Non-viral *in vivo* mRNA delivery for cancer research, vaccination or gene therapy

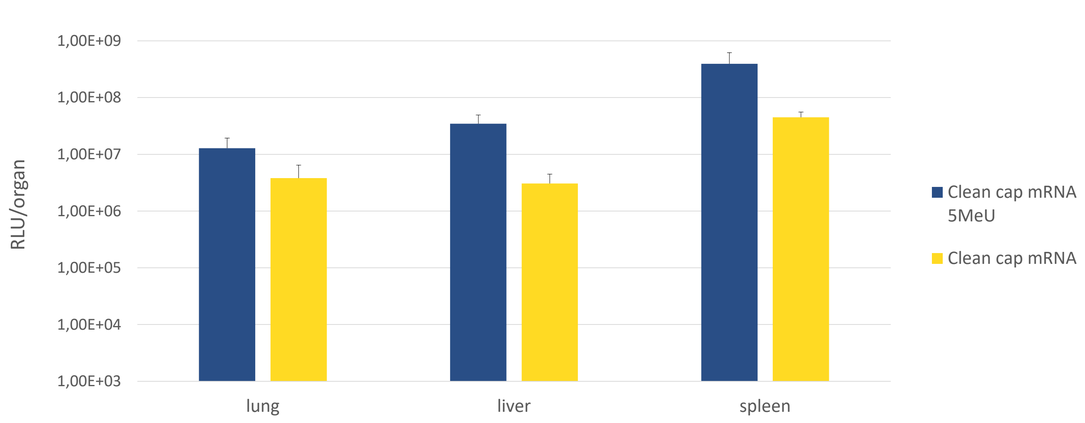
Valérie Toussaint-Moreau, Thibaut Benchimol, Fanny Prémartin, Perrine Friedel, Géraldine Guérin-Peyrou, Maxime Dumont, Fabrice Stock, Patrick Erbacher

Polyplus-transfection, Bioparc, 850 Boulevard S. Brant, 67400 Illkirch, France

Abstract

A major challenge to the use of nucleic acids in both Vaccination and Gene therapy is its efficient *in vivo* delivery to the targeted cells or site of action. Delivery of mRNA *in vivo* is even more challenging, because mRNA is less stable, and can elicit an immune response. Therefore, we developed a non-viral delivery reagent that efficiently encapsulates mRNA to i) protect mRNA from degradation and ii) prevent triggering of an unwanted immune response. Here we demonstrate that our cationic reagent can be used to directly inject mRNA via local and systemic administration routes to efficiently reach a wide range of tissues (including spleen and lymph nodes). Furthermore, the transient nature of mRNA transfection can be beneficial for a number of other applications, including cellular reprogramming, genome editing (CRISPR/Cas9) and vaccines.

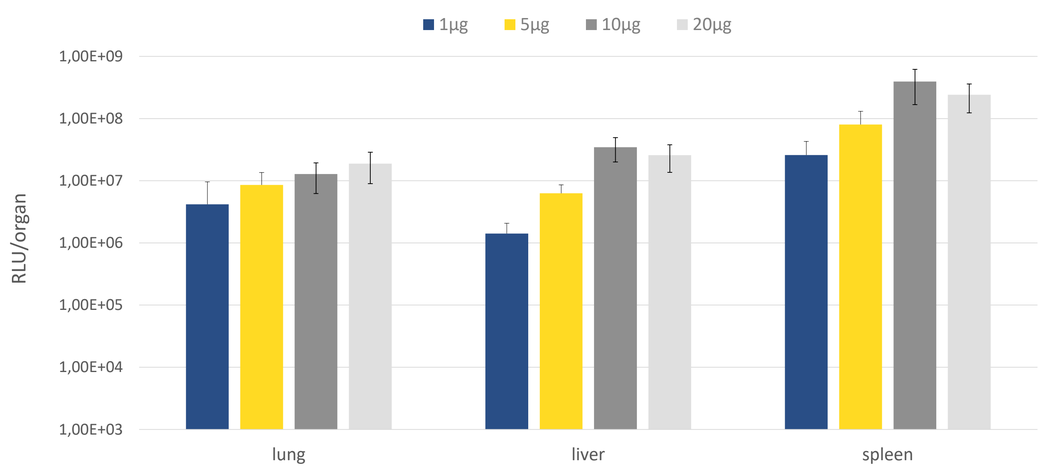
Chemical modifications of mRNA improve gene expression



mRNA encoding Luciferase injected into mice using *in vivo*-jetRNA® through retro-orbital injection. Complexes were formed using 10 µg of mRNA and 10 µL of *in vivo*-jetRNA® (ratio mRNA/*in vivo*-jetRNA® of 1:1 - μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer. Luciferase expression was assessed 24 h post-injection.

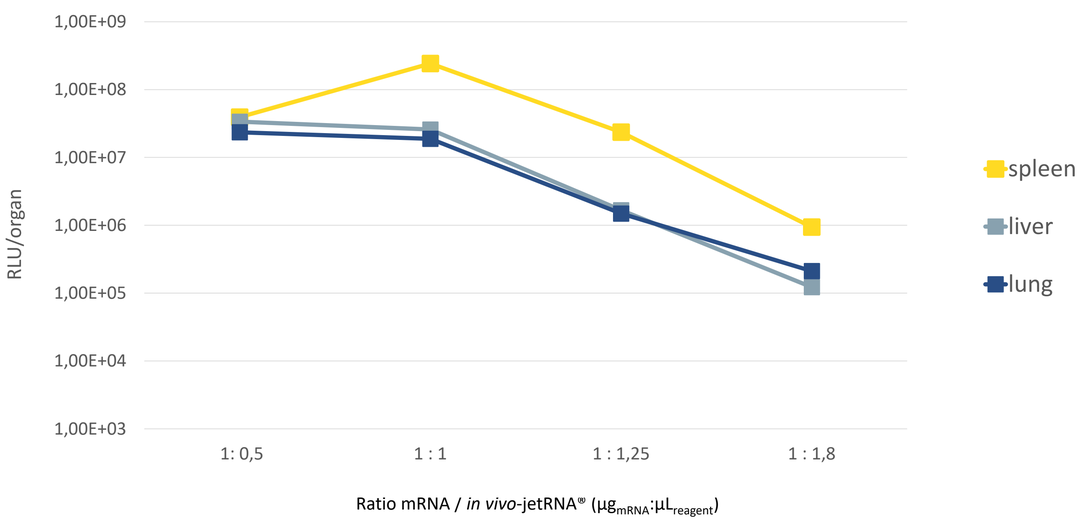
Protocol optimization

Determination of the optimal mRNA amount



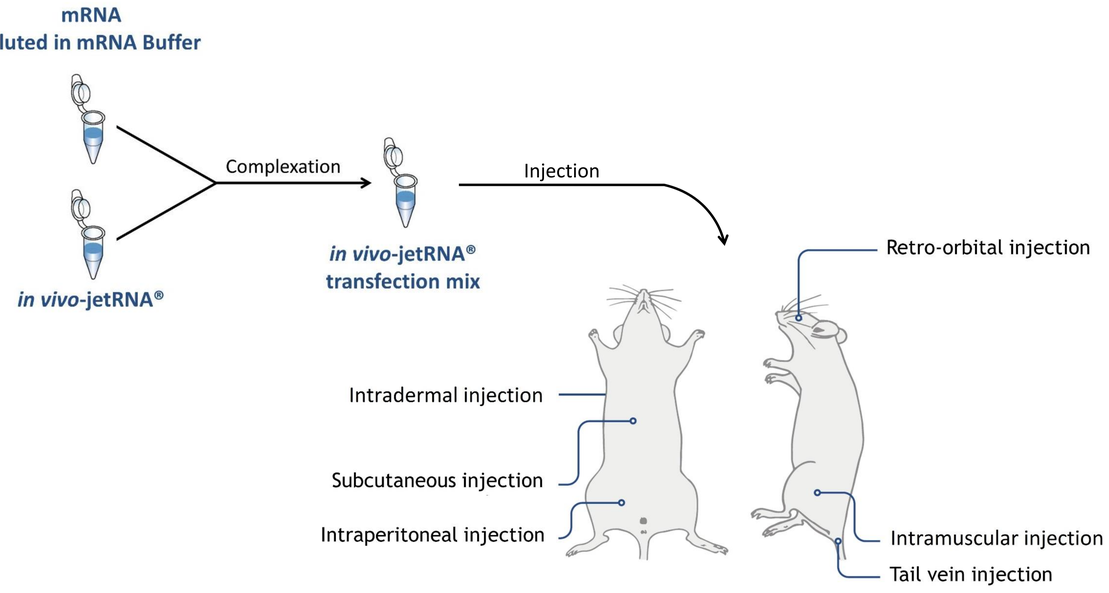
Different amounts of mRNA encoding Luciferase were injected into mice using *in vivo*-jetRNA® through retro-orbital administration route. Complexes were formed using different quantities of mRNA with a mRNA/*in vivo*-jetRNA® ratio of 1:1 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer. Luciferase expression was assessed 24 h post-injection.

Determination of the optimal volume of *in vivo*-jetRNA®



mRNA encoding Luciferase was administered into mice using *in vivo*-jetRNA® through intravenous injection (retro orbital injection). Complexes were formed using 20 µg of mRNA and different volume of *in vivo*-jetRNA®. Luciferase expression was assessed 24 h post-injection.

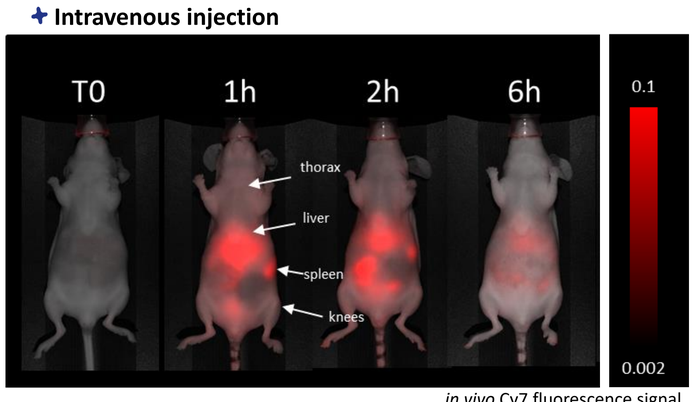
Easy and fast protocol



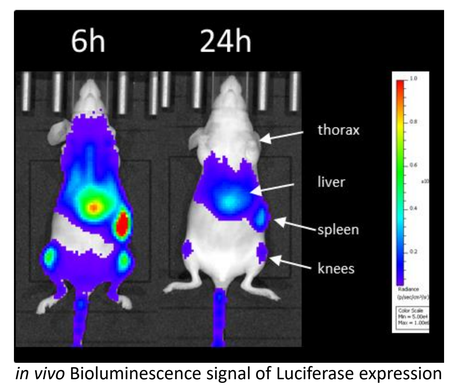
Examples of delivery routes using in vivo-jetRNA® in mice

Biodistribution of mRNA/*in vivo*-jetRNA® complexes

✚ **Intravenous injection**

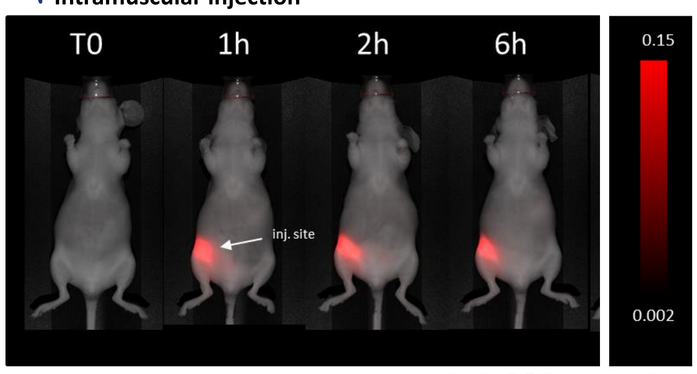


in vivo Cy7 fluorescence signal

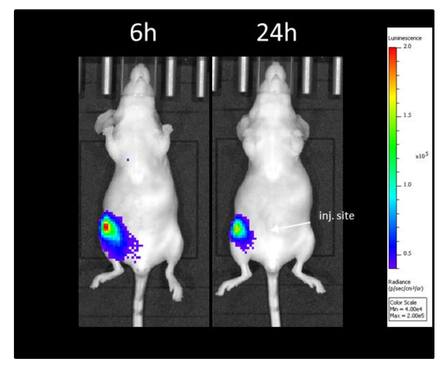


in vivo Bioluminescence signal of Luciferase expression

✚ **Intramuscular injection**

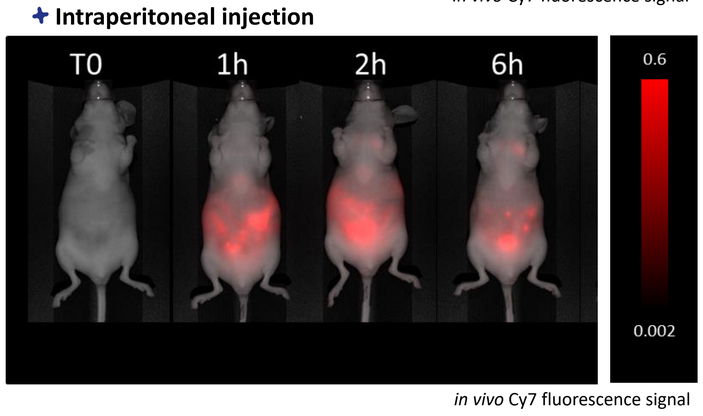


in vivo Cy7 fluorescence signal

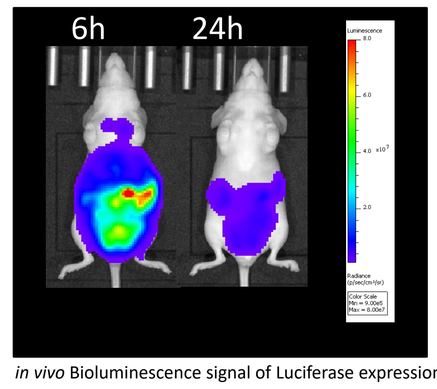


in vivo Bioluminescence signal of Luciferase expression

✚ **Intraperitoneal injection**



in vivo Cy7 fluorescence signal



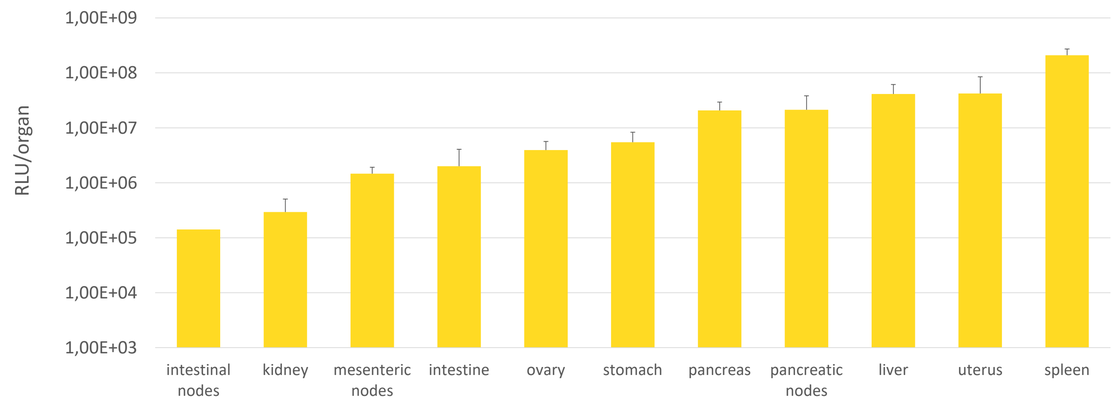
in vivo Bioluminescence signal of Luciferase expression

Cy7 labeled mRNA encoding for Luciferase was injected into mice using *in vivo*-jetRNA® through different administration routes. Complexes were formed with a mRNA/*in vivo*-jetRNA® ratio of 1:1 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer using either 2.5 µg, 10 µg or 20 µg of mRNA for respectively intramuscular, intravenous or intraperitoneal injections. Fluorescence signals were observed with Bioluminescence imaging using *IVIS system (PerkinElmer)* for both Cy7 (on the left) and Luciferase (on the right)

Plateforme OPTIMAL, Imagerie optique du petit animal, INSERM U1209 – Université Grenoble Alpes

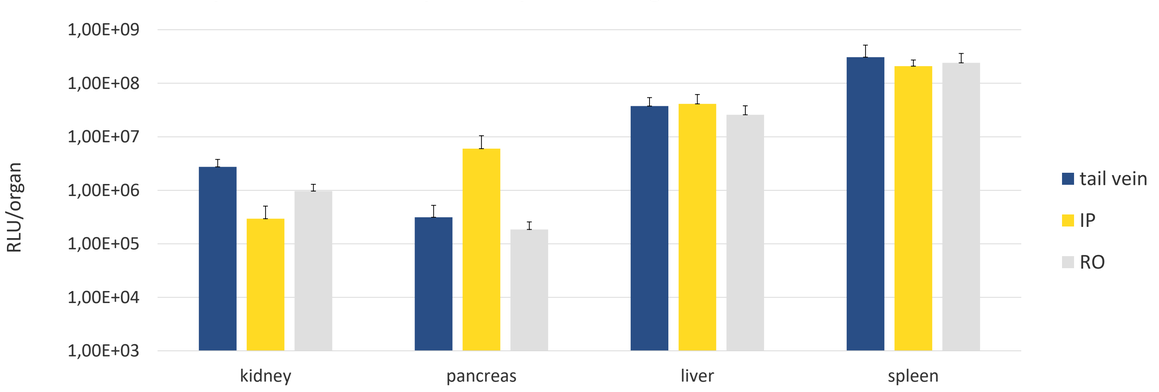
mRNA expression profile using *in vivo*-jetRNA®

mRNA delivery via intraperitoneal administration route



mRNA encoding Luciferase was injected into mice using *in vivo*-jetRNA® through intraperitoneal injection. Complexes were formed using 40 µg of mRNA with an mRNA/*in vivo*-jetRNA® ratio of 1:1 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer. Luciferase expression was assessed 24 h post-injection.

Comparison of mRNA expression profiles using different administration routes



mRNA encoding Luciferase was injected into mice using *in vivo*-jetRNA® through different administration routes. Complexes were formed with an mRNA/*in vivo*-jetPEI ratio of 1:1 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer using either 10 µg mRNA for intravenous injection (retro-orbital injection – RO or tail vein injection) or 20 µg mRNA for intraperitoneal (IP) injection. Luciferase expression was assessed 24 h post-injection.

Conclusion: advantages of *in vivo*-jetRNA®

- ✚ **Efficient: High gene expression with low amount of mRNA**
- ✚ **Adaptable: Suited for any injection route to target any organ**
- ✚ **Time-saving: Ready-to-use reagent with an easy protocol**
- ✚ **Tailored: Customized protocols from *in vivo* delivery expert**

Nous contacter

Service technique
Réactifs : 01 34 60 60 24 - tech@ozyme.fr
Instrumentation : 01 30 85 92 88 - instrum@ozyme.fr