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Mechanistic insights into interactions between ionizable lipid-containing lipid nanoparticles and the endosomal membrane

Yeon Ju Go, Erkhembayar Jadamba,* and Hyunjin Shin*

MOGAM Institute for Biomedical Research (MIBR)

**Corresponding author: hyunjin.shin@mogam.re.kr*

Ionizable lipid-containing lipid nanoparticles (LNPs) have emerged as effective RNA carriers in RNA-based therapeutics. In particular, ionizable lipids (ILs), with their pH-sensitive properties, play a critical role in endosomal escape and lipid-mediated RNA delivery. Thus, the development of ILs that enhance endosomal escape—a major bottleneck in successful cytosolic RNA delivery—is of great importance. However, the molecular-level mechanisms and dynamics of ILs during the endosomal escape process remain poorly understood. To address this, we employed coarse-grained (CG) molecular dynamics (MD) simulations. We designed ALC-0315–containing LNPs and D-Lin-MC3-DMA (MC3)–containing LNPs, both clinically validated LNP systems, under pH conditions representative of the early and late endosomes. Correspondingly, lipid bilayers were constructed to reflect the compositions of early and late endosomal membranes, enabling investigation of the merging process between LNPs and endosomal membranes. Across both membrane compositions and LNP types, we identified common, characteristic dynamics of ILs that underlie endosomal escape at the molecular scale. These mechanistic insights into IL-mediated endosomal escape provide valuable guidance for the rational design of more effective ILs, thereby advancing the development of next-generation RNA-based therapeutics.