Elucidation of Lipid Nanoparticle Fusion Mechanisms Using Artificial Membranes

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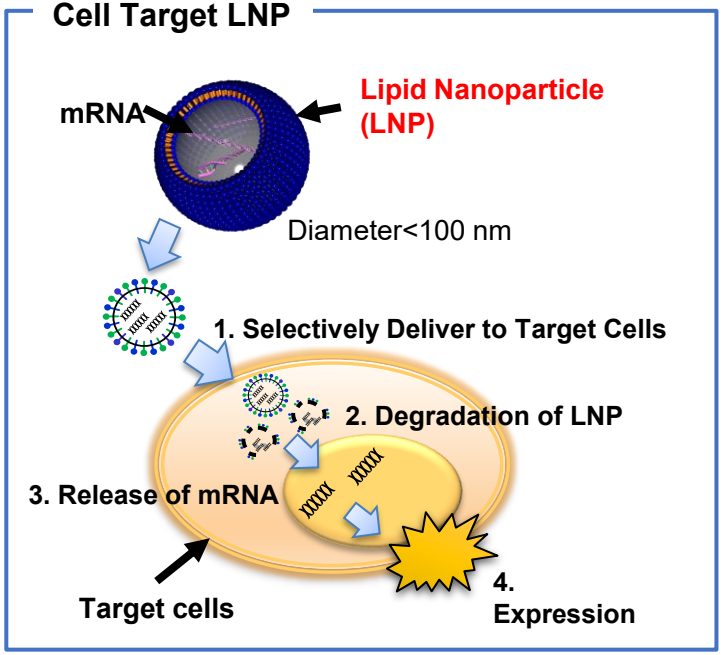
NEXT-GENERATION DELIVERY INNOVATIONS

Lipid Nanoparticle (LNP) Problems and “Designable LNP” Concept



Problem

Targeted delivery to specific cell types is essential for advancing LNP clinical applications.

Conventional Solution: Active Targeting

Conjugate specific antibodies or ligands to LNPs

⇣

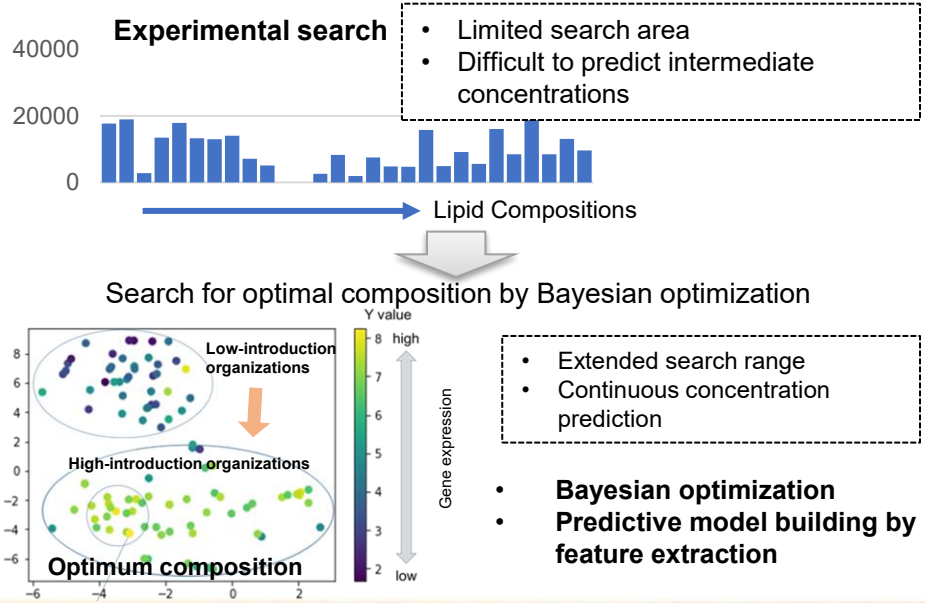
Suppress immunogenic responses and enhance application versatility.

Our Solution

We engineered “Designable LNP” as an LNP design platform to achieve cell-tropism by **optimizing lipid composition**.

”Designable LNP” Platform

Optimized design method for LNP composition

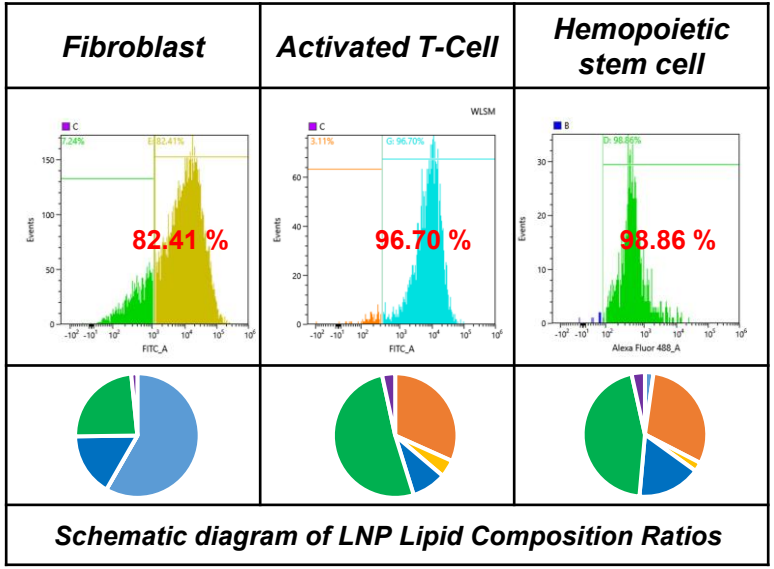


- Limited search area
- Difficult to predict intermediate concentrations

- Extended search range
- Continuous concentration prediction

- **Bayesian optimization**
- **Predictive model building by feature extraction**

Design of Cell-tropic LNP

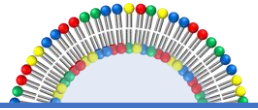


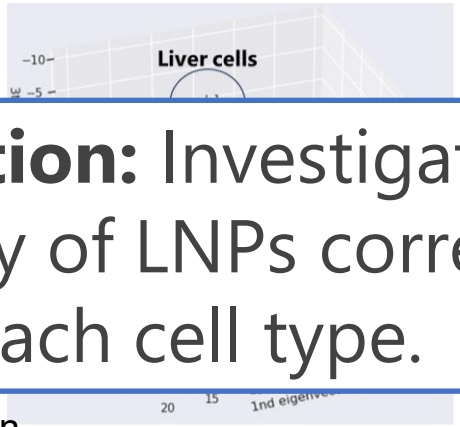
Schematic diagram of LNP Lipid Composition Ratios

From Screening to Design: Optimal LNP Design with AI

Why LNPs have cell tropism?

Cells exhibit distinct lipid compositions





Lipid-lipid interaction

- **AI-driven lipid composition optimization cannot identify the underlying causes of cell specificity.**

- **Research Question:** Investigate how the cell-tropic delivery efficiency of LNPs correlates with the lipid composition of each cell type.

distinct lipid profiles of each cell type can be separated.

Objective

- Elucidate the cell-tropic delivery mechanism of "Designable LNP".

- Verify the cell-tropic delivery mechanism based on lipid-lipid interactions, excluding non-lipid membrane components.
- Construct a cell-tropic delivery analysis using cell-mimic nanoparticles.

Materials

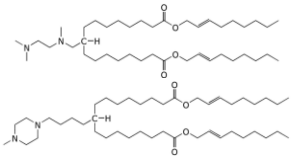
Lipid Nanoparticles

LNPs

Ionized lipids

FFT-10

FFT-20

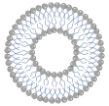


Chol = Cholesterol **PEG = DMG-PEG-2000**

	LNP1	LNP2	LNP3	LNP4	LNP5	LNP6
FFT10	26%	15%	41%	28%	22%	0%
FFT20	0%	0%	0%	0%	0%	27%
DOPE	0%	0%	0%	0%	0%	5%
DOTAP	18%	21%	15%	10%	31%	10%
Chol	53%	60%	42%	58%	44%	55%
PEG	4%	4%	3%	4%	3%	4%
z-ave(nm)	89.8	70.44	111.7	78.78	84.89	71.02
ζ(mV)	19.0	13.3	21.6	4.71	19.9	11.1

Cell mimic nanoparticles

SUVs



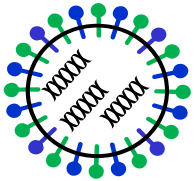
	A	B	C	D	E	F	G	H	I
DOPC	63%	53%	43%	58%	48%	38%	53%	43%	33%
DOPE	0%	0%	0%	5%	5%	5%	10%	10%	10%
DOPS	5%	5%	5%	5%	5%	10%	10%	10%	10%
Chol	30%	40%	50%	30%	40%	50%	30%	40%	50%
PEG	1%	1%	1%	1%	1%	1%	1%	1%	1%

	J	K	L	M	N	O	P	Q	R
DOPC	58%	48%	38%	53%	43%	33%	48%	38%	28%
DOPE	0%	0%	0%	5%	5%	5%	10%	10%	10%
DOPS	10%	10%	20%	20%	20%	20%	20%	30%	30%
Chol	30%	40%	50%	30%	40%	50%	30%	40%	50%
PEG	1%	1%	1%	1%	1%	1%	1%	1%	1%

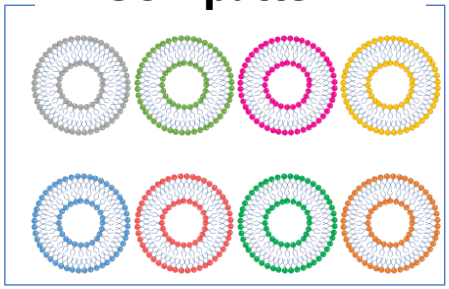
Methods

Mixing NPs

LNP pattern



SUV pattern



Fusion

Dynamic Light Scattering

z(nm) average Size measurement By Dynamic Light Scattering

Particle size UP

Measured by Zetasizer (Malvern. Inc.)

Statistical Analysis

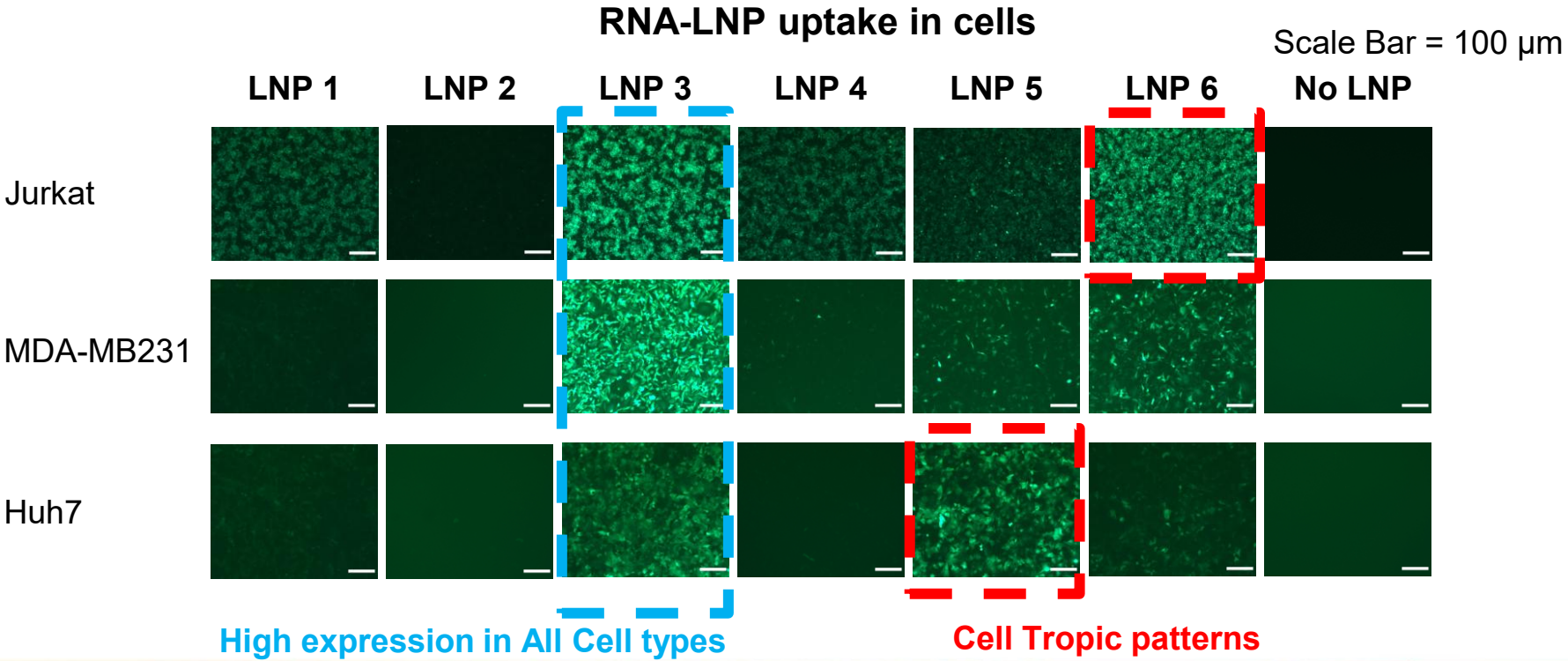
$$R = \frac{D_{\text{mix}}}{\sqrt{D_{\text{LNP}}^2 + D_{\text{SUV}}^2}}$$

- D_{LNP}: Diameter of the liposome before mixing
- D_{SUV}: Diameter of the small unilamellar vesicle (SUV) before mixing
- D_{mix}: Diameter of the particle after mixing
- R: Particle size change rate

Model1: SUV composition + LNP composition model

Model2: SUV composition + LNP composition + LNP-SUV interaction model

Characteristics of mGFP LNP

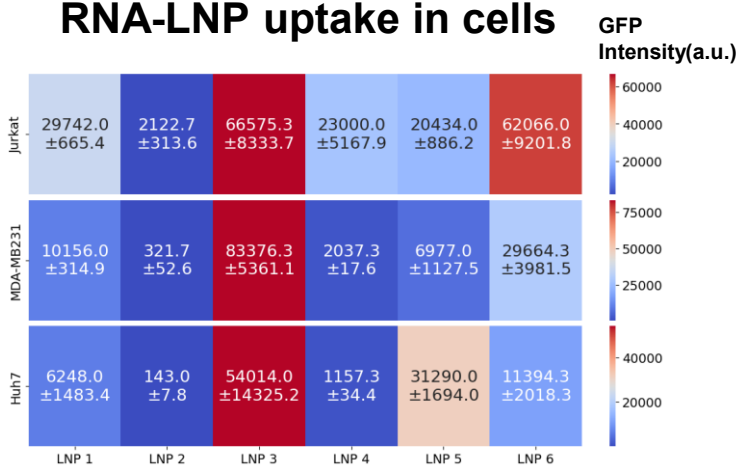


LC-MSMS Cell lipid compositions analysis

LC-MSMS Cell lipid compositions analysis

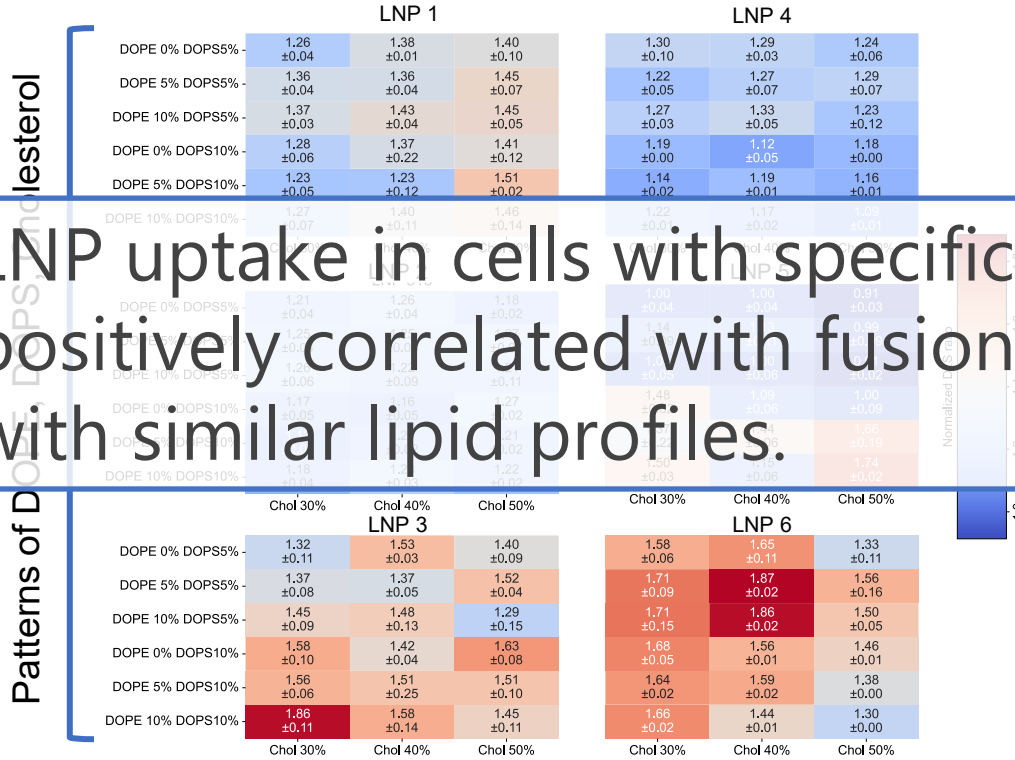
Lipids(mol %)	Jurkat	MDA-MB-231	Huh-7
PC	48.8	50.1	48.9
PS	1.1	5.1	2.3
PE	2.9	13.4	6.1
PI	2.7	2.3	7.9
SM	17.2	6.8	8.2
Cholesterol	27.2	22.3	26.6
Total	100.0	100.0	100.0

RNA-LNP uptake in cells



LNP1 ⇒ Standard
LNP2,4 ⇒ Lower uptake
LNP3 ⇒ High uptake in All types
LNP5 ⇒ High uptake in **Huh7** ⇒High anionic/cholesterol?
LNP6 ⇒ High uptake in **Jurkat** ⇒Low anionic?

DLS result of mixing combination of LNP and SUV



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Cholesterol	27.2	22.3	26.6
Total	100.0	100.0	100.0

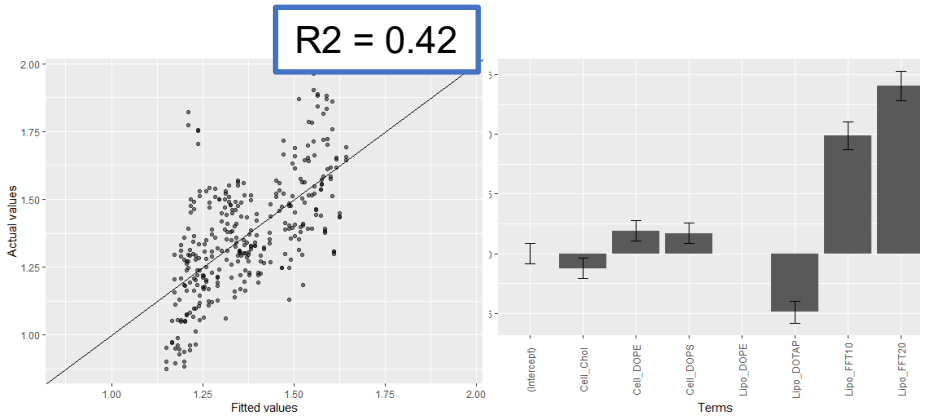
LNP uptake in cells with specific lipid compositions was positively correlated with fusion efficiency toward SUVs with similar lipid profiles.

LNP5：Higher fusion ratio in Higher in DOPS, Cholesterol
⇒Similar to Huh7 compositions

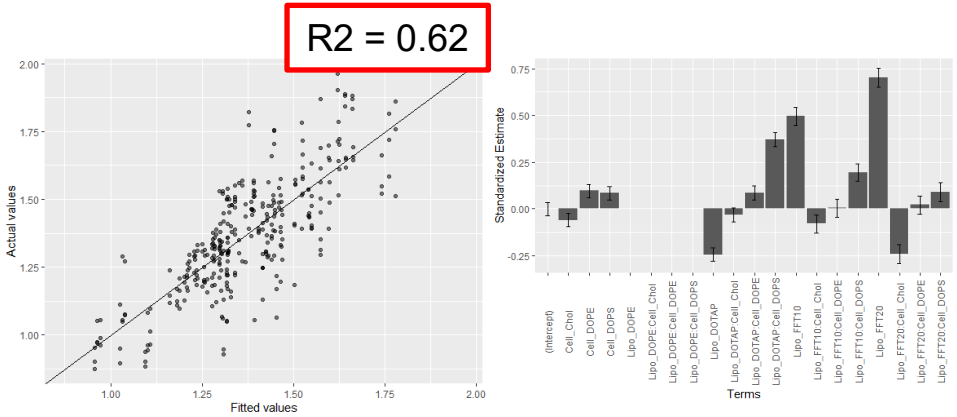
LNP6：Higher fusion ratio in Lower in DOPS, Cholesterol
⇒Similar to Jurkat/MDA-MB231 compositions

Statistical Analysis of Particle Size Change Rate Using Linear Regression

Model 1: SUV Composition + LNP Composition



Model 2: SUV Composition + LNP Composition + LNP–SUV Interaction



Introducing the **LNP–SUV interaction** led to improved performance.

Conclusion and Future Prospects

Conclusion

- We confirmed a cell-tropic delivery mechanism based on **lipid-lipid interactions**, excluding the influence of non-lipid molecules.
- We constructed a cell-tropic delivery analysis model using cell-mimic nanoparticles.

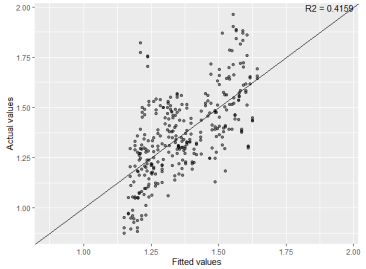
Future Prospects

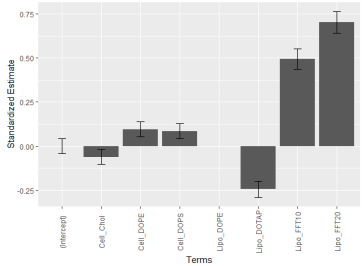
- Employing various nanoparticles, we will comprehensively explore factors involved in cell-tropism.

APPENDIX

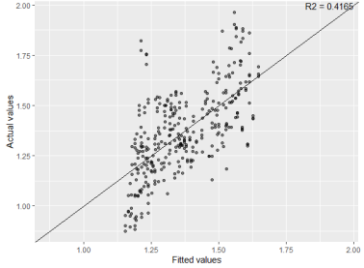
Statistical Analysis of Particle Size Change Rate Using Linear Regression

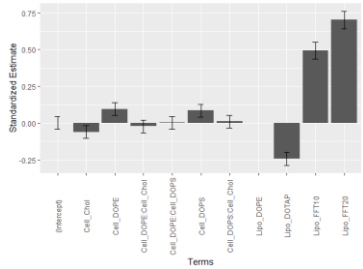
(1) SUV composition + LNP composition model



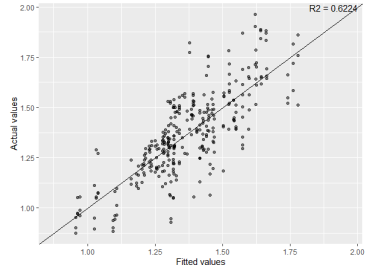


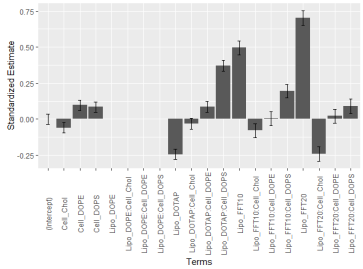
(2) SUV composition + LNP composition + intracellular interaction model



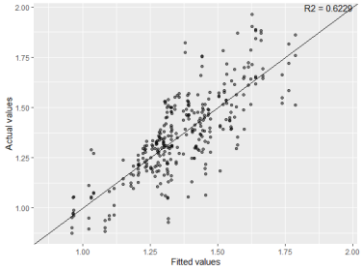


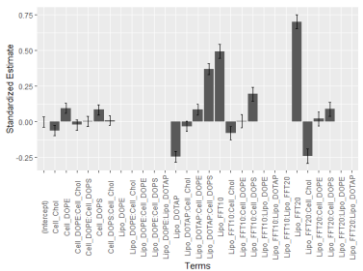
(3) SUV composition + LNP composition + LNP-SUV interaction model





(4) SUV composition + LNP composition + LNP-SUV interaction + intracellular interaction model

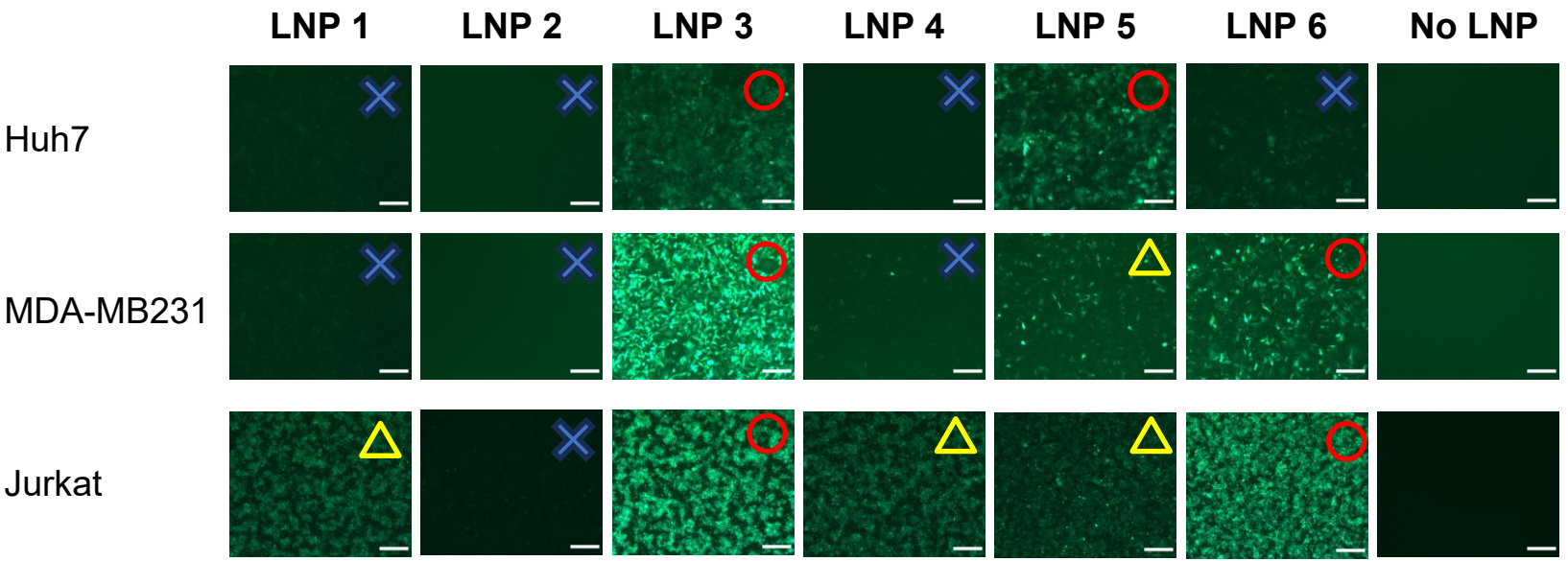




Property of LNPs

	z (nm)	PdI	ζ potential(mV)
LNP1	89.8	0.145	19
LNP2	70.44	0.229	13.3
LNP3	111.7	0.106	21.6
LNP4	78.78	0.148	4.71
LNP5	84.89	0.168	19.9
LNP6	71.02	0.133	11.1

Characteristics of LNP



Scale Bar = 100 μm