



Capping, poly(A) tail and LNP analysis for mRNA vaccine product development

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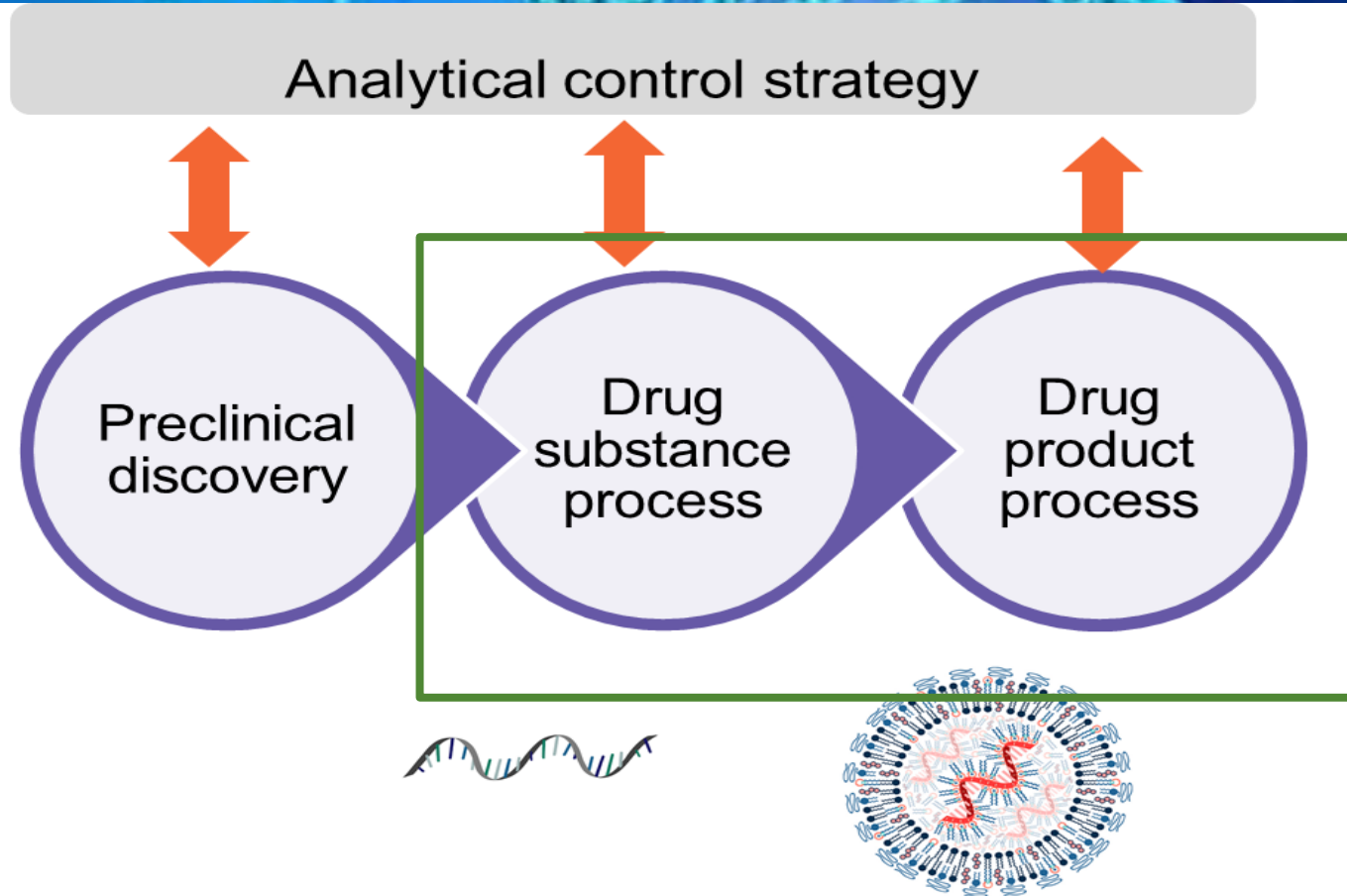


Disclaimer

This work was sponsored by GlaxoSmithKline Biologicals SA.
Qi Zeng is an employee of the GSK group of companies

CMC Analytical method development

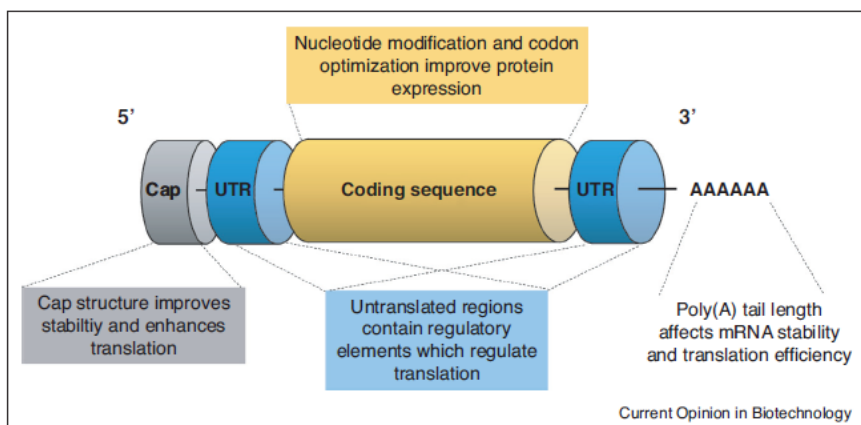
A critical role across the entire drug/vaccine development process



CMC

Chemistry, Manufacturing and Controls

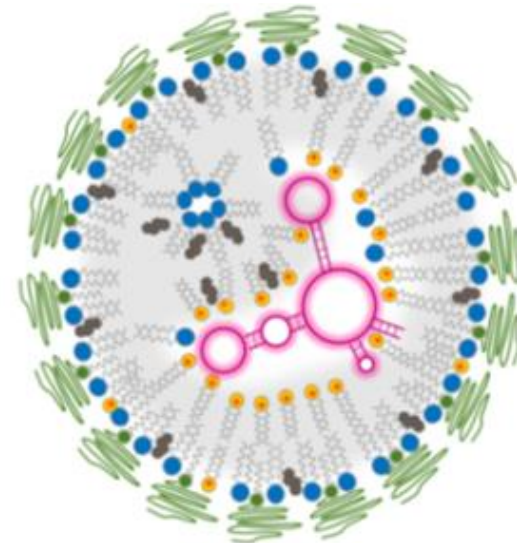
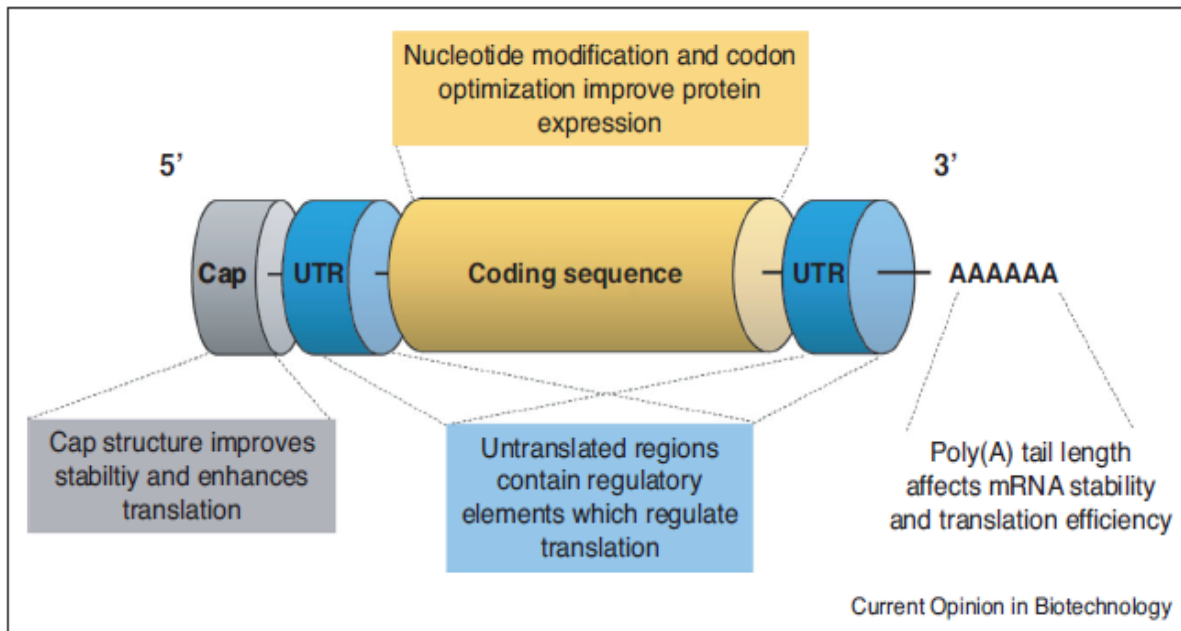
USP Guideline for mRNA vaccine analytical control strategy



Quality	Attribute	Method
Identity	Sequence confirmation	Next generation sequencing (NGS)
		Sanger sequencing
		Reverse Transcriptase – PCR
Content	RNA content	RT-qPCR and RT-dPCR, Ultraviolet Spectroscopy
Integrity	Percentage of intact mRNA and fragment mRNA	Capillary gel electrophoresis
	5' cap	IP-RP-HPLC
	3' poly(A)	RP-HPLC
	mRNA Integrity	Gel electrophoresis
Purity	Product related impurities - dsRNA	Immunoblot
	Residual DNA template	qPCR
Safety	Endotoxin	USP <85>
	Bioburden	USP <61>, <62>, <1115>
	Sterility	USP <71>
Other	Appearance	USP <1>, <790>
	pH	USP <791>

Critical Quality Attributes

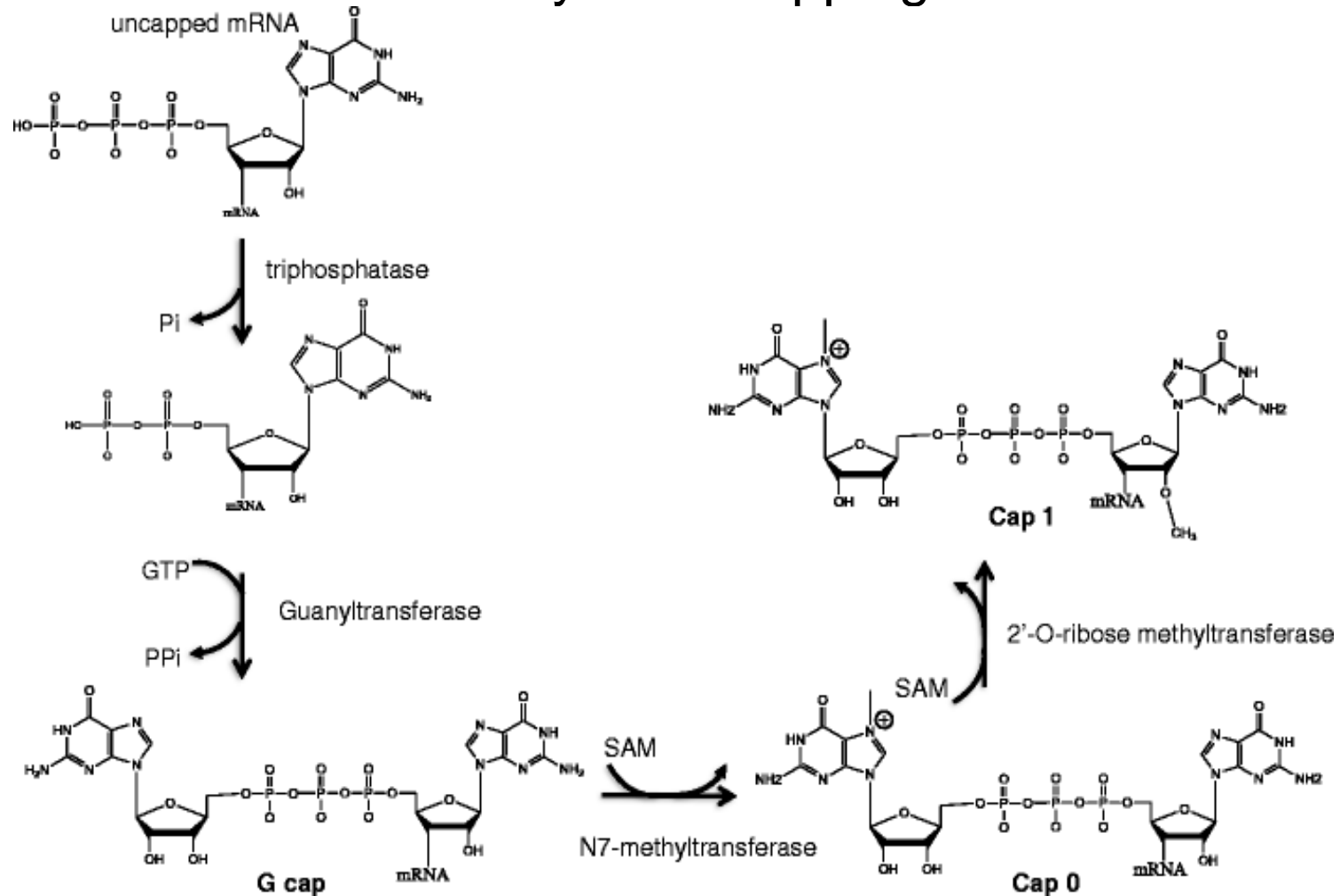
- 5'capping: improves mRNA stability and enhances translation.
- Poly(A) tail: mRNA stability and translation efficiency.
- Cationic lipid: counter ion to stabilize mRNA molecule.



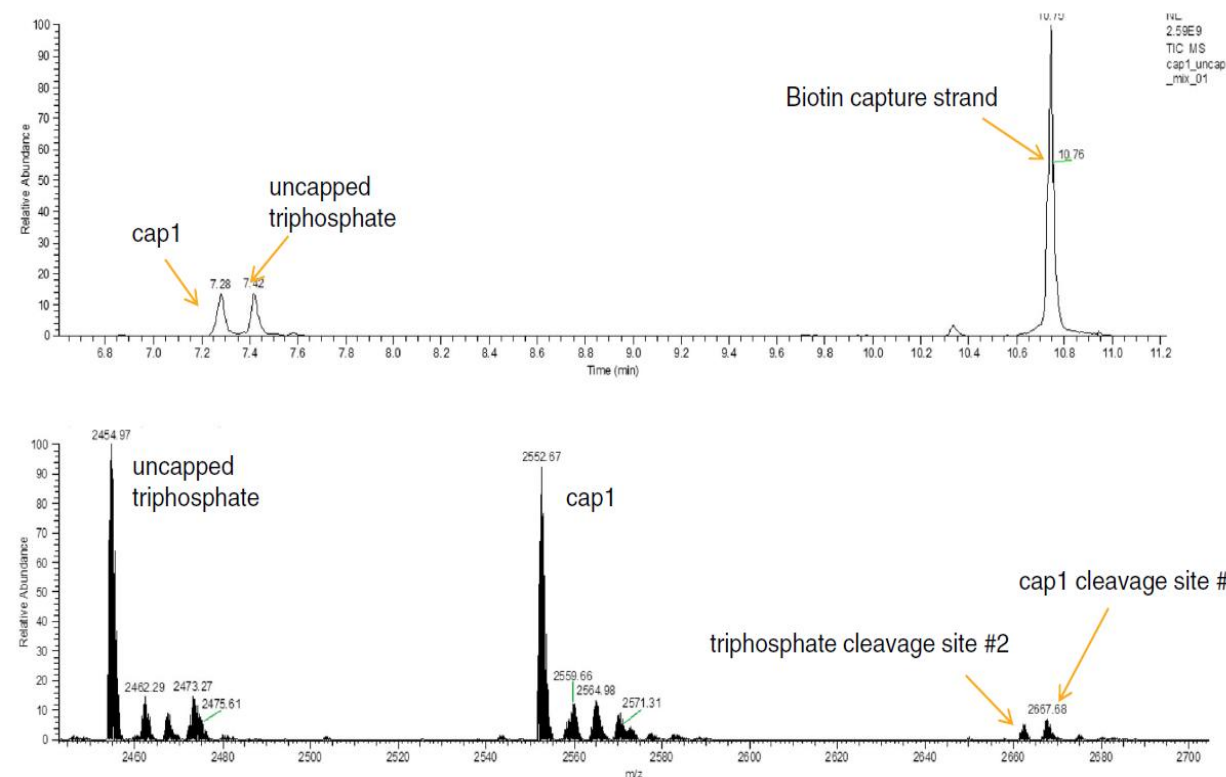
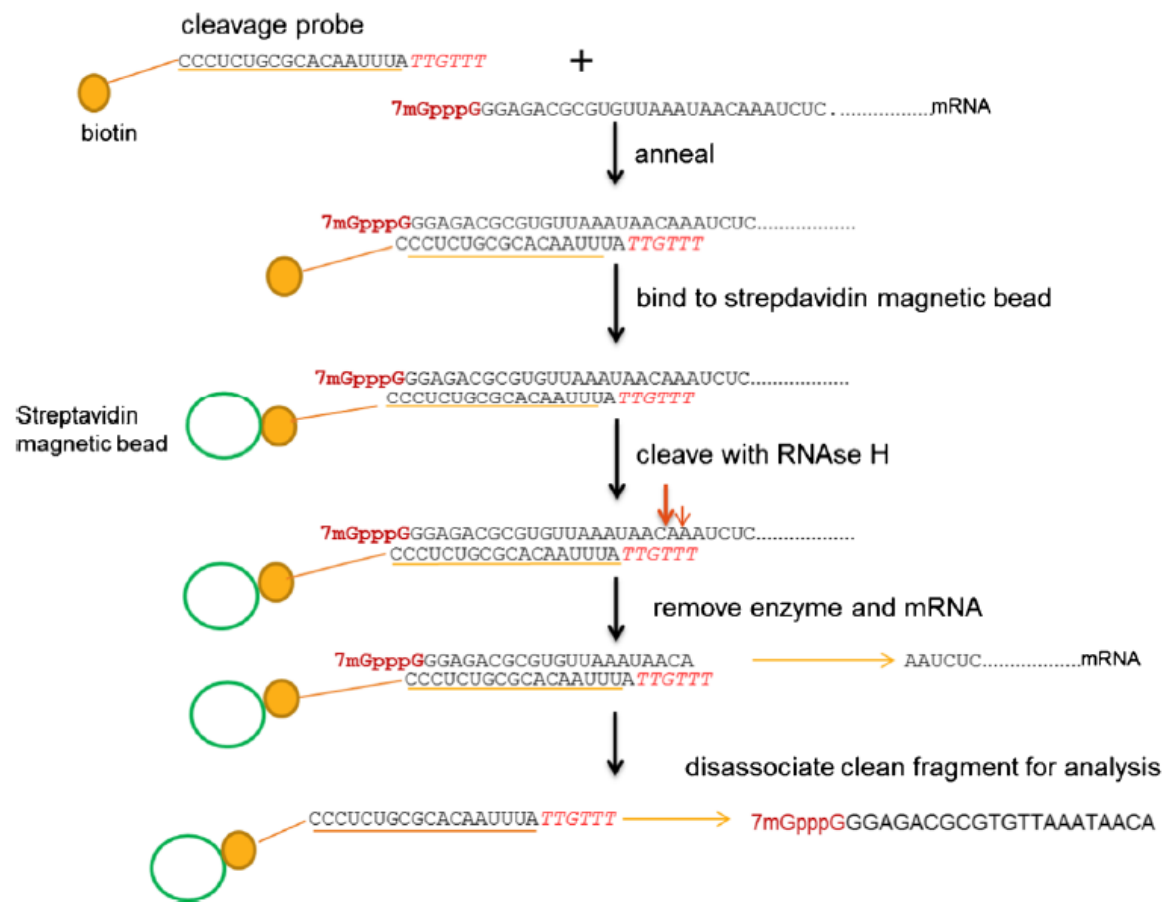
Kon E, Elia U, Peer D. Principles for designing an optimal mRNA lipid nanoparticle vaccine. Curr Opin Biotechnol. 2022 Feb;73:329-336. doi: 10.1016/j.copbio.2021.09.016. Epub 2021 Oct 26. PMID: 34715546; PMCID: PMC8547895.

5'capping reaction by Vaccinia enzyme system

Vaccinia enzymatic capping reaction.

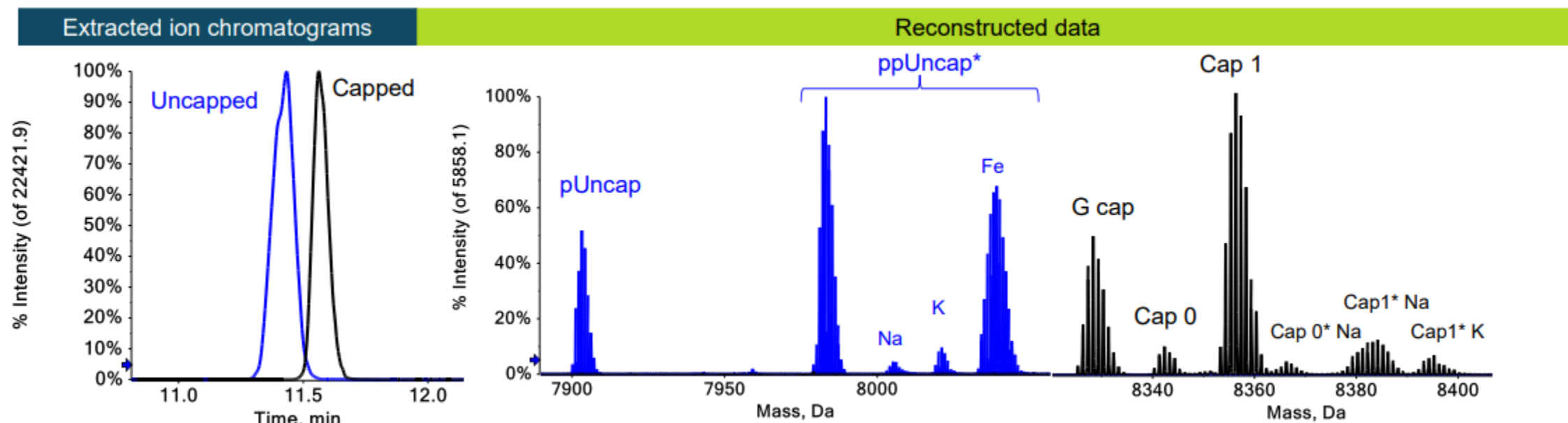


Capping analysis with customized probe and RNaseH



Beverley et al, 2016 Anal Bioanal Chem (2016) 408:5021–5030

Separation and identification of different capping stages.

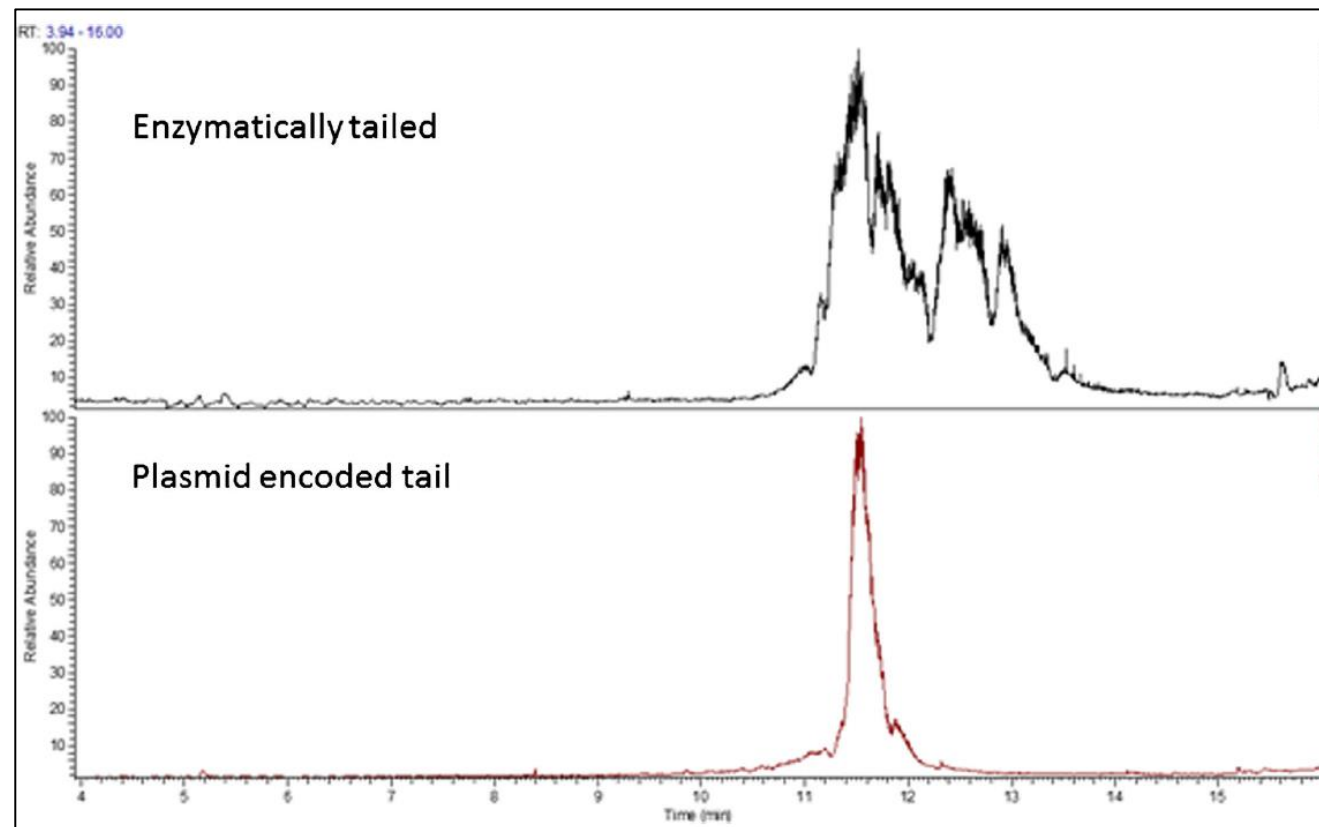


SCIEX technical application notes "Characterization and relative quantification of mRNA 5'-capping"

► Poly (A) tail synthesis approaches

- Enzymatic adding poly (A) tail.
- Encoded in plasmid DNA template

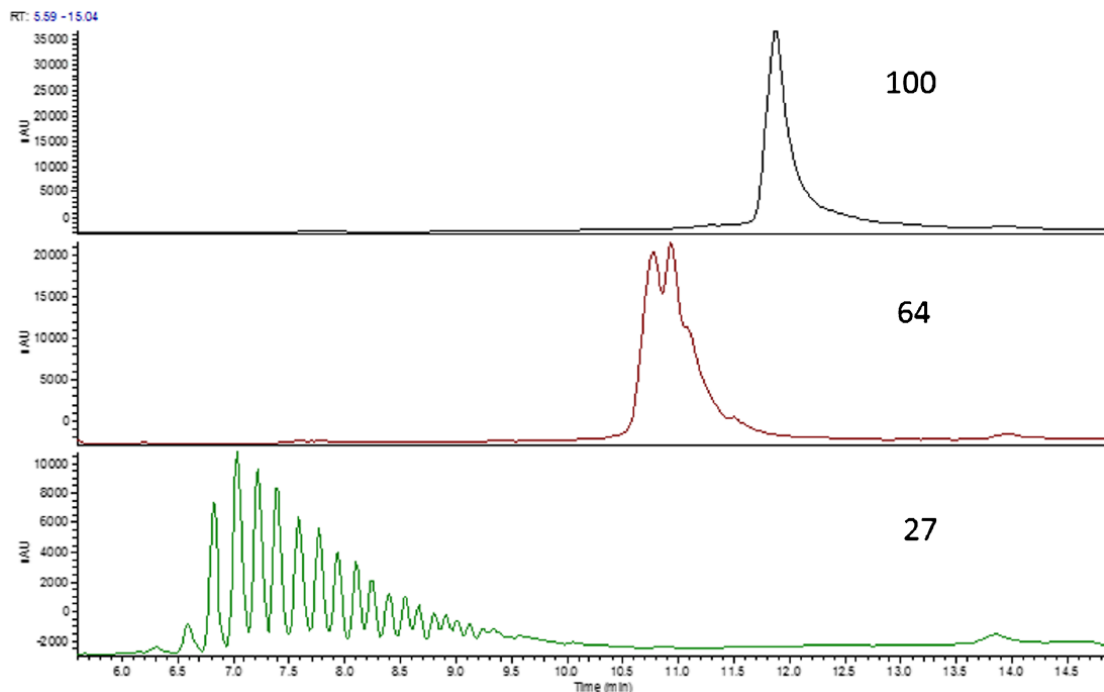
- Heterogeneity



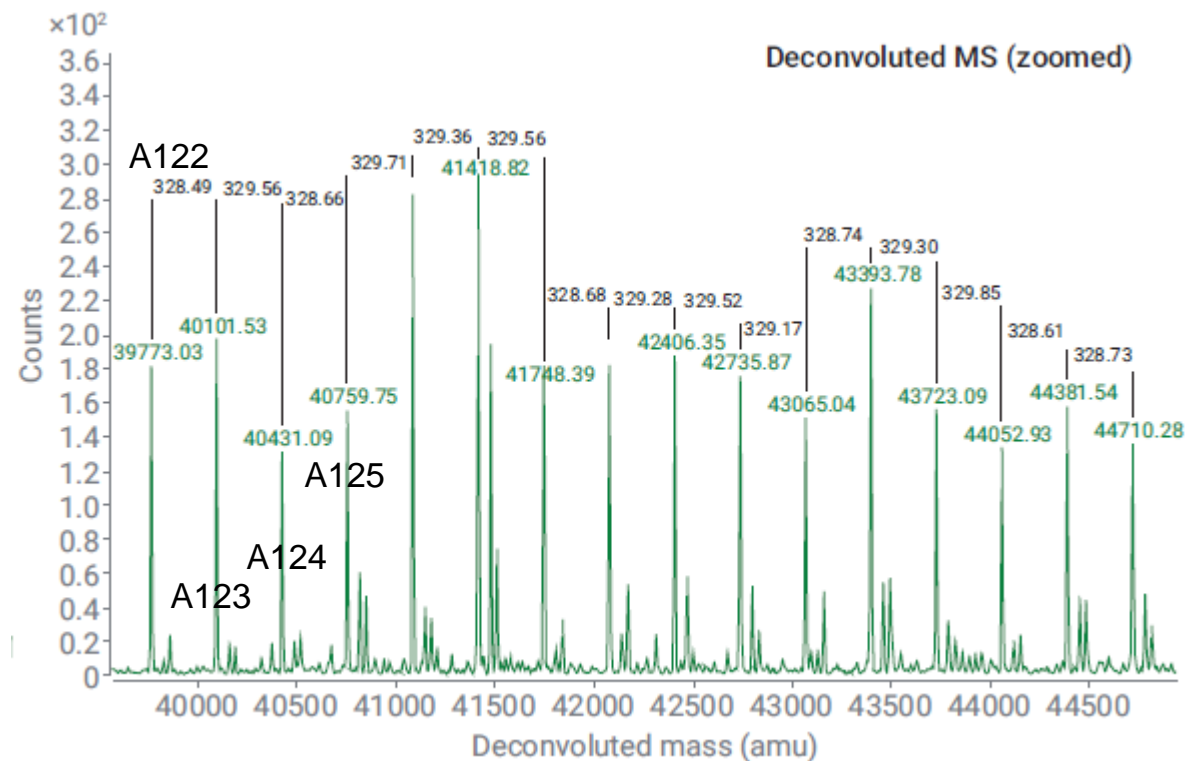
Beverley et al. 2018 Analytical and Bioanalytical Chemistry (2018) 410:1667–1677

Poly (A) tail analysis by LC-MS

LC chromatogram of polyA tail at various length



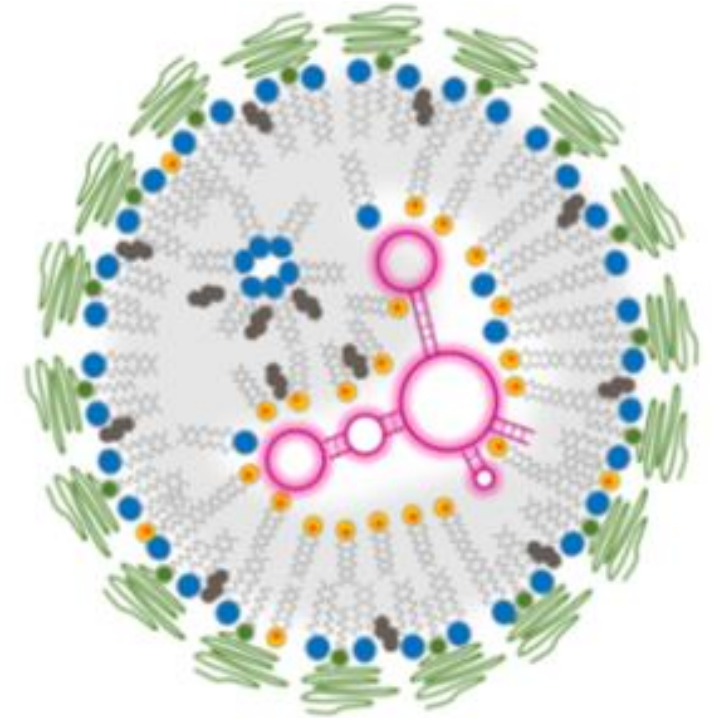
High resolution mass spectra of long polyA tails



Agilent application notes "Analysis of mRNA Poly-A Sequence variants by High-Resolution LC/MS".

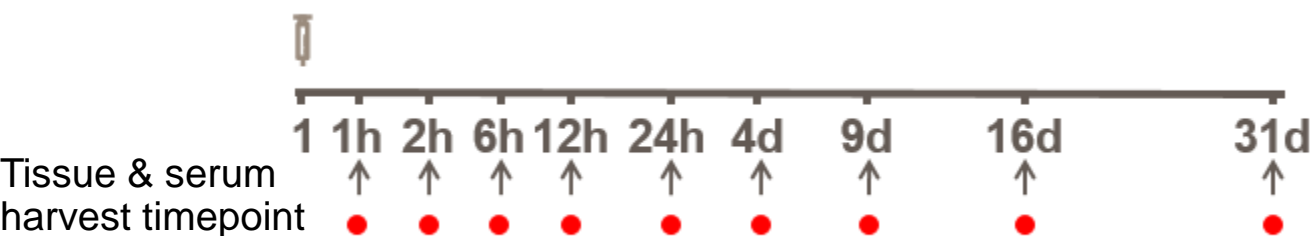
LNP biodistribution and pharmacokinetic analysis by LC-MS

Component	Function
Cationic lipid	RNA entrapment and intracellular release
Zwitterionic lipid (DSPC) Phosphatidylcholine	as lipid base for particle assembling
Cholesterol	Stabilizes particles by affecting mechanical strength and elasticity, and increasing lipid packing density
PEGylated lipid	Prevents particle aggregation during the formulation process, increases circulation lifetime.



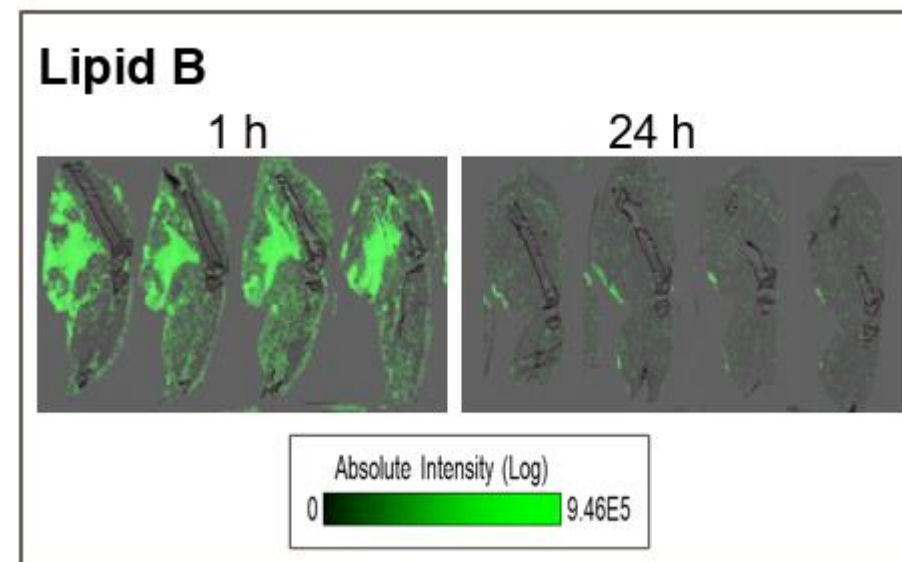
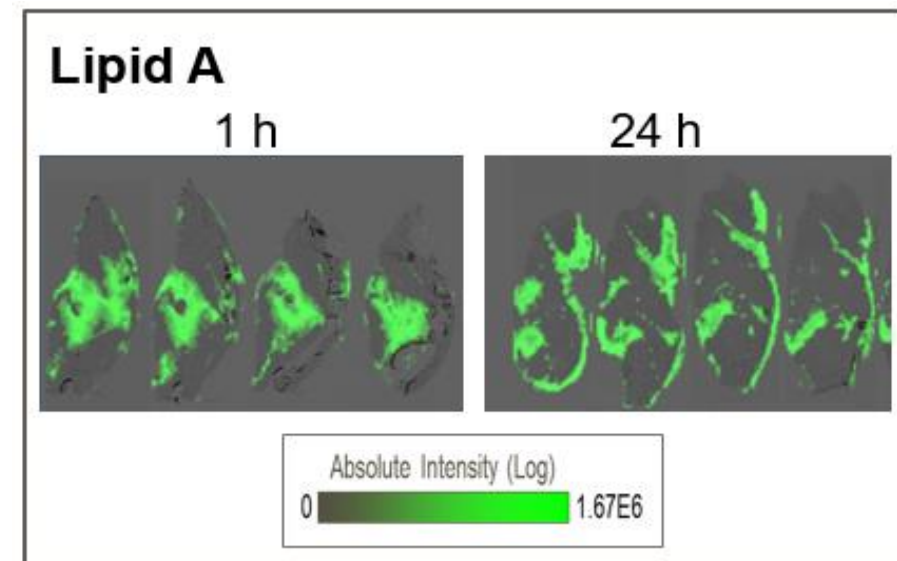
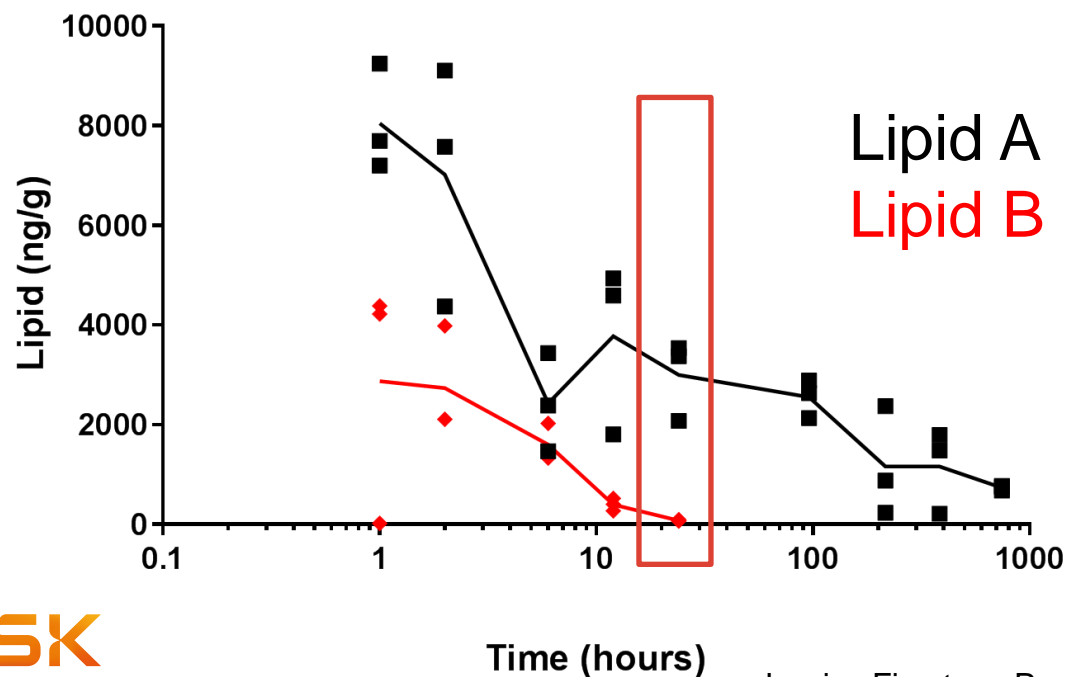
Pharmacokinetic analysis of cationic lipids in mouse model

Lipid biodistribution by MALDI MS imaging



Cationic lipid quantification by LC-MS

Cationic Lipid in Hind Limb



Conclusion

- An overview of LC-MS analytical methods development for several critical quality attributes in mRNA vaccine development: 5'Capping, 3'PolyA tail and LNP biodistribution and pharmacokinetic analysis.
- LC-MS has demonstrated the high-resolution power to support full understanding of mRNA characteristics and robustness to provide quantitative analysis for lipid pharmacokinetic study.



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