Synthetic Lipid Particle Models Illuminate Lipid Interactions Driving LNP-Membrane Fusion

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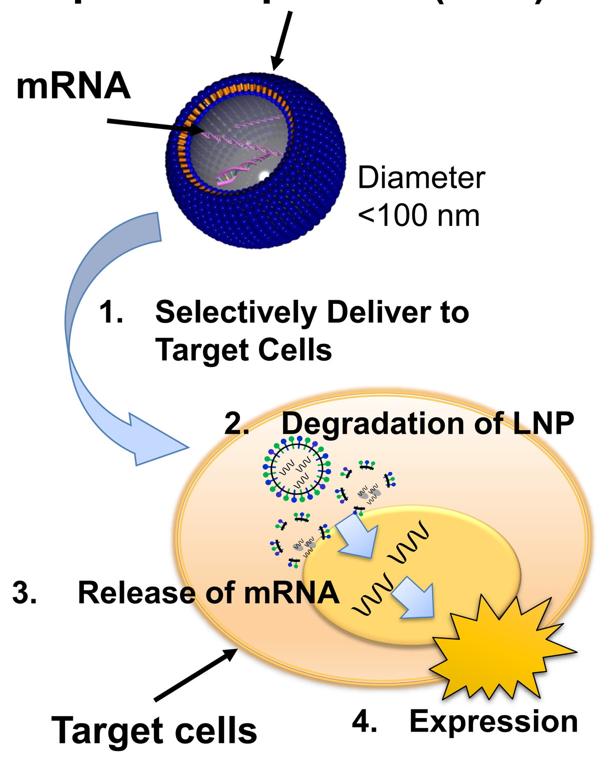
TOSHIBA

"Designable LNPs" Concept



Problem

Achieving cell-type-specific delivery remains challenging

Conventional Solution

Conjugate specific antibodies or ligands to LNPs

Challenges: Immunogenic responses and limited application versatility.

Our Solution

"Designable LNPs"

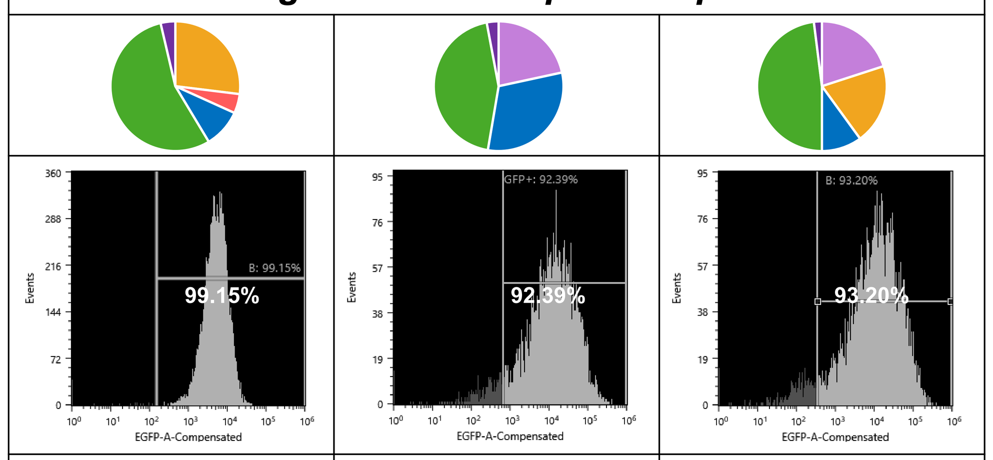
An LNP design platform that enables cell tropism through **optimized lipid composition using AI**

We applied AI-driven Bayesian optimization to identify optimal lipid compositions

Nozaki,E. et al., J. Nanomedicine, 2021

These optimized LNPs exhibited improved delivery efficiency in target cells.

Schematic diagram of LNP Lipid Composition Ratios

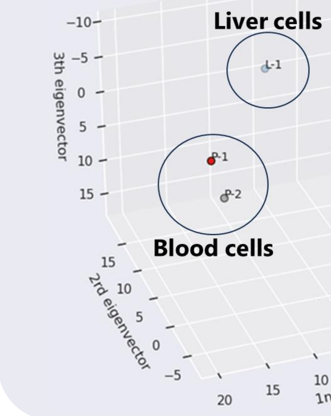


Leukemia cell line | *Liver cancer cell line* | *Breast cancer cell line*

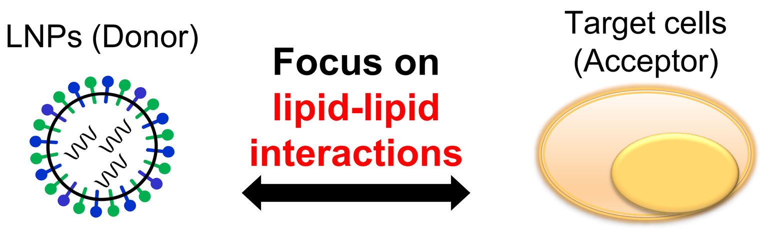
Our Hypothesis

LNP cell tropism depends not only on composition, but also on interactions with target cell membrane lipids

We analyzed the lipid profiles of more than 300 species using LC-MS/MS.



t-SNE showed clear **separation of lipid profiles by cell type.**

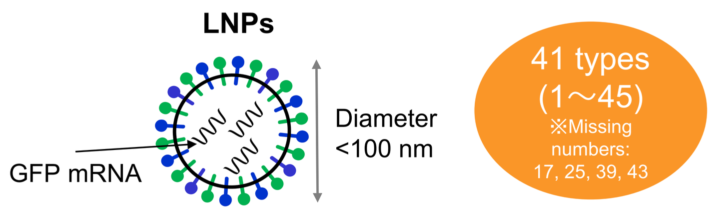


Objective

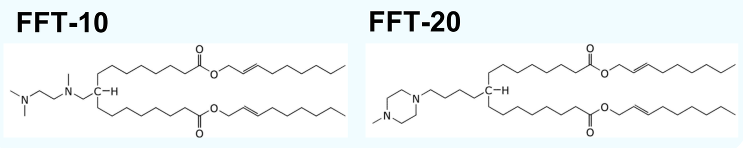
To validate that incorporating lipid-lipid interactions improves the predictive accuracy of LNP delivery

Materials & Methods

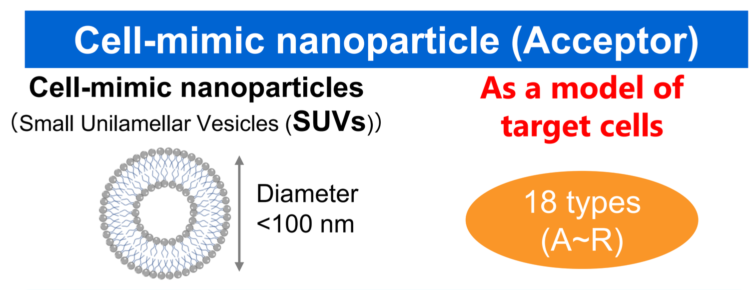
Lipid Nanoparticle (Donor)

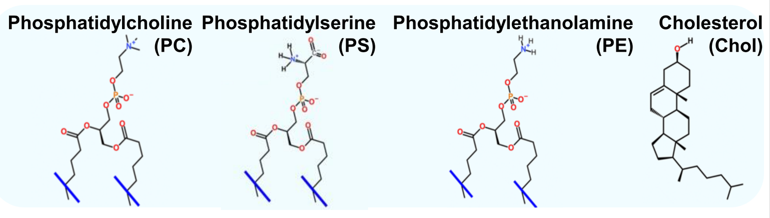


Our proprietary ionizable lipid

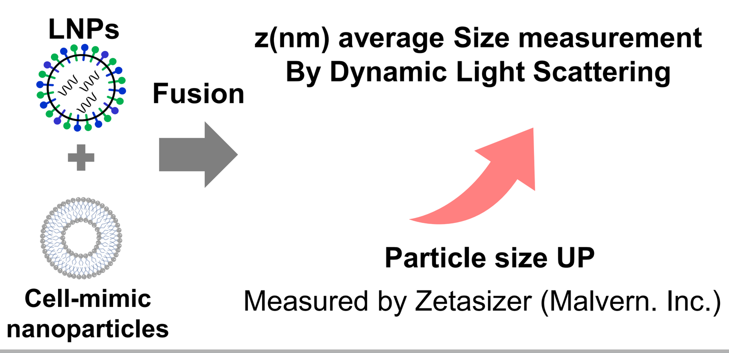


Cell-mimic nanoparticle (Acceptor)





Preparing LNP-SUV mixtures



Advantages

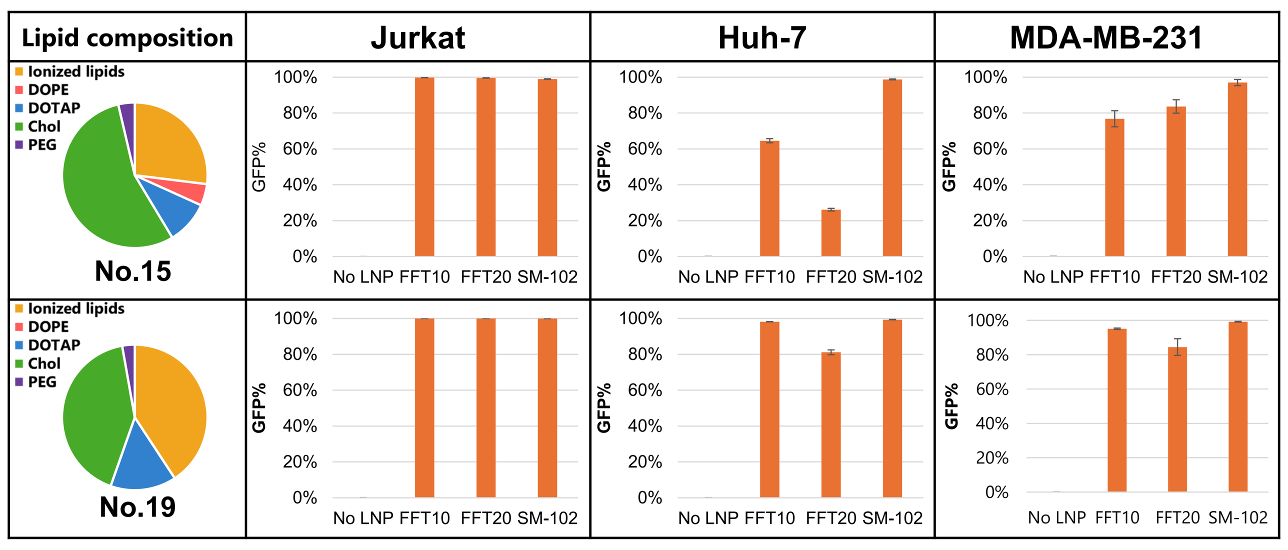
- Enables assessment of lipid–lipid interactions independent of ligands or glycan structures.
- Facilitates precise control of lipid composition

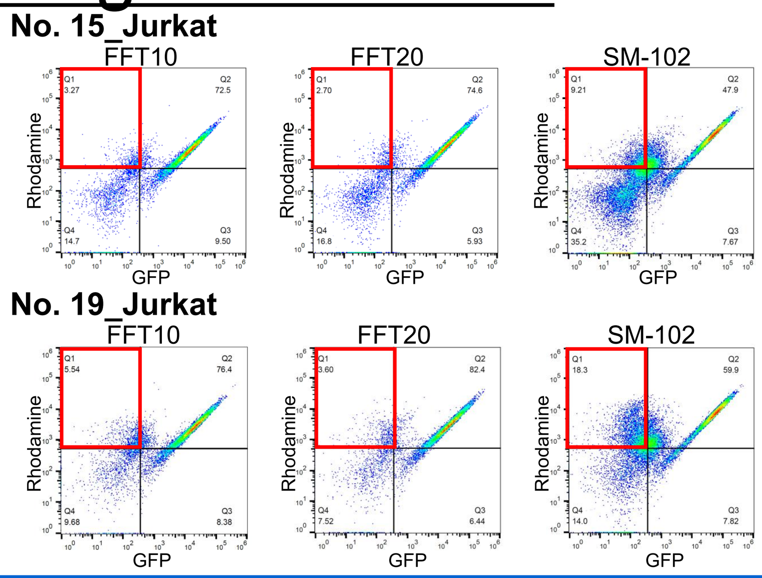
Post-fusion particle size changes

$$R = \frac{D_{mix}}{\sqrt{D_{LNP} D_{SUV}}}$$

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

Comparison of FFT and SM-102 in "Designable LNPs"





SM-102

- Showed more uniform GFP expression across conditions
- Exhibited a higher frequency of Rhodamine-high, GFP-negative events outside the FSC/SSC-defined cell population

⇒ Produced signals across cell types and compositions, but included non-productive events

Broad applicability across multiple cell types

FFT

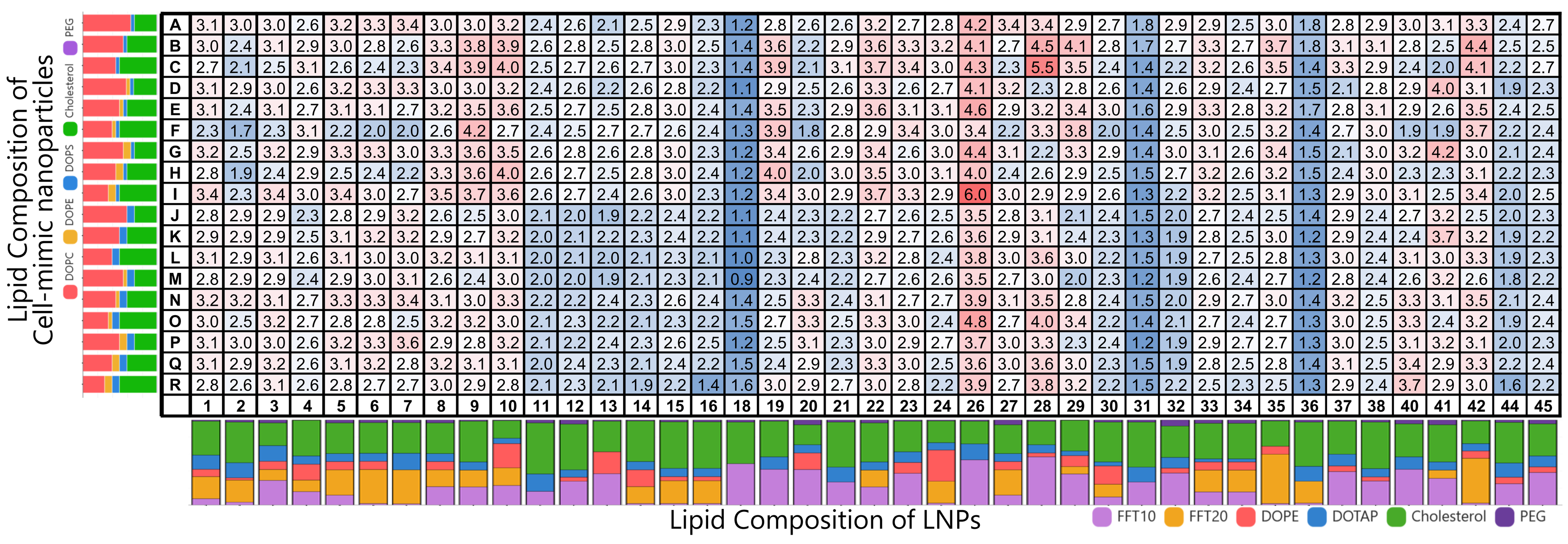
- Showed cell- and composition-dependent GFP expression
- Exhibited fewer Rhodamine-high / GFP-negative events outside the FSC/SSC-defined cell population

⇒ Displayed a more tunable delivery profile, depending on both cell type and lipid composition

Tunable control of cell tropism

Analysis of lipid-lipid Interactions in a Model System

Particle Size Change Rates (R) for LNPs and Cell-mimic nanoparticles



Statistical Analysis of Particle Size Change Rate Using Linear Regression

Model 1: Basic model (Lipid composition only)

$$y = \beta_0 + \sum_{j \in SUV} \beta_j x_j + \sum_{k \in LNP} \beta_k x_k$$

Model 2: Interaction Model

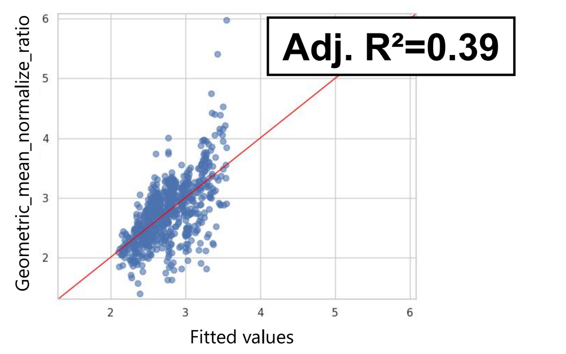
$$y = \beta_0 + \sum_{j \in SUV} \beta_j x_j + \sum_{k \in LNP} \beta_k x_k + \boxed{\sum_{j \in SUV} \sum_{k \in LNP} \gamma_{jk}(x_j \cdot x_k)} + \boxed{\sum_{j<l \in LNP} \delta_{jl}(x_j \cdot x_l)}$$

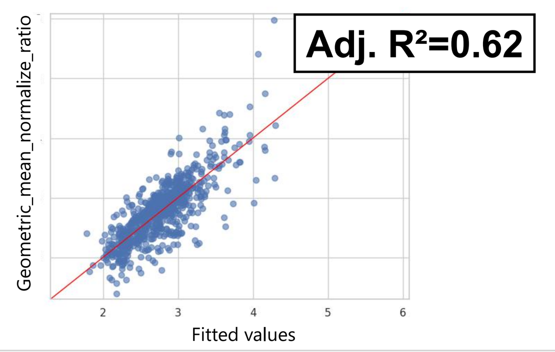
+LNPs-Cell-mimic nanoparticles interactions

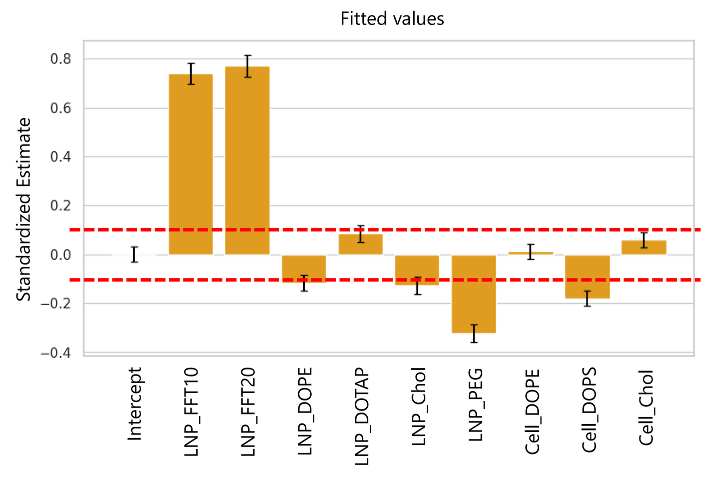
+ Intra-LNPs lipid interactions

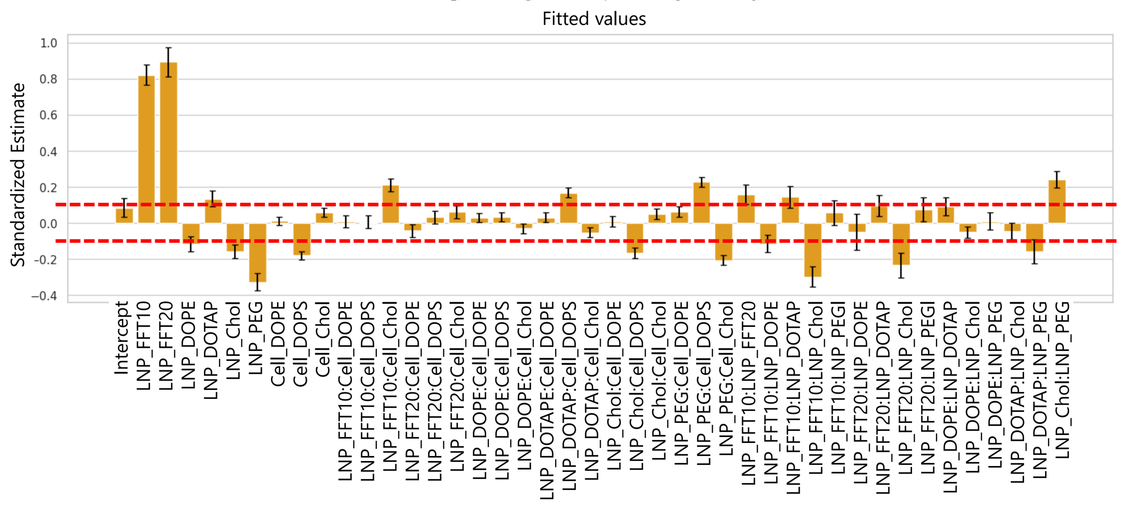
Top: Measured vs. predicted particle size change rates (R).

Bottom: Regression coefficients; |value| ≥ 0.1 indicates major contribution.







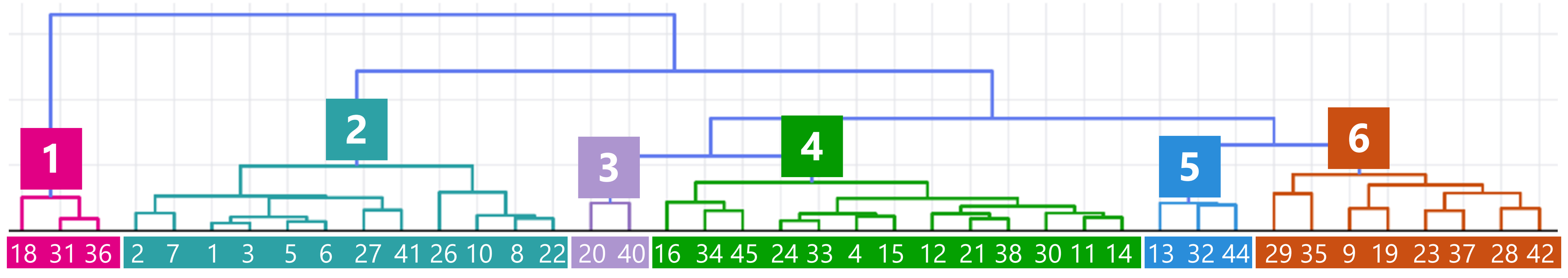


- FFT showed the largest contribution in the basic model
- Including lipid interactions improved model accuracy (0.39 → 0.62)

⇒ Prediction depends not only on FFT, but also on lipid interactions

Cell-mimic–based clustering classifies delivery behavior across cell types

Clustering of LNPs from particle size change rates (R) data



Scaled MFI mapping after EGFP transfection of all LNPs

- Incubation: 24 h at 37 °C (5% CO_2)
- Fluorescence measured by plate reader or flow cytometry

		Mammary gland		Nerve		Pancreas		Lungs		Liver	Gastrointestinal	Blood	
Cluster	LNP No.	MCF7	MM231	SK-N-SH	T98G	Panc-1	MIA-Paca-2	A549	HCI-2122	Huh-7	SW480	Jurkat	MOLT4
1	18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	31	0.25	0.09	0.20	0.29	0.08	0.40	0.28	0.18	0.11	0.51	0.01	0.01
	36	0.25	0.04	0.18	0.58	0.17	0.20	0.34	0.19	0.07	0.44	0.02	0.01
2	1	0.80	0.78	0.43	1.00	1.00	0.41	0.62	0.44	0.75	0.60	0.24	0.23
	2	0.41	0.50	0.16	0.60	0.56	0.20	0.15	0.10	0.75	0.43	0.43	0.28
	3	0.80	0.59	0.92	1.00	0.79	0.58	0.77	0.74	0.48	0.67	0.18	0.11
	5	0.57	0.90	0.26	0.68	0.81	0.67	0.26	0.26	0.55	0.34	0.86	0.83
	6	0.57	0.95	0.31	0.65	0.85	0.67	0.29	0.24	0.67	0.29	1.00	1.00
	7	0.43	0.49	0.28	0.52	0.88	0.31	0.24	0.13	0.62	0.42	0.41	0.19
	8	0.48	0.66	0.23	0.42	0.67	0.69	0.22	0.30	0.46	0.33	0.88	0.85
	10	0.53	0.77	0.31	0.49	0.67	1.00	0.39	0.56	0.59	0.65	0.03	0.05
	22	0.30	0.36	0.13	0.40	0.31	0.23	0.17	0.12	0.30	0.32	0.66	0.49
	26	0.78	0.44	0.96	0.73	0.83	0.31	0.75	0.81	0.29	0.73	0.22	0.02
	27	0.18	0.07	0.08	0.39	0.30	0.15	0.09	0.13	0.31	0.06	0.50	0.39
	41	0.06	0.10	0.01	0.05	0.04	0.14	0.03	0.02	0.23	0.09	0.70	0.28
3	20	0.20	0.32	0.13	0.23	0.26	0.29	0.14	0.19	0.29	0.07	0.36	0.40
	40	0.10	0.12	0.11	0.19	0.28	0.32	0.10	0.07	0.81	0.15	0.84	0.56
4	4	0.52	0.79	0.21	0.52	0.39	0.73	0.31	0.45	0.33	0.49	0.20	0.18
	11	0.19	0.11	0.10	0.06	0.18	0.21	0.09	0.01	0.18	0.25	0.04	0.02
	12	0.02	0.01	0.04	0.01	0.02	0.03	0.01	0.01	0.11	0.03	0.32	0.18
	14	0.37	0.93	0.23	0.78	0.37	0.42	0.16	0.36	0.04	0.41	0.30	0.18
	15	0.37	0.48	0.15	0.59	0.29	0.39	0.11	0.17	0.17	0.20	0.57	0.44
	16	0.35	0.30	0.13	0.47	0.10	0.40	0.07	0.16	0.11	0.18	0.67	0.35
	21	0.56	0.36	0.71	0.50	0.53	0.29	0.33	0.51	0.71	0.61	0.25	0.10
	24	0.44	1.00	0.18	0.52	0.49	0.91	0.14	0.42	0.13	0.74	0.15	0.03
	30	0.02	0.12	0.02	0.06	0.05	0.31	0.01	0.06	0.12	0.12	0.11	0.05
	33	0.12	0.12	0.03	0.28	0.11	0.28	0.03	0.13	0.41	0.05	0.08	0.07
	34	0.08	0.03	0.02	0.25	0.02	0.16	0.02	0.05	0.21	0.05	0.17	0.06
	38	0.17	0.09	0.08	0.27	0.24	0.21	0.12	0.12	0.05	0.20	0.23	0.15
	45	0.41	0.18	0.27	0.49	0.32	0.28	0.52	0.36	0.35	0.64	0.10	0.05
5	13	0.02	0.11	0.01	0.00	0.15	0.47	0.11	0.01	0.54	0.23	0.11	0.06
	32	0.11	0.05	0.09	0.22	0.14	0.22	0.00	0.08	0.25	0.17	0.07	0.07
	44	0.61	0.26	0.46	0.80	0.27	0.49	0.94	0.43	0.05	0.80	0.02	0.00
6	9	0.90	0.66	0.40	0.84	0.69	0.91	0.85	0.59	0.74	0.95	0.63	0.41
	19	0.75	0.45	1.00	0.66	0.48	0.47	0.53	0.85	0.32	0.68	0.38	0.16
	23	1.00	0.66	0.86	0.60	0.74	0.90	1.00	1.00	0.77	0.81	0.15	0.09
	28	0.59	0.51	0.51	0.65	0.56	0.47	0.50	0.61	0.31	0.58	0.10	0.02
	29	0.70	0.95	0.38	0.51	0.68	0.96	0.79	0.85	0.47	0.70	0.16	0.03
	35	0.00	0.00	0.03	0.00	0.06	0.13	0.07	0.00	0.52	0.01	0.00	0.00
	37	0.60	0.66	0.58	0.77	0.13	0.36	0.00	0.75	0.33	0.75	0.05	0.02
	42	0.37	0.67	0.44	0.59	0.51	0.29	0.84	0.51	1.00	0.64	0.11	0.03

Cell-mimic–based clustering is consistent with delivery patterns in real cells, despite being derived solely from lipid interaction data

Conclusions

- ➢ FFT lipids are suitable components for cell-targeted LNP with tunable delivery specificity.
- ➢ Cell tropism depends on LNP composition and target membrane properties, not FFT alone.
- ➢ Target membrane lipid profiles provide a novel parameter for cell-targeted LNP design.

Please contact !

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LNP HP

https://www.global.toshiba/ww/products-solutions/lnp.html