Bioengineering Day Poster Addendum (ABET questions)

Submit one file per person. You may borrow materials from each other in your group.
FILE NAMING CONVENTION -- Examples:
01BENGLastName.pdf
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To help the department to prepare for the next cycle of ABET accreditation, please prepare a one-page, bullet style summary of answers to the questions posed below. This addendum will be displayed at BE Day on the web site for judges to see. (In the past, printed versions were attached to the poster.) Give as much detail as you can while staying within the one-page limit. Use 12 pt font.

In what follows, "device" is broadly interpreted, including computer program, biotechnology process, mechanical or electrical device – i.e. everybody's project.

For each, describe how your team addressed or used the following over the course of your work:

1. List two to four **Desired Needs** of your project that led to your final design objectives.
   a) A need exists to understand the molecular mechanisms underlying endothelial dysfunction in PAH, particularly the role of Connexin 40 in gap junction-mediated intercellular communication. Current treatments only manage symptoms without addressing the root cause.
   b) A translational need exists for a reliable, reproducible computational-experimental pipeline to identify and validate modulators of Cx40 function, enabling the development of targeted therapeutics that restore endothelial communication in PAH
   c) A research need exists for standardized tools and constructs that accurately reproduce physiologic Cx40 behaviour in heterologous expression systems, which current models fail to achieve, limiting the study of Cx40's contribution to endothelial dysfunction
   d) A clinical need exists for PAH patients, as no approved therapeutics can restore or enhance Cx40 channel function; improved mechanistic understanding of Cx40 structure, trafficking and permeability is essential to guide future therapy development

2. List the major **Constraints** on your design/project
   e) Safety/Regulatory Affairs: All wet lab work was conducted under standard biosafety protocol in approved laboratory settings; recombinant DNA use (plasmid transfections in HeLa/HEK293T cells) followed institutional biosafety guidelines. Future therapeutic applications would require FDA 21 CFR Part 58 compliance for preclinical drug screening and IND submission requirements
   f) Risks: Key risks included (1) technical risk of incorrect use of bioinformatics tools (mitigated by dedicated training/task specialization to BINF majors) (2) limited lab resources such as reagent supply chain delays (mitigated by proactive inventory monitoring) (3) Cx40's inherently low recombinant expression compressing signal-to-noise in all functional assays; and (4) computational model bias from AlphaFold3 training data, requiring experimental validation of all in silico predictions
   g) Quality control/marketability: ALL computational predictions have not been validated with experimental assays (immunofluorescence, flow cytometry). Coexpression metrics were validated across three different independent scRNA-seq datasets and permeability biosensors were designed as bicistronic expression vectors to achieve reliable co-expression. This demonstrates iterative quality improvement; the pipeline addresses an unmet market need given zero FDA-approved Cx40-targeted PAH therapies.

3. List the major **Engineering Standards** on your design/project

a) **Components/assays**: ISO 5725 applied to fluorescence quantification in both the cAMP biosensor and immunofluorescence gap junction assays. FAIR data principles and MIAME standards guided scRNA-seq data processing and reporting
b) **Performance constraints**: AlphaFold3 structural predictions are constrained by model confidence metrics (pLDDT, PAE, ipTM) which we used as pass/fail thresholds. Flow cytometry data was analyzed using geometric mean fluorescence intensity as required for log-normally distributed data. This is consistent with established flow cytometry reporting guidelines (MIFlowCyt)

4. Explain **Ethical, Environmental, or Societal concerns** for practical applications of your project.

a) **Ethical:** All cell-based experiments used established immortalized cell lines with no animal or human subject involvement at this stage. Future therapeutic screening must ensure equitable access to any resulting treatments, as PAH disproportionately affects women and underserved populations. Responsible AI use is considered, given reliance on machine learning models (AlphaFold3, Protein MPNN) whose predictions carry inherent uncertainty and must not be over-interpreted without experimental validation.
b) **Environmental:** Computational methods were prioritized to reduce the volume of wet lab experiments, minimizing chemical and biological waste. Standard lab disposal protocols were followed for all cell culture and reagent disposal
c) **Societal:** Advancing Cx40 as a therapeutic target could provide a curative rather than palliative option for PAH patients, reducing diseases burden and healthcare costs. The scalable pipeline framework also contributed broadly to the scientific community by providing reproducible tools for connexin research.

5. Describe **Active Teamwork** and **Leadership** in your design group

a) **Collaboration**: Each teamember contributed distinct computational and experimental expertise (scRNA-seq, MD simulations. Mutagenesis, imaging, biosensor design) requiring constant cross-functional collaboration. Weekly meetings integrated diverse perspectives from dry lab and wet lab members.
b) **Delegation:** Leadership was distributed across subprojects according to expertise: Aiden led scRNA-seq coexpression analyses, Ben led immunofluorescence imaging, Anirudh led MD/AF simulations, Curtis led Cx40 expression optimization, Satvik led mutational mapping and Protein MPNN, and Nishant led biosensor development. Each lead coordinated their subproject, made decisions within their domain and reported progress to the group.
c) **Goals and deadlines**: The team developed a Gantt chart with clear milestones. Weekly meetings tracked progress against these milestones and allowed real-time scope adjustment when timelines were at risk.
d) **Constructive feedback:** The team received ongoing feedback from Dr. Kufareva during formal and informal lab meetings. After advisor feedback on early biosensor co-expression results (only 2.45% double-positive cells), the team redesigned the vector architecture with an insulator sequence, achieve 42.6% co0expression. Team members also gave each other peer feedback on analytical choices (e.g. metric selection), leading to empirically validated decisions.

6. What were the most significant motivating factors that led you to

a) **New knowledge:** The gap between PAH patients' unmet therapeutic needs and the current mechanistic understanding of Cx40 motivated the team to master entirely new tools (AlphaFold3, Protein MPNN, BioEmu, scRNA-seq pipelines). The complexity of connexin biology required continuous literature review and upskilling throughout both quarters.
b) **Self-initiating**: Team members independently identified and pursued methodological improvements without being directed to do so. For example, the ProteinMPNN scoring pipeline was iteratively upgraded from BLOSUM-based to AlphaMissense scoring to PMIbased independence scoring, each upgrade driven by individual members recognizing limitations in prior approaches.
c) **Persist against challenges:** Cx40's notoriously low expression presented a bottleneck. Rather than abandoning the experimental approach, the team iterated through multiple solutions: codon optimization, rational PTM-site mutagenesis via site-directed mutagenesis, and ProteinMPNN-guided structural stabilization mutants.

7. What are your most **innovative and/or entrepreneurial ideas** for this project

cAMP transcriptional biosensor

The bicistronic cAMP-EGFP biosensor design, using a CRE-EGF-degron reporter in receiver cells and an A2AR driven cAMP pathway in donor cells, enables quantitative, connexin-specific permeability measurement. By achieving 42.6% co-expression with the redesigned vector, this assay is on a path toward 96-well format screening of small molecule GJC modulators—a capability that does not currently exist for Cx40.