Group 14

Advancing Connexin 40 as a Therapeutic Target in Pulmonary Hypertension

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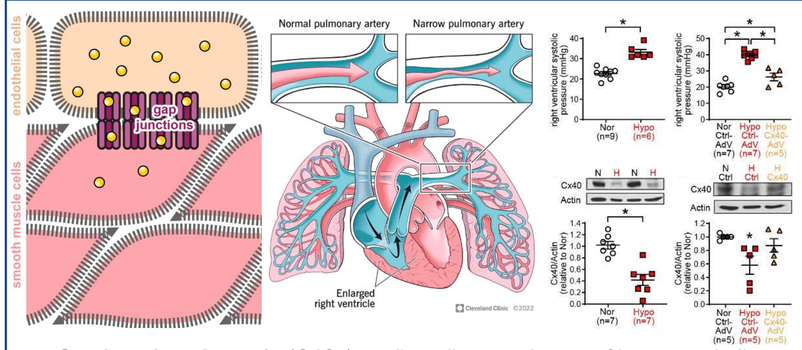
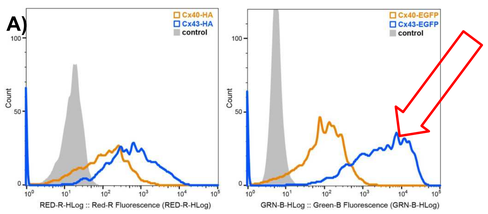
Background



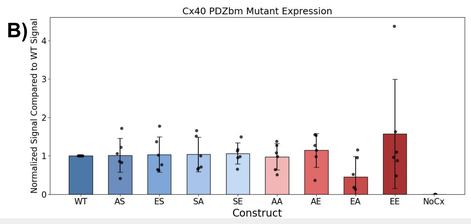
- **Gap junction channels** (GJCs) mediate direct exchange of ions, metabolites, and messengers between adjacent cells [1], critical for coordinated functions.
- **Connexin 40 (Cx40)**, highly expressed in endothelial cells, is essential for coordinating endothelial signaling and vessel function.
- **Pulmonary arterial hypertension (PAH)** is a cardiovascular disease characterized by elevated pressure in the pulmonary arteries. This eventually leads to reduced right ventricle function and heart failure.
- **Cx40 is downregulated in PAH** patients and animal models.
- **Restoring Cx40 function rescues disease phenotypes**, highlighting it as a promising therapeutic target [3].
- Therapeutic development is limited by incomplete mechanistic understanding.

How do we increase Cx40 Expression?



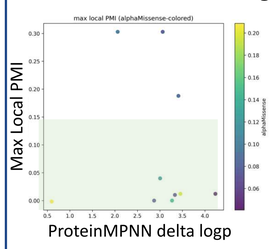
- Currently, Cx40 does not express very well
- High expression is important for our permeability assays to test possible GJC modulators

- We employ predictions of C-terminal post-translational modifications and global structural improvements by performing site-directed mutagenesis
- Measure Cx40 mutant expression through flow cytometry

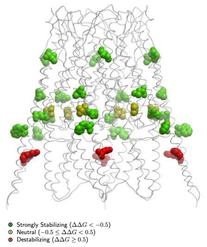


(A) HA and EGFP tagged Cx40 and Cx43 expression where red fluorescence corresponds to HA and green to EGFP tagged respectively. **(B)** C-terminal PTM site mutants expression normalized by the base fluorescence.

Structure-aware modeling reveals candidate mutations for Cx40 stabilization



ProteinMPNN was used to identify probable stabilizing mutations, while PMI analysis assessed whether mutations were independent or statistically coupled.

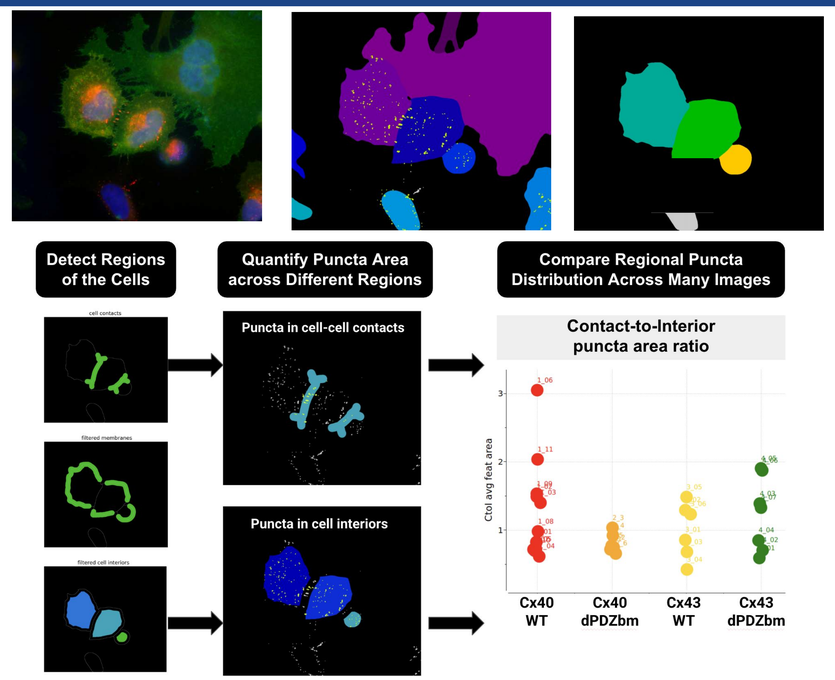


Using ICM, we determined how biophysically stabilizing each mutation was in terms of binding energy (DDG)

	ORIGINAL	MUTATION
G230A (ΔΔG = −1.05 kcal/mol)		
A216L (ΔΔG = −1.13 kcal/mol)		
F173L (ΔΔG = −2.04 kcal/mol)		

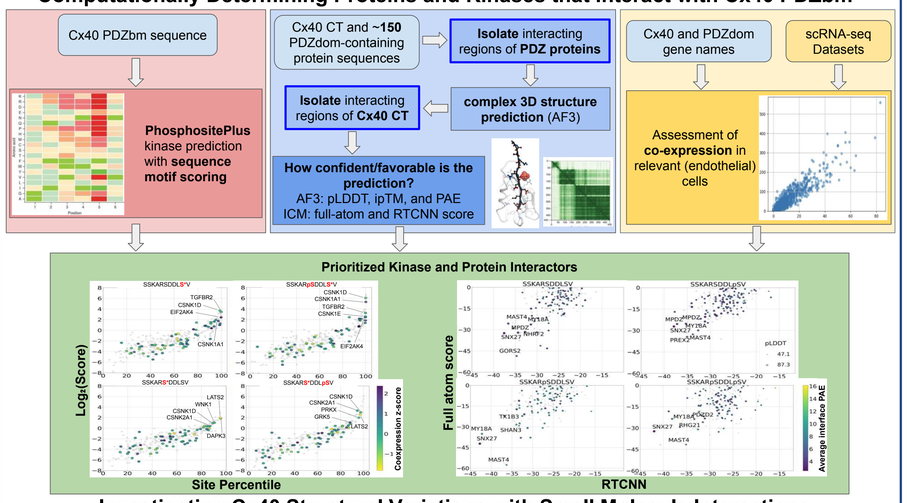
ICM was used to view structural conformations of the various stabilizing mutations between the original and mutated residues.

Based on multiple IF images, how well does a connexin variant form GJ plaques at cell-cell contacts?

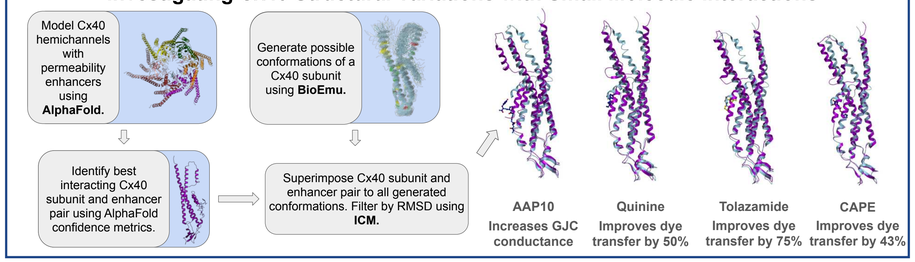


How does Cx40 interact with proteins and small molecules?

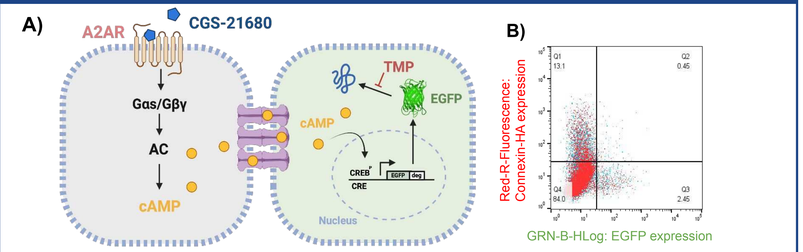
Computationally Determining Proteins and Kinases that Interact with Cx40 PDZbm



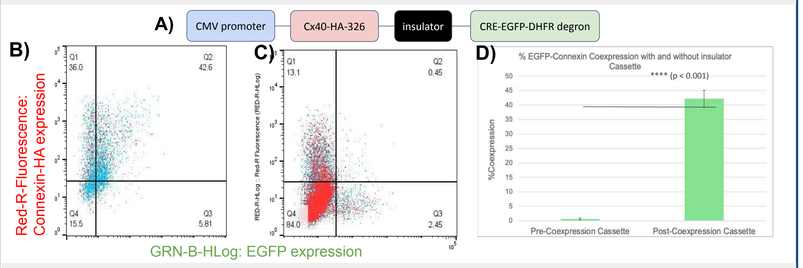
Investigating Cx40 Structural Variations with Small Molecule Interactions



How can we detect permeability and use it as a proxy for Connexin function?



Schematic of transcriptional cAMP biosensor with (**A**) donor (left) population transfected with A2AR pathway for cAMP production and recipient (right) population transfected with CRE-EGFP-DHFR cassette **(B)** Displays expression bottleneck in which HeLa cells only express Connexin (red only) or EGFP (green only) due to transfection of two different plasmids. Forcing genes onto the same plasmid is hypothesized to increase the coexpression signal



(A) Design of coexpression cassette with EGFP biosensor and Connexin-HA with insulator sequence to prevent promoter crosstalk between genes. **(B)** Coexpression plasmid displays increased Green-Red signal when compared to **(C)** single expression on two separate plasmids. Statistical significance with p-value < 0.001 is shown in **(D)**

Final Workflow

Cx40 Expression

PTM Site and Stabilizing Mutations

Computational Interaction Prediction

PPI prediction with AF3, PsP, ICM and scRNA-seq coexpression

Small Molecule Interactions

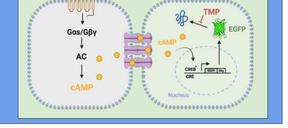
Increasing Cx40 expression allows for better analysis of IF and permeability assays

Experimental Validation

Inhibit/overexpress top scoring proteins or small molecules, and observe the effect with IF and permeability assays

IF Imaging & Quantification to measure GJ trafficking & formation

GJ permeability assay



Future Directions

- Improve on permeability assay functionality and reproducibility
- Reduce variability in IF data and obtain more biological repeats
- Measure how PDZ-binding and Ser phosphorylation affect GJC permeability
- Experimentally validate top PDZdom and kinase candidates

Acknowledgements & References

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References: