## Methodological aspects – isolation, characterization, cargo, clinical significance of ncRNAs and applications in AD diagnosis

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Extracellular vesicles (EVs) are a heterogeneous group of lipid bilayer-delimited particles that are naturally released from all cells and into most biological fluids (Blood, CSF, ISF). EVs facilitate intercellular communication through protection of protein, lipid and RNA cargo. Interactions of EVs with recipient cells can have various effects on the target cell impacting multiple physiological and pathological processes. There has been increased attention to understand how the specific content of EVs impact cellular physiology and human pathology. We will present limitations in many methodological aspects of studying EVs including: tissue preparation, isolation and characterization, analysis of the cargo, and applications in neurodegenerative disease. A better understanding of their biogenesis, the content of their cell specific cargo, their release and trafficking mechanisms, including through blood brain barrier, is particularly important in better understanding neuronal development, aging and neurodegeneration. We will also present recent challenges in better understanding the importance of microglial EVs in neuroinflammation and pathogenesis of neurodegenerative disease.