Solution biophysics reveals the polydisperse structure of RNA lipid nanoparticles

We applied emerging label-free biophysical techniques to map the size, composition and shape of RNA lipid nanoparticles. By linking these physical measurements to gene expression in human T cells and mice, we uncovered structure–activity relationships that guide RNA delivery.

The problem

Lipid nanoparticles (LNPs) have transformed the field of RNA medicine by providing protection and enabling transfection of RNA cargo[1]. Over one billion doses of mRNA–LNP SARS-CoV-2 vaccines have been manufactured[2], yet the physicochemical characteristics of LNPs are poorly defined. The lack of data on distinct structure–activity relationships (SARs) has hindered rationale LNP design. Standard tools such as dynamic light scattering or fluorescence-based RNA assays provide only average size or encapsulation values and cannot resolve LNP subpopulations or distinguish RNA-loaded LNPs from empty LNPs[3]. As LNPs are inherently polydisperse, there is an urgent need for high-resolution, solution-based characterization methods to guide rational LNP design and improve RNA delivery[4]. Our goal was to combine emerging biophysical techniques with biological assays to elucidate LNP properties and their impact on RNA delivery.

The solution

To probe the complexity of LNPs, we formulated a small library of the gold-standard LNPs MC3, C12-200, SM-102 and ALC-0315, using both bulk pipette mixing and microfluidic devices. We paired traditional LNP analytical measurements with sedimentation velocity analytical ultracentrifugation (SV-AUC), field-flow fractionation followed by multiangle light scattering (FFF–MALS) and size-exclusion chromatography in line with synchrotron small-angle X-ray scattering (SEC–SAXS). Each biophysical approach uncovered a unique aspect of LNP heterogeneity and provided physicochemical and structural information that traditional techniques were unable to resolve (Fig. 1a). We complemented these data with biological studies in primary human T cell cultures and in mice to build SARs that identified the features that strongly correlate with successful RNA transfection. We then examined the association between the various LNP formulations and endosomal LNP escape in T cells.

We observed extensive heterogeneity across the LNP formulations tested. SV-AUC indicated that many particles had little or no RNA cargo, and multiple LNP subpopulations that either sedimented or floated were present in each batch. FFF–MALS showed that microfluidic LNPs were smaller, were more monodisperse and contained more RNA than bulk-mixed LNPs. Using SEC–SAXS with our integrated data pipeline[5], we resolved distinct scattering components, confirming that each LNP formulation comprised multiple subpopulations with unique characteristics. Finally, reconstructions of the scattering data demonstrated that LNPs are prolate ellipsoids rather than spheres (Fig.1b). Mixing methods produced LNP formulations of varied efficacy, depending on lipid composition: microfluidic mixing enhanced transfection for FDA-approved MC3, SM-102 and ALC-0315 LNPs, whereas bulk-mixed C12-200 outperformed the microfluidic counterpart. These results strongly correlated with the ordering of RNA–lipid interactions within the LNP, as reflected in the SAXS profiles.

Next, we investigated endosomal escape of LNP-delivered RNA by inhibiting the various endosomal pathways in T cell cultures. We found that bulk-mixed C12-200 LNPs escaped caveola-mediated endocytosis, whereas the other LNPs, including microfluidic-mixed C12-200 LNPs, remained stuck in this pathway. This indicates that preparation method might influence the degree to which LNPs escape certain endosomal pathways.

The implications

Our results indicate that multiple high-resolution methods are needed to deconvolve polydisperse formulations and extract meaningful SARs. As the most predictive physical parameters vary with biological context, design rules should be developed for each target tissue or delivery route, instead of a 'one-size-fits-all' approach. The ability to resolve empty and loaded particles and anisotropic shapes provides an important foundation for optimizing encapsulation, RNA delivery and safety.

The findings were limited by the small set of excipients and formulation conditions and by the focus on models using luciferase reporters. Our mechanistic studies centered on endosomal escape in T cells and did not assess other biological models or processes, such as protein corona formation. Therefore, studies with larger LNP libraries, diverse RNA cargo and additional mechanistic assays will be needed to further explore our findings. We plan to expand our library of LNPs and RNA cargoes, integrate additional biological readouts beyond luciferase and refine microfluidic devices to tune particle shape and internal structure. Such efforts should help translate high-resolution biophysical insights into new LNP designs.

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EXPERT OPINION

"This integrative approach represents a notable advance in LNP characterization, offering a more mechanistic understanding of how structural and biophysical attributes influence functional performance. By establishing a framework to predict transfection outcomes, this strategy has the potential to accelerate the rational design of next-generation LNPs, providing valuable insight for researchers developing mRNA therapeutics." **Wei Tao, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.**

FIGURE

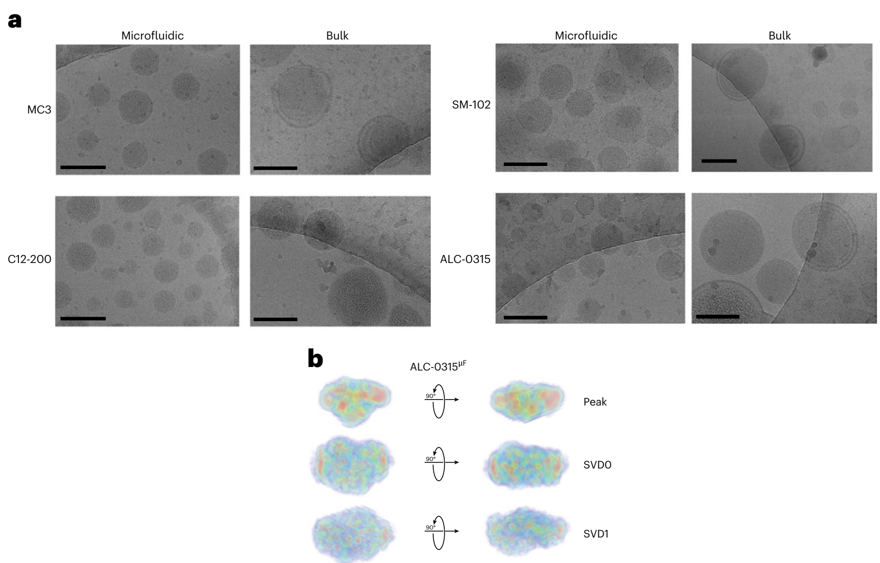

Fig. 1 | Shape of lipid nanoparticles. a, Cryogenic transmission electron microscopy of microfluidic- and bulk-formulated MC3, C12-200, SM-102 and ALC-0315 LNPs shows spherical particles and no major variations in morphology. **b**, Ab initio reconstructions using the algorithm DENSS (density from solution scattering) applied to SEC–SAXS data reveal that microfluidic-formulated ALC-0315 LNPs are prolate ellipsoidal rather than spherical. © 2025, Padilla, M. S. et al.

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BEHIND THE PAPER

Sophisticated biophysical and analytical equipment is unavailable in most research laboratories, with certain techniques, such as SEC–SAXS, only accessible in a few locations around the world. This manuscript wouldn't have been possible without the incredible collaborations between academia, industry and national laboratories. Each party contributed their expertise, and together, we overcame the most significant obstacle in our analysis: handling polydisperse mixtures in a rigorous manner. For example, assessing statistical uniqueness for the deconvolved SEC–SAXS samples required taking a cue from economics. We employed a volatility-of-ratio (V_R) metric to compare individual profiles to ensure that our deconvolution strategy resulted in statistically significant differences, indicating that we were observing multiple species within a single LNP batch. I look forward to seeing more cross-disciplinary and cross-institutional collaborations, particularly in the nanotechnology and biophysical realms.
M.S.P.

FROM THE EDITOR

"Lipid nanoparticles are a promising technology for delivering therapies to specific tissues in the body. This study shows how to improve the effectiveness of lipid nanoparticles by measuring their biophysical properties more rigorously than is usually done today." **Editorial Team, *Nature Biotechnology*.**