Non-Viral Nanocarriers for CRISPR-Based Genome Editing Tool Delivery

Yuyuan Wang[1, 2], Shaoqin Gong[1, 2 *]

1. Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI 53715, USA
2. Wisconsin Institute for Discovery, University of Wisconsin-Madison, Madison, WI 53715, USA

*: Correspondence should be addressed to SG (shaoqingong@wisc.edu)

Statement of Purpose: The CRISPR genome editing technique is a powerful tool, which can be used to treat many genetic disorders. However, clinical translation of CRISPR-based genome editing techniques has been slow largely due to the lack of safe and efficient delivery systems. We sought to develop customizable and efficient nanoplatforms to deliver CRISPR-based genome editing tools *in vitro* and *in vivo*. Our design criteria include: (1) good biocompatibility, (2) high loading content and loading efficiency, (3) excellent stability before reaching the target cell, (4) high specificity to target tissue/cell, (5) high cellular uptake, (6) efficient endosomal escape capability, (7) rapid release of the cargo inside the cytosol via a stimuli-responsive release mechanism, and (8) efficient transport to nucleus.

Methods: We engineered and optimized various types of multifunctional nanocarriers that meet our design criteria for safe and efficient delivery of various payloads (e.g., RNP and nucleic acids), including polyplexes, polymeric nanocapsules and silica-metal-organic framework hybrid nanoparticles (SMOF NP) (**Figure 1**). The *in vitro* and *in vivo* genome editing efficiency of these nanocarriers were studied, and the biocompatibility was investigated.

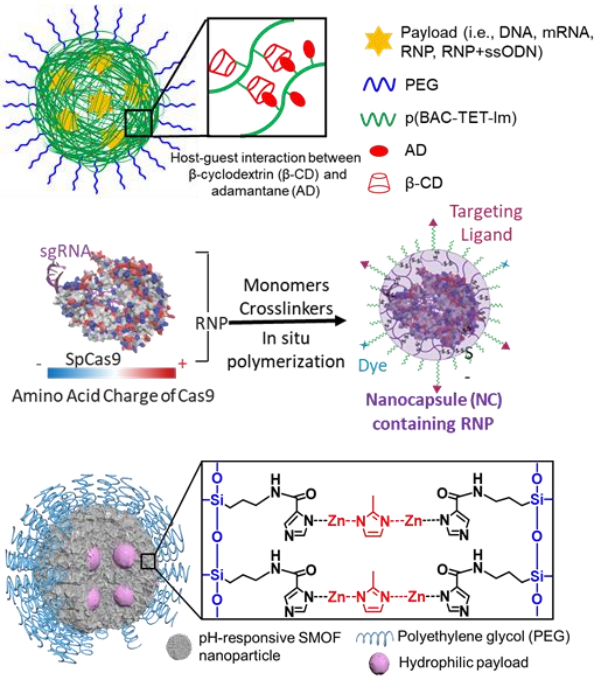

Figure 1. Multifunctional nanocarriers for the delivery of CRISPR systems.

Results: Our judiciously designed nanocarriers are able to encapsulate and deliver different forms of CRISPR-Cas genome editing tools (i.e., RNP, DNA and mRNA) with high loading contents and high loading efficiencies, therefore producing robust genome editing *in vitro* and *in vivo* in different cells/tissues without apparent toxicity. **Figure 2** shows *in vivo* genome editing in murine retinal pigment epithelium (RPE) tissue induced by RNP-encapsulated SMOF NPs.

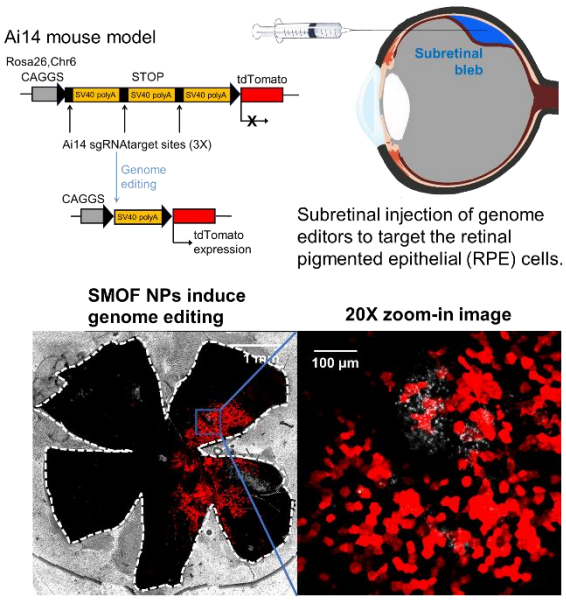

Figure 2. SMOF NPs induced efficient genome editing *in vivo* in Ai14 mice via subretinal administration.

Conclusions: Our delivery nanocarriers demonstrated efficient genome editing capability and good biocompatibility. Moreover, a key feature of these nanoplatforms is their customizability that allows for incorporation of different targeting ligands and cargoes. Thus, we anticipate that they could be further tailored to enable gene therapy and genome editing for the treatment of many different types of diseases.

References:

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