# Nanoparticles for Transmucosal Delivery of CRISPR-based Gene Editing Tools in the Lung

Belal Tafech

Supervisor: Dr. Sarah Hedtrich

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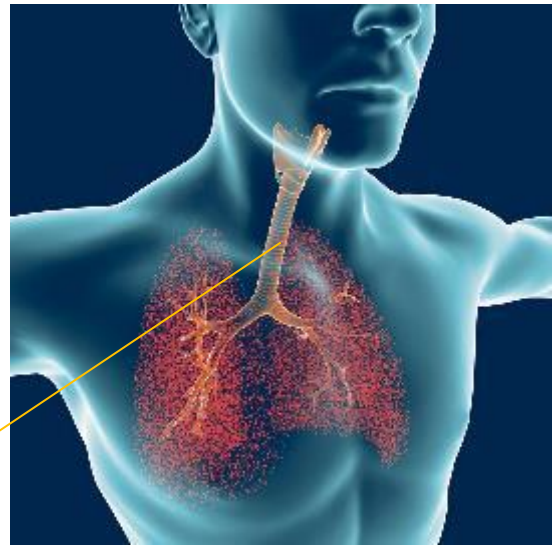
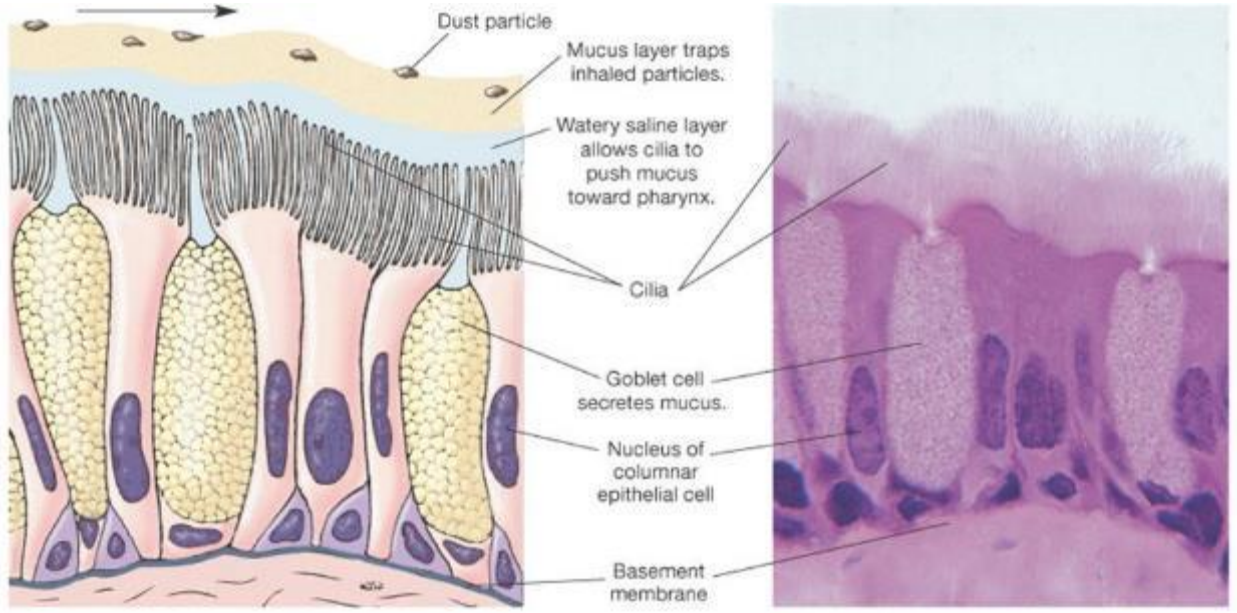
# Background: The airway mucus layer

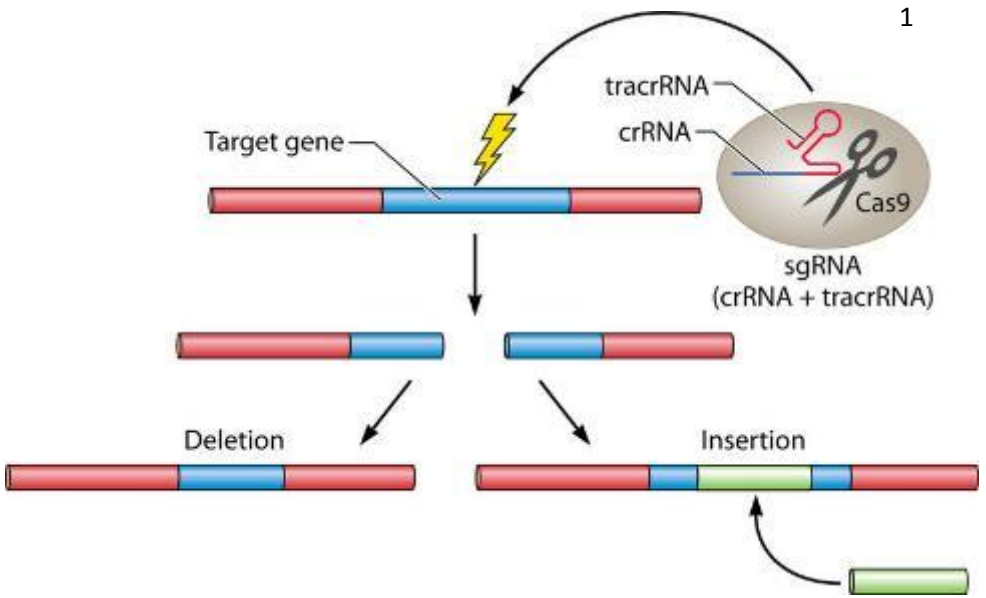
- A thin layer of fluid covering the lung epithelium
- Traps inhaled particles from penetrating into the epithelium
- Subdues delivery of promising therapeutics for pulmonary diseases
    - Cystic Fibrosis





# A promising therapy for Cystic Fibrosis

- Cystic Fibrosis (CF)
  - Monogenetic disorder
  - CFTR gene
  - Current medication
  - Median age of death is 33yrs in Canada
- CRISPR-Cas9: A revolutionary therapy



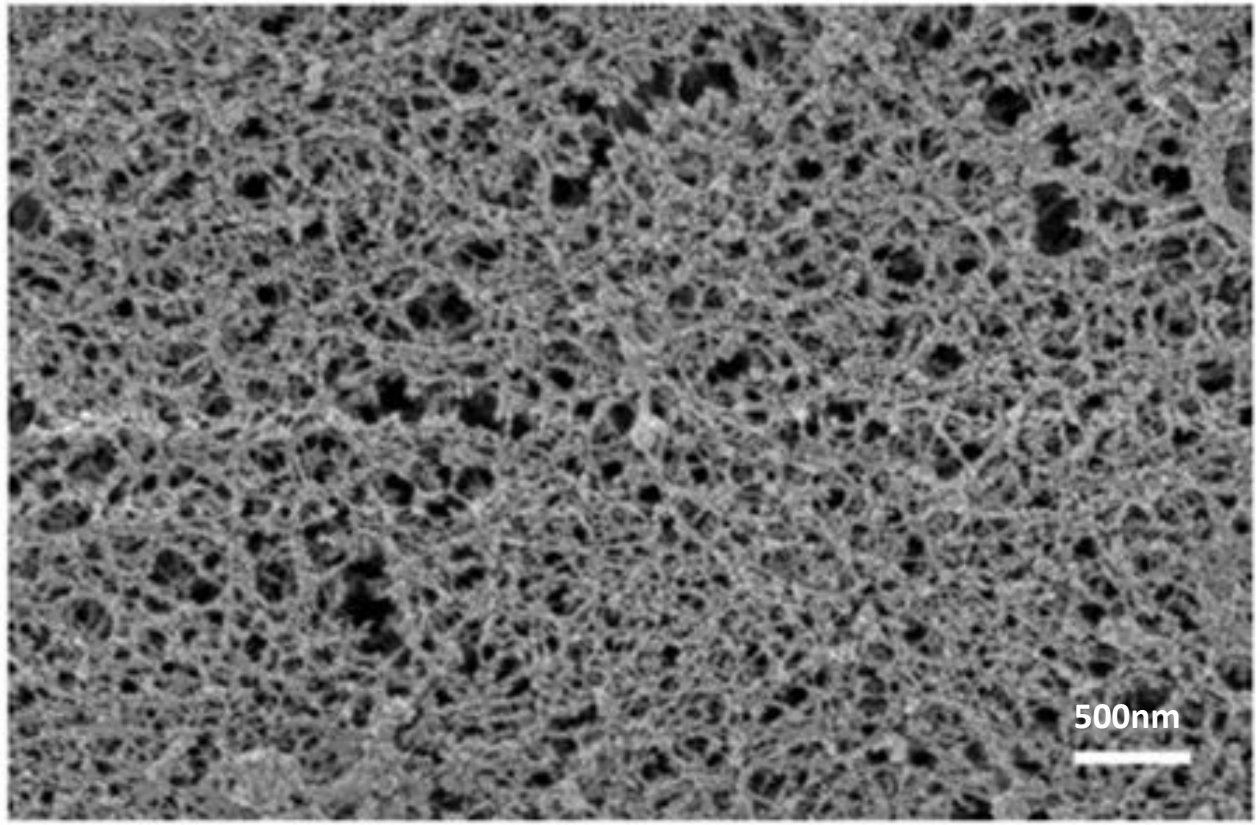

**The challenge remains in efficiently delivering genetic cargo across the mucus**



[1] Ishino, Yoshizumi & Krupovic, Mart & Forterre, Patrick. (2018). History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. Journal of Bacteriology. 200.

# Airway mucus barrier mechanisms

- **Physical barrier**
  - Mucin polymers and other mucus contents form a porous network



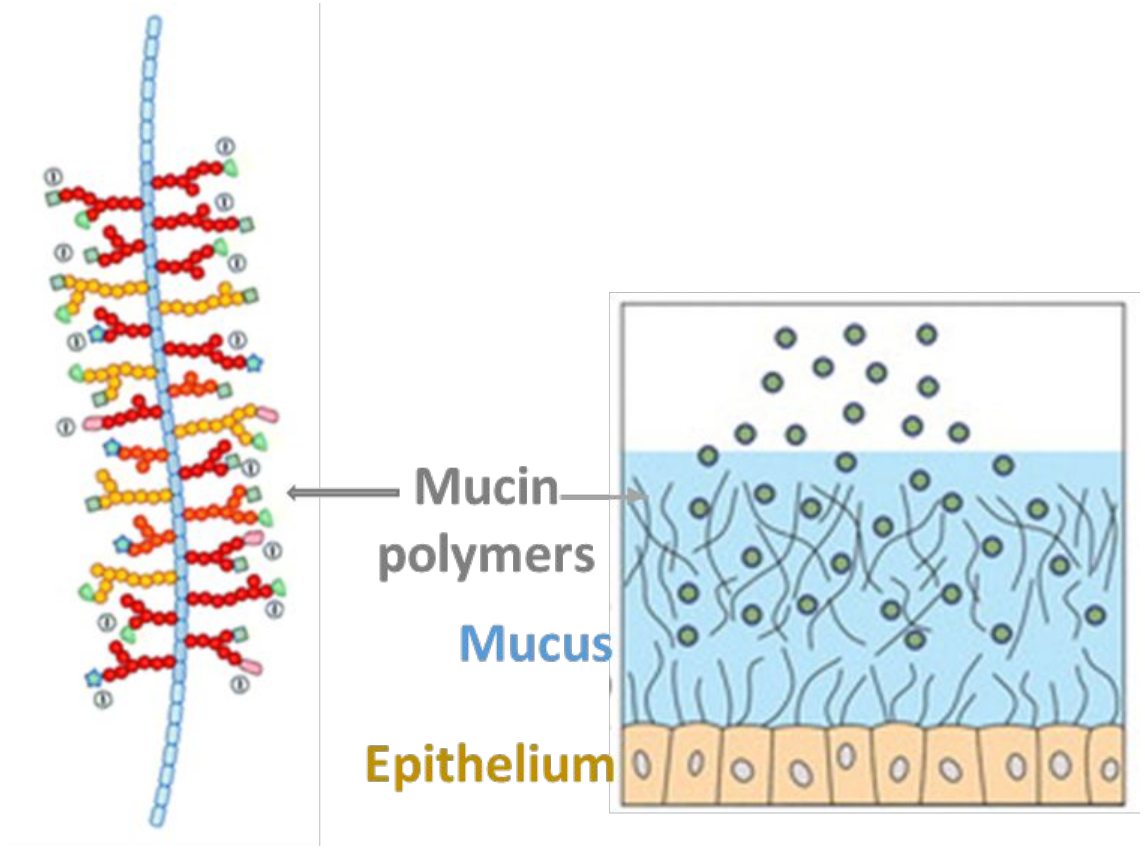

Average pore size

- Healthy: 200nm
- CF: < 150nm

Schuster BS, Suk JS, Woodworth GF, Hanes J. Nanoparticle diffusion in respiratory mucus from humans without lung disease. Biomaterials. 2013;34(13):3439-3446. doi:10.1016/j.biomaterials.2013.01.064

# Airway mucus barrier mechanisms

- **Biochemical barrier**
  - Glycan chains that terminate in negatively charged residues such as sialic acid (green)






Petrou G & Crouzier T. Biomater. Mucins as multifunctional building blocks of biomaterials. Sci. 2018(6): 2282-2297

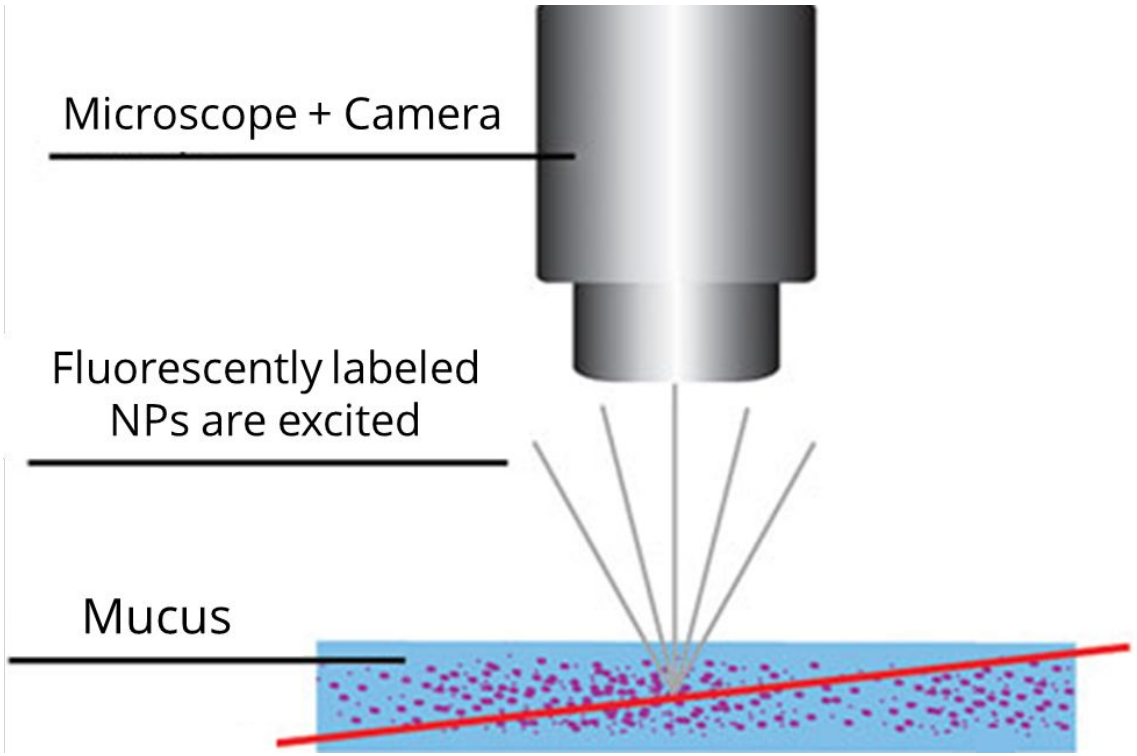
# Overall Research Objectives

Research goals:

1. Determining nanoparticle (NP) characteristics for efficient transmucosal gene delivery in healthy and disease states of the lung

2. Assessing NPs uptake and gene editing efficacy in primary lung epithelial cells

# Nanoparticle Tracking Analysis (NTA)



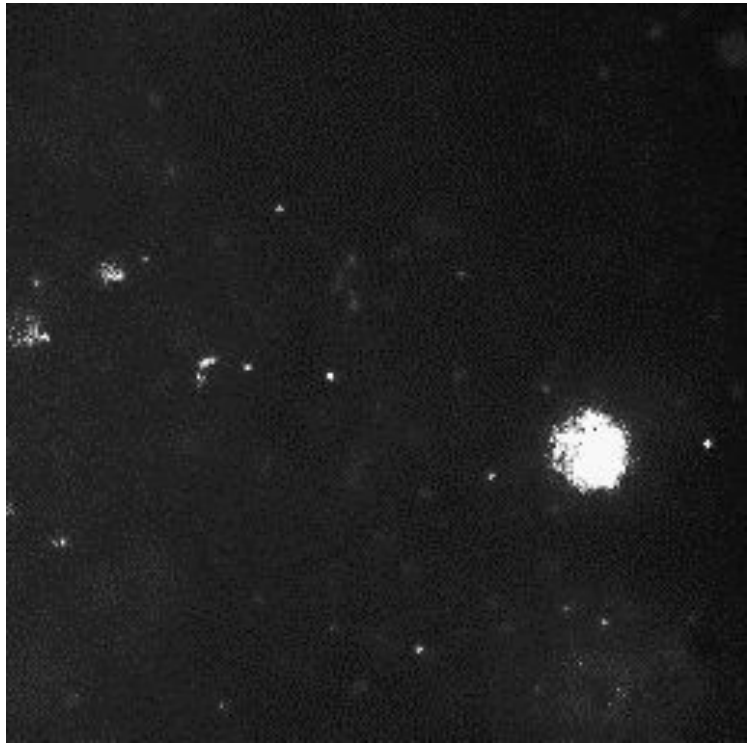

- **The NTA method relies on capturing videos of live NP movement in mucus**
- **The videos recorded are further analyzed using computer software to obtain median diffusion coefficients of NPs in mucus**

# Assessing NP + gene editing cargo diffusivity in mucus

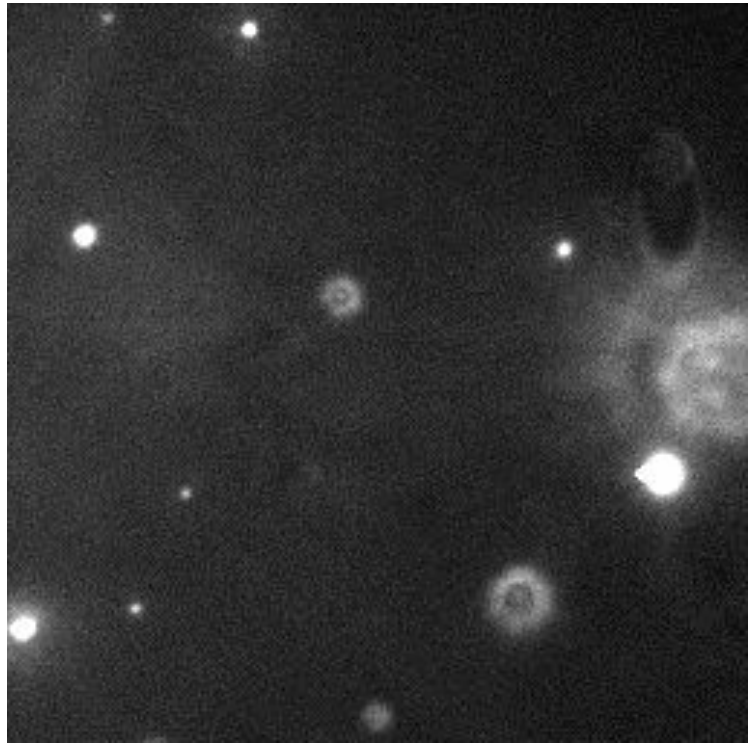
- Lipid nanoparticles (LNPs) encapsulating CRISPR-Cas9 in the form of either
  - mRNA: LNP-mRNA (~50nm)
  - Ribonucleoprotein (RNP) complex: LNP-RNP (~260nm)

- Compared LNP-mRNA vs LNP-RNP diffusivity in mucus using NTA method

# LNP-mRNA vs LNP-RNP in lung mucus



LNP-mRNA
In mucus

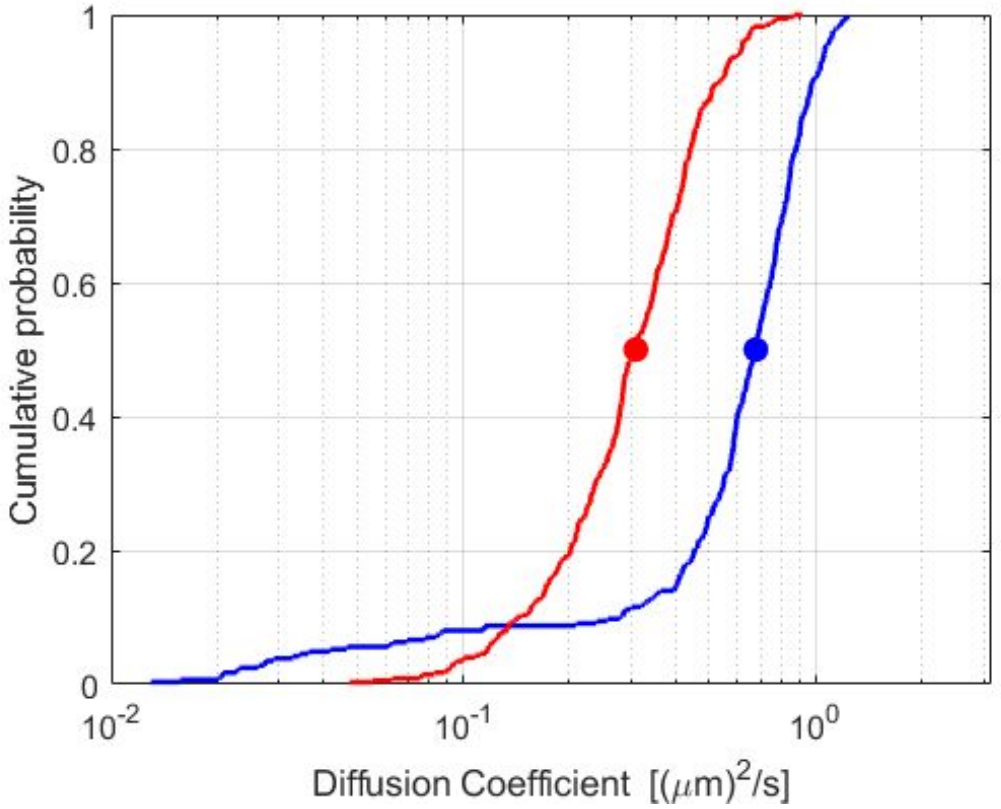


LNP-RNP
In mucus

# LNP-mRNA (Blue) vs LNP-RNP (Red)

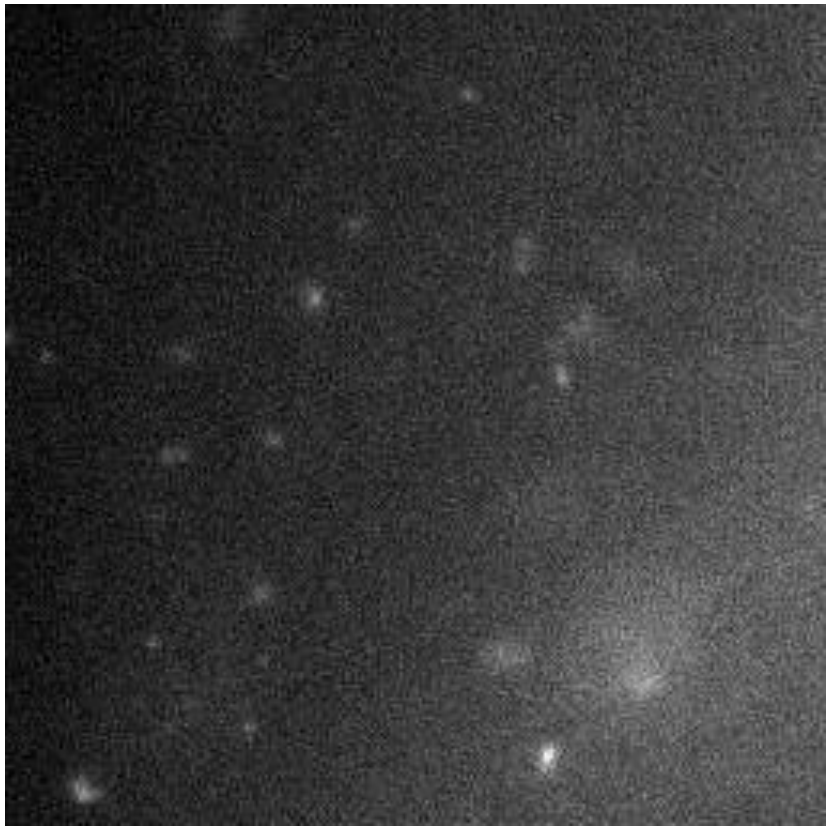
| Name | Median Diffusion Coefficient |
|---|---|
| LNP-mRNA | 0.68 $(\mu m)^2/s$ |
| LNP-RNP | 0.31 $(\mu m)^2/s$ |

- LNP-mRNA has a 2.19 fold increase in median diffusion coefficient
- At least partially due to LNP-mRNA (~50nm) smaller size than LNP-RNP (~260nm)

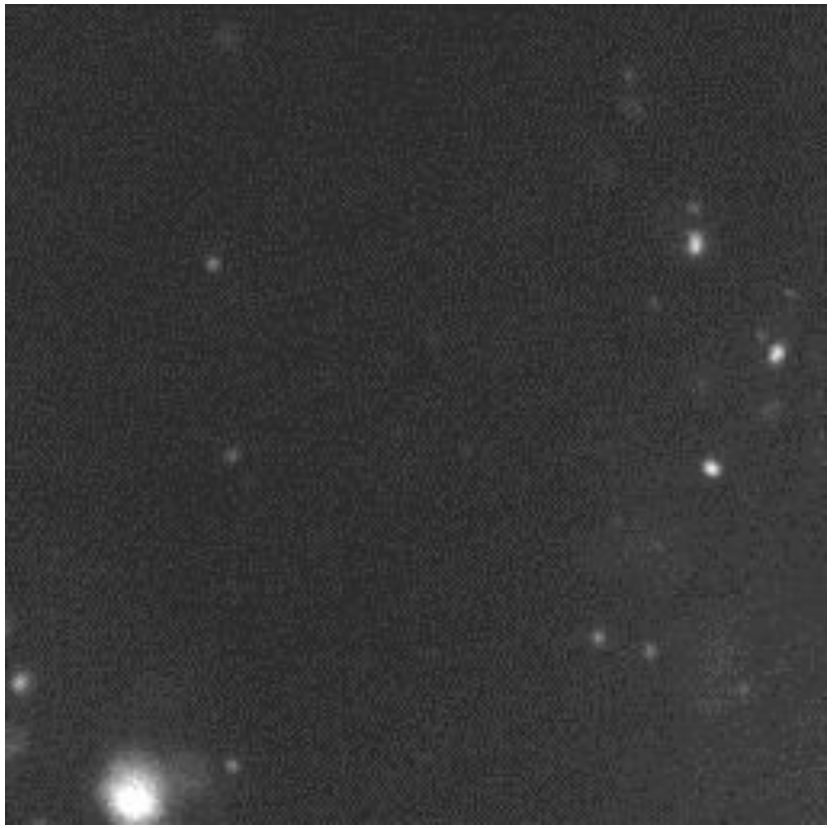


# Testing LNP transport in Cystic Fibrosis mucus

# LNP-mRNA vs LNP-RNP in Cystic Fibrosis (CF) mucus



LNP-mRNA

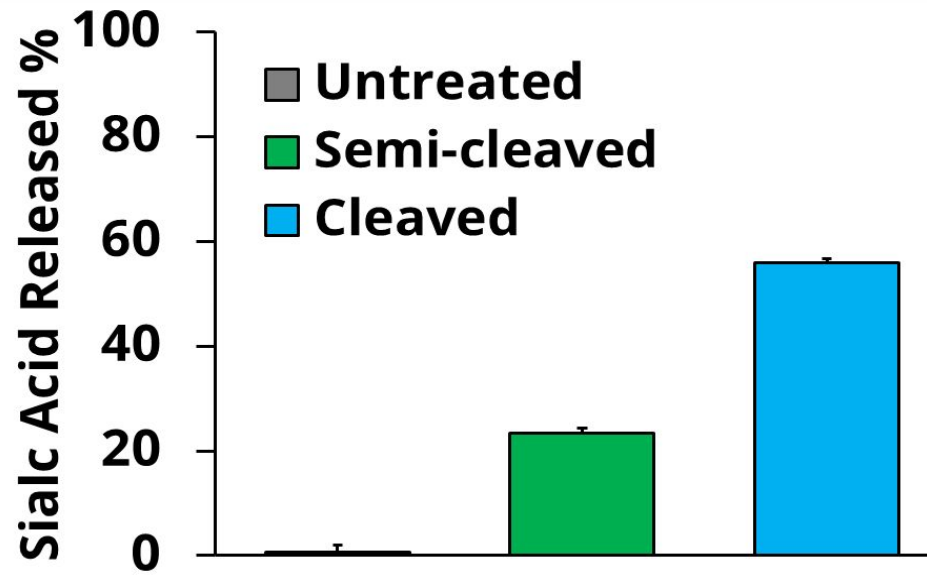


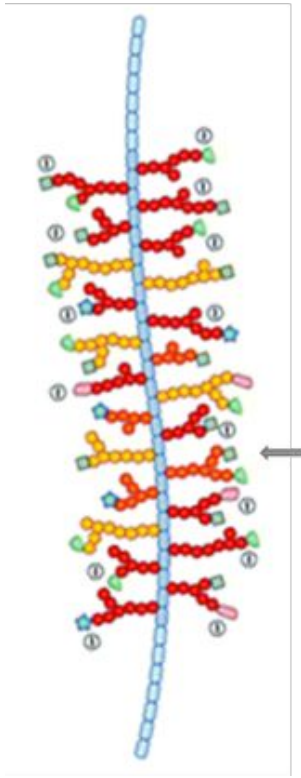
LNP-RNP

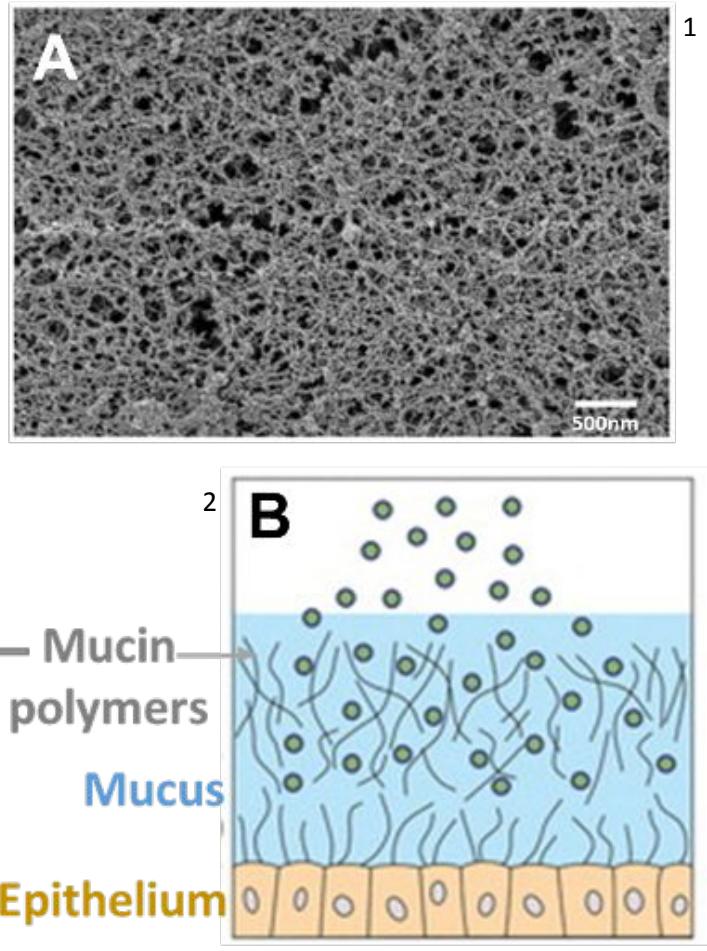
- The movement of the LNPs was drastically halted in CF mucus
- Factors other than size must be considered

# Impact of sialic acid on capsid diffusivity in mucus

- Mucin has glycan chains that terminate in negatively charged sialic acid residues (green)
- Enhances the trapping of charged particles
- Removed sialic acid from mucin using neuraminidase (NA) at different levels:



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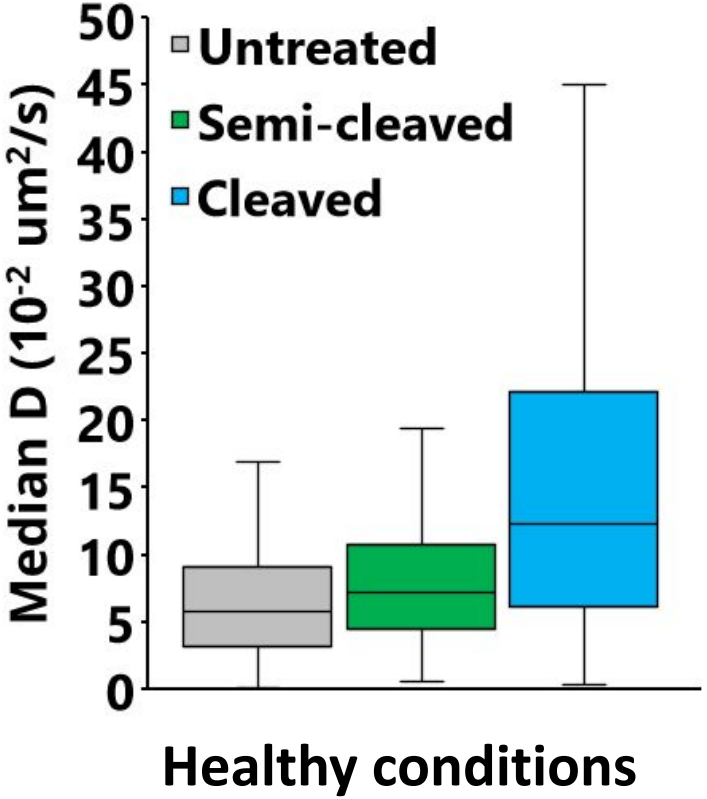
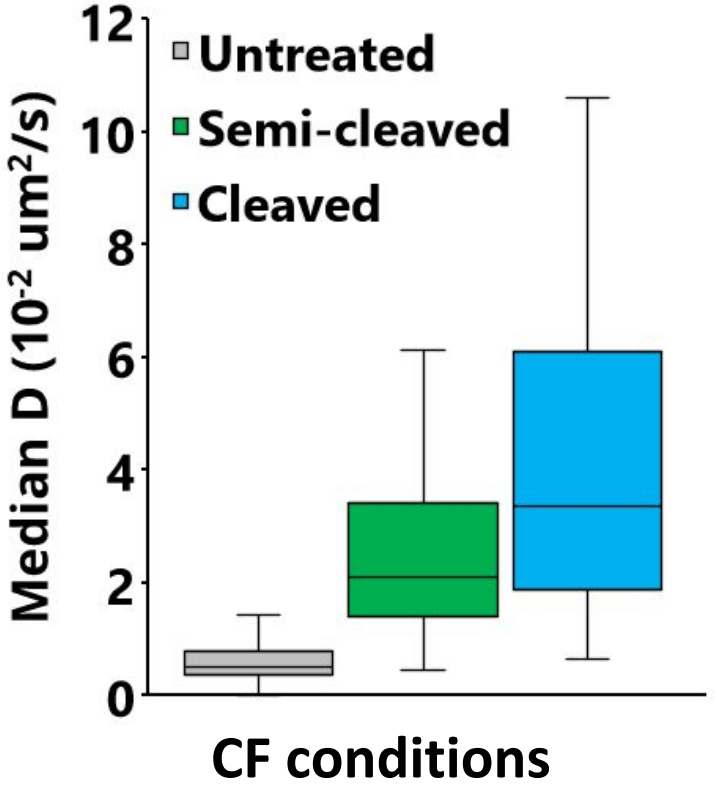
[1]Schuster BS *et al*. Biomaterials. 2013;34(13):3439-3446
[2]Petrou G & Crouzier T. Biomater. Sci. 2018(6): 2282-2297

# Impact of sialic acid removal on diffusivity

- Capsids with small sizes (~20nm) were added to mucus & NTA was performed



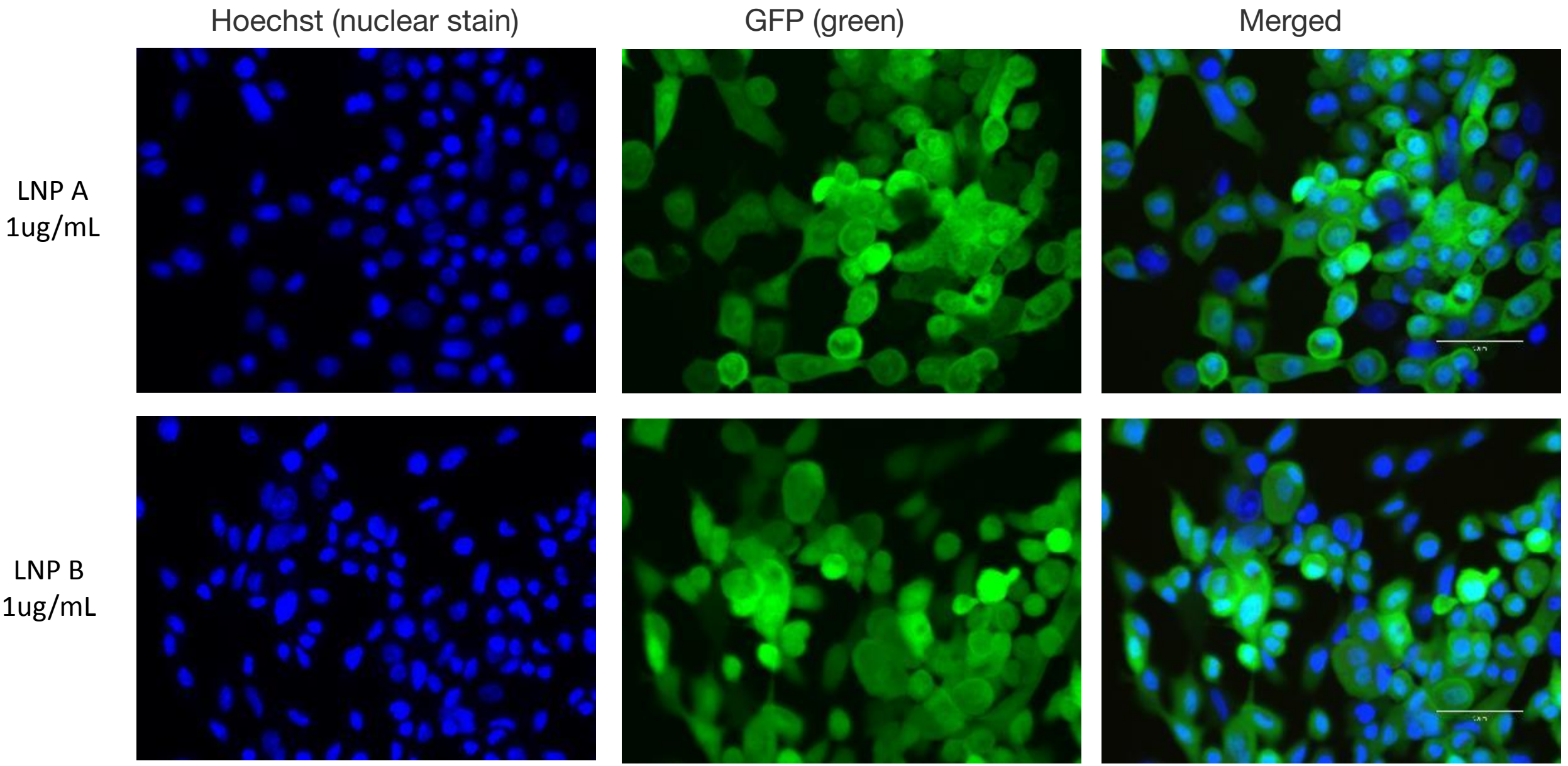

- Increase in NP diffusivity with higher % of SA removed in both healthy and CF mucus conditions

# Goal 2. Assessing LNP uptake and gene editing efficacy in primary lung epithelial cells

# Assessing the transfection efficiency of LNPs in primary lung epithelial cells

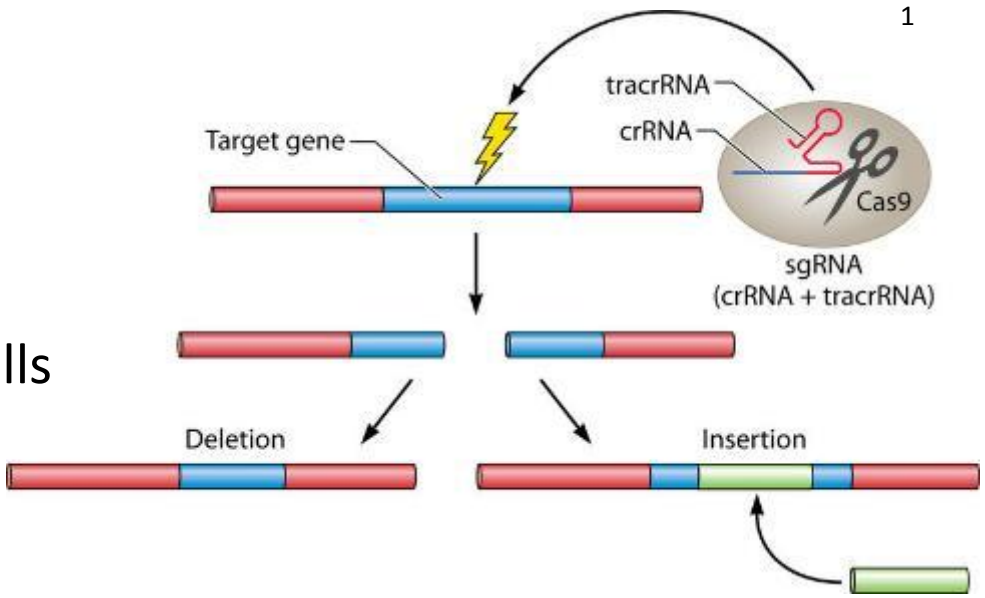
- Lipid nanoparticles (LNPs) loaded with mRNA encoding GFP
- Transfected into normal human bronchial epithelial (NHBE) cells
- Successful transfection results in:
  - GFP-mRNA is translated into a GFP protein
  - A green florescence can be detected
  - Imaged using florescence microscope

# NHBEs transfected with LNPs + eGFP-mRNA



# Gene editing efficacy in primary lung epithelial cells

- LNPs are loaded with
  - CRISPR-Cas9 mRNA or protein
  - SgRNA against HPRT
- Transfected in the primary lung cells
  - Normal human bronchial epithelial (NHBEs) cells
- Real-time PCR: Quantify relative HPRT Indel (insertion or deletion) percentages

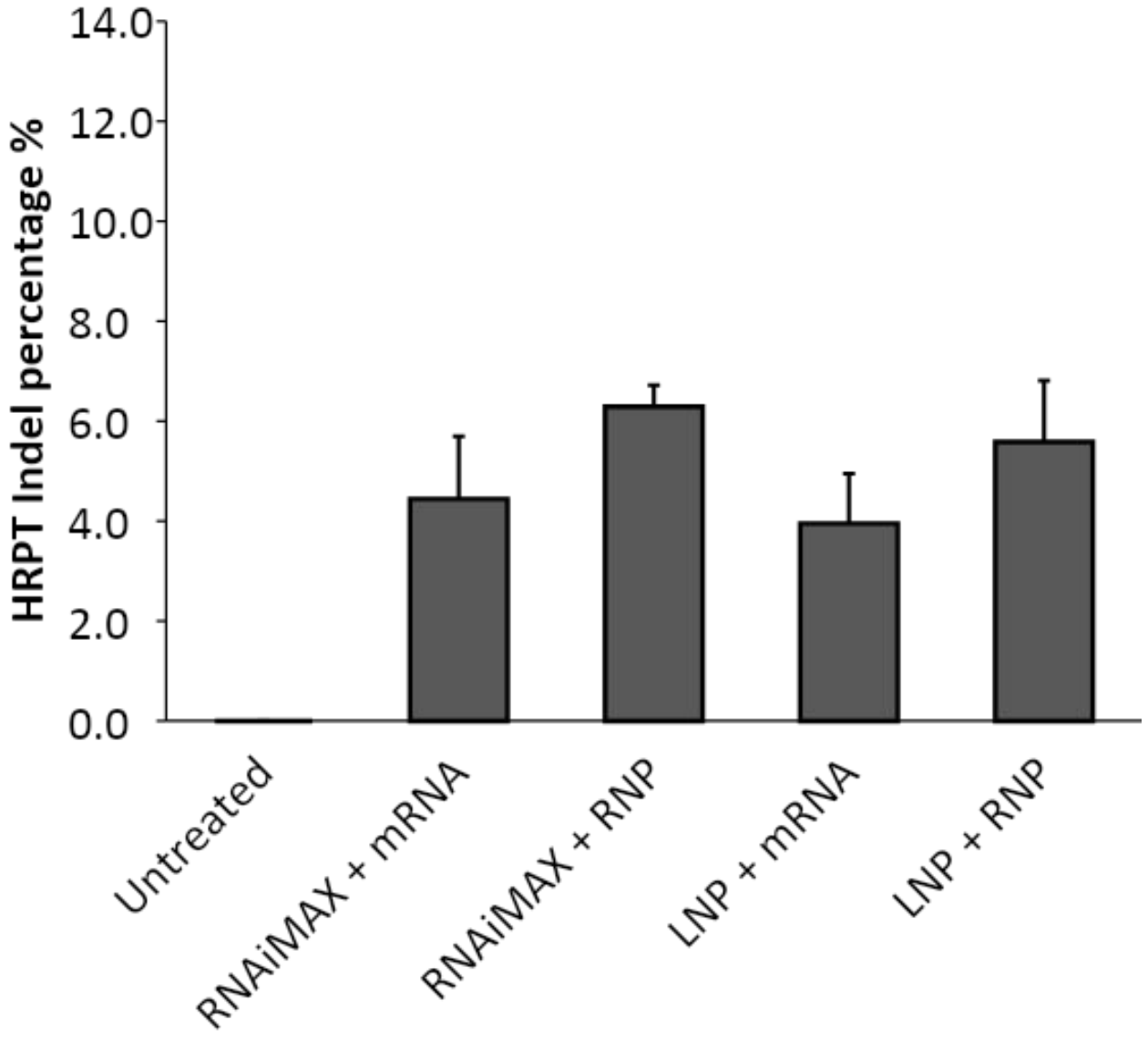


[1]



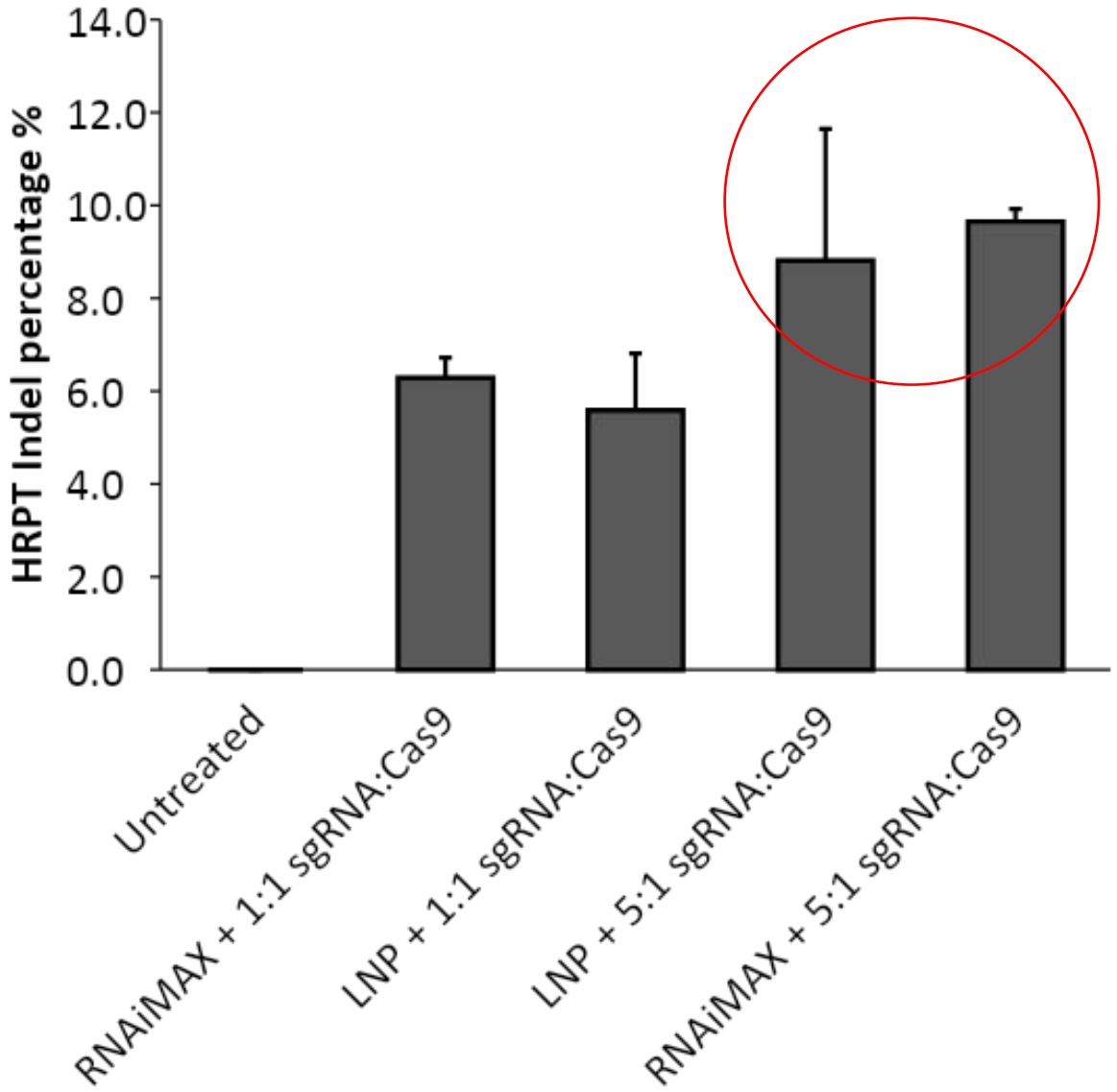
[1] Ishino, Yoshizumi & Krupovic, Mart & Forterre, Patrick. (2018). History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. Journal of Bacteriology. 200.

# Treating NHBE cells with LNPs + CRISPR-Cas9 mRNA or RNP




- Around 5% gene editing only
- Cas9 RNP appears slightly more potent than Cas9 mRNA

# Treating NHBE cells with CRISPR-Cas9 mRNA or RNP




- Increasing sgRNA to Cas9-protein ratio to 5:1
- Around 10% gene editing
- As sgRNA is degraded faster than Cas9 protein
  - More sgRNA helps overcome this

# Conclusions

- LNP-mRNA showed the highest diffusion in isolated healthy lung mucus
- Mucus isolated from Cystic Fibrosis patients halts the diffusion of LNPs
- Sialic acid removal improves NP diffusivity in healthy and CF conditions
- On the cellular level, LNPs show high uptake in primary lung epithelial cells
- Increasing ratio of sgRNA to Cas9 protein (5:1) improves gene editing

## Acknowledgement: Thank you!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# Thank you!

## Questions