Biological barriers to lipid nanoparticle mediated messenger RNA delivery to the retina

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CRS 2025 ANNUAL MEETING & EXPOSITION

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JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

CHALLENGES IN OPHTHALMOLOGY

280.10^6 Retinal degeneration

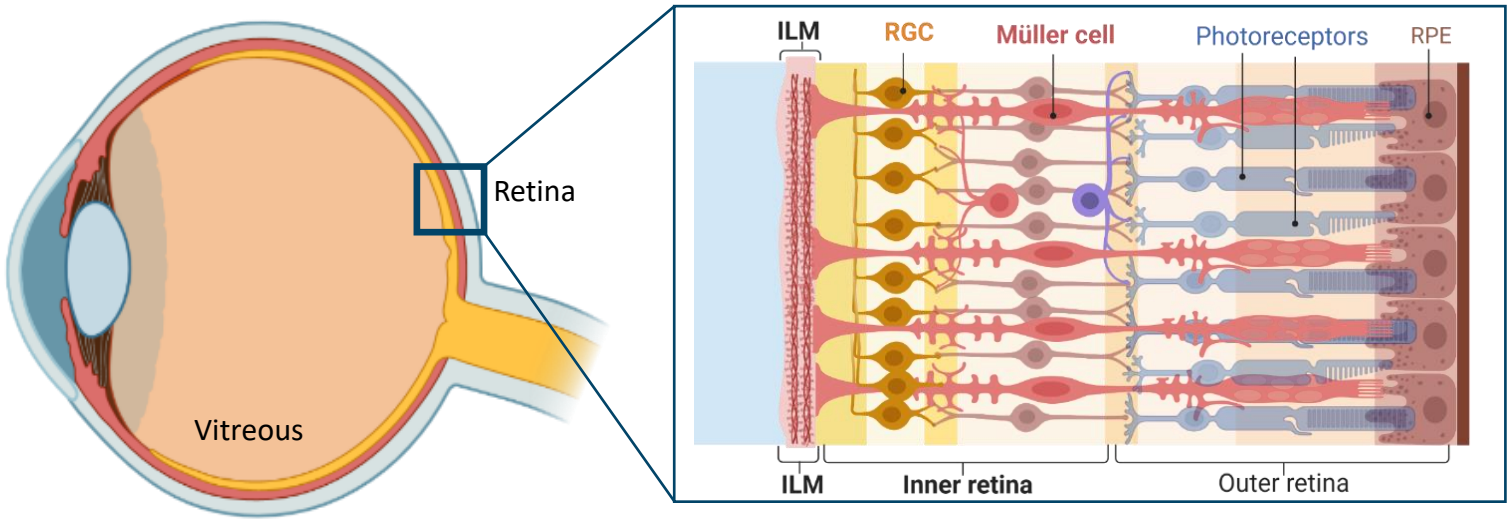
Transient protein expression
Cell reprogramming, genome editing

1

Retinal gene therapy
Luxturna®: AAV for long-term expression

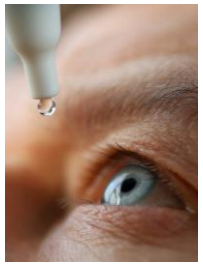
Messenger RNA

mRNA vaccins breakthrough 2021



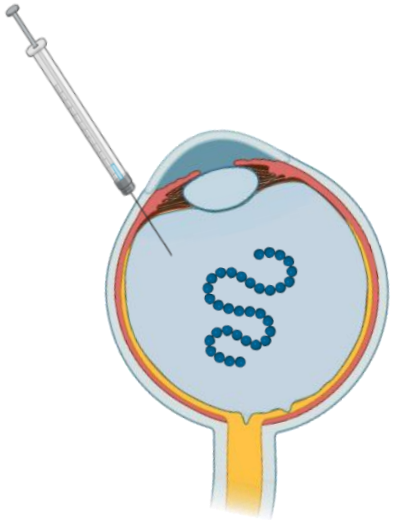
mRNA delivery currently unsuccessful

CHALLENGES IN OPHTHALMOLOGY



1900

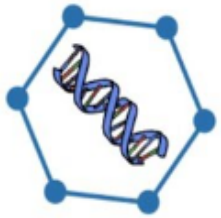
Small molecules



2007

Proteins

Extracellular targets



2018

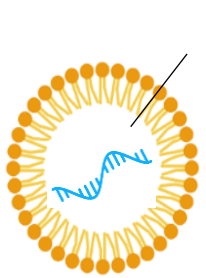
Viral vector

Long-term protein expression

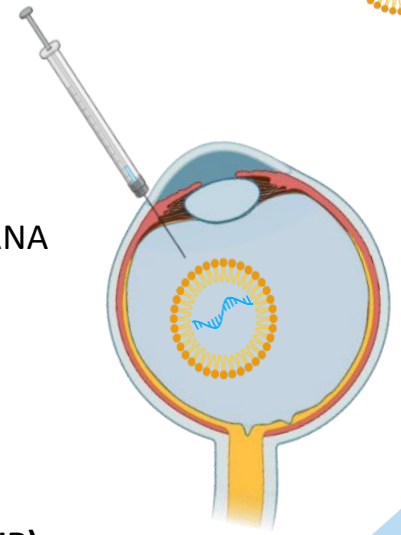
2021

mRNA vaccines

mRNA-LNP breakthrough



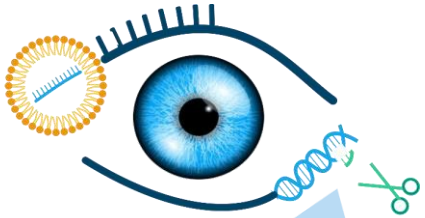
Lipid nanoparticle (LNP)



2028

Non-viral messenger RNA

Transient protein expression



20xy

mRNA-LNP

Cell reprogramming

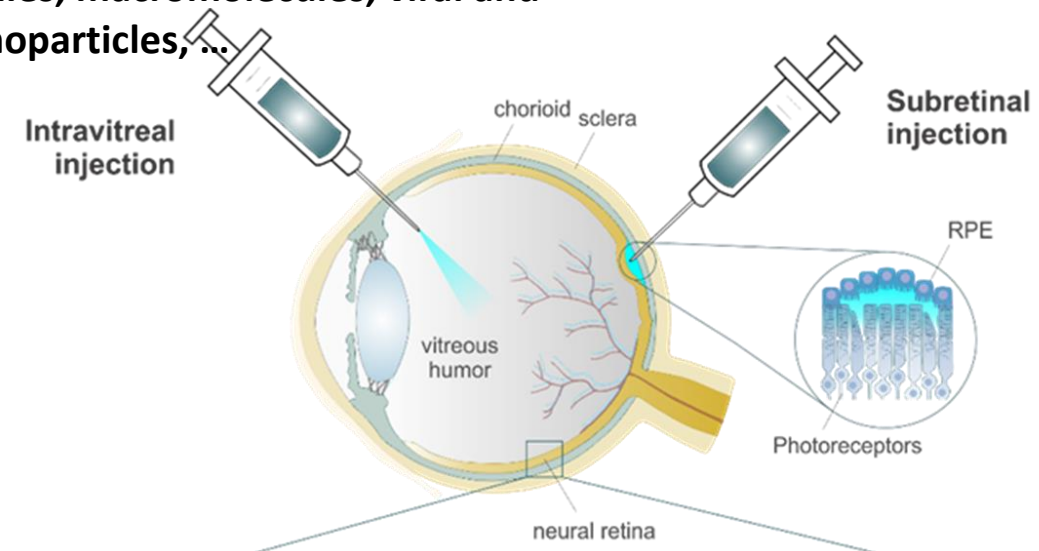
Genome editing

Current treatment options

Emerging strategies

DELIVERY ROUTES

Small molecules, macromolecules, viral and non-viral nanoparticles, …

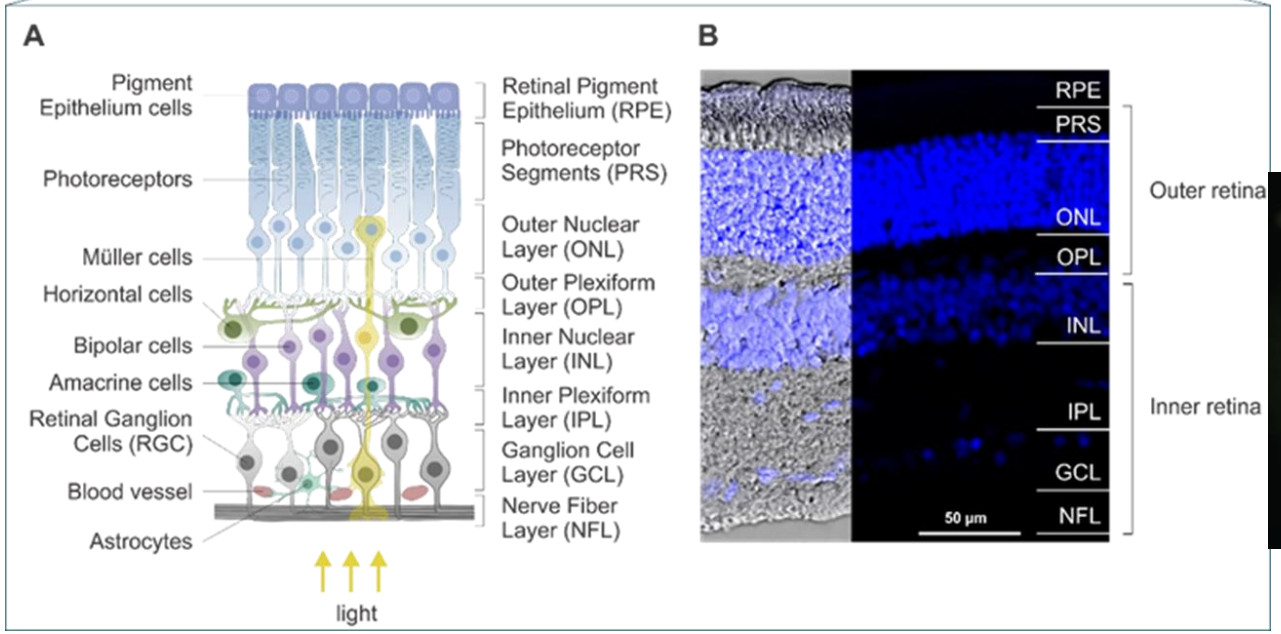


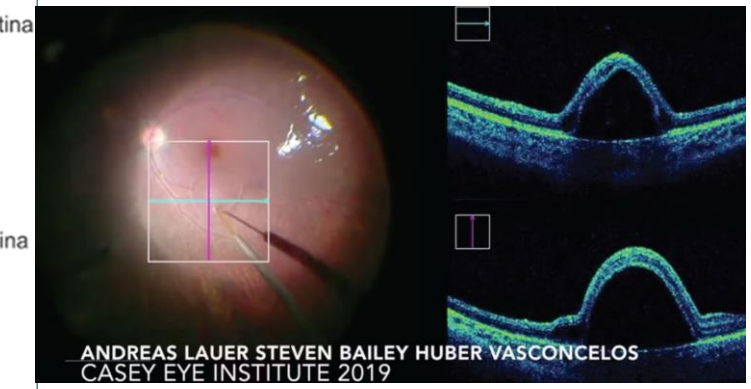
IVT injection:

- In the vitreous humor of the eye
- minimally invasive
- Relative easy to perform
- Safe, multiple administration possible
- More barriers to encounter

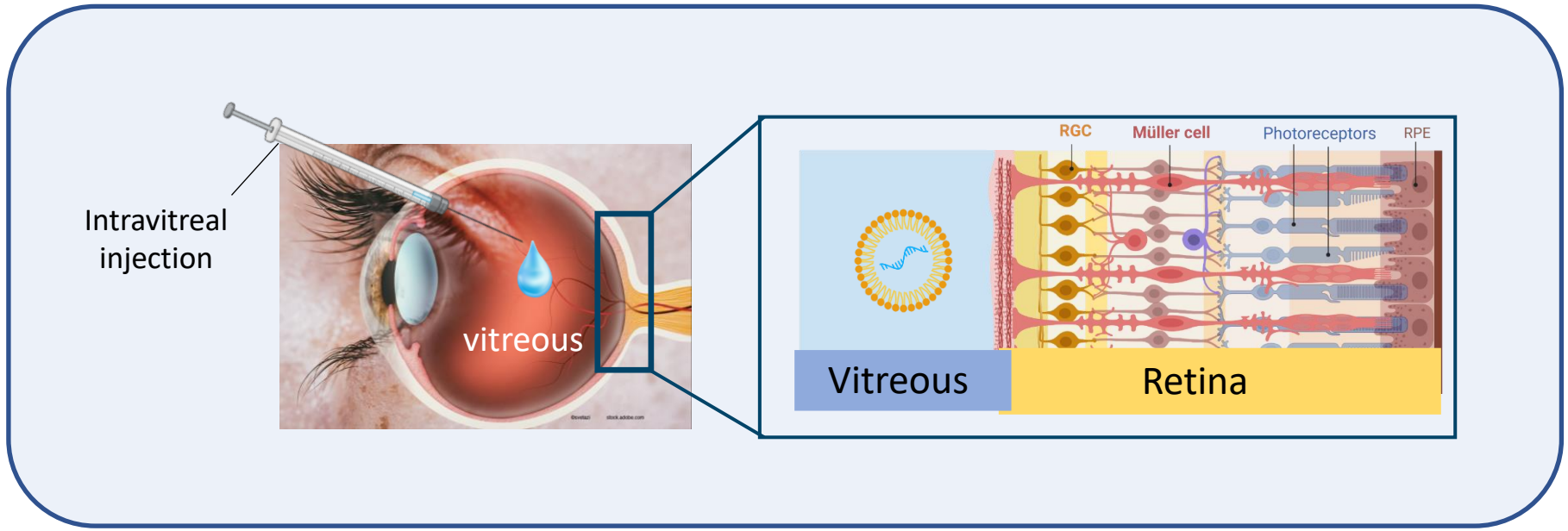
SR injection:

- Between RPE cells and photoreceptors
- More difficult to perform
- Risk of retinal detachment
- Mainly local effects
- Difficult to repeat

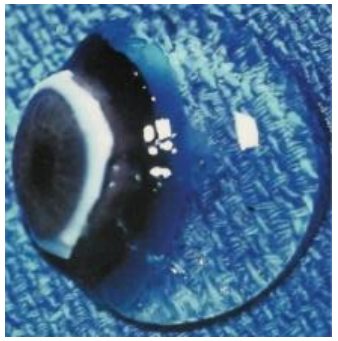




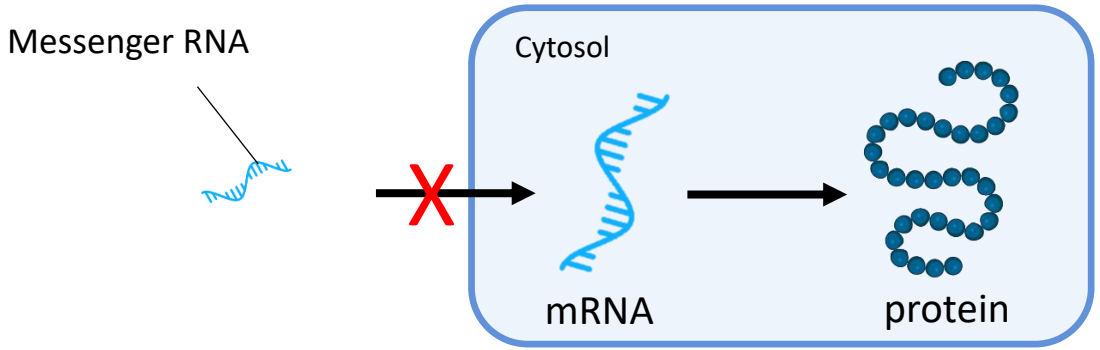
THE OPPORTUNITY: mRNA-LNPs



Vitreous



Retinal cell



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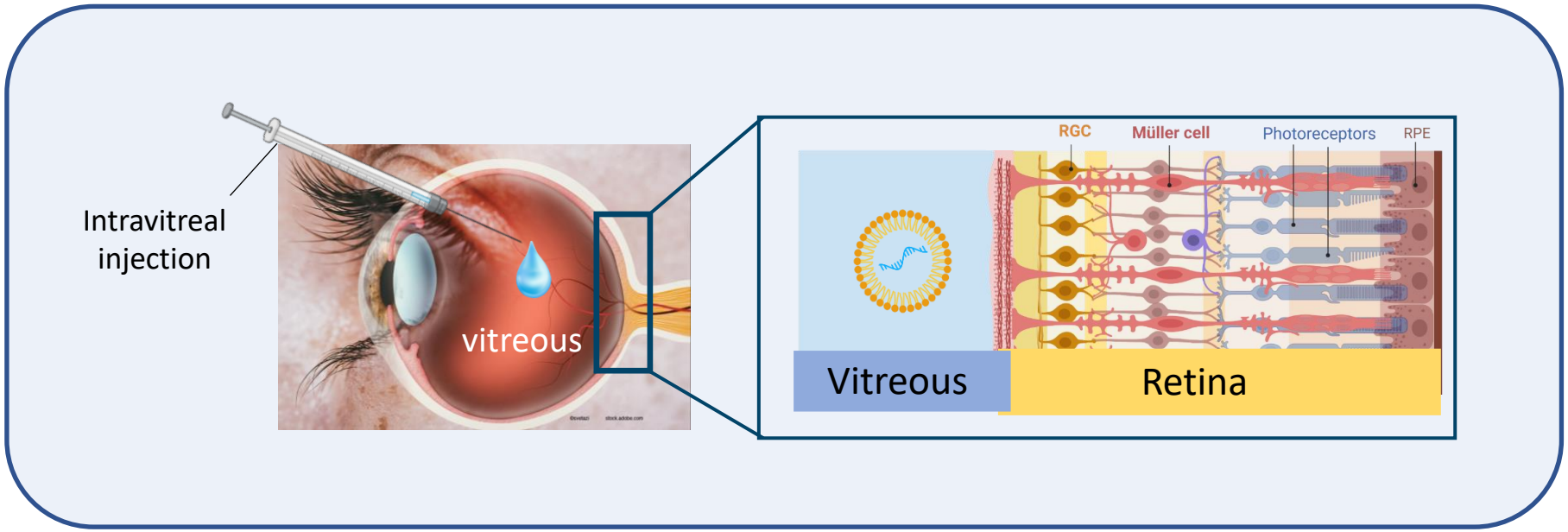
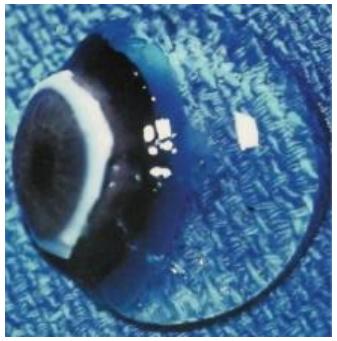
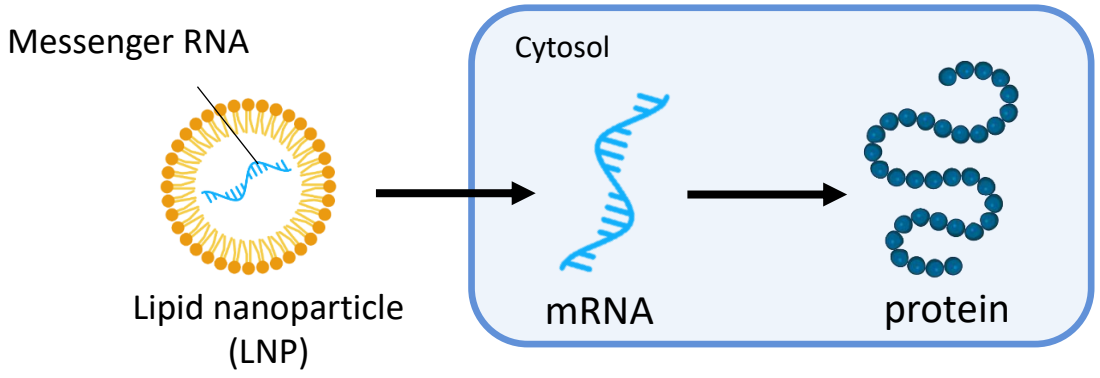


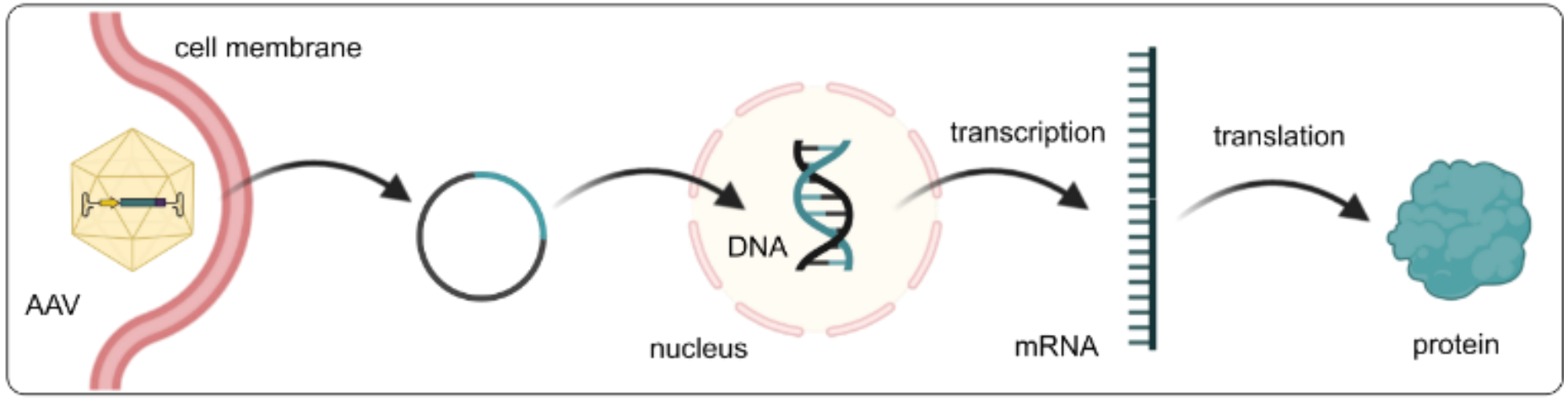
Vitreous



Retinal cell



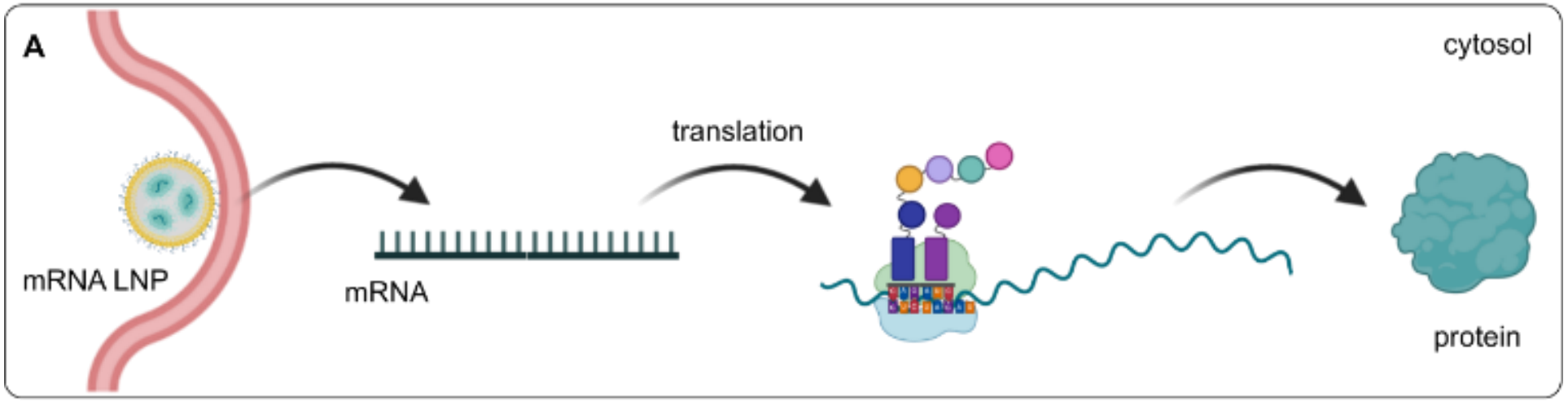
DNA delivery: often viral



Onset 14 days

Last years

mRNA delivery: often non-viral



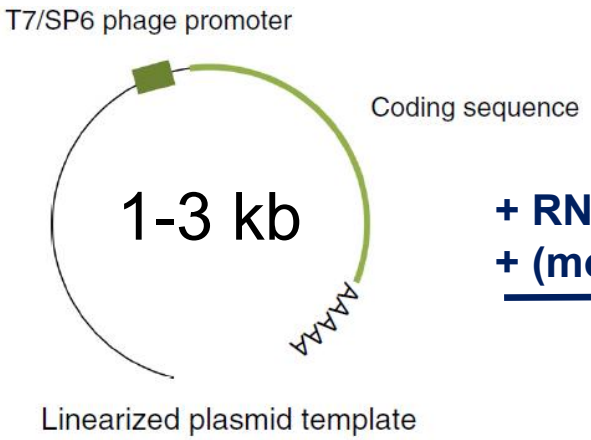
Onset 2-24 hours

Last 2-3 weeks

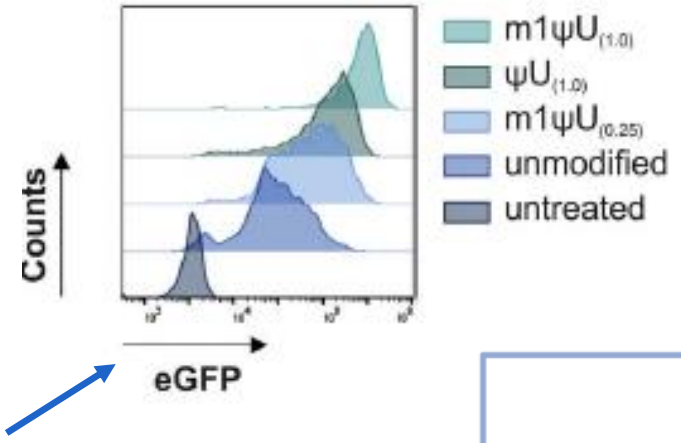
Zhang et al. Chem. Soc. Rev., 2024,53, 317-360

mRNA

Modified mRNA

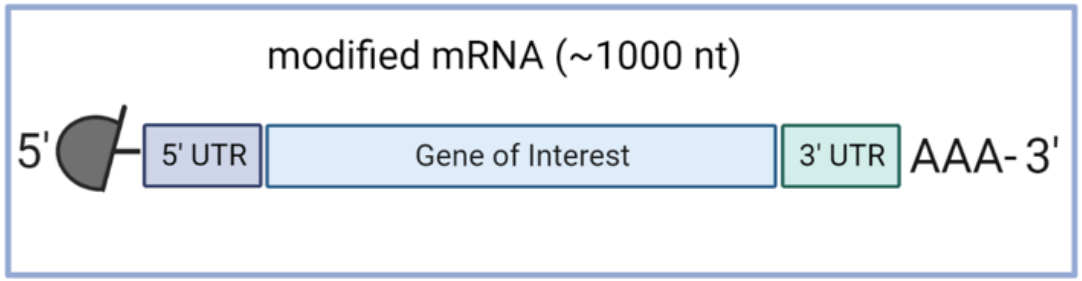


+ RNA polymerase
+ (modified) ribonucleotides A C G U



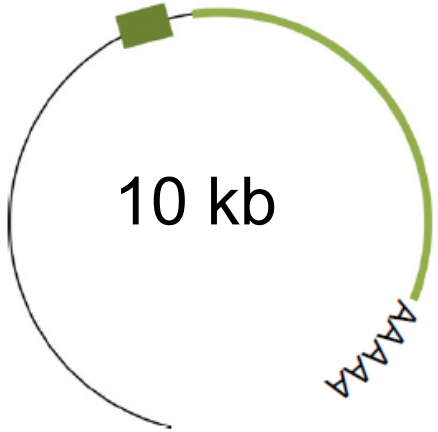
Stability!
Innate immunity!

Minnaert et al. 2021 ADDR
Devoldere et al. 2016 Drug Discov. Today



Modified mRNA

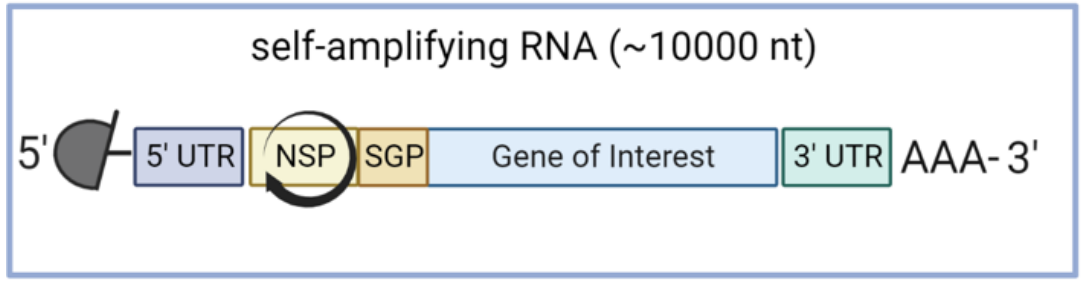
Self-amplifying mRNA



+ RNA polymerase
+ only non-modified ribonucleotides

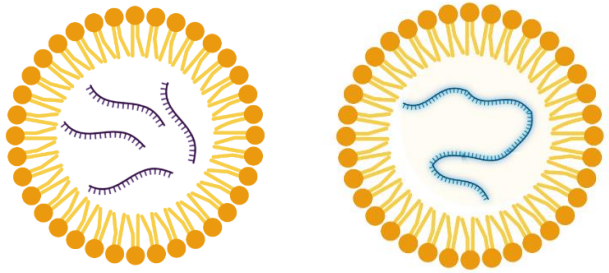
Based on the genome of an alphavirus:

- Non-structural proteins → replicase complex
- Structural proteins replaced by GOI



Self amplifying mRNA

LNPs

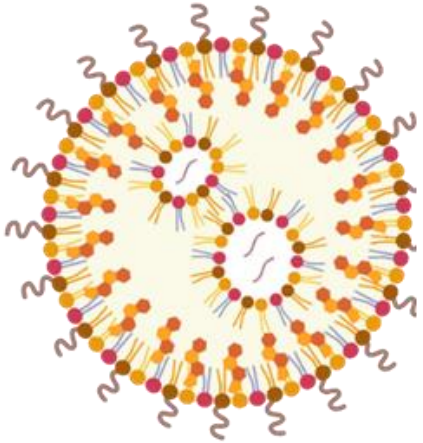


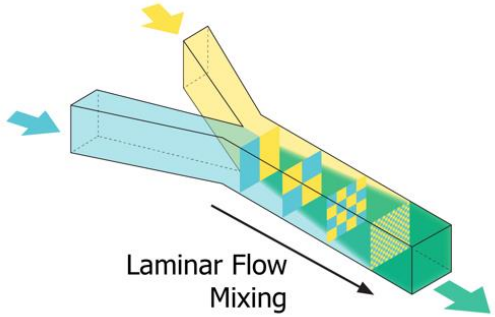
5-8 Modified mRNA strands

1-2 Self-amplifying mRNA strands

400 nm

80 – 180 nm





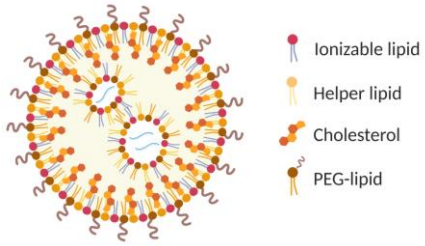
Ionizable lipid

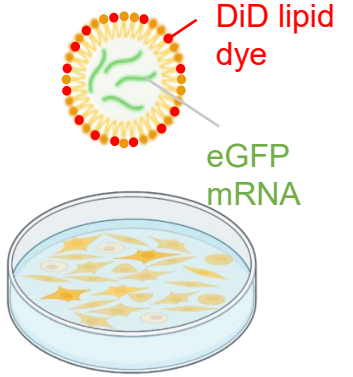
Helper lipid

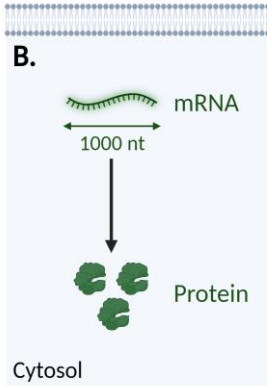
Cholesterol

PEG-lipid

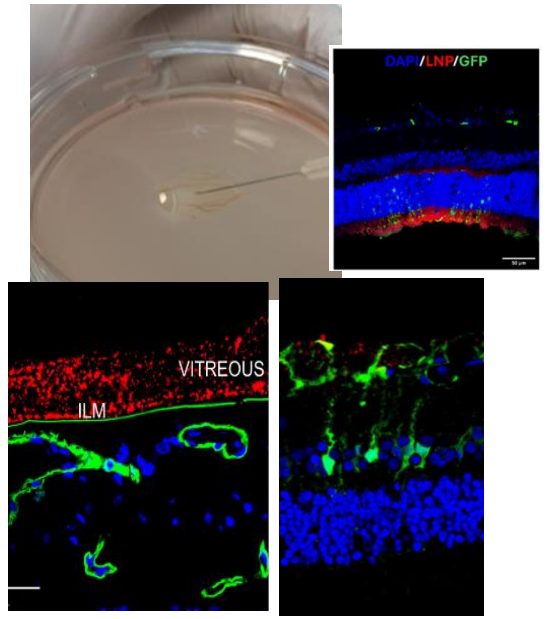
IN VITRO/IN VIVO SCREEN: lipid nanoparticles

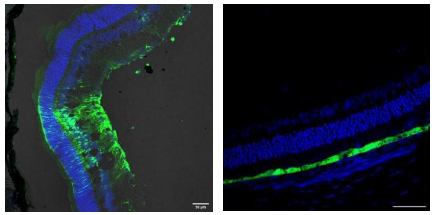


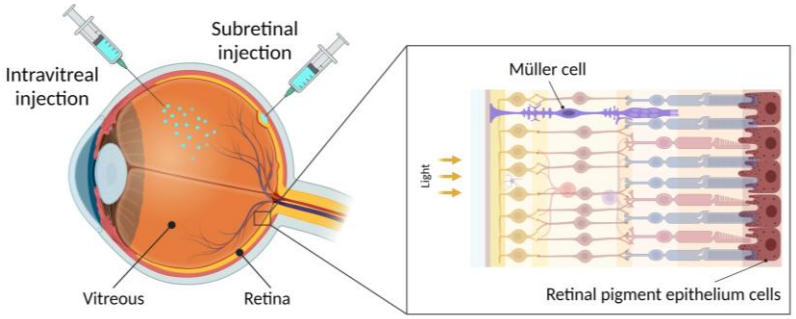




Retinal explant for IVT injection







Workflow

LNP preparation → **In vitro screen of LNP formulations** → **Ex vivo screen of LNP formulations** → **In vivo screen of LNP formulations**

- Size
- Charge
- Encapsulation efficiency
- Number of mRNA per LNP

- Müller cells (MIO-M1 cell line)
- RPE cells (ARPE cell line)
- 661W (photoreceptors)

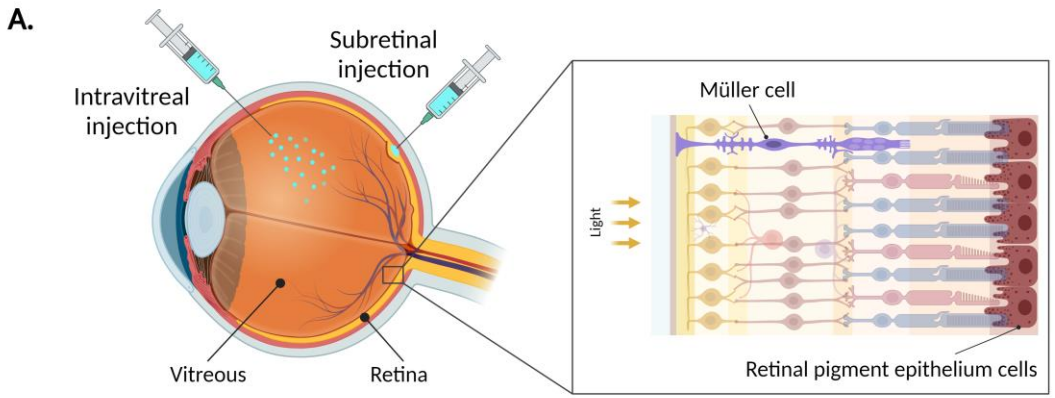
- Uptake
- Transfection efficiency
- Innate immunity

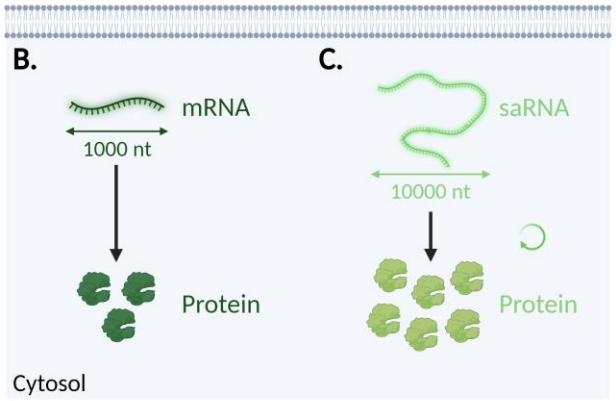
- Intravitreal mobility of LNP formulations
- ILM penetration in ex vivo retinal explants
- Uptake and eGFP expression in ex vivo retinal explants
- Flow of digested retinal explants
- ILM perforation technologies

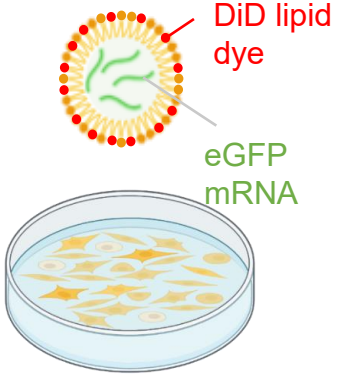
- Distribution of fluorescently labeled mRNA LNPs
- Immunogenicity of mRNA LNPs
- Expression efficiency of mRNA LNPs

IN VITRO SCREEN: mRNA vs saRNA with Lipofectamine

Vanluchene et al, Eur J Pharm Biopharm 2024

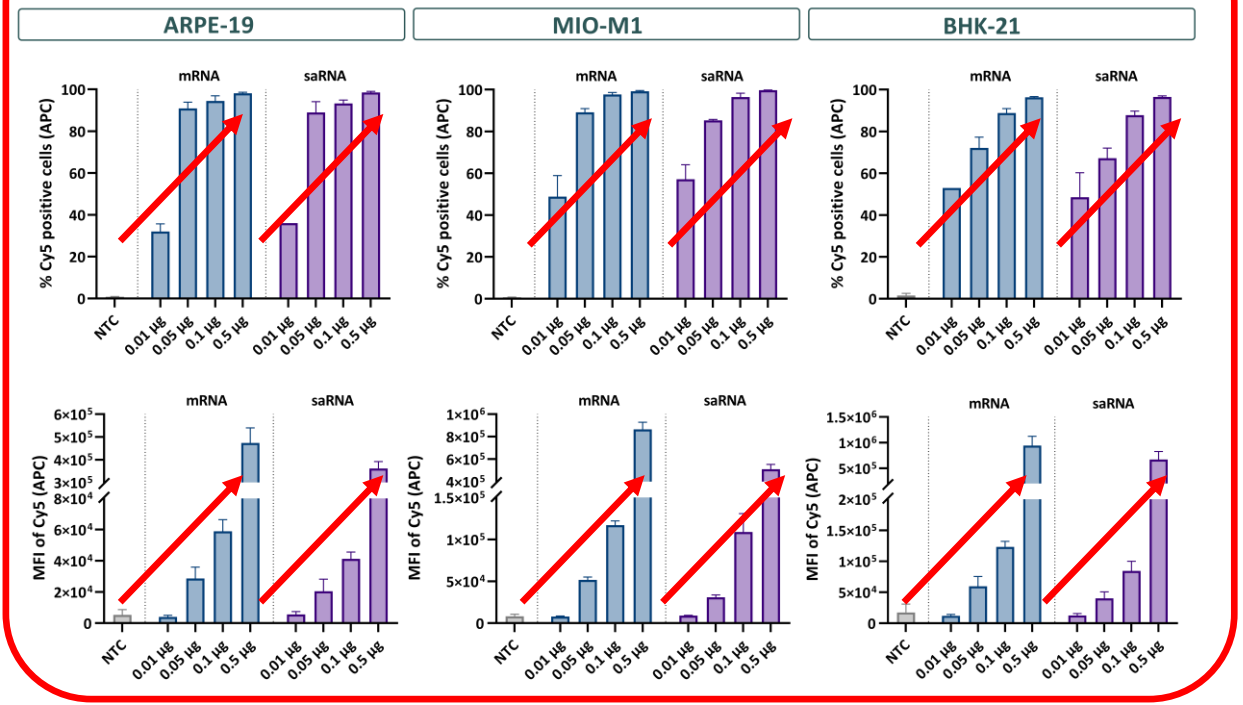




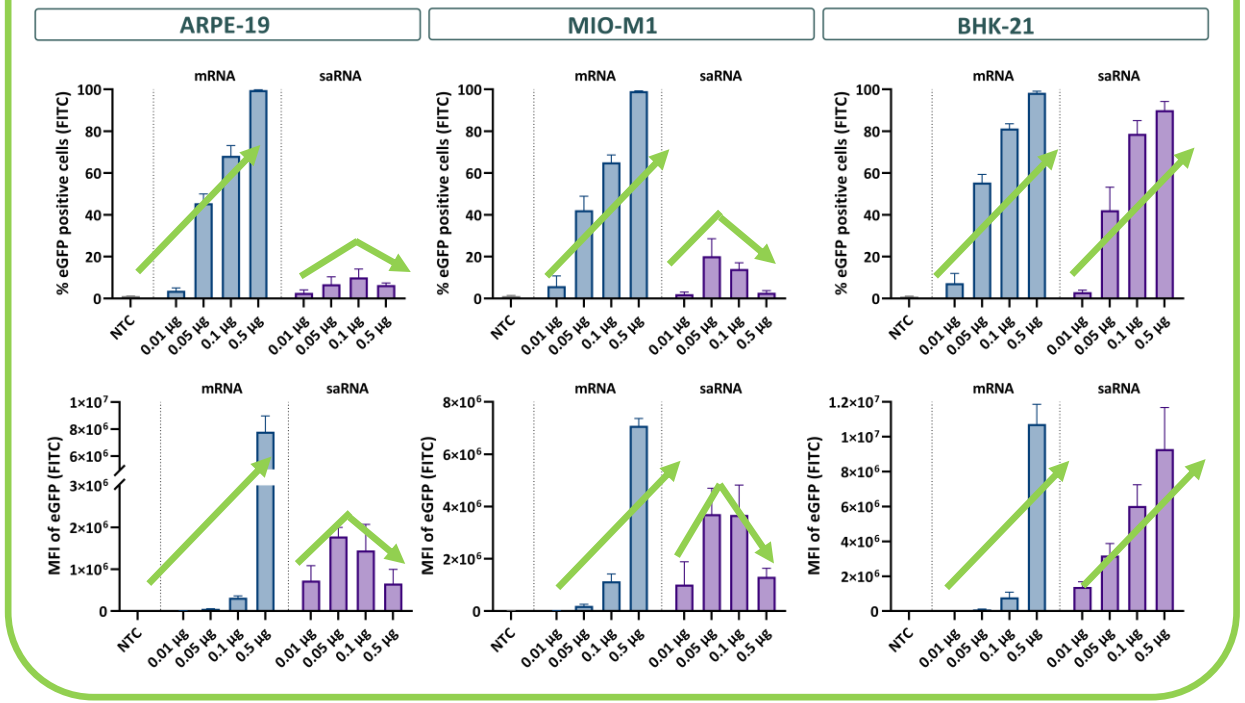


Cell culture: ARPE, Müller, BHK-21 cells

Uptake

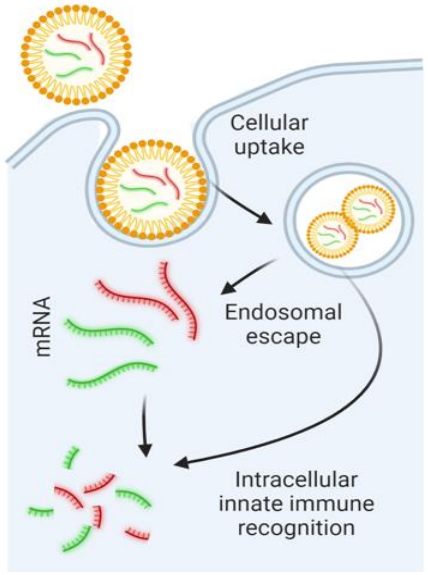


Transfection efficiency



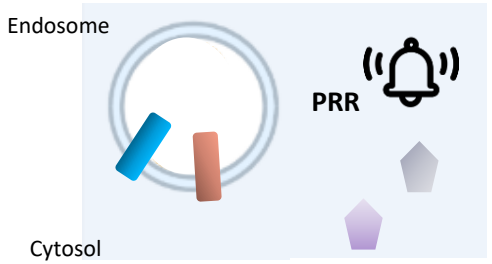
IN VITRO SCREEN: mRNA vs saRNA with Lipofectamine

Vanluchene et al, Eur J Pharm Biopharm 2024



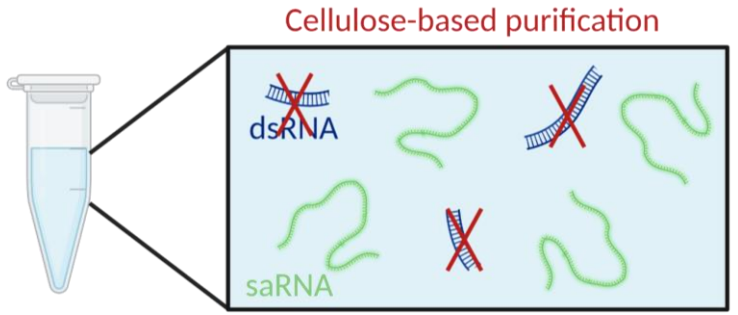
ARPE cell/Müller cell

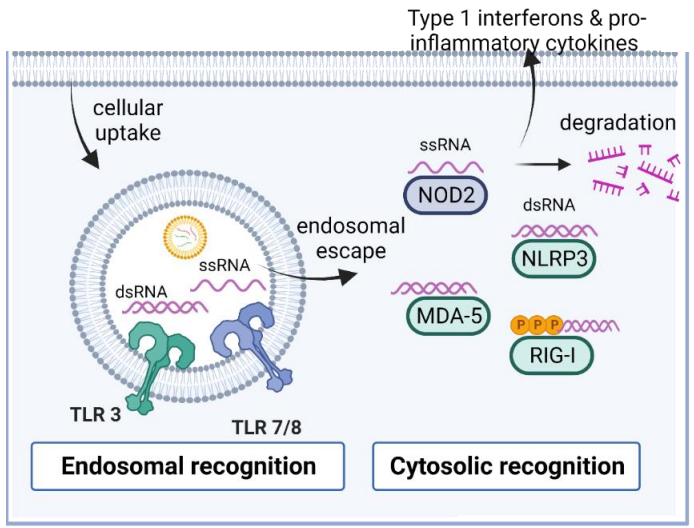
Immune competent

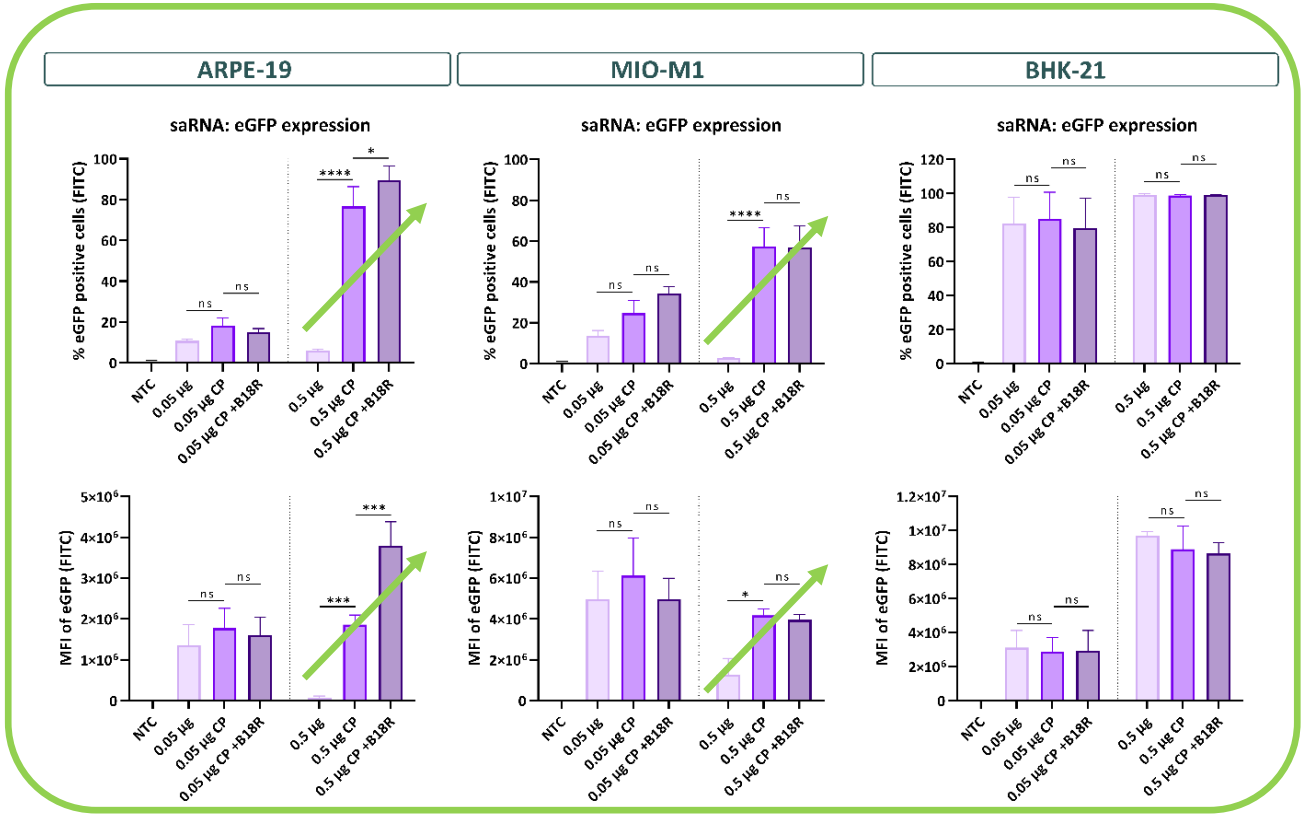


BHK cell

Immune deficient







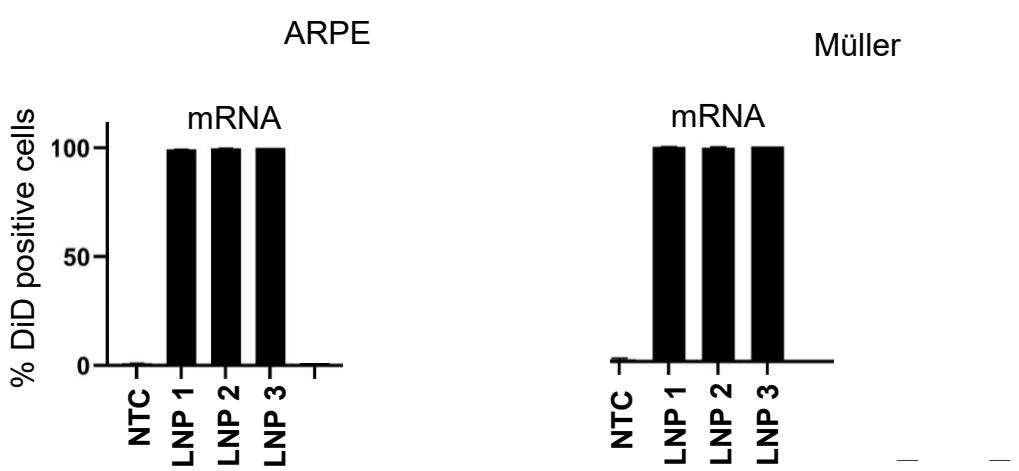
The mRNA cargo: Immunogenicity should be reduced to enhance transfection efficiency in immune competent retinal cells

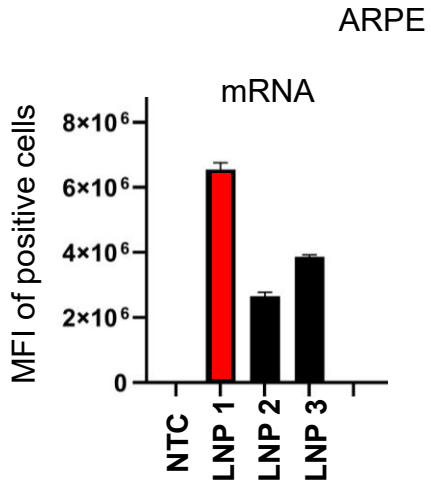
IN VITRO SCREEN: lipid nanoparticles

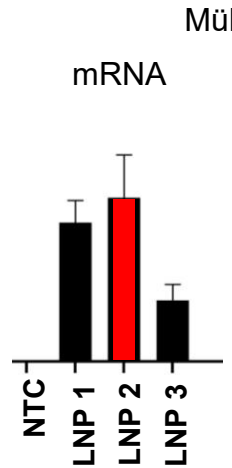
In vitro screen of LNP formulations

- Müller cells (MIO-M1 cell line)
- RPE cells (ARPE cell line)

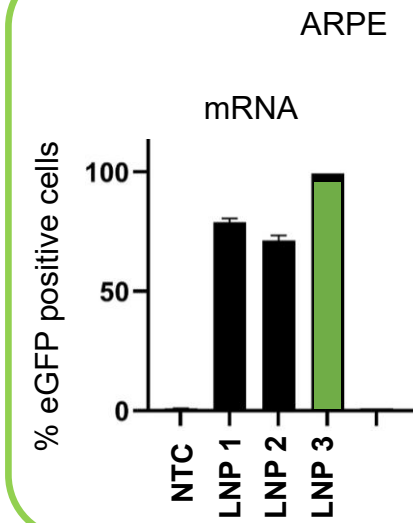
Uptake

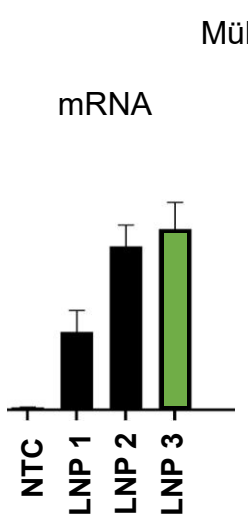


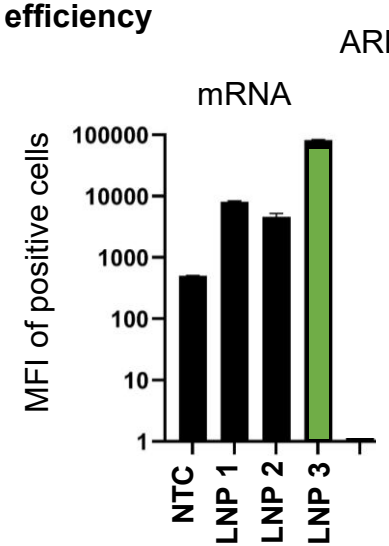


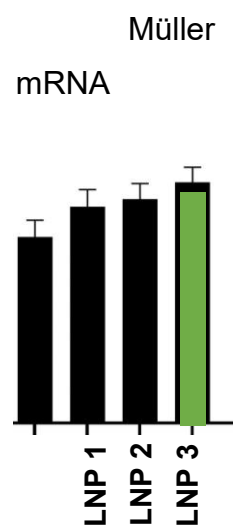


Transfection efficiency





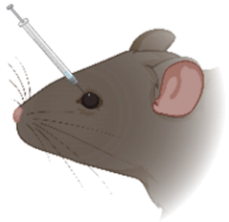


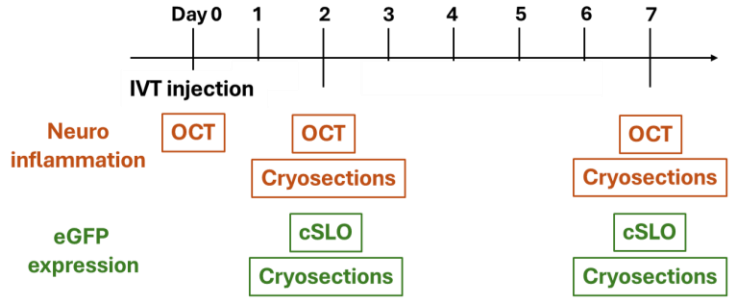


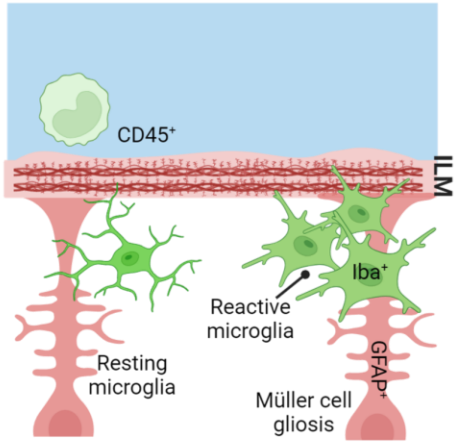
Cell type and LNP composition (+ batch!) dependent differences in uptake and transfection seen.

IN VIVO SCREEN: lipid nanoparticles

Unpublished - confidential



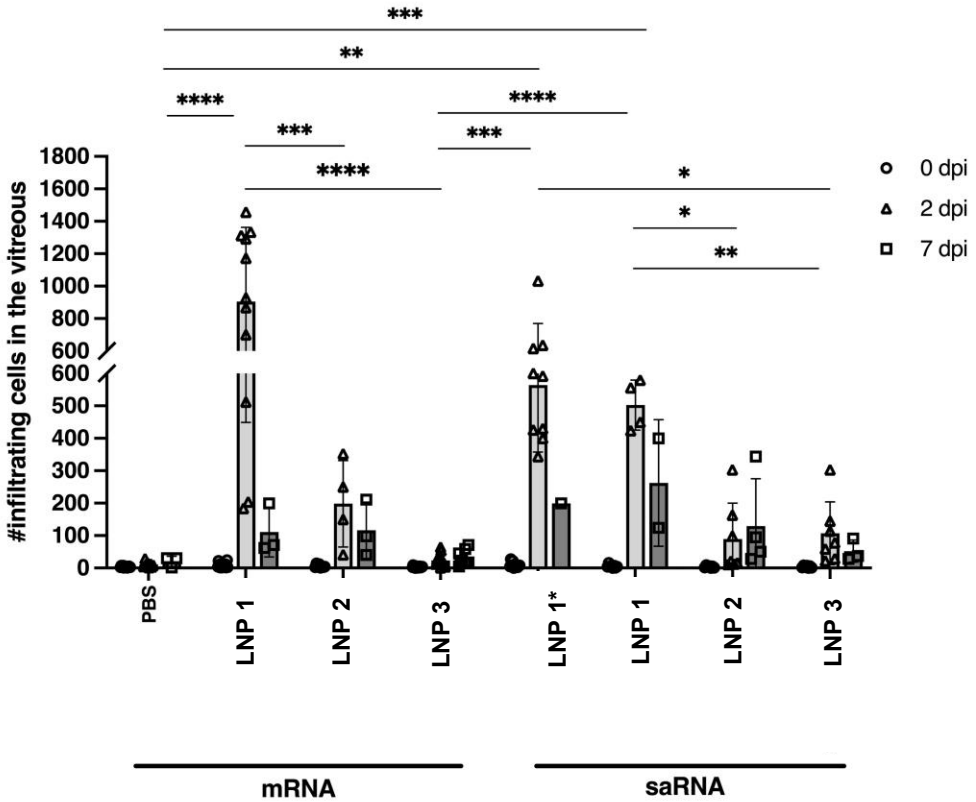


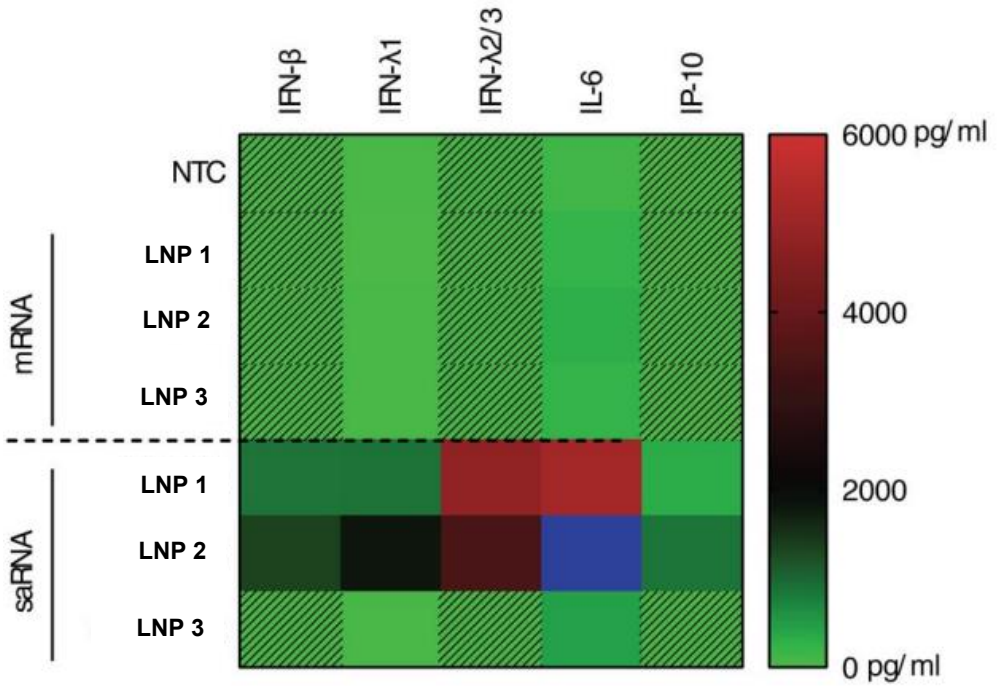


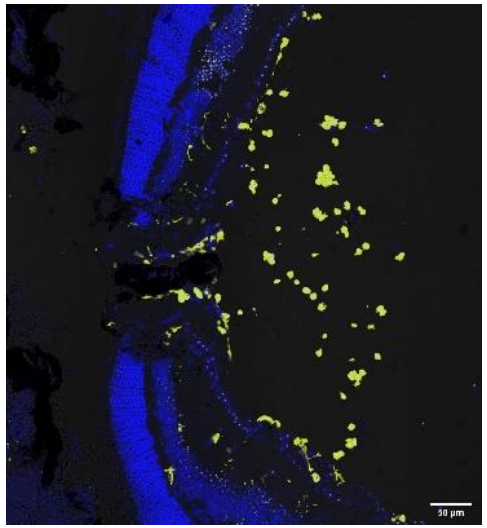
In vivo screen of LNP formulations

- **Immunogenicity of mRNA LNPs**
- Expression efficiency of mRNA LNPs

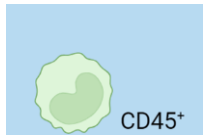
Immune cells in vitreous

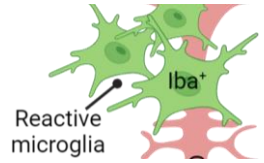


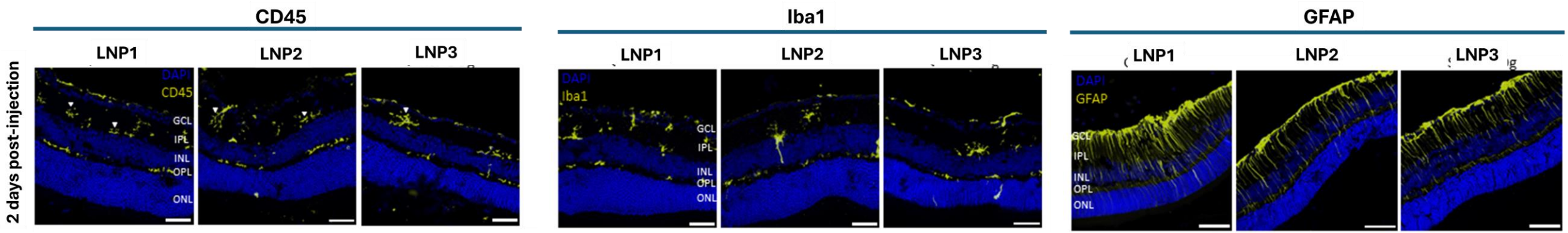




IN VIVO SCREEN: lipid nanoparticles

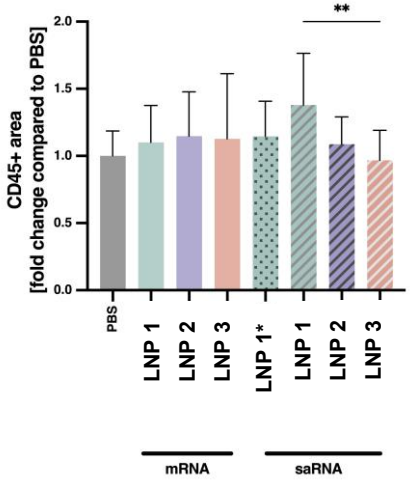


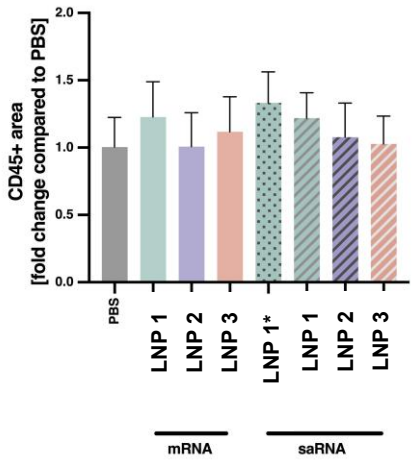


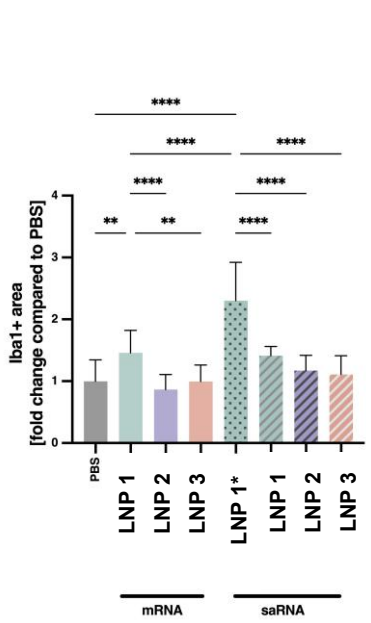


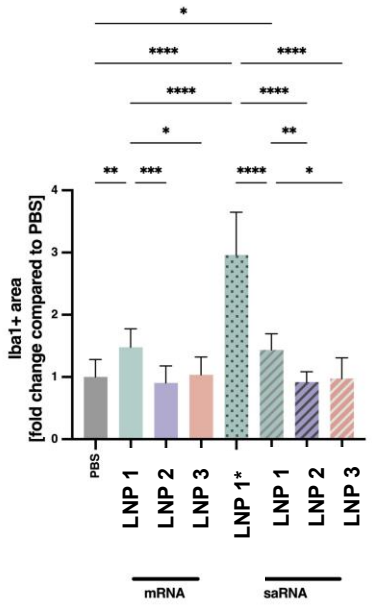
2 days

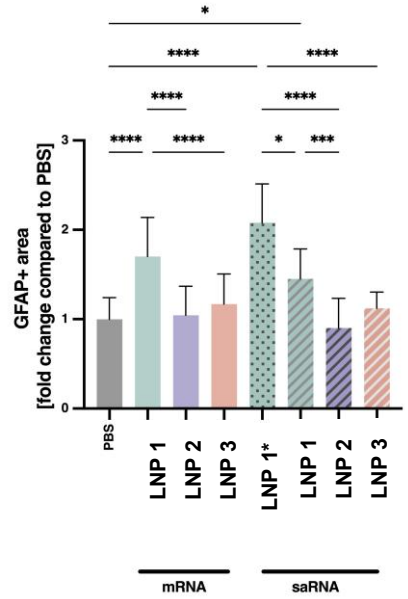
7 days

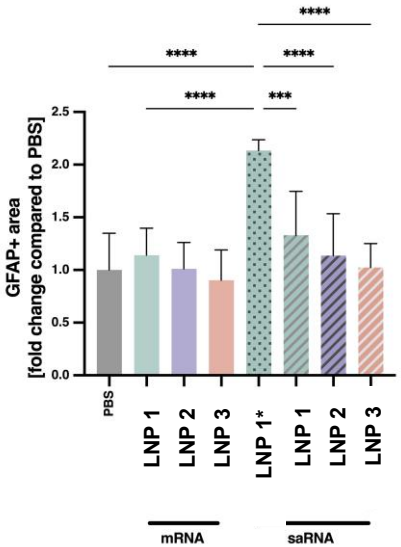












2 days

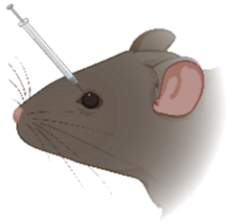
7 days

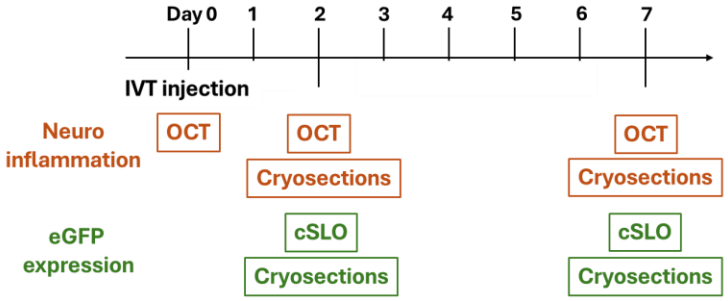
2 days

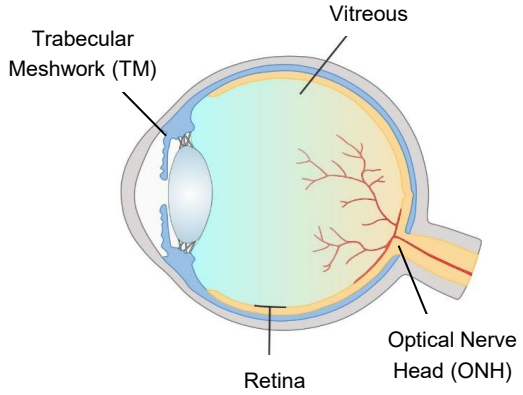
7 days

LNP1 is most and LNP3 is least immunogenic

IN VIVO SCREEN: lipid nanoparticles



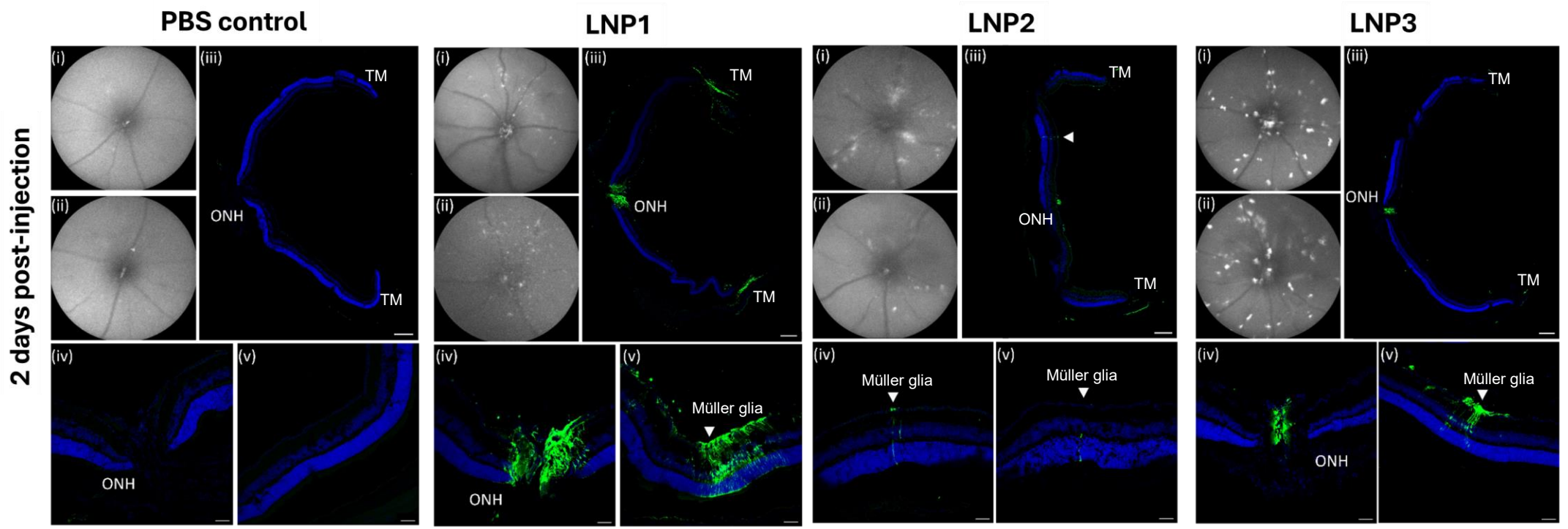


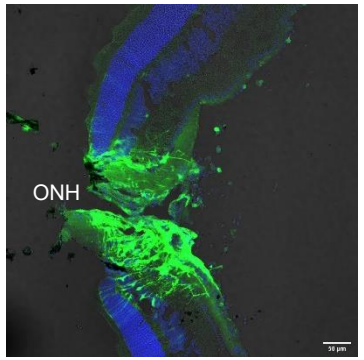


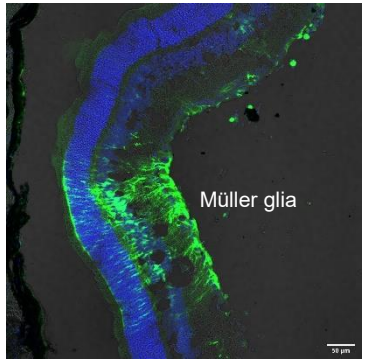
In vivo screen of LNP formulations

- Immunogenicity of mRNA LNPs
- **Expression efficiency of mRNA LNPs**

EGFP expression in retina

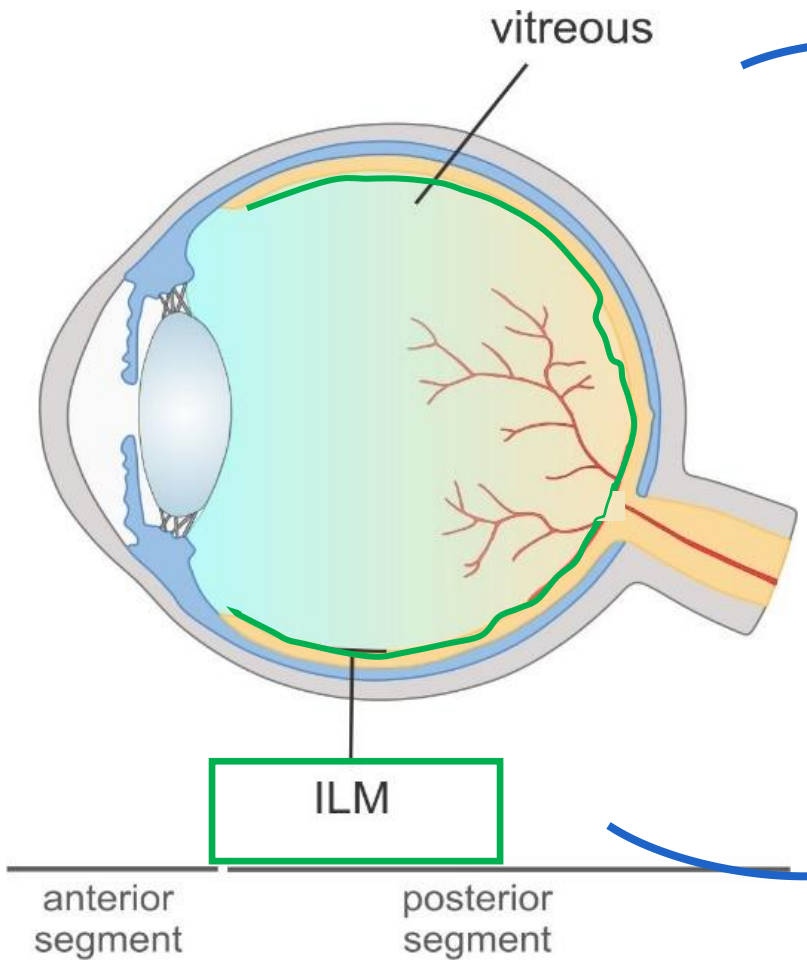




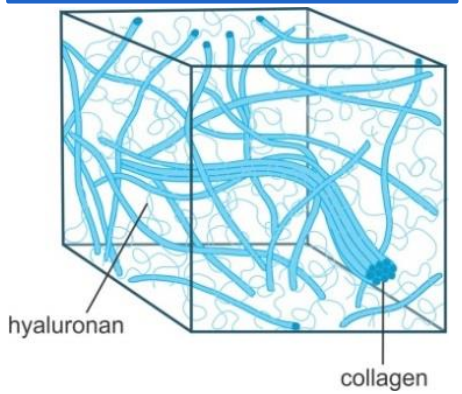


LNP1 and LNP3 give best results. Transfection limited to optical nerve head and few Müller glia.

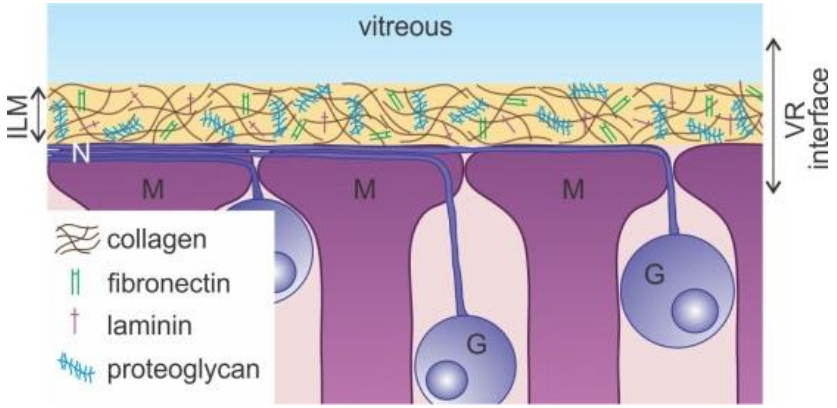
DELIVERY CHALLENGES



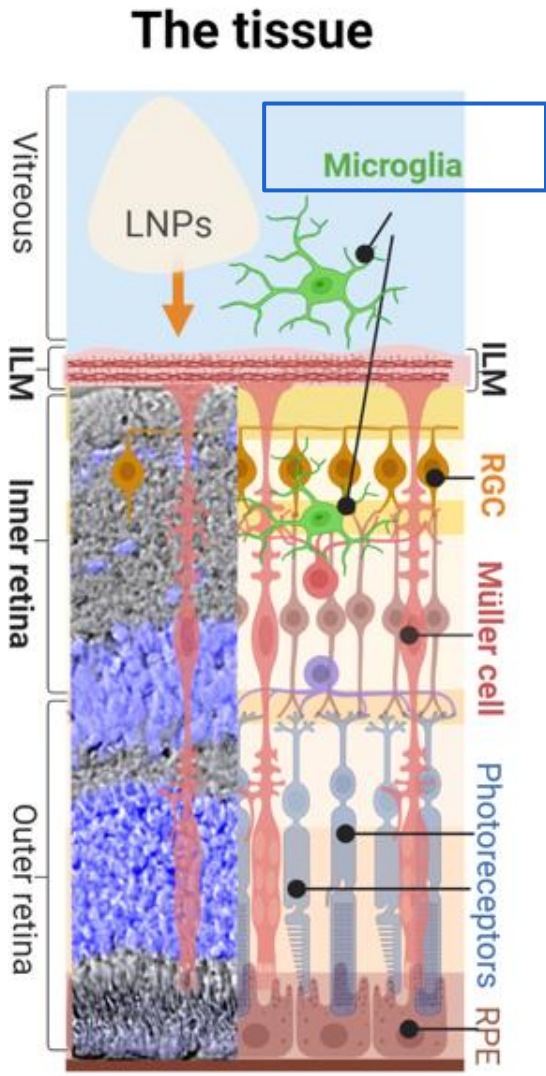
Vitreous



Inner Limiting Membrane



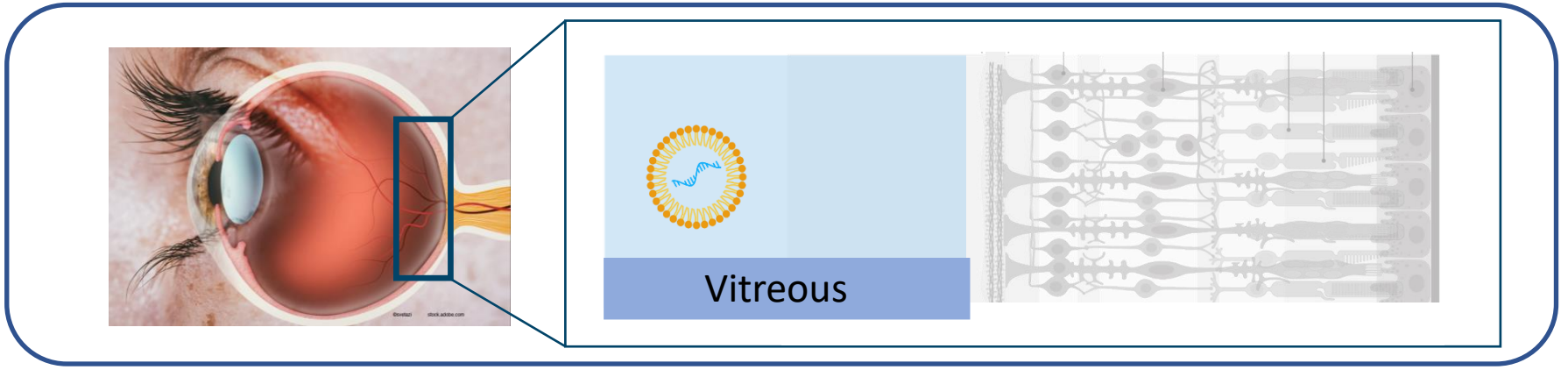
Retina



Peynshaert et al. 2017 Adv. Drug Deliv. Rev.

GHENT UNIVERSITY

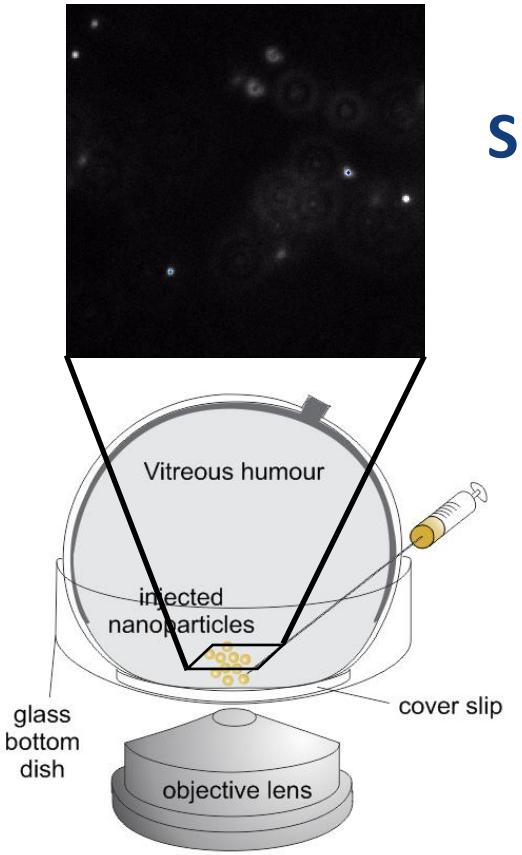
VITREOUS

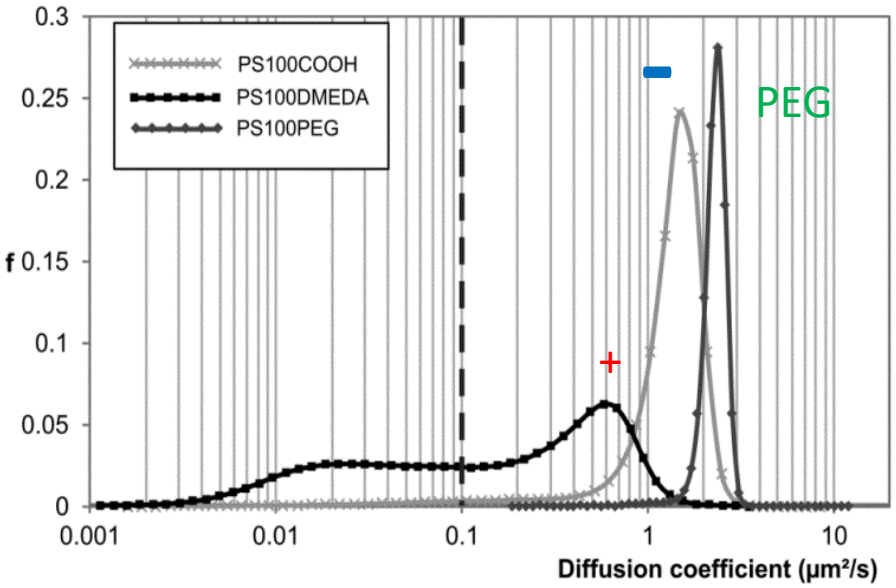


Negative or neutral charge best for mobility

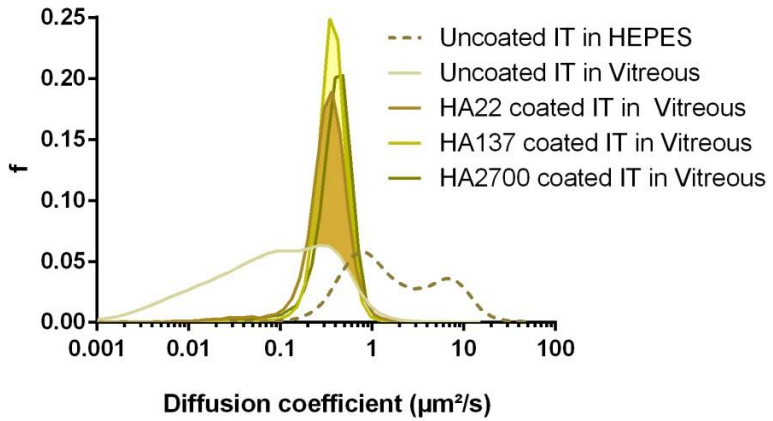
< 500 nm

SPT



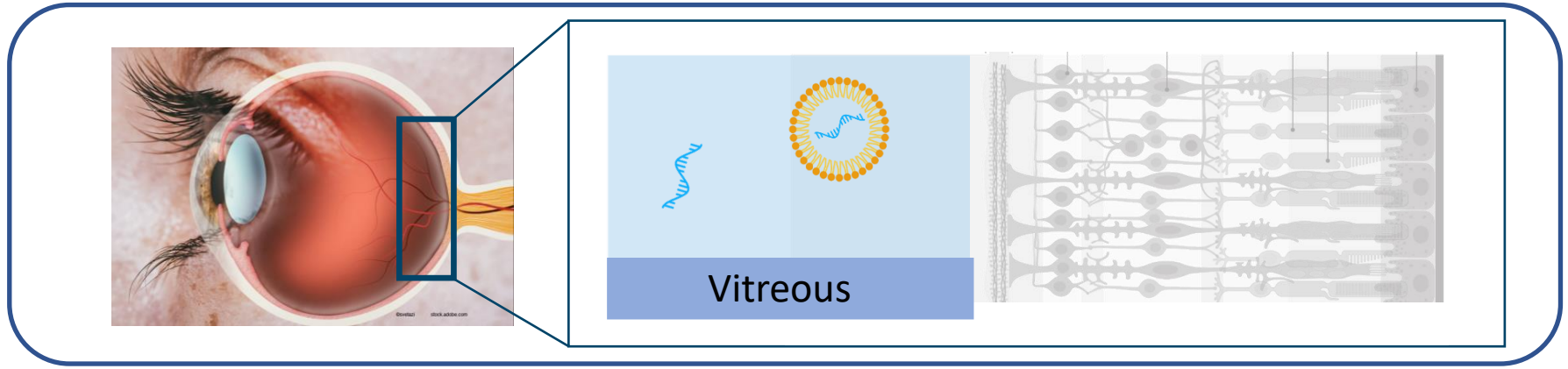


Martens et al. 2013 Nanomedicine

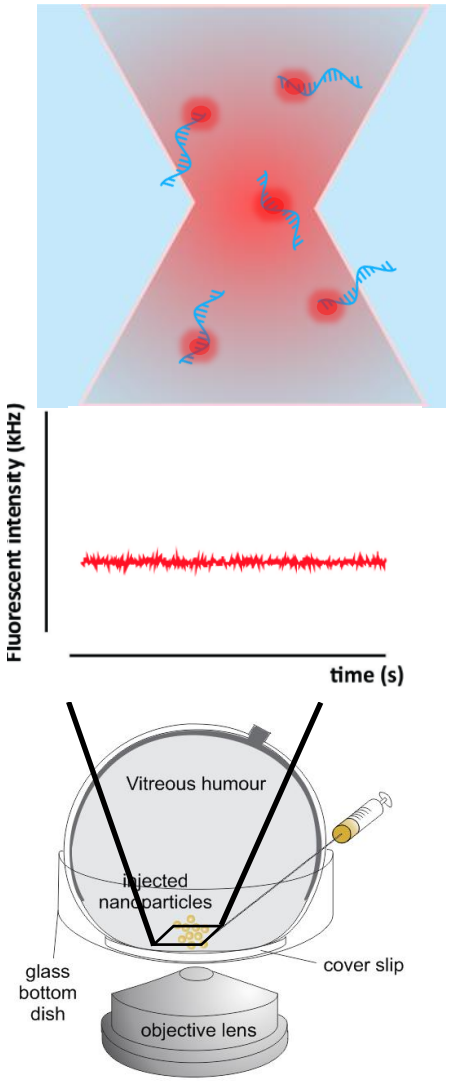


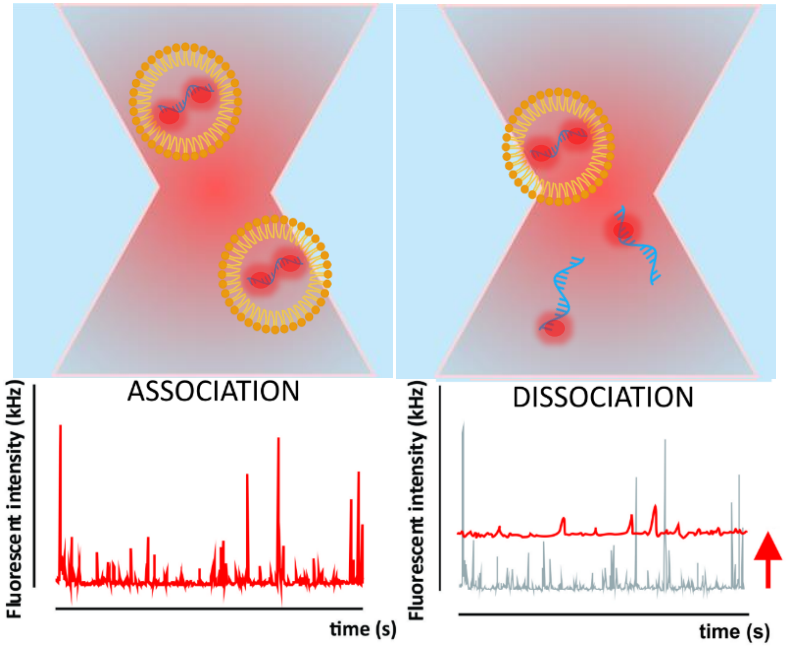
Devoldere et al. 2019, Eur J Pharm Biopharm

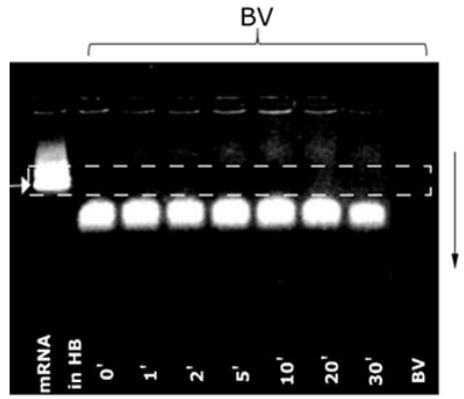
VITREOUS

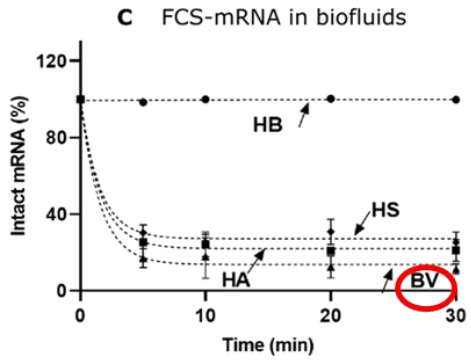


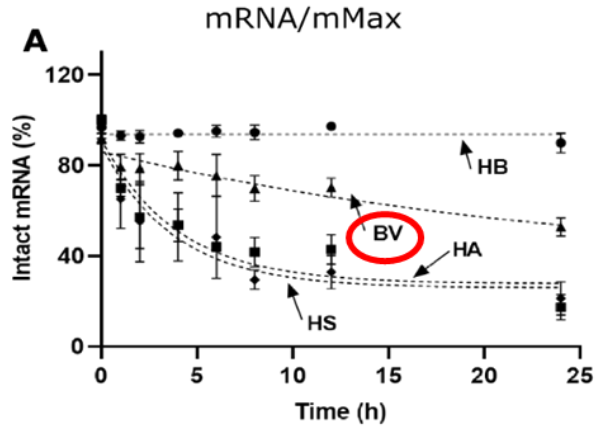
FCS

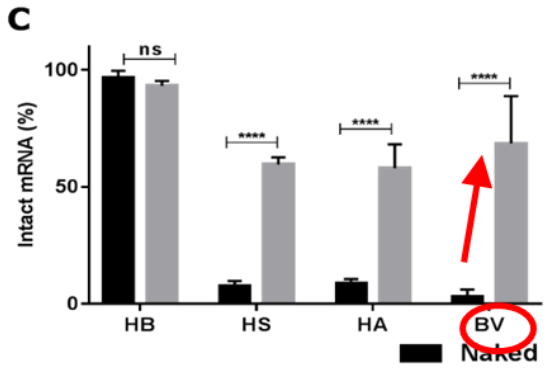










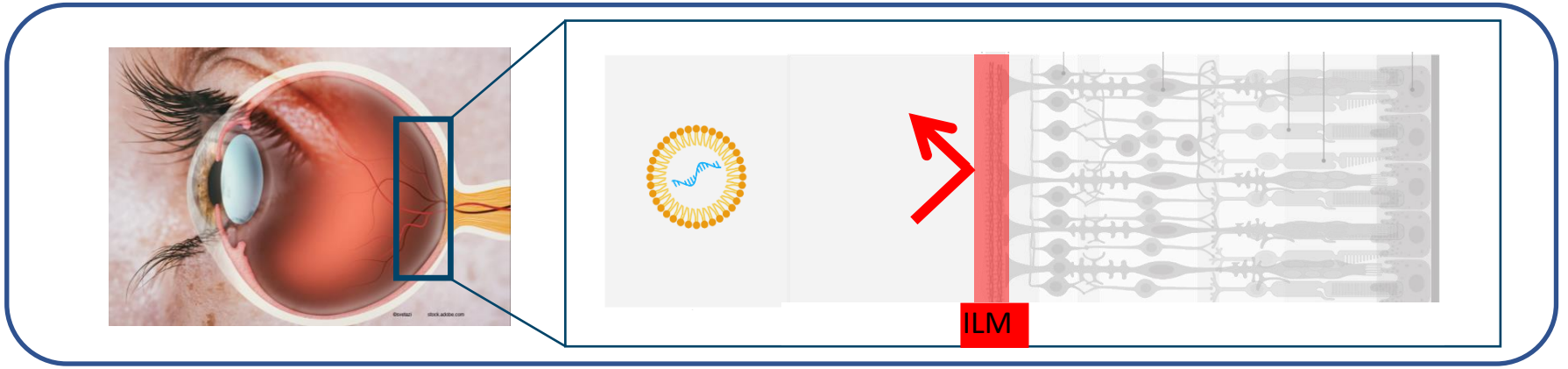


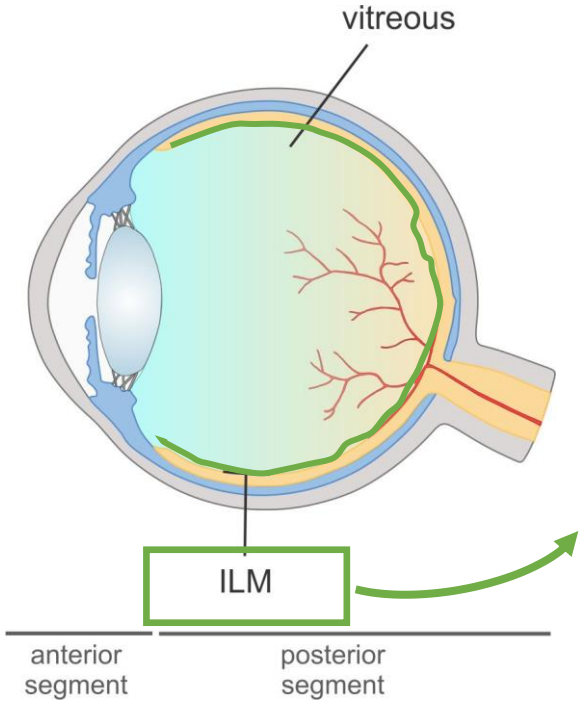
- Naked mRNA rapidly degraded
- LNPs give protection

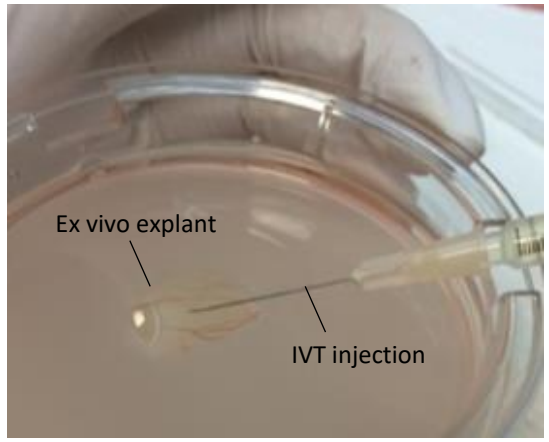
Zhang et al. 2020, Advanced Biosystems

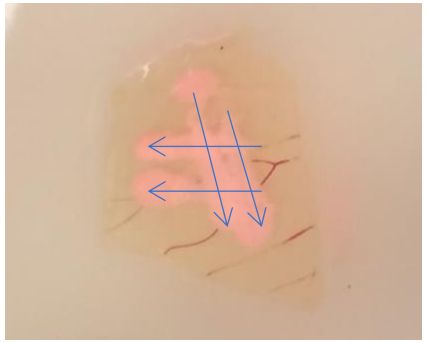
INNER LIMITING MEMBRANE

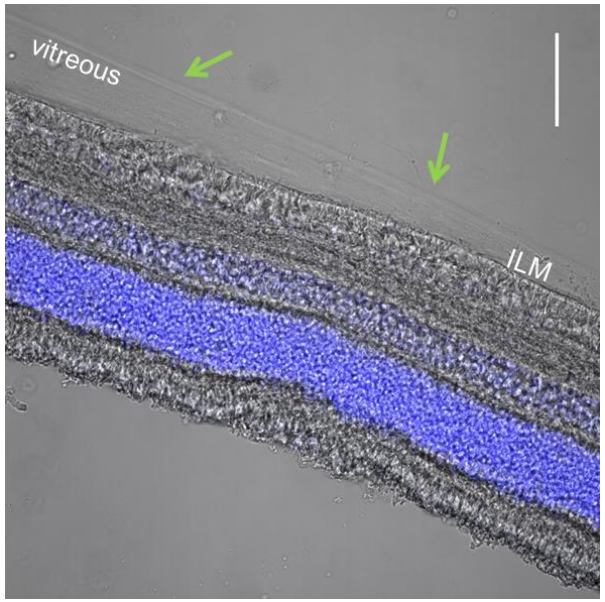
Peynshaert et al, Drug Delivery 2017,
Peynshaert et al, Adv. Drug Deliv. Rev. 2017,
Peynshaert et al, Curr Eye Res 2019,
Peynshaert et al, JCR 2022
De Clerck et al, Pharmaceutics 2022

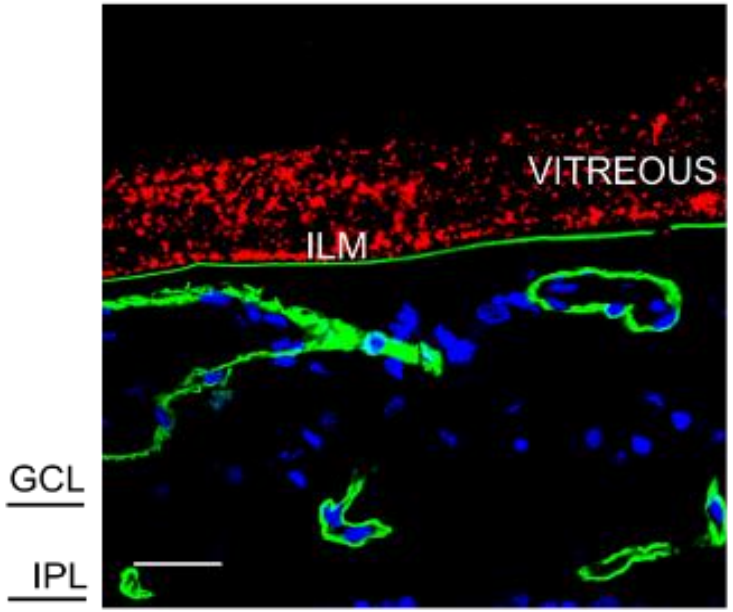






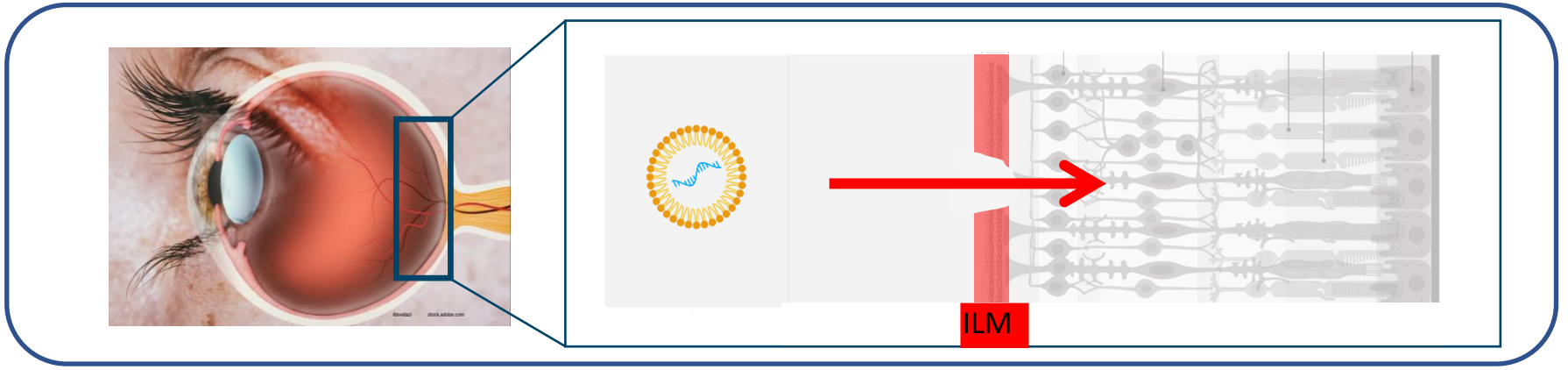






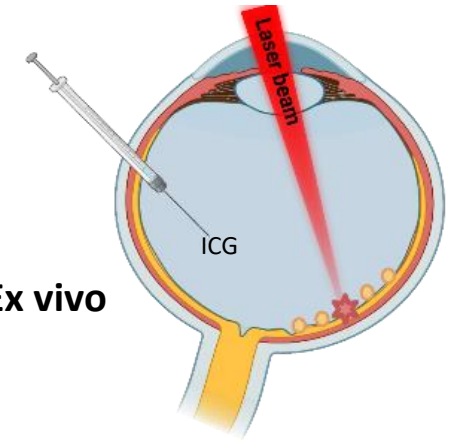
ILM huge barrier to LNP penetration

INNER LIMITING MEMBRANE



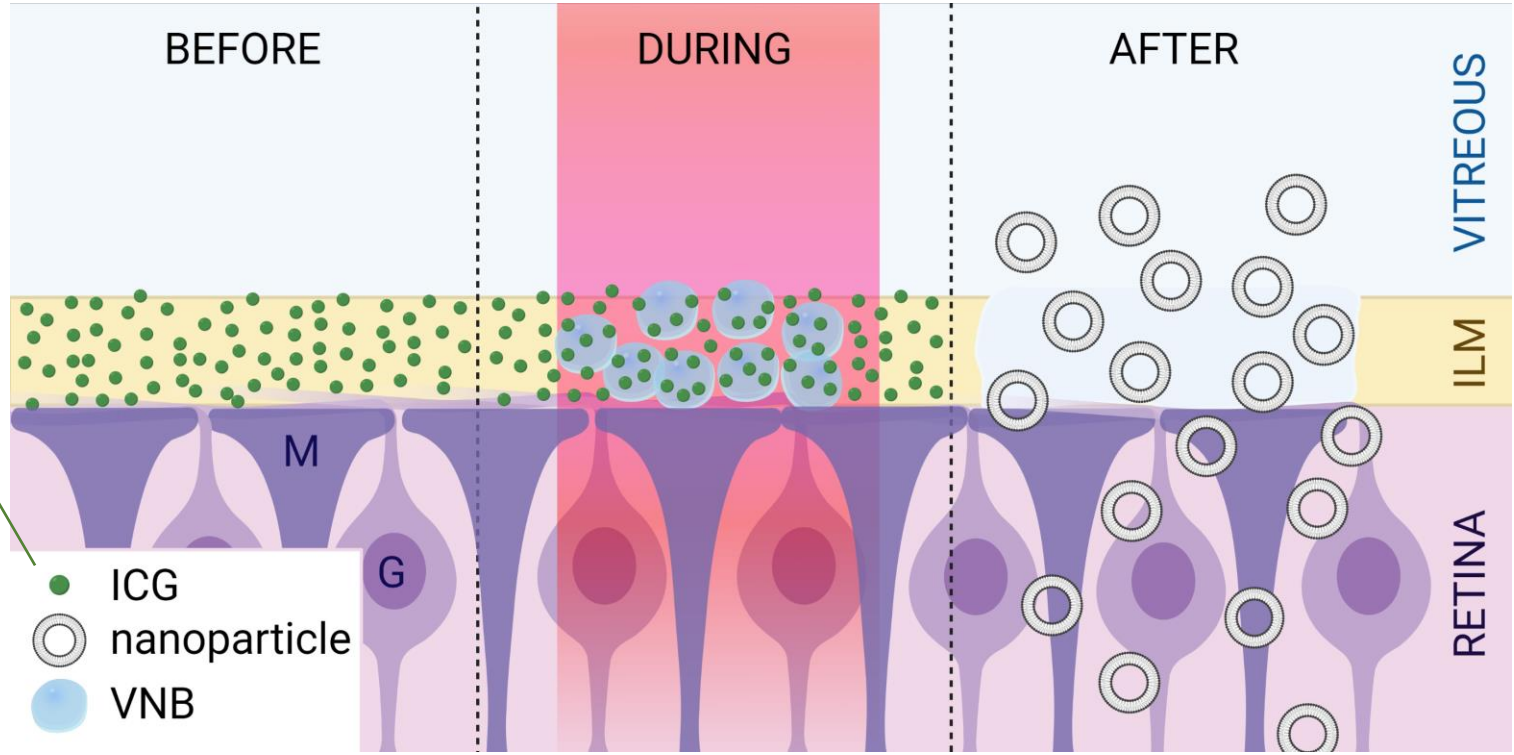
Peynshaert et al. 2022
Journal Controlled Release

ILM photoablation

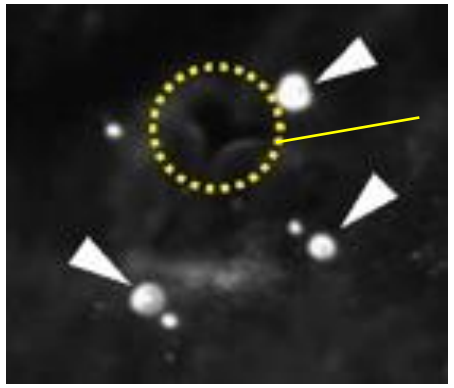


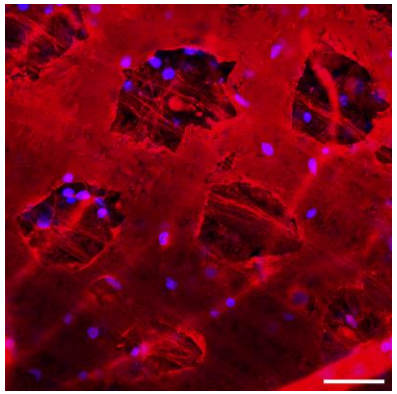
Ex vivo

FDA approved
Has affinity for ILM
Absorbs in NIR



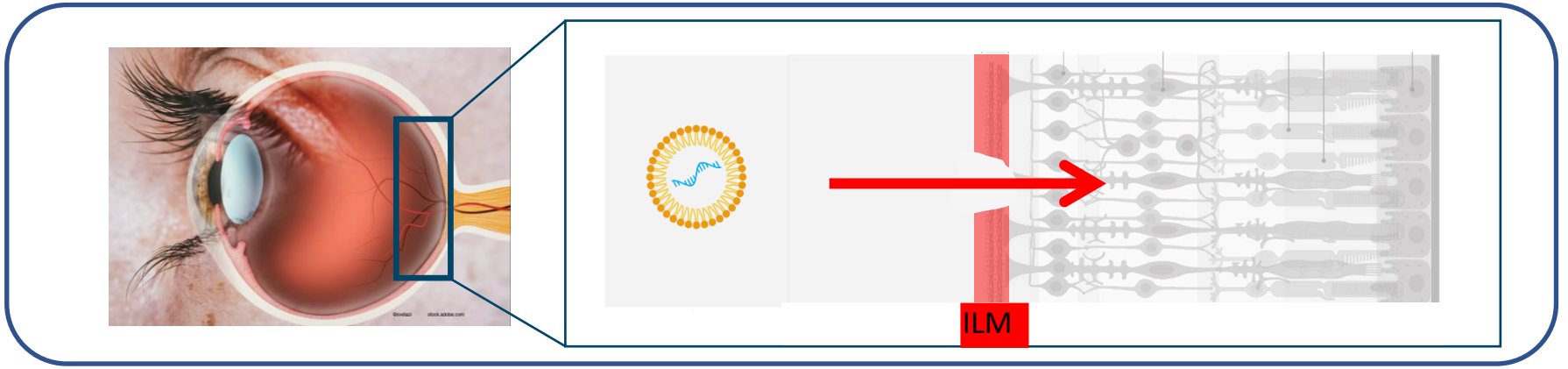
ILM pore formation



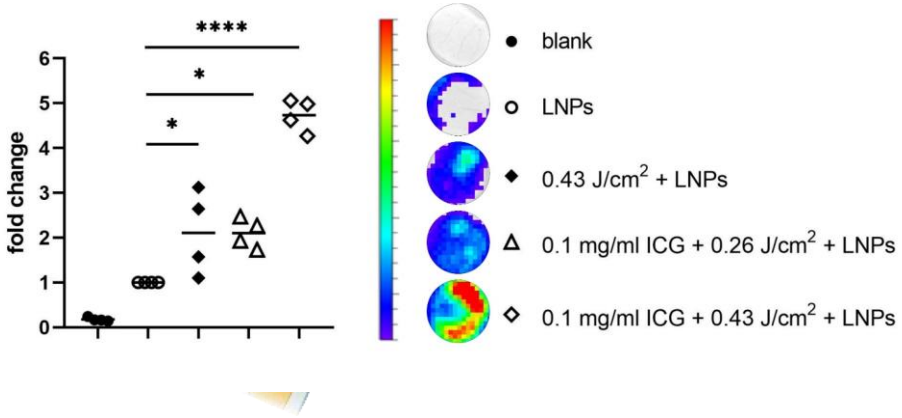


INNER LIMITING MEMBRANE

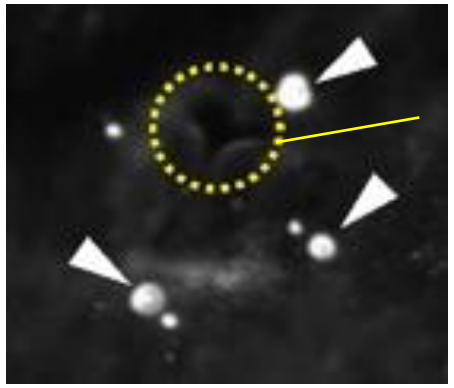
Peynshaert et al. 2022
Journal Controlled Release

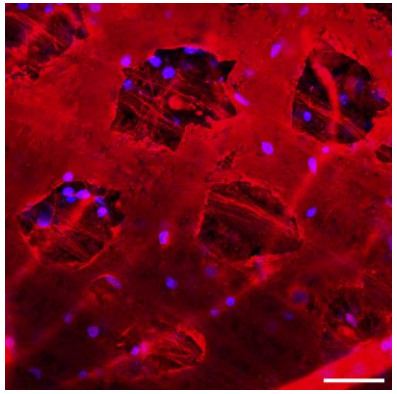


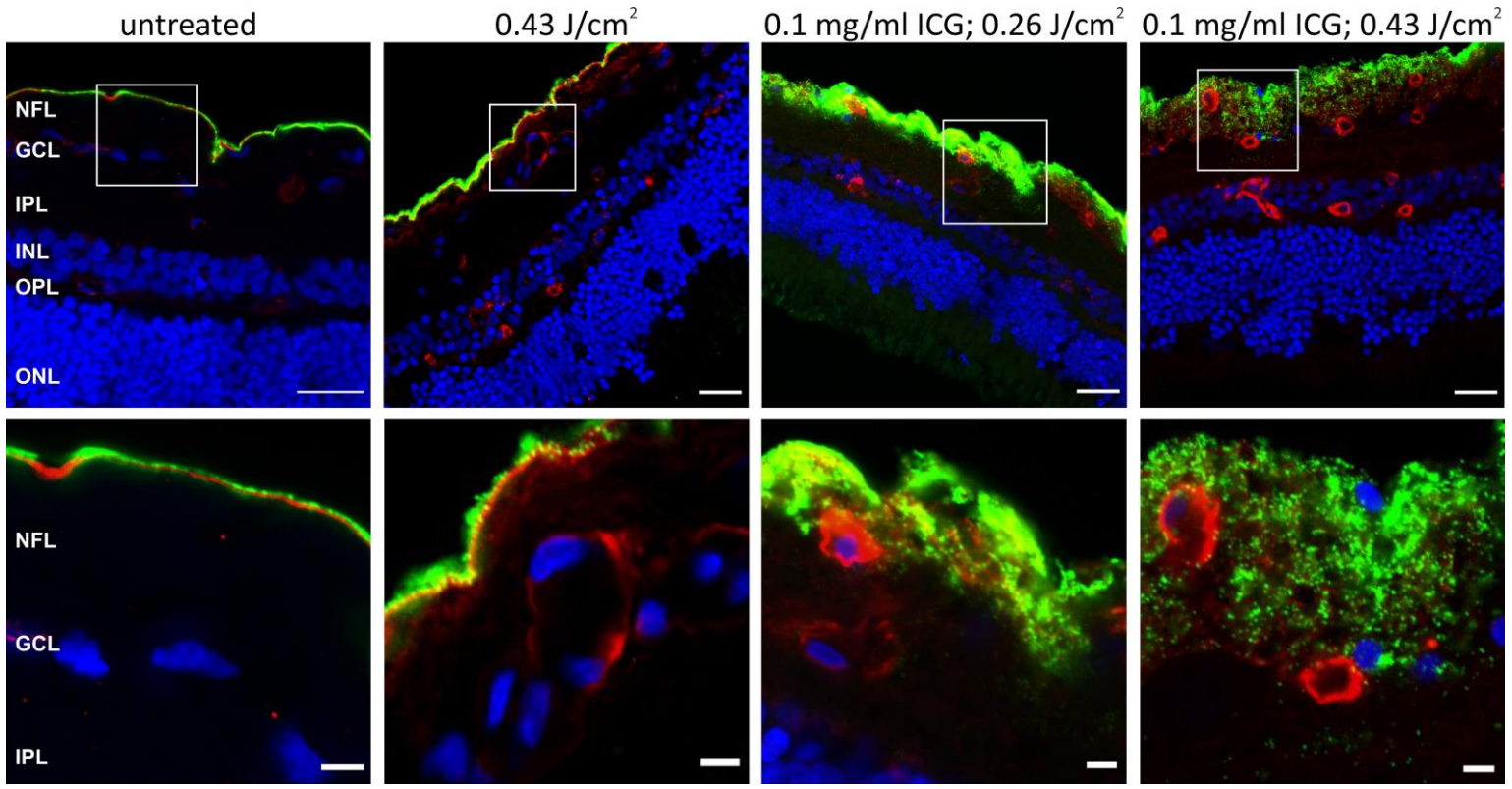
ILM photoablation



ILM pore formation







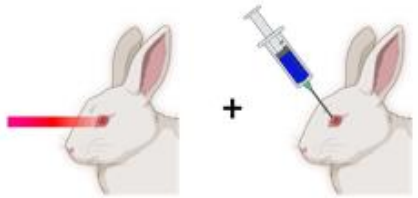
ILM
100nm Fluospheres (PEG)
nuclei

INNER LIMITING MEMBRANE

In vivo unpublished

Photoporation as promising technique to perforate the ILM

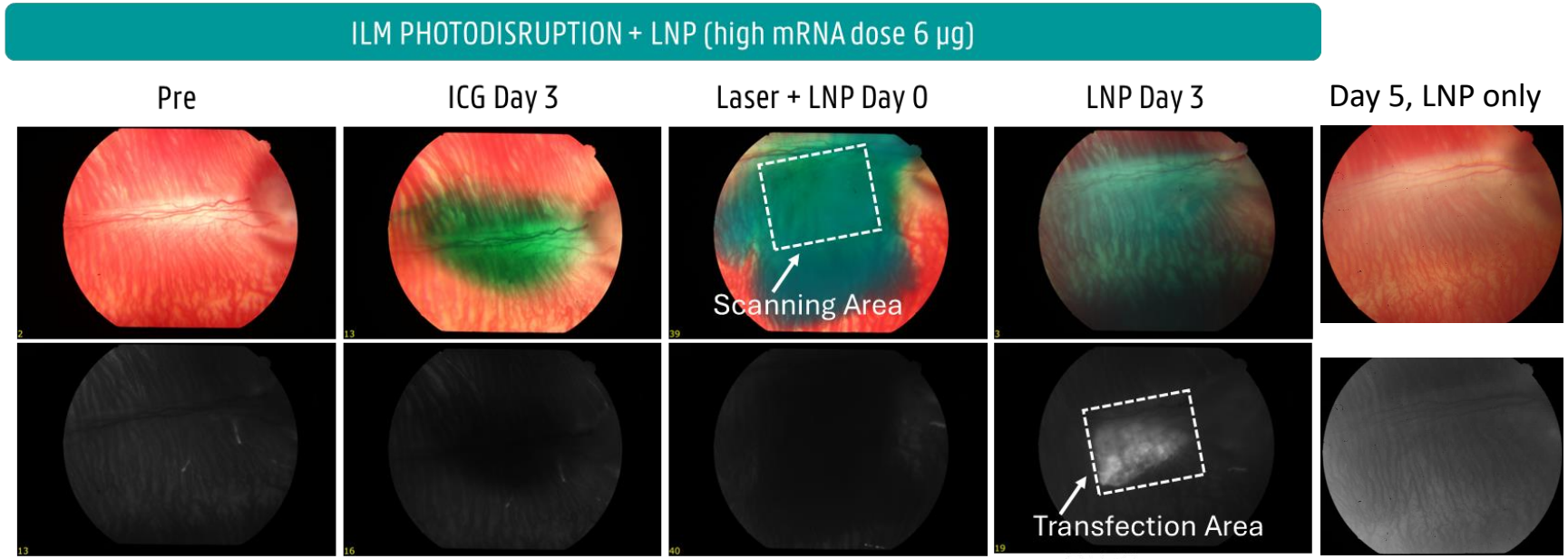
DAY 0: ICG injection

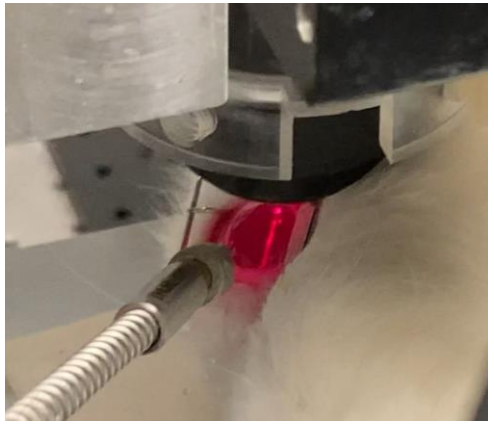


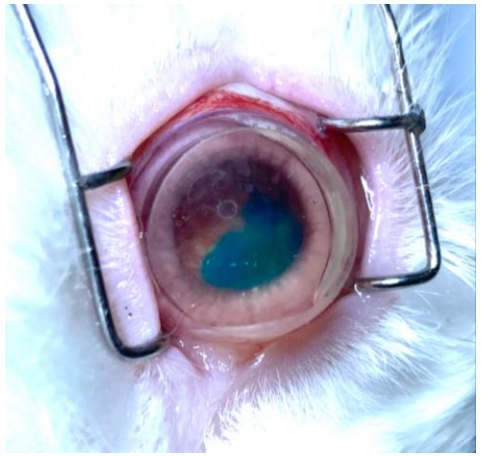
DAY 3: laser treatment + AAV/ LNP injection

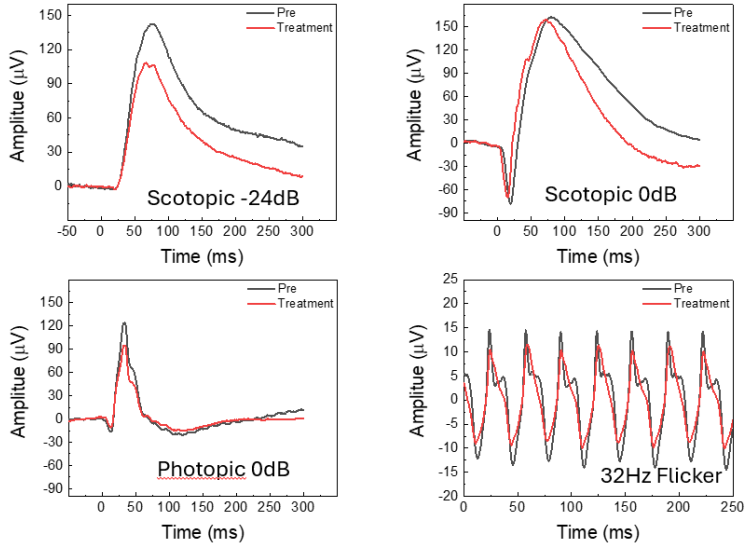
Color fundus

Fluorescent imaging
(GFP expression)







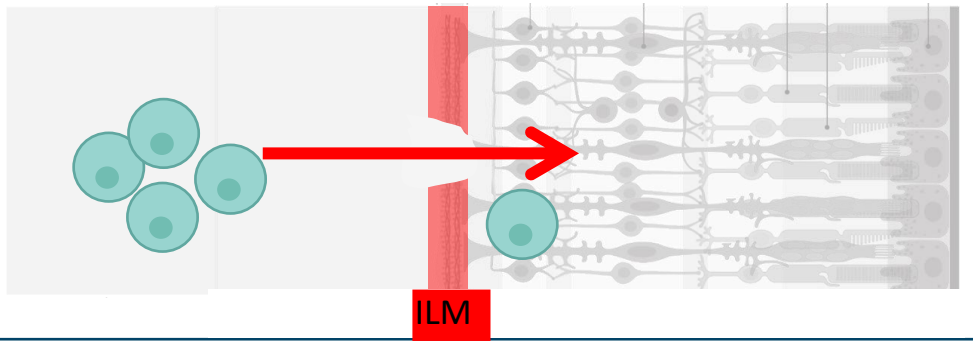


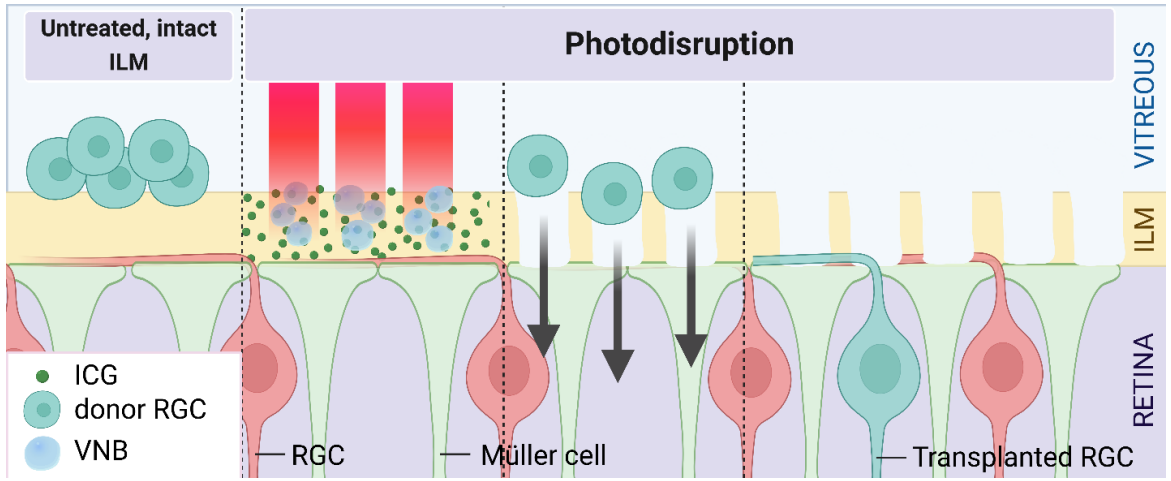
KELLOGG EYE CENTER
MICHIGAN MEDICINE

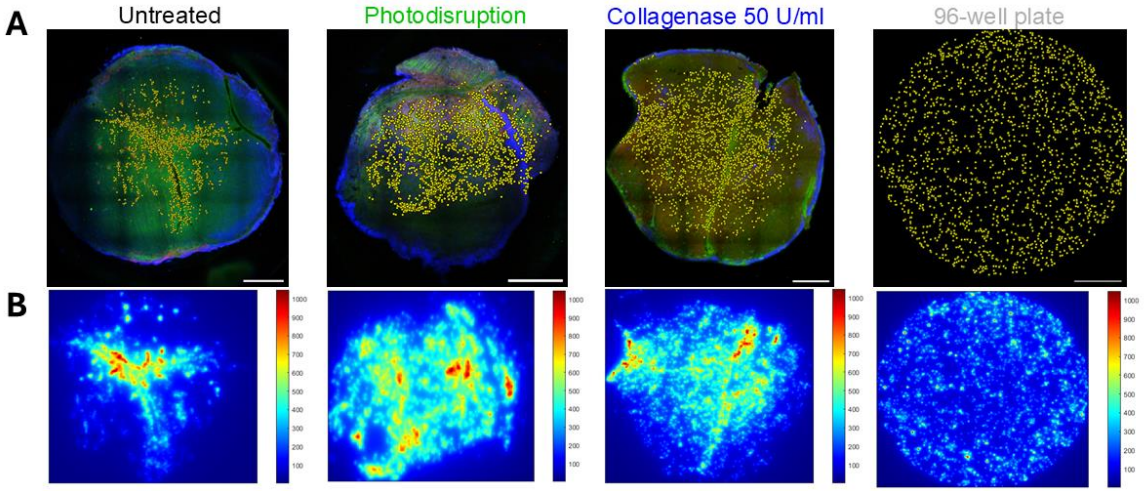
Prof. Yannis Paulus

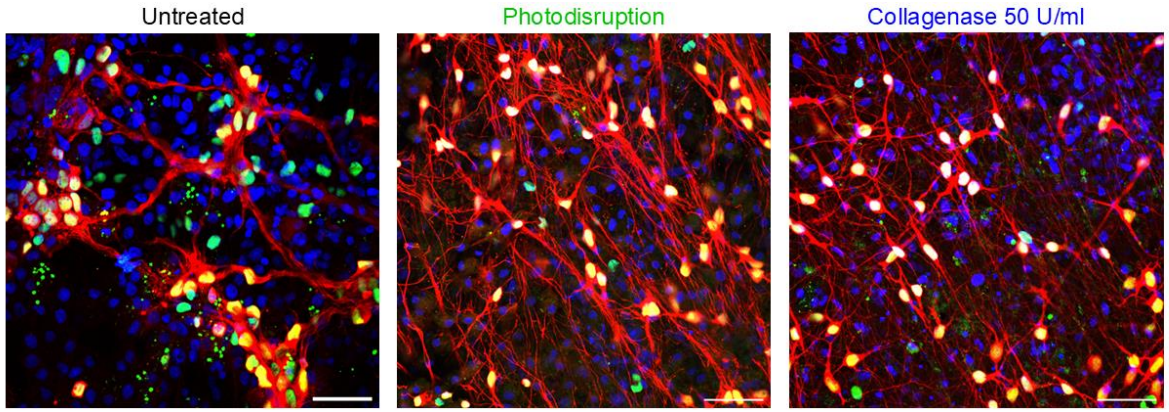
INNER LIMITING MEMBRANE

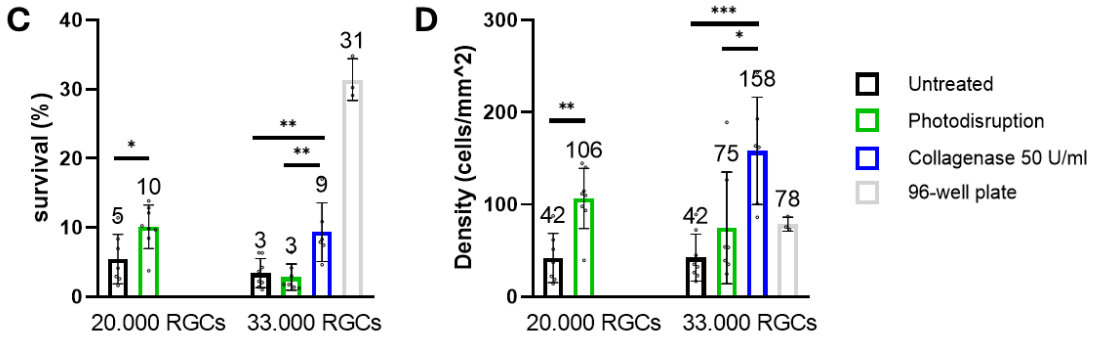
De Coster E et al. 2025
BioRXiv
https://doi.org/10.1101/2025.04.14.648093







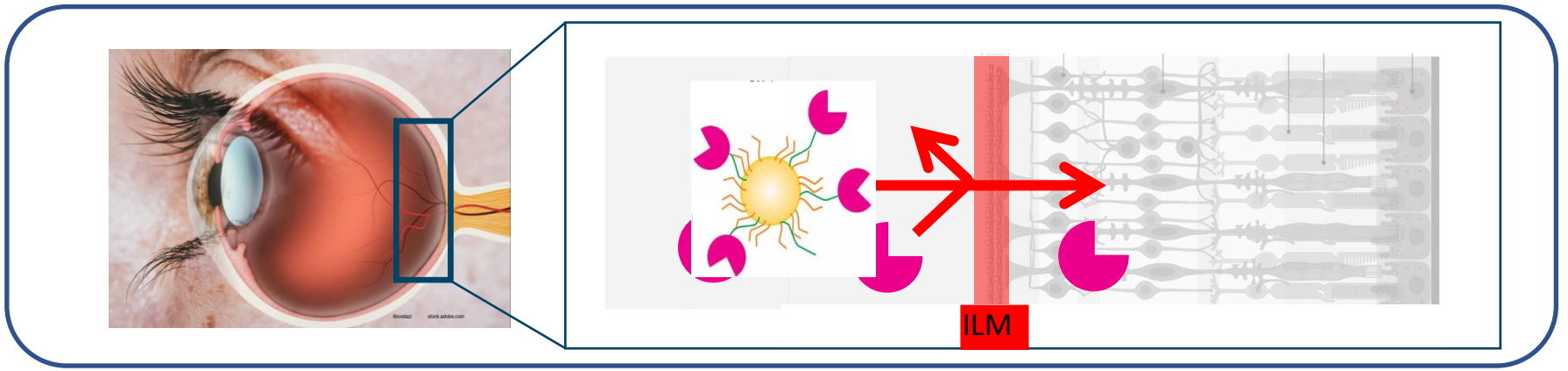


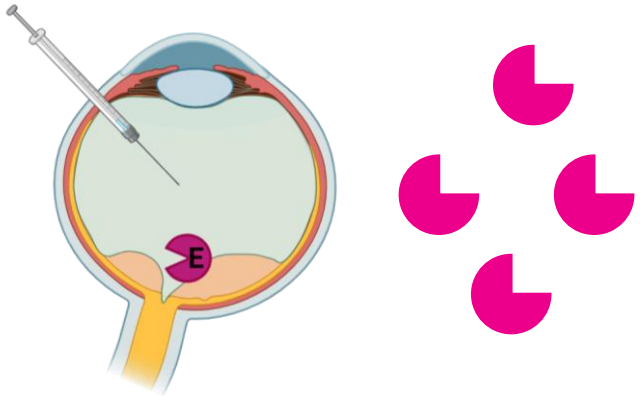


INNER LIMITING MEMBRANE

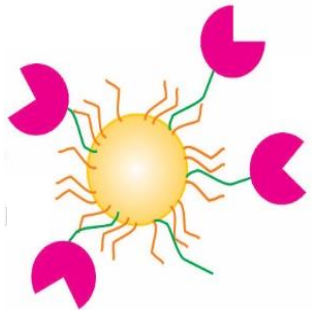
Minnaert et al, 2025
Journal Controlled Release

Turning the barrier into an advantage!

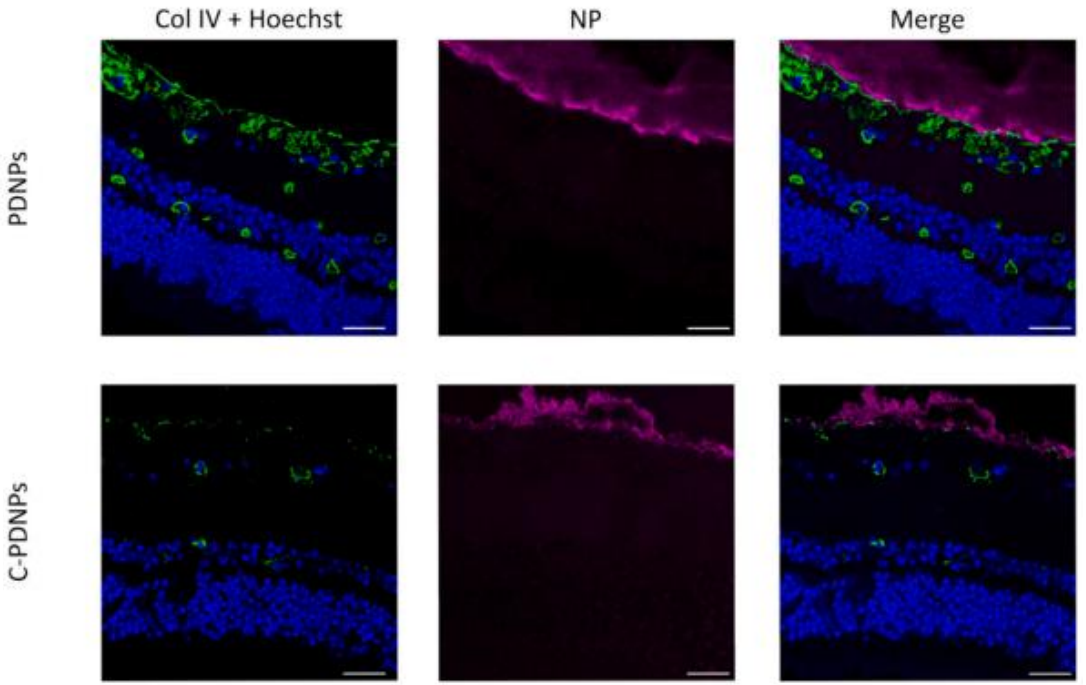


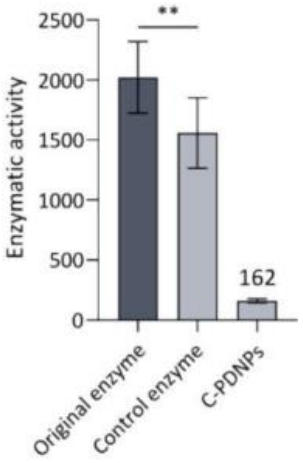


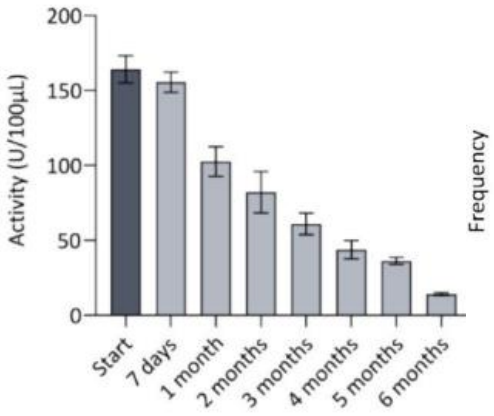
Free collagenase → toxic to the retina

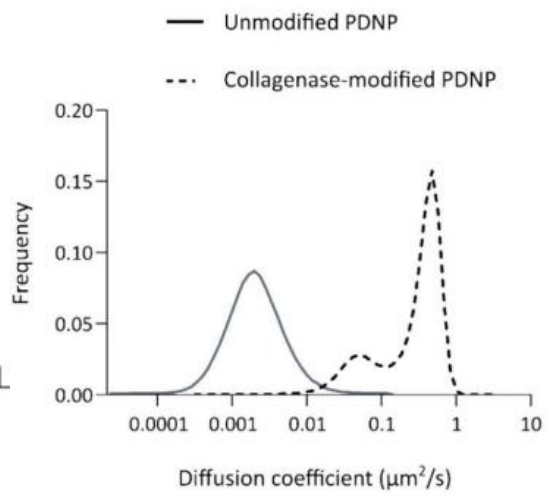


Collagenase coupled to nanoparticles
→ prevent penetration to the retina









INNER LIMITING MEMBRANE

Minnaert et al, 2025
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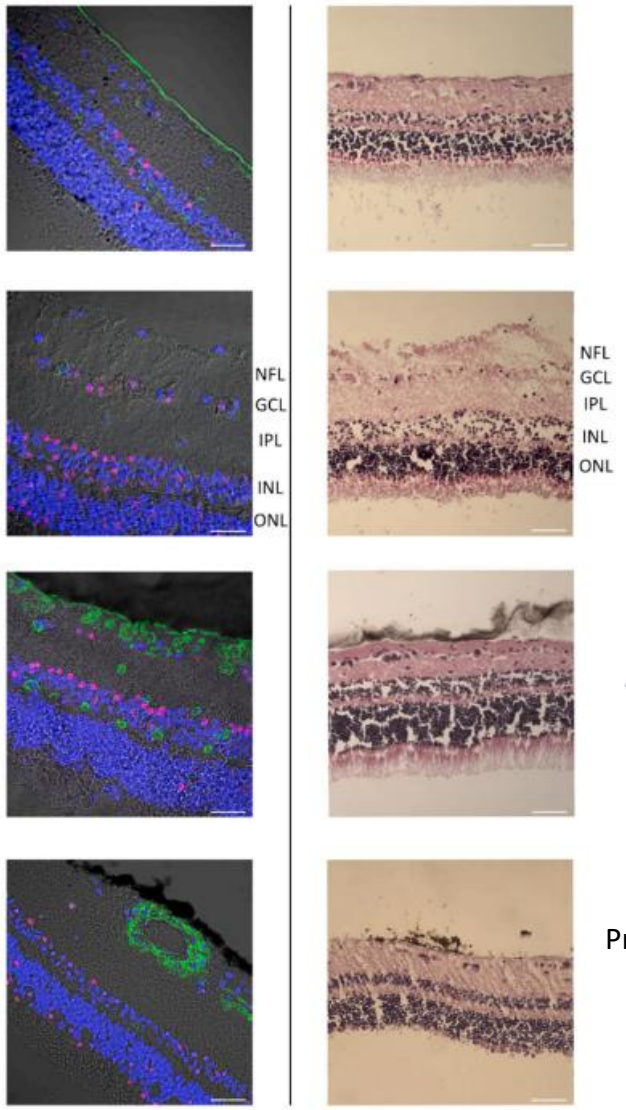
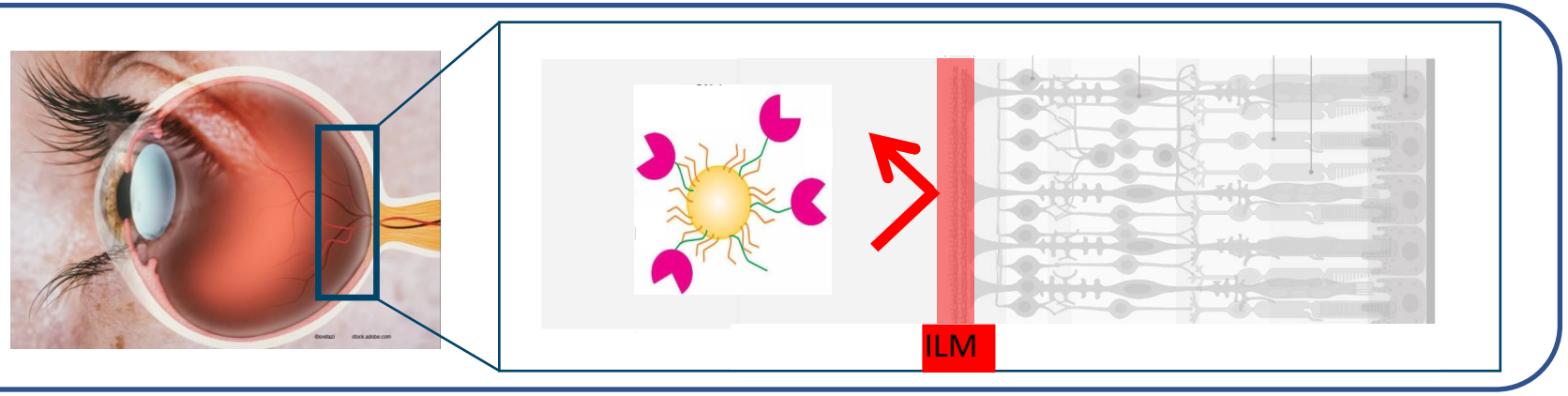
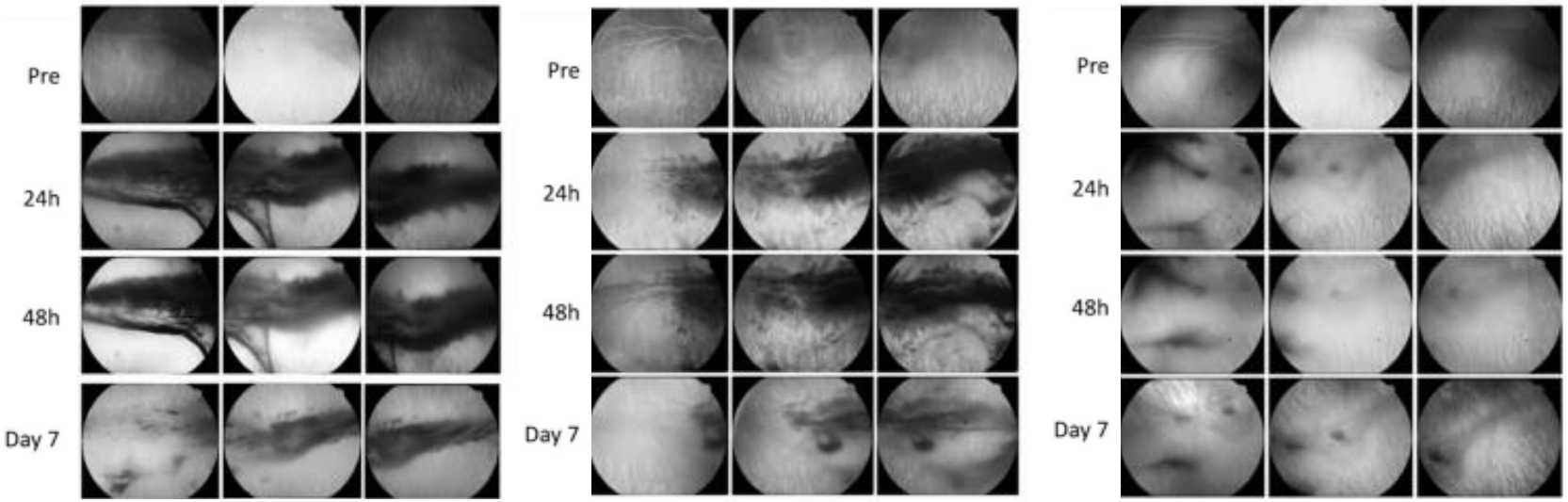
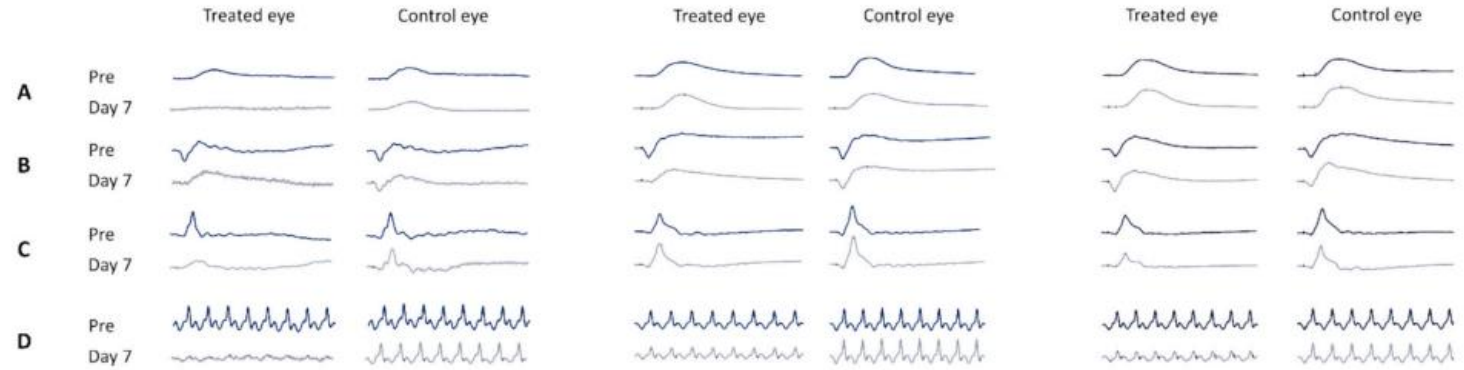
Turning the barrier into an advantage!



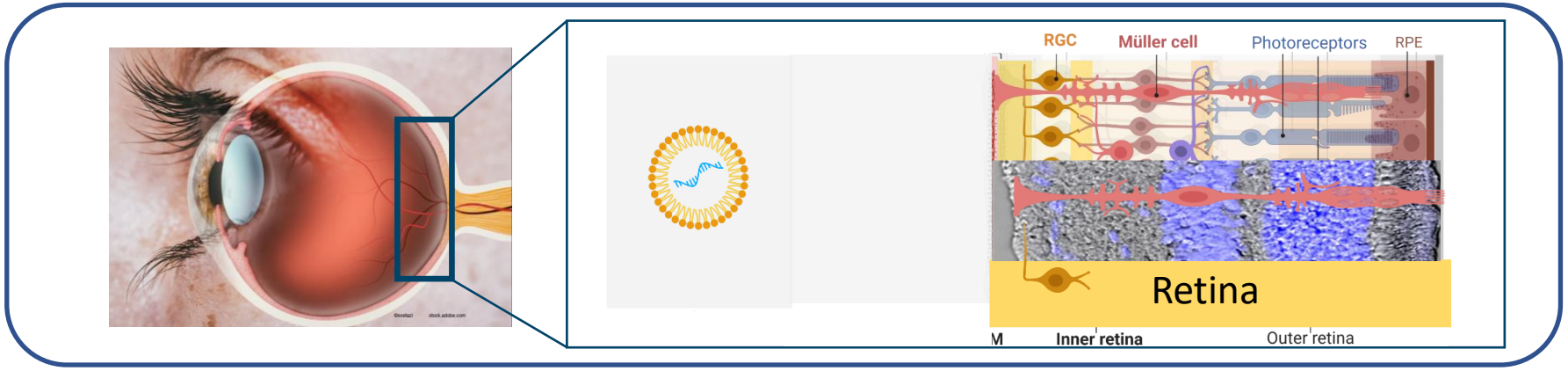
Prof. Yannis Paulus

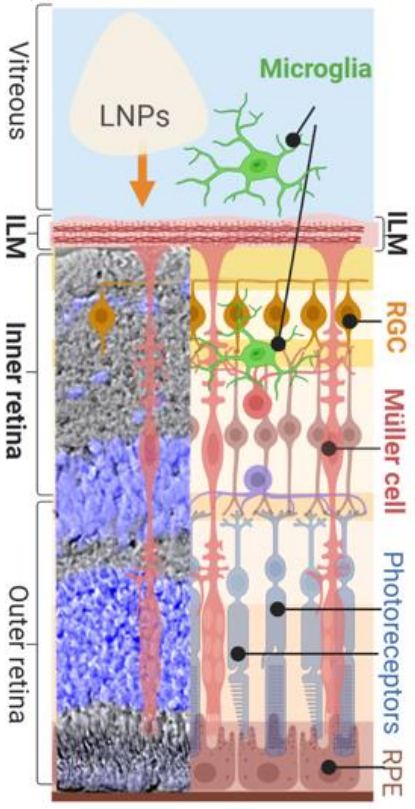




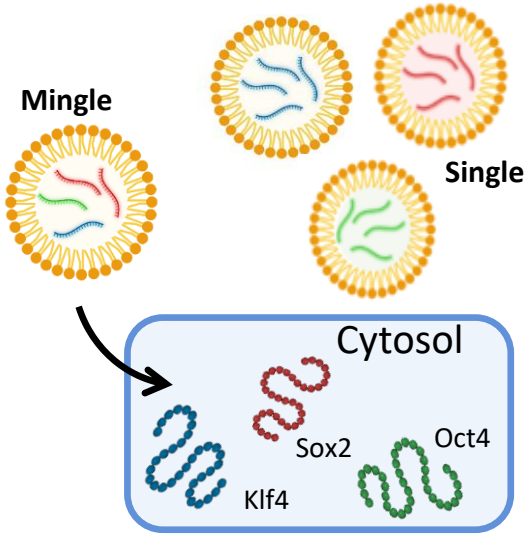


RETINA

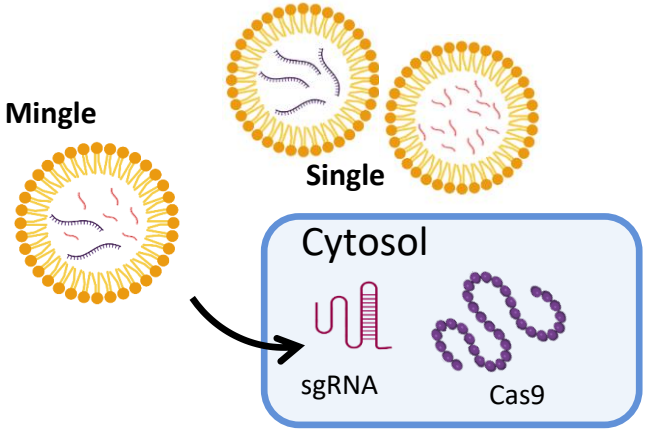




Cell reprogramming

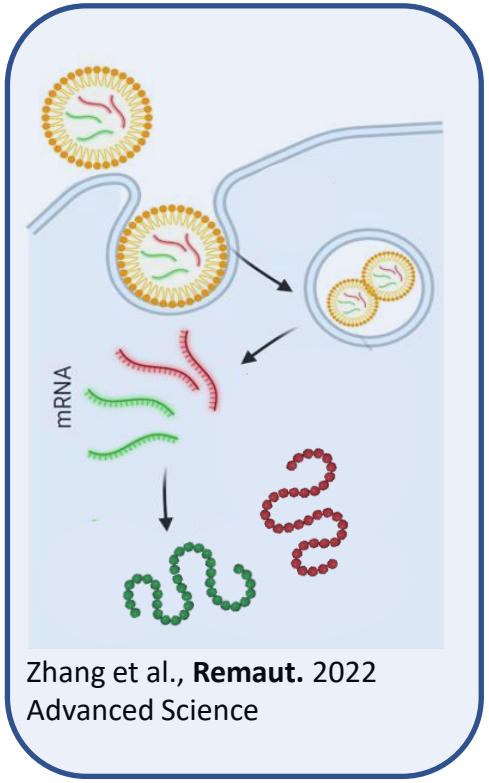


Genome editing



Co-delivery of different RNA molecules

RETINA



Zhang et al., **Remaut.** 2022
Advanced Science

MINGLE

mRNA1
+
mRNA2

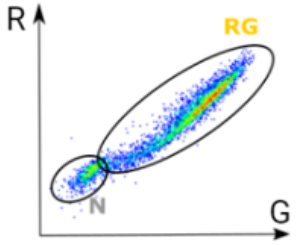
equal mRNA dose

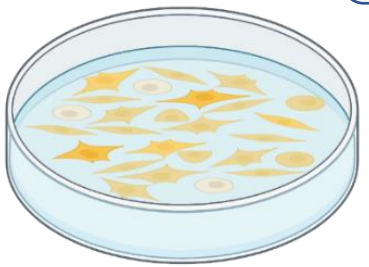
SINGLE

mRNA1 mRNA2

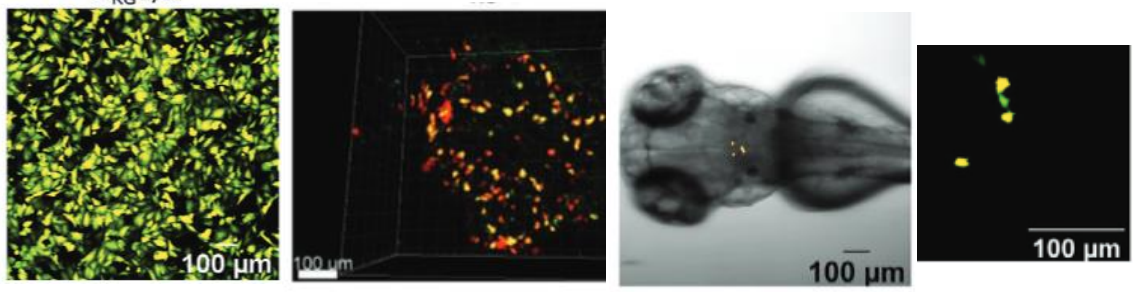
at **SINGLE CELL** level

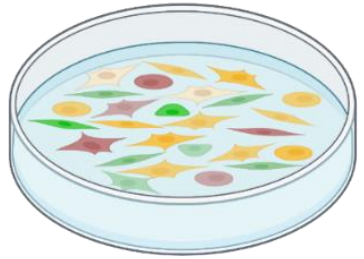
R^2=0.95



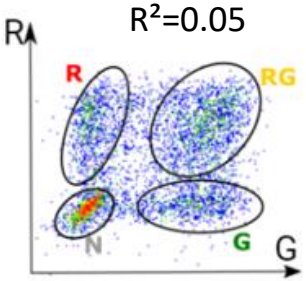


Well correlated expression of green and red protein in **each cell**

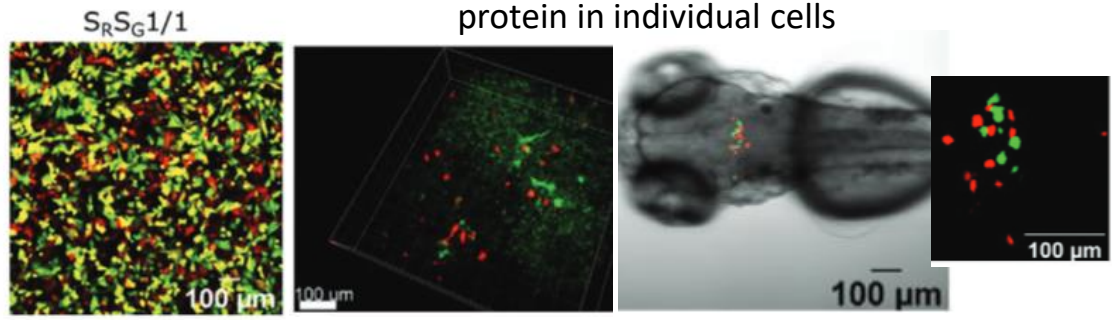




R^2=0.05

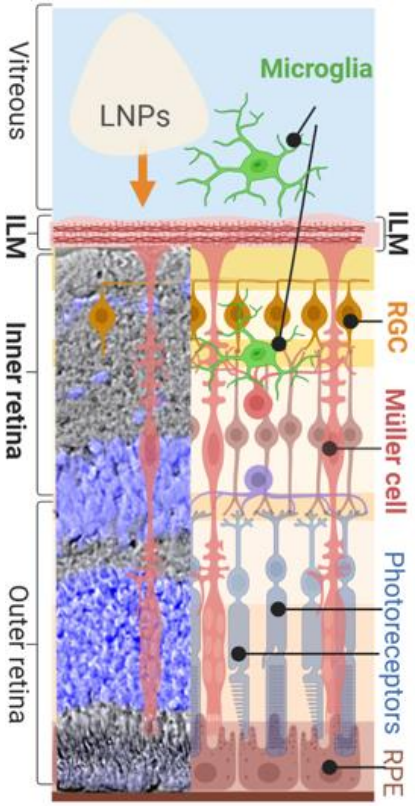


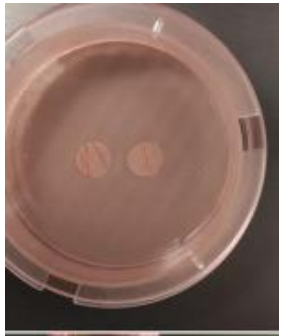
Highly random expression of green and/or red protein in individual cells

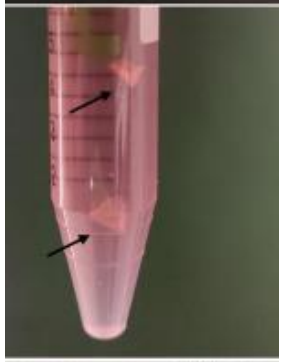


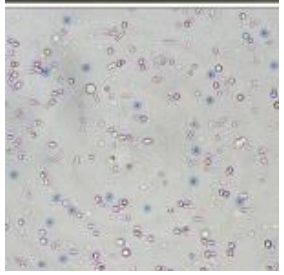
Co-delivery of different RNA molecules → co-encapsulation!

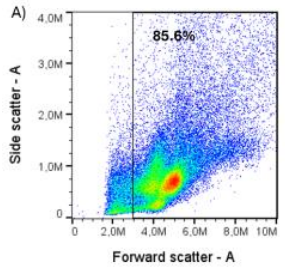
RETINAL EXPLANTS











Retinal explant

↓

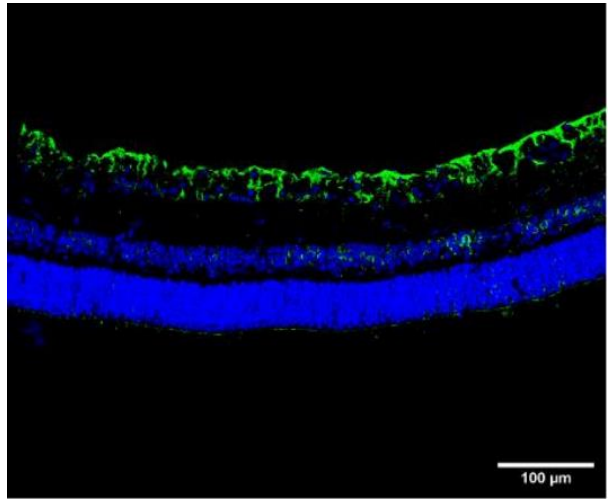
Enzymatic digestion

↓

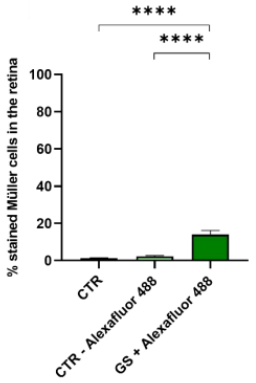
Cell suspension

↓

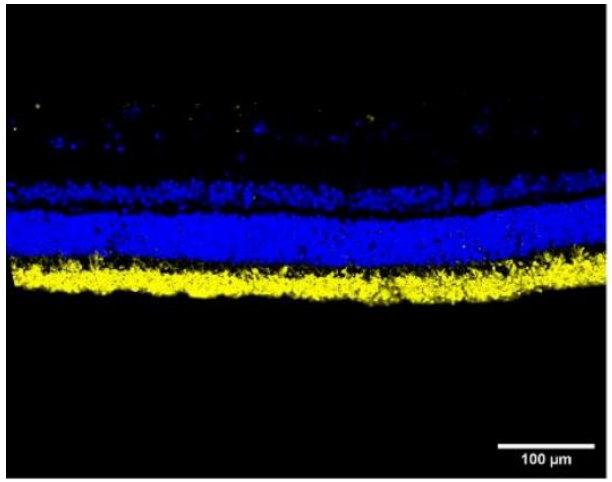
Flow cytometry



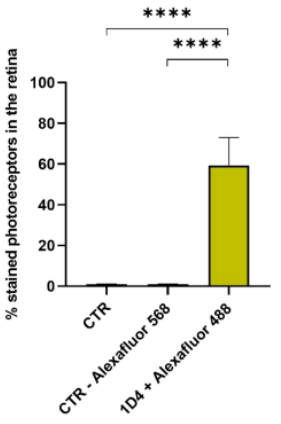
Muller cell staining
(GS: glutamine synthetase)



16% of digested retina are Muller cells



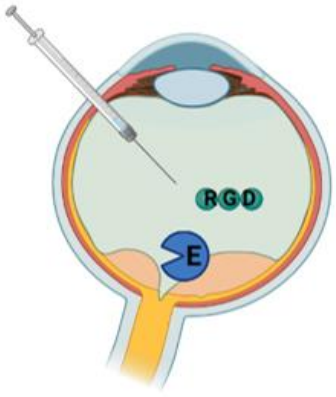
Photoreceptor staining
(1D4: rhodopsin)



60% of digested retina are PRs

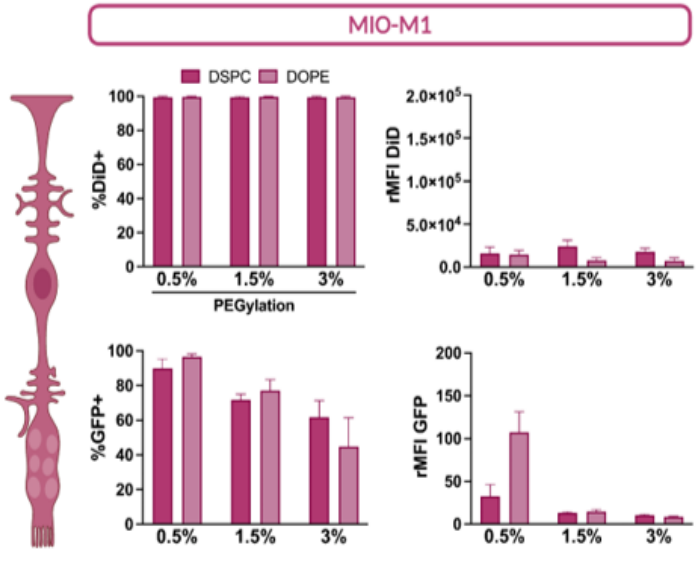
Retinal cell targeting

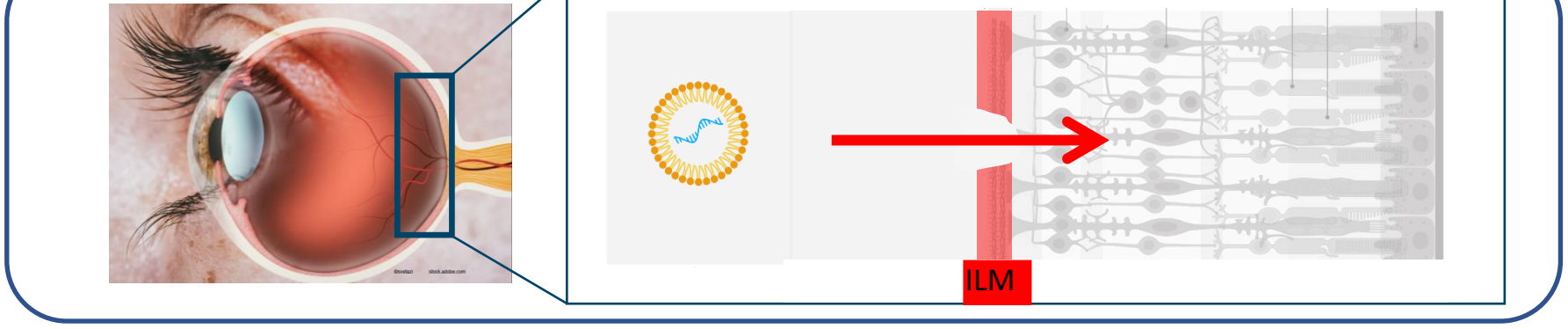
RETINAL EXPLANTS



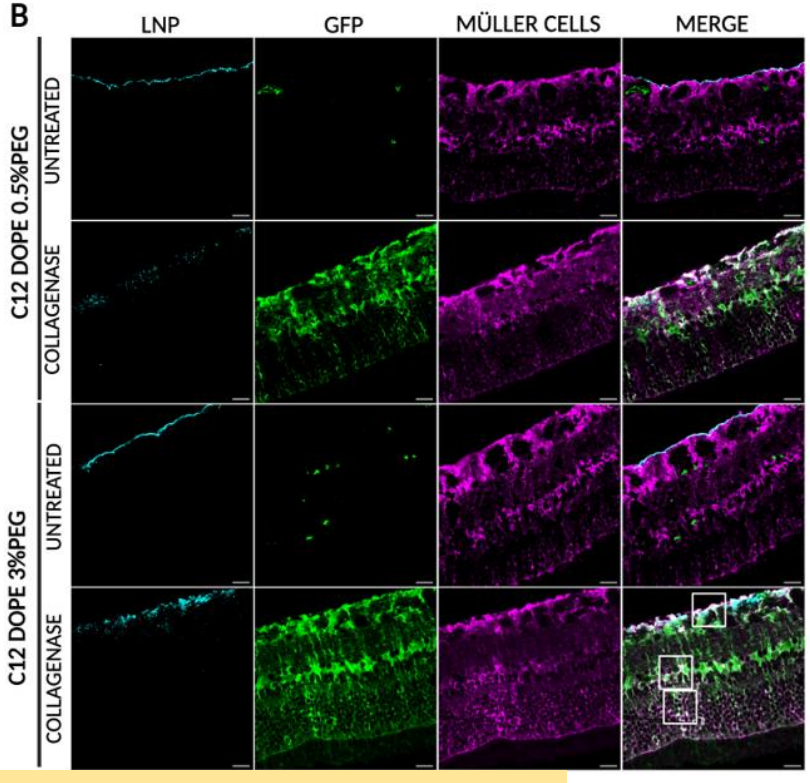
Vitreolytic strategies

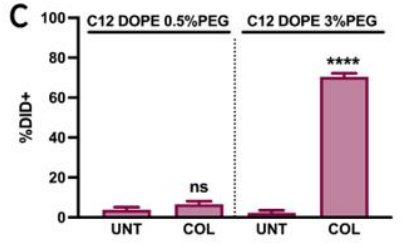
SIZE!

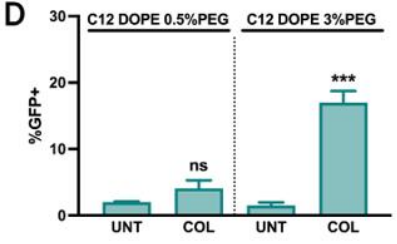


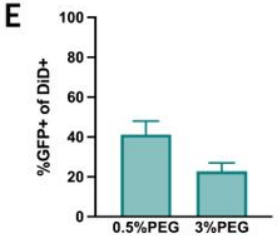


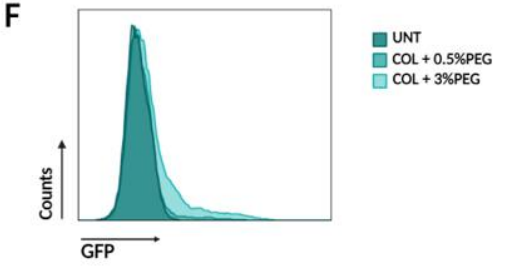
Kaat De Clerck, unpublished

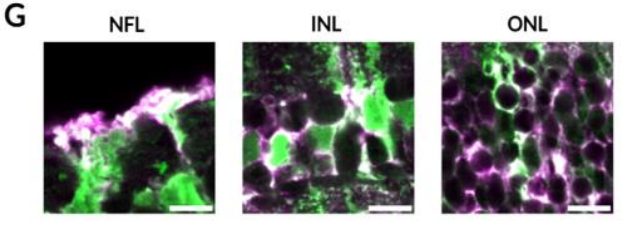






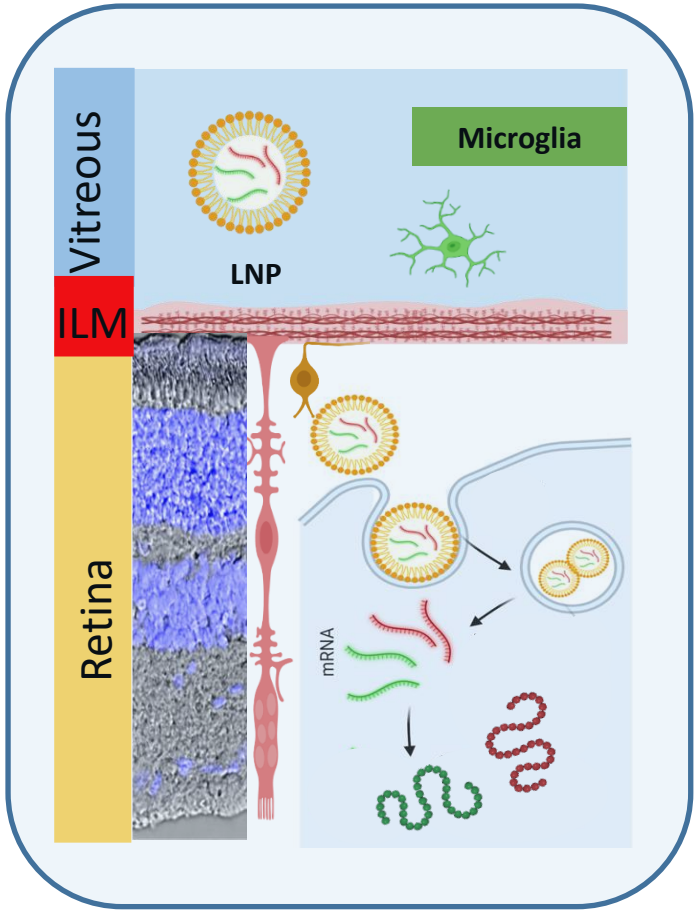






In vitro behavior different from ex vivo LNP behavior

CONCLUSIONS



- ☑ Ex vivo models available for intravitreal diffusion measurements and penetration of NPs over the ILM
- ☑ Applicable to all sorts of fluorescent materials: NPs, antibodies, nucleic acids, …
- Vitreous is a diffusion barrier for positively charged particles
- ☑ ILM is a penetration barrier for many NPs
- Age, disease and species dependent
- ☑ Intravitreal injection of LNPs is promising for retinal mRNA delivery
- ☑ Expression currently limited to optical nerve head and few Müller glia
- ☑ Innate immunity should be overcome & ILM penetration improved
- ☑ Diffusion in the retina and targeting the correct retinal cell type will remain serious hurdle as well
- ☑ Co-delivery will require additional formulation requirements

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