Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Silver Soldering of PNA:DNA duplexes: Assembly of triple duplex from bimodal PNAs with all-C on one face

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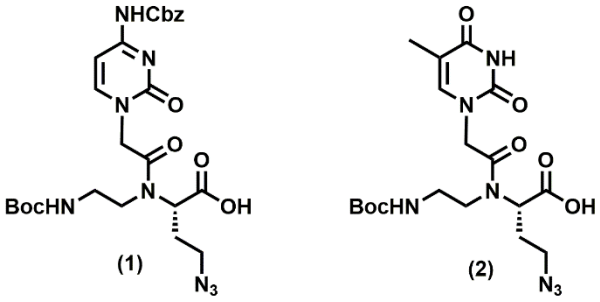
1.0 General material: The chemicals used were of laboratory or analytical grade. All the solvents used were distilled or dried to carry out different reactions. Reactions were monitored by thin-layer chromatography (TLC). Usual workup involved sequential washing of the organic extract with water and brine followed by drying the organic layer over anhydrous sodium sulphate and evaporation of solvent under vacuum. TLC were carried out on pre-coated silica gel GF_{254} sheets (Merck 5554). TLC plates were analyzed under UV lamp, by iodine spray and by spraying with ninhydrin solution, followed by heating of the plate. Column chromatographic separations were performed using silica gel (60-120 or 100-200 mesh).

All ^{1}H and ^{13}C NMR were recorded using Bruker AC-400 (400 MHz) or JEOL 400 MHz NMR spectrometers in $CDCl_3$ and DMSO-d_6. Chemical shifts are reported in parts per million (ppm, δ scale). Melting points were determined in open-end capillary tubes and are uncorrected. UV-Visible spectrophotometry (Perkin Elmer Lambda 45 double beam UV-Vis spectrophotometer) data were collected for compound/peptide characterization. Mass spectra for reaction intermediates were obtained by HRMS and the integrity of PNA oligomers was checked by Nano-ESI Spectrometer. PNA oligomers were purified by reverse phase HPLC system using semipreparative Phenomenex C18 (10 X 250 mm) column. DNA oligonucleotides were obtained commercially from Integrated DNA Technologies (IDT). Salts and reagents used in buffer preparation such as MOPS, $NaNO_3$, $AgNO_3$ etc. were obtained from Sigma-Aldrich. The pH of the buffer solutions was adjusted using HCl, from Sigma Aldrich.

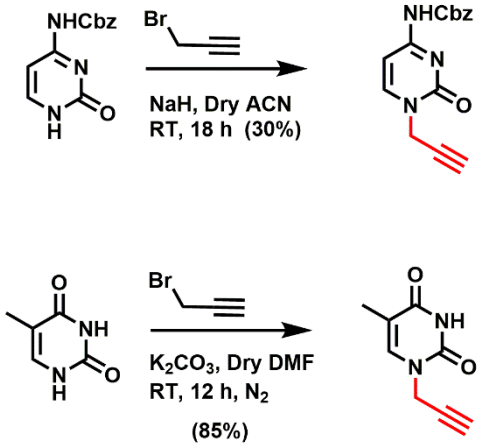
2.0 Monomer synthesis:

Each monomers were synthesized, characterized and purified according to standard reported protocol.[1]



Synthesis of N-propargyl nucleobases (C/T)

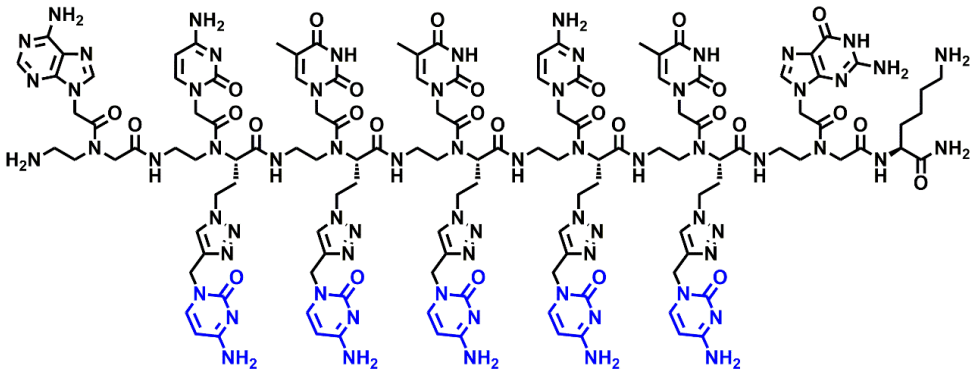
N-Propargyl nucleobases synthesized according to standard literature protocols.[2,3]



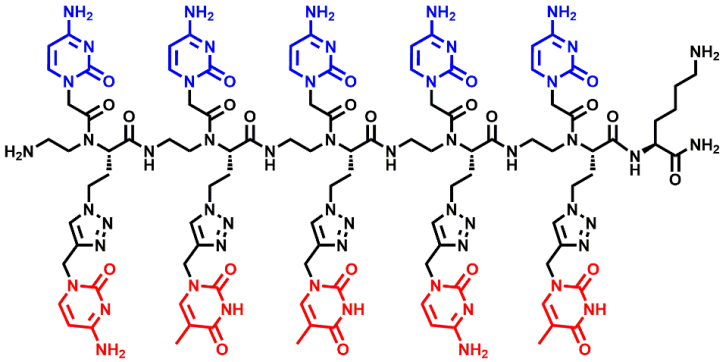
3.0 Solid-phase synthesis of PNA oligomers

aeg-PNA, iso-PNA and bimodal PNA oligomers were synthesized by standard solid-phase synthesis protocol.[1]

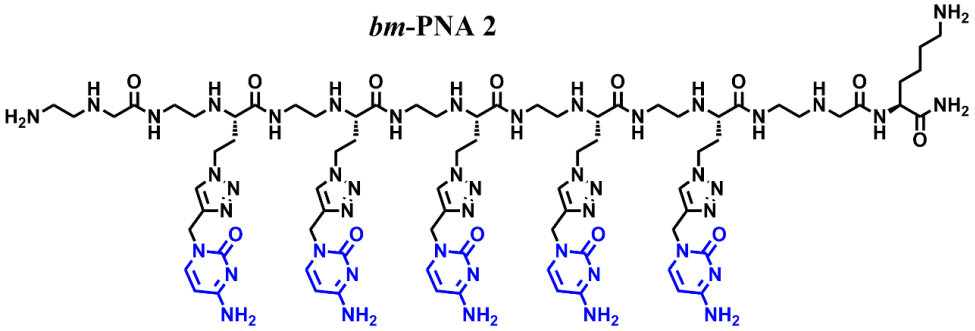
PNA oligomer sequences



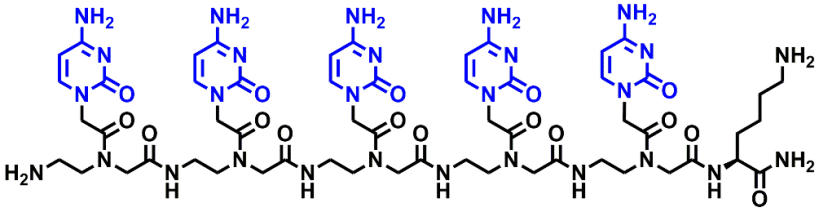
***bm*-PNA 1**



***bm*-PNA 2**

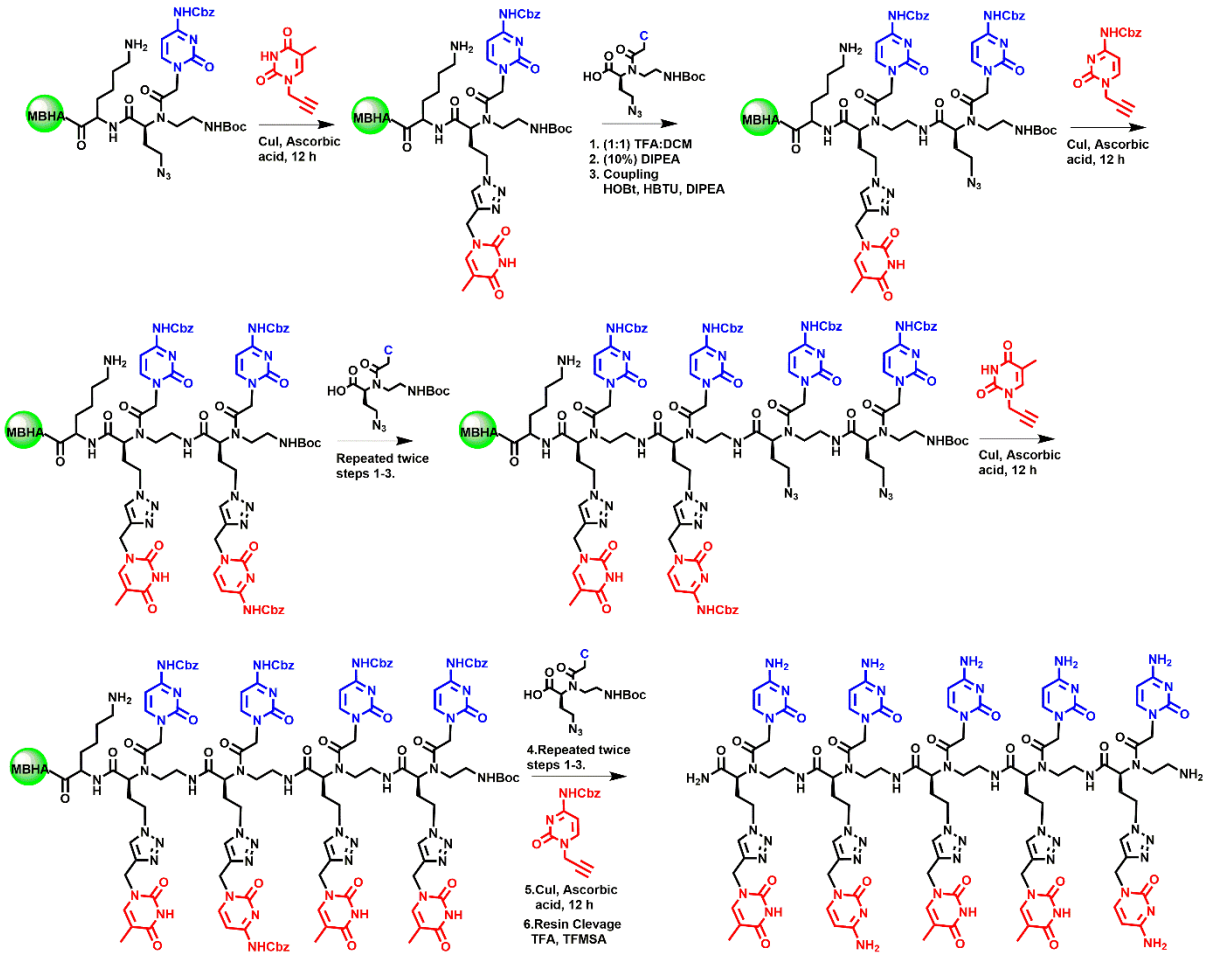


***iso*-PNA-C_5**



***aeg*-PNA-C_5**

Scheme S1 for Solid Phase Peptide Synthesis (SPPS) strategy for *bm*-PNA 2



bm-PNA **2**

4.0 RP-HPLC Chromatograms of PNA Oligomers

The purification of PNAs were carried out on Agilent Infinity 1200 HPLC system with semi-preparative Phenomenex C18 (10×250 mm) column using solvents water and acetonitrile with composition A: 0.1% TFA in CH_3CN:H_2O (5:95) and B= 0.1% TFA in CH_3CN:H_2O (1:1). The gradient for elution was 100% A to 100% B in 20 min, with flow rate of 2 mL/min. The elution's were monitored at 220 and 254 nm wavelength.

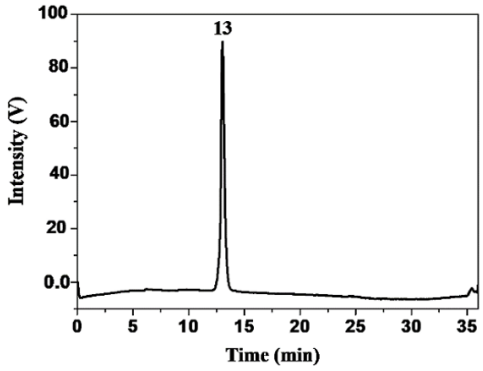


Figure S1. HPLC trace of iso-PNA-C_5

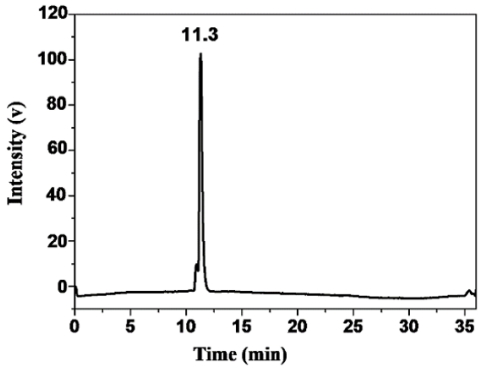


Figure S2. HPLC trace of *aeg*-PNA-C_5

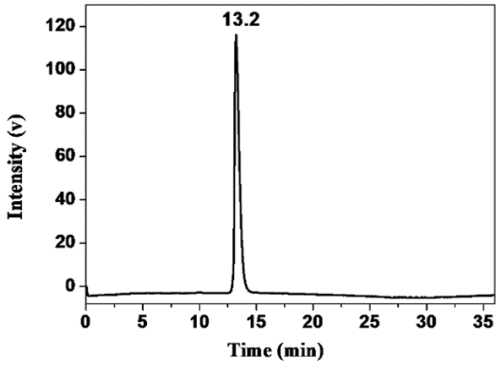


Figure S3. HPLC trace of *bm*-PNA-1

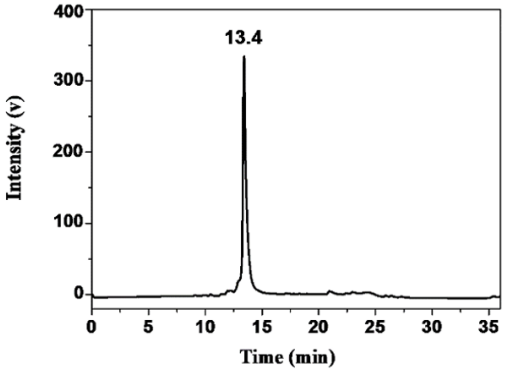


Figure S4. HPLC trace of *bm*-PNA-2

5.0 Nano-ESI and MALDI -TOF analysis of PNA Oligomers

All PNA oligomers analyzed using a Synapt G2 HD mass spectrometer (WatersCorporation) Nano ESI-MS spectra were collected in positive ion mode. A HPLC purified 5µM oligomer injected for analysis. Parameters of mass analysis as follows. Source temperature of 70 ºC, Capillary voltage of 1.5 kV, Cone voltage of 60 V Extraction cone 4.0 V.

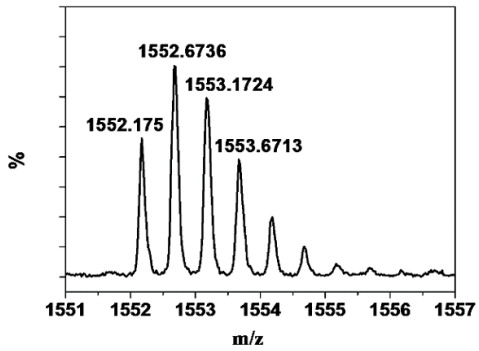


Figure S5. Nano-ESI spectrum of *bm*-PNA 1

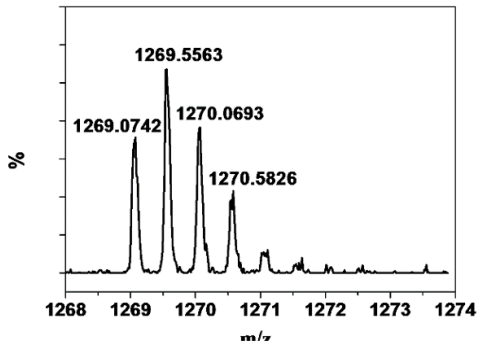


Figure S6. Nano-ESI spectrum of *bm*-PNA 2

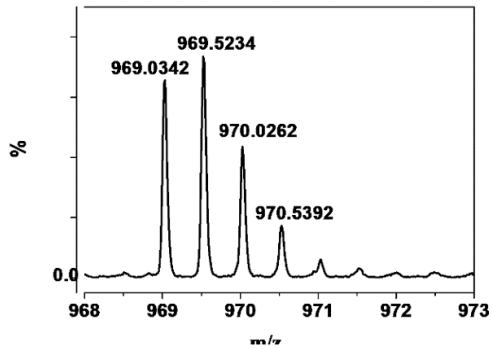


Figure S7. Nano-ESI spectrum of *iso*-PNA

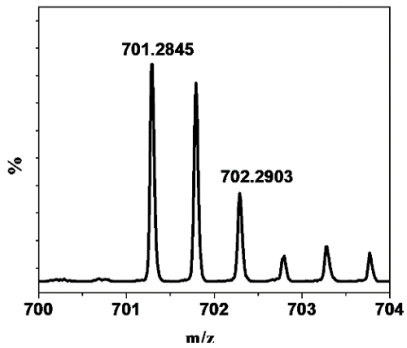


Figure S8. Nano-ESI spectrum of *aeg*-PNA

Table S1. Nano-ESI analysis of various PNA oligomers

Entry	Oligomers	Mol. formulae	Calcd. Mass	Obs. Mass	Charge Z
1	*bm*-PNA 1	$C_{126}H_{160}N_{69}O_{29}$	$[M]^+$3103.3166	$[M]^{2+}$ 1552.6736	2
2	*bm*-PNA 2	$C_{104}H_{133}N_{55}O_{24}$	$[M]^+$2536.0878	$[M]^{2+}$ 1269.5563	2
3	*iso*-PNA-C_5	$C_{79}H_{121}N_{47}O_{13}$	$[M]^+$1937.1530	$[M]^{2+}$ 969.5234	2
4	*aeg*-PNA-C_5	$C_{56}H_{80}N_{28}O_{16}$	$[M]^+$1400.6307	$[M]^{2+}$ 701.2845	2

MALDI-TOF analysis of PNA Oligomers

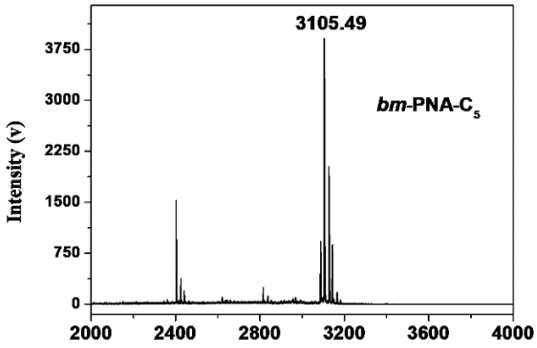


Figure S9. MALDI-TOF of *bm*-PNA 1

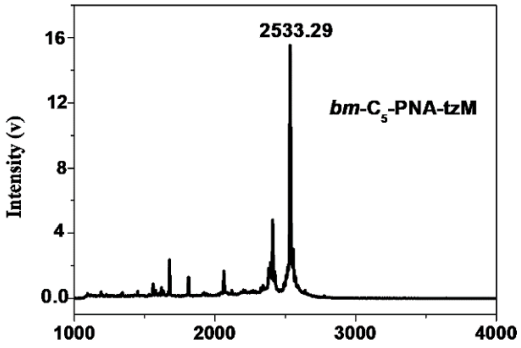


Figure S10. MALDI-TOF of *bm*-PNA 2

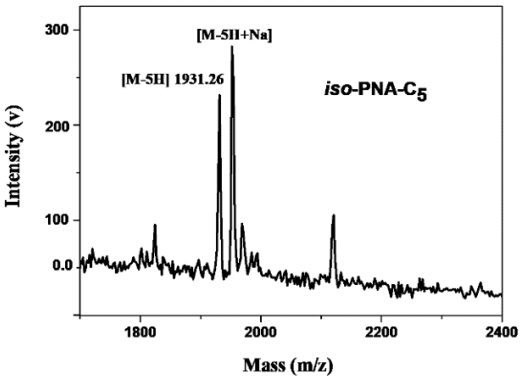


Figure S11. MALDI-TOF of *iso*-PNA

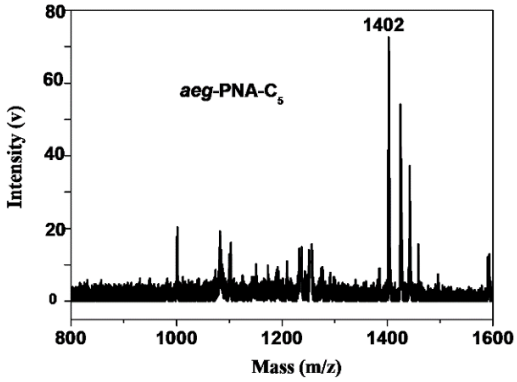


Figure S12. MALDI-TOF of *aeg*-PNA

Table S2. MALDI-TOF analysis of various PNA oligomers

Entry	Oligomers	Mol. formulae	Calcd. Mass	Obs. Mass
1	*bm*-PNA 1	$C_{126}H_{160}N_{69}O_{29}$	$[M]^+$3103.3166	[M+2H] 3105.49
2	*bm*-PNA 2	$C_{104}H_{133}N_{55}O_{24}$	$[M]^+$2536.0878	[M+3H] 2533.29
3	*iso*-PNA-C_5	$C_{79}H_{121}N_{47}O_{13}$	$[M]^+$1937.1530	[M-6H] 1931.26
4	*aeg*-PNA-C_5	$C_{56}H_{80}N_{28}O_{16}$	$[M]^+$1400.6307	[M+2H] 1402.0

6.0 DNA oligonucleotides used for biophysical studies

Table S2. DNA oligos used for biophysical studies

Entry	DNA	Sequence (5' to 3')
1	DNA **1**	CAGAAGT
2	DNA **2**	TAGAAG
3	dTC_5	TCCCCC

7.0 UV-T experiments: Temperature-UV Absorbance Measurement

UV-melting experiments were carried out on Varian Cary 300 UV spectrophotometer equipped with a peltier. The samples for T_m measurement were prepared by mixing the calculated amounts of respective oligonucleotides in the stoichiometric ratio (1:1, duplex) in MOPS buffer (10 mM) pH 7 and $NaNO_3$ (10 mM); to achieve a final strand concentration of 2.5 μM for each strand. The samples were annealed by heating at 90 °C for 10 min. followed by slow cooling to room temperature for 8 to 10 h and then kept at 4°C for 12 h. The samples (500 μL) were transferred to quartz cell and equilibrated at the starting temperature for 5 min. The OD at 260 nm was recorded in steps from 20-90 °C with temperature increment of 1 °C. Each melting experiment was repeated at least twice. The normalized absorbance at 260 nm was plotted as a function of the temperature. The data were fitted by sigmoidal curve, function Boltzmann for one face and bidose response for two face binding, where R square value in range of 0.96 to 0.99. The T_m was determined from the first derivative of normalized absorbance with respect to temperature and is accurate to ± 1.0 °C. The data were processed using Origin Pro 8.5, [The concentration of all oligonucleotides were calculated on the basis of absorbance from the molar extinction coefficients of the corresponding nucleobases i.e. T = 8.8 $cm^2/\mu mol$; C = 6.6 $cm^2/\mu mol$; G = 11.7 $cm^2/\mu mol$ and A = 13.7 $cm^2/\mu mol$].[4]

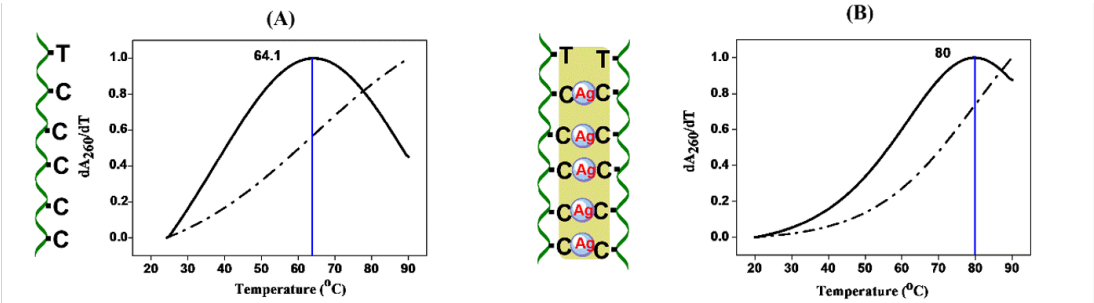


Figure S13 UV-Tm at 260 nm abs. of dTC_5 (A) Self melting of dTC_5 (B) dTC_5:Ag^+:dTC_5 duplex, Buffer: MOPS,[10 µM] (pH 7), $NaNO_3$ [10 µM], $AgNO_3$ [12.5 µM], Tm values are accurate to ±1.0 °C

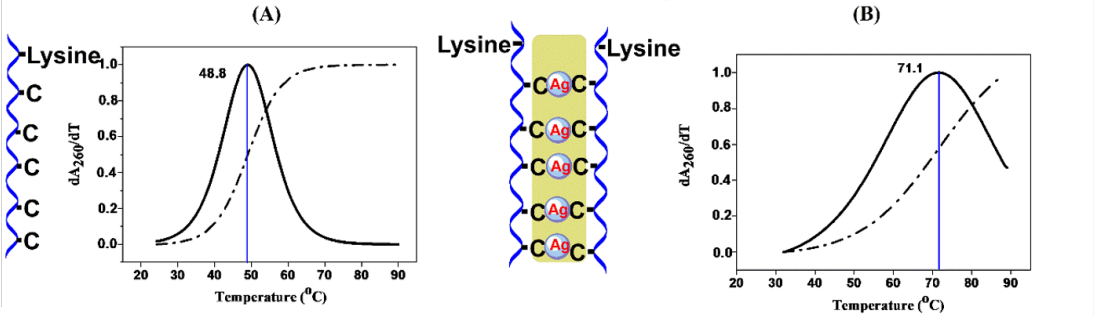


Figure S14 UV-Tm at 260 nm abs. of *aeg*-PNA-C_5 (A) Self melting of *aeg*-PNA-C_5 (B) *aeg*-PNA-C_5:Ag^+:*aeg*-PNA-C_5 duplex, Buffer: MOPS,[10 µM] (pH 7), $NaNO_3$ [10 µM], $AgNO_3$ [12.5 µM], Tm values are accurate to ±1.0 °C

Table S3. UV-T_m (°C) of dTC_5 and *aeg*-PNA-C_5 with and without Ag^+

Entry	Duplex	T_m (°C)	ΔT_m (°C) (stability with Ag^+)
1	dTC_5 (self melting)	64.1	-
2	dTC_5:Ag^+:dTC_5	80.0	+15.9
3	*aeg*-PNA-C_5 (self melting)	48.8	-
4	*aeg*-PNA-C_5:Ag^+: *aeg*-PNA-C_5	71.1	+22.3

8.0 Field emission scanning electron microscopy (FESEM) studies

A freshly prepared $AgNO_3$ solution was added to an aqueous solution of PNA, to get complex with a desired concentration of cytosine and Ag^+. From this prepared solution, 2 µl aliquot then drop cast on to clean silicon wafer. The solvent from the drop was allowed to evaporate under high vacuum and the films were then left to stand for 8 h under ambient conditions. Before FESEM imaging, the samples were coated with gold prior to imaging with a ZEISS ULTRA PLUS electron microscope. FESEM imaging was done in two slots. In first case, aliquots (2 µL) were taken out from a PNA/$AgNO_3$ mixture within the first 30 min (data not shown) and deposited on a clean Si wafer. Further, after 7 days aliquots were drop cast on si-wafer and after solvent evaporation subjected to imaging (Fig. S11)

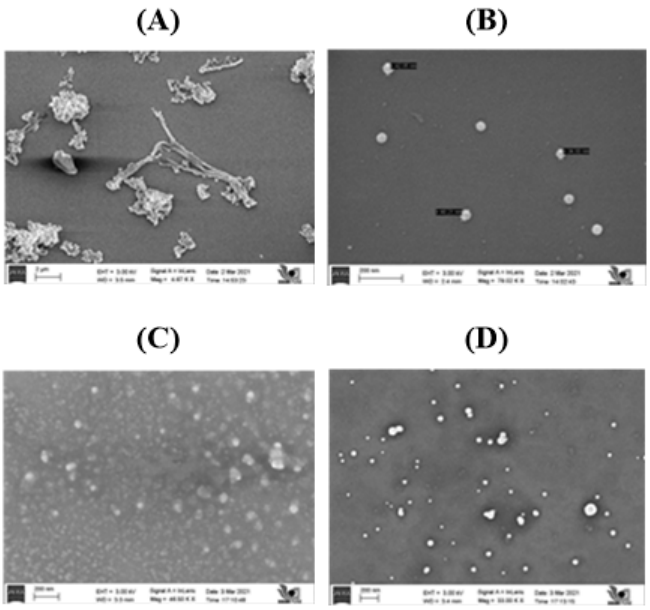
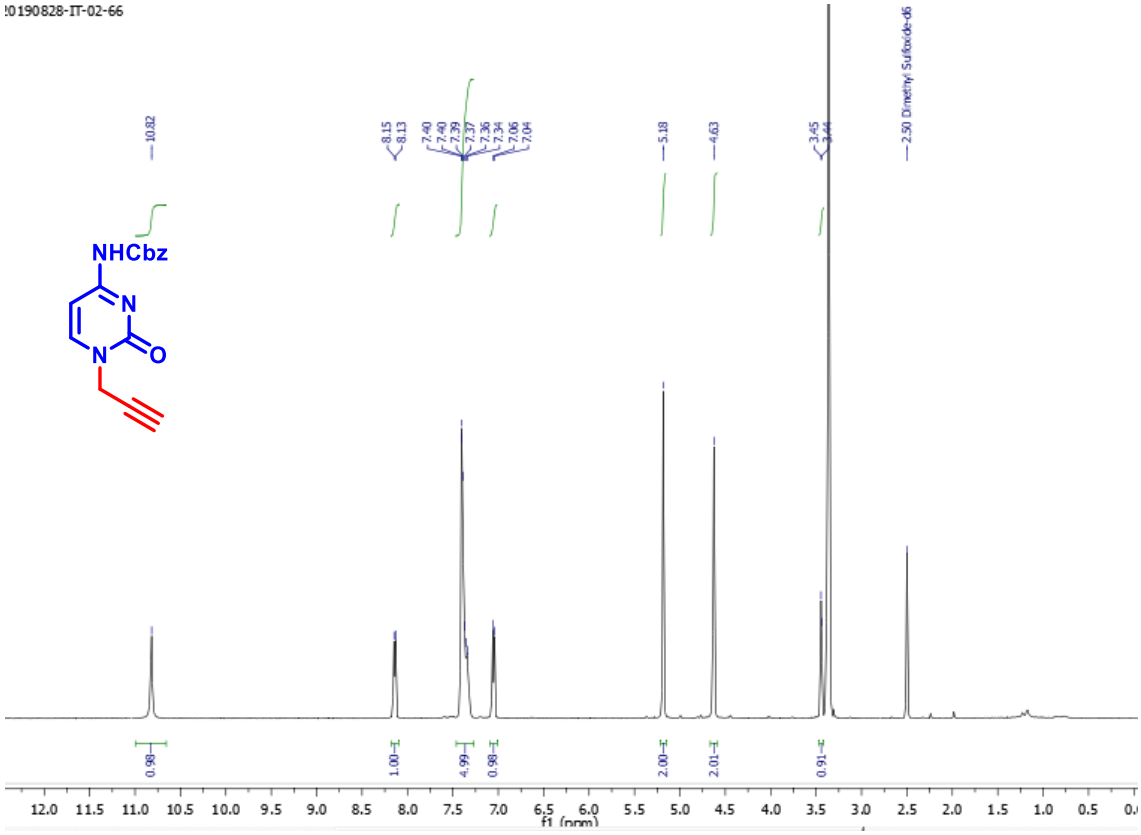
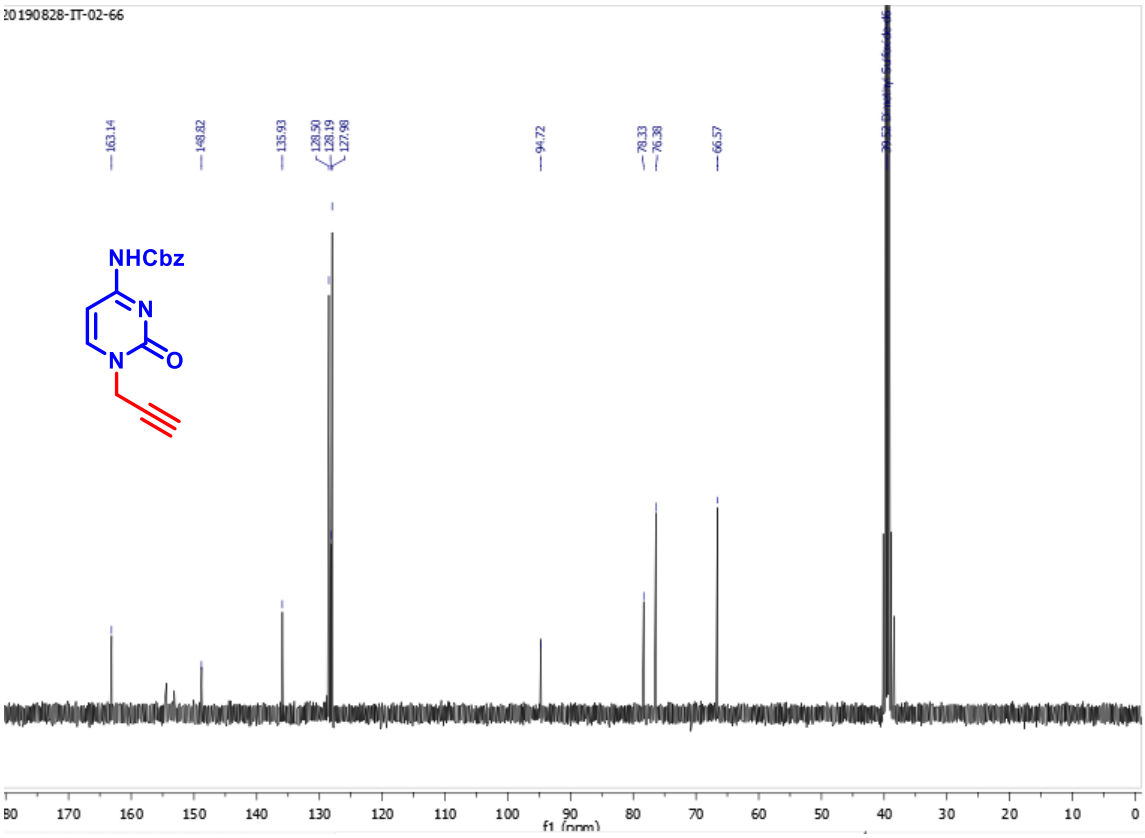


Figure S15. FESEM images of (A) dTC_5 (B) dTC_5:Ag^+:dTC_5 (C) *aeg*-PNA-C_5, and (D) *aeg*-PNA-C_5:Ag^+:*aeg*-PNA-C_5, Conc. of DNA/PNA [5 μM], $AgNO_3$ [25 μM].

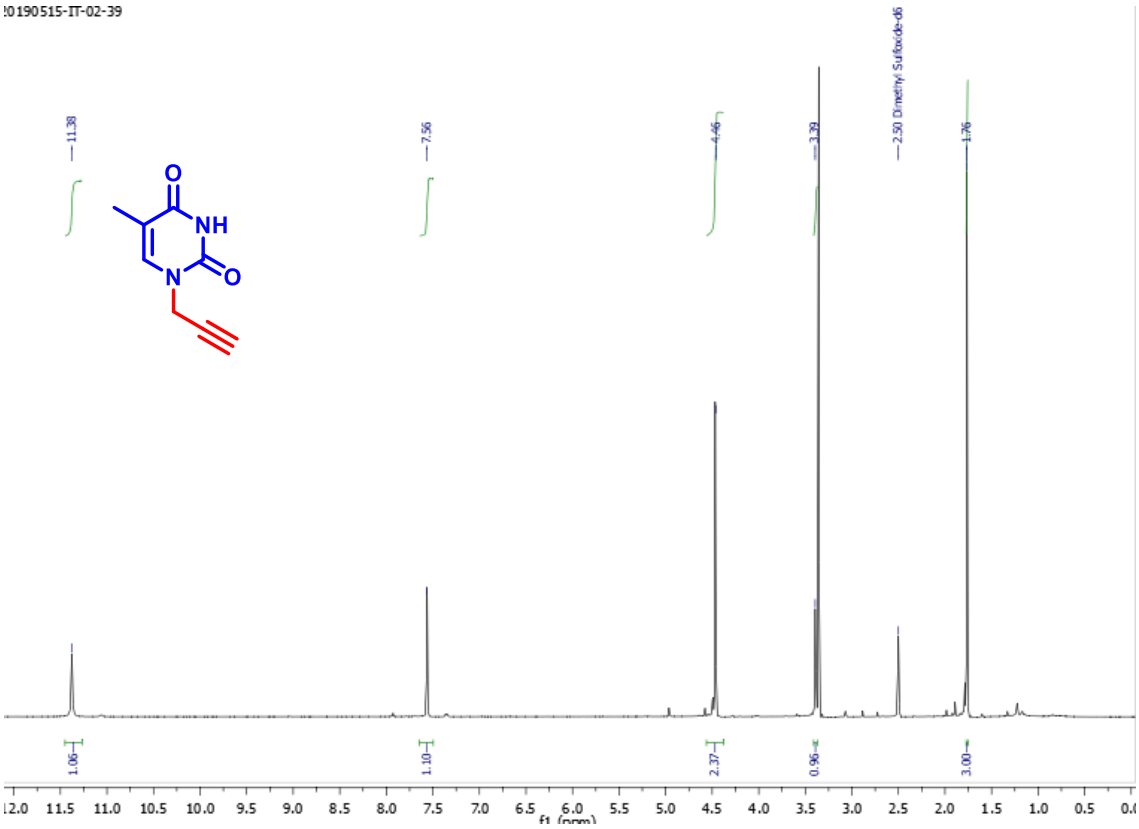
^{1}H NMR Spectrum of Propynyl Cytosine



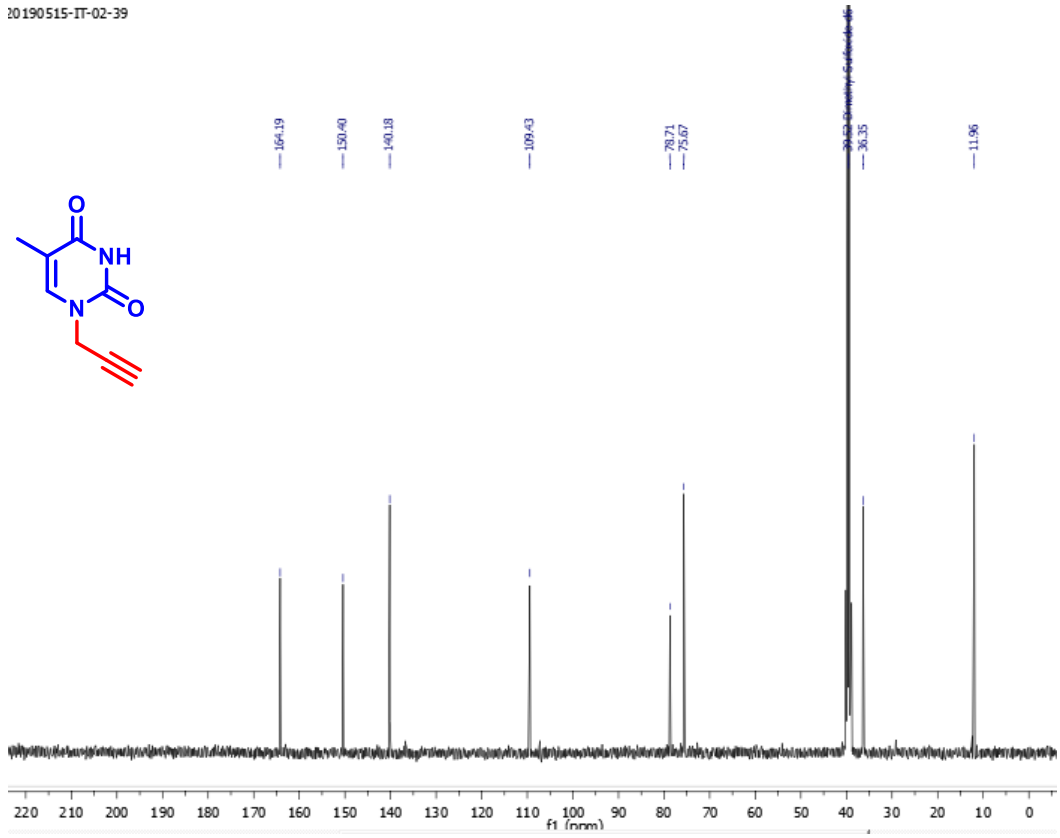
^{13}C NMR Spectrum of Propynyl Cytosine



^{1}H NMR Spectrum of N1-Propynyl Thymine



^{13}C NMR Spectrum of N1-Propynyl Thymine



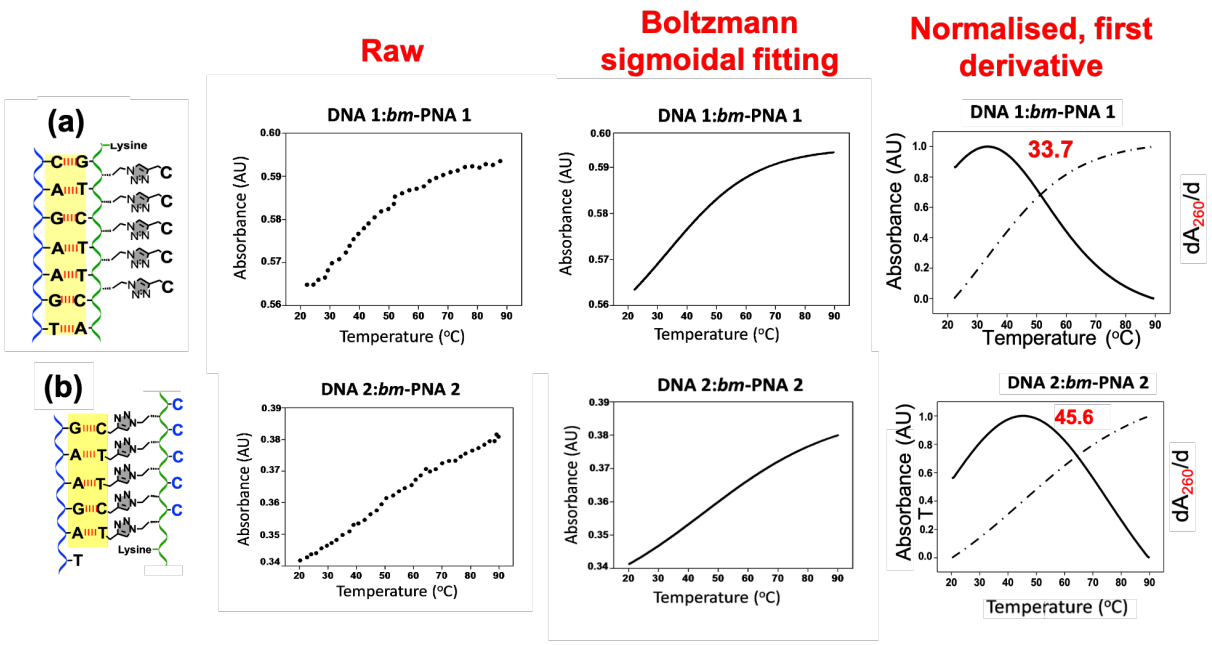


Figure S16. Comparison of raw data, smoothened and normalised first derivative UV-T plots along with cartoon structures of DNA:*bm*-PNA hybrids (a) DNA **1**:*bm*-PNA **1** and (b) DNA **2**:*bm*-PNA **2**

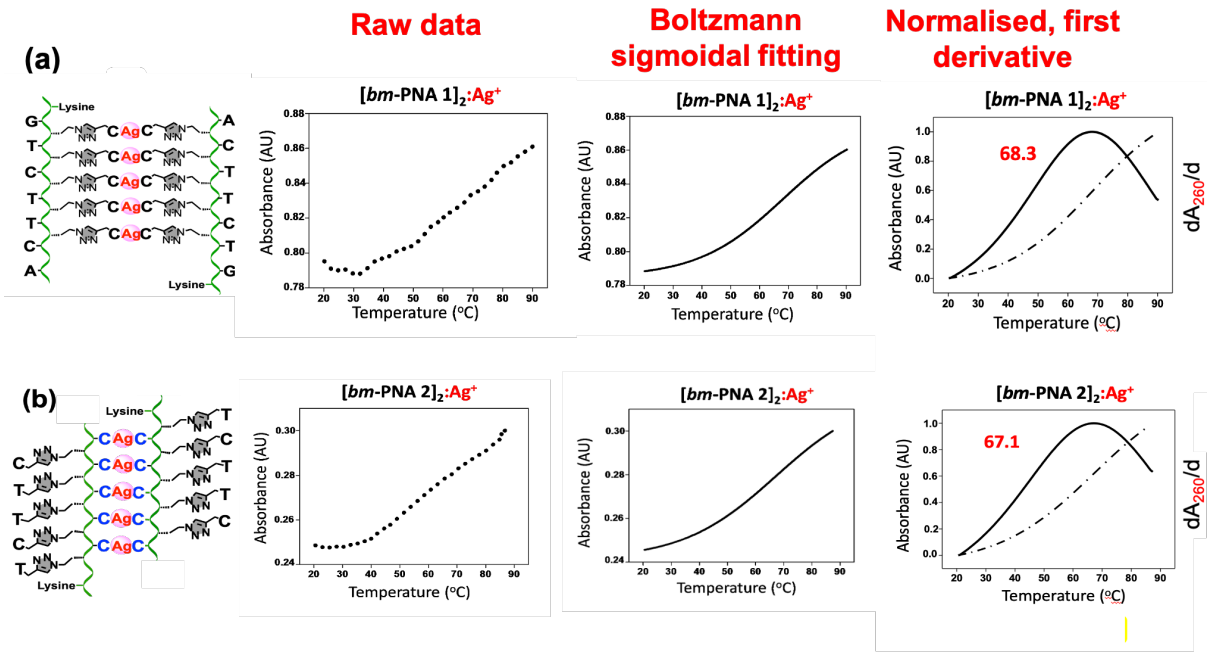


Figure S17. Comparison of raw data, smoothened and normalised first derivative UV-T plots along with cartoon structures of silver complexes (a) [*bm*-PNA **1**]$_2$**:** Ag^+ and (b) [*bm*-PNA **2**]$_2$**:**Ag^+

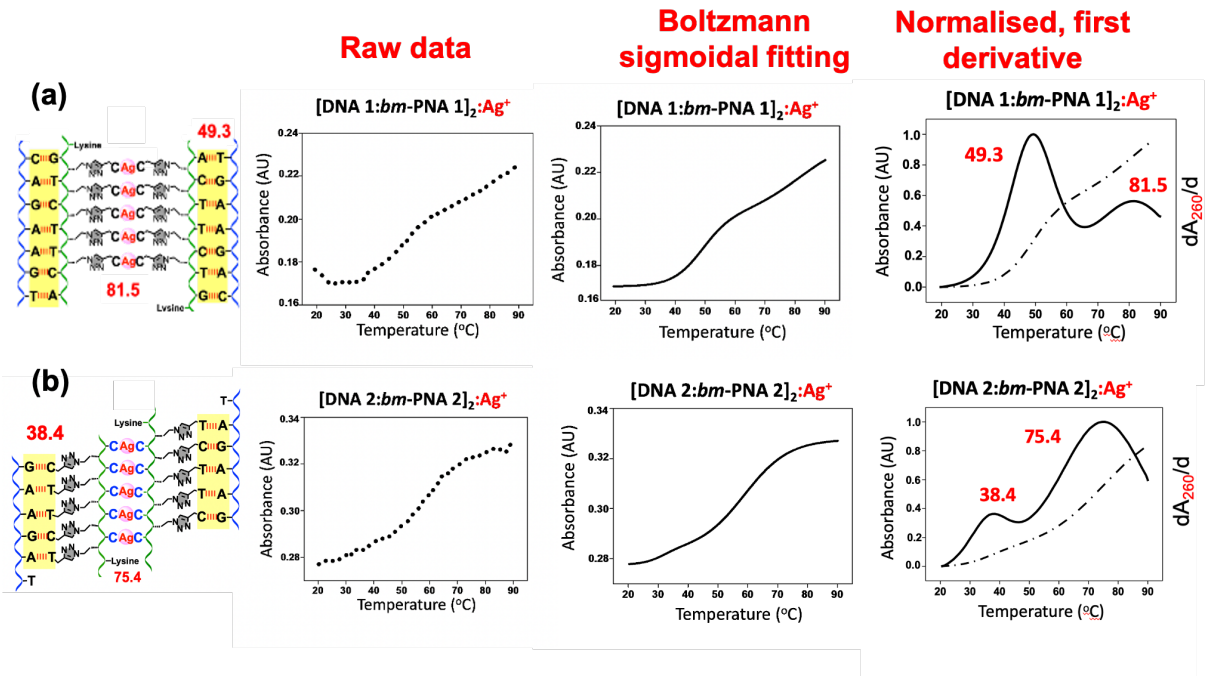


Figure S18. Comparison of raw data, smoothened and normalised first derivative UV-T plots along with cartoon structures of triple duplex silver complexes (a) [DNA **1**:*bm*-PNA **1**]$_2$**:** Ag^+ and (b) [DNA **2**:*bm*-PNA **2**]$_2$**:**Ag^+

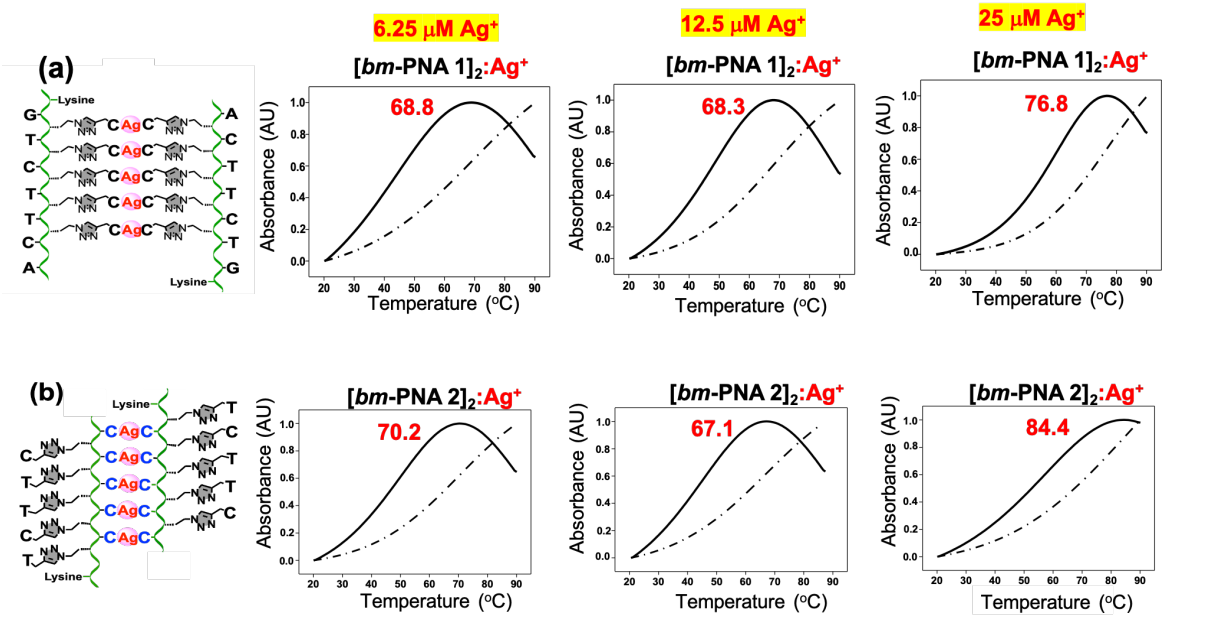


Figure S19. UV-T plots and T_ms of silver complexes and (a) [*bm*-PNA **1**]$_2$**:**Ag^+ and (b) [*bm*-PNA **2**]$_2$**:**Ag^+ at different concentrations of $AgNO_3$ uM). The buffer conditions: DNA/*bm*-PNA (2.5 μM), MOPS (10 mM, pH 7.0), $NaNO_3$ (10 mM) in presence of $AgNO_3$ 6.25 uM, 12.5 uM and 25.0 uM.

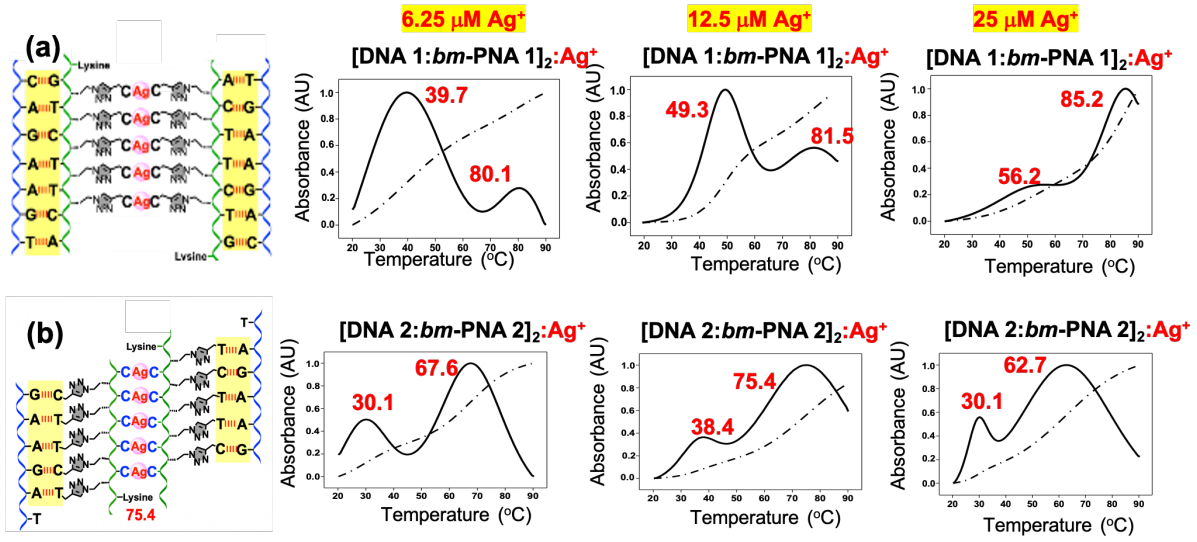


Figure S20. UV-T plots and T_ms of silver complexes (a) [DNA **1**:*bm*-PNA **1**]$_2$**:**Ag^+ and (b) [DNA **2**:*bm*-PNA **2**]$_2$**:**Ag^+ at different concentrations of $AgNO_3$. The buffer conditions: DNA/*bm*-PNA (2.5 μM), MOPS (10 mM, pH 7.0), $NaNO_3$ (10 mM) in presence of $AgNO_3$ 6.25 uM, 12.5 uM and 25.0 uM.

References

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