Supporting Information for "Mechanism of Cellular Uptake of Highly Fluorescent Conjugated Polymer Nanoparticles"

*Lawrence P. Fernando, Prakash Kandel, Jiangbo Yu, Jason McNeill, P. Christine, Ackroyd, and Kenneth A. Christensen**

Department of Chemistry, Clemson University, Clemson, SC 29634

*Corresponding Author

SUPPORTING INFORMATION EXPERIMENTAL

Absorbance and fluorescence emission spectra. Absorbance spectra of PFBT nanoparticle solutions were acquired using a Genesys 10UV (Thermo) spectrophotometer which has spectral resolution of 5 nm. Solutions were diluted to below 0.5 absorbance units in quartz cuvettes prior to measuring absorbance and below 0.05 absorbance units for fluorescence measurements. Fluorescence emission spectra were acquired using a scanning fluorometer (Photon Technology International; QM-4) with 75 W Xe arc lamp excitation and photon counting detection. Both excitation and emission monochromator slits were set to achieve an 8 nm band pass.

Phalloidin staining of actin filaments. Cells were plated at 100K/dish in glass bottom dishes and incubated with 2 nM PFBT nanoparticles for 8 h or vehicle alone as a control. The cells were fixed at 37°C with 4% paraformaldehyde in RB for 10 minutes then washed 3X in RB. Next, the cells were permeabilized for 3-5 min in RB + 0.1% Triton-X. Two (2) units of Alexa fluor 594 phalloidin was added to the cells for 20-30 min in RB + 1% BSA and finally washed 3X with this same buffer and images acquired on the microscope.

SUPPORTING INFORMATION FIGURES

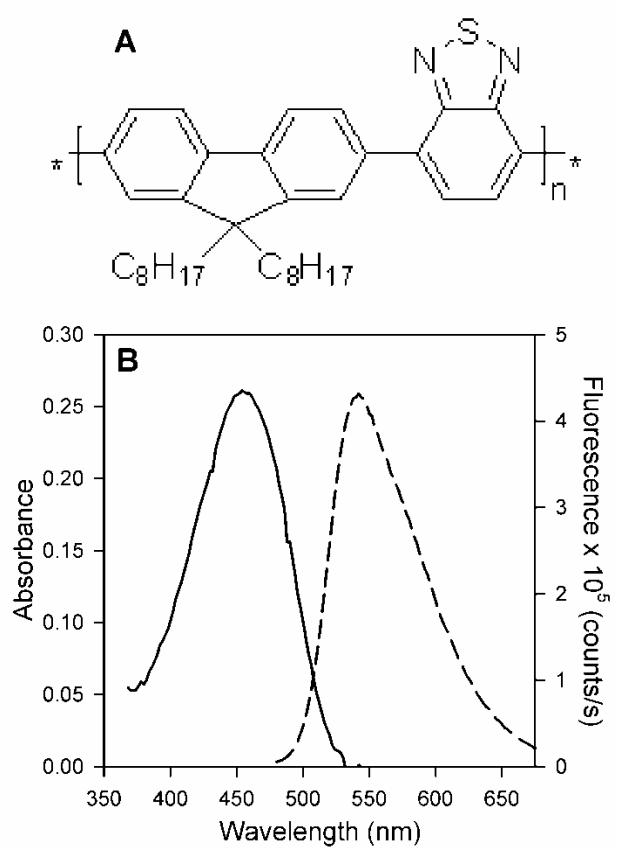

Figure S1 A) Chemical structure of PFBT. B) Absorbance spectrum and fluorescence emission spectrum of PFBT nanoparticles. An absorbance spectrum (solid line) was acquired using a ~4 nM solution of PFBT nanoparticles in water. A fluorescence emission spectrum (dashed line) was acquired using a ~300 pM solution of PFBT nanoparticles in water (λ_{ex} = 460 nm). The fluorescence emission was recorded from 480 – 700 nm.

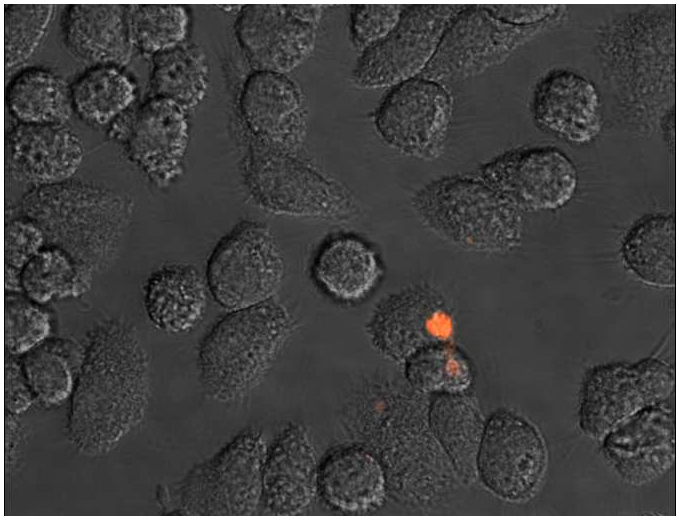

Figure S2 Cell viability after 8 h incubation on ice. An image overlay of DIC and propidium iodide (PI) fluorescence for cells following an 8 h incubation on ice. Cell morphology is normal for J774A.1 cells and only 1 cell in the microscope field shows strong nuclear staining with PI which only stains dead cells. All images were acquired at 37°C using a 60X/1.45NA objective. PI fluorescence measured using (λ_{ex} = 494 nm/λ_{em} = 624 nm).

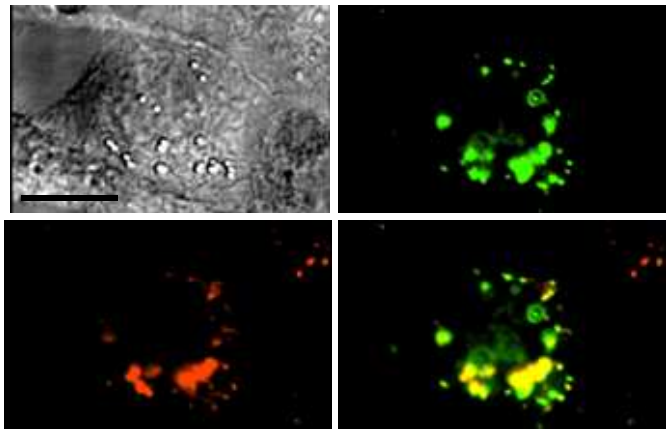

Figure S3 Colocalization of PFBT nanoparticles with TR-dex. CHO-K1 cells were grown in 35 mm glass bottom dishes as indicated in materials and methods. Cells were pulsed with 2 nM (4 ppm) PFBT nanoparticles and 250 nM TR-dex overnight in DMEM + 10% FBS at 37°C and 5% CO_2 then washed 3X in RB prior to imaging. All images were acquired at 37°C using a 60X/1.45NA objective. A) DIC (Scale bar = 10 µm); B) PFBT nanoparticle fluorescence (λ_{ex} = 494 nm/λ_{em} = 531 nm); C) TR-dex fluorescence (λ_{ex} = 575 nm/λ_{em} = 624 nm); D) Merged image; yellow color indicates probable colocalization.

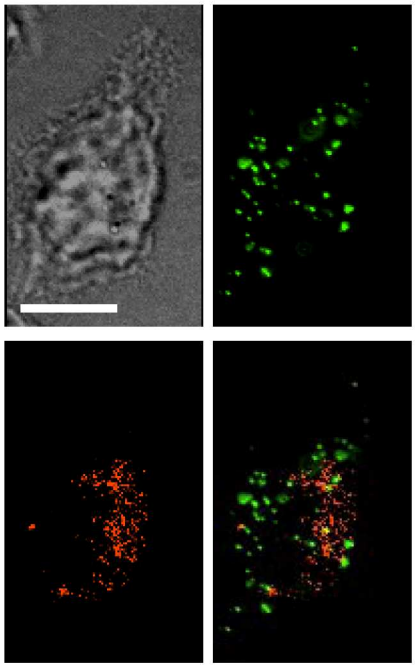

Figure S4 No Observed Colocalization of PFBT nanoparticles with Clathrin Heavy Chain. Cells were pulsed with 2 nM PFBT nanoparticles for 2 hours in DMEM + 10% FBS at 37°C and 5% CO_2 then immediately paraformaldehyde fixed, detergent permeabilized, and stained with an anti-clathrin heavy chain primary antibody followed by labeling with a Cy5 secondary Fab conjugate. Images were all acquired at room temperature using a 60X/1.45NA objective. A) DIC (Scale bar = 10 µm); B) PFBT nanoparticle fluorescence (λ_{ex} = 494 nm/λ_{em} = 531 nm); C) Cy5 (anti-clathrin heavy chain) fluorescence (λ_{ex} = 575 nm/λ_{em} = 624 nm); D) Merged image; yellow color indicates no observable colocalization.

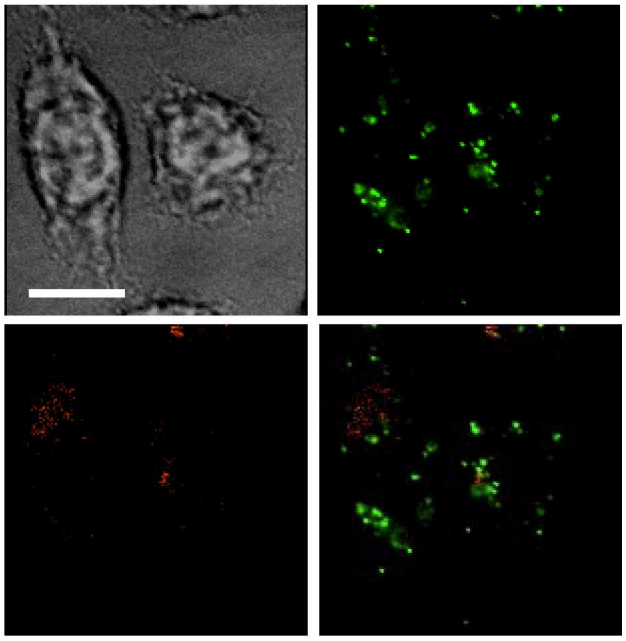

Figure S5 No Observed Colocalization of PFBT nanoparticles with Caveolin-1. Cells were pulsed with 2 nM PFBT nanoparticles for 2 hours in DMEM + 10% FBS at 37°C and 5% CO_2 then immediately paraformaldehyde fixed, detergent permeabilized, and stained with an anti-caveolin-1 primary antibody followed by labeling with a Cy5 secondary Fab conjugate. Images were all acquired at room temperature using a 60X/1.45NA objective. A) DIC (Scale bar = 10 μm); B) PFBT nanoparticle fluorescence (λ_{ex} = 494 nm/λ_{em} = 531 nm); C) Cy5 (anti-caveolin-1) fluorescence (λ_{ex} = 575 nm/λ_{em} = 624 nm); D) Merged image; yellow color indicates no observable colocalization.

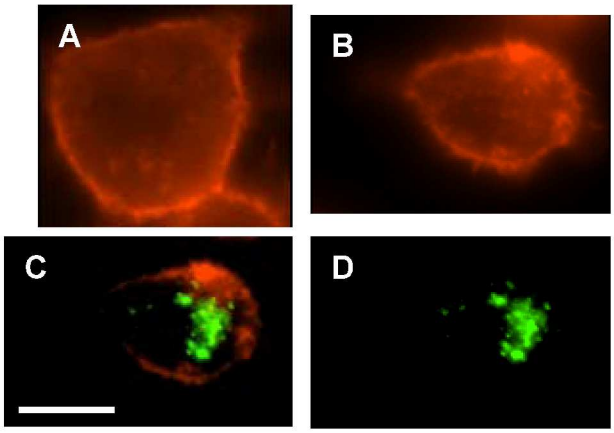

Figure S6 Normal actin filament distribution observed for cells treated with PFBT nanoparticles. Cells were pulsed with 2 nM PFBT nanoparticles for 8 hours in DMEM + 10% FBS at 37°C and 5% CO_2. Cells were then paraformaldehyde fixed, detergent permeabilized, and stained 2 units of Alexa fluor phalloidin. Images were all acquired at room temperature using a 60X/1.45NA objective. A) Alexa fluor 594 phalloidin (actin) fluorescence (λ_{ex} = 575 nm/λ_{em} = 624 nm; scale bar = 10 µm) for cells *not treated* with nanoparticles; B) Alexa fluor 594 phalloidin (actin) fluorescence (λ_{ex} = 575 nm/λ_{em} = 624 nm; scale bar = 10 µm) for cells *treated* with PFBT nanoparticles; C) Merged image; no observed differences between cells treated with nanoparticles and the untreated control; D) PFBT nanoparticle fluorescence (λ_{ex} = 494 nm/λ_{em} = 531 nm).