Structure and Function Study of Nucleic Acid Nanoparticles
For the Delivery of Peptide-siRNA Conjugates

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ABSTRACT SUMMARY
Herein we demonstrate the structure and function relation of nucleic acid nanoparticles for siRNA delivery. Holiday junction and tetrahedron nanostructures are efficiently prepared by the programmable self-assembly of DNA strands. Cell penetrating peptide (CPP) conjugated siRNA can be site-specifically hybridized on either holiday or tetrahedron nanostructure to form CPP-siRNA/DNA nanoparticles. These nucleic acid nanoparticles are evaluated for the GFP gene silencing and they exhibit remarkable differences in gene silencing efficiency. This study clearly provides strong evidences for the importance of nanostructure design on the intracellular delivery of RNA therapeutics.

INTRODUCTION
siRNAs have tremendous therapeutic potential, since they can selectively regulate target mRNA expression and protein synthesis. However, due to their innate physiochemical characteristics, the systemic delivery of naked siRNAs fails to achieve therapeutically relevant gene silencing. This problem can be overcome by formulating naked siRNAs with various cationic polymers and lipids to prepare nanoparticle systems. Cationic nanoparticles not only improve the intracellular uptake of siRNAs, but also provide an enhanced therapeutic gene silencing.

Although cationic polymers and lipids have been successfully employed to prepare gene delivery nanoparticles, they cannot provide a single uniform population in terms of size and shape for gene delivery applications. In terms of the delivery of ligand-siRNA conjugates (i.e: Fol-siRNA, Gal-siRNA, Peptide-siRNA), cationic delivery carriers often hamper the advantages of these ligand conjugated systems, since they do not have a control over the ligand density nor the orientation of the ligands on the single nanoparticles.

The present study has employed self-assembled nucleic acid nanoparticles for the delivery of peptide-conjugated siRNAs. Non-charge CPP has been introduced to the system evaluating the efficacy of CPP mediated delivery of siRNA/DNA nanoparticles as well as investigating the structure and function relation of nucleic acid nanoparticles with different nanostructures. Here two distinct shapes, holiday junction and tetrahedron, has been tested for the delivery of CPP-siRNAs and their in vitro gene silencing efficacy is evaluated in detail.

EXPERIMENTAL METHODS
1. Self-assembled oligonucleotide annealing. Holiday junction (Holiday) (IDT, USA) and tetrahedron (Tetra) DNA nanostructure were annealed in following condition. DNA strands (final strand concentration, 2 mM each) were mixed in an equal molar ratio in MgCl₂ 40 mM and NaCl₂ 135 mM. The solution was heated to 95°C then slowly cooled to 4°C for 2 h. Hybridization of Holiday and Tetra nanostructure was analyzed by native-PAGE.

2. Conjugation of CPP with siRNA
For annealing of siRNAs, 10 mM of each RNA strands were incubated in phosphate buffer for 2 h incubation at RT. Hybridized siRNA were introduced into cells using Lipofectamine RNAiMAX (Invitrogen, CA, USA). The hybridized siRNAs containing thiol groups were employed for the MAL-thiol conjugation. 20 molar excess CPP were reacted with siRNAs for 2 h to form CPP-siRNA conjugates.
3. Cell culture and treatment of CPP-siGFP conjugates
GFP-HeLa cells and Luc-HeLa cells were grown DMEM supplemented with 10% FBS. CPP-siRNA conjugates were added in various concentrations to the cell culture media for 24 h. Lipofectamine RNaiMAX was used as a positive control. GFP gene silencing was monitored by flow cytometry. Luciferase silencing in HeLa cells was evaluated using luciferase assay kit (Promega).

RESULTS AND DISCUSSION
Holiday was successfully hybridized in condition of MgCl2 20-40 mM and NaCl 134 mM (Fig. 1a). Tetra also was prepared in same condition (Fig. 1b). Native Page image showed that the was well achieved (Fig. 1c). CPP-siGFPs efficiently hybridized with the DNA core nanostructures in RT (Fig. 1d).

The gene silencing efficiency of CPP-siGFP conjugates was investigated in GFP expressing HeLa cells (Fig. 2). Lipofectamine-delivered siRNAs (35 nM) decreased GFP expression by over 50 %. The CPP-siGFP conjugates decreased the GFP by over 60% at dose dependent manner. These results indicate that the CPP-siGFP conjugates are highly potential for intracellular delivery of RNA therapeutics.

The synergistic gene silencing effects of the delivery of CPP-siGFP conjugates with nucleic acid core was evaluated. Both Holiday and Tetra nanostructure provided enhanced dose responses down to 10 nM in vitro (data not shown).

CONCLUSION
The synergistic gene silencing effects are achieved by combining the delivery of CPP-siRNA conjugates using nucleic acid nanoparticles with different structures. Systemic delivery of our system will provide a practical solution for the current drawbacks of cationic gene delivery systems.

REFERENCES
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