DNA-templated borononucleic acids self assembly : A study of minimal complexity

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ELECTRONIC SUPPLEMENTARY INFORMATION

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General

All reagents were purchased from Aldrich or local suppliers and used without purification. All unmodified oligonucleotides used for this study were purchased from Eurogentec. Synthesized 5’ borono-oligonucleotides were purified by RP-HPLC (Dionex Ultimate 3000) with a Nucleodur 100-7 C18 column (125 x 8 mm; Macherey-Nagel) and analyzed with a Nucleodur 100-3 C18 column (75 x 4.6 mm; Macherey-Nagel) and by MALDI-TOF MS (Voyager PerSeptive Biosystems) using trihydroxyacetophenone (THAP) as matrix and ammonium citrate as co-matrix. Thermal denaturation experiments were performed on a VARIAN Cary 300 UV spectrophotometer equipped with a Peltier temperature controller and a thermal analysis software.

Syntheses of 5’ boronooligonucleotides

Syntheses were performed in 1μmol scale using an ABI 381A DNA synthesizer by phosphoramidite chemistry with conditions described in Table S1. dT^{bn}-phosphoramidite was synthesized and incorporated at the 5'-end of an oligonucleotide according to previous records.[1,2]

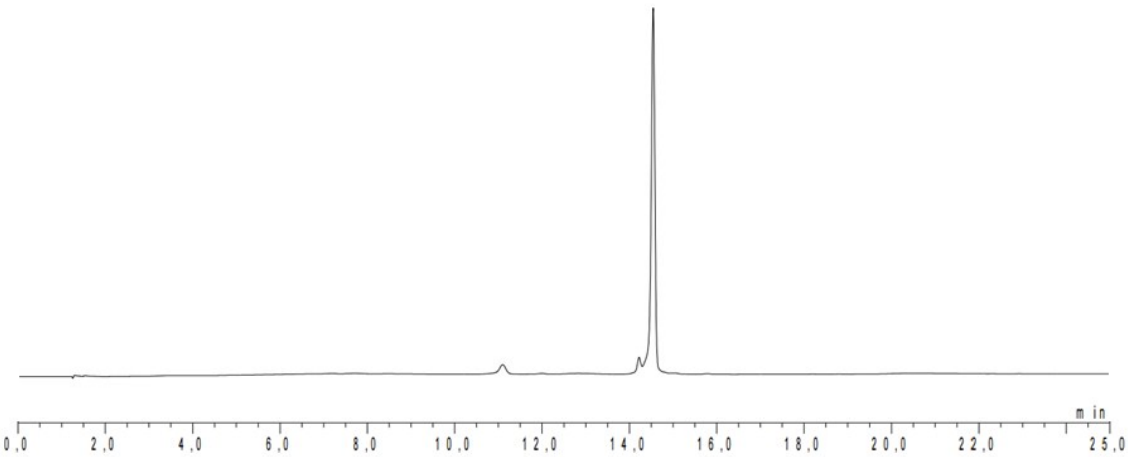
Table S1. Coupling conditions for oligonucleotides syntheses.

Step	Reaction	Reagent	Time (s)
1	Deblocking	3% TCA in DCM	35
2	Coupling	0.1M amidite in CH_3CN + 0.3M BMT in CH3CN	20
3	Capping	Ac_2O/THF/Pyridine + 10% NMI in THF	8
4	Oxidation	0.1M I_2 in THF/H_2O/Pyridine	15

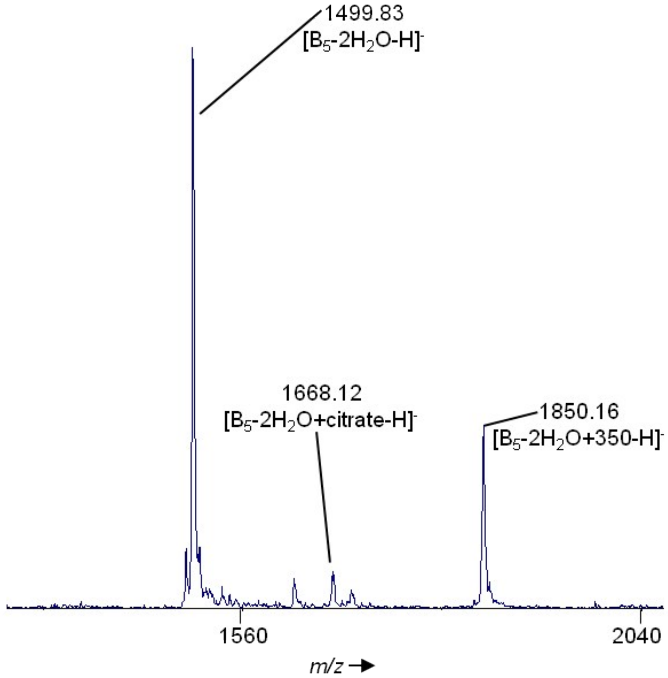
[1] D. Luvino, C. Baraguey, M. Smietana, J. J. Vasseur, *Chem. Commun.* **2008,** 2352.
[2] A. R. Martin, I. Barvik, D. Luvino, M. Smietana, J. J. Vasseur, *Angew. Chem.* **2011,** *50,* 4193.

Analyses of 5' boronooligonucleotides

*HPLC and MALDI-TOF analysis of B_5 5'-T^{bn}ATG**U**-3'*

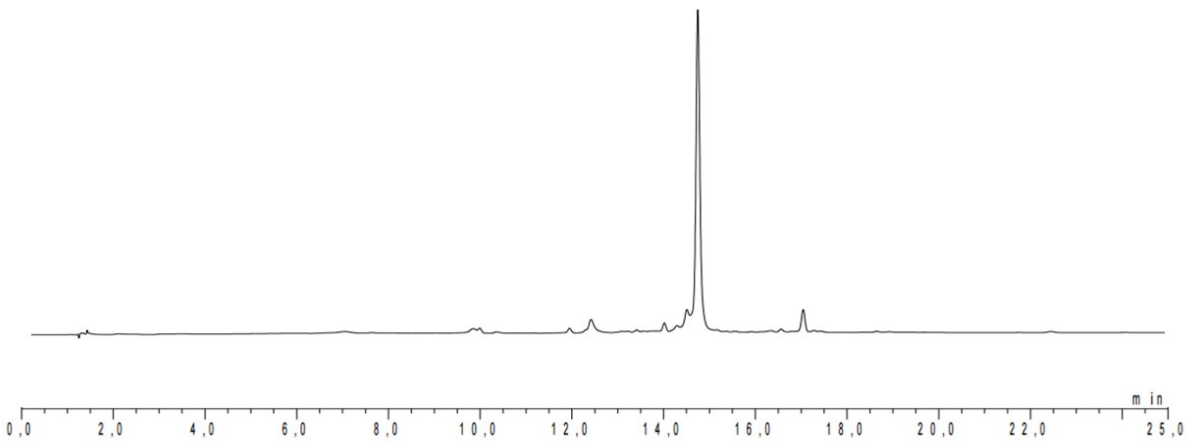


HPLC conditions analysis: Column Nucleodur C18, 100 Å, 3 µm, elution with a linear gradient of 0 to 20% CH_3CN in triethylammonium acetate buffer, pH 7, in 25 min, Flow rate 1 mL.min^{-1}, λ 260 nm.

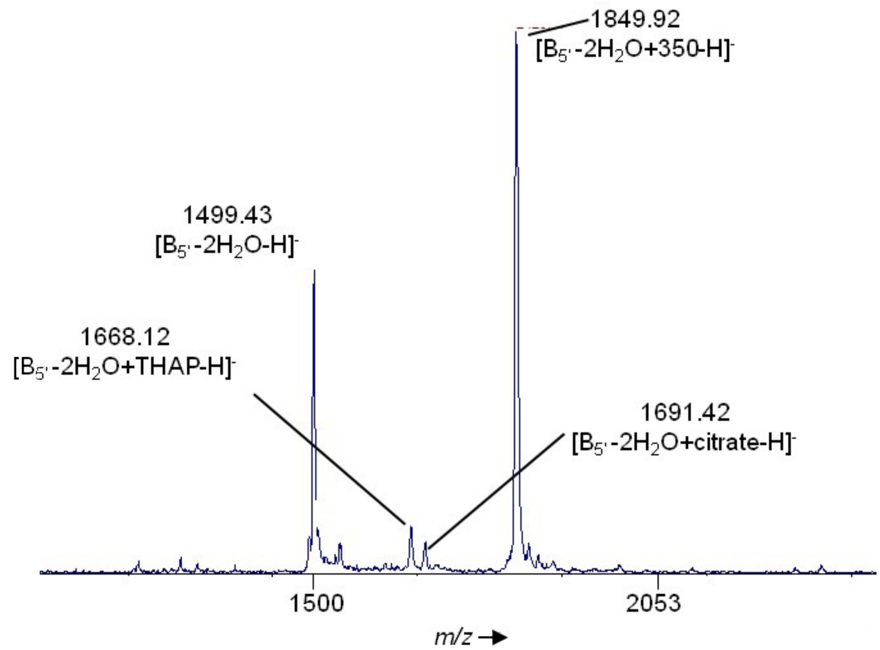


MALDI-TOF MS conditions analysis: ionization in negative mode, THAP (MW= 168.15 g.mol^{-1}) as matrix and ammonium citrate (MW= 243.2 g.mol^{-1}) as co-matrix, delay time 100 ns and an acceleration voltage of 24 kV.

*HPLC and MALDI-TOF analysis of $B_{5'}$ 5'-T^{bn}GTA**U**-3'*

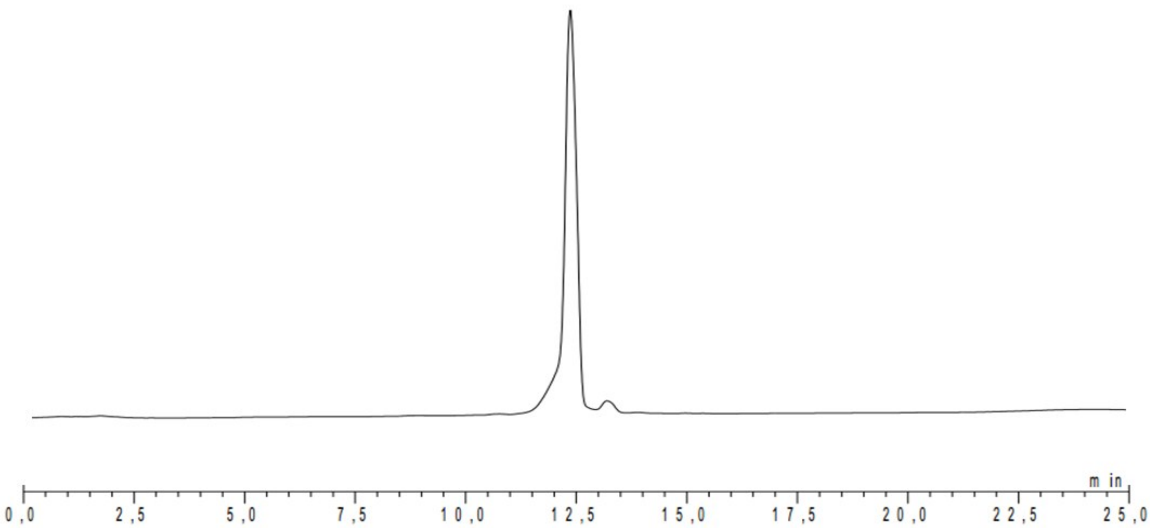


HPLC conditions analysis: Column Nucleodur C18, 100 Å, 3 µm, elution with a linear gradient of 0 to 20% CH_3CN in triethylammonium acetate buffer, pH 7, in 25 min, Flow rate 1 mL.min^{-1}, λ 260 nm.

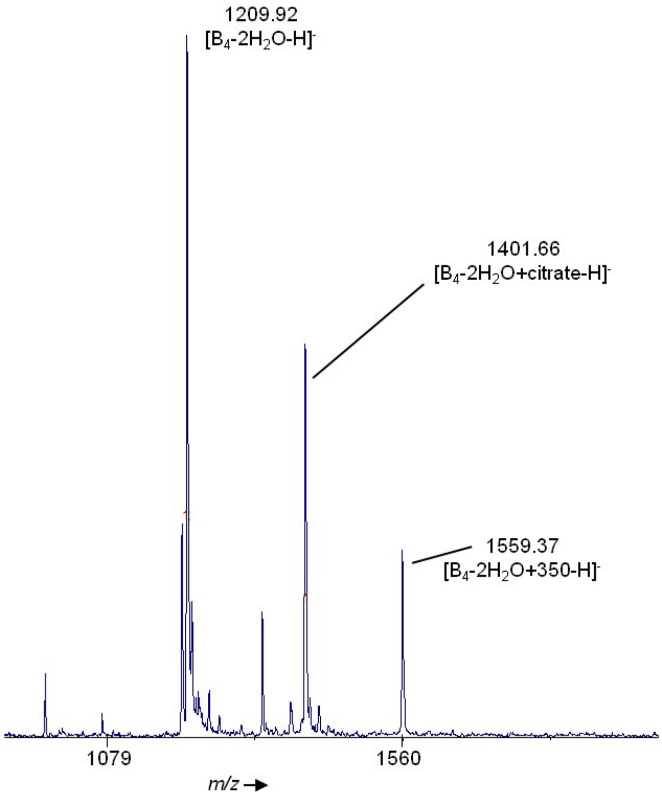


MALDI-TOF MS conditions analysis: ionization in negative mode THAP (MW= 168.15 g.mol^{-1}) as matrix and ammonium citrate (MW= 243.2 g.mol^{-1}) as co-matrix, delay time 100 ns and an acceleration voltage of 24 kV.

*HPLC and MALDI-TOF analysis of B_4 5'-T^{bn}GT**A**-3'*

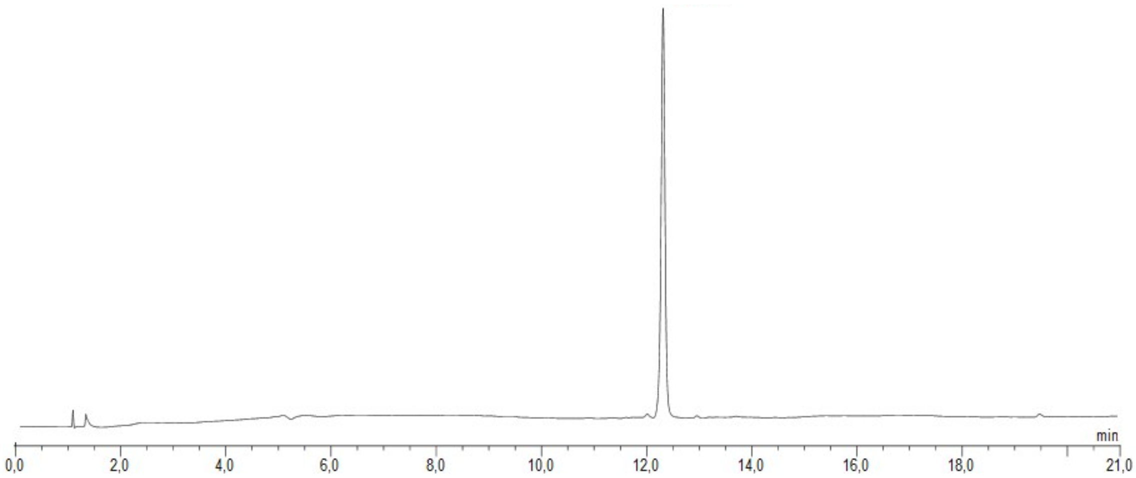


HPLC conditions analysis: Column Nucleodur C18, 100 Å, 3 µm, elution with a linear gradient of 0 to 25% CH_3CN in triethylammonium acetate buffer, pH 7, in 20min, Flow rate 1 mL.min^{-1}, λ 260 nm.

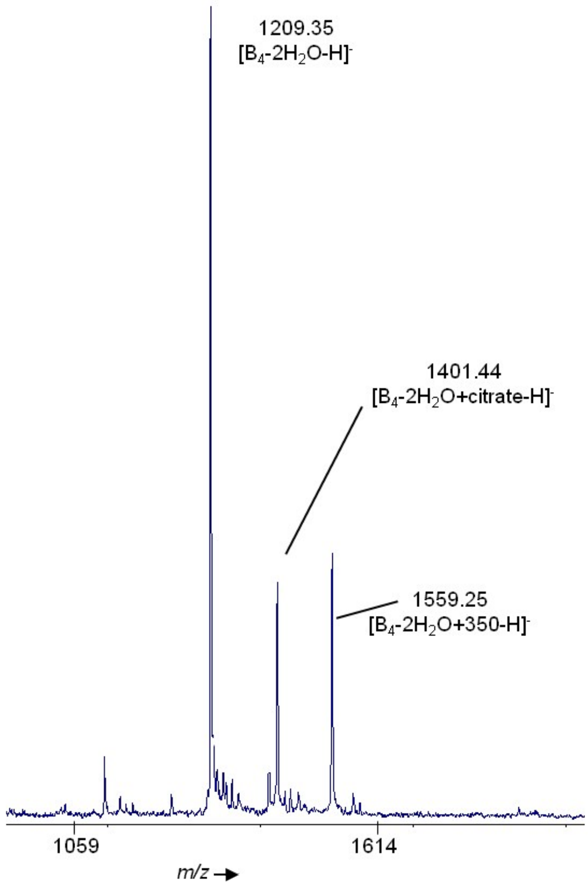


MALDI-TOF MS conditions analysis: ionization in negative mode, THAP (MW= 168.15 g.mol^{-1}) as matrix and ammonium citrate (MW= 243.2 g.mol^{-1}) as co-matrix, delay time 150 ns and an acceleration voltage of 24 kV.

*HPLC and MALDI-TOF analysis of $B_{4'}$ 5'-T^{bn}TG**A**-3'*

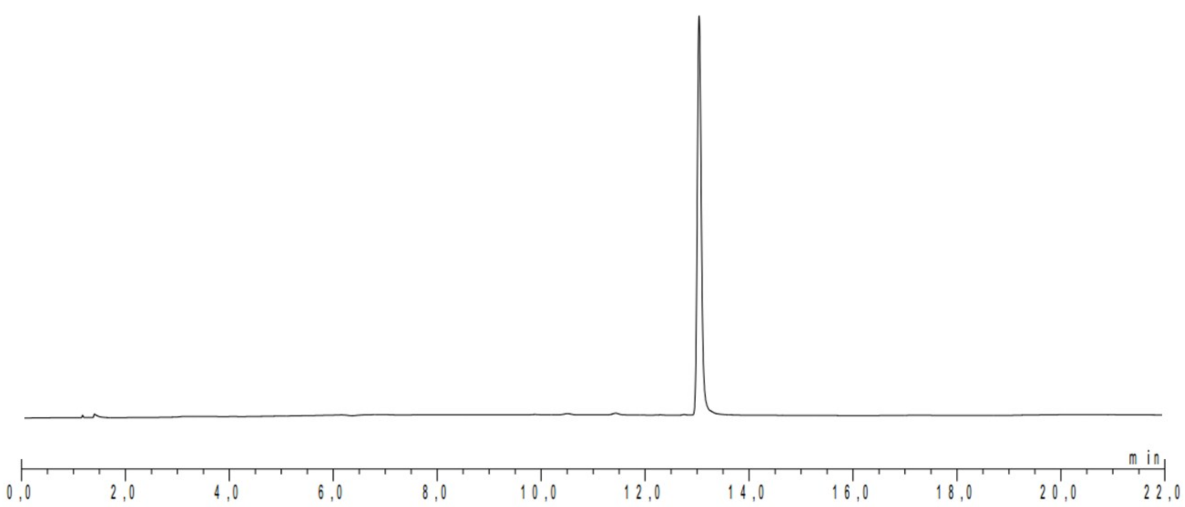


HPLC conditions analysis: Column Nucleodur C18, 100 Å, 3 µm, elution with a linear gradient of 0 to 25% CH_3CN in triethylammonium acetate buffer, pH 7, in 20min, Flow rate 1 mL.min^{-1}, λ 260 nm.

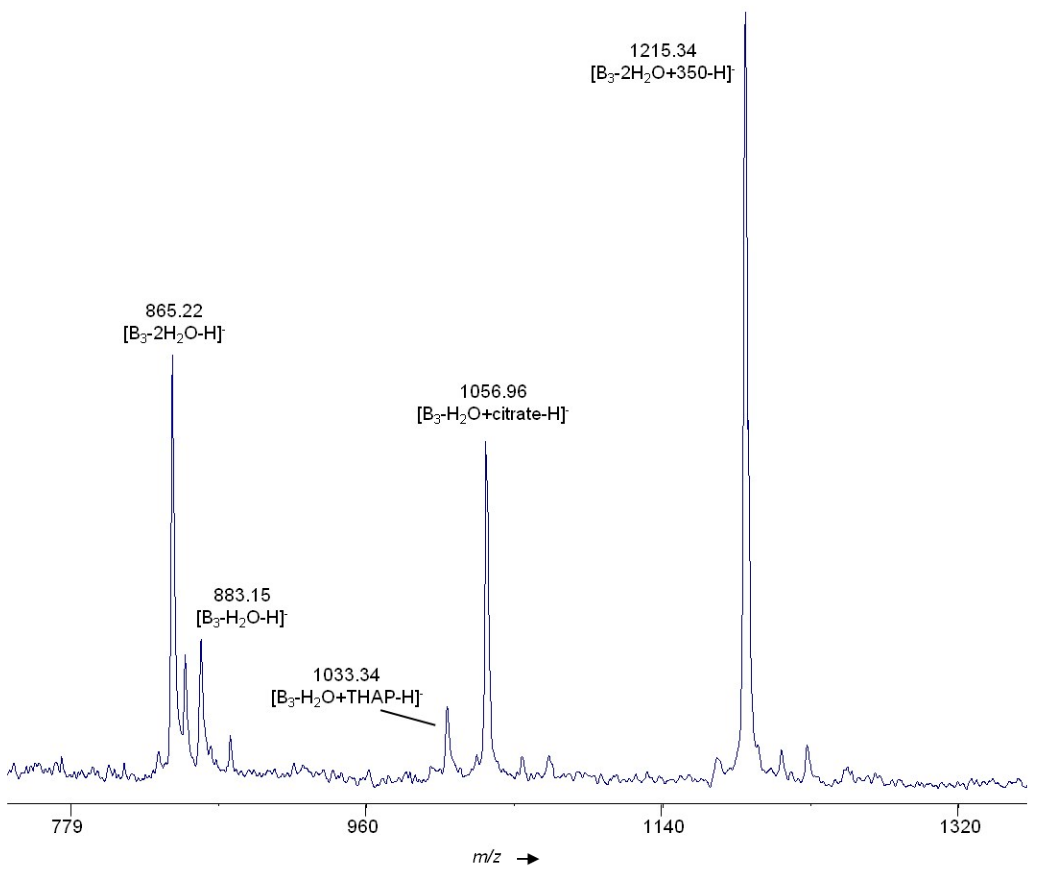


MALDI-TOF MS conditions analysis: ionization in negative mode, THAP (MW= 168.15 g.mol^{-1}) as matrix and ammonium citrate (MW= 243.2 g.mol^{-1}) as co-matrix, delay time 150 ns and an acceleration voltage of 24 kV.

*HPLC and MALDI-TOF analysis of B_3 5'-T^{bn}C**A**-3'*



HPLC conditions analysis: Column Nucleodur C18, 100 Å, 3 µm, elution with a linear gradient of 0 to 16% CH_3CN in triethylammonium acetate buffer, pH 7, in 20min, Flow rate 1 mL.min^{-1}, λ 260 nm.



MALDI-TOF MS conditions analysis: ionization in negative mode, THAP (MW= 168.15 g.mol^{-1}) as matrix and ammonium citrate (MW= 243.2 g.mol^{-1}) as co-matrix, delay time 150 ns and an acceleration voltage of 24 kV.

Denaturation experiments

Unless otherwise stated, the samples were prepared by mixing 3 μM of the template with stoichiometric amounts of complementary strands. Denaturation experiments were performed in a 1M NaCl, 10mM sodium cacodylate buffer at pH 7.5 or 9.5. A heating-cooling-heating cycle in the 0-90°C temperature range with a gradient of 0.5°C/min was applied.

Tm values were determined from the maxima of the first derivative plots of absorbance at 260 nm versus temperature.

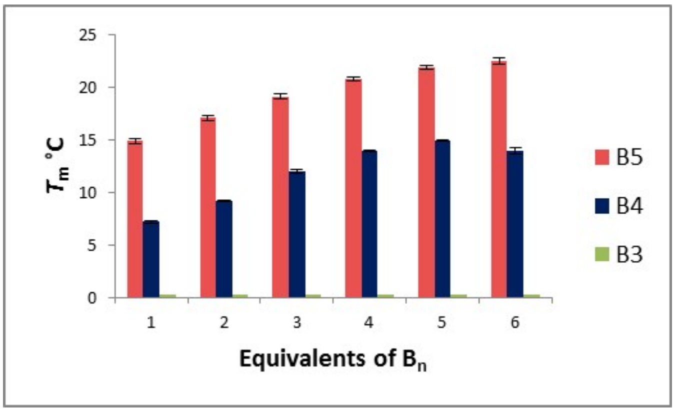
Melting curves and their derivatives

Table S2 : $\boldsymbol{T_m}$ values from Figure 6.

Entry	Bifunctionnal Strand[a]	T_m[b] [°C] according to the excess of B_n					
		1 eq	2 eq	3 eq	4 eq	5 eq	6 eq
1	B_5 T[bn]ATG**U**	14.9 ±0.3	17.1 ±0.3	19.1 ±0.3	20.8 ±0.2	21.9 ±0.2	22.5 ±0.4
2	B_4 T[bn]GT**A**	7.2 ±0.2	9.2 ±0.1	12.0 ±0.2	14.0 ±0.1	14.9 ±0.1	14.0 ±0.3
3	B_3 T[bn]C**A**	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]
4	B_5 with primer	29.0 ±0.3	29.0 ±0.2	29.0 ±0.2	n.d.[d]	n.d.[d]	n.d.[d]
5	B_4 with primer	21.0 ±0.3	25.0 ±0.1	26.0 ±0.1	25.8 ±0.2	n.d.[d]	n.d.[d]
6	B_3 with primer	10.7 ±0.1	13.6 ±0.2	15.3 ±0.4	15.8 ±0.2	n.d.[d]	n.d.[d]

[a] T[bn] refers to boronothymidine and bold letters represent RNA residues. [b] Melting temperatures are obtained from the maxima of the first derivatives of the melting curve (A_{260} vs temperature) recorded in a buffer containing 1 M NaCl and 10 mM of sodium cacodylate, Template concentration 3 μM. Curve fits data were averaged from fits of three denaturation curves. Uncertainties were estimated from standard deviations of experimental melting temperatures. [c] T_m lower than 5 °C. [d] Not determined.

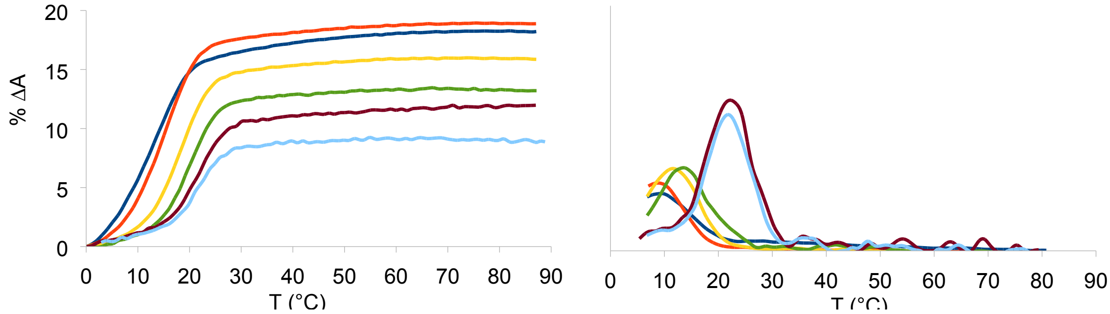
Figure S1. Bar-chart representation of Table 2.



Melting temperatures are obtained from the maxima of the first derivatives of the melting curve (A_{260} *vs* temperature) recorded in a buffer containing 1 M NaCl and 10 mM of sodium cacodylate, Template concentration 3 μM. Curve fits data were averaged from fits of three denaturation curves.

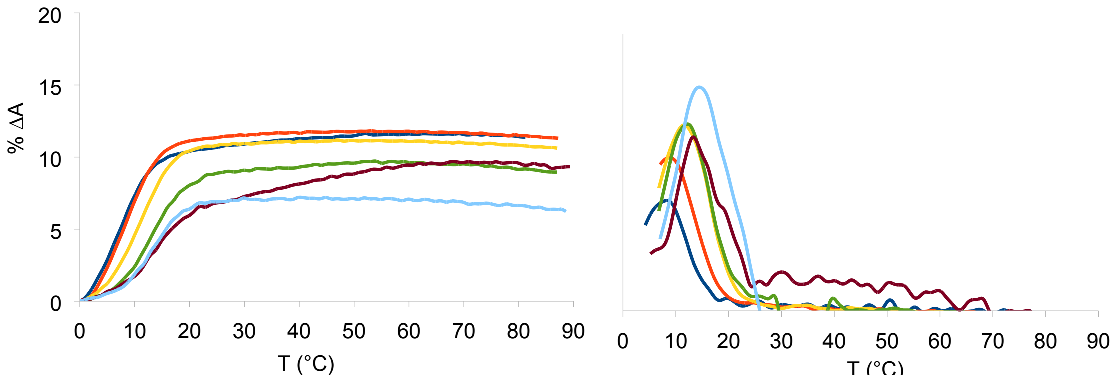
Melting curves and derivatives from Table S2.

Table S2, entry 1 :



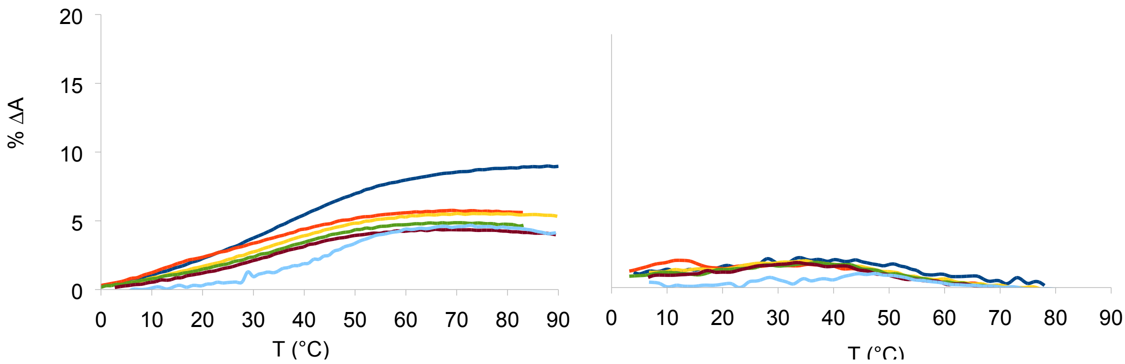
Melting curves and their derivatives at pH 9.5 of the complex 3'-CC(ATACA)$_3$CC with 5'-T^{bn}ATGrU 1eq (blue) ; 2eq (orange) ; 3eq (yellow) ; 4eq (green) ; 5eq (brown) and 6eq (cyan).

Table S2, entry 2 :



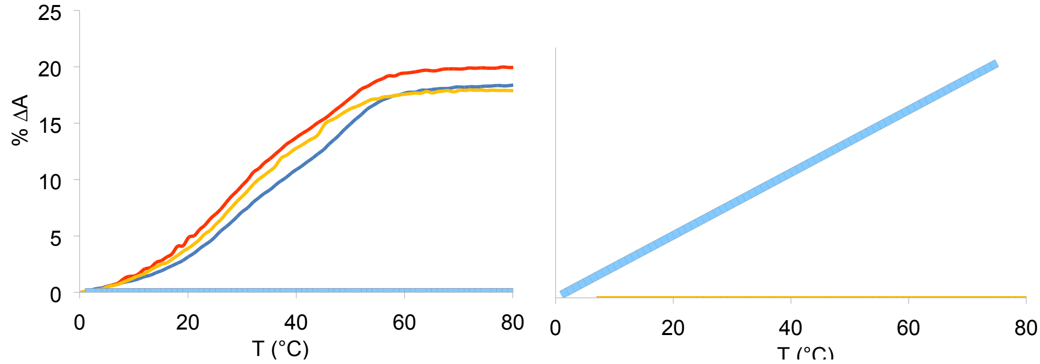
Melting curves and their derivatives at pH 9.5 of the complex 3'-CC(ACAT)$_3$CC with 5'-T^{bn}GTrA 1eq (blue) ; 2eq (orange) ; 3eq (yellow) ; 4eq (green) ; 5eq (brown) and 6eq (cyan).

Table S2, entry 3 :



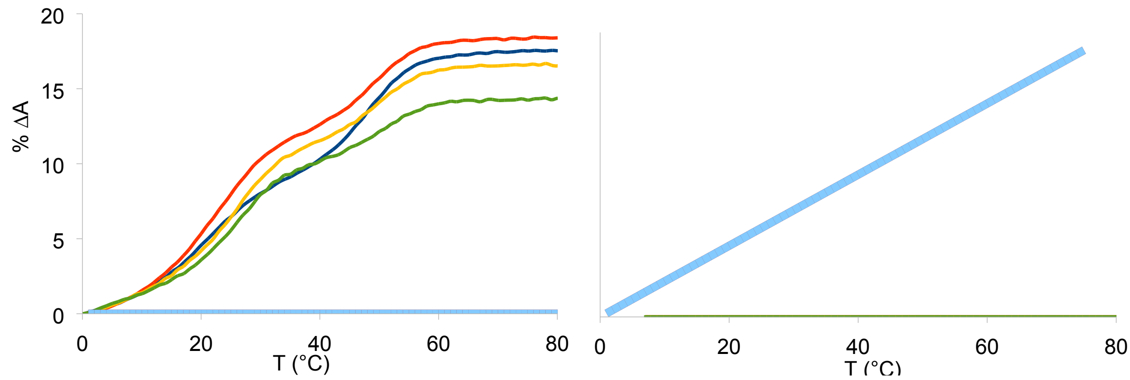
Melting curves and their derivatives at pH 9.5 of the complex 3'-CC(AGT)$_3$CC with 5'-T^{bn}CrA 1eq (blue) ; 2eq (orange) ; 3eq (yellow) ; 4eq (green) ; 5eq (brown) and 6eq (cyan).

Table S2, entry 4 :



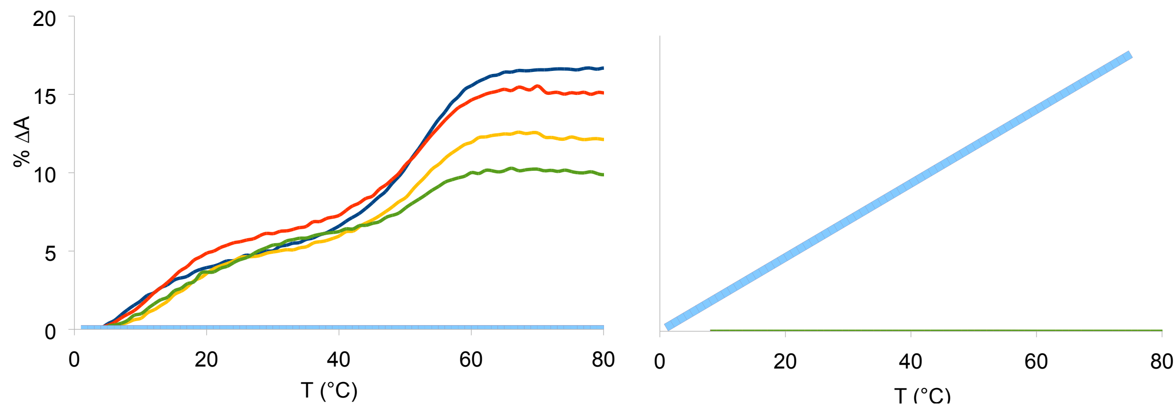
Melting curves and their derivatives at pH 9.5 of the complex 3'-CC(ACATA)$_3$(AGT)$_3$CC/5'-GGTCATCATCrA/5'-T^{bn}ATGrU 1eq (blue) ; 2eq (orange) ; 3eq (yellow).

Table S2, entry 5 :



Melting curves and their derivatives at pH 9.5 of the complex 3'-CC(ACAT)$_3$(AGT)$_3$CC/5'-GGTCATCATCrA/5'-T^{bn}GTrA 1eq (blue) ; 2eq (orange) ; 3eq (yellow) ; 4eq (green).

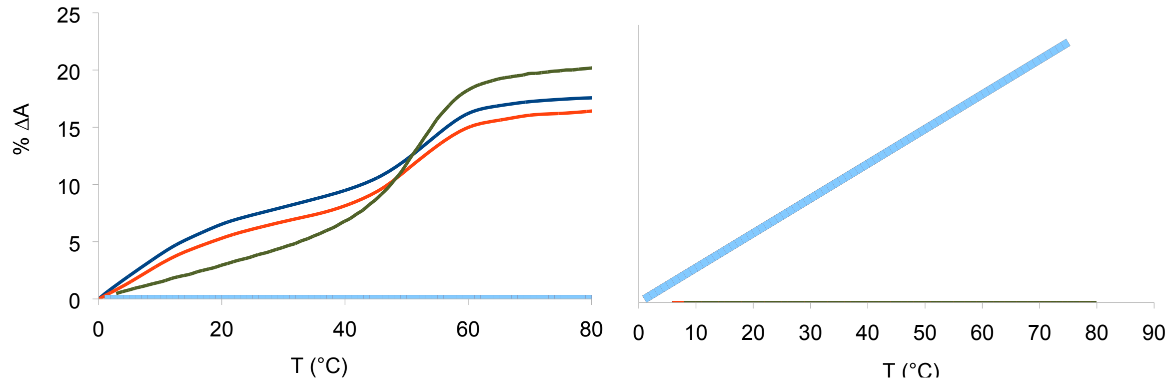
Table S2, entry 6 :



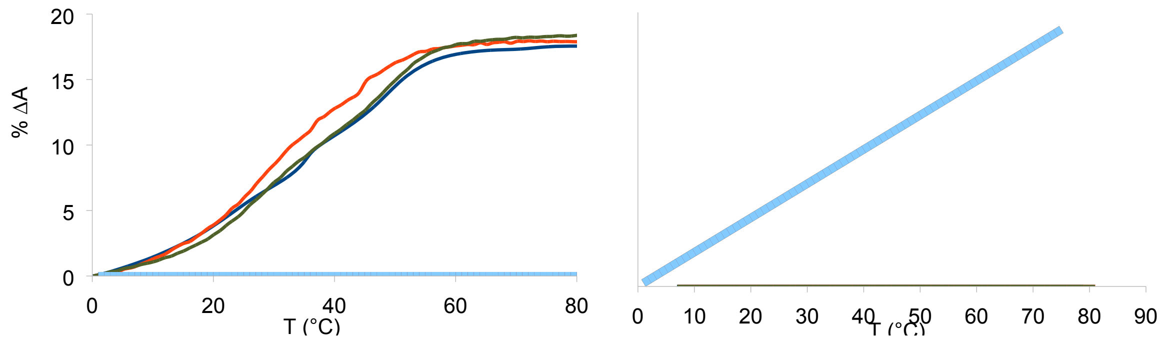
Melting curves and their derivatives at pH 9.5 of the complex 3'-CC(AGT)$_3$(AGT)$_3$CC/5'-GGTCATCATCrA/5'-T^{bn}CrA 1eq (blue) ; 2eq (orange) ; 3eq (yellow) ; 4eq (green).

Melting curves and derivatives from Table 3.

Table 3, entry 1 :

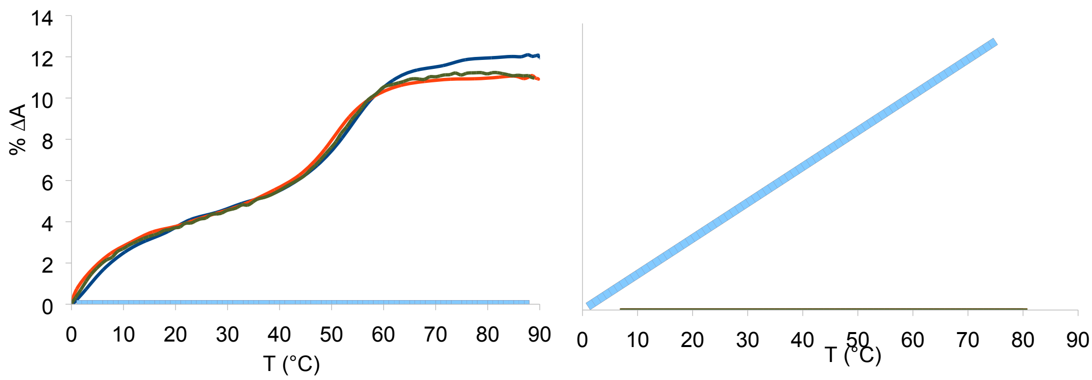


Melting curves and their derivatives of the complex 3'-CC(ATACA)$_3$(AGT)$_3$CC/5'-GGTCATCATCrA/5'-TATGrU at pH 7.5 (blue) ; pH 9.5 (orange) ; pH 7.5 3mM CN^- (green).

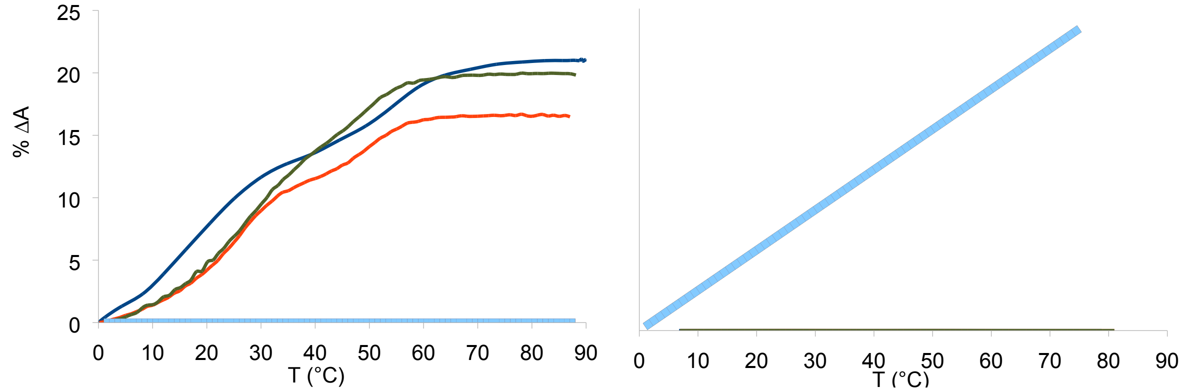


Melting curves and their derivatives of the complex 3'3'-CC(ATACA)$_3$(AGT)$_3$CC/5'-GGTCATCATCrA/5'-T^{bn}ATGrU at pH 7.5 (blue) ; pH 9.5 (orange) ; pH 7.5 3mM CN^- (green).

Table 3, entry 2 :

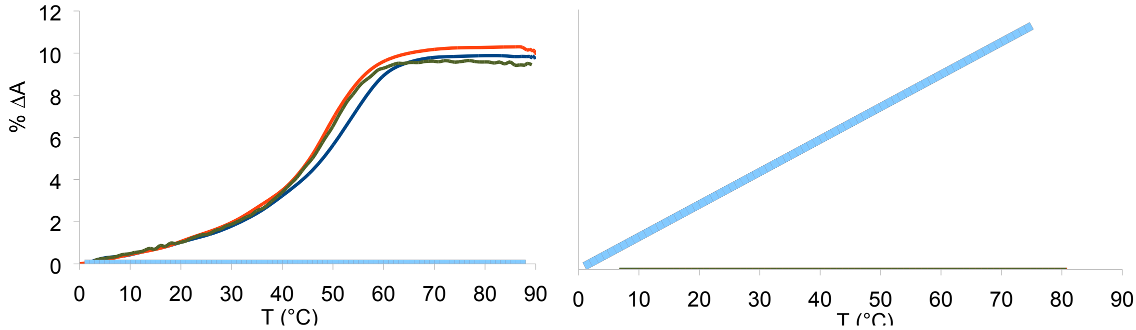


Melting curves and their derivatives of the complex 3'-CC(ACAT)$_3$(AGT)$_3$CC/5'-GGTCATCATCrA/5'-TGTrA at pH 7.5 (blue) ; pH 9.5 (orange) ; pH 7.5 3mM CN^- (green).

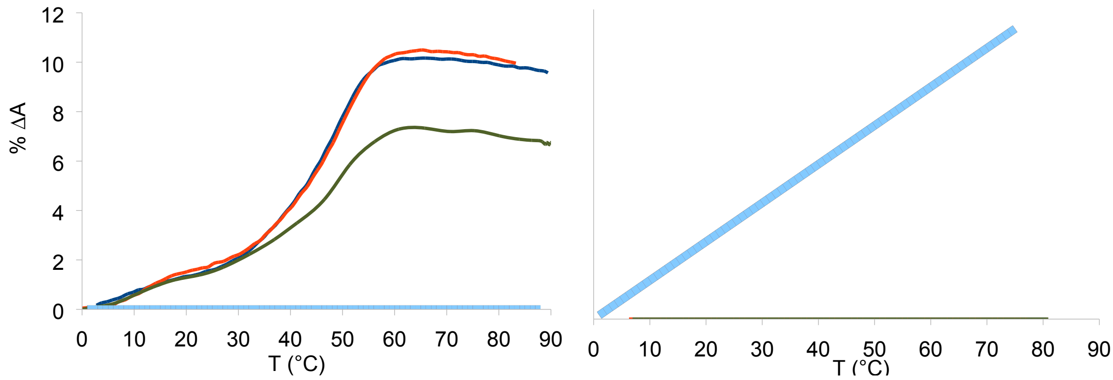


Melting curves and their derivatives of the complex 3'-CC(ACAT)$_3$(AGT)$_3$CC/5'-GGTCATCATCrA/5'-T^{bn}GTrA at pH 7.5 (blue) ; pH 9.5 (orange) ; pH 7.5 3mM CN^- (green).

Table 3, entry 3 :



Melting curves and their derivatives of the complex 3'-CC(AGT)$_6$CC/5'-GGTCATCATCrA/5'-TCrA at pH 7.5 (blue) ; pH 9.5 (orange) ; pH 7.5 3mM CN^- (green).



Melting curves and their derivatives of the complex 3'-CC(AGT)$_6$CC/5'-GGTCATCATCrA/5'-T^{bn}CrA at pH 7.5 (blue) ; pH 9.5 (orange) ; pH 7.5 3mM CN^- (green).

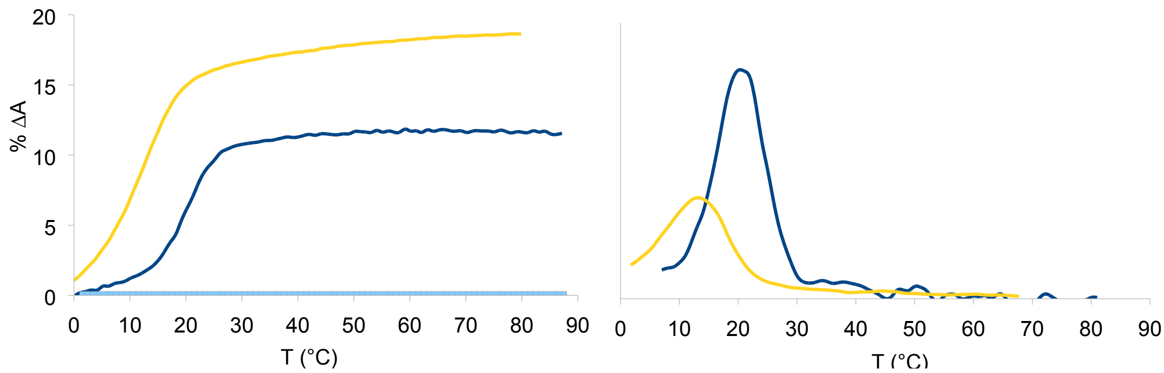
Table S3. Results not included in paper tables.

Entry	Template	Template sequence (5'-3')	Sequences	T_m [°C][a]
1	T_5	CC-(ACATA)$_3$-CC	B_5 5eq	pH 7.5 3mM CN^-: 20.2
	T_4	CC-(TACA)$_3$-CC	B_4 5eq	pH 7.5 3mM CN^-: 13.0
2	$T_{5'}$	CC-(ACATA)-ATACA-(ACATA)-CC	B_5	-[c]
			$B_{5'}$	-[c]
			B_5+$B_{5'}$	pH 9.5: 20.0
3	$T_{4'}$	CC-(TACA)-TCAA-(TACA)-CC	B_4	-[c]
			$B_{4'}$	-[c]
			B_4 + $B_{4'}$	pH 9.5: 13.5

[a] Melting temperatures are obtained from the maxima of the first derivatives of the melting curve (A260 vs temperature) recorded in a buffer containing 1 M NaCl and 10 mM of sodium cacodylate, Template concentration 3 µM. Curve fits data were averaged from fits of three denaturation curves.

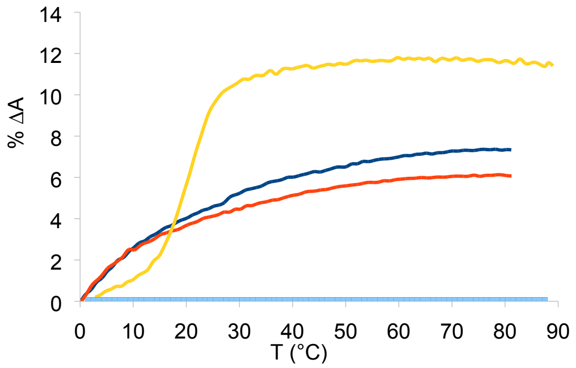
Melting curves and derivatives from Table S3.

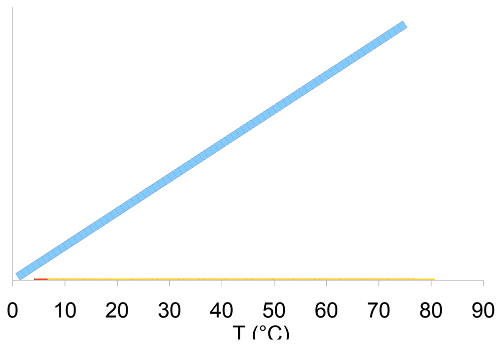
Table S3, entry 1 :



Melting curves and their derivatives at pH 7.5 with 3mM NaCN of complexes 3'-CC(ATACA)$_3$CC/5'-T^{bn}ATGrU (blue) and 3'-CC(ACAT)$_3$CC/5'-T^{bn}GTrA (yellow).

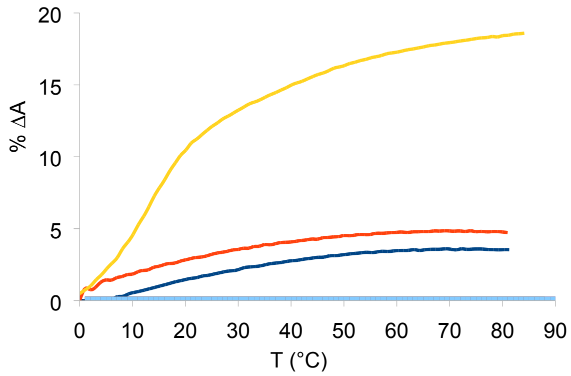
Table S3, entry 2 :

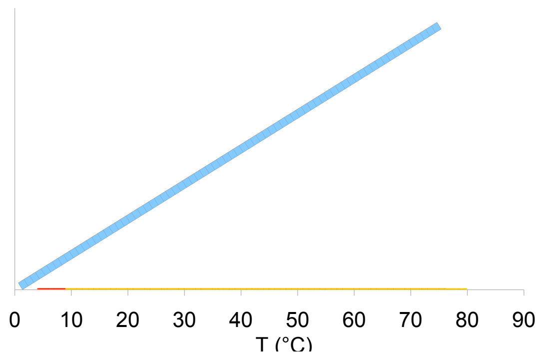




Melting curves and their derivatives at pH 9.5 of template CC-(ACATA)-ATACA-(ACATA)-CC with 5'-T^{bn}ATGrU (blue), 5'-T^{bn}TAGrU (orange) and both bifunctionnal strands (yellow).

Table S3, entry 3 :





Melting curves and their derivatives at pH 9.5 of template CC-(ACAT)-AACT-(ACAT)-CC with 5'-T^{bn}GTrA (blue), 5'-T^{bn}TGrA (orange) and both bifunctionnal strands (yellow).