

# Les oligonucléotides bioconjugués; systèmes supramoléculaires et applications thérapeutiques

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philippe.barthelemy@inserm.fr

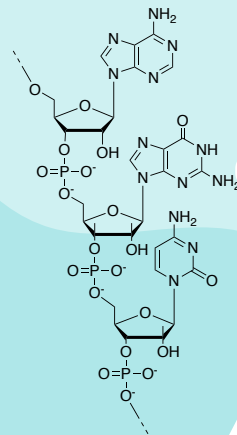
ARNA

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Inserm



Unité ARNA, INSERM U1212 / UMR CNRS 5320  
<https://arna.cnrs.fr>  
Equipe ChemBioPharm  
<http://chembiopharm.fr>



# Déclaration des liens d'intérêt

- Moderna
- Presans
- Amgen
- Novartis

**Cette présentation peut contenir des données hors AMM**





# Résumé

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**ASO DELIVERY**

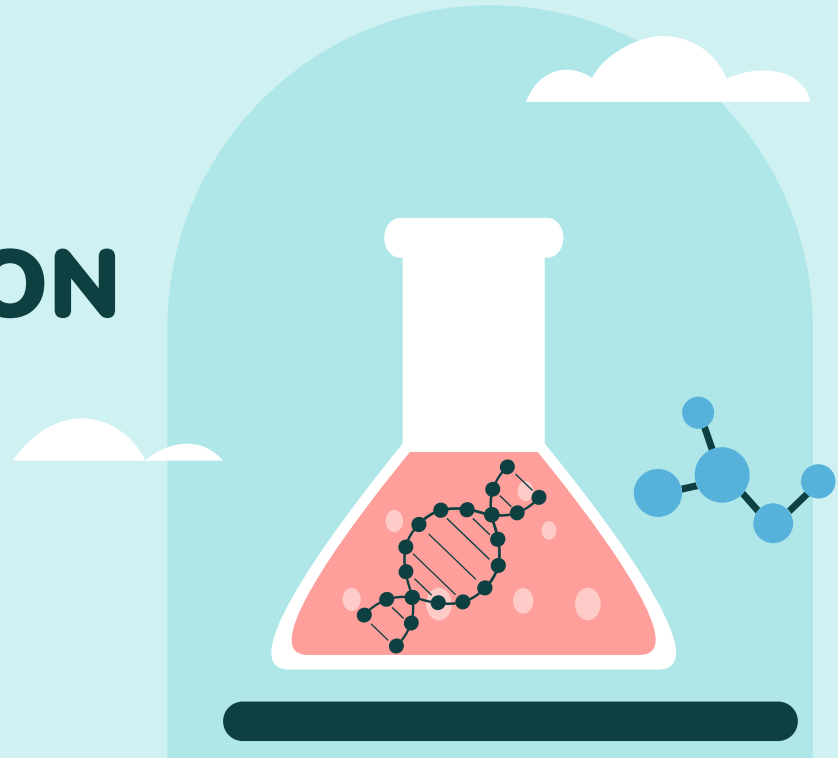
5

**CHIMIE  
SUPRAMOLAIRES DES  
CONJUGUES**



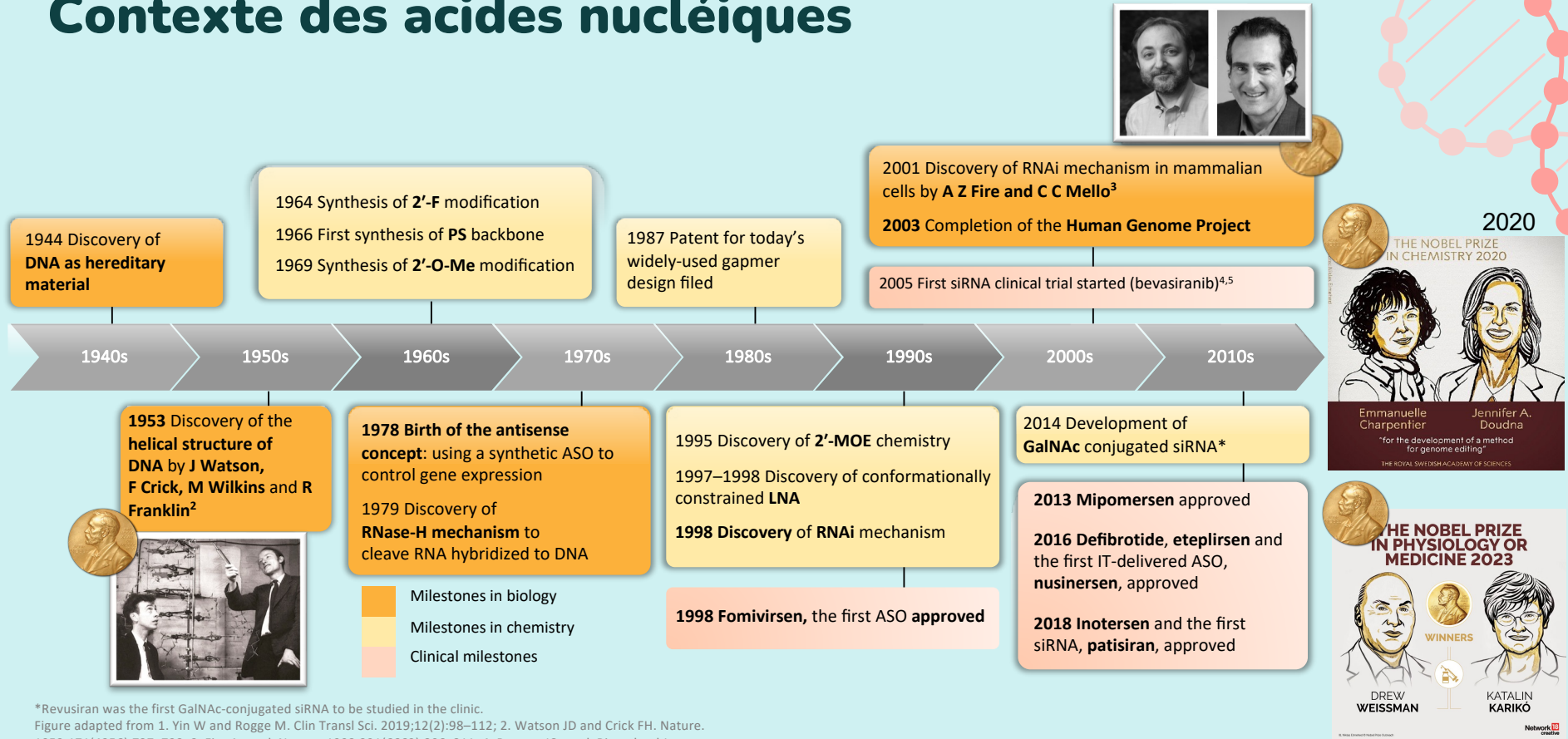
01

# INTRODUCTION CONTEXTE



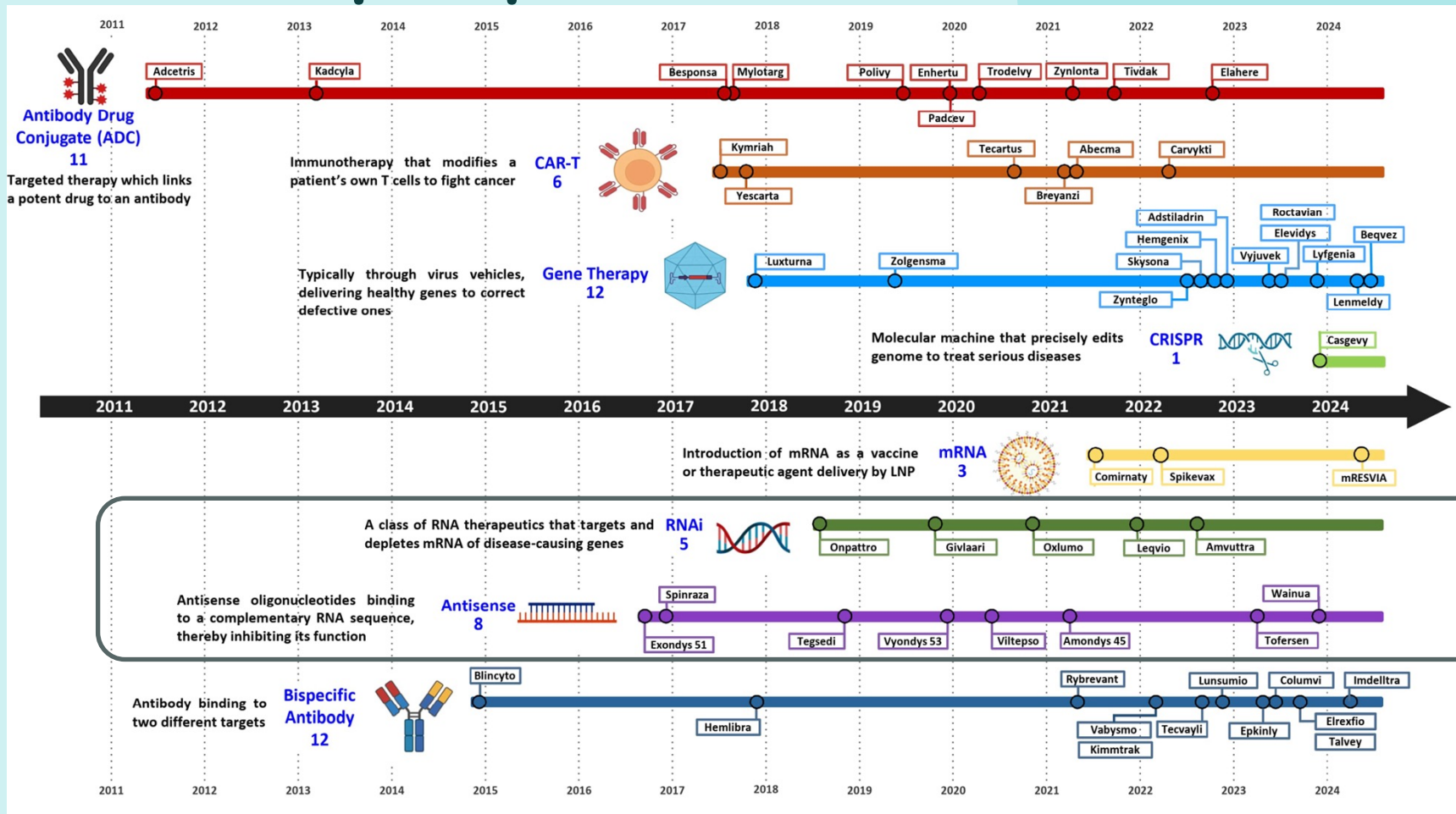


# Contexte des acides nucléiques

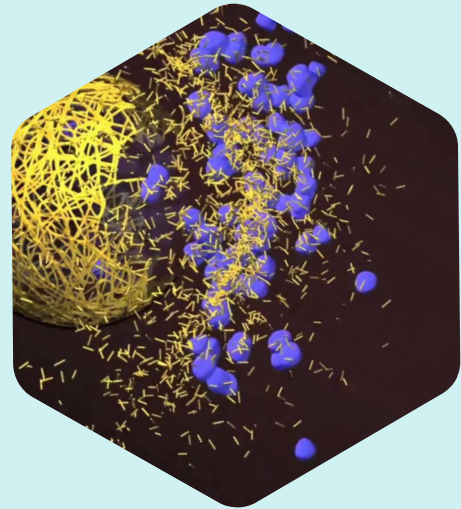


\*Revusiran was the first GalNAc-conjugated siRNA to be studied in the clinic.  
Figure adapted from 1. Yin W and Rogge M. Clin Transl Sci. 2019;12(2):98–112; 2. Watson JD and Crick FH. Nature. 1953;171(4356):737–738; 3. Fire A, et al. Nature. 1998;391(6669):806–811; 4. Burnett JC, et al. Biotechnol J. 2011;6(9):1130–1146; 5 <https://clinicaltrials.gov/ct2/show/NCT00259753>. (Accessed 20 Sept, 2022).

# Modalités thérapeutiques

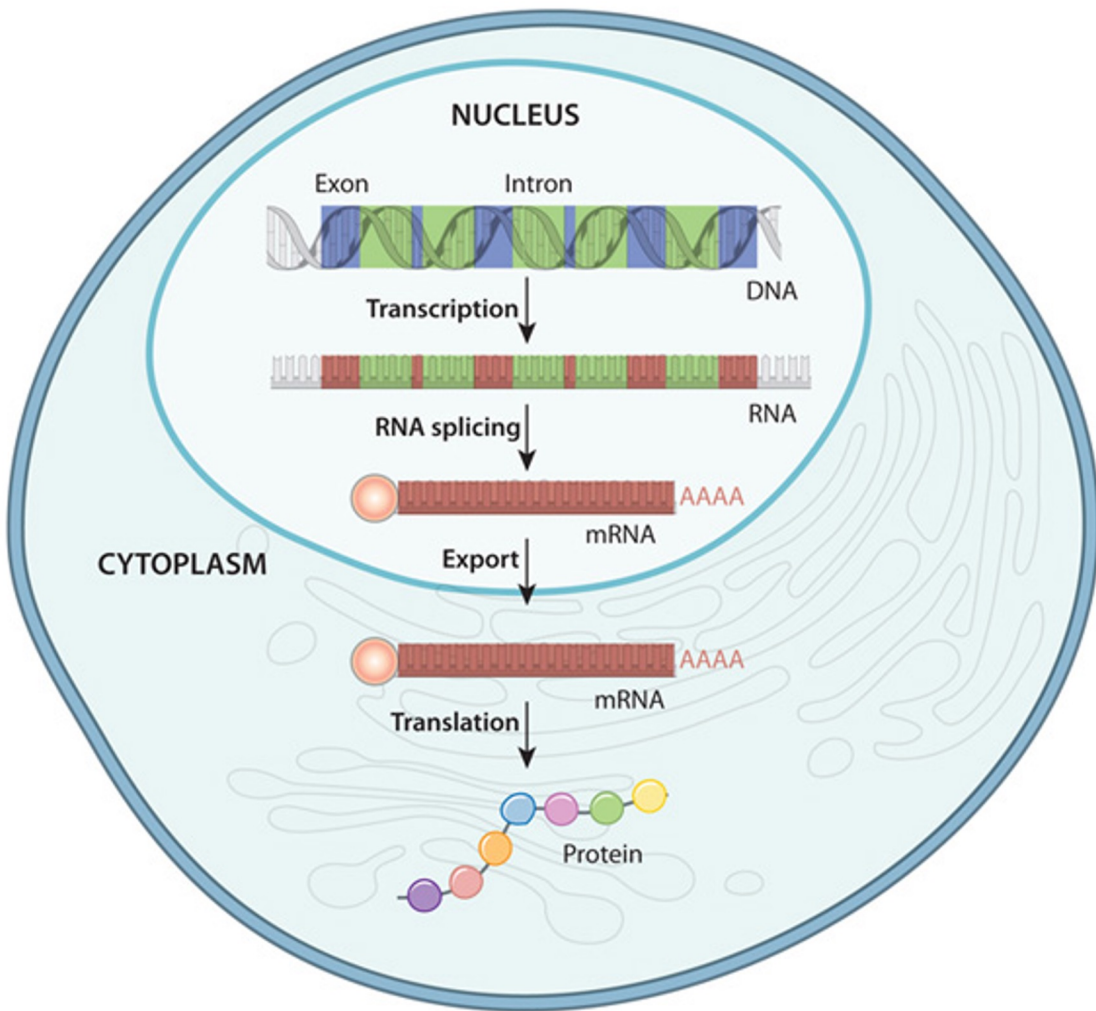


# MECANISMES BIOLOGIQUES



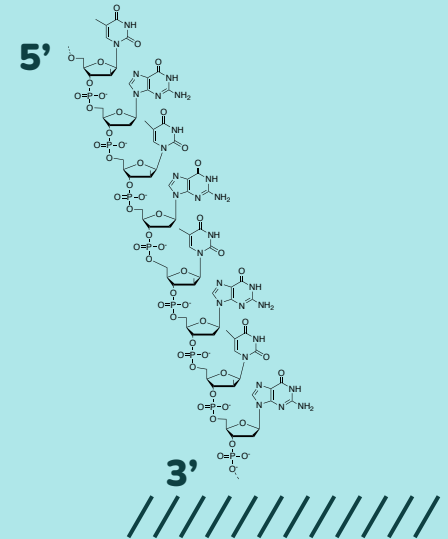
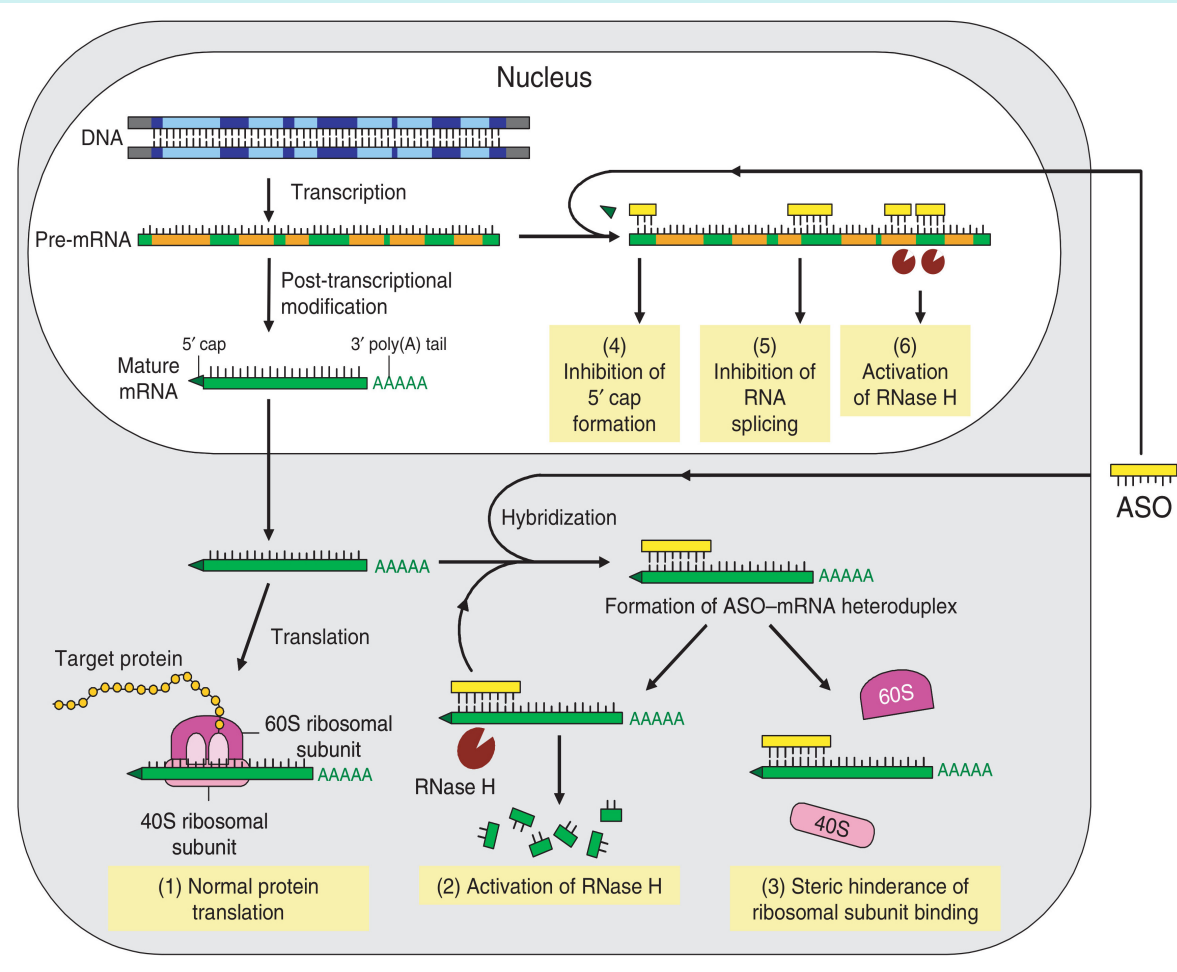
# Expression génétique chez un eucaryote

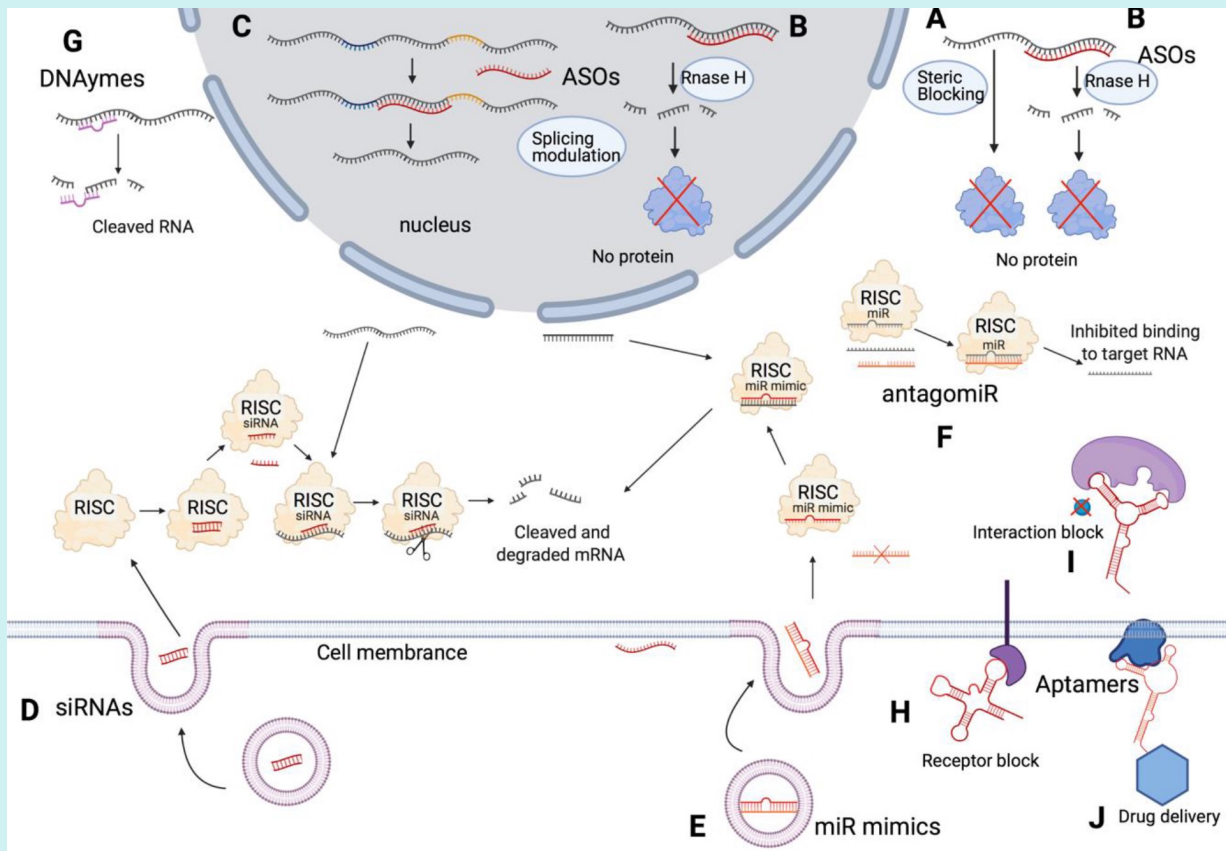
=> l'ADN est transcrit en ARNm. Certaines régions sont supprimées (introns) lors du traitement initial de l'ARNm. Les exons restants sont ensuite épissés ensemble et la molécule d'ARNm épissée (rouge) est préparée pour être exportée hors du noyau grâce à l'ajout d'un embout (sphère) et d'une queue polyA. Une fois dans le cytoplasme, l'ARNm est traduit en protéine via les ribosomes



# ANTISENS (ASO)

=> Un antisens est un oligonucléotide simple brin spécifique de la séquence de l'ARN messager qu'il cible





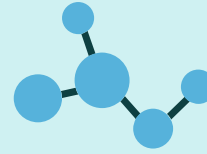
**Nombreux  
mécanismes  
Possibles !**

**Exemples : Oligonucléotides Thérapeutiques en Oncologie**

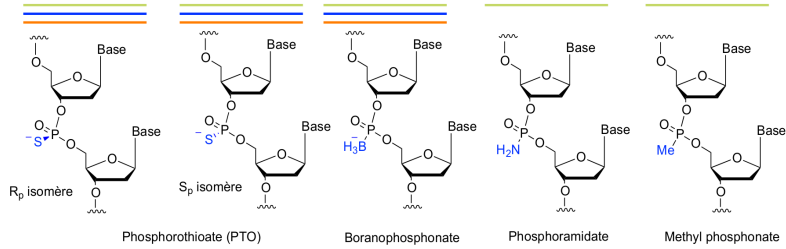




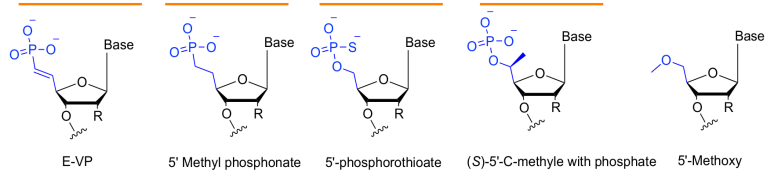
# Modifications chimiques



Phosphate backbone modification



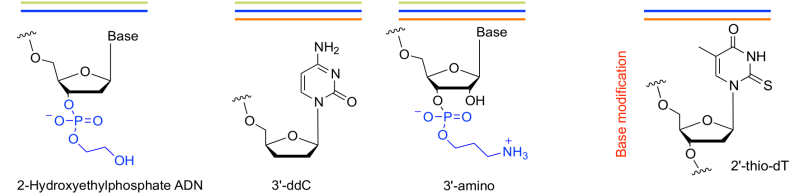
5' phosphate stabilization



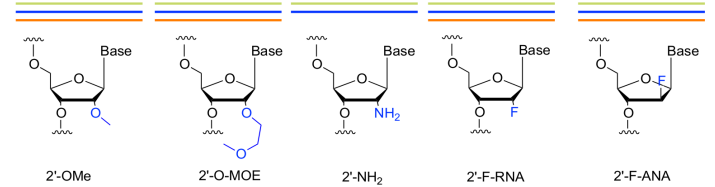
— Steric blockers  
— RNase H  
— RNAi

5

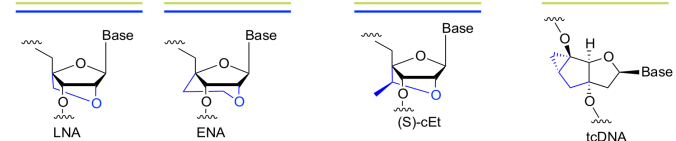
3' phosphate stabilization



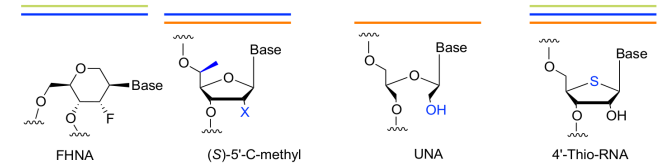
2'-modifications



Sugar modification  
Constrained nucleotides



Other modified sugars



⇒ **Stabilité**  
 ⇒ **Modulation du mécanisme d'action**  
 ⇒ **« Delivery »**

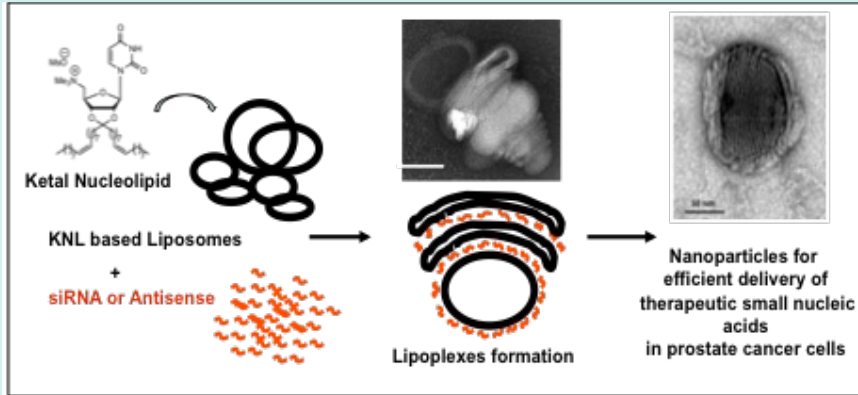




# ⇒ « Delivery »

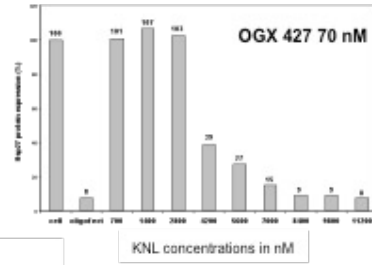
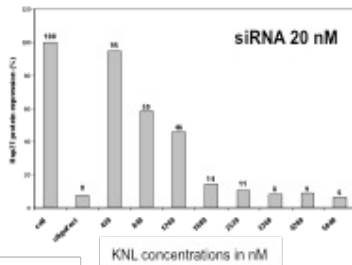
**Target: Prostate Cancer**

**Therapeutic oligonucleotides : siRNA, ASO**



Hsp27 protein, 27 kD

Hsp27 protein, 27 kD



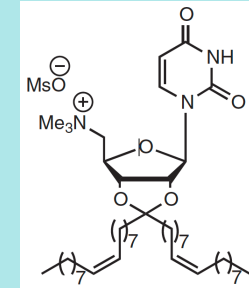
Heat Shock Protein 27 (Hsp27) is overexpressed in Castrate-Resistant Prostate Cancer

**High efficacy of Transfection siRNA / ASO for Hsp27 Low toxicity**

*Luvino et al. Journal of Controlled Release 172 (2013) 954–961*

## LNPs

**Formulations Conjugués Nucleolipides cationiques**



## SNALPs\*

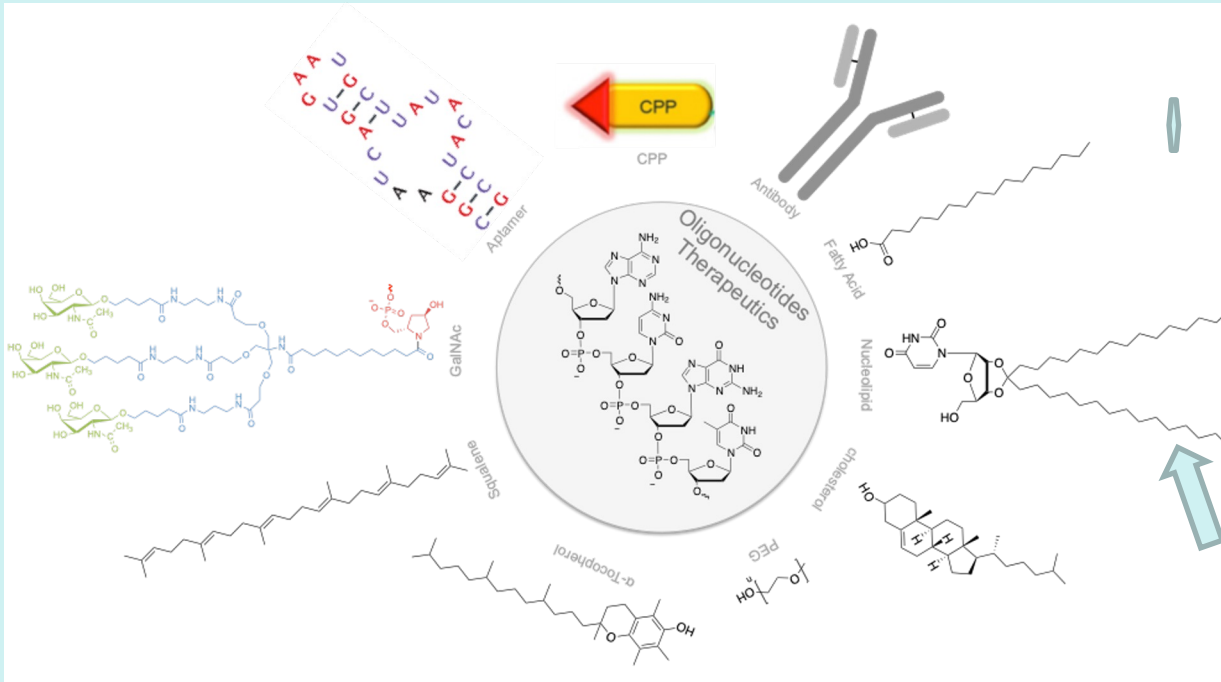
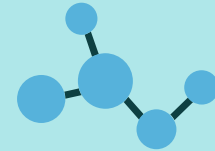
**\*Stable Nucleic Acid Lipid Particles**



# ⇒ « Delivery »

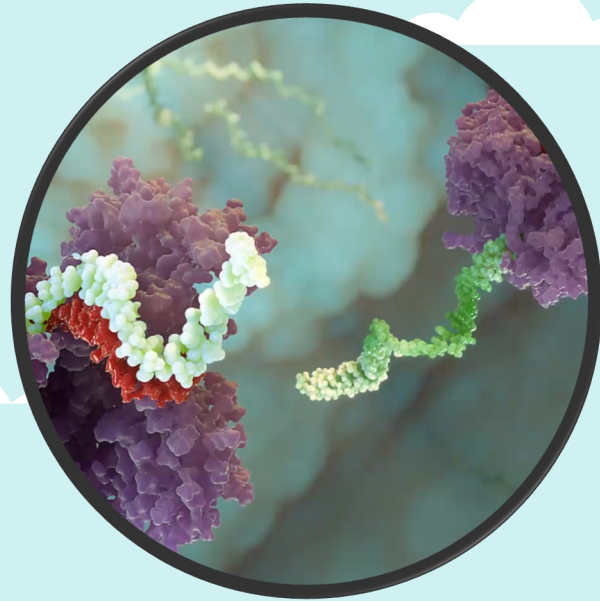
# Bio-conjugués

## Molécules hybrides de synthèse



02

# CHIMIE DES CONJUGUÉS

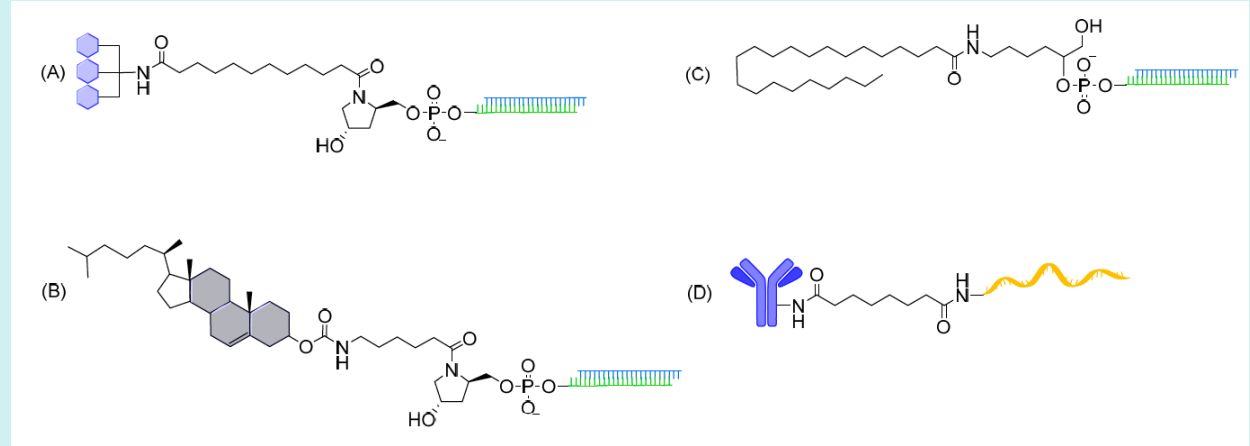


# CHIMIE DES CONJUGUÉS

## Challenges et opportunités

- ✓ - Biodélivrance
- ✓ - Ciblage
- ✓ - Multimodalités
- ✓ - Stabilité

## Exemples de structures

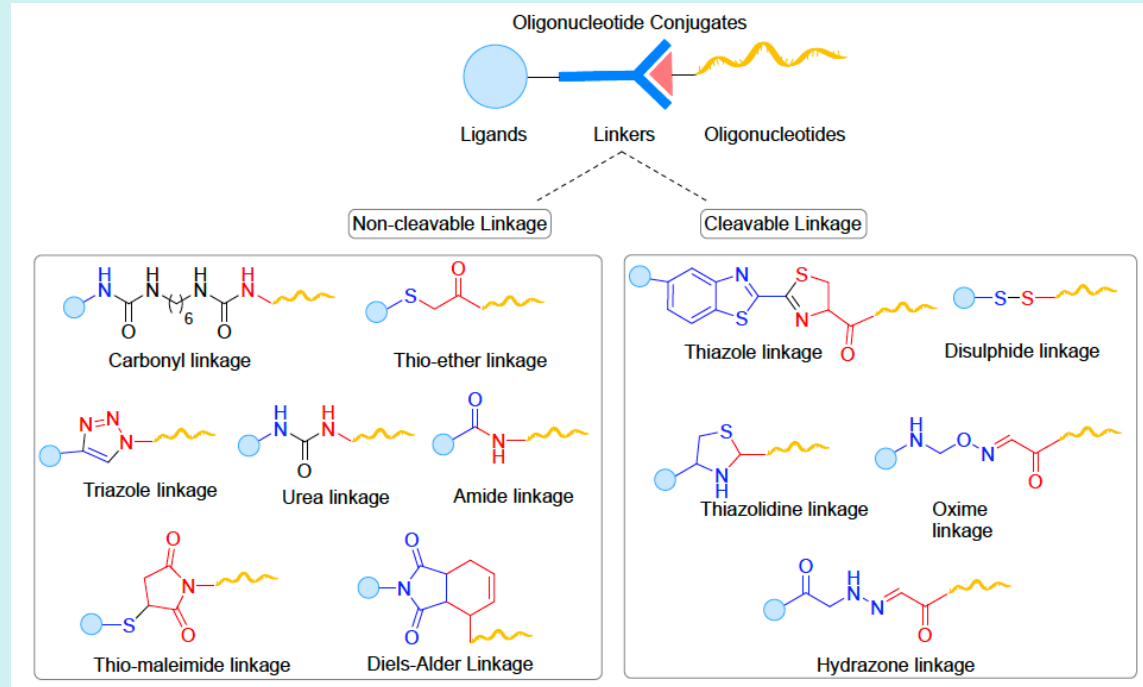


# CHIMIE DES CONJUGUÉS

## Choix du linker

### Challenges et opportunités

- ✓ - Biodélivrance
- ✓ - Ciblage
- ✓ - Multimodalités
- ✓ - Stabilité

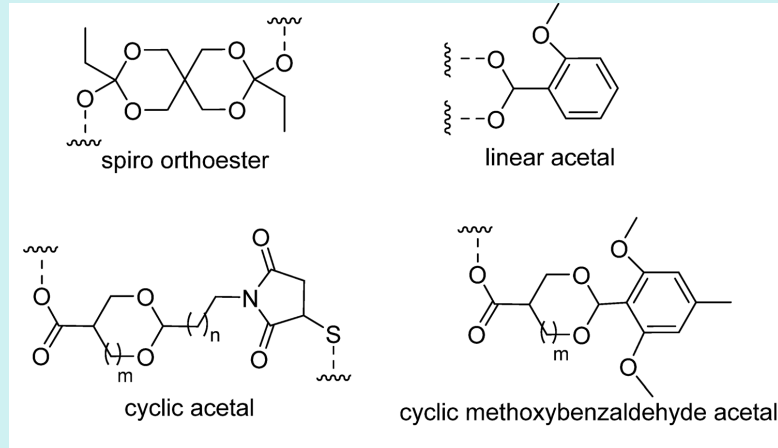
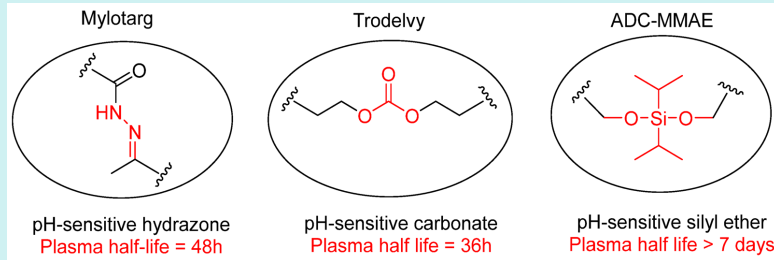


# CHIMIE DES CONJUGUÉS

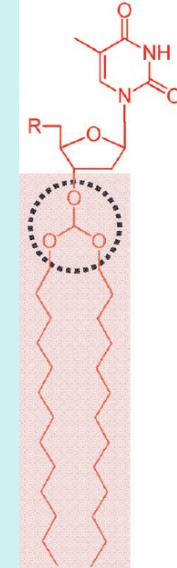
## Challenges et opportunités

- ✓ - Biodélivrance
- ✓ - Ciblage
- ✓ - Multimodalités
- ✓ - Stabilité

## Chimie pH sensible



## Choix du linker



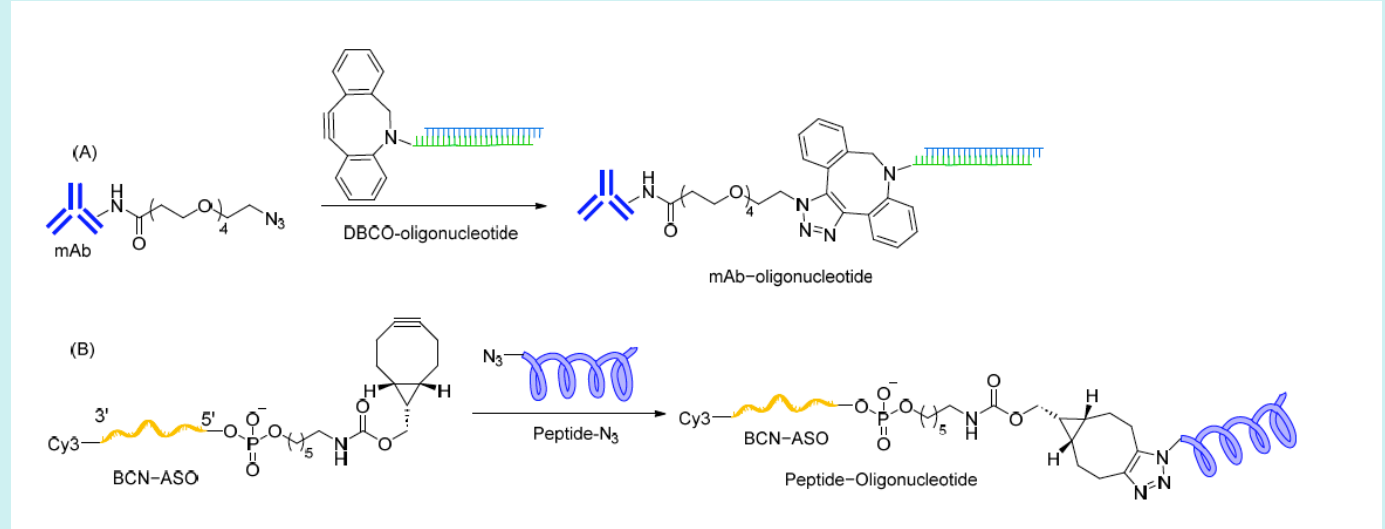
Barthélémy. *et al. PCT* 2015,  
Oumzil *et al. ChemMedChem* 2015

# CHIMIE DES CONJUGUÉS

## Challenges et opportunités

### Chimie click (DBCO)

- ✓ - Biodélivrance
- ✓ - Ciblage
- ✓ - Multimodalités
- ✓ - Stabilité

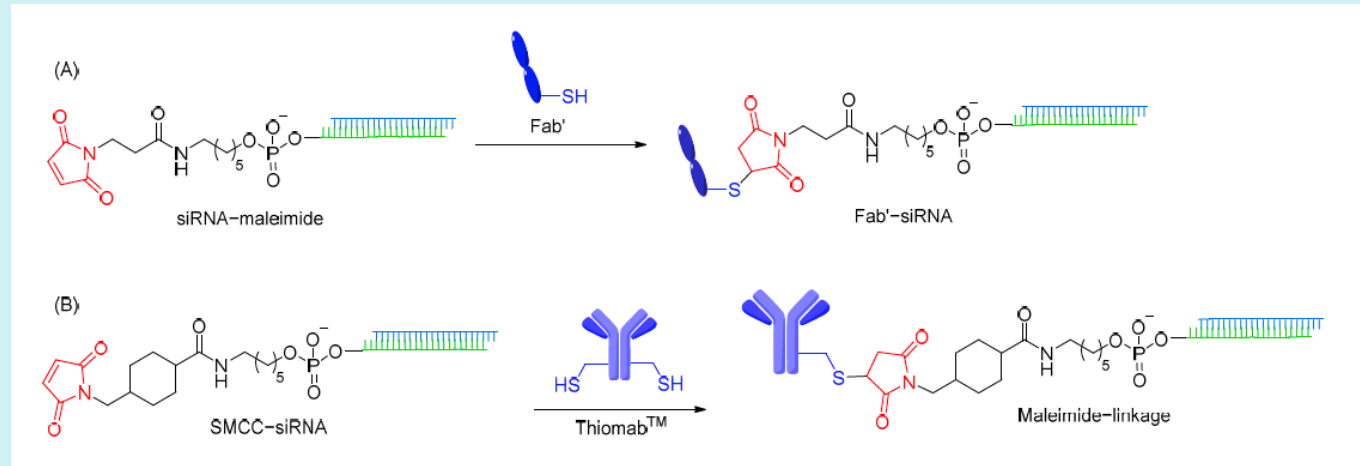


# CHIMIE DES CONJUGUÉS

## Challenges et opportunités

- ✓ - Biodélivrance
- ✓ - Ciblage
- ✓ - Multimodalités
- ✓ - Stabilité

### Maléimide



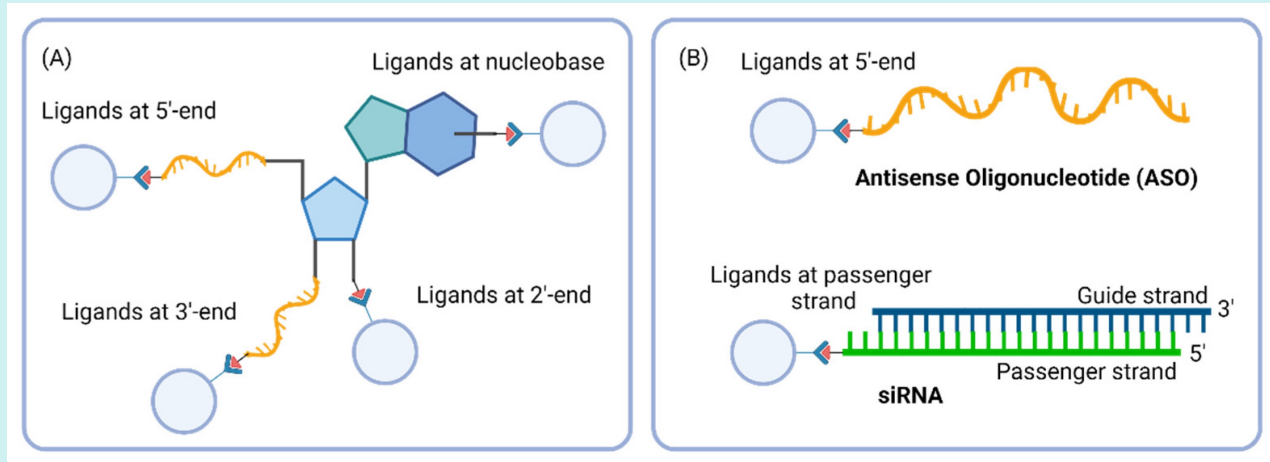


# CHIMIE DES CONJUGUÉS

## Challenges et opportunités

- ✓ - Biodélivrance
- ✓ - Ciblage
- ✓ - Multimodalités
- ✓ - Stabilité

Position ?



# CHIMIE DES CONJUGUÉS

## Challenges et opportunités

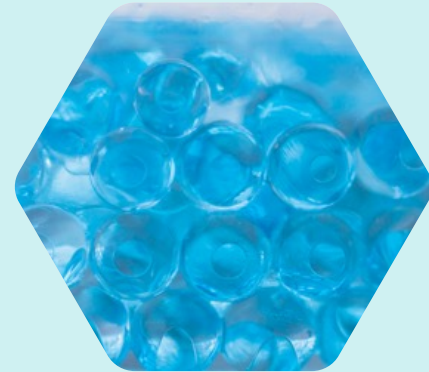
## Avantages / Inconvénients

- ✓ - Biodélivrance
- ✓ - Ciblage
- ✓ - Multimodalités
- ✓ - Stabilité

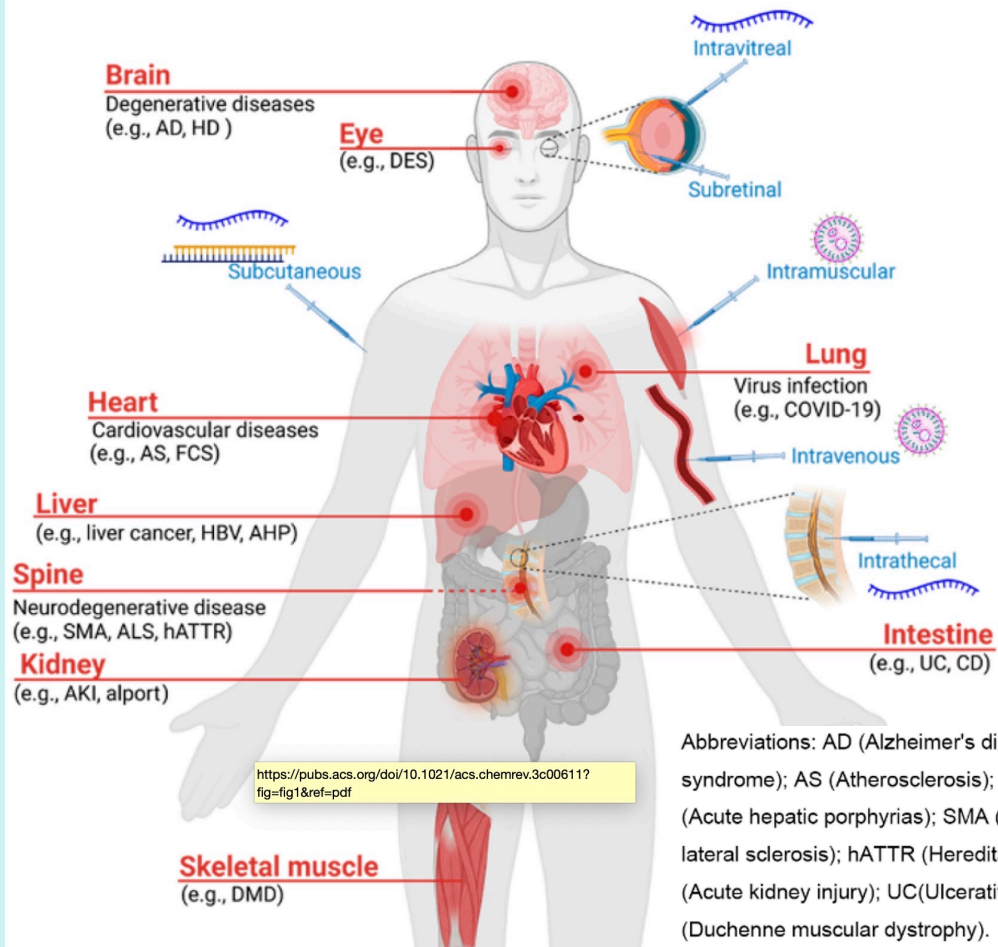
Stepwise Solid-Phase Synthesis		
Conjugation via	Advantages	Disadvantages/Limitations
Bifunctional or trifunctional linker	<ul style="list-style-type: none"><li>• Absence of time-consuming isolation/purification of both peptide (P) and oligonucleotide (O) fragments</li><li>• No excess of either P or O fragment—less solubility problems</li><li>• May be convenient for peptide-PNA conjugates (P-PNAs) due to protecting group compatibility</li></ul>	<ul style="list-style-type: none"><li>• Poor compatibility of P and O chemistries: the need to design a suitable protecting group scheme.</li><li>• Attachment of limited number of amino acids without side-chain protection</li><li>• Difficulty synthesizing longer than medium-length conjugates</li></ul>
Post-Synthetic Conjugation		
Conjugation via	Advantages	Disadvantages/Limitations
Thioether or disulfide bond Native ligation Oxime, thiazolidine, or hydrazone linkage Amide bond formation Click chemistry Diels-Alder reaction	<ul style="list-style-type: none"><li>• Many suitable conjugation procedures available</li><li>• Many reagents for functionalization of either fragment available</li><li>• No problem with incompatibility of the two chemistries</li><li>• Conjugation of peptides with any amino acid composition</li><li>• Conjugation of peptides of almost any length (up to proteins)</li></ul>	<ul style="list-style-type: none"><li>• Separate multistep preparation and purification of both fragments</li><li>• Reaction in aqueous solvents</li><li>• Solubility problems with polycationic or highly hydrophobic peptides</li></ul>

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# APPLICATIONS THERAPEUTIQUES

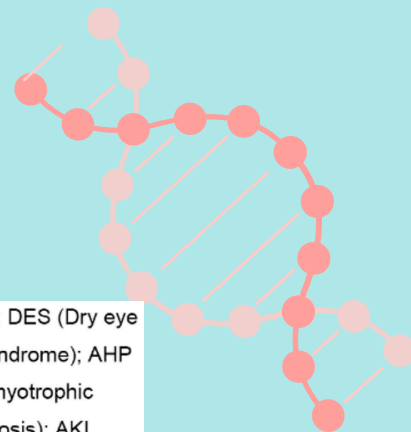


# Chemical modifications for better RNA therapeutics

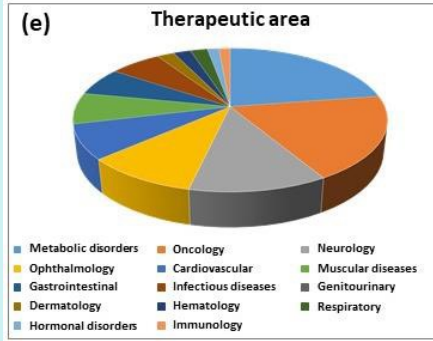
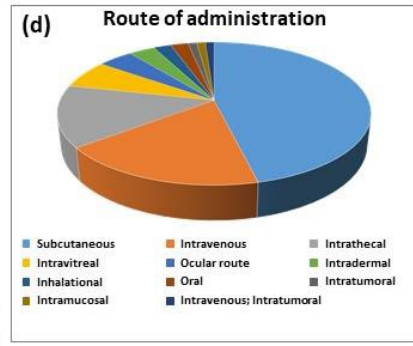
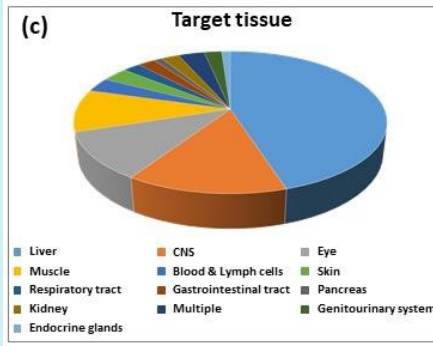
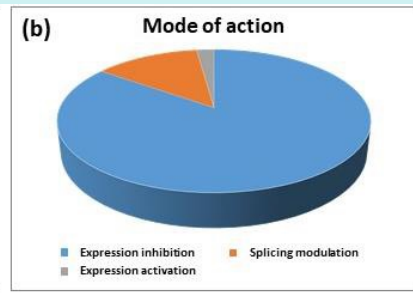
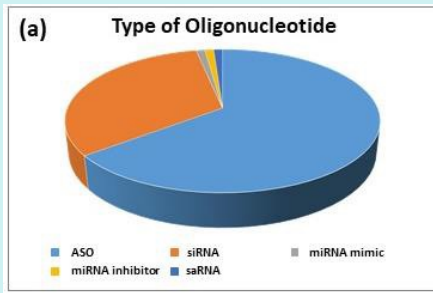
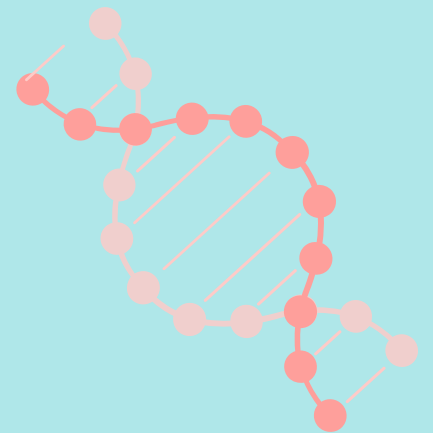


Abbreviations: AD (Alzheimer's disease); HD (Huntington's disease); DES (Dry eye syndrome); AS (Atherosclerosis); FCS (Familial chylomicronemia syndrome); AHP (Acute hepatic porphyrias); SMA (Spinal muscular atrophy); ALS (amyotrophic lateral sclerosis); hATTR (Hereditary transthyretin-mediated amyloidosis); AKI (Acute kidney injury); UC (Ulcerative colitis); CD (Crohn's disease); DMD (Duchenne muscular dystrophy).

## Applications thérapeutiques



# Applications thérapeutiques



Plus de 40 oligonucleotides en développement clinique

# Médicaments



**ASO 1998**  
CMV Retinitis



**Aptamer 2004**  
Age-Related Macular Degeneration (AMD)



**ASO 2013**  
Familial Hypercholesterolemia



**SSO 2016 SSO 2019**  
Duchenne Muscular Dystrophy



**SSO 2016**  
Spinal Muscular Atrophy



**Vaccine Adjuvant 2017**  
HBV



**siRNA 2018**  
TTR Polyneuropathy



**ASO 2018**  
TTR Polyneuropathy



**ASO 2019 EU**  
Familial Chylomicronemia Syndrome



**siRNA 2019**  
AHP Porphyria



**siRNA 2020**  
Primary Hyperoxaluria type 1



**SSO 2020**  
Duchenne Muscular Dystrophy



**SSO 2021**  
Duchenne Muscular Dystrophy



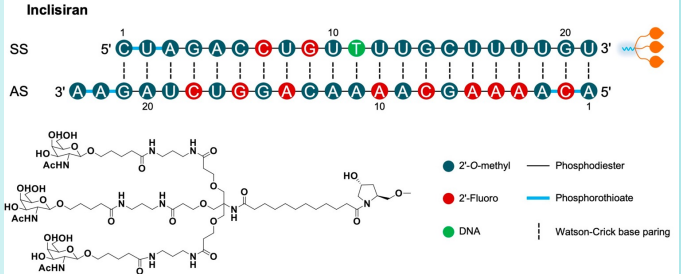
**siRNA 2021**  
LDL-C reduction "siRNA statin"



**siRNA 2022**  
TTR Polyneuropathy



**mRNAs 2021/2022**  
Covid-19 Vaccines



**siRNA targeting  
the expression of PSK9**





04

# ASO DELIVERY

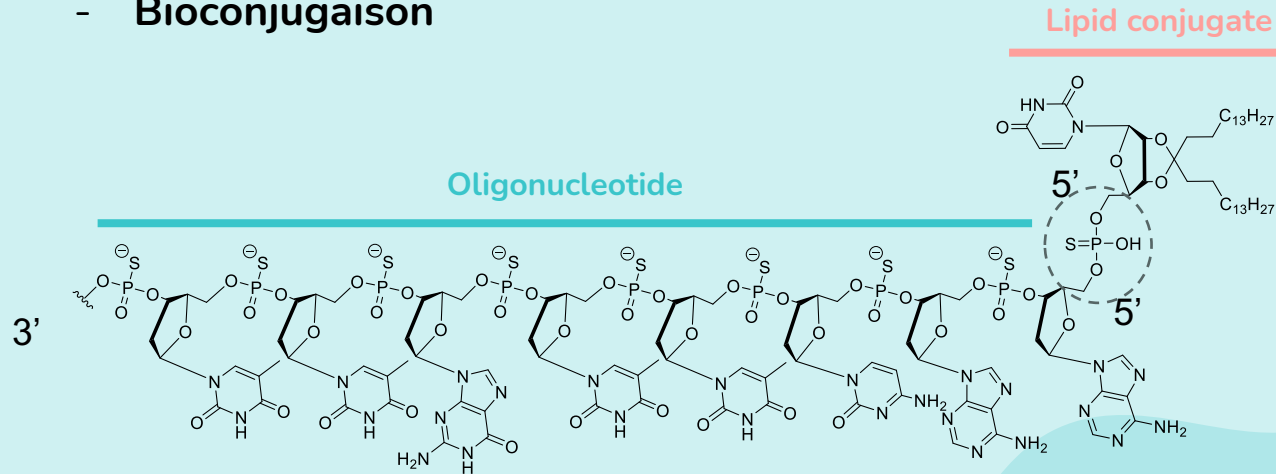


ARNA 

# LIPID ANTISENSE OLIGONUCLEOTIDE (L-ASO)

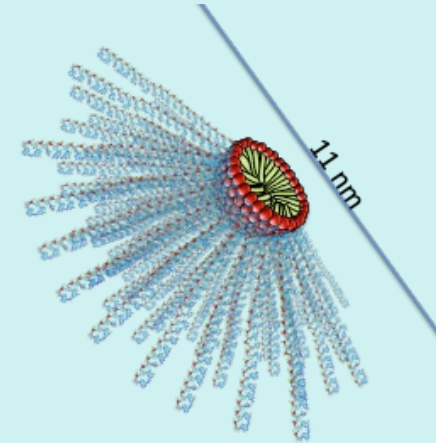
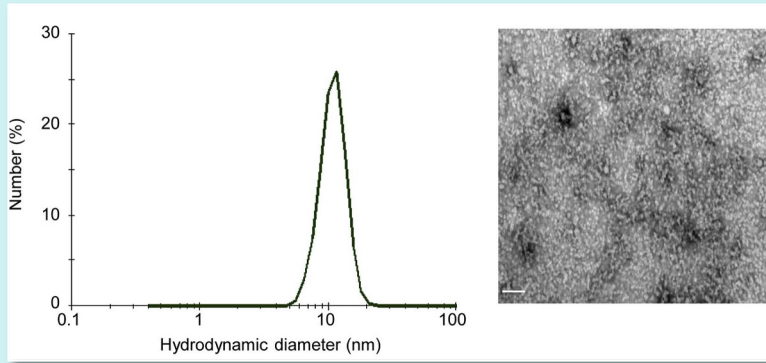
Modifications pour améliorer la biodélivrance et le ciblage cellulaire

- Conjugué lipidiques
- Bioconjugaison





# LIPID ANTISENSE OLIGONUCLEOTIDE (L-ASO)



LASO => **Inserm** **Transfert**

- Cancer
- Résistance aux antibiotiques
- Douleur
- Allergies, etc

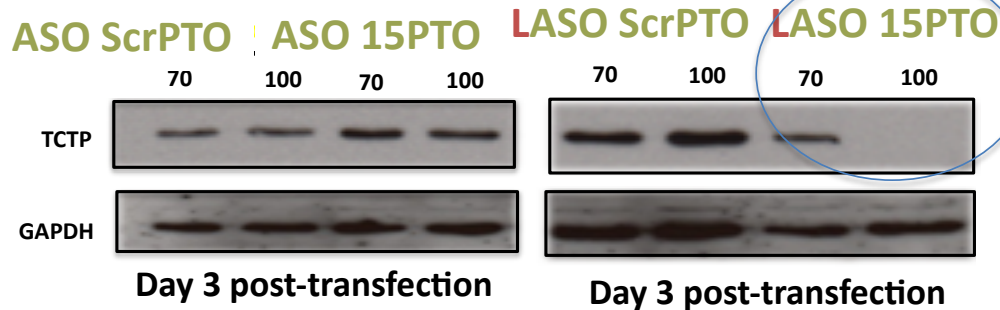
# PROSTATE CANCER

ASO => Translationally Controlled Tumor Protein (TCTP)  
TCTP est sur exprimé dans les cancers résistants CRPC



Collaboration  
Dr. Palma Rocchi  
Centre de Recherche en Cancérologie de Marseille  
CRCM, Inserm UMR 1068-CNRS UMR 7058

## LASO 15 PTO Inhibits TCTP expression



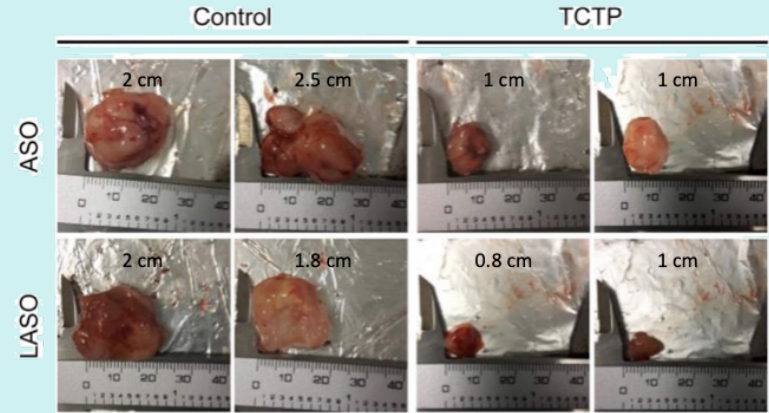
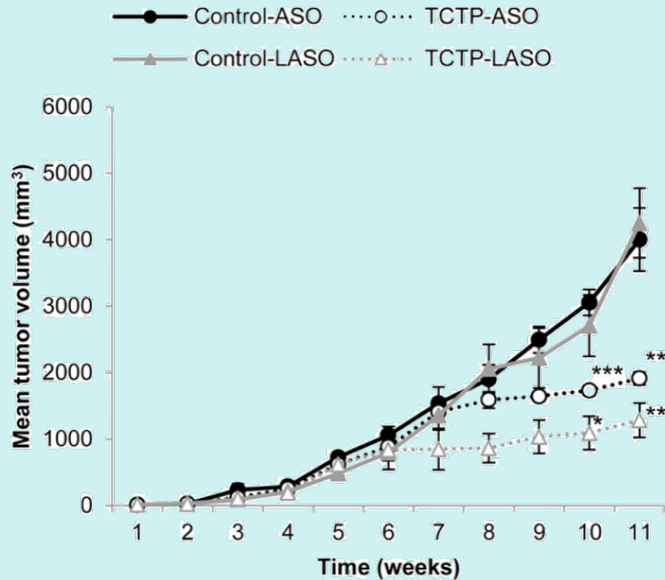
LASO inhibe l'expression de la TCTP sans agent de transfection

## Lipid-ASO

Barthélémy *et al.* PCT/IB2013/001516 (2013)  
Barthélémy *et al.* PCT/IB2013/001517 (2013)

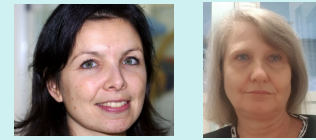
# PROSTATE CANCER

ASO => Translationally Controlled Tumor Protein (TCTP)  
 TCTP est sur exprimé dans les cancers résistants CRPC



**Lipid-ASO**

LASO inhibe la croissance tumorale *in vivo*

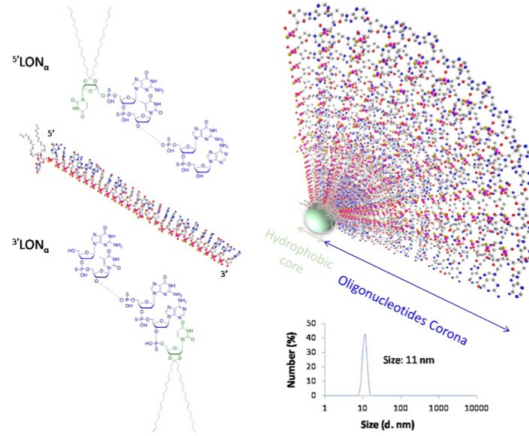
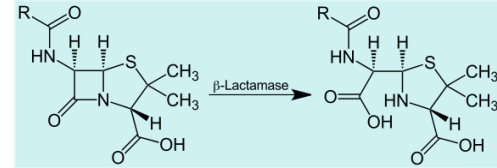


Kauss, T.; Arpin, C.

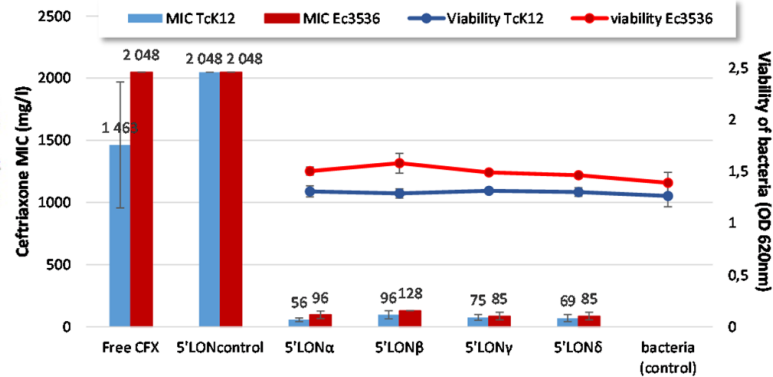
OPEN

## Lipid oligonucleotides as a new strategy for tackling the antibiotic resistance

Tina Kauss<sup>1\*</sup>, Corinne Arpin<sup>2\*</sup>, Léa Bientz<sup>2,3</sup>, Phouc Vinh Nguyen<sup>1,3</sup>, Brune Vialet<sup>1</sup>, Sebastien Benizri<sup>1</sup> & Philippe Barthélémy<sup>1\*</sup>



**B** Ceftriaxone MIC on laboratory (TcK12) and clinical (Ec3536) resistant strains of *E. coli*



- Biodélivrance des Oligonucleotides dans les cellules procaryotes
- Diminution des concentration minimale inhibitrice (MIC) des bactéries résistantes à la ceftriaxone (céphalosporine de troisième génération)

# Traitement de la douleur

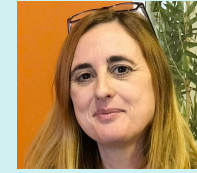
## 3 types de douleur chronique:

- Neuropathique,
- Nociceptive (inflammatoire),
- Dysfonctionnelle.

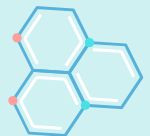
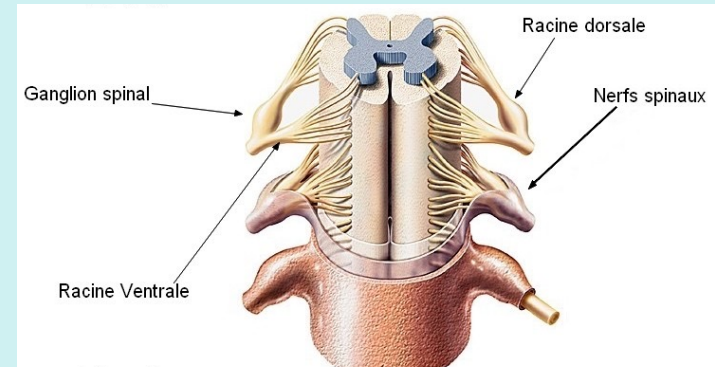
Le ganglion spinal stimule la racine dorsale jusqu'à la moelle épinière

**FXD2** est considéré comme un nouveau marqueur limité aux seuls mécanorécepteurs et nocicepteurs des neurones somato sensoriels primaires (Ventéo et al., 2012).

**La perte ou la diminution de FXD2 entraîne une réduction drastique ou la perte de douleur chez la souris**

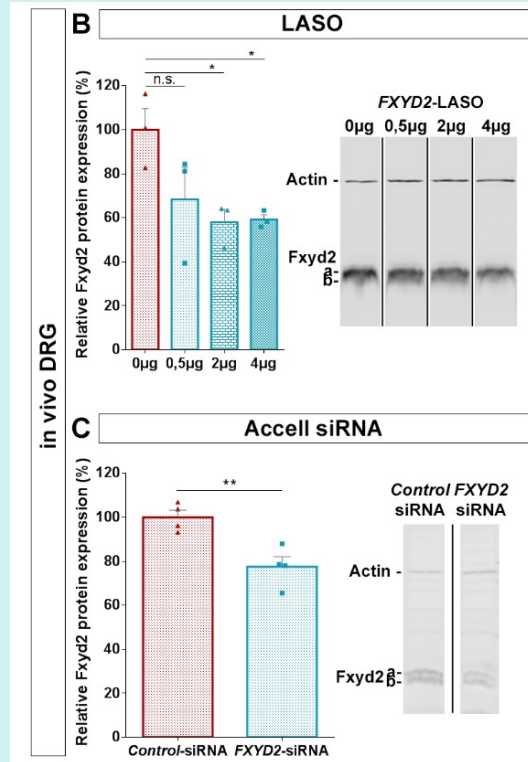
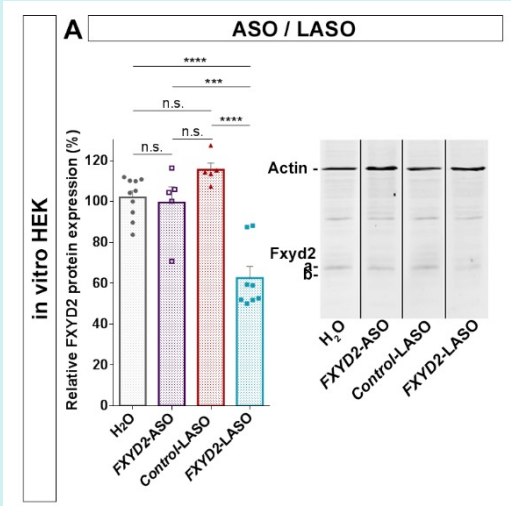


Collaboration Montpellier  
Dr Stéphanie Ventéo  
Dr Patrick Carroll



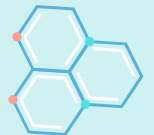
# Traitement de la douleur

Collaboration Montpellier  
Dr Stéphanie Ventéo  
Dr Patrick Carroll



L'expression de Fxyd2 peut être inhibée dans les ganglions de la racine dorsale par injection intrathécale d'oligonucléotides antisens sans l'utilisation de réactifs de transfection toxiques.

Western blot  
using  
a Fxyd2  
antibody



# Traitement de la douleur

Collaboration Montpellier  
Dr Stéphanie Ventéo  
Dr Patrick Carroll

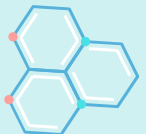
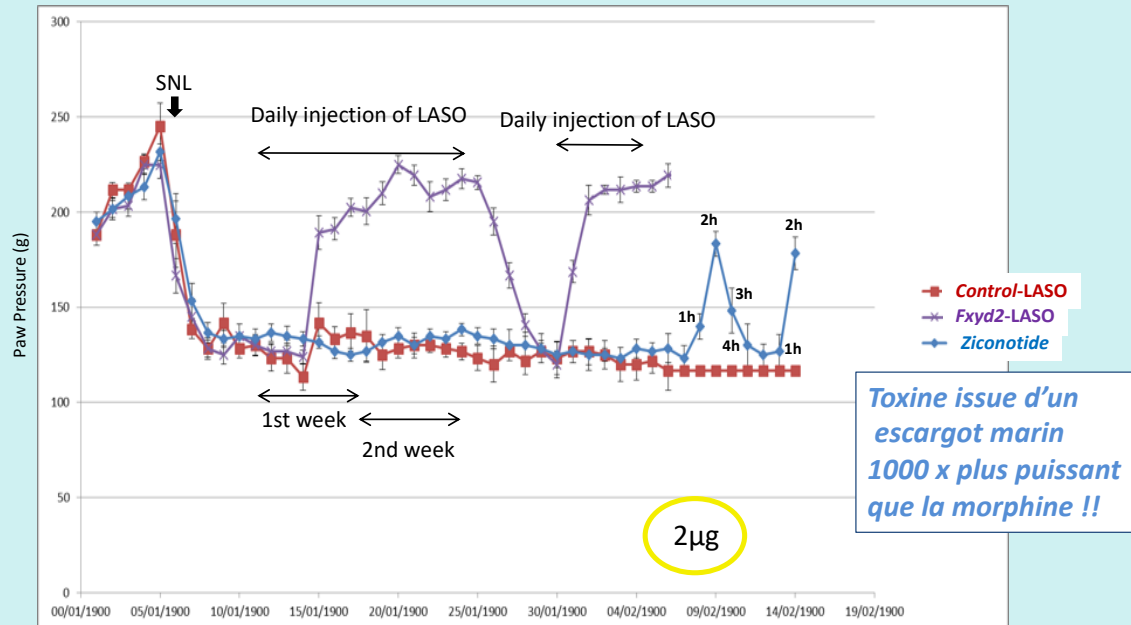


**NEUROPATHIC PAIN: LASO vs LScr (IT : 2  $\mu$ g)**  
Comparison with **Ziconotide** (PRIALT)

**NEUROPATHIC PAIN: Comparison, *in vivo*, of the analgesic effect of LASO75 at the best determined dose (2 $\mu$ g) versus ziconotide versus L-scramble control (2 $\mu$ g)**

(Daily injection in IT for 14 days, stop 7 days, resumption 7 days)

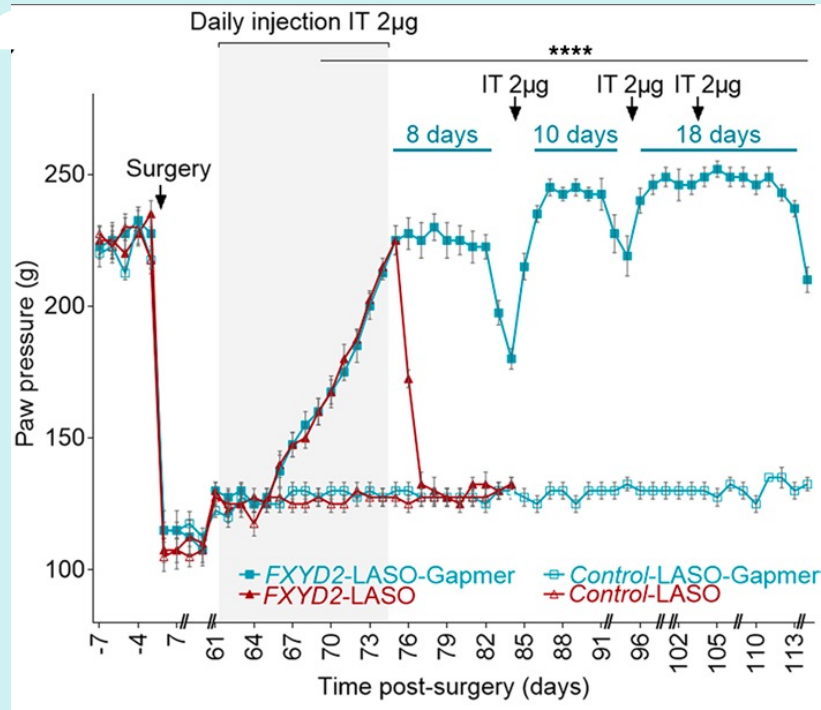
Randall



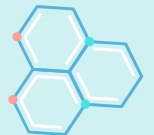
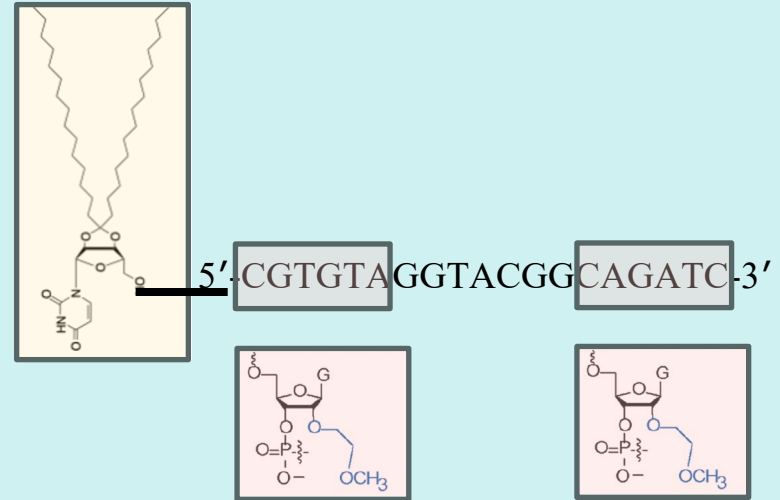


# Traitement de la douleur

Collaboration Montpellier  
Dr Stéphanie Ventéo  
Dr Patrick Carroll



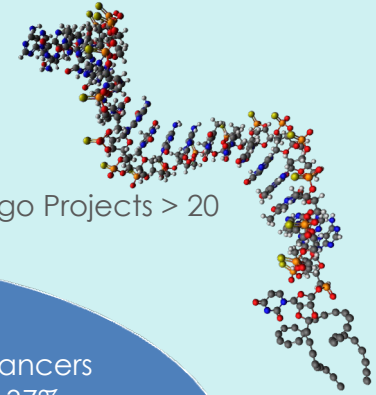
MOE-modified FXYD2-LASO-Gapmer provides long-lasting pain relief in neuropathic pain and inflammatory pain models



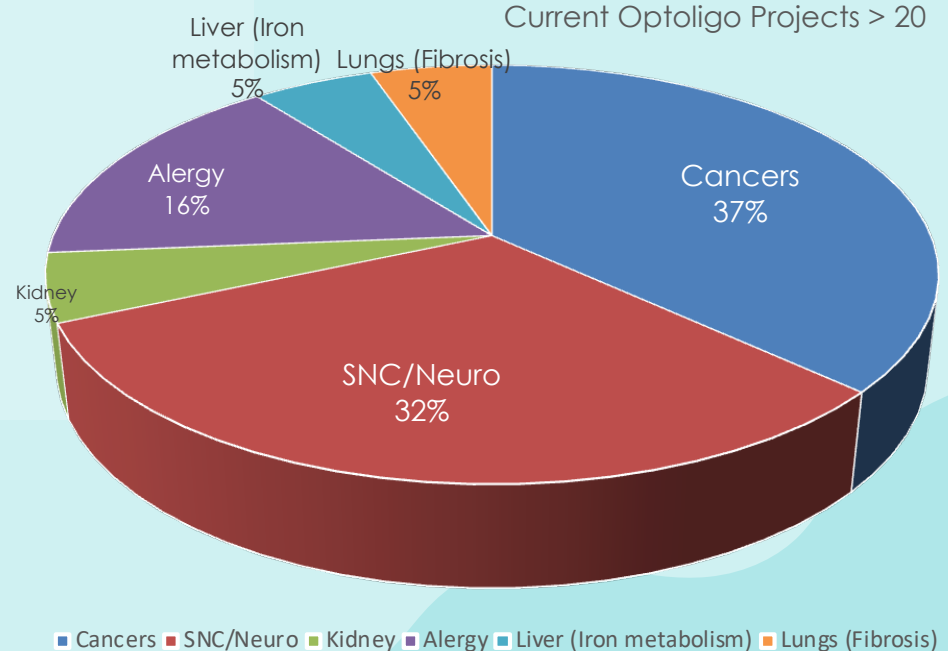


# Hub Optoligo

## LIPID ANTISENSE OLIGONUCLEOTIDE (L-ASO)

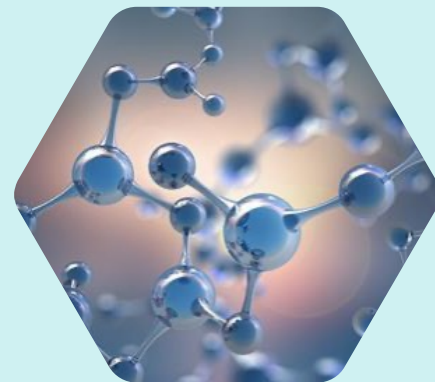


Porteur du projet	Type de pathologie	Organ ciblé	Cible biologique
	Métabolisme du fer (Anémie)	Foie	
	Biosensor, detection cellule souche tumorale	Cancer	
	Fibrose pulmonaire	Poumon	
	Inhibition de la sécrétion histaminique		
	Récepteur IgE		
	Douleur neuropathique et inflammatoire		
	Douleur neuropathique et inflammatoire		
	Cancer	Tumeurs gastro-intestinales	
	néphropathies	Rein	
	Asthme allergique		
		SNC	
	Lymphome T (cancer)	Sang	
	Maladie Parkinson	SNC	
	Antibiorésistance	Bactéries E. coli TCK12	
	Libération prolongée d'oligos		
	cancers of the B lymphoid	primary B cells and plasma cells	
	Cancer	Pancréas	
	Maladie des petits vaisseaux cérébraux	SNC	
	NPC. La maladie de Niemann-Pick de type C (« NPC SNC +		
	Cancer	Prostate	

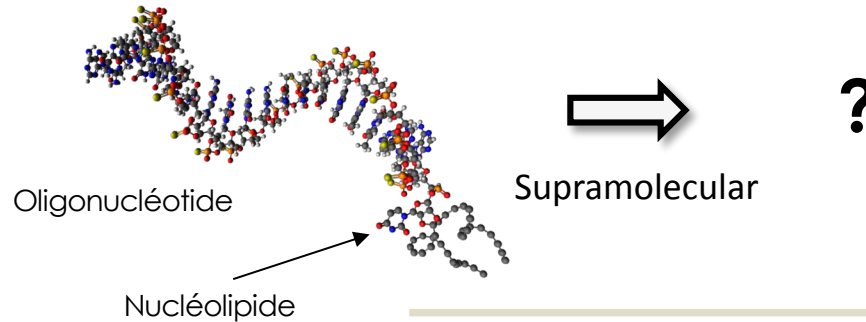


05

# CHIMIE SUPRAMOLAIRES DES CONJUGUES



## Bio-délivrance contrôlée ?



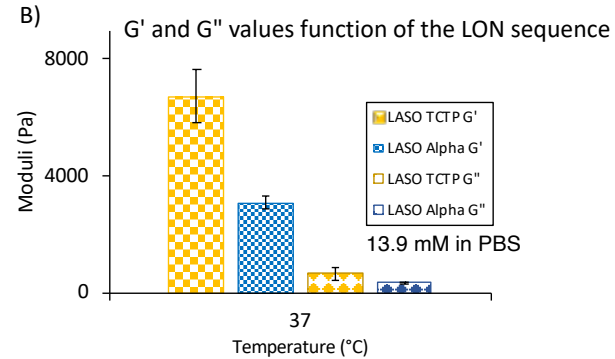
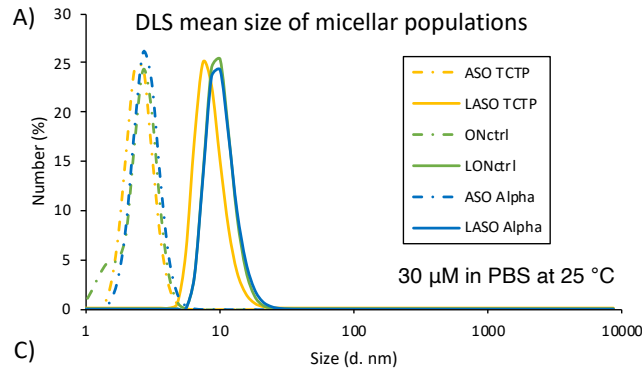
**Table 1** Sequences of tested ONs and LONs

Name <sup>a</sup>	Length (mers)	Sequence (5'→3')
ASO <sub>TCTP</sub>	20	5' AAC TTG TTT CCT GCA GGT GA 3'
LASO <sub>TCTP</sub>	21	5' (C <sub>15</sub> )U* AAC TTG TTT CCT GCA GGT GA 3'
ASO <sub>α</sub>	25	5' GCG CAG TGA TTT TTT AAC CAT GGG A 3'
LASO <sub>α</sub>	26	5' (C <sub>15</sub> )U*GCG CAG TGA TTT TTT AAC CAT GGG A 3'
ON <sub>Ctrl</sub>	19	5' CGT GTA GGT ACG GCA GAT C 3'
LON <sub>Ctrl</sub>	20	5' (C <sub>15</sub> )U* CGT GTA GGT ACG GCA GAT C 3'

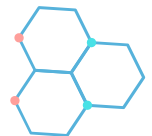
<sup>a</sup> LONs being 5' conjugates of the ON sequence with ketal bis-C<sub>15</sub> lipid.



## Etudes physico-chimique des ON/LON

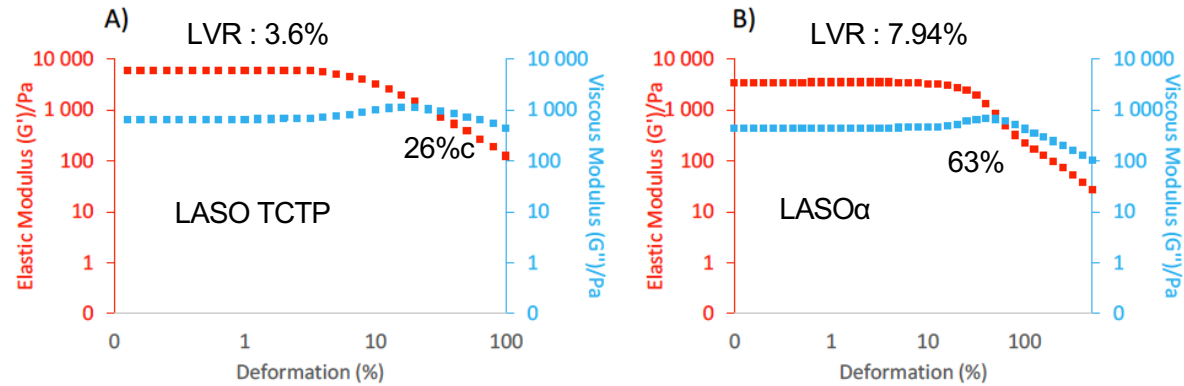


	Size (nm)	G' (Pa)	G'' (Pa)	LVR (%)	Breaking point (%)	Thixotropy
ASO <sub>TCTP</sub>	3.16 $\pm$ 0.41			No Gelation		
LASO <sub>TCTP</sub>	14.12 $\pm$ 0.17	6740 $\pm$ 927	684 $\pm$ 21	3.16	26 $\pm$ 5	Yes
ON <sub>ctrl</sub>	3.52 $\pm$ 0.55			No Gelation		
LON <sub>ctrl</sub>	14.35 $\pm$ 0.56			No Gelation		
ASO <sub><math>\alpha</math></sub>	3.91 $\pm$ 0.34			No Gelation		
LASO <sub><math>\alpha</math></sub>	15.05 $\pm$ 0.48	3088 $\pm$ 225	368 $\pm$ 2	7.94	63 $\pm$ 8	Yes



## Viscoelastic properties

### Amplitude sweep experiments



Amplitude sweep experiments of hydrogels at 13.9 mM ( $T^\circ$  37°C, 1 Hz, shear strain 0.01% to 100%).

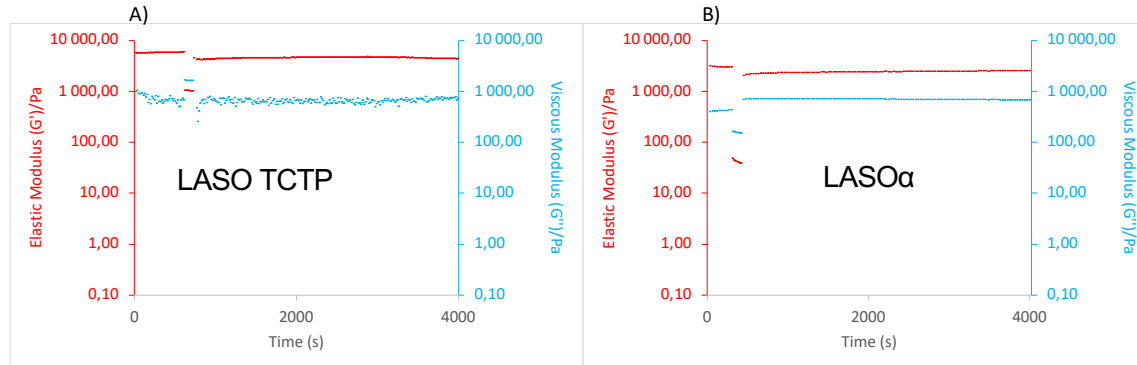
LASO $\alpha$  is a tougher material compared to LASO TCTP



Viscoelastic properties are sequence dependent



### Step-strain measurements

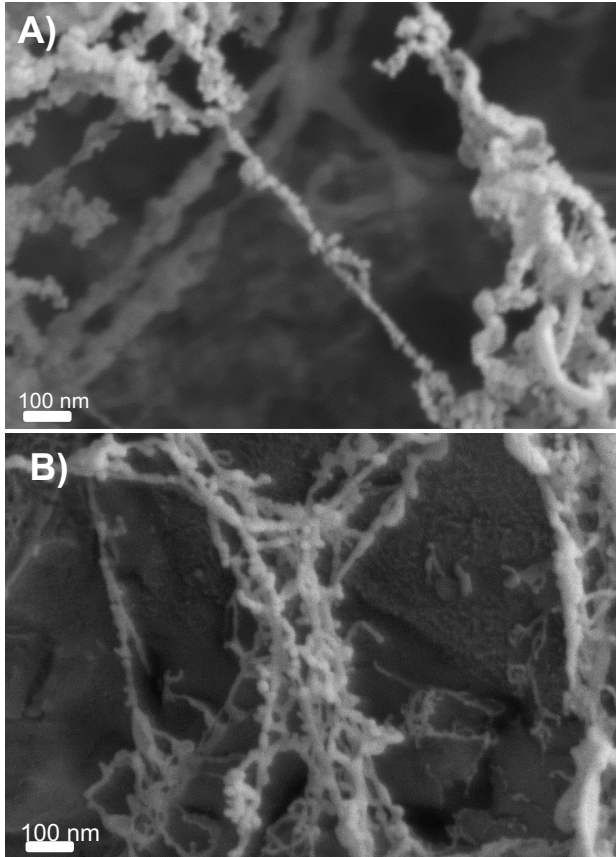


Step-strain experiments of A) LASOTCTP and B) LASO $\alpha$  hydrogels (13.9 mM) at 37°C and with a fixed angular frequency of 1 Hz. The gels were swept from 0.03% (structuration step) to 30% (destruction step) shear strain and then back to 0.03% (structuration step) shear strain.



Both LASO $\alpha$  and LASO TCTP biomaterials are **thixotropic**

## Gel morphologies

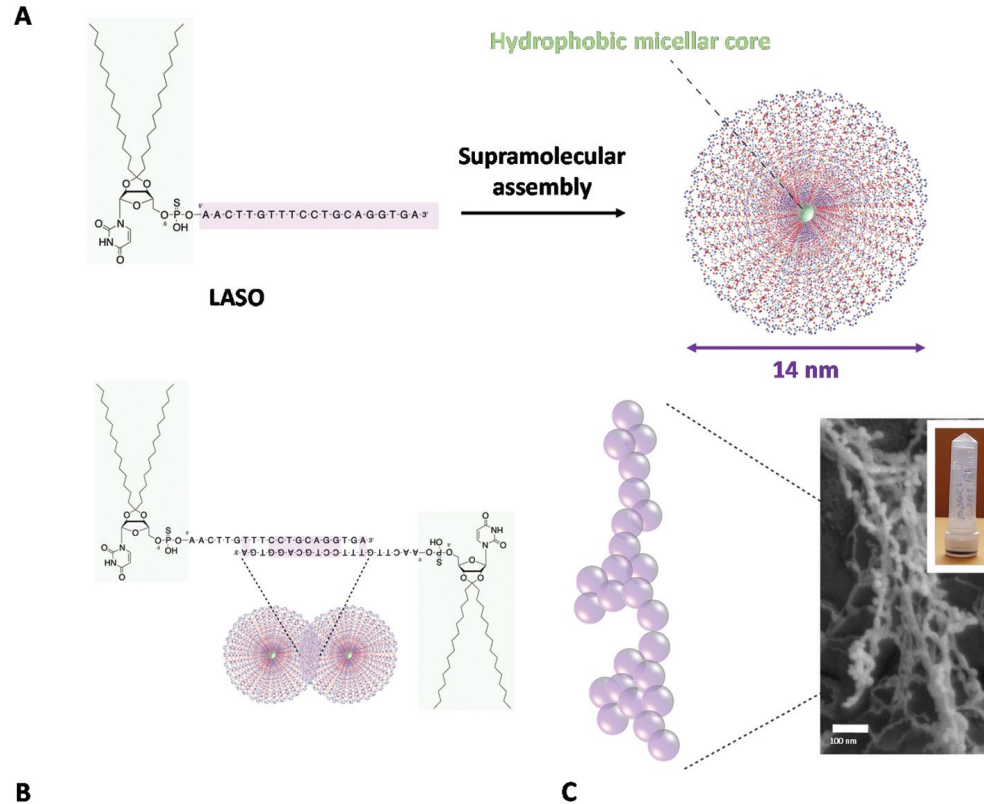


LASO $\alpha$  and LASO<sub>TCTP</sub> – based gel exhibited dense supramolecular networks with fibers of 10–20 nm in diameter.

Non-gelator molecules (LON<sub>Ctrl</sub>), cryo-SEM images showed simple micellar systems of 10–20 nm in size

Cryo-SEM images of (A) LASO $\alpha$ , (B) LASOTCTP at 13.9 mM in PBS 1 $\times$  (scale bar 100 nm)

## Supramolecular systems



Micelle-micelle supramolecular interactions stabilizing an entangled pearl-necklace network at the nanoscales



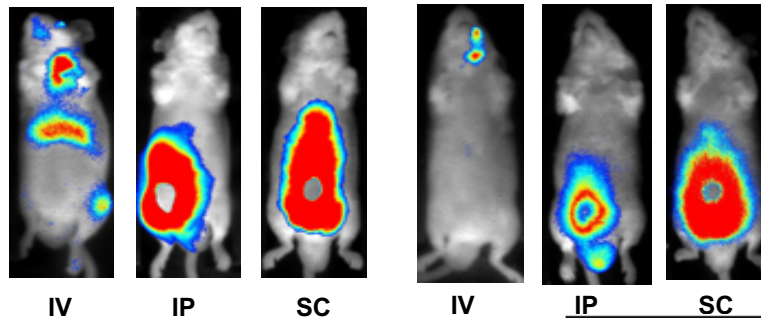
# Hydrogels

In vivo injection

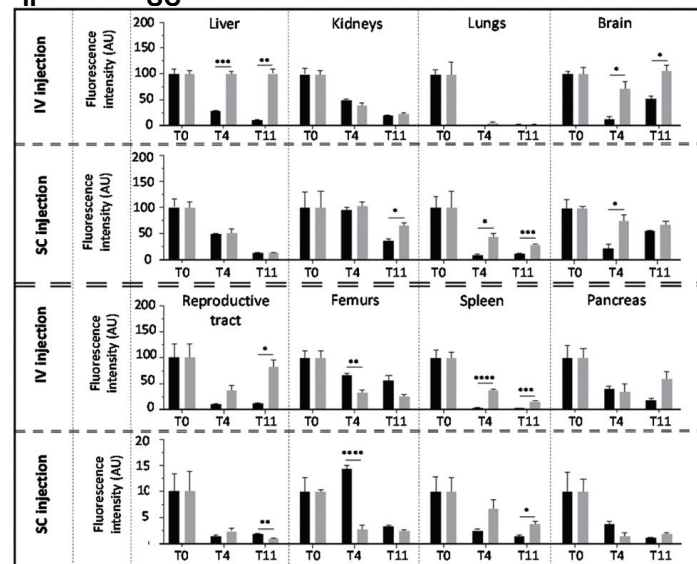
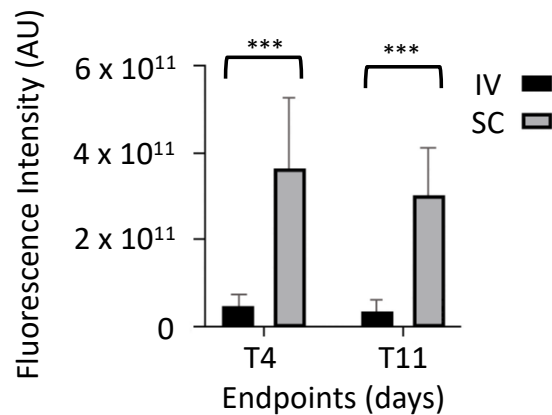
T0

T11

LASO<sub>TCTP</sub>

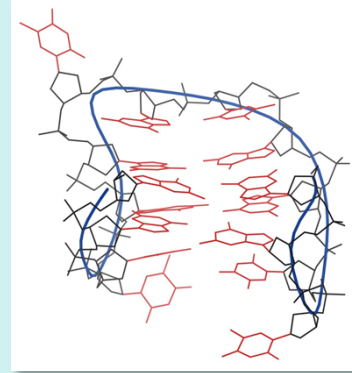


Whole mice LASO IV/SC

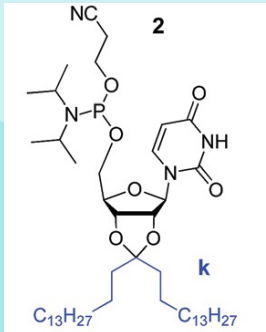


Fluorescence intensity on mice organs after IV and SC administrations of Cyanine 5 – LASOTCTP (grey)/ASOTCTP (black) (n = 5).

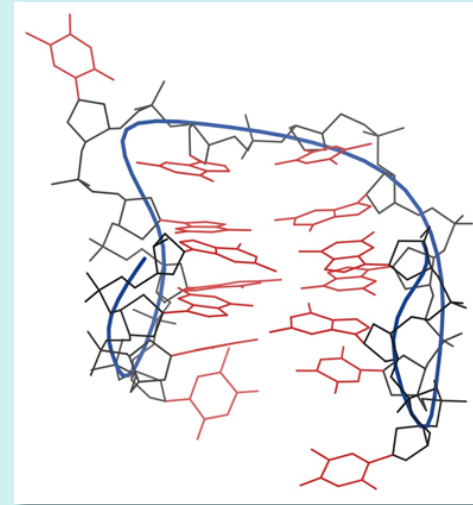
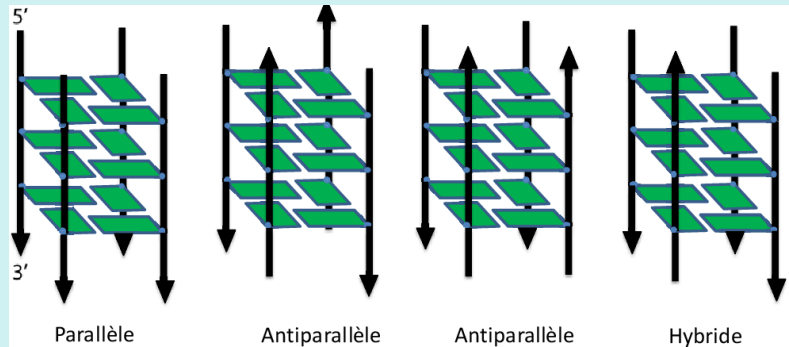
# Contrôle de la conformation ?



**Cas du TBA**  
**(aptamère de la thrombine, anticoagulant)**

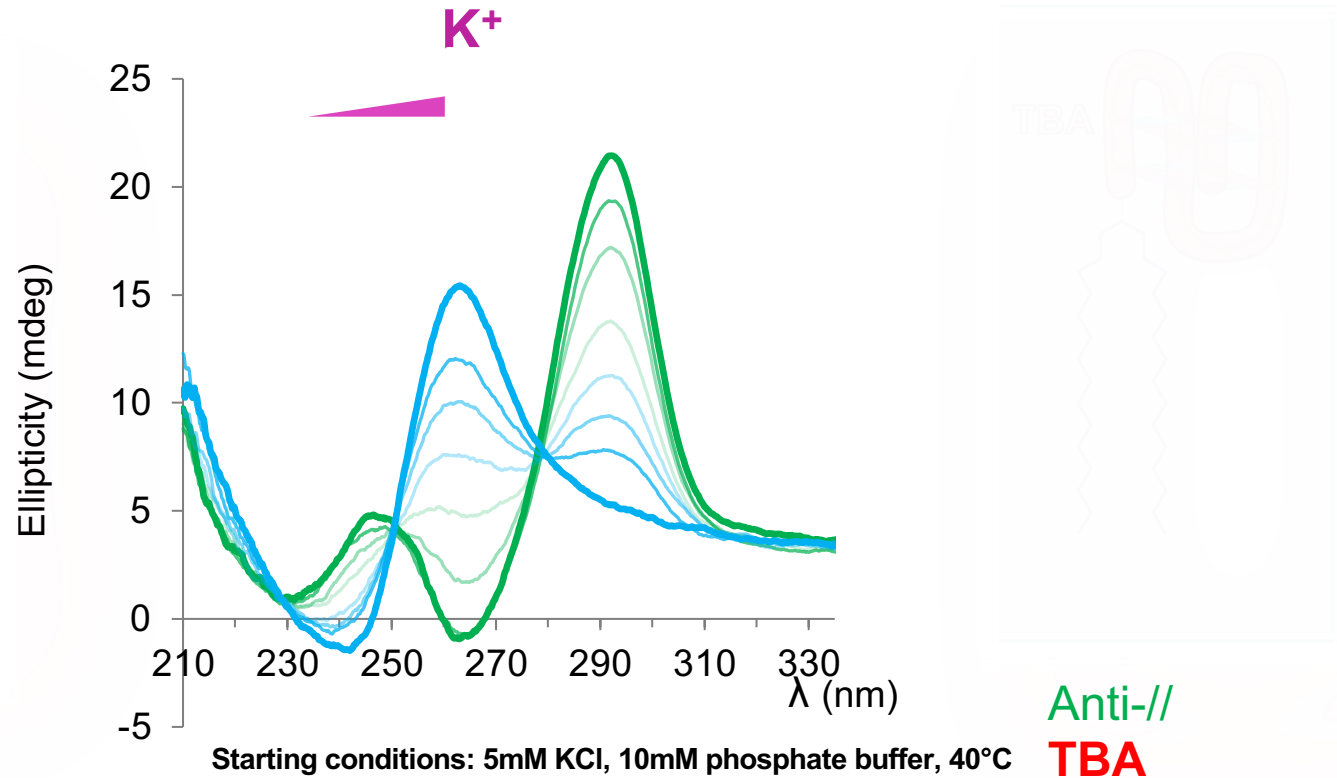


# Structure des G-quadruplexes parallèle, hybride et antiparallèle



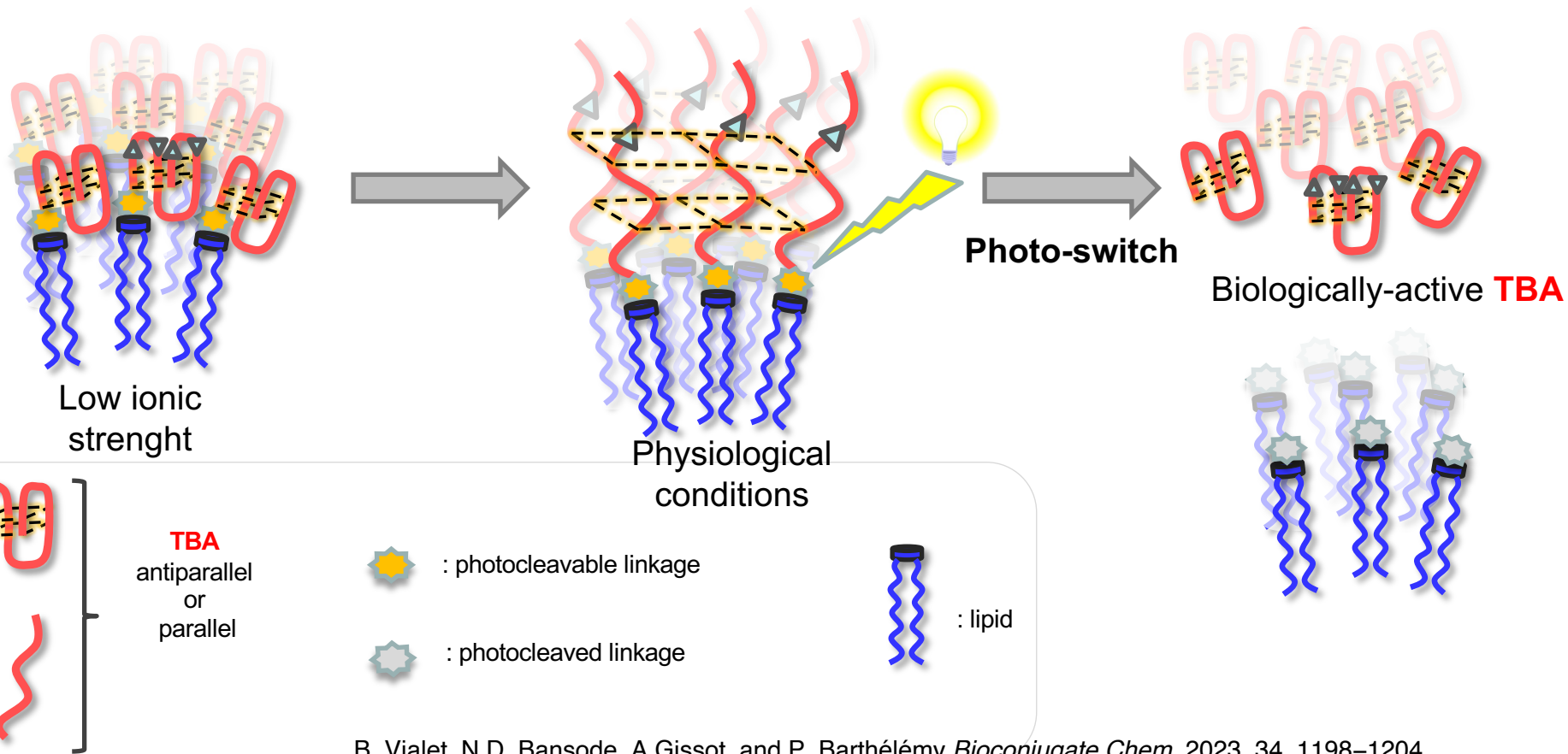
L'aptamère de la thrombine (TBA) est antiparallèle

# Contrôle des topologies de G4 ?

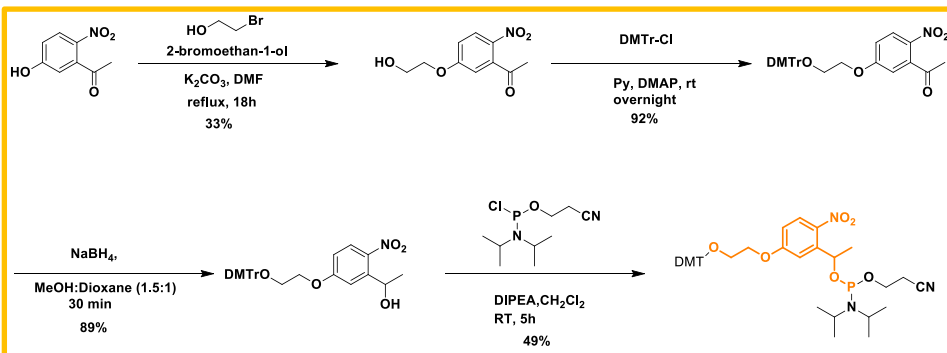
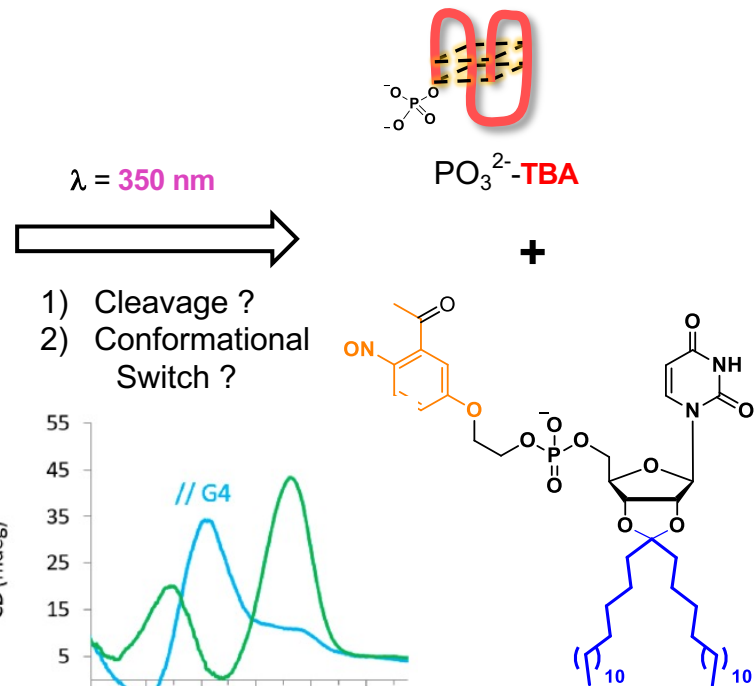
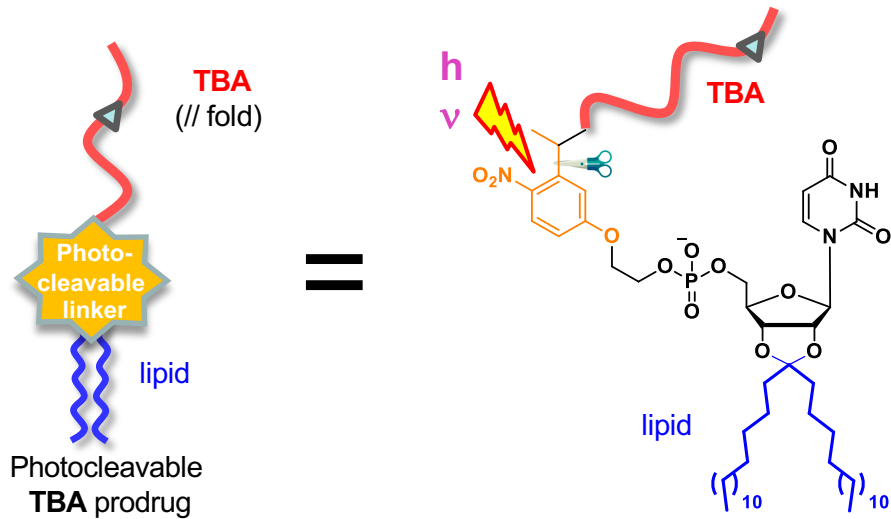



- The parallel form of TBA could act as a prodrug provided the process can be reversed

# Toward a photo cleavable TBA prodrug



# Toward a photo cleavable TBA prodrug



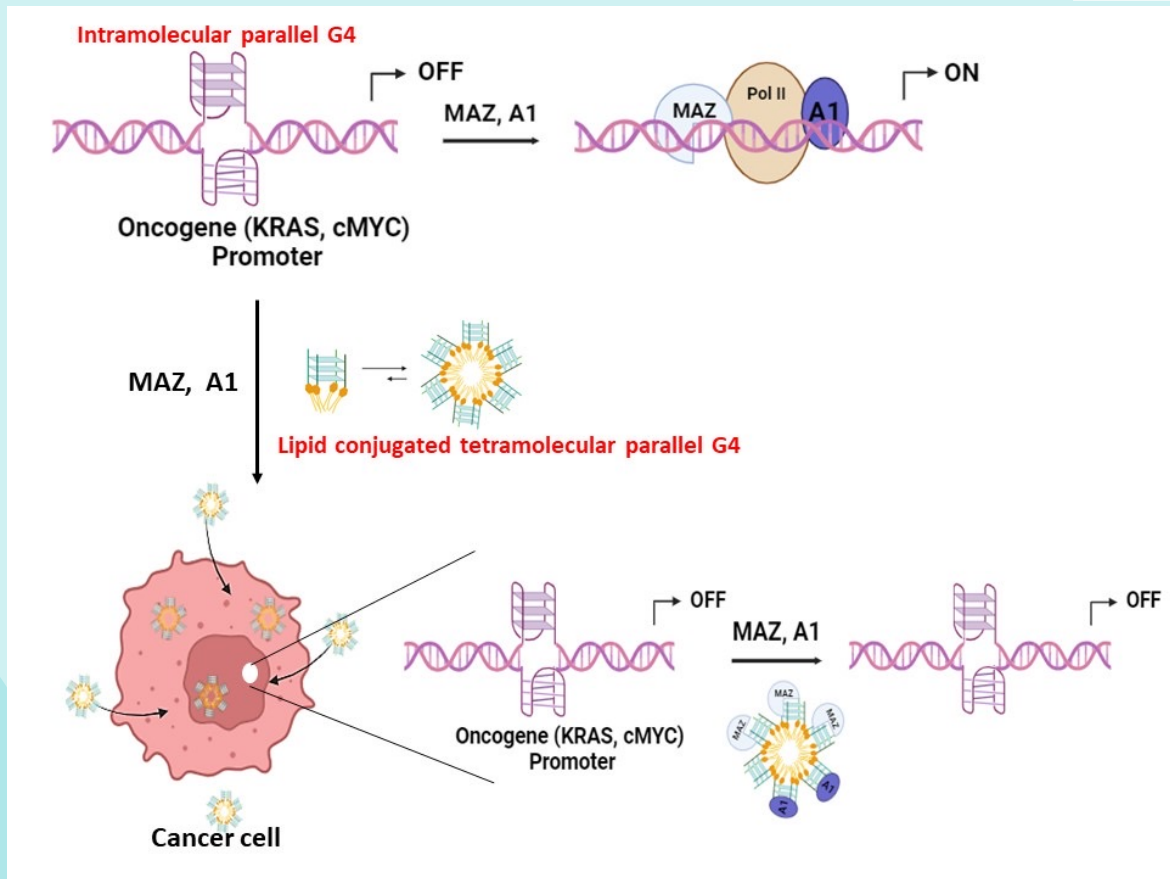


# **Contrôle de la conformation**



**Impact sur l'activité biologique ?**

# Nucleolipid-modified G4-decoy oligonucleotides as anticancer drug?

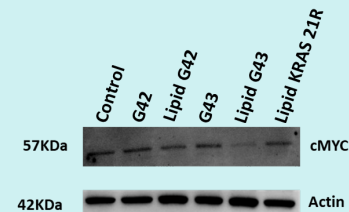
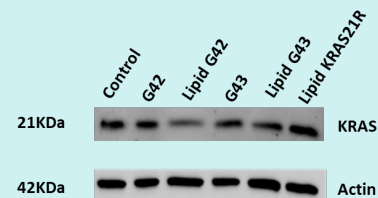
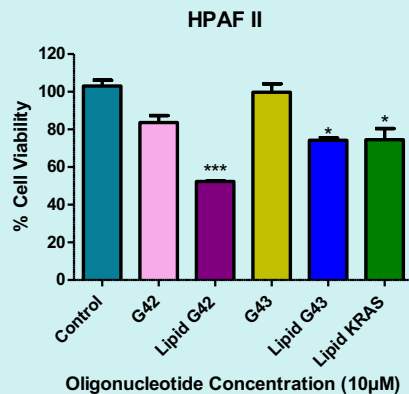
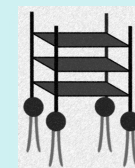
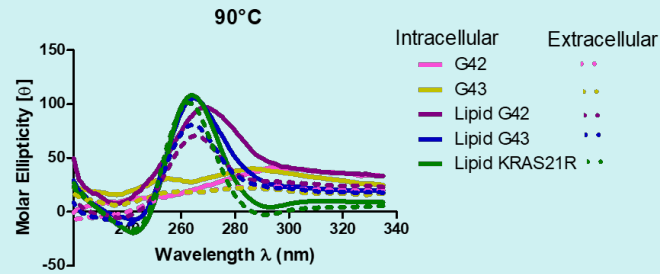
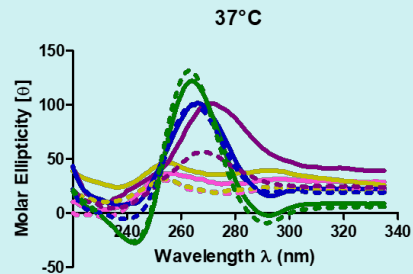
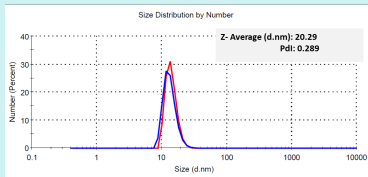
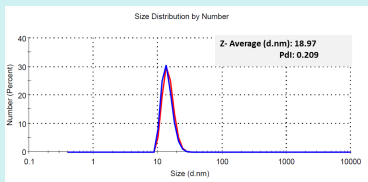
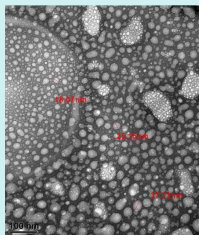


## Challenges

- ✓ Stability
- ✓ Controlling the topology
- ✓ Delivery into cells



# Nucleolipid-modified G4-decoy oligonucleotides as anticancer drug?



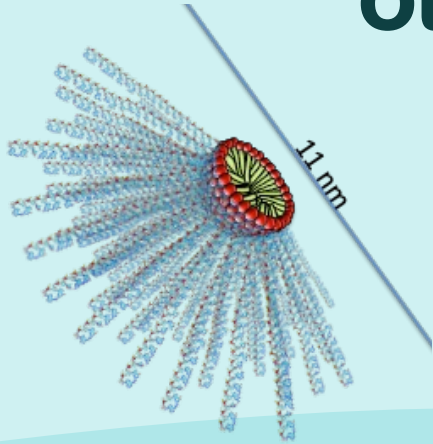
## Conjugués lipidique

- ✓ Stabilise des G4 parallèle dans les conditions extra et intra cellulaire
- ✓ Autorise l'internalisation dans les cellule
- ✓ Diminution de l'expression des Oncogènes KRAS et cMYC

# CONCLUSION



# Oligonucléotides bioconjugués



- ✓ “Self-delivery” des sequences ASOs *in vitro* et *in vivo* sans agents de transfection (pas de formulation)
- ✓ les LASOs sont des NCE (New Chemical Entities)
- ✓ API for Nano-formulation (co-delivery)
- ✓ Responsive nanomaterials
- ✓ Control of G4



**InsermTransfert**

# ARNA



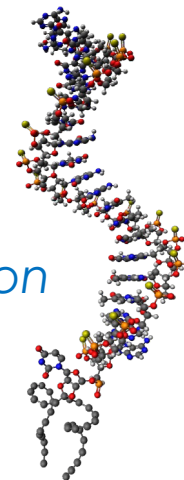
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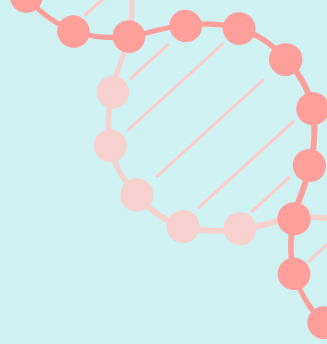
université  
de BORDEAUX



*Merci  
pour votre attention*

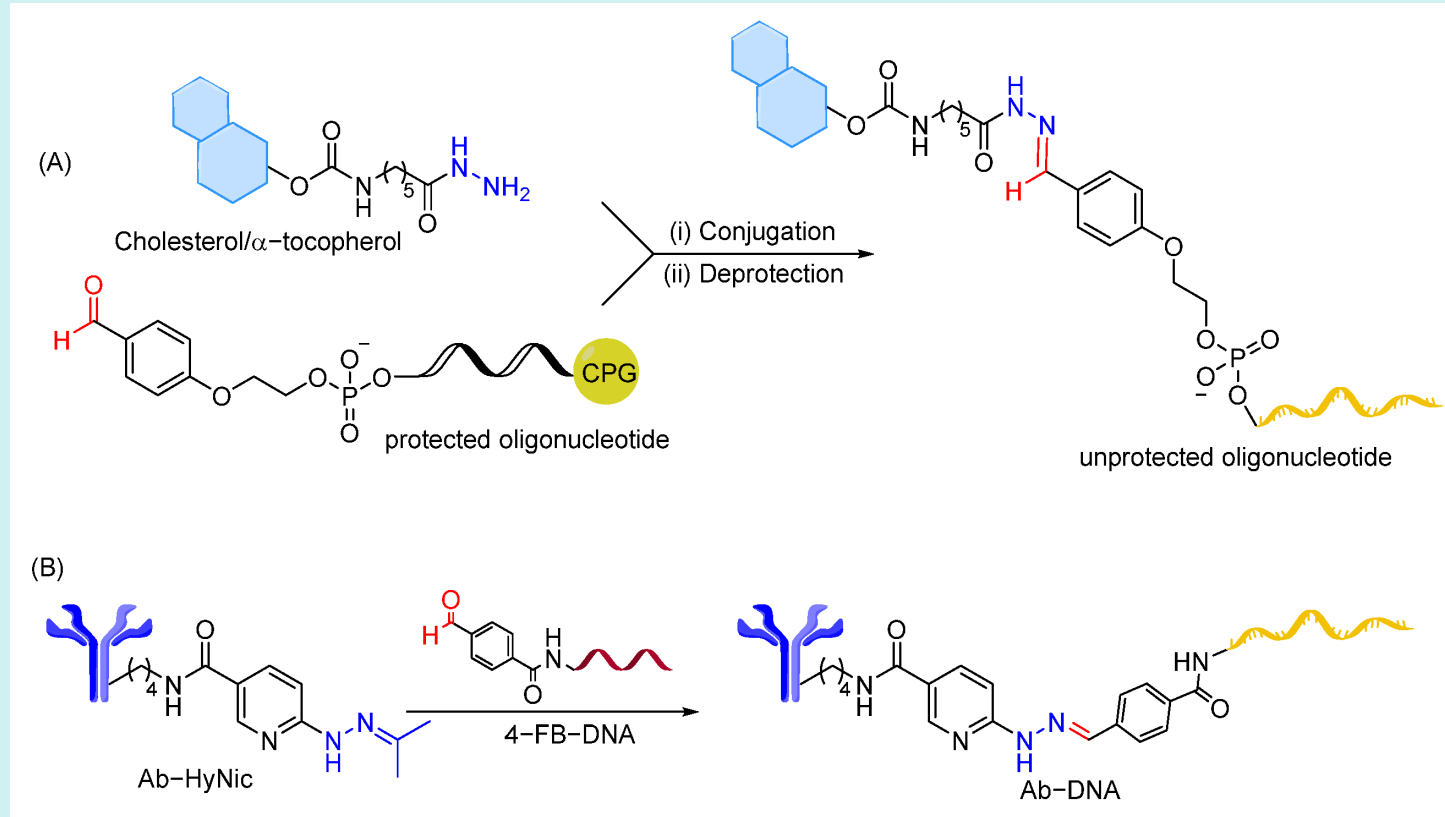


# ANNEXES



# Contexte des conjugués des acides nucléiques

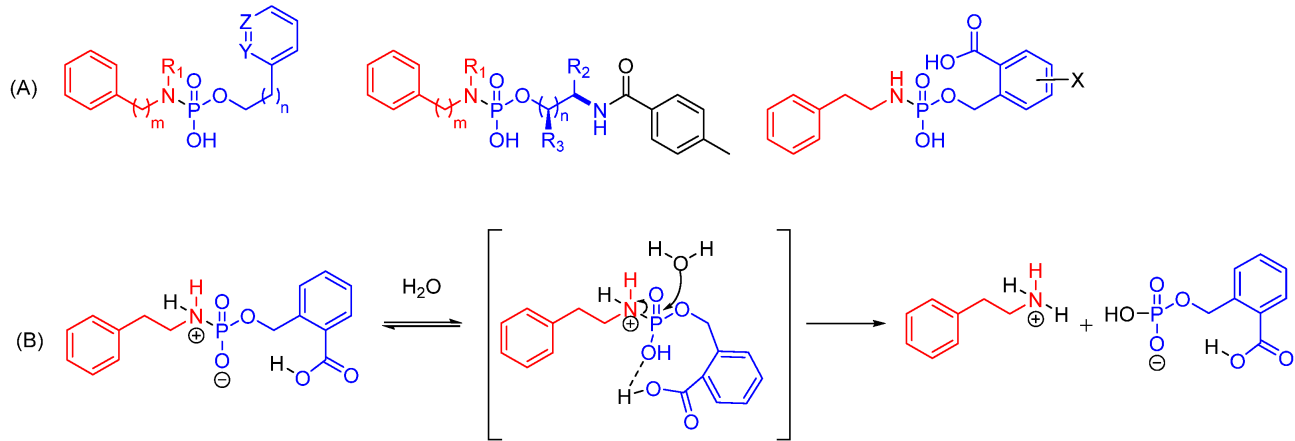
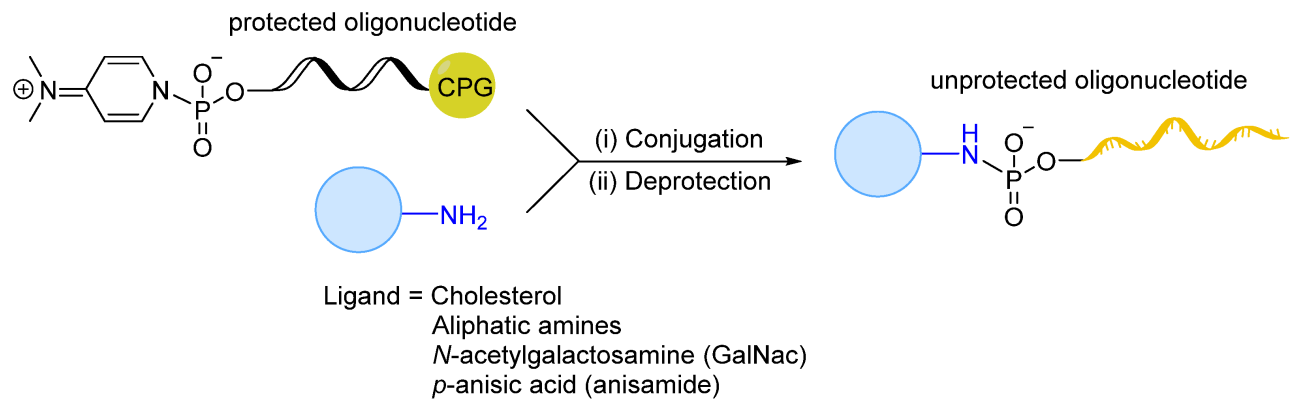
## Conjugués peptide-oligonucleotides



# Contexte des conjugués des acides nucléiques

## Conjugués peptide-oligonucleotides

pH sensitive linker

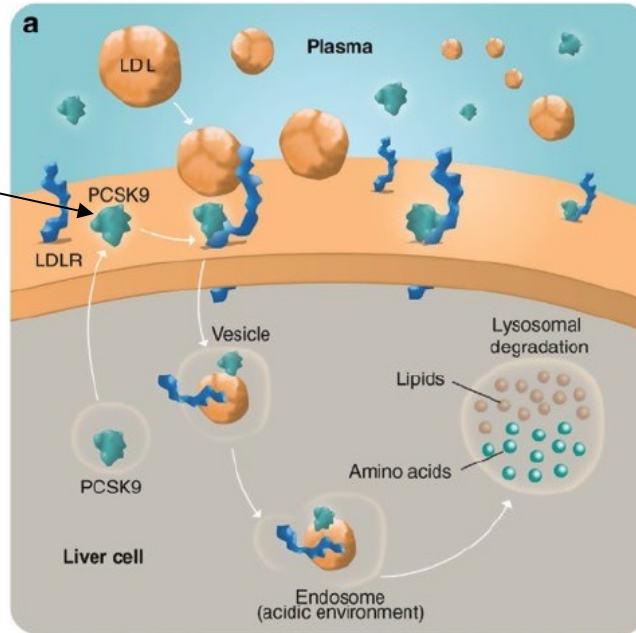


**The increased cardiovascular disease (CVD) risk is not satisfactorily addressed by current therapeutic approaches based on statins.**

=> PCSK9  
=> Apo(a)

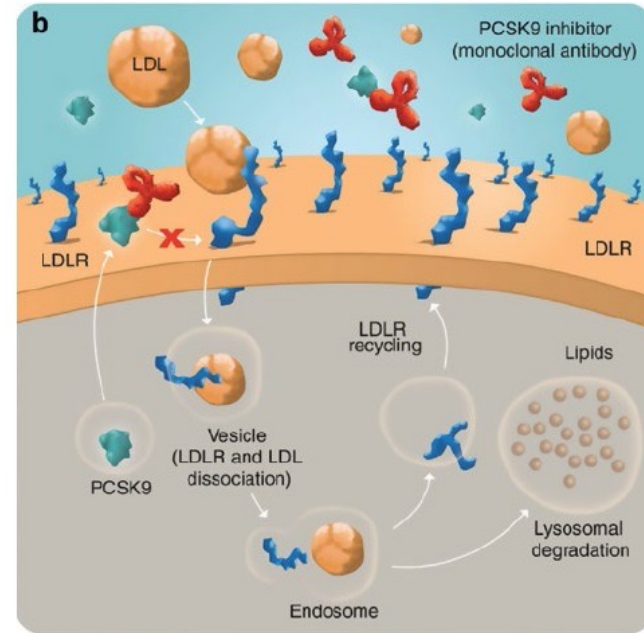
**PCSK9**

## How does PCSK9 work?



a) Secreted PCSK9 binds to LDLR on the liver cell surface and mediates the lysosomal degradation of the complex formed by PCSK9 - LDLR - LDL.

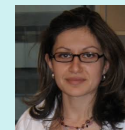
## How does Inhibitors work?



b) In the presence of a monoclonal antibody that binds to PCSK9, the PCSK9-mediated degradation of LDLR is inhibited, resulting in an increased uptake of LDL-cholesterol by LDLR as more LDLR are recycled at the cell surface.

Source: Krähenbühl S, et al. Unmet Needs in LDL-C Lowering: When Statins Won't Do! *Drugs*. 2016 Aug;76(12):1175-90





Palma Rocchi

*Article*

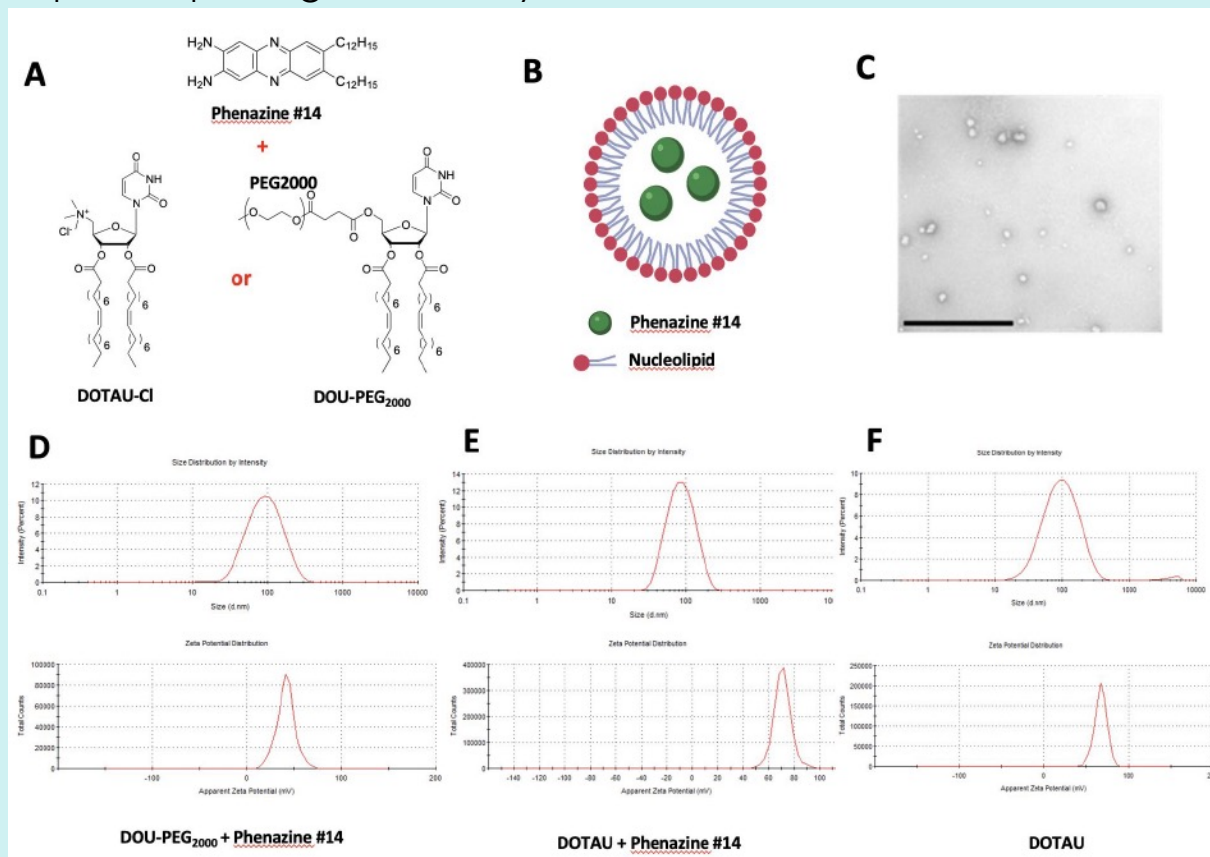
# Nucleoside-Lipid-Based Nanoparticles for Phenazine Delivery: A New Therapeutic Strategy to Disrupt Hsp27-eIF4E Interaction in Castration Resistant Prostate Cancer

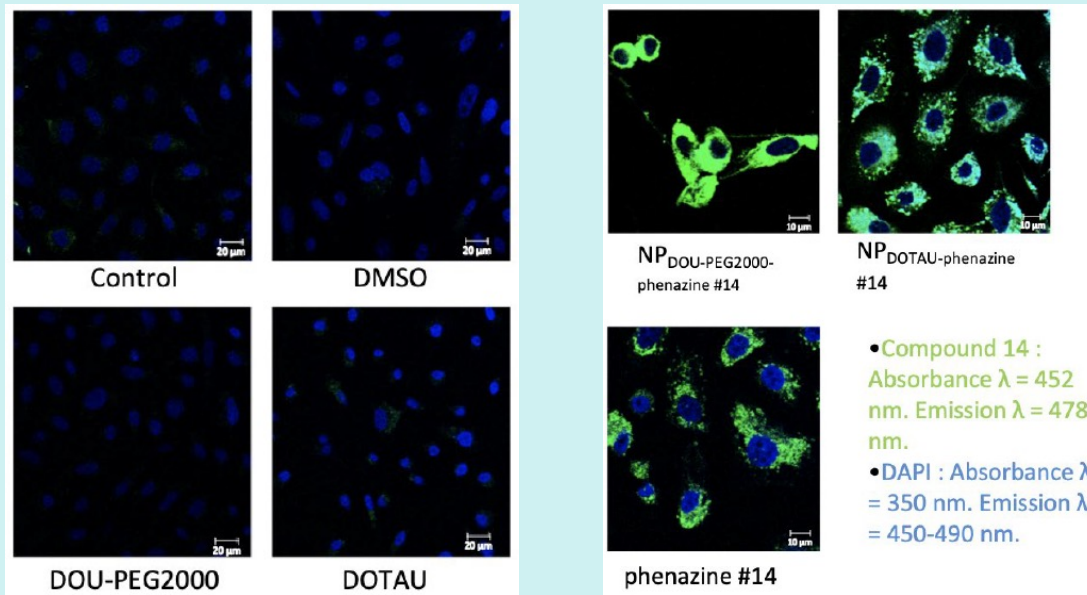
Hajer Ziouziou <sup>1,2,†</sup>, Clément Paris <sup>1,†</sup>, Sébastien Benizri <sup>3</sup>, Thi Khanh Le <sup>1</sup>, Claudia Andrieu <sup>1</sup>, Dang Tan Nguyen <sup>1</sup>, Ananda Appavoo <sup>3</sup>, David Taïeb <sup>1,4</sup>, Frédéric Brunel <sup>5</sup>, Ridha Oueslati <sup>2</sup>, Olivier Siri <sup>5</sup>, Michel Camplo <sup>5</sup>, Philippe Barthélémy <sup>3,\*</sup> and Palma Rocchi <sup>1,\*</sup>

Pharmaceutics **2021**, *13*, 623.

<https://doi.org/10.3390/pharmaceutics13050623>

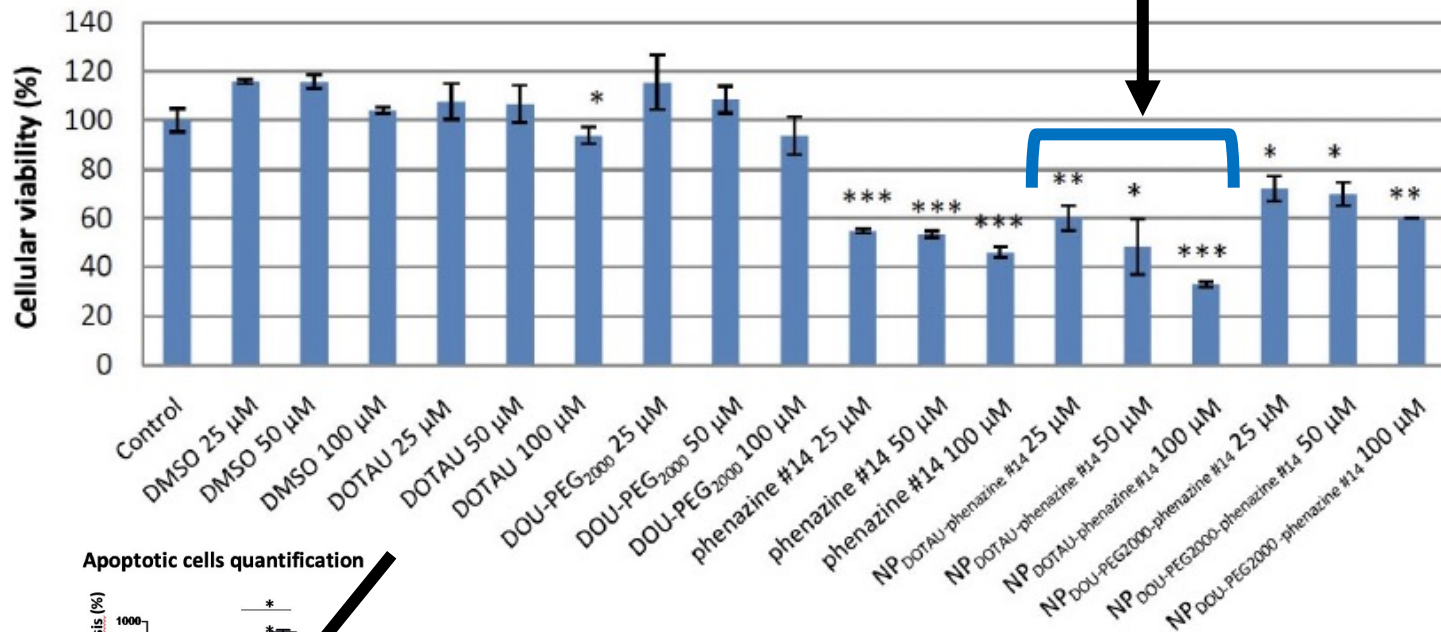
## Topic 2 Improving the delivery of APIs



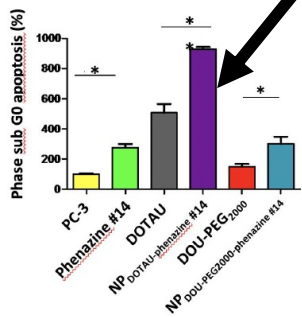


Confocal microscopic distribution of phenazine #14, NPDOU-PEG2000-phenazine #14, and NPDOTAUphenazine #14. PC-3 cells were treated at 100  $\mu$ M with phenazine #14 (last panel), NPDOTAU-phenazine #14 (right panel), and NPDOU-PEG2000-phenazine #14 (left panel) with DMSO (upper right panel) as control.

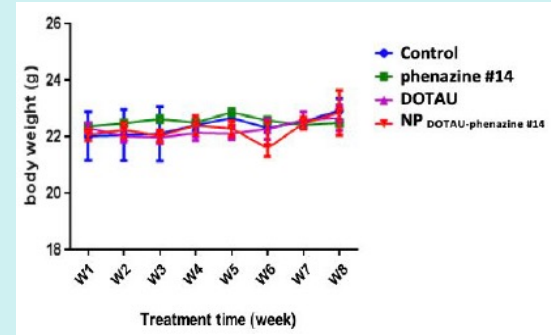
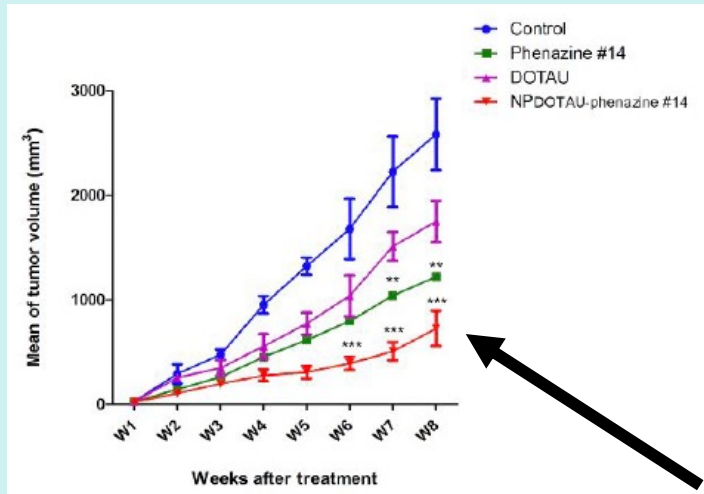
## PC-3 MTT viability Test



### Apoptotic cells quantification



NPDOTAU-phenazine #14 inhibits cell viability and increases apoptosis of PC-3 cells in vitro



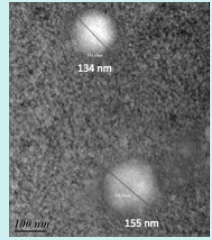
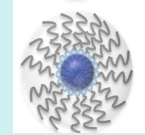
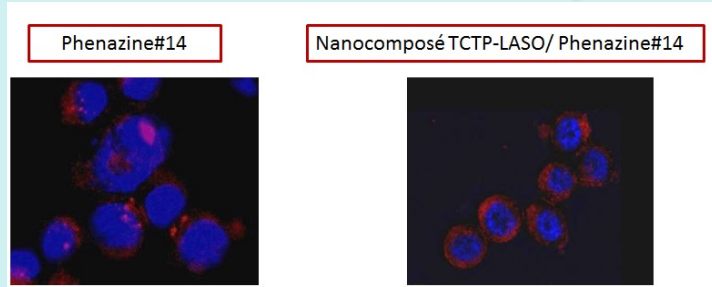
NP DOTAU-phenazine #14  
Impact on tumor volume evolution in vivo

## Conclusion

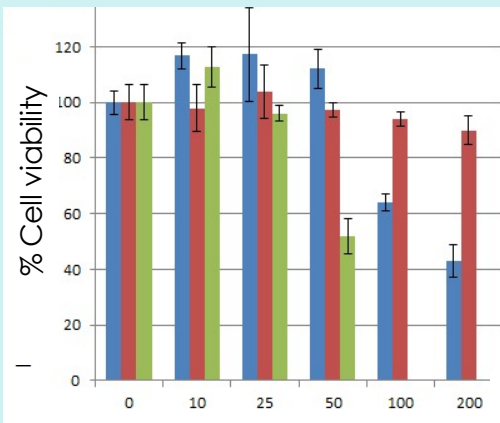
NP DOTAU-phenazine #14 can disrupt Hsp27/eIF4E interaction and treat CRPC with a good clinical tolerance of preclinical models

Eucaryote initiation factors -> therapeutic targets

# Alternative strategy



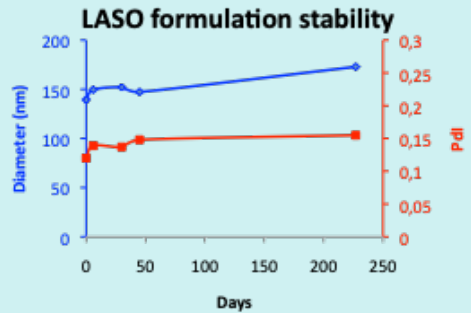
LASO + anticancer Drug  
(Phenazine, inhibitor interactions  
HSP27-eIF4E, inhibit translation)



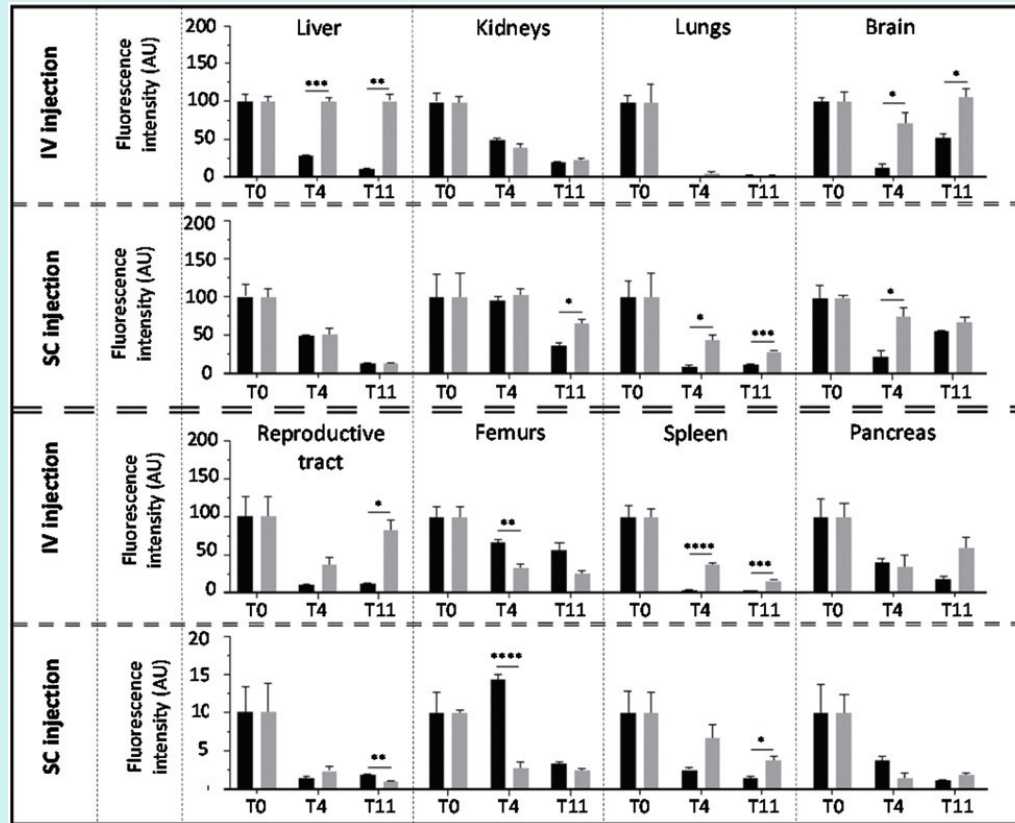
Cancer cells  
Prostate cancer PC3  
Resistant

■ Ph 14  
■ DMSO  
■ Nano ph 14/TCTP-LASO

Phenazine in uM



Strong synergism effect of  
the nanoformulations



Fluorescence intensity on mice organs after IV and SC administrations of Cyanine 5 – LASOTCTP (grey)/ASOTCTP (black) (n = 5). Twofactor ANOVA analysis followed by a Tukey test. \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001 and \*\*\*\*: p < 0.0001.