# Developing Lipid Nanoparticles for Improved Targeted Delivery of Self-Amplifying RNA and Messenger RNA to the Heart

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## Introduction

**Ischaemic heart disease (IHD) global burden**

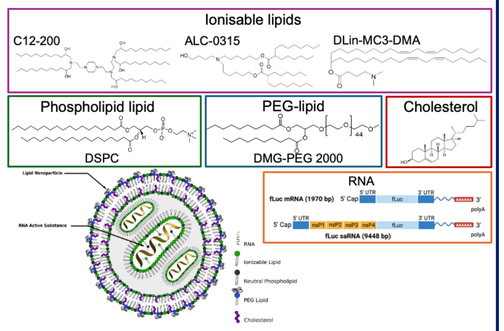
Ischaemic heart disease (IHD) affects 56.2 million individuals and is the leading global cause of mortality [1].

**Clinical challenges and opportunities**

Current therapies fail to prevent ischemia/reperfusion injury, with 15–21% 1-year mortality [2].
Messenger RNA (mRNA) is promising for treating IHD [3].
Self-amplifying RNA (saRNA) enhances protein expression compared to mRNA.
Currently, saRNA is utilised for vaccine applications [4].
Lipid nanoparticles (LNPs) are vital delivery platform for RNA agents [5].

**Cardiac delivery limitations**

1. Limited targeted delivery and accumulation.
2. Unlocking RNA therapeutic potential.



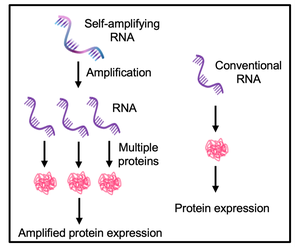

## Objectives and Overview of Experimental Design

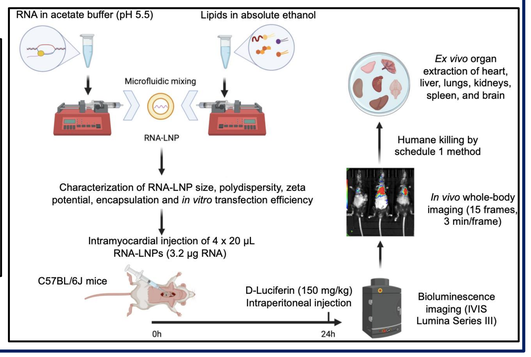
(I) Investigate the difference between LNPs delivering saRNA versus mRNA to the heart for **greater therapeutic potential and cardiac tropism.**

(II) Understand the physicochemical and biological properties of LNPs to optimise RNA-LNPs for **improved cardiac delivery and accumulation *in vivo*.**

(III) Study the limitations of cardiac-representative models to **increase the predictive performance of RNA-LNPs *in vivo*.**

(IV) Evaluate diverse ionizable lipids for constructing LNPs **to enhance transfection efficiency.**



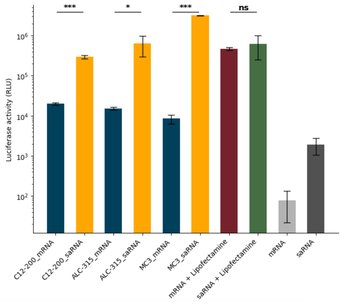

## Results and Discussion

### Physicochemical properties of RNA-LNPs

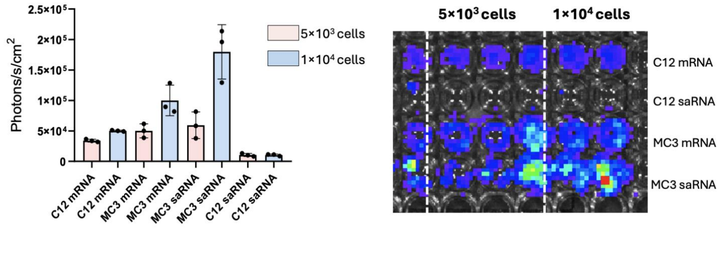
| Formulation | LNP components | | | | | Physicochemical RNA-LNP characteristics | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ionisable | DSPC | Cholesterol | PEG | RNA cargo | Size (nm) | Polydispersity index | Zeta potential | Encapsulation efficiency (EE) |
| **C12_mRNA** | 35 | 16 | 46.5 | 2.5 | mRNA | 161 ±10 | 0.25 ±0.006 | 0.98 ±0.5 | 90% |
| **C12_saRNA** | 35 | 16 | 46.5 | 2.5 | saRNA | 164 ±8 | 0.34 ±0.04 | 0.52 ±0.5 | 83% |
| **ALC_mRNA** | 50 | 10 | 38.5 | 1.5 | mRNA | 166 ±19 | 0.29 ±0.53 | -1.1 ±1 | 45% |
| **ALC_saRNA** | 50 | 10 | 38.5 | 1.5 | saRNA | 127 ±7 | 0.081 ±0.015 | -1.7 ±1 | 62% |
| **MC3_mRNA** | 50 | 10 | 38.5 | 1.5 | mRNA | 200 ±11 | 0.12 ±0.038 | -1.32 ±0.9 | 76% |
| **MC3_saRNA** | 50 | 10 | 38.5 | 1.5 | saRNA | 97 ±3 | 0.13 ±0.024 | -1.8 ±1 | 86% |

### *In vitro* transfection efficiency of RNA-LNPs

saRNA-LNPs showed higher transfection efficiency *in vitro* compared to mRNA-LNPs in HEK293T cells.
C12-mRNA expression was evident in Human Umbilical Vein Endothelial Cells (HUVECs), albeit roughly half the MC3-mRNA level.
MC3-saRNA outperformed MC3-mRNA by ~2-fold in HUVECs compared to ~400-fold increase in HEK293T cells.
The differential expression pattern across cell models suggests that LNP function is likely cell-type specific.




*In vitro* transfection efficiency of RNA-LNPs at 100 ng in HEK293T cells measured at 24 hrs post-transfection, values normalized to control. Statistical significance determined using a Z-test, with * p <0.05, ** p < 0.01, *** p < 0.001, and n.s. indicating no significant difference.



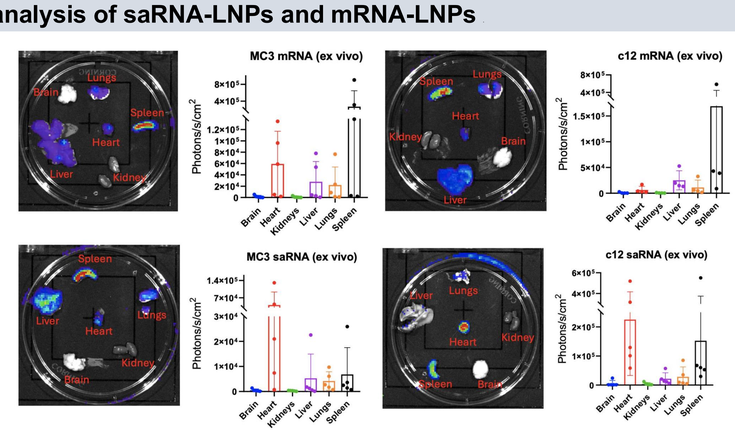




*In vitro* transfection efficiency of mRNA-LNP and saRNA-LNP at a 100 ng dose in different concentration of HUVECs, measured at 24 hours post-transfection.

### *In vivo* and *ex vivo* analysis of saRNA-LNPs and mRNA-LNPs

MC3 and C12-200-LNPs exhibited the highest *in vitro* transfection efficiency; thus, selected for *in vivo* studies.
The highest firefly luciferase (FLuc) expression in the heart was observed for C12-saRNA and lowest for the C12-mRNA group.
Cardiac-specific tropism was pronounced for MC3-saRNA, despite lower absolute bioluminescence compared to the C12-saRNA group.
Both mRNA-LNP groups showed spleen-specific expression.
MC3-mRNA and MC3-saRNA groups exhibited comparable cardiac expression levels, suggesting that substituting mRNA with saRNA reduced splenic tropism.
The observed shift in LNP tropism remains to be elucidated and requires further mechanistic studies.




*Ex vivo* bioluminescence imaging of FLuc mRNA-LNPs and saRNA-LNPs. C57BL/6 mice were sacrificed after *in vivo* imaging, and the brain, heart, kidney, liver, lung, and spleen were extracted for organ-specific imaging.

## Conclusions and Future Directions

This study demonstrates the potential of LNPs for improved delivery of saRNA and mRNA to the heart.
Future work includes expanding the ionisable lipid library for LNP engineering and evaluating LNP thermostability.
Additional experimental models are required to validate the RNA-LNP technology, including LNP evaluation in endothelial cells, cardiomyocytes, cardiac fibroblasts, macrophages, and human myocardial slices.
Additionally, *in vivo* LNP distribution could be assessed using a mouse model with myocardial infarction.
It is envisioned that the amendable LNPs can be decorated with targeting ligands to increase cardiac specific delivery.
Currently, an external BHF PhD studentship application for Ting Sun (MRes student) is in submission and a major UKRI bid is in preparation.

## Acknowledgements

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## References

[1] Khan, M.S. *et al. Nat. Rev. Cardiol.* **2024**, *21*, 717-734; [2] Heusch, G. *Nat. Rev. Cardiol.* **2020**, *17*, 773-789; [3] Evers, M.J.W. *et al. J. Control. Release* **2022**, *343*, 207-216; [4] McKay, P.F. *et al. Nat. Commun.* **2020,** *11*, 3523; [5] Hou, X. *et al. Nat. Rev. Mater.* **2021**, *6*, 1078-1094.