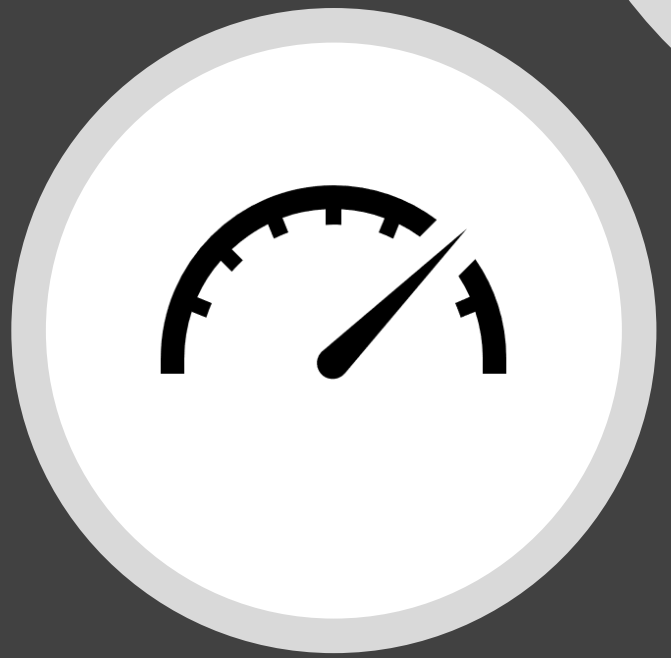
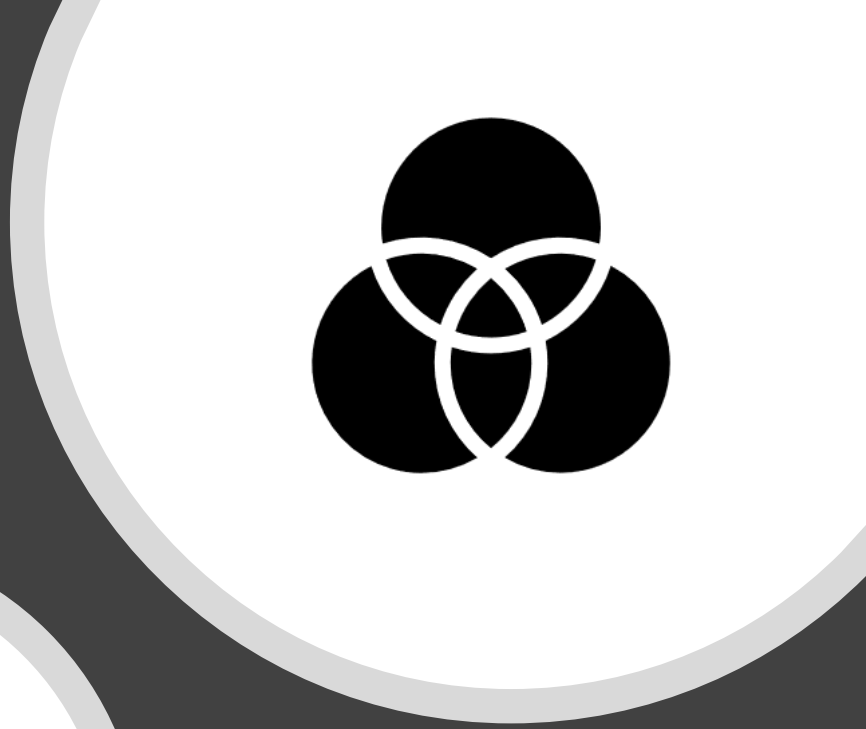
# Delivering mRNA Vaccines.

Yvonne Perrie et al. CRS 2022 11- 14 July 2022

# Research objectives

| | |
|---|---|
| Apply | Apply a scale-independent platform for RNA-delivery systems |
| Compare | Compare the route of administration |
| Investigate | Investigate different nanoparticle delivery platforms |



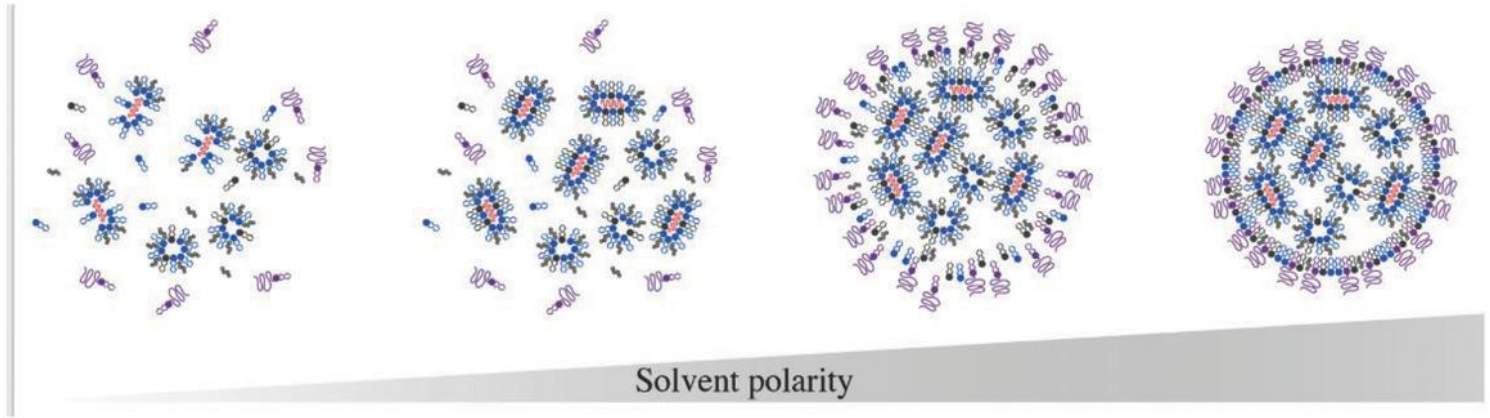




# LNP production method

mRNA

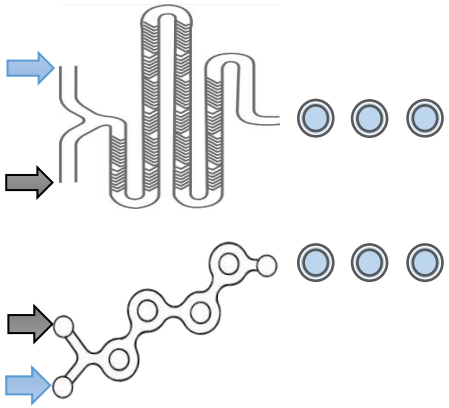
Lipids in solvent




LNPs

pH adjusted so that the ionisable lipid is cationic and binds to the RNA

The pH is then raised to 7.4, resulting in the outside of the LNPs being neutral.



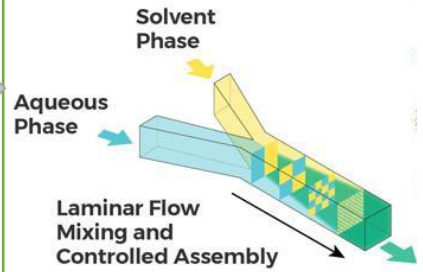
Staggered herringbone mixer

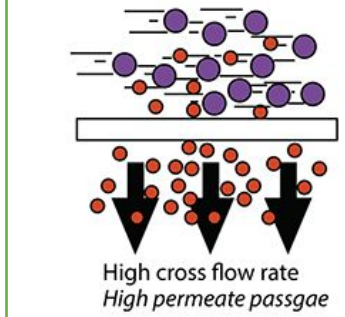
Toroidal mixer

Small Methods, Volume: 2, Issue: 9, First published: 26 April 2018, DOI: (10.1002/smtd.201700375)

# Production Protocols

| Substances | Process step |
| --- | --- |
| Lipids in ethanol | Manufacture using microfluidics |
| mRNA in buffer (Citrate buffer, 50 mM, pH 4) | |
| PBS | Purification using cross-flow filtration |

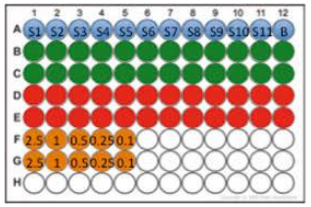



# CQAs measured

**Physico-chemical**
Size
PDI
Zeta Potential

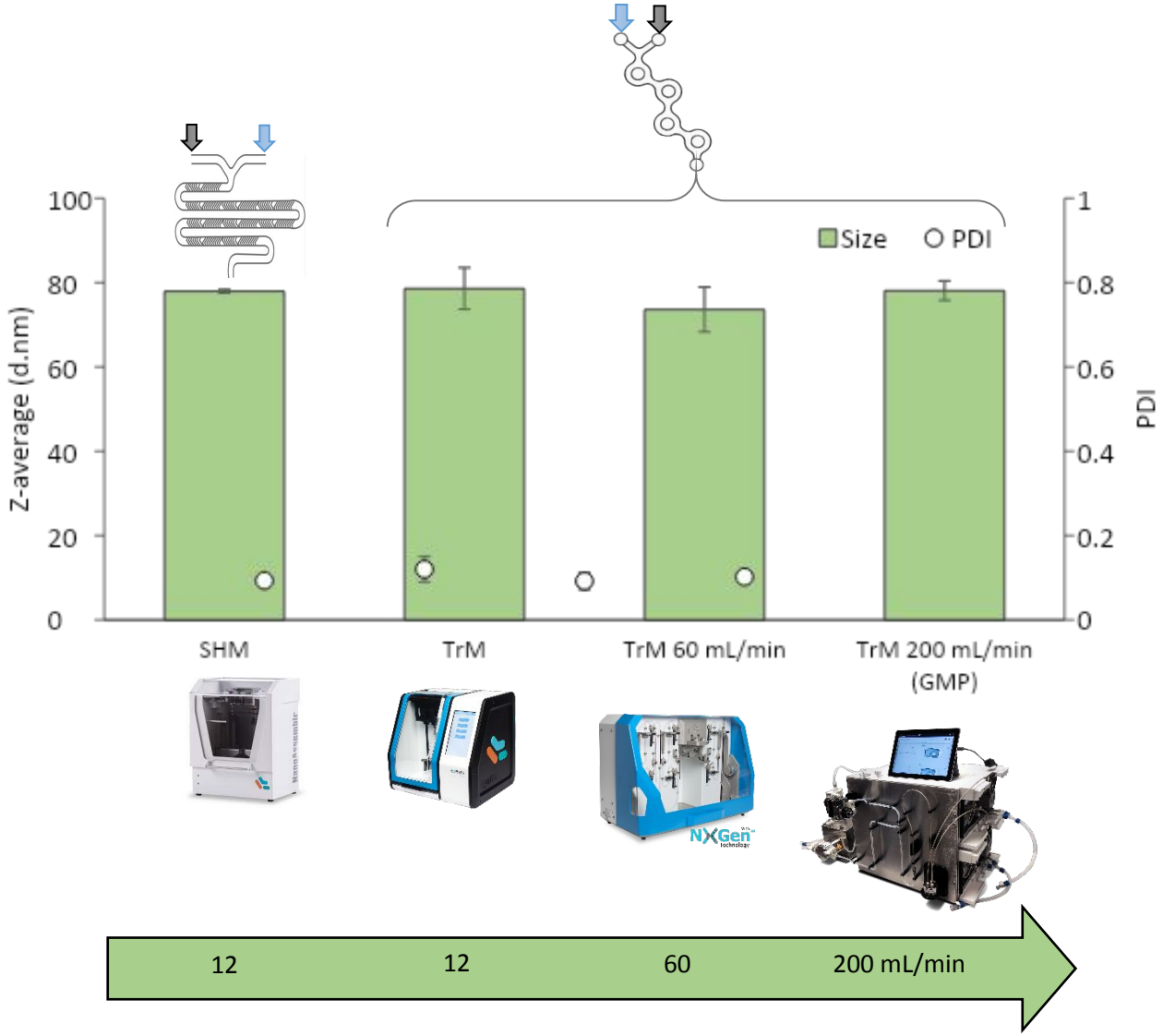
**mRNA content** (Ribogreen Assay)
mRNA loading (%EE)
Mass Balance/Yield (%MB)

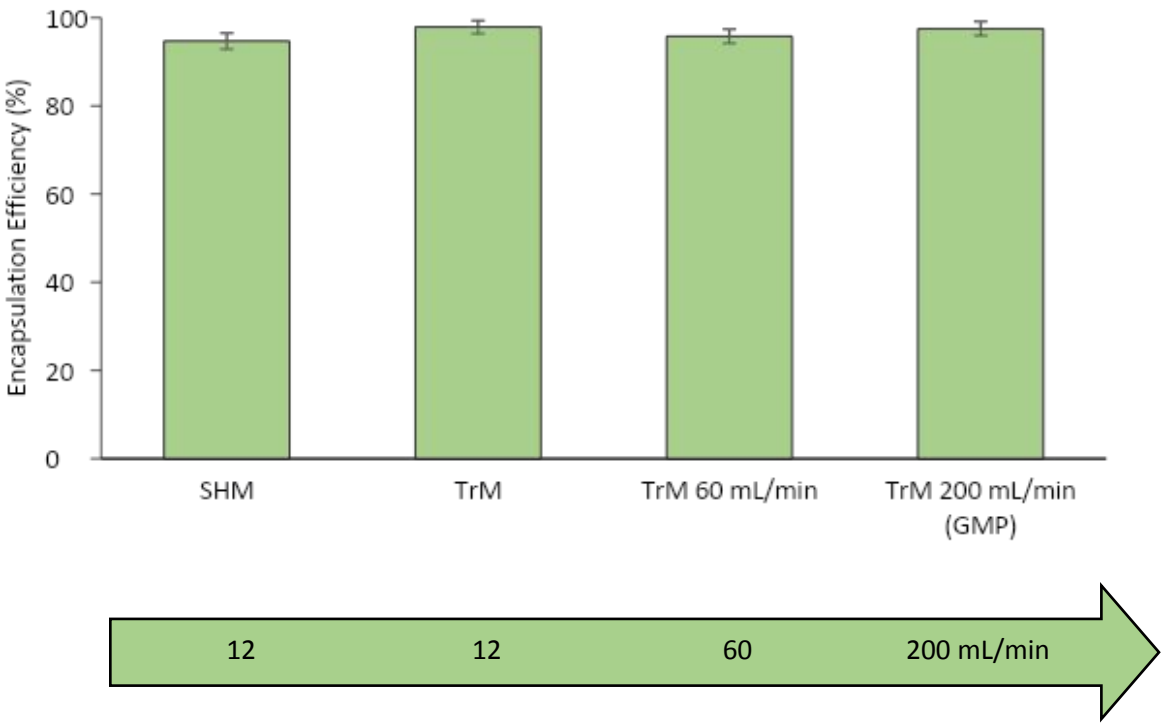


**In vitro potency**
Protein expression (24 h)

**In vivo potency**
Luciferase expression in Balb/c (6 h)
mRNA expression in vivo (6 weeks)

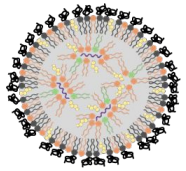
# Scale-independent production from bench to GMP



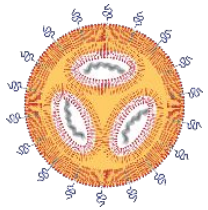




Manufacturing Considerations for the Development of Lipid Nanoparticles Using Microfluidics. Roces CB, Lou G, Jain N, Abraham S, Thomas A, Halbert GW, Perrie Y. *Pharmaceutics* 2020, *12*(11), 1095;

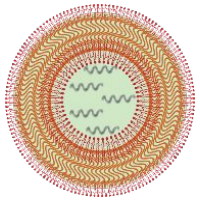
# Alternative nanoparticles



LNPs



Solid lipid nanoparticles



Polymeric nanoparticles

| Process Parameters | | Physico-chemical Characteristics | | | |
|---|---|---|---|---|---|
| Mix ratio (Aq:Solv) | Total flow rate | Size (d.nm) | PDI | Zeta-potential (mV) | saRNA E.E. (%) |
| 3:1 | 15 mL/min | 74 ± 1 | 0.09 ± 0.01 | 2.8 ± 1 | 97 ± 1 |
| 3:1 | 15 mL/min | 64 ± 2 | 0.14 ± 0.01 | 7.3 ± 7 | 97 ± 1 |
| 1:1 (DMSO) | 15 mL/min | 76 ± 7 | 0.16 ± 0.01 | 29.6 ± 19 | 98 ± 1 |

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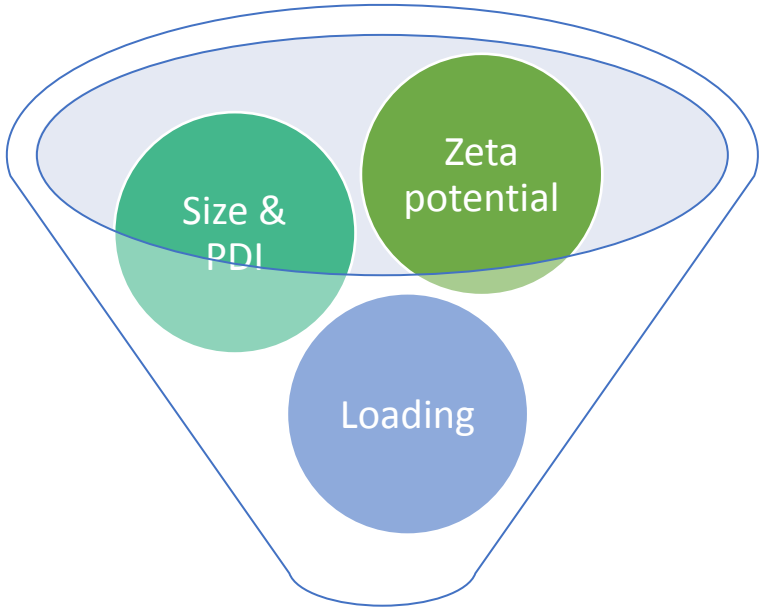
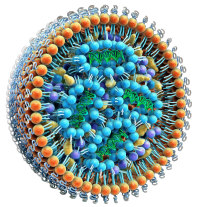
# saRNA-LNP vaccines

## Impact of formulation

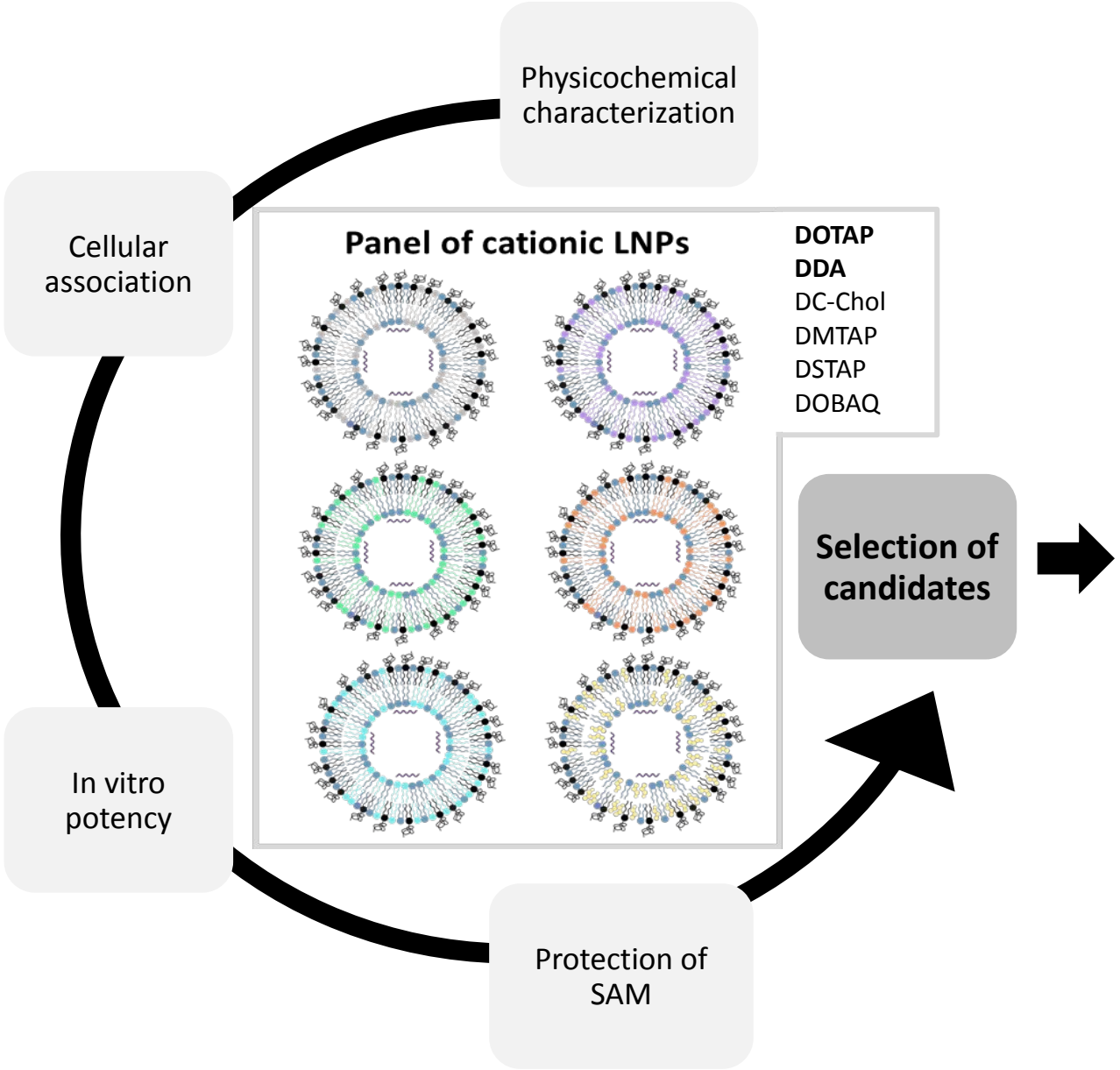



Physical characterisation



Tracking LNP distribution (fluorescence)


vaccine potency (immune response)




- Despite ionizable lipids recognised ability to deliver mRNA, they may be more expensive than existing cationic lipids (e.g. DOTAP).
- From a regulatory and safety perspective, there is less clinical data available on the use of novel ionizable lipids.
- Hence, formulations based on well-established lipids could be a useful option.

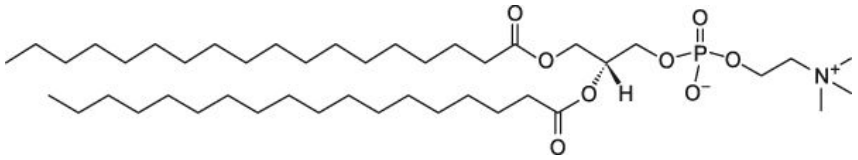
LNP formulation panel:

- Choice of cationic lipid: 6 screened
- DOPE:Cationic:DMG-PEG2000 (49:49:2 molar ratio)
- DSPC:Chol:cationic:DMG-PEG2000 (10:48:40:2 molar ratio)

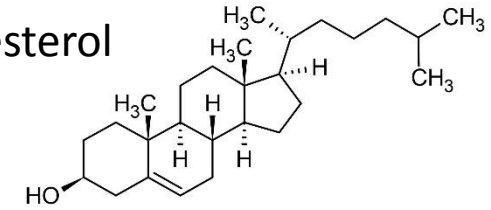
Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: The impact of cationic lipid selection. Lou G, Anderluzzi G, Tandrup Schmidt S, Woods S, Gallorini S, Brazzoli M, Giusti F, Ferlenghi I, Johnson R, Roberts CW, O'Hagan DT, Baudner BC, Perrie Y. J Control Release. 2020 Jun 30:S0168-3659(20)30362-X. doi: 10.1016/j.jconrel.2020.06.027. Online ahead of print.

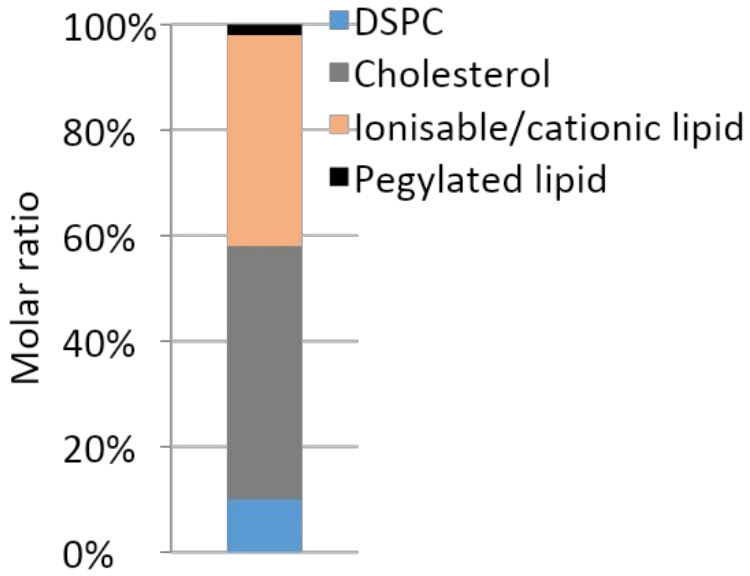
# Stabiliser lipid

DSPC



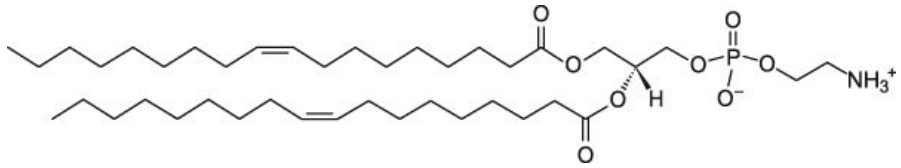
Cholesterol

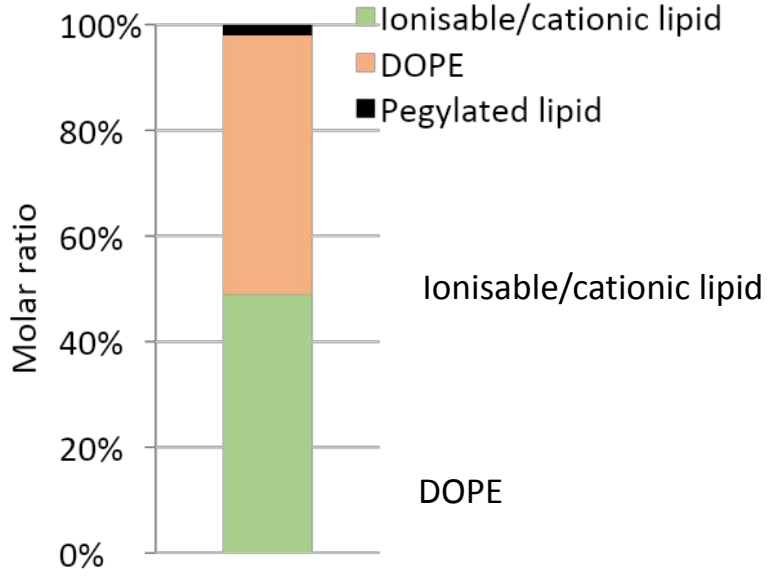





# Fusogenic lipid

DOPE



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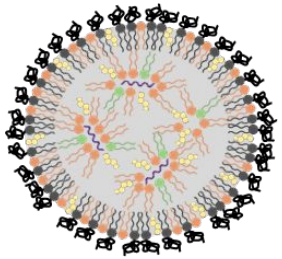
# Formulations that progressed:

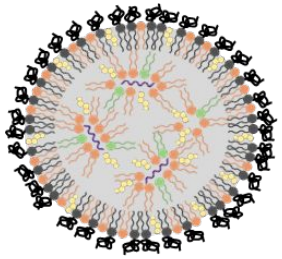
| Cationic lipid | Composition (molar ratio) | Size (d.nm) | PDI | ZP (mV) | SAM E.E. (%) |
|---|---|---|---|---|---|
| DOTAP | 49:49:2 | 83 ± 6 | 0.17 ± 0.05 | 3.1 ± 0.6 | 97 ± 2 |
| | 10:48:40:2 | 92 ± 5 | 0.23 ± 0.02 | 2.7 ± 0.4 | 99 ± 2 |
| DDA | 49:49:2 | 81 ± 9 | 0.13 ± 0.02 | 2.9 ± 0.7 | 98 ± 2 |
| | 10:48:40:2 | 80 ± 1 | 0.17 ± 0.02 | 2.4 ± 0.4 | 99 ± 1 |
| DC-Chol | 49:49:2 | 88 ± 6 | 0.16 ± 0.04 | 2.2 ± 1.9 | 91 ± 6 |
| | 10:48:40:2 | 88 ± 6 | 0.17 ± 0.03 | 1.3 ± 0.7 | 96 ± 4 |
| DMTAP | 49:49:2 | 86 ± 9 | 0.16 ± 0.02 | 2.2 ± 1.5 | 96 ± 3 |
| | 10:48:40:2 | 72 ± 2 | 0.15 ± 0.05 | 1.8 ± 0.6 | 98 ± 3 |
| ~~DSTAP~~ | 49:49:2 | 331 ± 70 | 0.89 ± 0.13 | 3.2 ± 0.3 | 70 ± 3 |
| | 10:48:40:2 | 472 ± 117 | 0.45 ± 0.10 | 3 ± 0.7 | 74 ± 4 |
| DOBAQ | 49:49:2 | 77 ± 2 | 0.22 ± 0.04 | 2.7 ± 1.0 | 85 ± 3 |
| | 10:48:40:2 | 66 ± 2 | 0.24 ± 0.02 | 1.9 ± 0.9 | 85 ± 2 |
| MC3 | 10:48:40:2 | 102 ± 4 | 0.10 ± 0.04 | 1.5 ± 1.3 | 98 ± 1 |

Out of the various formulations tested 3 LNPs were taken forward in vivo.

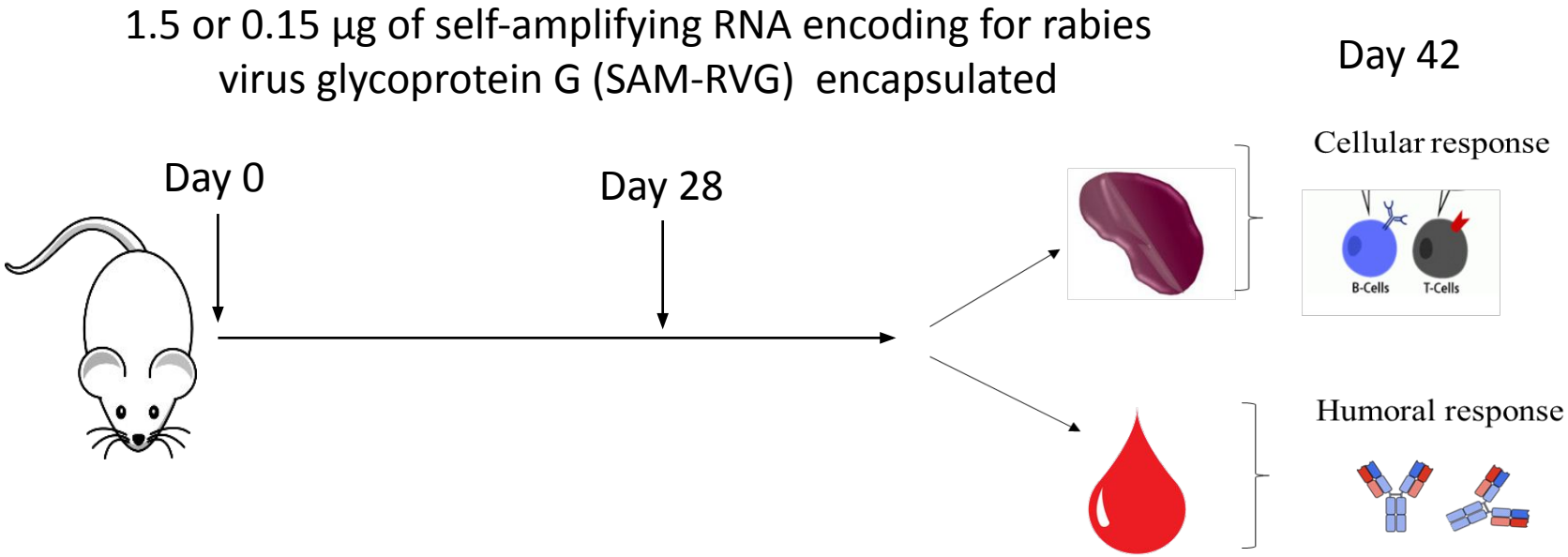


- DOPE:Cationic:DMG-PEG2000 (49:49:2 molar ratio)
- DSPC:Chol:cationic:DMG-PEG2000 (10:48:40:2 molar ratio)

# Impact on potency



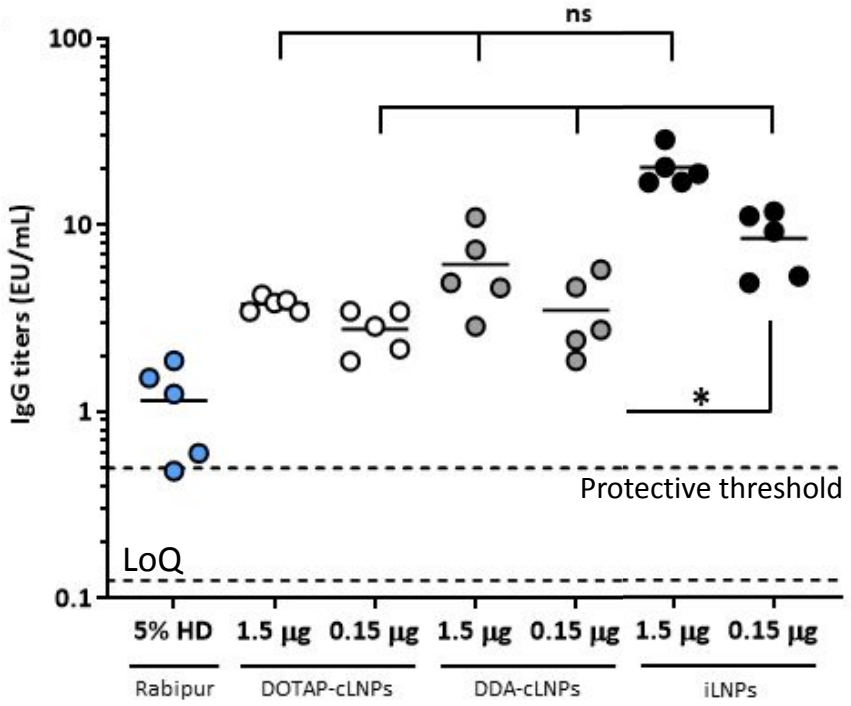
1. DOPE:DOTAP:DMG-PEG
2. DOPE:DDA:DMG-PEG
3. DSPC:Chol:MC3:DMG-PEG




Groups of ten BALB/c mice were immunized i.m. on days 0 and 28 with either 1.5 or 0.15 µg of self-amplifying RNA encoding for rabies G protein encapsulating DOTAP polymeric nanoparticles (NPs), DOTAP Liposomes or DDA Liposomes and compared with the commercial vaccine Rabipur (1/20 of human dose).

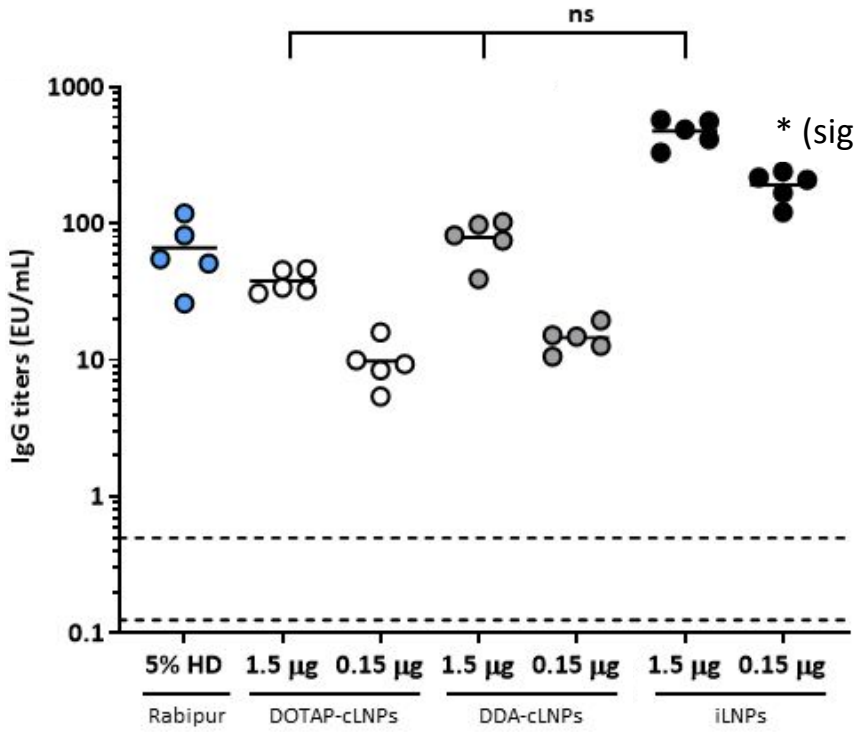
# Immune profiles: cLNP vs iLNP

2 weeks after 1st injection




- ✔ No sig difference between iLNPs and DOTAP and DDA-cLNPs
- ✔ All promote anti-RVG IgGs above the correlate of protection two weeks after a single vaccination.

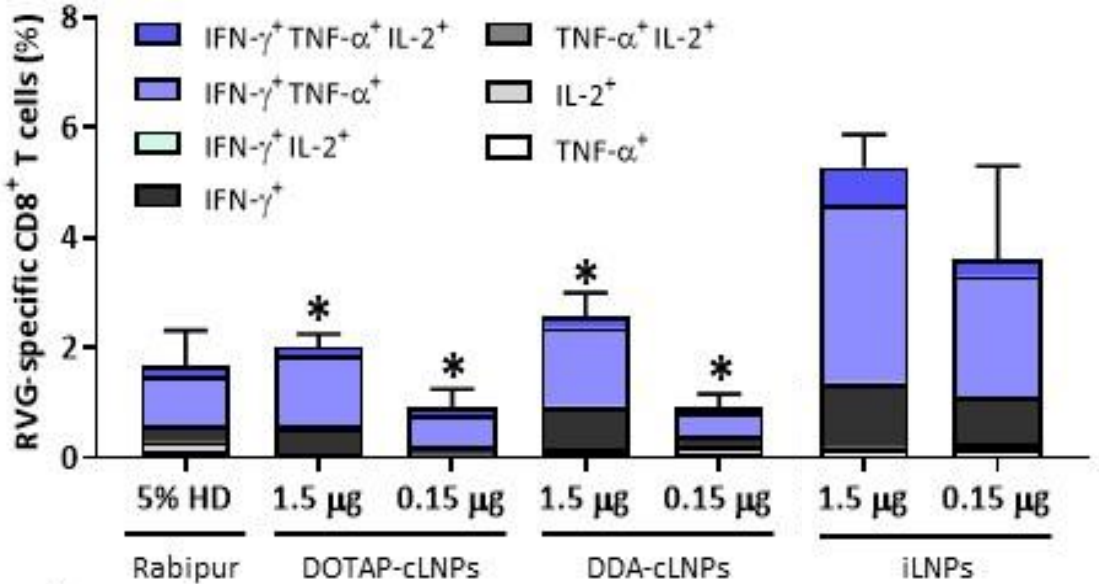
2 weeks after 2nd injection




* (sig higher at lower dose)

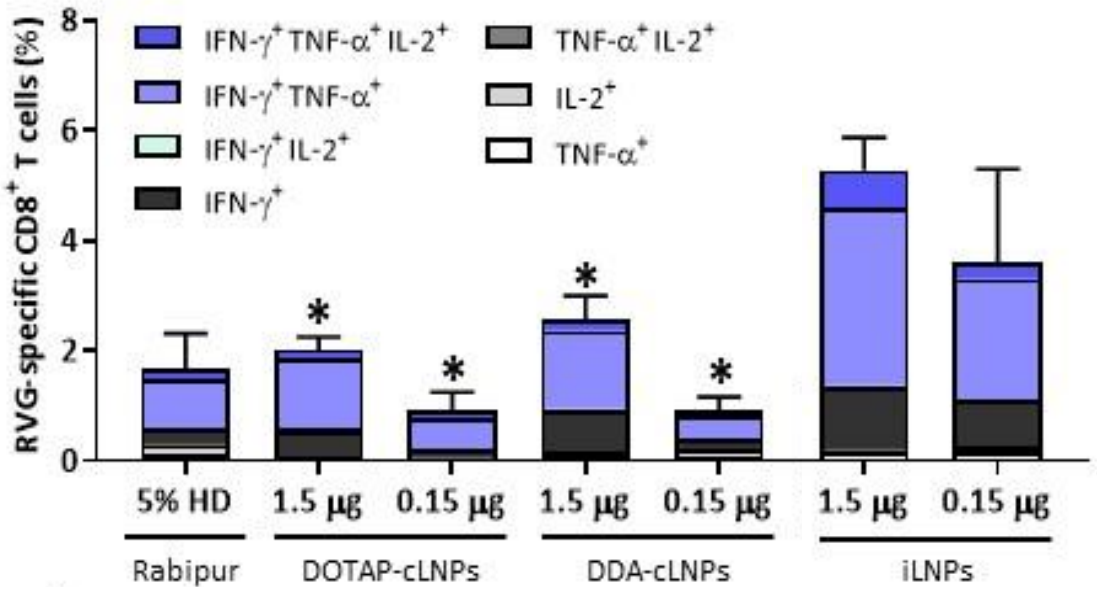
- ✔ titers increased up to 20-fold
- ✔ sig. difference between the cLNP and iLNP at lower dose.

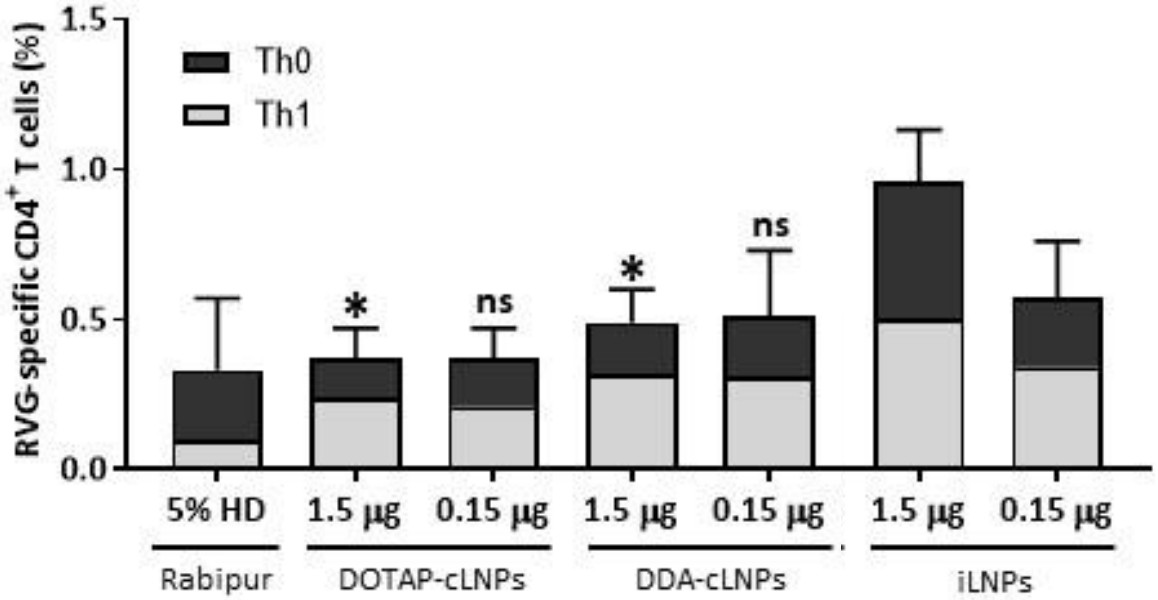
# Immune profiles: cytokine responses




- ✔ Most RVG-specific $CD8^+$ T cells had an effector Th1 phenotype (produce IFN-γ alone or in combo with TNF-α and/or IL-2).
- ✔ iLNP gave a sig. higher frequency of $CD8^+$ T cells.

# Immune profiles: cytokine responses



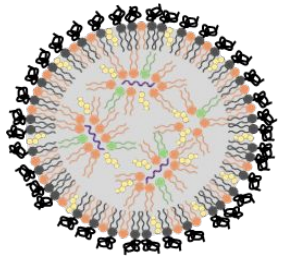




- ✔ Most RVG-specific $CD8^+$ T cells had an effector Th1 phenotype (produce IFN-γ alone or in combo with TNF-α and/or IL-2).
- ✔ iLNP gave a sig. higher frequency of $CD8^+$ T cells.
- ✔ Only at 1.5 µg iLNP dose induced sig. higher frequencies of RVG-specific $CD4^+$ T cells

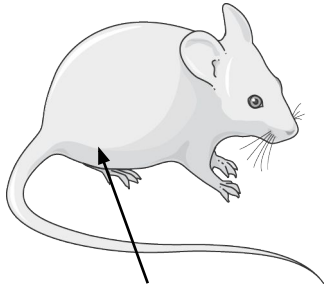
# Does biodistribution differ?

MC3>DOTAP=DDA

**lipid nanoparticles**

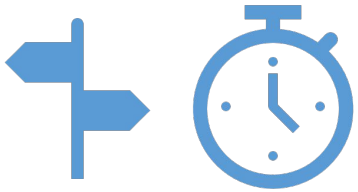


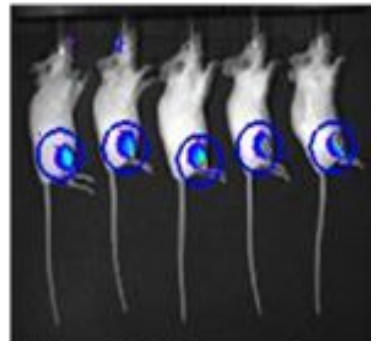
Vaccination



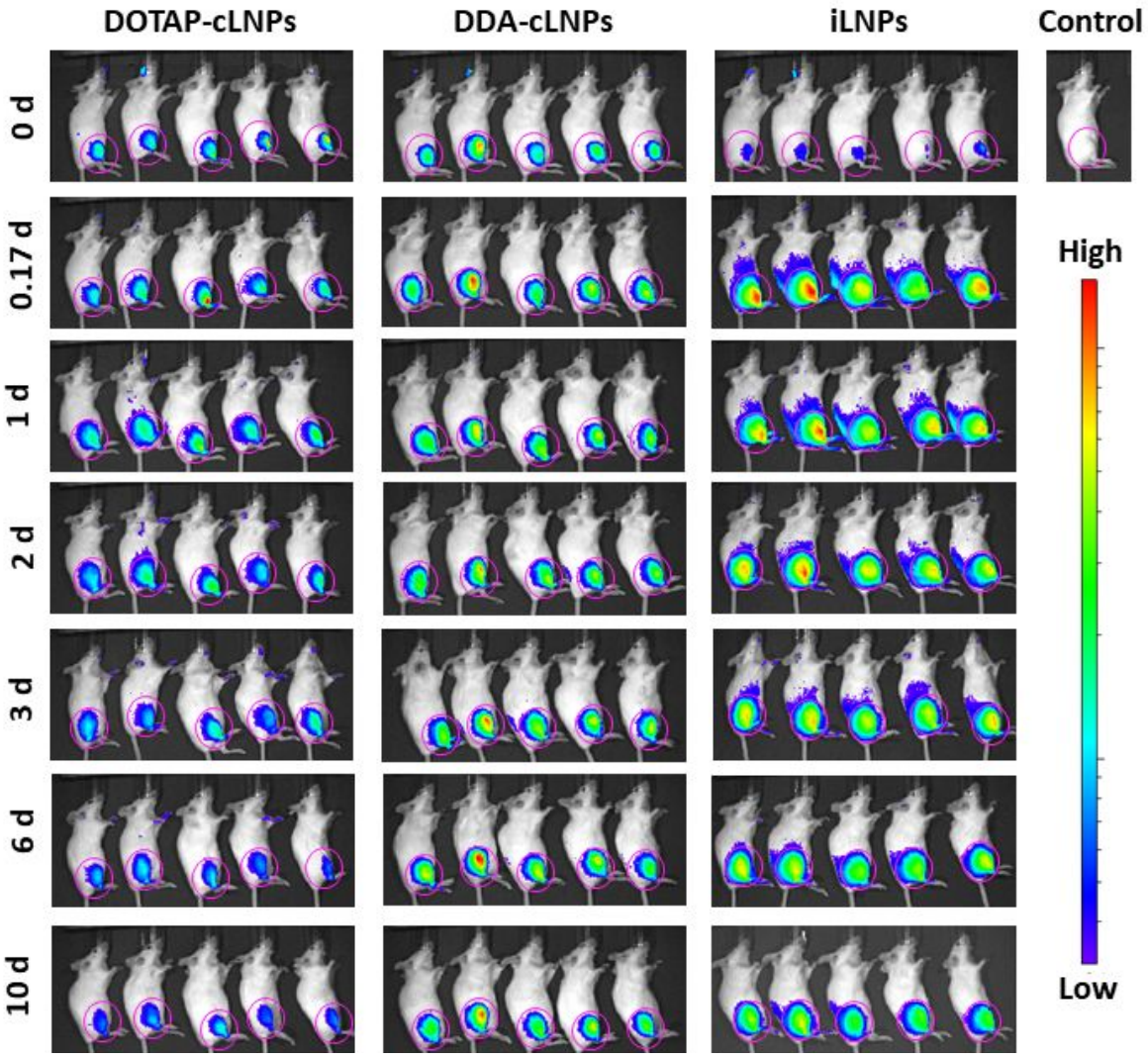
Intramuscular

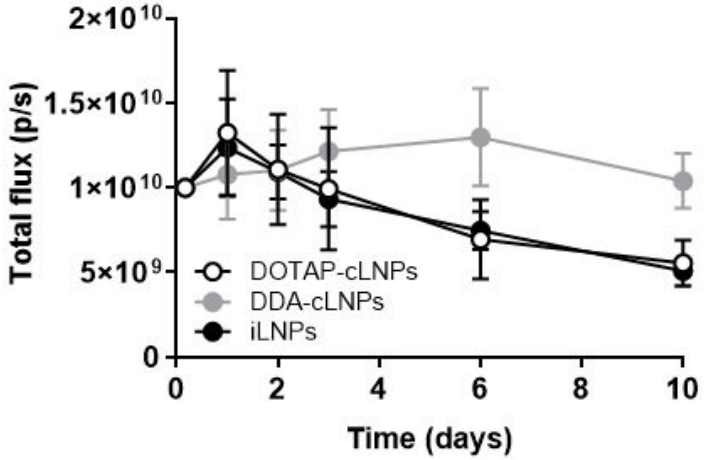
IVIS Spectrum In Vivo Imaging System

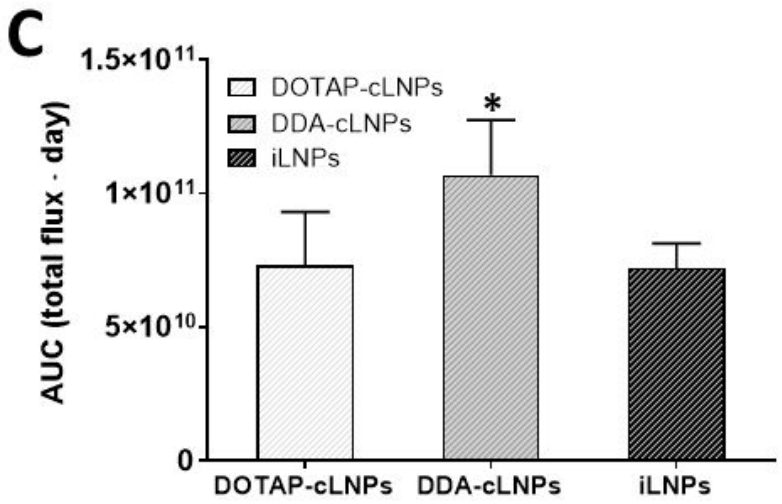




# Does biodistribution differ? Yes




DOTAP and DDA cLNP – different

DOTAP cLNP and iLNP – similar

But does not give insight into preferred biodistribution

# The impact of ionisable lipid choice:

Burcu Eryilmaz

| | DOTAP | DODAP | D-LIN-MC3-DMA | SM-102 | ALC-0315 |
|---|---|---|---|---|---|
| Size (nm) | 49.8 ± 6 | 69.0 ± 3 | 60.8 ± 1 | 65 ± 5 | 75 ± 6 |
| PDI | 0.24 ± 0.04 | 0.04 ± 0.02 | 0.11 ± 0.02 | 0.04 ± 0.03 | 0.09 ± 0.02 |
| Zeta Potential (mV) | 3.7 ± 1.1 | -1.9 ± 0.8 | -2.0 ± 1.3 | -1.3 ± 0.8 | -1.5 ± 0.5 |
| %EE | 99.6 ± 0.1 | 90.8 ±0.6 | 93 ± 1.6 | 97 ± 0.8 | 90 ± 1.6 |

# The impact of ionisable lipid choice:

| | DOTAP | DODAP | D-LIN-MC3-DMA | SM-102 | ALC-0315 |
|---|---|---|---|---|---|
| Size (nm) | 49.8 ± 6 | 69.0 ± 3 | 60.8 ± 1 | 65 ± 5 | 75 ± 6 |
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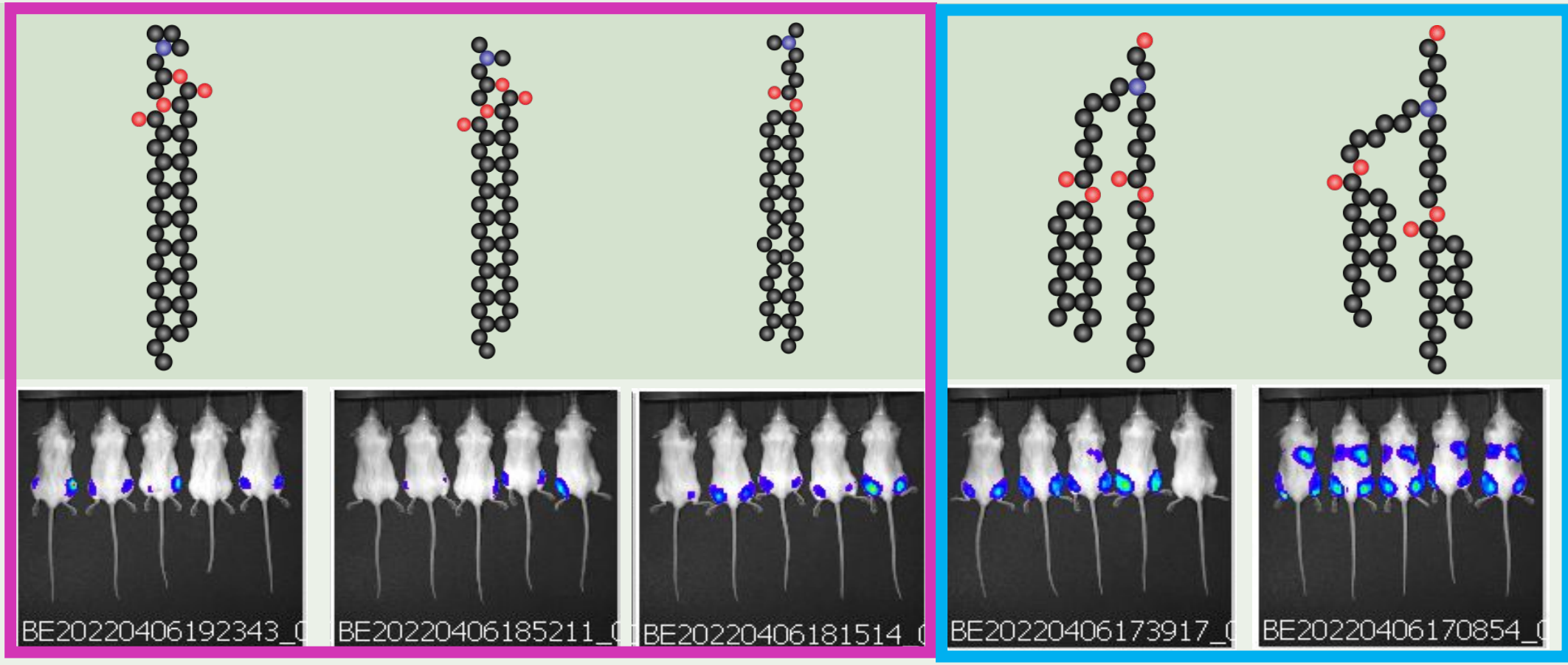


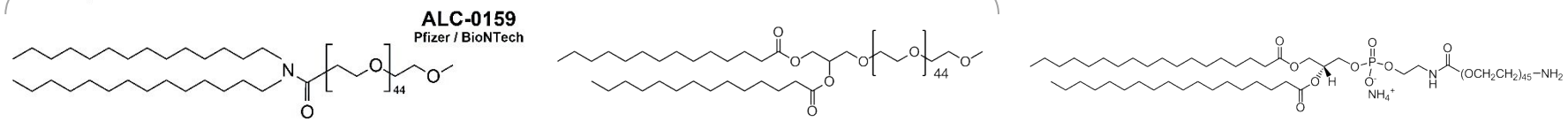

In vivo expression (Fluc)
(6h)

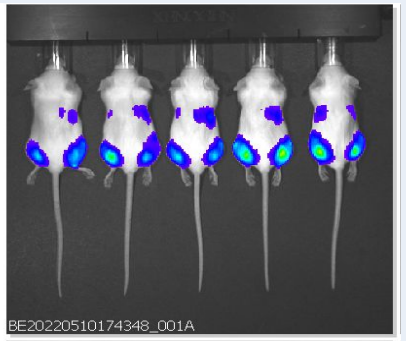
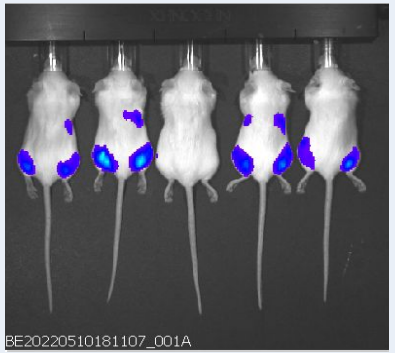
5 ug mRNA per leg

Burcu Eryilmaz

14C tail

18C tail

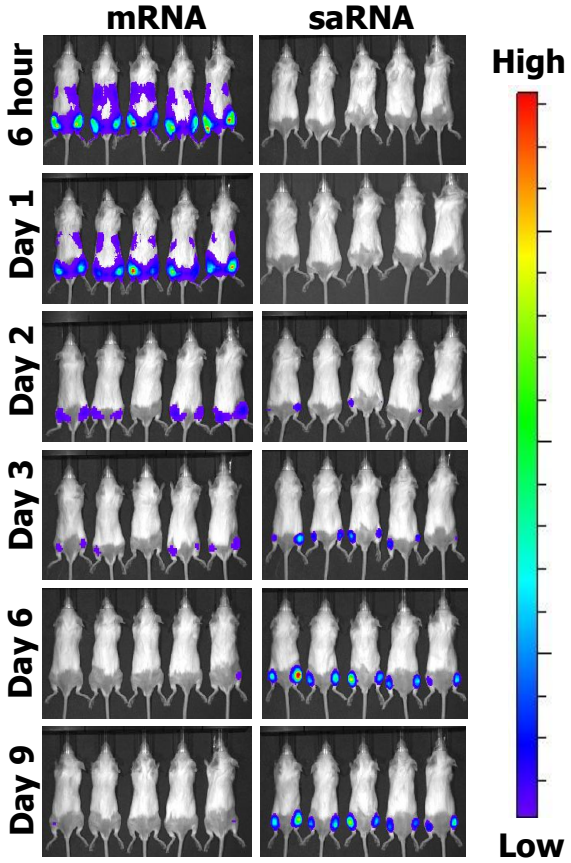



| ALC-0315 | ALC-0159 | DMG-PEG2k | DSPE-PEG2k |
|---|---|---|---|
| Size, PDI | 75 ± 5 nm, 0.08 ± 0.04 | 57 ± 3 nm, 0.11 ± 0.03 | 57 ± 1 nm, 0.04 ± 0.01 |
| ZP | -3 ± 1 mV | -3 ± 2 mV | -3 ± 2 mV |
| Loading (%EE) | 92 ± 3 % | 93 ± 2 % | 95 ± 4 % |
| LNP expression (6 h) |  |  |  |

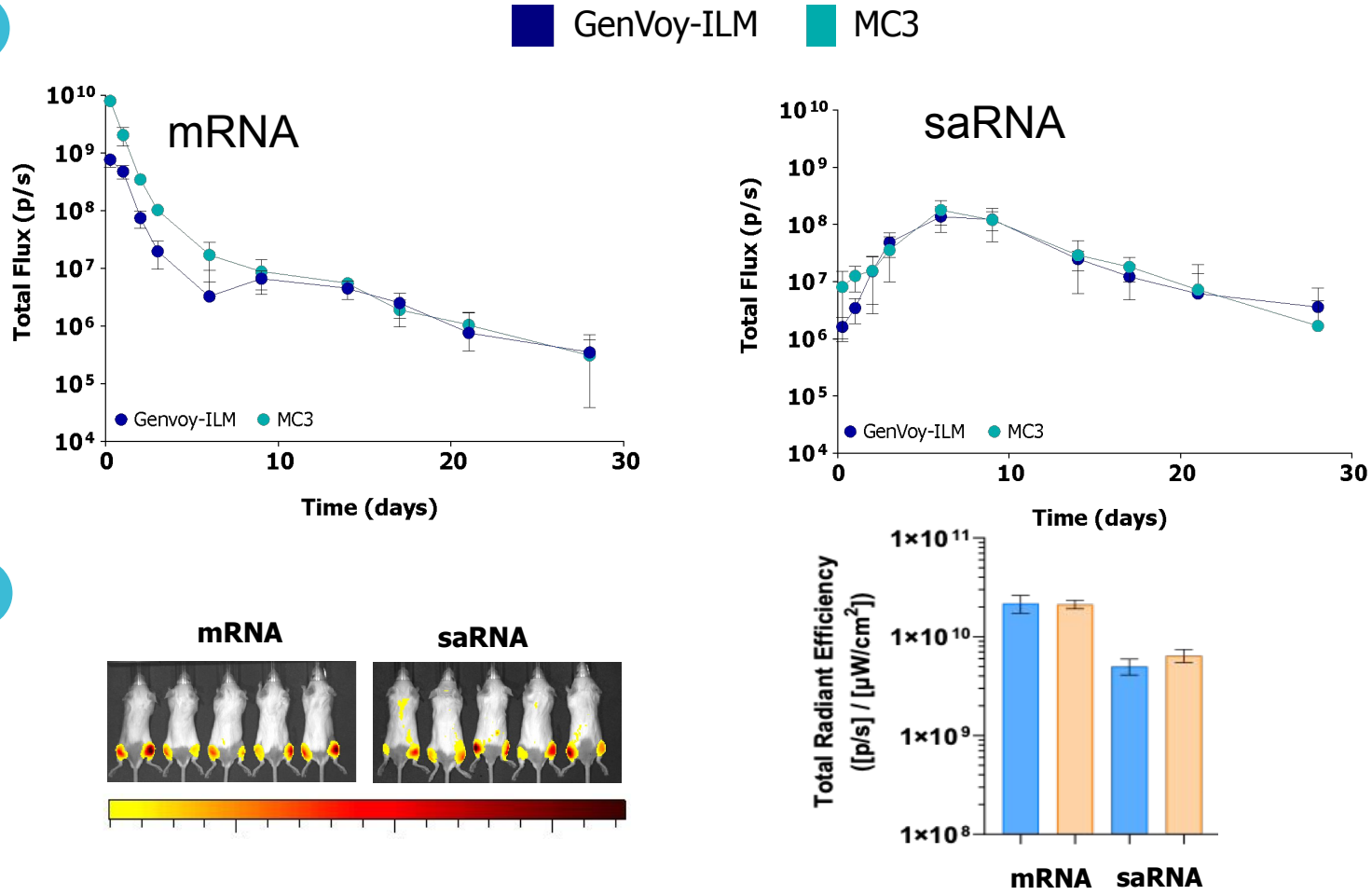
# GenVoy-ILM™ LNPs are an Effective *In Vivo* Delivery Vehicle for Both mRNA and saRNA: Protein Expression & LNP distribution

PRECISION NANOSYSTEMS
Create Transformative Medicines™

A




B




C

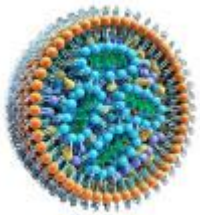




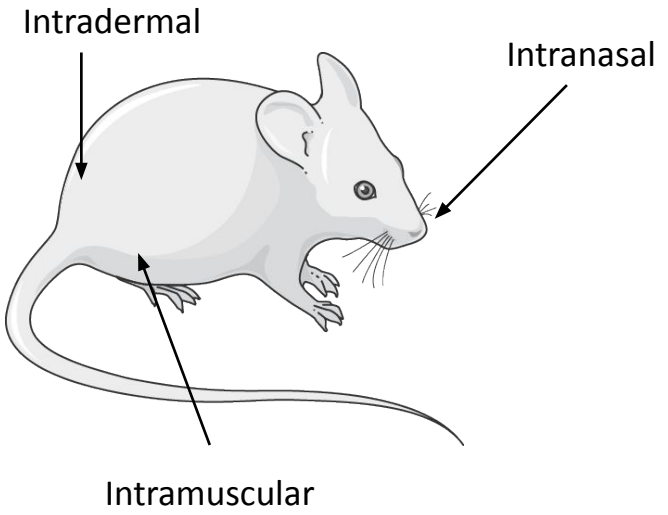


GenVoy-ILM and MC3 LNPs were prepared with 0.1 mol% DiD, encapsulating mRNA (5 µg/leg) or saRNA (1 µg/leg) encoding for FLuc. Female BALB/c mice (n=5) were injected IM with LNPs, and protein expression was determined using luminescence imaging (IVIS® Spectrum) over 28 days. Mice were injected IP with D-luciferin (150mg/kg) 15 minutes before imaging. (A) shows representative luminescence images of mice injected with GenVoy-ILM LNPs over 9 days. (B) shows the change in luminescence (total flux p/s) over 28 days post-IM injection with LNPs containing mRNA (left) and saRNA (right). Results are shown as the mean ± SD.

# Impact on route and platform



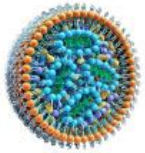
| Nanoparticle Composition | |
|---|---|
| SLNs | DOTAP, tristearin and DMG-PEG2000 |
| PNPs | DOTAP, PLGA and DMG-PEG2000 |
| cLNPs | DOTAP, DOPE and DMG-PEG2000 |
| iLNPs | Dlin-MC3-DMA |



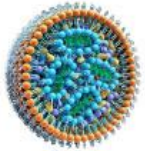
# In vitro screening:

Polymeric nanoparticles

Solid lipıd nanoparticles

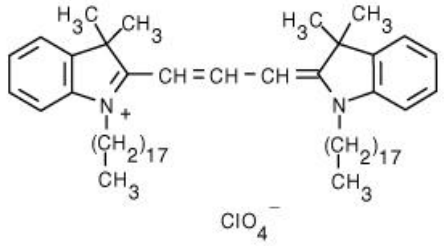


cLNPs



iLNPs

Dil Stain
(1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate ('DiI'; $DiIC_{18}(3)$))




Flu-labelled particles

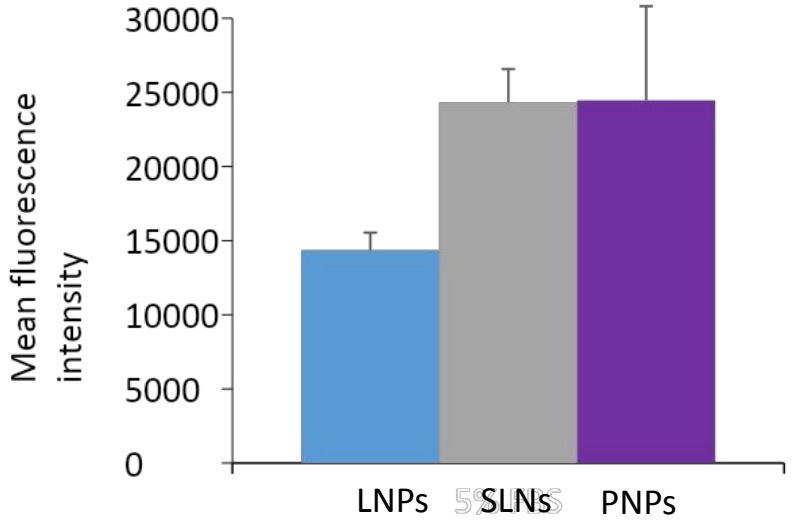
BHK cells

Investigating the Impact of Delivery System Design on the Efficacy of Self-Amplifying RNA Vaccines. Anderluzzi G, Lou G, Gallorini S, Brazzoli M, Johnson R, O'Hagan DT, Baudner BC, Perrie Y. Vaccines (Basel). 2020 May 8;8(2):E212. doi: 10.3390/vaccines8020212. PMID: 32397231

Cell viability up to 33 ug/mL

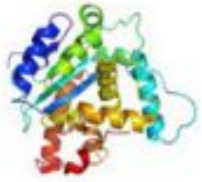
Viability

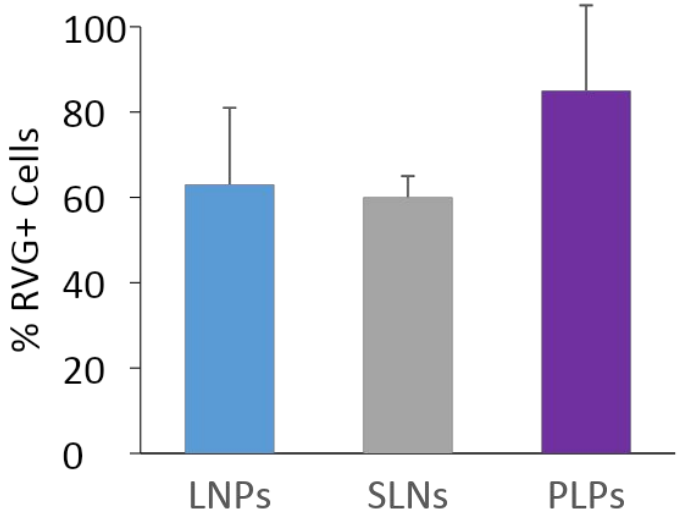
✔ DOTAP high conc tolerated in vitro, irrespective to the delivery platform




Uptake

✔ Solid lipid nanoparticles and polymeric nanoparticles tended to have higher cell uptake



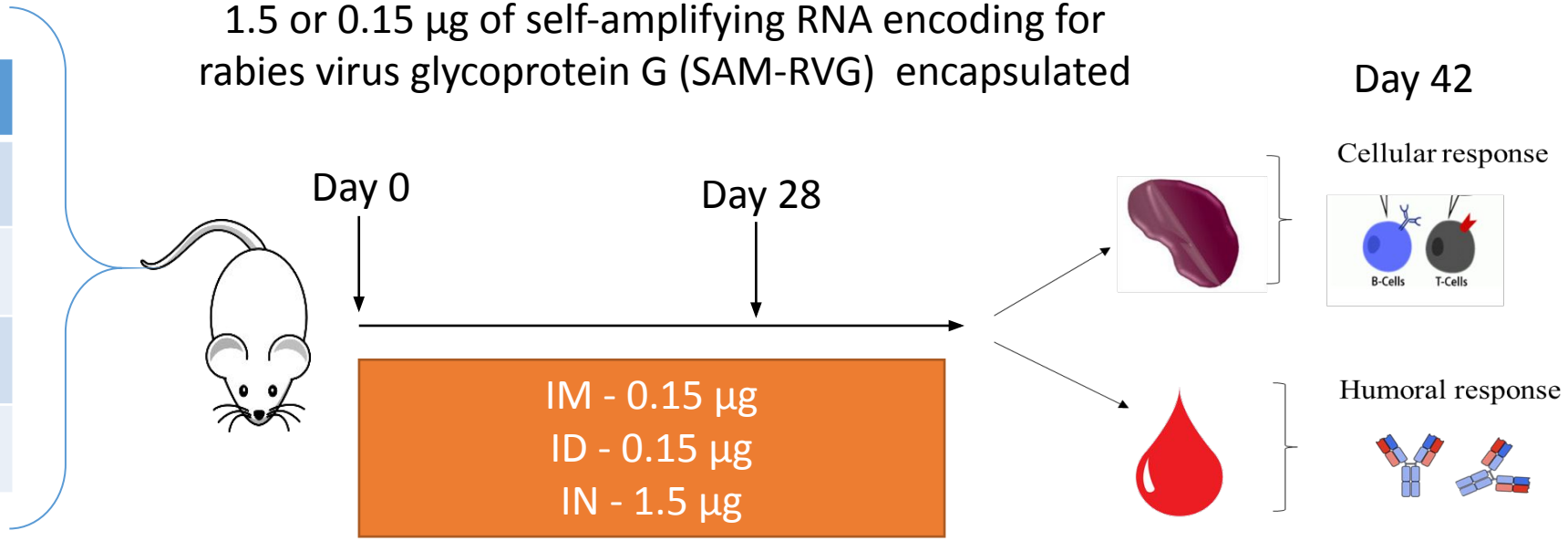



Potency

✔ No notable differences in transfection

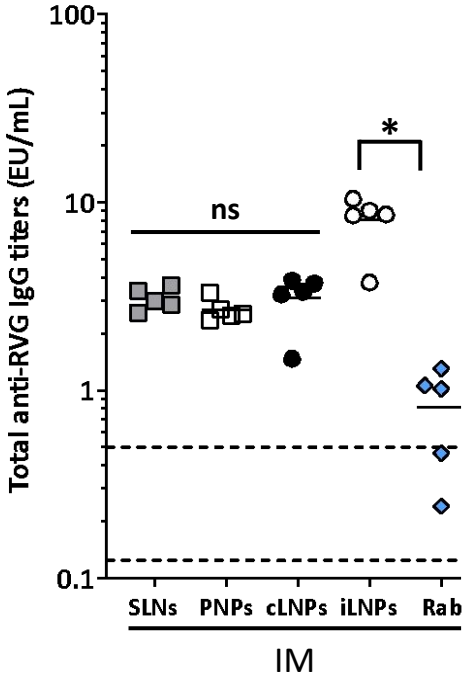
# Formulations selected and protocol

| Nanoparticle Composition | |
|---|---|
| SLNs | DOTAP, tristearin and DMG-PEG2000 |
| PNPs | DOTAP, PLGA and DMG-PEG2000 |
| cLNPs | DOTAP, DOPE and DMG-PEG2000 |
| iLNPs | Dlin-MC3-DMA |

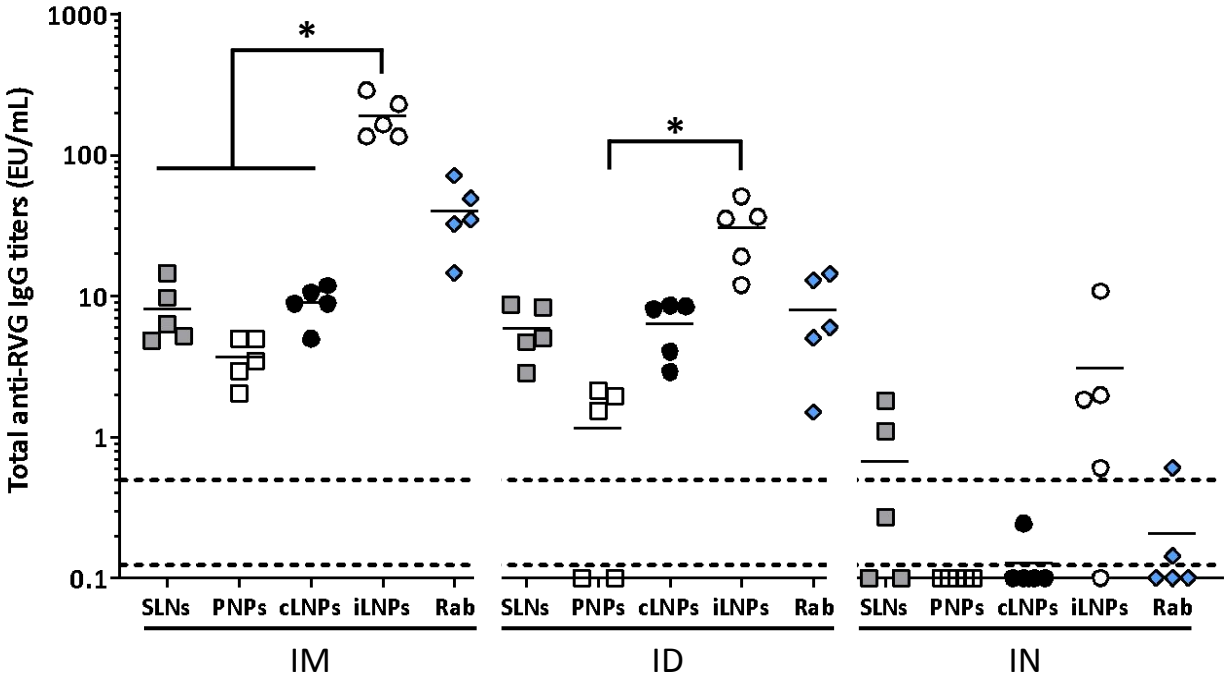



Groups of ten BALB/c mice were immunized i.m. on days 0 and 28 with either 1.5 or 0.15 µg of self-amplifying RNA encoding for rabies G protein encapsulating DOTAP polymeric nanoparticles (NPs), DOTAP Liposomes or DDA Liposomes and compared with the commercial vaccine Rabipur (1/20 of human dose).

A) 4 weeks




B) 6 weeks (2 weeks post 2nd dose)

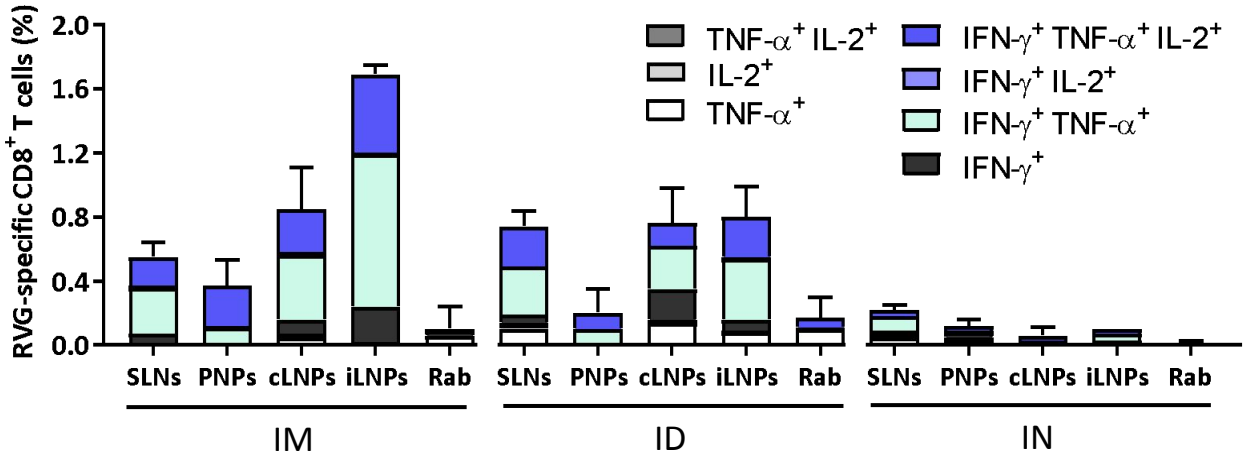



IM: no significant diff across the nanoparticles used
ID: polymeric nanoparticles giving lower response
IN: no notable responses

IM: As previously, at this low dose iLNPs higher response
ID: Similar to IM
IN: again, no notable responses

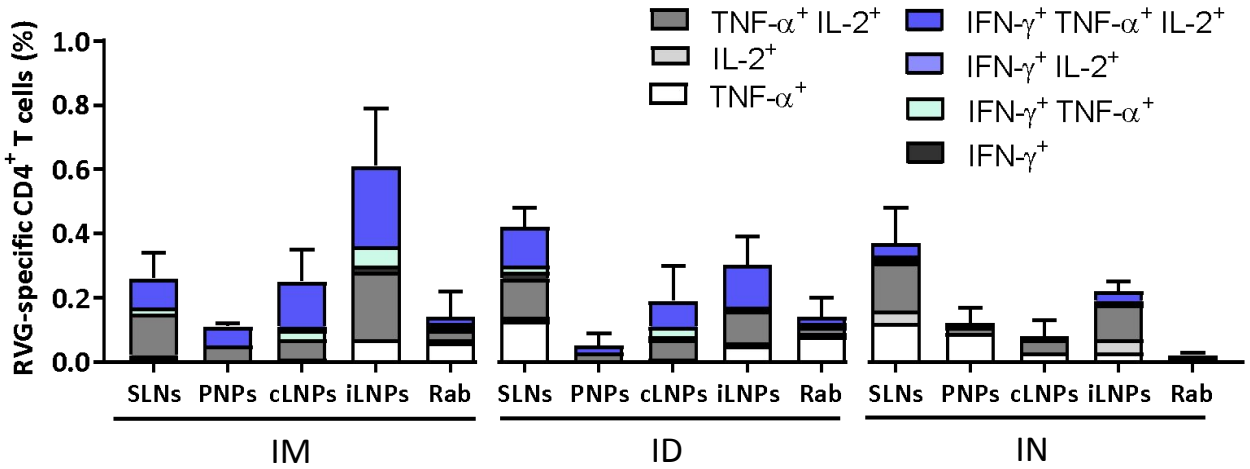
Splenocytes were collected two weeks after the second vaccination and re-stimulated in vitro with an RVG peptide pool.

A) Frequencies of cytokine-producing CD8+ T cells




- ✔ Frequencies of cytokine-producing CD8+ T were greater for iLNPs (similar to IgG)
- ✔ The majority of CD8+ T cells expressed IFN-γ in combo with TNF-α and/or IL-2, irrespective of the route of administration (generally associated with a mature effector phenotype).

B) Frequencies of CD4+ T cells

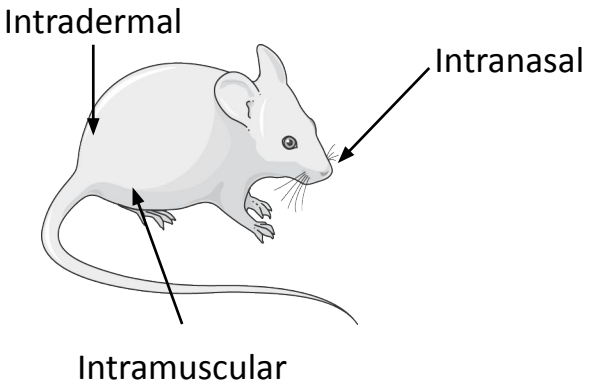



- ✔ iLNPs injected either IM or ID induced the highest frequencies of cytokines-producing RVG-specific splenic CD4+ and CD8+ T cells

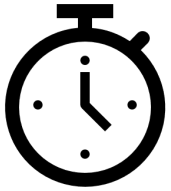
# Biodistribution protocol

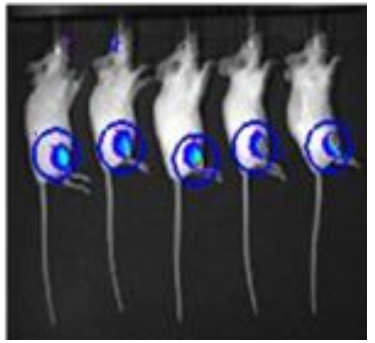
- Cationic SLNs
- Cationic PLNs
- Cationic LNPs
- ionisable LNPs

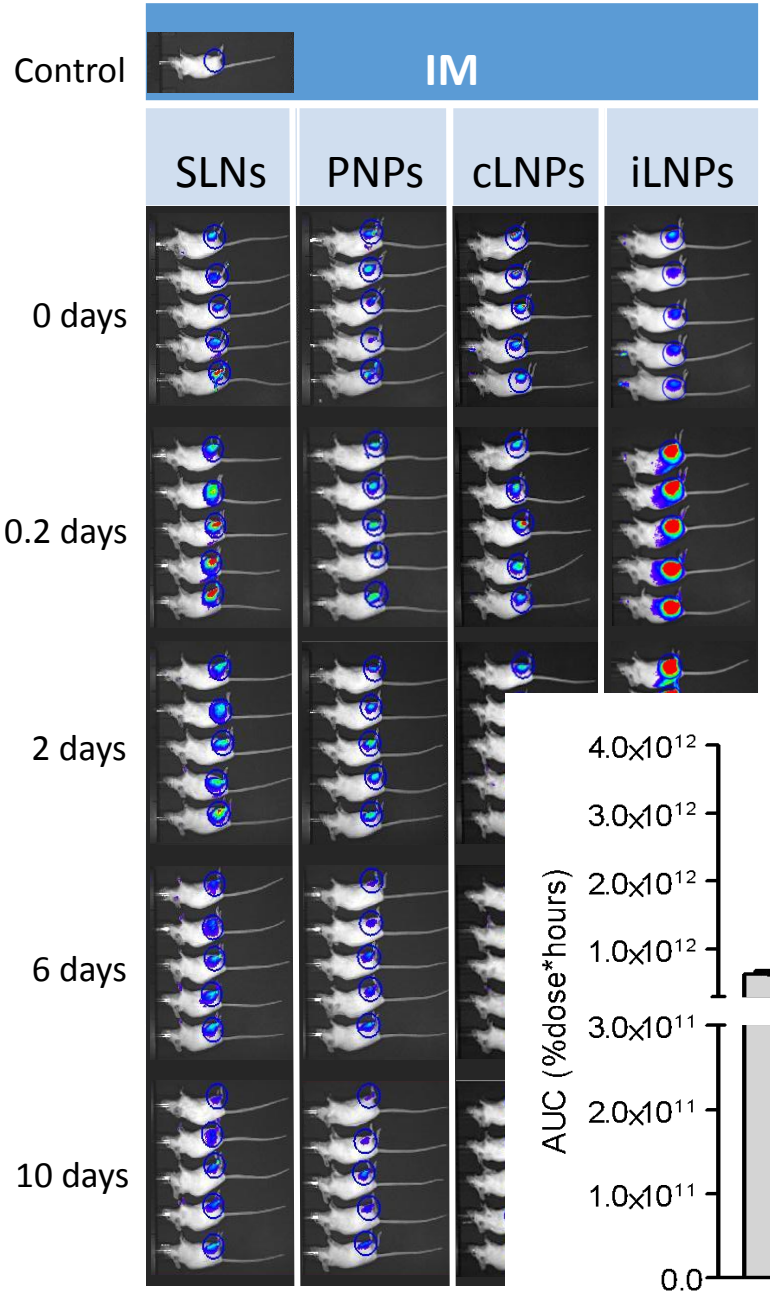
Vaccination

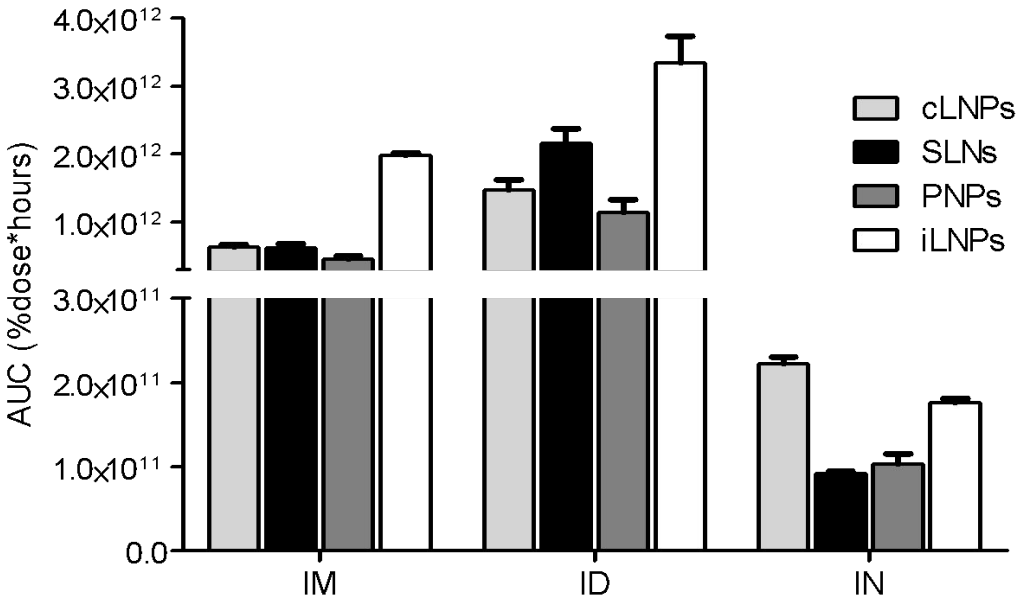


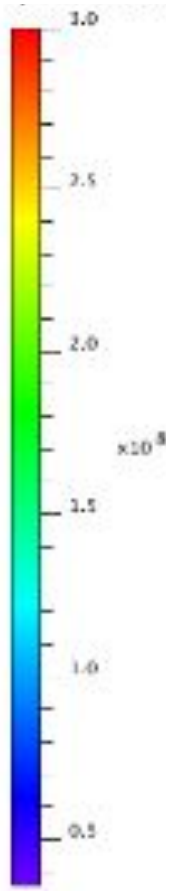

IVIS Spectrum In Vivo Imaging System



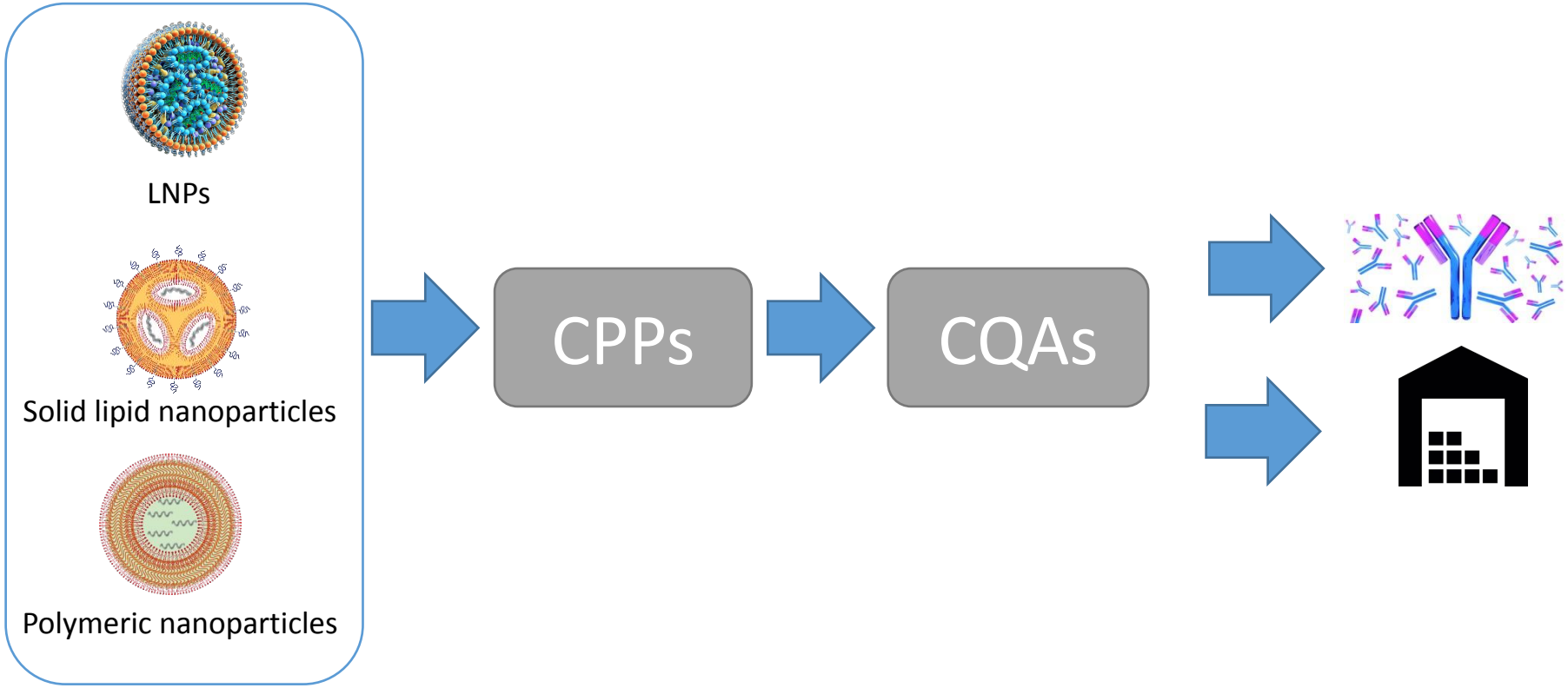



Control
IM
SLNs
PNPs
cLNPs
iLNPs
0 days
0.2 days
2 days
6 days
10 days


AUC (%dose*hours)
4.0x10^12
3.0x10^12
2.0x10^12
1.0x10^12
3.0x10^11
2.0x10^11
1.0x10^11
0.0
IM
ID
IN
cLNPs
SLNs
PNPs
iLNPs



# Summary:




**Current Team**
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Gillian Berrie
Dr Ankita Borah
Greg Chambers
Burcu Eryilmaz
Valeria Giacobbo
Dr Muattaz Hussain
Sarah Lindsay
Ashish Muglikar
Agata Ugorenko

**Previous members**
Edward Grahame
Natalie Orr
Giulia Anderluzzi
Gustavo Lou Ramirez
Cameron Webb
Carla Roces
Signe Schmidt