Cornelia de Lange Syndrome (CdLS) is a developmental disorder characterized by skeletal abnormalities, hirsutism, mental retardation, and heart defects [1]. CdLS is caused by mutations that affect the cohesin complex [1]. Cohesin changes chromatin structure to mediate transcription as evidenced by CdLS indivuals who experience transcriptional dysregulation in hundreds of genes [1]. Fifty-five percent of CdLS cases are caused by mutations in Nipped B-like Protein (NIPBL) which loads cohesin onto DNA [2]. NIPBL protein has been shown to affect heart development in a NIPBL deficient zebrafish model [3]. It is **currently unknown** how mutations in conserved amino acids of NIPBL effect heart development. **My primary goal** is to determine which amino acids in NIPBL are most critical during heart development and why. I will use zebrafish as a model because it can be used to screen chemical libraries and heart formation can be easily observed during development [3]. **I hypothesize** that single amino acid mutations in NIPBL will have effects on heart development unique to the amino acid mutated. **My long-term goal** is to determine the role of NIPBL in mediating heart development.

**Specific Aims:**

**Aim 1: Determine NIPBL amino acids critical to heart development**

Rationale: Amino acids of NIPBL found in organisms with hearts and not in organism without hearts may be critical to heart development.

Approach: I will use the MUSCLE algorithm and align NIPBL sequences of organisms with hearts and align NIPBL sequences of organisms without hearts to determine which amino acids are unique to organisms with hearts. Then I will use the CRISPR-Cas system to mutagenize zebrafish at the unique, conserved amino acids found previously. Heart development of mutant and wild type (WT) zebrafish will be observed throughout development.

Hypothesis: Zebrafish with mutations in amino acids critical to heart development will have strong phenotypes.

**Aim 2: Find small molecules with amino acid specificity**

Rationale: Small molecules may exist in chemical libraries capable of rescuing heart defects and interact with NIPBL at specific amino acids. Previously characterized roles of these amino acid specific compounds can link mutated amino acids to cellular phenomena.

Approach: Using a broad chemical library I will use small molecule microarrays to identify molecules which bind to mutagenized and wild-type NIPBL. GFP will be appended to the C terminus of NIPBL. Hits from the biochemical assay will be screened again by exposing zebrafish to the compound at different stages of development. Compounds whose effect changes between mutants will be searched on PubChem to find their previously characterized roles.

Hypothesis: Potential therapeutics may act via amino acid specific mechanisms.

**Aim 3: Discover amino acid specific interactions**

Rationale: Determining how the protein-protein interactions of NIPBL change between site-specific amino acid mutants will indicate which amino acids are protein binding. Gene ontology (GO) analysis of the discovered interacting partners will indicate how NIPBL directly controls heart development.

Approach: I will attach a TAP-tag onto the less conserved C’ terminus of NIPBL and extract the protein from zebrafish heart tissue of WT and each amino acid mutant. NIPBL complexes will then be captured using tandem affinity purification and characterized using mass spectrometry and bioinformatic analysis. Then I will document which interacting partners had GO terms for heart development.

Hypothesis: Interacting partners that have GO terms for heart development that appear in WT but not amino acid mutants are likely pathways critical to heart development. Consequently the amino acids that interact with these binding partners are critical to the role of NIPBL in heart development.

This research will investigate how NIPBL mediates heart development, search for therapeutics capable of reducing heart defects in CdLS patients, discover currently unknown interacting partners of NIPBL, and further annotate the structure of the NIPBL gene.

# References

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