Purification of LA-Related Protein 7 for Biophysical Characterization

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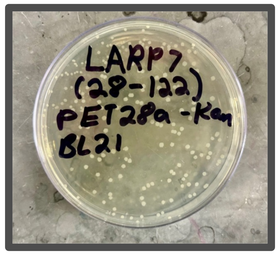
Introduction

LARP-7 is a member of the RNA-binding protein family and plays a crucial role in controlling the elongation of RNA Polymerase II, a vital enzyme used in gene expression during the transcription phase. LARP-7 can regulate the production of Polymerase II by forming a complex with the 7SK snRNP, which effectively inactivates the positive transcription elongation factor b (P-TEFb). P-TEFb is responsible for initiating RNA Polymerase II elongation and transitioning it from a paused state to an active elongation state. The inactivation of P-TEFb leads to the suppression of RNA Polymerase II transcription elongation, thereby affecting gene expression.

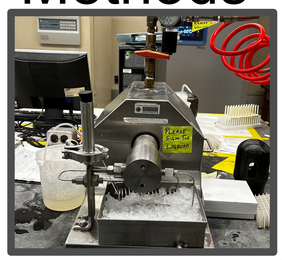
We are studying this protein to better understand its dynamics. Knowing which regions are flexible and which ones are rigid. In addition to knowing the conformational state of LARP-7 in a solution.

Methods



First a transformation was performed on E. Coli (BL21) competent cells with plasmid DNA.
The Cells take in the plasmid which contains gene that codes for LARP-7 and provides antibiotic resistance.

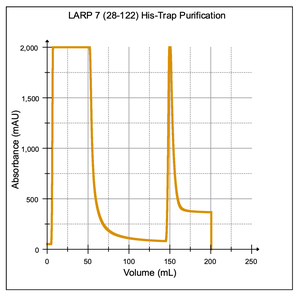
The cells were grown in M9 minimal medium supplemented with ^{15}Nitrogen and ^{13}Carbon until the desired density of cells was achieved. Expression was induced with IPTG, an allolactose analog.

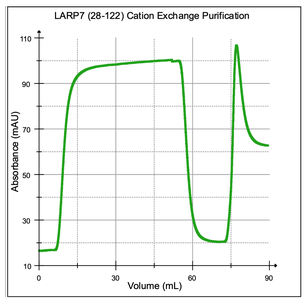


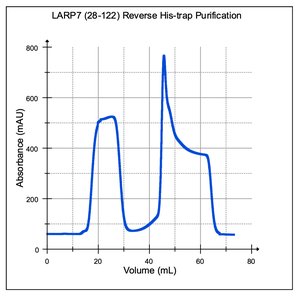
The cells were lysed using a microfluidizer. Then they were centrifuged to separate insoluble material from the cell lysate and the supernatant was collected and filtered.

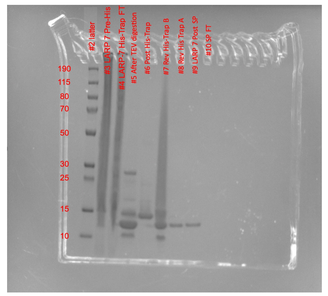
Protein was then purified from the cell lysate using the following techniques:
1.His-Trap
2.Dialysis/TEV Protease digestion
3.Reverse His-Trap
4.Cation Exchange
5.Desalting Column
Gel samples were taken at every step.

An NMR spectrometer with a three-channel probe was used to collect spectra containing chemical shift values of atomic nuclei in the protein. A unique chemical shift indicates a unique chemical environment.



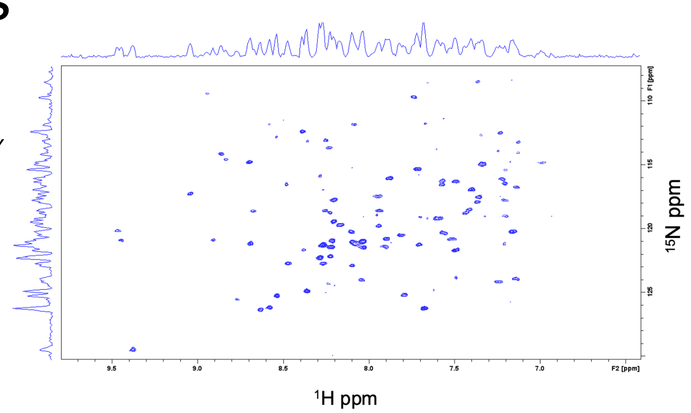






Results

^{1}H-^{15}N BEST-TROSY
@ 700 MHz
100 µM Protein
15 mM MES pH 6.5,
50 mM KCL
10% D_2O and 0.01% DSS



References
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Discussion

The NMR spectrum displays a peak for each nitrogen-hydrogen bond in a unique chemical environment. Because every amino acid (with the exception of proline) has an amide N-H, there is a corresponding peak for every amino acid in the protein. The peaks in this spectrum of LARP7 (amino acids 28-122) are well dispersed. This is indicative of a structured, globular protein domain, meaning the protein is likely folded correctly and the domain boundaries we selected are correct.

In future experiments, each amino acid will be assigned to its corresponding peak which will enable the study of the protein's dynamics via relaxation rates. The relaxation rate refers to the decrease in signal intensity over time. With spin-spin relaxation (R_2), resonances in more ridged regions will have a faster relaxation rate, while resonances in more flexible regions will have a slower relaxation rate.