

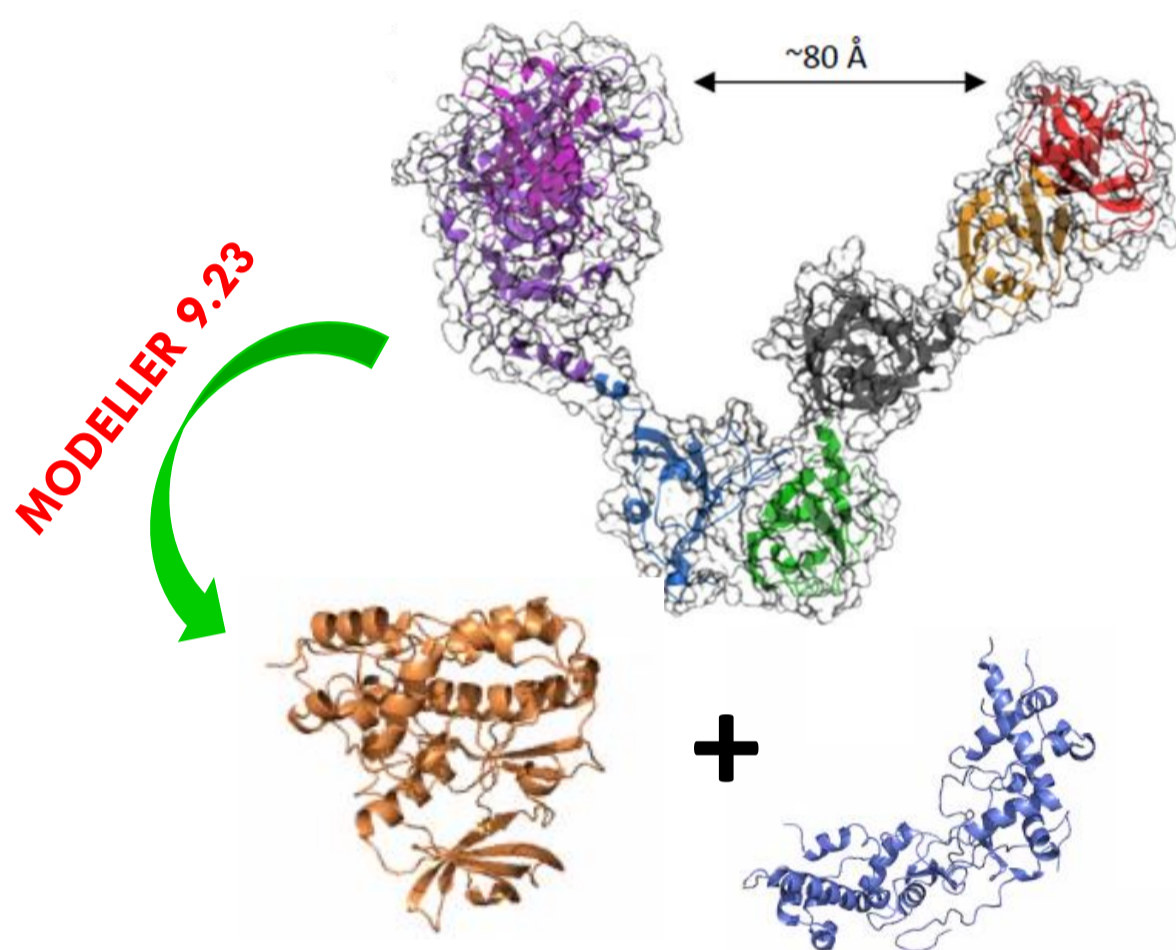
ABSTRACT

Under the purview of Human-viral infections, Human ubiquitin-specific protease 7 (USP7)- the most crucial deubiquitinating enzyme (DUB) in Ubiquitin Proteasome Pathway- is often manipulated by viruses such as Herpes Simplex Virus, Human Immunodeficiency Virus, Epstein-Barr Virus, Cytomegalovirus, Kaposi's Sarcoma Herpesvirus & many more, because it stabilizes proteins that play critical roles in the anti-viral responses. USP7 is also vital for regulation, stabilization, activity or localization of substrate proteins involved in epigenetics, tumor suppression, the DNA damage response & other pathways. Though several studies in the recent past have focused on manipulation of USP7 by viral proteins (would allow viral infection & favor its persistence by dampening/ deregulating the cellular processes), still a considerable gap in knowledge exists regarding how the USP7 is modified. Complete understanding of underlying molecular mechanisms is absolutely necessary to advance our knowledge of virus survival & host-interactions.

USP7 (~130 kDa) is composed of seven domains including N- terminal TRAF-like domain followed by the catalytic domain (CD) and five ubiquitin-like domains. Remarkably, even small C-terminal truncations diminish the activity of the enzyme, suggesting that the C-terminus regulates USP7 activity via a yet unknown mechanism. According to the current model, USP7 adopts an L-shaped structure with N- and C-termini separated by 80-100 Å. This model, however, fails to explain regulation of CD by C-terminus of USP7. Speculatively, C-terminus and CD are brought together in close proximity to create an active conformation, although conclusive structural evidences are still missing, while the available biochemical and structural data are contradictory. Despite successful crystallography studies of various fragments of USP7, structural studies of the full length (FL) protein have been unsuccessful. Here we report our progress in structural and functional characterization FL-USP7 enzyme using integrational approach combining NMR spectroscopy with Small Angle X-ray Scattering and computational molecular docking. A newly developed docking algorithm in HADDOCK allowed us to incorporate SAXS-derived spatial restraints into the modeling of the FL enzyme. Mapping of the inter-domain interaction sites using NMR chemical shift perturbation experiments validated the HADDOCK models. In our integrated FL-USP7 model, the catalytic domain and the regulatory C-terminal region of USP7 are located in close proximity, supporting previously hypothesized molecular mechanism of USP7 auto-activation.

METHODOLOGY and RESULTS

Homology Modelled structure of FL-USP7



MODELLER 9.23

Model was cut into 5 domains (TRAF, CD, UBL12, UBL3 & UBL45) for individual docking

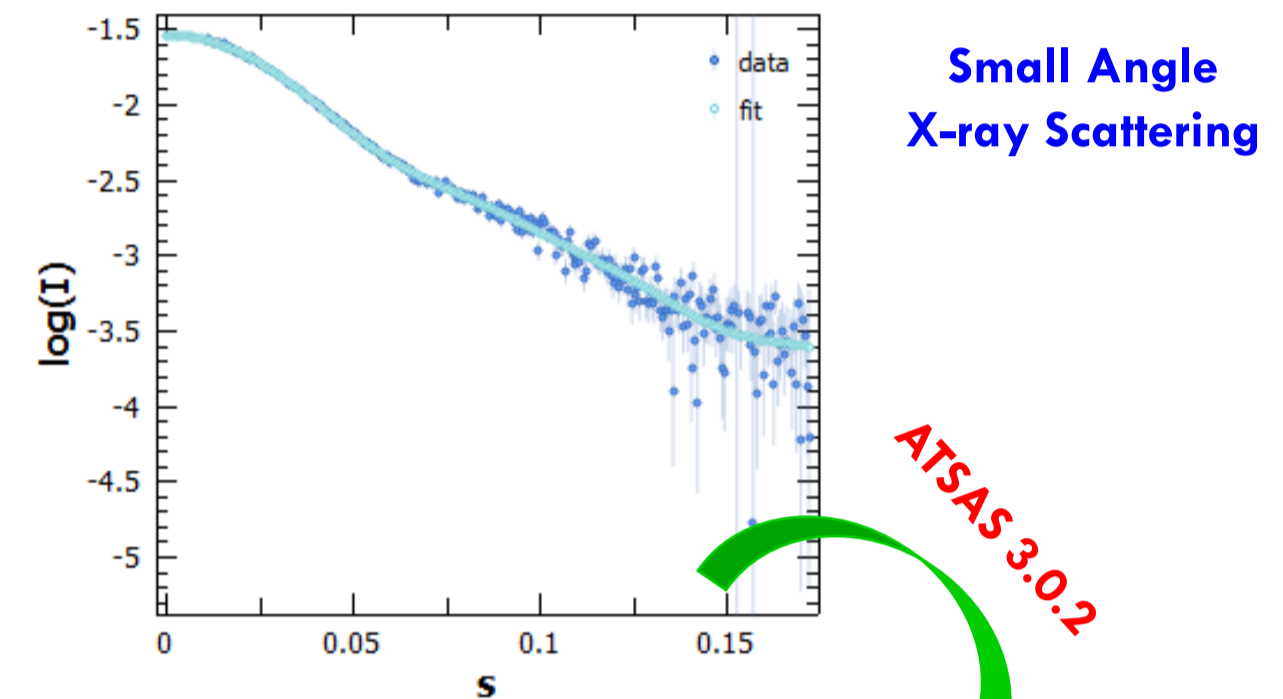
12 models from top 17 ($\chi^2 < 2.0$) with C-N distances < 6.0 were submitted to the local version of HADDOCK (where we can adjust the protein break detection cutoff to 6.0Å in order to restore the C-N bonds between the five pieces)

Restraints were added

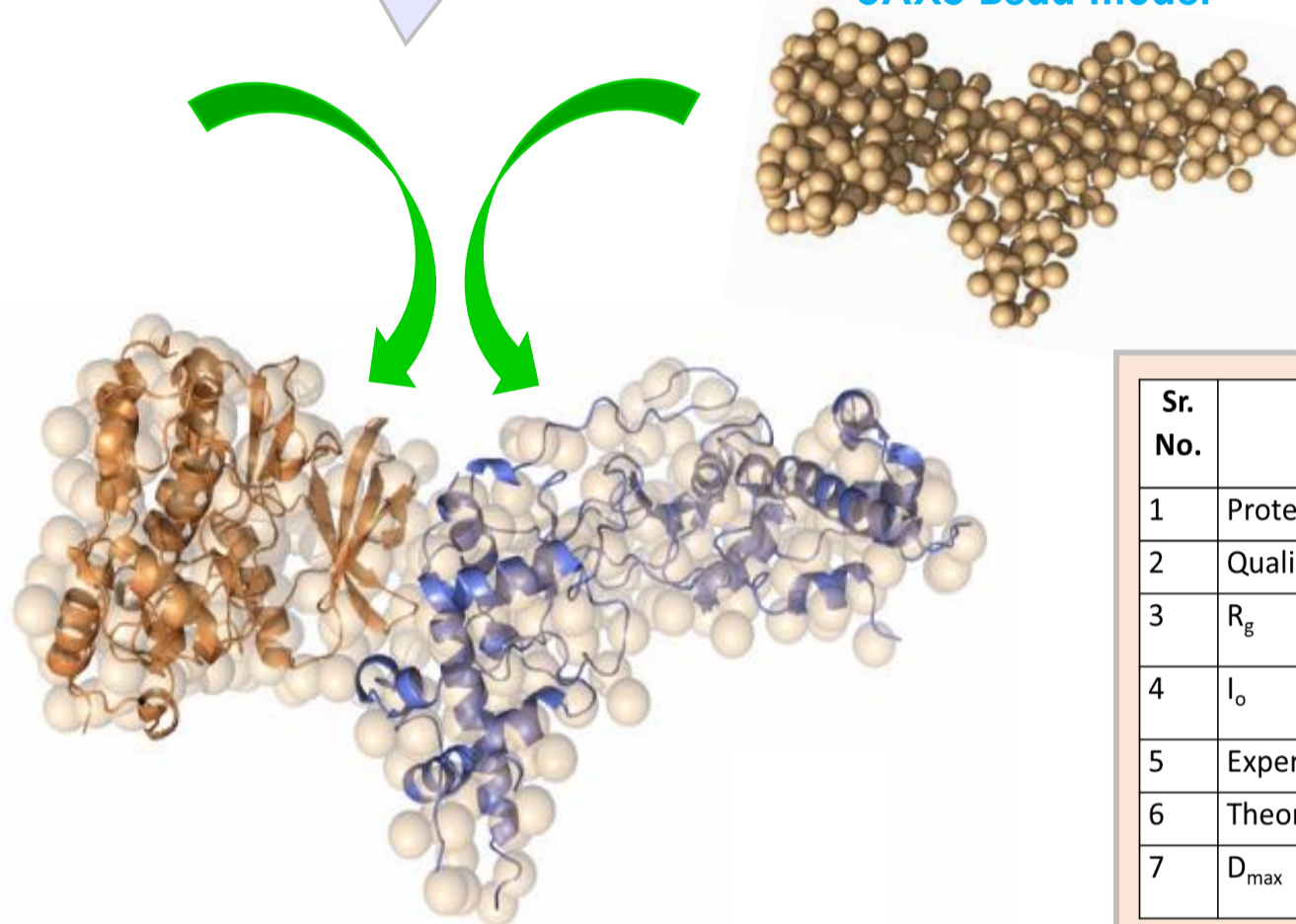
Shape restraint: between CA atoms to any SHA atom (DAMFILT beads) at 5Å

Connectivity restraints: between "termini" that are created by cuts (decreasing from 10Å to 5Å and finally to 1.3Å),

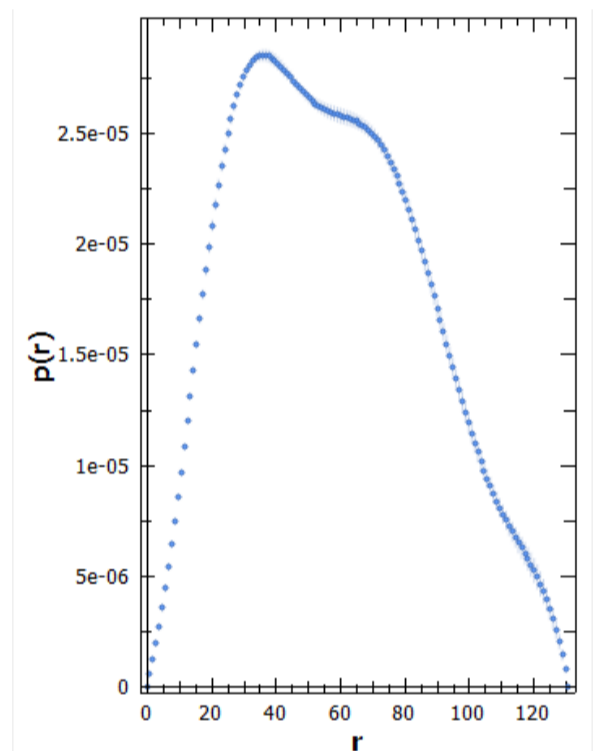
C-terminal restraints: between the last residue and its binding region within Catalytic domain to achieve proper fold



SAXS Bead model



Sr. No.	Factor	Value
1	Protein Concentration	0.4 μM
2	Quality of Data	89%
3	R _g	46.42 Å
4	I ₀	0.0292
5	Experimental Mol. Wt.	133.6 kDa
6	Theoretical Mol. Wt.	130.3 kDa
7	D _{max}	165.85 Å



SAXS Bead model docking with Homology modelled domains

HADDOCK 2.4

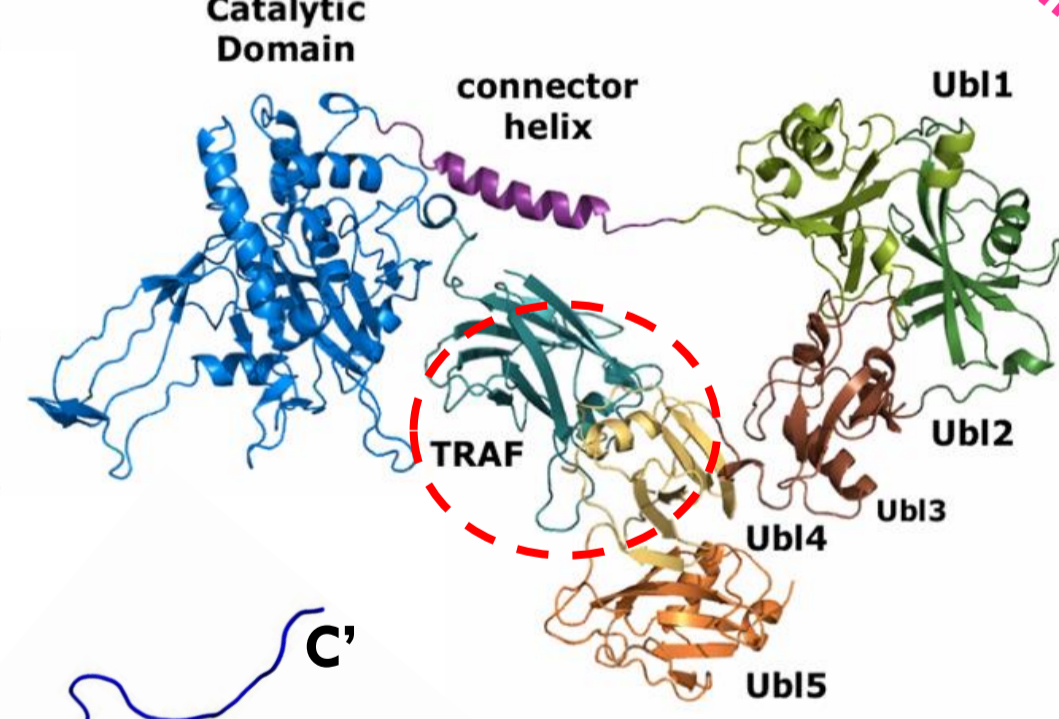
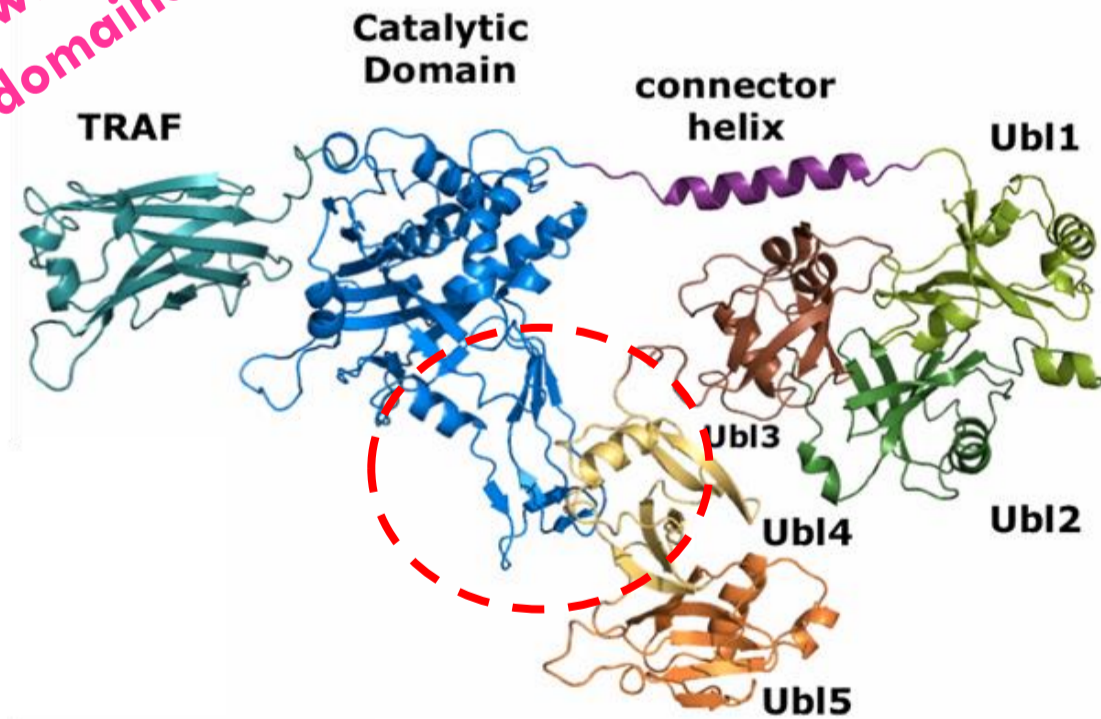
Model 1
 $\chi^2 = 1.54$

Docking Refinement

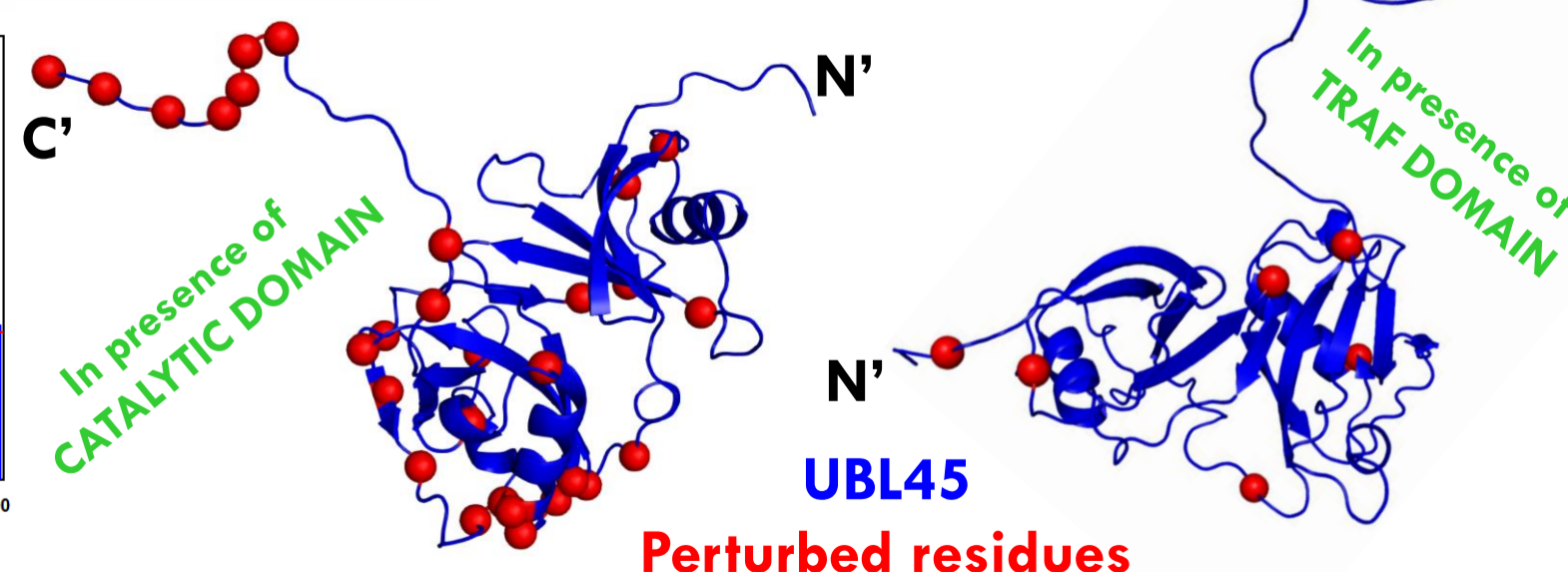
Model 2
 $\chi^2 = 1.41$

Catalytic domain (beta fingers) is in close proximity with C-terminal domains

TRAF domain is in close proximity with C-terminal domains



Nuclear Magnetic Resonance based Model Validation



CONCLUSION

ACKNOWLEDGEMENTS

Molecular weight of USP-7, calculated using SAXS technique (M.W. 133.6 kDa), was found to be in close proximity to theoretical molecular weight (M.W. 130.3 kDa); suggesting the amenability of the data to calculate envelope using ATSAS. Homology model of FL-USP7 obtained via MODELLER 2.0 was then fragmented into 5 domains for individual domain docking through a newly developed docking algorithm in HADDOCK, which allows incorporation of SAXS-derived spatial restraints into the conventional modeling approach. Docking refinement revealed two probable models with almost similar χ^2 values (1.54 for Model-1 and 1.41 for Model-2) as compared to experimental SAXS envelope. Thus, to validate obtained results at atomic precision, we performed NMR based titration experiments. Interaction of ¹⁵N labeled UBL45 with unlabeled CD & TRAF enabled mapping of the inter-domain interaction sites and selection of suitable integrated FL-USP7 model. Accordingly, the catalytic domain and the regulatory C-terminal region of USP7 are located in close proximity (i.e. Model-1), supporting previously hypothesized molecular mechanism of USP7 auto-activation.