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“Design of carriers for delivery of short and long RNAs: from miRNA replacement therapy to CRISPR/Cas gene editing”

Abstract
CRISPR/Cas is a revolutionary gene editing technology with wide-ranging utility. The safe, non-viral delivery of CRISPR/Cas components would greatly improve future therapeutic utility. The synthesis and development of zwitterionic amino lipids (ZALs) that are uniquely able to (co)deliver long RNAs including Cas9 mRNA and sgRNAs will be presented. ZAL nanoparticle (ZNP) delivery of low sgRNA doses (15 nm) reduces protein expression by >90% in cells. In contrast to transient therapies (such as RNAi), ZNP delivery of sgRNA enables permanent DNA editing with an indefinitely sustained 95% decrease in protein expression. ZNP delivery of mRNA results in high protein expression at low doses in vitro (<600 pM) and in vivo (1 mg/kg). Intravenous co-delivery of Cas9 mRNA and sgLoxP induced expression of floxed tdTomato in the liver, kidneys, and lungs of engineered mice. ZNPs provide a chemical guide for rational design of long RNA carriers, and represent a promising step towards improving the safety and utility of gene editing.

Biosketch
Dr. Daniel J. Siegwart is currently an Assistant Professor in the Simmons Comprehensive Cancer Center and Department of Biochemistry at the University of Texas Southwestern Medical Center. He received a B.S. in Biochemistry from Lehigh University in 2003, and a Ph.D. in Chemistry from Carnegie Mellon University (CMU) in 2008 under the supervision of University Professor Krzysztof Matyjaszewski. During his graduate studies, he received the Joseph A. Solomon Memorial Fellowship in Chemistry at CMU and was a National Science Foundation East Asia and Pacific Summer Institutes Fellow at The University of Tokyo in 2006 with Prof. Kazunori Kataoka. He then completed a National Institutes of Health NRSA-sponsored Postdoctoral Fellowship at Massachusetts Institute of Technology with Institute Professor Robert Langer (2008-2012). He began his independent research career in 2012. The central goal of the Siegwart Lab is to use materials chemistry to solve challenges in cancer therapy and diagnosis. In particular, they are focused on the development of new materials that can deliver RNAs to improve cancer outcomes. An array of coding and non-coding RNAs can now be used as cancer therapeutics (siRNA, miRNA, mRNA, CRISPR RNAs) because they are able to manipulate and edit expression of the essential genes that drive cancer development and progression. Although great advances have been made in the delivery of short RNAs, the ideal chemical and formulation composition is largely unknown for longer RNA cargo (mRNA, sgRNA). The Siegwart Lab aims to discover and define the critical physical and chemical properties of synthetic carriers required for therapeutic delivery of small (e.g. ~22 base pair miRNA) to large (e.g. ~5,000 nucleotide mRNA) RNAs. Their research is grounded in chemical design and takes advantage of the unique opportunities for collaborative research at UTSW Medical Center. They ultimately aspire to utilize chemistry and engineering to make a beneficial impact on human health.