction with 1 mM TCEP solution at 37 ºC.

The untemplated Staudinger reaction between the PNA probes was taking place down to 1 μM azide-PNA concentration. At 500 nM and below no background reaction was observed within the experiment times. The templated process was investigated in terms of sensitivity over mutations introduced to the DNA sequence and fidelity of the reaction in both directions of hybridization (PNA may hybridize with DNA both directions.

Table S1. DNA template sequences.

Symbol	DNA Sequence (5'-3')	
T1	GGG TAT CCG GCA CGT CGG	perfect match
T2	GGG TAT CCG **AA** GCA CGT CGG	match with 2 abasic sites
T3	GGG TAT CCG **AAC A** GCA CGT CGG	match with 4 abasic

		sites
T4	GGG TAT CCG **AAC AAC** GCA CGT CGG	match with 6 abasic sites
T5	GGC TGC ACG GCC TAT GGG	reversed perfect match
T6	GGC TGC ACG **GG** GCC TAT GGG	reversed match with 2 abasic sites
T7	GGC TGC ACG **GGG** GCC TAT GGG	reversed match with 3 abasic sites
T1mG	GGG TAT **G**CG GCA CGT CGG	match with a C->G mutation
T1mA	GGG TAT **A**CG GCA CGT CGG	match with a C->A mutation
T1mT	GGG TAT **T**CG GCA CGT CGG	match with a C->T mutation
T2mG	GGG TAT **G**CG **AA** GCA CGT CGG	2 abasic sites and a C->G mutation
T2mA	GGG TAT **A**CG **AA** GCA CGT CGG	2 abasic sites and a C->A mutation
T2mT	GGG TAT **T**CG **AA** GCA CGT CGG	2 abasic sites and a C->T mutation
T1mN	GGG TAT CCG GCA **G**GT CGG	match with other C->G mutation
T2mN	GGG TAT CCG **AA** GCA **G**GT CGG	2 abasic sites and other C->G mutation
Tx	A**A** CCG G**T**A CG	random template

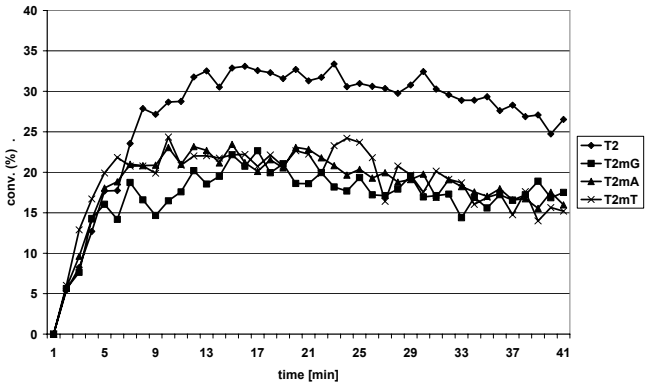


Figure S-1. Selectivity of the octameric probes in 1mM PBS buffer (37ºC, 250nM azide-PNA) with the perfect match template and templates containing single nucleotide mismatch (20% template load).

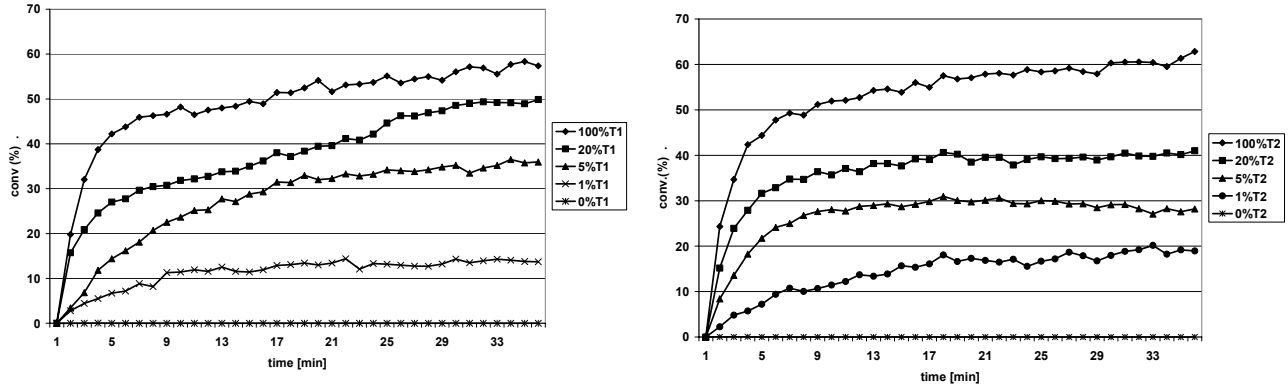


Figure S-2. Conversion as a function of time for various DNA concentrations (PNA-octamers, 500 nM in 50% formamide, matching **T1** (left) and **T2** (right) templates)

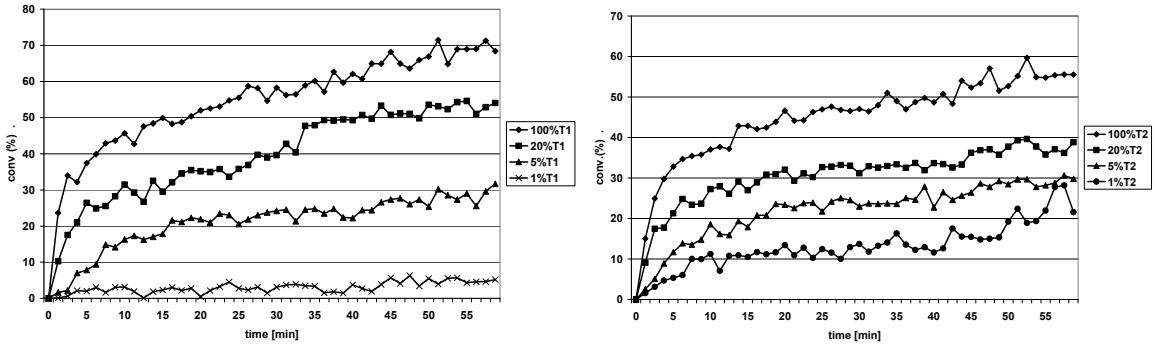


Figure S-3. Conversion as a function of time for various DNA concentrations (PNA-octamers, 100 nM in 50% formamide, matching **T1** (left) and **T2** (right) templates).

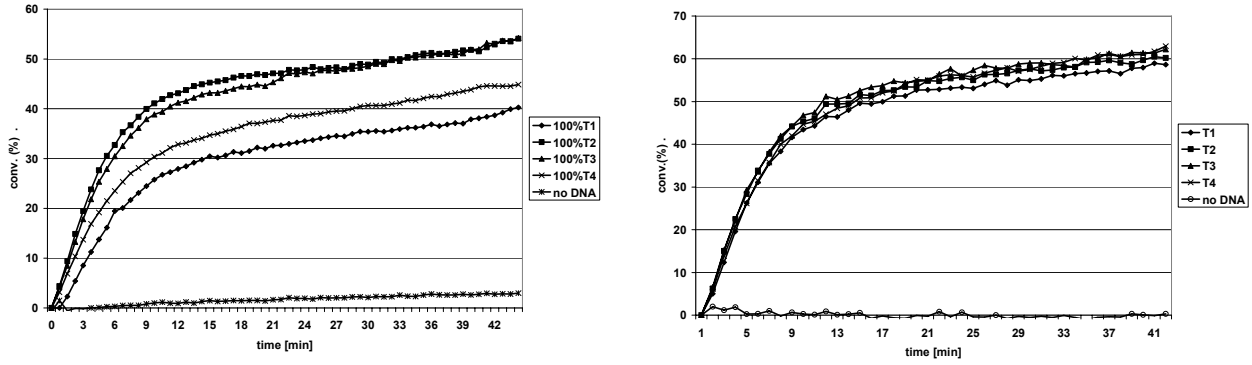


Figure S-4. Influence of abasic sites for hybridization of hexameric (left) and octameric (right) PNA probes on DNA templates (500 nM probes, 100% DNA, 50% formamide, 37 ºC)

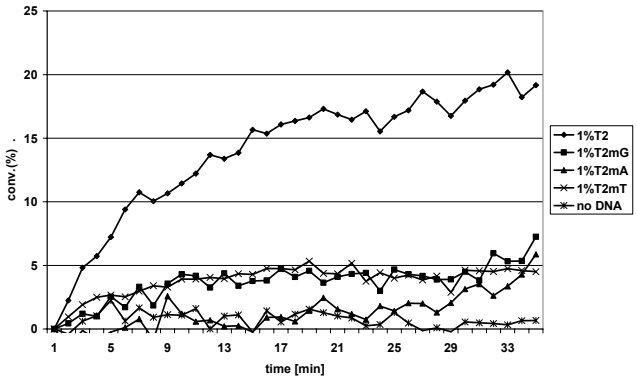


Figure S-5. Conversion as a function of time for matching **T2** DNA (1% DNA) and single mutations (**T2mG, T2mA, T2mT**) (PNA-octamers, 500 nM in 50% formamide)

1%T2	0.088
1%T2mG	0.023
1%T2mA	-
1%T2mT	0.028

Table S-2. The initial reaction rates[3] (t < 15 min.; v [nmol/s]) for matching **T2** DNA (1% DNA) and single mutations (**T2mG, T2mA, T2mT**) (PNA-octamers, 500 nM in 50% formamide)

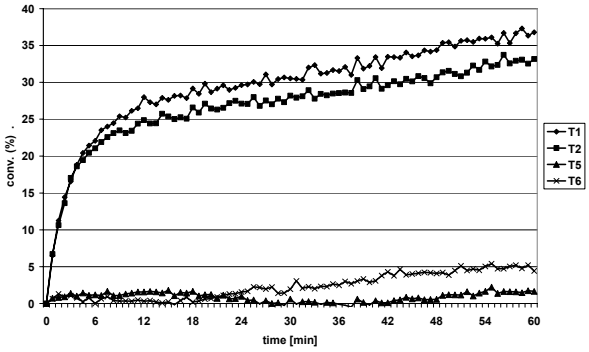


Figure S-6. Conversion as a function of time for matching templates **T1** and **T2** as well as reversed templates of the same sequences **T5** and **T6** (20% DNA, 500 nM PNA-octamers in 50% formamide)

T1	0.63
T2	0.61
T5	0.054
T6	0.059

Table S-3. The initial reaction rates[3] (t < 3 min.; v [nmol/s])for the octameric PNA probes on matching and reversed templates (20% DNA, 500 nM PNA-octamers in 50% formamide)

[1] F. Debaene, J. Da Silva, Z. Pianowski, F. Duran, N. Winssinger *Tetrahedron* **2007**, *in press* doi:10.1016/j.tet.2007.03.033
[2] D. J. Maly, F. Leonetti, B. J. Backes, D. S. Dauber, J. L. Harris, C. S. Craik, J. A. Ellman *J. Org. Chem.* **2002**, *67*, 910-915;
[3] The initial reaction rates were calculated using the approximation valid at the initial, linear part of the kinetic plot:

$$v = -d[A] / dt \approx \Delta[A] / \Delta t$$