Supporting Information

Photosensitizer-encapsulated ferritins mediate photodynamic therapy against cancer associated fibroblasts and improve tumor accumulation of nanoparticles

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MATERIALS AND METHODS

Expression and purification of ferritin and anti-FAP scFv We followed our published protocol to express and purify ferritins (FRT).[1] In brief, polymerase chain reaction (PCR) was used to amplify FRT DNA segment from cDNA using respective primers to introduce NcoI and XhoI restriction sites flanking the normal start and stop codons. The double digested PCR product was ligated to the NcoI/XhoI digested plasmid pRSF under T4 DNA ligase and the product was used to transform competent cells of E. coli XL1-Blue by standard procedures. The resulting pRSF/FRT plasmids were screened by appropriate restriction digestions, verified by DNA sequencing, and used to transform E. coli BL21 (DE3). For protein expression, a 1 L LB-ampicillin (50 μg/mL) culture of E. coli BL21(DE3)/FRT was grown at 37 °C until an OD600 reading of 0.8 was reached. IPTG (final concentration: 1 mM) was added to induce the production of proteins and the culture was carried out at 37 °C for 4 h. The harvested bacteria were sonicated and the resulting solution was centrifuged at 10,400 rpm (12,930 g) for 30 min to remove cell debris. The supernatant was incubated at 60 °C for 10 min and centrifuged at 10,400 rpm for 30 min to remove precipitates. The raw product was purified by HPLC on a Superose 6 size exclusion column. The concentration of FRT was determined by Bradford protein assay. The purified FRT was stored at -80 °C.

The anti-FAP scFv sequence was first reported by Brocks B. et al.[2] NcoI and HindIII restriction sites were introduced to the heavy chain, flanking the normal start and stop codons. MluI and NotI were introduced to the light chain. The resulting segment was inserted into a pOPE101 plasmid and transformed into E. coli JM109 with ampicillin-resistance. A PelB signal peptide sequence was inserted to the N-terminus of the scFv. The sequence was added to direct the translated scFv to the bacteria periplasm, where the scFv was folded into an active

architecture. To produce anti-FAP scFv, a 1 L LB containing ampicillin (25 μg/mL) culture of E. coli JM109 was grown at 37 ºC until an OD600 reading of 0.8 was reached. IPTG (final concentration: 0.5 mM) was added to induce the production of proteins. The bacteria were incubated at 37 °C for another 4 hours. The bacteria were harvested by centrifugation at 4,000 g at 4 °C. After sonication, the cell lysate was centrifuged at 12,930g for 30 min to remove cell debris and the supernatant was filtered through a 0.2 μm filter. A Ni-NTA cartridge was connected to HPLC and it was thoroughly washed with binding buffer NPI-10. The filtered supernatant was then loaded onto the Ni-NTA cartridge at 1 mL/min. NPI-20 washing buffer (10-fold column volume, i.e. 10 mL) was applied to the cartridge subsequently. Elution buffer of NPI-250 was then applied to elute the scFv from the column. The collections were dialyzed against 1x PBS (pH 7.4) at 4 °C for 48 hours. The concentration of scFv was determined by Bradford protein assay. The purified scFv was stored at -80 °C. 12% SDS-PAGE was used to analyze the size of the products.

Photosensitizer loading and anti-FAP scFv coupling The protocol for $ZnF_{16}Pc$ loading was published by us.[3] Briefly, the pH of a FRT solution (PBS, pH 7.4) was reduced to 2.0 using 1N HCl. A $ZnF_{16}Pc$ DMSO solution (5 mg/mL) was dropwise added, and the final FRT/$ZnF_{16}Pc$ weight ratio was tuned between 0.5-1.5. After gently shaking at room temperature for 30 minutes, the pH of the mixture was slowly increased back to 7.4 by adding 1 M NaOH. The resulting Z@FRT was purified on a NAP-5 column to remove the unloaded $ZnF_{16}Pc$. The anti-FAP scFv was coupled onto FRT using bis(sulfosuccinimidyl) suberate (BS3) as a crosslinker. Briefly, scFv and BS3 were mixed at a molar ratio of 1:30 for 30 min in PBS (pH 7.4) at room temperature. A centrifugal filtration unit (Amicon, MWCO = 10k) was used to remove the unbound BS3 molecules. The resulting intermediate was added to a FRT PBS

solution at a molar ratio of 20:1, and the mixture was incubated at room temperature for 30 min. A centrifugal filter (Amicon, MWCO = 100k) was used to purify the raw product and to obtain scFv-FRT.

Cell culture The 4T1 cell line was obtained from the American Type Culture Collection (ATCC). The cells were cultured in a RMPI 1640 medium (Corning®) supplemented with 10% fetal bovine serum (Corning®) and 1% penicillin-streptomycin (MediaTech, USA). The cells were incubated at 37 °C in 5% CO_2.

Animal models Athymic nude mice were purchased from the Envigo Laboratories. The animal model was established by subcutaneously injecting ~ 10^6 4T1 cells per site to both left and right hind limbs of each mouse. All of the experimental procedures were conducted following a protocol approved by the University of Georgia Institutional Animal Care and Use Committee.

***In vivo* imaging** The in vivo experiments started when tumors reached a size of ~100 mm^3. Briefly, scFv-Z@FRT (1.5 mg $ZnF_{16}Pc$/kg) was i.v. injected. The right-hand-side tumors were irradiated at 24 h post injection (p.i.) by a 671 nm laser (300 mW/cm^2, over a ~1 cm diameter beam) for 15 min. Forty eight hours later, mice were i.v. injected with IRDye800-labeled BSA (5 mg/kg), 10 nm QDs, or 50 nm QDs (n = 5). In vivo imaging was performed on a Maestro II imaging system at 5 min, 10 min, 30 min, 1 h, 4 h, and 24 h post BSA or QD administration. After the 24 h imaging, the animals were euthanized and the tumors were harvested for histology analyses.

Histology Cryogenic slices of 8 μm thickness were fixed with ice-cold acetone for 15 min and washed with 1x PBS (pH 7.4) for 5 min twice. Subsequently, 10% goat serum was applied at 37 °C for 1 h to block the slides. Phycoerythrin-labeled anti-CD31 antibody (ab25644) was

incubated with the slides at 4 °C overnight. After gently rinsing with PBS, the slides were mounted with a DAPI-containing mounting medium. Images were acquired on an Olympus IX71 microscope. Tricrhome staining kit was purchased from abcam (ab150686) and the staining was conducted by following a vendor-provided protocol.

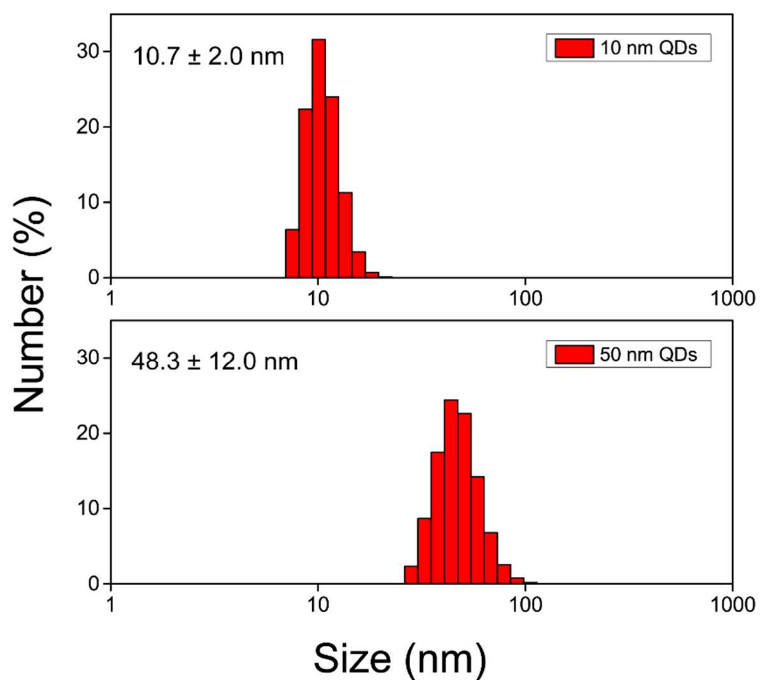

Figure S1. DLS analysis of 10 nm and 50 nm QDs.

References:

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