

## **AFM of single vesicles: a multiparametric morpho quantitative analysis**

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Extracellular vesicles (EVs) are secreted by almost all cell types reflect the physiopathological state of the parental cell, EVs circulate in all body fluids, reaching distant cell targets and delivering different bioactive cargoes. EVs are heterogeneous in terms of size and composition, depending on cell type and exposure to stimuli, and different methods have been developed to characterize their morphological, biophysical, and biochemical features. Among them, electron microscopy (EM) is the main technique used, however, the lack of standardized protocols makes it difficult to characterize EVs with a good reproducibility, thus using multiple approaches may represent a way to obtain more precise information. Furthermore, the relationship between architecture and function, not only in a molecular, but also in a cellular level, is gaining growing emphasis, characterizing morphometric parameters may represent a distinct, but effective approach to study the physiopathological state of the cell. Atomic force microscopy (AFM), may represent a versatile method to study in detail single EVs dynamics throughout the cell surface and its variations related to the physiological state, overcoming the limits of EM, and providing more reliable information.