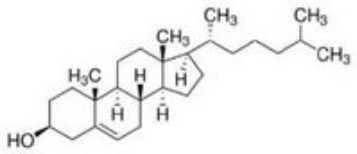
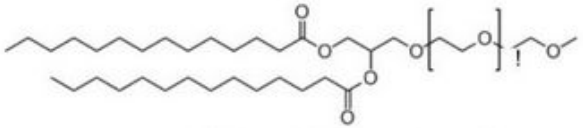
Synthetic, biodegradable lipid nanoparticles for gene therapy and gene editing

Rebecca Boiarsky & Vik Varma
2/8/2022
MIT 6.881/20.S948 Machine Learning Based Therapeutic Design

Lipid Nanoparticles (LNPs): Lipid Components

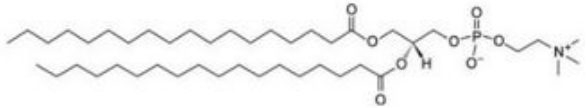


Cholesterol
(Cho)



1,2-dimyristoyl-rac-glycero-3-
methoxypolyethylene glycol-2000
(DMG-PEG 2000)
N = number of polyethyl glycol (PEG)

1,2-dilinoeyloxy-3-dimethylaminopropane
(Dlin-MC3-DMA) or (Ionizable MC3 lipid)



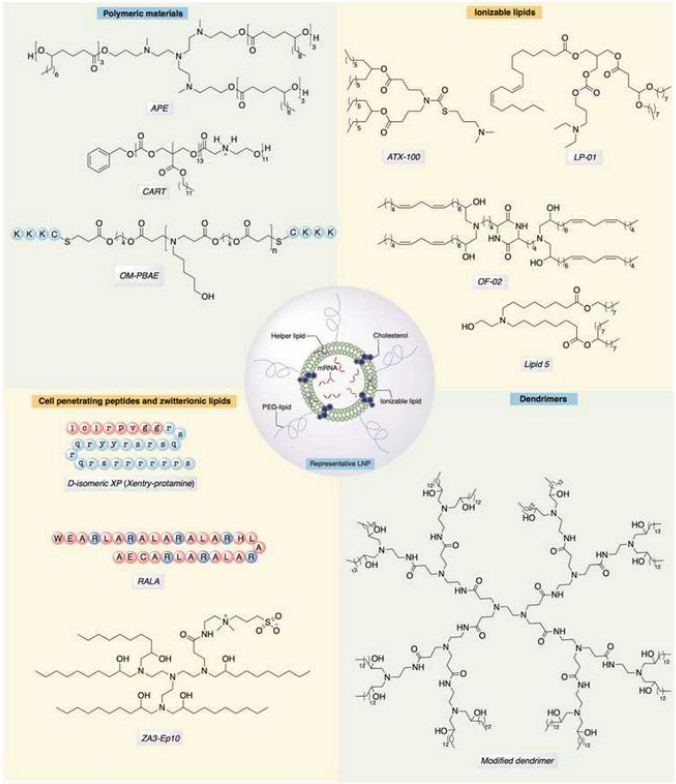
1,2-distearoyl-sn-glycero-3-phosphocholine
(DSPC) or PC(18:0/18:0)

LNPs consist of four lipid components (ionizable cationic lipid, distearolyphosphatidycholine or DSPC, cholesterol, and PEG-lipid)

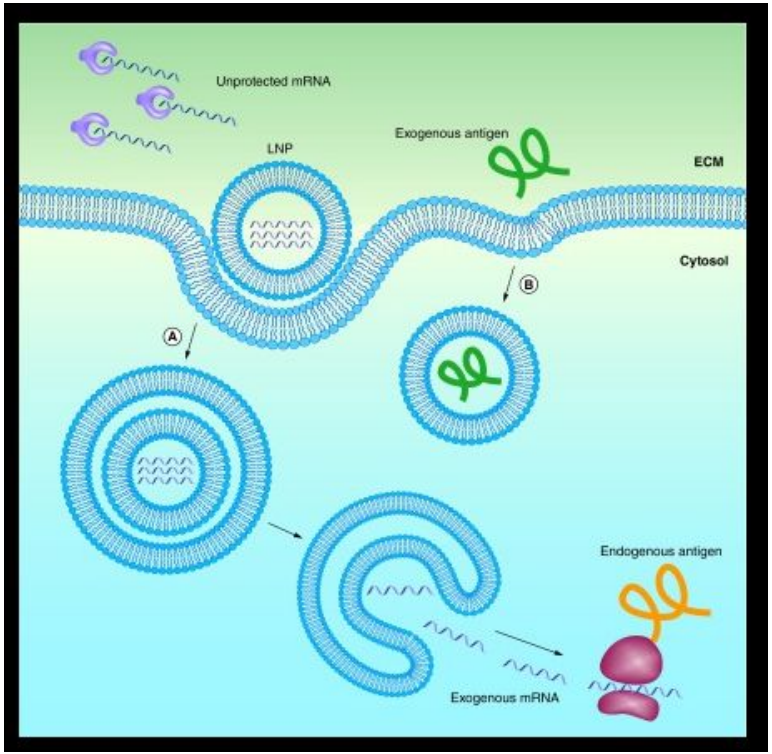
Lipid Nanoparticles (LNPs)

- Largest lipid component is **ionizable/cationic lipid** that drives potency
- Second largest lipid component is **cholesterol** for stability and membrane fusion
- **PEG-lipids** have been engineered to regulate LNP size and transfection potency
- **DSPC**’s role is less clear, but it is a “helper” lipid that is believed to help stability

Lipid Nanoparticles (LNPs)



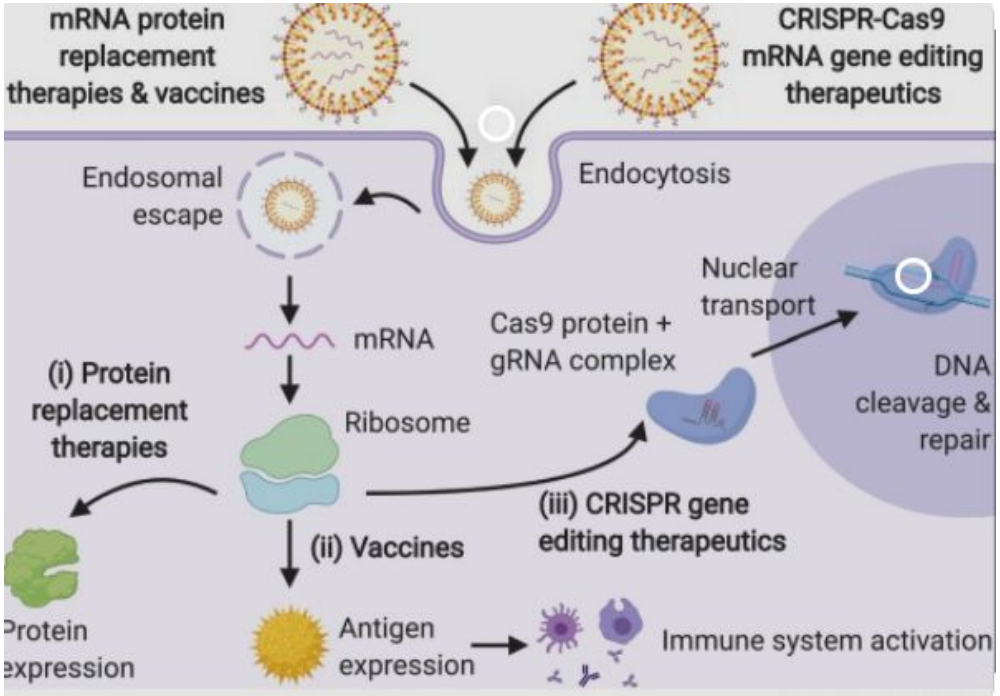
LNP Delivery



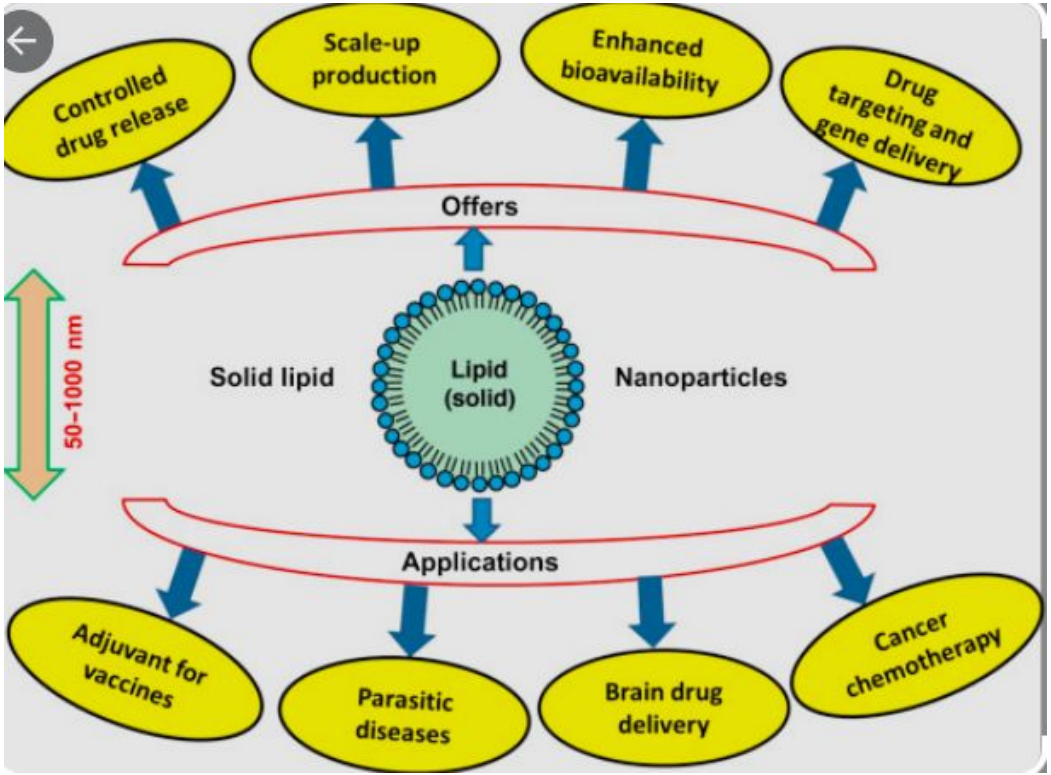
(a) A positively charged LNP with drug delivery at the negatively charged cell membrane. For mRNA delivery, the mRNA must escape both the LNP and the endosome.

(b) Extracellular proteins based vaccines are endocytosed in a similar manner, but do not need to escape from the endosome

LNP Delivery



LNP Applications



3 Different Applications of LNPs

1. Delivery of Cas9 mRNAs
2. Cancer vaccines
3. Delivery of cargo across blood-brain-barrier

Paper overview: Qui et al. 2021

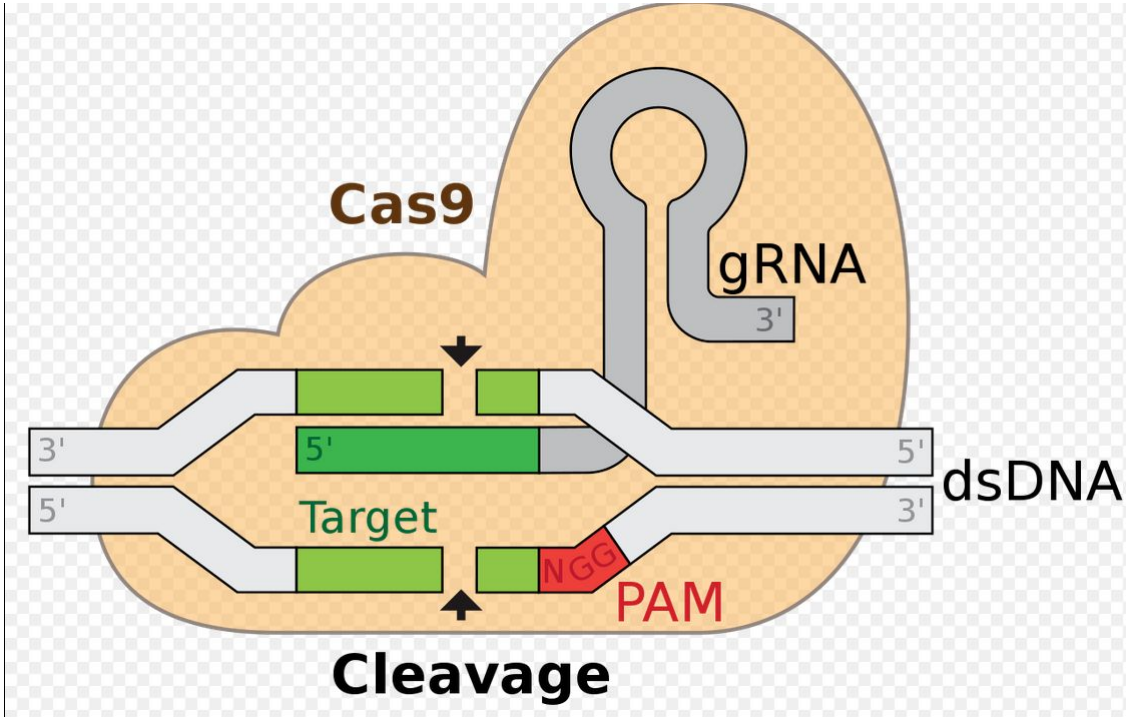
Lipid nanoparticle-mediated codelivery of Cas9 mRNA and single-guide RNA achieves liver-specific in vivo genome editing of Angptl3

Background

- CRISPR / CAS9 gene editing is based on a simplified version of the bacterial CRISPR / CAS9 antiviral defense systems

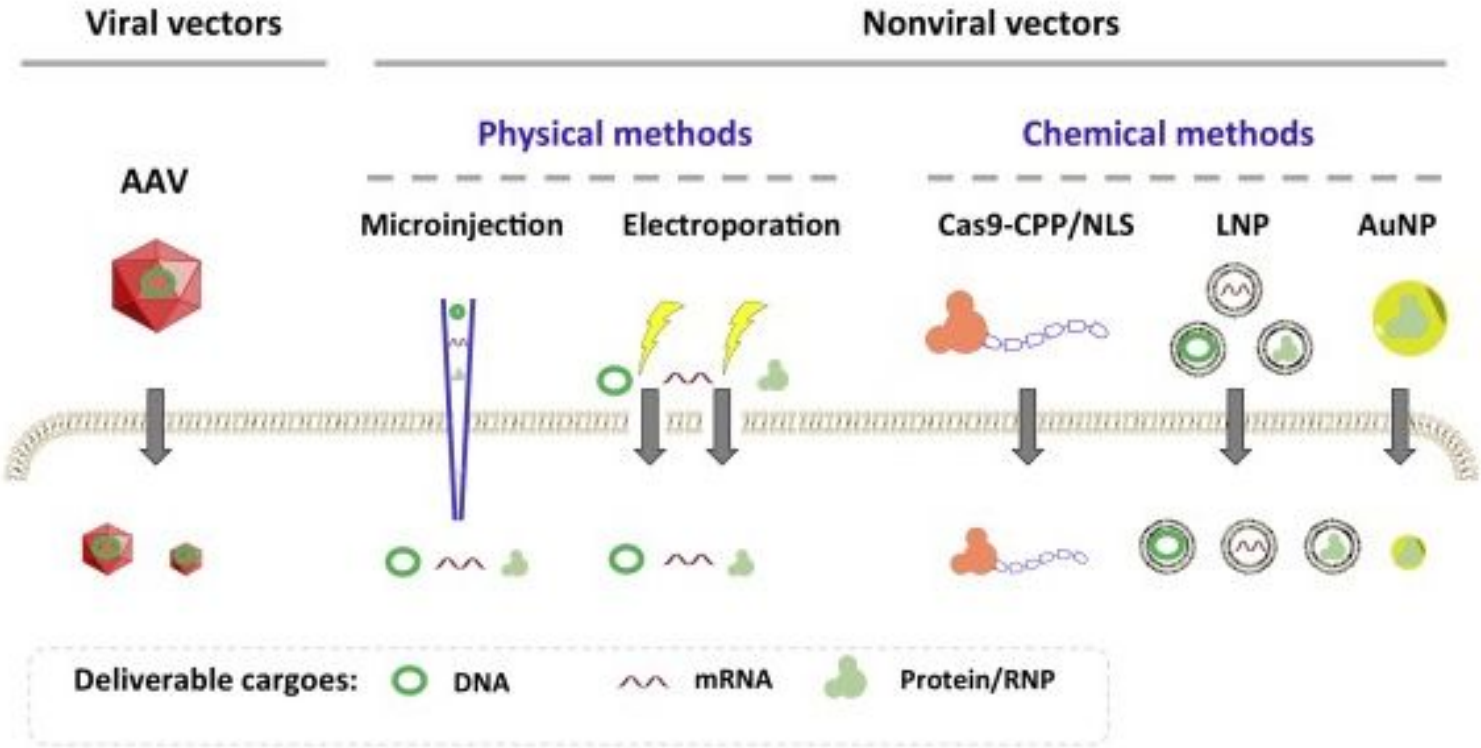
- By delivering the Cas9 nuclease complexed with a synthetic guide RNA into a cell, the cell's genome can be cut at a desired location, allowing existing genes to be removed and/or new ones added

Background: CRISPR/CAS9



Paper overview: Qui et al. 2021

Delivery Methods for CRISPR/CAS9



Delivery Methods for CRISPR/CAS9: AAVs

- AAVs are small (~20nm), non-pathogenic viruses
- Advantages: Not inserted into the host cell genome, and host immune responses are typically milder
- Disadvantages: The use of AAVs is limited by the size of cargo that can be packaged within them. May require two AACs to co-transfect cells

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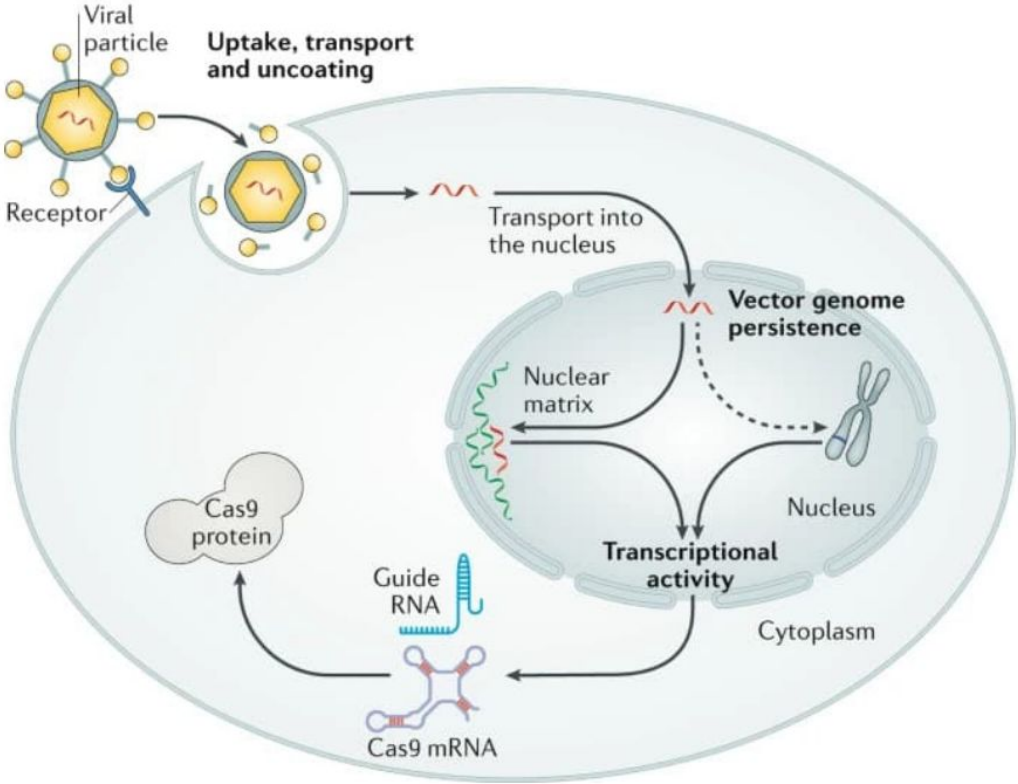


Delivery Methods for CRISPR/CAS9: Electroporation

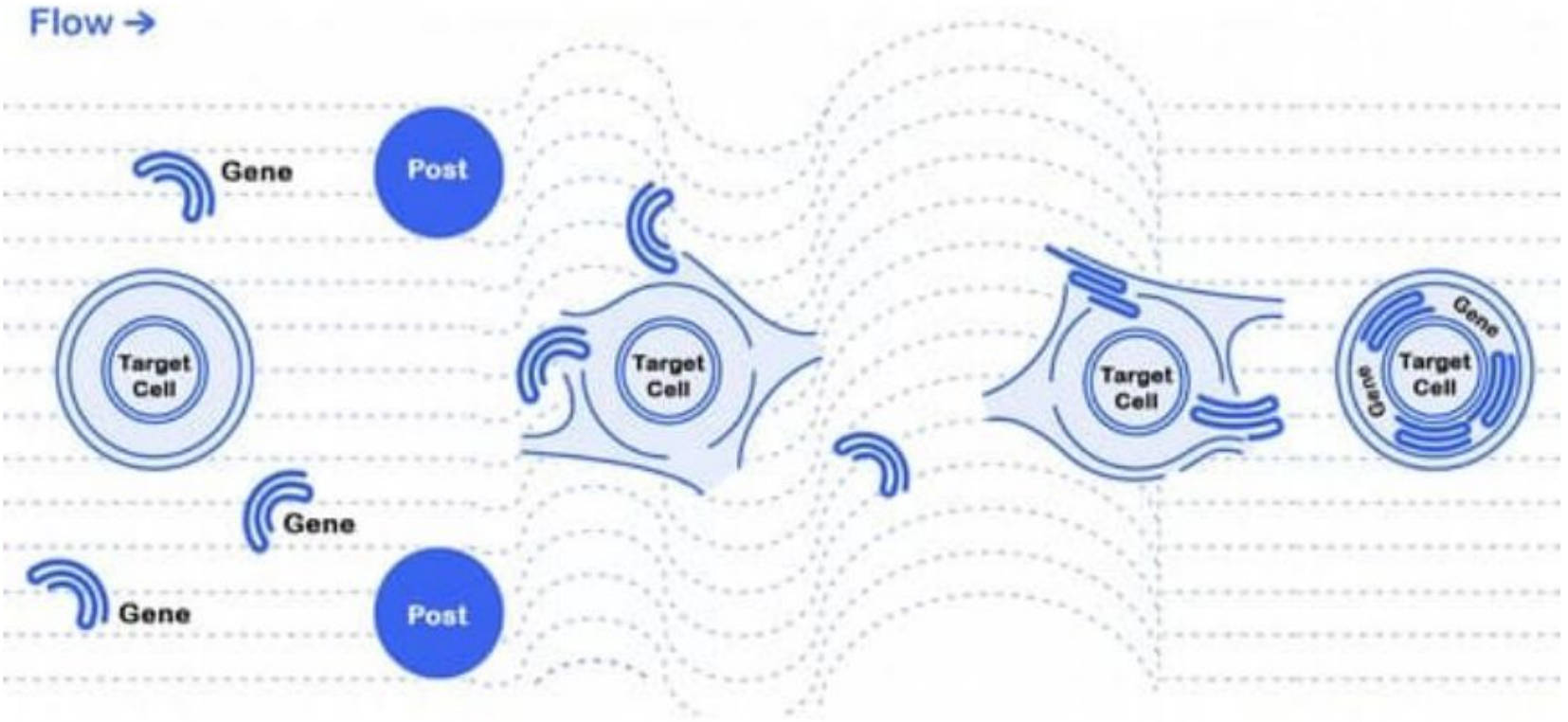
- Opening transient pores in the membrane of cells via high-voltage electrical currents, allowing CRISPR cargo to gain entry
- Advantages: Electroporation can be used for many different cell types, including those that are otherwise difficult to transfect
- Disadvantages: Mammalian cells are sensitive to electrical currents and electroporation can change the cell state

Delivery Methods for CRISPR/CAS9: Microfluidics

- Using microscopic channels to manipulate fluids and deliver cargo into cells
- Advantages: More gentle on cells than electroporation, and can be more efficient
- Disadvantages: Microfluidics is not yet a well-established method for CRISPR delivery, and as such the limitations are not well known. However, many microfluidic chips can only accommodate relatively small numbers of cells

Paper overview: Qui et al. 2021

Delivery Methods for CRISPR/CAS9: Microfluidics



Delivery Methods for CRISPR/CAS9: Microinjection

- Delivers cargo into individual cells by piercing the membrane with a microscopic needle
- Advantages: This method can deliver cargo of any molecular weight, at known quantities, to either the cytoplasm or nucleus of the cell
- Disadvantages: This method can be a challenging and laborious process

Delivery Methods for CRISPR/CAS9: LNPs

- Cationic liposomes are used to overcome the difficulties associated with delivering unstable mRNA
- Advantages: LNPs can be used with minimal safety and immunogenicity concerns due to lack of viral components
- Disadvantages: LNPs are usually encased by endosomes, and must ‘escape’ to avoid being directed into the lysosomal pathway and degraded, as well as gain access to the nucleus of the cell. These problems typically result in low transfection efficiencies.

Delivery Methods for CRISPR/CAS9: AuNPs

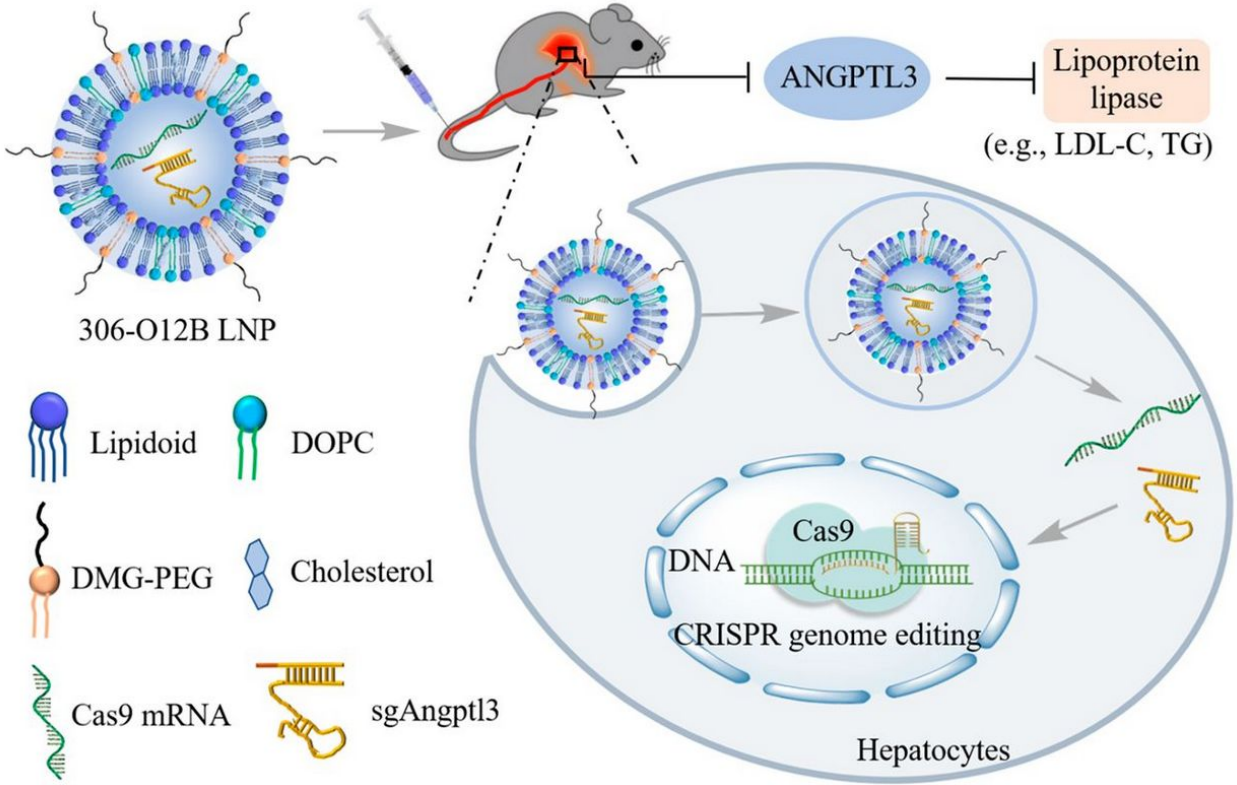
- Inorganic nanoparticles (NPs), such as gold nanoparticles (AuNPs)
- Advantages: High efficiency, relatively low cost, reduced off-target effects, high loading capacity, and lack of immune response and mutagenesis
- Disadvantages: AuNPs have a key disadvantage of being toxic at high concentrations, and since they are relatively new methods, there are some concerns regarding the long-term toxicity and accumulation of other NPs, such as magnetic NPs.

Creating new LNP to deliver genome editing

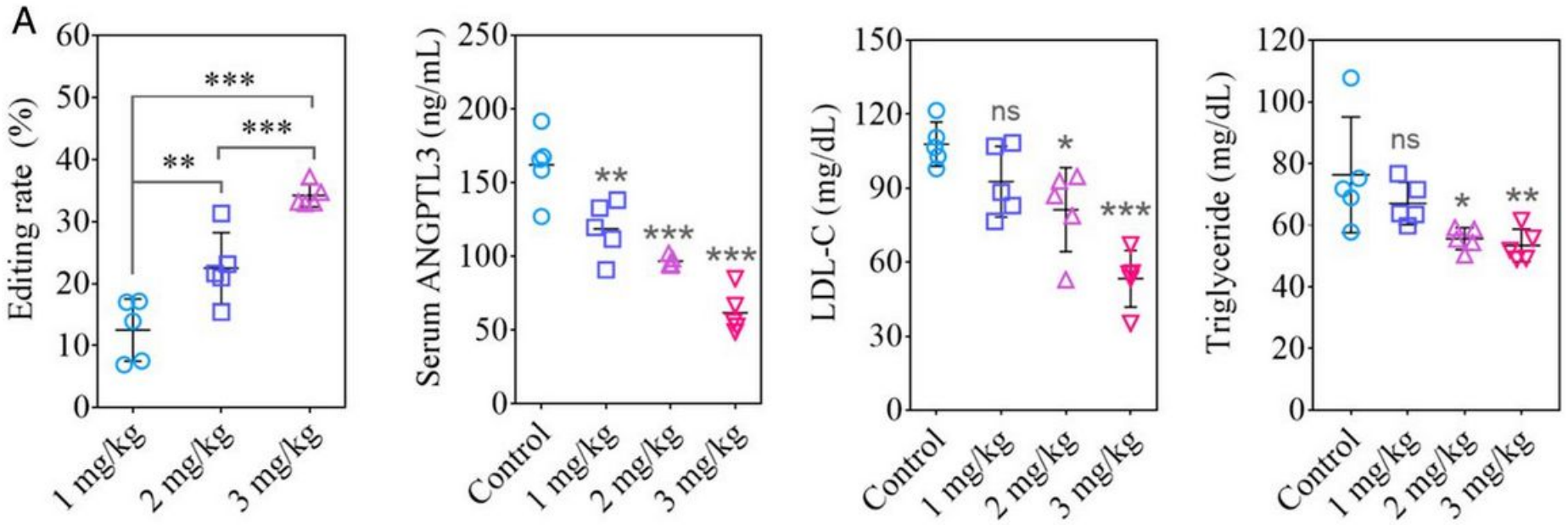
- Loss-of-function mutations in Angiopoietin-like 3 (*Angptl3*) are associated with lowered blood lipid levels, making *Angptl3* an attractive therapeutic target for the treatment of human lipoprotein metabolism disorders

- Paper describes a highly potent nonviral LNP-mediated CRISPR-Cas9 delivery system for the liver delivery of Cas9 mRNA and demonstrate its efficacy by targeting the *Angptl3* gene

Creating new LNP to deliver genome editing



Creating new LNP to deliver genome editing

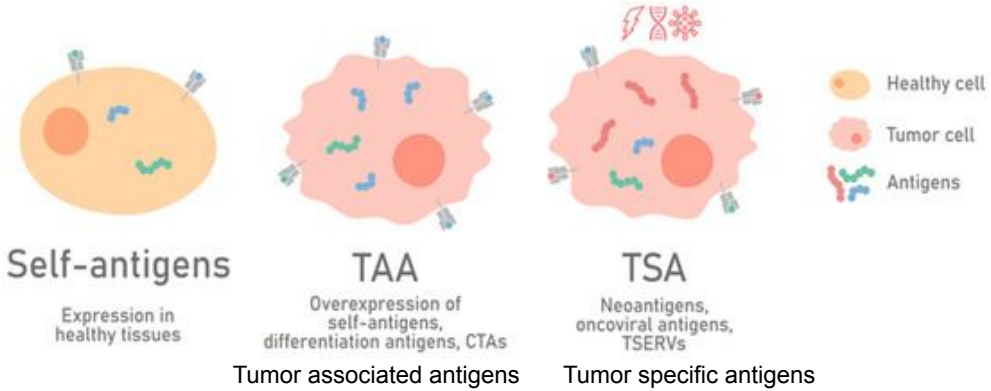


Paper overview: Chen et al. 2021

In situ cancer vaccination using lipidoid nanoparticles

To create a cancer vaccine, we want to:

1. Access the antigens of the cancer cells present in a particular tumor
   a. these may vary between individuals, or even between tumor cells at different sites or moments in time
2. Enhance the cross-presentation of tumor antigens
3. Overcome the immunosuppressive tumor microenvironment and TURN UP the immune response



Any molecule capable of being recognized by the immune system is considered an antigen. Many tumor cells produce antigens, which may be released in the bloodstream or remain on the cell surface.

(Figure: Feola et al. *Cancers* 2020)

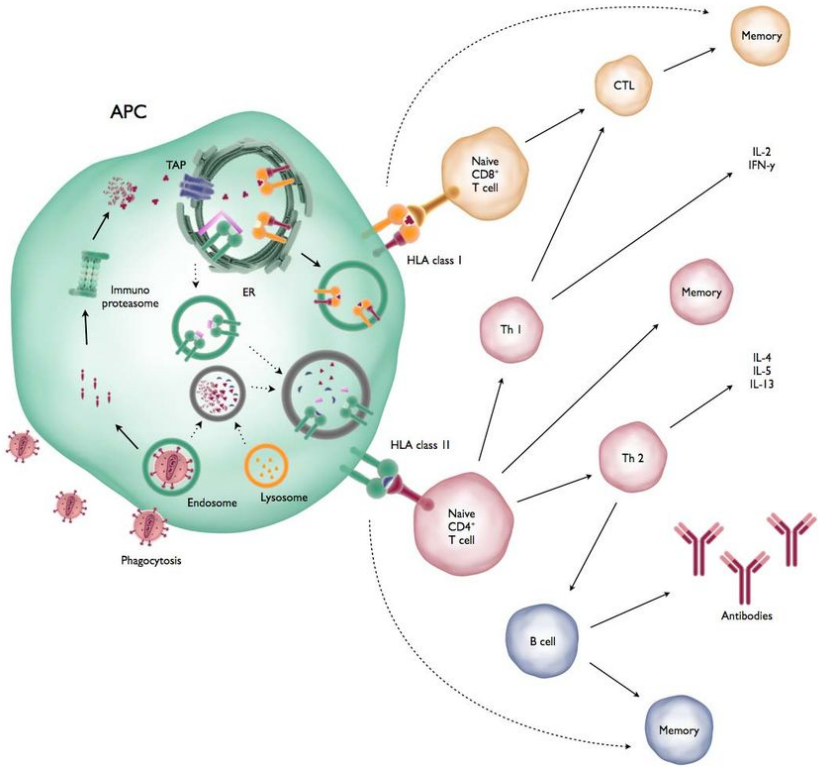
Definitions

- In situ: “in the original place”
- TAA: tumor associated antigens
- In situ vaccination:
  - Inducing and stimulating an immune response specifically at the tumor site[1]
  - Exploits TAAs available at a tumor site to induce a TAA-specific adaptive immune response[2]
  - TAAs are commonly released upon tumor cell death and may be subsequently processed and presented by antigen presenting cells[2]

1. https://www.frontiersin.org/articles/10.3389/fimmu.2021.650486/full
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5528727/

Immunology background: cross-presentation

- All nucleated cells express MHC class I molecules, which present peptides derived from endogenous proteins that are degraded in the cytosol by the proteasome
- MHC class I antigen presentation pathway enables the immune system to detect transformed or infected cells displaying peptides from modified-self or foreign proteins
- Naive antigen-specific CD8+ T cells, however, cannot directly eliminate transformed or infected cells. To become effector cytotoxic T lymphocytes (CTLs), they need to be activated by 'professional' antigen-presenting cells (APCs).
- When the APCs are not directly infected, they need to acquire exogenous antigens from the infectious agent and present them on MHC class I molecules, by a mechanism known as **cross-presentation**.



Joffre, O., Segura, E., Savina, A. et al. Cross-presentation by dendritic cells. Nat Rev Immunol 12, 557–569 (2012). https://doi.org/10.1038/nri3254
Figure: Arcanjo et al. Acta Biochim Pol, 2014

Cross-presentation continued

- Free antigens, such as OVA, may be internalized by APCs, degraded by enzymes in the lysosome and bound to the MHC class II complex
    - Upon presentation on the surface of the APC, would stimulate CD4+ T cells and generate a primarily antibody-based immune response.
- If the antigen can be **delivered to the cytosol of the APCs**, then it could instead be degraded by the proteasome and incorporated into the **MHC class I** molecules, where it is **cross-presented to cytotoxic CD8+ T cells** instead.
- The CD8+ T cell response is known to be critical to cancer immunotherapy.

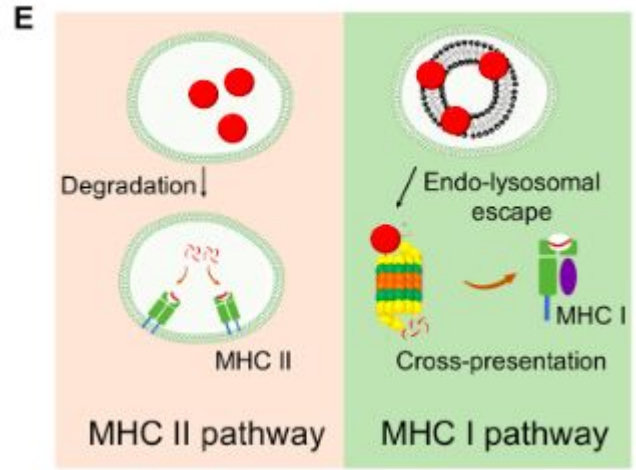


Figure 2 (Chen et al. 2021)

To create a cancer vaccine, we want to:

1. Access the antigens of the cancer cells present in a particular tumor

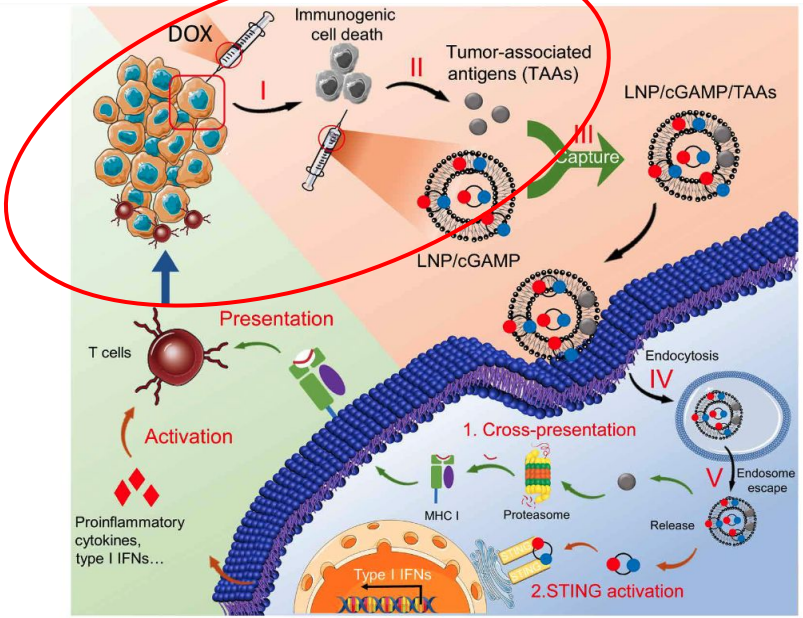
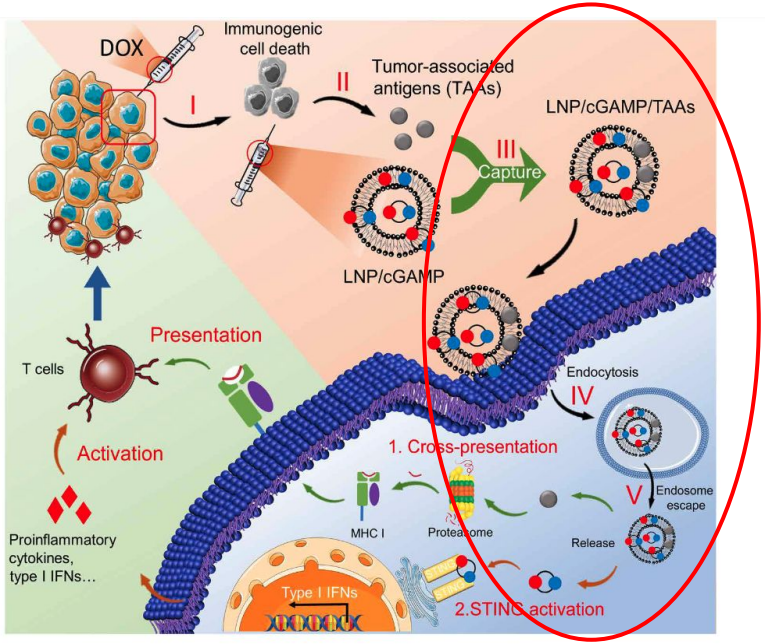


Fig. 1. The scheme illustration of LNP system–mediated antigen capturing, cross-presentation, and STING activation. (**I**) Low dose of DOX-induced immunogenic cancer cell death. (**II**) TAAs were released after the administration of low dose of DOX. (**III**) The released TAAs were captured by lipidoid nanoparticle (LNP)/2′5′-3′5′ cyclic guanosine monophosphate-adenosine monophosphate (cGAMP). (**IV**) The TAAs and cGAMP encapsulated in LNPs were delivered into APCs via endocytosis. (**V**) The TAAs and cGAMP escaped from endo/lysosomes to cytoplasm for further cross-presentation and STING activation.

To create a cancer vaccine, we want to:

2. Enhance the cross-presentation of tumor antigens



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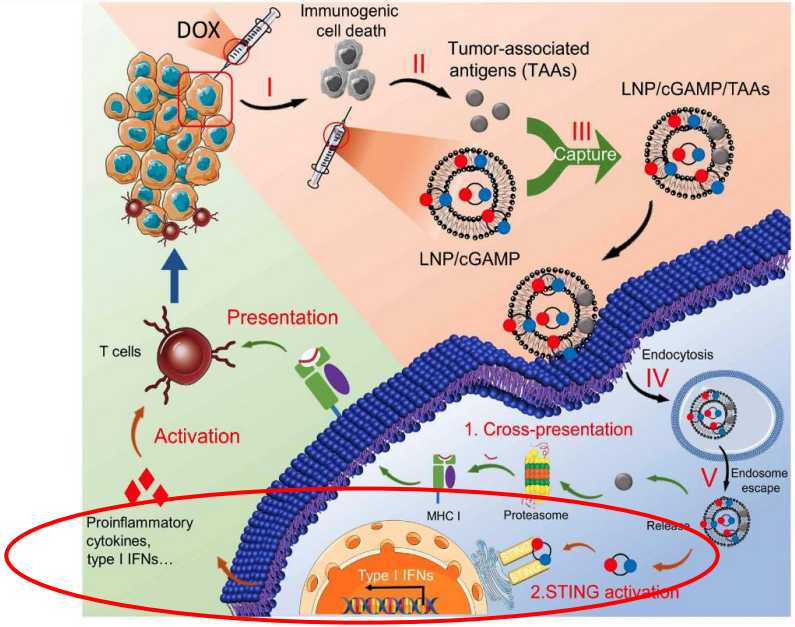
To create a cancer vaccine, we want to:

3. TURN UP the immune response



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Other approaches to in situ vaccination

- Oncolytic viruses
  - = viruses that preferentially infect and kill cancer cells
  - Safety concerns in patients - cytokine release syndrome
  - Not easy to produce
- Non-viral based in situ vaccination
  - Does not capture the antigens present in situ
  - Does not enhance cross presentation

Choosing the lipidoid

- Position and structure of lipidoid greatly influence the adjuvant (immune stimulating) effect of the LNP
- An ideal lipid nanoparticle for cancer immunotherapy should be able to
  - (i) capture the released tumor antigen and deliver into APCs with enhanced cross-presentation and
  - (ii) generate immunostimulatory effect

Choosing the lipidoid with greatest immunostimulatory effect

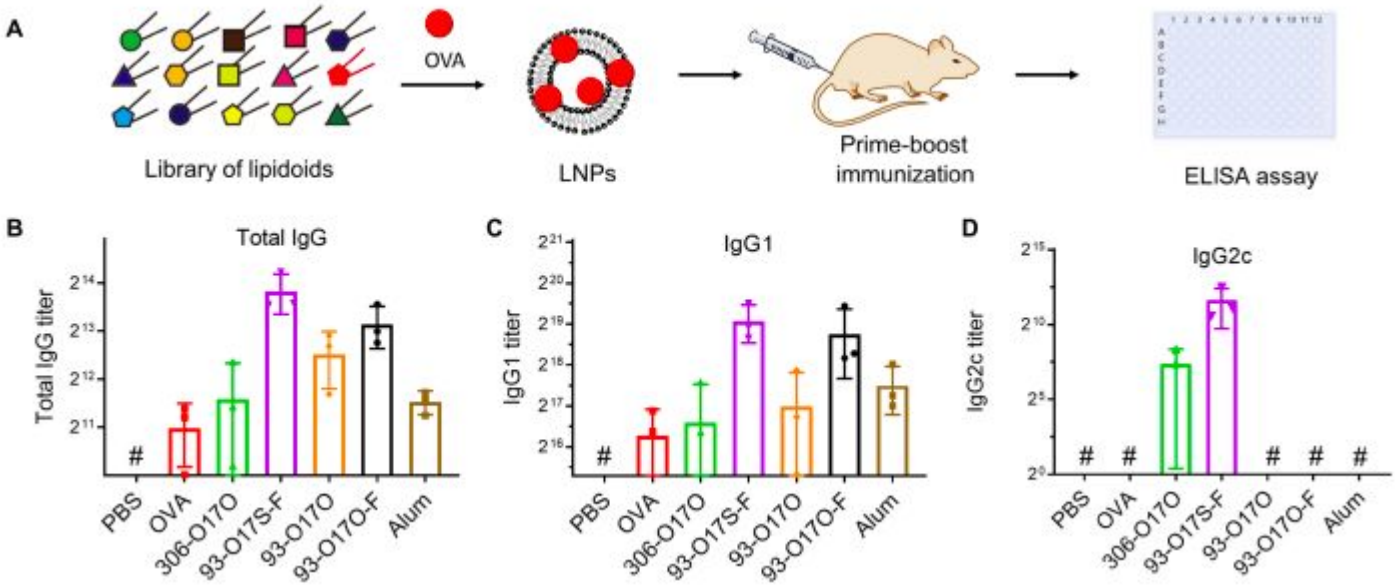

Figure 2

Intracellular delivery of cGAMP by LNP stimulates STING pathway

- The protein STING (stimulator of interferon genes) turns up the innate immune response
- cGAMP activates STING
  - results in the activation of APCs, the production of IFNs, and the priming of CD8+T cells against tumor antigens
- However, cGAMP itself is not able to freely cross the cell membrane to reach the STING promoters on the endoplasmic reticulum.

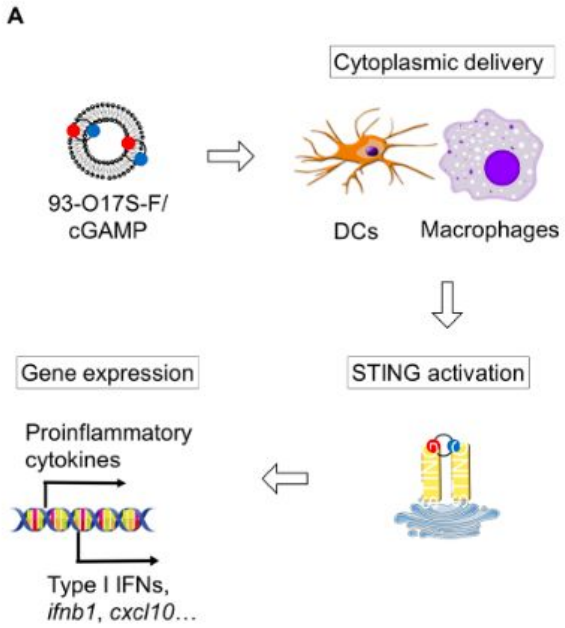

Figure 3A

Intracellular delivery of cGAMP by LNP stimulates STING pathway

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- However, cGAMP itself is not able to freely cross the cell membrane to reach the STING promoters on the endoplasmic reticulum.
- **LNPs can serve as the carrier for cGAMP!**

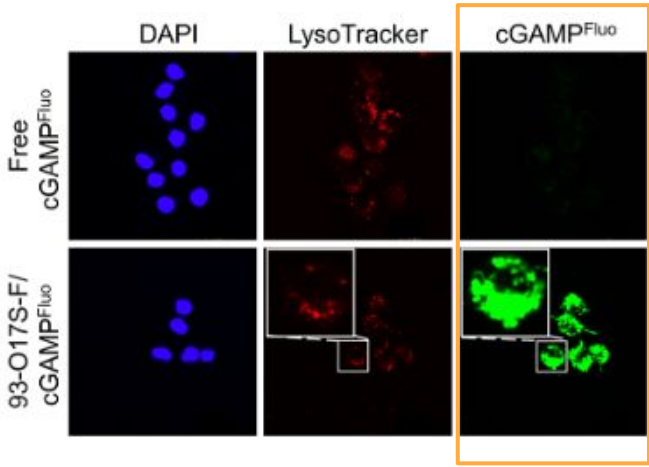
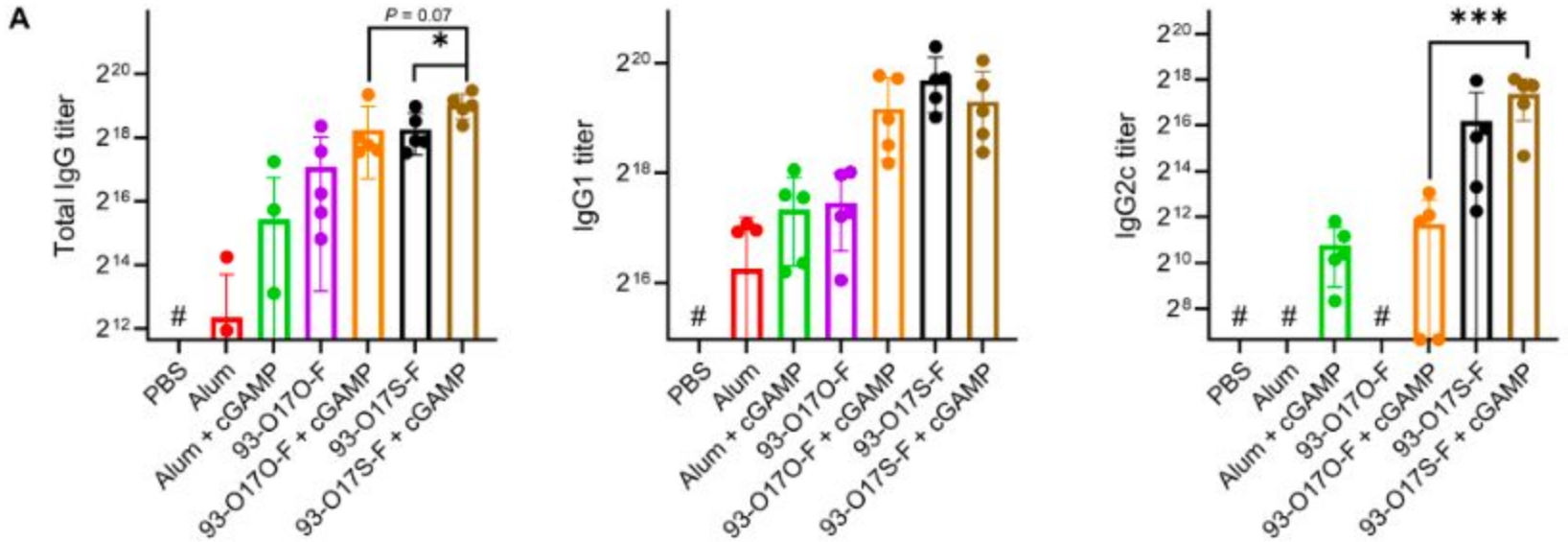


Figure 3B

Fig. 4. Enhanced humoral and cellular immune response by codelivery of cGAMP & OVA



(OVA, the reference antigen, is included in all the groups except for PBS)

In vivo antigen uptake and immune activation by 93-O17S-F/cGAMP

- Mice were injected with $OVA^{Alexa\text{-}647}$ (fluorescent), then either 93-O17S-F/cGAMP or PBS control
- When the second injection contained only PBS, $OVA^{Alexa\text{-}647}$ was mainly found in the bladder, suggesting the rapid clearance of the soluble protein through urine.
- By contrast, when the second injection contained 93-O17S-F/cGAMP, $OVA^{Alexa\text{-}647}$, was found in the draining lymph nodes

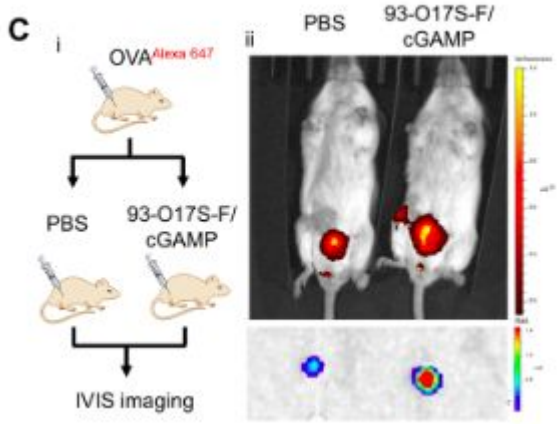
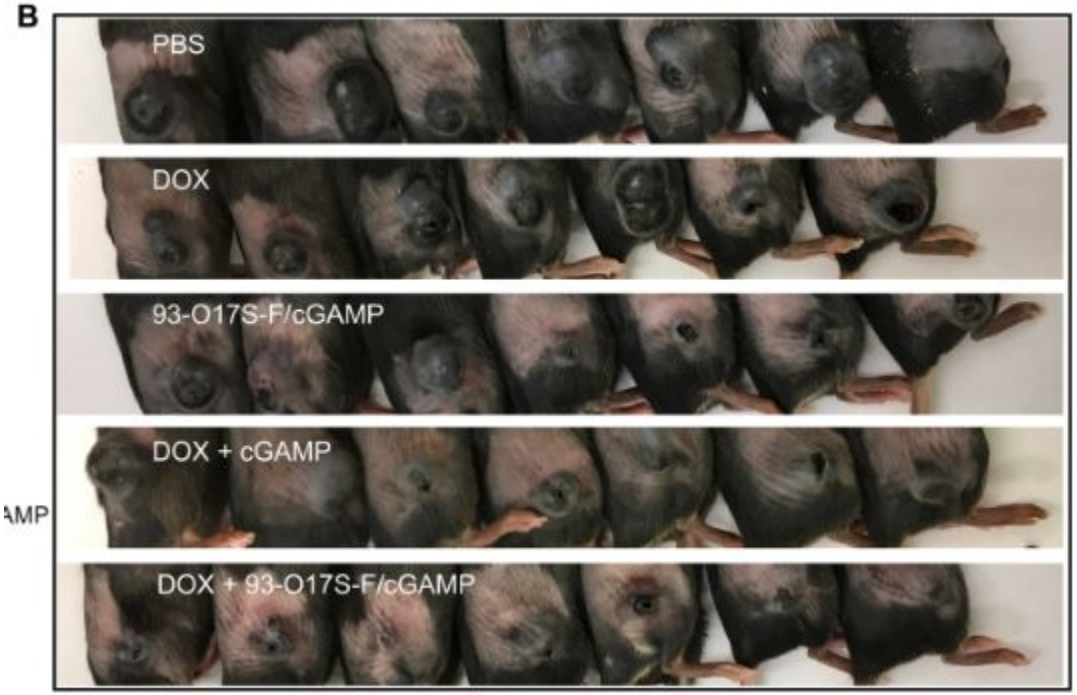
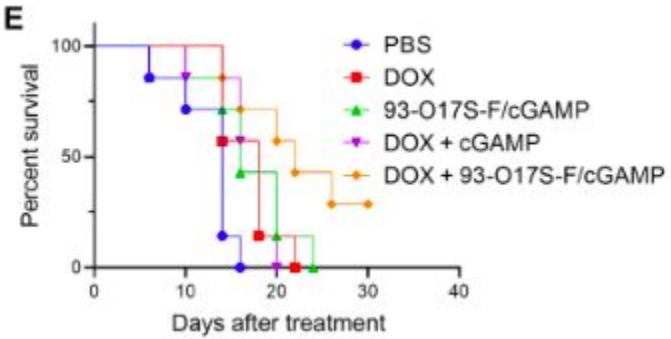


Figure 5C

Therapeutic effect by in situ vaccination of 93-O17S-F/cGAMP (Figure 6)



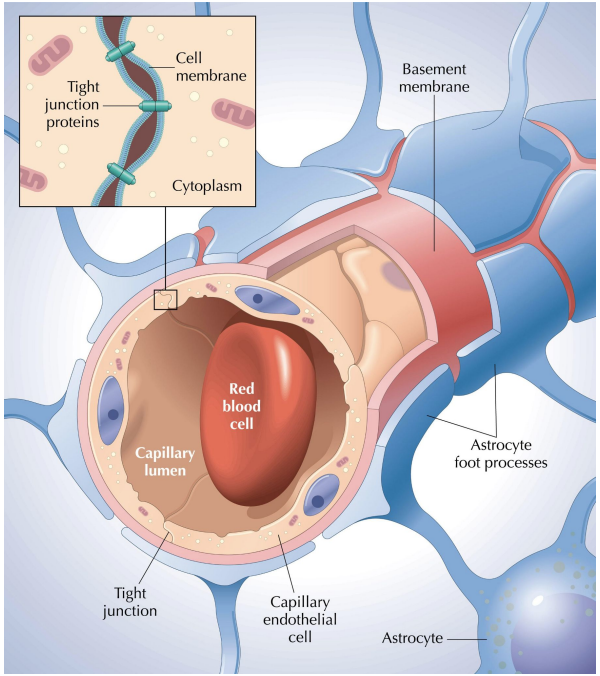


Paper overview: Ma et al. 2020

Neurotransmitter-derived lipidoids (NT-lipidoids) for enhanced brain delivery through intravenous injection

Background & Motivation

- Blood brain barrier (BBB): a highly selective semipermeable border of endothelial cells that prevents solutes in the circulating blood from non-selectively crossing into the extracellular fluid of the central nervous system (CNS) where neurons reside.
- The treatment of CNS diseases, such as neurodegenerative disorders, brain tumors, brain infections, and stroke, is severely constrained by the BBB because it prevents the transfer of most of small-molecule drugs and macromolecules (e.g., peptides, gene drugs, and protein drugs) into the brain



(photo credit: James Perkins)

Existing approaches to brain delivery

- direct central nervous system (CNS) administration
  - Invasive
  - may cause infection and tissue damage
  - limited by diffusion distance and rapid efflux of drugs out of the CNS within hours
- disruption of the BBB
  - BBB openings also allows for the leakage of plasma proteins into the brain, leading to neurotoxicity, vascular pathology, and chronic neuropathologic changes in the brain
- **carrier vehicle–mediated delivery** – but what carrier to use?
  - Viral vectors are effective but have limitations such as production cost and safety concerns
  - Exosomes have been used, but there still exist many challenges such as cargo loading procedure & in vivo toxicity
  - …more carriers and their shortcoming are detailed in the intro
  - **neurotransmitter (NT)–derived synthetic lipids**

This paper's big hypothesis

- Neurotransmitters (NTs) = endogenous chemicals that enable neurotransmission
- Notably, some NTs have been demonstrated to cross the BBB
- **Hypothesis: synthetic lipids derived from these NT derivatives will retain their capability for crossing BBB and may be useful as drug carriers for brain delivery**

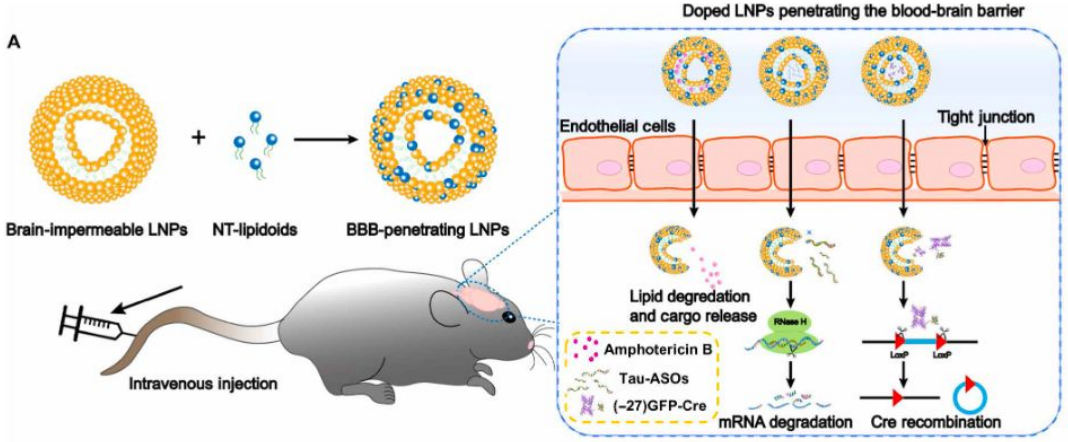


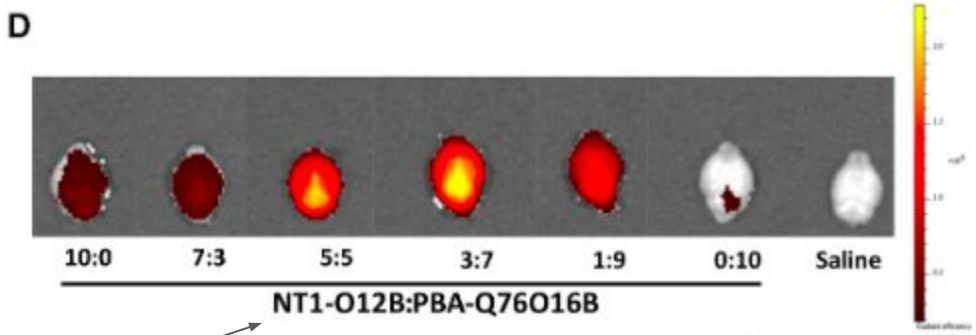
Figure 1

Overview of Results

- Synthesized lipidoid from three neurotransmitters
  - tryptamine (NT1)
  - phenethylamine (NT2)
  - phenylethanolamine (NT3)
- Doping the **NT1-lipidoids** into BBB-impermeable LNPs resulted in LNPs that can cross BBB effectively
  - (“dope” = add a substance to improve the properties of something)
- Successfully delivered three classes of cargos
  - small molecule
  - nucleic acid
  - protein

Delivery of small-molecule AmB into the mouse brain

- AmB is an antifungal drug and is the gold standard for the treatment of severe systemic fungal infections
- However, unable to treat brain infections because BBB impermeable
- If formulated in synthetic LNPs: cannot permeate BBB
- Here, formulated in NT1-lipidoids:



(ratio of two different lipidoids to control overall size of LNP)

Figure 2

Delivery of nucleic acid Tau-ASOs into the mouse brain for gene knockdown

- ASO = antisense oligonucleotide = compound that is able to bind messenger RNAs (mRNAs) to inhibit protein expression
- ASO-mediated tau reduction has shown promising results in the treatment of Alzheimer's disease after the local injection of the Tau-ASO using an intracerebroventricular (ICV) pump
  - invasive injection directly into the cerebrospinal fluid
- Needs to cross two barriers: BBB & cell membrane
  - ASOs must be present inside a cell expressing the target mRNA to functionally knock down the expression of that target

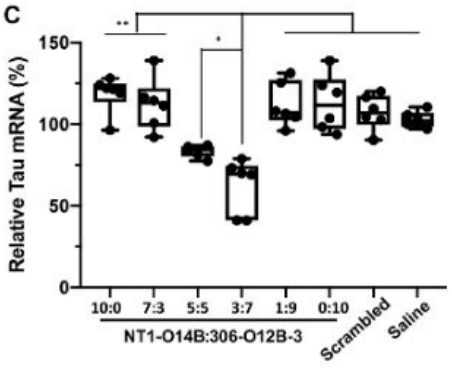


Figure 3

Delivery of GFP-Cre fusion protein for gene recombination in the Ai14 mouse brain

- Delivery of genome-editing proteins for genome modification in the brain has therapeutic potential for the treatment of CNS disorders.
- Current protein delivery strategies are mainly through local injection to the brain, which typically requires invasive drilling through the skull to access the tissue
- The successful intracellular delivery of Cre protein into the cells of Ai14 mouse leads to the gene recombination and turns on the tdTomato expression, which can be directly visualized as red fluorescence signal without additional staining
- Genome editing was observed in various different regions of the mouse brain, including cerebral cortex, hippocampus, and cerebellum

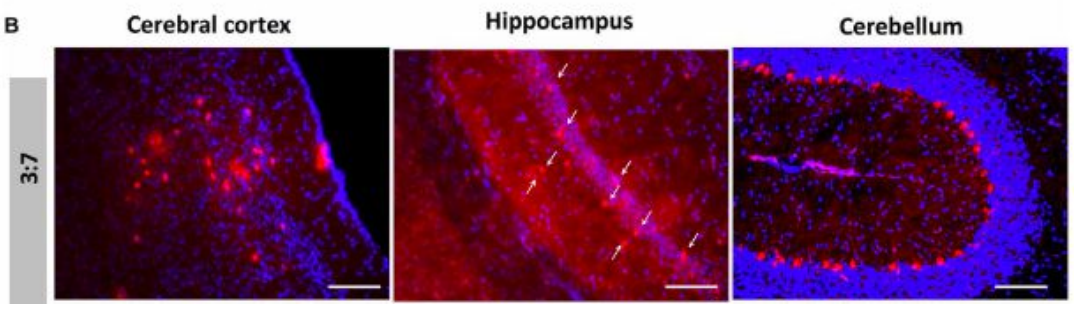


Figure 4