Direct CRISPPR/Cas9 ribonucleoprotein delivery to the retina: Surface modifications to increase diffusion and efficacy

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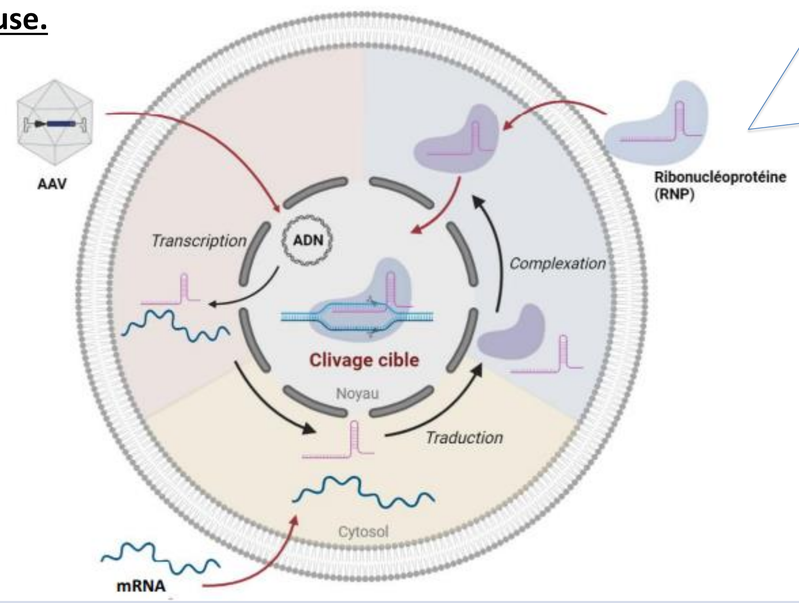
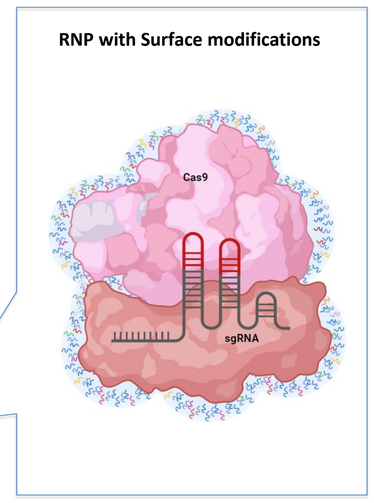
Inherited retinal diseases (IRDs) result from mutations in over 300 genes, causing retinal pathologies and potential blindness. Gene therapy has shown its potential to halt or even reverse retinal degeneration through the introduction of functional genes.

In general, viral vectors have demonstrated effective gene delivery. But concerns about long-term transgene expression and immune responses have led to the exploration of transient non-viral vectors approaches.

Genome editing mediated by CRISPR/cas9 or base editors has shown promise for treating retinal dystrophies. (Maeder et al., 2019). Successful delivery of Cas9 protein and its guide RNA as ribonucleoprotein (RNP) complexes has been reported in the retinal pigment epithelium *in vivo* (Kim et al., 2017 and Holmgaard et al., 2019) but not into photoreceptors, the main target for treating early-stage retinal degeneration.

While non-viral vectors hold promise in reducing safety concerns associated with viral vectors, addressing the challenges of efficient gene delivery is essential. This requires a concerted effort to **optimize existing non-viral vector technologies and develop innovative solutions for the transfer of gene editors to the retina for future therapeutic use.**





2. Hypothesis and aims

Hypothesis: Adding peptide modifications on the surface of RNPs to guide them to photoreceptor cells could improve the efficiency of gene therapy for inherited retinal diseases

Aims:

1 **Design and subretinal injections of modified CRISPR/Cas9 RNP**

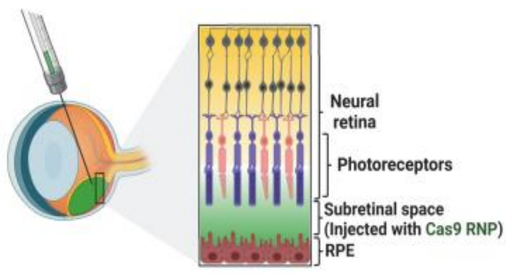
2 **Determine the cellular localization of the Cas9 protein**

3 **Investigate the gene editing efficiency in the neural retina and RPE**

Aim 1: Design and subretinal injections of modified RNP (by my tutor)

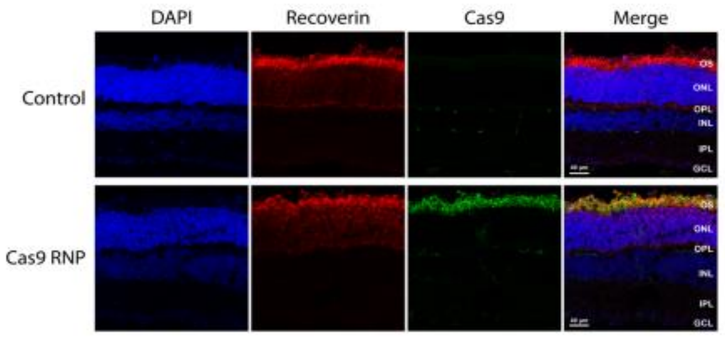
- Designed and tethered different surface modifications to **CRISPR/Cas9 RNP**
- *In vivo* injections, both subretinally and intravitreally, in a adult mouse model

	RNP modifications
1	RNP linked with PEG and peptide A
2	RNP-linked with peptide A or B
3	RNP-linked with peptide A and B
4	RNP with no link peptide
5	Buffer



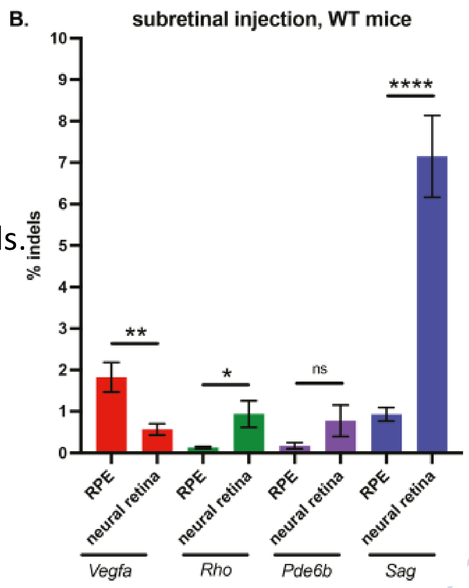
Aim 2: Determine the cellular localization of CAS9 protein (by me)

- Dissections of neural retina and RPE
- Prepared neural retina sections using Cryostat.
- Performed immunostaining with Cas9 protein antibodies and recoverin as photoreceptor markers.
- Confirmed CRISPR/Cas9 RNP delivery to photoreceptor cells through confocal microscopy.



Aim 3: Investigate the gene editing efficiency in the neural retina and RPE (by me)

- Dissections of neural retina and RPE
- DNA/RNA extractions
- PCR amplification of the targeted region.
- Asses editing activity via NGS analysis of indels.



Conclusion

Taken together with this work we hope to **improved the delivery efficiency** of the **CRISPR/Cas9 RNP in the neural retina** to provide a safe and efficient gene therapy method to patients with Inherited retinal diseases IRDs.

Perspective

Successful improvement in the delivery and gene editing o photoreceptor cells Will allow us to continue to target specific gene involved in different types of IDRs and see visual restoration in animal models.

References

Catherine Botto, Juliette Pulman, Hugo Malki, Duohao Ren, Paul Oudin, Anne De Cian, Marie As, Charlotte Izabelle, Bruno Saubamea, Stéphane Fouquet, Camille Robert, Aziz El-Amraoui, Sylvain Fisson, Jean-Paul Concordet, Deniz Dalkara bioRxiv 2023.10.16.562239; doi: https://doi.org/10.1101/2023.10.16.562239

Gautam, M., Jozic, A., Su, G.LN. et al. Lipid nanoparticles with PEG-variant surface modifications mediate genome editing in the mouse retina. Nat Commun 14, 6468 (2023). https://doi.org/10.1038/s41467-023-42189-3

Maeder, M. L., Stefanidakis, M., Wilson, C. J et al. (2019). Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nature medicine, 25(2), 229–233. https://doi.org/10.1038/s41591-018-0327-9