Structural Characterization of Two-Compartment Lipid Nanoparticles Using Small-Angle X-Ray/Neutron Scattering

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Lipid nanoparticles (LNPs) are one of the most successful engineered synthetic carriers to encapsulate and deliver siRNA, mRNA, DNA, or small molecules. They are typically composed of ionizable cationic lipids, phospholipids, cholesterol, and polyethyleneglycol (PEG)-lipids. Their overall architecture is a core-shell structure in which the core contains cationic lipids and cargo molecules, while the shell contains phospholipids and PEG-lipids. Cholesterol is thought to be distributed throughout the LNP. There are many outstanding questions about the structural organization of LNPs. In this presentation, we will present our recent work using a combination of structural characterization techniques to study LNPs with encapsulated large RNA molecules. Cryogenic electron microscopy (cryo-EM) showed that lipid nanoparticles had two-compartments surrounded by an external shell. We hypothesized this morphology is determined by lipid- and aqueous RNA-rich phase- separated cores, both surrounded by a DSPC/PEG-rich lipid shell. Using SAXS and SANS, we were able to obtain quantitative structural information about the structure of these LNPs.

SAXS data indicates that encapsulated RNA changes its conformation and becomes more compact than free RNA. To capture the overall morphology of the LNP observed in the cryo- EM, we develop a simpler cylinder core-shell model that consists of two adjoining cylinders within a shell. In the model, RNA and cationic lipids-rich compartments are constrained by the radius and length of the cylinder, and DSPC lipid shell is constrained by the thickness of a cylindrical shell. The model provided information about the overall size of the aqueous RNA/Lipid-rich compartments, volume fraction of solvent in the aqueous RNA compartment,

and overall dimensions of the LNPs. However, the model was not able to capture well structural features of the DSCP lipid shell. The advantages and limitations of this fitting approach will be described in the context of simpler fitting approaches. In addition, prospects and strategies for applying SANS in combination with isotopic labeling to characterize lipid nanoparticles will also be discussed. These findings provide insights toward understanding the mechanism of LNP formation with large RNA molecules and the rational design of LNPs for new RNA delivery systems.