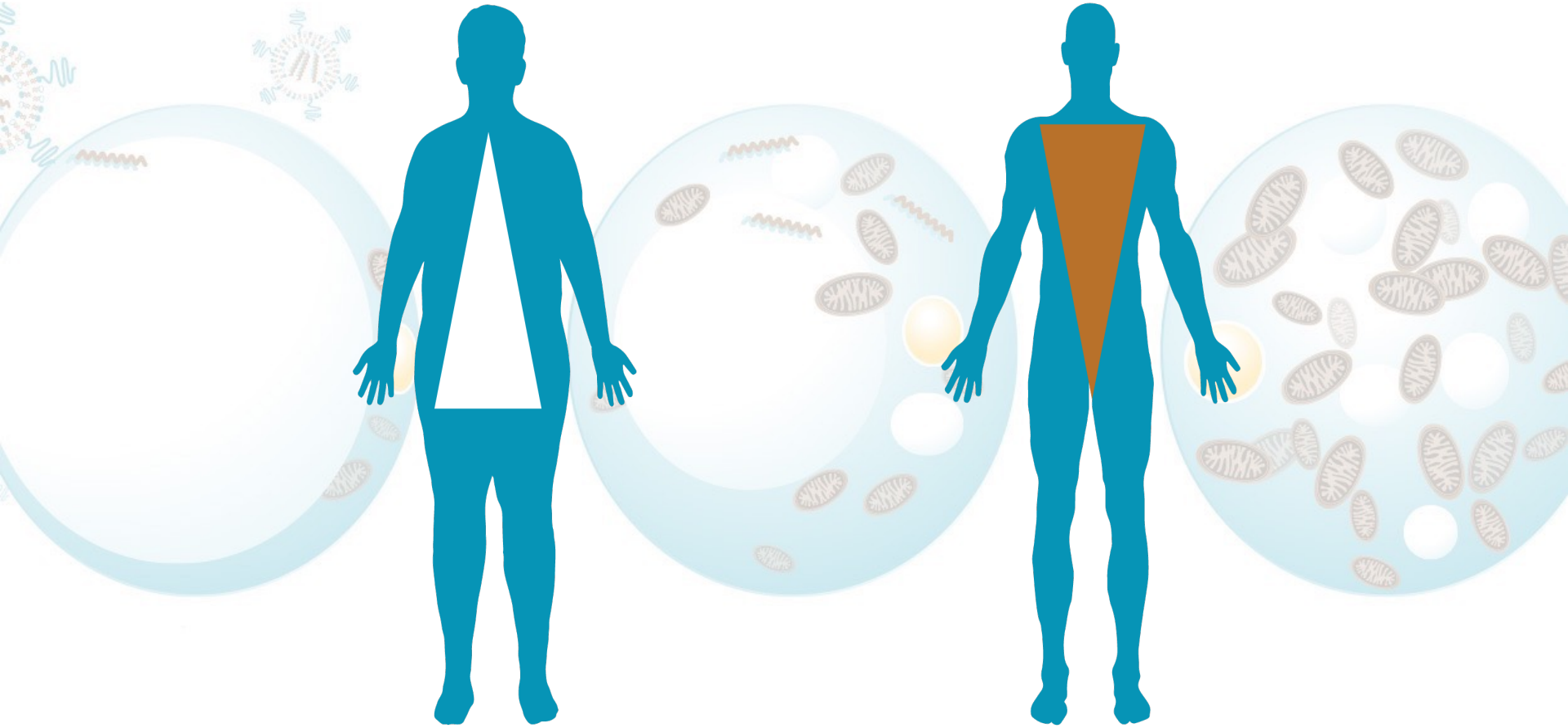
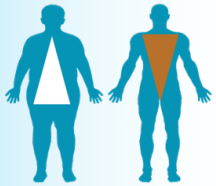
# Oligonucleotide Therapeutics with a Peptide Nucleic Acid Backbone to Treat Metabolic Pandemics



*Marc Thibonnier, M.D., M.Sc.*
*Founder & President, AptamiR Therapeutics, Inc.*

**2021 Oligonucleotide & Peptide Therapeutics TIDES USA Meeting, September 20-23, 2021**



# The Medical Problem

- **Obesity and Metabolic Dysfunction Associated Fatty Liver Disease** (MAFLD) are growing and costly **chronic pandemics** in need of safe, effective and convenient therapies

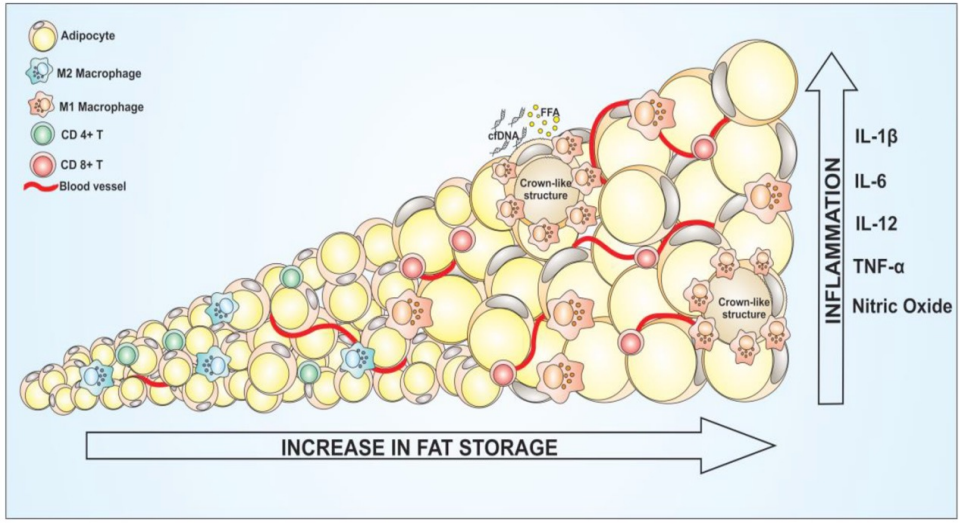
- **Obesity**
  - **Affects one third of the world population, including millions of children**
  - The economic burden of obesity on the healthcare systems is significant and growing (e.g. $190 billion or nearly 21% of annual medical spending in the United States)
  - Currently approved drugs offer limited efficacy with significant side effect profile and adverse events
  - Pharmacotherapy treatment rates of obesity are very low (<10%)
  - **Obesity worsens morbidity and mortality from COVID-19** (www.cdc.gov/obesity/data/obesity-and-covid-19.html)
  - **Adipocyte membranes are rich in the ACE2 docking protein for the SARS-CoV2 virus**
  - **The adipose tissues are a very large site of replication, refuge and shedding for that virus**

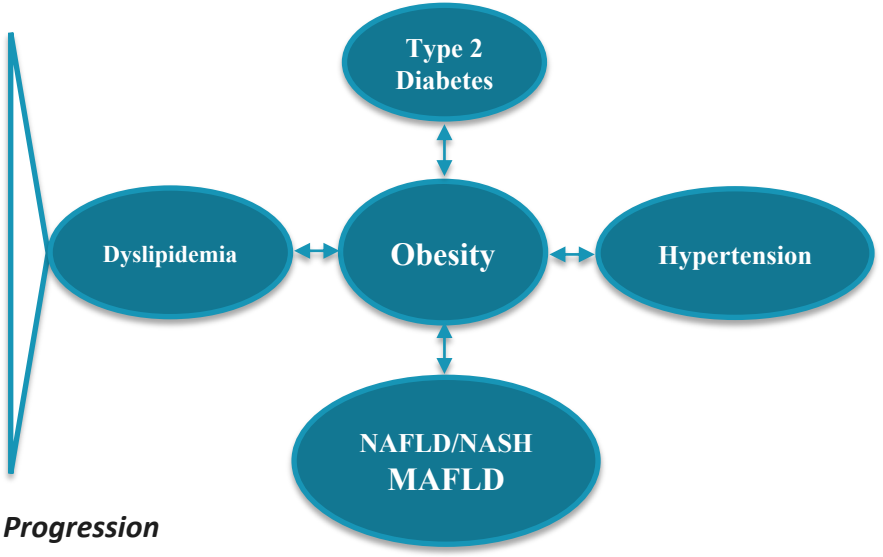
- **MAFLD**
  - **Affects 25% of the global adult population**, ranging from 14% in Africa to 32% in the Middle East
  - Prevalence of liver steatosis is 76% in obese patients
  - Lifetime costs of treatment of NASH in the United States in 2017 was $223 billion
  - Currently there is no approved drug for MAFLD in the context of multiple recent clinical failures of drug candidates

APTAMIR

# The Pathophysiology

**Fat accumulation, hypertrophy, inflammation and necrosis** cause several cardio-metabolic disorders including lipotoxicity, dyslipidemia, insulin resistance, diabetes, liver steatosis, inflammation and fibrosis

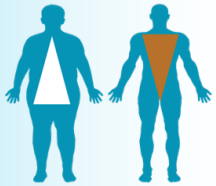



***The Impact of the Adipose Organ Plasticity on Inflammation and Cancer Progression***
*Cells 2019, 8, 662; doi:10.3390/cells8070662*

- Obesity and associated cardio-metabolic disorders are **multifactorial** diseases that cannot be easily controlled by classical therapeutic agents (Mechanism of Action: one drug-one target or one drug-two/three targets)
- Several small molecule drug candidates have failed to reach the market for obesity or MAFLD
- AptamiR's approach uses a **pleiotropic** concept (One Drug-Multiple Targets) by developing oligonucleotide therapeutics targeting **microRNAs,** as they simultaneously modulate many target genes involved in lipid oxidation, mitochondrial activity and energy expenditure.

APTAMIR



# The Market

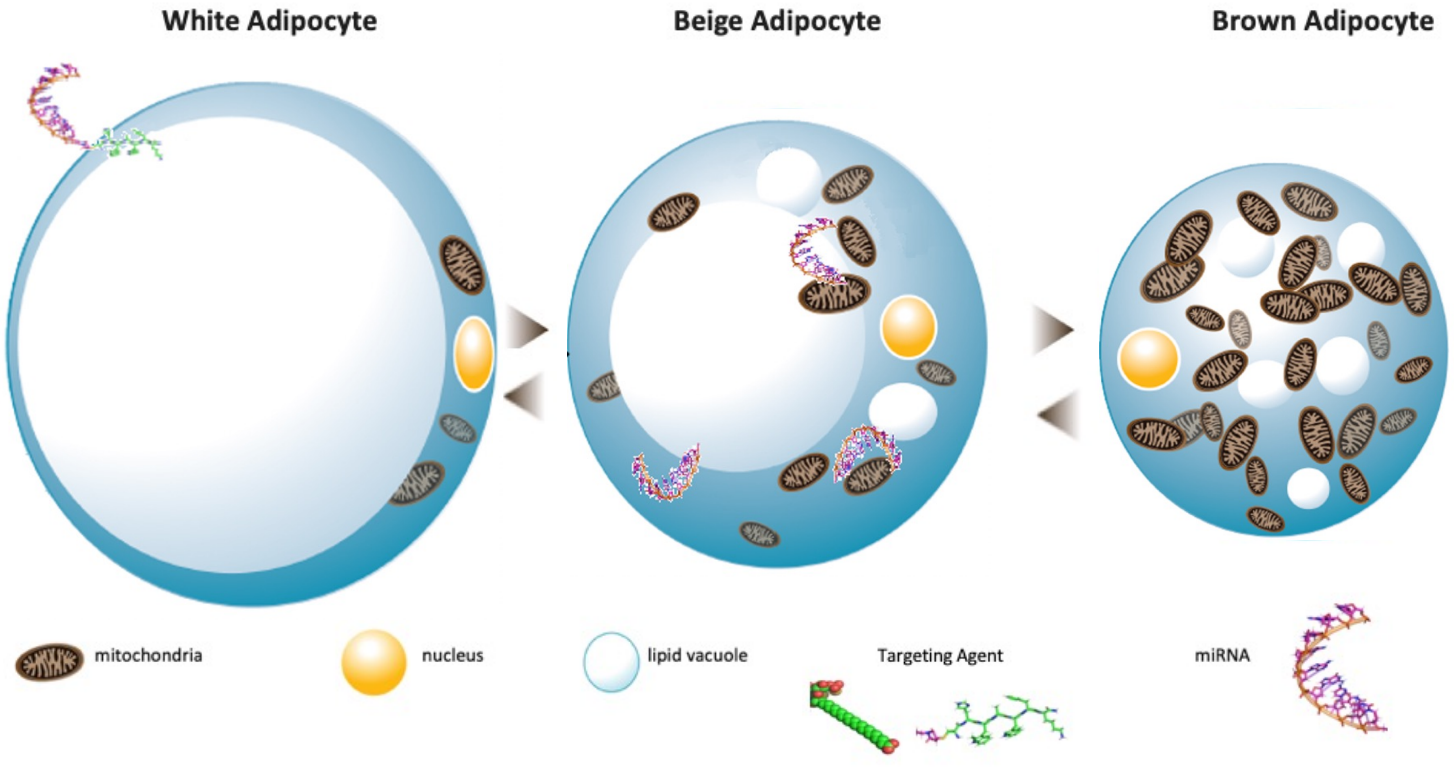
- **Obesity**
  - The US weight loss and diet control market was estimated to be **$66 Billion in 2017**
  - Annual **low productivity costs** in the US related to obesity are estimated to be **$75 Billion**
  - A safe and effective therapy to treat obesity (>10% weight loss) could capture a significant share of the global market, estimated to be ~**$20 Billion**

- **MAFLD**
  - Significant unmet medical need for a safe and effective treatment
  - The MAFLD market is expected to reach **$21 Billion globally by 2025**

# AptamiR Strategy & Technology

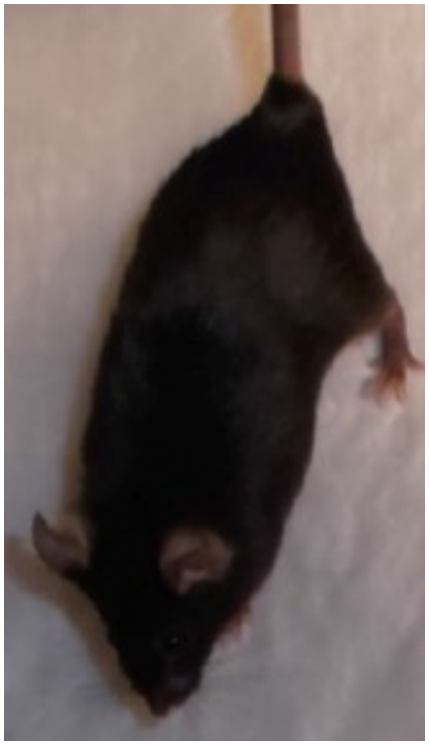
**Development of microRNA-derived Drugs (Oligonucleotide Therapeutics, ONTs):**

- Designed to be preferentially delivered to adipocytes and hepatocytes via the membrane Fatty Acid Translocase (FAT) transporter to induce lipolysis and increase energy expenditure
- Transforming subcutaneous lipid-storing fat cells (White Adipocytes) into calories-burning fat cells (”Browning Effect”)
- Reducing fat accumulation and body weight
- Correcting  lipotoxicity and dyslipidemia
- Improving insulin sensitivity and Type 2 Diabetes Mellitus
- Reversing liver steatosis, inflammation and fibrosis



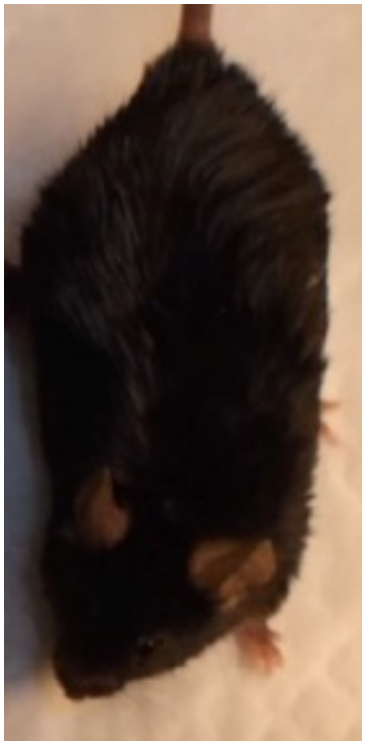
# AptamiR first drug candidate reduces fat mass and improves metabolic parameters in obese mice

Normal diet



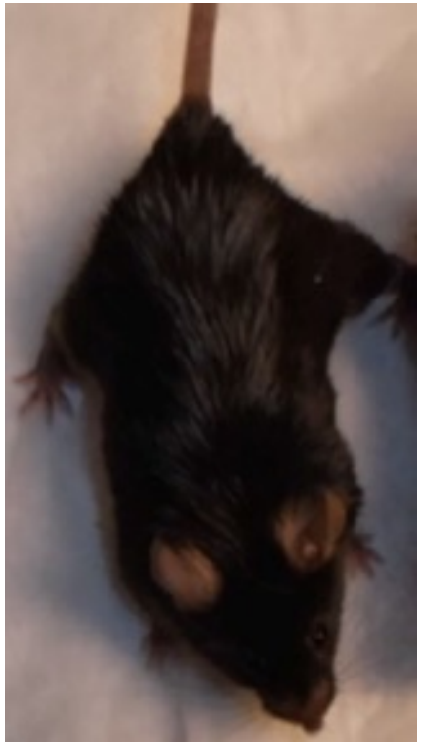
32.08 gm.

High-fat diet (60%)



45.85 gm.

High-fat diet (60%) **+ anti-miR Rx for 8 week**



35.51 gm.

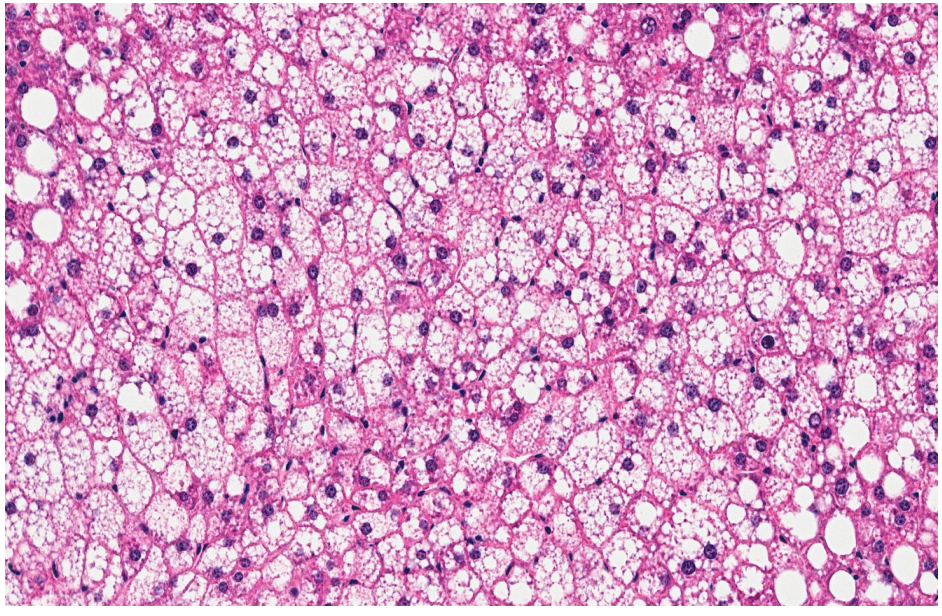
***Mean Body Weight at week 8 of study***

| Results | |
|---|---|
| Body Weight | **Reduced** |
| Fat Mass | **Reduced** |
| Lean Mass | **Unchanged** |
| Cholesterol | **Improved** |
| Glucose | **Improved** |
| Insulin Sensitivity | **Preserved** |
| Liver Tests | **Normal** |
| Temperature | **Normal** |
| Appetite | **Unchanged** |
| Energy Expenditure | **Increased** |
| Liver Steatosis | **Reduced** |

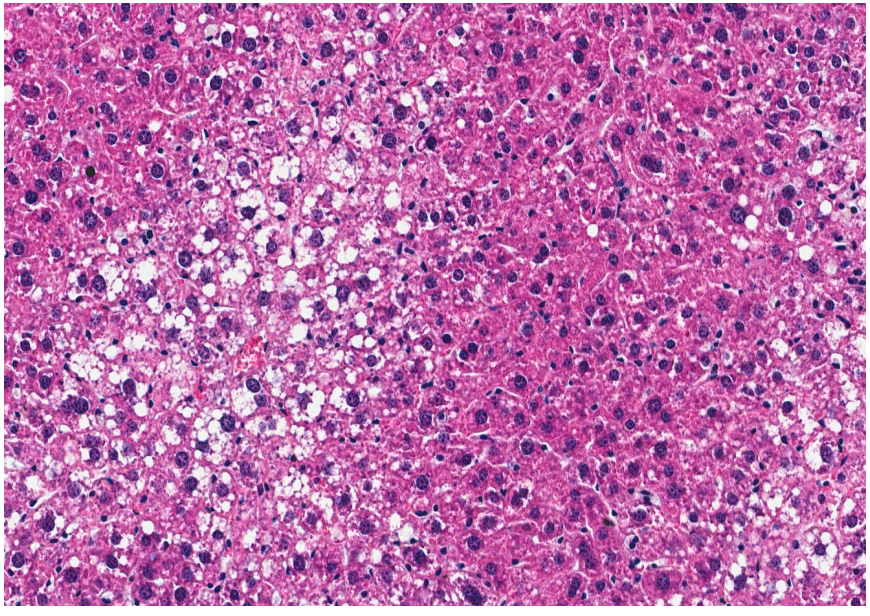
APTAMIR

# AptamiR miR-22-3p Antagomir Reduces Liver Fat Accumulation in DIO Mice

- Treatment for **12 weeks** with the miR-22-3p antagomir APT-110 produced a marked reduction in fatty infiltration of the liver (H&E staining):



**Saline**



**APT-110**


- Thibonnier, M. et al. Metabolic Benefits of MicroRNA-22 Inhibition, Nucleic Acid Ther., 30(2), 104-116, 2020
- Thibonnier, M. et al. Metabolic and Energetic Benefits of MicroRNA-22 Inhibition, BMJ Open Diabetes Res. Care, 8, 2020

- Elevated circulating miR-22-3p is a biomarker of NAFLD, in relation to activity score (NAS) and Steatosis Activity Fibrosis (SAF): Scientific Reports, 8:10606, 2018; BMC Genomics, 19:188, 2018
- Hepatic miR-22 overexpression enhances diet and alcohol-induced steatosis whereas reducing miR-22 level improves alcoholic steatosis in mouse models: JHEP Rep. 2020;2(2), 100093
- miR-22 promotes the development of liver cirrhosis through BMP7 suppression: Cell Physiol. Biochem. 2015;36:1026-1036
- miR-22 modulates the expression of lipogenesis-related genes and promotes hepatic steatosis in vitro: FEBS Open Bio 2021;322-332. "***miR-22 inhibitors may have potential as candidate drugs for NASH and obesity***"

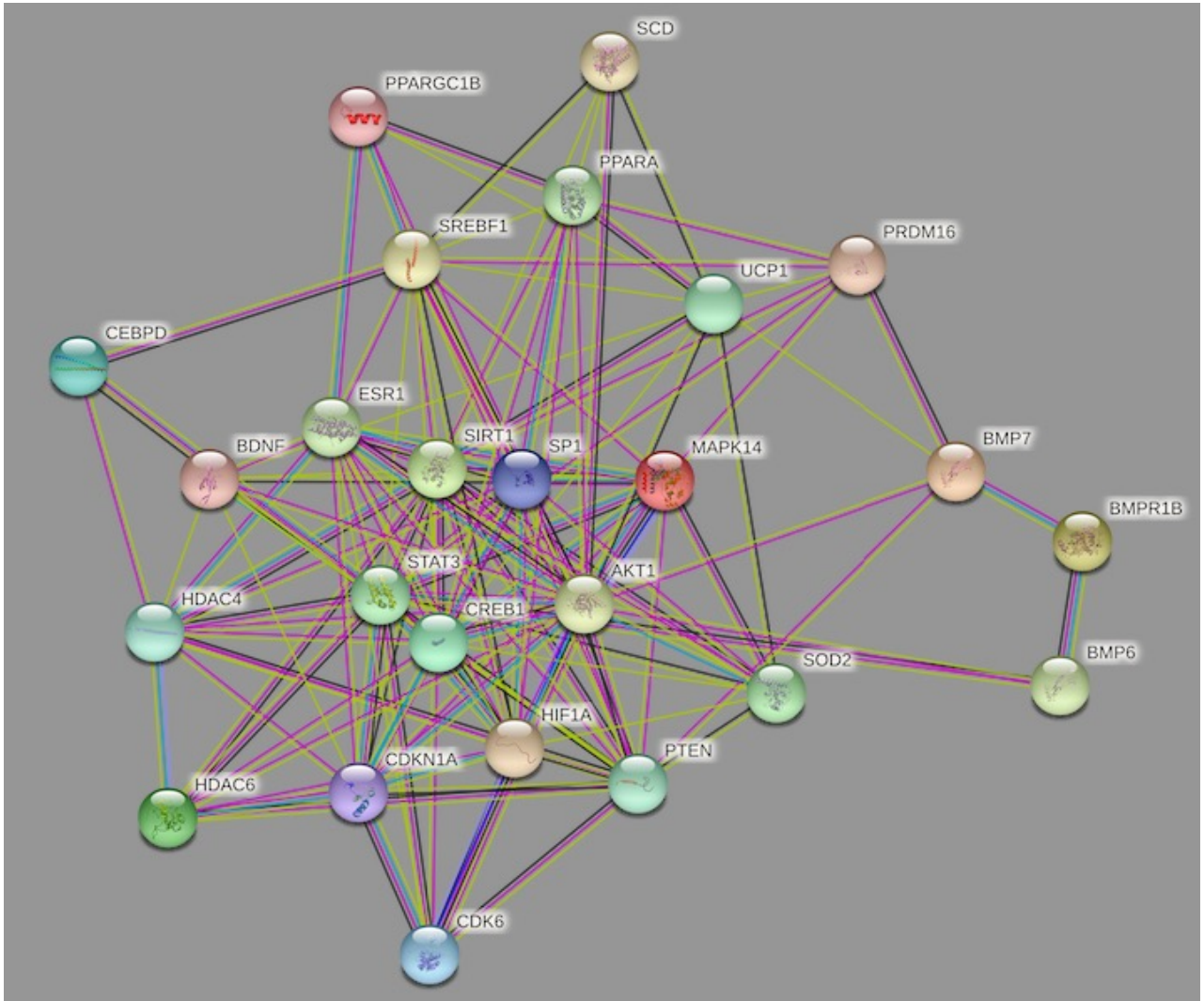
# Validation of miR-22-3p as a “metabolic target”

- Using various *in silico, in vitro* and *in vivo* tools, we demonstrated that miR-22-3p, a unique, conserved and universal miRNA, is an excellent metabolic target that modulates several genes as illustrated below with metaMIR (http://rna.informatik.uni-freiburg.de) which ranks miRNAs in relation to gene networks:

| miRNA | Final Score | Positive Combo | Positive Score | Positive Group |
|---|---|---|---|---|
| hsa-miR-22-3p | 12.11 | AKT1,BDNF,CDKN1A,CREB1,ESR1,HDAC4,HDAC6,KDM3A,KDM6B,KLF6,MAPK14,MECP2,PPARA,PPARGC1B,PRDM16,PTEN,RUNX2,SIRT1,SOD2,SP1,STAT3 | 12.112 | 21 |
| hsa-miR-1587 | 3.42 | AKT1,BDNF,CREB1,ESR1,HDAC6,KDM6B,MAPK14,PPARGC1B,PRDM16,RUNX2,SOD2,SP1,STAT3 | 3.416 | 13 |
| hsa-miR-30a-5p | 4.1 | AKT1,BDNF,CREB1,KDM3A,KDM6B,KLF6,PPARGC1B,PRDM16,PTEN,RUNX2,SIRT1,SOD2 | 4.1 | 12 |
| hsa-miR-519c-3p | 3.55 | AKT1,CDKN1A,CREB1,ESR1,HDAC4,KDM6B,PPARA,PRDM16,PTEN,RUNX2,SP1,STAT3 | 3.548 | 12 |
| hsa-miR-520c-3p | 4.58 | AKT1,CDKN1A,ESR1,HDAC4,KDM6B,KLF6,MECP2,PPARA,PPARGC1B,PRDM16,PTEN,RUNX2,SP1,STAT3 | 4.581 | 14 |
| hsa-miR-93-5p | 3.01 | AKT1,CDKN1A,ESR1,KDM6B,KLF6,MECP2,PPARA,PPARGC1B,PRDM16,PTEN,SIRT1,STAT3 | 3.006 | 12 |
| hsa-miR-520a-3p | 3.95 | AKT1,CREB1,ESR1,HDAC4,KDM6B,KLF6,MECP2,PPARGC1B,PRDM16,PTEN,RUNX2,SOD2,STAT3 | 3.95 | 13 |
| hsa-miR-1470 | 4.35 | AKT1,CREB1,HDAC4,KDM6B,KLF6,MAPK14,MECP2,PPARA,PPARGC1B,PTEN,RUNX2,SOD2,STAT3 | 4.351 | 13 |
| hsa-miR-5089-3p | 4.29 | AKT1,CREB1,KLF6,MAPK14,MECP2,PRDM16,PTEN,SIRT1,SOD2,STAT3 | 4.287 | 10 |
| hsa-miR-520b | 3.83 | AKT1,ESR1,HDAC4,KDM6B,KLF6,MECP2,PPARA,PPARGC1B,PRDM16,PTEN,RUNX2,SOD2,STAT3 | 3.834 | 13 |

# Validation of miR-22-3p as a “metabolic target”

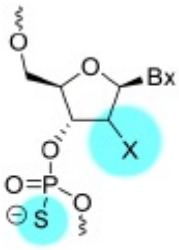
- Illustration of the interactions within the network of proteins related to miR-22 using the Protein-Protein Interaction Functional Enrichment Analysis Tool STRING (https://string-db.org) confirming the pleiotropic mode of action of our drug candidate



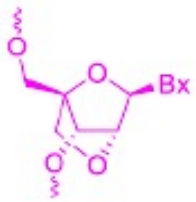
# Toxicity of the 1st Generation of ONTs

**The First Generation of ONTs (ASOs, siRNAs & miRNAs) developed by several Biotechnology Companies were found to trigger at supra-therapeutic doses in Non-Human Primates**:

- Transient activation of blood platelets and of the complement pathway in relation to Phosphorothioate (PS) backbone modifications
- Hepatic and renal toxicity in relation to Locked Nucleic Acid (LNA) sugar modifications



PS



LNA

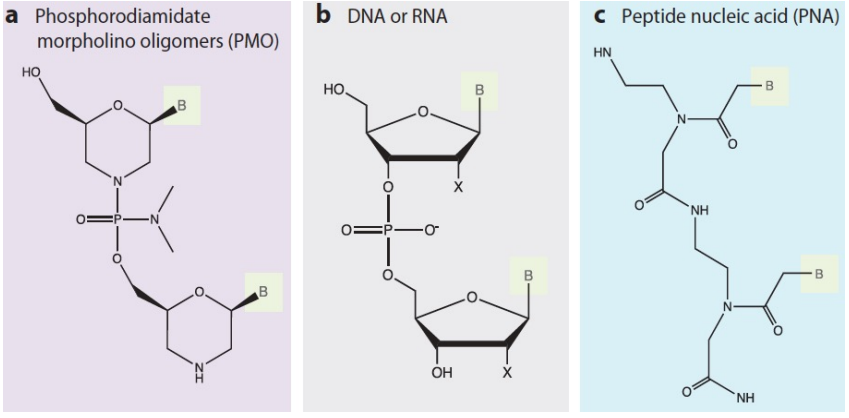
**Pre-IND toxicology and safety studies of our first miR-22-3p antagomir APT-110** (a "naked" single stranded 18-mer miR-22-3p antagomir containing PS and LNA modifications, ***5'-ln5MeC*-lnT*-dT*-mC*-dT*-dT*-ln5MeC*-dA*-dA*-mC*-dT*-lnG*-dG*-ln5MeC*-lnA*-dG*-ln5MeC*-lnT-3'***)** were completed in mice and non-human primates according to FDA guidance.

- In mice, APT-110 administered subcutaneously at 15, 60, and 240 mg/kg/dose on Days 1, 3, 5, 8, 15, 22, and 29 was tolerated in vivo and was associated with atrophy of adipose tissues at all dose levels
- In cynomolgus monkeys, APT-110 was administered subcutaneously at 3.75, 15, 37.5 and 60 mg/kg/dose on Days 1, 3, 5, 8, 15, 22, and 29 of the study. A transient activation of blood platelets and of the complement pathway was observed right after administration of supra-therapeutic doses. Kidney and liver histologic alterations were also noted.

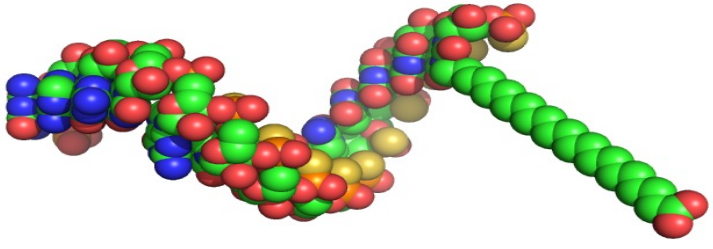
# Design of the 2nd Generation of ONTs

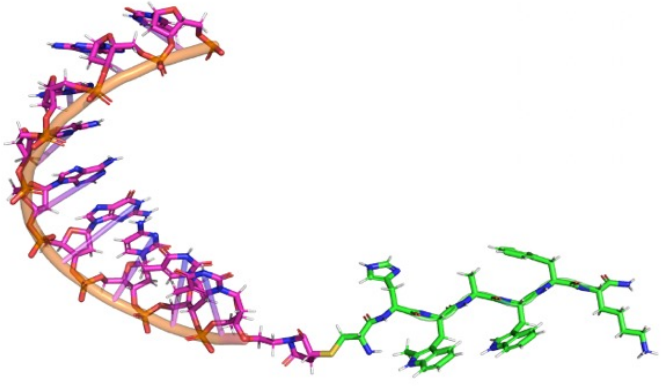
**Our Second Generation of ONTs was designed:**

- **To eliminate potential toxicity** by replacing PS and LNA modifications by a **PNA backbone**




- **To maintain resistance to nucleases and proteases/peptidases**
- **To conjugate the ONT** to a fatty acid or a short peptide for enhanced targeted delivery to adipocytes of a greatly reduced effective dose with an extended duration of action in target tissues





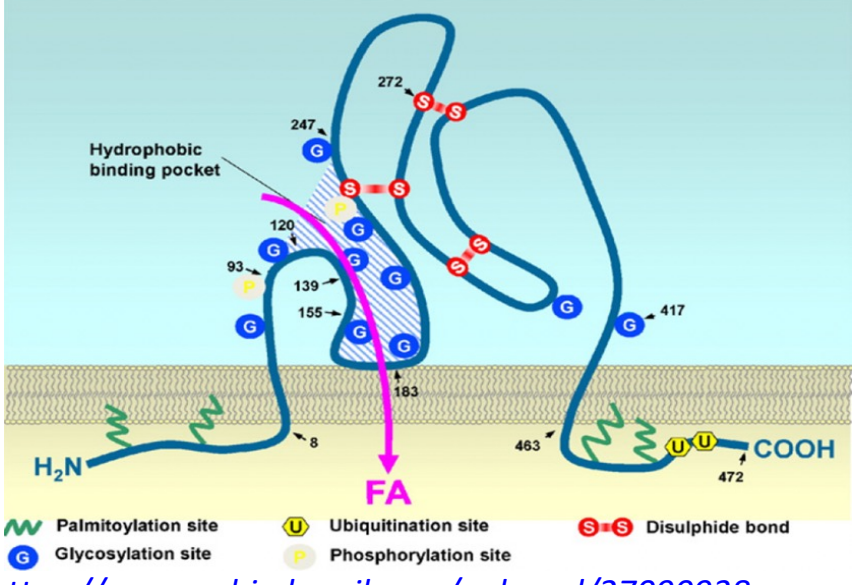
- **To avoid chirality**
- **To avoid protein binding**

APTAMIR

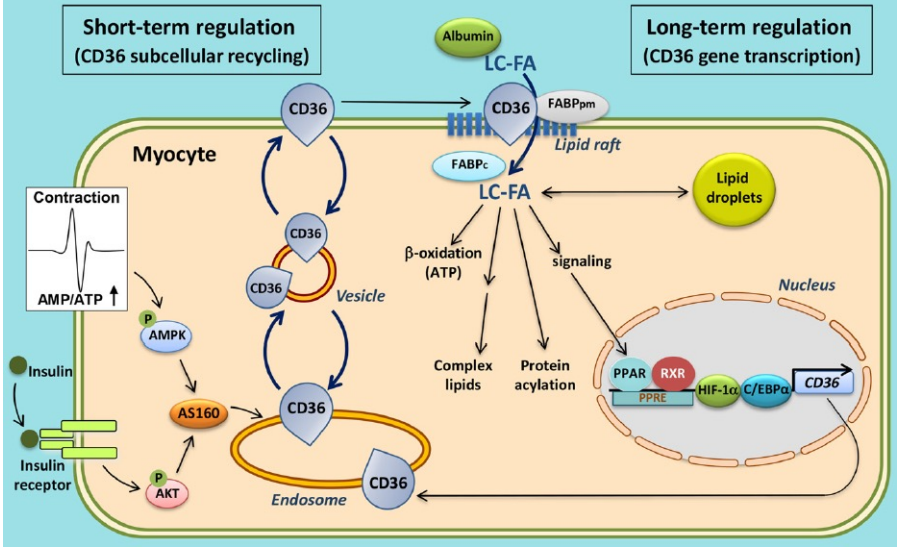
# Targeted Delivery of the 2nd Generation of ONTs

**Selection of the membrane Fatty Acid Translocase (FAT) transporter for targeted delivery of ONTs to adipocytes and metabolic organs**

- The membrane transporter FAT/CD36/SCARB3 is the main route of uptake by adipose tissues of long-chain fatty acids as well as short peptides like Hexarelin, Prohibitin and Thrombospondin Peptide-1
- FAT is expressed in cells and tissues sensitive to metabolic dysfunctions, such as adipocytes, hepatocytes, skeletal and cardiac myocytes, pancreatic β-cells, kidney glomeruli and tubules cells, monocytes and macrophages
- As the average obese male patient weighs around 200 lbs. with 40% being adipose tissue, there is a huge amount of FAT available at the surface of adipose tissues to transport inside the adipocytes our second generation of miR-22-3p antagomir coupled to a fatty acid or a peptide
- Consequently, the effective dose of our 2nd generation of ONT will be significantly reduced with a greatly improved safety and PK/PD profile, especially the mean residence time inside the targeted cells




https://www.ncbi.nlm.nih.gov/pubmed/27090938



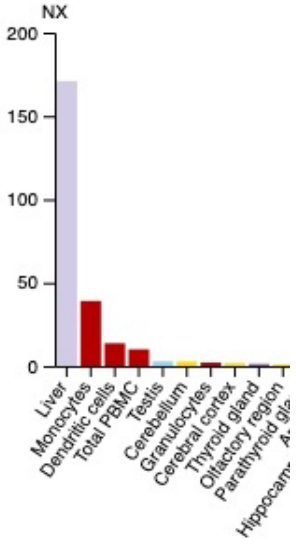

https://www.ncbi.nlm.nih.gov/pubmed/29687890

APTAMIR

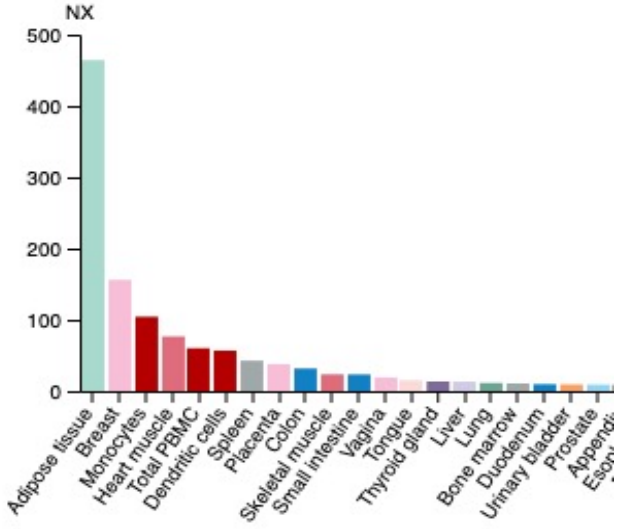
# Comparison of ASGR1 and FAT Expression

- **mRNA expression levels** (www.proteinatlas.org):

**ASGR1**

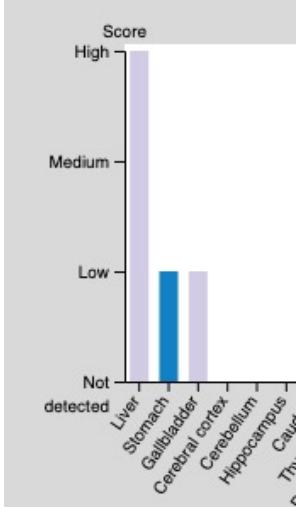



**FAT**

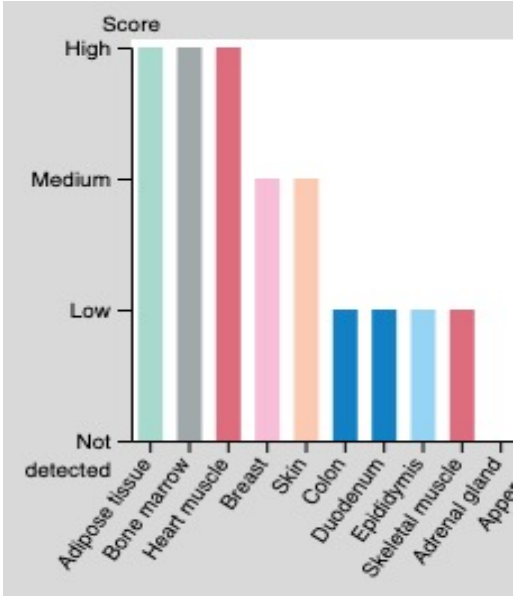



- **Protein expression levels:**

**ASGR1**



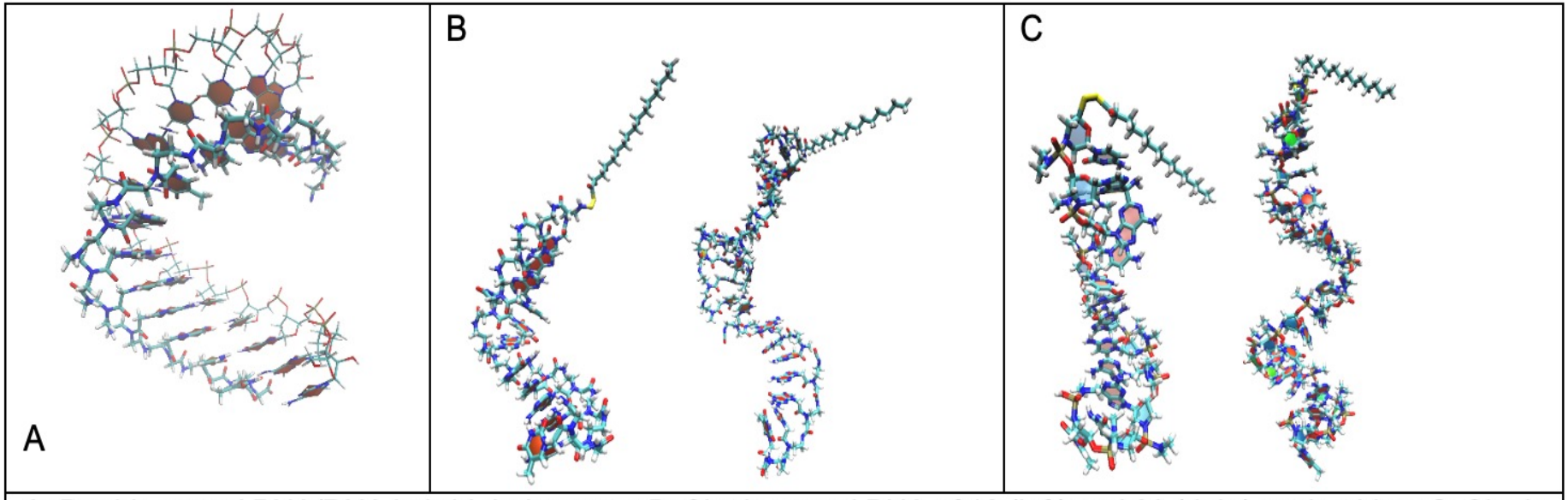

**FAT**




APTAMIR

# High Performance Molecular Dynamics Modeling on Graphics Processing Units of miR-22-3p Antagomirs

## Targeted Delivery via a Fatty Acid

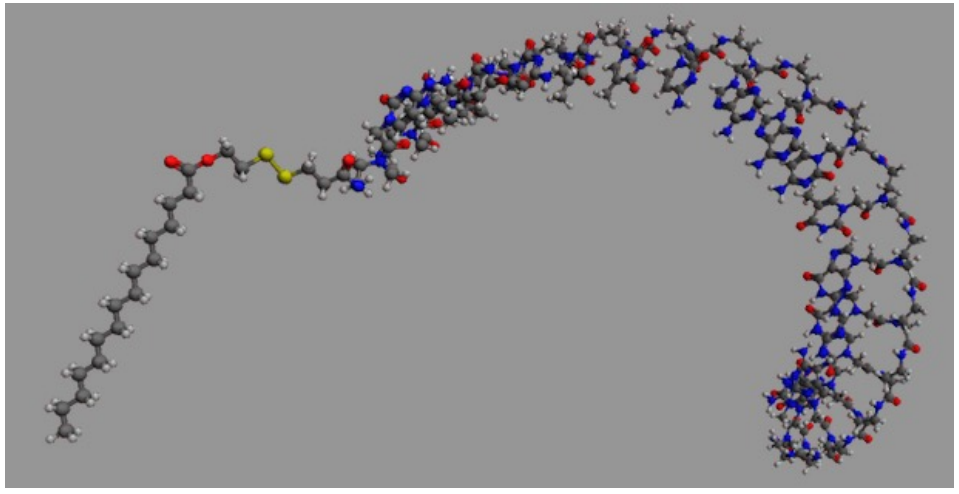



A: Double strand PNA/RNA hybrid dodecamer; B: Single strand PNA of 12 (left) and 20 (right) nucleotides; C: Single strand PMO of 12 (left) and 20 (right) nucleotides. The 5' terminals of (B) and (C) are capped with palmitic acid (C16)-S-S bond.

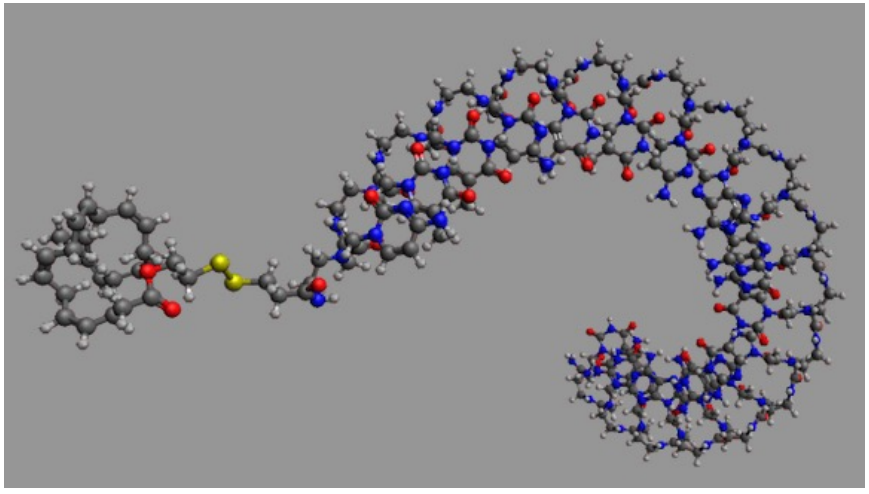
**Work performed by Dr. Chengwen Liu, Ph.D. in Computational Chemistry, Laboratory of Pr. Pengyu Ren, Ph.D., Department of Biomedical Engineering at the University of Texas at Austin**

*Molecular Dynamics Study of the Hybridization between RNA and Modified Oligonucleotides, Z. Jing, R. Qi, M. Thibonnier and P. Ren, J Chem Theory Comput 2019 Vol. 15 Issue 11 Pages 6422-6432*
https://www.ncbi.nlm.nih.gov/pubmed/31553600

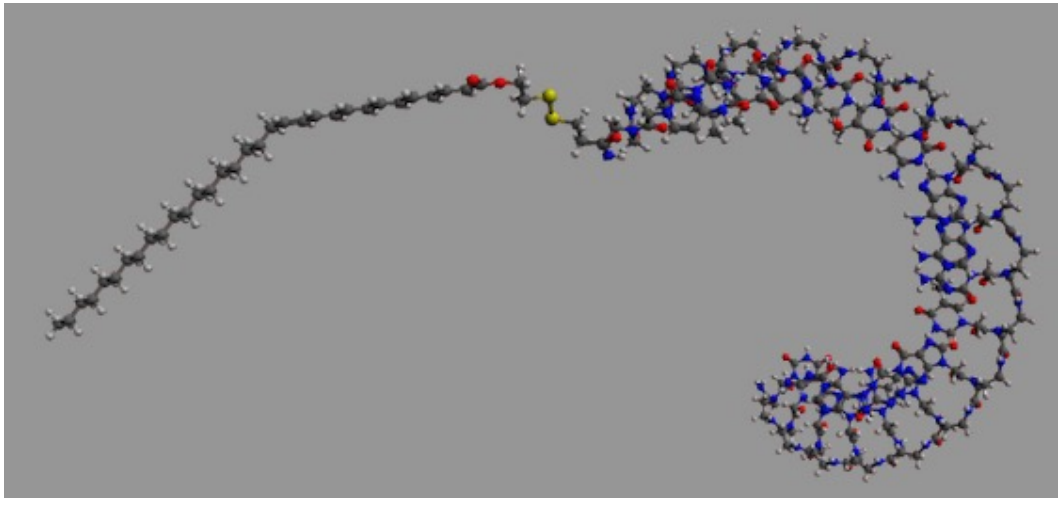
APTAMIR



C16 Palmitic Acid-SS-PNA 18 mer



C22:6 Docosahexaenoic Acid-SS-PNA 18 mer

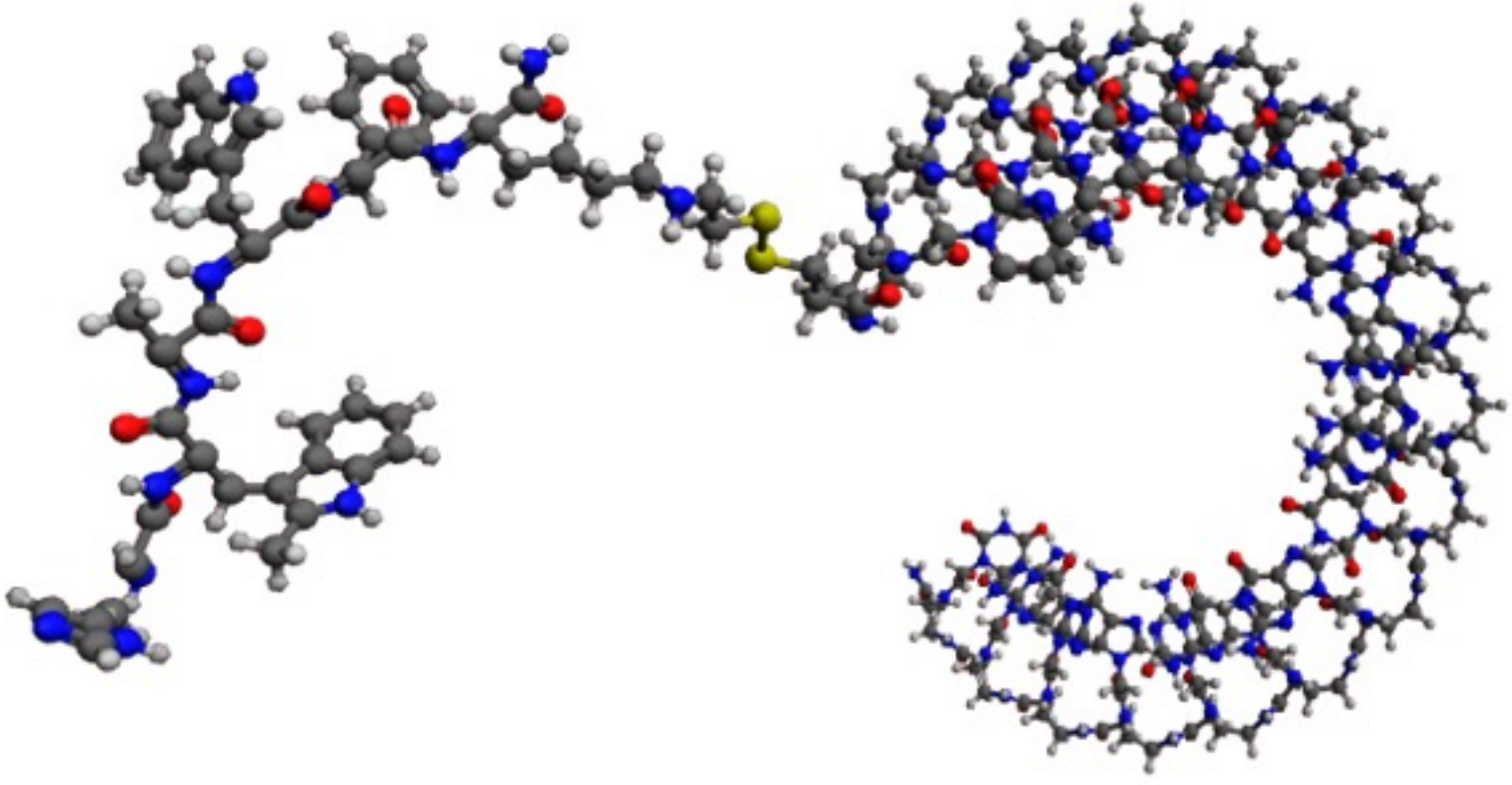


C32:6 Dotriacontahexaenoic Acid-SS-PNA 18 mer

# High Performance Molecular Dynamics Modeling on Graphics Processing Units of miR-22-3p Antagomir

## Targeted Delivery via a Short Peptide

### Hexarelin-SS-PNA 18-mer



APTAMIR

# Selection of Hexarelin for targeted delivery of ONTs to adipocytes and metabolic organs

- Hexarelin (His-D-2MeTrp-Ala-Trp-D-Phe-Lys-NH2), a stable analog of GHRP-6, is a high-affinity ligand for FAT/CD36
- The interaction of hexarelin with FAT promotes the transcriptional activation of nuclear receptor PPARγ and target gene profiling involved in metabolism
- In macrophages, hexarelin induces a molecular cascade involving nuclear liver X receptor LXRα and expression of apolipoprotein E (apoE) and sterol transporters ABCA1 and ABCG1
- Such activation of the PPARγ-LXRα-ABC metabolic pathway increases cholesterol efflux, resulting in enhanced HDL reverse cholesterol transport and regression of atherosclerosis

*André Tremblay et al. Int J Mol Sci 2018 Vol. 19 Issue 5, https://www.ncbi.nlm.nih.gov/pubmed/29883404*