Two-photon active polymeric nanoparticles for high contrast in-vitro imaging

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1. Materials and methods:

Materials:

Chloroform ($CHCl_3$), Tetrahydrofuran (THF), 1-propanol acetone and an anionic surfactant (sodium dodecyl sulphate (SDS)) were purchased from Sigma Aldrich. The fluorescent PURET was synthesized following the procedure previously reported. Poly(3,3'''-didodecyl quarter thiophene) (ADS12PQT) was acquired from American Dye Source, Inc. For cellular studies, THP-1 (Human acute monocyticleukemia) cells (TIB-202), MTT cell viability kit were purchased from American Type Culture Collection.

Instrumentations:

The formations of the nanoparticles were monitored using UV-vis spectroscopy and confirmed by dynamic light scattering (DLS) and scanning electron microscopy (SEM). UV-vis absorption spectra were recorded using a Perkin-Elmer Lambda-9 spectrophotometer. Fluorescence spectra were recorded using a Perkin-Elmer LS 55 Luminescence Spectrometer. The DLS measurement

of the nanoparticles was carried out using a custom-built Brookhaven laser light scattering goniometer. A JEOL JSM-7401F FE-SEM was also used to examine the formation of the nanoparticles. A Quantronix mode-locked Ti:sapphire laser at 800 nm with 100 femtosecond pulse-width and 1 kHz repetition rate was used as the excitation source for two-photon fluorescence (TPF) measurements. The 800 nm laser pulses were incident in the sample cuvette and the fluorescence signal was collected at right angles using a CCD array detector in conjunction with a monochromator. A short-pass filter at 750 nm was placed in front of the monochromator to remove the excitation. A 4.5 cm focal length lens was used to collect the fluorescence. The two-photon (TP) images were taken using a Zeiss LSM 710 NLO laser scanning confocal microscope with a Plan-Apochromat 63x 1.4NA oil lens and Zen 2009 imaging software was utilized for image capturing and analysis. A Coherent Chameleon Vision II femtosecond laser operated at 800 nm was employed as excitation for TP imaging.

2. Experimental:

2a. Fabrication of polymer nanoparticles:

The fluorescent polymer nanoparticles were fabricated using miniemulsion technique utilizing the anionic surfactant, SDS. SDS was first dissolved in distilled water and 1-propanol was added to the aqueous surfactant solution as a co-surfactant. PURET and ADS12PQT polymers were first dissolved in $CHCl_3$. The nanoparticles were then fabricated by adding the each of the polymer solution to the aqueous SDS solution under probe sonication and followed by heating at 65 ˚C for 20 min.

2b. Cell line preparation, cytotoxicity test and two-photon imaging

THP-1 cells were maintained in RPMI 1640 medium containing 10% heat-inactivated FBS, penicillin G 50 U/mL, and streptomycin sulfate 50 µg/mL. The cells were grown and maintained in 75 cm^2 cell culture flasks at 37 $^{\circ}C$ in 5% CO_2 humidified incubator. When the confluent THP-1 cells were centrifuged, and seeded in 12 or 96-well plates at 5 X 10^5 cells (total volume 200µL/well), in presence of 20 µg/mL PhorbolMyristate Acetate in order to differentiate them into mature macrophage like cells. For MTT assay, the 96 wells plated cells were incubated with different concentrations of the nanoparticles for 24 h at 37 °C. Cell morphology observations were made using a phase contrast microscope. The relative viability of the cells incubated with the nanoparticles to untreated cells was determined by measuring the MTT-formazan absorbance

on a Kinetic microplate reader Spectra Max M5, Molecular Devices, move all to instrumentation section and what was used for at 570 nm. For confocal microscopy, THP-1 cells were grown on coverslips in 12 wells plate (described above). Cells were fixed with 4% paraformaldehyde and coverslips were mounted on a glass slide for microscopy. The TPF image data were taken using the Zeiss laser scanning confocal instrument. The TP emission signal from the PURET and ADS12PQT nanoparticles was measured at 600nm and 650nm, respectively.

3. Additional Figures:

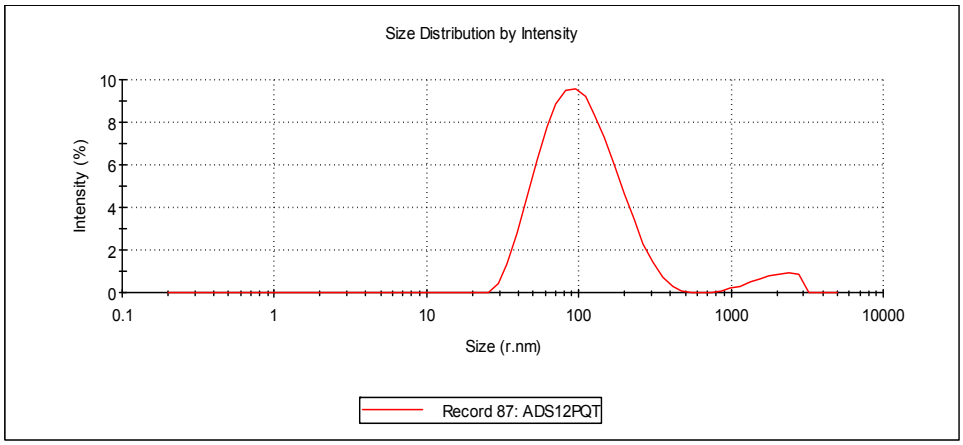


Figure S1. Particle size distribution for the ADS12PQT nanoparticles determined by DLS.

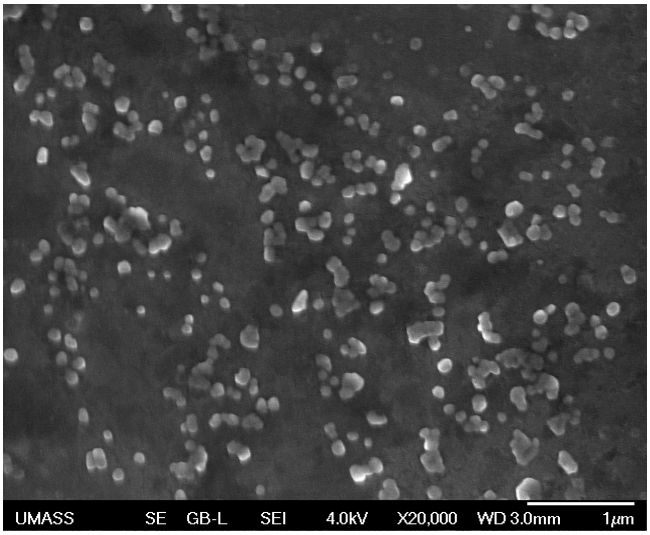
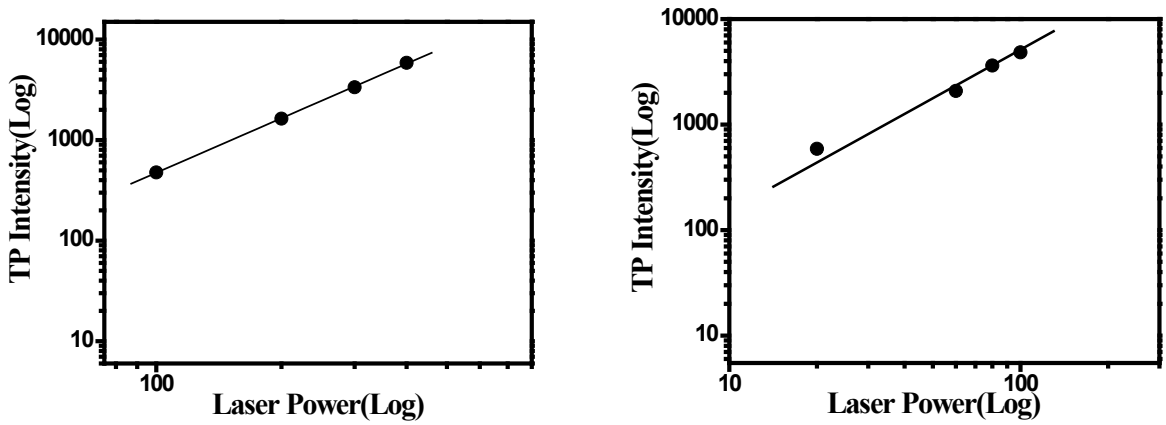


Figure S2. SEM image of the ADS12PQT nanoparticles.



(a) (b)

Figure S3. Quadratic dependence of the TPF from (a) the PURET nanoparticles and (b) the ADS12PQT nanoparticles on the incident laser power.

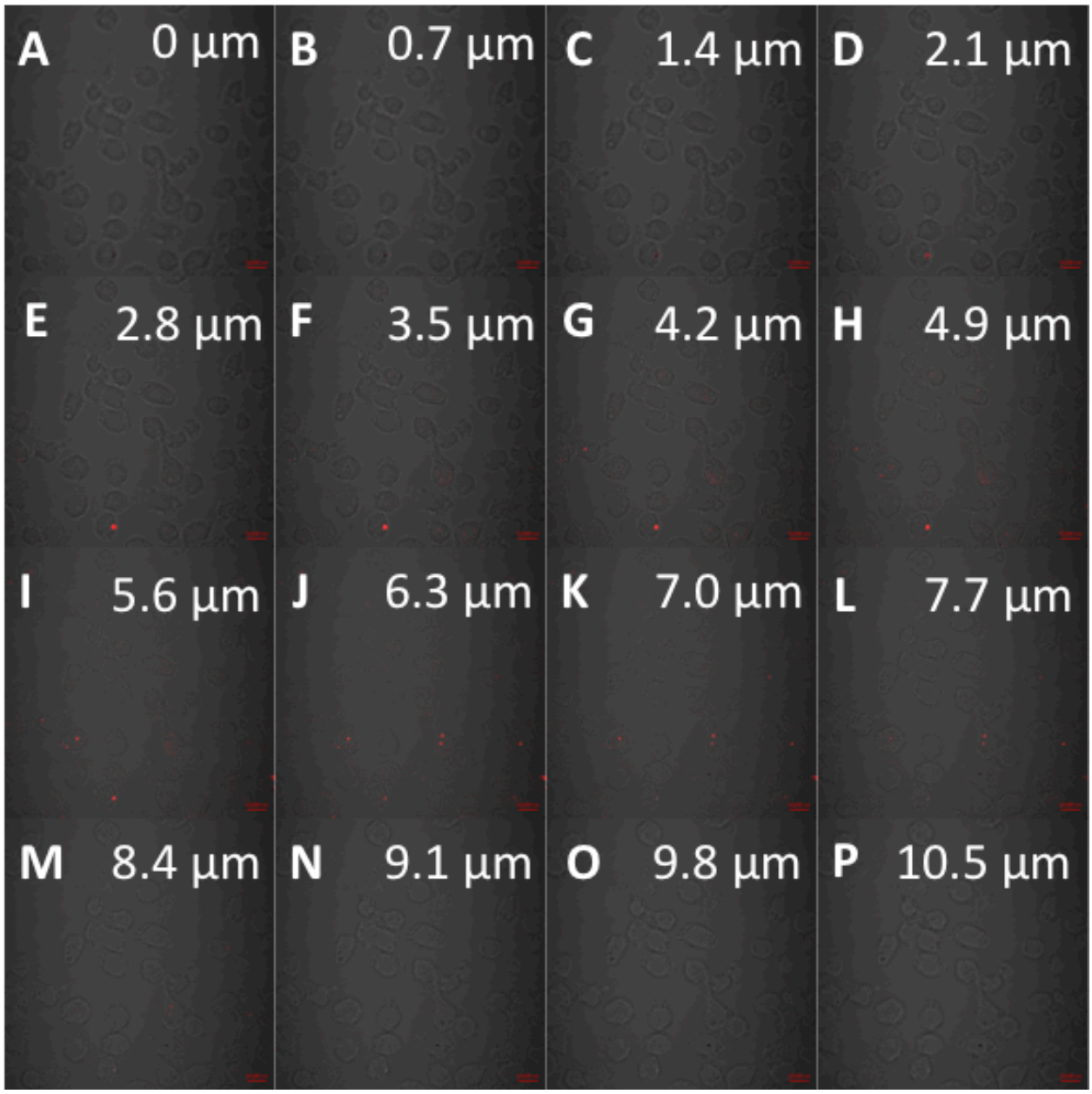


Figure S4. PURET nanoparticles (Red color) cellular uptake and Z-stacking. Images were collected at 0.7 µm intervals with the 800 nm laser to create a stack in the Z-axis. Red color indicates the fluorescence signal from internalized two photon active nanoparticles.

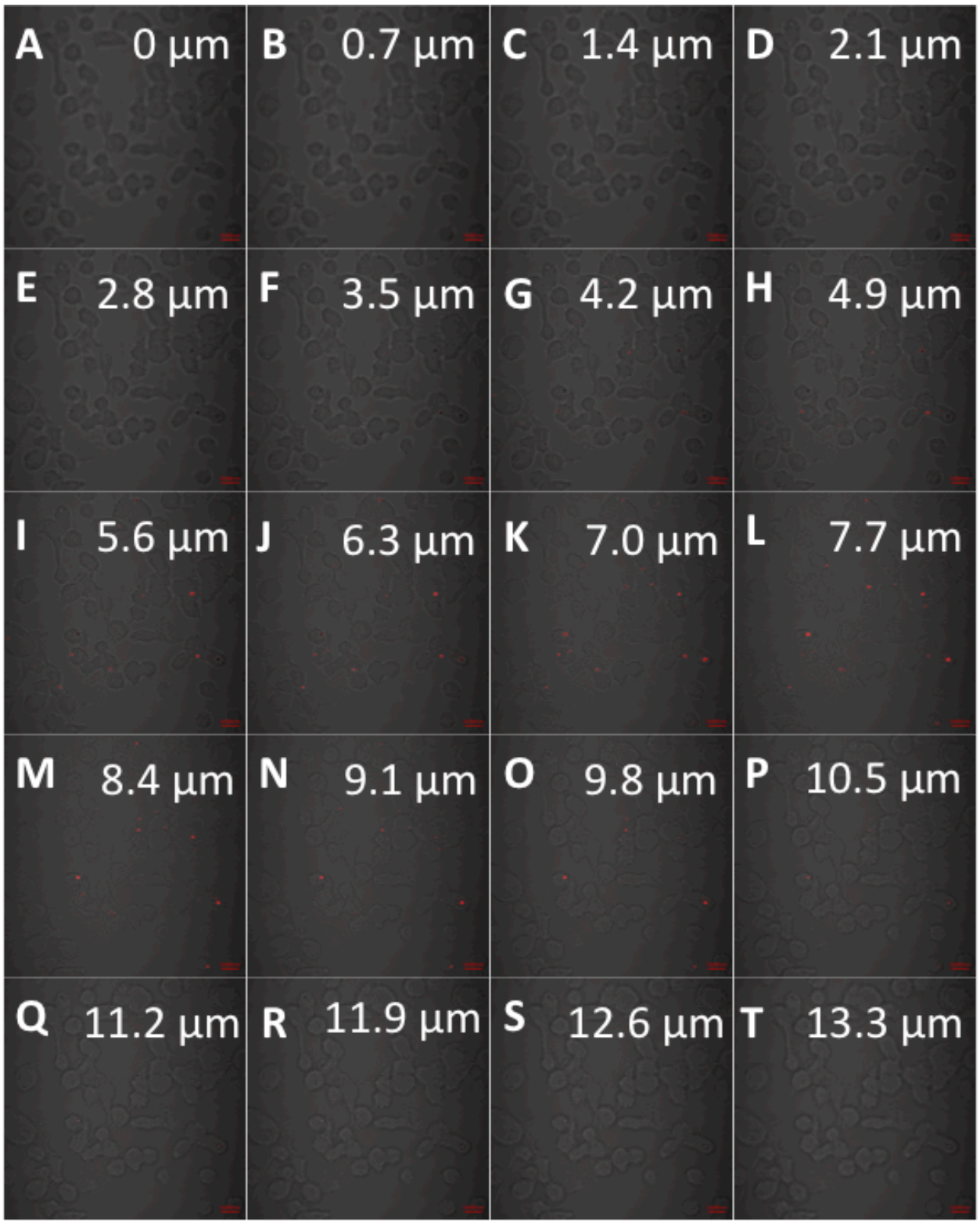


Figure S5. ADS12PQT nanoparticles cellular uptake and Z-stacking. Images were collected at 0.7 µm intervals with the 800 nm laser to create a stack in the Z-axis. Red color indicates the fluorescence signal from internalized two photon active nanoparticles.