Combinatorial, High-throughput Synthesis of Core-shell Nanoparticles for siRNA Delivery

Daniel J. Siegwart, Kathryn Whitehead, Shan Jiang, Minglin Ma, Hao Cheng, Gaurav Sahay, Arturo Vegas, Patrick Fenton, Kevin T. Love, Christopher G. Levins, Robert S. Langer, and Daniel G. Anderson
Massachusetts Institute of Technology, David H. Koch Institute for Integrative Cancer Research, Cambridge, MA 02139

Statement of Purpose: Nanoparticles are routinely used for drug and gene delivery. However, it remains challenging to predict the optimal material properties for delivery of a specific drug or biomolecule. High-throughput (HTP), combinatorial methods can aid in the discovery of new nanoparticles for delivery, while simultaneously elucidating the important design parameters of a given system for a targeted application. We will present a "modular" approach that applied combinatorial CRP[1] for the formation, characterization, and screening of more than 1,500 core cross-linked hairy nanoparticles for siRNA[2] delivery comprised of cationic cross-linked cores and variable shells with precise control over the particle size, chemical composition, and architecture. To achieve the desired core-shell structure,[3] we employed a method of cross-linking block copolymers prepared by RAFT polymerization. We cross-linked via the reaction of amines with epoxides[4] due to the availability of a large number of amines that can incorporate cationic charge into the core, and the efficiency and high rate of catalyst-free reaction.

Methods: Robotic, HTP synthesis was performed on a customized Symyx Core Module. Library Studio was used to design the libraries. The Ribogreen assay was performed on a Tecan Freedom EVO 200. For nanoparticle synthesis, a solution of amine in DMSO was added to a stirring solution of block copolymer in DMSO such that the mole ratio of epoxides to amine was 1:1. The resulting mixtures were stirred and heated at 55° C for 24 hours. *In vitro* siRNA transfection assays and *in vivo* Factor VII Silencing in Mice experiments were performed as previously described.[5]

Results We synthesized more than 1,500 core-shell nanoparticles via core-cross linking of precursor block copolymers containing epoxide groups with a library of chemically diverse amines (Figure 1).

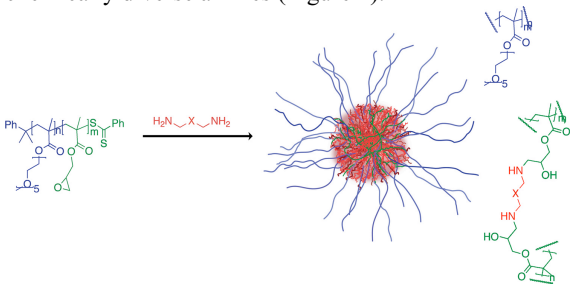

Figure 1. Formation of core-shell nanoparticles with a cationic core for siRNA delivery. "X" is a cationic moiety selected to complex siRNA and to enable endosomal escape.

We found that the block length, concentration, temperature, and choice of cross-linker are important factors in the formation of the nanoparticles. After preliminary studies, the synthesis were scaled up by reacting 16 different blocks copolymers (not shown due to space and structural resolution limitations) with more than 100 amines (not shown) in a combinatorial manner on a Symyx fluid handling robot to yield 1,536 structurally distinct core-shell nanoparticles. We characterized these nanoparticles using HTP GPC. We then screened them for siRNA complexation, siRNA delivery, and pDNA delivery in vitro (Figure 2). Many nanoparticles were able to complex tightly to siRNA (shown in red in 2a) and effectively deliver siRNA *in vitro*, silencing more than 75% of luciferase expression. The best performing nanoparticles were tested *in vivo* (not shown) and were capable of silencing more than 45% of Factor VII in mice.

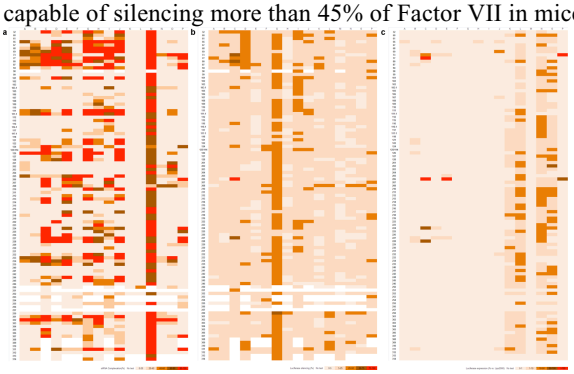

Figure 2. (a) siRNA complexation was quantified using the Ribogeen assay. (b) HeLa cells stably expressing both firefly and Renilla luciferases were treated with firefly targeting siRNA-nanoparticles complexes and the average % reduction in firefly luciferase activity after treatment is shown. (c) HeLa cells were treated with pDNA(luc)-nanoparticle complexes. The average % expression versus Lipofectamine2000 after treatment is shown.

Conclusions: This one pot synthetic method enables parallel generation of large libraries of chemically distinct core-shell materials. Notably, a number of materials were identified with both *in vitro* and *in vivo* utility and the common structural features of these materials suggest certain design criteria for creating future intracellular delivery agents. These trends, full synthetic details and characterization, and *in vivo* biodistribution and silencing data will be presented.

References: 1. Braunecker et al. *Prog. Poly. Sci.* **2007**, *32*, 93. **2.** Whitehead et al. *Nat. Rev. Drug. Disc.* **2009**, *8*, 129-138. **3.** O'Reilly et al. *Chem. Soc. Rev.* **2006**, *35*, 1068-1083. **4.** Tsarevsky et al. *Macromolecules* **2007**, *40*, 4439-4445. **5.** Love, et al. *Proc. Natl. Acad. Sci.* **2010**, *107*, 1864.