Desired Needs:

- PAH kills ~22,000 people annually with no curative treatment, establishing a clear need for disease-modifying therapy.
- Connexin 40 (Cx40) is downregulated in PAH and its upregulation is partially curative in mice, but poor in vitro expression has blocked modulator screening, necessitating an improved construct.

Constraints:

- **Safety/Regulatory**: Cell line and lentiviral work conducted under BSL-2/UCSD IBC approval; small molecules handled per SDS requirements.
- **Risks:** Stabilizing mutations may alter biological function; computational predictions may not reflect in vivo biology; Connexin-EGFP co-expression was a persistent technical bottleneck.
- **Global Impact:** PAH disproportionately affects underserved populations; a small molecule Cx40 enhancer could be far more accessible than current biologics.
- **Manufacturability/QC:** Single-vector co-expression plasmid uses standard cloning and open-source tools; expression quantified by flow cytometry, GJ formation by contact-to-interior puncta ratio from IF imaging.

Engineering Standards:

- AlphaFold3 and ProteinMPNN are community standards for structure prediction and sequence design, ensuring outputs are comparable to published literature.

Ethical/Environmental/Social Concerns:

- In vitro platforms reduce upstream reliance on animal models; equitable global access to any resulting therapy must be considered given the high cost of current PAH treatments.

Active Teamwork/Leadership:

- **Collaboration:** Project spanned wet lab, computational biology, and assay development, requiring continuous cross-subteam coordination.
- **Delegation:** Each member had their own connected subproject, with examples being Nishant leading permeability assay development, and Satvik with structural mutation predictions
- **Goals/Feedback:** Weekly lab meetings with Dr. Kufareva served as structured checkpoints; internal dry-runs provided peer feedback before the final presentation.

Motivating Factors:

- **New knowledge:** PAH's clinical severity drove self-directed learning in connexin biology; tools like BioEmu required hands-on learning from documentation and primary literature.
- **Self-initiating/Persistence:** The kinase/PDZ pipelines and co-expression plasmid were student-initiated solutions. Cx40's expression challenges drove a year-long, multi-pronged effort rather than abandonment.

Innovative/Entrepreneurial

- The modular platform connecting structure prediction, interactor prioritization, IF quantification, and small molecule analysis is broadly applicable to other connexin isoforms and gap junction diseases beyond PAH.