Directed evolution of RimJ For N-terminal acetylation with broad substrate specificity

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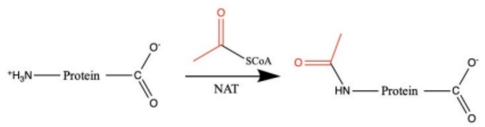
Background

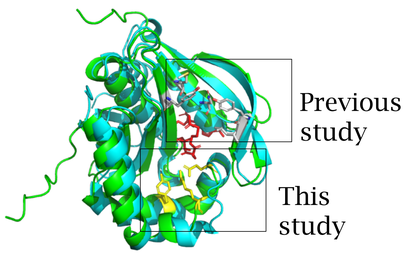
- N-terminal acetylation is essential for the stability, activity, and targeting of proteins in eukaryotes.
- However, most eukaryotic proteins are not acetylated when expressed in bacteria.
- RimJ is an N-terminal acetyltransferase known to acetylate many recombinant proteins with a narrow substrate specificity in E. coli, including the Z domain.
- N-terminal acetylation by RimJ has two requirements:
  - Initiator methionine cleavage
  - N-terminal amino acid sequence



Objective

- To increase the applicability of RimJ for the N-terminal acetylation of a broad range of recombinant proteins.
- Increase active site size to accommodate larger proteins, using a mutation to alanine.
- Determined from the Alpha Fold-predicted structure of E. coli RimJ, the following amino acids are of interest:

Y35A
E46A
R49A
Y106A
Y170A
L171A

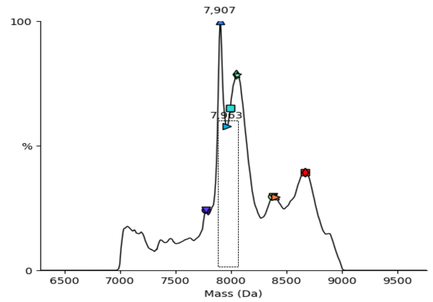


Methods

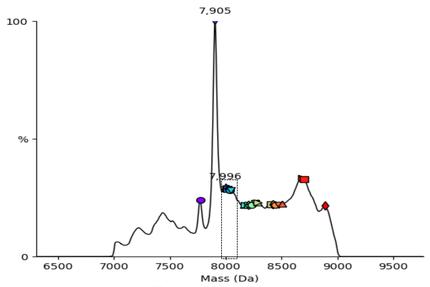
- Site-directed mutagenesis to create RimJ variants at these sites with mutation to alanine, a small neutral amino acid.
- Co-expression of the RimJ variant with Z-domain variants, T2I and S3K, which will not acetylate by wild type RimJ.
- Purification of protein by Ni-NTA affinity chromatography.
- Analyze the N-terminal acetylation proteins with mass spectrometry.

Mass Spectrometry Results

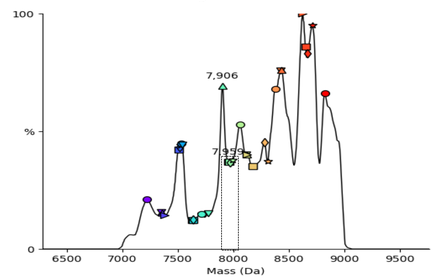
Z domain T2I



RimJ Y35A



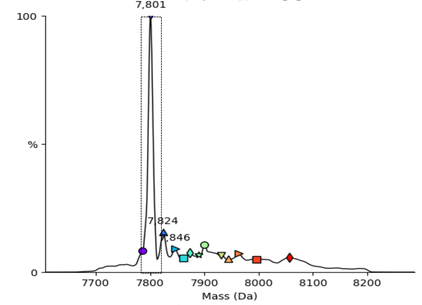
RimJ E46A



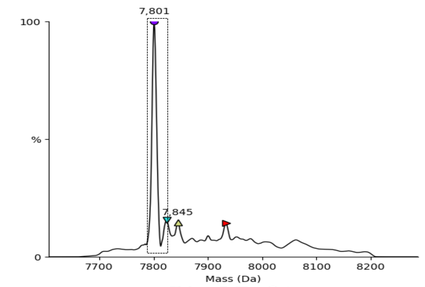
RimJ R49A

7905.69 Da (nonacetylated)
7947.63 Da (acetylated)

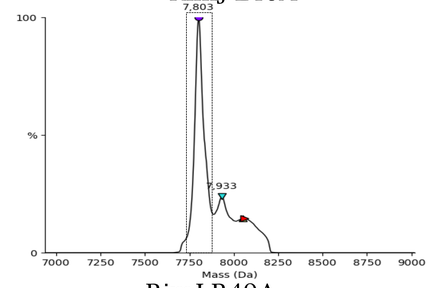
Z domain S3k



RimJ Y35A



RimJ E46A



RimJ R49A

7804.54 Da (nonacetylated)
7845.57 Da (acetylated)

Conclusion

- Peaks show nonacetylation (negative results) for single mutants Y35, E46, R49 for Z domain S3K.
- Accompanying peaks suggests impurities.
- Potential acetylation for single mutants Y35, E46, and R49 for Z domain T2I in broad peaks, although repeated purification is necessary.

Future Directions

- Further purification of Y35, E46, and R49 for expression in Z domain T2I with different chromatography method.
- Continue protein analysis of RimJ double and triple mutants:
  - Y35A E46A
  - Y35A R49A
  - Y35A Y106A
  - Y35A Y170A L171A

References

Perez, L. and Ryu. Y. (2015) RimJ-Catalyzed Sequence-Specific Protein N-terminal Acetylation in *Escherichia coli. Advances in Bioscience and Biotechnology*, 6, 182-193.

Acknowledgements

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