Electronic Supplementary Information (ESI)

Nucleic Acid Binding Properties of Thyminyl and Adeninyl Pyrrolidine–Amide Oligonucleotide Mimics (POM)

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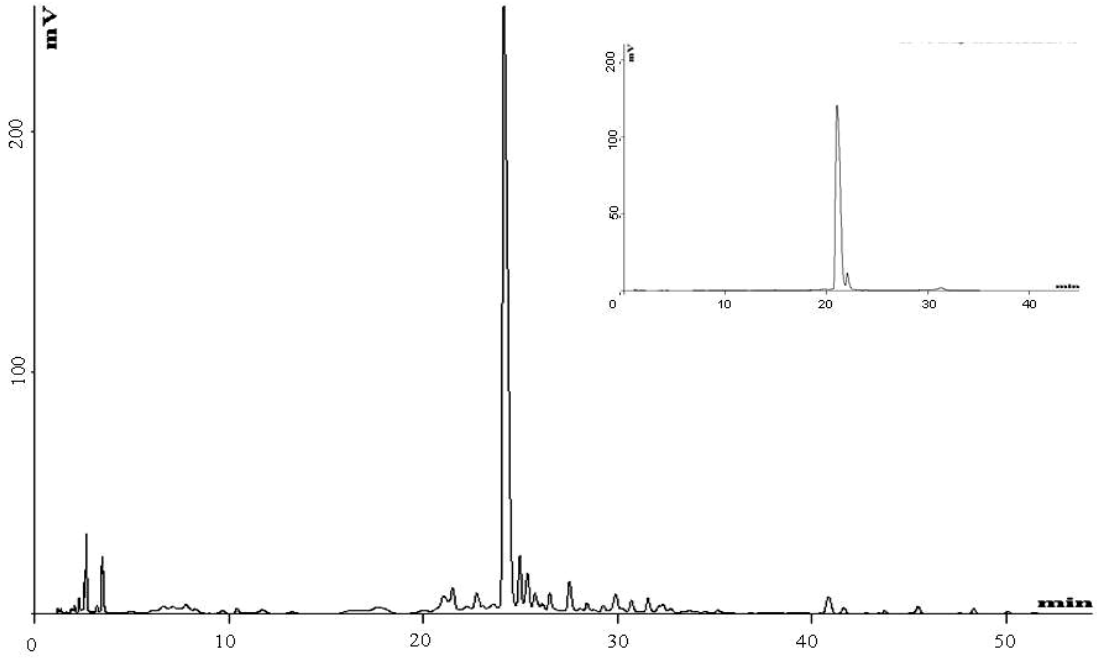

Figure 1. Reverse-phase HPLC chromatogram of Lys-POM(T)$_5$-LysNH$_2$: Kromasil C8, 3.5 u (250 x 4.6 mm) column; solvent A, 0.1% formic acid in H_2O; solvent B, CH_3CN; Flow rate 1 mLmin^{-1}; 100% A for 5min. then a gradient elution of 0% to 5% B in A over 30 min. Inset: HPLC chromatogram of pure Lys-POM(T)$_5$-LysNH$_2$.

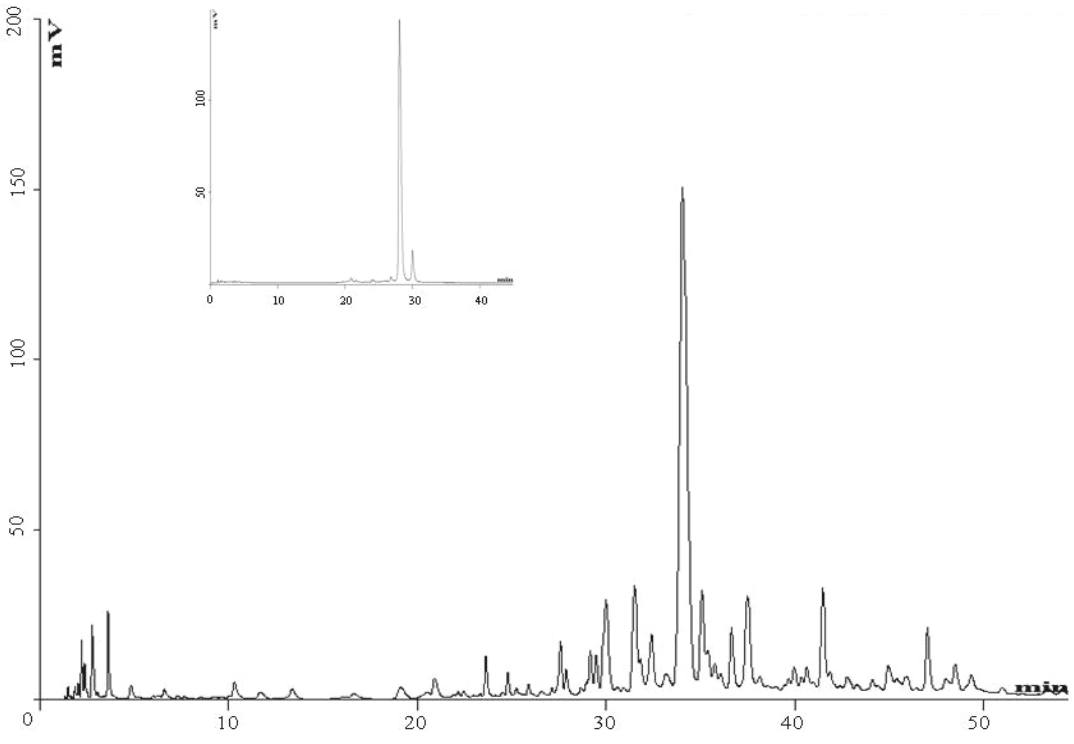

Figure 2. Reverse-phase HPLC chromatogram of crude Lys-POM(A)$_5$-NH$_2$ after on-resin adenyl deprotection. HPLC conditions as above. Inset: HPLC chromatogram of pure Lys-POM(A)$_5$-NH$_2$.

Table 1. Mass to charge ratios observed by ES-MS for POM oligomers.

POM	Observed *m/z*	Required *m/z*	Corresponding peaks
	819.9	819.9	$[M+2Na]^{2+}$
	808.9	808.9	$[M+H+Na]^{2+}$
Lys-POM(T)$_5$-LysNH$_2$	797.9	797.9	$[M+2H]^{2+}$
$C_{72}H_{107}N_{25}O_{17}$	545.3	544.9	$[M+2H+K]^{3+}$
MW: 1593.83	532.3	532.3	$[M+3H]^{3+}$
	409.7	410.4	$[M+2H+2Na]^{4+}$
	344.6	344.6	$[M+4Na+K]^{5+}$
	778.8	778.4	$[M+2Na]^{2+}$
	767.4	767.4	$[M+H+Na]^{2+}$
	756.8	756.4	$[M+2H]^{2+}$
Lys-POM(A)$_5$-NH$_2$	504.6	504.6	$[M+3H]^{3+}$
$C_{66}H_{90}N_{38}O_6$	517.5	517.3	$[M+2H+K]^{3+}$
MW: 1510.79	388.9	388.2	$[M+3H+K]^{4+}$
	378.7	278.7	$[M+4H]^{4+}$
	324.4	323.9	$[M+H+3Na+K]^{5+}$
	316.4	314.2	$[M+2H+3Na]^{5+}$

ESI spectra of all POM oligomers show multiply charged quasi-molecular ions (e.g. $[M+zH]^{z+}$) and exhibit the expected molecular ion charged state distribution. The reason for observing this distribution pattern is because of the relatively little fragmentation caused by the electrospray ionisation technique. The width of the distribution and most abundant multiply charged state is usually at about half the value of the highest charge state.[1] That is to say the total number of charged state possible for Lys-POM(T)$_5$-LysNH$_2$ is 8+ and thus the most abundant charged state for this oligomer should be about 4+ (Figure 3, *m/z* $[M+2H+2Na]^{4+}$: 409.7). The most abundant charged states observed for POM oligomers by ES-MS are hence in good agreement with the total number of sites possible for protonation under acidic conditions. $^{13}C/^{12}C$ isotopic relationships of Lys-POM(T)$_5$-LysNH$_2$ is illustrated in Figure 3. The separation of 0.5 amu by $[M+2H]^{2+}$ and $[M+H+Na]^{2+}$ ion peaks, 0.33 amu by $[M+3H]^{3+}$ ion peaks and 0.25 amu by $[M+2H+2Na]^{4+}$ ion peaks agrees with the actual charge count further supporting the identity of this oligomer.

[1] R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga, H. R Udseth,. *Anal. Chem.* **1990**, *62*, 882-899.

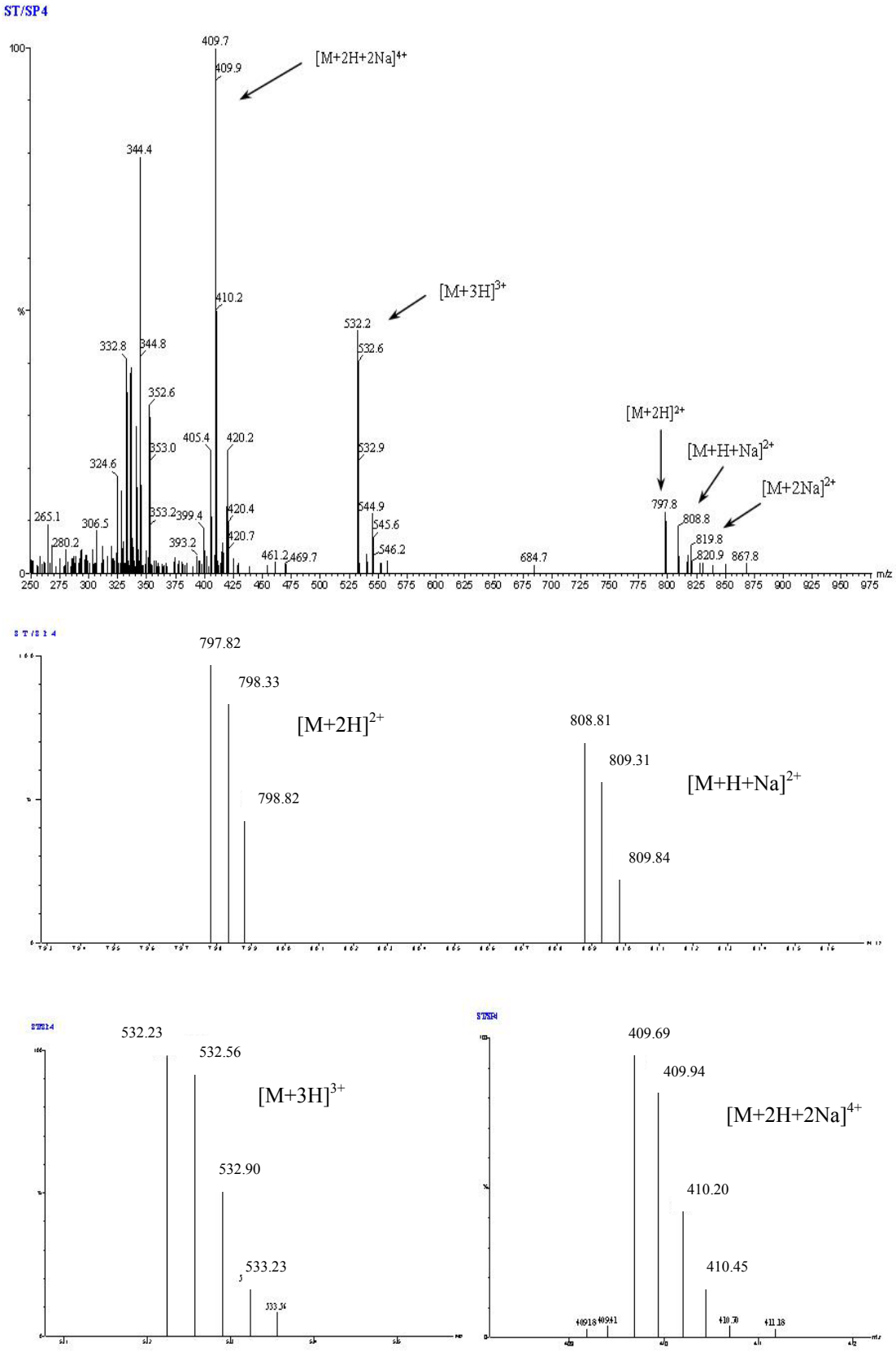

Figure 3. ES-MS spectra of Lys-POM(T)$_5$-LysNH$_2$ and expansion of peak clusters for doubly, triply and quadruply charged regions

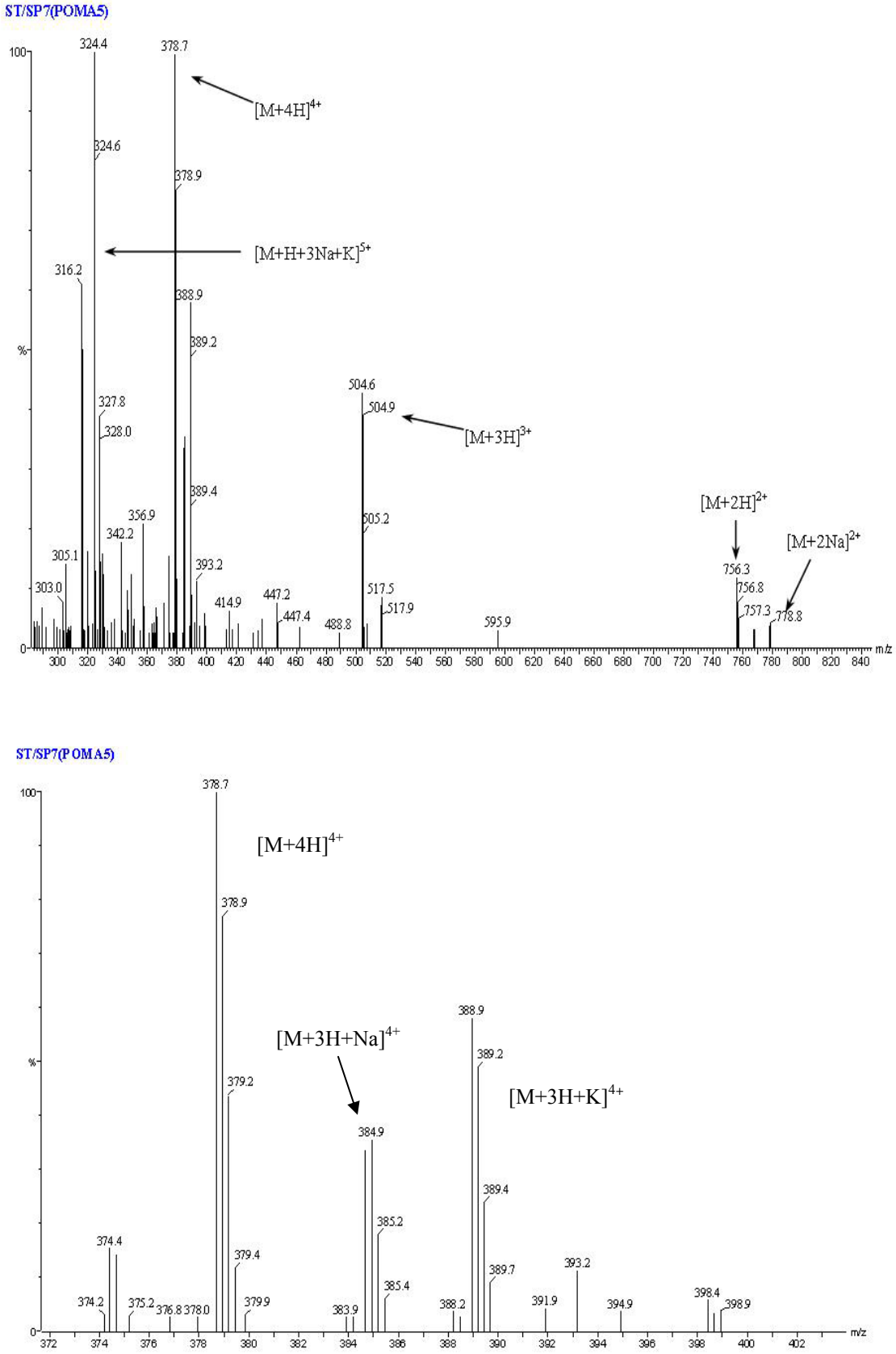

Figure 4. ES-MS spectra of Lys-POM$(A)_5$-NH_2 and expansion of peak clusters for quadruply charged region.

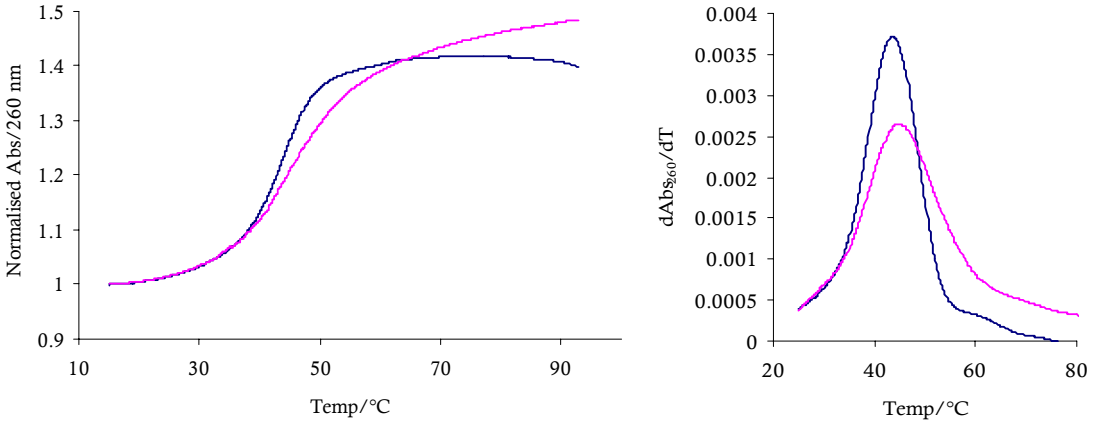

Figure 5. Lys-POM(T)$_5$-LysNH$_2$ *vs.* poly(rA) at 42 μM in bases for each strand, 10 mM K_2HPO_4, 0.12 M K^+ and pH 7.0 (these conditions were used through out). T_m (Renaturation) = 43.6 °C; T_m (Denaturation) = 44.8 °C.

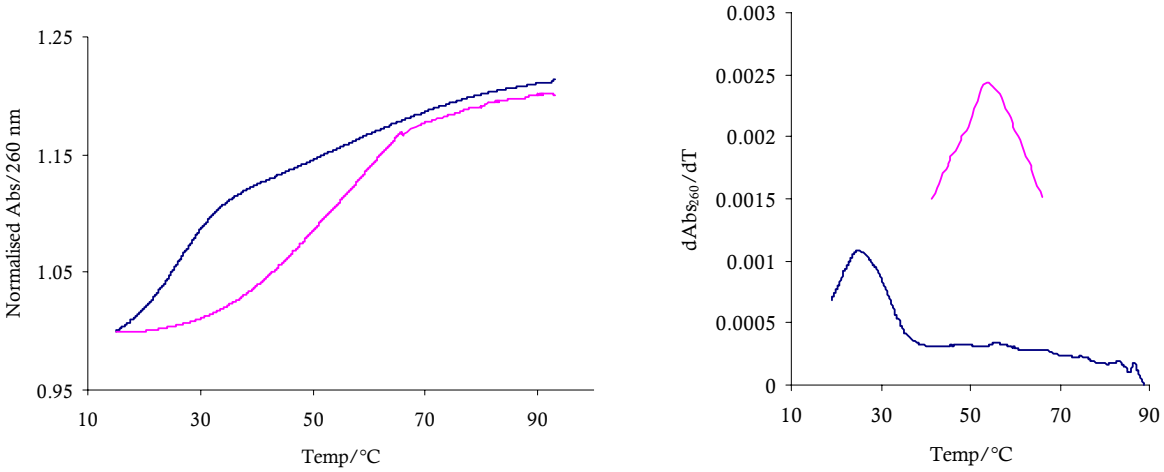

Figure 6. Lys-POM(T)$_5$-Lys NH$_2$ *vs.* poly(dA). T_m (Renaturation) = 25.1 °C; T_m (Denaturation) = 54.4 °C.

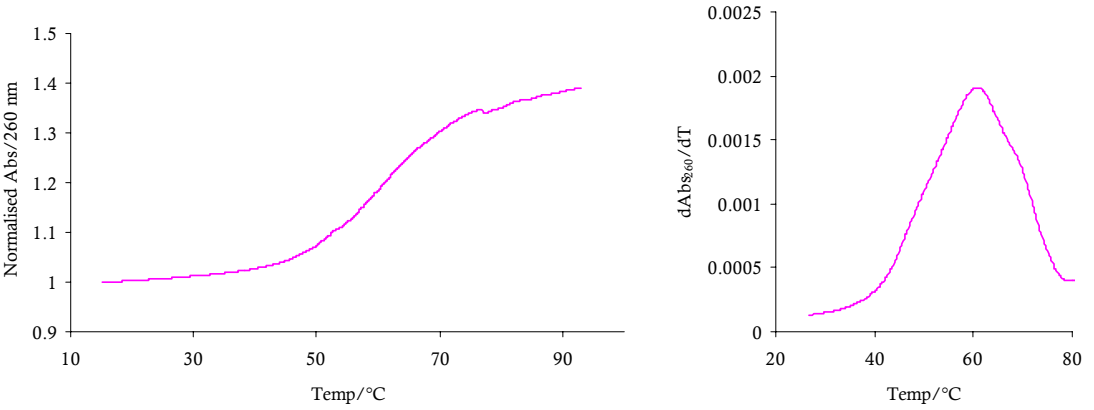

Figure 7. Lys-POM(T)$_5$-Lys NH$_2$ *vs.* poly(dA). T_m (Denaturation) after 48 h incubation = 65.0 °C.

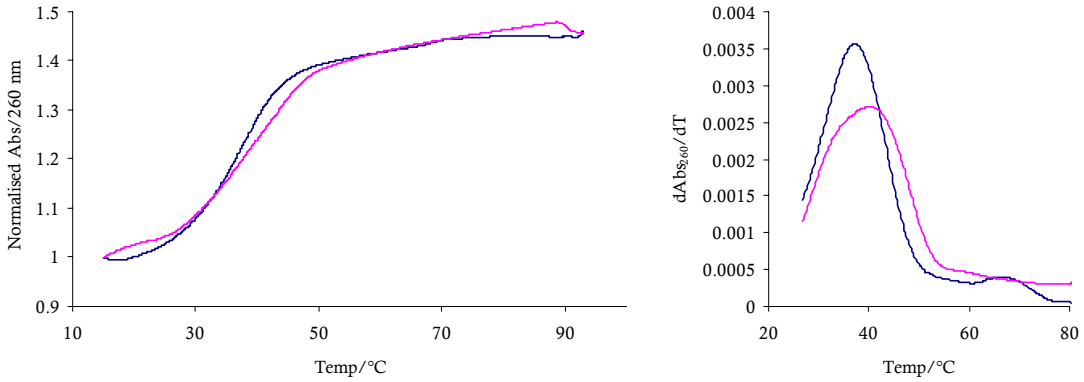

Figure 8. Lys-POM(T)$_5$-Lys NH_2 *vs.* r(A)$_{20}$. T_m (Renaturation) = 37.2 °; T_m (Denaturation) = 40.2 °C.

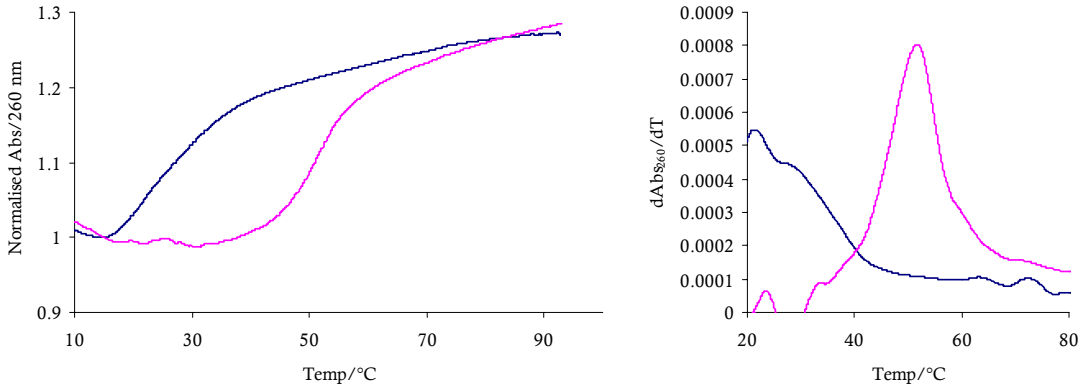

Figure 9. Lys-POM(T)$_5$-LysNH_2 *vs.* d(A)$_{20}$.T_m (Renaturation) = 51.6 °C; T_m (Denaturation) = 21.3 °C.

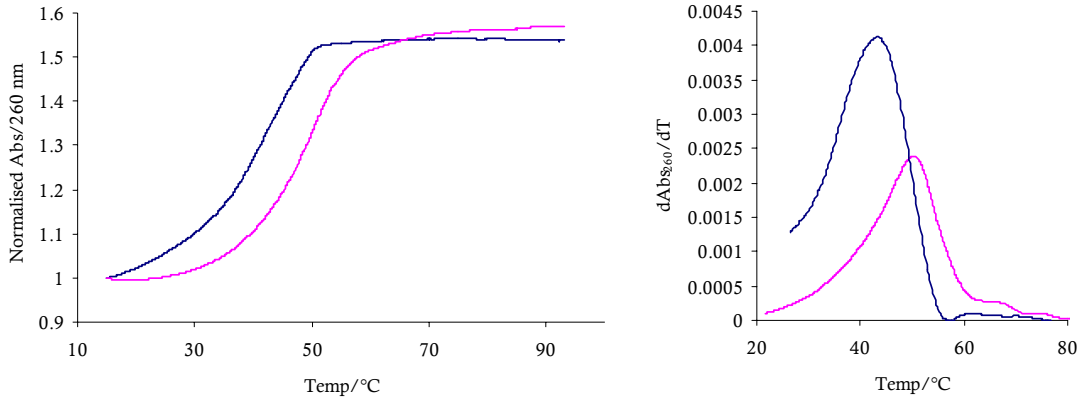

Figure 10. Lys-POM(A)$_5$-NH_2 *vs.* poly(rU). T_m (Renaturation) = 43.3 °C; T_m (Denaturation) =50.2 °C

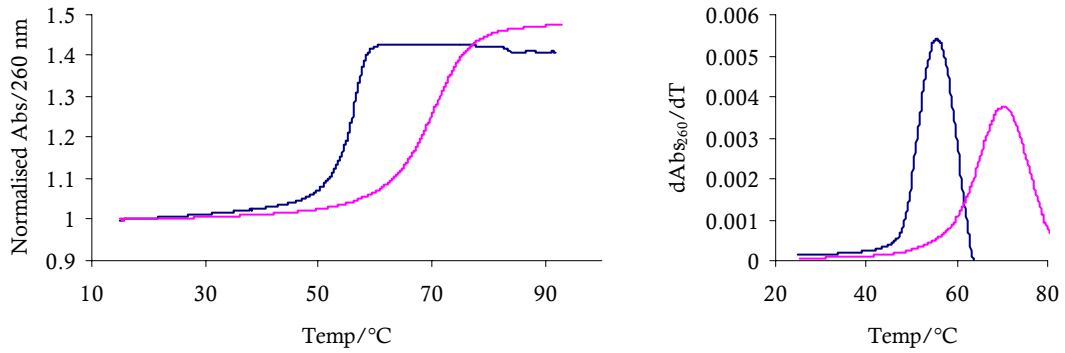

Figure 11. Lys-POM(A)$_5$-NH$_2$ *vs.* poly(dT). T_m (Renaturation) = 55.6 °C; T_m (Denaturation) = 70.3 °C.

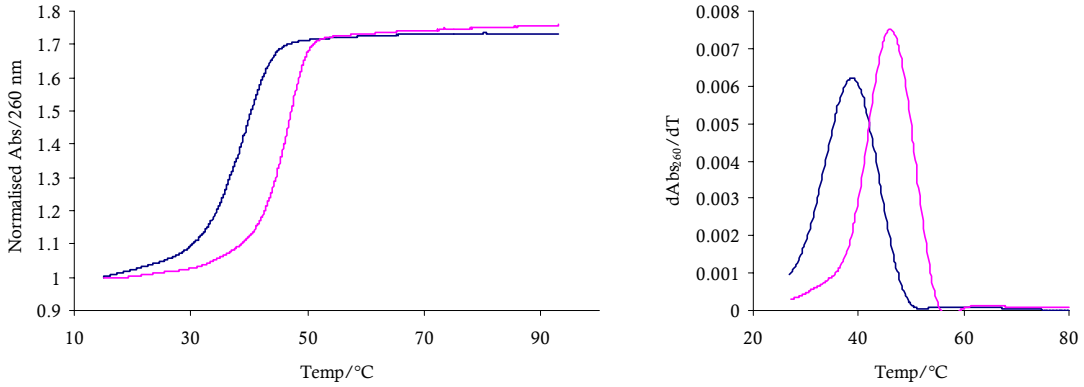

Figure 12. Lys-POM(A)$_5$-NH$_2$ *vs.* r(U)$_{20}$. T_m (Renaturation) = 38.8 °C; T_m (Denaturation) = 46.0 °C.

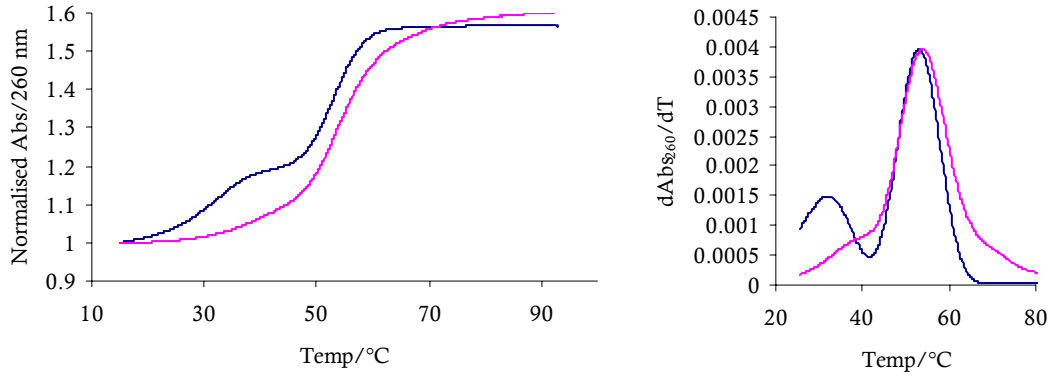

Figure13. Lys-POM(A)$_5$-NH$_2$ *vs.* d(T)$_{20}$. T_m (Renaturation) = 31.8 & 53.0 °C; T_m (Denaturation) = 54.0 °C.

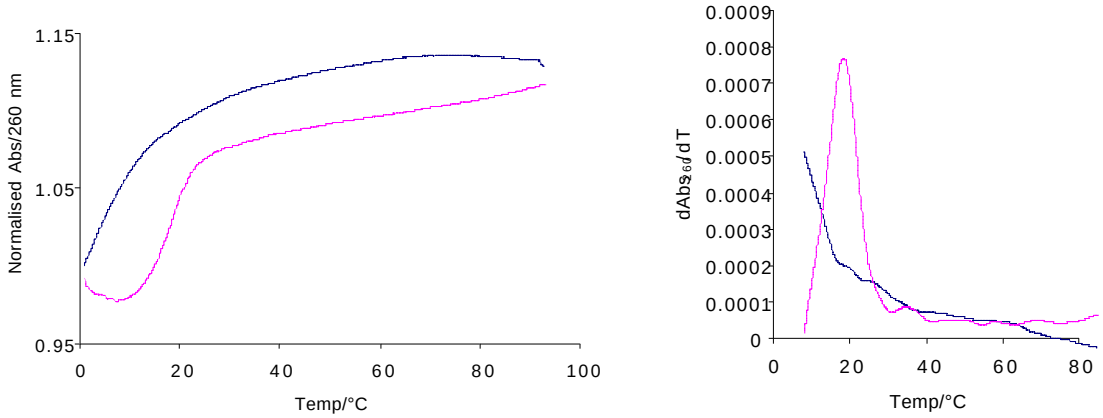

Figure 11 Lys-POM(A)$_5$-NH$_2$ *vs*. r(U)$_5$. T_m (Renaturation) <10 °C; T_m (Denaturation) = 18.3 °C

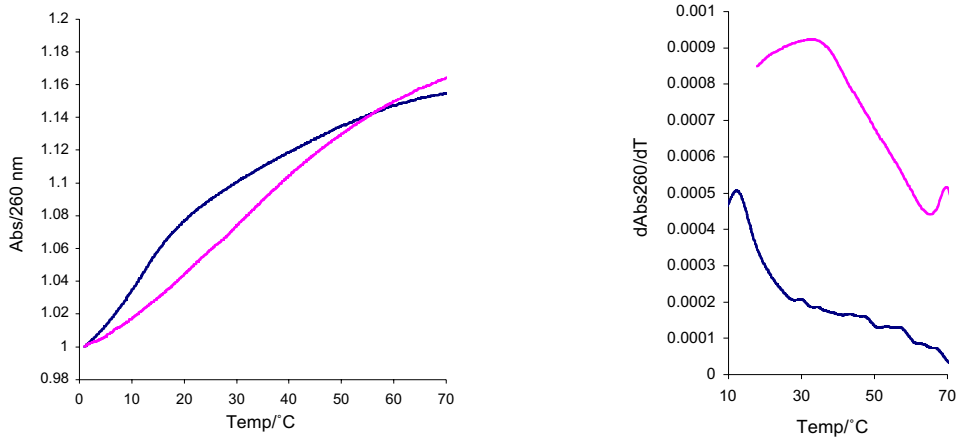

Figure 12 Lys-POM(T)$_5$-LysNH$_2$ *vs*. r(A)$_5$. T_m (Renaturation) 13.6 °C; T_m (Denaturation) = 34.5 °C

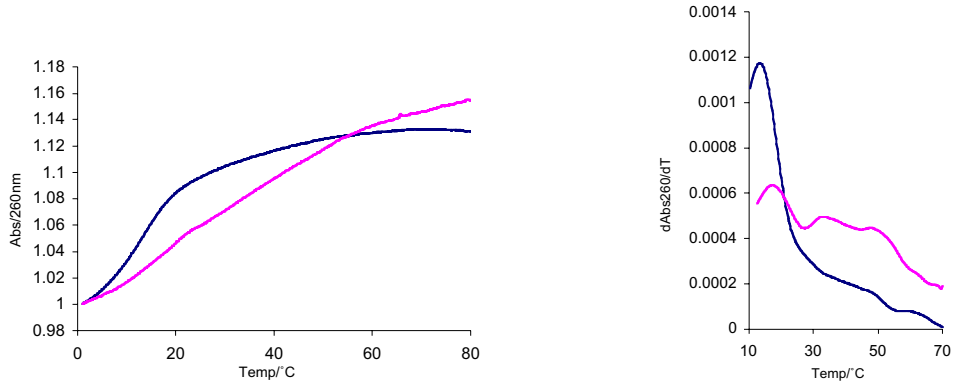

Figure 13 Lys-POM(T)$_5$-LysNH$_2$ *vs*. r(AAGAA). T_m (Renaturation) 14.2 °C; T_m (Denaturation) = 18.2 °C.

	T_m/˚C (% hypochromic/hyperchromic shift)	
	$d(T)_{20}$	
	cooling	heating
Poly(rA)	43.3 (29)	43.7 (31)
Poly(dA)	48.4 (30)	48.8 (32)
$r(A)_{20}$	40.6 (25)	41.2 (26)
$d(A)_{20}$	46.9 (23)	47.5 (30)
	$d(A)_{20}$	
	cooling	heating
Poly(rU)	34.5 (24)	35.0 (24)
Poly(dT)	49.6 (30)	50.1 (31)
$r(U)_{20}$	20.3 (25)	21.3 (26)
$d(T)_{20}$	46.9 (29)	47.5 (31)
$r(U)_5$	n.b.	n.b.

Table 2. T_ms for DNA 20mers, $d(T)_{20}$ and $d(A)_{20}$, with complementary DNA and RNA. Experiments were carried out as described in table 1 of the main manuscript.