Integrated diagnostic workflow for blood and urinary Extracellular Vesicles by Membrane Sensing Peptides and digital detection

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Extracellular Vesicles (EV) are gaining increasing importance as potential biomarkers in many pathological conditions. This area of research face big challenges due to EV small size, low refractive index, huge heterogeneity and high sensitivity demand in detecting low abundant disease-specific sub-populations. Such need can be met by innovative affinity-probes and digital detection, namely capable to reach the single-molecule sensitivity. Small EVs present fairly distinctive lipid membrane features that could be considered as a 'universal' marker, alternative or complementary to traditional characteristic surface- associated proteins. Our recent work has identified Membrane Sensing Peptides (MSP) as a novel class of molecular ligands for integrated small EV isolation and analysis. The membrane recognition and binding mechanisms are based on complementary electrostatic interactions between the peptide and the phospholipids on the outer membrane leaflet, that subsequently can lead to the insertion of hydrophobic residues into the membrane defects. MSP can be used for general capturing of all small EVs and subsequent unbiased immune-phenotyping of surface antigens. Here we present the integration of MSP onto bead-based digital platform for Single Molecule Immunoassays and their application for isolation-free EV analysis from complex bio-samples. Specifically, we will first show comparison of different bio- conjugation strategies of MSP onto beads for efficient EV capturing from urine, serum and plasma, demonstrating feasibility of our workflow directly in clinical samples. Then, we will report on a study on patient's stratification by profiling of serum EV specific surface antigens known to be relevant for cardio vascular risk assessment (CD62p, CD42a, CD31) on MSP-modified beads by digital immune-phenotyping.