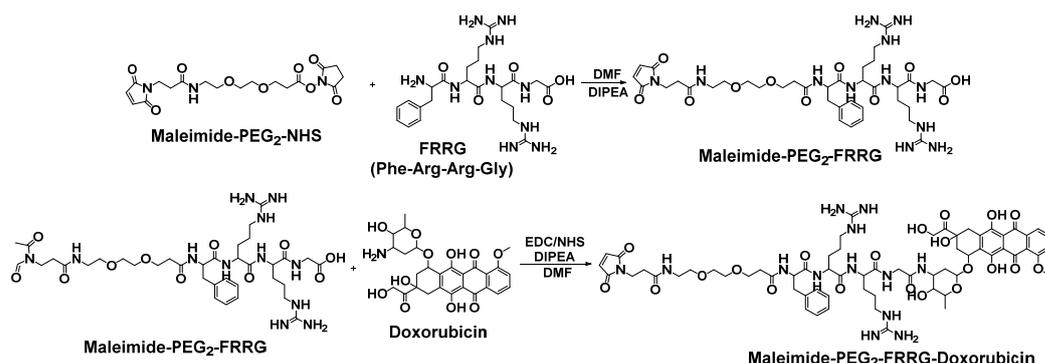
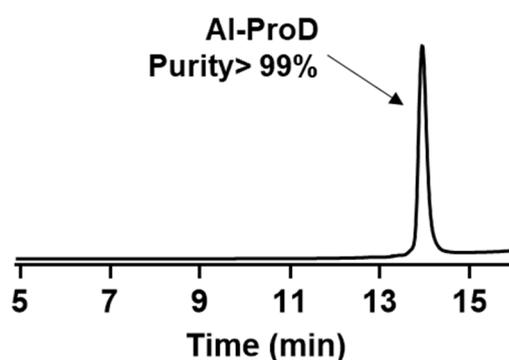


# Supplementary Materials: Cathepsin B-overexpressed Tumor Cell Activatable Albumin-Binding Doxorubicin Prodrug for Cancer-Targeted Therapy

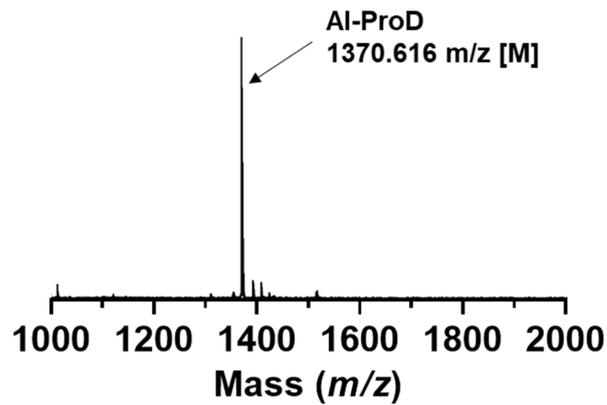
Hanhee Cho, Man Kyu Shim, Suah Yang, Sukyung Song, Yujeong Moon, Jinseong Kim, Youngro Byun, Cheol-Hee Ahn and Kwangmeyung Kim



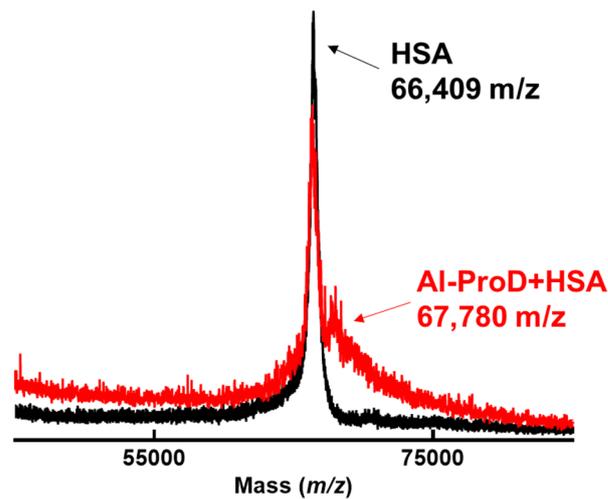
**Figure S1.** Synthetic scheme to prepare cathepsin B-overexpressed tumor cell activatable albumin-binding doxorubicin prodrug (AI-ProD). First, maleimide-PEG<sub>2</sub>-NHS (100 mg, 1 equiv) was reacted with NH<sub>2</sub>-FRRG-COOH (251.4 mg, 2 equiv) in anhydrous DMF (10 mL) at 37 °C for 12 h, and maleimide-PEG<sub>2</sub>-FRRG-COOH was purified using HPLC. Second, subsequent synthesis of maleimide-PEG<sub>2</sub>-FRRG-DOX (AI-ProD) was performed by dissolving maleimide-PEG<sub>2</sub>-FRRG-COOH (150 mg, 2 equiv), doxorubicin (DOX; 48.2 mg, 1 equiv), EDC (44.1 mg, 4 equiv) and NHS (40.9 mg, 4 equiv) in anhydrous 10 mL DMF, while stirring at 37 °C for 24 h. Then, the AI-ProD was further purified via HPLC with equal solvent condition, and lyophilized at -90 °C to obtain as a red powder (Freeze Dryer, ilShinBioBase, Republic of Korea).



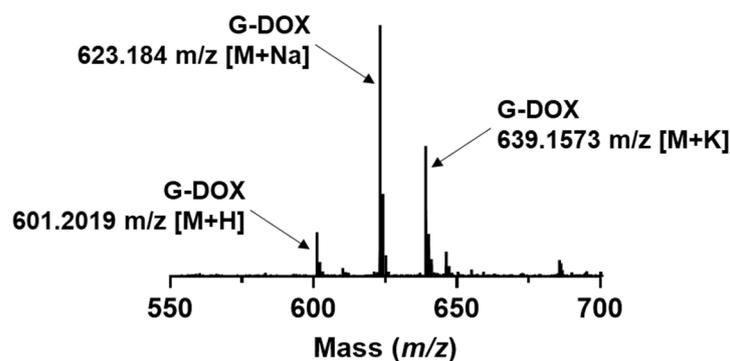
**Figure S2.** The purity (> 99%) of AI-ProD was confirmed by high performance liquid chromatography (HPLC).



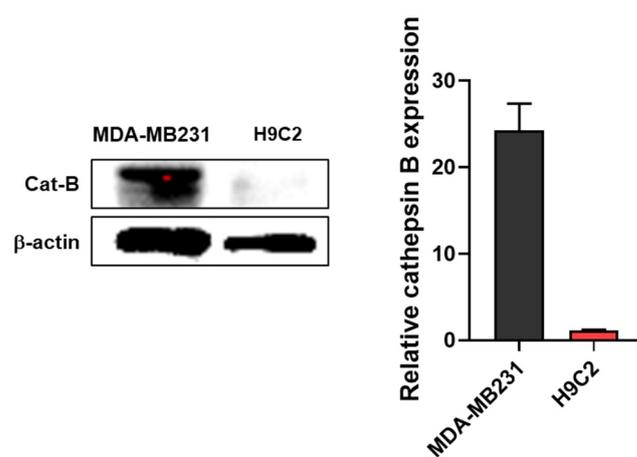
**Figure S3.** The molecular weight of Al-ProD was confirmed via MALDI-TOF mass spectrometer. The molecular weight of Al-ProD was calculated to be 1370.44 Da for  $C_{64}H_{83}N_{13}O_{21}$ , and measured to be 1370.616  $m/z$ .



**Figure S4.** MALDI-TOF analysis results of human serum albumin and human serum albumin-bound Al-ProD.



**Figure S5.** Metabolite assay of Al-ProD. Glycine-conjugated doxorubicin (G-DOX) release from Al-ProD was confirmed via MALDI-TOF mass spectrometer. For this analysis, Al-ProD was incubated with MES buffer containing cathepsin B (50  $\mu$ g), and enzyme reaction buffer was analyzed. As a result, the molecular weights of G-DOX were measured to be 601.2019  $m/z$  [M+H], 623.184 [M+Na] and 639.1573 [M+K].



**Figure S6.** Cathepsin B expression levels of MDA-MB231 and H9C2 cells (left) Western blot analysis of cathepsin B of MDA-MB231 cancer cells and H9C2 normal cells. (right) Relative expression levels of cathepsin B in each cell; MDA-MB231 cells expressed  $24.26 \pm 3.08$ -fold higher amount of cathepsin B than H9C2 cells.