TRIPLE RESONANCE NMR BACKBONE ASSIGNMENT OF THE LIGAND BINDING DOMAIN OF THE $\alpha_x\beta 2$ INTEGRIN

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ABSTRACT

The $\alpha_x\beta_2$ Integrin is a heterodimeric cell surface receptor exclusively expressed in leukocytes and plays a vital role in monocyte adhesion and atherosclerosis development. It functions in cellular trafficking, phagocytosis, and T-cell proliferation. Importantly, drugs targeted to $\alpha_x\beta_2$ could potentially have an anti-inflammatory effect without causing global immune suppression associated with steroids. The ligand binding domain of αXβ2, called the αX I-domain, transitions from closed/low affinity to open/high affinity states upon binding to a ligand.

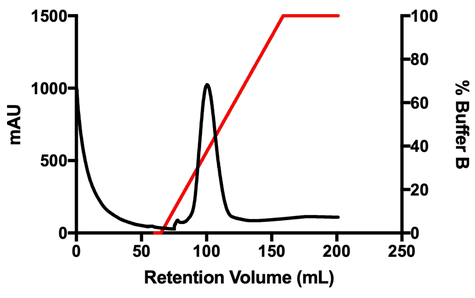
In order to design medications targeted to this protein and understand the mechanism of its transition between the open and closed conformational states, structural studies must be undertaken. Of particular interest are structural studies of the ligand-binding αI-domain, which undergoes drastic conformational changes in the transition between open and closed states. While there are published crystal structures of the α_x I domain in the open and closed states, these structures fail to show how the transition between these two states occurs, and the flexibility of the domain. Nuclear Magnetic Resonance (NMR) studies can bridge this gap by allowing for probing molecular motions at the nanosecond timescale.

Hence, as a preliminary project, we have conducted an NMR backbone assignment of the α_x I-domain. The αX I-domain plasmids are transformed to *E. coli* Rosetta cells. $^{15}N/^{13}C/^{2}H$ and $^{15}N/^{13}C$ labelled samples were purified by affinity and size exclusion chromatography. Differential Scanning Fluorimetry (DSF) was used to determine the NMR buffer in which the protein was most stable. Triple resonance experiments such as HNCACB, HNCOCACB, HNCO, and HNCACO were used to assign residues in the HSQC spectra.

RESULTS

Protein Purification

A) **Ni-NTA $^{2}H/^{13}C/^{15}N$ labeled α_x I Domain**



B) **Size Exclusion $^{2}H/^{13}C/^{15}N$ labeled αx I Domain**

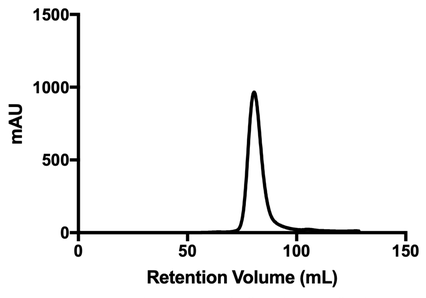


Figure 1: A) Affinity chromatography purification of triple labeled α_x I domain using a Histrap 10mL column. Protein was eluted by increasing the concentration of Buffer B relative to Buffer A by a linear gradient. **B)** Size Exclusion Chromatography (Superdex 75 Hiload 16/60) of triple labeled α_x I domain. Both purification steps were performed at 4 °C, with flow rates of 1mL/min and 2 mL/min respectively.

Assigned HSQC Spectra

$^{2}H/^{13}C/^{15}N$ labeled α_X I Domain

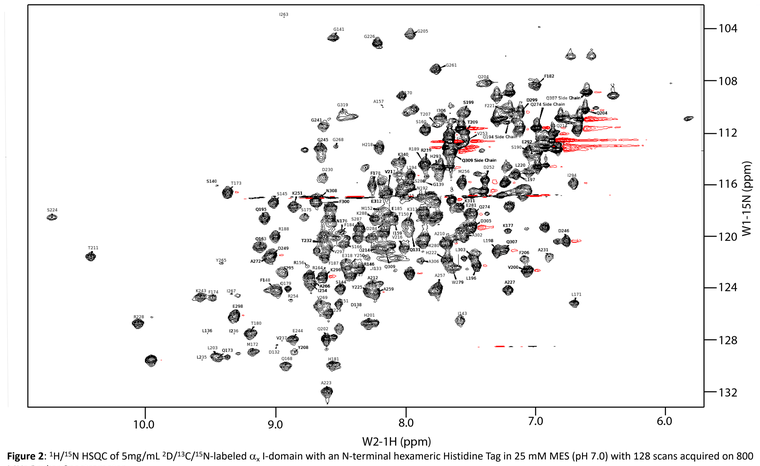


Figure 2: $^{1}H/^{15}N$ HSQC of 5mg/mL $^{2}D/^{13}C/^{15}N$-labeled α_x I-domain with an N-terminal hexameric Histidine Tag in 25 mM MES (pH 7.0) with 128 scans acquired on 800 MHz Bruker Spectrometer.

Strip Plots for Residues K280-D284

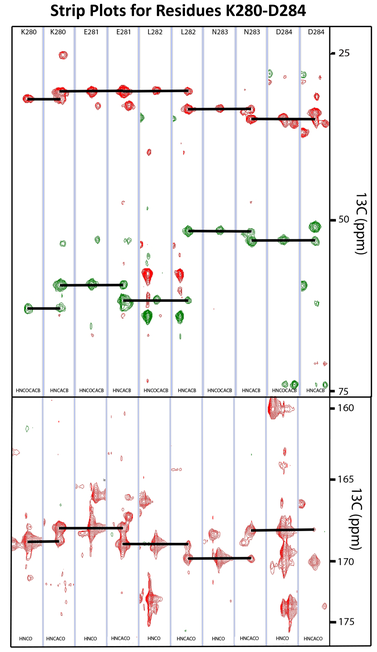


Figure 3: Strip plots from HNCOCACB and HNCACB spectra acquired at 298 K with 5 mg/mL $^{2}D/^{13}C/^{15}N$-labeled α_x I-domain in 10% D_2O illustrating the sequential through bond connectivities of $^{13}C\alpha$ and $^{13}C\beta$. Strip plots from HNCO and HNCACO spectra acquired at 298 K with 5 mg/mL $^{2}D/^{13}C/^{15}N$-labeled α_x I-domain in 10% D_2O illustrating the sequential through bond connectivities of ^{13}CO.

α_X I Domain Sequence Coverage

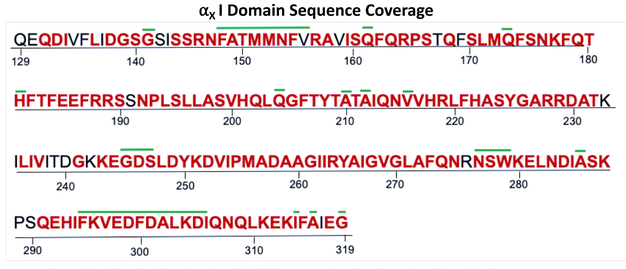


Figure 4: Residues of α_x I domain assigned in HSQC spectra. Assigned residues colored in red. Residues not assigned in black. Residues that showed peak splitting in HNCACB and HNCO spectra are labeled with a green lie. 165/186 (88.7%) of assignable residues were assigned.

Peak Splitting Present in Strip Plots

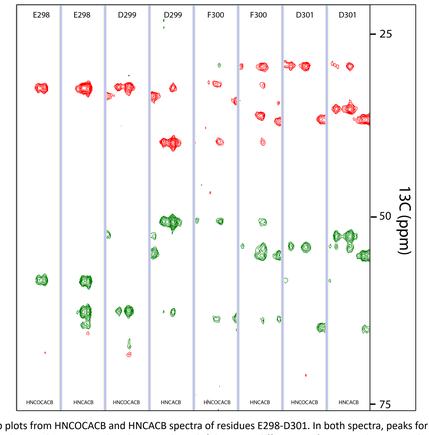


Figure 5: Strip plots from HNCOCACB and HNCACB spectra of residues E298-D301. In both spectra, peaks for the Cα and Cβ show splitting. This suggests that these residues shift between different conformational states, and are thus represented by more than on chemical shift.

Overlaid Crystal Structures with Residues Showing Peak Splitting Highlighted

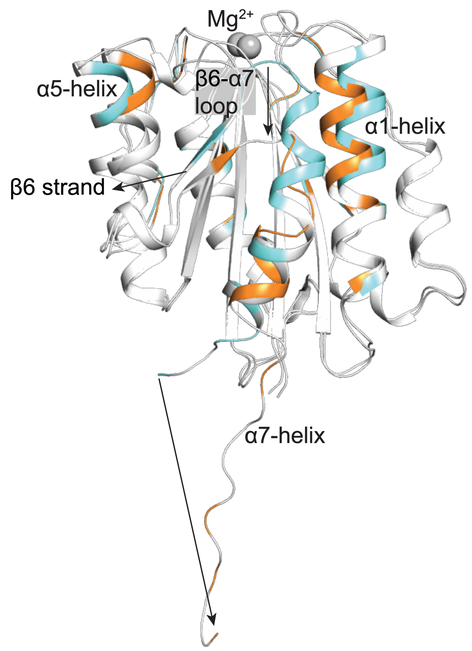


Figure 6: Overlaid X-ray crystal structures of the α_x I Domain in the active (PDBID: 4NEH) and closed (PDBID: 5ES4) conformation. Residues that showed peak splitting in the HNCACB and HNCO spectra are colored. The open conformation is colored in orange. The closed conformation is colored in blue. Residues not colored did not show peak splitting.

CONCLUSIONS

- $^{15}N/^{13}C/^{2}H$ and $^{15}N/^{13}C$ labeled α_X I Domain expressed in E. Coli Rosetta Cells
- Isotope labeled protein purified by size exclusion and Ni-NTA chromatography
- Acquired HSQC, HNCACB, HNCOCACB, HNCO, HNCACO, HNCA, HNCOCA spectra
- 165/186 (88.7%) of assignable residues assigned in HSQC (not including proline)
- Peak splitting present for many residues in allosteric regions of the protein (including the β_6-α_7 loop)
  - This suggests that the protein takes on more than one ensemble of conformations in solution
- The α_X I Domain may take on the open conformation in solution
  - This is especially biologically relevant, as it suggests that the protein becomes activated without the need of intracellular activation

REFERENCES

Curtis, J.L., and Morrison, V.L. (2017). β 2 integrins as regulators of dendritic cell, monocyte, and macrophage function. Frontiers Immunology *8*, 1–11.

Johnson, B. (2004) Using NMRView to visualize and analyze the NMR spectra of macromolecules In A. K. Dowling Protein NMR techniques. Humana Press. 313–352.

Lee, W., Tonelli, M., and Markley, J.L. (2015). Structural bioinformatics NMRFAM-SPARKY : enhanced software for biomolecular NMR spectroscopy. Bioinformatics *31*, 1325–1327.

Sen, M., Yuki, K., and Springer, T.A. (2013). An internal ligand-bound, metastable state of a leukocyte integrin,aXb2. Journal of Cell Biology *203*, 629–642.

Sen, M., & Springer, T. A. (2016). Leukocyte integrin α L β 2 headpiece structures : The α I domain , the pocket for the internal ligand , and concerted movements of its loops. Proceedings of the National Academy of Sciences *113*(11), 2940–2945.

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