Polymun Scientific Immunbiologische Forschung GmbH

LNP Process Development and Analytics for mRNA Vaccines and Targeted LNPs

Andreas Wagner

Levitronix Bioprocessing Conference
June 18th 2026

Polymun Scientific Immunbiologische Forschung GmbH

- **A PRIVATE COMPANY**
  Developing and Manufacturing Biopharmaceuticals and Liposomal Formulations for Human Application

  - CEO: Dr. Dietmar Katinger
  - Founded: 1992
  - Employees: 98

- regularly inspected by the Austrian regulatory authority AGES on behalf of EMA, last inspection in December 2025
- inspected by FDA in October 2013 / July 2023
- numerous audits by clients (~10 per year)

Core Activities

- ***Contract Development & Manufacturing of Biopharmaceuticals***
  for human application with focus on mammalian cell culture, process development & GMP production
- ***Contract Development & Manufacturing of LNPs and Liposomal Formulations***
  LNP & liposomal formulation development for APIs and vaccine antigens & GMP production
  - ***Formulation of mRNA and oligonucleotides in liposomes/LNPs***
    siRNA, saRNA, miRNA and mRNA formulated up to 300 g API input per batch
  - ***Liposomal adjuvants, liposomal vaccines***
    liposomal formulation of MPLA as well as other TLR4 agonists in combination with other adjuvants like saponins, CpG,..
  - ***Covid-19 mRNA vaccine collaborations with:***
    - BioNTech/Pfizer
    - CureVac
    - Imperial College London
    - Arcturus Therapeutics
- ***Research Reagents***
  manufacturing and distribution of HIV antibodies and antigens
- ***Own R&D Projects***
  funded by revenues from contract development and contract manufacturing

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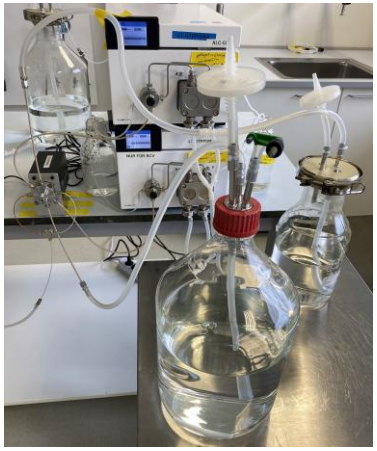
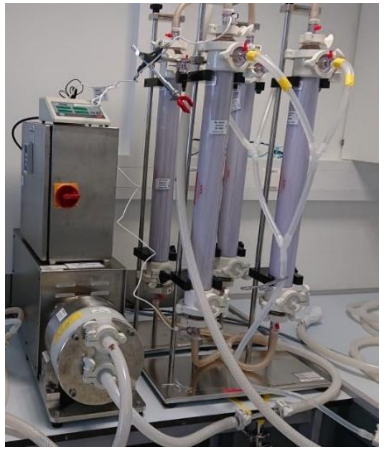
At a Polymun laboratory in Klosterneuburg, Austria, the size distribution of lipid nanoparticles is measured. MARYLISE VIGNEAU FOR THE WALL STREET JOURNAL

mRNA Vaccines – Achievements within < 1 year

- Process set-up, optimization and scale-up
- Production of 10 different vaccines for tox studies
- Production of 5 different vaccines to initiate clinical trials
- Production of 2 different vaccines for phase 3 (> 40 000 subjects)
- Tech transfer to BioNTech/Pfizer network
- Analytical method validations
- (multi-center) process validation
- Regulatory support
- Production of 15 million doses to be used in EUA program in late 2020 / early 2021
- Continued bulk DP production in 2021





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Production History

- 200+ GMP productions runs for oligo formulations
- 100+ GMP productions for
  - mRNA vaccines (incl. market product)
  - Gene editing formulations (mRNA/gRNA)
  - mRNA therapeutics (IV and pulmonary application)
- Intermediate batch volume > 500 L
- mRNA input amounts > 50 g / batch
- High reproducibility, High yields

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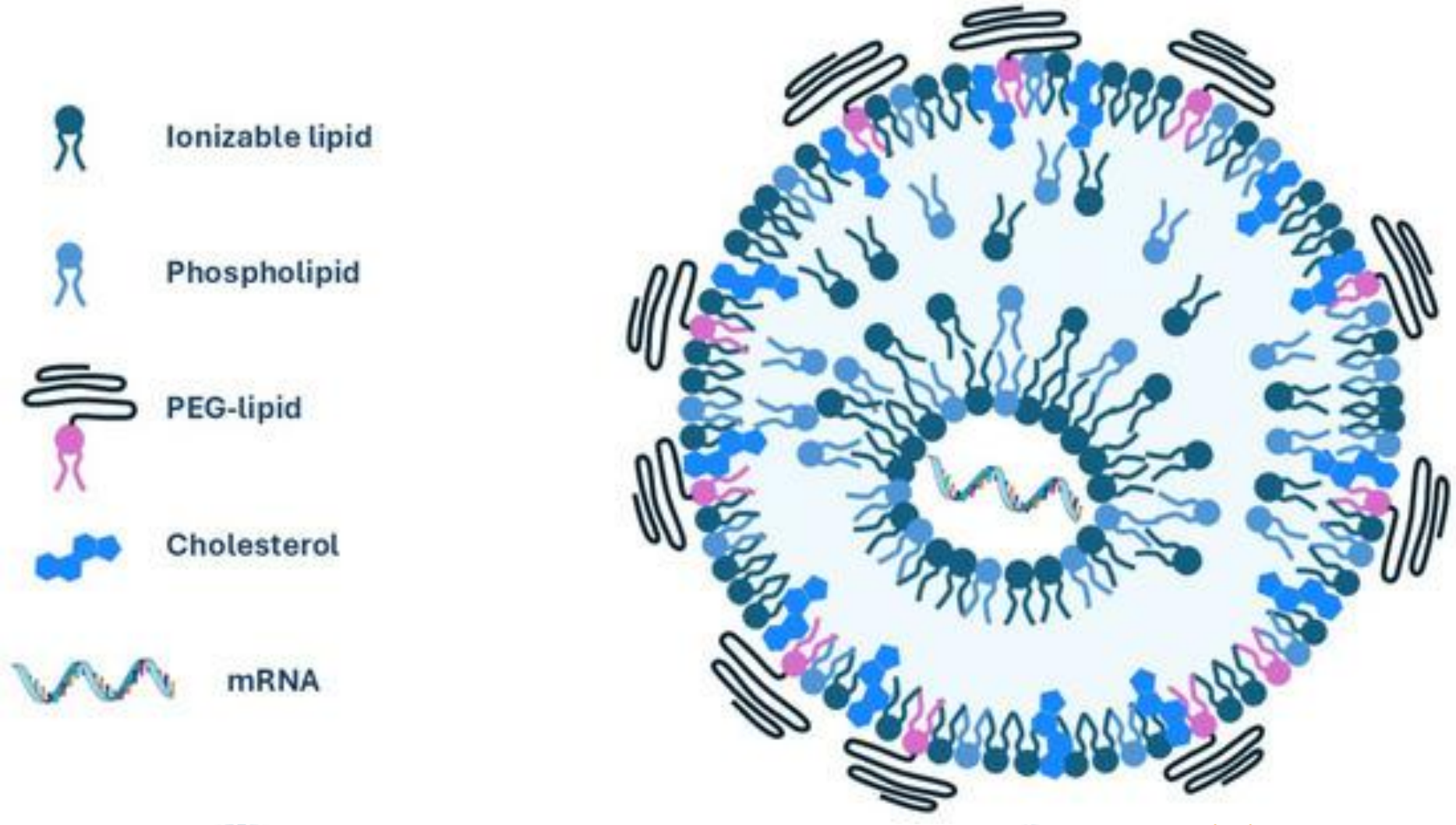
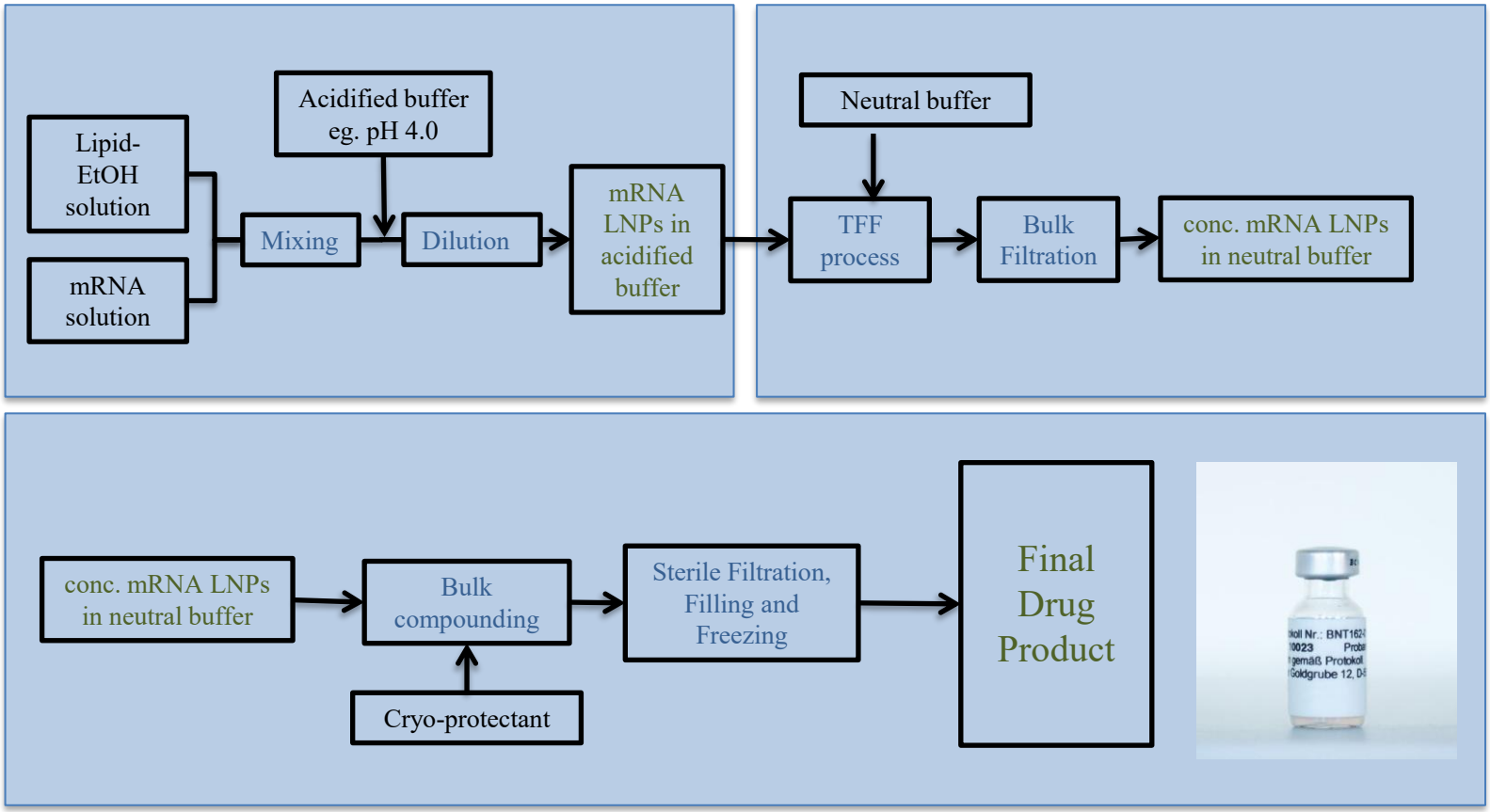
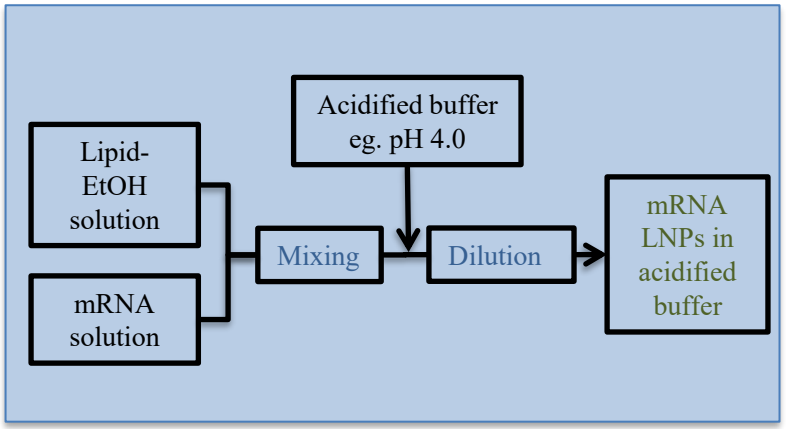
Components of mRNA LNPs

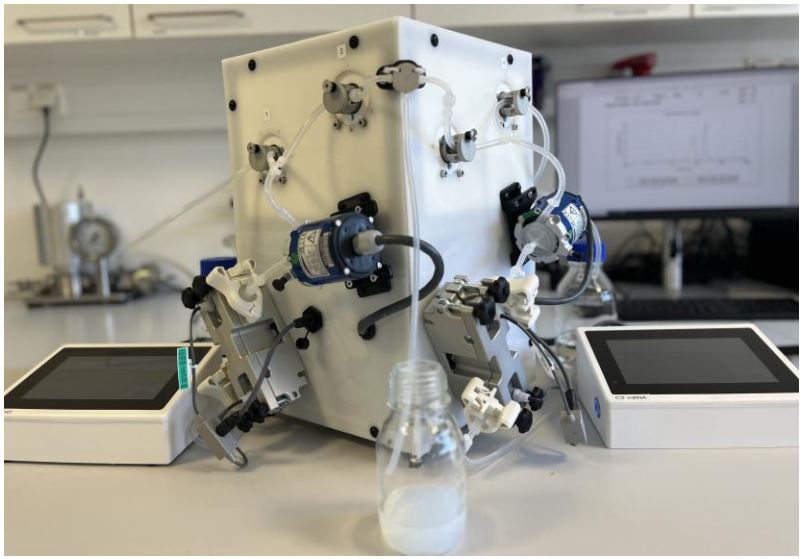


Production of mRNA LNP Vaccines

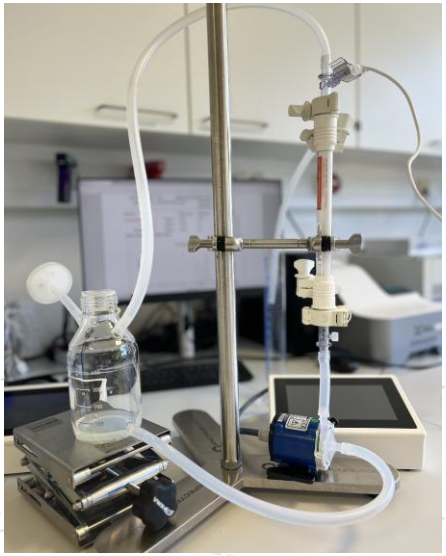


Production of mRNA LNP Vaccines

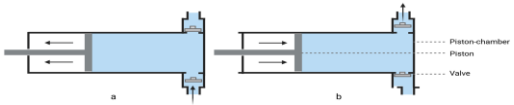


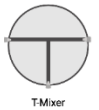


- Process optimization
- Equipment optimization
- Scale-up; Scale-down



Mixing

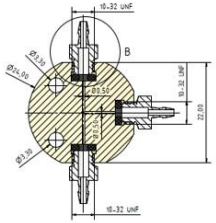

Piston-chamber
Piston
Valve
a
b

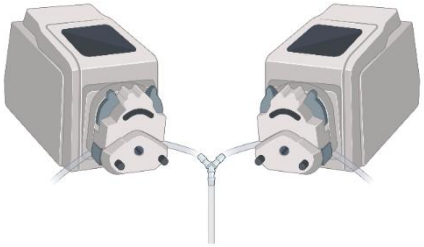

T-Mixer

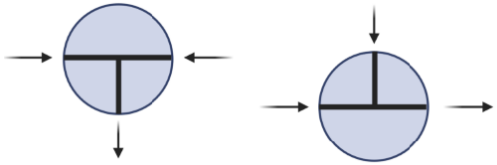

T-Piece


Y-Piece


BASF


10-32 UNF
B
10-32 UNF





Development and optimization of a mixing process for the preparation of lipid nanoparticle drug delivery systems for nucleic acids using magnetic levitation pumps

Master´s thesis for obtaining the academic degree

Master of Science

in the study programme: **Clinical Research**

submitted by

Daniel Kumar, B.Sc.

Department for Health Sciences, Medicine and Research

at University for Continuing Education Krems

Advisor: Dr. Andreas Wagner

Vienna, July 2025

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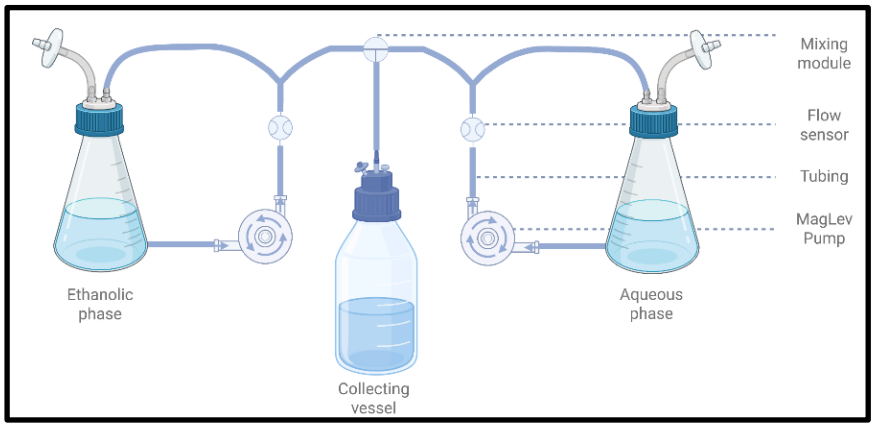
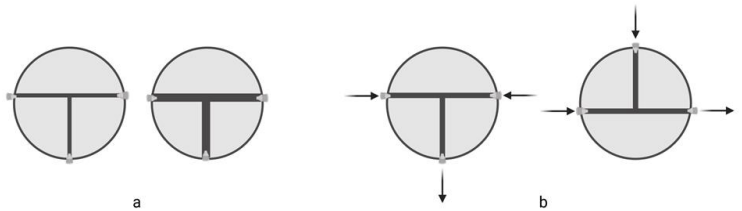
LNP processing – ongoing activities / optimization

- **Comparison:** piston pump batch

  with MagLev pump batches



- **Variations tested**:
  - Module size (0.5 mm vs. 1.0 mm)
  - Flow rates (low, standard, high)
  - Mixing angle (180° vs. 90°)



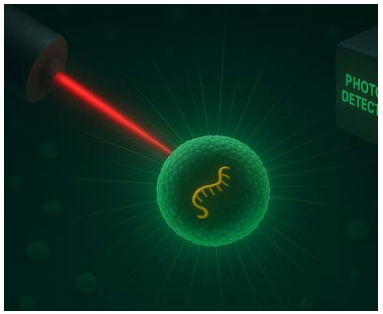
Analytics

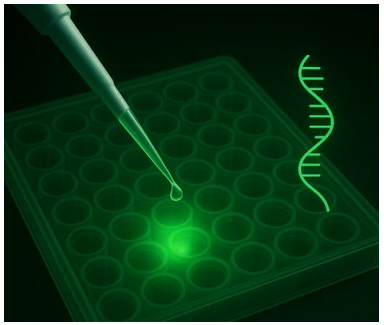
- **CQAs**
  - Particle size & Size distribution (PdI):

    Dynamic Light Scattering (DLS)

  - Encapsulation efficiency:

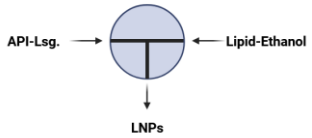
    RiboGreen assay





Baseline Comparison Piston vs. MagLev

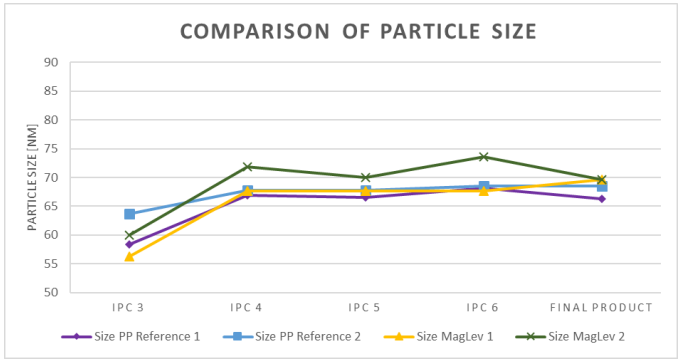
- Flowrates of: 40/120 ml/min
- 180° mixing / 0.5 mm standard module

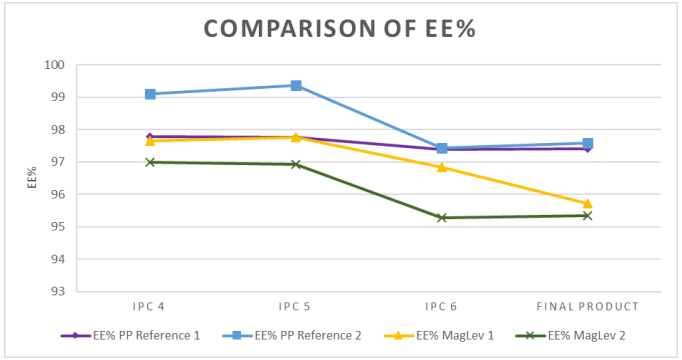


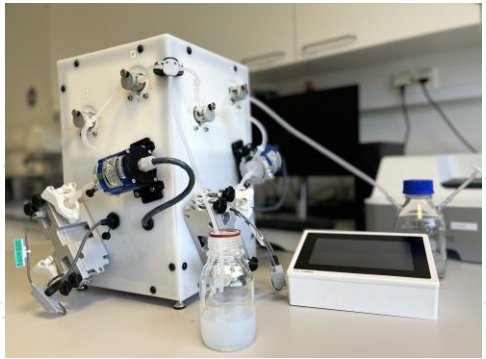
Piston pump (reference):

- Particle size: 66–71 nm
- PdI: 0.11–0.15
- Encapsulation efficiency: 97-98%

- **MagLev pump:**
- Particle size: 69-70 nm
- PdI: 0.16–0.17
- EE: 95–96% (≈2% lower)

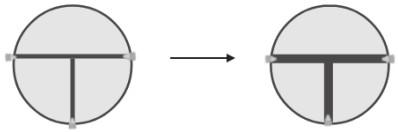




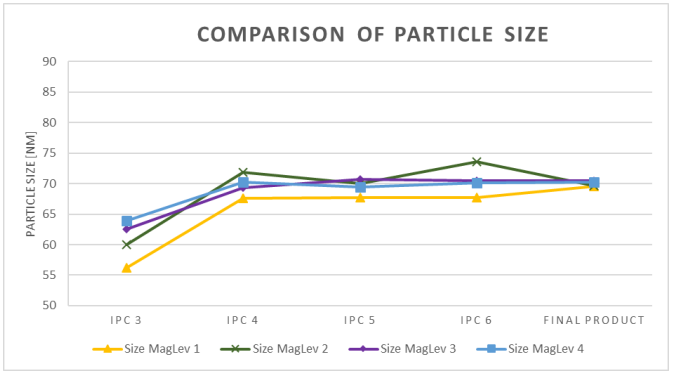


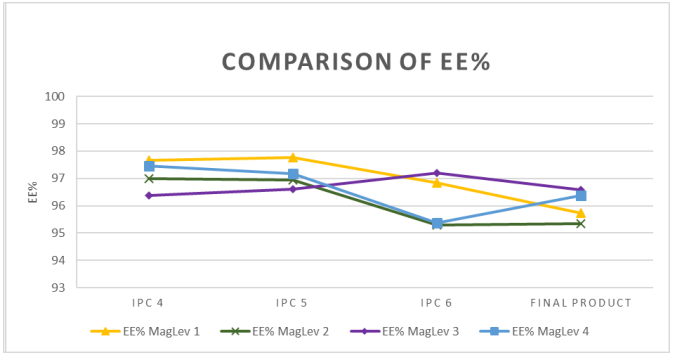
Larger module

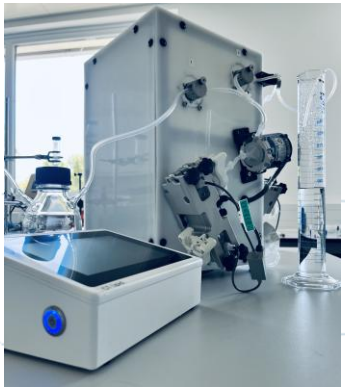
- Flowrates of: 40/120 ml/min
- 180° mixing / 1 mm module



- Larger module (1.0 mm): stable, less backpressure
- Particle size : 70-71 nm
- EE: 96-97%
- PdI: 0,14-0,15

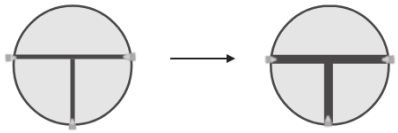




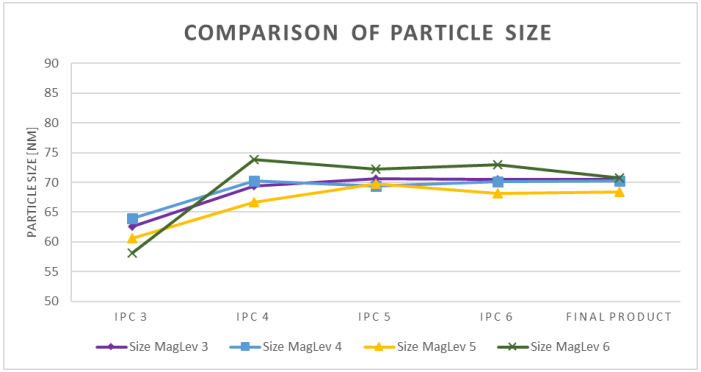


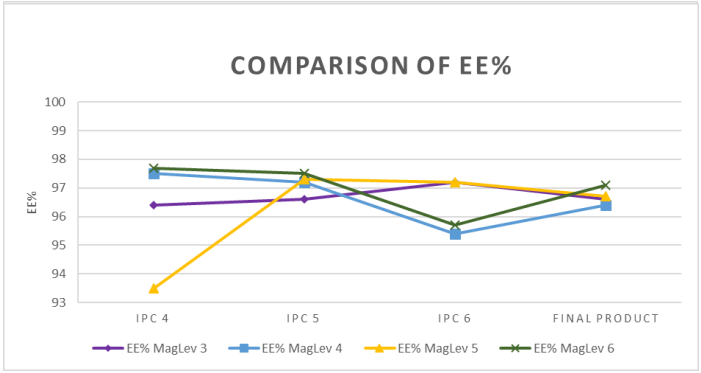
Larger module – increased flow rates

- Flowrates of: 80/240 ml/min
- 180° mixing / 1 mm module



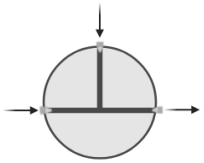
- Doubling flow rates possible only with 1.0 mm module - enables successful scale-up
- Particle size: 68-70 nm
- PdI: 0,15-0,16
- EE: 96-97%



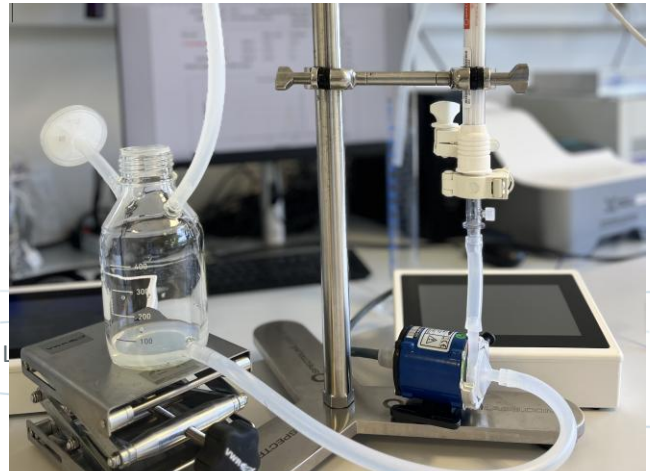


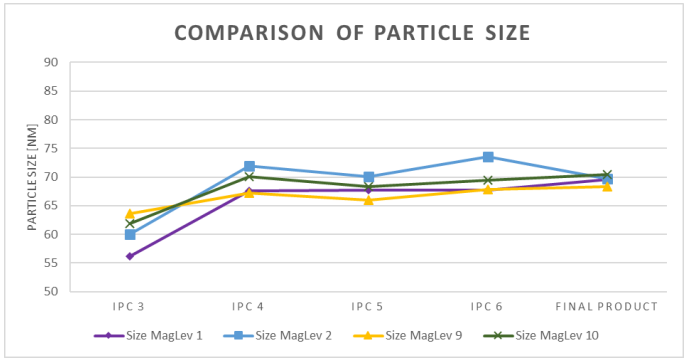
90° mixing

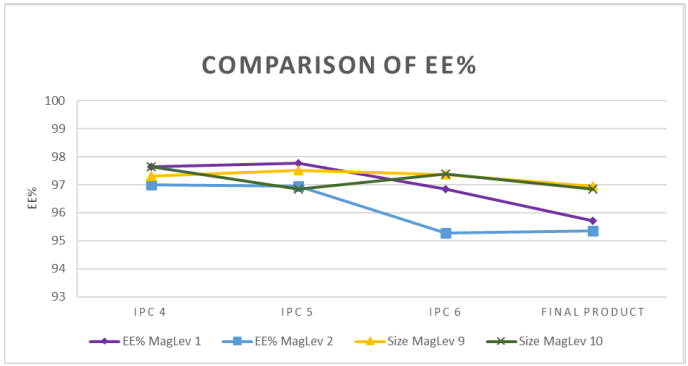
- Flowrates of: 40/120 ml/min
- 90° mixing / 0.5 mm standard module



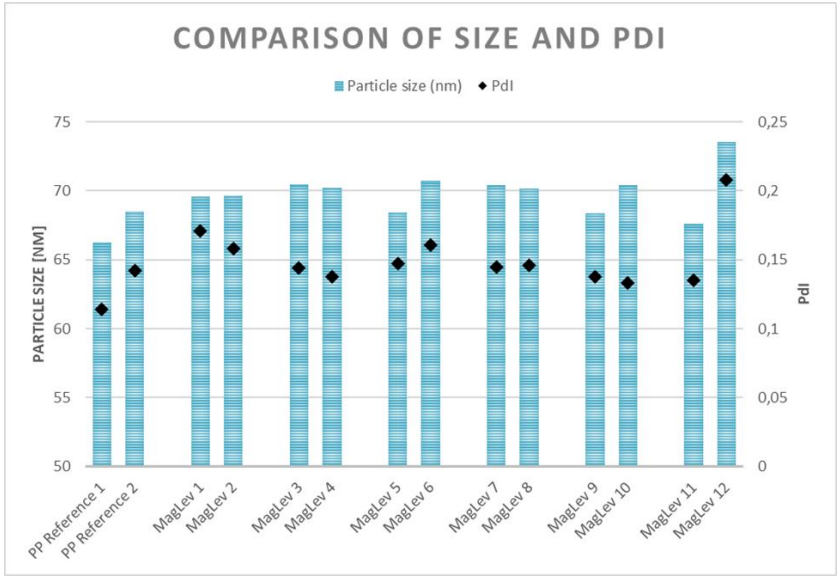
- Particle size: 68–70 nm
- PdI slightly improved 0.13–0.14
- EE stable at 96–97%







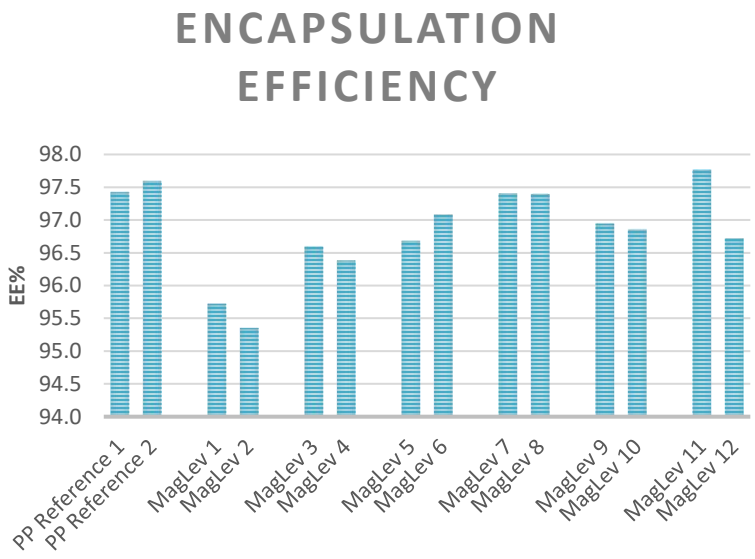
Summary



Key Observations:

- All batches yielded particle sizes around 70 nm → high reproducibility
- MagLev batches: comparable sizes (~68–72 nm), PdI <0.2
- Optimized MagLev settings (low flow / larger module / 90° angle): PdI similar to piston pumps
- Only weak point: small module + high flow = back pressure → process not feasible
- Takeaway: MagLev delivers robust and reproducible sizes and PdI values, with only minor differences compared to piston references.

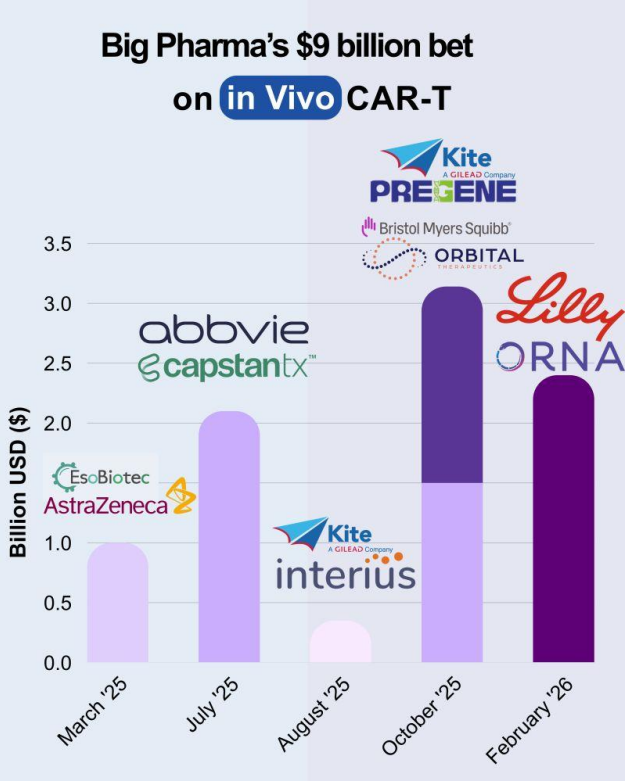
Summary

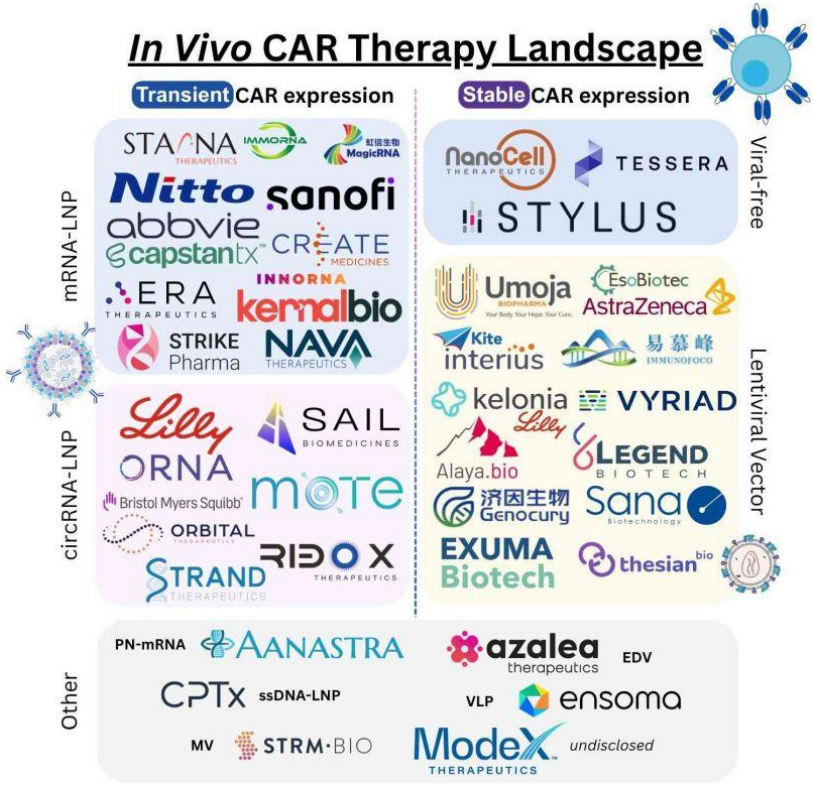


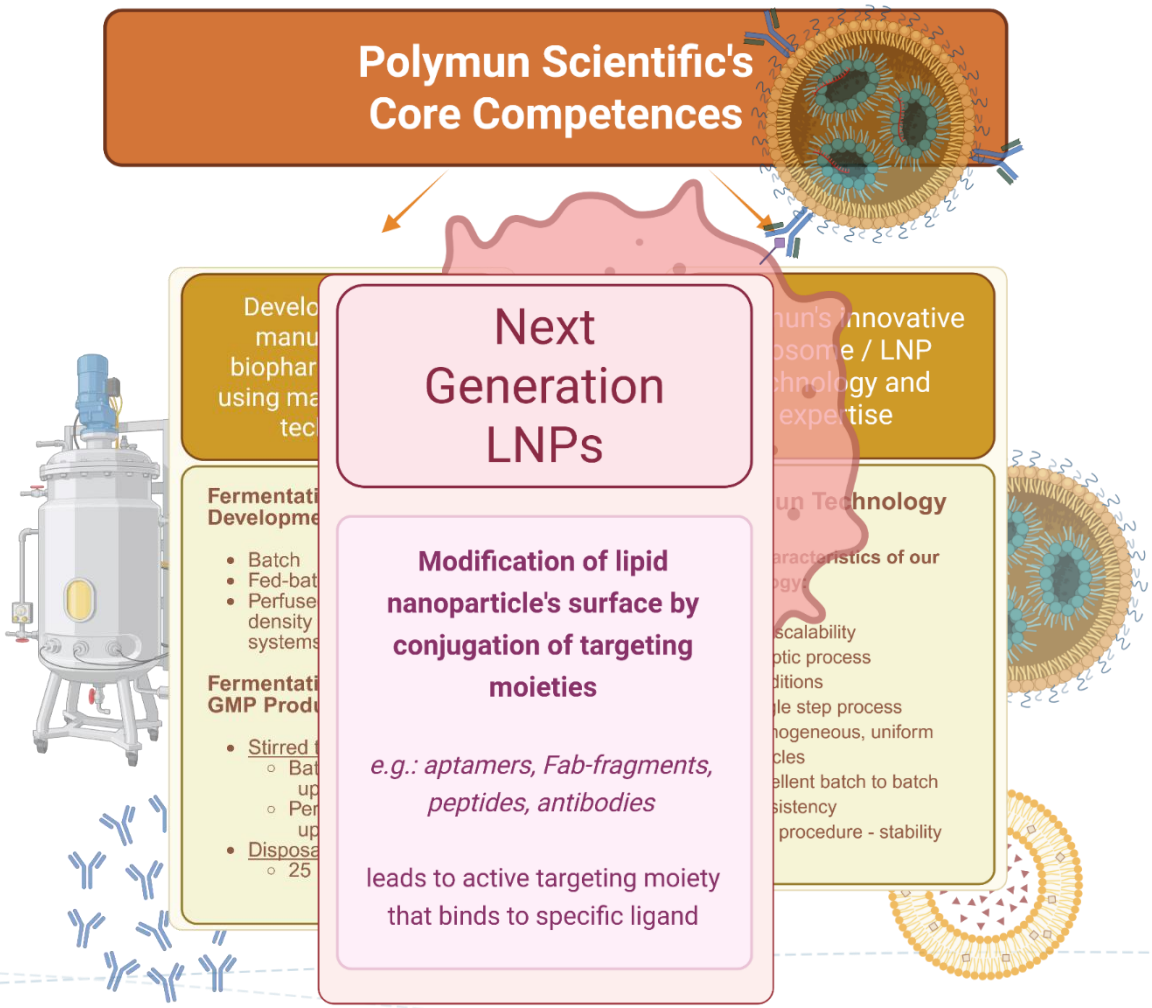
Key Observations:

- Piston references: consistently > 97% EE across all runs
- MagLev batches: Direct comparison (MagLev 1–2): slightly lower, ~95–96% final product
- Optimized conditions (larger module, reduced flow, 90° mixing): improved back to 96–98%
- Takeaway: MagLev pumps achieve high EE comparable to piston pumps when operated under optimized conditions.

Emerging Trends in the LNP space – in-vivo CAR-Therapy

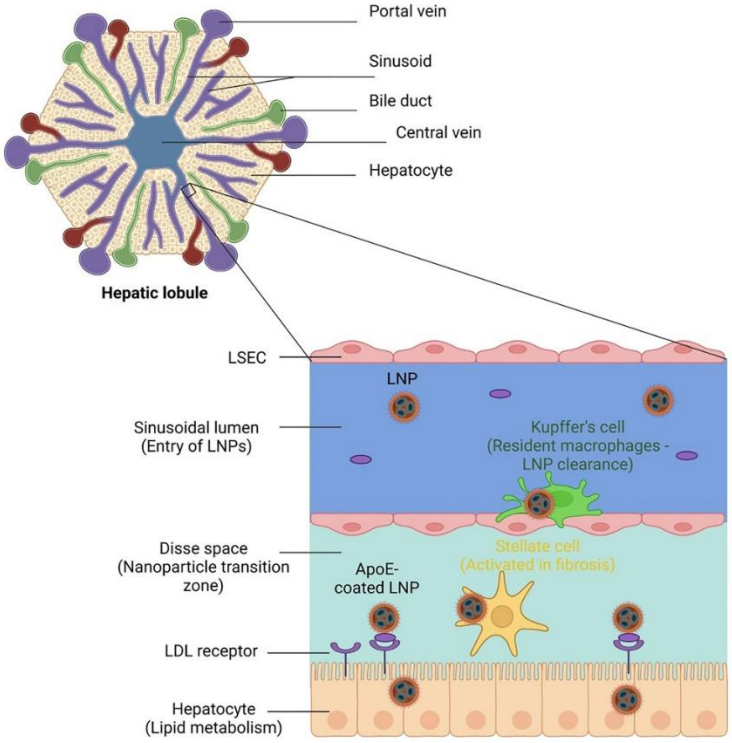





Polymun Scientific's Core Competences
Next Generation LNPs
Modification of lipid nanoparticle's surface by conjugation of targeting moieties
e.g.: aptamers, Fab-fragments, peptides, antibodies
leads to active targeting moiety that binds to specific ligand

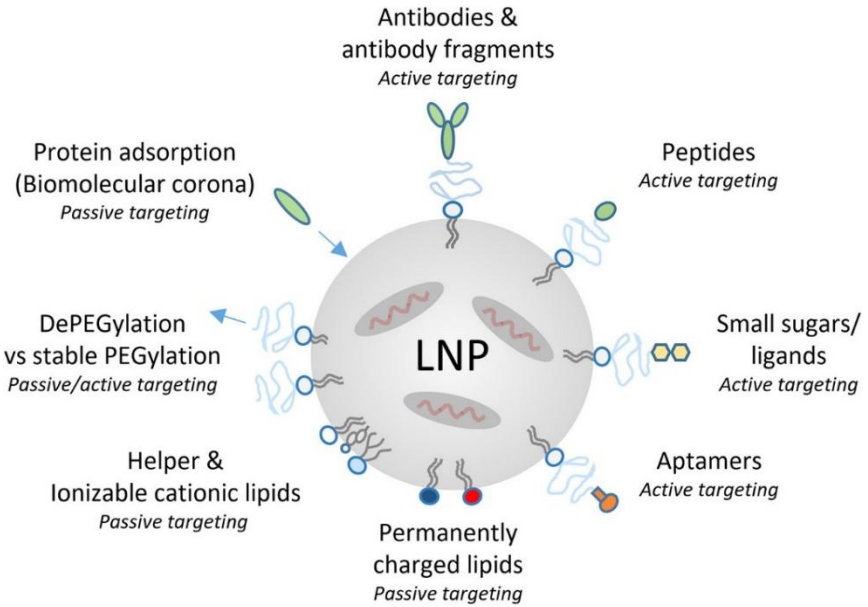
Why is targeting important?

Passive targeting (or non-targeting)
LNP uptake mechanism



https://doi.org/10.1016/j.omtm.2025.101436

Active targeting
LNP uptake mechanism
"extrahepatic delivery"



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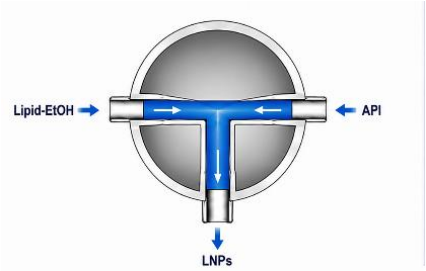
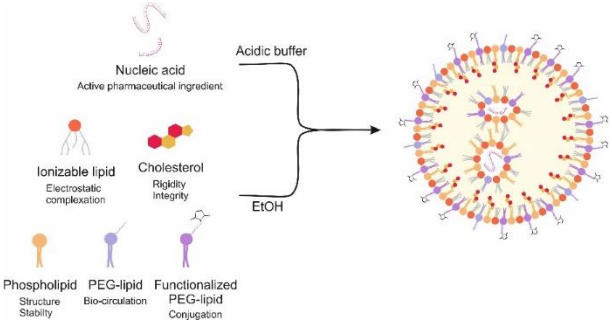
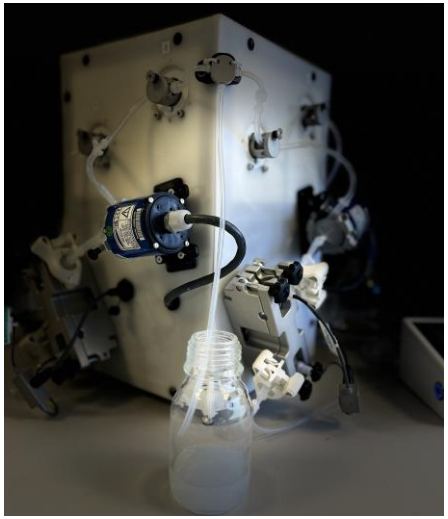
Lipid nanoparticles – Targeted delivery

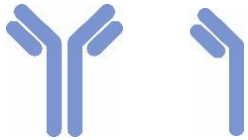
- LNP preparation



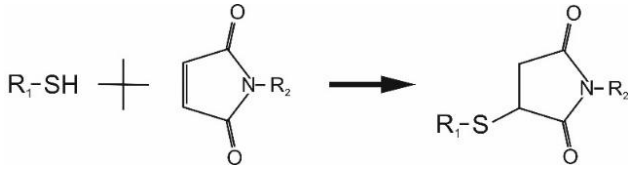


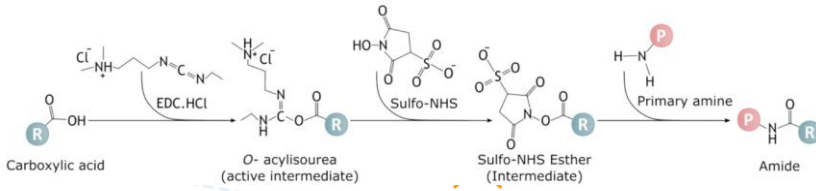


- Targeting moieties:
  - *Antibodies, antibody fragment, peptides, etc.*



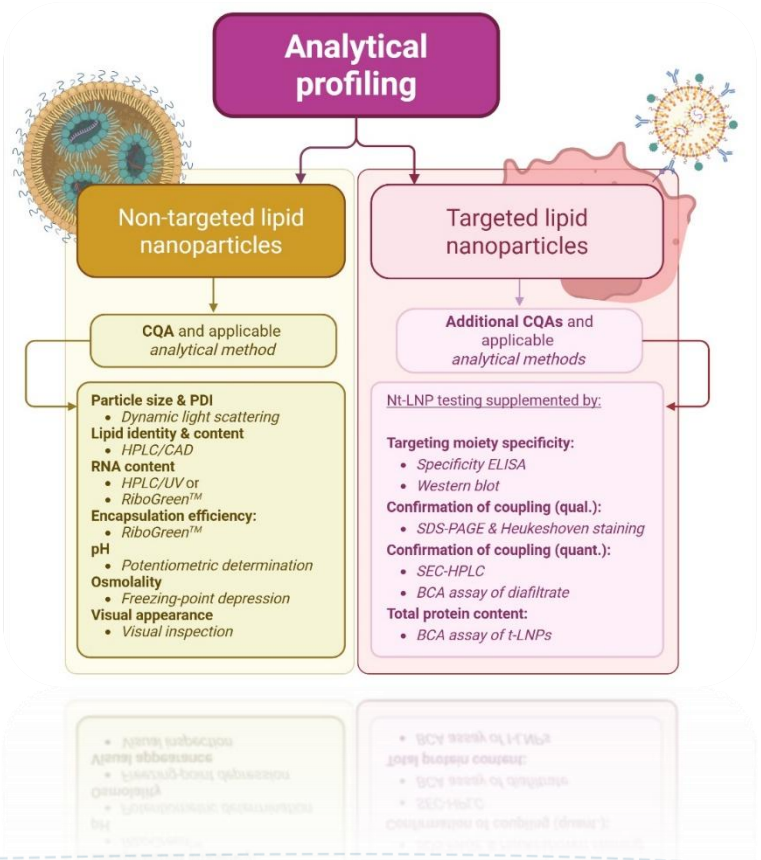
- Conjugation techniques
  - *Maleimide-thiol binding*
  - *EDC/NHS coupling*
  - *Biotin-avidin*





The merge of Polymun‘s analytical expertise

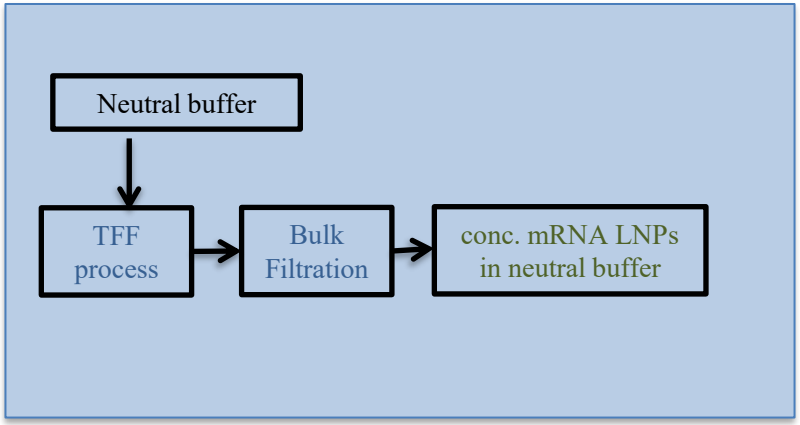
- **How can we prove successful conjugation?**
  - Characterization using standard analytical methods for LNP release testing
  - Analytical profiling of tLNPs needed to prove successful conjugation and efficiency (and stability!)
  - Use of orthogonal methods

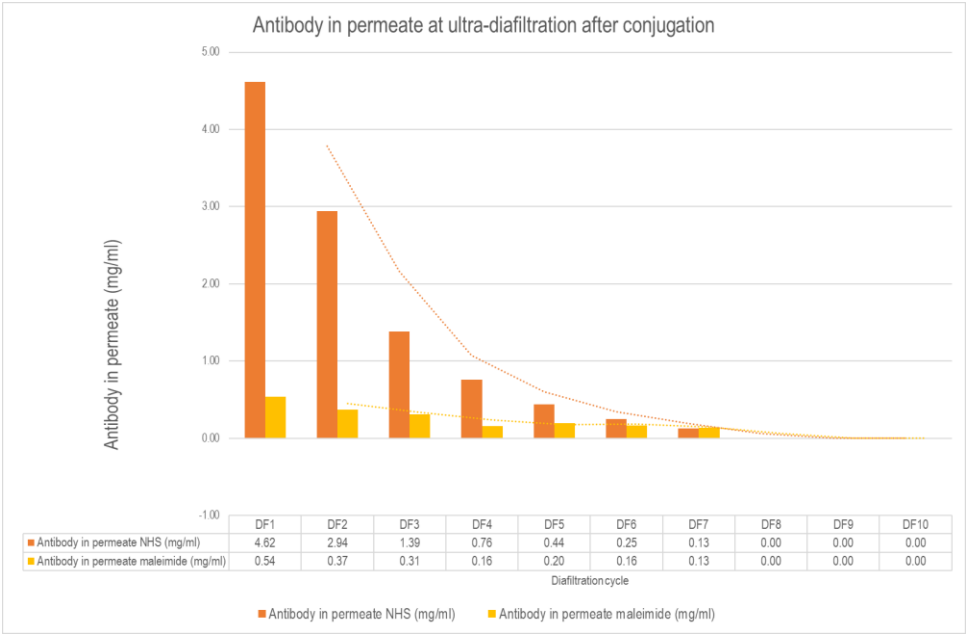


Standard Quality Control

- *mRNA content and EE% by Ribogreen Assay*
- *LNP size / size distribution (DLS)*
- *mRNA identity and integrity (Capillary electrophoreses)*
- *lipid identity and quantity (HPLC-CAD)*
- *mRNA content and ratio by IPRP HPLC*
- *pH*
- *Osmolality*
- *Bioburden testing*
- *Sterility testing*
- *Endotoxin testing*
- *Subvisible particles*
- *residual ethanol (GC)*

Last step after conjugation: Tangential Flow Filtration of tLNPs





- **Why?**
  - To remove unbound targeting moieties
  - To remove organic residues from LNP preparation
  - Exchange to desired buffer including cryoprotectants for frozen storage conditions

Comparison of permeates during ultra-/diafiltration of different conjugation strategies

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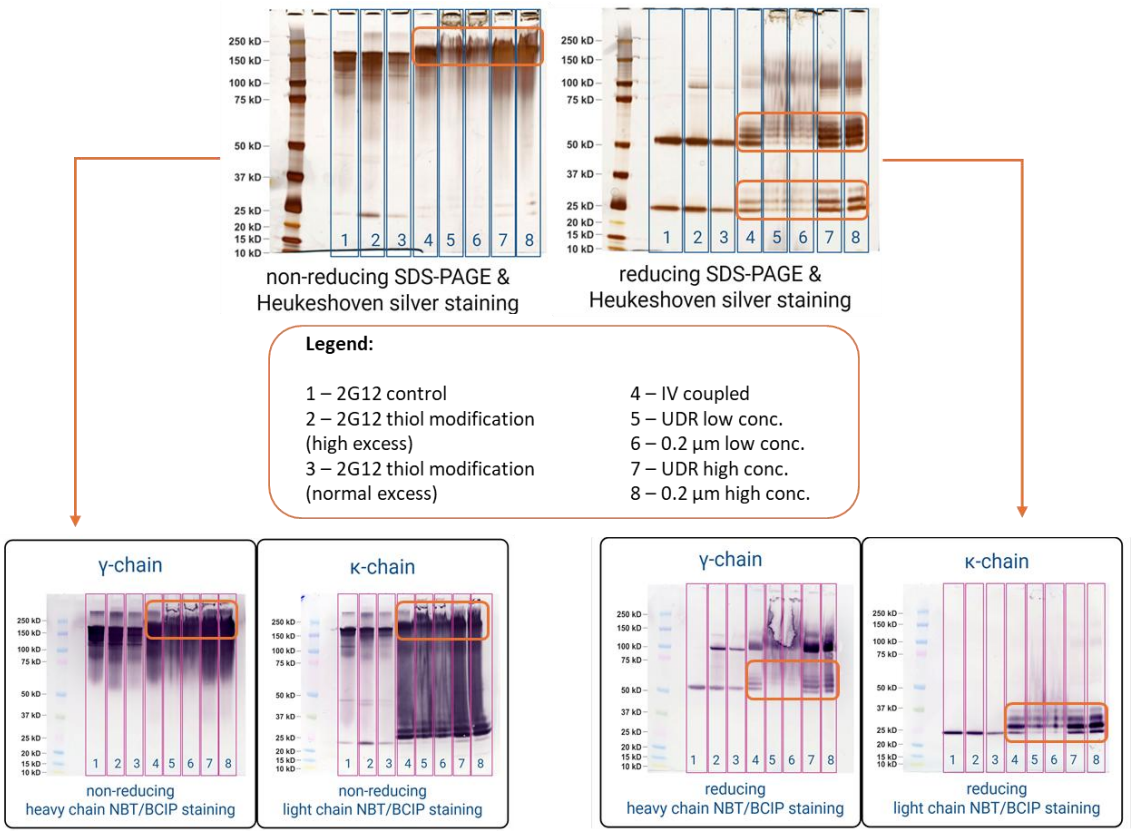
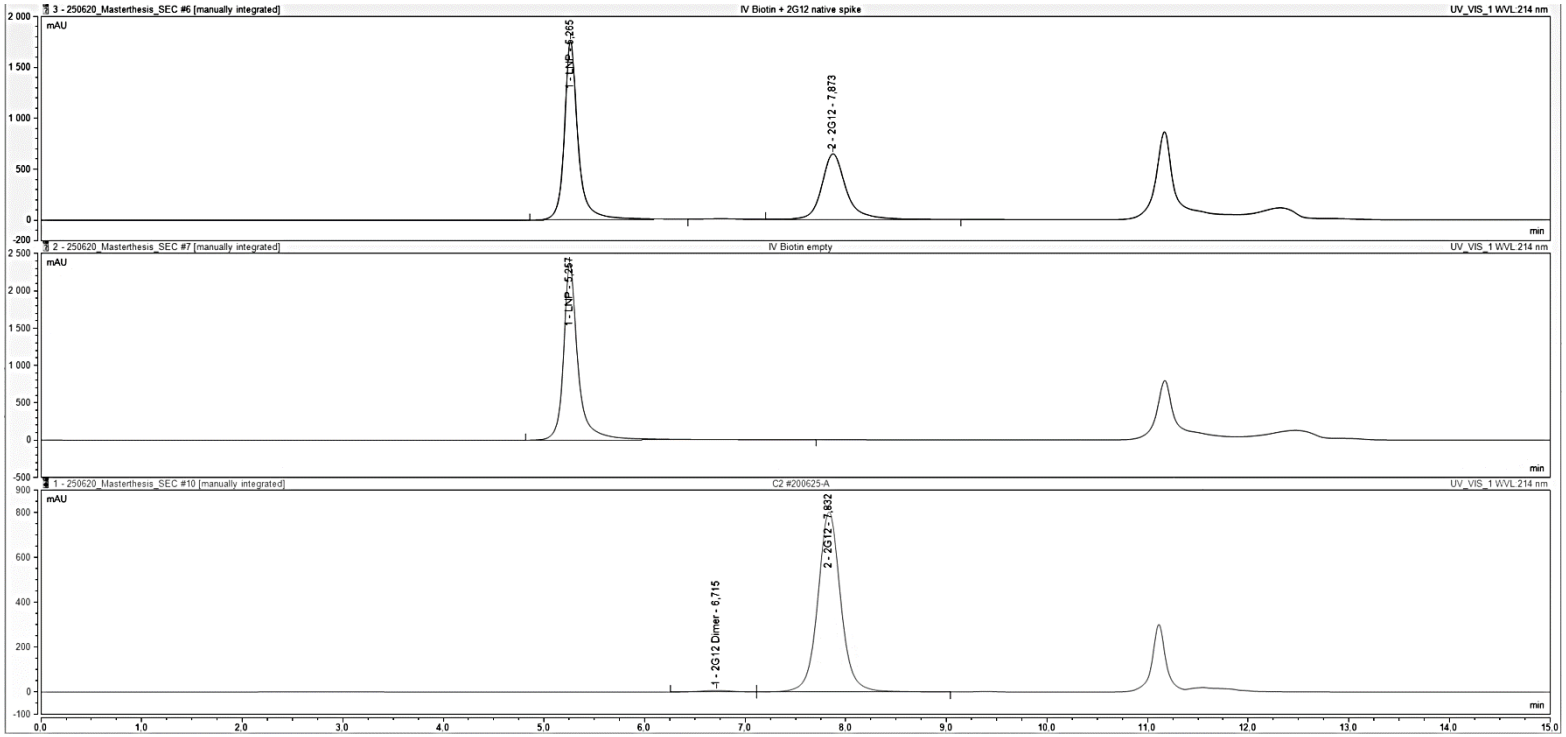
Lipid nanoparticles with targeting antibody moiety (2G12)



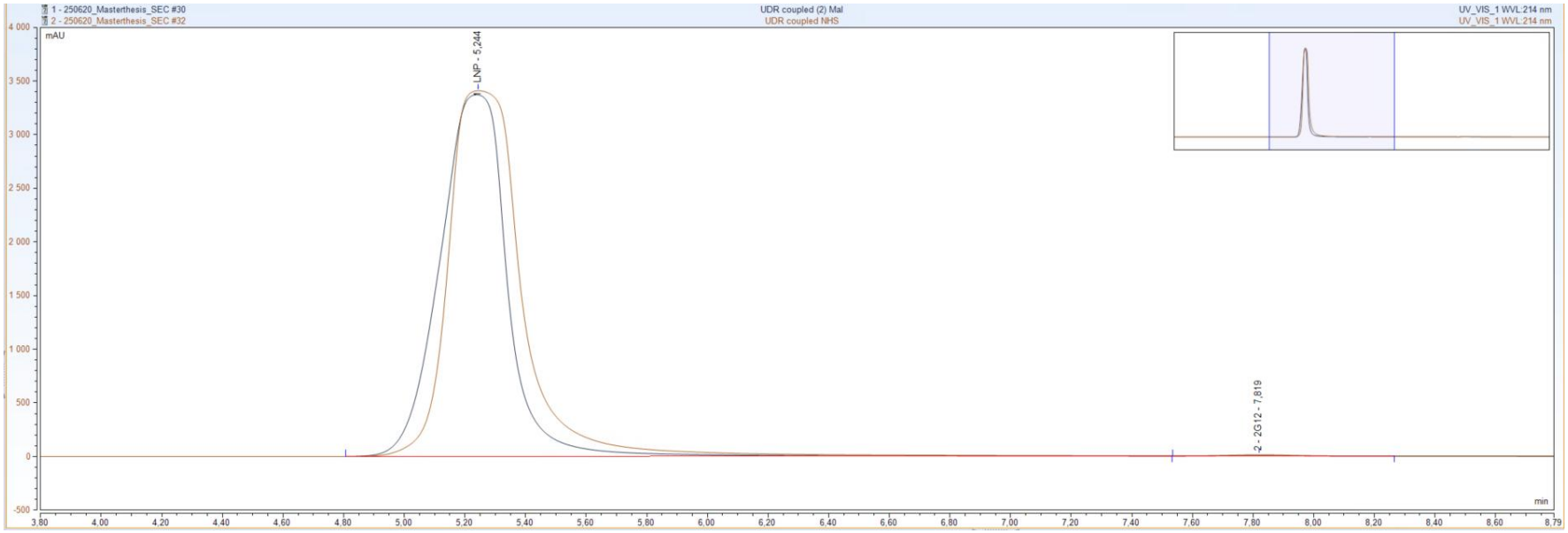
- ✓ **Coupling of LNP and targeting moiety successful**
- ➢ Particle sizes around 65 nm, PDI ≤ 0.07
- ➢ %EE ≥ 95 %
- ➢ **Clear upwards shift** (antibody coupled to PEG-MAL) in SDS-PAGE and Western blot

SEC-HPLC with UV detection



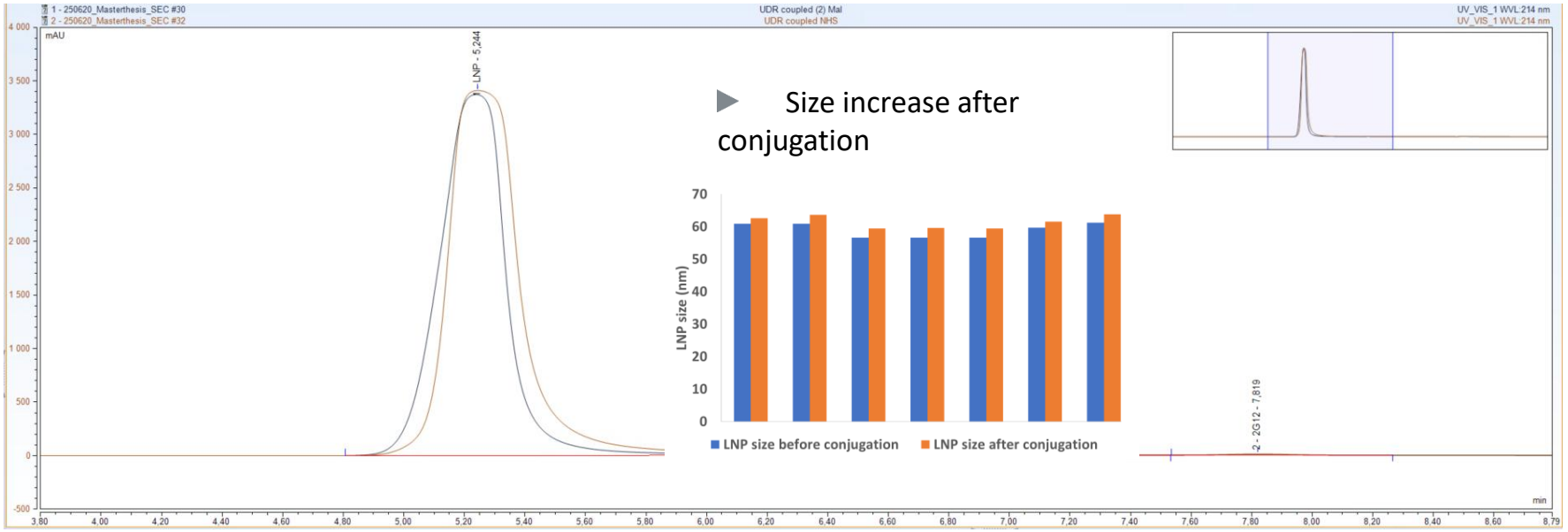
- **From top to bottom:**
  1) not conjugated intermediate volume with antibody spike to show resolution between LNP and antibody (not-conjugated control)
  2) not conjugated intermediate volume (LNP control)
  3) not conjugated antibody (2G12) (Antibody control)

SEC-HPLC with UV detection



- Blue: conjugated
- Orange: not conjugated

SEC-HPLC with UV detection



Thank you

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