The importance of loop length on the stability of i-motif structures

Article

Accepted Version

The importance of loop length on the stability of i-motif structures

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Supporting information

Experimental

All chemicals and oligonucleotides were purchased from Sigma-Aldrich. Oligonucleotides were purified by reverse phase column by Sigma-Aldrich and characteristic mass spectroscopy data is provided in table S1. UV spectroscopy was carried out using an Agilent Cary 100 with a temperature controlled 6 x 6 cell changer. CD spectra were collected on beamline B23 at Diamond Light Source.

Table S1 – Characteristic mass spectrum data for oligonucleotides used in this study.

Name	Sequence 5'-3'	Theorectical MW	Observed MW
C3T333	d(CCC**TTT**CCC**TTT**CCC**TTT**CCCT)	6452.64	6460.86
C3T444	d(CCC**TTTT**CCC**TTTT**CCC**TTTT**CCCT)	7365.30	7361.40
C3T555	d(CCC**TTTTT**CCC**TTTTT**CCC**TTTTT**CCCT)	8277.96	8288.85
C3T666	d(CCC**TTTTTT**CCC**TTTTTT**CCC**TTTTTT**CCCT)	9190.62	9205.65
C3T777	d(CCC**TTTTTTT**CCC**TTTTTTT**CCC**TTTTTTT**CCCT)	10103.28	10104.34
C3T888	d(CCC**TTTTTTTT**CCC**TTTTTTTT**CCC**TTTTTTTT**CCCT)	11015.94	11019.20
C3T338	d(CCC**TTT**CCC**TTT**CCC**TTTTTTTT**CCC)	7669.52	7673.01
C3T383	d(CCC**TTT**CCC**TTTTTTTT**CCC**TTT**CCC)	7669.52	7674.29
C3T833	d(CCC**TTTTTTTT**CCC**TTT**CCC**TTT**CCC)	7669.52	7674.29
C3T883	d(CCC**TTTTTTTT**CCC**TTTTTTT**CCC**TTT**CCC)	9190.62	9190.70
C3T838	d(CCC**TTTTTTTT**CCC**TTT**CCC**TTTTTTTT**CCC)	9190.62	9193.90
C3T388	d(CCC**TTT**CCC**TTTTTTTT**CCC**TTTTTTTT**CCC)	9190.62	9189.10
C3T8AA38	d(CCC**TTTAATTT**CCC**TTT**CCC**TTTTTTTT**CCC)	9208.94	9216.12
C3T838AA	d(CCC**TTTTTTTT**CCC**TTT**CCC**TTTAATTT**CCC)	9208.94	9212.72

Oligonucleotide Concentrations

The concentration of all oligonucleotides were calculated from the absorbance value at 260 nm using extinction coefficients calculated using the nearest neighbour method.

UV melting experiments

Oligonucleotides were dissolved in 50 mM sodium cacodylate buffer of the appropriate pH to a final concentration of 1 µM, and were annealed by heating to 90°C and slowly cooling to room temperature. UV spectra were recorded at 260 and 295 nm at 1°C intervals between 20-90 °C, with a temperature change rate of 1°C/min in a 1cm pathlength cuvette.

UV pH titrations

Oligonucleotides were dissolved in 50 mM sodium cacodylate at pH 8, and a spectrum recorded between 200-350 nm. The pH was adjusted by the addition of small aliquots (approximately 1 µL) of HCl and measured in the cuvette using a Thermo Scientific Orion Star A11 pH meter equipped with a

small diameter pH electrode. A UV spectrum was recorded between 200-350 nm wavelengths at 25°C for each pH point.

CD Spectroscopy

Oligonucleotides were dissolved in 20 mM sodium cacodylate buffer of the appropriate pH to a final concentration of 5 µM, and were annealed by heating to 90°C and slowly cooling to room temperature. CD spectra were recorded at 20 °C between 200 and 350 nm wavelengths with 1 nm wavelength increments in a 1cm pathlength cuvette on beamline B23 at Diamond Light Source.

UV melting data for C3T333-C3T888 at 295 nm

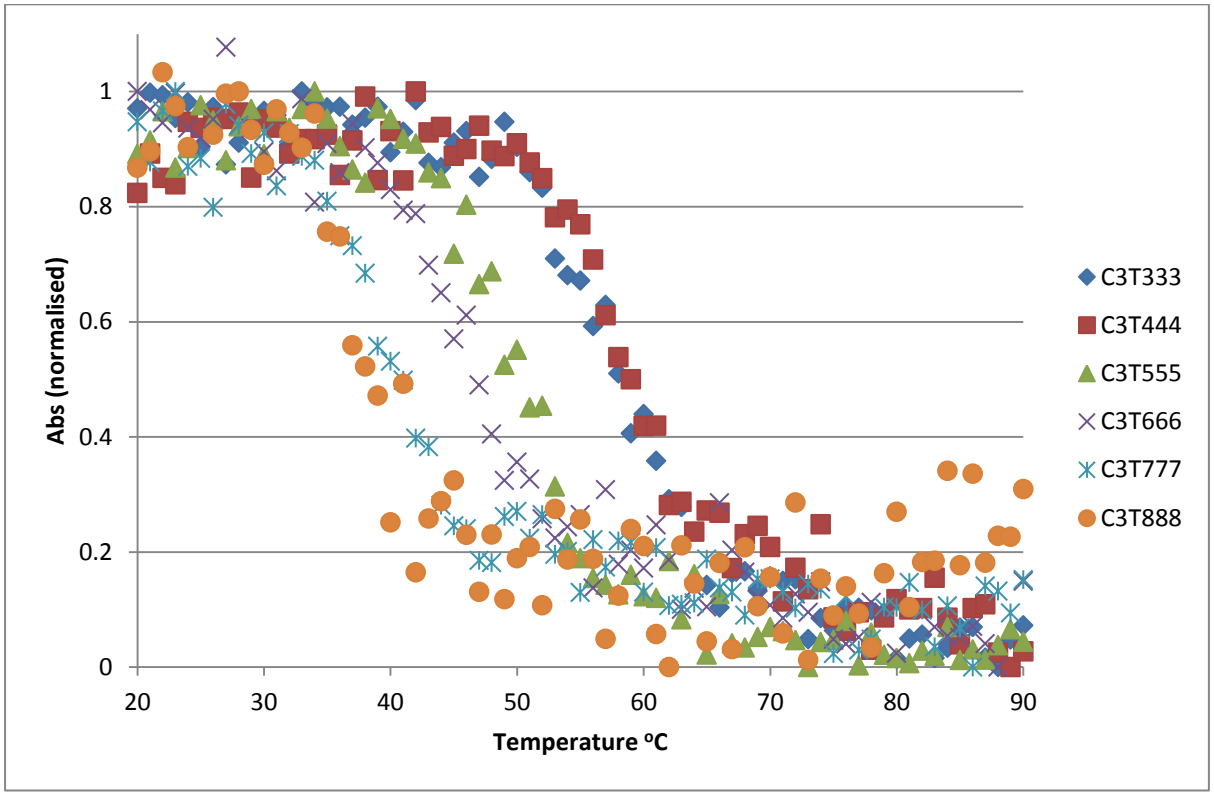

Figure S1 – UV melting curves for C3TXXX at 295 nm where X = 3-8

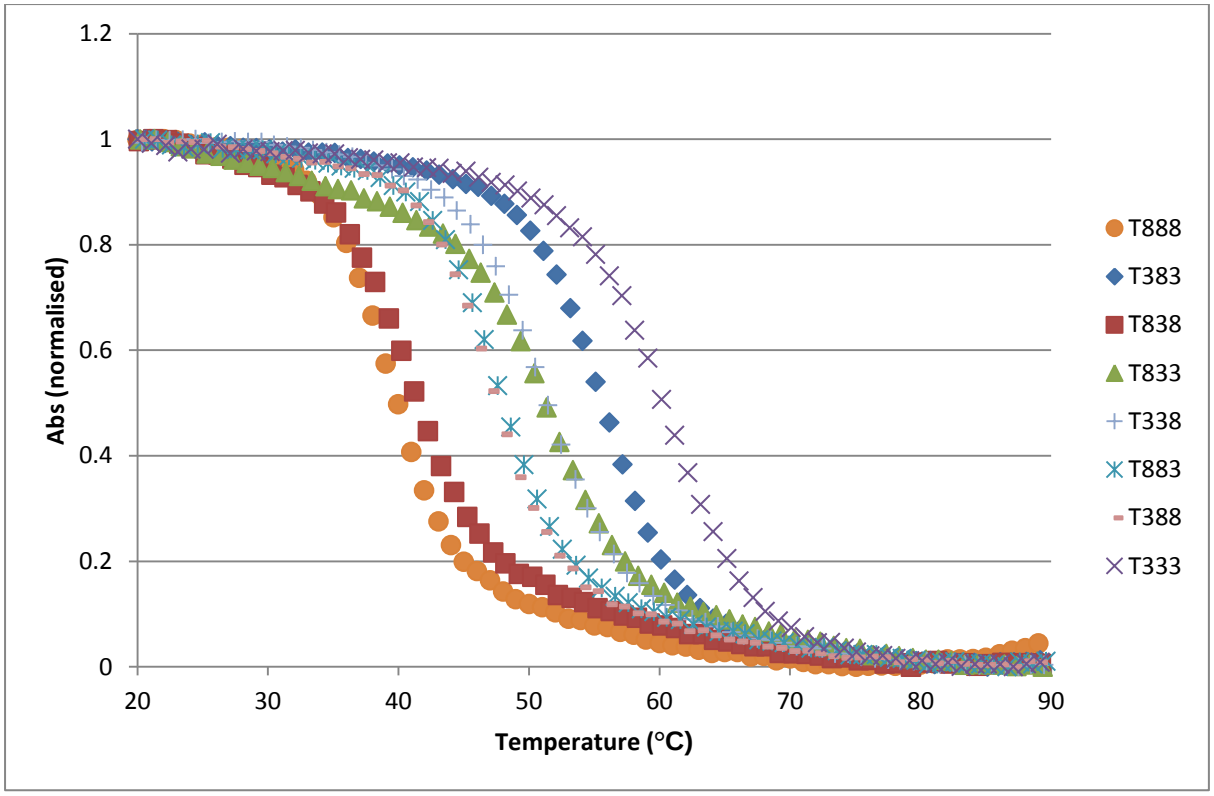

Figure S2 – UV melting curves for C3TXYZ at 295 where X, Y and Z = 3 or 8

UV pH titrations

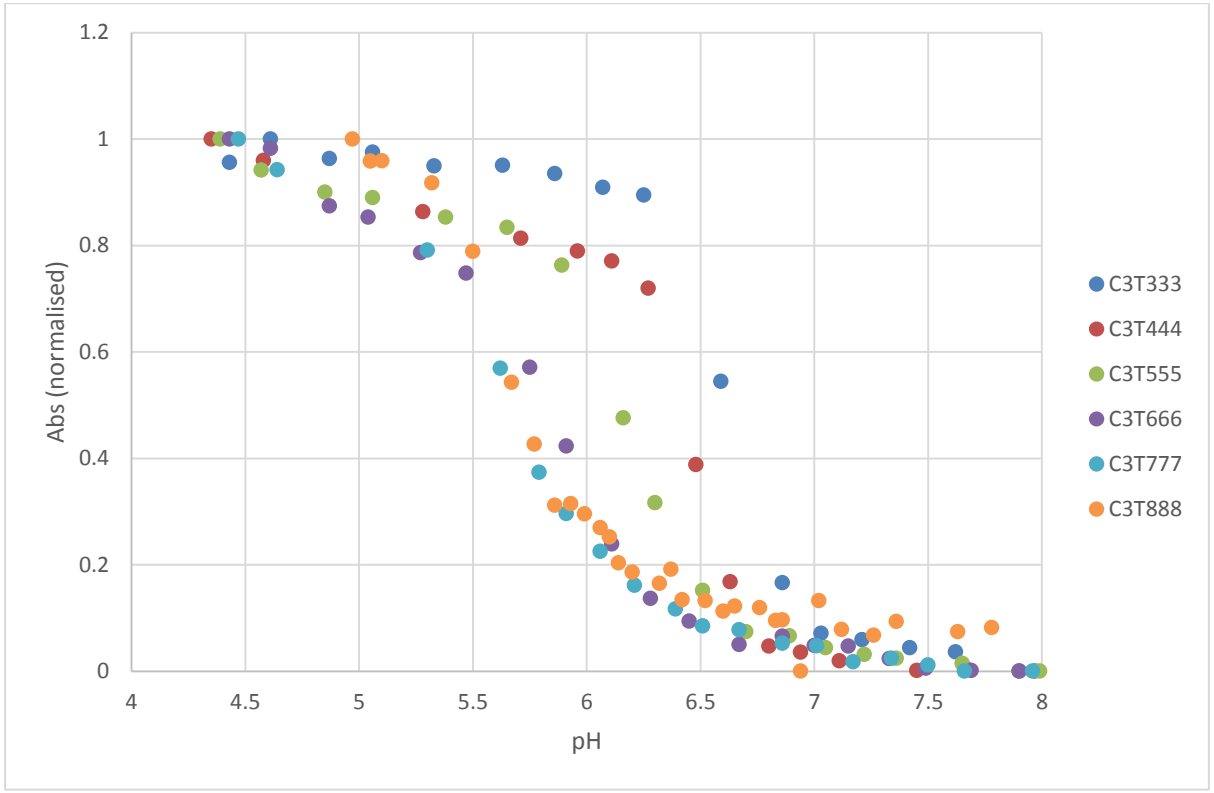

Figure S3 – UV pH titrations of C3TXXX at 295 where X = 3-8

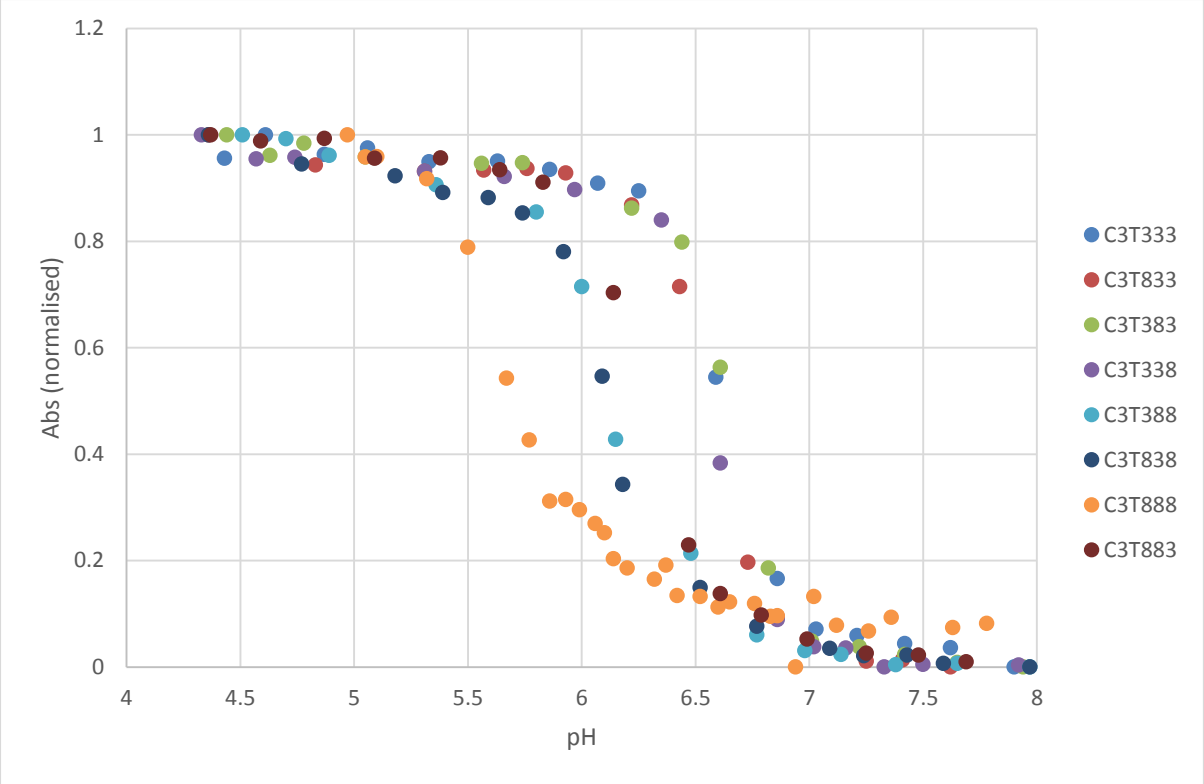

Figure S4 - UV pH titrations of C3TXYZ at 295 where X, Y and Z = 3 or 8

UV melting for sequences containing AA loops

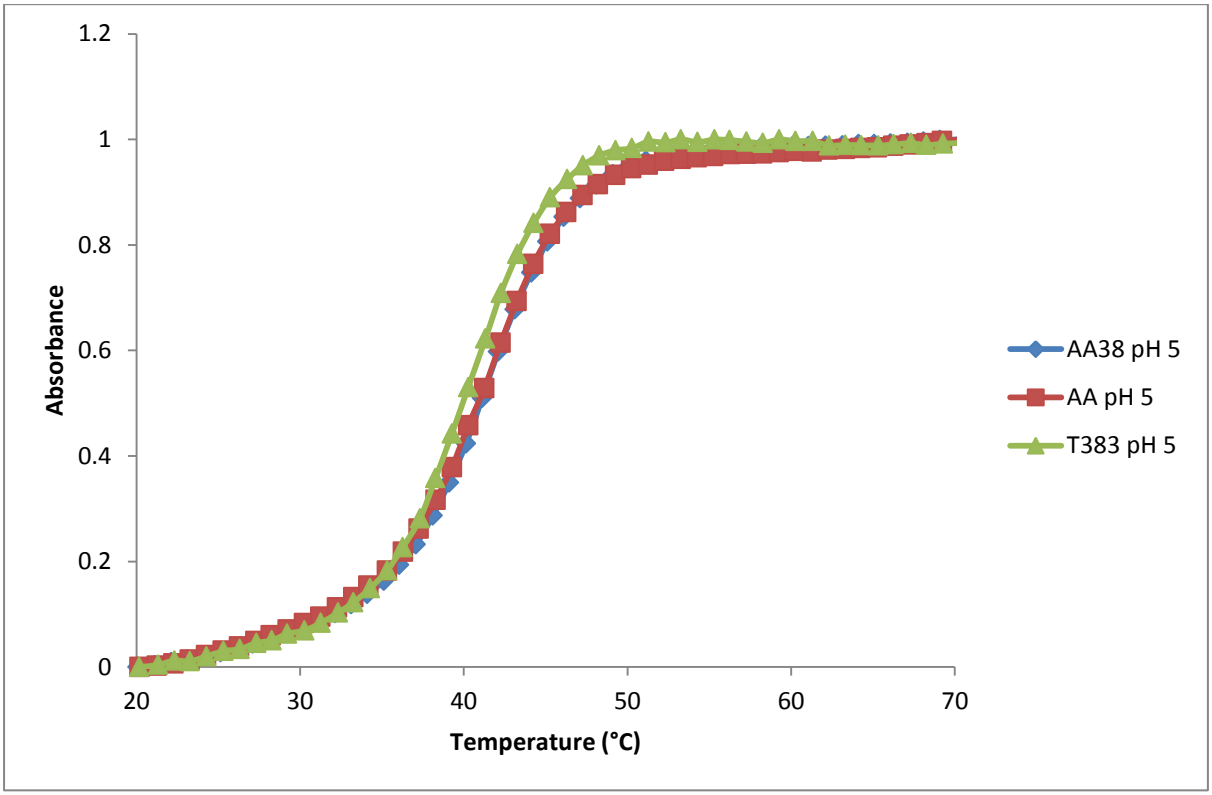

Figure S5 - UV melting curves for C3(AA)T38 (d(C_3TTTAATTT$C_3T_3C_3T_8C_3$), C3T83(AA) (d($C_3T_8C_3T_3C_3$TTTAATTTC_3) and C3T838 at 260nm. 50 mM sodium cacodylate pH 5

UV melting at different salt concentrations

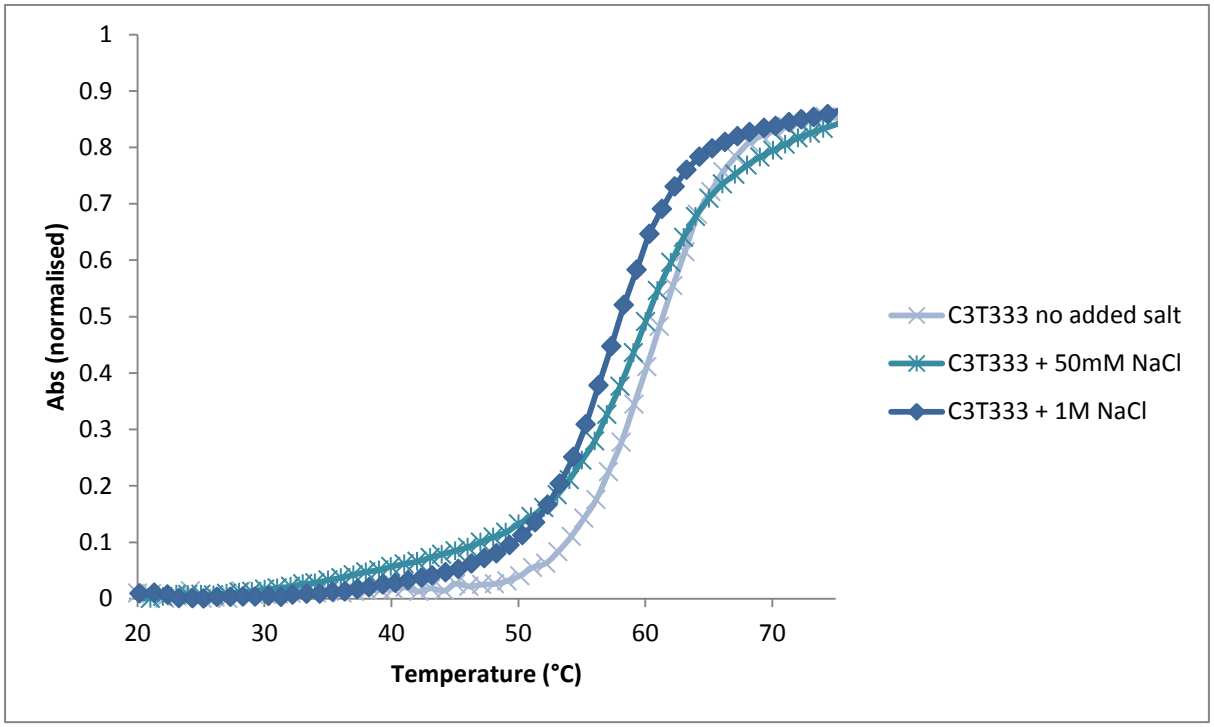

Figure S6 - UV melting curves of C3T333 with increasing salt concentration.

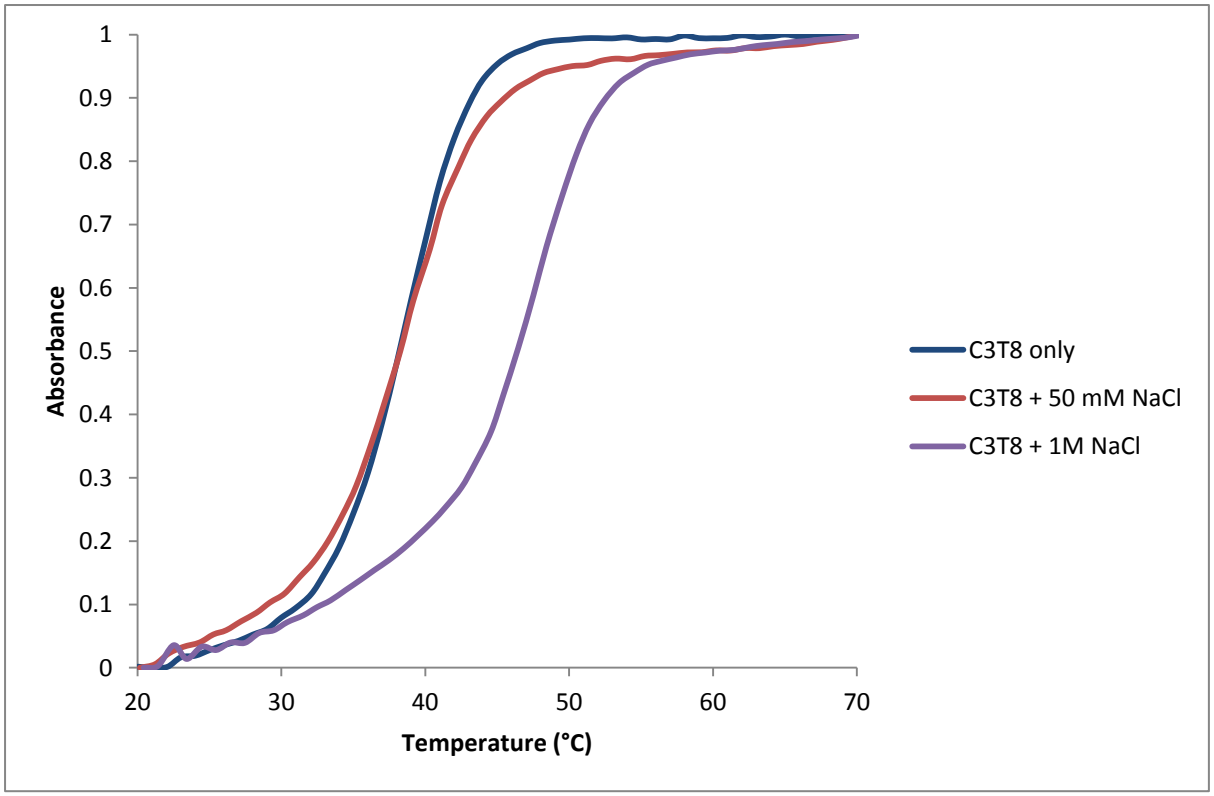

Figure S7 - UV melting curves of C3T888 with increasing salt concentration.

CD Spectra

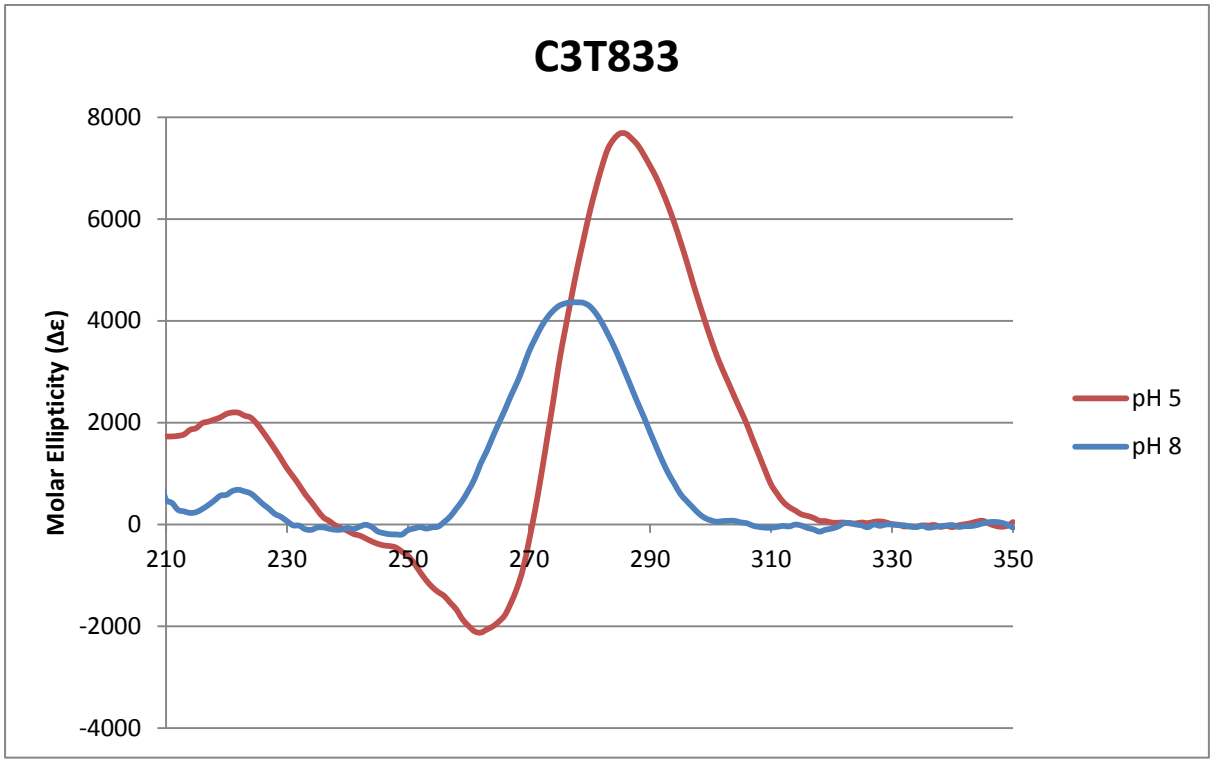

Figure S8 – CD Spectra of C3T833 at pH 5 and 8

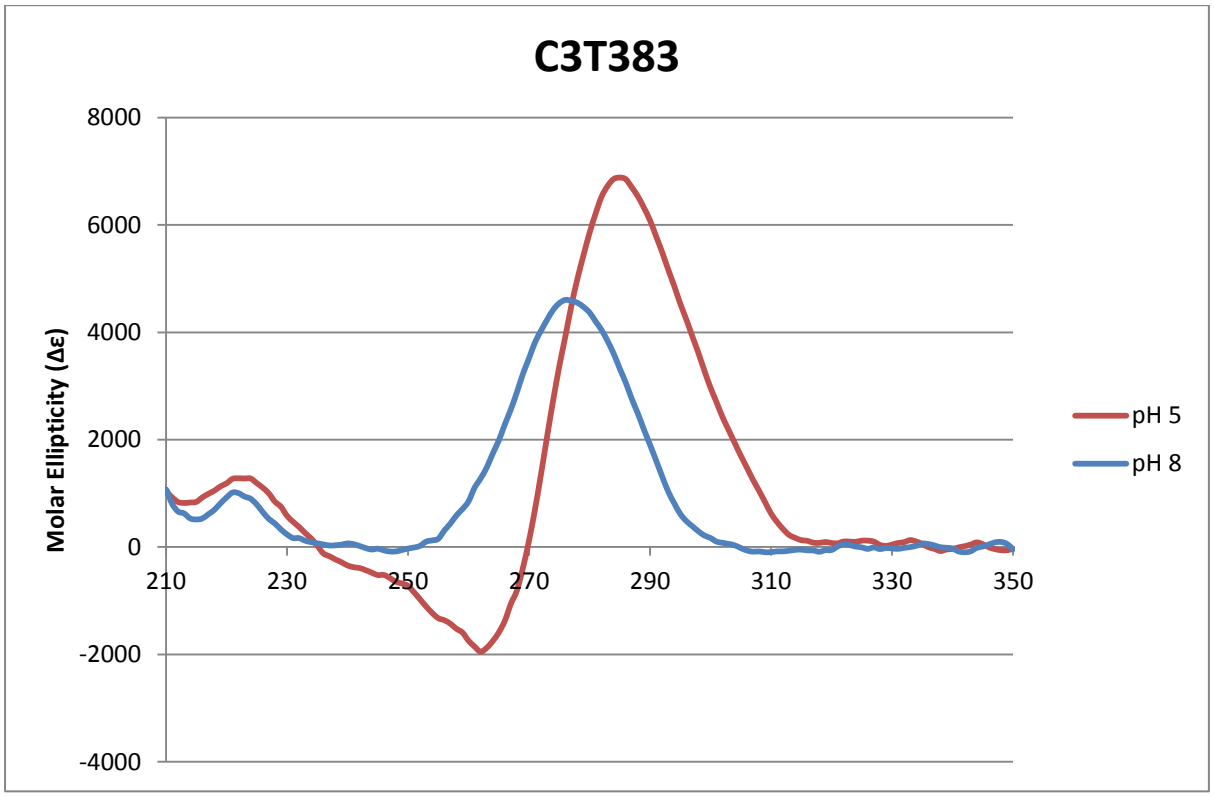

Figure S9 – CD Spectra of C3T383 at pH 5 and 8

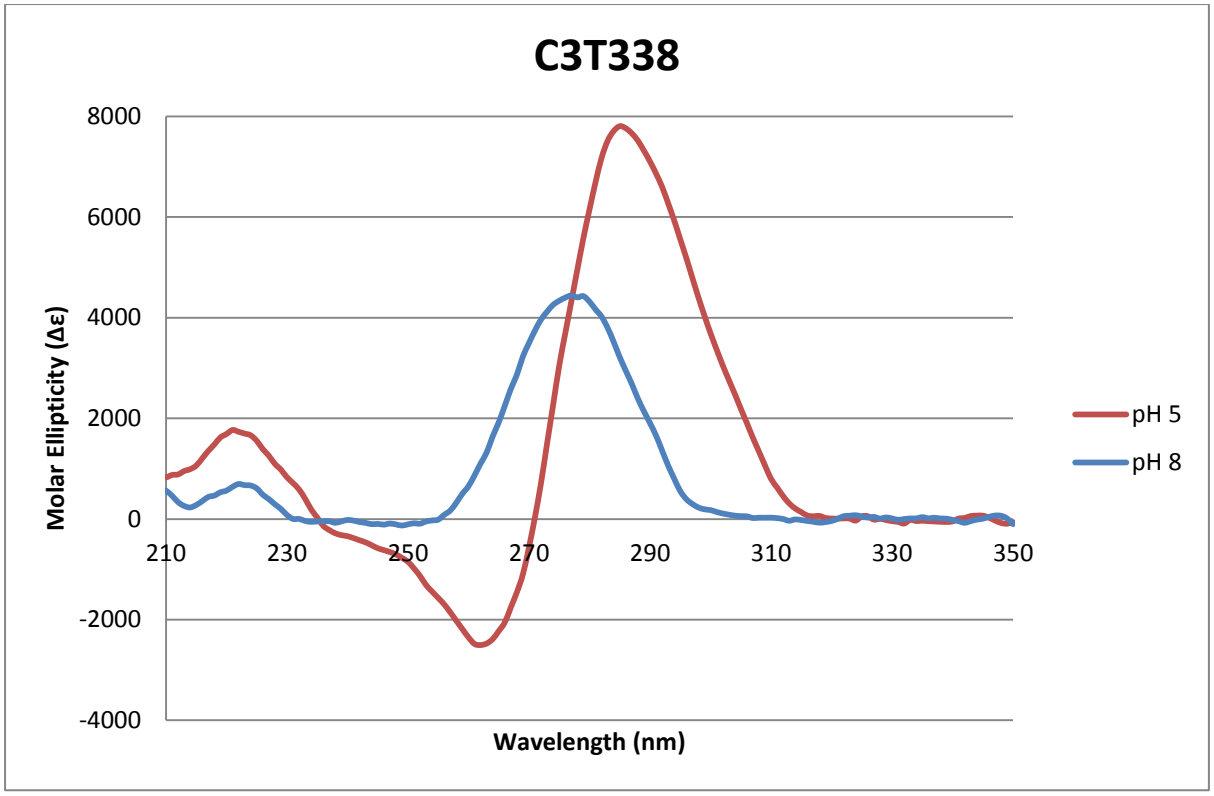

Figure S10 – CD Spectra of C3T338 at pH 5 and 8

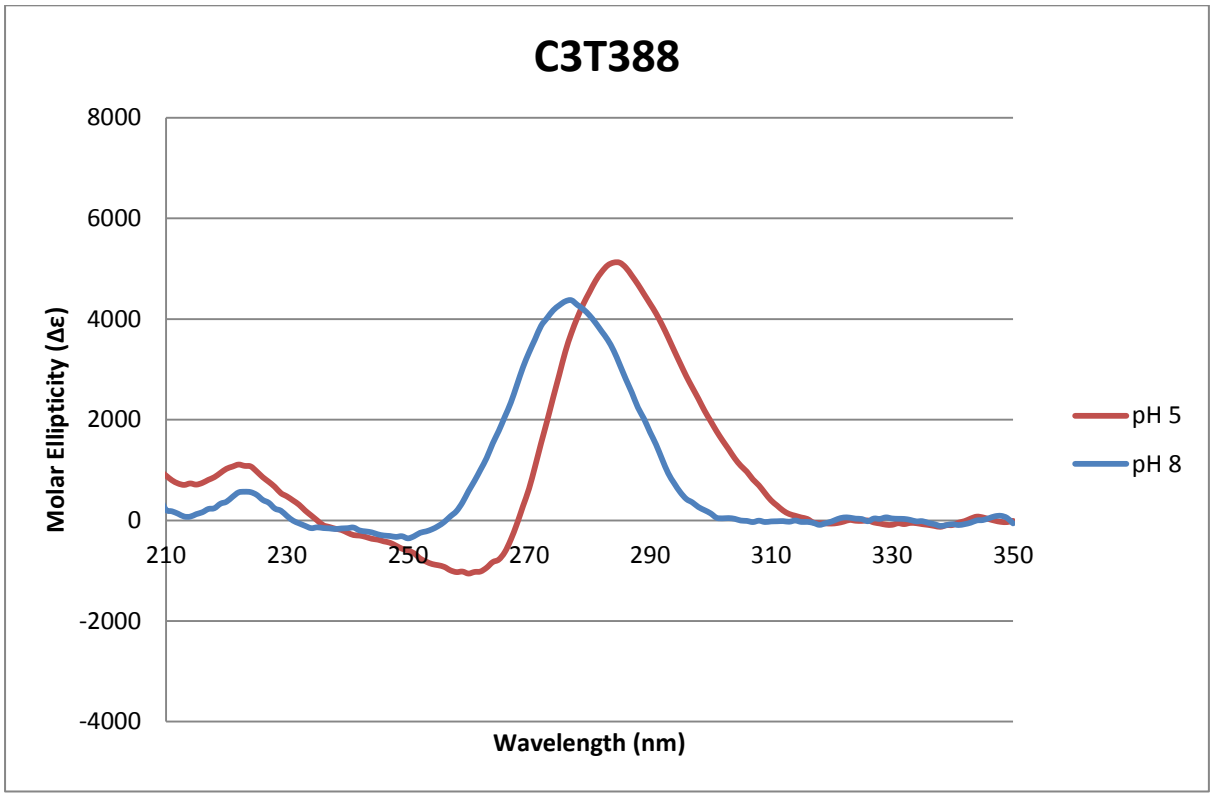

Figure S11 – CD Spectra of C3T388 at pH 5 and 8

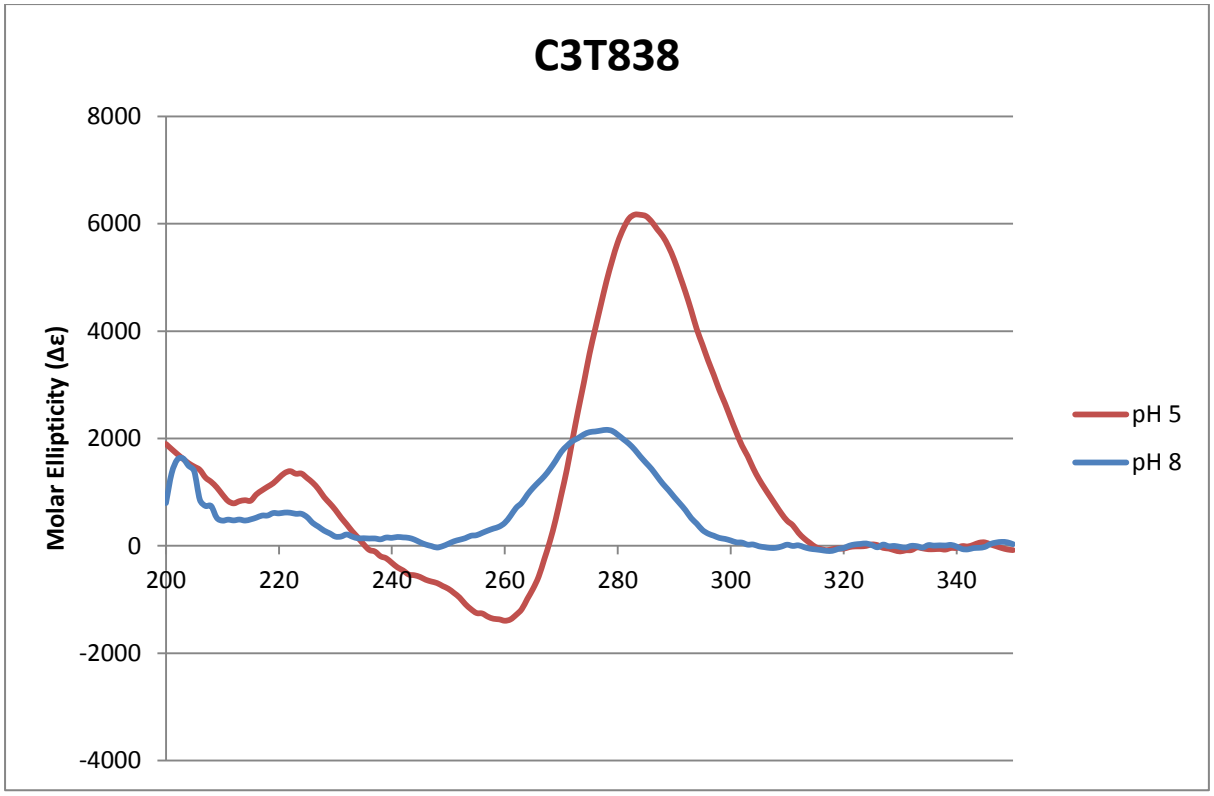

Figure S12 – CD Spectra of C3T838 at pH 5 and 8

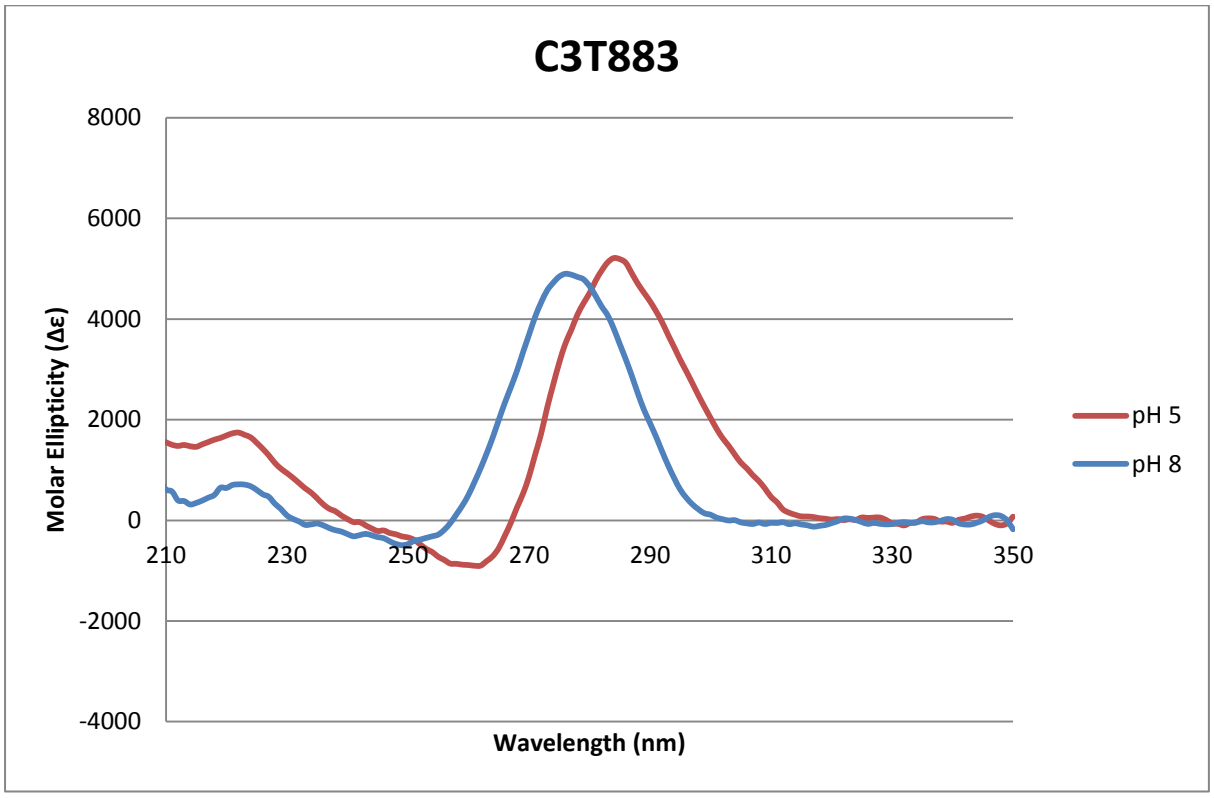

Figure S13 – CD Spectra of C3T883 at pH 5 and 8