Non-Viral Delivery Nanoplatforms for Brain-Targeted Genome Editing

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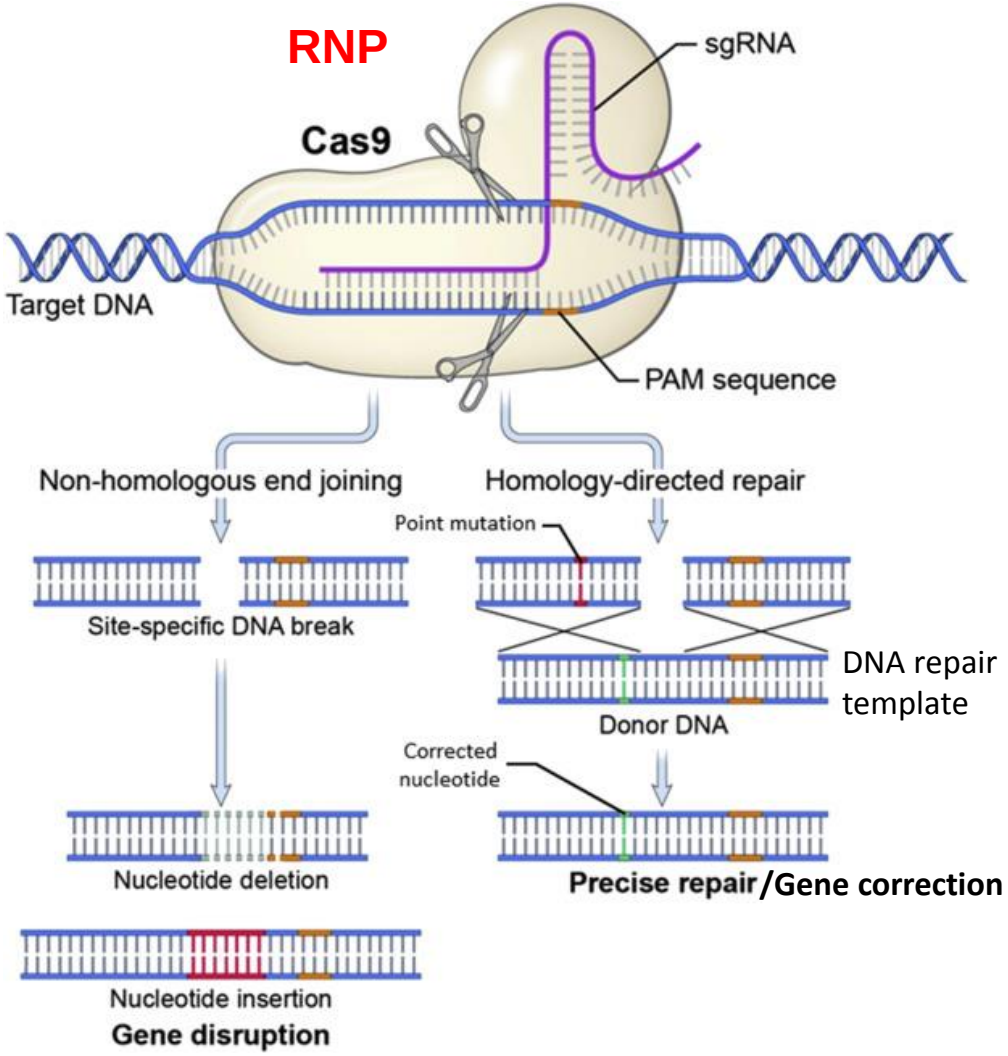
CRISPR-Cas9 Genome Editing

Gene deletions, insertions, or alterations

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

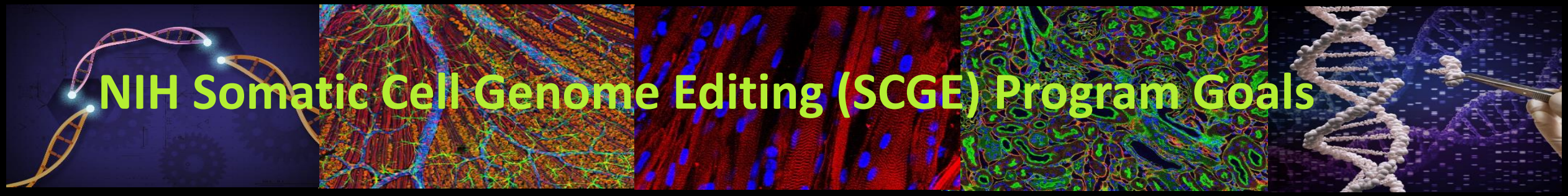


CRISPR Associated Proteins 9 (Cas9)

- Cas9/sgRNA **R**ibo**N**ucleo**P**rotein (**RNP**)
- The RNP homes in on the target DNA site encoded in the gRNA.
- Once RNP is activated, it causes a double-strand breakage (DSB) at the target site.
- The cell attempts to repair the DSB, causing either imprecise or precise editing.

***Challenge*: Safe and efficient delivery of the CRISPR genome editing machinery.**

Journal of Investigative Dermatology, 136(9), e87-e93, 2016.



Lower the Barriers for New Genome Editing Therapies by:

- Testing genome editing reagents and delivery systems in better animal models
- Assessing unintended biological effects
- Improving *in vivo* delivery of genome editing machinery
- Expanding the human genome engineering toolkit
- Coordinating partnerships and disseminating information

Our Collaborative Team

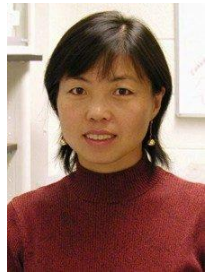
Dr. **Shaoqin Sarah Gong**
Vilas Distinguished Professor
Dept. of Biomedical Engineering, UW-Madison
Focus: Nanomedicine; targeted delivery

Dr. **Marina E. Emborg**
Professor, Dept. of Medical Physics, UW-Madison
Focus: Neurodegenerative disorders; translational neuroscience

Dr. **Jon Levine**
Professor, Wisconsin National Primate Research Center, UW-Madison
Focus: Gene silencing in neural populations; mouse and monkey models

Dr. **Subhojit Roy**
Professor, Dept. of Neuroscience, UW-Madison
Focus: Neuron cell and brain models; CRISPR tools editing APP

Dr. **Krishanu Saha**
Assistant Professor, Dept. of Biomedical Engineering, UW-Madison
Focus: Novel CRISPR technologies



Dr. **Xinyu Zhao**
Professor, Dept. of Neuroscience, UW-Madison
Focus: Neural stem cell and brain development

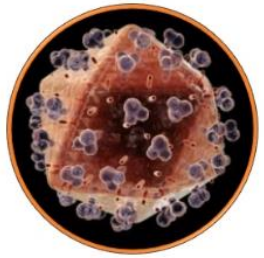
Non-Viral vs. Viral Vectors

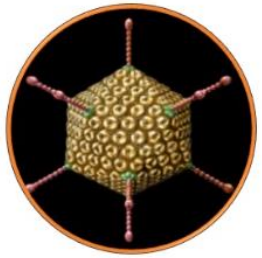
Viral Vectors

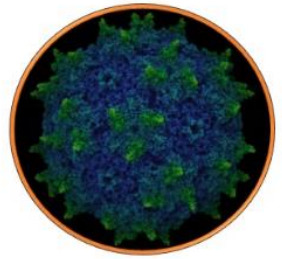
√ High efficiency
X Harder to scale up
X Immunogenicity
X Mutagenesis

Non-Viral Vectors

√ Better safety profile
√ Versatile chemistry
√ Easier to scale up
X Low efficiency

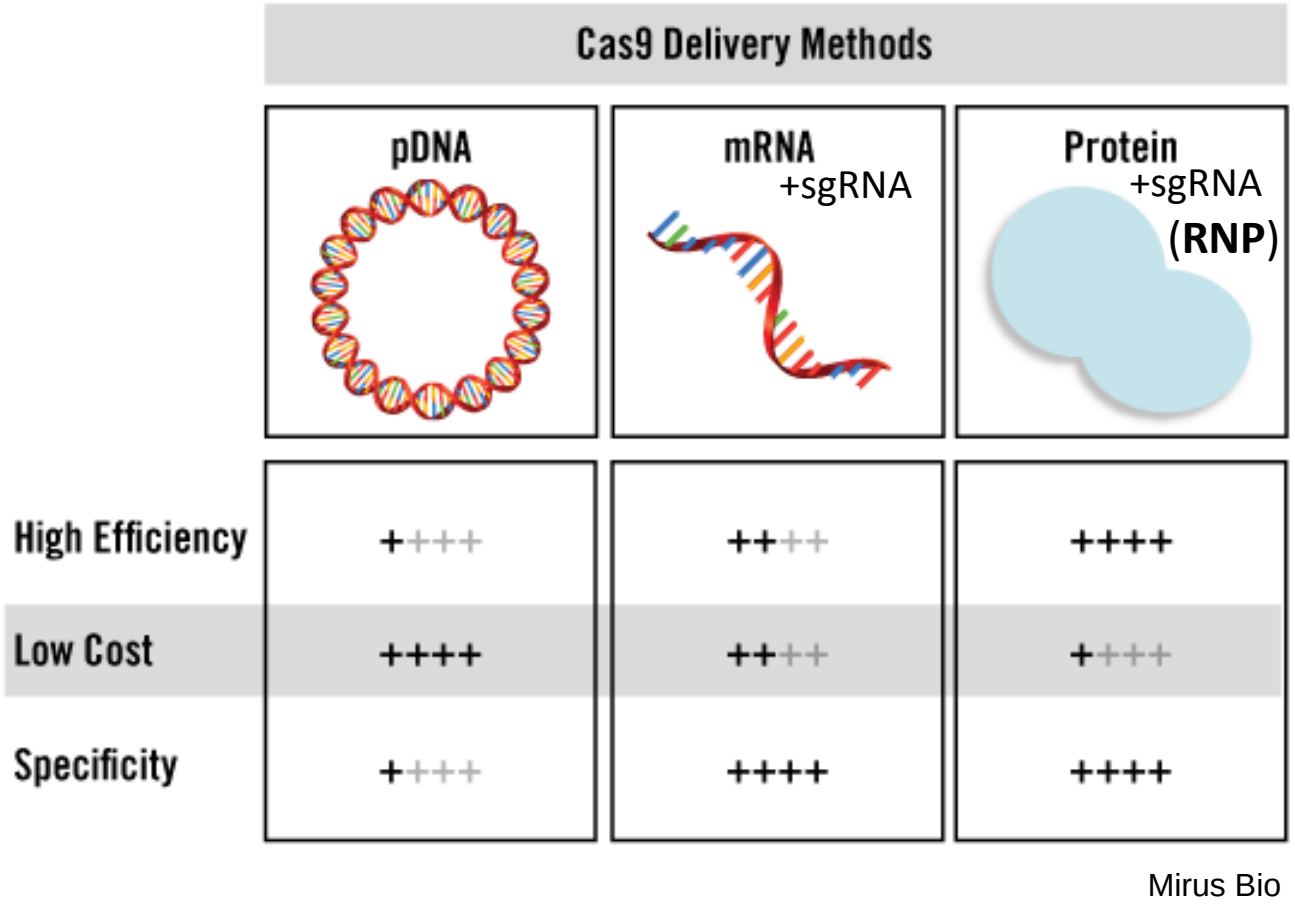






Features	Lentivirus	Adenovirus	AAV
Packaging Capacity	8-9 kb	8-9 kb	3-4 kb
Integrating	Yes	No	Rare
Immune Response	**	****	**

Delivery of Genome Editing Machinery



Mirus Bio

Protein

- Precise control over the Cas9/sgRNA ratio
- Rapid editing (no lag time)
- Lower off-target effect

pDNA or mRNA

- Variable copy number
- Delayed editing

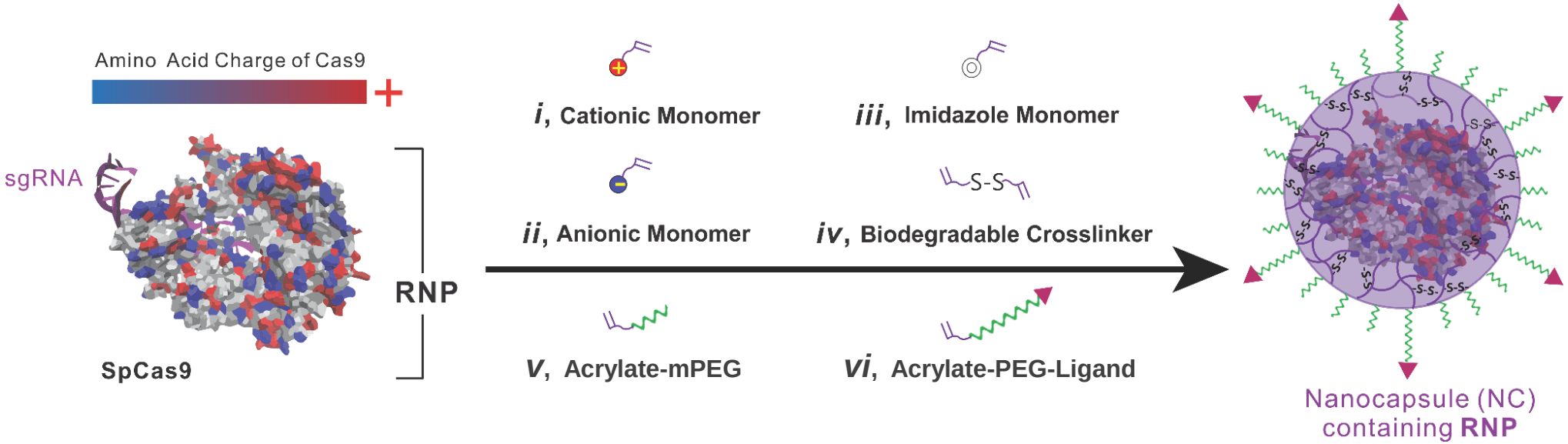
pDNA

- Higher off-target effect

Direct delivery of RNP requires non-viral vectors.

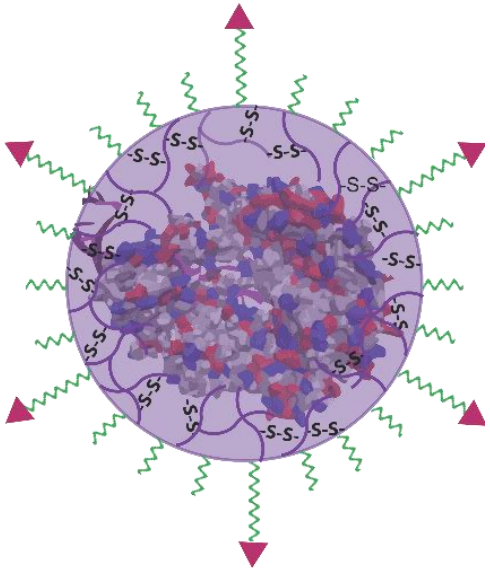
RNP Nanocapsule (NC)

Data presented in this talk have not been published.

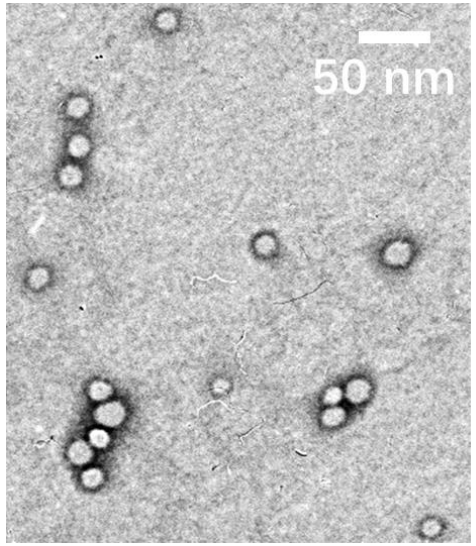


- Formation of the covalently-crosslinked, yet intracellularly biodegradable, nanocapsule (NC) for the delivery of the Cas9 RNP complex
- The surface of the RNP NCs can be conveniently functionalized with various types of targeting ligands.

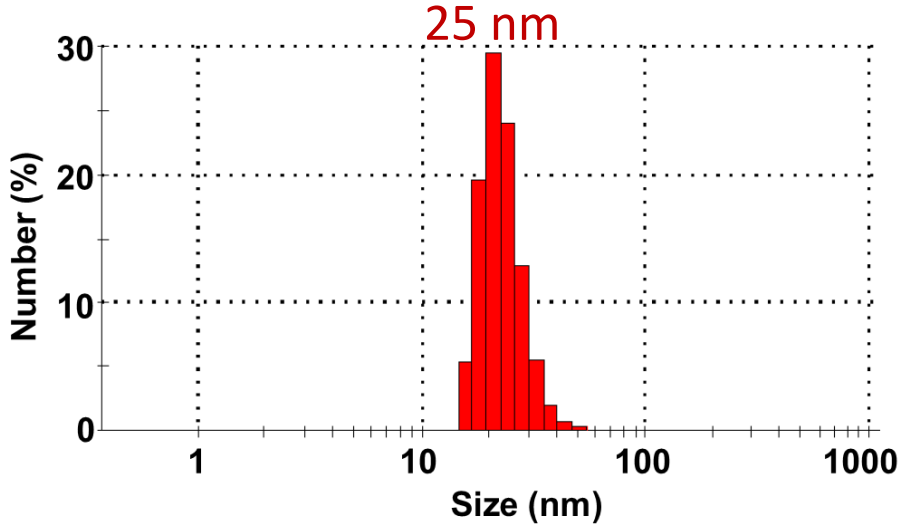
Characterizations of RNP NCs



TEM image



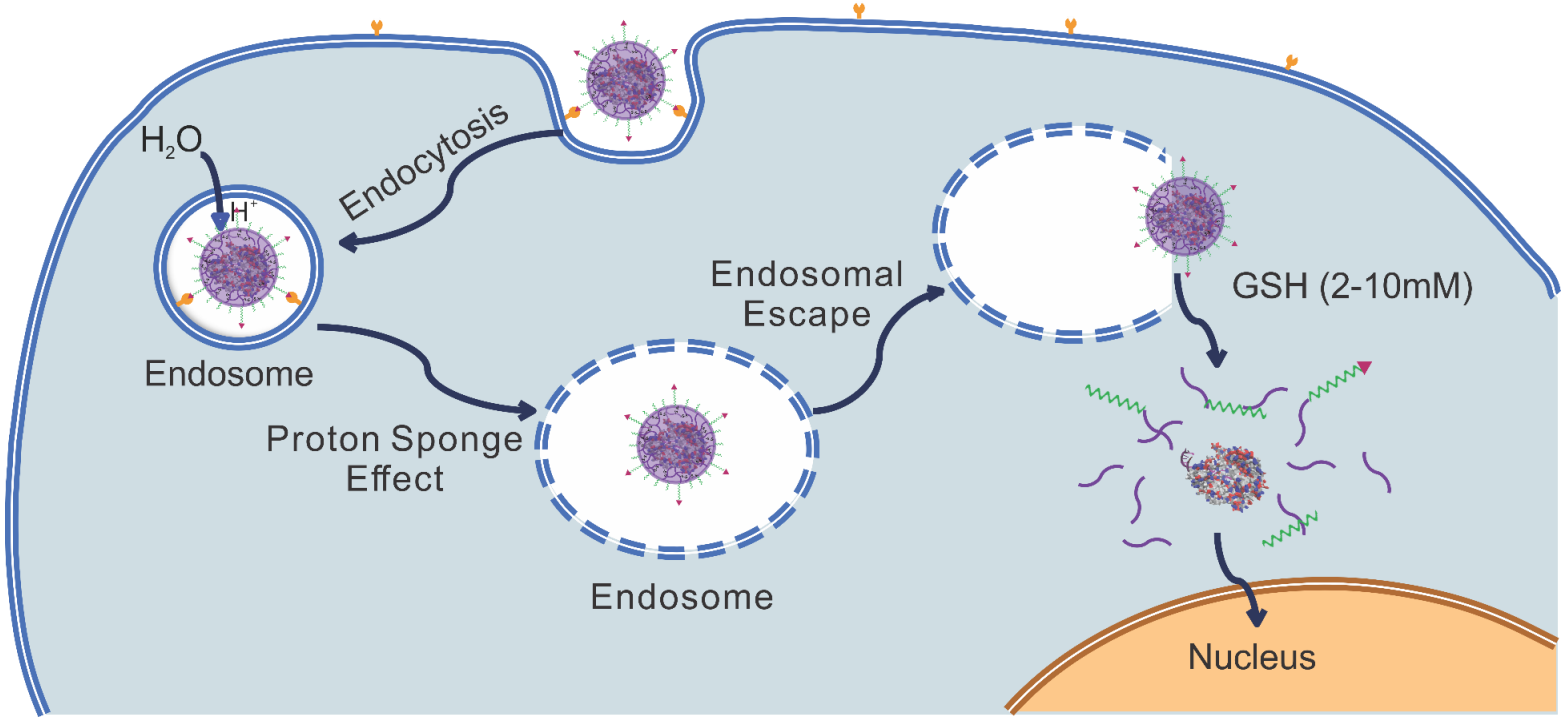
Dynamic light scattering (DLS)



- Hydrodynamic diameter ~ 25 nm
- Neutral surface charge (-4 mV)

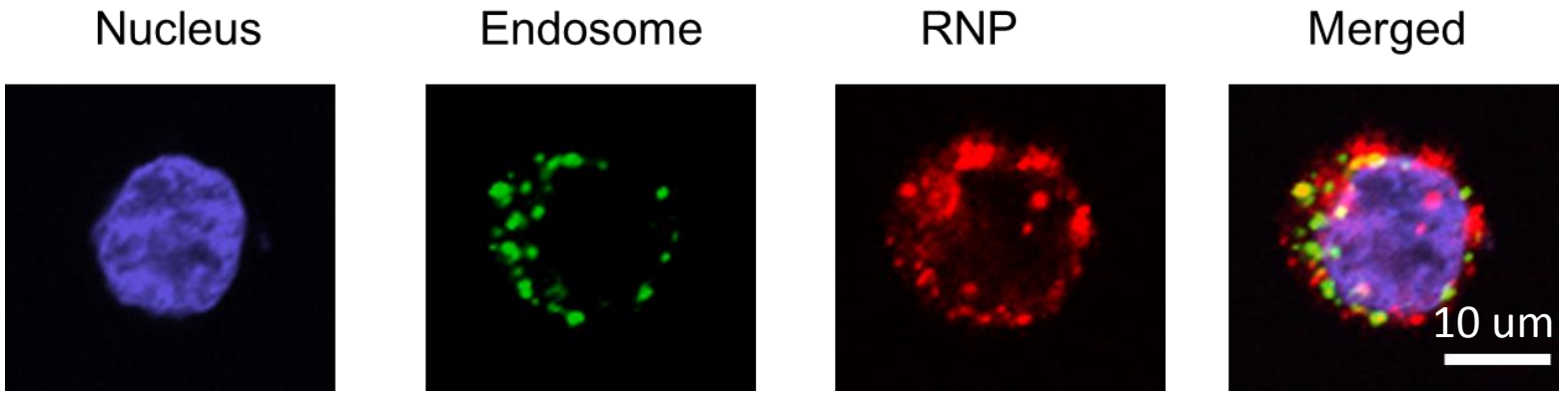
	Cas9 protein	RNP	**NC**
Size (nm)	7 ± 1	9 ± 1	25 ± 6
Zeta potential (mV)	13 ± 3	-20 ± 5	-4 ± 1

Intracellular Pathway of RNP NCs



Cellular uptake and subcellular trafficking of the RNP NCs:

- High cellular uptake
- Efficient endosomal escape
- Rapid release of the RNP inside of the cytosol
- Efficient nuclear transport

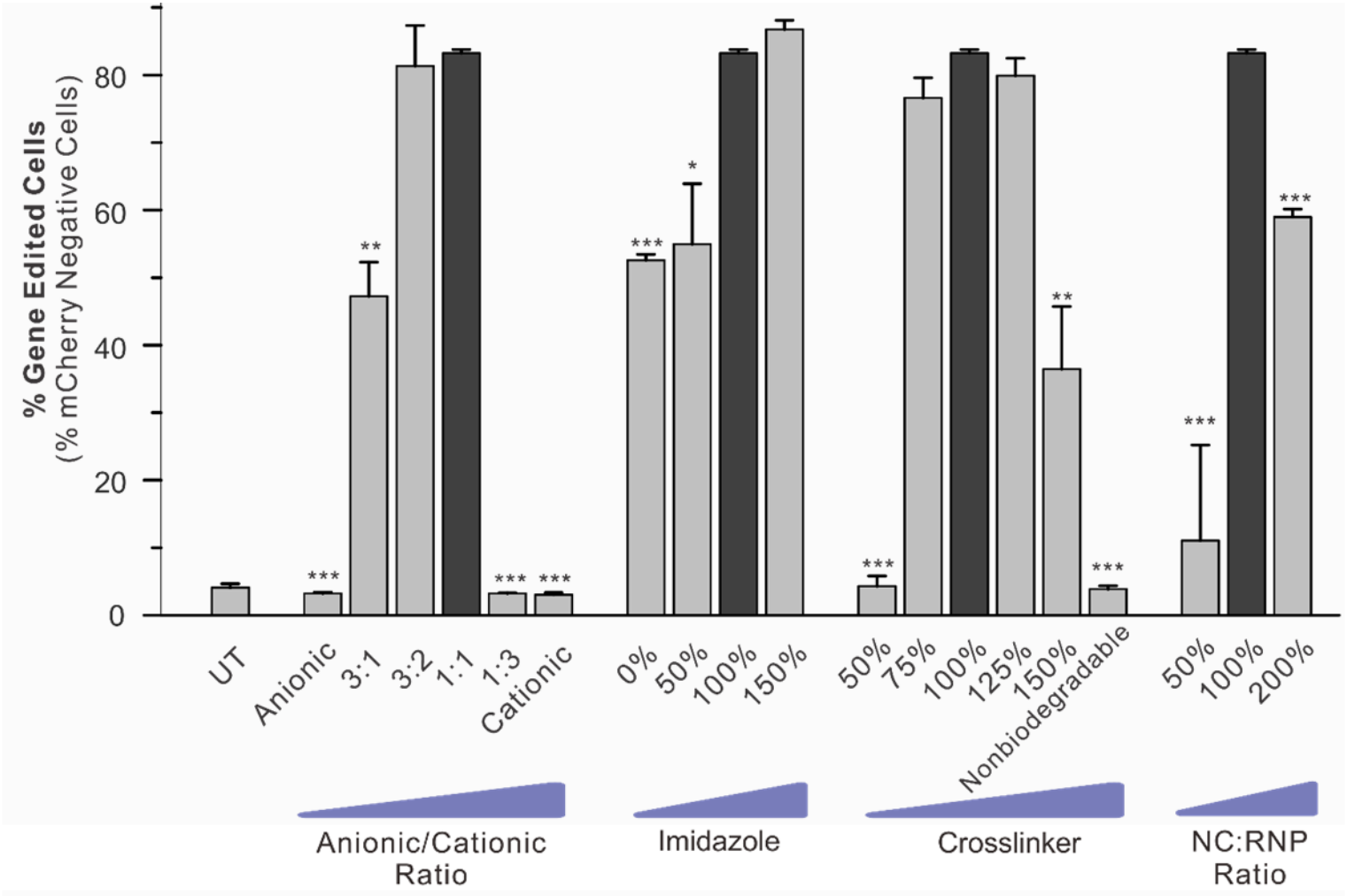


RNP NCs can effectively escape from endosomes.

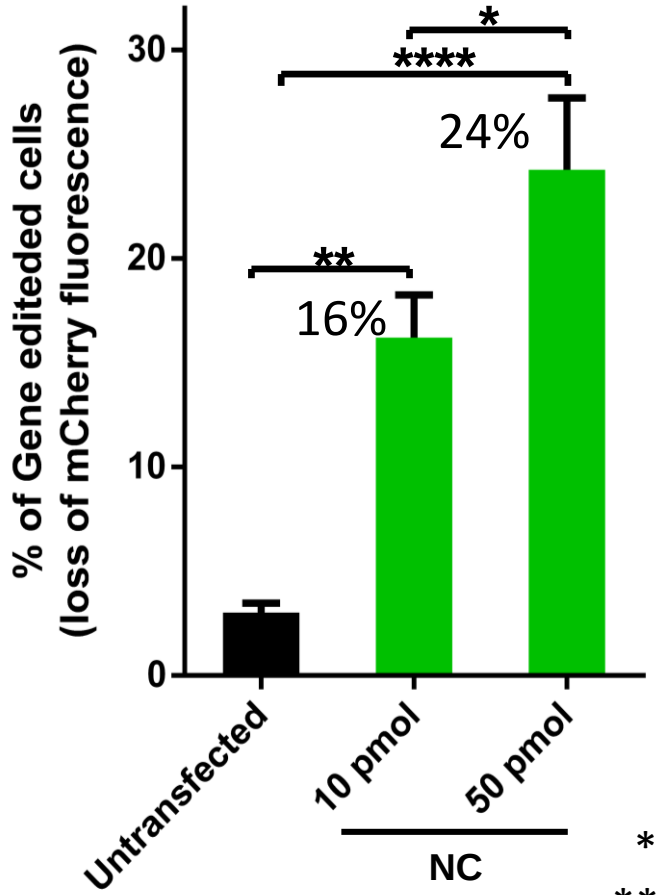
Blue: DAPI Green: Lysotracker Red: ATTO-labeled RNP 6 hr post-incubation

Optimization of RNP NCs

mCherry-HEK 293 cells
gRNA targets the mCherry gene



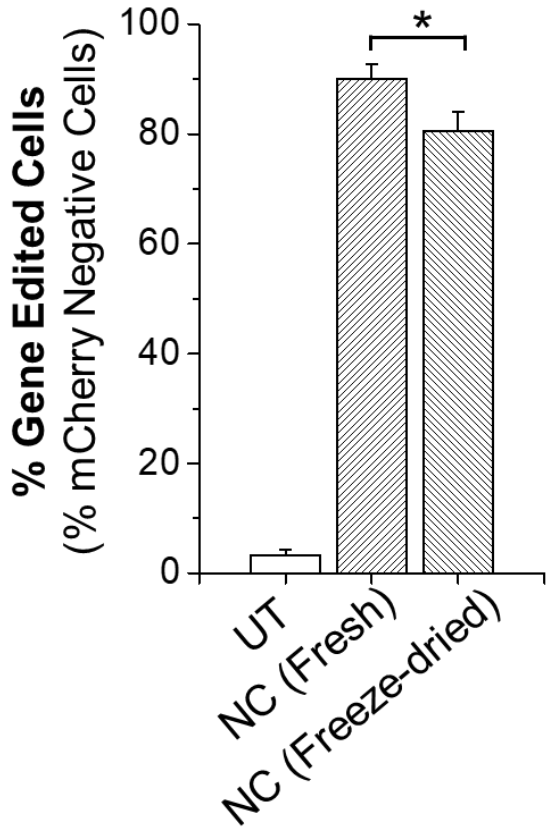
mCherry-human neural progenitor cells (mCherry-NPCs)



*: $p<0.05$
**: $p<0.01$
***: $p<0.001$
****: $p<0.0001$

Effect of Lyophilization on Gene Editing and Cytotoxicity of NCs

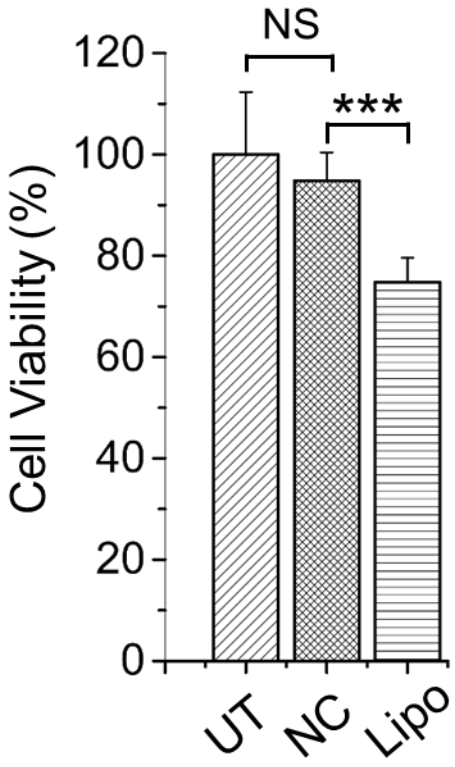
Gene editing after lyophilization



*: $p<0.05$;

- Over 90% of gene editing capability was retained after lyophilization of NCs.

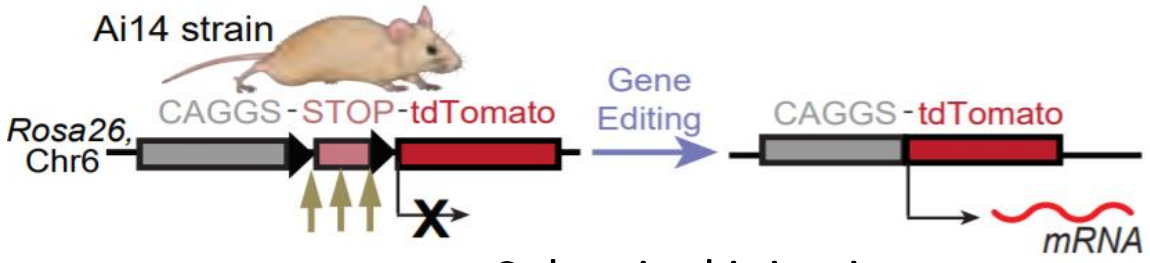
Cytotoxicity



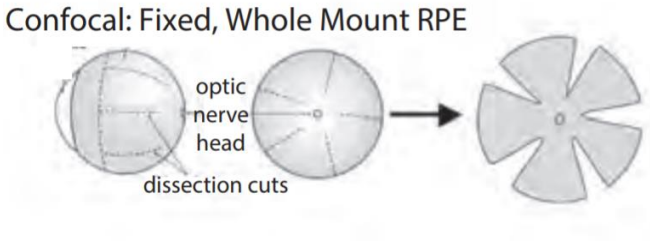
***: $p<0.001$

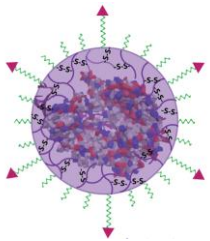
- NCs did not induce significant cytotoxicity.
- Lipo2000, a commercially available agent to deliver RNP, caused significant cell death (>25%).

RNP NCs: *In Vivo* Gene Editing With RPE Cells



Subretinal injection

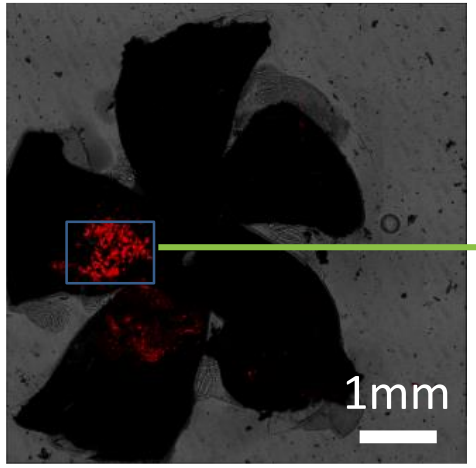


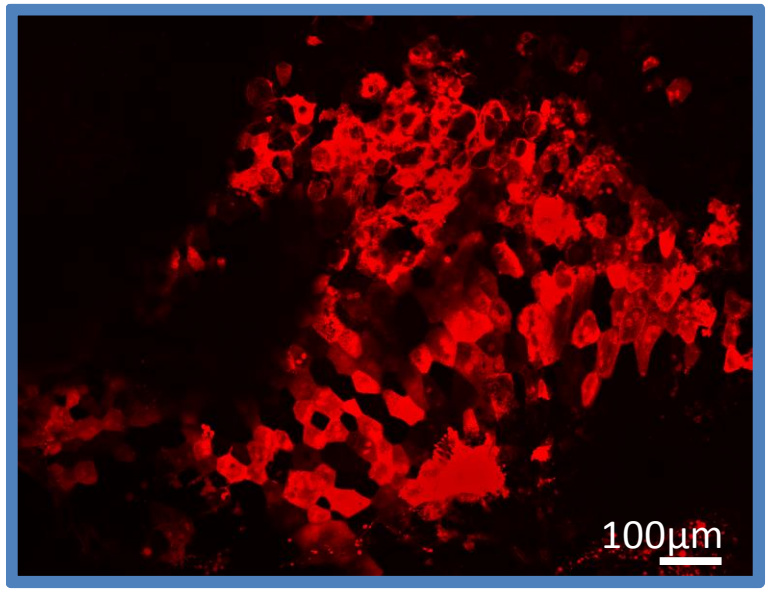


- The Ai14 mice harbor a *LoxP*-flanked stop cassette that prevents expression of the tdTomato fluorescent protein.
- RNP-guided excision of the stop cassette leads to tdTomato expression.

PBS

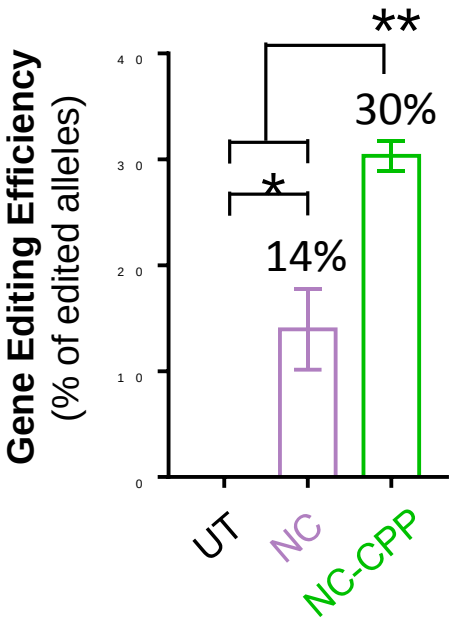
NC-RPE targeting ligand

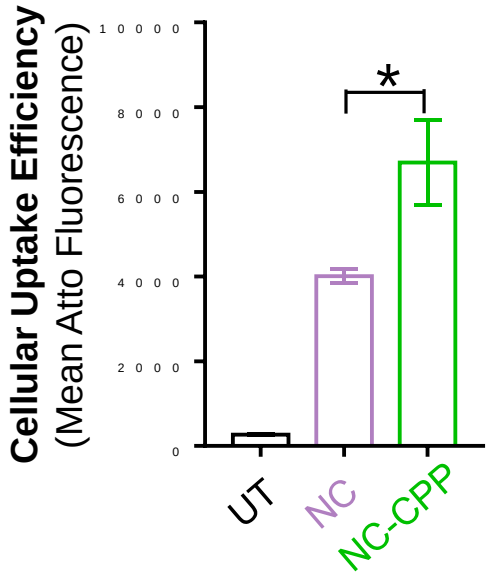


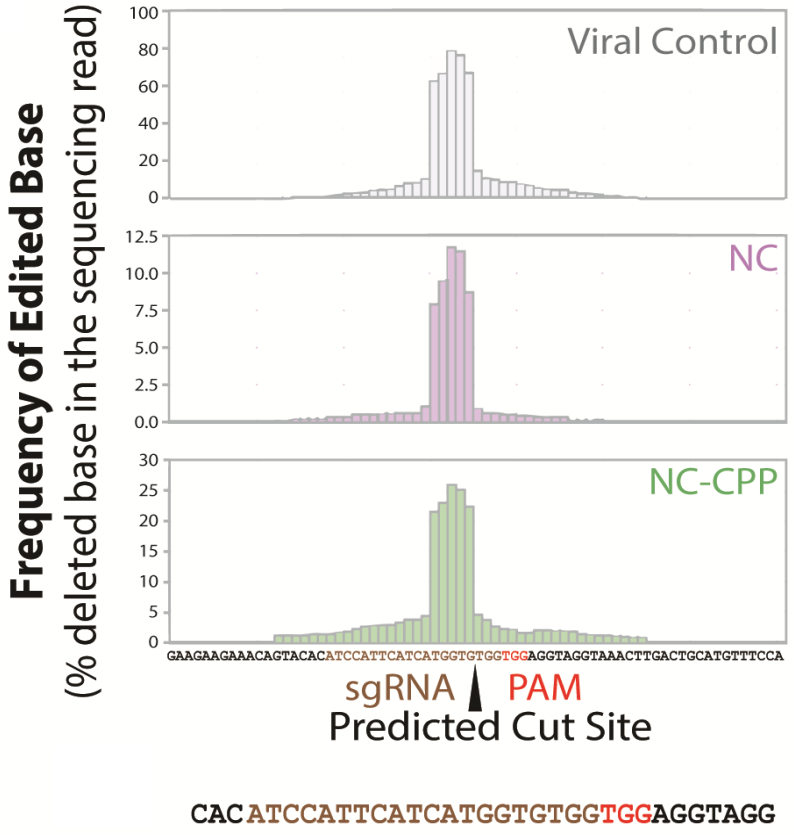


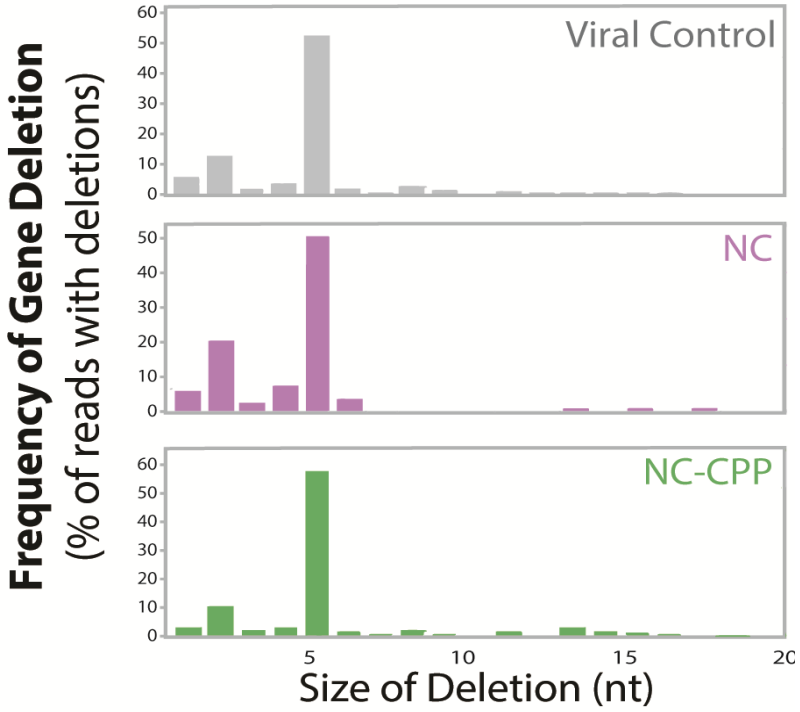
Dr. Bikash R. Pattnaik, UW-Madison

RNP NCs Targeting Human APP Gene in HEK cells







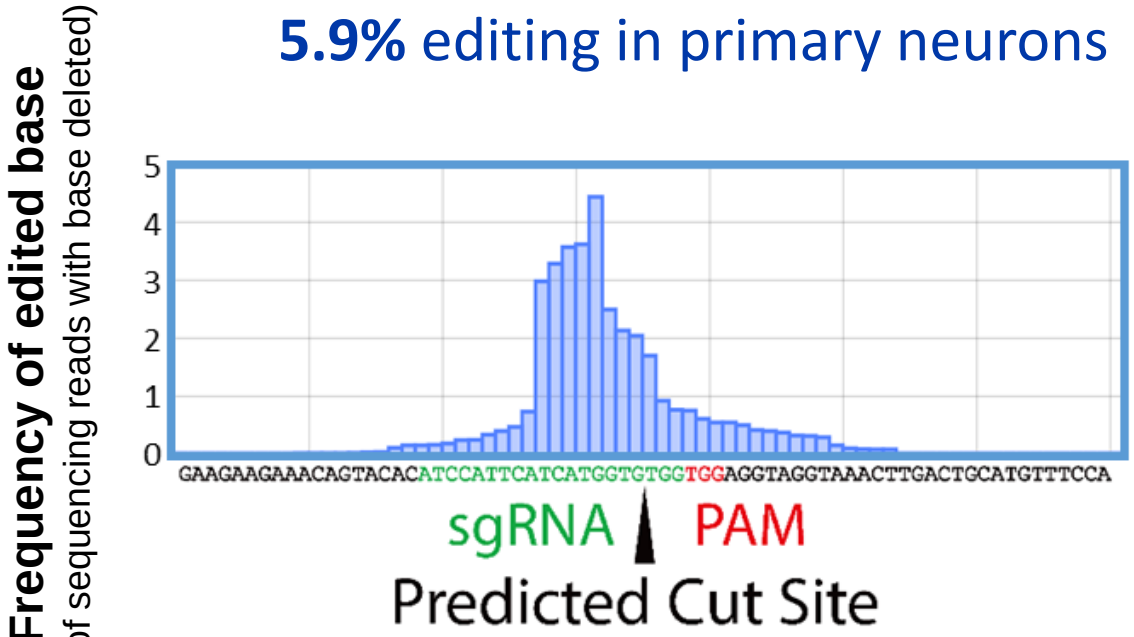


APP: amyloid precursor protein
CPP: cell-penetrating peptide

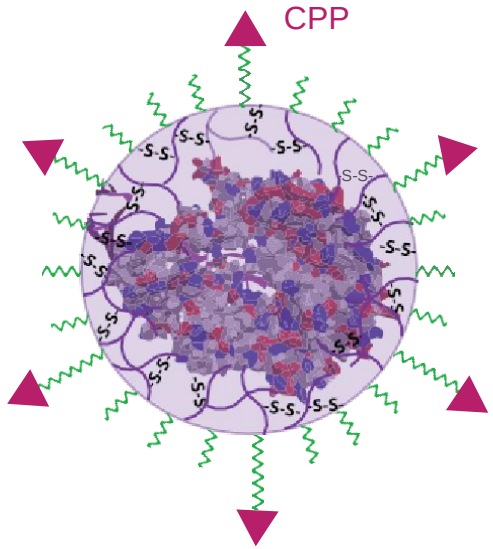
Viral control from Sun, Saha, Roy et al. *Nature Communications*, 2019

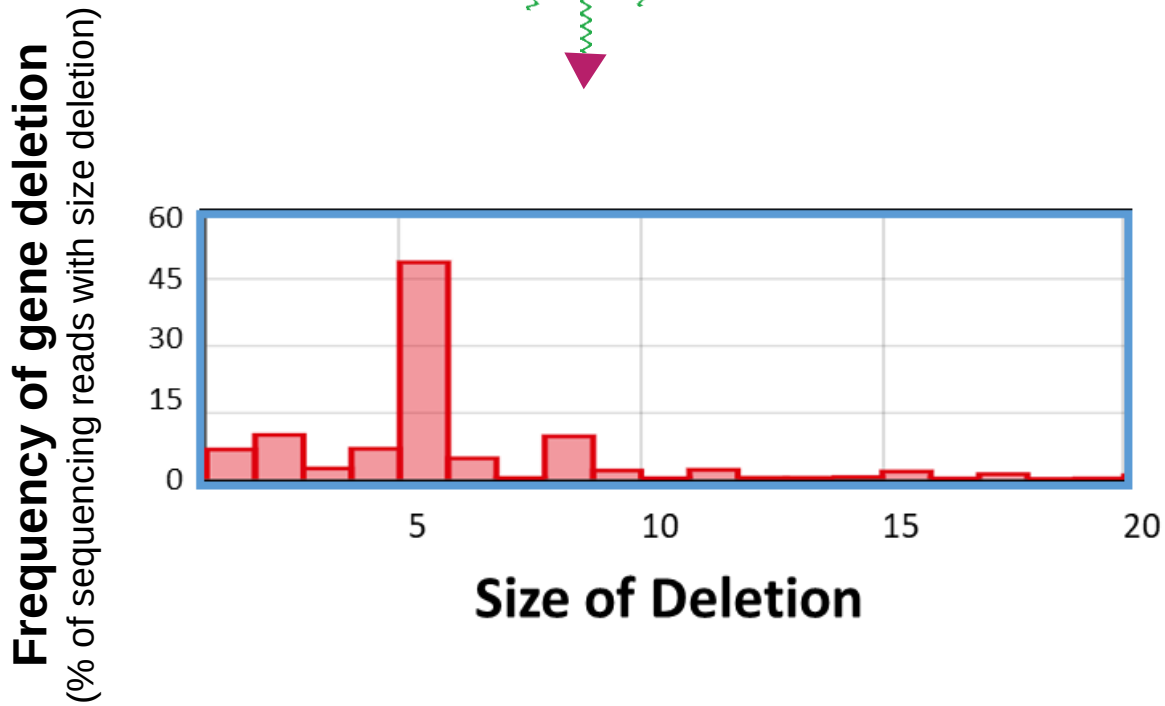
RNP NC-CPP Targeting Mouse APP Gene in Primary Mouse Neurons

5.9% editing in primary neurons



APP: amyloid precursor protein

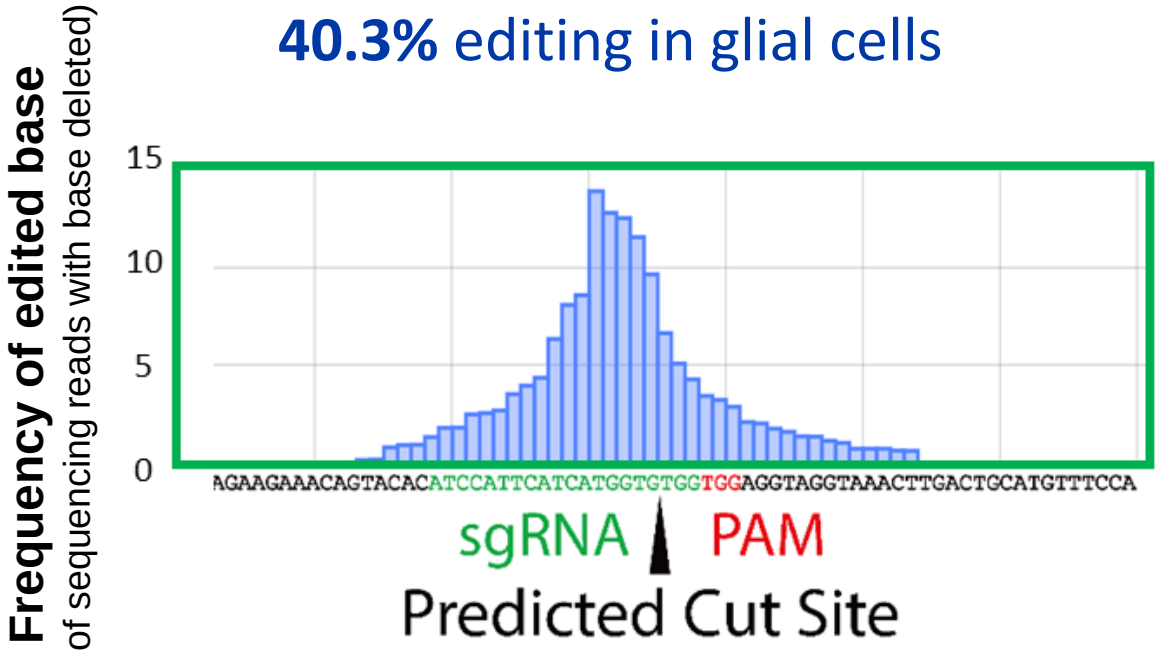




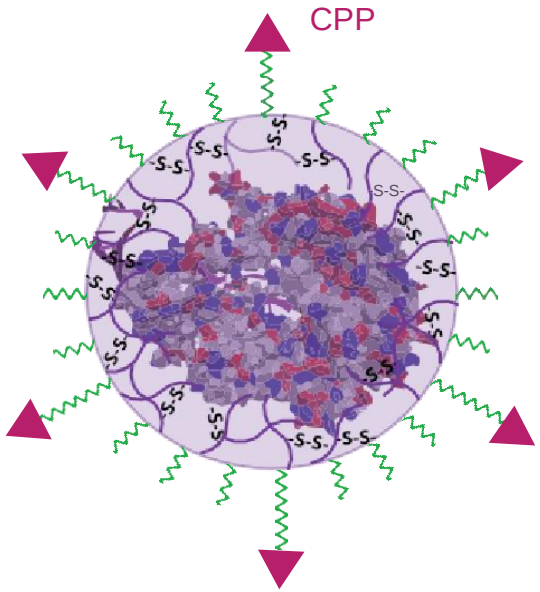
Preliminary data

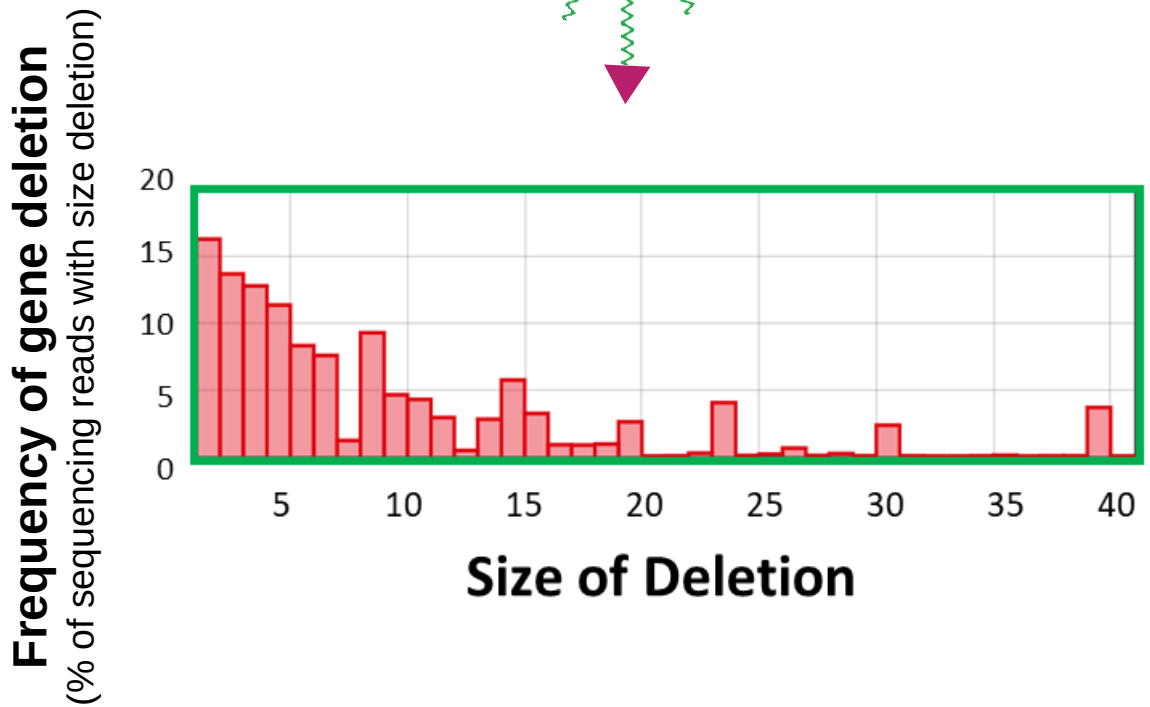
RNP NC-CPP Targeting Mouse TH Gene in Mouse Glial Cells

40.3% editing in glial cells



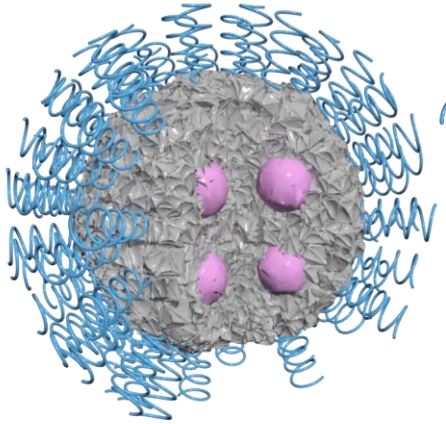
TH: tyrosine hydroxylase





Preliminary data

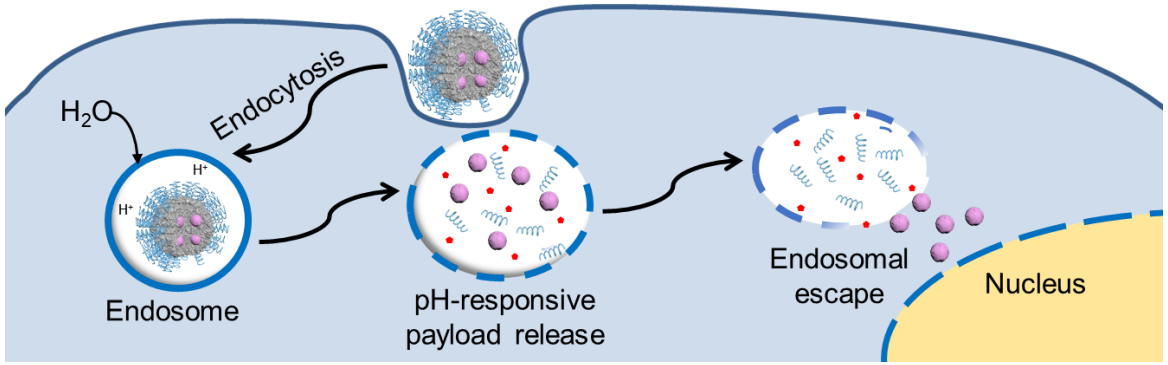
pH-Responsive Nanoparticles Enabling Both Gene Disruption and Gene Correction



Polyethylene glycol (PEG)

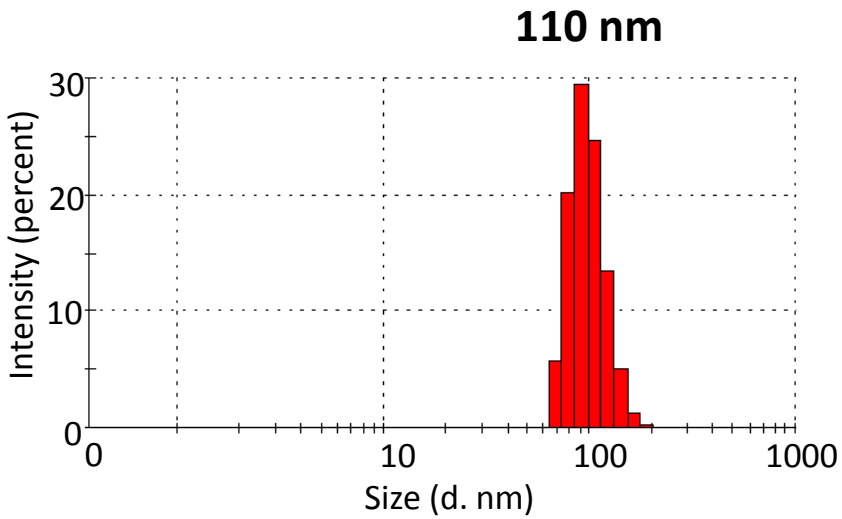
pH-responsive nanoparticle

Cas9 RNP

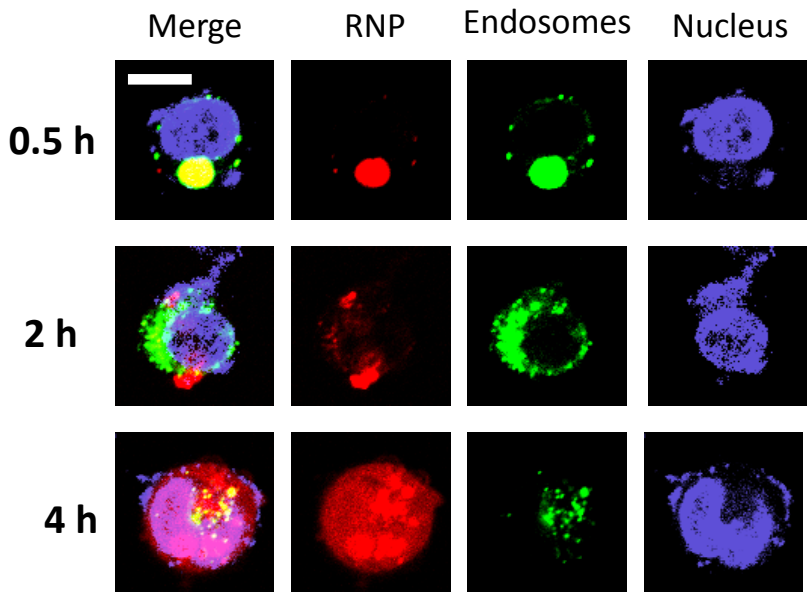


Hydrophilic payloads including DNA, mRNA, proteins, RNP, RNP + ssODN, hydrophilic small molecular drug

DLS



CLSM image



Payload: Cas9/sgRNA-ATTO550 RNP

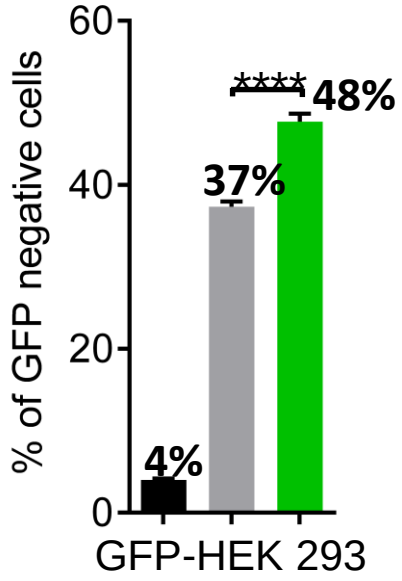
Cell line: HEK293 cells

Scale bar: 10 µm

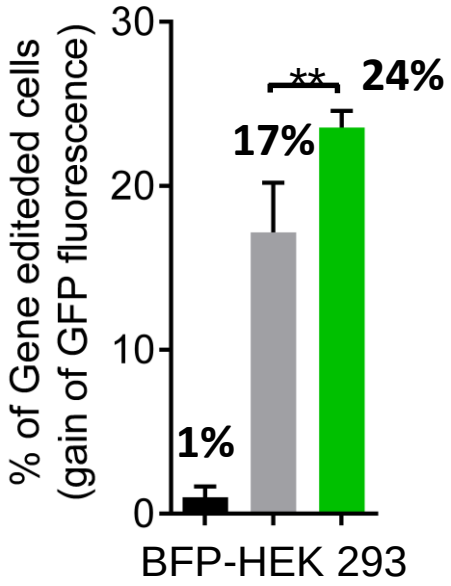
pH-Responsive Nanoparticles Enabling Both Gene Disruption and Gene Correction

Control
Lipo 2000
Hybrid NP

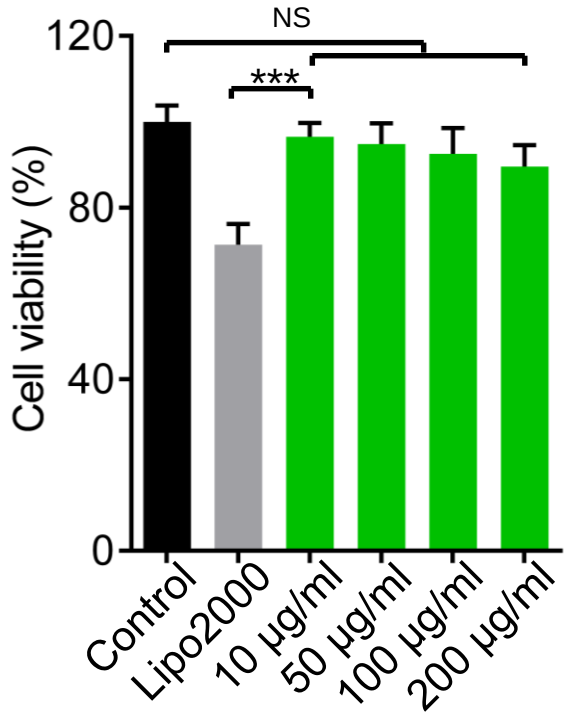
Payload: RNP
Gene Disruption



Payload: RNP + ssODN
Gene Correction/Precise editing



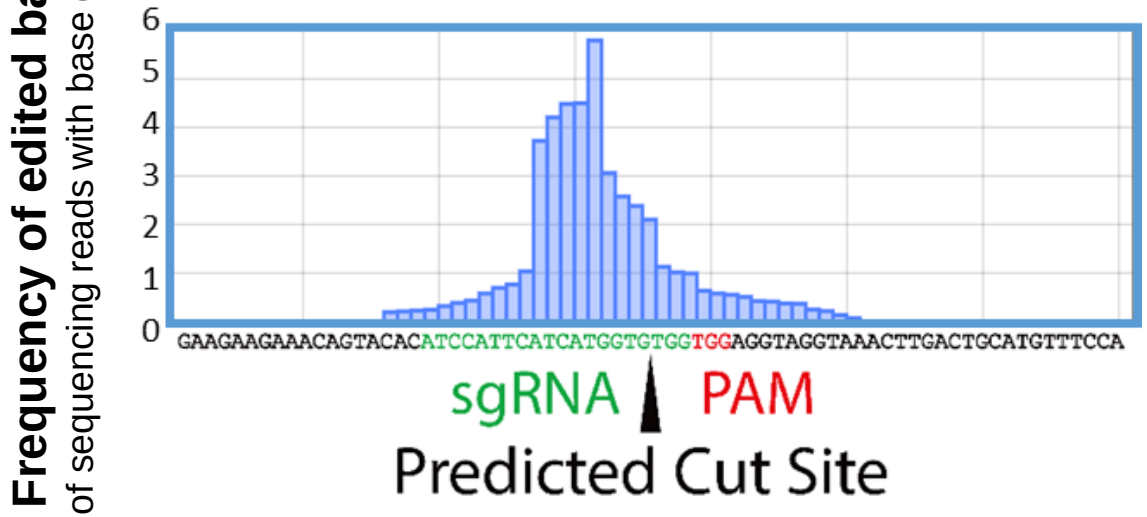
Cytotoxicity



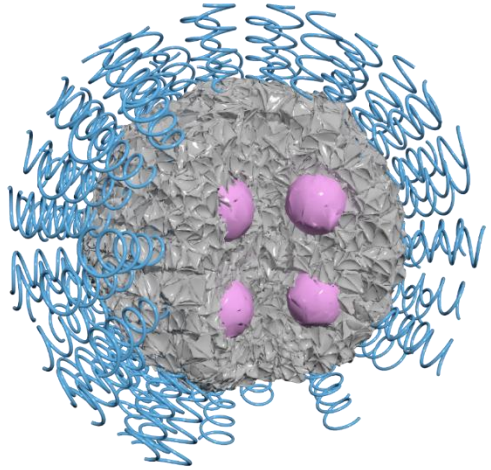
- Low cytotoxicity

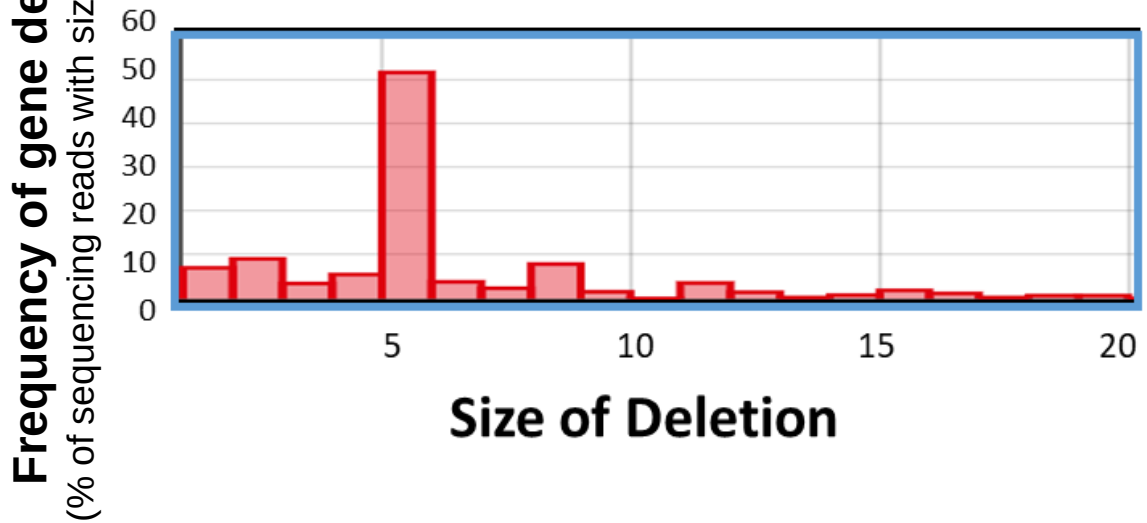
pH-Responsive Nanoparticles Targeting Mouse APP Gene in Primary Mouse Neurons

7.2% editing in primary neurons



APP: amyloid precursor protein

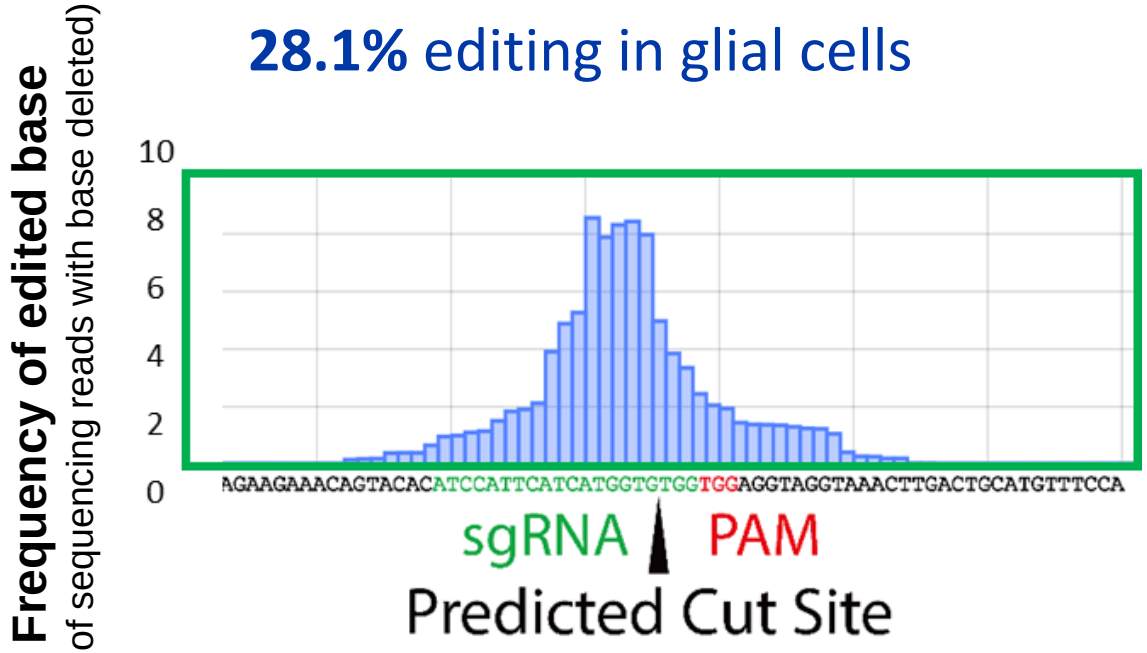




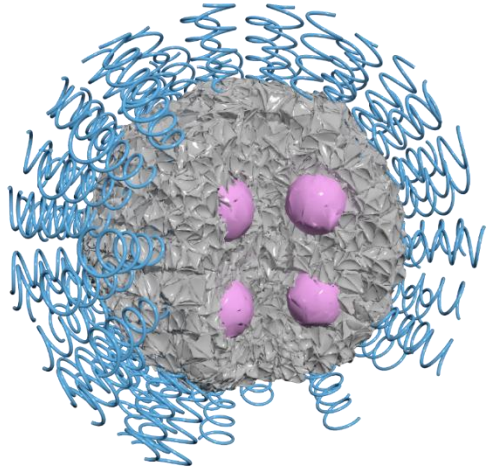
Preliminary data

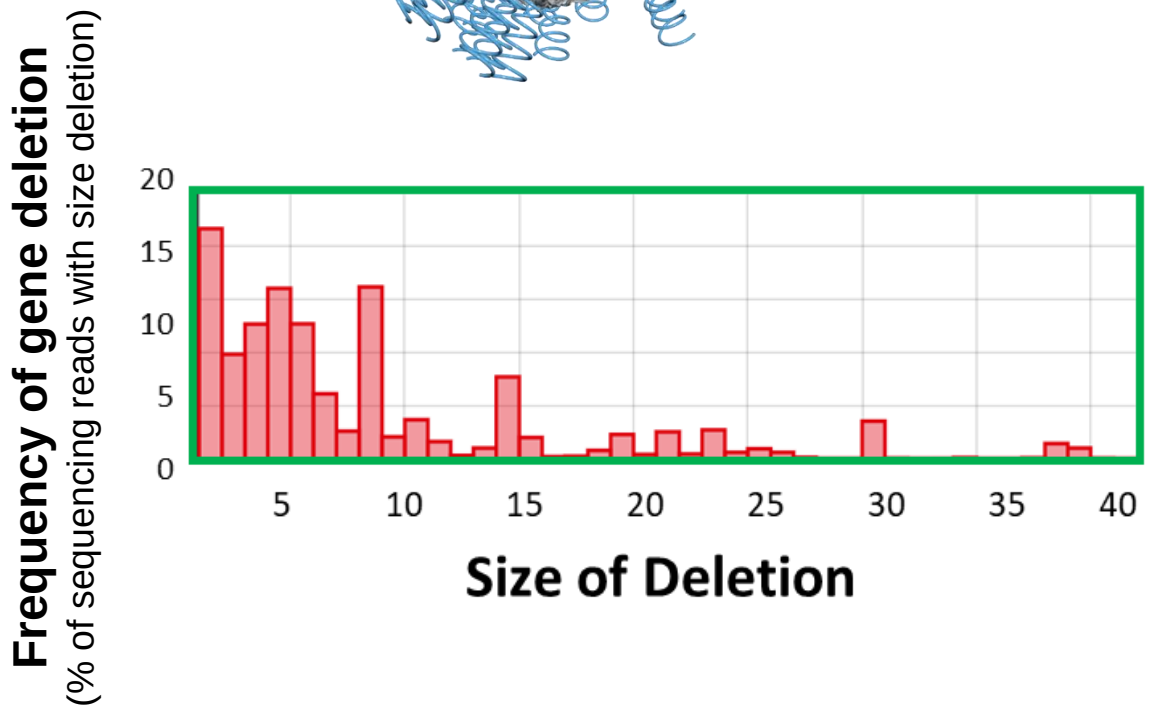
pH-Responsive Nanoparticles Targeting Mouse TH Gene in Mouse Glial Cells

28.1% editing in glial cells



TH: tyrosine hydroxylase

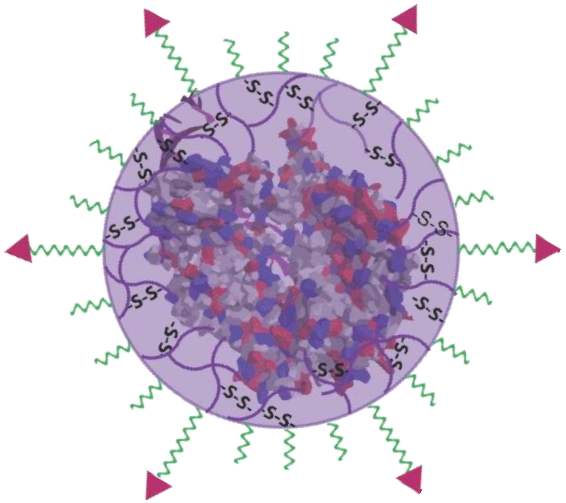




Preliminary data

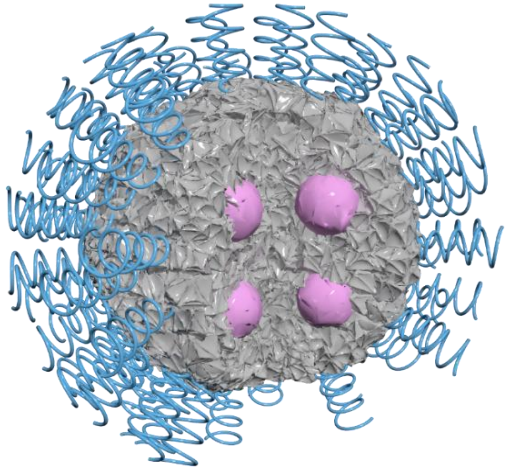
Summary

- CRISPR-Cas9 genome editing offers great promise to the field of neuroscience-both as a research tool and a treatment for various brain-related disorders.



~25 nm
RNP
Nanocapsule

- Capable of gene disruption
- Low cytotoxicity



~110 nm
pH-responsive nanoparticle
RNP, RNP + ssODN

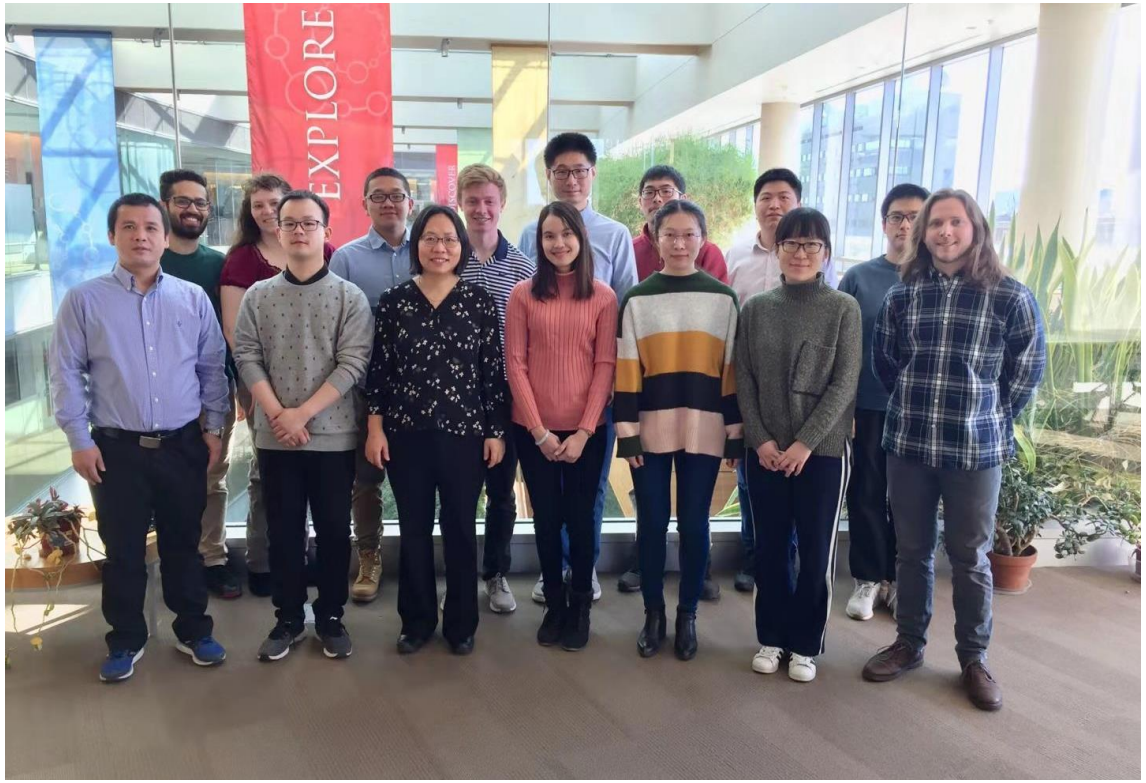
- Capable of gene disruption and gene correction
- Low cytotoxicity

- Future research will focus on further optimizing the performance of these nanoplatforms *in vivo*

Acknowledgments

The Gong Lab

Yuyuan Wang; Dr. Guojun Chen; Ruosen Alex Xie; Joseph Kraft; Dr. Xianghui Xu



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