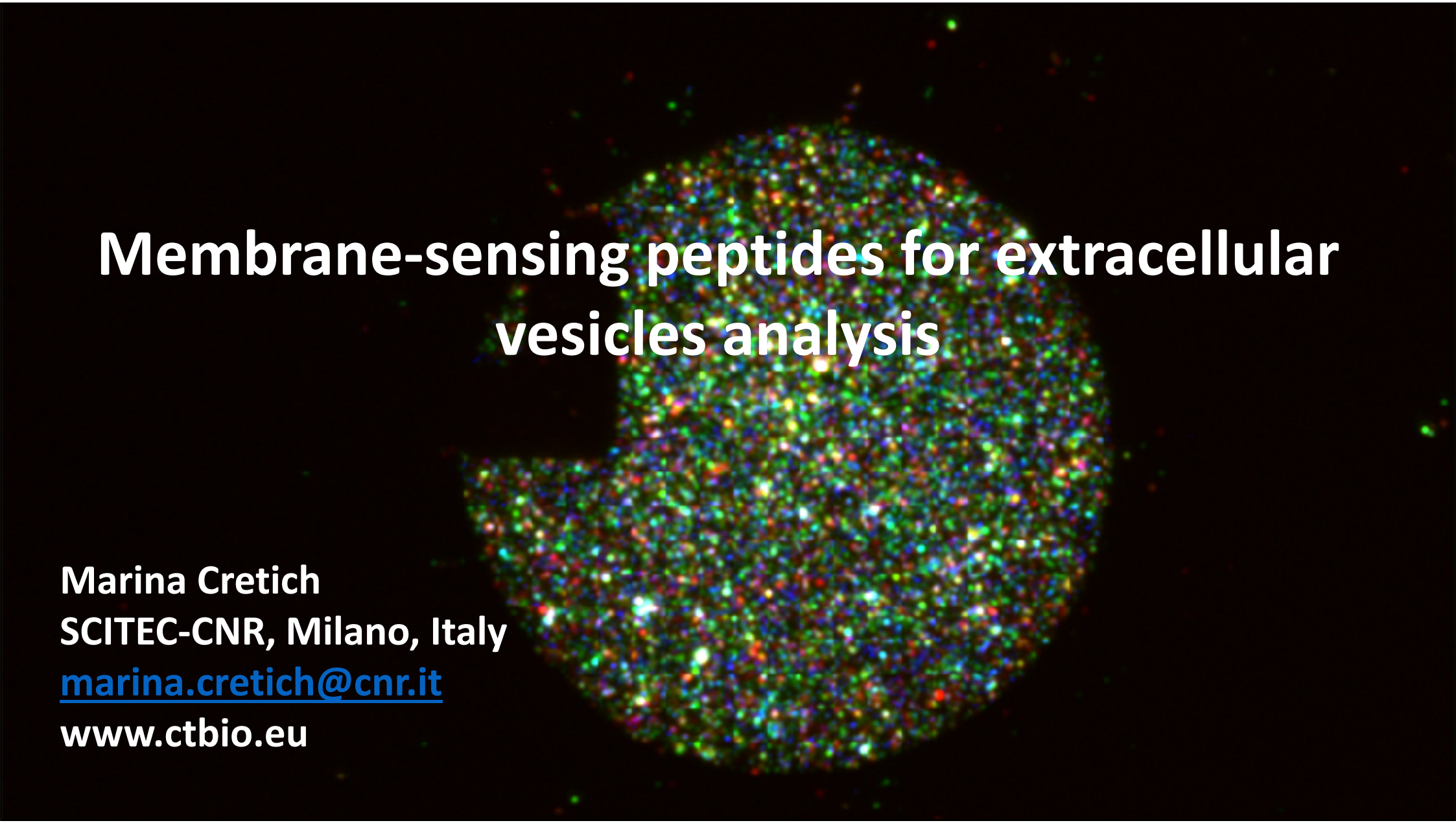
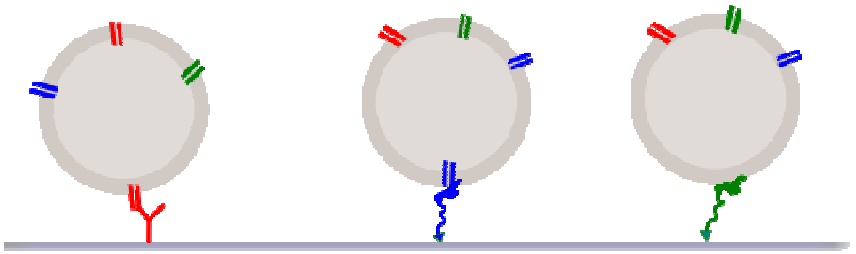
Membrane-sensing peptides for extracellular vesicles analysis



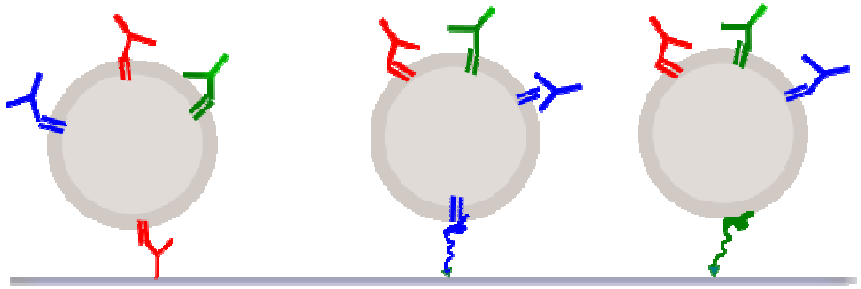
Marina Cretich
SCITEC-CNR, Milano, Italy
marina.cretich@cnr.it
www.ctbio.eu

➢ The context: EV microarrays



Antibody microarrays

Peptide microarrays



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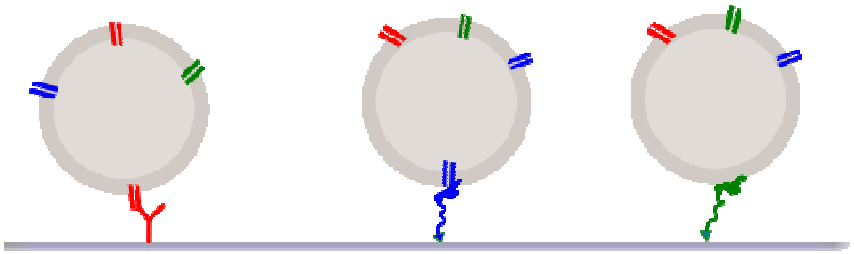
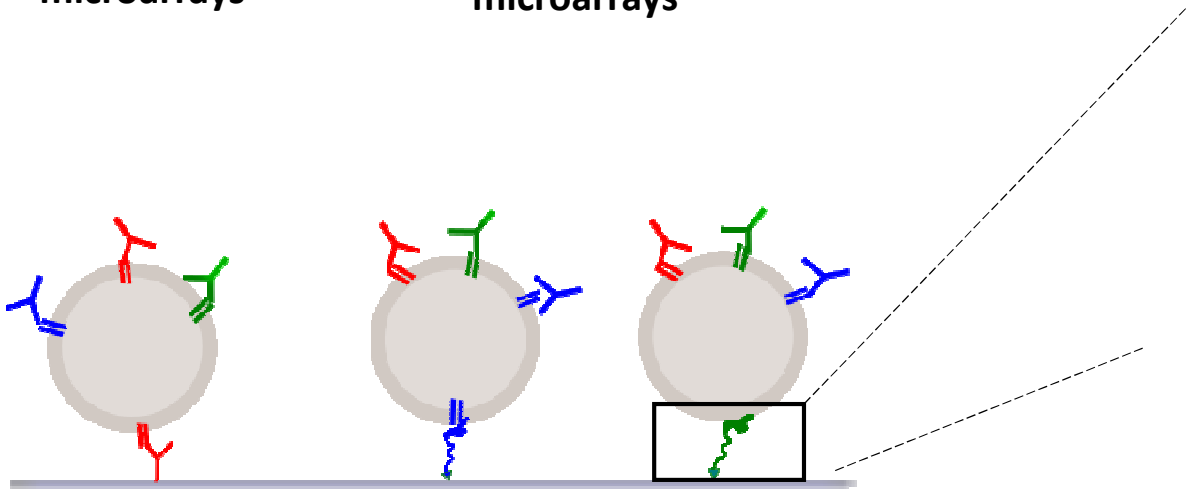


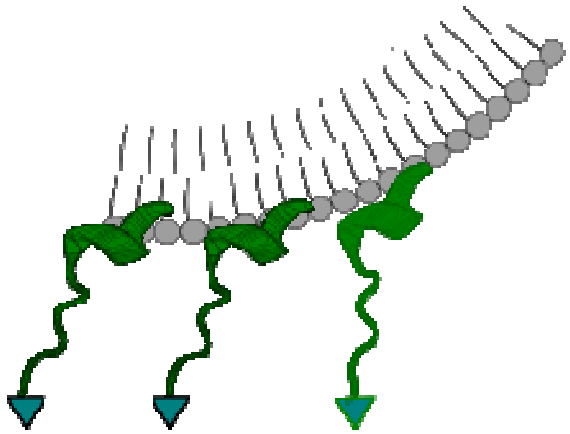
Antibody microarrays

Peptide microarrays



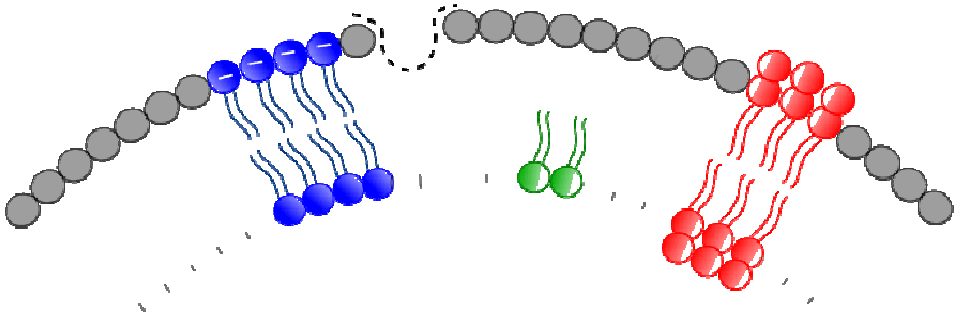
Membrane sensing peptides

EV



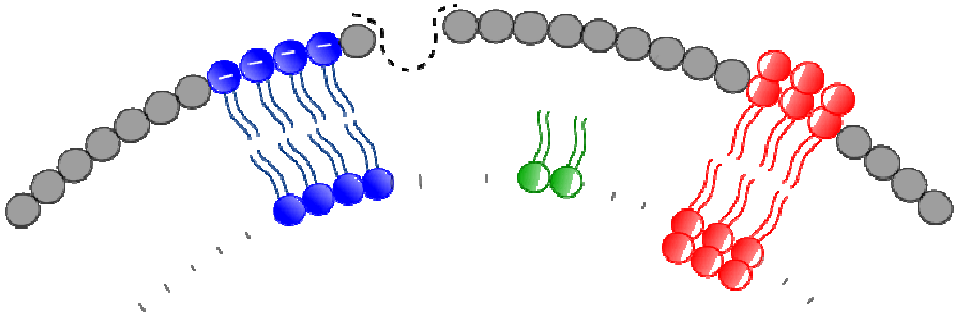
General baits unbiased by differential protein expression

➢ Membrane sensing peptides



- **Small EV membrane -> general yet specific marker:** High curvature; abundance of unsaturated anionic phospholipids in the ext. leaflet; lipid packing defects

➢ Membrane sensing peptides



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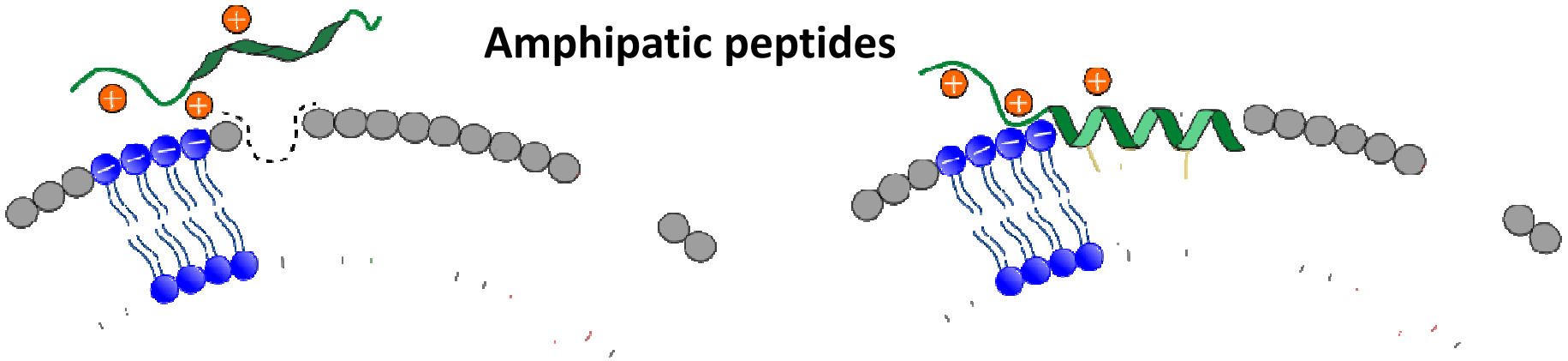
Amphipatic peptides

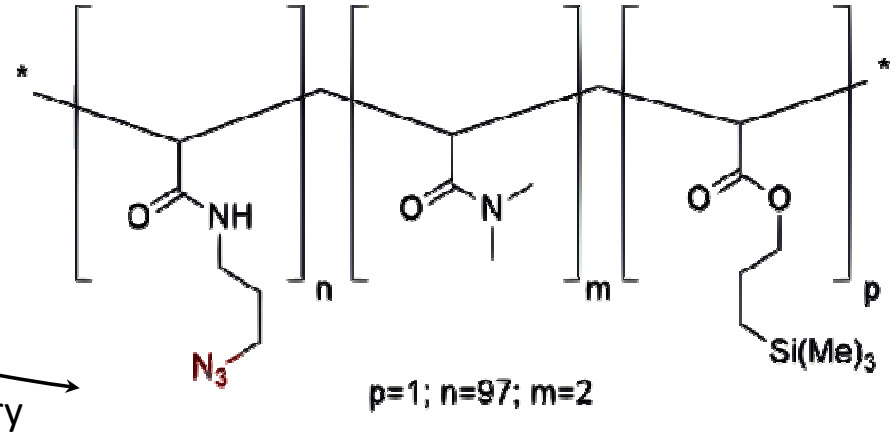


➢ Membrane sensing peptides

BP: RPPGFSPFR-K-G- $(O_2Oc)_2$-Prg

BPn: EPPGFSPFE-K-G- $(O_2Oc)_2$-Prg

Click chemistry



Copoly Azide

Saludes J.P. et al. Mol Biosyst, 2013, 9, 2005-2009

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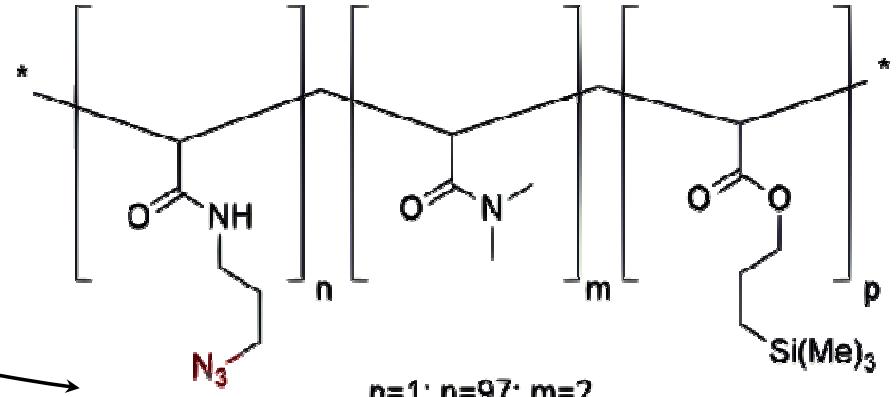

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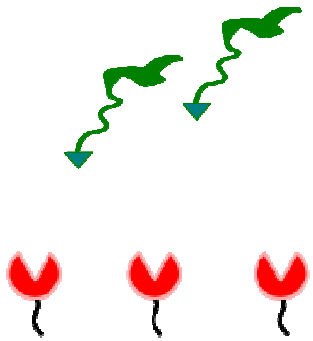
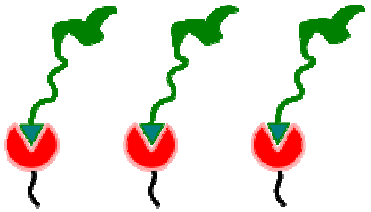


Clickable chip

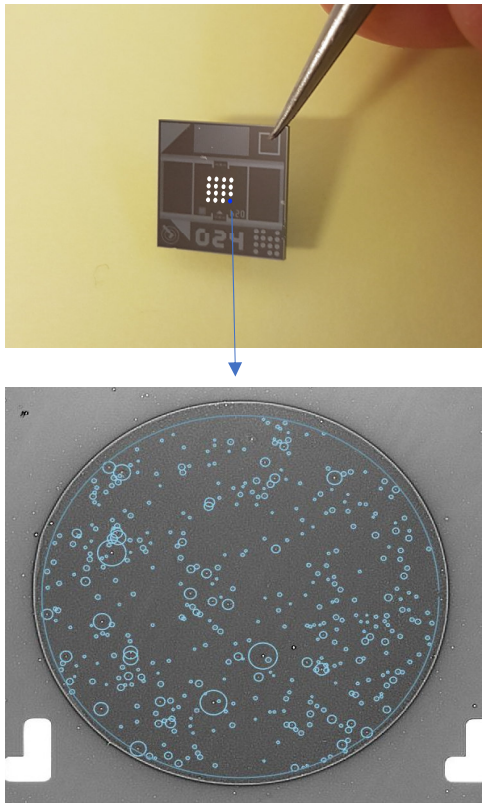
CuAAC



Gori A. et al JEV, 9:1, 1751428 *(2020)*

Experimental

Single Particle Interferometric Reflectance Imaging Sensor (SP-IRIS) by Nanoview ExoView™ R 100



G. Daaboul, Et al. Scientific Reports (2016) 6: 3746

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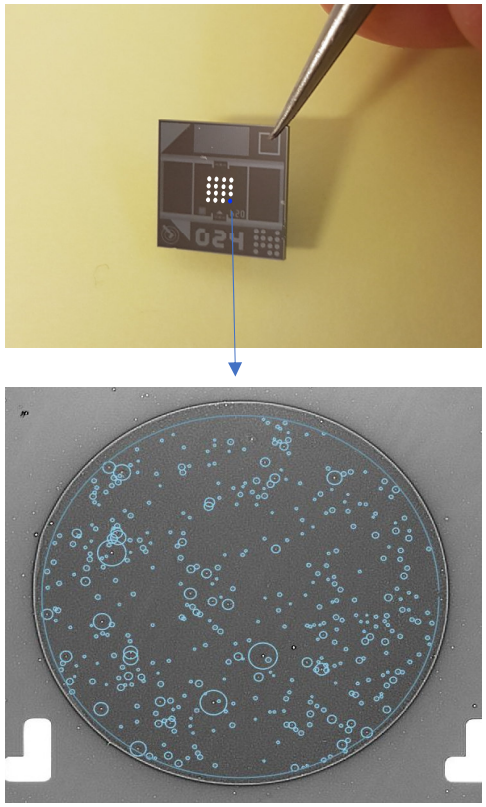
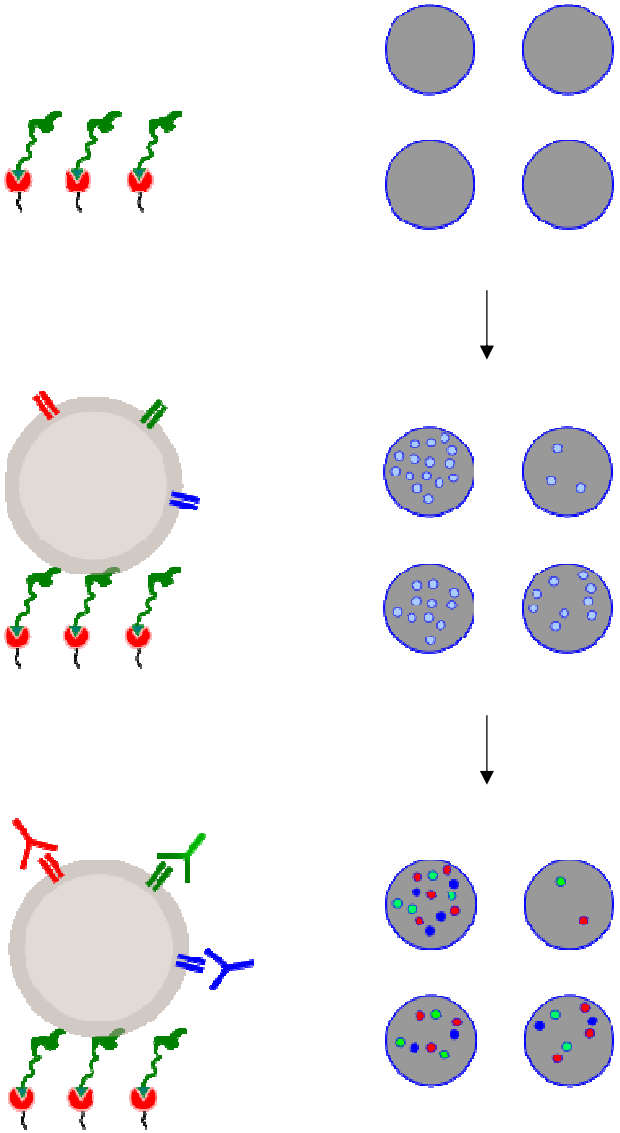




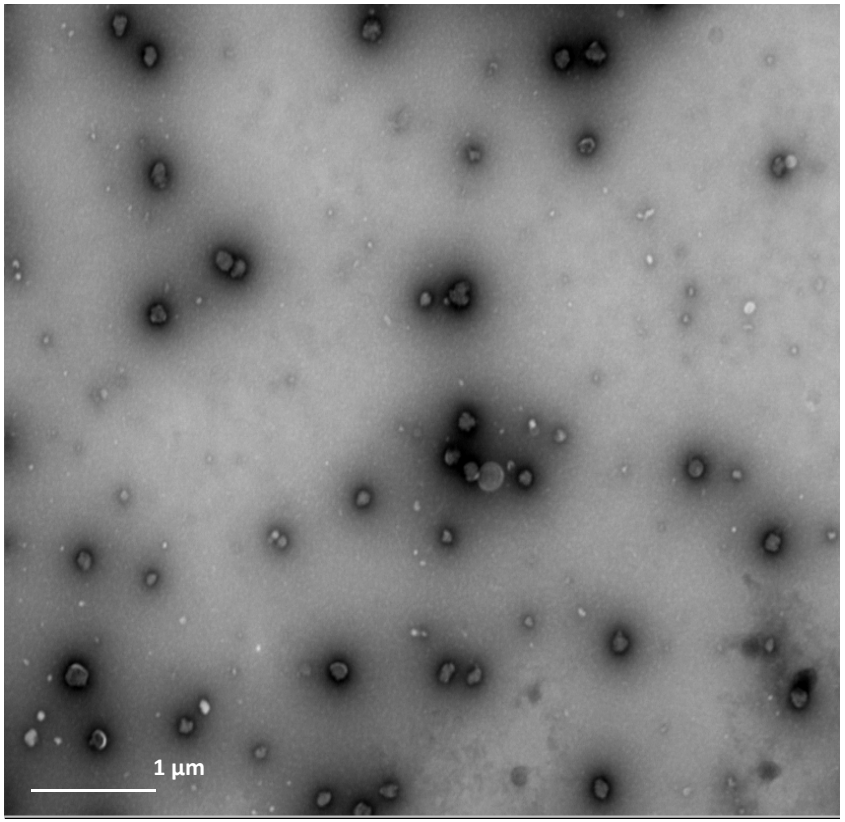
Peptide microarrays on clickable surfaces

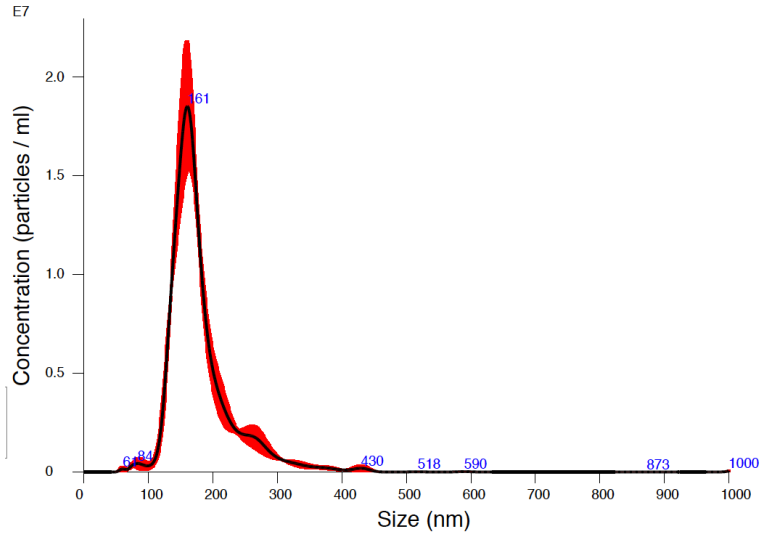
On-spot label-free EV counting and sizing by SP-IRIS

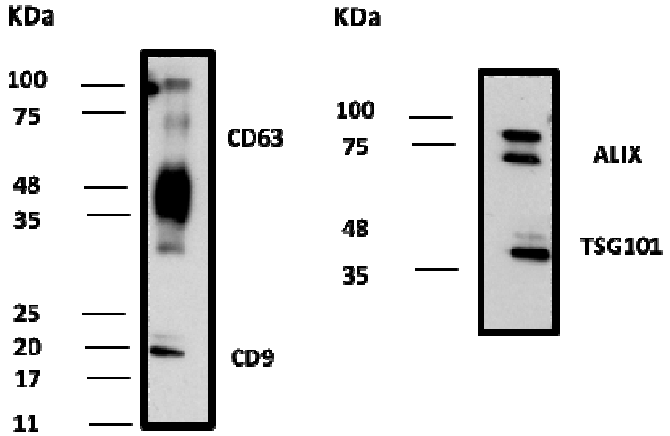
Fluorescence co-localization of protein markers by immune-staining (optional)

G. Daaboul, Et al. Scientific Reports (2016) 6: 3746

➢ UC isolated EVs from HEK cells: characterization according to MISEV2018







Gori A. et al JEV, 9:1, 1751428 *(2020)*

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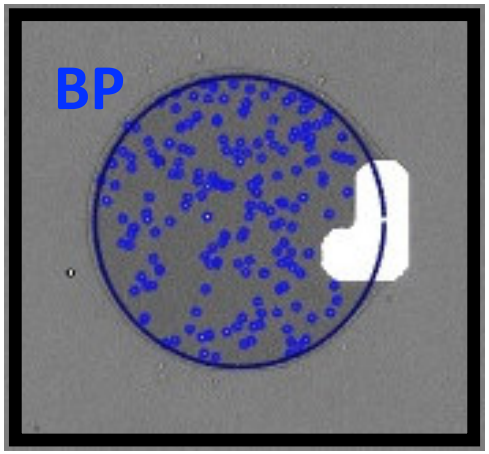

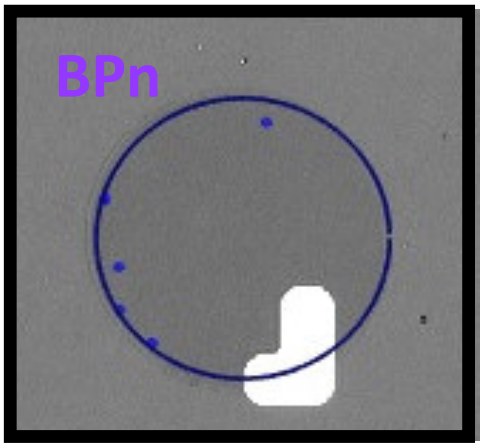

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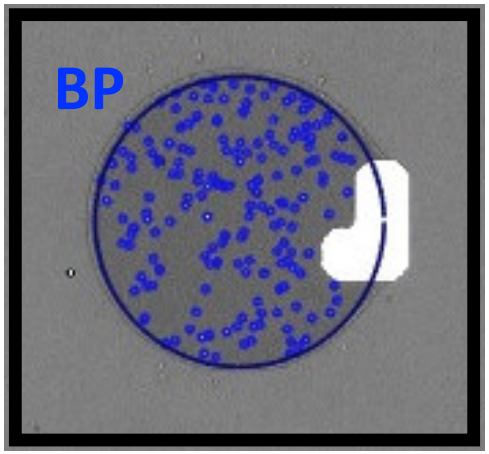

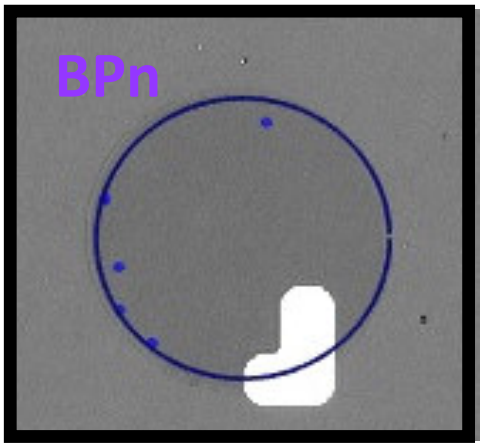

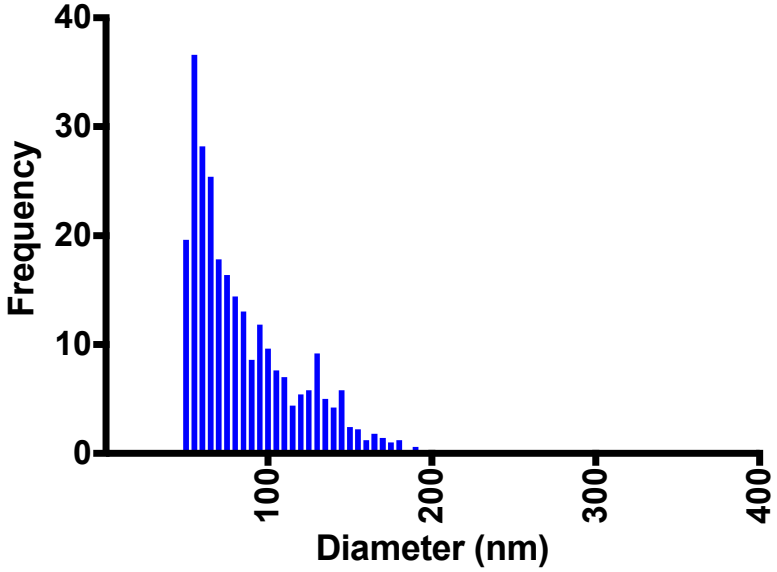

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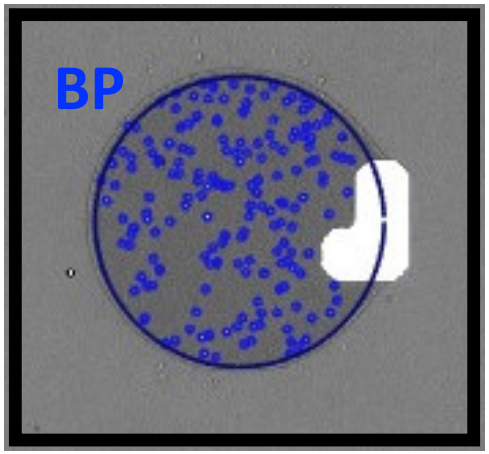

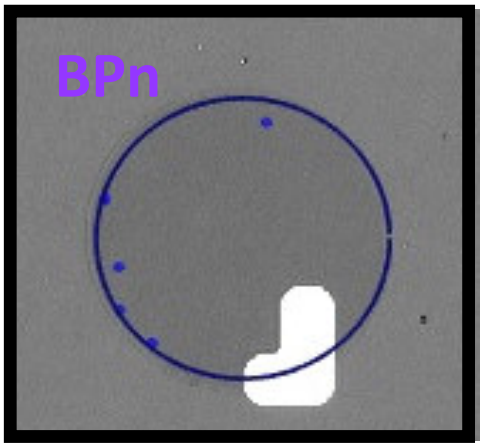

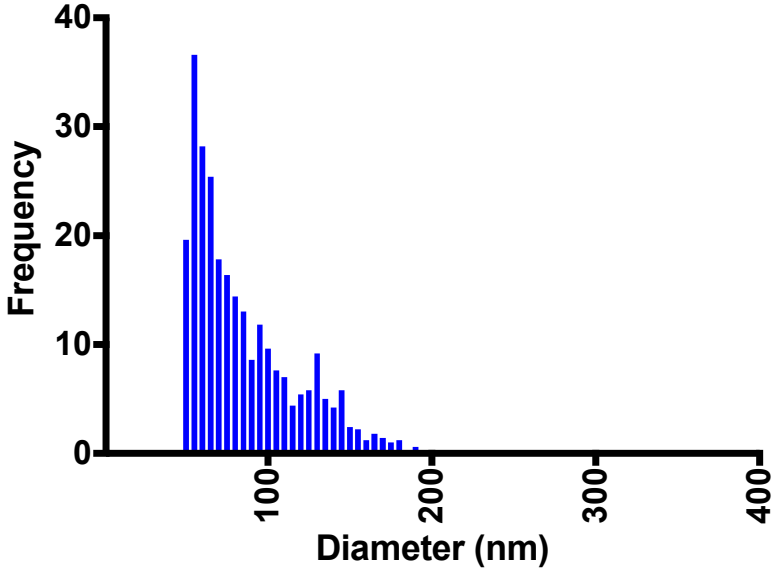

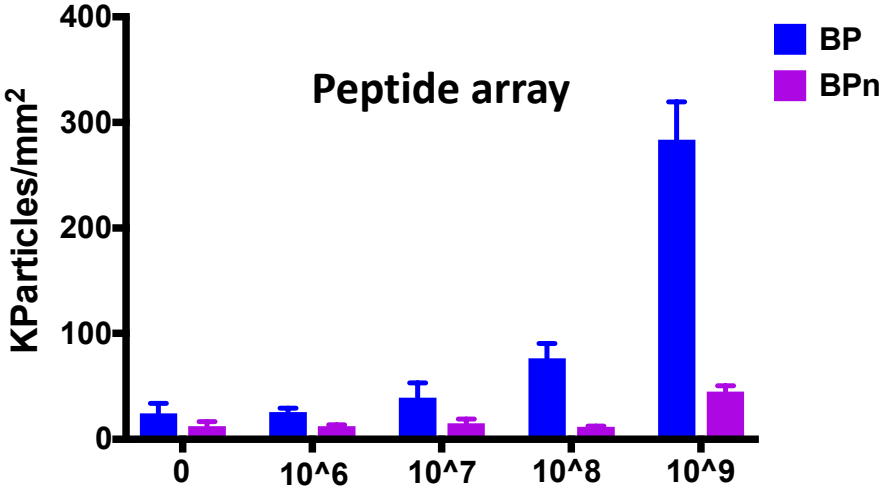

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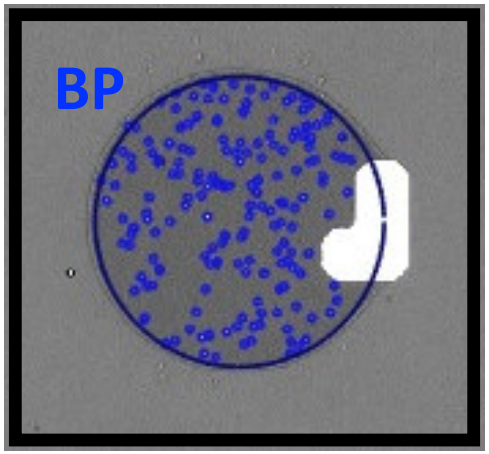
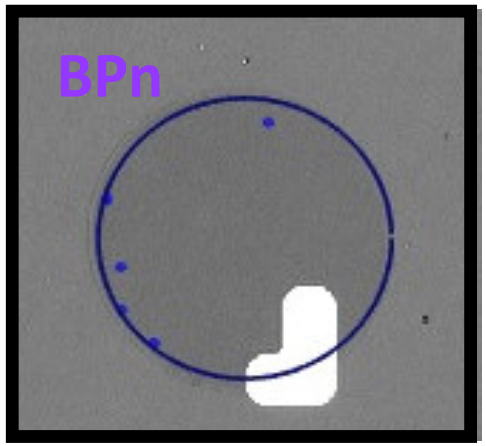
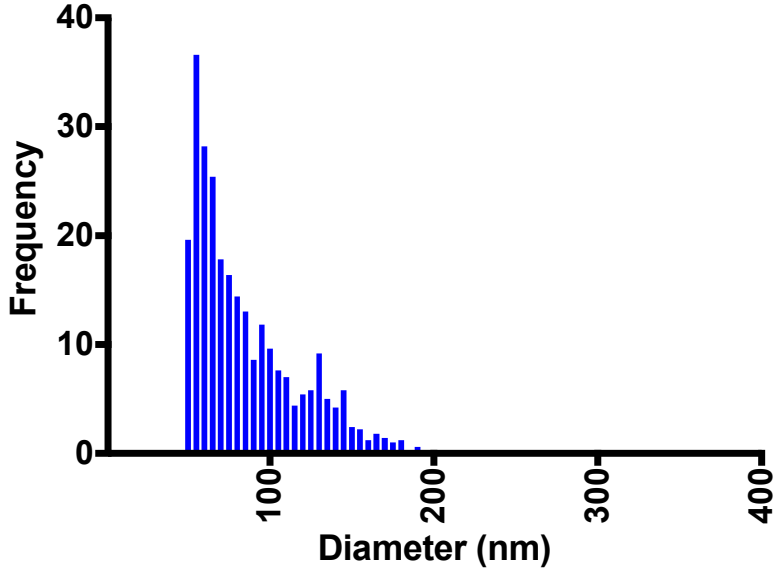
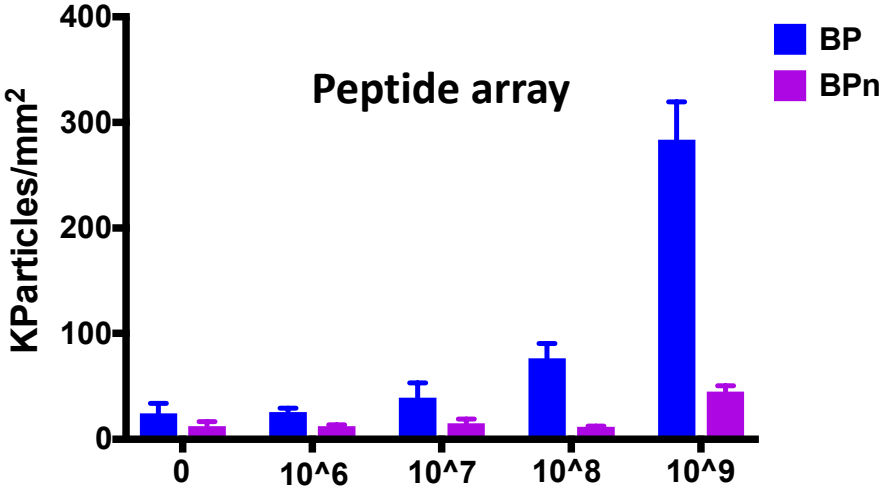
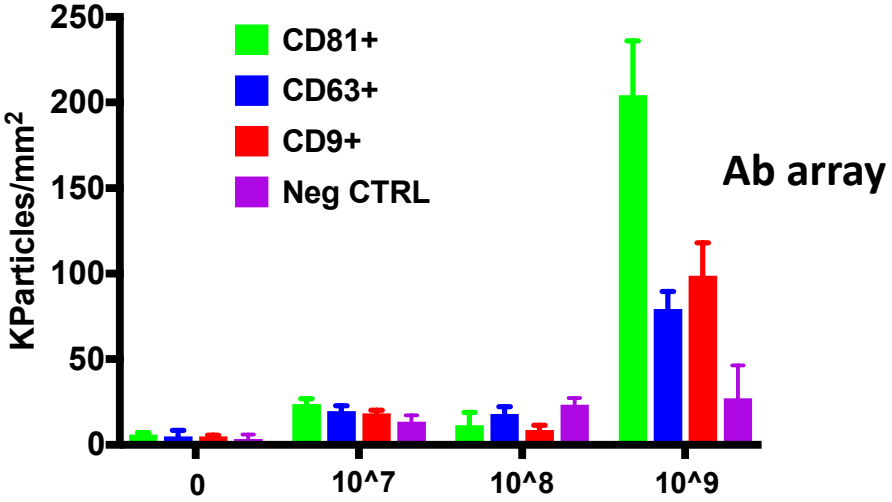




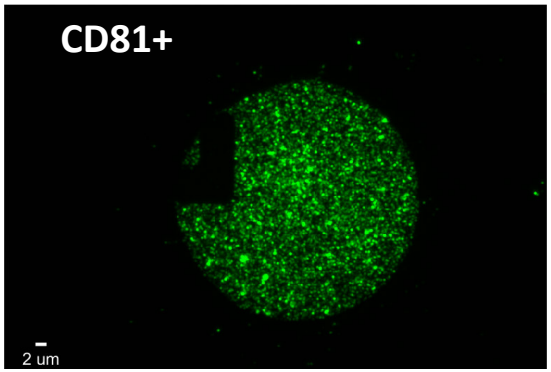


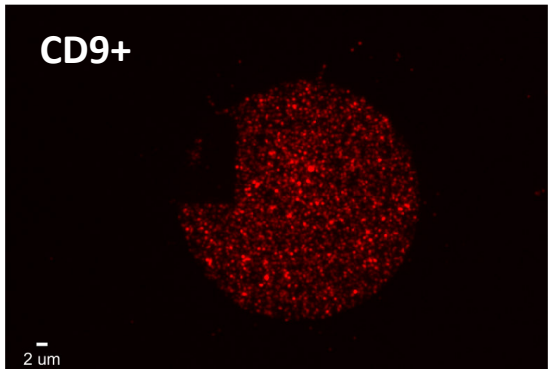


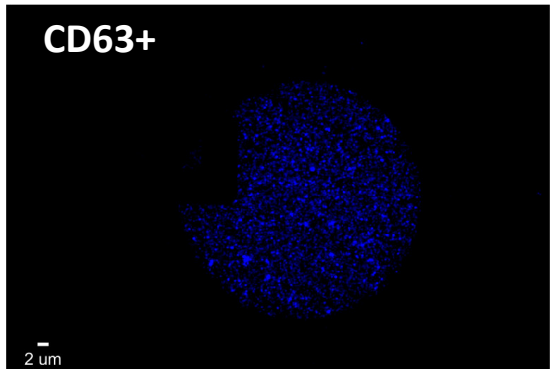


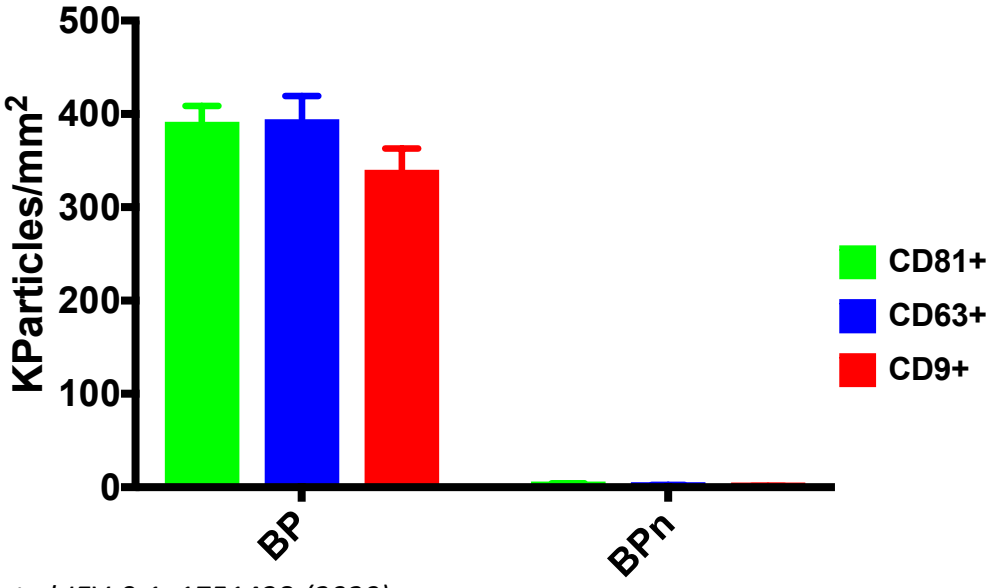
Gori A. et al JEV, 9:1, 1751428 *(2020)*

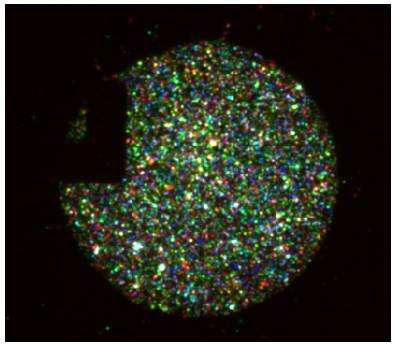
On-spot staining of CD9/CD63/CD81









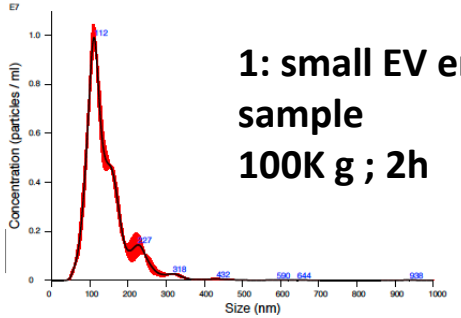


BP

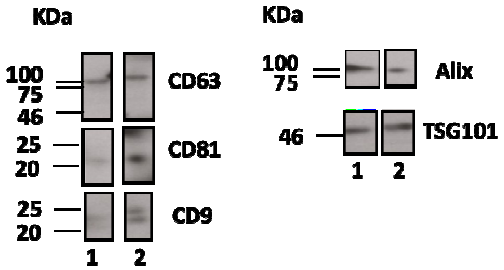
BPn

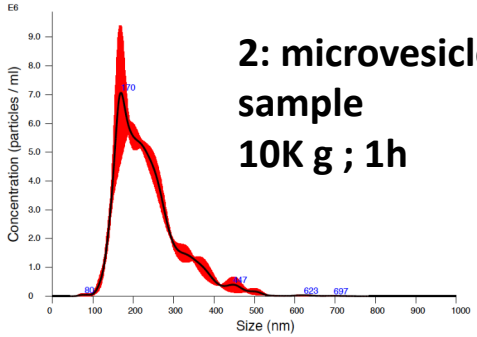
Gori A. et al JEV, 9:1, 1751428 *(2020)*

➢ Size dependancy of peptide capturing using EVs from platelet free human plasma



1: small EV enriched sample
100K g ; 2h





2: microvesicles enriched sample
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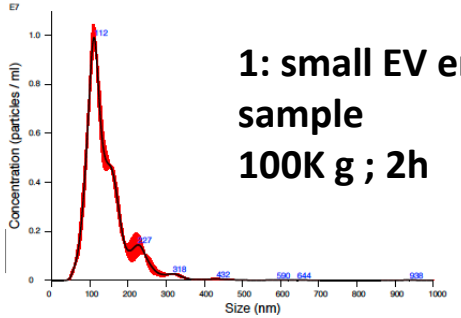

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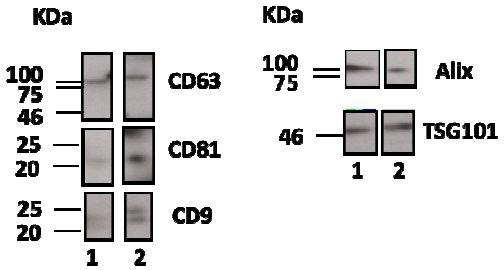

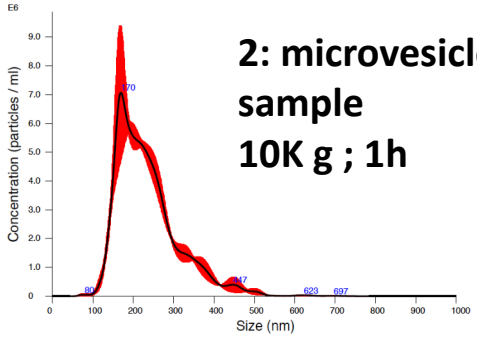

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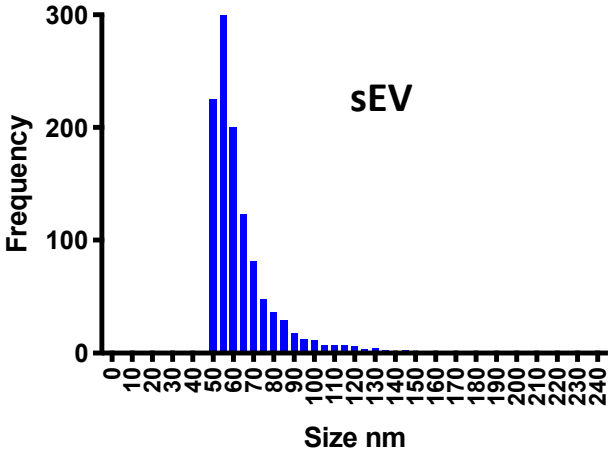

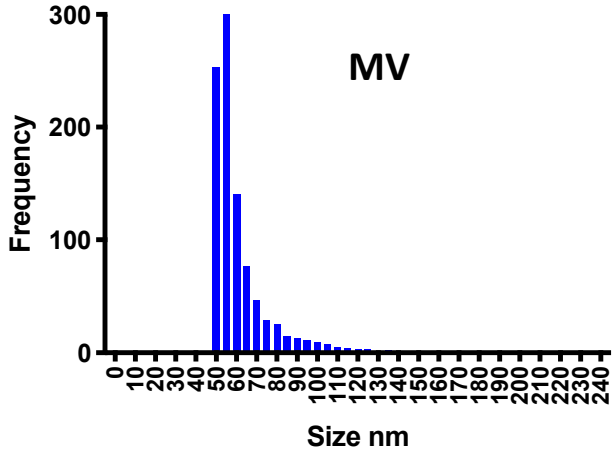

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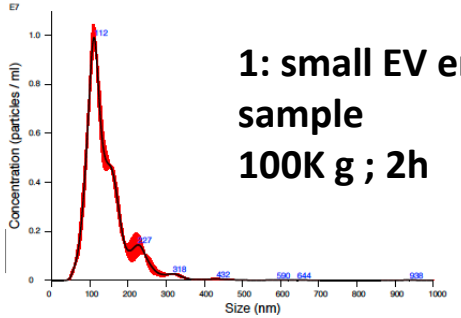

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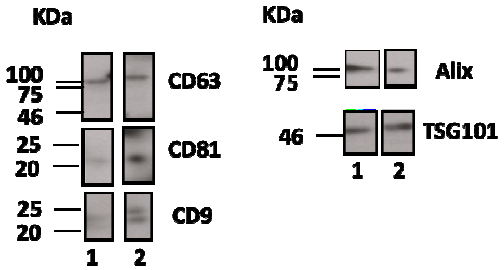

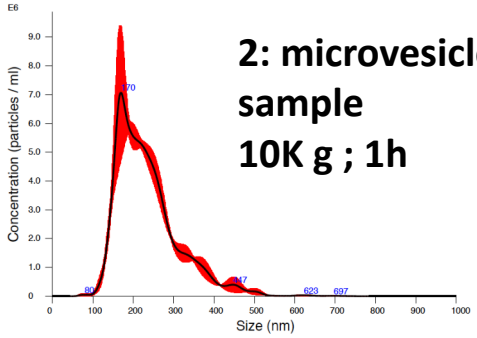

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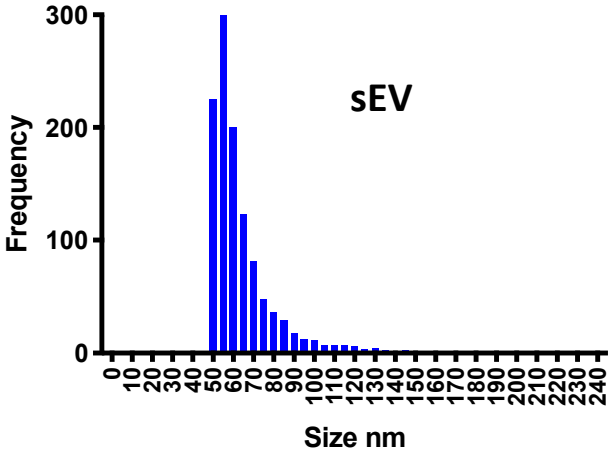
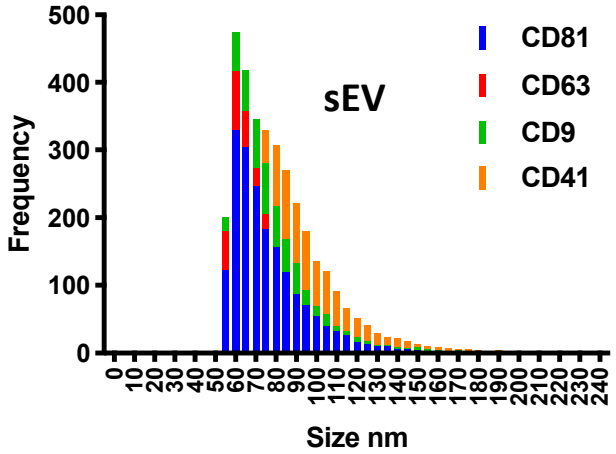
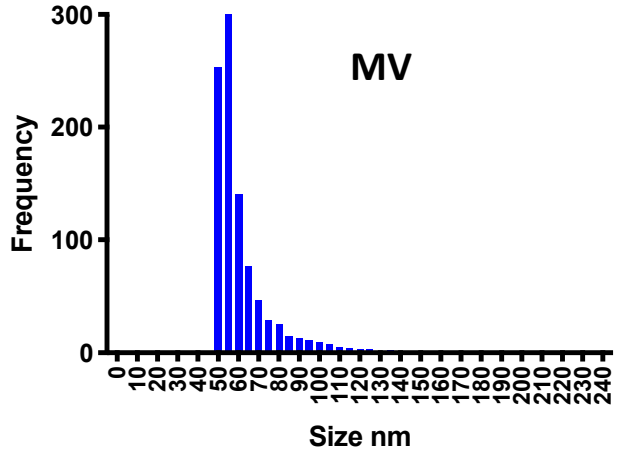
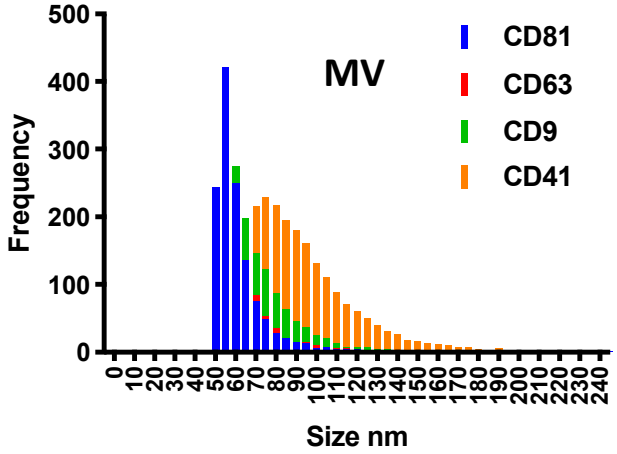


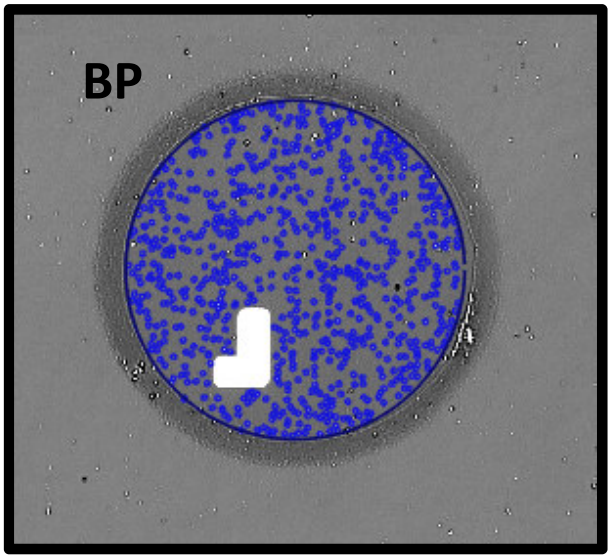


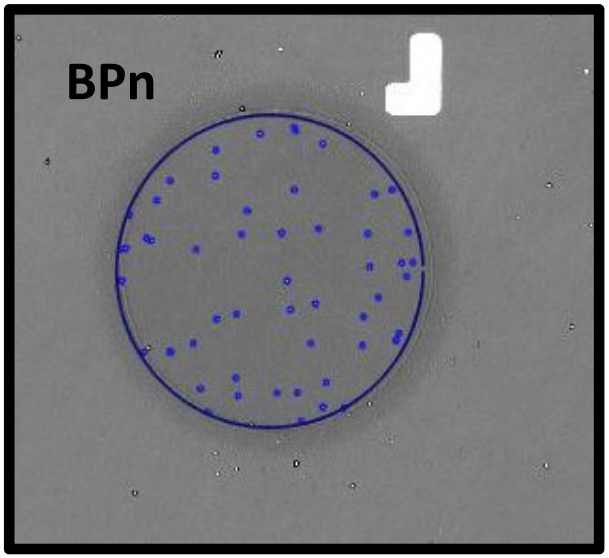


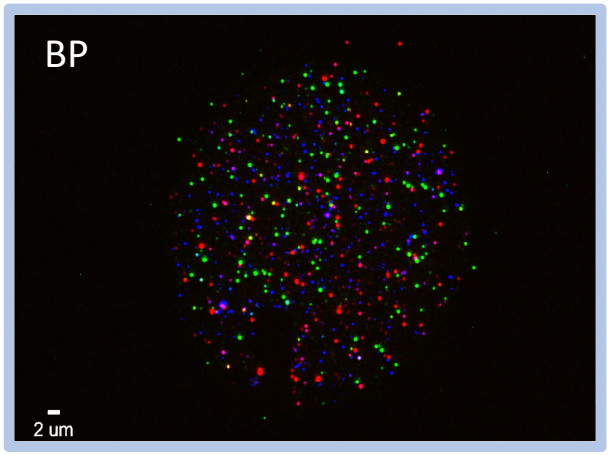


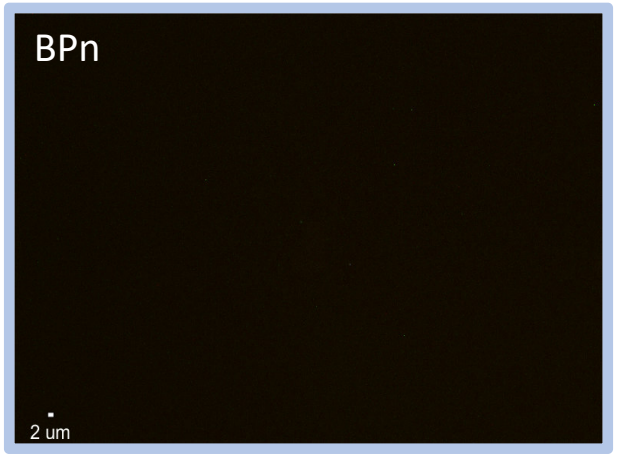
Gori A. et al JEV, 9:1, 1751428 *(2020)*

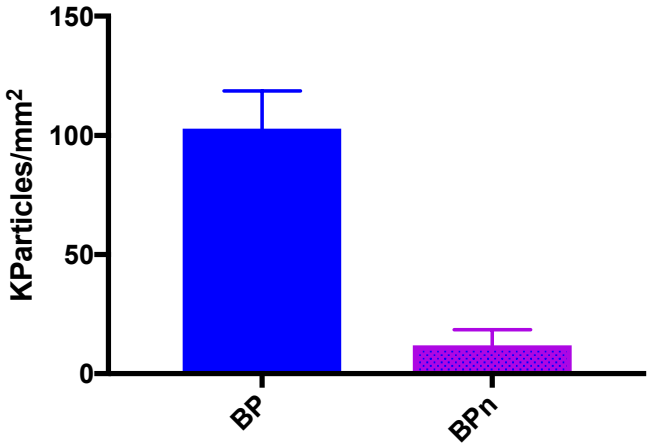
EVs captured from un-treated serum

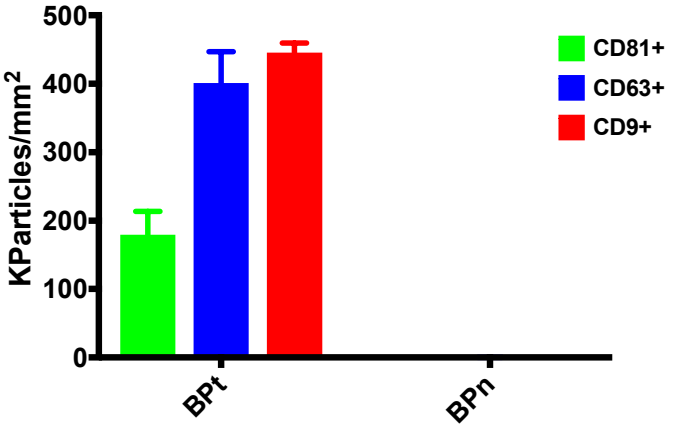




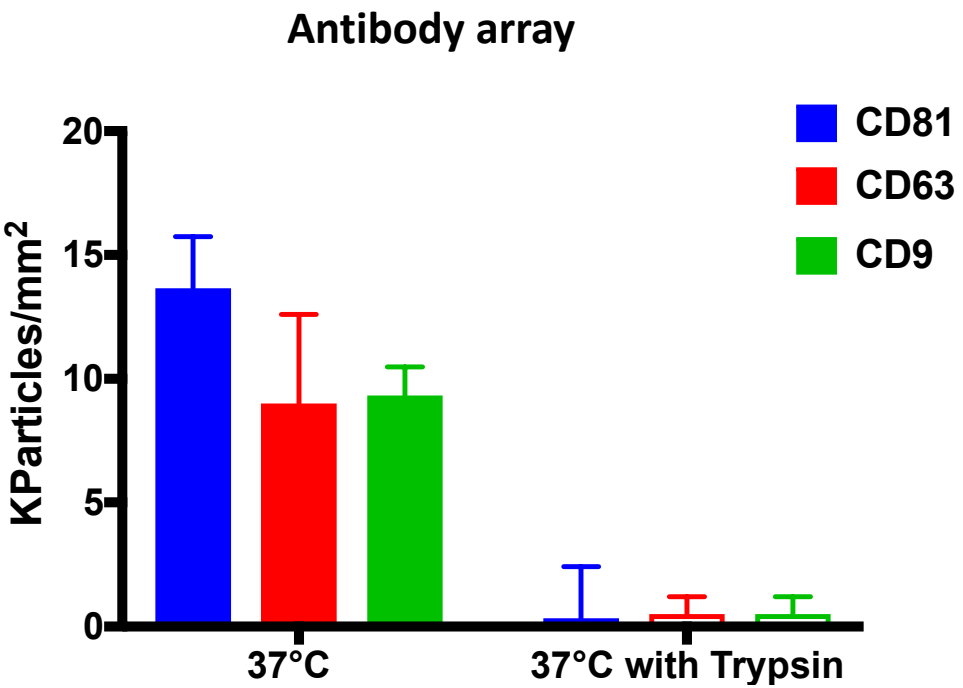








➢ Trypsin shaving of EVs

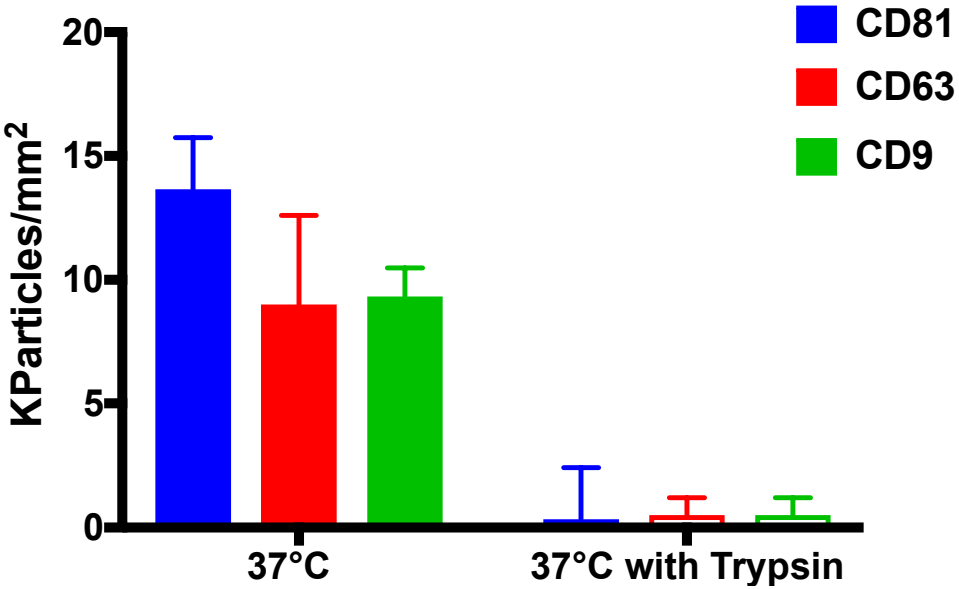


Binding is ABOLISHED by protein digestion

Gori A. et al JEV, 9:1, 1751428 *(2020)*

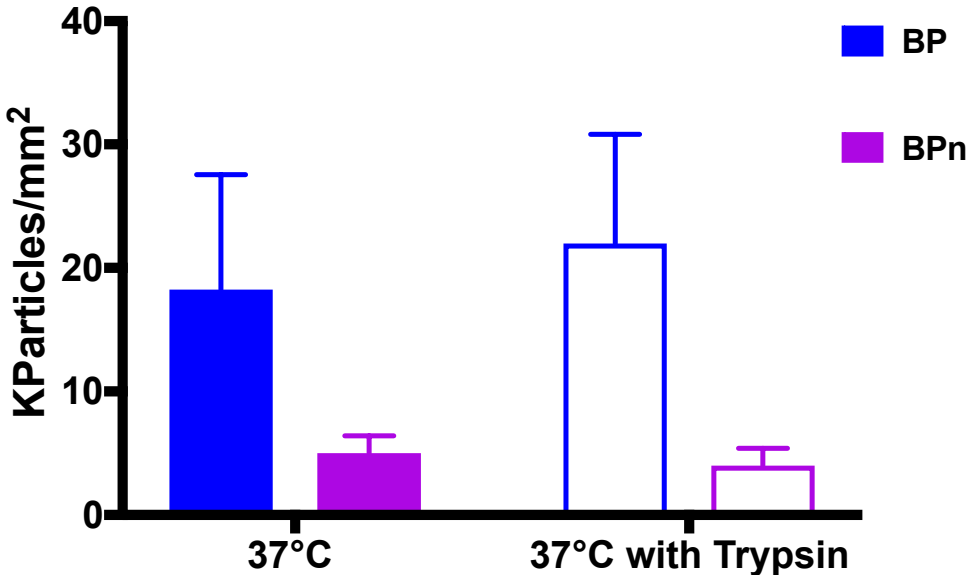
➢ Trypsin shaving of EVs

Antibody array



Binding is ABOLISHED by protein digestion

Membrane sensing peptide array



Binding is NOT influenced by protein digestion

Gori A. et al JEV, 9:1, 1751428 *(2020)*

➢ Summary and perspectives

- **First example of peptide microarrays applied to EVs**
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  - **High stability**
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New directions: →

- **Use as decorating and reporting agents for EVs**
- **Use as isolation tools**

Acknowledgements

CTB
CHEMISTRY & TECHNOLOGY FOR BIOSCIENCE

Alessandro Gori
Marcella Chiari
Roberto Frigerio
Angelo Musicò
Dario Brambilla
Greta Bergamaschi
Alessandro Strada
Francesco Damin
Laura Sola
Alessandro Mussida

NanoView BIOSCIENCES

George Daaboul
David Freedman

Fondazione Don Carlo Gnocchi Onlus

Silvia Picciolini

OSPEDALE SAN RAFFAELE

Riccardo Vago
Silvia Galbiati

HansaBioMed Life Sciences

exosomics

Natasa Zarovni

EVCIta
SOCIETÀ ITALIANA PER LE VESCICOLE EXTRACELLULARI

Fundings

MARVEL: Evolving reversible iMmunocapture by membrAne sensing peptides: towaRds scalable extracellular VEsicLes isolation
H20202-FETPROACT-2019-2020

INDEX: Integrated nanoparticle isolation and detection system for complete on-chip analysis of exosomes
H2020 FETOPEN-2016-2017

A Peptide Hydrogel Platform for Extracellular Vesicles Isolation and Multimodal Analysis
Bando Materiali Avanzati 2018

INTERSLA - INnovazione, nuovi modelli TEcnologici e Reti per curare la SLA

➢ The end and further info

Thank you for the kind attention!

More details here:

JOURNAL OF EXTRACELLULAR VESICLES
2020, VOL. 9, 1751428
https://doi.org/10.1080/20013078.2020.1751428

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Membrane-binding peptides for extracellular vesicles on-chip analysis

Alessandro Gori [a], Alessandro Romanato [a], Greta Bergamaschi [a], Alessandro Strada [a], Paola Gagni [a], Roberto Frigerio [a], Dario Brambilla [a], Riccardo Vago [b], Silvia Galbiati [c], Silvia Picciolini [d], Marzia Bedoni [d], George G. Daaboul [e], Marcella Chiari [a] and Marina Cretich [a]

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