One probe to bring them all: integrated separation and analytical workflow for extracellular vesicles

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Extracellular Vesicles (EV), including a subtype called exosomes, are nano-sized biogenic particles that are released by almost any type of cells in various bodily fluids like blood, urine, and cerebrospinal fluid. These particles contain and expose different types of molecules like proteins, lipids, glycans and nucleic acids, which makes them highly attractive potential biomarkers for several diseases, such as cancer, neurological disorders, and infectious diseases. However, their small size, low refractive index, inherent heterogeneity, and high sensitivity requirements make it difficult for them to become a prominent choice for liquid biopsy. To address these challenges, new affinity-probes and digital techniques capable to detect disease-specific sub-populations with low abundance are urgently needed. In our recent work [1], we have introduced a new type of molecular ligands for integrated small EV isolation and analysis called membrane-sensing peptides (MSP). These peptides are derived from Bradykinin and are capable of recognizing and binding the outer membrane leaflet of small EVs through complementary electrostatic interactions while leading to subsequent insertion of hydrophobic residues into the membrane lipid packing defects. Small EVs have indeed unique lipid membrane features in the extracellular environment that can be used as a "universal" marker.
MSP outperforms antibodies in terms of capturing capacity while being pan-specific, interspecies, and interkingdom, resulting in a versatile class of ligands with additional advantages in terms of stability and synthetic versatility. They can also be applied to synthetic lipid nanoparticles.
Our research has integrated MSP into different platforms for EV analysis and isolation including paramagnetic beads for Single Molecule Immunoassays (SiMoA). We have applied these methods to different workflows in urine and blood providing examples of clinically relevant feasibility in EV phenotyping and liquid biopsy in various biological media.

References

[1] A. Gori, A. Romanato, G. Bergamaschi, A. Strada, P. Gagni, R. Frigerio, et al., Membrane-binding peptides for extracellular vesicles on-chip analysis, J. Extracell. Vesicles. 9 (2020) 1751428. doi:10.1080/20013078.2020.1751428.

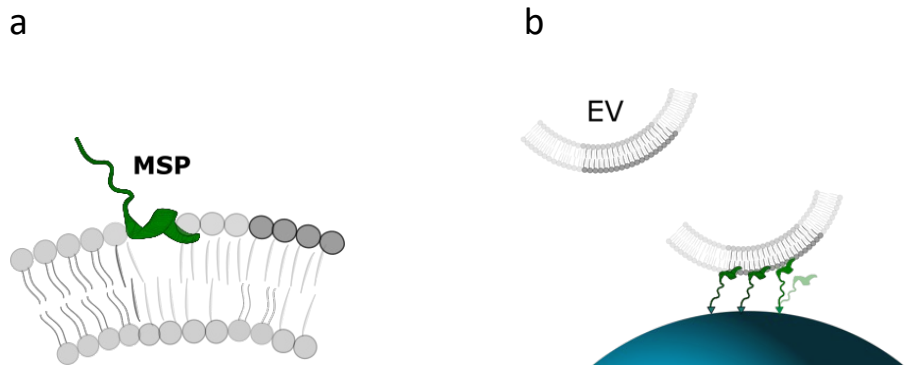

Figure 1: a) MSP are universal baits with affinity to small EV membrane and b) have been integrated into SiMoA paramagnetic beads for complete isolation and analysis workflows