Membrane Sensing Peptides for Extracellular Vesicles On-chip Analysis

EXTRACELLULAR VESICLES LAB

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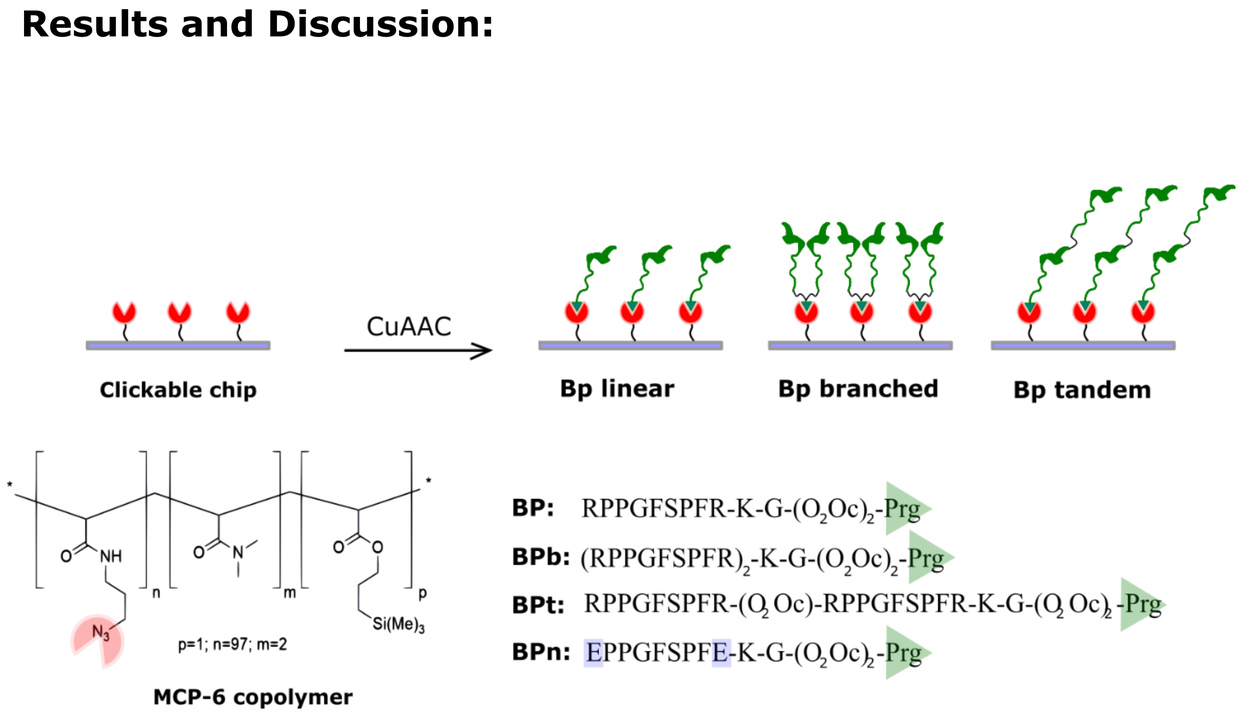
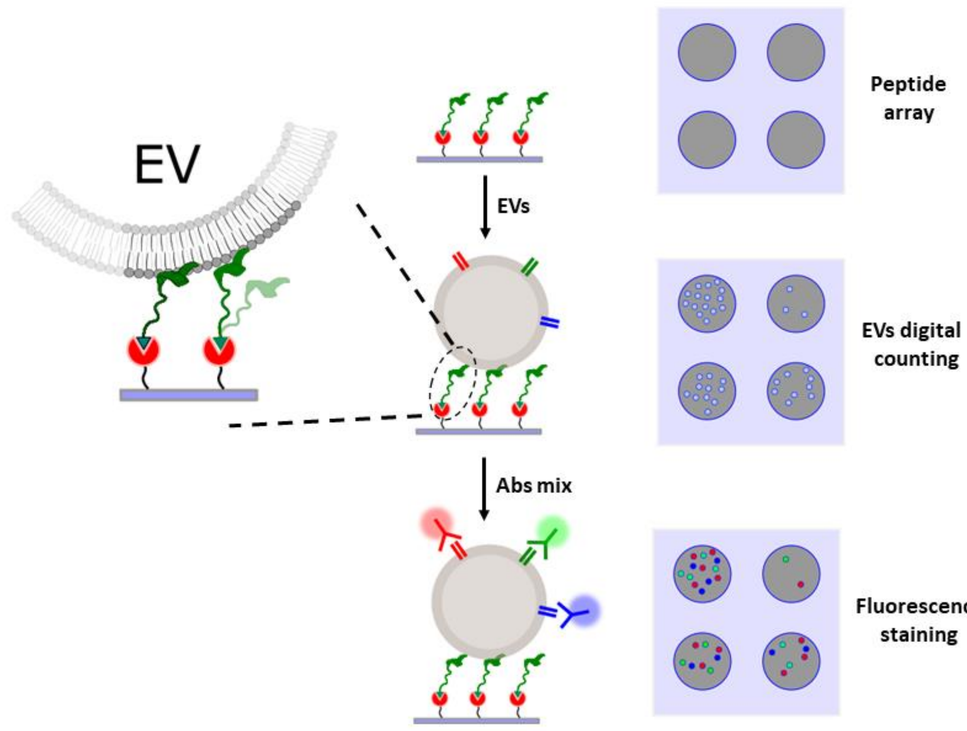
Highlights:

- Membrane-sensing peptides as general affinity probes for small EV
- Highly efficient and selective capturing of sEV even from complex biological samples (e.g. untreated human serum)
- First application of membrane sensing peptides to sEV profiling and first reported example of sEV peptide microarrays.

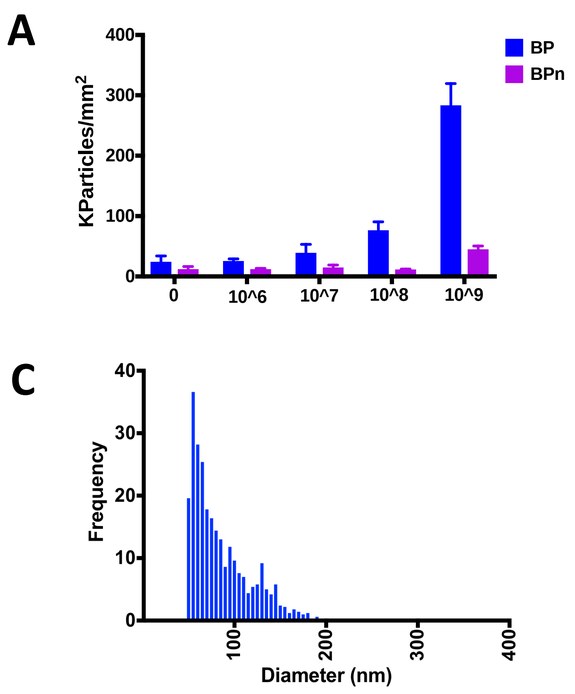
Results and Discussion:

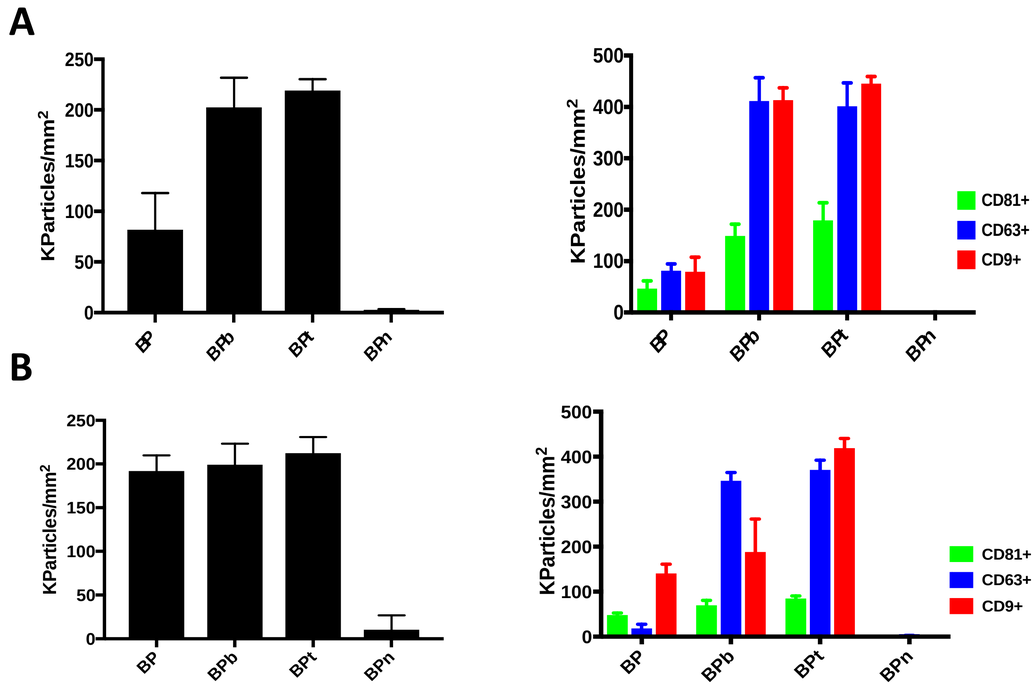




Bradykinin-derived peptides known to sense highly curved membranes (1) were immobilized on microarray chips through a chemoselective click-based strategy. Peptides baits were synthesized in a linear form (**BP**) and in two multivalent presentation: branched (**BPb**) and tandem (**BPt**). As a negative control (**BPn**), a peptide where arginine residues were mutated to (oppositely charged) glutamic acid residues was synthesized.

A silicon chip is arrayed with spots of capturing peptides and incubated with the EV sample. SP-IRIS platform images the chip and provides a label-free counting and sizing of the captured EV. The same chip can then be further incubated with fluorescent antibodies for immune-staining of EV associated proteins (anti-tetraspanins).





A) HEK UC particle density per mm^2 detected on BP and BPn peptide spots in a blank sample (filtered PBS) and in $1x10^6$ - $1x10^9$ particles/mL concentrations range. A clear dose-response effect is visible. Signal on BPn peptide is negligible. B) Representative images of BP and BPn peptide spots incubated with $1x10^7$ - $1x10^9$ particles/mL: blue dots indicate detected particles. C) Observed size distribution of captured particles reported as the number of counts detected in each 5nm bin D)HEK UC particle density per mm^2 detected on antibody microarray (anti CD81/CD63/CD9). Only $1x10^9$ particles/mL concentration provides on CD antibodies spots a signal distinguishable from that on the negative control antibody.

Analysis of EV isolated by ultracentrifugation from human serum incubated on peptide microarrays at $1x10^9$ particles/mL concentration (panel A). Analysis performed on unpurified human serum diluted 1:8 (panel B).

Density of particles captured by BP peptides (left panels) are confirmed by fluorescence staining using CD81/CD/63/CD9 fluorescent antibodies (right panels).

Conclusions and Futures Perspectives:

- ✓ We here demonstrated the use of membrane sensing peptides as a novel class of highly efficient molecular ligands for integrated sEV isolation and analysis;
- ✓ Our results greatly enrich the toolbox of affinity ligands for EV
- ✓ Ease of chemical manipulation, low cost of preparation and stability will allow peptides to be integrated in other EV analytical and isolation platforms

References:

[1] Saludes, J. P.; Morton, L. A.; Coulup, S. K.; Fiorini, Z.; Cook, B. M.; Beninson, L.; Chapman, E. R.; Fleshner, M.; Yin, H. Molecular BioSystems, (2013). http://doi:10.1039/c3mb70109c.

[2] Membrane-Binding Peptides for Extracellular Vesicles On-Chip Analysis. ChemRxiv. Preprint. *https://doi.org/10.26434/chemrxiv.9885167.v1*

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