

# INCRECENT<sup>®</sup>



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**MULTIMERIC OLIGONUCLEOTIDES for  
ENHANCED POTENCY AND PRECISION**

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# Summary

The major challenge in oligonucleotide therapeutics is delivery – getting sufficient active material into a target cell.

The current “Gold Standard” involves subcutaneous (SC) administration of a single oligonucleotide conjugated to a GalNAc ligand, which binds specifically to the ASGP receptor on hepatocytes. A key reason for this system’s success is in the numbers: SC administration is effectively a slow-release mechanism, hepatocytes are the most abundant cell type in the liver, the ASGP receptor is present on the cell surface at a high copy number of  $\sim 10^6$  per cell, and the turnover time for the receptor is very fast at approximately 15 minutes.

When attempting to target oligo conjugates to other cell types using different ligand-receptor pairs, the numbers are far less advantageous. The well-known receptors for many cell types implicated in disease are present at a copy number level that is smaller by an order of magnitude or more relative to ASGPr. Further, these same receptors turn over more slowly than ASGPr. All of this means that when attempting to target cells other than hepatocytes, the numbers are not favorable.

Our approach to beating the numbers is simple and effective. We conjugate multiple copies of therapeutically active oligo (“multimers”) to a cell-targeting ligand. As compared to a monomer-conjugate, our multimer-conjugates are 4-6 times larger resulting in dramatically longer serum half-life, meaning more opportunity for the conjugate to bind with the target cell. And once the binding occurs, the conjugate delivers multiple copies of active oligo to the cell for enhanced bioactivity. Put simply, our multimeric-conjugates deliver more shots on goal.

Even in a GalNAc-ASGPr test system, we have demonstrated that our multimer-conjugates improve delivery and performance over a monomer-conjugate. In our most recent work, intravenous (IV) administration of a GalNAc-siRNA heterohexamer bearing among the six siRNAs in the conjugate only one siRNA targeting the TTR transcript results in TTR knockdown equal to that exhibited by the same GalNAc-siRNA monomer delivered via SC administration. Given that conjugates delivered via IV do not enjoy the benefits of the SC compartment’s slow-release phenomenon, this is a profound result. Normally, monomeric-conjugates delivered via IV are rapidly cleared by the kidneys, but our heterohexamer-conjugate was large enough to avoid kidney clearance.

The multimers are prepared in high yield and purity under neutral aqueous conditions and no toxic effects have been observed in any of our experiments to date.

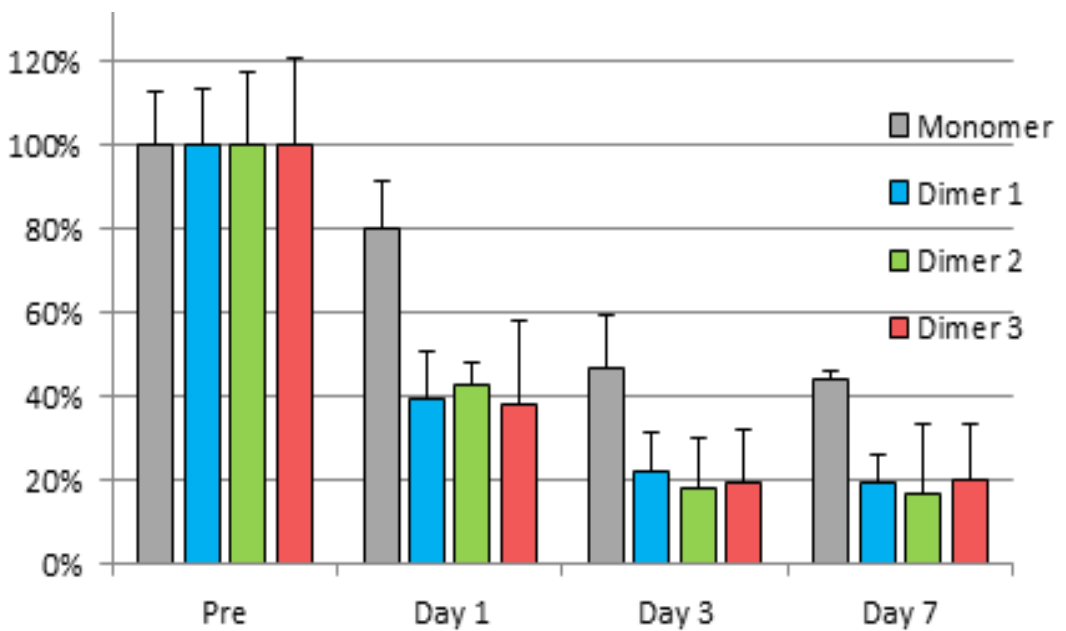
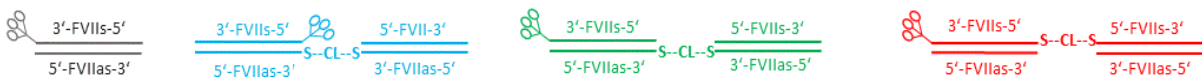
We believe our multimer-conjugates open up opportunities to address new disease targets that are currently unreachable by monomer-conjugates.

# Increased Knockdown Per Unit Ligand

In homomultimers.....

Knockdown of FVII activity in serum by FVII siRNA per mole GalNAc ligand:

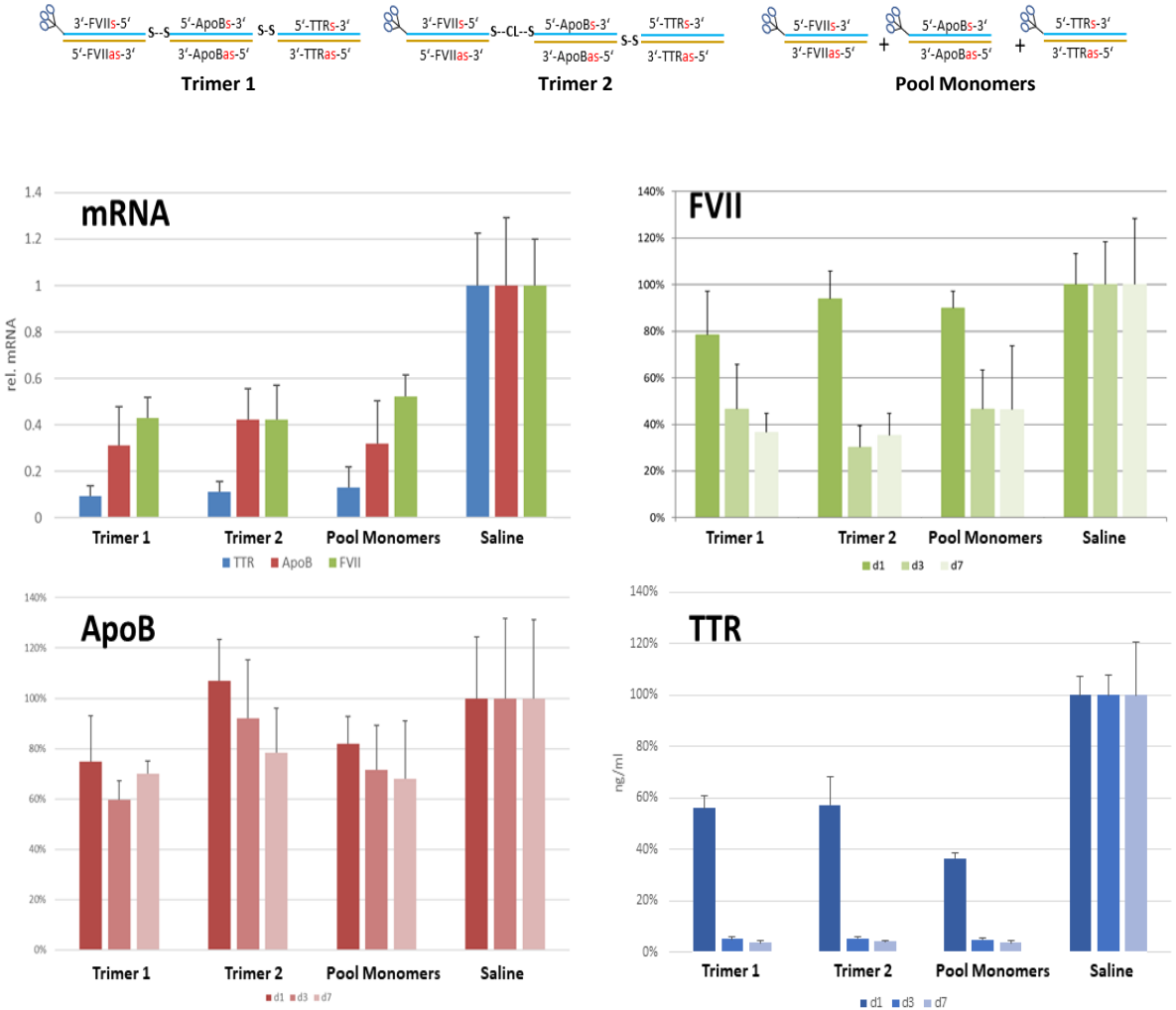
Comparison of Three GalNAc-FVII Homodimers vs GalNAc-FVII Monomer



**Determination of In Vivo FVII Gene Knockdown by Homodimeric GalNAc Conjugates.** GalNAc-conjugated homodimeric siRNAs targeted against FVII and a GalNAc-conjugated monomeric siFVII control were administered to mice subcutaneously at 25 mg/kg in a volume of 0.2 mL. Group sizes were n=4 mice/group for treated animals and n=5 for saline controls. Blood was collected 1 day prior to treatment and at 1-, 3-, and 7-days post-treatment, and analyzed for FVII enzyme activity. Homodimers outperformed control when normalized to the amount of GalNAc administered. When normalized to siRNA dose, homodimers and control were equivalent.

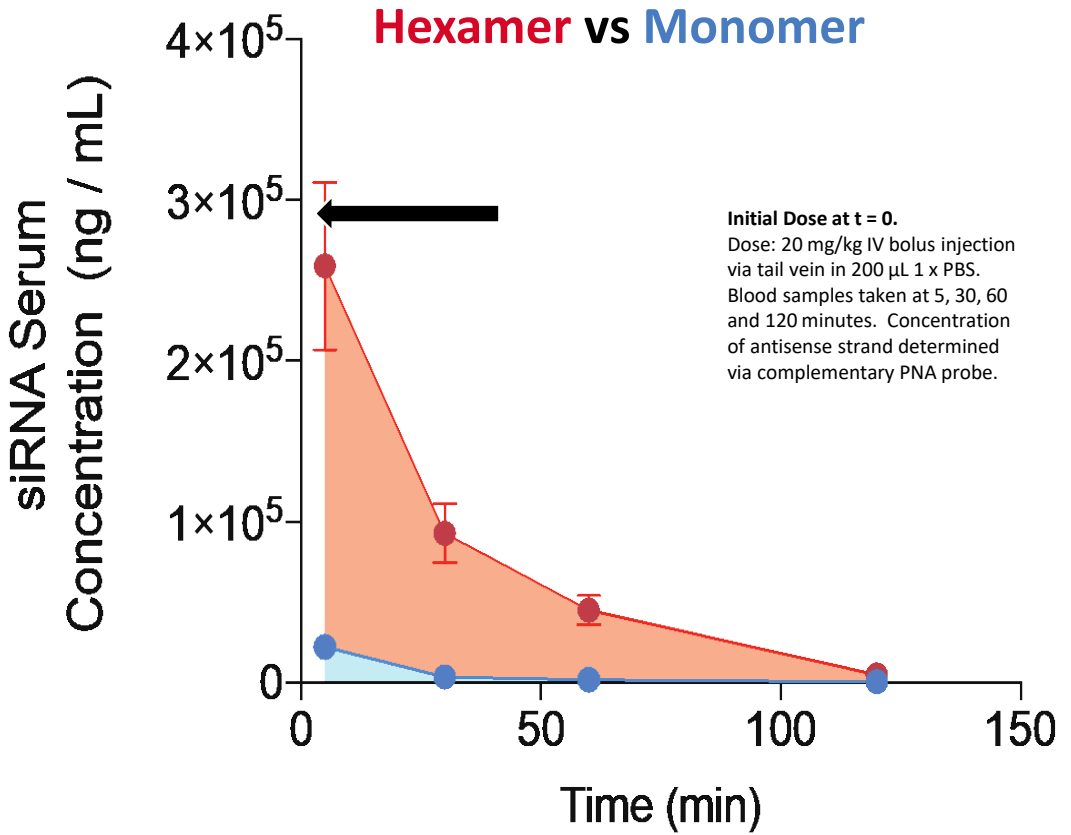
# ....and in heteromultimers

## Knockdown of FVII, ApoB and TTR in serum: Comparison of Two GalNAc-Heterotrimers vs GalNAc-Monomers

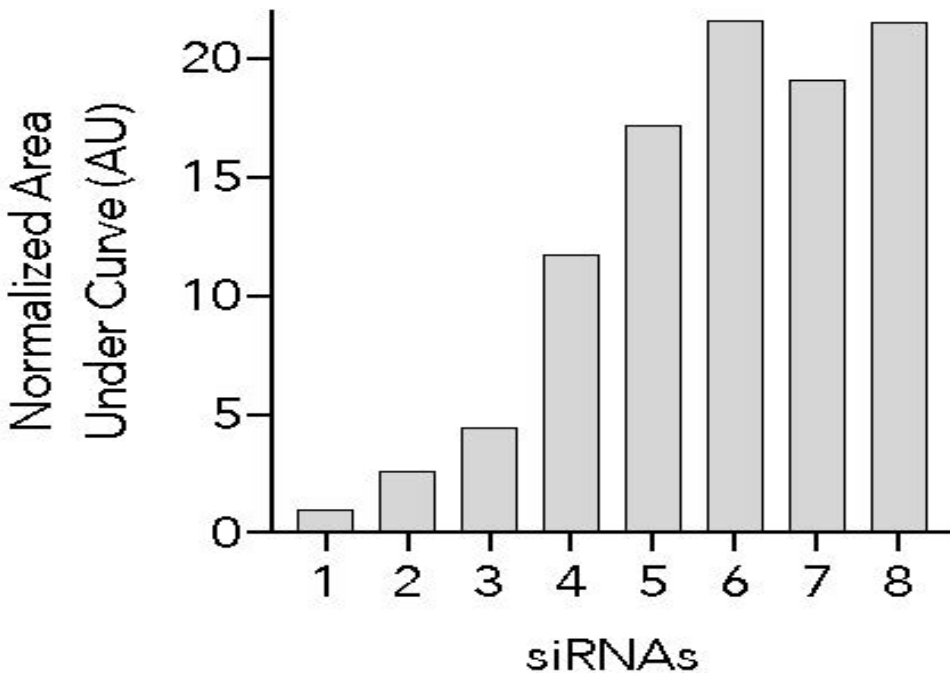


**Determination of In Vivo FVII/ApoB/TTR Gene Knockdown by Heterotrimeric GalNAc Conjugates.** Heterotrimers 1 and 2 and a pool of 3 monomeric GalNAc-conjugated siRNAs separately targeting FVII, TTR and ApoB were injected subcutaneously (0.1 mL volume) at a concentration of 50 mg/kg total RNA for the trimers and 17 mg/kg for each of the monomeric conjugates. Group sizes were n=4 mice/treatment group and n=5 for saline controls. Blood was collected 1 day prior to treatment and at 1-, 3- and 7-days post-treatment, and serum levels of FVII, ApoB and TTR were measured. Levels of FVII, ApoB and TTR mRNA were determined in liver lysates collected at day 7.

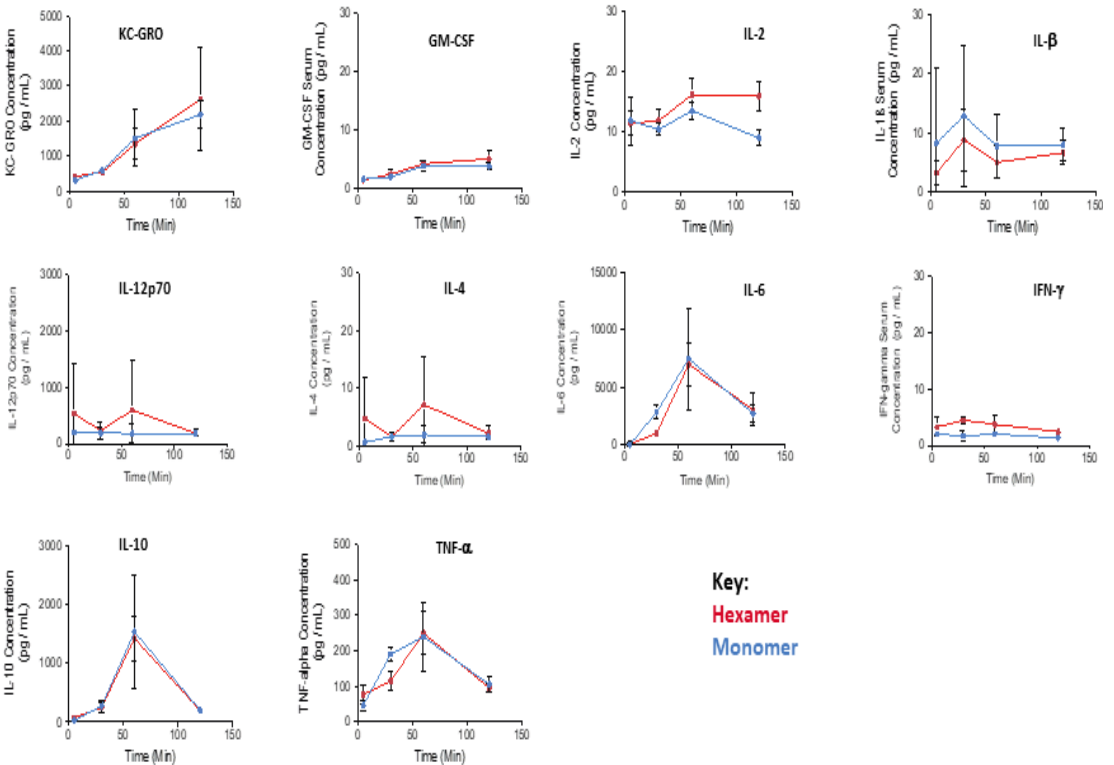
# Large, Unconjugated Multimers Have Increased Serum Half-lives.....



## Serum Half-lives 1 – 8 mers



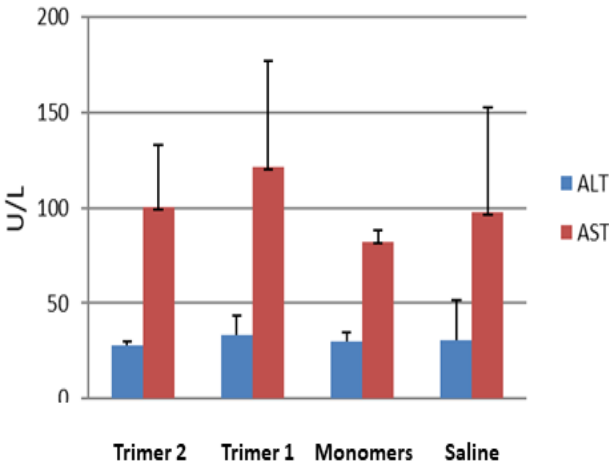
# .....With No Toxic Effects Observed



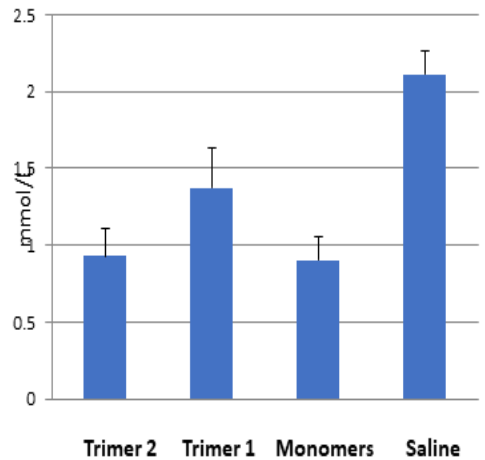
Determination of levels of 10 cytokines in blood samples taken at t = 5-, 30-, 60-, and 120-minutes using MSD U-Plex platform.

Key: Hexamer Monomer

## Serum Transaminases – day 7



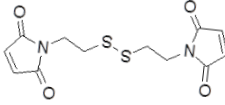
## Serum Cholesterol – day 7



Transaminase and cholesterol levels in serum after administration of heterotrimers 1 and 2 and the pool of monomeric controls vs saline at day 7.

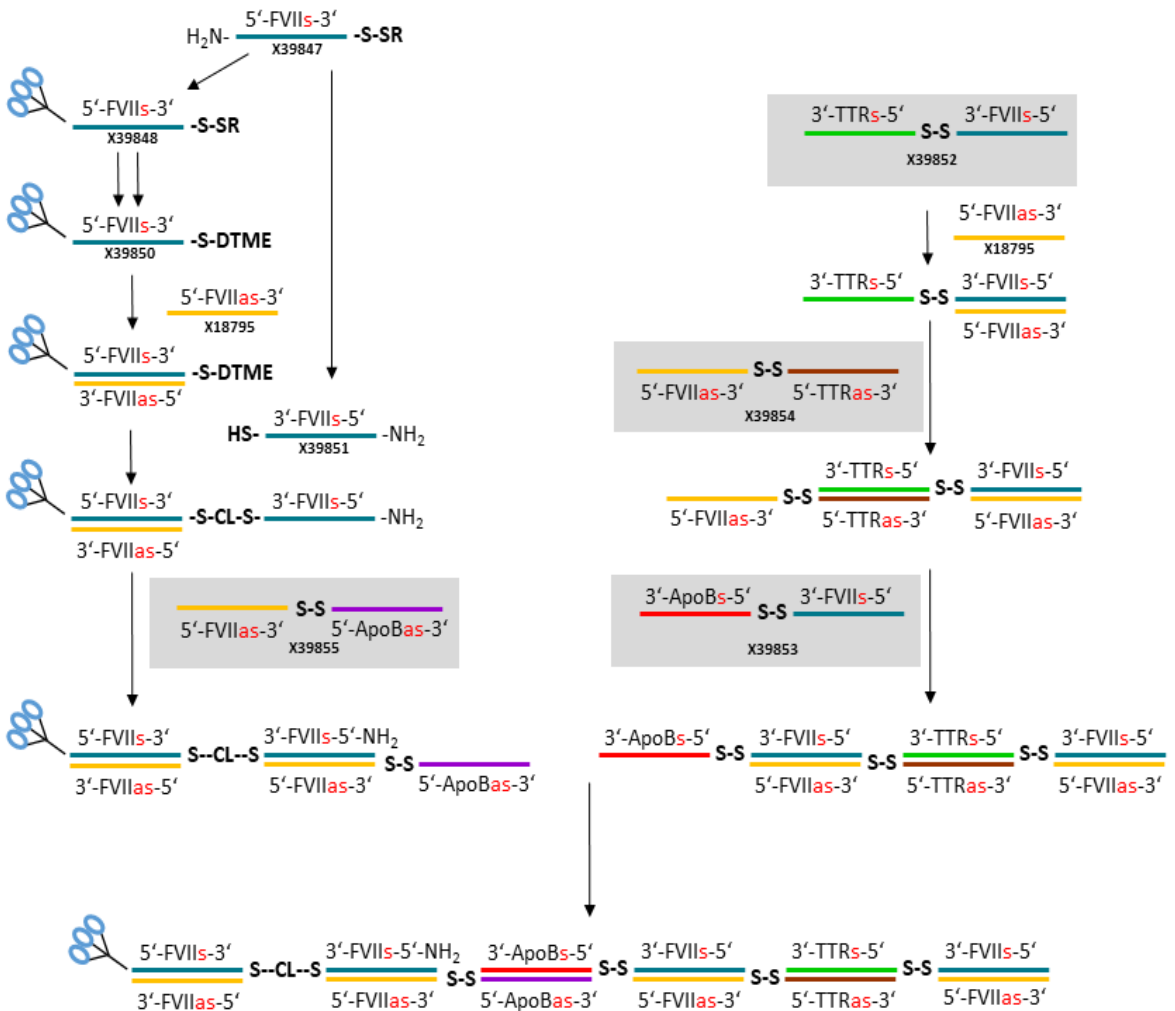
# Multimers are Efficiently Synthesized Under Neutral, Aqueous Conditions at Room Temperature.....

Reaction sequence involves mono-addition of DTME.....



.... to thiolated oligo and/or asymmetric annealing steps

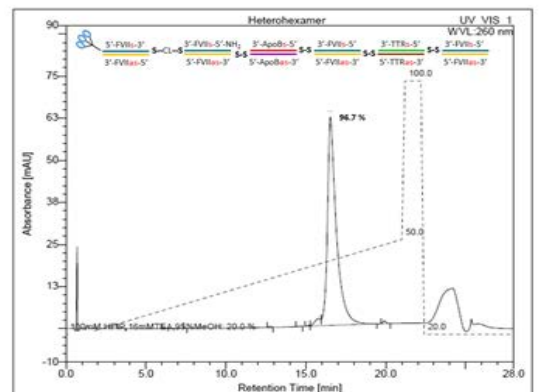
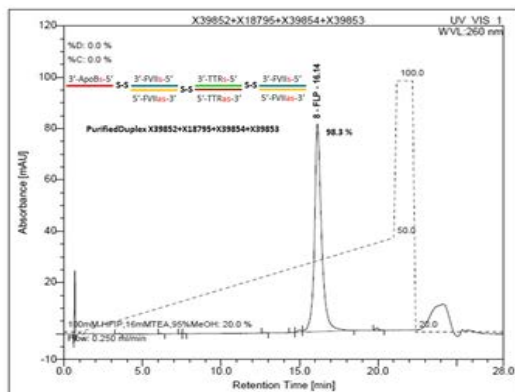
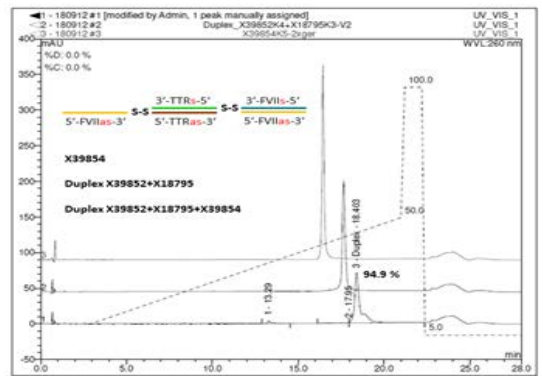
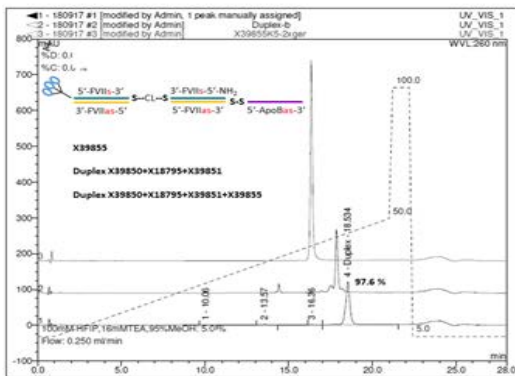
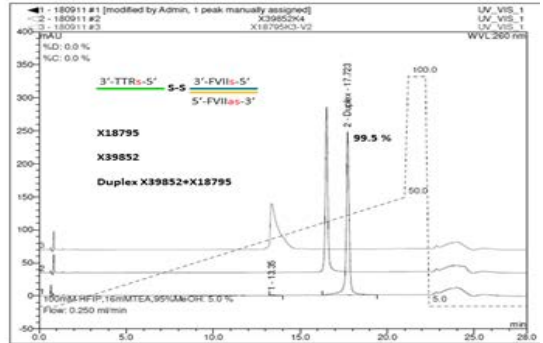
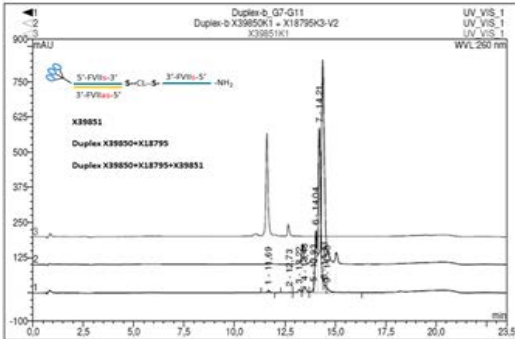
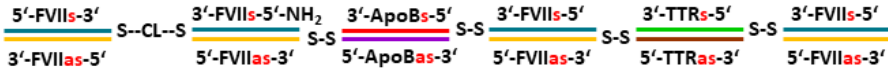
## Synthesis of 4:1:1 GalNAc-siFVII:siApoB:siTTR heterohexamer



Scheme for synthesis of a 4:1:1 GalNAc-siFVII:siApoB:siTTR hexamer via one mono-DTME derivative and 4 asymmetric annealing steps. Materials in gray boxes were obtained ex-synthesizer. All other steps were performed in aqueous solution at neutral pH and at room temperature.



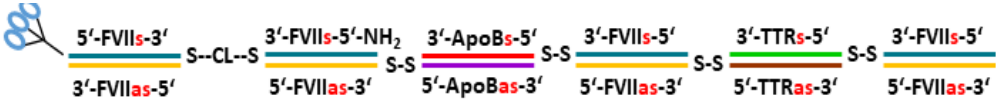
# .... In High Yield and Purity



Reverse phase hplc traces of annealing steps in synthesis of 4:1:1 GalNAc-siFVII:siApoB:siTTR heterohexamer

# Increased Bioactivity

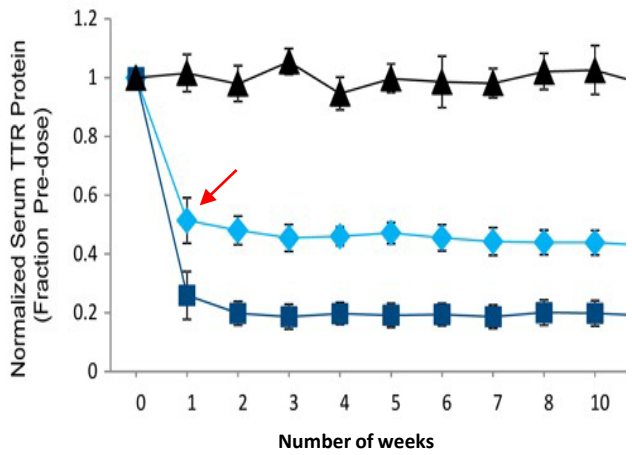
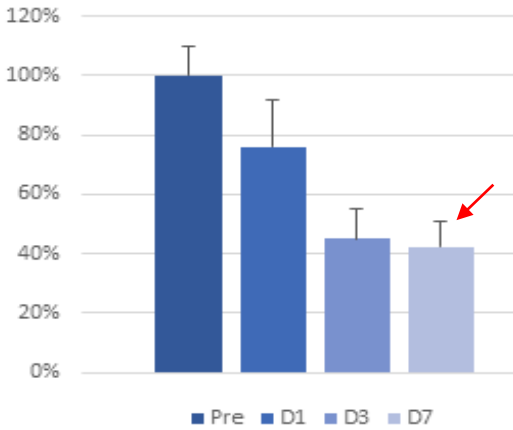
Knockdown of TTR and FVII by the 4:1:1 GalNAc-siFVII:siApoB:siTTR Hexamer @ 6 mg/kg via IV or SC administration vs corresponding GalNAc monomers via SC



## TTR protein levels in serum

GalNAc 4:1:1 hexamer  
1mg/kg equivalent siTTR via IV

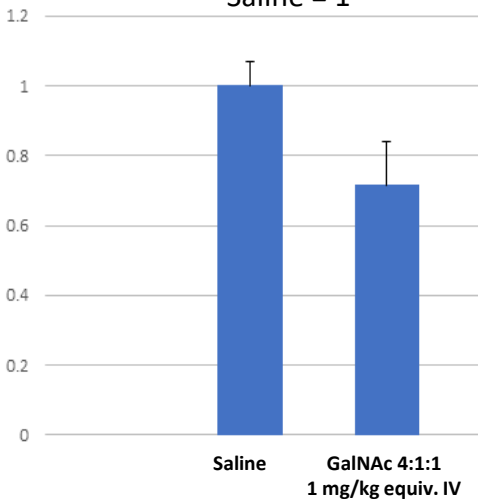
GalNAc siTTR monomer  
1 mg/kg SC weekly (Alynlam data)



Nair, J.K., et al; *J. Am. Chem. Soc.*, 2014, 136 (49), pp 16958-16961

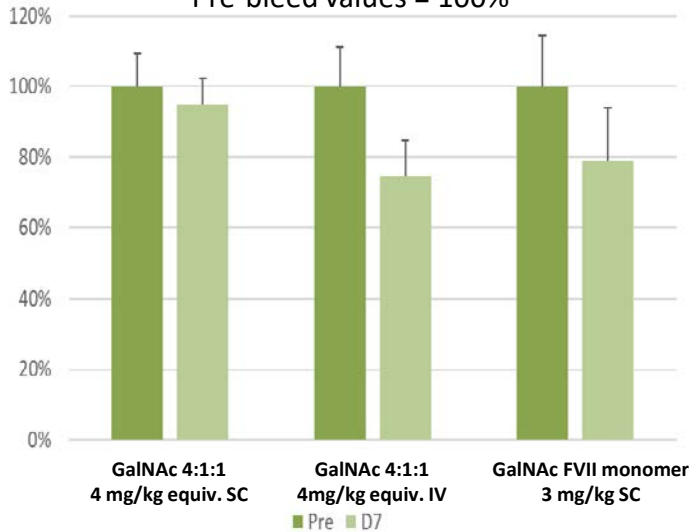
## mRNA levels in liver tissue at day 7

Saline = 1



## FVII activity in serum

Pre-bleed values = 100%



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