Effects of buffers on lipid nanoparticles (LNP) core structure on mRNA transfection efficiency

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Lipid nanoparticles (LNPs) are liposome-like structures with a complex lipid-based core known for their use in drug delivery [1]. Specifically, they have been used to encapsulate nucleic acids, such as messenger ribonucleic acid (mRNA), to develop mRNA-based vaccines against COVID-19 [2]. LNPs core is constituted by ionizable lipids and cholesterol that self-assemble to create inverse micellar phases where mRNA is encapsulated. It has been proposed that the mechanism of mRNA release is due to a change of pH in the endosomes that results in phase transition of the LNPs core from inverse cubic (Fd3m) to inverse hexagonal (H_{II}) structure due to the protonation of MC3 cationic lipid which results in a change of interface curvature (Figure 1). The effect of weak electrolytes, and their conjugate species used as pH buffers, on the phase transition of mRNA-based lipid structures is usually neglected. Different pH buffers can be used in LNPs synthesis during the last dialysis step, and buffer choice is based on pK_a value only without considering the chemical nature of the involved ions. However, buffers influence biological systems in a specific way that strongly depends on the intrinsic identity of the buffer [3]. The aim of the work is to go through the formation of the cholesterol-cationic lipid mesophase and to evaluate the buffer effects by mean of synchrotron SAXS measurements to understand the action mechanism of mRNA-LNPs vaccines. Buffer type (citrate, acetate, phosphate) and concentration (50 mM) affect the liquid crystal phase transition. The transition pH from Fd3m to H_{II} structure is found to be buffer specific, occurring at a pH decreasing long the series citrate > acetate > phosphate. The buffer species interact with the cationic ionizable lipid DLin-MC3-DMA used for mRNA transport. Future work will employ theoretical calculations to determine the stability of and therefore the transitions pH between different phases.

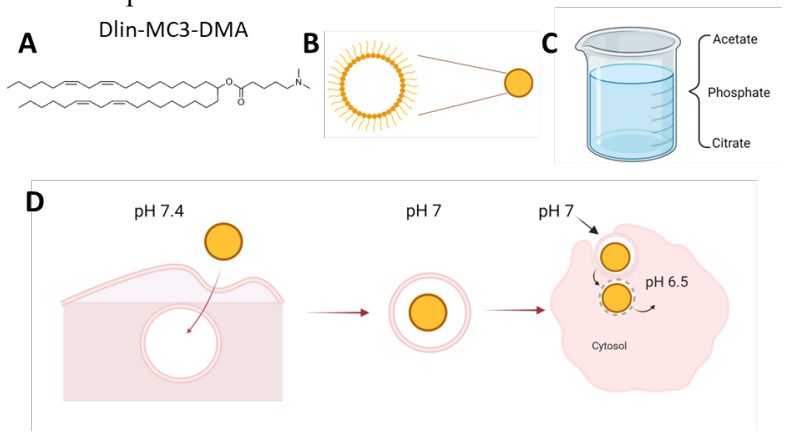

Figure 1 A) Cationic ionizable lipid DLin-MC3-DMA B) Inverse micelle as a possible structure composing bulk phase of LNPs C) Chosen buffers used during bulk phase formation for the present study D) pH dependent LNPs internalization and endosomal release

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