Protease-sensitive siRNA delivery vehicles

International Symposium on Polymer Therapeutics

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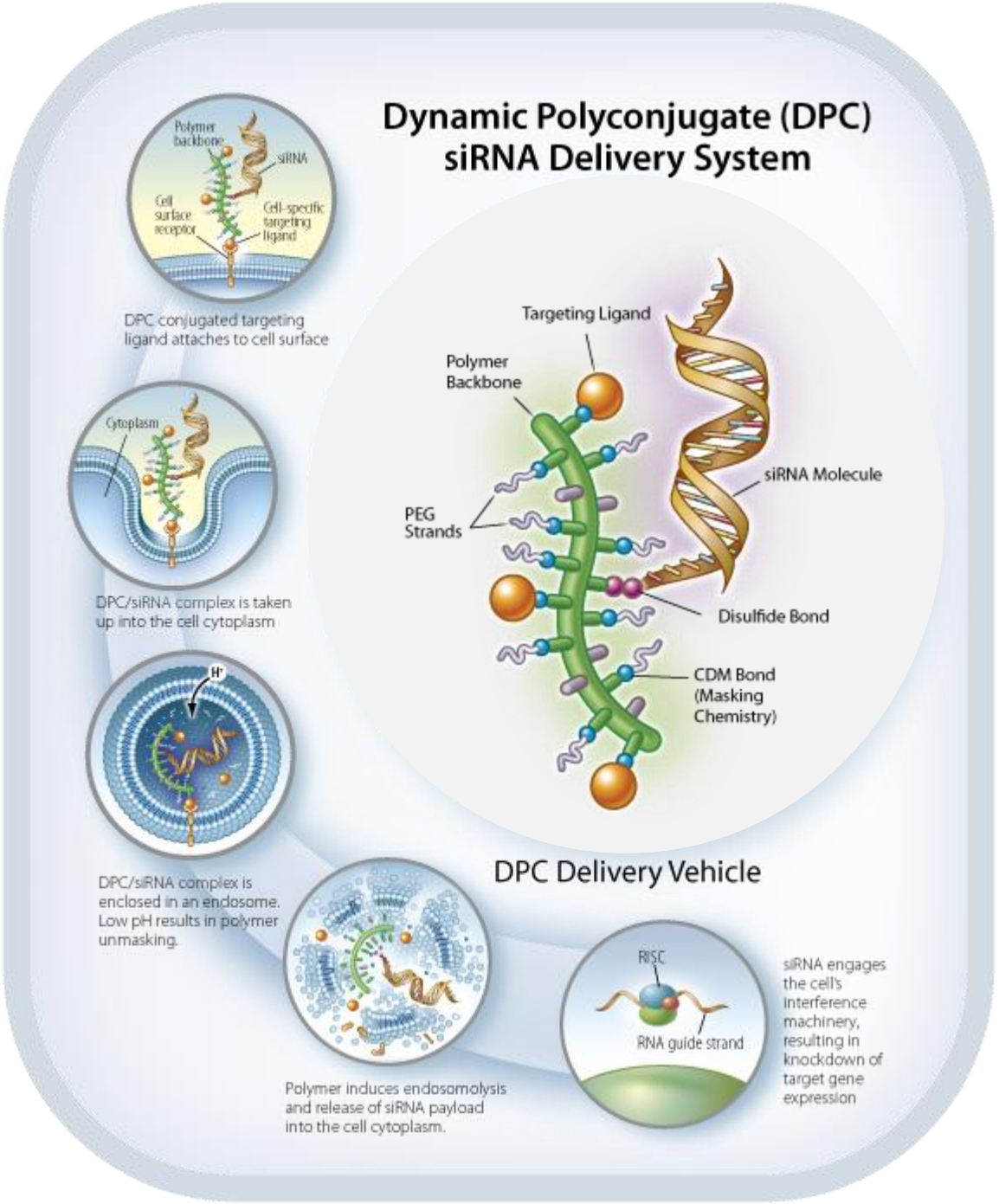
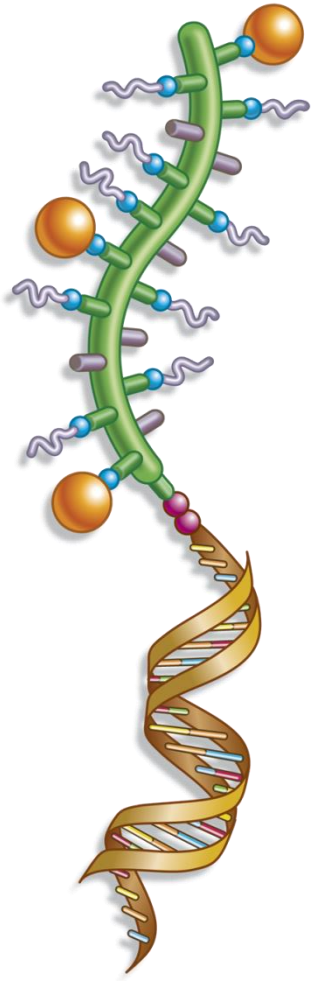
David Rozema

Arrowhead Madison



- DPCs
  - 5-20 nm in size
  - Contains endosomolytic polymer
  - Polymeric amines are “masked” with hydrophilic PEG or targeting ligand.
  - siRNA is attached to polymer
- Cellular uptake is ligand-driven
- Intracellular environment drives polymer unmasking
- Unmasked polymer disrupts endosomal membrane
- siRNA released to cytoplasm

Dynamic PolyConjugate (DPC) technology for siRNA delivery



Components of DPC

- Amphipathic polymer
  - Polyvinyl ether
  - Peptide
  - Polyacrylate
- siRNA conjugate
  - Polymer
  - Targeting ligand
- Targeting/masking
  - Polyethylene glycol
  - N-acetylgalactosamine (NAG)
- Reversible bond
  - pH-labile
  - Protease cleavable

DPC iterations

- pH labile masking of polyvinylether-siRNA conjugate
  - Rozema et al *PNAS* (**2007**) 104: 12982-12987.
- pH labile masking of peptide for delivery of siRNA-cholesterol conjugate
  - Wong et al *Nucleic Acid Therapeutics* (**2012**) 22: 380-390.
  - Wooddell et al *Molecular Therapy* (**2013**) 21:973-985. (Formulation for HBV)

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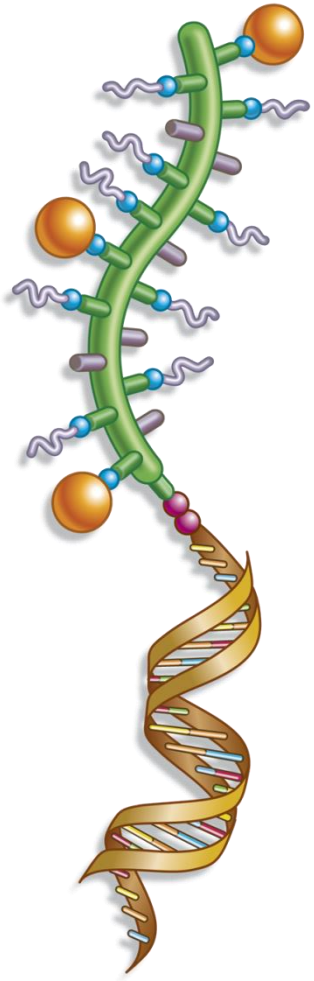

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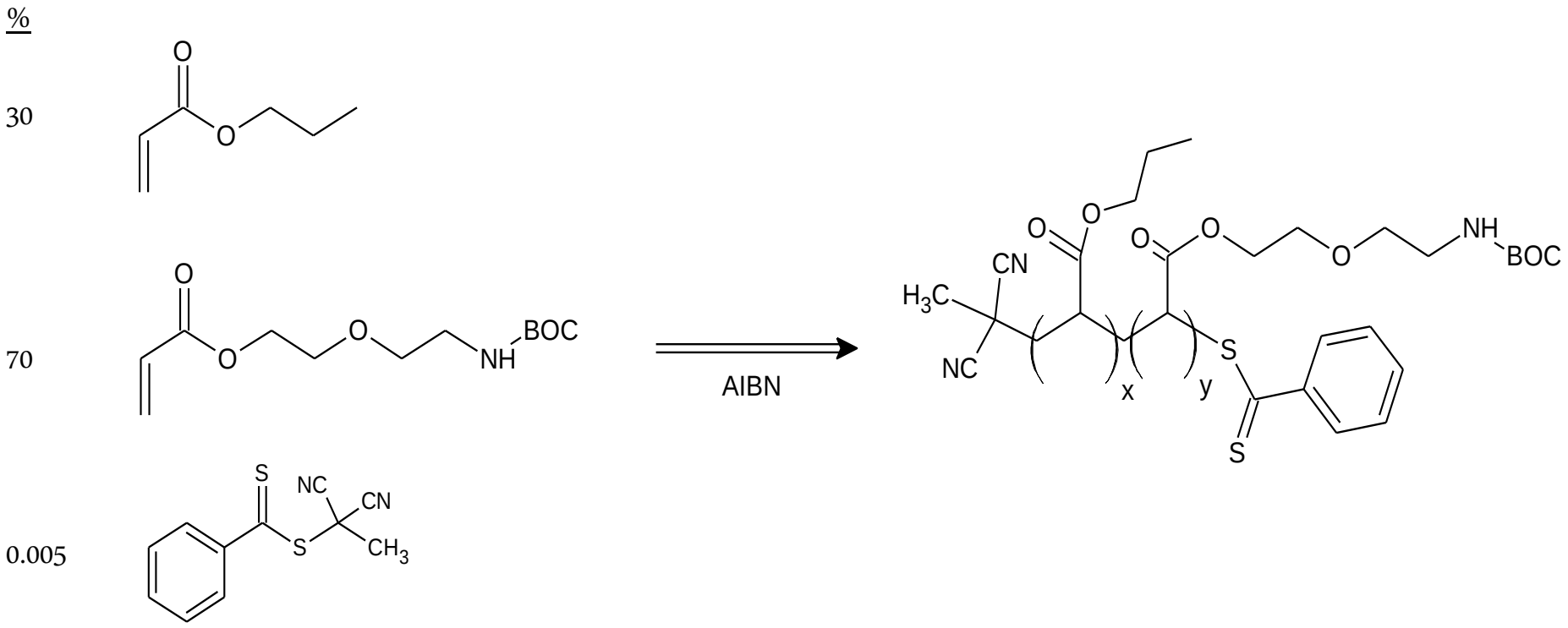
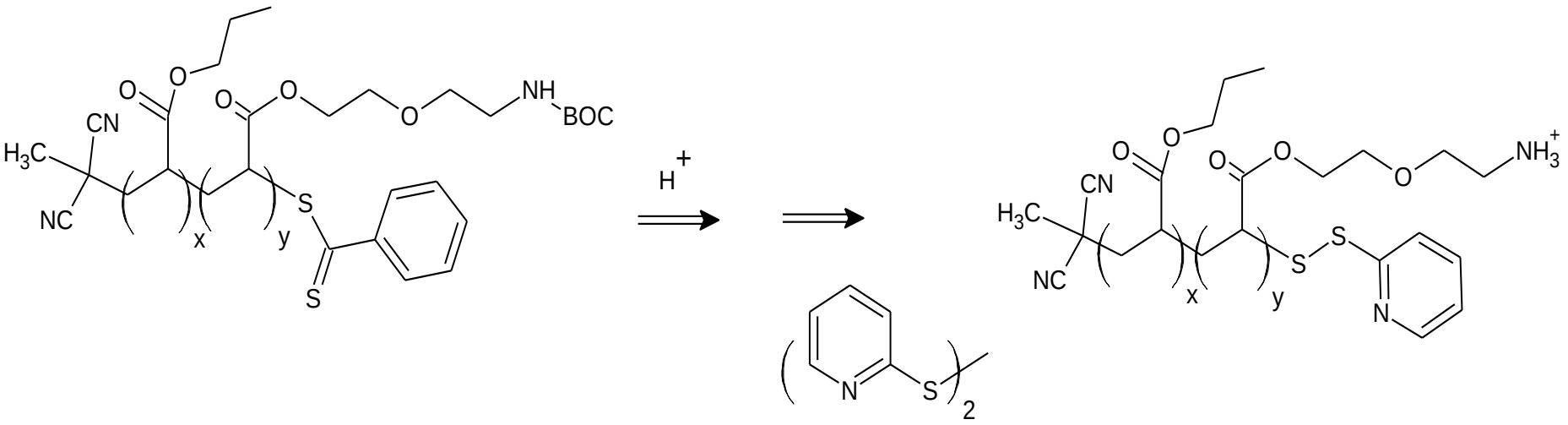
Polyacrylate Synthesis :

random copolymerization of alkyl and amino monomers in presence of **Reversible Addition-Fragmentation Transfer (RAFT)** agent



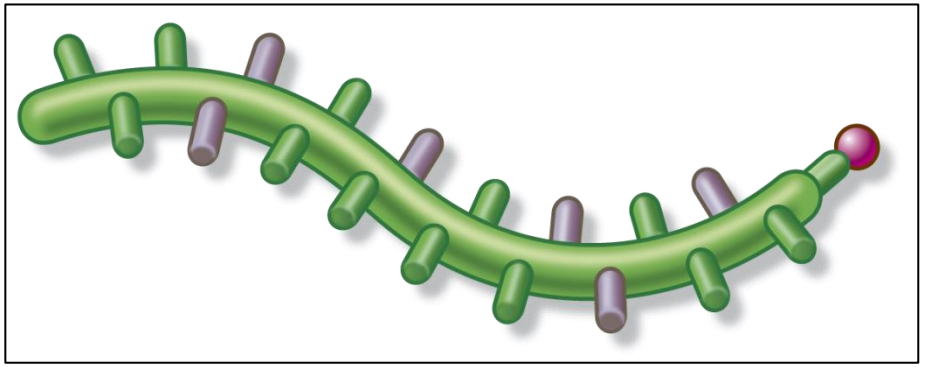
Advantages of RAFT polymerization

- Narrow polydispersity
- Uniform composition
- Scalable



- Treatment with acid
  - -liberates amino groups
  - -generates terminal thiol

- thiol converted to pyridyl disulfide



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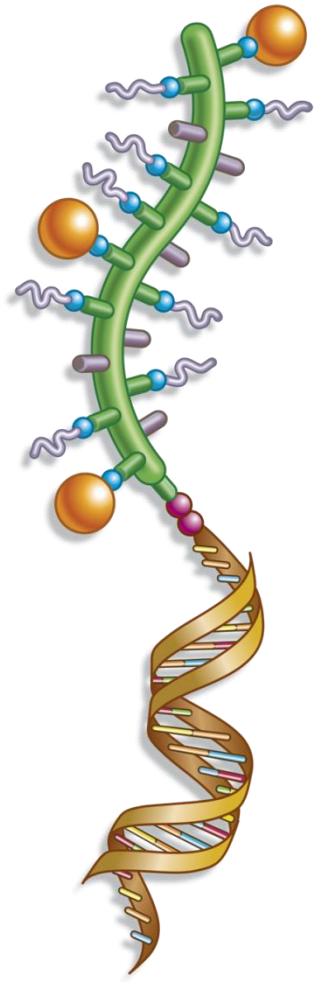

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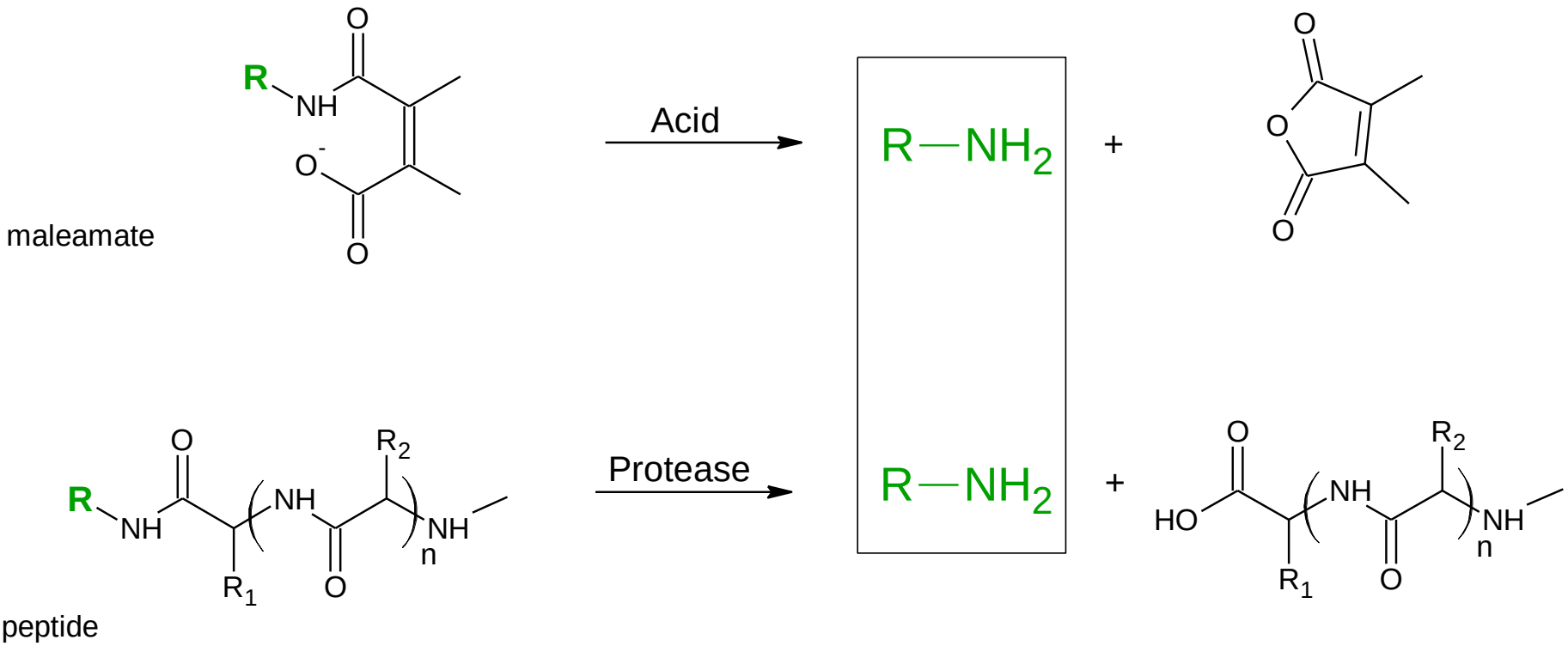
Masking Chemistry

-acid vs protease

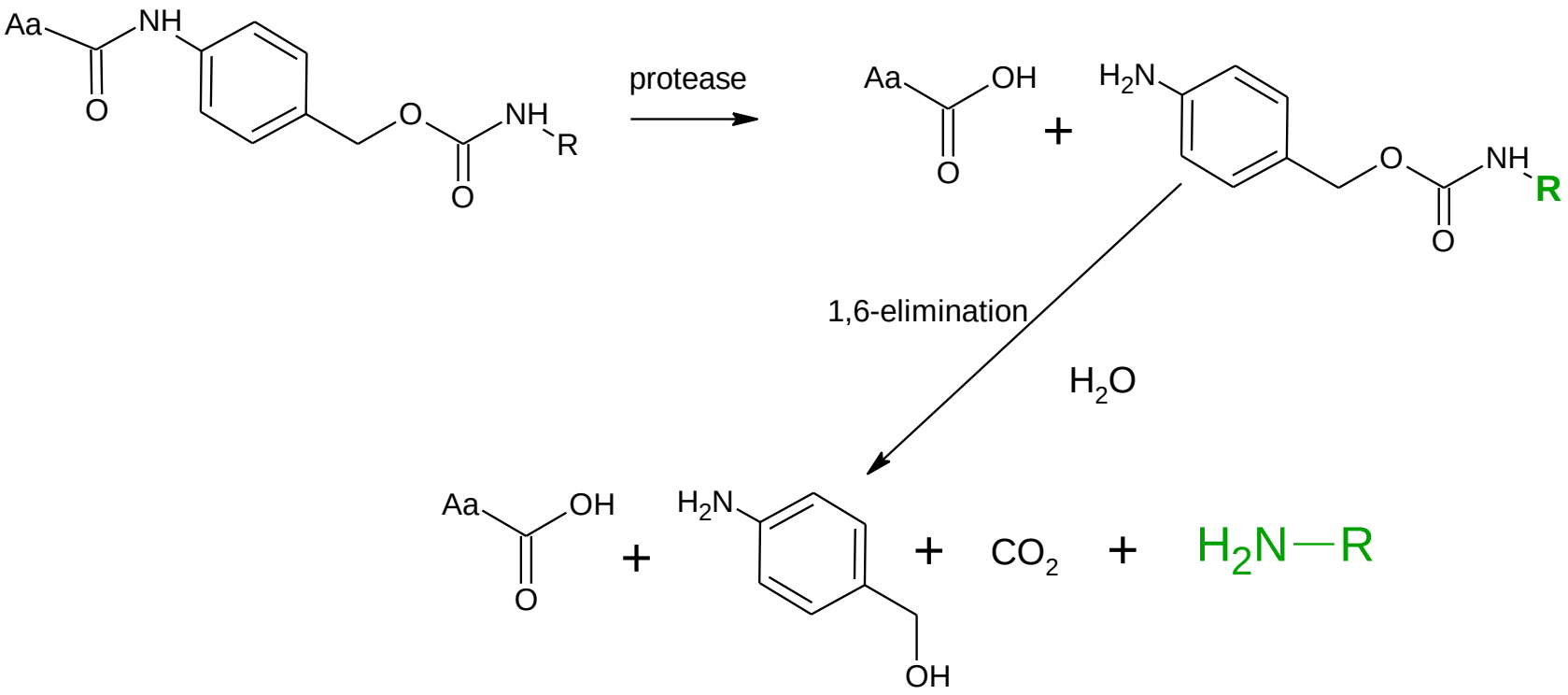


where **R** is membrane-active, amphipathic polyamine.

Protease-cleavable linker *p*-aminobenzylcarbamate (PABC)

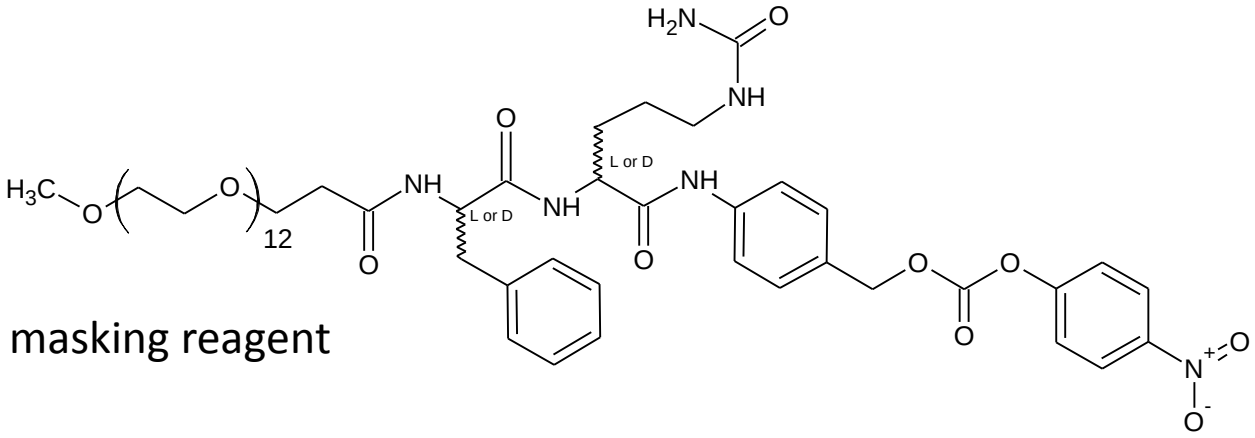
-known protease substrate

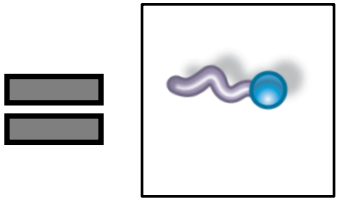
-extends cut site from released R



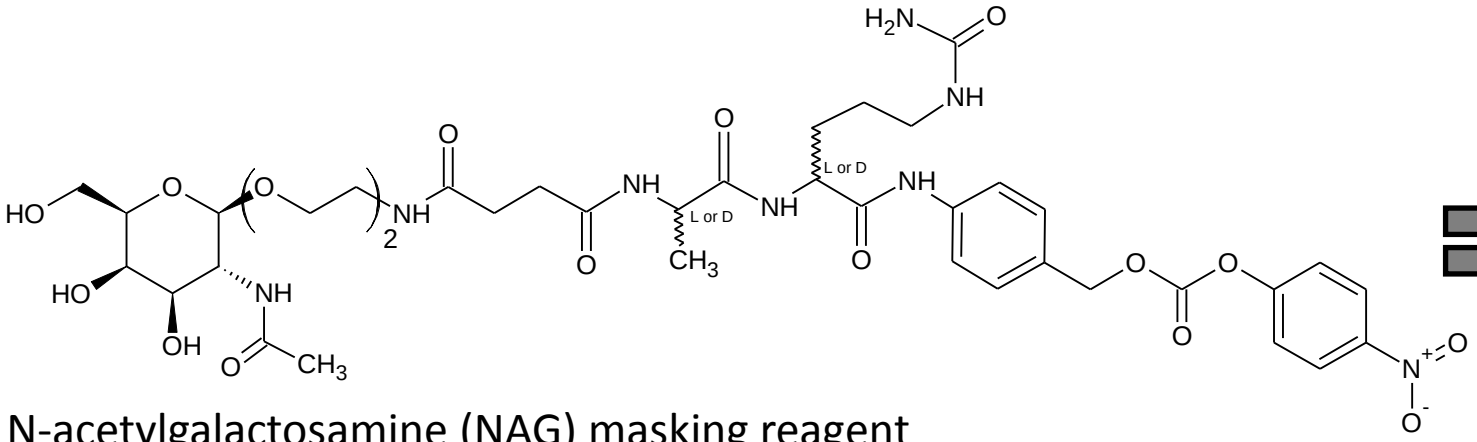
Protease-cleavable PEG and NAG

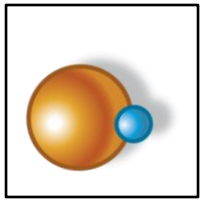
L and D diaminoacids derivatives synthesized as
p-nitrophenol carbonates for modification of amines





PEG masking reagent

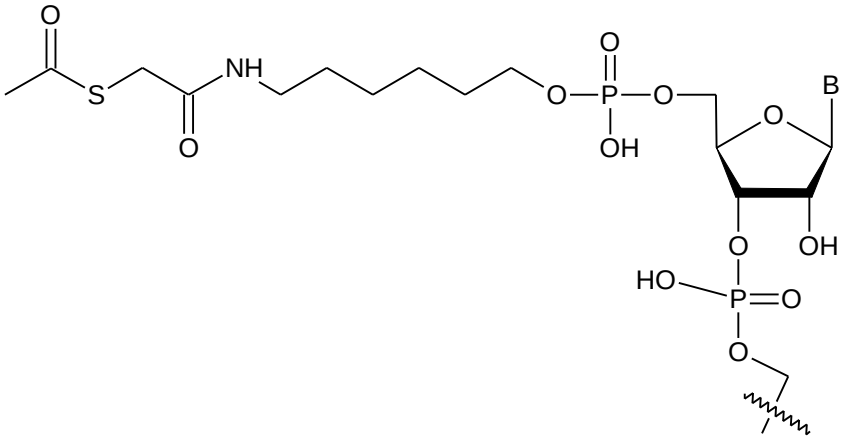


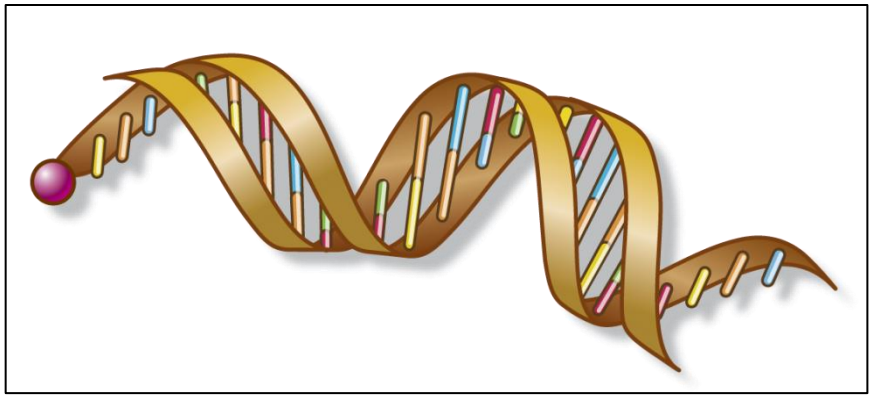


N-acetylgalactosamine (NAG) masking reagent

Thio siRNA

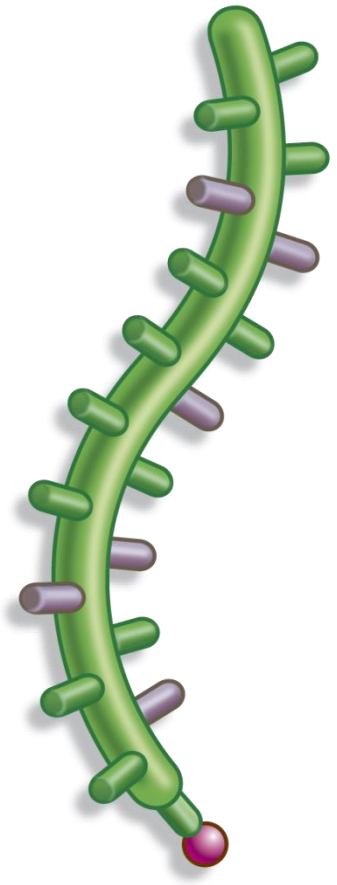
Duplex siRNA is synthesize using standard oligonucleotide chemistry, and a thioacetyl group (S-Ace) is placed at 5’ end of sense strand





Formulation Steps

- Modify with protease-labile reagents
  - Addition of masking reagents to polymer in aqueous solution
  - N-acetylgalactosamine ligand, then
  - PEG masking reagent
- Attach siRNA
- Inject iv
- 7 days postinjection, collect serum and liver for fVII activity and mRNA quantitation



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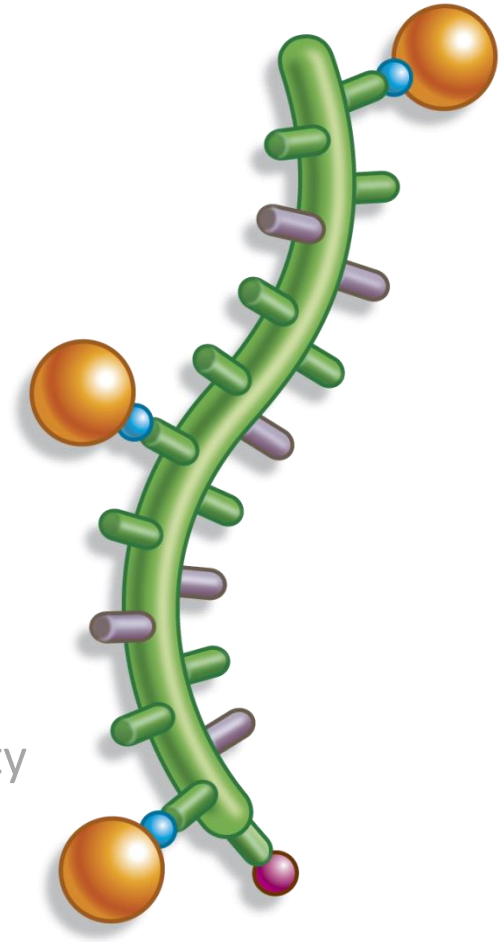

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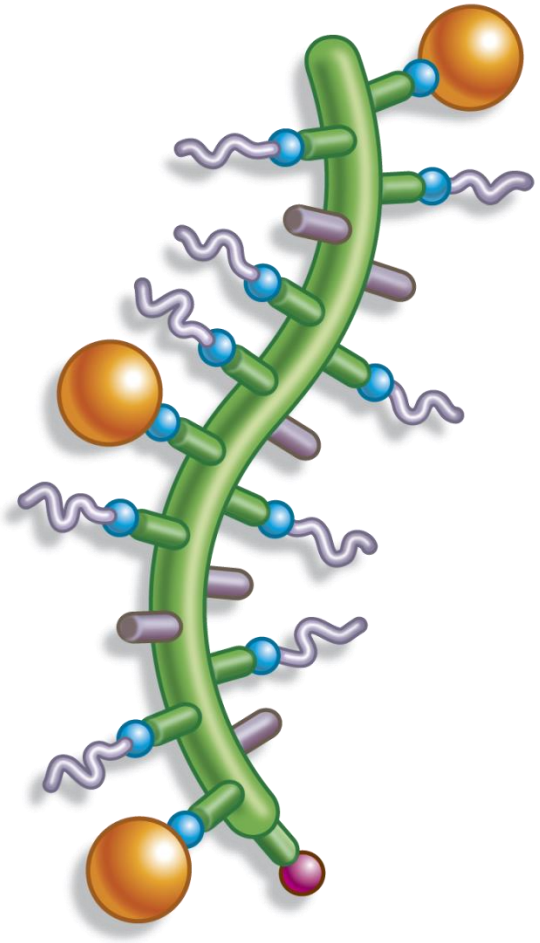
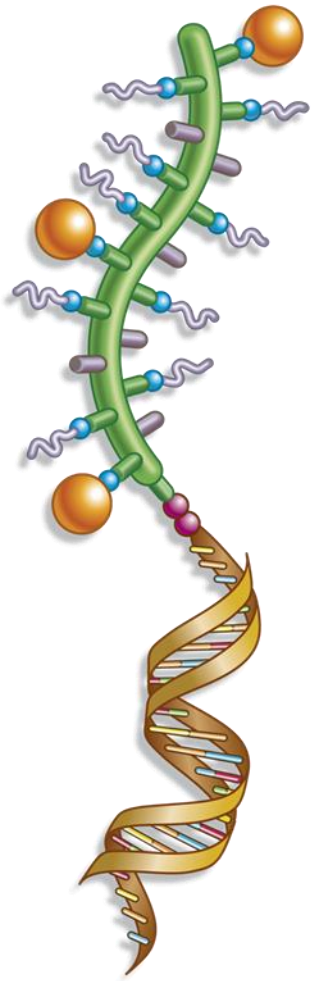


Formulation Steps

- Modify with protease-labile reagents
- Attach siRNA
  - Deprotection of thiol group on siRNA, which reacts with pyridyl disulfide group to form siRNA-polymer conjugate
- Inject iv
- 7 days postinjection, collect serum and liver for fVII activity and mRNA quantitation



Composition and size of injected formulation

Attribute	Value
siRNA:polymer (wt:wt / mol:mol)	1:10 / 1:5
Size	20 nm
ζ-potential	-5 mV

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- Inject iv

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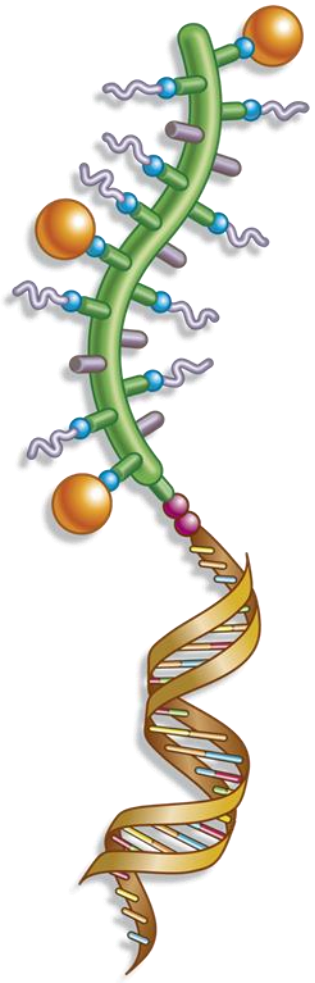
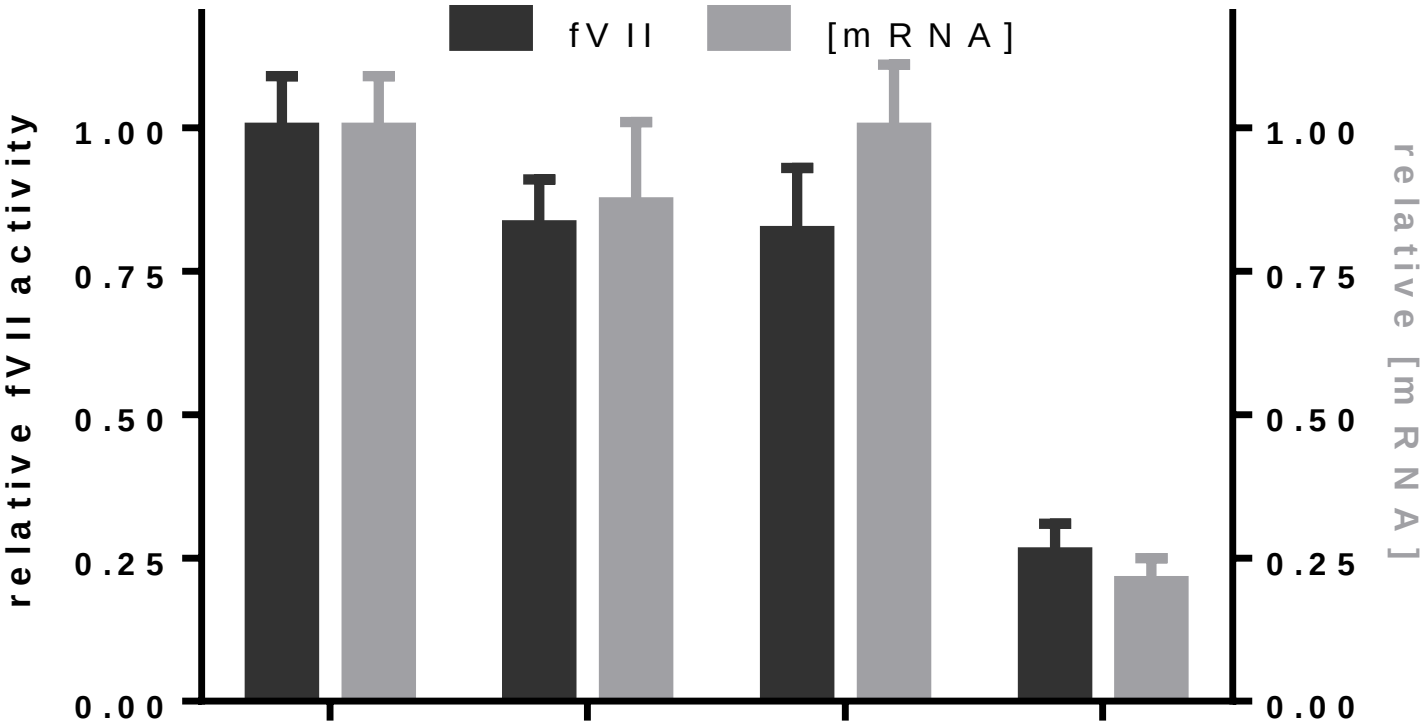


Protease-labile siRNA delivery vehicles

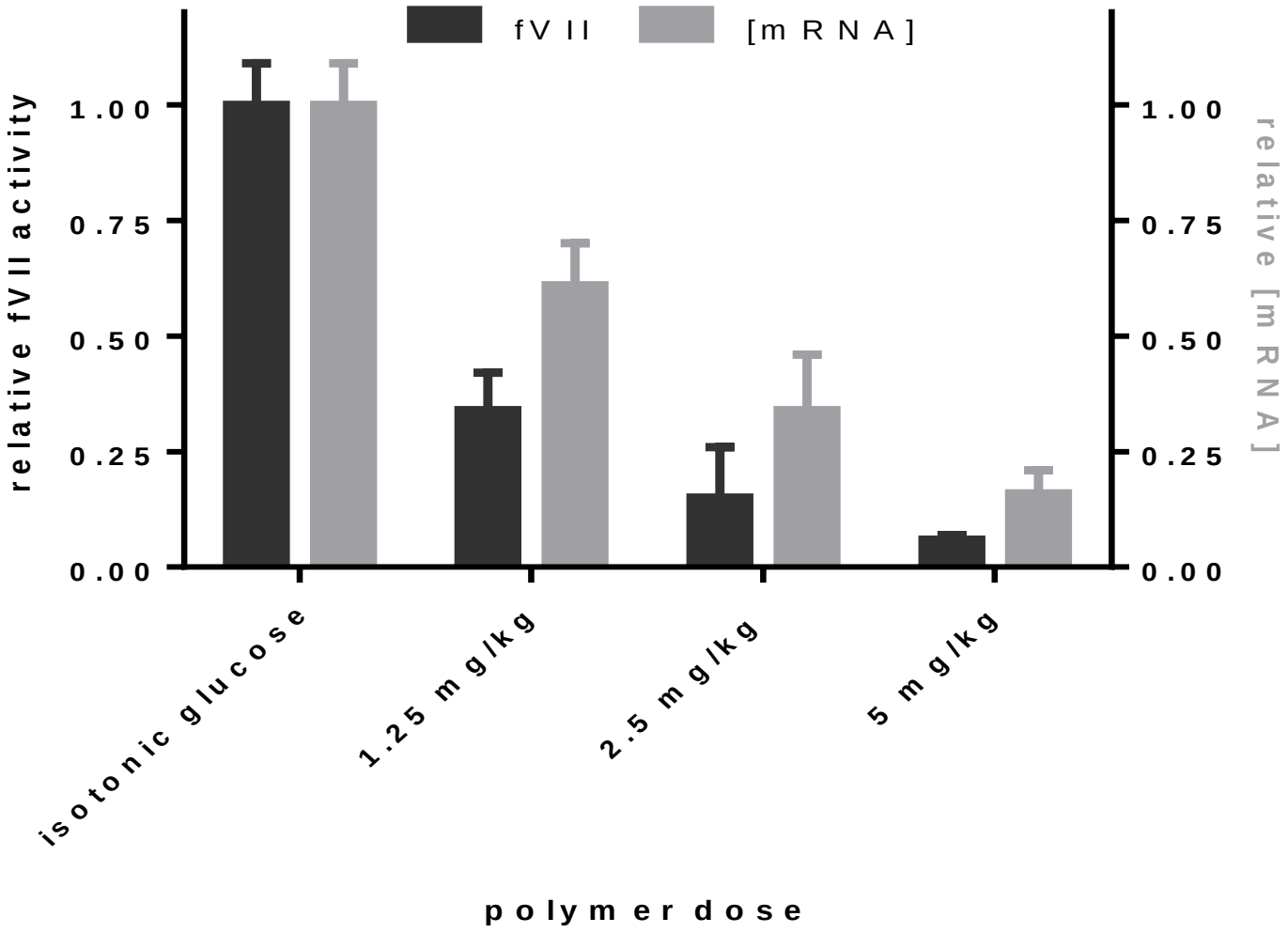


	Vehicle control	Non-targeted	D-amino acids	L-amino acids
siRNA	-	fVII siRNA	fVII siRNA	fVII siRNA
PEG	-	FCit-PEG	fcit-PEG	FCit-PEG
NAG	-	-	acit-NAG	ACit-NAG

0.25 mg/kg siRNA formulated with 2.5 mg/kg polymer. Tail vein injection in mice, n=4

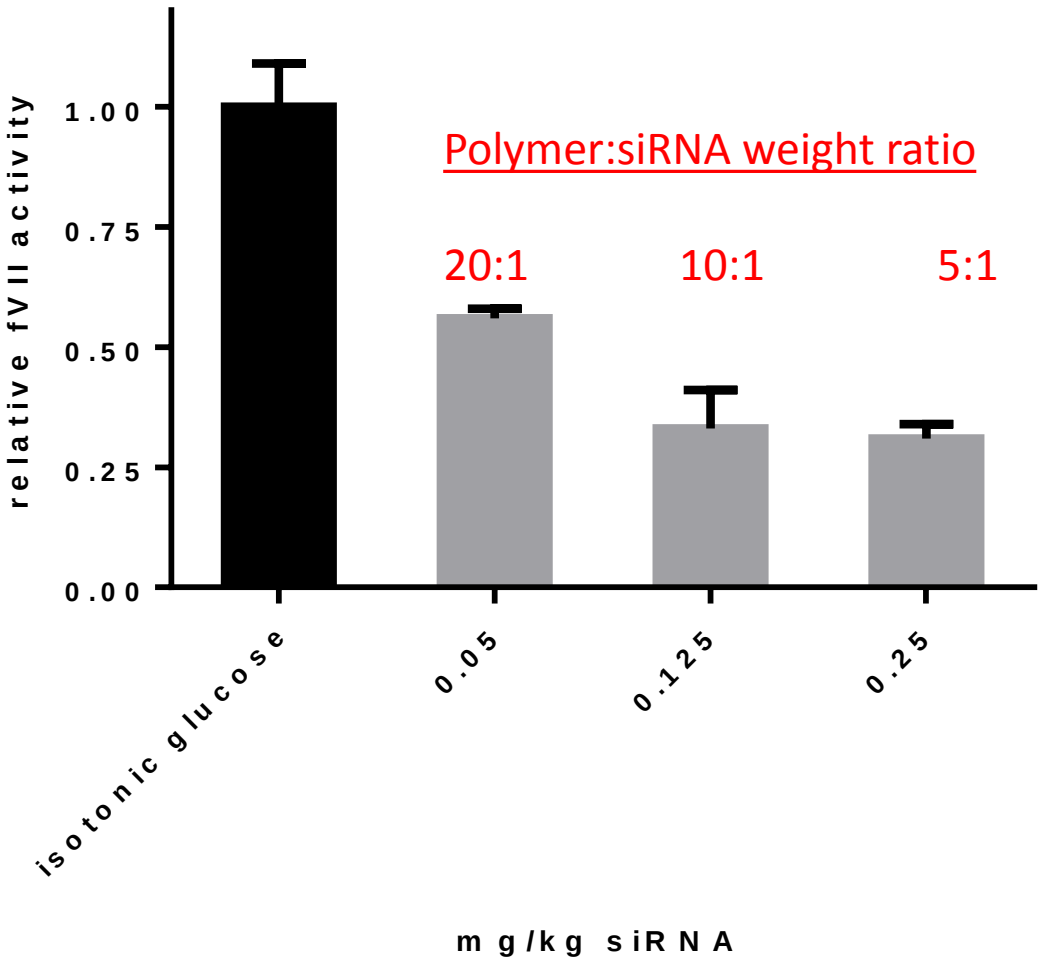
Dose titration

10:1 polymer to siRNA ratio



ED_{50} ≈1.25 with respect to polymer

Dose titration of siRNA with constant polymer dose of 1.25 mg/kg.



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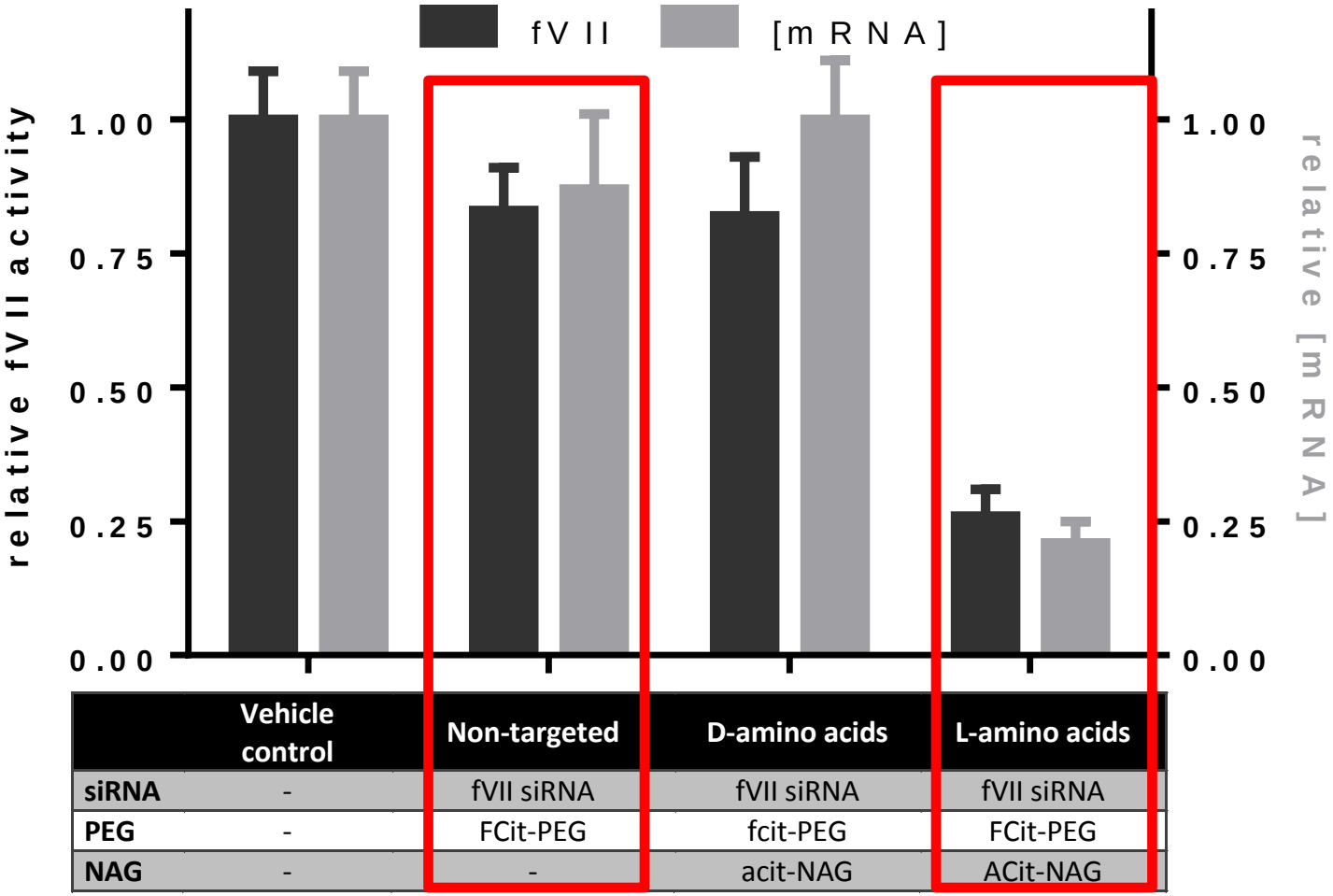

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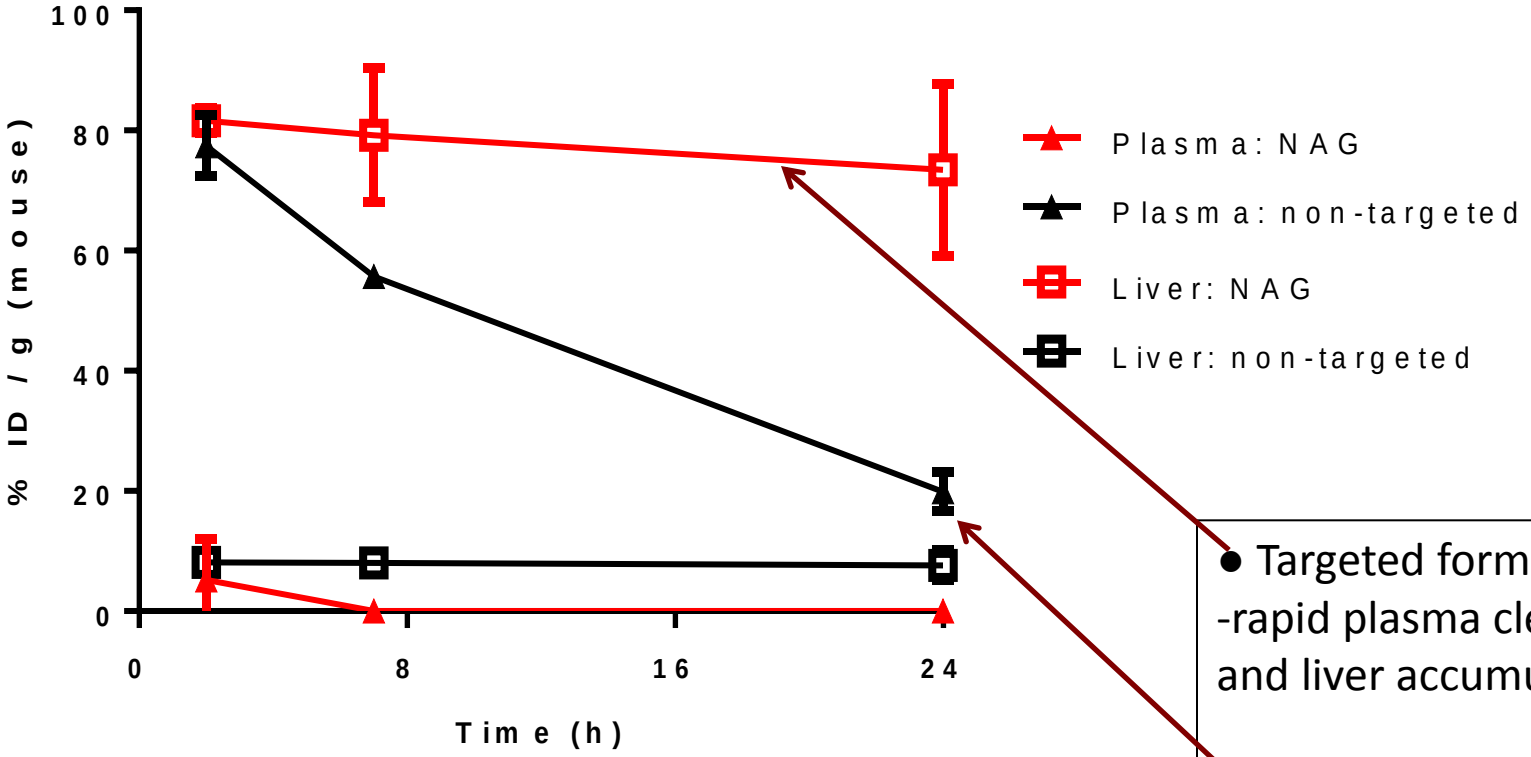
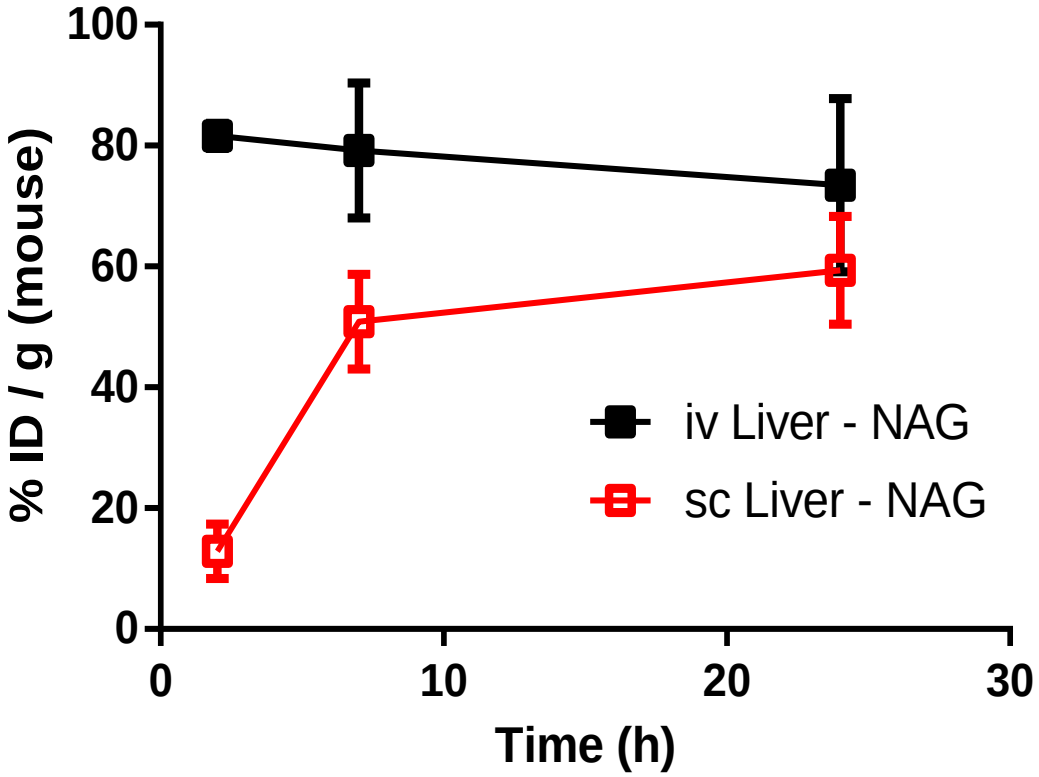
Plasma clearance and liver accumulation of targeted (NAG) and untargeted (PEG) formulations



- Targeted formulation:
  -rapid plasma clearance, and liver accumulation
- Untargeted formulation:
  -relatively long circulating

5 mg/kg of I125 radiolabeled polymer. IV injection, n=3

Liver accumulation:
subcutaneous vs intravenous administrations



- Targeted formulation accumulates in liver approximately 8 h postinjection
- 24 h liver accumulation similar to iv injection

5 mg/kg of I125 radiolabeled polymer, n=3

Subcutaneous Primate Formulation

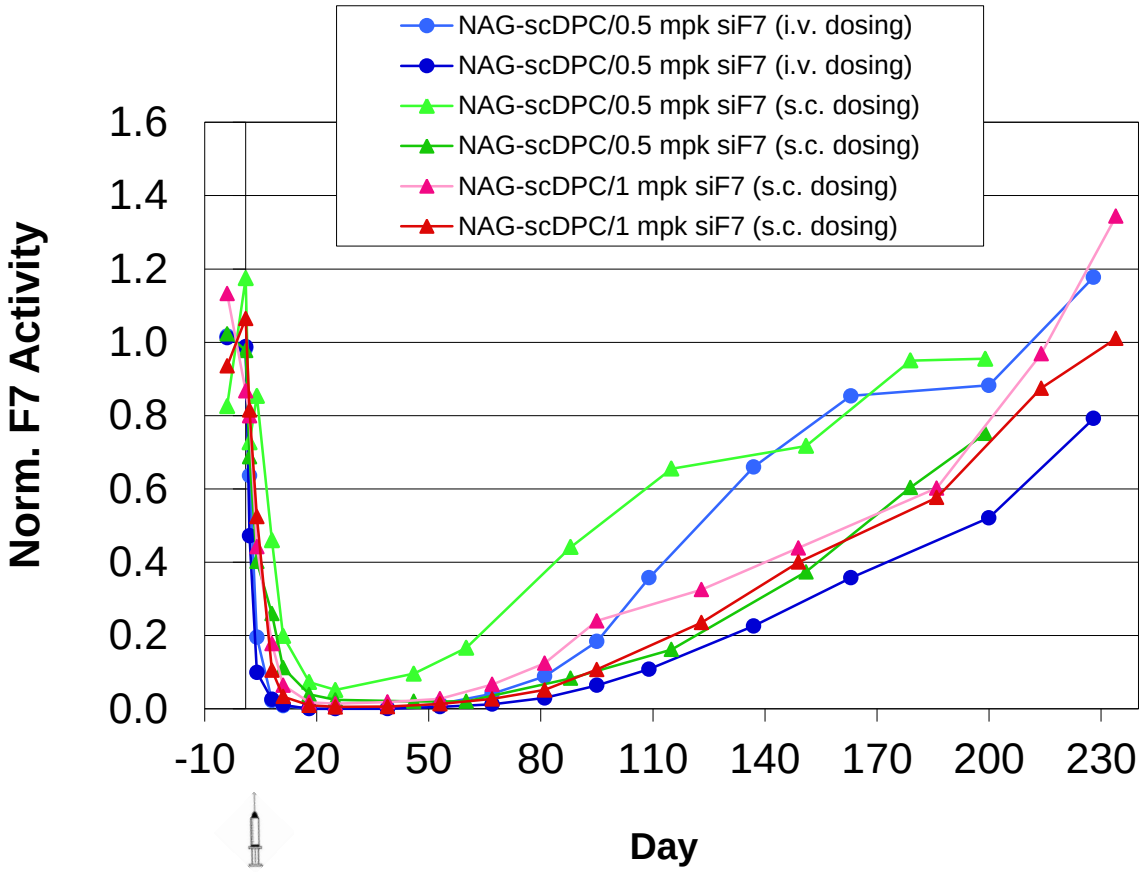
- Modify with protease-labile reagents
- Conjugate siRNA and polymer
- Lyophilize purified formulation and reconstitute at 20 mg/mL siRNA in isotonic glucose
- Subcutaneous injection (300 µl)

- collect serum for fVII and clinical markers

Efficacy/toxicity of NAG-scDPCs in NHPs

Target: Coagulation Factor 7 (F7)

- **Highly efficacious**
  - Single dose
  - i.v. and s.c. efficacy
  - >99% F7 KD at 1 mg/kg siRNA
  - Slightly slower onset when administered s.c. as expected
  - **>3 month duration of effect (>80% KD) after a single dose**



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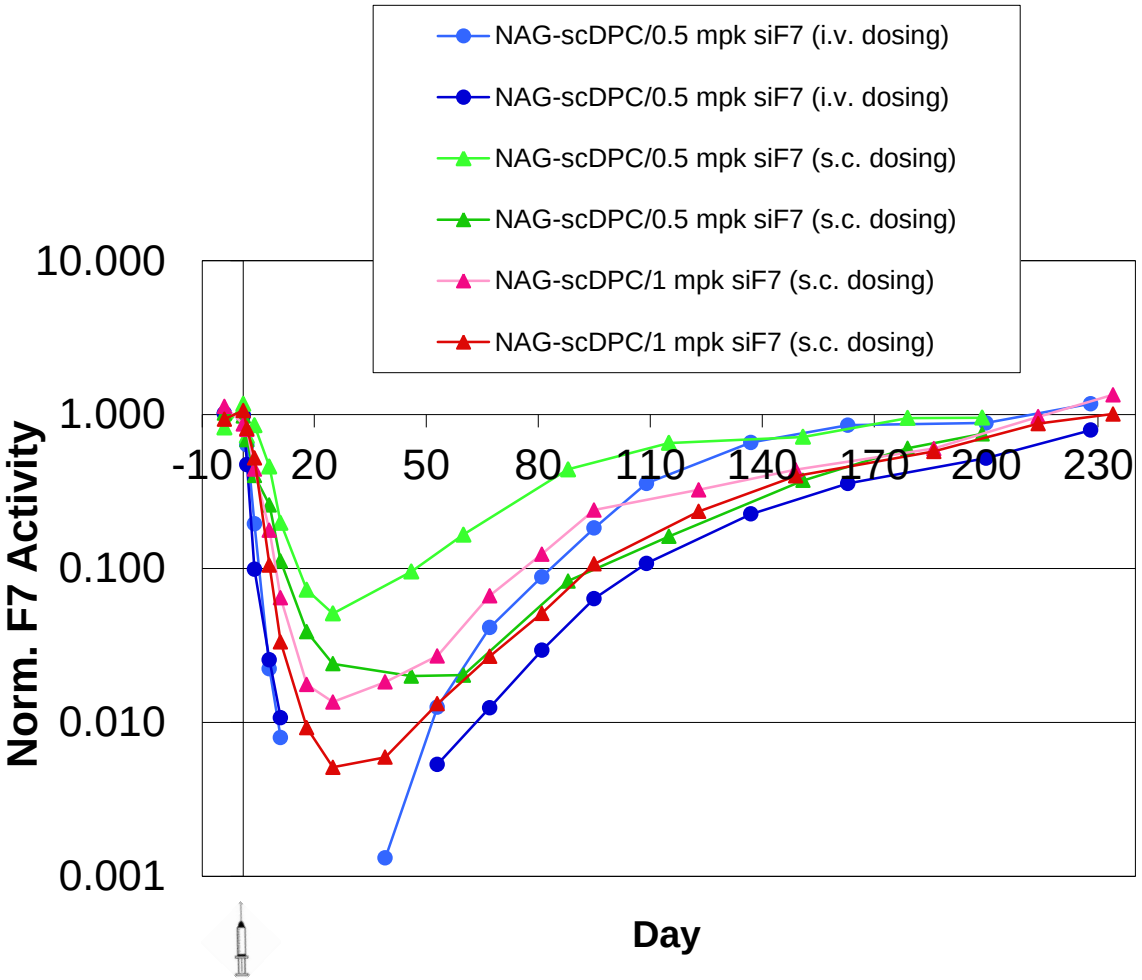

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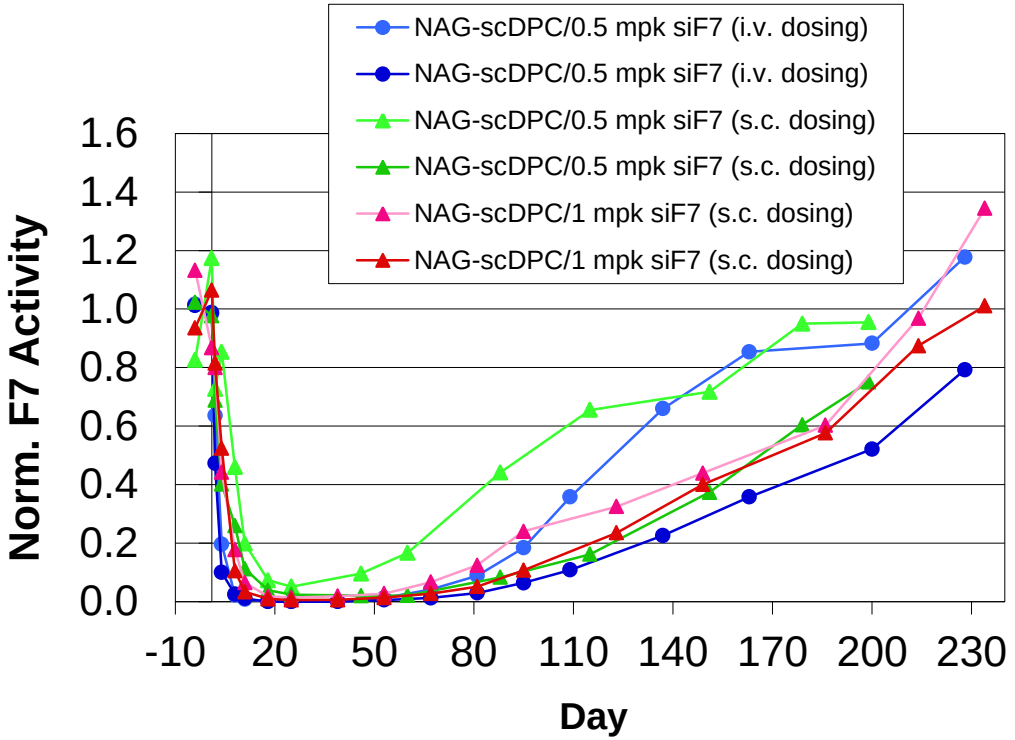
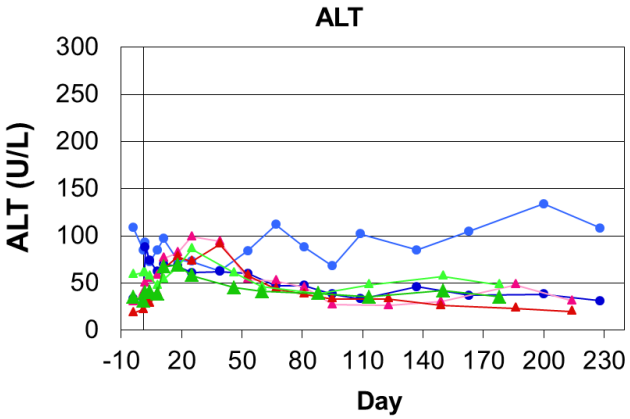
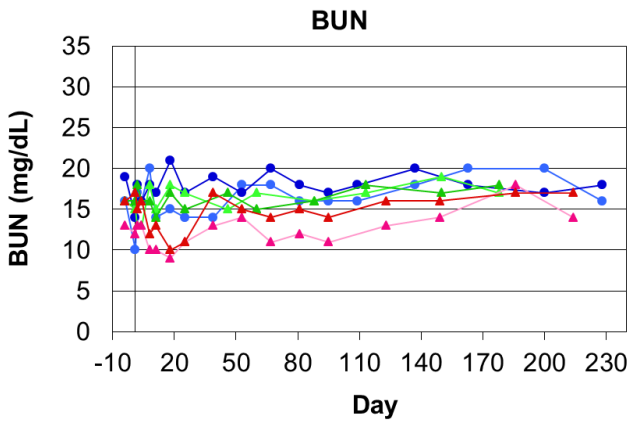


- **Toxicity not observed**
  - No changes in clin chem markers
  - No changes in hematology





Conclusions

- Novel protease-sensitive masking reagent has been developed for DPCs
  - DPC endosomolytic activity only activated after proteolysis of masking reagent
  - Very stable in serum with long-circulation times when untargeted
- DPC masked with protease-sensitive reagents are fully targetable
  - Attachment of NAG ligand results in hepatocyte targeting
  - Attachment of other ligands for targeting extra-hepatic tissues is possible
- Amenable to subcutaneous administration (scDPC)
  - Highly efficient siRNA delivery in mice and NHPs (sub mg/kg, single dose)
  - Deep target gene knockdown with very long duration of effect as demonstrated in NHPs with single dose
- Circulation times of non-targeted vehicles suggest that vehicles may be targeted to tissues other than liver

Contributors

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