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

SUPPORTING INFORMATION

Ferrocene as an electrochemical reporting surrogate of abasic sites in DNA

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S1. **Additional gel images:**

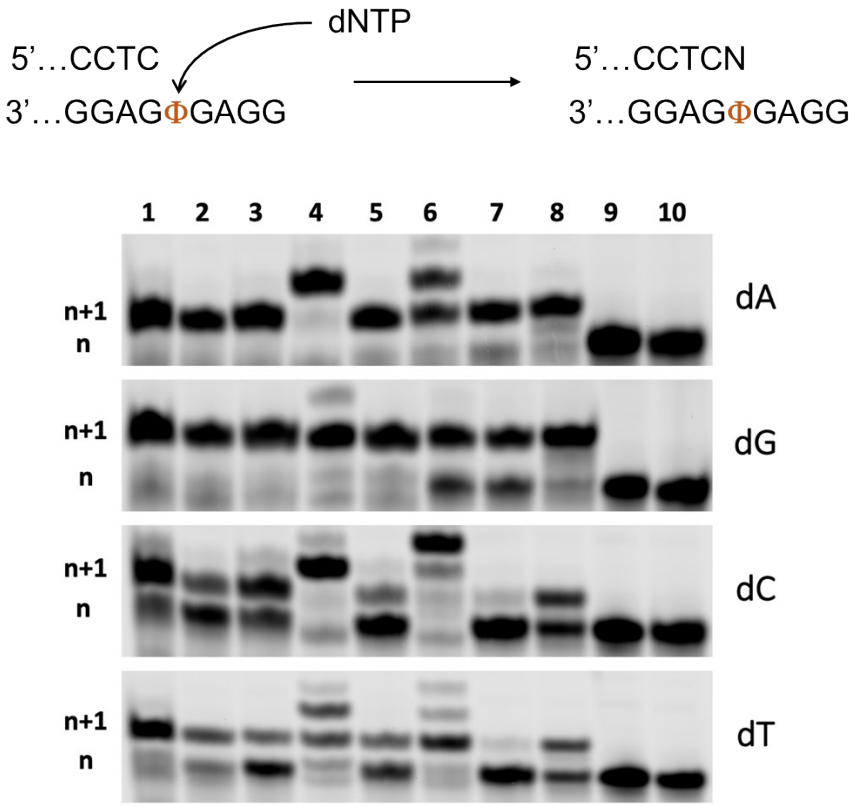


Figure S1. Gel image (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and template **AP1** (15 pmol) in the presence of the individual natural dNTPs (20 μM) and different DNA polymerases. All reactions were carried out for 1h. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Therminator (2 U), 5. Vent (*exo*$^-$) (2 U), 6. Kf *exo*$^-$ (5 U), 7. Dpo4 (2 U), 8. Deep Vent (2 U). The negative controls are: 9. No polymerase, 10. No dNTP. The label n is connected to the length of the primer, while n+1 is referred to the length of the primer with the addition of one dNMP.

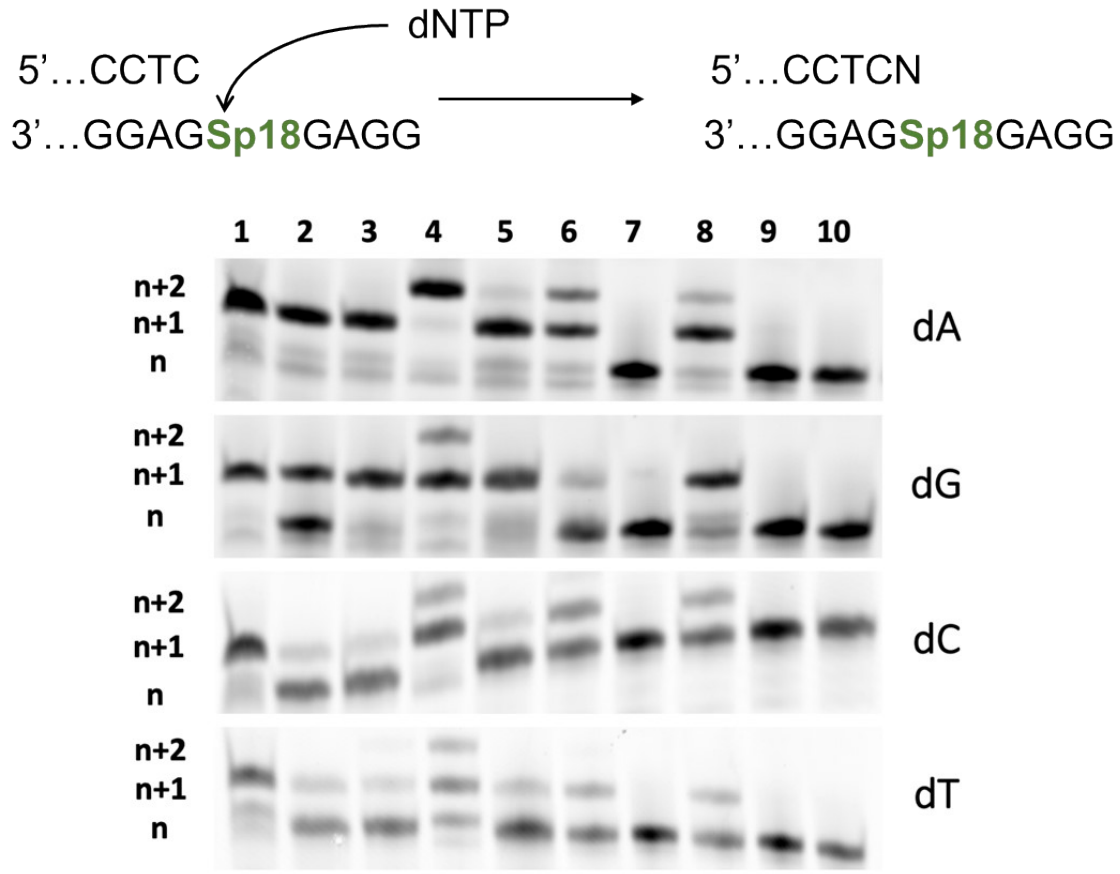


Figure S2. Gel image (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and template **M1-Sp18** (15 pmol) in the presence of the individual natural dNTPs (100 μM) and different DNA polymerases. All reactions were carried out for 1h. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Therminator (2 U), 5. Vent (*exo*$^-$) (2 U), 6. Kf *exo*$^-$ (5 U), 7. Dpo4 (2 U), 8. Deep Vent (2 U). The negative controls are: 9. No polymerase, 10. No dNTP. The label n is connected to the length of the primer, while n+1 is referred to the length of the primer with the addition of one dNMP while n+2 is referred to the length of the primer with the addition of two dNMPs.

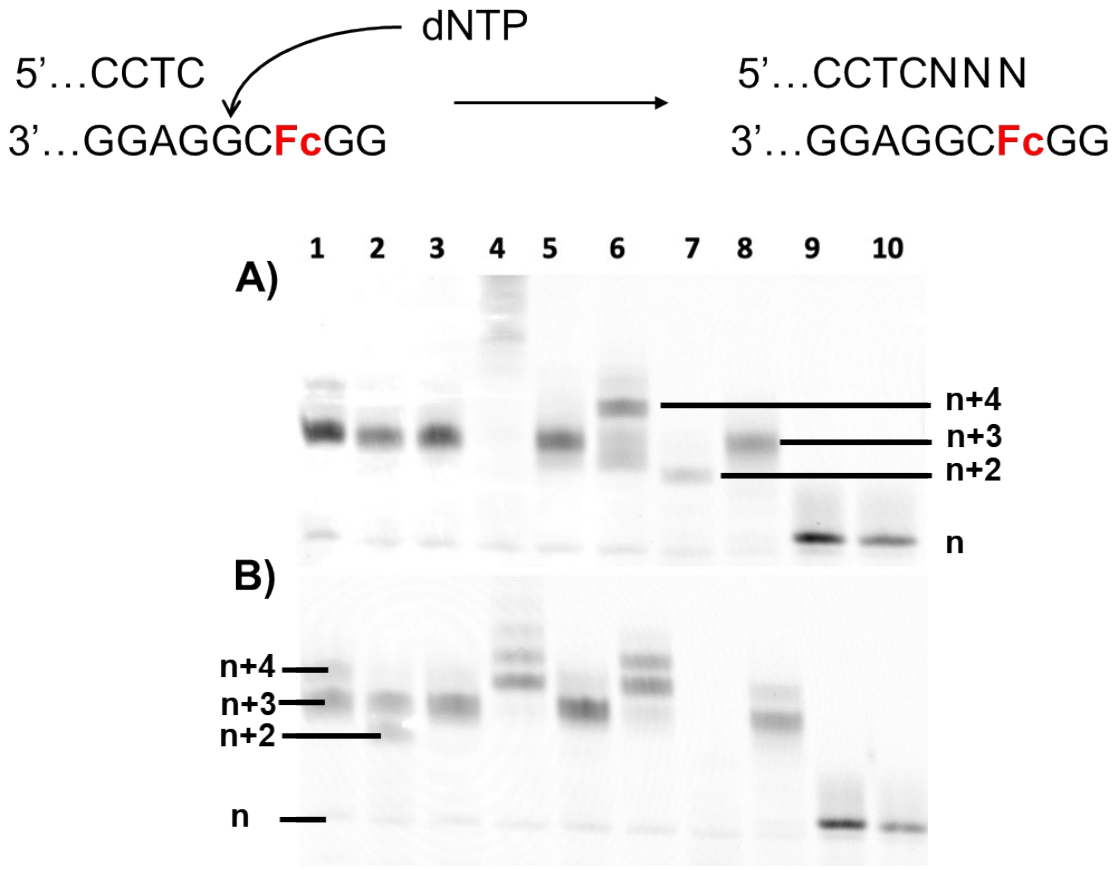


Figure S3. Gel image (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and template **M3** (15 pmol) and either A) dGTP and dCTP (100 μM) or B) dGTP, dCTP, and dATP (100 μM). All reactions were carried out for 1h. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Therminator (2 U), 5. Vent (*exo*$^-$) (2 U), 6. Kf *exo*$^-$ (5 U), 7. Dpo4 (2 U), 8. Deep Vent (2 U). The negative controls are: 9. No polymerase, 10. No dNTP. The label n is connected to the length of the primer, while n+2 is referred to the length of the primer with the addition of two dNMPs, n+3 corresponds to the product with three additions, and n+4 the product with four added nucleotides.

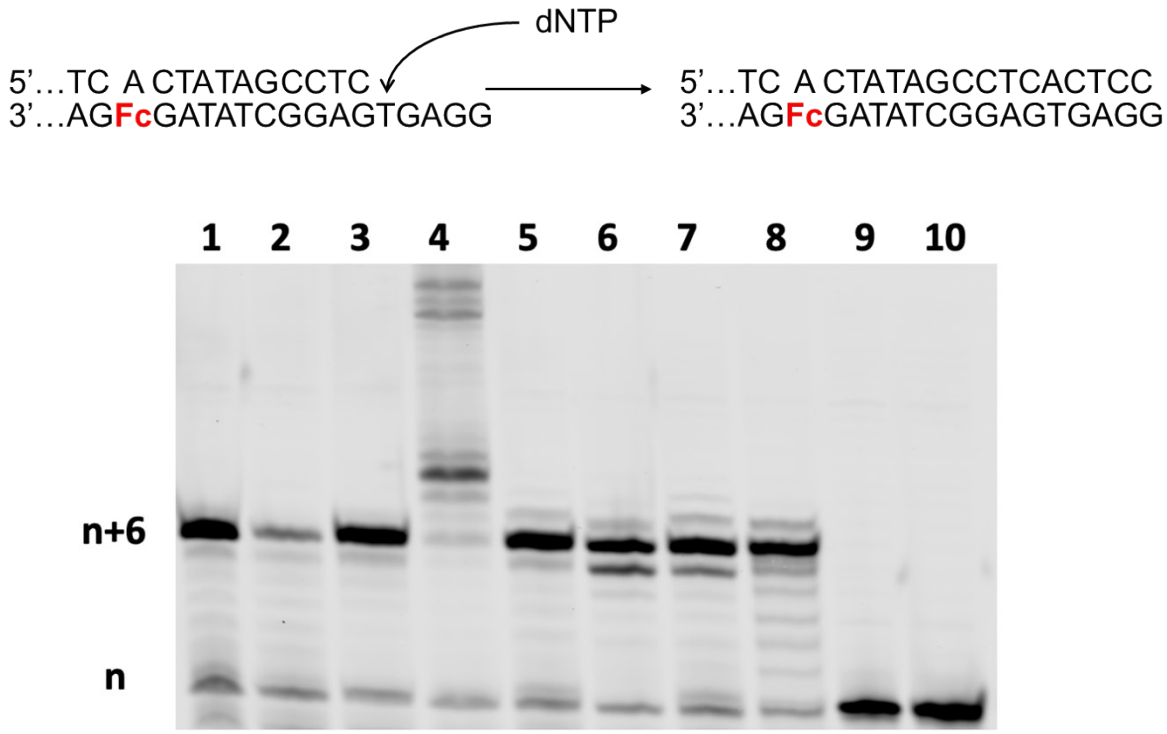


Figure S4. Gel image (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and template **M4** (15 pmol) in the presence of all four natural dNTPs (20 μM) and different DNA polymerases. All reactions were carried out for 1h. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Therminator (2 U), 5. Vent (*exo*$^-$) (2 U), 6. Kf *exo*$^-$ (5 U), 7. Dpo4 (2 U), 8. Deep Vent (2 U). The negative controls are: 9. No polymerase, 10. No dNTP. The label n is connected to the length of the primer, while n+6 is referred to the length of the primer with the addition of six dNMPs.

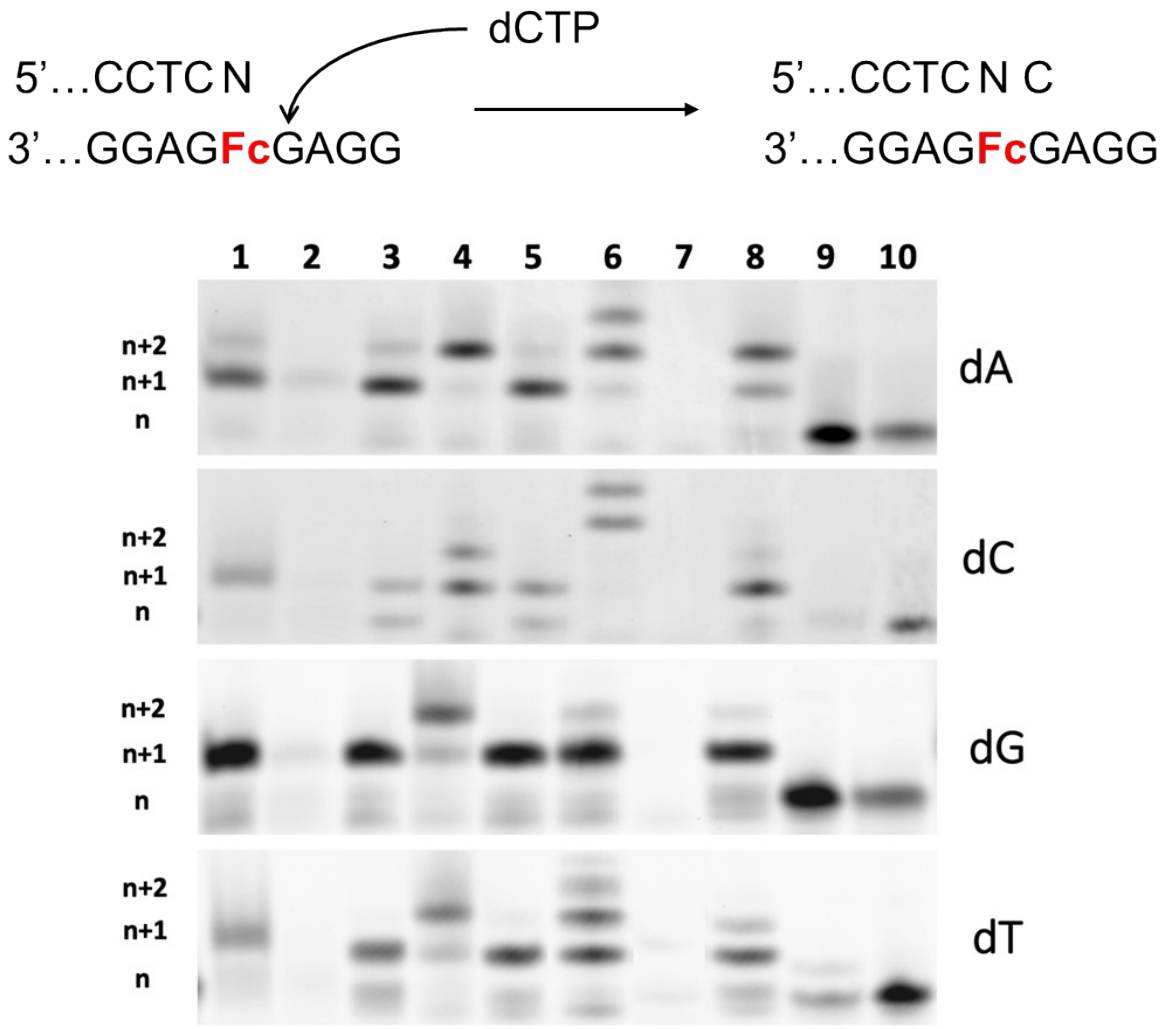


Figure S5. Gel image (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and template **M1** (15 pmol). A first nucleotide was incorporated opposite Fc by PEX reactions with dATP (100 μM, 20 min), dGTP (100 μM, 60 min), dCTP (100 μM, 120 min), or dTTP (100 μM, 120 min). After installation of a first nucleotide, the extended product was incubated with dCTP (20 μM, 60 min). List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Therminator (2 U), 5. Vent (*exo*$^-$) (2 U), 6. Kf *exo*$^-$ (5 U), 7. Dpo4 (2 U), 8. Deep Vent (2 U). The negative controls are: 9. No polymerase, 10. No dNTP. The label n is connected to the length of the primer, while n+2 is referred to the length of the primer with the addition of two dNMPs.

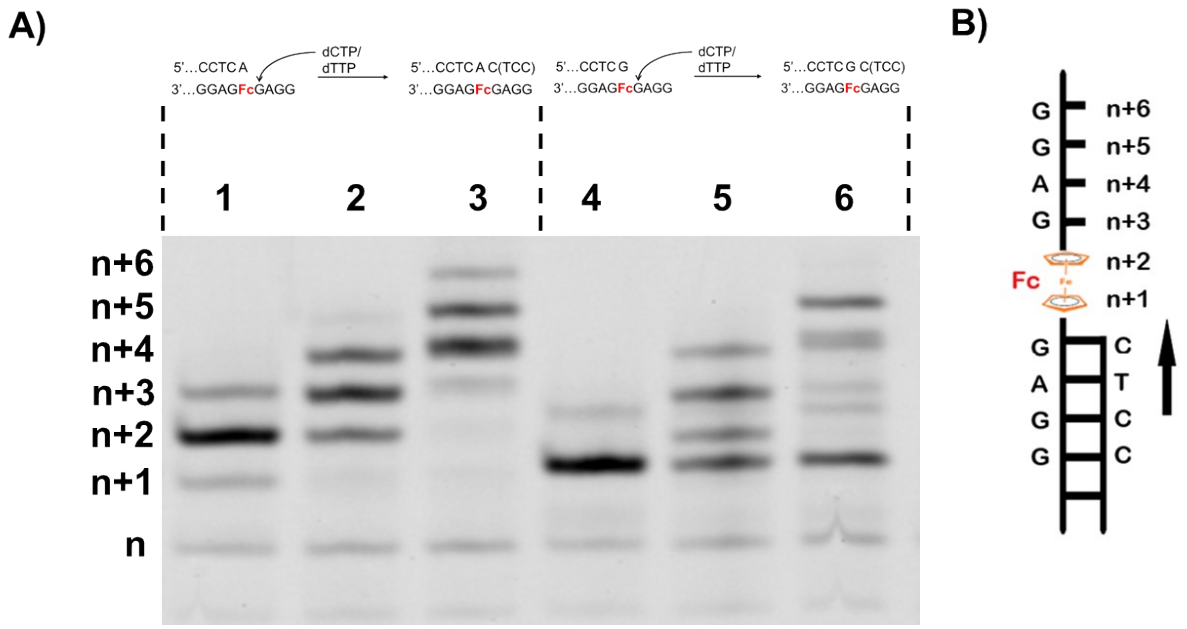


Figure S6. A) Gel image (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and template **M1** (15 pmol) and Dpo4. A first nucleotide was incorporated opposite Fc by PEX reactions with dATP (100 μM, 20 min, lane 1) or dGTP (100 μM, 60 min, lane 4). After this first addition, dCTP (20 μM, 60 min, lanes 2 and 5) or dCTP and dTTP (20 μM, 60 min, lanes 3 and 6) were added. The label n is connected to the length of the primer, while n+2 is referred to the length of the primer with the addition of two dNMPs, n+3 for three additions, n+4 for four and n+5 for five added nucleotides. B) Schematic representation for a tentative explanation for the formation of the n+6 product.

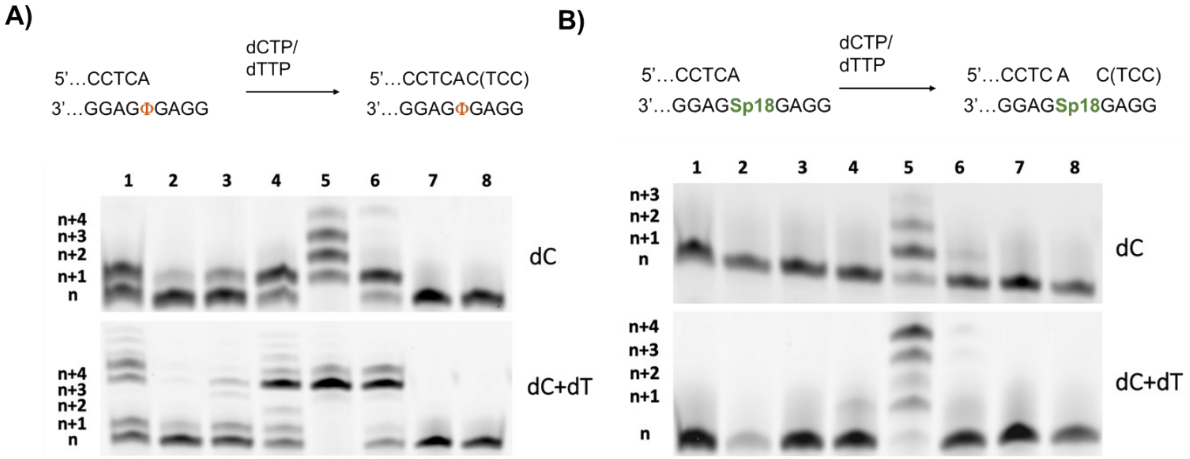


Figure S7. Gel images (PAGE 20%) of PEX reactions with primer **P2** (10 pmol) and A) template **AP1** (15 pmol) and B) template **M1-Sp18** with dCTP alone (dC, 100 μM) or with dCTP and dTTP (dC+dT, 100 μM) for 1h. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Vent (*exo*$^-$) (2 U), 5. Dpo4 (2 U), 6. Kf *exo*$^-$ (5 U). The negative controls are: 7. No polymerase, 8. No dNTP. The label n is connected to the length of the primer, while n+1 is referred to the length of the primer with the addition of one dNMP while n+2 is referred to the length of the primer with the addition of two dNMPs, n+3 and n+4 to three and four added nucleotides.

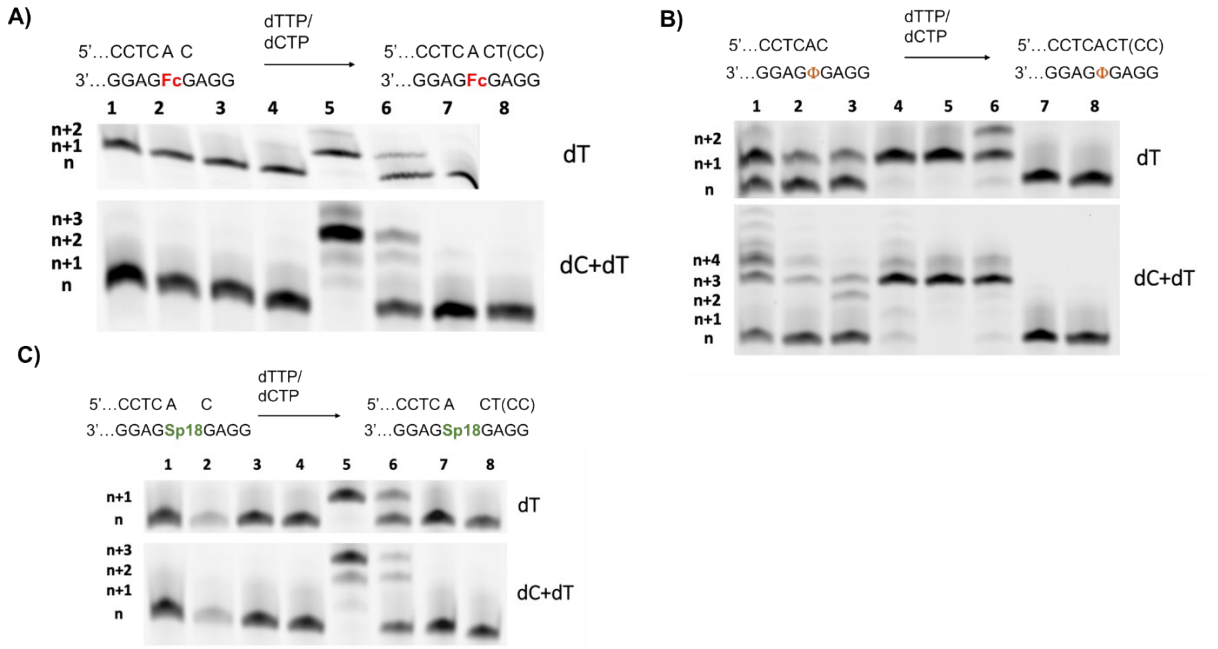


Figure S8. Gel images (PAGE 20%) of PEX reactions with primer **P3** (10 pmol) and templates A) **M1**, B) **AP1**, and C) **M1-Sp18** (all 15 pmol) with dTTP alone (dT, 100 μM) or with dCTP and dTTP (dC+dT, 100 μM) for 1h. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Vent (*exo*$^-$) (2 U), 5. Dpo4 (2 U), 6. Kf *exo*$^-$ (5 U). The negative controls are: 7. No polymerase, 8. No dNTP. The label n is connected to the length of the primer, while n+1 is referred to the length of the primer with the addition of one dNMP while n+2 is referred to the length of the primer with the addition of two dNMPs, n+3 and n+4 to three and four added nucleotides.

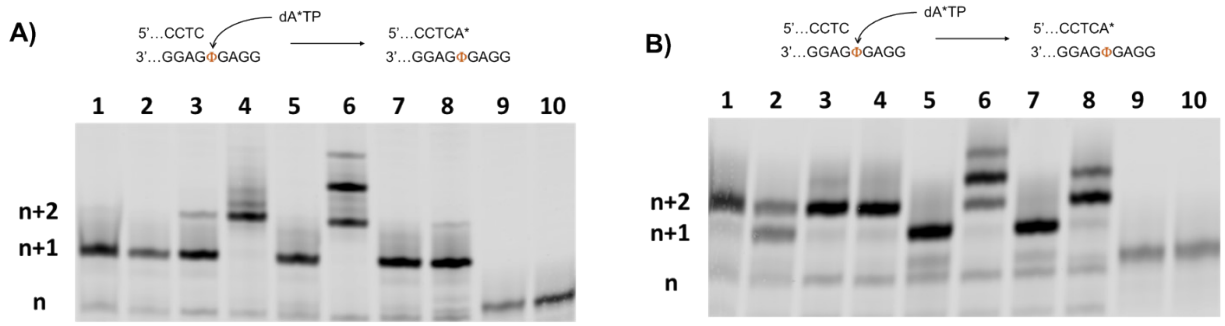


Figure S9. Gel images (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and template **AP1** (15 pmol) with modified dATP nucleotides A) **3** or B) **4**. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Therminator (2 U), 5. Vent (*exo*$^-$) (2 U), 6. Kf *exo*$^-$ (5 U), 7. Dpo4 (2 U), 8. Deep Vent (2 U). The negative controls are: 9. No polymerase, 10. No dNTP. All reactions were carried out with 200 μM modified nucleotides for 1h. The label n is connected to the length of the primer, while n+1 and n+2 refer to the length of the primer with the addition of one or two dNMPs, respectively.

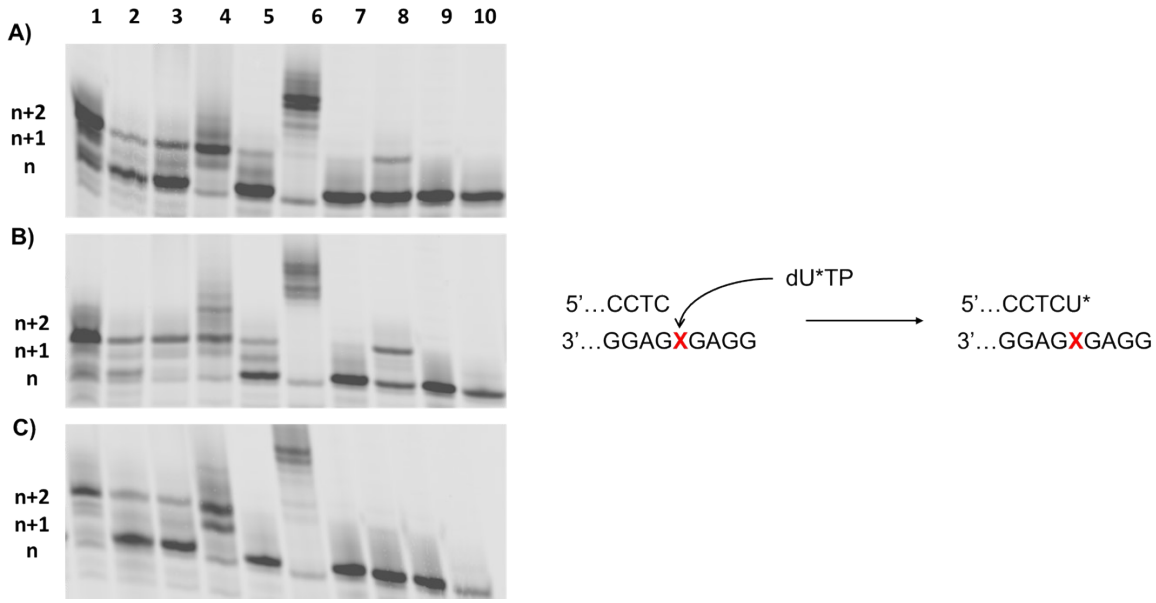


Figure S10. Gel images (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and 5-propargylamino-dUTP **5** and templates (all 15 pmol) A) **M1**, B) **AP1**, and C) **M1-Sp18**. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Therminator (2 U), 5. Vent (*exo*$^-$) (2 U), 6. Kf *exo*$^-$ (5 U), 7. Dpo4 (2 U), 8. Deep Vent (2 U). The negative controls are: 9. No polymerase, 10. No dNTP. All reactions were carried out with 200 µM modified nucleotides for 1h. The label n is connected to the length of the primer, while n+1 and n+2 refer to the length of the primer with the addition of one or two dNMPs, respectively.

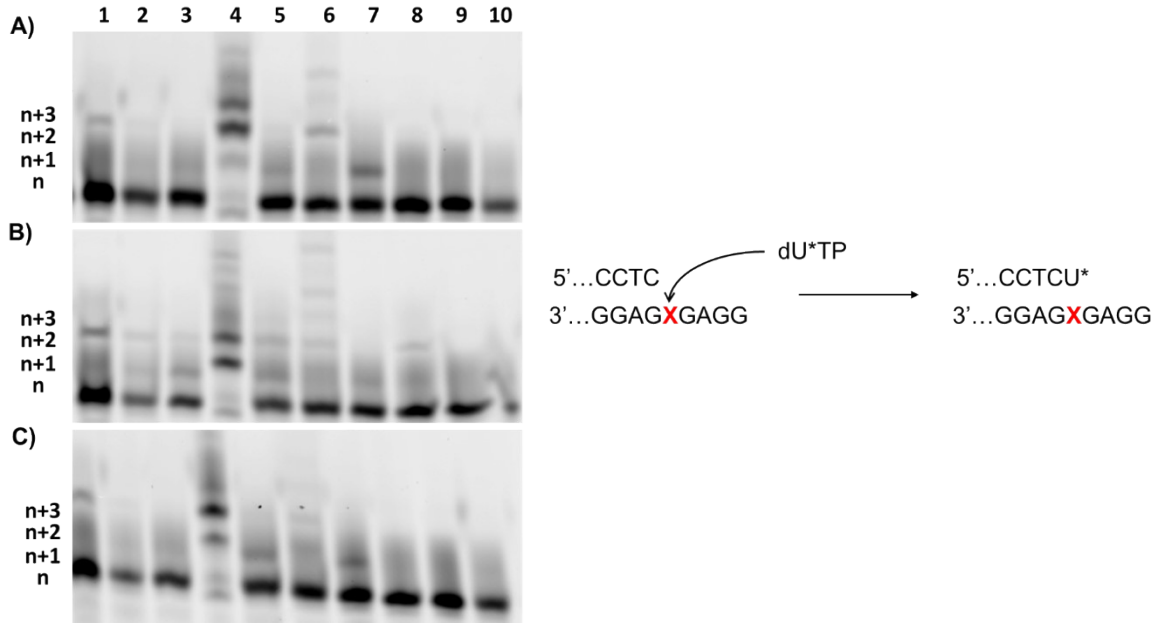


Figure S11. Gel images (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and 5-benzyl-modified-dUTP **7** and templates (all 15 pmol) A) **M1**, B) **AP1**, and C) **M1-Sp18**. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Therminator (2 U), 5. Vent (*exo*$^-$) (2 U), 6. Kf *exo*$^-$ (5 U), 7. Dpo4 (2 U), 8. Deep Vent (2 U). The negative controls are: 9. No polymerase, 10. No dNTP. All reactions were carried out with 200 µM modified nucleotides for 1h. The label n is connected to the length of the primer, while n+1, n+2, and n+3 refer to the length of the primer with the addition of one, two, or three dNMPs, respectively.

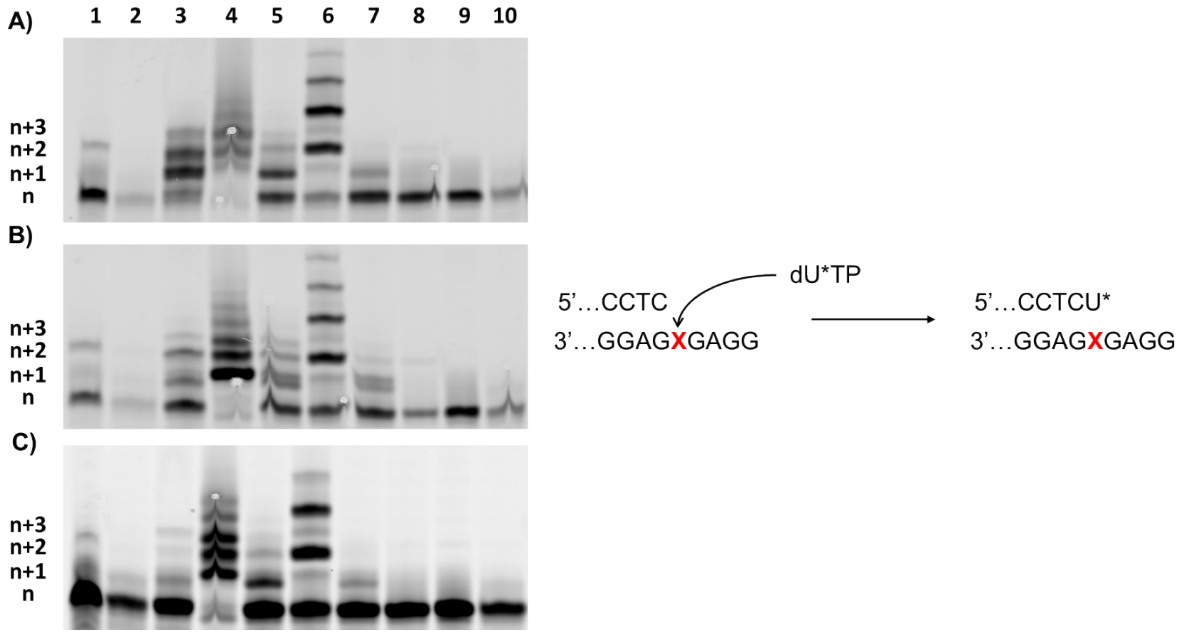
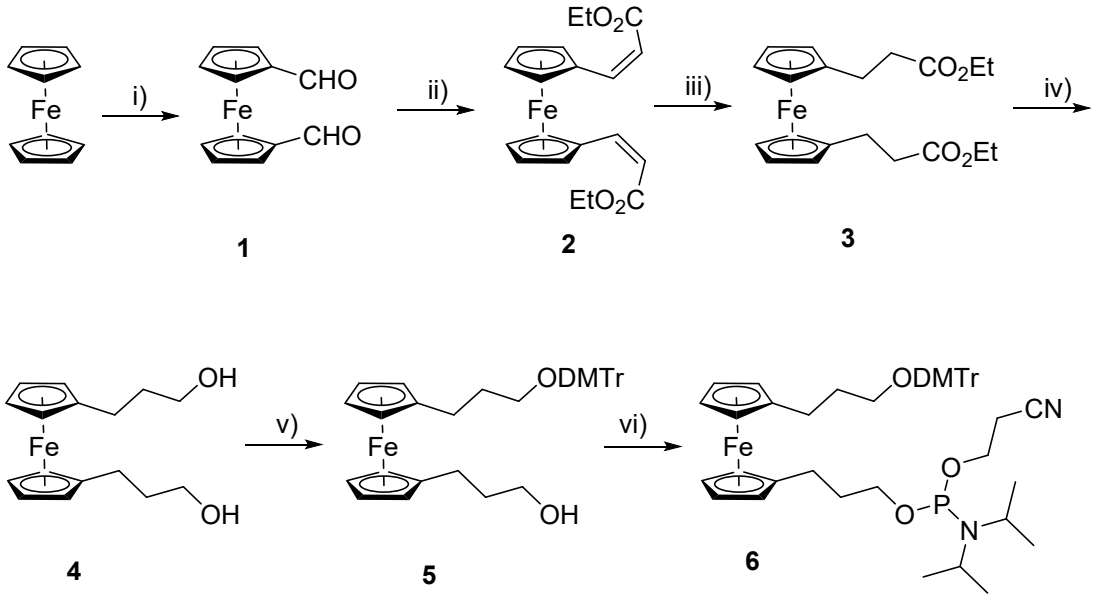


Figure S12. Gel images (PAGE 20%) of PEX reactions with primer **P1** (10 pmol) and 5-ferrocene-modified-dUTP **8** and templates (all 15 pmol) A) **M1**, B) **AP1**, and C) **M1-Sp18**. List of polymerases used: 1. Hemo Klem Taq (8 reactions), 2. Taq (5 U), 3. Bst (8 U), 4. Therminator (2 U), 5. Vent (*exo*$^-$) (2 U), 6. Kf *exo*$^-$ (5 U), 7. Dpo4 (2 U), 8. Deep Vent (2 U). The negative controls are: 9. No polymerase, 10. No dNTP. All reactions were carried out with 200 µM modified nucleotides for 1h. The label n is connected to the length of the primer, while n+1, n+2, and n+3 refer to the length of the primer with the addition of one, two, or three dNMPs, respectively.

S2. Chemical synthesis of Fc phosphoramidite monomer

A ferrocene phosphoramidite with two propyl linkers (compound **7**, Scheme S1), was synthesised according to a literature procedure.[1] The route started from ferrocene and was achieved in six steps in an overall yield of 12%.



Scheme S1. Synthesis of ferrocene phosphoramidite (compound **7**). Reagents and conditions: i) a) *n*-BuLi, TMEDA, 16h, b) DMF, -78°C, 10 min, c) RT, 2h, 70%; ii) triethylphosphonoacetate, NaH, RT, 25h, 51%; iii) $Pd(OH)_2/C$, H_2, RT, 22h, 96%; iv) $LiAlH_4$, 0°C, 2h, 87%; v) DMTr-Cl, DMAP, DIPEA, RT, 23h, 57%; vi) CEP-Cl, DIPEA, RT, 2h, 70%.

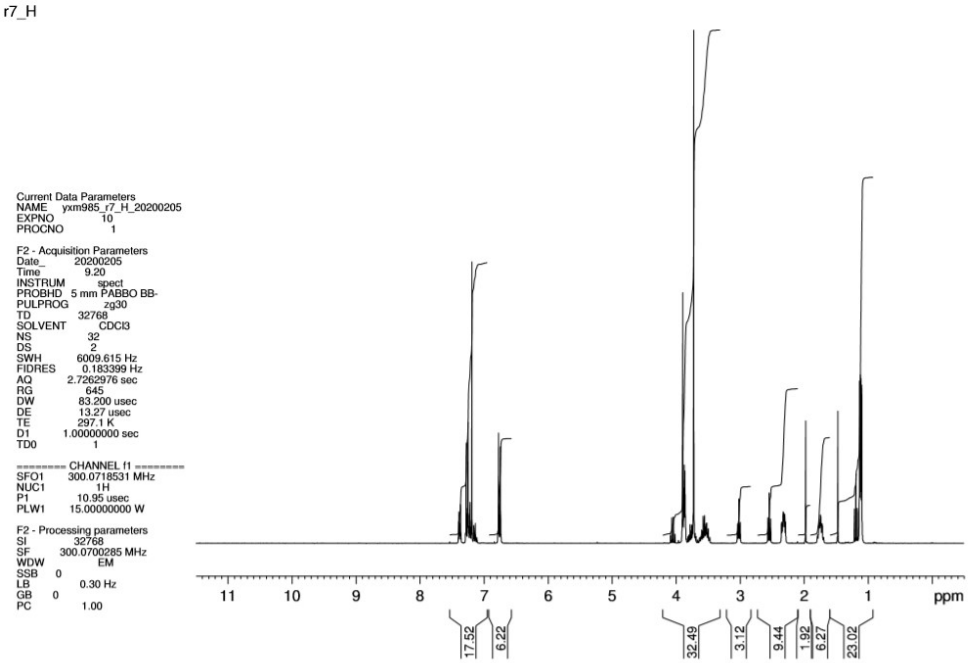


Figure S13. ^1H NMR spectrum of Fc-phosphoramidite **6** in $CDCl_3$.

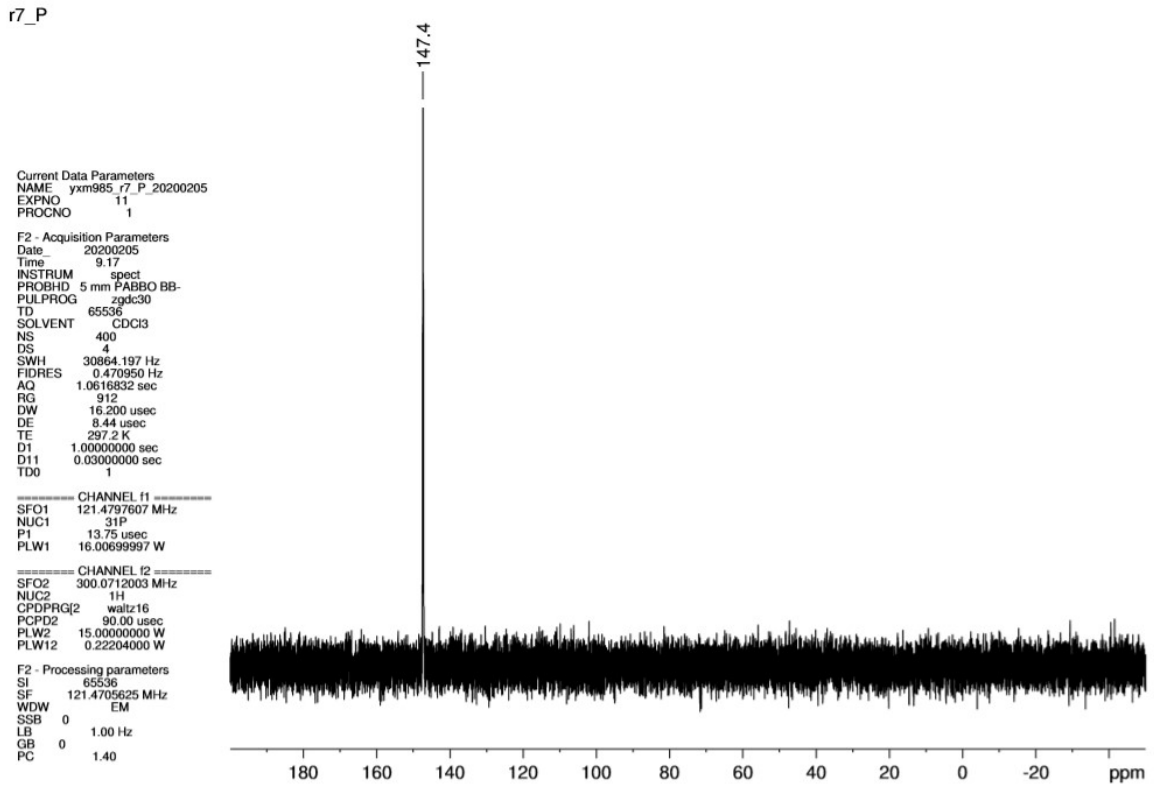


Figure S14. ^{31}P NMR spectrum of Fc-phosphoramidite **6** in $CDCl_3$.

S3. Oligonucleotide synthesis and characterisation data.

PacdA, iPr-Pac-dG, Ac-dC and dT phosphoramidites were purchased from LGC Genomics. The abasic nucleotide were purchased from Glen Research. The unmodified and the C18-spacer modified oligonucleotides were purchased from Integrated DNA Technologies. The dNTPs, all the DNA polymerases (*Hemo Klem Taq*, *Taq*, *Bst*, *Therminator*, *Vent (exo$^-$)*, *Klenow* fragment of DNA polymerase I exo-*(Kf(exo$^-$))*, *Sulfolobus*, *Deep Vent*) and the related reaction buffers were purchased from New England Biolabs.

Oligonucleotide synthesis

Oligonucleotides (see list in Table S1) were synthesized using an Applied Biosystems ABI 394 synthesizer. All phosphoramidites were dissolved in anhydrous acetonitrile (0.1 M) prior to synthesis. Strands were synthesised at a 1 µmol scale. Removal of 4,4'-dimethoxytrityl (DMTr) was achieved using trichloroacetic acid in dichloromethane (DCM). Phosphoramidites were activated with 5-ethylthio-1*H*-tetrazole (0.25 M) in acetonitrile prior to coupling and coupling times of 25 seconds were used. Unreacted material was capped using acetic anhydride and methylimidazole. Iodine (0.02 M) in THF/pyridine/water (7:2:1) oxidised the phosphotriesters formed. Upon completion, each oligonucleotide was treated with aqueous ammonia (30%) for 1 hour at room temperature followed by heating at 60°C for 6 hours, to cleave the strands from the resin and remove protecting groups. The solvent was removed using a Thermo Scientific speed vac and the crude was redissolved in Milli-Q water (0.9 mL) ready for purification. Strands A_1-A_4 were purchased from Merck and were used as received.

Oligonucleotide Purification

Semi preparative HPLC purification was performed on an Agilent Technologies 1260 Infinity system using a Phenomenex Clarity 5 µm Oligo-RP LC 250 x 10 mm column. The column was heated to 60 °C and the UV/vis absorbance of each run was monitored at 260 nm. Buffer D was the buffer of choice and contained 0.1 M triethylammonium acetate (TEAA) in HPLC grade H2O. 1 ml of sample was injected for each sample, at a flow rate of 3 ml/min. Oligonucleotides were purified using a method of gradient 5–18% Acetonitrile in Buffer D over a period of 45 minutes. Collected fractions were evaporated to dryness, diluted to 1 ml in Milli-Q water, and desalted using a NAP-10 column (GE Healthcare), whilst eluting to 1.5 ml.

Purity of oligonucleotides was determined by analytical HPLC using a Phenomenex Clarity 5 µm Oligo RP LC 250 x 4.6 mm column on an Agilent Technologies 1260 Infinity system. The column was heated to 60 °C and the UV/vis absorbance of each run was monitored at 260 nm. Buffer D was the buffer of choice and contained 0.1 M triethylammonium acetate (TEAA) in HPLC grade H2O. 20 µl of sample was injected with a run time of 45 minutes for each sample, at a flow rate of 1 ml/min. Solvent gradients used were identical to semi preparative HPLC. Samples showing >95% purity by analytical HPLC were deemed sufficiently pure for use in experiments.

Mass Spectrometry

Masses of the DNA oligonucleotides were determined using a Waters Xevo G2-XS system with a Time of Flight (TOF) detector using negative mode electrospray ionization mass spectrometry (ESI-MS). Deconvolution of the raw data was accomplished using Promass and molecular mass prediction was obtained using Chemdraw software.

Table S1. Sequences and masses of the oligonucleotides synthesised

	Sequence	Predicted Exact Mass	MS Found
AP1	GGA GAPG AGG CTA TAG TGA GTC GTA	7385.26	7384.77
AP2	GGA GAPAPG AGG CTA TAG TGA GTC GTA	7565.36	7565.81
M1	GGA GFcG AGG CTA TAG TGA GTC GTA	7569.29	7569.48
M2	GGA GFcFcG AGG CTA TAG TGA GTC GTA	7933.34	7933.48
M3	GGFc GCG AGG CTA TAG TGA GTC GTA	7545.28	7545.43
M4	GGA GTG AGG CTA TAG FcGA GTC GTA	7569.29	7569.81

The higher masses observed in the MS data (see figures below) are largely attributed to TEA adducts.

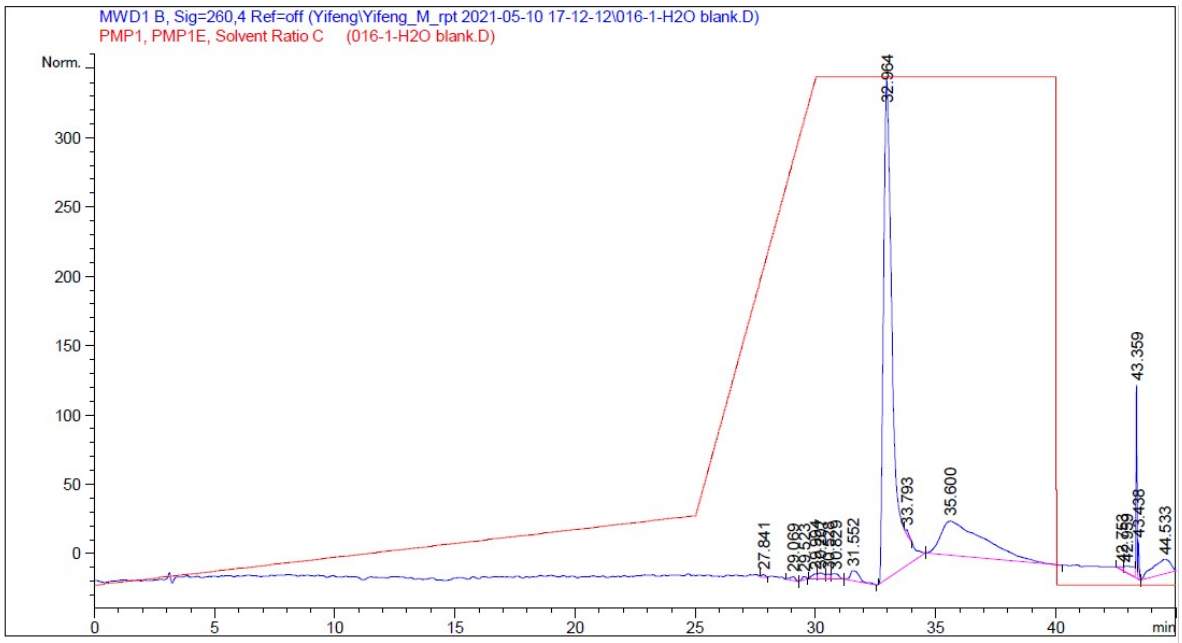


Figure S15. Analytical HPLC chromatogram of the blank.

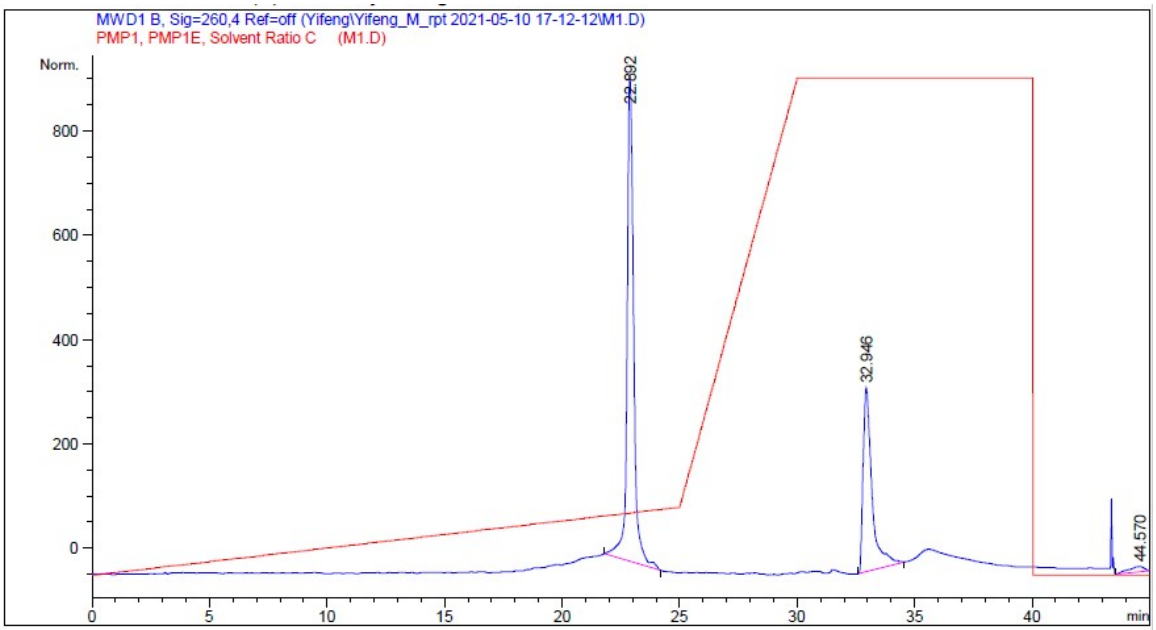


Figure S16. Analytical HPLC chromatogram of **M1**.

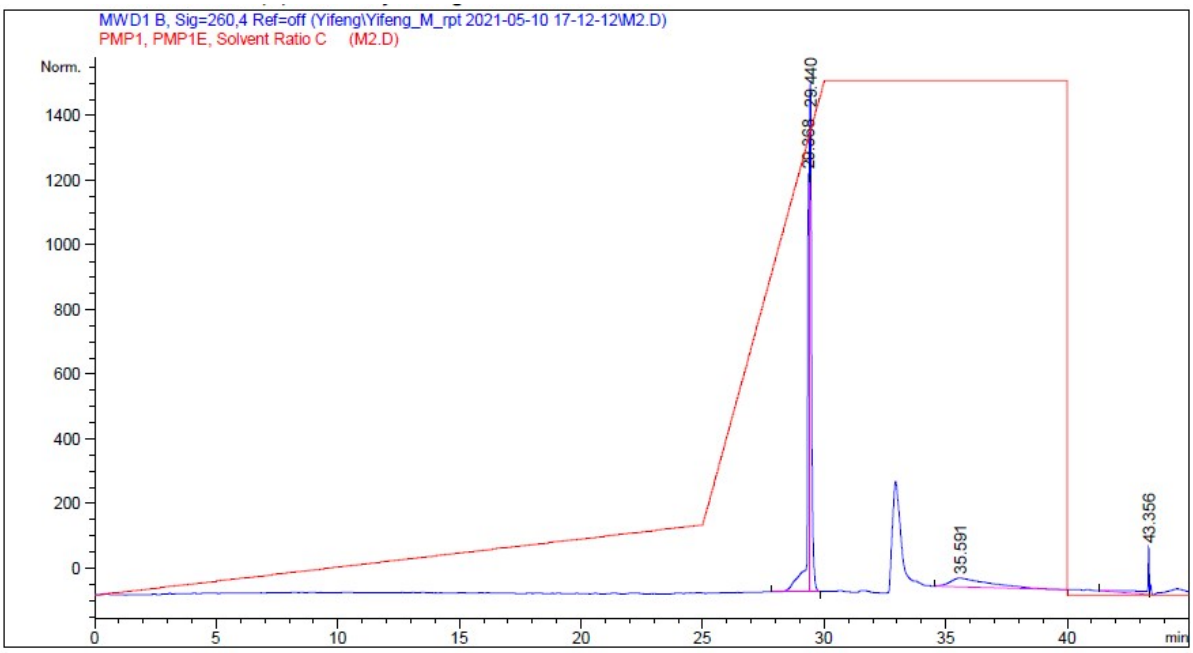


Figure S17. Analytical HPLC chromatogram of **M2**.

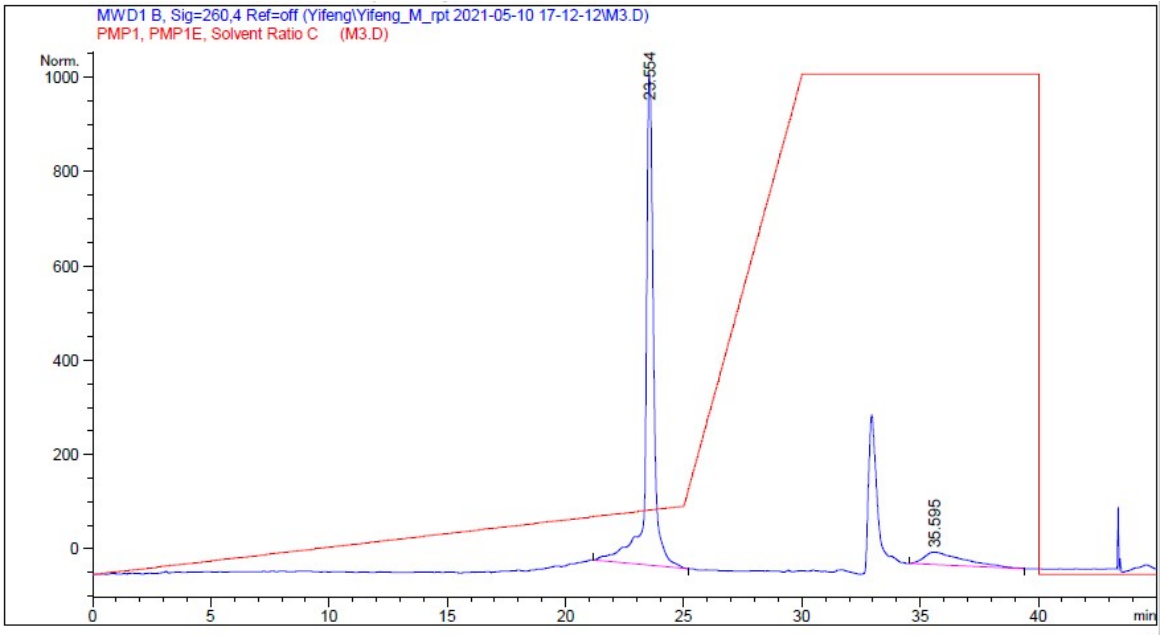


Figure S18. Analytical HPLC chromatogram of **M3.**

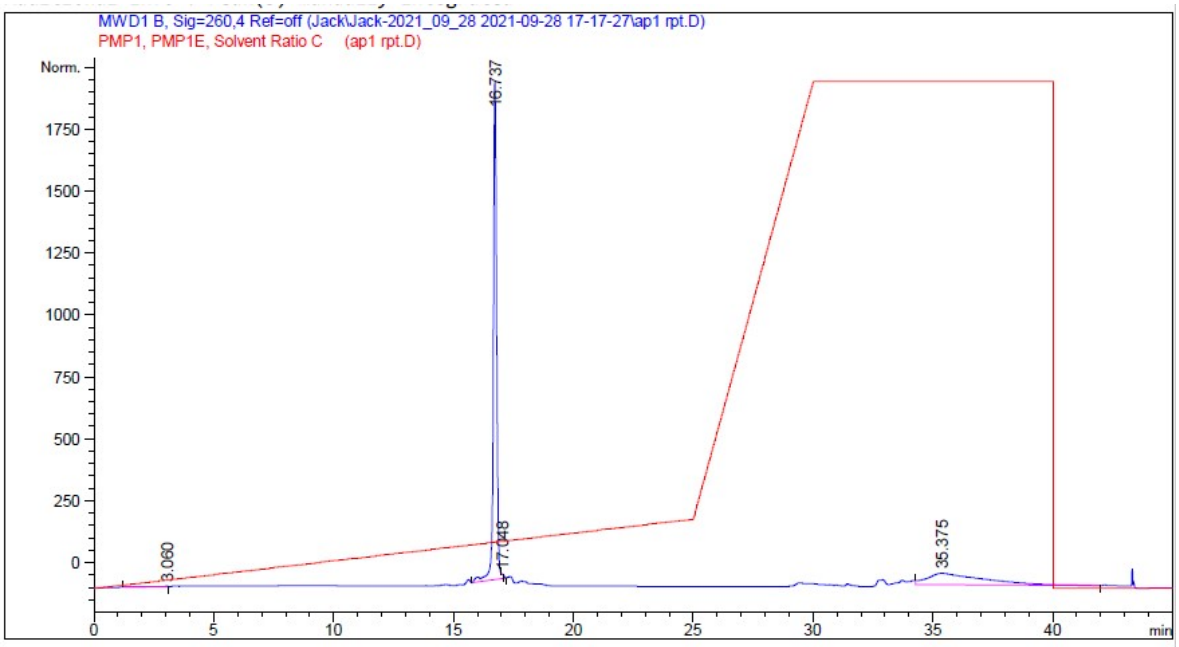


Figure S19. Analytical HPLC chromatogram of **AP1**.

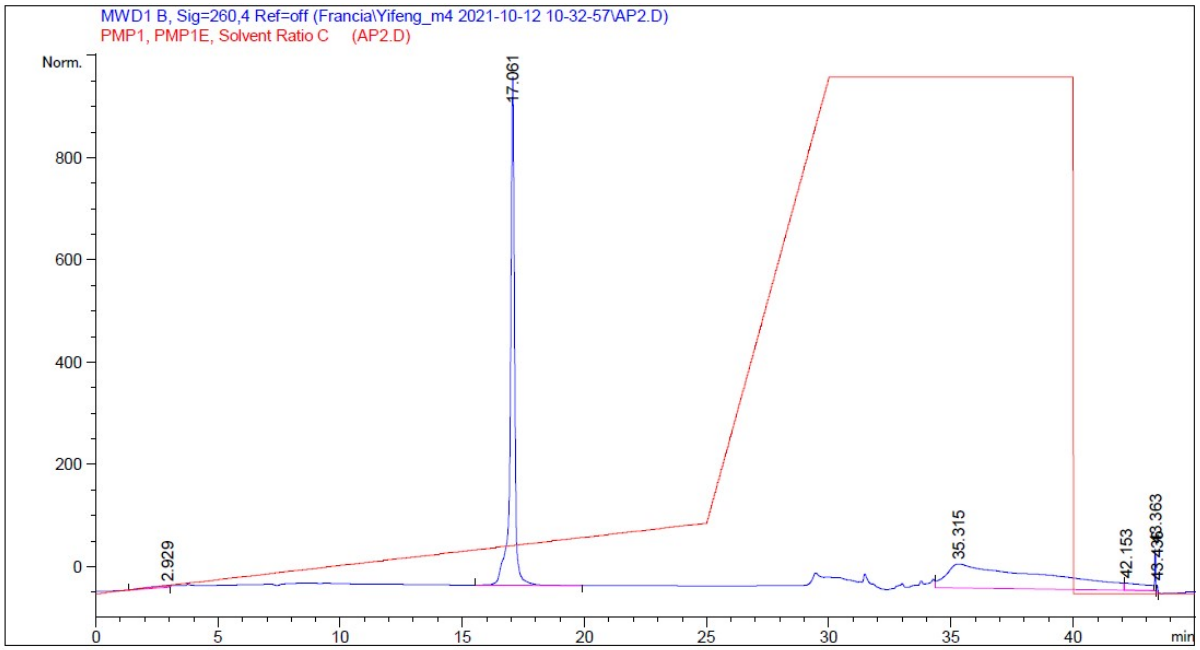


Figure S20. Analytical HPLC chromatogram of **AP2**.

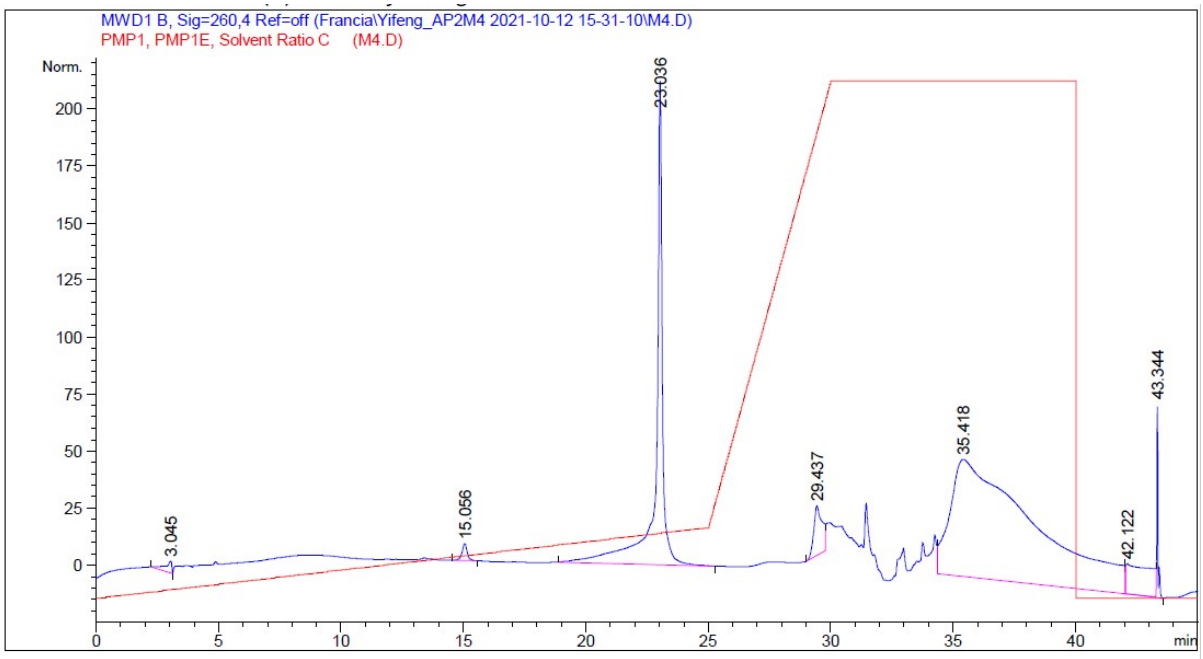


Figure S21. Analytical HPLC chromatogram of **M4**.

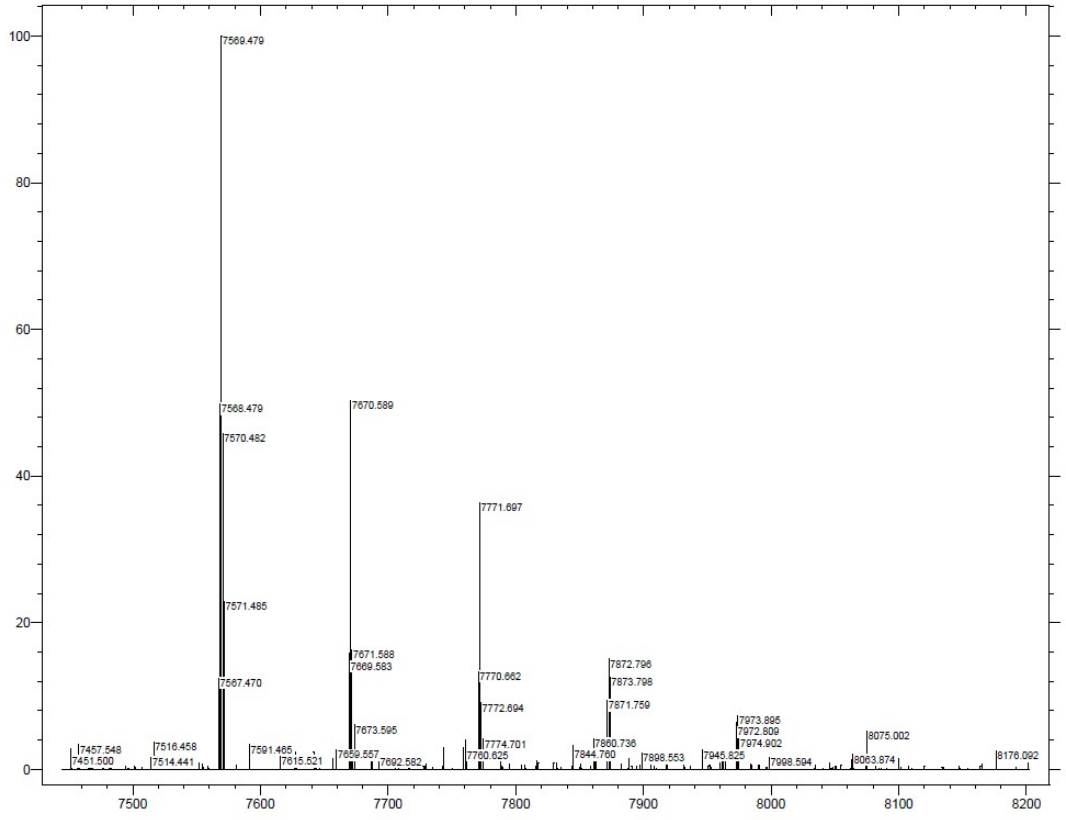


Figure S22. Mass spectrum of **M1**.

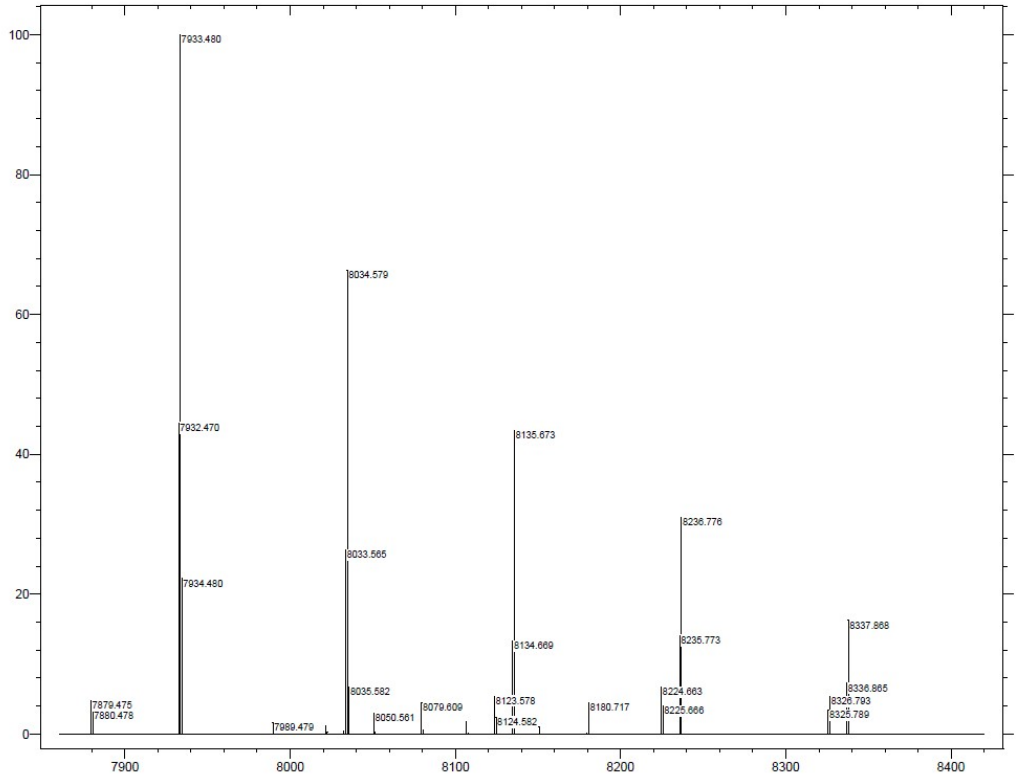


Figure S23. Mass spectrum of **M2**.

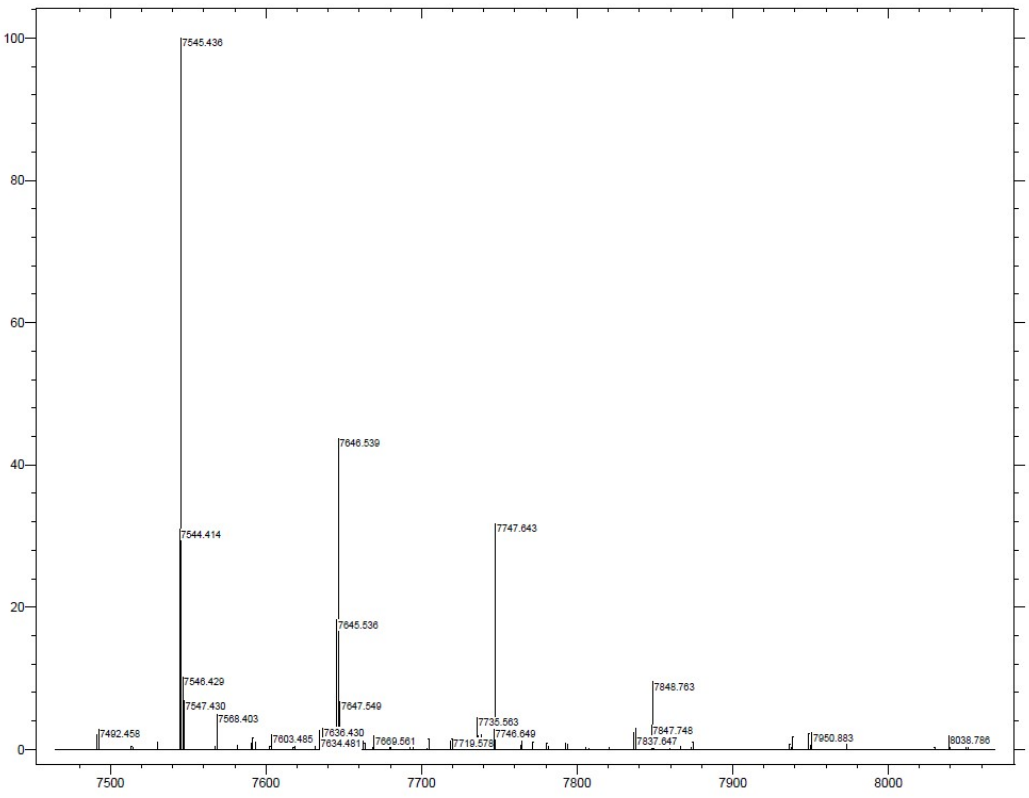


Figure S24. Mass spectrum of **M3**.

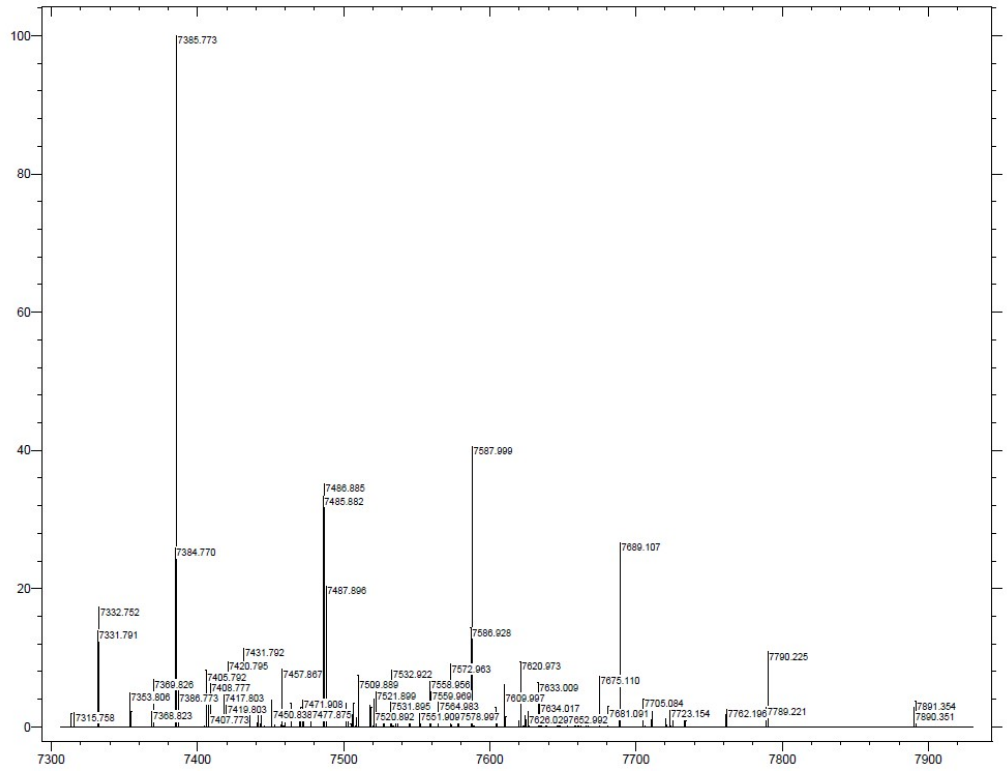


Figure S25. Mass spectrum of **AP1**.

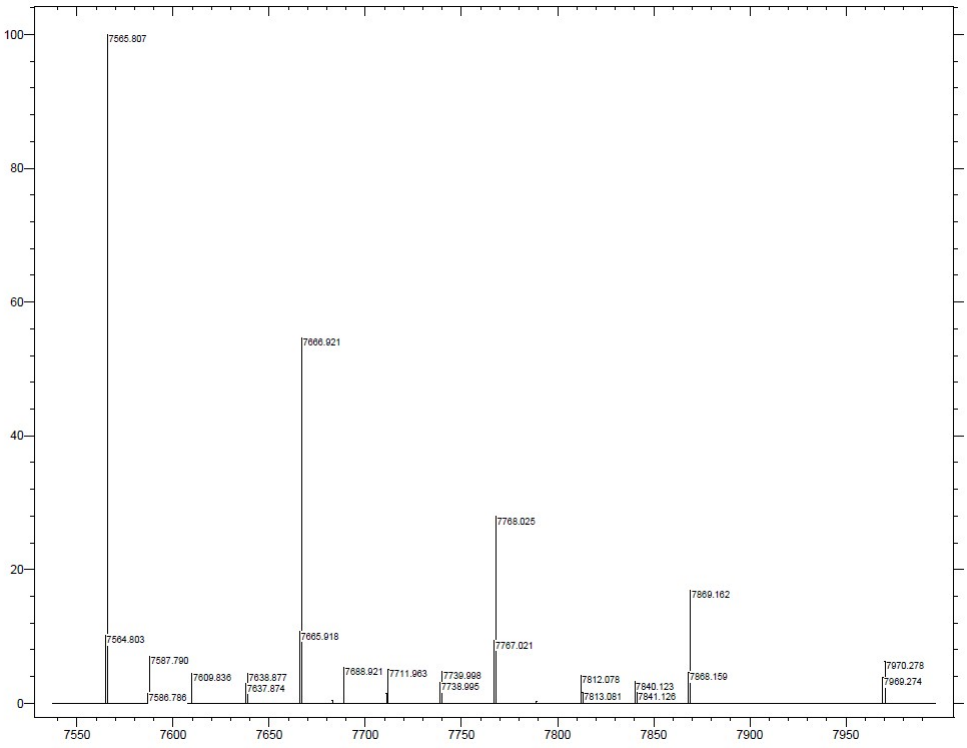


Figure S26. Mass spectrum of **AP2**.

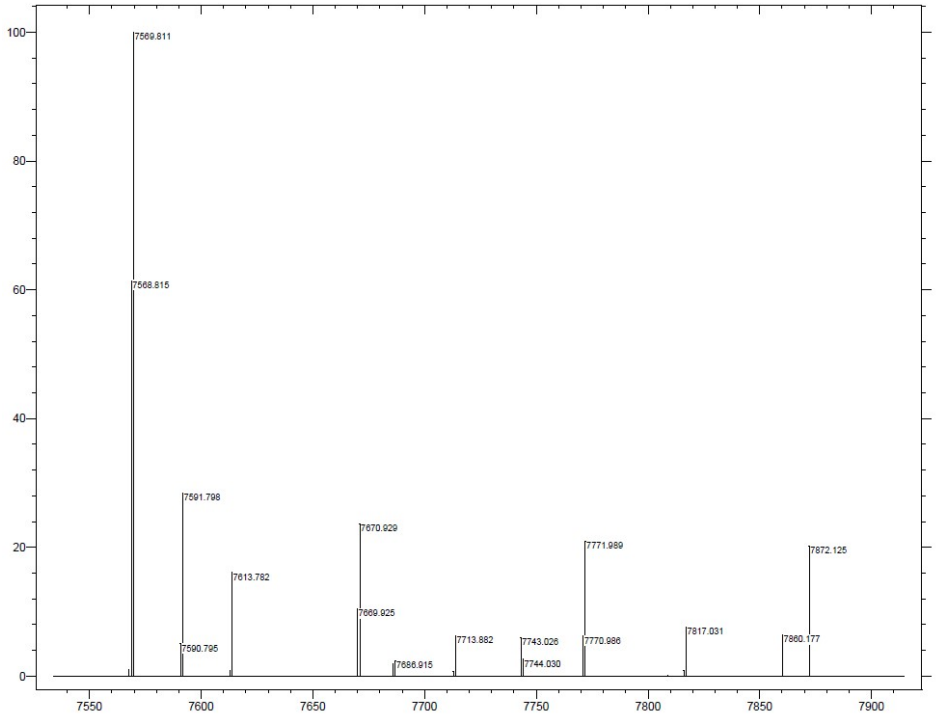


Figure S27. Mass spectrum of **M4**.

Reference:

1. Gupta NK, Wilkinson EA, Karuppannan SK, et al. Role of Order in the Mechanism of Charge Transport across Single-Stranded and Double-Stranded DNA Monolayers in Tunnel Junctions. *J. Am. Chem. Soc*. **2021**, *143*, 20309−20319 and references cited theirin)