The NanoBioAnalytical Platform : a tunable tool for deep characterization of extracellular vesicles

Alain Rouleau[1], Pauline Maes[2], David Rageot[2], Annie Frelet-Barrand[1*], Céline Elie-Caille[1*], Wilfrid Boireau[1*]

[1] FEMTO-ST Institute, CNRS-Université de Bourgogne-Franche-Comté, Besançon, France

2 CLIPP, Université de Bourgogne, Dijon, France

www.clipproteomic.fr; https://teams.femto-st.fr//BioMicroDevices/

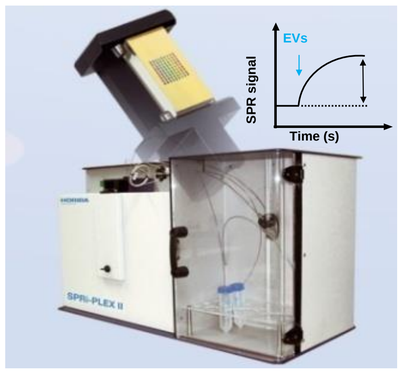
Context of the study

The NanoBioAnalytical (NBA) platform combines different complementary highly sensitive biophysical technologies for in-depth **label-free** investigation of biological samples at the nanoscale level. It is mainly devoted to the qualification of biological samples including extracellular vesicles (EVs), allows biodetection, phenotyping and sizing of EVs subsets by **multiplexed** immunocapture on biochips monitored by **Surface Plasmon Resonance** (SPR) on biochip, followed by a subsequent investigation by **Atomic Force Microscopy** (AFM). Moreover, a proteomic analysis of biological samples specifically captured on biochip is further achieved through nano-liquid chromatography-tandem **mass spectrometry** (MS). In parallel, a characterization in solution, giving size and concentration of the biological species of interest, helps to normalize the conditions of sample injection process on the NBA platform. This label-free system allows the qualification of biological samples including the difficult EVs samples, without limitation in size, from diverse origins [1, 2] and at a dynamic range from 10^6 to 10^9 particles/mL. The utility of the NBA platform was also recently highlighted by the EVs community in the latest MISEV guidelines [3]. This combination of techniques allows understanding features of EVs in different physiological and pathological mechanisms.

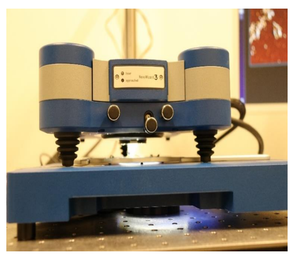
The NanoBioAnalytical Platform : combination of 3 techniques for analytical solution of EVs studies

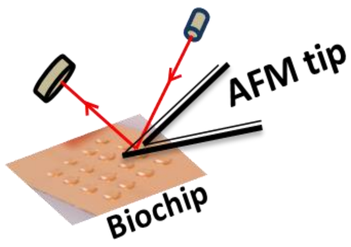
Homemade biochip
Femto-Engineering

SPRi Analysis
Horiba Scientific



AFM analysis
JPK Nanowizard 3





NanoLC-MS/Ms analysis

Ultimate 3000 nanoLC system
Thermofisher Scientific

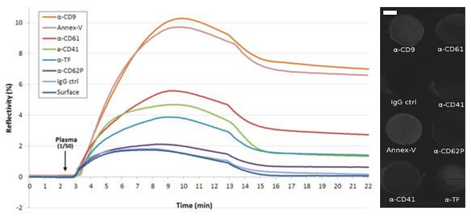
Orbitrap Elite
Thermofisher Scientific

Protein identification using Mascot/ ProlineStudio and Data interpretation

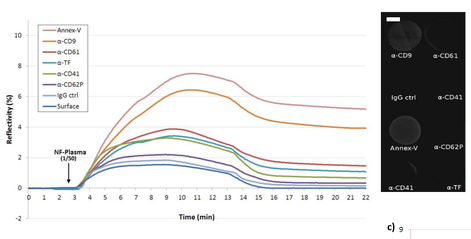
Biological applications of this NBA platform

SPRi for EVs phenotyping

a) Plasma before nanofiltration

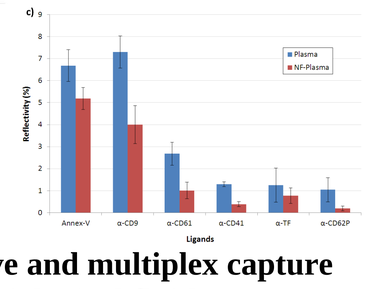


b) Plasma after nanofiltration (NF plasma)



Sensorgrams of the injection of plasma (a) and nanofiltered plasma (NF) (b) on multiplexed biochips with SPR image of the different spots after the injections. Collab. T. Burnouf, TMU, Taipei (Taiwan)

(c) Histogram of the capture level of EVs on the different spots.

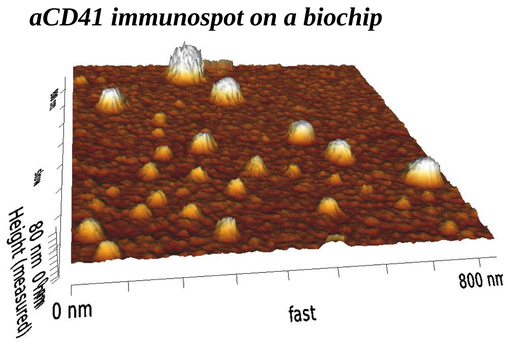


SPRi allows selective and multiplex capture of EVs in biological fluids

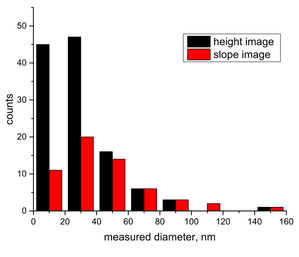
Physiological conditions
Dynamic range from 10^6 to 10^9 particles/mL
EVs subpopulations / multiplex biochips

AFM for EVs morphomechanical analysis

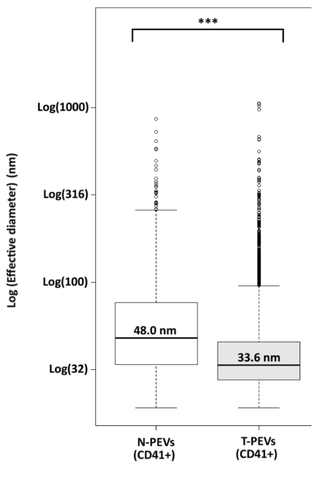
Platelet derived EVs captured on aCD41 immunospot on a biochip



EVs characterization : Slope image in QI gives more accurate results



Differential studies of native and thrombin activated platelet derived Evs Collab. T. Burnouf, TMU, Taipei (Taiwan)

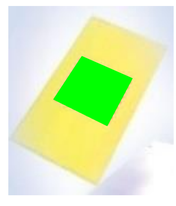


AFM allows metrology of different EVs subsets

QI mode, in liquid & unfixed :
ideal to visualize EVs in their intact shape and size

MS for EV proteome differential analysis

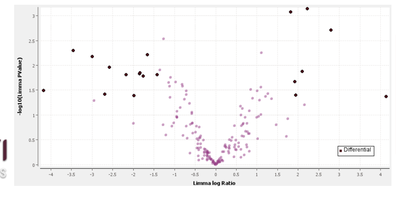
1 macrospot



On-chip Reduction / Digestion , Manual peptide extraction

ImagePrep
Bruker Daltonics

Vulcano plot of platelets derived EVs



ProFI PROTEOMICS

Identification
Quantification
Bioinformatics

MS allows differential quantitative proteomic studies based on the analysis of hundreds of ng of captured EVs on a biochip

LC-MS/MS, Orbitrap, MALDI-TOF

Current on-going projects in collaborative programs

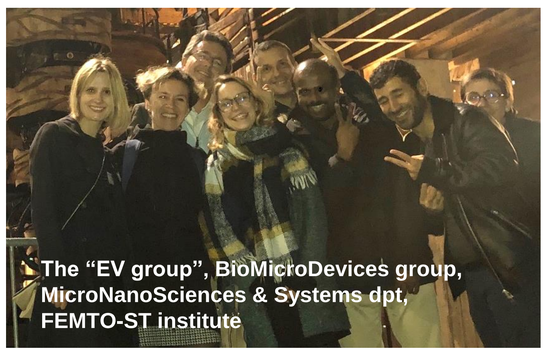
Eukaryotic EVs

✓ EVs from cancer cell lines and patients
INSERM UMR1231, Dijon (C. Garrido)
UMR1098, Besançon (C. Borg)
✓ Platelet and blood derived Evs
TMU, Taiwan (T. Burnouf)
HUG, Genève (P. Fontana)

Bacterial EVs

✓Recombinant OMVs from Gram negative bacteria
IRSD, Toulouse (E. Oswald)
✓ EVs secreted by Gram positive bacteria
UMR PAM, Dijon (J. Guzzo)
✓ Recombinant Gram positive EVs

Funds : FEDER MiMédI (2017-2021), French ANR Madness 2017, regional projects (Micro-MPs 2017, NanoLacto 2016)



https://www.youtube.com/watch?v=L_TKKsjgQLo

MiMédi
Microtechniques pour les Médicaments Innovants

UNION EUROPEENNE
RÉGION BOURGOGNE FRANCHE COMTÉ
avec le Fonds européen de développement régional (FEDER)

Conclusion

NBA platform = modular, versatile and upgradable for deep investigation of EVs and their subsets from diverse origins in term of size and phenotype qualification

3 calibration particles developed:
VLPs (50 nm), biofunctionalized beads (140 , 480 and 920 nm)

Platform open to new collaborations

Perspectives/Future developments

✓ Spectral (IR/Raman) analysis of EVs and subsets
✓ Sorting and/or manipulation of nanoobjects
✓ Cell culture upstream NBA
✓ Multi-omics approaches
✓ Integration of biodetection system upstream and/or directly on lab-on-chip

Club nanoMétrologie

French Scientific Initiative in Nanometrology of soft nanoparticles

LNE NanoSciences FRANCE

C. Elie-Caille joins office of C'Nano Grand Est for Nanometrology in June 2019, nominated as Nanometrology contact (CorrNanoMet).
Coordinator of a group focused on nanoanalysis in “complex media” and especially EVs
Contact: **celine.caille@femto-st.fr**

References

[1] S. Obeid et al., *Biosensors and Bioelectronics* 93, 250–259, 2017
[2] S. Obeid et al., *Nanomedicine: Nanotechnology, Biology, and Medicine*, 20, 101977, 2019
[3] C. Thery et al. *Journal of Extracellular Vesicles* 7(1) , Article Number: 1535750, 2018