

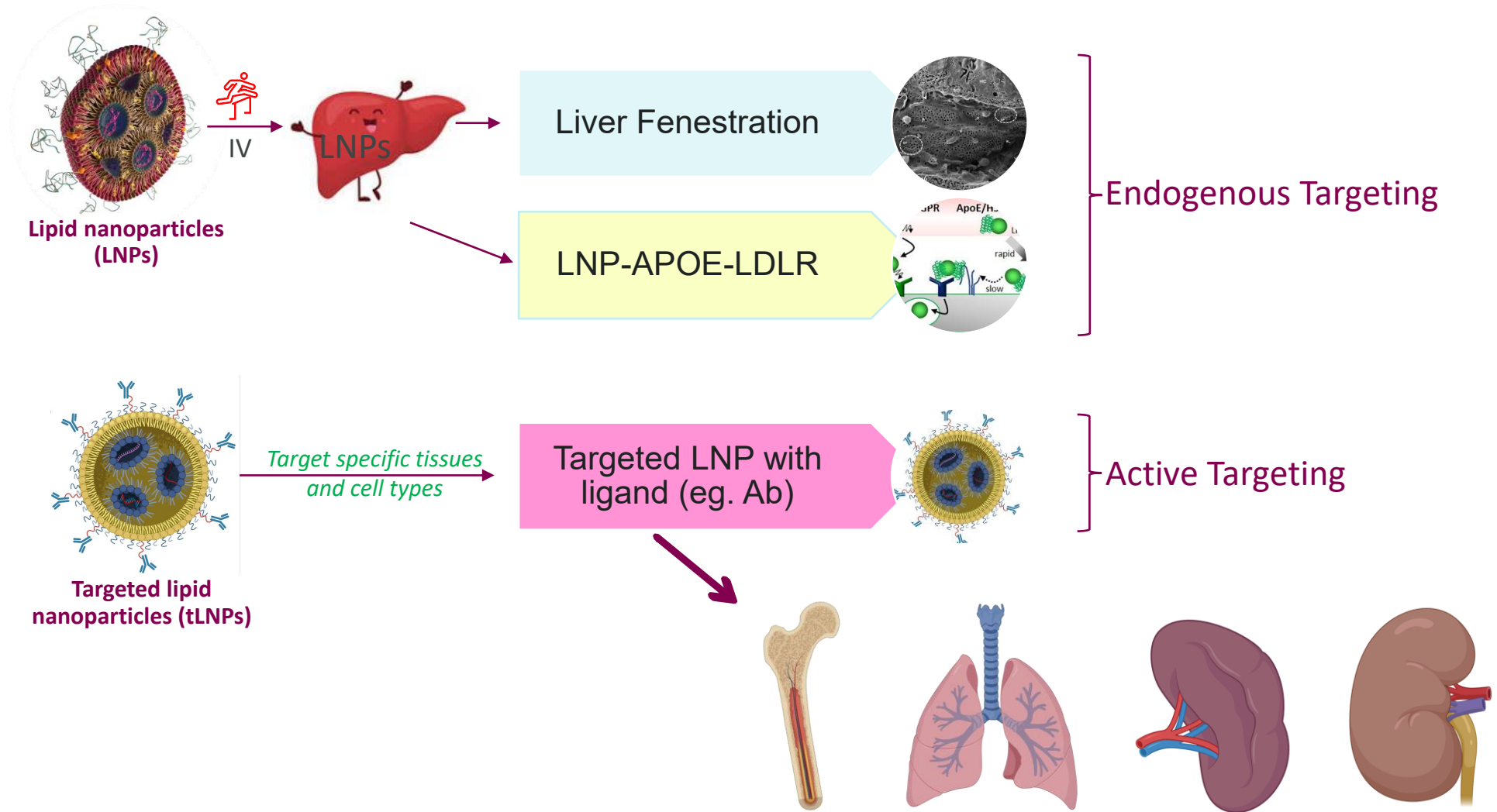
Antibody-conjugated lipid nanoparticles to enhance extrahepatic nucleic acid delivery

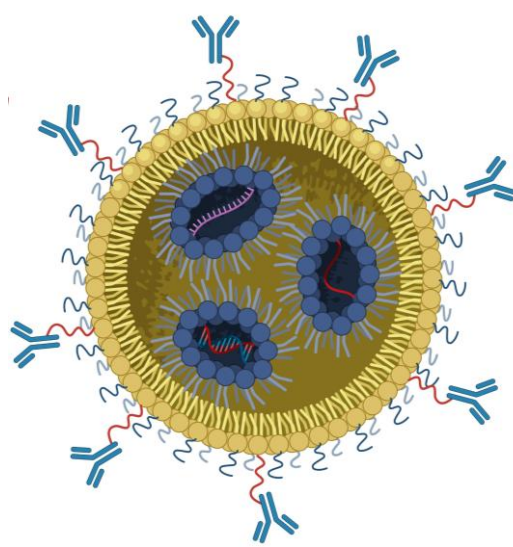
Sanjib Saha, Bei Cheng, Sabrina Khan,
Liping Zhou, Annette Bak

Advanced Drug Delivery, AstraZeneca

07/18/2025

Targeted Lipid Nanoparticles (tLNPs) for Extrahepatic mRNA Delivery

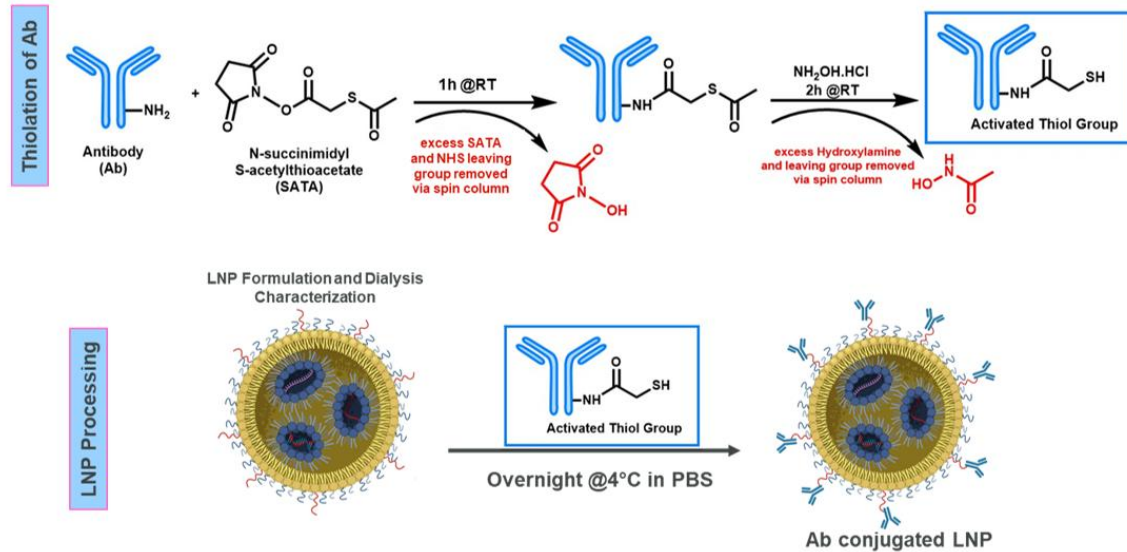




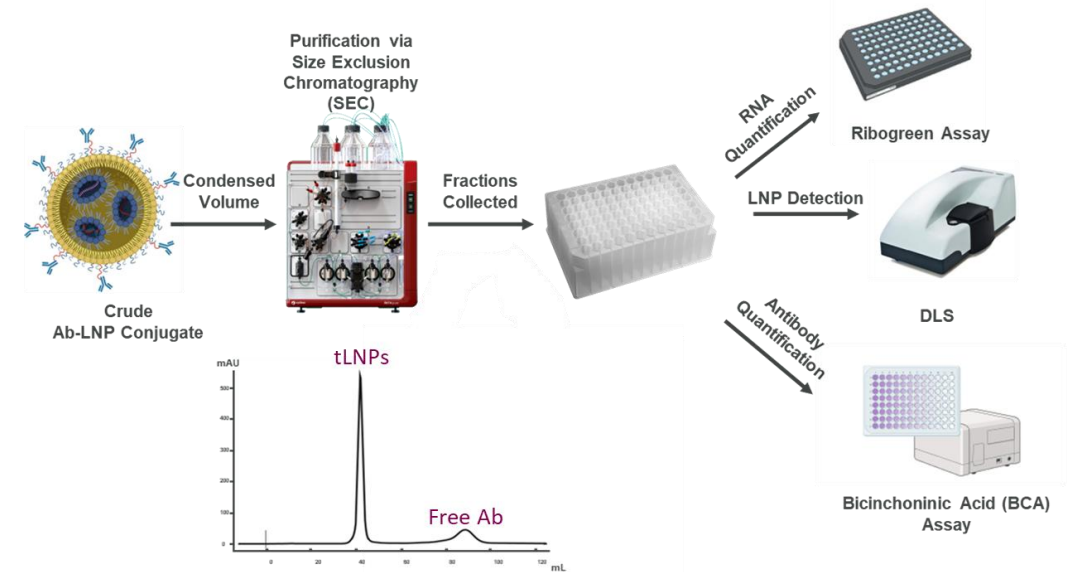
Platform case study with tool LNP and antibody Part I

Anti-CD117 tLNP Synthesis and Methods to Purify & Characterize

Antibody Conjugation



tLNP Purification & Characterization



- The antibody was thiolated and subsequently conjugated to LNP via a thiol-maleimide reaction, resulting in the generation of tLNP.
- Good separation between tLNP and antibody using size-exclusion chromatography (SEC).

Uniformly distributed small particles

LNPs	Size (nm)	PDI	%EE
Unconjugated LNPs	77	0.09	99
Anti-CD117 tLNPs	99	0.10	98

Breda et al. 2023; Science 381:436; Shi D. Nano Lett. 2023, 23, 7, 2938-2944.

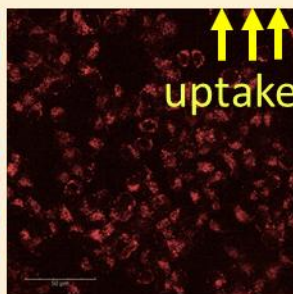
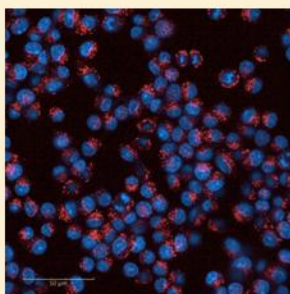


Anti-CD117 tLNPs Specifically Enhanced Uptake & Transfection in CD117⁺ Cells

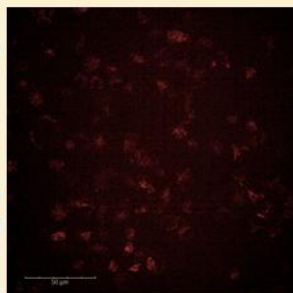
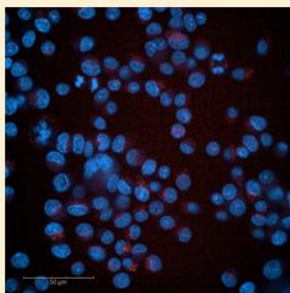
Anti-CD117 LNP increased cell uptake of Cy5 labelled mRNA in CD117⁺ cells

Nucleus/Cy5-mRNA

Cy5-mRNA



Anti-CD117
tLNP

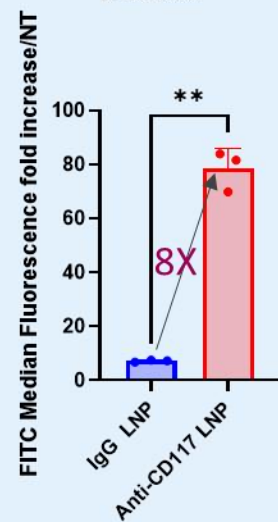
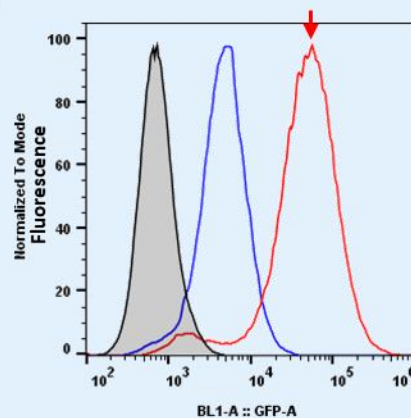


LNP

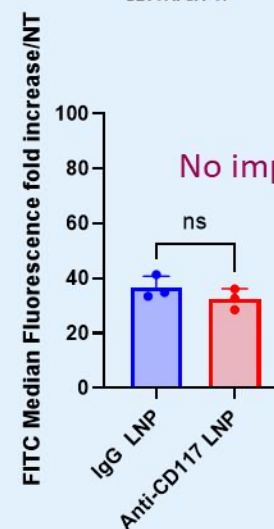
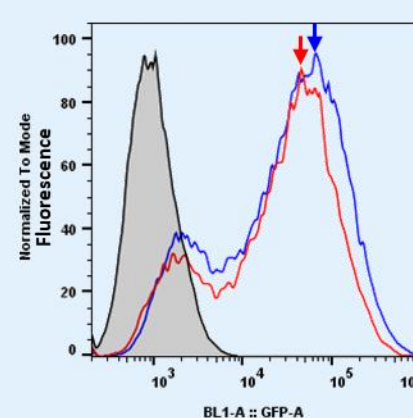
Cell uptake study with Cy5 labelled mRNA in CD117⁺ cells

8X improvement of transfection by Anti-CD117 tLNP in CD117⁺ cells

P815 cells (CD117⁺)



HepG2 cells (CD117⁻)

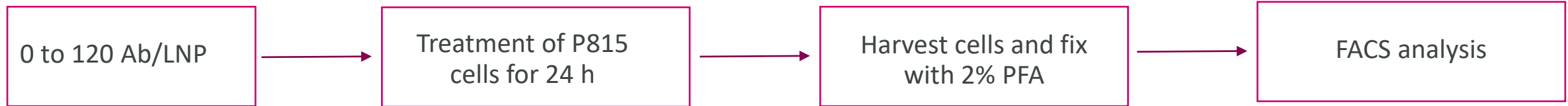


No improvement in CD117⁻ Cells

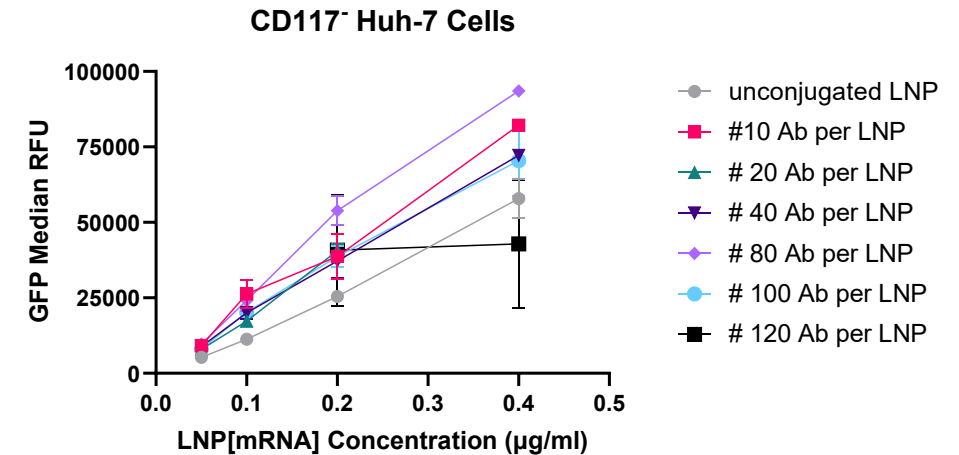
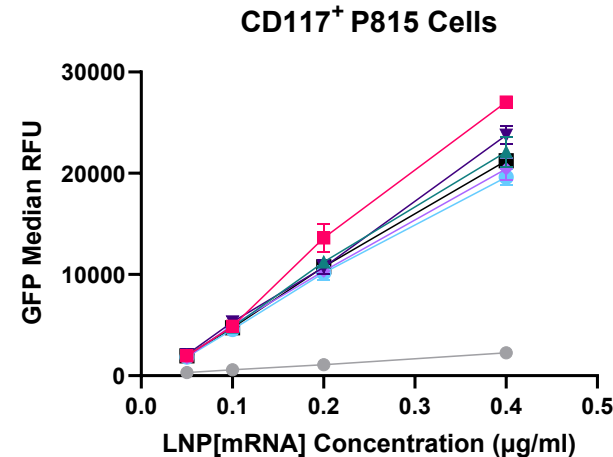
Cell transfection study with eGFP-mRNA



10 Antibody per LNP is Sufficient for Optimum *in vitro* Transfection



Antibody/LNP	Antibody: DSPE-PEG-Mal molar ratio
0	NA
10	0.12
20	0.24
40	0.48
80	0.95
100	1.19
120	1.52

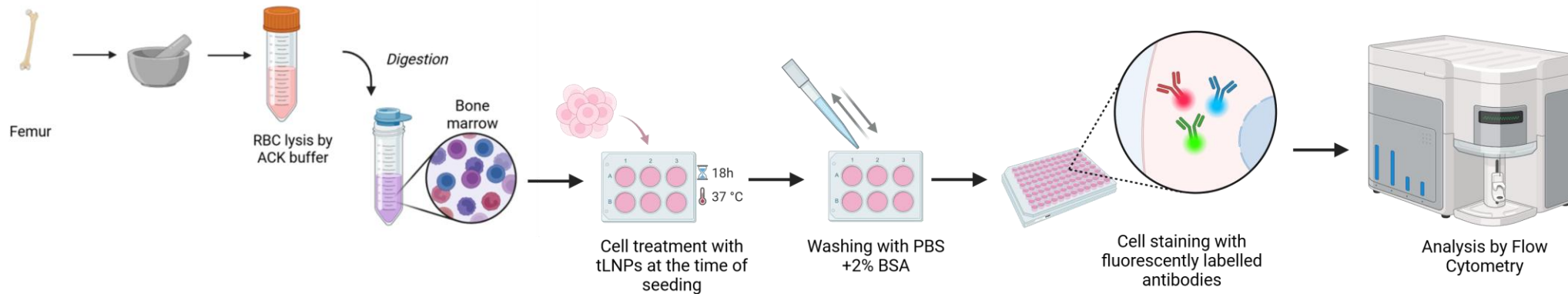


In this study, there was no significant improvement of transfection when antibody density increased from 10 to 120 per LNP.

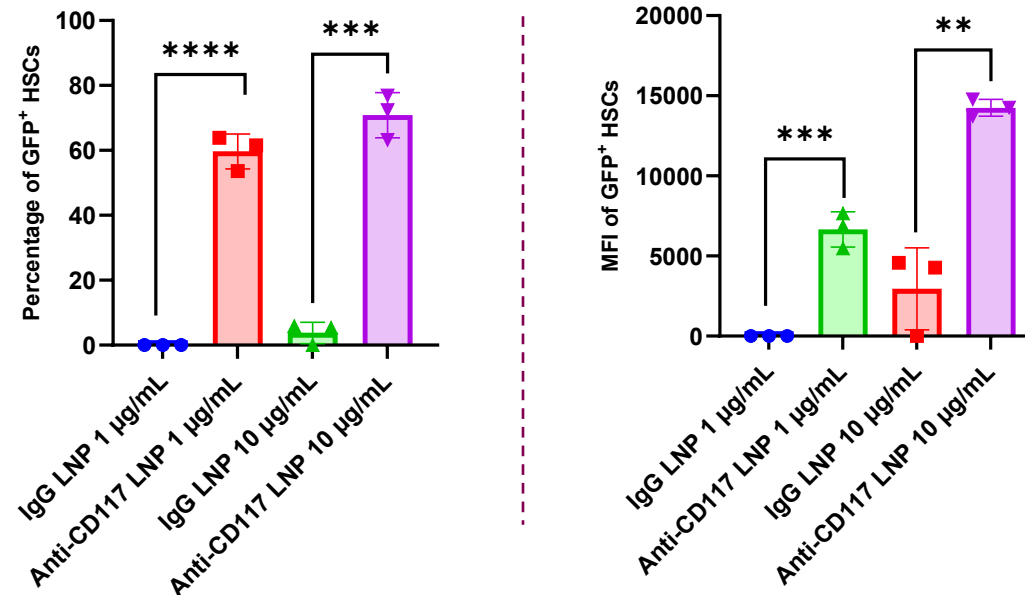


Anti-CD117 tLNPs Enhanced eGFP mRNA Expression in CD117⁺ HSCs in *Ex Vivo* Study

Workflow to *ex vivo* study with BM primary cells and analysis by flow cytometry

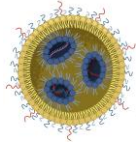


Anti-CD117 tLNPs enhanced eGFP mRNA expression in CD117⁺ HSCs

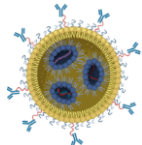


tLNPs are Stable at 4°C and -80°C Over 4 Weeks

Two formulations



Without Ab-LNP



Anti-CD117-LNP
(tLNP, 20 Ab/LNP)

Two storage conditions

- LNP in PBS at 4°C
- LNP in 20 mM Tris/8% sucrose at -80°C

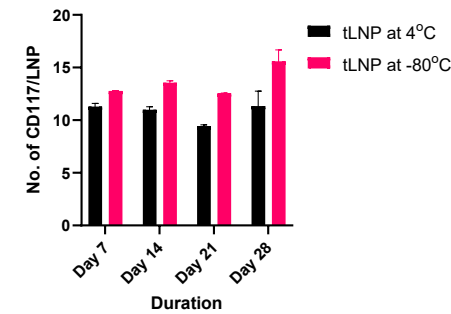
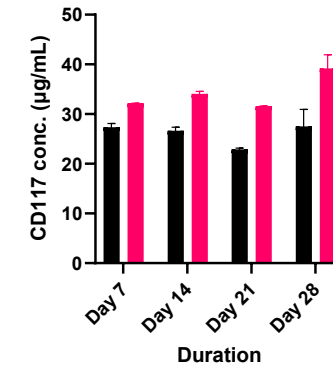
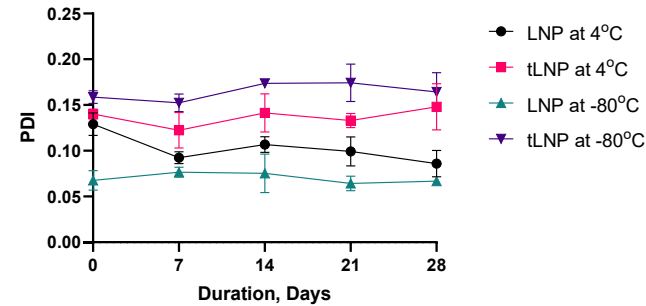
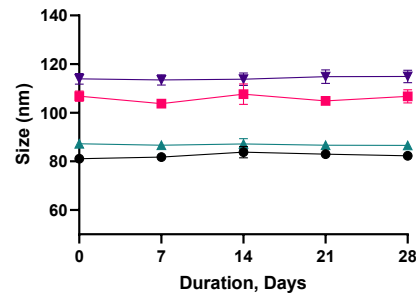
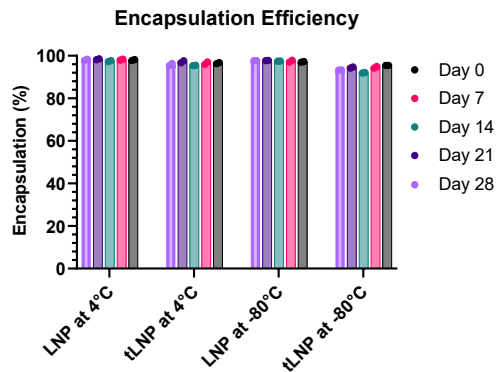
Readouts

Day 0

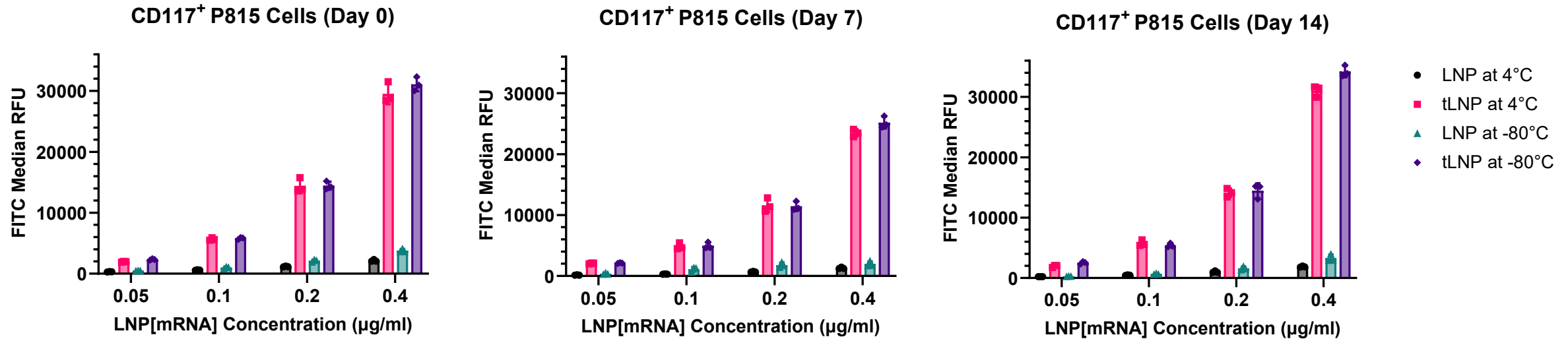
- Size and PDI by DLS
- % EE by Ribogreen
- *In vitro* transfection in P815 by FACS

Day 7, 14, 21 and 28

- Size and PDI by DLS
- % EE by Ribogreen
- Antibody quantification by BCA
- Antibody density/LNP
- *In vitro* transfection in P815 by FACS



tLNPs Retained Transfection Capability Throughout the Observation Period (2 Weeks)

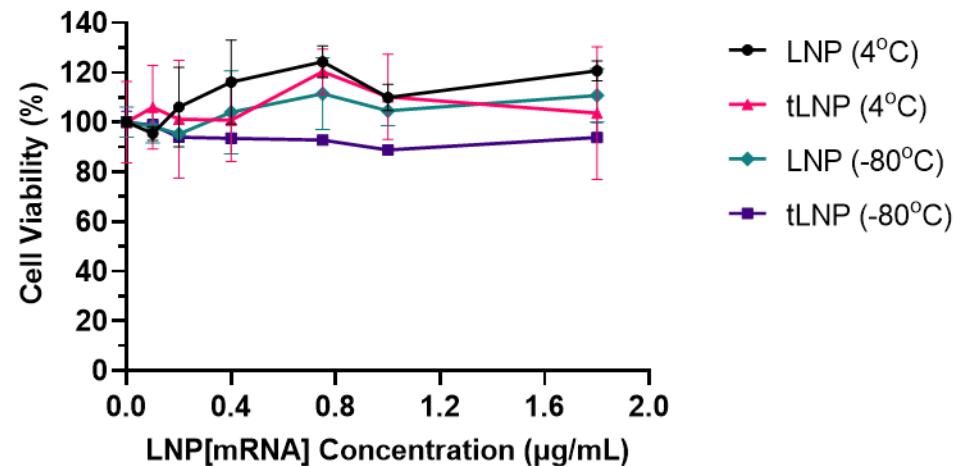
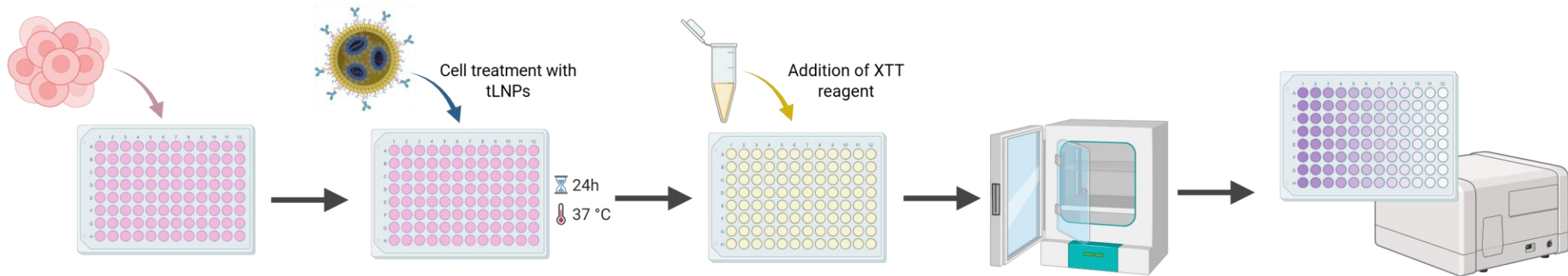


- There was no significant difference in transfection between tLNPs stored at 4 and -80°C over 2 weeks.
- Dose dependent transfection increase was observed for both tLNPs.



Anti-CD117 tLNPs were Not Toxic to P815 Cells

Workflow to measure cell viability using XTT assay



- The cytotoxicity of tLNP was evaluated in P815 cells for 24 h by XTT assay.
- The tLNP was not toxic when the dose was increased from 0.001 to 1.8 µg/mL.



Conclusion

- We have successfully synthesized anti-CD117 Ab conjugated tLNPs with desirable physiochemical properties and stability at 4 and -80 °C.
- tLNPs showed significant enhancement of uptake and transfection in CD117⁺ cells.
- 10 Ab/LNP was sufficient to have optimum *in vitro* transfection.
- tLNPs significantly enhanced mRNA expression in CD117⁺ HSCs in BM primary cells.
- tLNPs were not toxic to cells.

Acknowledgement

Laura Prickett, Flow Cytometry

