3rd RNAi-Based Therapeutics Summit

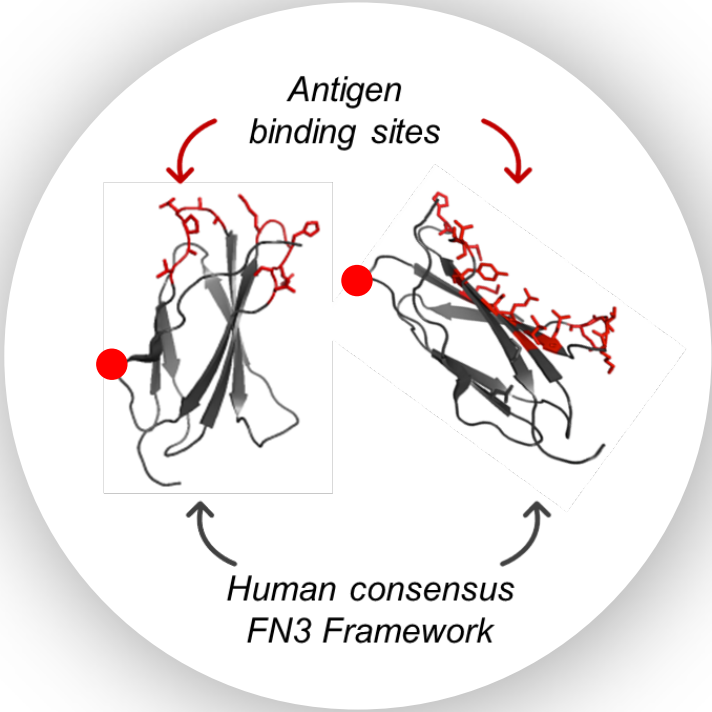
Zhanna Druzina, PhD
Sr. Director, Protein Engineering

Aro BIOTHERAPEUTICS

Centyrin overview

Rapid, iterative, flexible and chemically tractable platform for RNA drug targeting

- Proprietary antigen binding platform
- Built on a consensus human Tenascin C FN3 framework
- Exceptional stability and solubility
- ~1/15 size of standard monoclonal antibodies
- Readily expressed in E. Coli as multi-specific proteins
- Facile site-specific covalent conjugation to drug payloads
- No disulfide bonds; no glycosylation
- Not a known autoantigen



Ideal properties for targeted delivery of oligonucleotide therapies

● = Drug Conjugate Site

Centyrin Oligonucleotide Platform

1

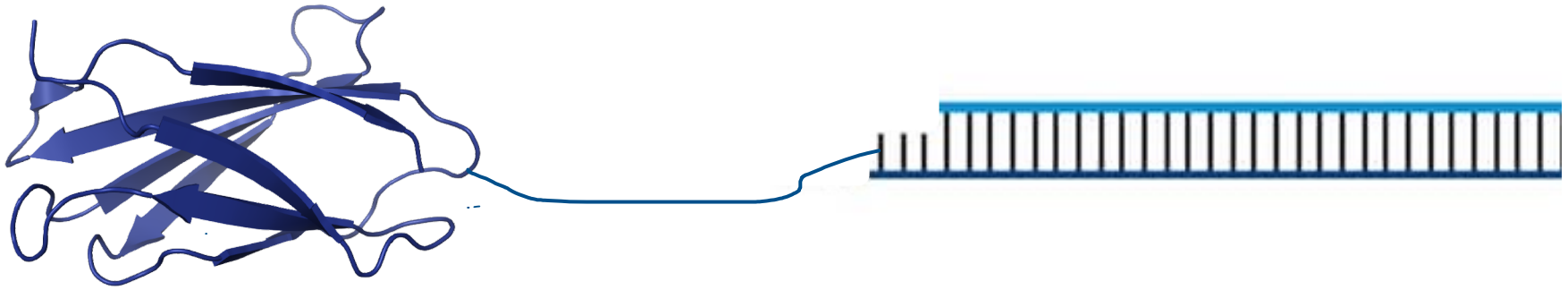
Proprietary
Tissue Targeting
Centyrin

2

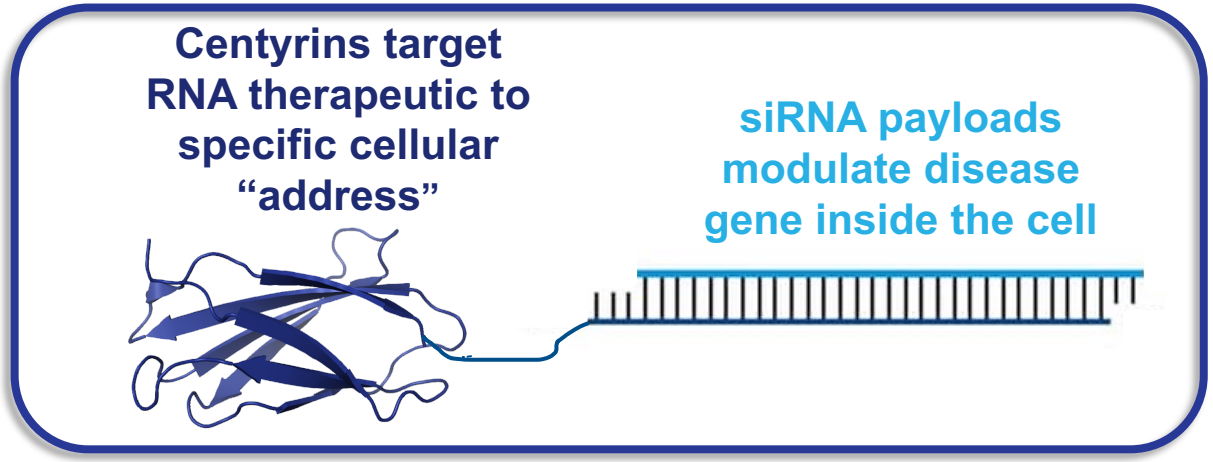
Established
Bioconjugation
Chemistry

3

Proprietary
Oligonucleotides



Aro's Centyrin platform enables tissue-targeted delivery of RNA medicines, unlocking vast therapeutic potential



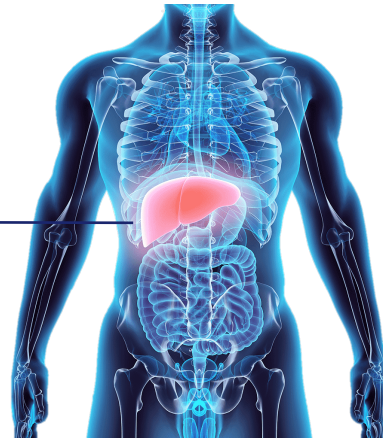
Current FDA approved RNA medicines target genes in the liver

Tegsedi® (inotersen) injection 284 mg/1.5 mL

onpattro® (patisiran) lipid complex injection 10 mg/5 mL

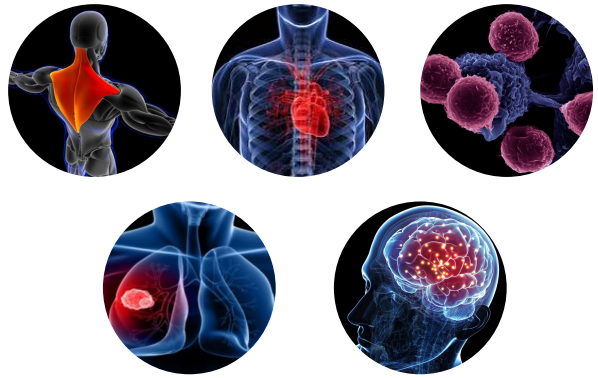
GIVLAARI® (givosiran) injection for subcutaneous use 189 mg/mL

OXLUMO® (lumasiran) for injection 94.5 mg/0.5 mL



Receptor mediated targeting enables tissue specific delivery of RNA medicines to many tissues/cell types:

- Skeletal muscle
- Cardiac muscle
- Immune cells
- Tumor tissue
- Additional tissues



Aro's Centyrin Discovery Engine Enables Rapid Creation of New Therapeutic Candidates

~6 Months from Target to Centyrin

In vivo POC

Target Selection

Identify cell surface protein(s) to drive cell-selective targeting

Parallel screens vs cyno antigen

Strong Binders

In vitro CIS display screening to identify high affinity binders from library ~10^{13}

Efficient Internalizers

Introduction of single cysteine & HTP creation of Centyrin-drug conjugates

Internalization Assay

Select Centyrins with best properties for efficient receptor mediated internalization

Secondary screens

Epitope

Specificity

Expression

Immunogenicity

Good Manufacturing Properties

Stability

Scale up in E coli

Homogeneous site – specific conjugation to drug payload

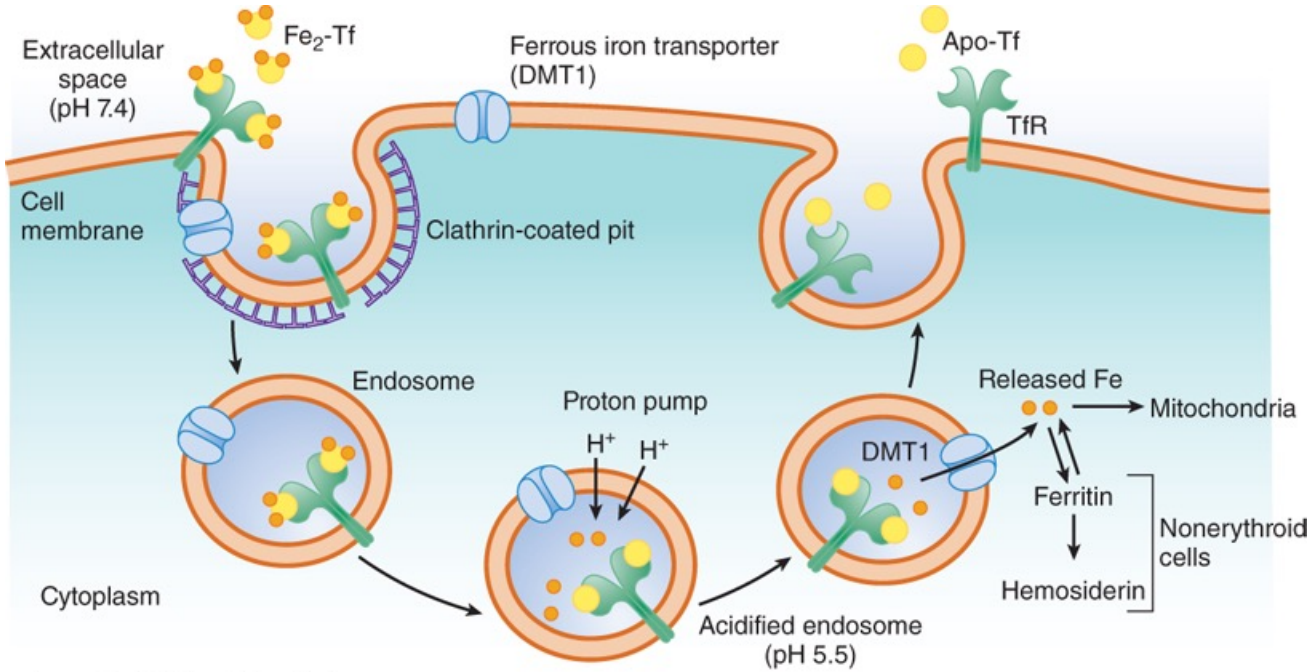
Disease models

Cyno studies

Final Lead Selection

Aro is developing an industry-leading position in targeting CD71

Customized CD71 Centyrins for different tissues to address a broad set of diseases



- Essential and ubiquitously expressed receptor responsible for iron transport into cells
- Efficient internalization on muscle, tumor cells, proliferating immune cells and endothelial cells at blood brain barrier
- We have generated a large diversity of CD71 Centyrins to enable efficient and customized targeting of various CD71+ cell types
- Demonstrated efficient targeting of CD71 Centyrin-oligo conjugates
  - Monovalent receptor binding does not block transferrin binding or CD71 surface expression
  - No evidence of agonist effect

Aro's Centyrin Discovery Engine Enables Rapid Creation of New Therapeutic Candidates

~6 Months from Target to Centyrin

In vivo POC

Target Selection

Identify cell surface protein(s) to drive cell-selective targeting

Strong Binders

In vitro CIS display screening to identify high affinity binders from library ~10^{13}

Parallel screens vs cyno antigen

Efficient Internalizers

Introduction of single cysteine & HTP creation of Centyrin-drug conjugates

Internalization Assay

Select Centyrins with best properties for efficient receptor mediated internalization

Secondary screens

Epitope

Specificity

Expression

Immunogenicity

Good Manufacturing Properties

Stability

Expression/

Scale up in E coli

Homogeneous site – specific conjugation to drug payload

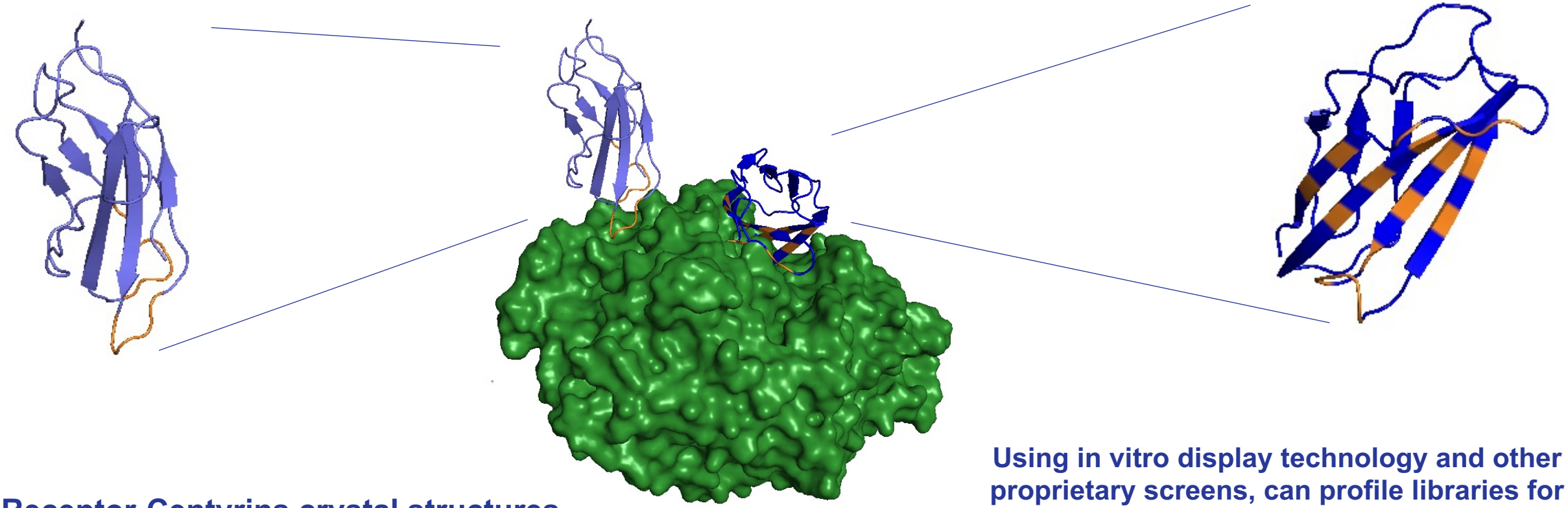
Disease models

Cyno studies

Final Lead Selection

Centyrins and mAbs have similar target affinity and specificity

Ability to rapidly and flexibly profile vast Centyrin libraries is a competitive advantage



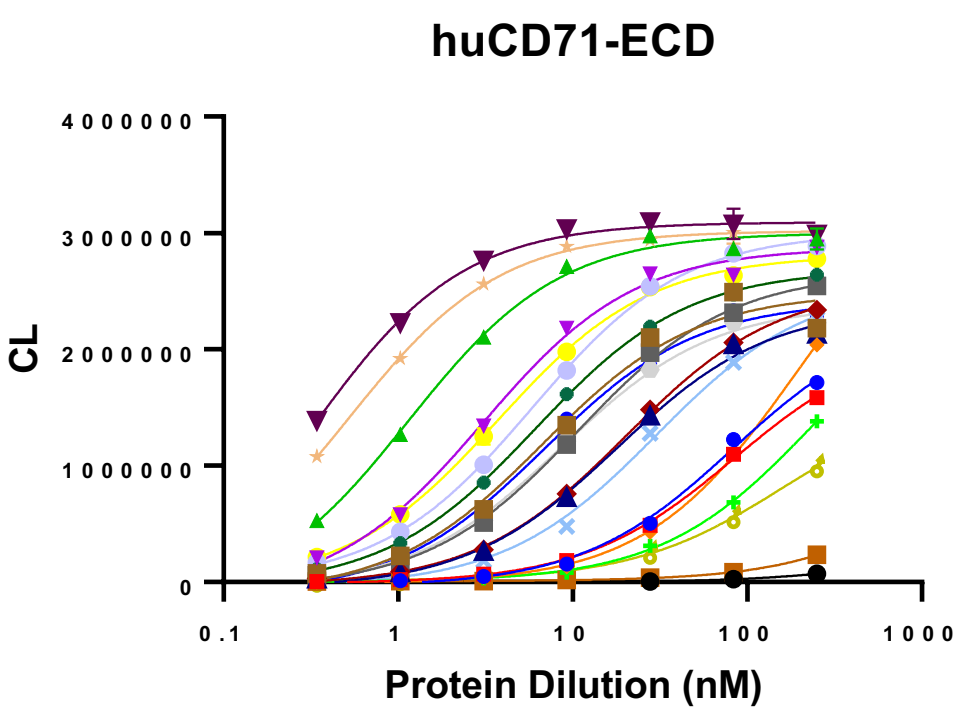
Receptor-Centyrins crystal structures

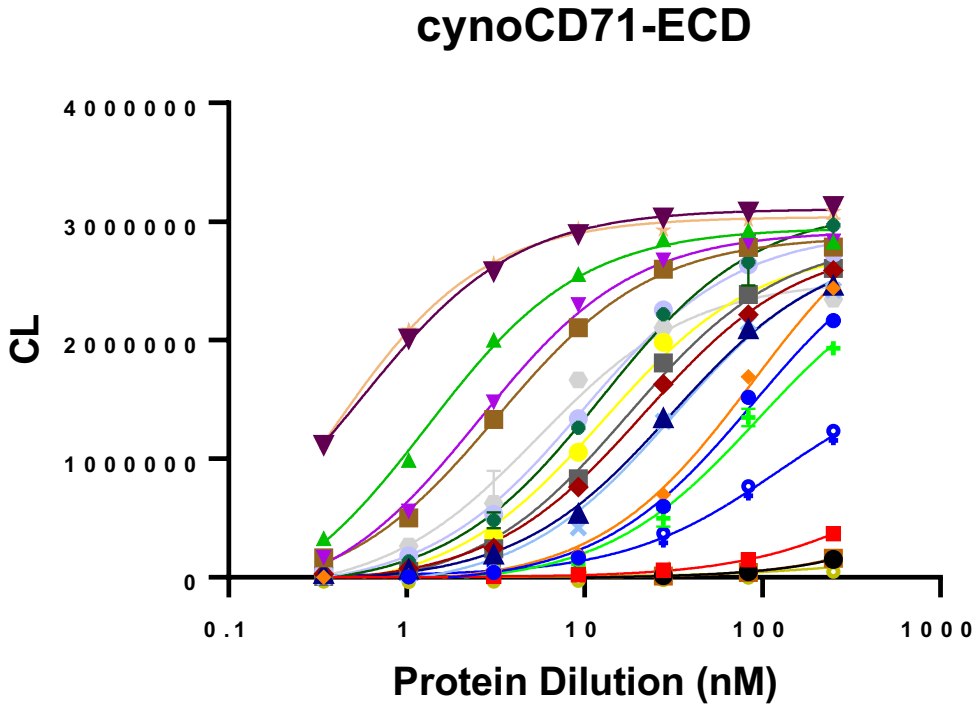
- ~20 Centyrin residues make direct contacts with Receptor
  - ~900 Å^2 buried on binding, similar to Fab-antigen
- Cell-Free Screening Technology
- High 10^{13} diversity

Using in vitro display technology and other proprietary screens, can profile libraries for

- Binders with varying affinity properties
- Binders with different epitopes
- Binders with different internalization characteristics
- Binders competitive / non-competitive with ligand

Multiple cross-reactive binders to Human and Cyno CD71with varying affinity properties

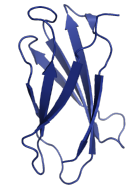




HuCD71-ECD - Extra Cellular Domain of hu TFR

Aro's Centyrin Discovery Engine Enables Rapid Creation of New Therapeutic Candidates

~6 Months from Target to Centyrin



In vivo POC

Target Selection

Identify cell surface protein(s) to drive cell-selective targeting

Strong Binders

In vitro CIS display screening to identify high affinity binders from library ~10^{13}

Parallel screens vs cyno antigen

Efficient Internalizers

Introduction of single cysteine & HTP creation of Centyrin-drug conjugates

Internalization Assay

Select Centyrins with best properties for efficient receptor mediated internalization

Secondary screens

Epitope

Specificity

Immunogenicity

Good Manufacturing Properties

Stability

Expression/

Scale up in E coli

Homogeneous site – specific conjugation to drug payload

Disease models

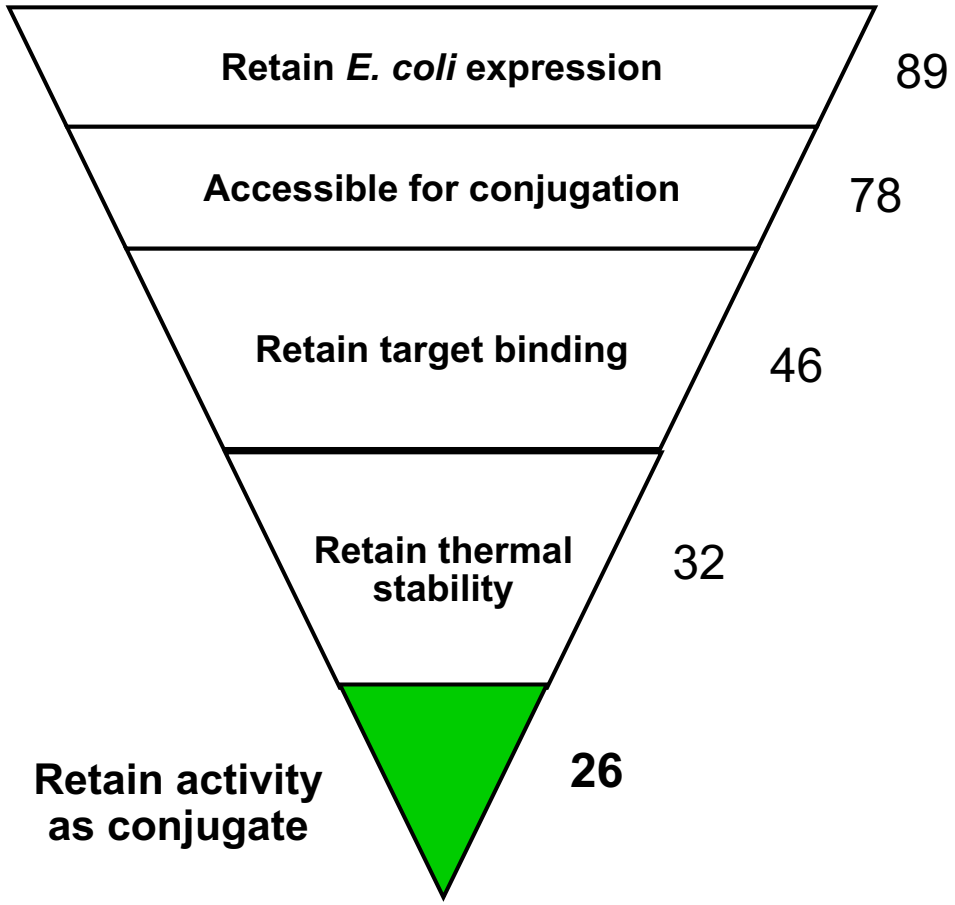
Cyno studies

Final Lead Selection

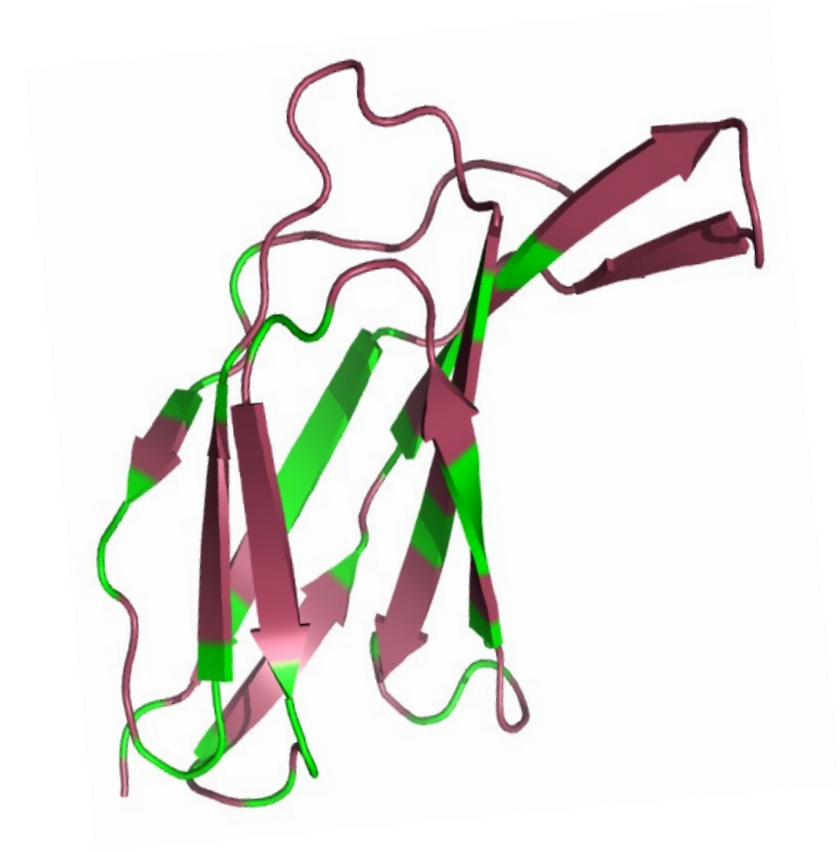
Optimized Sites for Cysteine Conjugation Identified

Adaptable to orthogonal conjugation chemistries

All 96 positions on the Centyrin scaffold were individually mutated to cysteine

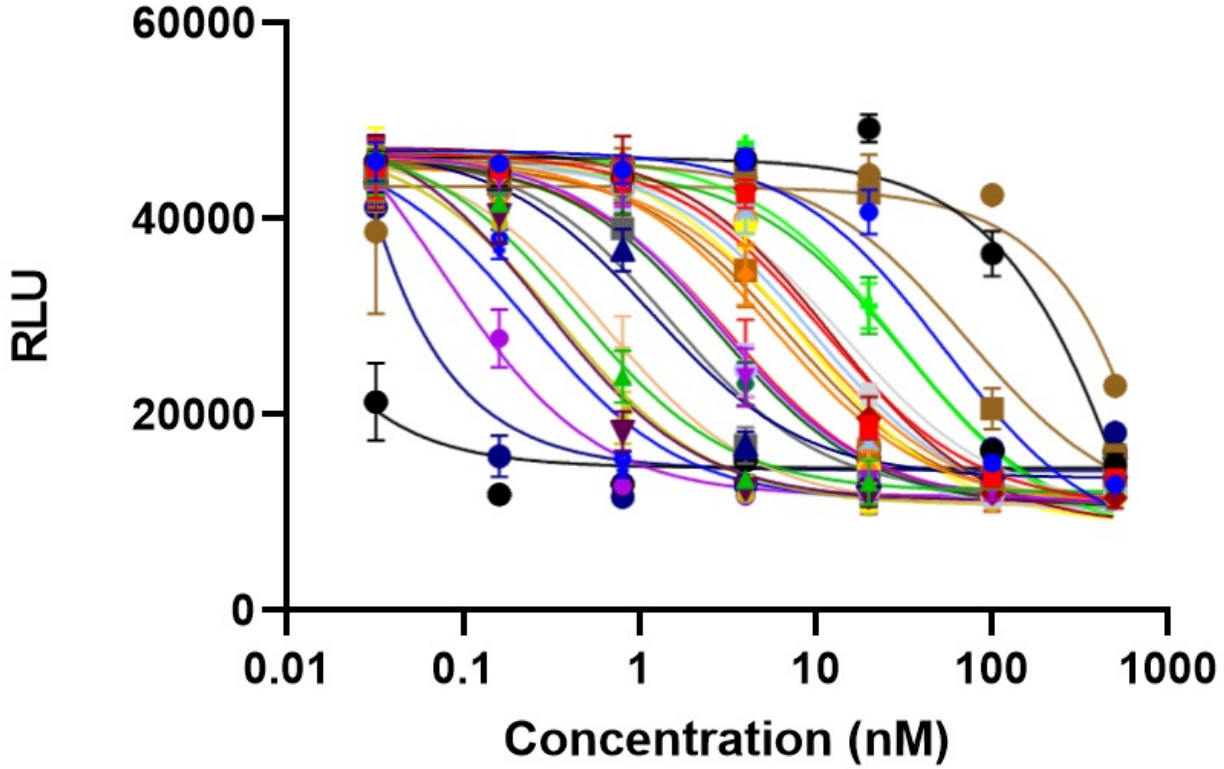


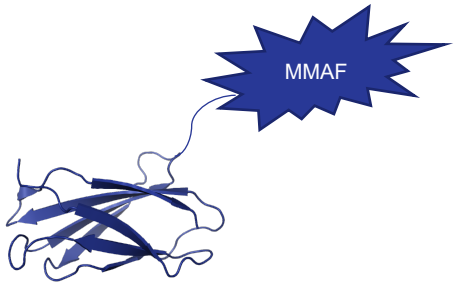
Green are tolerated positions for cysteine conjugation



Goldberg et al., PEDS 29 (12) 2016, 563-572

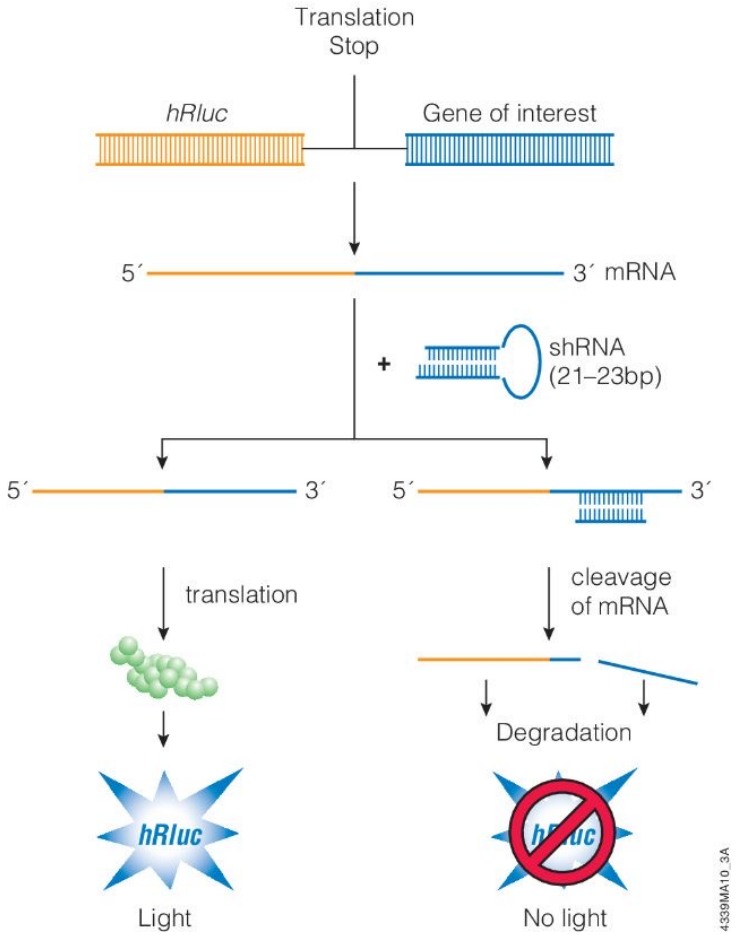
HTP Internalization assays demonstrate efficient toxin delivery

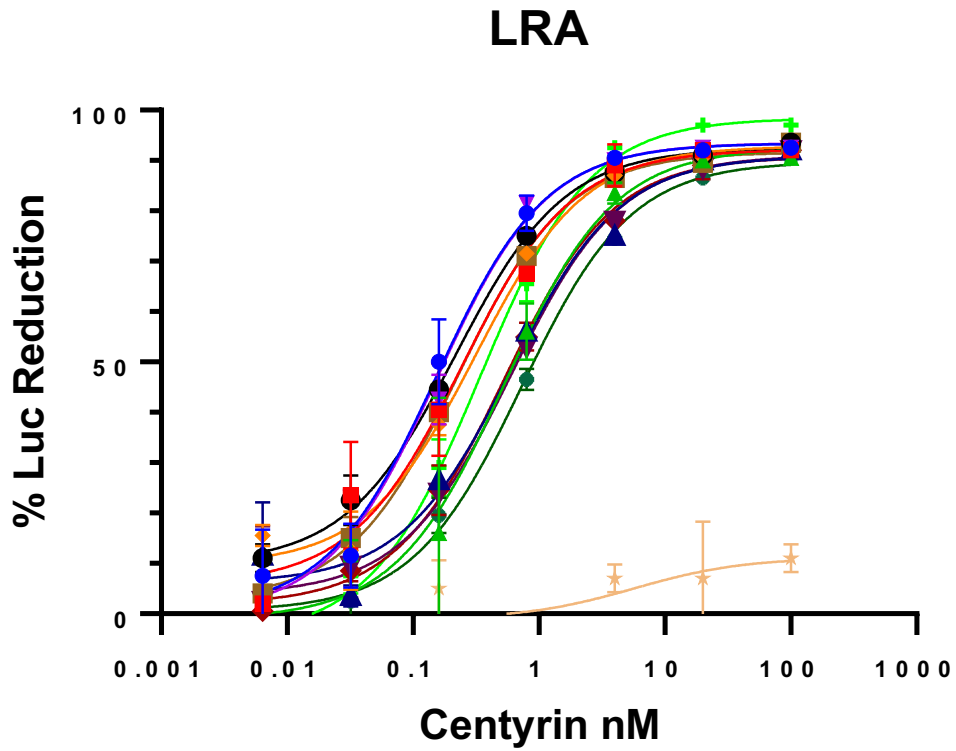




Cell Titer Glow™ Titer Internalization assay of CD71-centyrin-MMAF conjugate

Centyrin – siRNA conjugates with sub-nM potency identified in Luciferase Reporter Assay

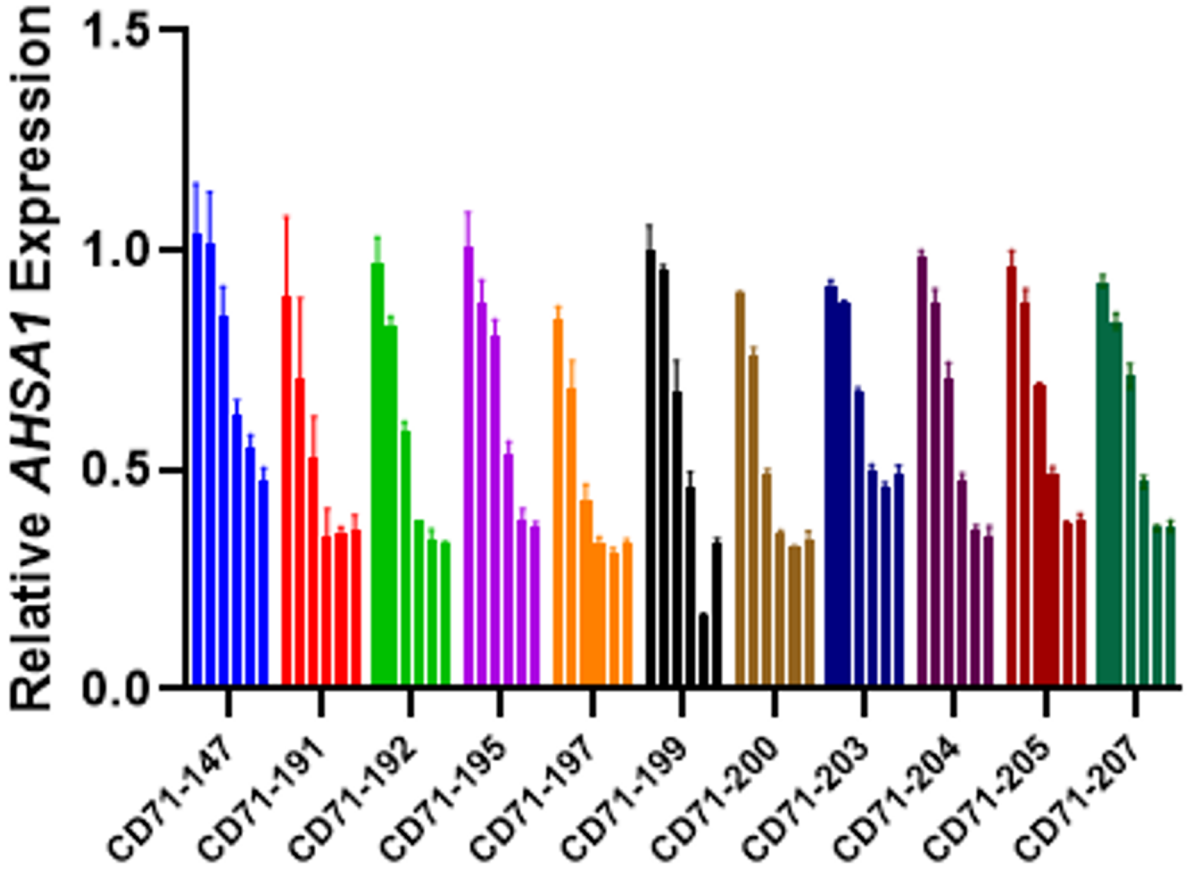




Luciferase Reporter Assay to screen for potent Centyrin-siRNA conjugates in gene knockdown

Efficient gene knockdown in human cells observed by qPCR

Centyrin-siRNA (*AHSA1*) conjugates



Aro's Centyrin Discovery Engine Enables Rapid Creation of New Therapeutic Candidates

~6 Months from Target to Centyrin

In vivo POC

Target Selection

Identify cell surface protein(s) to drive cell-selective targeting

Strong Binders

In vitro CIS display screening to identify high affinity binders from library ~10^{13}

Parallel screens vs cyno antigen

Efficient Internalizers

Introduction of single cysteine & htp creation of Centyrin-drug conjugates

Internalization Assay

Select Centyrins with best properties for efficient receptor mediated internalization

Secondary screens

Epitope

Specificity

Immunogenicity

Good Manufacturing Properties

Stability

Expression/

Scale up in E coli

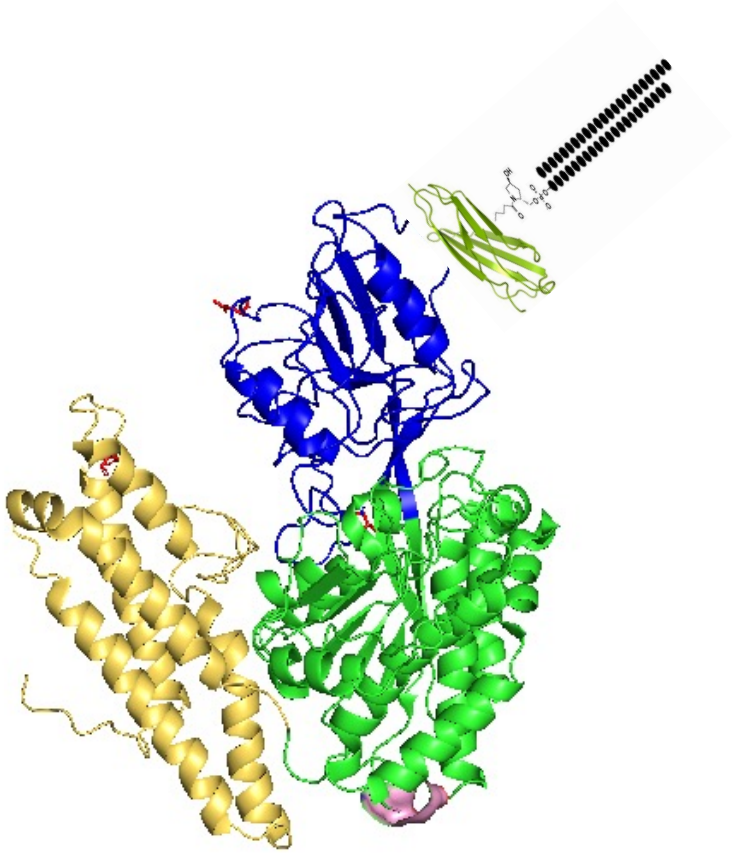
Homogeneous site – specific conjugation to drug payload

Disease models

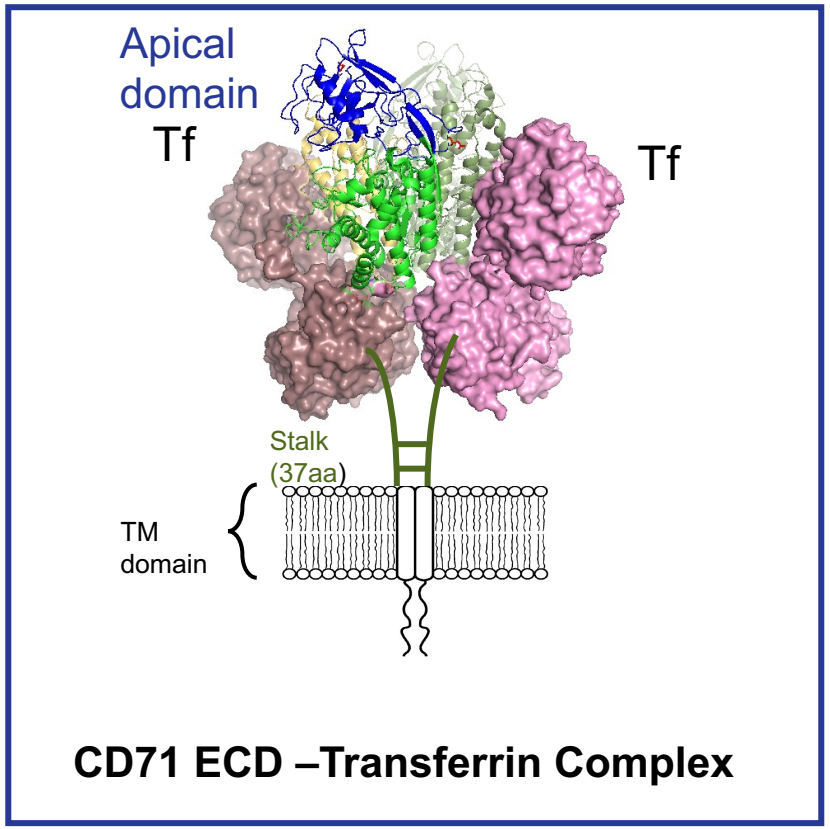
Cyno studies

Final Lead Selection

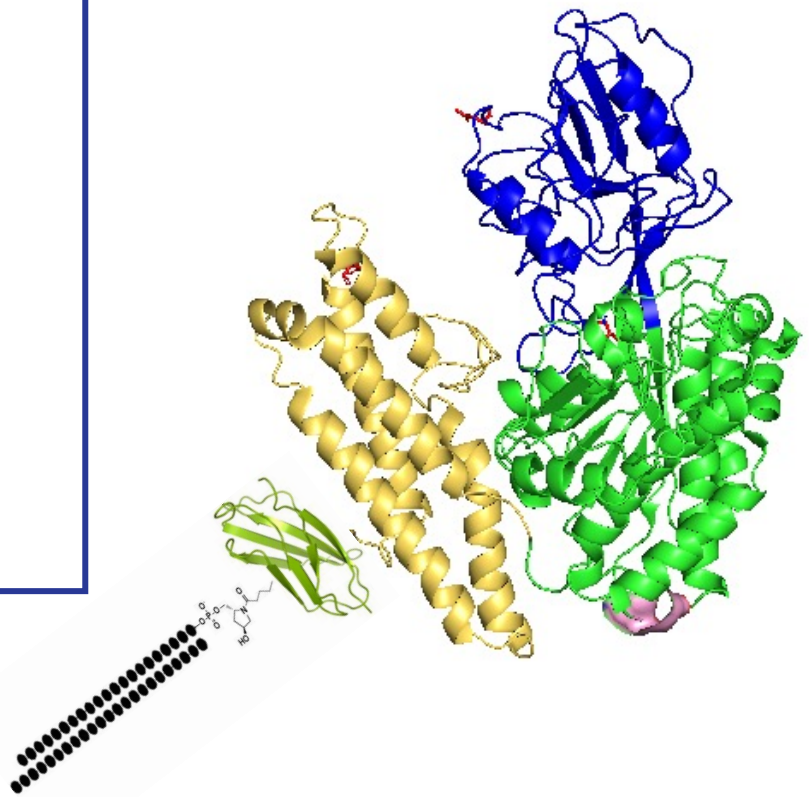
CD71 Targeting Centyrins: Selecting apical and non-apical binders



Apical Binding Centyrins

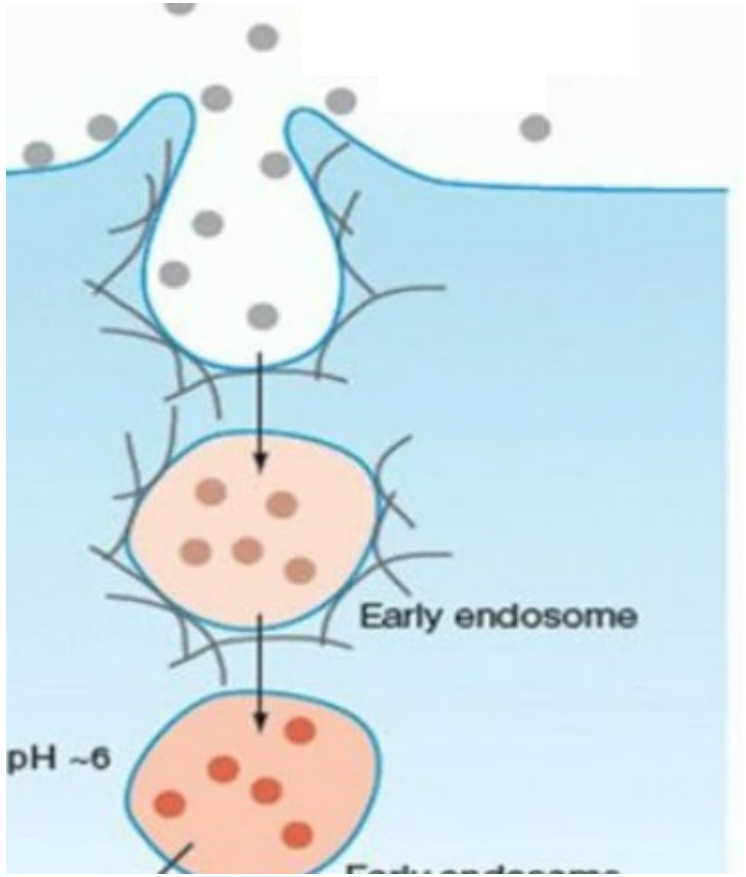


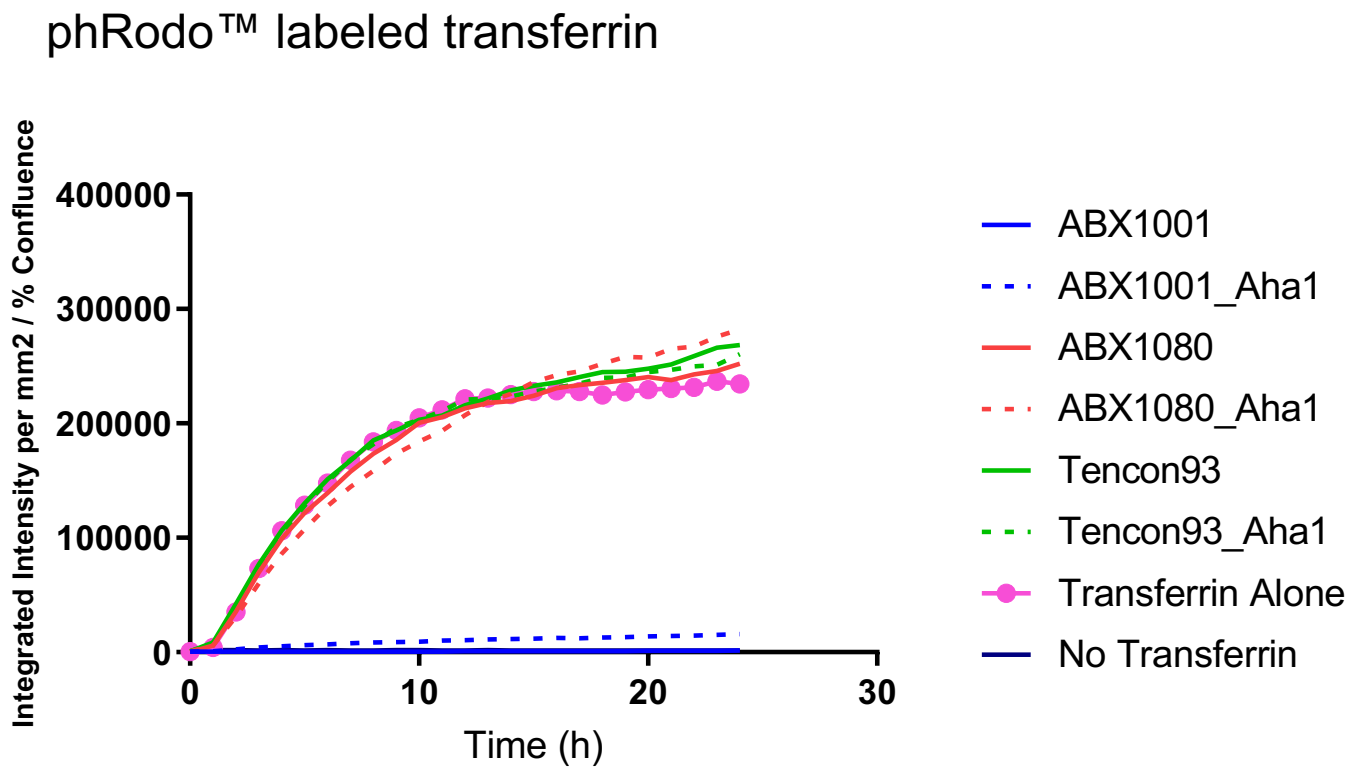
CD71 ECD –Transferrin Complex



Non-Apical Binding Centyrins

Selected CD71 Centyrins do not compete for transferrin uptake





Human SKBR3 cells

CD71 Centyrin has low immunogenicity potential and is highly selective for huCD71

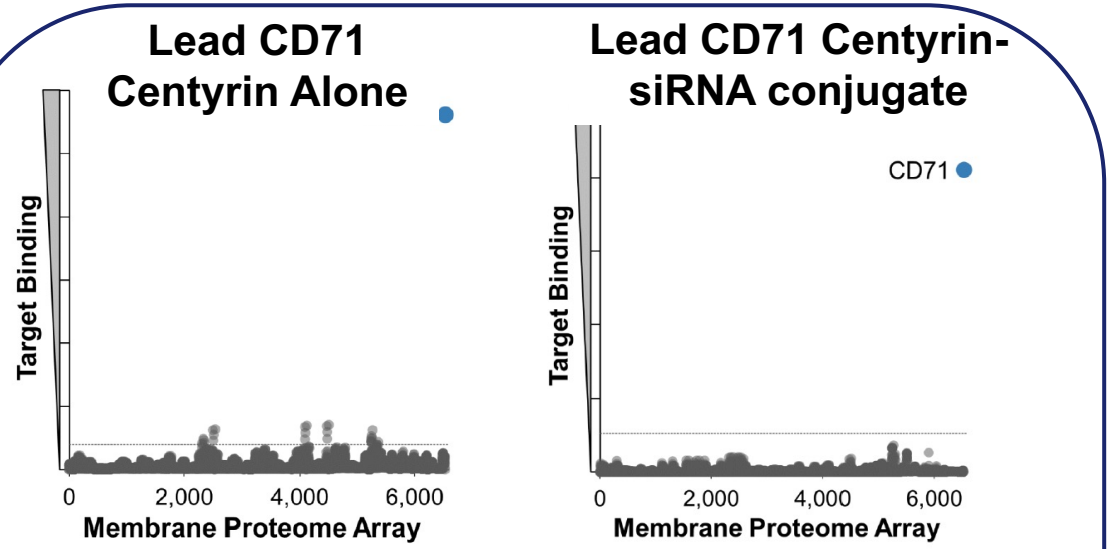
Low immune response index

Protein	Immune Response Index (RI)
PPD (Positive Control 1)	61.39
KLH (Positive Control 2)	32.05
Lead CD71 Centyrin	**0.19**

Most approved biologic drugs have RI from 0-1

- T cell activation assay
- 20 donor PBMC samples were HLA typed
- Allele distribution frequency of HLA class II resembled the global population
- T cell activation assessed after 7 days

PROIMMUNE
www.proimmune.com



- Membrane protein array (MPA) profiles the specificity of ligands that target human membrane proteins and identifies off-target effects
- CD71 was the only confirmed target for the lead CD71 Centyrin and Centyrin-siRNA conjugate

integral molecular

Aro's Centyrin Discovery Engine Enables Rapid Creation of New Therapeutic Candidates

~6 Months from Target to Centyrin

Target Selection

Identify cell surface protein(s) to drive cell-selective targeting

Strong Binders

In vitro CIS display screening to identify high affinity binders from library ~10^{13}

Parallel screens vs cyno antigen

Efficient Internalizers

Introduction of single cysteine & htp creation of Centyrin-drug conjugates

Internalization Assay

Select Centyrins with best properties for efficient receptor mediated internalization

Secondary screens

Epitope

Specificity

Immunogenicity

Good Manufacturing Properties

Stability

Expression/

Scale up in E coli

Homogeneous site – specific conjugation to drug payload

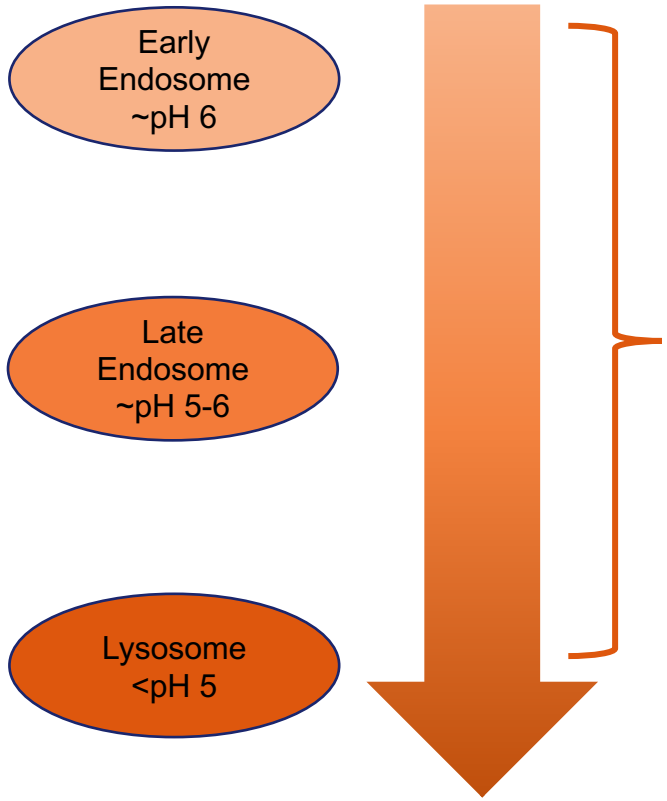
In vivo POC

Disease models

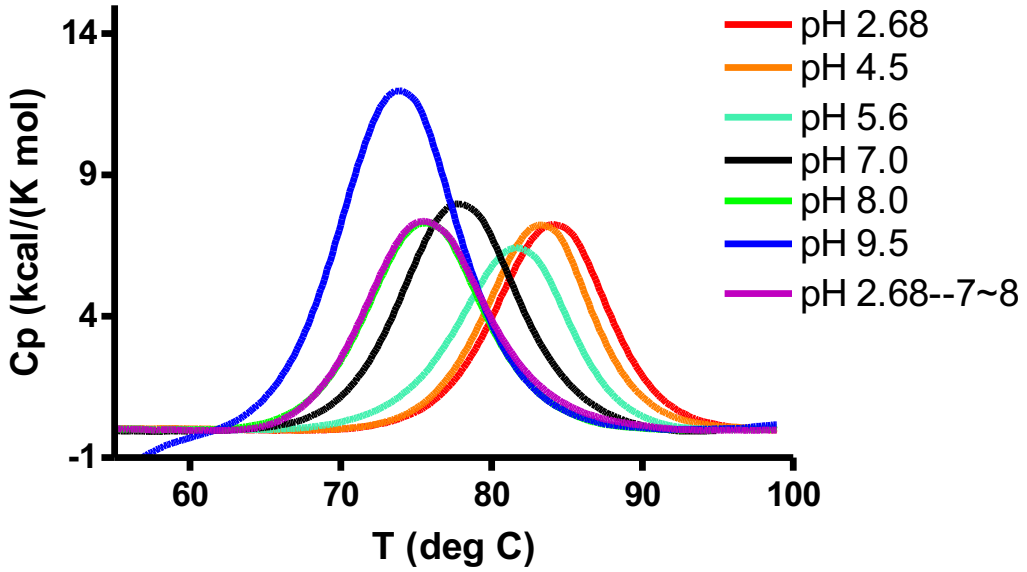
Cyno studies

Final Lead Selection

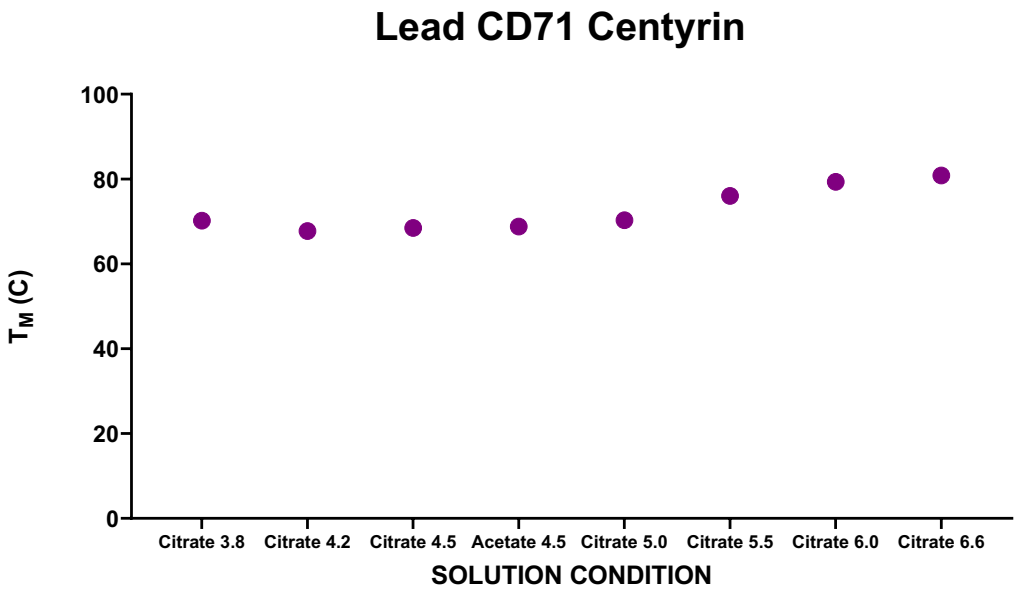
Centyrins remain folded at early endosome pH

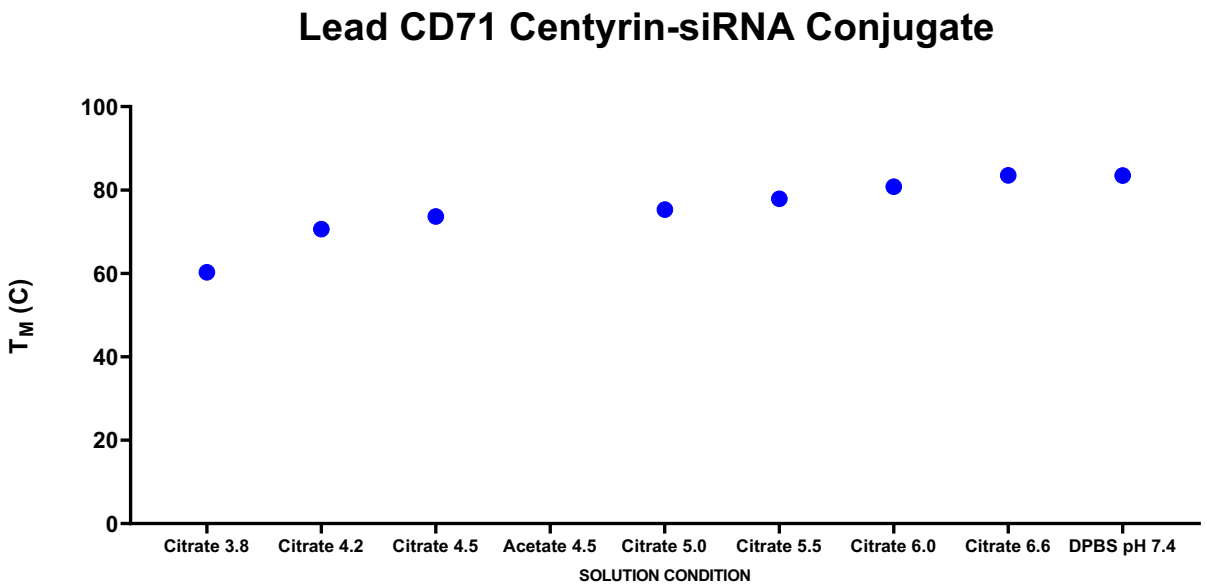


pH dependent melting temperatures support potential for Centyrin stability from early endosome to lysosome



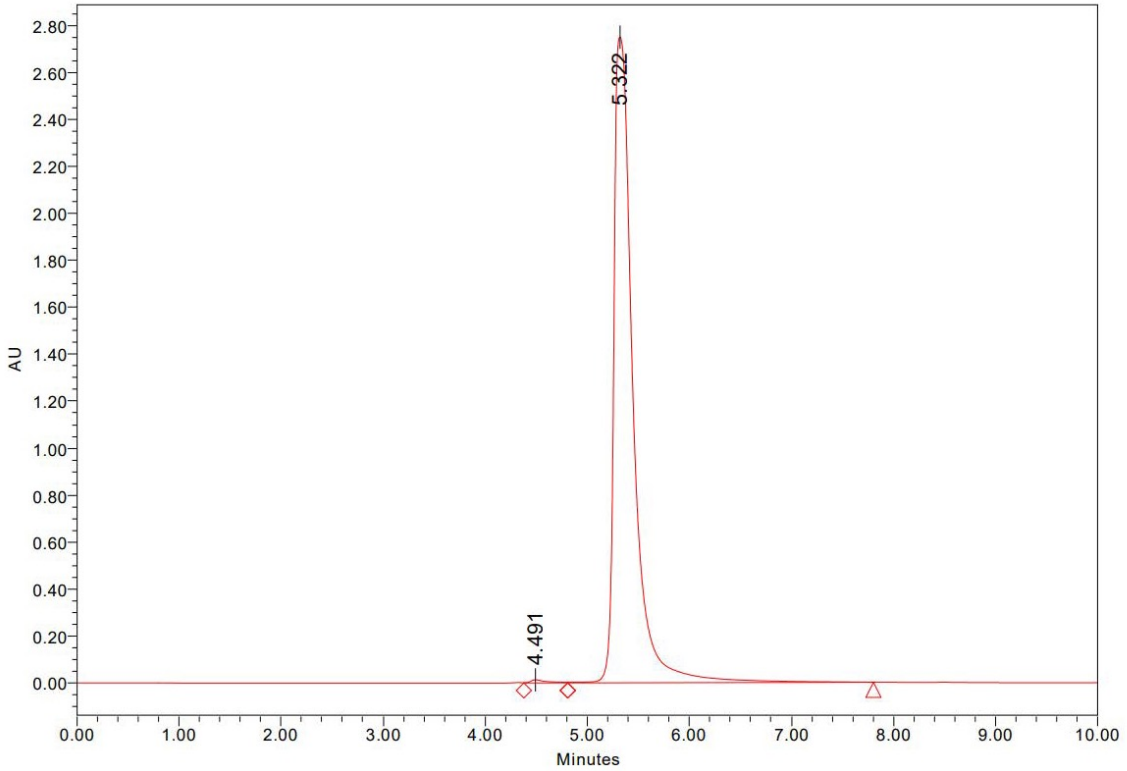
CD71 lead Centyrin and siRNA conjugate have high stability at large pH range

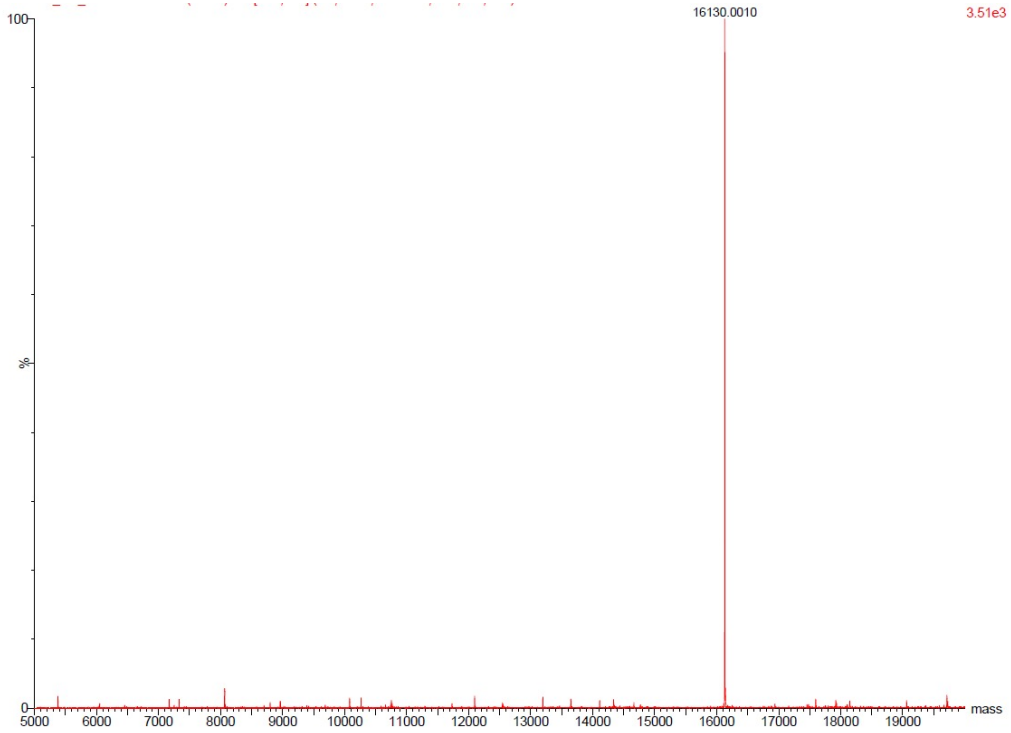




- Centyrins have high Tm's indicating extraordinary protein stability
- Stability is retained at low pH environments, such as the endosome
- Centyrin-siRNA conjugates retain high stabiligy in wide range of pH

Site-specific conjugation enable homogeneous 1DAR product





ABX1198-siRNA conjugate, 1.0 DAR

- Centyrins are highly expressed in *e.Coli*: 200-500 mg purified protein/1L culture
- Centyrins efficiently site-specifically conjugated to siRNA via a single Cys, 100% 1DAR

Aro's Centyrin Discovery Engine Enables Rapid Creation of New Therapeutic Candidates

~6 Months from Target to Centyrin

In Vivo POC

Target Selection

Identify cell surface protein(s) to drive cell-selective targeting

Strong Binders

In vitro CIS display screening to identify high affinity binders from library ~10^{13}

Parallel screens vs cyno antigen

Efficient Internalizers

Introduction of single cysteine & htp creation of Centyrin-drug conjugates

Internalization Assay

Select Centyrins with best properties for efficient receptor mediated internalization

Secondary screens

Epitope

Specificity

Immunogenicity

Good Manufacturing Properties

Stability

Expression/

Scale up in E coli

Homogeneous site – specific conjugation to drug payload

Disease models

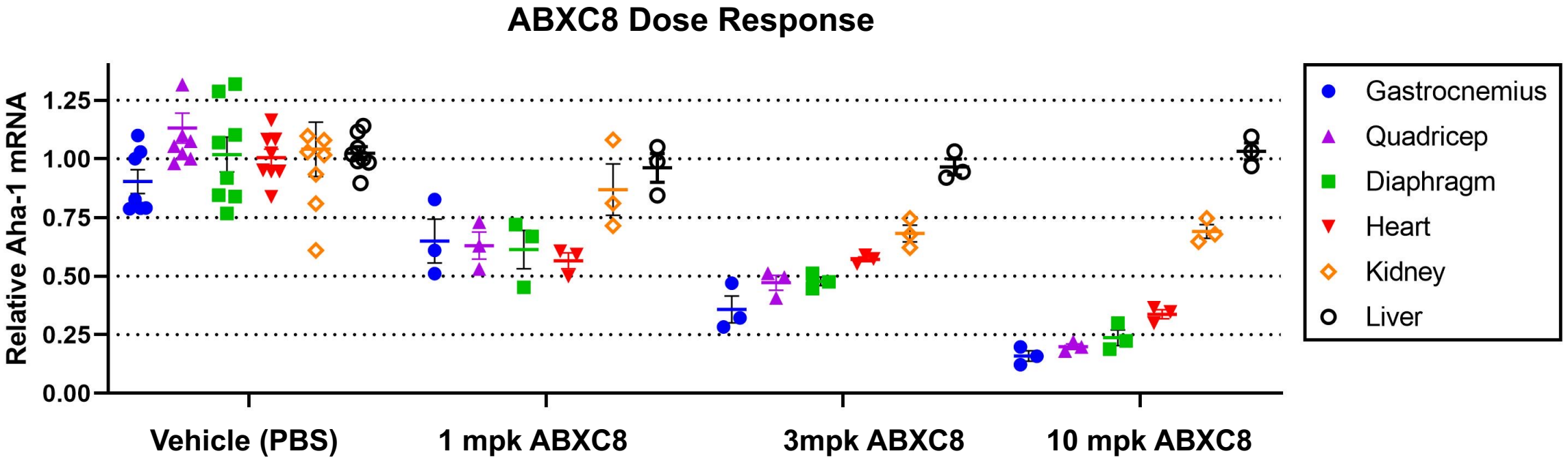
Cyno studies

Final Lead Selection

Robust, dose-dependent and selective gene knockdown in muscle

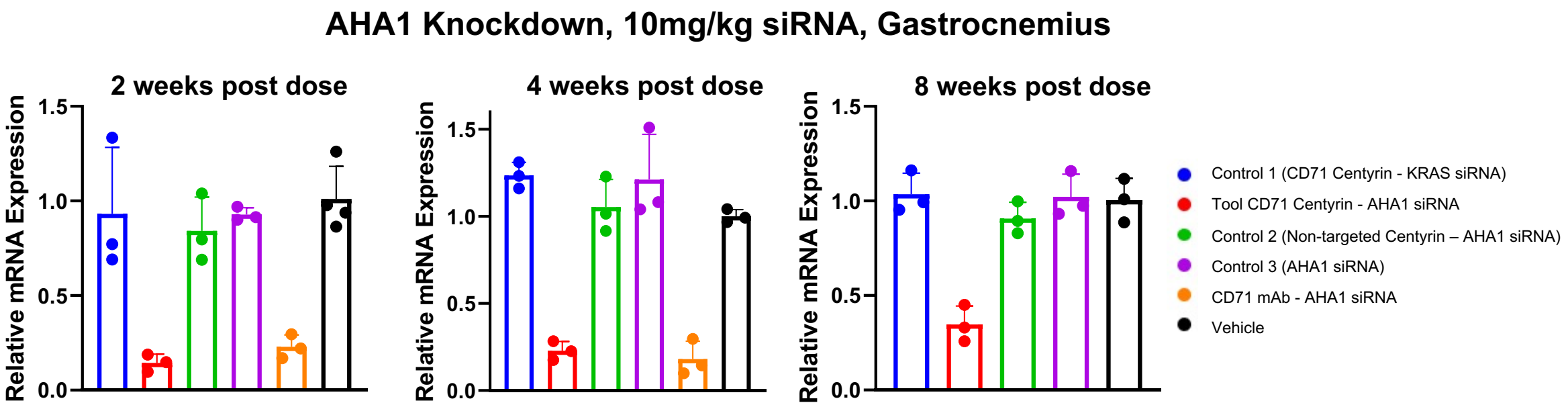
Tool mouse specific CD71 Centyrin-AHA1 siRNA conjugate

- **AHA-1 is a ubiquitously expressed housekeeping gene**
- **No/minimal gene knockdown observed in liver and kidneys**
- **Up to 80% gene knockdown observed 2 weeks after single dose**
- **Strong dose-response relationship observed**



- Mice dosed i.v. with PBS or 1, 3, or 10 mg/kg (siRNA) of ABXC8 (CD71-AHA1 conjugate). Tissues collected 2 weeks post single dose

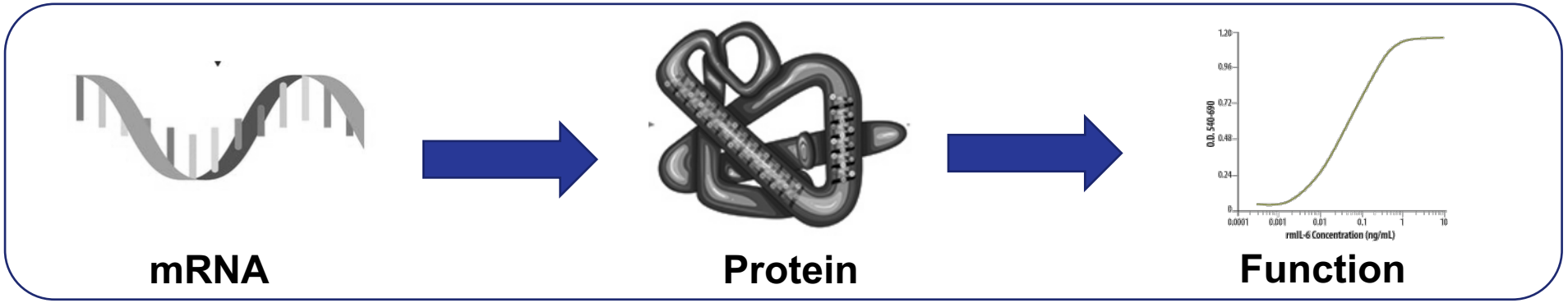
CD71 Centyrin-siRNA conjugate drives sustained gene knockdown at fraction of mAb conjugate dose in mice



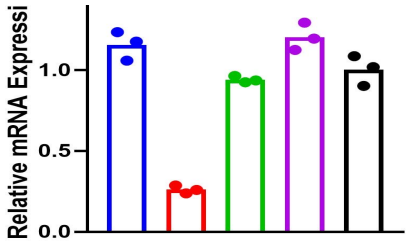
POC study with AHA1 housekeeping gene
C57/B6 mice received single dose of conjugates

	Centyrin – siRNA conjugate	mAb – siRNA conjugate
AHA1 knockdown wk2	86%	77%
AHA1 knockdown wk4	77%	82%
AHA1 knockdown wk8	65%	N/A
siRNA dose (mg/kg)	10 mg/kg	10 mg/kg
Conjugate dose (mg/kg)	~18 mg/kg	~120 mg/kg

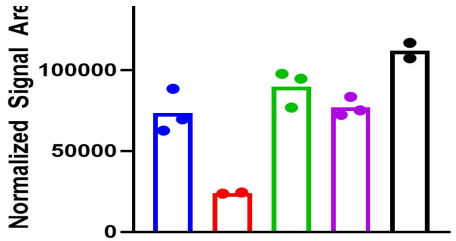
In vivo mRNA and protein knockdown are well correlated

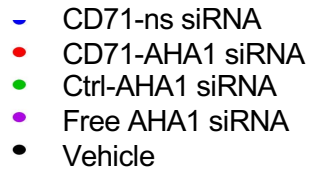


AHA1 mRNA K/d (Quadricep)



AHA1 Protein K/d (Quadricep)

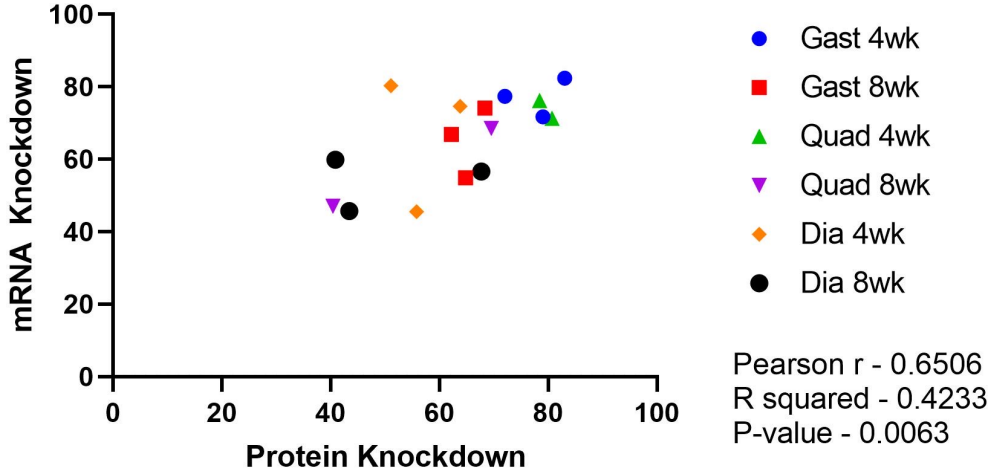




- C57/B6 mice
- 4 weeks following single dose (10mg/kg siRNA*)
- Similar data for other muscles (Gastrocnemius, Diaphragm,

**active siRNA = siRNA/2*

Long Term PD Study 2104081
Skeletal Muscle Correlation
4wk, 8wk post dose

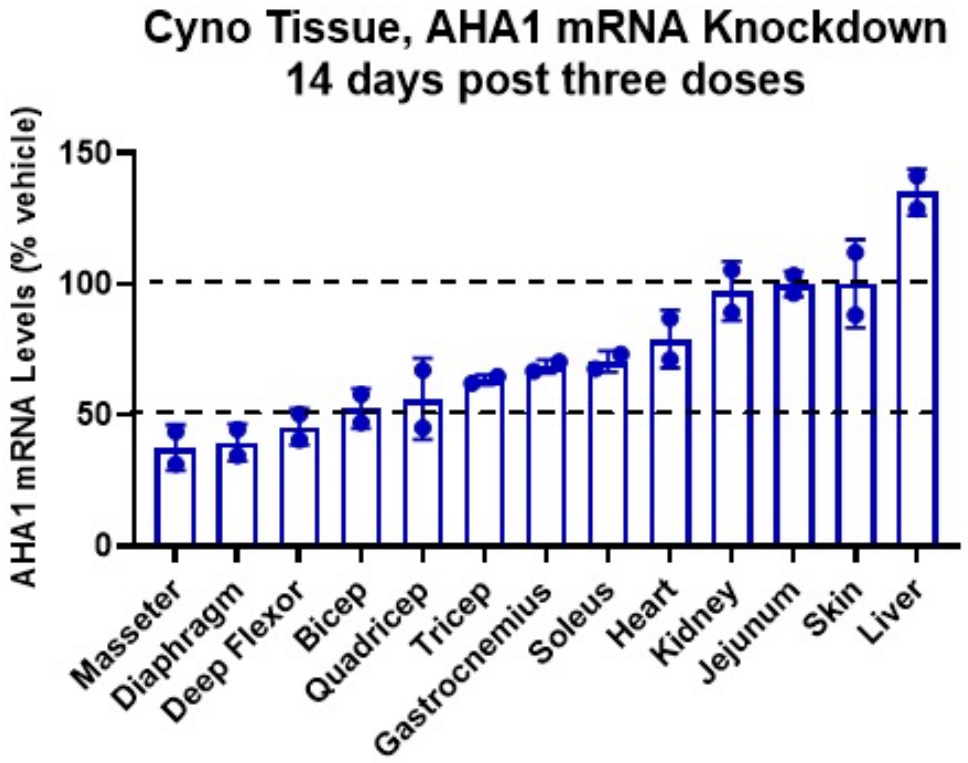


Pearson r - 0.6506
R squared - 0.4233
P-value - 0.0063

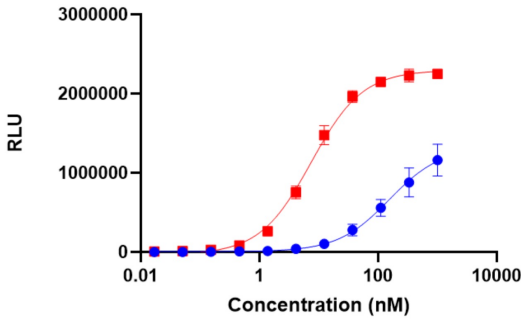
Centyrin affinity for cynoCD71 is reduced compared to human

However, binding to cyno CD71 enables toxicology studies

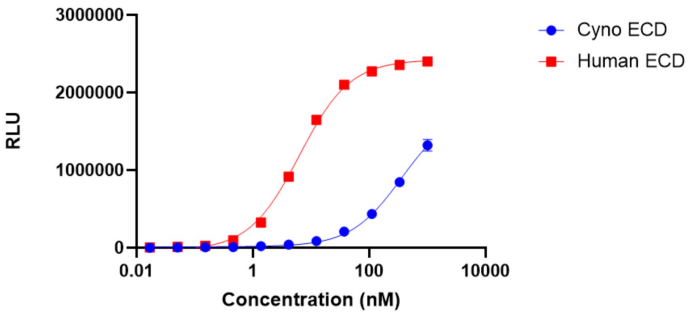
- ABXC-19 (3 doses, 3mg/kg siRNA)



CD71 centyrin



Centyrin-siRNA conjugate

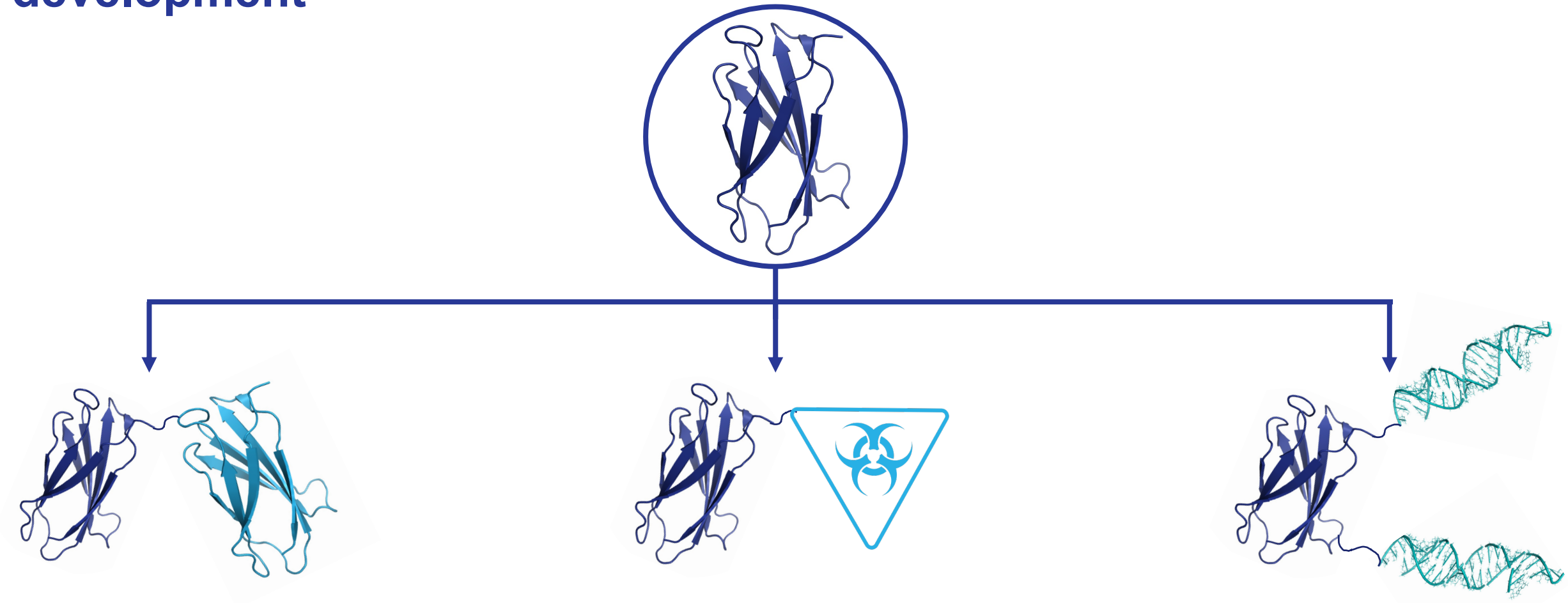


Domain	Centyrin EC50 (nM)	Conjugate EC50 (nM)
Hu CD71 ECD	7.4	> 6.3
Cyno CD71 ECD	> 150 nM	> 150nM

Centyrin-siRNA Conjugate Summary

- ✓ Panel of CD71 binding Centyrins have single digit nanomolar affinities and are highly specific for CD71
- ✓ CD71 Centyrin-siRNA conjugates demonstrate potent mRNA knockdown in mouse and cyno animal models
- ✓ Centyrin selection strategies can be tuned to achieve ideal binding to desired epitope
- ✓ Centyrin-siRNA conjugates employ simplified manufacturing processes with solubility properties that enable subcutaneous dosing
- ✓ Centyrin-Oligonucleotide conjugates are efficacious at a dose that is a fraction of the total drug dose of antibody (mAb or Fab) oligonucleotide conjugates

Centyrin modularity enables different therapeutic modality development



Bispecifics

Centyrin toxin conjugates

Dual, unique siRNA payloads

Summary: Centyrin targeted delivery of RNA medicines

Multiple advantages relative to other approaches

The Centyrin Advantage

Superior Drug Properties

- Receptor specific gene knockdown
- Low protein dose required to achieve gene modulation
- Site specific conjugation enabling homogeneous product
- Simplified and low-cost manufacturing in E. coli
- Low immunogenicity potential

Differentiated Product Opportunities

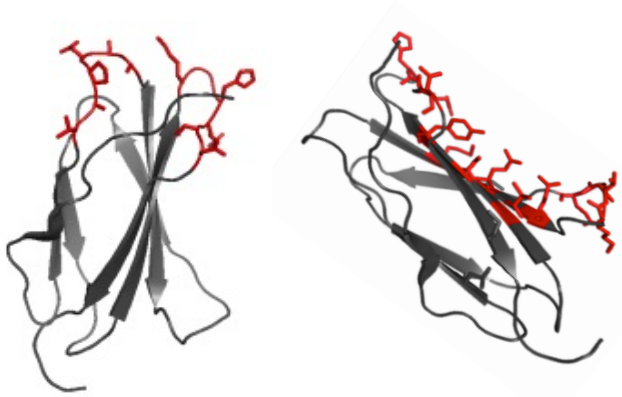
The Centyrin Advantage

Modularity and flexibility to optimize constructs

- Bispecific Centyrins for simultaneous targeting of two receptors
- Conjugation of multiple oligos enabling dual pathway inhibition
- Flexible formulation for diverse genetic payloads
- Tunable PK

Diverse Future Product Opportunities

Aro BIOTHERAPEUTICS



Thank You!