Supporting Information: Chemical characterization of extracellular vesicles at the sub-vesicle level

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Figure S1: Shift along the x and y axis after three single point spectra a and b show the shift along the x and y axis after three single point spectra and a new topography image were taken



Figure S2: **Point spectrum of the substrate with AnteoBind**[™]**Biosensor** AFM-IR spectra of AnteoBind[™]Biosensor (a, blue) smoothed using a Savitzky-Golay filter (20 points, second order). (b) sampling position marker in the AFM topography image



Figure S3: Alignment of the taken images using the SIFT algorithm Using the first image as reference image, all the other images have been compared to it. The overlayed image shows, how well the SIFT algorithm worked.



Figure S4: **Explained Variance of the PCA** Only the first four principal components were considered, as they contained the most meaningful information.