Electronic Supplementary Information (ESI)

for

Facile, Generic Capture and On-Fiber Differentiation of Exosomes via Confocal Immunofluorescence Microscopy using a Capillary-Channeled Polymer Fiber Solid-Phase Extraction Tip

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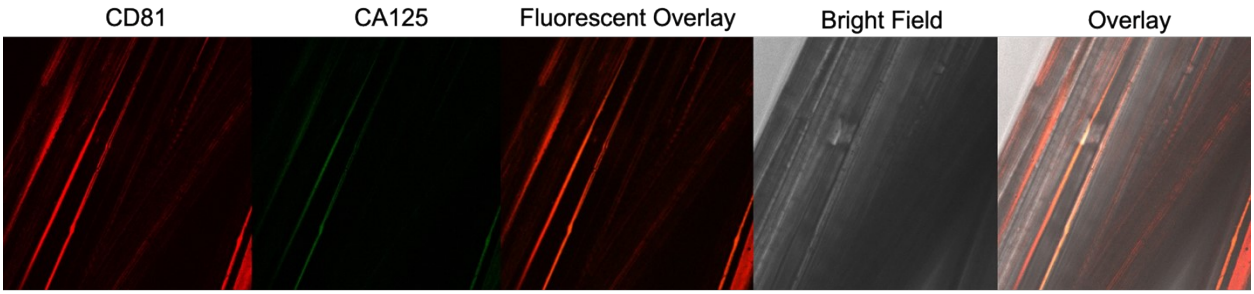
Standard (overnight) immunolabeling procedure

Following the HIC C-CP tip capture of EVs, the immobilized exosomes were exposed to a 5% BSA blocking solution in PBS to decrease non-specific binding of the primary and secondary antibodies. For this, 200 µL aliquots of 5% BSA were spun down 5 times at 300 *x g* for 60 s each. Then, the C-CP tips were submerged and incubated in 1 mL of blocking solution for 15 min on a shaker. After the blocking step, three 200 µl aliquots of PBS were spun down the tip (300 *x g* for 60 s each), then washed in 1 mL of PBS on a shaker for 15 min (employing 3 buffer changes where the PBS was replaced). Antibodies to the CD81 (mouse) and CA125 (rabbit) biomarker proteins were diluted 1:1000 in PBS, then 200 µL of the solution was applied to the C-CP tip, where it was allowed to wick down for 5 minutes, before centrifugation at 150 *x g* for 3 min. After the initial spin down, the C-CP tip was submerged and incubated in 1 mL of the primary antibody solution overnight at 4°C. Next, the washing and blocking steps were repeated as previously described. The AlexaFluor 488 (anti-rabbit) and Alexa Fluor 647 (anti-mouse) secondary antibodies were then diluted 1:1000 in PBS, and 200 µL of the secondary antibody solution was applied to the tip reservoir and allowed to wick for 5 minutes. Then the C-CP tip was centrifuged at 500 *x g* for 3 min before incubation in 1 mL of the secondary antibody for 1 hour at room temperature. Prior to confocal imaging, a final PBS wash was performed 5 times to remove the non-specifically bound secondary antibody.

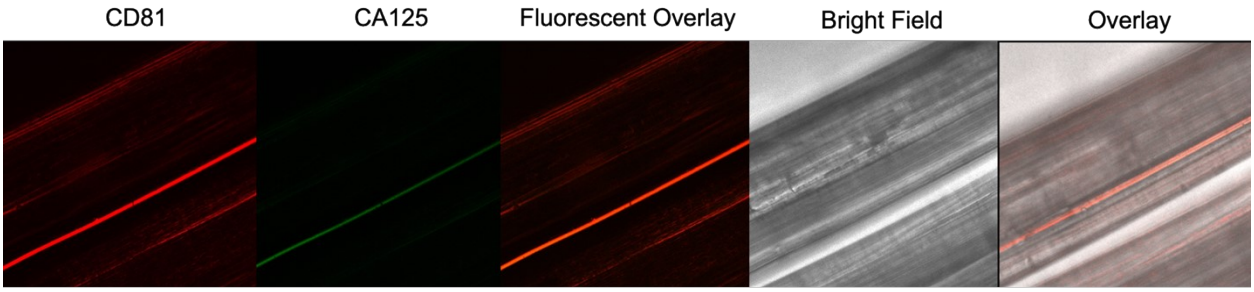
Negative control experiments

In order to demonstrate the essential concepts of generic exosome capture, with the ability to affect selective immunofluorescence imaging, it is essential to demonstrate

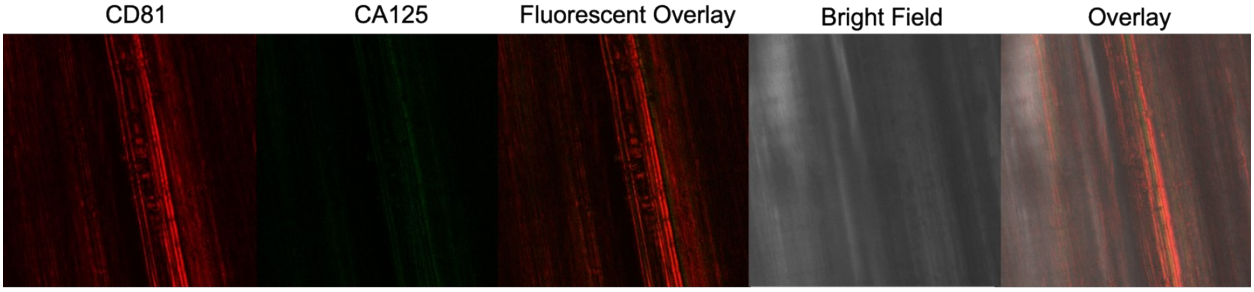
that non-specific interactions between the target proteins (and subsequently the labeled antibodies) and the fiber surfaces are not occurring. Shown below are immunofluorescence images of blank fibers treated with the antibody labels (SI Fig. 1a), exposed to purified CD81 and then the antibody labels (SI Fig. 1b), and the purified CD81 incubated with the antibody and applied to the fibers previously exposed to exosomes (SI Fig. 1c). Essential experimental details are provided with each. In each case, the desired response was realized, with virtually no indication of non-specific binding occurring at the fiber surfaces.



ESI Fig. 1a. Antibody interaction with blank PET Fiber: Blank PET fiber was washed with PBS, then the cleanup and labeling protocol was performed: 1-hour primary antibody incubation, 30 min. secondary antibody incubation, wash and block cycles between each incubation. Very minimal non-specific binding of the primary and/or secondary antibodies was observed.



ESI Fig. 1b. CD81 exposure to blank fiber: Purified CD81 protein was applied during the load step in 2M ammonium sulfate, then the protein cleanup and labeling steps were performed. Very minimal non-specific binding of the primary and/or secondary antibodies was observed.

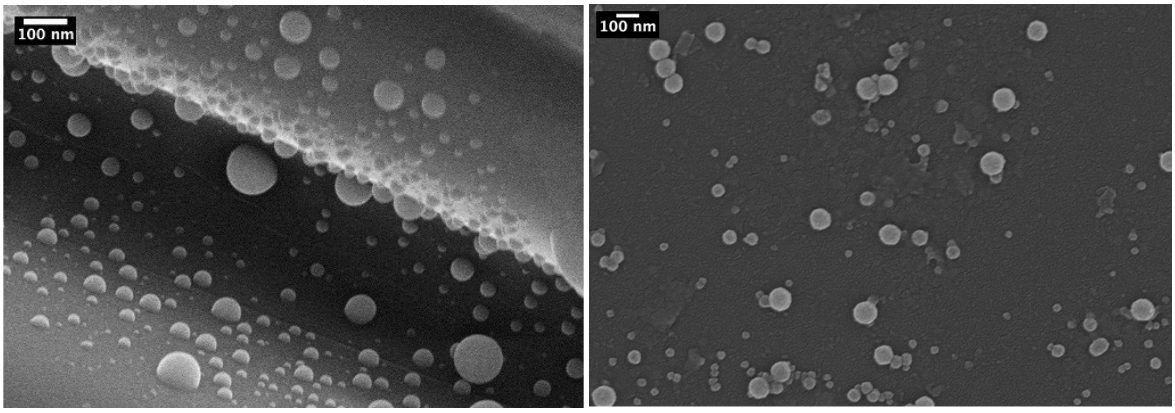


ESI Fig. 1c. CD81/antibody conjugate applied to fiber surface in presence exosomes: Exosome Standards (1:100 dilution, ~3 x 10^{10}) were loaded onto the fiber surface, and the protein cleanup step was performed, CD81 protein was incubated with the CD81 antibody (1 hour) before applying to tip, and labeling protocol was continued. Evidence is seen for excess antibody binding to captured exosomes, but very minimal non-specific binding of the antigen/antibody complex is observed.

Electron microscopy

In preparation, exosomes from exosome standards were isolated using the previously described C-CP tip method, then fixed by incubation with 1% osmium tetroxide for 1 hour and washed in microcentrifuge tubes on a shaker (3 times, 5

minutes each). After fixation, the samples were dehydrated using an ethanol-distilled water gradient from 0% to 100% ethanol, followed by three washes of 100% ethanol for 3 minutes each. Following dehydration, the sample was washed in a 50–50 hexamethyldisilazane (HMDS)-ethanol solution for 3 minutes and allowed to dry in a fume hood in 100% HMDS overnight. The dehydrated samples were sputter-coated with platinum at 70 mTorr argon for 2 minutes before EM imaging. For the SEM imaging, the prepared EV-coated C-CP fibers were placed directly on carbon tape and into the instrument. During the STEM imaging of the tip eluates, the liquid eluates were drop-casted onto a small piece of EM-grade silica wafer for 60 seconds, and the sample liquid was removed using a small piece of paper towel before imaging.

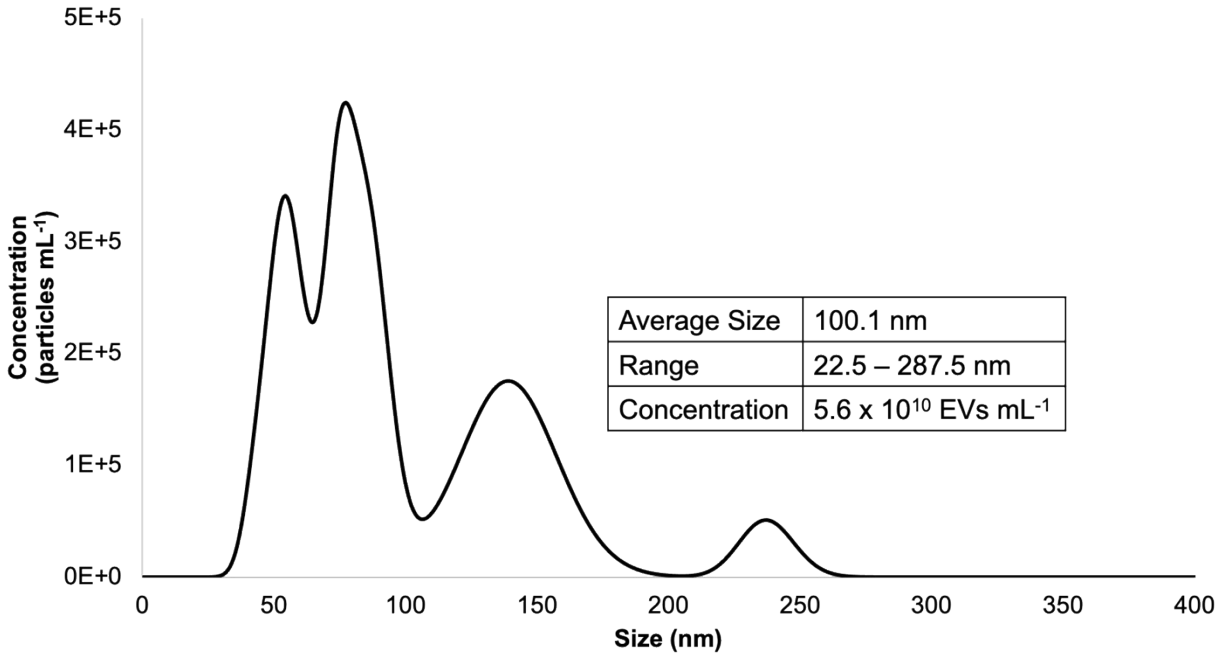


a) b)

Nanoparticle Tracking Analysis

ESI Figure 2: a) SEM micrograph of exosome standards (using the Hitachi S4800) captured on the C-CP fiber surface due to the HIC mode capture of the vesicles. b) STEM micrograph of exosome standards eluted from the C-CP fiber tip immobilized on a silica wafer. (taken using Hitatchi SU9000)

The NanoSight NS300 nanoparticle tracking analysis (NTA) system (Malvern Panalytical, Malvern, Worcestershire, United Kingdom) was used to determine the size distribution and concentration of EVs isolated from 100 µL of HEK293 cell culture media using the C-CP tip. The NTA determinations were performed as previously reported. Briefly, the NTA system was equipped with a 532 nm laser, and five replicate measurements were performed in 60-second intervals. Throughout experimentation, the focal plane was manually adjusted for the best optical field of view. The syringe pump was set to a constant flow rate of 50 µL per minute, the camera level was set to 14, and the detection threshold was set to 3. Before NTA measurement, the EV recoveries were diluted 1:1000 to be compatible with the working concentration range of the NanoSight instrument (10^7–10^9 particles per mL). The EV concentration value presented in ESI Fig. 3 compensates for this dilution factor.



ESI Figure 3: Size distribution and concentration of EVs isolated using the C-CP tip from HEK293 cell culture milieu, as determined using the NanoSight NS300 NTA instrument. The average of five 60-seconds runs is presented. A 1:1000 dilution of each EV aliquot was made prior to NTA, and the determined EV concentration accounts for this dilution factor.