1. **Desired Needs**
    a. Need to understand why Cx40 is downregulated/poorly trafficked in PAH and how that can affect endothelial dysfunction.
    b. Need a reproducible model that effectively measures Cx40 expression, membrane localization, gap junction plaque formation, and permeability.
2. **Major Constraints**
    a. **Safety/Regulatory Affairs:** future therapeutic development would require biocompatibility, avoidance of unintended side effects, and compliance with FDA validation.
    b. **Risks:** Computational predictions may be inaccurate due to model bias. Wet lab assays may be limited by low Cx40 expression and transfection efficiency.
    c. **Global Impact:** PAH is a terminal disease with no cure, and restoring endothelial communication could support future therapies beyond symptom management.
    d. **Manufacturability:** Reagents for the wet lab assays and computational resources for the prediction models may be expensive.
    e. **Quality Control/Marketability:** Need to sequence cloned mutants to check they are correct, use flow cytometry to quantify expression, and normalize IF puncta measurements across different microscopes.
3. **Major Engineering Standards**
    a. ISO 5725 guided the need for accurate and precise measurement of fluorescence-based biosensor and IF results
    b. MIAME and FAIR principles informed reproducible processing, annotation, and reporting of transcriptomic data
    c. FDA 21 CFR Part 58 Good Laboratory Practices will apply to later validation studies
    d. ISO 13485 and ISO 10993 may become relevant if the screening workflow advances toward medical-device or preclinical use
4. **Ethical, Environmental, and Societal Concerns**
    a. **Ethical**: avoid overinterpretation of AI-generated structural prediction without experimental validation
    b. **Environmental:** responsible reagent use, waste disposal, and minimizing repeated failed experiments
    c. **Societal:** addresses an unmet clinical need by targeting a possible root mechanism of PAH as opposed to just treating symptoms
5. **Active Teamwork and Leadership**
    a. Team members divided work by subproject (protein prediction, IF imaging, MD modeling, PTM mutagenesis, permeability, and ProteinMPNN mutation design), with some overlap, reusing some code and reagents

    b. We met once a week as a group with our PI, where we would update her and other team members on the work we were able to accomplish that week. This allowed us to complete tasks before deadlines and keep everyone accountable for their work
    c. We also had a group chat where we would actively express our thoughts and opinions on the direction of the project. No one was ignored, and everyone was respectful
6. **Motivating Factors for Learning, Initiative, and Persistence**
    a. The content that I was focused on was incredibly interesting to me. I think protein modeling is fascinating, and it kept me engaged while I was working and motivated me to learn more
    b. The need to find a cure for PAH also motivated me to work as quickly as possible, so fewer people have to suffer through such a terrible disease
    c. My PI and teammates were very encouraging and made me feel good when I got a decent chunk of work done for the project
7. **Innovative and Entrepreneurial Ideas**
    a. I think it would be somewhat difficult to commercialize this pipeline as it is very specific to Cx40, but I could see the incorporation of all the computational tools into an app that can output a lot of information about domains/interactions/structure of under-researched proteins
    b. This pipeline can be used to create a drug for PAH that targets Cx40, which can then be sold to PAH patients