FSU FLORIDA STATE UNIVERSITY

# USING CO-PRECIPITATION TO STUDY HOW PHOSPHORYLATION AFFECTS A-ACTININ-2 BINDING TO CARDIAC ACTIN

| Nicolas Viana Santana | Biochemistry | Solis Lab | Christopher Solís, PhD, MBA | Helene Tigro, PhD |

FSU UNDERGRADUATE RESEARCH OPPORTUNITY PROGRAM

## INVESTIGATING HOW PROTEIN MODIFICATION REGULATES HEART MUSCLE ADAPTATION

## BACKGROUND & RESEARCH QUESTION

Cardiac Muscle Structure

- Heart muscle contraction depends on organized units called sarcomeres, where actin must be aligned and stabilized for efficient contraction.

Role of **α-Actinin-2 (ACTN2)**

- ACTN2 is a Z-disc structural protein that crosslinks actin filaments, helping heart cells maintain structure during repeated mechanical stress.

Why Modification Matters

- Phosphorylation (the addition of a phosphate group) can **change a protein's shape and function.**
- that phosphorylation within ACTN2's actin-binding domain may alter how strongly it binds actin.
- Direct biochemical comparison of specific phosphorylation-related variants remains limited.

**Research Question**

How do phosphorylation-related modifications of ACTN2 influence its binding to F-actin?

**Hypothesis**

Phosphorylation-mimicking ACTN2 variants will show increased binding to actin compared to wild-type and phospho-null controls.

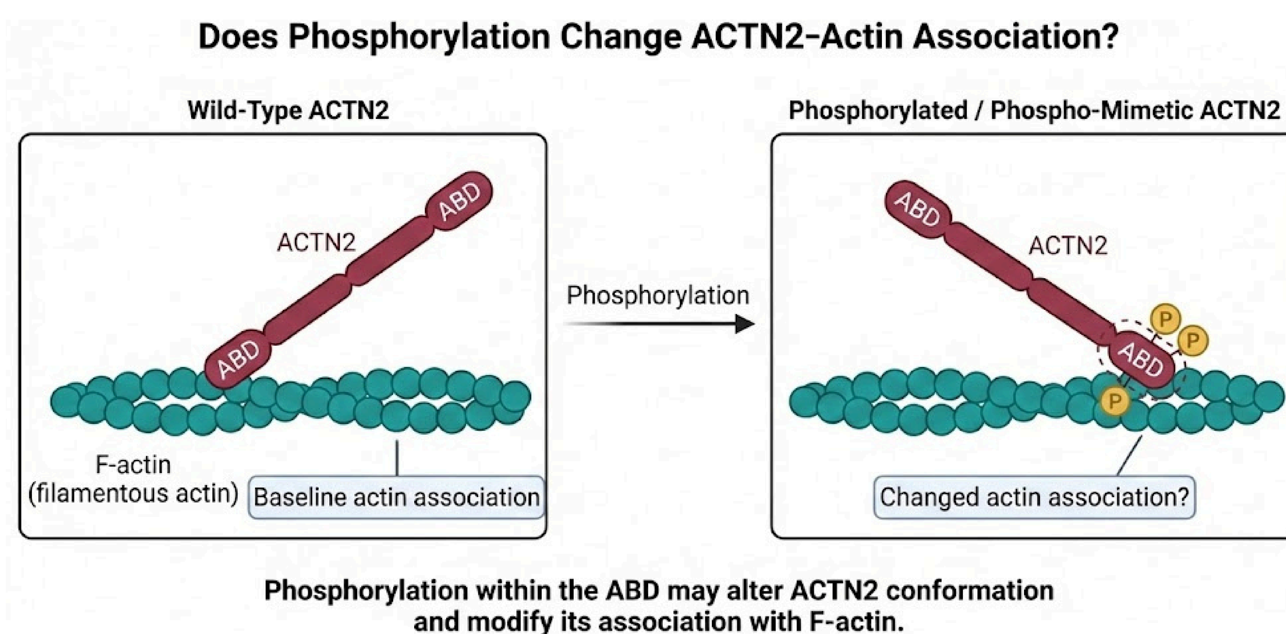
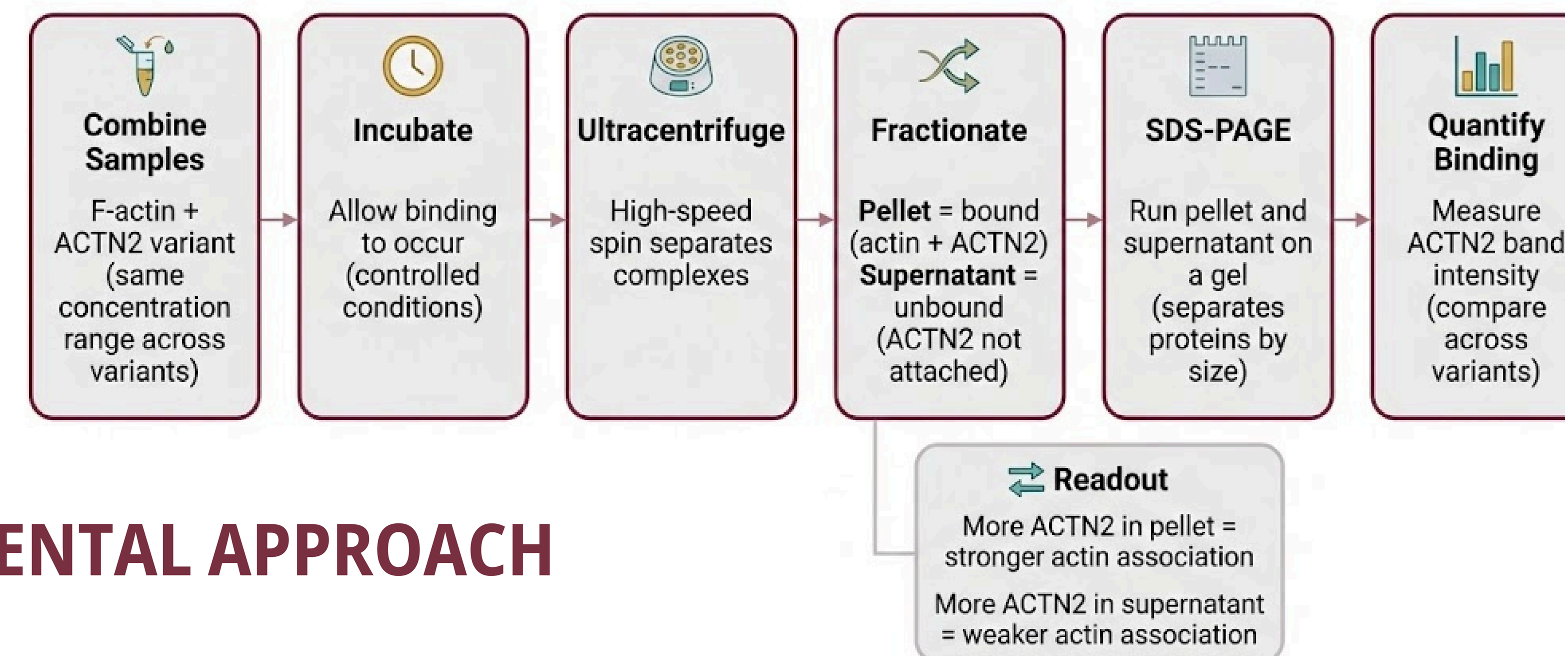



FIGURE 1. CONCEPTUAL MODEL ILLUSTRATING HOW PHOSPHORYLATION WITHIN THE ACTIN-BINDING DOMAIN (ABD) MAY ALTER ACTN2 ASSOCIATION WITH F-ACTIN.

FIGURE 2. OVERVIEW OF THE CO-PRECIPITATION WORKFLOW USED TO EVALUATE ACTN2–ACTIN BINDING.




## EXPERIMENTAL APPROACH

**Actin Preparation**

- Actin was purified from pig heart tissue to provide a cardiac-relevant binding model.
- Purity and concentration were confirmed using SDS-PAGE and Bradford assay.

**ACTN2 Variant Production**

- The actin-binding domain (ABD) of human ACTN2 was expressed in E. coli.
- Three variant types were generated: Wild-type, Phospho-mimetic (imitate phosphorylation), and Phospho-null (prevent phosphorylation)

**Co-Precipitation Assay**

- Equal protein concentrations were used across variants to allow direct comparison of binding strength.
- Relative association was determined by comparing ACTN2 distribution between pellet and supernatant fractions.

**Quantitative Analysis**

- Pellet and supernatant fractions were analyzed by SDS-PAGE.
- Band intensity was quantified using image analysis software (ImageJ).
- Relative binding was compared across variants to determine the effect of phosphorylation-related modification.

## RESULTS

Co-precipitation assay conditions were successfully optimized to separate actin-bound (pellet) and unbound (supernatant) ACTN2 fractions. Representative SDS-PAGE analysis demonstrates clear partitioning of proteins between fractions, confirming functional assay performance.

Initial comparative experiments suggest that phosphorylation-related ACTN2 variants may alter relative actin association compared to wild-type. **Quantitative densitometry analysis is ongoing to determine whether statistically significant differences exist across modification states. These findings are preliminary and based on early experiments, and additional data are needed to confirm the trends observed.**

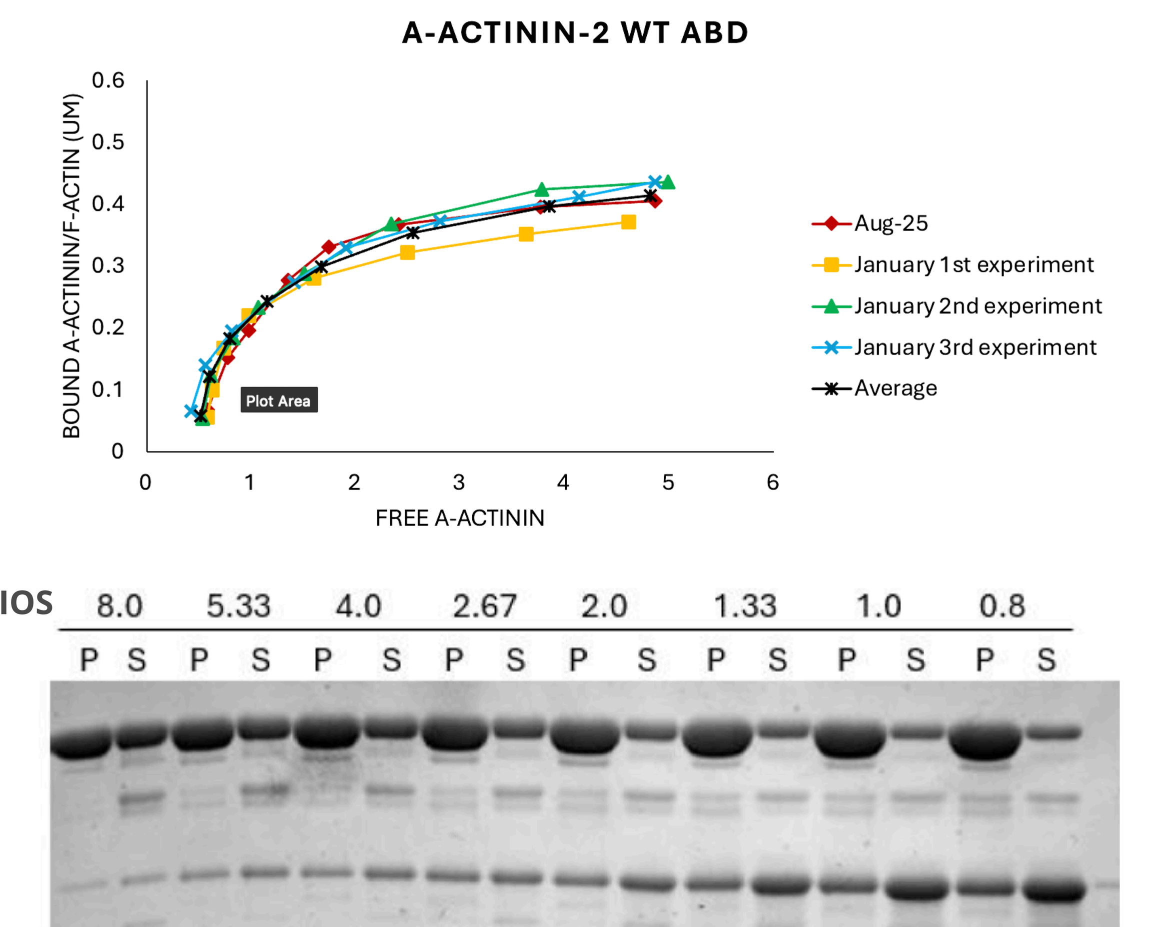



FIGURE 3. CO-PRECIPITATION ASSAY DEMONSTRATING BINDING OF A-ACTININ-2 WT ABD TO F-ACTIN. PELLET (P) AND SUPERNATANT (S) FRACTIONS WERE ANALYZED BY SDS-PAGE AFTER ULTRACENTRIFUGATION AT INCREASING ACTIN: A-ACTININ RATIOS. A-ACTININ ENRICHMENT IN PELLET FRACTIONS INDICATES CO-PRECIPITATION WITH F-ACTIN. DENSITOMETRIC ANALYSIS OF BAND INTENSITY WAS USED TO QUANTIFY BOUND A-ACTININ AND GENERATE THE BINDING CURVE SHOWN.

## CONCLUSION

This study establishes a co-precipitation approach to examine how phosphorylation-related changes affect the interaction between ACTN2 and actin. Early results suggest that modifications within the actin-binding domain may influence how strongly ACTN2 associates with actin in cardiac muscle. Further analysis will clarify the extent of these effects across different modification states.

## REFERENCES