**Enhancing Gene Therapy through Targeted Delivery of CRIPSR/Cas9 in a Novel, Inexpensive Lipid Nanoparticle**

**Abstract**
CRISPR/Cas9 has an enormous potential to treat chronic and genetic diseases through its versatility and efficiency in editing DNA. However, this technology has yet to be implemented because of the body's vigorous immune defenses against Cas9. Thus, the goal of the project is to develop a novel lipid nanoparticle that allows safe, targeted, and inexpensive delivery of Cas9.

**Methods/Materials**
Lipids nanoparticles were designed with a novel combination of lipids to allow immune evasion, dissociation within cells, and nontoxic metabolic degradation. Furthermore, a simple, inexpensive synthesis procedure was developed by optimizing thermodynamic favorability of the nanoparticle state. Polar headgroups of amphiphilic lipids adsorb to the polar Cas9 protein in a nonpolar environment, forming an inverse micelle. Upon dilution into an aqueous environment, nonpolar sites on inverse micelles attach to nonpolar sites on lipid tails to form the final nanoparticle with polar headgroups facing outwards.

Fluorescent targeting antibodies were conjugated to the nanoparticle surface in a convenient nucleophilic substitution reaction. Encapsulation efficiency was tested by measuring Cas9 concentration before and after the synthesis procedure. Antibody conjugation efficiency was tested by measuring fluorescence before and after an antibody wash. Transfection efficiency was tested by adding the nanoparticles to cell lines and quantifying DNA editing after 48 hours.

**Results**
The nanoparticle shows an excellent ability to encapsulate Cas9, with an encapsulation efficiency of 55.6%. Further, antibody conjugation efficiency was measured at 79.9%. Editing efficiency was measured at 3.9%, more than 100 times higher than the editing efficiency of unencapsulated Cas9, and around 5 times higher than commercial transfection methods.

**Conclusions/Discussion**
The proposed nanoparticle design exhibits nontoxic transfection and immune evasion. The novel synthesis procedure eliminates the need for expensive machinery, allowing a projected cost of only $30 per treatment. The nanoparticle shows a robust ability to transfect and edit DNA within cells, paving the way for effective gene therapy towards diseases from diabetes to cystic fibrosis.