Optimization of Ionizable Lipid Structure to Modulate Apoe Binding to Enable Stealth Lipid Nanoparticles

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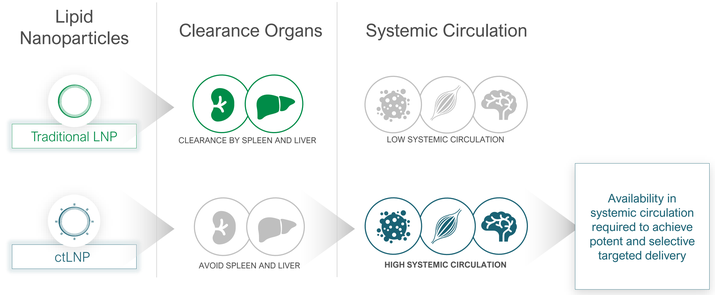
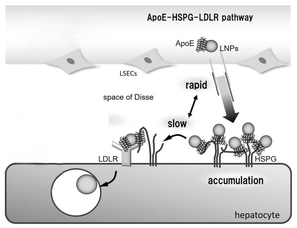
Untargeted stealth ctLNPs enable selective *in vivo* delivery by avoiding major clearance organs, resulting in extended systemic circulation

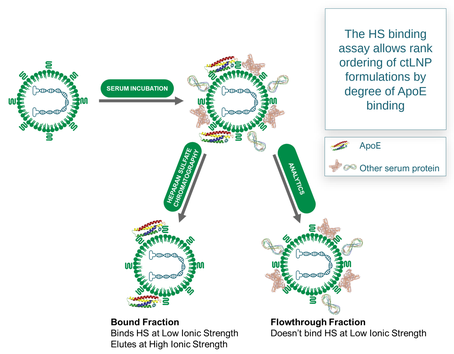


We developed a heparin sulfate (HS) binding assay to assess ApoE binding of serum-incubated LNPs

ApoE bound to LNP surface drives LDLR engagement and hepatic uptake

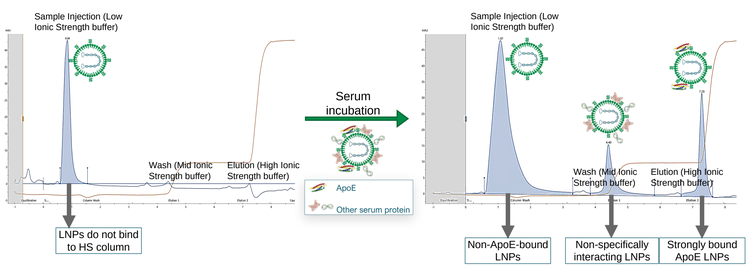


Adapted from: *Y. Sato, et al., J. of Controlled Release, 2020, 322, 217-226*



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

ApoE
Other serum protein



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 and HS binding

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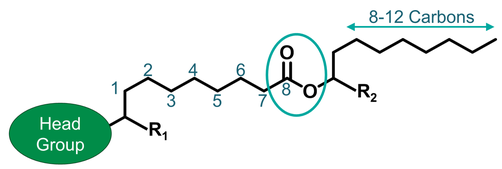
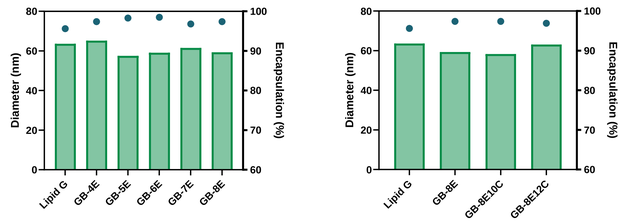
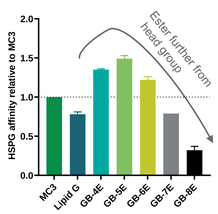


Modifications made to the parent ionizable lipid have no significant impact on standard particle analytics

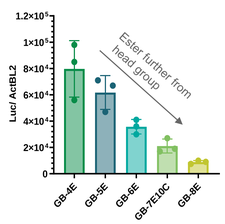


Ester away from the head group decreases HSPG binding after incubation in cyno serum, correlates to *in vitro* uptake in ARPE19 (LDLR dependent cell)

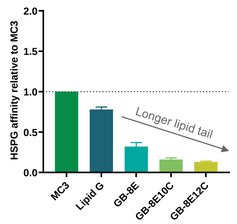
Ester further from the head group decreases HS affinity of ctLNP



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



Increased tail length of ionizable lipid decreases HS affinity of ctLNP



- Modification of ester position and tail length of parent ionizable lipid 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

Ionizable lipid chemical design plays an integral role in blood circulation time due to liver-avoidance

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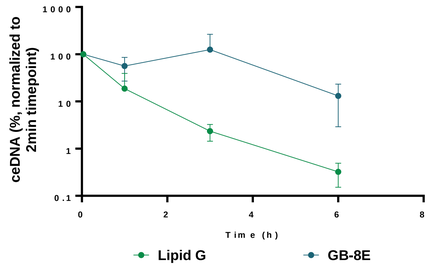
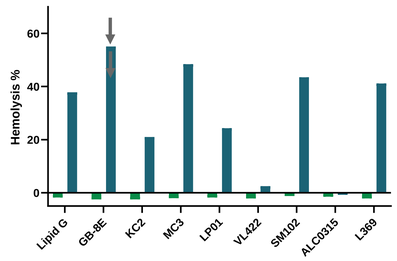


- Ionizable lipid structure can help prolong blood circulation time (mouse whole blood PK)
- "Stealth" lipid has significantly longer circulation time compared to an internal control (Lipid G)

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

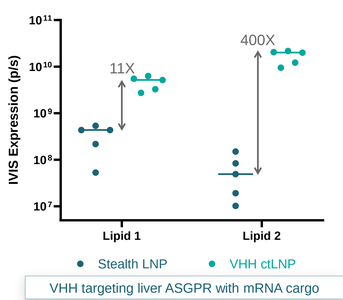
Stealth lipid's high membrane disruption activity indicative of endosomal escape potential



- High membrane disruption activity of "stealth" ionizable lipid is demonstrated with a hemolysis assay

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

***In vivo* expression comparing untargeted Stealth LNPs and ctLNPs**



- 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
- We're continuing to generate a larger pool of lipid chemistries to enable "stealth" while boosting potency