Supplementary Material:

Determination of Nanoparticle Localisation within Cellular Compartments in vitro Using Raman Spectroscopy

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S1. Cytotoxicity Evaluation

The toxicological characteristics of 40 nm carboxlylated and fluorescently labelled polystyrene particles were evaluated by the Alamar Blue (AB) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, in each case following manufacturer's instructions. Alamar Blue (AB) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Biosource (UK) and Sigma Aldrich Ltd. (Dublin, Ireland), respectively. Serial dilutions of the initial stock were made and nanoparticle solutions within the concentration range from $5x10^{12}$to $7.8x10^{9}$were prepared in order to evaluate the cytotoxic effect of nanoparticles on A549 cells.

A549 cells and 10% Dimethyl sulfoxide (DMSO)-90% medium solution were used as positive and negative controls, respectively. Cells were seeded in 96-well plates (Nunc, Denmark) with densities of 1 x 10^5 cells/ml, 7 x 10^4 cells/ml, 3 x 10^4cells/ml and 2 x 10^4 cells/ml for 24, 48, 72 and 96 hr exposures, respectively, to achieve the desired confluence

for each incubation period. Seeded cells were incubated for 24 hr for cell attachment. After 24 hr incubation, the medium was removed and the plates were rinsed with 100 µl/well phosphate buffered saline (PBS). Cells were exposed to different concentrations of nanoparticles ($5x10^{12}$, $1x10^{12}$, $5x10^{11}$, $2.5x10^{11}$, $1.25x10^{11}$, $6.25x10^{10}$, $3.13x10^{10}$, $1.56x10^{10}$, $7.80x10^{9}$ ppml) for the desired time period. Each cytotoxicity test was repeated 3 times as independent experiments with six replicates for each concentration and positive and negative control per micro plate.

The Alamar Blue and MTT assays were conducted on same plate. After the desired exposure time, the nanoparticle containing medium was removed and the plates were washed with 100 µl/well PBS. 100 µl of AB/ MTT solution (5% [v/v] solution of AB and 10% [v/v] of MTT dye) prepared in fresh medium without FBS and supplements were added to each well. The plates were incubated for 3 hr. AB fluorescence was measured by using a microplate reader (TECAN GENios, Grodig, Austria) at the excitation and emission wavelengths of 540 and 595 nm. For the MTT assay, the plates were washed with PBS to remove AB and 100 µl of DMSO were added to each well. The plates were shaken for 10 min at 200 rpm and the absorbance was quantified at 570 nm using the same microplate reader (reference filter 340 nm).

Both assays showed that the nanoparticles do not elicit a significant cytotoxic response over the applied concentration range. The elevated AB response (>100%) in Figure S1.A may be due to interaction of the nanoparticles with the assay at these high doses The results are consistent with previous reports for carboxylated, fluorescently labelled particles of polystyrene, which demonstrated negligible toxic response.

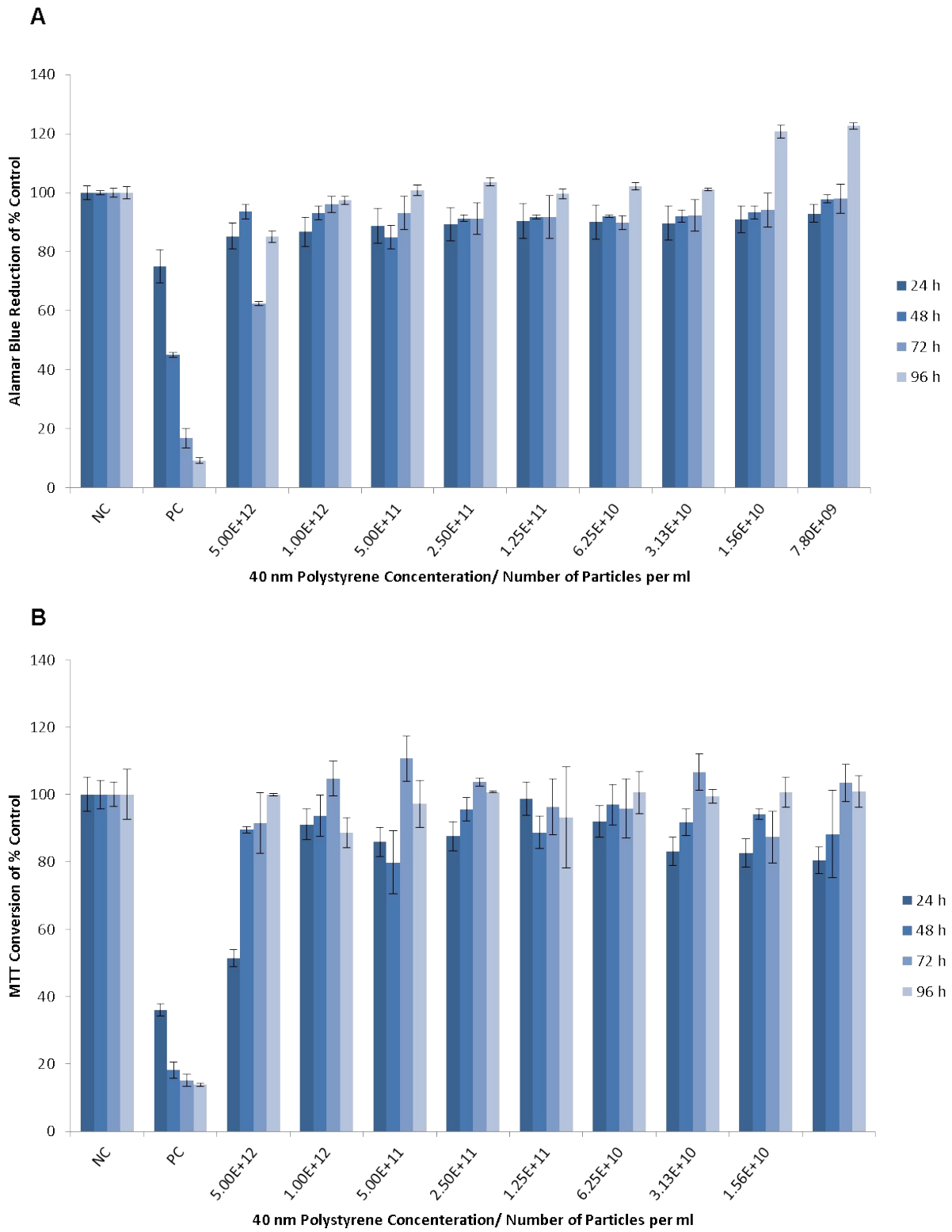

Figure S1 Cytotoxicity of 40 nm carboxlylated polystyrene nanoparticles after 24, 48, 72 and 96 hr exposures determined by the Alamar Blue (A) and MTT assay (B). Data are expressed

as % of control mean ± SD of three independent experiments. Negative control (NC): A549 cells; Positive control (PC): 10% Dimethyl sulfoxide (DMSO) 90 % media.

S2. Polystyrene Subtraction From Environment Cluster

In order to better differentiate clusters, the raw polystyrene spectrum was subtracted from the environment cluster by using CLS. Figure S2.1, Figure S2.2 and Figure S2.3 shows the scatter plots of PC and related loadings for 24 hr, 12 hr and 4 hr exposure data sets, respectively.

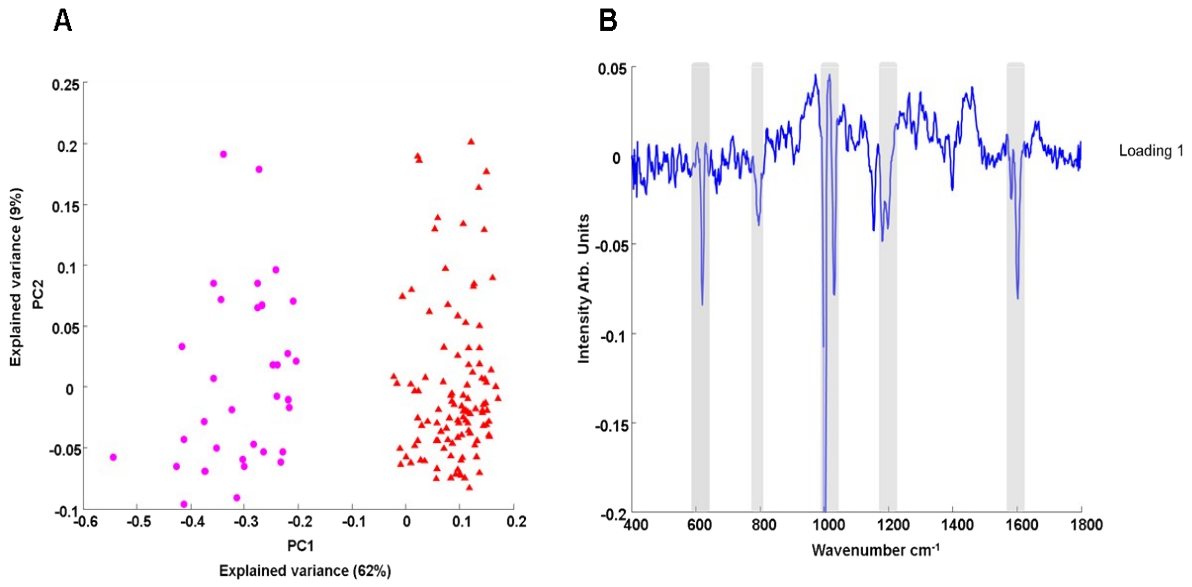

Figure S2.1 (A), Scatter plot of the PCA of spectra correspond to polystyrene nanoparticles and their environment after raw polystyrene spectrum subtracted for 24 hr particle exposure, same colours with K-means maps were used to identify scatter plots, (B) PC loading of polystyrene nanoparticles and polystyrene subtracted environment. The characteristic peaks of polystyrene were indicated with black circles in Loading 1.

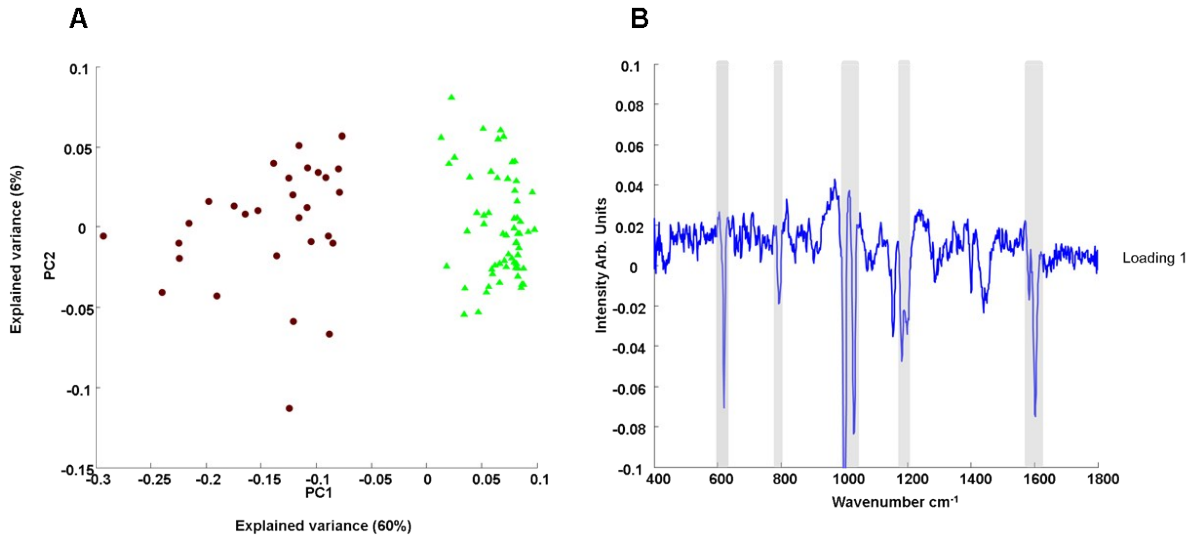

Figure S2.2 (A), Scatter plot of the PCA of spectra correspond to polystyrene nanoparticles and their environment after raw polystyrene spectrum subtracted for 12 hr particle exposure, same colours with K-means maps were used to identify scatter plots, (B) PC loading of polystyrene nanoparticles and polystyrene subtracted environment. The characteristic peaks of polystyrene were indicated with black circles in Loading 1.

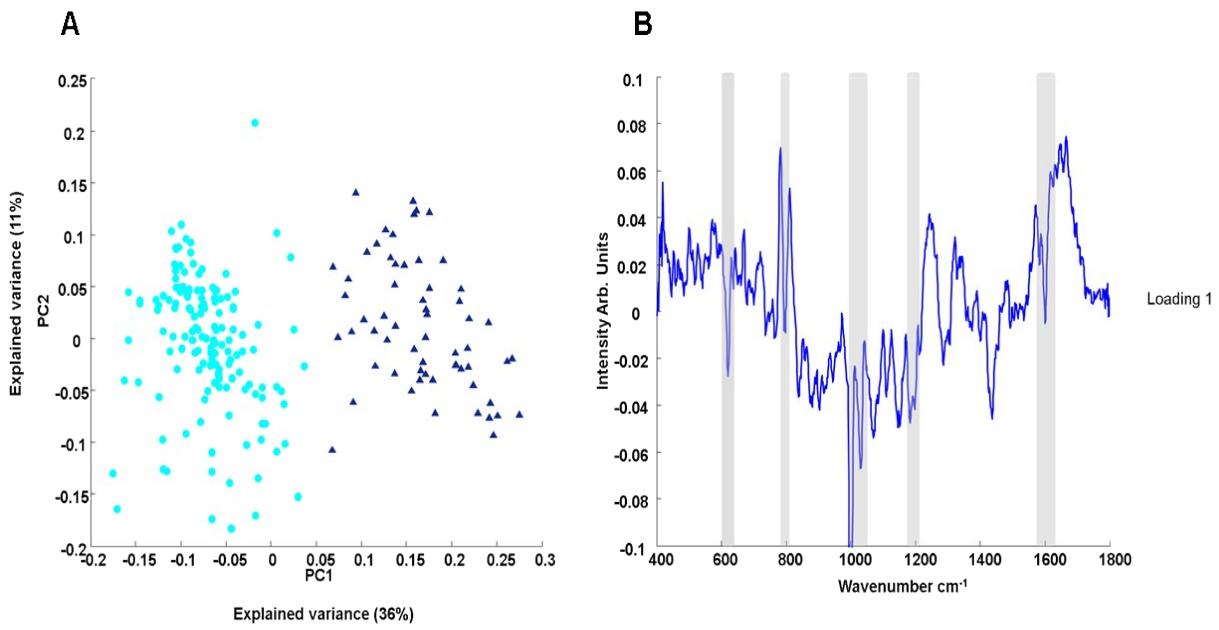

Figure S2.3 (A), Scatter plot of the PCA of spectra correspond to polystyrene nanoparticles and their environment after raw polystyrene spectrum subtracted for 4 hr particle exposure, same colours with K-means maps were used to identify scatter plots, (B) PC loading of polystyrene nanoparticles and polystyrene subtracted environment. The characteristic peaks of polystyrene were indicated with black circles in Loading 1.