Supporting Information

The Intracellular Destiny of the Protein Corona: A Study on its Cellular Internalization and Evolution

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Experimental Section

Dynamic light scattering

Dynamic Light Scattering (DLS) measurements were carried out at 25 °C using a Malvern Zetasizer Nano. Nanoparticle dispersions were placed in disposable cuvettes and loaded into the sample chamber. A 50 mW laser with a wavelength of 632.8 nm was used as the light source and the measurements were recorded at a detection angle of 173° (backscatter). An average of 3 measurements were recorded.

Transmission Electron Microscopy

TEM images of the nanoparticles were obtained on a FEI TECNAI Transmission Electron Microscope 120 kV instrument, operated at 100 kV with a beam current of about 65 mA. Samples for TEM were prepared by deposition and drying of a drop of the nanoparticle suspension in water onto a carbon-coated 300-mesh copper grid.

Artificial Lysosomal Fluid

In order to study the effect of the lysosomal environment on the protein corona of the nanoparticles accumulating in these organelles, an artificial lysosomal fluid was prepared following the method described by Michael Philip Beeston et al.[1] (main components: NaCl, NaOH, Citric acid, Glycine; pH 4.5).

MTS Assay

Cell viability was checked using the CellTiter 96 AQueous One Solution Reagent Kit and protocol (Promega). Cells were briefly seeded on a 96 well plate (15000 cells per well) and allowed to adhere for 24 hours prior to incubation with the nanoparticle dispersions at the desired concentration for 24 h. The medium was then discarded and cells were incubated with the CellTiter 96® AQueous One Solution Reagent according to the manufacturer´s instructions. The absorbance was recorded at 490 nm using a 96-well plate reader VarioSkan Flash (Thermo, USA). The interference of the nanoparticles with the assay was also tested to exclude eventual artefacts due to the nanoparticles.

Western Blotting

To detect the separated proteins, the gels were stained for 1 hour in Coomassie blue staining (50% methanol, 10% acetic acid, 2.5% (w/v) brilliant blue) and de-stained overnight in 50% methanol, 10% acetic acid. When higher sensitivity of detection was required, gels were stained using the silver staining Daiichi kit according to the protocol given by the supplier. Gels were scanned using a Biorad GS-800 calibrated densitometer scanner. For the western blotting procedure, after separation of the recovered proteins by SDS PAGE, the proteins were

transferred to a nitro cellulose membrane at 100 mV for 1 hour using a wet transfer method. Then, the membrane was incubated with a 2% BSA blocking solution in PBS TWEEN 0.01% for 1 hour at room temperature. The membrane was then incubated with the antibody of interest (LAMP-1, monoclonal, mouse, from Abcam) in blocking solution (1:4000) overnight at 4˚C. Finally, the membrane was washed 4-5 times with PBS TWEEN 0.01% and incubated with the secondary antibody (anti mouse HPR, Abcam) at a dilution of 1:8000 in blocking solution for 1 hour. The membrane was then washed with PBS TWEEN 0.01%, 4 times, incubated with the substrate solution for the chemiluminescent reaction (ECL PIERCE) for 1 minute, and finally developed on X-Ray film in a dark room.

Fluorescence quantification of nanoparticles/protein corona in isolated fractions

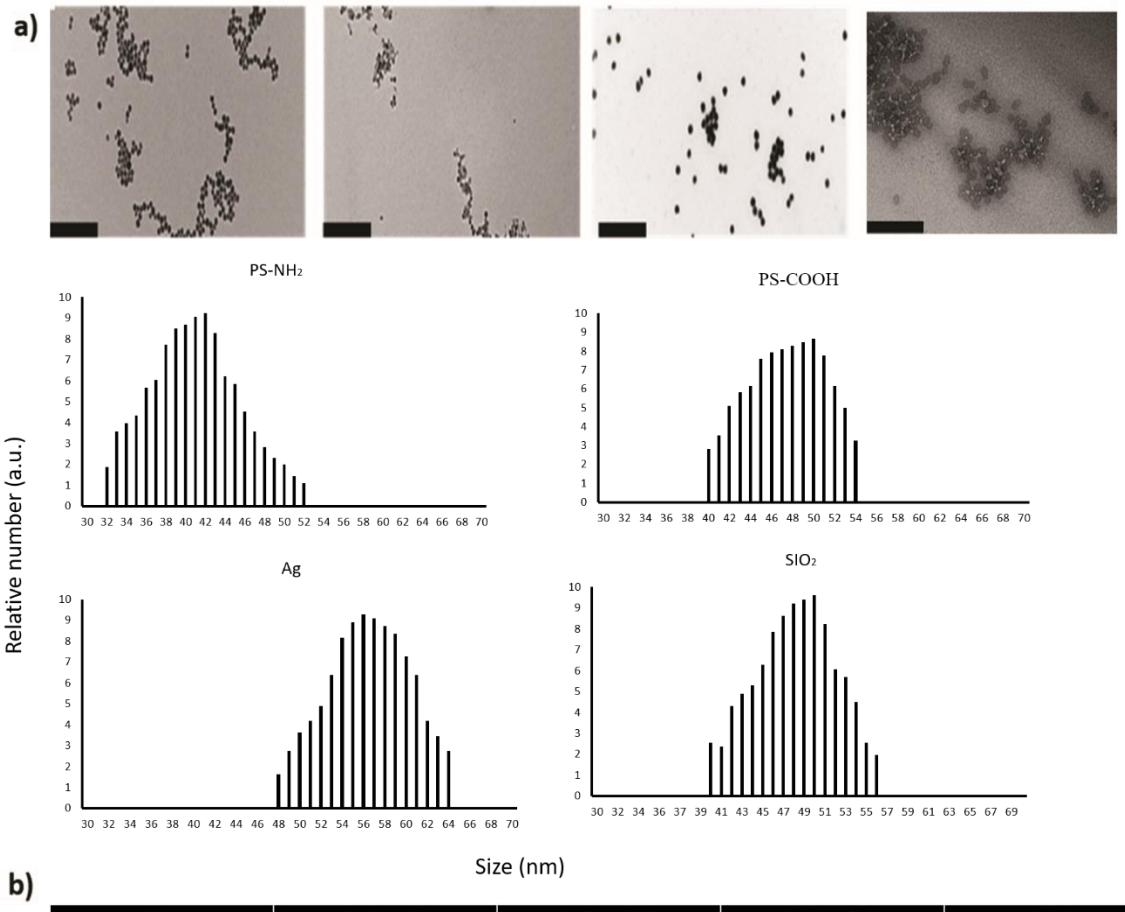
In order to quantify the amount of nanoparticles in different fractions, the recovered fractions (when possible) were suspended in 600 µl PBS and aliquoted in 96 well plates (Grenier). The fluorescence emissions (at 405 excitation and 450 nm emission for nanoparticles and 488 excitation and 520 nm emission for corona) were then recorded using a 96-well plate reader VarioSkan Flash (Thermo, USA).

Mass Spectrometry

In order to identify the recovered proteins by mass spectrometry analysis, after separation by SDS PAGE performed as described above, the bands of interest were excised from the gel and digested in-gel with trypsin (porcine trypsine, Promega), according to the method of Shevchenko et al.[2] The resulting peptide mixtures were resuspended in 0.1% formic acid and analysed by electrospray liquid chromatography mass spectrometry (LC MS/MS) using a HPLC (Surveyor, ThermoFinnigan, CA) interfaced with an LTQ Orbitrap (ThermoFinnigan, CA). Chromatography buffer solutions (Buffer A, 0.1% formic acid; Buffer B, 100% acetonitrile and 0.1% formic acid) were run using a 72 minute gradient. A 150 µl/min flow rate was used at the electrospray source. Spectra were analysed with Bioworks Browser 3.3.1 SP1

(ThermoFisher Scientific) using Sequest Uniprot/Swiss-Prot database (www.expasy.org). An exclusion filter was applied to reduce false positives, where peptides with P<0.001, and X correlation scores of 1.9, 2.5 and 3.2, for single, doubly and triply charged peptides, were retained.

Supplementary Data:

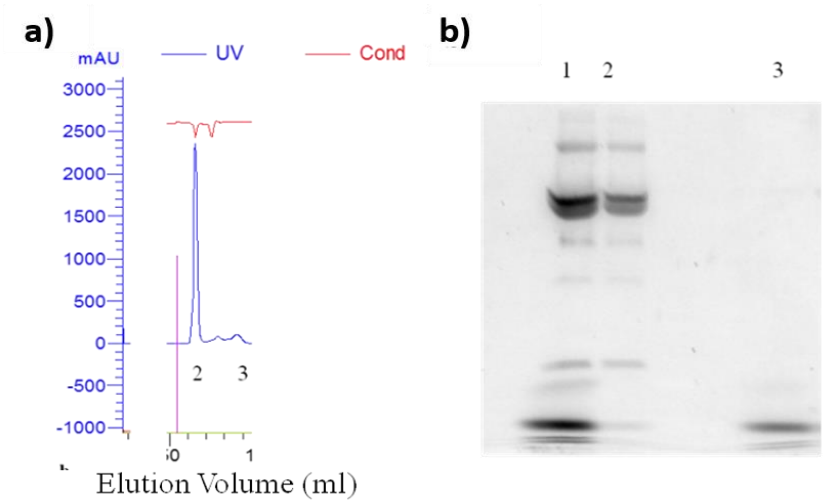


b)

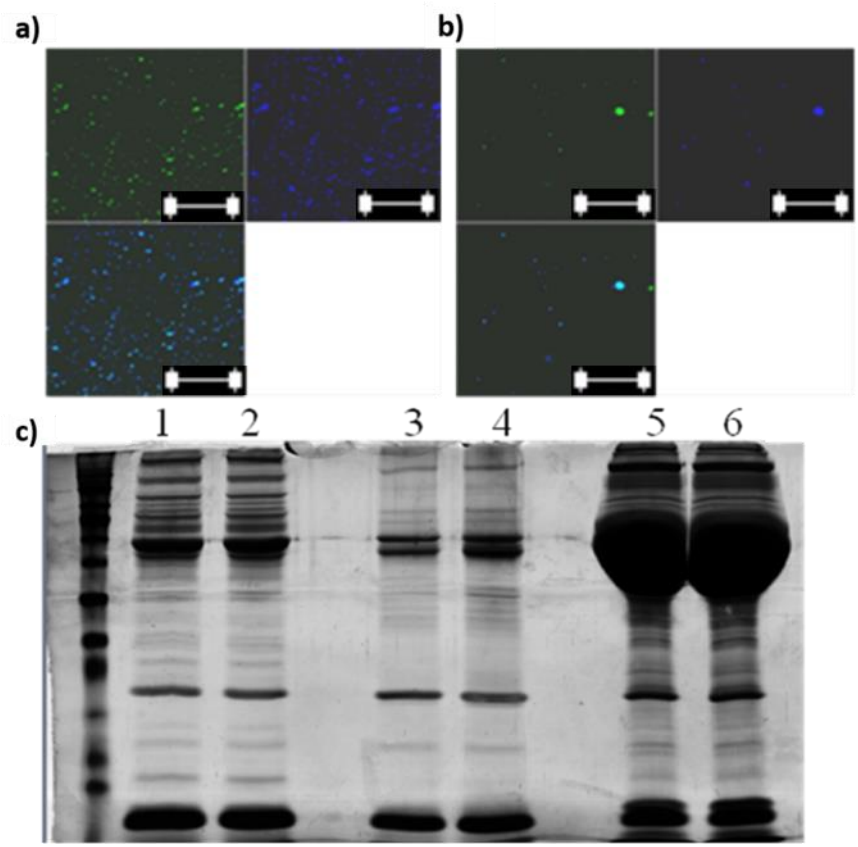
NPs	Z-Avg (I)	Z-Avg (N)	Z-Avg (V)	PDI
SiO_2	58.2 ± 0.4 nm	58.2 ± 0.4 nm	58.2 ± 0.4 nm	0.064 ± 0.01
PS-COOH	59.1 ± 0.1 nm	59.1 ± 0.1 nm	59.1 ± 0.1 nm	0.087 ± 0.01
$PS-NH_2$	54.7 ± 0.1 nm	54.7 ± 0.1 nm	54.7 ± 0.1 nm	0.12 ± 0.04
Ag	67 ± 0.1 nm	67 ± 0.1 nm	67 ± 0.1 nm	0.15 ± 0.06

Supplementary Figure S1: Nanoparticle characterization. **a:** TEM images of the nanoparticles utilized in the study (from left to right, $PS-NH_2$, PS-COOH, Ag and SiO_2 nanoparticles, scale bar, 500 nm). Histogram representation (below) with size distribution of the nanoparticles as by TEM analysis of 100

nanoparticles **b:** Nanoparticles were diluted in water to a final concentration of 100 µg/ml and characterized by DLS. Z-Average is the hydrodynamic diameter of the nanoparticles extracted by cumulated analysis of the data obtained by Dynamic Light Scattering (respectively for intensity (I), number (N) and volume (V), the uniformity of the measures suggests that the nanoparticles are well dispersed); PDI indicates the polydispersity index of the suspensions. Results show that the labelled hard nanoparticles are well dispersed and with a comparable size of around 50 nm.

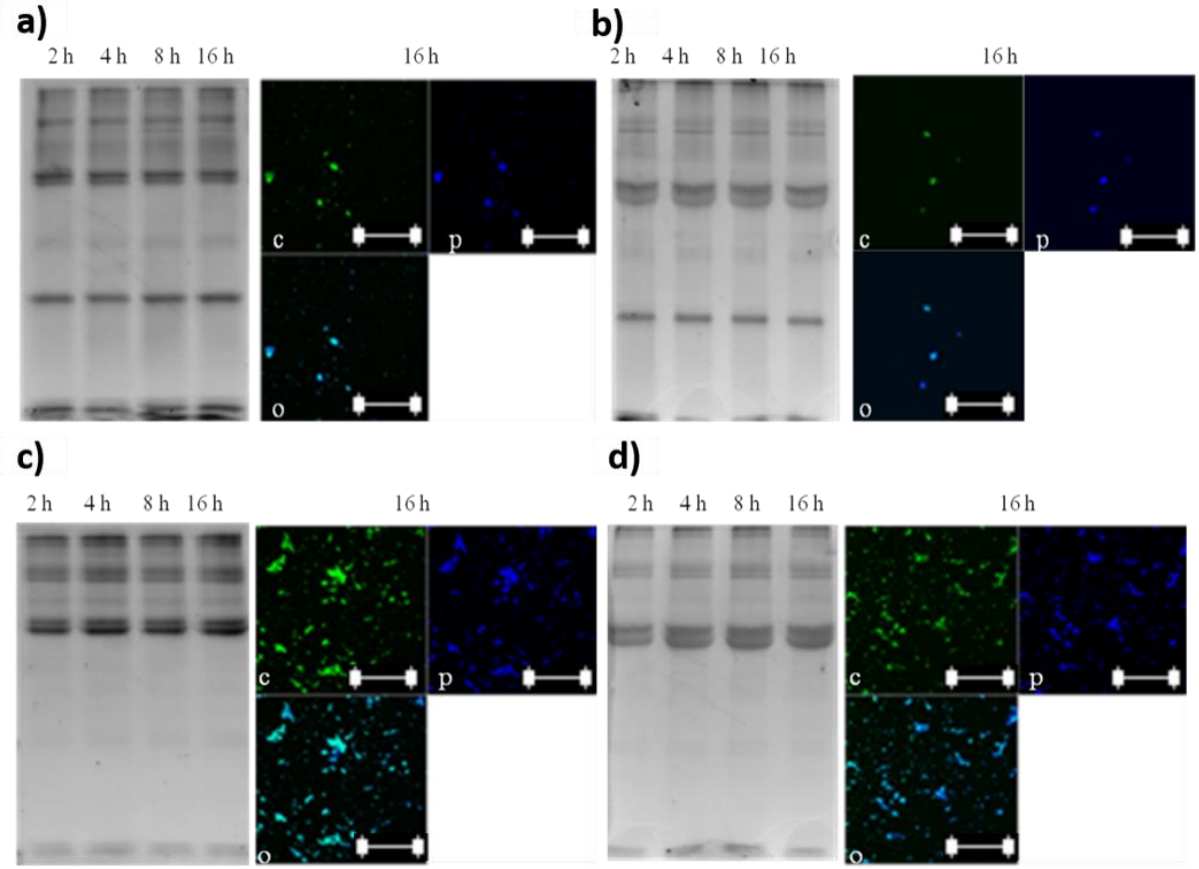


Supplementary Figure S2: Cleaning of the labelled serum. The unreacted free dye was removed from the serum with a G25 column using a FPLC apparatus. **a:** elution of the serum through the column showing the peak for the serum proteins (2) and the residual free dye (3). **b:** The collected samples were then run on a gel together with uncleaned serum (1) and the fluorescent proteins and dye were imaged. The results show that the free dye is removed from the serum fraction (2) and elutes differently from the proteins (3).



Supplementary Figure S3: Characterization of the fluorescently labelled protein corona-nanoparticle complexes. **a-b:** confocal images of labelled corona nanoparticle complexes (carboxyl- (a) and amino- (b) modified polystyrene nanoparticles). The labelled proteins always colocalize with the nanoparticles (in blue: nanoparticles and in green: corona), confirming that labelled hard corona complexes were formed. **c:** silver staining image of 1D SDS PAGE comparing the corona formed on carboxyl modified nanoparticles in unlabelled serum´(1) with the corona formed in labelled serum (2). The labelling procedure did not affect

the corona composition. The same results were obtained for amino-modified nanoparticles (3 and 4) and for the serum itself (5: unlabelled and 6: labelled).

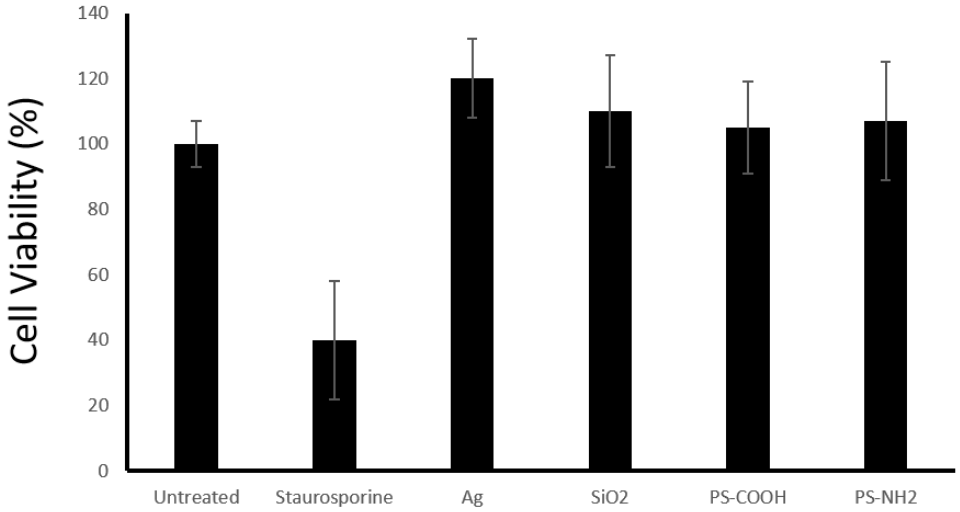


Supplementary Figure S4: Extracellular evolution of labelled protein corona-nanoparticle complexes. Labelled corona nanoparticle complexes were incubated for different times (2h, 4h, 8h and 16h) in extracellular media with unlabelled serum (cMEM, a and c) and in artificial lysosomal fluid (Alf, b and d). 1D SDS PAGE of the proteins recovered at different times, and confocal imaging (after 16 hours) were used to monitor eventual exchange of proteins from the original corona. **a-b:** corona formed on amino-modified nanoparticles. **c-d:** corona formed on carboxyl-modified polystyrene. Confocal imaging shows that the fluorescence of the proteins is still colocalizing with the nanoparticles (in blue: nanoparticles and in green: corona proteins) after 16 hours in unlabelled serum. SDS PAGE gels show that the pattern of the fluorescent

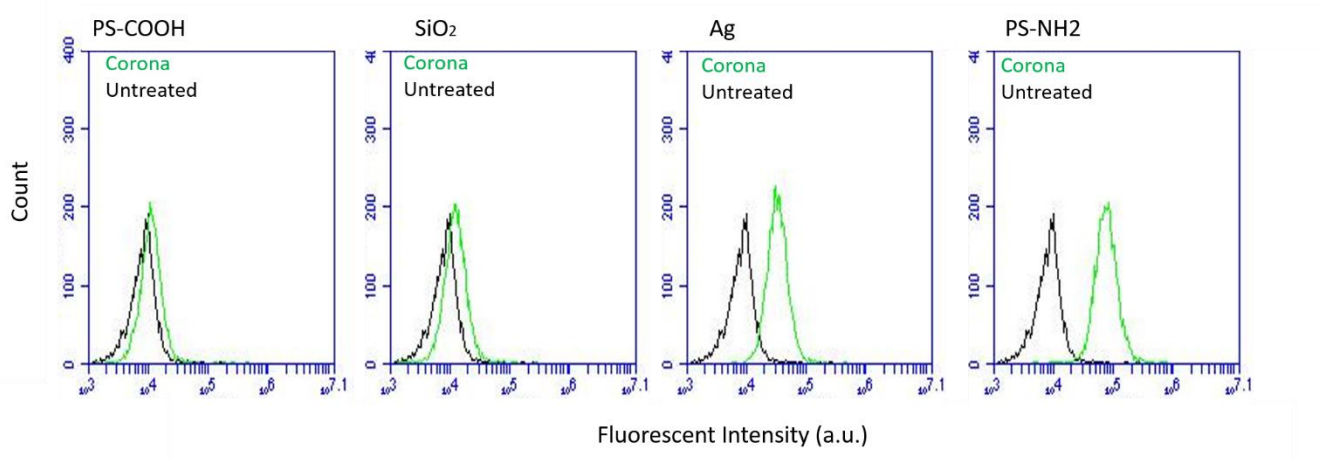
hard corona proteins is still the same over the 16 hours of incubation of the labelled corona complexes in unlabelled serum or Alf.

NPs	Z-Avg (I)	Z-Avg (N)	Z-Avg (V)	PDI	Z-Pot.
PS-COOH	76 ± 0.1 nm	76 ± 0.1 nm	76 ± 0.1 nm	0.15 ± 0.05	- 8.9 ± 1.1 mV
PS-NH_2	67 ± 0.1 nm	67 ± 0.1 nm	67 ± 0.1 nm	0.17 ± 0.05	-9.4 ± 0.6 mV
Ag	88 ± 0.1 nm	88 ± 0.1 nm	88 ± 0.1 nm	0.22 ± 0.07	-8.5 ± 0.8 mV
SiO_2	72 ± 0.5 nm	72 ± 0.5 nm	72 ± 0.5 nm	0.17 ± 0.02	-9.6 ± 1.4 mV

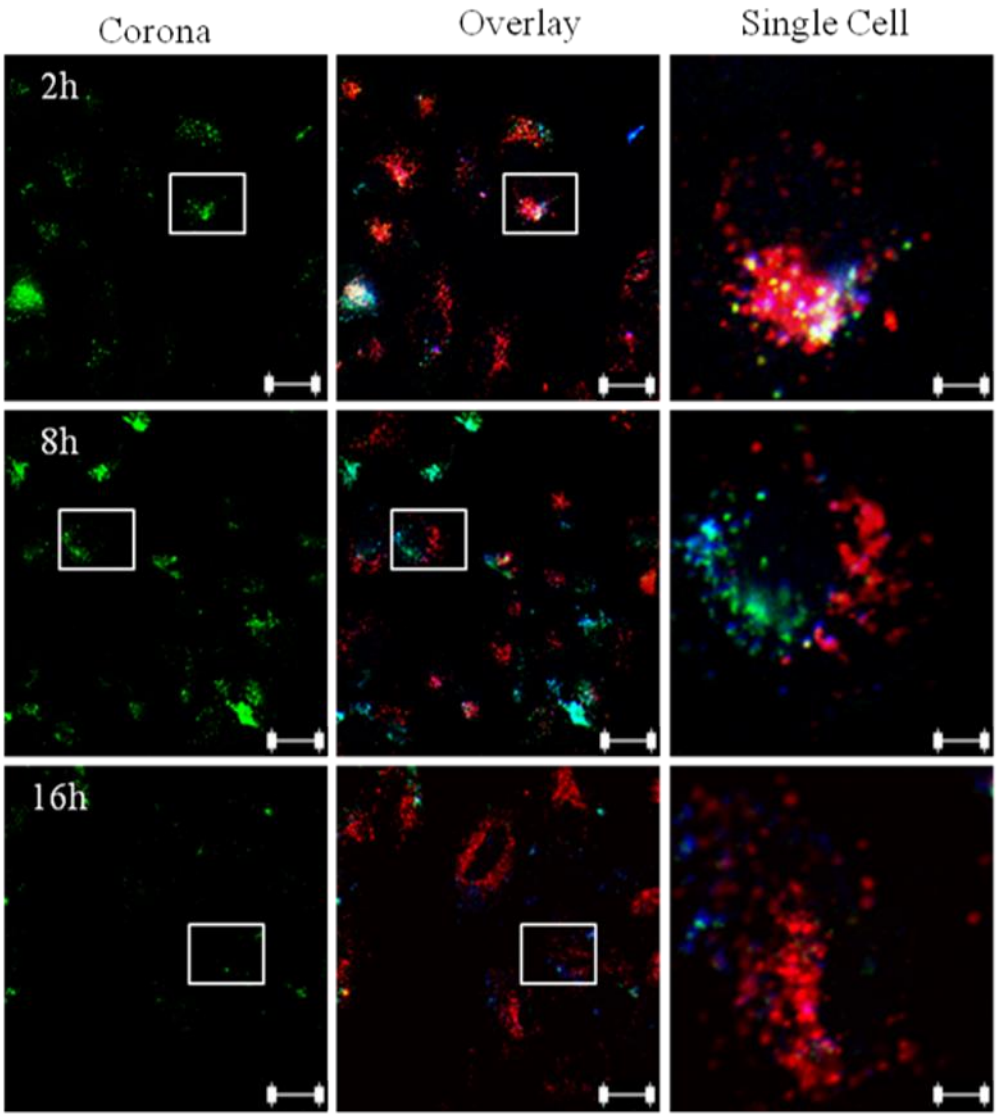
Supplementary Figure S5: Nanoparticle-corona dispersions. Nanoparticle-corona complexes were prepared as detailed in the methods section, then were diluted in unlabelled complete cell culture medium to a final concentration of 100 µg/ml and characterized by DLS. Z-Average is the hydrodynamic diameter of the nanoparticles extracted by cumulated analysis of the data obtained by Dynamic Light Scattering (respectively for intensity (I), number (N) and volume (V); Z potential indicated the zeta potential of the nanoparticles' dispersions. PDI indicates the polydispersity index of the suspensions. Results show that after isolation and resuspension in cMEM, the labelled hard corona-nanoparticle complexes are well dispersed. The values obtained by zeta potential could suggest that the surface of the nanoparticles is covered by proteins, however a contribution from the free proteins in solutions cannot be excluded.



Supplementary Figure S6: Toxicity evaluation of nanoparticle-corona complexes: A549 cells were incubated with 100 µg/ml of nanoparticle-corona complexes (p-: carboxyl-modified, p+: amino-modified) for 2 hours followed by growth in nanoparticle-free medium for 16 hours (see details in the method section), then their viability was measured with an MTS assay and compared with untreated cells (c-, negative control) and with cells treated with staurosporine (c+, positive control for cell death) (see methods section for details). The results showed that none of the concentrations of nanoparticles used induced a decrease in the cell viability**.**

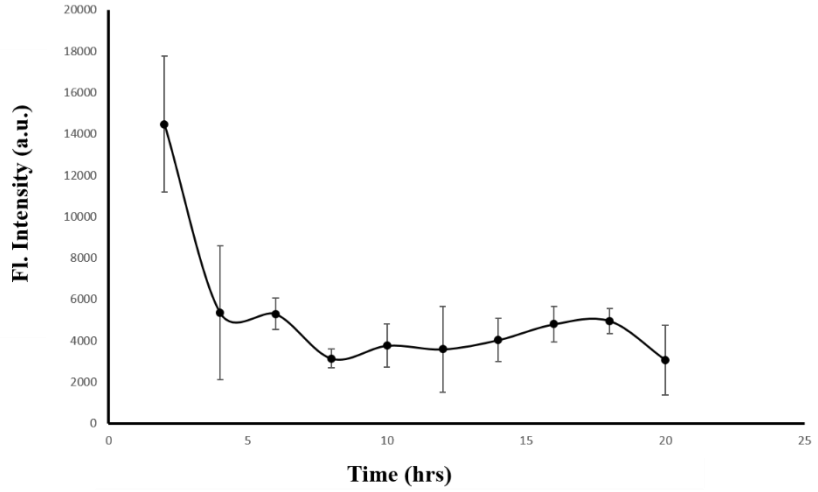


Supplementary Figure S7: Raw flow cytometry distributions indicating the uptake of fluorescent corona from different nanoparticles as seen in Figure 1 c. In green, samples treated with fluorescent corona nanoparticle complexes, in black untreated cells, as a reference.

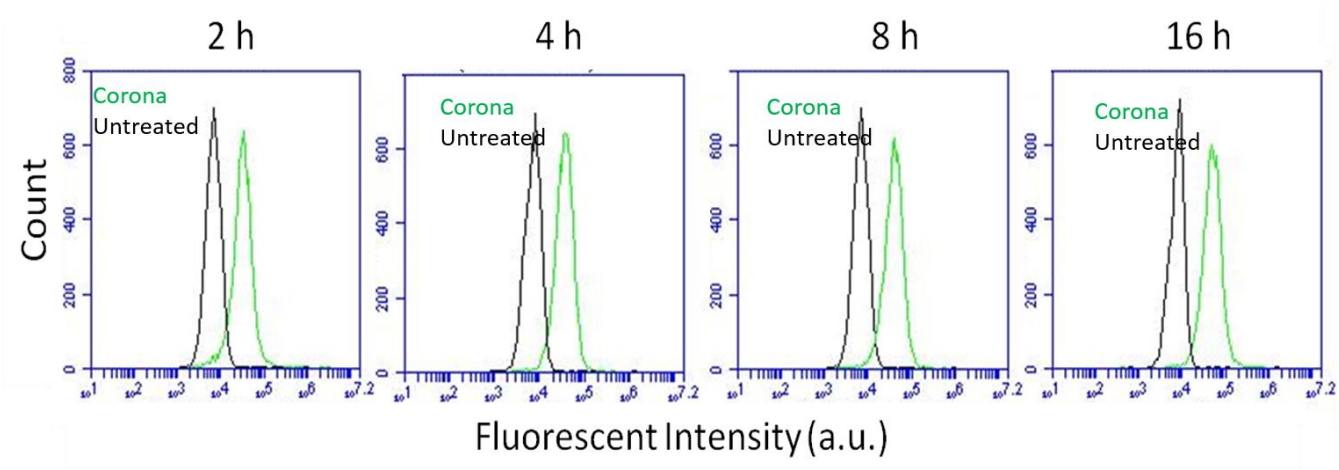


Supplementary Figure S8: Intracellular destiny of the protein corona associated with amino-modified polystyrene: A549 cells were exposed to 100 µg/ml labelled corona amino-modified nanoparticle complexes for 2 hours in unlabelled complete medium; the medium was then substituted with nanoparticle-free unlabelled medium and cells were allowed to grow for different times (2, 4, 8, and 16 hours) before high content analysis, confocal imaging and

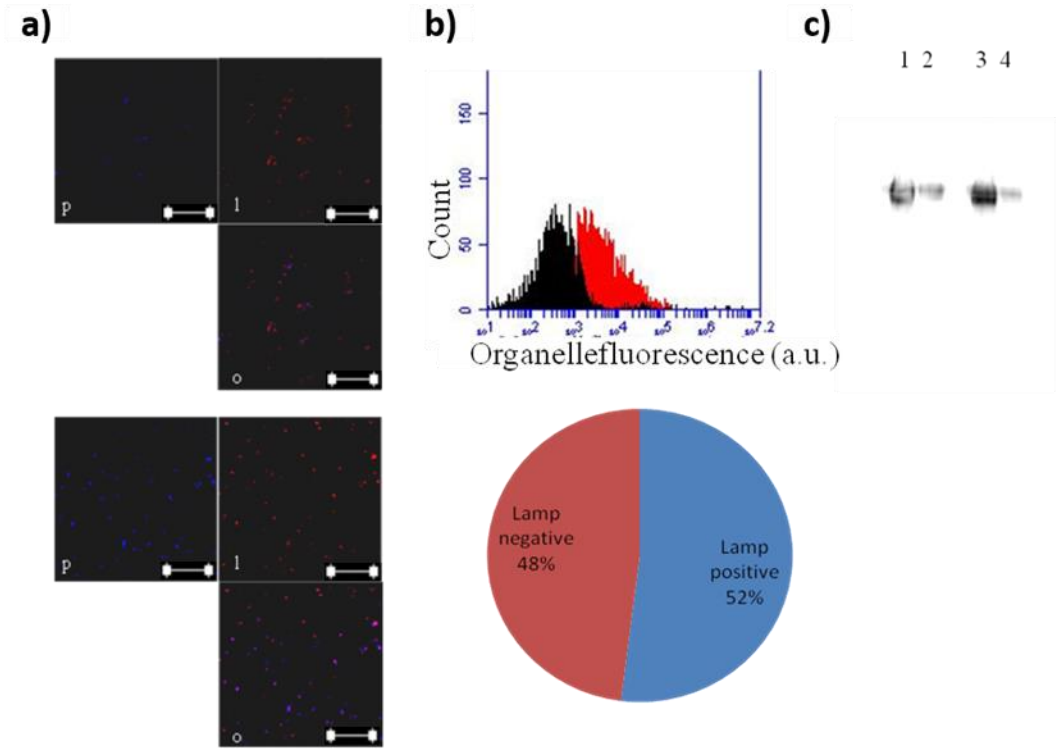
organelle isolation. Confocal images of A549 cells after exposure to nanoparticles as explained above (from the left to right: green corona channel; overlay of green corona, blue nanoparticles and red (LAMP-1 stained) lysosomal channels; magnification of a single cell, indicated in the previous figures by a white rectangle). The results show that labelled corona proteins enter the cells and reach the lysosomes, always colocalising with the nanoparticles. After 8 hours, fluorescent corona proteins on the nanoparticles decrease. At later times (16 hours) the majority of nanoparticles are inside the lysosomes and the fluorescent signal associated with the corona has almost vanished.



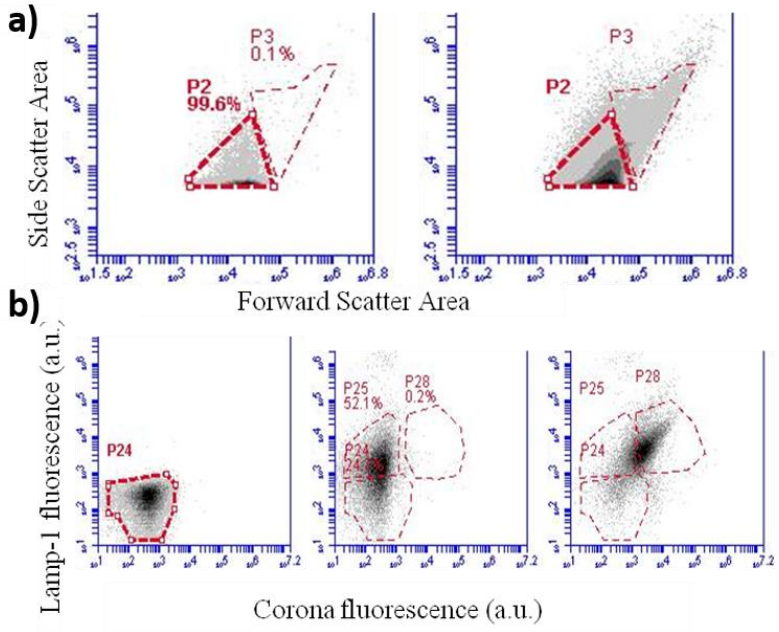
Supplementary Figure S9: Uptake and evolution of fluorescent corona on THP-1 cells. Briefly THP-1 cells were treated as described in Methods and the mean fluorescence intensity distributions of cells at different times after nanoparticle removal was obtained by live cell high content analysis. After 2 hours, fluorescence signal from the labelled corona proteins is detected and later decreases over time. However, some fluorescence signal is still measured even after 16 hours, suggesting that the labelled proteins (or proteins fragments) are still localised inside the cell. It is possible to note, however, that the kinetics of the process are vastly different than the ones obtained in A549 cells.



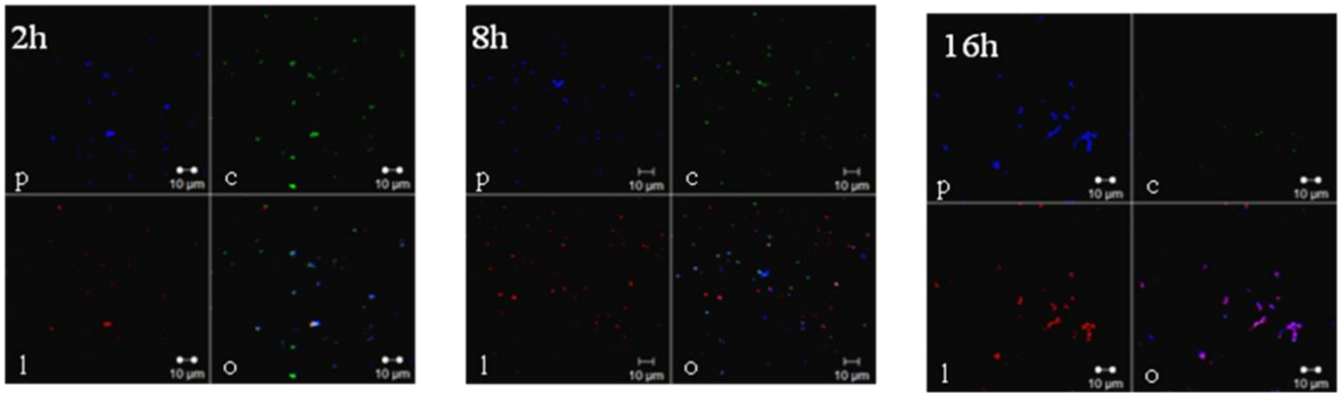
Supplementary Figure S10: Raw flow cytometry distributions indicating the uptake of fluorescent corona at different chase times as seen in Figure 2 d. In green, samples treated with fluorescent corona nanoparticle complexes, in black untreated cells, as a reference.



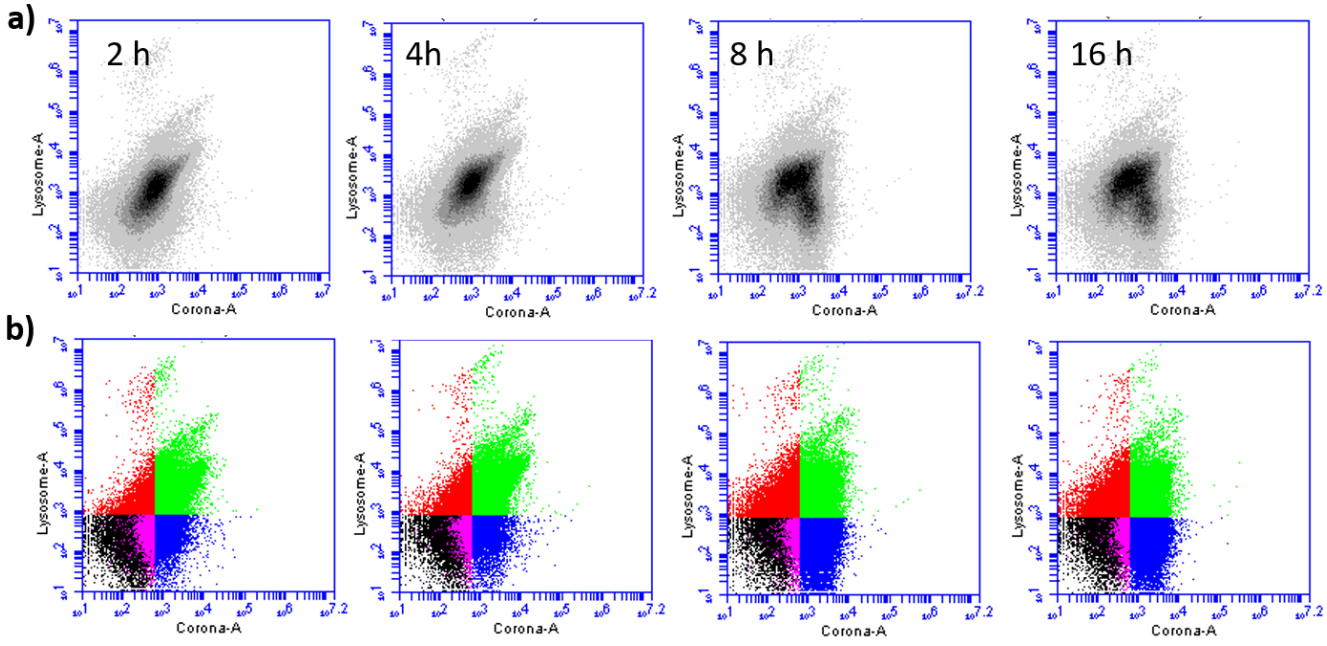
Supplementary Figure S11: Characterization of the CLF fraction: the enrichment in lysosomes in the crude lysosome fraction (CLF) was tested with three different techniques. **a:** confocal images of CLF obtained from cells treated with amino modified (top) and carboxyl modified (bottom) polystyrene nanoparticles (shown in blue, indicated by the letter p) show the presence of red LAMP-1 positive bodies (l) and that the majority of the nanoparticles colocalize with the lysosomes (the overlapped image, o, is also shown). **b:** flow cytometry fluorescence distributions of untreated (black) and LAMP-1 stained (red) CLF confirms the presence of lysosomes (red distribution). Quantification of the LAMP-1 positive population (bottom graph) shows that roughly the 52% of the analysed bodies are lysosomes. **c:** western blot of LAMP-1 in the CLF recovered from cells treated with the two nanoparticles (1: amino-modified and 3: carboxyl-modified) and the corresponding post nuclear supernatant (2 and 4, i.e. supernatant collected after pelleting the nuclei, see Methods for details) confirms an enrichment in lysosomes in the CLF.



Supplementary Figure S12: Gating procedure used for the flow cytometry analysis of lysosomes. **a:** First CLF fractions were analysed against the background given by PBS and a population of organelles was gated (P3, a). **b:** The Lamp-1 positive organelles were gated (P25, b) and the fluorescence of the corona was analysed (P28, b third panel).

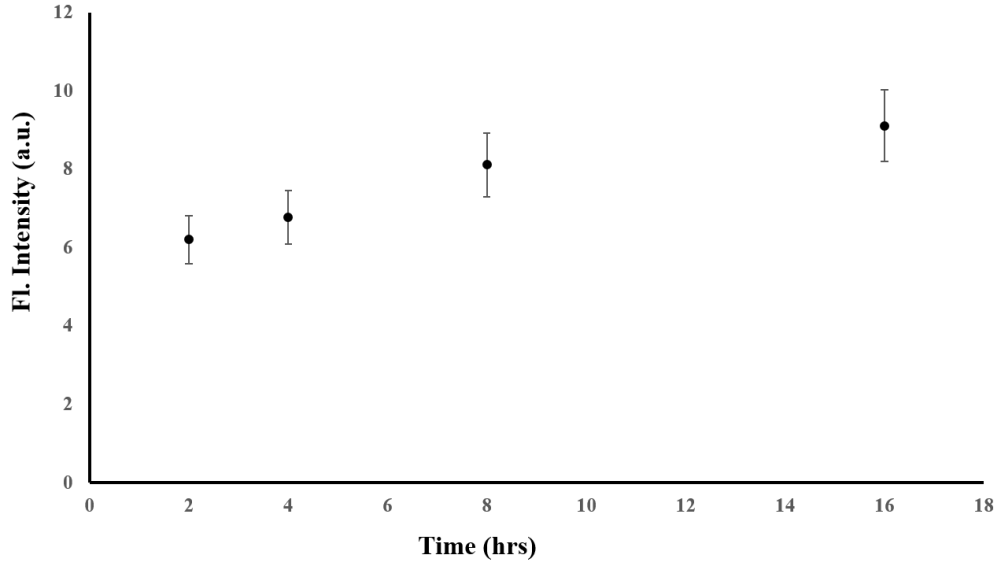


Supplementary Figure S13: Characterization of the intracellular corona in the isolated CLF by confocal microscopy. Confocal images of the labelled corona proteins (c, green) associated with the nanoparticles (p, blue) inside LAMP-1 stained lysosomes (l, red) isolated as described in the methods section. The overlapped image is also shown (o).

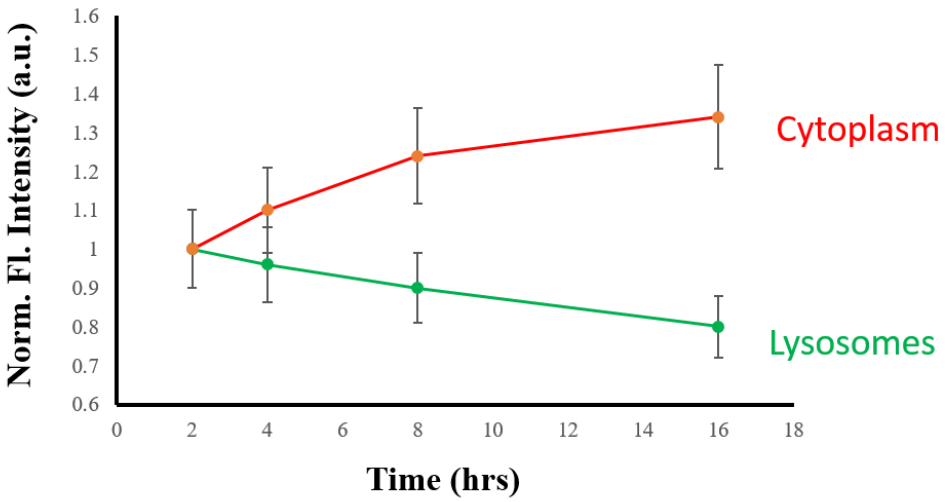


Supplementary Figure S14: Raw flow cytometry distributions of the plots analysed in Main Figure 3. Briefly one hundred thousand lysosomes were analysed as described in the methods section. **a:** Distributions as obtained by flow cytometry of isolated lysosomes at 2, 4 , 8 and 16 hours chase time after nanoparticle removal. It is possible to observe that the total green

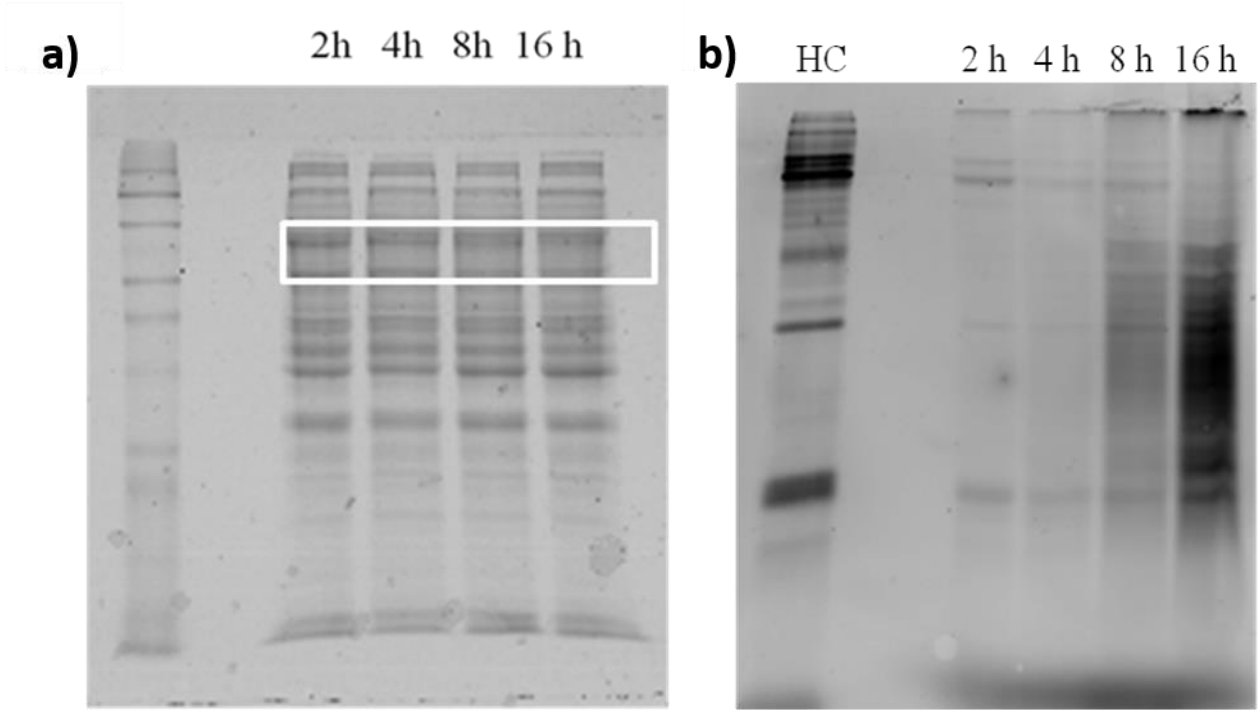
fluorescence associated with the corona proteins (x axis, as opposed to the lysosomal fluorescence, y axis) decreases slightly with time. **b:** The distributions presented can be color coded using the gating previously shown in Figure S9. The empty lysosomes are presented in red, the corona proteins inside the lysosomes are presented in green and the non-lysosomal population of nanoparticles are presented in blue.



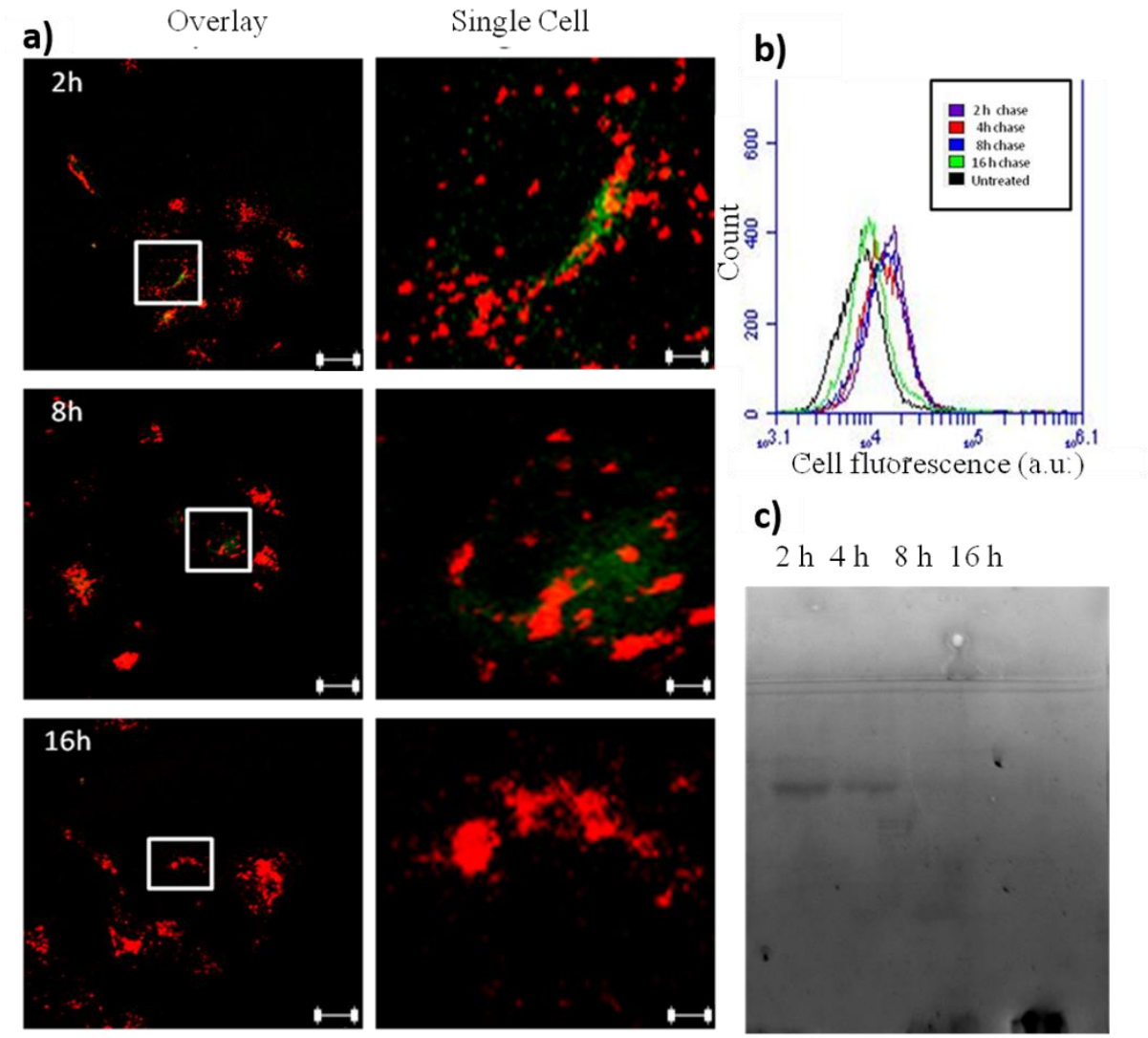
Supplementary Figure S15: Quantification of nanoparticles in the CLF after different chase times. The CLF obtained as described in the methods section, after 2 hours pulse and different chase times (2, 4, 8 and 16 hours) of PS-NH2 fluorescent corona complexes were dispersed in PBS after normalization for protein concentration by BSA (as described in the methods section) and the fluorescence relative to the nanoparticles was acquired to evaluate the amount of nanoparticles inside the lysosomes (see Supplementary Method). The results illustrate how more nanoparticles reach the lysosomes as a function of chase time.



Supplementary Figure S16: Quantification of corona in the CLF and in the lighter fraction after different chase times. The CLF obtained as described in the methods section after 2 hours pulse and different chase times (2, 4, 8 and 16 hours) of PS-NH2 fluorescent corona complexes was dispersed in PBS after normalization for protein concentration by BSA (as described in in the methods section) and the fluorescence relative to the corona was acquired to evaluate the amount of corona inside the lysosomes (see Supplementary Method). The fluorescent intensities were then normalized against the total intensity for the nanoparticles inside each fraction (see Supplementary Figure S14) and the same procedure was then applied to the lighter fraction (here indicated as cytoplasm, in red). The results illustrate how, as a function of time, less signal is recovered from the lysosomes and more signal is recovered from the cytoplasm, after normalization, confirming the findings in Figure 2.

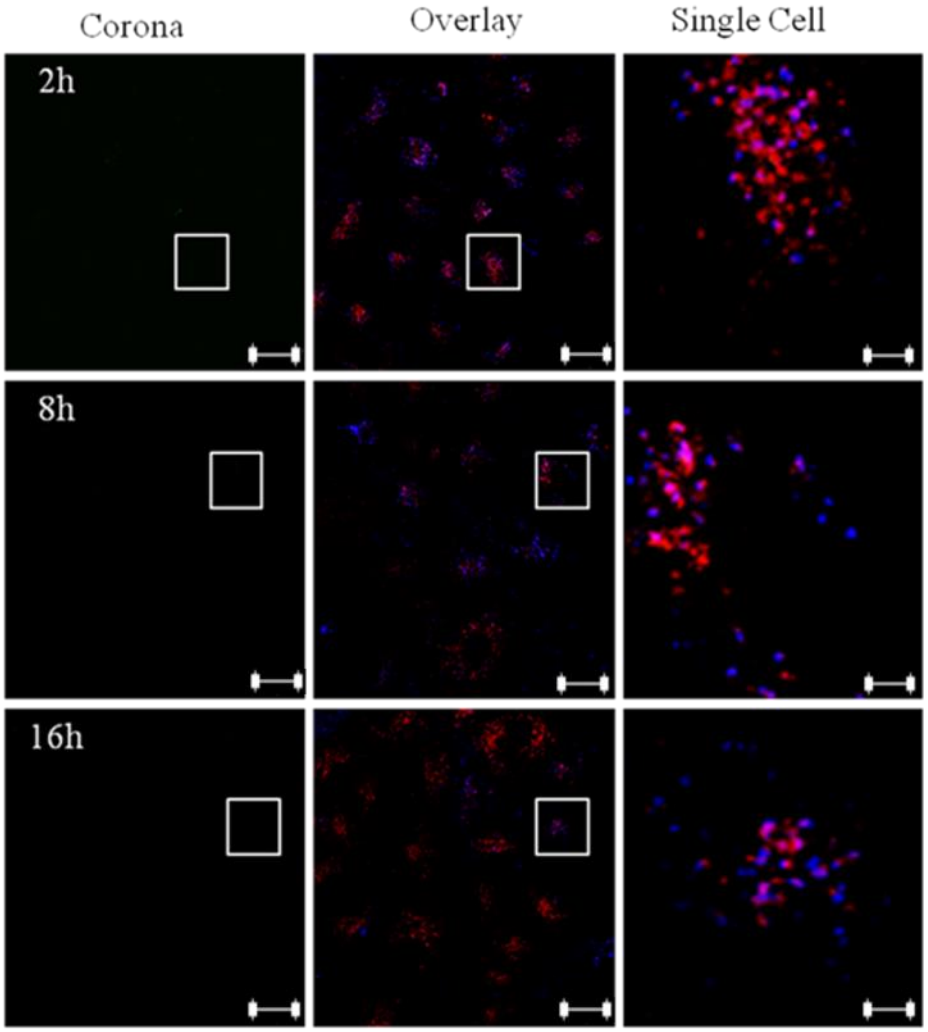


Supplementary Figure S17: Original gels from Main Figure 4. **a:** Coomassie staining of total proteins in the gel showed in Main Figure 4a (the bands analysed by mass spectrometry are indicated by a white rectangle) **b:** Fluorescence image of a SDS PAGE gel of the extracellular hard corona proteins (HC) and the proteins recovered in the crude lysosomal fractions (CLF) isolated at different times as showed in Figure 4b. In agreement with confocal images and high content imaging results, fluorescent proteins could be recovered in the CLF at early times, but later (8 and 16 hours) protein degradation is observed (appearance of lower molecular weight fragments and disappearance of high molecular weight proteins).

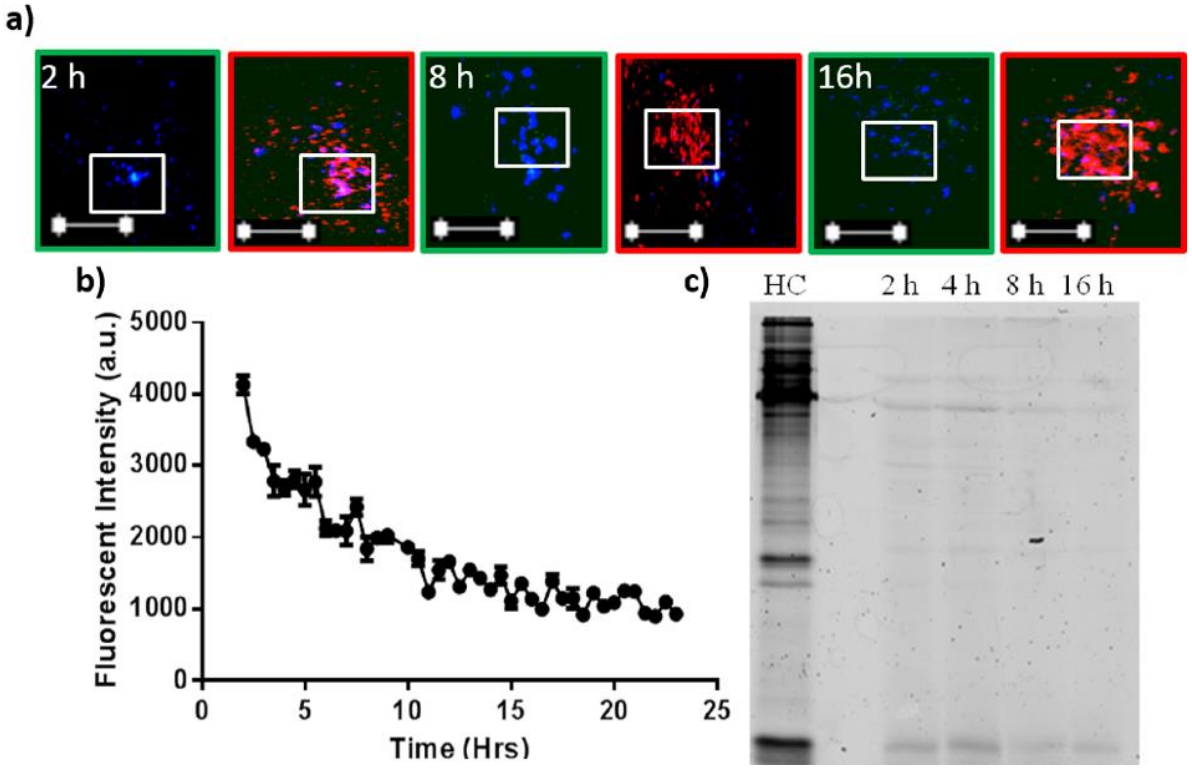


Supplementary Figure S18: Uptake and intracellular destiny of labelled bovine serum proteins: A549 cells were exposed to 5% labelled proteins mixed with 5% unlabelled proteins (total amount of serum bovine proteins: 10%) for 2 hours, then the medium was discarded and cells exposed to unlabelled complete medium for different times (2, 4, 8 and 16 hours) before flow cytometry measurements, confocal imaging and organelle isolation. **a:** Confocal images of A549 after exposure to labelled serum performed as explained above (left column: 63X images at different times after labelled serum removal. Scale bar: 20 µm; right column: magnification of a single cell from the previous images, indicated in the left picture with a white rectangle). The results show presence of small amounts of labelled proteins at earlier

times (2 hours) inside cells. At longer times (16 hours) the signal disappears. Green: labelled proteins. Red: LAMP-1 stained lysosomes. **b:** Mean fluorescence intensity distributions of organelles recovered from cells exposed to fluorescent serum for 2 hours and further growth in unlabelled serum, as determined by flow cytometry. A small fluorescence signal could be detected after 2 hours and decreases with time. **c:** fluorescence image of SDS PAGE of crude lysosome fraction recovered from cells exposed to labelled serum for 2 hours and further grown in unlabelled serum for different times (2, 4, 8 and 16 hours). The results show very low signal from labelled proteins localized into the recovered lysosomes, and this signal disappears at later times (8 and 16 hours) thereby confirming flow cytometry results

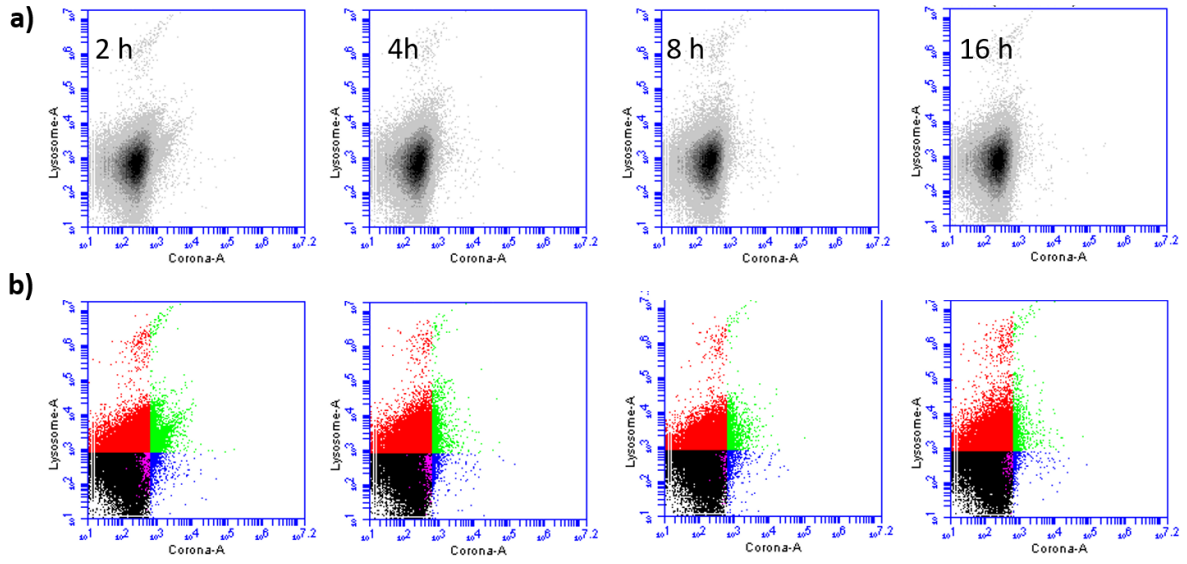


Supplementary Figure S19: Intracellular destiny of the protein corona associated with carboxyl-modified polystyrene: Confocal images of A549 after exposure to nanoparticles as explained above (from the left to right: green corona channel, overlay of green corona, nanoparticles (in blue) and red (LAMP-1 stained) lysosomes and magnification of a single cell indicated in the previous figure by a white rectangle). While nanoparticles are observed inside cells, very little amount of labelled corona proteins could be detected on the internalized nanoparticles, already after 2 hours, while at later times no signal is visible. At longer times (8 and 16 hours) more nanoparticles colocalize with the lysosomes.

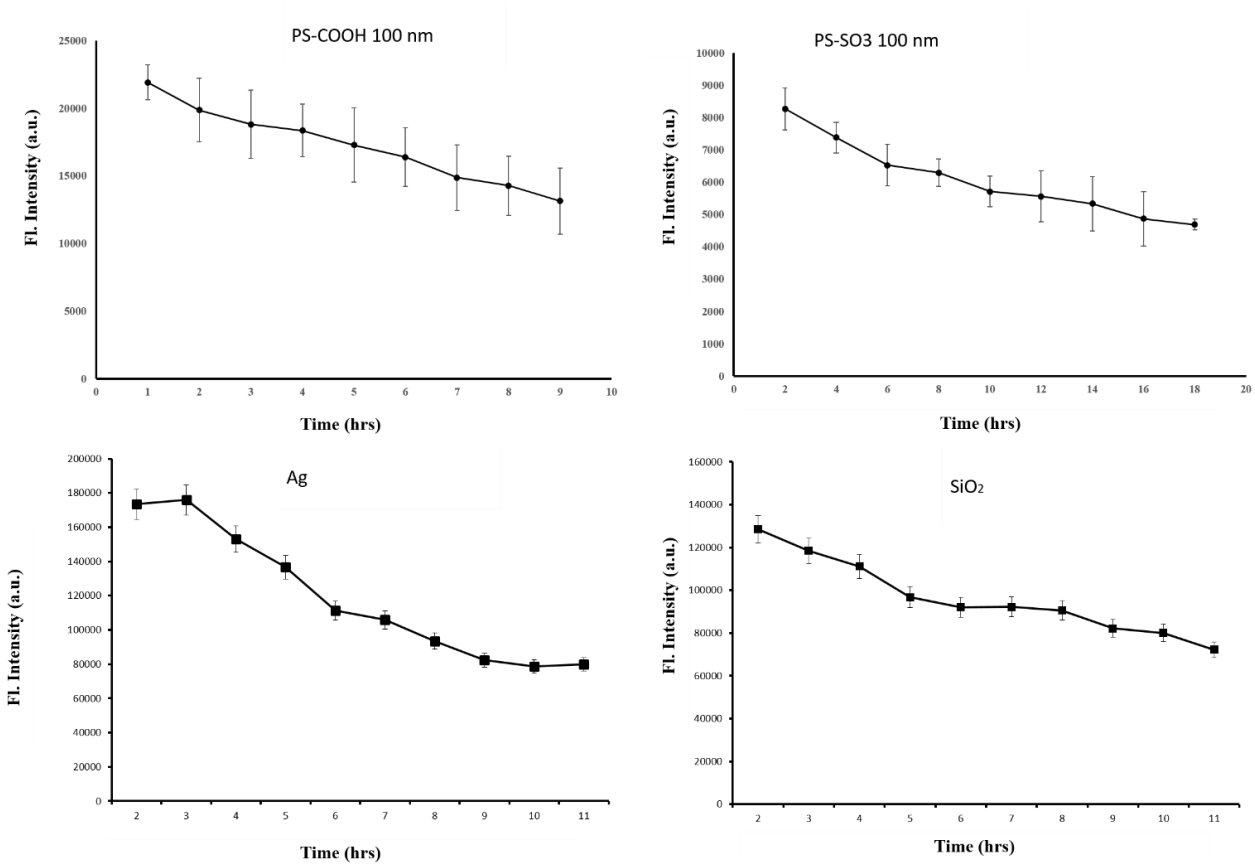


Supplementary Figure S20: Intracellular destiny of the protein corona associated with carboxyl-modified polystyrene: A549 cells were exposed to 100 µg/ml labelled corona carboxyl-modified nanoparticles for 2 hours in unlabelled complete medium; the medium was then substituted with nanoparticle-free unlabelled complete medium and the cells were allowed to grow for different times (2, 4, 8 and 16 hours) before live cell high content imaging, confocal

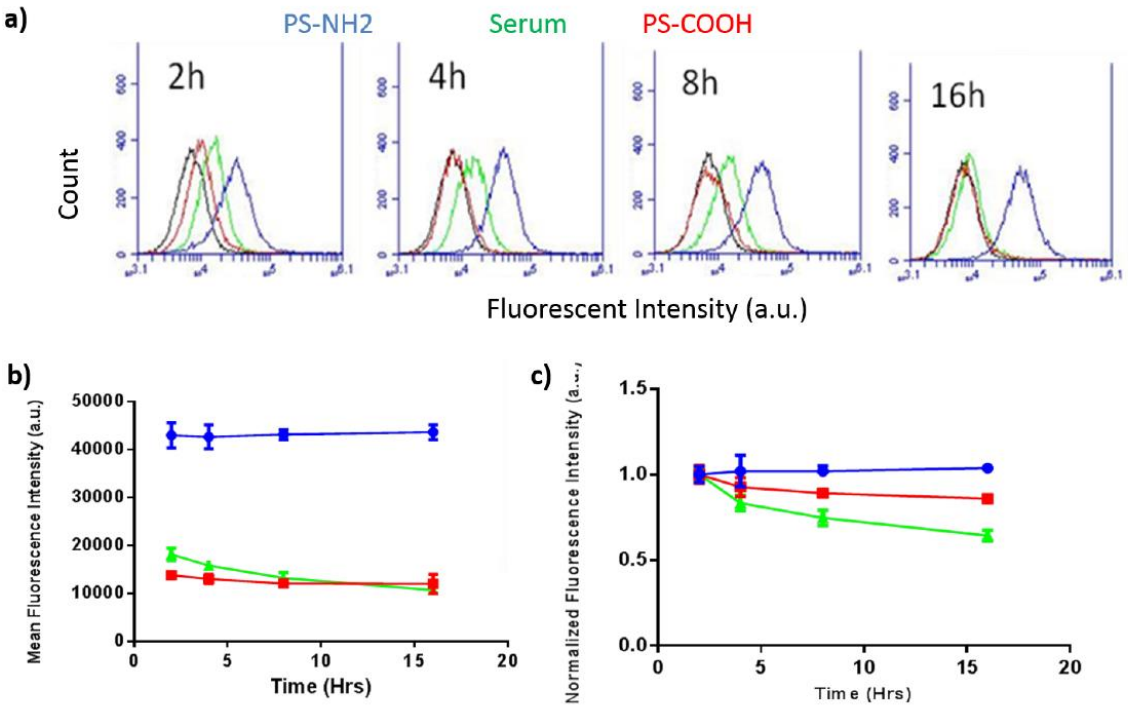
imaging and organelle isolation. **a:** Confocal images of A549 after exposure to nanoparticles as explained above (left panel, in green, corona and nanoparticles and right panels, in red, corona nanoparticles and lysosomes; green corona channel, nanoparticles in blue and red, lysosomes; scale bar 5 μm). While nanoparticles are observed inside cells, very small amount of labelled corona proteins could be detected on the internalised nanoparticles, already after 2 hours, while at later times no signal is visible. At longer times (8 and 16 hours) more nanoparticles colocalize with the lysosomes. **b**: Mean fluorescence intensity distributions of cells at different times after particle removal as obtained by live cell high content analysis. The results show that the signal of fluorescent proteins inside cells is smaller than the one obtained with PS-NH_2 nanoparticles, but decreases with a similar kinetics. It is however important to note that at longer times the fluorescence from the cells is equal to autofluorescence. **c**: Fluorescence image of a SDS PAGE gel of the labelled hard corona proteins (HC) and proteins recovered in the CLF fractions isolated at different times. The results show a very weak signal from the corona proteins already at 2 hours which quickly disappears at later times.



Supplementary Figure S21: Raw flow cytometry distributions of the plots analysed in Supplementary Figure S19. Briefly, one hundred thousand lysosomes were analysed as described in the methods section. **a:** Distributions as obtained by flow cytometry of isolated lysosomes at 2, 4 , 8 and 16 hours chase time after nanoparticle removal. It is possible to observe that the low total green fluorescence associated with the corona proteins (x axis, as opposed to the lysosomal fluorescence, y axis) decreases with time. **b:** The distributions presented can be colour coded using the gating previously shown in Figure S9. The empty lysosomes are presented in red, the corona proteins inside the lysosomes are presented in green and the non-lysosomal population of nanoparticles are presented in blue.

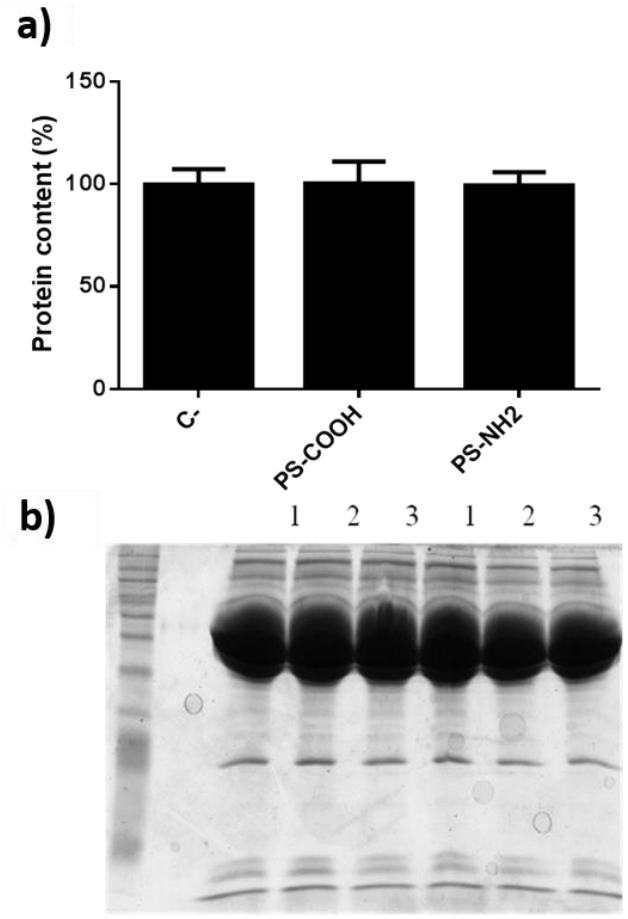


Supplementary Figure S22: Cellular processing of the fluorescently labelled protein corona associated with different core nanoparticles, different sizes and different surface chemistry (100 nm PS-COOH and PS-SO3, 60 nm silver nanoparticles and 50 nm silica nanoparticles). Briefly, after formation, nanoparticle fluorescent corona complexes were incubated for 2 hours (pulse time), and then the media was replaced with fresh media without nanoparticles (chase time). The mean fluorescence intensity distributions of cells at different times after particle removal as obtained by live cell high content analysis illustrates how the intralysosomal fluorescence decreases with time, indicating degradation of the corona. The results also show that the signal of fluorescent proteins inside cells is smaller than the one obtained with PS-NH_2 nanoparticles in all cases, but varieties greatly from particle to particle.

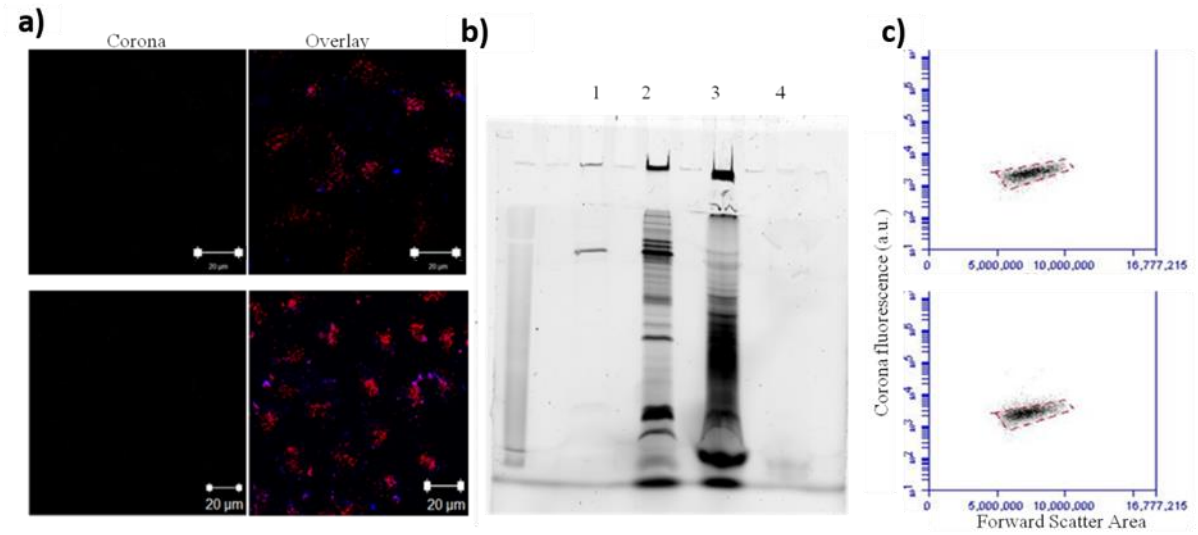


Supplementary Figure S23: Comparison of uptake of labelled corona and serum inside cells. **a:** Mean fluorescence intensity distributions obtained by flow cytometry on cells exposed for

2 hours to the different nanoparticle corona complexes (PS-COOH and PS-NH_2) and cells exposed to cell culture medium containing 5% labelled serum (see also Supplementary Fig. S9), followed by replacement with unlabelled (nanoparticle-free) medium and further growth for different times. Overlap of the distributions clearly shows that the highest uptake of fluorescent proteins is obtained when cells are exposed to labelled corona formed on PS-NH_2, while this is much smaller in the case of PS-COOH, and at a comparable level for cells exposed to simple labelled serum. At later times the proteins (or fragments of them) of the corona on the PS-NH_2 nanoparticles seem to persist inside the cells, while the proteins associated with the other samples have either completely degraded or exported. **b:** Mean cell fluorescence intensity as a function of time, from data shown in panel a, clearly showing how PSNH_2 corona proteins are internalized in high quantity by the cells in comparison to other nanoparticles or free proteins. **c**: Normalized evolution of the fluorescent signal associated with nanoparticle corona and free serum proteins, showing that the proteins associated with the nanoparticles are processed with different kinetics in comparison to what is observed for free (labelled) serum proteins.



Supplementary Figure S24: Quantification of serum proteins in the protein corona. **a:** The protein concentration of the supernatant recovered after protein corona formation on 100 µg/ml nanoparticles (Snp+ for amino modified nanoparticles and Snp- for carboxyl modified) was tested with BCA assay. The results show that the amount of proteins adsorbed on the nanoparticles in the corona is so small that it is difficult to determine a decrease in concentration in the recovered supernatant. **b:** the samples from the previous experiment (a) were also run into a 1D SDS PAGE and stained with coomassie (1: complete medium, 2: supernatant from amino modified nanoparticles, 3: supernatant from carboxyl modified nanoparticles). No differences were observed in the pattern of recovered proteins. This suggests that overall the amount of proteins adsorbed on the nanoparticles is very small, however the amount that enters cells on the nanoparticles is much higher than what is observed for cells exposed directly to 5% labelled serum.



Supplementary Figure S25: Controls for background fluorescence in untreated cells. In order to ensure that the fluorescent signal detected in Figure 1 and 2 was due to the labelled corona proteins, cells were treated with unlabelled corona nanoparticle complexes prepared in the same way and analysed using the same settings. The results confirm no green signal was detected in confocal imaging (a, top: amino modified, bottom: carboxyl modified, scale bar: 20 µm), 1D SDS PAGE (b, 1: unlabelled corona on amino-modified nanoparticles; 2: labelled corona on amino-modified nanoparticles; 3: labelled corona on amino-modified nanoparticles after 8 hours in cells and 4: unlabelled corona on amino-modified after 8 hours on cells) and flow cytometry (c , mean green fluorescence intensity versus forward scattering of cells exposed to unlabelled corona complexes formed on amino-modified (top) and carboxyl-modified (bottom) nanoparticles

Supplementary References

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