

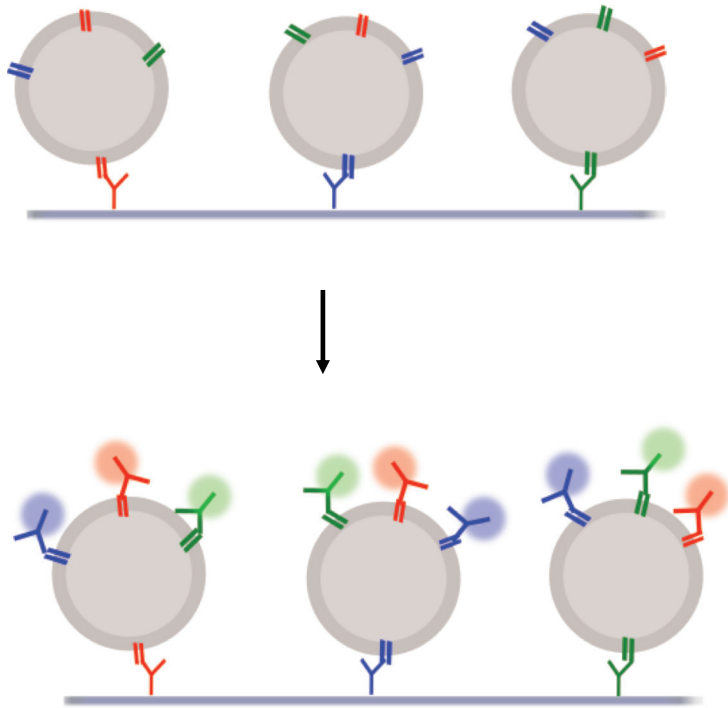


# Membrane sensing peptide microarrays for Extracellular Vesicles

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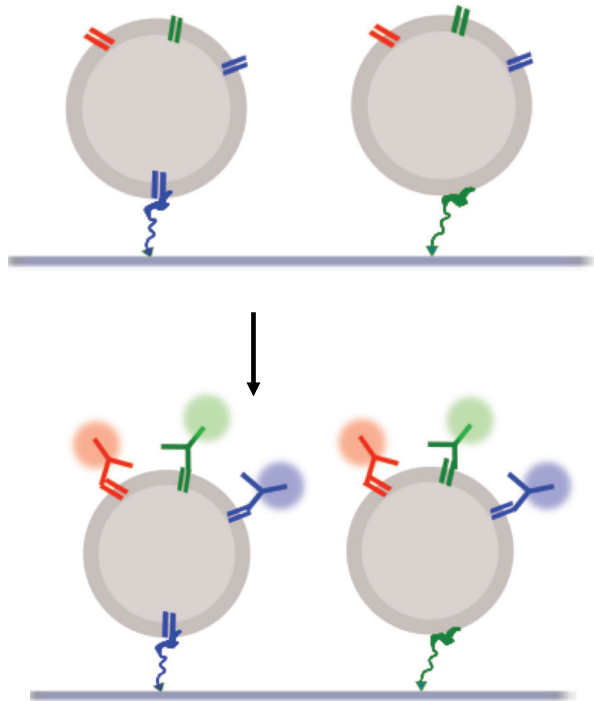
## ➤ The context: EV microarrays

### Antibody based capturing of EVS



## ➤ The context: EV microarrays

### Peptide microarrays for EVs

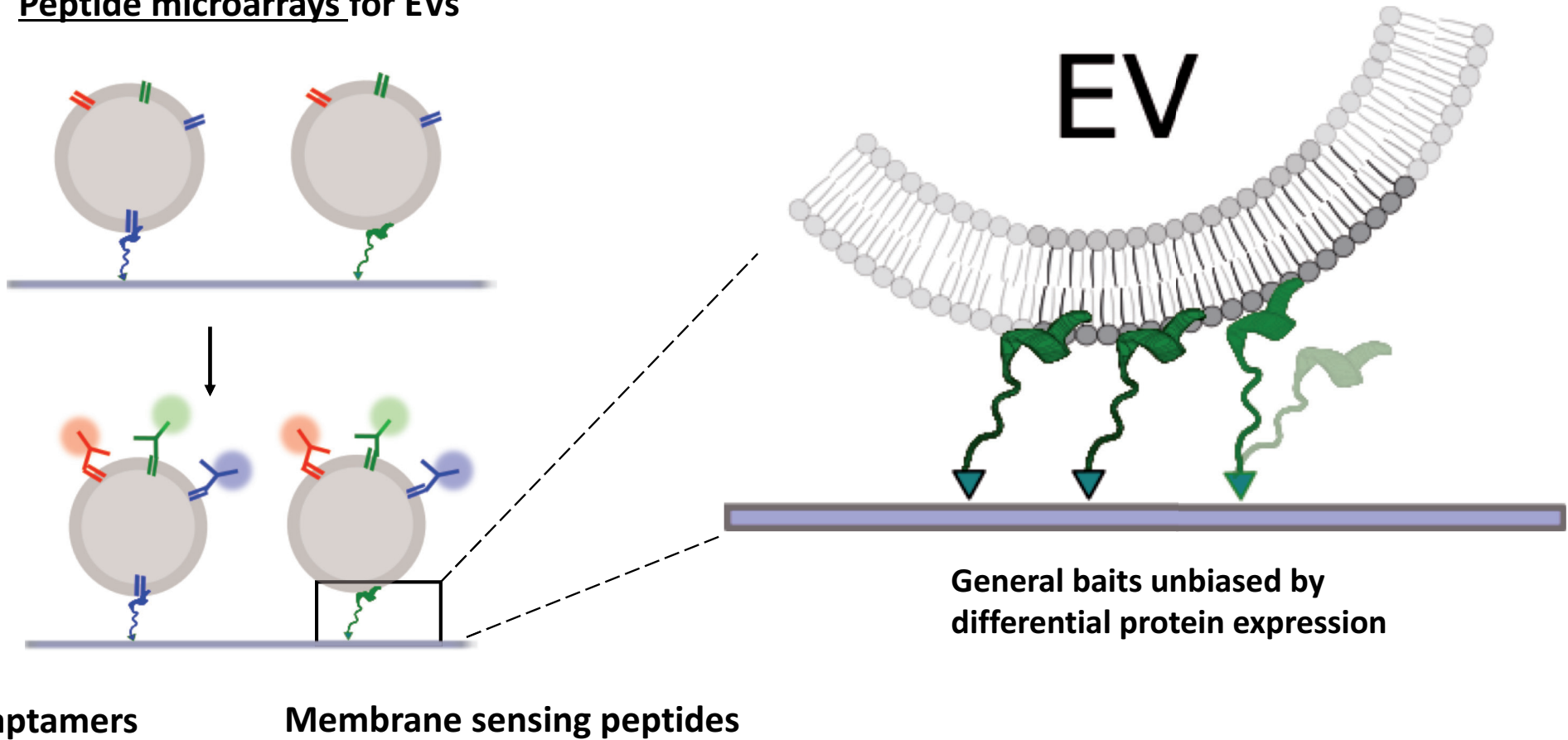


Peptide aptamers

Membrane sensing peptides

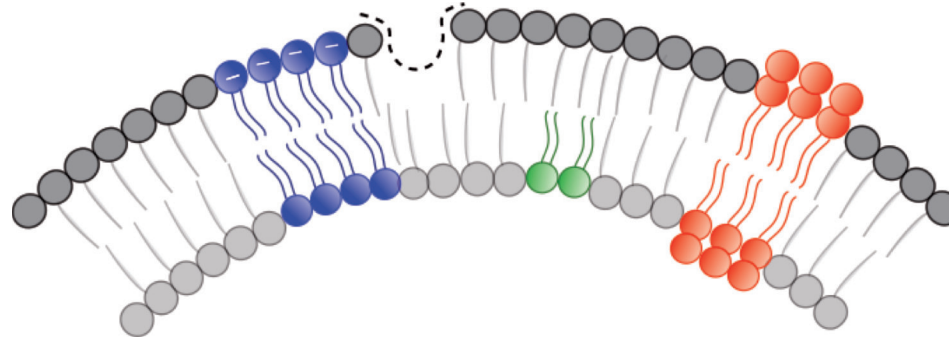
## ➤ The context: EV microarrays

### Peptide microarrays for EVs

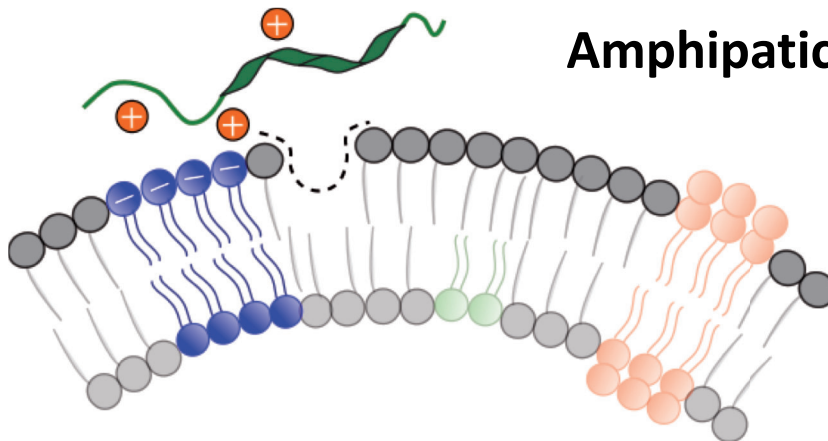




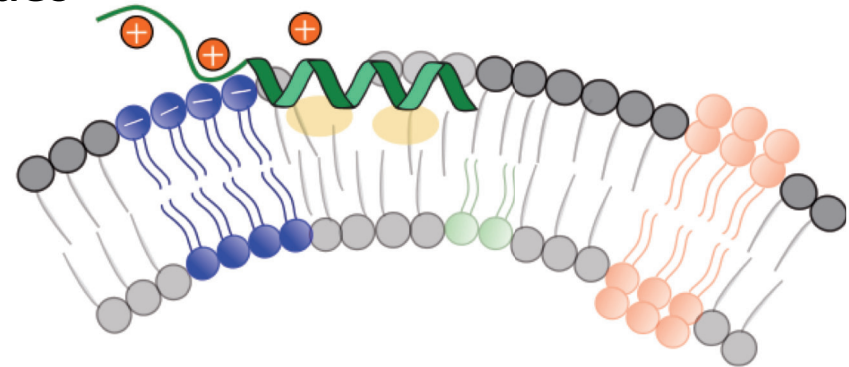
## ➤ Membrane sensing peptides



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



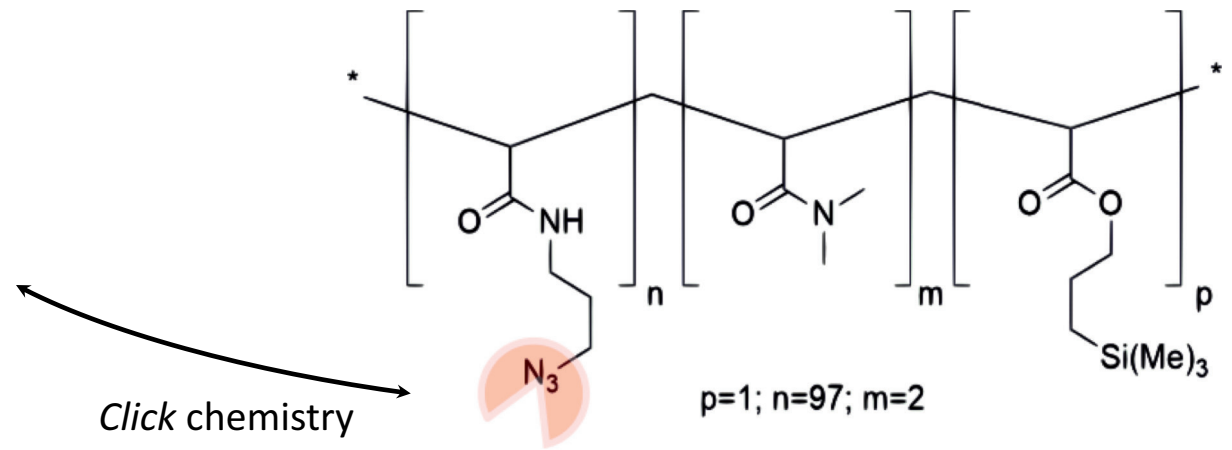
**Amphipathic peptides**



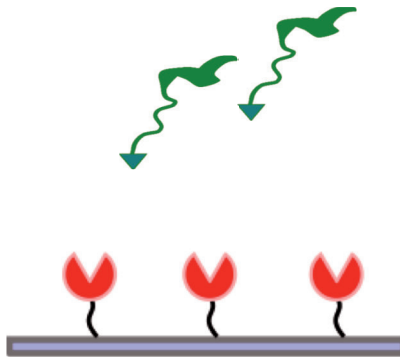
## ➤ Membrane sensing peptides

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

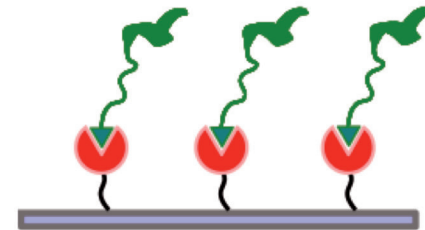
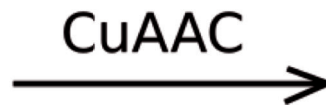
BPn: **E**PPGFSP**F**E-K-G- (O<sub>2</sub>Oc)<sub>2</sub>-Prg



**Copoly Azide**

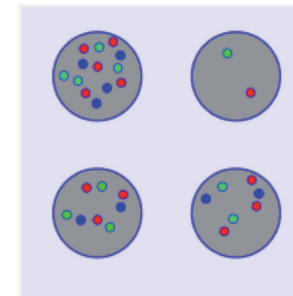
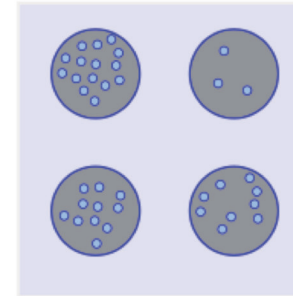
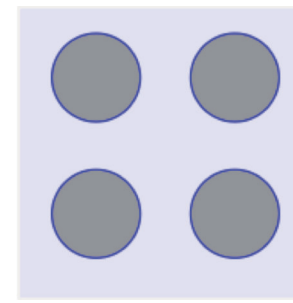
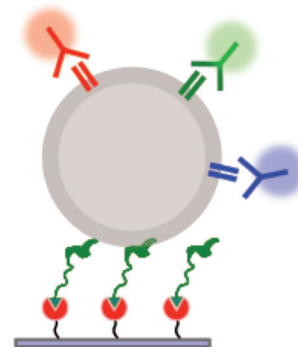
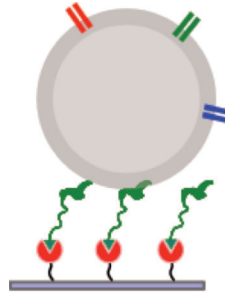
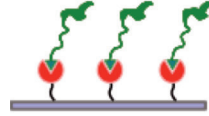
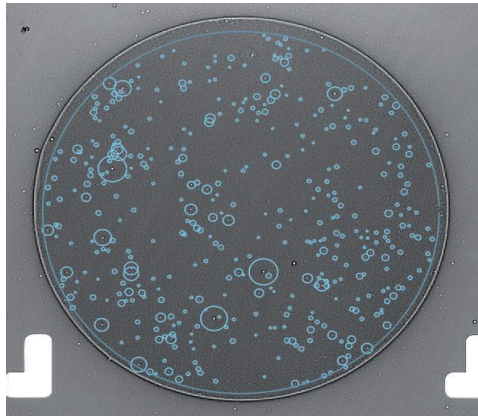
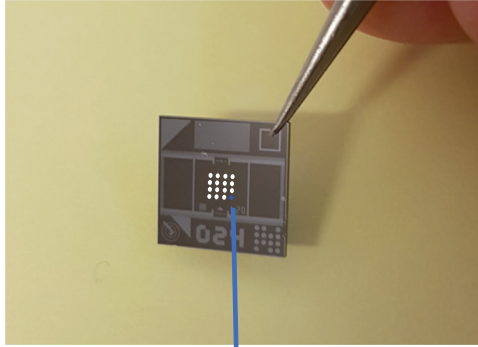


**Clickable chip**



## ➤ Experimental

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

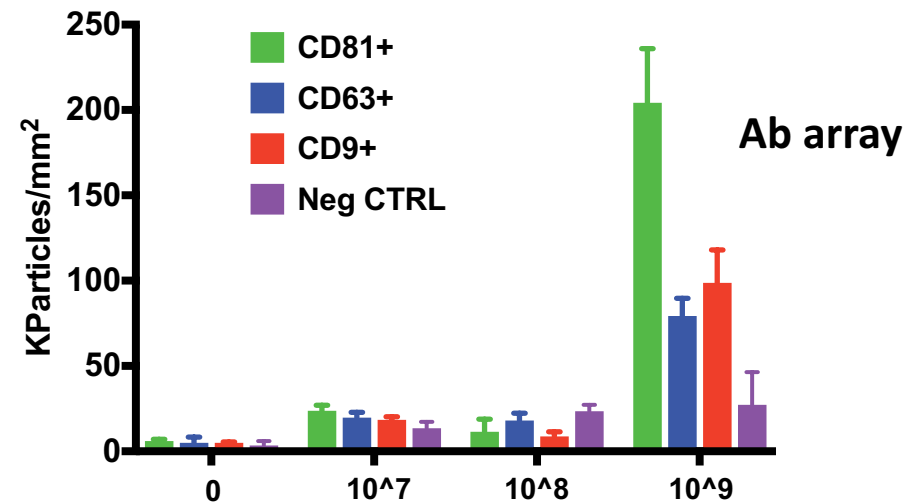
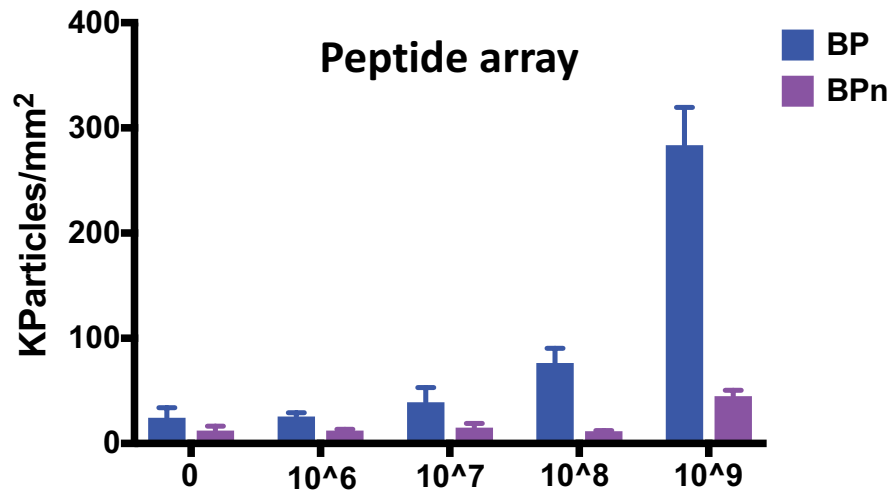
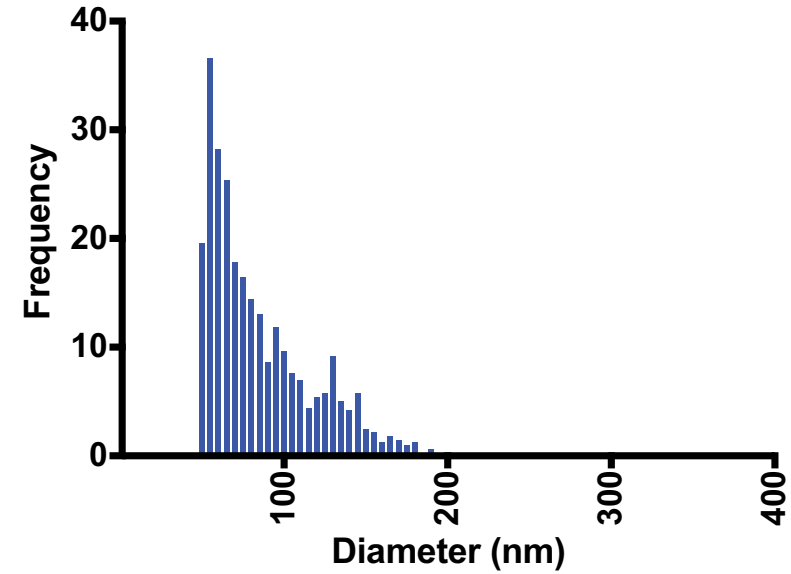
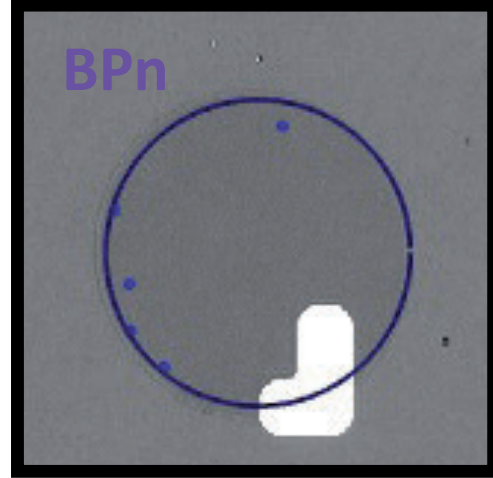
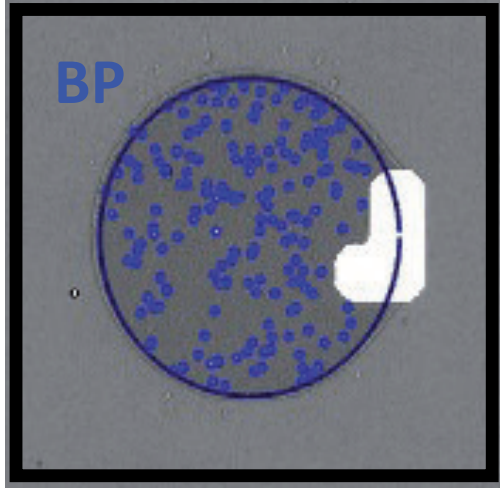


**Peptide microarrays on  
clickable surfaces**

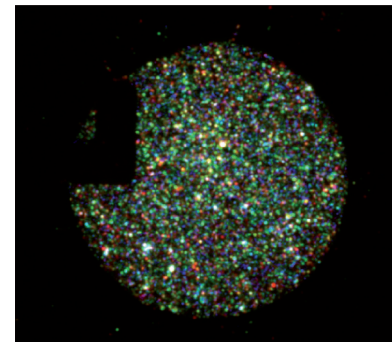
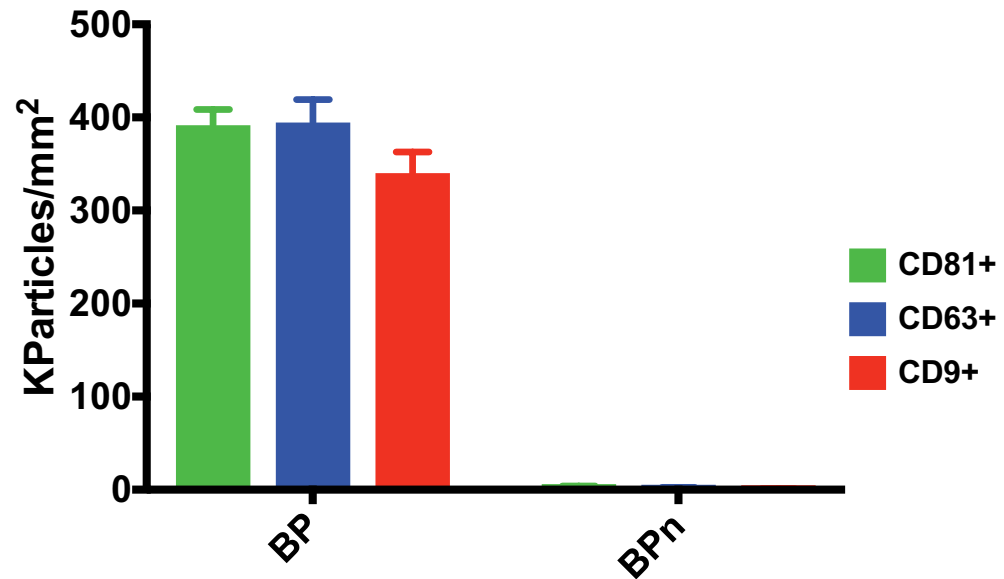
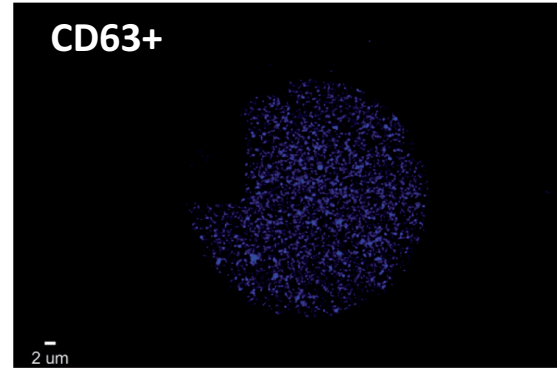
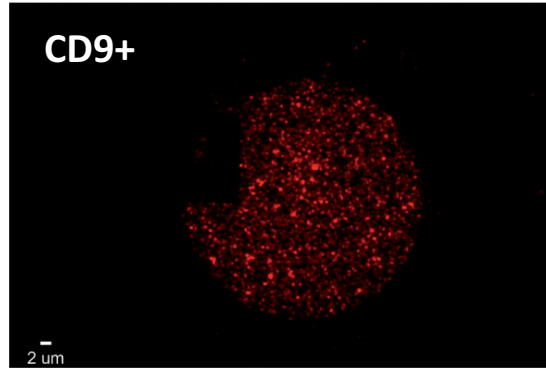
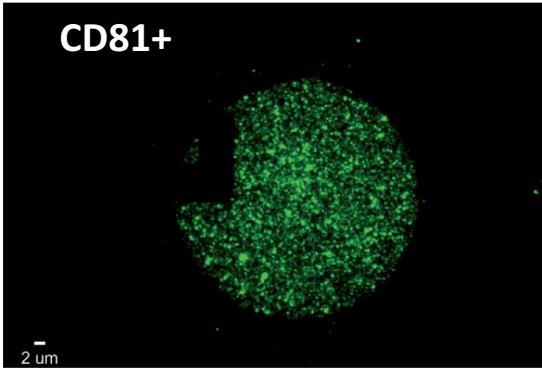
**On-spot label-free EV  
counting and sizing  
by SP-IRIS**

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

➤ UC isolated EVs from HEK cells captured by peptide spots



➤ On-spot staining of CD9/CD63/CD81

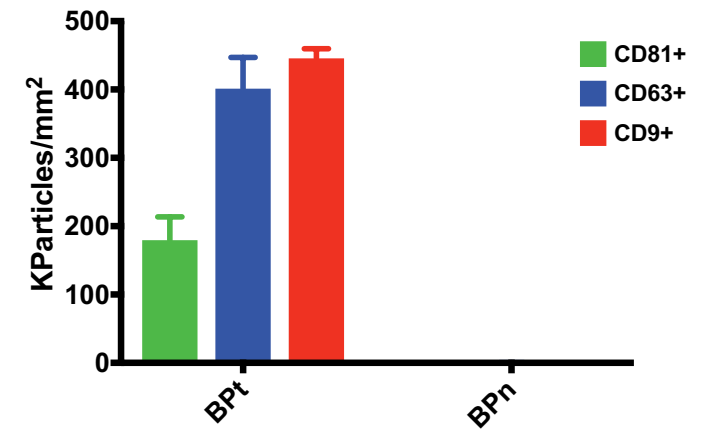
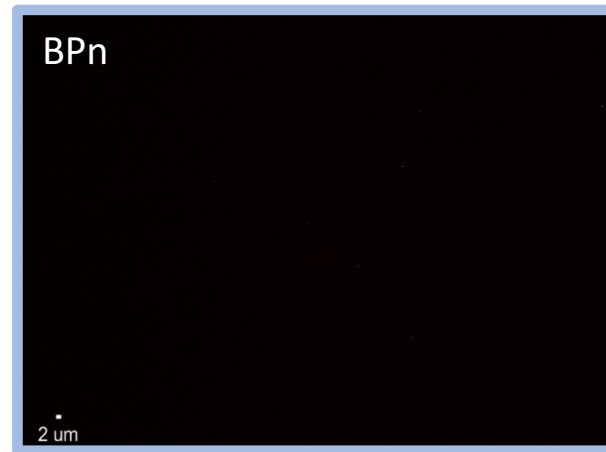
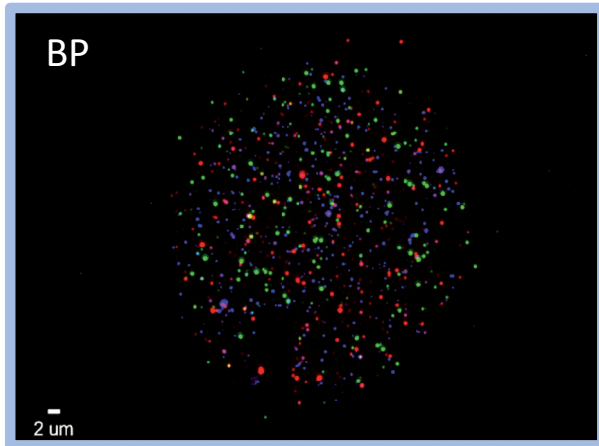
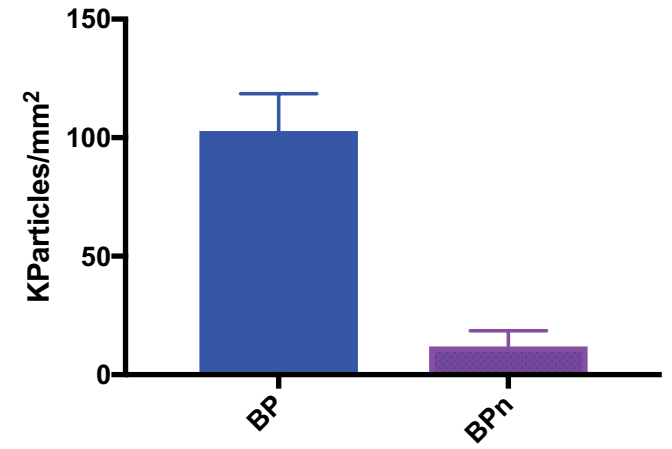
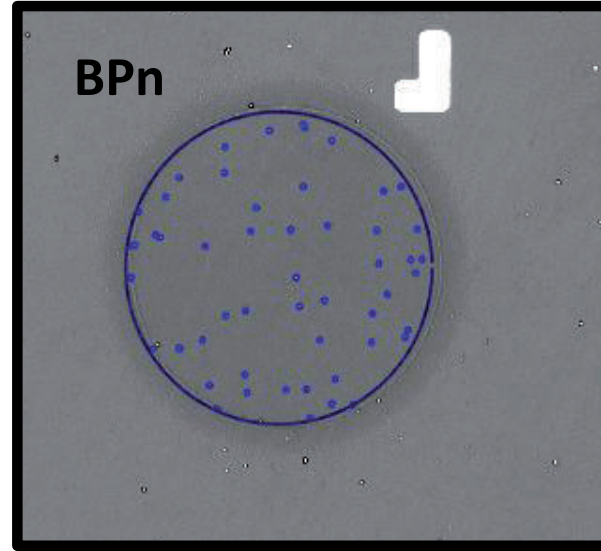
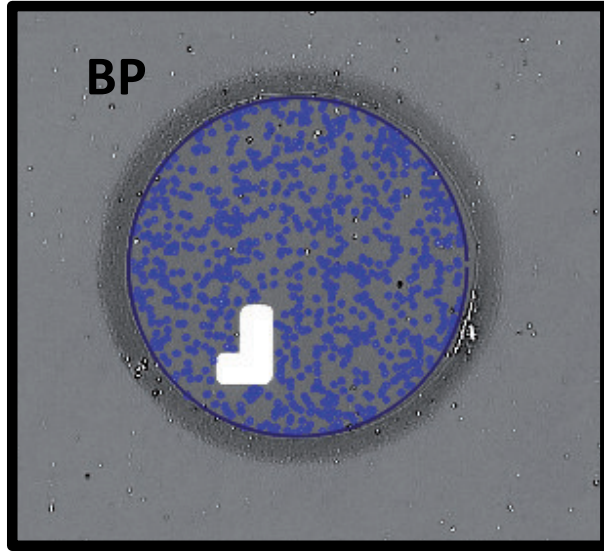


BP



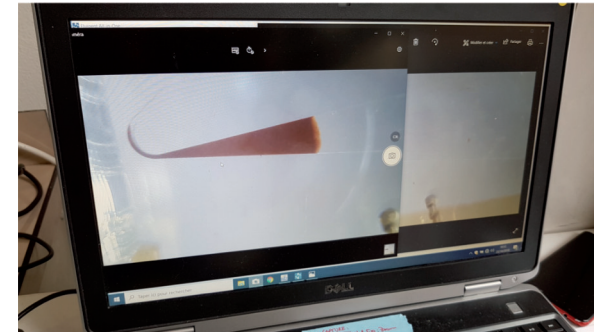
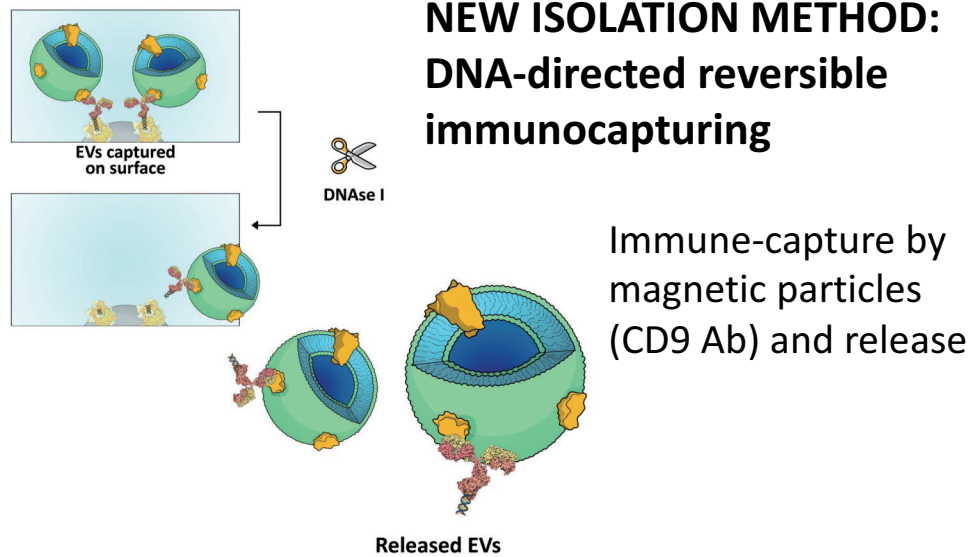
BPn

# ➤ EVs captured from un-treated serum



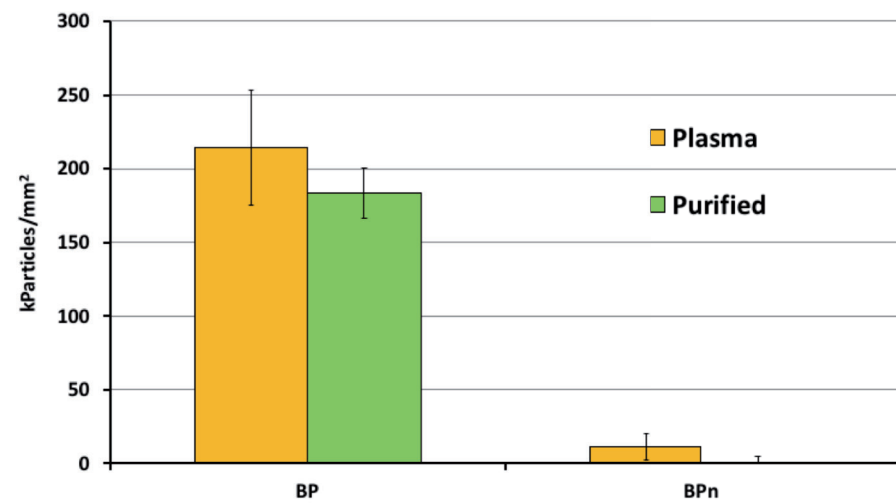


# ➤ EVs captured downstream microfluidic capture and release



**Fluidized bed  
chip**

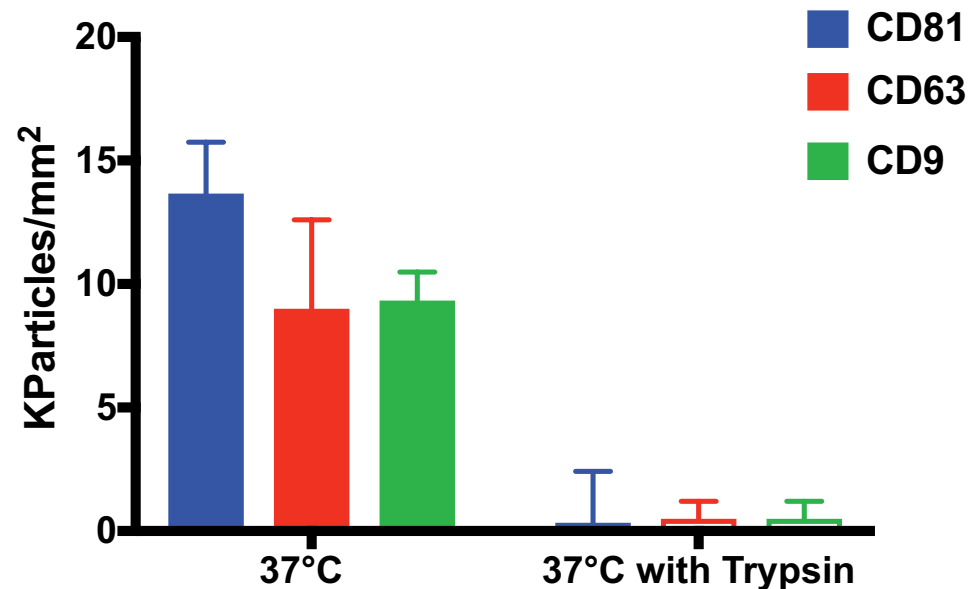
**Institut Curie – Paris**



**Poster P1  
presented by Dario Brambilla**

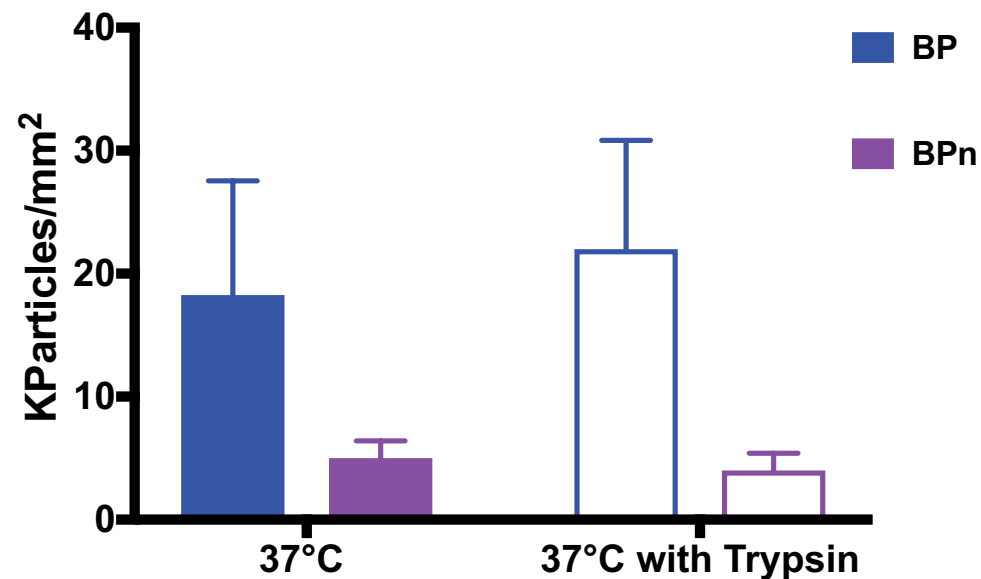
➤ Trypsin shaving of EVs

Antibody array



Binding is ABOLISHED by protein digestion

Membrane sensing peptide array

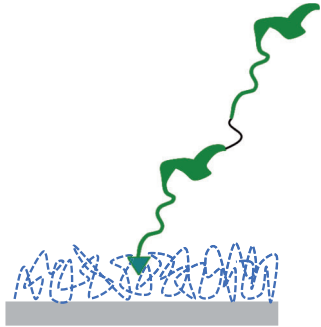


Binding is NOT influenced by protein digestion

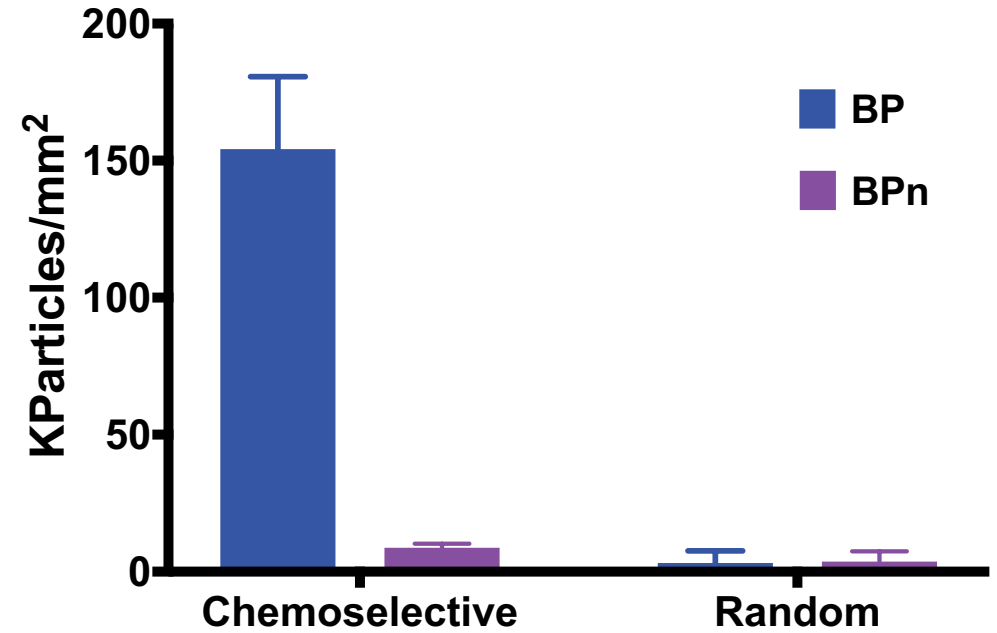
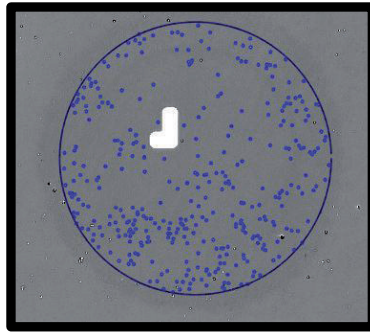
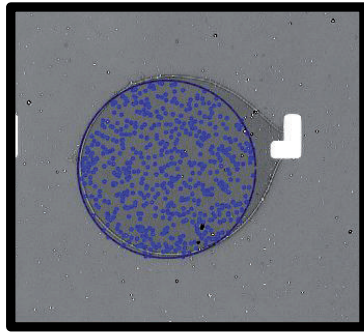
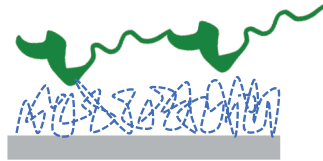


## ➤ Role of surface chemistry

Chemoselective



Random



## ➤ Summary and perspectives

- First example of **peptide microarrays** applied to EVs
  - **Peptides as low cost** alternatives to Abs
  - High stability; **no batch to batch variation**
  - High synthetic versatility → **virtually unlimited possibilities for functionalization**
- 
- Membrane sensing peptides: **universal ligands** for EVs unbiased by differential protein expression
  - Possibility to **combine general and specific** EVs capturing on a single array: **reference and standardization tool**
  - High capturing capacity due to **intrinsic multivalency and cooperative binding**

**New directions:**



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

## ➤ Acknowledgements



**Riccardo Vago**  
**Silvia Galbiati**



**George Daaboul**  
**David Freedman**



**LABION**  
**Silvia Picciolini**



**Natasa Zarovni**



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**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**



**Thank you for the kind attention**  
**Grazie a tutti per l'attenzione**



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