Defined Multimeric Oligonucleotides for Enhanced Therapeutic Effect.

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Summary

Modulating gene expression using therapeutic oligonucleotides has the potential to improve disease treatment and *in vivo* studies of complex biological processes. However, it remains difficult to deliver oligonucleotides in quantities sufficient to achieve a desired biological result into a particular cell or tissue type.

To address this issue, we have developed defined "multimeric" oligonucleotides, where a defined number of oligonucleotides (e.g., 2 or more siRNAs) are conjugated to a single targeting ligand.

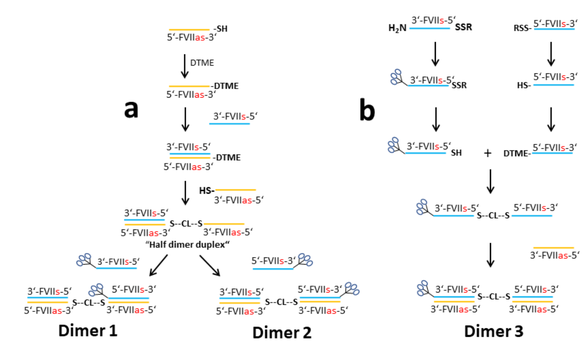
The individual units can be synthesized using the normal procedures and then assembled into multimers via a novel thiol/maleimide intermediate and/or asymmetric annealing under neutral aqueous conditions at room temperature in high yield and purity. The linkages employed are sufficiently stable in serum to enable prolongued bioavailability but are readily cleaved after entry into the target cell.

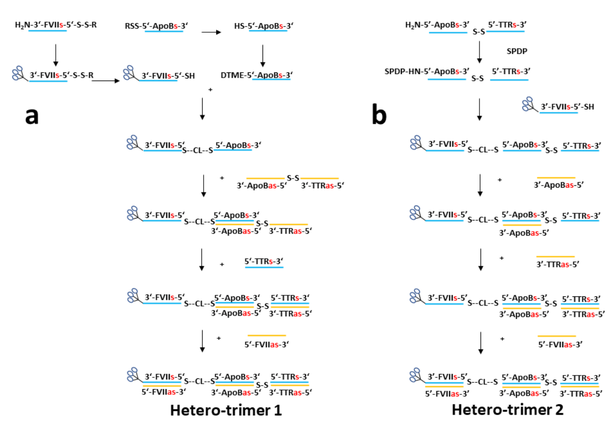
The resulting multimers exhibit the same activity per unit of oligonucleotide, but enhanced activity per unit of ligand as the latter is enabling the uptake of multiple cargos by the target cell per ligand/binding event.

Further, large multimers are big enough to resist clearance via the kidney and in consequence have a greatly increased serum half-life, in the case of hexamers by a factor of 20 or more relative to the corresponding monomer. Such large multimers are therefore even more potent, not only delivering more cargo per ligand/receptor binding event but enabling many more such events, resulting in a synergistic enhancement in activity such that a single siRNA unit in a hexamer delivered via IV exceeded the reported activity of the same siRNA in monomeric form delivered via SC.

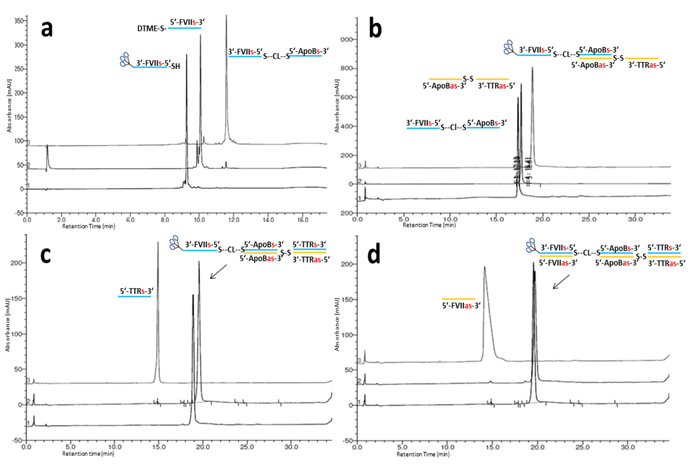
Here we present data obtained using a triantennary GalNAc ligand and three siRNAs targeting FVII, ApoB and TTR as a model system. All the methods described are compatible with a wide range of targeting ligands such as peptides, carbohydrates and aptamers, and can be used to deliver any combination of siRNAs, shRNAs, micro-RNAs, and ASOs.

Easy Synthesis
Mono-DTME + Assymetric Annealing

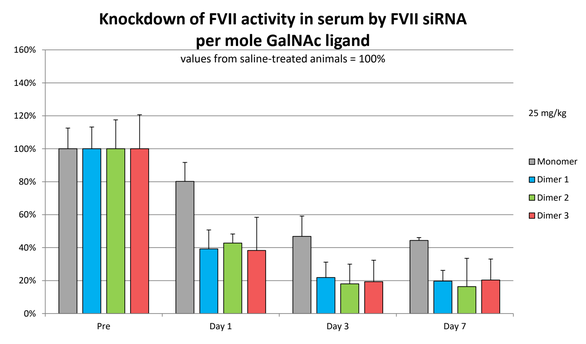




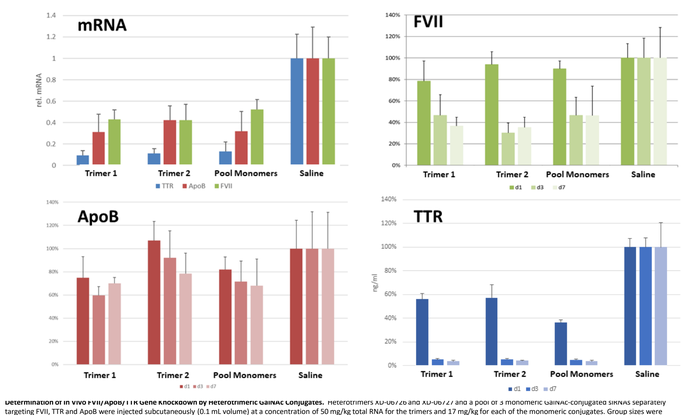
All Steps – high yield, high purity



Silencing per unit siRNA unchanged, per unit Ligand increased



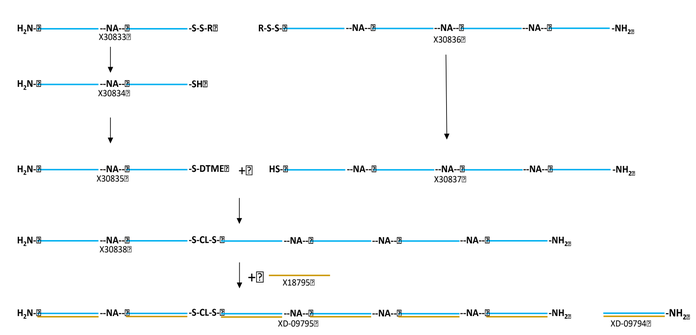
GalNAc-conjugated homodimeric siRNAs targeted against FVII and a GalNAc-conjugated monomeric siFVII control were administered to mice subcutaneously at 25mg/kg or 50 mg/kg in a volume of 0.2 mL. Group sizes were n=4 mice/group for treated animals and n=5 for saline controls. Blood was collected 1 day 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 controls were equivalent.

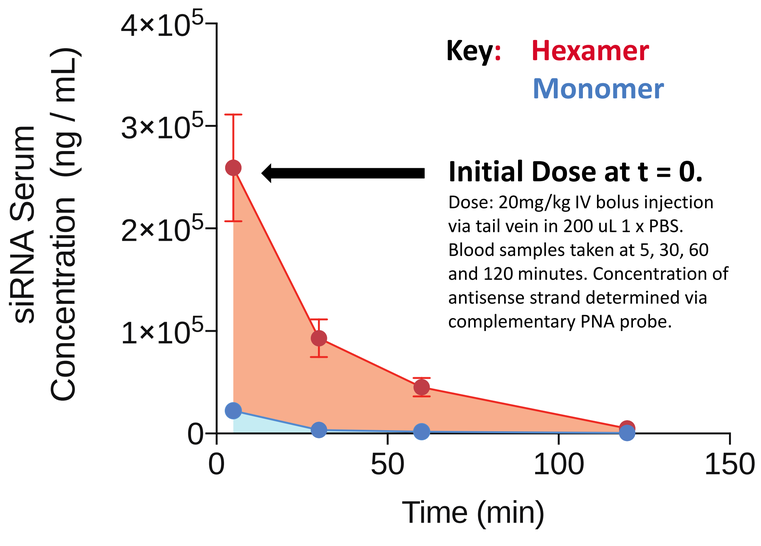


Determination of in vivo FVII/ApoB/ TTR Gene Knockdown by Heterotrimeric GalNAc Conjugates. Heterotrimers XD-09729 and XD-09794 and a pool of 3 monomeric GalNAc-conjugated siRNAs separately targeting FVII, TTR and ApoB were injected subcutaneously (0.1 mL volume) at a concentration of 50 mg/kg total 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 as collected 1 day prior to treatment and at 1, 3 and 7 days post-treatment, and serum levels of FVII, ApoB and TTR measured.

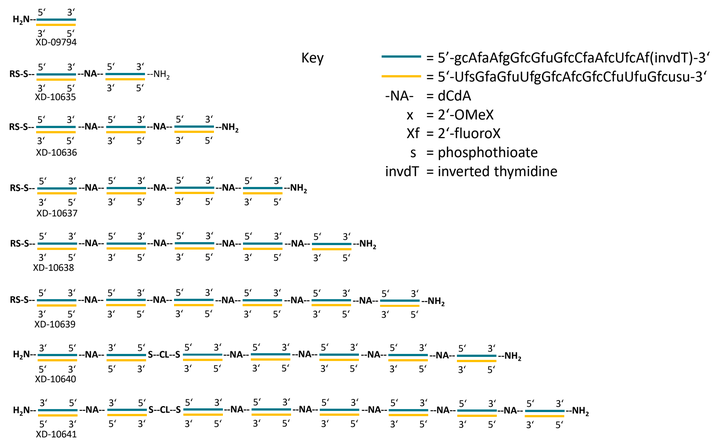
Large Multimers have Enhanced Serum Half-lives and Bioactivity wrt Monomers

Serum Half-lives:
FVII Hexamer vs monomer……

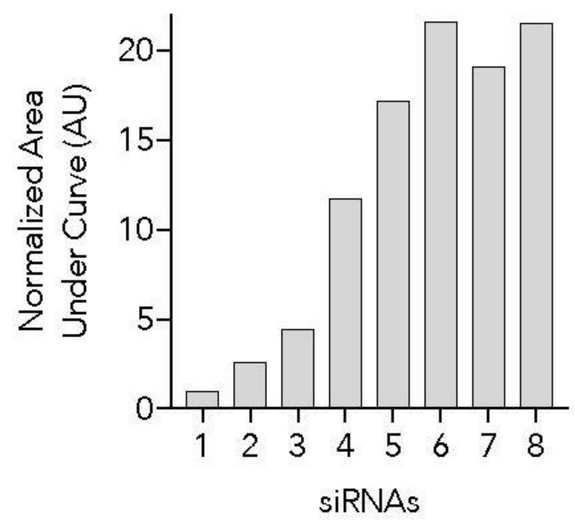




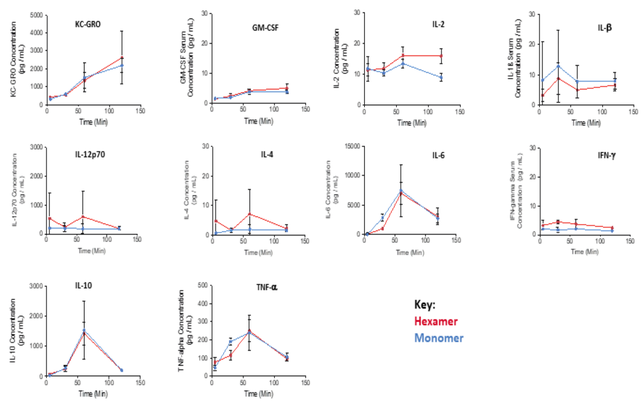
Comparison 1-8 mers:
Synthesis



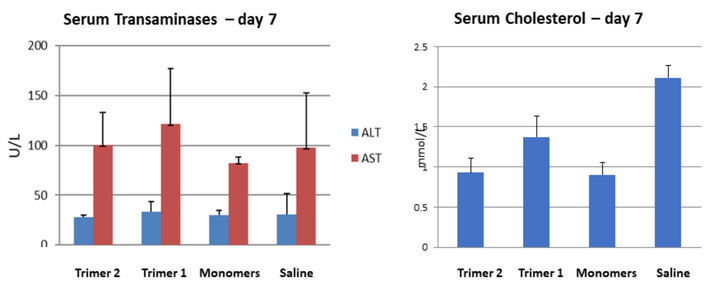
Serum Half-lives



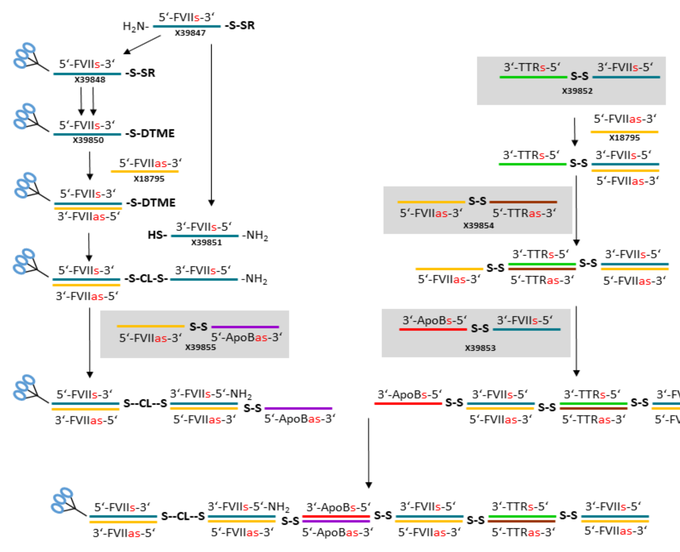
…all with minimal increase in toxicity

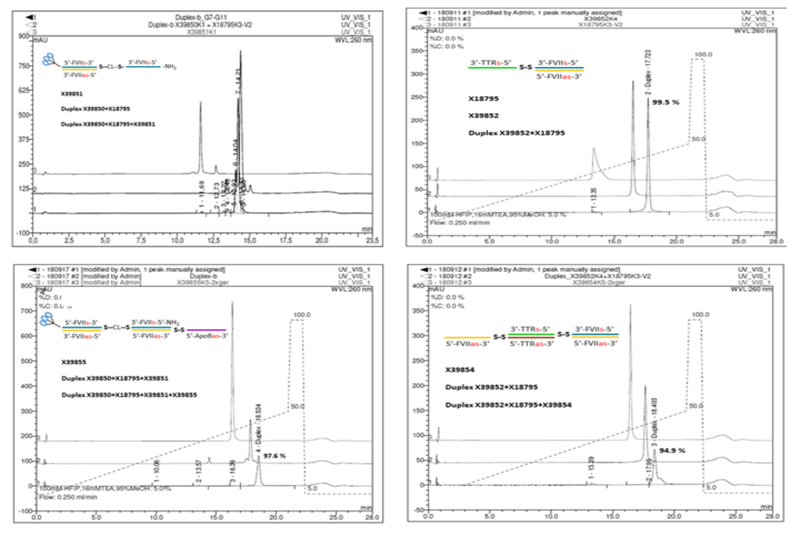


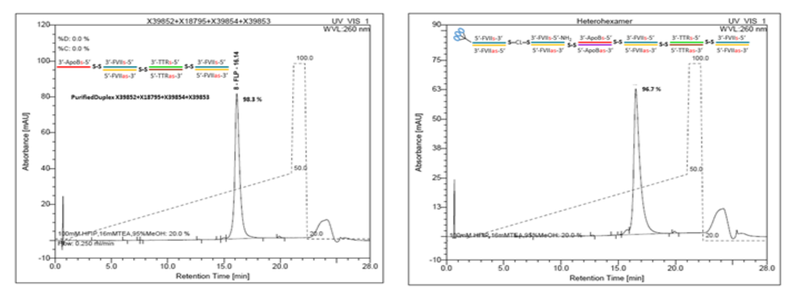
Determination of Levels of 10 Cytokines in blood samples taken at t= 5, 30, 60, and 120 minutes using MSD U-Plex platform.
Key: Hexamer Monomer



Bioactivity
4:1:1 FVII-ApoB-TTR Hetero-hexamer



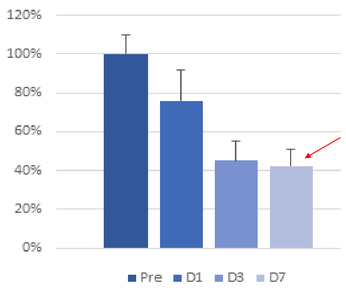




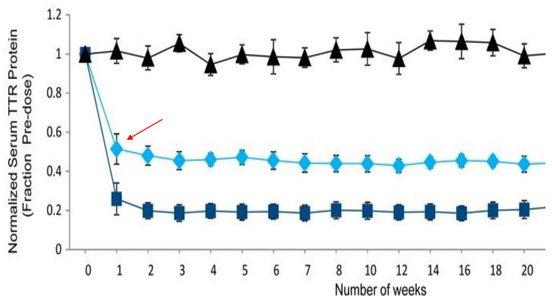
IV Knockdown by Hexamer exceeds SC Knockdown by Monomer

TTR protein levels in serum

GalNAc 4:1:1 Hexamer 1mg/kg equiv. TTR via I.V.

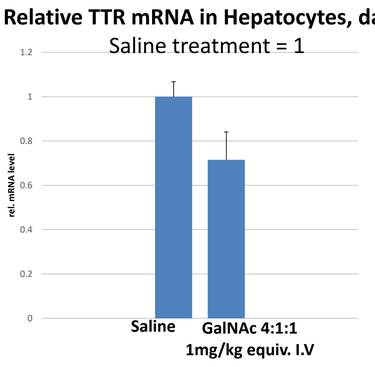


GalNAc TTR monomer 1 mg/kg S.C. weekly (Alnylam data)

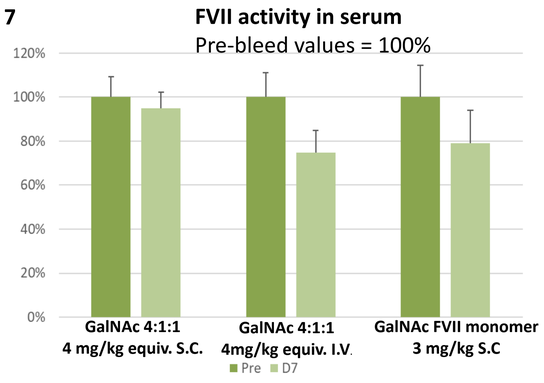


Nair, J.K., *et al*; *J. Am. Chem. Soc.*, **2014**, *136* (49), pp 16958–16961

Relative TTR mRNA in Hepatocytes, day 7
Saline treatment = 1



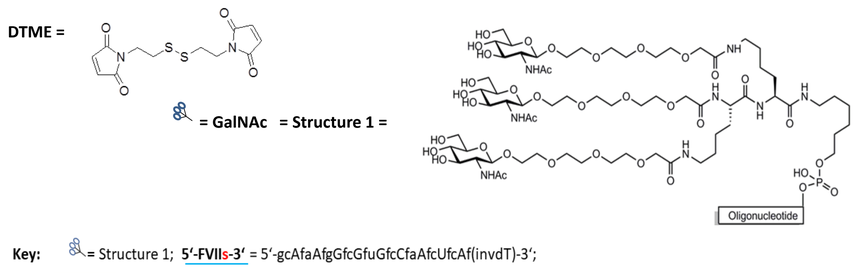
FVII activity in serum
Pre-bleed values = 100%



Conclusions

The potency of a ligand-oligonucleotide therapeutic agent can be greatly enhanced by preparing the oligonucleotide in multimeric form. This has the potential to enable oligonucleotide therapeutics to be effective against hitherto intractable targets involving low receptor copy numbers and internalization rates, and/or diseases requiring multiple knockdowns or IV delivery.

Key



Key: = Structure 1; **5'-FVIIs-3'** = 5'-gcAfaAfgGfcGfuGfcCfaAfcUfcAf(invdT)-3';
3'-FVIIas-5' = 3'-usucGfuUfuCfcGfcAfcGfgUfuGfaGfsUf-5'; **5'-ApoBs-3'** = 5'-cuAfuUfuGfgAfgAfgAfaAfuCfgAf(invdT)-3';
3'-ApoBas-5' = 3'-usugAfuAfaAfcCfuCfuCfuUfuAfgCfsUf-5'; **5'-TTRs-3'** = 5'-AfsasCfaGfuGfuUfCfUfuGfcUfcUfaUfaAf(invdT)-3';
3'-TTRas-5' = 3'-ususuUfgUfcAfcAfagaAfcGfaGfaUfasUfsu-5'; **S--CL--S** = C_6-alkyl-S-DTME-S-C_6-alkyl; **-S-S-** = C_6-alkyl-S-S-C_6-alkyl;
Xf = 2'-deoxy-2'-fluoronucleoside, x = 2'-OMe-nucleoside, invdT = inverted thymidine, s = phosphothioate