

Supporting Information

Characterization of G-quadruplexes in *Chlamydomonas reinhardtii* and the effects of polyamine and magnesium cations on structure and stability

W. Andrew Vinyard, Aaron M. Fleming, Jingwei Ma, and Cynthia J. Burrows*

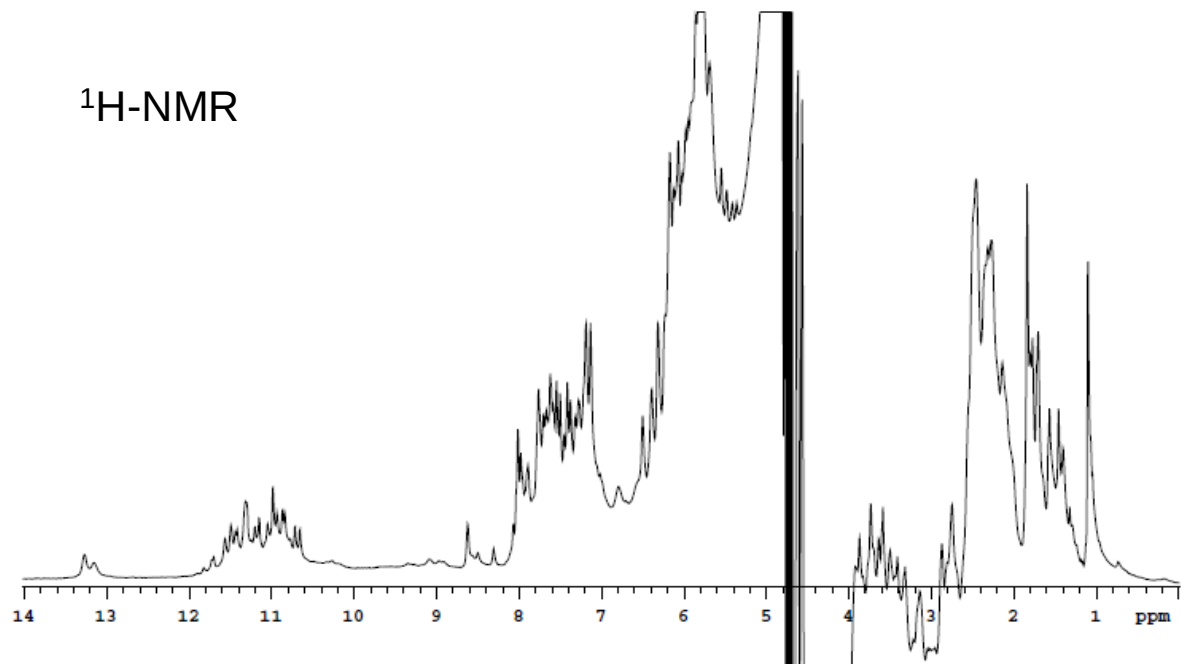
Department of Chemistry, University of Utah, Salt Lake City, Utah 84112-0850, United States

Item	Page
Figures S1–S19. PQS characterization data	S2–S20
Figure S20. Complete T_m values measured with the polyamines and the structures of the polyamines.	S21
Table S1. Comparison of all structural characterization data collected for each PQS	S22

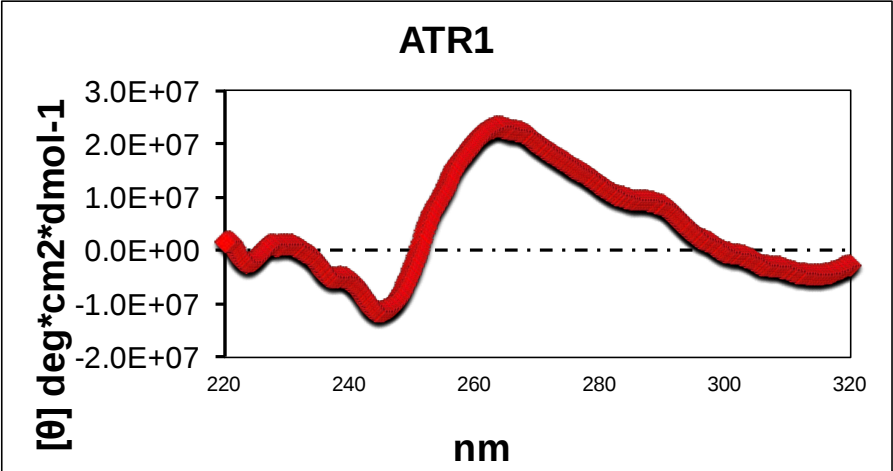
Figure S1. Data for ATR1

ATR1

Sequence 5`-GCGGGTGCGGGTGCGGGTGCGGGTG-3`



CD Spectrum



T_m
Value
 73.0 ± 1.0 °C

ThT Data

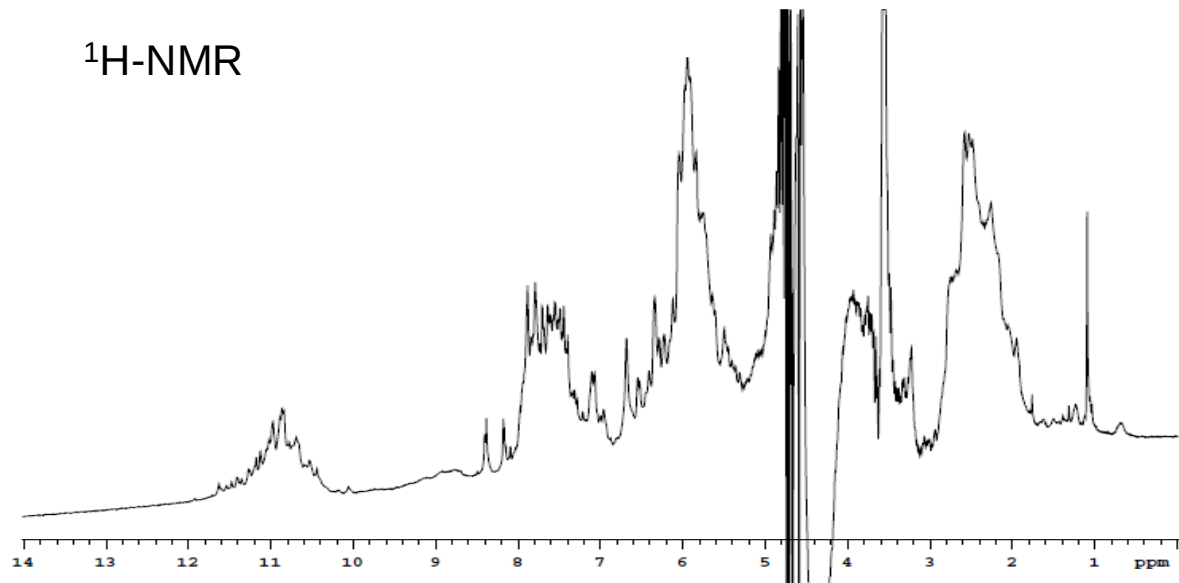
Sample	ThT	ssDNA	cMYC G4	DMC1 G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	49.2 ± 2.2

Figure S2. Data for CYC3

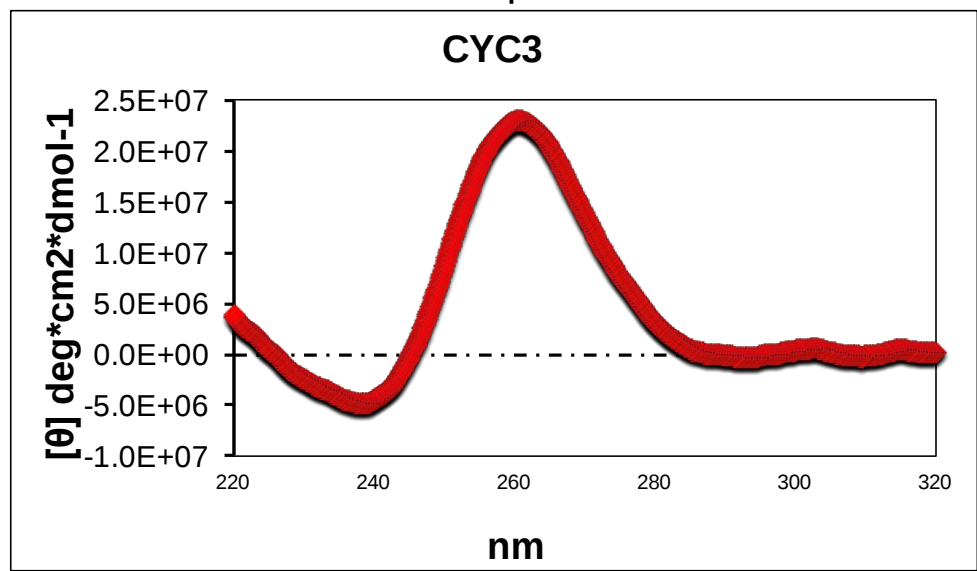
CYC3

Sequence 5`-GCGGGGAGGGGAGGGGGAGGGCA-3`

¹H-NMR



CD Spectrum



T_m
Value
 $70.9 \pm 1.7\text{ }^{\circ}\text{C}$

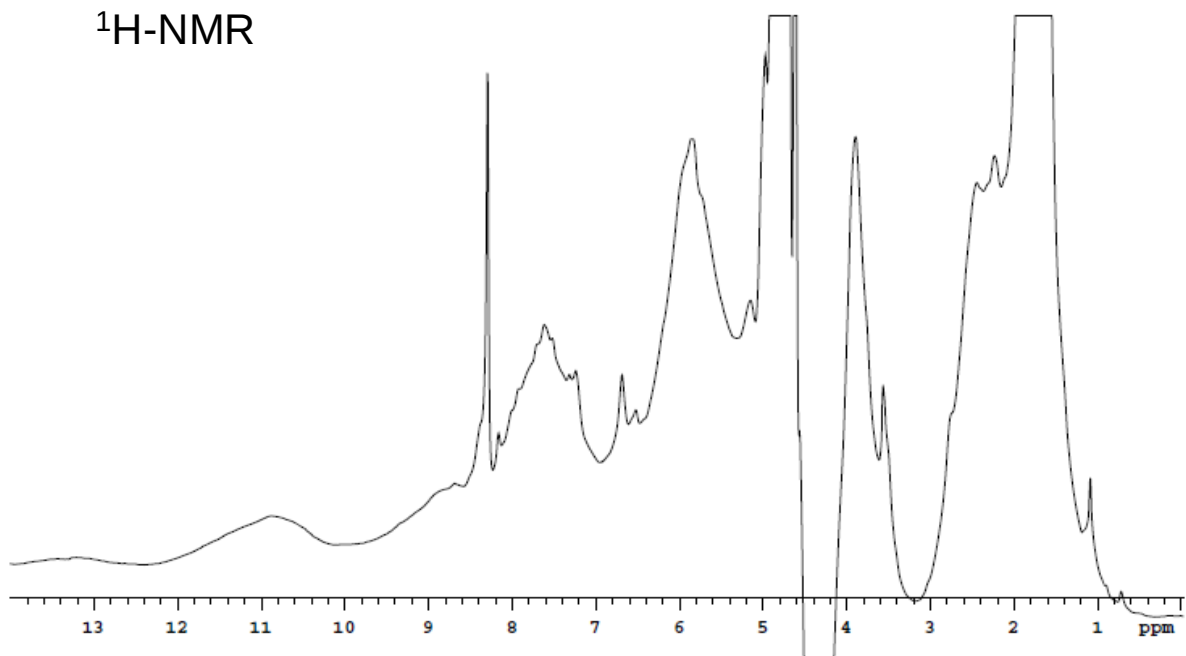
ThT Data

Sample	ThT	ssDNA	cMYC G4	CYC3 G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	94.6 ± 21.4

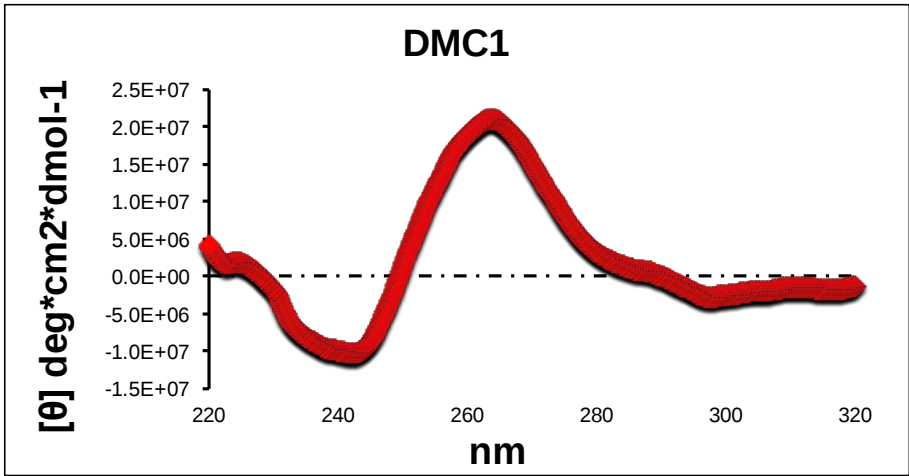
Figure S3. Data for DMC1

DMC1

Sequence 5`-GTGGGTGGGTGTGGGTGGGT-3`



CD Spectrum



T_m
Value
 $85.3 \pm 0.7 \text{ }^{\circ}\text{C}$

ThT Data

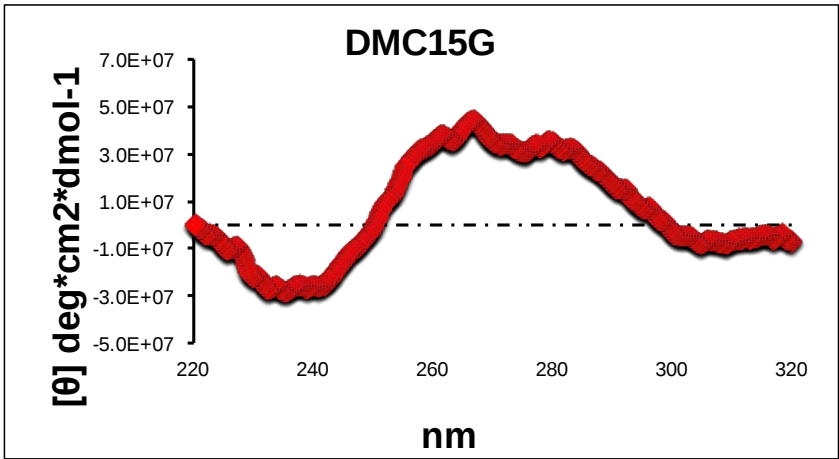
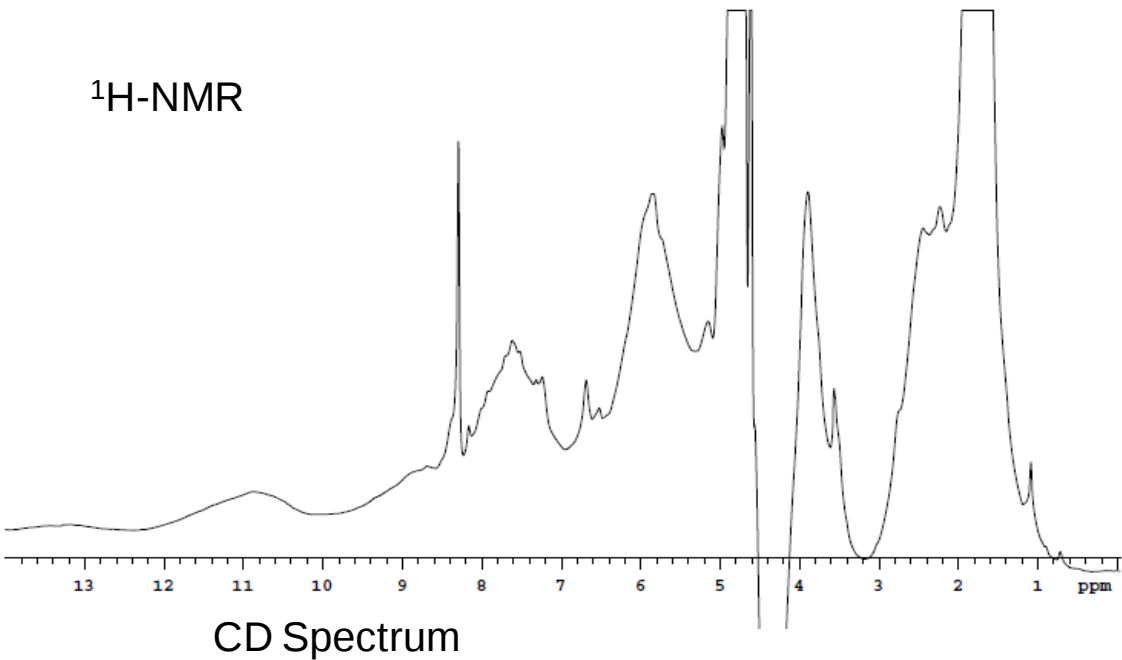
Sample	ThT	ssDNA	cMYC G4	DMC1 G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	92.6 ± 4.0

Figure S4. Data for DMC15G

DMC15G

Sequence

5`-GCGGGTGGGTGCGTGTCGTGTGGGTGGGTGTGGGTGGGTT-3`

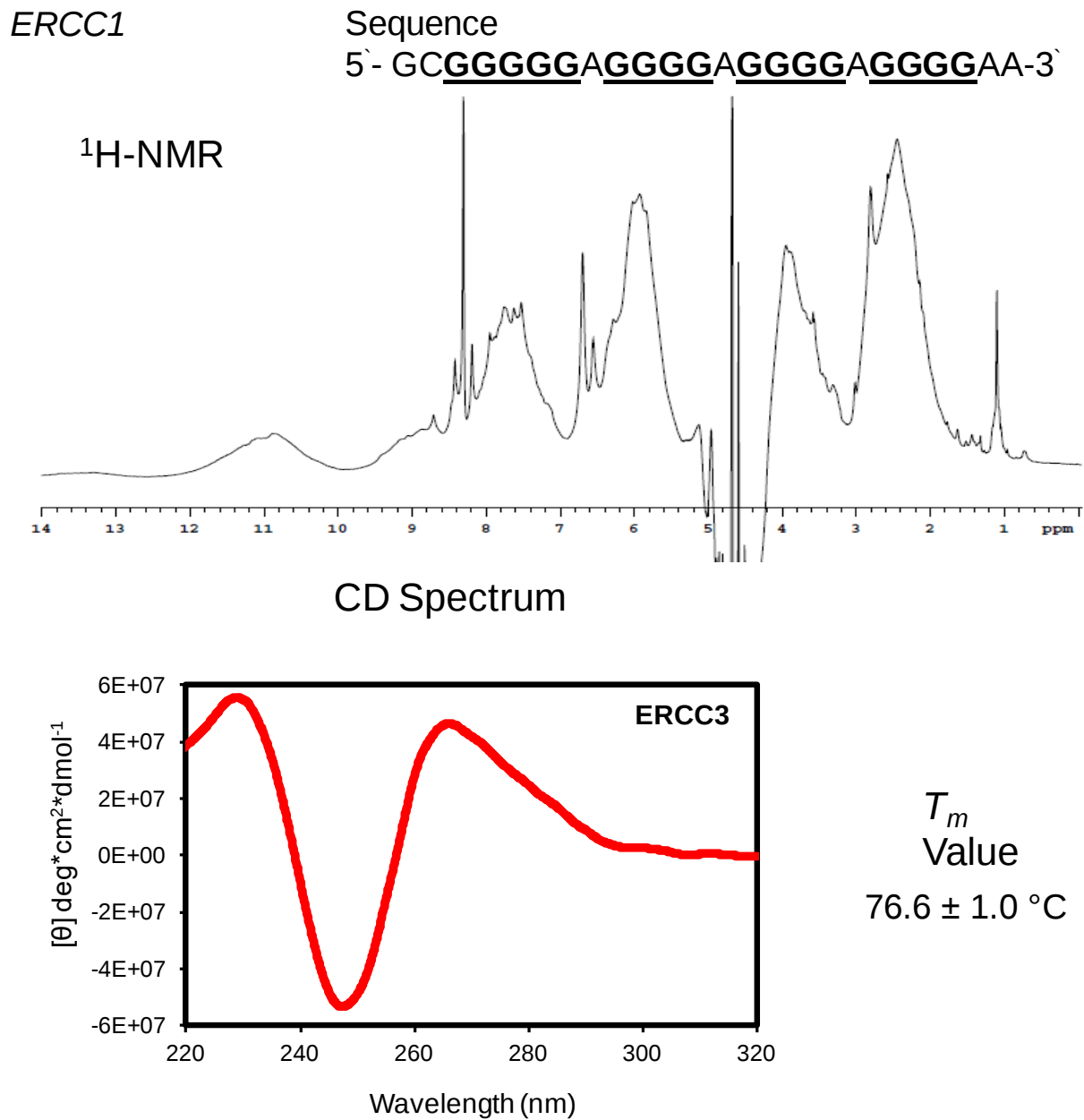


T_m
Value
 $64.4 \pm 0.8^\circ\text{C}$

ThT Data

Sample	ThT	ssDNA	cMYC G4	DMC15G G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	37.1 ± 1.1

Figure S5. Data for ERCC1



ThT Data

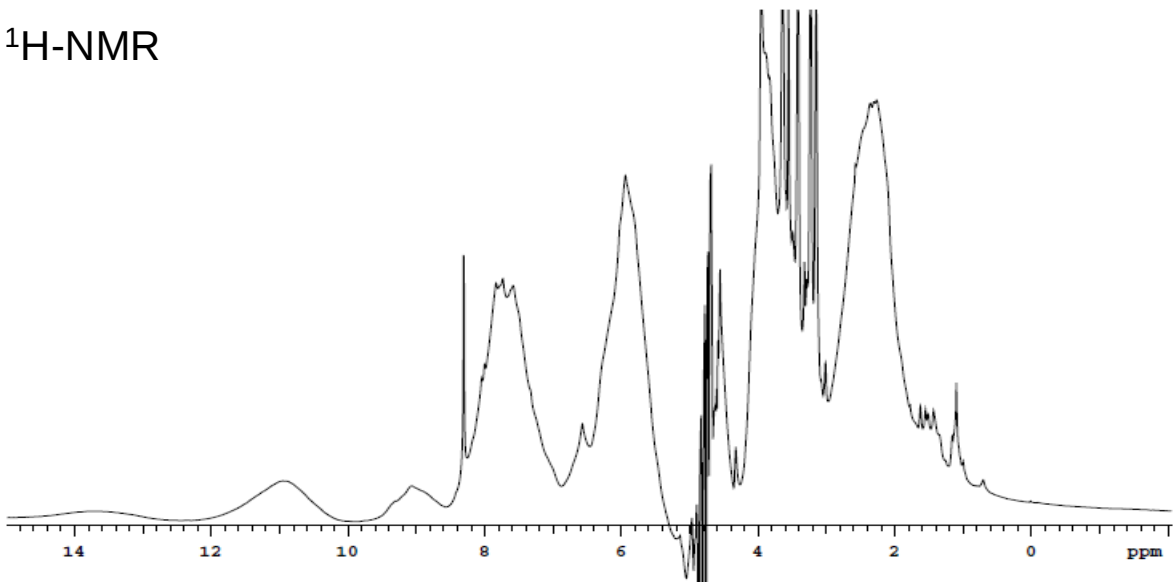
Sample	ThT	ssDNA	<i>cMYC</i> G4	<i>ERCC1</i> G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	107.9 ± 2.2

Figure S6. Data for ERCC3

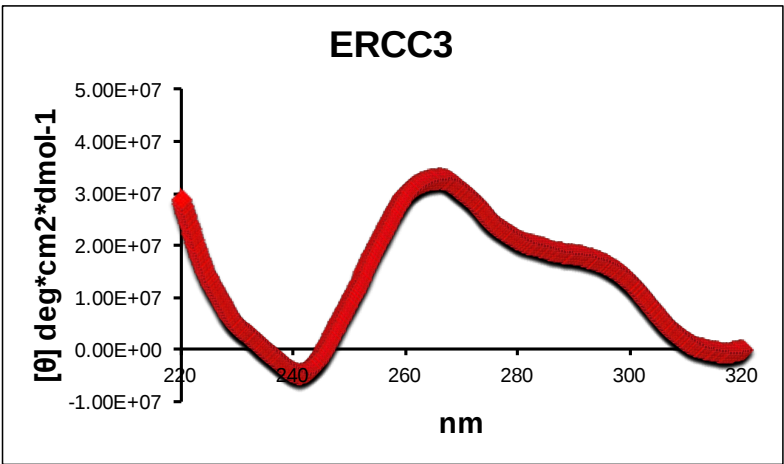
ERCC3

Sequence 5`-AAGGGGAGAGGGGAAAAGGGAGAAGGGGTT-3`

¹H-NMR



CD Spectrum



T_m
Value
52.2 ± 0.3 °C

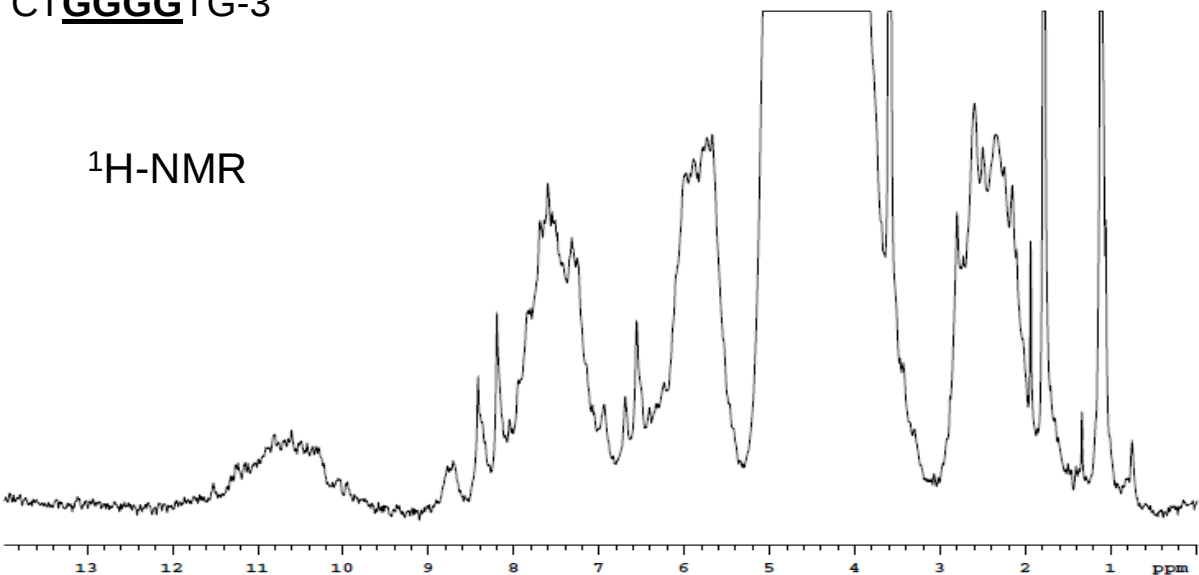
ThT Data

Sample	ThT	ssDNA	cMYC G4	ERCC3 G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	245.4 ± 3.3

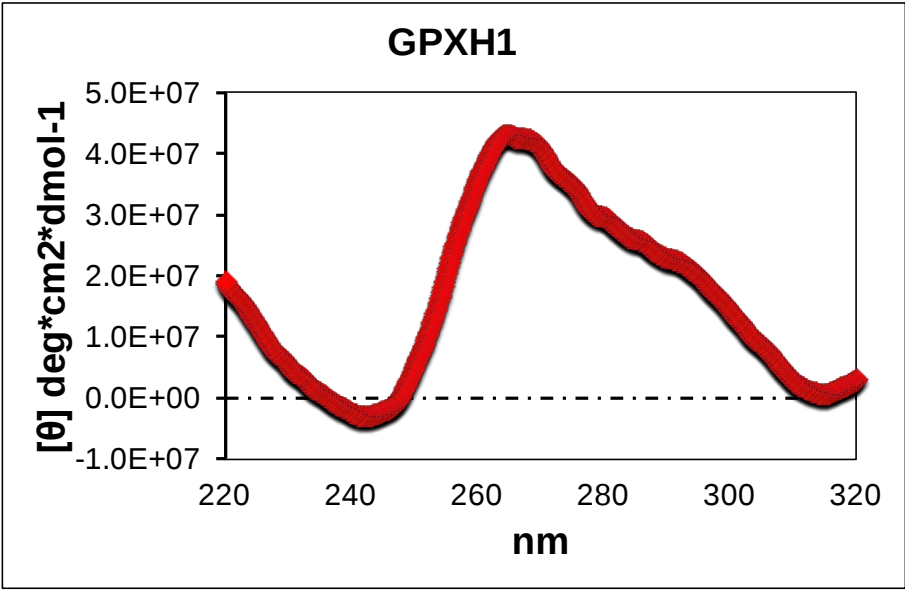
Figure S7. Data for GPXH1

GPXH1

Sequence 5'-AAGGGTGGGCAGGCGGAGGGGGCCGGGCGGG
CTGGGGTG-3'



CD Spectrum



T_m
Value
 60.9 ± 4.2 °C

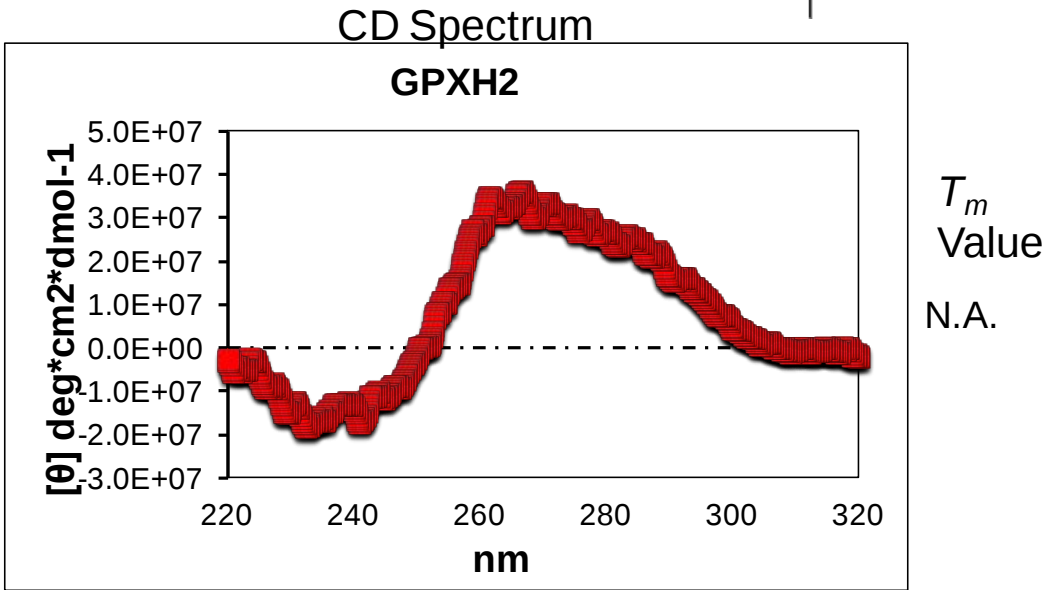
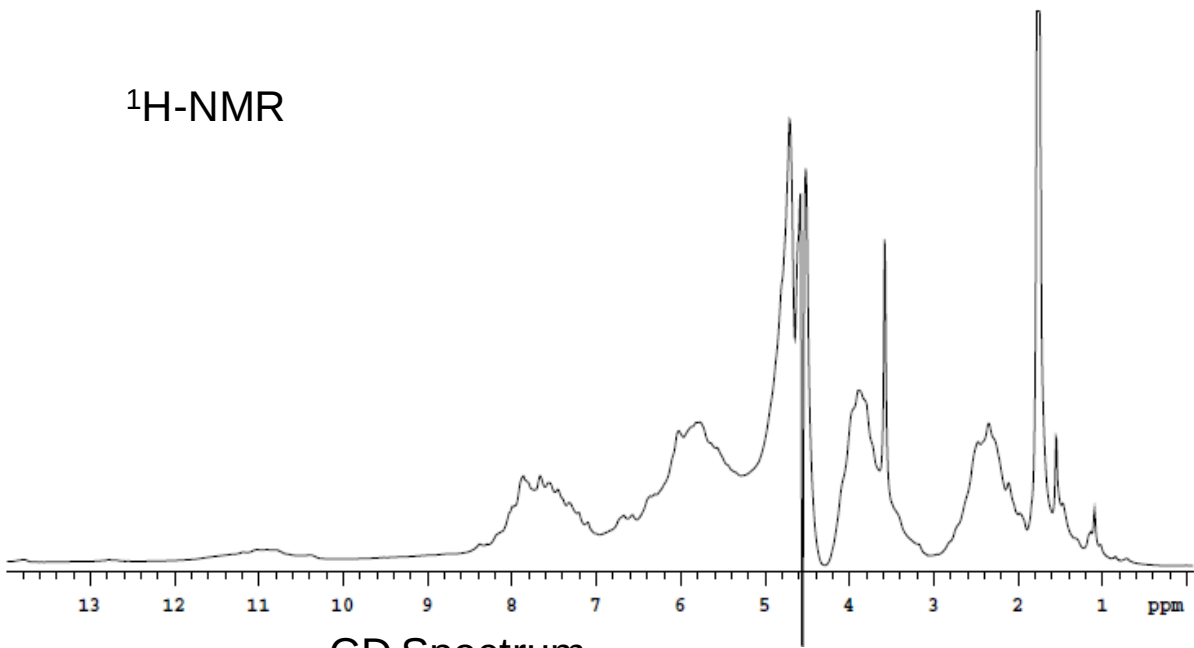
ThT Data

Sample	ThT	ssDNA	cMYC G4	GPXH1 G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	26.4 ± 1.3

Figure S8. Data for GPXH2

GPXH 2

Sequence 5`-CAGGGTGCAGCCGGGTTGGGTGCGGCCGGGTC-3`



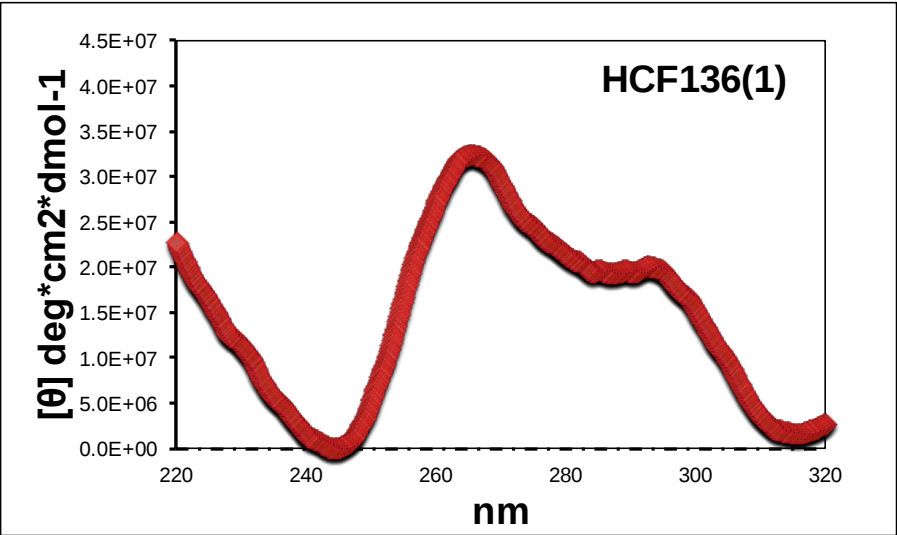
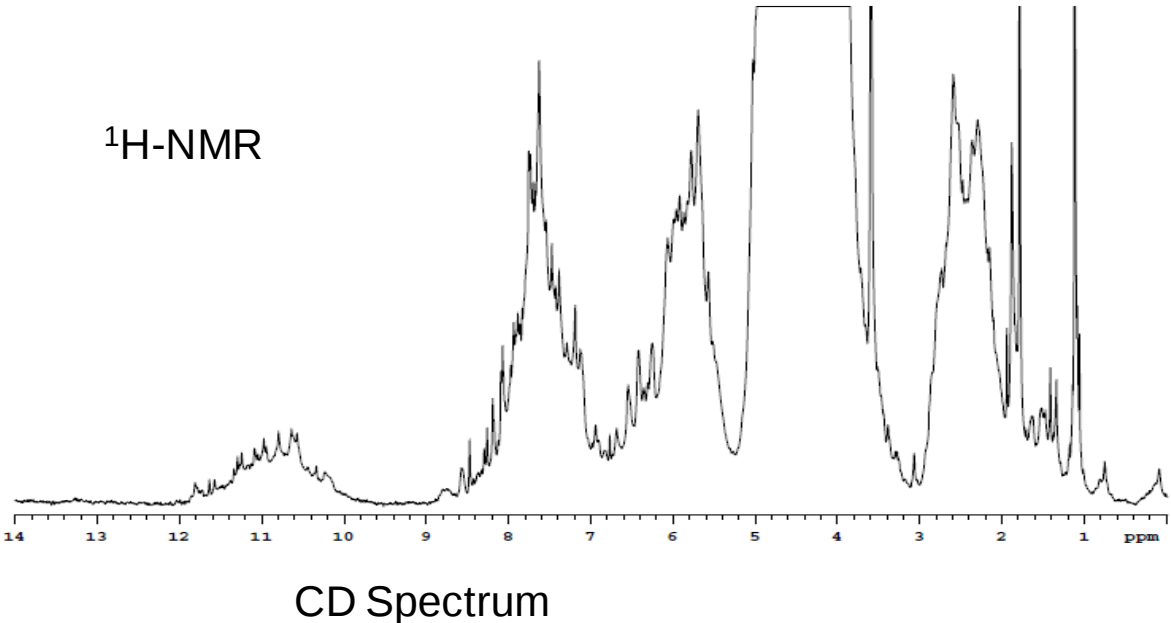
ThT Data

Sample	ThT	ssDNA	cMYC G4	GPXH2 G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	29.5 ± 0.4

Figure S9. Data for HCF136(1)

HCF136(1)

Sequence 5`-TTGGGGGAA GGGGGGGAGGG GGAGGGGGTG-3`



T_m
Value

66.9 ± 3.4 °C

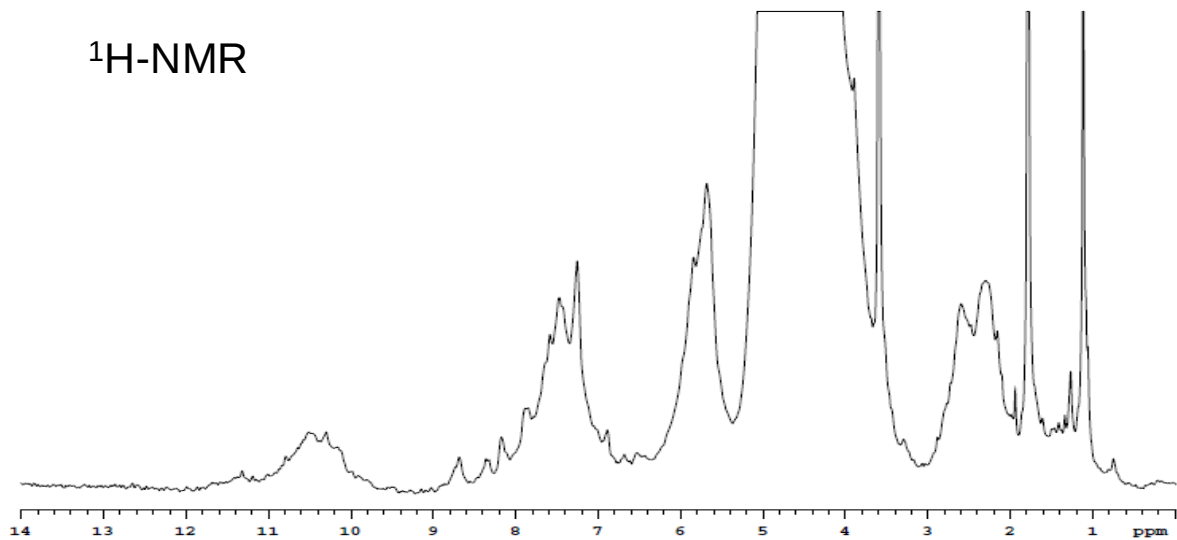
ThT Data

Sample	ThT	ssDNA	cMYC G4	HCF136(1) G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	319.6 ± 20.8

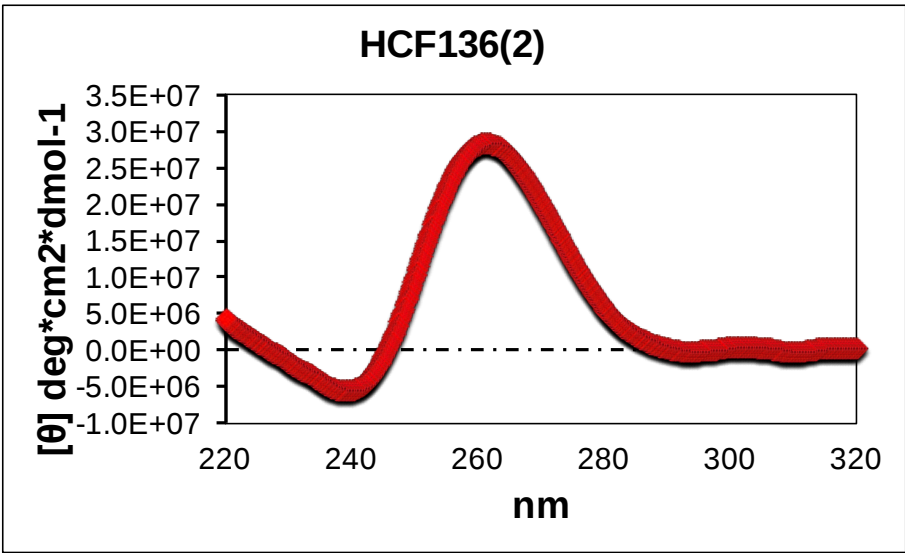
Figure S10. Data for HCF136(2)

HCF136(2)

Sequence 5-GAGGGGAGAGGGGTGGGAGAGGGGTG-3



CD Spectrum



T_m
Value

77.8 ± 1.2 °C

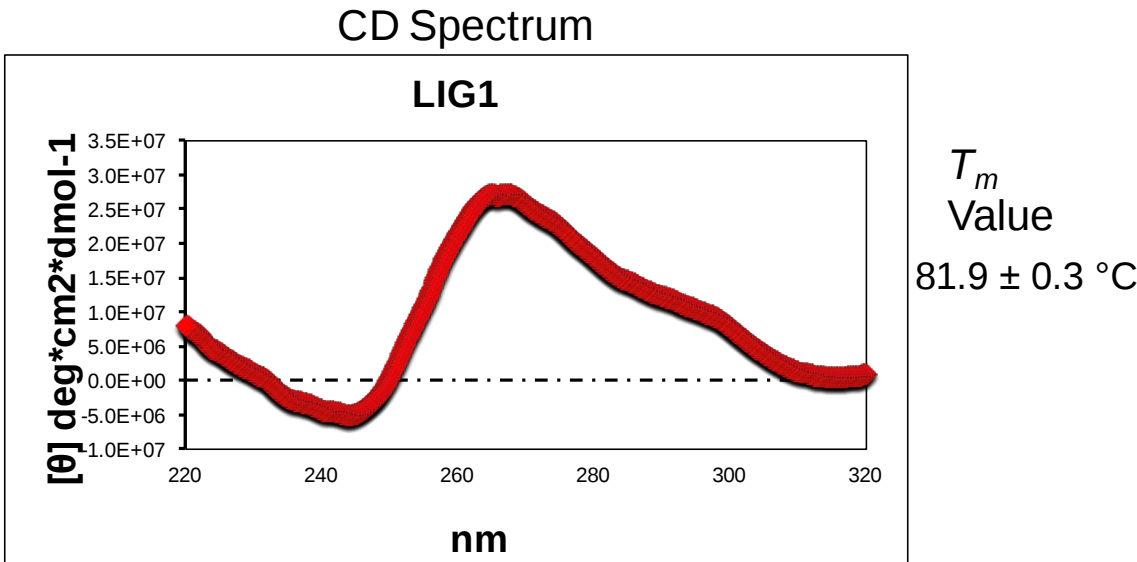
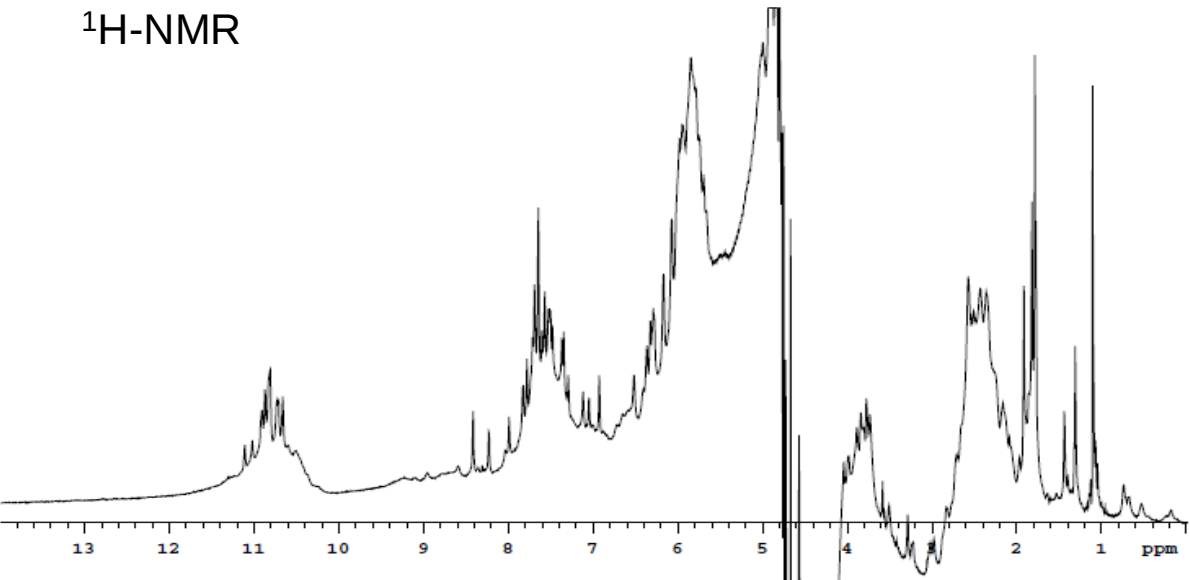
ThT Data

Sample	ThT	ssDNA	cMYC G4	HCF136(2) G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	335.4 ± 82.4

Figure S11. Data for LIG1

LIG1

Sequence 5`-GCGGGGTGGGTTGGGGTGGGGTC-3`



ThT Data

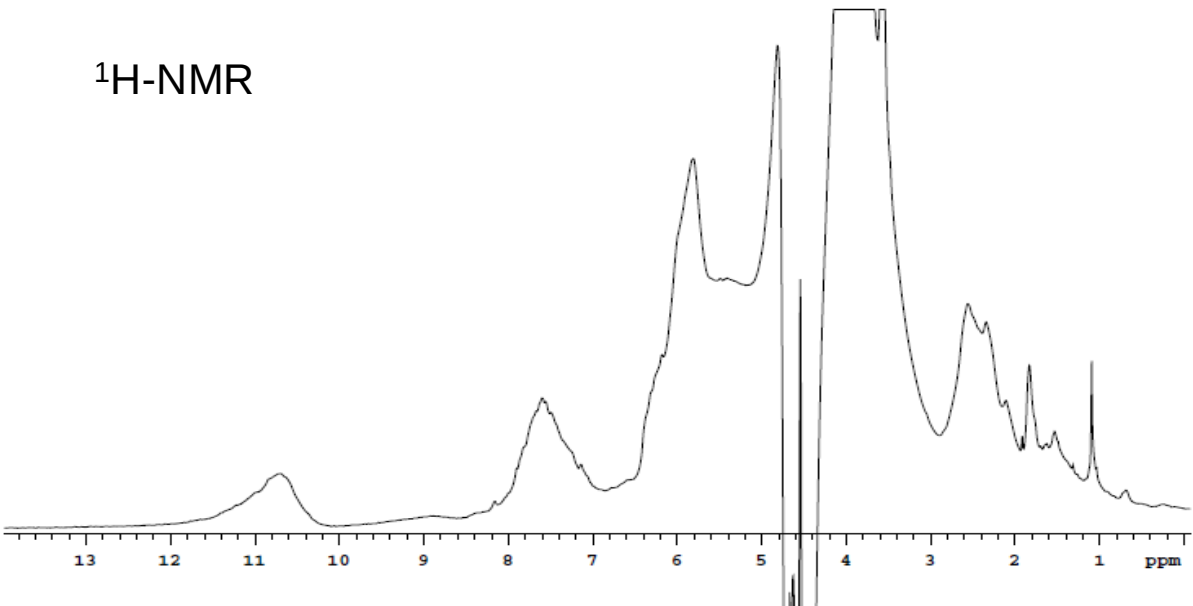
Sample	ThT	ssDNA	cMYC G4	LIG1 G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	158.6 ± 11.9

Figure S12. Data for LIG15G

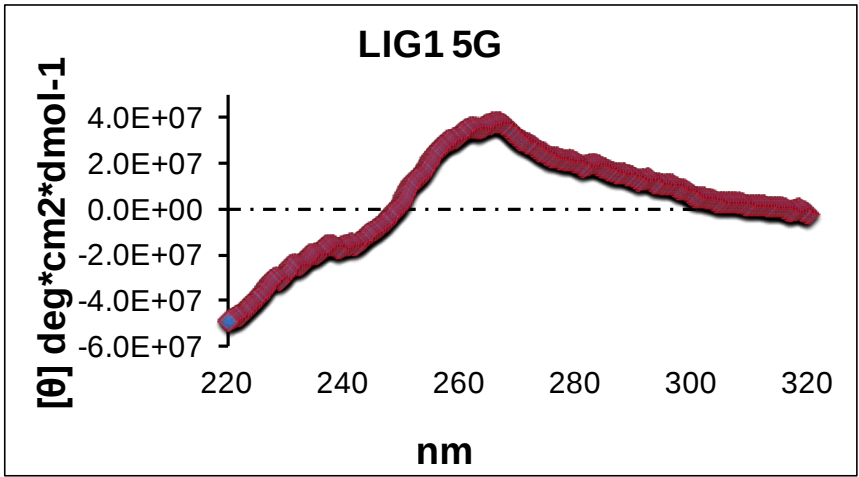
LIG15G

Sequence

5`-GCGGGGTGGGTTGGGGTAGGGGTCGCTCTGCGGGGTT-3`



CD Spectrum



T_m
Value

64.4 ± 4.3 °C

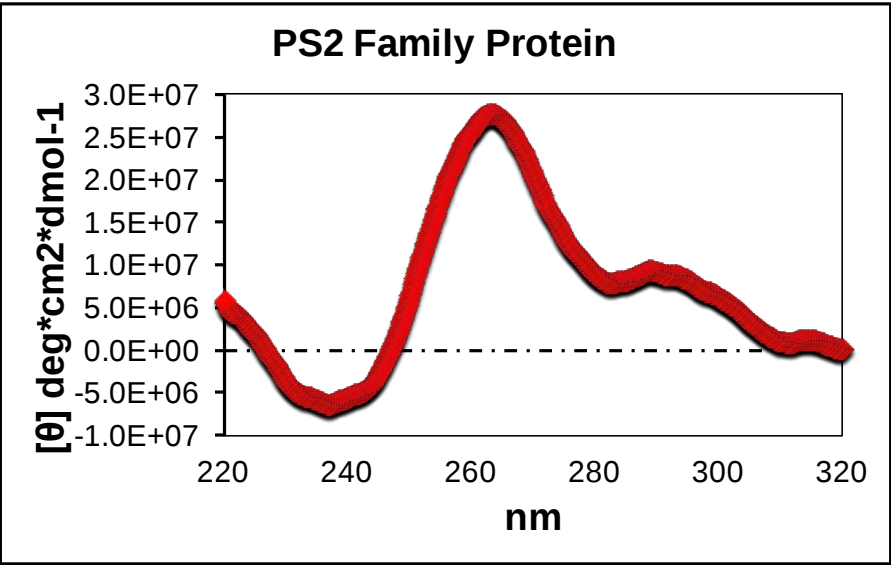
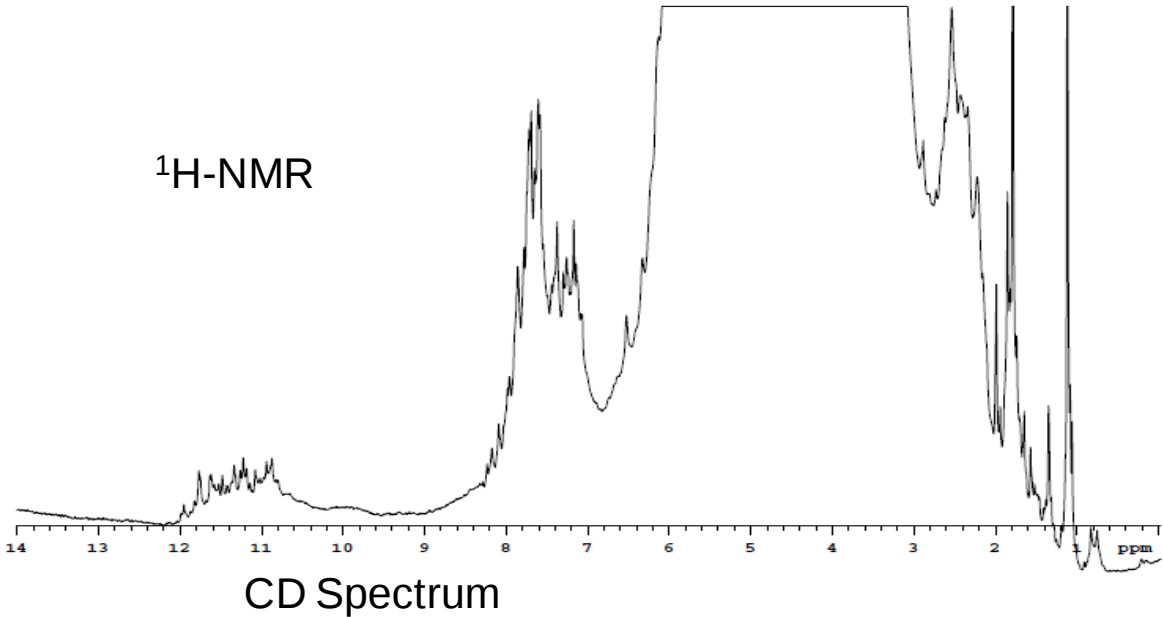
ThT Data

Sample	ThT	ssDNA	cMYC G4	LIG15G G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	19.8 ± 3.6

Figure S13. Data for PS3 Family Protein

PS2 Family Protein

Sequence 5'-GCGG GGAGTGGGTG AGGGCTGGGG CG-3'



T_m
Value
 $72.9 \pm 0.8\text{ }^{\circ}\text{C}$

ThT Data

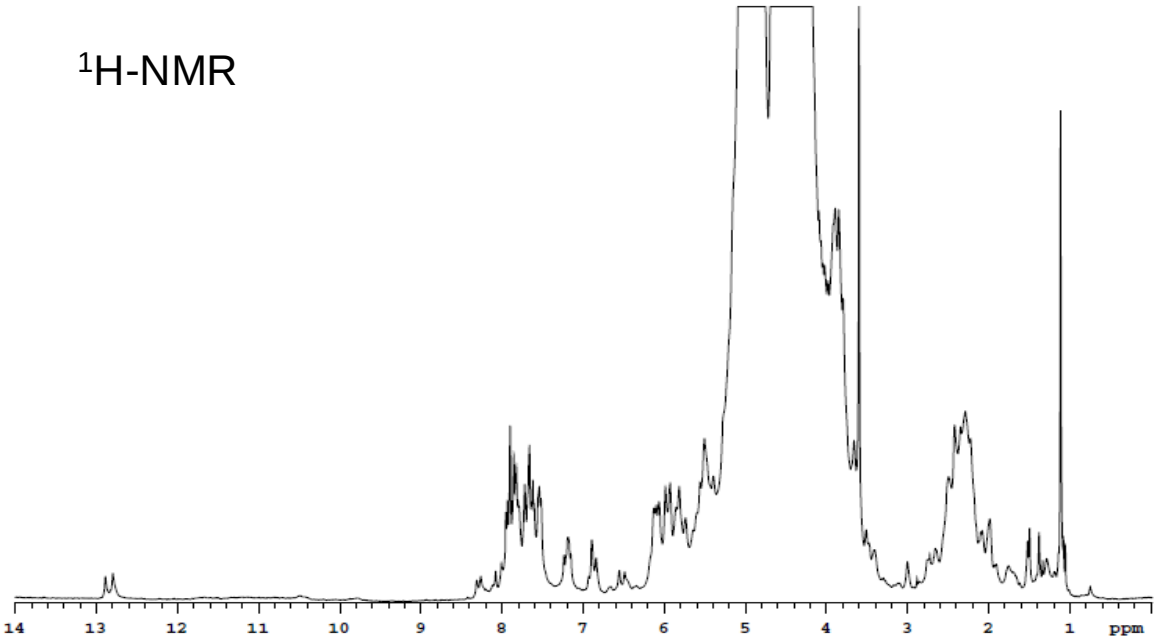
Sample	ThT	ssDNA	cMYC G4	PS2 G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	139.9 ± 59.6

Figure S14. Data for PS2 Subunit 1

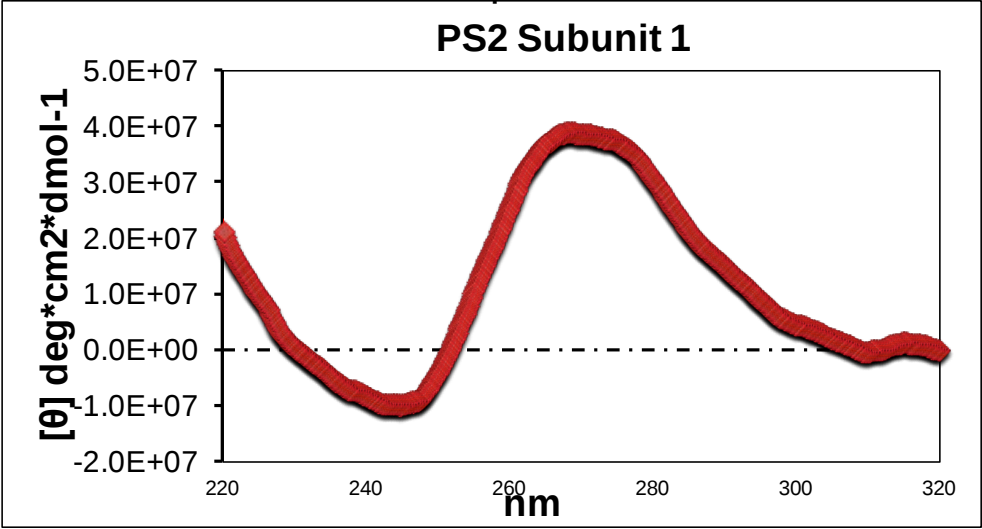
PS2 Subunit 1

Sequence 5`-GTGGGAGCGGGAGCGGGAGCGGGAG-3`

¹H-NMR



CD Spectrum



T_m
Value

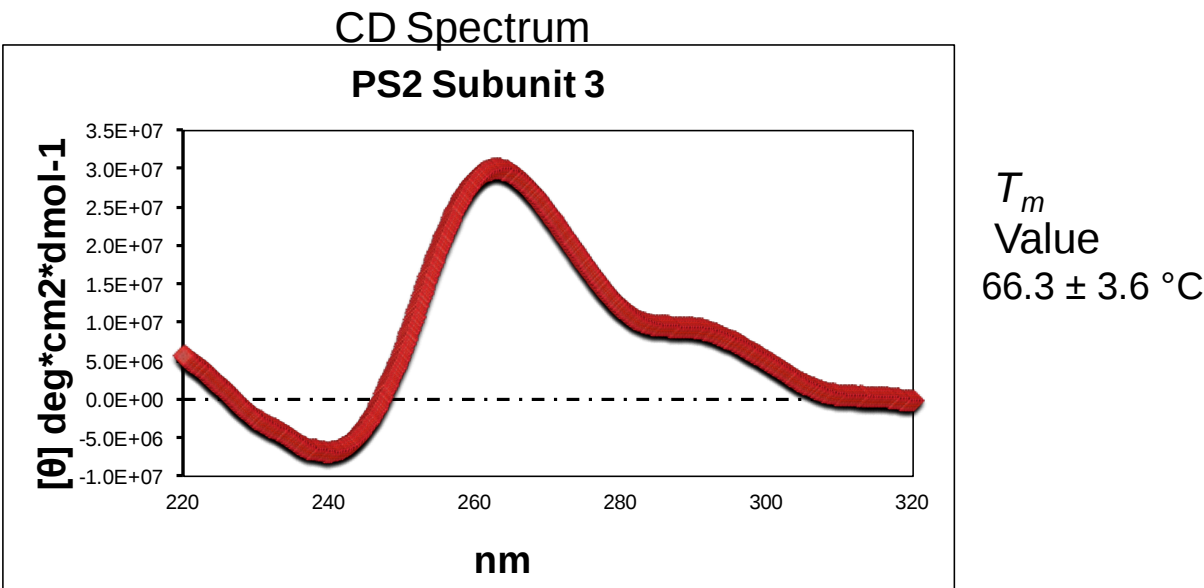
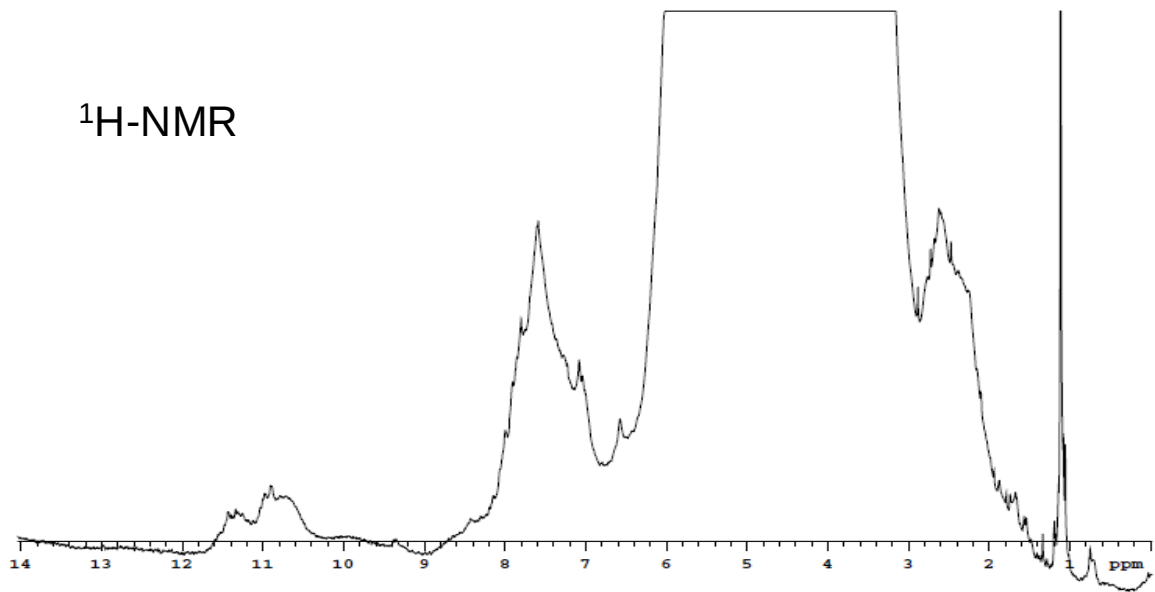
64.3 ± 3.8 °C

ThT Data

Sample	ThT	ssDNA	cMYC G4	PS2 Subunit 1 G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	3.34 ± 3.3

Figure S15. Data for PS2 Subunit 3

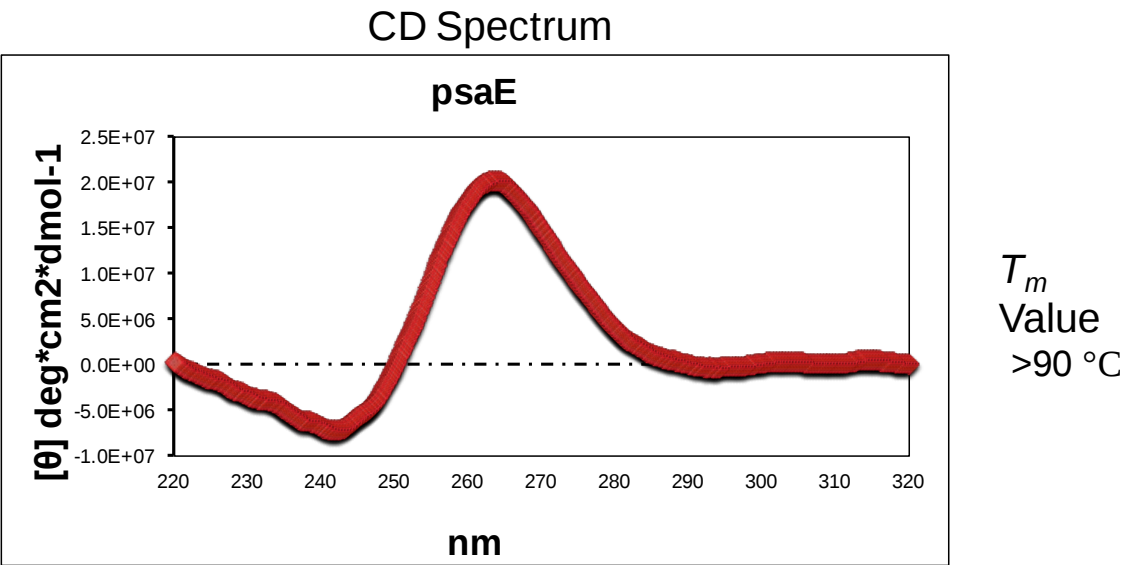
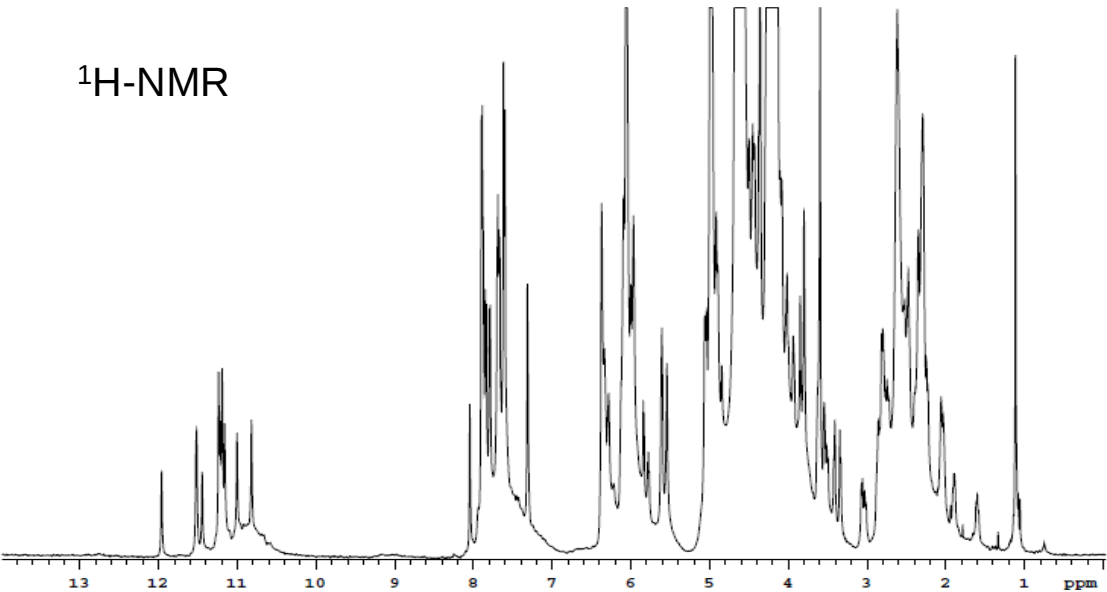
PS2 Subunit 3
Sequence 5'-CA GGGGGCAGGGGCTAGGGGGCAGGGGCA-3'



ThT Data	Sample	ThT	ssDNA	cMYC G4	PS2 Subunit 3 G4
	λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	259.5 ± 88.0

Figure S16. Data for psaE

psaE Sequence 5'-ACGGGCGGGCGGGCGGGCG-3'



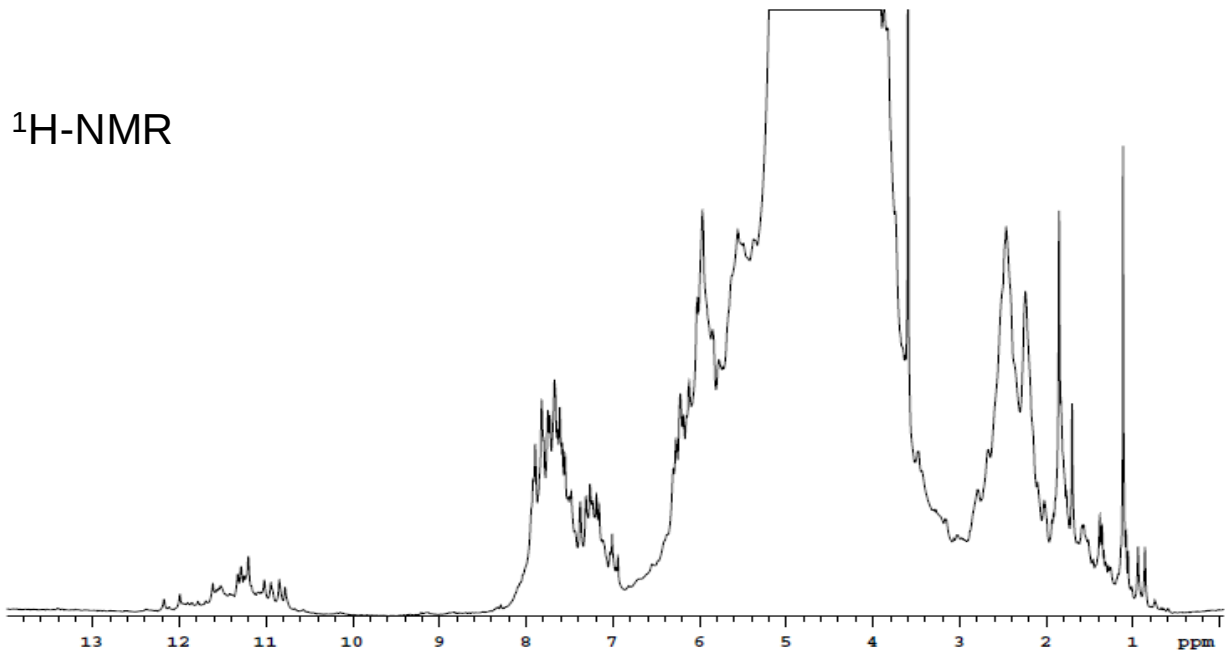
ThT Data

Sample	ThT	ssDNA	<i>cMYC</i> G4	<i>psaE</i> G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	53 ± 7.6

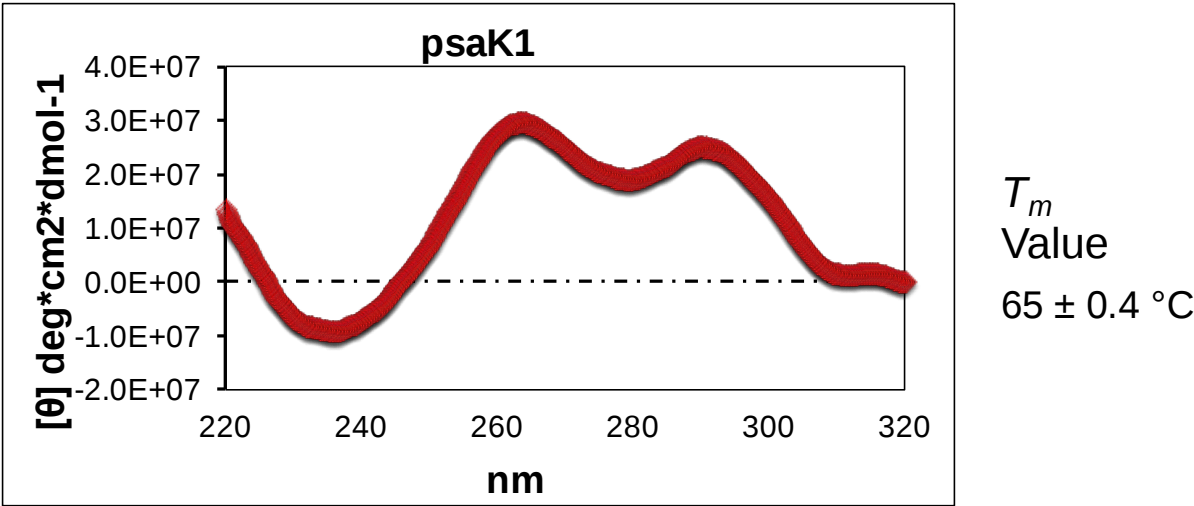
Figure S17. Data for psaK1

psaK1
Sequence 5`-ATGGGCCTGGGCGTGGG TCTAGGGGGAG-3`

¹H-NMR



CD Spectrum



ThT Data

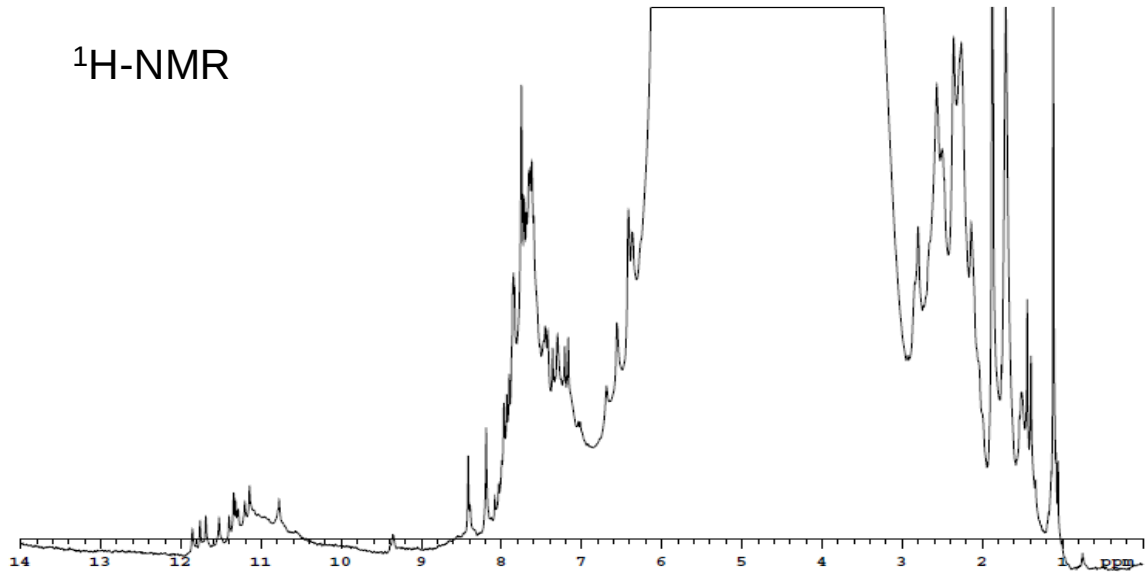
Sample	ThT	ssDNA	<i>cMYC</i> G4	<i>psaK1</i> G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	240.3 ± 36.5

Figure S18. Data for UQCC1(1)

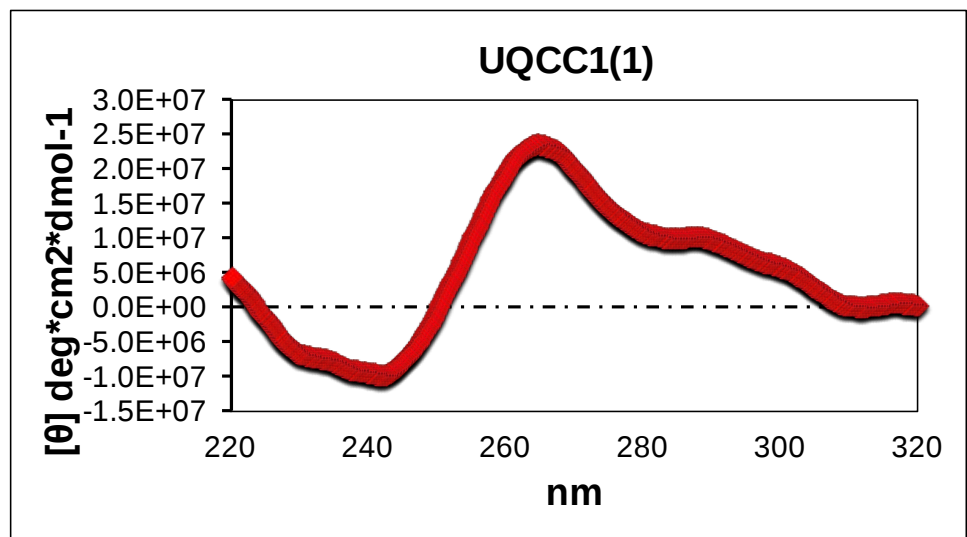
UQCC1(1)

Sequence 5'-TTGGGTGGGAGGGTGGGAG-3'

¹H-NMR



CD Spectrum



T_m
Value
 $56.3 \pm 0.4 \text{ } ^\circ\text{C}$

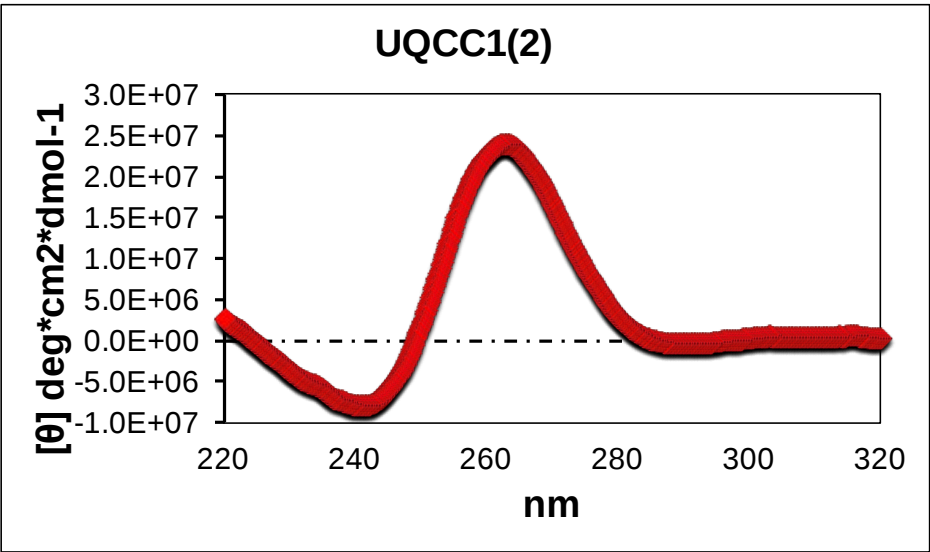
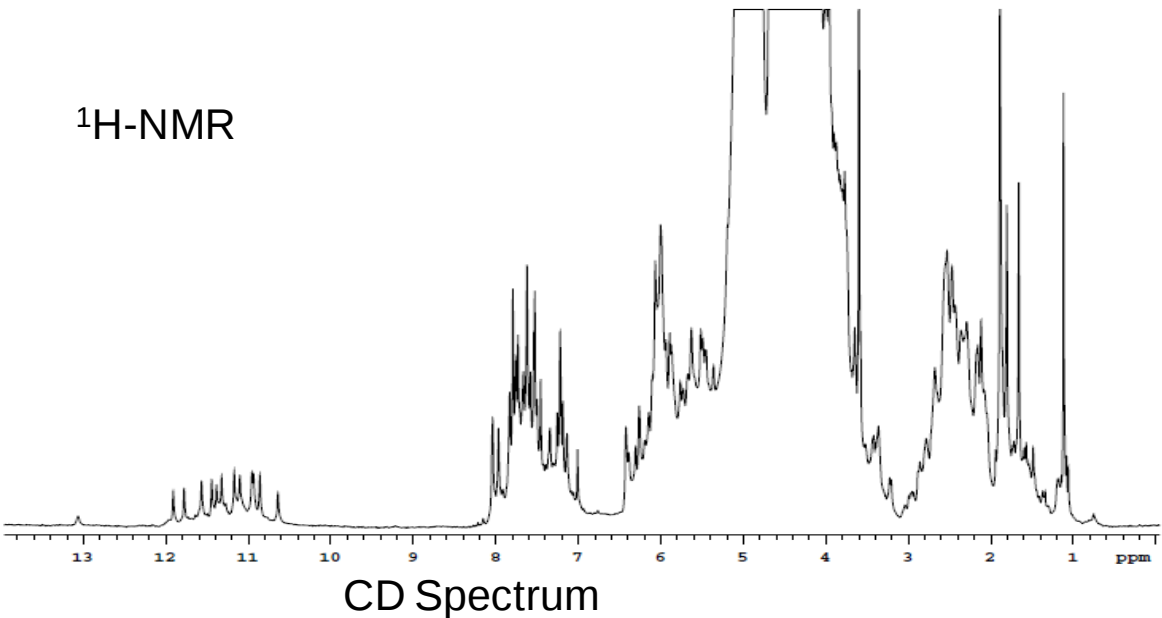
ThT Data

Sample	ThT	ssDNA	cMYC G4	UQCC1(1) G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	129.9 ± 16.2

Figure S19. Data for UQCC1(2)

UQCC1(2)

Sequence 5'-AC GGGTGGGTTGC GGGTCCGGGG CT-3'



T_m
Value
69.8± 1.5 °C

ThT Data

Sample	ThT	ssDNA	cMYC G4	UQCC1(2) G4
λ_{em} at 490 nm	0.9 ± 0.1	5.15 ± 1.0	51.2 ± 7.4	49.6 ± 14.0

Figure S20. Complete T_m values measured with the polyamines and the structures of the polyamines.

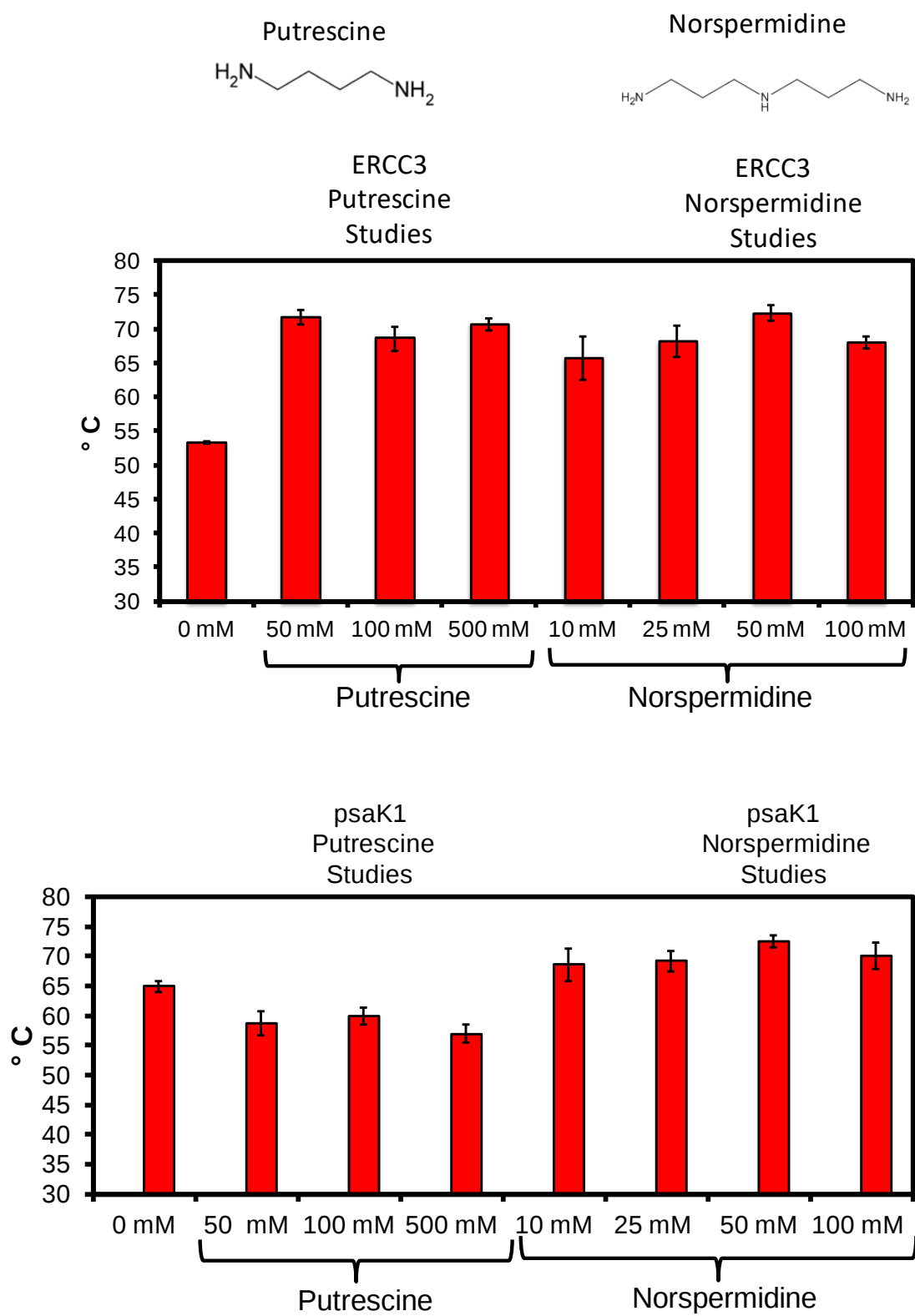


Table S1. Comparison of all structural characterization data collected for each PQS

PQS	¹ H-NMR (Hoogsteen Imino protons)	ThT > 20	CD (topology)	T _m
ATR1	+	+, 51.2 ± 7.4	Parallel	73.0 ± 1.0 °C
CYC3	+	+, 94.6 ± 21.4	Parallel	70.9 ± 1.7 °C
DMC1	+	+, 92.6 ± 4.0	Parallel	85.3 ± 0.7 °C
DMC15G	+	+, 37.1 ± 1.1	Parallel	>90 °C
ERCC1	+	+, 107.9 ± 2.2	Parallel	76.6 ± 1.0 °C
ERCC3	+	+, 245.9 ± 3.3	Mixed	52.2 ± 0.3 °C
GPXH1	+	+, 26.4 ± 1.3	Parallel	60.9 ± 4.2 °C
GPXH2	+	+, 29.5 ± 0.4	Single stranded	N/A
HCF136 (1)	+	+, 319.6 ± 20.8	Parallel	66.9 ± 3.4 °C
HCF136 (2)	+	+, 335.4 ± 82.4	Parallel	77.8 ± 1.2 °C
LIG1	+	+, 156.6 ± 11.9	Parallel	81.9 ± 0.3 °C
LIG1 5G	+	-, 19.8 ± 3.6	Parallel	64.4 ± 4.3 °C
PS2 Fam. Pro.	+	+, 139.9 ± 59.6	Parallel	72.9 ± 0.8 °C
PS2 Subunit 1	+	-, 3.34 ± 3.3	Parallel	64.3 ± 3.8 °C
PS2 Subunit 3	+	+, 259.5 ± 88.0	Parallel	66.3 ± 3.6 °C
psaE	+	+, 53 ± 7.6	Parallel	>90 °C
PsaK1	+	+, 240.3 ± 36.5	Hybrid	65 ± 0.4 °C
UQCC1 (1)	+	+, 129.9 ± 16.2	Parallel	56.3 ± 0.4 °C
UQCC1 (2)	+	+, 49.6 ± 14	Parallel	69.8 ± 1.5 °C