Optimization of Lipid Nanoparticles for Self-Amplifying RNA Expression & Cellular Activation Using a DoE Approach

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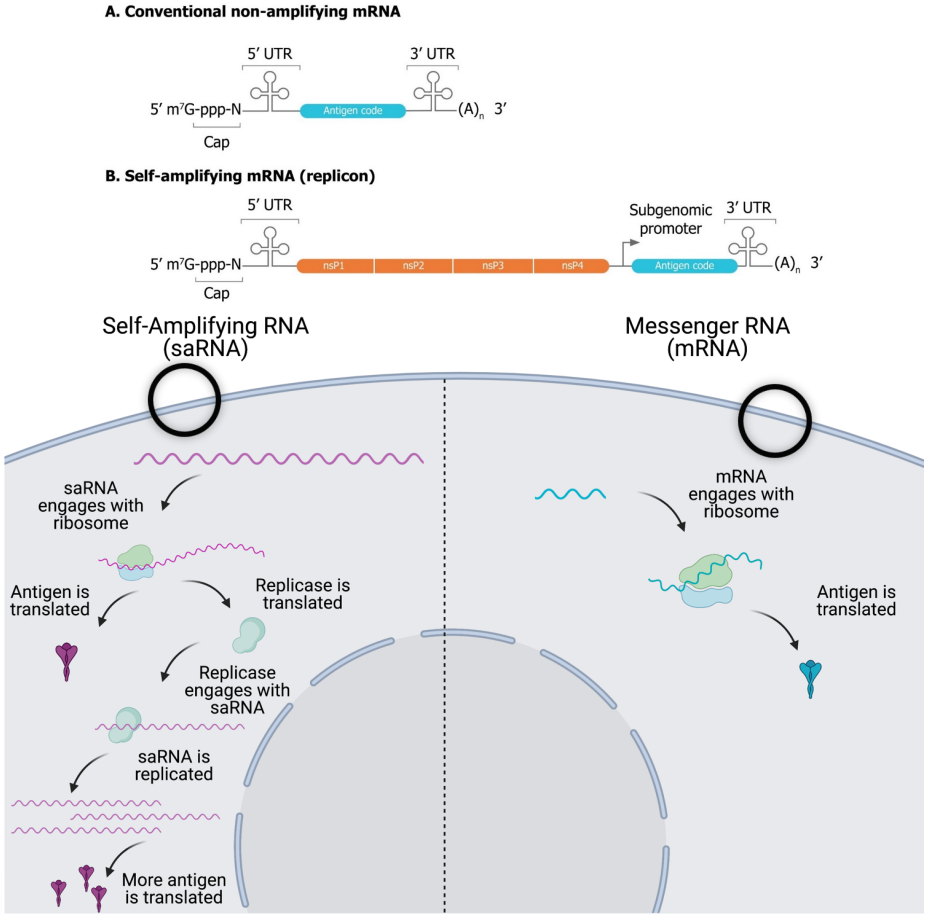
Advanced Delivery Science

Outline

- Delivery approaches for saRNA
- Effect of formulation on expression and immunogenicity of saRNA
- Optimization of LNP delivery for mRNA vs. saRNA

Conflict of interest: co-founder of VaxEquity

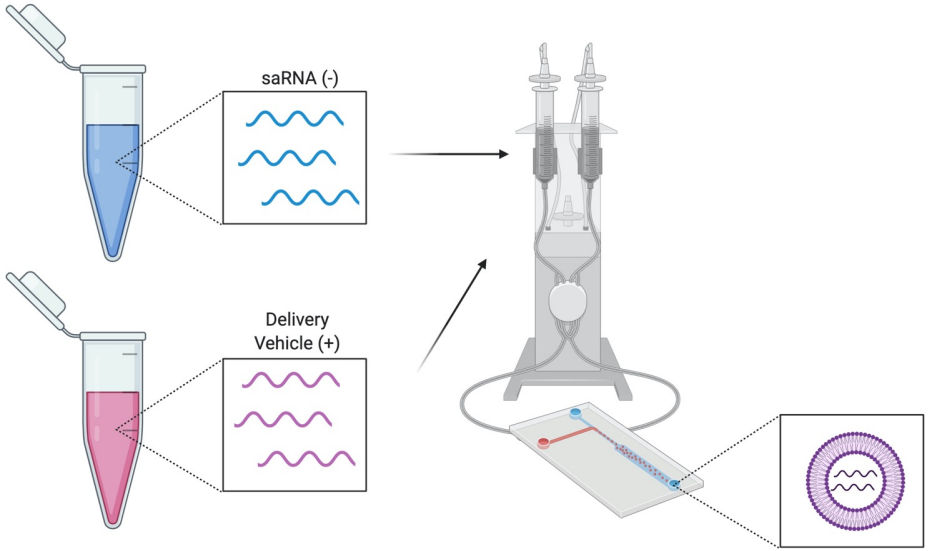
How is saRNA different from mRNA?



Blakney et al. Vaccines. 2021.

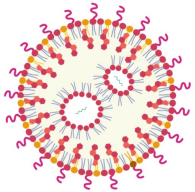
RNA Delivery Vehicles

Preparation of RNA formulations:

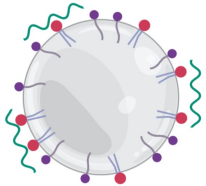


Polymeric nanoparticles

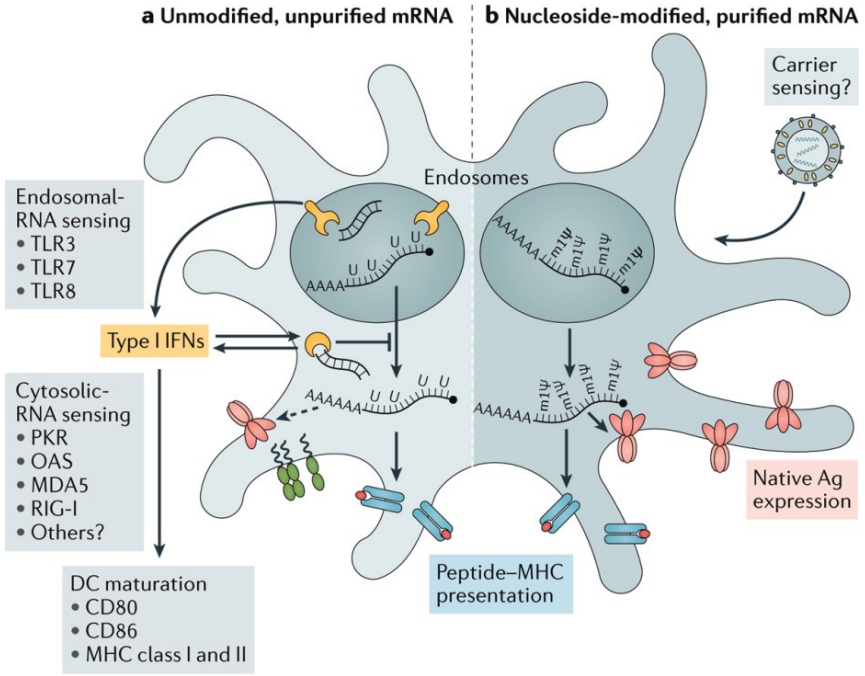
Lipid nanoparticles



Nanoemulsion

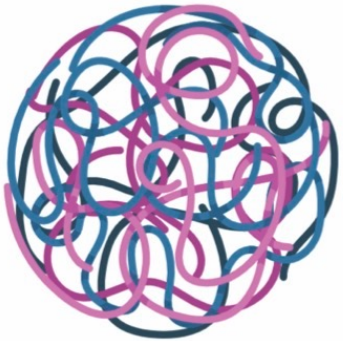


How is the delivery vehicle sensed intracellularly?



Pardi et al. Nature Reviews Drug Discovery. 2018.

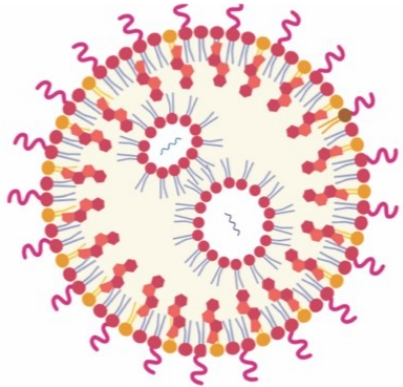
Goal: head-to-head comparison of polymeric and LNP formulations of saRNA



saRNA

pABOL

pABOL: bioreducible, cationic, linear polymer optimized in Stevens Lab at ICL



saRNA

Ionizable lipid

Cholesterol

Phospholipid

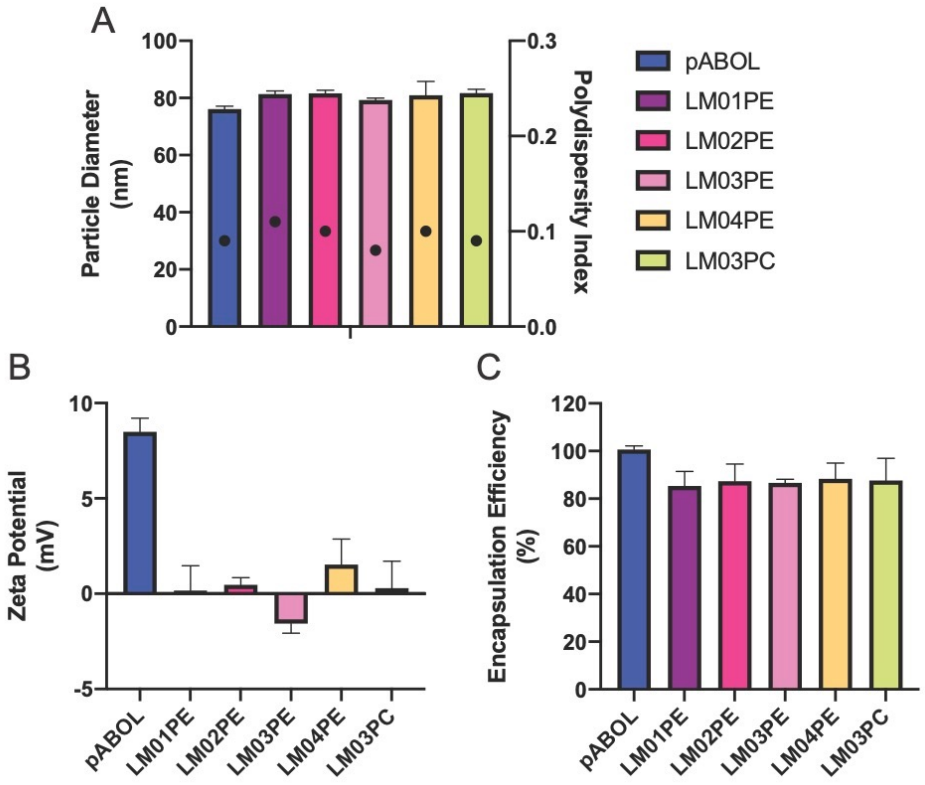
PEGylated lipid

Phospholipids: DO**PE** or DS**PC**

PRECISION NANOSYSTEMS

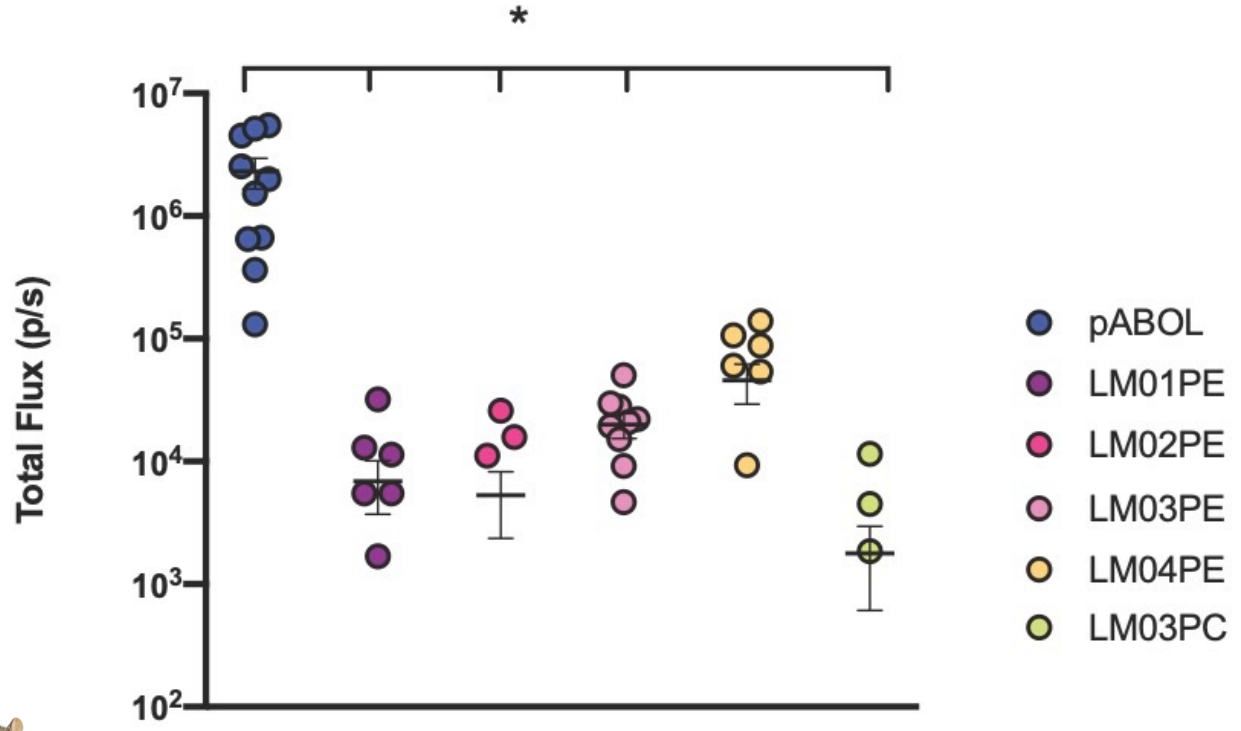
Blakney et al. Journal of Controlled Release. 2021.

pABOL and LNP formulations exhibit similar size and encapsulation efficiency, but not surface charge



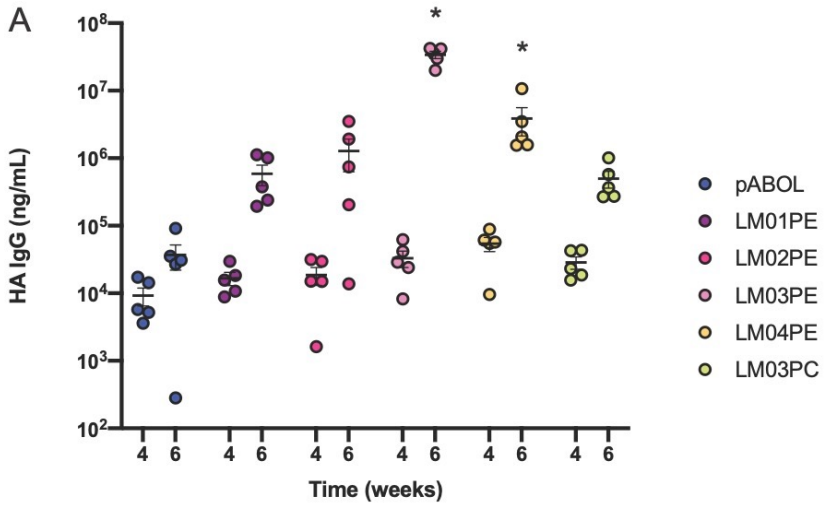
Blakney et al. Journal of Controlled Release. 2021.

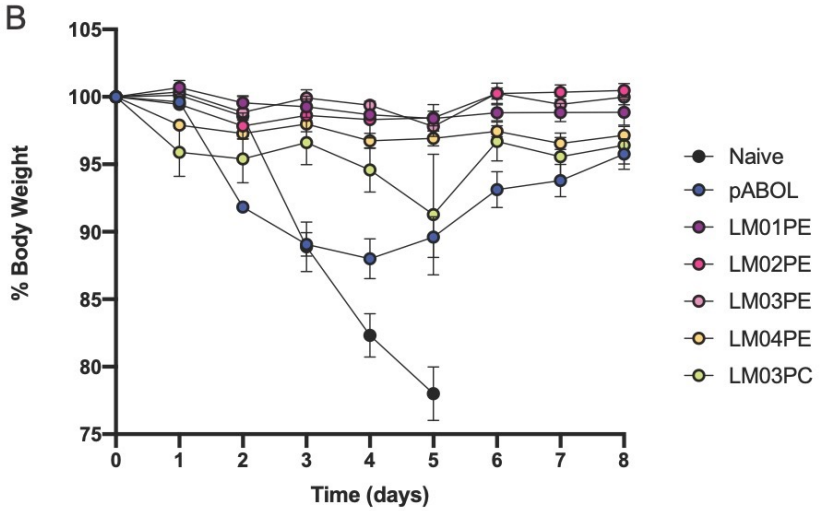
pABOL formulations result in 100-fold higher protein expression of saRNA than LNP *in vivo*



Blakney et al. Journal of Controlled Release. 2021.

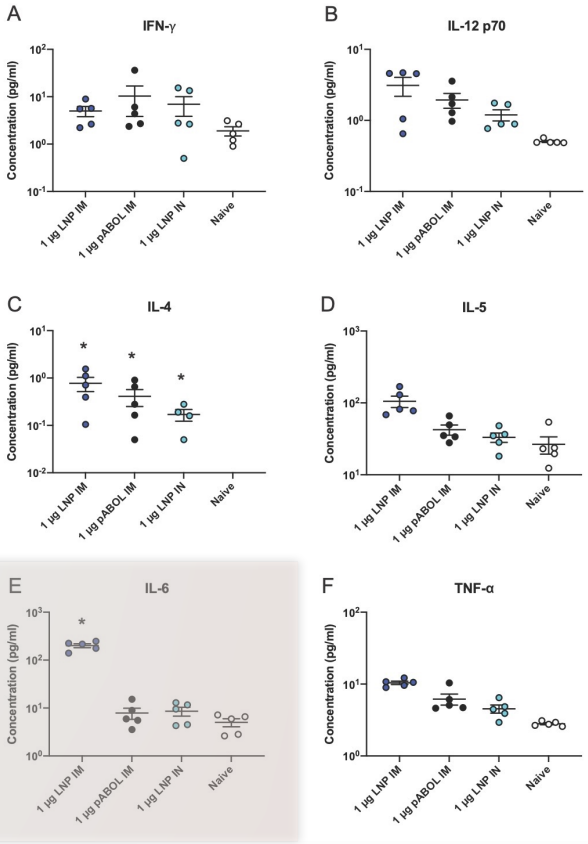
LNP formulations confer higher immunogenicity of saRNA against influenza HA than pABOL





Blakney et al. Journal of Controlled Release. 2021.

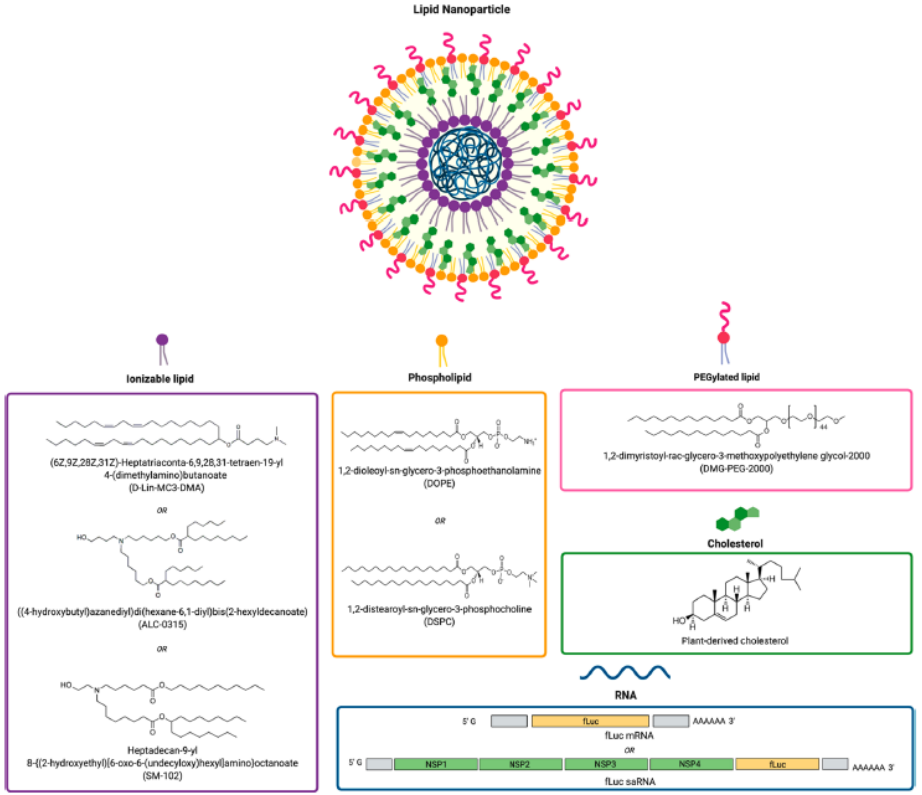
LNPs induce superior Th2 activation and reactogenicity compared to pABOL



- Measured systemic cytokine levels 4 hours after administration
- No detectable levels for GM-CSF, IL-1β, IL-2, IL-13 or IL-18
- Main difference for pABOL and LNP was IL-6

Blakney et al. Journal of Controlled Release. 2021.

LNP delivery of mRNA vs. saRNA

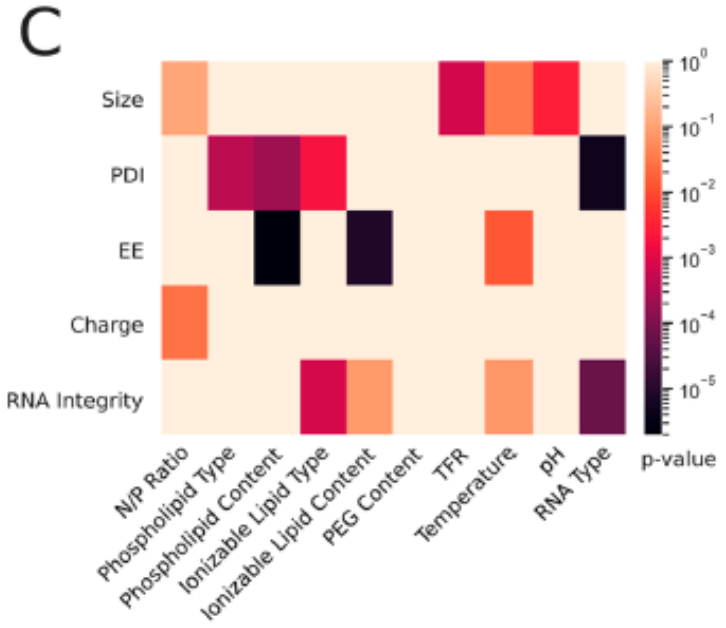


- What are optimal formulation parameters for maximizing and minimizing cellular activation?
- Are optimal parameters for saRNA also optimal for mRNA?

Ly et al. Molecular Pharmaceutics. 2022.

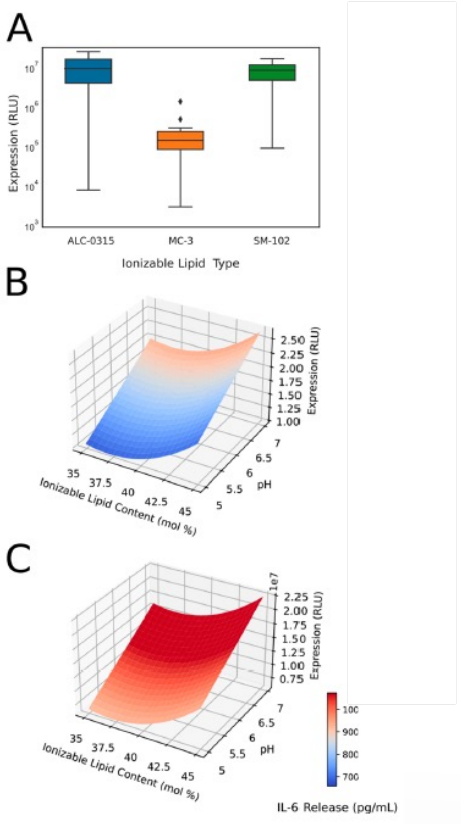
Design of Experiments optimization of LNP mRNA and saRNA formulations

experimental inputs			
factor		levels	
N/P ratio	5	10	15
phospholipid type		DOPE	DSPC
phospholipid content (mol %)	10	15	20
ionizable lipid type (corresponding pK_a)	DLin-MC3-DMA (6.4)	ALC-0315 (6.09)	SM-102 (6.75)
ionizable lipid content (mol %)	30	40	50
DMG-PEG-2000 content (mol %)	0	1.25	2.5
total flow rate	2 mL/min	9 mL/min	16 mL/min
ambient temperature during formulation (°C)	4	20	37
aqueous-phase pH	3	5	7
RNA type		mRNA	saRNA
experimental outputs			
critical quality attributes	analytical method		
size	dynamic light scattering		
PDI	dynamic light scattering		
EE	RiboGreen assay		
charge	dynamic light scattering		
% filled particles	RiboGreen assay/nanoparticle tracking analysis		
% full RNA transcripts	BioAnalyzer		



Ly et al. Molecular Pharmaceutics. 2022.

Optimized formulations for maximizing and minimizing cellular activation



input	optimal value 1: minimize cellular activation	optimal value 2: maximize cellular activation	optimal value 3: optimize CQAs
phospholipid content (mol %)	15.9	17.5	17.5
aqueous-phase pH	4.53	6	5.25
ionizable lipid type	ALC-0315	SM-102	ALC-0315
ionizable lipid content (mol %)	45	45	35

Fixed Parameters

N/P ratio	10
phospholipid type	DOPE
DMG-PEG-2000 content (mol %)	1.25
total flow rate (mL/min)	16
ambient temperature during formulation (°C)	20

Ly et al. Molecular Pharmaceutics. 2022.

DoE accurately predicts values for CQAs, protein expression and cellular activation

output	optimal 1			optimal 2			optimal 3		
	predicted	mRNA	saRNA	predicted	mRNA	saRNA	predicted	mRNA	saRNA
EE (%)	81.42	76.73	77.97	80.27	82.00	87.91	92.03	86.10	92.29
size (nm)	104.83	82.62	87.72	92.83	85.56	85.51	100.00	81.70	81.45
RNA integrity (% full transcript)	48.74	73.87	54.80	42.27	73.10	47.65[a]	49.43	84.23	52.30
IL-6 release (pg/mL)	780	872	771	1424	1324	1478	881	823	782
protein expression (RLU)	2.01×10^7	5.65×10^4	2.04×10^7	2.42×10^7	3.61×10^5	1.17×10^7	1.80×10^7	3.55×10^4	1.32×10^7
overall desirability	0.8228			0.9165			0.9203		

- Changing formulation parameters can impact cellular activation (i.e. IL-6 secretion)
- Optimal formulations for saRNA are also suitable for mRNA
- Size of RNA great affects integrity after encapsulation
- saRNA protein expression is 100-1000X higher than mRNA

Ly et al. Molecular Pharmaceutics. 2022.

Conclusions

FORMULATION STRATEGIES

- Polyplexes = 100X higher intramuscular protein expression, LNPs= 100X higher immunogenicity

OPTIMIZATION OF mRNA & saRNA LNPs

- Tuning LNP formulations can directly impact the cellular activation
- Formulations optimized for saRNA delivery and activation are equivalent for mRNA
- Large RNA is highly susceptible to degradation during encapsulation

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Innovate UK

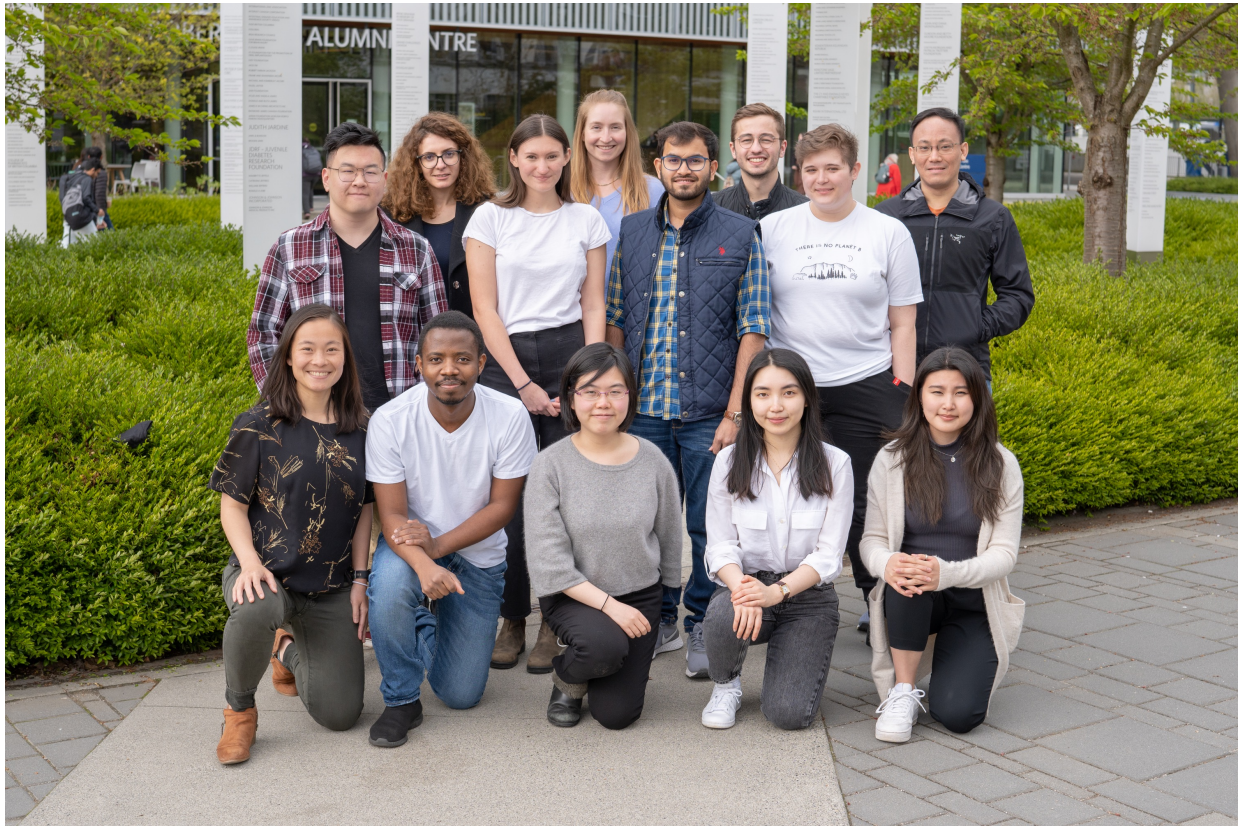
CIHR IRSC

NSERC CRSNG

EPSRC
Engineering and Physical Sciences Research Council

MARIE CURIE ACTIONS

Questions?



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