1302
doi:10.1017/S1431927622005347
Microsc. Microanal. 28 (Suppl 1), 2022

Cryo-Electron Microscopy of Extracellular Vesicles

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Extracellular vesicles (EVs) are nanoscale membrane-enclosed vesicles containing proteins, mRNAs, non-coding RNAs, and lipids derived from producing cells, which are now increasingly recognized as important vehicles of intercellular communication and circulating biomarkers for cell communication and physiology [1-4]. As a cutting-edge methodological approach, cryo-electron microscopy (cryo-EM) allows us to visualize a large spectrum of extracellular vesicles of various sizes and diverse morphologies. The uniqueness of extracellular vesicles is most noticeable in the differences to the external lipid bilayer shell and internalized vesicular structures (Figure 1). Purified solutions of extracellular vesicles are applied to EM grids and then rapidly-frozen to produce samples where the vesicles are preserved in a near-native frozen-hydrated state.

The UW-Madison Cryo-Electron Microscopy Research Center (CEMRC) provides the UW-Madison research community with access to cryo-EM instrumentation, technical assistance, and training. In this study, high-resolution cryo-EM images of purified extracellular vesicles were obtained on the 200 kV Talos Arctica with a Gatan K3-bioquantum energy filter direct electron detector. The images were acquired in CDS-counting mode using the SerialEM software package. SerialEM is flexible and may be used to collect EM imaging data on a range of sample types using a number of collection schemes, including montage maps, single or dual-axis electron tomography, and high-throughput single particle approaches [5]. For imaging samples with irregular particle sizes or sparse distribution, such as vesicles and viruses, SerialEM allows users to iteratively pick targets from maps and then navigate precisely to those designated points.

The diverse morphologies present in a purified sample of extracellular vesicles provides information about external structures that may be embedded in the vesicle’s membranes, and the encapsulation and internalization of cargo (Figure 1). This information may further our general understanding of the biological function of extracellular vesicles.

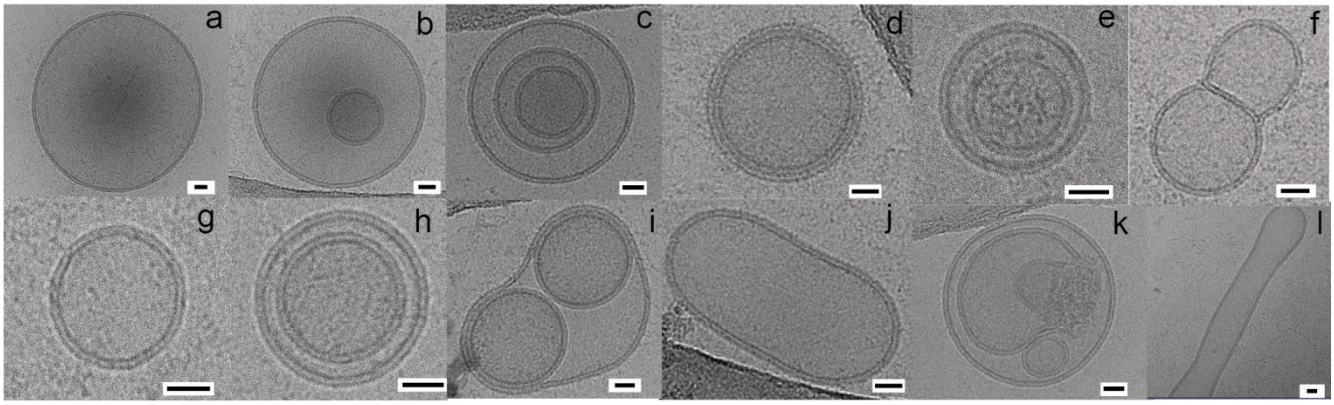

Figure 1. Cryo-EM images of EVs: (a, g) Single vesicle; (b, h) Double vesicles; (c, i) Triple vesicles; (d) Double-membrane vesicle; (e) Vesicles with electron dense cargo in lumen; (f) Joint vesicles; (k) Vesicles with broken membrane; (j) Short tubular; (l) Long tubular. (scale bar: 20 nm)

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https://doi.org/10.1017/S1431927622005347 Published online by Cambridge University Press