Improved accuracy from joint X-ray and NMR refinement of a protein-RNA complex structure

Azzurra Carlon,[1] Enrico Ravera,[1] Janosch Hennig,[2,3,4] Giacomo Parigi,[1] Michael Sattler,[2,3*] Claudio Luchinat[1*]

[1]Magnetic Resonance Center "CERM" and Department of Chemistry "Ugo Schiff", University of Florence and Magnetic Resonance Consortium (CIRMMP), Via L. Sacconi 6, 50019 Sesto Fiorentino, Italy
[2]Center for Integrated Protein Science Munich (CIPSM) at Department Chemie, Technische Universität München, 85747 Garching, Germany
[3]Institute of Structural Biology, Helmholtz Zentrum München, 85764 Neuherberg, Germany
[4]Present address: Structural and Computational Biology Unit, EMBL Heidelberg, 69117 Heidelberg, Germany

Supporting Information

PDB code: 4QQB – Resolution: 2.80 Å					
Parameters	Original structure	**Three tensors** Sxl (Nter), Sxl (Cter), and CSD1		**Two tensors** Sxl and CSD1	**Single tensor** Sxl-CSD1 complex
		– NMR	+ NMR	+ NMR	+ NMR
R-value	0.198	0.198	0.201	0.201	0.201
R-free	**0.236**	**0.234**	**0.236**	**0.236**	**0.235**
RMSD bond length	0.006	0.006	0.009	0.009	0.009
RMSD bond angles	1.113	1.260	1.592	1.591	1.595
RMSD chiral volume	0.074	0.099	0.097	0.097	0.097
Q-factor RDC	**0.440**	**-**	**0.124**	**0.131**	**0.144**
RDC weight	-	-	Sxl H^N-N = 0.015 (tol = 1.0) Sxl C-N = 0.50 (tol = 2.0) CSD1 H^N-N = 0.009 (tol = 1.0)	Sxl H^N-N = 0.015 (tol = 1.0) Sxl C-N = 0.50 (tol = 2.0) CSD1 H^N-N = 0.009 (tol = 1.0)	Sxl H^N-N = 0.015 (tol = 1.0) Sxl C-N = 0.50 (tol = 2.0) CSD1 H^N-N = 0.009 (tol = 1.0)
Weight dihedral angles* (*pep1, pep2, ω*)	-	2.0, 0.8, 2.0	2.0, 0.8, 2.0	2.0, 0.8, 2.0	2.0, 0.8, 2.0
Weight matrix	0.01	0.002	0.002	0.002	0.002
Weight refined_atoms, Weight other_atoms	1.0, 1.0	1.0, 25.0	1.0, 25.0	1.0, 25.0	1.0, 25.0

[*] REFMAC-NMR additional restraints defined in the CCP4 library:

- *pep1* value of 180° with a tolerance of 1.0°
- *pep2* value of 180° with a tolerance of 1.0°
- *ω* value of 180° with a tolerance of 5.0°

Table S1: Force constants and tolerances used for the different refinement calculations performed for the Sxl-Unr complex.

Original structure – Tensor parameters				
	A	R	D^{HN} (Hz)	Q_{RDC}
SXL (chain A) and CSD1 (chain X)	-0.00189	-0.186	-21.644	0.392
SXL (chain B) and CSD1 (chain Y)	-0.00186	-0.188	-21.403	0.465

Table S2: Alignment tensors calculated for the original X-ray structures of the two complexes A-P-X and B-C-Y found in the asymmetric unit of the crystal.

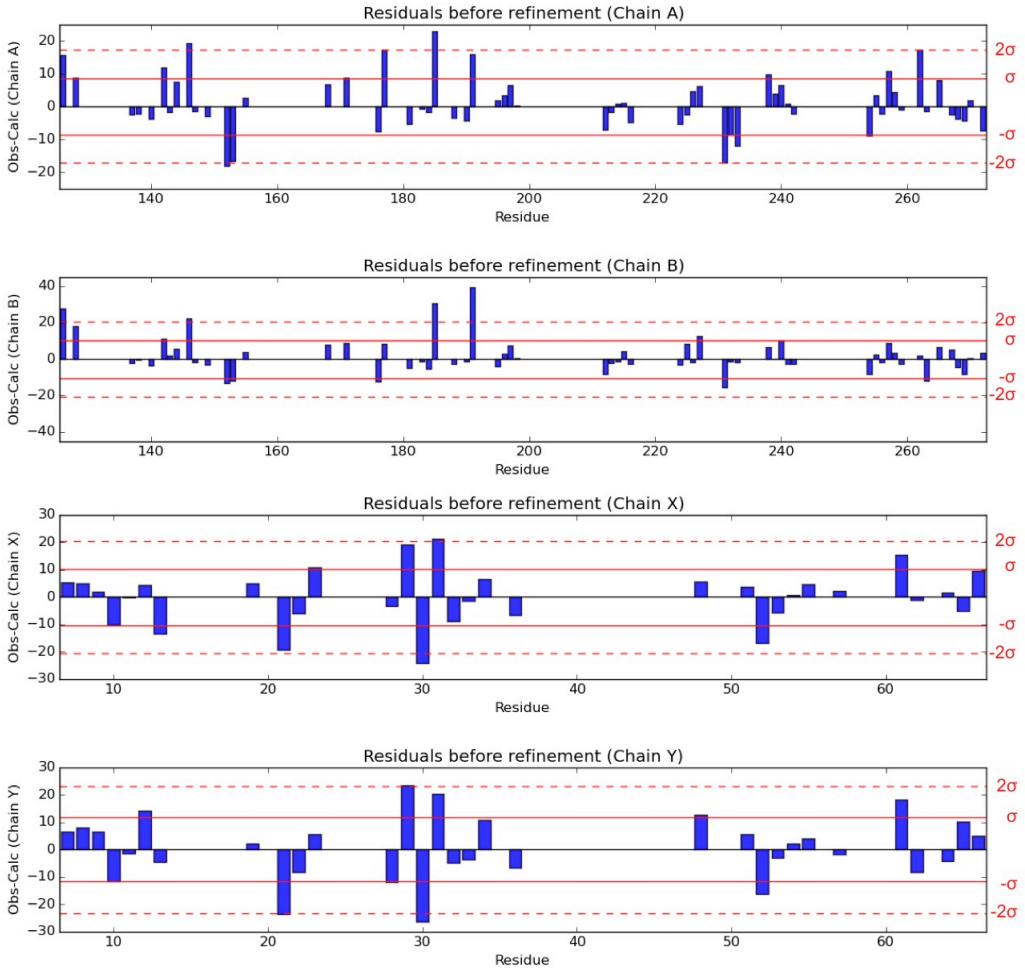

Fig.S1a: RDC residuals compared to their standard deviation before refinement.

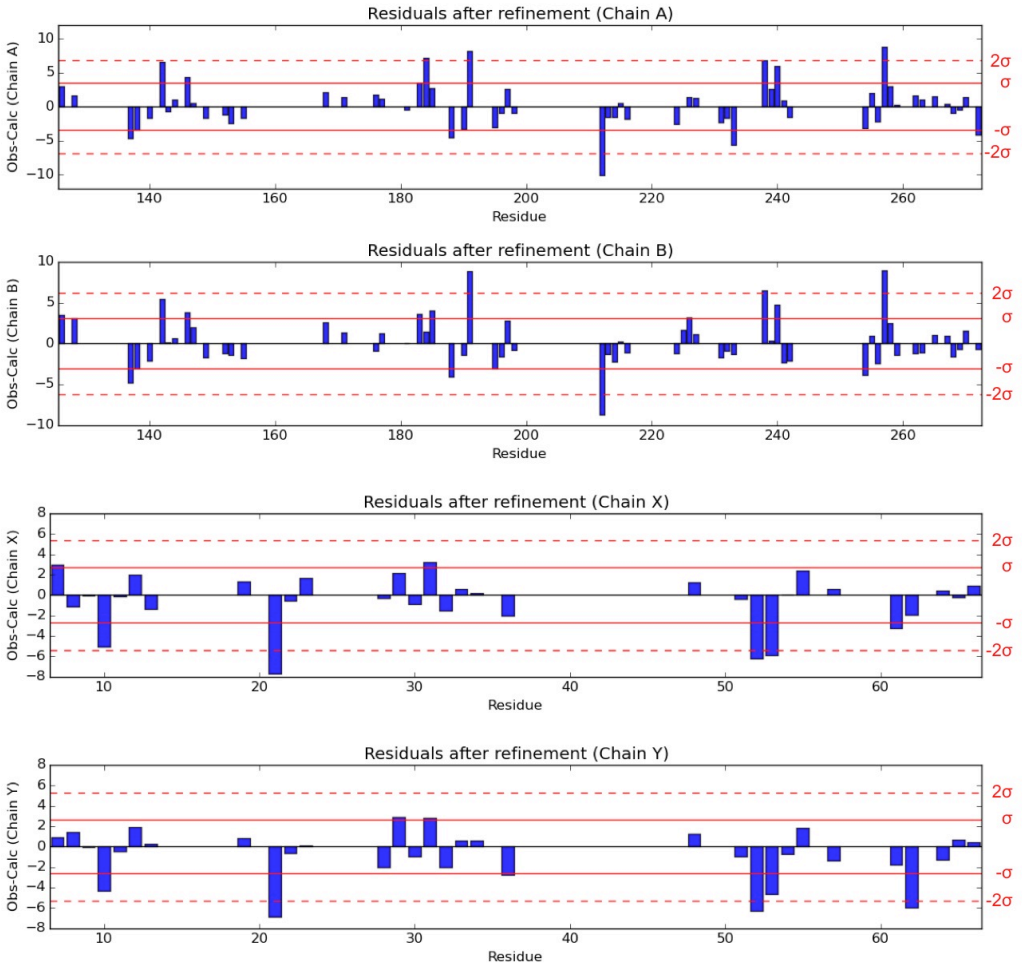

Fig.S1b: RDC residuals compared to their standard deviation after refinement.

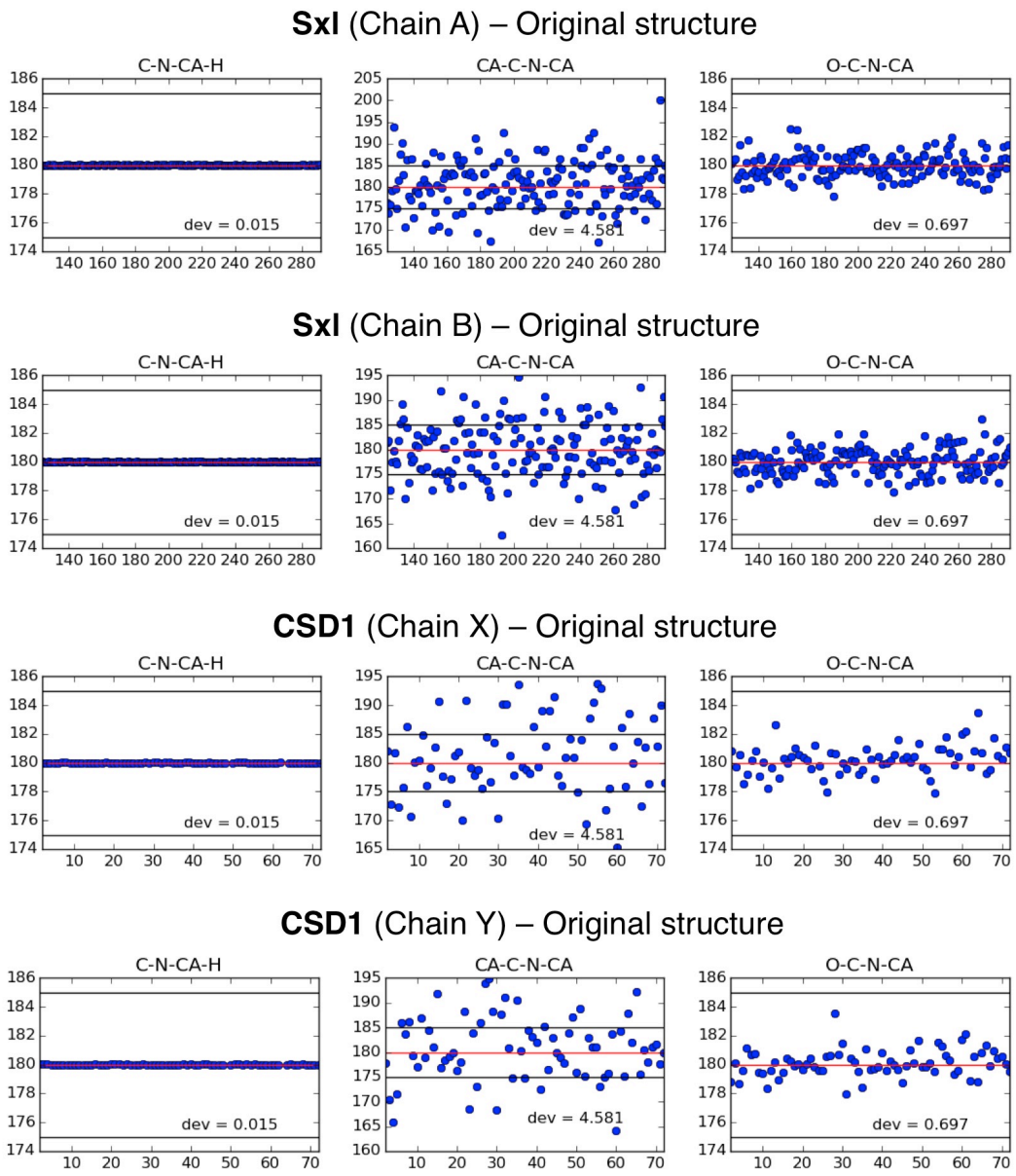

Fig.S2a: Reported values of pep2 (C_{i-1}-N_i- C^{α}_i-H_i), ω (C^{α}_i-C_i-N_{i+1}- C^{α}_{i+1}) and pep1 (O_i-C_i-N_{i+1}-C^{α}_i) for the original structures of SxI (Chains A and B) and CSD1 (Chains X and Y).

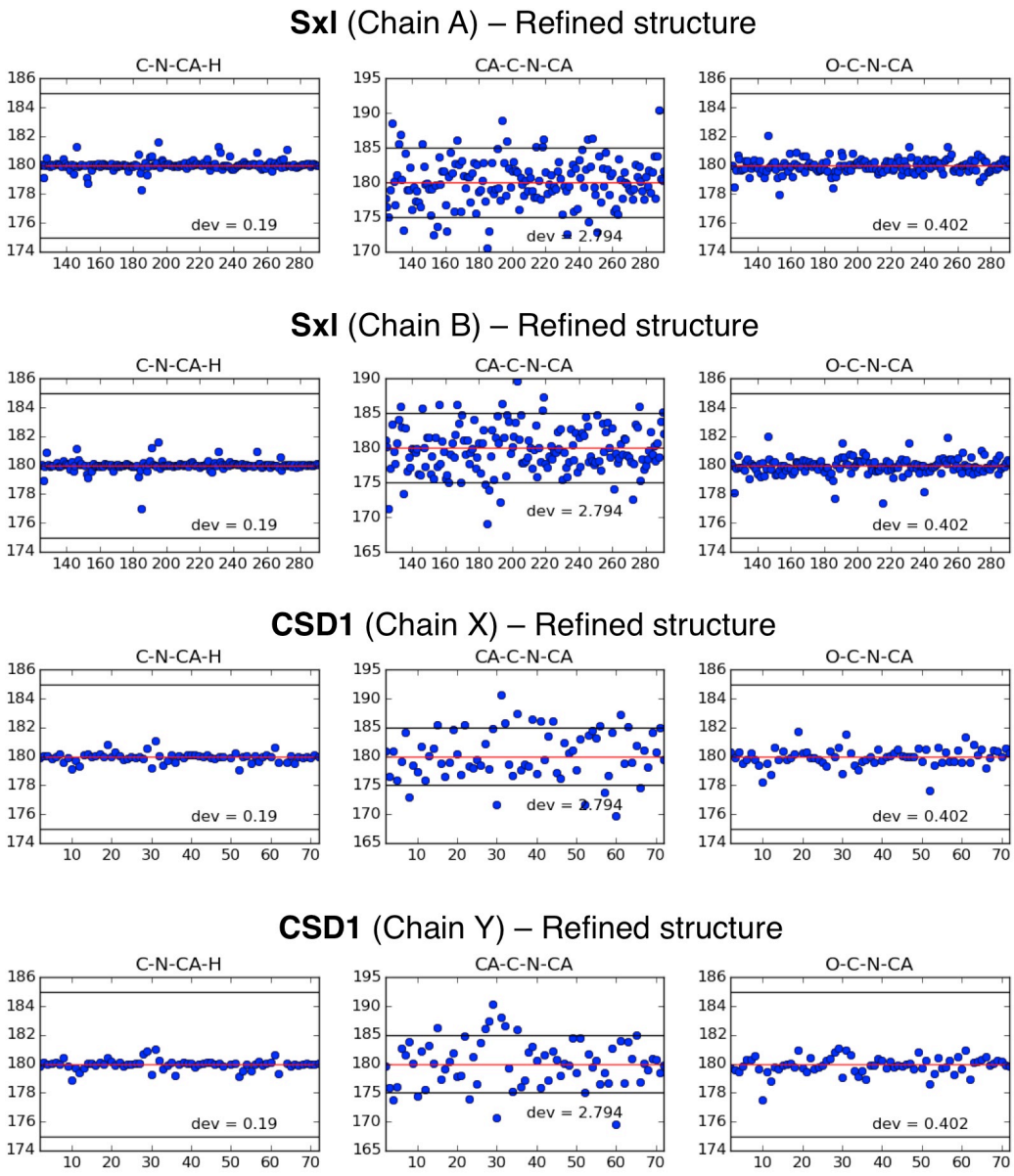

Fig.S2b: Reported values of pep2 (C_{i-1}-N_i- C^{α}_i-H_i), ω (C^{α}_i-C_i-N_{i+1}- C^{α}_{i+1}) and pep1 (O_i-C_i-N_{i+1}-C^{α}_i) for the REFMAC-NMR refined structures of SxI (Chains A and B) and CSD1 (Chains X and Y).

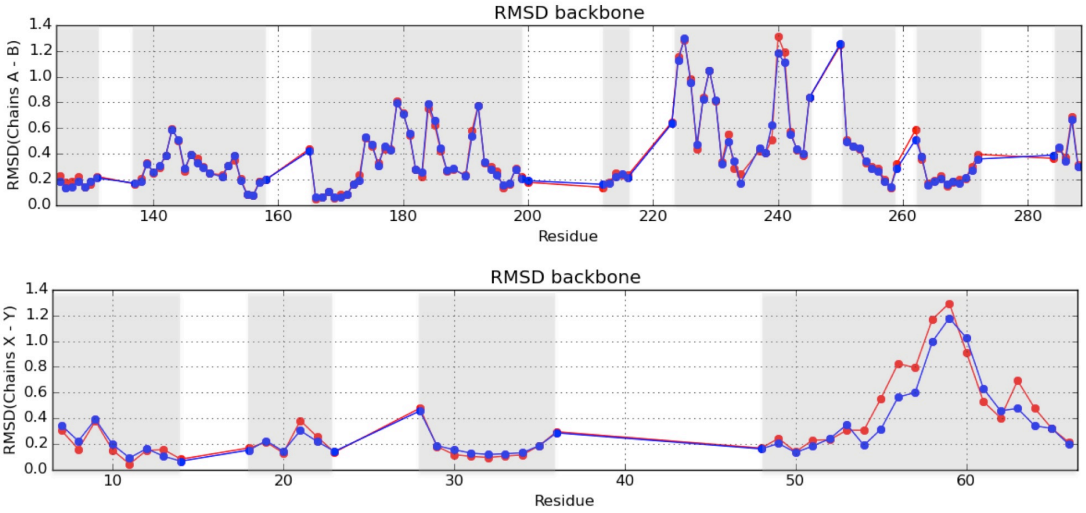

Fig.S3: Residue-by-residue backbone RMSD (for secondary structure elements or for residues not belonging to secondary structure elements for which RDCs were measured) between the two complexes in the asymmetric unit before (red) and after (blue) the refinement, for chains A and B (top), and chains X and Y (bottom) for the .

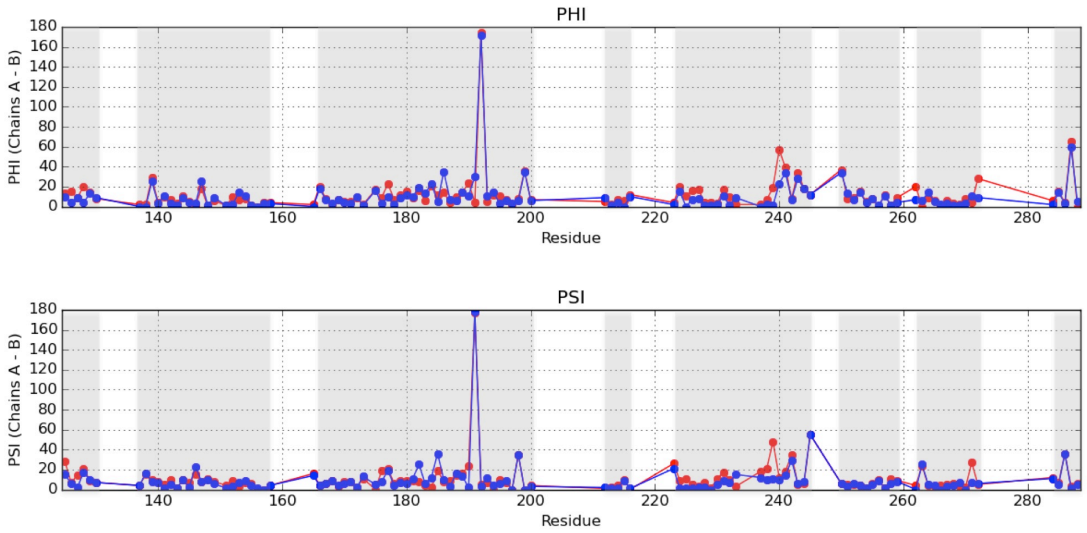

Fig.S4a: Residue-by-residue angle RMSD (for secondary structure elements or for residues not belonging to secondary structure elements for which RDCs were measured) between the chains A and B before (red) and after (blue) the refinement.

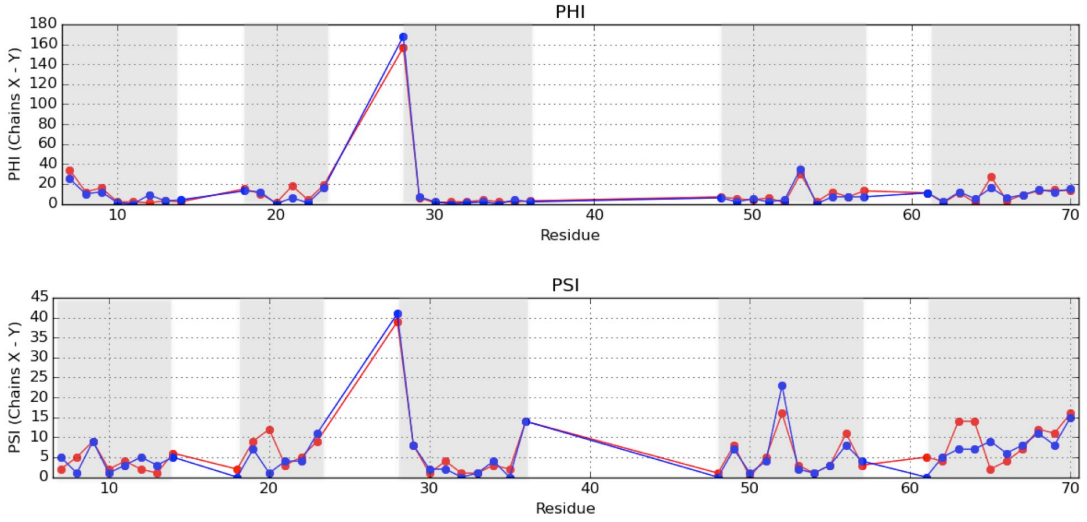

Fig.S4b: Residue-by-residue angle RMSD (for secondary structure elements or for residues not belonging to secondary structure elements for which RDCs were measured) between the chains X and Y before (red) and after (blue) the refinement.

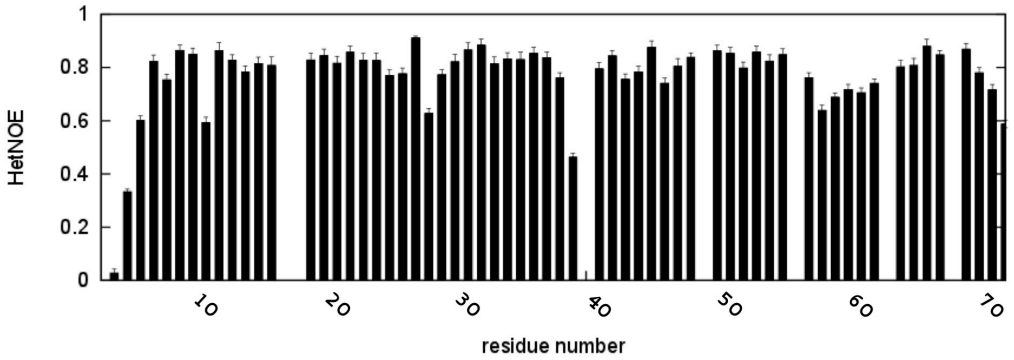

Fig.S5: Heteronuclear {^{1}H}-^{15}N NOE values for the free CSD1. The region around and including residues R58-R59 exhibits a lower NOE value, indicating a higher flexibility.

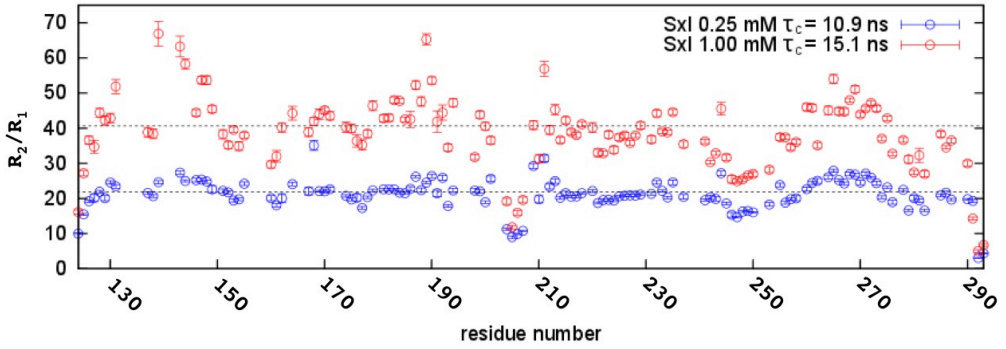

Fig.S6: ^{15}N relaxation data R_2/R_1) of free SxI at 0.25 mM (blue) and 1 mM (red) concentrations. At higher concentration, SxI has a higher rotational correlation time (τ_c = 15.1 ns at 1 mM compared to τ_c = 10.9 ns at 0.25 mM), indicating a weak dimerization propensity, which maybe mediated by residues around I189 and K246.