# On-chip detection, sizing and proteomics of extracellular vesicles

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ISEV 2018, 3-5 may 2018, Barcelona

# Challenges in EVs analysis :

- CRUDE sample (with « contaminating » biological objects)
- NO labelling
- COMPLETE & GLOBAL EVs subpopulations

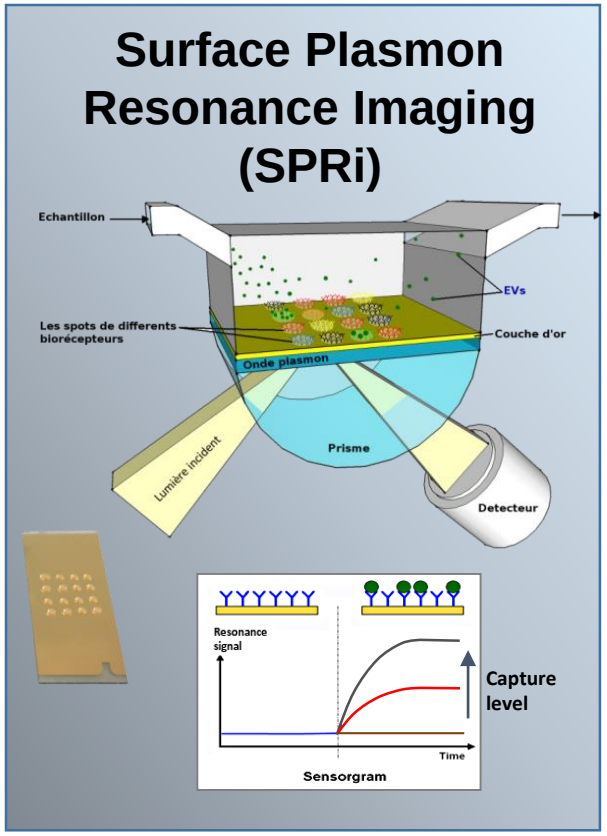
**!! Need to combine technologies !!**

- In PHYSIOLOGICAL conditions,

**!! to keep them functional !!**

# Our technological combination : the NBA platform

## Surface Plasmon Resonance Imaging (SPRi)




MPs phenotyping & spatialization On microarrays

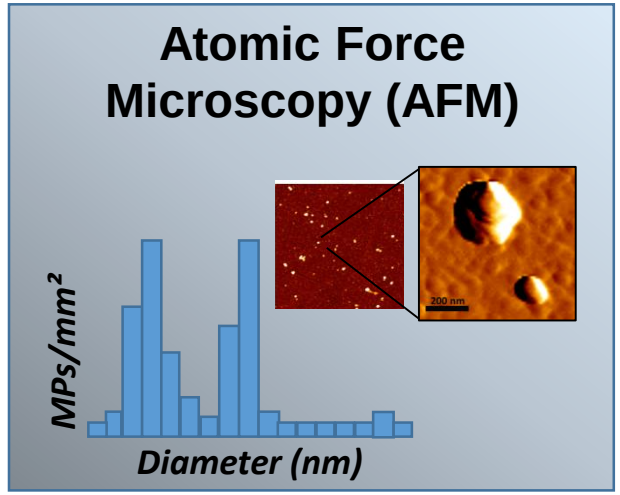
HOME-MADE BIOCHIPS

The NanoBioAnalytical (NBA) platform

IN ORIGINAL SAMPLES

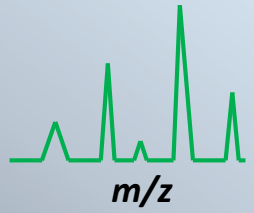
LABEL-FREE

## Atomic Force Microscopy (AFM)




MP viewing, sizing and counting

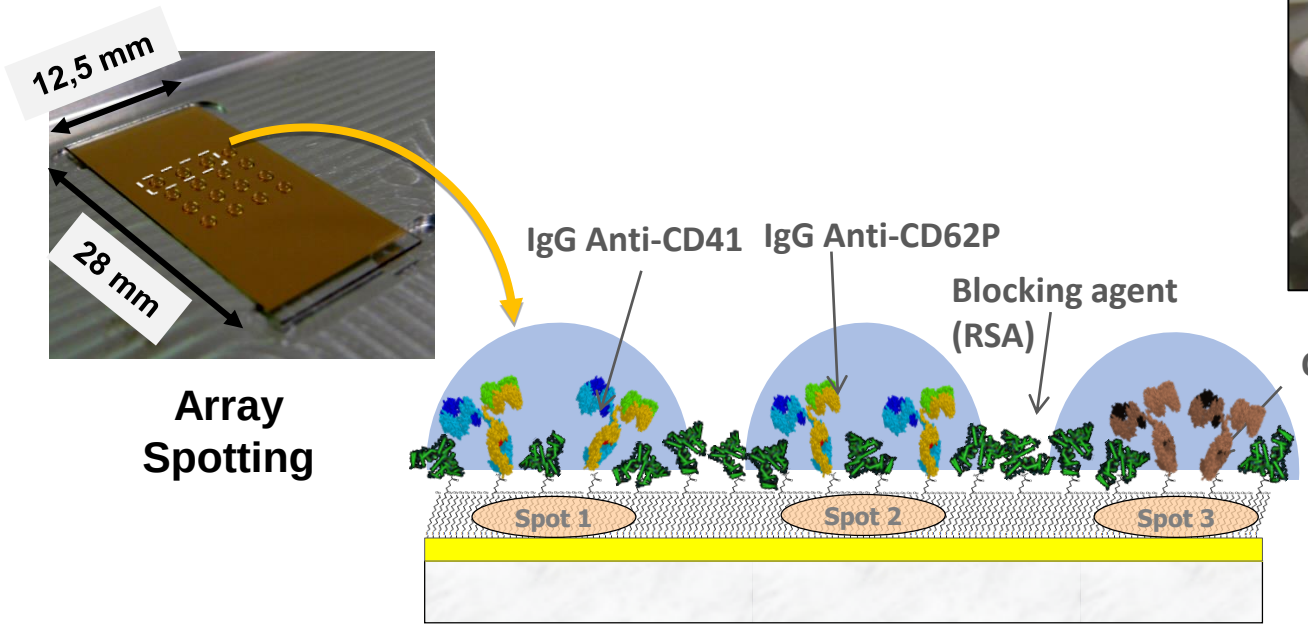
## Mass Spectrometry (MS)

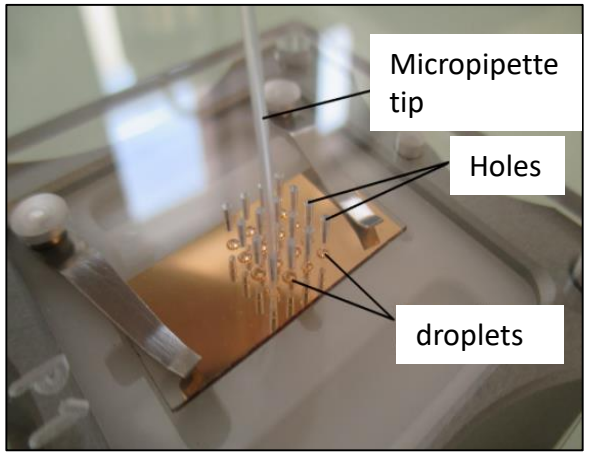


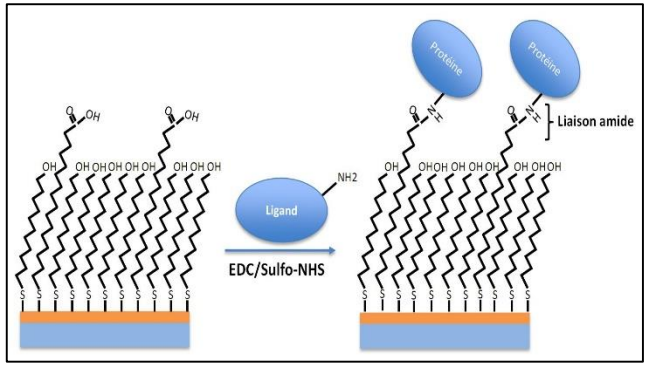

MPs proteomic profiles

# The gold biochip: the corner stone of NBA

Design of the biochip for EVs capture:

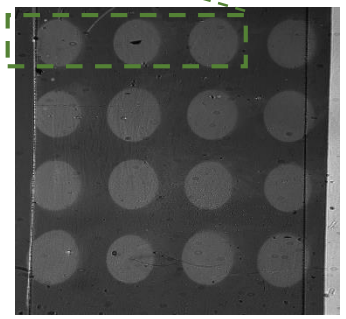


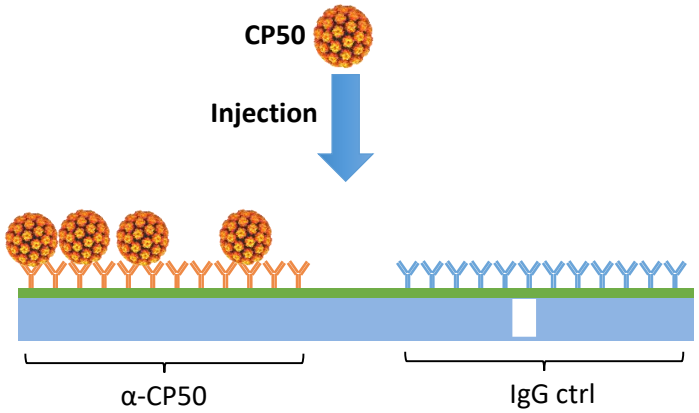

**Modular biointerface:**
1 to 96 spots

**Surface coverage:**
Until 15 000 Ab/µm²



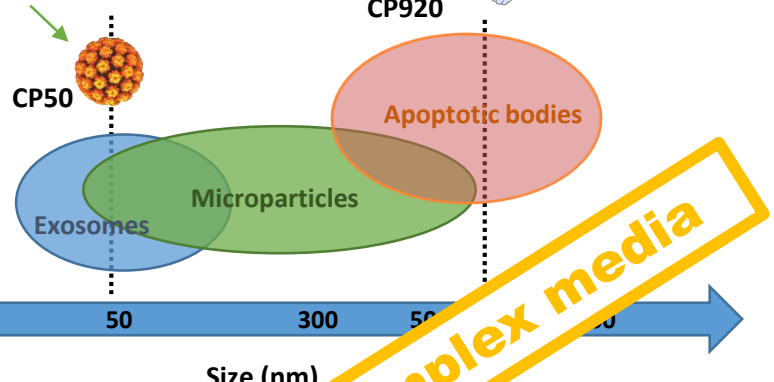
**Phase contrast image of the biochip in SPRi-Plex II (Horiba)**

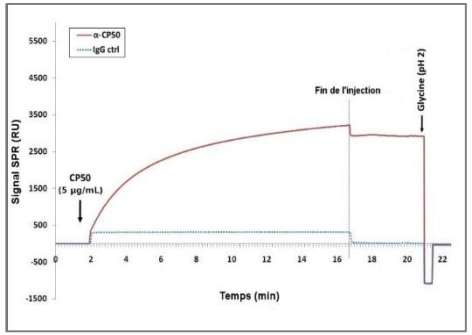
# Calibration of NBA: nanoparticles covering 50 nm to 1µm

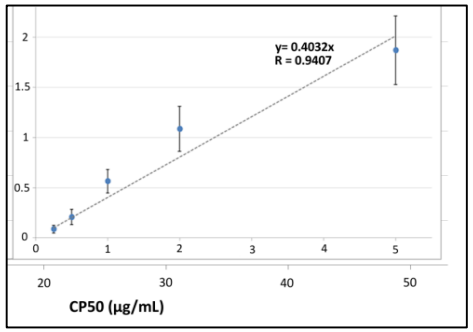



- VP1 capsid protein of norovirus
- 50 nm
- Anti-CP50

- Biofunctionnalized bead
- 920 nm
- Anti-CP920



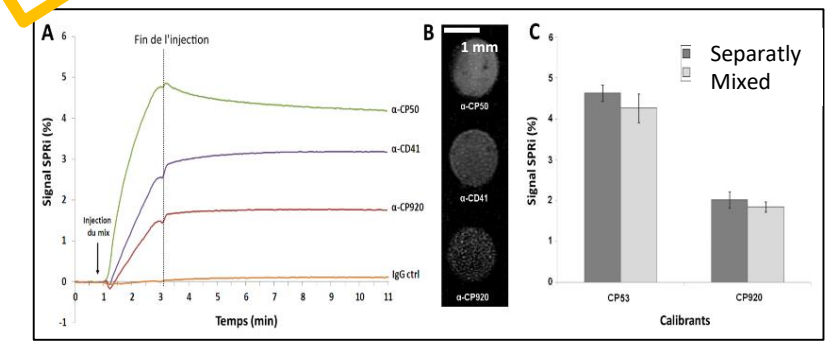



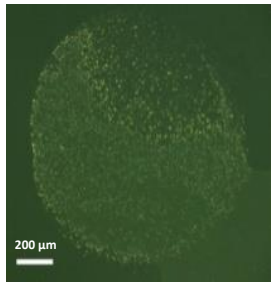



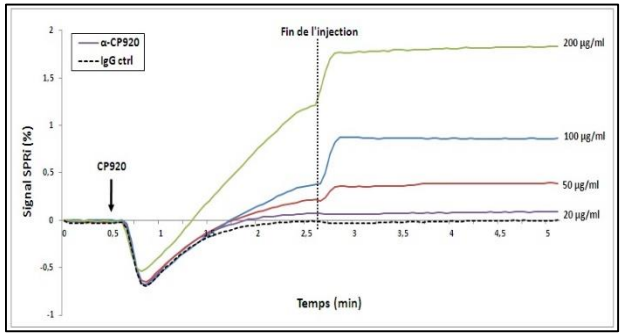

**Specific & stable CP50 capture**

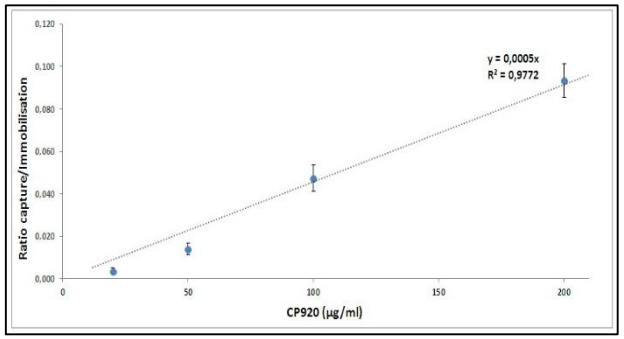
**Dynamic range for CP50 :**

**$2{,}5x10^{9}$ – $2x10^{11}$ CP50/mL**




**Specific & stable CP920 capture**

**Dynamic range for CP920 :**

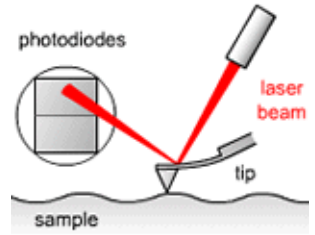
**$3{,}25x10^{7}$ – $3{,}25x10^{8}$ CP920/mL**

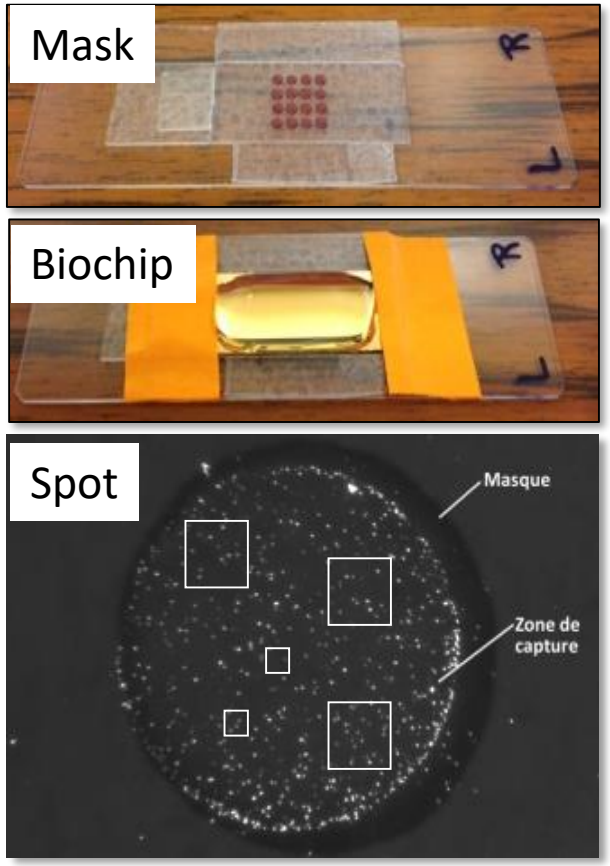
**NBA efficient in complex media**

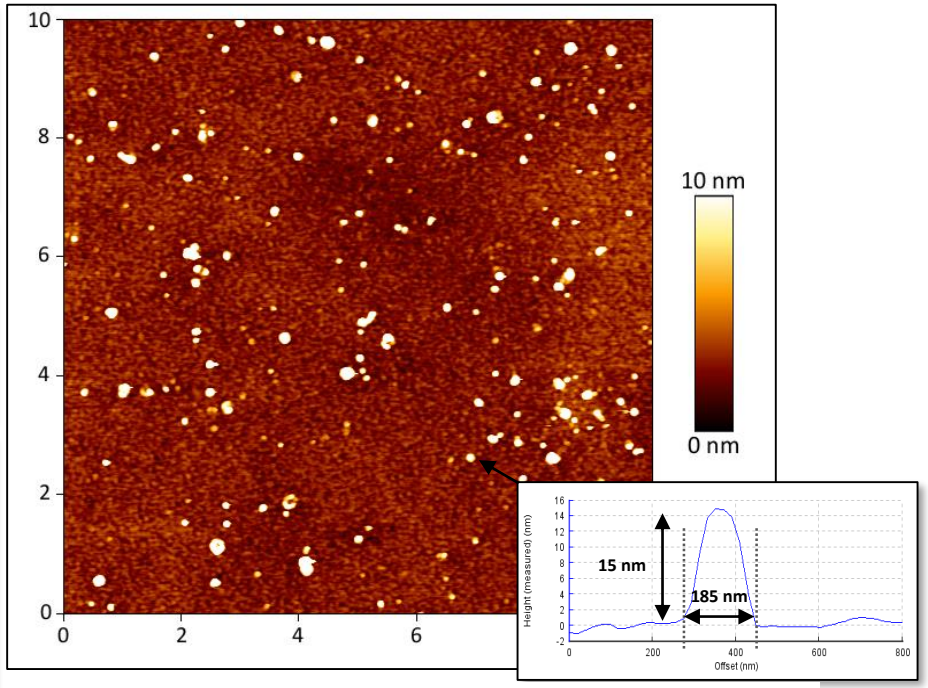
# AFM to achieve EVs size subsets and morphology

Platelet-derived EVs

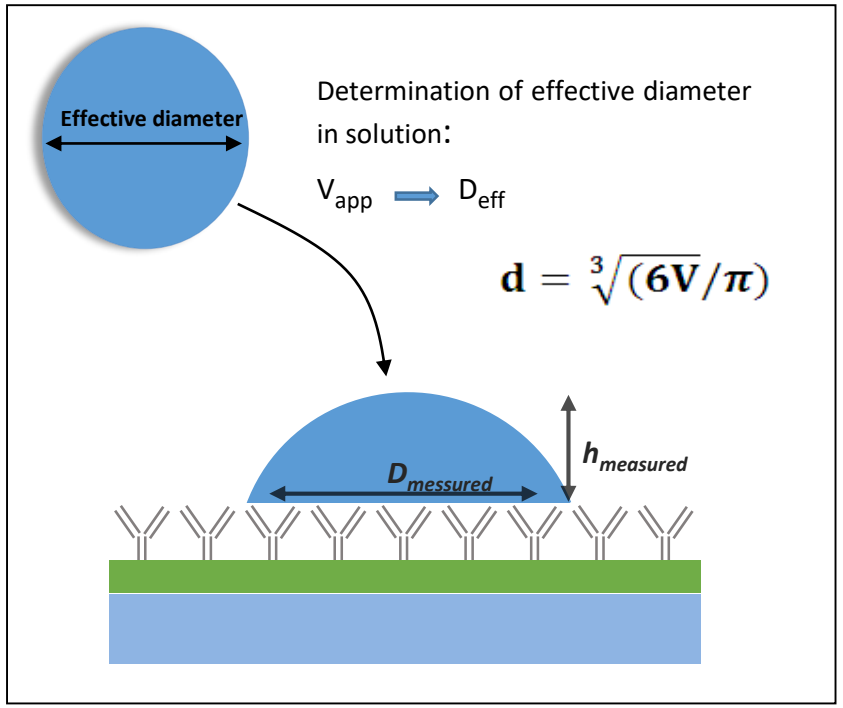
**EVs imaging after fixation, in air …**




**Gold biochip after EVs immunocapture. AFM image (contact mode) in air after EVs glutaraldehyde fixation.**



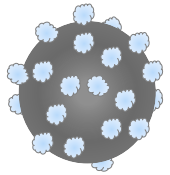

(Kanno et al. 2002; Oropesa et al. 2013; Sebaihi et al. 2017).

**-Several area imaged on each spot**
**-At least 1000 EVs counted/spot**

- **EVs = Individual « flatten » objects**
  **→ Recalculation of <u>effective diameter</u>**

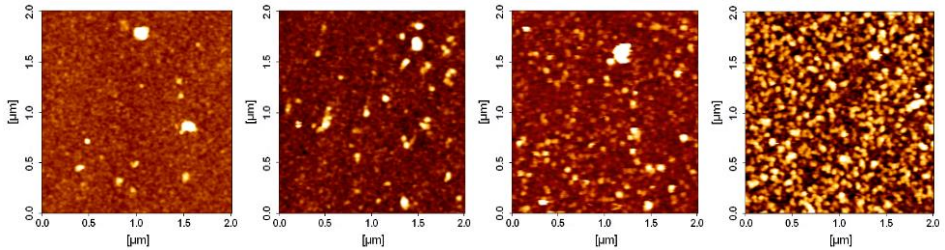
# Correlation between EVs SPRi biodetection and AFM counting

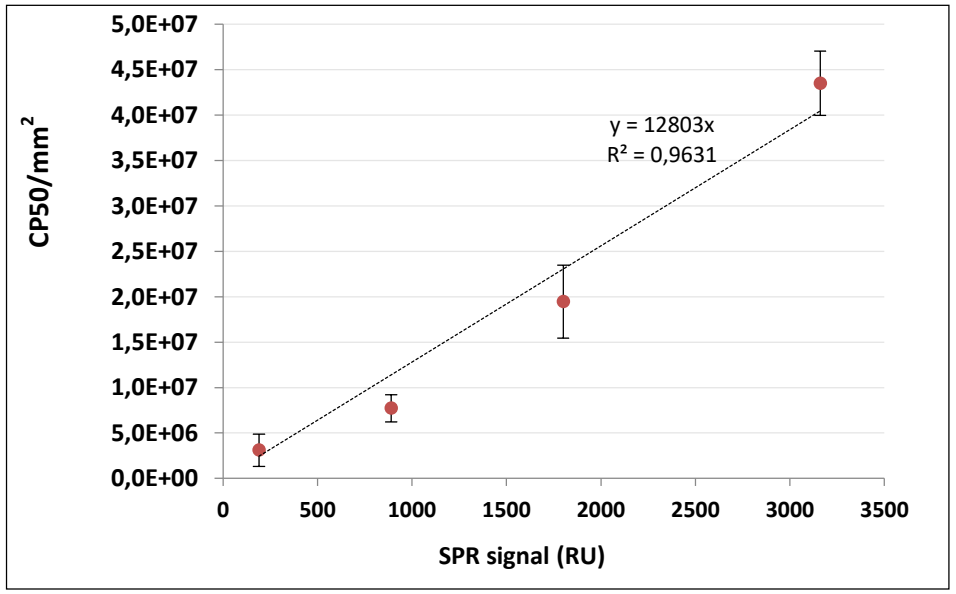
**CP50**



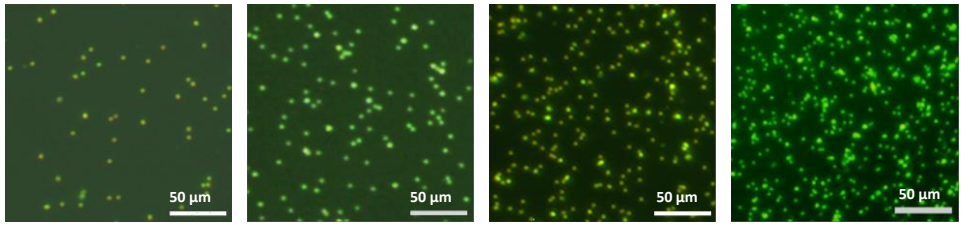
**CP920**

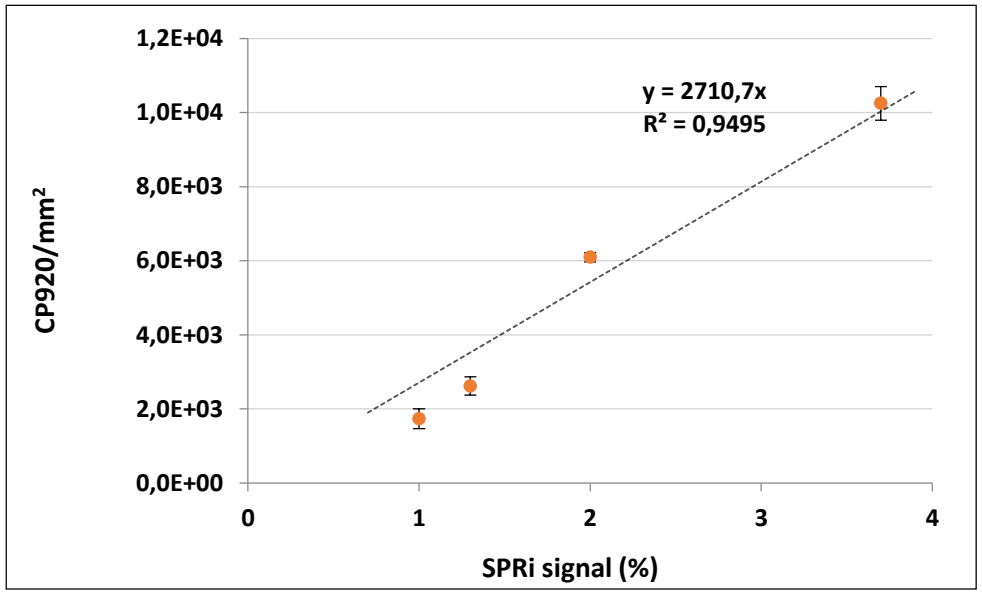
**190 RU** **890 RU** **1800 RU** **3160 RU**




**1 %** **1,3 %** **2 %** **3,7%**

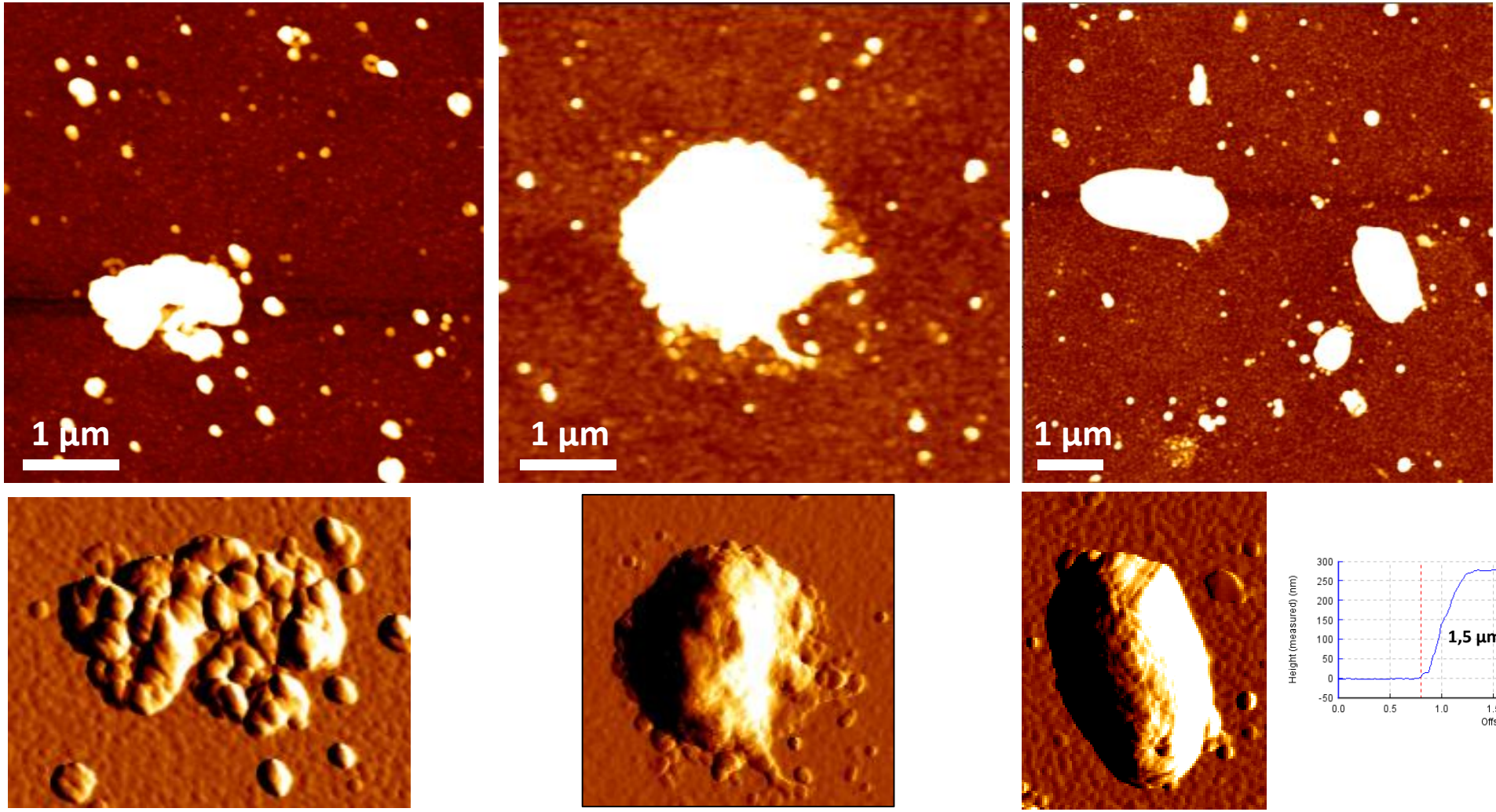



➢ **SPRi/AFM correlation in EVs analysis, from nm to µm**

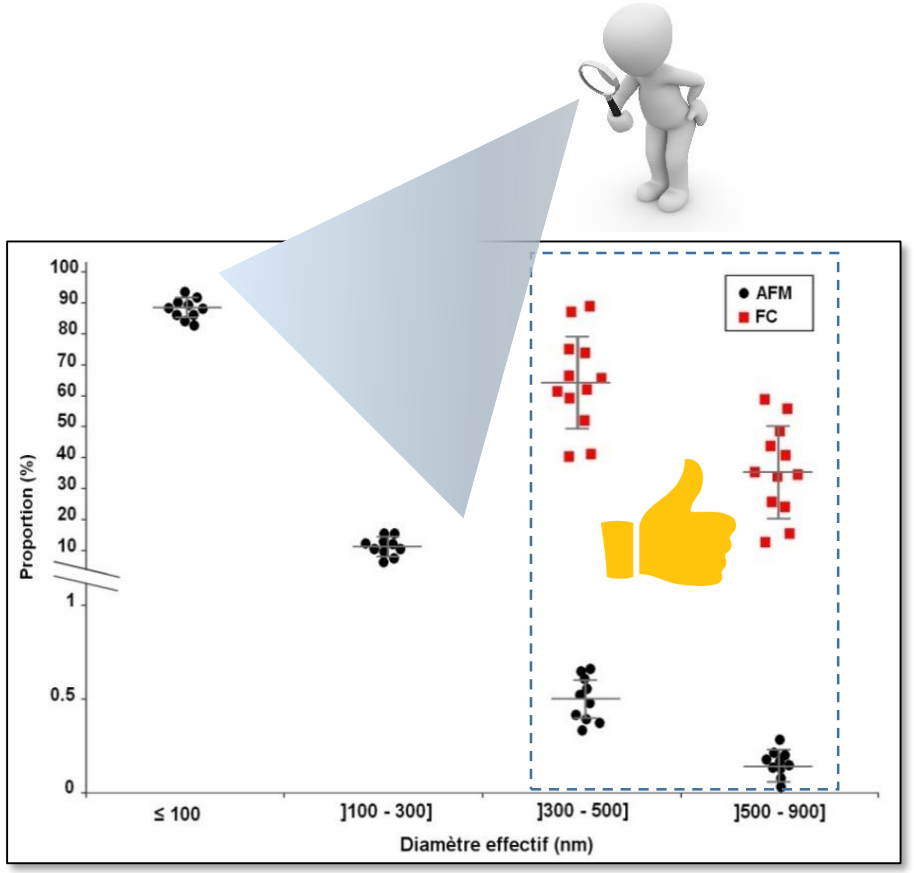
# AFM : size, but also morphology

Platelet-derived EVs




➢ **Discrimination between aggregates & individual big EVs**

# NBA for EVs biodetection, sizing and morphology




**Size distribution of immunocaptured EVs by AFM on gold biochip (black circles) or in solution by FC (red squares) on the same sample.**

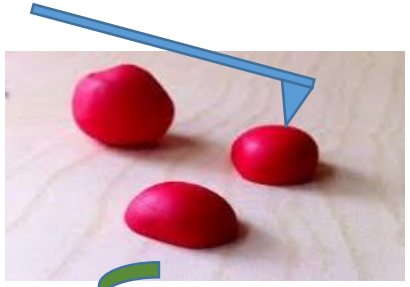
➢ **NBA efficient to dose, select, spatialize, evaluate size and morphology of EVs subsets**

*Obeid S., Ceroi A., Mourey G., Saas P., Elie-Caille C. Boireau W.,*
*Biosensors & Bioelectronics, 2017, 93, 250-259*

# EVs : soft and deformable vesicles…

***How to keep them spherical while captured on substrate ?***

**Impact of AFM imaging mode?**

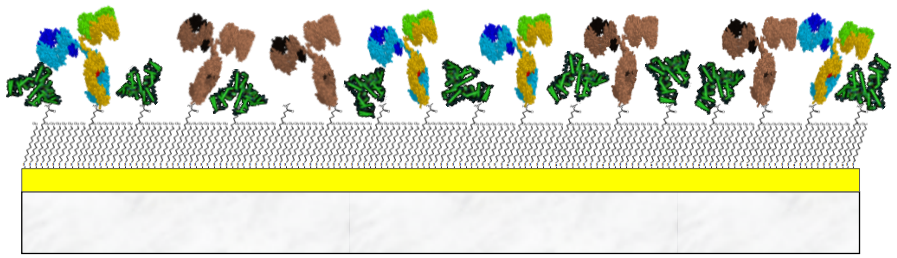


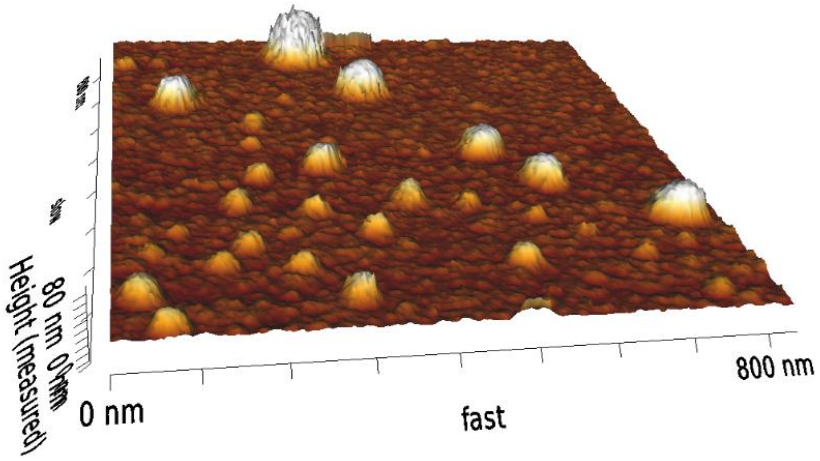
**Impact of the biointerface ?**

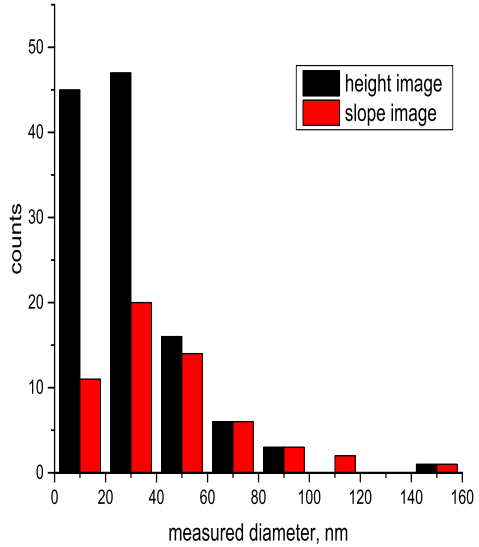
**Quantitative Imaging mode**

w/o EVs fixation, in liquid…

**On « diluted » IgG biointerface**






**→ Dilution of the specific Ab and soft afm imaging mode = spherical EVs**

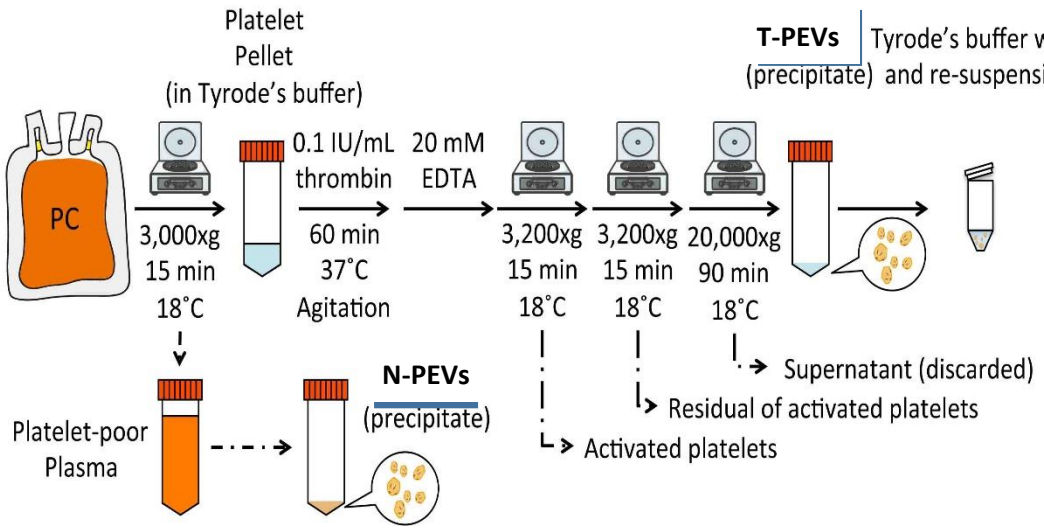
***Poster 09.01 of Ksenia Maximova – Saturday***

# Application of NBA to a biological model: platelet derived EVs effect on monocytes

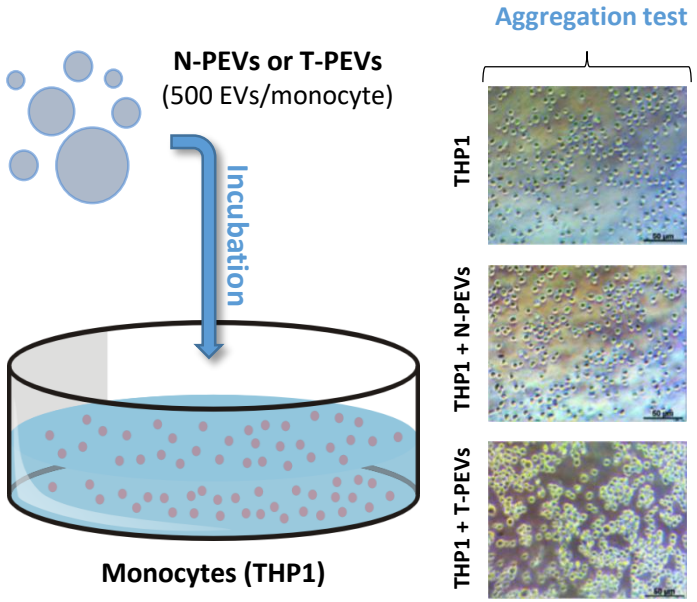
*Collaboration : T. Burnouf, Taiwan*

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

## EVs preparation



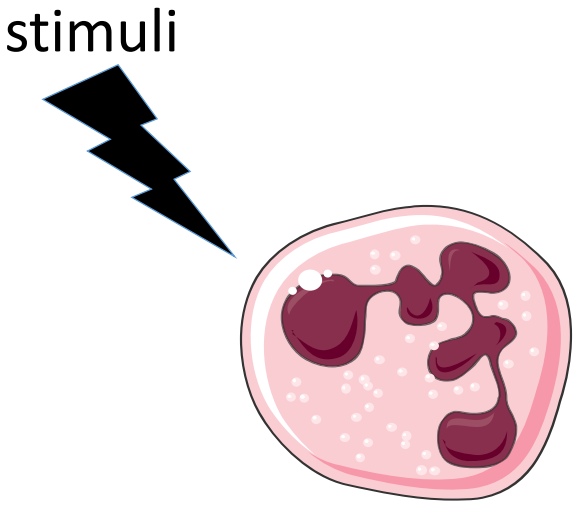

## EVs function on monocytes




Lin et al. Transfus Apher Sci. 2015

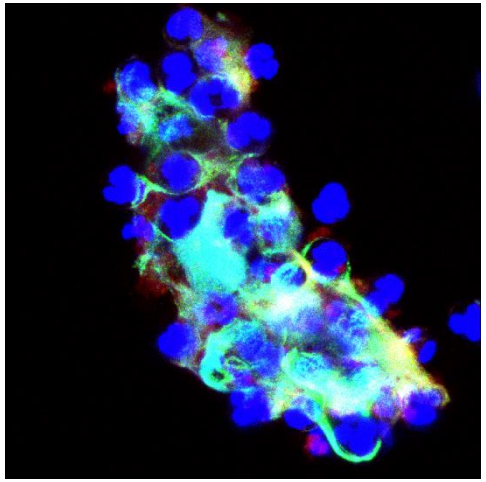
**T-PEVs induce aggregation of THP1…**

# Neutrophil aggregation and extracellular traps (NETs)

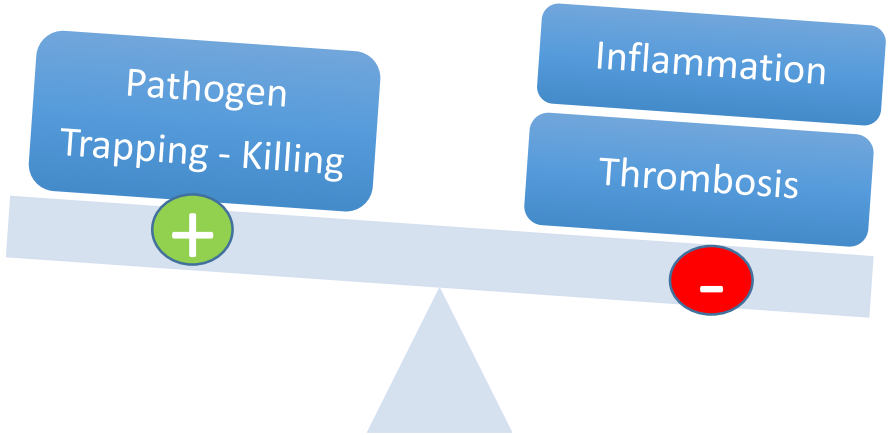



neutrophils release granule proteins and chromatin to form an extracellular fibril matrix known as NETs

Pathogen trapping & killing

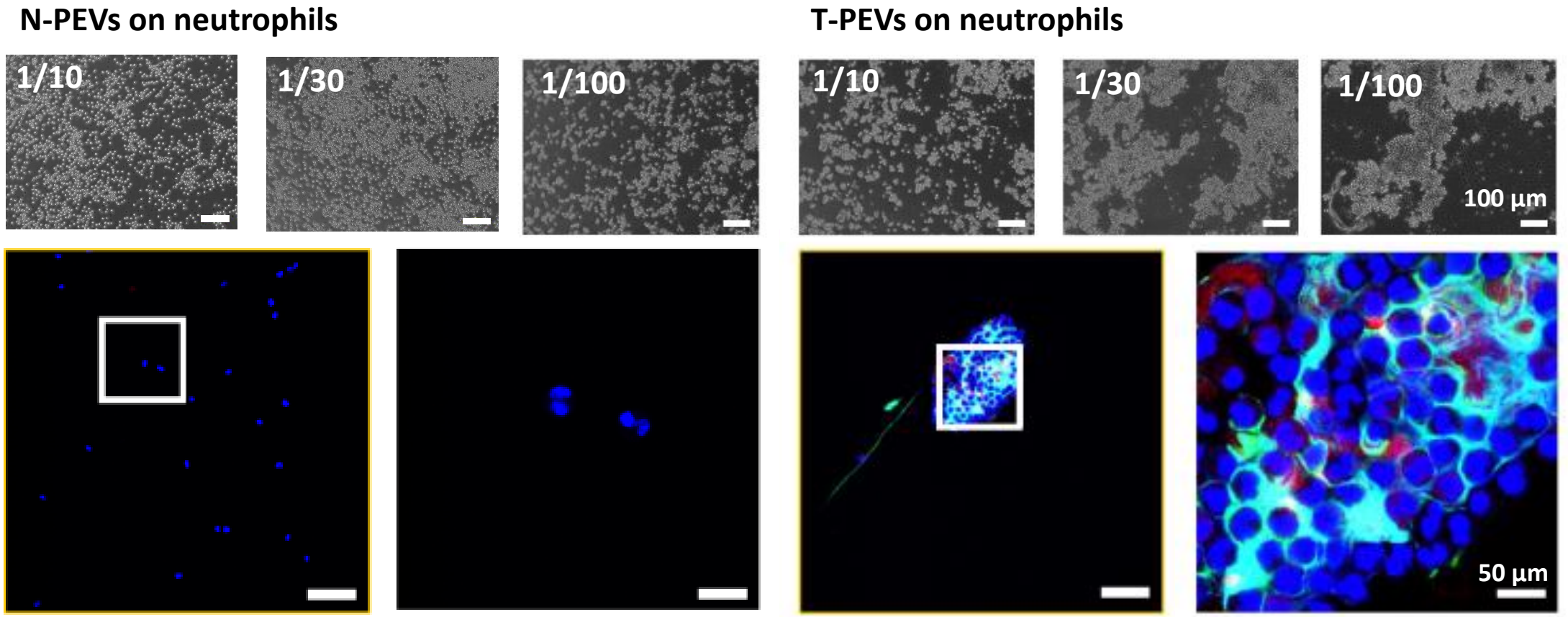


## NETs: paradoxical physiological impact ?




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

# Neutrophil aggregation and extracellular traps (NETs)



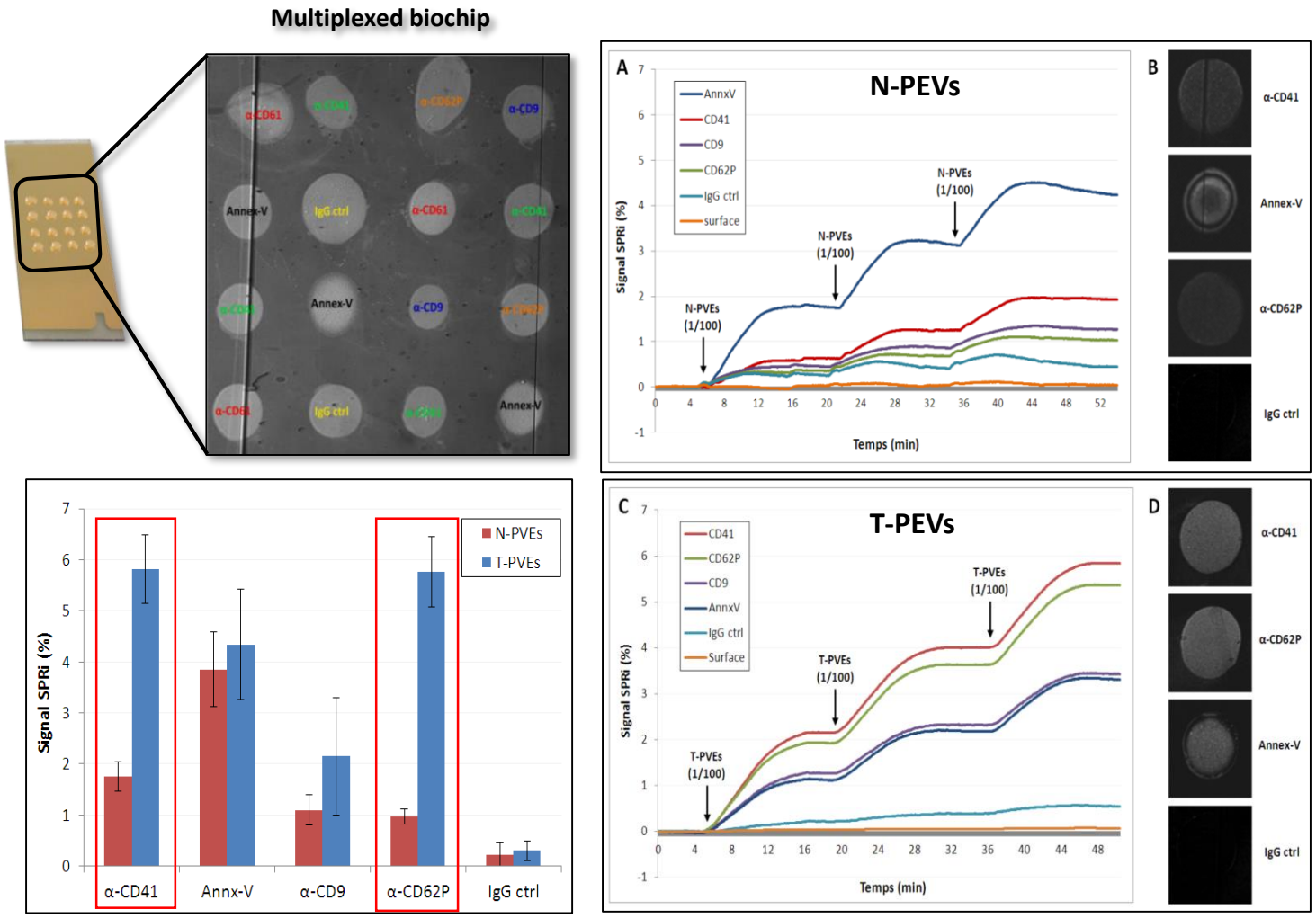

*DNA (blue), histone H1 (green), and MPO (red)*

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

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

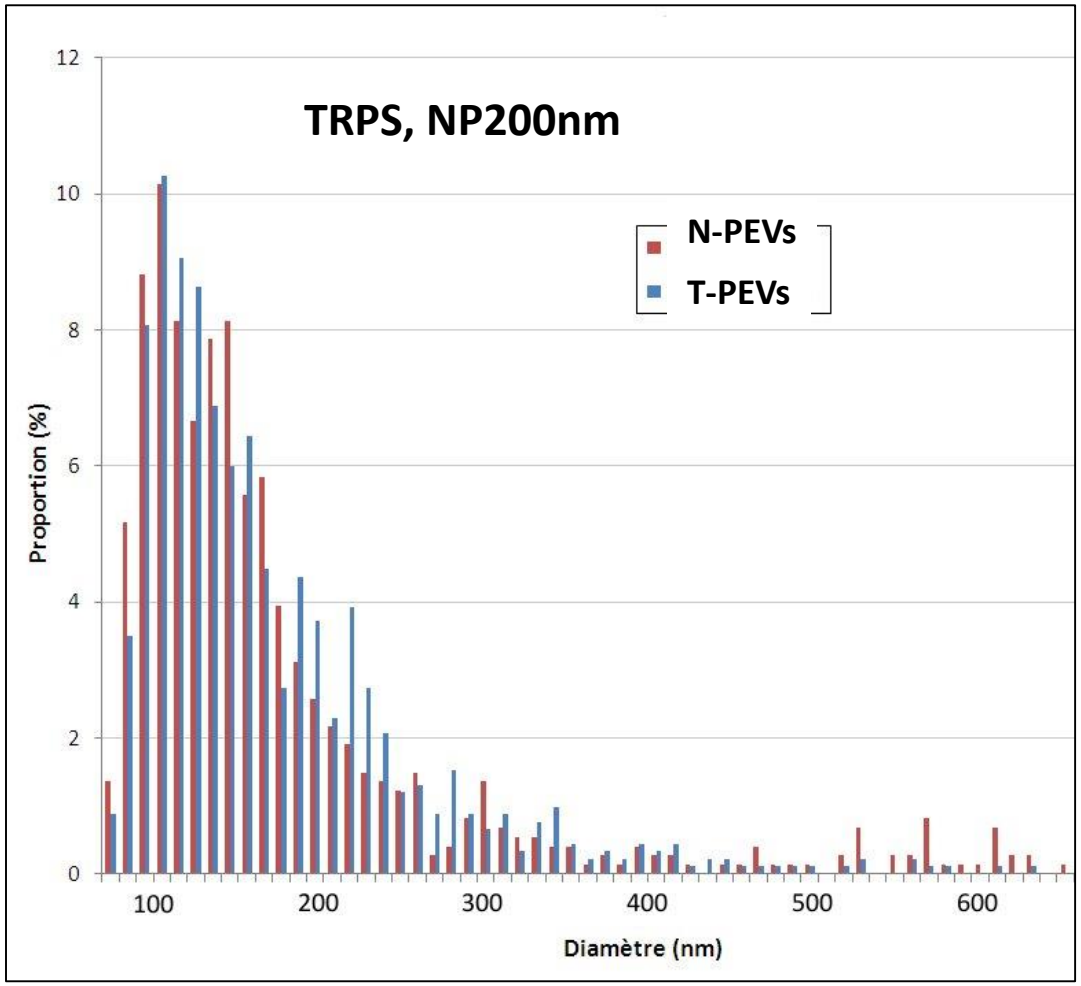
# EVs subsets biodetection on the chip

*Collaboration :*
*T. Burnouf,*
*Taiwan*




- **T-PMP : capture +++ on aCD41 and aCD62P**
  **EVs concentration, size, protein expression level ??**

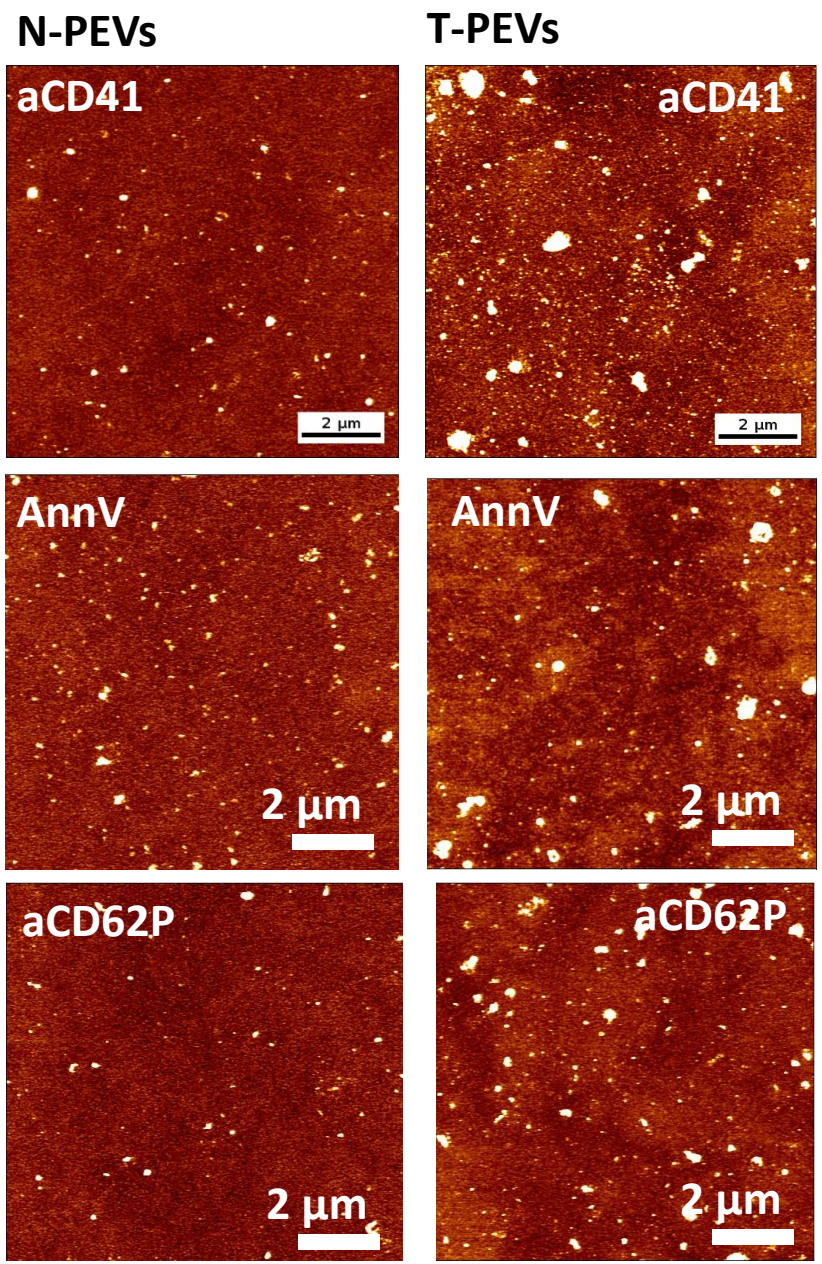
# Concentration, in solution ? no




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

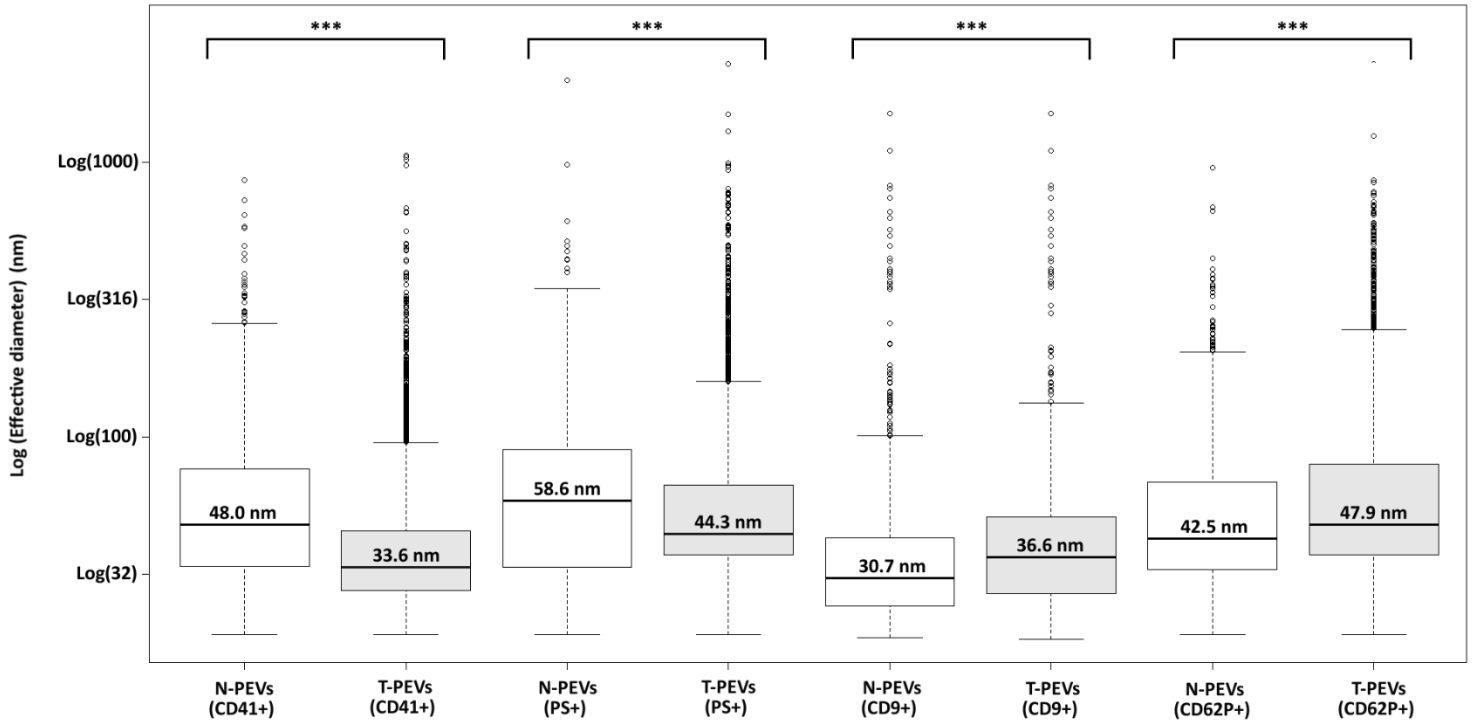
→ N-PEV sample : higher concentration !! / no apparent difference in size

# Size ? … partially




Gwiddion image data processing
(more than 1000 EV counted/spot)

**EV effective diameter**

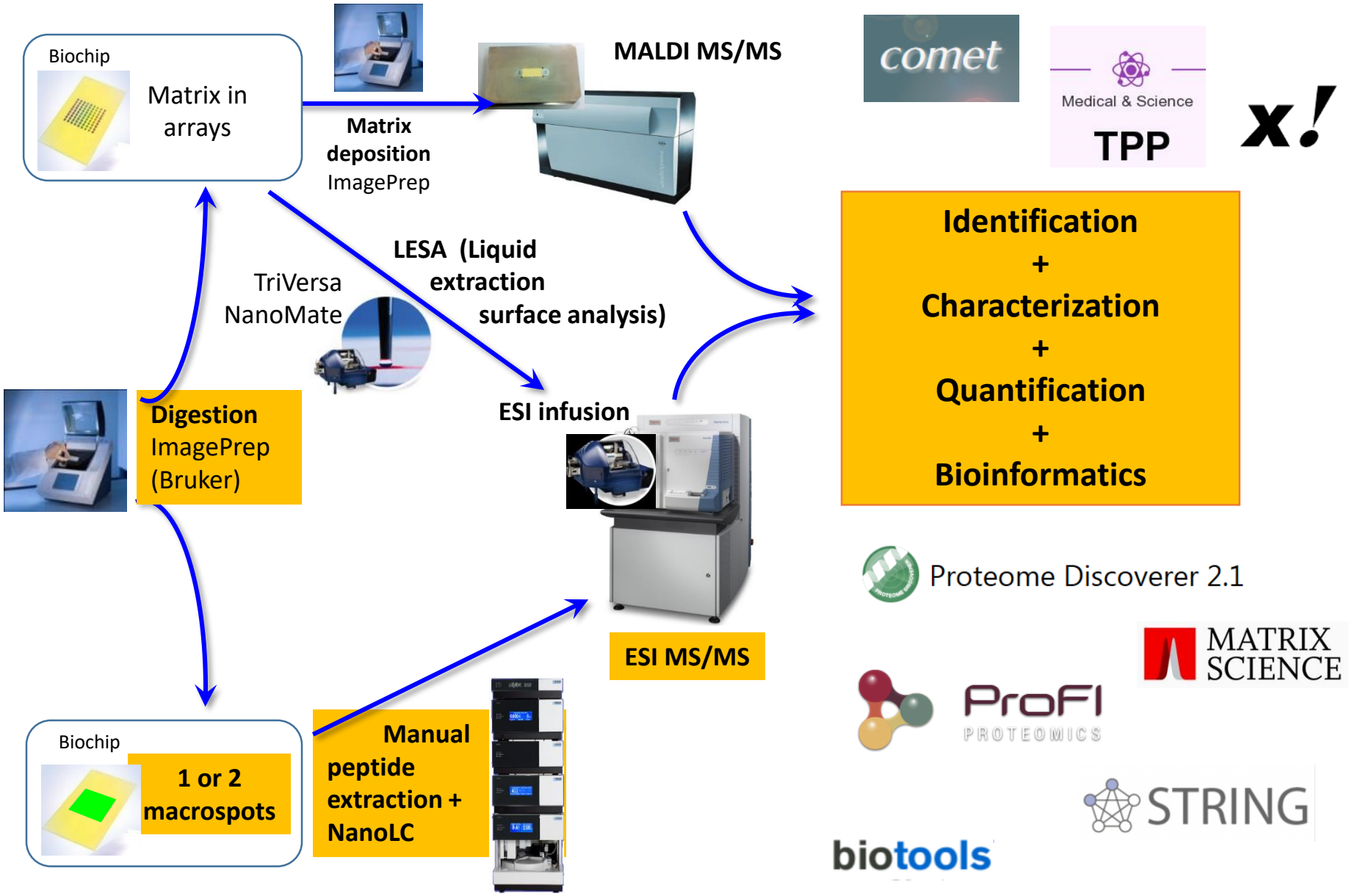



→ T-PEV CD41+ and PS+ : slightly smaller than N-PEVs

→ T-PEV CD9 and CD62P : slightly bigger than N-PEVs

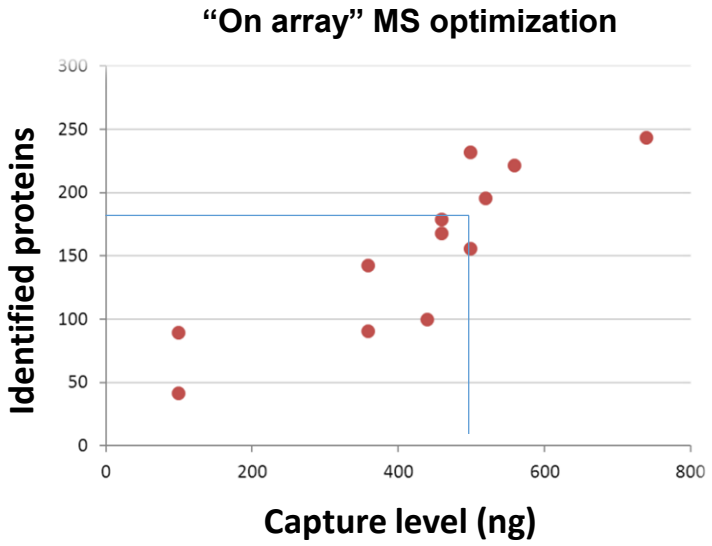
# Protein expression level ? …

MS workflow in detail for NBA



# Protein expression level ? …

## EVs proteomics « on arrays »

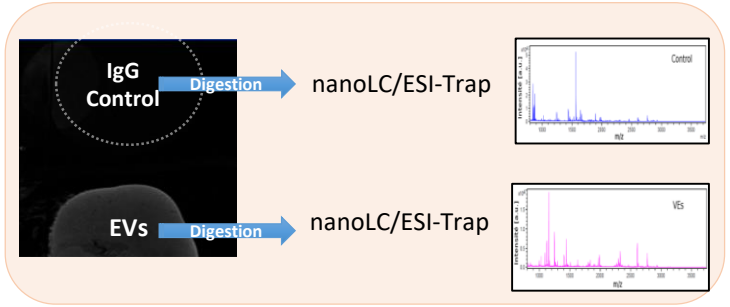


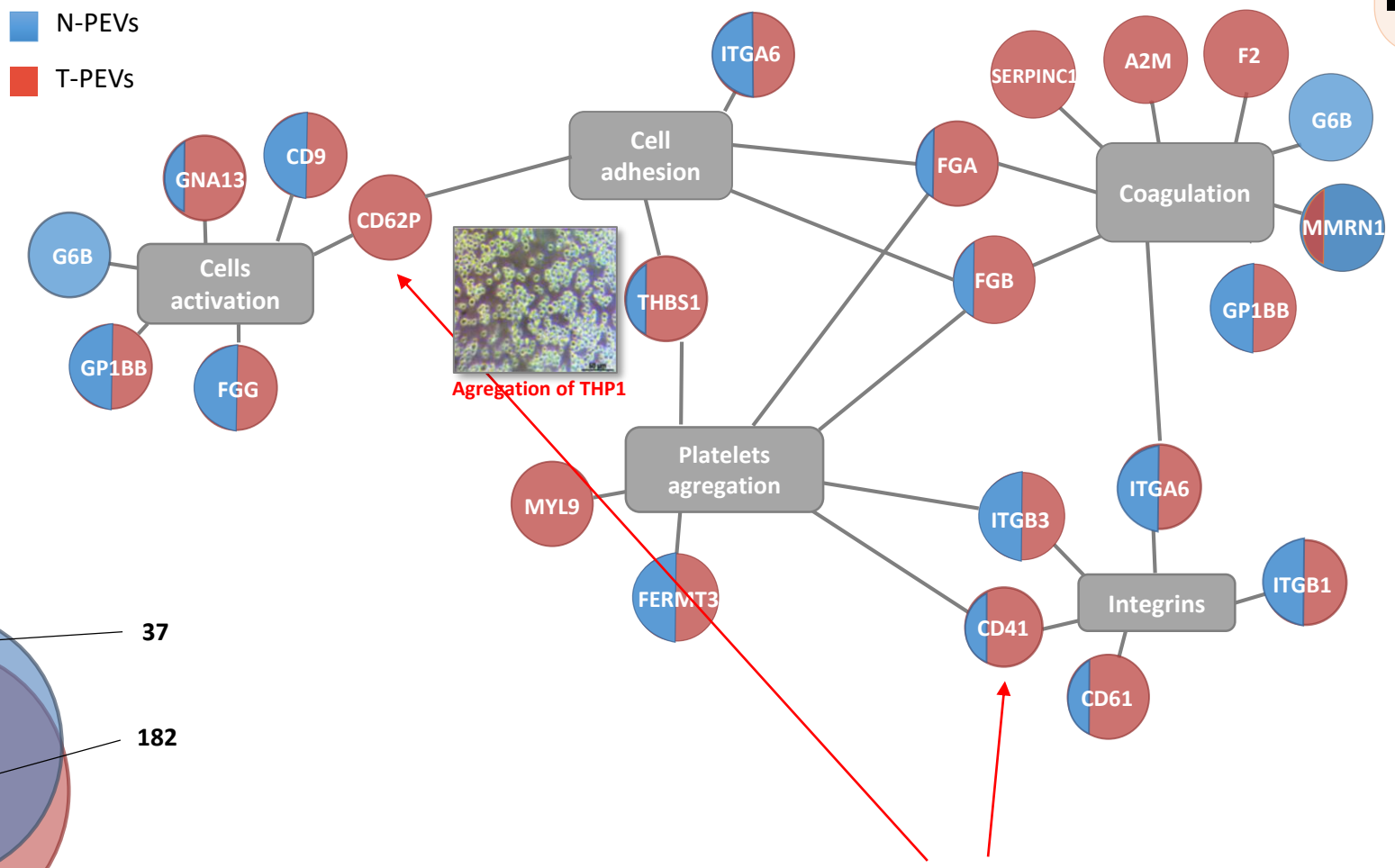

| | | T-PEVs | | N-PEVs | |
|---|---|---|---|---|---|
| Protein Set | Description | Score T-PMP | Peptides T-PMP | Score N-PMP | Peptides N-PMP |
| Q9Y490 | Talin-1 | 3232,74 | 49 | 3943,33 | 61 |
| P21333 | Filamin-A | 2805,99 | 47 | 3778,84 | 62 |
| A0A024QZN4 | Vinculin, isoform CRA_c | 1275,97 | 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 |
| ..... | ..... | ..... | ..... | .... | ..... |
| ..... | ..... | | | | |

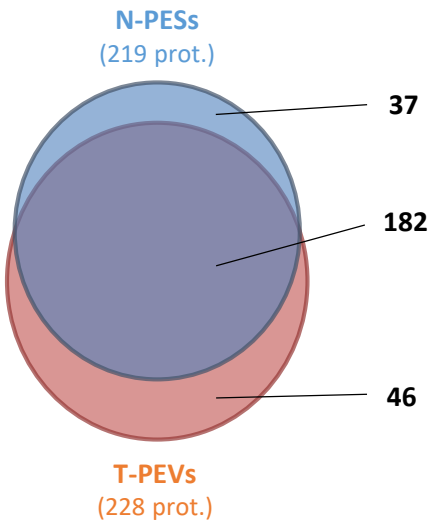
**~ 200 proteins identified from ~ 500 ng of captured EV and several differential proteins …**

*Collaboration : T. Burnouf, Taiwan*

# Protein expression level ? Yes …



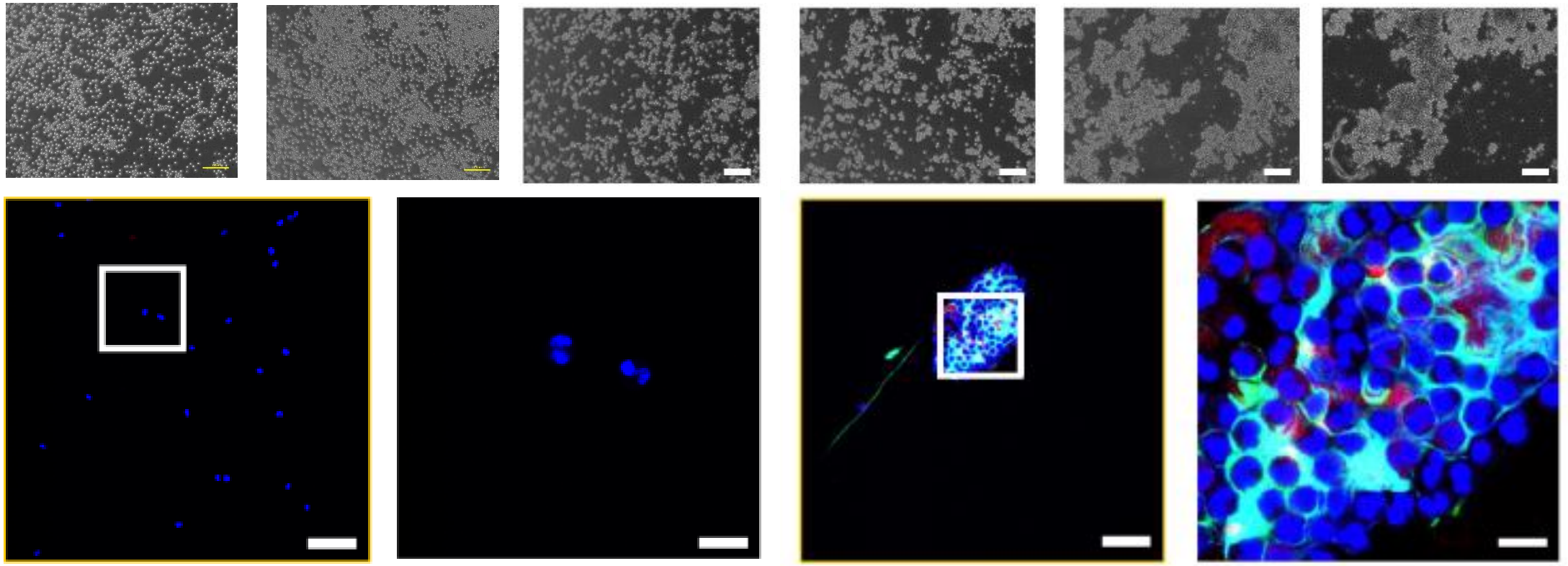

**Differencial MS profiles**

*Obeid S. et al, unpublished data*

# Neutrophil aggregation and extracellular traps (NETs)

N-PEVs on neutrophils

T-PEVs on neutrophils



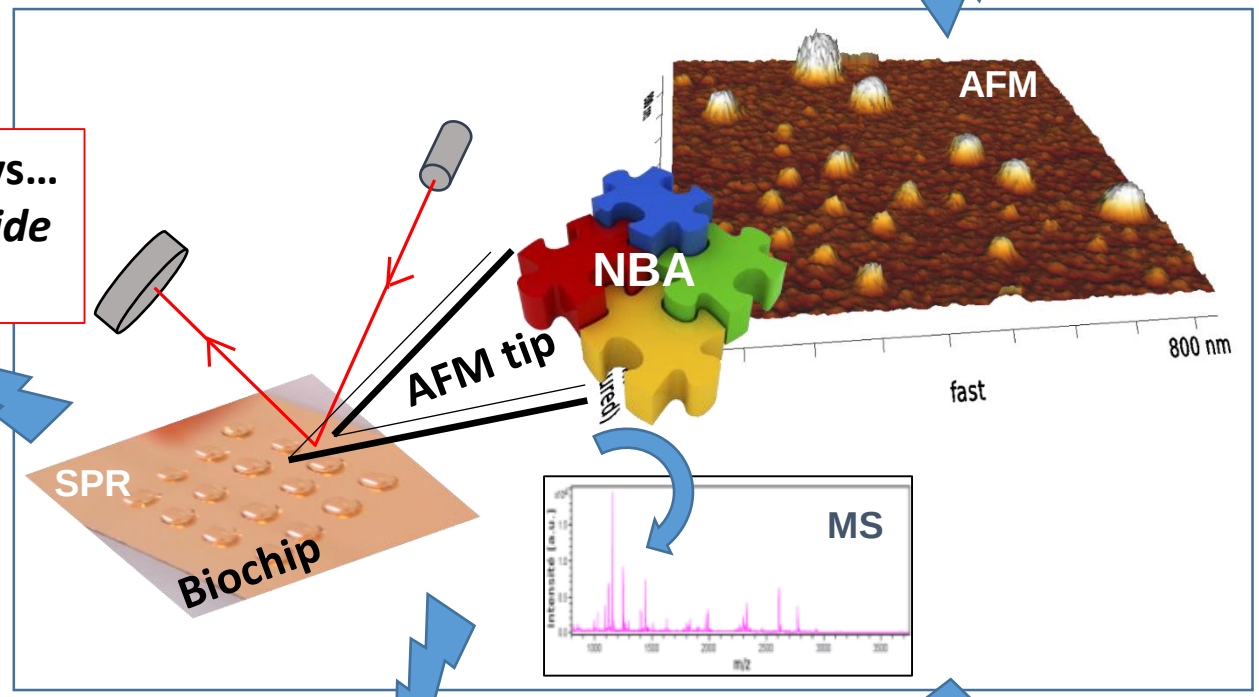
→ T-PEVs induce aggregation of neutrophil aggregation and NETs formation…

| T-PEVs effect on monocytes : | concentration ?? | size ?? | Protein expression ??? |
|---|---|---|---|
| | NO | partially | YES |

# Conclusions and perspectives

Molecular mapping & elasticity

Sandwich assays…
*EVs subsets inside each spot*




Additional modules for
- *EVs production*
- *Sorting*

- Whole protein…
- Multiomics…

# Acknowledgments


### FEMTO-ST Institute & CLIPP

S. Obeid
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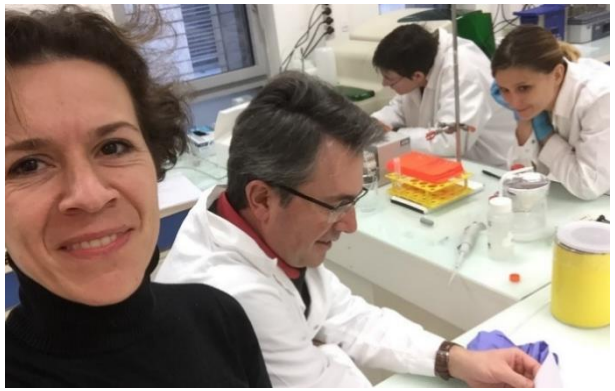
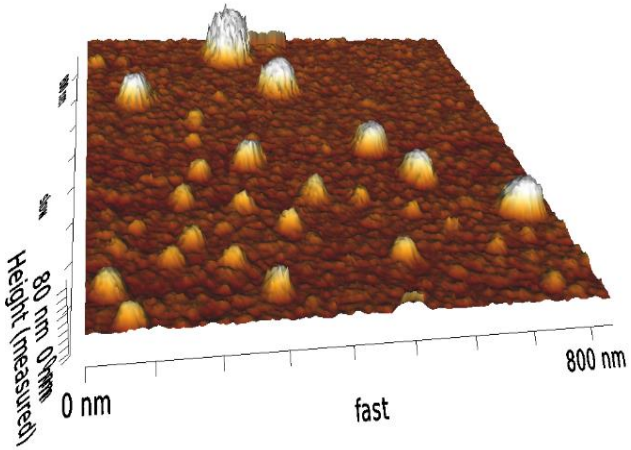



### Collaborators

P. Saas
T. Burnouf

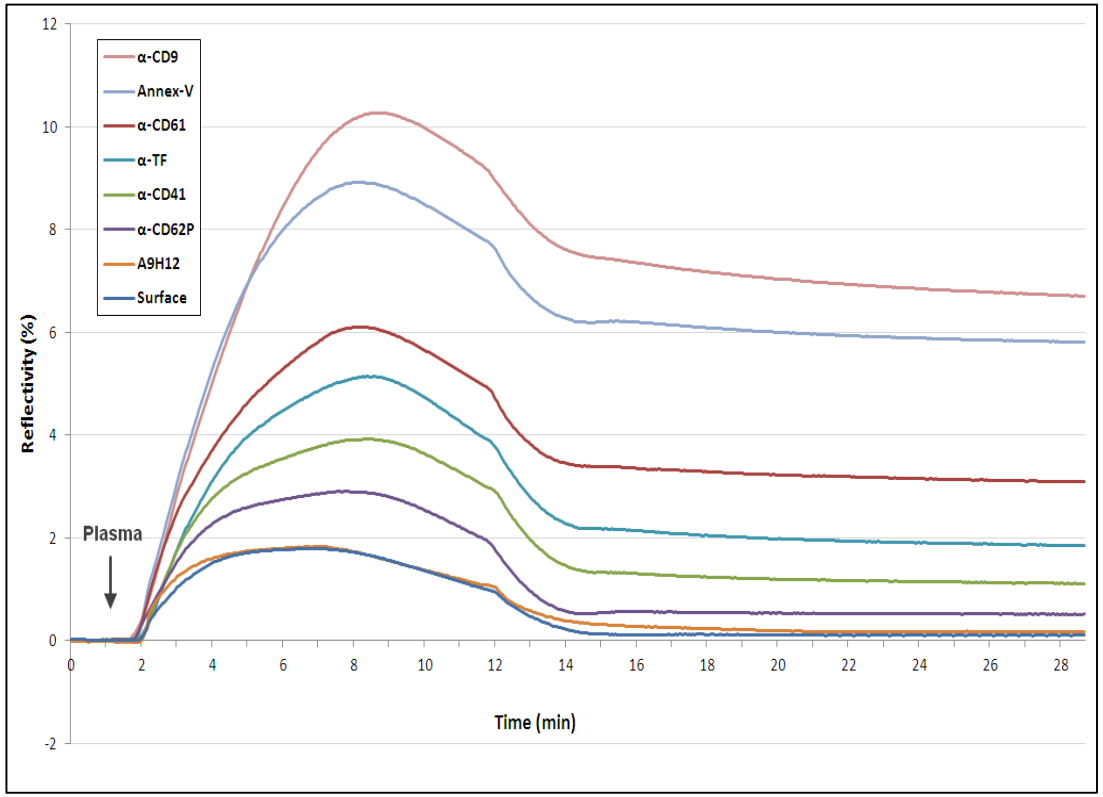
### Fundings

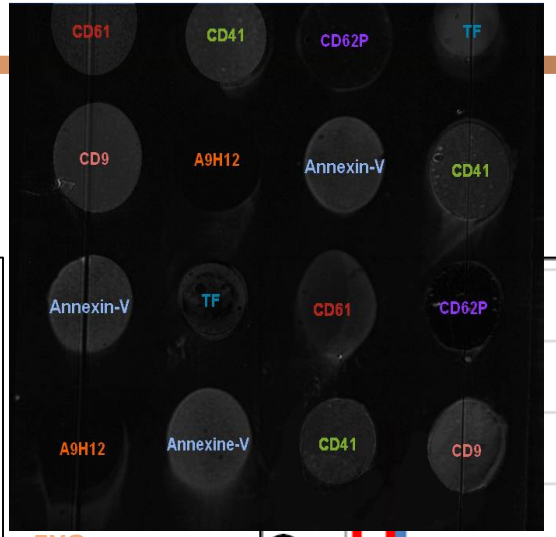
Région Franche Comté (2013, 2017)
CNRS : Defi Nano 2013
CNRS : Défi instrum. aux limites 2017
FEDER « MIMEDI » 2018
ANR 2017 « MADNESS »


**THANK YOU !**

# plasma EVs analysis



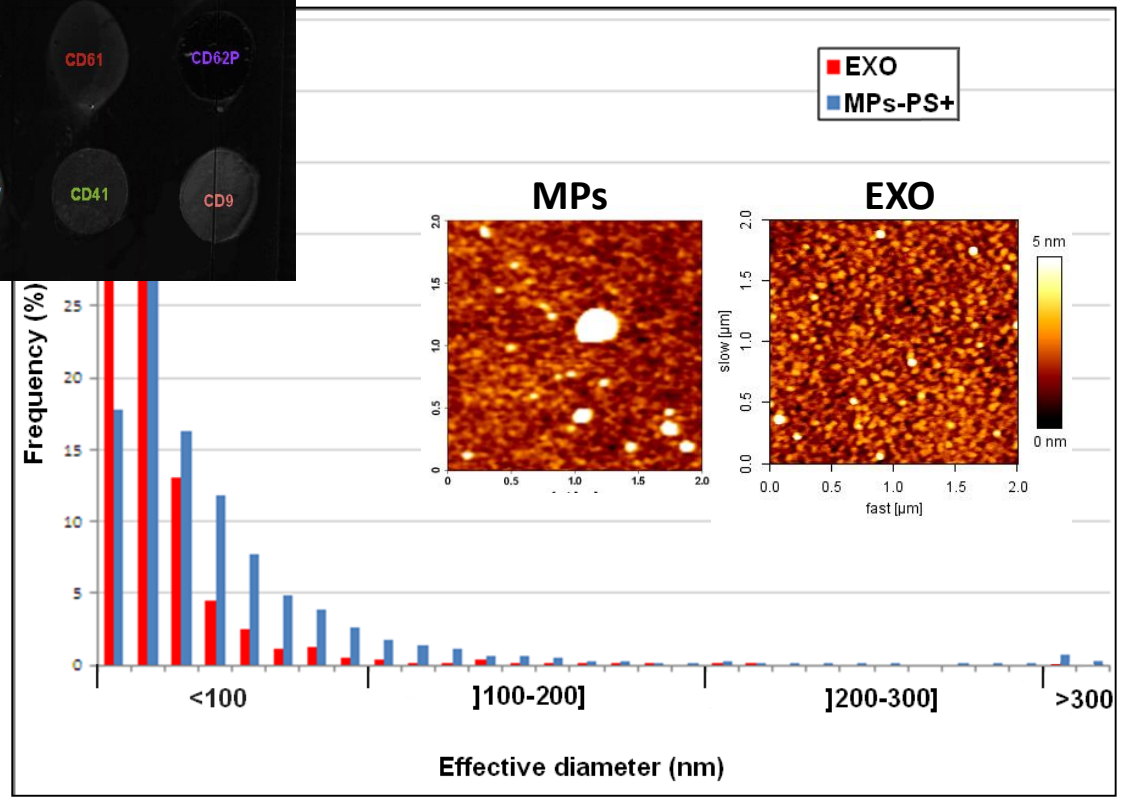

EXO

MP-PS+

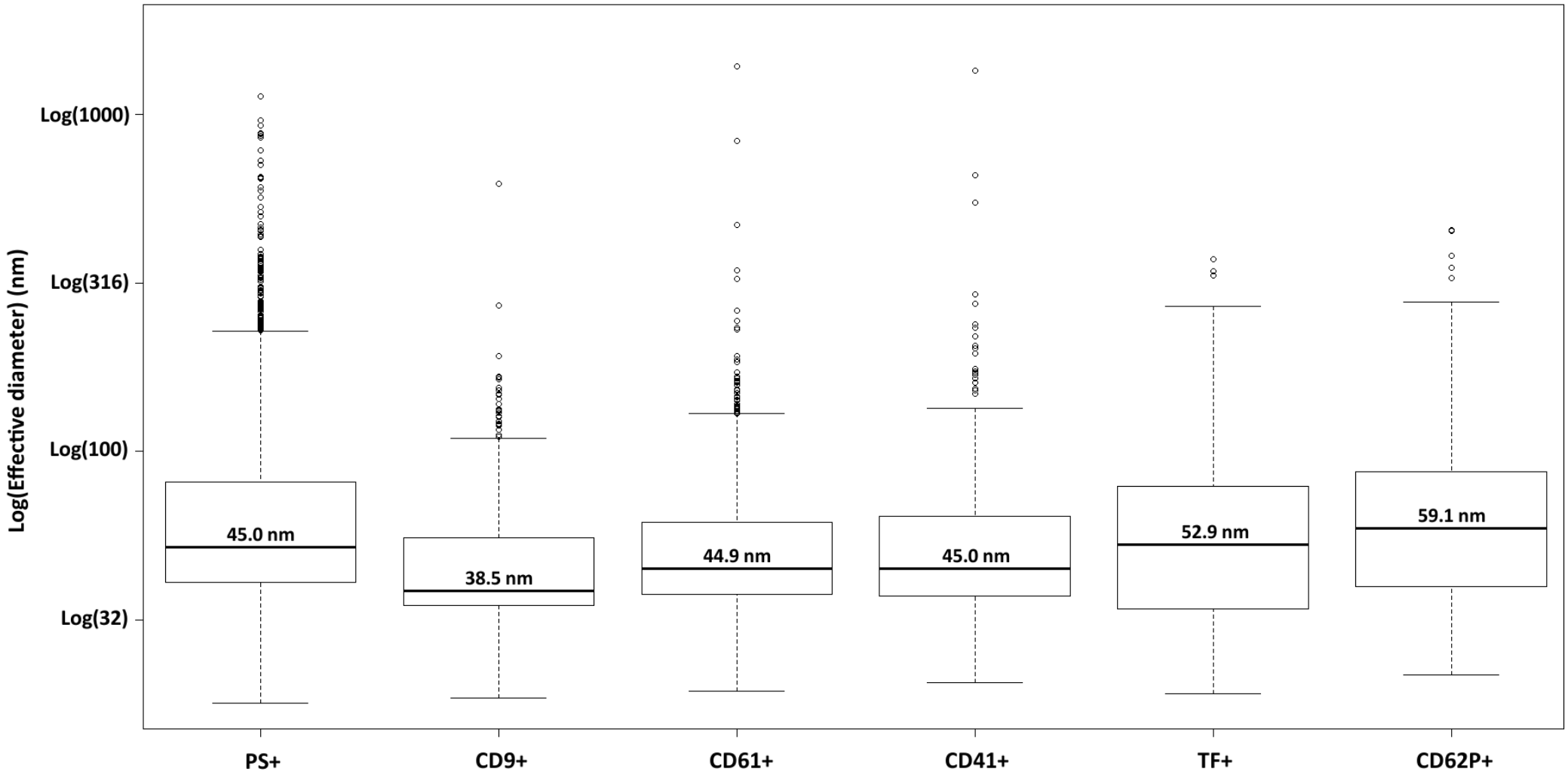
PMPs & EMPs

MP-TF+ PMPs

Activated PMPs




- Selective detection and quantification of the different EV subsets.
- Discrimination between EVs and exosomes


Log(Effective diameter) (nm)
Log(1000)
Log(316)
Log(100)
Log(32)
45.0 nm
38.5 nm
44.9 nm
45.0 nm
52.9 nm
59.1 nm
PS+
CD9+
CD61+
CD41+
TF+
CD62P+