Conformational Study of the RNA-binding *human* La (Lupus antigen) protein through NMR Spectroscopy and structural basis of La HCV IRES recognition

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Introduction

Lupus Antigen (La) a 408 amino acid protein and a member of an RNA-binding protein family, known as La-Related Proteins. La is a multi-domain protein, consisting of a La Motif (LaM), two RNA Recognition Motifs (RRM1 and RRM2) and a C-terminal region (Figure 1)[1]. La protein mainly found in nucleus but can also be found in cytoplasm having distinguish biological roles[2]. In the nucleus, La's N-Terminal Domain (LaM-RRM1) binds to relatively all nascent transcripts of RNA Polymerase III (e.g., pre-tRNA) during their maturation process[1]. In the cytoplasm La protein stimulates the translation of different cellular and viral mRNAs. The translation of these mRNAs is Internal Ribosome Entry Site (IRES) – mediated. La binds to the IRES sequence and stimulates the translation. In this occasion, the RRM2 of C-Terminal Domain of the protein is required for IRES binding. Hepatitis C Virus (HCV) is an RNA virus and its genome's translation is IRES-mediated. La binds to IV domain of HCV's IRES, where the start codon is located and stimulates the translation[3].

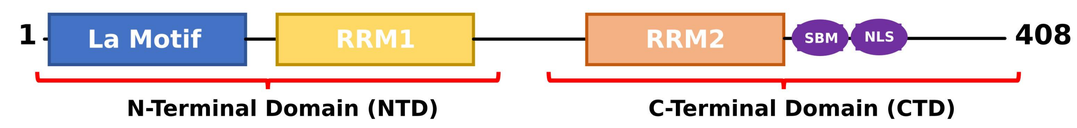

Figure 1: Domain organization of *human* La protein

Aim of the Study : To interpret the structural and the dynamical properties of the La protein through NMR Spectroscopy and to shed light on La-HCV IRES interaction via NMR Spectroscopy and Isothermal Titration Calorimetry (ITC)

Results and Discussion

i) NMR Assignment

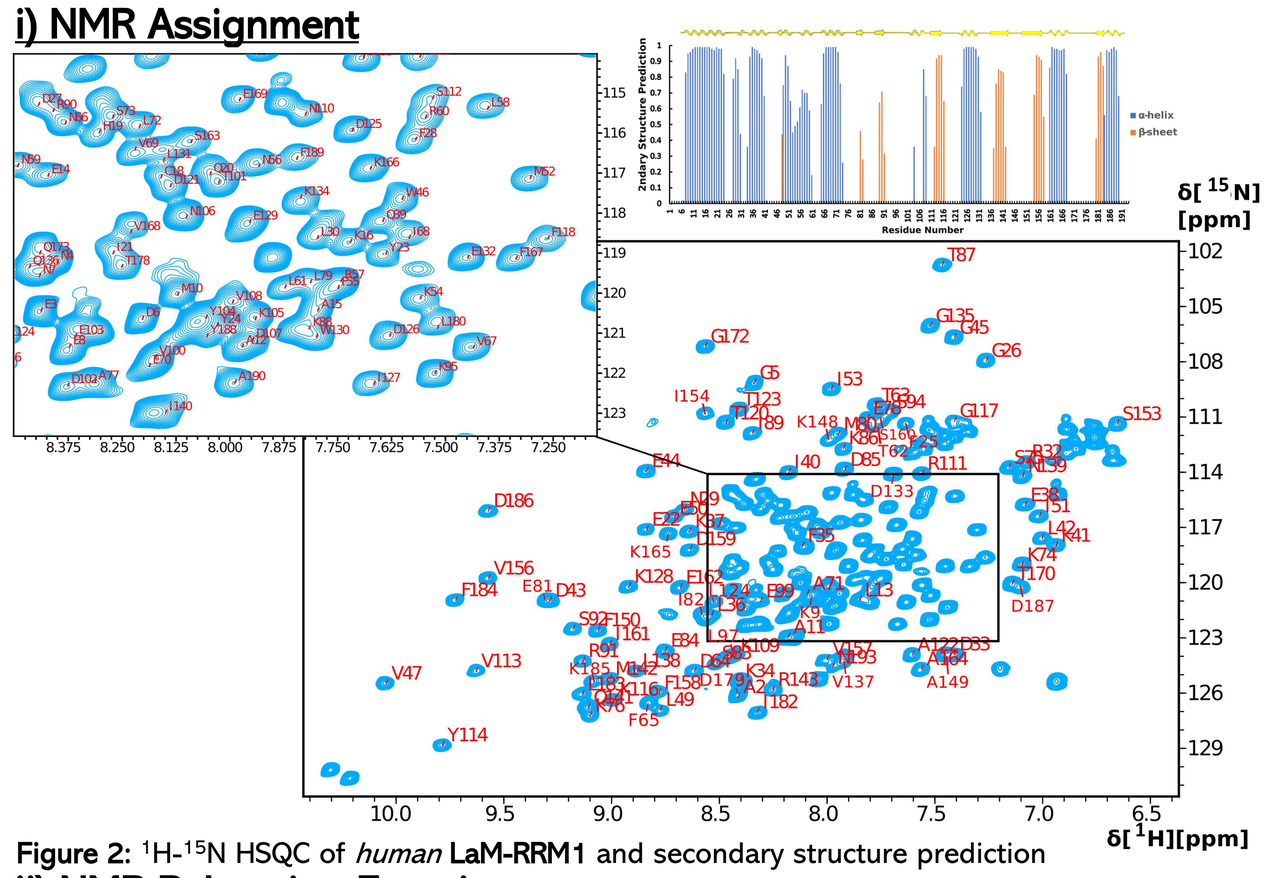

Figure 2: ^{1}H-^{15}N HSQC of *human* **LaM-RRM1** and secondary structure prediction

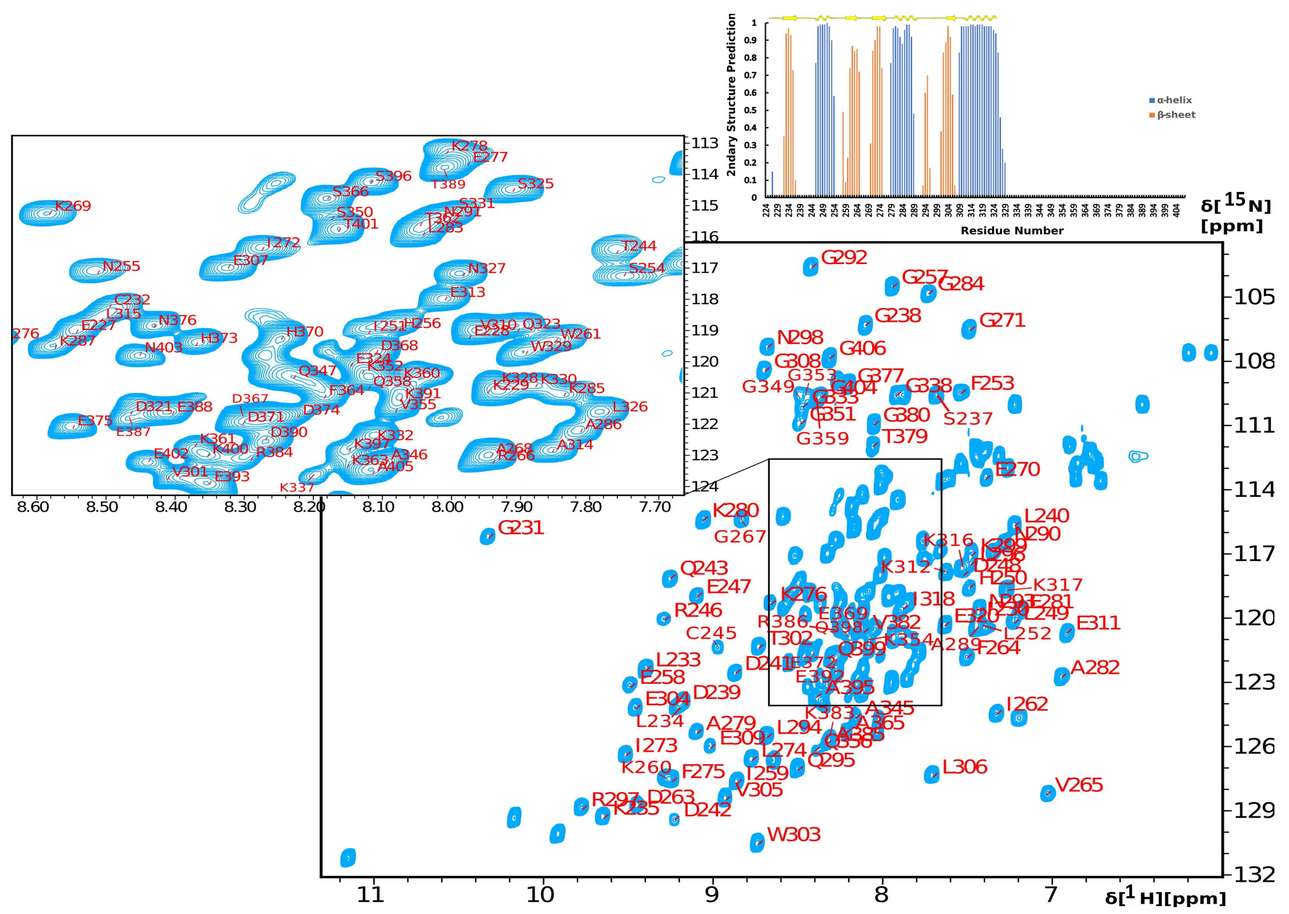

Figure 3: ^{1}H-^{15}N HSQC of *human* **RRM2-Cter** and secondary structure prediction

ii) NMR Relaxation Experiments

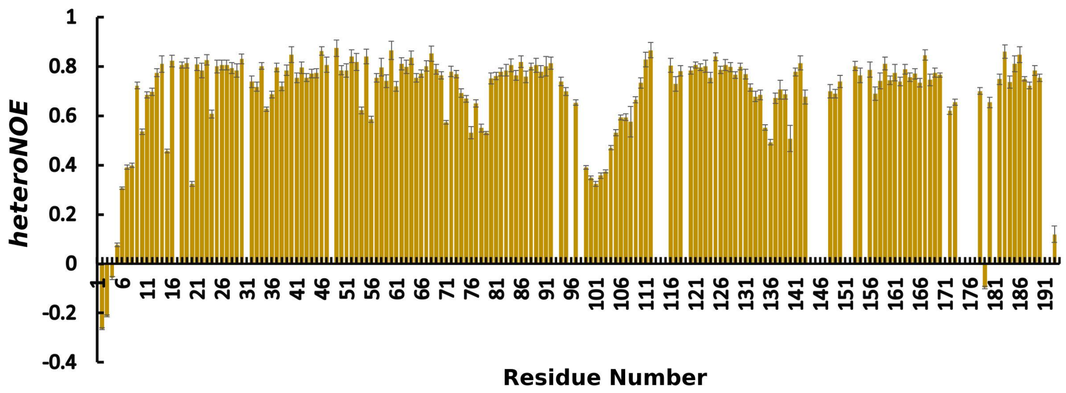

Figure 4: heteroNOE values of *human* La 1-194 (LaM-RRM1) free state

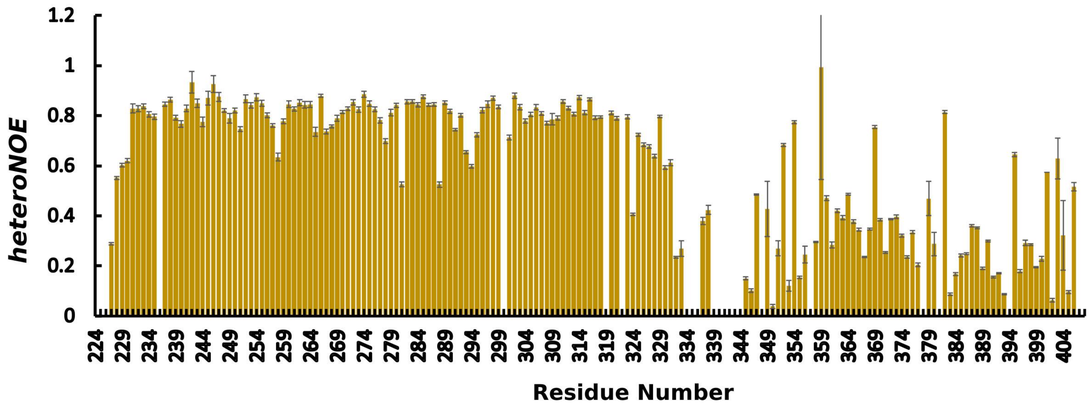

Figure 5: heteroNOE values of *human* La 224-408 (RRM2-Cter) free state

iii) La protein – HCV IRES interaction

La 225-359 (RRM2 SBM) – IV Domain HCV IRES

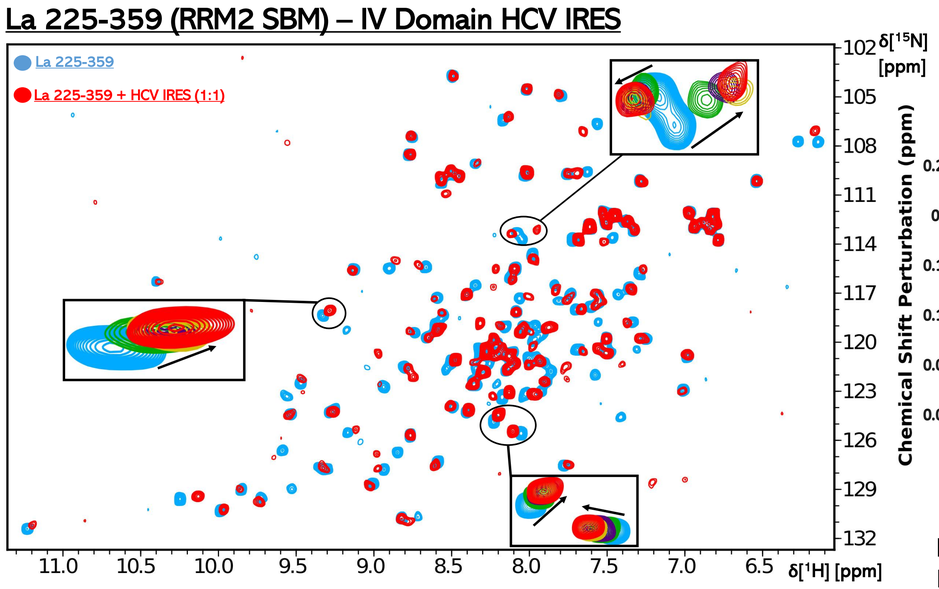

Figure 6: HSQC Spectrum of *human* La 225-359 (RRM2 SBM) (Blue) overlayed with HSQC Spectrum of La 225-359 in 1:1 ratio with Domain IV of HCV IRES (Red).

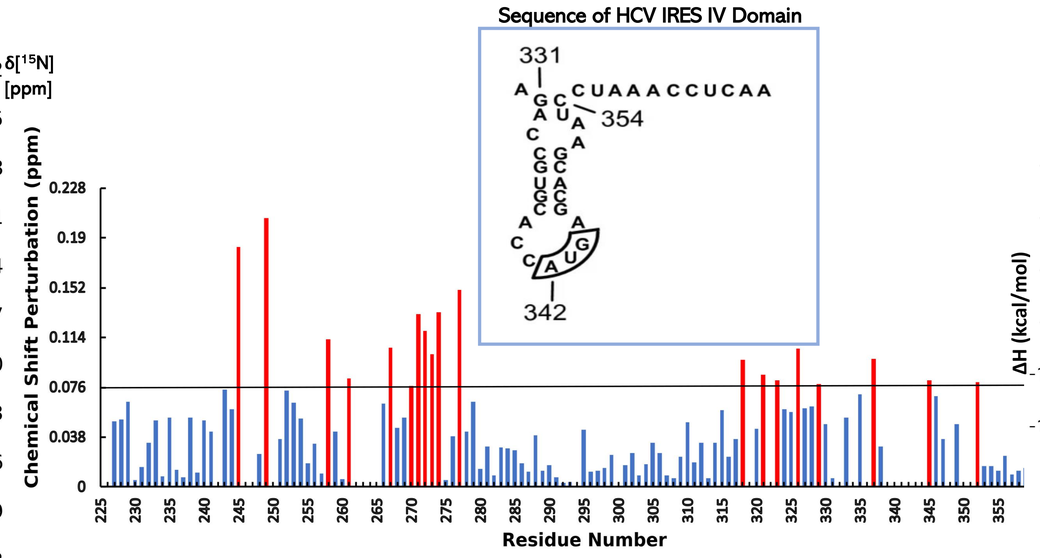

Figure 7: Chemical Shift Perturbation of La 225-359 in 1:1 ratio with Domain IV of HCV IRES. Perturbated residues (Red) unperturbated residues (Blue)

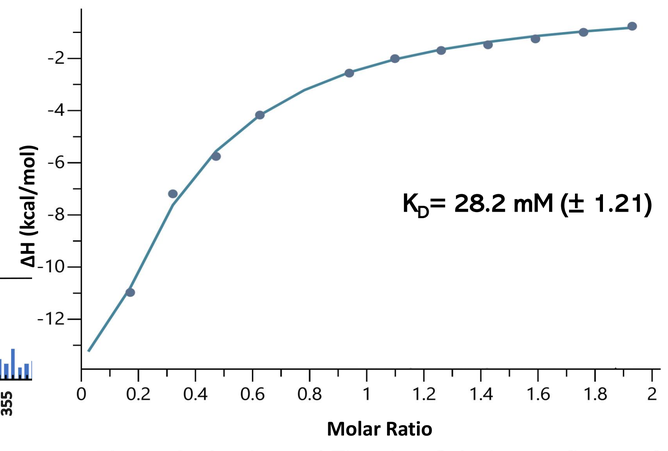

Figure 8: Isothermal Titration Calorimetry figure of La 225-359 interaction with Domain IV of HCV IRES.

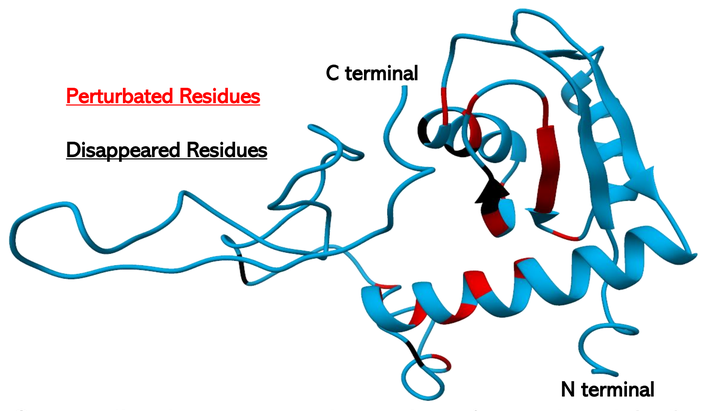

Figure 9: RRM2-Cter predicted structure and mapping of perturbated (red) and disappeared (black) residues at 1:1 ratio of **La 225-359** with **IV domain of HCV IRES**

Acknowledgments

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3. Martino, L. et al. Nucleic Acids Res. 2012, 40, 1381–1394.