Analysis of the alpha-actinin-2 actin-binding domain using AI programs

FSU | FLORIDA STATE UNIVERSITY

Keyse Kelly Ferraz and Rhiannon Crawford

FSU UNDERGRADUATE RESEARCH OPPORTUNITY PROGRAM
CENTER FOR UNDERGRADUATE RESEARCH & ACADEMIC ENGAGEMENT

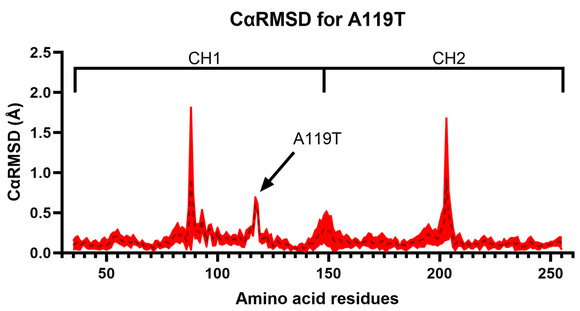
Introduction

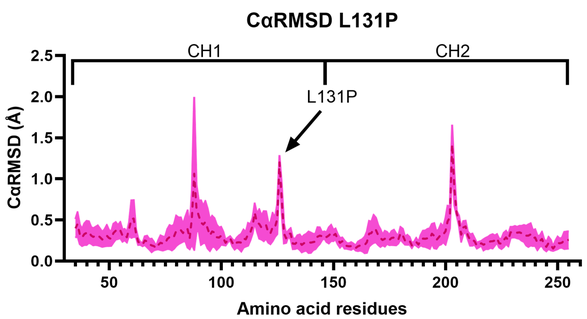
Sarcomeres are the fundamental contractile units of cardiac muscle cells, and their structural organization is essential for proper force generation and heart function. Disruption of Z-disc proteins, particularly α-actinin-2 (ACTN2), has been strongly associated with cardiomyopathies and altered cardiac mechanics (Wadmore et al., 2021; Noureddine et al., 2025). Because protein function is tightly linked to three-dimensional conformation, even single amino acid substitutions may alter structural positioning and binding interactions within the sarcomere.

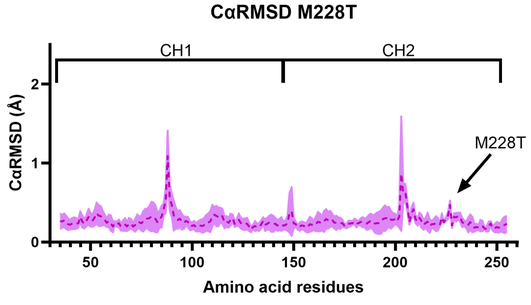
We hypothesize that certain disease-associated mutations in ACTN2 promote a more open conformation of the actin-binding domain, increasing binding accessibility and activity at the Z-disc. This conformational shift may contribute to altered sarcomere mechanics and provide a potential mechanistic explanation for abnormal cardiac growth observed in cardiomyopathy.

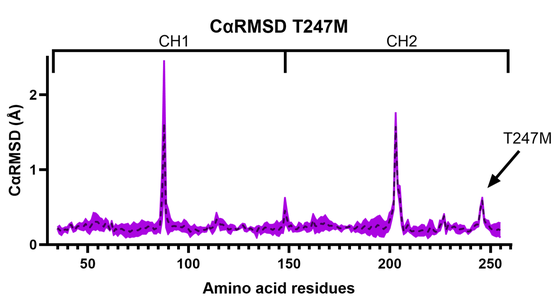
To investigate this, we used AlphaFold to generate structural predictions of wild-type and mutant ACTN2 proteins. Predicted structures were aligned and analyzed in ChimeraX to quantify conformational differences, including Cα root mean square deviation (CaRMSD) and domain orientation changes. These structural comparisons allowed us to evaluate whether specific mutations shift the protein toward a more open configuration. While AI-based tools such as SarcAsM provide powerful approaches for quantitative sarcomere analysis (Härtter et al., 2025), the present study focuses on structural modeling to establish a molecular basis for mutation-induced sarcomeric dysfunction.

CαRMSD for each mutation

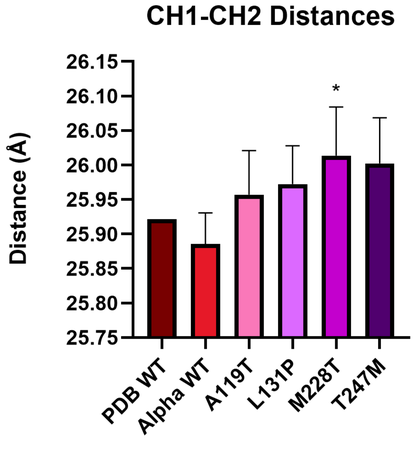


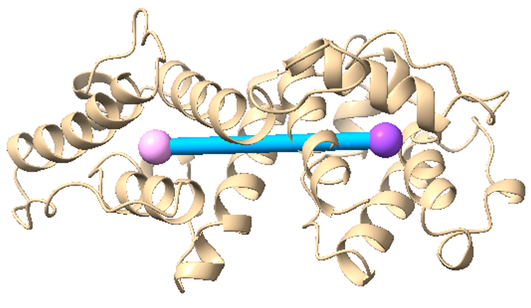






Mean center of mass distances for CH1 and CH2 domains





Conclusion

- Disease-associated mutations in ACTN2 produce measurable structural changes in the actin-binding domain of α-actinin-2.
- CaRMSD analysis revealed localized structural deviations near mutation sites compared to the predicted wild-type structure.
- Mutant proteins showed slightly increased CH1–CH2 center-of-mass distances, suggesting greater domain separation.
- This structural shift is consistent with a more open actin-binding conformation.
- Increased domain accessibility may alter actin binding at the Z-disc and affect sarcomere mechanics.
- These findings suggest that ACTN2 mutations may contribute to cardiomyopathy by disrupting sarcomere organization and structural stability.
- AI-based structural tools such as SarcAsM provide an effective approach for identifying mutation-induced structural changes in cardiac proteins.

Ackowlegdements & References

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