Supporting Information

Cellular delivery of bioorthogonal pretargeting therapeutics in PSMA-positive prostate cancer

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1. MALDI-TOF analysis of components.

Samples of components were prepared following standard protocol for MALDI analysis using sinapinic acid as the matrix. MALDI-TOF spectra were taken following standard protocol optimized for protein analysis. Figure S1 shows embedded MALDI-TOF spectra of initial protein and protein after modification.

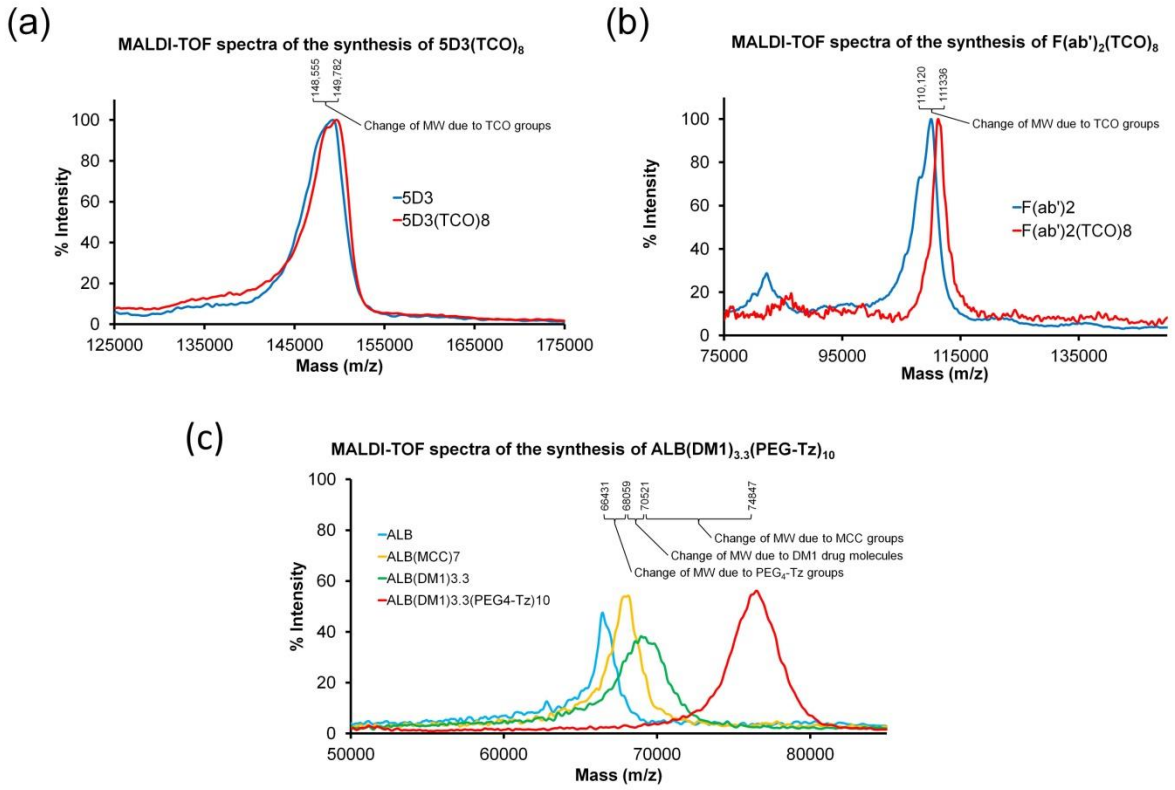

Figure S1. MALDI-TOF analysis of components. Embedded MALDI-TOF spectra of (a) 5D3 mAb and 5D3(TCO)$_8$, (b) F(ab')$_2$ fragment and F(ab')$_2$(TCO)$_8$, and (c) Albumin (ALB), ALB(MCC)$_7$, ALB(DM1)$_{3.3}$, and ALB(DM1)$_{3.3}$(PEG$_4$-Tz)$_{10}$.

2. Dynamic light scattering (DLS) analysis of components

Samples of components (~1.0 mg/mL) were prepared in PBS (pH 7.2) and hydrodynamic diameter of components was measured using a MALVERN-Nano series Zetasizer. Slight changes of hydrodynamic diameter were observed in all components after modification but were not statistically significant. Graphs of Volume % distribution of sizes (nm) are shown in Figure S2.

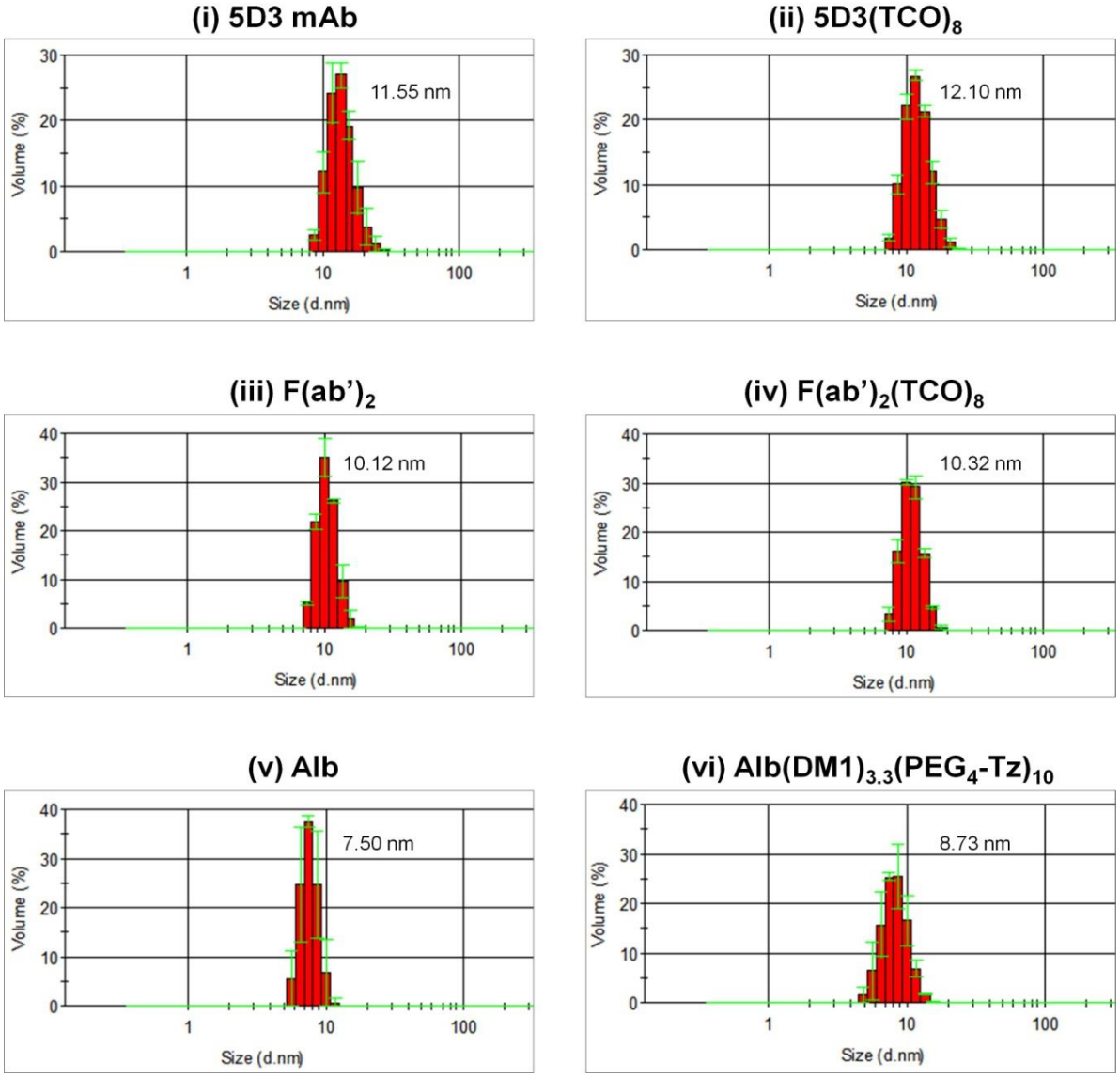

Figure S2. DLS analysis of components.

3. Internalization of anti-PSMA 5D3 mAb and F(ab')$_2$

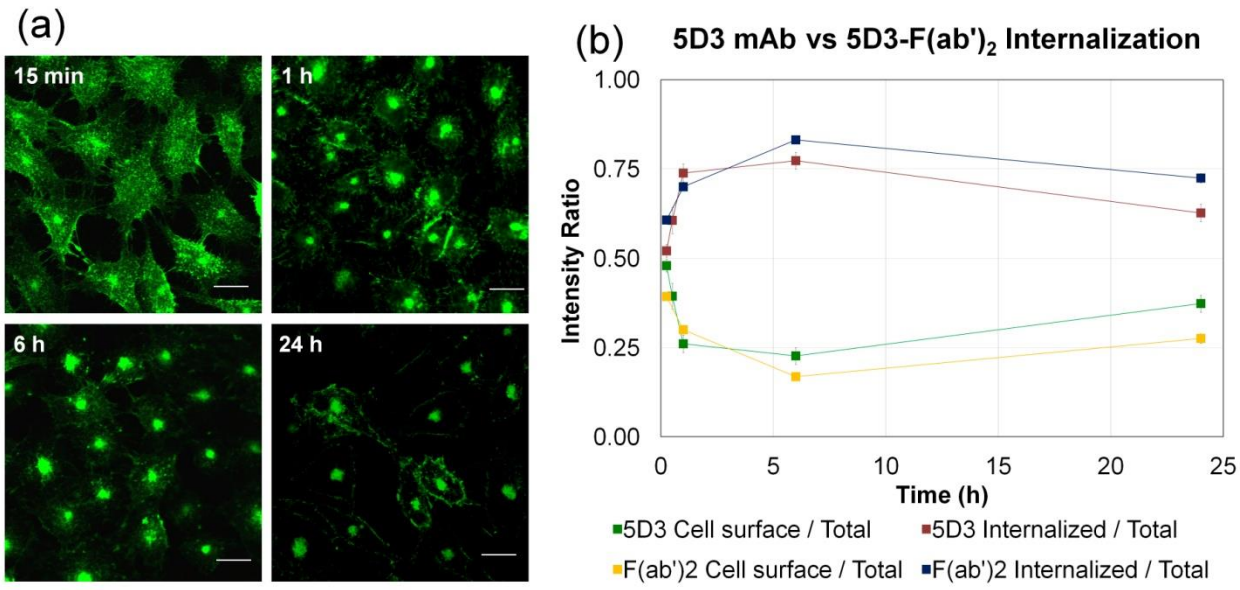

Figure S3. Internalization of anti-PSMA 5D3 mAb. (**a**) Fluorescence images of 5D3(AF-488)$_2$ internalization in PSMA(+) PC3-PIP cells (Scale bar: 20 μm). (**b**) Change of the fluorescence intensities of 5D3(AF-488)$_2$ and F(ab')$_2$(AF-488)$_2$ in the cytoplasm and cell surface.

4. ALB(PEG$_4$-Tz)$_{10}$(Rhod)$_2$ in PC3-PIP cells

PC3-PIP cells grown in 4-well chamber slides as describes in the Method 2.8 were treated with ALB(DM1)$_{3.3}$(PEG$_4$-Tz)$_{10}$(Rhod)$_2$ (150 μL of 50 μg/mL in DPBS) at 37 °C for 30 min. After the washing step, DPBS was replaced by the growth medium and the incubation was continued for 2 h. After cells were fixed by 4% PFA for 20 min at 4 °C and washed with deionized H_2O. Cells were counterstained with Hoechst 33342 and imaged using the Zeiss AxioObserver confocal fluorescence microscope with LSM700 confocal module and analyzed using NIH ImageJ.

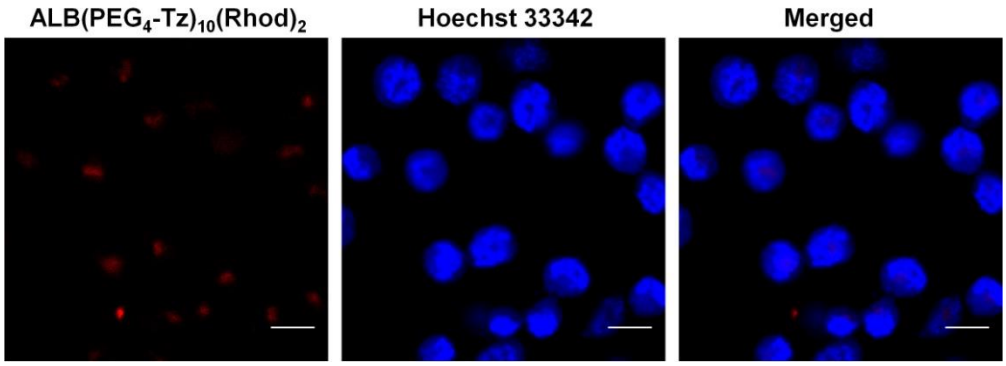

Figure S4. Fluorescence images of PSMA(+) PC3-PIP cells treated with ALB(PEG$_4$-Tz)$_{10}$(Rhod)$_2$ without pretargeting components.