Reversible aptamer-directed immobilization of antibodies and its application in extracellular vesicles separation

Dario BRAMBILLA – *SCITEC, CNR, Milano*

Extracellular vesicles (EVs) emerged as a powerful source of biomarkers. Unfortunately, a single platform capable of interrogating the complex biology of EVs does not exist. EVs are insufficiently characterized to support their clinical implications. Immunoaffinity approaches offer unmatched selectivity and the possibility to separate subpopulations of EVs. However, current immunoaffinity bead-based assays lack solutions for the release of intact EVs, limiting immunoaffinity isolation's range of applications. We introduce a novel approach that integrates two fundamental steps for EV analysis: i) affinity isolation of target EVs from plasma, and ii) digital, multiparametric analysis of EVs.

Antibodies that target a specific EV subpopulation are conjugated to an oligonucleotide composed of two domains: a streptavidin aptamer region, and a "barcode" sequence. The aptamer is used for the reversible immobilization of antibodies on magnetic beads. Upon incubation with biotin, which competes for streptavidin with the aptamer, the conjugates are released from beads. The barcode sequence is used for the recapture of EVs-antibody complexes on the surface of a DNA microarray allowing multiplexed detection.

The proposed barcoding strategy provides the following advantage: i) efficient EV release in mild conditions, preserving EVs integrity, ii) flexibility and customizable capture of a large number of EV targets, iii) unique features for integrated in-line EV isolation and detection.