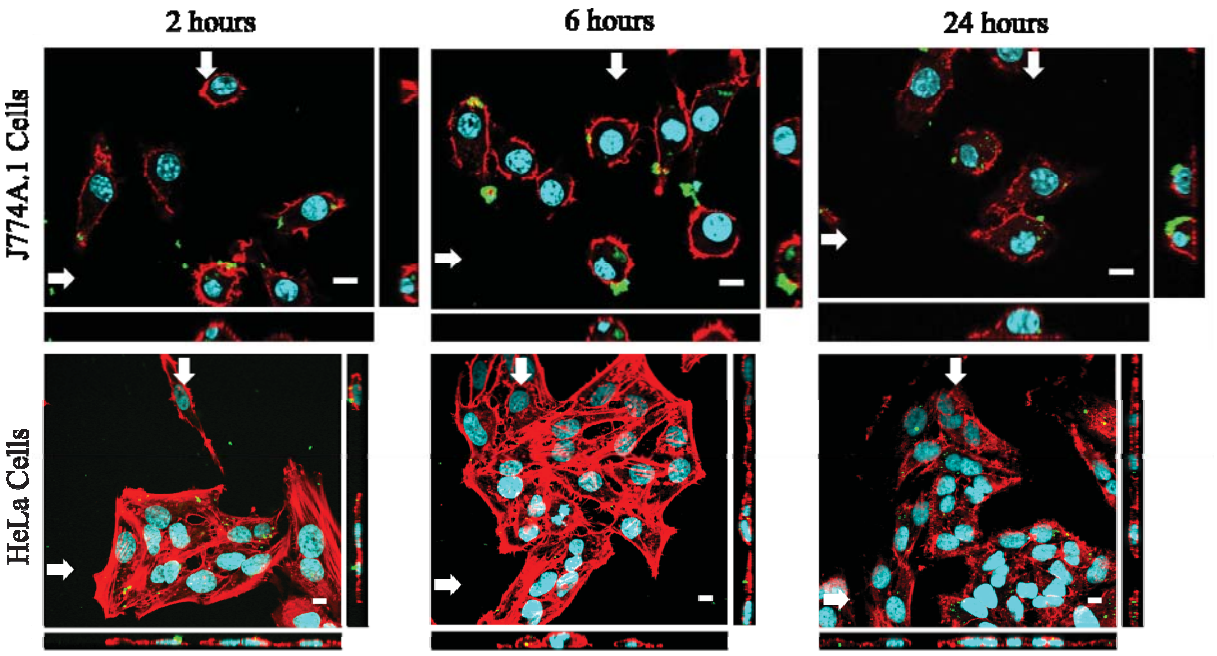
Supporting Information

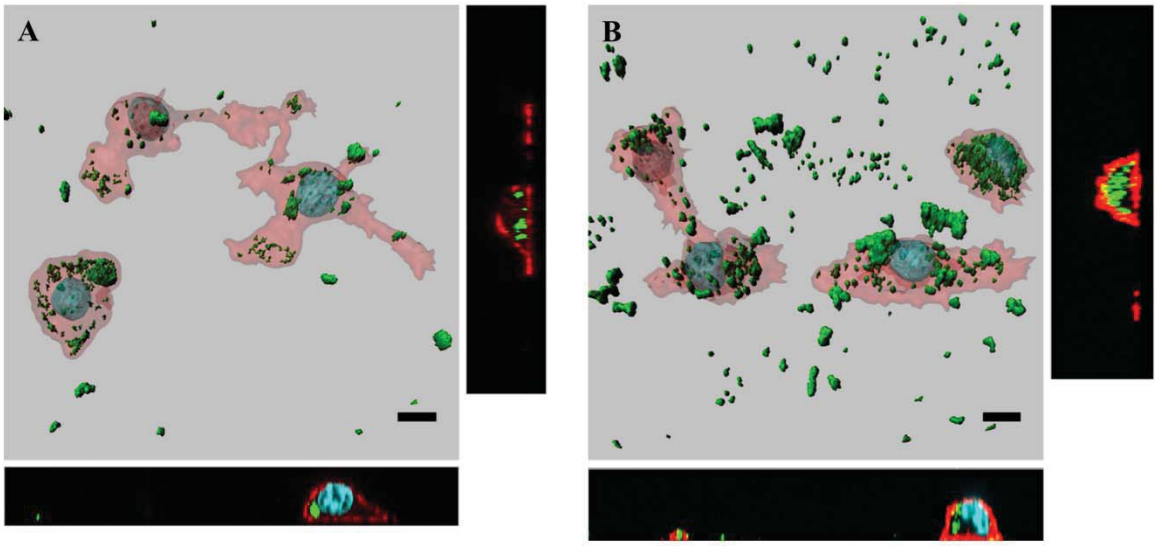
for *Part. Part. Sys. Charact.*, DOI: 10.1002/ppsc.201400152

Uptake and intracellular fate of peptide surface functionalized silica hybrid magnetic nanoparticles *in vitro*

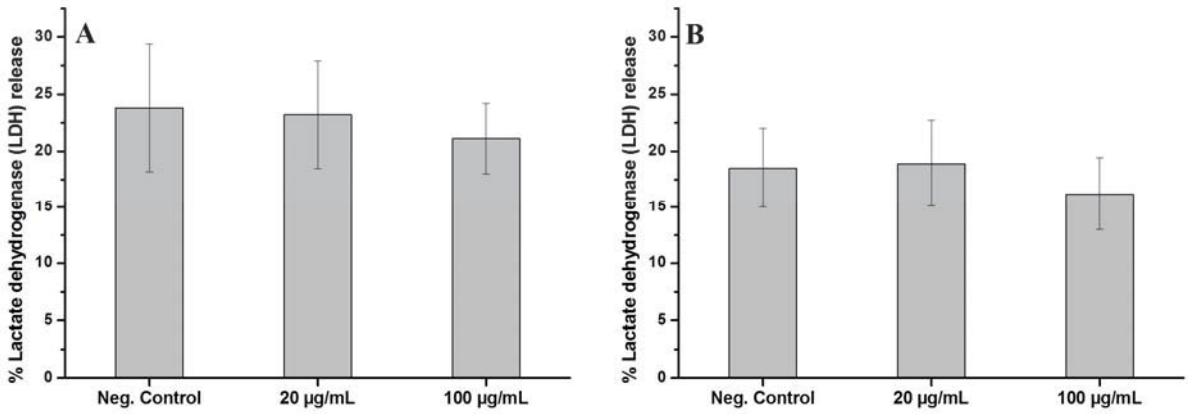
Reinaldo G. Digigow, Dimitri Vanhecke, Barbara Rothen-Rutishauser, Martin J. D. Clift and Alke Petri-Fink**



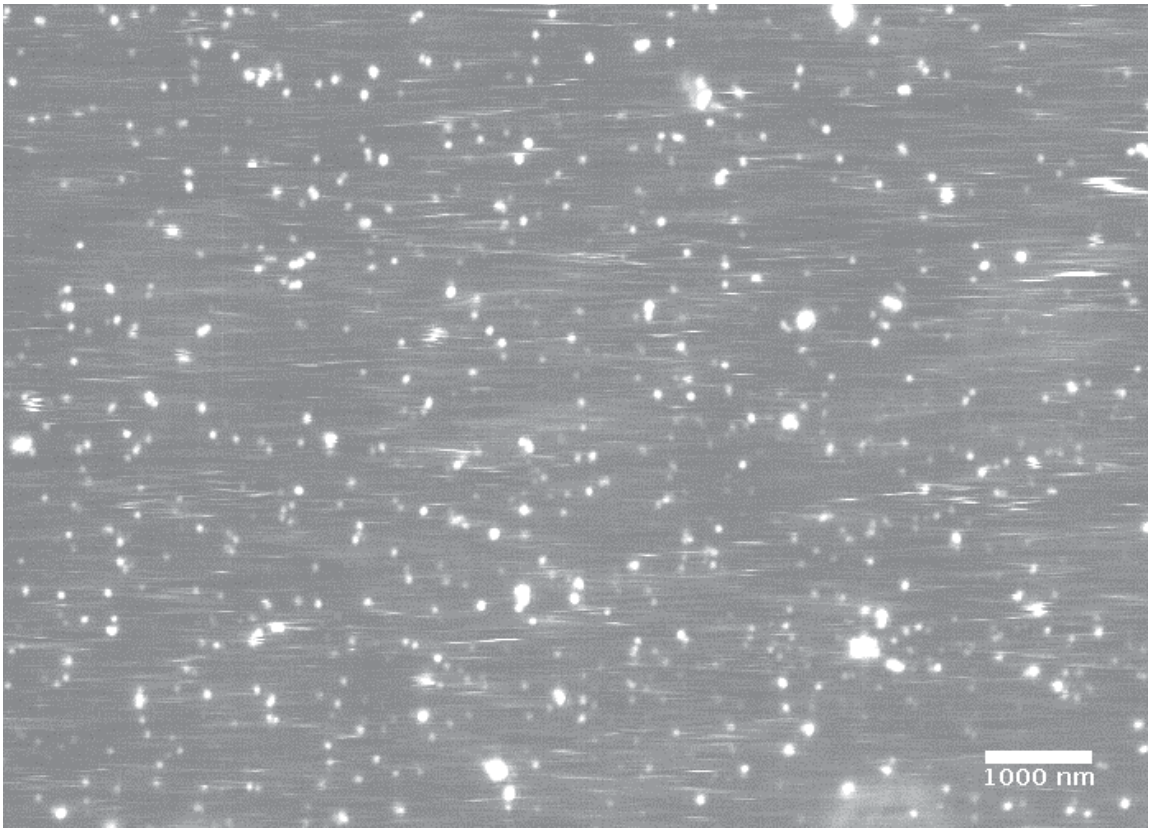
SI-Figure 1: Laser scanning confocal microscopy (LSM) images showing the internalization of silica coated iron oxide nanoparticles (SHMNPs) coupled with a nuclear targeting peptide sequence on their surface (SHMNPs-PEG-cRGD-NLS) in J774A.1 mouse 'macrophage-like' and HeLa cells after 2, 6, and 24 hours exposure to 20 µg/mL in an environment of 37 °C, 5% CO_2. The images show single optical xy-projections (pinhole at 1 Airy unit) with representative orthogonal side views in xz (bottom) and yz (right). The position of the orthogonal side views is denoted by the arrows in xy. Red colour corresponds to the F-actin stained with Phalloidin-rhodamine. Cyan colour indicates the nuclear region of cells (DAPI stain). Green fluorescence corresponds to the SHMNPs-PEG-cRGD-NLS (Alexa488 fluorophore present on the surface of the NPs). Scale bars represent 10 µm.



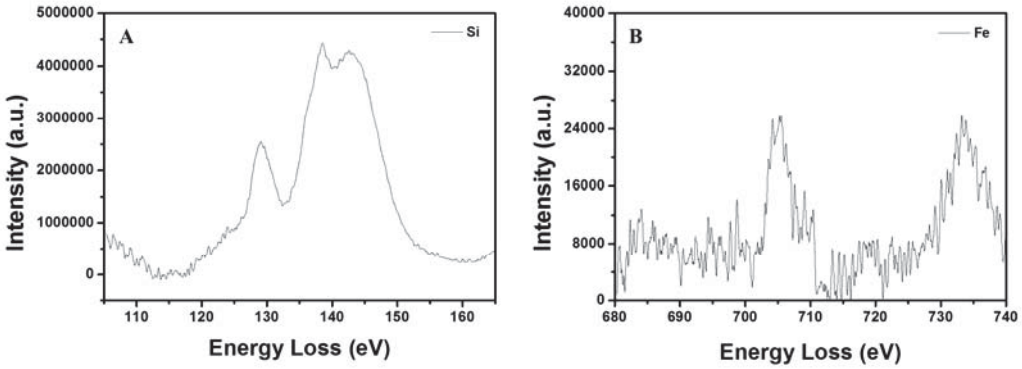
SI-Figure 2: Laser scanning confocal microscopy images (LSM) showing the internalization of silica coated iron oxide nanoparticles (SHMNPs) coupled with a nuclear targeting peptide sequence on their surface (SHMNPs-PEG-cRGD-NLS) in J774A.1 mouse 'macrophage-like' cells after 24 hours exposure at (**A**) 20 µg/mL and (**B**) 100 µg/mL in an environment of 37°C, 5% CO_2. Images show single optical xy-projections with representative side views in xz (bottom) and yz (right) direction with a 63x magnification. Red colour corresponds to the cell membrane (F-actin) stained with Phalloidin-rhodamine. Cyan colour indicates the nuclear region of cells (DAPI stain). Green fluorescence corresponds to the SHMNPs-PEG-cRGD-NLS (Alexa488 fluorophore present on the surface of the NPs). Scale bars represent 10 µm.



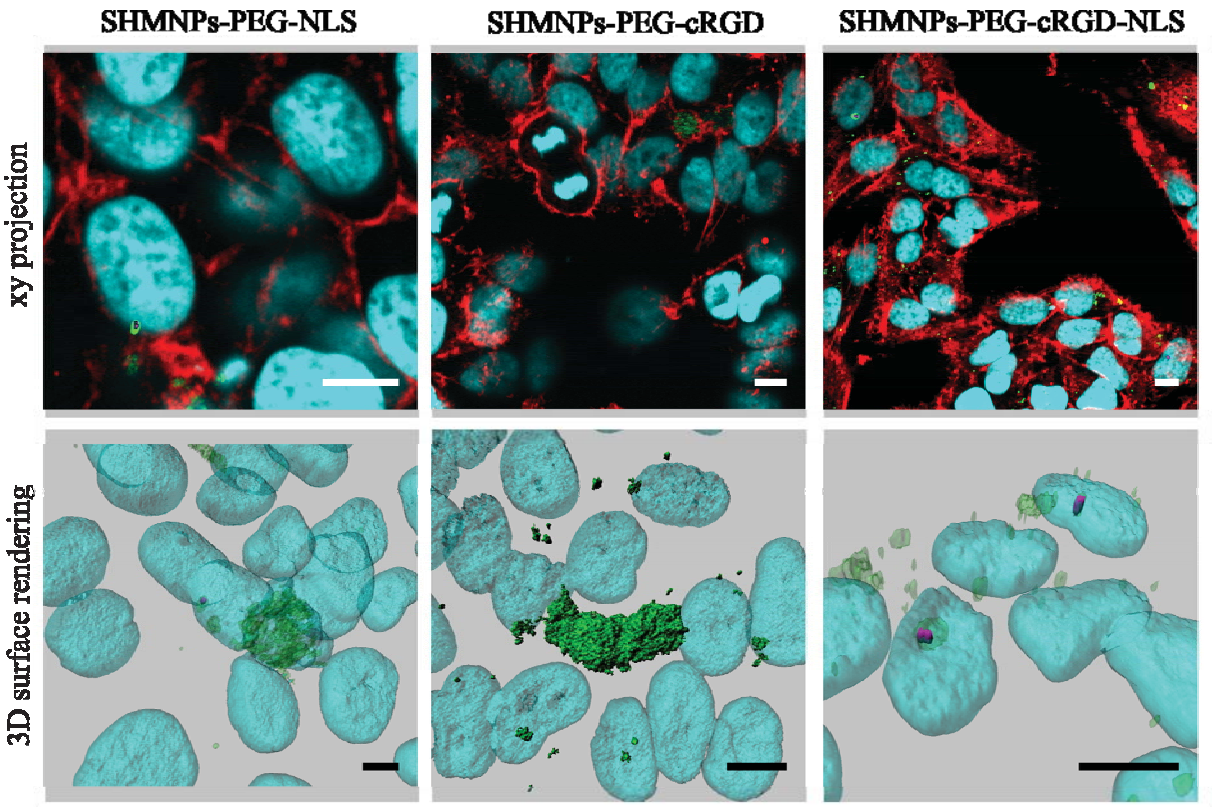
SI-Figure 3: Percentage lactate dehydrogenase (LDH) release from (**A**) J774A.1 macrophage and (**B**) HeLa cells after 24 hours exposure to SHMNPs-PEG-cRGD-NLS at 20 and 100 µg/mL. All data is relative to the positive control used (0.2% Triton X100). Specific cell culture media to each cell type was employed as the negative control. Data is presented as the mean ± standard error of the mean (SEM) (n=3 in triplicate).



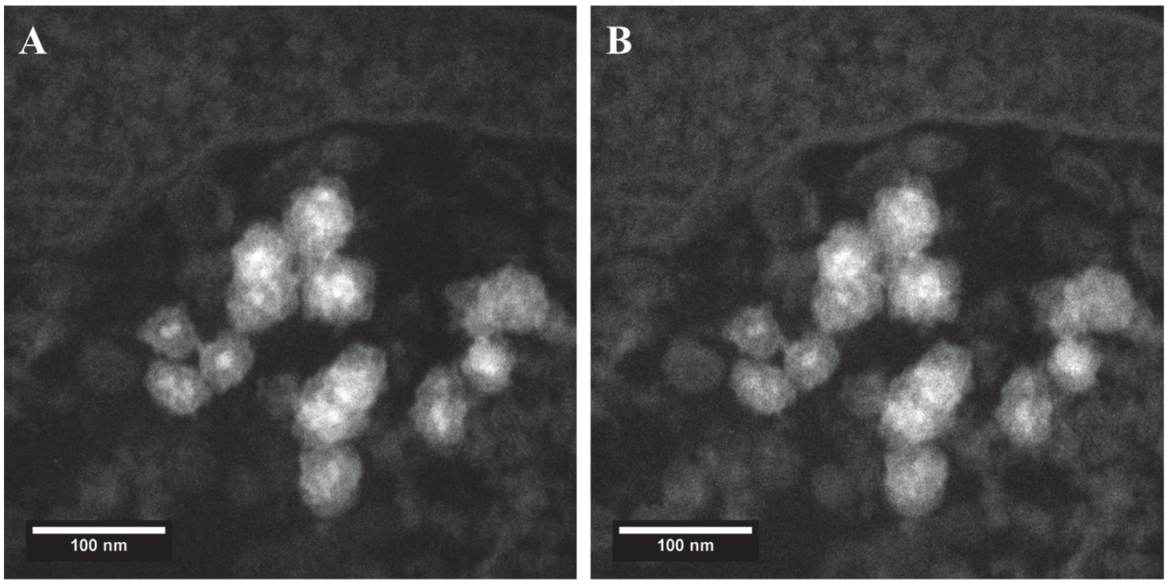
SI-Figure 4: Hyperspectral image of silica coated iron oxide nanoparticles (SHMNPs) in solution, recorded at 556 nm. For each pixel, the signal is recorded between 400 nm and 1000 nm with a 1 nm resolution. The resulting spectrum of randomly chosen SHMNPs is used to build a library representing a spectral fingerprint of the SHMNPs and subsequently used to detect the SHMNPs in Figure 3 (main text). The streaks in the background are due to SHMNPs that have not settled on the glass slide yet and are subject to Brownian motion. The exposure time for the entire cube was 625 seconds.



SI-Figure 5: Electron energy loss spectrum (EELS) of (**A**) Silicon (Si) and (**B**) Iron (Fe) from the field of view shown in Figure 5C (main text).



SI-Figure 6: Laser scanning confocal microscopy (LSM) images showing the internalization of silica coated iron oxide nanoparticles (SHMNPs) coupled with a nuclear targeting peptide sequence on their surface (SHMNPs-PEG-cRGD-NLS) in Hela cells after 24 hours exposure to either (**i**) SHMNPs-PEG-NLS, (**ii**) SHMNPs-PEG-cRGD or (**iii**) SHMNPs-PEG-cRGD-NLS at 20 µg/mL in an environment of 37 °C, 5% CO_2. In the xy-projection, the red colour corresponds to the cell membrane (F-actin) stained with Phalloidin-rhodamine. The cyan colour indicates the nuclear region of cells (DAPI stain). In the 3D surface rendering of the xy-projections, the green fluorescence corresponds to the SHMNPs-PEG-cRGD-NLS (Alexa488 fluorophore present in the NP complex). The cyan colour represents the cell nuclei, whilst the green the different peptide sequences. The purple colour evident in the 3D surface rendering images indicates an overlap between the cyan and green channels, indicative of NP association with the cell nuclei. Scale bars represent 10 µm.



SI-Figure 7: (**A**) The post-edge recording of the same field of view of Figure 5C (main text). (**B**) The first pre-edge recording of the same field of view of Figure 5C (main text).