Inverse Cubic and Hexagonal Mesophase Evolution within Ionizable Lipid Nanoparticles Correlates with mRNA Transfection in Macrophages

Haitao Yu, Joshua Iscaro, Brendan Dyett, Yiran Zhang, Susanne Seibt, Natalia Martinez, Jacinta White, Calum J. Drummond, Steven Bozinovski*, Jiali Zhai**
School of Science, STEM College
RMIT University,
Melbourne, Victoria 3000, Australia

Haitao Yu: haitao.yu@rmit.edu.au
Jiali Zhai: maggie.zhai@rmit.edu.au
Steven Bozinovski: steven.bozinovski@rmit.edu.au
Calum J. Drummond: calum.drummond@rmit.edu.au

mRNA lipid nanoparticle (LNP) technology presents enormous opportunities to prevent and treat various diseases. Here, we developed a novel series of LNPs containing ionizable amino-lipids showing a remarkable array of tunable and pH-sensitive lyotropic liquid crystalline mesophases including the inverse bicontinuous cubic and hexagonal phases characterized by high-throughput synchrotron radiation X-ray scattering. Furthermore, with an interest to develop mRNA therapeutics for lung macrophage targeting, we discovered that there is a strong correlation between the mesophase transition of the LNPs during acidification and the macrophage association/transfection efficiency of mRNAs. The slight molecular structural differences between the SM-102 and ALC-0315 ionizable lipids are linked to the LNP's ability to transform their internal structures from an amorphous state to the inverse micellar, hexagonal, and finally cubic structures during the endosomal maturation. SM-102 LNPs showed exceptionally improved transfection efficiency due to its ability to form the cubic structure at lower pH than the ALC-0315 analogues, which remained within the hexagonal structure, previously attributed to promote endosomal escape of the ionizable LNPs. Overall, the new knowledge draws our attention to the important role of mesophase transition in endosomal escape and the novel LNP libraries reported herein have broad prospects for advancing mRNA therapeutics.

References:

(1) Yu, H.; Iscaro, J.; Dyett, B.; Zhang, Y.; Seibt, S.; Martinez, N.; White, J.; Drummond, C. J.; Bozinovski, S.; Zhai, J. J. Am. Chem. Soc. **2023**, 145 (45), 24765–24774.
(2) Yu, H.; Angelova, A.; Angelov, B.; Dyett, B.; Matthews, L.; Zhang, Y.; El Mohamad, M.; Cai, X.; Valimehr, S.; Drummond, C. J.; Zhai, J. Angew. Chemie **2023**, 135 (35), e202304977.
(3) Yu, H.; Dyett, B.; Kirby, N.; Cai, X.; Mohamad, M. El; Bozinovski, S.; Drummond, C. J.; Zhai, J. Small **2024**, 2309200, 1–10.