Polymun Scientific Immunbiologische Forschung GmbH

Pioneering a versatile LNP production process for mRNA vaccines, therapeutics and gene editing – Unveiling the proof of concept

Andreas Wagner / Polymun Levitronix Bioprocessing Conference, May 2024

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/BASG on behalf of EMA, last inspection in April 2024
- Inspections by other authorities: US FDA, October 2013, July 2023; Russia, June 2018 & January 2021; Korea, November 2018; Brazil, November 2022
- 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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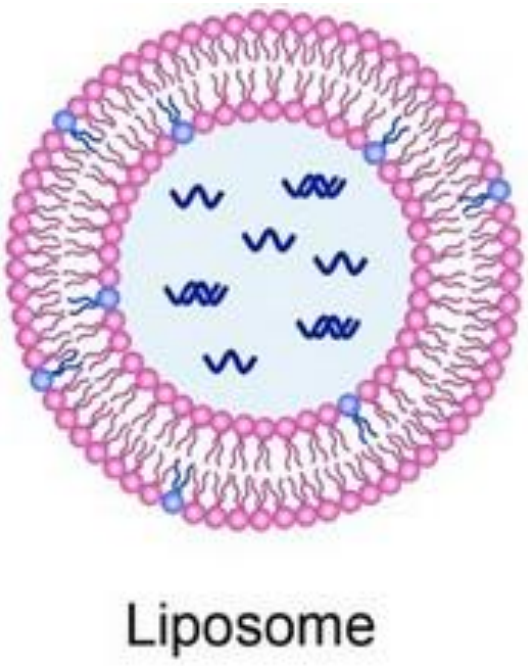
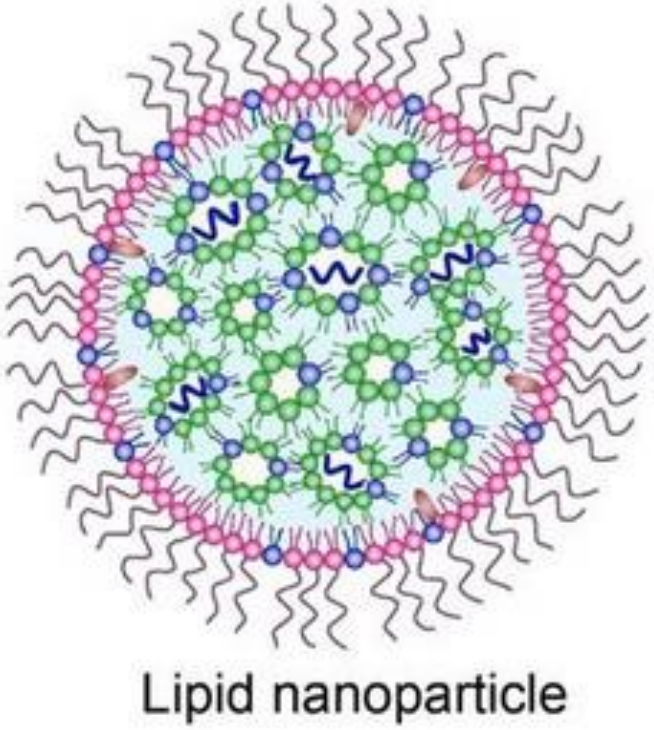
At a Polymun laboratory in Klosterneuburg, Austria, the size distribution of lipid nanoparticles is measured. MARYLISE VIGNEAU FOR THE WALL STREET JOURNAL

Comparison of Liposomes and Lipid-Nanoparticles





From:
Delivering the right message: Challenges and opportunities in lipid nanoparticles-mediated modified mRNA therapeutics—An innate immune system standpoint
Granot & Peer, Seminars in Immunology 2017, 34

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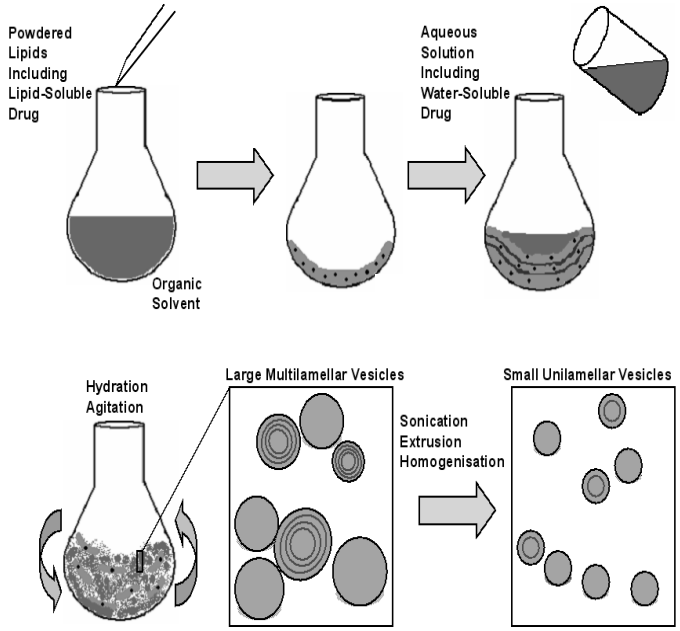
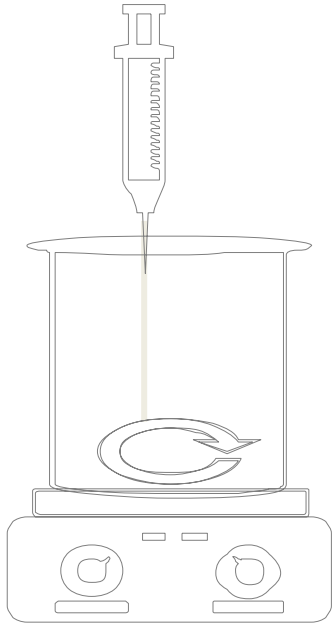
Liposome formulation processes

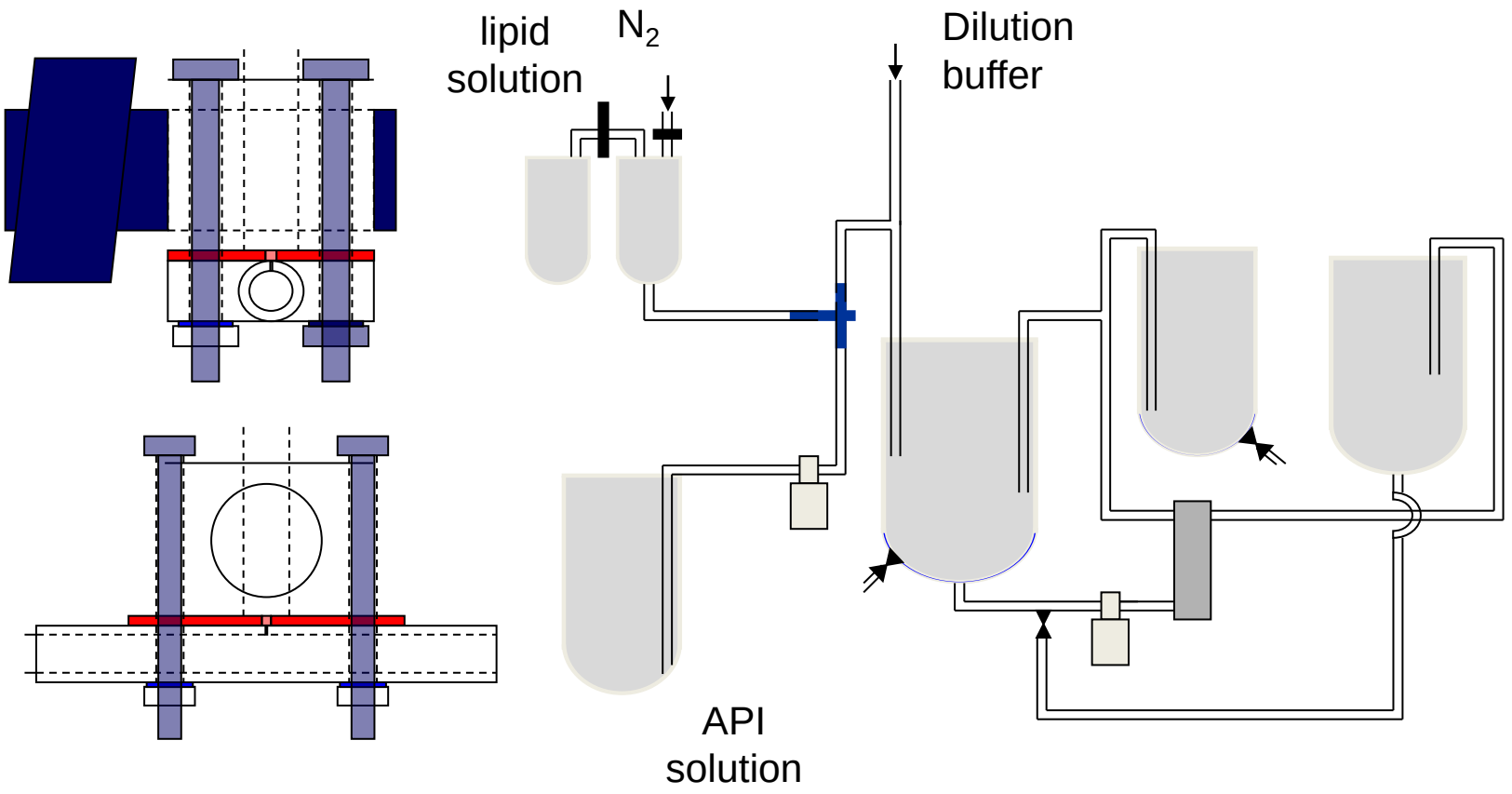
- Film method – most frequently used lab scale liposome formulation technique



- Lab scale ethanol injection method according to Batzri et al.



The Liposome Technology



Wagner et al., 2006, GMP Production of Liposomes - A New Industrial Approach. J Liposome Res 16(3):311-9

How it started

JOURNAL OF LIPOSOME RESEARCH
Vol. 12, No. 3, pp. 259–270, 2002

THE CROSSFLOW INJECTION TECHNIQUE: AN IMPROVEMENT OF THE ETHANOL INJECTION METHOD

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ENHANCED PROTEIN LOADING INTO LIPOSOMES BY THE MULTIPLE CROSSFLOW INJECTION TECHNIQUE

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ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 54 (2002) 213–219

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Liposomes produced in a pilot scale: production, purification and efficiency aspects

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Received 21 January 2002; accepted in revised form 26 April 2002

GMP Production of Liposomes—A New Industrial Approach

ANDREAS WAGNER,[1] MIRKO PLATZGUMMER,[1] GÜNTHER KREISMAYR,[1] HERIBERT QUENDLER,[2] GABRIELA STIEGLER,[1] BORIS FERKO,[2] GABRIELA VECERA,[1] KAROLA VORAUER-UHL,[2] AND HERMANN KATINGER PROF[1,2]

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Review Article

Liposome Technology for Industrial Purposes

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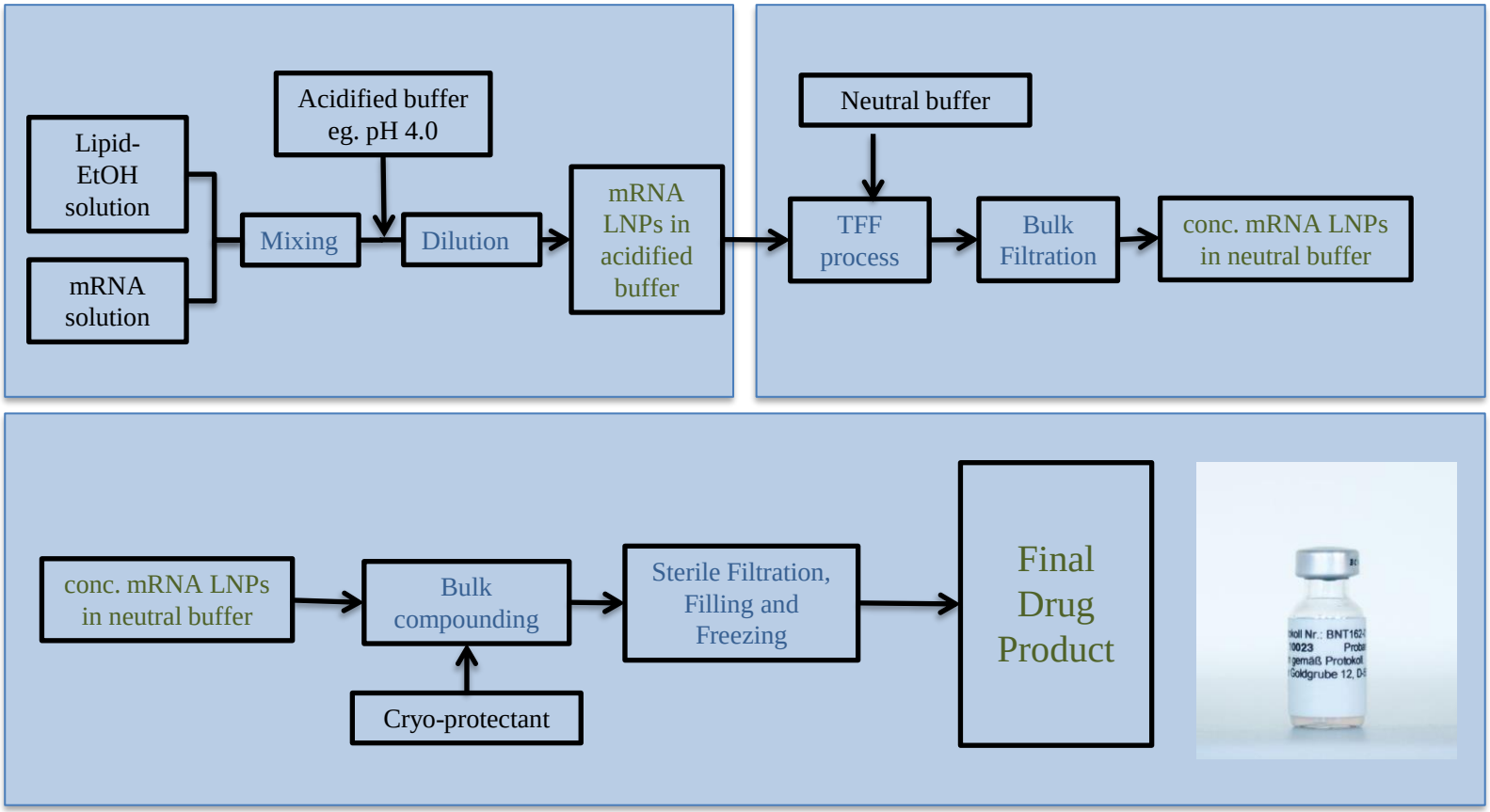
Production of mRNA LNP Vaccines



mRNA LNP process development – key formulation parameters

– *Formulation development*

** lipid composition (ratio ionizable lipid, PEG-lipid, PC, cholesterol)*

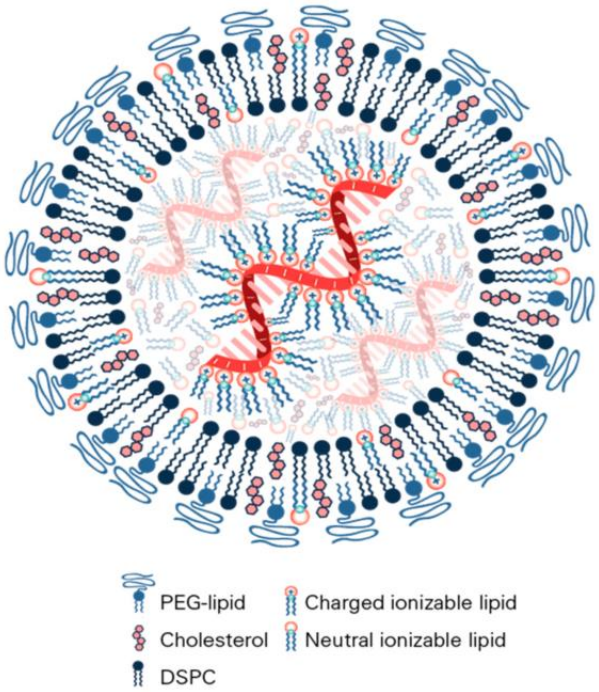
** drug substance type, size*

** ratio ionizable lipid to mRNA (N/P ratio)*

** raw material quality/purity*

** RNA-buffer/dilution buffer: pH, ionic strength, viscosity*

** Ratio aqueous phase vs. solvent and solvent type*



mRNA LNP process development – critical process parameters

- *LNP formation step:*

  ** concentration of mRNA in acidified buffer and lipids in EtOH*

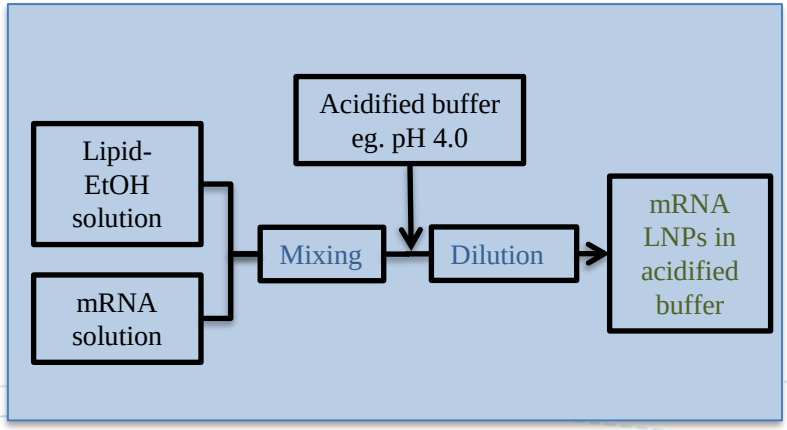
  ** flow rates and flow rate ratios*

  ** inline dilution: type of buffer, time of particle maturation, EtOH concentration reduction*

  ** aqueous phase: pH, ionic strength, viscosity*

  ** process temperature – impacts mRNA as well as particle quality*

  ** pump types – pulsation, cavitation,….*



Critical process parameters – Concentration lipids/mRNA & flow rate

Experiment:	mRNA concentration [mg/mL]	Total lipid concentration [mg/mL] *	Flow rate total [mL/min]	Size [nm] / PdI
1	0.6	45	480	69.5 / 0.155
2	0.6	45	320	63.6 / 0.128
3	0.6	45	160	59.0 / 0.100
4	0.4	30	320	52.7 / 0.103
5	0.2	15	480	53.3 / 0.170
6	0.2	15	160	50.1 / 0.075

** lipids at 60 mg/ml in EtOH precipitate at RT*

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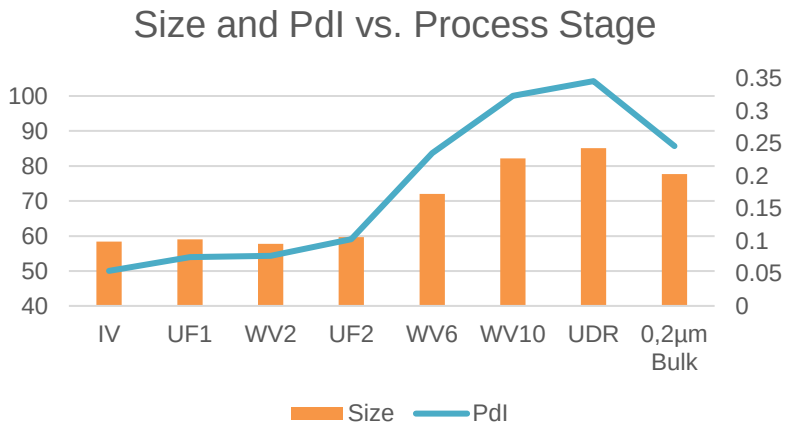
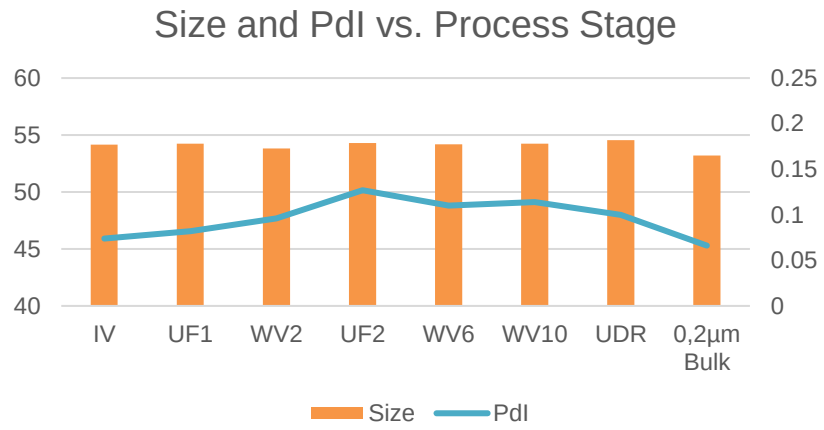
Critical process parameters – Ionic strength and pH of formulation buffer

- Higher pH of formulation buffer improves colloidal stability during TFF and 0,2 µm filtration process (acetate buffer pH 4.0 vs pH 6.0)





- Increasing ionic strength improves encapsulation efficiency

Acetate buffer pH 4.0, 30 mM EE% = 84

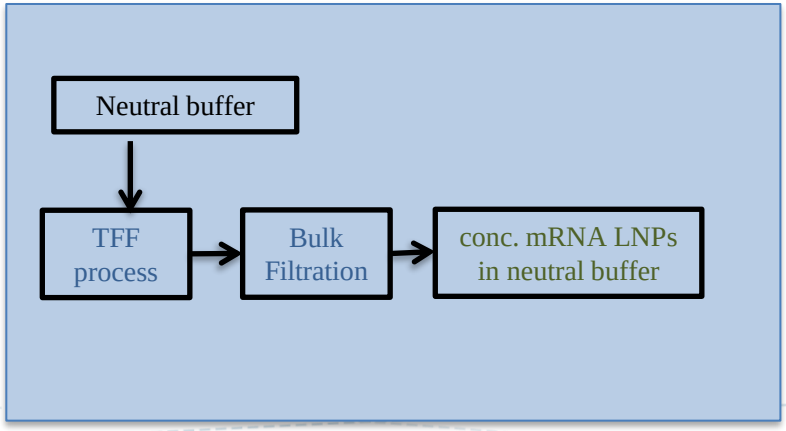
Acetate buffer pH 4.0, 120 mM EE% = 94

mRNA LNP process development – critical process parameters

- *TFF process:*
  - ** loading: DP per membrane area*
  - ** shear rate, TMP, HF-length*
  - ** TFF sequence: eg. DF1 – UF1 – DF2 – UF2*

    UF1 – DF1 – UF2

    (ultrafiltration – concentration factor); (diafiltration – number of volume exchanges)
  - ** process temperature*



- *bulk filtration process:*
  - ** filter type: material, cut-off*
  - ** loading: DP per filter membrane area*
  - ** flow rate, pressure*
  - ** pump/flow type*

mRNA LNP process development – critical process parameters

- *sterile filtration process and filling:*

  ** filter type: material, cut-of*

  ** pump type: impact on DP quality to avoid generation of particulates*

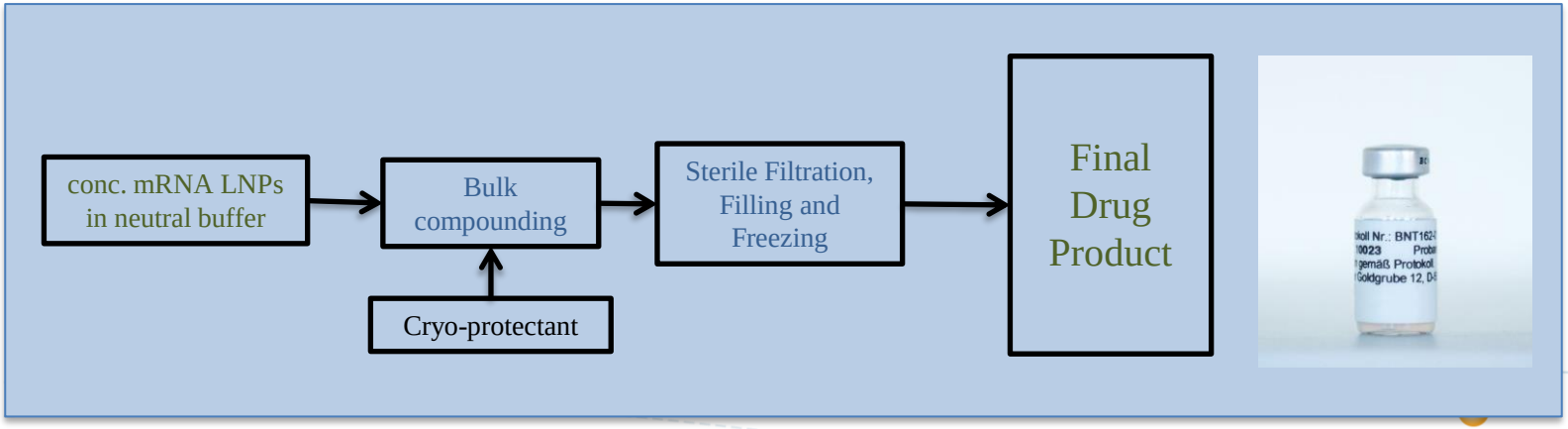
  ** loading: DP per filter membrane area*

  ** flow rate, pressure*

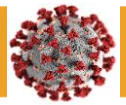
  ** pump/flow strategy: vacuum, positive pressure, pump (type)*

  ** process temperature*

  ** primary packaging material; CCIT @- 80° C storage*



mRNA Vaccines – Scale up



- *Initial LNP formation process was designed to formulate 1 g mRNA- LNPs within 45 min*
- *Target: formulation of 1 g mRNA ≤ 1 min*
- *Scale up strategies:*

LNP formation

* *Increase of concentration of mRNA in acidified buffer and lipids in EtOH*
* *Increase of flow rates*
* *Multiple mixing lines*

TFF process

* *Increase of filter membrane area at constant shear rate*
* *Optimizing the TFF sequence*

Sterile filtration process

* *Increase of filter membrane area at constant pressure*

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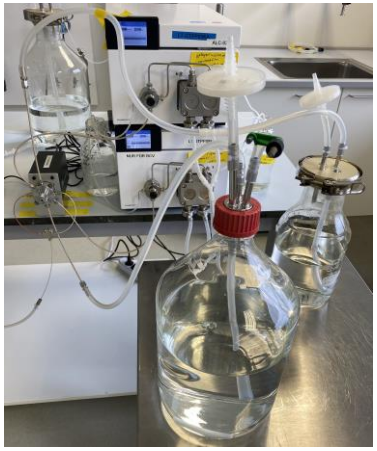
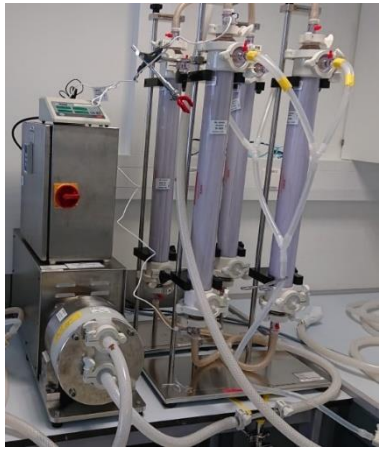
mRNA Vaccines – Achievements within < 1 year

- *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*





LNP processing – ongoing activities / optimization

- *LNP formation step:*
  - * *mRNA buffer optimization*
  - * *concentration of mRNA in acidified buffer and lipids in EtOH*
  - * *flow rates and flow rate ratios*
  - * *mixing unit, mixing angles*
  - * *pump types – pulsation, cavitation,….*
  - * *inline dilution: type of buffer, time of particle maturation, EtOH concentration reduction*
  - * *aqueous phase: pH, ionic strength, viscosity*
  - * *process temperature – impacts mRNA as well as particle quality*

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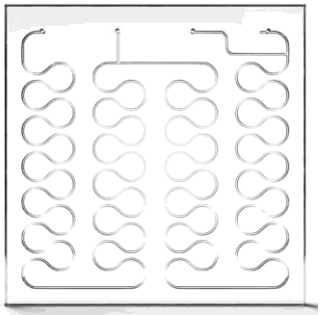
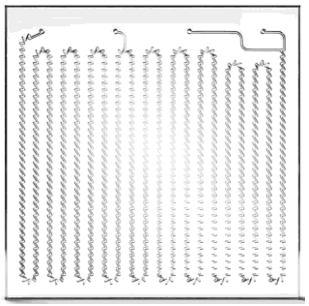
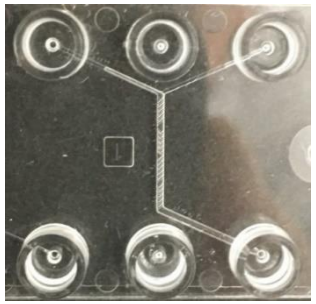
LNP processing – ongoing activities / optimization

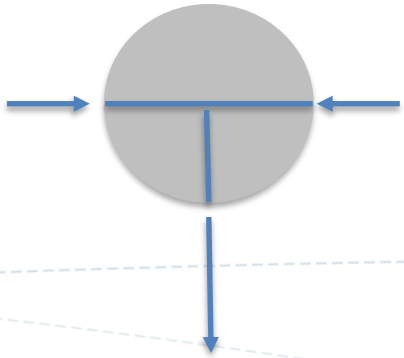
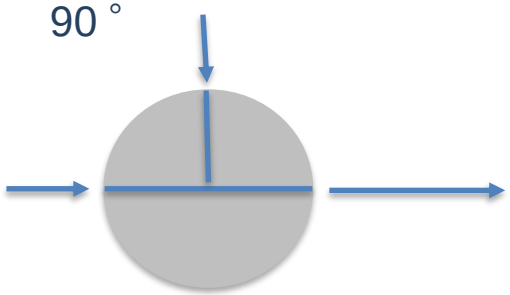
- *Mixing unit:*
  - * *microfluidic mixing*
  - * *T-mixer, Y-mixer, X-mixer*
  - * *Polymun cross-flow mixer*


- *Mixing angels:*

180 °

90 °

- *Pump types:*

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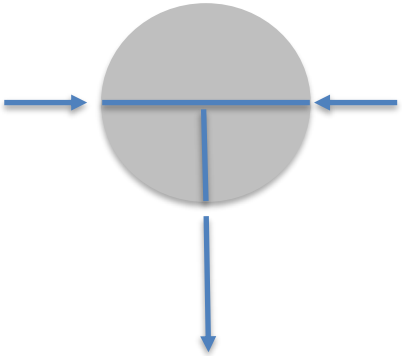
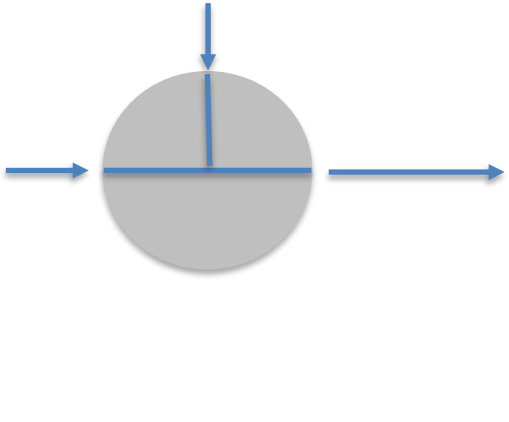
LNP processing – ongoing activities / optimization

Mixing angles and pump type:

180 °



90 °



Input [mg]	Pump	Flowrates [mL/min]	Angle	IV Size/PdI		UDR Size/PdI		0.2 m filtr.		CBS		F/T Vial	
				Size	PdI	Size	PdI	Size	PdI	Size	PdI	Size	PdI
80	Pump A	40/120	180°	55,68	0,052	61,40	0,101	60,48	0,085	60,30	0,087	64,74	0,093
80	Pump A	20/60	90°	58,83	0,064	62,96	0,085	62,26	0,079	64,53	0,096	67,5	0,103
80	Pump A	20/60	180°	57,41	0,057	78,53	0,127	78,51	0,113	78,17	0,128	91,7	0,103
30	Pump B	20/60	90°	61,11	0,067	67,37	0,075	66,45	0,059	67,33	0,070	70,08	0,076
30	Pump B	20/60	180°	93,78	0,135	92,67	0,121	91,50	0,118	94,09	0,109	94,09	0,109

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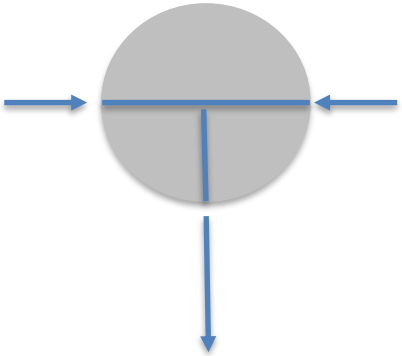
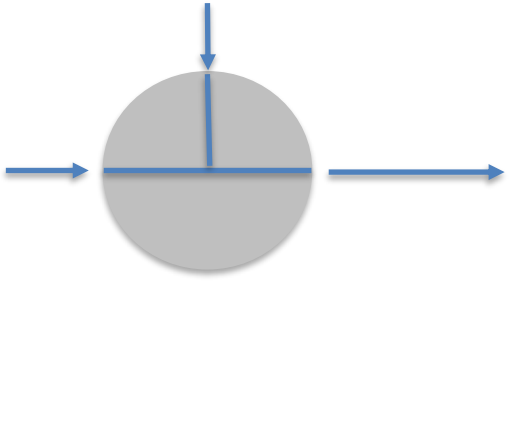
LNP processing – ongoing activities / optimization

Mixing angles and pump type:

180 °



90 °



Input [mg]	Pump	Flowrates [mL/min]	Angle	IV Ribogreen		UDR Ribogreen		F/T Ribogreen	
				Total [µg/ml]	EE%	Total [µg/ml]	EE%	Total [µg/ml	EE%
80	Pump A	40/120	180°	113,83	N.A.	N.A.	N.A.	544,53	95,11
80	Pump A	20/60	90°	109,95	N.A.	1223,20	96,43	574,21	90,44
80	Pump A	20/60	180°	111,30	N.A.	1355,42	95,97	610,57	97,87
30	Pump B	20/60	90°	108,10	N.A.	1185,66	97,55	573,54	97,09
30	Pump B	20/60	180°	104,12	N.A.	1220,88	95,24	542,53	94,97

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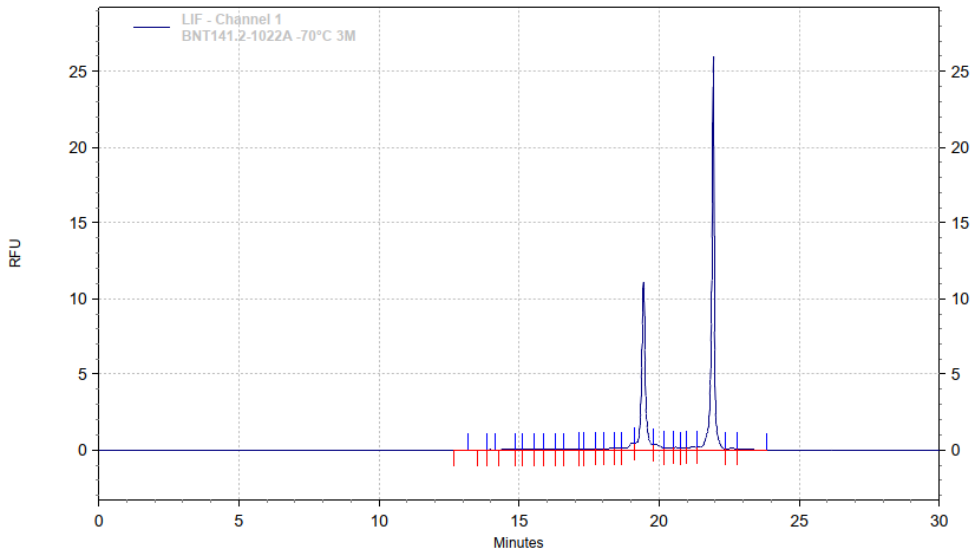
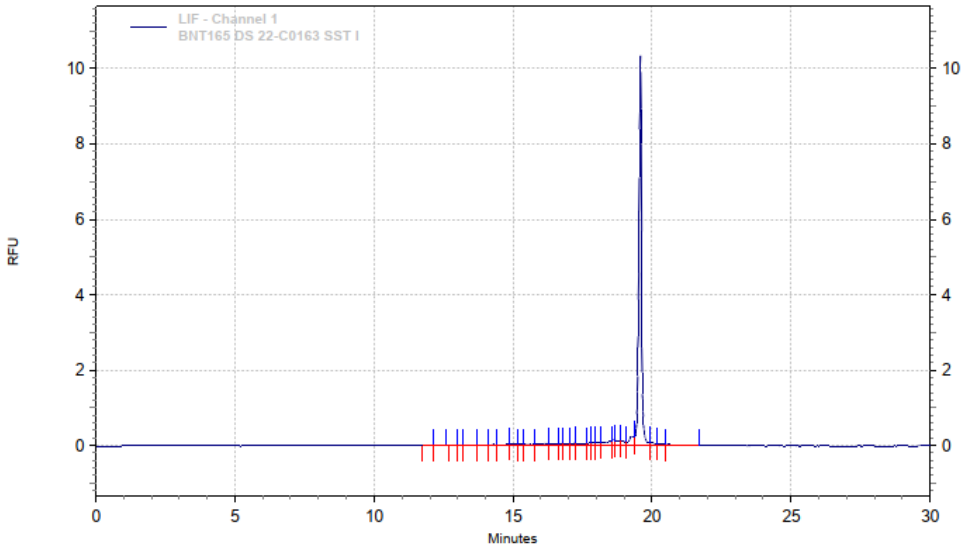
Quality Control

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

RNA Integrity by Capillary Electrophoreses





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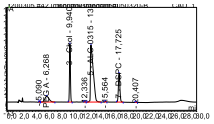
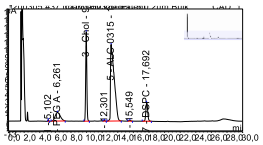
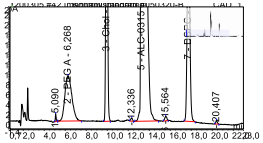
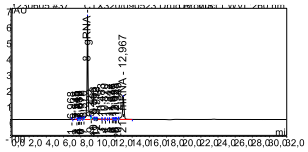
Lipid Identy and Quantity by rp-HPLC-CAD

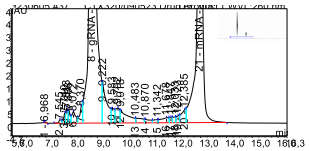






RNA – Quantity and Identity of gRNA and mRNA by IPRP





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