Assessing Phosphorylation-induced Conformational Changes in the α-actinin2 Actin-binding Domain Through Analysis of Small-Angle X-ray Scattering (SAXS) Data

FSU | FLORIDA STATE UNIVERSITY

Martina Madrzak [1], Shellsea Ibarra Carreón [1], Helene Tigro [2], Christopher Solís [2]

[1] Florida State University Undergraduate Research Program [2] Florida State University Anne's College

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

ABSTRACT

α-Actinin-2 (ACTN2) is a sarcomeric protein essential for maintaining cardiac muscle structure and contractile function. Dysregulation of ACTN2 is associated with cardiomyopathies. Phosphorylation is thought to regulate ACTN2 interaction with actin, but its structural effects remain unclear. Particularly within the actin binding domain (ABD). AlphaFold3 predictions visualized in ChimeraX suggested that phosphorylation may induce a large-scale "opening" of the ABD. We therefore hypothesize that phosphorylation promotes conformation expansion of the ACTN2 ABD in solution.

To test this hypothesis, recombinant human ACTN2 wild-type and phosphomimetic ABD constructs were expressed in *E. coli* and purified using affinity and ion-exchange chromatography. Purity (>98%) was confirmed by SDS-PAGE and colorimetric assays. Small-Angle X-ray Scattering (SAXS) data was used to characterize solution-state structural differences between variants. Global structural parameters, including radius of gyration and molecular envelopes were modeled and analyzed using BioXTAS RAW and the ATSAS software suite.

Preliminary results indicate that phosphomimetic variants exhibit increased structural expansion relative to wild-type, supporting the hypothesis that phosphorylation alters ACTN2 ABD conformation. These findings provide insight into how post-translational modification may regulate sarcomeric architecture and contribute to cardiac disease mechanisms.

METHODS

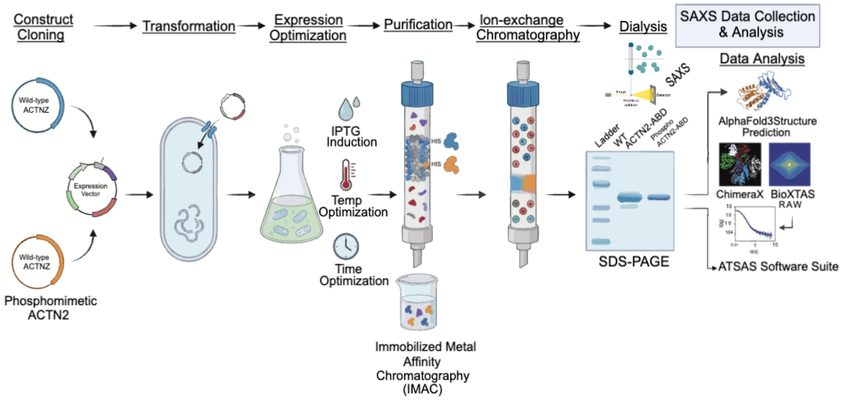

Figure 1: Expression and purification process of recombinant human ACTN2 ABD in *E. coli.*

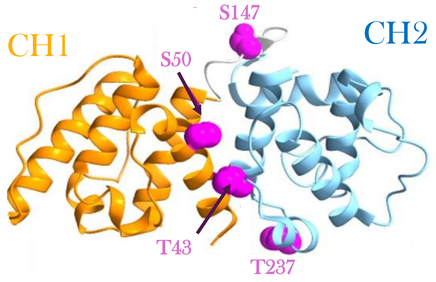

Figure 2: Structural prediction of the ACTN2 ABD reveals phosphorylation sites at the calponin homology domain (CH1–CH2) interface, affecting the CH1-CH2 separation.

RESULTS

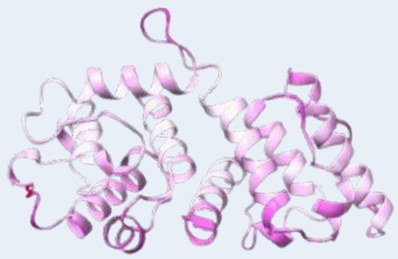

Figure 3: α-actinin-2 ABD wild-type vs A-actinin-2 ABD T43D S50D S147D T237D predicted by AlphaFold3 and analyzed with ChimeraX.

Cα RMSD

0.04 0.69 1.34

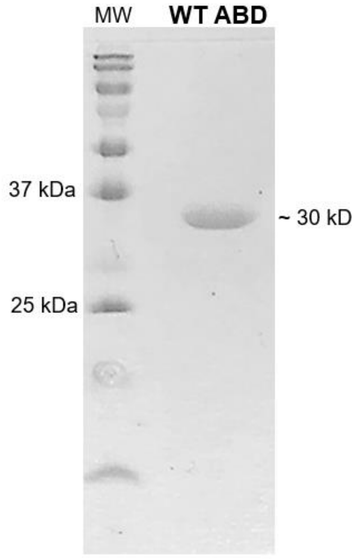

Figure 4: SDS-PAGE to assess purity and molecular weight (MW).
- Colorimetric assays were additionally used for protein quantification.
- Optimization to achieve **>98%** purity for SAXS analysis Small-Angle X-ray Scattering (SAXS).

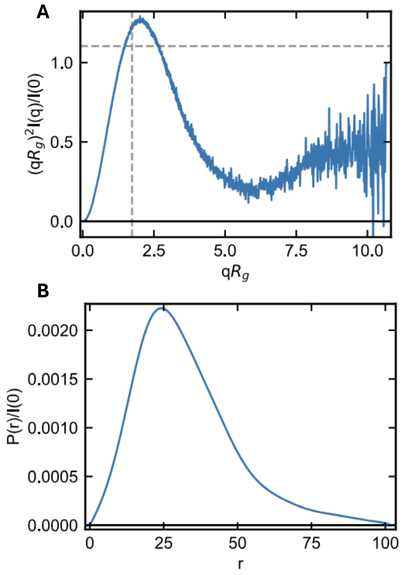

Figure 5: SAXS data summary figure for recombinant human ACTN2 WT ABD.
A) Dimensionless Kratky plot. Dashed lines show where a globular system would peak.
B) P(r) function(s), normalized by I(0).

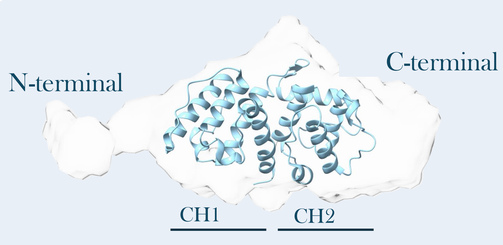

Figure 6: Computational Analysis of Solution-state SAXS performed on purified α-actinin-2 WT ABD.

DISCUSSION

Does phosphorylation induce structural expansion of the ACTN2 actin-binding domain?

SAXS structural Validation:

Wild-Type (WT) α-actinin-2 ABD exhibits:
- Compact scattering profile.
- Well-defined Kratky peak.
- Low apparent flexibility.

CH1 and CH2 domains remain in close proximity based on size parameters. Thus, Isolated WT ABD behaves as a compact and stable monomer in solution.

Our SAXS data show that the purified human α-actinin-2 ABD is well-folded and stable in solution and closely matches previously reported structures. This demonstrates that SAXS is a powerful method for visualizing domain conformation and CH1-CH2 separation under near-physiological conditions.

Phosphorylation has been proposed to regulate α-actinin-2–actin interaction. Establishing that the WT ABD is compact provides a structural baseline for determining whether phosphomimetic variants exhibit phosphorylation-induced expansion.

Research on the α-actinin-2 ABD conformational changes

Strengths
- High-purity recombinant protein (>98%)
- Solution-state structural analysis (physiologically relevant)
- Integration of SAXS and computational modeling.

Limitations
- SAXS provides low-resolution structural information (changes to the ABD CH1-CH2 separation might be difficult to visualize).
- Phosphorylation is modeled using phosphomimetic substitutions.

FUTURE DIRECTIONS

Phosphomimetic SAXS analysis is ongoing

- Complete the purification of each phosphomimetic variant: **T43D**, **S50D**, **S147D**, **T237D**, and **T43D S50D S147D T237D**.
- Complete SAXS analysis of phosphomimetic variants.
- Quantify changes in Rg, Dmax, and CH1-CH2 separation.
- Integrate molecular dynamics simulations to model domain opening.

RESOURCES

- **AlphaFold 3** server and **ChimeraX** software for protein structure predictions.
- **Argonne National Laboratory** for the SAXS analysis.
- **BioXTAS RAW** and **ATSAS** software for data analysis.
- **FigureLabs.AI** for protocol visualization.