Supplementary Material for

Multimodal Bacteriochlorophyll Theranostic Agent

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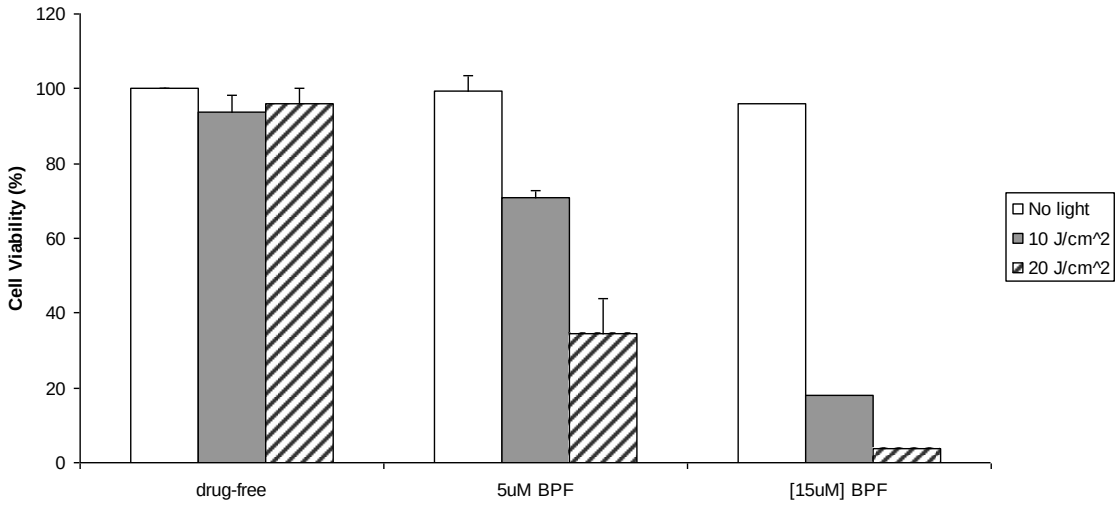

Figure S1. *In vitro* PDT efficacy of 5 μM and 15 μM BPF in KB (FR positive) cells using 10 J/cm2 and 20 J/cm2 light doses. Data are expressed as mean values ± SEM of three independent experiments.

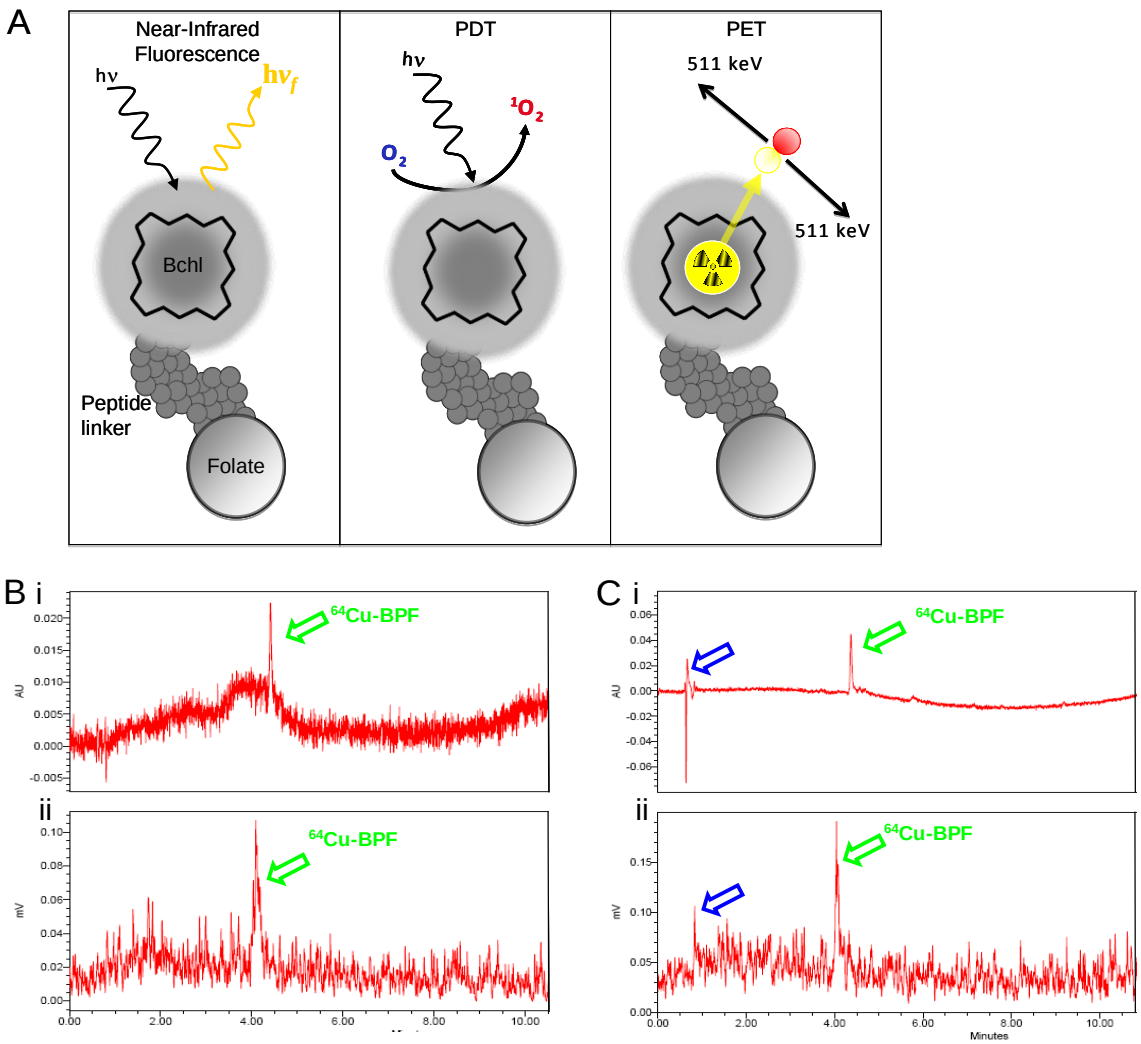

Figure S2. A) Tri-modal BPF: 1) Fluorescence imaging, 2) PDT and 3) PET imaging. ^{64}Cu radiolabeling of BPF showing B) quality control of ^{64}Cu-labeled BPF by radio-UPLC using an i) UV 310nm channel and ii) radioactivity channel and C) Stability of ^{64}Cu-PPF in saline was evaluated using an i) UV 254 nm channel and ii) radioactivity channel. Blue arrow depicts minimal amount of ^{64}Cu labeled degradation of BPF after 24h.

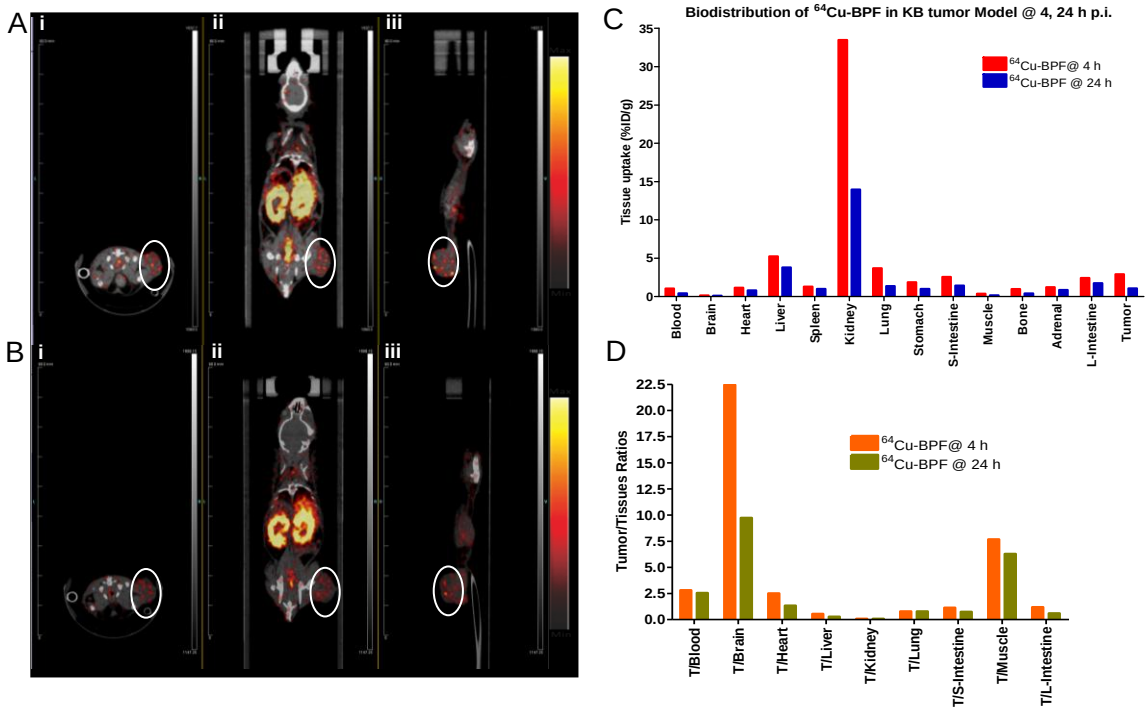

Figure S3. PET imaging and Biodistribution studies of ^{64}Cu-BPF in mice bearing KB xenografts. Representative PET images showing a single i) coronal, ii) sagittal and iii) axial slice of animals at A) 4h and B) 24h after a 500 µCi i.v. injection of ^{64}Cu-BPF. Biodistribution of ^{64}Cu-BPF at 4 and 24h post injection where D) tissue uptake measured as % injected dose/ gram of tissue (% ID/g) and D) tumor-tissue ratio of ^{64}Cu-BPF biodistribution.

Radiolabeling BPF: In a 1.5 mL eppendorf tube, 2 µL DMSO was added to dissolve 50 µg (~30 nmol) of BPF. 0.1 mL of 0.1 M NH_4OAc buffer (pH = 5.5) was added and vortexed producing a dark green solution. 0.10 mL of $^{64}Cu(Acetate)_2$ solution (0.5 - 5.0 mCi, Sherbrooke, Quebec) was then added and the reaction mixture was heated in a water bath at 60°C for 20min. After cooling to room temperature, a sample of resulting solution was analyzed by radio-UPLC. The radiolabeling yield was > 99.9% and the radiochemical purity of ^{64}Cu-BPF was > 98% (this depends on the purity of the starting material BPF) and the specific activity was 2.66 × 106 GBq/mol. The radio-UPLC method used the Acquity UPLC system (Waters Corp., Milford, MA) equipped with PDA detector, Bioscan radioactive detector and Acquity BEH C18 column (2.1 × 100 mm, 1.7 µm; Waters). The flow rate was 0.8 mL/min. The mobile phase was isocratic with 80% solvent A (0.1 M TEAA, pH 7) and 20% solvent B (acetonitrile) at 0min, followed by a gradient mobile phase shifting from 20% solvent B at 0min to 100% solvent B at 12min and back to 20% solvent B at 15min. Purification of ^{64}Cu-BPF used a Sep-Pak C18 cartridge according to the following procedure: 1) Attach a syringe to the Sep-Pak C18 cartridge. 2) Flush the column with 5 mL of ethanol and flush the column with 10 mL of saline to equilibrate the column. 3) Load the sample onto the column and wash the

sample with 10 mL of saline. 4) Elute with 400 μl of 80% ethanol, collect the fractions of purified sample. 5) Dry samples using a speed-vacuum and resuspend in saline. A certain amount of radioactivity is washed down in step 3 if unlabeled free ^{64}Cu is observed in the system. With the natural dark purple color of Bchl, the elution of Bchl-conjugate can be easily and directly monitored visually in step 4, and the fractions with the deepest color contain the highest concentration of labeled and unlabeled Bchl-conjugate.

***In vivo* PET imaging studies:** ^{64}Cu-BPF was prepared and administered without any further purification. The dose solution was prepared by dissolving the radiotracer in saline to a concentration of 2.5 - 5.0 mCi/mL for MicroPET imaging, and diluted to a concentration of 0.1 - 0.5 mCi/mL. The resulting solution was filtered with a 0.20 μMillex-LG filter before being administered to the animals. Each tumor-bearing mouse was injected via the tail vein with 0.1 - 0.2 mL of the filtered dose solution. MicroPET imaging was performed using a MicroPET Siemans Focus 220 (Concorde Microsystems, Knoxville, TN). KB tumor-bearing mouse was anesthetized with 2% isoflurane in oxygen, and injected with ~500 μCi of ^{64}Cu-BPF via the tail vein, and placed near the center of the FOV where the highest resolution and sensitivity are obtained. A 10min static PET image was obtained at 4h post injection and 30-45min static PET images were acquired at 24h post injection. Throughout the imaging, the animal was kept anesthetized and directly transferred to the scanner, together with the supporting bed, without any movement. CT scanning was carried out immediately after each PET imaging session. The static PET images were then acquired with same parameters at 4 and 24h post injection.

Biodistribution Studies: Biodistribution studies were performed using athymic nude mice bearing KB xenografts. The ^{64}Cu-BPF (~12.5 μCi in 0.1 mL saline) was administered into each animal via the tail vein. Four animals were euthanized by with 2% isoflurane, exsanguinationed, and opening of the thoracic cavity at 4 or 24h post injection. Blood samples were withdrawn from the heart through a syringe. Organs were excised, washed with saline, dried with absorbent tissue, weighed and counted on a γ-counter (Perkin-Elmer Wizard-1480). Organs of interest included the tumor, heart, spleen, lungs, liver, kidneys, adrenal, stomach, intestine, muscle, bone and brain. Organ uptake was calculated as a percentage of the injected dose per gram of tissue (%ID/g). The biodistribution data and target-to-background (T/B) ratios are reported as the mean and standard deviation based on results from three animals at each time point.