Supplementary Information

Supplementary Tables

Supplementary Table S1: Experimental parameters used for assignment of Cse4 in 5 M urea

Experiment	No. of TD points			Spectral width (ppm)			Acquisition time (ms)			No. of scans	Total acquisition time
	t_3	t_2	t_1	ω_3	ω_2	ω_1	t_{3max}	t_{2max}	t_{1max}		
HNCO	2048	40	112	12	24	20	113.6	11	14.8	16	1d
HN(CA)CO (NUS)	698	56	96	12	24	14	38.7	15	24.2	24	21h
HNCACB	2048	48	112	12	24	75	113.6	13.15	3.9	32	20h
HN(CO)CACB	2048	48	112	12	24	66	113.6	13.15	4.5	40	18h
TOCSY-HSQC	2048	48	112	12	26	12	113.6	12.14	6.2	16	1d

Supplementary Table S2: Experimental parameters used for assignment of Cse4ΔC (Cse4 N-terminus)

Experiment	No. of TD points			Spectral width (ppm)			Acquisition time (ms)			No. of scans	Total acquisition time
	t_3	t_2	t_1	ω_3	ω_2	ω_1	t_{3max}	t_{2max}	t_{1max}		
HNCO	2048	48	128	12	32	24	113.5	9.86	14.1	8	16h
HN(CA)CO	2048	56	96	14	32	22	97.5	11.5	11.5	16	1d 5h
HNCA	2048	48	128	12	32	32	113.6	9.8	10.6	8	16h 36h
HN(CO)CA	2048	56	128	12	32	32	113.6	11.5	10.6	16	1d 15h
CBCA(CO)NH	2048	40	128	12	32	80	113.6	8.22	4.24	16	1d 4h
H(CCO)NH	2048	44	132	12	32	16	113.6	9	5.6	16	1d 8h

Supplementary Table S3: Parameters used for comparison of HSQC spectra of Cse4 in native state and Cse4+H4 samples

Experiment	No. of TD points		Spectral width (ppm)		Acquisition time (ms)		No. of scans	Total acquisition time
	t_2	t_1	ω_2	ω_1	t_{2max}	t_{1max}		
Cse4 in 4M urea	2048	256	14	32	97.48	52.6	56	23.5 min
Cse4+H4 in 4M urea (BEST-HSQC)	2048	196	12	32	113.6	40.3	80	60 min
Cse4 in 2M Arg (Native)	2048	256	12	28	113.6	60	80	32 min
Cse4+H4 (Native) co-folded (BEST-HSQC)	2048	256	12	32	113.6	52.6	256	192 min

Supplementary Table S4: **Comparison of fluorescence intensity decay parameters of Cse4-Ala mutants: N-terminus (W178A) and C-terminus (W7A) in buffer and in presence 8 M urea (D)**

	Mutant	τ_1 (ns) (α_1)	τ_2 (ns) (α_2)	τ_3 (ns) (α_3)	τ_m (ns)
N-terminus	W178A	0.49(0.41)	1.75(0.42)	4.7(0.17)	1.78±0.25
	W178A+D	0.5(0.33)	1.75(0.47)	4.7(0.2)	1.88±0.19
C-terminus	W7A	0.3(0.42)	1.5(0.48)	4.7(0.1)	1.3±0.08
	W7A+D	0.46(0.44)	1.72(0.41)	4.65(0.15)	1.68±0.14

Supplementary Table S5: **Comparison of fluorescence anisotropy decay parameters of Ala mutants of Cse4 N-terminus (W178A) and C-terminus (W7A)**

	Mutant	ϕ_1 (ns)	β1	ϕ_2 (ns)	β2
N-terminus	W178A	0.11±0.01	0.5±0.04	>15	0.5±0.04
C-terminus	W7A	0.19±0.02	0.53±0.06	14.8±3	0.47±0.1

Supplementary Figures

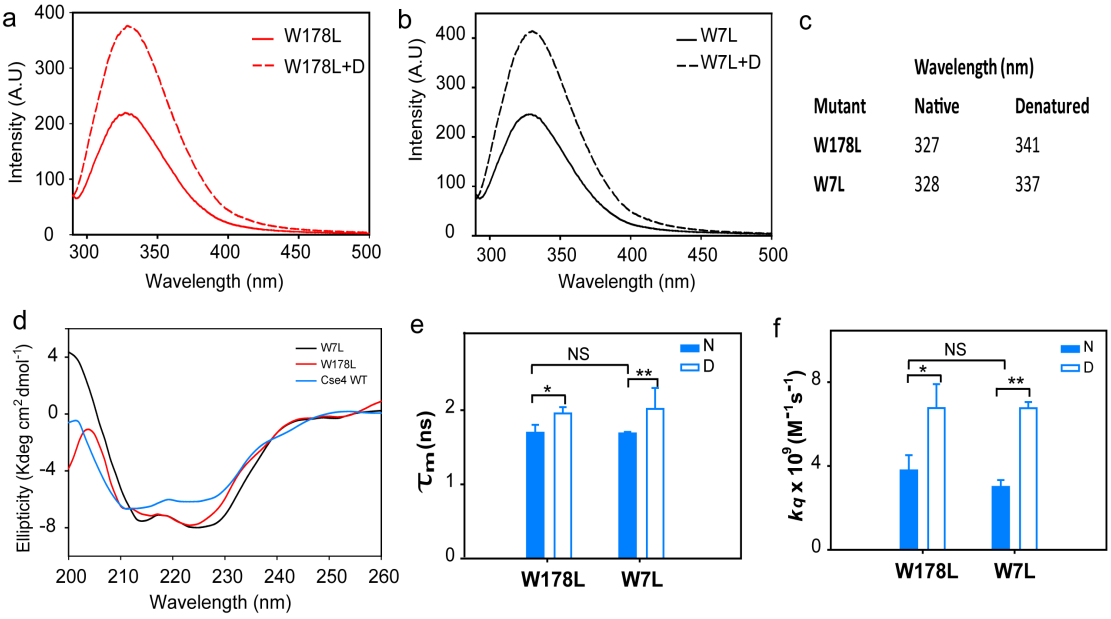

Fig S1. Characterization of Cse4-Leu mutants. a, b. Fluorescence spectrum of and W178L (red) and W7L (black) respectively, in denaturant (+D, 8 M Urea) (dotted line) and in native state (solid line), the change in the fluorescence intensity and shift in the maxima indicates protein folding transitions; **c.** The fluorescence maxima for the folded and denatured proteins exhibit a distinct shift in wavelength indicating a drastic change in Trp environment in the two states; **d**. The CD spectra of the folded mutant proteins indicate alpha helical conformation similar to WT Cse4 indicating that the mutations do not change the secondary structure of Cse4 (blue), the slight difference in the ellipticity is due to lower concentration of WT Cse4; **e, f.** fluorescence life time and solvent accessibility of the two domains in the native (N) and denatured (D) state (8 M urea), note the significant change between the denatured and native states.

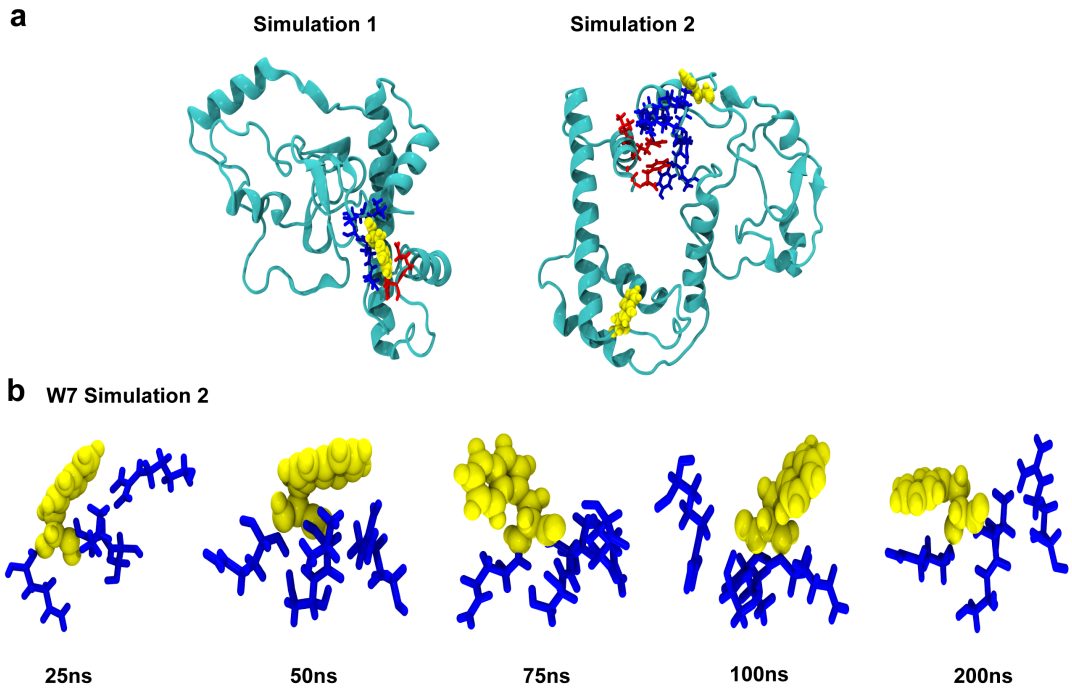

Fig S2. a. Cse4 conformations in different simulations. Two representative simulations showing different structures of Cse4 at the end of 300 ns simulation, the different simulations ended in a different conformation basins where the W7 interacts with different residues, but in both structures there is a clear interaction between the two domains; **b. Change in the conformation of W7 throughout the simulation-2**. The conformation of W7 at the end of 300 ns in Simulation 2 differs from Simulation 1 (Fig 2e), however the flexibility of the Tryptophan side chain is retained in both simulations.

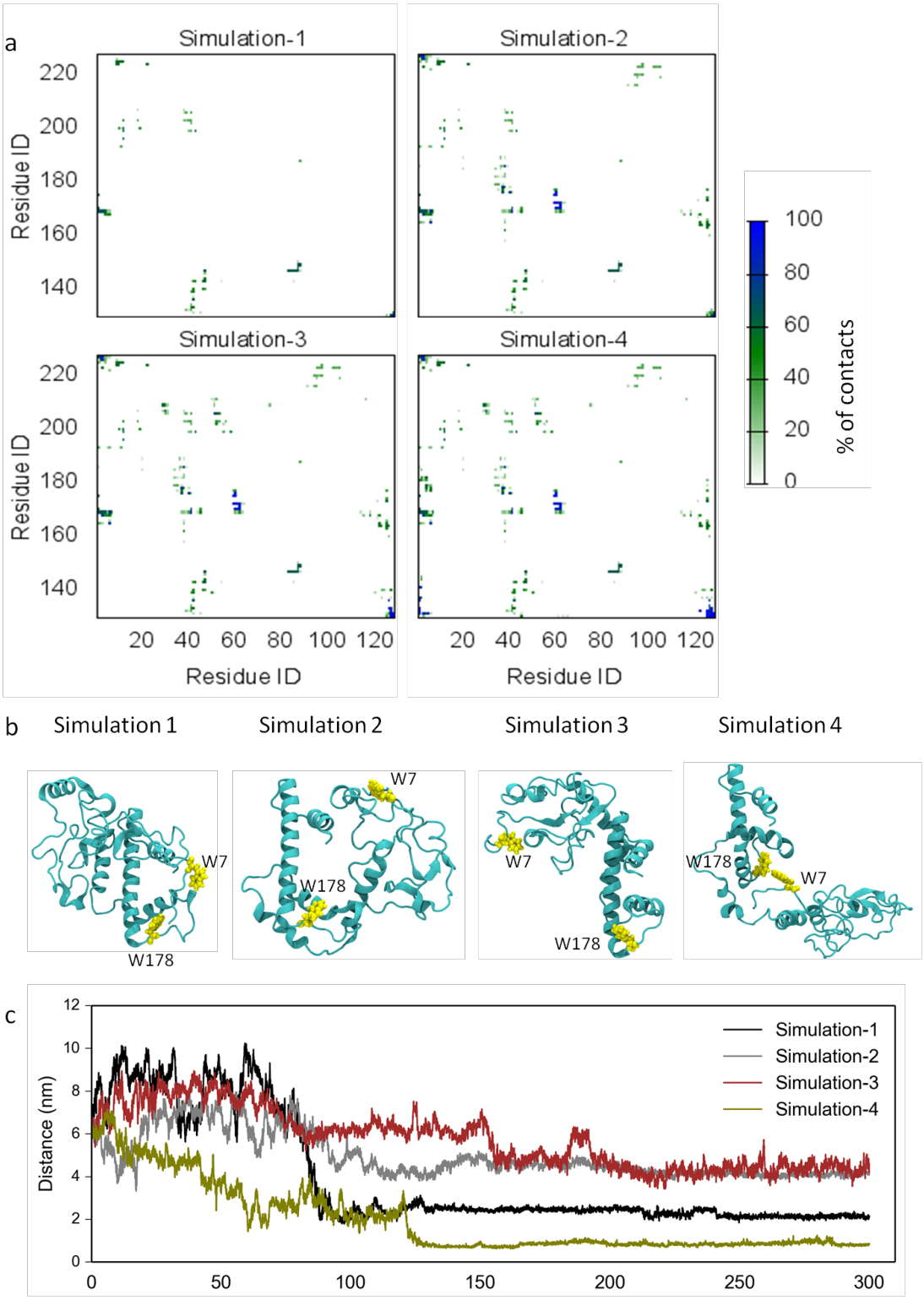

Fig S3. Interaction between the N and C-terminus of Cse4. **a**. Contacts within 4 Å between the N-terminus domain and C-terminus domain from 250-300 ns of each simulation of Cse4 are shown. Due to the difference in all conformations, many unique contacts are seen in all four maps, however the regions 1-7, 10-13, 22, 38-42, 44, 83-89 are interacting with the 165-178, 146-150, 188, 195-199, 201-203, 205-206, and 223-226 in all four simulations. **b.** The conformation of Cse4 at the end of 300 ns simulations, Trp residues are highlighted

(yellow). **c.** The change in the distance between the Cα atoms of the two Trp residues throughout the 300 ns is plotted. The distance reduces from ~6.7 nm to 2.1±0.1 nm, 4.1±0.1 nm, 4.4±0.3 nm, 0.9±0.1 nm (averages are over last 50 ns data i.e. 250-300 ns) in the four simulations respectively, which supports the fluorescence results of interaction between the two domains.

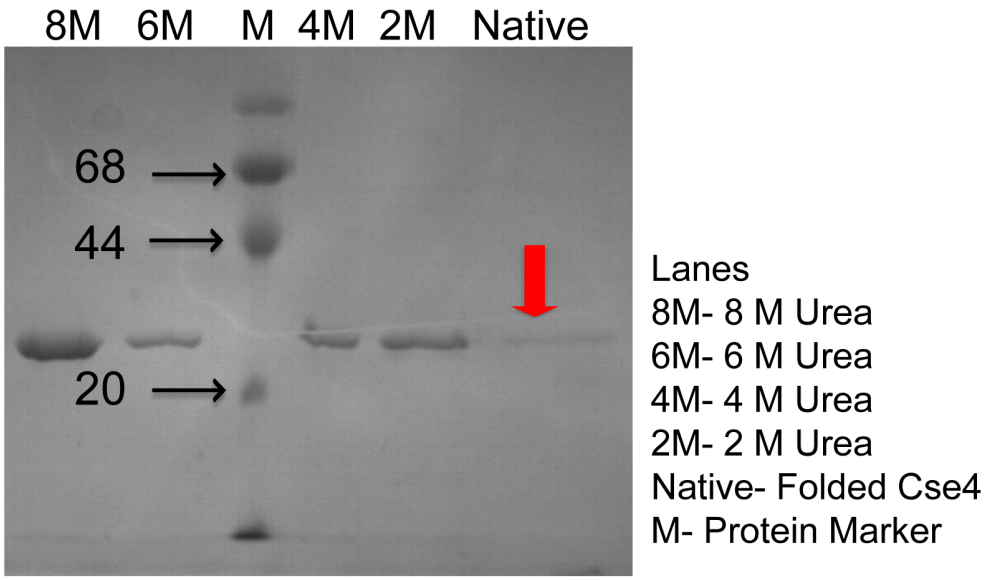

Fig S4. Folding of full length Cse4. The amount of protein in the soluble fraction reduced as the denaturant was diluted; the red arrow indicates the faint band of Cse4 that remained in soluble fraction after complete removal of urea. On concentrating the fraction of Cse4 with no urea, the sample was not enough for NMR experiments, however the fluorescence experiments could be performed.

Various methodologies as well as additives were used in an attempt to increase the concentration in the soluble fraction, like different redox couples, buffer solutions and additives like Arginine and glycerol (data not shown). The protein solubilized at extremely acidic or basic environments but is unusable for biological studies under these conditions. We attempted to remain as close to the physiological pH as possible resulting in lower yield of the protein.

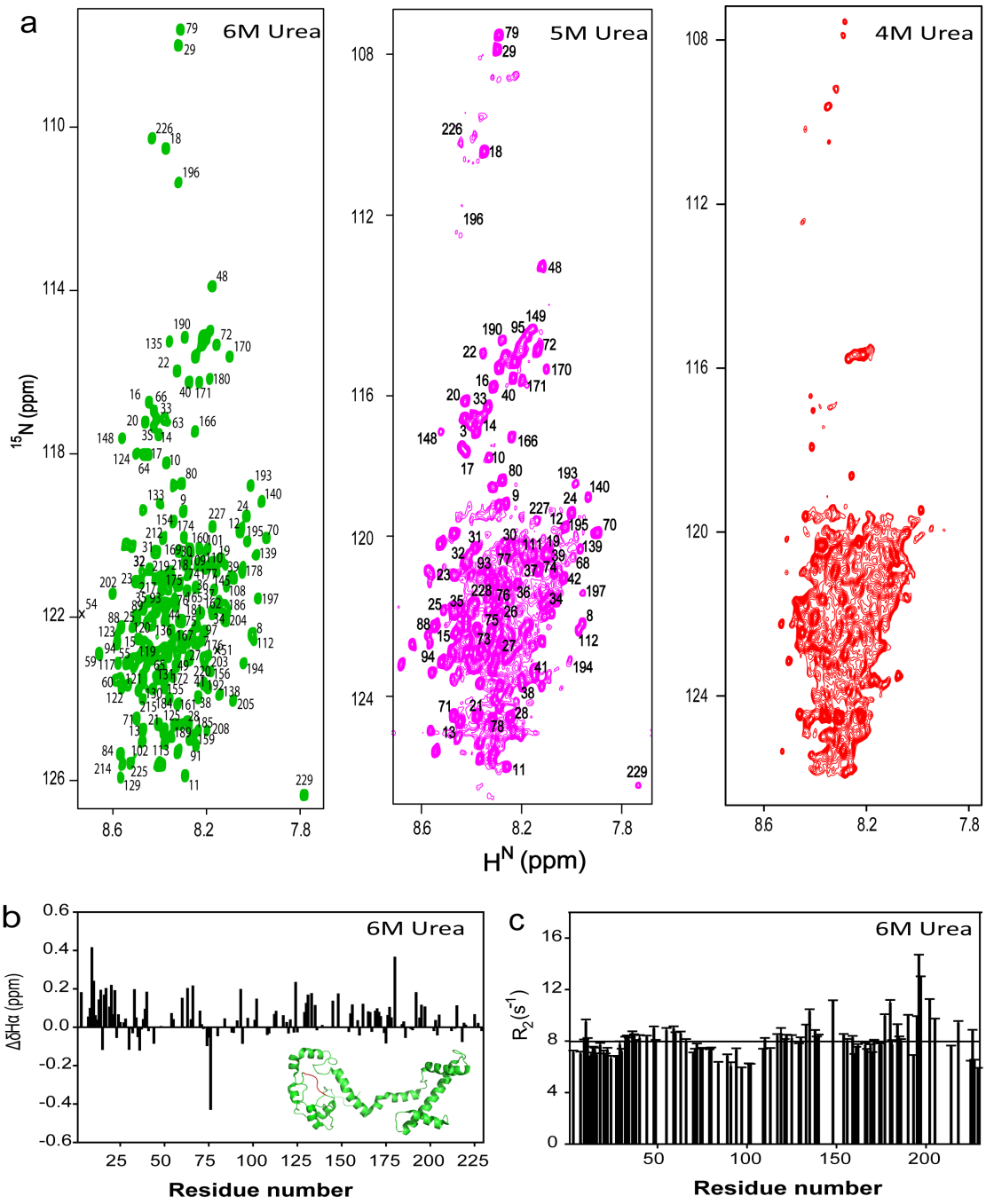

Fig S5. a. The ^{15}N-HSQC spectra of Cse4 protein equilibrated in different urea concentrations (6 M-green, 5 M- megenta, 4 M-red). The peaks have considerably shifted from 6 M to 5 M urea concentration. The assigned residue numbers are labeled in the 6 M and 5 M spectra. For Cse4 at 5 M urea concentration unambiguous assignment of 70 residues could be completed. Almost half the peaks for Cse4 protein have disappeared from 8 M to 5 M urea concentration. At 4 M urea concentration, peaks have broadened considerably. **b**. Residue wise plots of secondary chemical shifts from ΔδHα, the residues W7-S14, S16-S22, F169-K172 show extended conformation and the residues T72-D76 indicated alpha helical propensity at 6 M urea concentration; inset: Residues showing helical propensity are highlighted (red) on the Cse4 modeled structure. The structural propensities do not deviate much from the reported propensities of the denatured protein (8 M urea); **c.** Residue-wise dynamics (^{15}N transverse relaxation rates R_2) for Cse4 protein in 6 M urea, the dotted line represents the average R_2.

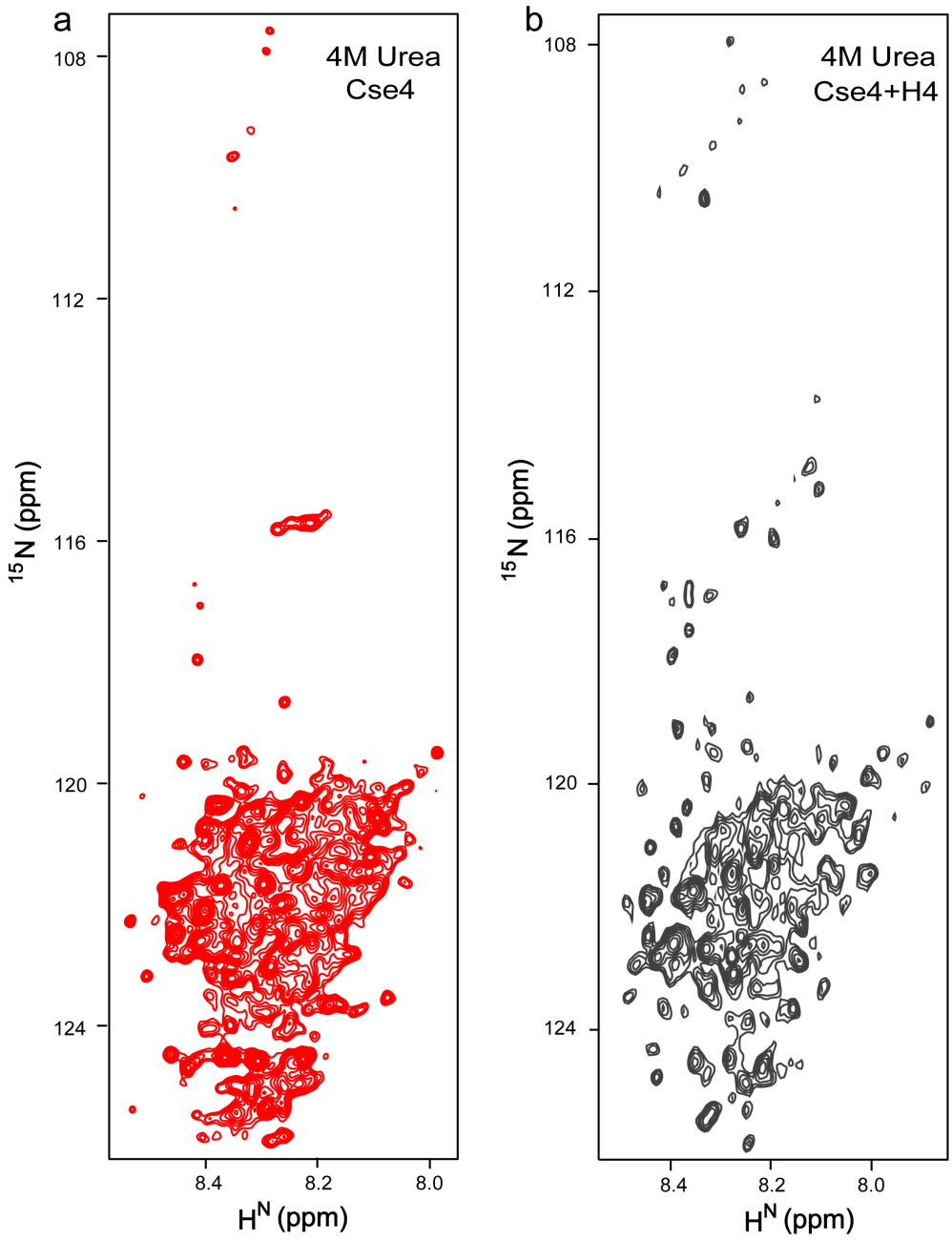

Fig S6. Stabilizing effect of H4 on Cse4 protein. ^{15}N-HSQC spectra of (a) ^{15}N labeled Cse4 protein in 4 M urea, (b) ^{15}N-Cse4 co-folded with H4 (unlabeled) in 4 M urea. The spectrum with H4 is more resolved and the broadened peaks have started to reappear.

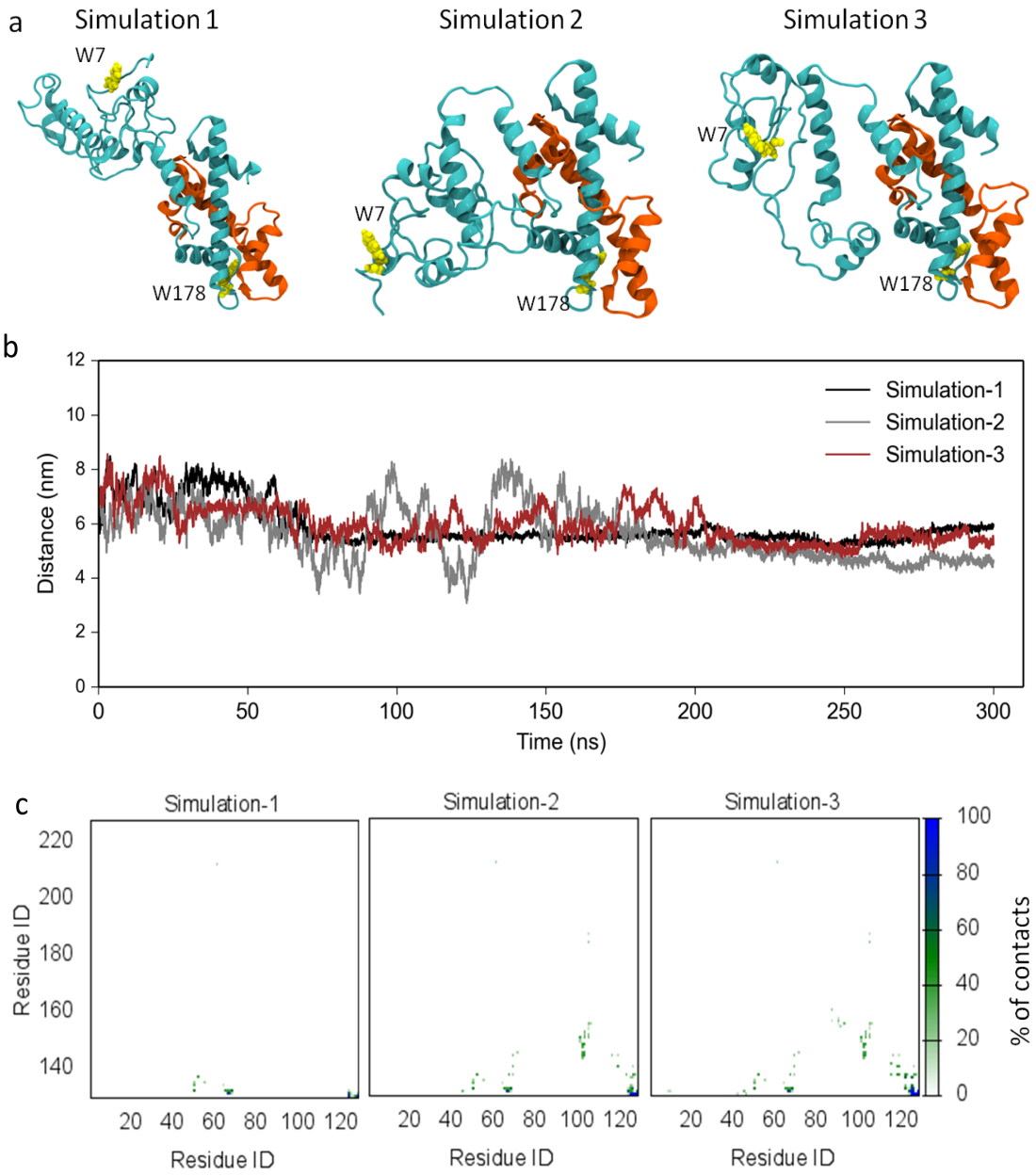

Fig S7. **a**. **Distance between W7 and W178 in Cse4+H4 complex**. The structures represent different conformations adopted by the Cse4+H4 complex at the end of 300 ns simulation respectively. Although the conformation of N-terminus of Cse4 was different in all three simulations, the interaction between the two domains was not observed in any simulation, the Cse4 protein is represented in Cyan, H4 in orange and both Trp residues of Cse4 in yellow; **b**. The change in the distance between the Cα atoms of the two Trp residues of Cse4 in complex with H4 throughout the 300 ns is shown. In all three simulations, the Trp residues did not come closer than 4.8 nm; **c**. **Interaction between the NTD and CTD of Cse4 in complex with H4.** Contacts within 4 Å between the N-terminus domain and C-terminus domain from 250-300 ns of each simulation of Cse4+H4 are shown. In Cse4+H4 complex simulations, the NTD residues 51, 53, 56, 62, 65-70, 102-104, 106-107, 117, 120, and 123-129 are in contact with CTD residues 130-137, 140-155, 184, 187 and 212.

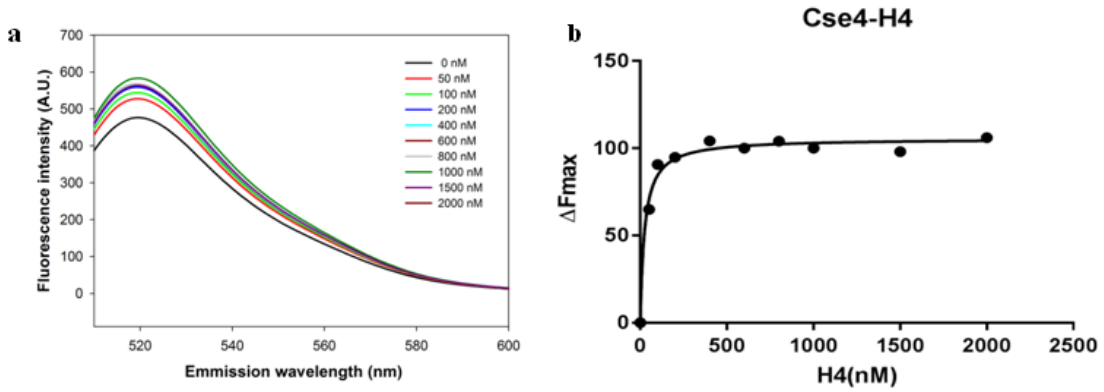

Fig S8: Strong interaction between Cse4 and H4. **a.** The fluorescence intensity of 500 nM Cse4 was monitored with increasing concentrations of H4 (0- 2000 nM), and fluorescence intensity of FITC-Cse4 was monitored at 520 nm. The intensity increases with the concentration of H4 and saturated at 200 nM H4, indicating strong binding. b Representative graph (experiment done in triplicates) showing the fitted curve to obtain Kd.

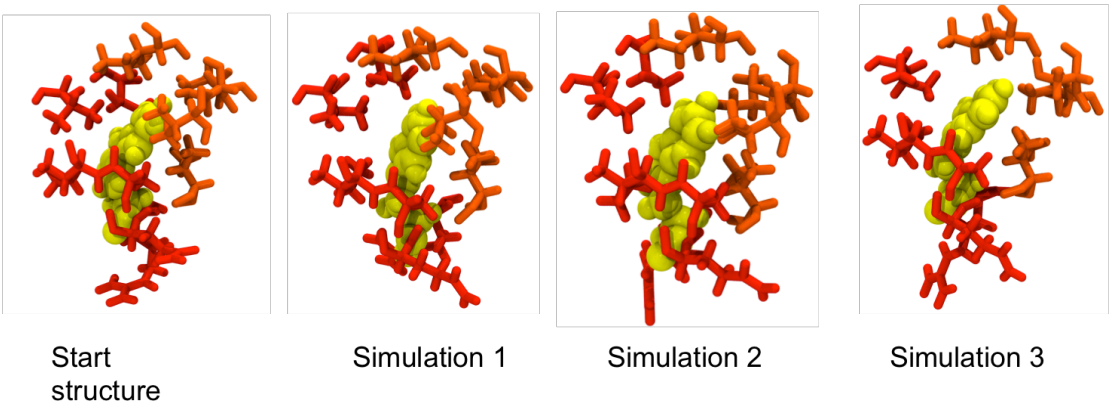

Fig S9: C-terminus Trp is restricted in Cse4+H4 structure. Comparison of W178 conformation at the start of the simulation with the structures at the end of 300 ns simulation reveal that the W178 is restricted by side chains of residues from Cse4 (Red) as well as H4 (Orange) in all three simulations. Since, the flexibility of this residue does not vary, it can explain a minor change observed in anisotropy for C-terminus on addition of H4.

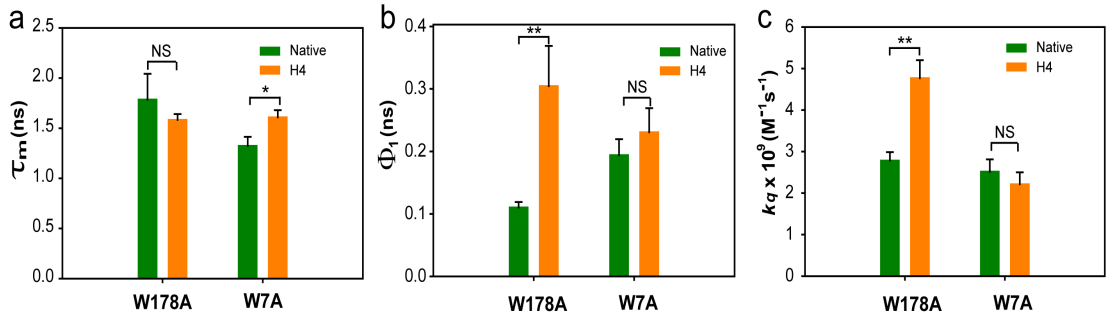

Fig S10: Comparison between the fluorescence parameters between Cse4 and Cse4+H4 for the two domains in Ala mutants: **a.** fluorescence lifetime; **b**. Change in conformational flexibility of the Trp; **c**. Solvent accessibility of Trp residues. The statistical significance was calculated by one-way analysis of variance: *, p <0.05; **, p <0.01; NS (not significant), p >0.05; error bars represent SD.

Supplementary Movie legends

Supplementary Movie_1:
Cse4 forms a closed conformation: 300 ns simulation of the Cse4 monomer shows that the flexible N-terminus interacts with C-terminus and the two domains did not dissociate once they are bound till the end of 300 ns. The grey (surface representation) shows the initial conformation and the blue (ribbon form) depicts the conformation change throughout the simulation, the tryptophan residues are shown as yellow.

Supplementary Movie_2:
H4 binding results in open conformation: The simulation of Cse4 monomer bound to histone H4 shows that the Cse4 inter-domain interaction is prevented in presence of H4 and NTD did not fold back on CTD through the 300 ns. The NTD remains flexible and free to interact with other components. The grey (surface representation) shows the initial conformation of Cse4+H4, red (ribbon form) represents H4 during the course of simulation and the blue (ribbon form) depicts the conformation change in Cse4 throughout the simulation.