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## Analytical toolbox for reliable characterization of extracellular vesicles

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Robust and well-established orthogonal techniques for biophysical characterization of individual EVs are required to utilize them as therapeutic and diagnostic tools. We have established capabilities to reliably characterize EV samples using three different orthogonal techniques; 1) Nanoparticle tracking analysis, a scattering and fluorescence-based technique to determine the size and concentration of nanoparticles. 2) Flow Cytometry, a fluorescence-based technique with similar principles as flow cytometry with critical enhancements to enable the effective detection of smaller particles. 3) Analytical HPLC, a semi-quantitative technology to analyze the EV samples using three different detectors: Multi angle light scattering, UV and fluorescence.

We propose performing fundamental studies using standard samples and models for both EVs and protein contaminations in the background. We comprehensively, evaluated the 3 technologies in our analytical toolbox, and compared the results with other gold standard techniques such as Cryo-EM. The relevant ratio of EVs compared to other non-EV components of the EV samples is a critical factor in reliably analyzing a crude EV samples. Here, we provide tips and guidelines to the researchers in the field to identify the limitations of any analytical/single particle analysis technology to ensure reliable characterization.

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