LABORATORY of PHARMACEUTICAL MICRO- and NANOTECHNOLOGY

Université de Montréal

Chaire en Biothérapie de l'Association Canadienne du Médicament Générique (ACMG) et Biosimilaires Canada

Davide Brambilla, PhD

3rd Scientific committee, August 2020

Biosimilars Canada
The Voice of Biosimilar Medicines in Canada

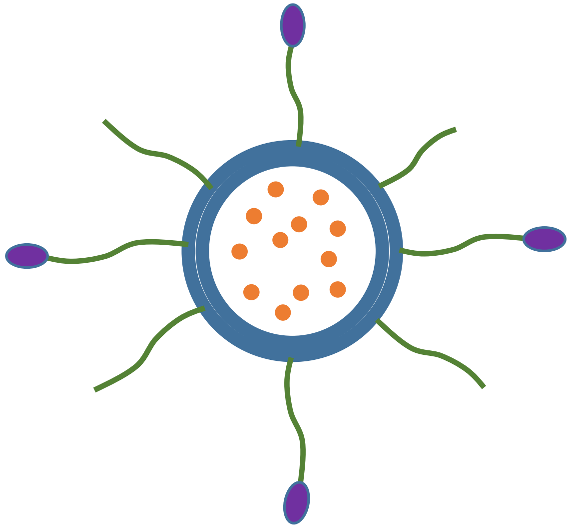
CGPA
GENERIC DRUGS
SAME QUALITY
LOWER PRICE

Projects

- **Carolina**: NETs-targeting DNAse-loaded nanoparticle
- **Fatma**: Tips-releasing microneedles for anti-psoriasis drug intradermal depot / mRNA loaded lipid particles
- **Sam**: Fluorescent microtattoo for diagnostic applications
- **Philippe**: Polymeric porous microparticles for biomedical applications
- **Elise**: Superabsorbent polymeric microneedles for ISF samples for heart failure monitoring
- **Cloé**: Dissolving microneedles for non-invasive administration of azapeptides

Nanoformulations for targeting NET

- Target NET (DNAse and NETosis inhibitors)
- Increase the half-life of DNAse or NETosis inhibitors



SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

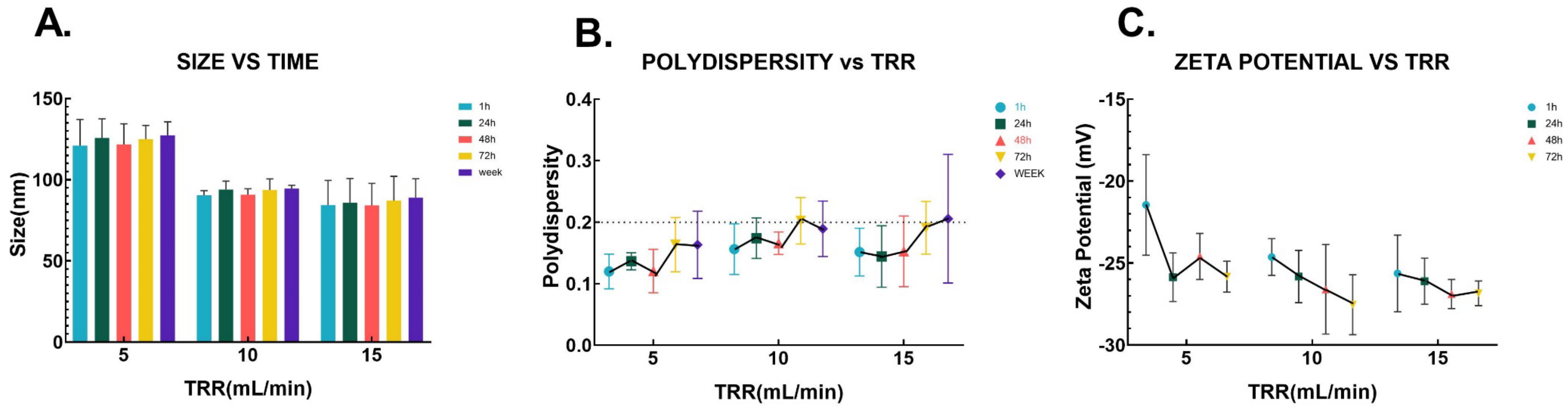
Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps

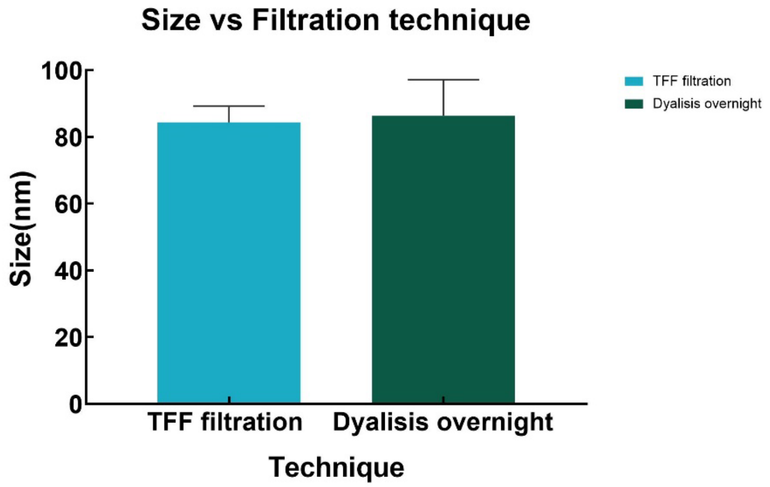
Juwon Park,[1*†] Robert W. Wysocki,[1,2,3*] Zohreh Amoozgar,[4*‡] Laura Maiorino,[1,5*] Miriam R. Fein,[1,3] Julie Jorns,[6] Anne F. Schott,[6] Yumi Kinugasa-Katayama,[1] Youngseok Lee,[7] Nam Hee Won,[7] Elizabeth S. Nakasone,[1,5] Stephen A. Hearn,[8] Victoria Küttner,[1] Jing Qiu,[1] Ana S. Almeida,[1] Naiara Perurena,[1§] Kai Kessenbrock,[9] Michael S. Goldberg,[4,10] Mikala Egeblad[1||]

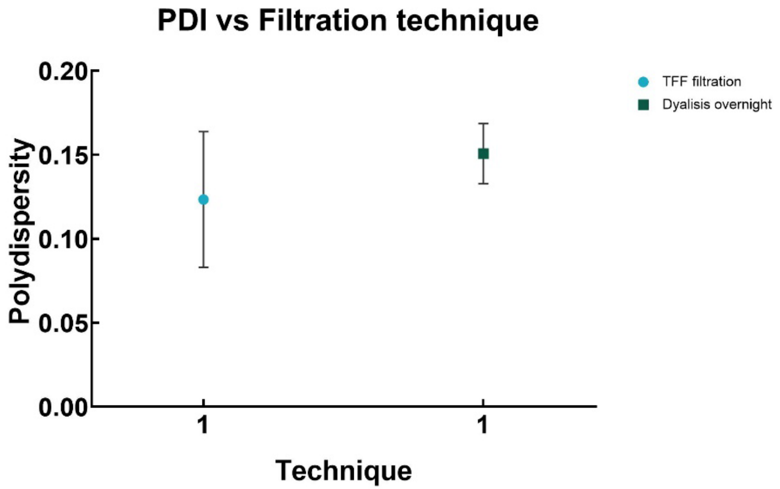
No target - half-life? - Stability?

Last year

Liposome generation optimization

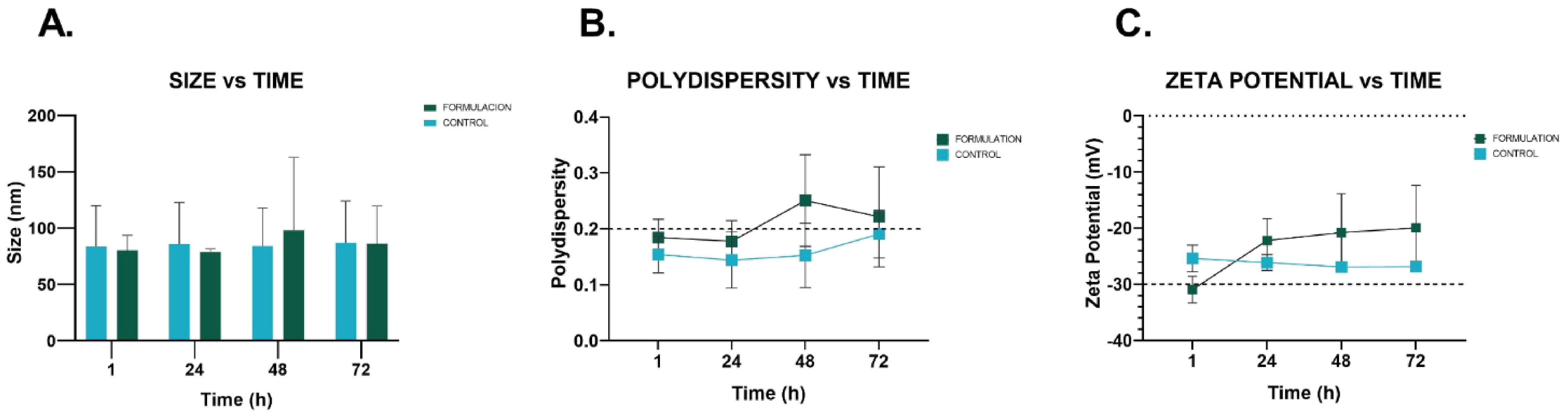






Last year

RGD-functionalized liposomes

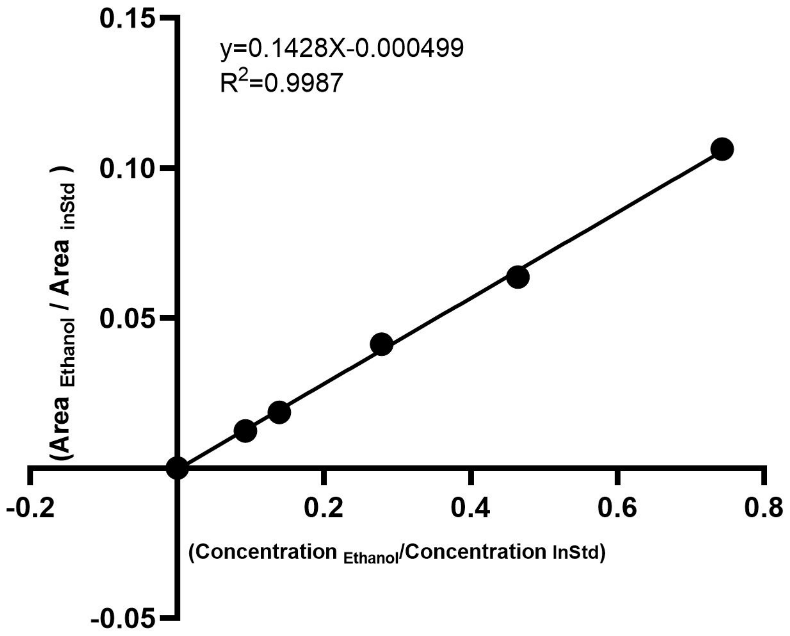


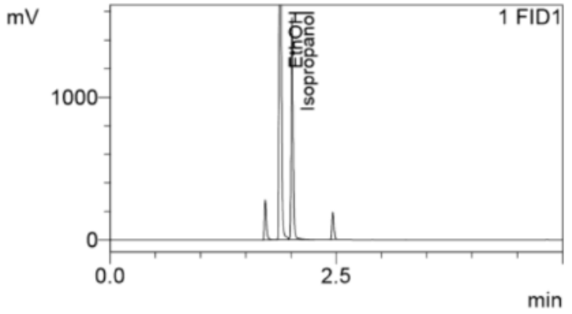
Kruskal Wallis Test ci=99.9% p<0.0001
n=3

Last year

RESIDUAL ETHANOL QUANTIFICATION BY GC and HPLC

Calibration curve residual Ethanol by GC





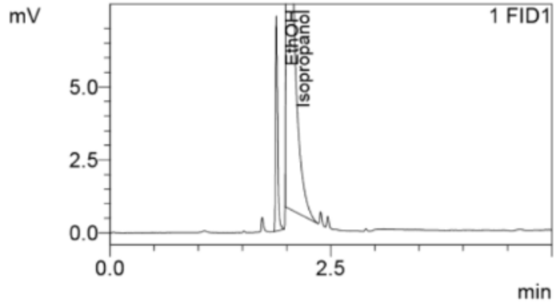
FID1

Peak#	Ret. Time	Area	Height	ID#
1	1.715	416169	276226	
2	1.879	5421556	3753049	1
3	2.011	2347038	1534460	2
4	2.461	297083	188334	
Total		8481845	5752069	

FID1

ID#	Name	Conc.	Unit
1	EthOH	63.920	ppm
2	Isopropanol	27.671	ppm

Before: 174327 ppm



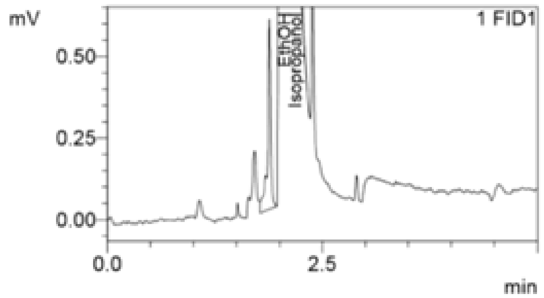
FID1

Peak#	Ret. Time	Area	Height	ID#
1	1.884	11706	7288	1
2	2.012	2466077	1619181	2
Total		2477783	1626469	

FID1

ID#	Name	Conc.	Unit
1	EthOH	0.472	ppm
2	Isopropanol	99.528	ppm

TFF: 395 ppm



FID1

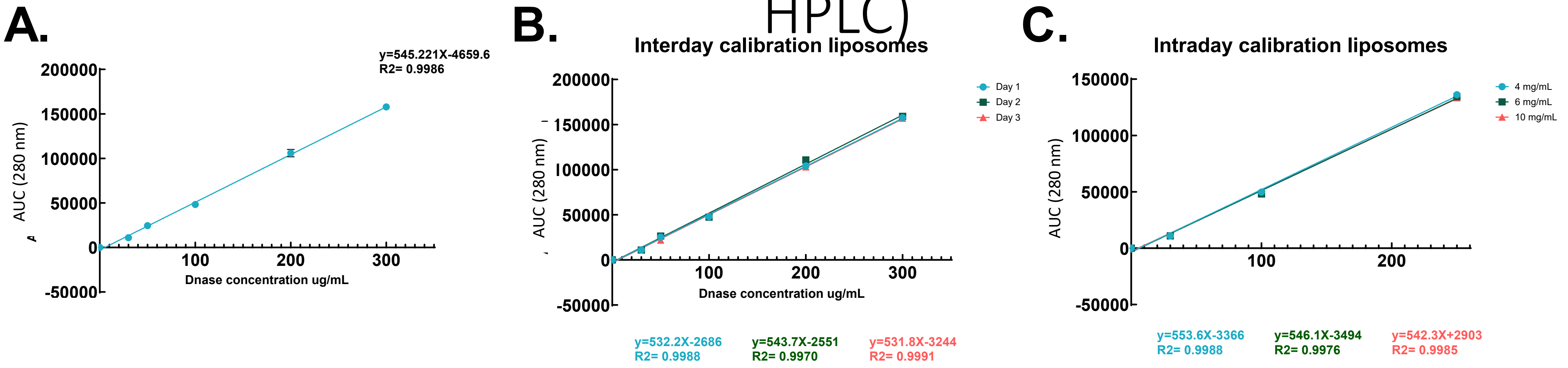
Peak#	Ret. Time	Area	Height	ID#
1	1.884	1426	577	1
2	2.010	2504571	1672428	2
Total		2505997	1673005	

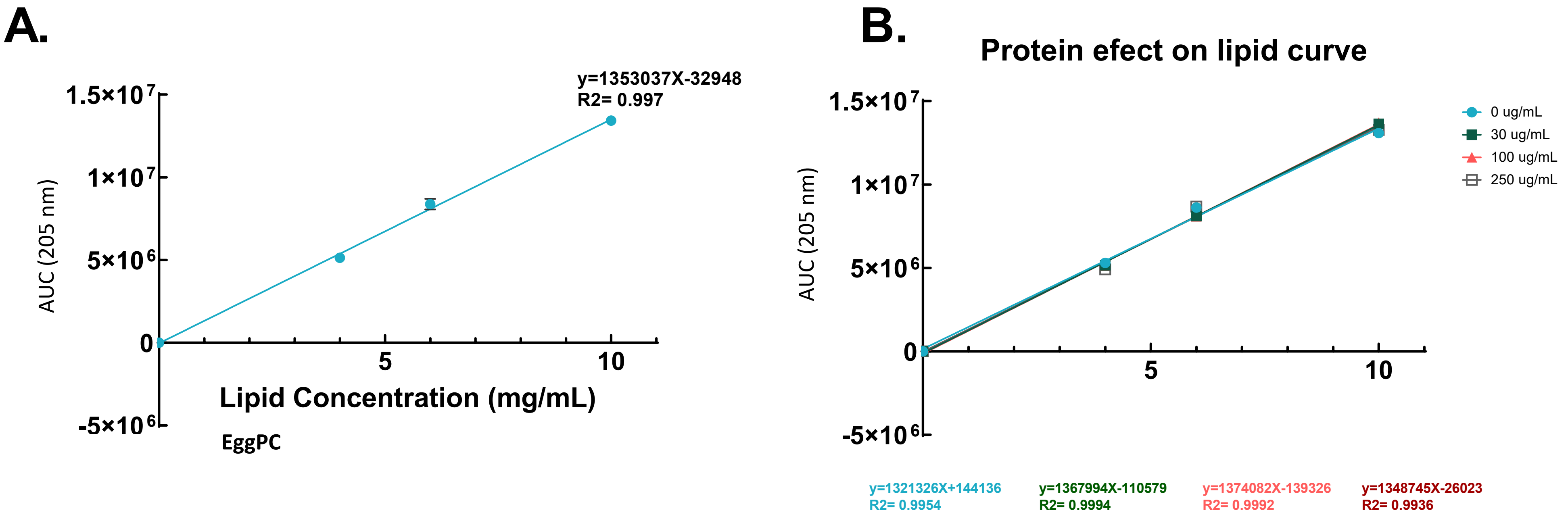
FID1

ID#	Name	Conc.	Unit
1	EthOH	0.057	ppm
2	Isopropanol	99.943	ppm

Dialysis: 0 ppm??

Analytical method, HPLC: lipids, DNAse (couple of suppliers) HPLC)

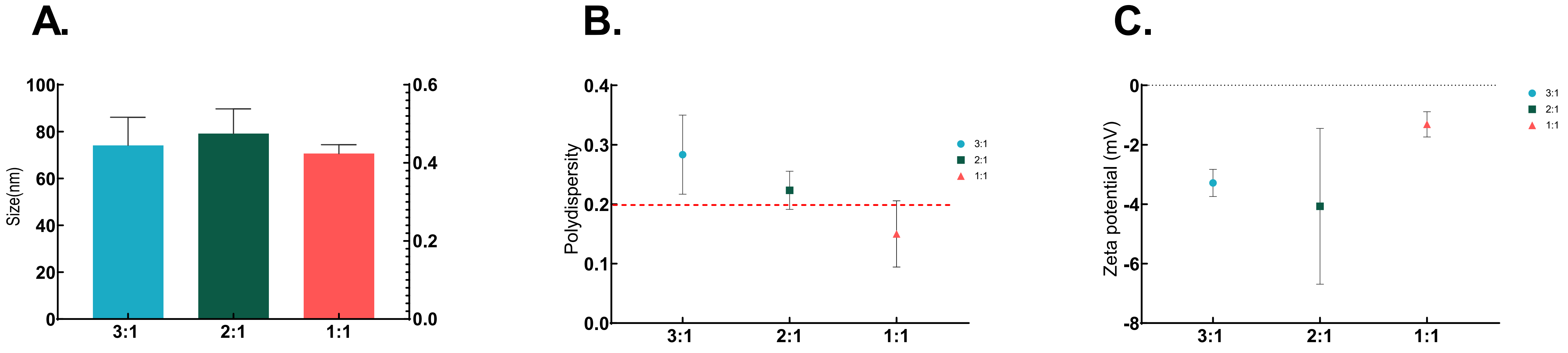




EggPC:Chol:DSPE-PEG : 50.5:44.5:5, FFR: 1:1 - TFR: 12mL/min, pH:4 (sodium acetate, 20mM), i[Dnase]: 250 µg/mL. i[lipid]: 10mg/mL-14.26mM. Solvent: Ethanol 95%. Aqueous phase: Sodium acetate pH: 4 ± 0.5, Purification method: TFF. Buffer exchange to PBS pH: 7± 0.5 (Forbes, Hussain et al. 2019).

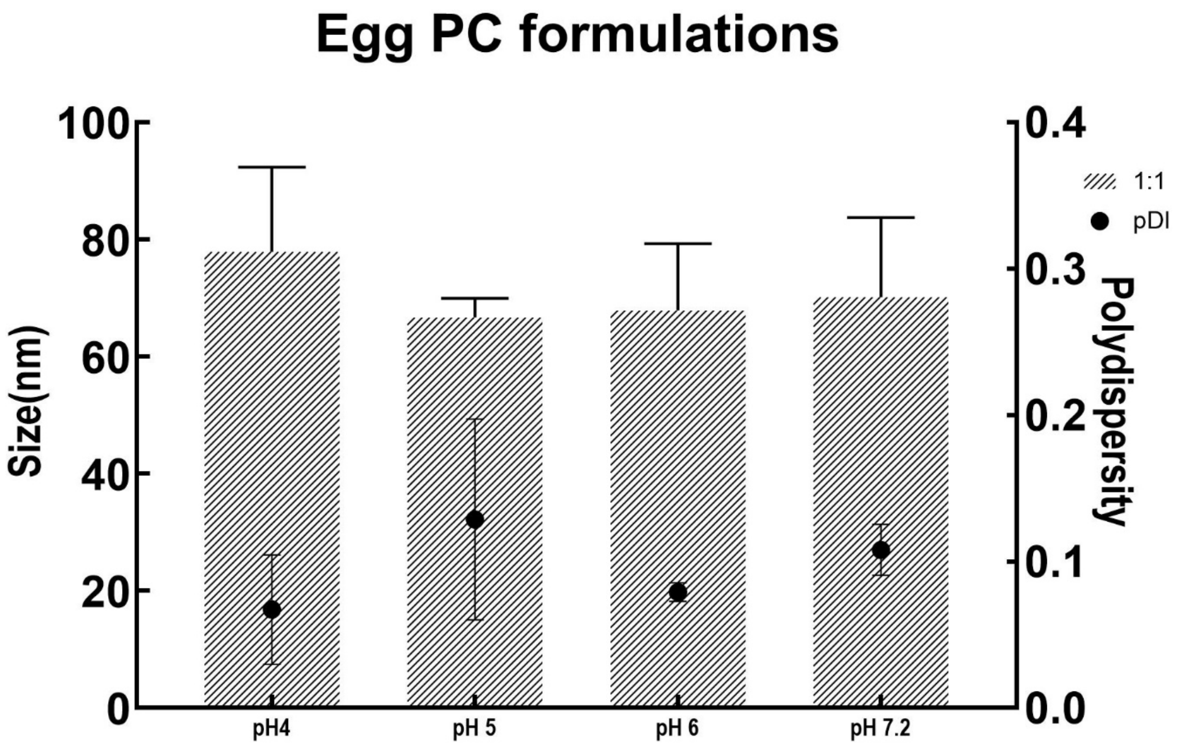
DNAse loading into liposomes: FRR effect

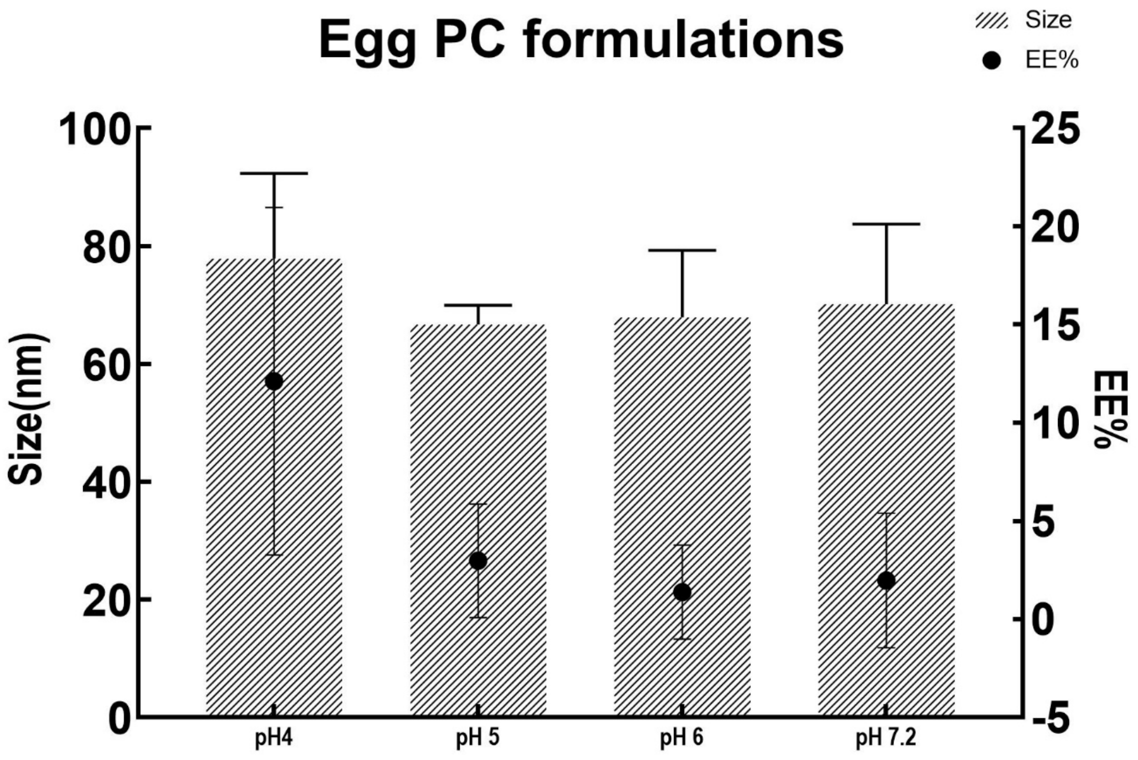
Nanoassembler
10 mg/mL lipid
DNAse 250 microg/mL
Different FRR (3:1, 2:1, 1:1):
TFF purification
no encapsulation



DNAse loading into liposomes: pH effect

10mg/mL lipid
250microg/mL DNAse

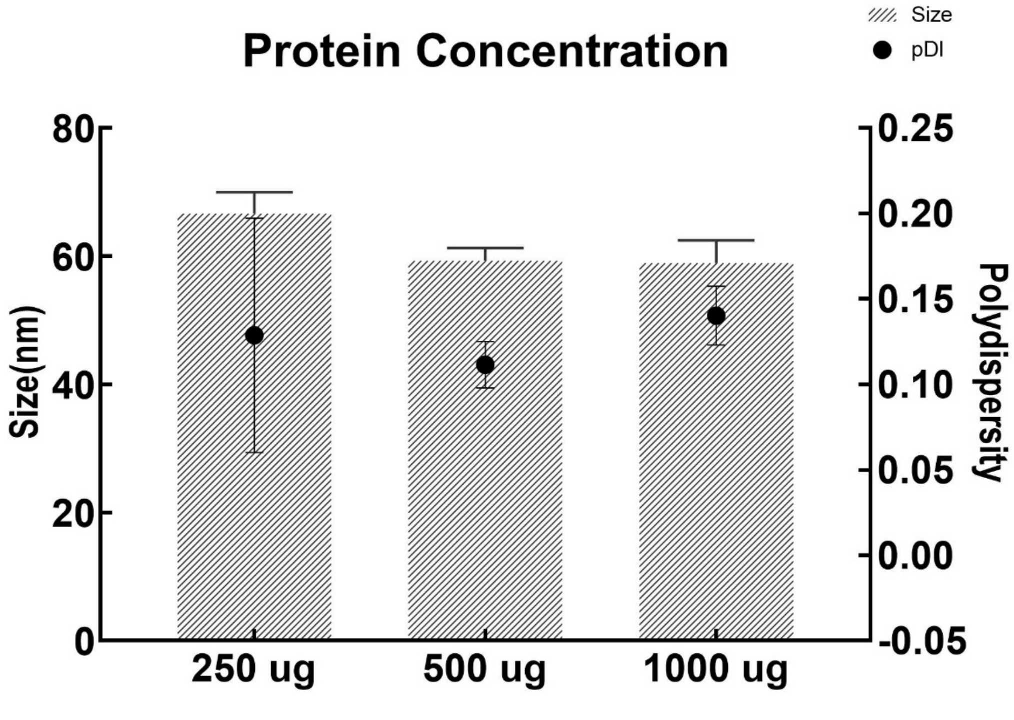


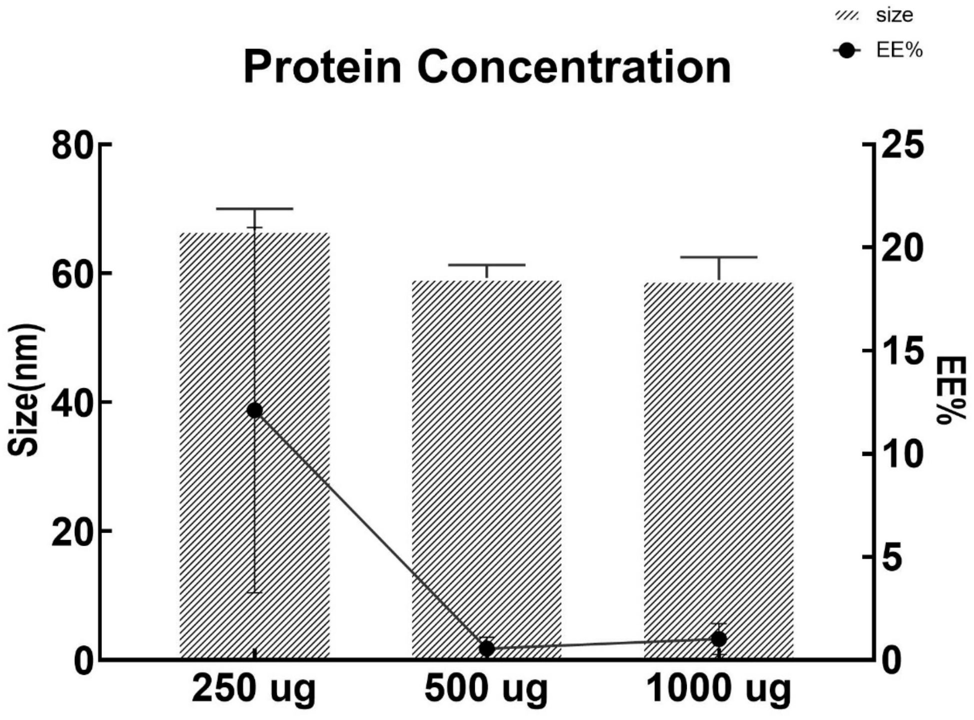


pH 4 seems better (DNAse IP: 5,22) positive at pH 4

DNAse loading into liposomes: DNAse concentration effect

pH5 for potential issues of DNAse activity
10mg/mL lipid





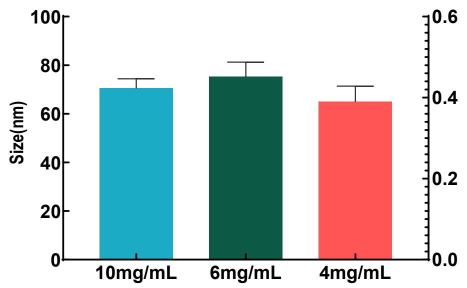
Problem reproducibility in loading (better/faster/less lost purification TFF)

No impact of Calcium and Magnesium during liposome production

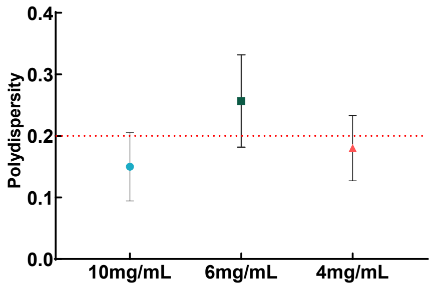
IP DNAse 5.22
Lipid not really charged

Effect of lipid concentration DNAse 250 microg/mL

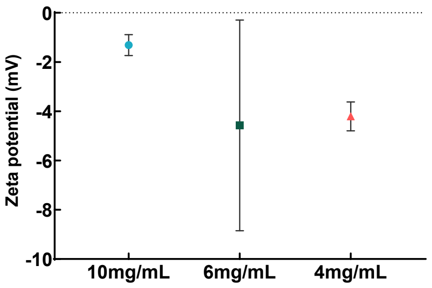
A.

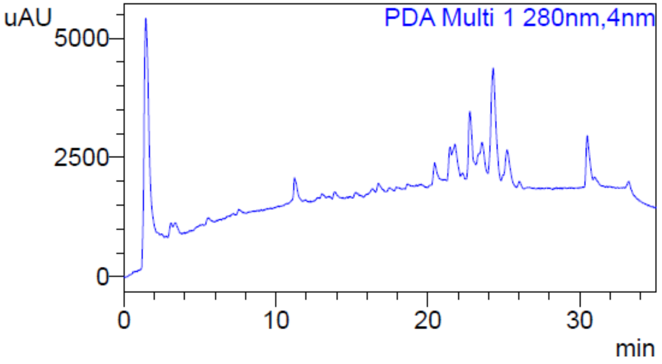


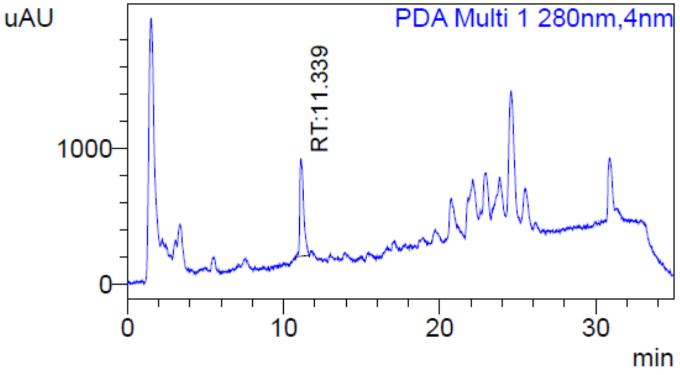
B.

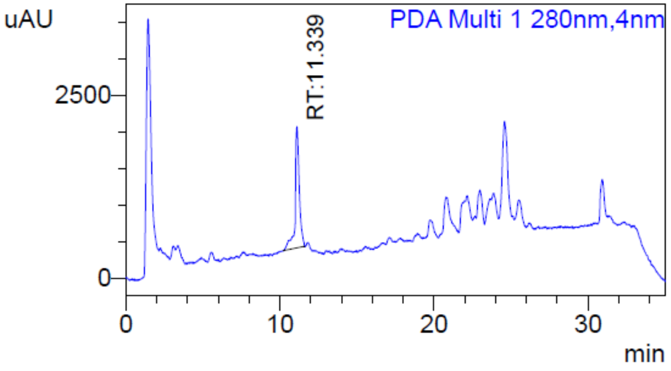


C.







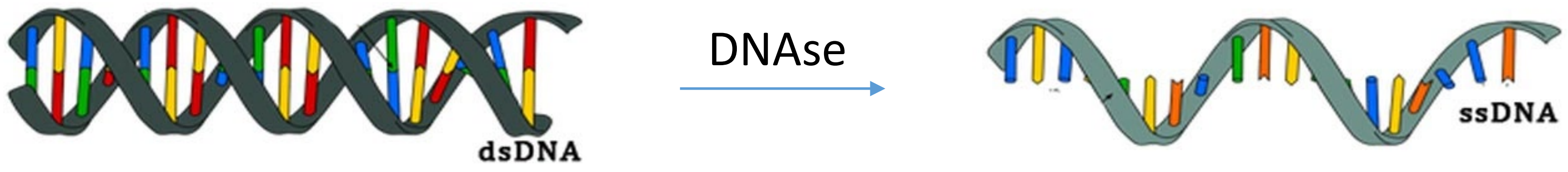


pH 4 for loading

i Lipid concentration (mg/mL)	EE%	Drug loading %
4	9.3 ±2.6	0.92 ± 0.24
6	3.27 ± 4.68	0.34 ± 0.47
10	0	0

DNAse loaded Liposomes enzyme activity

Quant-iT™ PicoGreen™



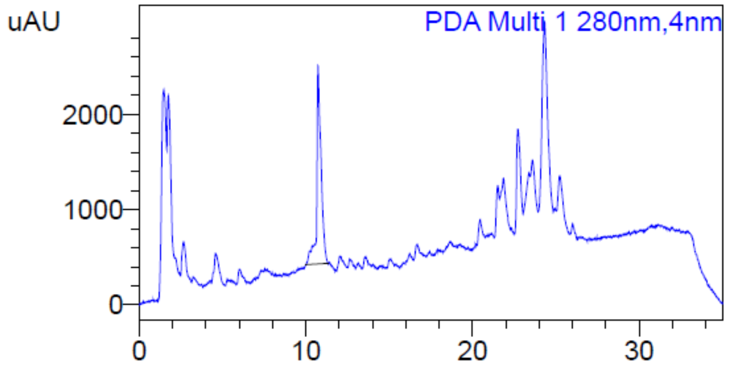
	1	2	3
A	2X Unloaded Liposomes + PBS	2x Unloaded liposomes + Free Dnase	2x liposomal DNAse + pbs
B	**50µL TE 25µL C- 25µL DNA 100 µL Picogreen**	**50µL TE 25µL C+ 25µL DNA 100 µL Picogreen**	**50µL TE 25µL sample 25µL DNA 100 µL Picogreen**
C	**50µL TE+Tritonx 25µL C- 25µL DNA 100 µL Picogreen**	**50µL TE +Tritonx 25µL C+ 25µL DNA 100 µL Picogreen**	**50µL TE +Tritonx 25µL sample 25µL DNA 100 µL Picogreen**

Results fluorescence: λ_{EX}: 480nm , λ_{Em}: 520nm.

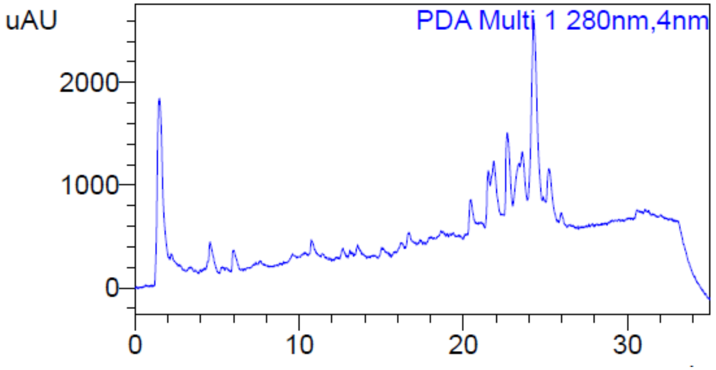
2	1	7
2335	599	2106
2451	503	1834
2055	**265**	**372**
1891	**207**	**383**

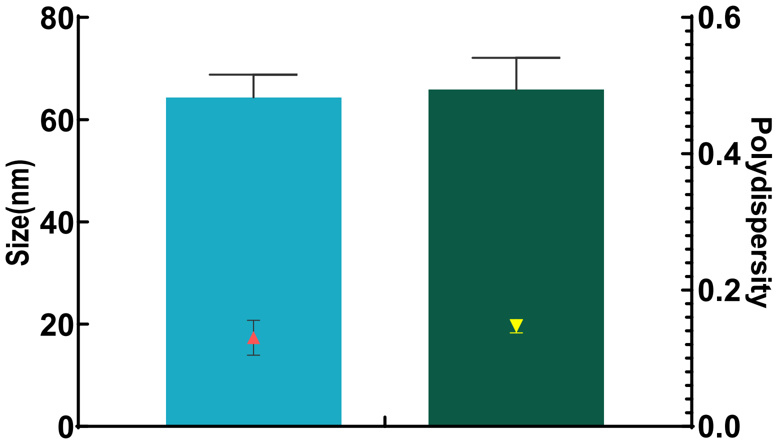
DNAse release

LCP 0017 QUANTIFICATION OF LIPOSOMAL DNAse RELEASE BY HPLC AFTER AMICON CENTRIFUGATION		Version LCP0017.3	Page 1/1
Objectives	Measure the release of DNAse from liposomal formulation: purifying free protein with Amicon and evaluating liposomal DNAse by HPLC.		Issue Date: 12/06/2020
Notes	100 µL Liposomes at 10mg/mL + 3.9 mL of PBS. System configuration: 4000g for 18 min, column prewash with Milli Q water		Implementation Date: 10/07/2020



After 1h at 37°C

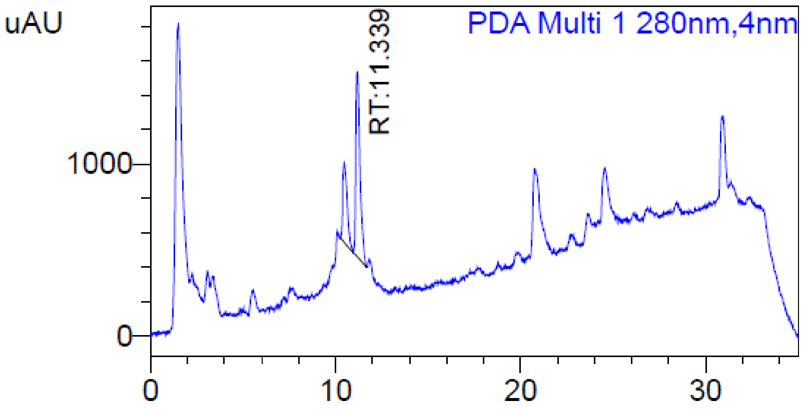




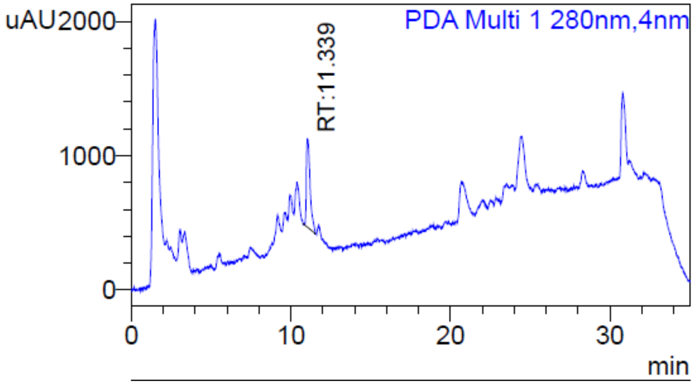
DOTAP formulation (charge effect)

pH 7 (DNAse -; DOTAP +)

	DOTAP	Egg PC	Chol	DSPE-PEG	Size (nm)	PDI	Zeta Potential (mV)
F1	50.5		44.5	5	117.6±6.88	0.25±0.03	4.99±2.77
F2	45	10	40	5	110.6±0.75	0.35±0.03	4.85±0.70
F3	31.4	10.2	53.5	4.9	125.52±11.81	0.4±0.001	6.53±1.40
F1.2	40.5	10	44.5	5	115.02±3.70	0.41±0.01	4.32±3.19



F1

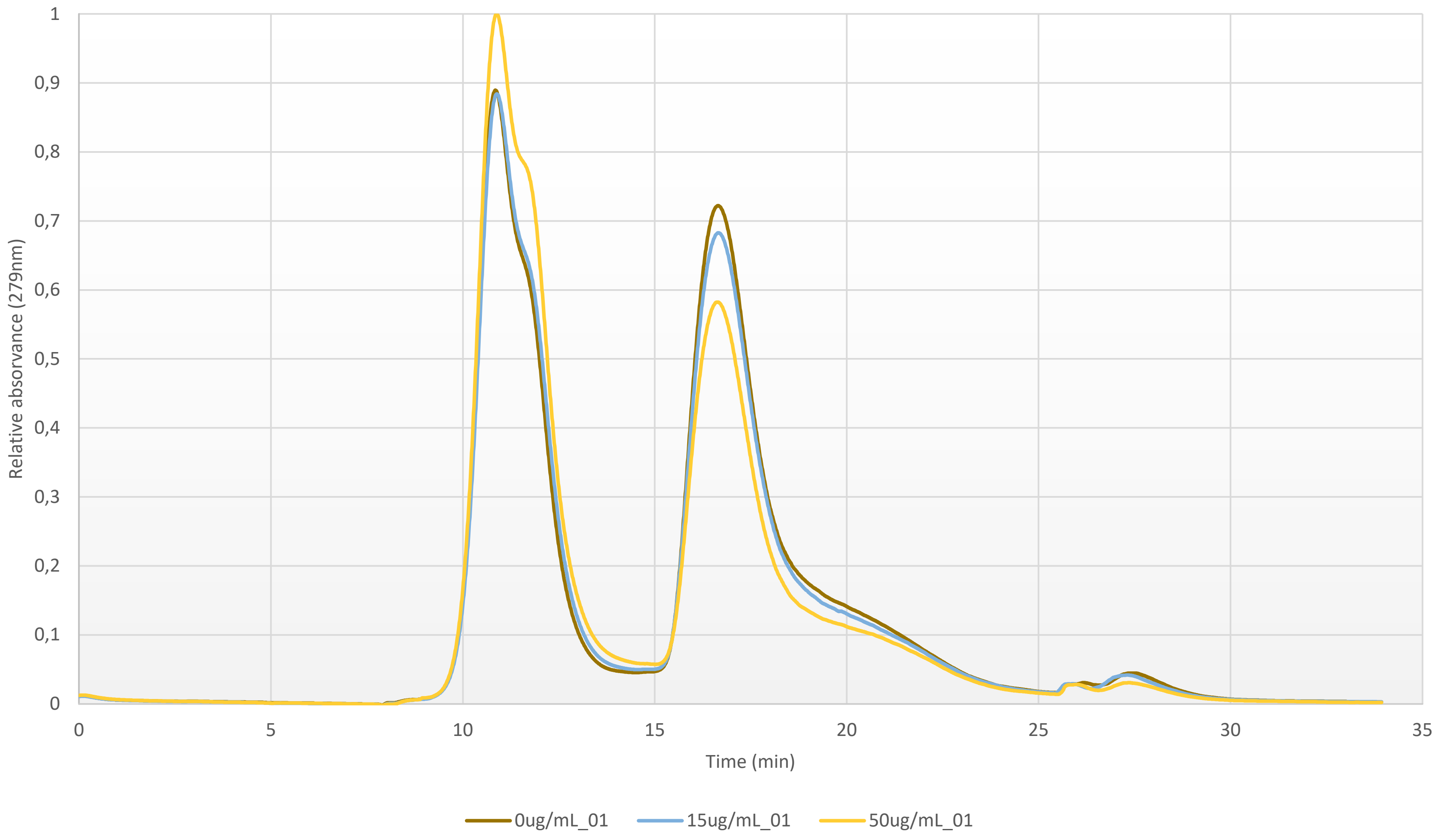


F3

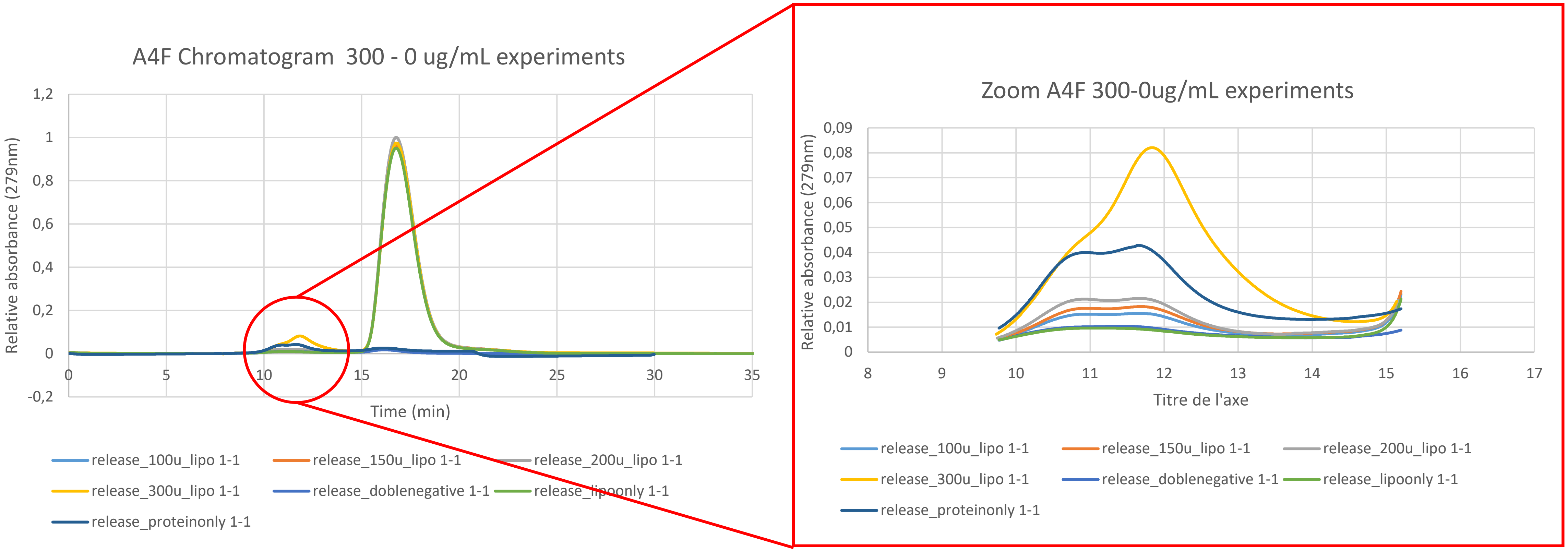
Analytic problem with DNAse/DOTAP

Asymmetrical flow field-flow fractionation (AF4): for release

DNAse + liposomes physical mixture

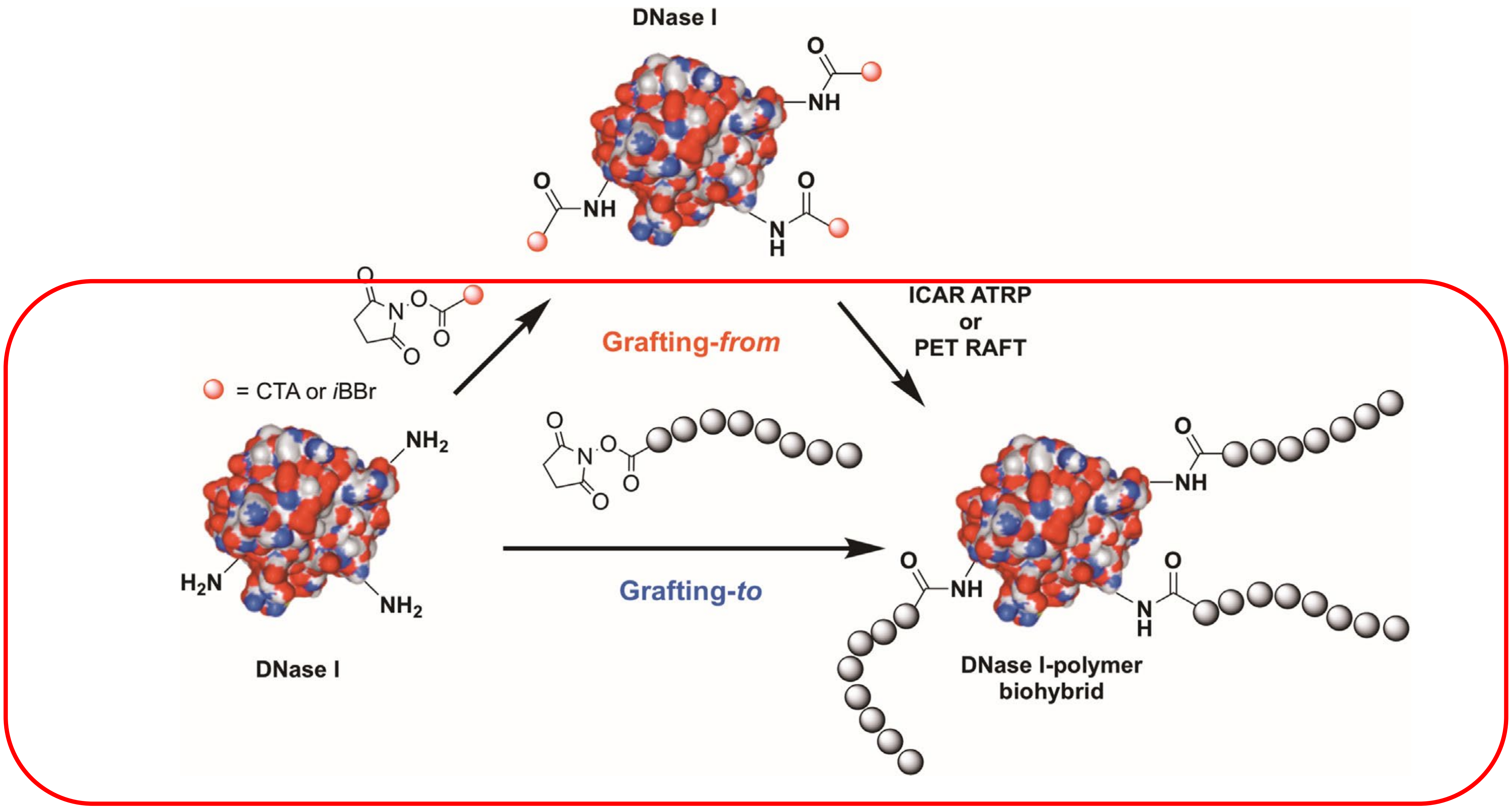


Asymmetrical flow field-flow fractionation (AF4): for release

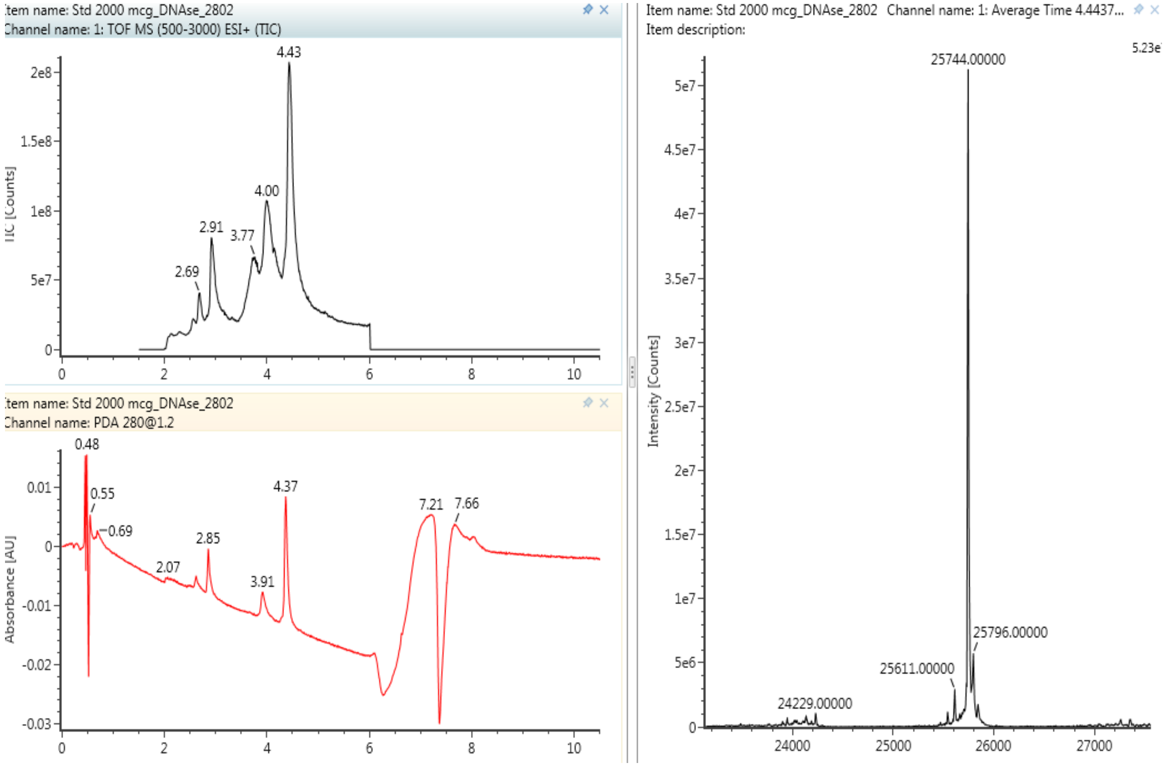


Good resolution; low sensitivity

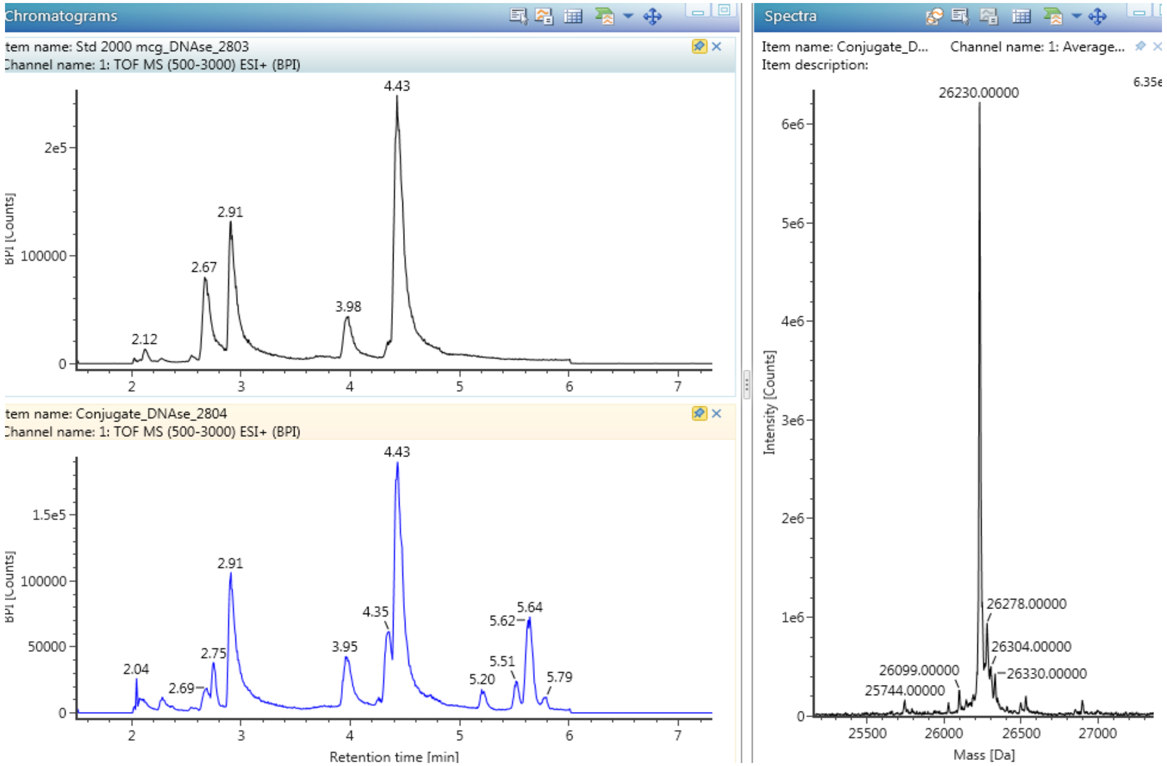
DNAse Hydrophobization



DNAse Hydrophobization: C18 chains



Masses 25744 Da



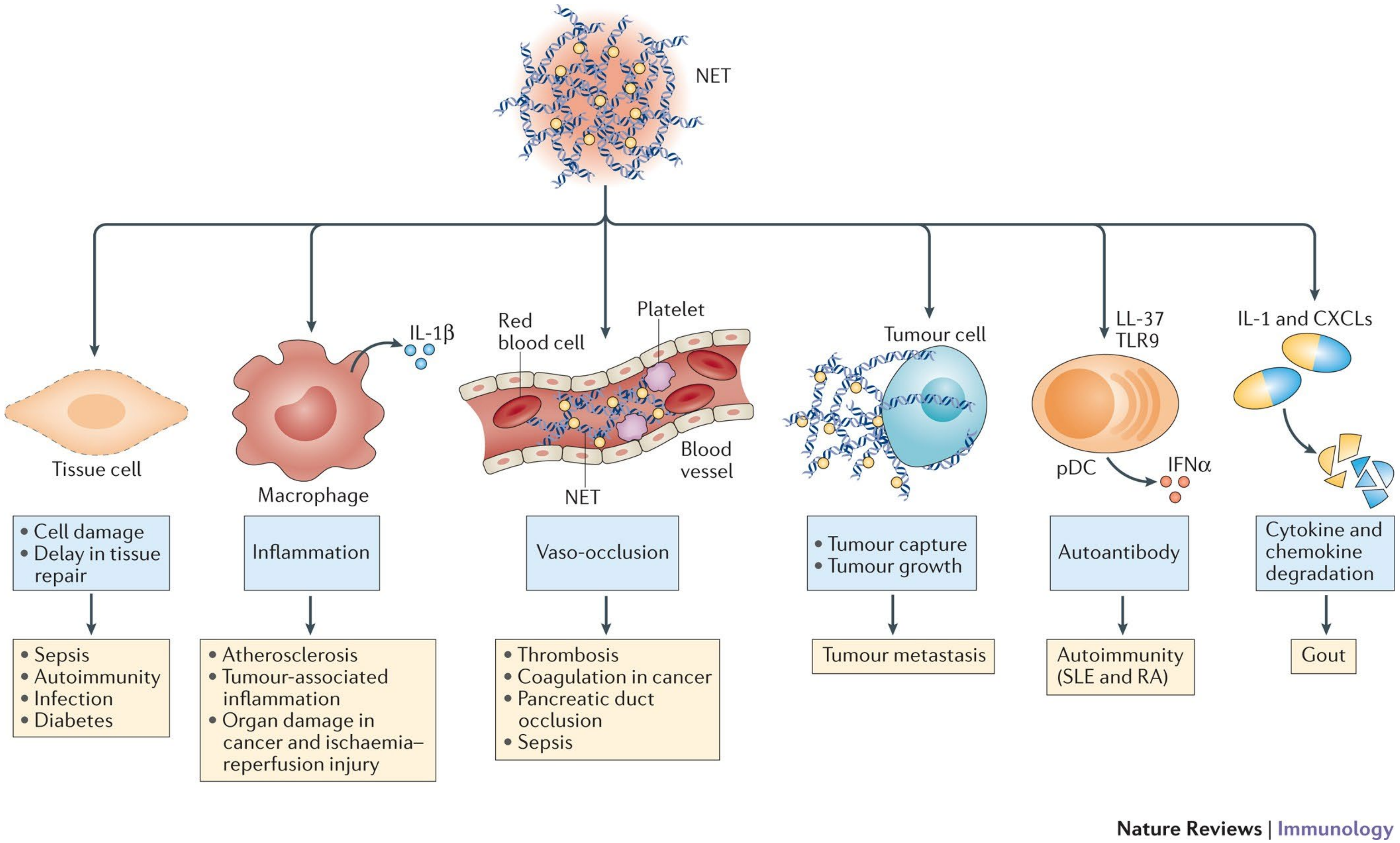
Masse derive 26230 Da

(+ 486 Da / 3 = 3 x 162 Da)

Next months

- Complete characyerization of a few formulations
- Complete release of lead formulation
- In vitro activity (NETs) of lead formulation
- Write publication on formulation optimization
- Move on: overall low loading; DNAse makes everything complex/expensive

NETs updates



NETs and Covid19

JCI The Journal of Clinical Investigation

Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis

Panagiotis Skendros, … , John D. Lambris, Konstantinos Ritis

blood

American Society of Hematology
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Washington, DC 20036
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editorial@hematology.org

Neutrophil Extracellular Traps (NETs) Contribute to Immunothrombosis in COVID-19 Acute Respiratory Distress Syndrome

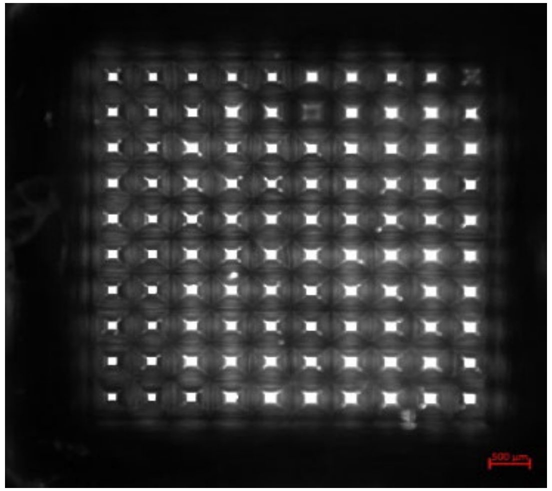
Table 1: Summary of trials using Pulmozyme (DNAse I) in COVID-19-positive patients

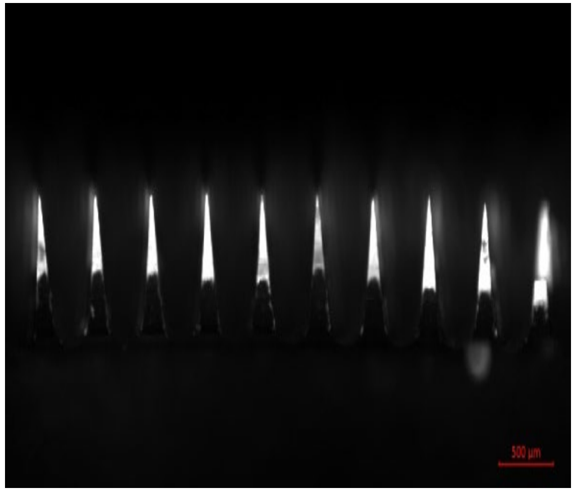
Trial name and location	Study design	Primary endpoints	Estimated primary completion
NCT04402944 Boston Children's Hospital, Boston, USA	60 participants, intubated and mechanically ventilated Treated vs. placebo Dose: 2.5 mg inhaled BID x up to 28 days	Ventilator-free days at 28 days	End of May 2021
NCT04432987 Acibadem Univeristy, Istanbul, Turkey	60 participants (a) newly diagnosed (b) monitored by mechanical ventilation) Treated vs. control Dose: 2.5 mg inhaled BID x 7 days	Response to treatment (patient complaints, blood inflammatory markers, intubation for group a, extubation for group b)	End of August 2020
NCT04445285 DAMPENCOVID University of South Alabama, Alabama, USA	44 participants on high-flow oxygen or mechanical ventilation Treated vs. placebo Dose: 2.5 mg inhaled daily x 5 days	Mortality at 28 days Systemic therapeutic response	End of July 2020
NCT04387786 DACOVID Feinstein Institute of Medical Research and CHSL, New York, USA	5 participants, mechanically ventilated Case series Dose: 2.5 mg inhaled BID x 25 days	Participants discharged from the ICU Participants who survived COVID-19	April 24, 2020 (*completed*)
NCT04359654 COVASE University College, London, UK	50 participants, on supplemental oxygen Treated vs. control (best available care)	Change in inflammation (CRP)	Early August 2020

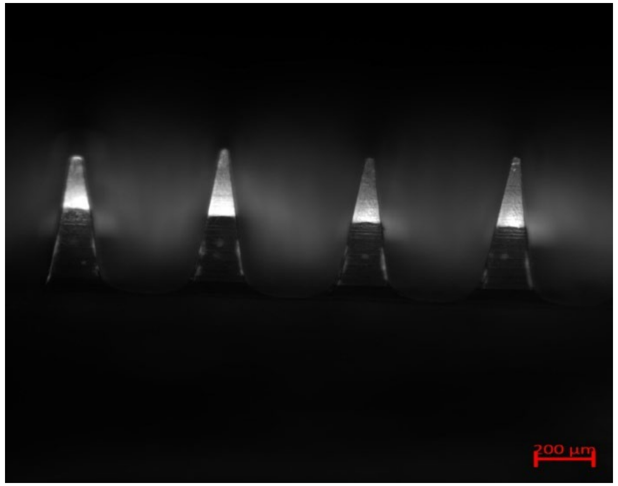
	Dose: 2.5 mg inhaled BID x 7 days		
NCT04355364 COVIDORNASE Hopital Fondation Ophtalmologique Adolphe de Rothschild, Strasbourg, France	100 participants, intubated and on mechanical ventilation in the ICU Treated vs. control Dose: 2.5 mg inhaled BID x 7 days	Efficacy of intratracheal administration: occurrence of at least one grade improvement	End of August 2020
NCT04402970 DORNASESARS2 University of Missouri-Columbia, Missouri, USA	20 participants, mechanically ventilated Treated vs. standard Dose: 2.5 mg inhaled BID x 3 days	Improvement in PaO2/FiO2	May 31, 2021
RO-IIS-2020-20631 Brazil	60 participants, ventilatory support Dose: 2.5 mg inhaled daily x 6 days	Need and duration of non-invasive and invasive ventilatory support	N/A
EudraCT 2020-001849-39 NETS-C-19 Lund, Sweden	100 participants, on supplemental oxygen Dose: 1mg/mL inhaled x 28 days	Time (days) until the study patient has an oxygen saturation of >93% without supplemental oxygen for 24 hours or until the patient is discharged from the hospital	N/A

Results

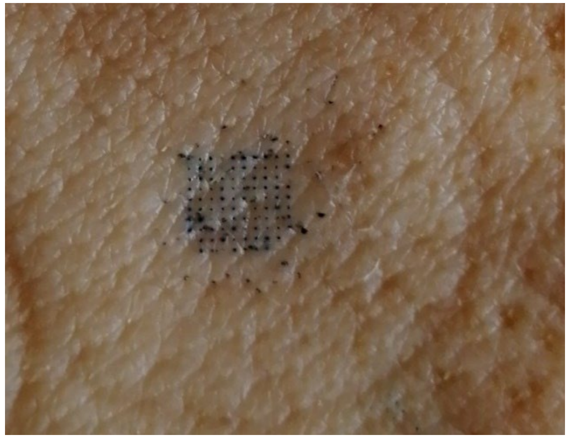
1. Morphological characterization



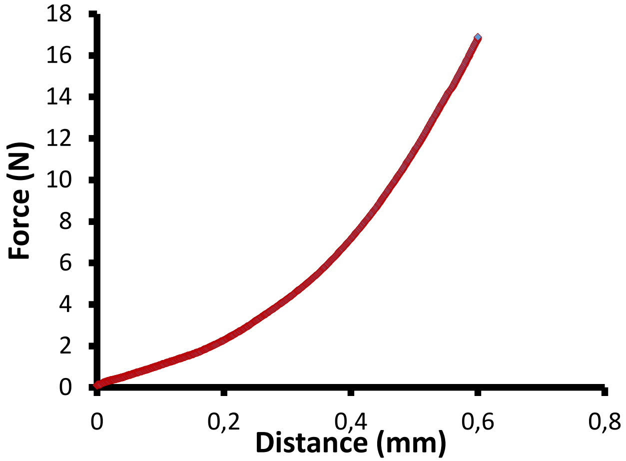




2. Insertion capability



3. Mechanical properties



Lipid particles loaded with mRNA coding for peptides anti PCSK9

Proprotein convertase subtilisin/kexin type 9, involved in coronary artery diseases (Pr. Gaetan Mayer)

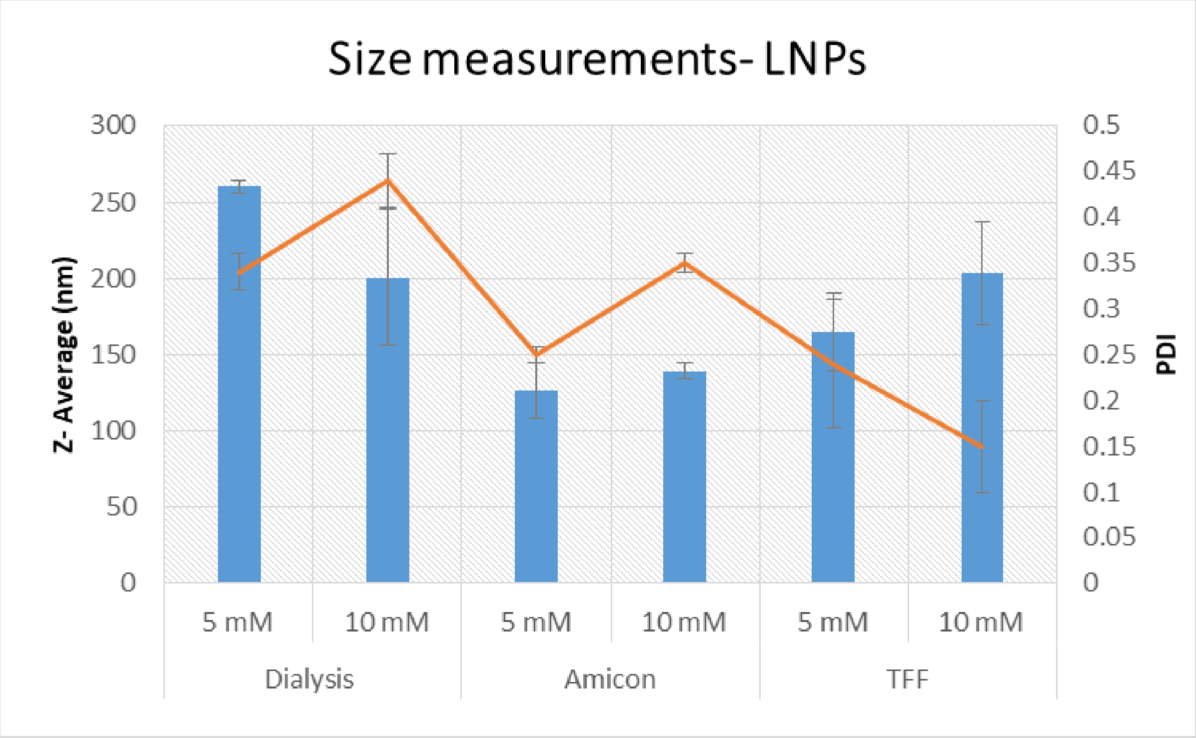
Compare with CSL3 (Prof. Leblond) as ionisable lipod

DODMA: DOPE: Cholesterol: DSPE-PEG2000, molar ratio of 50: 10: 38.5: 1.5

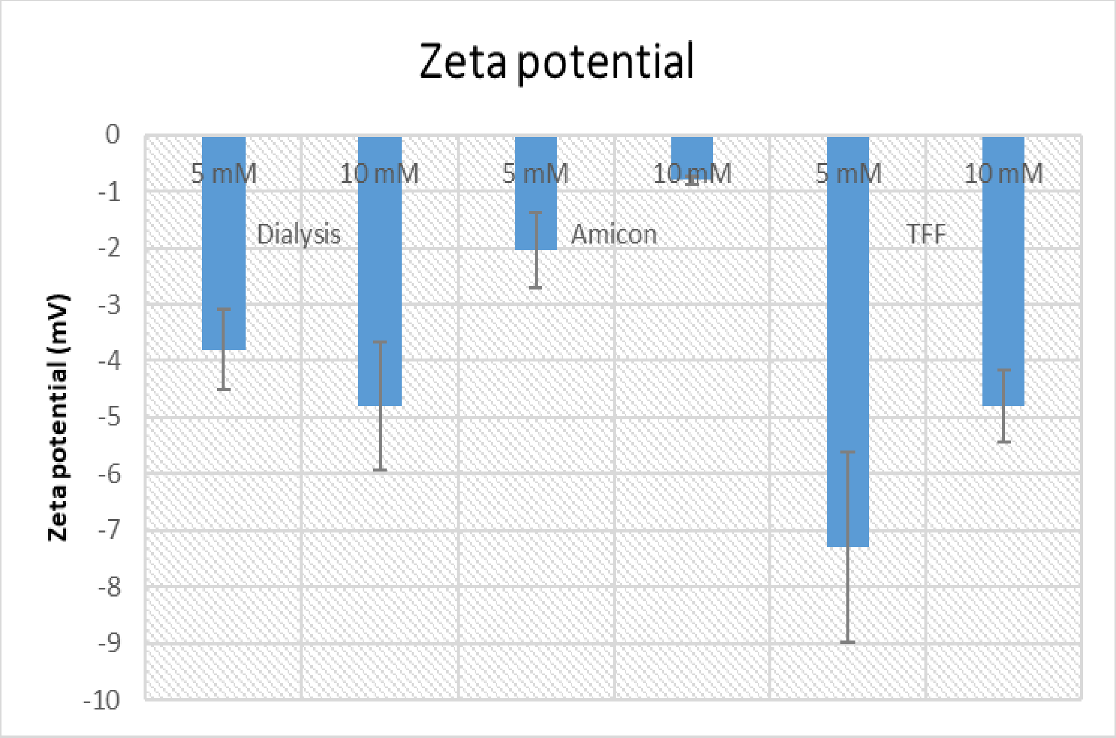
*N/p ratio 4 (pDNA GFP)

*working conditions: Total flow rate: 12 ml/min; Flow ratio: 3:1 aqueous to organic

1. Particle size and polydispersity



2. Zeta potential



Project mostly funded by RQRM

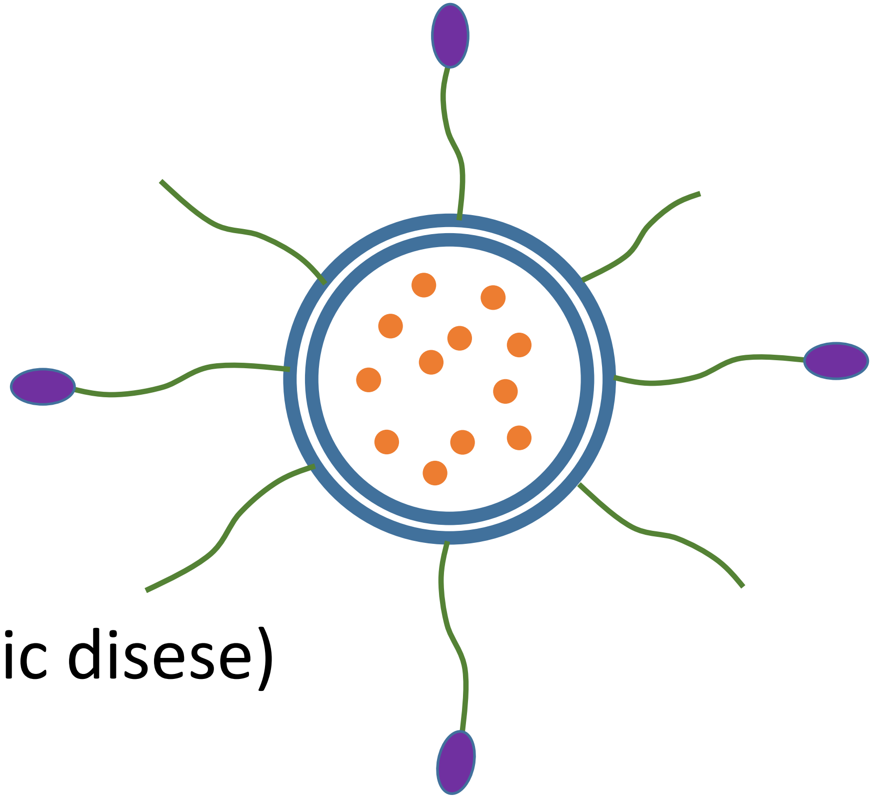
Next year

- **Nastaran** (NETs-targeting PAD4 Inhibitors-loaded nanoparticle)
- **Matthias** (mRNA/metabolic enhancer loaded lipid particles)
- **Naghme** Superabsorbent polymeric microneedles for ISF samples for heart failure monitoring
- **Alfonso/Vivienne** (upconverting NPs for light-induced release of drugs)

- **Ahmed** (biocompatibility of new dental biomaterials, McGill)

Projects for 2020-2021

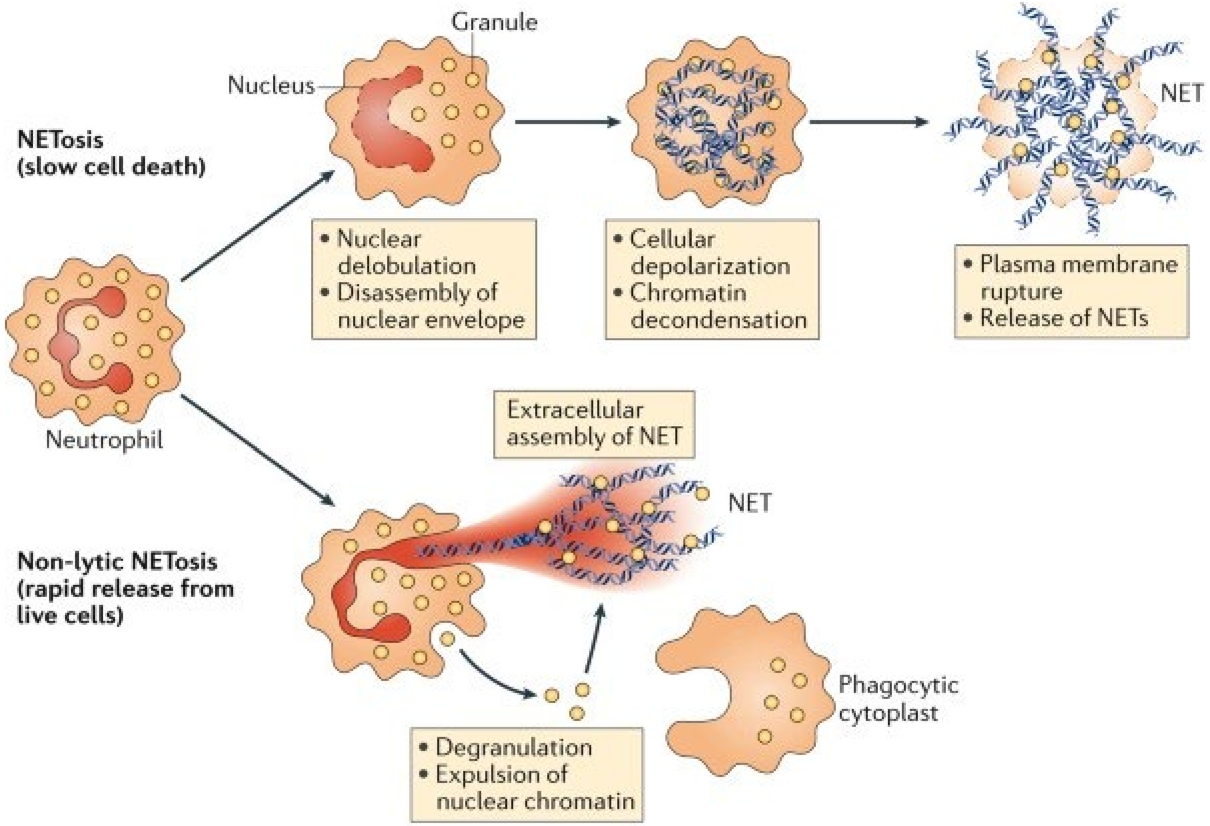
- PAD inhibitors into liposomes



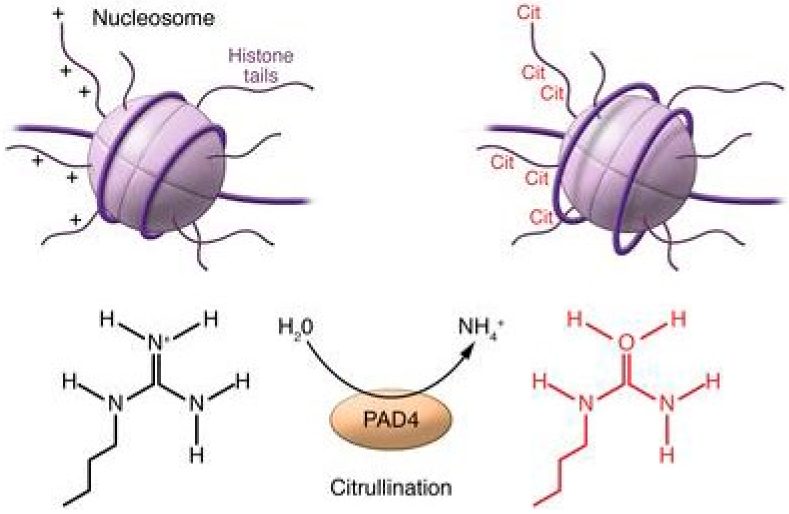
- mRNA and metabolism enhancers (rare genetic disese)

PAD4 inhibitors

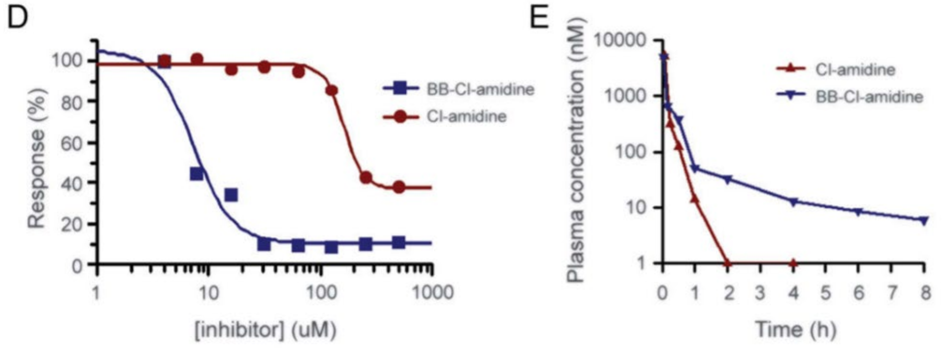
Protein-arginine deiminase type 4 (**PAD4**): nuclear enzyme that citrullinates arginine residues, converts amine groups to ketones
Generation of anti-citrullinated protein antibodies (ACPAs) in several autoimmune diseases

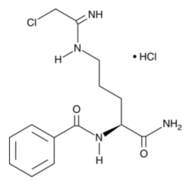


Nature Reviews | Immunology

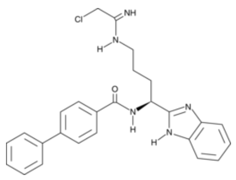


PAD inhibitors loaded liposomes





CL-amidine



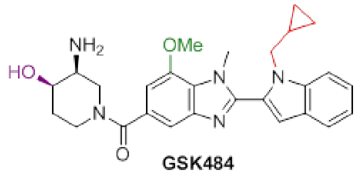
BB CL-amidine

- Loading optimization
- Functionality in vitro (NETs)

2 Year:

- PK
- Activity

- Improve PK
- Accumulate in inflammed sites (NETs)



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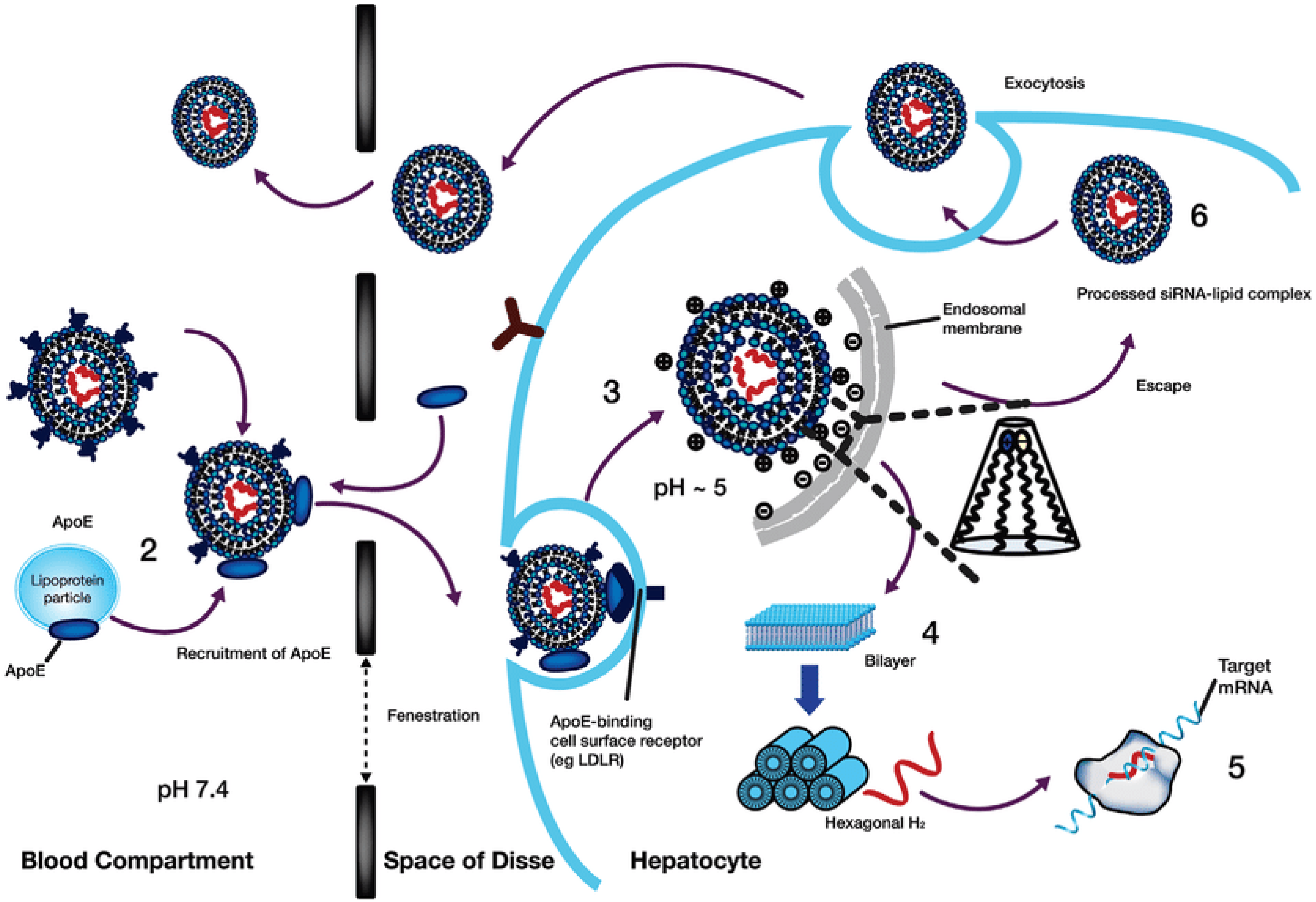
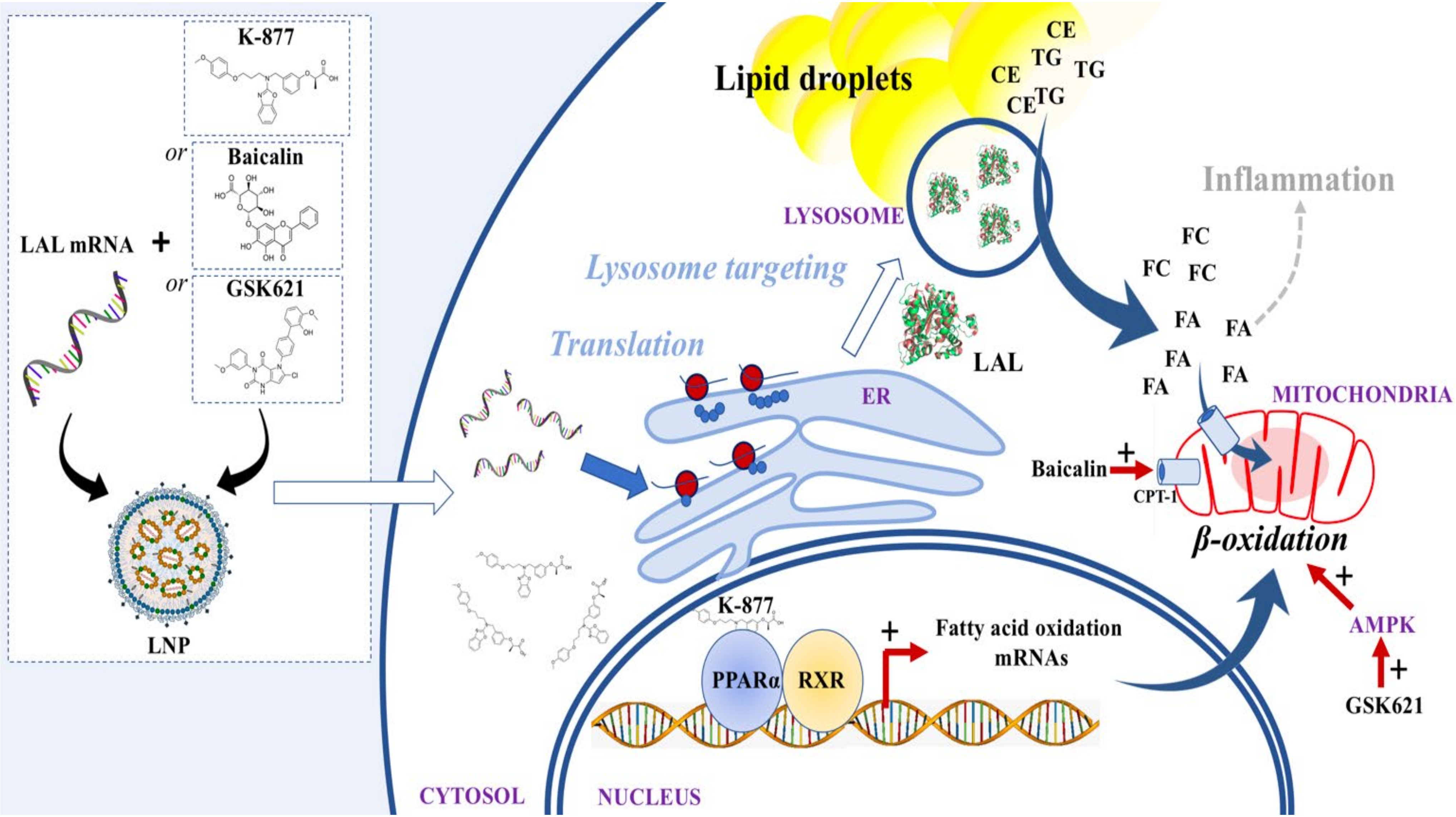
Squalene-based multidrug nanoparticles for improved mitigation of uncontrolled inflammation

Flavio Dormont[1], Romain Brusini[1], Catherine Cailleau[1], Franceline Reynaud[1,2], Arnaud Peramo[1], Amandine Gendron[1], Julie Mougin[1], Françoise Gaudin[3,4], Mariana Varna[1], Patrick Couvreur[1]*

Lysosomal acid lipase (LAL) mRNA and metabolism enhancers



Schematic representation



Project steps

- Loading of mRNA optimization (Opattro formulation, **DMPG-PEG**)
- Internatilazion/transfection in HepG2 cells (FACS, Co-focal, ELISA)
- Loading of adenosine monophosphate kinase (AMPK) activators (key metabolic enzyme whose activation blocks lipogenesis and promote lipid oxidation)

Following years

- In vitro LAL deficiency generation in HepG2 cells (CRISPR-Cas9) (Pr. Gravel)
- Formulation functionality *in vitro*
- Formulation funcitonality *in vivo*