



# Segmental isotope labelling of the multidomain La protein for NMR structural study

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# La protein (SS-B)



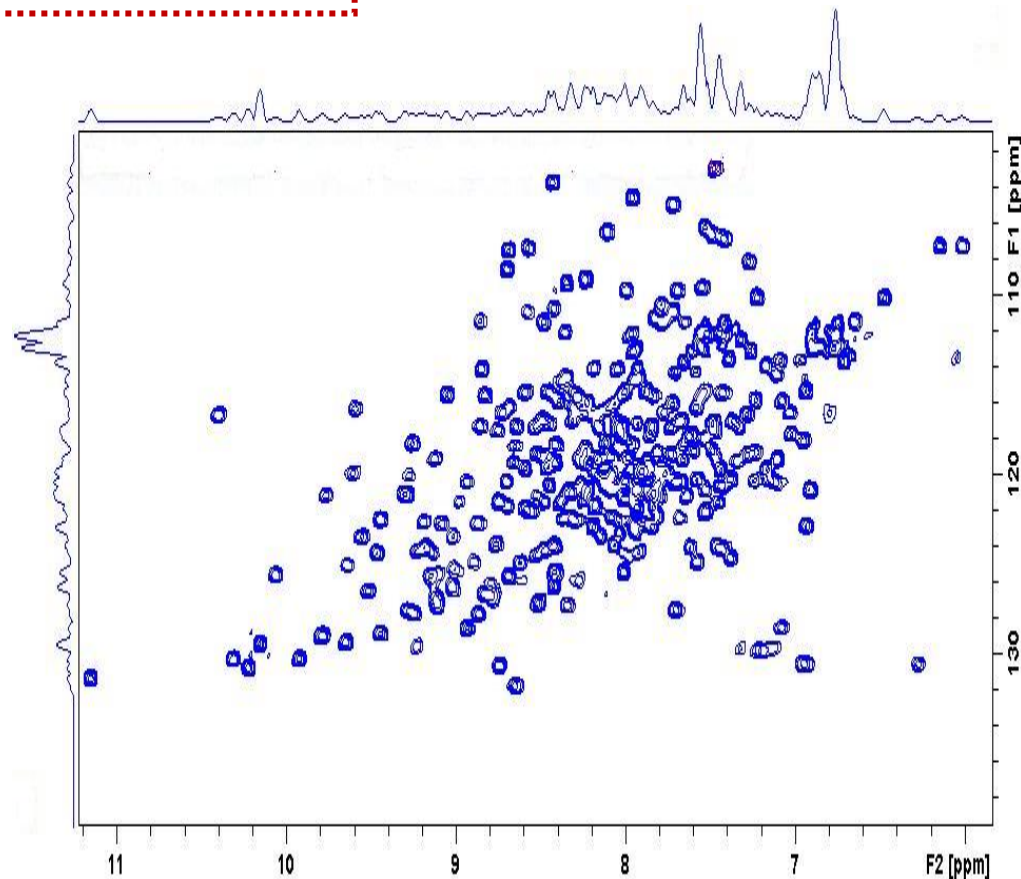
- La (Lupus antigen) / SS-B (Sjogren's Syndrome antigen B)
- first described in patients suffering from Rheumatic Systemic Lupus Erythematosus and Sjogren's syndrome
- nuclear phosphoprotein
- associates with all synthesized RNA polymerase III transcripts like pre-tRNA, pre-5S rRNA, snRNA, RNase P RNA, Y RNA
- exhibits high affinity for 3'UUU-OH

Absence of La → changes in tRNA amount, size and folding

# NMR structures of LaM-RRM1-RRM2 (334 a.a.)- Segmental Labeling

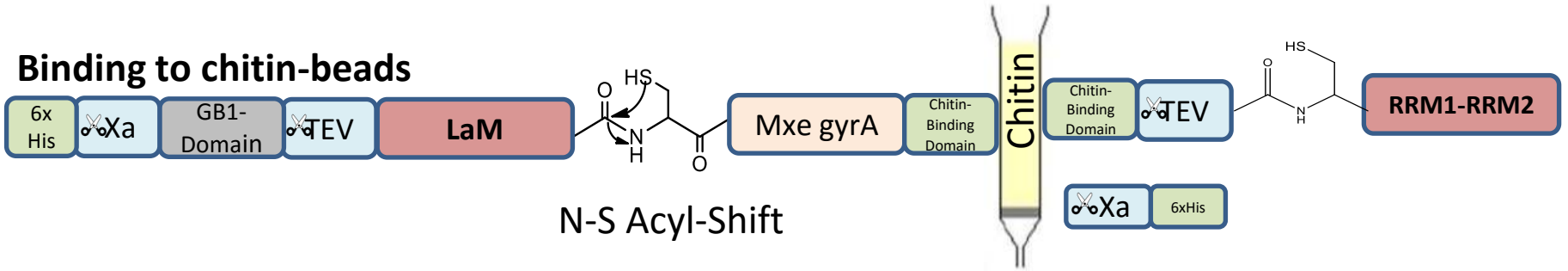


- Allows for specific segment(s) within a protein to be selectively examined by NMR.
- Used for multi-domain proteins
- Simplify the multi-dimensional heteronuclear NMR spectra



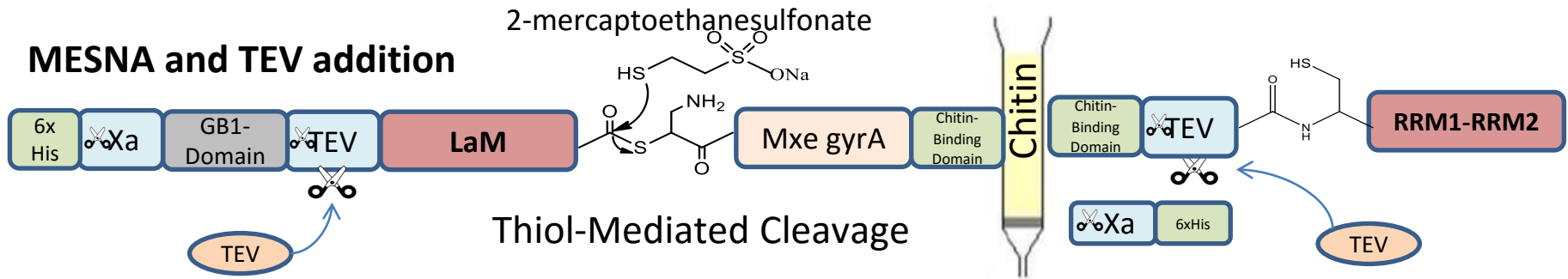
# The ligation strategy for LaM and RRM1-RRM2 domains of La protein

## Binding to chitin-beads



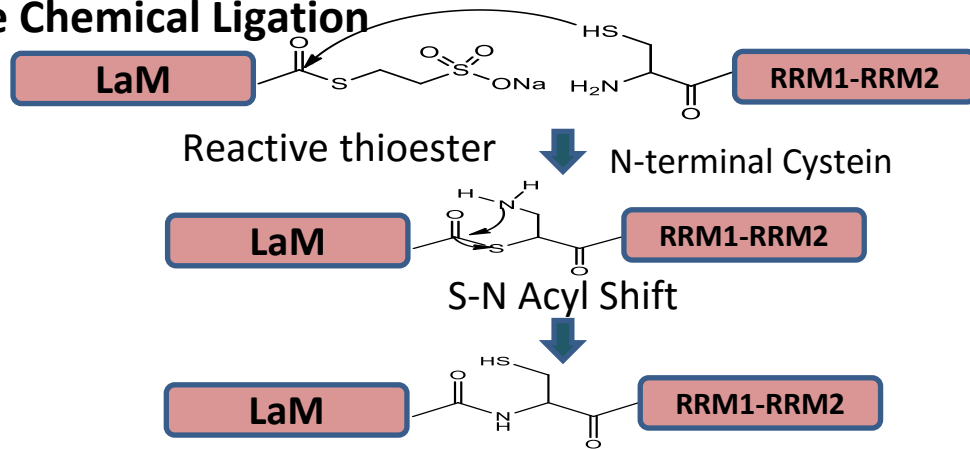
N-S Acyl-Shift

## MESNA and TEV addition



Thiol-Mediated Cleavage

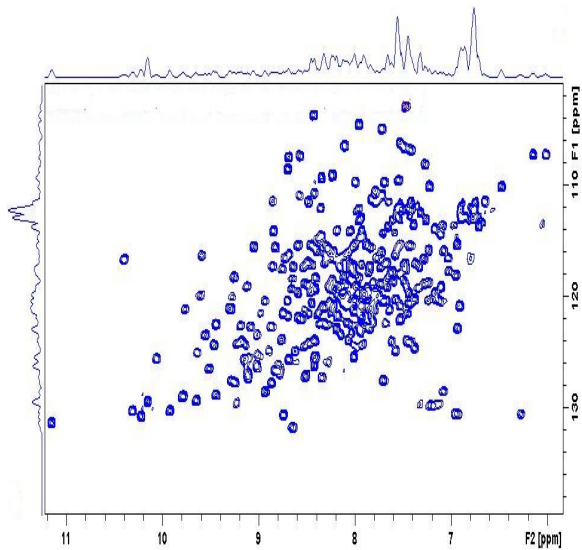
## Native Chemical Ligation



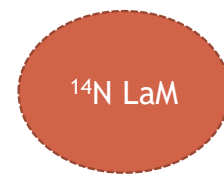
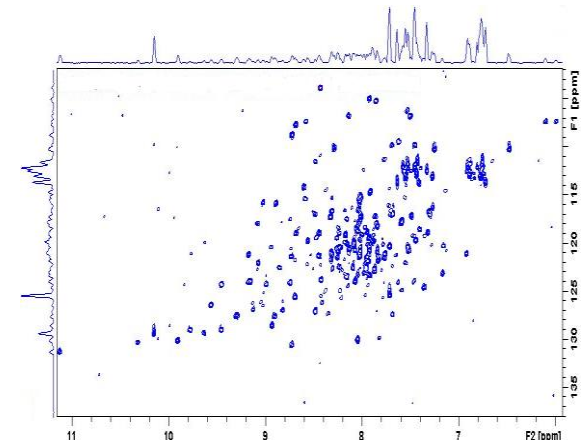
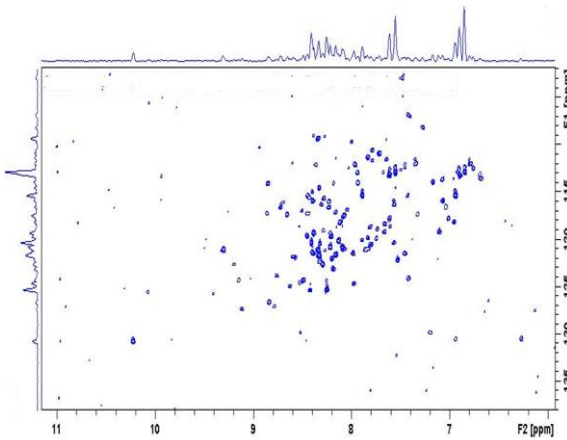
Ligated Domains

# Segmental labelling on LaM-RRM1-RRM2 domain of La protein

$^{15}\text{N}\{\text{LaM-RRM1-RRM2}\}$  (334 a.a.)



$^{15}\text{N}\{\text{LaM}\}$ - $^{14}\text{N}\{\text{RRM1-RRM2}\}$  (334 a.a.)     $^{14}\text{N}\{\text{LaM}\}$ - $^{15}\text{N}\{\text{RRM1-RRM2}\}$  (334 a.a.)



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## Supervisor

Professor **Giorgos Spyroulias**-Department of Pharmacy, University of Patras

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