Supplementary Data

Thermodynamic Characterization of Specific Interactions between the Human Lon Protease and G-quartet DNA

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FIGURES LEGENDS

Figure S1. Heat denaturation and base substitution of the LSP sequences.

LSPas and TG_6T were incubated at 120°C for 2 hr, and briefly chilled on ice. The samples were analyzed on 20% native PAGE at 20°C. LSPasGA is a variant of LSPas in which the third and fourth guanines were substituted with adenines.

Figure S2. Native gel electrophoresis of G-rich DNA sequences.

The G-rich oligonucleotides as shown were analyzed on 20% PAGE at 20°C.

Figure S3. Electrophoresis mobility shift assay for the competitive binding of DNA to hLon.

hLon (6 μM) was incubated with fluorescently labeled LSPas (1 μM) in the presence of unlabeled DNA competitors (50 μM) for 20 min at the temperature indicated. The reaction mixtures were resolved on 6% polyacrylamide gels in TGE buffer at 25°C (A) or at 37°C (B). hLon binding to the LSPas probe was competed not only by the addition of unlabeled LSPas and LSPas18, but also by unlabeled LS2as, LS4as, LS5as, and LS6as, all of which formed high-ordered structures on native PAGE (Fig. S2). By contrast, TG_6T did not compete well for hLon. The discrepancy in competitiveness between TG_6T and other high-order oligonucleotides may be due to the length of DNA. The addition of LSPs, LSPs18, and AC_6A saturated LSPas differently (control experiment, not shown), and the availability of LSPas quadruplex for hLon was significantly reduced in the presence of LSPs and LSPs18.

Figure S4. Circular dichroism spectra of hLon in the presence and absence of DNA.

(A) The CD spectra of hLon (2.5 μM) were scanned from 20 to 90°C. (B) hLon mixed with LSPas at a molar ratio of 1:1 was monitored spectroscopically from 20 to 70°C. (C) The CD spectra of the hLon in complex with TG_6T were obtained similarly.

Figure S5. Circular dichroism spectra of DNA in the presence of hLon.

(A) LSPas and (B) TG_6T complexed with hLon at 10:1 molar ratios were scanned with circular dichroism. The molar ellipticities of LSPas and TG_6T were slightly reduced in the presence of hLon, but no significant structural transition was found.

Figure S6. Circular dichroism analysis of intermolecular G-quadruplex DNA with known structures.

Two DNA sequences known to form intermolecular G-quartets were subjected to CD analysis in buffer containing 10 mM sodium cacodylate (pH 7.5), 100 mM NaCl, 5 mM $MgCl_2$, and 0.1 mM EDTA (compare with results shown in Figure 2). The CD spectra of TG_4T (A) and TG_3CG_2T (B) peaked at 261 nm and 258 nm, both of which manifested the structural pattern of parallel G-quartets. The PDB ID for the structures of TG_4T and TG_3CG_2T are 2O4F and 1EVO, respectively. (C) TG_4T and TG_3CG_2T were analyzed on 20% native PAGE.

TABLES

Table S1. Sequence summary of G-quadruplex motifs found in human mtDNA

Analysis tool: Quadfinder ver. 1.0 (http://miracle.igib.res.in/quadfinder/)
Sequence input: NC_001807 (the H strand of human mtDNA)
Pattern: $G_{3-8}L_{2-20}G_{3-8}L_{2-20}G_{3-8}L_{2-20}G_{3-8}$
Search parameters: G stretch from 3 to 8, N stretch from 2 to 20

Location	Sequence (3'→5')	Sequence found within the region
435-471	GGGGGGTTGATTGTGTAATAAAAGGGGAGGGTGAGGG	LSPas
436-471	GGGGGTTGATTGTGTAATAAAAGGGGAGGGTGAGGG	
437-471	GGGGTTGATTGTGTAATAAAAGGGGAGGGTGAGGG	
438-471	GGGTTGATTGTGTAATAAAAGGGGAGGGTGAGGG	
536-572	GGGGTATGGGGCTTGGTTGGTTTGGGGTTTCTGTGGG	
536-573	GGGGTATGGGGCTTGGTTGGTTTGGGGTTTCTGTGGGG	
536-574	GGGGTATGGGGCTTGGTTGGTTTGGGGTTTCTGTGGGGG	
536-575	GGGGTATGGGGCTTGGTTGGTTTGGGGTTTCTGTGGGGGG	
537-575	GGGTATGGGGCTTGGTTGGTTTGGGGTTTCTGTGGGGGG	
3485-3530	GGGGATTTTGGGCGGTGTAGATGGTAGTGGGAGATGTAGTGGCGGG	
3485-3531	GGGGATTTTGGGCGGTGTAGATGGTAGTGGGAGATGTAGTGGCGGGG	
3486-3531	GGGATTTTGGGCGGTGTAGATGGTAGTGGGAGATGTAGTGGCGGGG	
3567-3588	GGGGGGAGGGGTATGGGTTGGG	
3567-3589	GGGGGGAGGGGTATGGGTTGGGG	
3567-3590	GGGGGGAGGGGTATGGGTTGGGGG	
3568-3590	GGGGGAGGGGTATGGGTTGGGGG	
3569-3590	GGGGAGGGGTATGGGTTGGGGG	
3570-3590	GGGAGGGGTATGGGTTGGGGG	
4431-4464	GGGTATGGGGCTTTTACAACCAATATGGGAAGGG	
4431-4479	GGGTATGGGGCTTTTACAACCAATATGGGAAGGGCATGATTAATTAGGG	
4431-4480	GGGTATGGGGCTTTTACAACCAATATGGGAAGGGCATGATTAATTAGGGG	
4437-4491	GGGGCTTTTACAACCAATATGGGAAGGGCATGATTAATTAGGGGACCGGGTTGGG	
4438-4491	GGGCTTTTACAACCAATATGGGAAGGGCATGATTAATTAGGGGACCGGGTTGGG	
4457-4491	GGGAAGGGCATGATTAATTAGGGGACCGGGTTGGG	
4462-4491	GGGCATGATTAATTAGGGGACCGGGTTGGG	
4796-4837	GGGGGAAAGTGAAGACTCAGGGTCTCCAATGGGTTCCGTGGG	
4796-4838	GGGGGAAAGTGAAGACTCAGGGTCTCCAATGGGTTCCGTGGGG	
4797-4838	GGGGAAAGTGAAGACTCAGGGTCTCCAATGGGTTCCGTGGGG	
4798-4838	GGGAAAGTGAAGACTCAGGGTCTCCAATGGGTTCCGTGGGG	
5194-5235	GGGAATTAAGGTAGGTGGGAGGAGAGGGATCCTCCGGACGGG	
5194-5236	GGGAATTAAGGTAGGTGGGAGGAGAGGGATCCTCCGGACGGGG	
5194-5237	GGGAATTAAGGTAGGTGGGAGGAGAGGGATCCTCCGGACGGGGG	
5194-5256	GGGAATTAAGGTAGGTGGGAGGAGAGGGATCCTCCGGACGGGGGCGATTGGCCGAAAAACGGG	
5210-5256	GGGAGGAGAGGGATCCTCCGGACGGGGGCGATTGGCCGAAAAACGGG	
5434-5464	GGGTGGGGTAAGGAGGGGTGTGAGTAGCGGG	
8208-8265	GGGTAGCAGGATCTTAATTAAGGGGATTTTTAGAAACTTTATCCCGGGCATAAATGGG	LS2as
8208-8275	GGGTAGCAGGATCTTAATTAAGGGGATTTTTAGAAACTTTATCCCGGGCATAAATGGGATATCGTGGG	
8208-8276	GGGTAGCAGGATCTTAATTAAGGGGATTTTTAGAAACTTTATCCCGGGCATAAATGGGATATCGTGGGG	
8208-8277	GGGTAGCAGGATCTTAATTAAGGGGATTTTTAGAAACTTTATCCCGGGCATAAATGGGATATCGTGGGGG	
8229-8296	GGGGATTTTTAGAAACTTTATCCCGGGCATAAATGGGATATCGTGGGGGAGATGGGGGAGATCTCGGG	
8230-8296	GGGATTTTTAGAAACTTTATCCCGGGCATAAATGGGATATCGTGGGGGAGATGGGGGAGATCTCGGG	
8253-8296	GGGCATAAATGGGATATCGTGGGGGAGATGGGGGAGATCTCGGG	
8263-8296	GGGATATCGTGGGGGAGATGGGGGAGATCTCGGG	
9244-9271	GGGTCGGGTACTGGGGATTGTCCCCGGG	
9244-9280	GGGTCGGGTACTGGGGATTGTCCCCGGGAGAGTCGGG	
9249-9280	GGGTACTGGGGATTGTCCCCGGGAGAGTCGGG	
9527-9574	GGGGATGGGGGGTTGATCCTCCCGTGACCGGGGGTTGTCCGTAGTGGG	
9527-9575	GGGGATGGGGGGTTGATCCTCCCGTGACCGGGGGTTGTCCGTAGTGGGG	
9528-9575	GGGATGGGGGGTTGATCCTCCCGTGACCGGGGGTTGTCCGTAGTGGGG	
9533-9596	GGGGGGTTGATCCTCCCGTGACCGGGGGTTGTCCGTAGTGGGGCGATTTAGGGGATCTTCAGGG	
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9536-9596	GGGTTGATCCTCCCGTGACCGGGGGTTGTCCGTAGTGGGGCGATTTAGGGGATCTTCAGGG	
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9557-9596	GGGGTTGTCCGTAGTGGGGCGATTTAGGGGATCTTCAGGG	
9558-9596	GGGTTGTCCGTAGTGGGGCGATTTAGGGGATCTTCAGGG	
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10163-10208	GGGGAATGCTCACGCCGAAGCTGGGATATAGGGGGCGGGCGCAGGG	
10164-10208	GGGAATGCTCACGCCGAAGCTGGGATATAGGGGGCGGGCGCAGGG	
10185-10208	GGGATATAGGGGGCGGGCGCAGGG	
12019-12087	GGGTGGTGTAATTGTTGTATTTTGGGAGTAAGTGTGCTCTTTTGTGGGAGTACAAGTATGTGGATAGGG	
12019-12088	GGGTGGTGTAATTGTTGTATTTTGGGAGTAAGTGTGCTCTTTTGTGGGAGTACAAGTATGTGGATAGGGG	
12019-12089	GGGTGGTGTAATTGTTGTATTTTGGGAGTAAGTGTGCTCTTTTGTGGGAGTACAAGTATGTGGATAGGGGG	
12042-12105	GGGAGTAAGTGTGCTCTTTTGTGGGAGTACAAGTATGTGGATAGGGGGTAAGAGGAGGATAGGG	
12042-12112	GGGAGTAAGTGTGCTCTTTTGTGGGAGTACAAGTATGTGGATAGGGGGTAAGAGGAGGATAGGGAGTTGGG	
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12363-12389	GGGATTGGGACTGAAGGGATTAAGGGG	
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13755-13808	GGGGGCGTAGGGGGAAGGTTTGTTGTTAGGGGGAGATGGATTTTGAGTGTCGGG	
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14228-14285	GGGTATTAGTATGTTTCGGGGGCGTGGTTATCCTAGGAGGGCTTAGTTGGGACTGGGG	
14245-14285	GGGGGCGTGGTTATCCTAGGAGGGCTTAGTTGGGACTGGGG	
14246-14285	GGGGCGTGGTTATCCTAGGAGGGCTTAGTTGGGACTGGGG	
14247-14285	GGGCGTGGTTATCCTAGGAGGGCTTAGTTGGGACTGGGG	
15503-15540	GGGTCTGTTAATATGGGATCGGTTGGGGAATTTGTGGG	LS6as
15503-15541	GGGTCTGTTAATATGGGATCGGTTGGGGAATTTGTGGGG	
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15503-15557	GGGTCTGTTAATATGGGATCGGTTGGGGAATTTGTGGGGAGGGGTGTAGTTCGGG	
15517-15557	GGGATCGGTTGGGGAATTTGTGGGGAGGGGTGTAGTTCGGG	
15527-15557	GGGGAATTTGTGGGGAGGGGTGTAGTTCGGG	
15528-15557	GGGAATTTGTGGGGAGGGGTGTAGTTCGGG	
16168-16191	GGGTTGGGTGTAGTTTGGGGGGGG	
16168-16192	GGGTTGGGTGTAGTTTGGGGGGGGG	
16168-16193	GGGTTGGGTGTAGTTTGGGGGGGGGG	
16168-16194	GGGTTGGGTGTAGTTTGGGGGGGGGGG	
16168-16195	GGGTTGGGTGTAGTTTGGGGGGGGGGGG	
16355-16397	GGGAAGAGCAGGGGTACCTACTGGGGGGAGTCTATCCCCAGGG	

FIGURES

Figure S1.

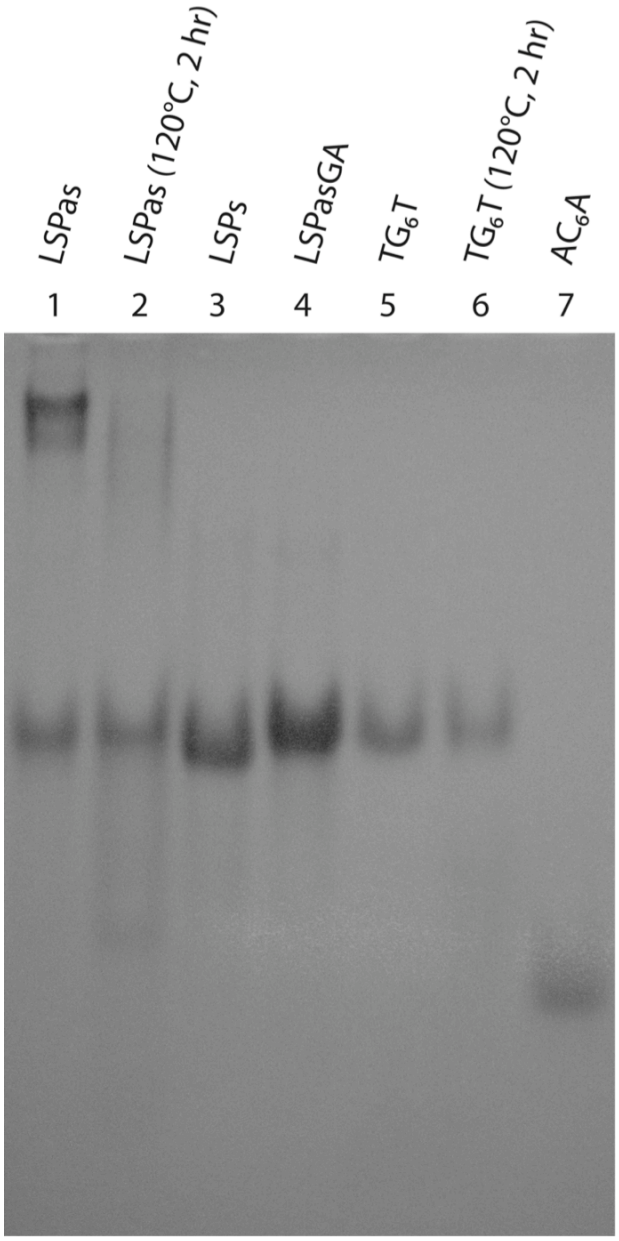


Figure S2.

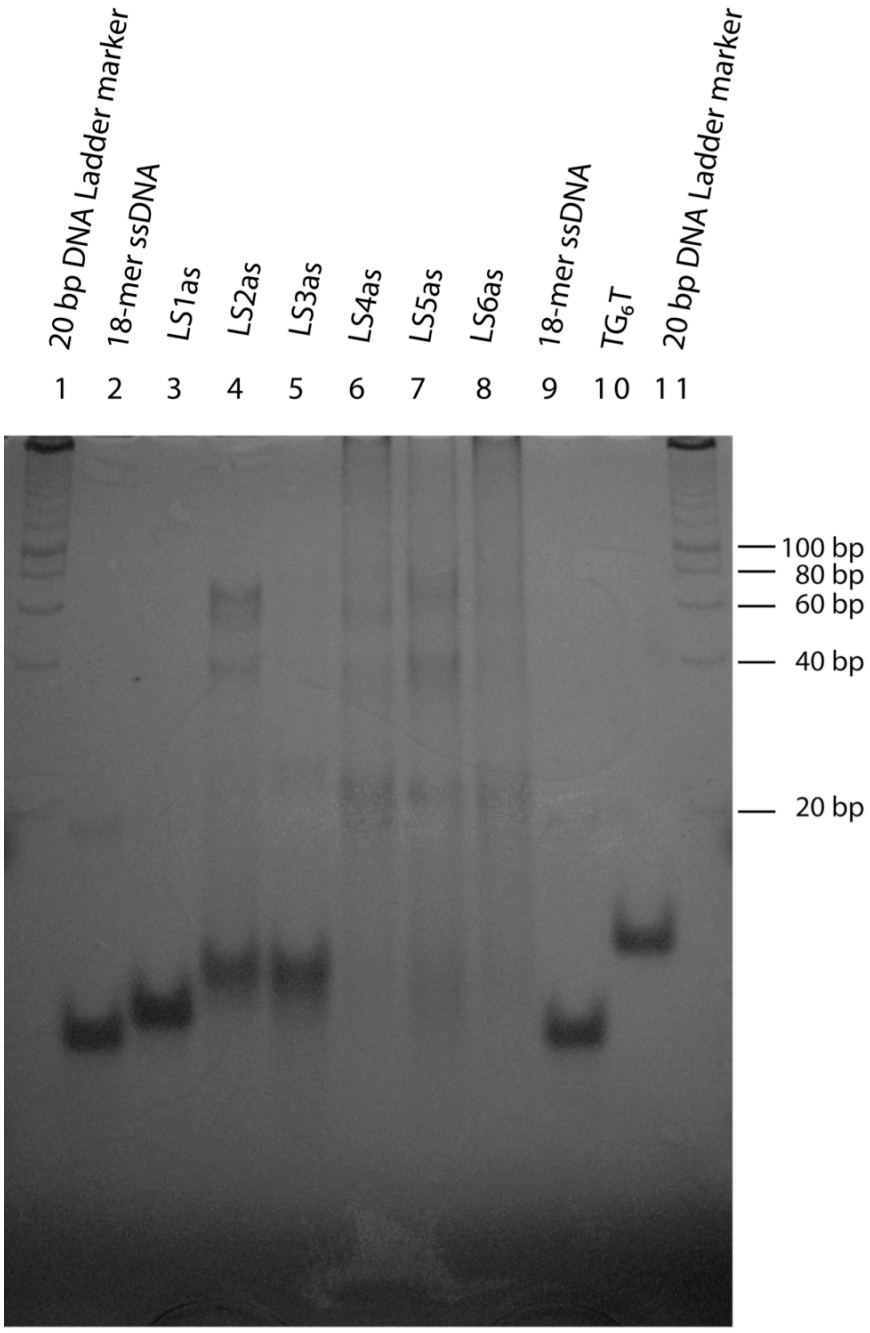

20 bp DNA Ladder marker
18-mer ssDNA
LS1as
LS2as
LS3as
LS4as
LS5as
LS6as
18-mer ssDNA
TG6T
20 bp DNA Ladder marker
1 2 3 4 5 6 7 8 9 10 11
100 bp
80 bp
60 bp
40 bp
20 bp

Figure S3.

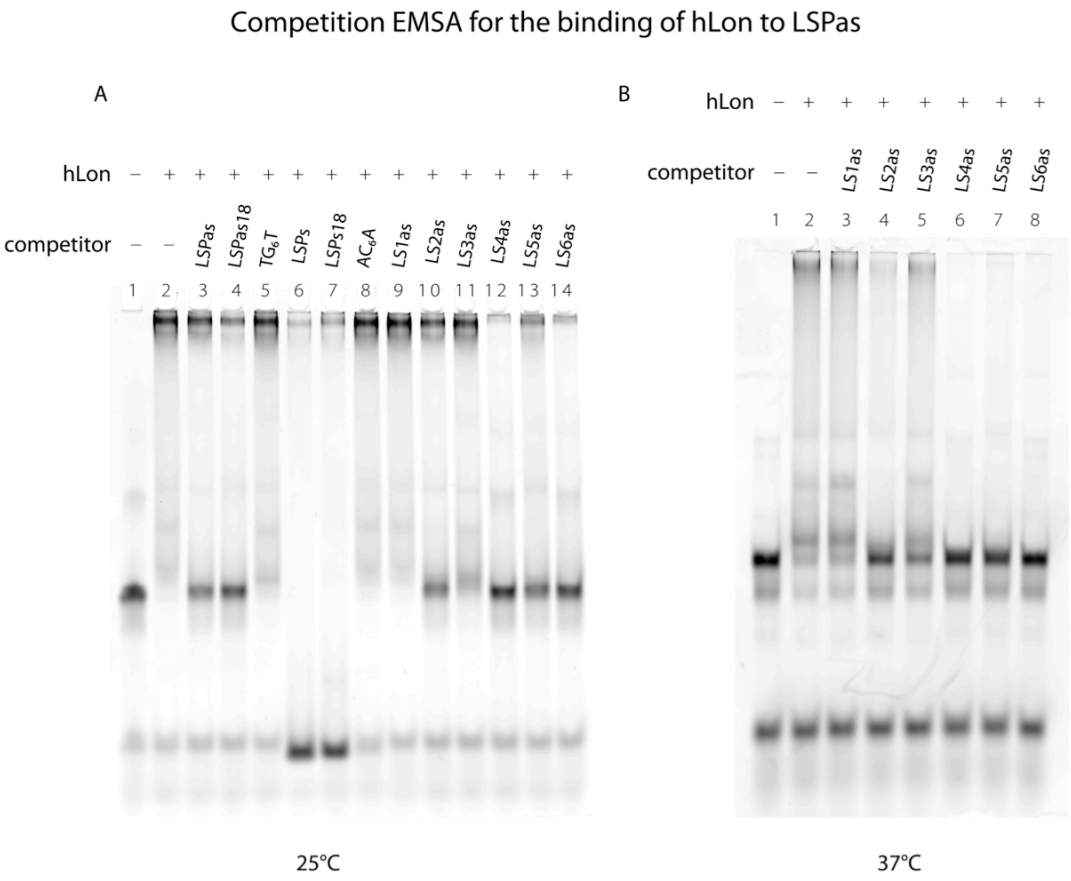

Competition EMSA for the binding of hLon to LSPas
A
hLon
competitor
LSPas
LSPas18
TG6T
LSPs
LSPs18
AC6A
LS1as
LS2as
LS3as
LS4as
LS5as
LS6as
1 2 3 4 5 6 7 8 9 10 11 12 13 14
25°C
B
hLon
competitor
LS1as
LS2as
LS3as
LS4as
LS5as
LS6as
1 2 3 4 5 6 7 8
37°C

Figure S4.

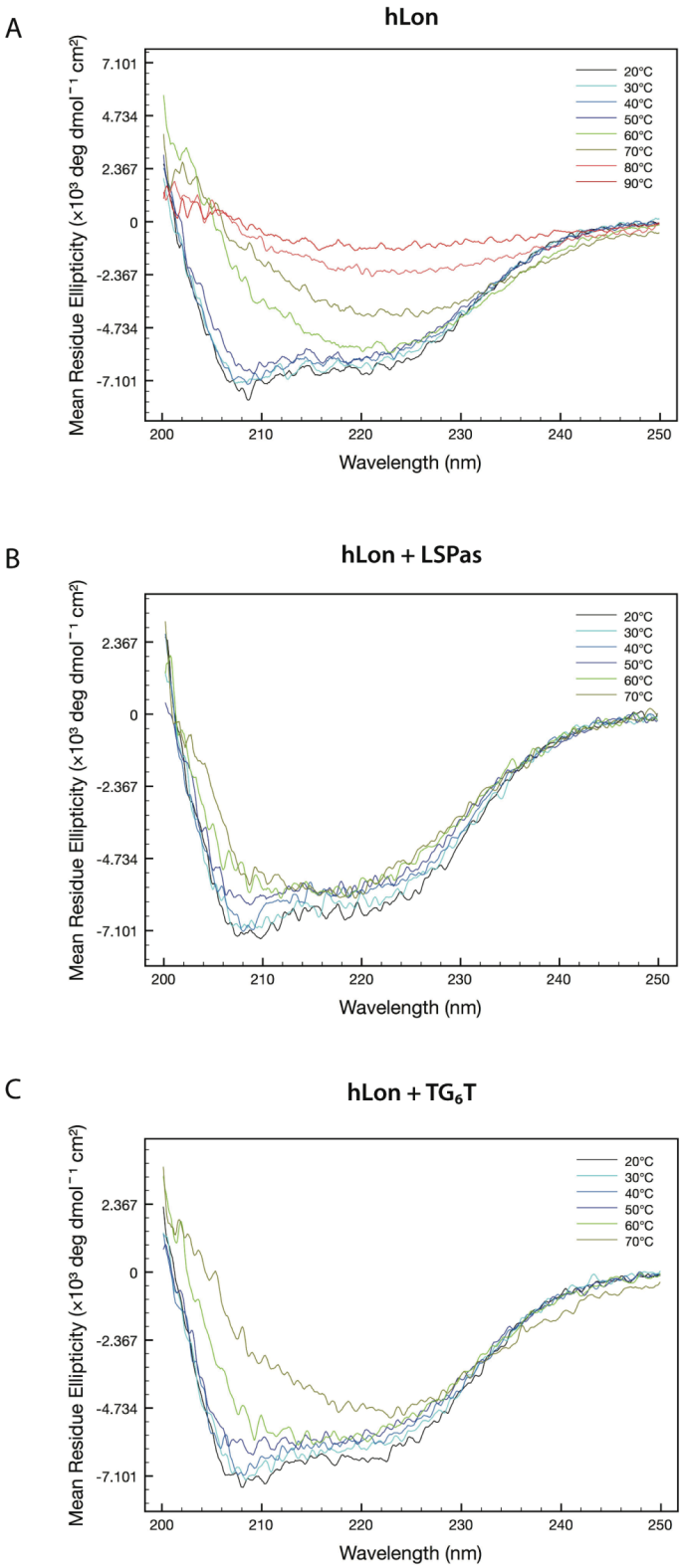
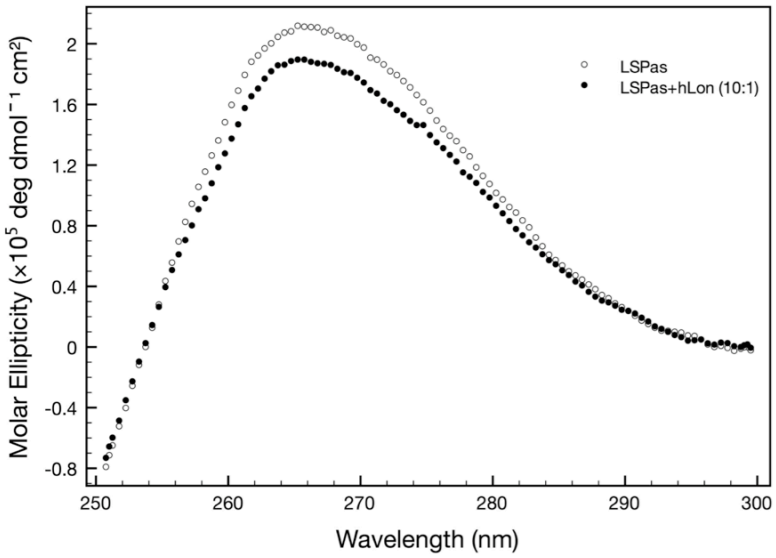

A
hLon
Mean Residue Ellipticity (×10³ deg dmol⁻¹ cm²)
7.101
4.734
2.367
0
-2.367
-4.734
-7.101
200
210
220
230
240
250
Wavelength (nm)
20°C
30°C
40°C
50°C
60°C
70°C
80°C
90°C
B
hLon + LSPas
Mean Residue Ellipticity (×10³ deg dmol⁻¹ cm²)
2.367
0
-2.367
-4.734
-7.101
200
210
220
230
240
250
Wavelength (nm)
20°C
30°C
40°C
50°C
60°C
70°C
C
hLon + TG6T
Mean Residue Ellipticity (×10³ deg dmol⁻¹ cm²)
2.367
0
-2.367
-4.734
-7.101
200
210
220
230
240
250
Wavelength (nm)
20°C
30°C
40°C
50°C
60°C
70°C

Figure S5.

A


LSPas
LSPas+hLon (10:1)
Molar Ellipticity (×10⁵ deg dmol⁻¹ cm²)
Wavelength (nm)
2
1.6
1.2
0.8
0.4
0
-0.4
-0.8
250
260
270
280
290
300

B

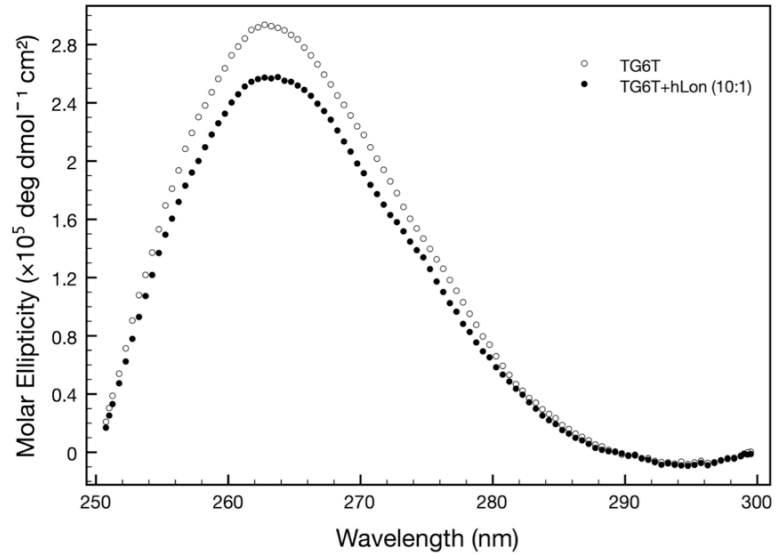
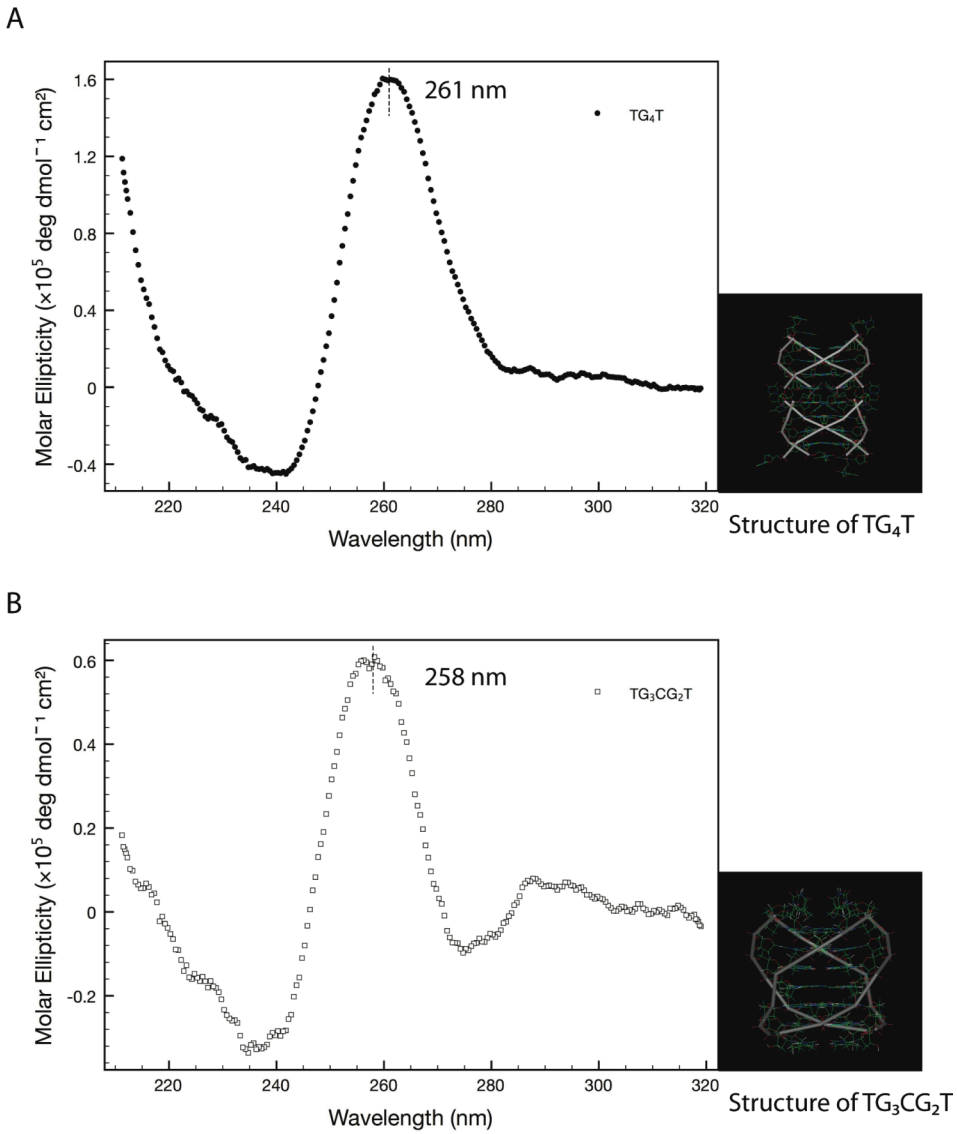

TG6T
TG6T+hLon (10:1)
Molar Ellipticity (×10⁵ deg dmol⁻¹ cm²)
Wavelength (nm)
2.8
2.4
2
1.6
1.2
0.8
0.4
0
250
260
270
280
290
300

Figure S6.


A
261 nm
TG4T
Molar Ellipticity (×10^5 deg dmol^-1 cm^2)
Wavelength (nm)
Structure of TG4T
B
258 nm
TG3CG2T
Molar Ellipticity (×10^5 deg dmol^-1 cm^2)
Wavelength (nm)
Structure of TG3CG2T
C
AC6A
TG6T
TG3CG2T
TG4T
1
2
3
4