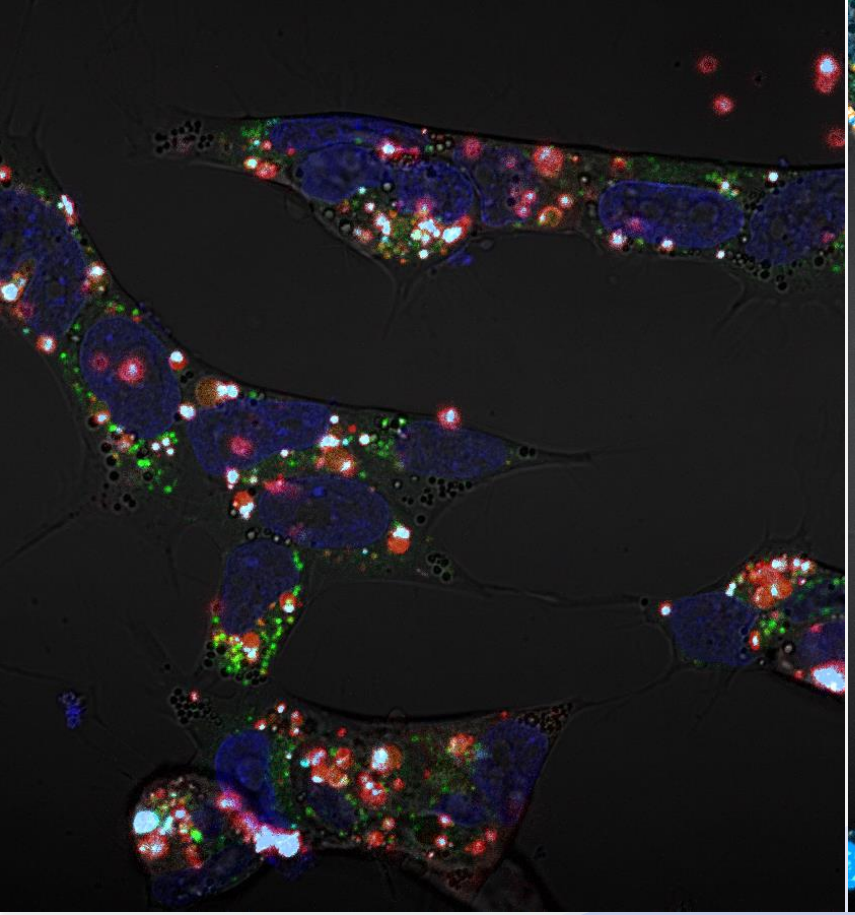
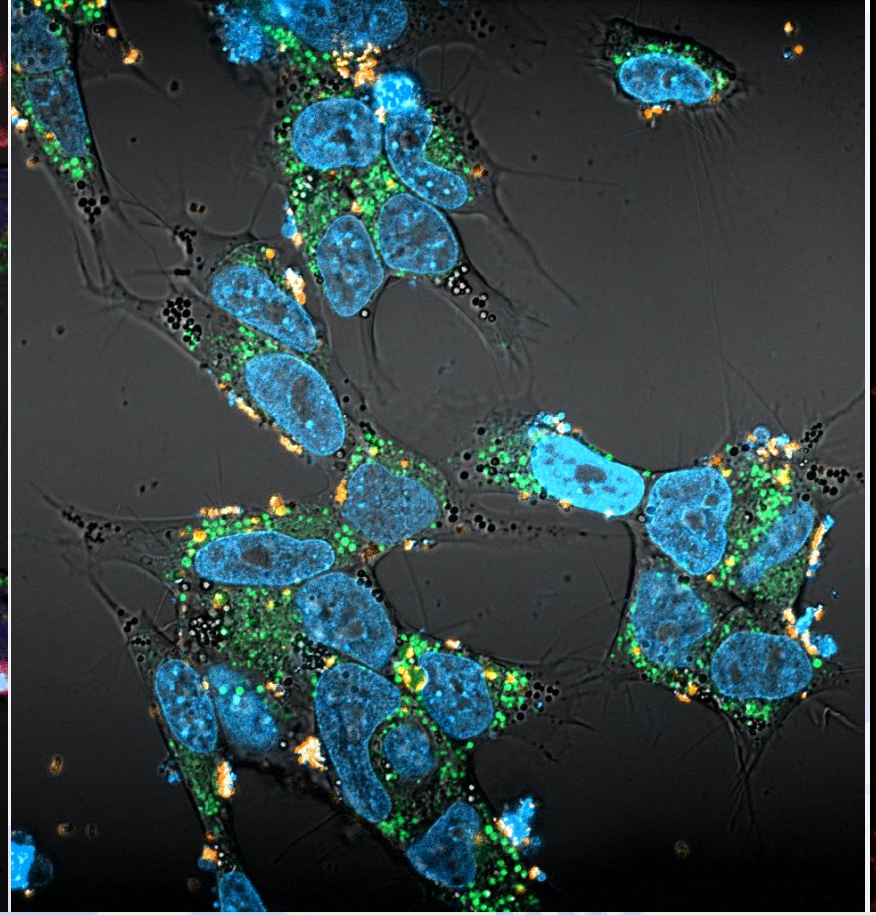
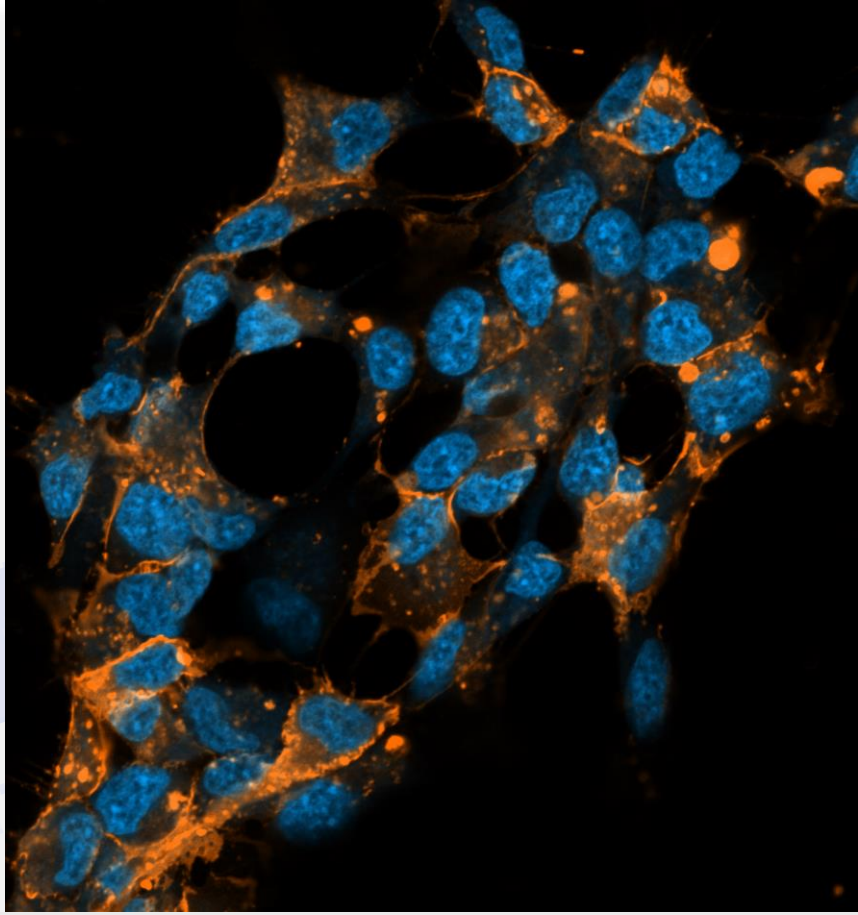




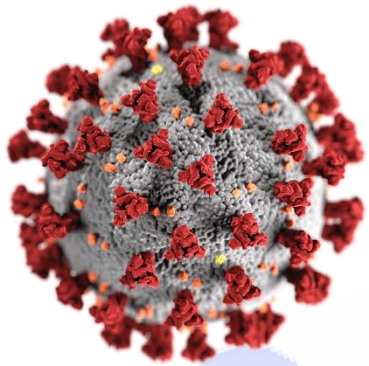
QUATSOMES AS A NOVEL NANOCARRIER FOR CLINICAL DELIVERY OF SMALL RNAs

Ariadna Boloix, PhD

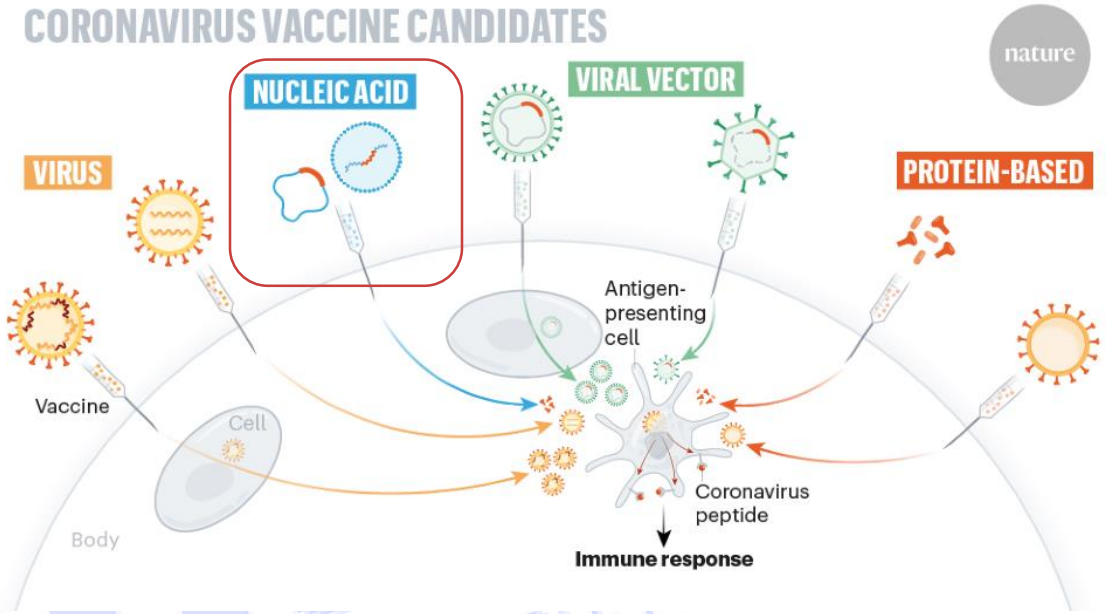
21.10.21

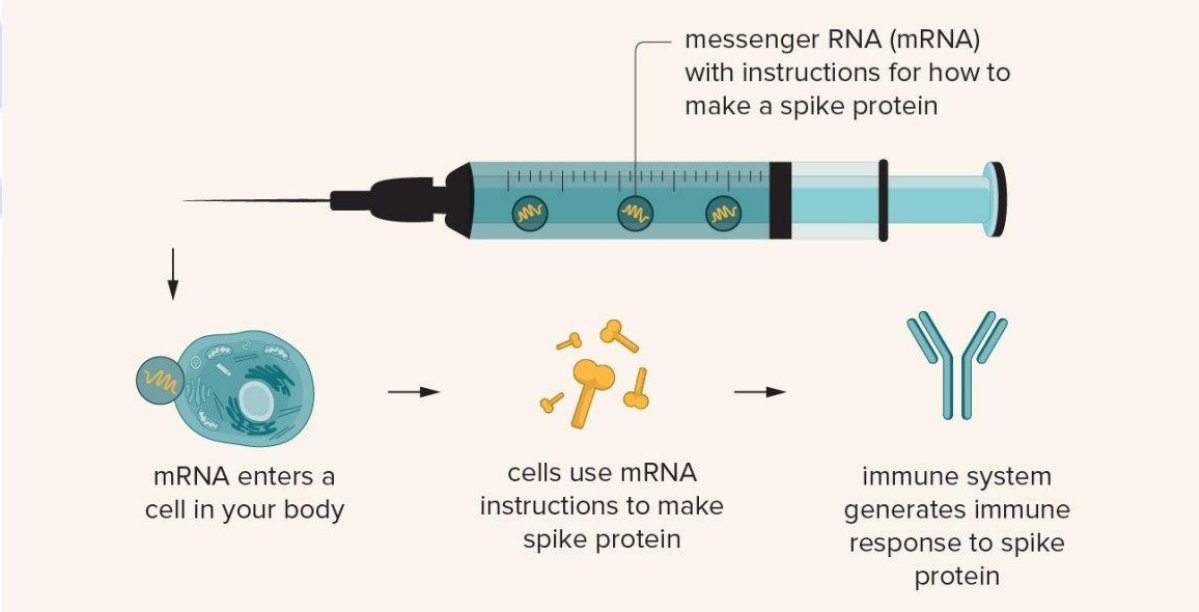
ICMAB
INSTITUT DE CIÈNCIA DE MATERIALS DE BARCELONA
EXCELENCIA SEVERO OCHOA
CSIC
Vall d'Hebron
UNIVERSITAT DE BARCELONA
Facultat de Farmàcia i Ciències de l'Alimentació

RNA-based therapies & COVID-19

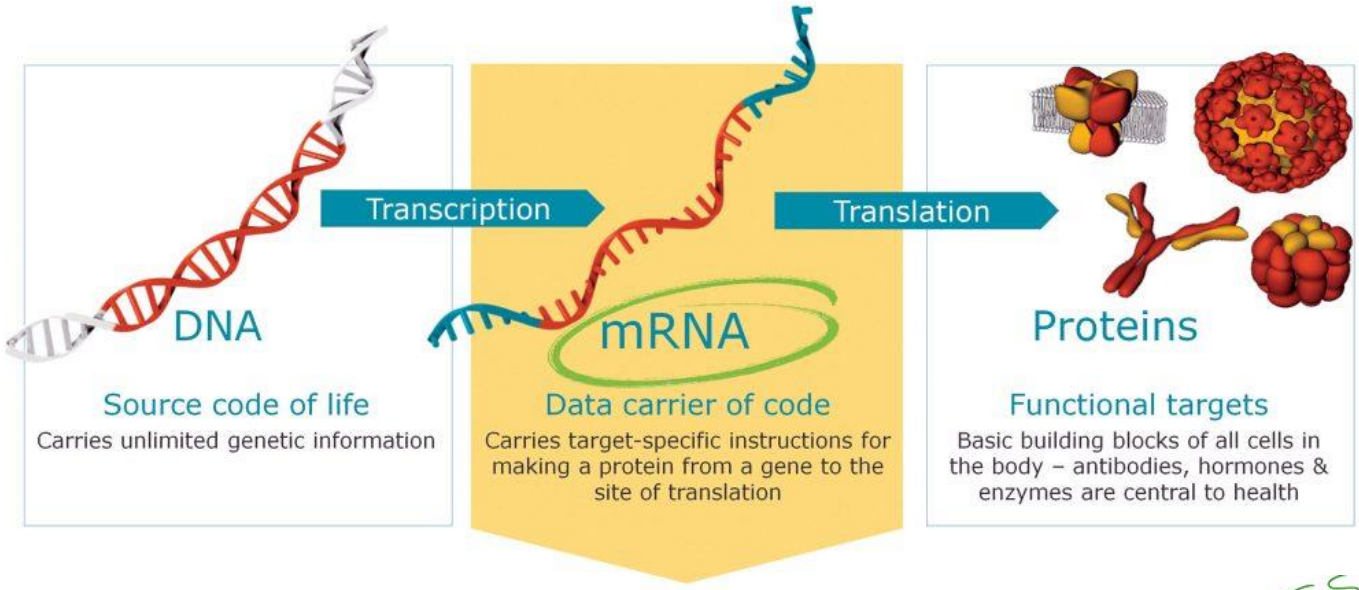


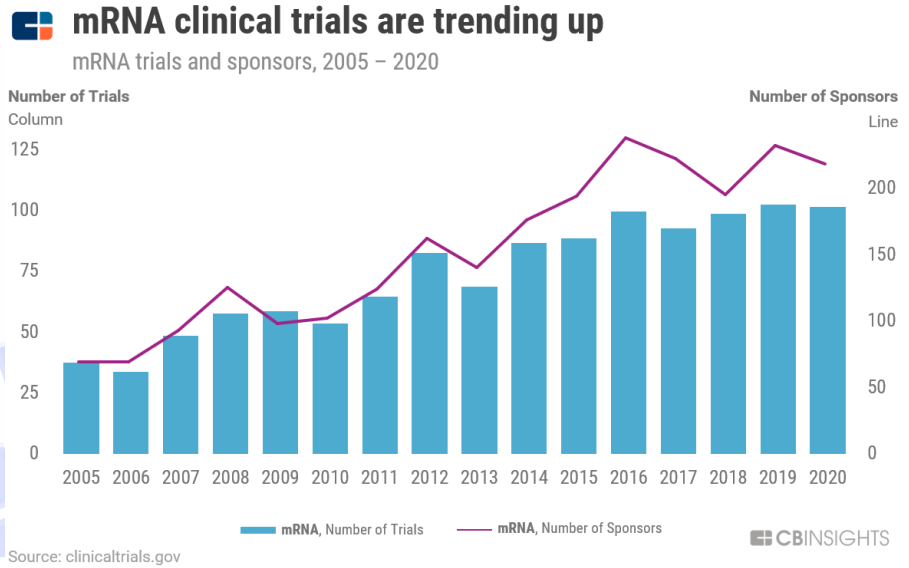
SARS-COV-2

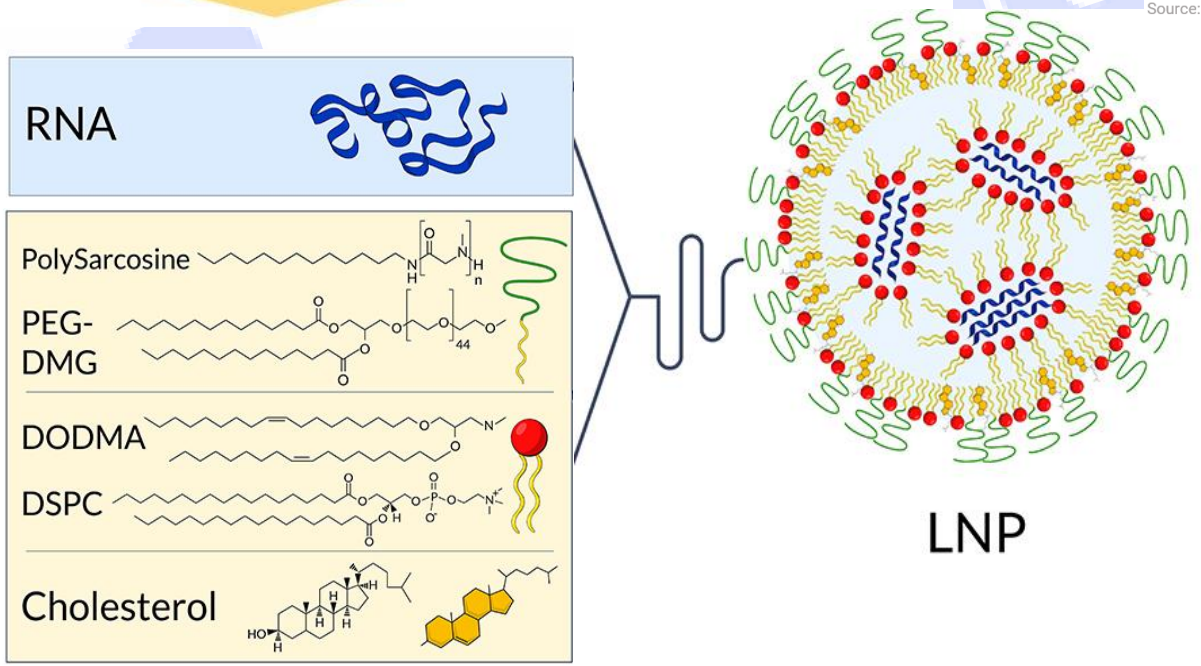




RNA-based therapies & COVID-19

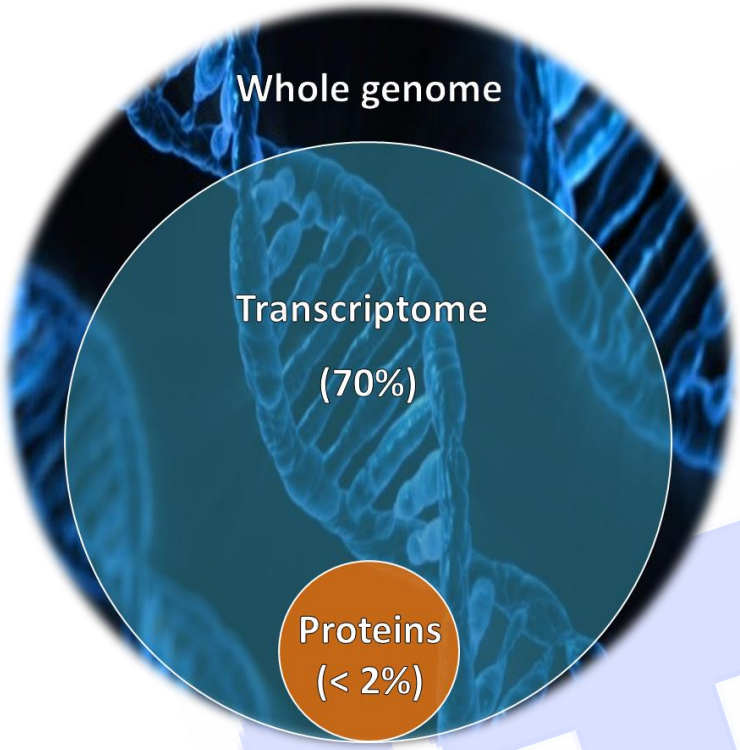




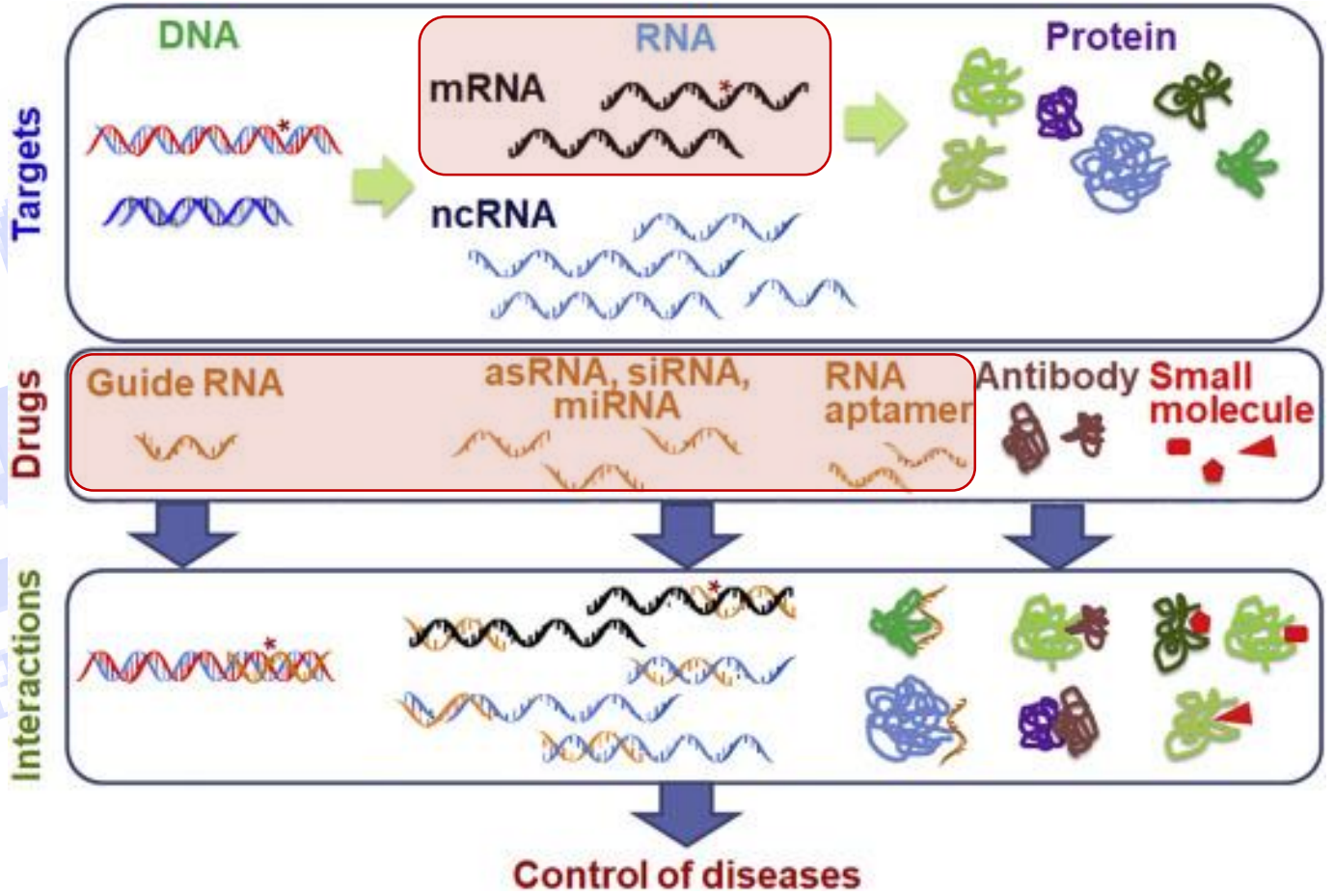


Sorry, this is not a COVID-19 seminar!

RNA-based therapies expand the druggable targets

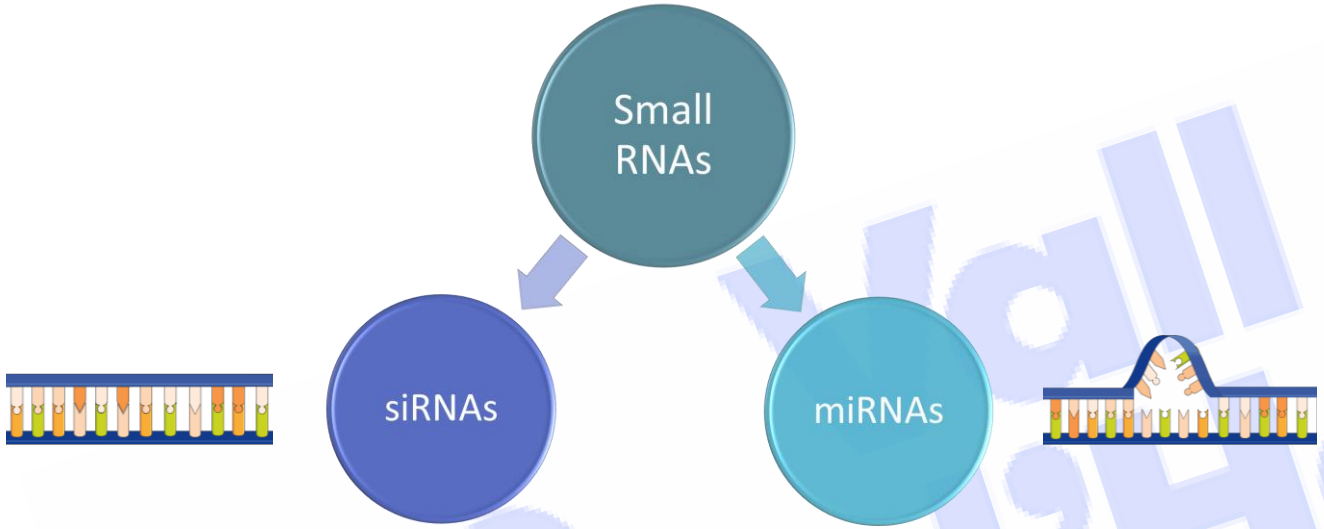


Only **0.05%** of the human genome is **druggable**



Yu AM et al., Pharmacol Ther. 2019

Use of small RNAs as therapeutic tools

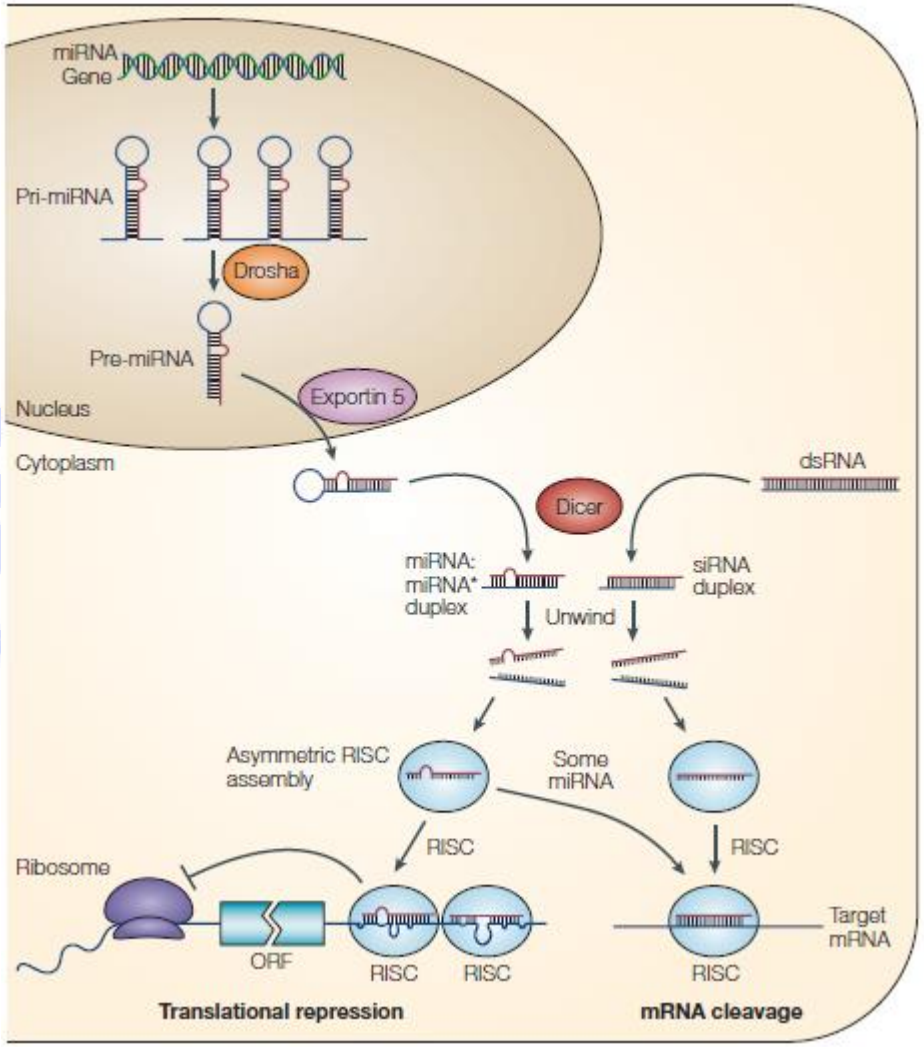


1. Similar structure (~20 nt in length)

2. Both share the processing machinery

3. Similar effects over translation repression or mRNA cleavage

4. Both use the complementarity of bases to repress target expression

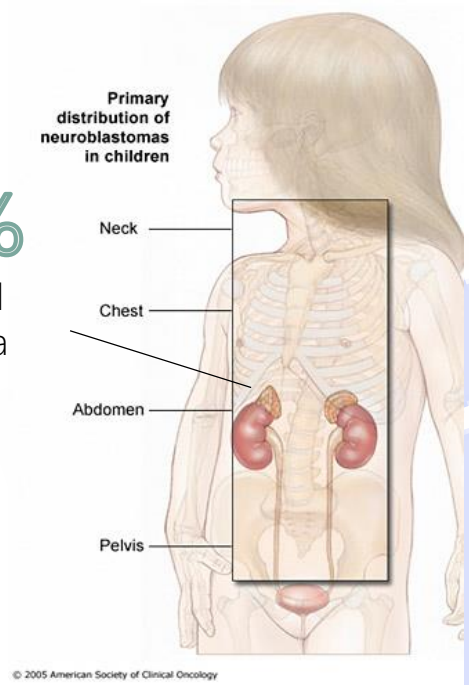


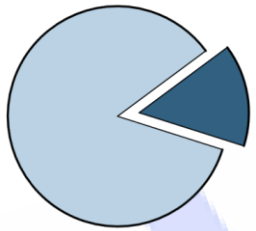
He & Hannon, 2004, *Nature Reviews Genetics*

Proof of Concept: Developing RNA therapies for Neuroblastoma

Currently, there is no approved therapies for refractory or relapsed Neuroblastoma

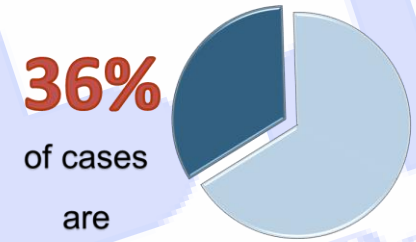
50% adrenal medulla





15% of childhood cancer **deaths**

67% of **survival** rates



36% of cases are **high-risk** neuroblastoma

50% of high-risk neuroblastoma patients **relapse**

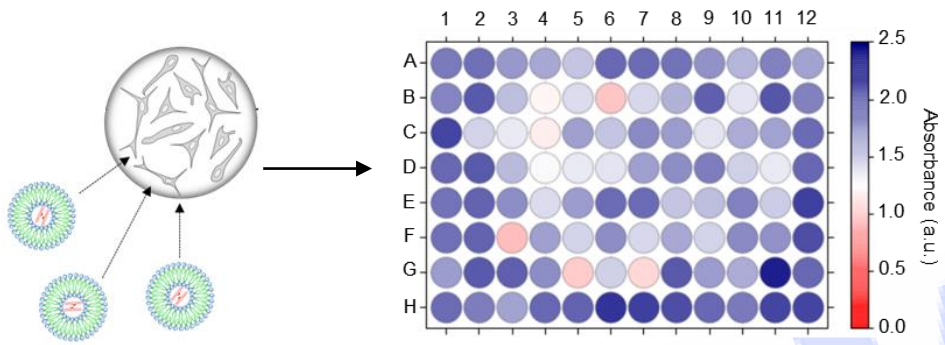
But current therapies lead to:

Chemoresistance

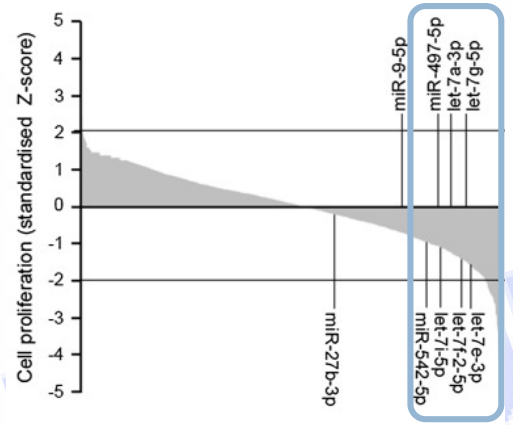
Permanent **side effects**

Urgent need for more efficient and less toxic treatments, such as RNA-based therapies

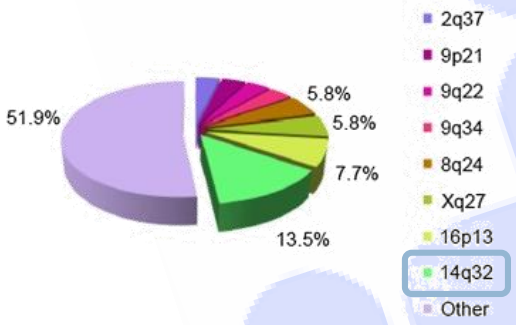
Tumor suppressive miR-323a-5p, a miRNA restoration therapy for NB



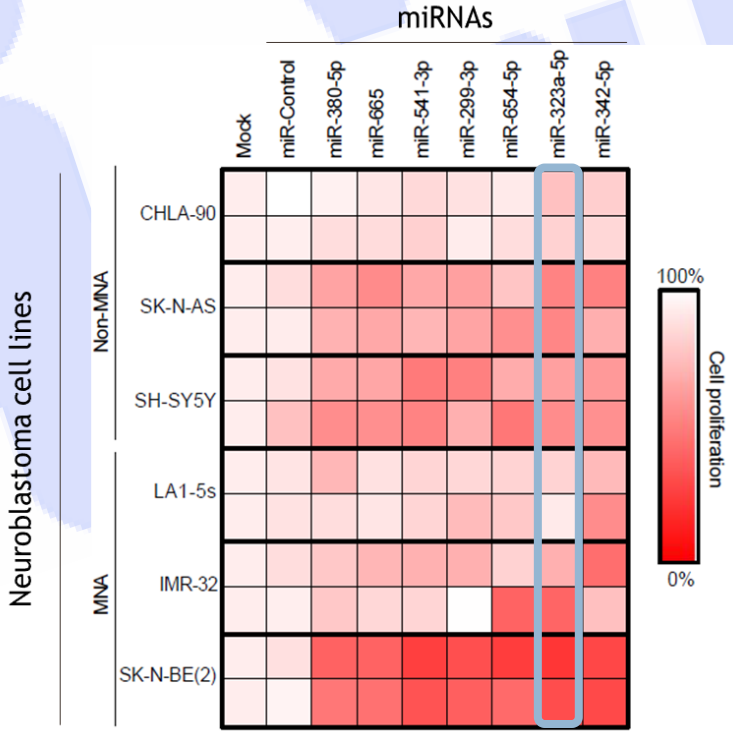
High throughput screening of 2048 miRNAs in neuroblastoma cells

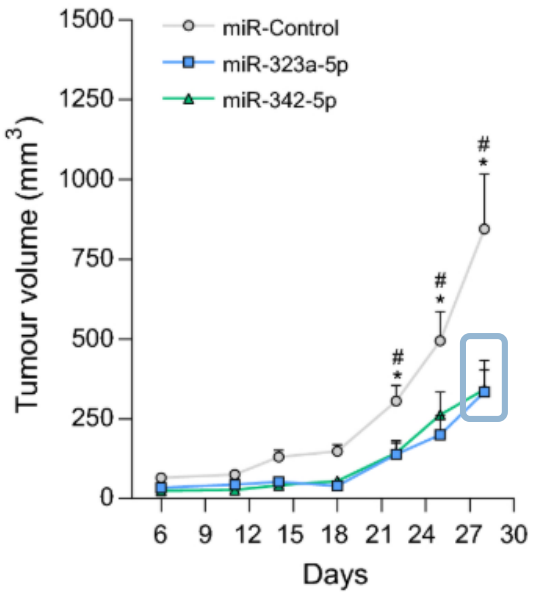


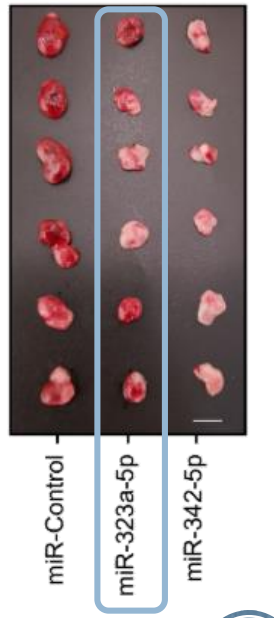
52 miRNAs reduced >50% of SK-N-BE(2) proliferation



7 of 52 miRNAs (13.5%) are located in the 14q32 locus





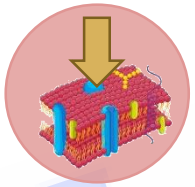


Soriano A et al. 2019; Cell Mol Life Science

Challenges of RNA-based therapies

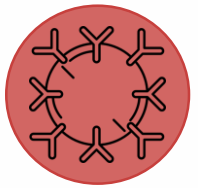
Naked RNA-based therapies

Low bioavailability



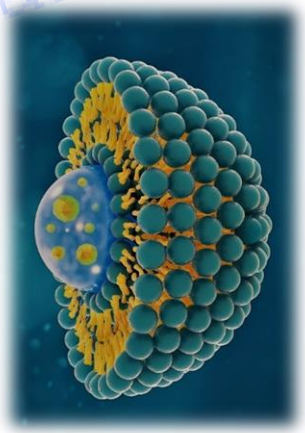
Low internalization

Low endosomal escape



Immune response

But for RNA administration a nanovehicle is required



Until now the main bottleneck of RNA-based therapies is the lack of a standard formulation for clinical administration, because nanocarriers under development present:

Toxicity

Poor stability

Difficulties in scale up

Challenges of RNA-based nanotherapies

Table 1 | **Selected RNA therapeutics approved and in development**

Drug	Company	Indication	Status
ASO			
Eteplirsen (Exondys 51)	Sarepta	DMD	Approved (2016)[a]
Nusinersen (Spinraza)	Ionis/Biogen	SMA	Approved (2016)[a]
Inotersen (Tegsedi)	Ionis/Akcea/PTC	hATTR	Approved (2018)[a]
Volanesorsen (Waylivra)	Ionis/Akcea/PTC	FCS	Approved (2019)[b]
Golodirsen (Vyondys 53)	Sarepta	DMD	Approved (2019)[a]
Viltolarsen	NS Pharma	DMD	NDA
Casimersen (SRP-4045)	Sarepta	DMD	NDA
TQJ230 (AKCEA-APO(a)-L_{Rx})	Ionis/Akcea/Novartis	Hyperlipoproteinaemia with cardiovascular risk	Phase III
Tofersen	Ionis/Biogen	SOD1-driven ALS	Phase III
IONIS-HTT_{Rx}	Ionis/Roche	Huntington disease	Phase III
Trabedersen (OT-101)	Mateon (Oncotelic)	Brain cancer	Phase III
Volanesorsen	Ionis/Akcea	FPL	Phase III
siRNA			
Patisiran (Onpattro)	Alnylam	hATTR	Approved (2018)[a]
Givosiran (Givlaari)	Alnylam	AHP	Approved (2019)[a]
Lumasiran	Alnylam	Hyperoxaluria	NDA
Inclisiran	Alnylam/Novartis (The Medicines Company)	Dyslipidaemia/ hypercholesterolaemia	NDA
QR-110	ProQR	Leber's congenital amaurosis	Phase III
Vutrisiran	Alnylam	ATTR/hATTR	Phase III
QP-1002	Quark	Renal disease/failure, delayed graft function	Phase III
Tivanisiran (SYL1001)	Sylentis	Dry eye	Phase III
Fitusiran	Alnylam/Sanofi Genzyme	Haemophilia A and B	Phase III

AHP, acute hepatic porphyria; ALS, amyotrophic lateral sclerosis; ASO, antisense oligonucleotide; DMD, Duchenne muscular dystrophy; FCS, familial chylomicronaemia syndrome; FPL, familial partial lipodystrophy; hATTR, hereditary transthyretin amyloidosis; NDA, new drug application; SMA, spinal muscular atrophy. [a]This refers to approval in the USA by the FDA, which was the first major market approval for all agents. [b]Volanesorsen (Waylivra) is approved in the EU, but is still at NDA stage in the USA. Source: Biomedtracker, Informa, June 2019; updated January 2020.

Local administration

Hepatic diseases

High cost

Approved by FDA in 2018

Published: 17 January 2010

Rational design of cationic lipids for siRNA delivery

Sean C Semple, Akin Akinc, [...] Michael J Hope

Nature Biotechnology **28**, 172–176(2010) | Cite this article

4445 Accesses | **812** Citations | **16** Altmetric | Metrics

AdisInsight Report | Published: 24 September 2018

Patisiran: First Global Approval

Sheridan M. Hoy

Drugs **78**, 1625–1631(2018) | Cite this article

1851 Accesses | **82** Citations | **4** Altmetric | Metrics

› Angew Chem Int Ed Engl. 2012 Aug 20;51(34):8529-33. doi: 10.1002/anie.201203263. Epub 2012 Jul 10.

Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo

Muthusamy Jayaraman [1], Steven M Ansell, Barbara L Mui, Ying K Tam, Jianxin Chen, Xinyao Du, David Butler, Laxman Eltepu, Shigeo Matsuda, Jayaprakash K Narayanannair, Kallanthottathil G Rajeev, Ismail M Hafez, Akin Akinc, Martin A Maier, Mark A Tracy, Pieter R Cullis, Thomas D Madden, Muthiah Manoharan, Michael J Hope

Affiliations + expand

PMID: 22782619 PMCID: PMC3470698 DOI: 10.1002/anie.201203263

› Nucleic Acids Res. 2019 Feb 20;47(3):1082-1096. doi: 10.1093/nar/gky1239.

Diverse lipid conjugates for functional extra-hepatic siRNA delivery in vivo

Annabelle Biscans [1 2], Andrew Coles [1 2], Reka Haraszti [1 2], Dimas Echeverria [1 2], Matthew Hassler [1 2], Maire Osborn [1 2], Anastasia Khvorova [1 2]

Affiliations + expand

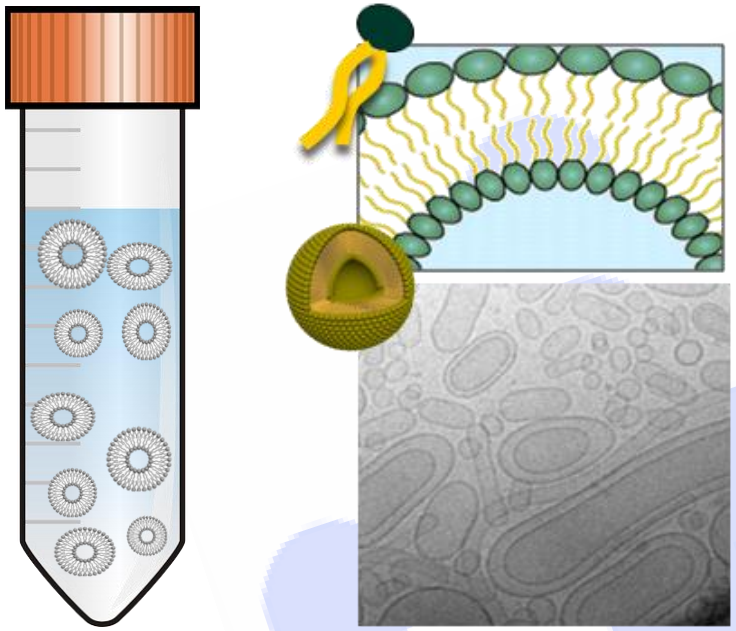
PMID: 30544191 PMCID: PMC6379722 DOI: 10.1093/nar/gky1239

Until now the research of a suitable nanocarrier to deliver RNA therapies is still a challenge in the field

Nanocarriers from the past to the future

Liposomes

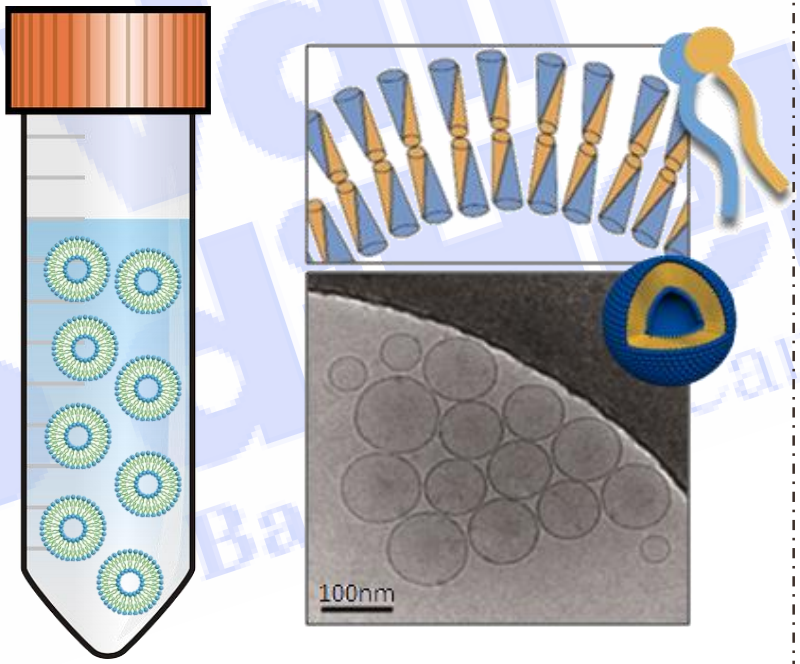
Nanovesicles made by the self-assembly of amphiphilic molecule



- High polidispersity
- High aggregation

Quatsomes (QS)

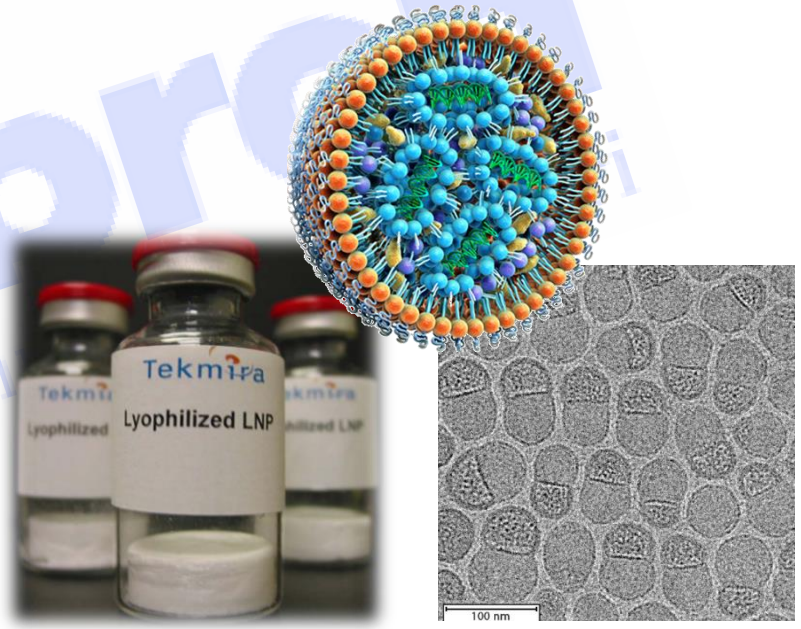
New nanovesicles resulting from the self-assembling of quaternary ammonium surfactants and sterols



- High homogeneity
- High colloidal stability

Lipid nanoparticles (LNP)

Solid or liquid nanoparticles made of ionizable and neutral lipids

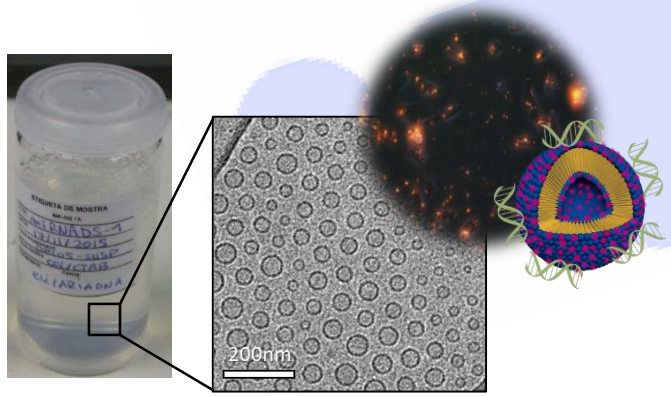


- High homogeneity
- Stability under certain conditions

Quatsomes nanocarrier

To implement RNA-based therapies into clinical practice, they will be conjugated with the outstanding Quatsome nanocarrier.

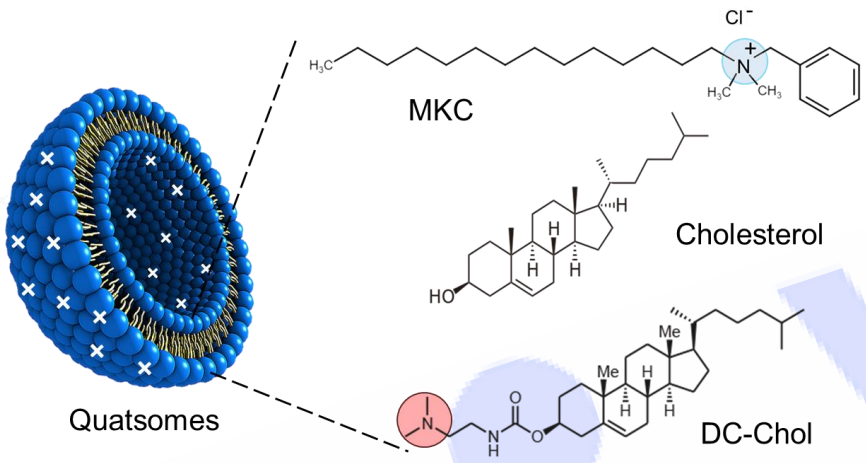
- High homogeneity
- High stability
- Conjugation of RNAs
- Non-toxic in vivo
- Targeted therapy
- Scale up
- Patented technology



Nanovesicles and its use for nucleic acid delivery
PCT Nº: PCT/EP2020/063195 filed in May 2020; WO2020229469

Depressurization of a Expanded Liquid Organic Solution - Suspension (DELOS-Susp) (WO2006079889)

Quatsomes are homogeneous, spherical & nanometric vesicles

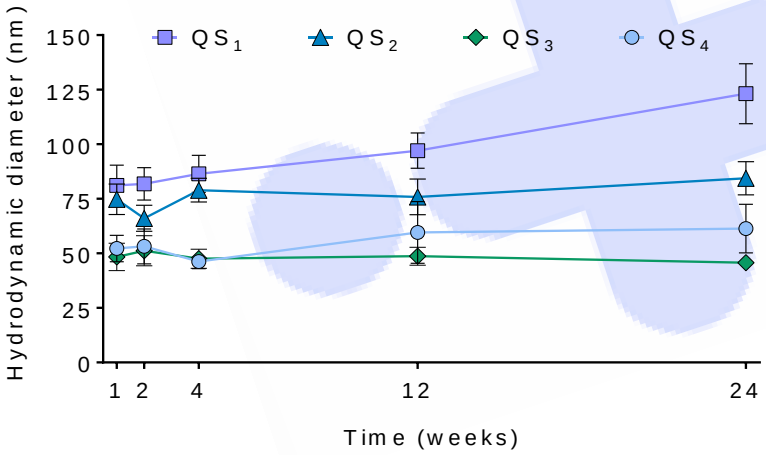


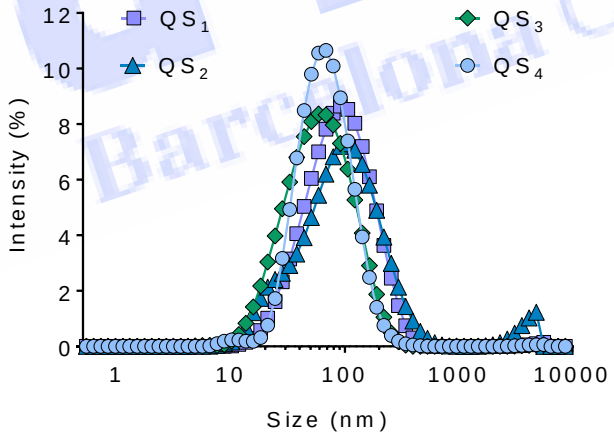
QS_1 (100%Chol/0%DC-Chol):MKC

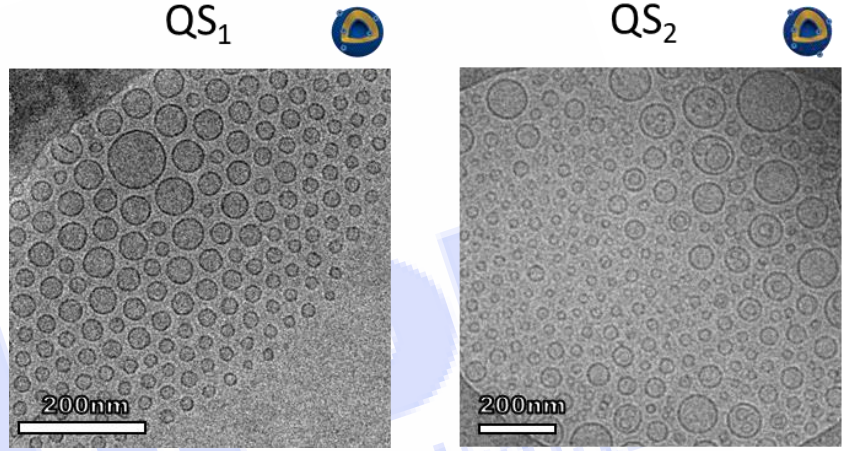
QS_2 (90%Chol/10%DC-Chol):MKC

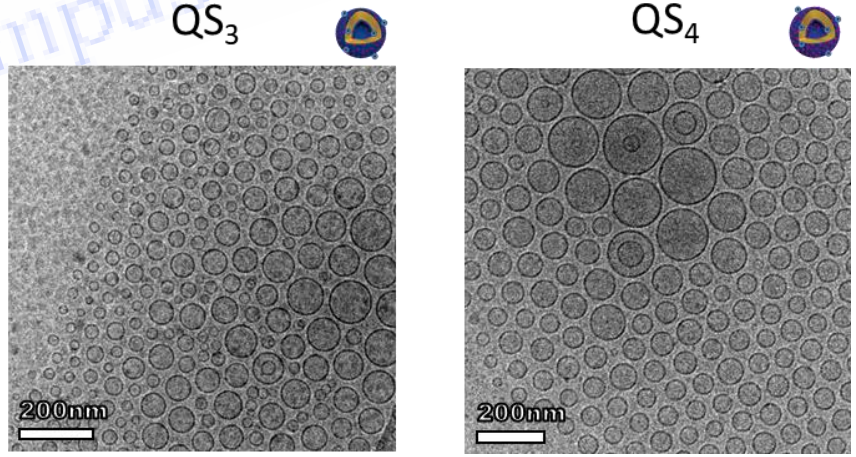
QS_3 (50%Chol/50%DC-Chol):MKC

QS_4 (0%Chol/100%DC-Chol):MKC



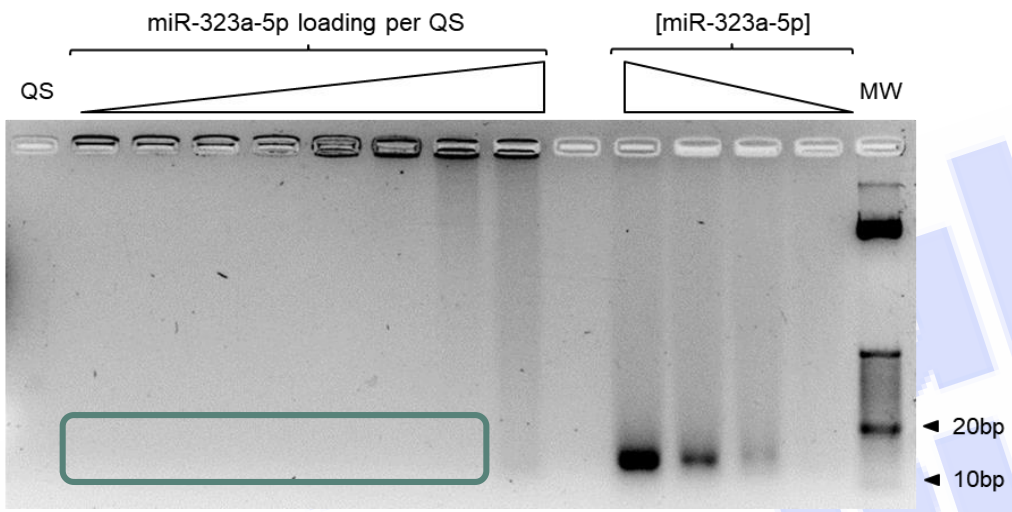


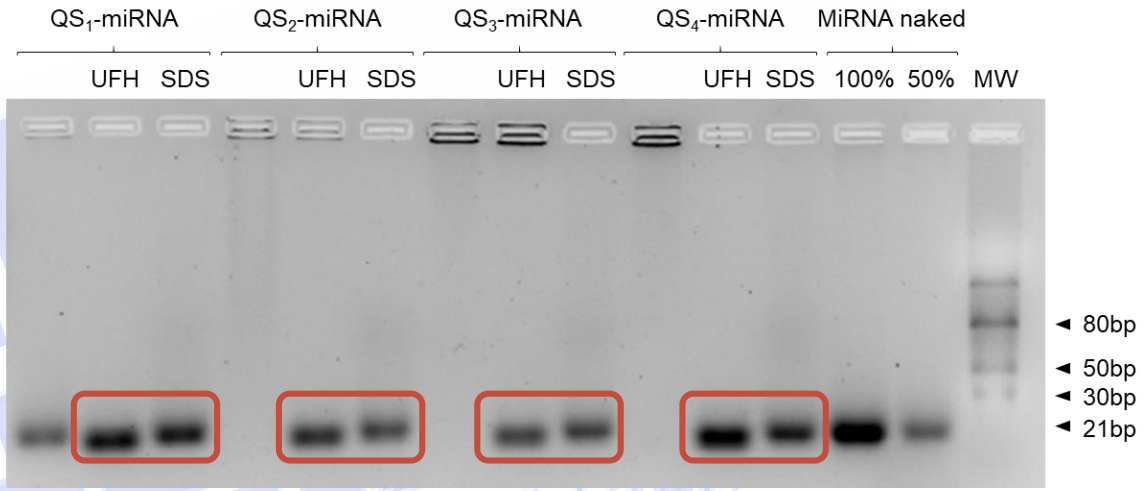


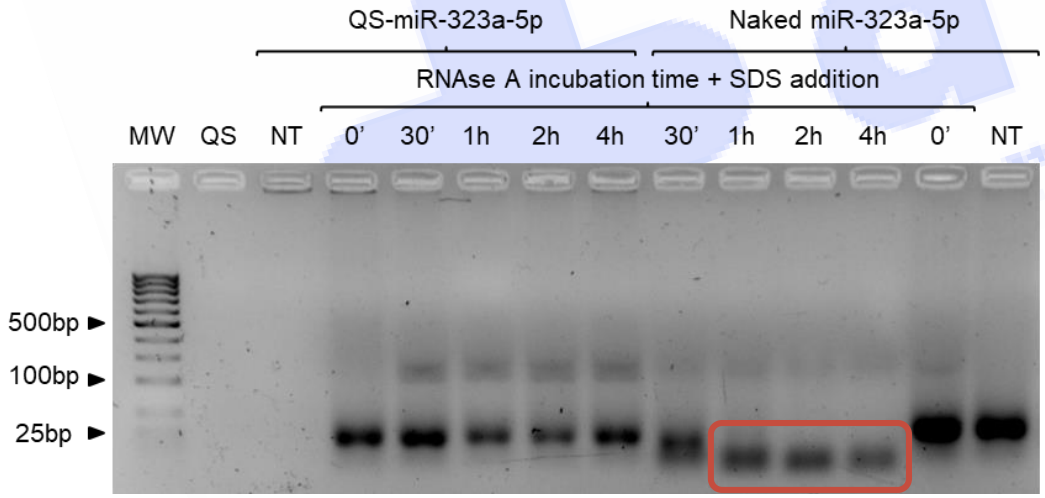


Boloix A *et al. Small. Under minor revisions*

QS can conjugate miRNAs and protect them form RNAses degradation







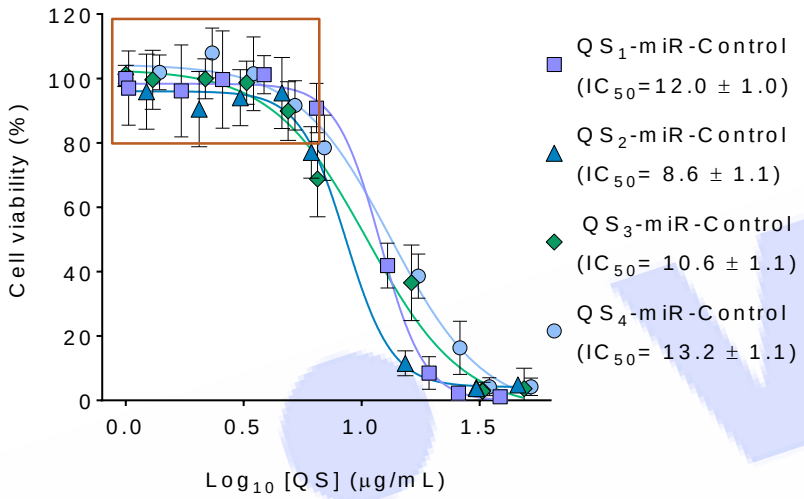
- High loading of sRNAs
- Reversible conjugation
- Protection from RNAses

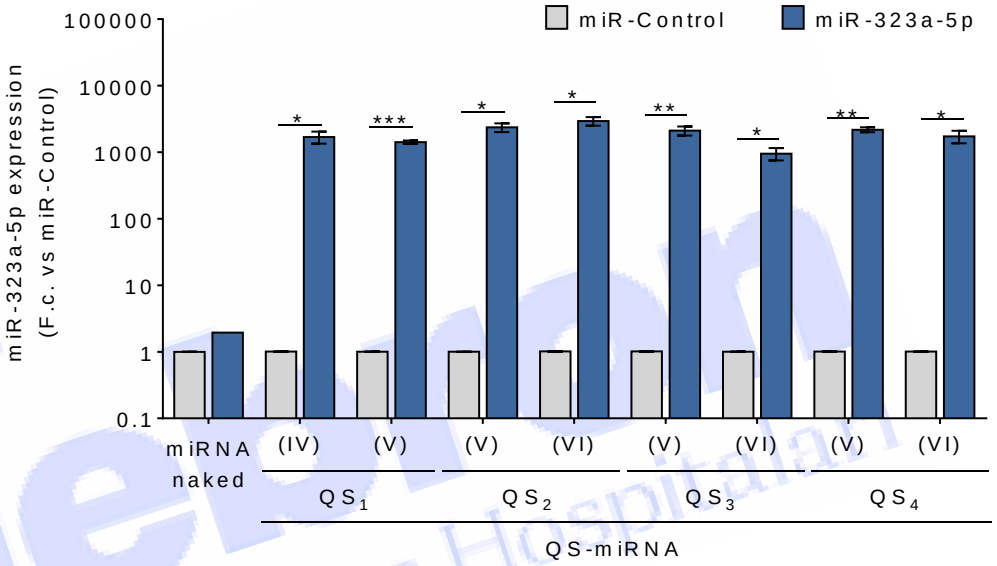
Boloix A et al., **2021,** *Manuscript submitted*

Boloix A *et al. Small. Under minor revisions*

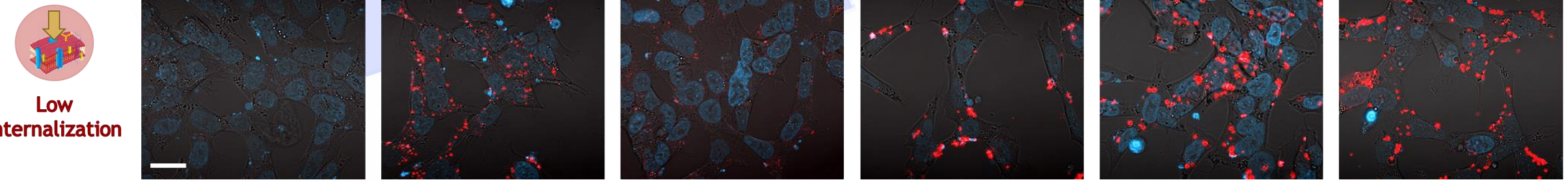
Quatsomes can increase miRNA levels with very low cell toxicity

Toxicity





Low internalization

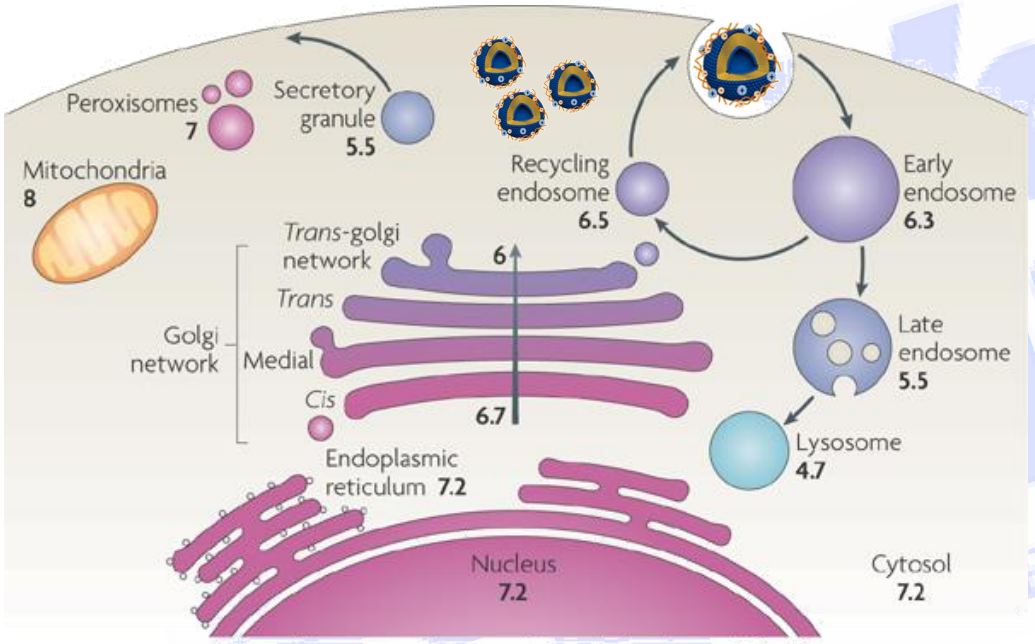


Scale bar represents 20 µm.

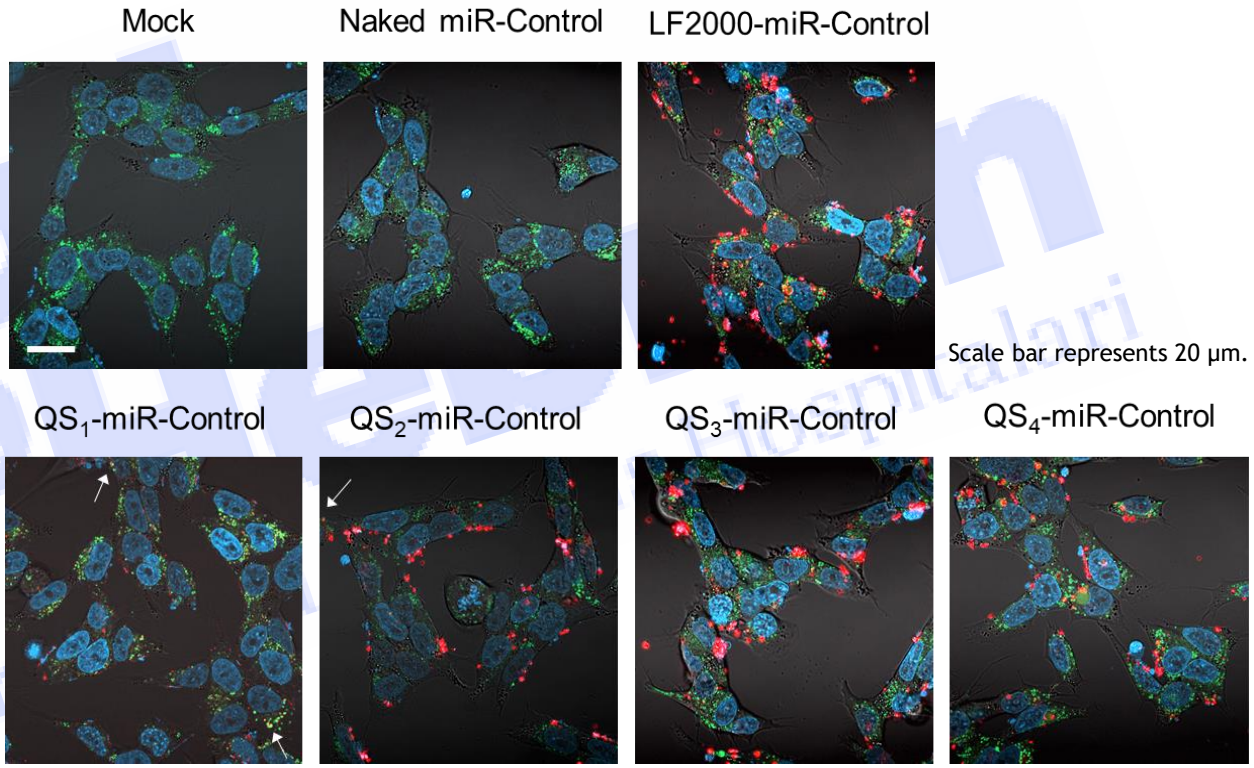
Boloix A *et al. Small. Under minor revisions*

QS-miRNA conjugates internalize by non-endocytic pathways

Low endosomal escape



Casey JR, *Nature Reviews Molecular Cell Biology, 2010*

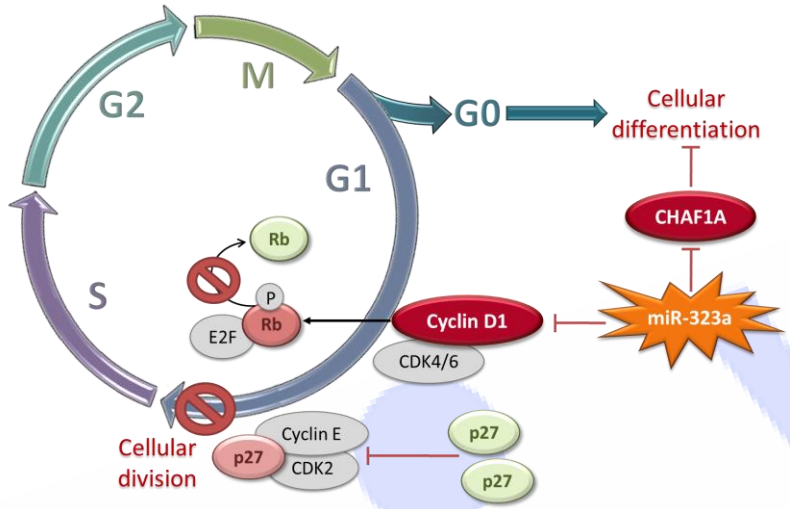


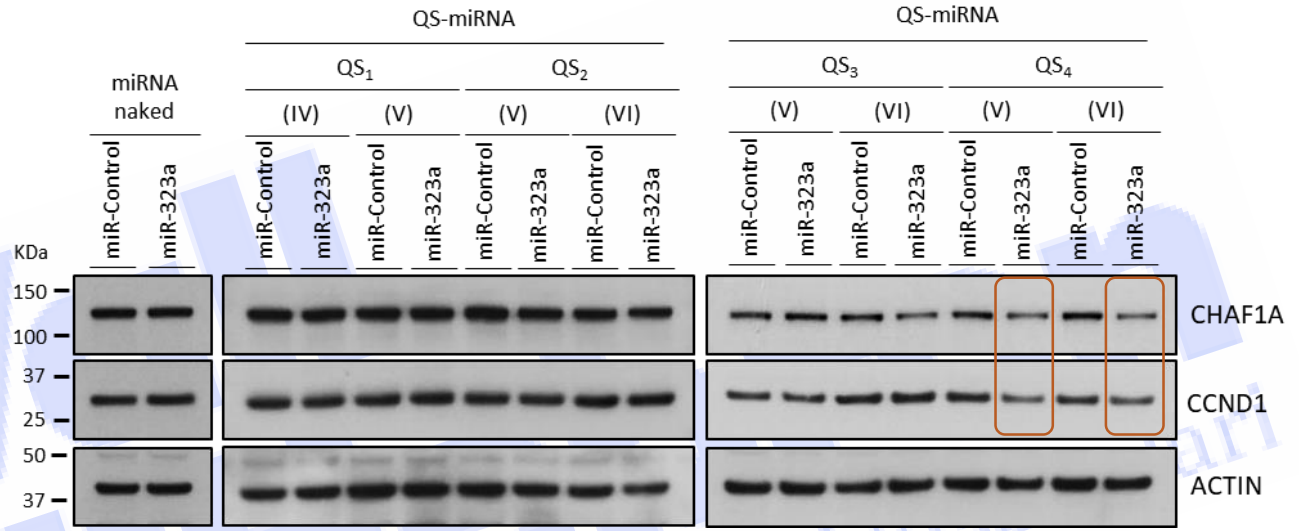
Scale bar represents 20 µm.

But... Is the miRNA arrival in the citosol sufficient to induce molecular effects on its target genes?

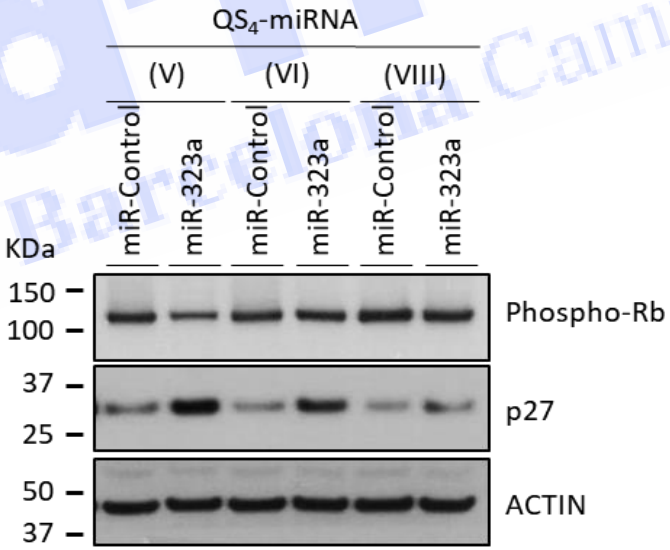
Boloix A *et al. Small. Under minor revisions*

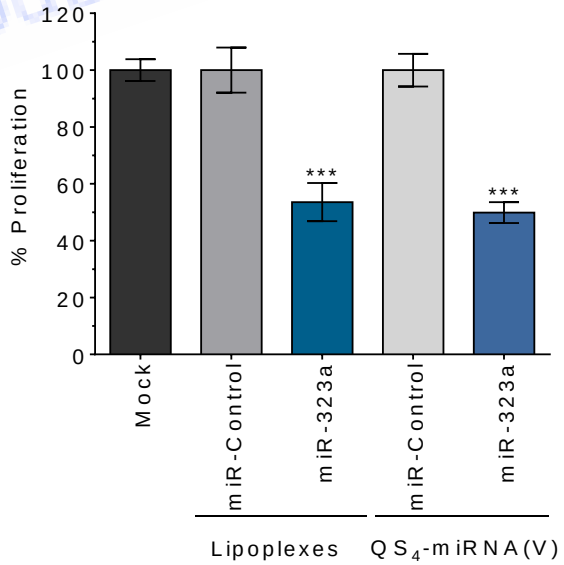
QS4–miR-323a-5p reduce neuroblastoma cells proliferation





Only QS_4-miR-323a-5p modulate miR-323a-5p target expression



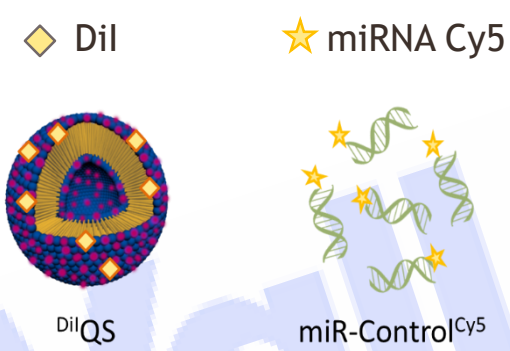


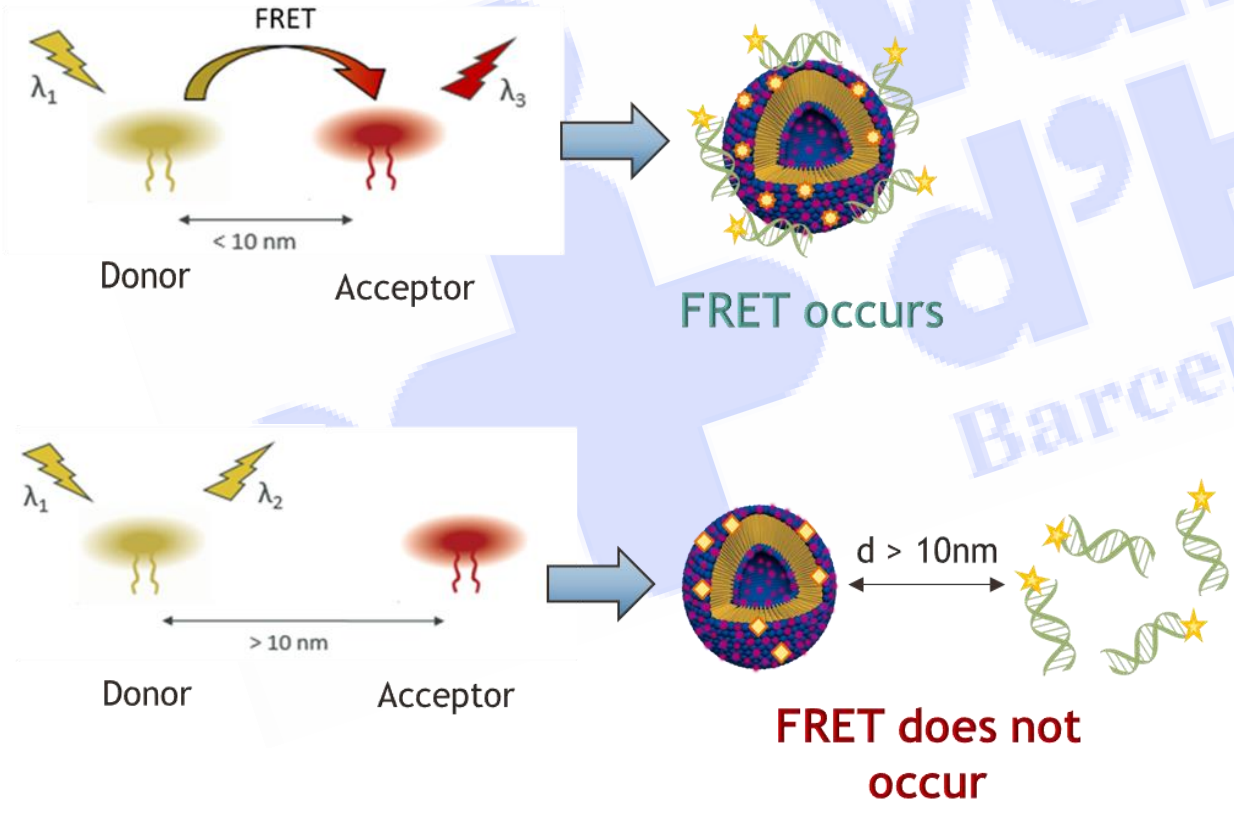
Boloix A *et al. Small. Under minor revisions*

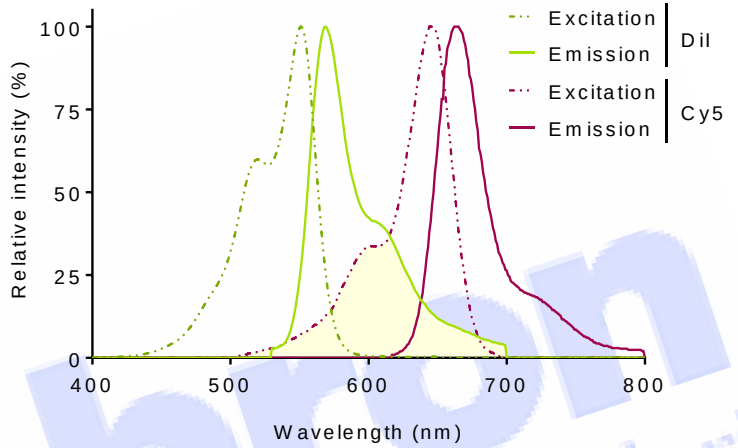
MiRNAs are released from QS?

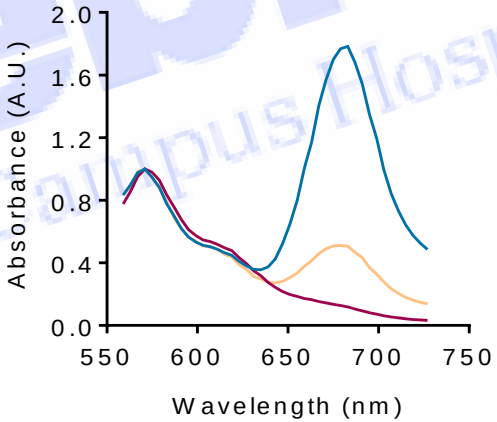
Cargo release

The labelling of Quatsomes and miRNAs allow the intracellular tracking of conjugates using FRET technique









$^{DiI}QS_4$-miR-Control^{Cy5}

$^{DiI}QS_4$-miR-Control + QS_4-miR-Control^{Cy5}

$^{DiI}QS_4$-miR-Control^{Cy5} + SDS

In collaboration with Dr. Albertazzi

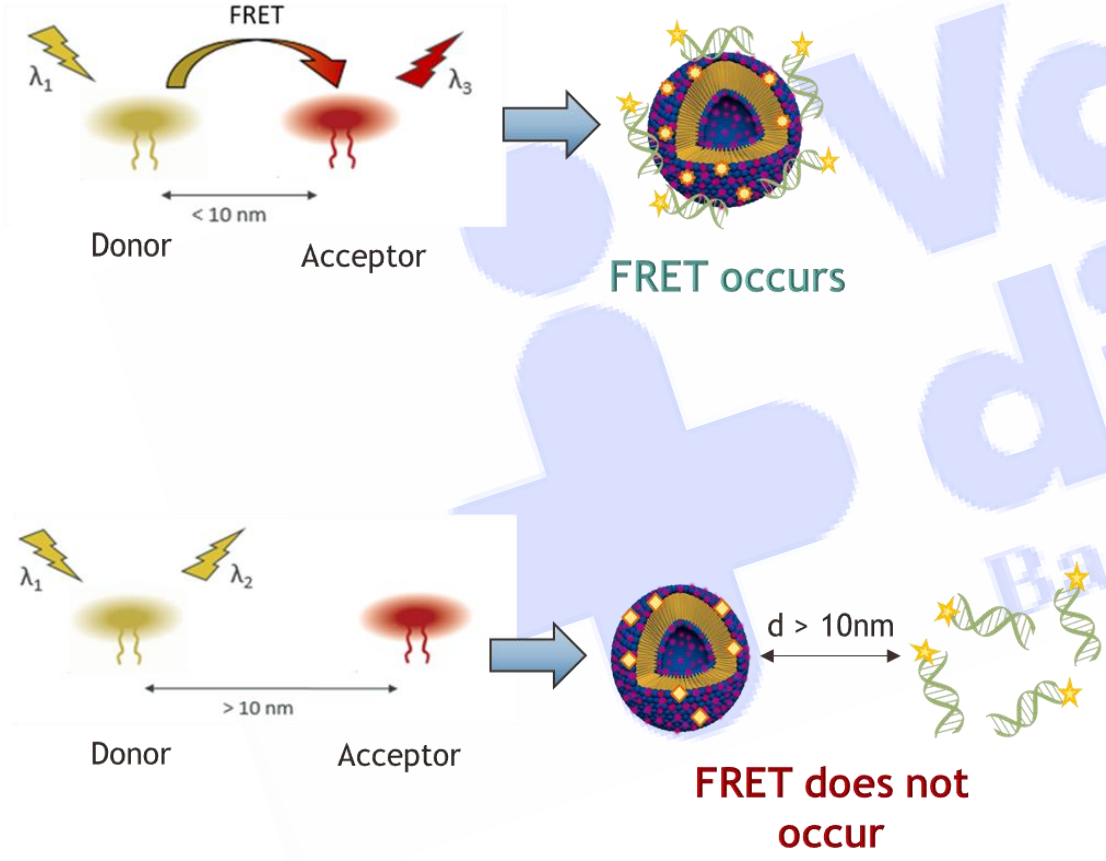
TU/e EINDHOVEN UNIVERSITY OF TECHNOLOGY IBEC

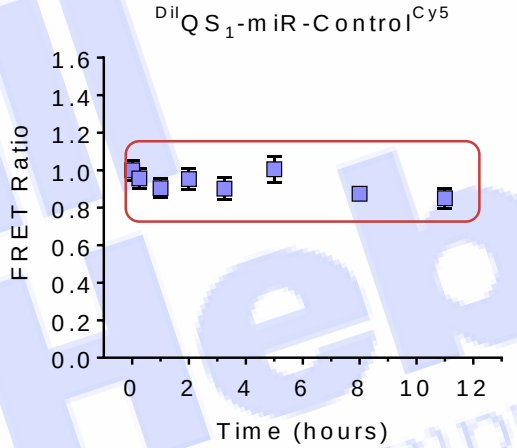
Boloix A *et al. Small. Under minor revisions*

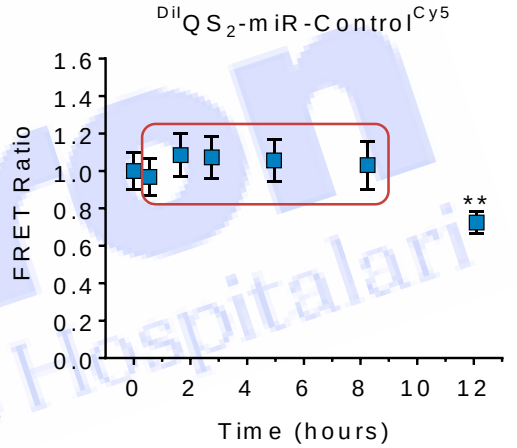
Only QS_4 release miRNAs into the cytosol

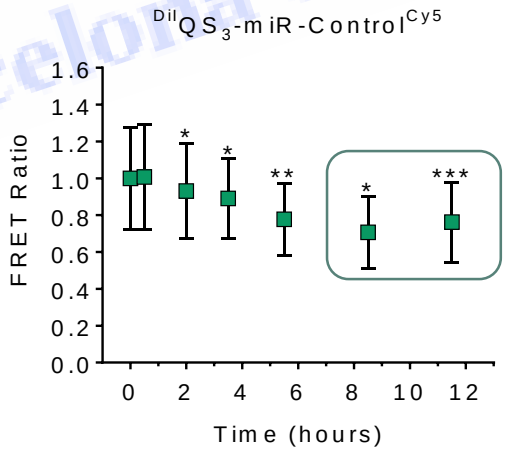
In collaboration with Dr. Albertazzi

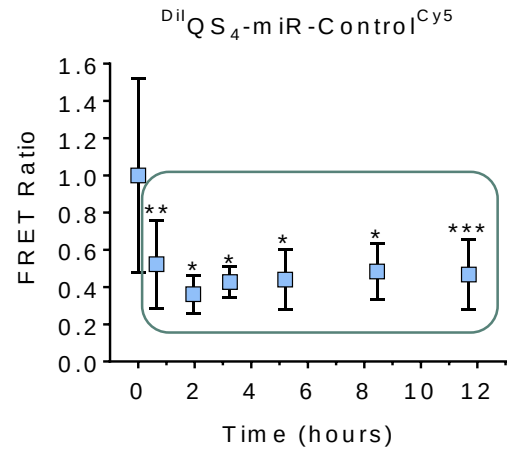
Cargo release











Boloix A *et al. Small. Under minor revisions*

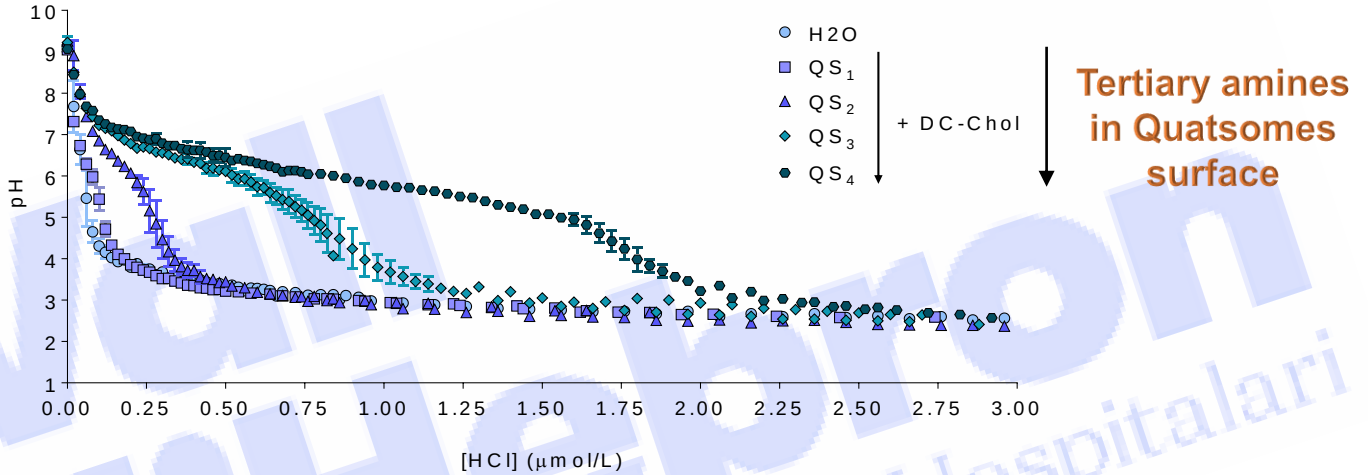
MiRNA is released from QS_4 by a pH-dependent mechanism

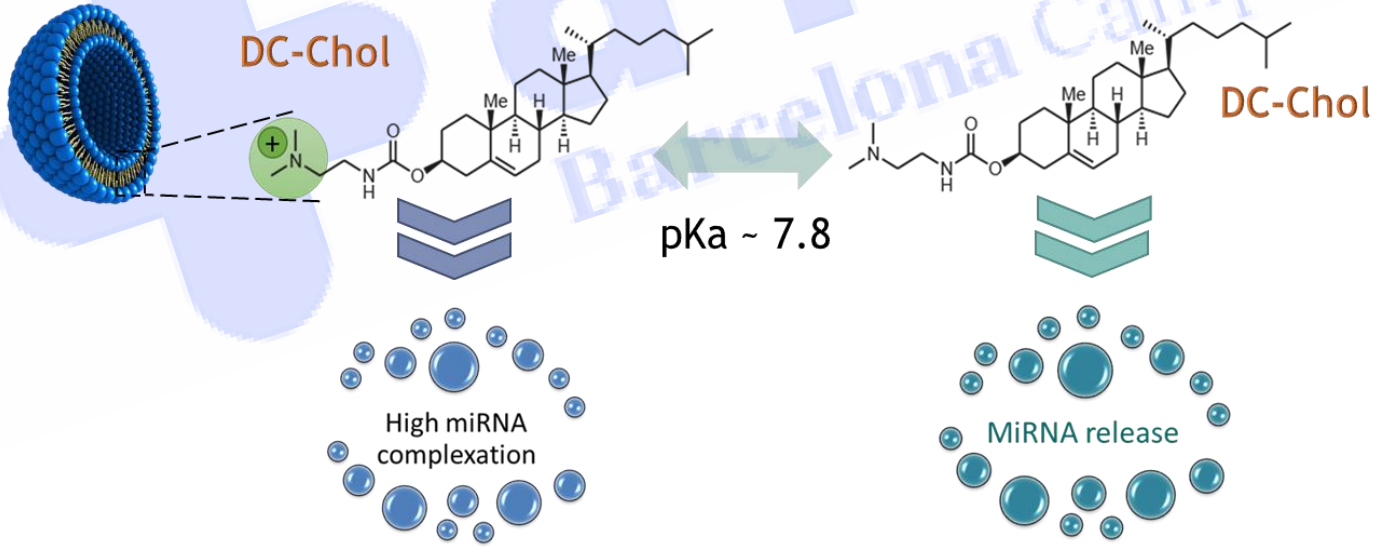
QS_1 (100%Chol/0%DC-Chol):MKC

QS_2 (90%Chol/10%DC-Chol):MKC

QS_3 (50%Chol/50%DC-Chol):MKC

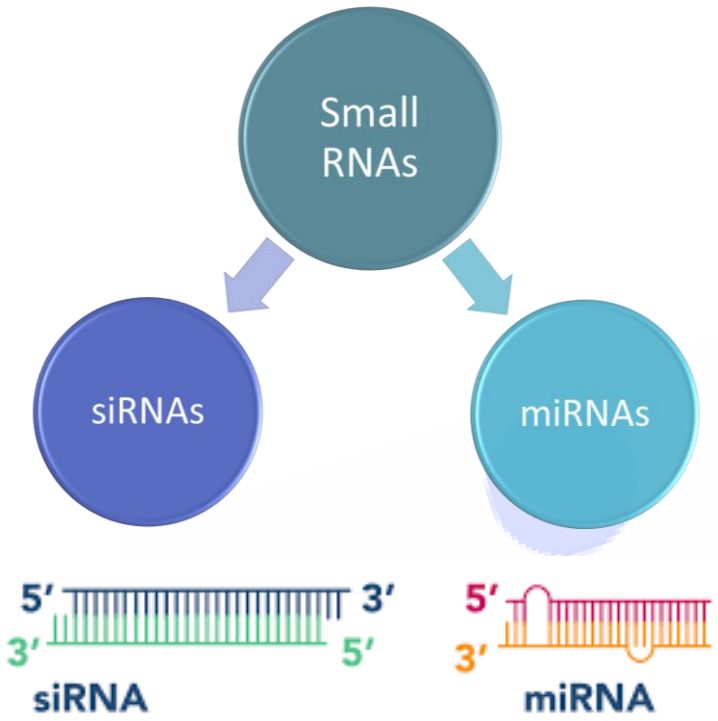
QS_4 (0%Chol/100%DC-Chol):MKC

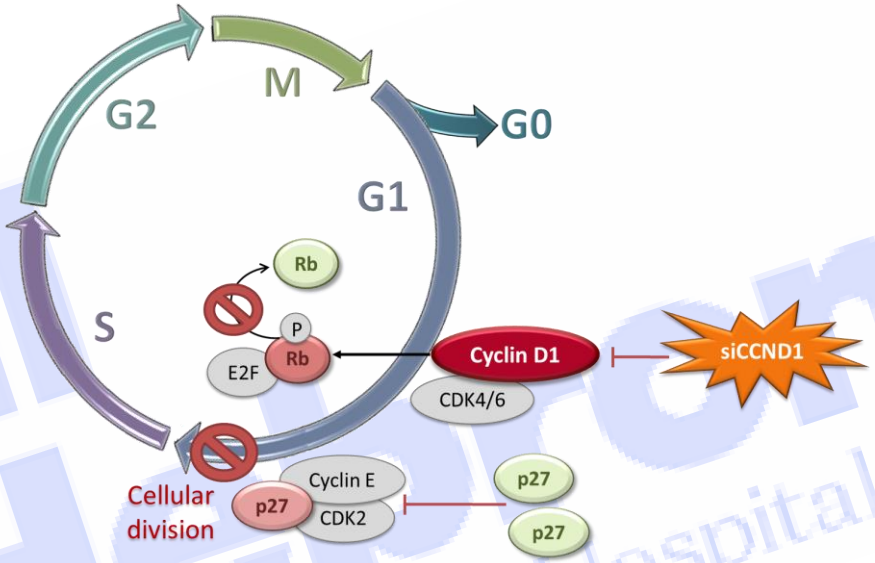


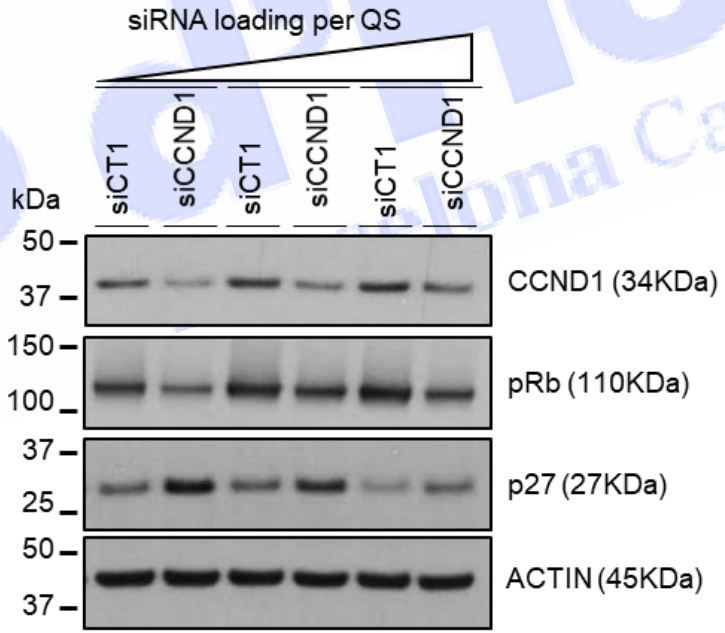


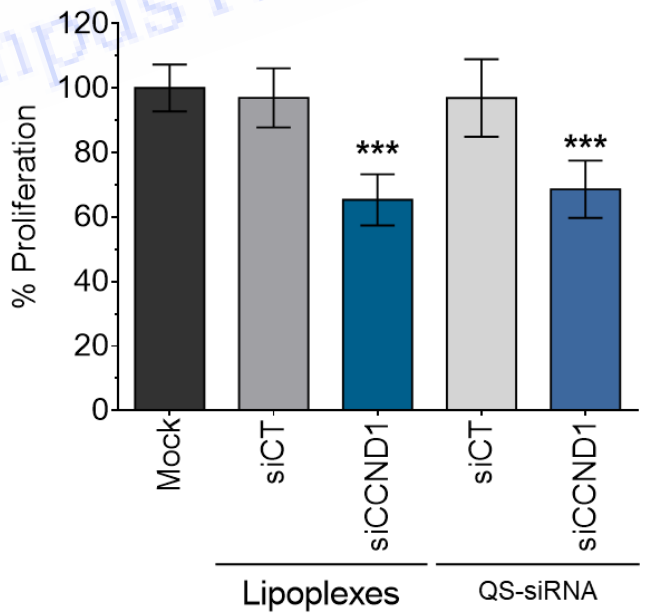
Boloix A *et al. Small. Under minor revisions*

Quatsomes can also conjugate and deliver siRNAs



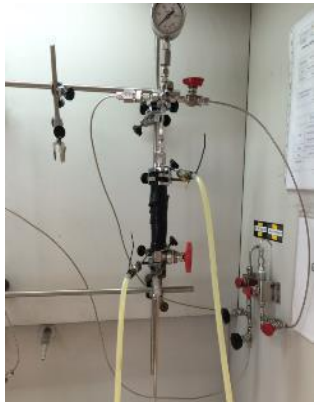






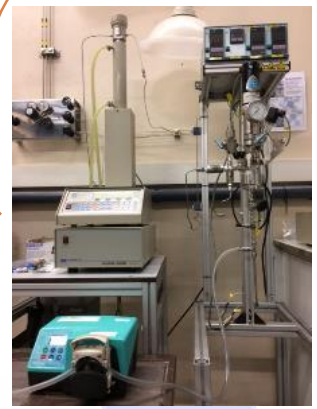
Boloix A *et al. Small. Under minor revisions*

But…Can QS production be scaled-up?



P6

***25-75 ml** of nanovesicles*



P50

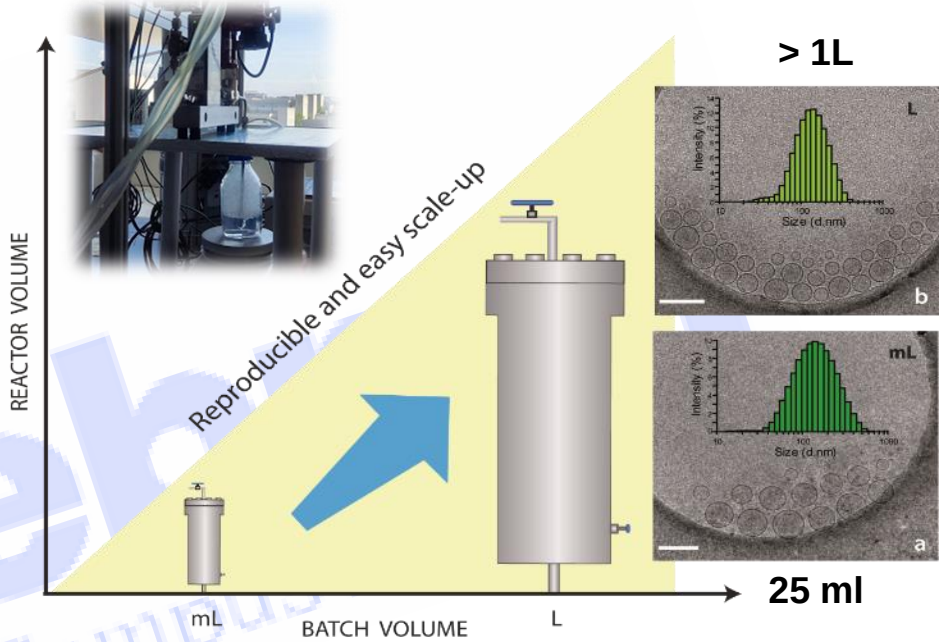
***250-750 ml** of nanovesicles*

P300

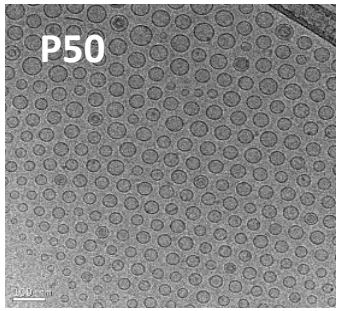
***> 1 L** of nanovesicles*

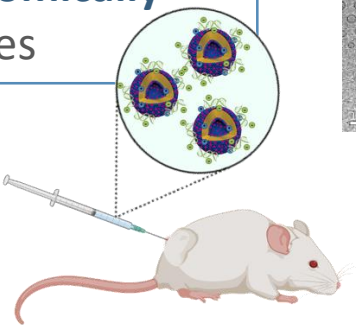
P2000

***> 8 L** of nanovesicles*



- **GMP compatibility** positively evaluated for both batch and continuous system
- **Quality by Design** approach.
- Cost study at industrial scale available for DELOS production of APIs particles. **Economically and Environmentally Viable** in comparison to conventional manufacturing processes

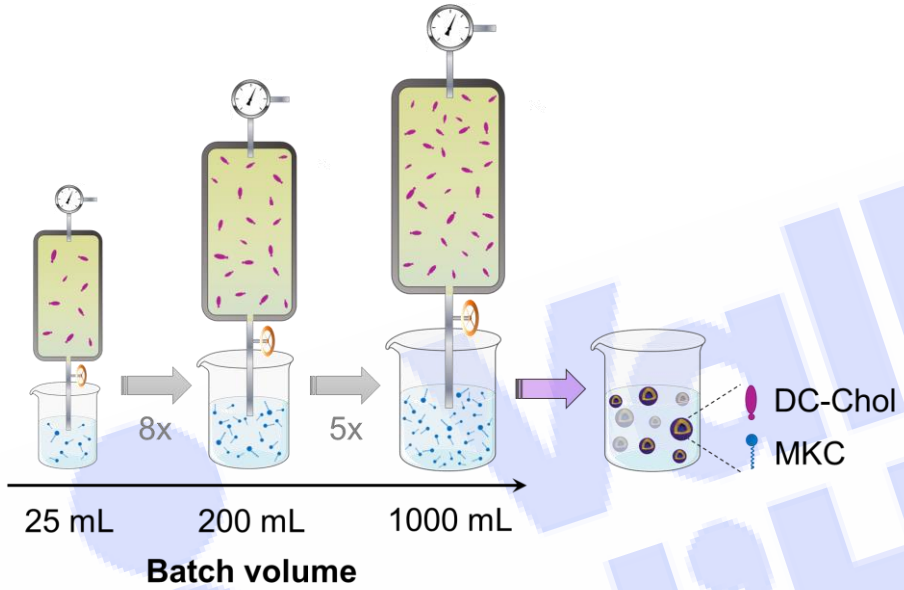


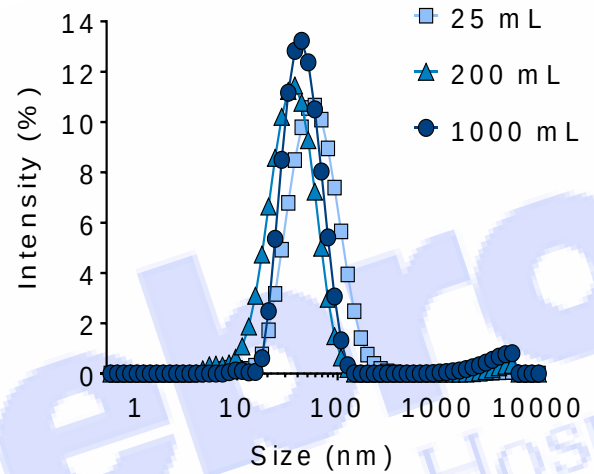


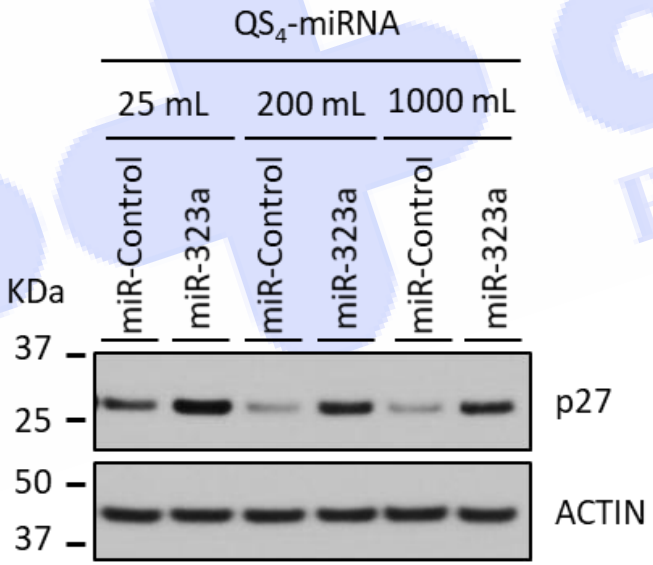
I Cabrera et al. *Nano Lett.*, **2013,** *13*, 3766–3774

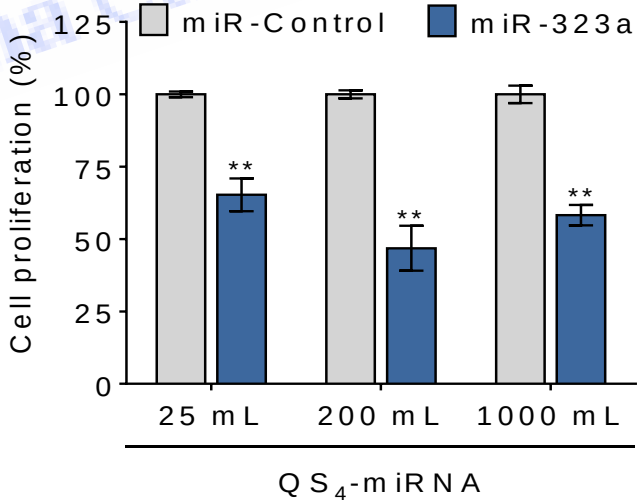
Scaled–up QS_4 have reproducible properties and similar *in vitro* effects

Difficulties in scale up









Boloix A *et al. Small. Under minor revisions*

MiR-323a-5p is released from QS_4 after *in vivo* administration

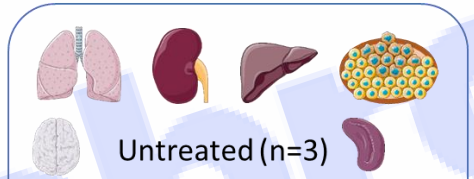
[CmiRNA] = 2 mg/kg or 42.55 µM
[$C^{Dil}QS_4$] = 1,52 mg/mL or 0,75mM

Untreated (n=3)
$^{Dil}QS_4$-miR-Control (n=3)
$^{Dil}QS_4$-miR-323a-5p (n=3)

$5{\cdot}10^6$ cells SK-N-BE(2) explant injection (15-21 days before)

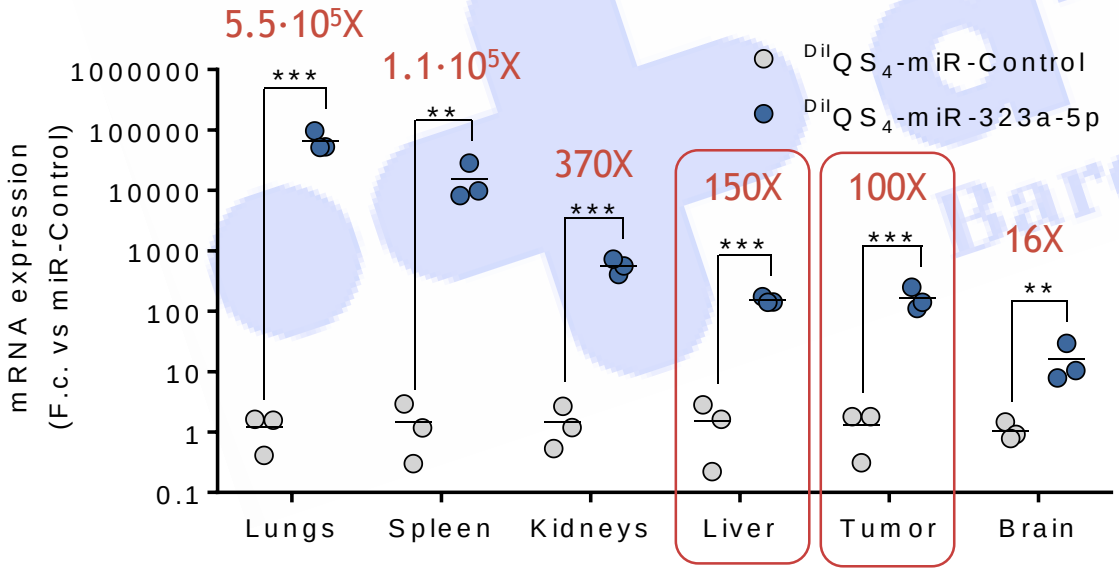
0 h

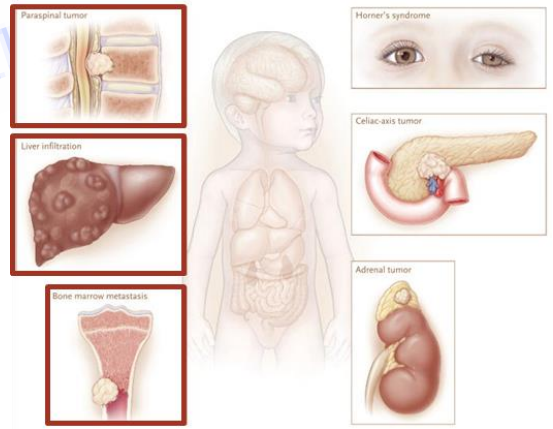
24 h



Untreated (n=3)
$^{Dil}QS_4$-miR-Control (n=3)
$^{Dil}QS_4$-miR-323a-5p (n=3)

- IVIS
- qPCR

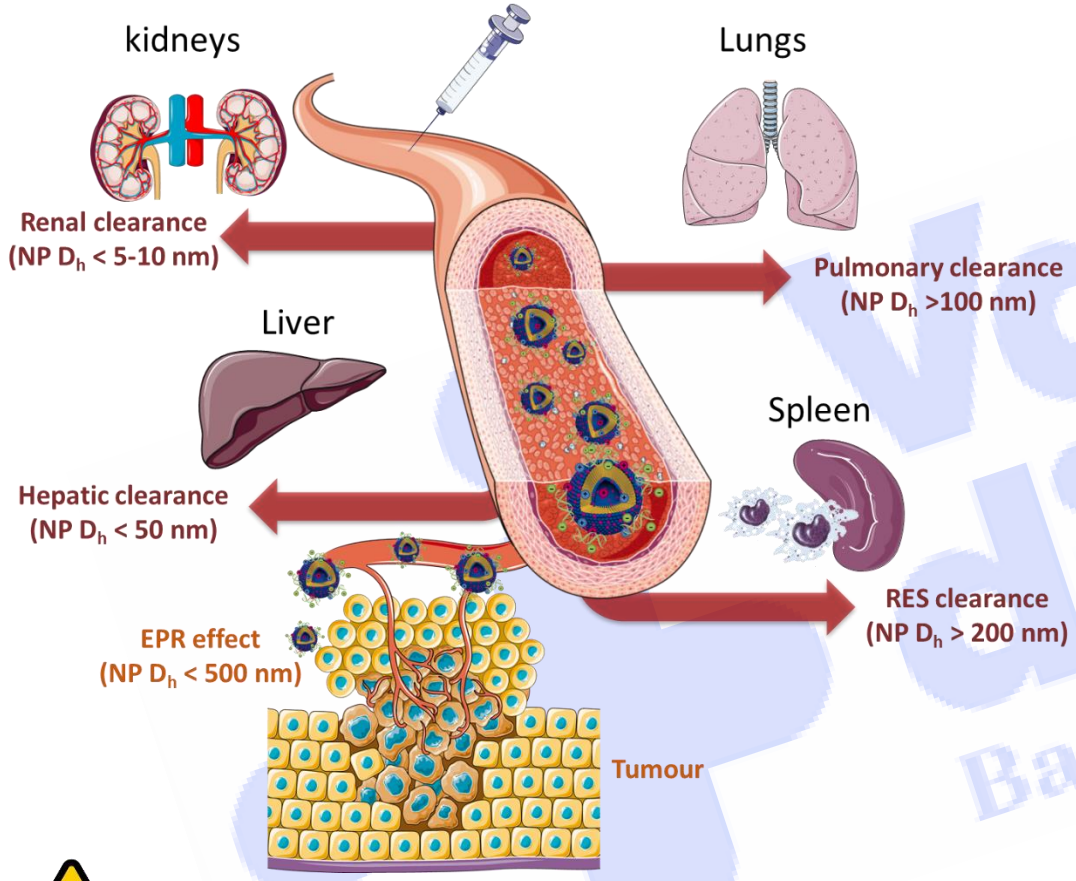




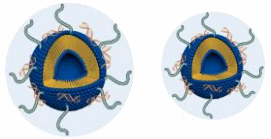
High-risk neuroblastoma metastasize into lymph nodes, bone marrow and **liver**

Boloix A *et al. Small. Under minor revisions*

Perspectives: How can we improve QS-miRNA biodistribution?



1. Surface decoration with **polyethylene glycol (PEG)**



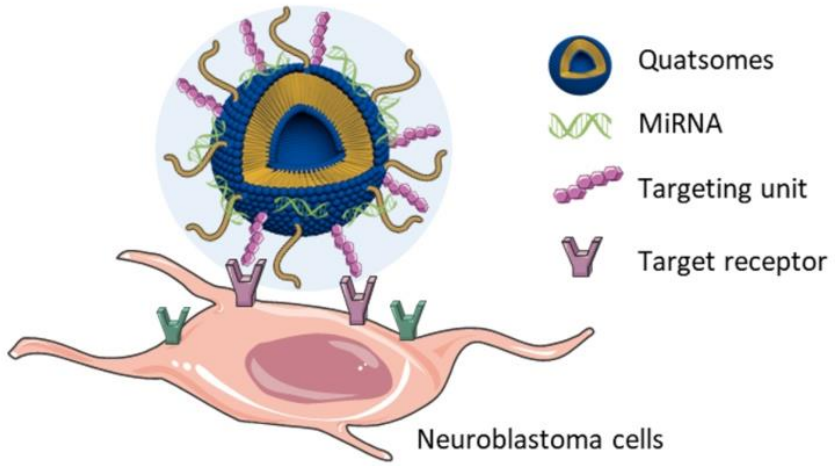
Less aggregation and RES uptake

Immunogenicity

2. Morphology, composition and charge modification

Improved delivery

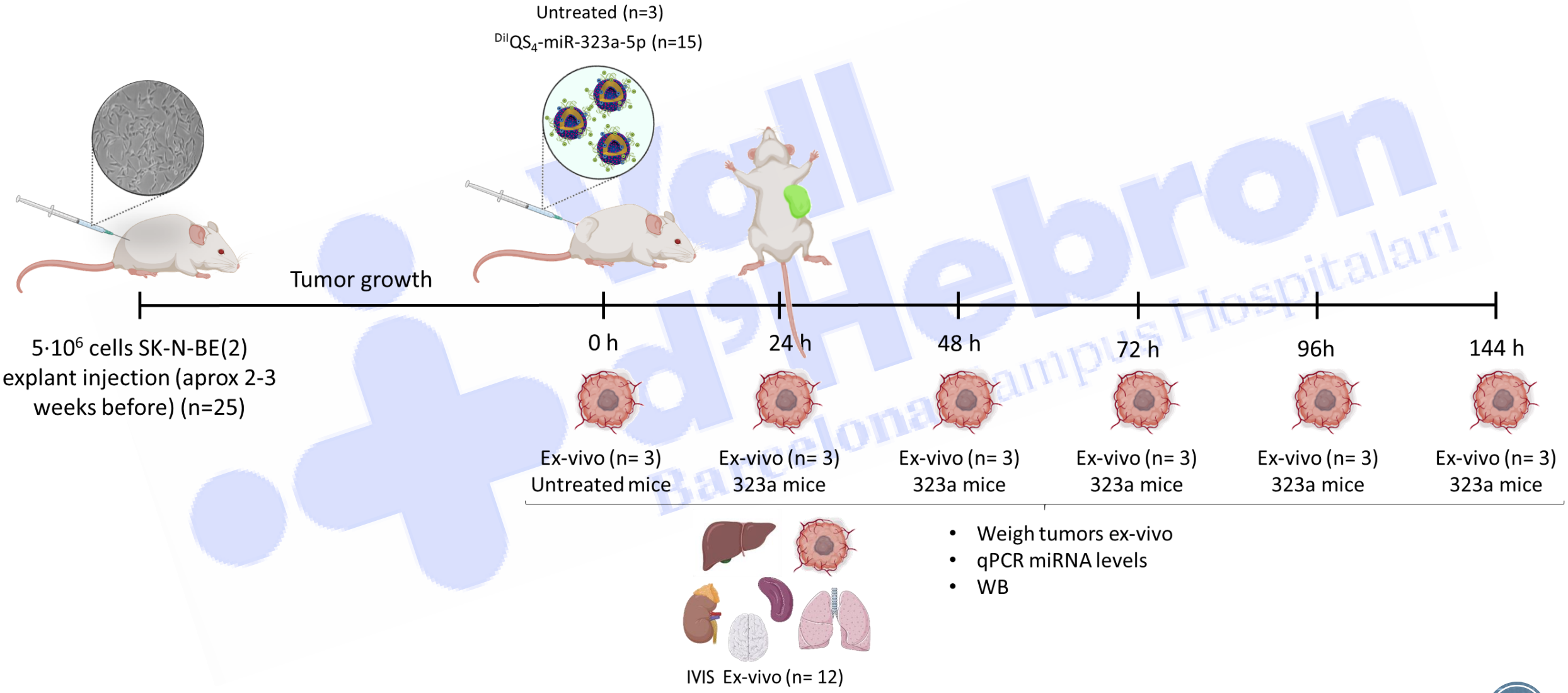
3. **Active targeting** with targeting peptides



Targeted therapy

For cancer treatment, the local administration of RNA therapies is preferred to ensure efficacy

In the near future: Intratumoral efficacy of QS_4-miR-323a



Acknowledgements

Laboratory of Translational Research in Child and Adolescent Cancer

Dr. Miguel Segura

Vall d'Hebron Institut de Recerca

Nanomol Group

Dra. Nora Ventosa

NANOMOL CENTRE OF NANOTECHNOLOGY AND MOLECULAR MATERIALS

ICMAB INSTITUT DE CIÈNCIA DE MATERIALS DE BARCELONA

EXCELENCIA SEVERO OCHOA

CSIC

ciber-bbn Centro de Investigación Biomédica en Red Bioingeniería, Biomateriales y Nanomedicina

Collaborators:

TU/e EINDHOVEN UNIVERSITY OF TECHNOLOGY

Molecular Biosensing for Medical Diagnostics

Dr. Lorenzo Albertazzi

IBEC Institute for Bioengineering of Catalonia

EXCELENCIA SEVERO OCHOA

Nanoscopy for nanomedicine group

iQAC CSIC Consejo Superior de Investigaciones Científicas

Multivalent systems for nanomedicine

Dr. Míriam Royo

ciber-bbn Centro de Investigación Biomédica en Red Bioingeniería, Biomateriales y Nanomedicina

Synthesis of Peptides Unit

Funding:

MARIE CURIE ACTIONS

Ministerio de Economía y Competitividad · Instituto de Salud Carlos III · Unión Europea · Fondo Europeo de Desarrollo Regional

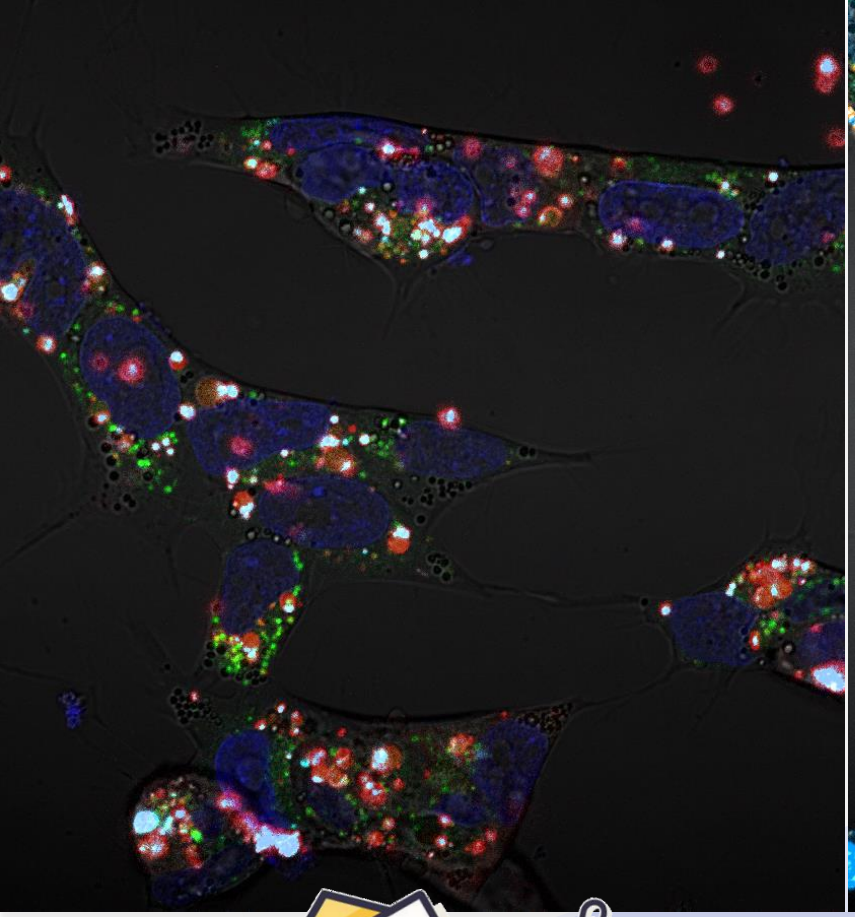
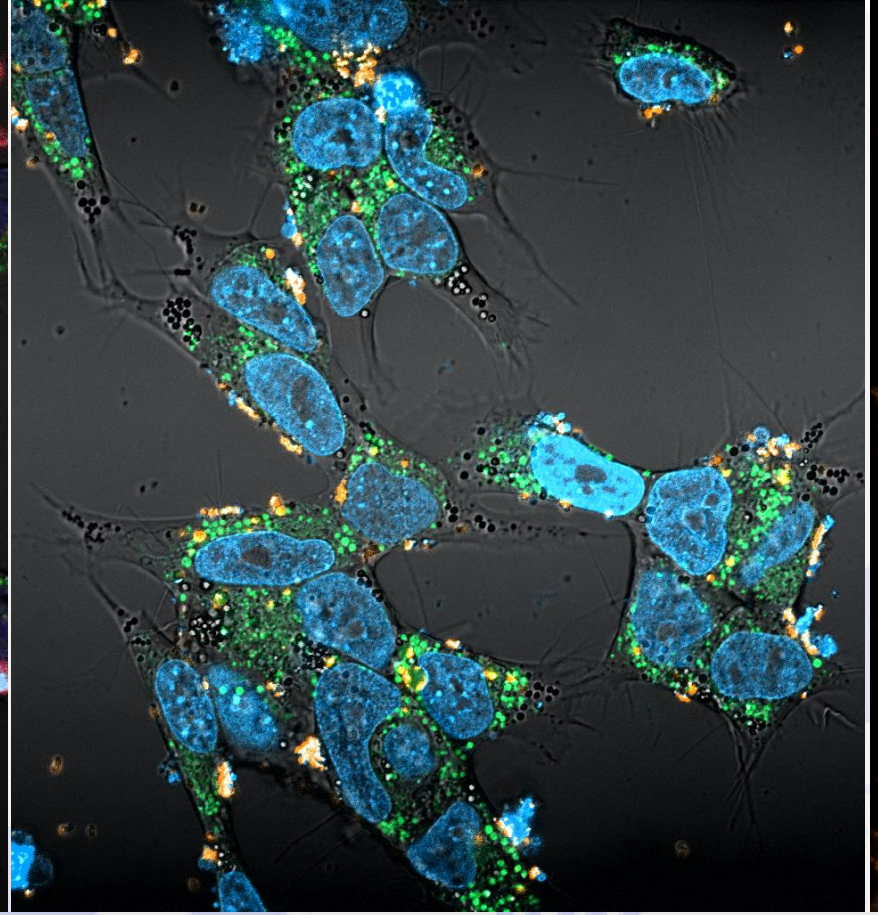
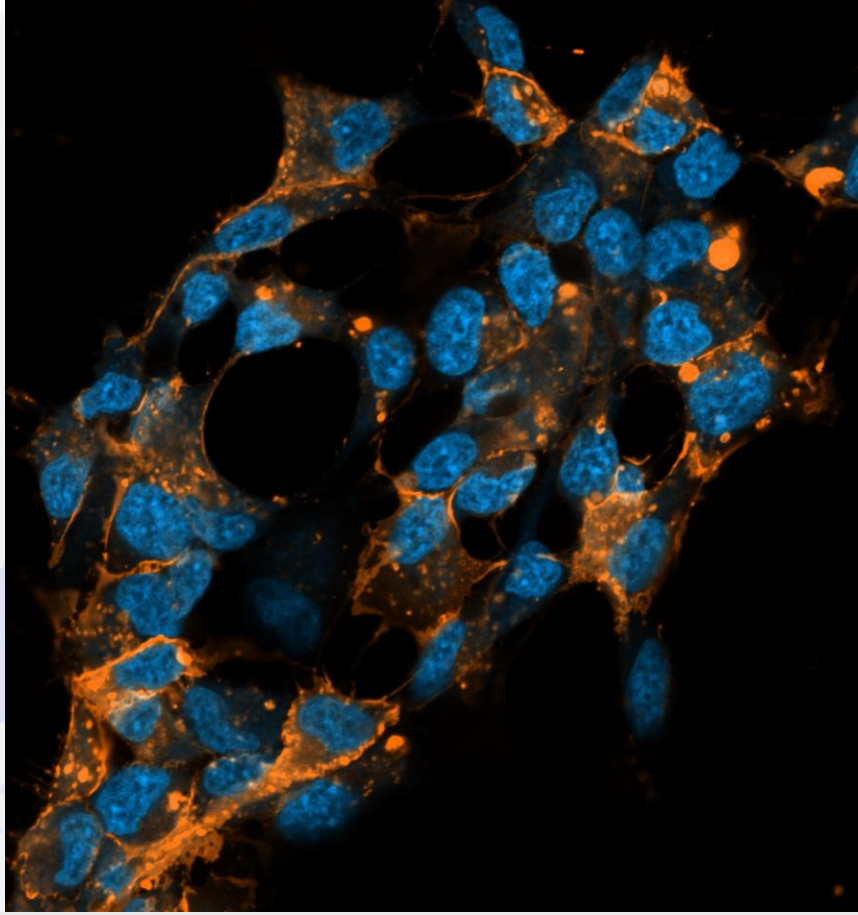
AGAUR Agència de Gestió d'Ajuts Universitaris i de Recerca

aecc Contra el Cáncer

Vilalta AMICS JOAN PETIT VENS AMB CÀNCER

NEN

Instituto de Salud Carlos III

Dr. Ariadna Boloix

Post-doctoral researcher & technology developer

Laboratory of Translational Research in Child and Adolescent Cancer
Vall d'Hebron Institut de Recerca (VHIR)
Collserola Building, Lab 207-208
Passeig Vall d'Hebron 119-129
08035 Barcelona, Spain
+34934894067
ariadna.boloix@vhir.org

ICMAB
INSTITUT DE CIÈNCIA DE MATERIALS DE BARCELONA
EXCELENCIA SEVERO OCHOA
CSIC

Vall d'Hebron

UNIVERSITAT DE BARCELONA
Facultat de Farmàcia i Ciències de l'Alimentació