NanOZ-LNP™, Lipid NanoParticles Results

1. Description

Lipid Nanoparticles (LNPs) represent the most effective and safe delivery systems for the translational success of nucleic acid drugs. OZ Biosciences has developed a Microfluidics Production Platform for the reproducible development of safe & potent drug delivery vehicles (LNP) for pharmaceutical applications.
OZ Biosciences can support every stage of your mRNA-LNP production, from mRNA synthesis to LNP formulation development and manufacturing.
For any of RNA (mRNA, siRNA), DNA or APIs encapsulation, you can provide us with your molecule of interest and we will formulate it into LNPs.

2. Storage and shipping condition

Storage & Shipping: LNP must be stored at -80°C. We recommend to minimize freeze-thaw cycles to preserve LNPs integrity. LNPs are shipped with Dry Ice.

Physico-chemical characterization of LNPs

NanOZ-LNPs are formulated through pressure-driven controlled flow microfluidics systems. Once collected and purified, NanOZ-LNPs physico-chemical properties are fully characterized in terms of size distribution, charge surface, structure integrity, encapsulation efficiency and stability.

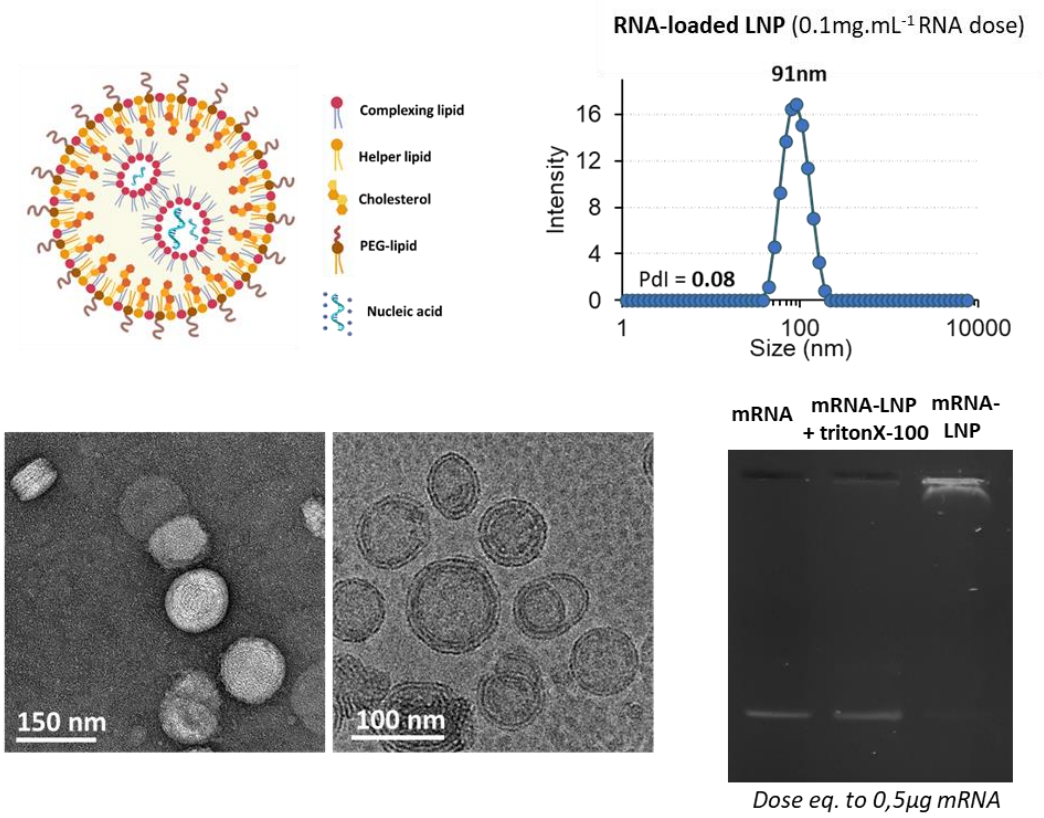


Fig. 1: Physico-chemical characterizations of NanOZ-LNP/mRNA: Size distribution and Polydispersity Index measurements by DLS, morphology and monodispersity observed by colour negative TEM and CryoTEM micrographies and mRNA integrity after formulation inside LNP monitored by agarose gel electrophoresis at mRNA dose equals to 0.5µg. Data Source: The results obtained by OZB, Marseille, .

The NanOZ-LNP/mRNA are monodisperse nanoparticles of spherical morphology with size usually ranging between 80-150nm and PDI<0.2. Once encapsulated, the integrity of the mRNA is verified by gel electrophoresis.

NanOZ-LNP *in vivo* data

90 mice were subcutaneously injected 4 times (D0, D14, D28 & D68) by different OZB lipids in complexes with self-amplifying RNA (saRNA) (n=5 per group) to evaluate the immunogenicity and toxicity; max. vaccination vol of 100 µL/per injection at 2µg RNA. The body weight was recorded and spleen, lymph and blood were collected for analysis.

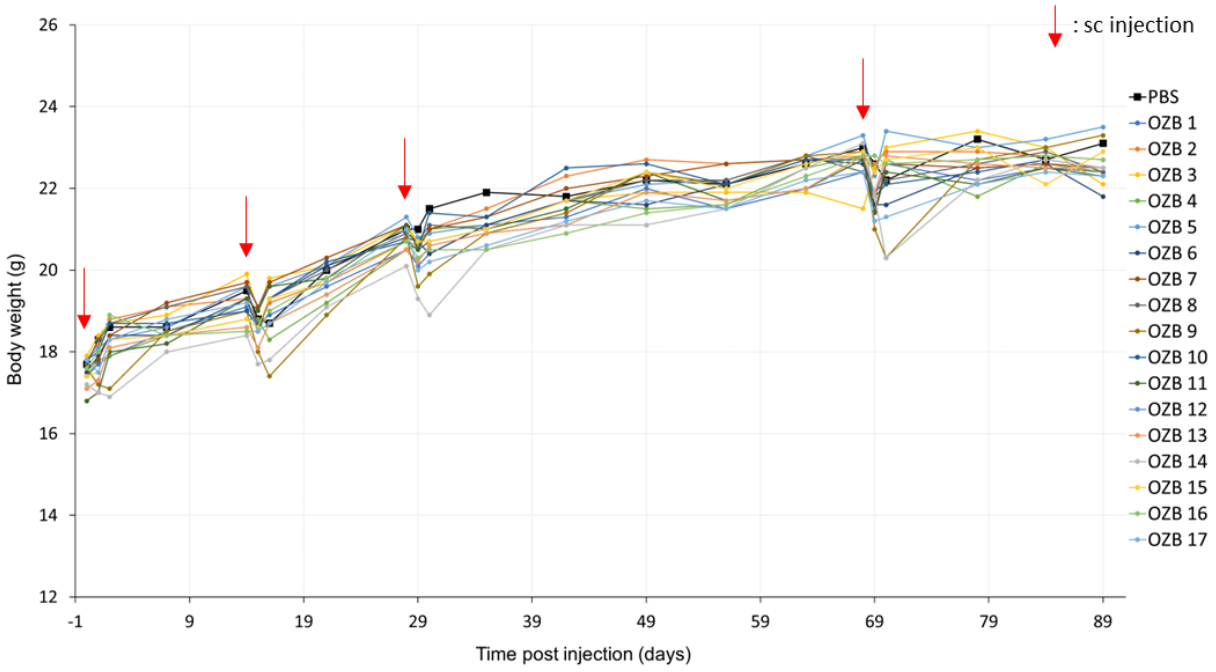


Fig. 2: Toxicity study of OZB complexes with Luc saRNA after four immunizations in mice. Data Source: The results were acheived by OZB, Marseille, whitin the UNIVAX consortium project: A "Universal" Influenza Vaccine through Synthetic, Dendritic Cell-Targeted, Self-Replicating RNA Vaccines. FP-7 (HEALTH-F3-2013-601738) supproted by the European Commission.

All the animals were in good health condition, no clinical symptoms of toxicity or other adverse reactions were observed after administration of four immunizations. All formulations were well tolerated by animals.

The biodistribution and transfection efficiency of NanOZ-LNP loaded with Firefly Luciferase mRNA (mRNA-Luc) was evaluated after i.p. injection of 10µg mRNA formulated in LNP in nude mice. Bioluminescence intensity was monitored by IVIS instrument over 25h.

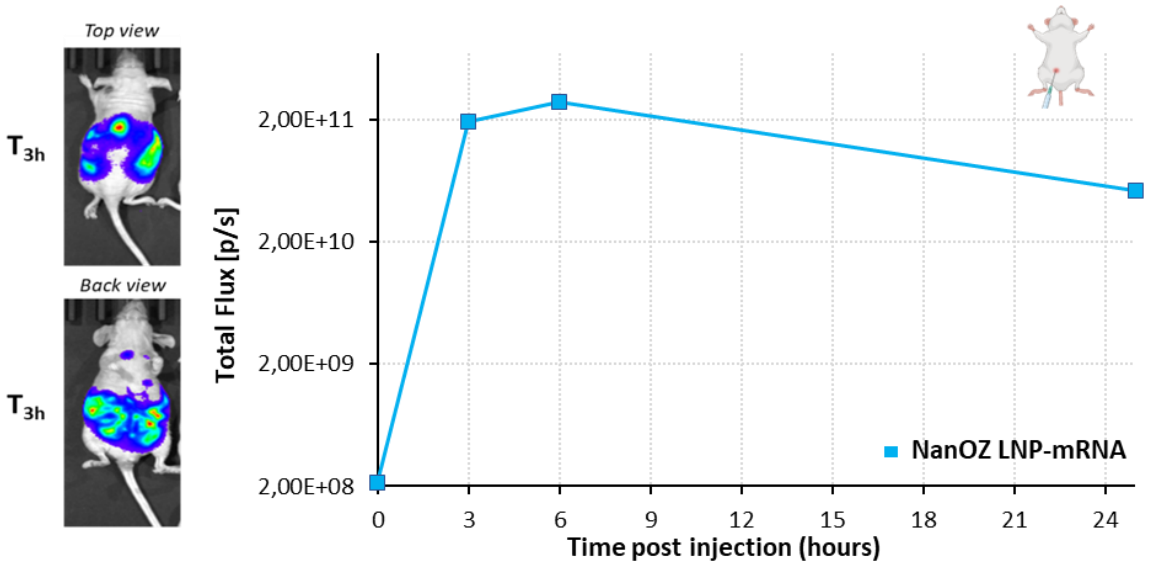


Fig. 3: IVIS bioluminescence signal 3h after i.p. administration of mRNA Luc-LNPs in nude mice. Kinetics of bioluminescence signal over 25h after i.p. administration of mRNA Luc-LNPs (dose equivalent to 10µg mRNA). Data Source: The results were acheived in collaboration with the TRACE PDX Platform, KU Leuven.

As observed a single intra-peritoneal (i.p.) injection of NanOZ-LNP/mRNA enables high bioluminescence signal and luciferase expression into surrounded organs for at least 25h (Figure 3).

The biodistribution and transfection efficiency of NanOZ-LNP loaded with Firefly Luciferase mRNA (mRNA-Luc) was evaluated after i.p. injection of 10µg mRNA formulated in LNP in nude mice with two formulations based on proprietary ionizable lipids compared with a LNP formulation based on DOTAP cationic lipid. Bioluminescence intensity was monitored by IVIS instrument over 25h.

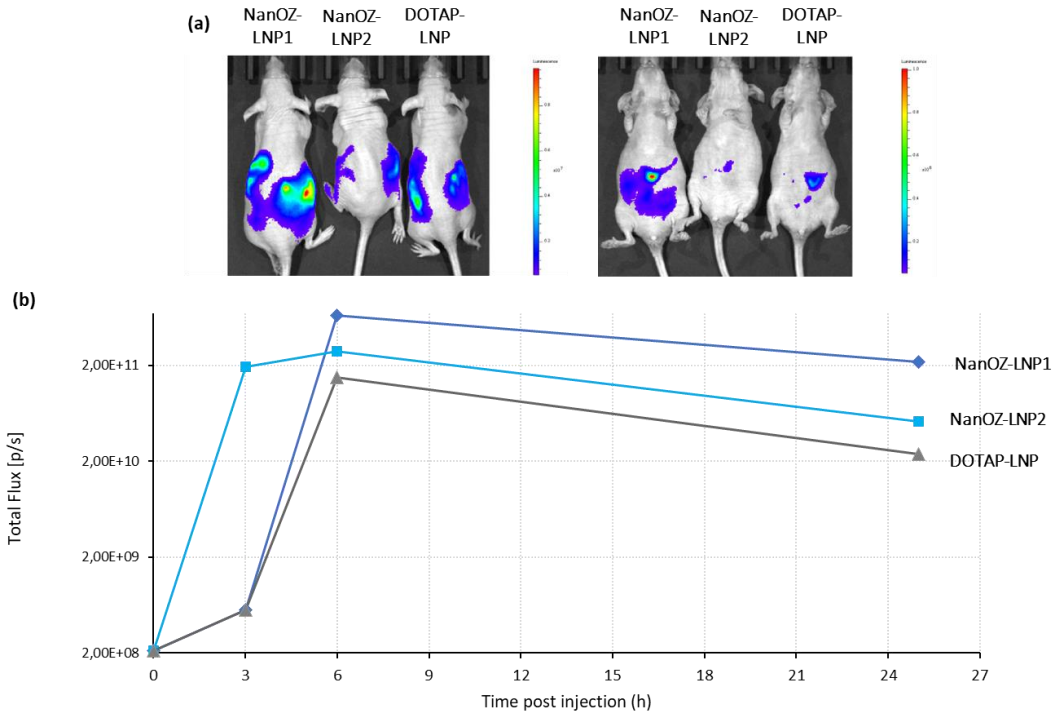


Fig. 4: IVIS images 6h after i.p. administration of 3 mRNA-LNPs formulation: NanOZ-LNP1, NanOZ-LNP2 and DOTAP-LNP in nude mice. Kinetics of bioluminescence signal over 25h after i.p. administration of 3 mRNA-LNPs formulation: NanOZ-LNP1, NanOZ-LNP2 and DOTAP-LNP in nude mice (dose equivalent to 10µg mRNA). Data Source: The results were acheived in collaboration with the TRACE PDX Platform, KU Leuven.

As observed, a single intra-peritoneal (i.p.) injection of Luc-LNPs enables high bioluminescence signal and luciferase expression into surrounded organs for at least 25h (Figure 4). The highest transfection efficiency was observed for the NanOZ-LNP1 formulation.

Nude mice were injected i.p. with Firefly Luciferase mRNA (mRNA-Luc) formulated into 3 different LNP formulations at 10µg RNA dose to evaluate the nucleic acid delivery into organs; The liver, lung, kidney and spleen were collected and analysed through RT-PCR.

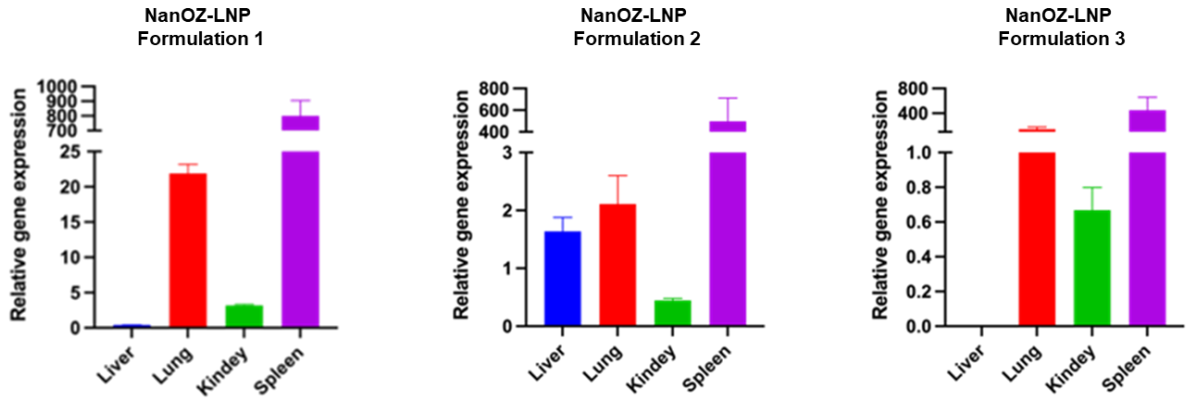
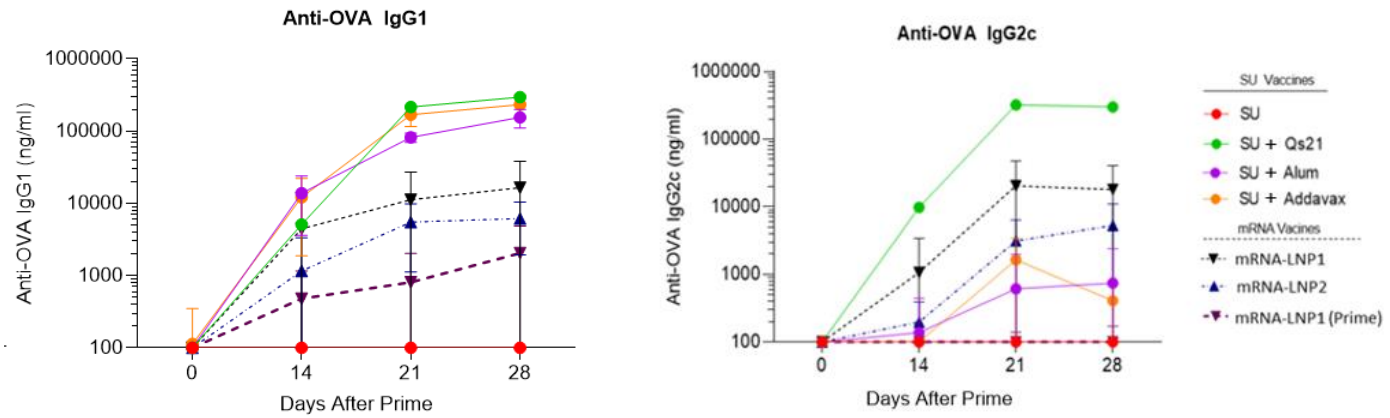


Fig. 5: Relative gene expression from firefly luciferase in liver, lungs, kidney and spleen after i.p. injection of 3 mRNA-LNPs (10µg mRNA dose) formulations. Data Source: The results were acheived in collaboration with the TRACE PDX Platform, KU Leuven.

The LNPs are efficient non-viral nanocarriers for the *in vivo* delivery of nucleic acids to different organs. Notably, the modulation of the formulation parameters (e.g. lipids nature, size, zeta potential…) enables the biodistribution to specific organ.

NanOZ-LNP *in vivo* immunization results

The comparison between mRNA-based vaccines versus subunit vaccine for immune efficacy were evaluated in C57BL6J mice using the ovalbumin model. Prime boost (D0-14) immunizations were evaluated with OVA antigen delivered by mRNA-based LNPs, where two different formulations were tested corresponding to LNP1 and LNP2, or by recombinant protein (alone or adjuvanted). To characterize the immuno-phenotyping of the mice the sera were collected at D0, D14, D21 and D28 and splenocytes were collected at D28 and analysed by ELISA, Elispot and Multiplex. The mice were immunized by s.c. route with 10µg OVA subunit (SU) or 10µg mRNA doses (100µL per injection).



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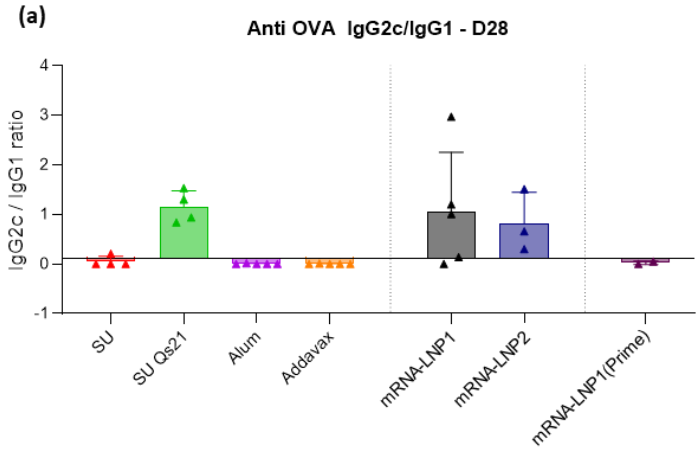

Fig. 6: Anti-OVA IgG production and (a) Th1 humoral biomarker to OVA subunit vaccine (10µg) in absence or presence of vaccines adjuvants (QS21 (5µg), Alum (Aluminum Hydroxide gel)1X or addaVax 1X) and to mRNA(OVA)-LNPs (mRNA dose equals to 10µg) upon sub-cutaneous prime boost injection (D0-14) in C57BL6J mice. Data Source: The results were acheived by OZ Biosciences in collaboration with Dr. Antoine Tanne, SaponiQx, Lexington, MA, USA.

RNA vaccine LNP formulations are efficient at priming a strong Th1 humoral response equivalent to the best TH1 adjuvants such as QS-21 upon sub-cutaneous prime boost injection (D0-14) in C57BL6J mice. In addition, no significative signal was observed when the OVA antigen is directly administrated. It is noteworthy, the necessity of two injections (boost at D14) to obtain a long-lasting immune response.

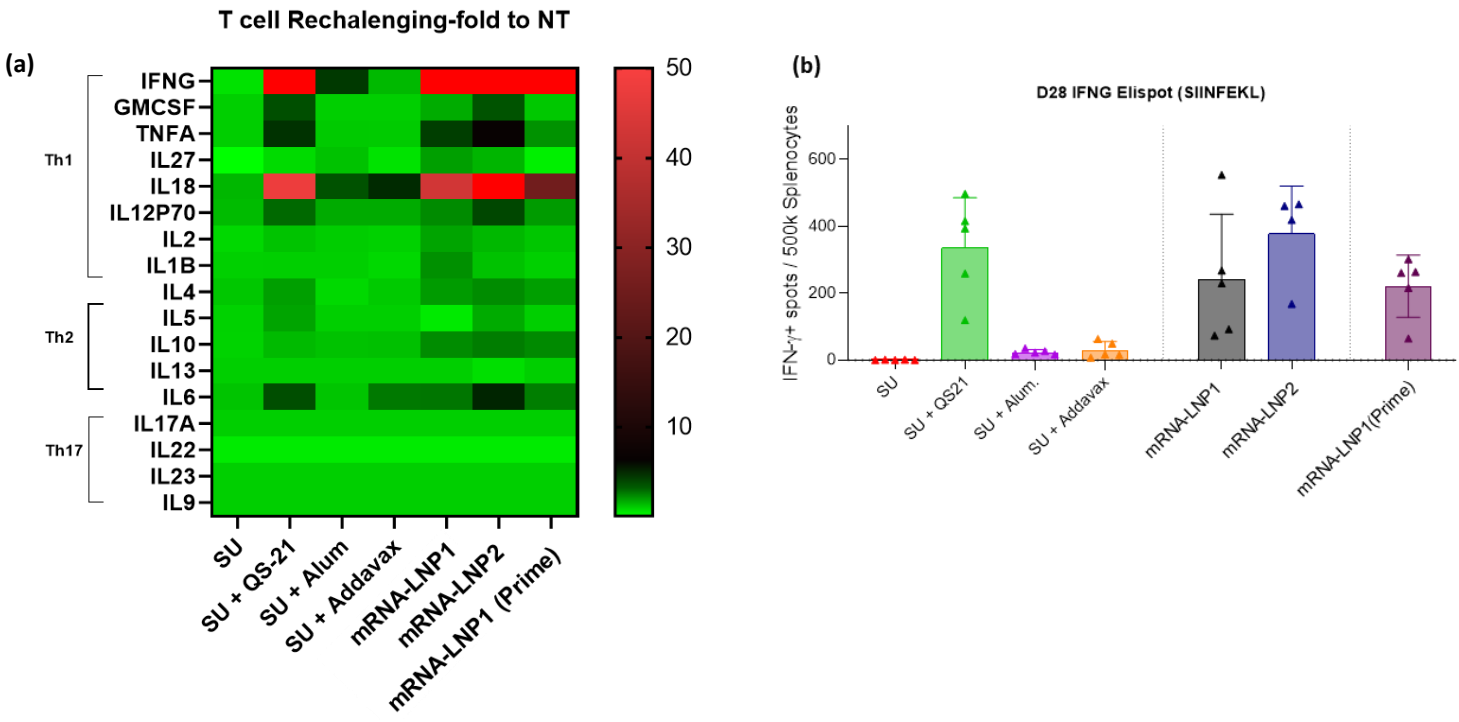

Fig. 7: (a) T cellular response and (b) CD8+ T cell response to OVA subunit vaccine (10µg) in absence or presence of vaccines adjuvants (QS1 (5µg), Alum 1X or addaVax 1X) and to mRNA(OVA)-LNPs (mRNA dose equals to 10µg) upon sub-cutaneous prime boost injection (D0-14) in C57BL6J mice. Data Source: The results were acheived by OZ Biosciences in collaboration with Dr. Antoine Tanne, SaponiQx, Lexington, MA, USA .

RNA vaccine LNP formulations are efficient at priming a strong TH1 humoral response and at stimulating IFN-γ and IL-18 production upon sub-cutaneous prime boost injection (D0-14) in C57BL6J mice. In addition, no significative immune response was observed when the OVA antigen is directly administrated.

In conclusion, NanOZ-LNP are promising candidates for developing of highly efficient mRNA-based vaccines by combined both strong humoral and T-mediated cell responses.

In vitro vs *in vivo* evaluation of LNPs

Comparison study between the transfection efficiency of Luciferase mRNA lipoplexes versus LNPs for both *in vitro* (HEK293 cells) and *in vivo* (nude mice) models.

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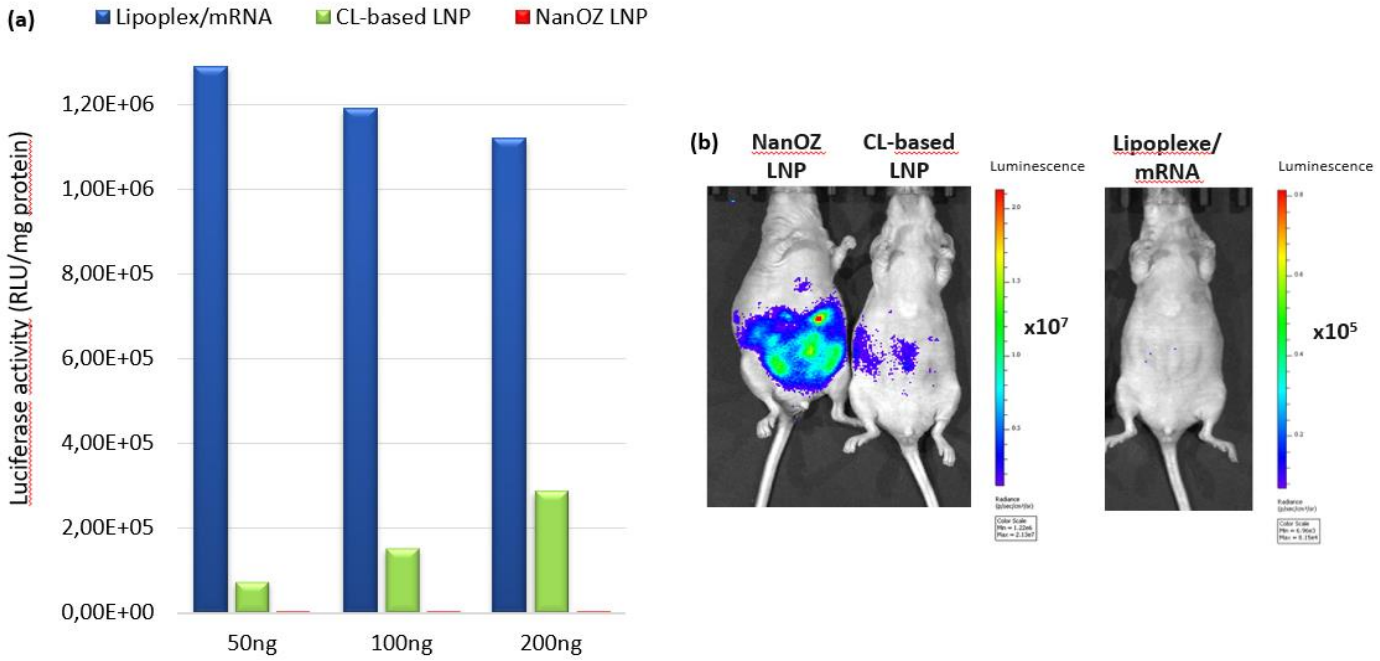


Fig. 8: Comparison of transfection efficiency. (a) *in vitro* evaluation: HEK293 Cells (1x10^5 cells/well) were transfected with different amount of Luc mRNA: 50ng, 100ng and 200ng formulated in Lipoplex (RmesFect), Cationic Lipid(CL)-based LNP and NanOZ-LNP and the Luciferase activity was monitored 24h after incubation of the formulations by Luciferase assay. (b) *in vivo* evaluation: IVIS images of bioluminescence signal 3h after i.p. injection of 10µg Luc mRNA formulated in Lipoplex (DOTAP), Cationic Lipid(CL)-based LNP and NanOZ-LNP in nude mice. Data Source: The results were acheived by OZB, Marseille and in collaboration with TRACE PDX Platform, KU Leuven.[A3]

For the *in vitro* study, the transfection outcome indicated that the highest transfection efficiencies were obtained with mRNA formulated into lipoplex (e.g. Rmesfect). The luciferase activity of transfection with NanOZ LNP was negligible mainly due to the presence of lipid-PEG that reduces *in vitro* efficiency while enhancing *in vivo* delivery. In addition, only a slight signal was obtained for cationic-lipid (CL)-based LNP where the cellular uptake is facilitated due to the permanent cationic charge. The reverse tendency was observed when the formulations were injected in nude mice with the highest transfection obtained when using LNP nanocarrier.

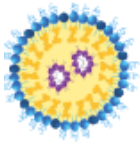
These results highlighted that LNPs are mainly designed and formulated for *in vivo* application and not really efficient for *in vitro* application in comparison to classical transfection reagents. Nonetheless, LNPs can be functionalized onto the surface (e.g. mAb, Apolipoprotein E, mannose…etc) to facilitate the entry of NPs into the cells and have been reported to be a promising option for some cells such as T lymphocytes.

OUR CUSTOM SERVICES

mRNA Synthesis

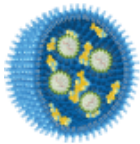
- Gene synthesis, Cloning & DNA template production.
- *In vitro* Transcription.
- Purification & Quality control.

NanOZ-LNP™ Design Platform



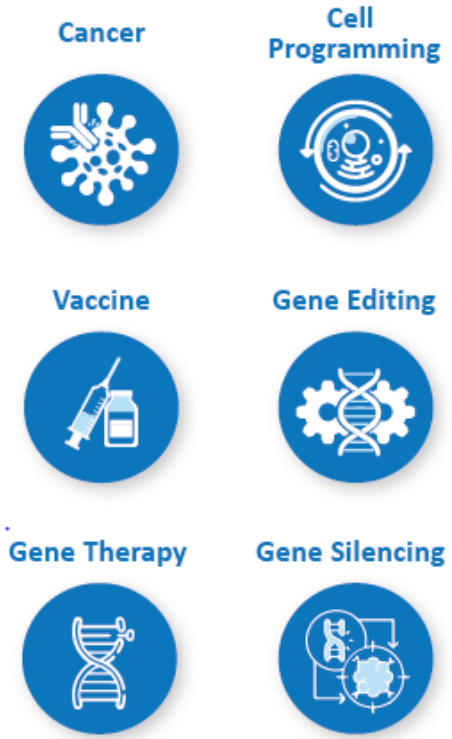
- Lipid Chemistry & Functionalization.
- Formulation Design & Manufactring.
- NanOZ-LNPs™ Custom.

Customer DNA, RNA, API



- Provide us with your molecule of interest and we will formulate it into LNPs

BIOMEDICAL APPLICATIONS



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The art of delivery systems

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