Structural and Dynamic Characterization of YAP and the YAP:TEAD Complex

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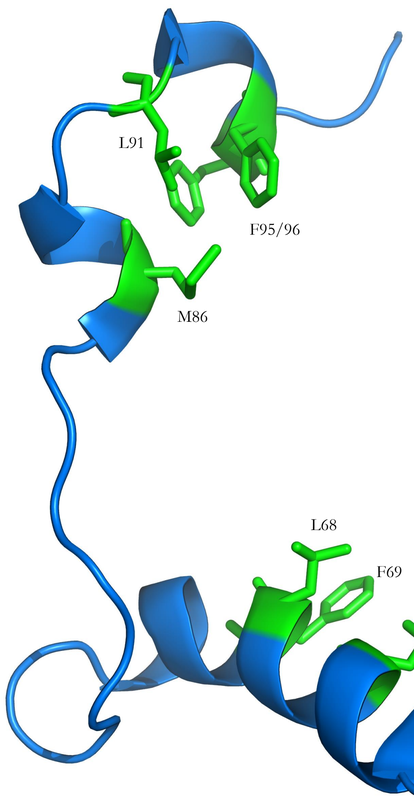
Introduction

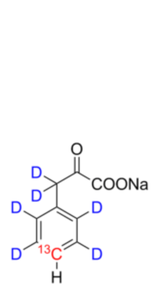
Yes-associated protein (YAP) contains intrinsically disordered protein (IDP) regions that play a major role in the Hippo pathway that regulates organ size, cell proliferation, apoptosis [1], and is associated with a wide range of cancers. Therefore, the binding between YAP and transcriptional enhanced associate domain (TEAD) proteins is an interesting target for cancer therapy [2]. The crystal structure of the TEAD-binding domain of YAP (50-171) bound to TEAD was recently solved [3]. Nevertheless, further studies revealed that various binding partners of TEAD access similar binding interfaces as YAP [4]. Therefore, our study focuses on the characterization of the intrinsically disordered apo state of YAP.

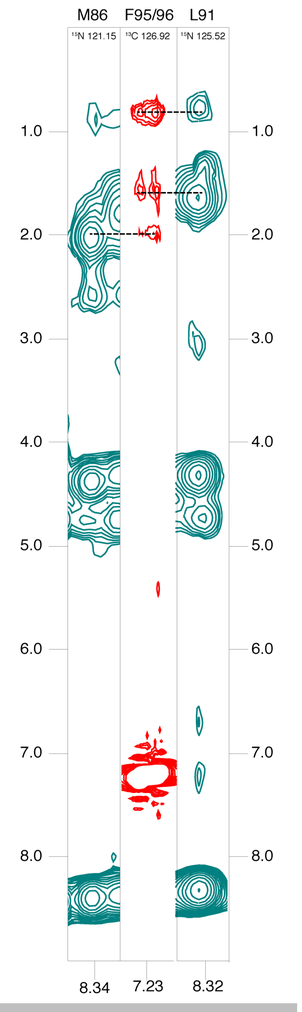


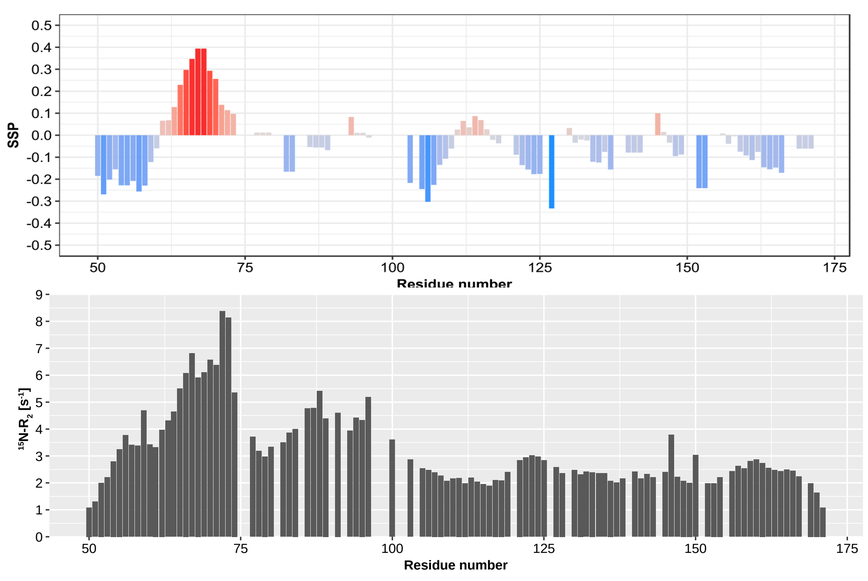
Results

1 Preformation of the secondary structure elements

The ^{15}N R_2 rates are increased in the the α-helix (61-73) and the Ω-loop (86-100) region. This hints at secondary structure formation. The preformation of the α-helix is further supported by the secondary structure propensity (SSP) score derived from the C^{α} and C^{β} chemical shifts [5]. To observe the preformation of the Ω-loop, we selectively labeled Phenylalanines with a late metabolic precursor [6] that enables us to detect NOEs between F95/96-H^{ζ} and L91-H^{γ}, L91-H^{δ}, and M86-H^{ε}. These contacts are sufficient to constitute the Ω-loop structure.

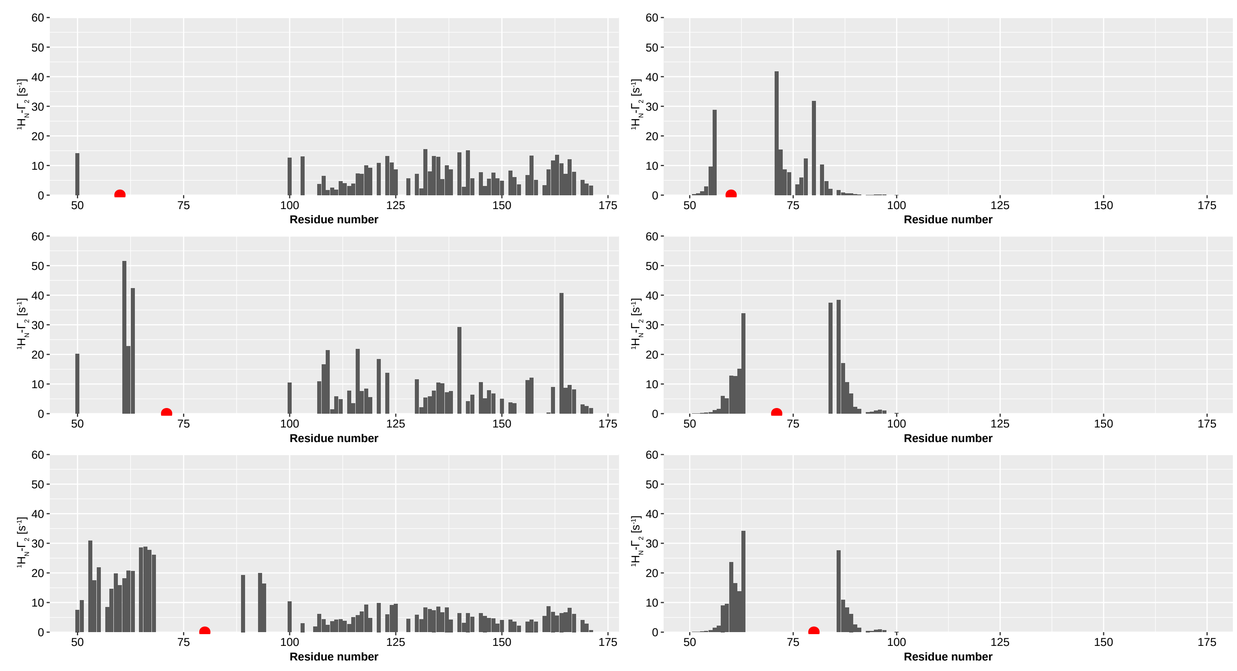






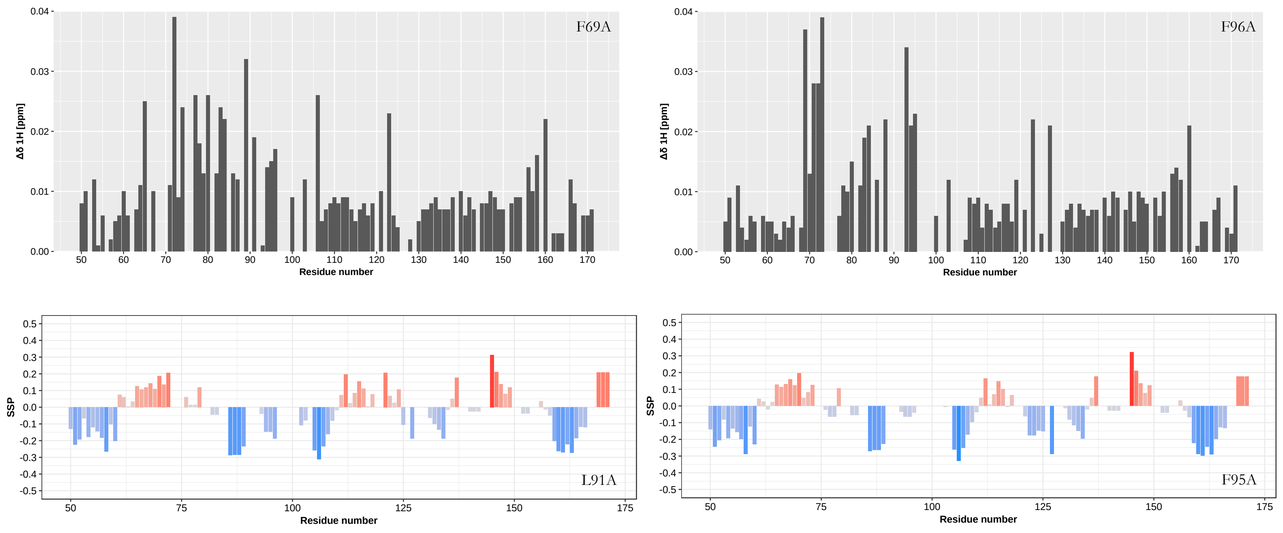
2 Paramagnetic relaxation enhancement (PRE)

The application of PREs via MTSL spin labels (red dots) to probe for long-range (approx. 10-35 Å) contacts indicates a close spatial proximity between the α-helix and the Ω-loop region. Furthermore, if the spin label is placed at position V80 that corresponds to the middle of the linker region connecting the α-helix and the Ω-loop, much less residues in the N-terminus relax too fast for signal detection. The comparison of experimentally derived PREs (left) with PREs calculated from the crystal structure of the YAP:TEAD complex (right) indicate that the apo structure is more compact than the structure of the bound state.

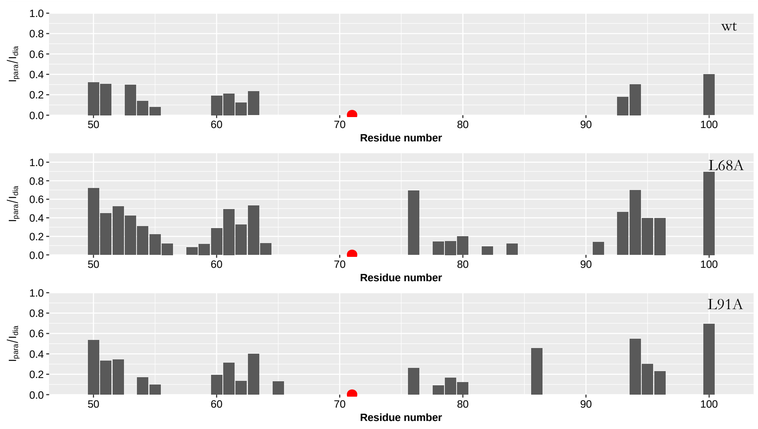


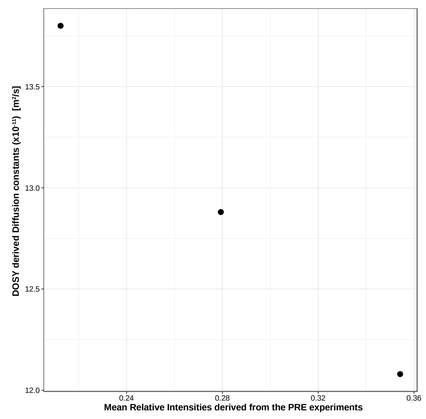
3 Interdependence of the α-helix and the Ω-loop region

On the basis of a recent dissection of the binding interface between YAP and TEAD [7], we chose crucial sites for mutagenesis. Though, we observe an interdependence between the preformation of the α-helix and the Ω-loop region. Mutations in the α-helix region affect the stability of the Ω-loop region and vice versa. In particular, if one of the crucial hydrophobic residues in the Ω-loop is mutated, we observe a significant decrease in the α-helical propensity. Therefore, we assume that there is a co-stabilization between the two elements via hydrophobic interactions (highlighted in green in the figure above).



4 Disrupting the compact state





The combination of a PRE spin label and mutations of residues that have been identified to be crucial for the YAP:TEAD binding indicate that YAP is de-compacting upon introduction of these mutations. These findings are further supported by DOSY derived diffusion constants that decrease upon these mutations. The DOSY data are in good agreement with the observations from the PRE measurements. Therefore, the de-compaction may have an influence on the kinetic behavior of the YAP:TEAD complex formation.

Conclusion

Our findings reveal a compact state in YAP that is even more compact than the bound form of YAP. The preformation of this super-compact state seems to facilitate the interaction with TEAD. Therefore, we suggest that YAP needs to de-compact to bind to TEAD.

References

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