Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information for

Conformational ensembles of neuromedin C reveal a progressive coil-helix transition within a binding-induced folding mechanism

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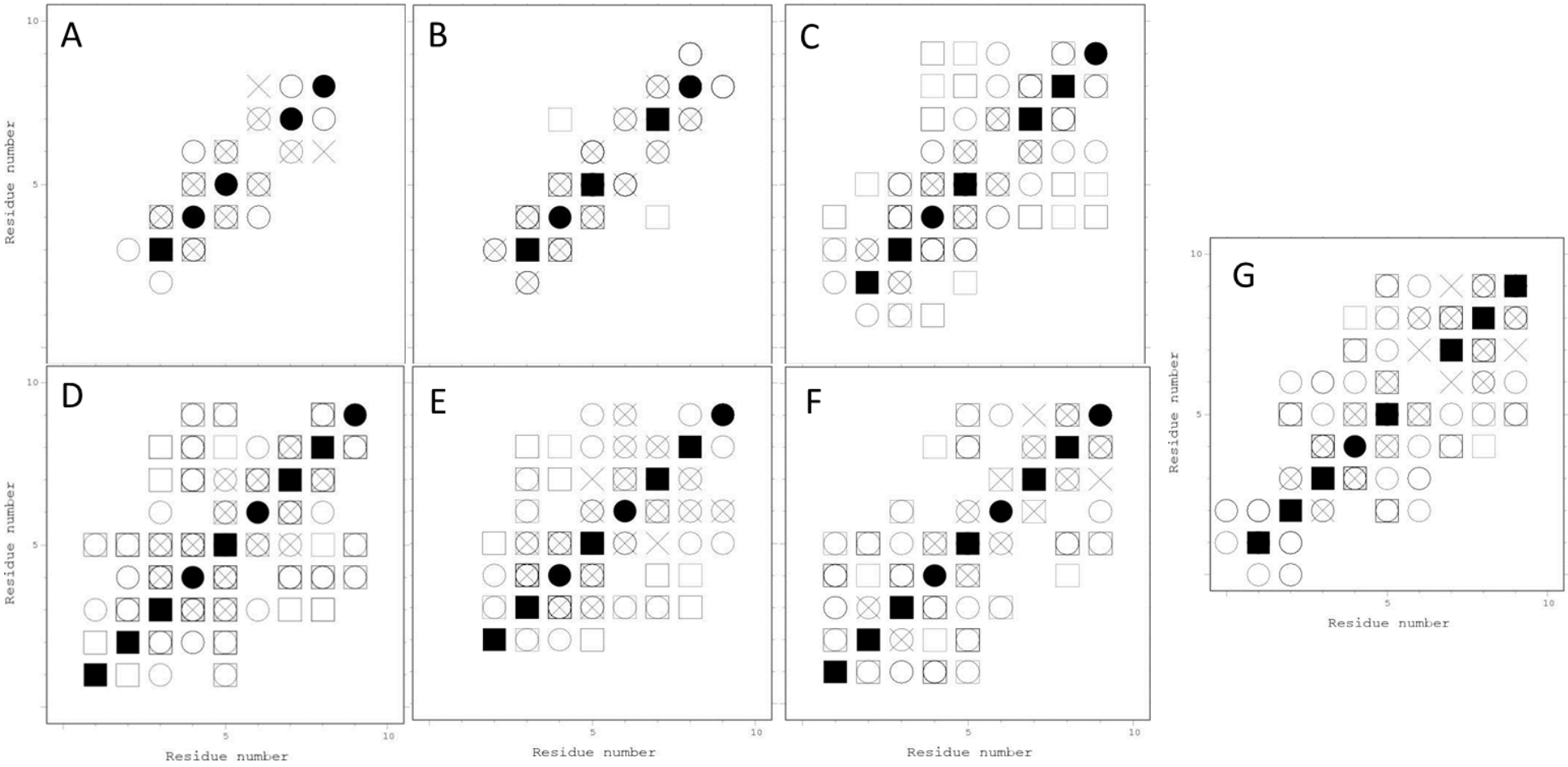

Figure S1. Contact maps for NMC structures calculated in the presence of (**A**) 0%, (**B**) 10%, (**C**) 25%, (**D**) 40%, (**E**) 60% and (**F**) 90% d_3-TFE, and in the presence of (**G**) 150mM d_{25}-SDS. The geometrical restraints yielding these contact maps were taken from the ^{1}H,^{1}H NOEs intensities using an automatic assignment process. X represents backbone-backbone interactions; □ represents backbone-side chain interactions (filled if intraresidue); □ represents side chain-side chain interactions (filled if intraresidue). The contact maps plots were obtained analyzing the solution structure through the Protein Structure Validation Server (PSV) (http://psvs-1_4-dev.nesg.org/).[69]

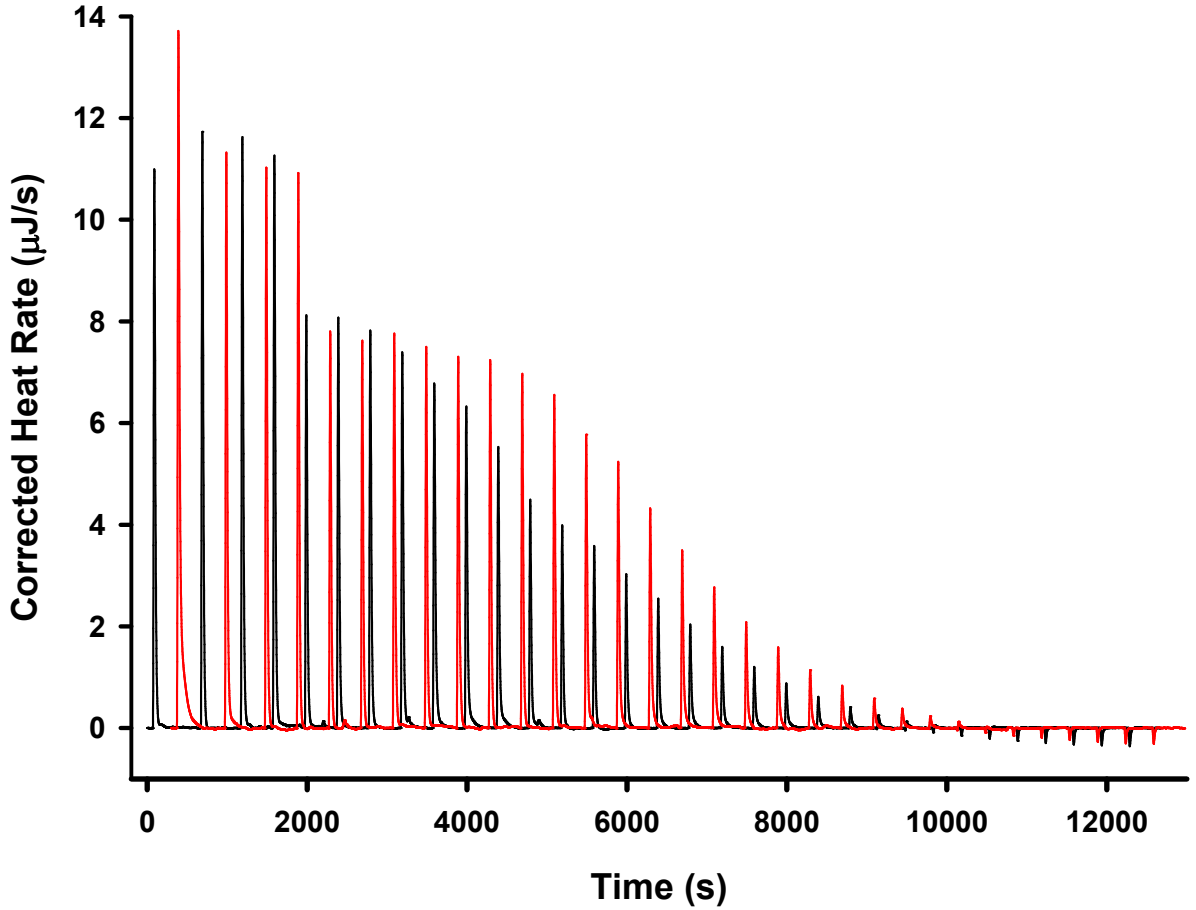

Figure S2. Heat flow versus time profiles resulting from injection of 2μl (the first four injections) or 1.5μl (the other injections) aliquots of a 10mM acetate buffered solution (pH 4.0) of SDS at 35mM concentration into a 171μl reaction cell containing a 10mM acetate buffer solution either alone (*black*) or in presence of 42 μM of NMC (*red*). The titration plot representing the experiment carried out on NMC has been shifted on the graph 300s towards higher injection times to have a better comparison between both plots.

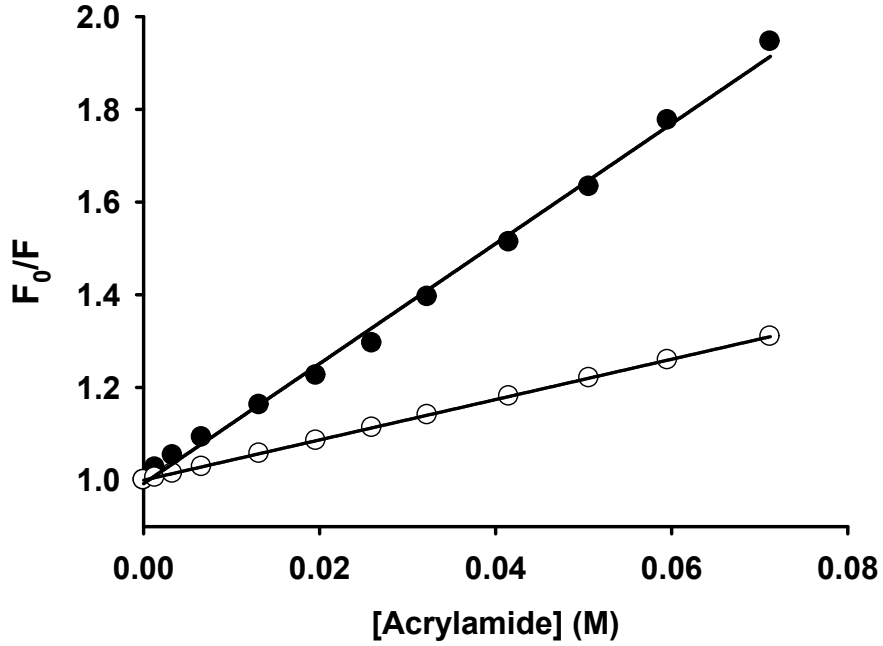

Figure S3. Stern-Volmer plots for the quenching of a 0.1mM NMC buffered solution solution alone (●) and in presence of 50mM SDS (□). *F* and F_0 are the fluorescence intensities in the presence and absence of acrylamide, respectively (λ_{exc} 280nm; λ_{em} 356nm in ● and λ_{em} 345nm in □).

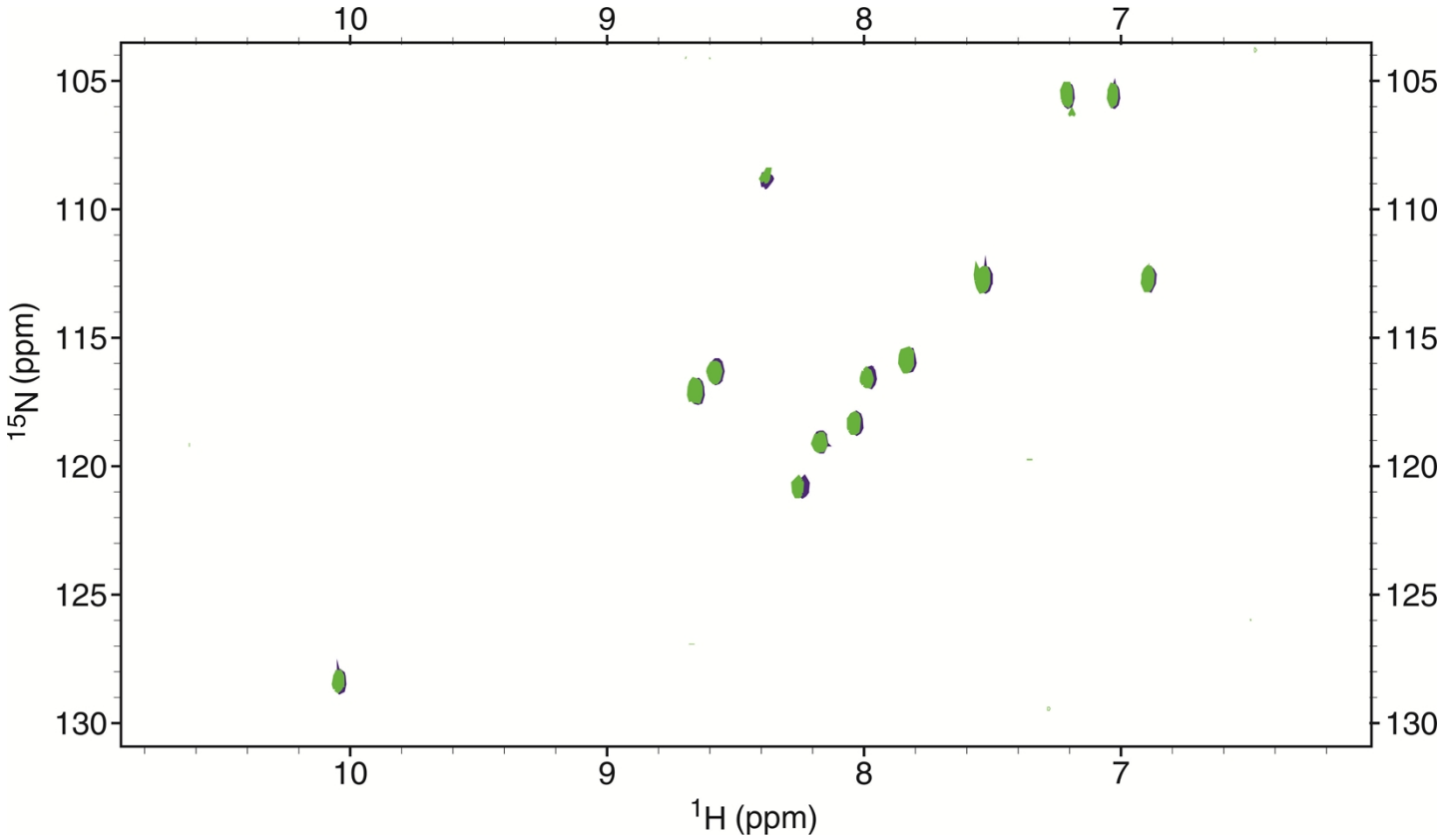

Figure S4. Overlapping of the ^{15}N-HSQC spectra of NMC recorded at 15ºC in 10mM acetate buffer (pH 4.0) in presence of 150mM d_{25}-SDS (*blue*) and in presence of 50mM SDS (*green*).

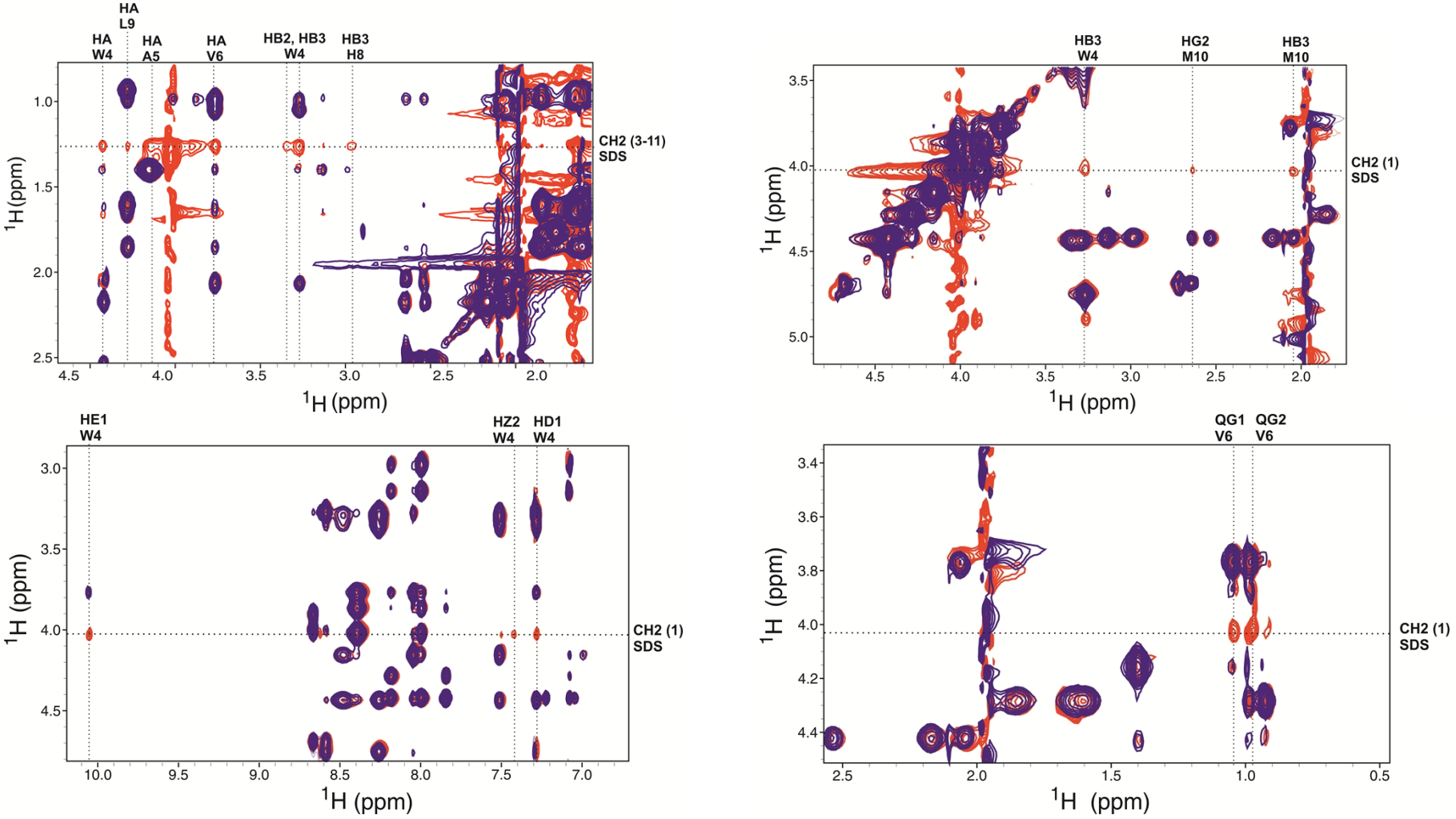

Figure S5. Overlap of the ^{1}H,^{1}H-NOESY spectra of NMC at different spectral regions when NMC was bound to SDS micelles (*red*) and when it was bound to d_{25}-SDS micelles (*blue*).

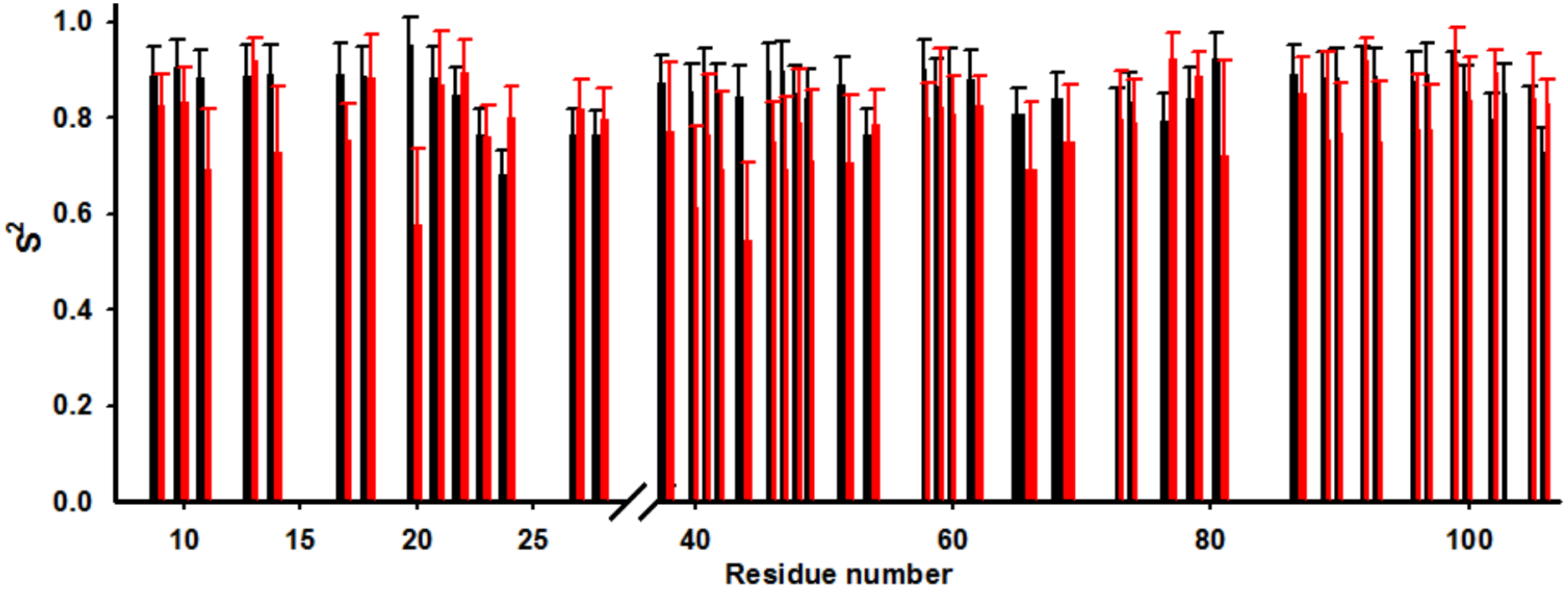

Figure S6. Generalized order parameters (S^2) of CyaY in 25mM Tris-HCl buffer at pH 8.3 (*black bars*) and in hen egg white (*red bars*) as a function of the residue number. S^2 values were calculated using previously published R_1, R_2 and HET-NOE data[59] by applying the Liparizi-Szabo model-free analysis.

Table S1. Secondary structure contents of NMC at different TFE percentages estimated from the corresponding CD spectrum acquired in 10mM acetate buffer (pH 4.0) at 15ºC.

% TFE	% Helix[a]	% Antiparallel β-sheet[a]	% Turns[a]	% Random Coil[a]
0	3.4	35.6	10.7	50.3
10	3.8	33.8	12	50.4
25	10.6	29.6	11.2	48.6
40	26.8	21.8	7.0	44.7
60	37.3	17.1	3.9	41.8
90	44.3	10.9	7.2	37.6
SDS	36.8	23.2	0.0	40.0

[a] The secondary structure contents were estimated by using the BeStSel on-line platform (http://bestsel.elte.hu/)

Table S2. Residue-specific dynamics parameters calculated from the Lipari-Szabo model-free formalism at 14.1T for NMC in 10mM acetate buffer at pH 4.0 and 15ºC.

Residue	Model	χ^2	S^2	τ_e, ps
2	2	2.76	0.23±0.03	80±16
3	2	0.19	0.37±0.03	173±31
4	2	0.055	0.47±0.02	137±22
5	2	0.082	0.62±0.01	22±7
6	2	0.117	0.48±0.02	80±9
7	2	0.054	0.54±0.01	82±10
8	2	0.178	0.53±0.02	50±9
9	2	0.093	0.41±0.02	60±7
10	2	0.651	0.37±0.02	140±15

Table S3. Residue-specific dynamics parameters calculated from the Lipari-Szabo model-free formalism at 14.1T and 15ºC for a NMC solution containing 60% TFE and 40% acetate buffer (10mM) at pH 4.0.

Residue	Model	χ^2	S^2	τ_e, ps	R_{ex}, s^{-1}	S^2_f [a]
3	4	0.14	0.68±0.15	510±110	3.65±0.67	-
4	2	0.31	0.74±0.01	112±15	-	-
5	2	0.83	0.79±0.02	71±16	-	-
6	2	1.58	0.68±0.01	98±12	-	-
7	2	2.85	0.75±0.01	59±12	-	-
8	2	0.006	0.75±0.02	99±17	-	-
9	5	0.000	0.43±0.10	1190±322	-	0.89±0.02
10	5	0.000	0.42±0.06	694±167	-	0.85±0.02

[a] $S^2 = S^2_f \cdot S^2_s$

Table S4. Residue-specific dynamics parameters calculated from the Lipari-Szabo model-free formalism at 14.1T for NMC under its SDS micelle bound state, at pH 4.0 and 15ºC.

Residue	Model	χ^2	S^2	τ_e, ps	R_{ex}, s^{-1}	S^2_f [a]
2	5	0.00	0.43±0.06	743±173	-	0.79±0.07
3	1	3.90	0.84±0.04	-	-	-
4	1	4.40	0.86±0.07	-	-	-
5	4	0.00	0.64±0.06	64±35	6.0±2.6	-
6	1	5.00	0.87±0.06	-	-	-
7	1	3.50	0.86±0.07	-	-	-
8	3	3.11	0.93±0.07	-	2.1±0.9	-
9	1	0.82	0.79±0.06	-	-	-
10	2	0.00	0.77±0.02	597±356	-	-

[a] $S^2 = S^2_f \cdot S^2_s$