

Enabling the next-generation non-viral gene therapy through Lipid-PEG alternatives based on bioinspired polymers

Aroa Duro Castaño, Curapath, Spain



Enabling next-generation Non-Viral Gene Therapy through custom design and end-to-end services in manufacturing of polymer and lipid-based therapeutics.

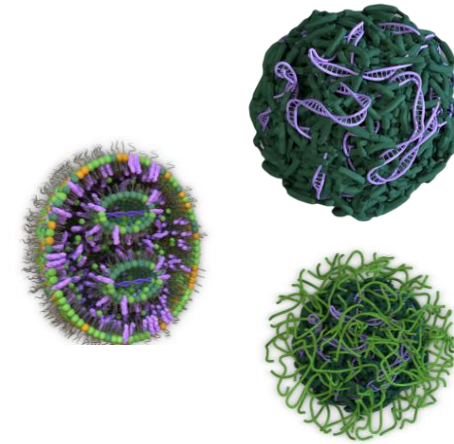
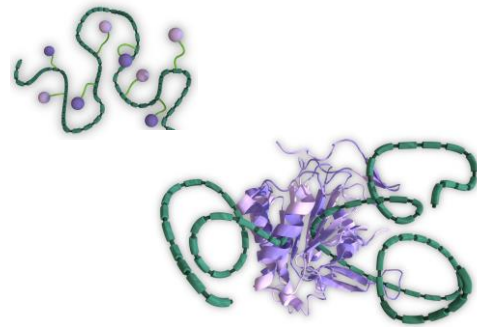
Our product categories include:



**Polymer & Lipid
Excipients**



**Chemical and
Biological
Conjugation**



**Lipid and Polymer
Nanoparticle
Formulation**



**Aseptic Fill
& Finish**

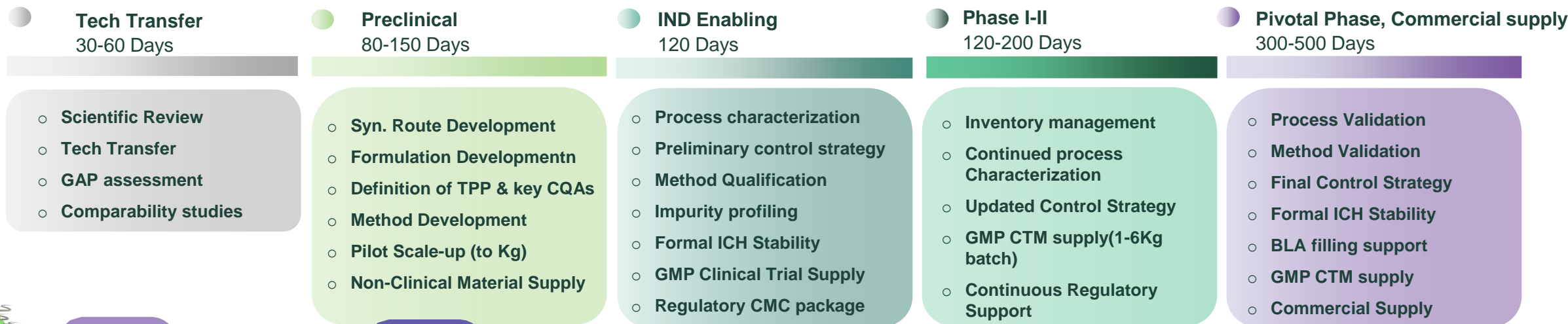


With our integrated partnership style, we support small and large clients and become a strategic partner to solve challenges in development and accelerate your speed to the clinic.



We smoothly navigate your therapeutic product throughout the clinical stages

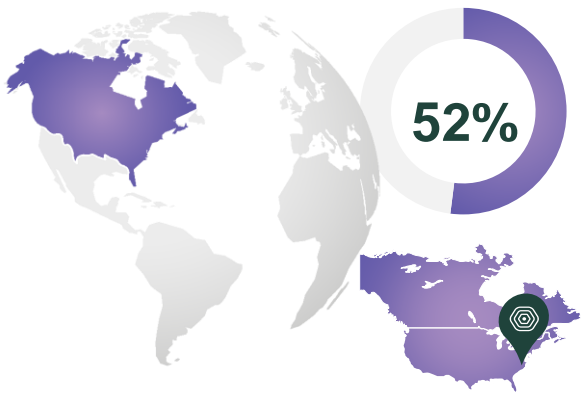
RA Based & Phase Appropriate CMC Development for Novel Excipients & Nanoparticle Drug Products



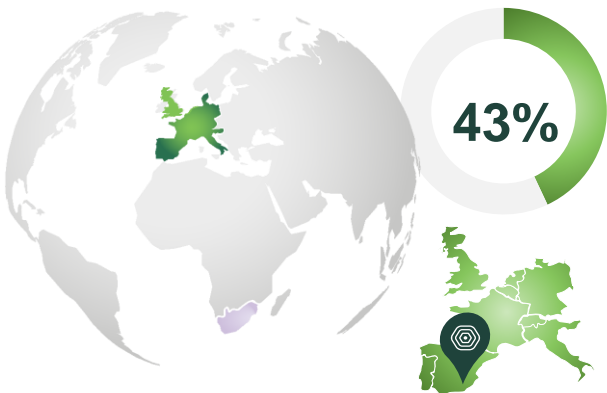
Collaborating with pharma and biotech companies worldwide



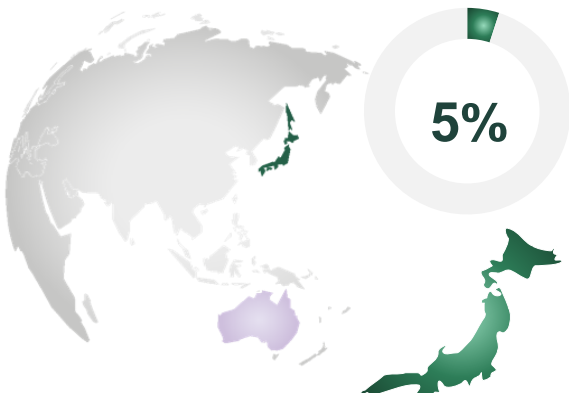
North America



Europe



Japan



+50 Active Customer Accounts, world leading innovator Biotechs and Pharmaceutical companies

Product Type %

PNP	51%
LNP	29%
Polymer Conjugate	17%
Polymer Drug	3%

Payload %

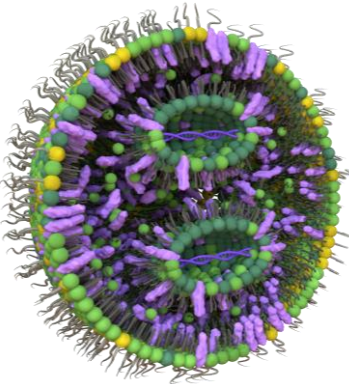
Nucleic Acid	35%
Small molecule	28%
Other	25%
Biologic	12%

Candidate Stage %

Discovery-preclinical	74%
Clinical Stage	12%
Pivotal Stage	8%
Commercial	6%



Beyond PEGylated lipids: Non-immunogenic shielding lipids in vaccines



Ionizable Lipid (*neutral/protonated*)

Aids in the encapsulation of nucleic acids through electrostatic interactions



Cholesterol

Decreases permeability of the LNP and enhances its stability



Nucleic Acid (*e.g. mRNA*)

Encodes protein of interest



'Helper' Lipid

Improves LNP stability and fusogenicity



PEG-Lipid

Prevents non-specific protein absorption, particle aggregation and controls LNP size

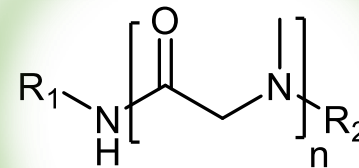
PEG Alternatives

PSar, Biocompatible, endogenous building blocks, overcome PEG immunogenicity

The key challenge is mimicking PEG physic-chemical properties while avoiding immunogenicity



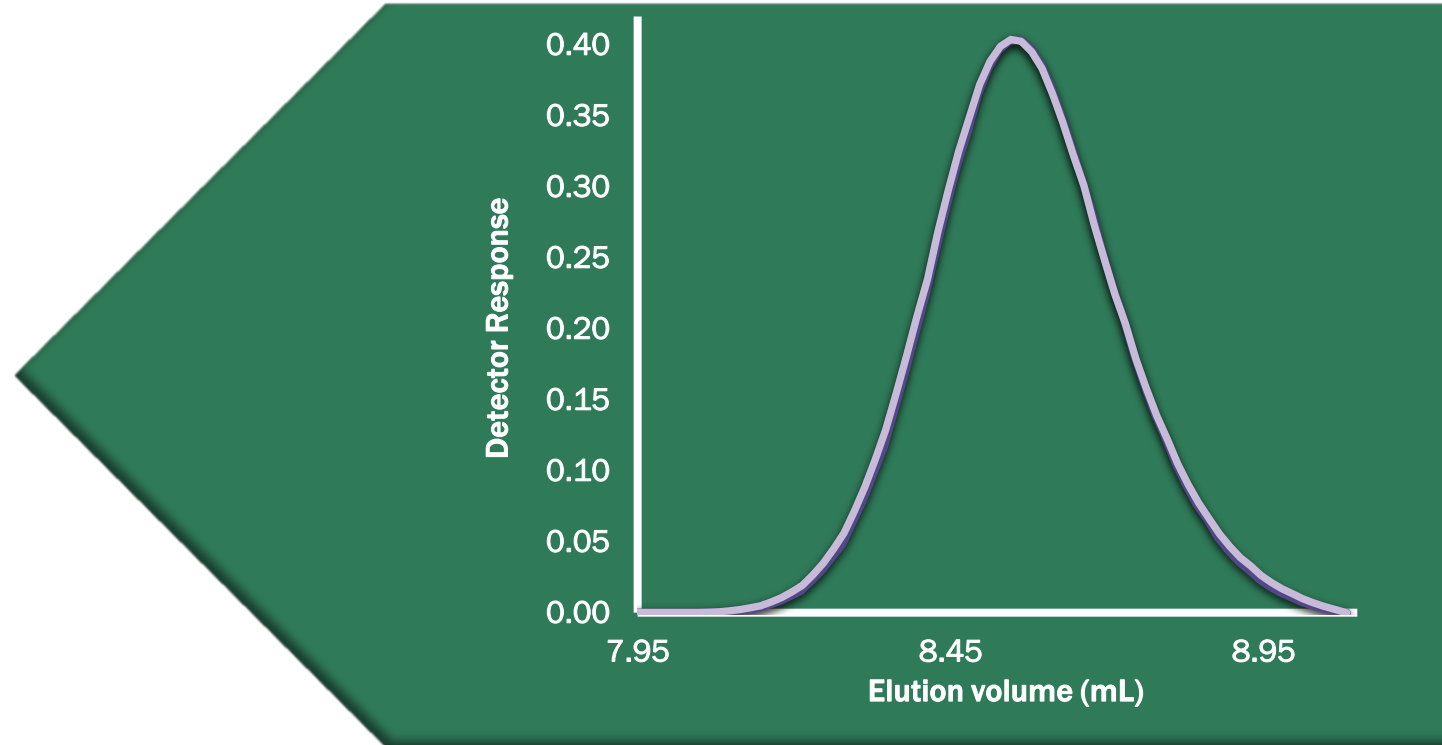
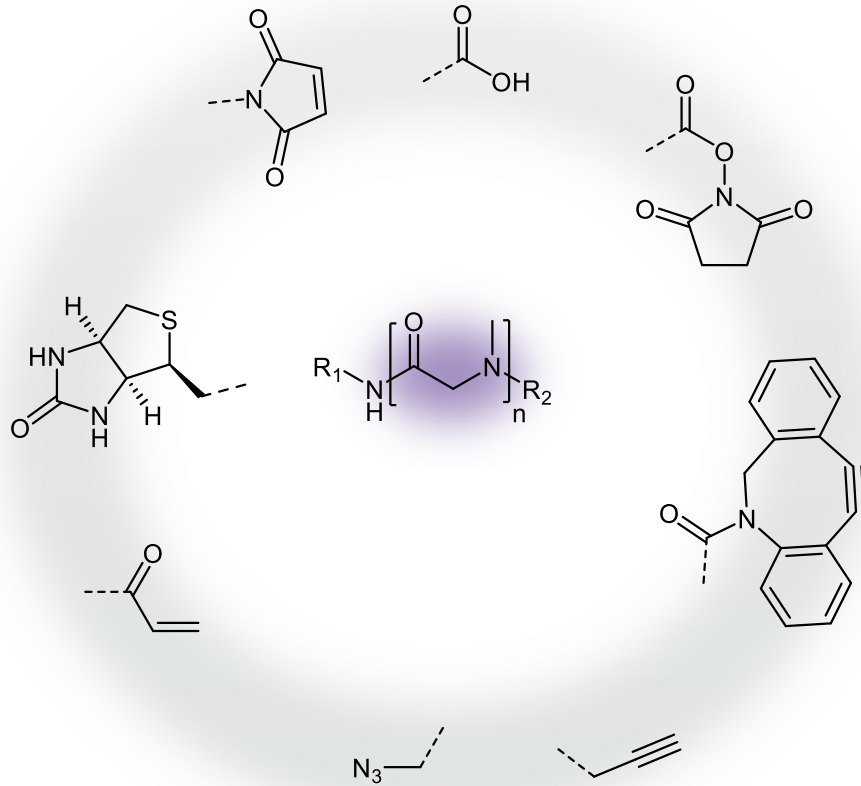
Psar-based Lipid



Beyond PEGylated lipids: Non-immunogenic shielding lipids in vaccines

From few grams to kg

R&D batch (100g)

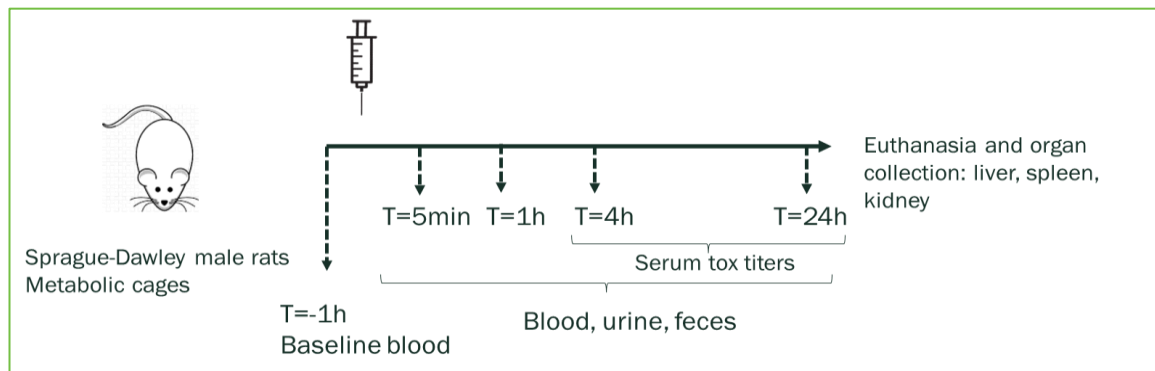


PSAR has analogue solubility and macromolecular properties and interactions with water compared to PEG, with a wide and versatile C-N terminus group functionality for (bio)conjugation and a proven GMP manufacturability.

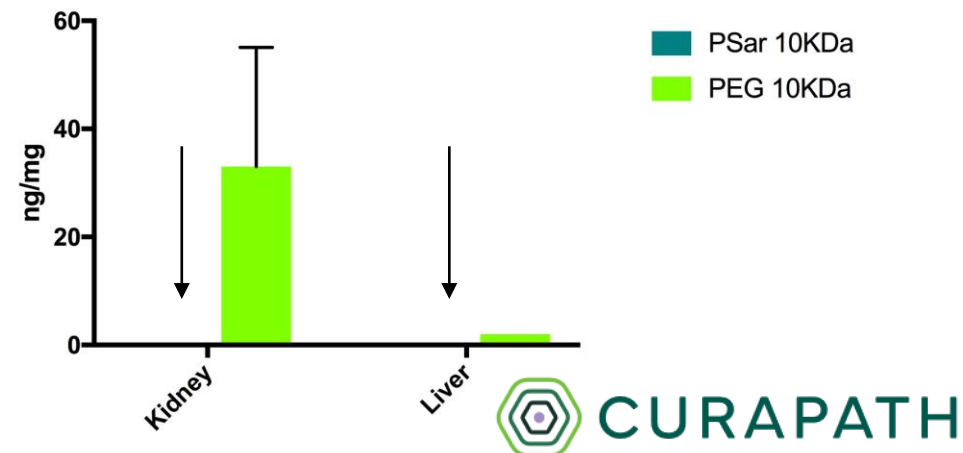
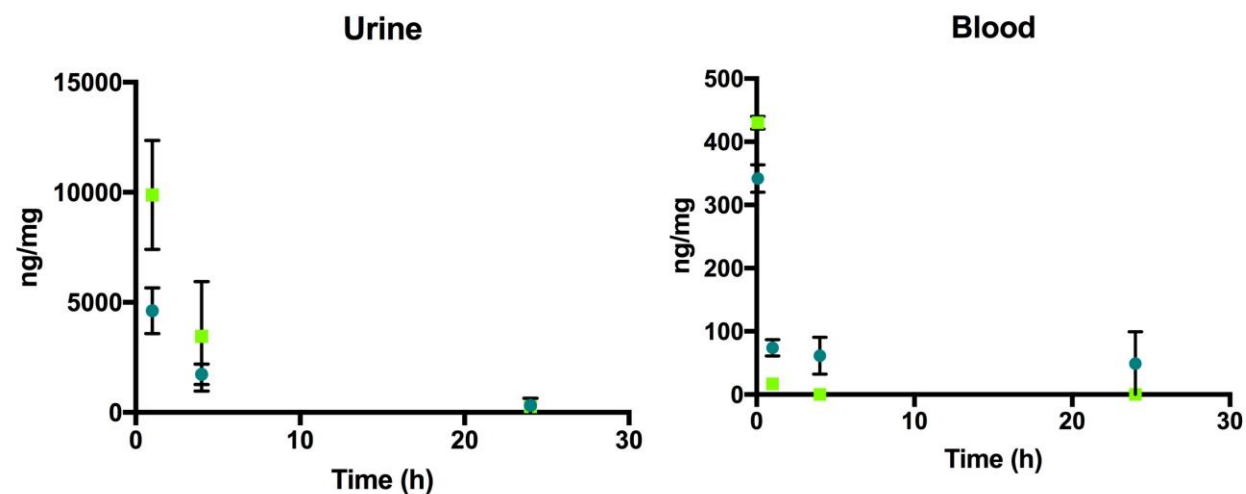


Superior Polysarcosine (Psar) Biological properties

PEG vs PSar Polymer Backbones TOX, Distribution, Elimination Study

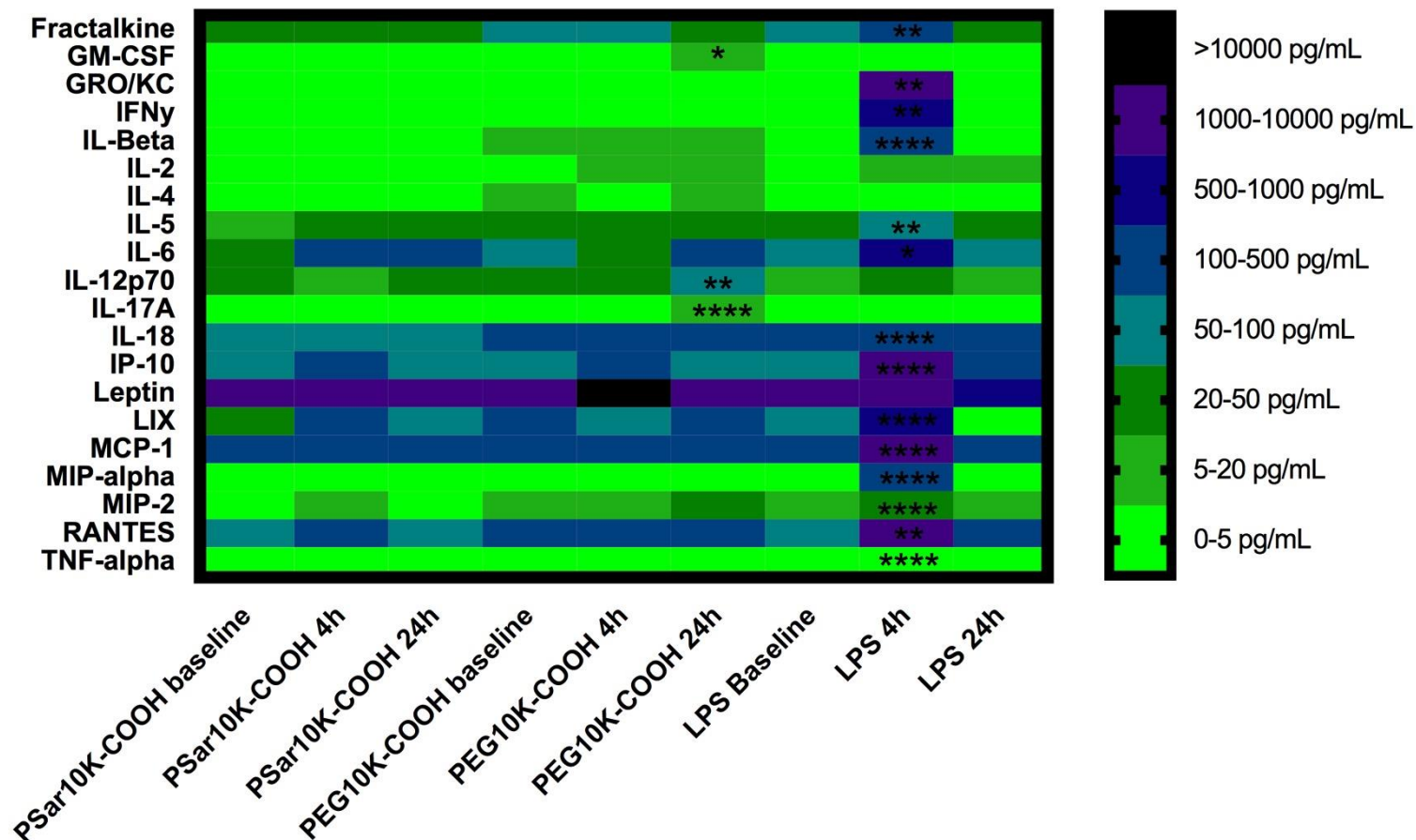


- Both PEG and PSar are eliminated through urine.
- Both PEG and PSar Not detected in feces or spleen.
- PEG is detected in Liver and Kidneys
- PSar is not detected in Liver and Kidneys suggesting possible biodegradation



Superior Polysarcosine (Psar) Biological properties

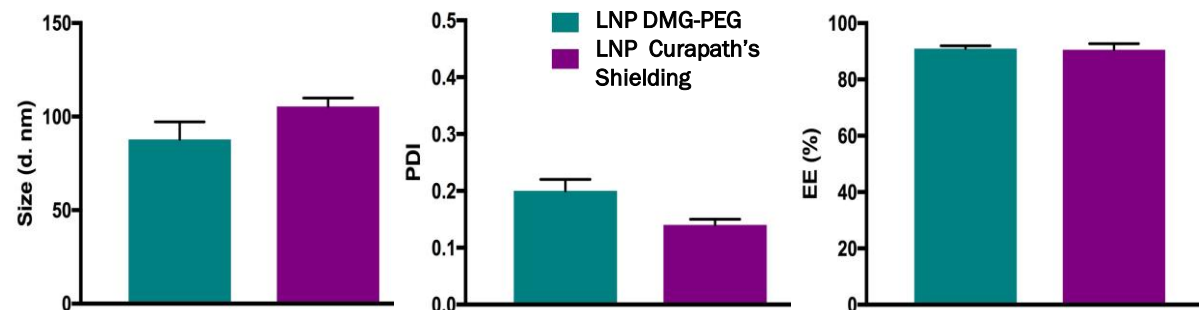
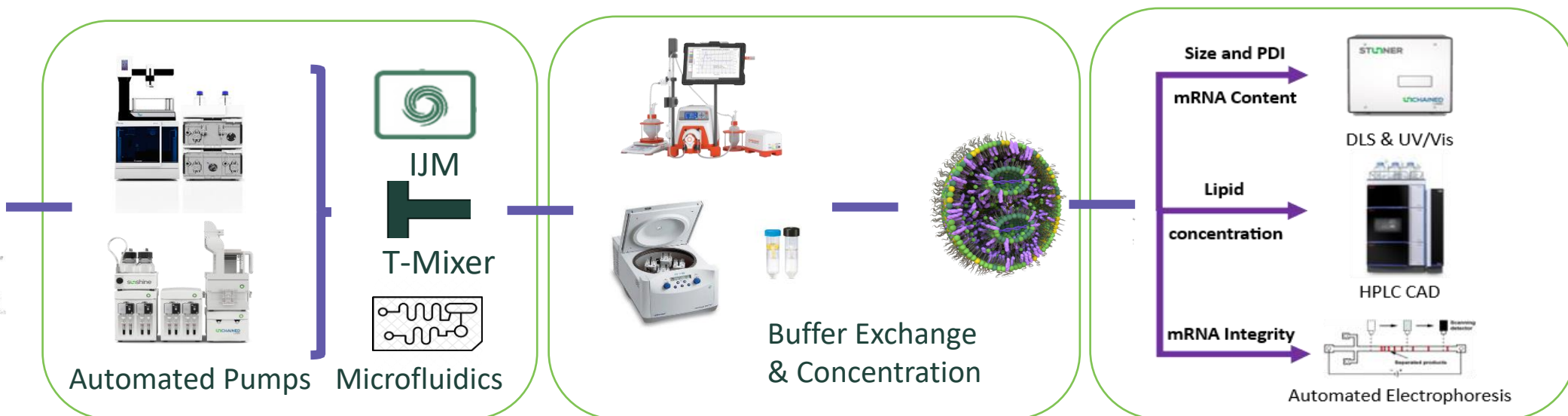
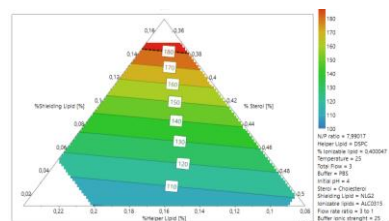
PEG vs PSar Polymer Backbones TOX, Distribution, Elimination Study



- The **MEA-PSAR140-COOH** polymer did not induce a systemic immune response since the level of inflammatory mediators stays the same as the baseline.
- However, there was an increase in few systemic inflammatory mediators 24h **after the administration of MEO-PEG10K-COOH**.
- Remarkably: **IL-17 regulates foreign body response to synthetic materials**
(L. Chung et al. Sci Traslatonal Med 2020, 12, 539)



Curapath's shielding lipids in LNP formulations



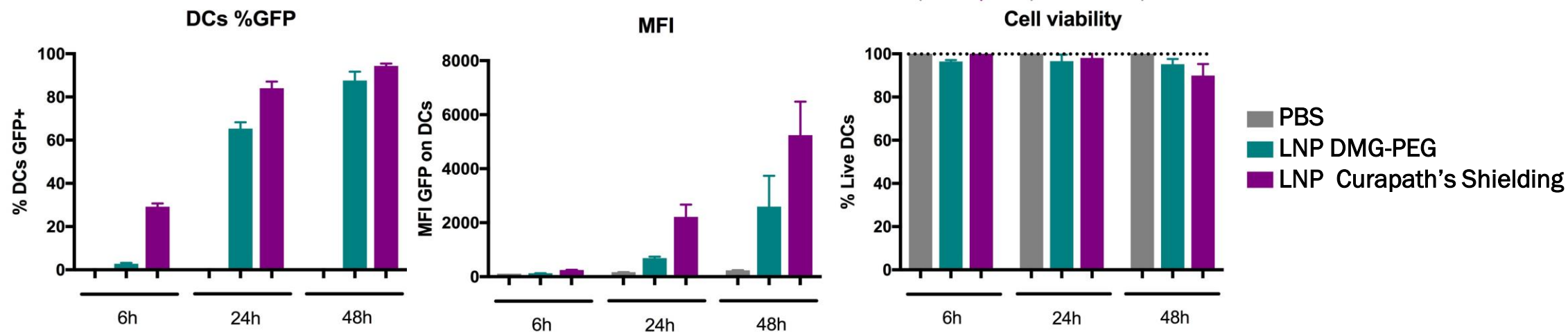
Curapath's shielding lipid yields LNPs with *adequate size in the 100 nm range and low PDIs*, with *>90% nucleic acid encapsulation efficiency* (commercial benchmark, mRNA-Luc)



Curapath's shielding lipids in LNP formulations



In vitro testing



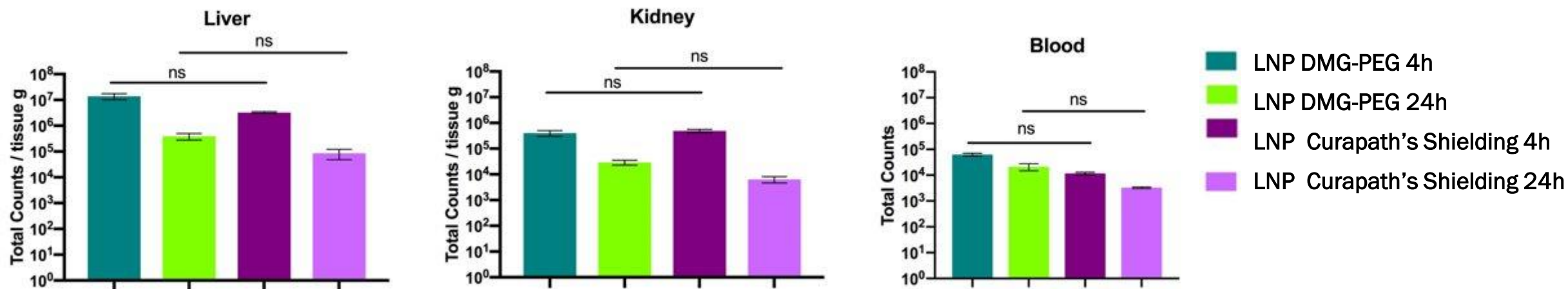
Curapath's shielding lipid perform equally as Benchmark in terms of transfection efficiency in vitro without affecting cell viability



Curapath's shielding lipids in LNP formulations



In vivo i.v. testing



Curapath's shielding lipid perform equally as Benchmark in terms of transfection efficiency in vivo without toxic effects



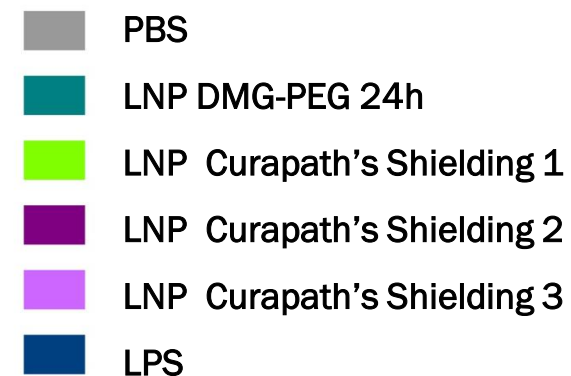
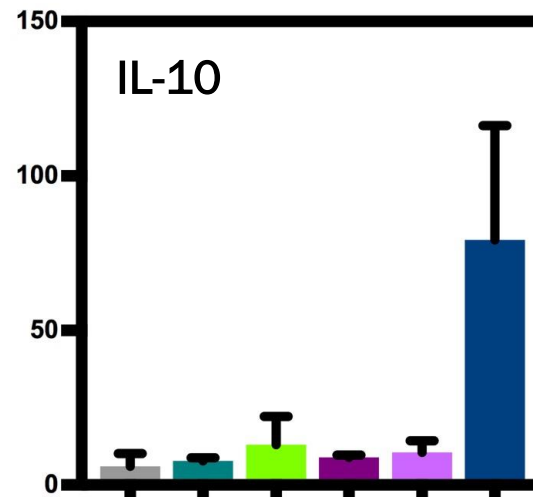
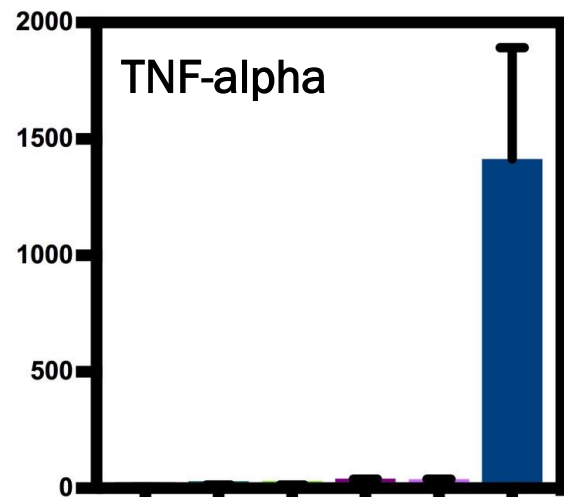
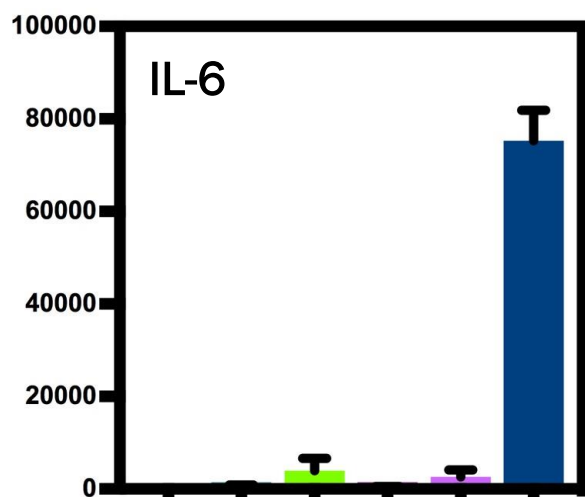
Curapath's shielding lipids in LNP formulations



In vivo i.v. testing



Pro-inflammatory markers and cytokine levels measured by ELISA in serum samples of Balb/C mice 2h after the i.v. injection



LNPs with proprietary shielding lipid do not induce significantly different release of pro-inflammatory markers and interleukines when compared to Benchmark



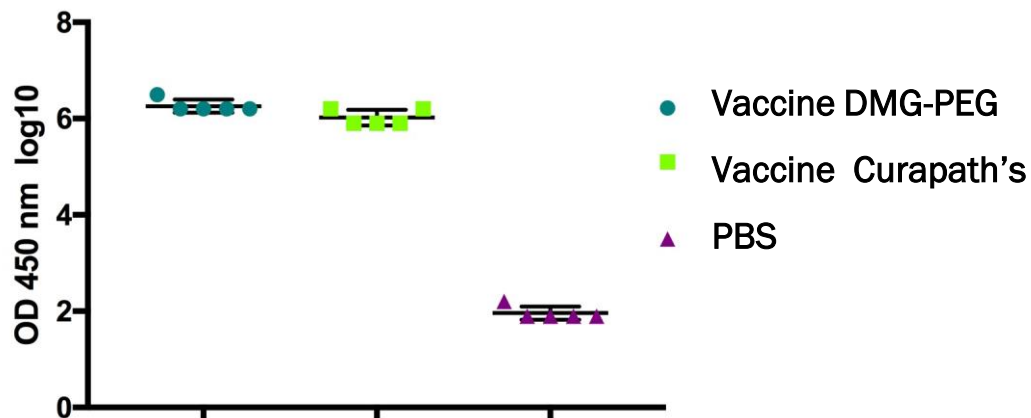
Curapath's shielding lipids in LNP formulations



In vivo i.m. testing



ELISA

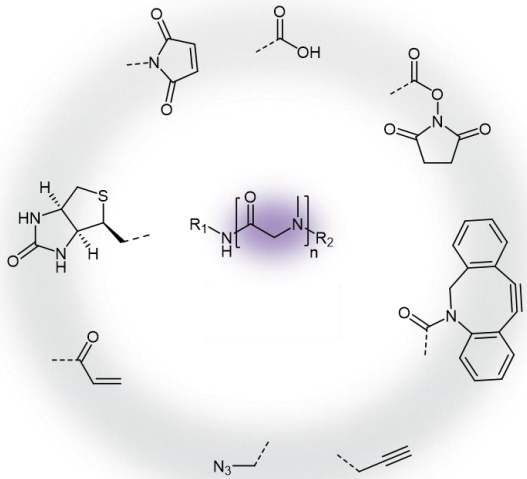


- Antibody titers by ELISA against a SARS-CoV-2 relevant protein measured as optical density (OD) at 450 nm at the end point of the experiment, 2 weeks after day 0 and day 14 intramuscular immunizations with Vaccine LNPs with DMG-PEG and Vaccine LNPs with Curapath's Psar shielding lipid.

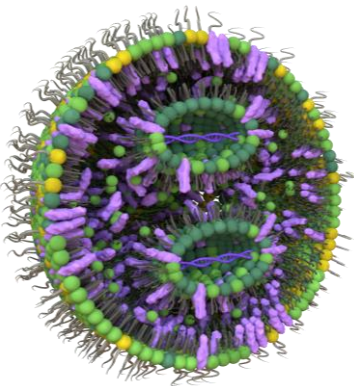
LNPs with proprietary shielding lipid perform equally as Benchmark in terms of immune response in vaccine applications



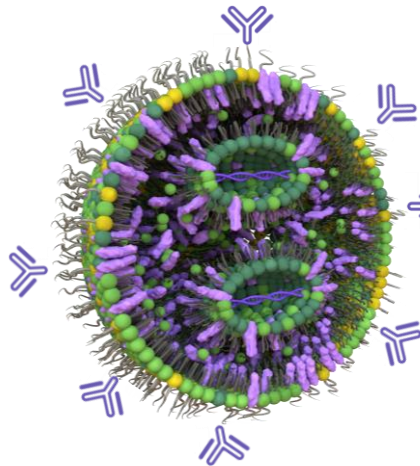
Advancing Precision Delivery: LNP Surface Modification Techniques



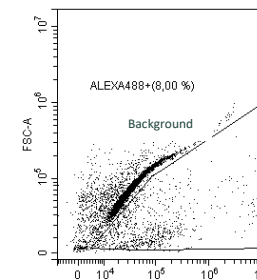
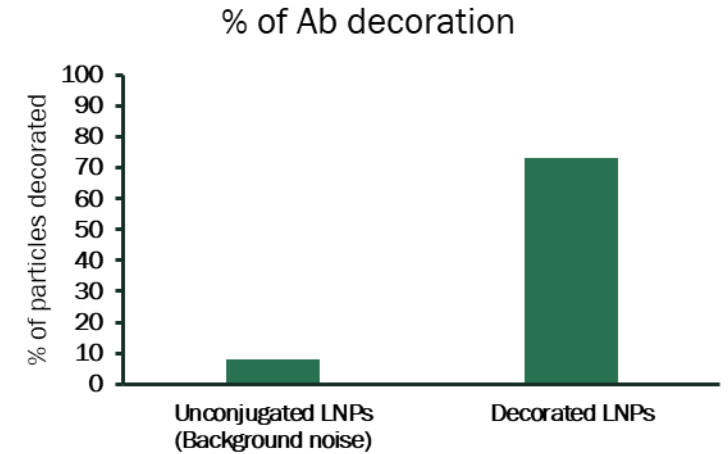
The chemical versatility and robust manufacturing procedure of Psar allows for controlled functionalizations for nanoparticles surface modification → targeting extra-hepatic tissues



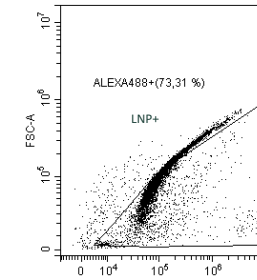
“Efficient – Simple – Clean”
“click-Chemistry”



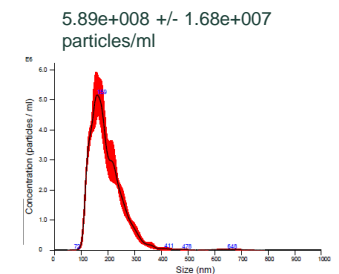
Antibody decoration assessment by Cytometry



Decorated



Particle concentration



Curapath's shielding lipids opportunities



Manufacturing feasibility

- Curapath's shielding lipids have been synthesized and scaled-up with batch-to-batch reproducibility.



GMP production

- GMP manufacturing of polymer-lipids in GMP multi-gran scale at Curapath's plant.



Strong patent protection

- Excellent patent position overcoming PEG components.
- Priorities from 2021/2022 & national phases 2024. Expected worldwide protection through 2041/2042.





LNP formulations

- Curapath's shielding lipid yield NPs with adequate size in the 100 nm range and low PDIs, with >90% nucleic acid encapsulation efficiency.




Brochures, Product Catalogue, Case Studies, Product Highlights, App Notes, Posters.....

Optimize Your Formulation Processes and Fill & Finish Requests with Robust Precision Lipid and Polymer Services

Precision | Manufacturing | Scalability



White Paper

Polysarcosine (pSar)–a safer, more effective alternative to poly-ethylene glycol (PEG)



ABSTRACT

Conjugation of polyethylene glycol (PEG) to therapeutic molecules can increase drug half-life, solubility, and therapeutic potency. However, an increasing number of healthy individuals develop anti-PEG antibodies. PEG immunogenicity can cause anaphylactic shock and dramatically reduce the efficacy of the treatment. Here we describe a potential solution to the problem. Polyaminoacid-based polymers such as polysarcosine (pSar), provide a non-immunogenic alternative to PEG. Non-toxic, biodegradable polymers are not recognized by the immune system and provide equal or better solubility and therapeutic potency compared to PEG. Polysarcosine, along with other polyaminoacid-based delivery systems, have been successfully developed & manufactured at Curapath oGMP manufacturing facility in Valencia, Spain.

Introduction

Polyethylene glycol is a hydrophilic polymer that has been frequently used in everyday products, including paints, cosmetics, food, and medicine. The PEG market reached 4.15 billion dollars in 2019 and is expected to grow at a CAGR of 10.8% from 2020 to 2026, primarily driven by strong demand from the pharmaceutical industry (<https://www.marketsandmarkets.com/industry-analysis/polyethylene-egcol-market>). In the pharmaceutical industry, polymers are widely used as a gold standard of bioconjugation to prolong biologics circulation in the blood, increase solubility and improve potency. Many PEGylated products, including peptides, proteins, small interfering RNAs, and even small molecules, were successfully tested in clinical trials over the past two decades. Sales of the two most successful products, Pegasys and Neulasta, exceeded \$5 billion in 2011 (1, 2). PEG polymer use grew steadily in the pharmaceutical, cosmeceutical, and food industries making it one of the most abundantly manufactured polymers. With the successes observed for lipid-based nanoparticle delivery systems, it was not surprising that the two leading vaccine-producing companies, Moderna and BioNTech/Pfizer, incorporated PEGylated lipids as part of the mRNA delivery of nanoparticles, in their race to stop COVID-19 pandemic. Both companies went on to manufacture and use hundreds of millions of doses to eradicate the coronavirus pandemic that claimed over 6 million human lives globally. That dramatic increase in the polymer use exposed a critical problem: PEG is immunogenic and should not be used for individuals with severe allergic reactions (3,4,5,6).

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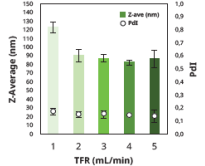
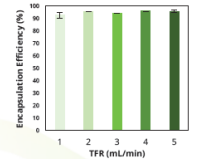
LNP Formulation and Analytical Services

Lipid nanoparticles (LNPs) are biocompatible, biodegradable, non-viral vectors that show high encapsulation efficiency. LNPs are used to deliver nucleic acids in clinical applications. Most notably, LNPs are being used as mRNA-delivery vehicles in the SARS-CoV-2 vaccines developed by BioNTech/Pfizer and Moderna.

The manufacture of mRNA-LNPs is a complex process in which the micromixer plays the critical role of mixing the organic phase containing lipids and the aqueous phase containing the nucleic acids. Tightly controlling the micromixer's operating parameters allows the system to produce mRNA-LNPs of reproducibly defined physical and functional properties.

Using the IJM NanoScaler, we have optimized key operating parameters, such as total flow rate (TFR).


As part of our end-to-end services, we at Curapath develop and optimize LNP formulations during pre-clinical R&D.


Encapsulation efficiency is greater than 90% across all conditions tested.

With the NanoScaler, we are able to formulate batches 50 times larger than R&D batches, obtaining mRNA-LNPs of similarly adequate characteristics. Pre-clinical studies can therefore be performed with the use of the NanoScaler, as several hundred mL of LNPs can be produced in a few hours.

The NanoScaler, KNAUER's new benchtop Impingement Jets Mixing (IJM) micromixer, is designed for low sample consumption to minimize waste, and can be used to produce from one to hundreds of milliliters of lipid-encapsulated nucleotides.



Case Study



Development of a Highly Efficient, Biodegradable, Polymeric, Non-viral Vector (NVV) Platform for Nucleic Acid Delivery

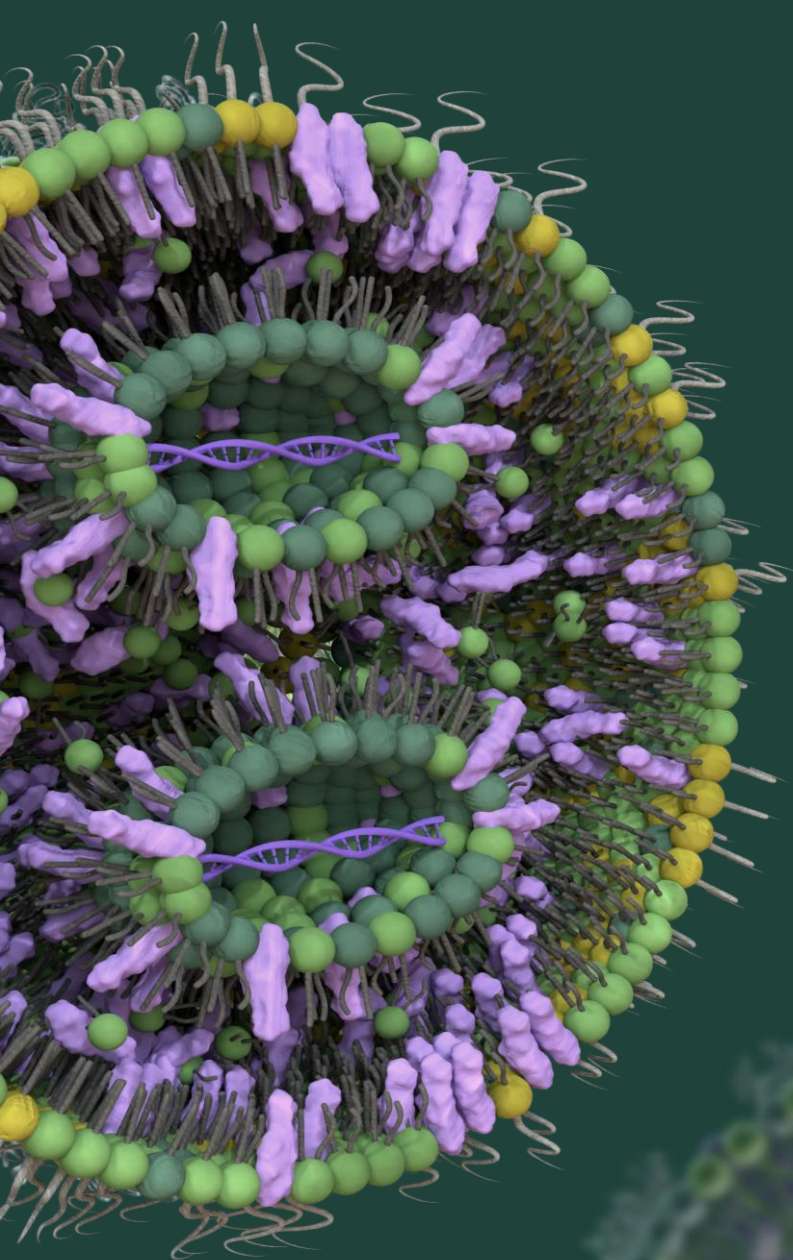
Emerging Biotechnology

Drug Candidate and Development Status

An emerging biotechnology company sought help in advancing novel nucleic-acid constructs to the market via an orphan drug designation pathway. This client approached Curapath to design and develop a suitable vehicle based on polymeric nanoparticles to bring its technology from R&D to the clinic as rapidly as possible. Moreover, the customer intention was to pave the way towards developing a novel polymeric platform for treating other genetic diseases in the future.

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... and more to come



GMP production site



📍 Av. Benjamín Franklin, 19
46980 Paterna, Valencia

Innovation & Development site



📍 Carrer d'Alexander Graham Bell, 6
46980 Paterna, Valencia

Commercial Offices



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