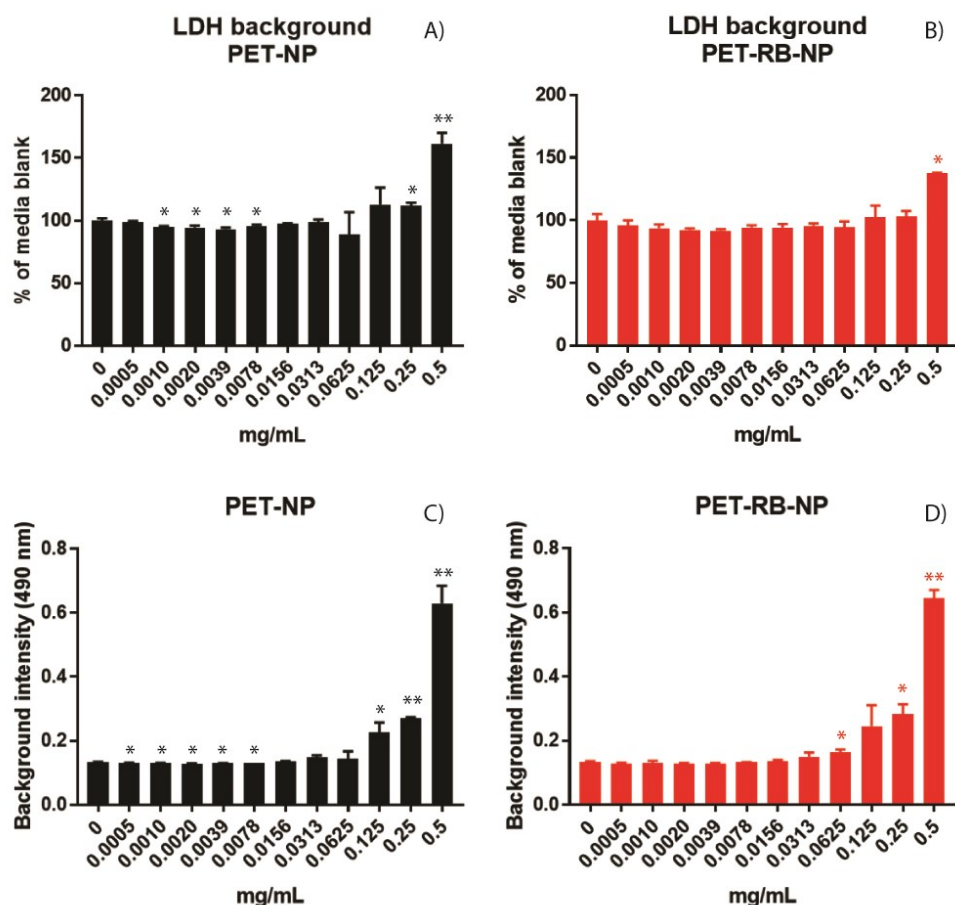


Supplementary Information

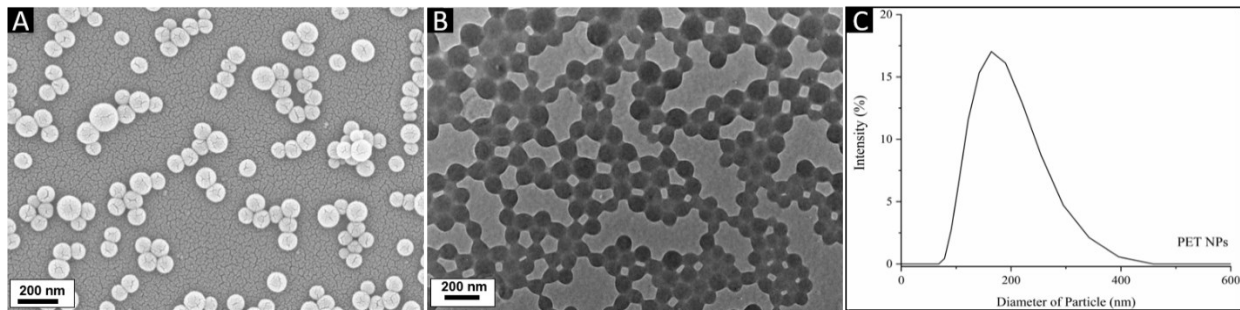
Fabrication of Polyethylene Terephthalate (PET) Nanoparticles with Fluorescent Tracers for Studies in Mammalian Cells

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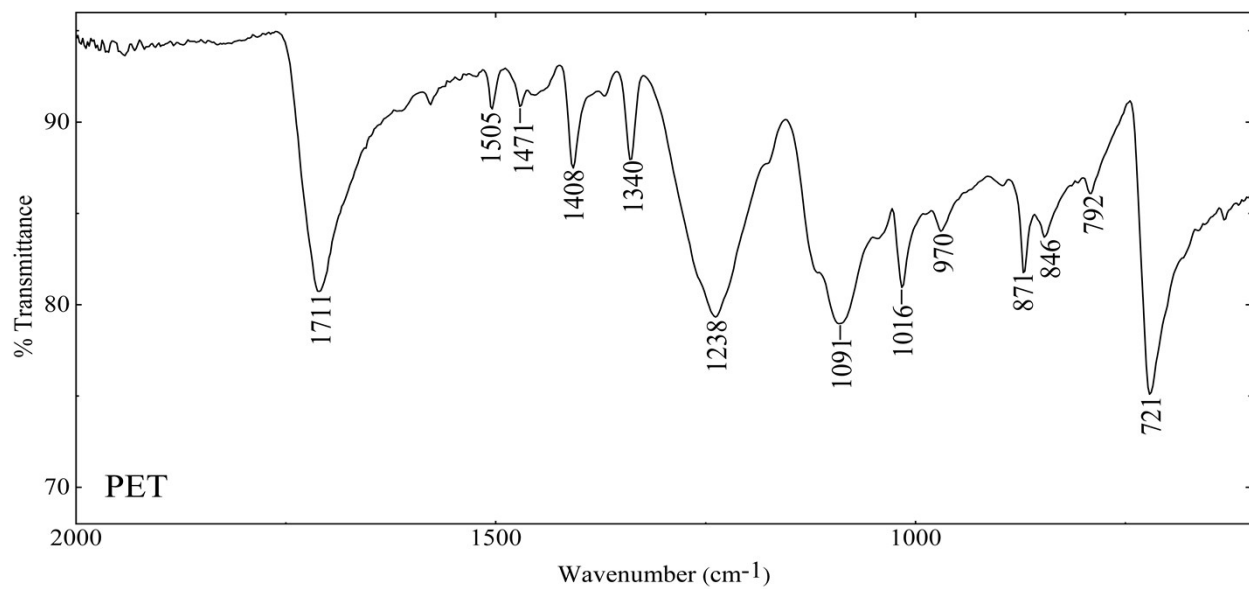
Supplemental Information 1. Control LDH experiment without cells was performed for PET-NP (A) and PET-RB NP (B) and calculated as % of media blank. The data show that the two highest tested concentrations of PET-NP (0.25 and 0.5 mg/mL) interfere with the LDH assay, but not to a level that can explain the cytotoxicity response found for the RAW264.7 cells. The interference by PET-RB NP was less than what was found for PET-NP. It should be mentioned that the PET NPs concentration between control experiment and RAW 264.7 assays may not be identical, since the control experiment does not take any NPs removed from the media due to cell uptake into consideration. The background intensity at 490 nm was similar for PET-NP (C) and PET-RB NP (D). The data showed a concentration-dependent increase in adsorption at 490 nm. The adsorption at 490 nm might confound the measured MTS response for the highest tested concentration and mask low levels of adverse impact on cell metabolism. Also, here it should be highlighted that the NP concentration in the RAW 264.7 assays are unlikely to be the same as for the control experiment, especially at the higher concentrations, since the number of NPs present in the MTS assays is what the cells have taken up.



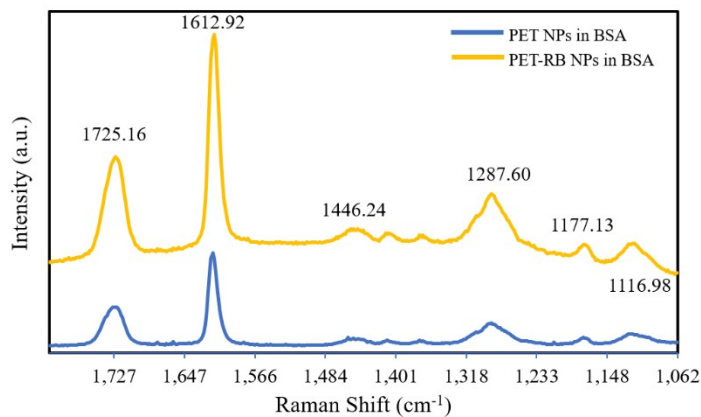
Supplemental Information 2. A) SEM, B) TEM, and C) the DLS curve for PET NPs without rhodamine B. The DLS profile is an average of three DLS runs.



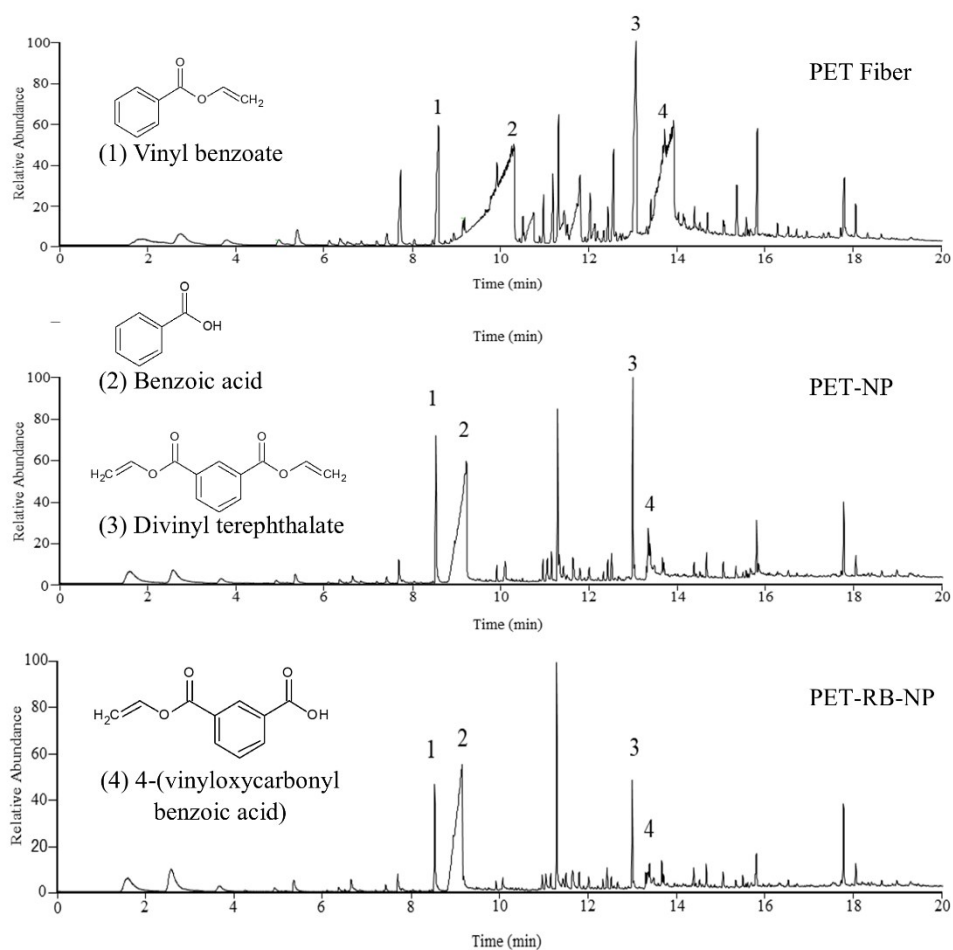
Supplemental Information 3. FT-IR spectra of PET starting material.



Supplemental Information 4. Raman spectra of PET NPs and PET-RB NPs in 0.5 mg/ mL of BSA. The main peak at 1612.92 cm^{-1} corresponded to Raman scattering from benzene rings in the PET structure. Other secondary peaks were located at 1725.16 cm^{-1} (carbonyl stretching), 1446.24 and 1287.60 cm^{-1} (weaker C-C bonds), and 1177 and 1116.98 cm^{-1} (weaker C-O-C asymmetric stretching vibrations). The spectra of all samples were measured at room temperature using a Horiba XploRA Raman Confocal Microscope (Horiba Scientific, Piscataway, NJ) at wavelength excitation of 532 nm with 1200 L mm^{-1} grating.



Supplemental Information 5. Pyrolysis-GC/MS chromatograms of PET fiber used for fabrication of PET-NP and PET-RB NP. Four of the characteristics peaks were identified as (1) vinyl benzoate, (2) benzoic acid, (3) divinyl terephthalate, and (4) 4-(vinylloxycarbonyl benzoic acid). Pyro-GC/MS chromatograms of PET NPs and PET showed peaks identified as vinyl benzoate, benzoic acid, divinyl terephthalate, and 4-(vinylloxycarbonyl benzoic acid)) which are all components of PET¹⁻². Pyrolysis was performed on a CDS Analytical 5250-T Trapping Pyrolysis Autosampler (Oxford, PA) connected to a Thermo Scientific Trace 1310 gas chromatograph coupled to a Thermo Q-Exactive mass spectrometer (Waltham, MA). Sample vials were comprised of a quartz rod inside a quartz tube with the top headspace was packed with quartz wool. Samples were transferred into the vial. An initial thermal desorption step was carried out at 50°C for 60 seconds which was sent to the GC-MS. Then a 350°C cleaning step for 20 seconds was utilized in which all sample contents that were sufficiently volatile were sent to an exhaust port to prevent unwanted material from reaching the column, finally a step of 50°C for 3 seconds and then ramped to 700°C at 10 °C/mSec and held for 60 seconds in which all material was sent to the column for analysis. Data analysis was performed using Xcalibur software version 4.1.31.9 (Thermo) and National Institute of Standards and Technology version 17 library (Gaithersburg, MD) and literature² to help identify spectral peaks of interest.

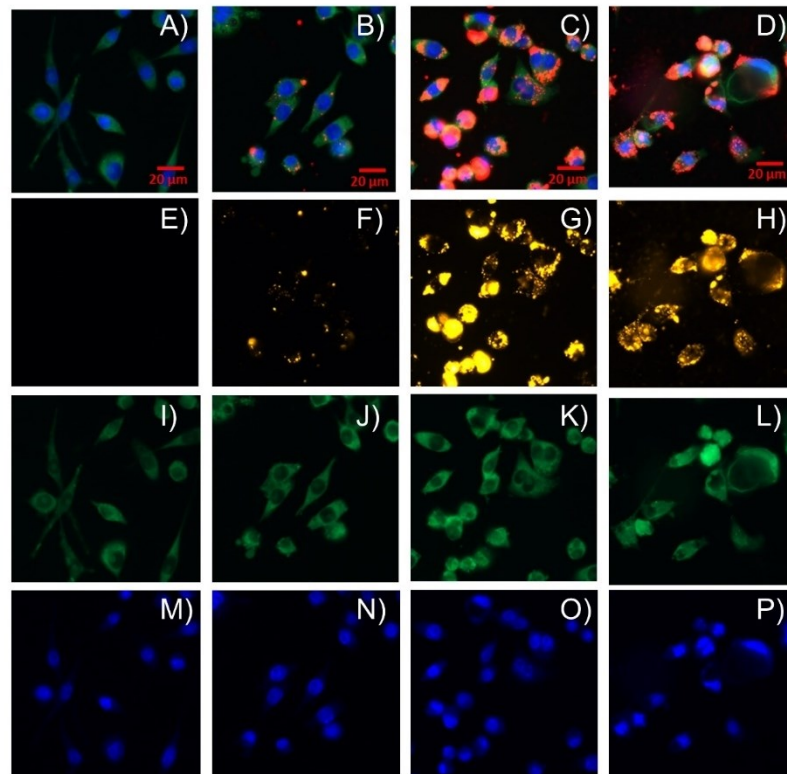


Supplemental Information 6. The surface chemical states in PET NPs in BSA with and without rhodamine B were investigated by XPS analysis. **Table S6** shows the binding energies of all the elements present in the samples. The shift in the binding energies for the C 1s, N 1s, O 1s, Zn 2p and S 2p spectra correspond to the difference in the interactions between the elements and PET structure. The peak centered at 284.4 eV for C 1s is present in both samples and is associated with phenyl carbons in the PET structure. A satellite peak centered around 291 eV is due to the π - π^* shake-up process in the aromatic ring within the structure³. The O 1s spectrum centered around 530.5 eV corresponds to C=O bond. There is also presence of a peak for N 1s centered around 399.5 eV is the result of C-N bonding between nitrogen and the aromatic PET ring⁴. In addition, Zn 2p peak with two spin-orbit splits of 2p_{3/2} and 2p_{1/2} with ~23 eV difference in the binding energy is observed. The 2p_{3/2} centered at 1021.3 eV confirms the presence of Zinc in the Zn⁺² chemical environment. No noticeable shift in the binding energies is observed in both samples. Lastly, there is peaks for S 2p_{3/2} in S 2p spectra around 163 eV in both samples⁵. Measurements were carried out on an Escalab Xi + XPS (Thermo Fisher Scientific, Waltham, MA). All scans were charge compensated. Survey scans were run at 200 eV pass energy with 1.0 eV step size and 10 ms dwell time. While single element scans were done at 50 eV pass energy with 0.1 eV step size and 50 ms dwell time.

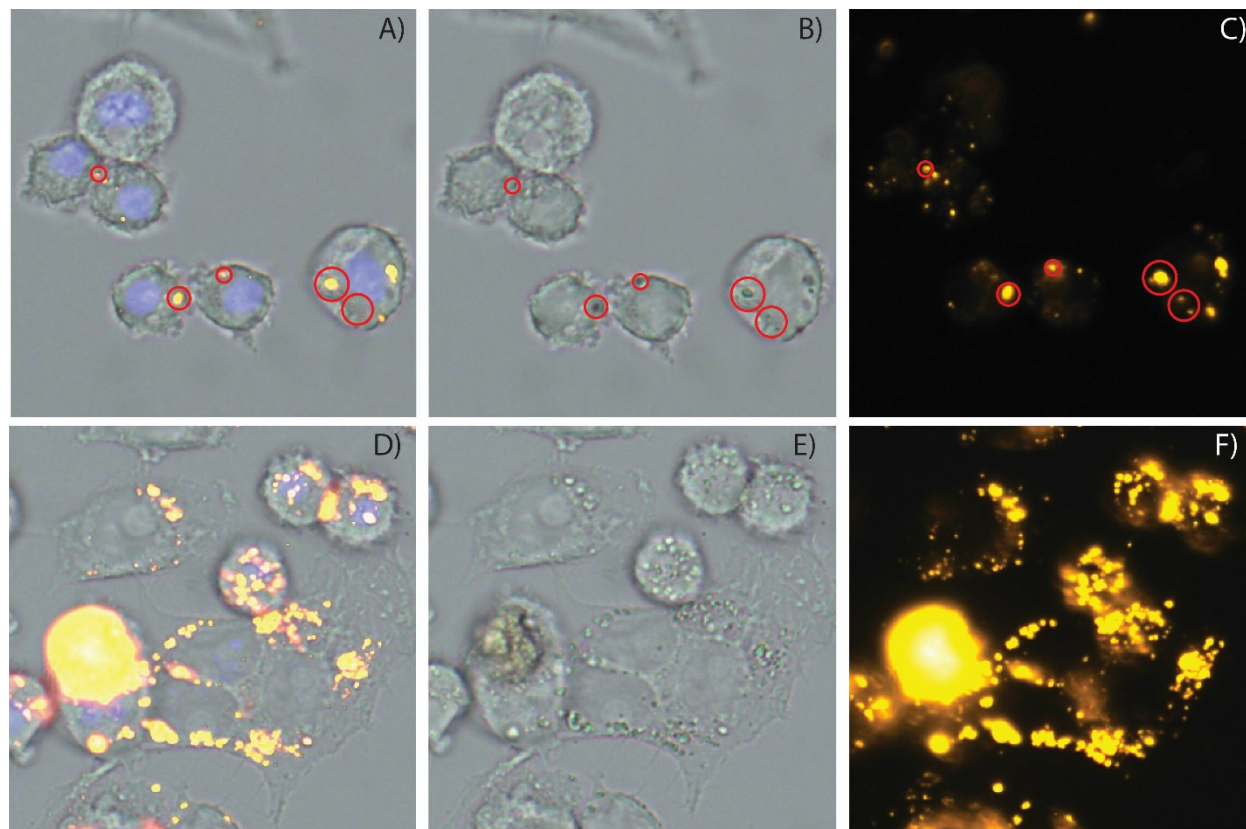
Table S6. Binding energies of major elements present in the PET samples

Sample	C 1s		O 1s	N 1s	Zn 2p		S 2p
	C-C	Satellite			Zn 2p _{3/2}	Zn 2p _{1/2}	
PET NP in BSA	284.4	291.2	530.5	399.5	1021.3	1044.4	162.8
PET-RB NP in BSA	284.4	291	530.6	399.8	1021.1	1045	163.1

Supplemental Information 7. Fluorescence microscopy of RAW 264.7 cells exposed to PET-RB NPs, showing the overlay images of the three fluorescence channels (A-D), PET-RB NPs (E-H), cell cytoplasm (I-L), and nuclei (M-P) for control (A+E+I+M), 0.005 mg/mL PET-RB NPs (B+F+J+N), 0.05 mg/mL (C+G+K+O), and 0.5 mg/mL PET-RB NPs (D+H+L+P).



Supplemental Information 8. Overlay images (A and D) of bright field (B and E), DAPI (nuclei), and rhodamine (PET-RB NP) (C and F) of RAW 264.7 cells exposed to 0.005 mg/mL (A-C) and 0.5 mg/mL (D-F) PET-RB NP. The microscopy images show a good overlay of particles observed in bright field and with fluorescence microscopy (rhodamine B). Examples for 0.005 mg/mL PET-RB NP exposed RAW 264.7 cells are indicated with red circles. These overlaid images support that cells contain these PET NPs. Future studies will be required to further ascertain the potential for tracer leaching from these NPs.



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