Nano can be Big(ger): bringing added value to milk fat

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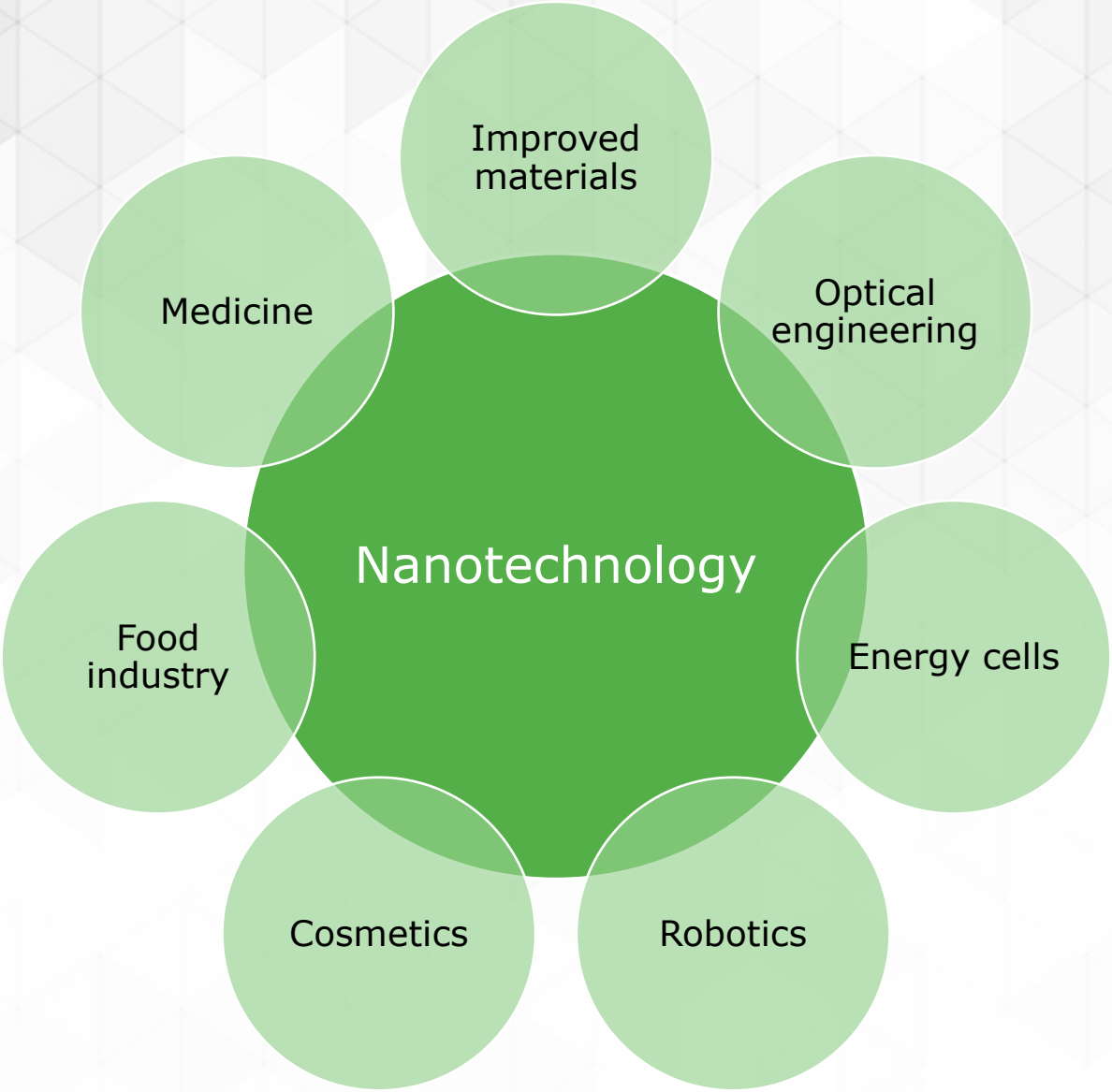
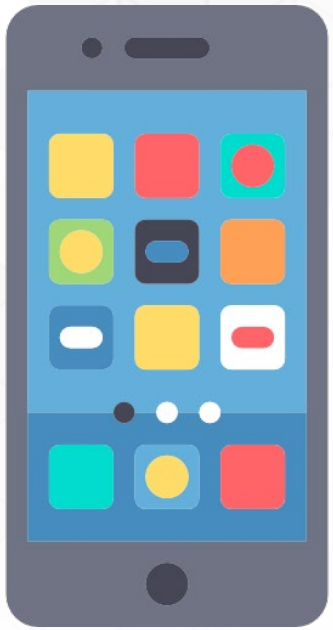
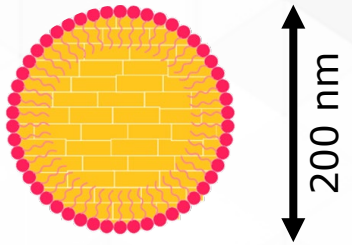
75th EAAP – September 2024 – Florence, Italy

Introduction



Introduction

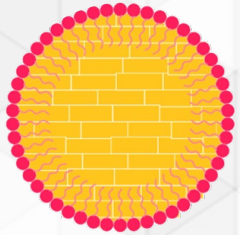




100 million times smaller

100 million times smaller

Introduction



Nano delivery systems

- Address bioavailability issues
- Address negative side effects
- Address stability issues

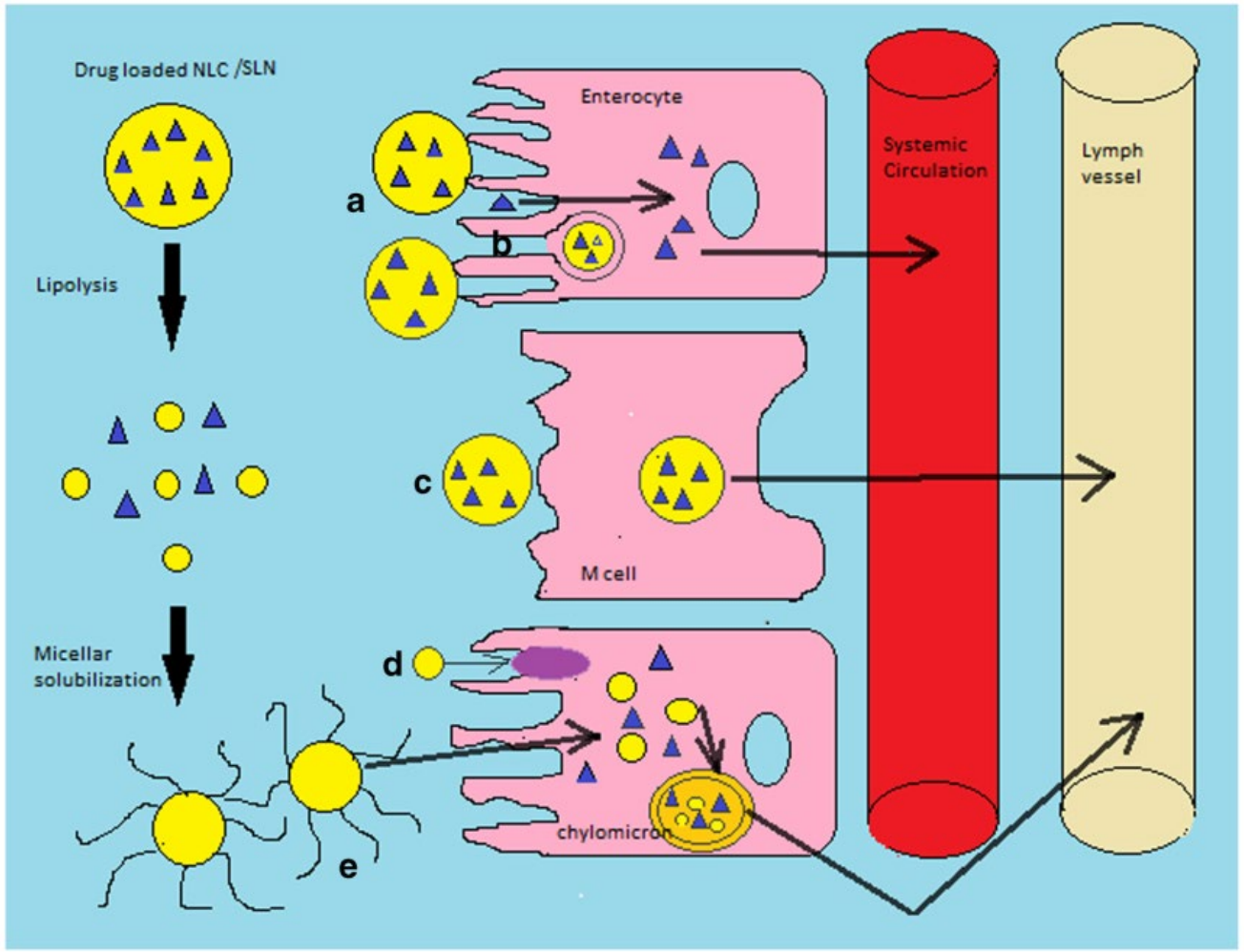
- **Lipid-based nanoparticles**
  - Generally regarded as safe components
  - Among the most easily scalable production methods

- **Vitamin D**
  - Essential for bone health and systemic regulation
  - Increasingly deficient with required supplementation
  - <u>Highly lipophilic</u>

Silva, M. C., & Furlanetto, T. W. (2018). Intestinal absorption of vitamin D: a systematic review. Nutr Rev, 76(1), 60-76. https://doi.org/10.1093/nutrit/nux034

Introduction

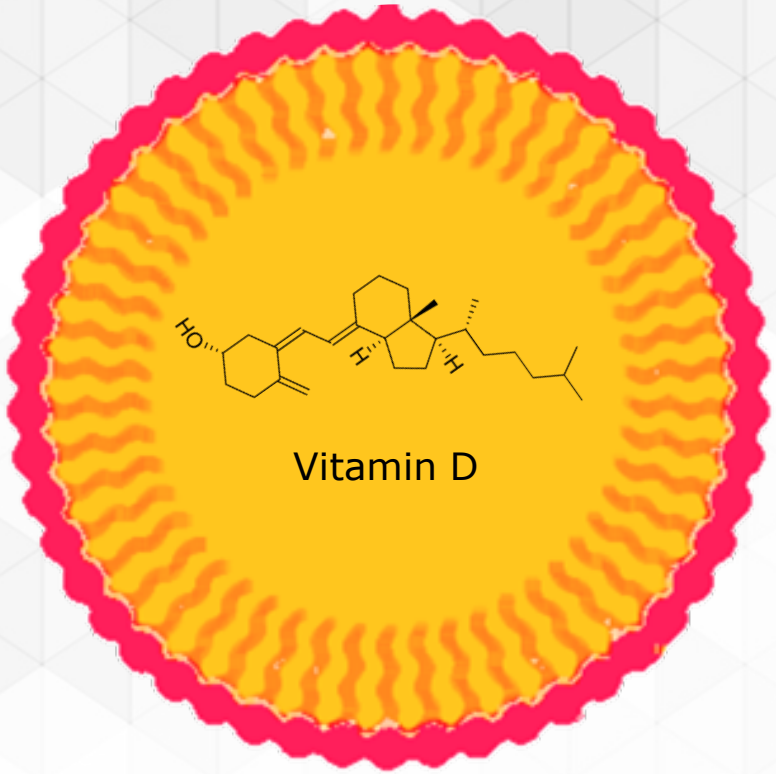
- **Uptake into the bloodstream**
  - Particles of ~500 nm can be absorbed by enterocytes and their cargo delivered to the bloodstream
- **Uptake into the lymph system**
  - Particles of ~200 nm are preferentially collected by M cells and then delivered to the lymphatic vessels
  - Lipophilic molecules can be transported via the chylomicrons produced in enterocytes



Talegaonkar, S. and A. Bhattacharyya, Potential of Lipid Nanoparticles (SLNs and NLCs) in Enhancing Oral Bioavailability of Drugs with Poor Intestinal Permeability. AAPS PharmSciTech, 2019. **20(3)**

Managuli, R.S., et al., Targeting the intestinal lymphatic system: a versatile path for enhanced oral bioavailability of drugs. Expert Opinion on Drug Delivery, 2018. **15(8)**

Objective

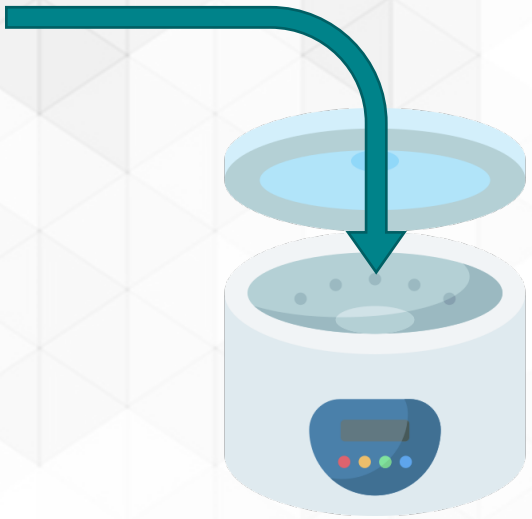


Lipid nanoparticles (LNP)

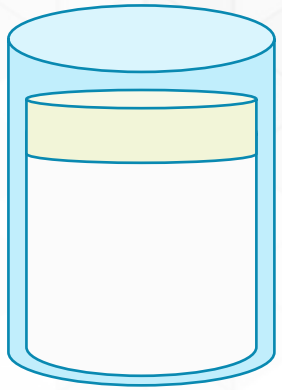
=

Nutraceutical

Milk cream separation - Protocol



Centrifuged at 14000 xg for 30 min at 4 ºC

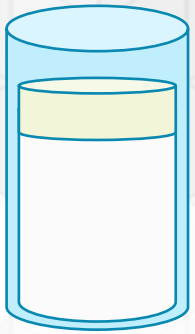


Milk Cream

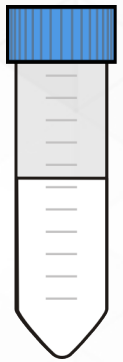
- 85% fat
- 6% protein
- 9% lactose

Nanoparticle production material

Nanoparticle production - Protocol



150 mg of milk fat



50 mg of surfactant

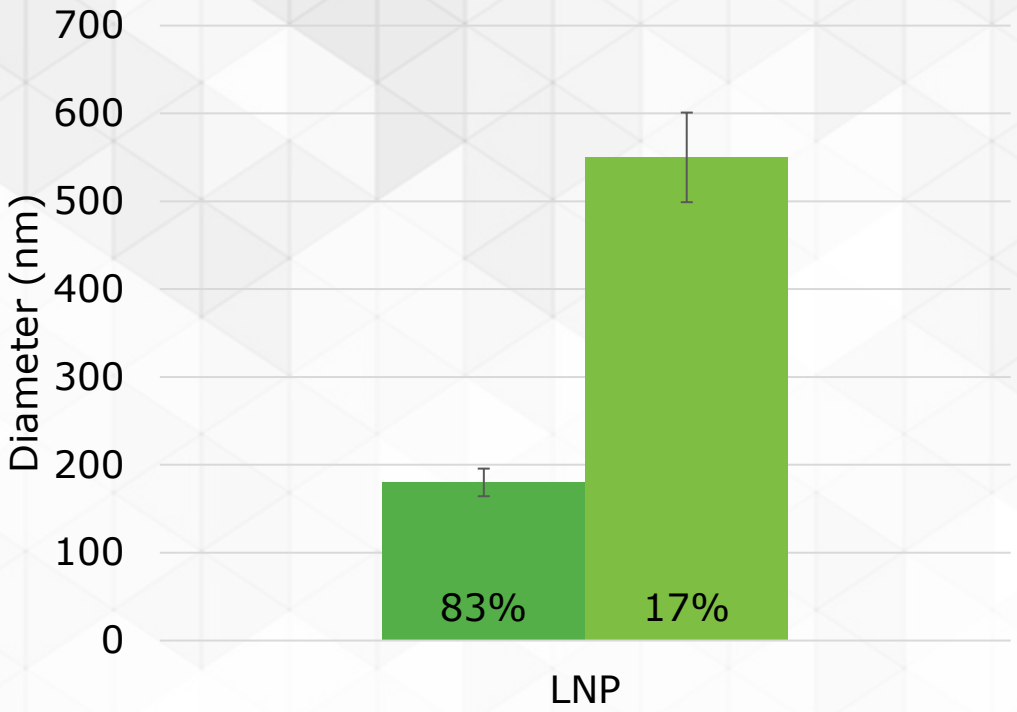
Water bath at 55 ºC
until lipid melting

Add 4.5 mL of water
bath at 55 ºC

Ultrasonicated for 5
min at 80% amplitude

Nanoparticle
formulations

Nanoparticle characterization

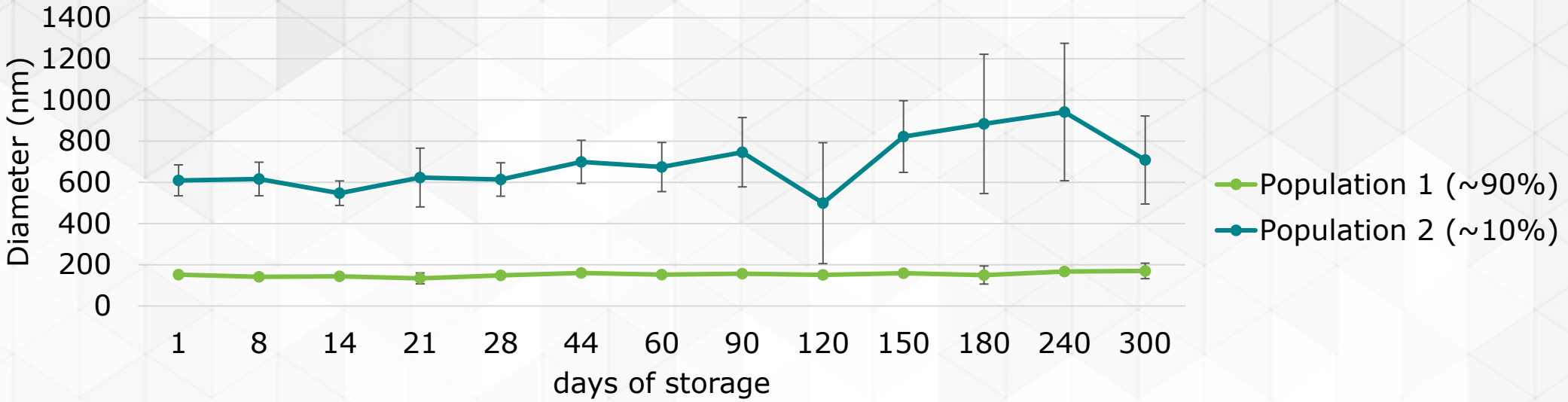


The diameters of LNP are optimal for intestinal absorption of lipid NP

Vitamin D was selected as a model molecule and was added at 1000x the DDR

	Mean ± SD
Polydispersity Index	0.28 ± 0.01
Zeta potential (mV)	-35.8 ± 3.3
Vitamin D loading (%)	10.2 ± 0.3

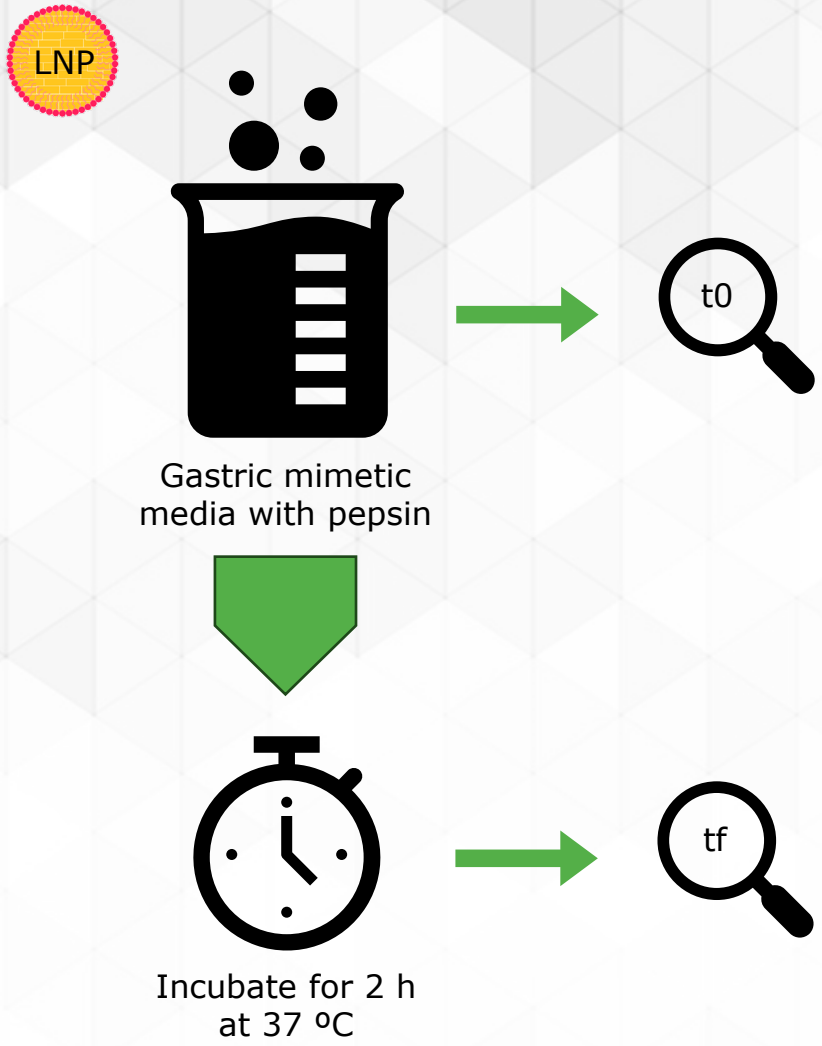
Nanoparticle characterization – shelf-life

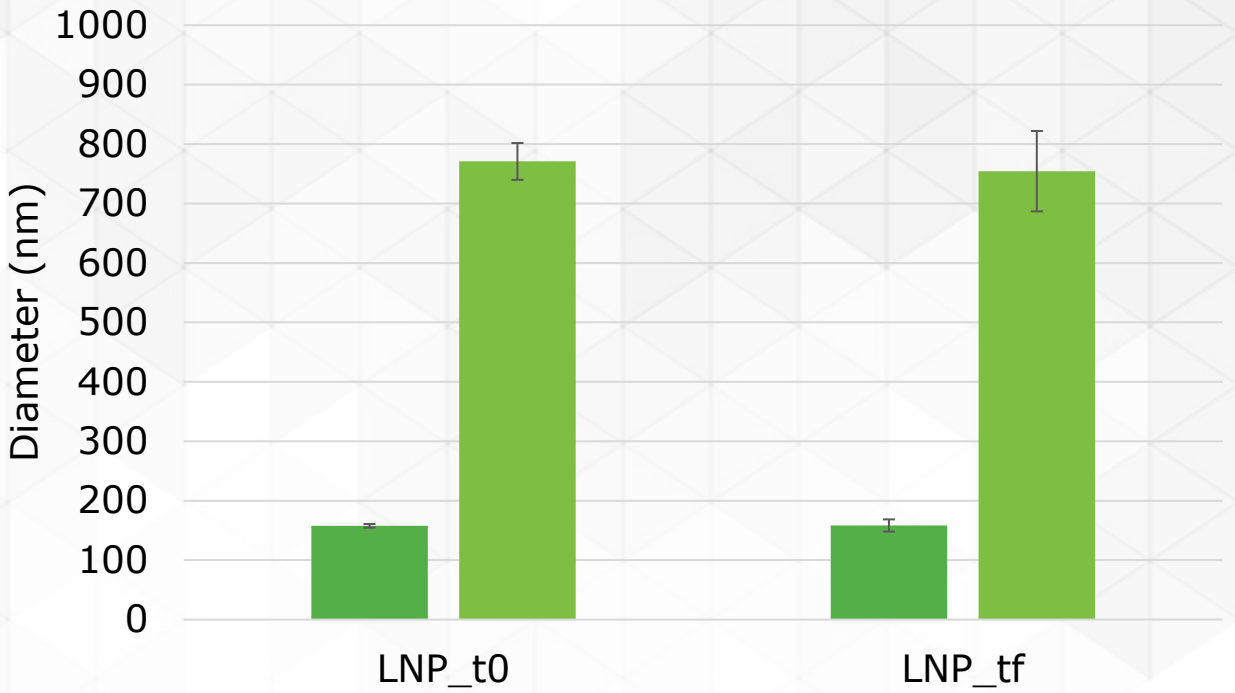


LNP are stable for at least 300 days!

PdI and zeta potential values also remained very similar over 300 days

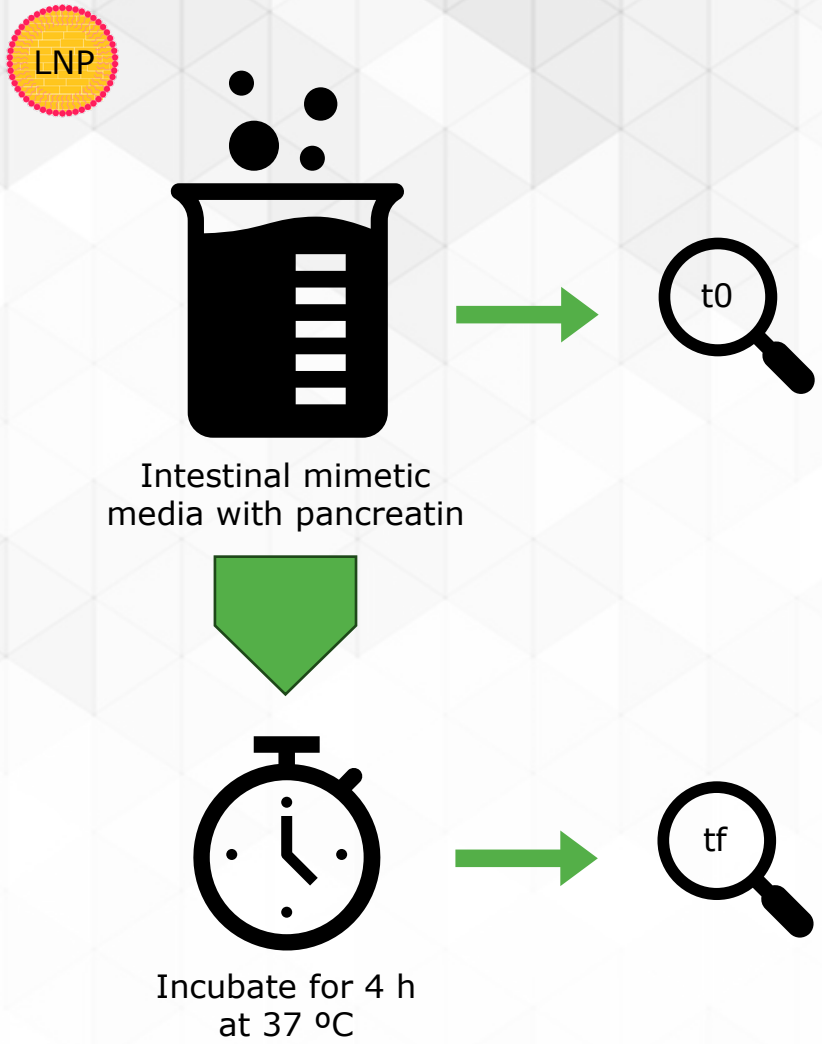
Nanoparticle characterization – gastric resistance

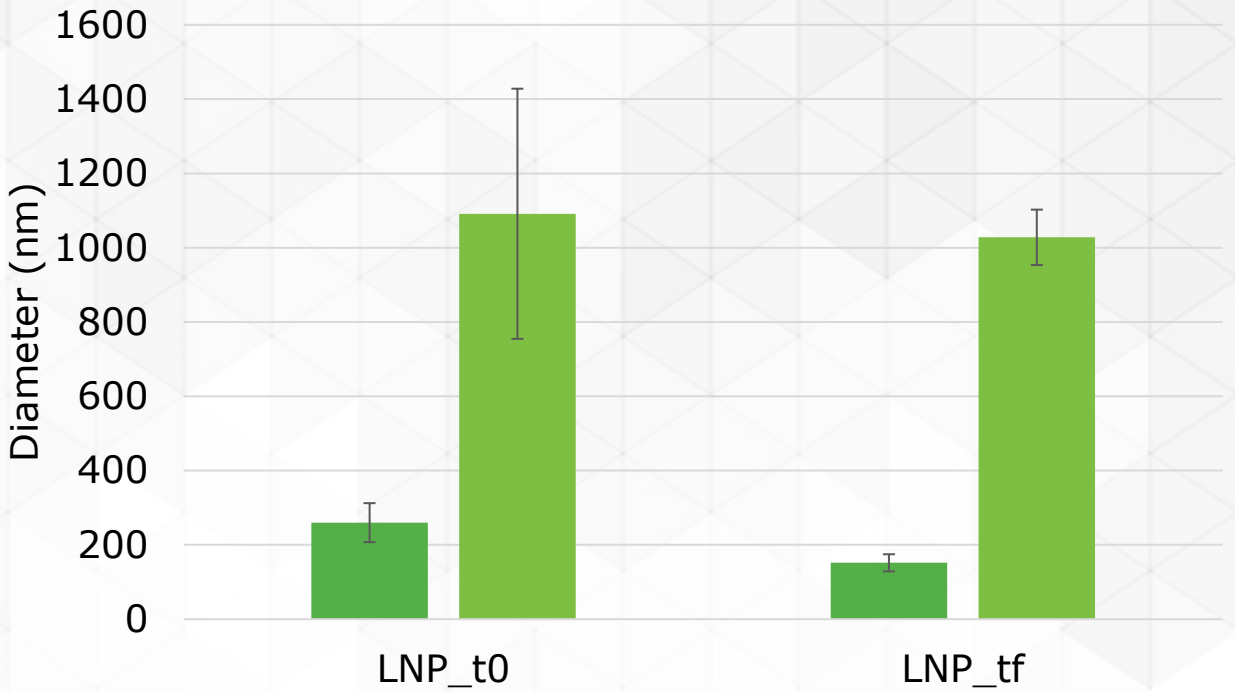




LNP resist gastric digestion!

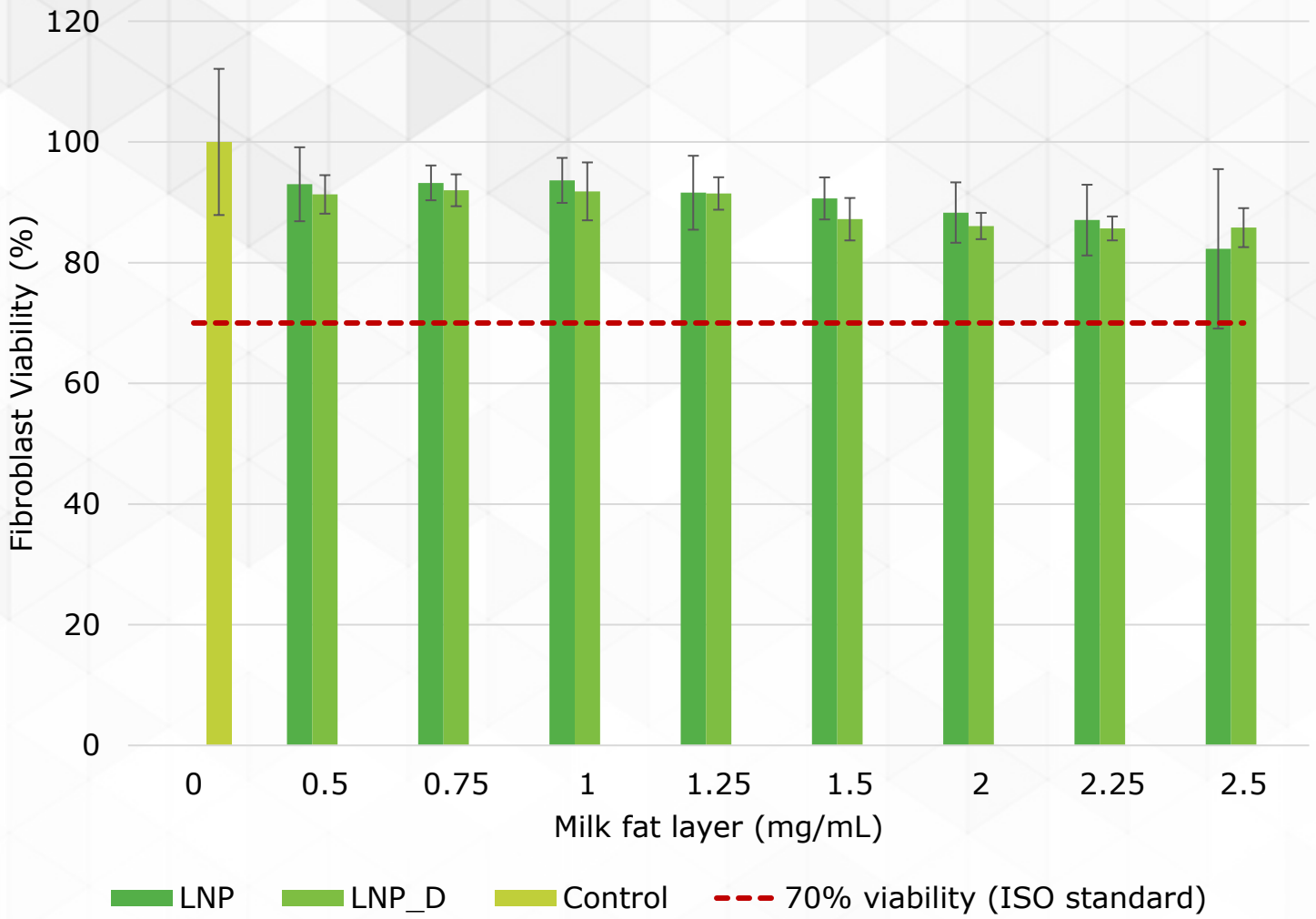
Nanoparticle characterization – intestinal resistance





LNP resist intestinal digestion!

Nanoparticle characterization – *in vitro* toxicity

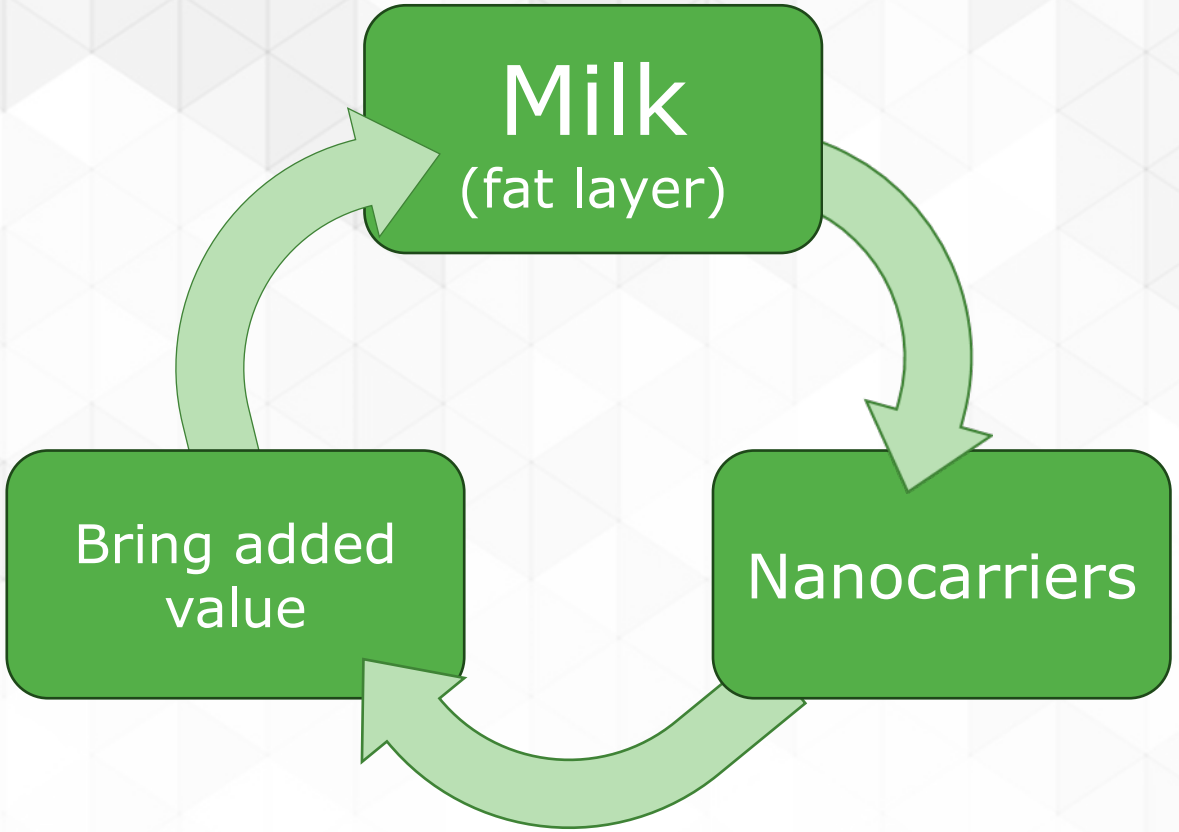


Results of MTT *in vitro* viability assay performed in Fibroblasts (L929)

The NP do not appear to be cytotoxic!

At the highest concentration, the NP provide more vitamin D than the DDR!

Conclusions



- ✓ Using organic-solvent free scalable methods
- ✓ With optimal properties for oral applications
- ✓ Great storage and gastrointestinal stability
- ✓ Excellent biocompatibility
- ✓ Enormous potential for vitamin D delivery

Future work

- **Short term**
  - Assess *in vitro permeability*
- **Long term**
  - Assess *in vivo* efficacy
  - Assess the Incorporation into food products

Acknowledgements

NanoProMilk Team

- Salette Reis
- Ana Rita Cabrita
- António Mira
- Cláudia Nunes
- Filipa Soares
- Maria Macedo
- Andreia Granja