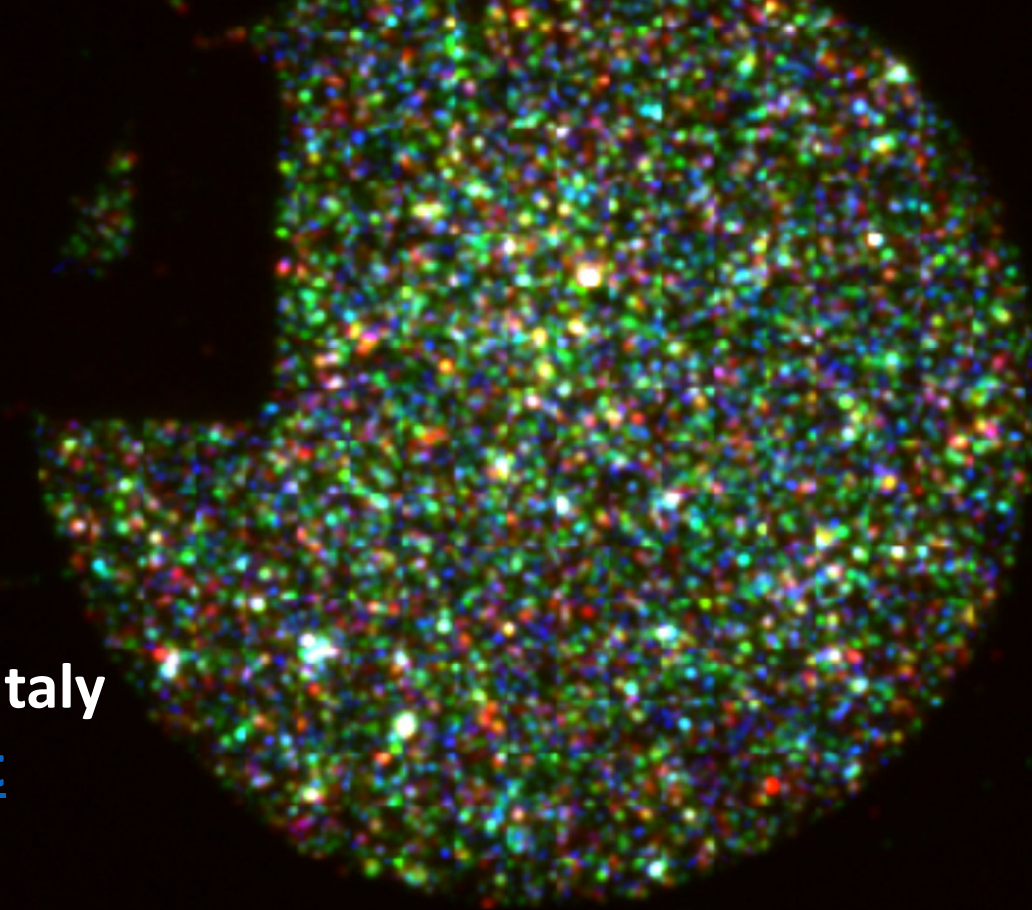


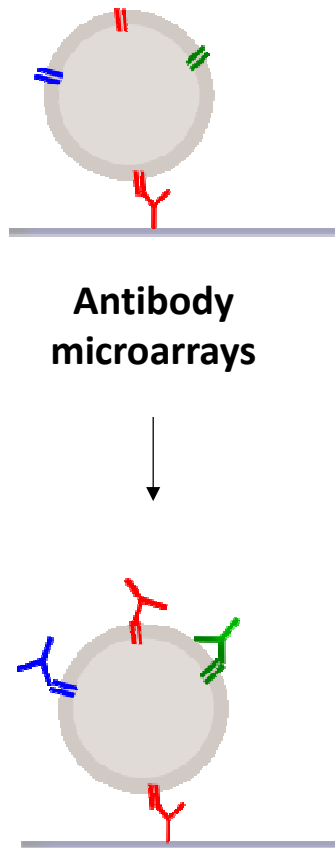
# Membrane-binding peptides for small extracellular vesicles on-chip analysis



Marina Cretich  
SCITEC-CNR, Milano, Italy  
[marina.cretich@cnr.it](mailto:marina.cretich@cnr.it)  
[www.ctbio.eu](http://www.ctbio.eu)

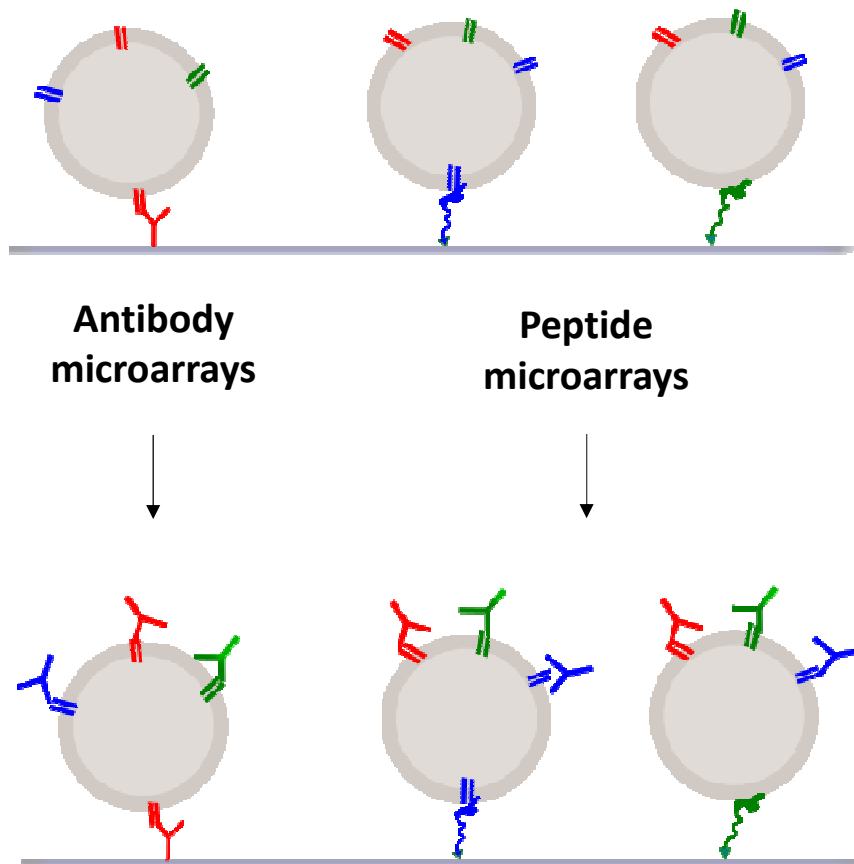
- 1. EV microarrays**
- 2. Membrane curvature sensing peptides for EV microarrays**
- 3. Insights into the binding mechanism**
- 4. The role of microarray surface chemistry**
- 5. Size dependancy of EV capturing**
- 6. Conclusion and perspectives**

## ➤ The context: EV microarrays



Jørgensen, M.; Bæk, R.; et al *JEV*, **2**, 1–9 (2013).

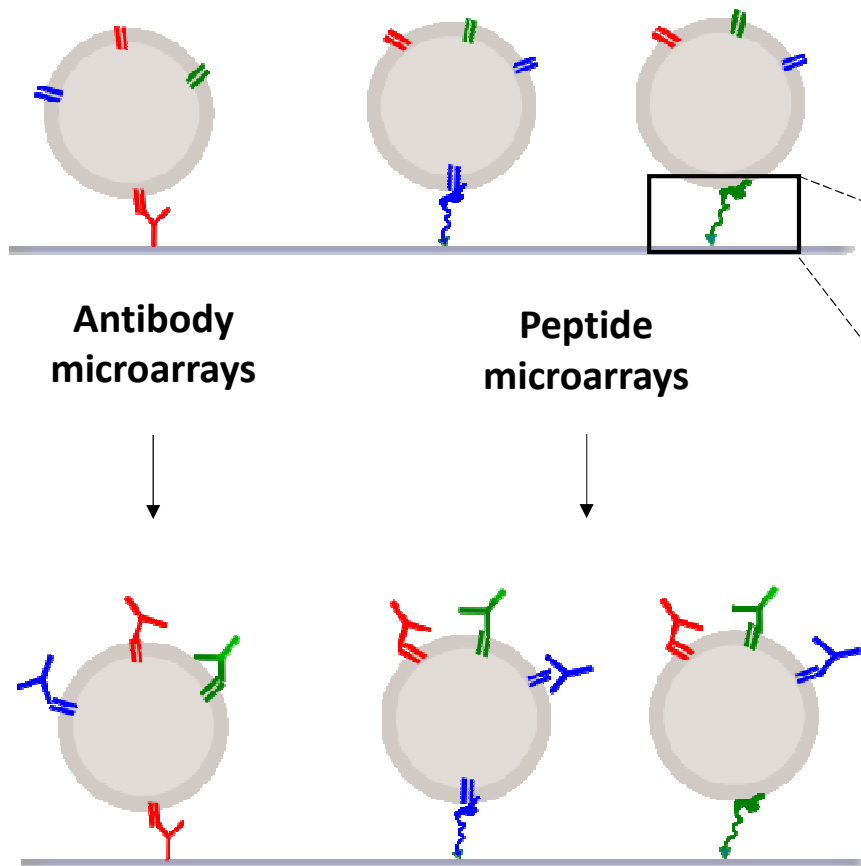
## ➤ The context: EV microarrays



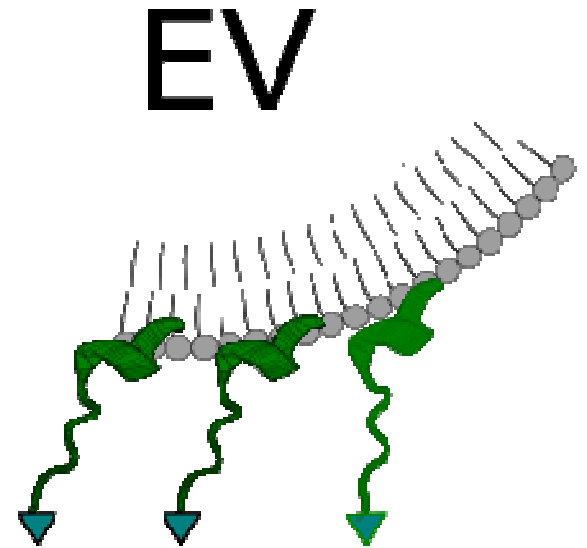
Jørgensen, M.; Bæk, R.; et al *JEV*, **2**, 1–9 (2013).

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

## ➤ The context: EV microarrays

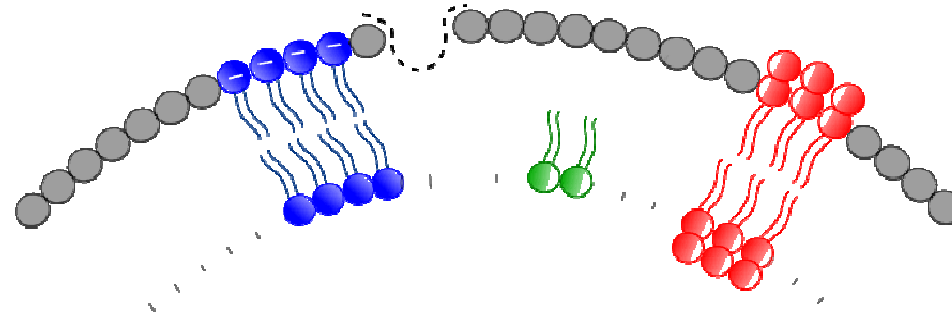


Membrane binding peptides



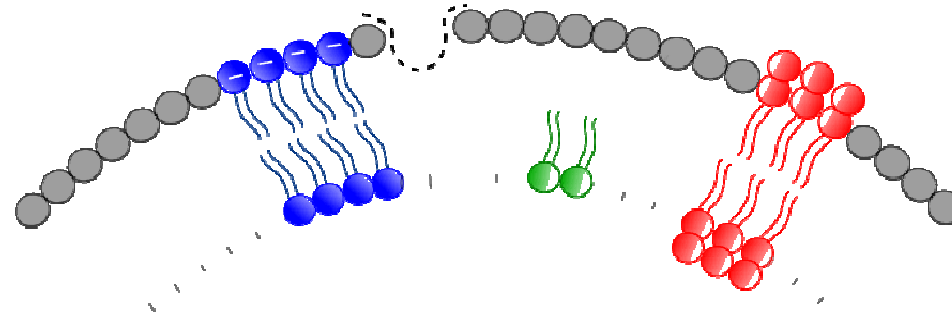
General baits unbiased by differential protein expression

## ➤ Membrane sensing peptides

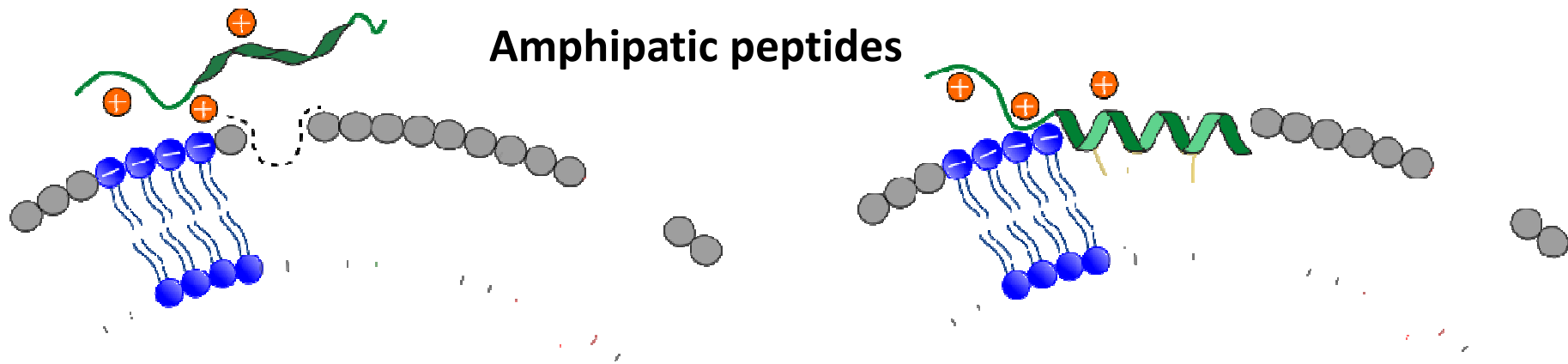


- **Small EV membrane -> general yet specific marker:** High curvature; abundance of unsaturated anionic phospholipids in the ext. leaflet; lipid packing defects

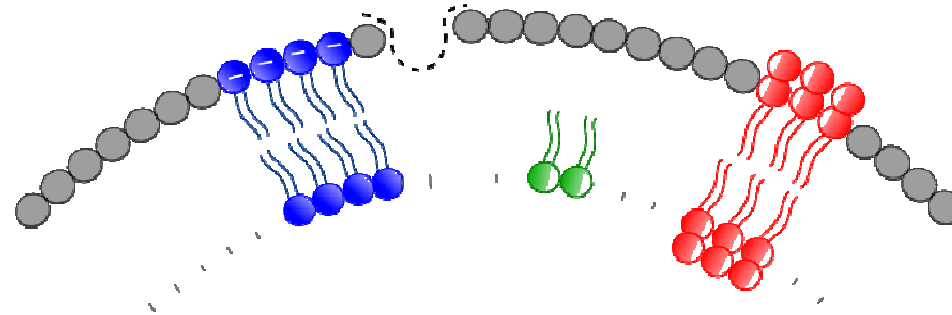
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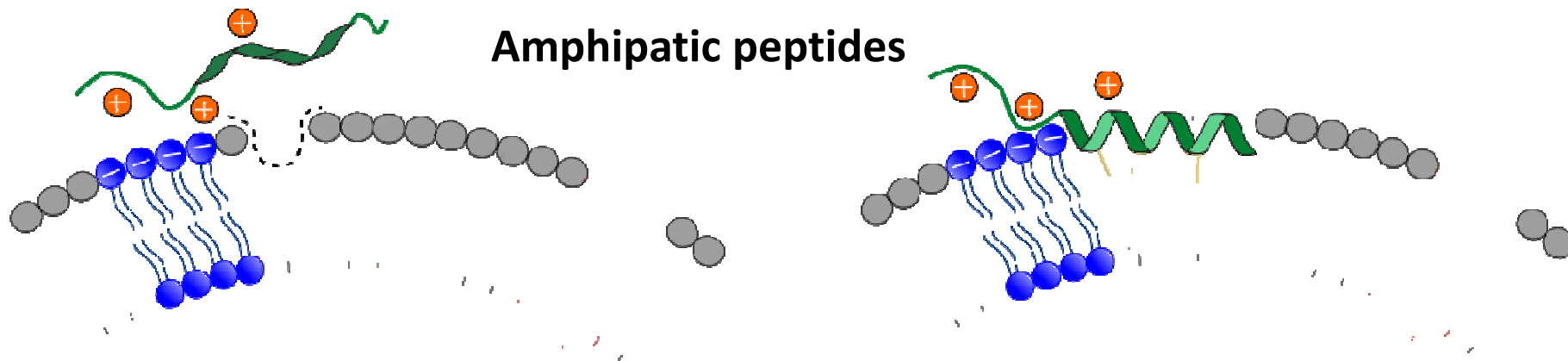


## ➤ Membrane sensing peptides



Prof. Hang Hubert Yin  
WebEVTalk series  
May 21<sup>st</sup> 2020

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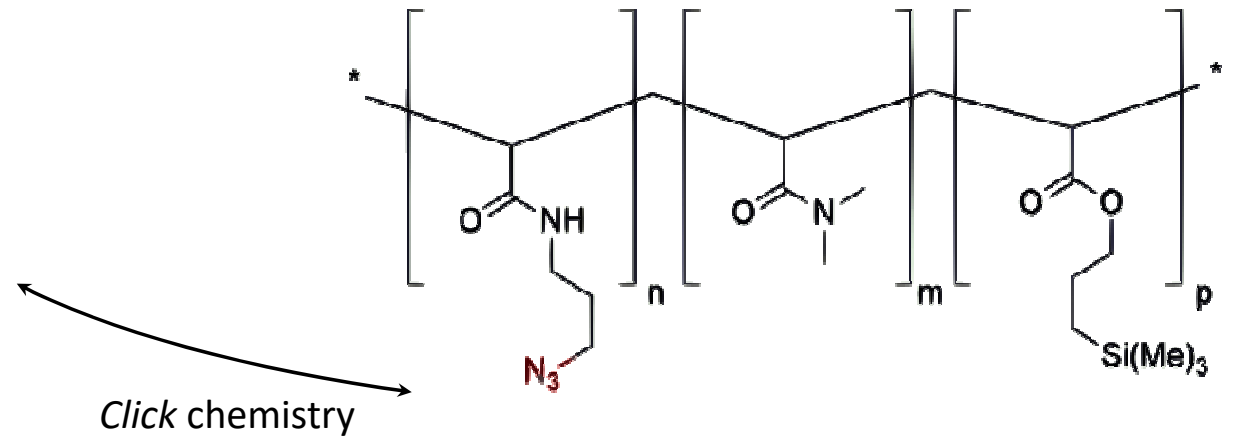




## ➤ Membrane sensing peptides

BP: RPPGFSPFR-K-G- (O<sub>2</sub>Oc)<sub>2</sub>-Prg

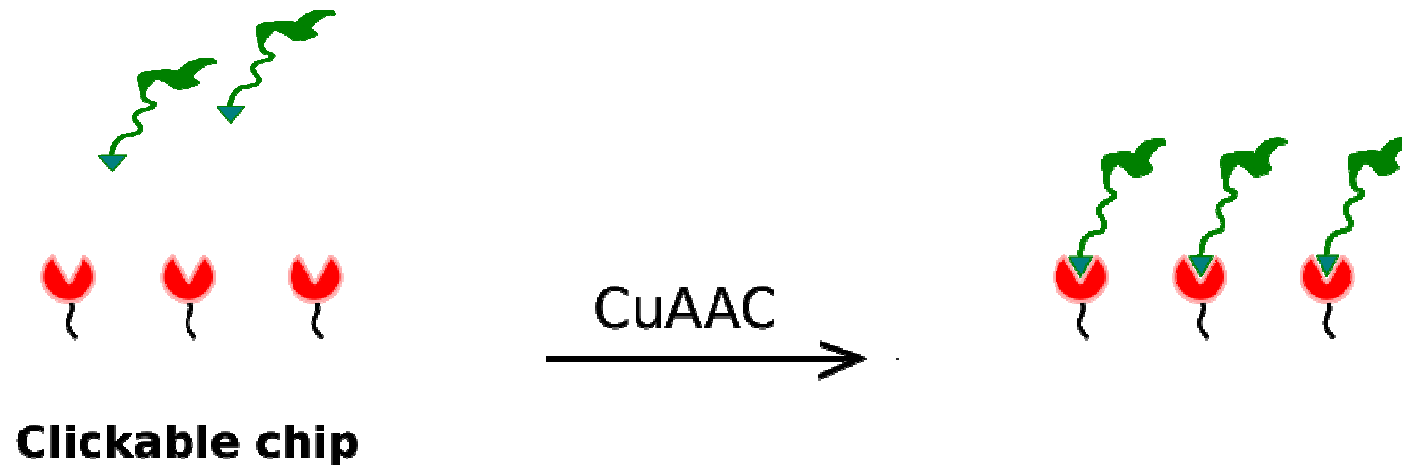
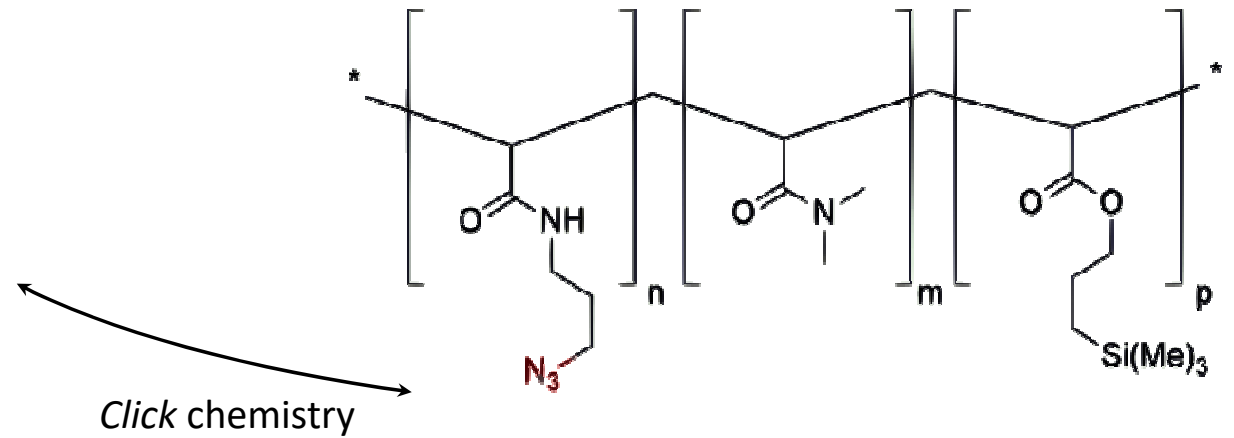
BPn: **E**PPGFSPF**E**-K-G- (O<sub>2</sub>Oc)<sub>2</sub>-Prg



## ➤ Membrane sensing peptides

BP: RPPGFSPFR-K-G- (O<sub>2</sub>Oc)<sub>2</sub>-Prg

BPn: **E**PPGFSPF**E**-K-G- (O<sub>2</sub>Oc)<sub>2</sub>-Prg

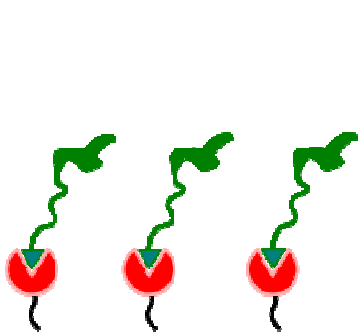
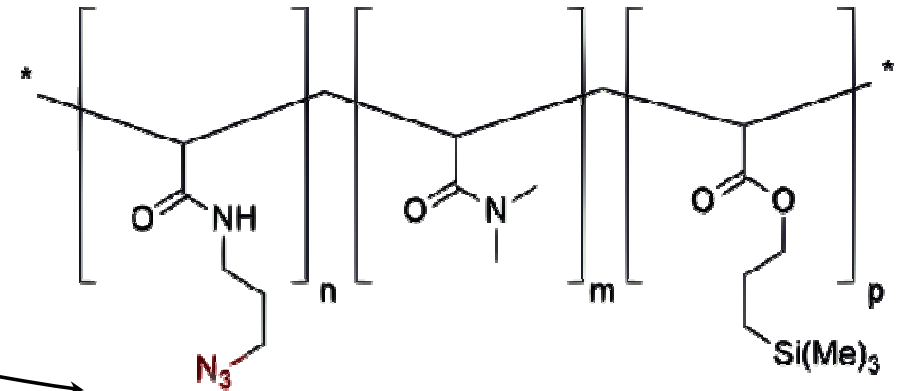


## ➤ Membrane sensing peptides

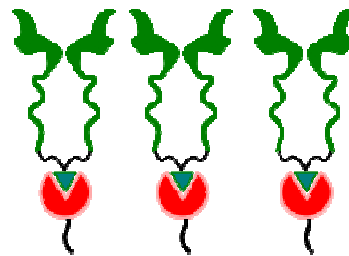
BP: RPPGFSPFR-K-G- (O<sub>2</sub>Oc)<sub>2</sub>-Prg

BPn: **E**PPGFSP**F**E-K-G- (O<sub>2</sub>Oc)<sub>2</sub>-Prg

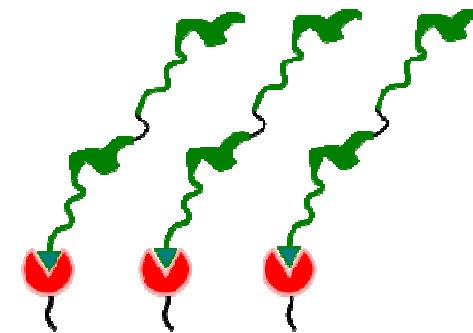
Click chemistry



**Bp linear**



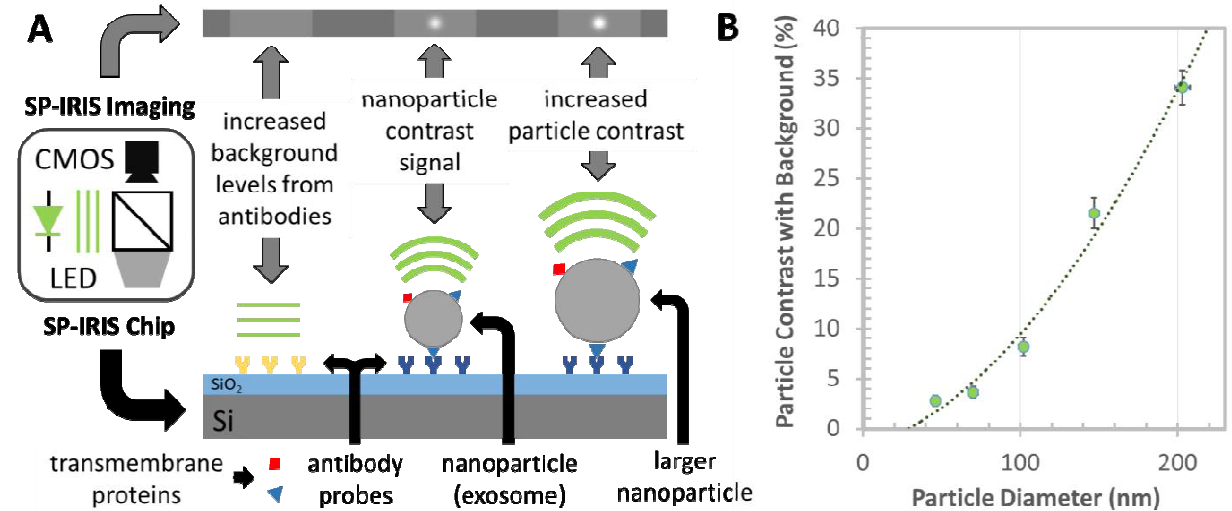
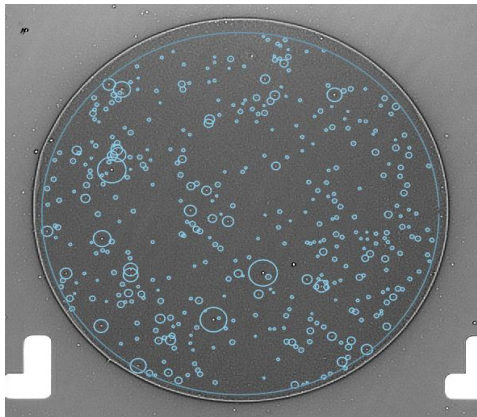
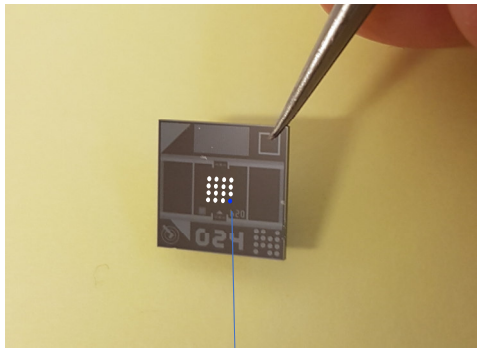
**Bp branched**



**Bp tandem**

## ➤ Experimental

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



#### SP-IRIS detection principle:

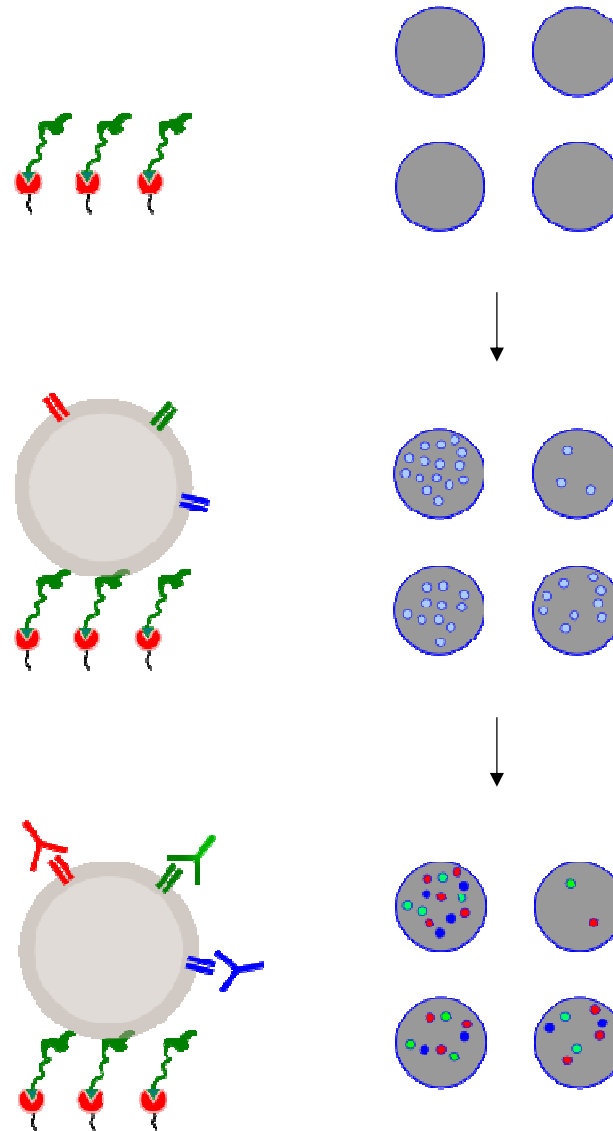
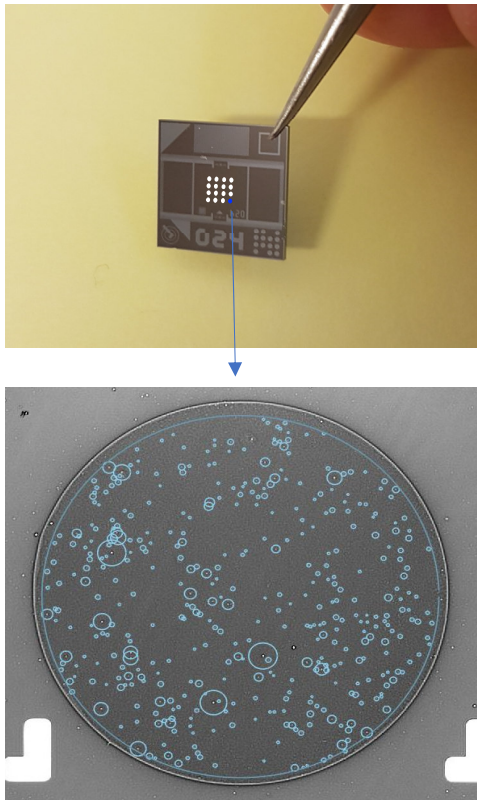
- 1) Monochromatic LED light illuminates the sensor surface
- 2) Nanoparticle scattering signature is enhanced interferometrically
- 3) And is captured on a CMOS camera.

Prof. Selim Unlu's  
group



## ➤ Experimental

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

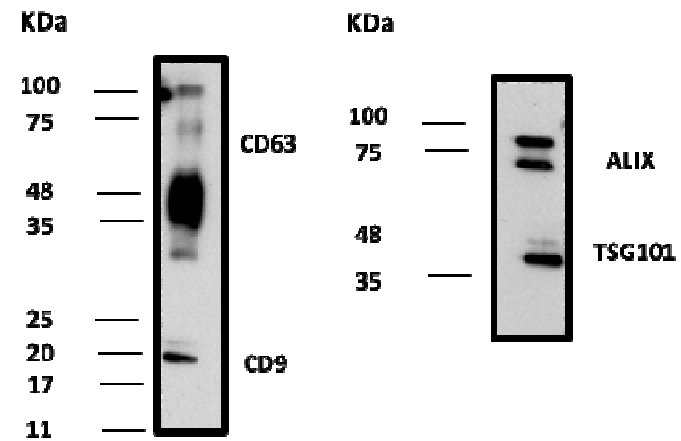
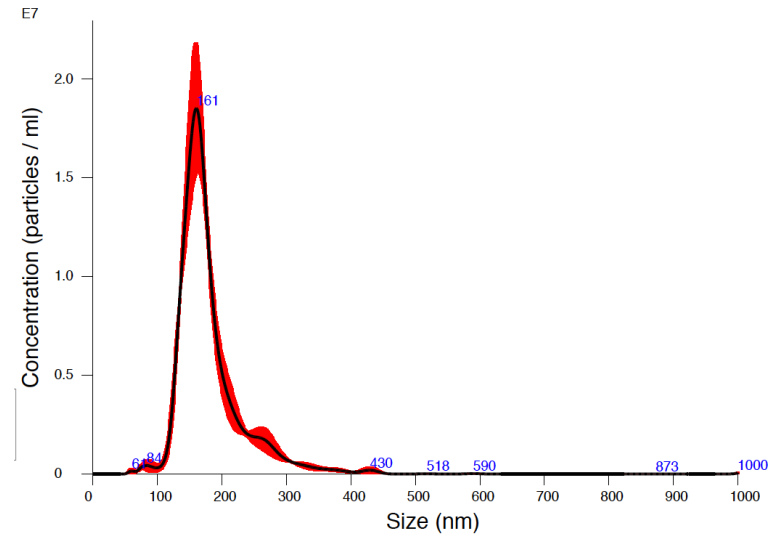
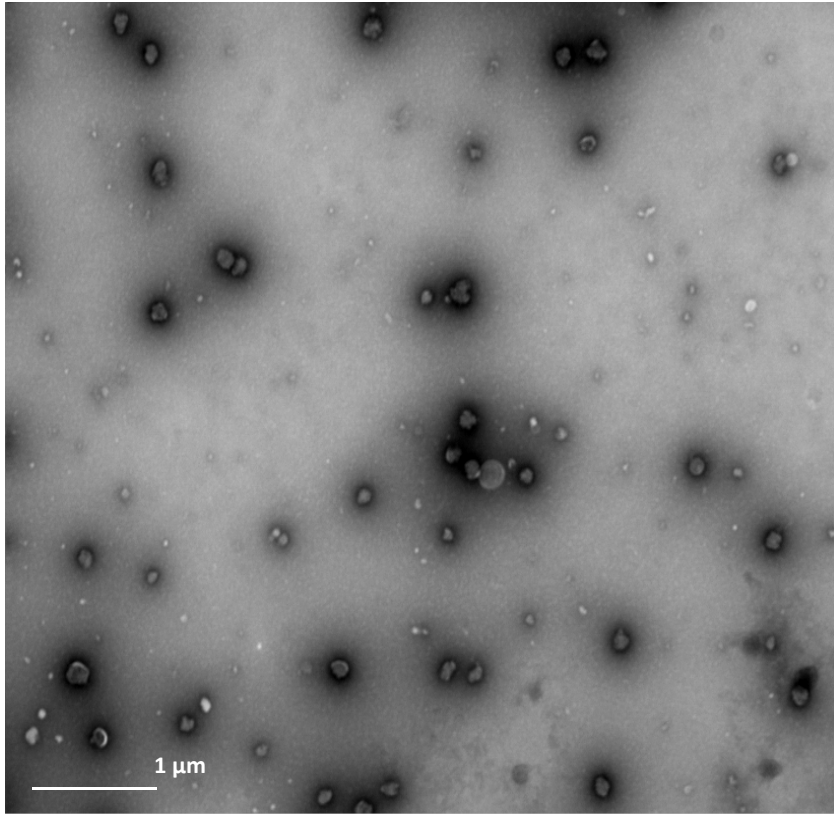


Peptide microarrays on  
clickable surfaces

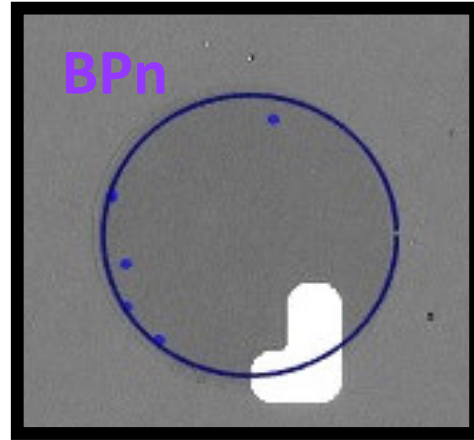
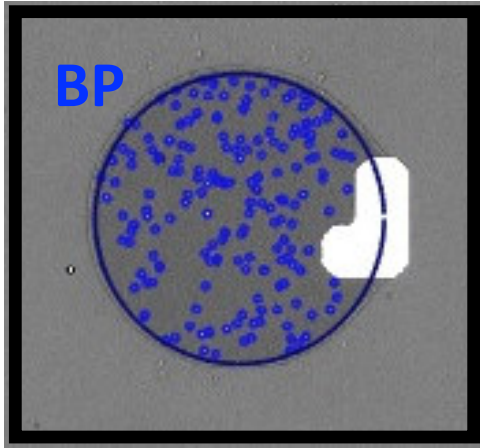
On-spot label-free EV  
counting and sizing  
by SP-IRIS

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

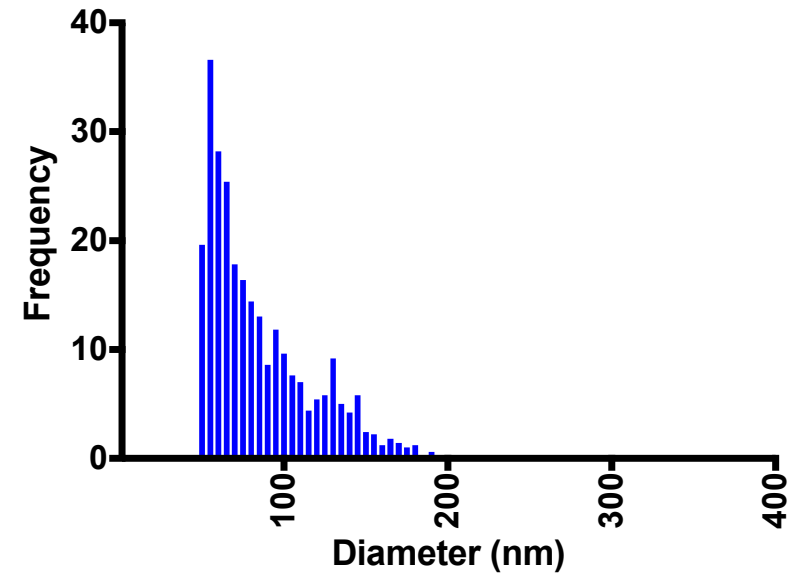
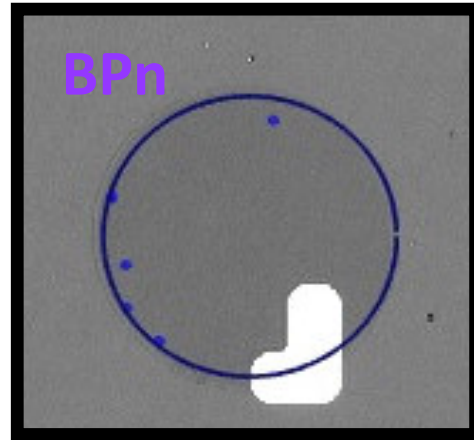
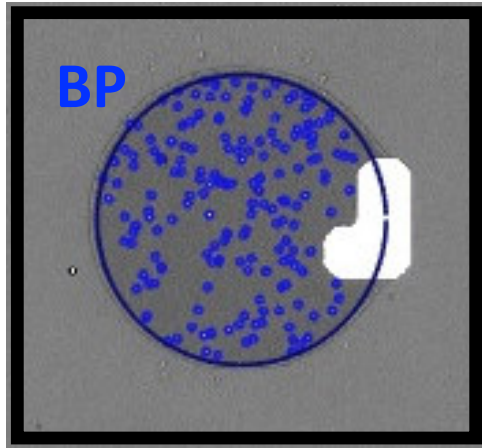
## ➤ UC isolated EVs from HEK cells: characterization according to MISEV2018



➤ UC isolated EVs from HEK cells captured by peptide spots

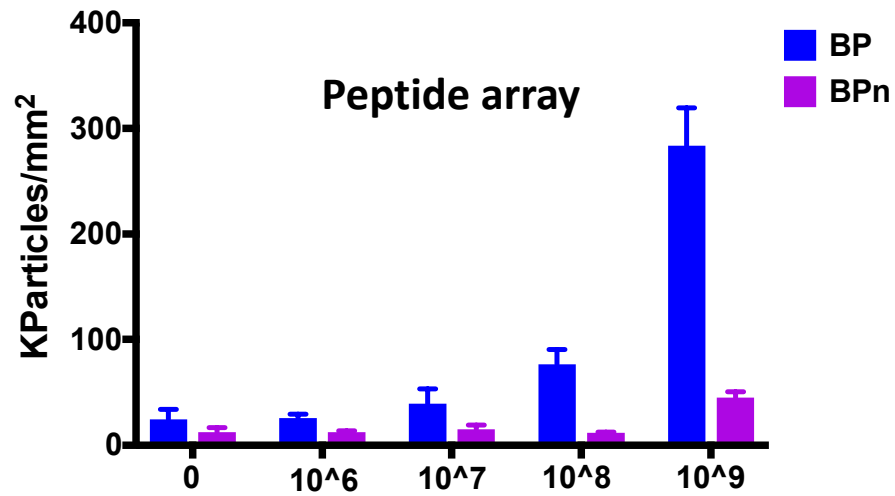
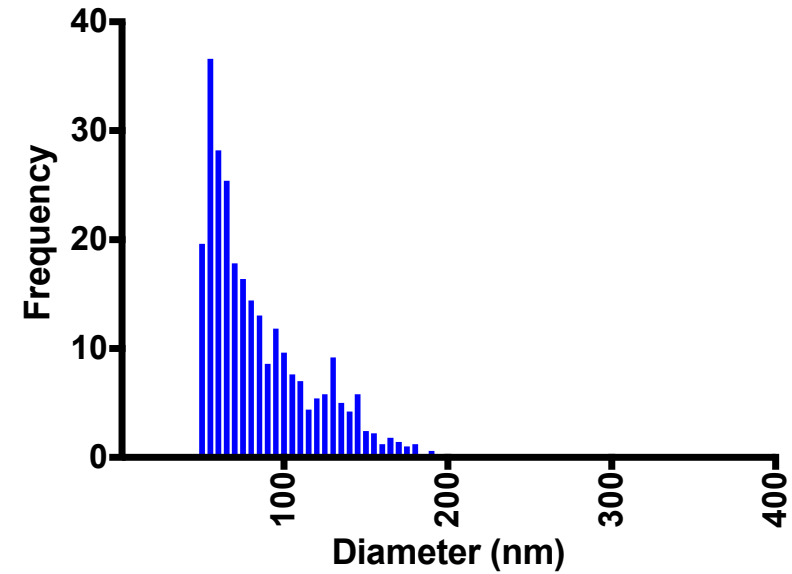
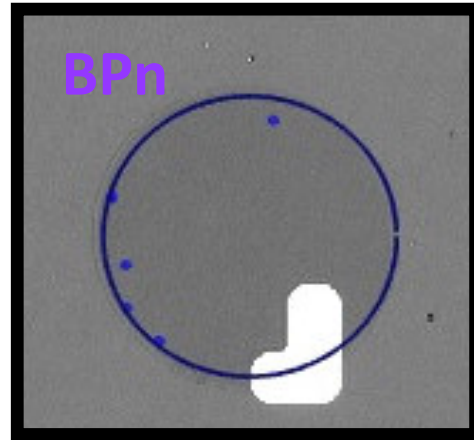
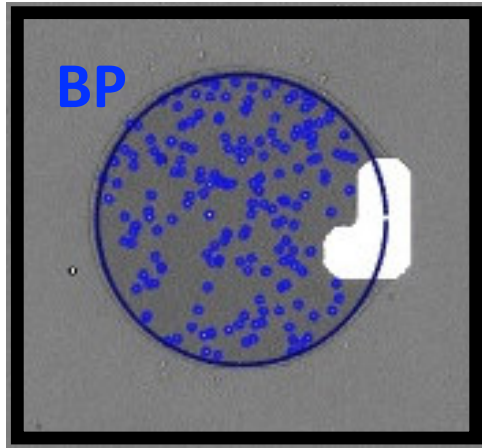


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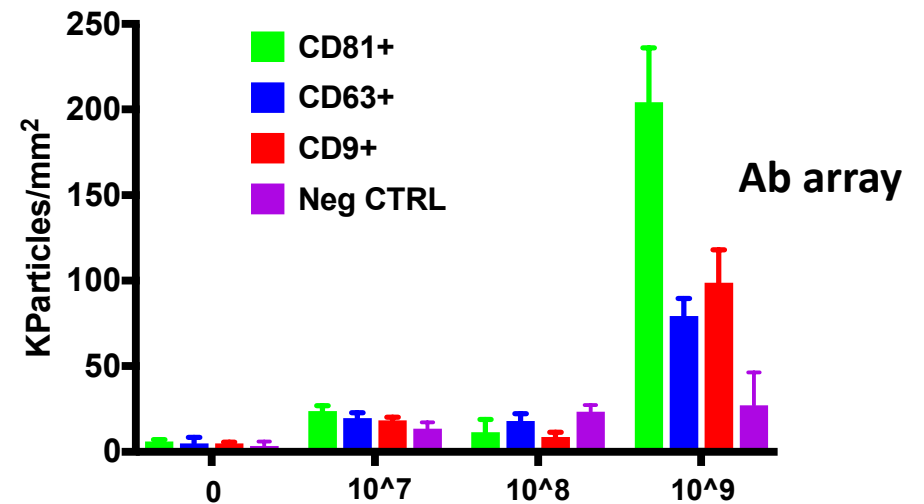
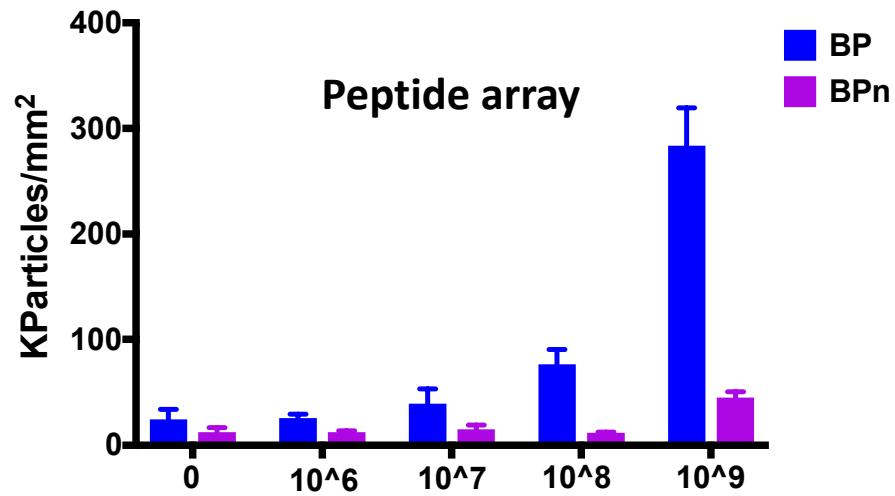
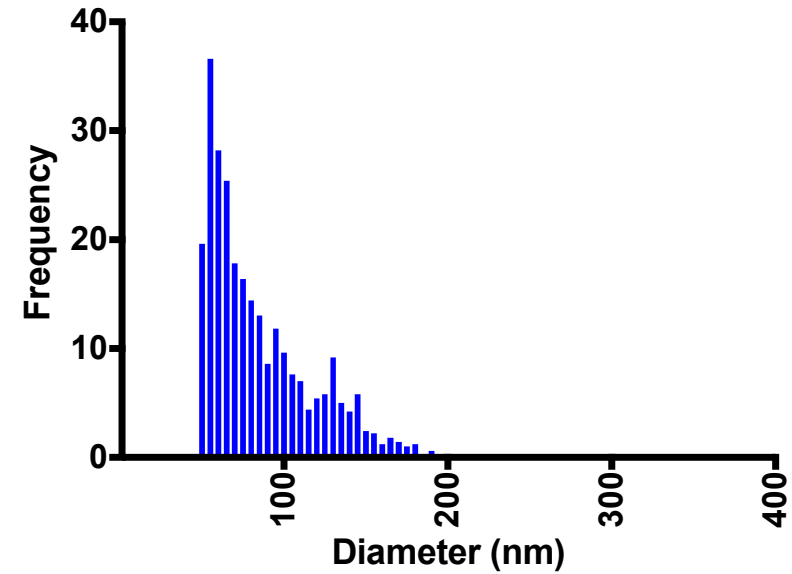
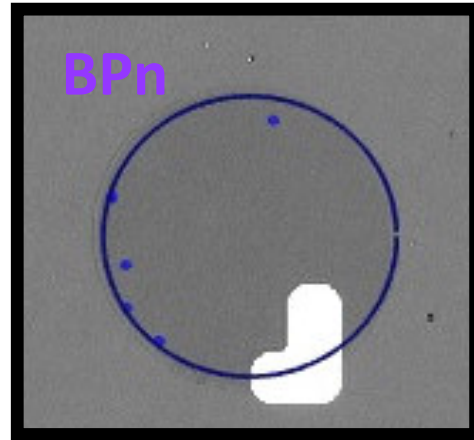
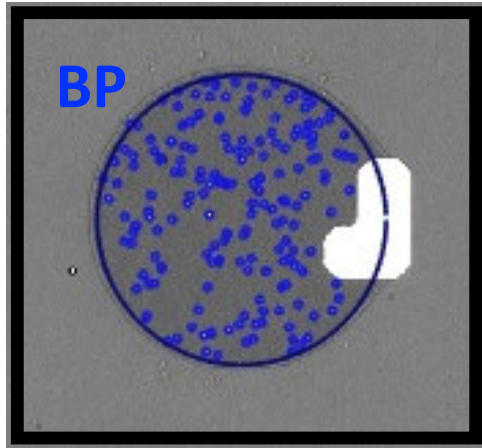




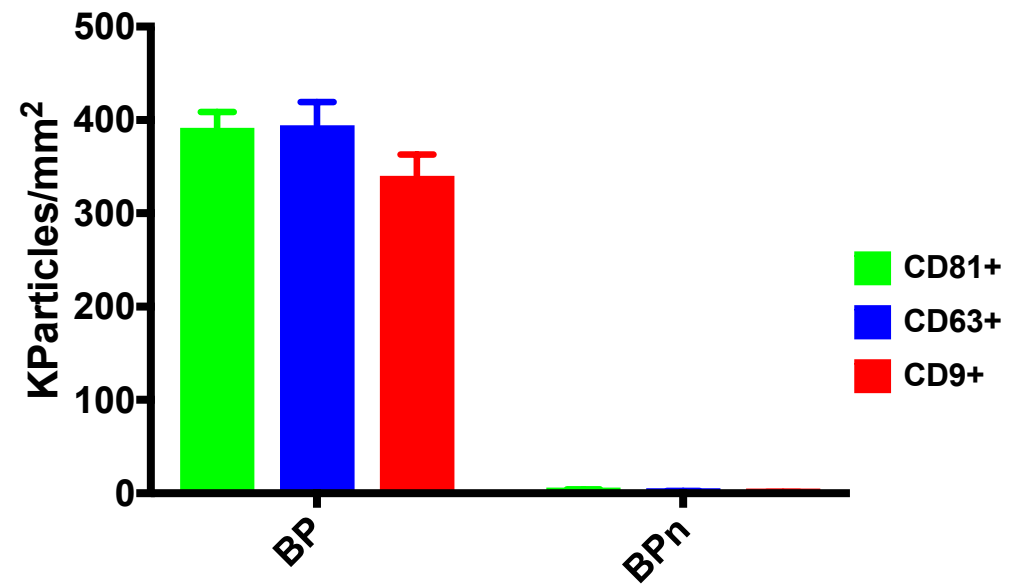
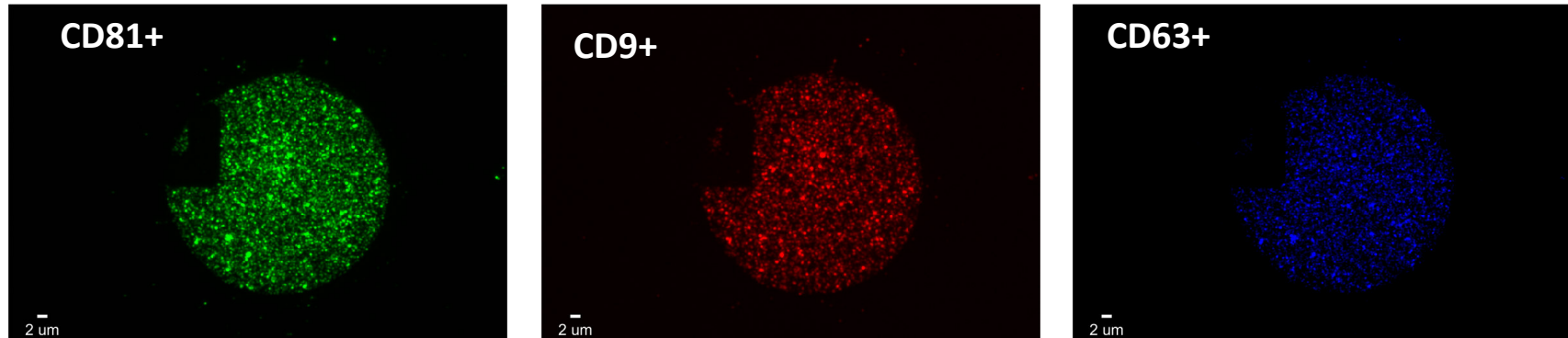
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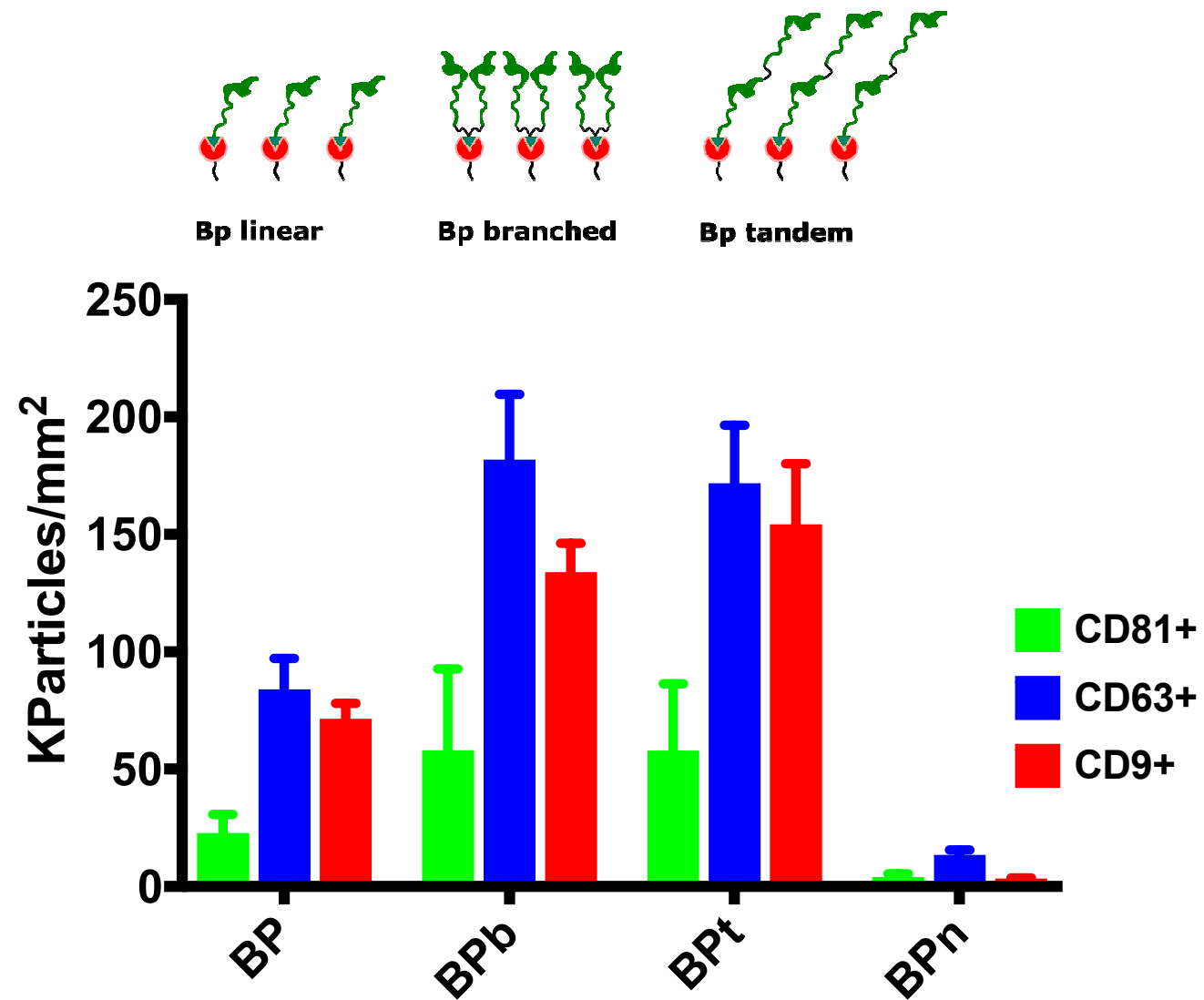
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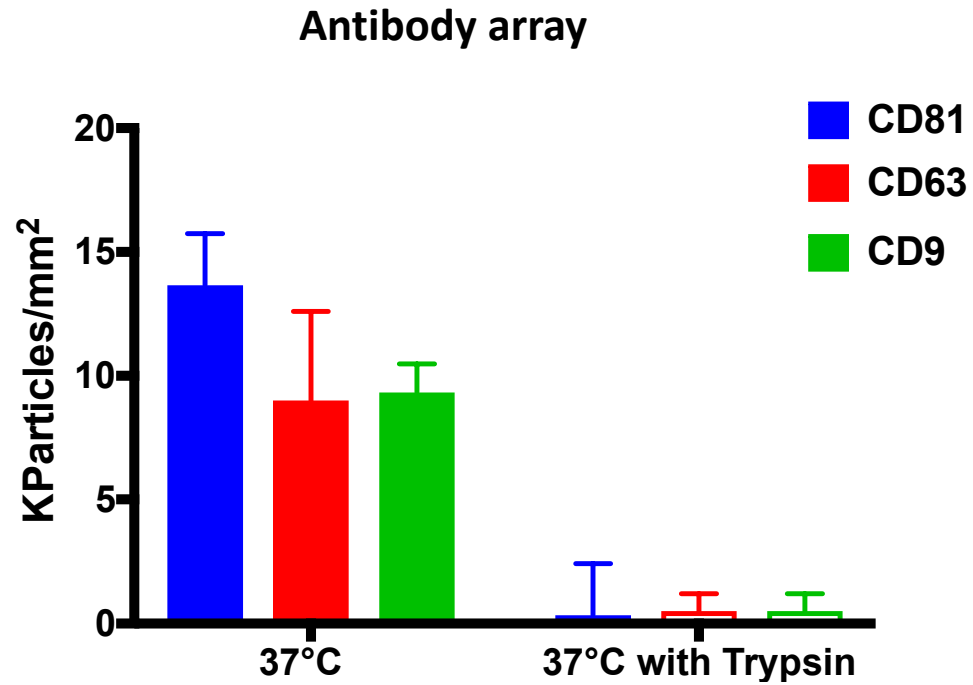
## ➤ On-spot staining of CD9/CD63/CD81



➤ EVs captured from un-treated serum

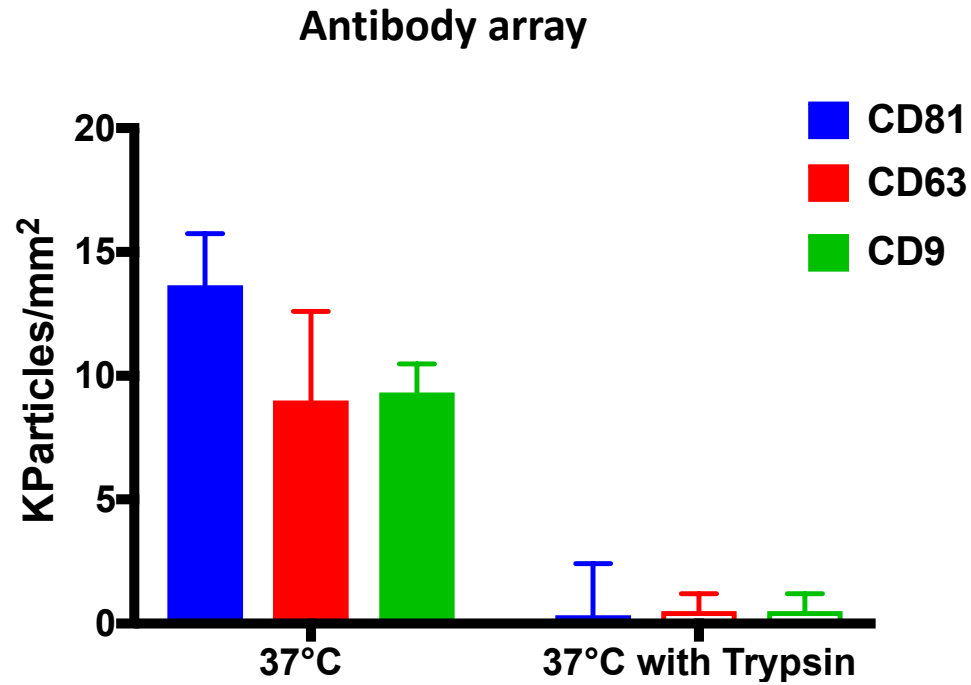


## ➤ Trypsin shaving of EVs

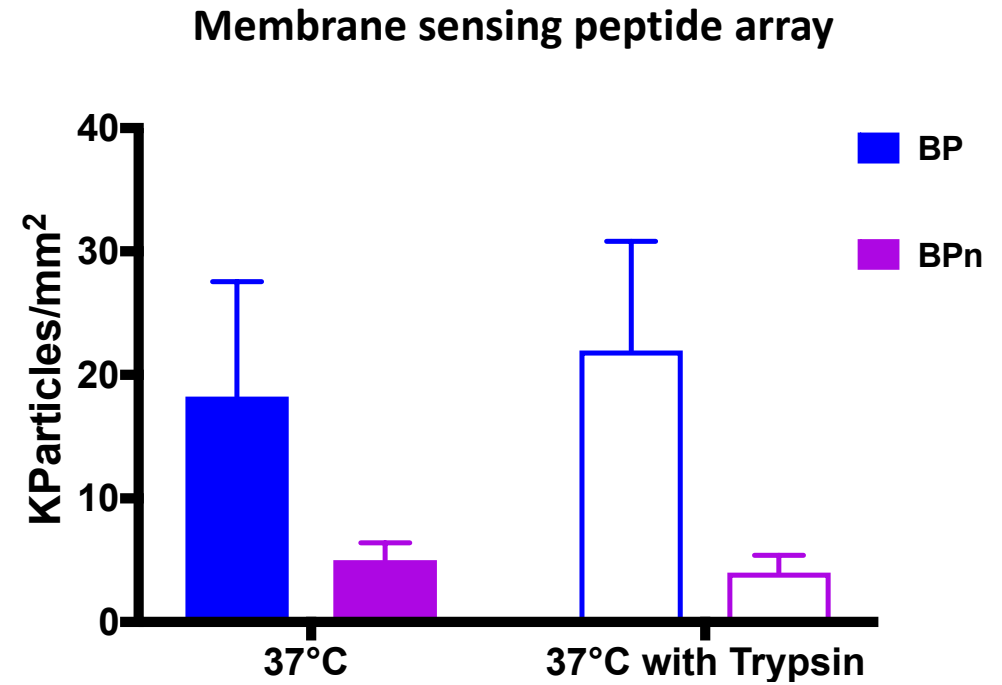


**Binding is ABOLISHED by protein digestion**

## ➤ Trypsin shaving of EVs



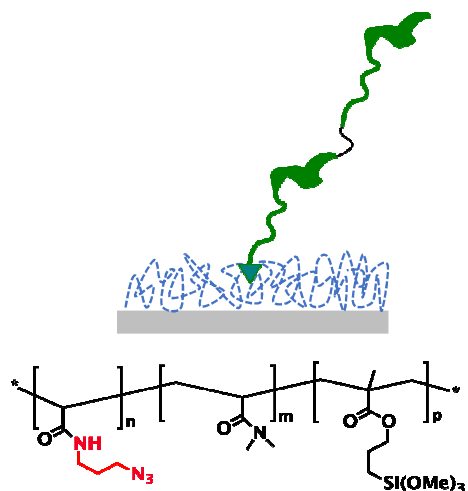
**Binding is ABOLISHED by protein digestion**



**Binding is NOT influenced by protein digestion**

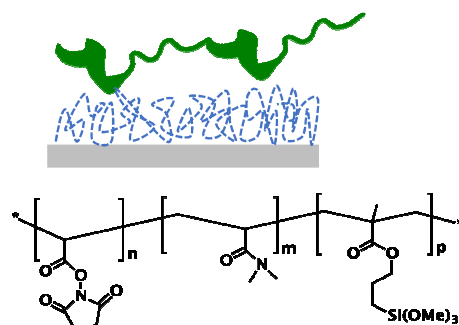
## ➤ Role of surface chemistry

### Chemoselective



MCP-6

### Random



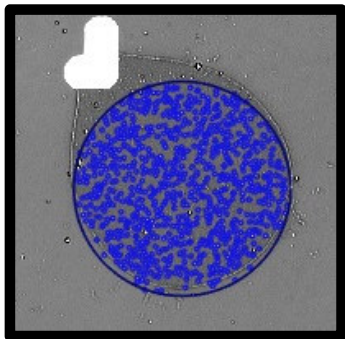
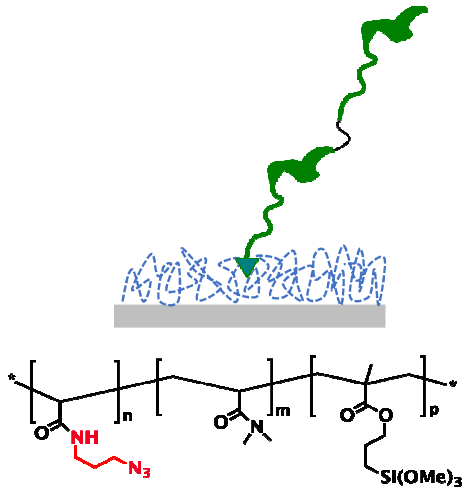
MCP-2

Pirri G et al Anal Chem, 2004, 76 (5), 1352-1358

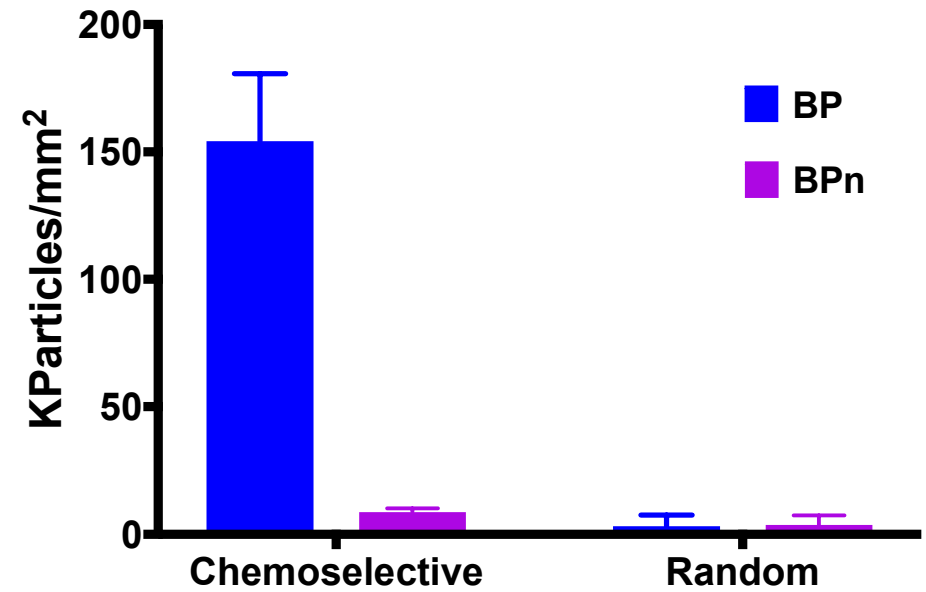
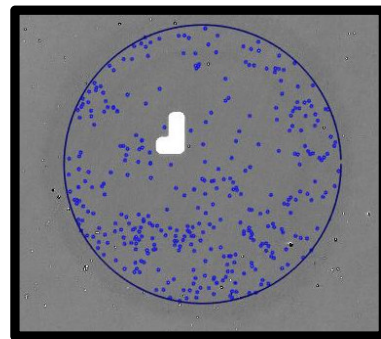
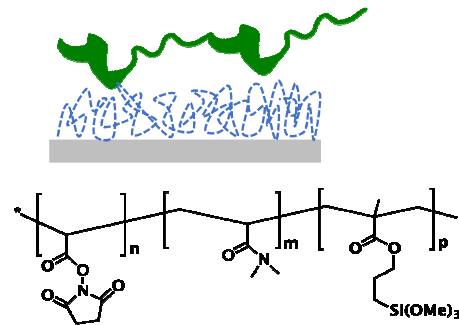
Sola L. et al. Langmuir, 2016, 32, 10284-10295

## ➤ Role of surface chemistry

### Chemoselective



### Random



*God made the bulk; surfaces were invented by the devil.*  
Wolfgang Pauli (1900-1958)



## ➤ Size dependancy of peptide capturing using liposomes of different size

### Pronanosome Lipo-N

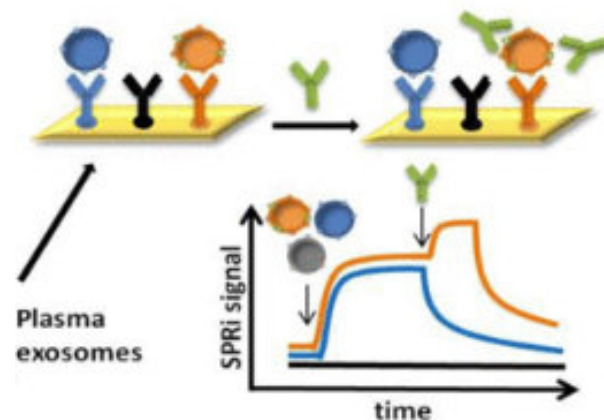
Sample	Size (Z-Average), nm	PDI
Lipo-N 150 nm	165, 4 ± 0,75	0,056 ± 0,014
Lipo-N 250 nm	255,6 ± 3,59	0,174 ± 0,038
Lipo-N 450 nm	425,0 ± 3,58	0,376 ± 0,011

## ➤ Size dependancy of peptide capturing using liposomes of different size

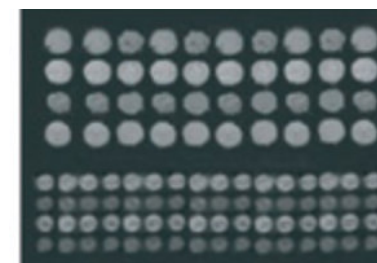
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### Surface plasmon resonance Imaging (SPRi)



Silvia Picciolini et al.  
Anal Chem 2018  
7;90(15):8873-8880

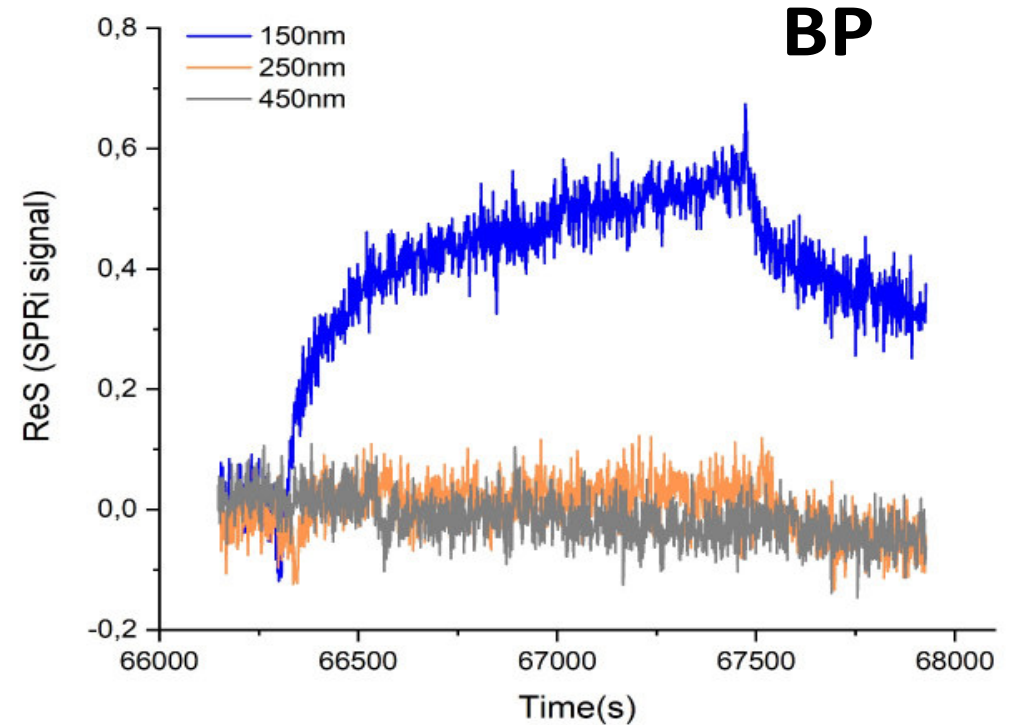


Gori, A., Cretich, et al Anal. Chim. Acta (2017) 983, 189–197

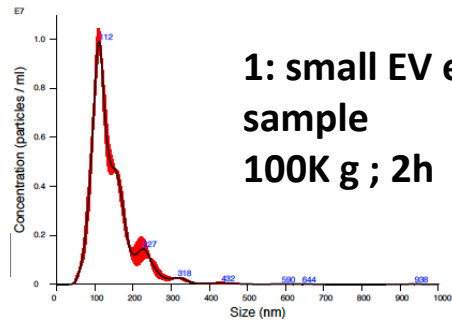
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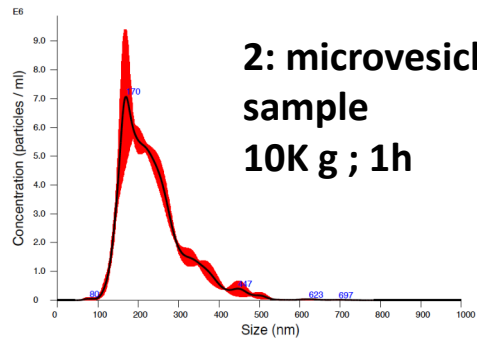
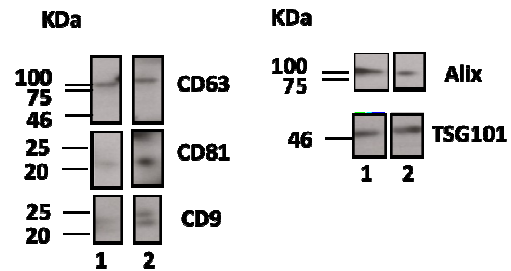
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## ➤ Size dependency of peptide capturing using EVs from platelet free human plasma

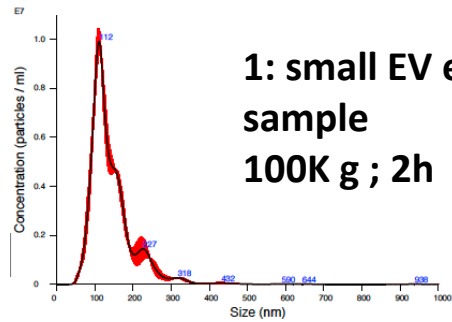


**1: small EV enriched  
sample  
100K g ; 2h**

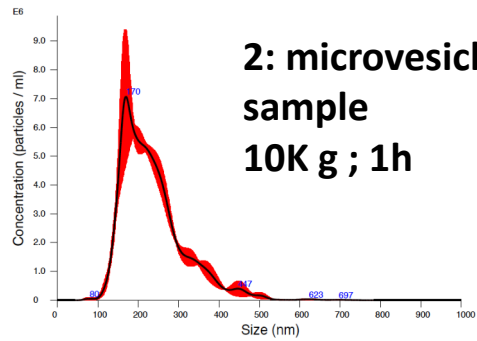
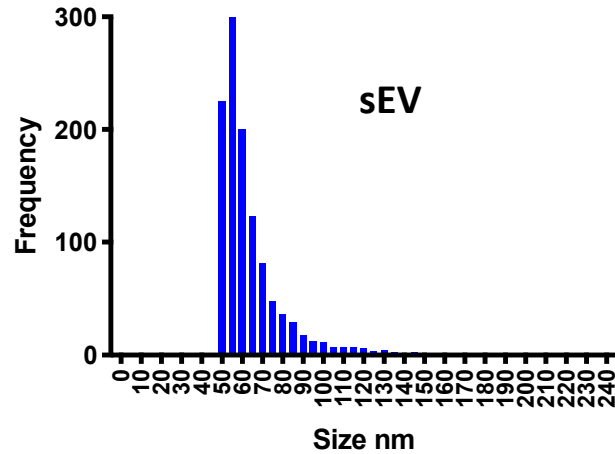
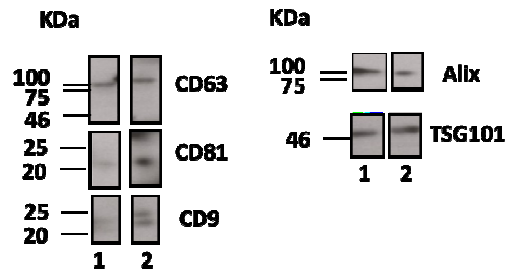


**2: microvesicles enriched  
sample  
10K g ; 1h**

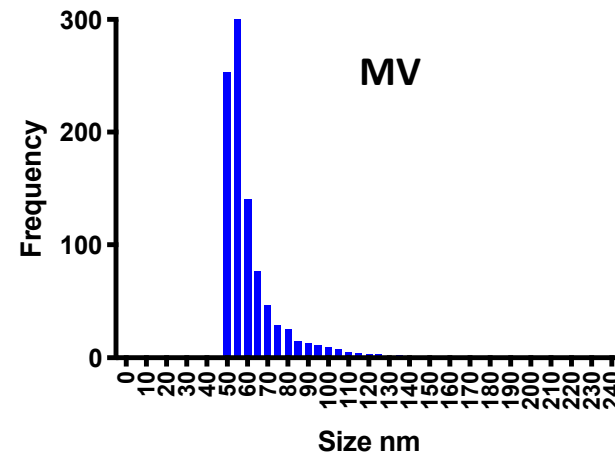
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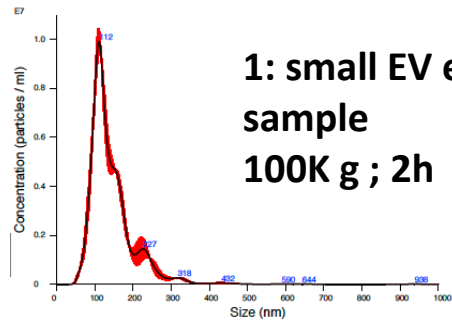
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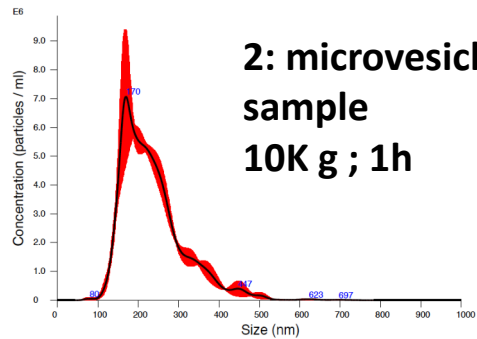
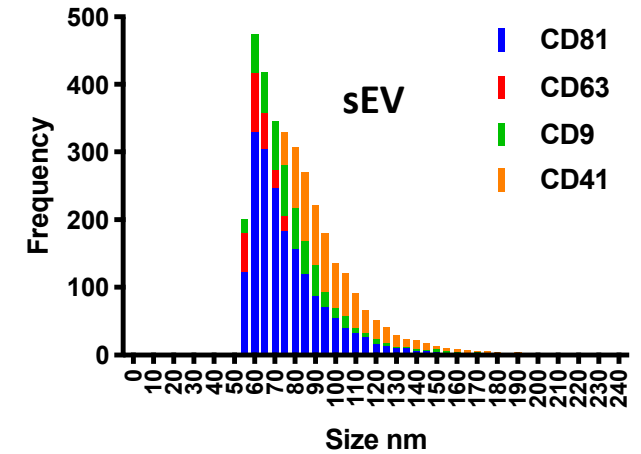
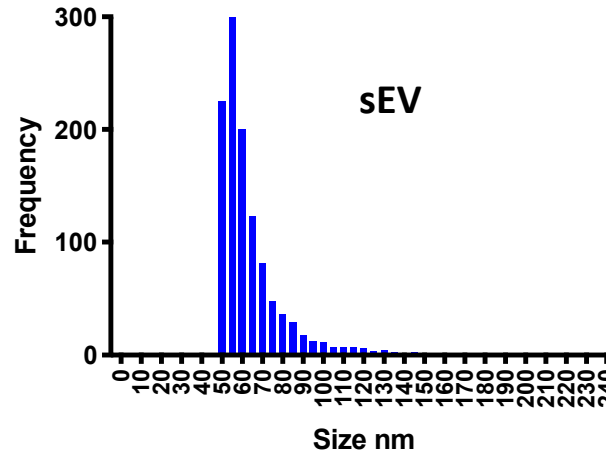
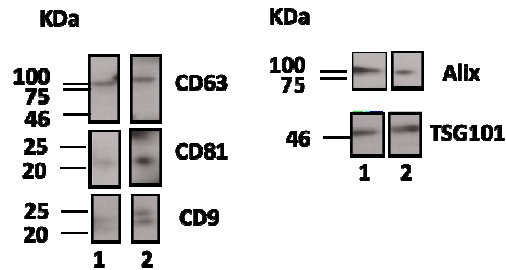
**2: microvesicles enriched  
sample  
10K g ; 1h**



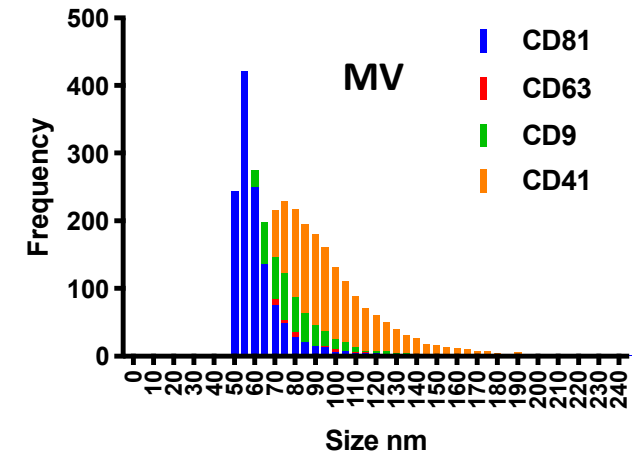
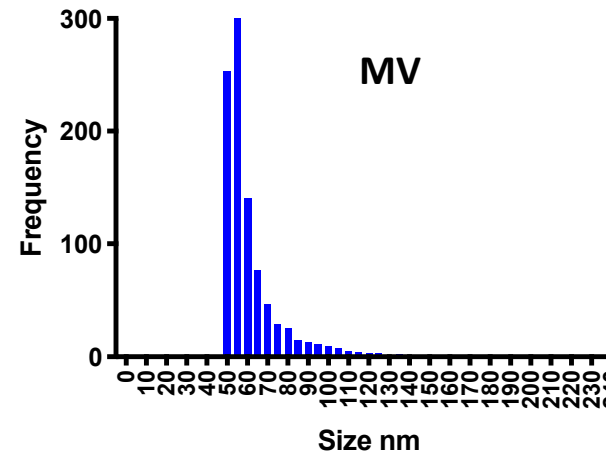
# ➤ Size dependancy of peptide capturing using EVs from platelet free human plasma



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



**2: microvesicles enriched sample**  
10K g ; 1h



## ➤ Summary

- **First example of peptide microarrays applied to EVs**
- **Peptides as alternatives to Antibodies:**
  - **Low cost**
  - **High stability**
  - **No batch to batch variation**
  - **High synthetic versatility → virtually unlimited possibilities for functionalization**

## ➤ Summary

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- **Peptides as alternatives to Antibodies:**
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  - **High stability**
  - **No batch to batch variation**
  - **High synthetic versatility → virtually unlimited possibilities for functionalization**
- **Membrane sensing peptides: universal ligands unbiased by differential EV protein expression**
- **Possible reference and standardization tool**
- **High capturing capacity due to intrinsic multivalency and cooperative binding**
- **Generic baits for both MVs and sEV within their overlapping size range (50–120 nm)**
- **Applicable to complex samples**



**New directions:**

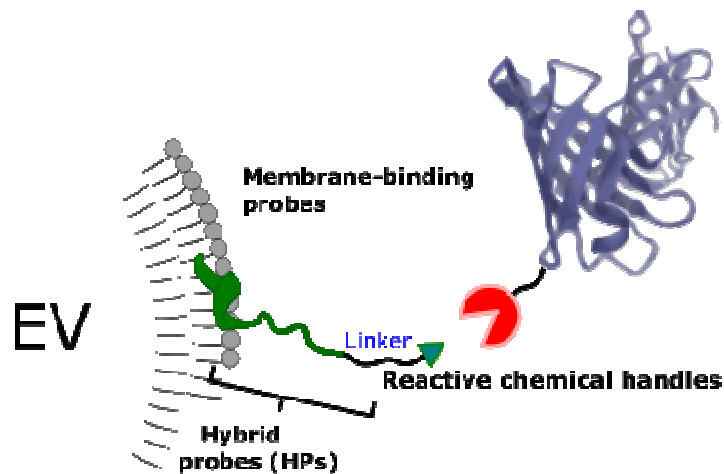


- Wider panel of membrane sensing peptides
- Peptidic probes for specific proteins (eg by Phage Display library screening)
- Use as decorating and reporting agents for EVs
- Use as isolation tools (capture and release)

**New directions:**



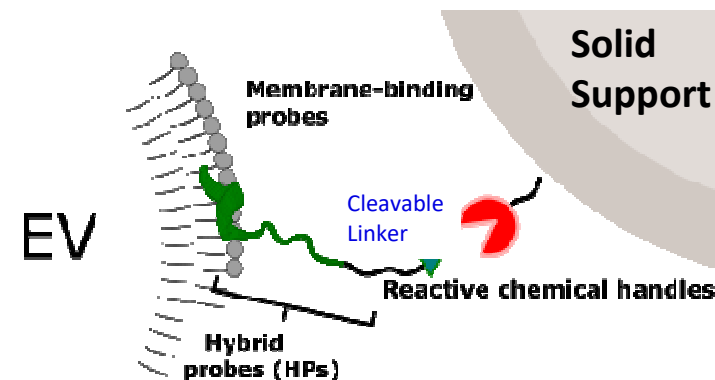
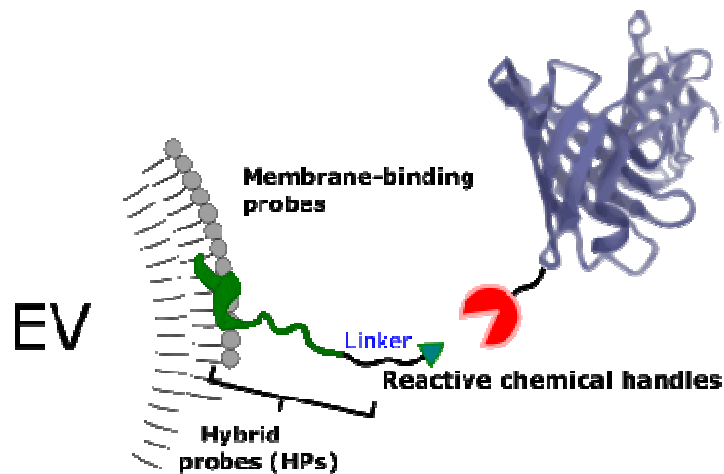
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- Wider panel of membrane sensing peptides
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- Use as isolation tools (capture and release)



➤ Merging peptide science and EVs: an opportunity arises

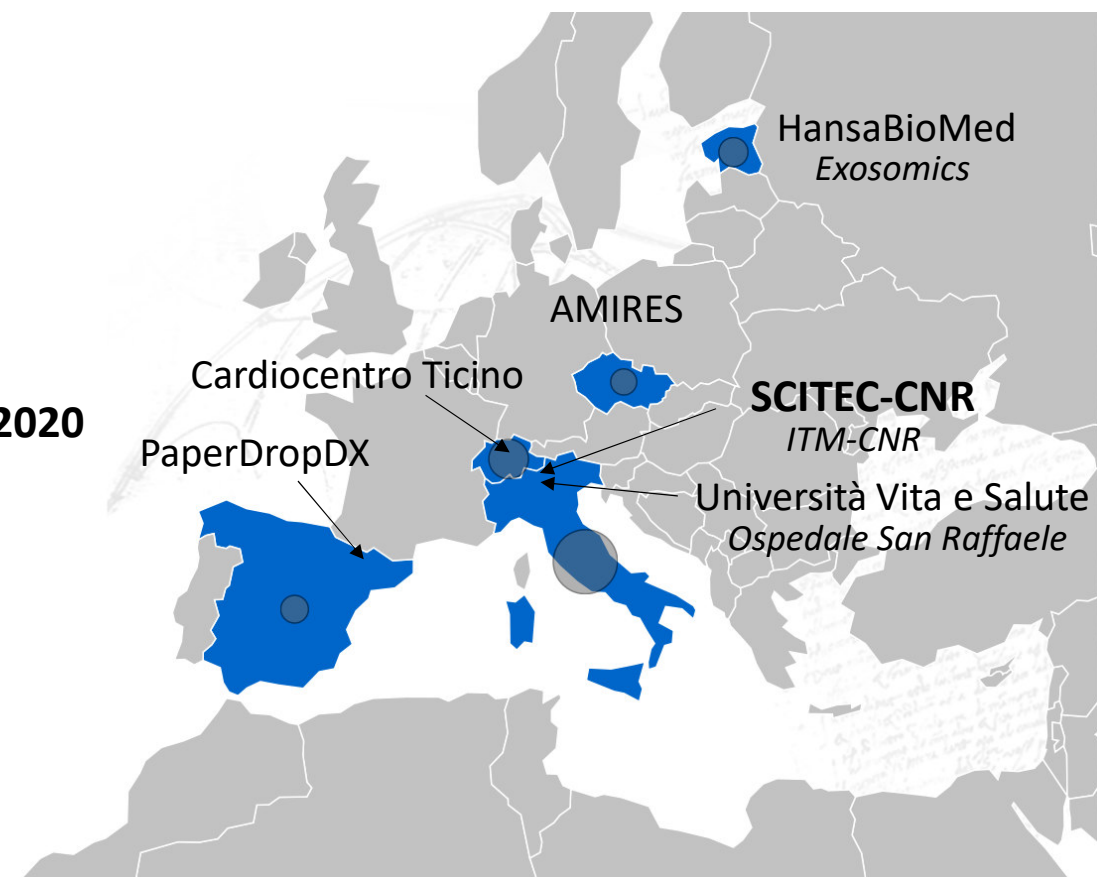


**Evolving reversible iMmunocapture by membrAne sensing peptides: towaRds scalable extracellular VEsicles isolation**

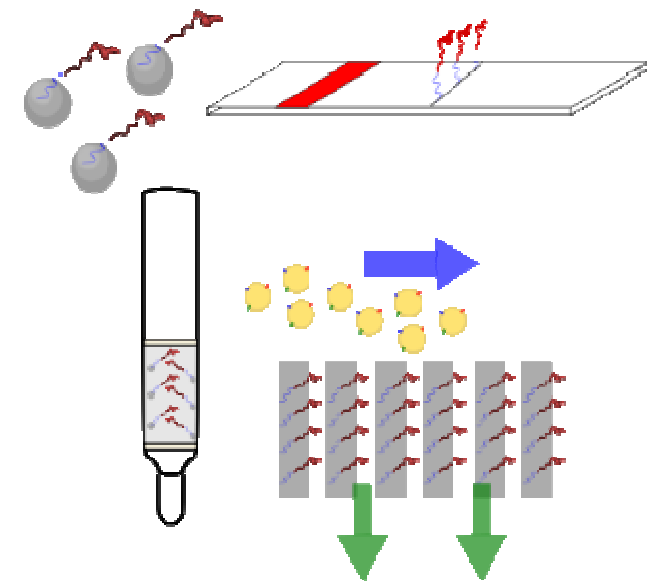
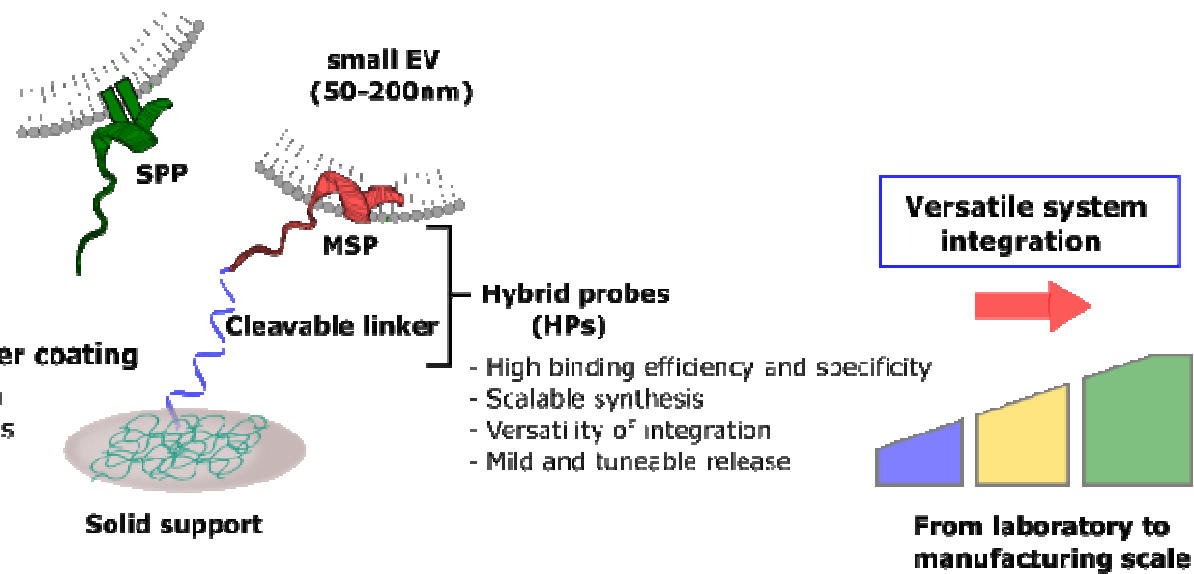


**Call: H20202-FETPROACT-2019-2020**  
**Topic: FETPROACT-EIC-06-2019**

**1/11/2020 – 31/10/2022**  
**Funding: 1.881.000 Euro**  
**5 partners**



## ➤ Merging peptide science and EVs: an opportunity arises



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## ➤ Acknowledgements



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## ➤ Fundings



**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**





*Thank you for the kind attention!*

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