Investigating the Interaction Between the Intrinsically Unstructured Region (IUR) and MLLE Domain of LARP1 Using Biophysical Approaches.

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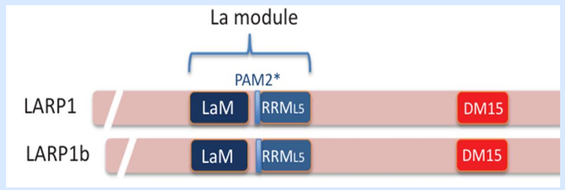
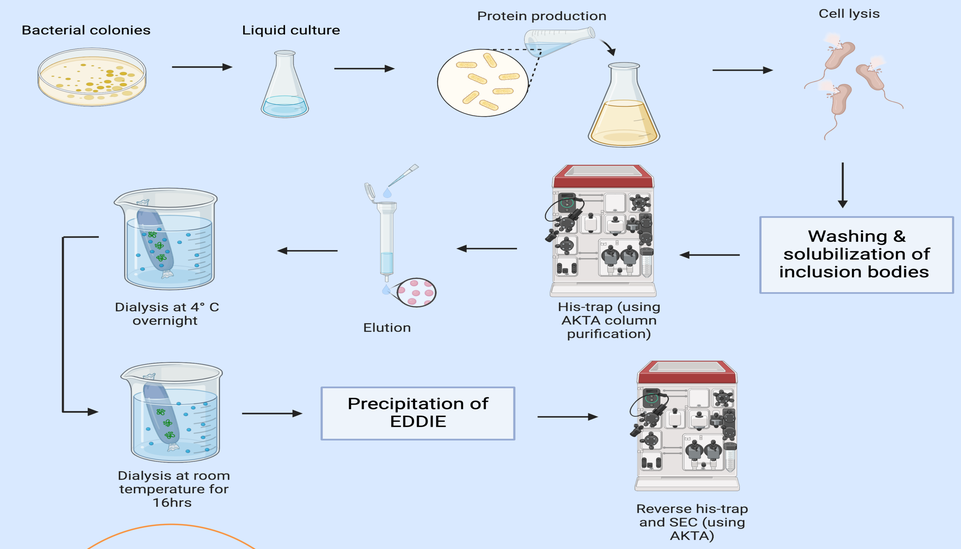
INTRODUCTION

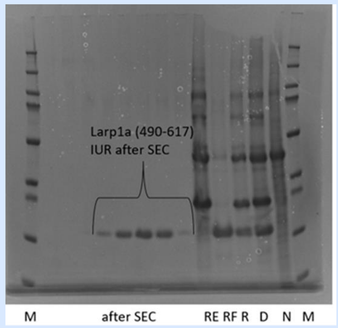
- LARP1 (Lupus antigen-related protein 1) is an RNA-binding protein involved in the post-transcriptional regulation of mRNAs that contain a 5' terminal oligopyrimidine (5'TOP) pathway.
- The intrinsically unstructured region (IUR) of LARP1 is believed to be essential for regulating the interactions between RNA and other proteins, including the MLLE domain, which is a binding site involved in LARP1's interactions.
- Understanding how they interact with each other is vital for gaining insights into LARP1's role in regulating protein synthesis and its broader implications for cellular function.
- In our research, we employed multiple biophysical techniques, including affinity and size exclusion chromatography, Electrophoretic Mobility Shift Assays (EMSA), and Nuclear Magnetic Resonance (NMR) spectroscopy, to investigate the interaction between IUR and MLLE domain.
- Through analyzing these interactions, we aim to provide new insights into LARP1's dynamic role in regulating the translation of mRNA.



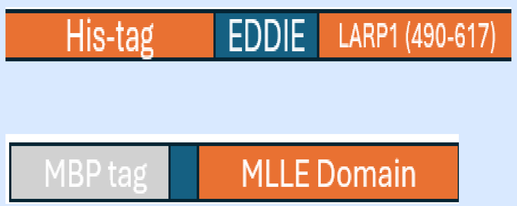
METHODS

Expression and Purification of LARP1 (IUR):

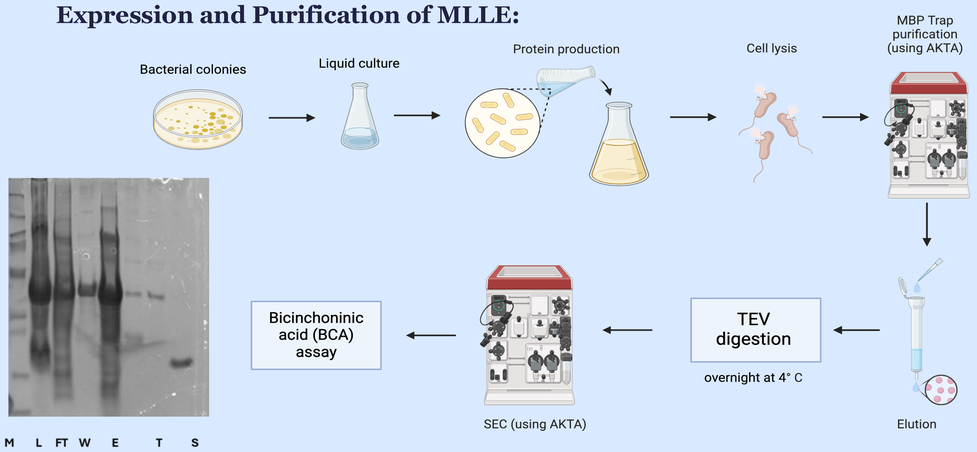




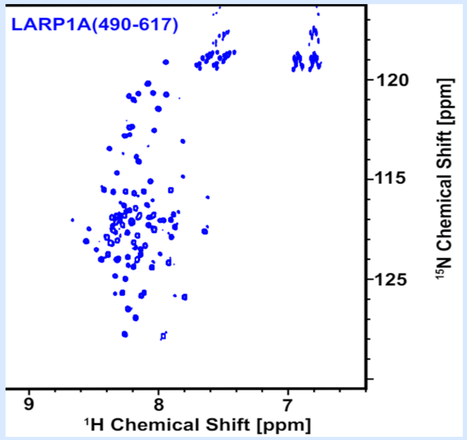
Protein Constructs:



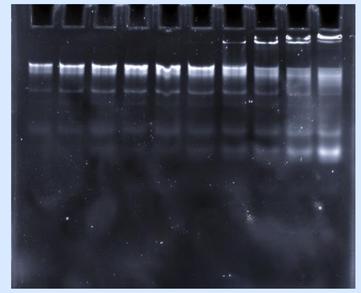
Expression and Purification of MLLE:



RESULTS



[15N-1H] HSQC of LARP1 IDR



EMSA:IDR LARP1 with TOP mRNA

DISCUSSION

- Both LARP1(490-617) and the MLLE Domain were successfully expressed and purified.
- 15N-1H HSQC confirmed the presence of purified IUR of LARP1.
- EMSA gel showed the shift, confirming the binding between IUR LARP1 with TOP mRNA(fluorescently labeled).

CONCLUSION

- EMSA suggests that the IUR influences RNA binding, so the current workflow involves studying its interaction with MLLE, providing new insights into the dynamic regulatory role of LARP1. We are implementing NMR Titration experiments between 15N labelled LARP1 IUR and MLLE domain.
- NMR experiments will help us to understand the mapping of how IUR of LARP1 binds with MLLE to get a detailed knowledge of LARP1's functionality.

REFERENCES

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2. Jia, J. J., Lahr, R. M., Solgaard, M. T., Moraes, B. J., Pointet, R., Yang, A. D.,& Fonseca, B. D. (2021). *Nucleic acids research*, 49(6), 3461-3489.

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