DryLabUpdates

The previous docx file I uploaded had all the progress I made till that point. This one has further updates. Progress was mainly made on LNP PK (which sums to LNP exposure to tissues) and PD (gene expression and efficacy). The following primary questions were dealt with:

1. What is the translation efficiency of our mRNA-LNPs?
2. What is the efficiency with which LNPs are taken up by the cells?
3. What is the general behaviour of LNPs with respect to phharmacokinetics?

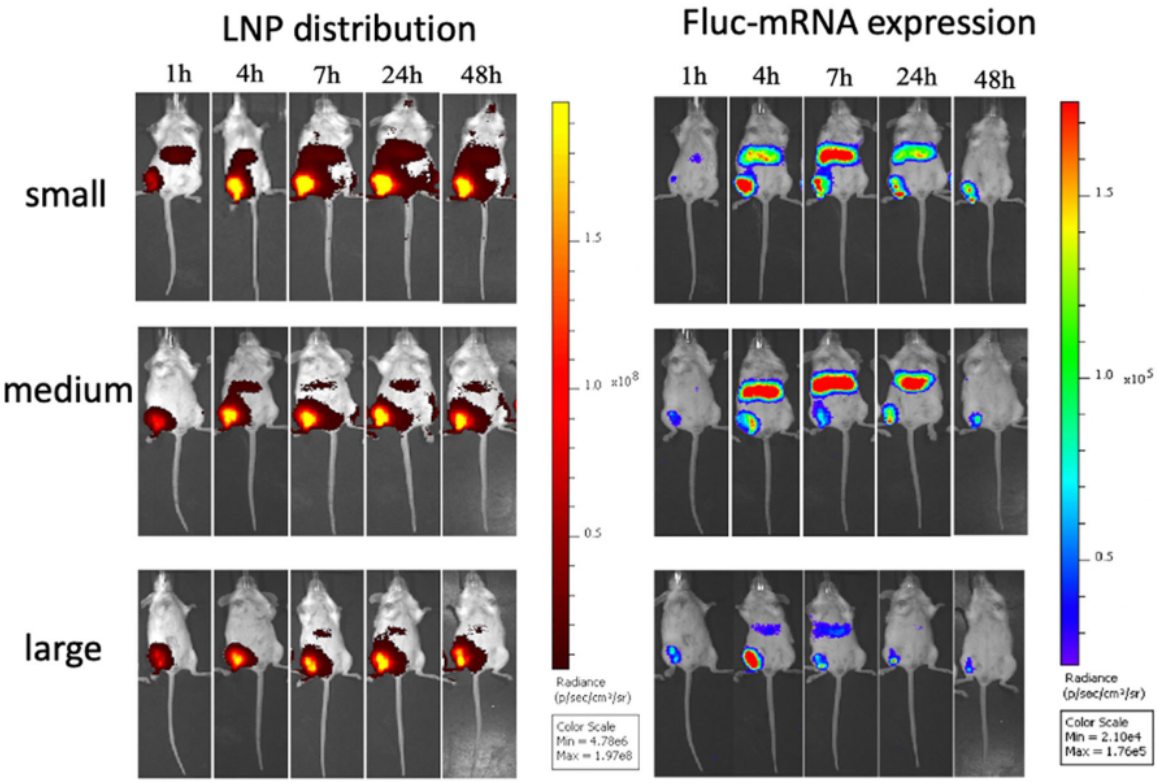
tl;dr: I didn't look into solid data pertaining to our **specific** EDB-LNP, however the studies do point out a lot about mRNA LNP PK and also emphaize the fact that there is no simple correlation between amount of LNPs taken up by cells and mRNA translation efficiency. The studies also point out the issues of trying to model PK without wet lab input.

LNP PK/PD:

- Two main papers deal with this:
  - Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes
  - Biodistribution and Non-linear Gene Expression of mRNA LNPs Affected by Delivery Route and Particle Size
- Both the papers modeled and recorded translation periods of mRNA-LNPs.
- Different translation periods and efficiency were obtained on administering LNPs through different routes.

1. Those injected through IVs had a very high protein production peak that occurred quite early. However, the translation stopped in a comparatively short period of time.
2. Intra-Peritoneal Injections gave a relativity smaller protein production peak, however the translation continued for a longer time.
3. All injection methods produced almost the same amount of total protein (IV, Intradermal, Intramuscular, Peritoneal, etc.)
4. Smaller LNPs have a tendency to circulate to places away from site of injection.

1. IV has the most amount of circulation
2. Subcutaneous injections have the least amount of transport away from injection site. However, subcutaneous injections consistently displayed poor level of mRNA translation and protein production.
3. A lot of LNPs get accumulated in the liver.



5.
6. Larger LNPs stay at the site of injection more.
    1. However, our LNPs is probably going to be ~100nm, which is a smaller size, so we can expect a lot of transport.

Statistical Analysis aspects:

- The paper on Expression Kinetics used a standard formula for half-life of mRNA:
- $$t_{1/2} = \Delta t \ln(2) / \ln(N_0/N_t)$$
- Δt is the time between measurements, N_0 is the starting value and N_t is the value at the end of time interval.
- However, as obvious, the required values can only be obtained through wet-lab observations.

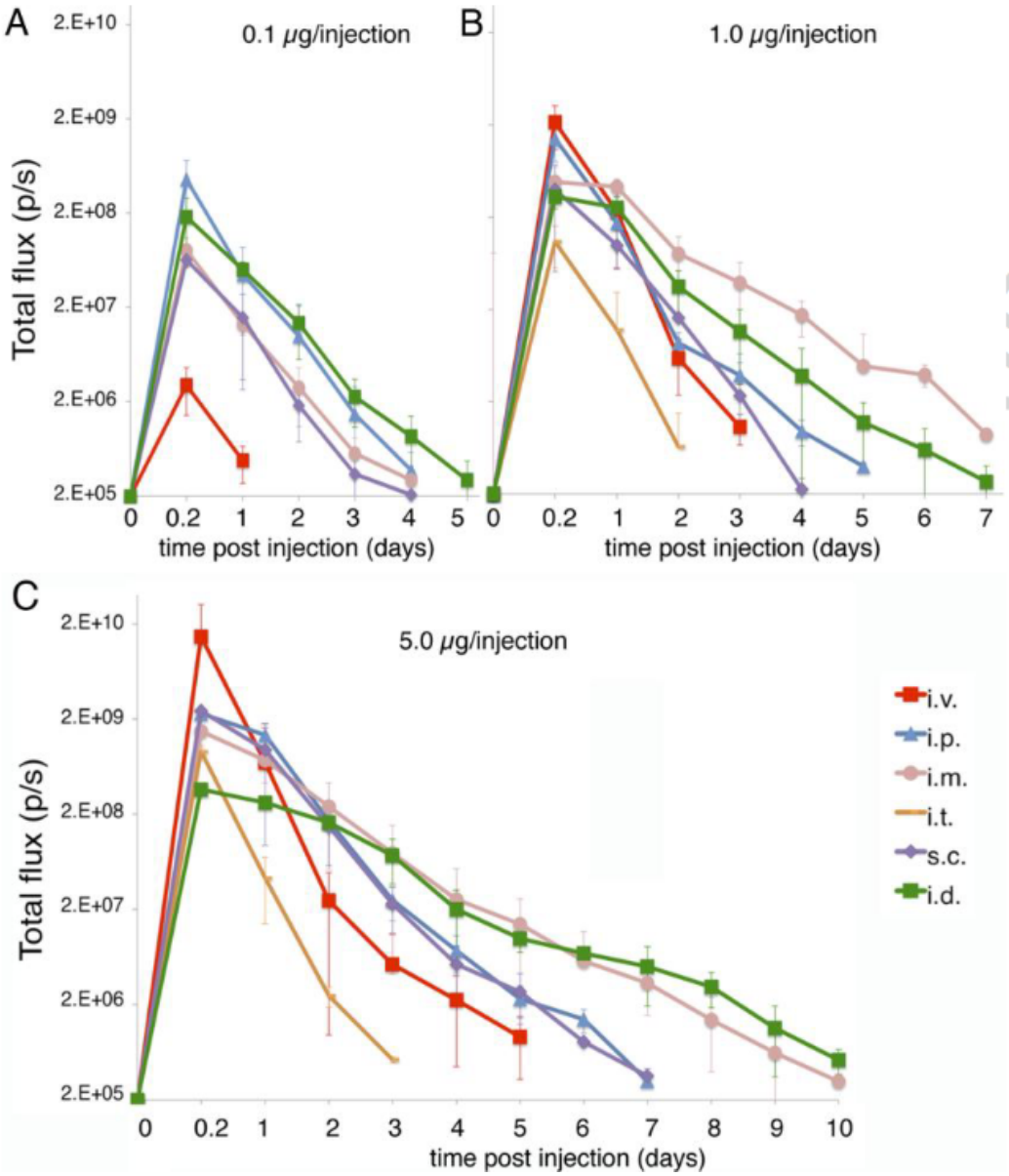
Why they're relevant:

- The studies were conducted on HEK293T cell lines.

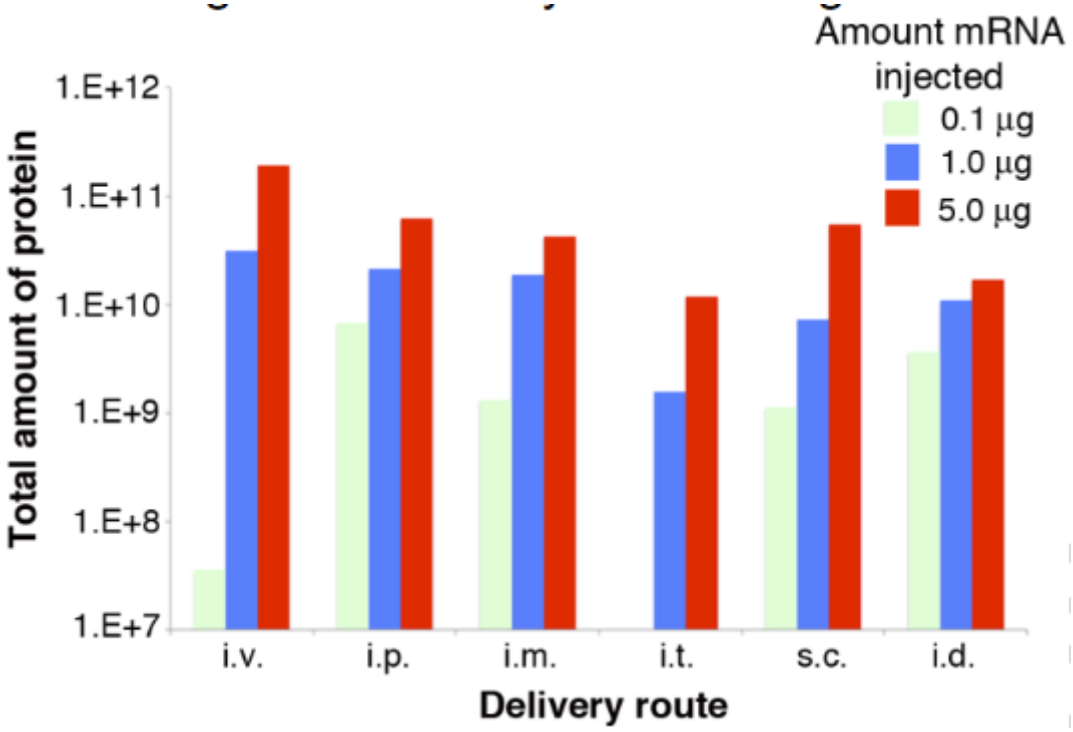
- From what I gather, LNP composition was a standard one too.
- They show that LNP sizes varying with mRNA concentration present.
- Both studies used luciferase to correlate amount to protein produced. Hence, wet lab input is essential for proper PK/PD modelling.

Relevant Conclusions:

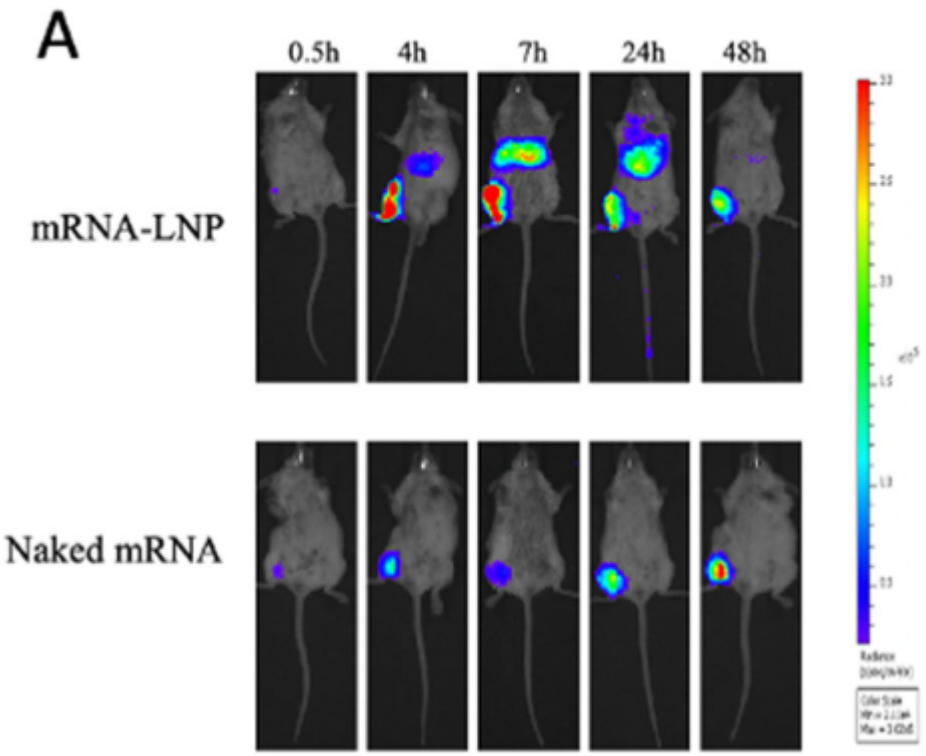
1. Higher concentration of mRNA-LNPs increased protein production efficiency through IVs, while other means of injection had similar efficiencies for lower as well as high concentration of mRNAs:



2. Total produced protein was similar for all methods:



3. All measurements were done using firefly luciferase encoding mRNA and subsequent fluorescence bioimaging. The authors recognized the fact that circulation of LNPs in the system and their different translational efficiencies in different systemic regions makes it difficult to come with a standard model for PK-PDs of LNPs.
4. The expression kinetics study suggests upto 10 days of protein production in case of luciferase.
5. The biodistribution paper shows, through observations that mRNA-LNPs are more efficient for protein production as compared to naked mRNA:



"PK-PD relationship of mRNA-LNPs is highly complex, making the prediction of gene expression and efficacy (PD) unlikely just based on the LNP exposures in tissues (PK). Nevertheless it is prudent to fully characterize the PK in order to optimize the PD and minimize the toxicities of a gene delivery product."