Targeted delivery of lipid nanoparticles

TIDES

May 2024

Di L. Bush, PhD

generation bio™

Disclosure statements

- Di L. Bush, Ph.D.
  - I am a current employee of Generation Bio Co.
  - I hold employee Incentive Stock Options (ISO) in Generation Bio Co.
  - I have not received a separate speaking fee for this learning activity

Forward looking statements

- Any statements in this presentation about future expectations, plans and prospects for the company, including statements about our strategic plans or objectives, technology platforms, research and clinical development plans, and preclinical data and other statements containing the words “believes,” “anticipates,” “plans,” “expects,” and similar expressions, constitute forward-looking statements within the meaning of The Private Securities Litigation Reform Act of 1995. Actual results may differ materially from those indicated by such forward-looking statements as a result of various important factors, including: uncertainties inherent in the identification and development of product candidates, including the conduct of research activities, the initiation and completion of preclinical studies and clinical trials and clinical development of the company’s product candidates; uncertainties as to the availability and timing of results from preclinical studies and clinical trials; uncertainties regarding our novel platforms and related technologies; whether results from preclinical studies will be predictive of the results of later preclinical studies and clinical trials; challenges in the manufacture of genetic medicine products; whether the company’s cash resources are sufficient to fund the company’s operating expenses and capital expenditure requirements for the period anticipated; as well as the other risks and uncertainties set forth in the “Risk Factors” section of our most recent annual report on Form 10-K, which is on file with the Securities and Exchange Commission, and in subsequent filings the company may make with the Securities and Exchange Commission. In addition, the forward-looking statements included in this presentation represent the company’s views as of the date hereof. The company anticipates that subsequent events and developments will cause the company’s views to change. However, while the company may elect to update these forward-looking statements at some point in the future, the company specifically disclaims any obligation to do so. These forward-looking statements should not be relied upon as representing the company’s views as of any date subsequent to the date on which they were made.

Two novel platforms – delivery and cargo – drive differentiated therapeutic opportunities

ctLNP

CELL-TARGETED DELIVERY

REDOSABLE

HIGHLY SELECTIVE

MULTI-TISSUE

In vivo delivery
to previously unreachable
cell types and tissues

iqDNA

IMMUNE-QUIET CARGO

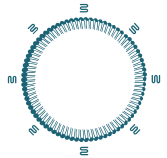
DURABLE

TITRATABLE

GAIN OF FUNCTION

Express or replace large genes

Two novel platforms – delivery and cargo – drive differentiated therapeutic opportunities



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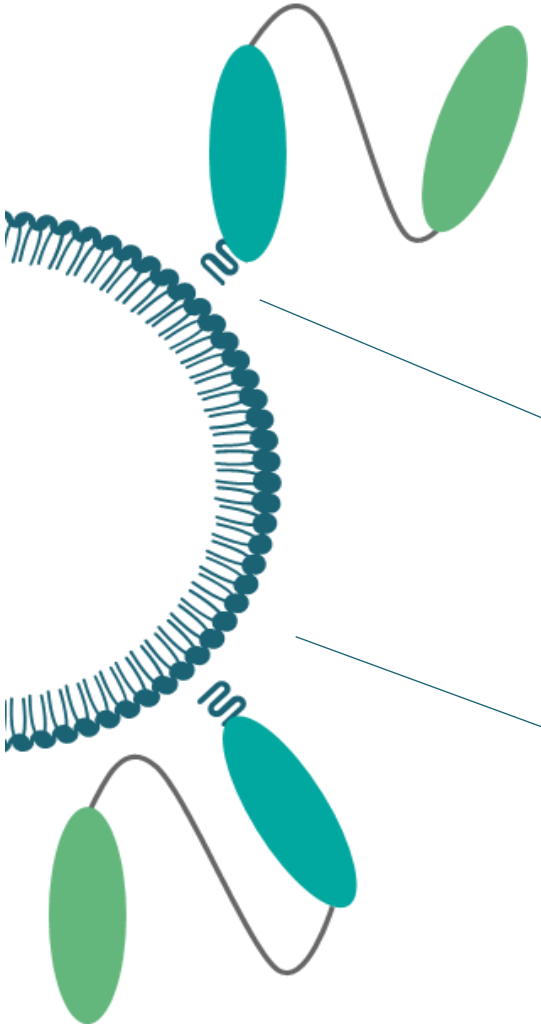
ctLNP is a modular proprietary platform based on stealth, linker, and targeting



LIGAND BIOLOGY

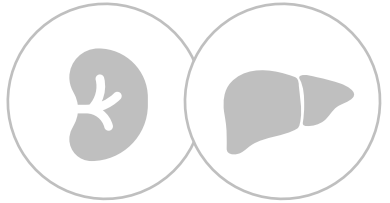
HIGHLY POTENT SELECTIVE TARGETING

LINKER BIOCONJUGATION

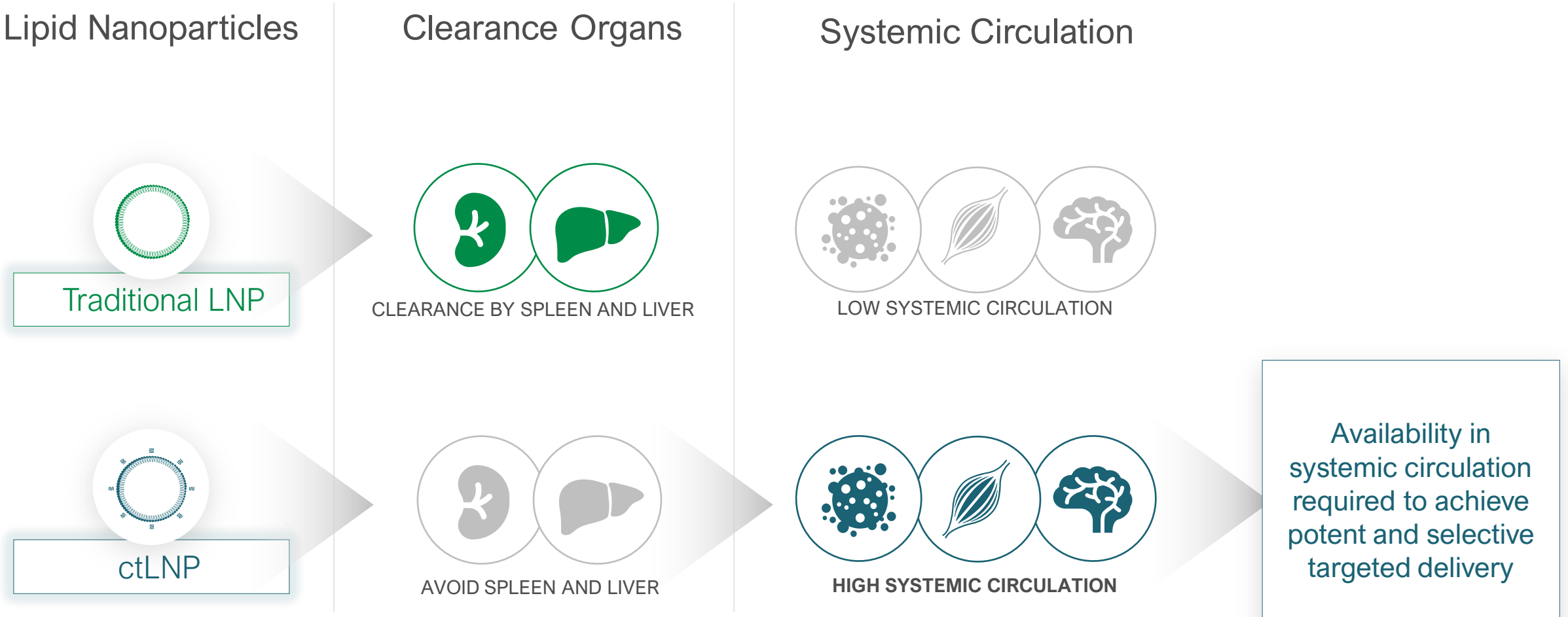
LINKER ENABLES MODULAR LIGAND ATTACHMENT

CORE STEALTH LNP

AVOIDS CLEARANCE, HIGH CONCENTRATION IN CIRCULATION

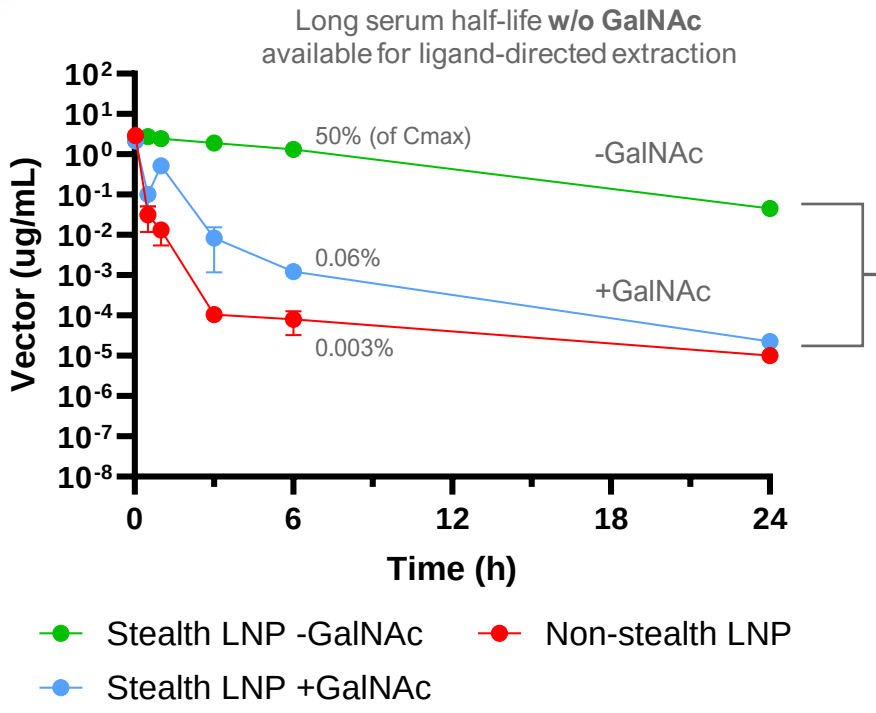


ctLNP avoids liver and spleen clearance, enables a platform approach to targeting previously unreachable cell types and tissues



Stealth profile of ctLNP supports targeting to cell types and tissues beyond the liver

Core stealth LNP persists in circulation and avoids liver and spleen uptake



Active, ligand targeting

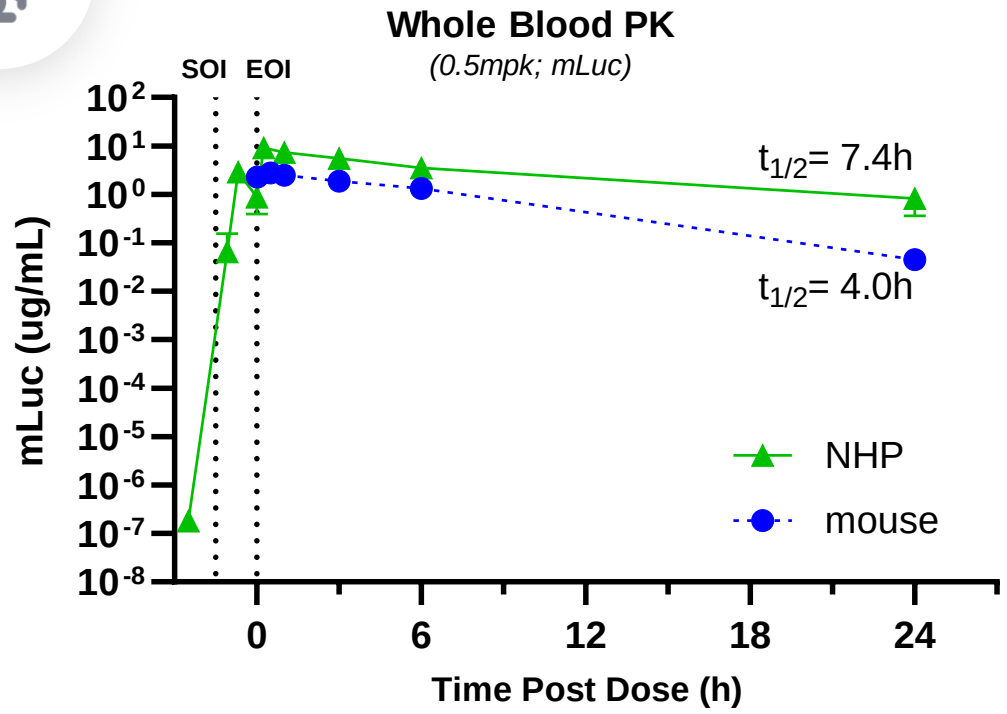
Setting the stage for cell and tissue-selective delivery

Access to extra-hepatic tissues

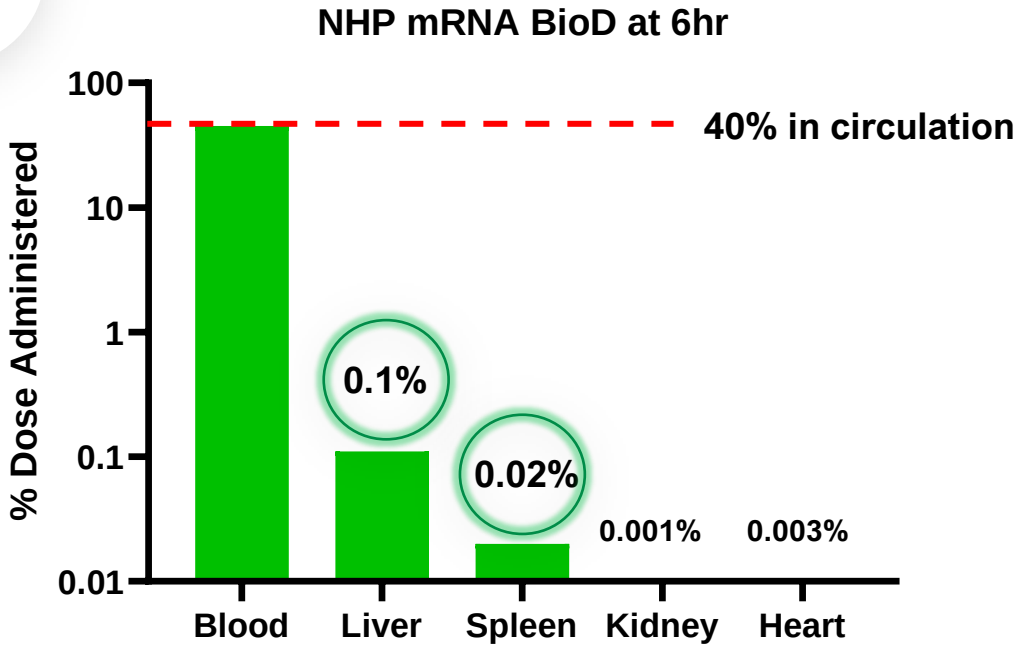
Data presented at ESGCT 2023 meeting

Untargeted ctLNP carrying mRNA demonstrates prolonged circulation and avoids clearance by liver and spleen in NHP

Long circulation time in NHP



Majority of drug remains in circulation, avoiding clearance by liver or spleen



Data presented at ESGCT 2023 meeting


Stealth LNP optimization
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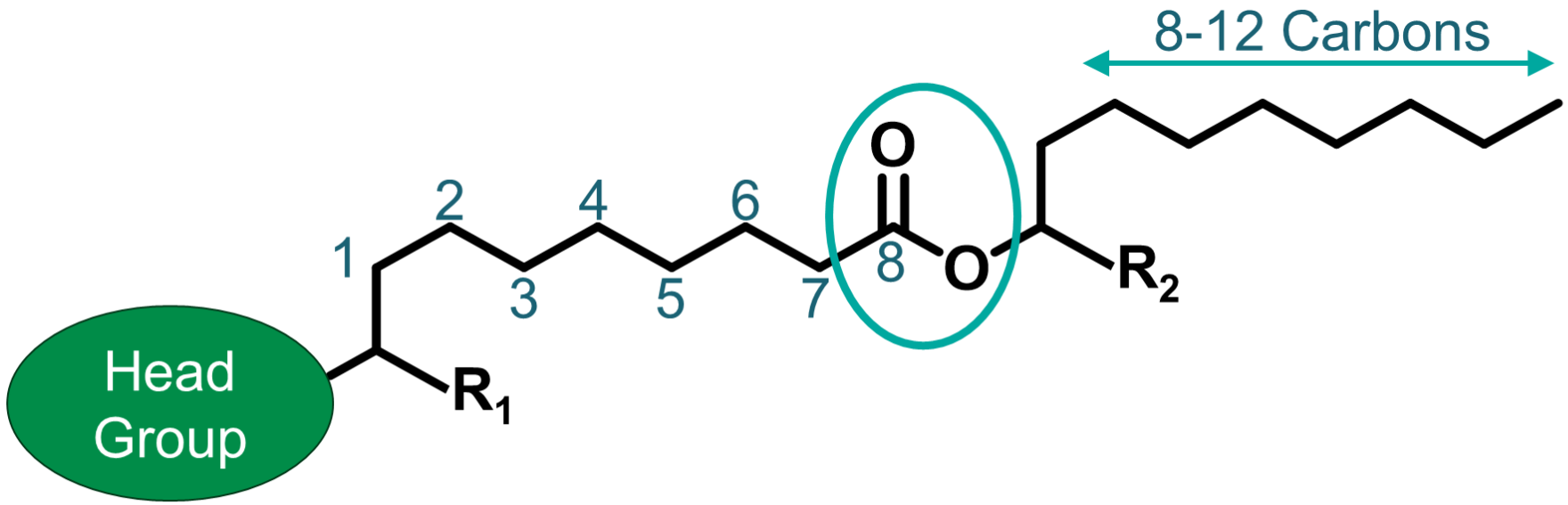
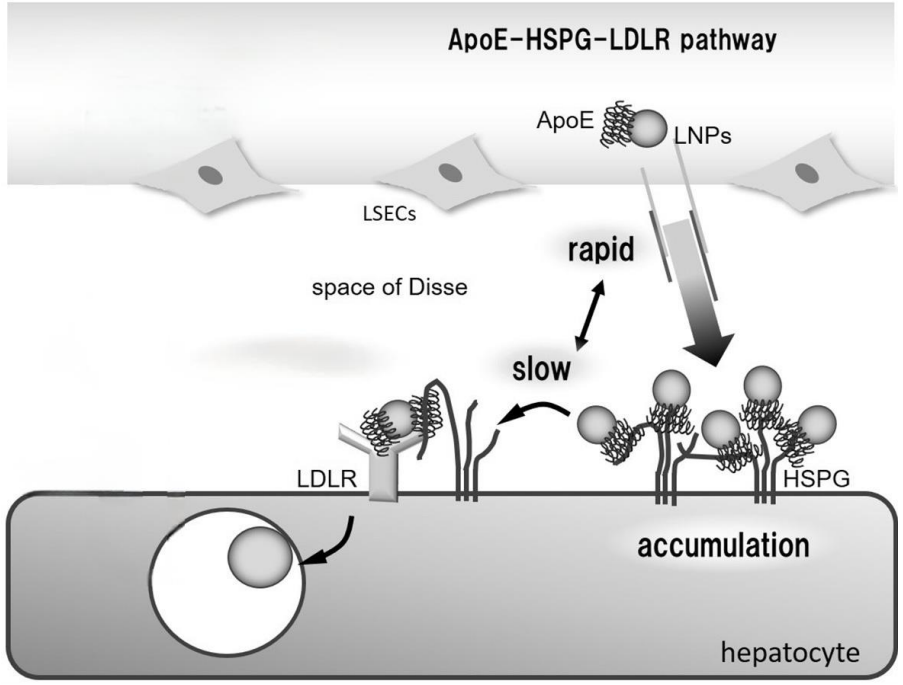
Structural modifications of a constituent ionizable lipid demonstrate clear relationships between structural elements & serum protein binding

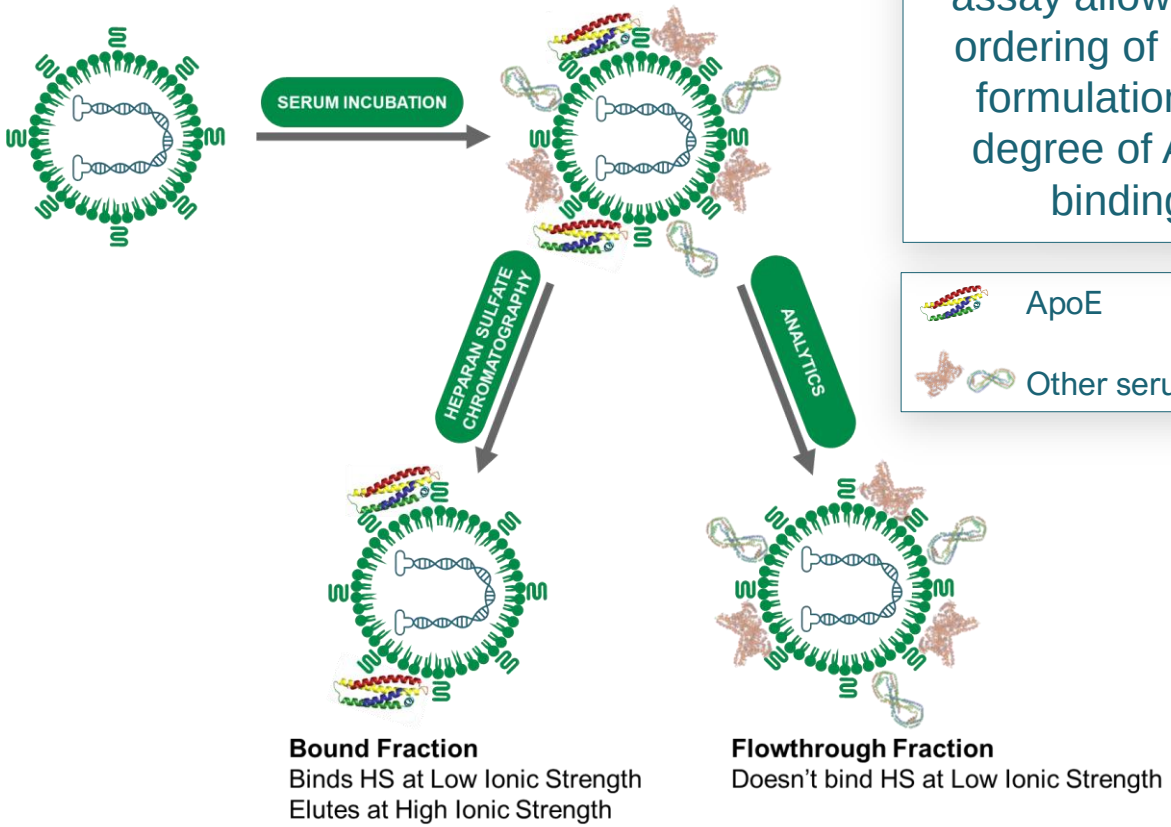


Development of a heparan sulfate (HS) binding assay to assess ApoE binding of serum-incubated LNPs

ApoE bound to LNP surface drives LDLR engagement and hepatic uptake



Adapted from: *Y. Sato, et al., J. of Controlled Release, 2020, 322, 217-226*

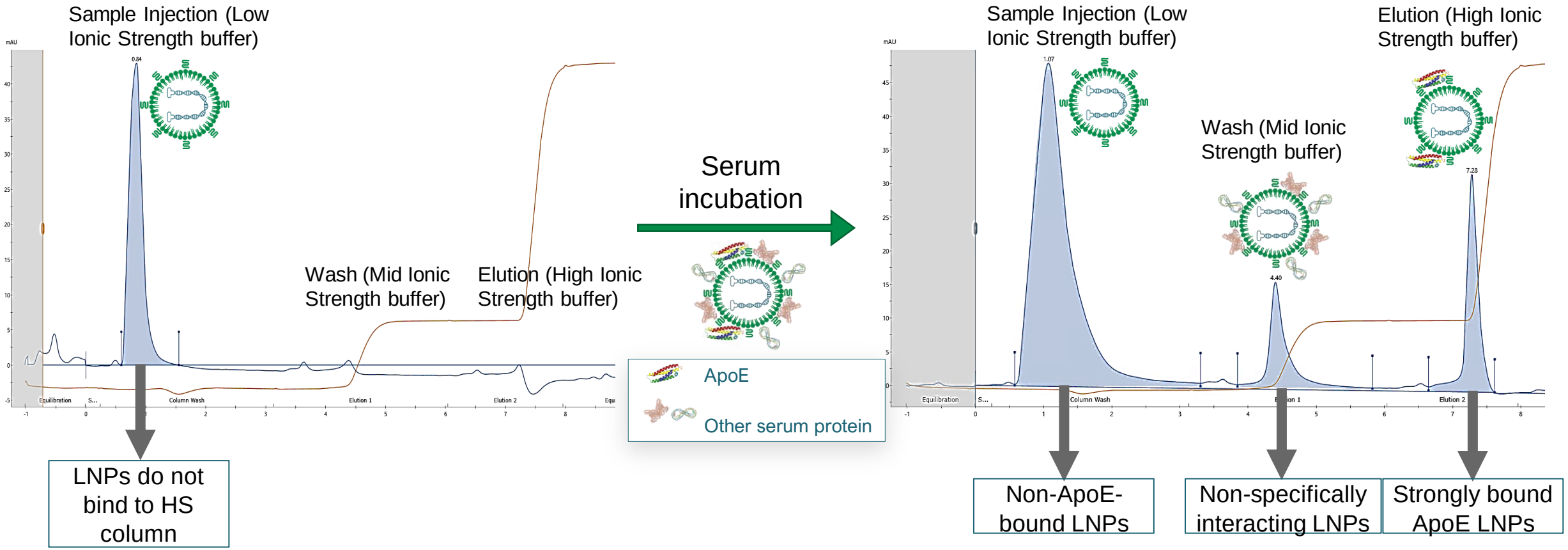


The HS binding assay allows rank ordering of ctLNP formulations by degree of ApoE binding

ApoE

Other serum protein

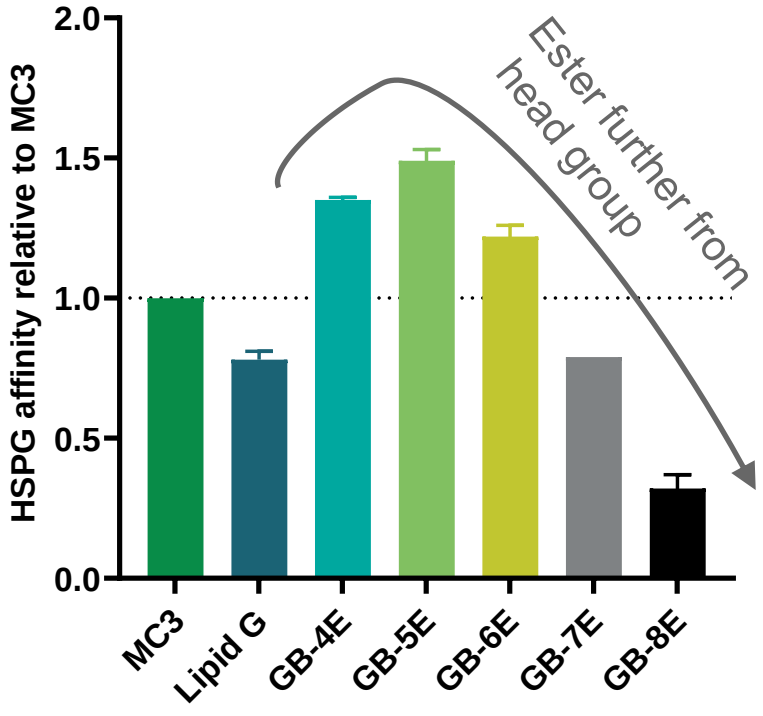
Development of a heparan sulfate (HS) binding assay to assess ApoE binding of serum-incubated LNPs



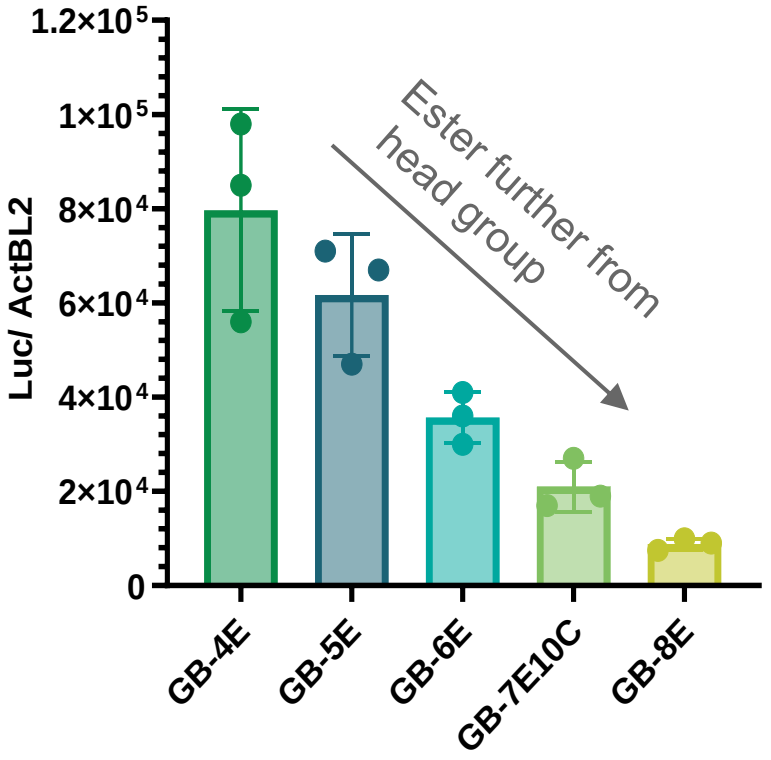
In principle, higher elution peak (more ApoE) is ideal for hepatic uptake; lower elution peak (less ApoE) is ideal for stealth (extrahepatic)

Structural modifications of a parental ionizable lipid demonstrate clear relationships between structural elements & HS binding

Ester further from the head group decreases HS affinity of ctLNP

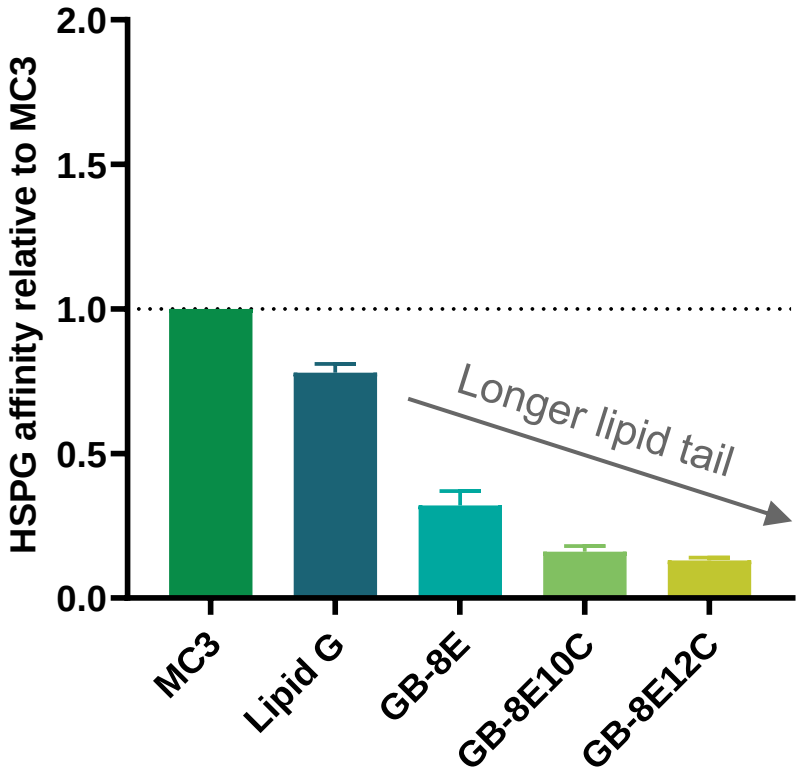


LDLr-mediated uptake correlates with HS affinity, i.e. ApoE binding



Structural modifications of a parental ionizable lipid demonstrate clear relationships between structural elements & HS binding

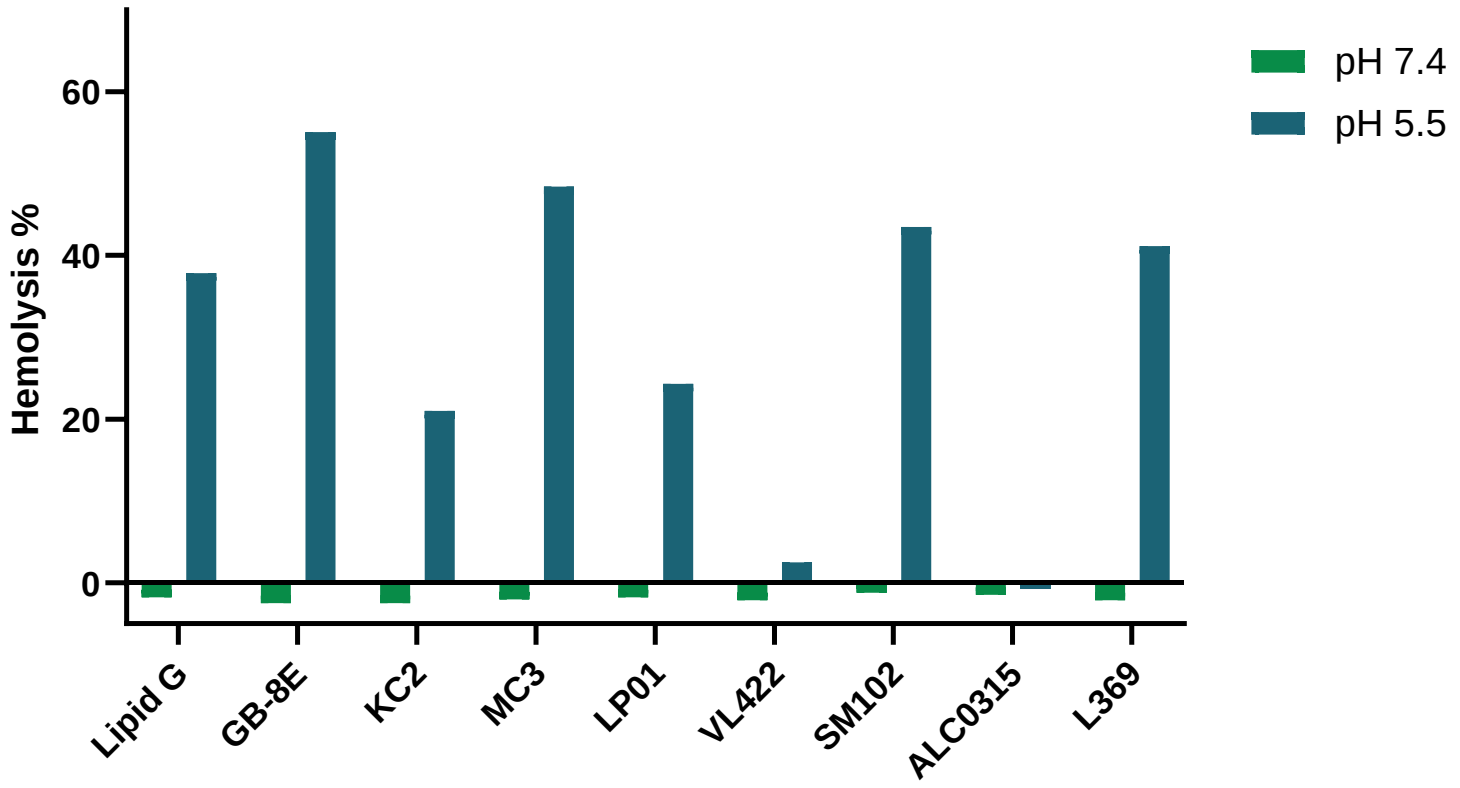
Increased tail length of ionizable decreases HS affinity of ctLNP



- Modification of ester position and tail length of parent ionizable allows control over ApoE affinity of formulated ctLNPs (as compared to MC3-based formulation)
- Correlation with in vitro cell uptake via ApoE-HSPG-LDLr pathway demonstrates ability for LDLr (hepatic) avoidance with ionizable lipid modification

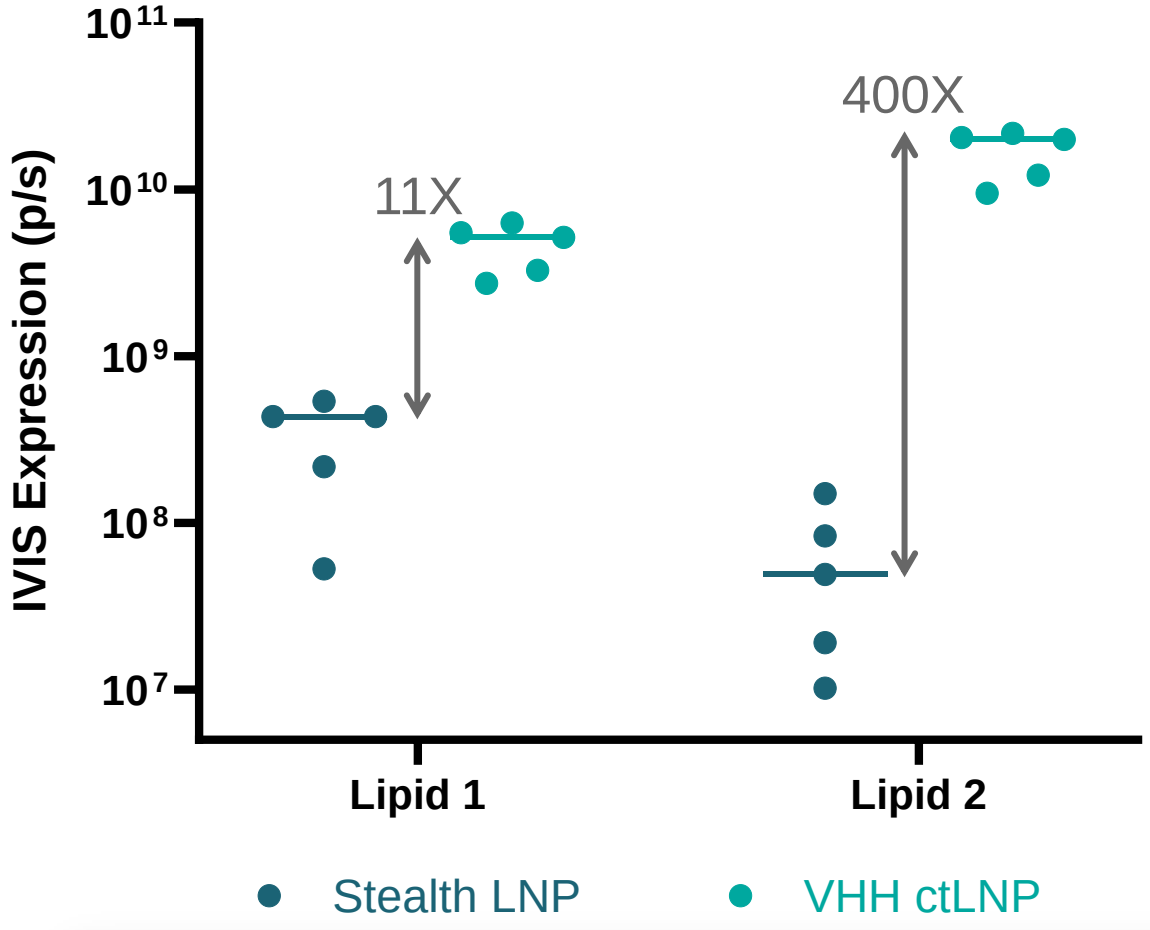
Structural changes in the ionizable lipid can improve stealth without sacrificing endosomal escape potential

Stealth lipid's membrane disruption activity indicative of high endosomal escape potential



- High membrane disruption activity of "stealth" ionizable is demonstrated with a hemolysis assay
- Outperforms an internal control, and several clinically used and commercially available ionizable lipids

Selection of the appropriate “stealth” ionizable can simultaneously improve stealth and expression upon conjugation with a targeting ligand



VHH targeting liver ASGPR with mRNA cargo

In vivo expression comparing untargeted Stealth LNPs and ctLNPs

- Dose = 0.05 mpk
- Ionizable lipid structure has a significant impact on ctLNP interaction with serum protein
- Chemical design can significantly improve both stealth and potency
  - Lipid 1 has an 11X expression difference, Lipid 2 has a 400X expression difference between targeted ctLNPs and untargeted stealth LNPs

Conjugation optimization

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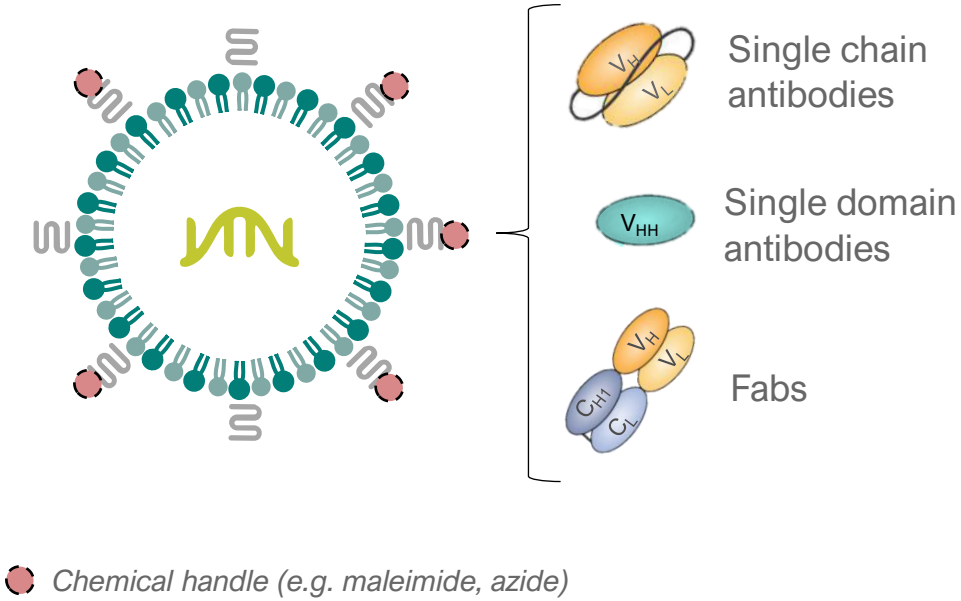
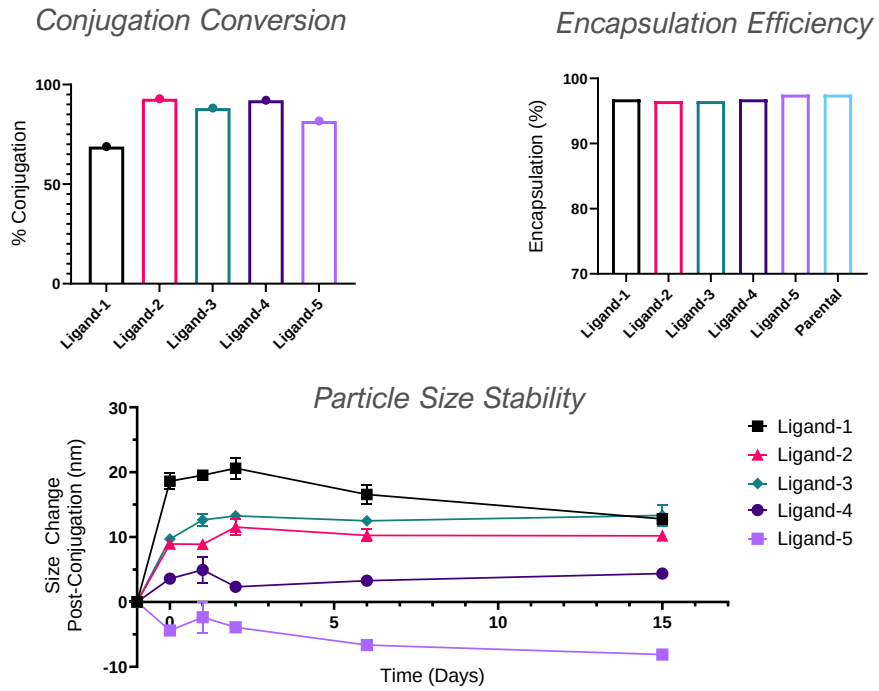
Bioconjugation platform enables active ligand targeting, leveraging site specific conjugation to generate stable, functional ctLNPs

Active ligand targeting through direct conjugation of functionalized LNPs



Site specific bioconjugation enables highly stable, selective ctLNPs



See Poster 1241

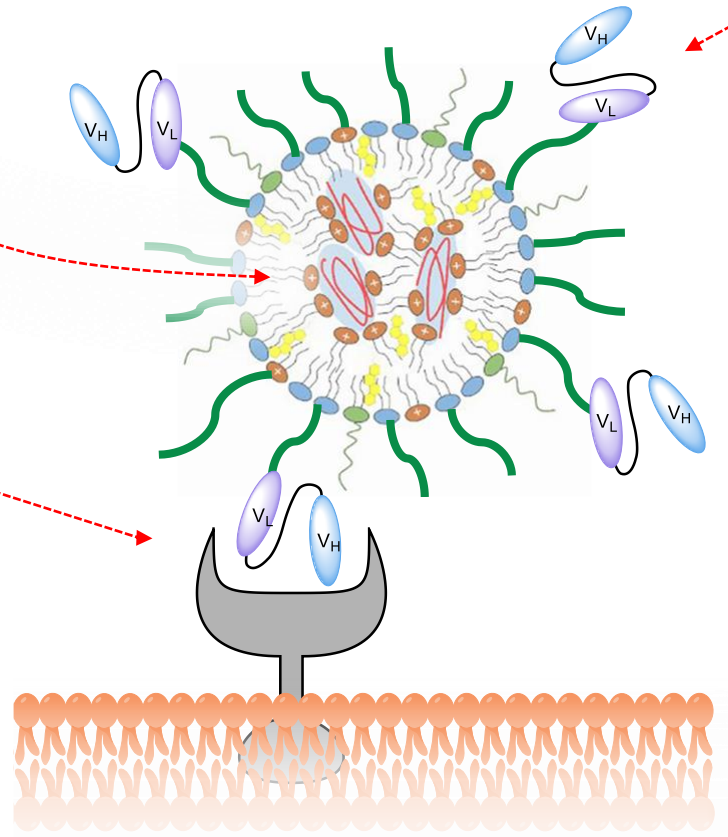
Release assay and enhanced characterization panels established to provide a clear picture of conjugate quality

Cargo Characterization

- ✓ Encapsulation (Ribogreen)
- ✓ Payload Concentration (IEX)
- ✓ Copy Number (ddPCR)
- ✓ Payload Purity (IPRP-HPLC)

Conjugate Functionality

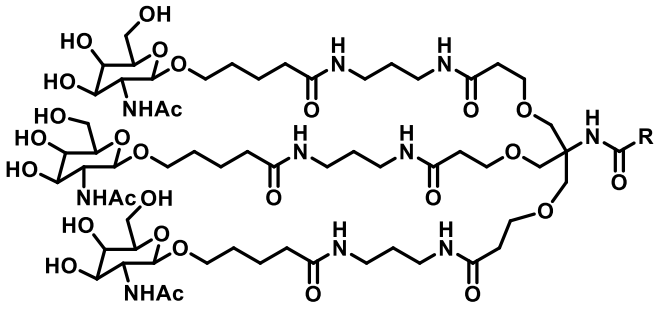
- ✓ Ligand Binding (LNP-Conjugate ELISA)
- ✓ Association, Internalization, & Expression in primary cells and cell-lines
- ✓ Companion Study in Mice



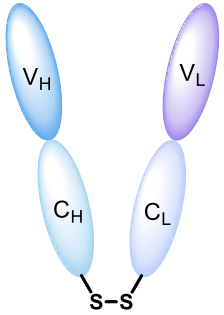
LNP-Ligand Conjugate Characterization

- ✓ Size & PDI (DLS & NTA)
- ✓ Particle Concentration (NTA)
- ✓ Zeta Potential
- ✓ Endotoxin (LAL)
- ✓ pH and Osmolality (Osmotech)
- ✓ Lipid Molar Ratio (UPLC-CAD)
- ✓ Conjugation Efficiency and Conjugate Species Purity (SDS-PAGE & LC-MS)
- ✓ FFF-MALS
- ✓ Cryo-EM

Comparing multiple targeting ligand formats with varying affinities to ASGPR and off-target receptors

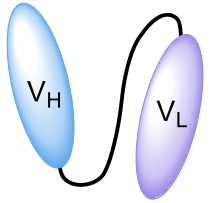


$(GalNAc)_3$
hASGPR (nM): 30.7
CD301 (nM): 7.6



Fab-1
hASGPR (nM): 94.6
CD301 (nM): N/A

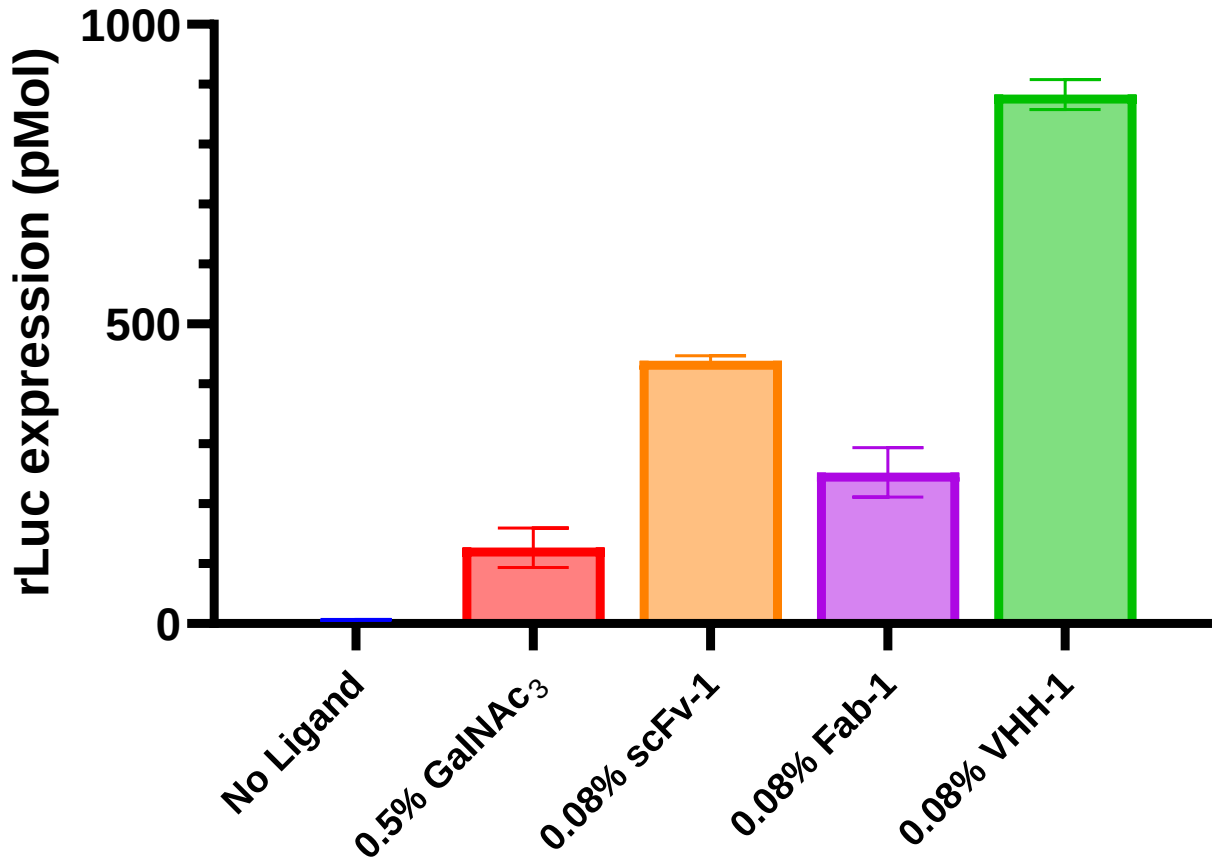
VHH-1
hASGPR (nM): 9.0
CD301 (nM): N/A



scFv-1
hASGPR (nM): 17.3
CD301 (nM): N/A

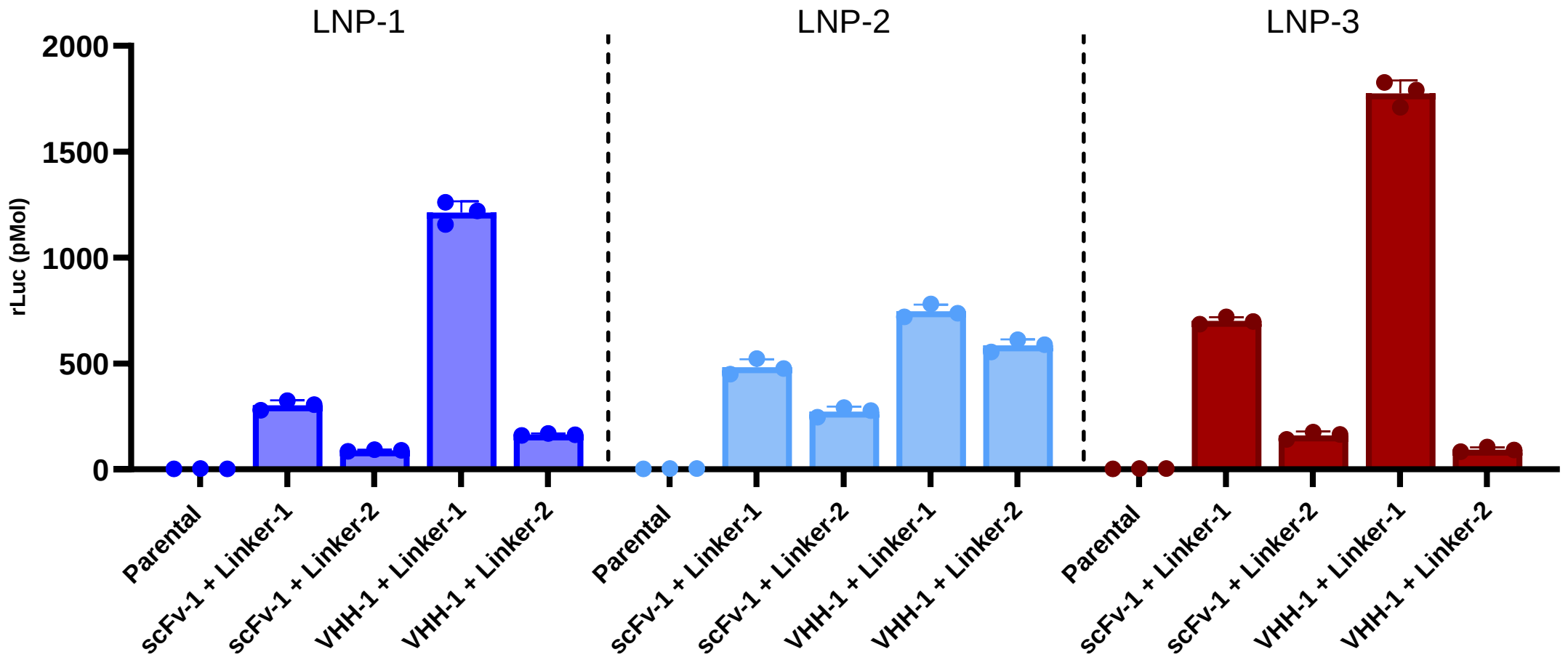
Comparing multiple targeting ligand formats with varying affinities to ASGPR and off-target receptors

mFLuc expression from ctLNPs demonstrates antibody-derived ligands superiority over $GalNAc_3$

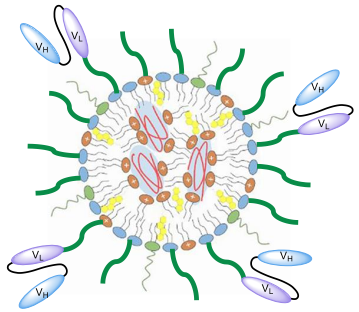


- VHH-1 and scFv-1 were selected for further optimization

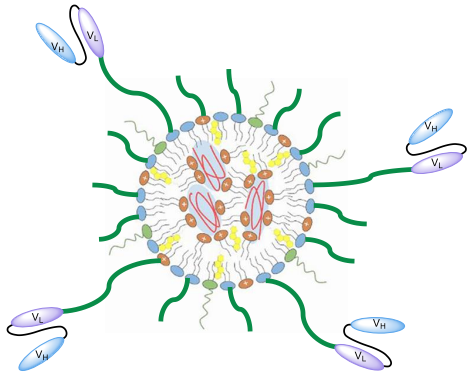
Linker chemistry 1 demonstrates higher activity across multiple stealth LNPs with both ScFv-1 & VHH-1



Longer ligand spacers facilitate higher uptake into and expression by primary hepatocytes

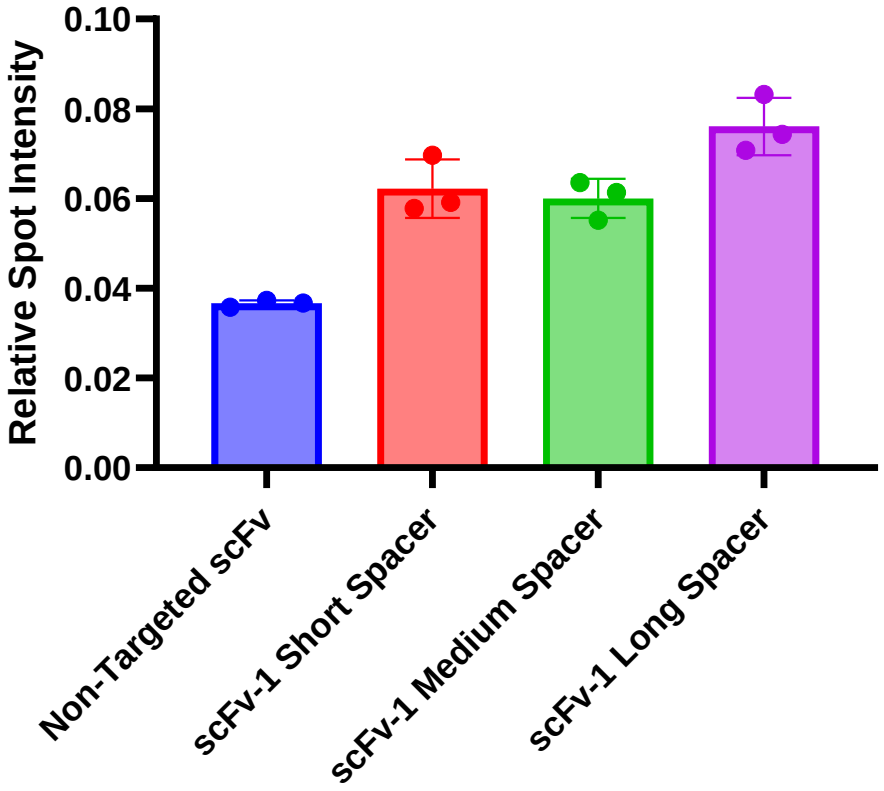


ctLNP +
Short Spacer

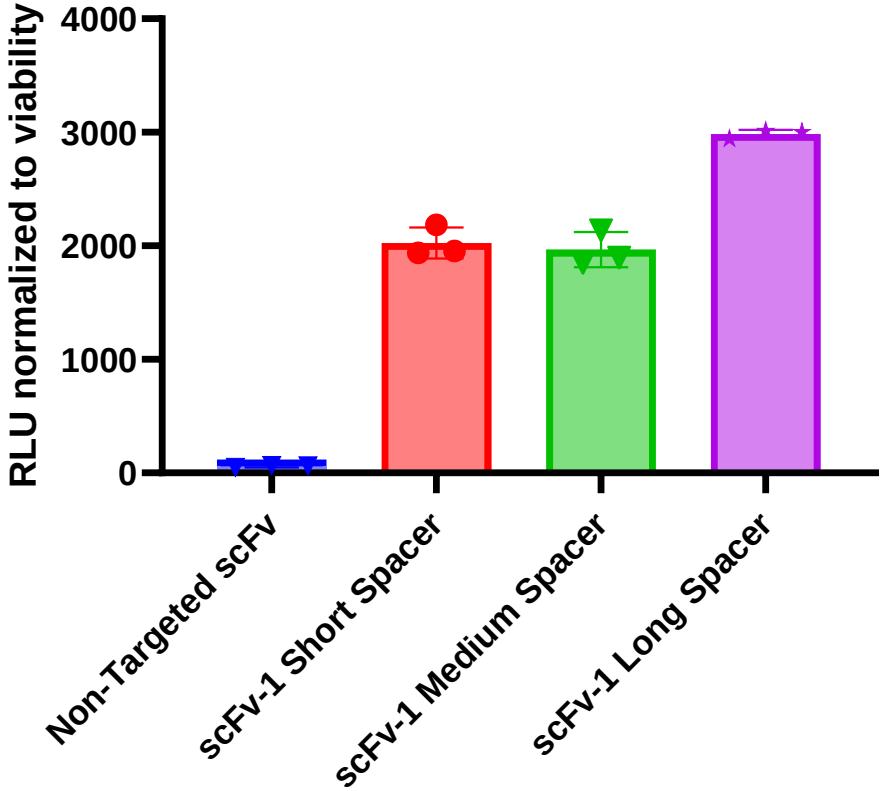


ctLNP +
Long Spacer

Uptake into primary hepatocytes is higher for ligands attached to a longer spacer



Higher uptake into hepatocytes leads to higher expression



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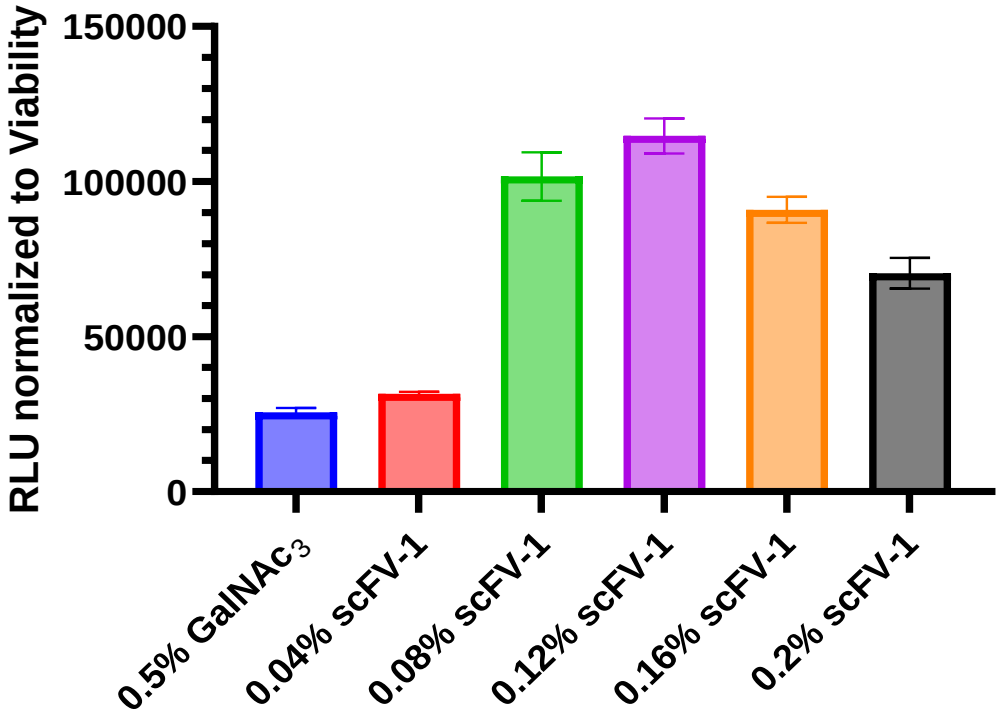
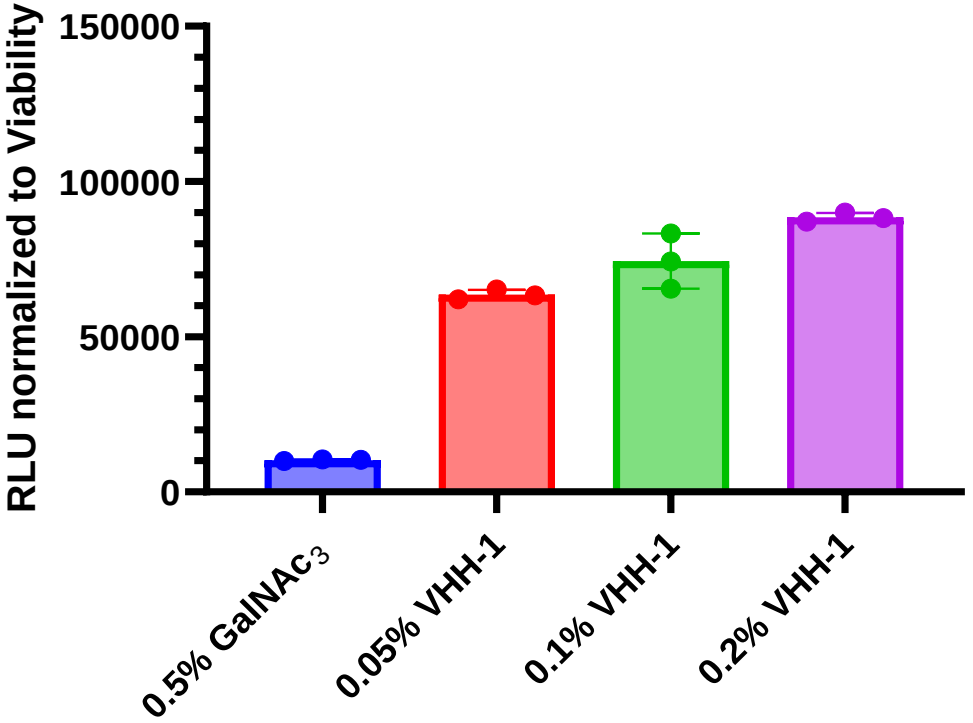
The number of ligands per LNP can be titrated for optimal expression *in vitro*

Optimal ligand density is 0.12% (125 ligands per particle) for scFv-1 in primary mouse hepatocytes



42 scFv per particle → 210 scFv per particle

Smaller format ligands show preferential in vitro activity at higher ligand densities



52 VHH per particle → 210 VHH per particle

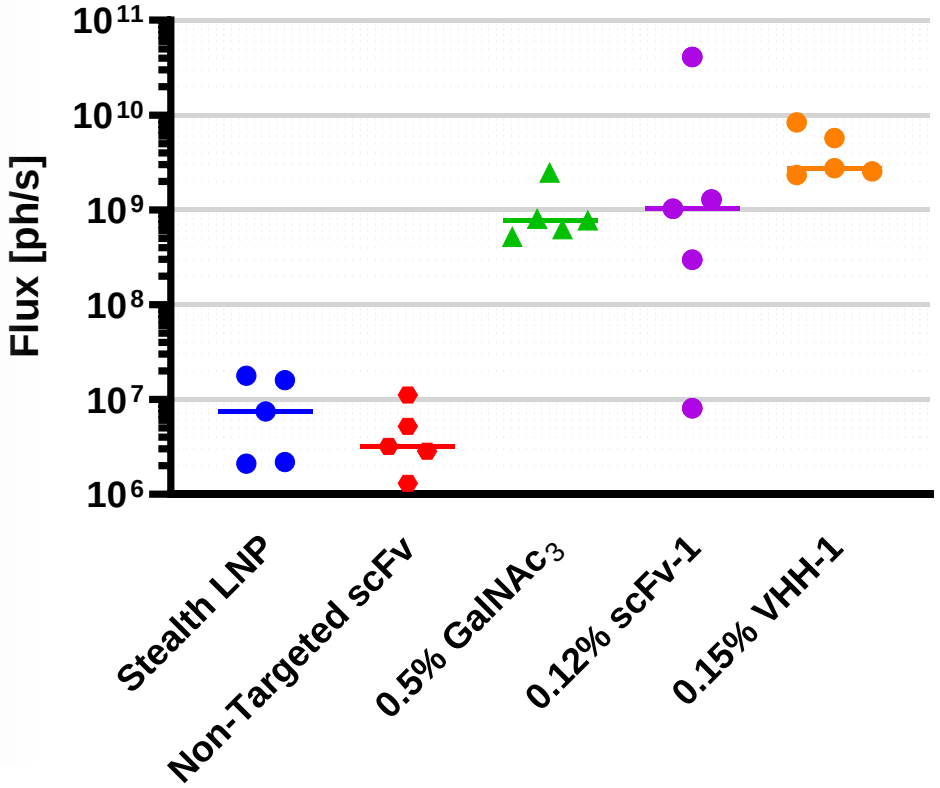
ASGPR targeting with protein ligands show higher liver delivery than GalNAc3 in mice

1.0 mg/kg i.v.
injection Luc ceDNA

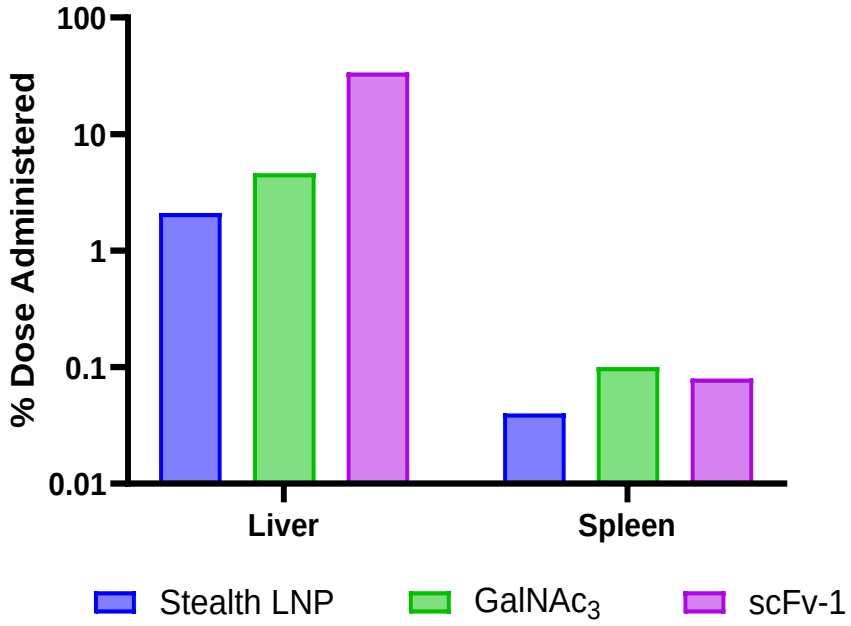
ApoE $^{-/-}$ Mice

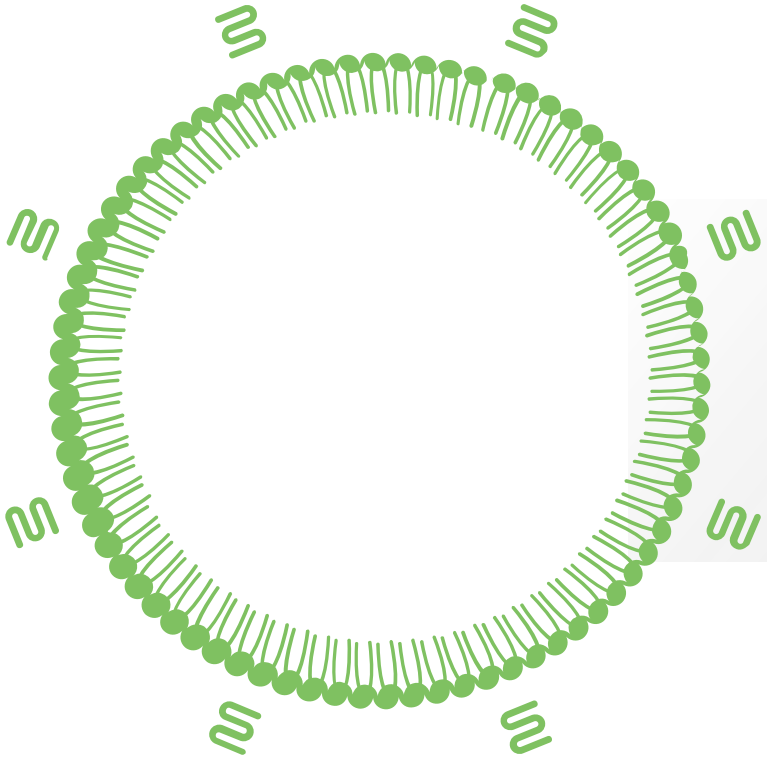
IVIS analysis: 7 days

Use of a VHH ligand led to a 0.4 log enhancement in potency over $GalNAc_3$



scFv targeting ligand delivers more cargo to the liver at 1 hour



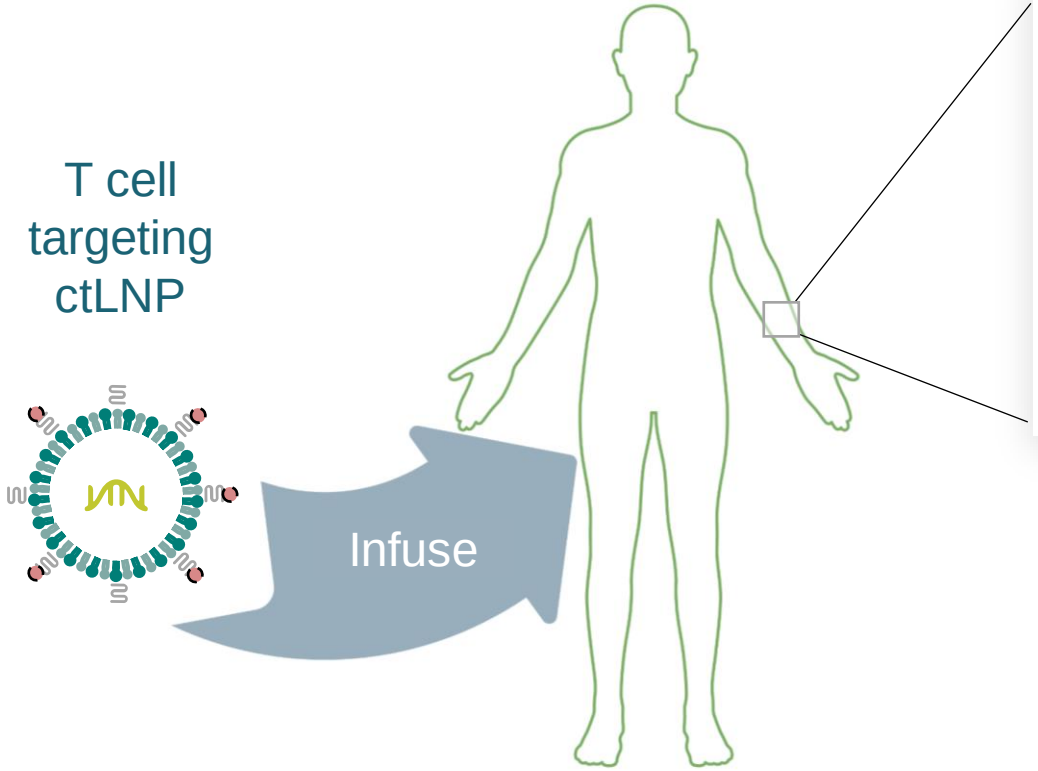


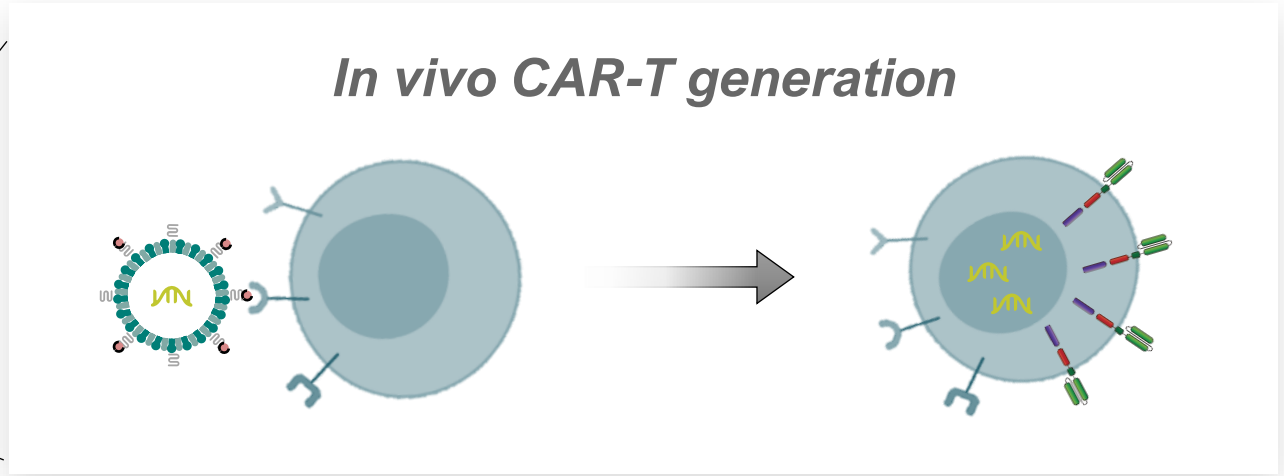
ctLNP

ctLNP enables access to new tissues and cell types

Developing ctLNP to build potent, selective in vivo T cell programs

ctLNP platform enables highly selective delivery to T cells *in vivo* for redosable CAR-T therapies



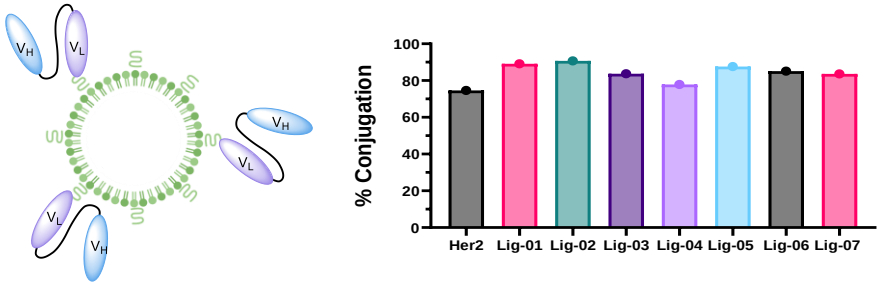


- *In situ therapy*
- *Scalable, off-the-shelf intervention*
- *Re-dosable CAR expression*
- *No lymphodepletion*

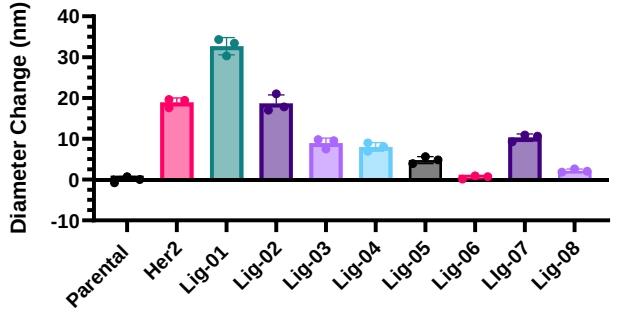
T cell ctLNPs demonstrate dose dependent, receptor specific uptake *in vitro*

Efficient conjugation of protein ligands maintains LNP stability

High Conjugation Efficiency

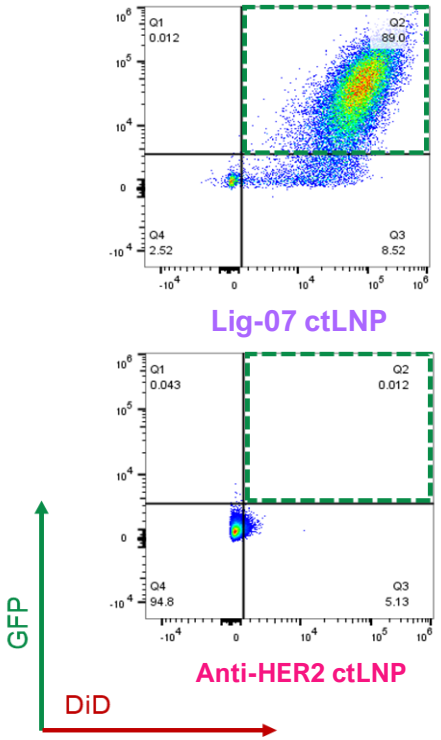


Pre/Post Conjugation Particle Size Stability

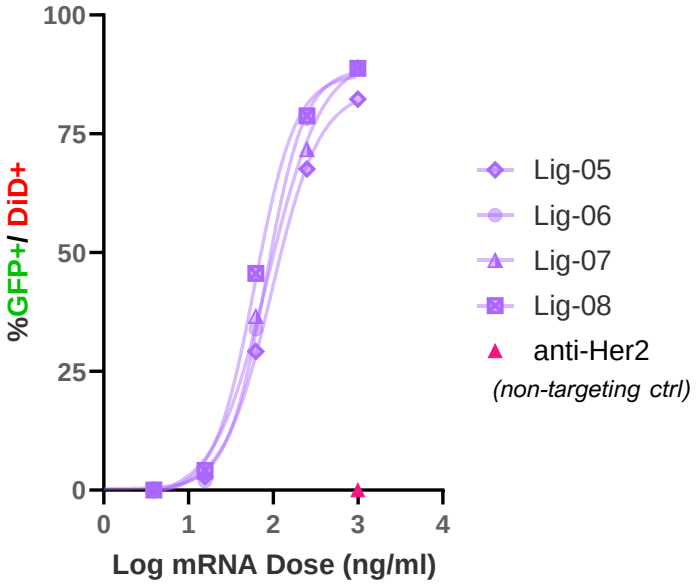


ctLNP uptake and expression is dose dependent and target specific

High Specificity



Dose Responsive uptake (DiD) *and* expression (GFP) in primary human T cells

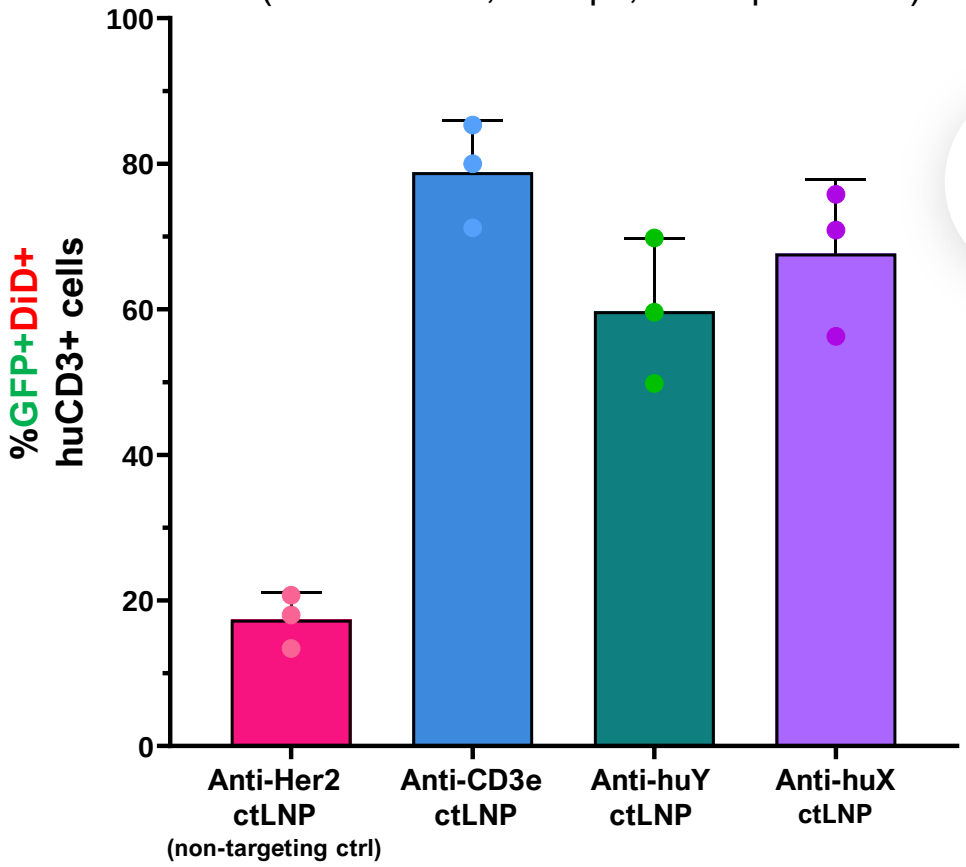


generation bio™ moderna

T cell ctLNPs demonstrate efficient uptake and expression of mRNA cargo *in vivo*

GFP expression in circulating T cells

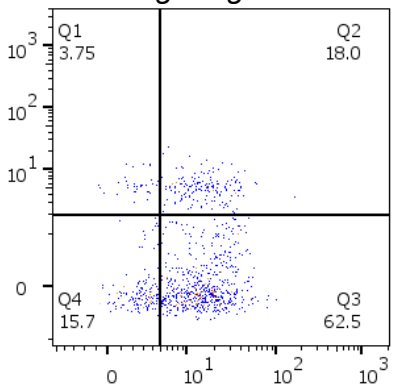
(hPBMC mice; 0.5mpk; 24hrs post dose)



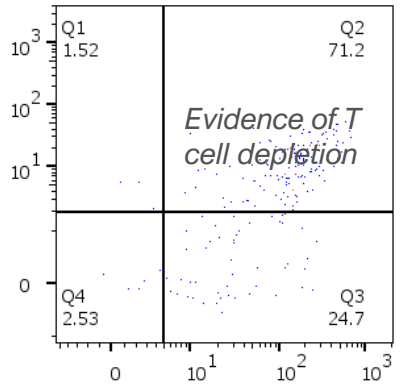
Similar results seen in splenic T cells

Anti-Her2-ctLNP

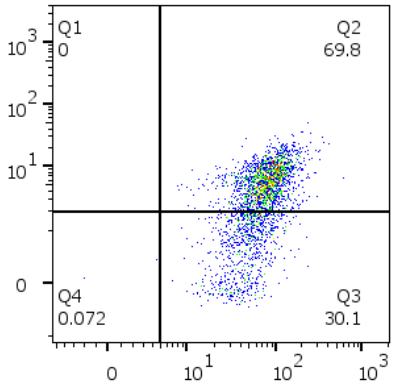
Non-targeting control



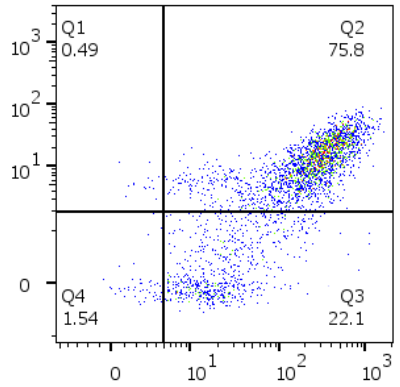
Anti-CD3ε-ctLNP



Anti-huY-ctLNP



Anti-huX-ctLNP



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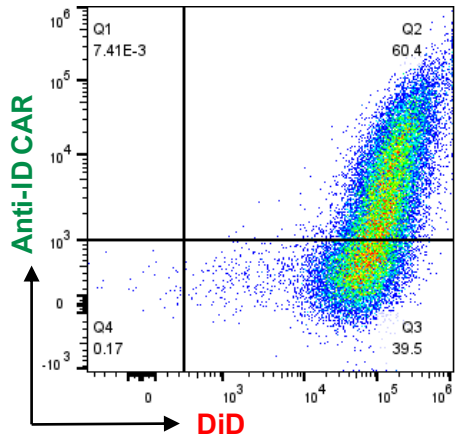
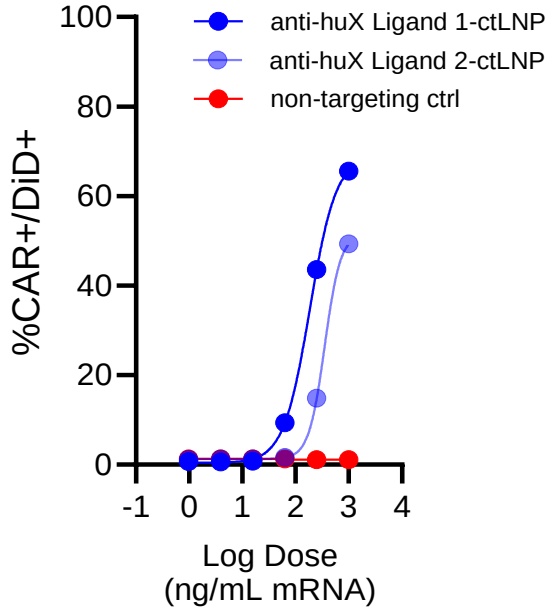
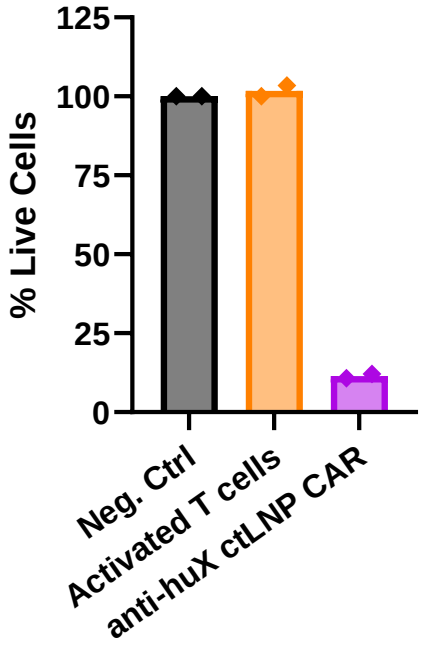
T cell ctLNP drives high level of functional CAR expression in T cells *in vitro*

Robust dose-dependent CAR expression





Functional CAR activity in cell killing assay

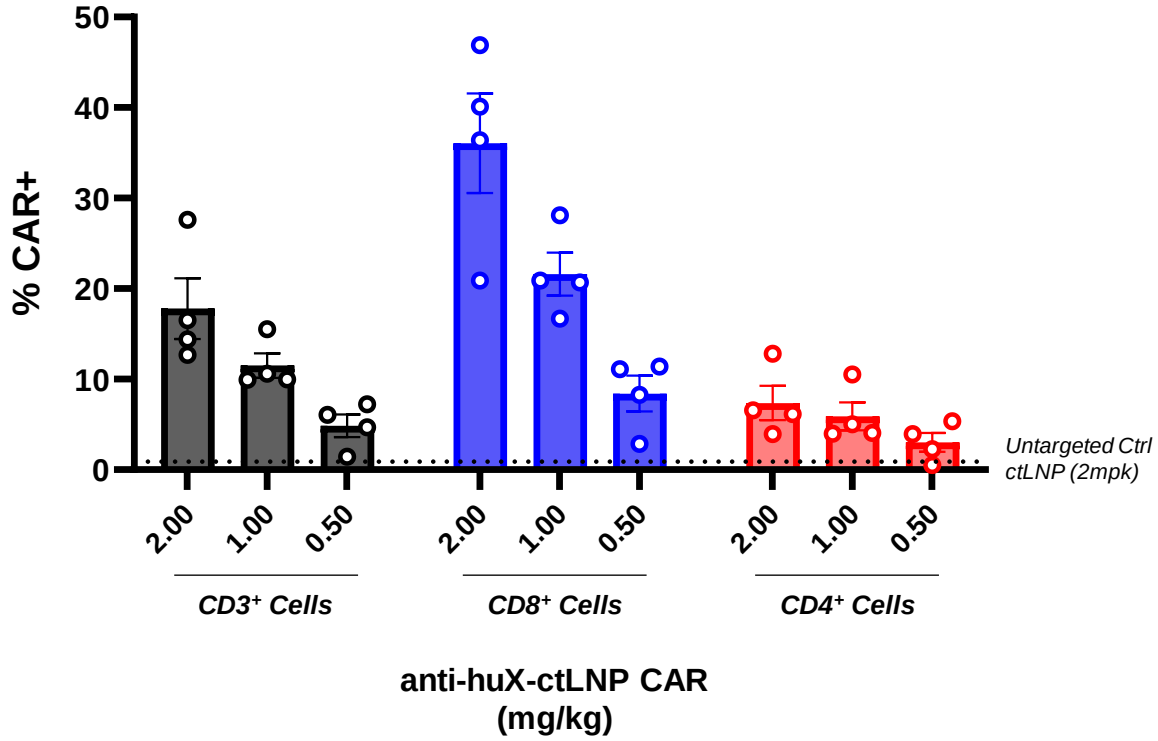


T cell ctLNPs show robust uptake and expression of CAR encoding mRNA *in vivo*

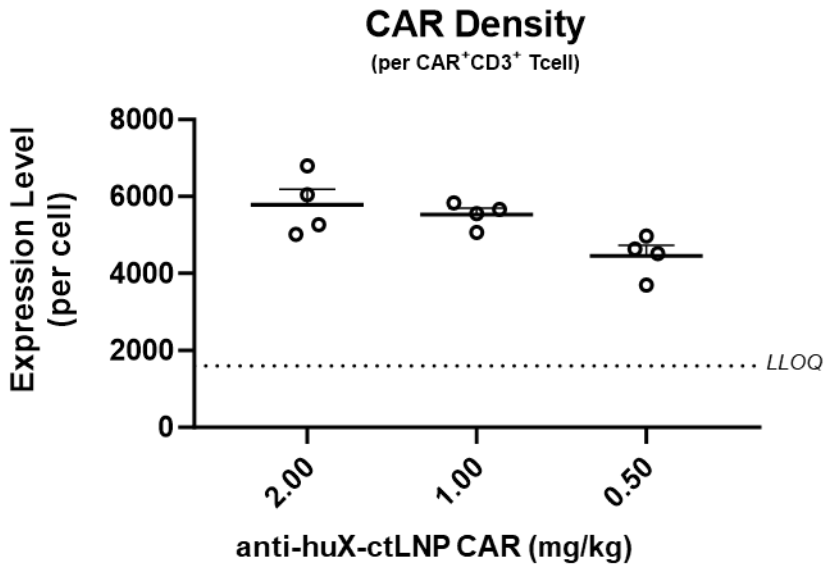
hPBMC

Efficient, dose responsive CAR expression

(hPBMC mice; splenocytes; 48hrs post dose)



Robust surface presentation on CAR-T cells



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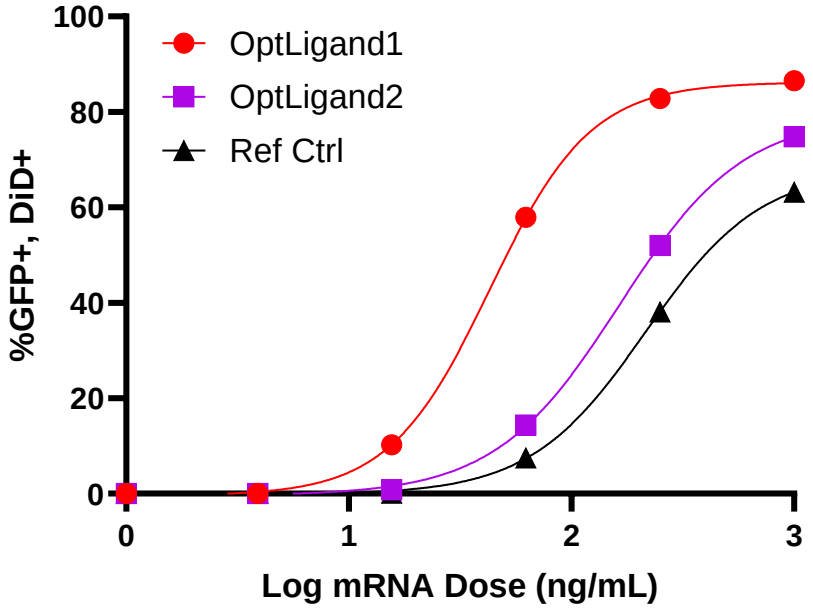
Next Steps: Optimization of ctLNP potency through ligand and process

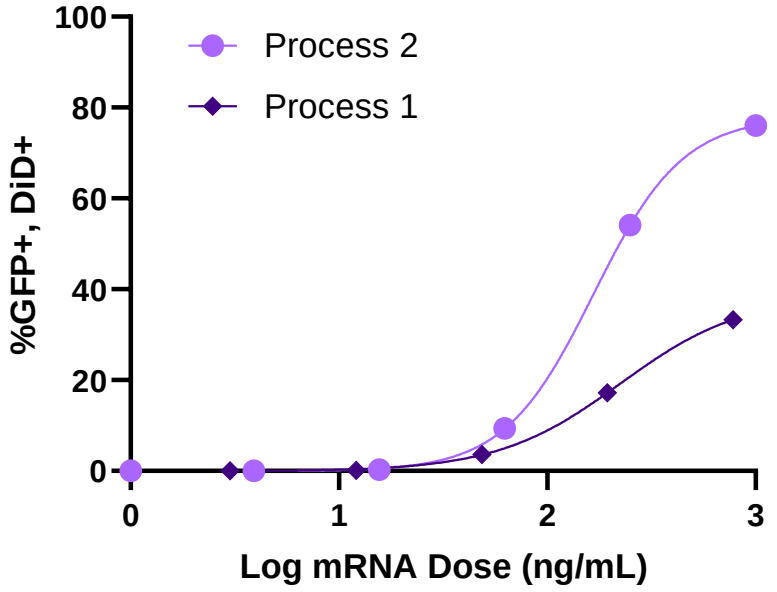
Optimized ligand conjugates enable more efficient delivery

(primary human T-cells)



Process improvements enhance delivery potency

(primary human T-cells)



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