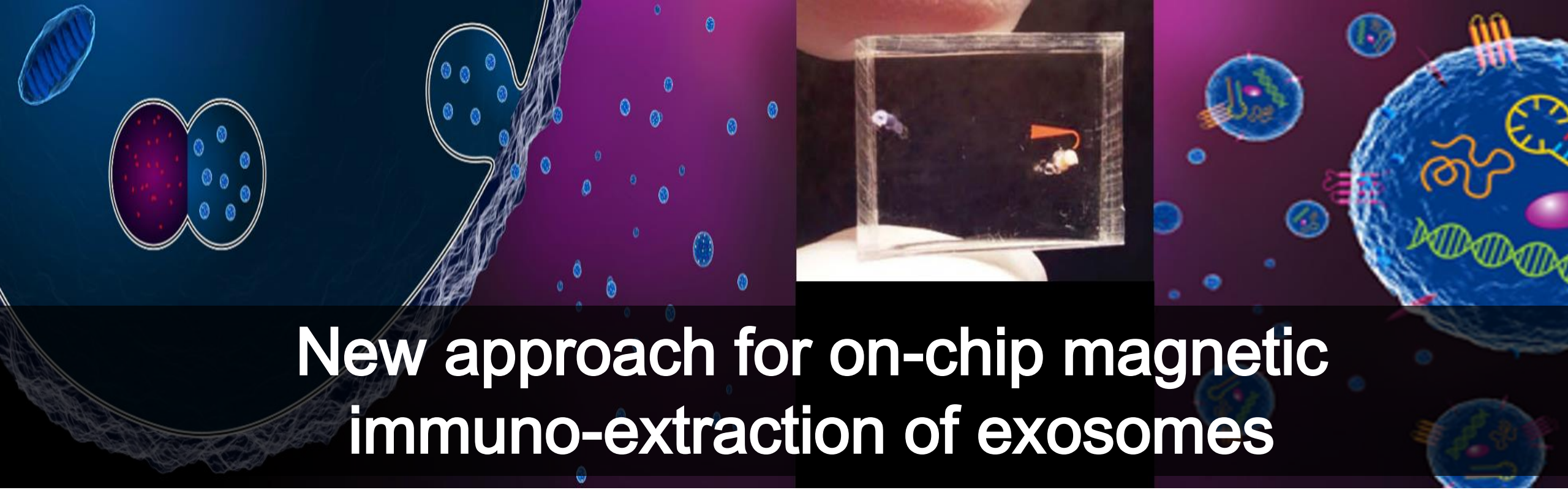


New approach for on-chip magnetic immuno-extraction of exosomes

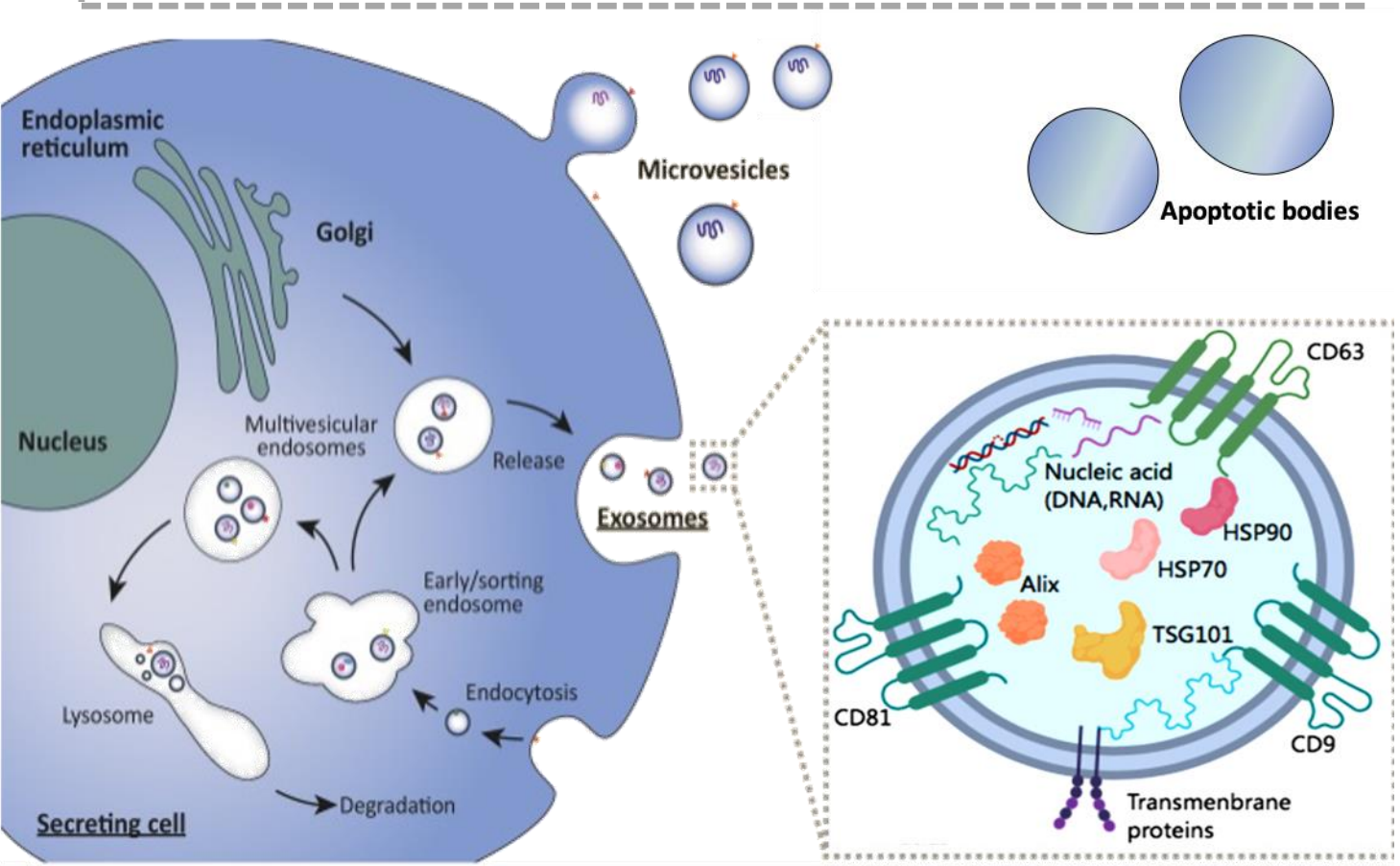
Monica Araya-Farias*, Dario Brambilla, Lucile Alexandre, Laura Trapiella-Alfonso, Giacomo Gropplero, Marine Verhursel, William Cesar, Marina Cretich, Marcella Chiari, Stéphanie Descroix

*Macromolecules and Microsystems in Biology and Medicine (MMBM)
Institut Curie – Research Center - UMR 168
Institut Pierre Gilles de Gennes pour la Microfluidique (IPGG)
Paris, France

September 27-30, 2020
Virtual edition
e-MSB 2020
36th International Symposium on Microscale Separations and Bioanalysis

Extracellular Vesicles (EVs)

Cells	Apoptotic bodies	Microvesicles	Exosomes
5-10 µm	1-5µm	100nm - 1µm	40-160 nm



Exosome Biogenesis and Molecular Composition

Modified from Holm et al. 2018. Trends in Neuroscience 41(6) 360-372 (doi.org/10.1016/j.tins.2018.03.006: 10.1016)

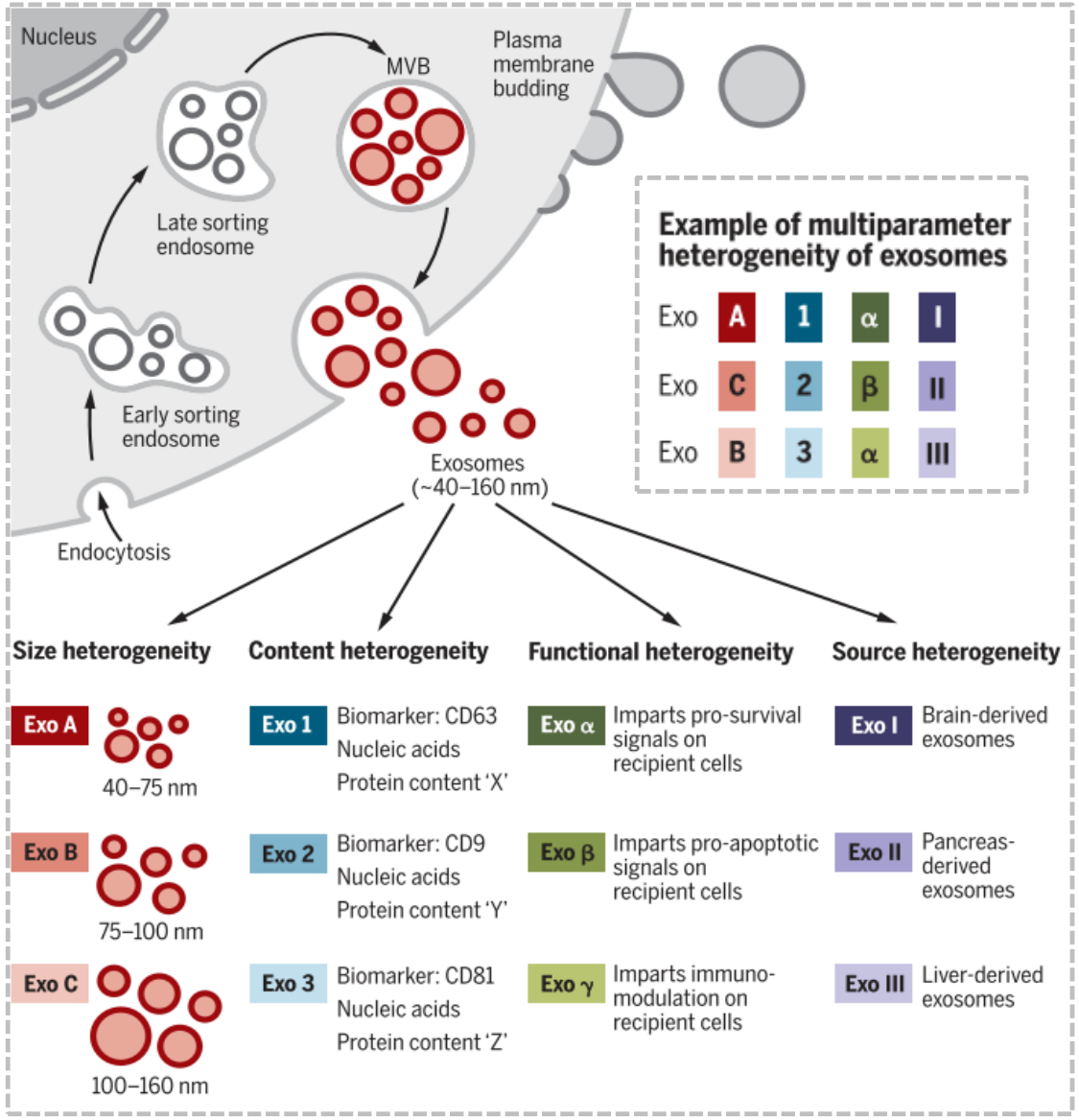
Hallmarks of exosomes

- smallest sized cell-cell communication EV
- circulate in peripheral blood and present in body fluids (plasma, urine, saliva CSF…)
- contain a large quantity of bioactive molecules
- participe in a multiple processes in health and disease
- are the mirror of the original cell

⇩

circulating biomarkers

Analysis of Exosomes : the CHALLENGES



« The small size is an obstacle for a clinical utilization of exosomes »

A single exosome is ~1/1000 times the size of cell

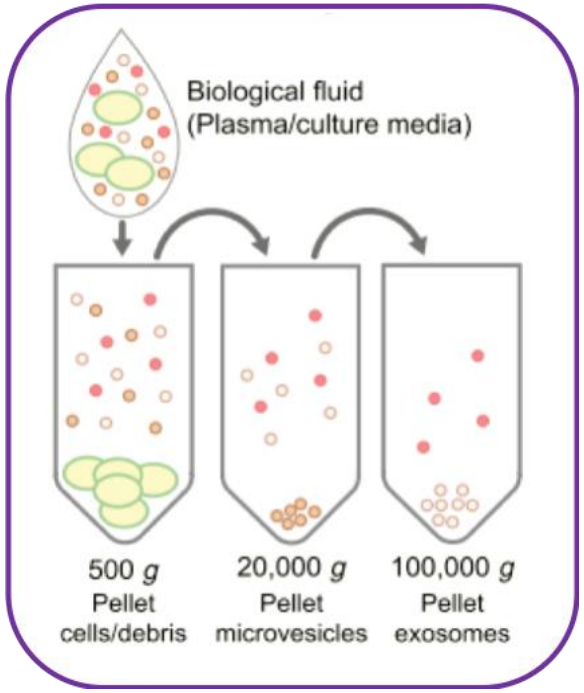
- Heterogenous population with small size
- Sensitivity needed for exosome subpopulations
- Complexity of information to decode
- Different exosome handling and analysis protocols

Modified from Kalluri and LeBlue 2020, Science 367, 1-15 ,M.Cretich, ESWB, 2018,

Conventional EVs isolation methods

A. DENSITY

Ultracentrifugation

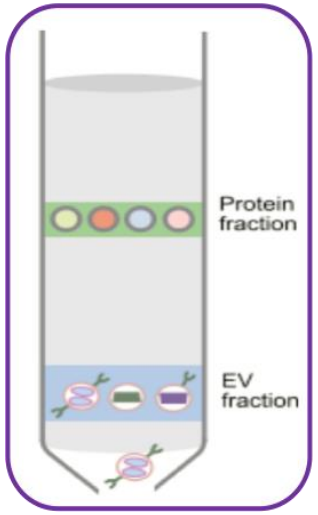


GOLD STANDARD TECNHIQUE

B. SIZE

Size Exclusion

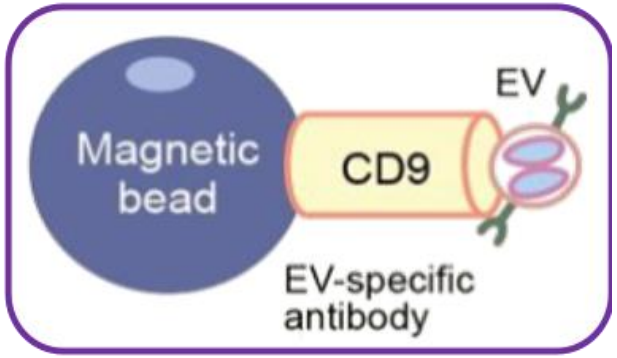
- Filters
- Chromatography



C. AFFINITY

Immunoaffinity isolation

- EV-specific antibodies immobilized on different media (beads, matrices, plates)



Need to develop novel methods and platforms for efficient isolation of EVs

Figures adapted from Akbar et al.,2019. Diabelogia 62(12): 2179-2187

Microfluidic-based technologies

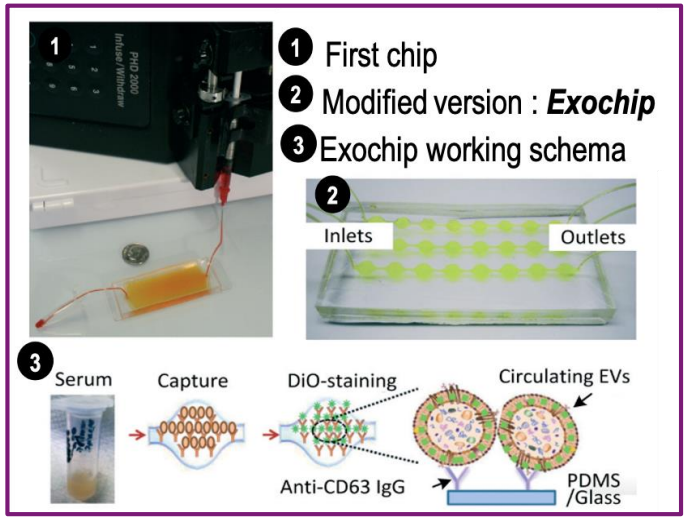
ADVANTAGES

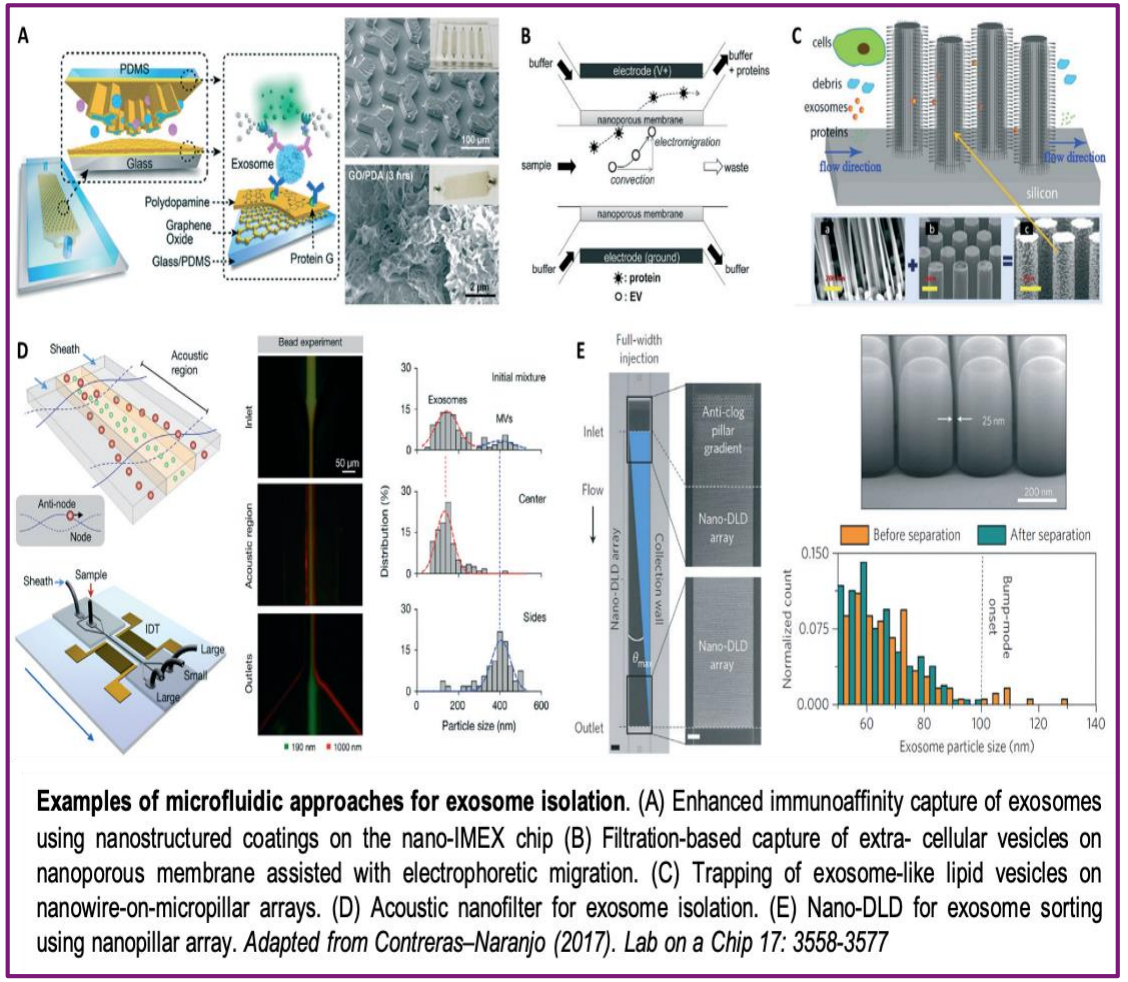
- Cost-effective technologies
- Possibility of automated and multiplexed analysis
- Reduced consumption of samples and reagents
- Faster analysis

Microfluidic strategies for exosome isolation

- Immunoaffinity
- Electroactive separation (Electrophoresis- driven filtration, Electrokinetic concentration isolation)
- Trapping based separation (membrane-based filtration, trapping on nanowires)
- Acoustic isolation (acoustic nanofiltration)
- Continuous- flow sorting (Deterministic Lateral Displacement (DLD), nano-DLD, viscoelastic-based flow sorting)

First microfluidic device for exosome isolation





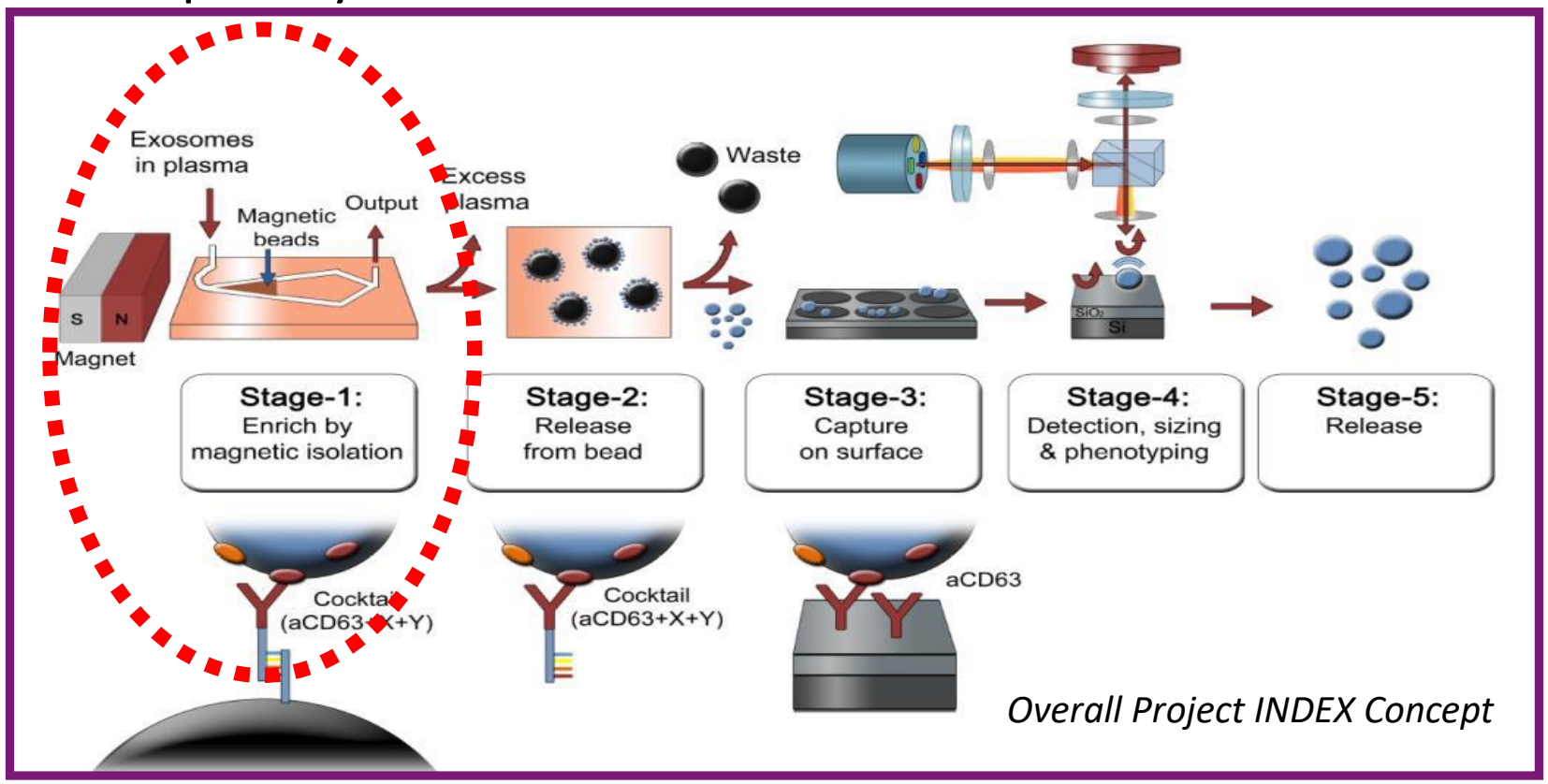
Examples of microfluidic approaches for exosome isolation. (A) Enhanced immunoaffinity capture of exosomes using nanostructured coatings on the nano-IMEX chip (B) Filtration-based capture of extra- cellular vesicles on nanoporous membrane assisted with electrophoretic migration. (C) Trapping of exosome-like lipid vesicles on nanowire-on-micropillar arrays. (D) Acoustic nanofilter for exosome isolation. (E) Nano-DLD for exosome sorting using nanopillar array. *Adapted from Contreras–Naranjo (2017). Lab on a Chip 17: 3558-3577*

« Microfluidics : science and technology of manipulating and controlling fluids in the range of microliters to picoliters »

Integrated nanoparticle isolation and detection system for complete on-chip analysis of Exosomes

Horizon2020 Framework Programme
FETOPEN-2016-2017-Grant 766466

Dr.Marcella Chiari
Project Coordinator
ICRM-CNR, Milan, Italy



Overall Project INDEX Concept

- **REVERSIBLE** Affinity Isolation
- ENRICHMENT
- CONCENTRATION
- PURITY

- **MULTIPARAMETER** Analysis

Count

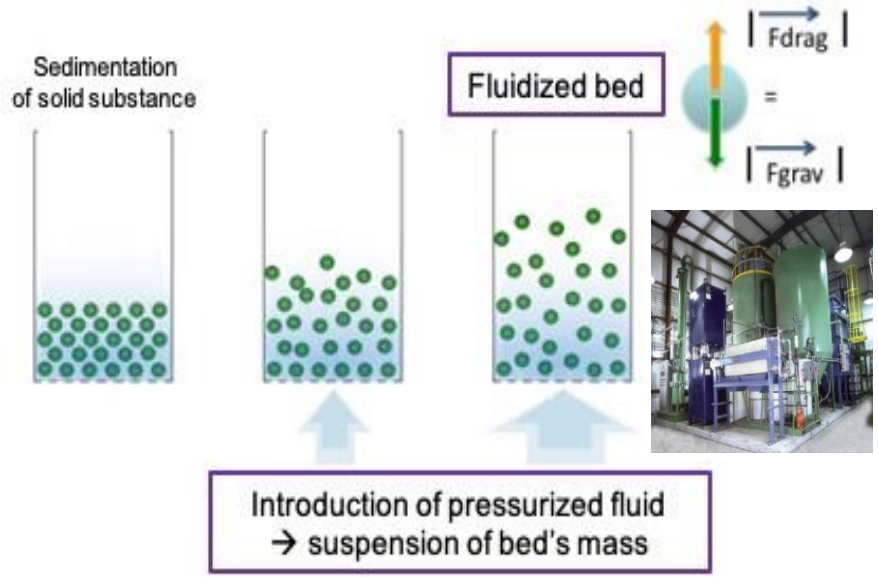
Size

Phenotype

www.indexproject.eu

EVs isolation module : Microfluidic Magnetic Fluidized Bed

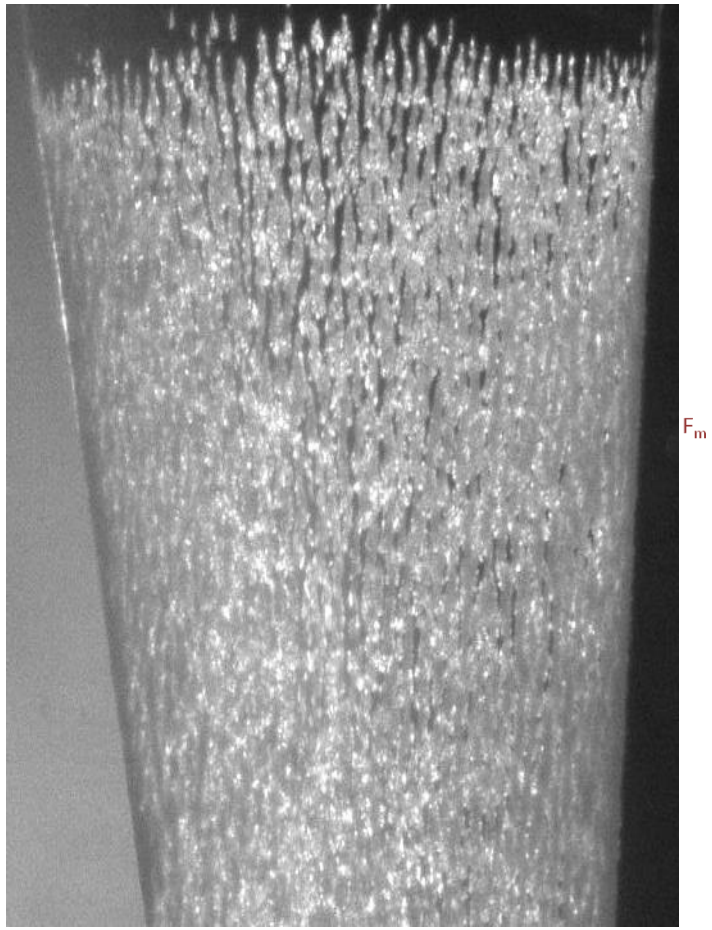
Macroscopic fluidized bed

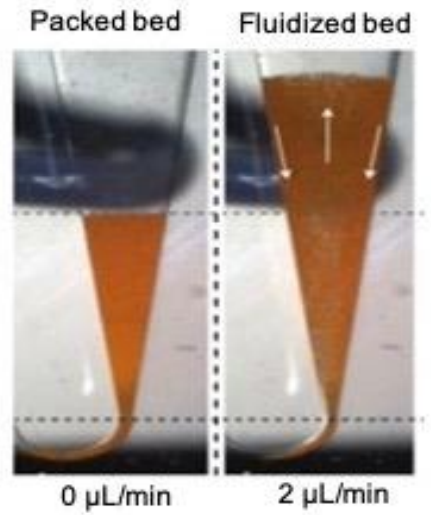


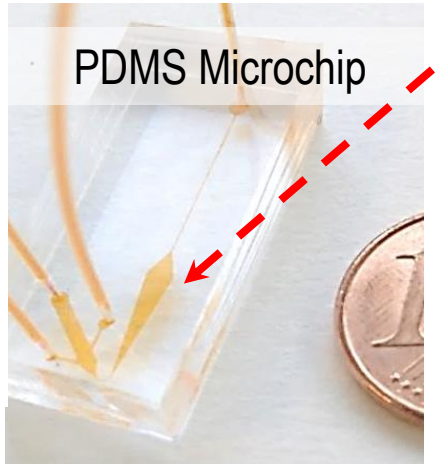
Advantages

- High surface area contact between fluid and solid
- High flow rate
- Continuous and uniform particle mixing

Microscopic fluidized bed







Chamber
Length ~ 2cm
Angle of 13°
Height =
FB 1.0 First generation
50 μm, 50 µg beads

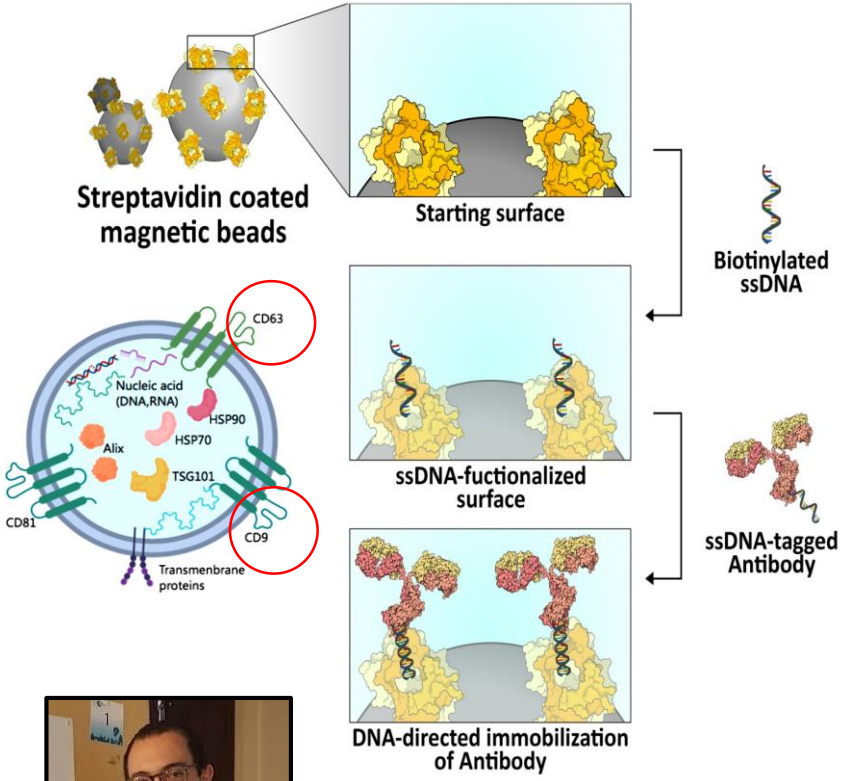
FB 2.0 First generation
250 μm, 250 µg beads

Magnetic beads recirculation !

Pereiro et al. 2017. Lab Chip 17, 1603–1615, *L.Alexandre*, PhD Thesis,2018

Experimental SET-UP

BEADS FUNCTIONALIZATION

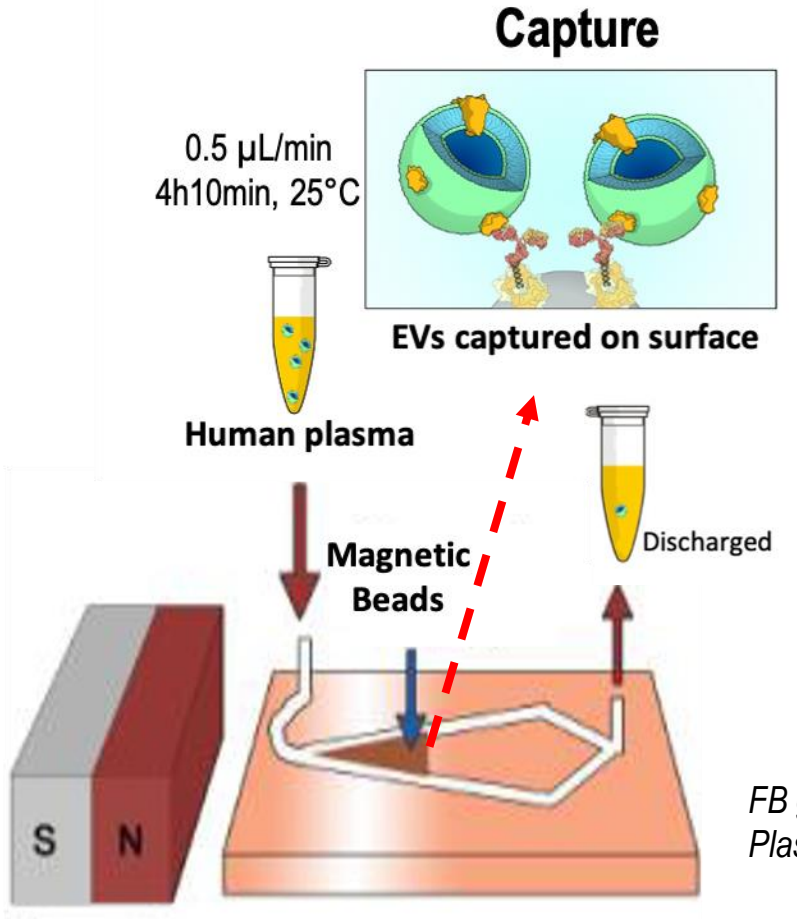


Dario Brambilla (ICRM-CNR, Milan, Italy)

ChemRxiv (doi.org/10.26434/chemrxiv.12234683.v1)

Talanta 222 (2021) 121542 (doi.org/10.1016/j.talanta.2020.121542)

FLUIDIZED BED



ANALYSES

Size distribution, particle concentration and density

- Nano Tracking Analysis (NTA)
- Microaarray platform

Surface protein markers

-Western Blot analysis

-ELISA tests

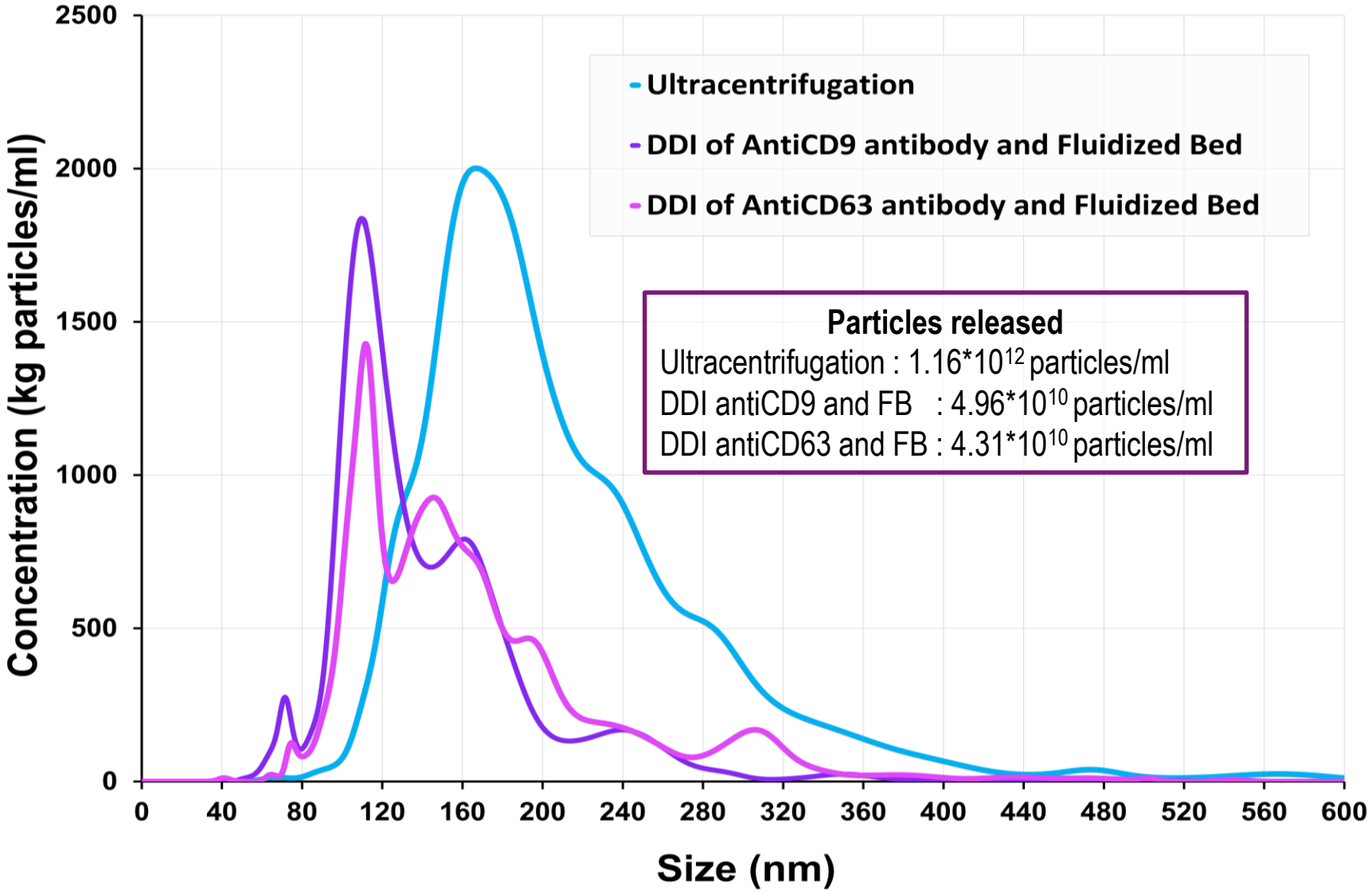
Microscopy Analysis

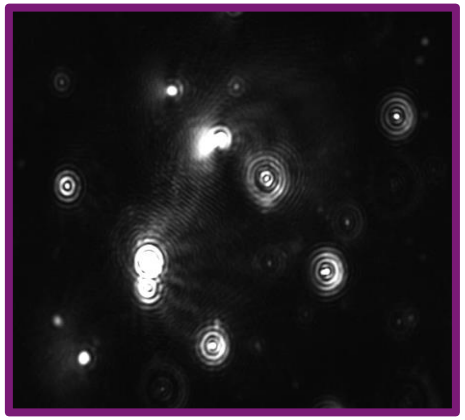
- Transmission Electron Microscopy (TEM)
- Cryo-TEM

FB generation 2.0 : microfluidic device 250 µm height

Plasma from Aalborg University Hospital

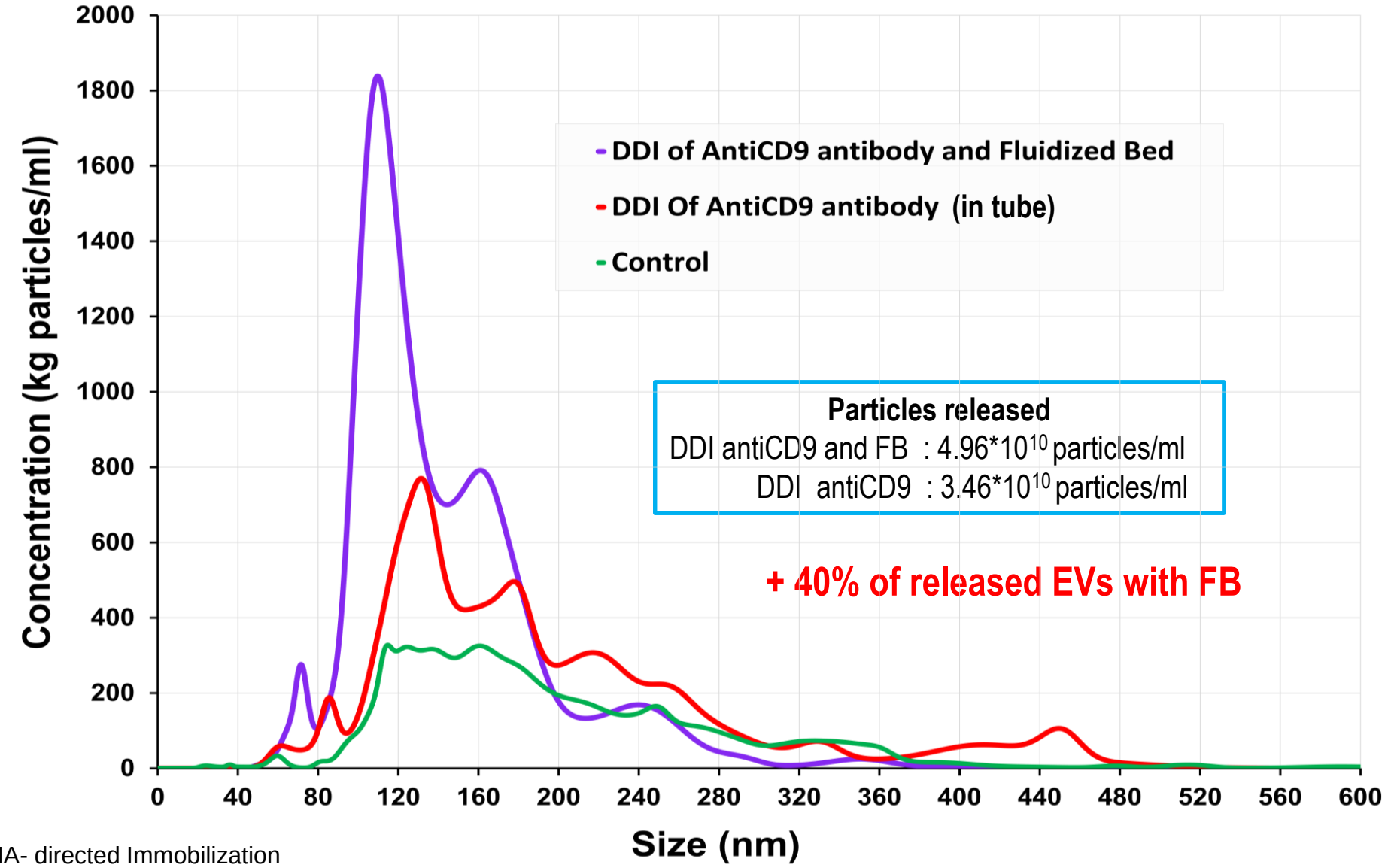
Characterization of Extracellular vesicles (EVs) : NTA





DDI : DNA- directed Immobilization
Ultracentrifugation : (10.000 x g 10 min, 150000 x g, 90 min at 4°C)
FB: Fluidized Bed

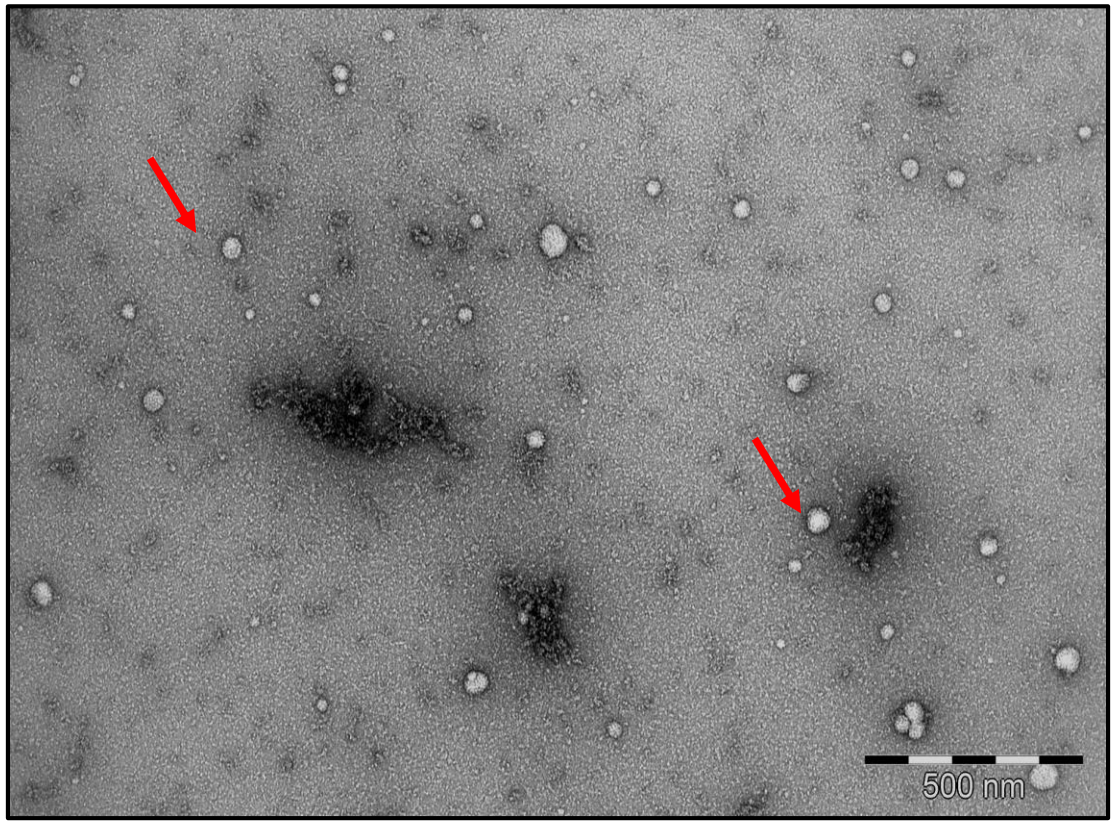
Characterization of Extracellular vesicles (EVs) : NTA



DDI : DNA- directed Immobilization

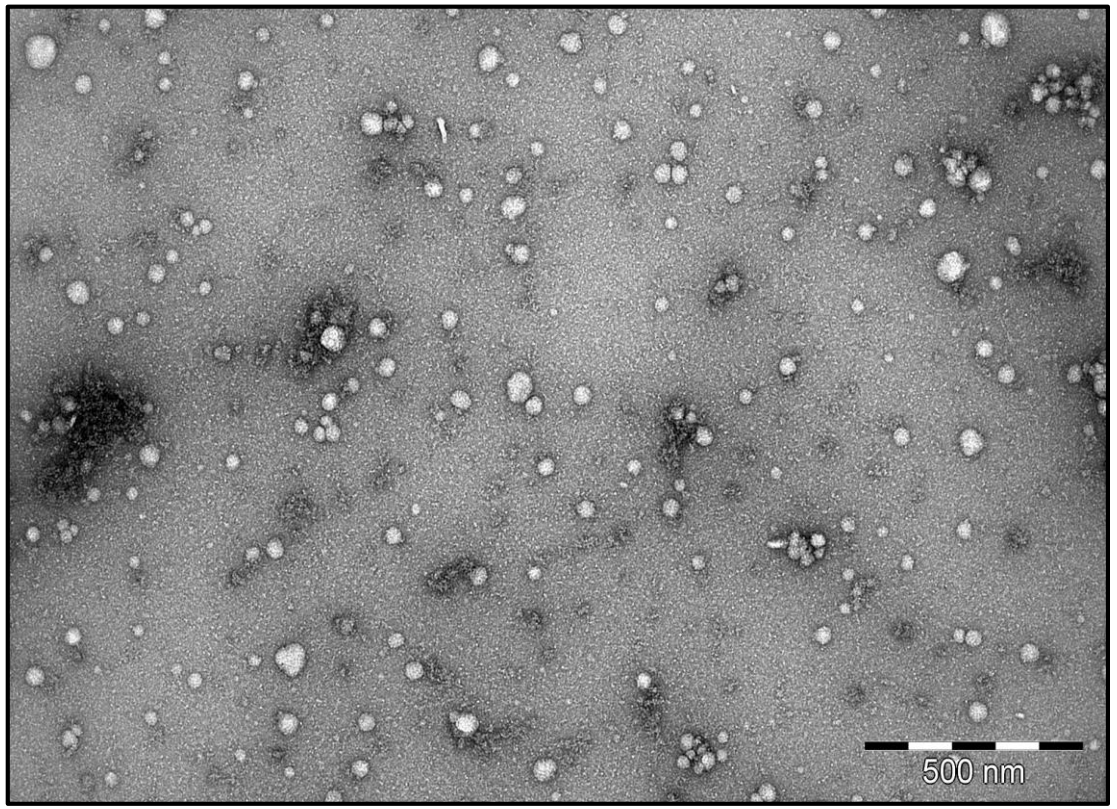
Characterization of Extracellular vesicles (EVs) : TEM analysis

TEM image of uranyl acetate stained Evs released after combined DDI and Fluidized Bed



Capture 0.5 µL/min (4h10 min) at 25°C Release 0.5 µL/min
1h40 min at 37°C, 125 µL of plasma

Optimization FB conditions : *ongoing work*

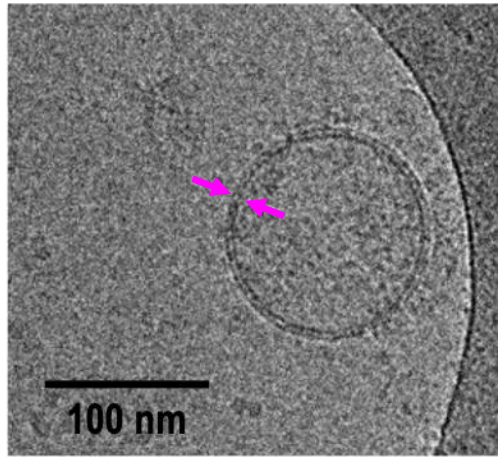


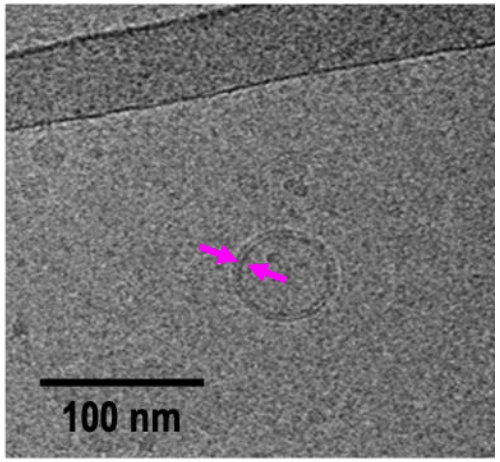
Capture 1.66µL/min (2h30 min) at 25°C Release 0.5 µL/min
1h40 min at 37°C, 250 µL of plasma

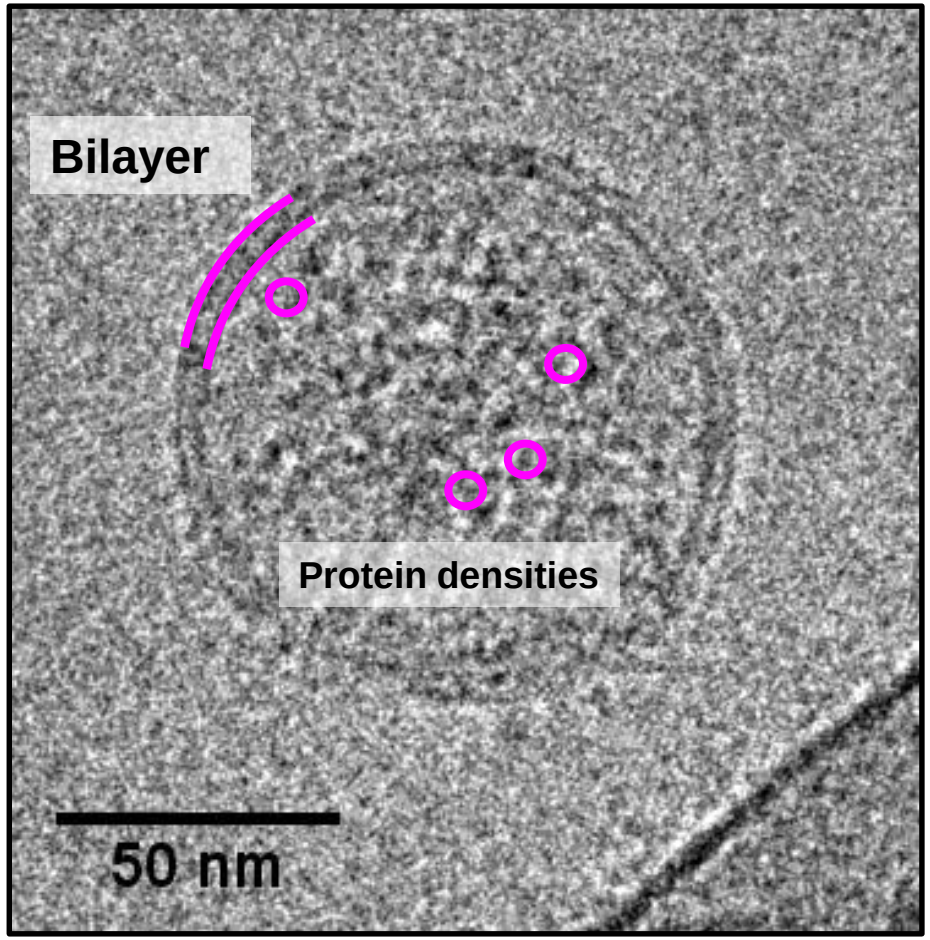
Cryo-Imaging

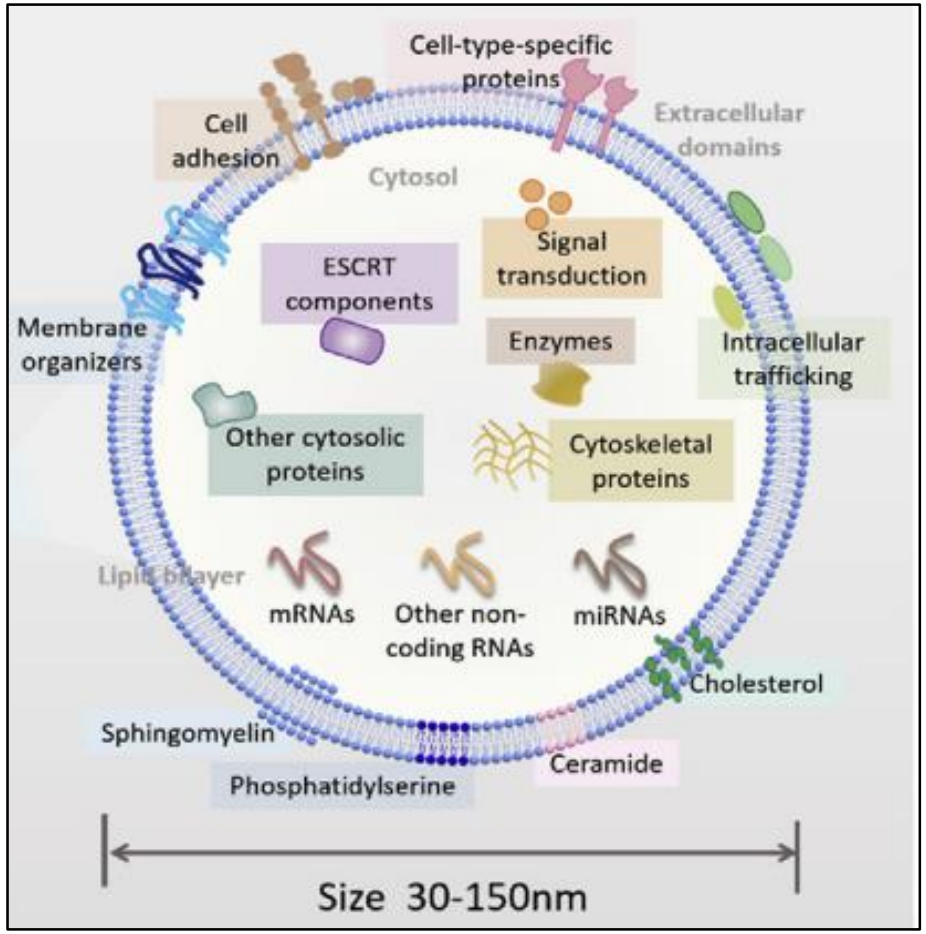
Characterization of Extracellular vesicles (EVs) : CRYO-TEM analysis

Intact small-sized EVs : Exosomes









Visible bilayer
Visible protein densities

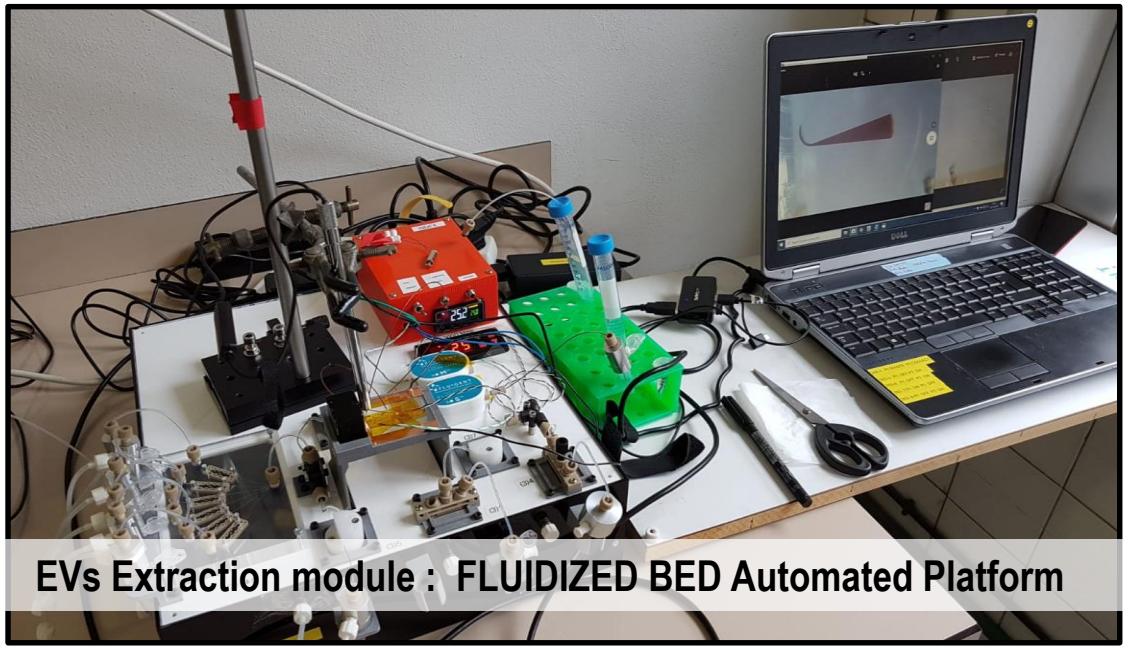
CONCLUSIONS & FUTURE

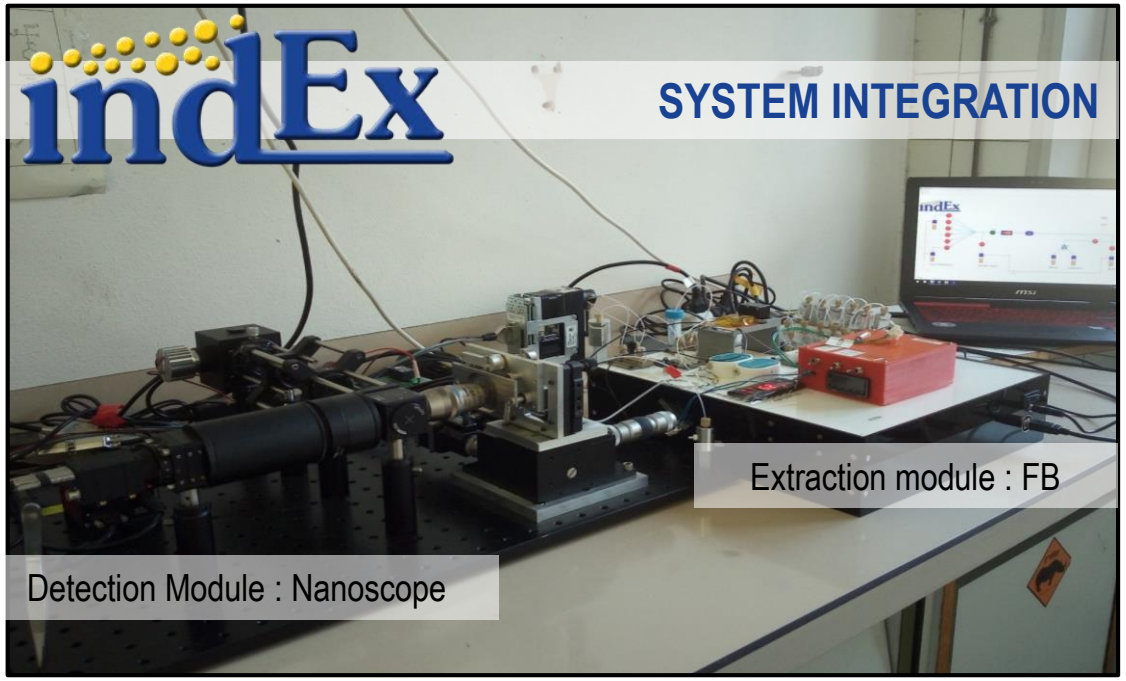
The combination of DNA-directed Immobilization (DDI) approach and Fluidized Bed technology allowed to efficiently extract exosomes from human plasma and to release them for further analysis.

NTA analysis revealed a size distribution ‘typical’ of an extracellular vesicles population characterized by an higher number of particles (40% higher than an experiment in tube) between 100 and 200 nm.

Cryo-TEM analysis confirmed the presence of intact small sized EVs (exosomes) isolated by our microfluidic strategy.

Perspectives :
Further experiments will be performed to assess how these fluidized bed devices can be sequentially coupled to extract different exosome subpopulation


EVs Extraction module : FLUIDIZED BED Automated Platform



ACKNOWLEDGEMENTS

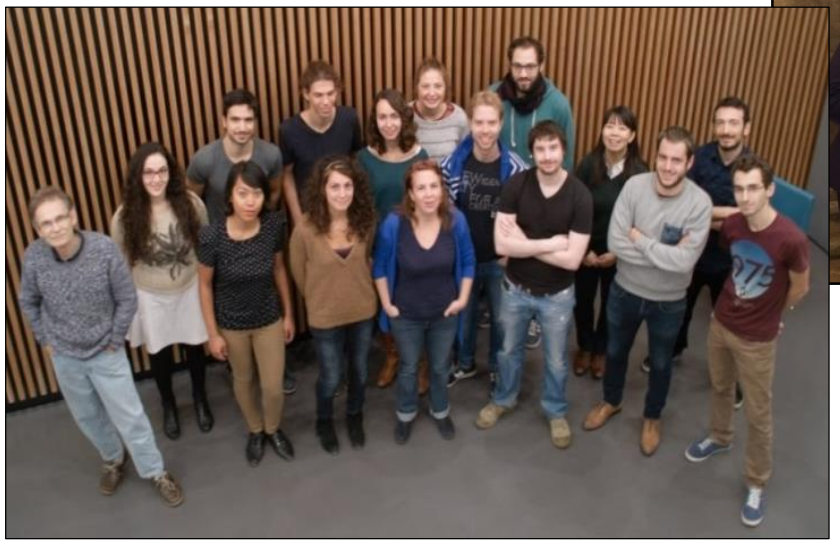
MARCELLA CHIARI
DARIO BRAMBILLA
MARINA CRETICH
LAURA SOLA
CNR-ICRM team

RIKKE BAEK
MALENE JOERGENSEN

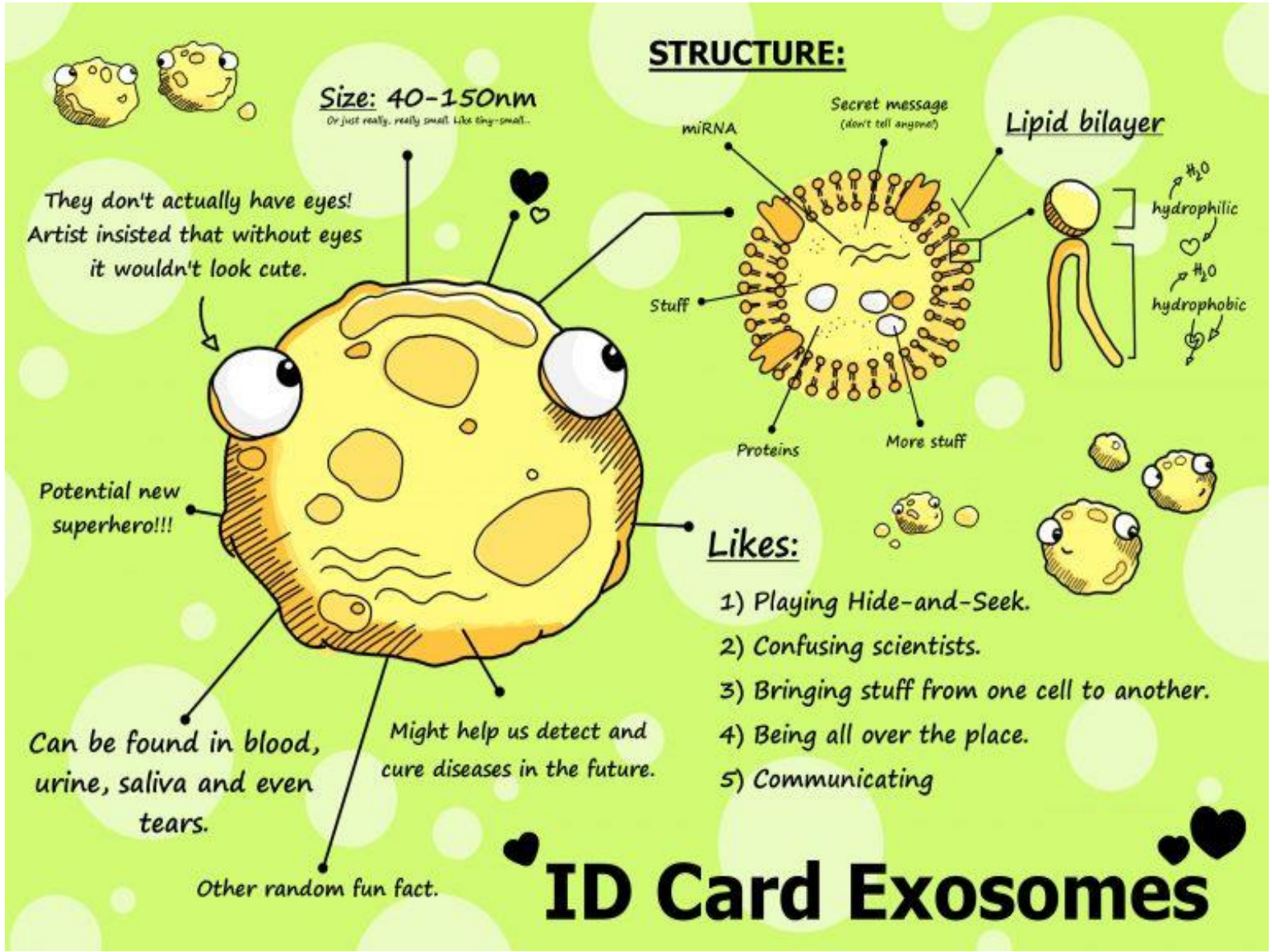
WILLIAM CESAR
MARINE VERHURSEL

STEPHANIE DESCROIX
GIACOMO GROPPLERO
LUCILE ALEXANDRE
DANIEL LEVY
AURELIE DI CICCO
MMBM team

LAURA TRAPIELLA-ALFONSO



Horizon2020 Framework Programme, H2020-FETOPEN-2016-2017



Thank you for your kind attention !

QUESTIONS ?