Arrowhead Research
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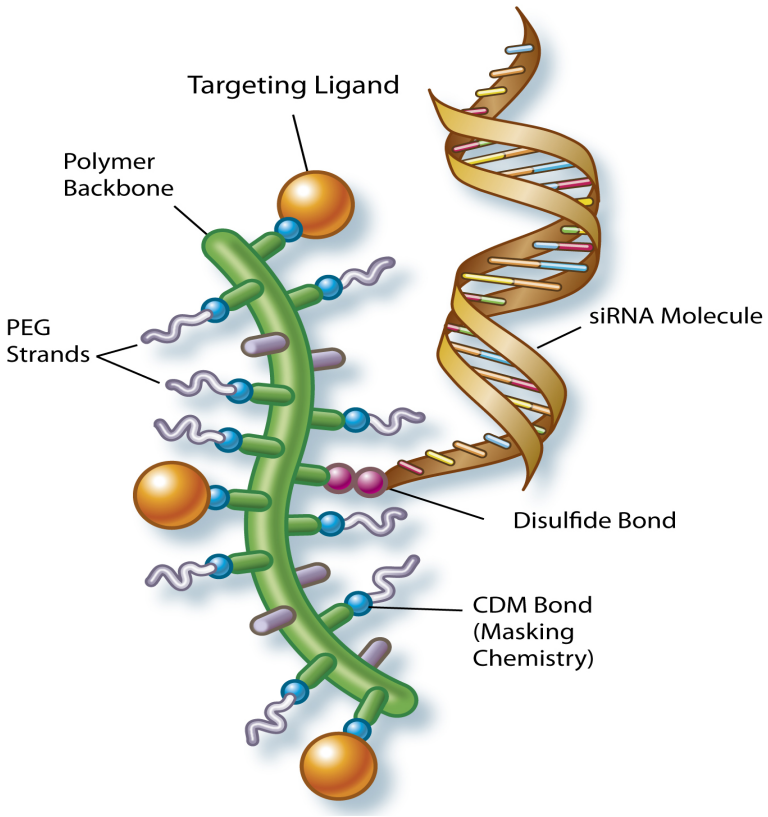
Next Generation Dynamic PolyConjugate (DPC) for siRNA Delivery *in vivo*

Aaron Almeida Ph.D.
Senior Scientist
Chemistry
Arrowhead Research Corporation

Dynamic Polyconjugate (DPC) technology for siRNA delivery *in vivo*



Components of a DPC

siRNA- Active Pharmaceutical Ingredient
RISC mediated gene silencing

Endosomolytic Polymer
Facilitates entry of siRNA into cytoplasm

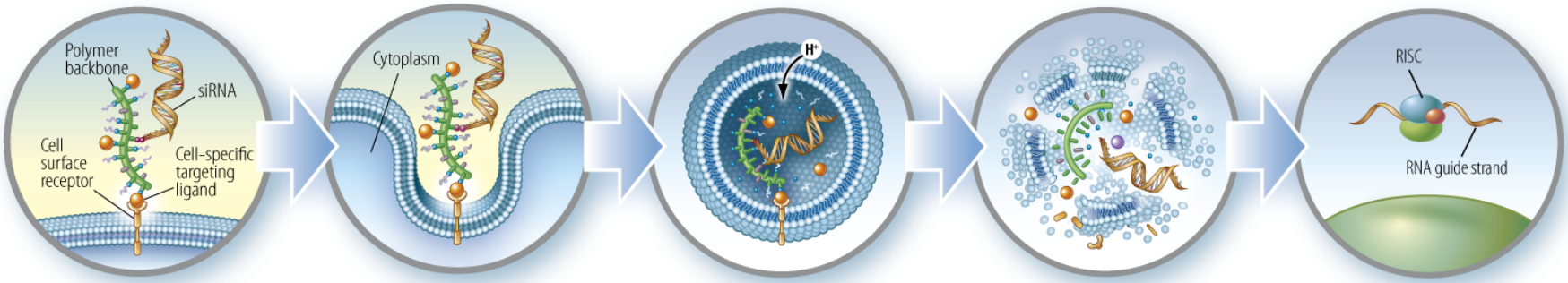
Poly Ethylene Glycol (PEG)
Inhibit membrane interactions of polymer during delivery

Targeting Ligand
Delivers siRNA and polymer to cells

Masking Chemistry
Reversible attachment chemistry which releases PEG and targeting ligands from polymer in endosomes

Rozema, Lewis et al. Proc Natl Acad Sci U S A. 2007 Aug 7;104(32):12982-7

Mechanism of DPC-mediated siRNA delivery



DPC conjugated targeting ligand attaches to cell surface

DPC and siRNA are taken up into the cell's endosome

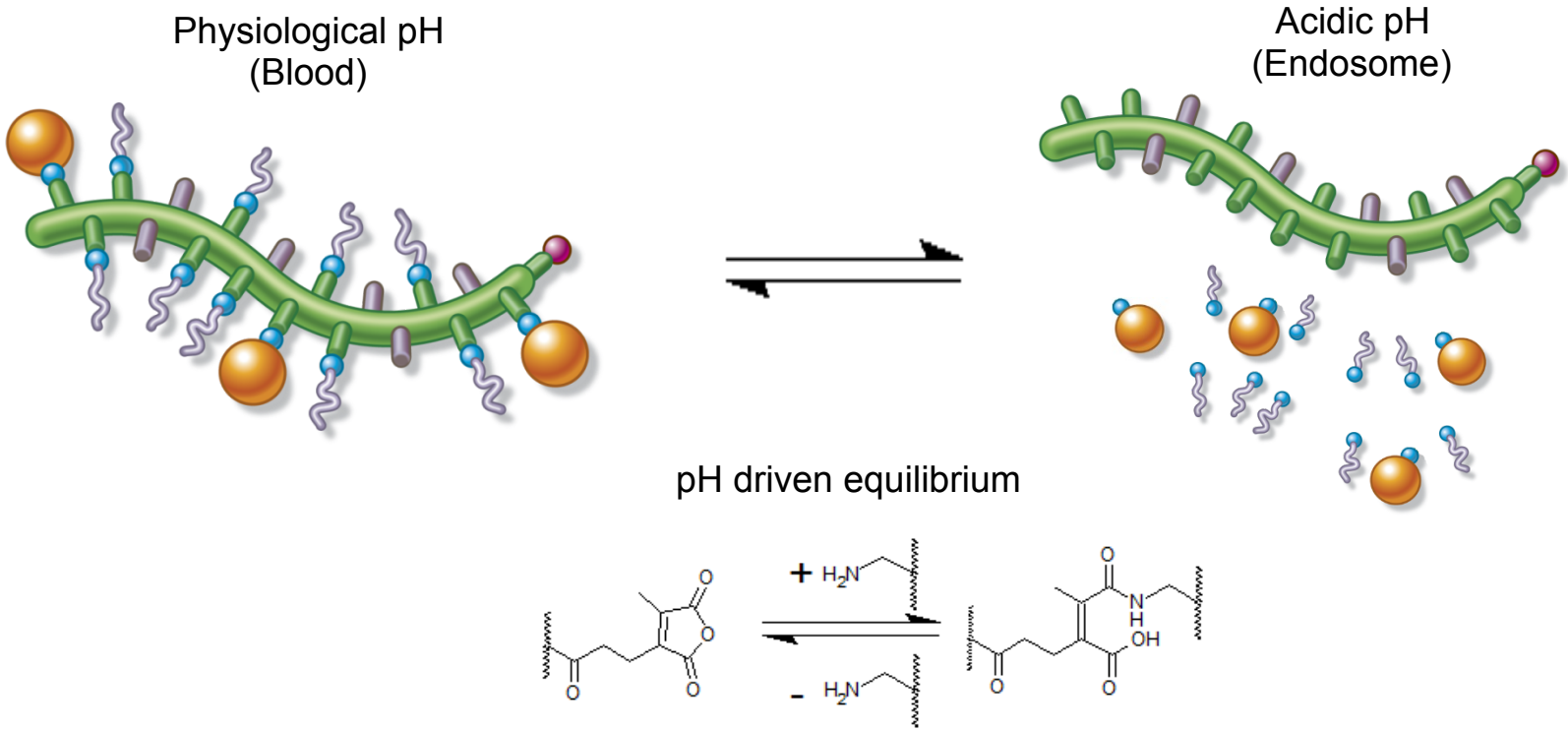
DPC/siRNA complex is enclosed in endosome low pH results in polymer unmasking

Polymer induces endosomolysis and release of siRNA into cell cytoplasm

RNA strand engages cell's interference machinery, resulting in knockdown of target gene expression

Rozema, Lewis et al. Proc Natl Acad Sci U S A. 2007 Aug 7;104(32):12982-7

Dynamic PolyConjugate (DPC) masking chemistry



Acidic pH of endosomes activates endosomolytic activity of polymer, facilitating release of siRNA to cytoplasm

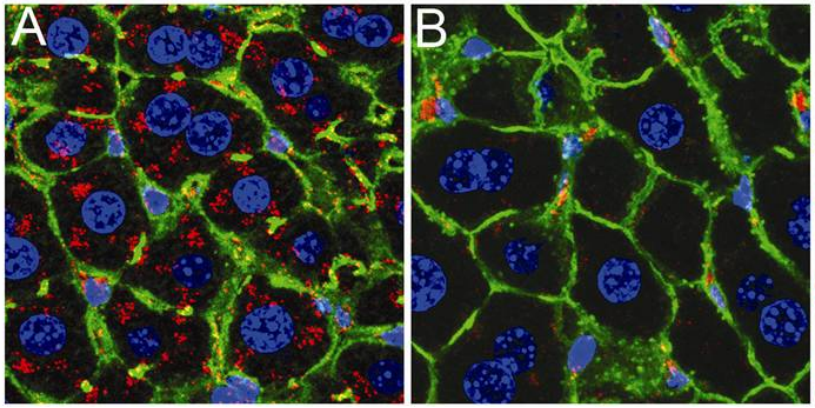
Rozema, Lewis et al. Proc Natl Acad Sci U S A. 2007 Aug 7;104(32):12982-7

DPCs for targeted siRNA delivery to hepatocytes

Ligand: N-acetyl galactosamine ligand (NAG)

NAG is a ligand for the asialoglycoprotein receptor on hepatocytes

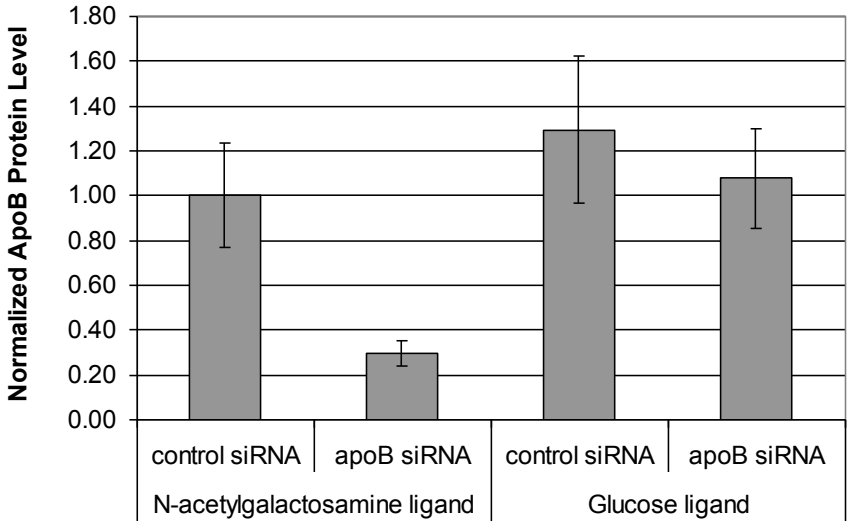
ICR mice, t=60'



NAG ligand (hepatocyte targeted)

glucose ligand (non-targeted)

DPC-siRNA
nucleus
cell membrane

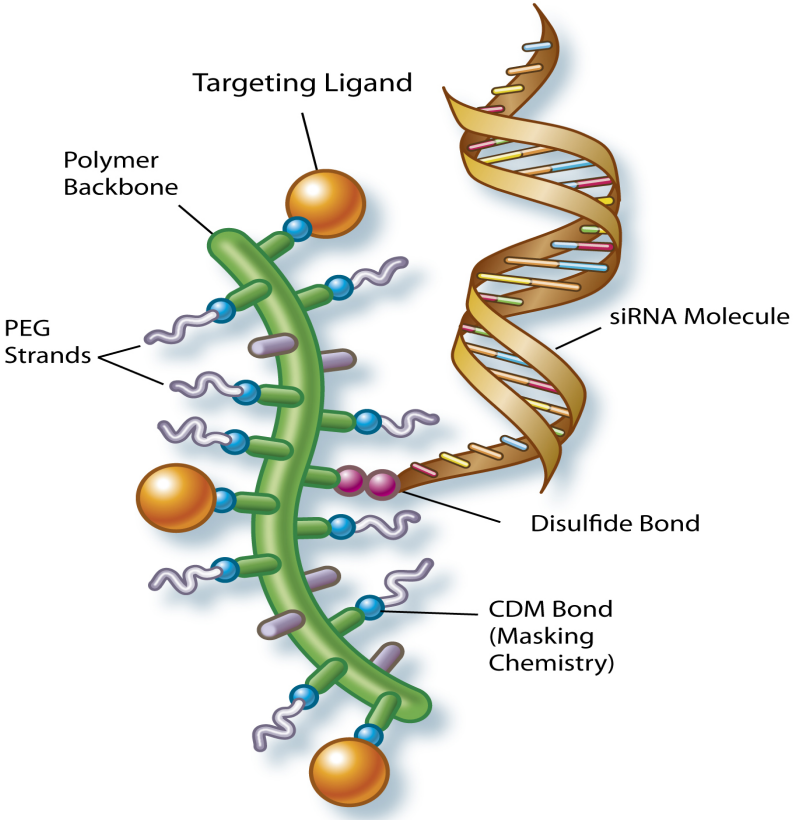


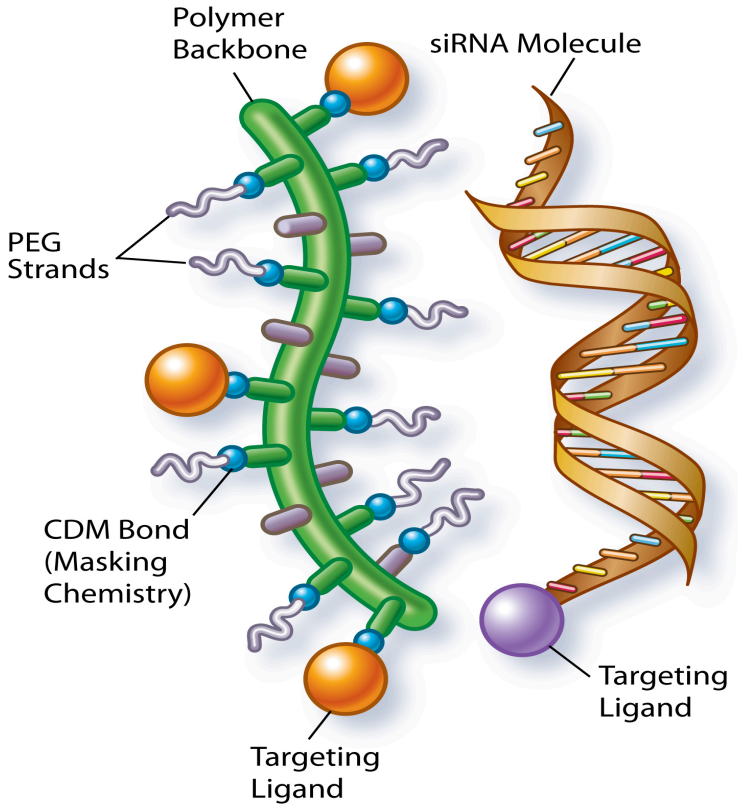
Hepatocyte-uptake of DPCs is ligand dependent

Target gene knockdown is ligand dependent

Rozema, Lewis et al. Proc Natl Acad Sci U S A. 2007 Aug 7;104(32):12982-7

DPC 2.0 – Separate targeting of the DPC polymer and the siRNA





Prototypical DPC

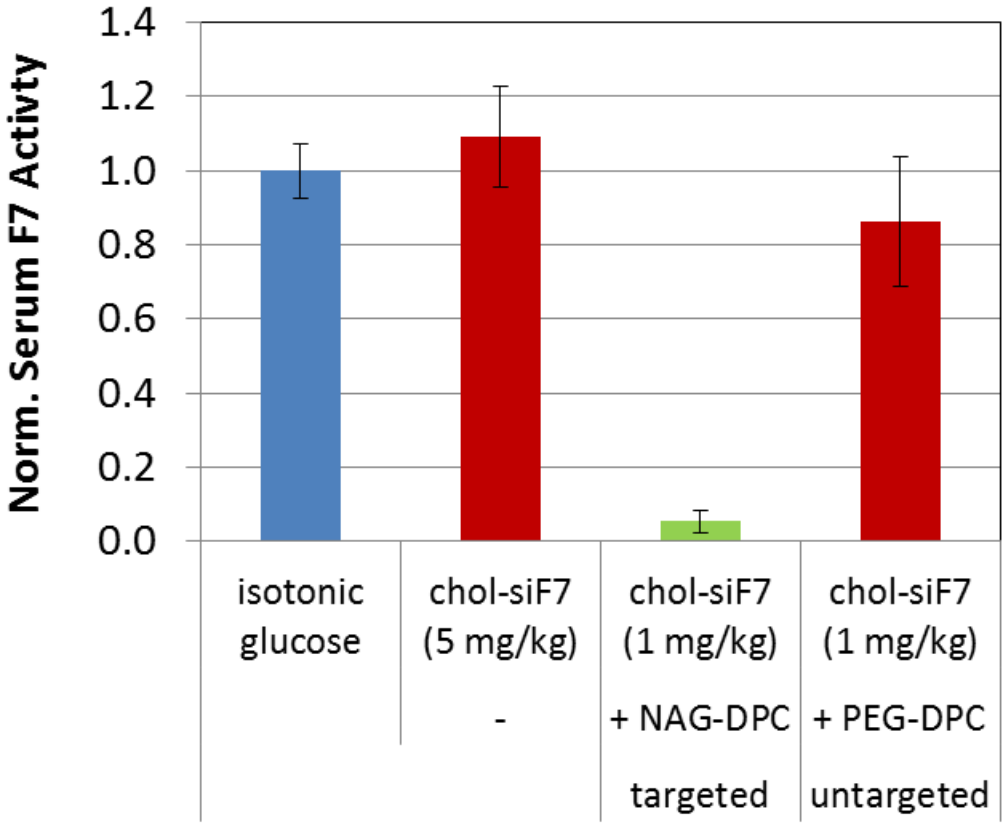
- Covalent attachment of siRNA to masked polymer

DPC polymer + targeted siRNA

- Masked polymer and siRNA are NOT attached and do NOT interact.
- Targeted independently to the same cell after co-injection
- Typically NAG-DPC and Chol-siRNA

Co-injection of hepatocyte-targeted NAG-DPC improves delivery of liver-tropic chol-siRNA

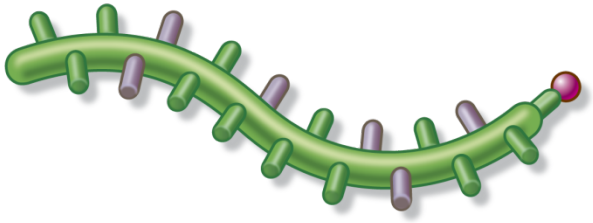
Target: Coagulation Factor 7



mice, single i.v. injection, 48 hr timepoint

Wong et al, Nucleic Acid Ther. 2012 Dec;22(6):380-90

Exploration of polymer space

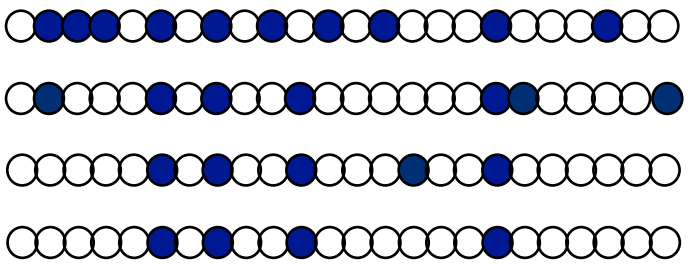


Polymer

Membrane lytic activity
Amphipathic, poly cationic

Reversible Masking of Membrane Lytic Activity
PEG to reduce membrane interactions during delivery
Targeting ligand to direct DPC to target ligand

DPC polymers:
Random co-polymerization of hydrophobic and hydrophilic monomers

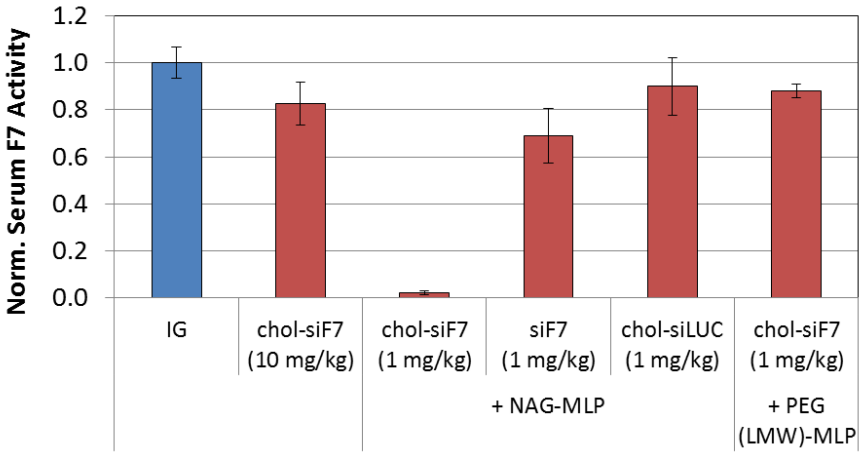


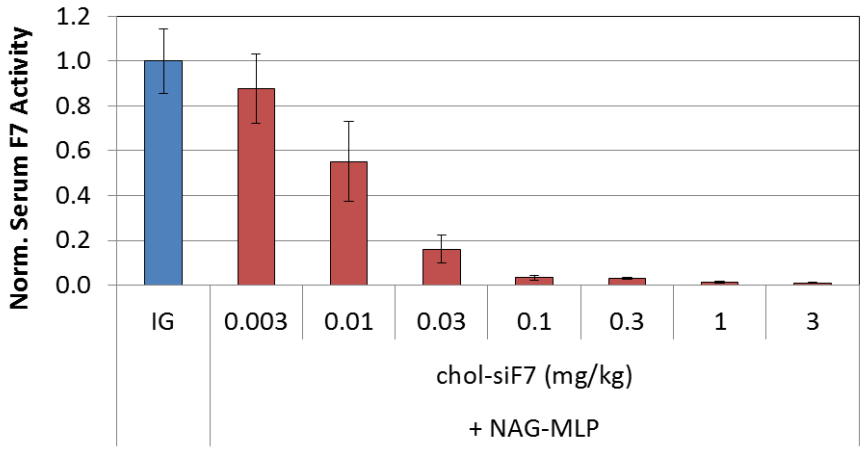
DPC peptides: Membrane Lytic Peptides (MLP)
Defined solid phase synthesis of hydrophobic and hydrophilic amino acids

Co-injection of NAG-MLP and chol-siRNA

Requirements for target gene KD and chol-siRNA titration in liver

single dose





<u>Target gene knockdown requires</u>:
Liver-tropic siRNA (cholesterol-siRNA) ***<u>and</u>*** hepatocyte-targeted DPC peptide (NAG-MLP)

Co-injection of NAG-MLP with chol-siF7 enables highly efficient delivery

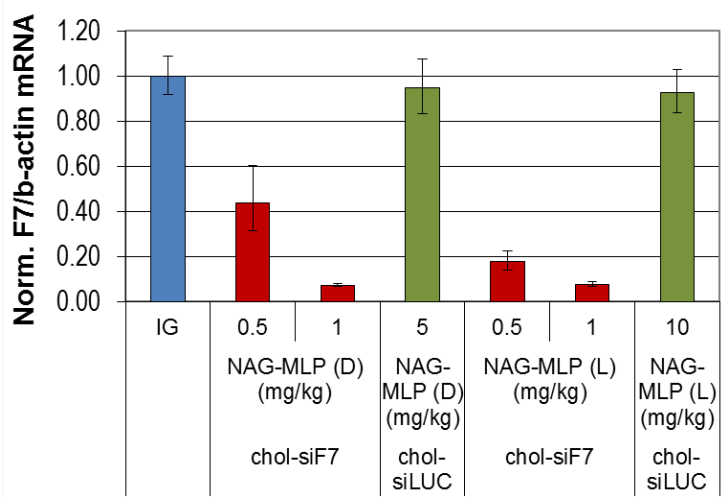
- ED_{50} = 0.01 mg/kg chol-siF7
- ED_{99} = 1 mg/kg chol-siF7

mice, 6 mg/kg NAG-MLP, 48 hr timepoint

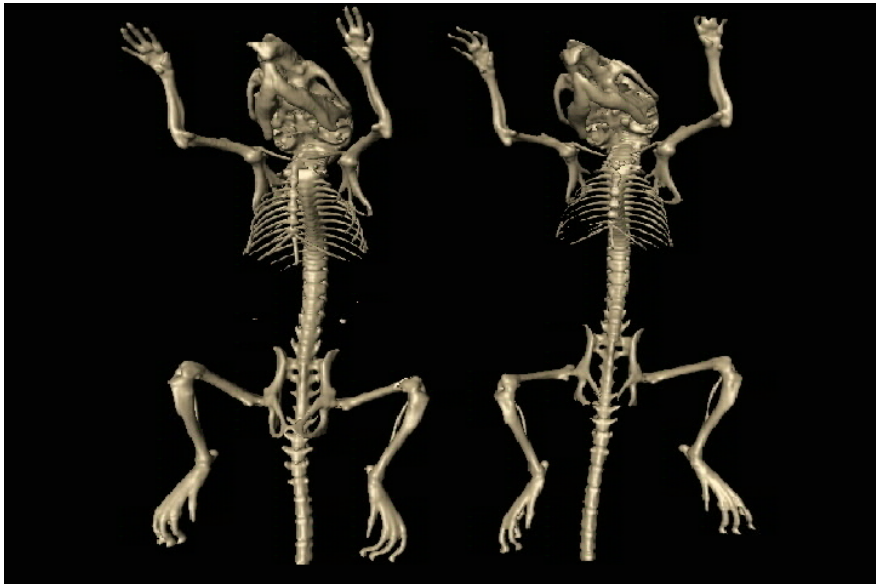
Wooddell et al, Mol Ther 2013 May; 21(5) 973-85

PET imaging of mice injected with ^{124}I-NAG-MLP

(L) NAG-MLP vs. non-biodegradable (D) enantiomer



NAG-MLP(L) NAG-MLP(D)



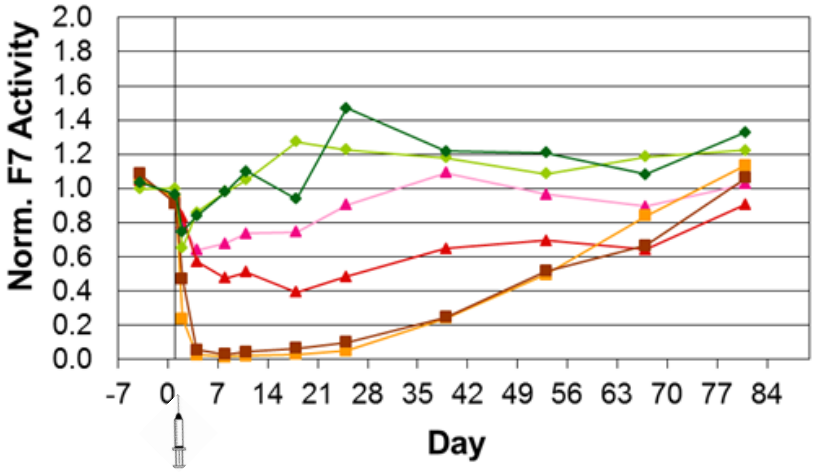
(L) and (D) forms are equally efficacious

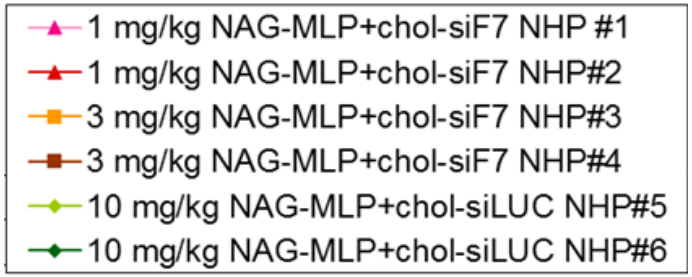
After siRNA delivery NAG-MLP (L) is rapidly metabolized in the liver and eliminated.

Efficacy in non-human primates

NAG-(L)-MLP dose titration + chol-siRNA, single iv dose

Target: Coagulation Factor VII





- **Highly efficacious**
  - *ED_{50} NAG-MLP = 1 mg/kg*
  - *>99% KD at 3 mg/kg NAG-MLP*
  - *>80% KD for 5 weeks*
  - *No change using chol-siLuc control*

2 mg/kg chol-siRNA

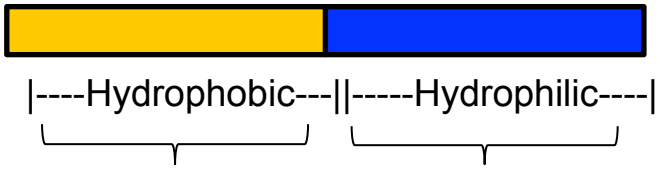
Modes of MLP Interactions with membranes

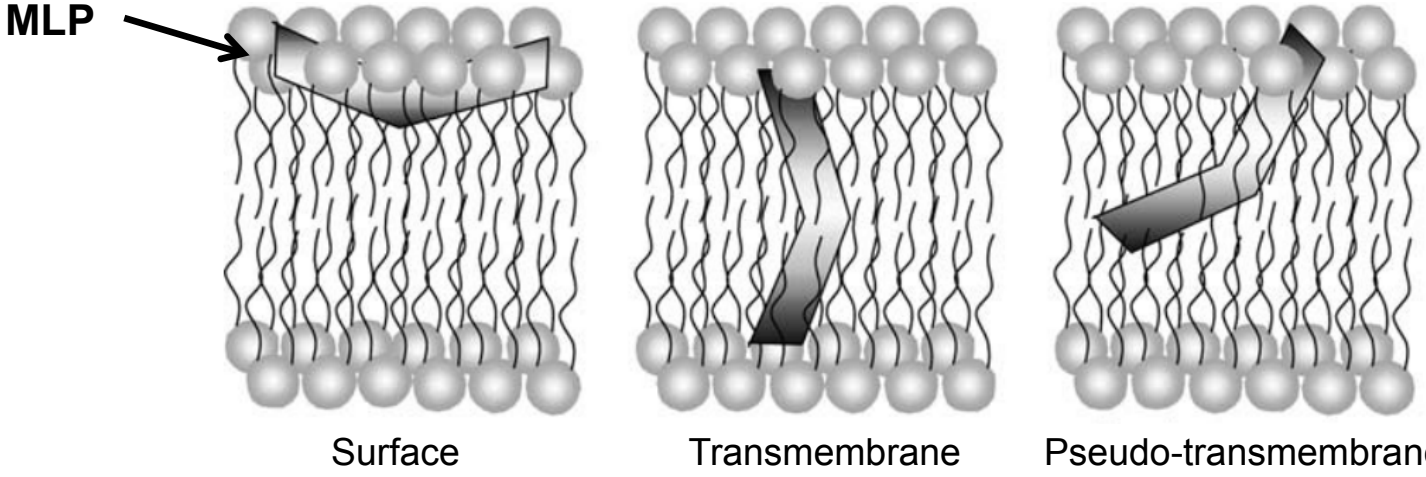
Two Main Characteristics of Peptides:

Cationic
strong interaction with lipids head-groups

Amphipathic
Block pattern enables transmembrane interactions

Membrane Lytic Peptide

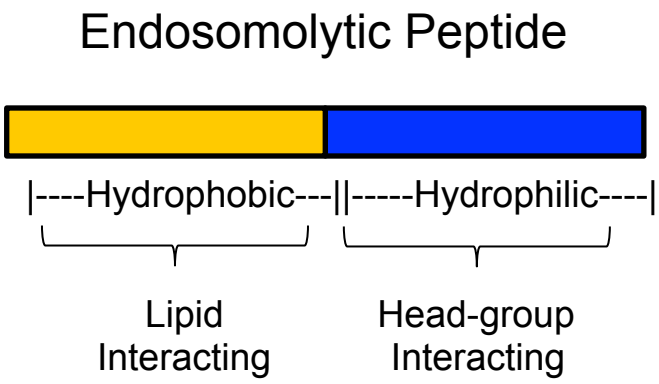




Transmembrane-like interactions provide potent membrane disruption

Raghuraman H. et al, Biosci Rep 2007 27: 189-223

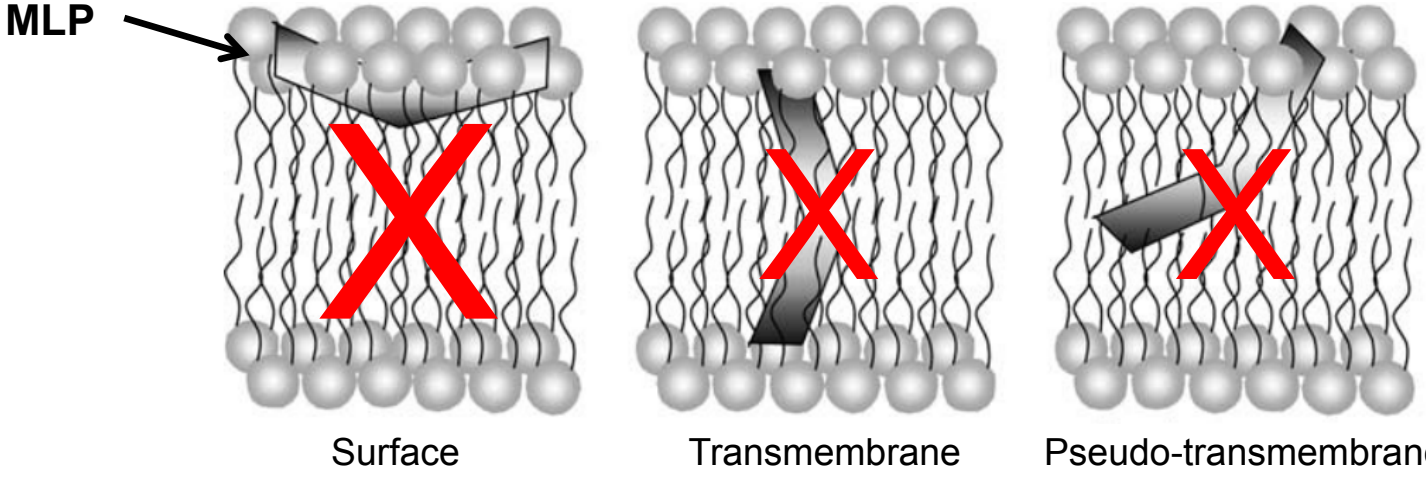
Influence of masking on MLP membrane interactions



Masking of MLP occurs at cationic amines primarily in hydrophilic region

Broad disruption of membrane interactions

Modes of MLP Interacting with Membrane



Raghuraman H. et al, Biosci Rep 2007 27: 189-223

Next generation delivery NAG-MLP DPCs

Hydrophilic extensions to further reduce hydrophobic interactions

- Introduce hydrophilic extension to reduce non-specific membrane interactions during delivery
- Inhibit transmembrane interactions of MLP during delivery
- Polyethylene Glycol (PEG) as a hydrophilic extension

Endosomolytic Peptide

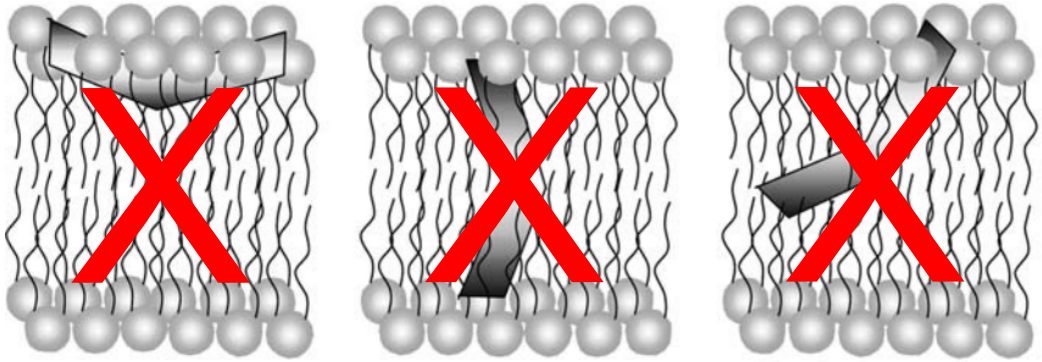
|----Hydrophobic---||-----Hydrophilic----|

Hydrophilic-Endosomolytic Peptide

|-----Hydrophilic----||----Hydrophobic---||-----Hydrophilic----|

CDM-Masking

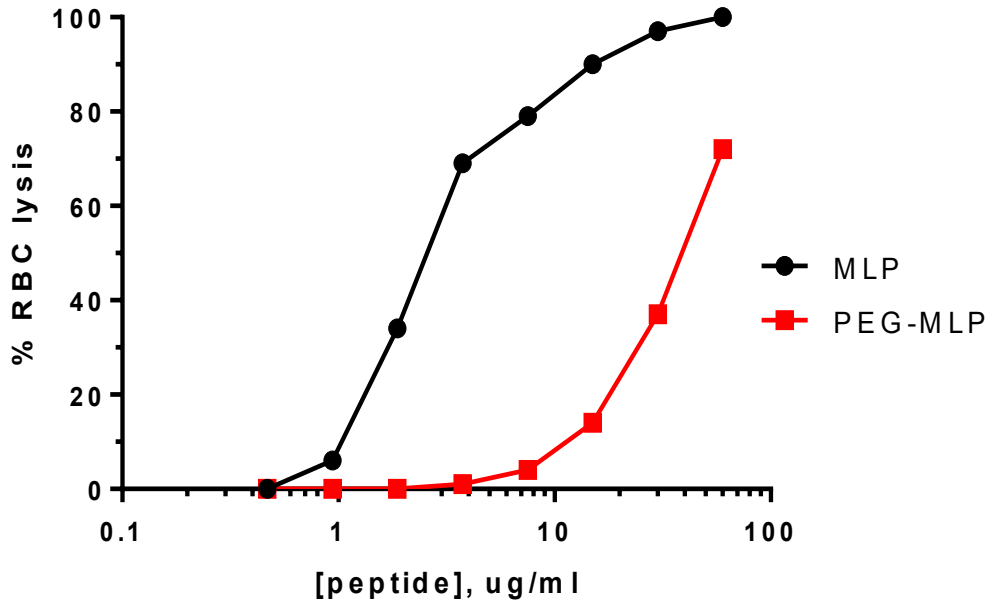
Hydrophilic Extension



Raghuraman H. et al, Biosci Rep 2007 27: 189-223

Attachment of PEG to amine terminus reduces hemolytic activity

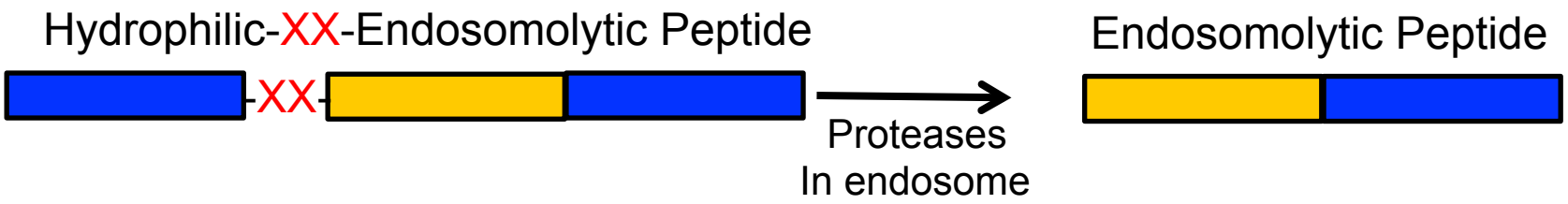
Red blood cell hemolysis assay shows significantly reduced lytic behavior for PEG-MLP's vs. MLP



Reduced Potency in siRNA knockdown, due to presence of PEG

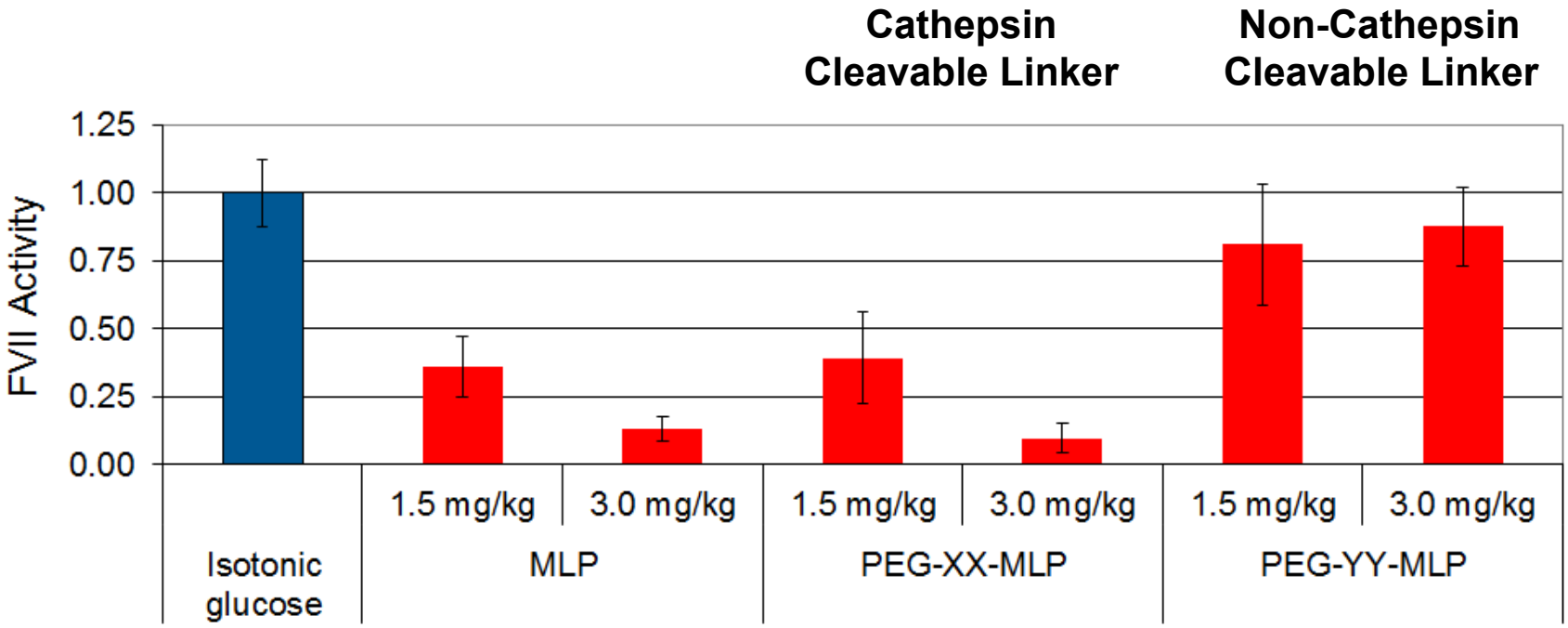
How do we keep in vivo potency for siRNA knockdown?

Protease sensitive linker used to attach hydrophilic extension



- Hydrophilic extension reduces membrane interactions
- Removal of hydrophilic extension necessary for siRNA delivery potency
- Addition of protease sensitive cut-site allows removal of hydrophilic extension in the endosome

Identity of protease cut-site influences potency of siRNA knockdown

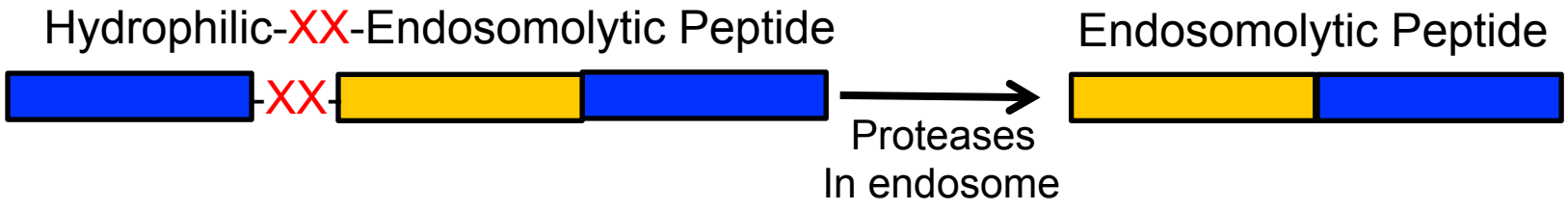


ICR Mice, 2 mg/kg chol-siRNA, 48 hr timepoint

PEG-XX-MLP shows similar efficacy to parent MLP

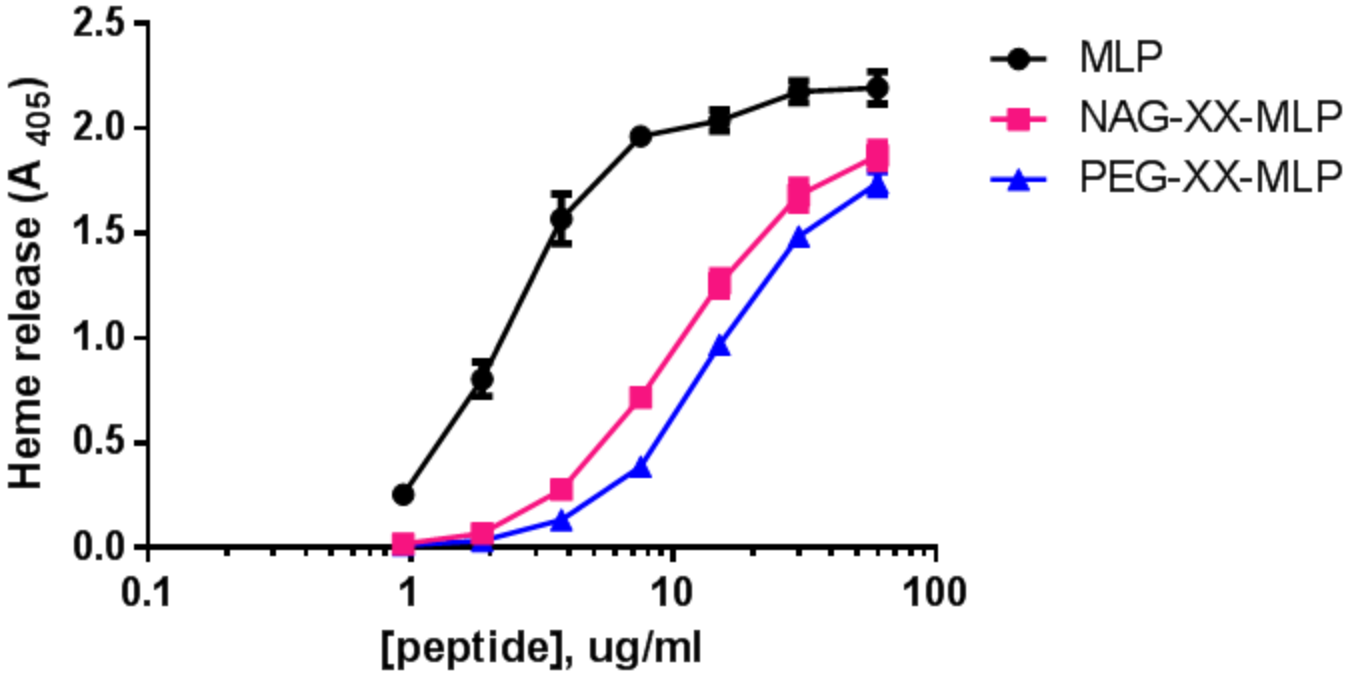
Can NAG be used as a hydrophilic extension?

- Decreased hemolysis with similar potency with PEG-XX-MLP's
  - Demonstrates protease cleavage to facilitate delivery with less lysis of base peptide due to N-terminal hydrophilic extension



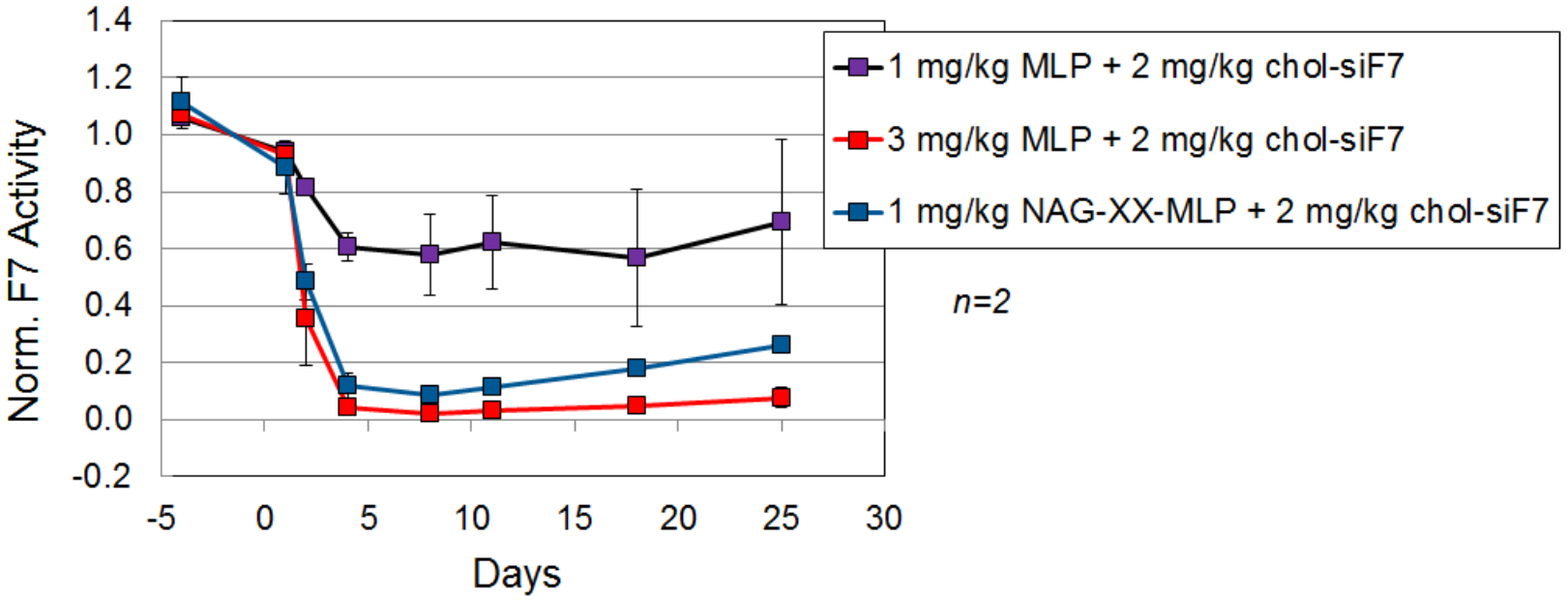
- Can NAG be used as hydrophilic extension?
  - Provide both hydrophilic modification and potentially enhanced targeting

Attachment of NAG reduces hemolytic behavior of MLP



NAG-XX-MLP shows hemolytic behavior similar to that of PEG-XX-MLP

NAG-MLP showed increased activity in non-human primates compared to MLP



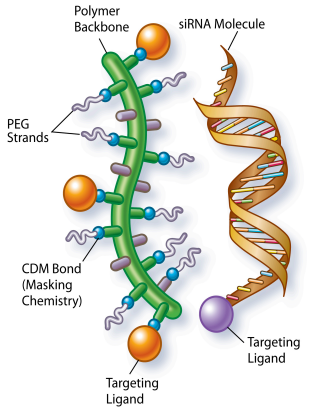
NAG-XX-MLP shows ~3-fold increased efficacy compared to MLP in non-human primates

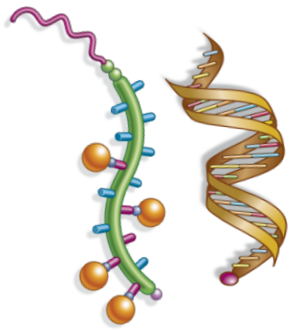
Dynamic PolyConjugates (DPC's) as a Platform for siRNA Delivery

- Peptides can be used as DPC polymers (e.g. NAG-MLP)
- Co-injection of NAG-MLP and chol-siRNA is highly effective
- NAG-MLP is well-tolerated & biodegradable

- Hydrophilic extensions modulate non-specific membrane interactions of MLP
- Inclusion of a protease cleavable linker allows functional siRNA delivery
- Use of NAG as hydrophilic extension increases potency of MLP while reducing non-specific membrane interactions

DPC polymer + targeted siRNA





Contributors

CSO - Dave Lewis

Biology

Chris Wooddell
So Wong
Qili Chu
Holly Hamilton
Tom Reppen
Stephanie Bertin
Jacob Griffin
Vladimir Subbotin
Vladimir Trubetskoy
Yinghua Bian
Jessica Montez
Hans Herweijer
Alex Sokoloff

Live Animal Research

Julia Hegge
Tracie Milarch
Linda Goth
Rachel Schmidt
Sheryl Ferger

Chemistry

Dave Rozema
Jason Klein
Darren Wakefield
Collin Hagen
Anthony Perillo-Nicholas
Lauren Carlson
Andrei Blokhin
Jeff Carlson
Jonathon Benson

University of Wisconsin

Jamie Weichert
Mohammed Farhoud
Benny Titz

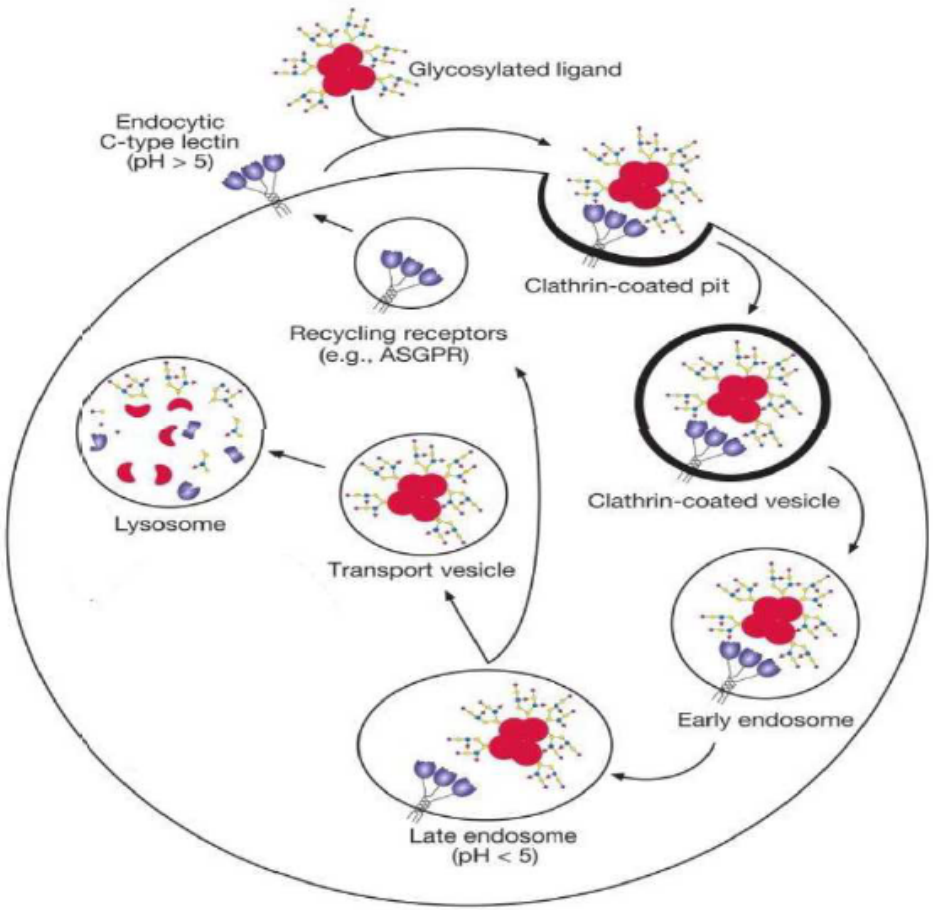
Thank you!

Questions?

Using NAG ligand to target DPCs to hepatocytes via the asialoglycoprotein receptor (ASGPr)

ASGPR

- Highly expressed in hepatocytes
  - » 0.5-1 million copies/cell
- Clears serum glycoproteins via clathrin-mediated endocytosis
- High rate of uptake
- Recycling time ~15 minutes
- Conserved across species



N-acetylgalactosamine (NAG) is a high affinity ligand for ASGPr

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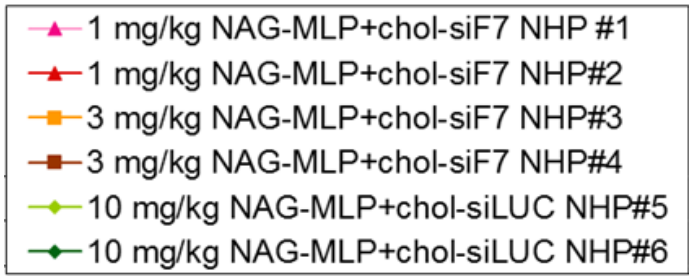
Well Tolerated in non-human primates

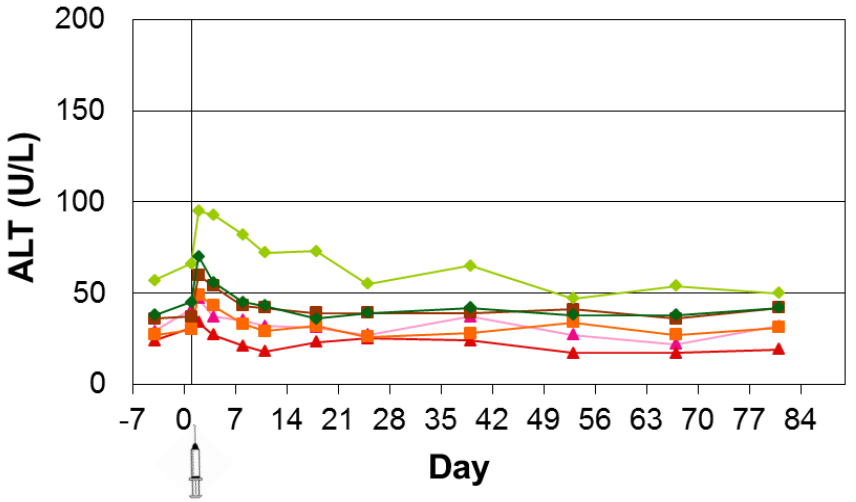
NAG-(L)-MLP dose titration + chol-siRNA, single iv dose

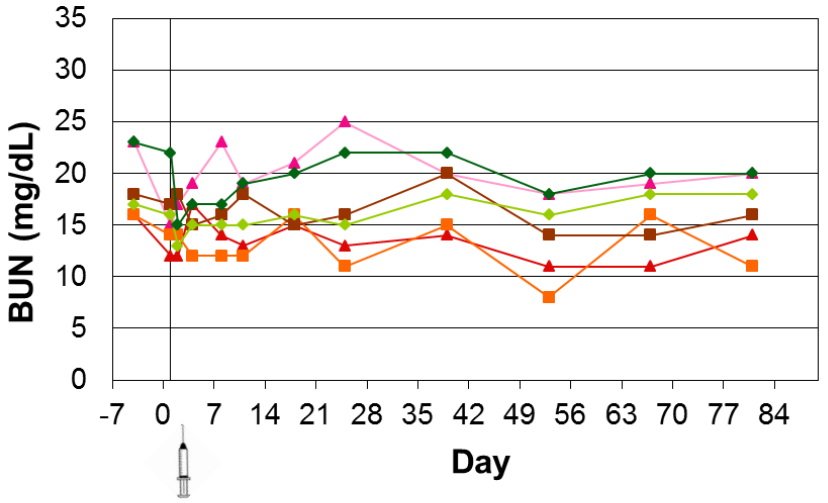
Target: Coagulation Factor VII



2 mg/kg chol-siRNA

- **No toxicity reached**
  - *No treatment-related changes in clin chem markers*
  - *No changes in hematology*
  - *No changes in cytokine levels (panel of 23)*





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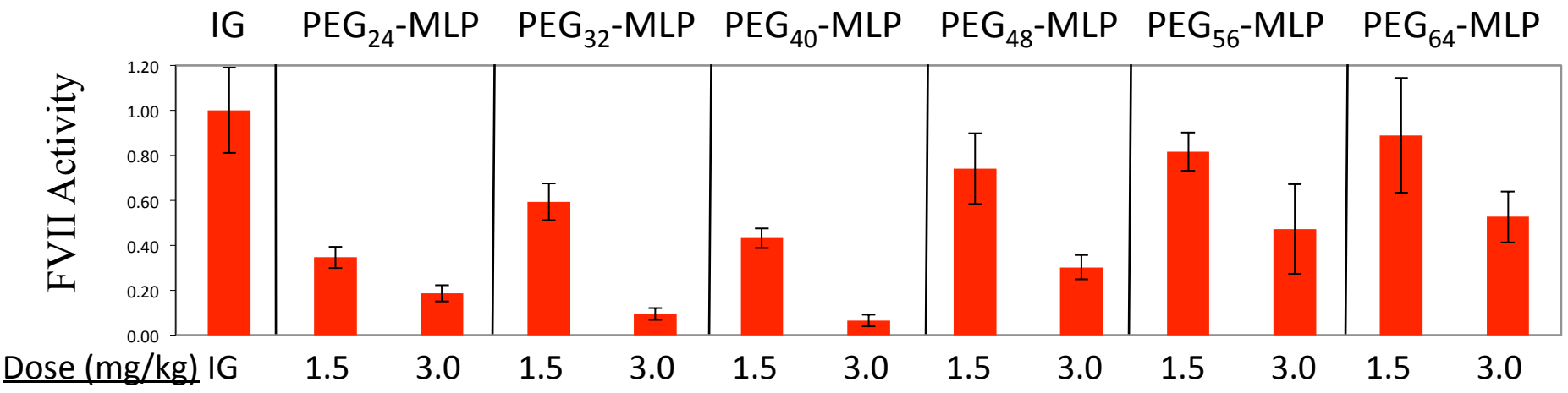
PEG-MLP loses potency with longer than ~40 PEG



Reduce number of examples

ICR Mice, 2 mg/kg siRNA, 48 hr timepoint