Supplementary Information

Fabrication and Modification of Dual-Faced Nano-Mushrooms for Tri-Functional Cell Theranostics: SERS/Fluorescence Signaling, Protein Targeting, and Drug Delivery

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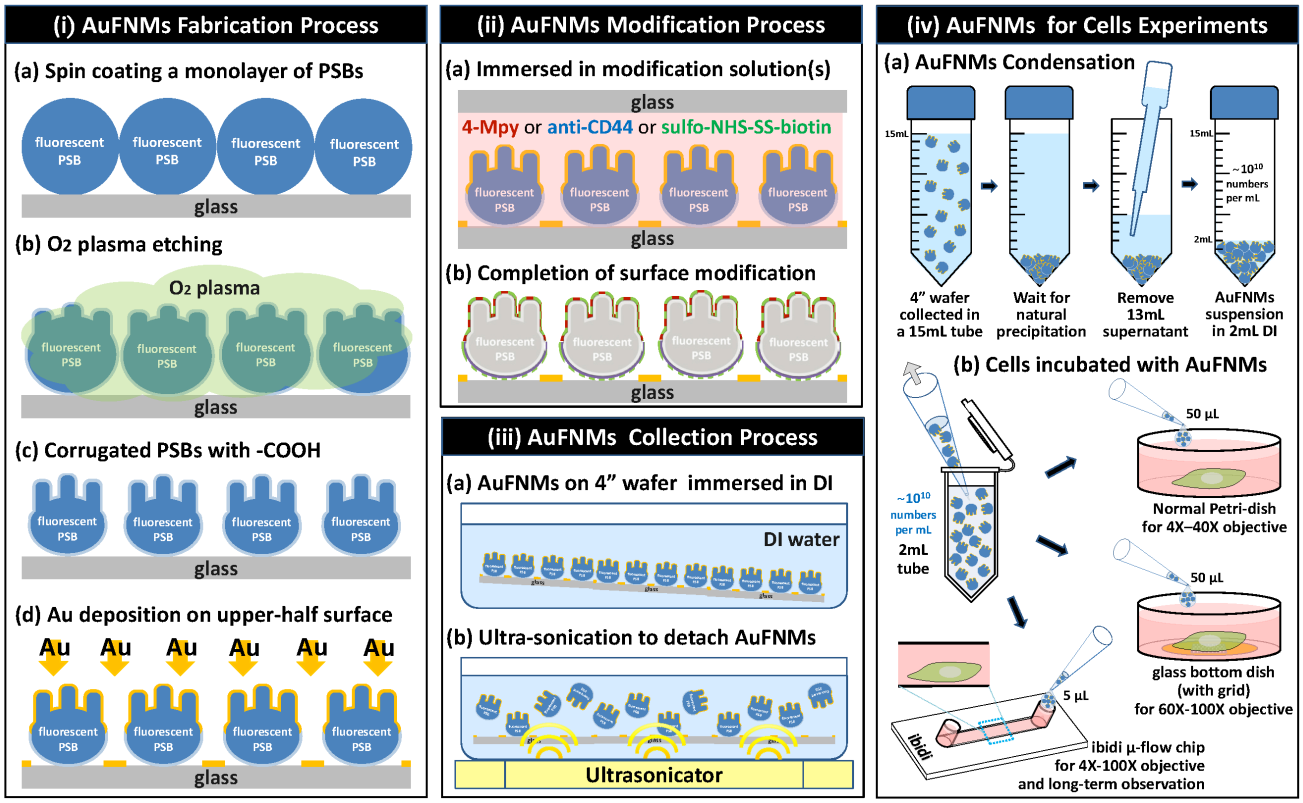

Figure S-1. Schematic illustration of the (i) particle fabrication, (ii) surface modification, and (iii) particle collection processes of the AuFNMs, and (iv) the processes for cancer cell recognition experiments. For the **(i)** ***AuFNMs Fabrication Process***: (a) Spin coating a condensed 1–5% PSB solution (in a solvent mixture of 75% alcohol and 25% original solution) under a three-step spin procedure (400 rpm/10 sec, 800 rpm/120 sec, and 3000 rpm/5 sec) to obtain a PSB monolayer on a 4" glass wafer. (b) Oxygen plasma etching (60 W/60 mtorr/20–220 sec) to shrink the particle diameter and obtain a corrugated surface. After the completion of plasma etching, (c) carboxylic functional groups are formed over the entire surface of the PSBs. Finally, (d) a gold layer with an adhesion of titanium is vertically deposited on to the corrugated PSBs to obtain AuFNMs. For the **(ii)** ***AuFNMs Modification Process***: (a) Add 200 µL of 100 µM 4-Mpy (in DI water), 1 µg/mL anti-CD44 (in PBS), or 1 mg/mL sulfo-NHS-SS-biotin (in PBS) one at a time onto the 4" glass wafer with the AuFNMs, and subsequently cover it with a blank glass wafer to spread the coating solution for 30 min, 1-2 hr, or 1 hr, respectively. While finishing the coating(s), gently wash the wafer containing AuFNMs 3 times with DI water and blow it dry using a weak compressed air flow to obtain (b) the selective surface modification on the AuNFMs. For the **(iii)** ***AuFNMs Collection Process***: (a) Immerse the 4" glass wafer with the AuFNMs into a cleaned crystallizing dish with 15 mL DI water. Then, (b) put the dish onto an ultrasonicator at full power at room temperature for 5–10 min to detach the AuFNMs from the glass wafer. Finally, remove the wafer and collect the AuFNM solution into a 15-mL centrifuge tube. For the **(iv)** ***AuFNMs for Cells Experiments***: (a) The AuFNMs collected from one 4" glass wafer are suspended in a centrifuge tube with15 mL DI water. To increase the AuFNM concentration, 13 mL DI water is removed after the natural precipitation of the AuFNMs. Therefore, monodispersed AuFNM solution with a final concentration of ~10^{10} numbers per milliliter is obtained. (b) To observe the reaction between the cells and the AuFNMs with various surface modifications, three types of dishes are used: a standard Petri dish, a 160–190-µm glass-bottom Petri dish with or without a grid, and an ibidi µ-flow chip with a flow channel of 17(L) × 3.8(W) × 0.4(H) mm and an 180-µm glass bottom. After 4–6 hr incubation in those dishes, 50 µL (5 µL) AuFNMs in 2 mL (200 µL) DMEM replaces the original medium in the standard and glass-bottom Petri dish (ibidi µ-flow chip) for cell-particle interaction.

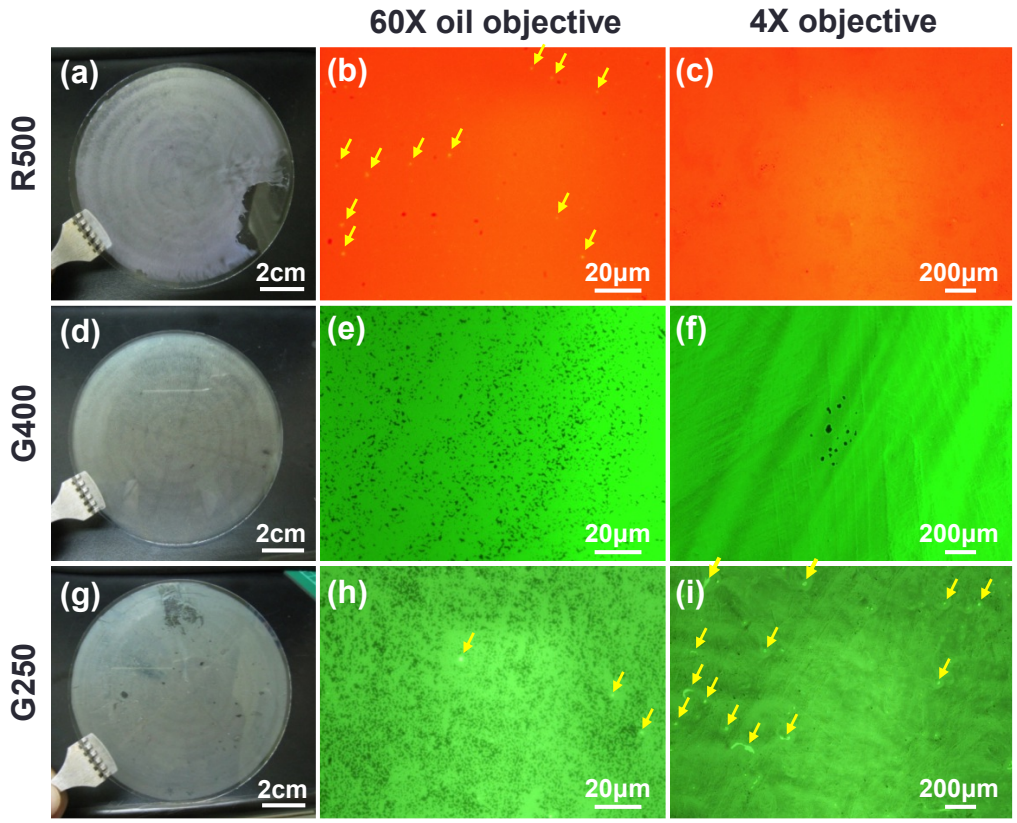

Figure S-2. The pictures and fluorescent images are the spin-coated (a)-(c) R500, (d)-(f) G400, and (g)-(i) G250 PSB monolayers on 4" glass wafers. The yellow arrows indicate the non-monolayer defects.

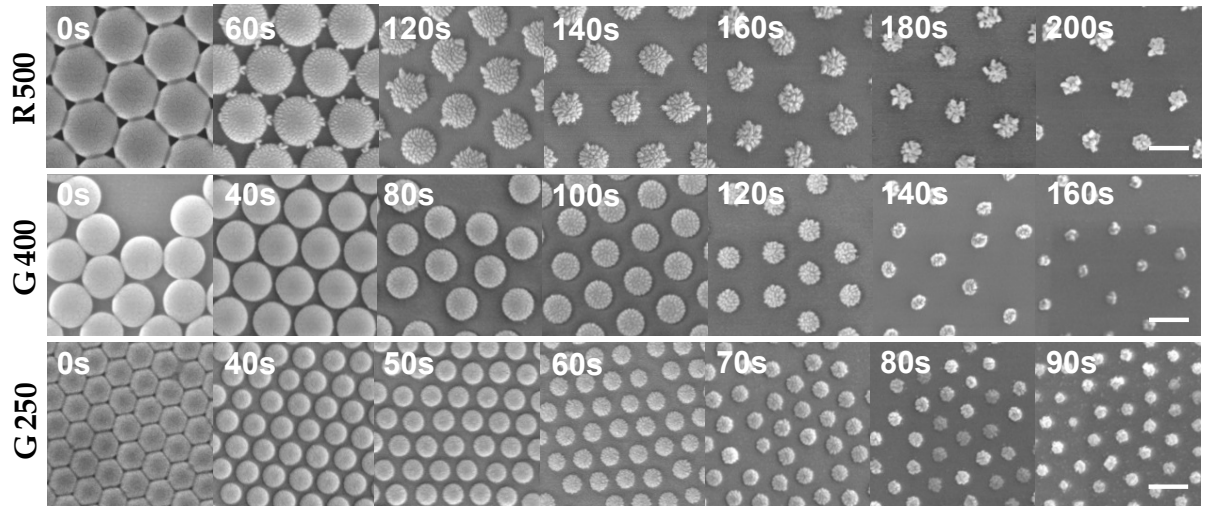

Figure S-3. The SEM images of the FNM arrays before and after a series of oxygen-plasma-etching time. (Scale: 300 nm)

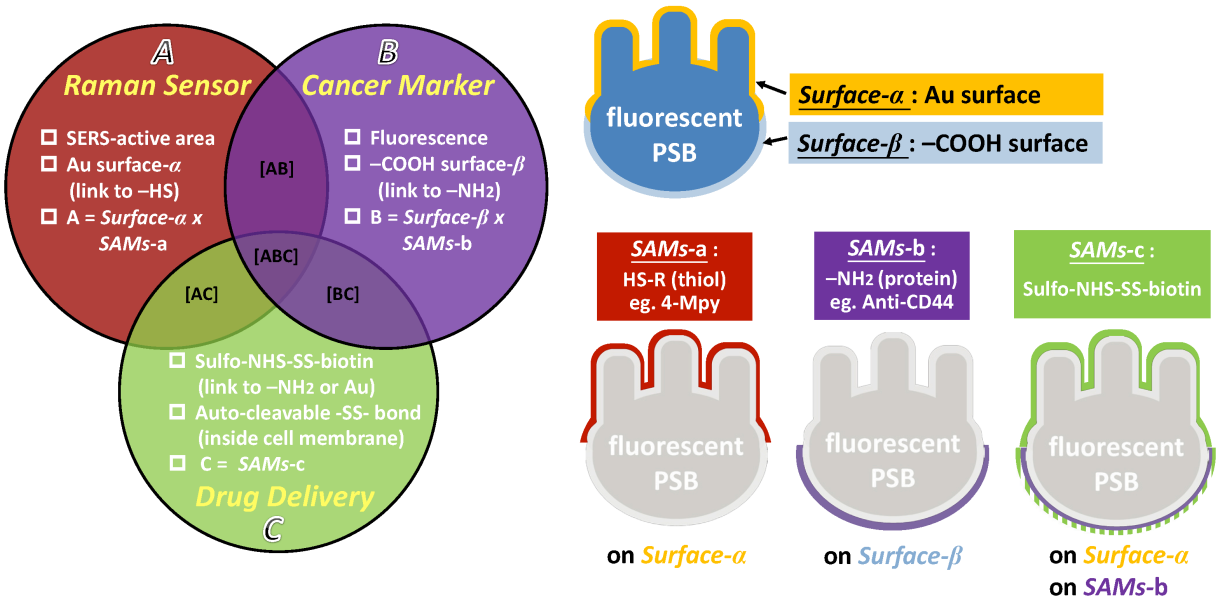

Figure S-4. The three circles represent three functions (***A*** for Raman sensor, ***B*** for cancer cell marker, and ***C*** for drug delivery) of the AuFNMs after 4-Mpy (***SAMs*-a**) and anti-CD44 (**SAMs-b**) modifications on the intrinsic dual surfaces of the corrugated Au (***Surface-α***) and –COOH (***Surface-β***), respectively, in cooperation with the extrinsic disulfide linker of the sulfo-NHS-SS-biotin (***SAMs*-c**).

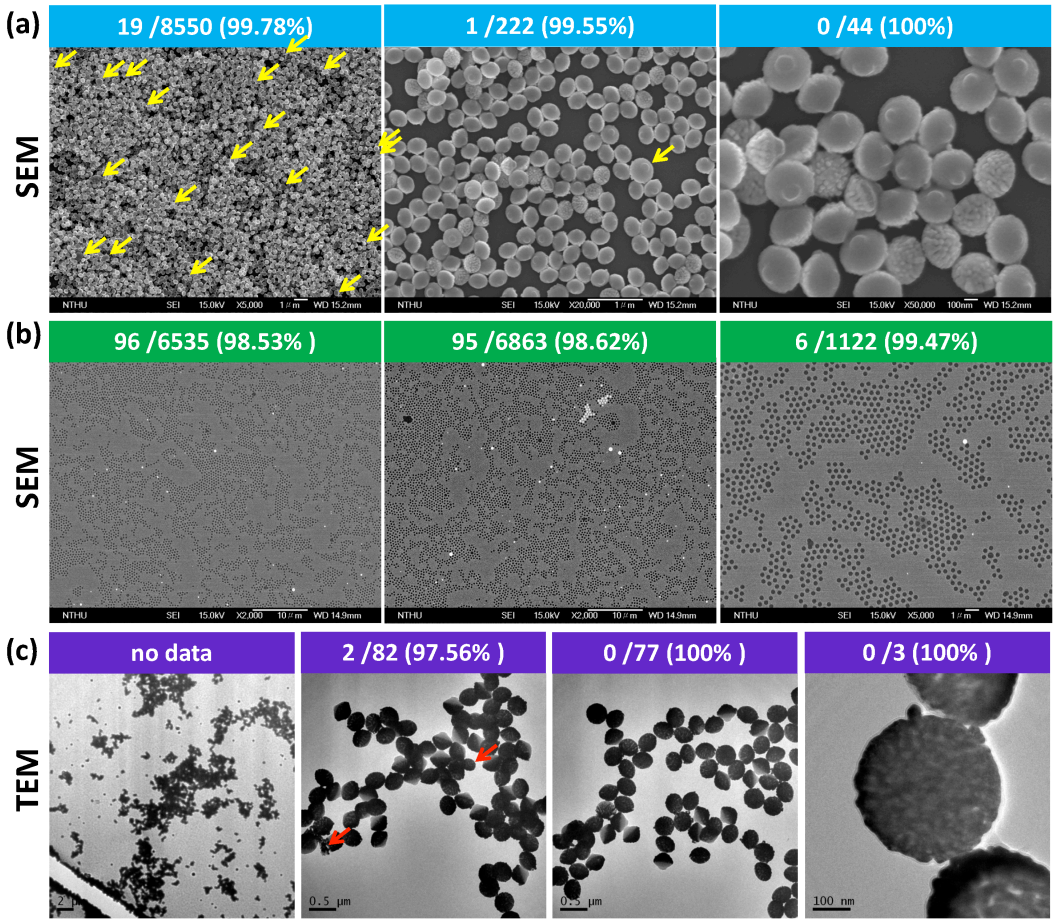

Figure S-5. The surface materials and morphologies, particle purification, the detachment ratio of the AuFNMs$_{(Au30\text{-}O140\text{-}R500)}$-antiCD44 from the 4" glass wafer. The numbers on each image represent the impurity or the non-detached/total particle ratio (the ratio of purification or detachment). (a) SEM images of a 1-μL AuFNM$_{(Au30\text{-}O140\text{-}R500)}$-antiCD44 solution dried onto a diced Si substrate. The minimum purification from these SEM images is >99%. (b) SEM images of the AuFNMs$_{(Au30\text{-}O140\text{-}R500)}$-coated glass wafer after ultrasonication for 5 min to detach the AuFNMs. The minimum detachment ratio is >98%. (c) TEM images of the AuFNMs$_{(Au30\text{-}O140\text{-}R500)}$-antiCD44. The darker portion of the AuFNM is the gold coating, and the brighter portion is the polystyrene. The yellow and red arrows indicate impurities or residues.

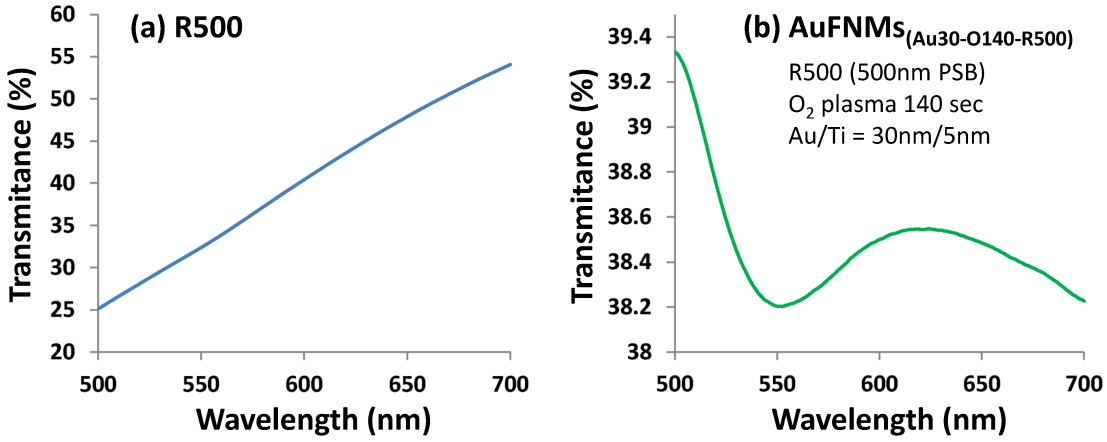

Figure S-6. The transmittance spectra of (a) the R500 PSB solution and (b) the AuFNM$_{(Au30\text{-}O140\text{-}R500)}$ solution.

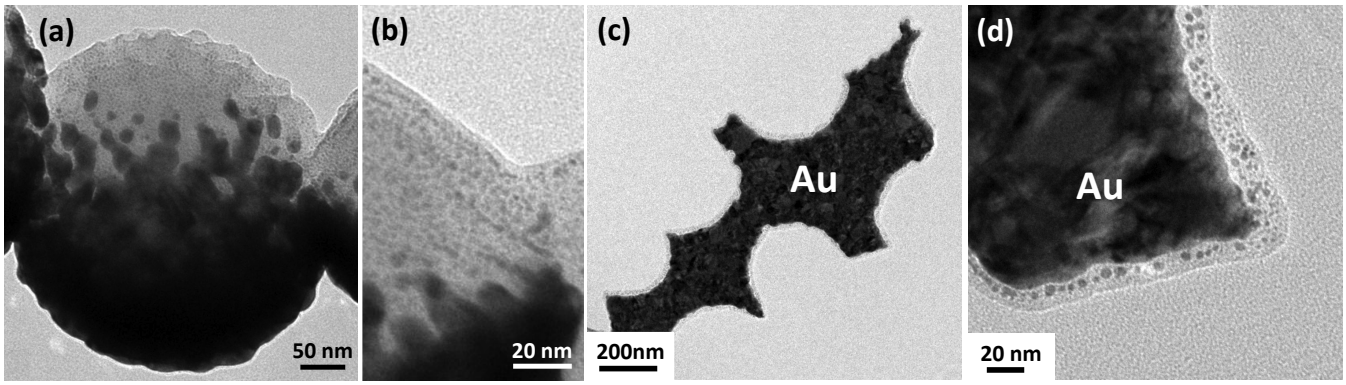

Figure S-7. TEM images of (a)-(b) AuFNMs$_{(Au20\text{-}O120\text{-}G400)}$-antiCD44-(sulfo-NHS-SS-biotin)/strep-QDs and (c)-(d) a gold film modified with sulfo-NHS-SS-biotin (***SAMs*-c**) and Qdot® 585 streptavidin (strep-QDs).

Movie S-1. The video shows z-axis scanning optical microscopy images under a combination of bright and fluorescent fields. The cells were pre-incubated with ~2.5×10^8 numbers/mL anti-CD44-treated G250 or AuFNMs$_{(Au20\text{-}O120\text{-}R400)}$-antiCD44 in normal DMEM for 30 min and washed 3−5 times with a PBS solution. A trypsin-EDTA solution (0.05%) was used in the dishes for a 5-min reaction, and normal DMEM medium was added to detach the cells. The detached cells were transferred onto an 8-well glass-bottom Petri dish for counting the particles on/inside the cell membrane using a 60×/1.25 NA objective. From left to right, there are HeLa cells with anti-CD44-treated G250, chondrocyte cells with AuFNMs$_{(Au20\text{-}O120\text{-}R400)}$-antiCD44, and HeLa cells with AuFNMs$_{(Au20\text{-}O120\text{-}R400)}$-antiCD44, in accordance with Figure 5(d), 5(e), and 5(f), respectively.

Movie S-2. The video shows the XYT images of live HeLa cells in both of bright (left) and fluorescent (right) fields. The HeLa cells are pre-incubated with AuFNMs$_{(Au30\text{-}O140\text{-}R500)}$-antiCD44 for 3 hr and washed 3-5 times with a PBS solution. The images are captured using a 60×/1.25 NA objective, and the video is in 5× accelerating speed. There is a time delay between the bright images and fluorescent images, so the related positions of AuFNMs are slightly different.

Movie S-3. The video shows the XYT images of two live HeLa cells under a combination of bright and fluorescent fields. The HeLa cells are pre-incubated with AuFNMs$_{(Au30\text{-}O140\text{-}R500)}$-antiCD44 for 10 min and washed 3-5 times with a PBS solution. The images are observed using a 60×/1.25 NA objective, and the video is in 10× accelerating speed. The 1-min intervals are due to the limited storage memory of the recording system. The total observation time in this video is 660 sec.

Movie S-4. The video shows the XYZ confocal images of two live HeLa cells. The HeLa cells are cultured in an ibidi μ-flow chip for 3−6 hr and treated with CellMask deep-red membrane dye (ex/em 649/666 nm). Then, the medium of the HeLa cells is replaced with DMEM containing both of AuFNMs$_{(Au20\text{-}O120\text{-}R400)}$-antiCD44 and AuFNMs$_{(Au20\text{-}O120\text{-}G400)}$-antiCD44. After incubating for 30 min, washing the flow channel with a PBS solution for 5 min, and adding no-phenol-red DMEM, the μ-flow chip is placed onto our confocal microscope for observation. The images are captured using a 100×/1.3 NA oil objective, and the step size of the z-axis is 100 nm. The RGB colors are converted from original gray scale intensity to red for cell membrane, green for AuFNMs$_{(Au20\text{-}O120\text{-}R400)}$-antiCD44, and blue for AuFNMs$_{(Au20\text{-}O120\text{-}G400)}$-antiCD44. This video is in 2.5× accelerating speed.

Movie S-5. The video shows the in-motion 3D reconstruction images of the confocal images in Movie S-4.