ACS Webinars
CLICK • WATCH • LEARN • DISCUSS

ACS Chemistry for Life®

Have Questions?

Type them into questions box!

"Why am I muted?"
Don't worry. Everyone is muted except the presenter and host.
Thank you and enjoy the show.

@AmerChemSociety

@AmericanChemicalSociety

@AmerChemSociety

LinkedIn https://www.linkedin.com/company/american-chemical-society

Check out the ACS Webinar Library!

An ACS member exclusive benefit

ACS Chemistry for Life®

Hundreds of presentations from the best and brightest minds that chemistry has to offer are available to you on-demand. The Library is divided into 6 different sections to help you more easily find what you are searching.

Professional Development

▶ View the Collection

Learn how to write better abstracts, deliver more engaging presentations, and network to your next dream job. Brush up on your soft skills and set a new career path by mastering what can not be taught in the lab.

Technology & Innovation

▶ View the Collection

From renewable fuels to creating the materials for the technology of tomorrow, chemistry plays a pivotal role in advancing our world. Meet the chemists that are building a better world and see how their science is making it happen.

Drug Design and Delivery

▶ View the Collection

The Drug Design Delivery Series has built a collection of the top minds in the field to explain the mechanics of drug discovery. Discover the latest research, receive an overview on different fields of study, and gain insight on how to possibily overcome your own med chem roadblocks.

Culinary Chemistry

▶ View the Collection

Why does food taste better when it is grilled or what molecular compounds make a great wine? Discover the delectable science of your favorite food and drink and don't forget to come back for a second helping.

Popular Chemistry

▶ View the Collection

Feeling burdened by all that molecular weight? Listen to experts expound on the amazing side of current hot science topics. Discover the chemistry of rockets, how viruses have affected human history, or the molecular breakdown of a hangover.

Business & Entrepreneurship

▶ View the Collection

How do ideas make it from the lab to the real world? Discover the ins and outs of the chemical industry whether you are looking to start a business or desire a priceless industry-wide perspective.

https://www.acs.org/content/acs/en/acs-webinars/videos.html

3

3

Learn from the best and brightest minds in chemistry! Hundreds of webinars on diverse topics presented by experts in the chemical sciences and enterprise.

Edited Recordings are an exclusive ACS member benefit and are made available once the recording has been edited and posted.

Live Broadcasts of ACS Webinars® continue to be available to the general public several times a week generally from 2-3pm ET!

A **collection of the best recordings** from the ACS Webinars Library will occasionally be rebroadcast to highlight the value of the content.

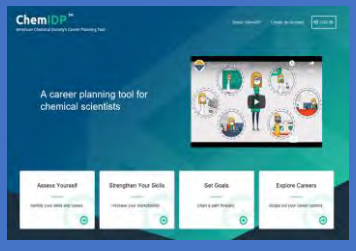
www.acs.org/acswebinars

4

4

Advance YOUR CAREER

ChemIDP™



ChemIDP.org

Discover ACS PUBLICATIONS

Publishing Resources

ACS REVIEWER LAB™

ACS axial

ACS AUTHOR UNIVERSITY

ACS Authoring Services

publish.acs.org

Connect WITH CHEMISTS AND OTHER SCIENCE PROFESSIONALS

CAS SciFinder Future Leaders

171 alumni, 35 countries and over 120 institutions

acsoncampus.acs.org/resources

ACS Chemistry for Life®

CAS A DIVISION OF THE AMERICAN CHEMICAL SOCIETY

ACS Publications Most Trusted. Most Cited. Most Read.

ACS ON CAMPUS

5

5

From ACS Industry Member Programs

- **Industry Matters Newsletter**

  ACS Member-only weekly newsletter with exclusive interviews with industry leaders and insights to advance your career.

  Preview & Subscribe: acs.org/indnews

- in ACS Innovation Hub

  Connect, collaborate, and stay informed about the trends leading chemical innovation

  Join: bit.ly/ACSinnovationhub

6

ACS Career Navigator: Your Home for Career Services

Whether you are just starting your journey, transitioning jobs, or looking to brush up or learn new skills, the **ACS Career Navigator** has the resources to point you in the right direction.

We have a collection of career resources to support you during this global pandemic:


Professional Education


Virtual Career Consultants

ACS Leadership Development System


Career Navigator LIVE!


ChemIDP


College to Career

ACS Webinars

Virtual Classrooms

Visit www.ACS.org/COVID19-Network to learn more!

7

7

8


NATIONAL CHEMISTRY WEEK
FAST OR SLOW
Chemistry Makes It Go!
OCTOBER 17–23, 2021
#NCW
ACS Chemistry for Life
American Chemical Society
Visit www.acs.org/ncw
On Sunday, August 1st, 2021 discover how to get a big reaction out of your community for National Chemistry Week!
#NCW

9


Join us in our efforts to increase the diversity of chemistry.
Valued donors like you have sustained ACS educational programs that are welcoming students from diverse backgrounds into our profession.
www.acs.org/donate
ACS Office of Philanthropy
Chemistry for Life®

10

A Career Planning Tool For Chemical Scientists

ChemIDP is an Individual Development Plan designed specifically for graduate students and postdoctoral scholars in the chemical sciences. Through immersive, self-paced activities, users explore potential careers, determine specific skills needed for success, and develop plans to achieve professional goals. **ChemIDP** tracks user progress and input, providing tips and strategies to complete goals and guide career exploration.

https://chemidp.acs.org

11

11

ACS Bridge Program

Are you thinking of Grad School?

If you are from an underrepresented racial or ethnic group, we want to empower you to get your graduate degree!

The ACS Bridge Program offers:

- **A FREE common application that will highlight your achievements to participating Bridge Departments**
- **Resources to help write competitive grad school applications and connect you with mentors, students, and industry partners!**

Learn more and apply at www.acs.org/bridge

Email us at bridge@acs.org

12

12

ACS Department of Diversity Programs

Advancing ACS's Core Value of Diversity, Inclusion & Respect

We believe in the strength of diversity in all its forms, because inclusion of and respect for diverse people, experiences, and ideas lead to superior solutions to world challenges and advances chemistry as a global, multidisciplinary science.

Contact Us:
https://app.suggestionox.com/r/DI_R
Diversity@acs.org

@ACSDiversity

ACS Diversity

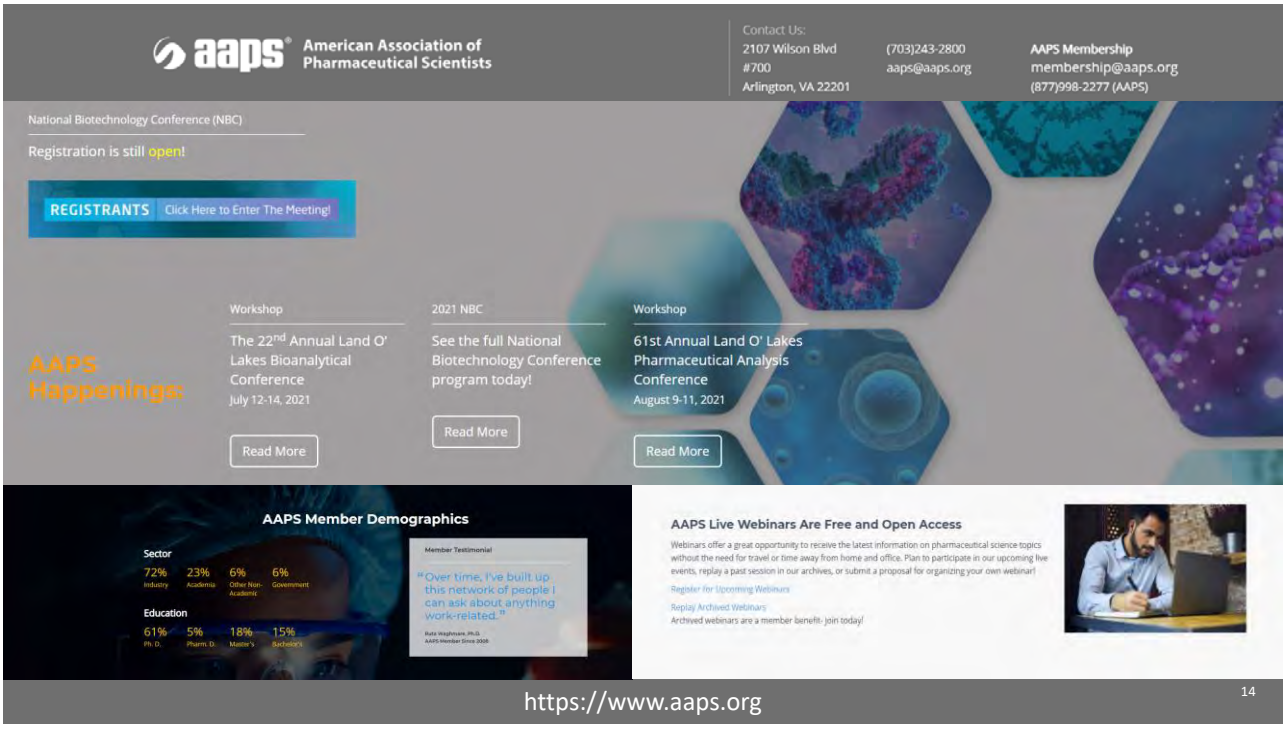
ACS Voices Diversity Podcast

acsvoices.podbean.com/

www.acs.org/diversity

13

13



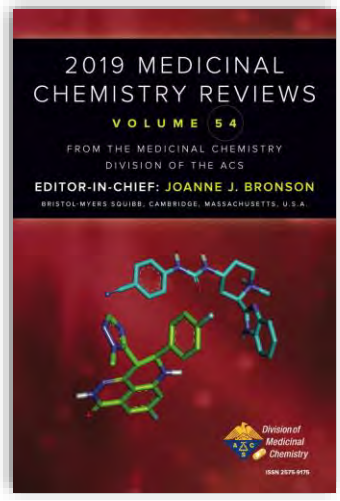
https://www.aaps.org

14

14

Join the Division Today!

ACS Technical Division
Medicinal Chemistry (MEDI)



For $25 membership ($10 for students)**, You Will Receive:**

- A free digital copy of our annual medicinal chemistry review volume (over 680 pages, $160 retail price)
- Abstracts of MEDI programming at national meetings
- Access to student travel grants and fellowships

Find out more about the ACS MEDI Division! www.acsmedchem.org

15

15

ACS Webinars
CLICK • WATCH • LEARN • DISCUSS

ACS Chemistry for Life®

ORGANIZERS


Annette Bak
AstraZeneca


Lorraine Clark
ACS Publications


Patrice L. Jackson-Ayotunde
Univ. of Maryland Eastern Shore


Anirban Mahapatra
ACS Publications


Nicholas Meanwell
Bristol-Myers Squibb


John Morrison
The Janssen Pharmaceutical Companies of Johnson & Johnson


Nurulain Zaveri
Astraea Therapeutics

2021 Drug Design and Delivery Series

We are continuing the theme of last year's symposium and will feature more of the most innovative and revolutionary ideas in drug design and delivery. **This year we have decided to increase the duration of each broadcast for an additional 30 minutes in the hope to dive deeper into each topic as well as answer more of your questions.** The details for upcoming broadcasts will be posted as they are finalized.

CO-PRODUCED WITH

ACS Technical Division Medicinal Chemistry (MEDI)

aaps American Association of Pharmaceutical Scientists

ACS Publications Most Trusted. Most Cited. Most Read.

SPEAKERS FROM

Bristol-Myers Squibb · GSK · CARMOT · Pfizer · HEPTARES therapeutics · Genentech A Member of the Roche Group · MERCK · MONASH University

https://www.acs.org/content/acs/en/acs-webinars/drug-discovery.html

16

16

ACS Webinars
CLICK • WATCH • LEARN • DISCUSS

ACS Chemistry for Life®

Date: Wednesday, August 4, 2021 @ 2-3pm ET
Speakers: Andre Argenton, Dow / Scott Collick, DuPont Mobility & Materials / Adwoa Baah-Dwomoh, W.L. Gore & Associates
Moderator: Rebekah Paul, American Chemical Society

Register for Free!

What You Will Learn:

- The variety of ways companies promote diversity, equity, inclusion, and respect
- What programs companies are using to drive change
- Opportunities for the next generation workforce within industry

Co-produced with: ACS External Affairs & Communications and ACS Industry Member Programs

Date: Thursday, August 5, 2021 @ 2-3pm ET
Speaker: Patricia Simpson, Game Changing Etiquette and the University of Illinois at Urbana-Champaign
Moderator: Matt Grandbois, DuPont Electronics & Industrial

Register for Free!

What You Will Learn:

- Why is business acumen important for all employees
- What are the core elements of a business acumen
- How to develop or enhance your business acumen

Co-produced with: ACS Division of Professional Relations

Date: Wednesday, August 11, 2021 @ 2-3pm ET
Speakers: Jim Skinner, Terregena Inc. and ACS SCHB and H.N. Cheng, 2021 ACS President and US Department of Agriculture
Moderator: Diane Grob Schmidt, 2015 ACS President

Register for Free!

What You Will Learn:

- How to organize your team during the early stages
- How to organize the structure of your company during the early stages
- How your team and organizational structure may evolve in the growth and maturity stages in order to address challenges and opportunities

Co-produced with: ACS Industry Member Programs, ACS President-Elect, ACS Board Committee on Corporation Associates, ACS Committee on Technician Affairs, the ACS Division of Small Chemical Businesses, and the ACS Division of Business Development and Management

www.acs.org/acswebinars

17

17

ACS Chemistry for Life®
ACS Technical Division Medicinal Chemistry (MEDI)
ACS Publications Most Trusted. Most Cited. Most Read.
aaps

Targeted Delivery of
RNA-targeted Therapeutics

FREE Webinar | TODAY at 2pm ET

ACS Webinars
CLICK • WATCH • LEARN • DISCUSS

THIS ACS WEBINAR WILL BEGIN SHORTLY...

18

18

ACS Webinars
CLICK • WATCH • LEARN • DISCUSS

ACS Chemistry for Life®

Targeted Delivery of RNA-targeted Therapeutics

PUNIT SETH
Vice President, Medicinal Chemistry,
Ionis Pharmaceuticals

CHARVI NANAVATI
Associate Director, Clinical Pharmacology
and Pharmacokinetics,
Ionis Pharmaceuticals

Presentation slides available now! Today's recording will be made available to all registrants for 24 hours before moving to www.acs.org/acswebinars as an exclusive member benefit.

www.acs.org/acswebinars

This ACS Webinar is co-produced with the ACS Division of Medicinal Chemistry, American Association of Pharmaceutical Scientists, and ACS Publications.

19

19

20

Nucleic acid drugs can work through multiple mechanisms

- Nucleic acid therapeutics which bind RNA by Watson-Crick base pairing *and*
  - Promote degradation of RNA
    - RNase H – single stranded (ss) DNA ASOs
    - siRNA – double stranded (ds) and ssRNA ASOs
  - Do not promote degradation of RNA
    - Splice modulation – ssASOs with variable chemistry
    - miRNA antagonists – ssASOs with variable chemistry
    - mRNA editing – ssASOs with variable chemistry
    - Translational arrest – ssASOs with variable chemistry
- Nucleic acid therapeutics which bind to DNA by Watson-Crick base pairing
  - CRISPR/CAS9 for gene editing
- mRNA that are translated to therapeutic proteins
  - Vaccines, protein replacement, gene editing
- Aptamers and immuno-modulatory oligonucleotides that bind to protein targets

21

Nucleic acid drugs approved by regulatory agencies – *many targets considered undruggable by traditional drug discovery platforms*

Name	Disease	Target	Mechanism	Chemistry	Sponsor	Approved
Vitravene™	CMV retinitis	Viral RNA	RNaseH1	PS DNA	Ionis/Novartis	1998
Macugen™	AMD	VEGF	Aptamer	F/OMe	Eyetech/Pfizer	2004
Kynamro™	High cholesterol	ApoB100	RNaseH1	PS MOE/DNA	Ionis/Genzyme	2013
Spinraza™	SMA	SMN2	RNA splicing	PS MOE	Ionis/Biogen	2016
Exondys™	DMD	Dystrophin	RNA splicing	PMO	Sarepta	2016
Tegsedi™	TTR amyloidosis	Transthyretin	RNaseH1	PS MOE/DNA	Ionis/Akcea	2018
Onpattro™	TTR amyloidosis	Transthyretin	siRNA	RNA/OMe/LNP	Alnylam	2018
Waylivra™	High triglycerides	ApoCIII	RNaseH1	PS MOE/DNA	Ionis/Akcea	2019
Givosiran™	Hepatic porphyria	ALAS1	siRNA	F/OMe/GalNAc	Alnylam	2019
Vyondys™	DMD	Dystrophin	RNA splicing	PMO	Sarepta	2019
Viltepso™	DMD	Dystrophin	RNA splicing	PMO	NS Pharma	2020
Oxlumo™	Hyperoxaluria	Glycolate oxidase	siRNA	F/OMe/GalNAc	Alnylam	2020
Leqvio™	High cholesterol	PCSK9	siRNA	F/OMe/GalNAc	Alnylam	2020
Vaccine	COVID	Virus antigen	mRNA	RNA/LNP	Moderna	2020
Vaccine	COVID	Virus antigen	mRNA	RNA/LNP	BioNTech/Pfizer	2020

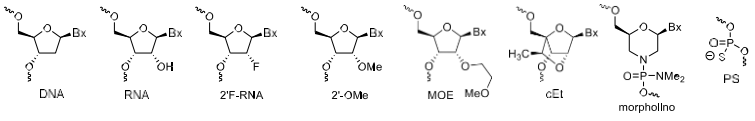
>100 nucleic acid-based drugs currently in clinical development

22

Representative oligonucleotide designs used in the clinic

Design	Compound	Design	Mechanism
	Spinraza	Full PS MOE	RNA splicing
	Exondys	Full PMO	RNA splicing
	Tegsedi	PS MOE gapmer	RNaseH1
	Gen 2.5	PS cEt gapmer	RNaseH1
	Onpattro	siRNA/LNP	Ago2
GalNac	Givlarri	siRNA/GalNAc	Ago2

PS PMO MOE Morpholino cEt DNA RNA OMe 2'F



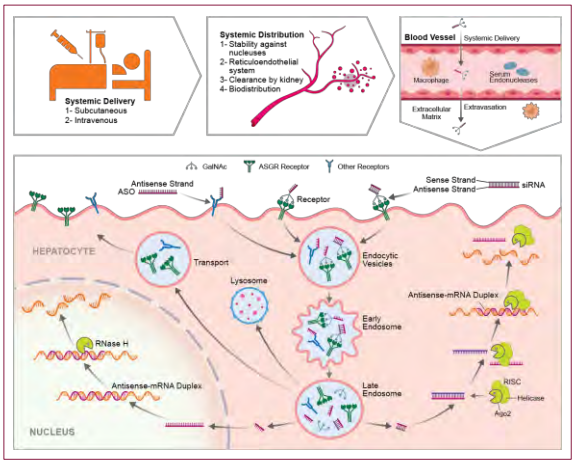
Gokirmak et al, *Trends Pharmacol. Sci.* 2021

23

Tissue and cellular barriers to effective delivery of nucleic acid therapeutics

- Nuclease-mediated degradation in plasma and tissue
- Kidney filtration
- Scavenging by RES
- Passage across the capillary endothelium
- Entry into cells
- Escape from endo-lysosomal compartments
- Bind to targeted RNA and recruit effector mechanism

Seth et al, *J Clin Invest.* 2019,*129*, 915-925



Gokirmak et al, *Trends Pharmacol. Sci*, 2021, 588

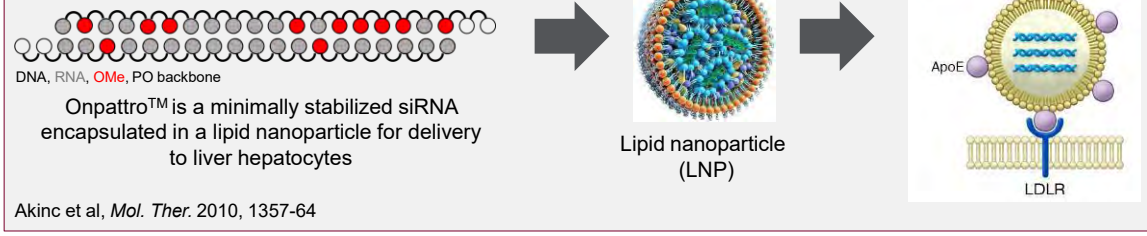
24

Strategies for effective delivery of nucleic acid therapeutics

- Delivery using lipid nanoparticles (LNPs)
  - Minimally modified siRNA and mRNA
- Delivery of chemically modified nucleic acids therapeutics
  - Passive delivery by enhancing association with plasma and cell-surface proteins
    - Delivery to the CNS following injection into the CSF
    - Delivery to the lung using aerosols
  - Active delivery by targeting specific cell-types
    - ASGR-mediated delivery to hepatocytes
    - GLP1R-mediated delivery to pancreatic beta cells
    - TfR1-mediated delivery to skeletal muscle and heart

25

Delivery of minimally modified siRNA and mRNA using LNPs



Akinc et al, *Mol. Ther.* 2010, 1357-64

- LNPs used to deliver mRNA vaccines for COVID
- LNPs also used to deliver chemically modified sgRNA and Cas9 mRNA for gene editing in the liver

Schoenmaker et al, *Int. J. Pharmaceutics*, 2021, 120586
Yin et al, *Nat. Biotechnol*. 2017, 1179

26

Quiz

- Which modifications are used to enhance the drug-like properties of therapeutic siRNA
  - 2'-*O*-Methyl
  - 2'-F
  - PS

- Correct answer – all of the above

27

27

Delivery of chemically modified nucleic acid therapeutics

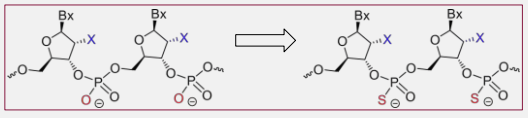
1. Passive delivery by enhancing association with plasma and cell-surface proteins
2. Active delivery by targeting specific cell-types

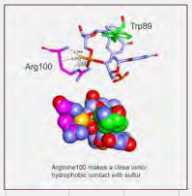
28

28

The phosphorothioate (PS) modification – essential component of nucleic acid therapeutics

- Phosphorothioate (PS) backbone
  - Improves metabolic stability for all classes of nucleic acid therapeutics
  - Supports RNaseH1 activity
  - Enhances binding to proteins
    - Plasma, cell surface and intra-cellular
- Proteins interact with anionic sulfur in PS by electrostatic and hydrophobic interactions
  - Often times with the same amino acid
- Protein binding is directly proportional to PS content
  - Suggests an avidity model where each PS contributes a small percentage to overall binding affinity





ASO	#PS
GoᵐCoAoToGoToToᵐCoToᵐCoAoᵐCoAoToToA	0
GsᵐCoAoToGoToToᵐCoToᵐCoAoᵐCoAoToTsA	2
GoᵐCsAoToGoTsToᵐCoToᵐCsAoᵐCoAoTsToA	4
GsᵐCoAsToGoTsToᵐCsToᵐCoAsᵐCoAsToToA	6
GsᵐCsAoToGsToTsᵐCsToᵐCsAoᵐCsAoToTsA	8
GsᵐCsAoTsGsToTsᵐCsToᵐCsAsᵐCoAsTsToA	10
GsᵐCsAsToGsTsTsᵐCoTsᵐCsAsᵐCoAsTsTsA	12
GsᵐCsAsTsGsTsTsᵐCsTsᵐCsAsᵐCoAsTsTsA	14
GsᵐCsAsTsGsTsTsᵐCsTsᵐCsAsᵐCsAsTsTsA	15

IC50 (nM)

	PO	PS2	PS4	PS6	PS8	PS10	PS12	PS14	PS16
NCL	1003.0	806.5	429.5	56.23	5.41	1.22	0.23	0.01	0.01
PC4	163.5	163.0	75.7	27.8	5.7	3.9	0.8	0.4	0.3
FUS	1003.0	1003.0	244.5	44.57	12.92	4.47	0.83	0.22	0.71
SFPQ	1003.0	1003.0	1003.0	89.9	15.5	6.2	1.4	0.5	1.0
SSBP	1003.0	544.7	64.5	13.7	5.8	7.2	0.4	0.3	0.7
RNAse H1	1003.0	1003.0	1003.0	316.8	77.1	12.8	2.2	0.4	1.0
La	1003.0	539.4	82.3	96.7	38.5	13.4	2.7	0.9	1.9
LRPPRC	1003.0	1003.0	1003.0	1003.0	1003.0	100.3	2.4	1.4	1.0
P54	1003.0	1003.0	1003.0	1003.0	495.7	158.4	36.0	7.5	15.5

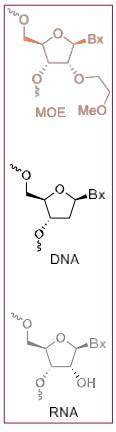
Hyjek, JACS 2020; Vickers, JACS, 2020; Crooke, JACS, 2020

29

29

PS ASOs bind to plasma proteins which facilitates distribution in animals

ASO Design and Sequence	K_d (μM)					
	Albumin	Transferrin	IgG	Fibrinogen	A2M	HRG
CTGCTAGCCTCTGGATTTGA	3.8	2.3	0.9	0.4	0.015	0.002
CTGCTAGCCTCTGGATTTGA	10.4	7.3	0.9	0.3	0.044	0.009
CTGCTAGCCTCTGGATTTGA	26.9	11.6	3.6	1.8	0.051	0.10
CTGCTAGCCTCTGGATTTGA	81.6	34.2	5.9	4.2	0.072	0.014
TTTTTTTTTTTTTTTTTTTT	0.94	2.6	1	0.26	0.019	0.017
AAAAAAAAAAAAAAAAAAAA	204.5	277.6	74.2	13.4	3.0	0.041
CTGCTAGCCTCTGGATTTGA GACGAUCGGAGACCUAAACU	762.9	460	>500	> 75	> 1	> 1
CTGCTAGCCTCTGGATTTGA GACGAUCGGAGACCUAAACU	130.2	34.4	1.8	0.7	> 1	0.045



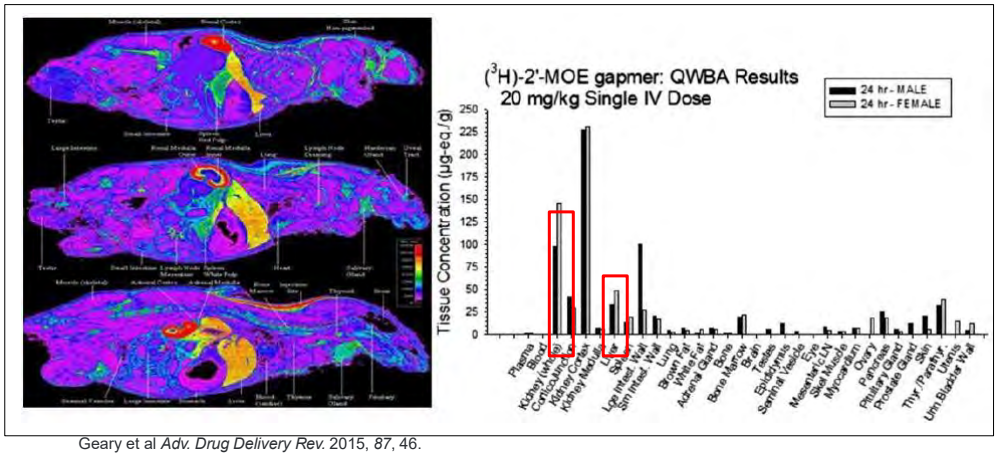
MOE, DNA, RNA, All ASOs have PS backbone except underlined letters which are PO

Gaus, et al (2018) *Nucleic Acids Res.*, **47**, 1110-1122.

30

30

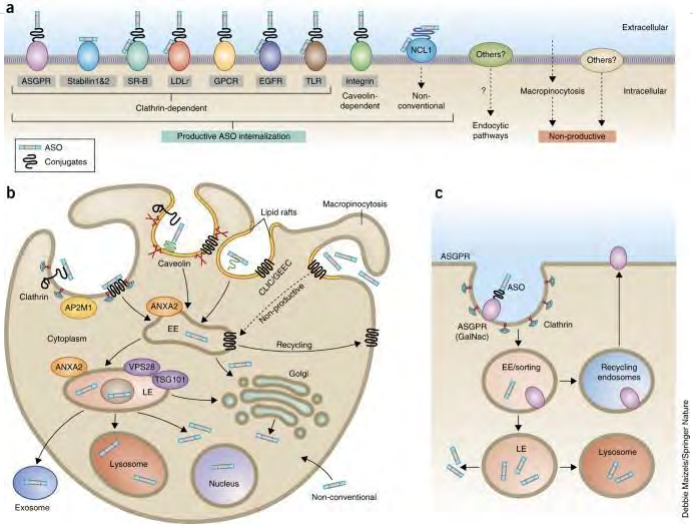
PS ASOs distribute broadly after systemic injection (but accumulate preferentially in the liver and the kidney)

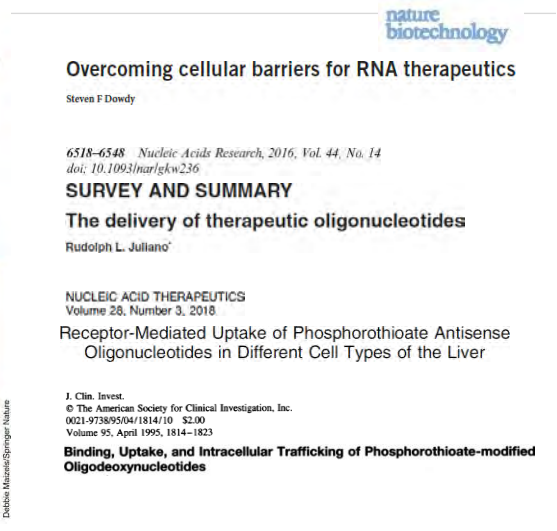


Geary et al *Adv. Drug Delivery Rev.* 2015, *87*, 46.

PS ASOs bind to plasma and cell-surface proteins which facilitates their distribution into tissues in animals

PS ASOs interact with several classes of cell-surface proteins which can facilitate cellular uptake




nature biotechnology

Overcoming cellular barriers for RNA therapeutics

Steven F Dowdy

6518–6548 *Nucleic Acids Research, 2016, Vol. 44, No. 14*
doi: 10.1093/nar/gkw236

SURVEY AND SUMMARY

The delivery of therapeutic oligonucleotides

Rudolph L. Juliano*

NUCLEIC ACID THERAPEUTICS
Volume 28, Number 3, 2018

Receptor-Mediated Uptake of Phosphorothioate Antisense Oligonucleotides in Different Cell Types of the Liver

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/95/04/1814/10 $2.00
Volume 95, April 1995, 1814–1823

Binding, Uptake, and Intracellular Trafficking of Phosphorothioate-modified Oligodeoxynucleotides

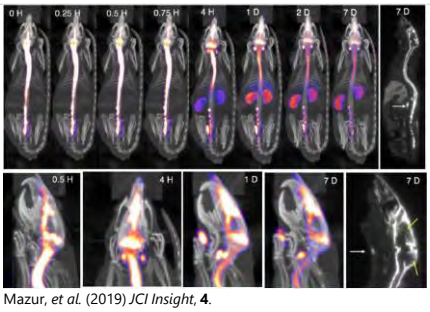
Crooke, (2017) Cellular uptake and trafficking of antisense oligonucleotides. *Nat Biotech*, **35**, 230-237.

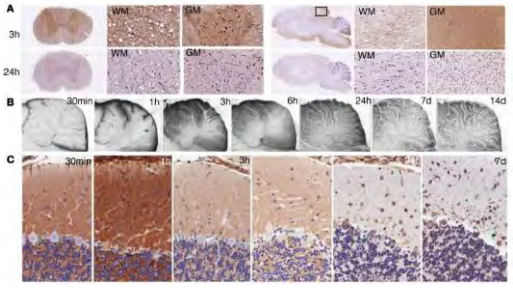
Passive delivery by enhancing association with plasma and cell-surface proteins

1. Delivery of Gen 2 ASO to CNS following injection into the CSF
2. Delivery of Gen 2.5 ASOs to lung following aerosol delivery

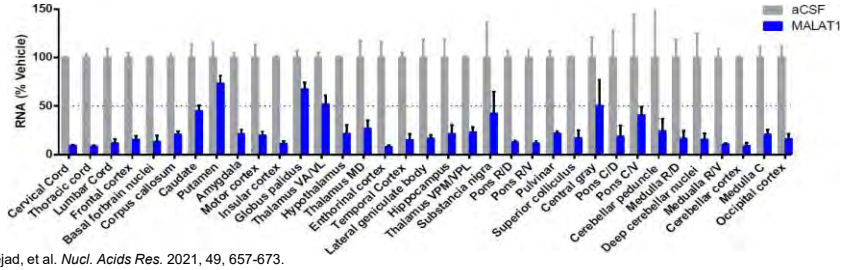
33

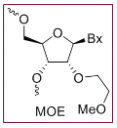
Protein binding properties of PS ASOs facilitate ASO distribution and uptake into the CNS following delivery into the CSF





Mazur, *et al.* (2019) *JCI Insight*, **4**.





Jafar-nejad, et al. *Nucl. Acids Res.* 2021, 49, 657-673.

34

Quiz

- What are the key determinants for interaction of PS oligonucleotides with proteins
  - Number of PS
  - Flexibility
  - Lack of bulky 2'-modifications

- Correct answer is all of the above

35

35

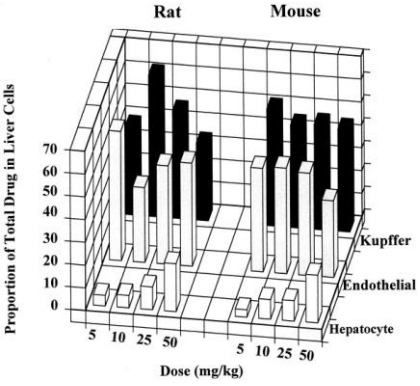
Active delivery by targeting specific cell-types

1. ASGR-mediated delivery to hepatocytes
2. GLP1R-mediated delivery to pancreatic beta cells
3. TfR1-mediated delivery to skeletal muscle and heart

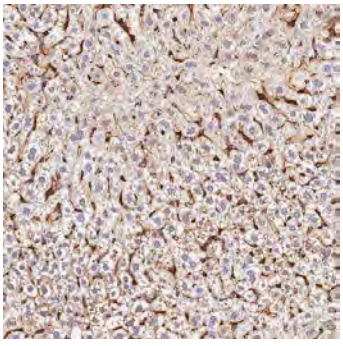
36

36

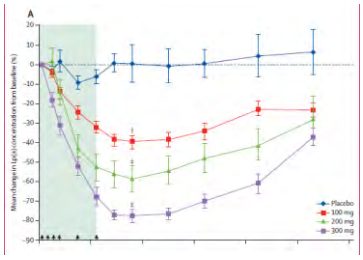
PS ASOs distribute to all cell-types in the liver but accumulate preferentially in non-parenchymal cells



Graham et al *Biochem. Pharmacol.* 2001, 62, 297
Butler et al *Lab. Invest.* 1997, 77, 379.
Bijsterbosch et al *Nucleic Acids Res.* 1997, *25*, 3290.
Donner et al *Nucleic Acid Ther.*, 2017, **27**, 209-220.



NPC's constitute <10% of liver mass
Hepatocytes account for 80% of liver mass



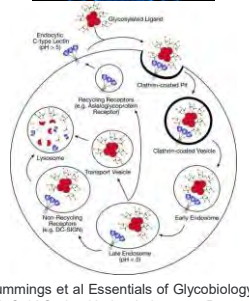
Dose-dependent reduction of hepatocyte expressed targets

PS ASOs are internalized into different cell types in the liver via receptors such as Stabilins, EGFR and ASGR

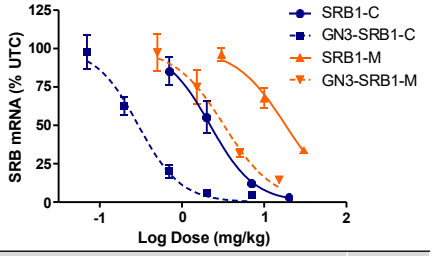
37

37

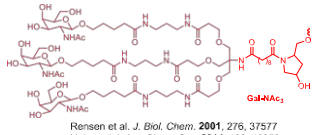
ASGR mediated delivery into hepatocytes enhances ASO potency 10-60 fold for gene targets expressed in hepatocytes



Cummings et al Essentials of Glycobiology. 2nd Ed. Cold Spring Harbor Laboratory Press; 2009. Chapter 31.



ASO (5'-3')	ED_{50} (mg/kg)
GCTTCAGTCATGACTTCCTT	18.3
GCTTCAGTCATGACTTCCTT-Tri-GalNAc	3.3
TCAGTCATGACTTC	2.2
TCAGTCATGACTTC-Tri-GalNAc	0.3



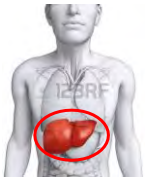
Rensen et al. *J. Biol. Chem.* **2001**, 276, 37577
Nair et al *J. Am. Chem. Soc.* **2014**, 136, 16958

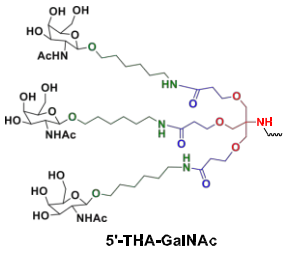
Prakash, *et al.* (2014) Targeted delivery of antisense oligonucleotides to hepatocytes using triantennary N-acetyl galactosamine improves potency 10-fold in mice. *Nucleic Acids Res.*, **42**, 8796-8807 – ***NAR Breakthrough Article***; Ostergaard et al (2015) *Bioconjug. Chem.*, **26**, 1451-1455; Prakash, *et al.* (2015) *Bioorg. Med. Chem. Lett.*, **25**, 4127-4130; Migawa, et al (2016) *Bioorg. Med. Chem. Lett.*, **26**, 2194-2197; Yu, *et al.* (2016) *Mol Ther Nucleic Acids*, **5**, e317; Prakash, *et al.* (2016) *J. Med. Chem.*, **59**, 2718-2733; Shemesh, *et al.* (2016) *Mol Ther Nucleic Acids*, **5**, e319; Prakash et al (2016) *Bioorg. Med. Chem. Lett.*, **26**, 2817-2820; Yu, *et al.* (2016) *Nucleic Acid Ther.*, **26**, 372-380; Kinberger, *et al.* (2016) *Bioorg. Med. Chem. Lett.*, **26**, 3690-3693; Schmidt et al (2017) *Nucleic Acids Res.*, **45**, 2294-2306; Tanowitz et al (2017) Nucleic Acids Res. **45**, 12388-12400.

38

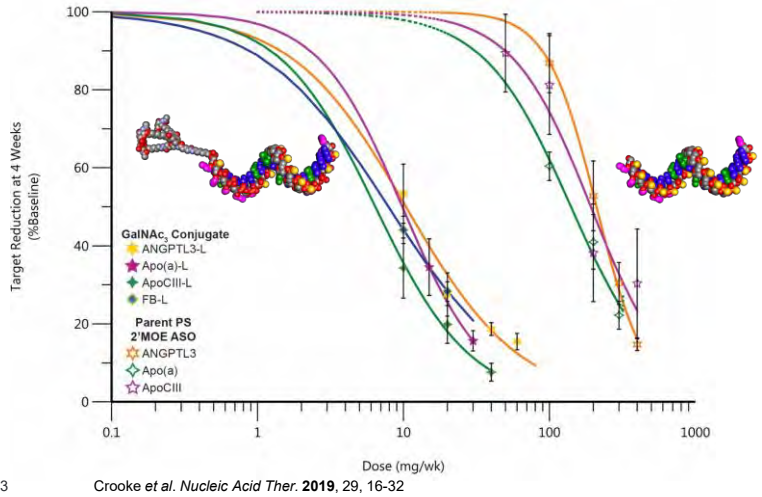
38

ASGR mediated delivery enhances ASO potency up to 30-fold in man for gene targets expressed in hepatocytes





Prakash, *et al.* (2016) *J. Med. Chem.*, **59**, 2718-2733



Crooke *et al. Nucleic Acid Ther.* **2019**, 29, 16-32

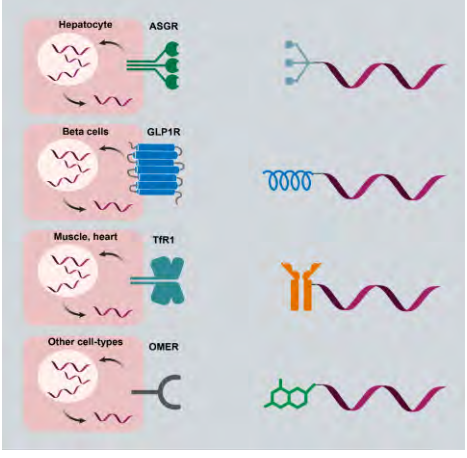
39

39

Ionis LICA Platform Continues to Expand

Multiple Tissues

- Liver
- Pancreas
- Muscle
- Many others in research stage



Multiple Ligands

- Carbohydrates
- Peptides
- Antibodies and FAB fragments
- Small molecules

40

40

Quiz

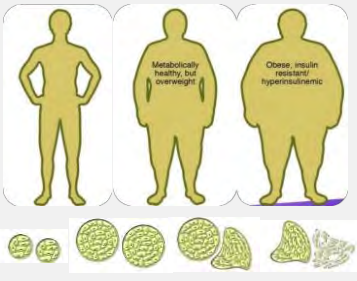
- What classes of receptors can be used for transport of oligonucleotides into cells
  - Lectins
  - GPCR
  - Nutrient
  - Scavenger

- Correct answer is all of the above

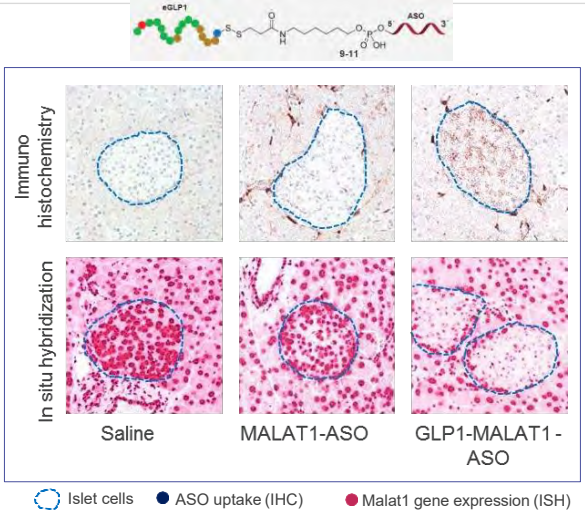
41

Targeted delivery of PS ASOs to pancreatic beta cells accomplished by targeting the GLP-1 receptor

Islets expand when insulin demand increases and when islets fail to adapt, diabetes develops



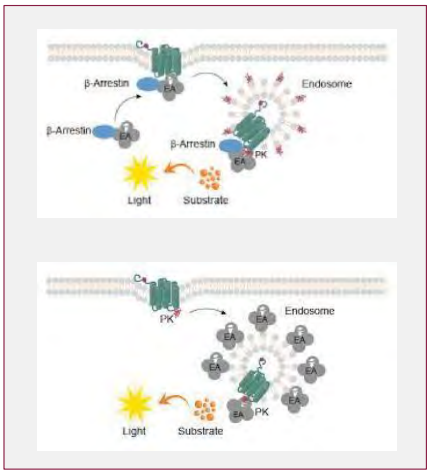
- Diabetes and obesity are global issues
- Majority of ᛒ cells destroyed when symptoms appear
- Blood glucose abnormalities = ᛒ cell ↓ 50%

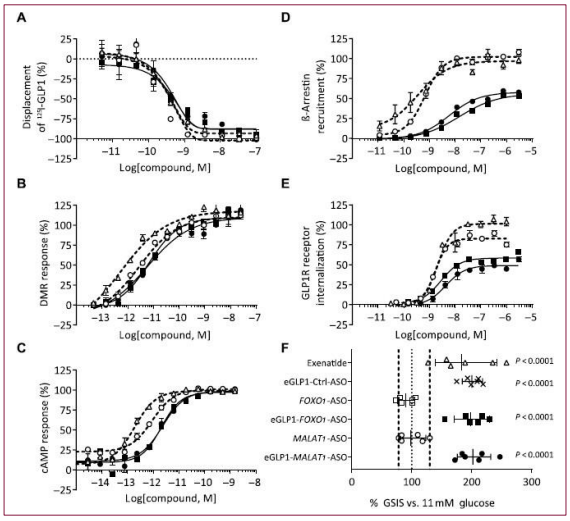


Ammala, *et al.* (2018) Targeted delivery of antisense oligonucleotides to pancreatic beta-cells. *Sci Adv*, **4**, eaat3386.

42

GLP1-peptide ASO conjugates behave as partial agonist of GLP1R





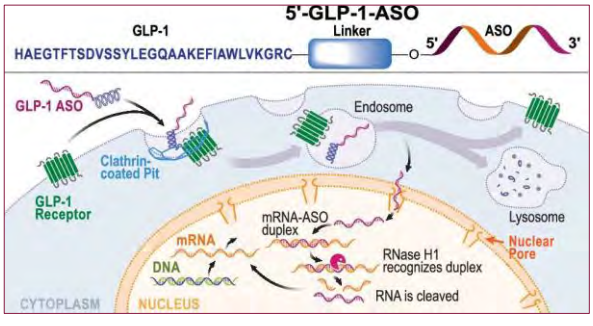
Peptide
ASO-Peptide

Ammala, *et al.* (2018) Targeted delivery of antisense oligonucleotides to pancreatic beta-cells. *Sci Adv*, **4**, eaat3386.

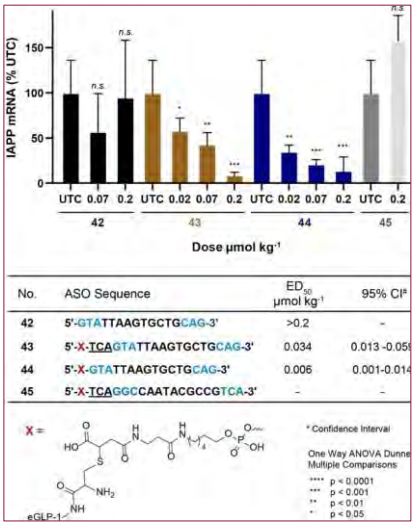
43

43

ASOs conjugated to GLP1 peptides show excellent potency for down regulating Islet Amyloid Polypeptide mRNA in pancreatic beta cells



Knerr et al, *JACS*, 2020, 3416



No.	ASO Sequence	ED_{50} µmol kg^{-1}	95% CI[a]
42	5'-GTATTAAGTGCTGCAG-3'	>0.2	-
43	5'-X-TCAGTATTAAGTGCTGCAG-3'	0.034	0.013 -0.05
44	5'-X-GTATTAAGTGCTGCAG-3'	0.006	0.001-0.01
45	5'-X-TCAGGCCAATACGCCGTCA-3'	-	-

X =

[a] Confidence Interval

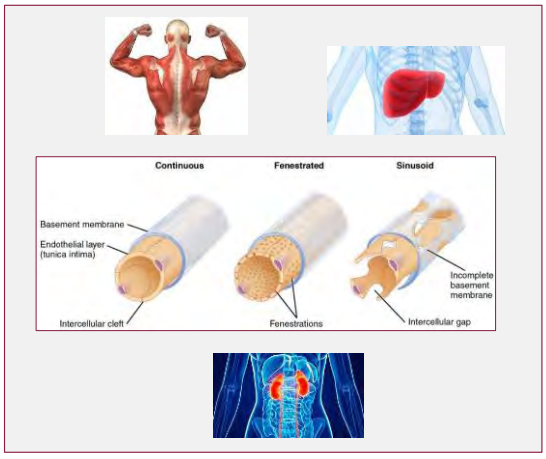
One Way ANOVA Dunnett Multiple Comparisons

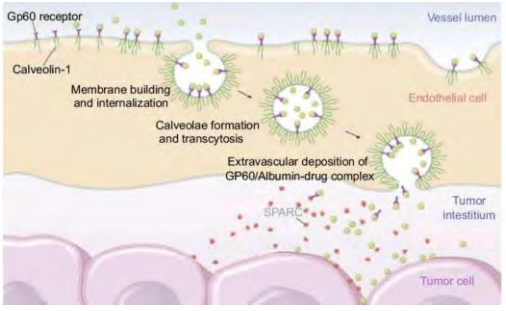
**** p < 0.0001
*** p < 0.001
** p < 0.01
* p < 0.05

44

44

Enhancing delivery to skeletal muscle tissues – traversing the capillary endothelium



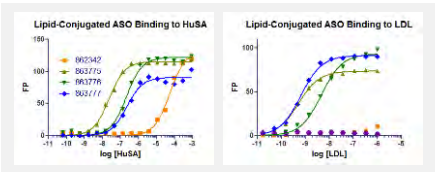


- Albumin and lipoproteins are efficiently transported across the capillary endothelium and 60% of total albumin is extra-vascular
- Albumin and lipoproteins bind lipids
- *Hypothesis – Can lipid conjugation enhance ASO association with albumin and lipoproteins and improve ASO potency in muscle tissues?*

45

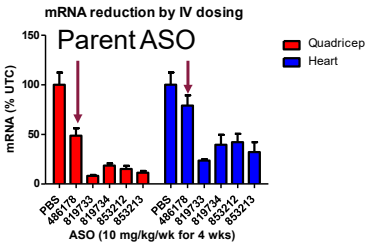
45

Lipid-conjugation enhances ASO activity in skeletal muscle and heart by facilitating ASO transport across the capillary endothelium

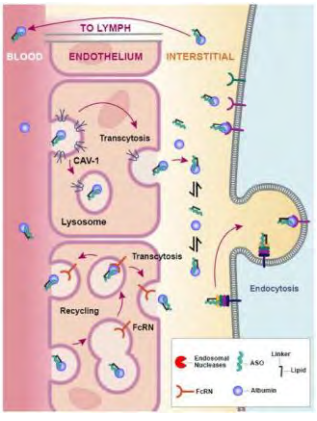


ION #	ASO (5' to 3')	HuSA Kd [nM]	LDL Kd [nM]
862342	GCATTCTAATAGCAGC	51,700	No binding
863775	Tocopherol-GCATTCTAATAGCAGC	23.8	0.5
863776	Palmitoyl-GCATTCTAATAGCAGC	217.9	4.6
863777	Cholesterol-GCATTCTAATAGCAGC-	227.1	0.6

Ostergaard, *et al.* (2019) *Nucleic Acids Res.*, **47**, 6045-6058.
Prakash, *et al.* (2019) *Nucleic Acids Res.*, **47**, 6029-6044.



ION No	Lipid
486178	none
819733	5'-TEG-Cholesterol
819734	5'-TEG-Tocopherol
853212	5'-TEG-C16
853213	5'-hexylamino-C16



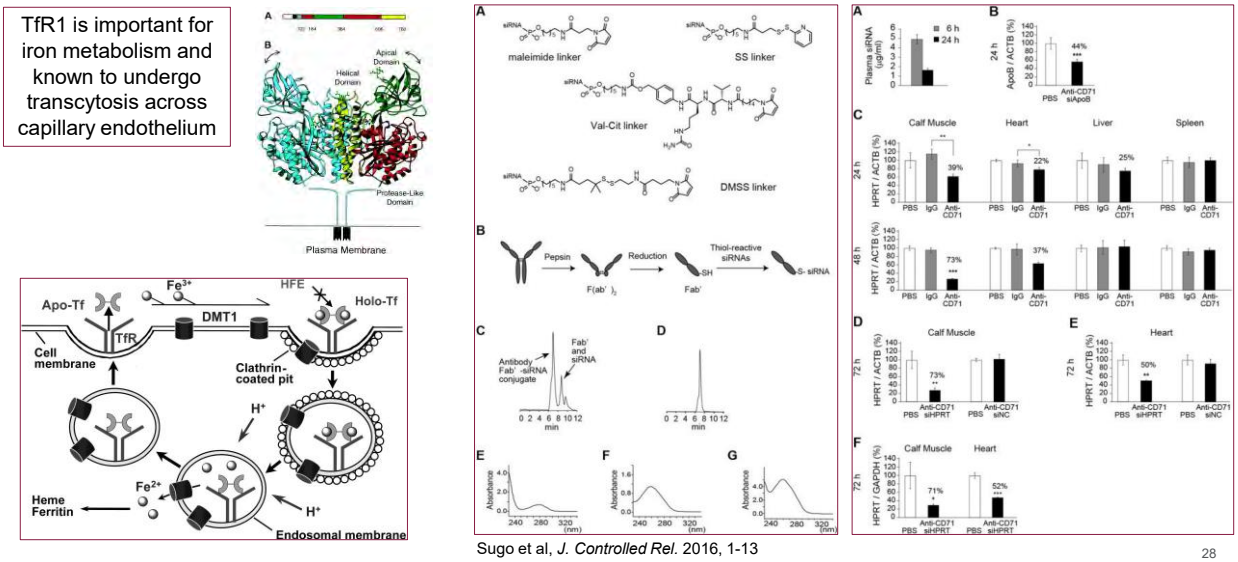
Chappell et al (2020) *Nucleic Acids Res.*, **48**, 4382

46

46

Targeting Transferrin receptor 1 for enhancing potency in skeletal muscle and heart tissues

TfR1 is important for iron metabolism and known to undergo transcytosis across capillary endothelium



Sugo et al, *J. Controlled Rel.* 2016, 1-13

28

47

Nucleic acid therapeutics need to traverse the capillary endothelium before uptake into parenchymal cells

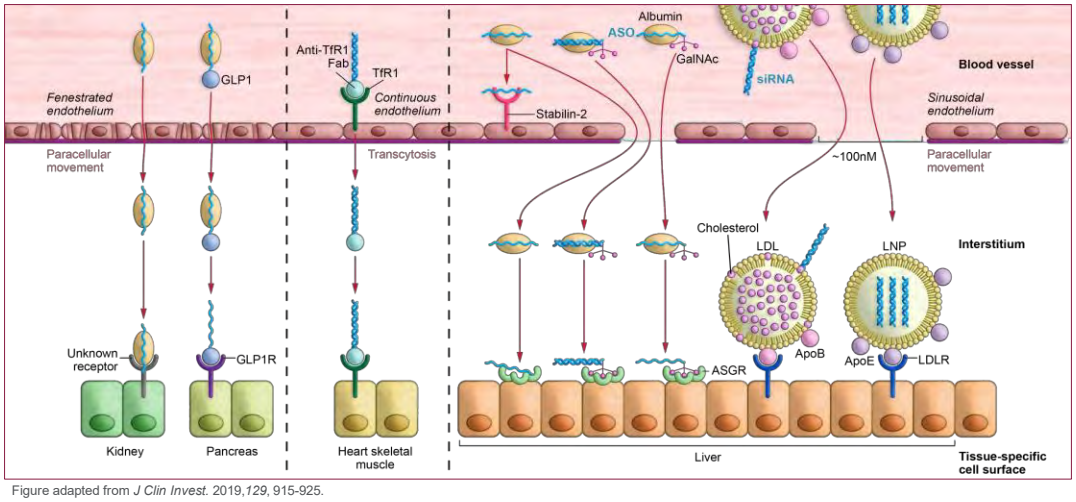


Figure adapted from *J Clin Invest.* 2019,*129*, 915-925.

48

48

Summary

- PS ASOs can be delivered to specific tissues and cell types by passive or active targeting strategies
- Protein binding properties of PS ASOs facilitates distribution and cellular uptake in animals
  - Plasma proteins that bind PS ASOs have been characterized
  - Several cell-surface proteins that internalize PS ASOs have been identified
- Targeted delivery to specific cell-types can be achieved using targeting ligands
  - GalNAc/ASGR; GLP1/GLP1R; antibodies/TfR1
  - Several additional receptors investigated for delivery of oligonucleotide cargo with promising initial results
- Actively advancing GLP1- and TfR1-targeting platforms into preclinical and late-stage drug discovery at Ionis

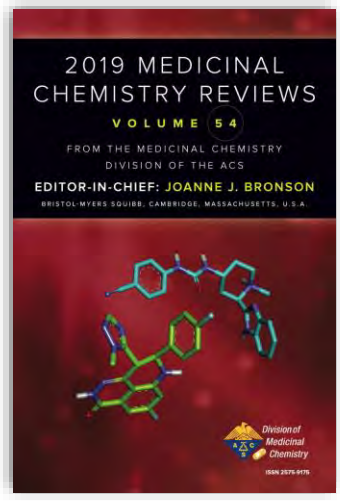
49

49

50

Join the Division Today!

ACS Technical Division
Medicinal Chemistry (MEDI)



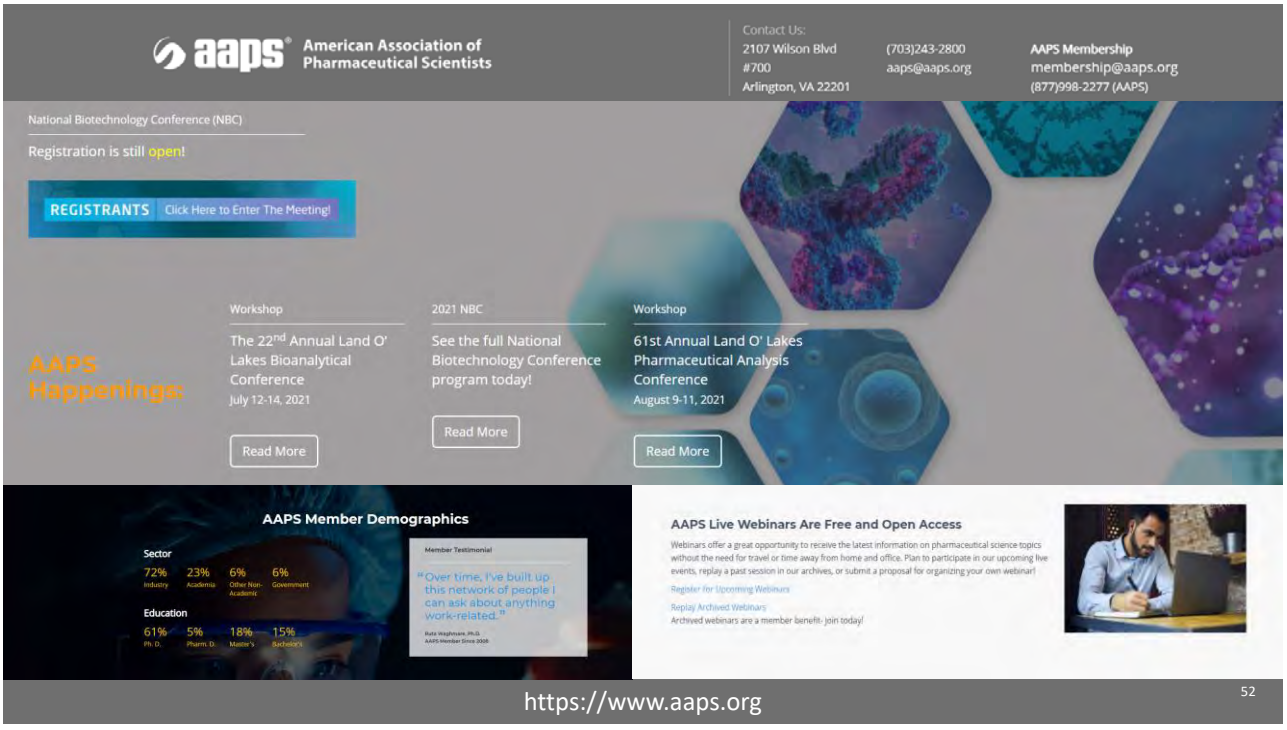
For $25 membership ($10 for students)**, You Will Receive:**

- A free digital copy of our annual medicinal chemistry review volume (over 680 pages, $160 retail price)
- Abstracts of MEDI programming at national meetings
- Access to student travel grants and fellowships

Find out more about the ACS MEDI Division! www.acsmedchem.org

51

51



https://www.aaps.org

52

52

ACS Webinars
CLICK • WATCH • LEARN • DISCUSS

ACS Chemistry for Life®

Targeted Delivery of RNA-targeted Therapeutics

PUNIT SETH
Vice President, Medicinal Chemistry,
Ionis Pharmaceuticals

CHARVI NANAVATI
Associate Director, Clinical Pharmacology and Pharmacokinetics,
Ionis Pharmaceuticals

Presentation slides available now! Today's recording will be made available to all registrants for 24 hours before moving to www.acs.org/acswebinars as an exclusive member benefit.

www.acs.org/acswebinars

This ACS Webinar is co-produced with the ACS Division of Medicinal Chemistry, American Association of Pharmaceutical Scientists, and ACS Publications.

53

53

Learn from the best and brightest minds in chemistry! Hundreds of webinars on diverse topics presented by experts in the chemical sciences and enterprise.

Edited Recordings are an exclusive ACS member benefit and are made available once the recording has been edited and posted.

Live Broadcasts of ACS Webinars® continue to be available to the general public several times a week generally from 2-3pm ET!

A **collection of the best recordings** from the ACS Webinars Library will occasionally be rebroadcast to highlight the value of the content.

www.acs.org/acswebinars

54

54

ACS Webinars
CLICK • WATCH • LEARN • DISCUSS

ACS Chemistry for Life®

Date: Wednesday, August 4, 2021 @ 2-3pm ET
Speakers: Andre Argenton, Dow / Scott Collick, DuPont Mobility & Materials / Adwoa Baah-Dwomoh, W.L. Gore & Associates
Moderator: Rebekah Paul, American Chemical Society

Register for Free!

What You Will Learn:

- The variety of ways companies promote diversity, equity, inclusion, and respect
- What programs companies are using to drive change
- Opportunities for the next generation workforce within industry

Co-produced with: ACS External Affairs & Communications and ACS Industry Member Programs

Date: Thursday, August 5, 2021 @ 2-3pm ET
Speaker: Patricia Simpson, Game Changing Etiquette and the University of Illinois at Urbana-Champaign
Moderator: Matt Grandbois, DuPont Electronics & Industrial

Register for Free!

What You Will Learn:

- Why is business acumen important for all employees
- What are the core elements of a business acumen
- How to develop or enhance your business acumen

Co-produced with: ACS Division of Professional Relations

Date: Wednesday, August 11, 2021 @ 2-3pm ET
Speakers: Jim Skinner, Terregena Inc. and ACS SCHB and H.N. Cheng, 2021 ACS President and US Department of Agriculture
Moderator: Diane Grob Schmidt, 2015 ACS President

Register for Free!

What You Will Learn:

- How to organize your team during the early stages
- How to organize the structure of your company during the early stages
- How your team and organizational structure may evolve in the growth and maturity stages in order to address challenges and opportunities

Co-produced with: ACS Industry Member Programs, ACS President-Elect, ACS Board Committee on Corporation Associates, ACS Committee on Technician Affairs, the ACS Division of Small Chemical Businesses, and the ACS Division of Business Development and Management

www.acs.org/acswebinars

55

55

ACS Webinars
CLICK • WATCH • LEARN • DISCUSS

ACS Chemistry for Life®

ACS Webinars® does not endorse any products or services. The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the American Chemical Society.

Contact ACS Webinars ® at acswebinars@acs.org

56

56

ACS Webinars
CLICK • WATCH • LEARN • DISCUSS

ACS Chemistry for Life®

Date: Wednesday, August 4, 2021 @ 2-3pm ET
Speakers: Andre Argenton, Dow / Scott Collick, DuPont Mobility & Materials / Adwoa Baah-Dwomoh, W.L. Gore & Associates
Moderator: Rebekah Paul, American Chemical Society

Register for Free!

What You Will Learn:

- The variety of ways companies promote diversity, equity, inclusion, and respect
- What programs companies are using to drive change
- Opportunities for the next generation workforce within industry

Co-produced with: ACS External Affairs & Communications and ACS Industry Member Programs

Date: Thursday, August 5, 2021 @ 2-3pm ET
Speaker: Patricia Simpson, Game Changing Etiquette and the University of Illinois at Urbana-Champaign
Moderator: Matt Grandbois, DuPont Electronics & Industrial

Register for Free!

What You Will Learn:

- Why is business acumen important for all employees
- What are the core elements of a business acumen
- How to develop or enhance your business acumen

Co-produced with: ACS Division of Professional Relations

Date: Wednesday, August 11, 2021 @ 2-3pm ET
Speakers: Jim Skinner, Terregena Inc. and ACS SCHB and H.N. Cheng, 2021 ACS President and US Department of Agriculture
Moderator: Diane Grob Schmidt, 2015 ACS President

Register for Free!

What You Will Learn:

- How to organize your team during the early stages
- How to organize the structure of your company during the early stages
- How your team and organizational structure may evolve in the growth and maturity stages in order to address challenges and opportunities

Co-produced with: ACS Industry Member Programs, ACS President-Elect, ACS Board Committee on Corporation Associates, ACS Committee on Technician Affairs, the ACS Division of Small Chemical Businesses, and the ACS Division of Business Development and Management

57

www.acs.org/acswebinars

57