Novartis Institutes for BioMedical Research

Extracellular Targeted Protein Degradation: An Emerging Therapeutic Modality

Thomas Smith, Ph.D., Novartis Institutes for Biomedical Research

2nd Next-Generation Conjugates Summit, Boston, MA, USA

February 23, 2023

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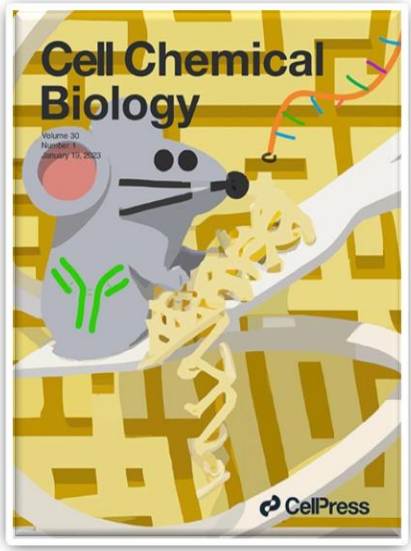
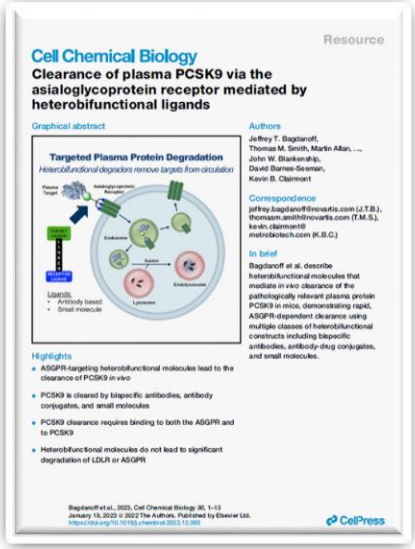
Declaration of Interests

The presenter is a current employee of the Novartis Institutes for BioMedical Research (NIBR), and is co-author of a published manuscript and inventor on patent applications, all related to this work

See Bagdanoff et al., 2023, Cell Chemical Biology 30, 1–13. January 19, 2023





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Novartis Institutes for BioMedical Research

Addressing unmet medical need with our Disease Areas, Institutes, Enabling Platforms

Immunology | Cardiovascular & Metabolism | Immuno-Oncology | Musculoskeletal | Neuroscience | Oncology | Ophthalmology | Respiratory Diseases

Novartis Biologics Center

Chemical Biology & Therapeutics

Global Discovery Chemistry

Translational Medicine

Friedrich Miescher Institute for Biomedical Research (FMI)

Novartis Institute for Tropical Diseases (NITD)

6 research locations around the globe

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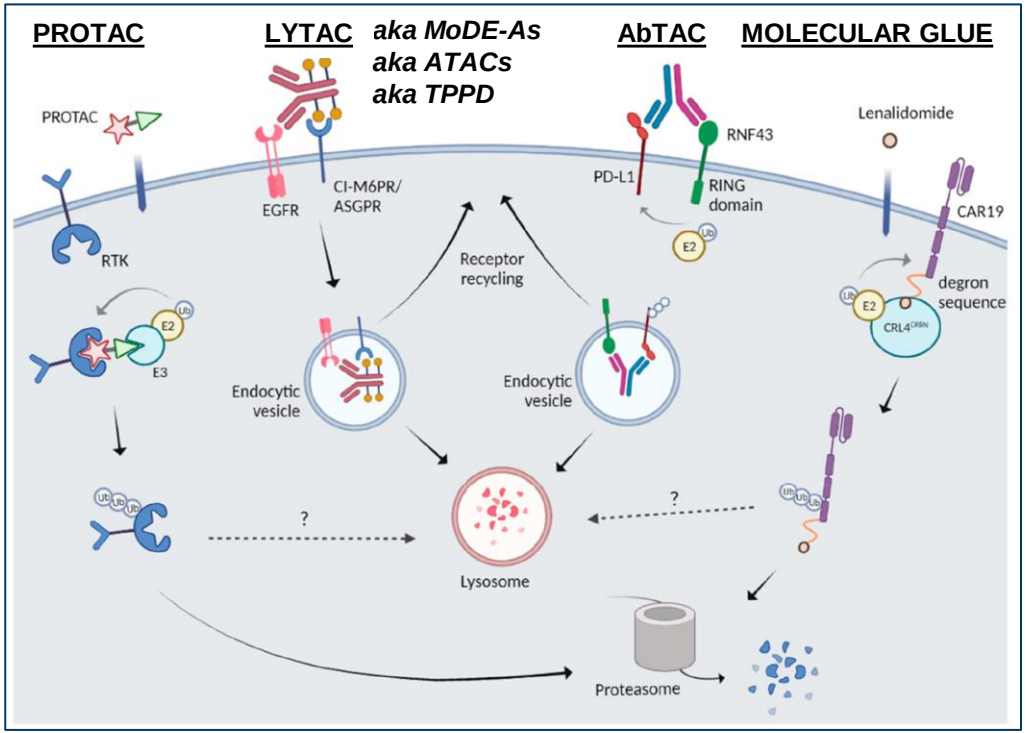
Degradation of Extracellular Protein Targets

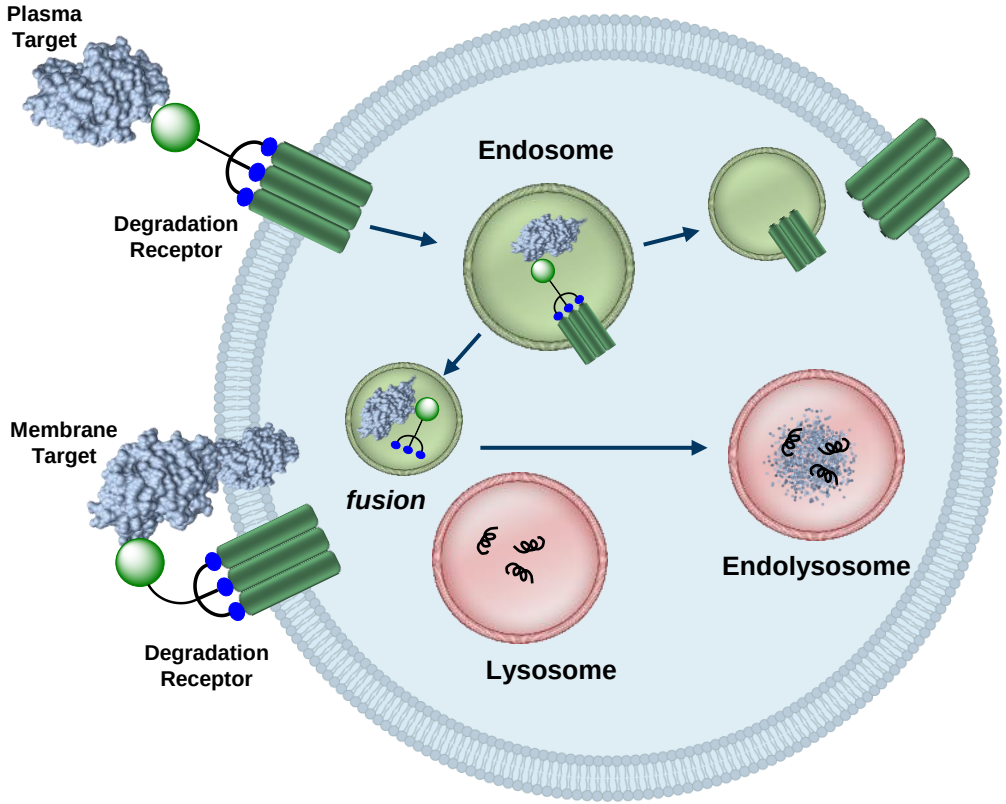
eTPD



Adapted from
Ruffilli et al., Proteolysis Targeting Chimeras (PROTACs): A Perspective on Integral Membrane Protein Degradation. ACS Pharmacol. Transl. Sci. 2022, 5, 10, 849–858.

Targeted Plasma Protein Degradation (TPPD)

Extracellular degraders tackle cell surface membrane and soluble plasma targets

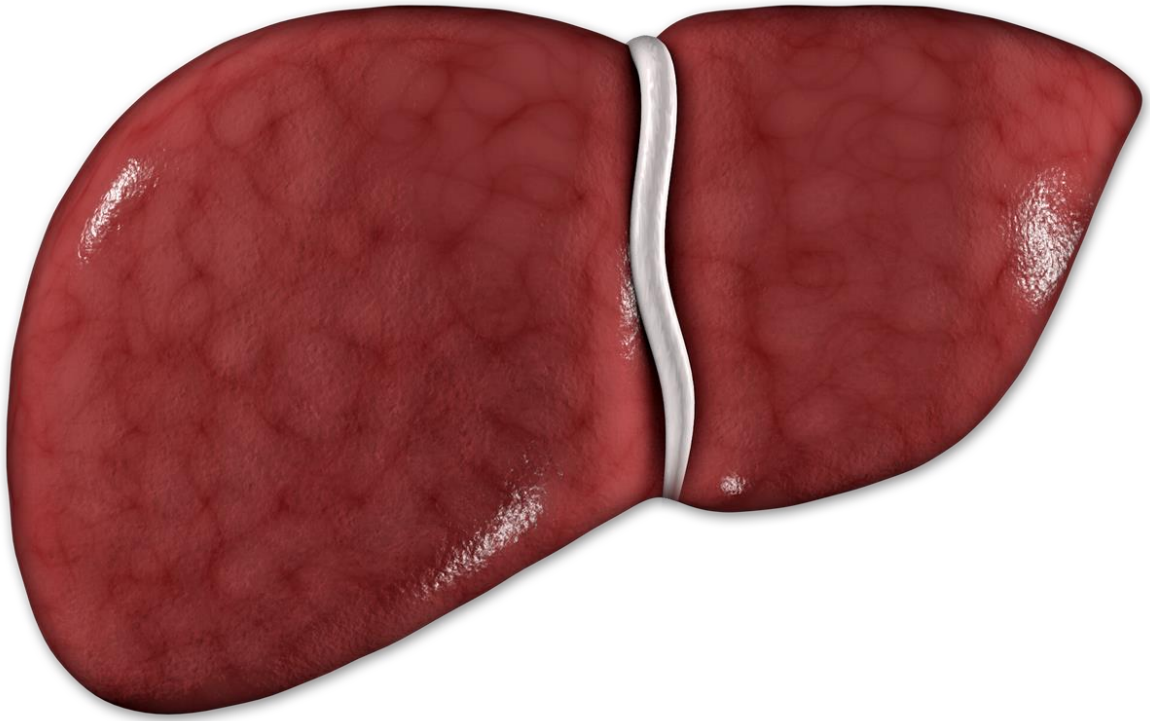


Ideal TPPD receptors will:

- Deliver target to endolysosomal system
- Not be degraded along with target
- Recycle constitutively and rapidly
- Have high capacity to internalize target
- Have low safety concern with reduced capacity (inhibition benign)

The Asialoglycoprotein Receptor (ASGPR)

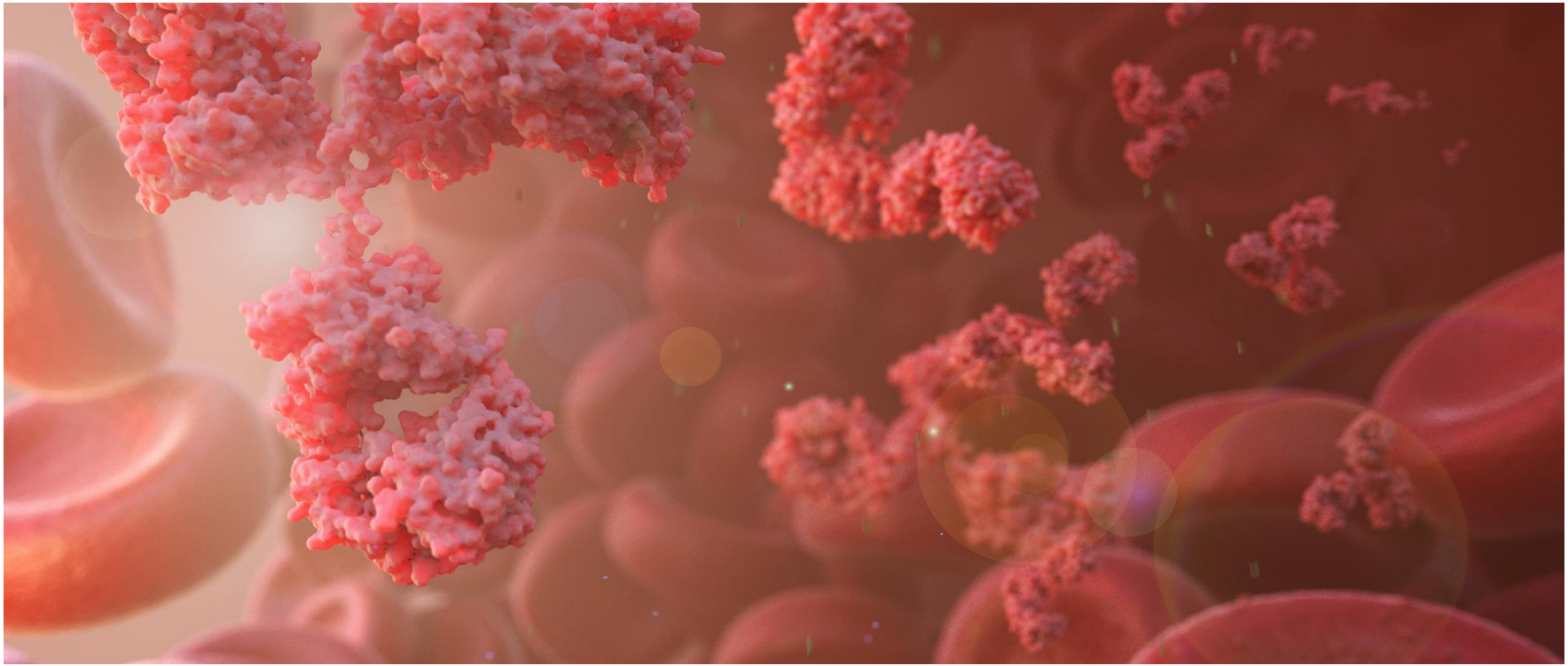
A high density, rapidly internalizing scavenger receptor of the liver



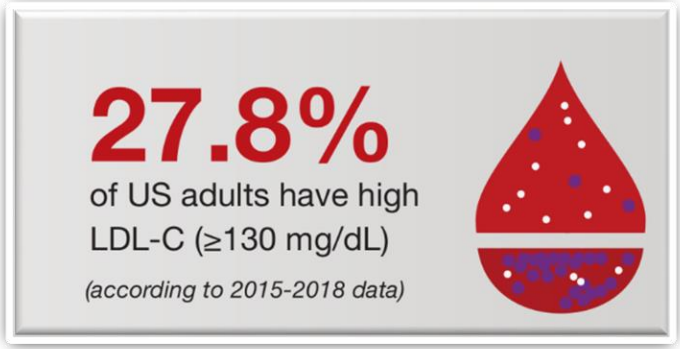
Das, S. et al. Asialoglycoprotein receptor and targeting strategies in “Targeted Intracellular Drug Delivery by Receptor Mediated Endocytosis”, Springer 2019

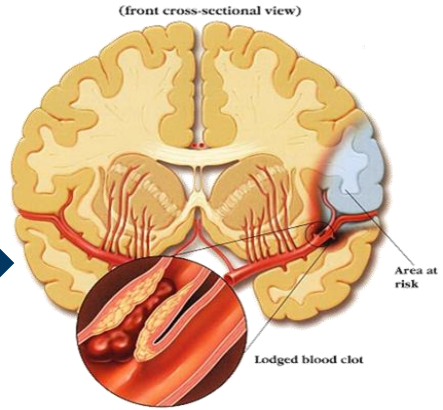
Searching For The Best Therapeutic Applications

Diseases driven by soluble circulating factors

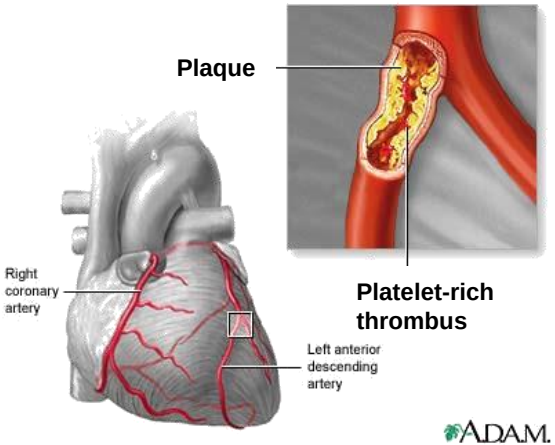


Cholesterol Levels Are One Of The Primary Risk Factors For Development Of Atherosclerosis & CVD





Ischemic Stroke



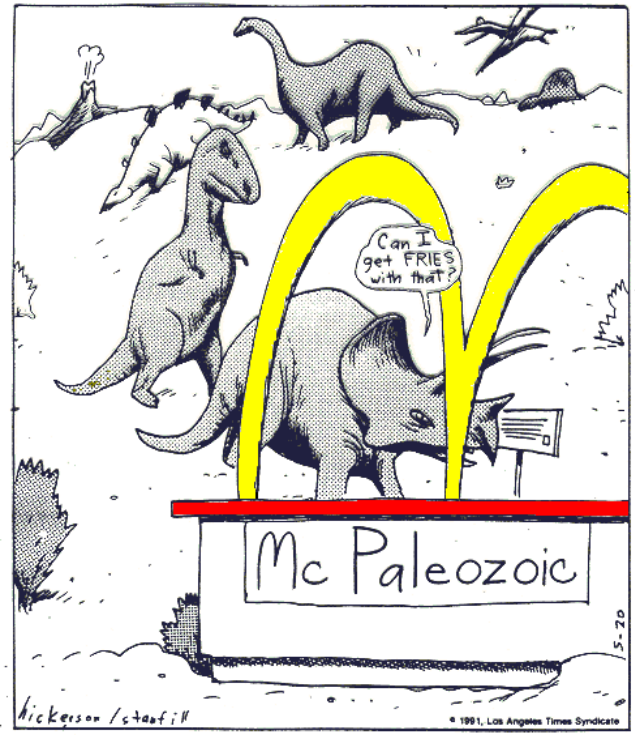
Acute Coronary Syndrome

Atherosclerosis is a progressive inflammatory and pro-thrombotic disease, exacerbated by high levels of LDL-cholesterol

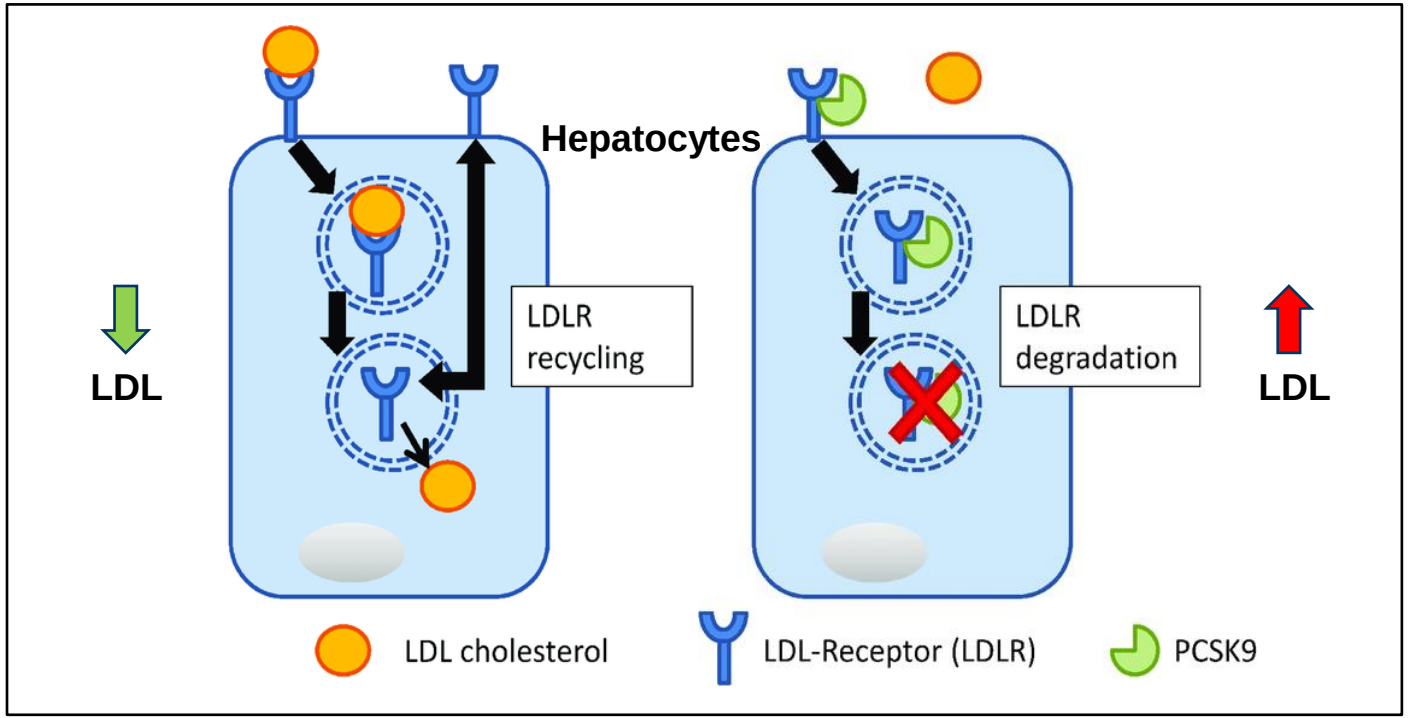
Tsao CW, et al. Heart Disease and Stroke Statistics-2022 Update: A Report From the American Heart Association. Circulation. 2022 Feb 22;145(8):e153-e639

Genetics, Lifestyle, And Diet All Play A Role In CVD

The REAL reason dinosaurs became extinct



The Role Of PCSK9 In Modulating LDL Levels

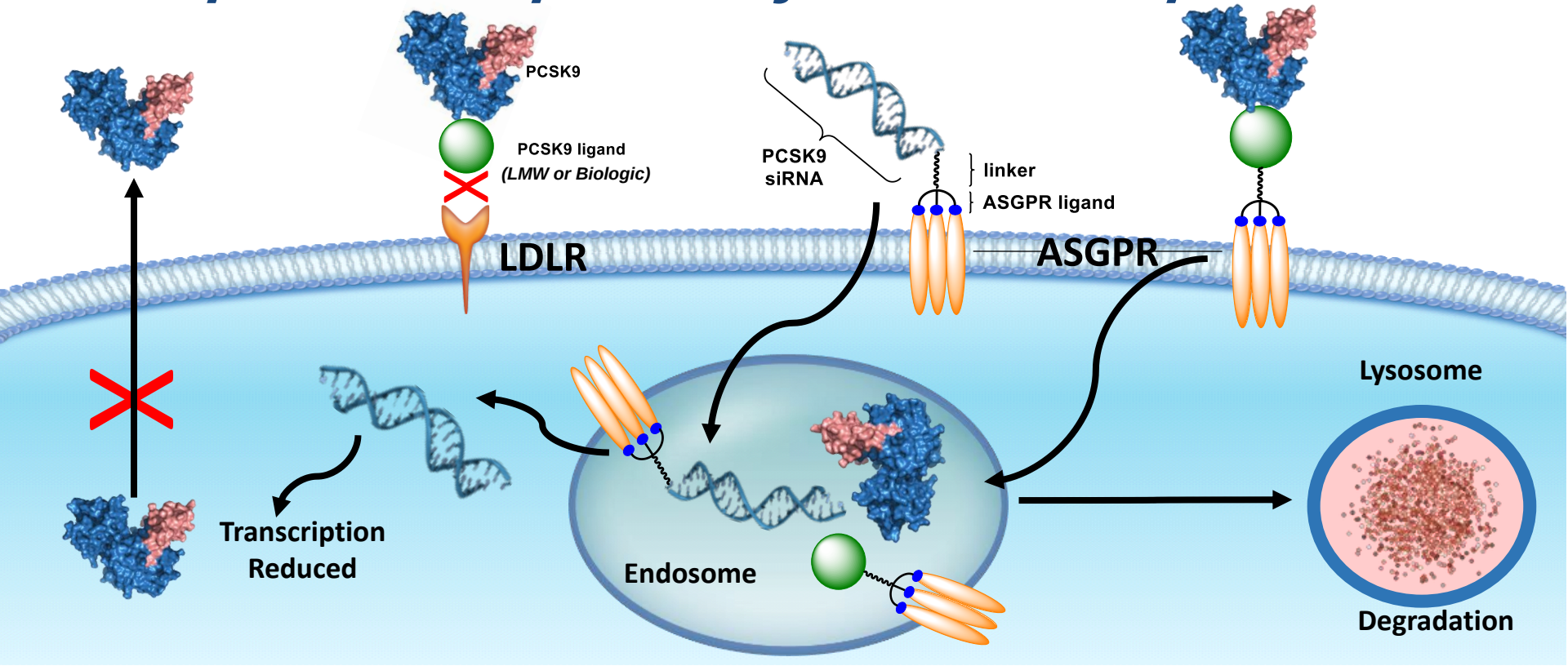


Statins, along with diet and exercise, are often not enough for many patients to reach their recommended LDL target level.

Schlegel V, et al. Low PCSK9 levels are correlated with mortality in patients with end-stage liver disease. PLoS One. 2017 Jul 20;12(7):e0181540.

Proving The Technology – PCSK9 As A Case Study

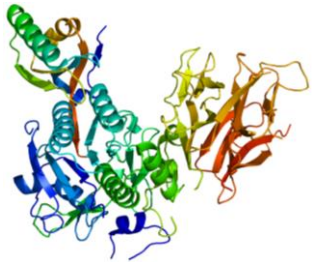
TPPD represents a unique modality for clearance of plasma PCSK9



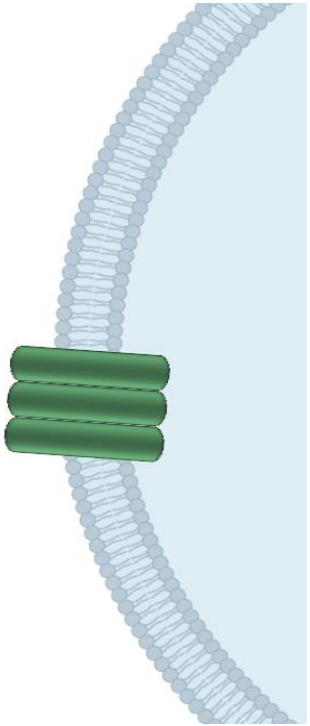
Design Principles of Extracellular Degraders

A modular bifunctional design enables access to diverse extracellular target space

TARGET



RECEPTOR



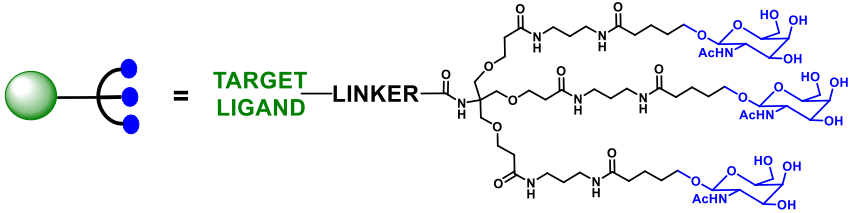
Target & Receptor Ligands:

- Protein or Peptide-based
- Aptamer-based
- LMW-based

Towards An Extracellular PCSK9 Degrader

- **Synthesize and characterize bifunctional ligands**
- Validate bifunctional binding
- Assess cellular uptake
- Evaluate PCSK9 target clearance *in vivo*

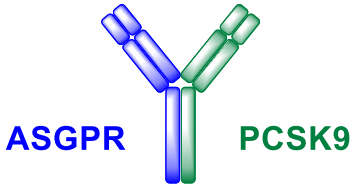
GalNAc trimer



Compound 1

Bifunctional Antibody

ASGPR Ab – PCSK9 Ab



Compound 5

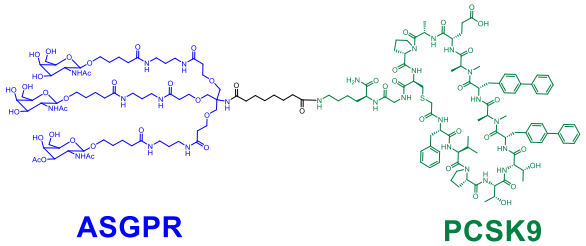
GalNAc trimer – Antibody

$[GalNAc]_3$ – PCSK9 Ab

Compound 10

GalNAc trimer – Macrocycle

$[GalNAc]_3$ – PCSK9 binder



Compound 15

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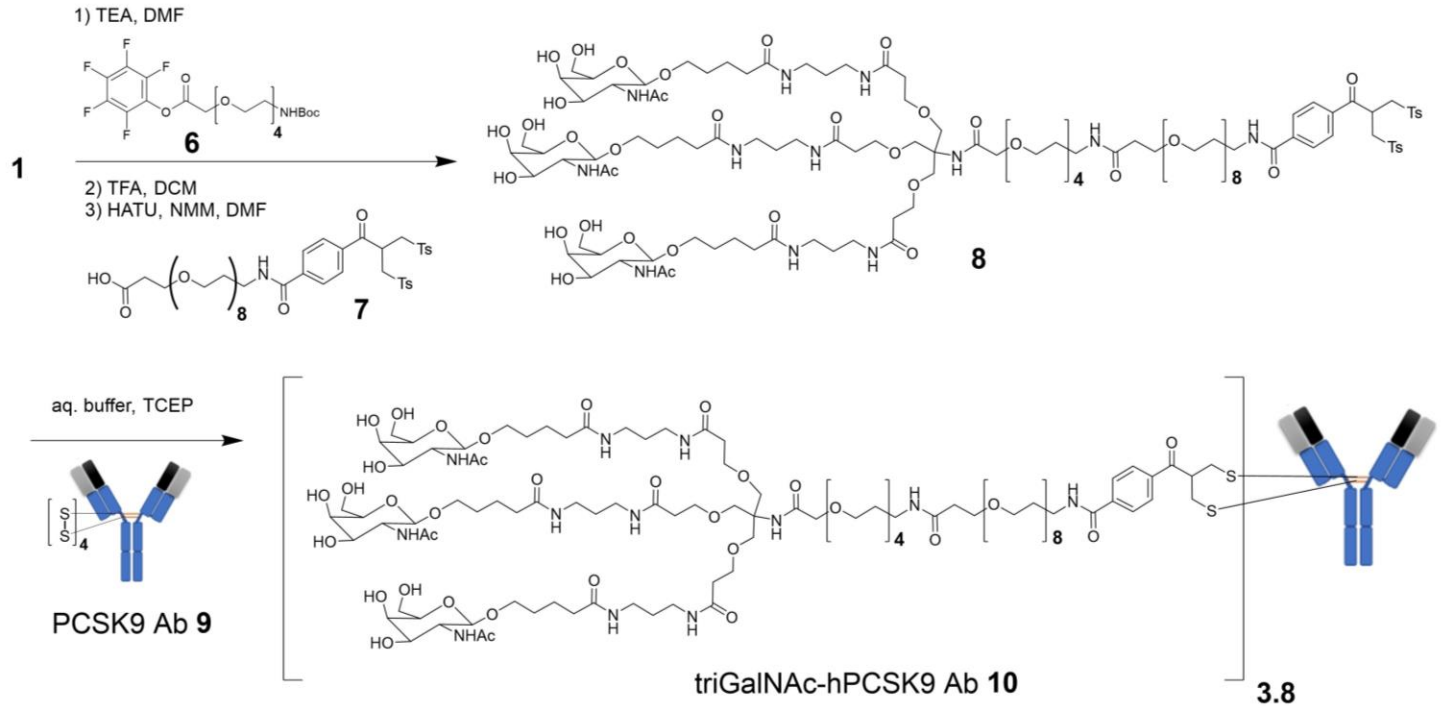
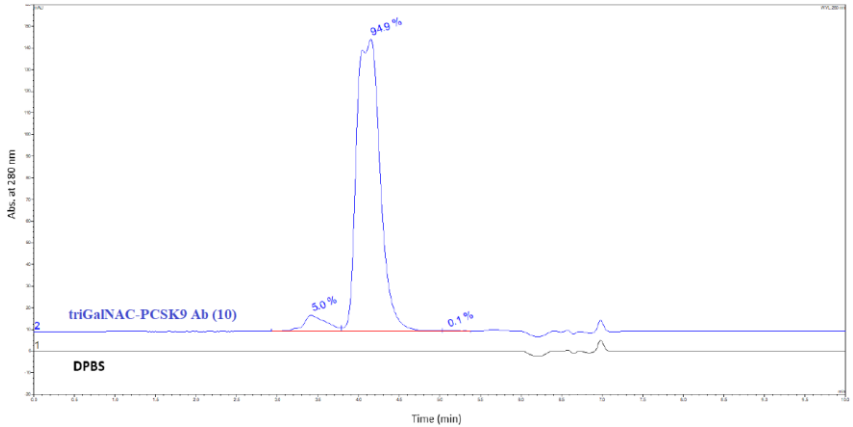
Compound 10 Synthesis



Compound 10 Characterization

ABZENA

A. Size exclusion chromatogram (λ = 280 nm) for triGalNAc-PCSK9 Ab (10) (blue trace); Dulbecco's PBS, pH 7.0 blank (black trace).



B. Analytical Summary for triGalNAc-PCSK9 Ab (10)

Analysis	Results
Appearance	Clear solution
DAR variants (LC-MS)	DAR 3: 5% DAR 4: 95% Average DAR: 4.0
% Purity (SEC)	94.9% monomeric
Endotoxin (EU/mg)	0.22
Concentration (UV)	8.3 mg/mL
Amount (by UV Analysis)	9.9 mg
Average MW	155,680 Da

Compound quality and integrity are paramount for further studies

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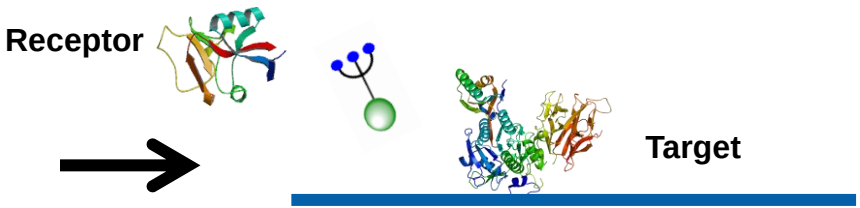
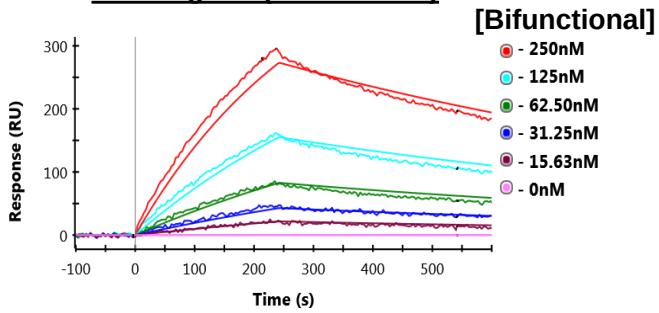
Biophysical Validation Of Bifunctionals

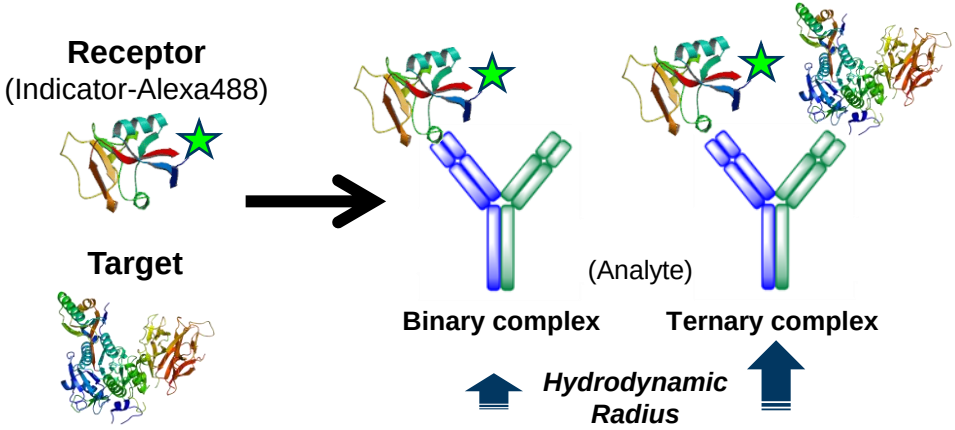
Surface Plasmon Resonance (SPR)

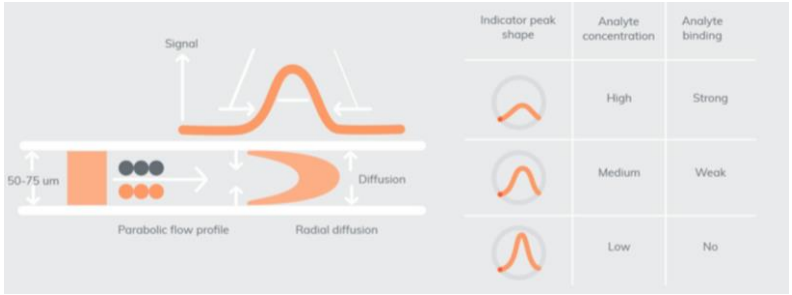


Sensorgram (SPR results)



Flow Induced Dispersion Analysis (FIDA)





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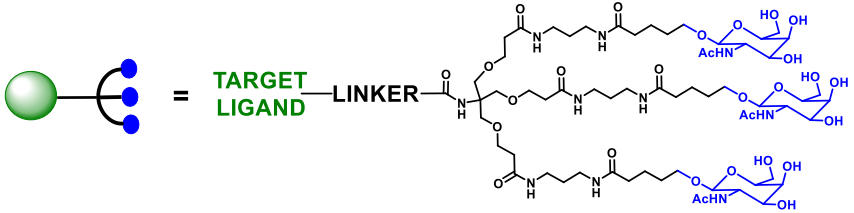
Towards An Extracellular PCSK9 Degrader

- Synthesize and characterize bifunctional ligands
- **Validate bifunctional binding**
- Assess cellular uptake
- Evaluate PCSK9 target clearance *in vivo*

GalNAc trimer

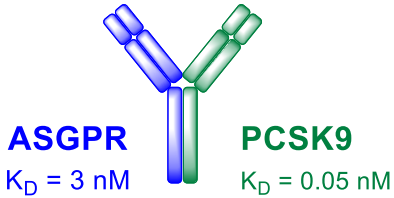
ASGPR K_D ~ 3 nM



Compound 1

Bifunctional Antibody

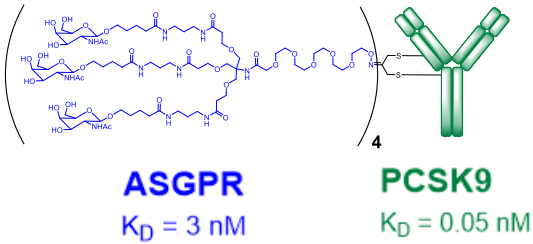
ASGPR Ab – PCSK9 Ab



Compound 5

GalNAc trimer – Antibody

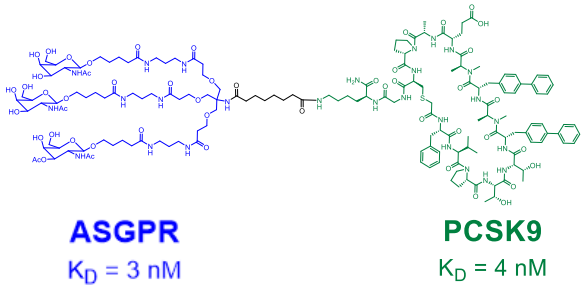
$[GalNAc]_3$ – PCSK9 Ab



Compound 10

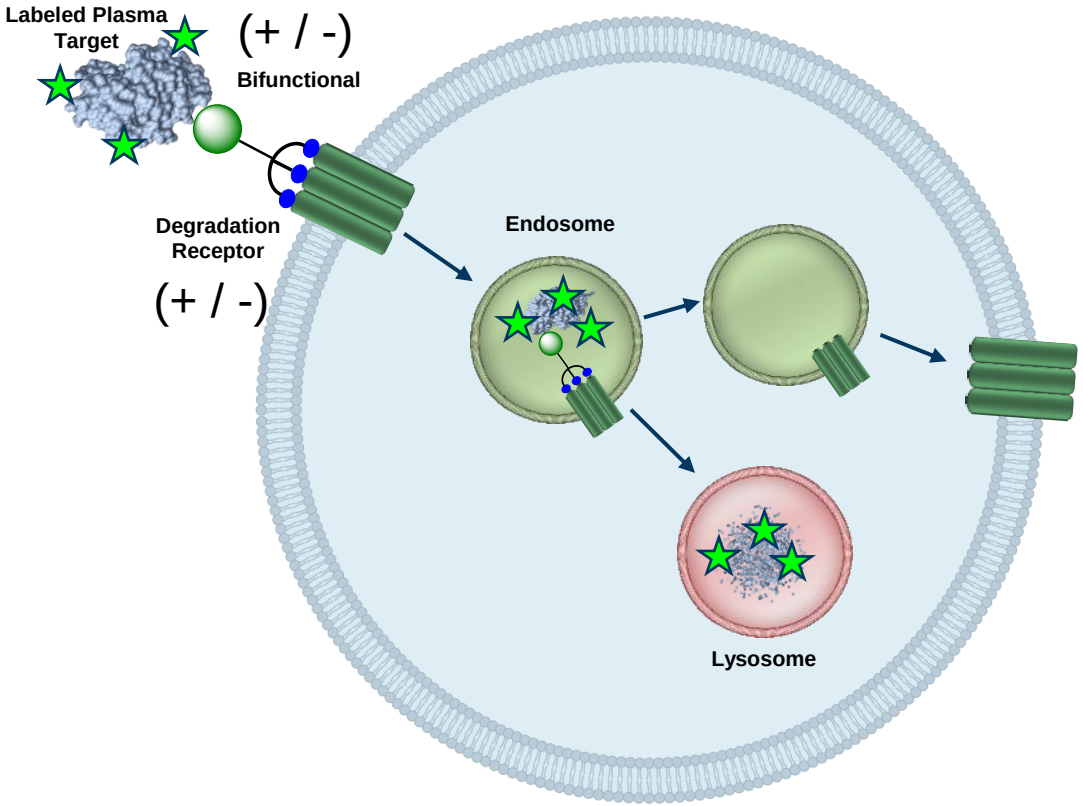
GalNAc trimer – Macrocycle

$[GalNAc]_3$ – PCSK9 binder



Compound 15

Cellular Validation Of Bifunctionals



FACS

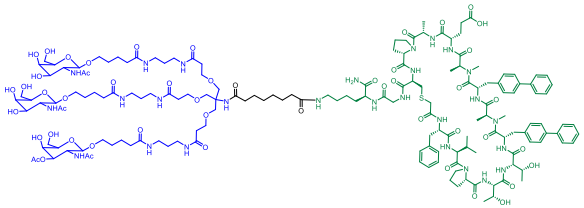
Imaging

Towards An Extracellular PCSK9 Degrader

- Synthesize and characterize bifunctional ligands
- Validate bifunctional binding
- **Assess cellular uptake**
- Evaluate PCSK9 target clearance

GalNAc trimer – Macrocycle

$[GalNAc]_3$ – PCSK9 binder



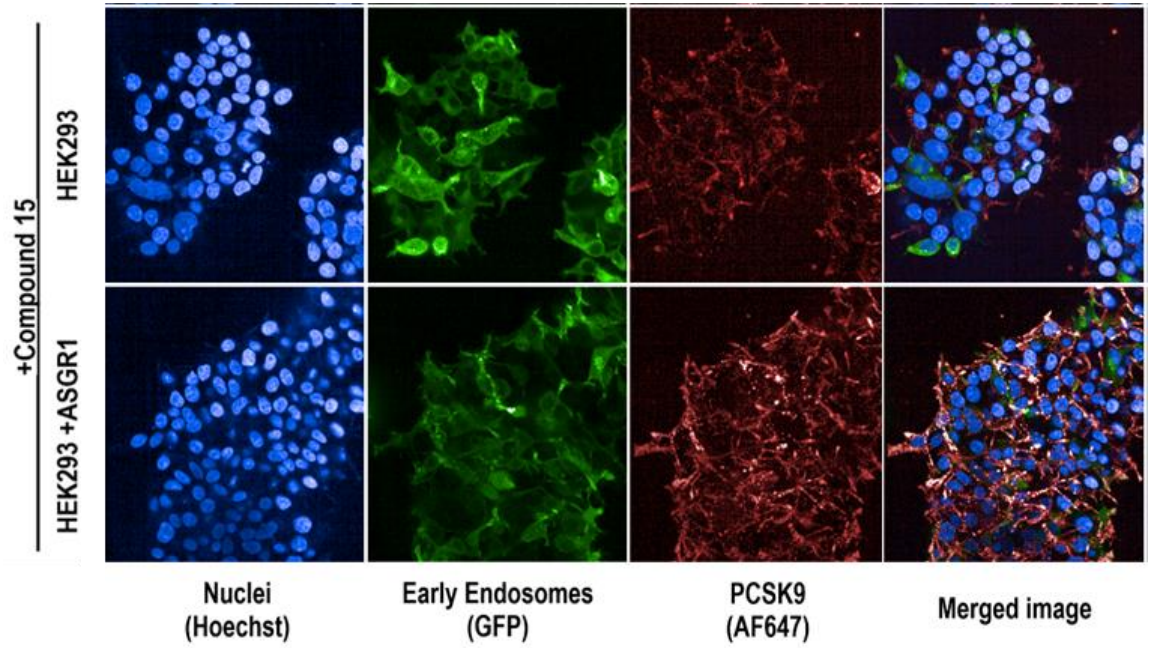
ASGPR

K_D = 3 nM

PCSK9

K_D = 4 nM

Compound 15



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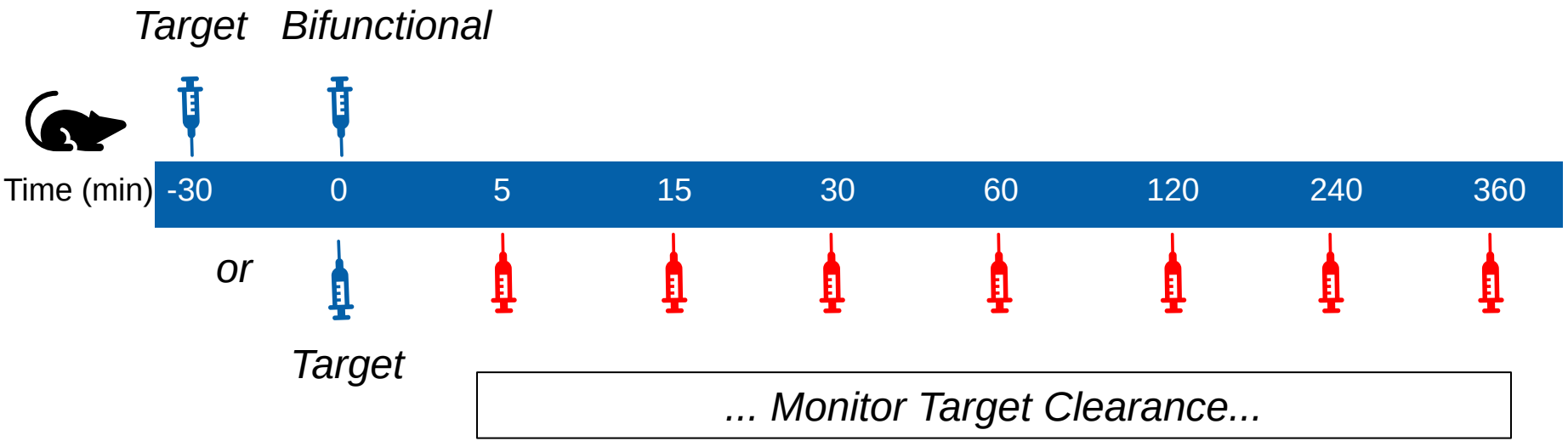
In Vivo Assessment Of Bifunctionals

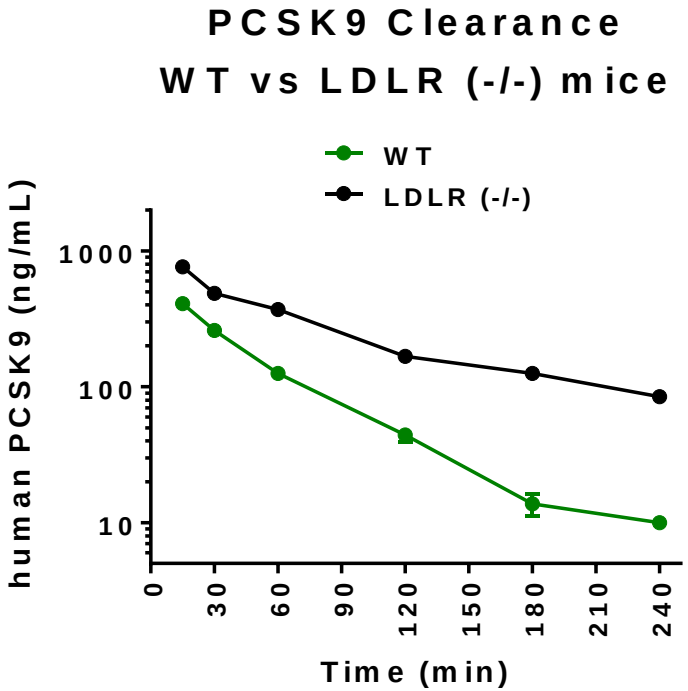


Towards An Extracellular PCSK9 Degrader

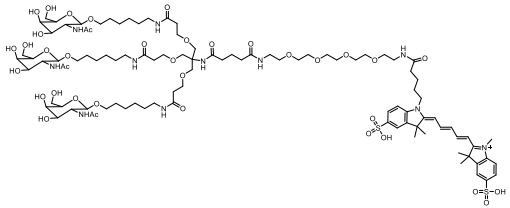
- Synthesize and characterize bifunctional ligands
- Validate bifunctional binding
- Assess cellular uptake
- **Evaluate PCSK9 target clearance *in vivo***

Challenge: PCSK9 clearance is largely through liver low density lipoprotein receptor (LDLR)

LDLR KO Animals Provide A Better Window To Detect Facilitated Clearance Of PCSK9



GalNac Trimer - Fluor is Rapidly Delivered to LDLR (-/-) Mouse Liver



Compound 24

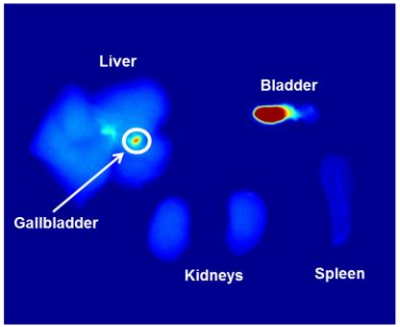
t = 0 min: Probe injection (tail vein)

t = 30 min; 8 hrs.

Fluorescent dye control Cy5.5 (2 mg/kg)
Or Cy5.5-GalNAc conjugate (2 mg/kg)

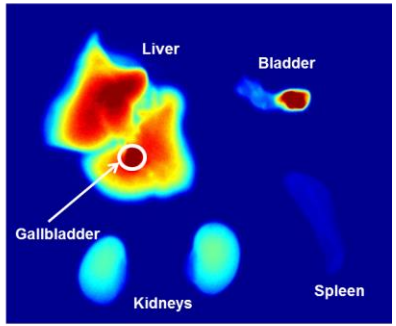
In vivo and *ex vivo* imaging

Cy5.5 dye control



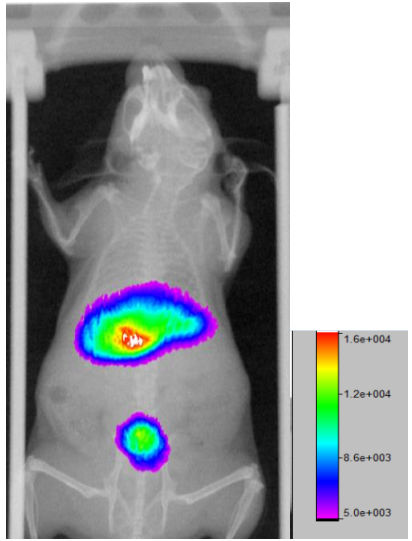
Indicator range: 0-0.554
Scale Factor: 2

GalNAc-Cy5.5 control ligand



***t* = 30min**

Cy5.5-GalNAc conjugate (2 mg/kg)



***t* = 8hr**

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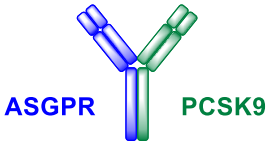
Plasma PCSK9 Clearance is Achieved With Antibody-Based Bifunctionals

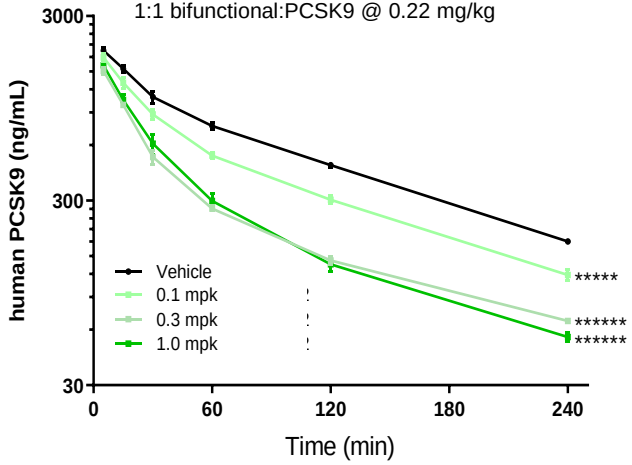
ABZENA

Bifunctional Antibody

ASGPR Ab – PCSK9 Ab

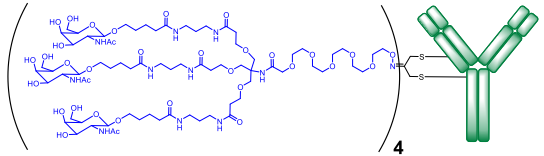


Compound 5

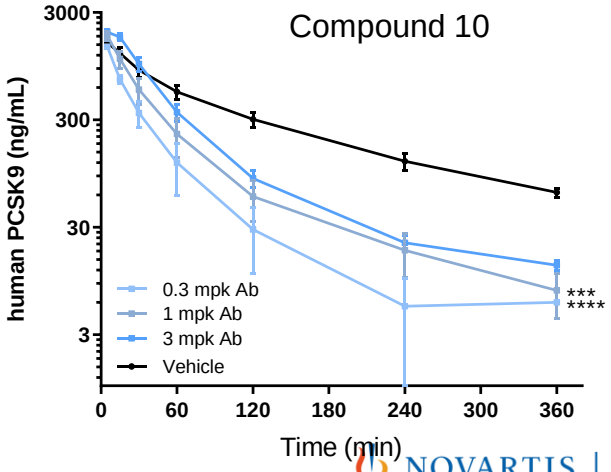


GalNAc trimer – Antibody

[GalNAc]$_3$ – PCSK9 Ab



Compound 10



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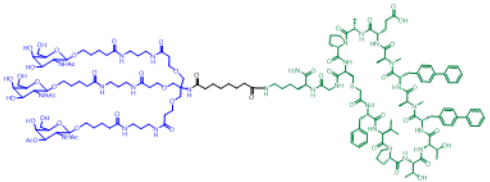
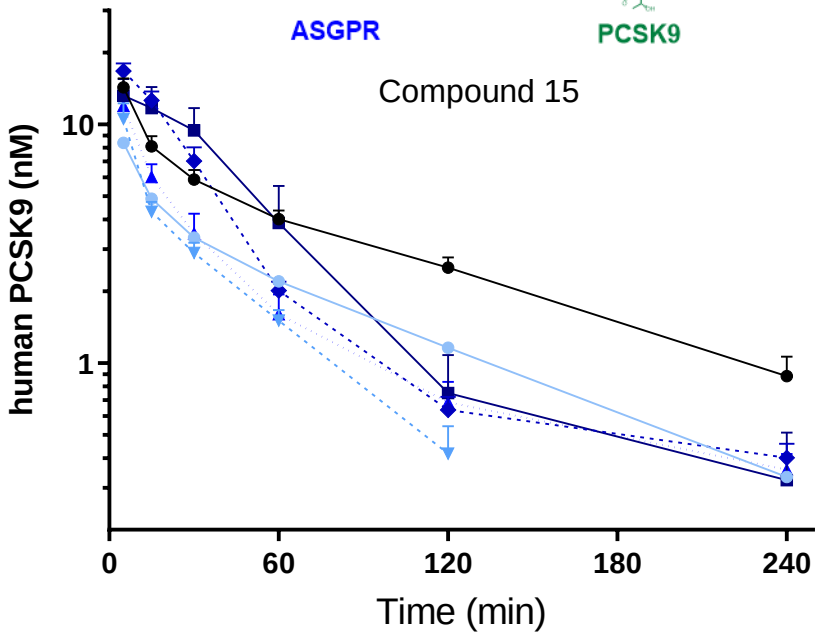
Plasma PCSK9 Clearance is Achieved With "LMW"-Based Bifunctionals

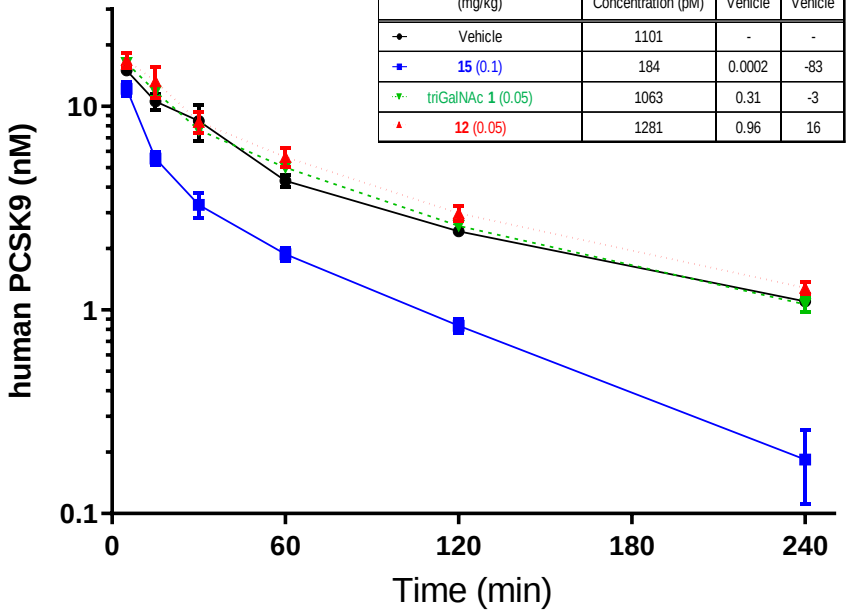


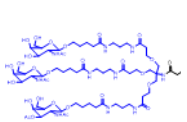


Compound **15** (mg/kg)	PCSK9 Concentration (pM) at T = 120 min	p vs. Vehicle	Δ% vs. Vehicle
0	2513	-	-
0.01	1160	0.0376	-54
0.1	417	0.0049	-83
0.3	684	0.0118	-73
1	636	0.0097	-78
3	748	0.0071	-70

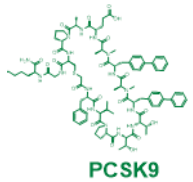
Plasma PCSK9 Clearance Does Not Occur with Only Target Ligand Or Receptor Ligand

Compound (mg/kg)	Terminal PCSK9 Concentration (pM)	p vs. Vehicle	Δ% vs. Vehicle
Vehicle	1101	-	-
15 (0.1)	184	0.0002	-83
triGalNAc **1** (0.05)	1063	0.31	-3
12 (0.05)	1281	0.96	16

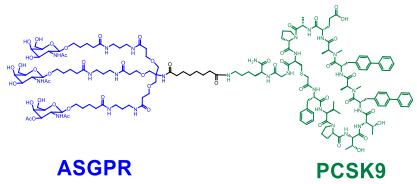




Compound 1



Compound 12



Compound 15

PCSK9 clearance requires formation of ternary complex

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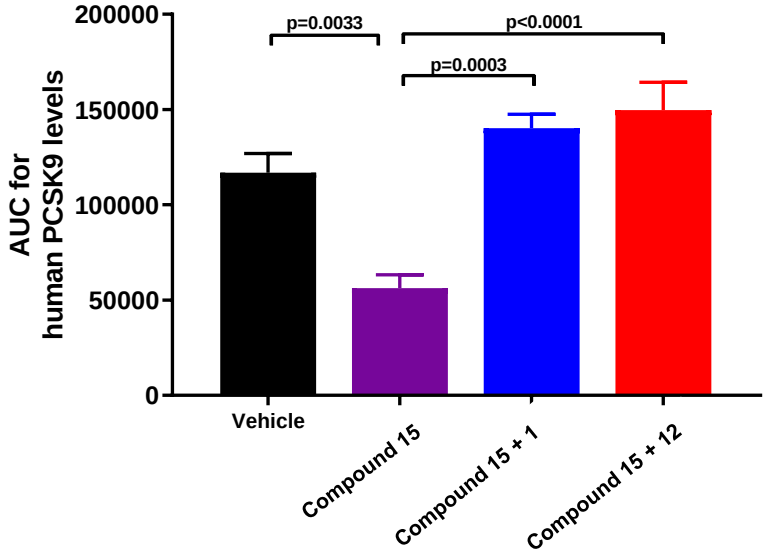
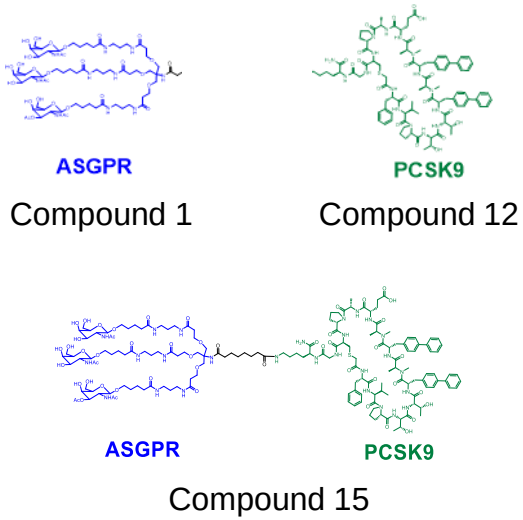
Plasma PCSK9 Clearance Is Inhibited By Excess Target Ligand Or Receptor Ligand





Monofunctional ligands will compete with bifunctional ligands to inhibit clearance.

In Conclusion

- Extracellular targeted protein degradation (eTPD)
  - Emerging approach for tackling membrane and soluble targets
- ASGPR
  - High-density, high-capacity shuttle to endolysosomal system
- Bifunctional compounds to ASGPR and PCSK9 feasible: *quality is paramount*
- Circulating PCSK9 cleared *in vivo* in mice by
  - bispecific antibodies
  - antibody conjugates
  - small molecules

TPPD Team Members and Key Contributors

CVM
Kevin Clairmont
Kevin Bean
Meg Brousseau
Shari Caplan
Pam Grewal
Alex Koch
Jen Lussier
Peter O'Donnell
Meihui Pan
Leeann Schreier
Vanitha Subramanian
Victoria Vera
Gerry Waters
Jian Xu
Qing Yang

NBC / ASI
John Blankenship
Regis Cebe
Christina Dornelas
Tony Fleming
Brian Granda
Thomas Huber
Ned Kirkpatrick
Alexandra Lavoisier
Crystal Shih
Bruno Tigani
Elisabetta Traggiai
William Tschantz

RD
Tim Benson
Katherine Mccauley
David Rowlands

GDC
Jeff Bagdanoff
Martin Allan
Frederic Berst
Chris Brain
Dirk Bussiere
Gregor Cremosnik
Stefanie Flohr
Ralf Glatthar
Rene Hersperger
Peter Meier
Lauren Monovich
Kenji Namoto
Elizabeth Ornelas
Dominik Pistorius (NPU)
Esther Schmitt (NPU)
Alok Singh
Thomas Vorherr

ATI
Frederic Bornancin
Dominik Buser
Thomas Calzascia
Isabelle Isnardi
Gautier Robert
James Rush
Richard Siegel
Helmut Sparrer
Max Warncke

DAx
Amy Berwick
Paola Capodieci
Bill Dietrich
Betsy George
Sue Stevenson

CBT
Thomas Smith
Rishi Arora
Jason Baird
Fred Bassilana
Steve Canham
Dominick Casalena
Chun-Hao Chiu
Raj Chopra
Feng Cong
Christoph Dumelin
William Forrester
Gabe Gamber
Celia Mendez Garcia
Matt Gerding
Patrick Hauck
Uli Hommel
Srinivas Honnappa
Dan King
Lukas Leder
Bo Lu
Sue Menon
Zachary Nguyen
Johannes Ottl
Josh Paulk
Nicole Renaud
Michael Romanowski
Jonas Schaefer
Philip Skaanderup
Claudio Thoma
Shuangxi Wang
Jonathan Whicher
Christian Wiesmann
Mikias Woldegiorgis
Lili Xie

Genesis Labs
Michael Chaffers
Sarah Cooper
Ian Hunt
Kathleen Kellogg
Aimee Reynolds

Scientific Advisory Board
Richard MacDonald
University of Nebraska
Natalie Dales (GDC)
Lloyd Klickstein (TM)
John Tallarico (CBT)

PK Sciences
Ann Brown
David Nettleton
Dan Wall

TPPD Innovation Postdoc
Elizabeth Moore

Drug Prototypes Committee
Rohan Beckwith
Kirk Clark
Rishi Jain
Lynn McGregor
Folkert Reck
Nicole Renaud
Isabel Zaror

NIBR Leadership
Jay Bradner
Christian Bruns
Karin Briner
Shaun Coughlin
Vishal Patel
Thomas Pietzonka
Jeff Porter
John Tallarico

Legal
Jana Harris
Linyu Mitra
Dan Raymond
Wei Zhang

Procurement
Joe Carvalho
Benoit Collin
Ellen Crawford

External
Lani Peterson, STORY
Aurigene
CEPiA Sanofi
GeneArt
LakePharma
Proteros Biostructures GmbH
Abzena UK Ltd.
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Thank you

Harnessing ASGPR-Dependent Degradation

Pioneering efforts in this space date back > 35 years

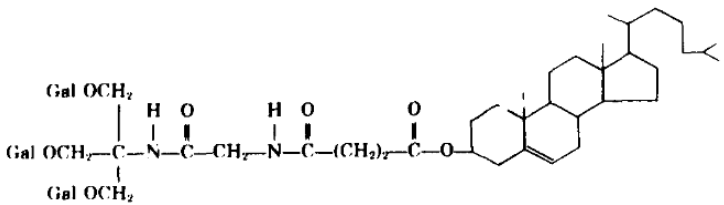
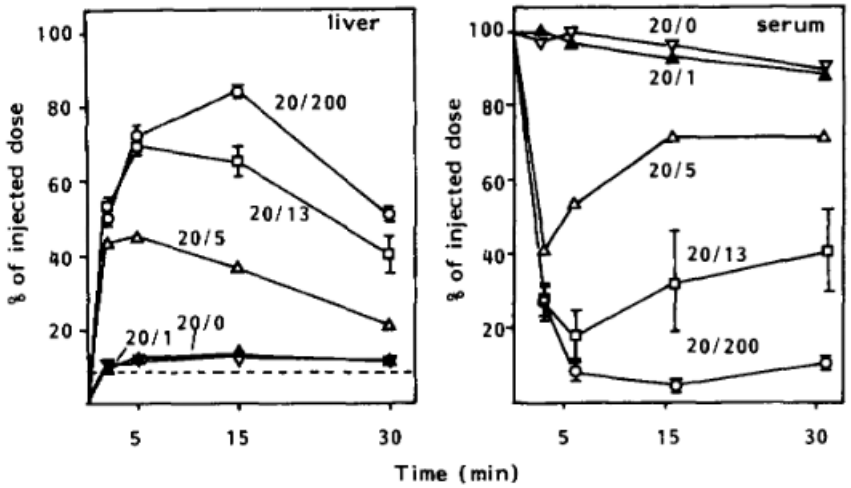


Diagram I. The structure of Tris-Gal-Chol.

Effect of Tris-Gal-Chol on the liver association and serum decay of ^{125}I-LDL



- **"It is concluded that Tris-Gal-Chol incorporation into LDL leads to a markedly increased catabolism of LDL by the liver which might be used for lowering serum LDL levels."**
  TJ van Berkel et al., 1985 (PMID: 2579071)