

# Journal of Materials Chemistry B

Materials for biology and medicine

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: R. Singh, K. Bruce, M. de Vries, S. Y. Heazlewood, J. White, B. W. Muir, B. Cao, X. Mulet, D. Layton and C. M. Doherty, J. *Mater. Chem. B*, 2025, DOI: 10.1039/D4TB02101K.



This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

## ARTICLE

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

Ruhani Singh<sup>a</sup>, Kerri Bruce<sup>b</sup>, Shen Y Heazlewood<sup>a, d</sup>, Jacinta F White<sup>a</sup>, Malisja de Vries<sup>a</sup>, Benjamin W. Muir<sup>a</sup>, Benjamin Cao<sup>a, d</sup>, Xavier Mulet<sup>c</sup>, Daniel Layton<sup>b, \*</sup>, and Cara M. Doherty<sup>a, \*</sup>

Received 00th January 20xx,  
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

mRNA vaccines have emerged as a highly effective strategy for the prevention and treatment of various diseases. A critical factor driving the success of mRNA vaccines is the development of advanced multicomponent lipid nanoparticles (LNPs) as a delivery system. As mRNA-LNP technology becomes increasingly integral to vaccine and therapeutic development, there is significant potential to enhance LNP efficiency and build upon the first generation of clinically approved mRNA-LNP products. This can lead to the development of superior formulations that achieve higher protein expression and improved therapeutic outcomes. In this study, we present a novel approach to enhance the transfection efficiency of mRNA-LNPs using ZIF-8 metal-organic framework (MOF). We demonstrate effective encapsulation of mRNA-LNPs within ZIF-8, with preserved structural integrity during dissociation and release. Remarkably, following MOF encapsulation and release, we observed a 3-fold and 8-fold increase in transfection efficiency of the mRNA-LNPs at 48 h in HEK-293 and HCT-116 cells, respectively. Our findings suggest that the presence of ZIF-8 materials with the mRNA-LNPs significantly contributes to their improved transfection and translation efficiency.

## Introduction

Over the past two centuries, the emergence of infectious diseases has driven vaccine innovation, culminating recently in the clinical success of mRNA vaccines during the SARS-CoV-2 pandemic. These vaccines demonstrated high safety and efficacy, showcasing the potential of mRNA technology to transform disease prevention and treatment. Their rapid development, precise targeting, and simplified manufacturing have drawn widespread attention and investment [1,2].

Efficient intracellular delivery of mRNA is fundamental to the success of mRNA vaccines and is predominantly achieved via lipid nanoparticles (LNPs), which protect mRNA from degradation and facilitate their cellular uptake [3,4]. Consequently, lipid-based delivery systems—including lipoplexes, liposomes, and LNPs—have gained prominence as versatile and effective carriers for mRNA therapeutics [4,5]. Despite these advances, significant challenges remain: the stringent requirement for cold-chain storage to maintain vaccine stability, the reliance on syringe- and needle-based administration methods, and the need for multiple booster doses to sustain long-term immunity continue to limit broad deployment, particularly in resource-limited settings [1,4].

Among emerging materials-based strategies, metal-organic frameworks (MOFs)—particularly zeolitic imidazolate framework-8 (ZIF-8)—have shown considerable promise in improving vaccine delivery and stability. One of the earliest studies in this field utilized ZIF-8 to co-encapsulate the model antigen ovalbumin alongside adjuvants, resulting in composite formulations capable of eliciting robust immune responses [6]. Other MOFs based on aluminium, zirconium, and potassium have similarly enabled oral antigen delivery and improved immunogenicity [7–9]. The ZIF-8 MOF remains particularly attractive due to its porosity, pH-responsive degradability, and chemical stability. In vitro, it exhibits low to moderate cytotoxicity, influenced by parameters such as particle size, surface chemistry, dose, and exposure time. Particularly, toxicity is cell line-dependent—for example, macrophages and epithelial cells show earlier oxidative stress responses than cancer cells [10]. The toxicity is primarily driven by zinc ion release and reactive oxygen species generation under acidic conditions [10]. Several strategies have been explored to enhance ZIF-8-based therapeutic delivery, notably surface functionalization with molecules like polydopamine, polyethylene glycol (PEG), hyaluronic acid, and silica to improve stability and biocompatibility [11–14]. Therefore, the effective assessment of ZIF-8-based platforms depends on both optimized particle design and the use of suitable cell models.

In addition to addressing toxicity concerns, preclinical animal studies have shown that ZIF-8 can also modulate immune responses to subunit vaccines, resulting in enhanced antibody titres. [6,15]. A recent study at the Massachusetts Institute of

<sup>a</sup> CSIRO Manufacturing, Private Bag 10, Clayton South, Victoria 3169, Australia

<sup>b</sup> CSIRO Health & Biosecurity, Australian Centre for Disease Preparedness, Geelong, Victoria 3220, Australia

<sup>c</sup> School of Science, RMIT University, Melbourne, Victoria 3000, Australia

<sup>d</sup> Australian Regenerative Medicine Institute, Monash University, Clayton, VIC, Australia

Supplementary Information available: See DOI: 10.1039/x0xx00000x



Technology has revealed that ZIF-8 degradation products activate endosomal toll-like receptors, potentiating spike protein immunogenicity [16]. Beyond protein antigens, MOF encapsulation has also been extended to whole-virus platforms. For instance, the Gassensmith group demonstrated that ZIF-8-encapsulated tobacco mosaic virus (TMV) exhibited enhanced thermal and chemical stability [17–19]. Building on this, our group applied ZIF-8 biomimetic mineralization to a commercially available live-viral vaccine—the V4 strain of Newcastle Disease Virus (NDV) and the WSN strain of Influenza A, significantly extending their stability from days to months [20].

More recently, MOFs have been explored for stabilizing liposomal formulations. Herbert et al. immobilized proteoliposomes within ZIFs, conferring exceptional resistance to thermal, mechanical, and chemical stressors [21]. Kumari et al. developed a ZIF-8-encapsulated liposomal system suitable for biolistic delivery, offering a viable alternative to needle-based injection [22]. Other studies have demonstrated successful intranasal delivery of ZIF-8-coated liposomes in mice without compromising biocompatibility [23]. These findings suggest that MOF encapsulation could enhance the stability and delivery of more complex lipid nanoparticle systems like the SARS-CoV-2 mRNA vaccines (e.g., BNT162b2, mRNA-1273).

In this study, we present the first demonstration of ZIF-8 biomimetic mineralization applied to mRNA-LNPs. We formulated LNPs containing enhanced green fluorescent protein (eGFP)-encoding mRNA with an ionizable lipid, helper lipid, PEGylated lipid, and cholesterol. These mRNA-LNPs were successfully encapsulated and released from ZIF-8 while maintaining structural integrity and biological function. Remarkably, ZIF-8 encapsulation significantly enhanced mRNA transfection efficiency, offering a promising strategy for the stabilization and delivery of mRNA-based vaccines.

## Results and discussion

### MOF Biomimetic Mineralization of LNP@mRNA

To prepare the LNPs used in this study, we vortex-mixed an ethanolic phase composed of the lipids ALC-0315, DSPC, cholesterol, and ALC-0159 with an aqueous phase containing eGFP mRNA, at a 1:3 volumetric ratio. The resulting mRNA-LNPs had an N to P ratio of 6, with an average mRNA encapsulation efficiency of 80%, determined by performing a ribogreen assay (S1). This is consistent with previous studies on LNP@mRNA systems, which typically report encapsulation efficiencies ranging between 70–95% [24,25].

Among the various metal-organic frameworks (MOFs) employed in the biomedical field, zeolitic imidazolate frameworks (ZIFs)—comprising an extended network of  $\text{Zn}^{2+}$  ions coordinated with 2-methylimidazole linker units—have been extensively explored for applications such as biomolecule encapsulation, drug delivery, and gene therapy. The mechanism of ZIF formation has been well studied and involves the initial association of positively charged zinc ions with the biomolecule, followed by the attachment of the imidazole organic ligands.

This process facilitates the formation of prenucleation clusters of ZIF-8, leading to controlled biomimetic mineralization on the surface of biomacromolecules [26].

We have previously applied ZIF-8 for the biomimetic mineralization of live-viral vaccines [20]. The virions in aqueous solution have a negative zeta potential due to their surface proteins, which facilitates interaction with  $\text{Zn}^{2+}$  ions, resulting in successful encapsulation and thermal stabilization of these vaccines. However, the scenario differs when mRNA is encapsulated within a multi-component lipid nanoparticle (LNP). The inclusion of an ionizable cationic lipid, such as ALC-0315 (theoretical pKa 6.09), in the LNP formulation used in this study hinders ZIF-8 biomimetic mineralization when the particles are resuspended in nuclease-free water. Cationic surface charge has been shown to hinder ZIF encapsulation [27]. Furthermore, mRNA-LNPs require carefully buffered conditions, as they are osmotically unstable in water [18]. We have previously addressed the challenge of cationic surface charge on proteins using a MOF biomimetic co-encapsulation technique with serum albumin [28]. Other approaches, such as those by Herbert et al, have utilized a solvent solution containing 100 mM NaCl, 1 mM TCEP, and 20 mM MOPS buffered to pH 7.0 to facilitate ZIF biomimetic mineralization of proteo-liposomes [21]. Herein, we used OptiMEM™, a reduced-serum media as the solvent, as it offers both the buffering capacity along with helper constituents to enable ZIF biomimetic co-encapsulation of the LNPs.

A variation in ZIF polymorph can be produced by varying the precursor concentrations [29]. While maintaining a constant precursor ratio of zinc acetate to 2-methylimidazole (1:4), we investigated three ZIF-8@LNP@mRNA formulations — A-ZIF-8@LNP@mRNA, B-ZIF-8@LNP@mRNA, and C-ZIF-8@LNP@mRNA, with progressively increasing concentrations of the precursors. Specifically, we prepared the formulations with zinc acetate at 4, 8, and 20 mM, and 2-methylimidazole at 16, 32, and 80 mM, respectively. The optimization of precursor concentrations aimed to maximize ZIF-8 encapsulation of LNPs while minimizing the potential toxicity associated with excess ZIF material, knowing that toxicity is dose-dependent. All ZIF-8@LNP@mRNA syntheses and subsequent post-processing were conducted in proprietary OptiMEM™ media.

The aqueous synthesis of ZIF-8 at a low molar ratio of precursors (1:4) typically favors the formation of an amorphous material (aZIF) [30,31]. Consistent with expectations, our synthesis conditions resulted in an amorphous coordination polymer. However, to confirm the composition of ZIF-8, it was necessary to obtain a crystalline phase. To achieve this, the amorphous A-ZIF-8@LNP@mRNA composite was washed with ethanol, which resulted in the formation of a crystalline ZIF-8 phase, as confirmed by X-ray diffraction (XRD) analysis shown in Figure 1b. Figure 1c (ii, iii) presents TEM and SEM images of the A-ZIF-8@LNP@mRNA precipitate, revealing a dense deposit of the amorphous flocculate. When ethanol post-processing was applied, the composite transitioned to crystalline ZIF-8@LNP@mRNA (ethanol washed) crystals, exhibiting a



truncated rhombic dodecahedral morphology, rather than the typical rhombic dodecahedron morphology of control ZIF-8 (S2).

The functional integrity of the ZIF-8 encapsulated LNPs was evaluated *in vitro* after releasing the mRNA-LNPs from their ZIF scaffolds using a sodium citrate buffer (pH 5.0, 50 mM). ZIF-8 is known to be unstable and dissociates rapidly in acidic sodium citrate buffer. Moreover, citrate ions chelate  $\text{Zn}^{2+}$ , further breaking the coordination bonds within the ZIF-8 MOF, facilitating the release of encapsulated mRNA-LNPs. We confirmed using TEM analysis, that the LNPs released from the MOF maintained their structural integrity. Additionally, we observed an increase in their hydrodynamic size from an average of 174 nm (Pdl: 0.3) to an average of 260 nm (Pdl: 0.2) using dynamic light scattering (DLS), as shown in Figure 1d.

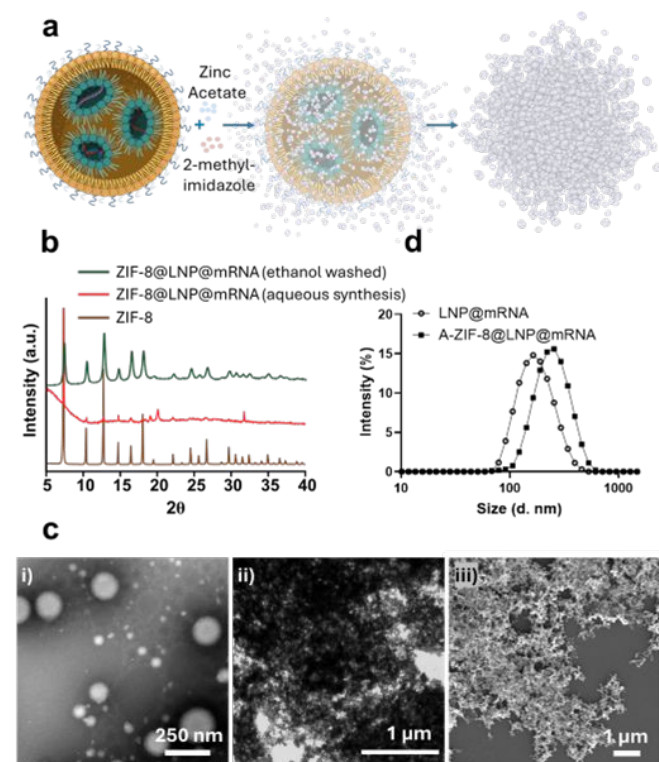


Figure 1. Synthesis and characterization of ZIF-8@LNP@mRNA prepared via the biomimetic mineralization technique. (a) Schematic illustration of the proposed mechanism: The presence of LNPs in a buffered medium increases the local concentration of precursors, facilitating the formation of ZIF-8 prenucleation clusters, leading to the biomimetic growth of ZIF-8 around the LNPs. (b) Powder X-ray diffraction (PXRD) spectra demonstrate the similarity between ZIF-8@LNP@mRNA (ethanol washed; crystalline) and the control ZIF-8 MOF, whereas the aqueous synthesis resulted in ZIF-8@LNP@mRNA (aqueous synthesis; amorphous) with an amorphous phase. (c) Transmission electron microscopy (TEM) images show LNP@mRNA (i) (scale – 250 nm) and ZIF-8@LNP@mRNA (ii) (scale – 1  $\mu\text{m}$ ), while the scanning electron microscopy (SEM) image of ZIF-8@LNP@mRNA (iii) (scale – 1  $\mu\text{m}$ ) highlights the structural characteristics. (d) The average hydrodynamic diameter of the LNP@mRNA increased from 174 nm to 260 nm after encapsulation and release from the ZIF-8@LNP@mRNA, as observed using dynamic light scattering (DLS).

Figure 2a shows Energy-dispersive X-ray spectroscopy (EDS) analysis of A-ZIF-8@LNP@mRNA. The EDS confirms the presence of expected elements in the mRNA-encapsulating LNP, including C, O, N, P, and Zn. Zinc from the ZIF-8 is

distributed throughout the sample but is especially concentrated around the spherical moieties in the flocculate, confirming the ZIF mineralized composition of the LNP formulations. The elemental composition (weight %) for each sample is detailed in the corresponding table in supplementary Figure S3. After sodium citrate dissolution of the ZIF, EDS of the sample showed a limited presence of zinc inside the emerging carbon-rich spherical nanoparticles, while much of the dissolved zinc and sodium salts were observed around these nanostructures (S4).

TEM, with its exceptional nanoscale resolution, was used to visualize the sequence of events from LNP biomimetic mineralization in the ZIF MOF to the subsequent release using citrate buffer prior to transfection assays. Figure 2b (i) shows the unilamellar LNPs revealed by negative contrast using a phosphotungstic acid negative-stain. The LNPs exhibited a wide size distribution, ranging from 50 nm to 500 nm, which reflects the variability introduced by manual vortex-mixing during preparation. The formation of ZIF-8@LNP@mRNA produced a floc, as shown in the electron image in Figure 2b (ii). Upon the addition of sodium citrate release buffer, the ZIF disintegrates, as captured in the unstained image in Figure 2b (iii), where LNPs are seen emerging from the partially dissolved, electron-dense ZIF material. After complete dissolution, the negatively stained LNPs in Figure 2b (iv) confirms that the structural integrity of the released LNPs remains intact following the encapsulation and release process.

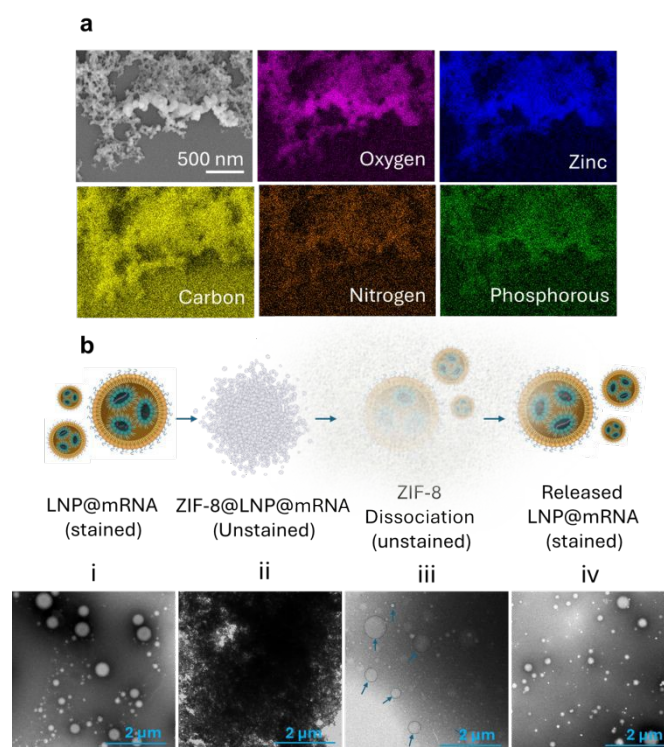


Figure 2. Elemental analysis of ZIF-8@LNP@mRNA and Transmission Electron Microscopy (TEM) visualization of ZIF-8 encapsulation and release of LNP@mRNA. (a) Energy-dispersive X-ray spectroscopy (EDS) analysis of A-ZIF-8@LNP@mRNA confirms the presence of expected elements in the mRNA-encapsulating LNP, including O, C, and





## ARTICLE

## Journal Name

N, along with Zn from ZIF-8 (blue) and P from the LNP@mRNA (green). These elements are distributed throughout the sample, with Zn particularly concentrated around the spherical moieties in the flocculate, confirming the ZIF mineralized composition of the LNP formulations (scale = 500 nm). The sequence of events is depicted in Schematic Steps (i) to (iv) and captured using TEM images: (i) LNP@mRNA are encapsulated within the ZIF-8 MOF, forming an amorphous composite (ZIF-8@LNP@mRNA); (ii) Sodium citrate buffer (pH 5.0, 50 mM) is then used to disintegrate the ZIF-8, with pointers highlighting individual nanoparticles released upon MOF disintegration. (iii), resulting in the release of structurally intact LNP@mRNAs (iv).

### Efficiency of the ZIF-8@LNP@mRNA Formulations

The transfection efficiency and mRNA translation of the three formulations — A-ZIF-8@LNP@mRNA, B-ZIF-8@LNP@mRNA, and C-ZIF-8@LNP@mRNA—were compared to LNP@mRNA in HEK-293 and HCT-116 cells. The experimental process is illustrated in Figure 3a. On the day of transfection, the culture media was removed from sub-confluent adherent cells plated in a 96-well plate and replaced with each of the formulation types (LNP@mRNA, A-ZIF-8@LNP@mRNA, B-ZIF-8@LNP@mRNA, and C ZIF 8@LNP@mRNA) diluted in reduced serum media (OptiMEM™) at a 2.5 µg/mL mRNA loading. The cells were incubated with these different formulations for 24 hours, after which the media was replaced with normal culture media containing 10% FBS. Cells were continuously imaged, every 3 hours for a total period of 48 hours. In parallel, a lactate dehydrogenase (LDH) assay was performed at the 24-hour time point to assess cytotoxicity, determined by the level of plasma membrane damage (Supplementary Figure S5).

Transfection efficiency (%) was quantified by measuring the green-fluorescent area as a fraction of the total phase area confluence, while protein expression (translation efficiency) was assessed by the total green integrated intensity. Figures 3b and 3d show the transfection efficiency of LNP@mRNA, A-ZIF-8@LNP@mRNA, B-ZIF-8@LNP@mRNA, and C-ZIF-8@LNP@mRNA in HEK-293 and HCT-116 cells, respectively. LNP@mRNA showed a delayed GFP expression, which began 12 hours post-incubation and increased sharply to a mean transfection efficiency of 45% at 24 hours. As shown in Figure 3c, the integrated green intensity, representing the amount of protein produced, also increased between 18 and 24 hours, reaching a peak of  $6.6 \times 10^6$  units at 24 hours. Upon the addition of serum-containing media at 24 hours (indicated by a dotted line), the number of cells treated with LNP@mRNA doubled over the next 24 hours (Supplementary Figure S6). However, the increase in GFP-expressing cells was not proportional to the increase in confluence, leading to a drop in transfection efficiency to an average of 25% at 48 hours. The GFP protein levels also decreased, as evidenced by a reduction in the integrated green intensity to  $4 \times 10^6$  units at 48 hours.

In contrast, A-ZIF-8@LNP@mRNA, B-ZIF-8@LNP@mRNA, and C-ZIF-8@LNP@mRNA exhibited more rapid GFP expression profiles in HEK-293 cells, with significantly higher mean transfection efficiencies of 57%, 51%, and 40% at 18 hours, and 74%, 66%, and 52% at 24 hours, respectively. Beyond 24 hours, the fraction of transfected cells plateaued for A-ZIF-8@LNP@mRNA and B-ZIF-8@LNP@mRNA, indicating that, unlike the stalled GFP expression in LNP@mRNA-treated cells, the number of green-fluorescent cells increased in proportion

to the phase area confluence for these ZIF-8 formulations. The green integrated intensity continued to increase, indicating consistently higher protein production for all three ZIF-8 formulations. Notably, A-ZIF-8@LNP@mRNA demonstrated a significantly higher amount of GFP, with a 5-fold increase compared to LNP@mRNA at 48 hours.

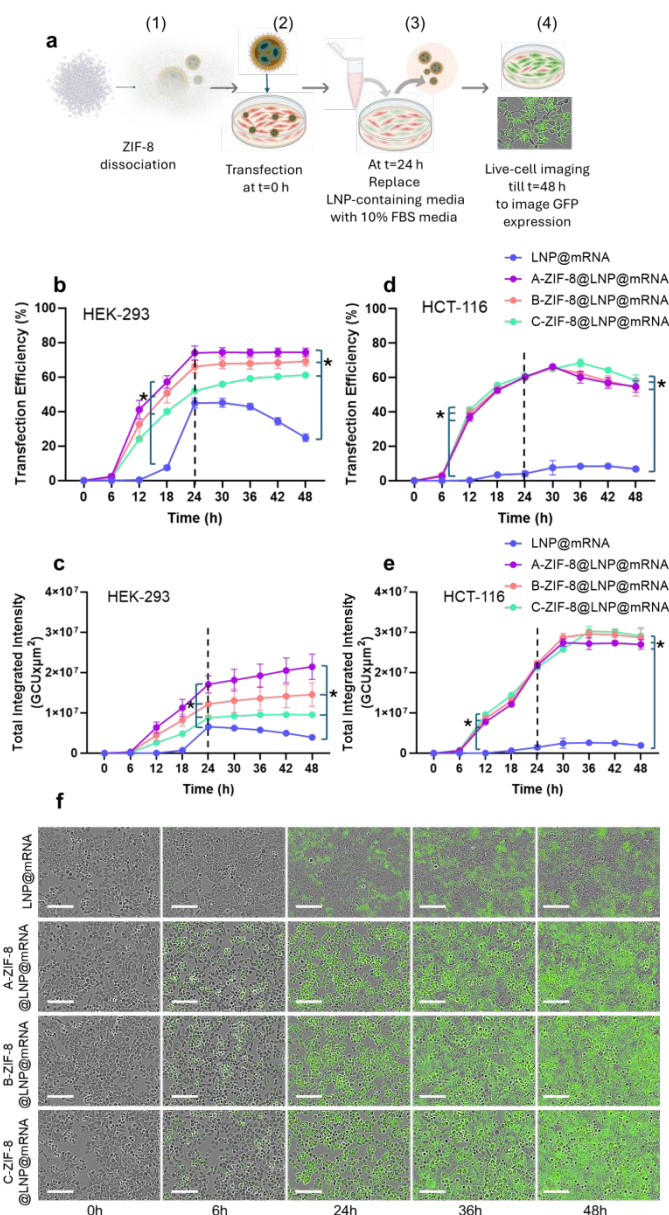


Figure 3. Transfection efficiency of LNP@mRNA and ZIF-8@LNP@mRNA formulations evaluated in HEK-293 and HCT-116 cells. (a) Schematic representation of the experimental methodology for transfection experiments. (1) ZIF-8@LNP@mRNA formulations were dissociated to release LNPs using a sodium citrate buffer (50 mM, pH 5.0) and then (2) added to adherent cells cultured in OptiMEM media. After a 24-hour incubation period, (3) the media containing the formulations was replaced with cell culture media supplemented with 10% serum. The cells were monitored continuously during the initial 24 hours and (4) for an additional 24 hours to assess phase area confluence, green fluorescent protein expression area, and intensity in the cells. The control and test formulations included: 1) OptiMEM media, 2) LNP@mRNA, 3) A-ZIF-8@LNP@mRNA, 4) B-ZIF-8@LNP@mRNA, and 5) C-ZIF-8@LNP@mRNA. Transfection efficiencies in HEK-293 cells (b) and HCT-116 cells (d), defined by the green area fraction of the total phase area in each well (n=3), were plotted as shown. Corresponding total integrated intensity values are presented in (c) and (e), respectively. The dotted line at 24 hours indicates the time point at which the media conditions were changed.



Chronological representative images from HCT-116 cells treated with each test LNP formulation are shown in (f) scale – 150  $\mu\text{m}$ . Data were analysed by ordinary two-way ANOVA, comparing mean values of the different formulations at a single time point (\*;  $p < 0.05$ ).

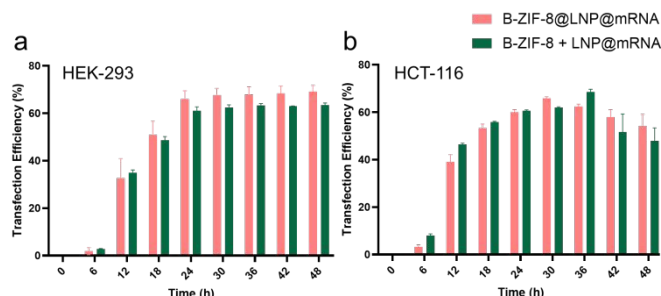
Overall, there was no significant difference in transfection efficiency between A-ZIF-8@LNP@mRNA and B-ZIF-8@LNP@mRNA. However, A-ZIF-8@LNP@mRNA consistently outperformed C-ZIF-8@LNP@mRNA from 18 hours onwards. A-ZIF-8@LNP@mRNA was the most optimal formulation, showing 7.6-, 1.6-, and 3-fold higher transfection efficiency than LNP@mRNA at 18, 24, and 48 hours, respectively.

Due to their epithelial origin, HCT-116 cells are considered difficult to transfect, which is evident in our results (Figure 3d), where the control LNP@mRNA achieved a maximum transfection efficiency of only 8.5% in HCT-116 cells, compared to 45% in HEK-293 cells. Recent efforts to address this limitation have focused on developing novel lipid formulations. For example, Qian et al. reported a newly synthesized lipid, S-1, which demonstrated significantly enhanced expression in HCT-116 cells, surpassing that in HEK-293 cells over a 24-hour period. Notably, our ZIF-8@LNP@mRNA formulation achieved comparable transfection efficiencies in both HCT-116 and HEK-293 cells, suggesting improved performance in hard-to-transfect cell lines [32]. While LNP-treated HCT-116 cells showed a mean transfection efficiency of 3.5% at 18 hours, over 50% of cells were successfully transfected using the ZIF-8 formulations at the same time point. All three ZIF-8@LNP@mRNA formulations significantly outperformed LNP@mRNA from 12 hours onwards, with no significant differences among A-ZIF-8@LNP@mRNA, B-ZIF-8@LNP@mRNA, and C-ZIF-8@LNP@mRNA at any time point. The maximum mean transfection efficiency reached 66% for A-ZIF-8@LNP@mRNA and B-ZIF-8@LNP@mRNA at 30 hours, and 69% for C-ZIF-8@LNP@mRNA at 36 hours. GFP protein production steadily increased to  $2.7 \times 10^7$ ,  $2.9 \times 10^7$ , and  $2.6 \times 10^7$  units for A-ZIF-8@LNP@mRNA, B-ZIF-8@LNP@mRNA, and C-ZIF-8@LNP@mRNA, respectively—at least 10 times higher than the  $2.5 \times 10^6$  units for LNP@mRNA at 30 hours. Between 30 and 48 hours, despite the recovery in cell density (Figure S6) and a plateaued intensity indicating further GFP expression, the transfection efficiency values dropped to 55%, 54%, and 58%, respectively, for A-ZIF-8@LNP@mRNA, B-ZIF-8@LNP@mRNA, and C-ZIF-8@LNP@mRNA, remaining significantly higher than the 7% mean for LNP@mRNA. Figure 3f shows representative images from each of the LNP@mRNA and ZIF-8@LNP@mRNA treated wells. (See corresponding images for HEK-293 cells in Supplementary Figure S8). A separate experiment with A549 cells using a shorter 5-hour incubation (compared to the 24-hour incubation used for HEK-293 and HCT-116 cells) showed a similar enhancement in transfection efficiency, consistent with observations in HEK-293 and HCT-116 cells (Supplementary Figure S7). However, the use of a different cell type and incubation condition led to an earlier plateau in GFP expression at the 12-hour time point.

## ZIF-8 attributed enhanced transfection is independent of ZIF-8 encapsulation

View Article Online  
DOI: 10.1039/D4TB02101K

The initial aim of our study was to apply biomimetic mineralization of mRNA-LNPs for developing needle-free and thermostable vaccine formulations. However, we discovered that the ZIF-8@LNP@mRNA formulations we developed significantly enhanced the transfection and translation efficiency of LNPs. To determine whether this enhancement is due to the ZIF-8 encapsulation or structural changes in the mRNA-LNPs upon release, we compared B-ZIF-8@LNP@mRNA with B-ZIF-8 + LNP@mRNA, where B-ZIF-8 was separately synthesized and added to LNP@mRNA. Both formulations were dissociated using sodium citrate buffer (50 mM, pH 5.0) before transfection experiments. Figures 4a and 4b display the transfection efficiency in HEK-293 and HCT-116 cells. No significant difference in transfection efficiency was observed between the two formulations in both cell lines, suggesting that the enhancement in transfection efficiency is independent of ZIF-8 encapsulation. We propose that the presence of ZIF-8 dissociates is responsible for the increased mRNA transfection and translation efficiency. Nonetheless, ZIF-8 encapsulation could offer additional benefits, such as enhanced thermostability and alternative administration routes, as



indicated by other studies [21,22,33,34].

Figure 4. A comparison of the transfection efficiencies of B-ZIF-8 + LNP@mRNA and B-ZIF-8@LNP@mRNA, formulations containing the ZIF-8 added to LNP@mRNA and ZIF-8 encapsulated LNP@mRNA, respectively in (a) HEK-293 and (b) HCT-116 cells.

Reviewing prior art in the field of ZIF-8-vaccine development, most studies have employed nanoZIF-8 as an intracellular delivery vehicle for intracellular delivery of non-replicating viral vaccines and sub-unit vaccines [6,15–17,19,33,35–37]. Conversely, ZIF-8 can also serve as a protective coating, to be released or dissociated just before vaccine administration [20]. This application depends on the cargo; while nucleic acids and proteins require a nanocarrier for intracellular delivery, live-viral and non-viral agents like LNPs are self-sufficient for cell invasion and endocytosis.

The ZIF-8 MOF structure comprises a lattice of tetrahedral units made of zinc ions coordinated with four imidazole molecules. The coordination bond can easily dissociate with changes in pH or the presence of a chelator. This dissociation can occur exogenously using sodium citrate buffer (50 mM, pH 5.0) or intracellularly after endocytosis in lysosomal and endosomal



compartments, releasing the cargo vaccine along with ZIF-8 disintegration products containing imidazolate and  $\text{Zn}^{2+}$ .

Both imidazole and zinc have been associated with enhancing vaccine response, and we postulate that they may have a synergistic effect in the context of ZIF-8. Imidazole is present in many commercial toll-like receptor (TLR) agonists [38,39], which are critical to the innate immune system. TLRs 3, 7, 8, and 9, located on endosomal membranes, trigger immune responses upon encountering viral or bacterial RNA. Interestingly, recent reports suggest that ZIF-8 induces TLR-3, 7, and 9 expressions, initiating proinflammatory responses and thereby enhancing immunogenicity [9]. This enhancement is mainly attributed to the 2-methylimidazole degradation products, with  $\text{Zn}^{2+}$  shown not to activate TLR-7, TLR-8, or TLR-9 in reporter cells. The study also compared ZIF-8 with mRNA-LNPs, suggesting that LNP@mRNA immune activation is driven by monocyte-derived dendritic cells and the cDC1 subset of conventional DCs, while TLR-7 activation by ZIF-8 is unique among other nanodelivery vehicles.

Zinc homeostasis is crucial for normal immune function [40]. The metalloimmunological effects of zinc have been extensively studied, showing that  $\text{Zn}^{2+}$  enhances the transfection efficiency of various polymeric and lipoplex-based transfection agents. These studies link  $\text{Zn}^{2+}$  to 1) increased endocytosis through cell membrane-metal coordination, 2) enhanced endosomal escape via strong interactions between zinc and phosphatidylserine in the endosomal membrane and increased 'proton-sponge' effect leading to endosome swelling and rupture, and 3) excellent anti-serum or low-protein binding capability [41–45]. These properties make  $\text{Zn}^{2+}$  advantageous for mRNA therapeutics, where only 2–8% of mRNA typically escapes the endosome to be released into the cytosol [46]. Additionally, zinc's coordination with nucleic acid phosphate groups aids in maintaining the integrity of the nucleic acids, leading to higher antigen production and improved immune response [47,48].

## Conclusion

Despite significant advancements in mRNA-LNP vaccines, challenges remain in maximizing LNP efficiency, which is critical for realizing the therapeutic potential of mRNA-based therapies. Transfection efficiency—defined as the percentage of cells successfully expressing the target protein after LNP delivery—depends on factors such as LNP formulation, cellular uptake, endosomal escape, and mRNA translation efficiency [49]. Optimizing these steps offers opportunities to enhance transfection efficiency and therapeutic outcomes.

Inspired by recent studies on ZIF encapsulation of liposomes and proteoliposomes, we explored the biomimetic mineralization of mRNA-loaded multi-lipid nanoparticles. During this investigation, we discovered that ZIF-8 significantly enhances LNP transfection efficiency. This effect is not linked to structural or compositional changes in the LNPs upon encapsulation but is attributed to the presence of ZIF-8 dissociates, which enhance transfection efficiency. Based on literature,  $\text{Zn}^{2+}$  aids in mRNA cytosolic delivery, boosting transfection efficiency and potentially increasing antibody

production in vivo, while imidazoles can trigger an innate immune response. We hypothesize that the imidazole and zinc components of ZIF-8 may work synergistically to enhance both transfection efficiency and immunogenicity.

The results of this preliminary study suggest that ZIF-8 is a safe, facile, and cost-effective additive that could improve vaccine efficiency. Additionally, when encapsulated within ZIF-8, formulations may be suitable for needle-free administration and ambient storage. Given that ZIF-8 dissociation is pH-dependent, encapsulated formulations could be adapted for single-dose, slow-release vaccines, which have been shown to elicit stronger immune responses. Overall, these findings warrant further investigation into the role and mechanism of ZIF-8 and its dissociates in improving the efficacy of mRNA-LNP vaccines, potentially utilizing these novel materials as additives to mRNA-LNP formulations.

## Experimental

### Materials

All materials, zinc acetate dihydrate ( $\geq 99\%$ ), 2-methylimidazole (99%), Tris Buffered Saline (TBS) tablets, sodium citrate dihydrate, citric acid, ethanol, DNase/RNase free water, OptiMEM media were purchased commercially. All buffers were sterilized by autoclaving at  $121^\circ\text{C}$ , 15 psi for 1 h.

Cell Culture: HEK-293 cells were maintained at  $37^\circ\text{C}$ , 5%  $\text{CO}_2$  in complete cell culture medium containing DMEM (Glutamax), 10% foetal bovine serum (FBS), 2mM L-glutamine (GlutaMAX), 1% NEAA. The HCT-116 cells were maintained in McCoy's 5a modified media, 10% heat inactivated FBS, 2mM L-glutamine (GlutaMAX). The A549 cells were maintained in F12 media, 10% heat inactivated FBS and glutamax. All the cells were subcultured every 3–4 d. One day prior to the transfection, cells were seeded at a density of  $2.0 \times 10^4$  cells per well in 96 well plates in complete cell culture media.

### Preparation of Lipid Nanoparticles (LNPs)

There are multiple comparable methods such as pipette mixing, vortex mixing, and microfluidic mixing that can be used to efficiently mix and manufacture multi-lipid nanoparticles. [50] Solution (a), A 90  $\mu\text{L}$  aqueous solution of eGFP mRNA was prepared by diluting 15  $\mu\text{L}$  of stock mRNA (ApexBio ARCA modified EGFP mRNA; #R1001, 996 nucleotides; supplied as 1 mg/ml in 1 mM Sodium Citrate, pH 6.4) in 75  $\mu\text{L}$  of sodium citrate buffer (pH 4.0, 10 mM).

Solution (b), A 30  $\mu\text{L}$  ethanolic solution containing, an ionizable lipid ALC-0315 (6-((2-hexyldecanoyl)oxy)-N-(6-((2-hexyldecanoyl)oxy)hexyl)-N-(4-hydroxybutyl)hexan-1-aminium), a helper lipid DSPC (Distearoylphosphatidylcholine), Cholesterol, and a PEGylated lipid ALC-0159 (Methoxypolyethyleneglycoloxy(2000)-N,N-ditetradecylacetamide) in a molar ratio of 46.3: 9.4: 42.7: 1.6 was prepared. The lipids and the molar ratios used herein are similar to those used for BNT162b2; Comirnaty i.e. the SARS-CoV-2 mRNA vaccine manufactured by the Pfizer-BioNTech collaboration [51]. For multiple experiments within a period of 2 months, we prepared this solution in bulk and stored at  $20^\circ\text{C}$





until further use. To prepare LNPs, Solution (b) is added to Solution (a) in a 1.5 mL tube with constant vortex mixing for 30 sec. The LNP solution formed herein is immediately dialyzed using a Pur-A-Lyzer™ dialysis kit (Sigma Aldrich, #PURN60030, capacity 10 250 µL, MWCO 6-8 kDa) against Tris Buffered Saline (50 mM, pH 7.6) for a period of 1 h. Dialysed LNP solution is collected and made to 150 µL using Tris Buffered Saline (50 mM, pH 7.6). The 150 µL LNP solution is further diluted 4 times in reduced serum media (OptiMEM media; ThermoFisher Scientific #31985062; containing sodium bicarbonate buffer system (2.4 g/L), insulin, transferrin, hypoxanthine, thymidine, and trace elements to allow for a reduction in serum supplementation) to make 600 µL of LNPs in OptiMEM media, Solution (c) for further use.

### Preparation of ZIF@LNP formulations

Stock solutions of the ZIF precursors, the organic ligand 2-methylimidazole and the metal salt, zinc acetate was prepared in OptiMEM media at 320 mM and 80 mM, respectively. To a 300 µL aliquot of LNP Solution (c) each, 150 µL of 32 mM, 64 mM and 160 mM of 2 methylimidazole were added and carefully mixed by pipetting for preparation of ZIF(A), ZIF(B), and ZIF(C) formulations, respectively. This was followed by an addition of 150 µL zinc acetate solution at a concentration of 8 mM, 16 mM, and 40 mM and soft pipette mixing for preparation of ZIF(A), ZIF(B), and ZIF(C) formulations, respectively. Flocs appeared immediately for ZIF(C) and slowly for ZIF(B) and ZIF(A). The solutions were left to sit over a period of 15 min at room temperature. The pellet was washed and collected by centrifugation at 14,000 x g for 5 min to give ZIF(A), ZIF(B), and ZIF(C) formulations, respectively. Prior to cell experiments, the LNPs from the ZIF formulations were recovered by exfoliating the ZIF using a sodium citrate buffer (50 mM, pH 5.0). To the above prepared pellets, 300 µL of the sodium buffer was added and gently mixed by pipetting, until the flocculation turned clear.

### In-Vitro Transfection Experiments

Solution (c) as prepared in section 4.1; LNPs in OptiMEM media and the LNPs recovered from ZIF formulations, as prepared in section 4.2 were all diluted 10 times in OptiMEM media to a final mRNA concentration of 2.5 µg/mL or 2500 ng/mL before adding to cells. Cell culture media was completely removed and 100 µL of all samples were added to each well (96 well plate). Cell culture plates were continuously imaged every 3 h using the Incucyte® Live Cell Analysis system to observe the phase area cell confluence and the green fluorescence signal from eGFP expression. For HEK-293 and HCT-116 cells, after a period of 24 h, the samples were removed and replaced with complete cell culture media containing 10% FBS. The plate was imaged every 3 hours on the Incucyte® for a period of 48 h. Experiments were performed in triplicate (n=3) and repeated twice. For the A549 cells, the samples were removed and replaced with complete cell culture media containing 10% FBS after 5 h. A549 cells were also continuously observed on the Incucyte® for a period of 48 h.

### Characterisation Techniques

#### Dynamic Light Scattering (DLS)

Dynamic-light scattering was measured with a Malvern Zeta-sizer Nano ZS. A disposable cuvette was used at 25°C with a 633 nm laser source, a medium refractive index of 1.33, a material refractive index of 1.51, and a scattering angle of 175°.

#### Transmission electron microscopy (TEM)

Carbon-coated grids (EMSCF200H –CU-TH, ProSciTech) were glow discharged to render them hydrophilic. A 10 µL drop of sample was applied to an upturned grid held in anti-capillary forceps, over moist filter paper, and left for 10 min to adsorb. The excess sample was then removed with filter paper. If stained, the grid was then inverted onto a drop of 2% PTA stain, pH 6.9 on Parafilm, for 1 min. The grid was removed, the stain wicked away with filter paper and allowed to dry before viewing in the microscope. The samples were examined using a Tecnai 12 Transmission Electron Microscope (FEI, Eindhoven, The Netherlands) at an operating voltage of 120 kV. Images were recorded using a FEI Eagle 4kx4k CCD camera and AnalySIS v3.2 camera control software (Olympus).

#### Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS)

Samples were mounted either on carbon tape or silicon wafer and then put on aluminium stubs. The samples were coated with conductive Gold using Cressington HR 208HR sputter coater for 20 s to give a 3 nm coating. Samples were imaged using a Zeiss Merlin FESEM at an accelerating voltage of 3 kV in the secondary electron or in lens mode depending on magnification. Magnification is indicated with the scale bars in each image. Elemental composition (EDS) was conducted using Oxford Instruments Extreme 100 mm2 windowless SSD detector at an accelerating voltage of 5 kV.

#### Statistical Analysis

All values reported are means ± SD. Data were analysed by ordinary two-way ANOVA comparing mean values of the different parameters at a single time point (p<0.05). Either Tukey's multiple comparison, or the Hol-Sidak multiple comparisons (\*, p<0.05) were performed with a single pooled variance and family-wise alpha threshold/confidence level of 0.05 (95% CI). Tables for all statistical comparisons and the corresponding adjusted P values are available in the supplementary information.

### Conflicts of interest

There are no conflicts to declare.

### Data availability

The data supporting this article have been included as part of the Supplementary Information.

### Acknowledgements

View Article Online  
DOI: 10.1039/D4TB02101K





## ARTICLE

## Journal Name

RS and CMD acknowledge CSIRO Strategic funding for this work. CMD acknowledges the Veski Inspiring Women fellowship for support. Brenda Williams, Manufacturing, CSIRO is acknowledged for her assistance in cell culturing and maintenance.

## References

- Pardi, N. et al. (2018). mRNA vaccines—a new era in vaccinology. *Nature Reviews Drug Discovery*. <https://doi.org/10.1038/nrd.2017.243>
- Sahin, U. et al. (2020). COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature*. <https://doi.org/10.1038/s41586-020-2814-7>.
- Sahin, U. et al. (2014). mRNA-based therapeutics — developing a new class of drugs. *Nature Reviews Drug Discovery*. <https://doi.org/10.1038/nrd4278>
- Hou, X. et al. (2021) Lipid nanoparticles for mRNA delivery. *Nat Rev Mater* **6**, 1078–1094 (2021). <https://doi.org/10.1038/s41578-021-00358-0>
- Verbeke, R. et al. (2021) The dawn of mRNA vaccines: The COVID-19 case. *Journal of Controlled Release*. 10.1016/j.jconrel.2021.03.043
- Zhang, Y. et al. (2016). Metal-Organic-Framework-Based Vaccine Platforms for Enhanced Systemic Immune and Memory Response. *Advanced Functional Materials*. <https://doi.org/10.1002/adfm.201600650>.
- Miao, Y.B. et al. (2019). Engineering a Nanoscale Al-MOF-Armored Antigen Carried by a “Trojan Horse”-Like Platform for Oral Vaccination to Induce Potent and Long-Lasting Immunity. *Advanced Functional Materials*. <https://doi.org/10.1002/adfm.201904828>.
- Qi, Y. et al. (2019). Antigen-enabled facile preparation of MOF nanovaccine to activate the complement system for enhanced antigen-mediated immune response. *Biomaterials Science*. <https://doi.org/10.1039/c9bm01145e>.
- Li, C. et al. (2021). Cyclodextrin metal-organic framework as vaccine adjuvants enhances immune responses. *Drug Delivery*. <https://doi.org/10.1080/10717544.2021.2012306>.
- Hoop, M., et al. (2018) Biocompatibility characteristics of the metal organic framework ZIF-8 for therapeutical applications, *Applied Materials Today*, <https://doi.org/10.1016/j.apmt.2017.12.014>.
- Peng, L. et al. (2022) Preparation of PEG/ZIF-8@HF drug delivery system for melanoma treatment via oral administration. *Drug Delivery*. <http://doi.org/10.1080/10717544.2022.2058649>
- Wang, Q. et al. (2023) ZIF-8 integrated with polydopamine coating as a novel nano-platform for skin-specific drug delivery. *Journal of Material Chemistry B*. <https://doi.org/10.1039/D2TB02361J>
- Yu, S. et al. (2021) Hyaluronic acid coating on the surface of curcumin-loaded ZIF-8 nanoparticles for improved breast cancer therapy: An in vitro and in vivo study. *Colloids and Surfaces B: Biointerfaces*. <https://doi.org/10.1016/j.colsurfb.2021.111759>
- Ren, S.-Z. et al. (2019) Nanoscale metal–organic-frameworks coated by biodegradable organosilica for pH and redox dual responsive drug release and high-performance anticancer therapy. *ACS Applied Materials and Interfaces*. 10.1021/acsami.9b04236
- Zhang, G. et al. (2021). Poly(ethylene glycol)-Mediated Assembly of Vaccine Particles to Improve Stability and Immunogenicity. *ACS Applied Materials and Interfaces*. <https://doi.org/10.1021/acsami.1c00706>.
- Alsaïari, S.K. et al. (2024). Zeolitic imidazolate frameworks activate endosomal Toll-like receptors and potentiate immunogenicity of SARS-CoV-2 spike protein trimer. *Science Advances*. <https://doi.org/10.1126/sciadv.adj6380>.
- Li, S. et al. (2018). Investigation of Controlled Growth of Metal-Organic Frameworks on Anisotropic Virus Particles. *ACS Applied Materials and Interfaces*. <https://doi.org/10.1021/acsami.8b01369>.
- Li, S. et al. (2016). Template-Directed Synthesis of Porous and Protective Core–Shell Bionanoparticles. *Angewandte Chemie*. <https://doi.org/10.1002/ange.201604879>.
- Luzuriaga, M.A. et al. (2019). Enhanced Stability and Controlled Delivery of MOF-Encapsulated Vaccines and Their Immunogenic Response in Vivo. *ACS Applied Materials and Interfaces*. <https://doi.org/10.1021/acsami.8b20504>.
- Singh, R. et al. (2022). Biomimetic metal-organic frameworks as protective scaffolds for live-virus encapsulation and vaccine stabilization. *Acta Biomaterialia*. <https://doi.org/10.1016/j.actbio.2022.02.002>.
- Herbert, F.C. et al. (2021). Stabilization of supramolecular membrane protein–lipid bilayer assemblies through immobilization in a crystalline exoskeleton. *Nature Communications*. <https://doi.org/10.1038/s41467-021-22285-y>.
- Kumari, S. et al. (2023). Biolistic delivery of liposomes protected in metal-organic frameworks. *Proceedings of the National Academy of Sciences of the United States of America*. <https://doi.org/10.1073/pnas.2218247120>.
- Kumari, S. et al. (2023). In vivo biocompatibility of ZIF-8 for slow release via intranasal administration. *Chemical Science*. <https://doi.org/10.1039/d3sc00500c>.
- Hassett, K. J., et al. (2019) Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines *Molecular Therapy*. doi: 10.1016/j.omtn.2019.01.013.



- 25 Buschmann, M. D., et al. (2021) Nanomaterial Delivery Systems for mRNA Vaccines. *Nature Reviews Materials*. 10.3390/vaccines9010065
- 26 Liang, K., et al. (2015) Biomimetic mineralization of metal-organic frameworks as protective coatings for biomacromolecules. *Nature Communications*. <https://doi.org/10.1038/ncomms8240>
- 27 Maddigan, N.K. et al. (2018). Protein surface functionalisation as a general strategy for facilitating biomimetic mineralisation of ZIF-8. *Chemical Science*. <https://doi.org/10.1039/c8sc00825f>.
- 28 Singh, R. et al. (2021). Stable MOF@enzyme composites for electrochemical biosensing devices. *Journal of Materials Chemistry C*. <https://doi.org/10.1039/d1tc00407g>.
- 29 Eddaoudi, M. et al. (2015). Zeolite-like metal-organic frameworks (ZMOFs): Design, synthesis, and properties. *Chemical Society Reviews*. <https://doi.org/10.1039/c4cs00230j>.
- 30 Falcaro, P. et al. (2020). Phase dependent encapsulation and release profile of ZIF-based biocomposites. *Chemical Science*. <https://doi.org/10.1039/c9sc05433b>.
- 31 Ogata, A.F. et al. (2020). Direct Observation of Amorphous Precursor Phases in the Nucleation of Protein-Metal-Organic Frameworks. *Journal of the American Chemical Society*. <https://doi.org/10.1021/jacs.9b11371>.
- 32 Qian R, et. al (2024) Synthesis of novel cholesterol-based ionizable lipids for mRNA delivery. *Colloids and Surfaces B: Biointerfaces* doi: 10.1016/j.colsurfb.2024.113980.
- 33 Wijesundara, Y.H. et al. (2024). The Promise and Potential of Metal-Organic Frameworks and Covalent Organic Frameworks in Vaccine Nanotechnology. *Chemical Reviews*. <https://doi.org/10.1021/acs.chemrev.3c00409>.
- 34 Kumari, S. et al. (2023). In vivo biocompatibility of ZIF-8 for slow release via intranasal administration. *Chemical Science*. <https://doi.org/10.1039/d3sc00500c>.
- 35 Wang, L. et al. (2022). Enhanced Delivery of TLR7/8 Agonists by Metal-Organic Frameworks for Hepatitis B Virus Cure. *ACS Applied Materials and Interfaces*. <https://doi.org/10.1021/acsami.2c11203>.
- 36 Riccò, R. et al. (2018). Metal-Organic Frameworks for Cell and Virus Biology: A Perspective. *ACS Nano*. <https://doi.org/10.1021/acsnano.7b08056>.
- 37 Ehrman, R.N. et al. (2024). A scalable synthesis of adjuvanting antigen depots based on metal-organic frameworks. *Chemical Science*. <https://doi.org/10.1039/d3sc06734c>.
- 38 Bhagchandani, S. et al. (2021). Evolution of Toll-like receptor 7/8 agonist therapeutics and their delivery approaches: From antiviral formulations to vaccine adjuvants. *Advanced Drug Delivery Reviews*. <https://doi.org/10.1016/j.addr.2021.05.013>.
- 39 Ma, F. et al. (2010). The TLR7 agonists imiquimod and gardiquimod improve DC-based immunotherapy for melanoma in mice. *Cellular & Molecular Immunology*. <https://doi.org/10.1038/cmi.2010.30>.
- 40 Chen, B. et al. (2024). Cellular zinc metabolism and zinc signaling: from biological functions to diseases and therapeutic targets. *Signal Transduction and Targeted Therapy*. <https://doi.org/10.1038/s41392-023-01679-y>.
- 41 Zhang, Y.-M. et al. (2020). Zinc( <sc>ii</sc> )-cyclen coordinative amphiphiles for enhanced gene delivery. *RSC Advances*. <https://doi.org/10.1039/D0RA08027F>.
- 42 Fang, H. et al. (2019). Zinc ion coordination significantly improved the transfection efficiency of low molecular weight polyethylenimine. *Biomaterials Science*. <https://doi.org/10.1039/C9BM00039A>.
- 43 Nie, X. et al. (2023). DPA-Zinc around Polyplexes Acts Like PEG to Reduce Protein Binding While Targeting Cancer Cells. *Advanced Healthcare Materials*. <https://doi.org/10.1002/adhm.202203252>.
- 44 Chu, C. et al. (2019). Zinc(II)-Dipicolylamine Coordination Nanotheranostics: Toward Synergistic Nanomedicine by Combined Photo/Gene Therapy. *Angewandte Chemie International Edition*. <https://doi.org/10.1002/anie.201812482>.
- 45 Zhao, R.-M. et al. (2021). Zn-Promoted gene transfection efficiency for non-viral vectors: a mechanism study. *New Journal of Chemistry*. <https://doi.org/10.1039/D1NJ02115J>.
- 46 Chatterjee, S. et al. (2024). Endosomal escape: A bottleneck for LNP-mediated therapeutics. *Proceedings of the National Academy of Sciences*. <https://doi.org/10.1073/pnas.2307800120>.
- 47 Zou, Z. et al. (2021). Zn<sup>2+</sup>-Coordination-Driven RNA Assembly with Retained Integrity and Biological Functions. *Angewandte Chemie - International Edition*. <https://doi.org/10.1002/anie.202110404>.
- 48 DeLong, R.K. et al. (2024). Enhancing RNA Payload and Temperature Stability and Activity with Cationic Peptide-Coated Zinc Oxide Nanoparticles. *ACS Pharmacology and Translational Science*. <https://doi.org/10.1021/acsptsci.3c00280>.
- 49 Sahin, U. et al. (2017). Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature*. <https://doi.org/10.1038/nature23003>.
- 50 Wang, X. et al. (2023). Preparation of selective organ-targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. *Nature Protocols*. <https://doi.org/10.1038/s41596-022-00755-x>.

View Article Online  
<https://doi.org/10.1039/D4TB02101K>



## ARTICLE

## Journal Name

- 51 Schoenmaker, L, et al. (2021) mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. International journal of Pharmaceutics  
<https://doi.org/10.1016/j.ijpharm.2021.120586>

View Article Online  
DOI: 10.1039/D4TB02101K





The data supporting this article have been included as part of the Supplementary Information.

[View Article Online](#)

DOI: 10.1039/D4TB02101K

