Student: Wesalo, Joshua (MS2)
Mentor: Alexander Deiters, PhD
Title: Improved photocaged cyclic morpholino oligonucleotides for microRNA knockdown and *in vivo* gene silencing
Date: 6/18/2015
Medical Scientist Training Program Annual Scientific Retreat

Synthetic oligonucleotides can control mRNA and miRNA function with high sequence specificity, making them useful tools for research and for disease treatment. In biological experiments, however, endogenous nucleases degrade exogenous oligonucleotides. To resist these enzymes, investigators have developed oligonucleotides with different backbone chemistry. Morpholino oligonucleotides (MOs), which have a backbone comprised of morpholine rings instead of pentoses, resist enzymatic degradation and retain the ability to hybridize to DNA and RNA via Watson-Crick base pairing. The Deiters Lab has been improving the functionality of MOs by generating caged MOs (cMOs) that do not bind target sequences until exposed to light of a specific wavelength. To accomplish this, we join the ends of a linear MO with a photo-cleavable linker. The resulting circular cMOs do not hybridize to target sequences effectively. Upon wavelength-specific irradiation, a bond in the linker is cleaved, opening the circular cMO into a linear molecule and restoring its capability to hybridize to complementary DNA and RNA.

Several problems plague the application of cMOs as research tools and as potential therapeutics. First, cMOs often show “leakiness” (background activity before light exposure). Second, cMOs do not penetrate cellular membranes due to their high molecular weight and neutral net charge. Third, the wavelengths of light required for activating cMOs are impractical for many biological uses. Improvements in linker design could solve all three problems. We seek to synthesize shorter, tighter linkers with various wavelength specificities. Additionally, we intend to incorporate alkyne “handles” into the linkers for conjugation to cell-penetrating peptides via “click” chemistry to improve cellular delivery. To apply these engineered oligonucleotides to biological systems, we plan to deliver cMOs to cancer cell lines and to have our collaborators inject cMOs into zebrafish embryos. In the former, we will target miR-125b, a negative regulator of p53; in the latter, we will target genes important for developmental patterning. We anticipate that cMOs with shortened diethylaminocoumarin (DEACM) and propargyl-6-nitroveratryloxy (PNVO) linkers will knock down target gene expression effectively upon exposure to light (405 nm and 365 nm, respectively).