Ionizable Small Molecule Drug-incorporated Lipid Nanoparticles for Treatment of Breast Cancer

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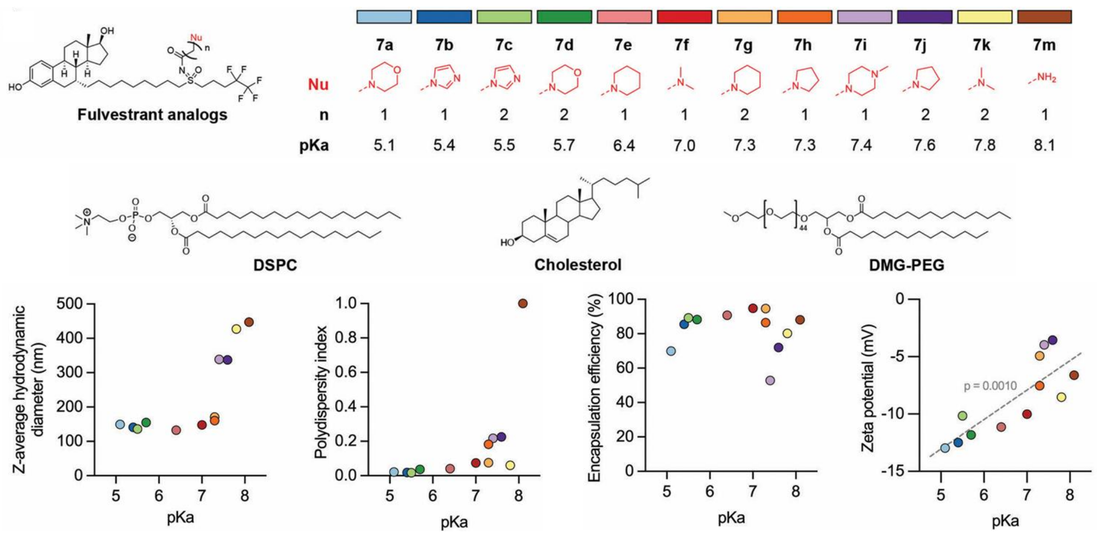
Introduction

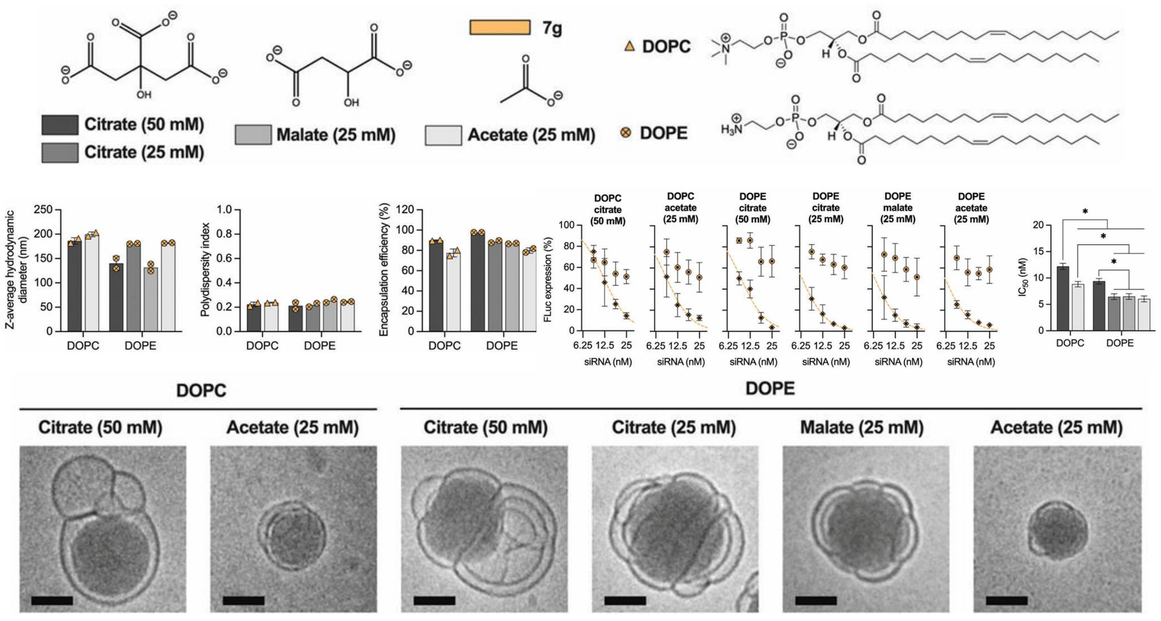
Co-delivery of small molecule drugs and RNA therapeutics in a single nanoparticle can align pharmacokinetics, ensure spatiotemporal co-localization at the tumor site, and enhance synergy against drug-resistant cancers. Ionizable fulvestrant analogs have recently enabled drug-rich, siRNA-loaded nanoparticles with efficient endosomal escape and potent gene silencing. Building on this platform, we hypothesized that combining fulvestrant with ionizable lipids would generate a novel “ionizable drug-lipid” nanoparticle with superior endosomal escape and siRNA-mediated gene silencing relative to conventional LNPs, leading to more effective therapeutic outcomes in breast cancer.

Formulation & Characterization of Ionizable Fulvestrant Analogs-incorporated LNP



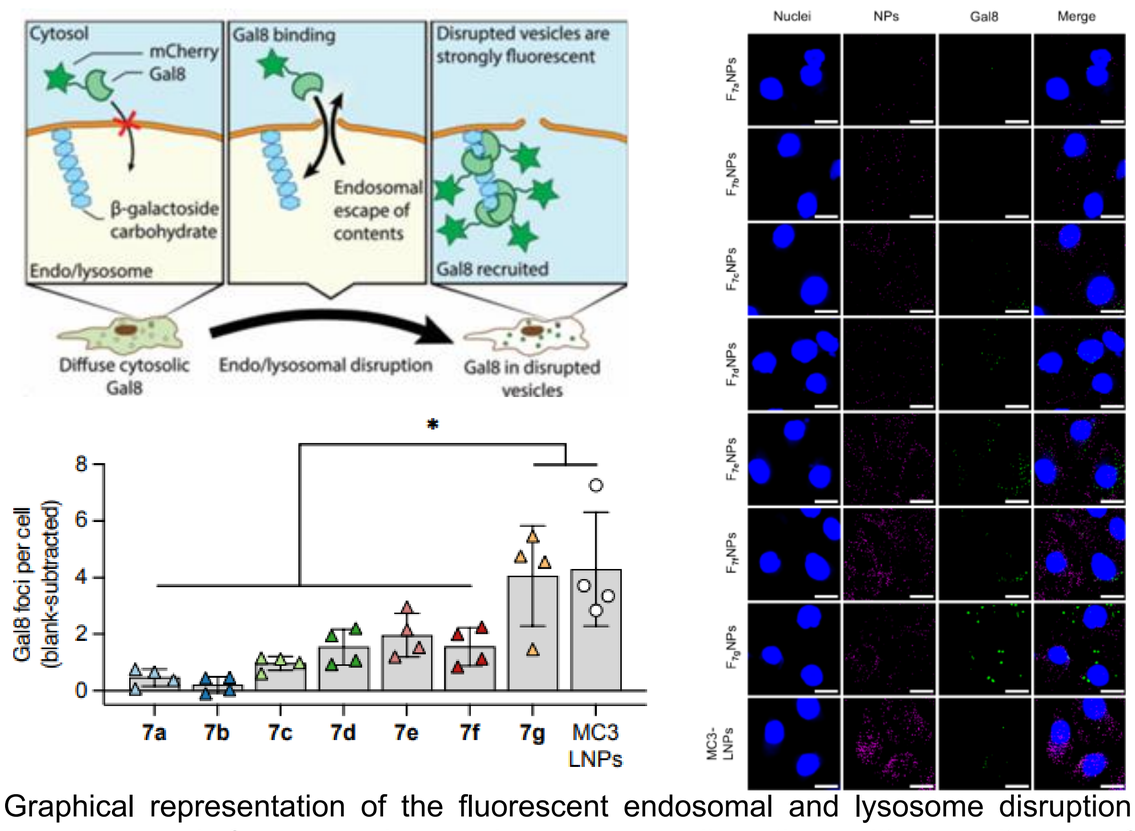
Ionizable fulvestrant analogs were formulated into LNPs with cholesterol, DSPC and DMG-PEG (50:38.5:10:1.5 mole ratio) to encapsulate siRNA. Average size, PDI, encapsulation efficiency (EE%) and zeta potential were measured for characterization.

Optimization of FxNP Phospholipid Selection & Buffer



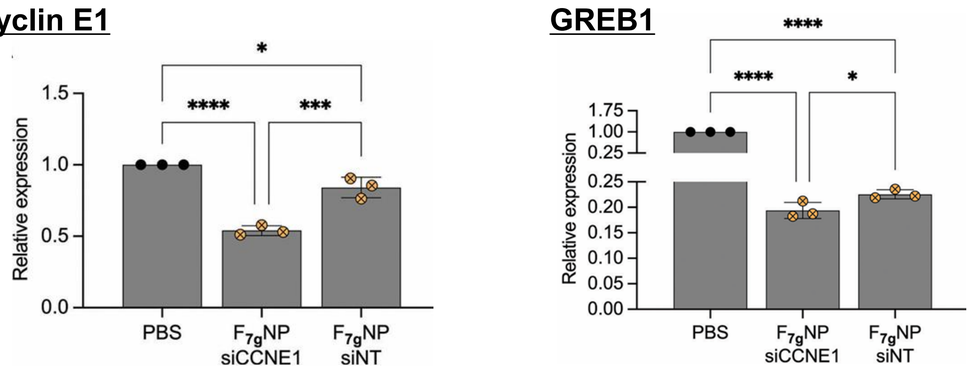
Optimization of phospholipid and buffer for LNP formulation. Size, PDI, EE(%), target gene knockdown effect, IC50 and morphology (scale bar: 50nm) were analyzed. The combination of DOPE and 25 mM Acetate yielded the most stable LNP formulation.

Endo-lysosomes Disruption Assay by FxNPs



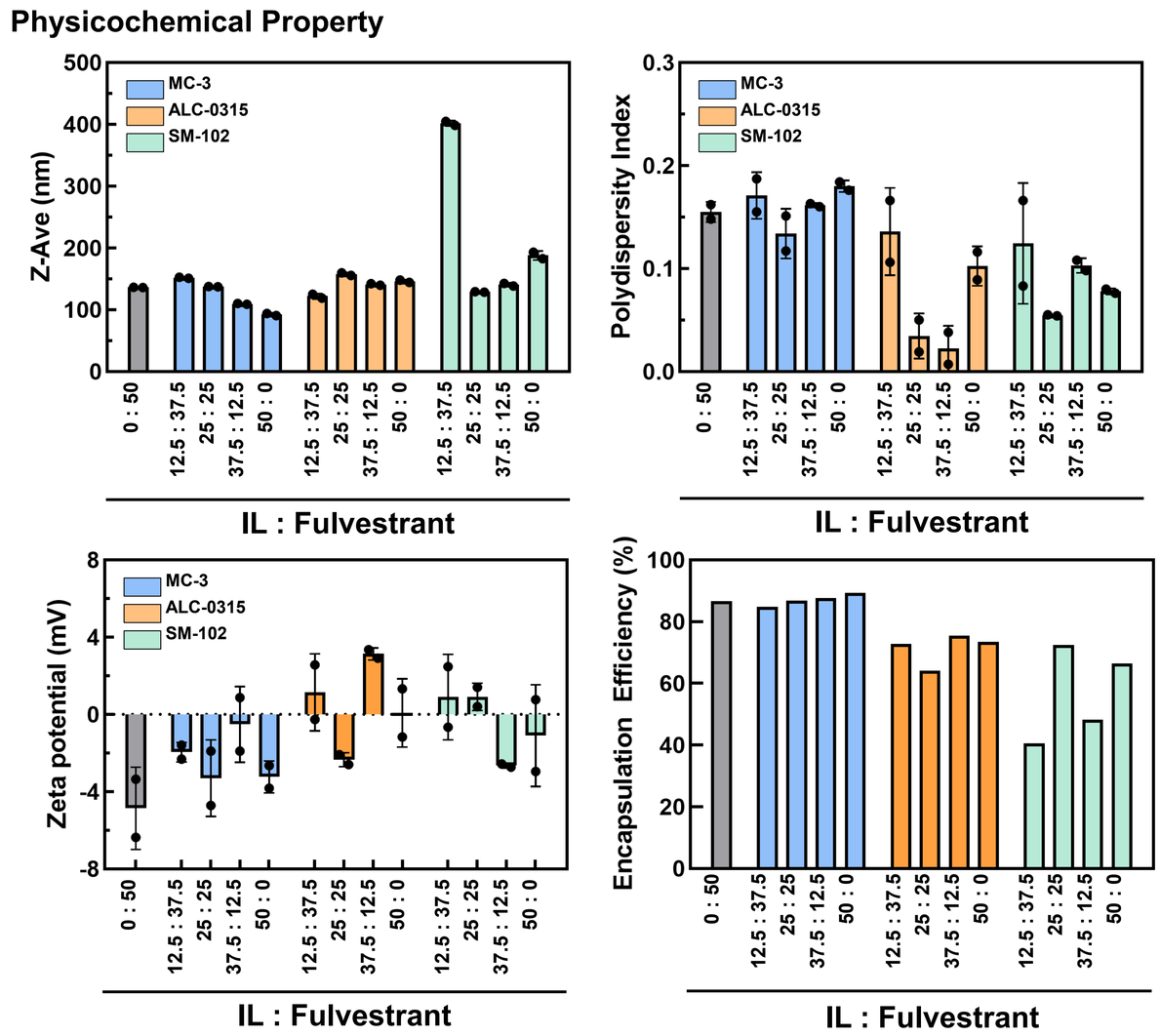
Graphical representation of the fluorescent endosomal and lysosome disruption reporter used for assessing endo-lysosomal disruption with recruitment of mCherry-Gal8 and representative images and quantitative data by FxNPs with DOPC in SKOV3-mChG8 cells (scale bar: 25μm). F_{7g}NP exhibited the highest efficiency in endosomal disruption.

Target Gene Knockdown (Cyclin E1 & GREB1) Analysis by qPCR

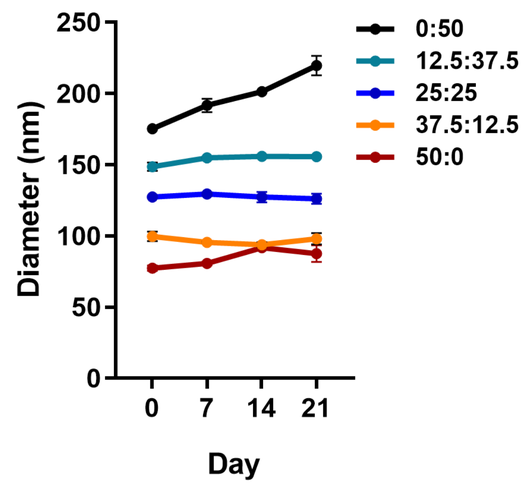


Knockdown effect of CCNE1 and GREB1 mRNA in T47D-R cells after by CCNE1-encapsulated F_{7g}NP.

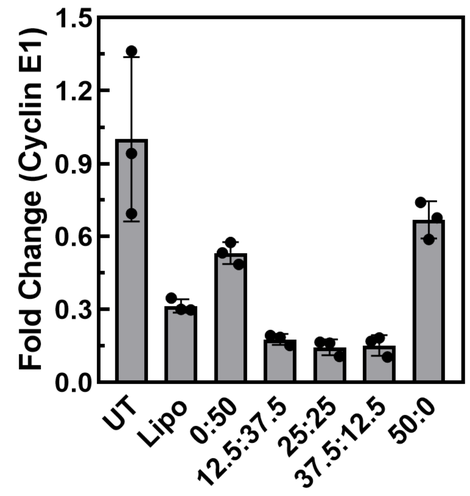
Adjustment of Ratio of Fulvestrant Analog & Ionizable Lipid



Storage Stability



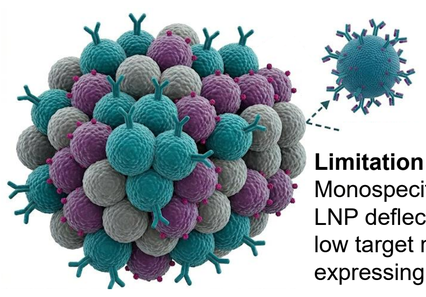
Cyclin E1 Knockdown



Various ratio of ionizable lipids to fulvestrant analog were incorporated to formulate LNPs and characterized by the measurement of size, PDI, zeta potential and EE(%). Furthermore, the storage stability of F_{7g}NPs in 4℃ and target gene (cyclin E1, CCNE1) were analyzed.

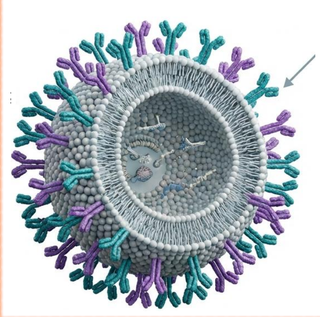
Dual-targeted FxNPs for Enhanced Cancer Specificity

Limitation of Monospecific Ab-LNP for Cancer Therapy



Limitation: Monospecific Ab-LNP deflection on low target receptor expressing cells.

Next-Gen Configuration: Dual-targeted LNP



Modular Surface Modification

1) Overcomes Heterogeneity
2) Enhanced Selectivity
3) Validated Synergy

Conclusions & Future work

Ionizable fulvestrant-incorporated LNPs (F-LNPs) support high drug loading with efficient siRNA encapsulation, improved endosomal escape and cellular uptake, resulting in potent gene silencing. Concurrent CCNE1 knockdown and GREB1 suppression in drug-resistant ER-positive breast cancer highlight this platform as a promising strategy for combination gene delivery and future gene-editing applications.

To overcome the tumor heterogeneity, dual-targeted F-LNPs will be developed to improve the cancer-specific delivery followed by enhanced therapeutic effectiveness, achieving low off-target effect as a future work.

Acknowledgements

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References:
[1] Kai V. Slaughter et al., *Advanced Materials* (2024)
[2] Eric N. Donders et al., *Advanced Science* (2023)