

**Thesis Title:** Development of a Structural Nucleic Acid Delivery Vector Technology  
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The safe, cell-specific delivery of biomacromolecules such as proteins and nucleic acids represents a major technical bottleneck in the translation of new medicines to the clinic today. An idealized delivery vector for these applications is safe, effective, scalable, and versatile. This thesis is focused on the development of a new delivery vector technology that is based on nucleic acid nanoparticles fabricated using the DNA origami technique. These materials are fabricated via the self-assembly of a long single-stranded nucleic acid 'scaffold' and many short oligonucleotide 'staples' into user-defined nanostructures determined by the sequences of the nucleic acids. Due to their well-defined composition and structure, biocompatibility, and ability to incorporate secondary molecules, nucleic acid nanoparticles serve as a candidate technology to build a delivery vector platform on.

The first section of my thesis focuses on the safety of nucleic acid nanoparticles. In wild-type mice, we administered a nucleic acid nanoparticle, approximately 40 nanometers in diameter, intravenously at a therapeutically relevant dose, and assessed the baseline non-clinical safety of these materials. We did not observe any signs of toxicity via animal behavior and body weight, we did not observe any abnormalities in the hematology or clinical biochemistry, and we did not observe any toxicity in the histopathology analysis. While we observed no toxicities in this experiment, it is also known that nucleic acids can be toxic via activation of the immune system. We further explored this direction in more detail, where we did not observe immune cell proliferation via blood cell counts, or pro-inflammatory cytokine induction. To investigate the immunogenicity of nucleic acid nanoparticles, we administered the same material into SJL/J mice which serve as a model for the generation of anti-DNA antibodies, where we did not observe the generation of anti-DNA antibodies. From this preliminary, single dose study, we conclude that nucleic acid nanoparticles are well tolerated; follow up safety studies are required when specific targeting ligands and therapeutic cargos are incorporated into the formulations.

The second section of my thesis is focused on integrating chemical and analytical techniques to fabricate diverse nucleic acid nanoparticles for broad application. We implemented strain-promoted azide-alkyne cycloaddition (SPAAC) chemistry to fabricate nucleic acid nanoparticles conjugated with diverse secondary molecules spanning protein, polymer, peptide, small molecule, and carbohydrate systems. We then extended this advancement by incorporating the conjugate handle at arbitrary positions within the nucleic acid nanoparticle which allows for higher density incorporation of secondary molecules, and enables higher precision spatial organization of the secondary molecules. Additionally, we implemented inverse electron-demand Diels-Alder (IEDDA) chemistry as an orthogonal chemistry to SPAAC chemistry to functionalize nucleic acid nanoparticles, which enabled the heterovalent covalent functionalization of these materials with near-arbitrary secondary molecules. During this research, we integrated two analytical techniques to quantify the extent of functionalization of nucleic acid nanoparticles with secondary molecules. First, we developed a liquid chromatography assay to monitor the covalent reaction occurring on the materials that was dependent on the hydrophobicity of the conjugate handle. We showed this technique allows for the quantification of the

functionalization efficiency for diverse secondary molecules conjugated using SPAAC chemistry. Then, we integrated a mass spectrometry technique termed charge-detection mass spectrometry (CD-MS). CD-MS enabled the precise measurement of nucleic acid nanoparticle molecular masses, and the quantification of secondary molecule incorporation into the materials. Combined, these implementations of chemistries and analytical techniques into the nucleic acid nanoparticle platform now enable near-arbitrary material fabrication to design delivery vectors using nucleic acid nanoparticles.

In the final section of this thesis, I describe work towards developing an infectious disease vaccine delivery vector using nucleic acid nanoparticles. We conjugated the receptor-binding domain (RBD) of the SARS-CoV-2 virus to nucleic acid nanoparticles using SPAAC chemistry with differing copy numbers of RBD per nanoparticle. In *in vitro* cell models of B cell activation and in wild-type mice, nucleic acid nanoparticles displaying thirty copies of the RBD performed better than either nucleic acid nanoparticles displaying six copies of the RBD or RBD alone without nanoparticulate scaffolding. Additionally, with T cell receptor knockout mice, we demonstrated T cell help is essential for the humoral immunity generated by these materials. Finally, in comparison to vaccine vectors fabricated with a protein scaffold, our nucleic acid-scaffolded vector led to the generation of similar levels of on-target antibodies (against the RBD), while not generating antibodies off-target (against the scaffold). In contrast, the protein-based vaccine vector generated a strong antibody response against both the target protein and against the scaffold itself.

The work in this thesis contributes to the development of nucleic acid nanoparticles as safe, effective, scalable, and versatile delivery vectors in broad applications by helping to elucidate their safety profile, integrating several chemistry and analytical techniques into the technology platform, and providing a proof-of-concept that nucleic acid nanoparticles can delivery biomacromolecular cargos *in vivo*.

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