Analytical Characterization of LNP-RNA Nanovaccines

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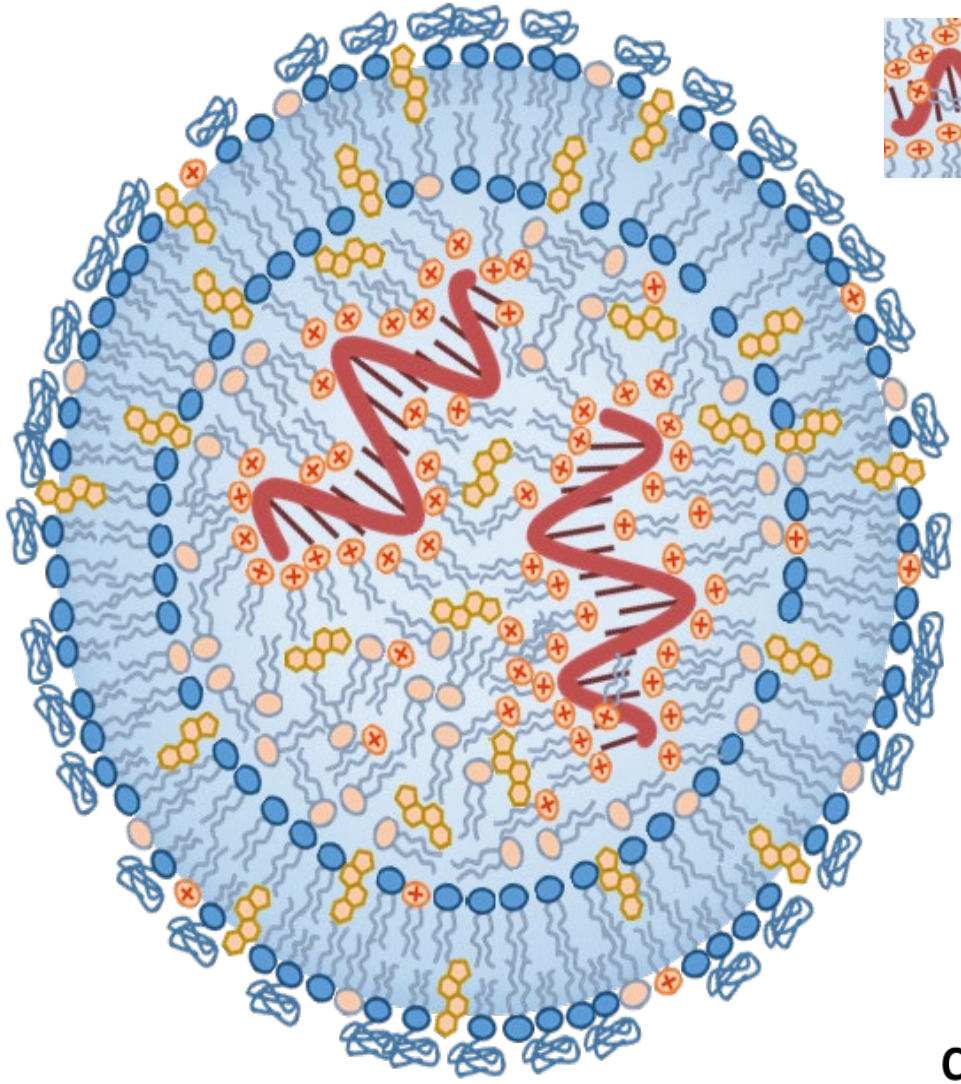
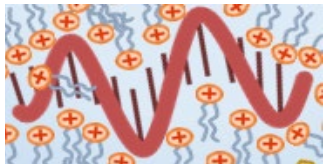
The views expressed are those of the presenter and should not be construed to represent European Commission views or policies

CCQM Workshop on Particle Metrology, 26th October 2022

LNP-mRNA nanovaccines for COVID-19

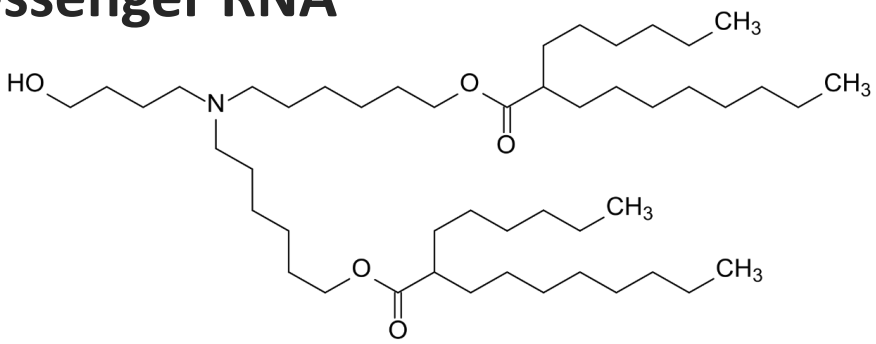
Pfizer/BionTech, Moderna



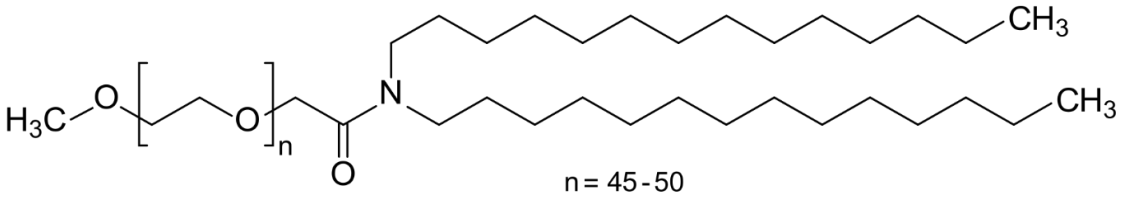


mRNA: messenger RNA

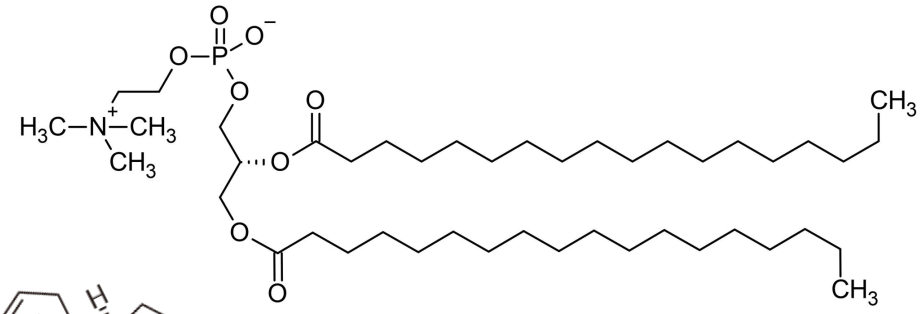
Charged ionizable lipid



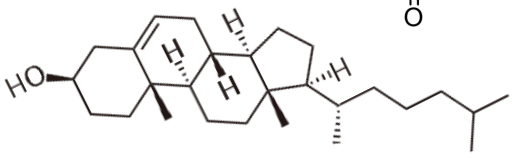
PEG-lipid



Phospholipid



Cholesterol



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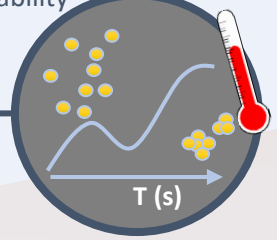
LNP-mRNA Nanovaccines vs. Nanomedicines

- There are strong similarities, but also key differences
- Main point: nanovaccines are given *intra muscular* (im), nanomedicines are *intra venous* (iv) administration.
- Messenger RNA much more complex biological molecule than small drugs usually delivered by nanomedicines.

Pre-Clinical Characterization of Nanovaccines

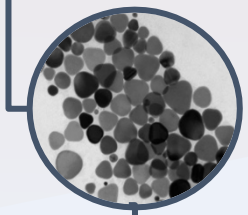
Physico-Chemical characterization

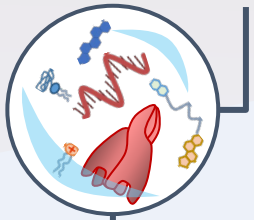
Stability
- Aggregation
- Carrier/cargo structure
- Time stability



Physical
- Size distribution
- Particle concentration
- Shape and morphology

Chemical
- Composition
- Surface charge and pKa
- Free/bound cargo
- Cargo release kinetic

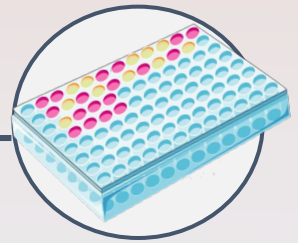


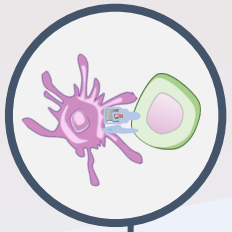


Batch-to-batch consistency

In vitro testing

***In vitro* toxicity**
- Endotoxin contamination
- Cytotoxicity
- Immunotoxicity





***In vitro* immunogenicity**
- Delivery efficiency
- Interaction with immune cells
- Immunomodulation

In-vivo

***In-vivo* pre-clinical testing**
- Immunogenicity
- Efficacy
- Safety

Clinical trials

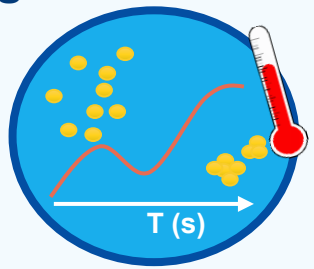
nature nanotechnology 2022 Jun;17(6):570-6. PERSPECTIVE
https://doi.org/10.1038/s41565-022-01129-w
Check for updates

Characterization of nanoparticles-based vaccines for COVID-19

Giuditta Guerrini[1,3], Davide Magrì[1,3], Sabrina Gioria[1], Donata Medaglini[2] ✉ and Luigi Calzolai[1] ✉

Key Physicochemical Properties of LNP-mRNA Nanovaccines

Stability
- Shelf life and storage
- Carrier/cargo structure
- Aggregation



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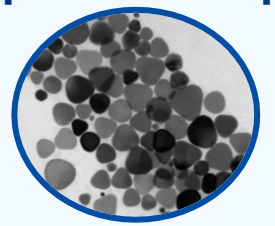
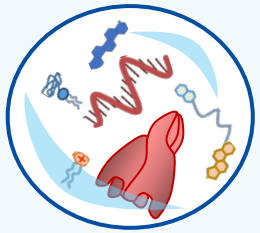
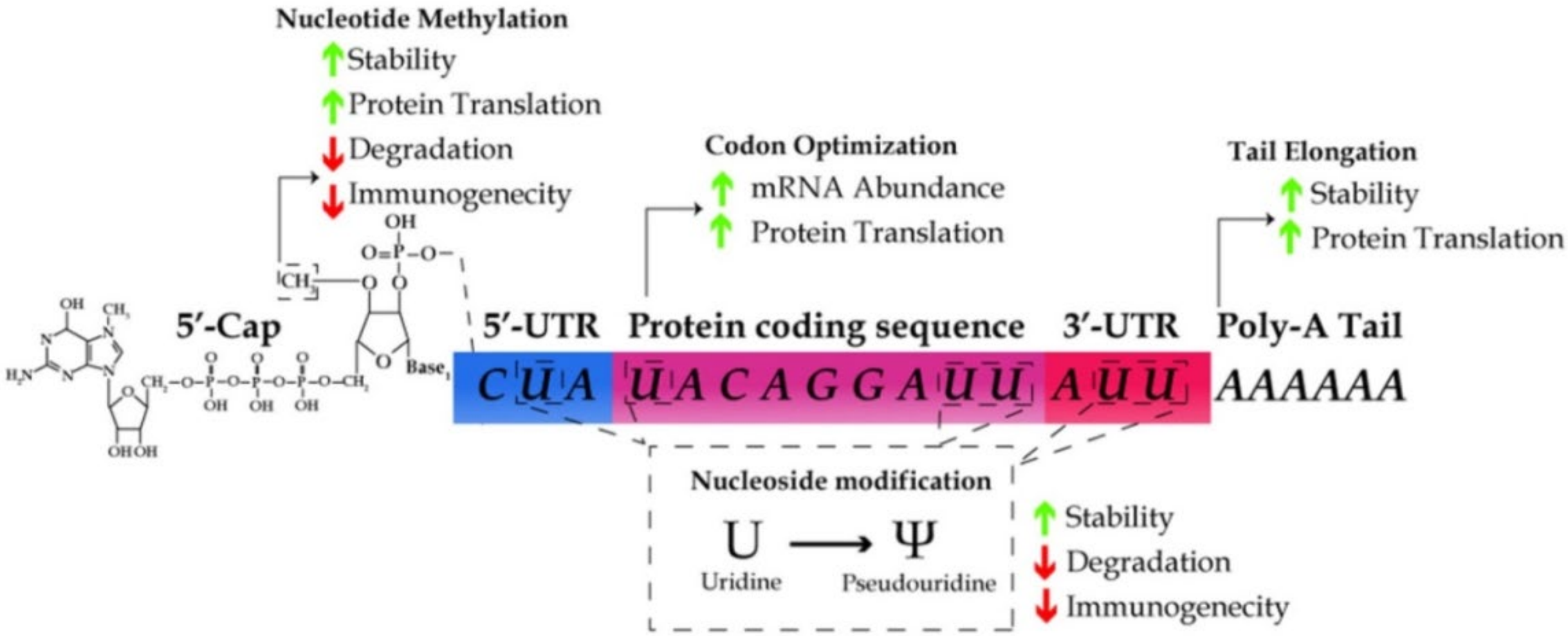




Chemical
- Composition
- Surface charge and pKa
- Free/bound cargo
- Cargo release kinetic

Composition: mRNA modifications



Park, Jung Woo, et al. "mRNA vaccines for COVID-19: what, why and how."
International journal of biological sciences 17.6 (2021): 1446.

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Composition Issues

- mRNA quantification and integrity
- 5’ capping and 3’ polyA-tail
- Purity of the mRNA
- Proportion of mRNA that is encapsulated (i.e. free/bound cargo)
- Lipid-related impurities
- Lipid-RNA adducts
- Actual structure and morphology of soft LNP-mRNA

Analytical Challenges

- Which analytical measurement available to measure each key property?
- Analytical capabilities for mRNA measurements somehow less advanced
- When to use orthogonal measurements?
- How to measure internal structures of these soft particles with techniques working in vacuum? (ex. TOF-SIMS, XPS)
- No reference materials and/or documentary standards available

Lipid-RNA adducts in LNP-mRNA systems

Packer, Meredith, et al. "A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems." *Nature communications* 12.1 (2021): 1-11.

Impurities formed through lipid:mRNA reactions:

Oxidation of the ionizable cationic lipid leads to the formation of covalent lipid-mRNA adducts. These lipid-mRNA species cannot be transcribed into protein

How they were identified:

- Reversed phase–ion pair HPLC (RP-IP HPLC)
- Can’t be detected using capillary electrophoresis, which is normally used to measure mRNA integrity

Lipid-RNA adducts in LNP-mRNA systems

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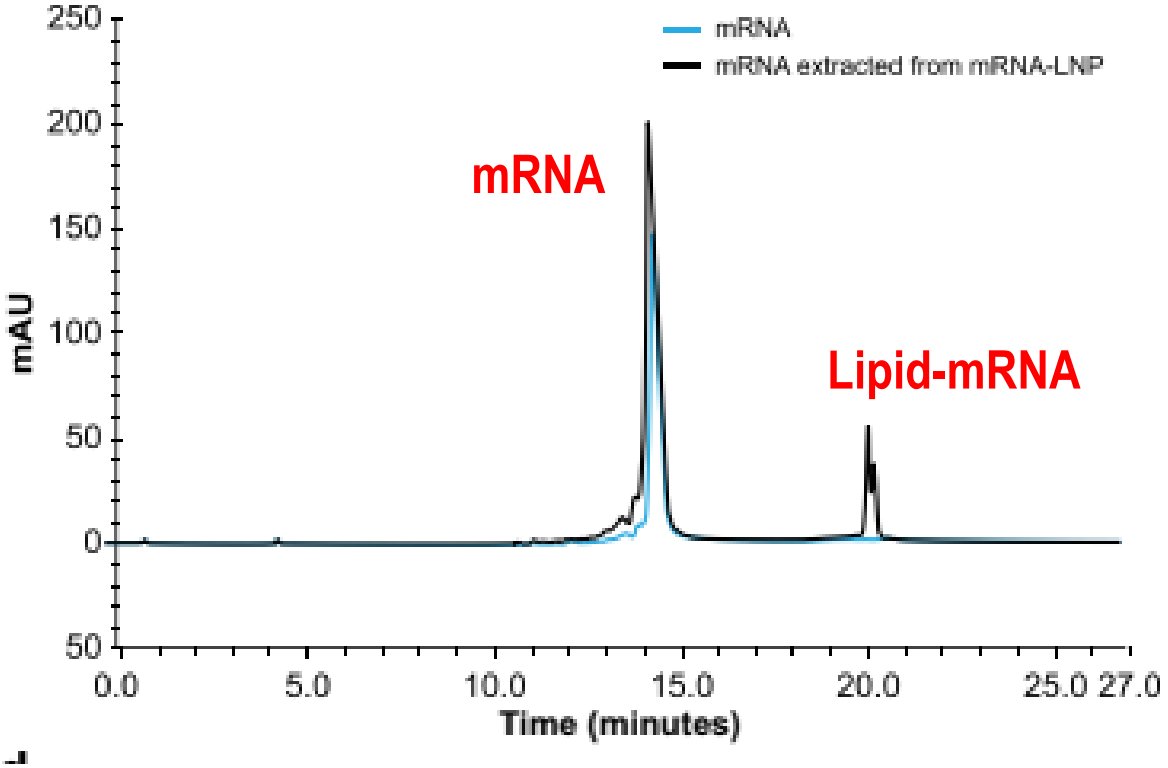
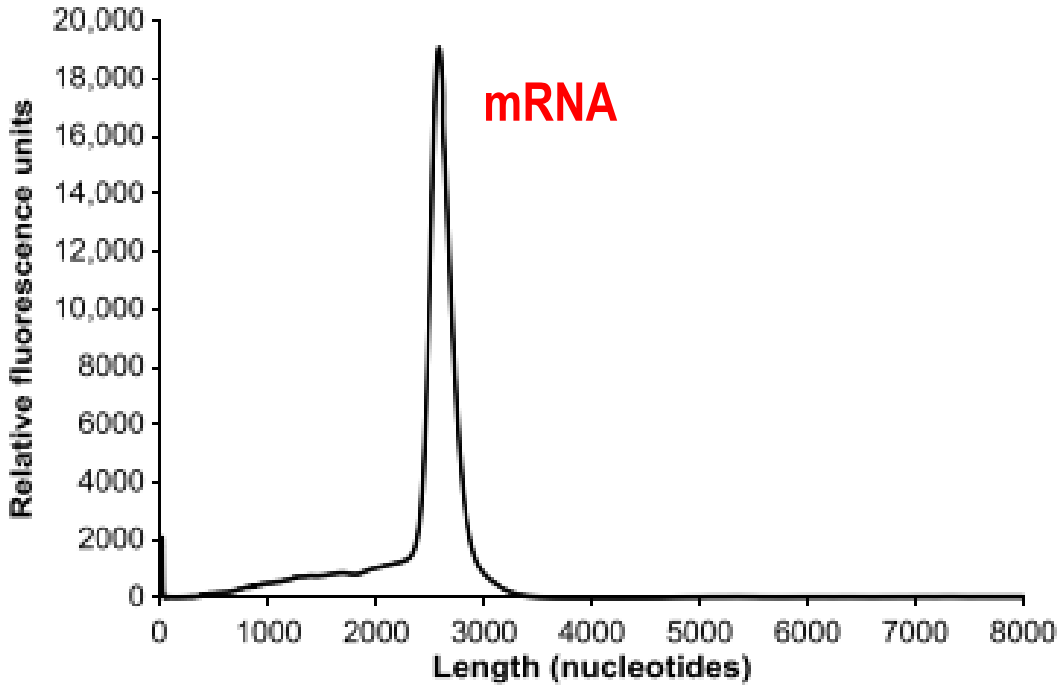
Reversed phase–ion pair HPLC: 2 peaks

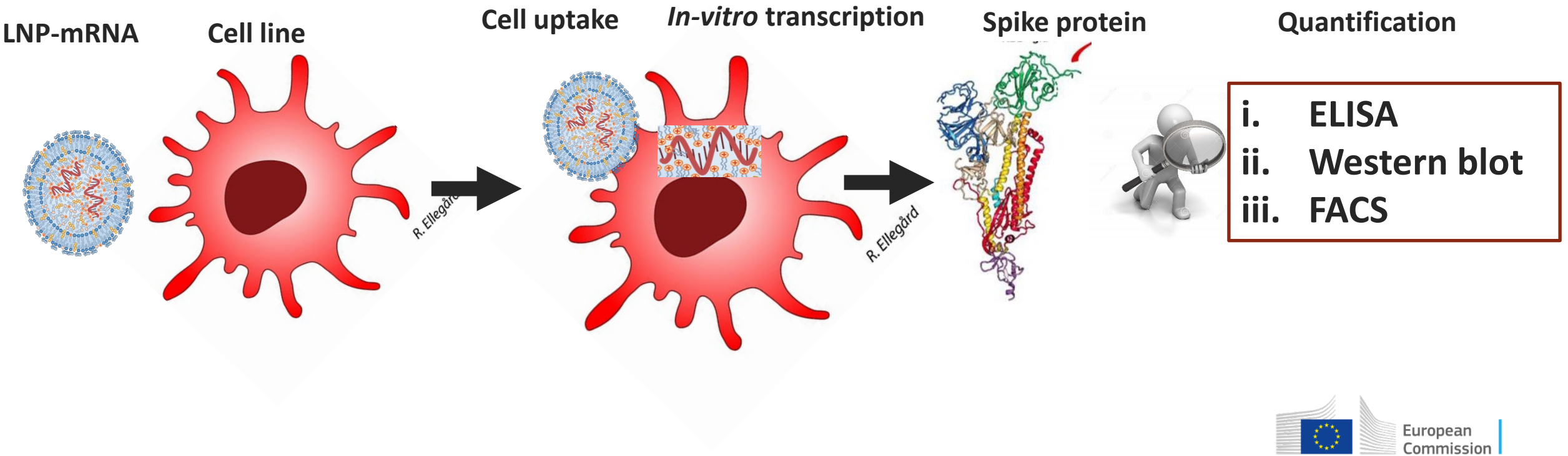


Capillary electrophoresis: single peak



In-vitro Potency of LNP-mRNA

- Depends on uptake of the LNP-mRNA by cells and the expression of the encoded antigen.
- It is a good surrogate end-point of the quality (and efficacy) of the formulation without use of animals.



How to Standardize Potency Measures

- Which LNP-mRNA. Reference material?
- Which cell line?
- Which read out measurement?
- How to ensure traceability?

The rapid expansion of RNA therapeutics

- The success of LNP-mRNA vaccines is leading to rapid expansion of RNA therapeutics in modern research and clinical development
- Infectious diseases and cancer show the largest growth and the greatest number of therapeutics in research phases
- **They will face similar issues for their accurate characterization**

	Preclinical	Active	Completed
Respiratory Disease	58%	33%	8%
Autoimmune Disease	50%	33%	17%
Blood Disease	50%	31%	13%
Liver Disease	46%	38%	8%
Infectious Disease	43%	25%	29%
Neurological and Neuromuscular	38%	38%	5%
Metabolic Disease	33%	24%	24%
Other*	33%	0%	67%
Wound healing	33%	0%	67%
Cardiovascular Disease	28%	34%	31%
Cancer	26%	48%	23%
Eye Disease	20%	35%	35%
Kidney Disease	14%	36%	36%
Transplantation	0%	0%	100%

https://www.cas.org/resources/cas-insights/biotechnology/rna-therapeutics-revolution

Conclusions

- Following the success of mRNA vaccines, LNP-RNA therapeutics are rapidly expanding in several therapeutic areas
- Analytical methods are needed to accurately measure key properties of these challenging LNP-RNA therapeutics
- Reference materials and documentary standard are needed to ensure the required confidence in the measurements


Thank you