Inverse cubic and hexagonal mesophase evolution within ionizable lipid nanoparticles correlates with mRNA transfection in macrophages

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mRNA lipid nanoparticle (LNP) technology presents enormous opportunities to prevent and treat various diseases. In a recent study using time-resolved small angle X-ray scattering, we demonstrated that the ionizable lipids ALC-0315 and SM-102 used in mainstream COVID-19 mRNA vaccines manifest pH-responsive formation of inverse lyotropic liquid crystalline mesophases including the cubic and the hexagonal mesophases.[1] In this study, we aimed to correlate their pH-responsive structures with mRNA delivery and transfection abilities in macrophage cells.[2]

Partial phase diagrams of LNPs as a function of ionizable lipid ratio and pH variation examined by small angle X-ray scattering were presented, showing pH-sensitive structural transitions. In terms of biological activities, the pH-dependent mesophase behavior of LNPs during the endosomal acidification process dominates the cell association, where a broader span of structural transition and earlier starting point (higher pH) in the SM-102 ionizable lipid LNPs are more favorable for macrophage MH-S cells compared to the ALC-0315-based LNPs. As a proof-of-concept application, the optimal mRNA@LNPs with desirable pH-dependent phase behavior was demonstrated as a nanocarrier for EGFP mRNA delivery into MH-S cells with a transfection efficiency over 70%. Interestingly, mRNA@LNPs comprising SM-102 lipid showed superior mRNA transfection than the ALC-0315 analogue, which was correlated to the lack of the ability to transform into the cubic phase at an acidic condition (e.g., pH 4).

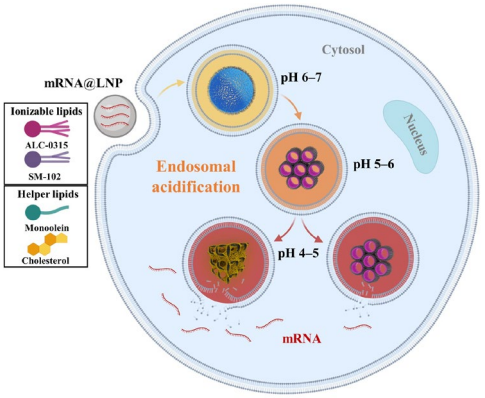

Figure 1: Sketch of the lyotropic liquid crystalline mesophase transition of ionizable LNPs (ionizable lipids/monoolein/cholesterol) during endosomal acidification and the correlation with mRNA cargo release.

References

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