Effect of the LNP Preparation Buffer on Their Adsorption and Fusion Behavior at a Late Endosomal Membrane (LEM) Mimic

Michela Tozzi[1], Eleftheria Kosta[2], Nastaran Fallah Randjbar[2], Nima Sasanian[2], Andrea Salis[1], Fredrik Höök[2], Tommy Nylander[3], Margaret Holmes[2], Cristina Carucci[1], Flaminia Cesare Marincola[1]

[1]*Department of Chemical and Geological Sciences and CSGI, University of Cagliari, Cittadella Universitaria, S.S. 554 bivio Sestu, Monserrato, 09042, CA, Italy*. michelatozzi6@gmail.com.
[2]Department of Physics, Division of Nano and Biophysics, Chalmers University of Technology, Gothenburg 41296, Sweden
[3]Department of Physical Chemistry, Lund University, SE-22100 Lund, Sweden

In recent years, lipid nanoparticles (LNPs) have attracted considerable attention because of their ability to efficiently deliver sensitive biomolecules such as RNA. In particular, LNPs have been used as drug delivery systems (DDS) in the COVID-19 vaccine to deliver mRNA.[1] Previous studies have shown how both the pH and the buffer used for their preparation affect the phase of the bulk phase of the LNPs and the transfection efficiency of the LNPs in the cells.[2] However, the effect of the preparation buffer on the interaction between LNPs and endosomal membranes, a crucial step for endosomal escape and cytosolic delivery, remains poorly understood. To better understand the endosomal escape, and whether this process is also influenced by the buffer, LNPs have been prepared using 3 different buffers at pH 4.5 and their interactions with a model of the late endosomal membrane (LEM) have been studied through different surface techniques. Phosphate and citrate buffers were prepared at an ionic strength of 0.1 M to allow direct comparison, while McIlvaine, a phosphate-citrate buffer prepared by mixing a 0.1 M of citric acid solution with a 0.2 M of disodium phosphate solution presented a higher ionic strength (0.2 M) as the concentration was not changed to avoid any modification of the original composition. LNPs have been prepared using a microfluidic system and two different lipid formulations: an Onpattro-like composition, corresponding to the first FDA- and EMA-approved LNP system for siRNA delivery, and a DSPC-rich formulation, known in literature to have an extended circulation lifetime and an increased transfection. [3] The lipids used for the nanoparticles are D-Lin-MC3-DMA, Cholesterol, DSPC and DMPE-PEG2000 in a molar ratio of 50:38.5:10:1.5 for the Onpattro composition and 50:10:38.5:1.5 for the High-DSPC composition. The LEM mimic has been prepared using POPC,DOPE,Cholesterol and $BMP_{18:1}$ in a 61:16:17:6 molar ratio.[4] All interaction studies were carried out in McIlvaine buffer at pH 5.5 to reproduce the acidic environment of late endosomes.[5] Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) experiments were performed by forming a supported LEM bilayer on the sensor surface, followed by LNP injection. LNPs prepared in phosphate buffer gave the highest adsorption (467 ± 67 ng/cm^2 and 437 ± 70 ng/cm^2 for High-DSPC and Onpattro formulation respectively), followed by citrate-LNPs (343 ± 35 ng/cm^2 for High-DSPC formulation and 290 ± 41 ng/cm^2 for Onpattro formulation) and McIlvaine-LNPs (243 ± 39 ng/cm^2 and 193 ± 42 ng/cm^2 for High-DSPC and Onpattro formulation respectively). Using QCM-D, the adsorption isotherm of LNPs prepared in citrate buffer pH 3 has been studied as a function of the LNPs concentration. The data has been fitted using a Freundlich isotherm, giving a K_F of 855 ± 85 mL/mg and an n value of 1.68 ± 0.09. Complementary Langmuir Blodgett (LB) experiments were carried out by depositing a LEM monolayer on the surface of the McIlvaine buffer pH 5.5 placed in the LB trough and the LNPs have been injected underneath the monolayer to study their influence on the surface pressure (SP). Phosphate-LNPs gave the highest rise in SP (43 mN/m and 47 mN/m for High-DSPC and Onpattro LNPs respectively), followed by McIlvaine-LNPs (33 mN/m and 37 mN/m for High-DSPC and Onpattro LNPs respectively) and Citrate-LNPs (29 mN/m and 32 mN/m for High-DSPC and Onpattro LNPs respectively). These results indicate that phosphate-LNPs exhibit the strongest fusion capability toward the endosomal membrane model, whereas citrate-prepared LNPs display the weakest interaction. The different trends in the strength of interaction between LEM-LNPs seen using QCM-D and LB might be attributed to the fact that the former accounts for all the interactions between the LNPs and the LEM (that is, binding and fusion), while the latter only shows fusion events. Neutron Reflectometry (NR) and Total Internal Reflection Fluorescence (TIRF) microscopy

measurements planned in the near future will help to further understand how the differently prepared LNPs interact with a LEM mimic.

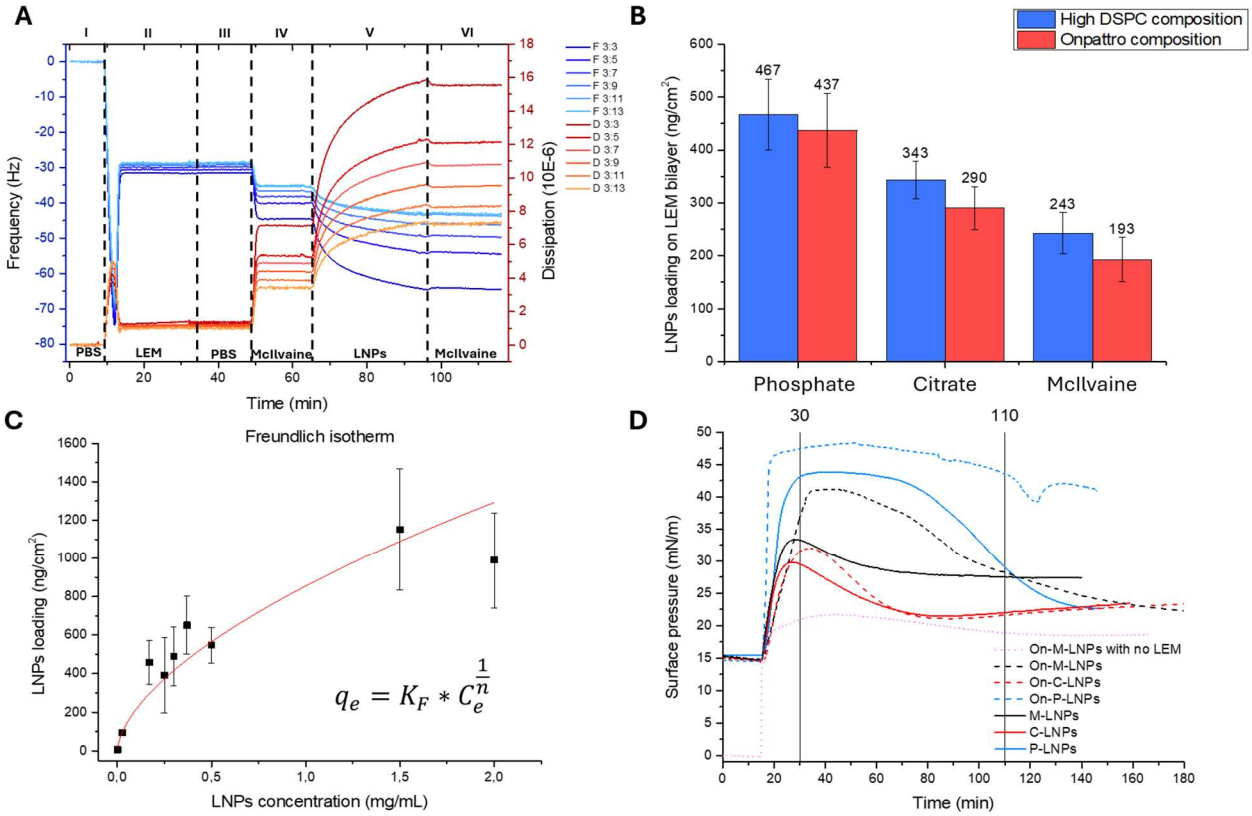

Figure 1 – LNP interaction with LEM. Example of QCM-D measurement (A). Areal mass of LNPs (High-DSPC and Onpattro) prepared in different buffers and adsorbed on a LEM bilayer in QCM-D analysis (B). Adsorption isotherm of citrate LNPs on a LEM bilayer in QCM-D analysis with increasing concentration of LNPs dispersions (C). Interaction of LNPs prepared with different buffers on a LEM monolayer on LB measurements (D).

REFERENCES

[1] Zhang H., Han S., Zhang X., Ma J., Jin L., Wang Y., Ma Y., Ma T., Yu F., Song G. (2026) "Novel Lipid Nanoparticle (LNP) Delivery Systems Enabling the Advancement of RNA Therapeutics". *Advanced Healthcare Materials*, 0:e05340. DOI 10.1002/adhm.202505340

[2] Carucci C., Philipp J., Müller J.A., Sudarsan A., Kostyurina E., Blanchet C. E., Schwierz N., Parson D.F., Salis A., Rädler J.O. (2025) "Buffer Specificity of Ionizable Lipid Nanoparticle Transfection Efficiency and Bulk Phase Transition". *ACS Nano 19* (11), 10829-10840. DOI 10.1021/acsnano.4c14098

[3] Chander N., Basha G., Cheng M.H.Y., Witzigmann D., Cullis P.R. (2023) "Lipid nanoparticle mRNA systems containing high levels of sphingomyelin engender higher protein expression in hepatic and extra-hepatic tissues" Mol Ther Methods Clin Dev. 2023 Jun 12;30:235–245. DOI 10.1016/j.omtm.2023.06.005

[4] Spadea A., Jackman M., Cui L., Pereira S., Lawrence M.J., Campbell R.A., Ashford M. (2022) "Nucleic Acid-Loaded Lipid Nanoparticle Interactions with Model Endosomal Membranes" ACS Appl. Mater. Interfaces 2022, 14, 30371−30384. DOI 10.1021/acsami.2c06065

[5] Aliakbarinodehi N., Niederkofler S., EmilssonG., Parkkila P., Olsén E., Jing Y., Sjöberg M., Agnarsson B., Lindfors L. (2024) "Time-Resolved Inspection of Ionizable Lipid-Facilitated Lipid Nanoparticle Disintegration and Cargo Release at an Early Endosomal Membrane Mimic". *ACS Nano* 2024, 18, 34, 22989–23000. DOI 10.1021/acsnano.4c04519