NanoBioAnalytical tools for blood-derived extracellular vesicles characterization

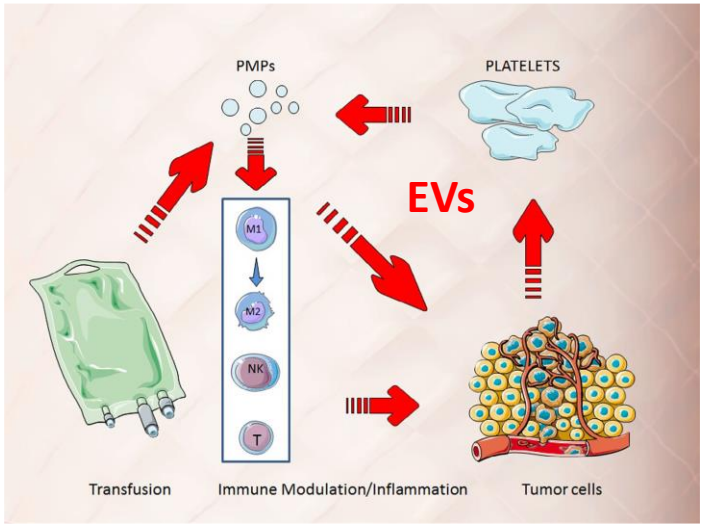
Celine Elie-Caille

FEMTO-ST Institute, Micro Nano Sciences and Systems (MN2S) department / BioMicroDevices group, Besançon (France)

Minisymposium, TMU, November the 16th 2018

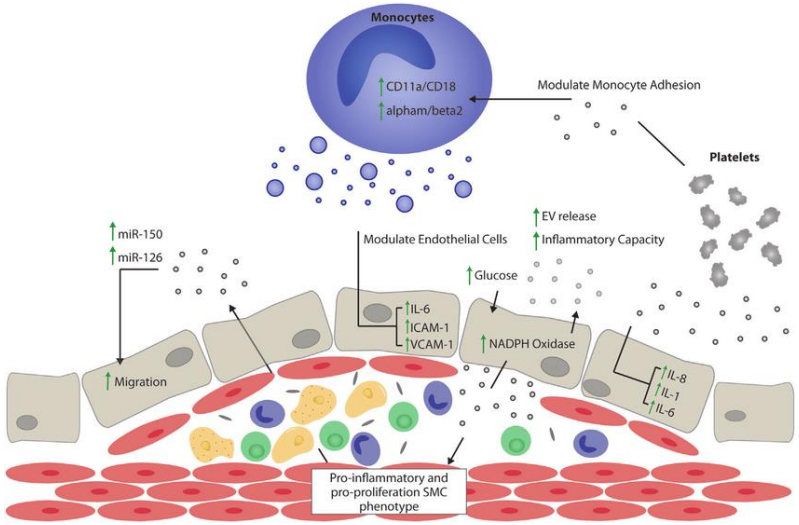
Blood EV content : multiple origins and functions

In Cancer



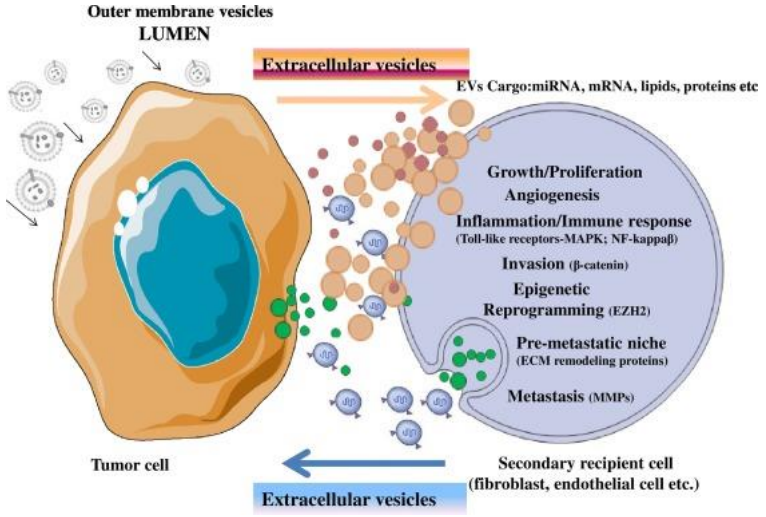
From Goubran (Burnouf T.) et al, Trans Aph 2015

In thrombosis



From Gustafson et al, 2017

In inflammation, etc…

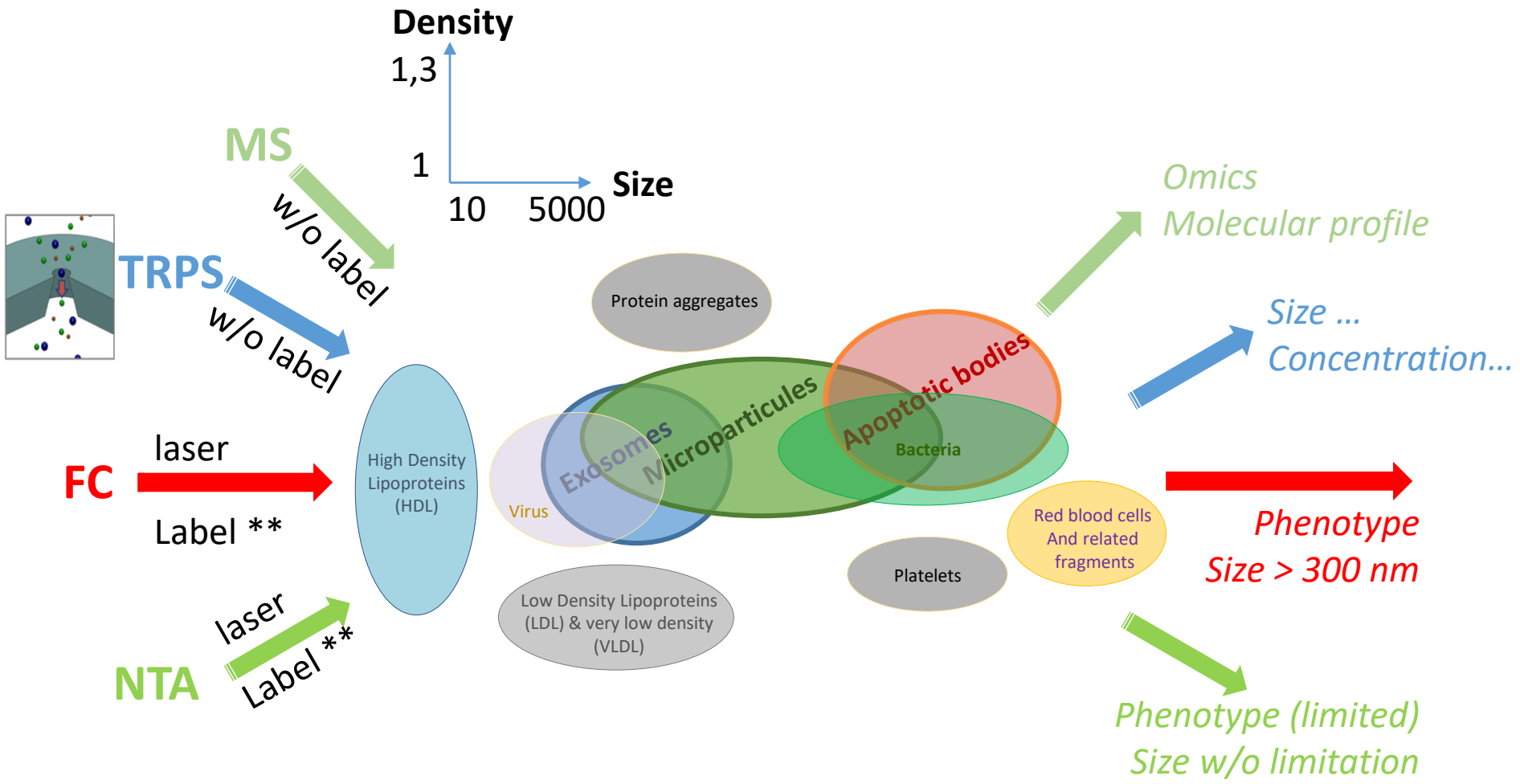


From Barteneva et al, 2017

- EVs produced by all cells
- EVs = potential circulating biomarker candidates
- 10^ to 10^6 EVs/ml in blood : High concentration

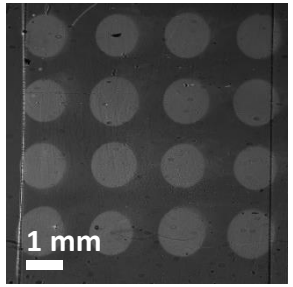
Blood circulating EVs :
at the height
of the cell-cell communication

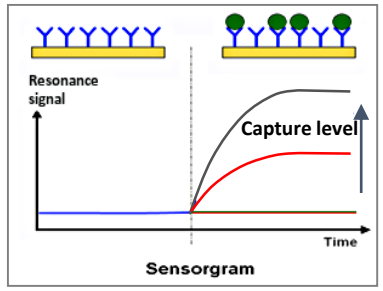
Limits surrounded EVs analytical methods



In solution : difficult, even impossible to have <u>concentration, size and phenotype, at once</u>

Our NanoBioAnalytical platform

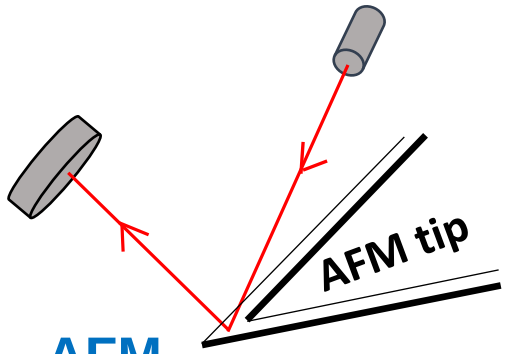


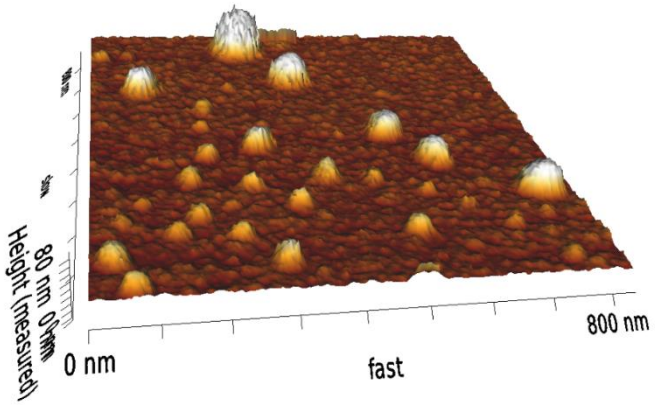


EVs phenotyping & spatialization on microarrays

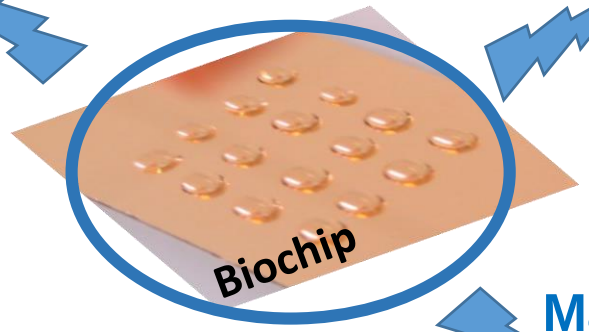
SPRi

AFM



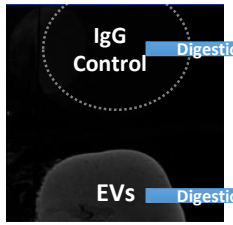


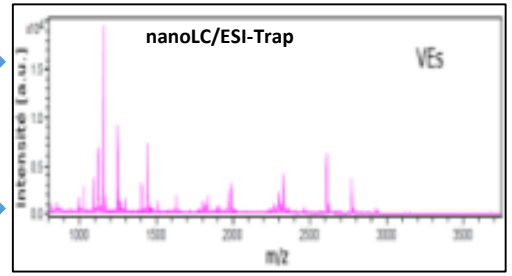
EVs viewing, sizing and counting

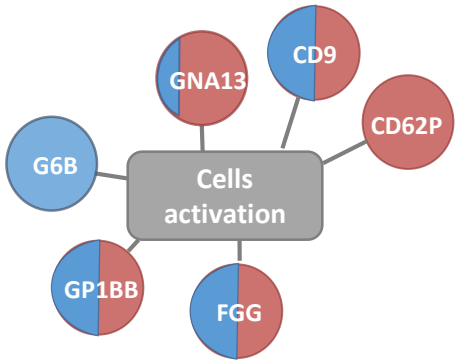


Mass spectrometry

Advantages of NBA:
-home-made biochips
-on original biological samples
-label-free methods

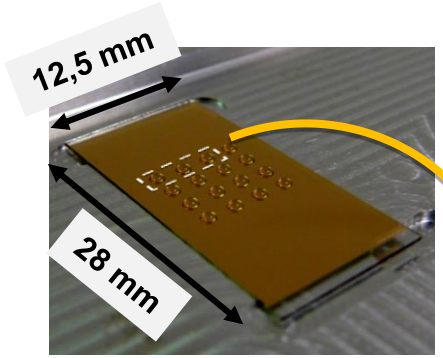




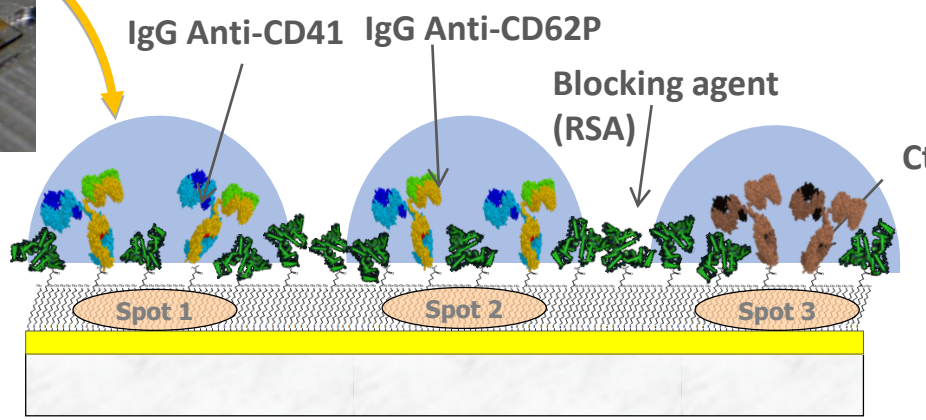


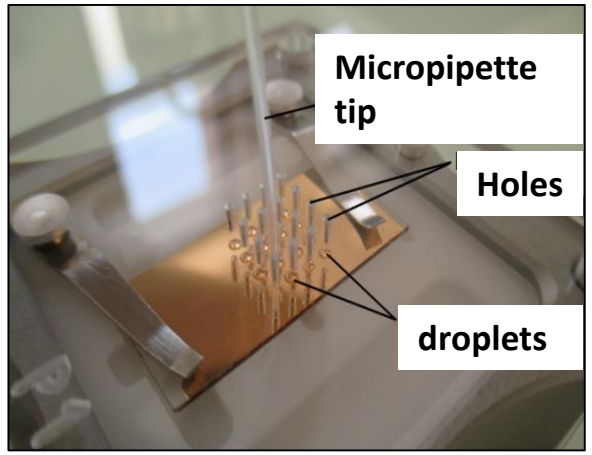
EVs proteomic profiles

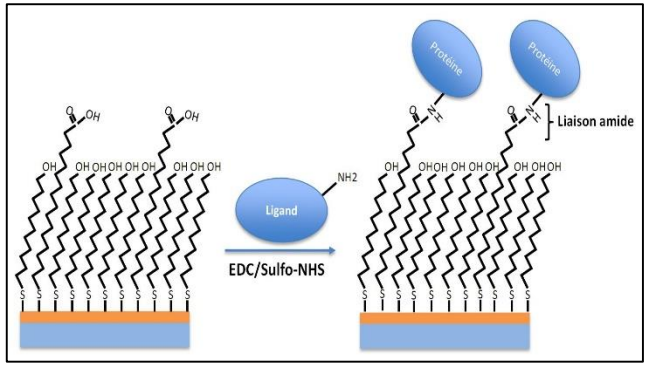
The gold biochip: the corner stone of NBA



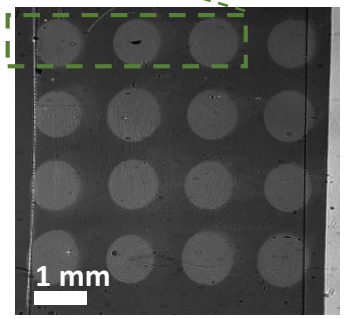
Array Spotting







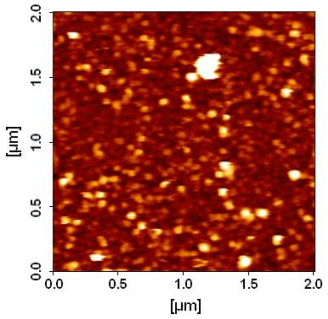
Modular biointerface:
1 to 96 spots
Surface coverage:
Until 15 000 Ab/µm²

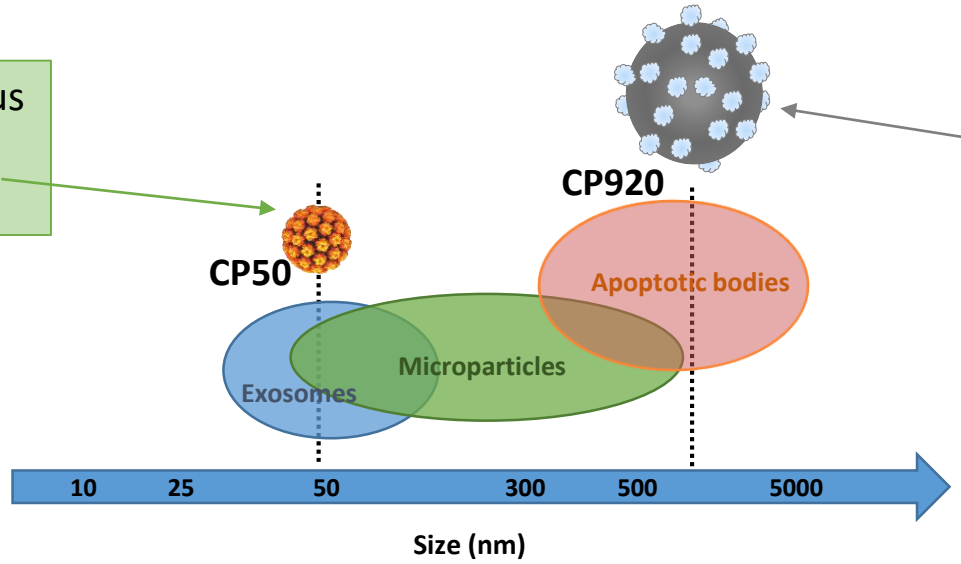


Phase contrast image of the biochip in SPRi-Plex II (Horiba)

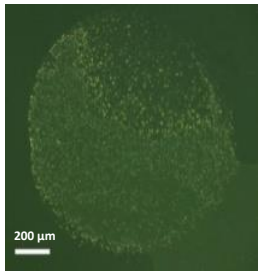
Calibration of NBA: nanoparticles covering 50 nm to 1µm

- VP1 capsid protein of norovirus
- 50 nm
- Anti-CP50





- Biofunctionnalized bead
- 920 nm
- Anti-CP920



CP50 particles :

Specific & stable CP50 capture

Dynamic range for CP50 :

$2{,}5 \times 10^{9}$ – 2×10^{11} CP50/mL

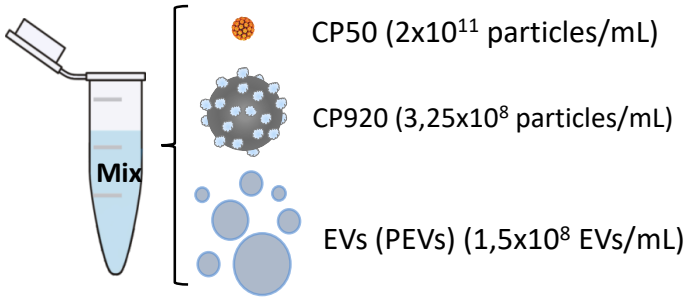
CP920 particles :

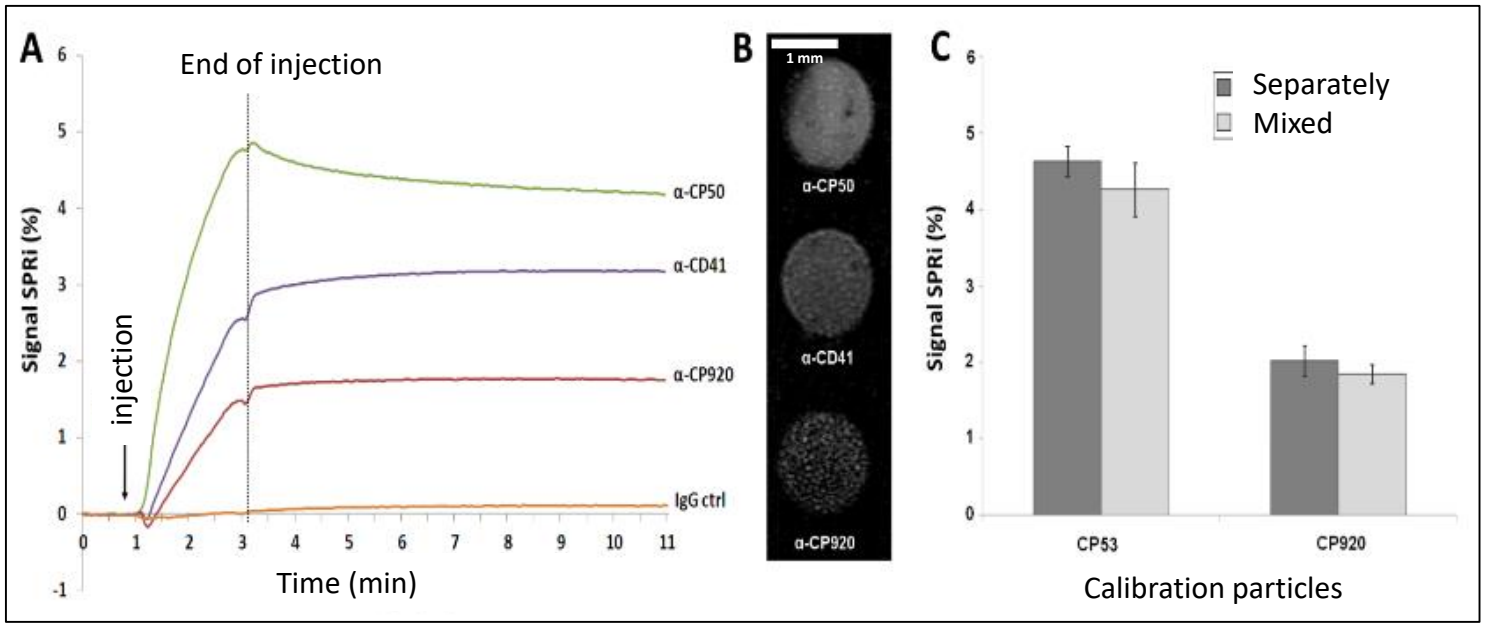
Specific & stable CP920 capture

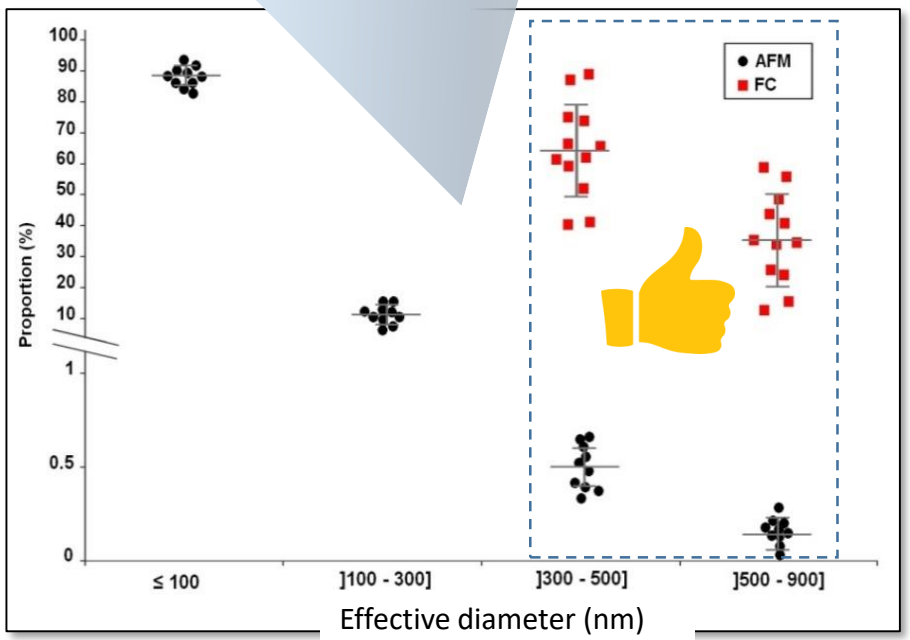
Dynamic range for CP920 :

$3{,}25 \times 10^{7}$ – $3{,}25 \times 10^{8}$ CP920/mL

NBA for EVs biodetection, sizing and morphology







EVs used : platelet-derived EVs

➢ **NBA efficient to dose, select, spatialize, evaluate size and morphology of EVs subsets**

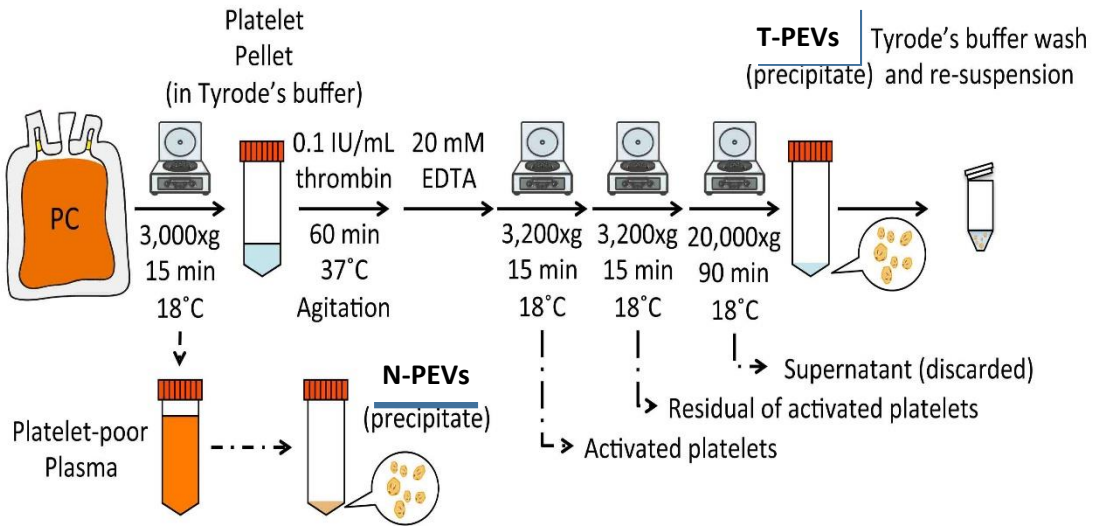
Obeid S., et al, Biosensors & Bioelectronics, 2017, 93, 250-259

Application of NBA to a biological model: Platelet-derived EVs effect on monocytes

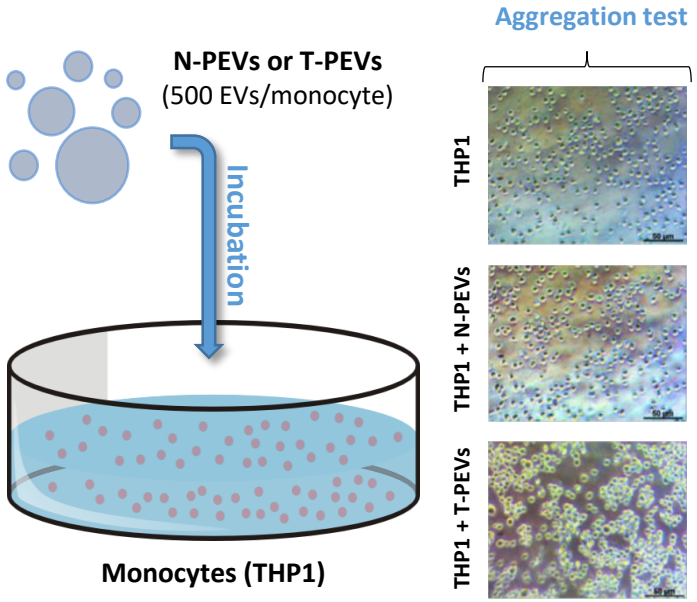
Collaboration : T. Burnouf, Taiwan

Objective : understand the **pro-inflammatory and pro-thrombotic « role »** of EVs from plasma or platelet concentrates in transfused patients

EVs preparation



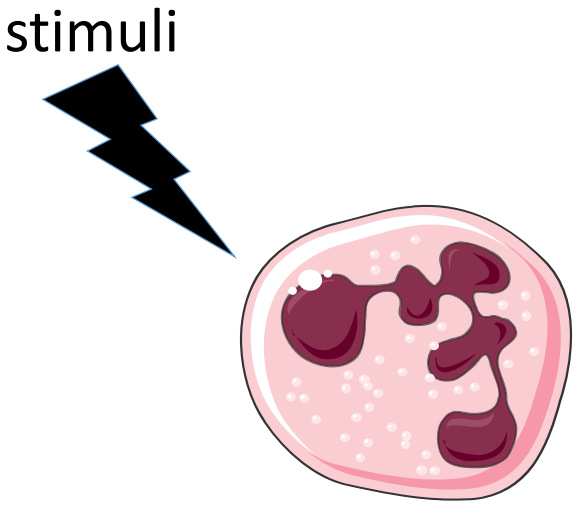
EVs function on monocytes



Lin et al. Transfus Apher Sci. 2015

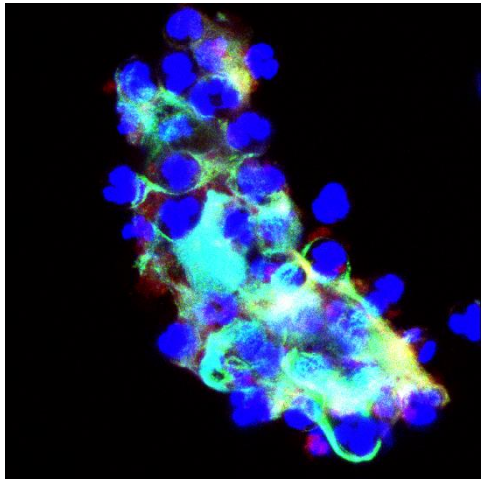
T-PEVs induce aggregation of THP1…

Neutrophil aggregation and extracellular traps (NETs)

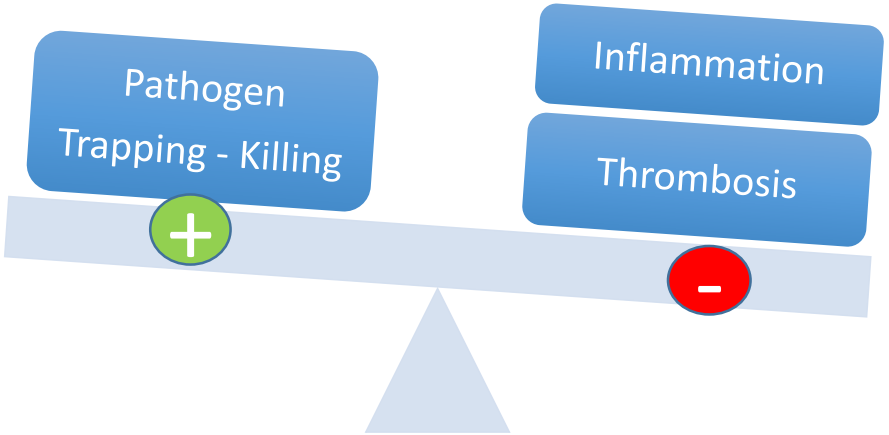


neutrophils release granule proteins and chromatin to form an extracellular fibril matrix known as NETs

Pathogen trapping & killing



NETs: paradoxical physiological impact ?

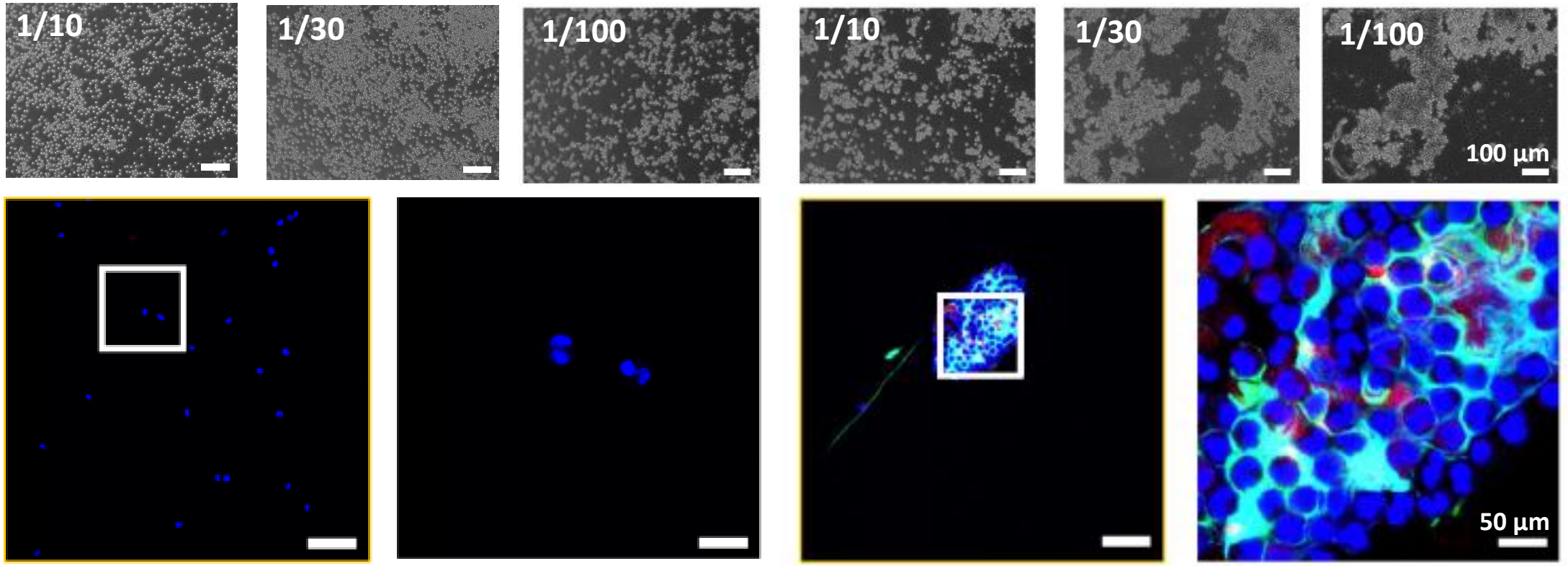


Hypothesis:
Thrombogenic risks of plasma for transfusion are mediated by PEVs through:
- direct thrombin generation
- and neutrophil activation

Neutrophil aggregation and extracellular traps (NETs)

N-PEVs on neutrophils

T-PEVs on neutrophils

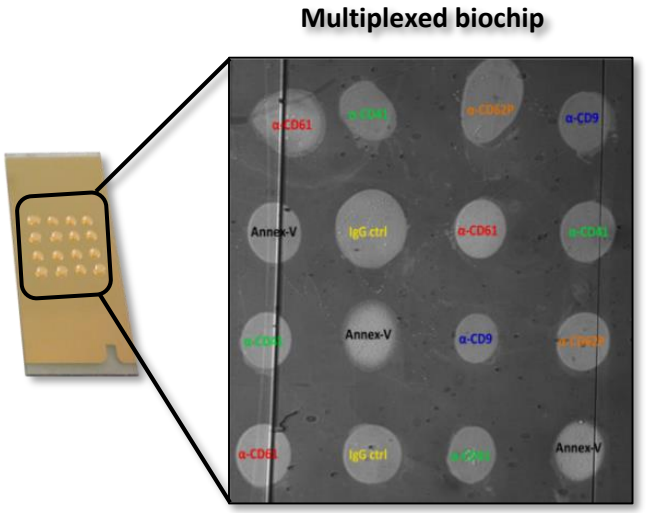


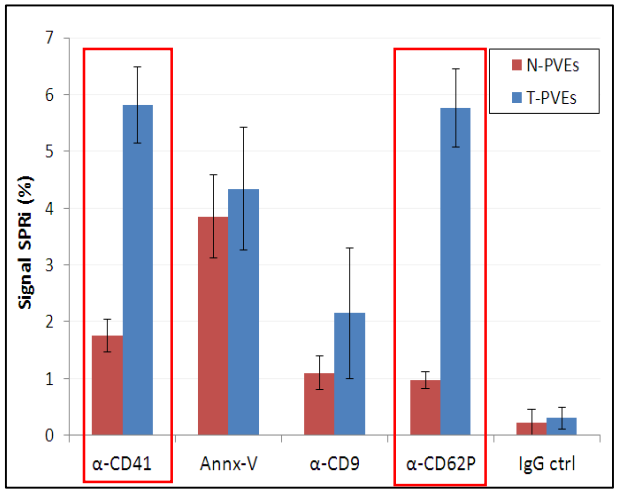
DNA (blue), histone H1 (green), and MPO (red)

→ T-PEVs induce aggregation of neutrophil aggregation and NETs formation…

T-PEVs effect on monocytes : concentration ?? size ?? composition???

Differential EVs subsets capture on the chip

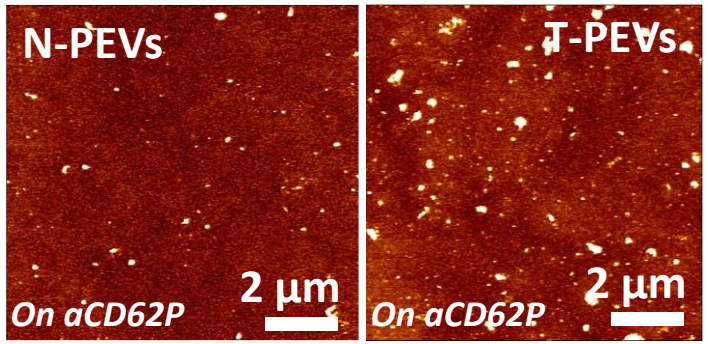




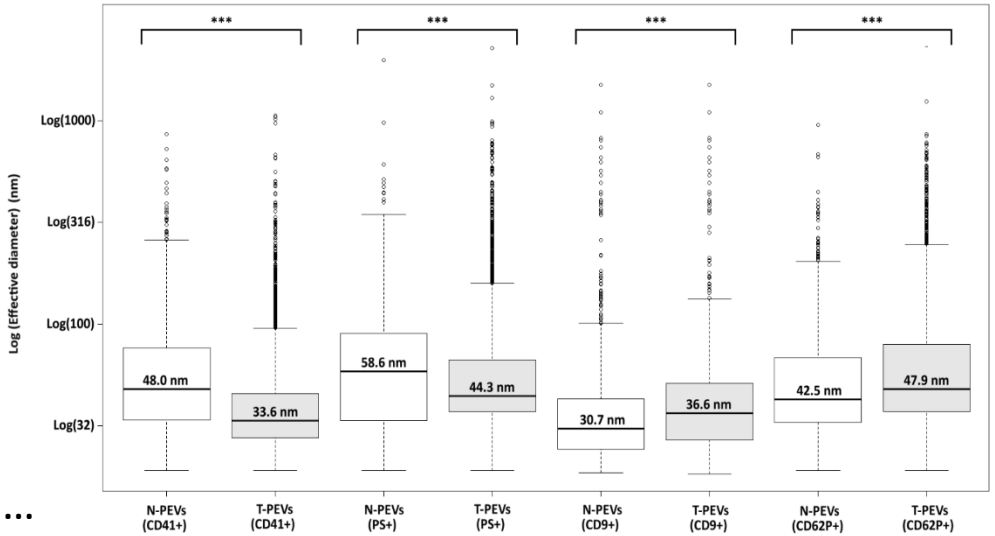
➢ **T-PEV : capture +++ on aCD41 and aCD62P**

	Concentration (EVs/mL)	
	Qnano	FC
N-PEVs	$3,7x10^{12}$	$2,1x10^{10}$
T-PEVs	$1,3x10^{11}$	$6,8x10^{9}$

…Not due to the EVs concentration



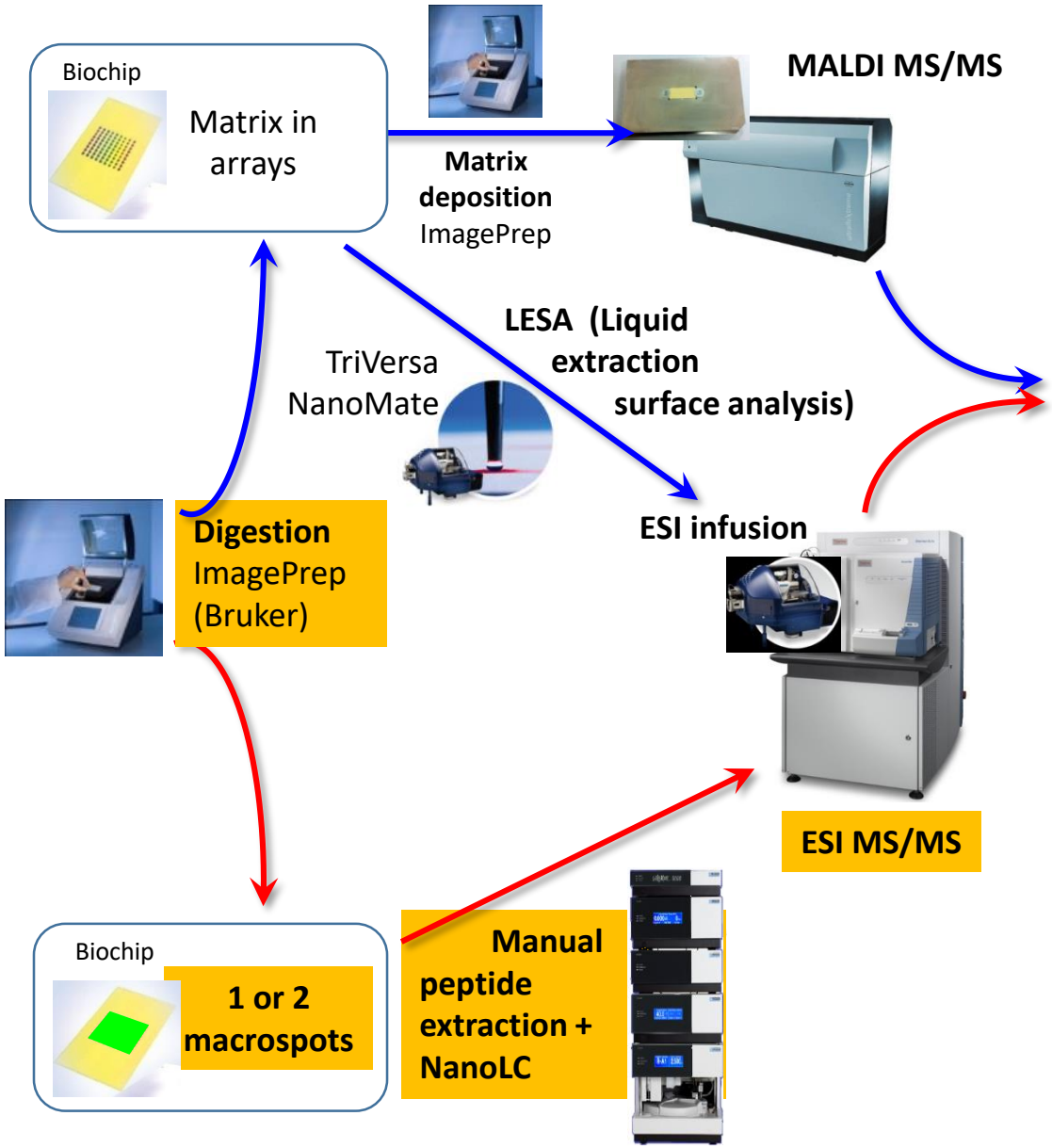
…Not due to the EVs size …



Obeid S. et al, Nanomedicine, Biology and medicine (submitted)

Proteomics of EVs subsets captured on the chip (1/3)

MS workflow in detail for NBA



Identification
+
Characterization
+
Quantification
+
Bioinformatics

comet

Medical & Science TPP

x!

Proteome Discoverer 2.1

MATRIX SCIENCE

ProFI PROTEOMICS

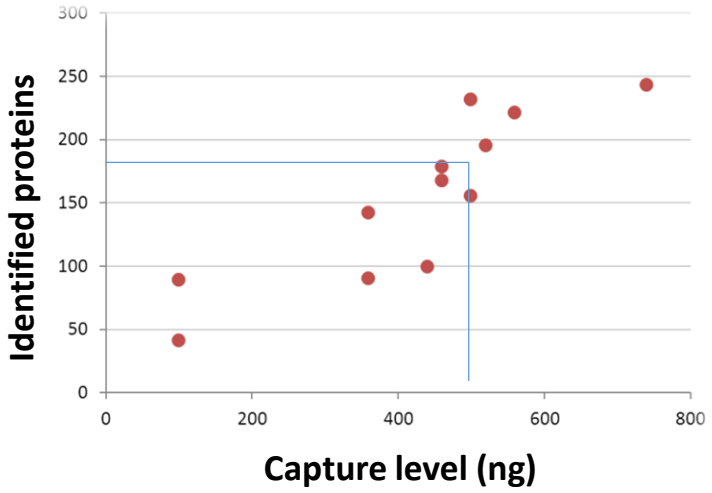
STRING

biotools

Proteomics of EVs subsets captured on the chip (2/3)

EVs proteomics « on arrays »

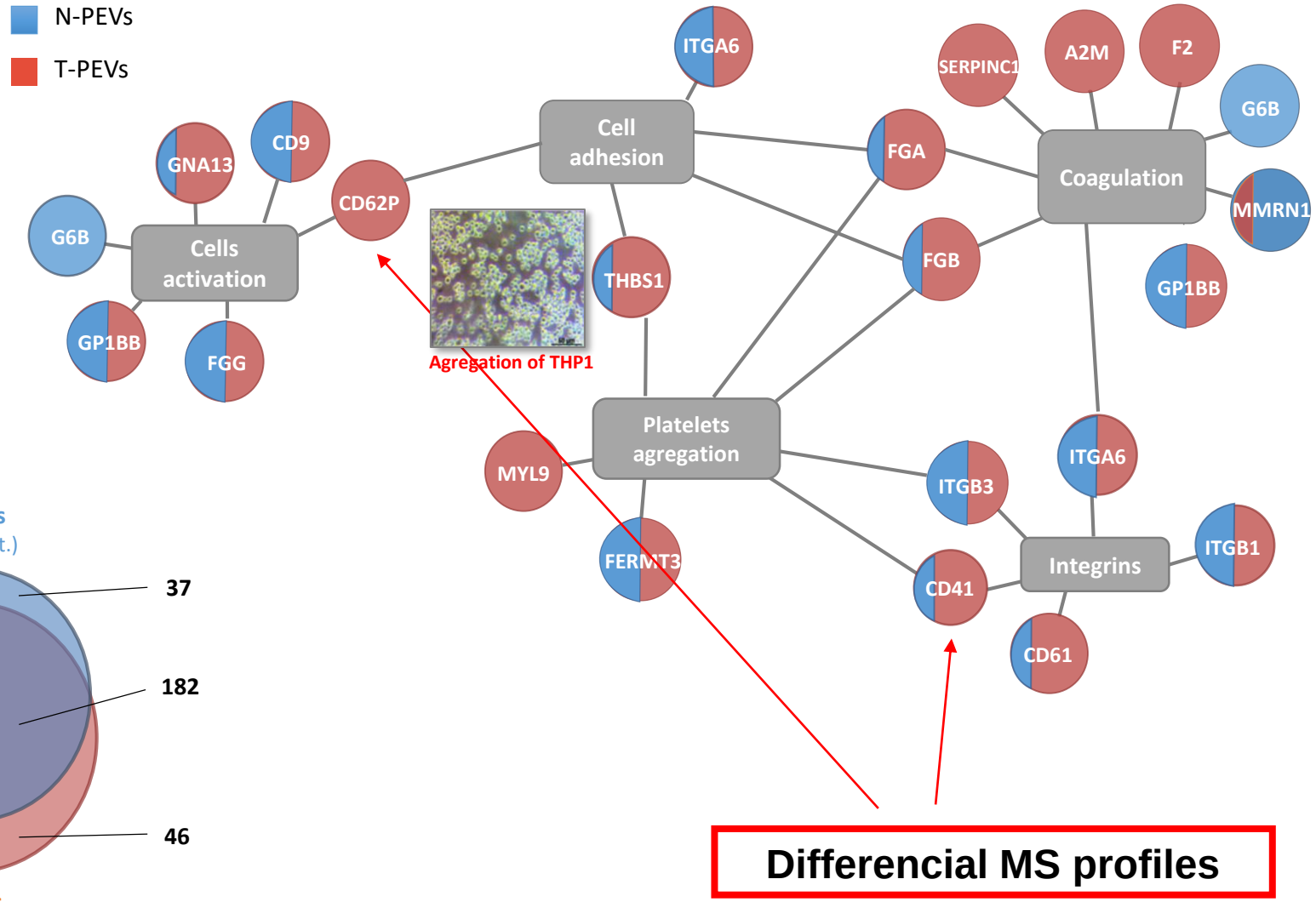
“On array” MS optimization

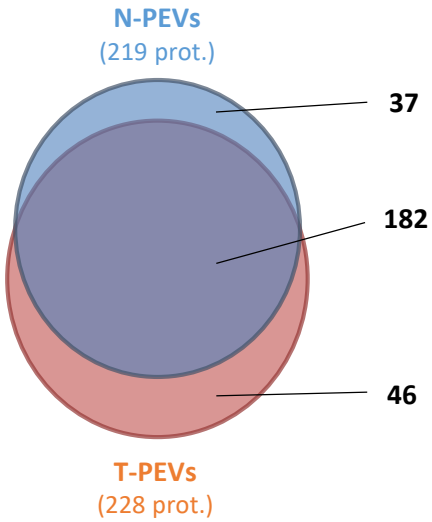


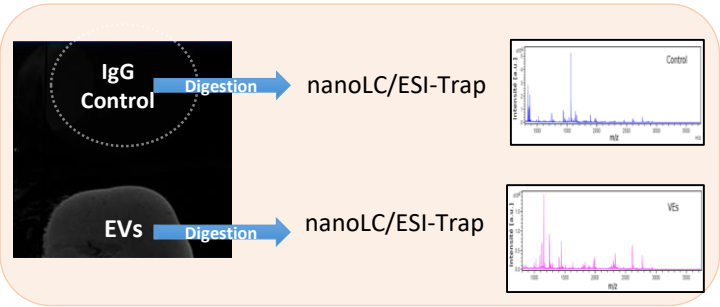
		T-PEVs		N-PEVs	
Protein Set	Description	Score T-PMP	Peptides T-PMP	Score N-PMP	Peptides N-PMP
Q9Y490	Talin-1	3232,74	49	3943,33	61
P21333	Filamin-A	2805,99	47	3778,84	62
A0A024QZN4	Vinculin, isoform CRA_c	1275,97	23	1157,94	24
P08514	Integrin alpha-IIb	1233,54	17	1653,09	25
P60709	Actin, cytoplasmic 1	1076,79	19	1345,02	25
A0A0A0MRJ7	Coagulation factor V	259,97	5	945,11	17
F6KPG5	Albumin (Fragment)	1031,38	17	608,76	10
A0A024R882	Stomatin, isoform CRA_a	808,04	10	921,68	12
E7EPG1	Multimerin-1	45,33	1	690,01	13
L7UUZ7	Integrin beta	611,35	9	604,56	9
P11142	Heat shock cognate 71 kDa protein	609,84	9	666,08	12
A0A024R1N1	Myosin, heavy polypeptide 9, non-muscle	576,45	10	550,12	8
P02671	Fibrinogen alpha chain	493,98	11	133,44	3
A0A0A0MS51	Gelsolin	489,27	9	823,74	13
A0A024R3E3	Apolipoprotein A-I, isoform CRA_a	463,22	9	300,03	5
A8K486	Peptidyl-prolyl cis-trans isomerase	457,41	8	355,44	6
A0A024R694	Actinin, alpha 1, isoform CRA_a	428,23	8	327,95	7
A0A024RB87	RAP1B, member of RAS oncogene family	390,9	5	527,1	7
P11021	78 kDa glucose-regulated protein	390,21	7	230,05	4
P04406	Glyceraldehyde-3-phosphate dehydrogenase	367,78	6	542,4	10
P13224	Platelet glycoprotein Ib beta chain	350,35	6	367,7	7
B4DE78	cDNA FLJ52141, highly similar to 14-3-3 protein gamma	344,31	7	332,17	6
Q86UX7	Fermitin family homolog 3	341,67	5	409,09	6
A0A024R5Z9	Pyruvate kinase	340,44	6	353,76	6
D0PNI1	Epididymis luminal protein 4	339,64	5	463,93	7
.....	.....	.....	.....	....	.....
.....	.....				

~ 200 proteins identified from ~ 500 ng of captured EV
and several differential proteins …

Proteomics of EVs subsets captured on the chip (3/3)







T-PEV : capture +++ on aCD41 and aCD62P…

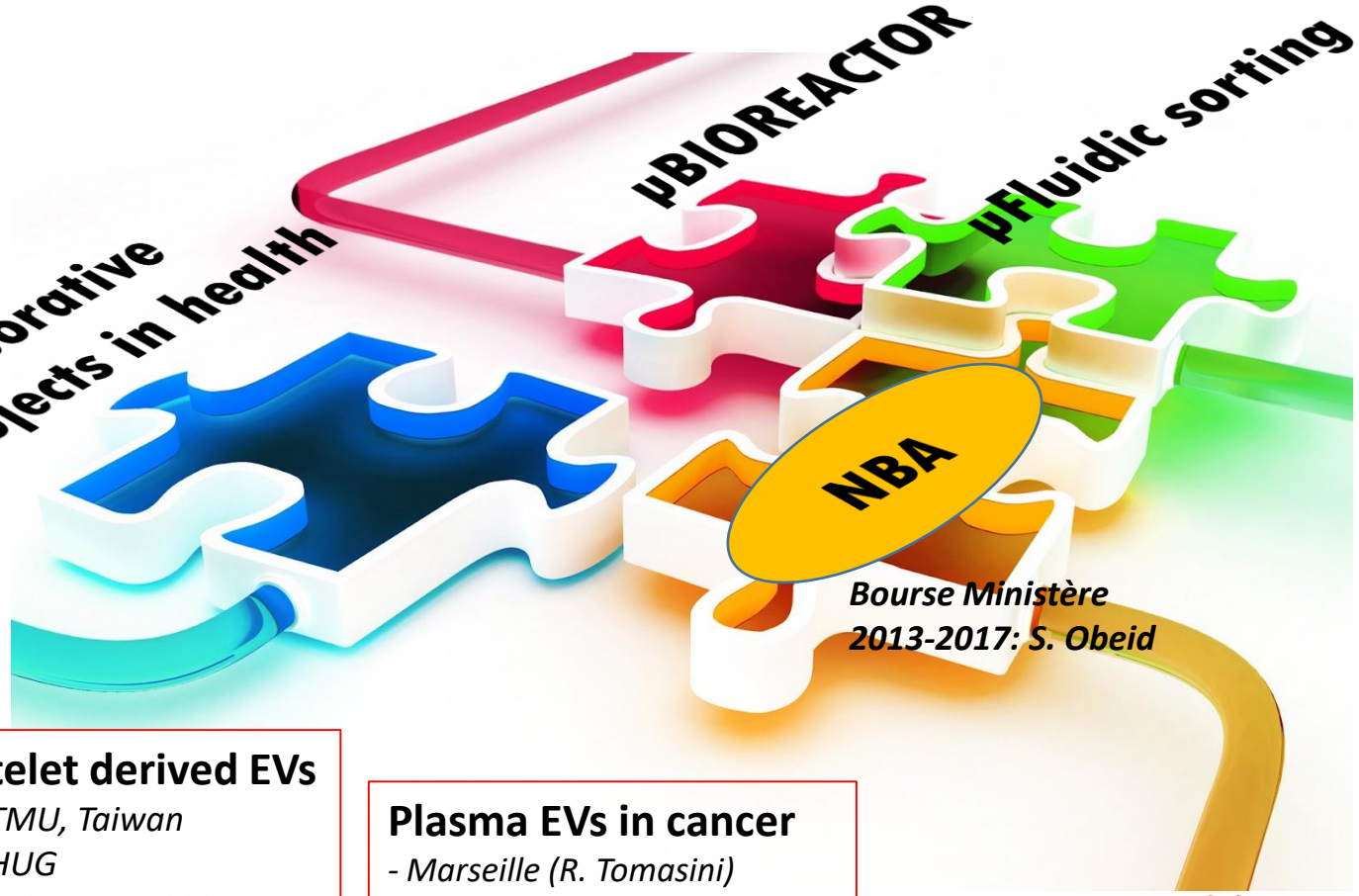
Due to protein expression level ? Yes !

Obeid S. et al, unpublished data

NBA: an upgradeable platform

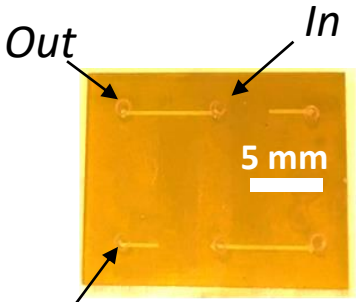
BOURSE REGION 2017-2020

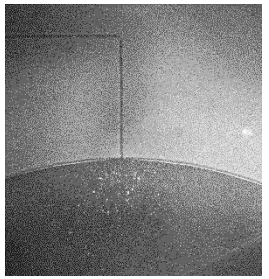
Inserts for cell coculture



Bourse Ministère 2013-2017: S. Obeid

ANR MADNESS with LAAS
Toulouse





Cut off = 700 nm

Platelet derived EVs
- *TMU, Taiwan*
- *HUG*
- *INSERM U1098*

Plasma EVs in cancer
- *Marseille (R. Tomasini)*
- *Dijon (C. Garrido)*
- *Besançon (C. Borg)*

Acoustofluidic sorting (Oct-Dec 2018)
A. Khelif (FEMTO-ST)

Acknowledgments

FEMTO-ST Institute & CLIPP

S. Obeid
K. Maximova
W. Boireau
A. Rouleau
K. Maximova
G. Lucchi, P. Maes

Collaborators

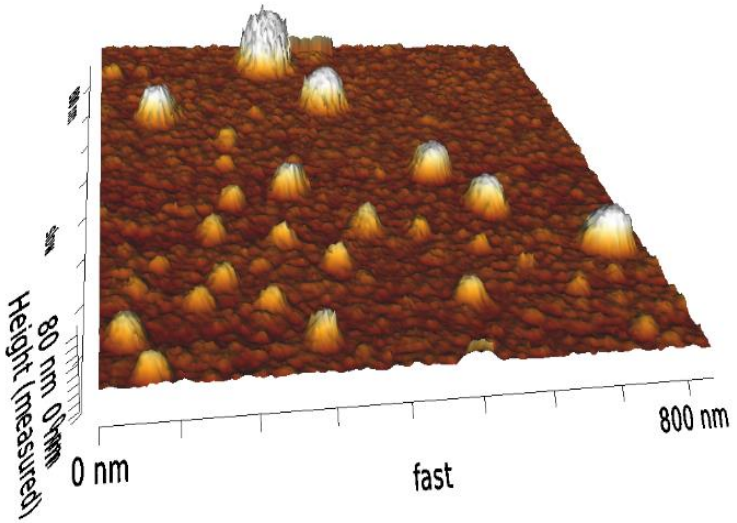
T. Burnouf
A-M Gué
T. Lecompte
P. Saas

Fundings

FEDER « MIMEDI » 2018
ANR 2017 « MADNESS »
Région Franche Comté (2017)
CNRS : Défi instrum. aux limites 2017

« EV group » in BioMicroDevice Team

May 2018, at FEMTO-ST Institute



THANK YOU !