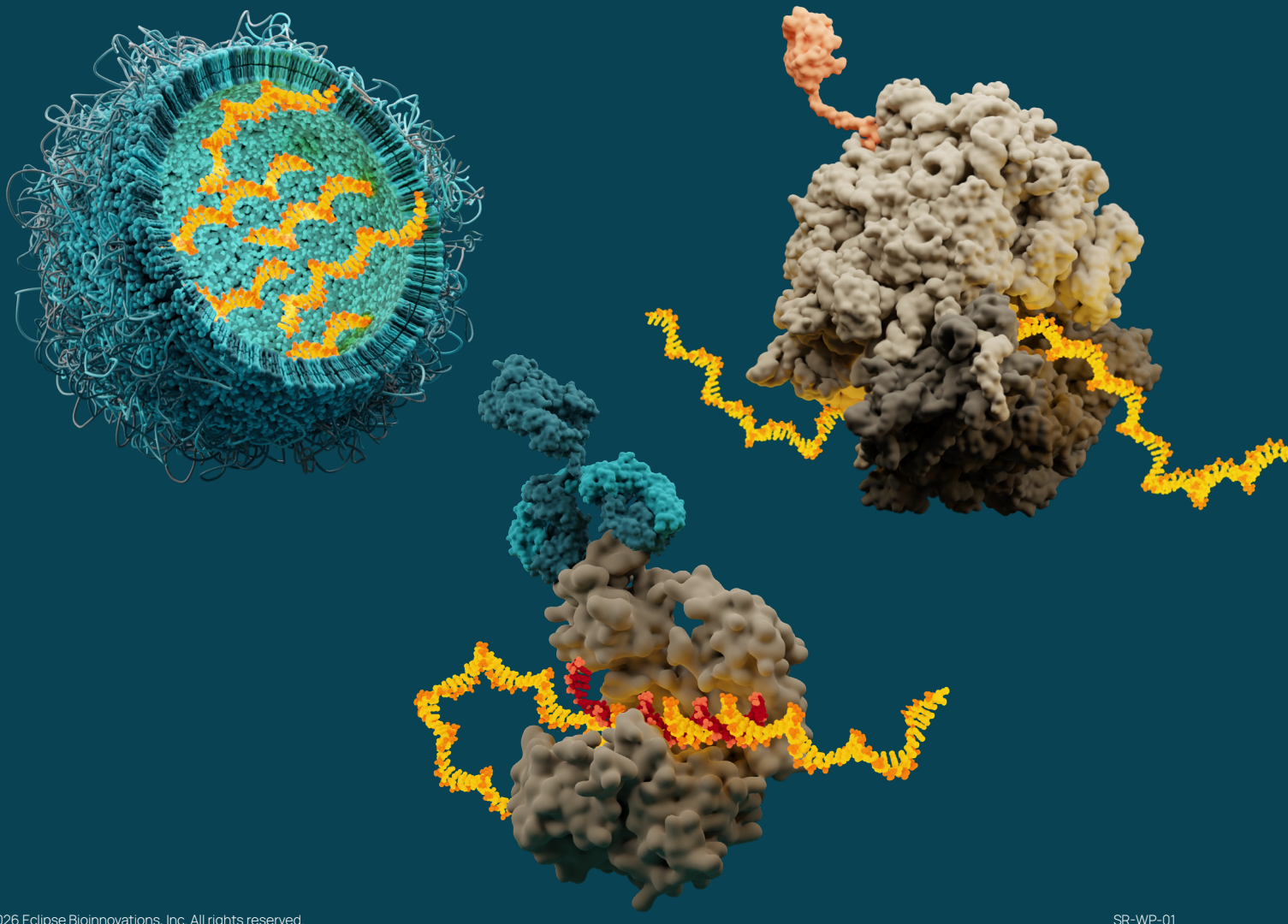
ECLIPSEBIO
THE RNA SUCCESS COMPANY

Whitepaper

5 safety risks for RNA therapeutic development



Introduction

RNA-based and RNA-targeting therapeutics are transitioning from a landscape of "unknown unknowns" to one where key safety risks can be directly measured and engineered. In 2026, the most significant safety challenges for developing RNA therapeutics center on two areas: controlling manufacturing-related impurities and designing drug products that minimize off-target effects. In this whitepaper, we explain five common safety risks and mitigation strategies within these two areas.

5 common safety risks in the RNA therapeutics space

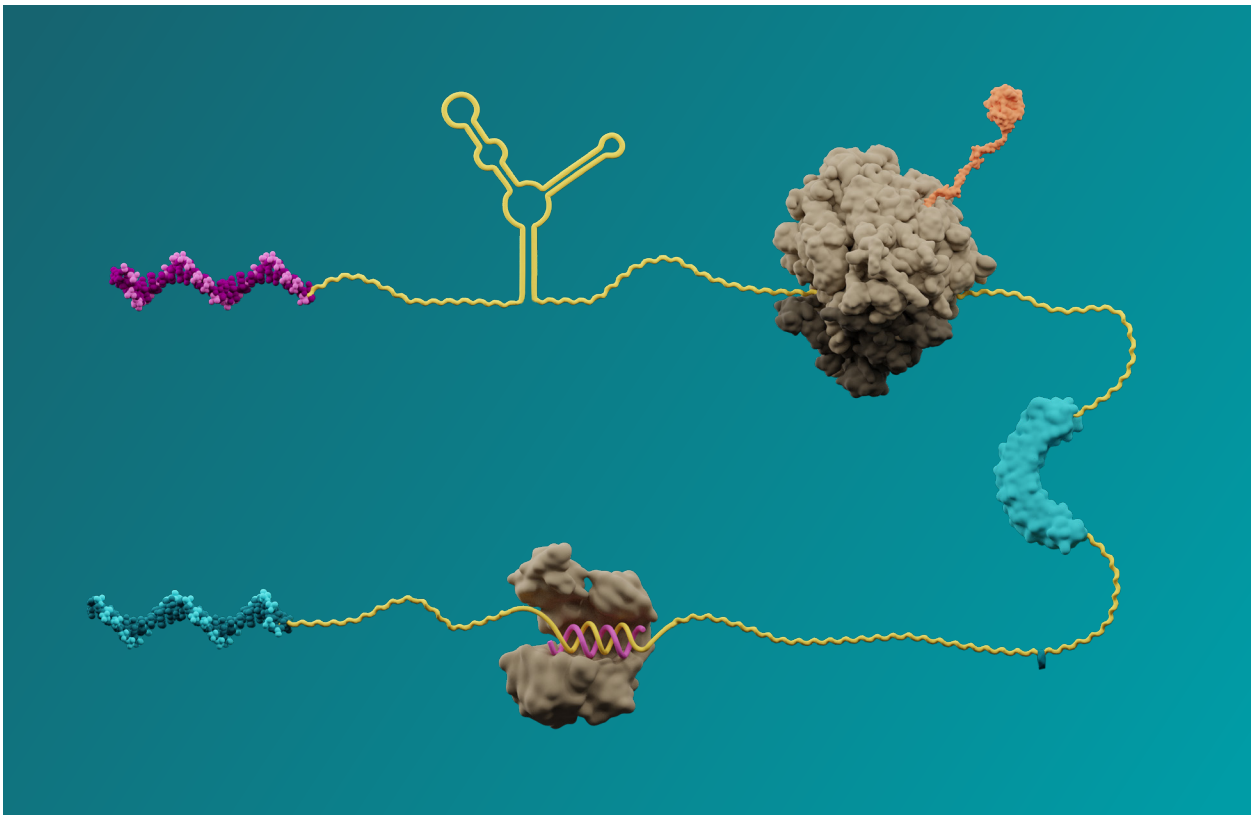
Risk 1: Cellular responses from manufacturing impurities

Risk 2: Delivery vehicle-related toxicity

Risk 3: Off-target activity

Risk 4: mRNA therapy stability

Risk 5: Inconsistent functional outputs



Risk 1: Cellular responses from manufacturing impurities

Although in vitro transcription (IVT) processes for long RNA therapeutics continue to improve, impurities such as double-stranded RNA and abortive transcripts are still generated. If carried into the final drug product, these impurities can activate innate immune pathways, reducing stability and translation while triggering cytokine signaling and other off-target effects. While low levels may be acceptable for vaccines, gene editing and other non-vaccine modalities typically require immune-silent drug products.

Mitigation efforts are converging on three complementary areas:

- Sequence design optimization to reduce impurity formation
- Improved manufacturing processes, including higher-fidelity polymerases and selective purification
- Advanced analytical approaches that identify and quantify specific impurity species to enable robust batch comparisons

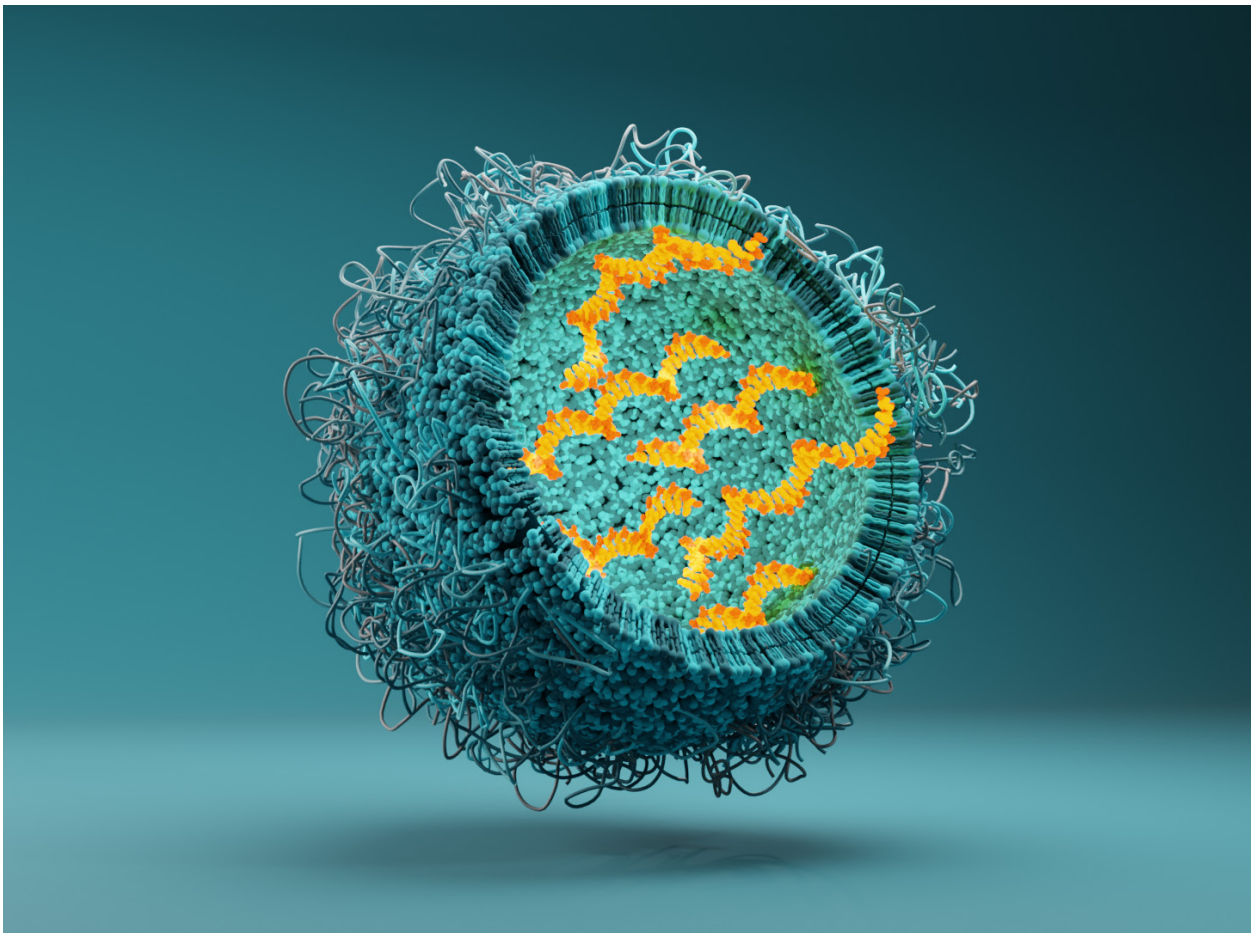
While much of the focus has been on mRNA and gene editing modalities, similar immune responses can also occur with siRNAs. Chemical modifications have reduced this risk, but immune activation remains an important consideration during development.

Risk 2: Delivery vehicle-related toxicity

Historically, most RNA delivery approaches have been optimized for the liver, including GalNAc conjugates for siRNAs and lipid nanoparticles (LNPs) for long nucleic acid therapies. To move beyond hepatic delivery, significant efforts are now focused on exploring new lipid chemistries and targeting moieties to enable tissue-specific uptake. While these strategies have begun to show promise, the introduction of novel excipients increases the need for more extensive safety evaluation.

To derisk these approaches, three complementary strategies are emerging in 2026:

- AI-powered engineering is being used to efficiently explore delivery design space and predict toxicity, reducing reliance on costly in vivo studies.
- Co-optimization of the drug substance and delivery vehicle is improving tissue specificity and translation efficiency, enabling lower therapeutic doses.
- Advanced analytical frameworks are being implemented to track biodistribution and pair it with safety biomarker panels, providing earlier and more quantitative insight into delivery-related risk.

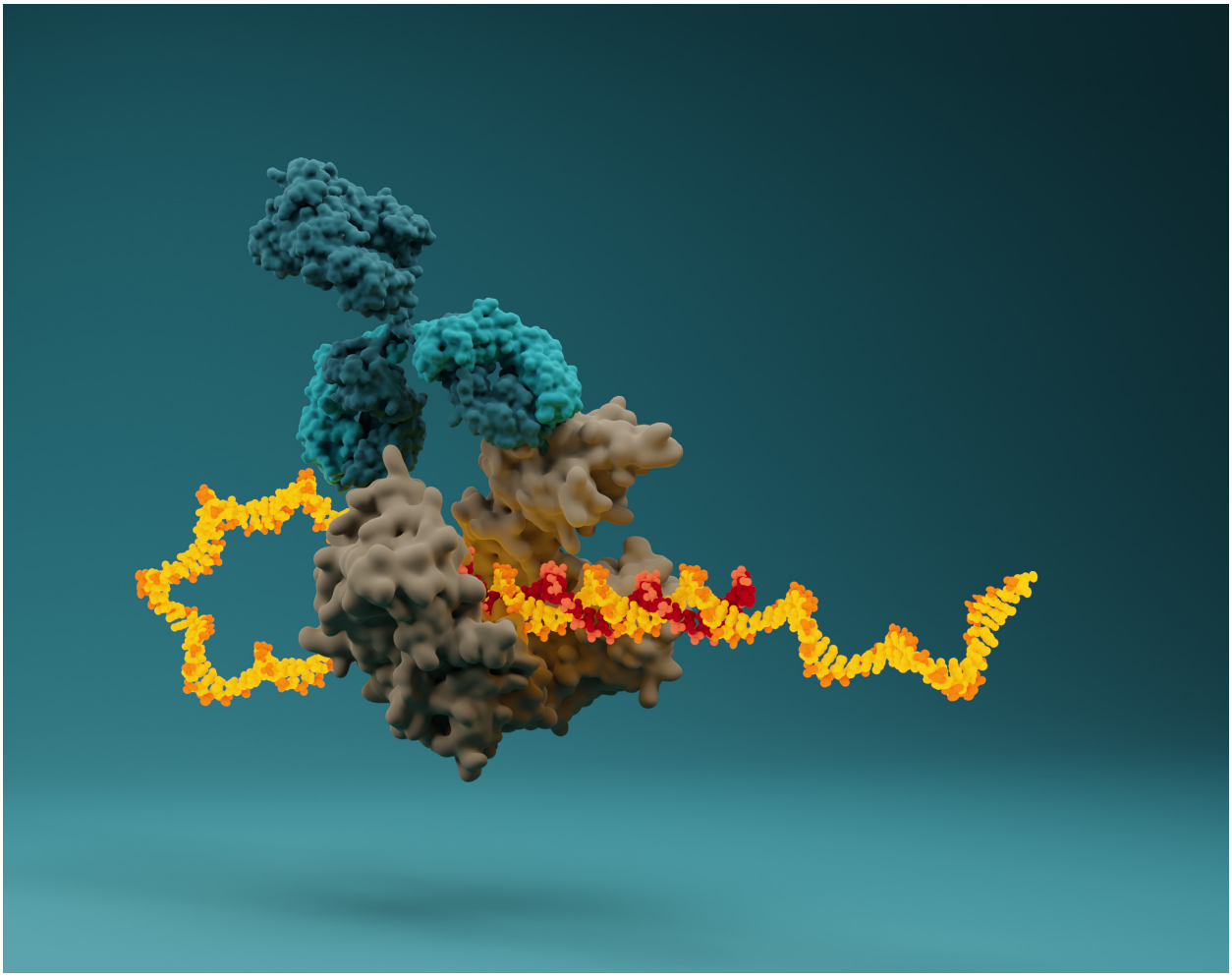


Risk 3: Off-target activity

Although delivery vehicle optimization can improve targeting to the intended tissue, off-target effects can still occur. These include miRNA-like activity from siRNAs, unintended genomic edits in gene editing therapies, and sequence-dependent effects in mRNA therapeutics.

To derisk off-target activity in 2026, developers are expected to take two complementary approaches:

- Using AI to improve drug design by predicting and minimizing off-target interactions is rapidly growing. This has been seen in gene editing development where multiple AI models are now being applied to optimize editor specificity and predict off-target events.
- While AI provides powerful guidance, empirical measurement remains essential to confirm model predictions. Sequencing-based assays are increasingly being adopted for this purpose, including approaches such as DISCOVER-Seq for genome editing and miR-eCLIP+ for direct profiling of siRNA off-target binding.

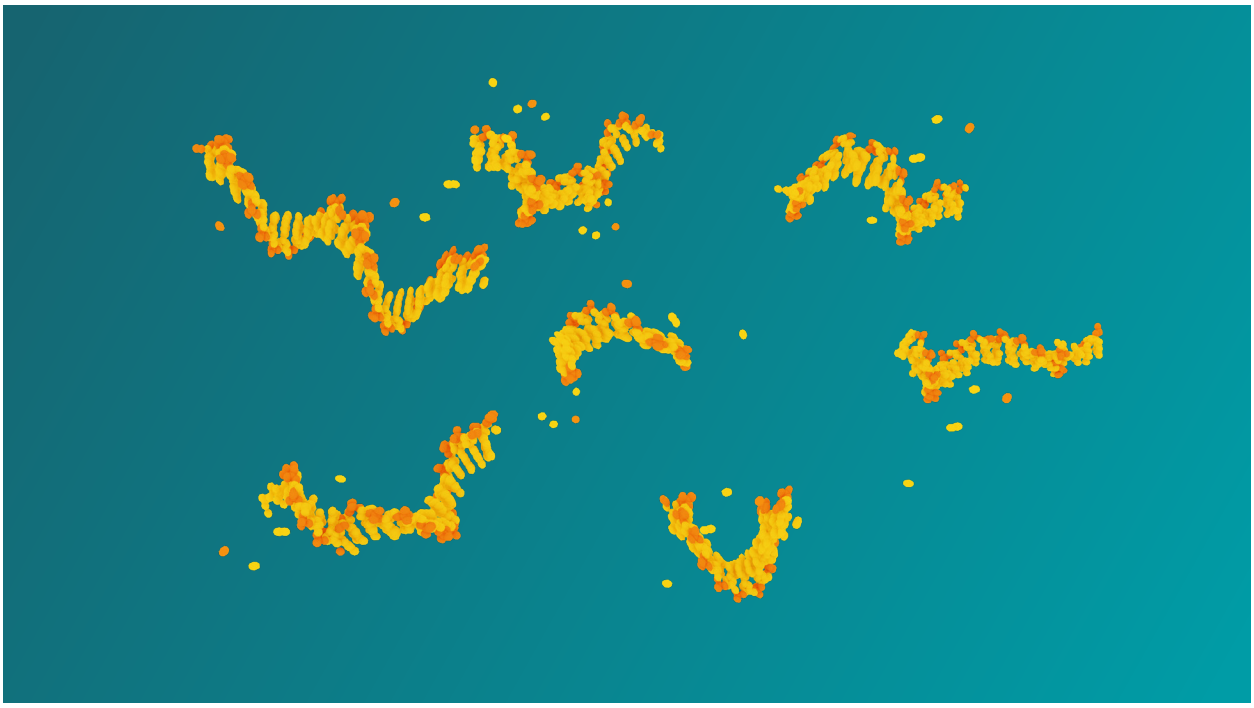


Risk 4: mRNA therapy stability

RNA is intrinsically unstable, and for long nucleic acid therapies, this instability can lead to loss of potency and increased batch-to-batch variability. The primary drivers of RNA instability are degradation by RNases, chemical hydrolysis, and oxidative damage.

To derisk mRNA therapies, developers will need to focus on three key areas:

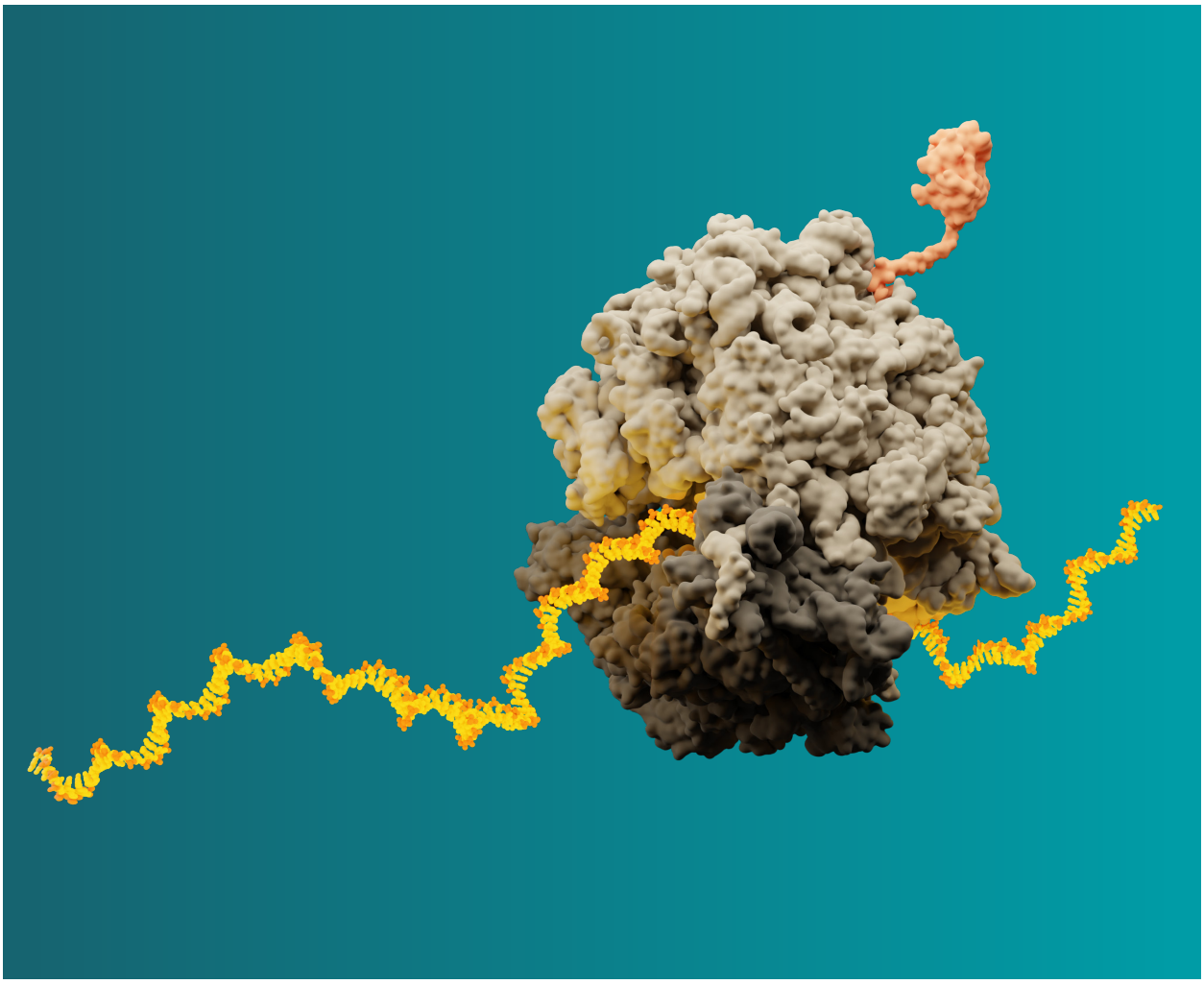
- Improving RNA sequence design can enhance stability, such as reducing unpaired or flexible regions that are more susceptible to hydrolysis. However, increased structural stability can come at the expense of translation efficiency, requiring multi-objective design strategies that balance stability with protein expression.
- Optimizing LNP formulations can preserve RNA integrity, including reducing reliance on strict cold-chain storage and minimizing the generation of reactive oxygen species that drive oxidative damage. While the role of excipients in oxidation has been widely studied, LNPs can also alter RNA secondary structure in ways that promote hydrolysis, making it essential to evaluate formulation effects across multiple dimensions of RNA stability.
- Establishing robust temperature and storage stability testing workflows using multiple orthogonal analytical measurements can help identify the best RNA candidates. Defining true stability boundaries under realistic handling and storage conditions will be critical, and combining complementary assays will help ensure that only the most stable and derisked candidates advance.



Risk 5: Inconsistent functional outputs

Even when an siRNA or mRNA therapeutic is derisked for manufacturing quality, delivery, off-target effects, and stability, it may still fail to demonstrate efficacy in patients. Some RNA targets are inherently resistant to siRNA-mediated knockdown for reasons that remain poorly understood, and certain genomic loci are challenging to edit efficiently despite optimal guide design.

In 2026, developers are increasingly expected to deepen their understanding of therapeutic function through multiomic validation strategies that combine multiple orthogonal measurements. For example, mRNA therapies are often evaluated using Western blots to assess protein expression, but this alone does not explain why expression is low. Reduced output could stem from inefficient LNP uptake, impaired endosomal escape, ribosome pausing, or RNA degradation. By integrating traditional assays with newer approaches such as LNP tracking, ribosome profiling, and direct RNA sequencing, developers can move beyond endpoint readouts and gain mechanistic insight into the drivers of inconsistent functional outcomes.



Reducing Safety Risks at Eclipsebio

At Eclipsebio, we use a combination of data-informed design and sequencing-based analytics to reduce these safety risks on RNA therapeutics. Our eNAVIGATE platform designs mRNA-based therapies using AI models that consider quality, stability, and manufacturability from the start. Our eVERSE database provides AI training data for key dimensions of RNA biology including RNA secondary structure and miRNA binding. Our eMERGE platform uses sequencing-based assays to analyze RNA quality, including identifying dsRNA impurities and measuring secondary structure in LNPs.

If you are ready to design or characterize stable, functional RNA therapeutics, contact Eclipsebio today.