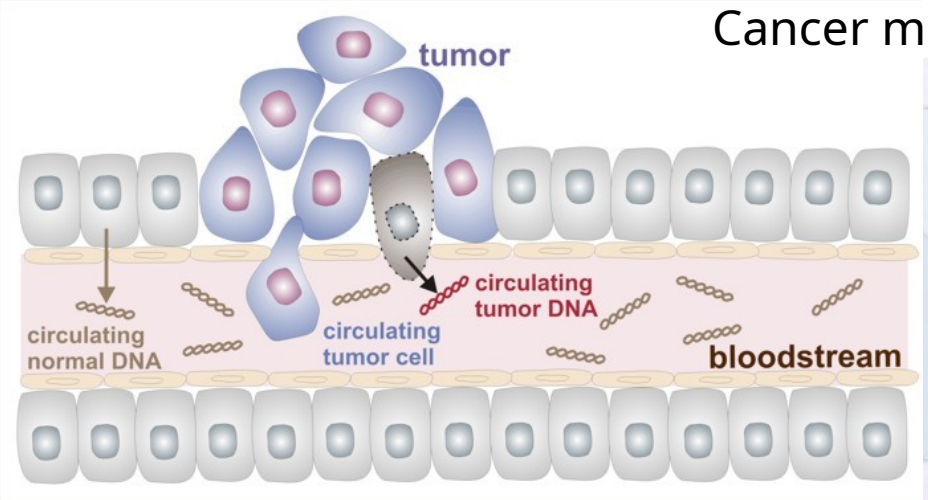
Cell-free DNA-based biomarker search using high-sensitivity sizing with µLAS technology

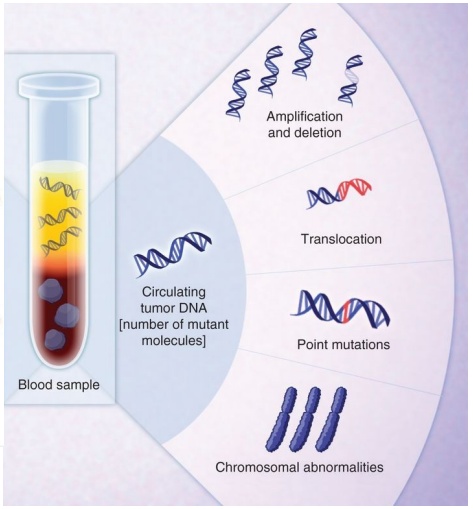
Aurélien BANCAUD (abancaud@laas.fr), Michaël TOUBLANC

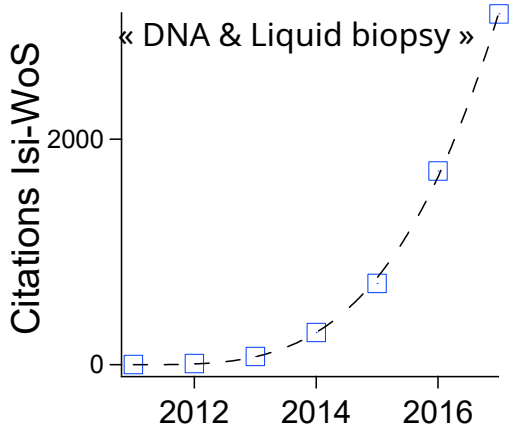
11/06/2025

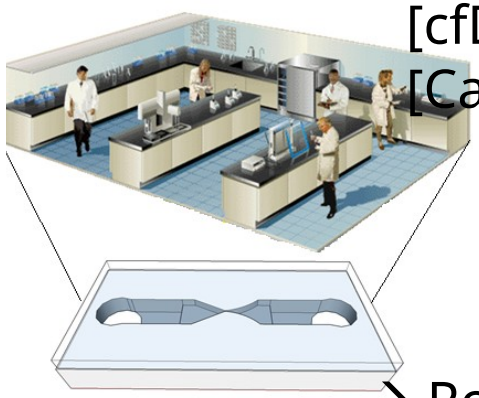
cfDNA as a biomarker in oncology



Cancer mutations and rearrangements







Challenges:

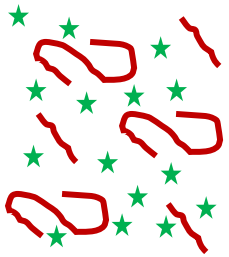
[cfDNA] = 10 – 100 ng/mL (3,000 to 30,000 genomes /mL)

[Cancer mutations] = 30 – 300 copies /mL (~1 attoMolar)

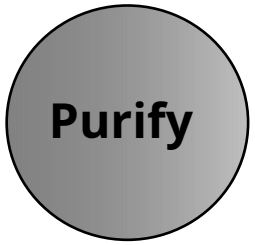
Purify **Concentrate** **Separate** **Identify**

→ Room for technological development for cfDNA analysis

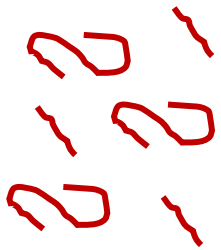
DNA processing from an analytical chemistry point of view



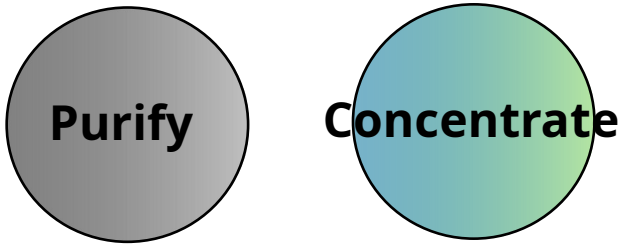
1- purification / dialysis



DNA analysis from an analytical chemistry point of view



2- concentration



DNA analysis from an analytical chemistry point of view



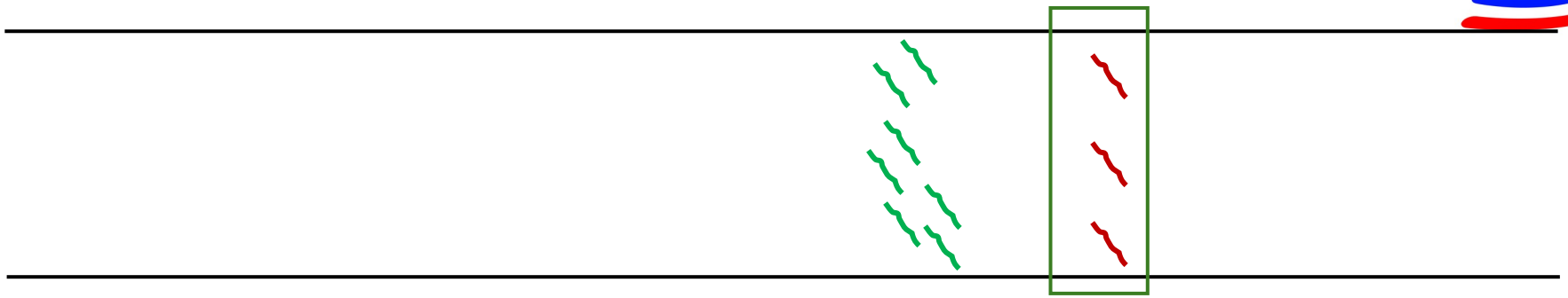
3- separation

Purify

Concentrate

Separate

DNA analysis from an analytical chemistry point of view





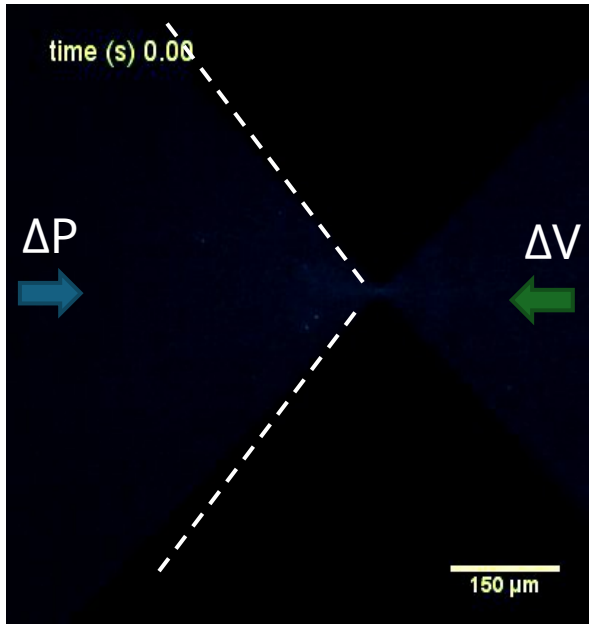
Control of DNA migration is a solution for advanced processing



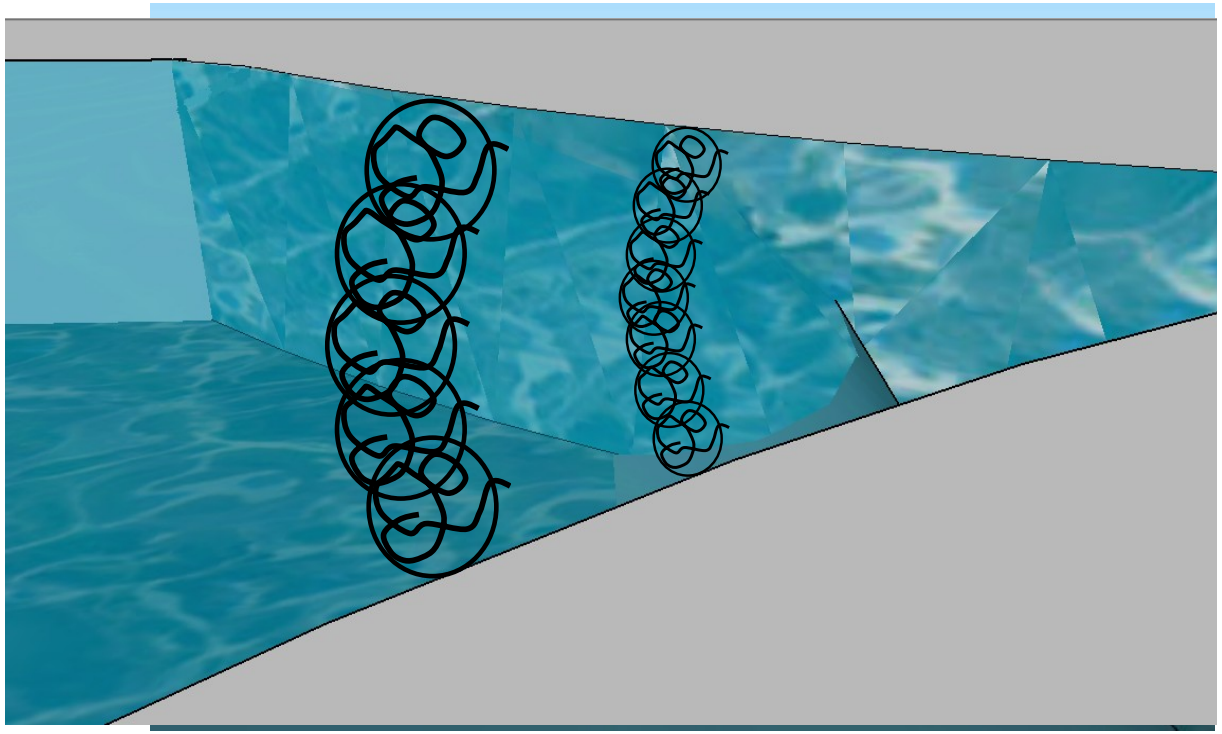
µLAS: micro-reactor for nucleic acids processing

Outline: - µLAS principle and on-line operations
- Application to cell-free DNA

µLAS operation: DNA Separation & concentration



PCT/EP2015/067826
Ranchon, Lab Chip, 2016
Chami, Soft Mat, 2018



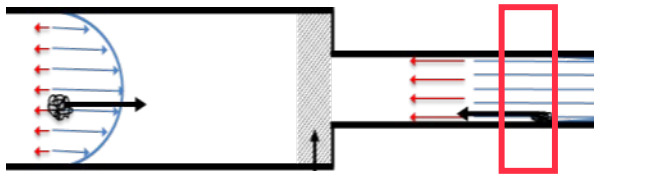
µLAS for target sequence detection

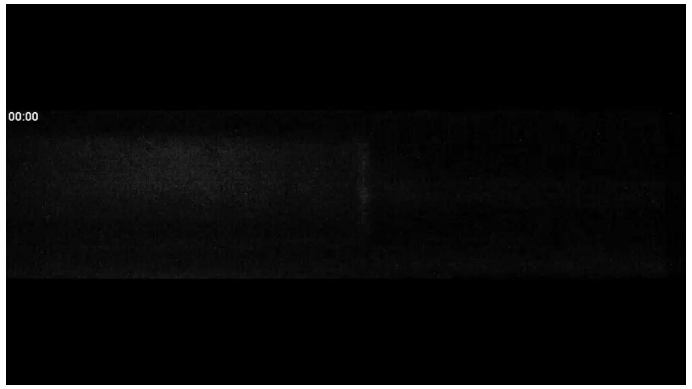
Tijunelyte, *Biosensors & Bioelectronics*, 2021
Tijunelyte, *Biomicrofluidics*, 2022
Teillet, *Biomed Microdevices*, 2025

µLAS for sample fractionation

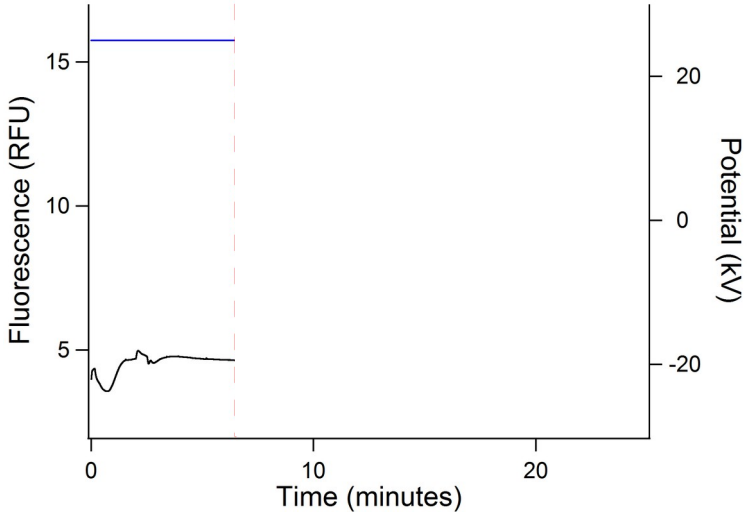
Milon, *Nucl Acid Res*, 2019
Milon, *Lab Chip,* 2020
Bruand, *Anal Chem*, 2023

BIABooster: µLAS in capillary electrophoresis systems









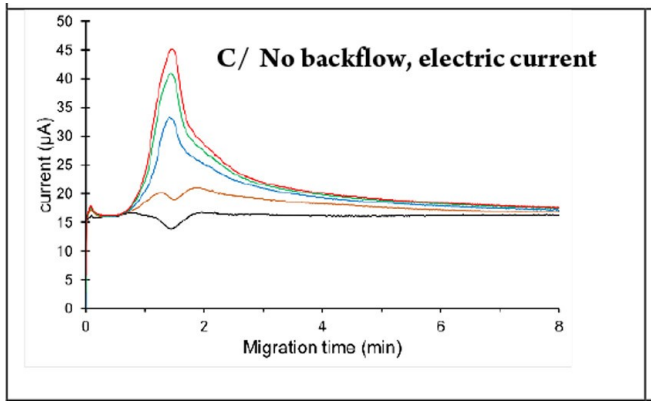
- 0.8 µl injected
- **LOD : 1 fg/µL at 1kb, 500 times more sensitive than current CE**
- Sizing reproducibility : 3 %
- Quantitation accuracy : +/- 10 %

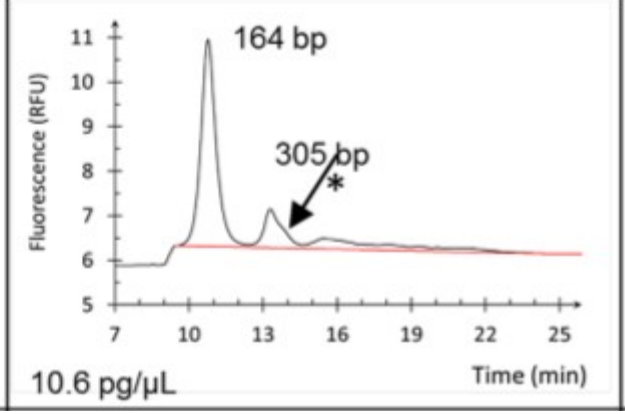
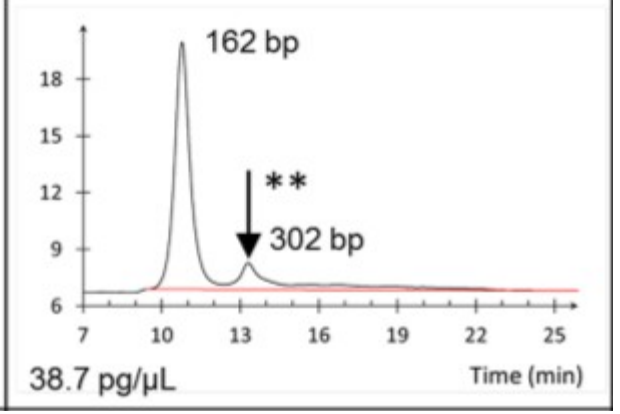
adelis ADVANCED SEPARATIVE TECHNOLOGIES

Andriamanampisoa, *Anal Chem* (2018)

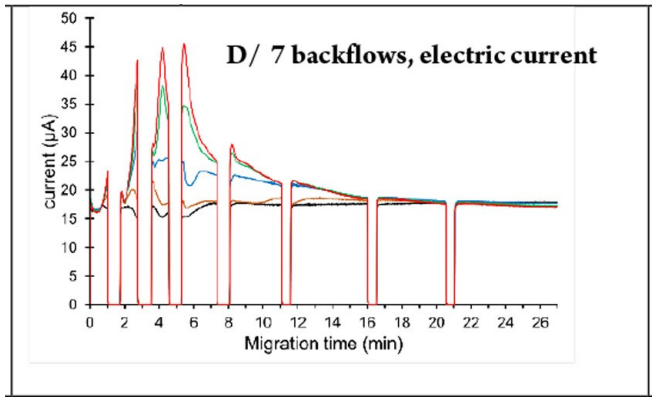
Direct analysis of cfDNA with BIABooster

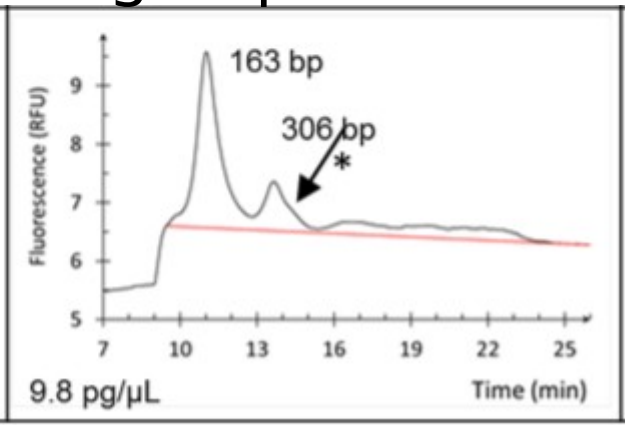
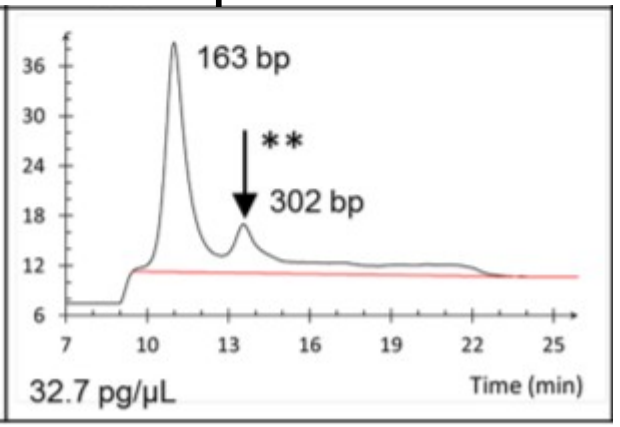
Direct analysis after DNA extraction with glass beads



	Healthy volonteer	Lung cancer patients
Purified cfDNA		

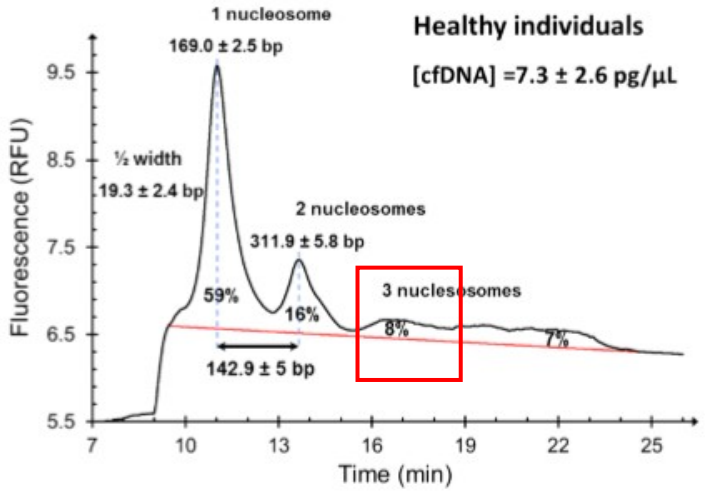
Direct plasma analysis with 7 washing steps to remove salts/proteins

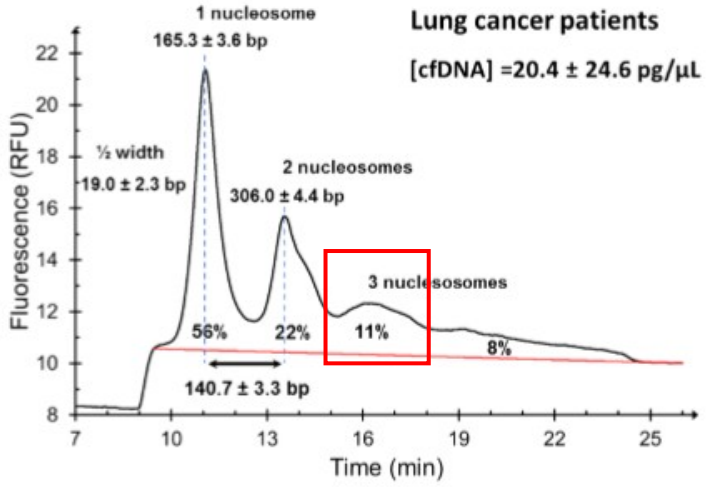


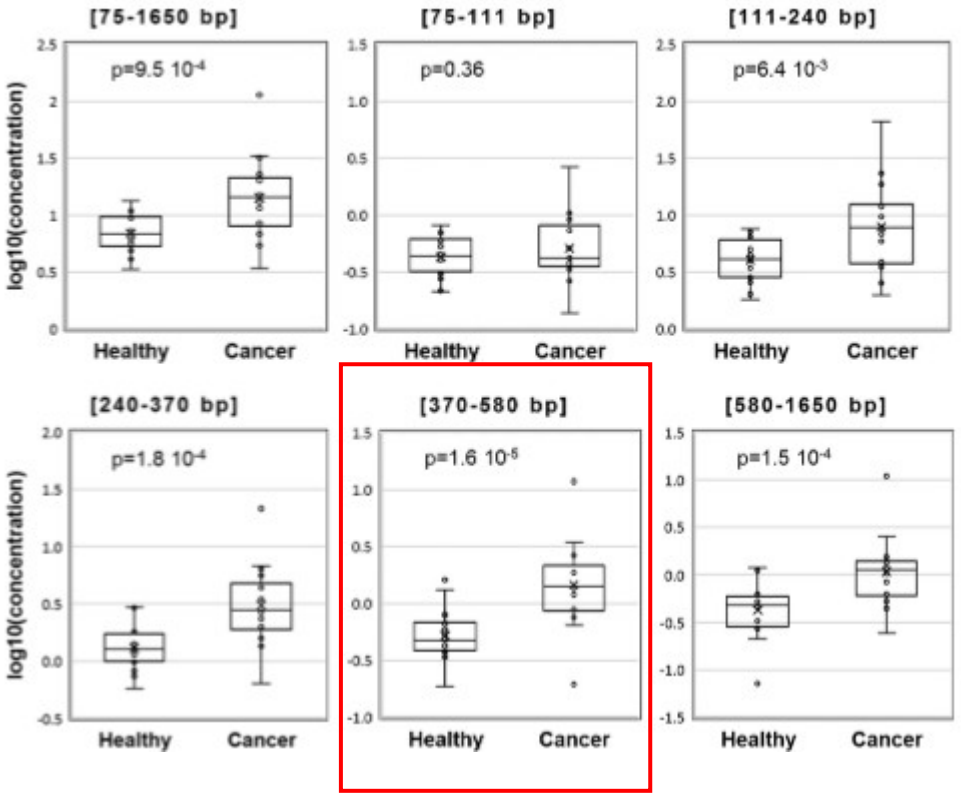
cfDNA in plasma		

Boutonnet, *Anal Chem*, 2023

cfDNA profile as a biomarker: best size range definition



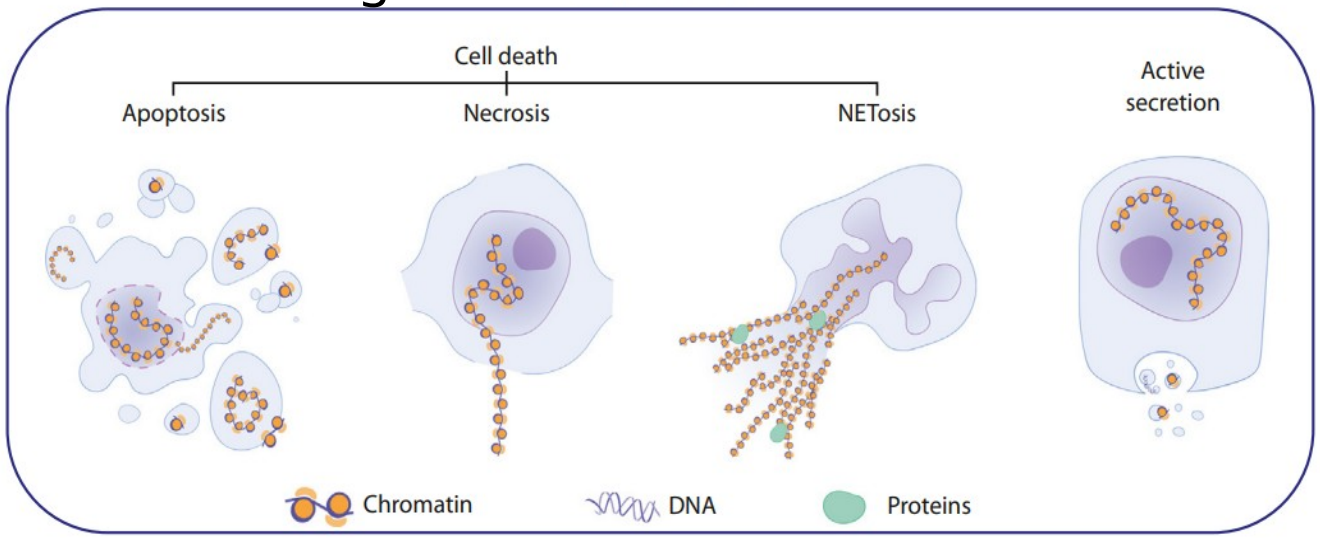




Boutonnet, *Anal Chem*, 2023

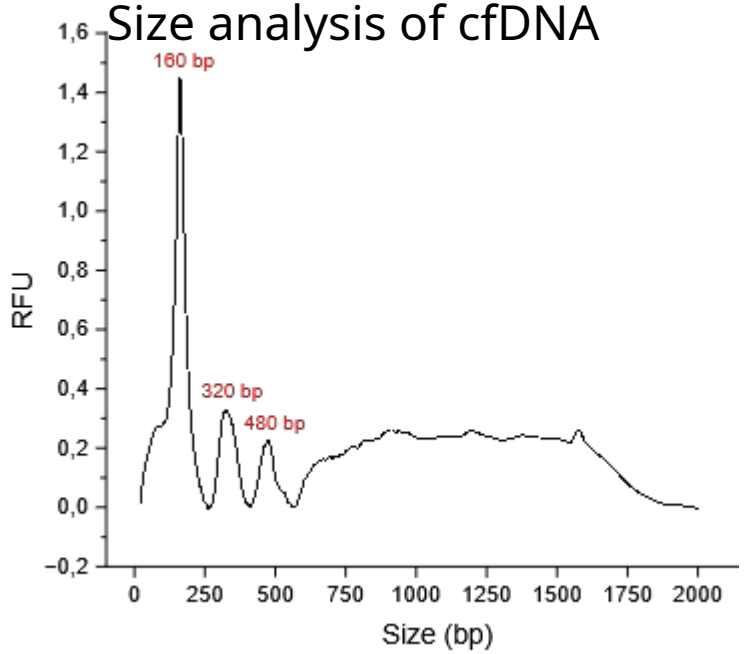
Advancing cfDNA biomarker search: Future directions

Origin and structure of cfDNA



Han, Trends in Genetics, (2021)

Size analysis of cfDNA

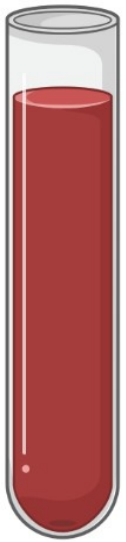


What is the structure of DNA in the blood?

Can this structure be targeted to enhance cfDNA-based diagnostics?

Challenge: Refined cfDNA analysis requires high sensitivity

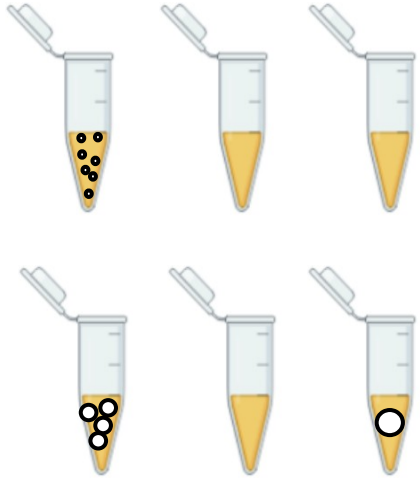
cfDNA screening in plasma : proposed workflow



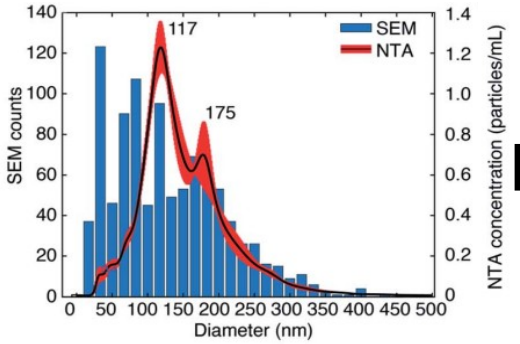
Whole blood



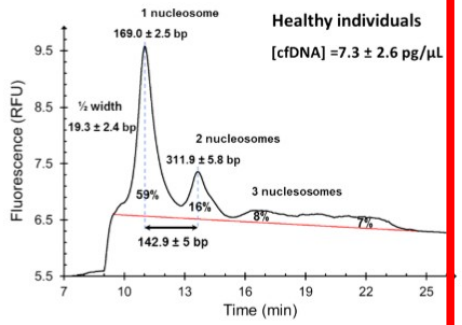
Plasma extraction



Plasma fraction : size/density separation



Size distribution/Proteins quantification



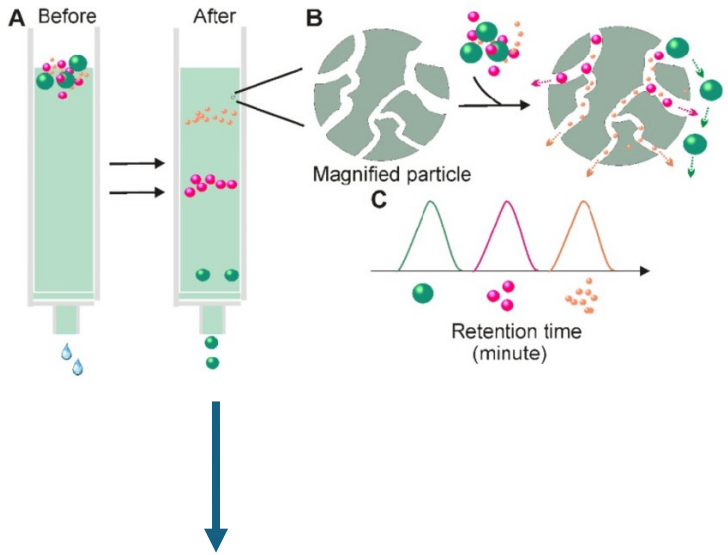
cfDNA analysis with BiaBooster

Anal. Chem. 2023, 95, 9263−9270

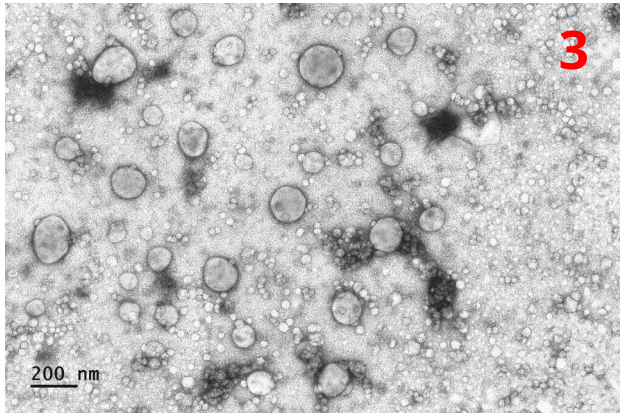
Nanoscale Adv., 2021, 3, 3053

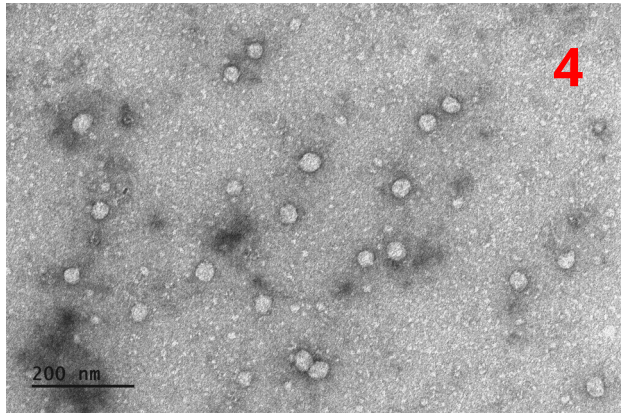
cfDNA after size exclusion chromatography: size matters

Size exclusion principle

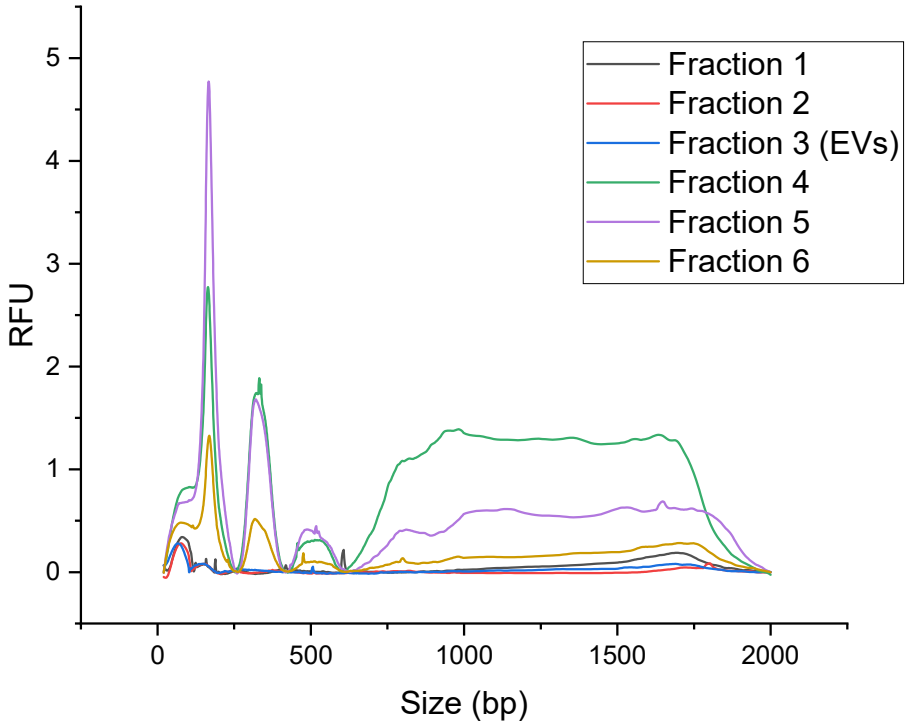


Collect 6 fractions





TEM images (70 keV) of fraction 3 & 4



Size profile of cfDNA after SEC separation

Conclusions and future prospects

1. µLAS: High performance DNA analysis (best performances in commercial instruments)
2. µLAS: Unique potential for in line purification and analysis
3. Agnostic cfDNA analysis seems to contain physiological readout, but not sufficiently discriminant

1. Exploiting purification technologies to target specific particles in plasma
2. Size, density, surface antibodies can be used to enhance diagnostics
3. Changes in healthy individuals and cancer patients?