# Generation of Antibody Oligonucleotide Conjugates Using a Conjugation Chemistry With Multiple Linkers

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Oligonucleotides, especially siRNAs, have been identified as a point of interest in recent research due to their capability for targeting of “undruggable” targets. siRNAs bind to and guide the RNA Induced Silencing Complex (RISC) in the cytoplasm, leading to degradation of specific targeted mRNAs. Despite advancements in the field, delivery to extrahepatic tissues remains a challenge. Current FDA-approved delivery systems such as lipid nanoparticles (LNPs) and trivalent N-Acetyl galactosamine (GalNAc), primarily target the liver. Antibodies, however, are emerging as a promising modality for targeted extrahepatic delivery of oligonucleotides. Antibody oligonucleotide conjugates (AOCs) leverage antibodies' receptor-based uptake for precise delivery of siRNA to targeted regions of the body. However, current chemistries for AOC generation are either inefficient or non-site specific. My research introduces a improvement on a previous method for generating AOCs. This method combines an enzymatic transglutaminase reaction with an Inverse Electron Demand Diels-Alder (IEDDA) reaction but uses a different linker. Specifically, a tetrazine linker is added to the antibody, followed by purification through size exclusion chromatography (SEC). The siRNA is conjugated to the antibody linker using IEDDA and purified through anion exchange chromatography (AEX), achieving a drug to antibody ratio (DAR) of ~1. The conjugate was characterized through multiple analytical methods. This new conjugation method successfully generates AOCs while significantly shortening conjugation time. Future applications include applying this method to significantly enhance the efficiency and scalability of AOC drug development as well as generate other modalities such as peptide siRNA conjugates.

**Awards Won:**