NANOLIPOSOME (NLS) TECHNOLOGY – A NEW INNOVATIVE APPROACH

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We make APIs perform

INTRODUCTION

Liposomes have been investigated since 1965 and have become of high interest for *oral delivery* in the last years e.g. for large Peptides/Peptide Hormones like Insulin (6 kDa), Exenatide (4 kDa) , Semaglutide (4 kDa), Calcitonine (3.5 kDa) or small Peptides like Desmopressin, Leuprolide and Octreotide

Source: Materials for oral delivery of proteins and peptides,
Mitragotri et.al., Nature Reviews, Vol.5, 02/2020

INTRODUCTION

For peptides, but also for low soluble active compounds two barriers have to be overcome in oral delivery:

- *Mucus barrier*

  (water, proteins, lipids, electrolytes)
- *Cellular barrier*

  (epithelial cells; different ways like

  transcellular or paracellular pathway;

  limit of 6 kDa for peptides proposed)

Source:
Materials for oral delivery of proteins and peptides,
Mitragotri et.al., Nature Reviews, Vol.5, 02/2020
A.Bernkop-Schnürch et. al., Advanced Drug Delivery Reviews,
142, 2019, 91-101

INTRODUCTION

To overcome these barriers several strategies have been followed:

- Use of protease inhibitors (e.g. Aprotinin)
- Use pH/acidity modifiers (e.g. Citric acid)
- Use of chelating agents (e.g. EDTA)
- Use of surfactants (e.g. SNAC, medium chain fatty acids)
- Use of bile salts (e.g. SGC, STC)
- Polymers (e.g. Chitosans, Hyaluronic acid)
- Microneedles, Microcontainer (e.g. LUMI)
- Micelles and Liposomes

Source: Materials for oral delivery of proteins and peptides,
Mitragotri et.al., Nature Reviews, Vol.5, 02/2020

However, all these approaches are lacking a sufficient general approach and providing the following disadvantages:

- Although permeation enhancers have been used, the oral bioavailability is still low (e.g. 0.4 – 1.0% for oral Semaglutide/SNAC in Rybelsus® or Octreotide/TPE in Mycappsa® with approx. 0.25%)
- Loading of active ingredients into delivery systems is still very limited (e.g. in microneedles, patches, microcontainers)
- Use of inhibitors or surfactants can cause significant side effects
- Scalability problem for most of the described processes

INTRODUCTION

Losan has decided to further develop the liposomal (***N**ano**L**ipo**S**ome*) or mixed micelle approach for the oral delivery of peptides/low soluble compounds due to the following reasons:

- The ***bead mill technology*** represents an implemented, ***safe technology*** that has widely been used in the manufacture of ***Nanocrystal suspensions*** over the last 10 years
- The use of the ***dual centrifugation method*** was shown *to be* ***predictive for Nanosuspensions*** in large scale and could be similar for the NLS technology (Hagedorn et al.)
- G.Fricker and U.Massing et.al. have already shown that Liposomes can be in principle screened in a lab-scale (1–10 mL) successfully. Further optimizations of this screening method have been performed
- Losan has the know-how and experience in the further ***downprocessing*** for dehydration of the liposomal or mixed micelle dispersions and formulation of final dosage forms like ***capsules, enteric coated capsules, tablets or stick packs***

INTRODUCTION

The advantages of this technology by initial formed VPGs (Vesicular Phospholipid Gels) and subsequent formed liposomes in the nanometer range are:

- the high encapsulation efficacy (EE) 45 – 60% (proven with Calcein inclusion) without organic solvents
- Liposomes are well tolerable after application
- the use of surface modifier/permeation enhancer like Sodium Glycocholate (SGC) enables the possibility of loaded liposomes to overcome the mucosal/ cell barrier, e.g. Insulin over 4 h at 37 °C in Pepsin / Trypsin media

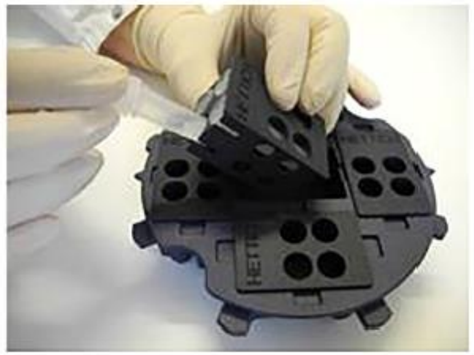
(Source: Wu et. al., Int. J. Nanomedicine, 2011, 1155-1166)

INTRODUCTION

- Liposomes should permeate intact through the mucosal and epithelial cell barrier and are enabling possibilities for the delivery of instable active substances like peptides or RNA
- The concept can be widened to low soluble compounds for oral delivery
- Enteric coated systems can be applied to avoid gastric instability
- The ***one-pot-system*** at Losan has major advantages compared to a high pressure homogenization or small scale nanoprecipitation microfluidics approaches (flow up to 4 ml/min!)

Source: Tirelli et. al., Int. J. of Pharmaceutics, 534, 2017 , 97-107

BEAD MILLING EQUIPMENT



Screening scale (2 – 10 mL)
DeltaVita 1 (Hettich/Netzsch)

Lab/Pilot scale (0.5 – 10 L)
DeltaVita 300Z

Production scale (30 – 800 L)
Pharma AlphaZetaVita Z10

EQUIPMENT FOR DOWNPROCESSING

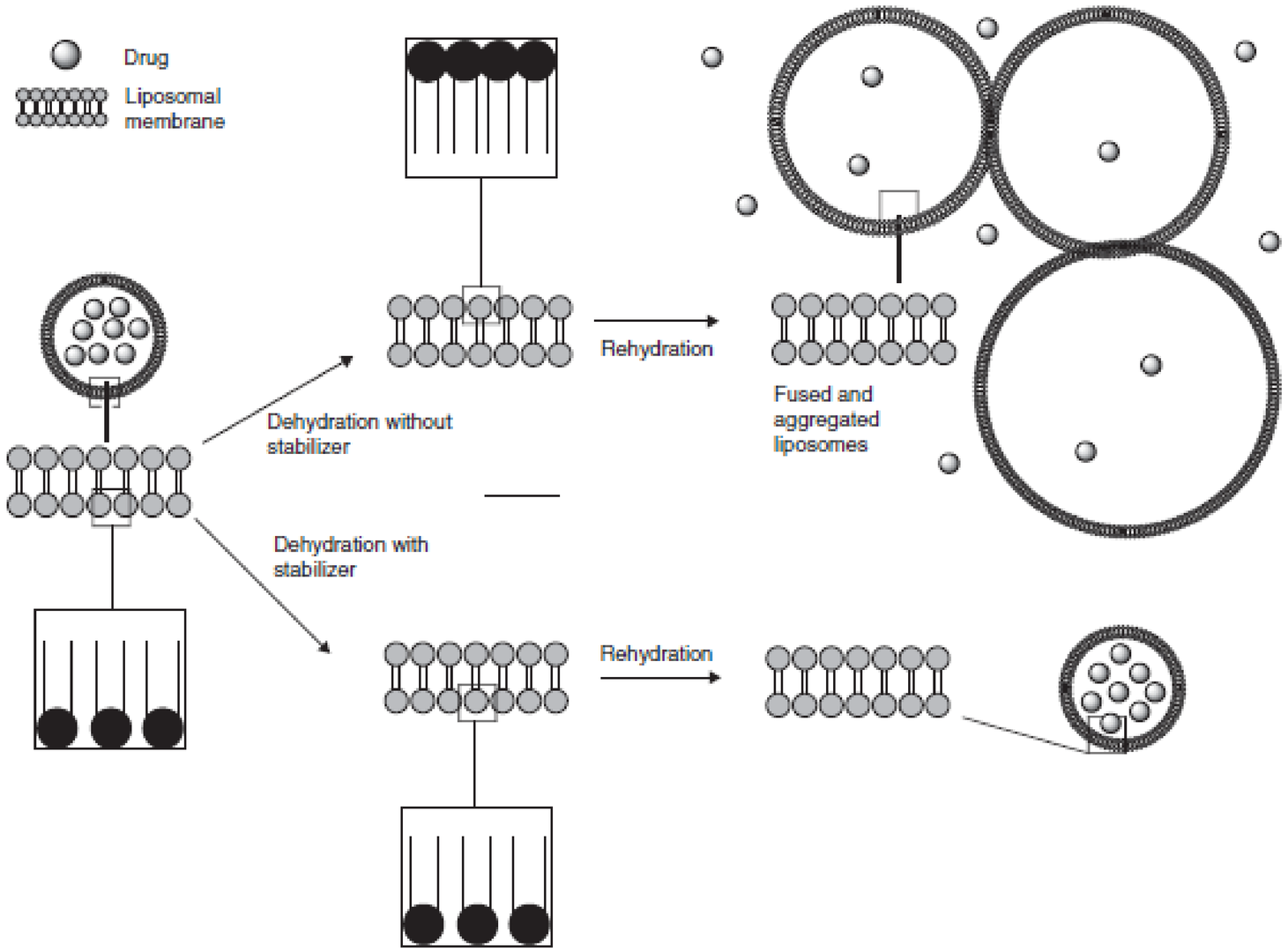
Mini/Midi Glatt
Lab-scale
10 g – 1000 g

Syntegon/Bosch Unilab
Scale-up
1 – 6 kg

Syntegon/Bosch Solidlab 2
Pilot-scale
2 – 14 kg

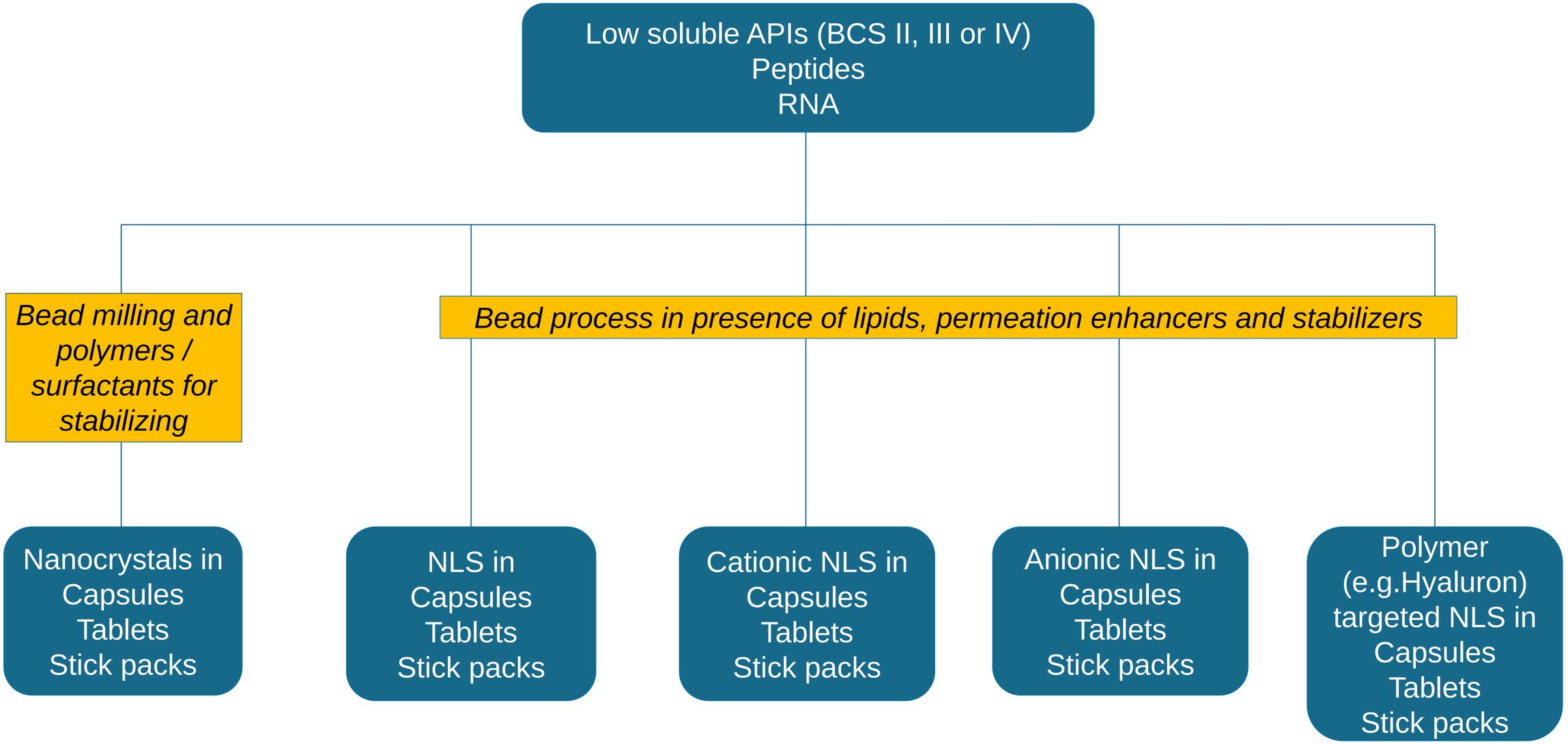
Syntegon/Bosch HKC
Production scale
120 – 400 kg

DEHYDRATION OF LIPOSOMES



Source: C. Foged et. al. Researchgate March 2011

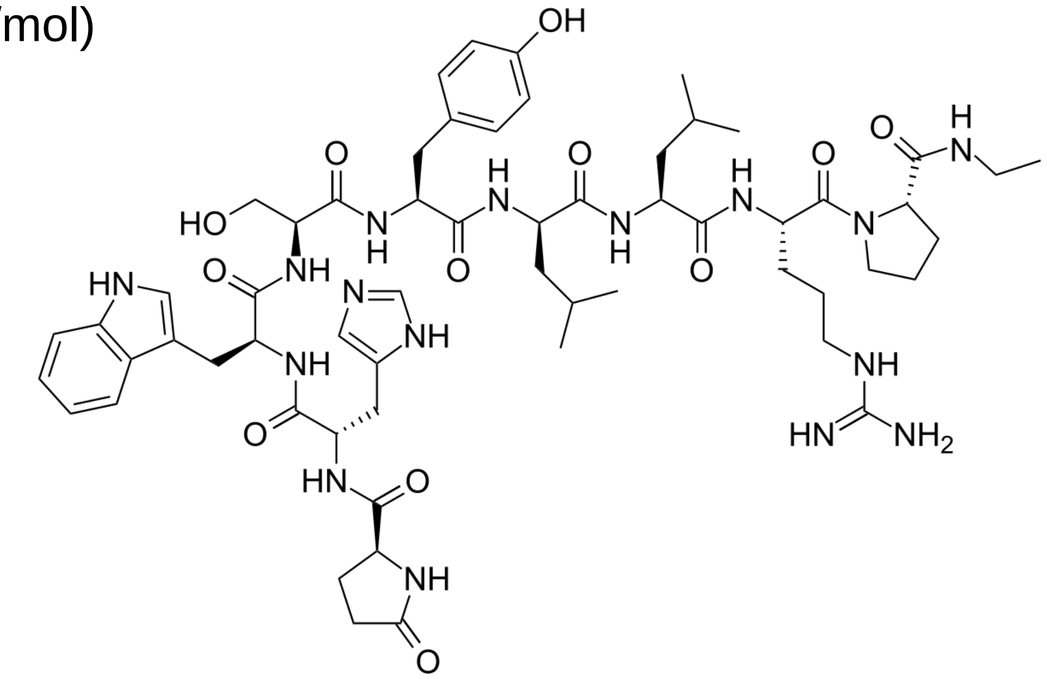
OVERVIEW FOR BEAD MILLING TECHNOLOGY AT LOSAN



MODEL COMPOUND LEUPROLIDE

Leuprolide was defined as model compound due to the following reasons:

- Leuprolide tablet under clinical evaluation by Enteris BioPharma (molecule is of interest for oral delivery)
- Fast and complete degradation in SIF media (less than 5 min)
- Nonapeptide with appropriate size (MW 1200 g/mol)
- Limited soluble in aqueous media
- Availability of analytical methods



5-Oxo-Pro-His-Trp-Ser-Tyr-d-Leu-Leu-Arg-Pro-NH-C2H5

NLS (PILOT SCALE TRIALS)

Milling process DeltaVita 300Z (30 – 40% lipid concentration, no active ingredient)
Batch size: 600 mL

30 min

60 min

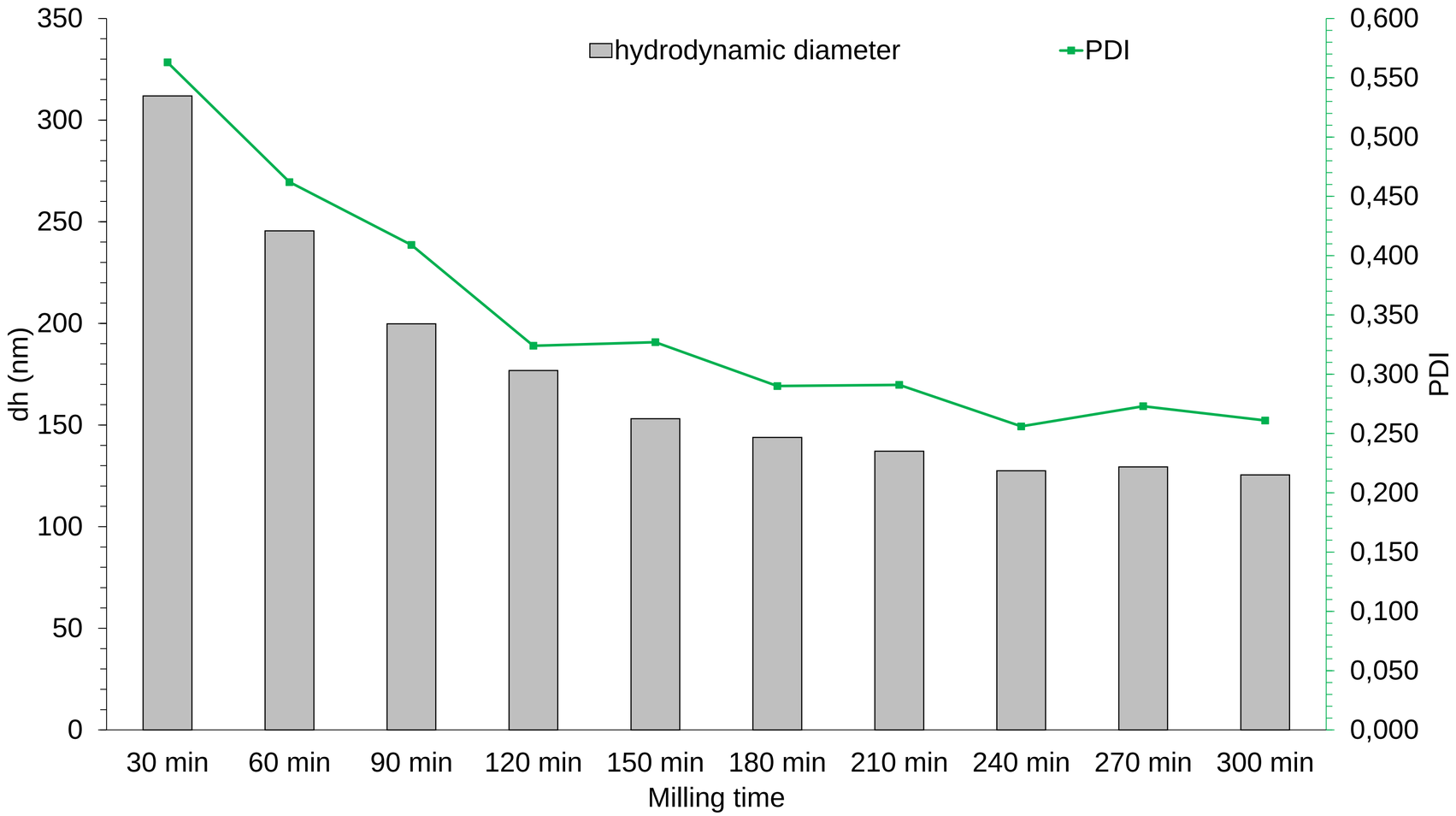
90 min

120 min

150 min

180 min

Particle size distribution (DLS; ZetaSizer) as IPC during process (30% lipid concentration)



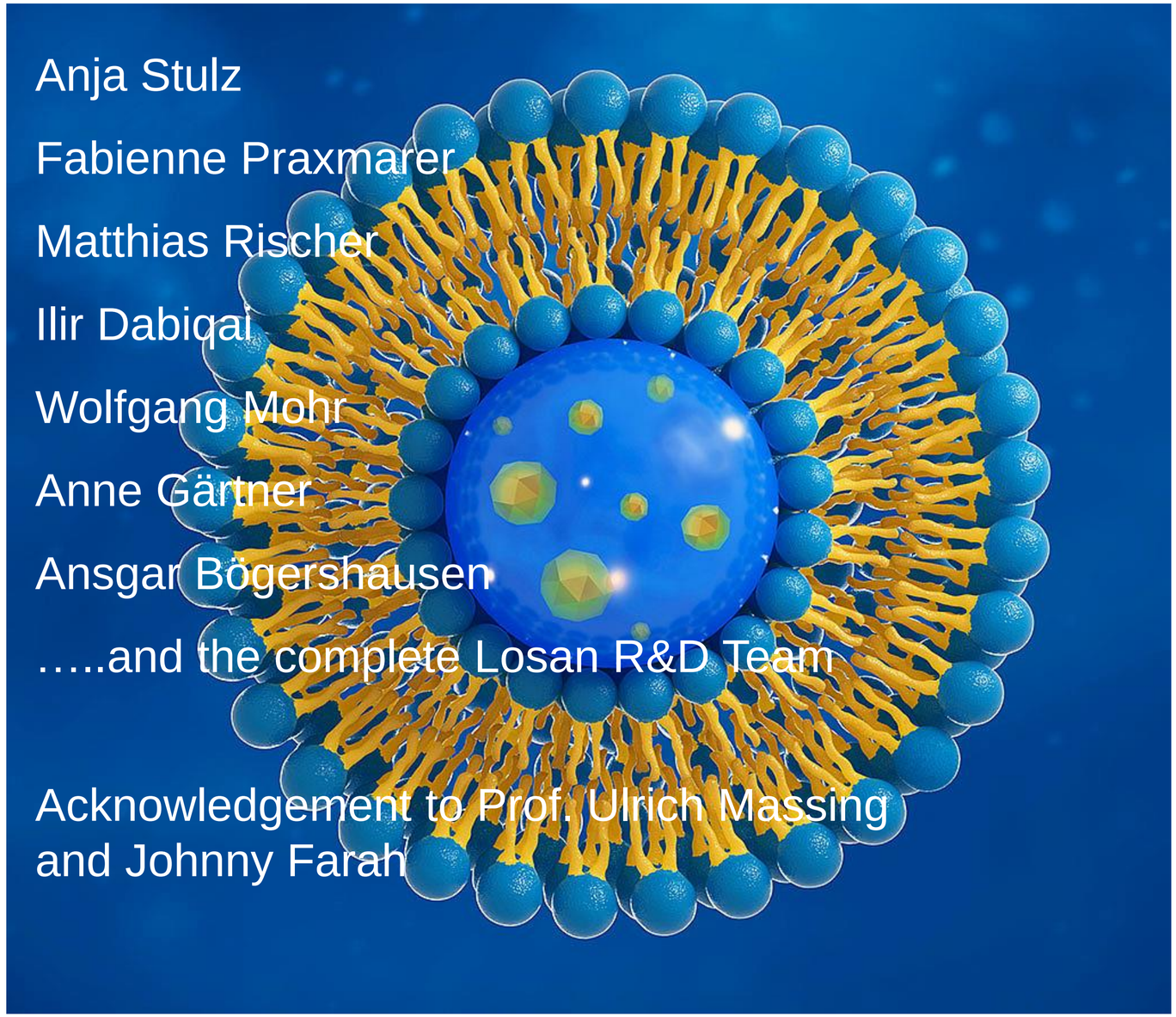
SUMMARY

- Screening & pilot scale process for NLS implemented, production process under implementation.
- Process is regarded to be superior compared to HPH, Microfluidics or any other technology with regard to EE, scalability and process time.
- NLS with/without modifications possible.
- Integrity of NLS in SIF shown over expected resorption window; peptide stays intact in NLS to be resorbed by epithel layer.
- Entrapment efficiency shown from 40 – 60% with Calcein.
- Downprocessing by FBD possible without significant degradation of NLS in the carrier. However, further stabilizers and carriers have to be tested to increase liposomal stability and release level

OUTLOOK

- Scale-up trials with Leuprolide as model compound
- Stability studies at different storage conditions
- Formulation of a capsule formulation (enteric protected) containing Leuprolide and selection of suitable carrier matrices
- Prove of concept in-vivo (rats) with Leuprolide with regard to bioavailability

THE NLS TEAM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