# Cell membrane penetration and mitochondrial targeting by platinum-decorated ceria nanoparticles

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**ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)**

## Mitochondrial targeting

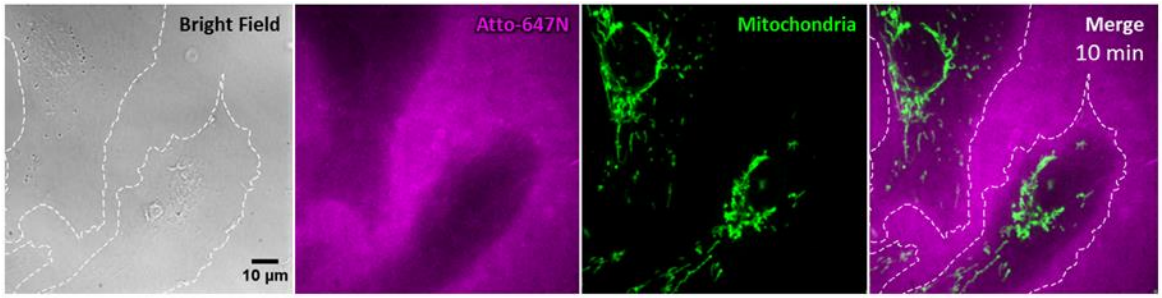


**Fig. S1** Live-cell imaging studies on the interaction between Atto 647N molecules and cells. HMEC−1 cells (left panel, dashed lines) were incubated with Atto 647N dye molecules free in solution (middle left panel, magenta). It can be observed that these molecules are not able to overcome the plasma membrane barrier and do not reach the cytosol (middle left panel, darker regions). Therefore, Atto 647N free in solution is not able to accumulate in mitochondria (middle right panel, green), as can be visualized in the merge image (right panel). Note the absence of colocalizing pixels in the mitochondrial region.

**Inhibitor studies**

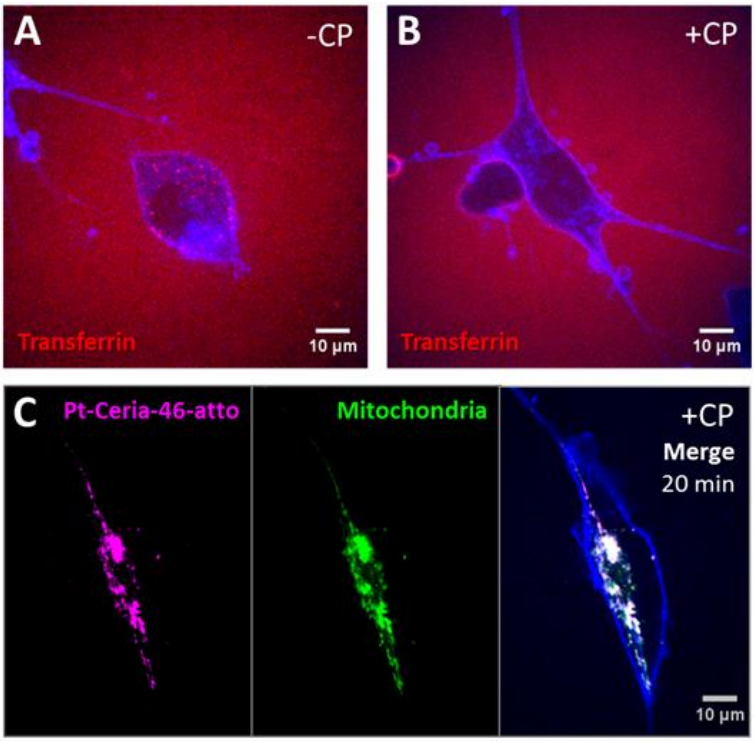


**Fig. S2** Live-cell confocal study on the role of clathrin-mediated endocytosis during the uptake of small Pt-ceria NPs by HMEC−1 cells. (A) Transferrin (red) is taken up into the cellular outlines (blue: CellMask) in the absence of chlorpromazine (-CP). Transferrin is present in the cell culture medium as well, leading to a staining of the extracellular space. (B) The clathrin-mediated pathway is inhibited by chlorpromazine (+CP), and transferrin (red) is no longer internalized by cells. (C) Inhibition with (+CP) under the same experimental conditions of B, however, does not hinder the entry of Pt-Ceria-46-atto NPs (left panel, magenta) into cells. After 20 min, a large number of NPs is already associated with mitochondria (middle panel, green: MitoTracker). Note the clear colocalization between NPs and mitochondria in the merge image (right panel, colocalization pixels in white; blue: CellMask).

**Inhibitor studies**

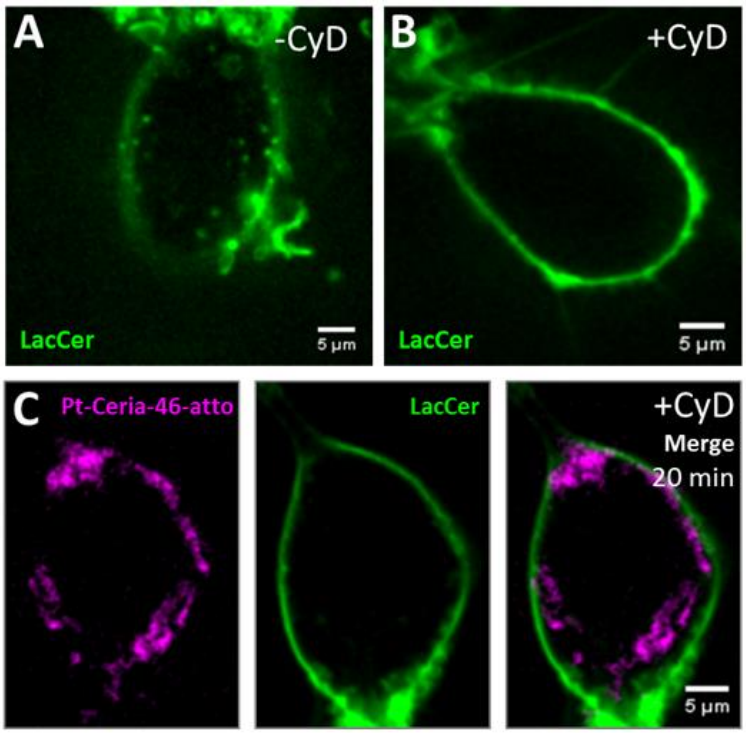


**Fig. S3** Live-cell confocal study on the influence of caveolin-dependent endocytosis during the uptake of Pt-ceria NPs by HMEC−1 cells. (A) In the absence of cytochalasin D (-CyD), lactosylceramide (LacCer; green) is internalized by caveolae vesicles that can be visualized inside the cell. The cellular outlines are evidenced by LacCer molecules. (B) Caveolin-mediated endocytosis is inhibited by cytochalasin D (CyD+), and LacCer is no longer internalized by cells. (C) Inhibition (CyD+), however, does not block the internalization of Pt-Ceria-46-atto NPs (left panel, magenta) into cells. After 20 min, a large number of NPs is found inside the cell. On the other hand, internalization of LacCer molecules (middle panel, green) was blocked by CyD. Note that Pt-Ceria-46-atto NPs are distributed in the characteristic filament-like structures of the mitochondrial network (merge image, right panel).

**Protein corona**

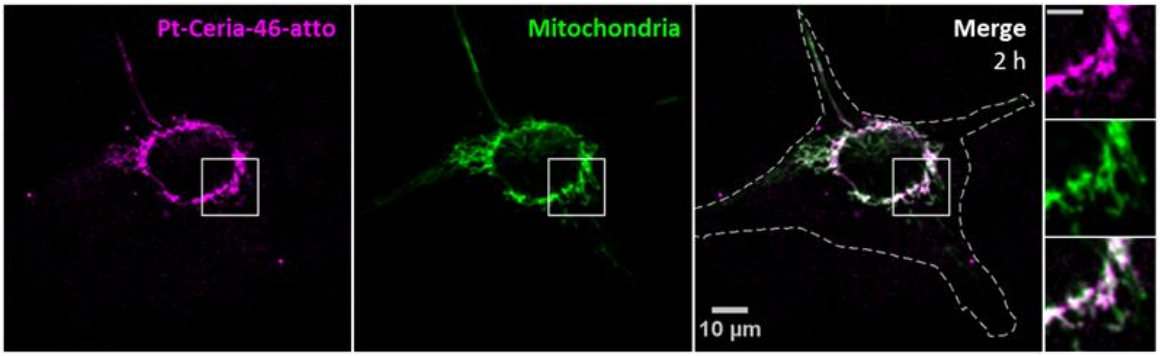


**Fig. S4** Influence of the protein corona on the cellular uptake behavior of small Pt-ceria NPs. The protein corona is believed to have a strong influence in the interactions between NPs and cells. In all uptake experiments of this work, except this one, cells were incubated in serum-containing cell medium. Here, however, HMEC−1 cells were incubated with Pt-Ceria-46-atto NPs for 2 h in serum-free medium. Under this special experimental condition NPs are not covered by adsorbed proteins. Interestingly, the absence of serum proteins did not alter the uptake route. Pt-Ceria-46-atto NPs (left panel, magenta) were rapidly internalized by HMEC−1 cells (dashed line) and attached to mitochondria (middle panel, green: MitoTracker). Colocalization between NPs and mitochondria can be clearly visualized in the merge image (right panel, white). Inset: boxed regions in detail. The typical filament-like structures of mitochondria and associated Pt-Ceria-46-atto NPs are evident. Scale bar = 5 µm.

## Cell membrane integrity

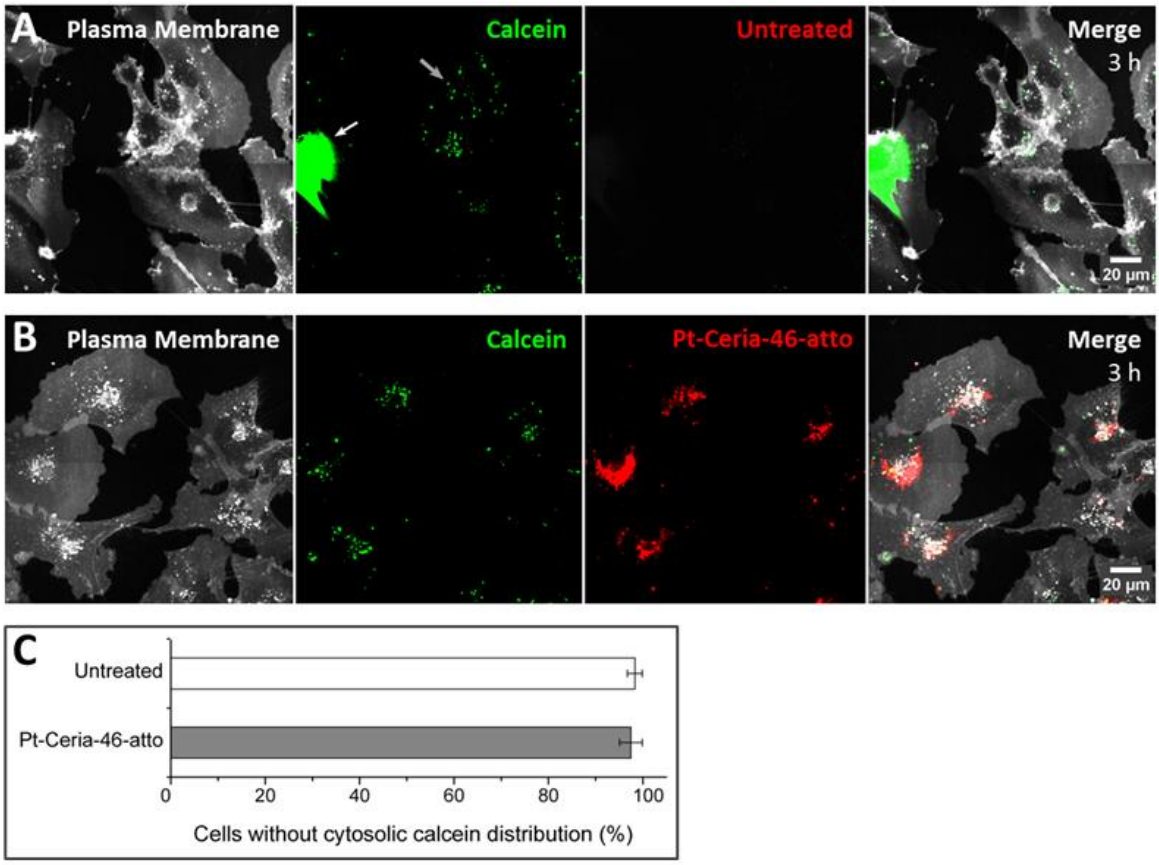


**Fig. S5** Impact of small Pt-ceria NPs on the membrane integrity: calcein leakage in assay. Z-projection of live-cell confocal images depicting HMEC−1 cells (left panels, CellMask) co-incubated with calcein (middle left panels, green) and (A) untreated or (B) treated with 100 µg/mL Pt-Ceria-46-atto NPs for 3 h (middle right panels, red). Calcein is a cell-impermeant fluorescent dye that is internalized *via* endocytosis by healthy cells and displays a punctuate pattern (gray arrow in A). However, when the cell membrane integrity is compromised, calcein dye molecules leak into the cell and a diffuse pattern of cytosolic calcein distribution is observed (white arrow in A). NP and calcein internalization by cells can be appreciated in the merge images (right panels). Scoring the percentage of cells without cytosolic calcein distribution is a conventional method to assess the impact of NPs on the membrane integrity and the cellular viability. (C) Percentage of cells without cytosolic calcein distribution for untreated and NP treated experiments (gray and white bars, respectively). In total, the plasma membrane integrity of more than 340 cells was evaluated. For both untreated and NP treated cells, the percentage of viable cells was above 97%. Hence, these results point to a cellular uptake process of small Pt-ceria NPs that does not impair the plasma membrane integrity. Data represent mean ± standard error of two independent experiments.

**Cell membrane integrity**

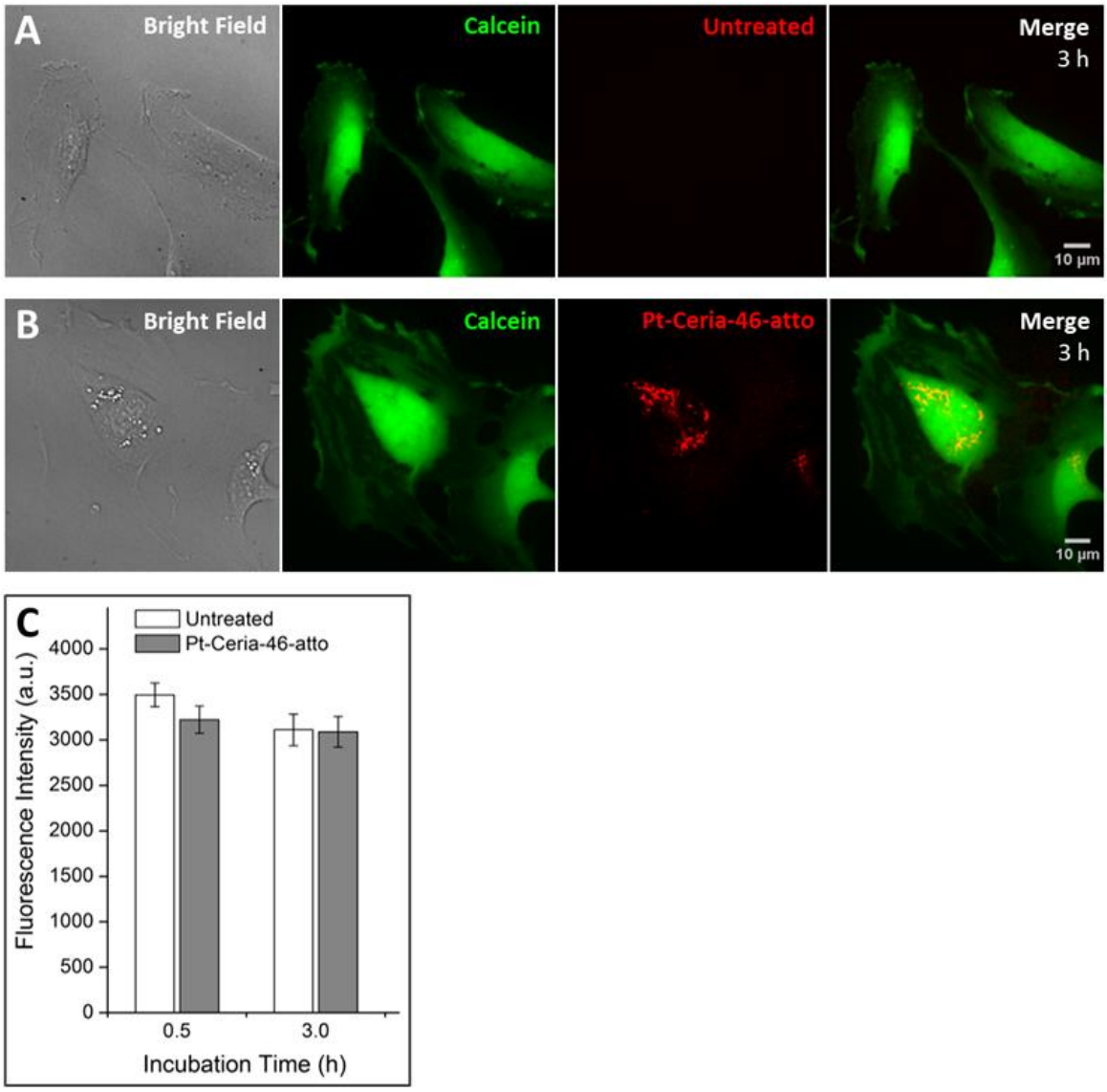


**Fig. S6** Impact of small Pt-ceria NPs on the membrane integrity: calcein leakage out assay. Bright field (left panels) and Z-projection of live-cell confocal images. HMEC−1 cells were pre-loaded with cytosolic calcein (middle left panels, green) and subsequently (A) untreated or (B) treated with 100 µg/mL Pt-Ceria-46-atto NPs for 0.5 and 3 h (middle right panels, red). In this assay, if the plasma membrane is disrupted, pre-loaded calcein dye molecules can leak out the cell and an overall reduction in the cytosolic fluorescence intensity occurs. Thus, a reduction in the mean fluorescence intensity can be correlated with particle-induced cell membrane damage. Intracellular NPs and cytosolic calcein can be clearly visualized in the merge images (right panels). (C) Mean fluorescence intensity of calcein measured in the cytosolic region of individual HMEC−1 cells. In total, more than 70 single cells were analyzed per time point. Remarkably, no significant difference ($p < 0.01$) between untreated and Pt-Ceria-46-atto NPs treated cells was detected (white and gray bars, respectively). Data represent mean ± standard error of two independent experiments (n = 35–40).

**Cell-penetrating Pt-ceria NPs**

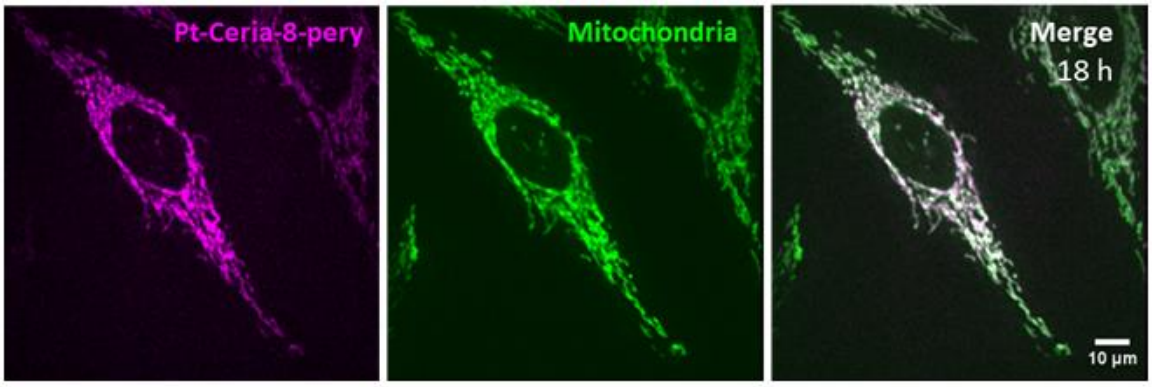


**Fig. S7** Live-cell imaging study on the uptake of Pt-ceria NPs by human cervical cancer cells (HeLa). HeLa cells were incubated with Pt-Ceria-8-atto NPs (left panel, magenta) for 18 h. NPs were internalized by cancer cells and attached to mitochondria (middle panel, green: MitoTracker). Note the high degree of colocalization in the merge image (right panel, white). Similar results were obtained with HeLa cells incubated with small Pt-ceria NPs for just a few minutes (data not shown).