Electronic Supplementary Material (ESI) for Nanoscale Advances.

Electronic Supplementary Information

X-ray tomography shows the varying three-dimensional morphology of gold nanoaggregates in the cellular ultrastructure

*Daniela Drescher, Tina Büchner, Peter Guttmann, Stephan Werner, Gerd Schneider, and Janina Kneipp**

Physicochemical properties of gold nanoparticles

The TEM images and histograms in Figure S1 show citrate-stabilized gold nanoparticles prepared according to a protocol of Lee and Meisel[1]. The gold nanoparticles have a particle size of 32 ± 5 nm. Compared to the UV-vis spectra of gold nanoparticles in water (Figure S1C, black line), the absorbance at 530 nm decreases progressively with increasing concentration of sodium chloride. A significant decrease in absorbance at 530 nm and at the same time an increase in the NIR range occurs after the addition of electrolyte solutions with a salt concentration > 100 mM as a result of the electrostatic destabilization of the particles. The position and shape of the extended plasmon band are determined by the size and morphology of the aggregates.[2] The shift of the extinction maximum to 850 nm suggests a change in the particle size distribution,[3] which is associated with the irreversible particle aggregation.

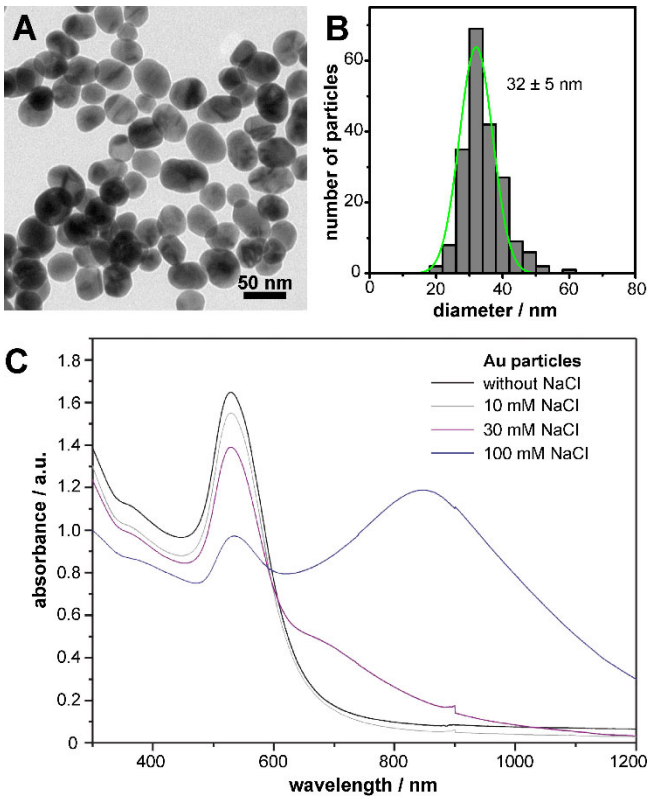

Figure S1. Characterization of gold nanoparticles. (A) TEM image and (B) particle size distribution of as-synthesized gold nanoparticles. (C) Absorbance spectra of citrate-stabilized gold nanoparticles in sodium chloride solution of different concentrations.

TEM investigation of gold particles in cells

Information on the localization of the particles inside the cell and their interaction with cellular compartments can be obtained by TEM images. In contrast to cryo-XM, it requires chemical fixation and staining of the cells and the preparation of electron-transparent ultramicrotome cuts. Due to its high spatial resolution, TEM can be used to visualize individual nanoparticles and small aggregates in cellular substructures (Figure S2). TEM images of fibroblast cells after 3h-exposure to gold nanoparticles confirm that gold nanoparticles are present exclusively in endosomes or lysosomes in the cytoplasm suggesting their endocytotic uptake. Most of the vesicles are located near the nucleus or mitochondria and contain between 1 and 10 nanoparticles. In the course of endosomal maturation, multivesicular structures are formed, leading to the occurrence of larger aggregates of up to 20 nanoparticles (Figure S2B, C). No nanoparticles were be found in the cell nucleus. To obtain information about the 3D distribution of nanoparticles and their aggregates in the cellular ultrastructure tomographic data are required, since TEM gives only 2D information of a few nm thick cell sections.

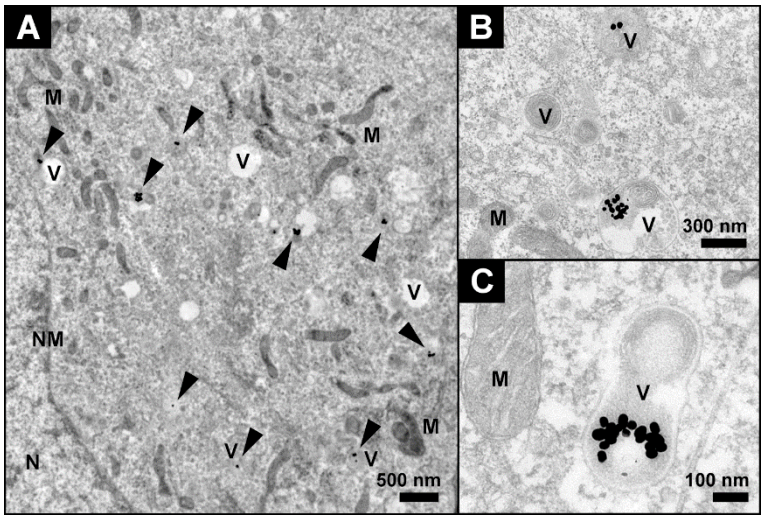

Figure S2. TEM images of 3T3 fibroblast cells after 3 hours of incubation with 100 pM gold nanoparticles. Abbreviations: N, cell nucleus; NM, nucleus membrane; M, mitochondrion; V, vesicle. The arrows indicate gold nanoparticles.

Additional XT data of gold particles in cells

X-ray microscopic images of macrophages incubated with gold nanoparticles for 24 hours show aggregates in the close proximity to the cell nucleus (Figure S3A, B). Aggregates consisting of several hundred nanoparticles with sizes up to 1 µm can be identified inside the cells. These observations can be explained by endosomal maturation and multivesicular fusion of particle-containing vesicles.[4]

After 24h-incubation of fibroblasts with gold nanoparticles (Figure S3C-G), a large amount of aggregates can be found, which are predominantly located in the perinuclear area. The size of these aggregates is much smaller compared to macrophages (Figure S3A, B). While the nuclear and plasma membranes are clearly visible on the projection images (Figure S3C, D), the membranes of the vesicles are only rarely visible. Tilt angle series were recorded for representative fibroblasts, which enable the reconstruction of the sample volume and thus the visualization of the distribution the gold nanoparticles in the native cellular structure.

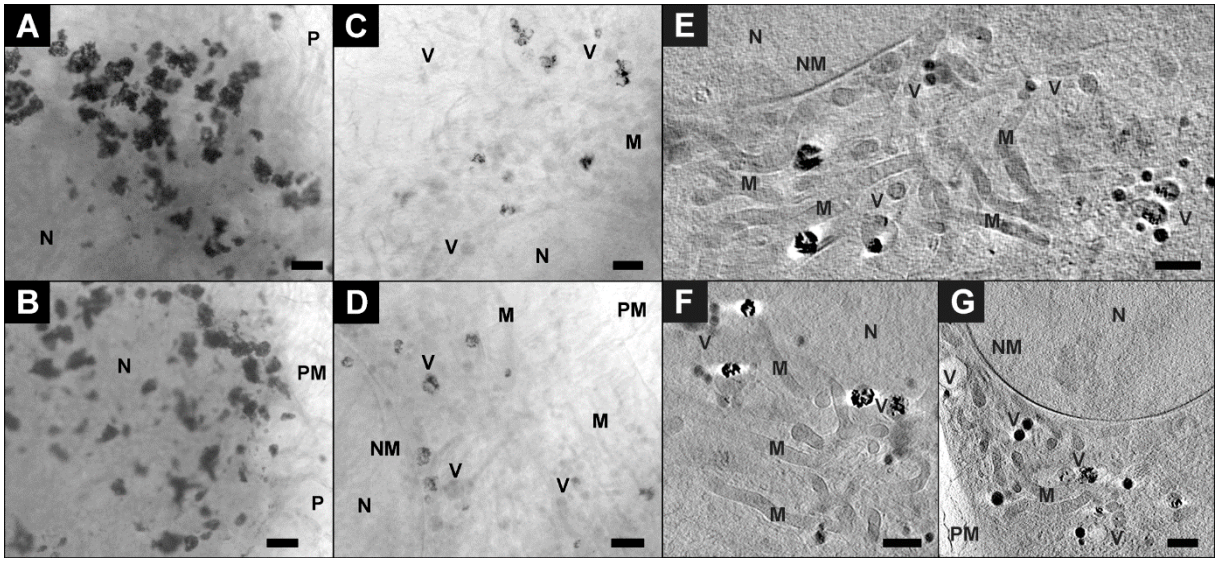

Figure S3. X-ray microscopic images of (A, B) macrophages and (C-D) fibroblasts after incubation with 100 pM gold nanoparticles for 24 hours. All images were acquired with a 25 nm zone plate (9.8 nm pixel size). Representative slices of a tomographic reconstruction in E-G confirm the localization of particles in vesicles. Scale bars: 1 µm. Abbreviations: N, cell nucleus; NM, nucleus membrane; M, mitochondrion; PM, plasma membrane; P, pseudopod; V, vesicle.

In Figure S4 exemplary sections of reconstructed X-ray tomograms of fibroblasts incubated with gold nanoparticles and nanoaggregates are shown (see also Movie S1 to S4). The tomographic slices demonstrate the 3D localization of single particles and aggregates in endosomes and lysosomes in the immediate vicinity of mitochondria or the nuclear membrane in different heights of the cell also above the nucleus.

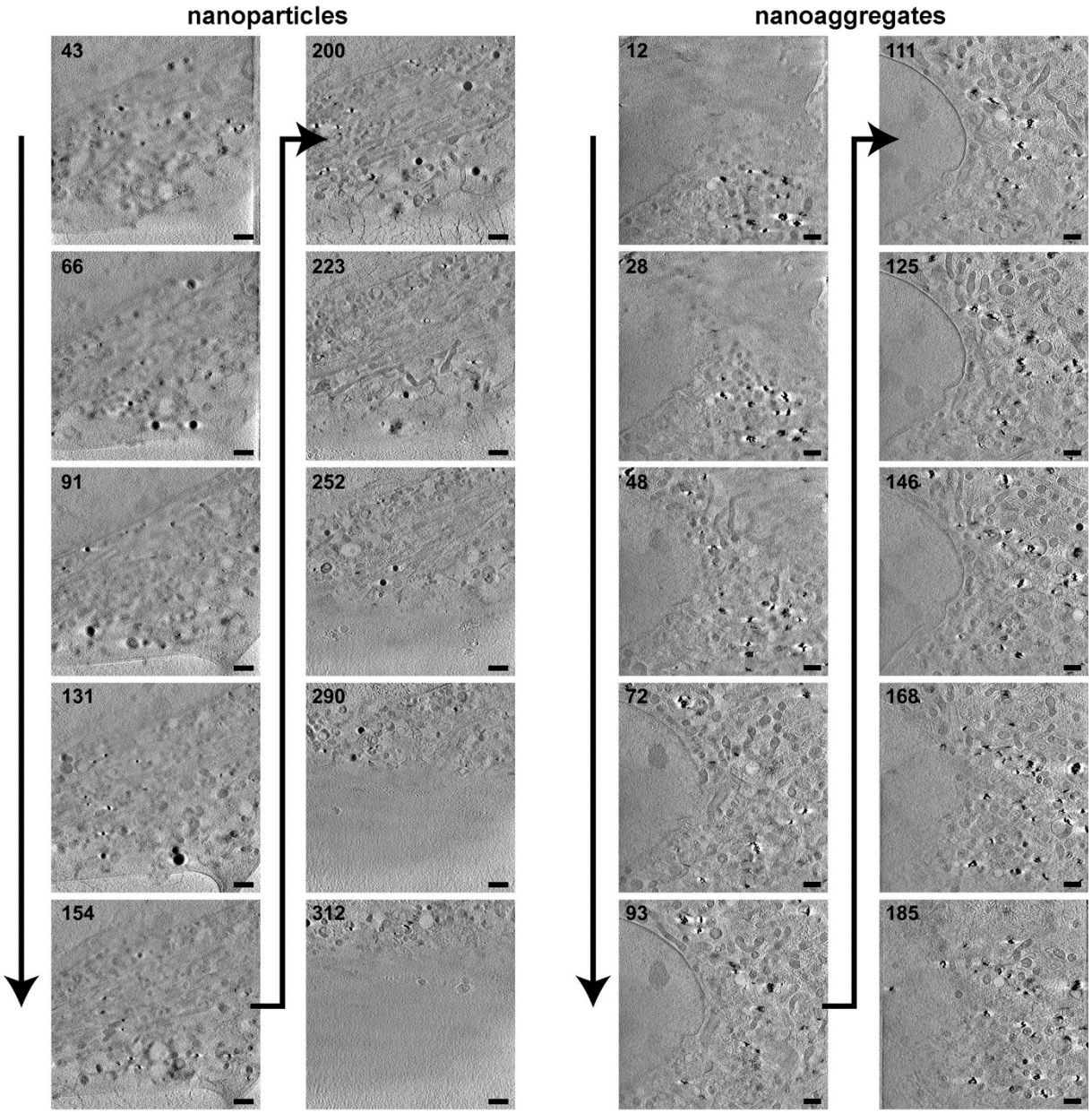

Figure S4. Representative slices of two X-ray tomograms of fibroblasts after 3h-incubation with gold nanoparticles and nanoaggregates. Tomographic reconstruction was performed using eTomo. Scale bars: 1 µm. A slice has a thickness of approximately 10 nm.

References

1 P. C. Lee and D. Meisel, *J Phys Chem*, 1982, **86**, 3391-3395.

2 M. Kerker, O. Siiman and D. S. Wang, *The Journal of Physical Chemistry*, 1984, **88**, 3168-3170.

3 T. Jensen, L. Kelly, A. Lazarides and G. C. Schatz, *Journal of Cluster Science*, 1999, **10**, 295-317.

4 J. Huotari and A. Helenius, *EMBO J*, 2011, **30**, 3481-3500.