Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information:

Description of the system	Number of nucleotides	Number of sodium counterions	Number of water molecules
Modelled MicroROSE	29	28	4081
ΔG10 MicroROSE	28	27	3573
Mutated A5U MicroROSE	29	28	4081
Mutated U25A MicroROSE	29	28	4081

Table ST1 Description of four starting structures investigated in this study.

Nucleotide numbers associated with steps for modelled MicroROSE	Nucleotide numbers associated with steps for ΔG10 MicroROSE	Nucleotide numbers associated with steps for mutated A5U MicroROSE	Nucleotide numbers associated with steps for mutated U25A MicroROSE
G1:C29-G2:C28	G1: C29-G2:C28	G1:C29-G2:C28	G1:C29-G2:C28
G2:C28-C3:G27	G2: C28-C3:G27	G2:C28-C3:G27	G2:C28-C3:G27
C3:G27-C4:G26	C3: G27-C34:G26	C3:G27-C4:G26	C3:G27-C4:G26
C4:G26-A5:U25	C4: G26-A5:U25	C4:G26-U5:U25	C4:G26-A5:A25
U8:A22-U9:G21	U8:A22-9U:21G	U8:A22-U9:G21	U8:A22-U9:G21
C11:G20-U12:A19	U9:G21-C11:G20	C11:G20-U12:A19	C11:G20-U12:A19
U12:A19-C13:G18	C11:G20-U12:A19	U12:A19-C13:G18	U12:A19-C13:G18
	U12:A19-C13:G1		

Table ST2 Presents nucleotide numbers associated with base paired dinucleotide steps for modelled MicroROSE, ΔG10 MicroROSE, mutated U5A MicroROSE and mutated U25A MicroROSE. (Considered only consecutive stacked base pairs are considered).

Nucleotide numbers associated with steps	VDW (kcal/mol)	ELEC (kcal/mol)	Total stacking energy at 315K	VDW (kcal/mol)	ELEC (kcal/mol)	Total stacking energy at 350K	VDW (kcal/ mol)	ELEC (kcal/mol)	Total stacking energy at 400K
G1:C29-G2:C28	-10.53	-4.41	-14.94±0.11	-10.30	-4.28	-14.58±0.44	-9.70	-3.78	-12.48±1.08
G2:C28-C3:G27	-10.43	-4.03	-14.46±0.18	-10.21	-3.90	-14.11±0.56	-9.62	-3.75	-13.37±1.11
C3:G27-C4:G26	-9.72	-3.80	-13.52±0.26	-9.61	-3.75	-13.36±0.37	-8.88	-2.71	-11.59±0.98
C4:G26-A5:U25	-9.03	-3.61	-12.64±0.31	-8.91	-3.54	-12.45±0.31	-8.18	-2.05	-11.23±1.11
U8:A22-U9:G21	-9.50	-2.80	-12.30±0.24	-9.38	-2.69	-12.07±0.45	-7.01	-1.77	-8.78±1.08
C11:G20-U12:A19	-9.20	-2.95	-12.15±0.27	-9.03	-2.81	-11.84±0.39	-6.59	-1.38	-7.97±1.23
U12:A19-C13:G18	-9.10	-2.91	-12.01±0.34	-8.95	-2.77	-11.72±0.45	-6.50	-1.25	-7.75±1.16

Table ST3 Average stacking energy (kcal/mol) of the constituent dinucleotide steps for modelled MicroROSE system where total stacking energy is composed of van der Waals and electrostatic energy and has been calculated over the last 25 ns of simulation run.

Nucleotide numbers associated with steps	VDW (Kcal/mol)	ELEC (Kcal/mol)	Total stacking energy at 315K	VDW (Kcal/mol)	ELEC (Kcal/mol)	Total stacking energy at 350K	VDW (Kcal/mol)	ELEC (Kcal/mol)	Total stacking energy at 400K
G1: C29-G2:C28	-10.56	-4.46	-15.02±0.11	-10.57	-4.36	-14.93±0.45	-10.30	-4.28	-14.58±0.76
G2: C28-C3:G27	-10.57	-4.32	-14.89±0.21	-10.50	-4.25	-14.75±0.48	-10.18	-4.01	-14.19±0.67
C3: G27-C34:G26	-9.82	-3.90	-13.72±0.08	-9.75	-3.85	-13.60±0.41	-9.61	-3.36	-12.97±0.71
C4: G26-A5:U25	-9.38	-3.82	-13.20±0.17	-9.30	-3.65	-12.95±0.51	-9.01	-2.98	-11.99±0.77
U8:A22-9U:21G	-9.78	-2.83	-12.61±0.21	-9.48	-2.75	-12.23±0.54	-8.61	-1.81	-10.42±0.68
U9:G21-C11:G20	-9.50	-2.74	-12.24±0.16	-9.40	-2.60	-12.00±0.52	-8.42	-1.67	-10.09±0.78
C11:G20-U12:A19	-9.24	-2.98	-12.22±0.25	-9.10	-2.83	-1.1.93±0.61	-7.75	-1.78	-9.53±0.72
U12:A19-C13:G1	-9.20	-3.00	-12.20±0.28	-9.05	-2.82	-11.87±0.65	-7.79	-1.72	-9.51±0.78

Table ST4 Average stacking energy (kcal/mol) of the constituent dinucleotide steps for ΔG10 MicroROSE system where total stacking energy is composed of van der Waals and electrostatic energy and has been calculated over the last 25 ns of simulation run.

Nucleotide numbers associated with steps	VDW (kcal/mol)	ELEC (kcal/mol)	Total stacking energy at 315K	VDW (kcal/mol)	ELEC (kcal/mol)	Total stacking energy at 350K	VDW (kcal/mol)	ELEC (kcal/mol)	Total stacking energy at 400K
G1:C29-G2:C28	-10.52	-4.41	-14.93±0.14	-10.28	-4.21	-14.49±0.61	-9.27	-3.58	-11.85±1.21
G2:C28-C3:G27	-10.41	-4.01	-14.42±0.17	-10.15	-3.80	-13.95±0.55	-9.43	-3.24	-12.67±1.34
C3:G27-C4:G26	-9.65	-3.55	-13.20±0.21	-9.55	-3.38	-12.93±0.48	-8.15	-2.10	-10.25±1.41
C4:G26-U5:U25	-8.88	-3.50	-12.38±0.24	-8.74	-3.35	-12.09±0.68	-7.70	-1.80	-9.50±1.28
U8:A22-U9:G21	-9.35	-2.68	-12.03±0.17	-9.12	-2.51	-11.63±0.63	-6.93	-1.68	-8.61±1.38
C11:G20-U12:A19	-9.08	-2.80	-11.88±0.29	-8.88	-2.55	-11.43±0.54	-6.43	-1.30	-7.73±1.35
U12:A19-C13:G18	-8.98	-2.77	-11.75±0.31	-8.71	-2.58	-11.29±0.71	-6.36	-1.18	-7.54±1.45

Table ST5 Average stacking energy (kcal/mol) of the constituent dinucleotide steps for mutated A5U MicroROSE system where total stacking energy is composed of van der Waals and electrostatic energy and has been calculated over the last 25 ns of simulation run.

Nucleotide numbers associated with steps	VDW (kcal/mol)	ELEC (kcal/mol)	Total stacking energy at 315K	VDW (kcal/ mol)	ELEC (kcal/mol)	Total stacking energy at 350K	VDW (kcal/mol)	ELEC (kcal/mol)	Total stacking energy at 400K
G1:C29-G2:C28	-10.51	-4.40	-14.91±0.21	-10.18	-4.16	-14.34±0.40	-8.48	-2.80	-11.28±1.11
G2:C28-C3:G27	-10.28	-3.97	-14.25±0.16	-10.11	-3.74	-13.85±0.54	-9.31	-3.02	-12.33±1.18
C3:G27-C4:G26	-9.58	-3.51	-13.09±0.23	-9.37	-3.40	-12.77±0.38	-8.01	-1.88	-9.89±1.03
C4:G26-A5:A25	-8.78	-3.45	-12.23±0.18	-8.52	-3.17	-11.69±0.65	-7.58	-1.75	-9.33±1.21
U8:A22-U9:G21	-9.30	-2.60	-11.90±0.28	-9.01	-2.32	-11.33±0.59	-5.80	-1.32	-7.12±1.18
C11:G20 - U12:A19	-8.98	-2.70	-11.68±0.13	-8.25	-2.49	-10.74±0.71	-5.32	-1.15	-6.47±1.31
U12:A19 - C13:G18	-8.92	-2.68	-11.60±0.31	-8.10	-2.38	-10.48±0.78	-5.11	-1.01	--6.12±1.35

Table ST6 Average stacking energy (kcal/mol) of the constituent dinucleotide steps for mutated U25A MicroROSE system where total stacking energy is composed of van der Waals and electrostatic energy and has been calculated over the last 25 ns of simulation run.

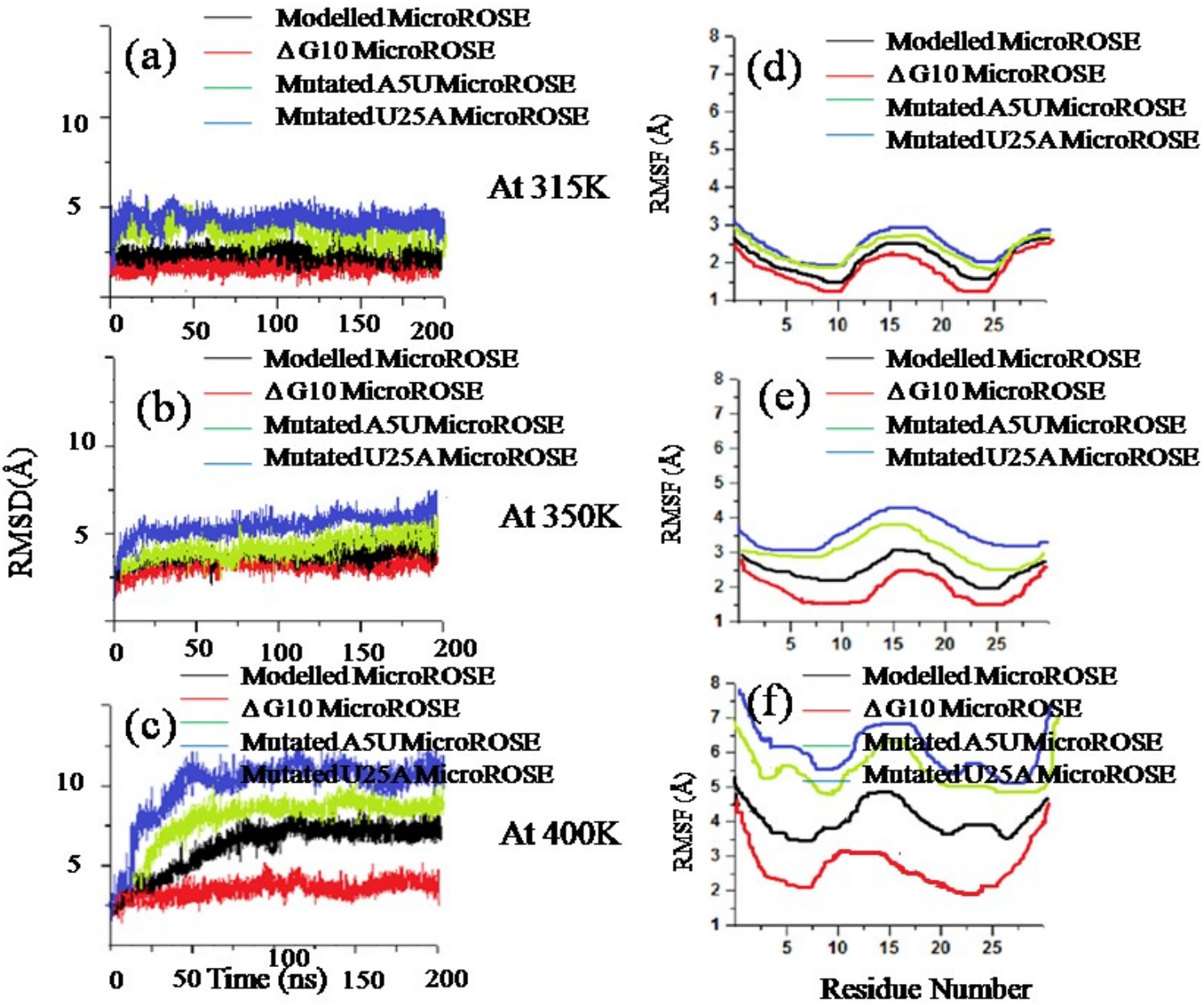

Fig. S1 Root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of four simulated systems with different starting velocities (S2 simulation). Backbone RMSD of four systems during the 200 ns simulation are calculated with respect to their equilibrated structures, plotted against time (a) At 315 K, (b) At 350 K and (c) At 400 K. Backbone RMSF of each residue for four systems is plotted (d) At 315 K, (e) At 350 K and (f) At 400 K.

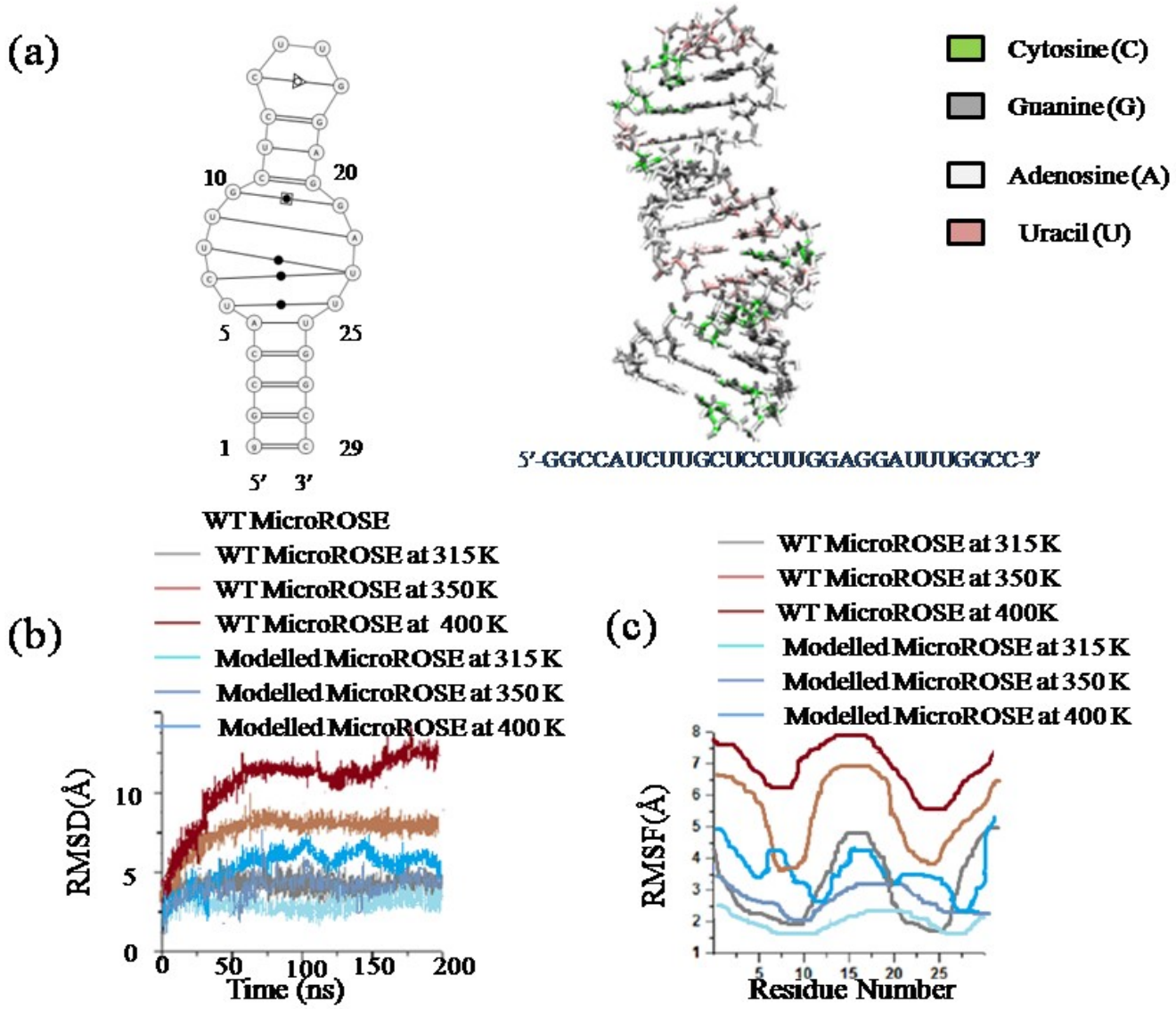

Fig. S2 (a) Dynamic bonds representation of WT MicroROSE system with sequence, schematic secondary structure representation of the system (left hand side) as determined by the RNA pdbee 2.0 programme. (b) Backbone RMSD of the WT MicroROSE and modelled MicroROSE systems during the 200 ns simulation are calculated with respect to the equilibrated structures, plotted against time at three temperatures (at 315 K, 350 K and 400 K). (c) Backbone RMSF of each residue of WT MicroROSE and modelled MicroROSE system is plotted against time at three temperatures (at 315 K, 350 K and 400 K).

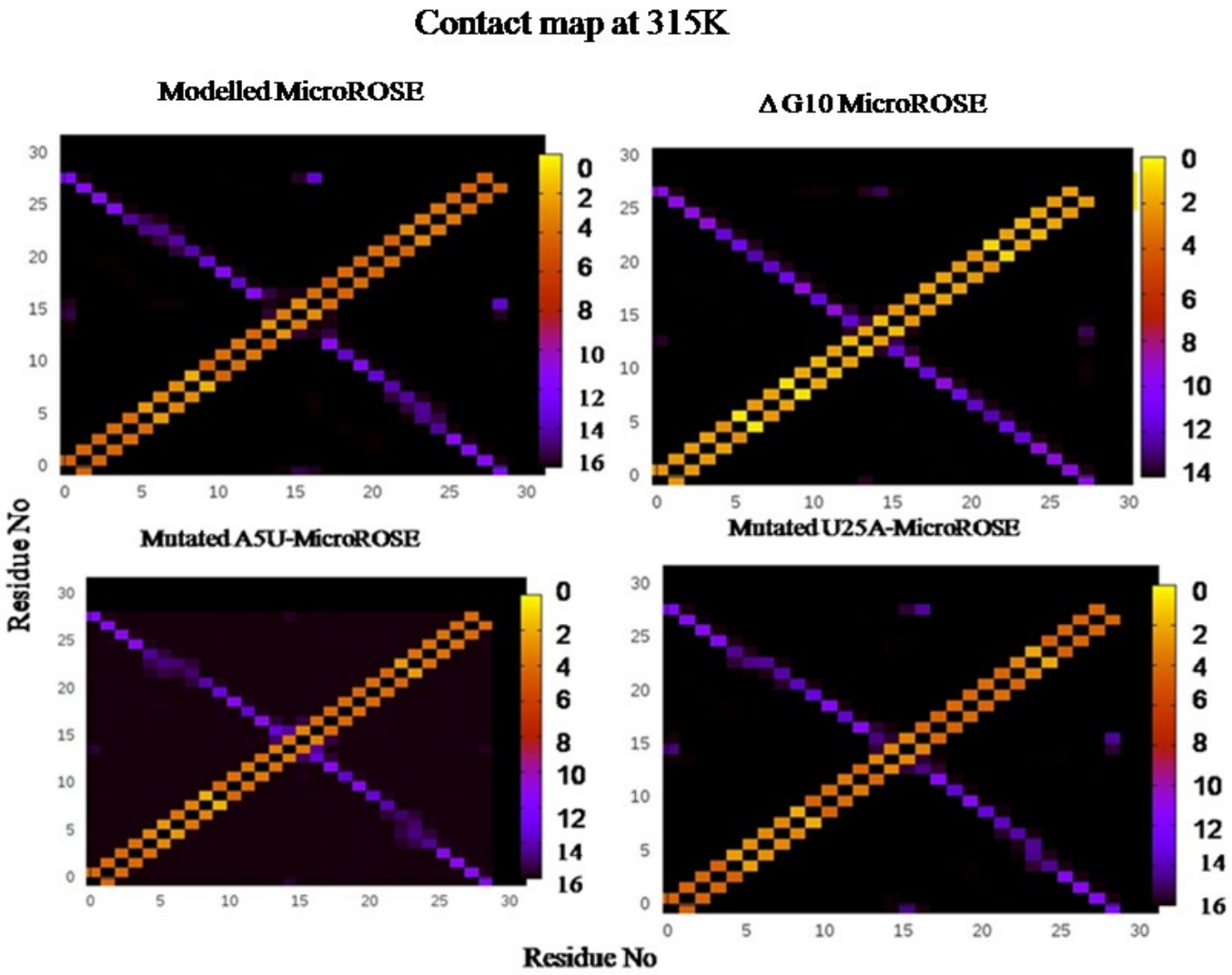

Fig. S3 Residue wise contact map for the four simulated systems (modelled MicroROSE, ΔG10 MicroROSE, mutated A5U MicroROSE and mutated U25A MicroROSE) at 315K.

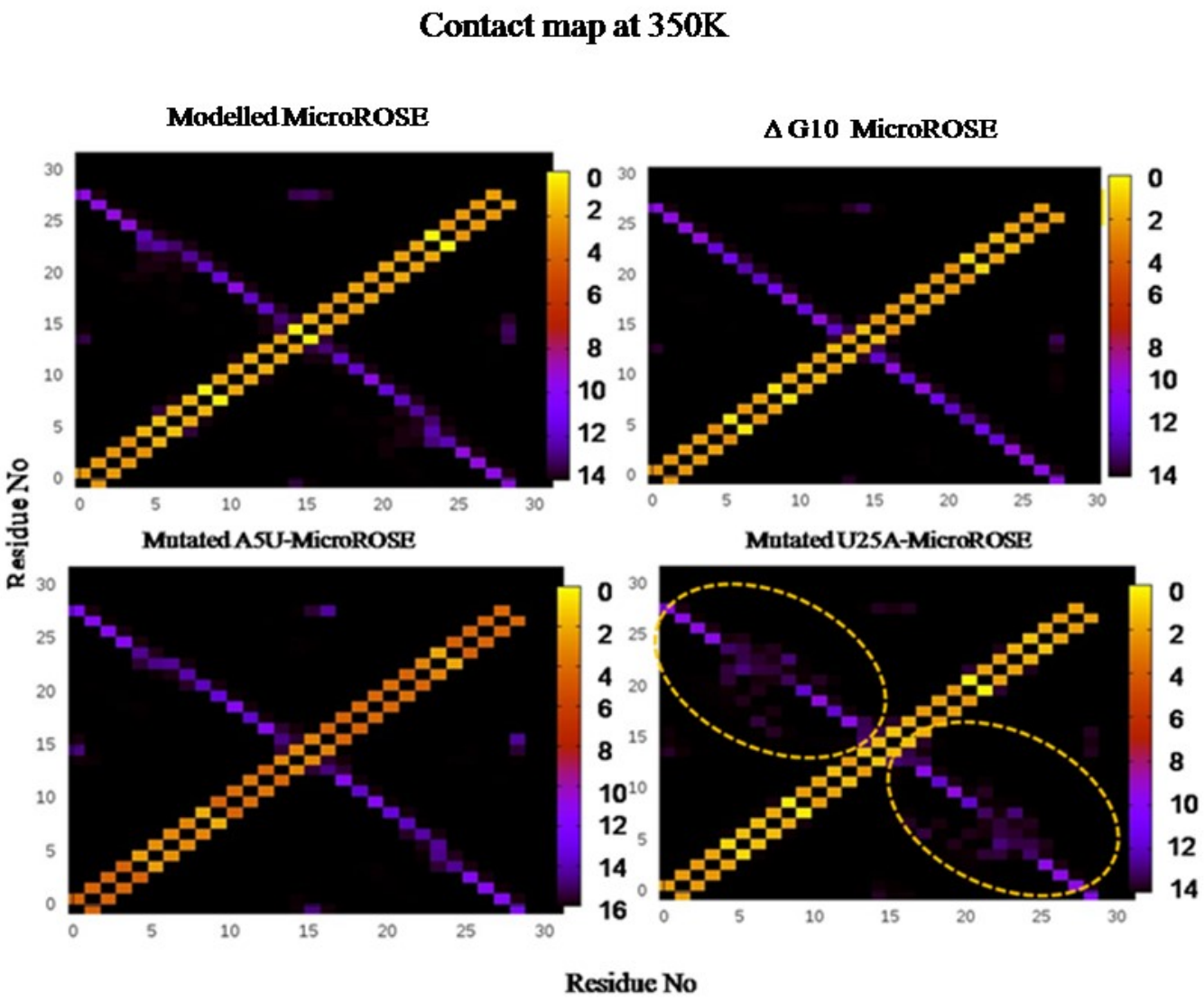

Fig. S4 Residue wise contact map for the four simulated systems (modelled MicroROSE, ΔG10 MicroROSE, mutated A5U MicroROSE and mutated U25A MicroROSE) at 350 K.

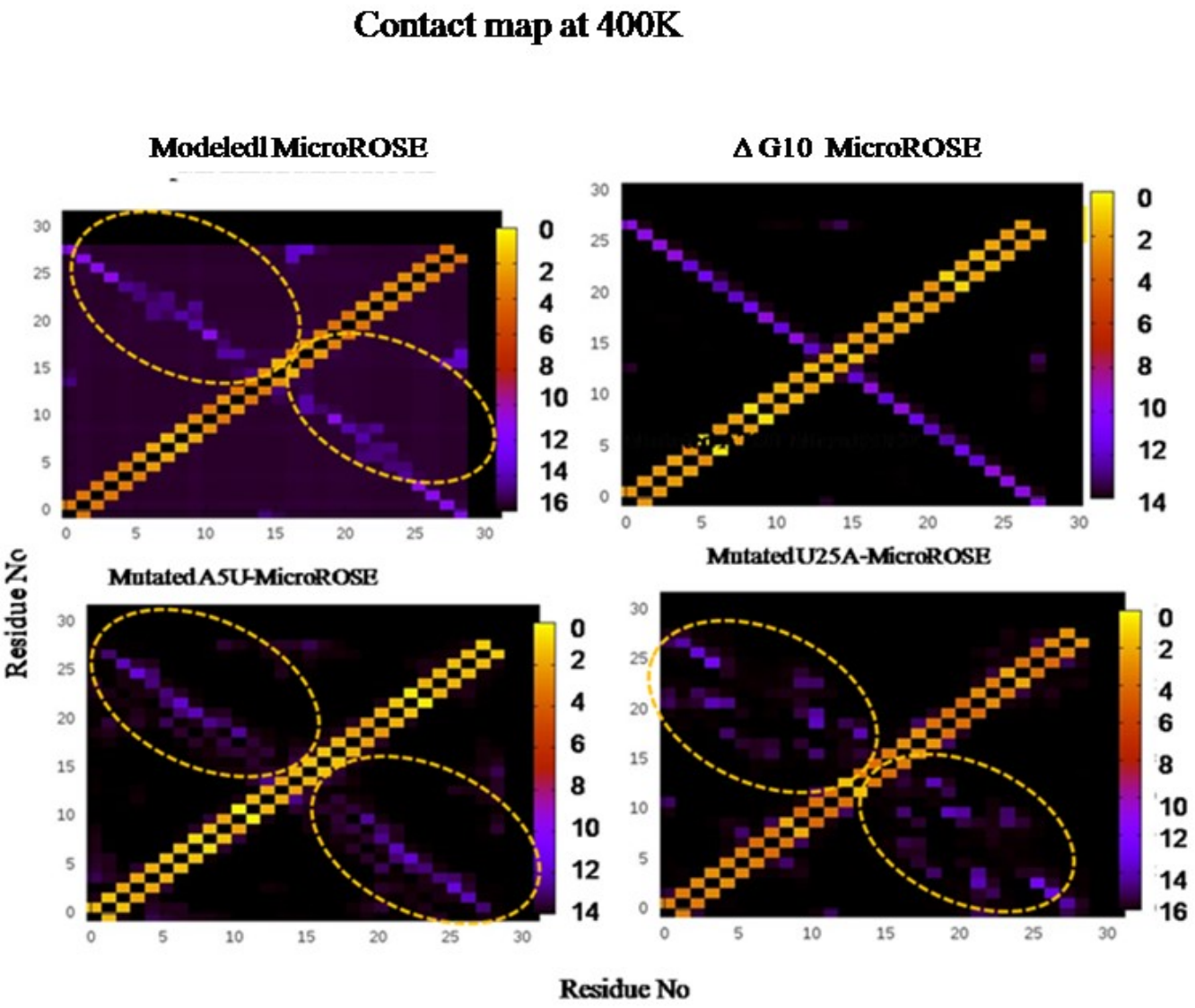

Fig. S5 Residue wise contact map for the four simulated systems (modelled MicroROSE, ΔG10 MicroROSE, mutated A5U MicroROSE and mutated U25A MicroROSE) at 400 K.

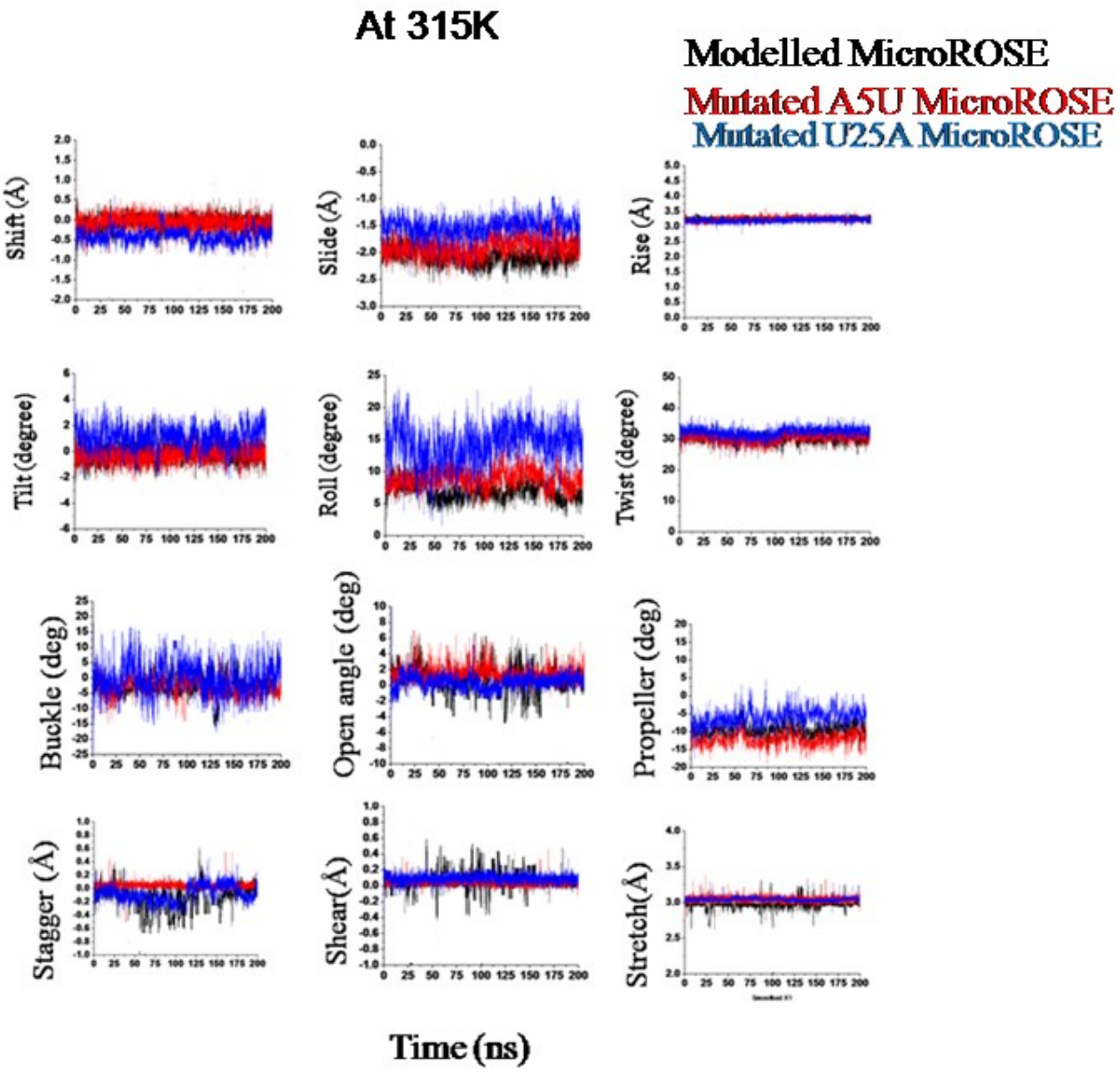

Fig. S6 Trajectory of the six inter-base pair step parameters and the six intra base pair parameters for bases constituting the C4:G26-A5:U25, C4:G26-U5:U25 and C4:G26-A5:A25 base pairs step for modelled MicroROSE and both mutated MicroROSE systems respectively at 315 K.

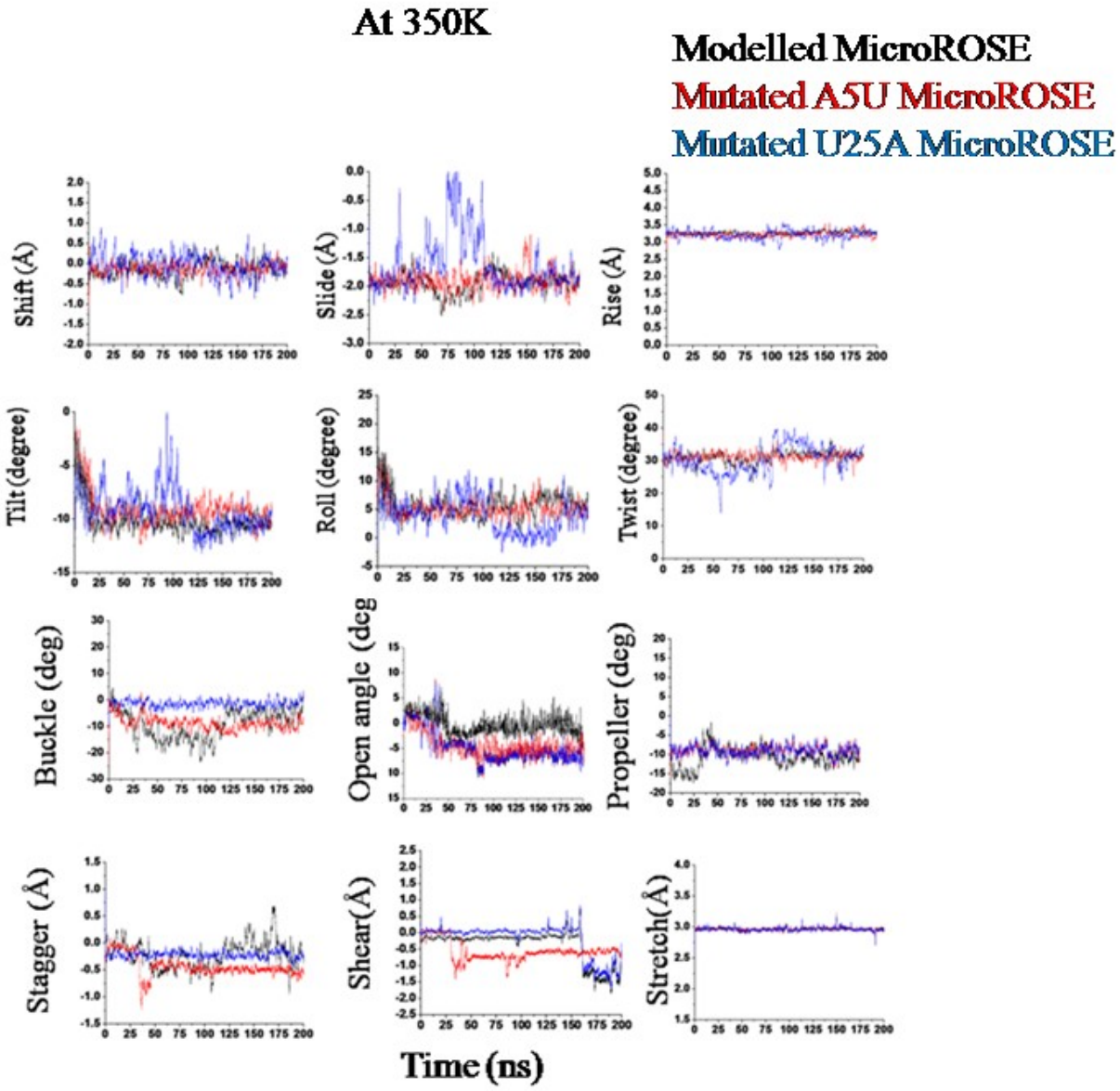

Fig. S7 Trajectory of the six inter-base pair step parameters and the six intra base pair parameters for bases constituting the C4:G26-A5:U25, C4:G26-U5:U25 and C4:G26-A5:A25 base pairs step for modelled MicroROSE and both mutated MicroROSE systems respectively at 350 K.

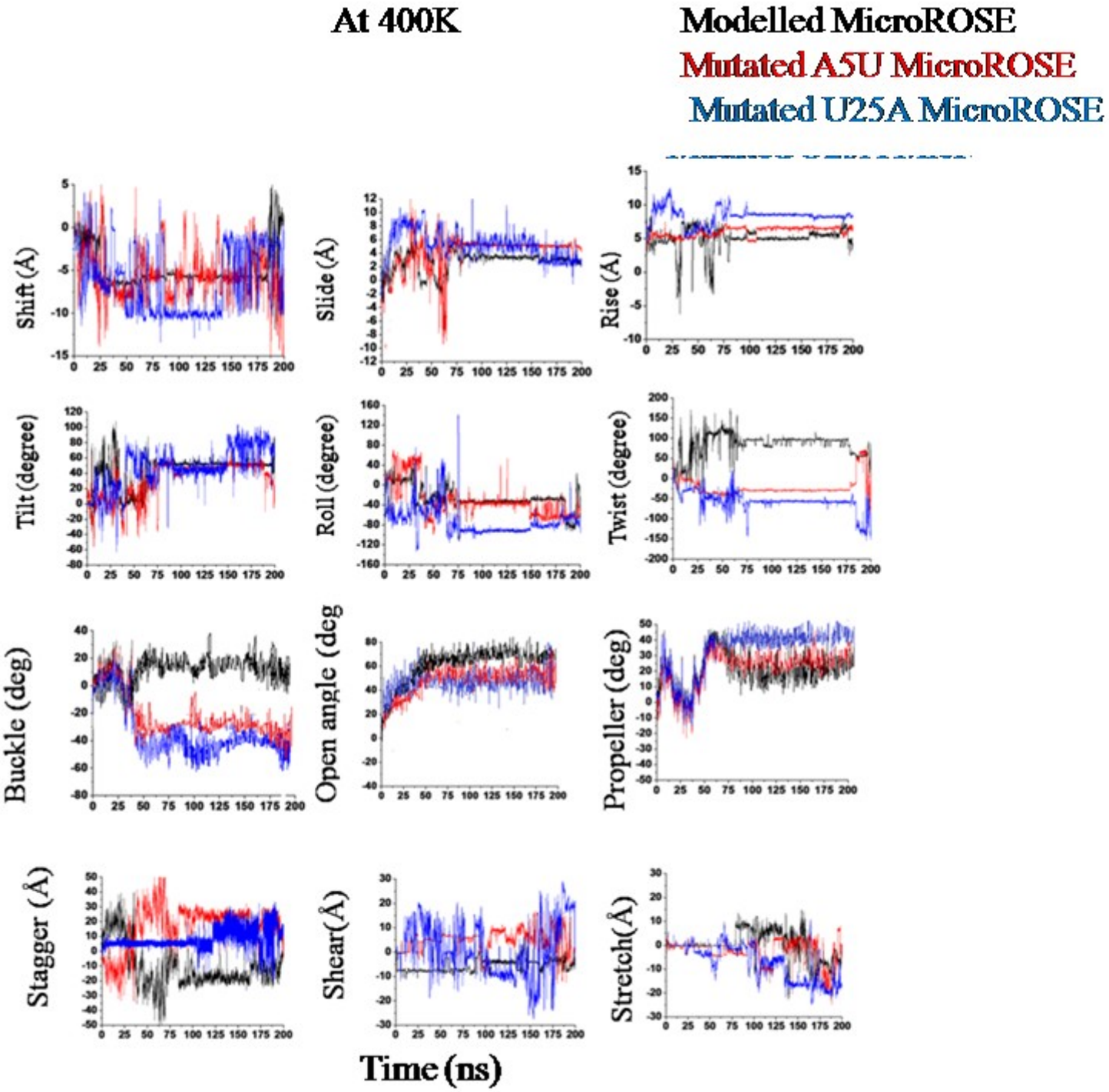

Fig. S8 Trajectory of the six inter-base pair step parameters and the six intra base pair parameters for bases constituting the C4:G26-A5:U25, C4:G26-U5:U25 and C4:G26-A5:A25base pairs step for modelled MicroROSE and both mutated MicroROSE systems respectively at 400 K.

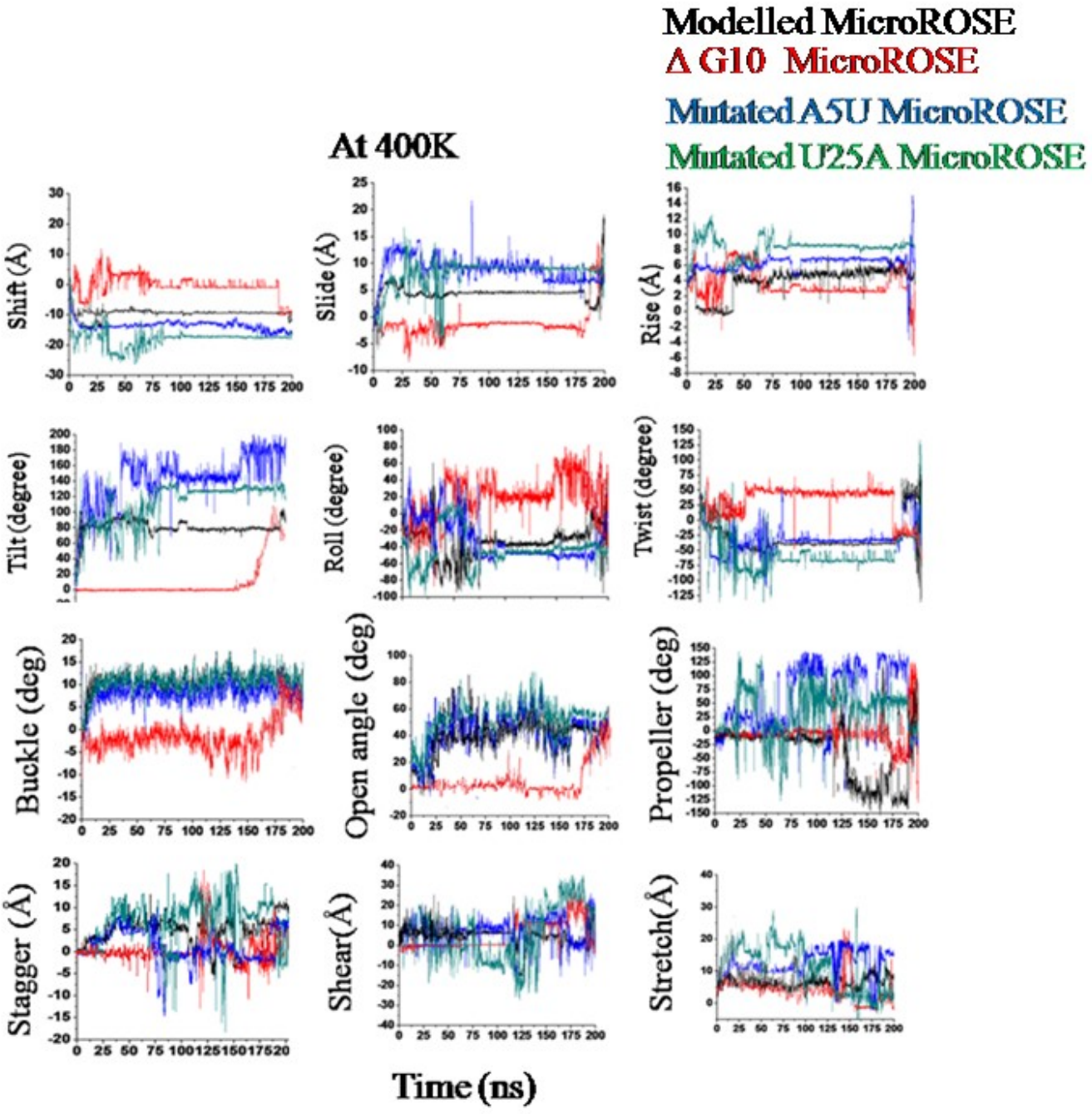

Fig. S9 Trajectory of the six inter-base pair step parameters and the six intra base pair parameters for bases constituting the U8:A22-U9:G21 base pairs step for modelled MicroROSE, ΔG10 MicroROSE, mutated A5U MicroROSE and mutated U25A MicroROSE) at 400 K.

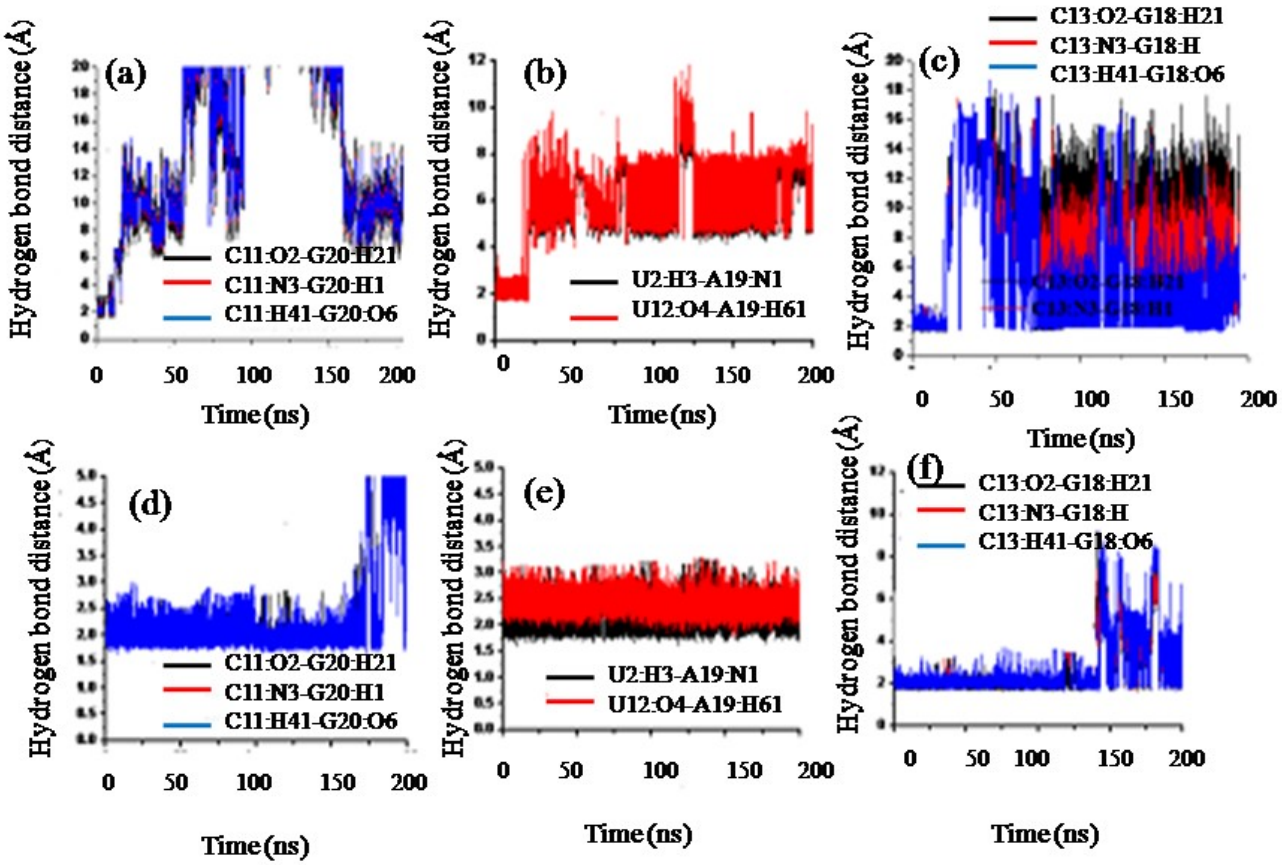

Fig. S10 Hydrogen bond distance plot with time of three Watson-Crick hydrogen bonds in modelled MicroROSE and ΔG10 MicroROSE.