Supplementary Information

Zeolitic Imidazolate Frameworks Enhanced Transfection Efficiency of mRNA Loaded Lipid Nanoparticles.

Ruhani Singh,[a] Kerri Bruce,[b] Shen Y Heazlewood,[a,d], Jacinta F White,[a], Benjamin W. Muir,[a] Benjamin Cao,[a,d] Xavier Mulet,[c] Daniel Layton,[b] and Cara M. Doherty,[a]**

[a]CSIRO Manufacturing, Private Bag 10, Clayton South, Victoria 3169, Australia
[b]CSIRO Health & Biosecurity, Australian Centre for Disease Preparedness, Geelong, Victoria 3220, Australia
[c]School of Science, RMIT University, Melbourne, Victoria 3000, Australia
[d]Australian Regenerative Medicine Institute, Monash University, Clayton, VIC, Australia
*cara.doherty@csiro.au, daniel.layton@csiro.au

1. Ribogreen assay to determine mRNA encapsulation efficiency in the lipid nanoparticles (LNPs)

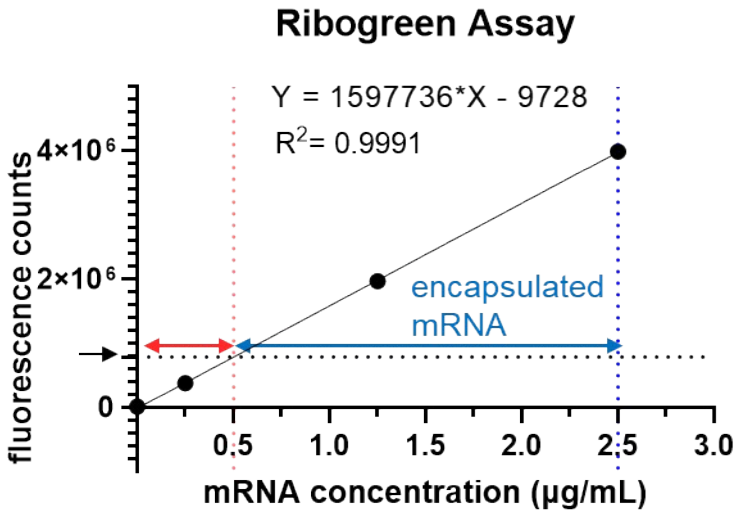


Figure S1. The mRNA-LNPs had an average mRNA encapsulation efficiency of 80% measured using a ribogreen assay. A standard curve for mRNA concentrations is used to indirectly derive the concentration of mRNA in the nanoparticles. The unencapsulated mRNA, present in the buffer is freely available to bind with the ribogreen agent giving an average fluorescence read-out of **792520** gcu (n=5); The corresponding mRNA conc. calculated from the standard curve = 0.5 µg/mL. The total mRNA concentration used, i.e. encapsulated and unencapsulated is 2.5 µg/mL. Therefore, the encapsulation efficiency = (2.5-0.5/2.5) *100 = **80%.** The mRNA-LNPs had an average mRNA encapsulation efficiency of 80% measured using a ribogreen assay.

2. Scanning electron microscopy (SEM) images for ZIF-8 and ZIF-8@LNP@mRNA

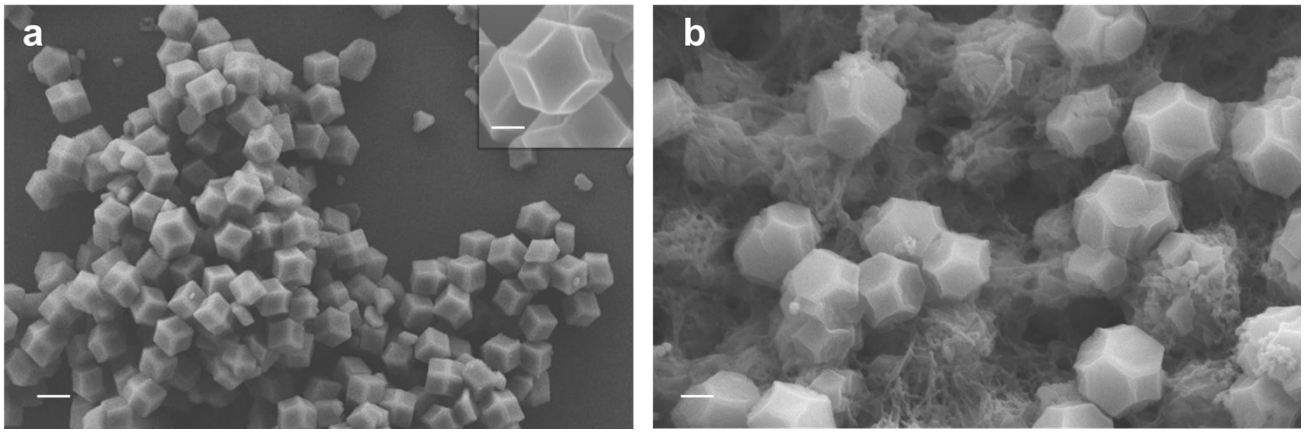
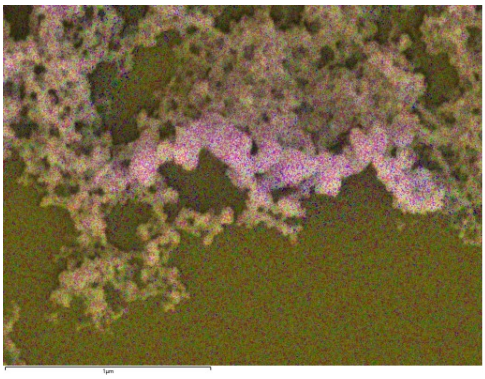


Figure S2. Shows (a) Rhombic dodecahedral ZIF-8 and (b) truncated rhombic dodecahedral ZIF-8@LNP@mRNA (ethanol washed) (scale – 200 nm, inset scale – 100 nm)

3. Elemental Analysis for A-ZIF-8@LNP@mRNA



Element	Weight %
C	39.8
Zn	28.6
O	17.9
P	6.5
Cl	3.1
Na	2.1
N	1.9

Figure S3. Energy Dispersive Spectroscopy (EDS) was performed to analyse the elemental composition (weight %) for A-ZIF-8@LNP@mRNA sample in image. The corresponding table shows presence of P and Zn, which are due to the the presence of encapsulated mRNA-LNPs and the ZIF-8 encapsulant.

4. Elemental Analysis following the ZIF-8 disintegration and Release of LNPs from A-ZIF-8@LNP@mRNA

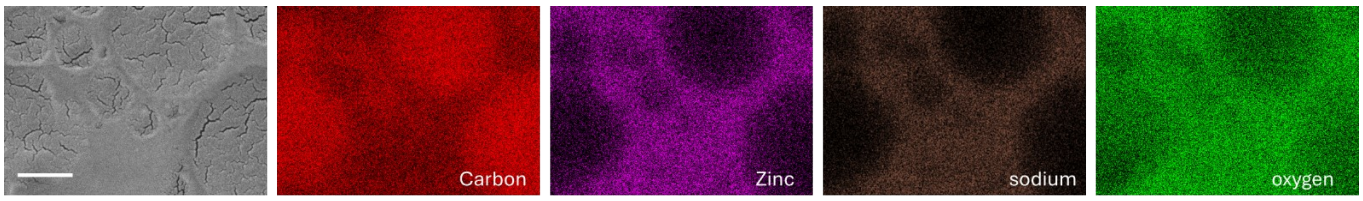


Figure S4. EDS performed on the LNP@mRNA released from the A-ZIF-8@LNP@mRNA shows emerging carbon rich spherical nanoparticles, limited in presence of zinc, majority of which is present around the nanoparticles along with sodium corresponding to the dissolute zinc and sodium salts (scale-500nm). The sample was too thin to confidently assess any trace elements like phosphorous.

5. Lactate Dehydrogenase Assay

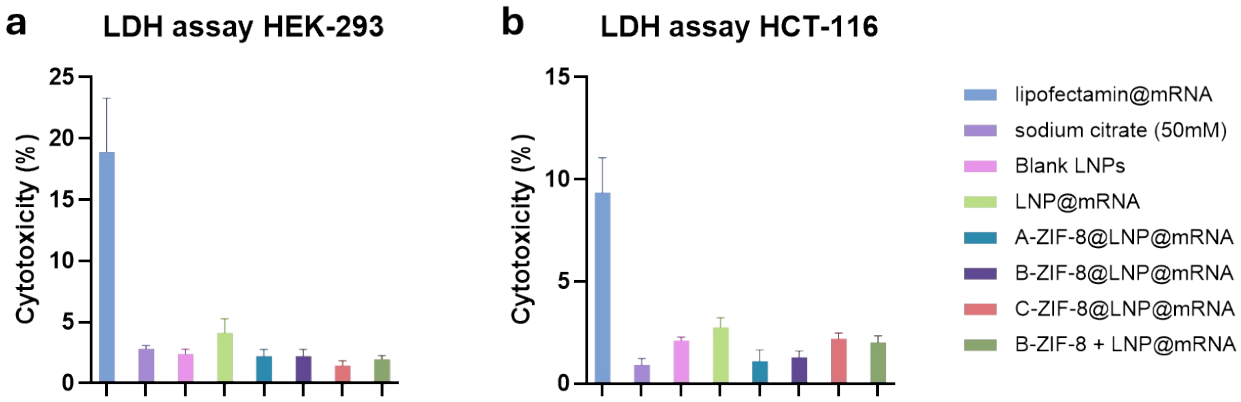


Figure S5 An LDH release assay was performed at 24 h after the incubation of the HEK-293 and HCT-116 cells with various mRNA transfection agents including a commercially available cationic lipid transfection agent, Lipofectamine™ 3000 LNPs, the LNP@mRNA and the three ZIF-8@LNP@mRNA formulations. A percentage of cytotoxicity is calculated, normalized to 100% toxicity for complete lysis of untreated cells. Figure (a) and (b) show cytotoxicity of each of the formulations in HEK-293 and HCT-116 cells, respectively.

6. Efficiency of the ZIF-8@LNP@mRNA formulations in A549 Cells

The transfection efficiency of the three formulations, A-ZIF-8@LNP@mRNA, B-ZIF-8@LNP@mRNA, and C-ZIF-8@LNP@mRNA was also investigated in the A549 cell line. However, the conditions used were different to HEK-293 and HCT-116 data presented in the manuscript. Cells were incubated with each of the test transfection formulation for a period of 5 h in OptiMEM media after which, the media was replaced with cell culture media containing 10% serum.

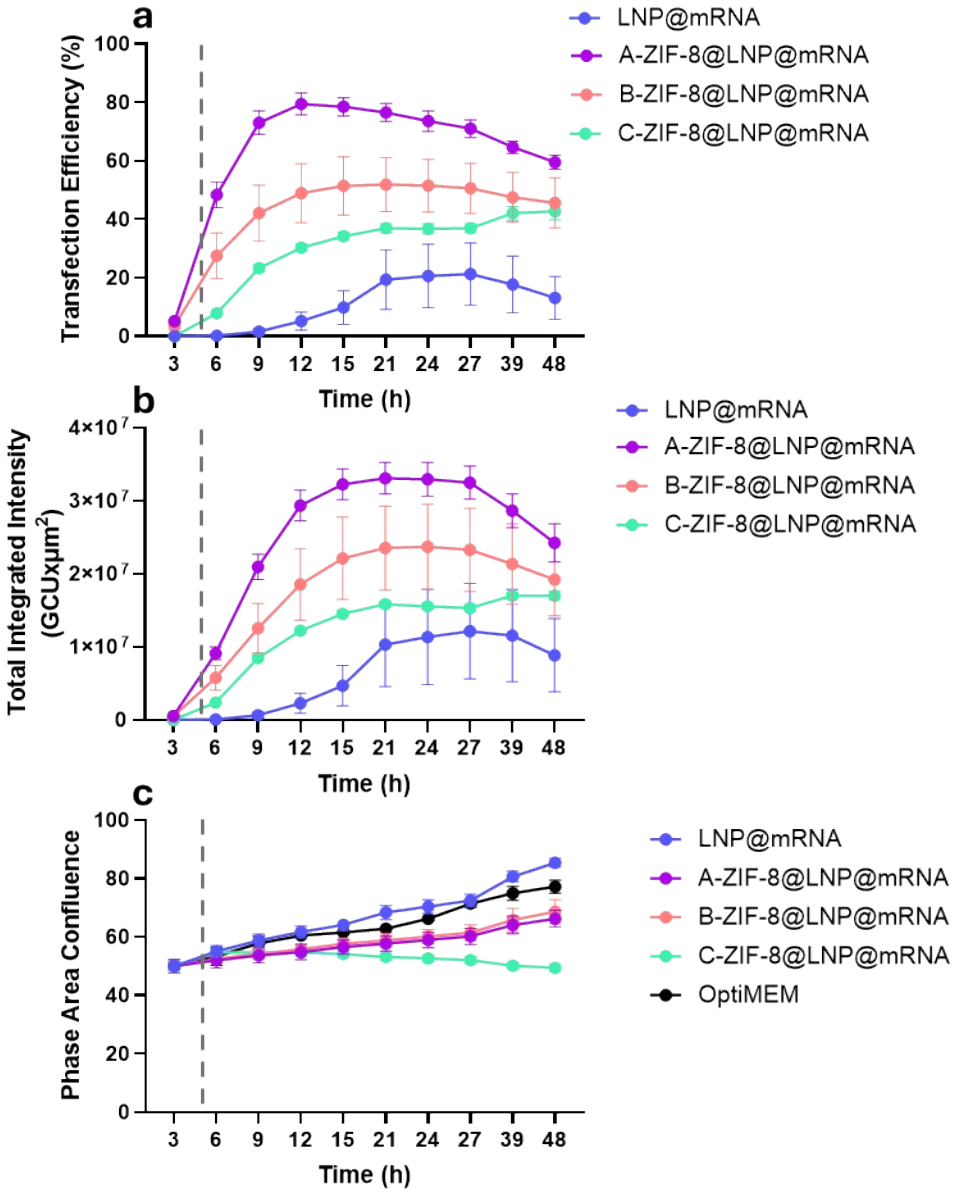


Figure S6 Shows (a) Transfection efficiency, (b) Total Integrated Intensity and the (c) phase area confluence of A549 cells when treated with the LNP@mRNA and ZIF-8@LNP@mRNA formulations. The dotted line indicates the 5 h point at which the media was replaced.

7. Phase Area Confluence (%) of the HEK-293 and HCT-116 cells.

Cell transfection can vary between cell lines. We tested both HEK-293 and HCT-116 cells for the ability of the LNPs to transfect following encapsulation. Considering the HCT-116 cells have a shorter doubling time, when $2x10^4$ cells were seeded per well, the HEK-293's were 50% confluent whilst the HCT-116 cells were nearly 80% confluent on the day of the experiment after overnight incubation. Figure S7 shows there was no difference in confluence between the untreated cells control and the LNP@mRNA sample in (a) and (b). Figure S7 (a) shows HEK-293 cells were more sensitive to serum deprivation as shown by the significant drop in confluence between the 18h-24h time points. Figure S7 (b) shows the HCT-116 cells maintained a steady confluence with no significant decrease during the serum deprivation conditions. Comparing the LNP@mRNA with the ZIF-8@LNP@mRNA formulations in the HEK-293 cells (Figure S7 (a)), the highest ZIF concentration, C-ZIF-8@LNP@mRNA, resulted in a significant drop in confluence with treatment and no apparent phase area recovery after the addition of serum containing media. Cells treated with B-ZIF-8@LNP@mRNA also demonstrated poor recovery after media replacement. Figure S7 (b) shows all three ZIF-8@LNP@mRNA formulations affected cell confluence which recovered nearly completely after media replacement by 48 h.

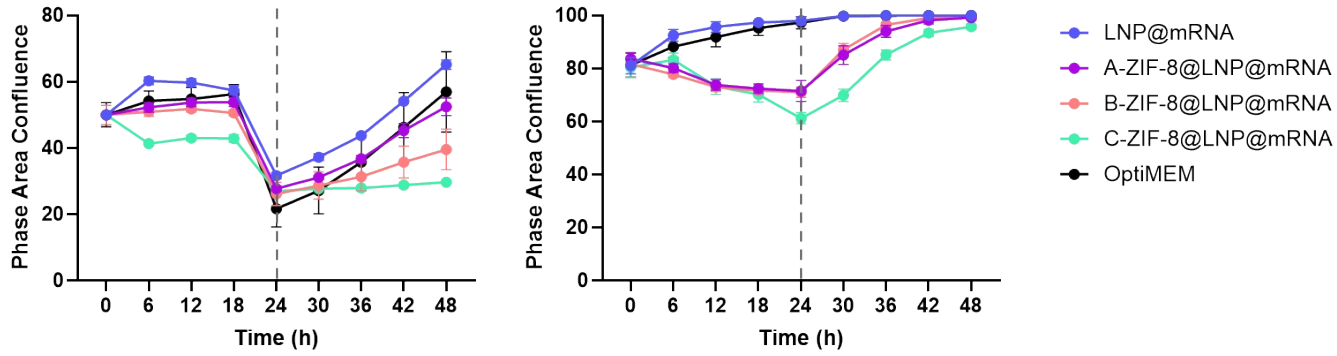


Figure S7. Phase Area Confluence (%) of the HEK-293 (a) and HCT-116 (b) cells.

8. eGFP expression in HEK-293 cells

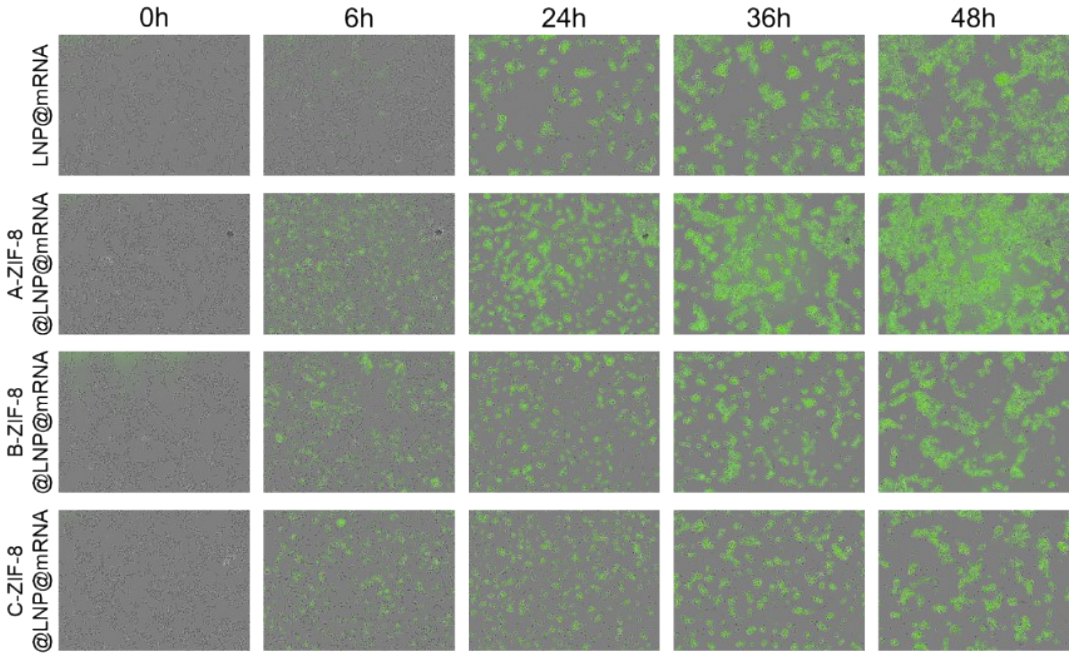


Figure S8. Chronological (time indicated on top) representational images from the HEK-293 cells transfected with each of the test LNP and ZIF-8@LNP@mRNA formulation as shown on left.