

## BioSys/BioISI Research Seminar

### Isolation, characterization and clinical implication of extracellular vesicles

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Extracellular vesicles (EVs) mediate communication among cells and organisms through local and distant transport of proteins, nucleic acids and lipids. They emerge as potential indicators for environmental conditions and human diseases. The recent expansion of the EV research field has yielded a tangle of isolation and characterization methods. We created an expandable open-source knowledgebase to map methodological specifications of EV-related publications. To date, 1226 research publications from 2010 till 2015 are included. The knowledgebase exposes extensive diversity in EV isolation and characterization methods and identifies gaps in methodology reporting and critical quality control experiments. These methodological issues jeopardize EV research, since they give rise to ambiguous results. In order for the field to mature and reach its full potential, our knowledgebase aims to create a sense of urgent mutual responsibility among researchers to improve the quality and reproducibility of EV research. In addition, we provide a comparative evaluation of four EV isolation protocols for their usability, yield and purity, and their impact on downstream omics approaches for biomarker discovery. OptiPrep density gradient centrifugation outperforms ultracentrifugation and ExoQuick and Total Exosome Isolation precipitation in terms of purity, as illustrated by the highest number of CD63-positive EVs, the highest enrichment in EV marker proteins and a lack of contaminating proteins such as Ago2. The purest EV fractions revealed a unique mRNA profile enriched for translation, ribosome, mitochondrion and nuclear lumen function. Our results demonstrate that implementation of high purification techniques is a prerequisite to identify EV-specific functions and biomarkers.

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