Impact of exposure conditions on the uptake of nanoparticles by cultured cells

Yalan Huang[1,2,3#], Xing Sun[2#], Sabine Vidal-Y-Sy[4], Yuanyuan Wang[4], Miao Feng[2], Ziyao Liu[2], Yang Liu[2], Bing Qi[2], Yanan Kang[2], Christian Gorzelanny[4], Wolfgang J. Parak[2*], Neus Feliu[2,5*]

[1] Department of Ultrasound, Shenzhen People's Hospital (The First Affiliated Hospital, Southern University of Science and Technology; The Second Clinical Medical College, Jinan University), Shenzhen 518020, China
[2] Center for Hybrid Nanostructures (CHyN), Universität Hamburg, 22607 Hamburg, Germany
[3] Post-doctoral Scientific Research Station of Basic Medicine, Jinan University, Guangzhou 510632, China
[4] Department of Dermatology, University Medical Center Hamburg-Eppendorf, 20251 Hamburg, Germany
[5] Fraunhofer Center for Applied Nanotechnology (CAN), 20146 Hamburg, Germany
# equal contribution
* corresponding authors: wolfgang.parak@uni-hamburg.de, neus.feliu@physnet.uni-hamburg.de

SUPPORTING INFORMATION

I Synthesis and characterization of gold nanoparticles

I.1 General description of synthesis

Spherical and rod shaped gold nanoparticles (Au NPs and Au NRs, respectively) of different sizes were synthesized as previously reported [1]. The NPs and NRs were purified by ultracentrifugation [1]. The NPs are referred to with their nominal diameter for which the synthesis conditions were used. The real diameter d_c was determined by TEM as described in section I.1. For example 5 nm Au NPs refers to Au NPs that were synthesized according to a protocol that should yield Au NPs of 5 nm core diameter. The here achieved "real" diameters were determined from TEM size-distributions, which in the case of 5 nm Au NPs was for example $d_c = 4.8 \pm 0.9$ nm (Table SI I-4). The NRs are referred to with their nominal length for which the synthesis conditions were used.

I.2 Transmission electron microscopy

Transmission electron microscopy (TEM) images were used to investigate the shape and size distribution of the all NPs/NRs as previously shown [1]. The spherical NPs were hereby characterized by their core diameter d_c. The rod-shaped NRs were characterized by their core diameter d_c and their core length L_c. Hereby "core" refers to the Au part of the NPs/NRs, *i.e.* without considering the organic surface coating. To determine the size distribution $N(d_C)$ of the core diameter of the different Au NP/NRs, d_c was determined from 100 NP/NRs by analyzing TEM images using the ImageJ software. The same procedure was applied for determining the distribution N(Lc) of the lengths of the NRs. Illustrative TEM images of the Au NP/NRs are displayed in Figure SI I-1and the obtained size distribution histograms can be seen in Figure SI I-2 and Table SI I-4.

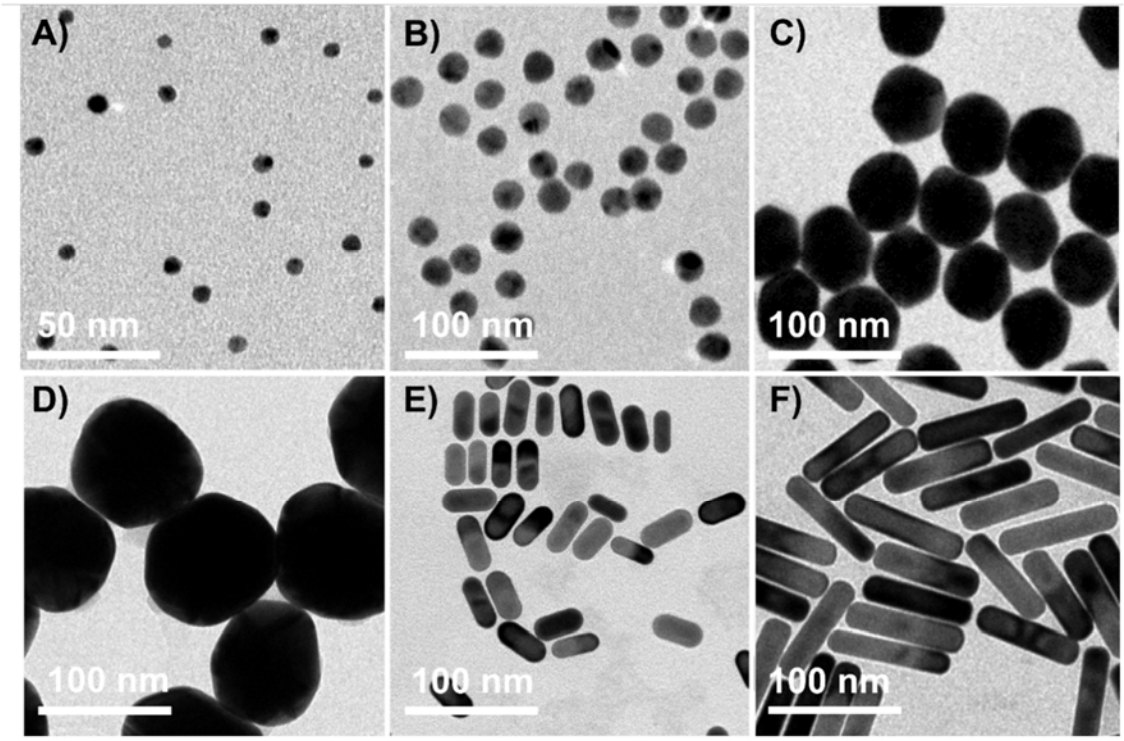

Figure SI I-1. Representative TEM images of Au NP/NRs. A) 5, B) 25, C) 50, D) 100 nm Au NPs and E) 40, F) 100 nm Au NRs, respectively. Additional detailed information of NP/NRs is shown in Table SI I-4.

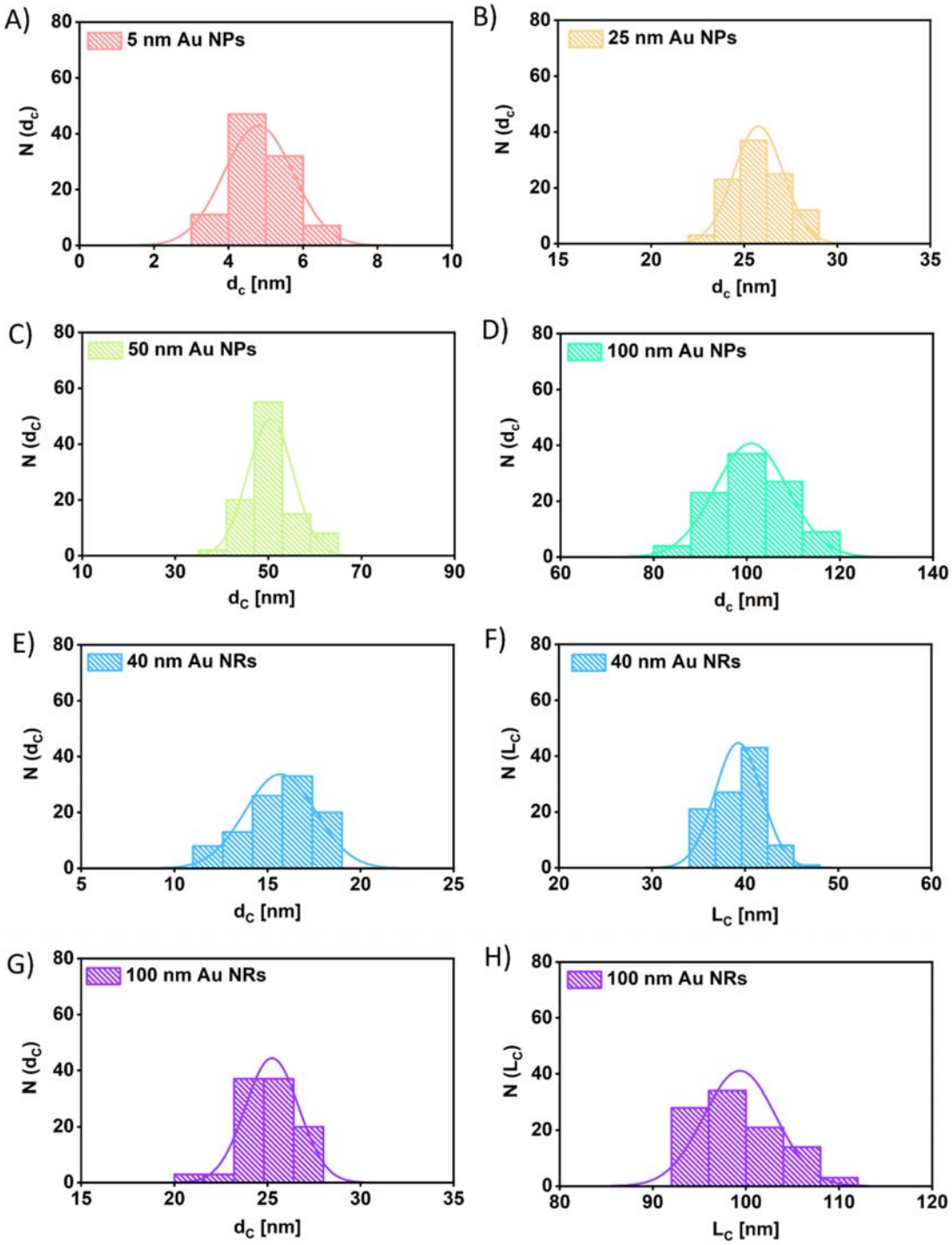

Figure SI I-2. Size distribution histogram Gaussian fit for the diameters d_c and lengths L_c of the Au NP/NRs. N describes the number of counted NPs/NRs of certain diameter/length. A) 5 nm Au NPs, B) 25 nm Au NPs, C) 50 nm Au NPs, D) 100 nm Au NPs, E), F) 40 nm Au NRs, and G), H) 100 nm Au NRs. The corresponding mean values and standard deviations as extracted from the fits are shown in Table SI I-4.

I.3 Estimation of the molar mass of the different NPs/NRs

The molar mass M_w of the NPs and NRs was calculated as previously reported by estimating the volume of one NP/NR from their geometry (V_{NP},V_{NR}) and by using the bulk density of gold ρ_{Au} [1] Hereby, the 5, 25, 50, and 100 nm Au NPs were assumed to be homogeneous spheres, and their volume (V_{NP}) was calculated based on Equation SI I-1. In the case of the 40 and 100 nm Au NRs, they were assumed to be cylinders, and their volume (V_{NR}) was calculated based on Equation SI I-2. The average diameter (d_c) of the NPs/NRs and the length (L_c) of the NRs were taken from the calculated TEM values as shown in Table SI I-4.

$$V_{NP} = \frac{4}{3} \cdot \pi \cdot (\frac{d_c}{2})^3 \qquad \text{Equation SI I-1}$$

$$V_{NR} = \pi \cdot \left(\frac{d_c}{2}\right)^2 \cdot L_c \qquad \text{Equation SI I-2}$$

Using Avogadro's number (N_A) the molar mass M_w of the NP and NR cores was calculated as:

$$M_{NP} = V_{NP} \cdot \rho_{Au} \cdot N_A \qquad \text{Equation SI I-3}$$

$$M_{NP} = V_{NR} \cdot \rho_{Au} \cdot N_A$$

The molar masses of the NP and NR cores (M_{NP}) as derived with this approach are listed in Table SI I-1 and Table SI I-4.

Sample	M_{NP} [g/mol]
5 nm Au NPs	$6.7 \cdot 10^5$
25 nm Au NPs	$1.04 \cdot 10^8$
50 nm Au NPs	$7.78 \cdot 10^8$
100 nm Au NPs	$6.24 \cdot 10^9$
40 nm Au NRs	$8.8 \cdot 10^7$
100 nm Au NRs	$5.8 \cdot 10^8$

Table SI I-1. Molar mass M_{NP} of the NPs and NRs as calculation by Equation SI I-3.

The molar concentration of the NP/NRs was calculated from the mass concentration of Au (C_{Au}) by Equation SI I-4.

$$c_{NP} = C_{Au}/M_{NP} \qquad \text{Equation SI I-4}$$

I.4 Concentration determination of the Au NPs/NRs

The concentration of the Au NPs/NRs in this study was done by UV/vis absorption measurements, using reported molar extinction coefficients from the literature. As afterwards mass concentrations C_{Au} and not molar NP concentrations c_{NP} were used as metrics, this turned out to be not a good choice. The resulting uncertainty in concentration determination will be discussed in the following.

The molar extinction coefficients at 450 nm for the different NPs were taken from Haiss et al. and are listed in Table SI I-2. By measuring the absorption A_{450} of a NP/NR solution in a cuvette of pathlength l at 450 nm, the molar NP/NR concentration $c_{NP(UV)}$ can be derived according to the Beer-Lambert law as reported (Equation SI I-5) [2].

$$c_{NP(UV)} = \frac{A_{450}}{\varepsilon_{450} \cdot l} \qquad \text{Equation SI I-5}$$

By using the molar masses M_{NP} of the different NP/NR types from Table SI I.1, the molar NP concentrations $c_{NP(UV}$ can be converted into mass concentrations of gold $C_{AU(UV)}$ according to Equation SI I-4: $C_{Au(UV)} = c_{NP(UV)} \cdot M_{NP}$. In Table SI I-2 the calculated concentrations $c_{NP(UV)}$ and $C_{Au(UV)}$ for examples of NP/NR solutions as derived from their absorption A_{450} are provided.

Sample	ε_{450} [L/(mol·cm)]	A_{450}	$c_{NP(UV)}$ [nM]	$C_{Au(UV)}$ [µg/L]	$C_{Au(ICP)}$ [µg/L]
5 nm Au NPs	$7.2 \cdot 10^6$	0.40·50	2800	$1.9 \cdot 10^6$	$1.1 \cdot 10^6$
25 nm Au NPs	$1.1 \cdot 10^9$	0.54·50	25	$2.6 \cdot 10^6$	$1.4 \cdot 10^6$
50 nm Au NPs	$9.9 \cdot 10^9$	0.40·50	2.1	$1.6 \cdot 10^6$	$1.2 \cdot 10^6$
100 nm Au NPs	$6.44 \cdot 10^{10}$	0.71·10	0.11	$6.9 \cdot 10^5$	$7.5 \cdot 10^5$
40 nm Au NRs	$1.42 \cdot 10^9$	0.45·25	7.9	$6.9 \cdot 10^5$	$4.6 \cdot 10^5$
100 nm Au NRs	$4.53 \cdot 10^9$	0.43·10	0.95	$5.5 \cdot 10^5$	$2.8 \cdot 10^5$

Table SI I-2. The molar extintion coefficients ε_{450} at 450 nm were taken from Haiss et al [2]. For different NP/NR solutions their absorption A_{450} at 450 nm was measured in a cuvette of pathlength l = 1 cm. As the optical density for absorption measurements should be well below 1, the solutions were appropriately diluted. A_{450} = 0.40·50 = 20 refers to a NP solution, which has been diluted with a factor 50, the absorption of the diluted solution was measured to be 0.40 and thus the absorption corresponding to the undiluted solution is 0.40·50. The molar NP/NR concentrations $c_{NP(UV)}$ were then determined according to Equation SI I-5. The molar concentration were converted in mass concentrations $C_{Au(UV)}$ according to Equation SI I-4. For the same sample solutions also the mass concentrations $C_{Au(ICP)}$ as determined with ICP-MS are provided. Comparison of $C_{Au(UV)}$ and $C_{Au(ICP)}$ provides insight in the experimental error of concentration determination. All values in the table are rounded.

The Au concentration in the NP/NR samples $C_{Au(ICP)}$ was also directly measured with elemental analysis by inductively coupled plasma-mass-spectrometry (ICP-MS, Agilent 7700, Germany). There is a discrepancy between the mass concentrations as determined by UV/vis absorption spectroscopy and ICP-MS, see Table SI I-2. According to Table SI I-2, the mass concentration of based on the ICP-MS measurements is always lower than the one as calculated from the UV/vis absorption data, except for the 100 nm Au NPs. This is mainly due to the fact that the NPs/NRs are not perfect spheres/tubes as assumed for the calculation of the molar masses M_{NP} (Table SI I-1). For this reason, the $C_{Au(UV)}$ values as derived from the UV/vis absorption measurements and the estimated M_{NP} values are affected by errors. Unfortunately this became evident only at the end of our study. Therefore, our metrics is based on $C_{Au(UV)}$ and unfortunately not on $C_{Au(ICP)}$, which would have been the better choice, not only due to the unreliability of M_{NP}, but also due to the fact that the amount of Au inside cells was also determined by ICP-MS and not by UV/vis absorption. For most of our studies with cells incubation concentrations of $C_{Au(UV)}$ = 10 µg/mL and 20 µg/mL were used. To get a better feeling for the error in concentration determination in Table SI I-3 the corresponding ICP-MS mass concentrations are listed. In the cellular uptake studies later the effect of the concentration determination errors (i.e. while cells have been incubated with the different NP/NR solutions at the same $C_{Au(UV)}$ values, the incubation concentrations in terms of $C_{Au(ICP)}$ were different for the different NP/NR samples), cf. section IV.6.

Sample	5 nm Au NPs	25 nm Au NPs	50 nm Au NPs	100 nm Au NPs	40 nm Au NRs	100 nm Au NRs
$C_{Au(UV)}$ [µg/mL]	10	10	10	10	10	10
$C_{Au(ICP)}$ [µg/mL]	5.9	5.3	7.2	10.8	6.6	5.1
$C_{Au(UV)}$ [µg/mL]	20	20	20	20	20	20
$C_{Au(ICP)}$ [µg/mL]	11.8	10.5	14.4	21.6	13.1	10.2

Table SI I-3. Comparison of exposure concentrations as calculated for UV/vis absorption ($C_{Au(UV)}$) and determined by ICP-MS ($C_{Au(ICP)}$).

I.5 Dynamic light scattering and zeta-potential measurements

To determine the hydrodynamic diameters d_h of the NPs, dynamic light scattering (DLS) was use as described before [1] Data are shown in Figure SI I-3. No DLS measurements were performed on the NRs. The zeta-potentials ζ for the different NPs/NRs were measured with laser Doppler anemometry [1] Data are presented in Figure SI I-4. Both measurements were carried out with a Malvern ZetasizerNano particle analyzer ZEN 3600 instrument in MilliQ water.

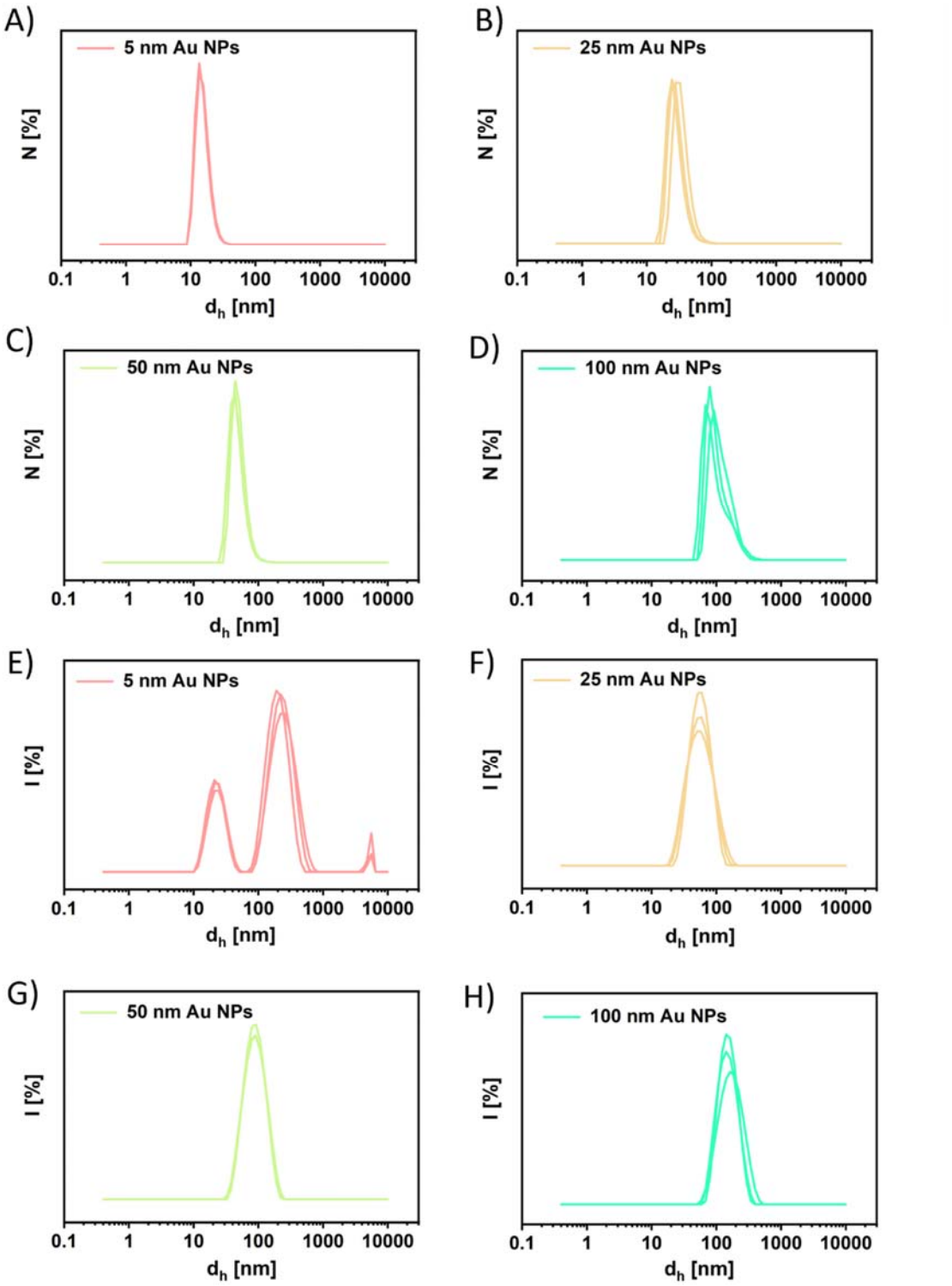

Figure SI I-3. Hydrodynamic diameter d_h of the different Au NPs/NRs as determined by DLS in terms of (A, B, C, D) number distribution and (E, F, G, H) intensity distribution. The mean hydrodynamic diameters as determined from these distributions are shown in Table SI I-4.

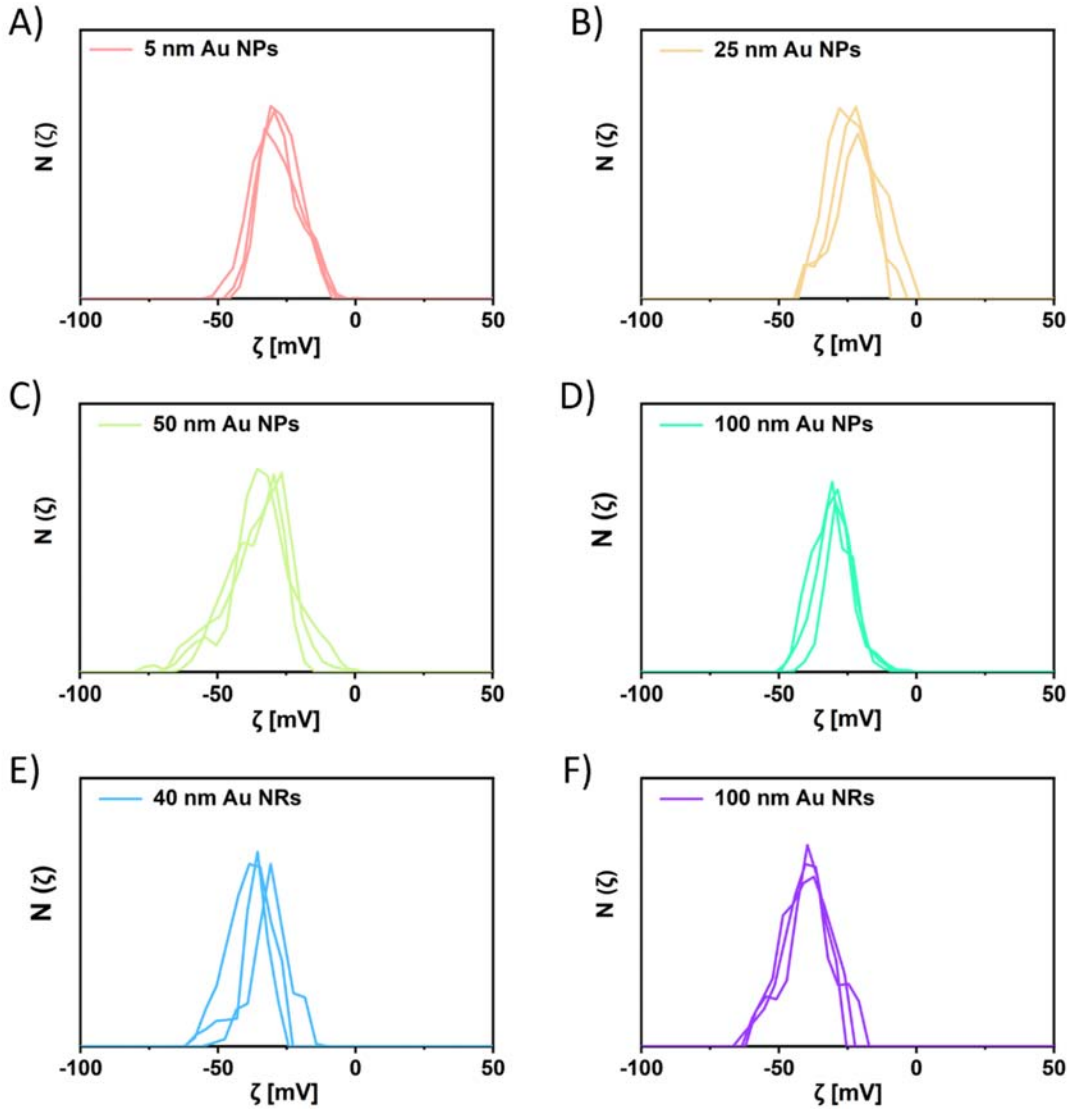

Figure SI I-4. Zeta potentials ζ of the different NPs/NRs. The mean zeta potentials as extracted from these distributions are shown in Table SI I-4.

I.6 UV/vis absorption spectroscopy

UV/vis absorption spectra of the Au NP and Au NR solutions were recorded in MilliQ water with an Agilent 8453 spectrometer, see Figure SI I-5. From the spectra the wavelength of the surface plasmon resonance peak λ_{SPR} was determined, see Table SI I-4.

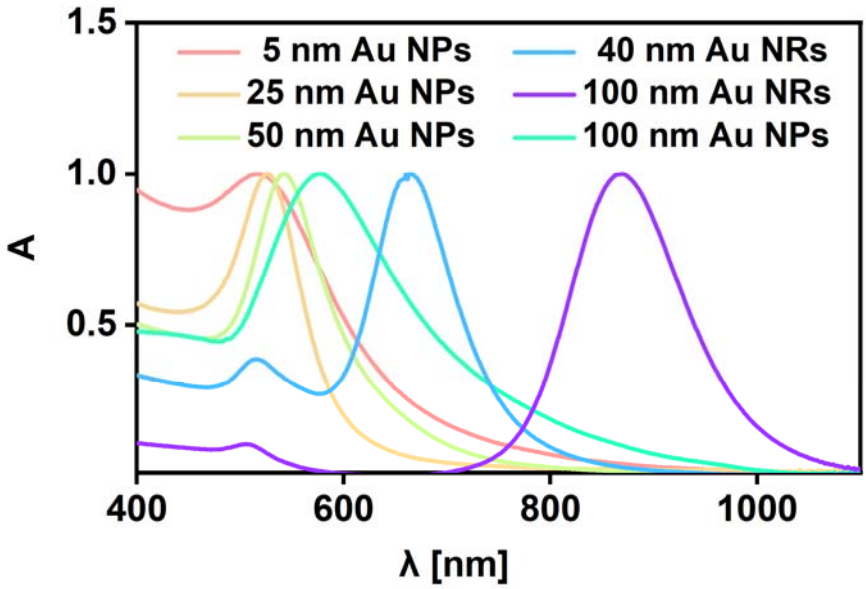

Figure SI I-5. Normalized UV/vis absorption spectra of the different NP/NR solutions.

Sample	λ_{SPR} [nm]	d_c [nm]	L_c [nm]	$d_{h(N)}$ [nm]	PdI	ζ [mV]
5 nm Au NPs	517	4.8 ± 0.9	-	15.5 ± 0.3	0.26 ± 0.04	-31.8 ± 2.3
25 nm Au NPs	526	25.8 ± 1.3	-	30.6 ± 3.5	0.23 ± 0.01	-40.6 ± 1.9
50 nm Au NPs	543	50.4 ± 4.9	-	47.9 ± 2.2	0.20 ± 0.00	-38.9 ± 0.9
100 nm Au NPs	578	101.0 ± 7.8	-	109.4 ± 8.7	0.11 ± 0.03	-47.1 ± 2.1
40 nm Au NRs	675	15.7 ± 1.9	39.3 ± 2.5	-	-	-39.1 ± 2.1
100 nm Au NRs	870	25.2 ± 1.4	99.3 ± 3.9	-	-	-41.0 ± 3.0

Table SI I-4. Characterization data of the different NP/NR samples: wavelength of surface plasmon resonance peak λ_{SPR}, core diameter as determined by TEM d_c, core length for the NRs as determined by TEM L_c, mean hydrodynamic diameter as determined from the DLS number distribution $d_{h(N)}$, DLS polydispersity index PdI, and zeta potential ζ. Data are given as mean value ± standard deviation (SD).

I.7 Interfacial tension measurements at the Au NP/NR toluene interface

The dynamic interfacial tension (IFT) at the Au NP/NR solution-toluene interface was determined by the pendant drop tensionmetry using a Drop shape analyzer (DSA25, Krüss, Germany), analogues to a previous report with an outer needle diameter of 1.85 mm and an inner need

diameter of 0.84 nm[3]. Au NP/NR solution at concentration $C_{Au(UV)}$ = 112 µg/mL was filled in a Hamilton syringe plugged to a stainless steel needle. A sample droplet of 50 µL of the NP/NR solution was injected within ca. 15 s into toluene phase. Injection of 50 µL of water served as control. The droplet was recorded by an ultrafast camera (Krüss, Germany) for 30 min in intervals of 1 s. Using the analysis software package for the set-up (Krüss, Germany) the surface tension γ was calculated and plotted versus time, see Figure SI I-6. There was almost no time dependence in the surface tension and thus mesoequilibrium was reached fast. The values for all NP/NR samples are similar within the experimental error and similar to the the surface tension γ ≈ 35 mN/m of the control water in toluene sample. This indicates that the surface of all NP/NR samples have similar hydrophilicity/hydrophobicity. This is expected, as all NP/NR samples had the same surface chemistry.

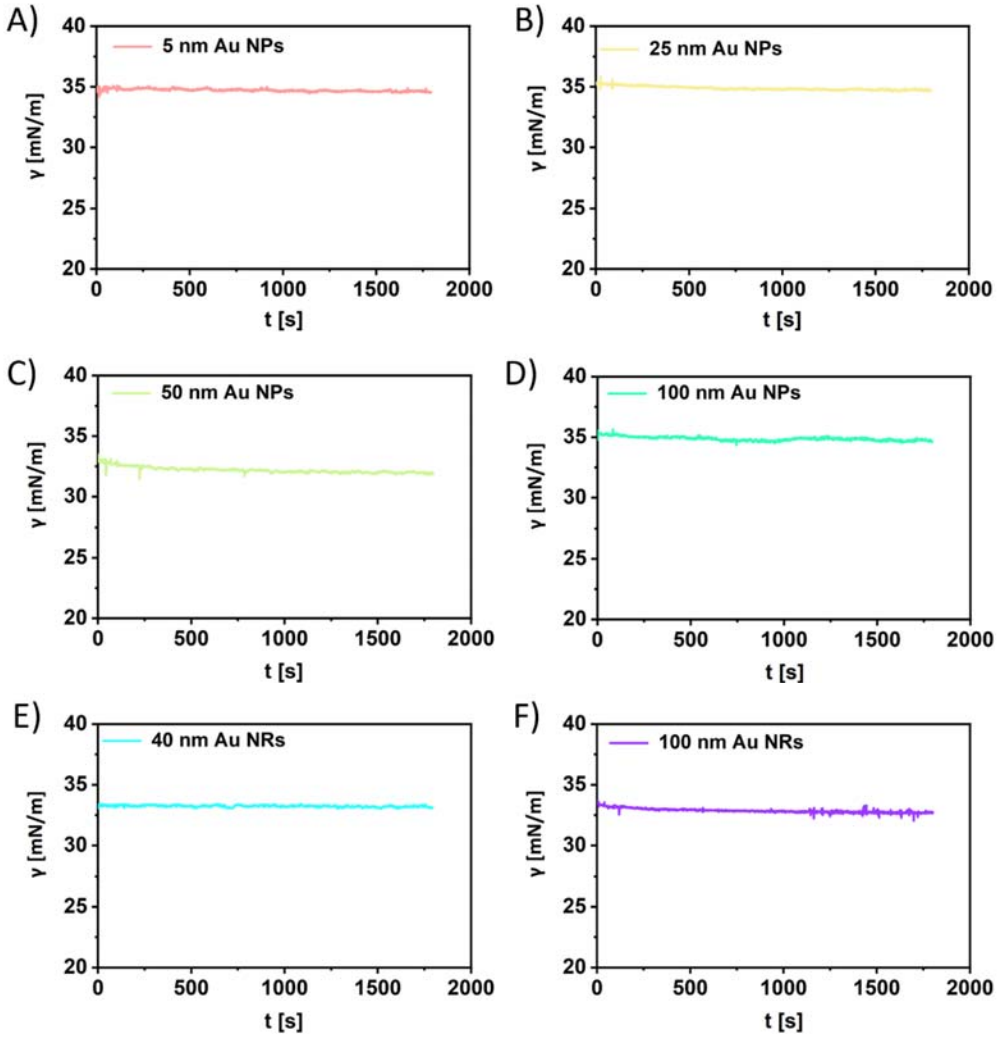

Figure SI I-6. Dynamic IFT at the aqueous Au NPs/Au NR solution-toluene interface during 30 min measurement time, plotting the surface tension γ over time. The graphs are representative for three parallel measurements that have been carried out per sample.

II Cell culture and exposure geometries

II.1 General cell culture protocols

Human cervical adenocarcinoma cells (HeLa cell line CCL2) were purchased from American Type Culture Collection (ATCC, Manassas, USA). Briefly, cells were maintained in complete cell culture media, which is Dulbecco's Modified Eagle's Medium (DMEM high glucose content, pyruvate, Gibco^{TM}, Germany) complemented with 10% of fetal bovine serum (FBS, Biochrom, Germany), and 1% of (penicillin-streptomycin (p-s), Fisher Scientific, Germany). Cells were incubated at 37 °C in a 5% CO_2 humid air atmosphere incubator. When cells had reached approximately 90% confluency, which was usually two days after cell passaging, cells were passaged. For this, cells were washed one time with 10 mL of phosphate buffered saline (PBS without Ca^{2+} and Mg^{2+}, Biochrom, Germany), and then dissociated with 1 mL of trypsin ethylenediaminetetraacetic acid (Trypsin-EDTA, 0.05%) solution (Fisher Scientific, Germany) after the removal of the PBS. When the cells had detached 10 mL complete cell culture media was added to stop the trypsin digestion. Then cells were gently pipetted in a centrifuge tube and were centrifuged at 300·g for 5 min. After that, cells were suspended in complete cell culture media and then counted and seeded in new 75 cm^2 flasks for further culture or seeded in culture plates for performing the *in vitro* experiments as described in the following sections.

II.2 Description of the different exposure geometries

The 4 different exposure geometries as used in this study are shown in Figures SI II-1 and SI II-2. The "static" system corresponds to the classical geometry of adherent cell cultures, where cells are adherently grown at the bottom of a cell culture flask with the medium above the cells. In the "dynamic" system cells were also adherently grown at the bottom of a cell culture flask, but the flask with the medium was rotated with 10 rpm. In the "fluid" system, cells were grown adherently at the surface of a flow channel (µ-slides I 0.2 Luer, Ibidi GmbH, Martinsried, Germany) and a perfusion set pump a continuous unidirectional flow of the medium above the cells was made. In the "suspension" system the cells and medium were immediately rotated in 15 mL Falcon tubes with 10 rpm, so that the cells did not adhere but remained suspended. In this system the suspended HeLa cells initially has spherical morphology, but after around 8 h of incubation, the cells had assembled to macroscopic tumor spheroids and thus had completely different morphology as in the 3 other systems. For incubation of cells with NP the medium was supplemented with the NPs at the desired concentration $C_{Au(UV)}$.

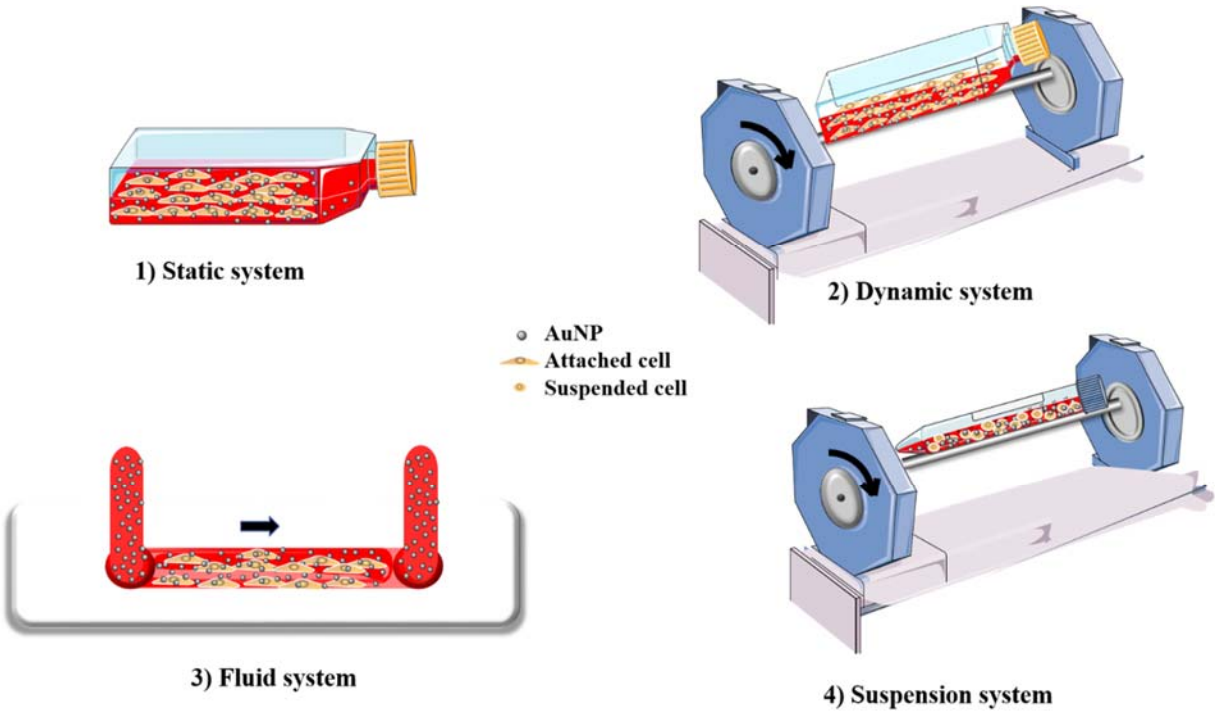

Figure SI II-1 Schematic graphs of the 4 incubation systems.

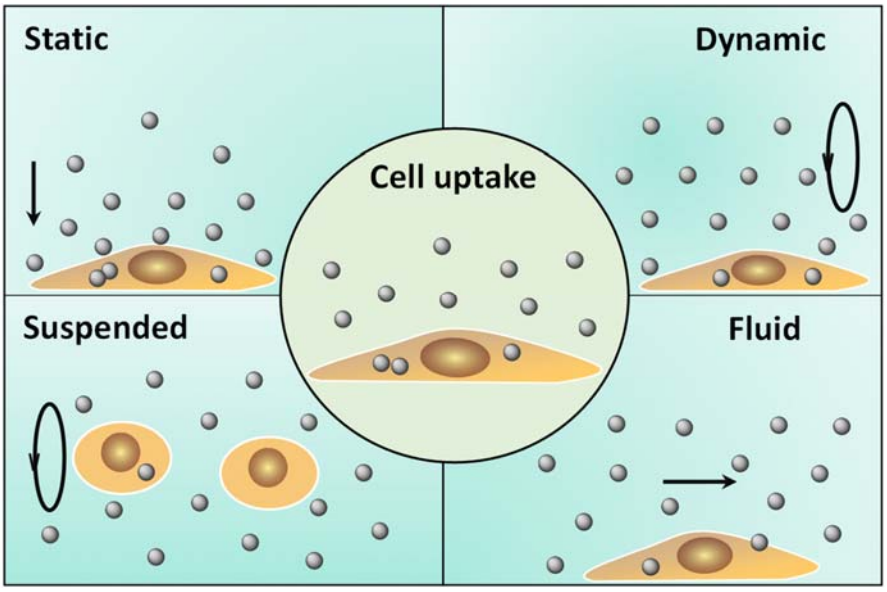

Figure SI II-2 Schematic figure of the cells and Au NPs/NRs and their movement in the four different incubation systems.

II.3 Quantification of cell proliferation

To investigate the effect of the incubation systems on cell growth, the number of cells was plotted over time. For the "static" and "dynamic" exposure geometry, 3×10^5 HeLa cells were seeded in the 12.5 cm^2 seeding area culture flasks ("T12.5 flask"; corresponding to a cell density of 24,000 cells/cm^2) with a volume of 3 mL or 6 mL complete cell culture media added. For the "fluid" exposure geometry, 1×10^5 cells were seeded per µ-slide (0.2 Luer, seeding area 2.5 cm^2) at a cell density of 40,000 cells/cm^2 and after attachment to the surface, the µ-slide was connected to a pump with 6 mL complete cell culture medium. For the "suspension" exposure geometry, 3×10^5 cells were seeded in 3 mL or 6 mL complete cell culture medium in 15 mL falcon tubes. Seeding in the different geometries was done with several flasks/slides/tubes in parallel. Every 24 h cells in one flask/slide/tube were counted (for the adherent cells the cells had to by trypsinyzed for this), so that at the end then number of cells at different time points of incubation could be determined, see Figure SI III-3. Data indicate that cell proliferation was similar in the "static", "dynamic", and "fluid" exposure geometry, while in the "suspension" geometry, the cell proliferation rate was much lower.

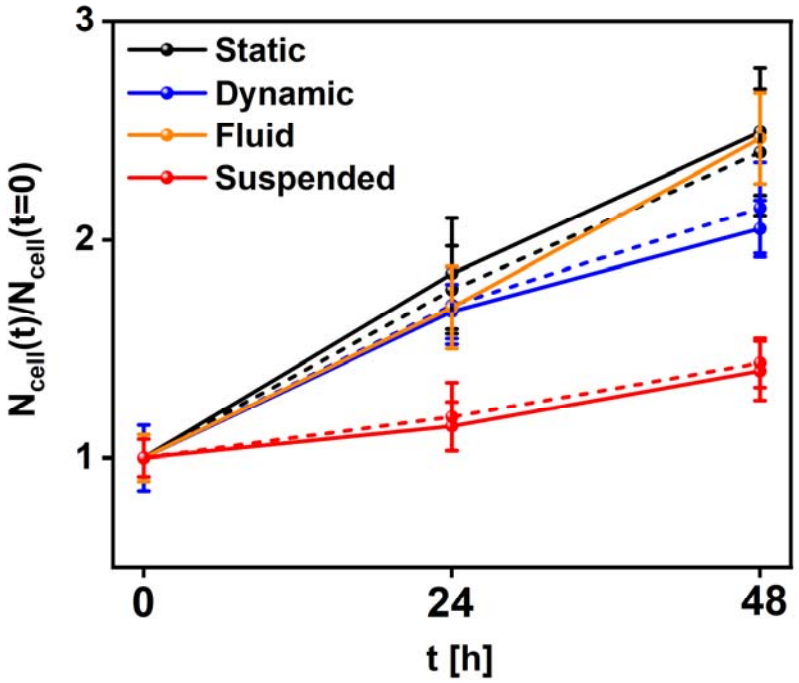

Figure SI II-3. HeLa cell growth curves for the "static", "dynamic", "fluid", and "suspension" geometries over time. The number of cells N_{cell} is plotted over time, normalized to the number of cells at time t = 0. The time point t = 0 here in case of the adherent cells does not refer to the time of seeding, but to a point of time when cells had become adherent to the surface. We note, that due to the normalization a shift in the time point t = 0 of a few hours will not change the nature of the here presented graph and thus the data are considered to be consistent, despite the fact that the

"time when cells had become adherent" is not precisely defined. The solid lines represent culture in 6 mL cell media, while the dashes lines represent systems culture in 3 mL cell media. The data shown correspond to mean values ± standard deviations (SD) from three independent experiments.

III Cell viability

III.1 Concentration-dependence of cell viability of cells exposed to NPs/NRs under "static" conditions based on the resazurin assay

The evaluate potential cytoxicity of Au NPs/NRs to HeLa cells, the resazurin assay was performed as previously reported [4]. To perform the resazurin assay, 7500 cells in 100 µL complete cell culture media were seeded in per well in 96 well plates (seeding area per well = 0.32 cm^2). The ells were kept overnight in a 37 °C humid incubator containing 5% CO_2. The next day, the media was discarded by aspiration and the cells were exposed to 100 µL Au NP/NR containing complete cell medium at concentrations $C_{Au(UV)}$ for 24 and 48 h. 100 µL fresh complete media (i.e. $C_{Au(UV)} = 0$) were used as control sample. After the desired exposure time, the medium was aspirated and the cells in the wells were washed twice with 100 µL PBS. Cells in each well were then stained with 100 µL of 0.025 mg/mL resazurin solution, which was prepared using 10% volume to volume percentage (V/V) resazurin solution of 0.25 mg/mL concentration (Sigma-Aldrich, R7017) mixed with 90% V/V complete culture media. After 4 h incubation in a 37 °C humid incubator, the fluorescence I_{FL} of the plates was read with a fluorescence meter (Fluorolog-3, from Jobin Yvon inc, USA, connected with a microplate reader stage). For the fluorescence read-out the excitation wavelength was λ_{exc} = 560 nm, and the emission spectra was collected from 580 nm to 600 nm. The cell viability V was calculated according to the following equation:

$$V(C_{Au(UV)}) = \frac{I_{FL}(C_{Au(UV)}) - I_{FL,blank}}{I_{FL}(C_{Au(UV)}=0) - I_{FL,blank}} \qquad \text{Equation SI III-1}$$

$I_{FL,blank}$, $I_{FL}(C_{Au(UV)}=0)$, and $I_{FL}(C_{Au(UV)}=0)$ refer to the mean fluorescence intensity between 580 nm to 582 nm from the wells of blank, control, and Au NP/NR samples. The blank sample was water in the wells. The results for the concentration-dependent viability are shown in Figure SI III-1.

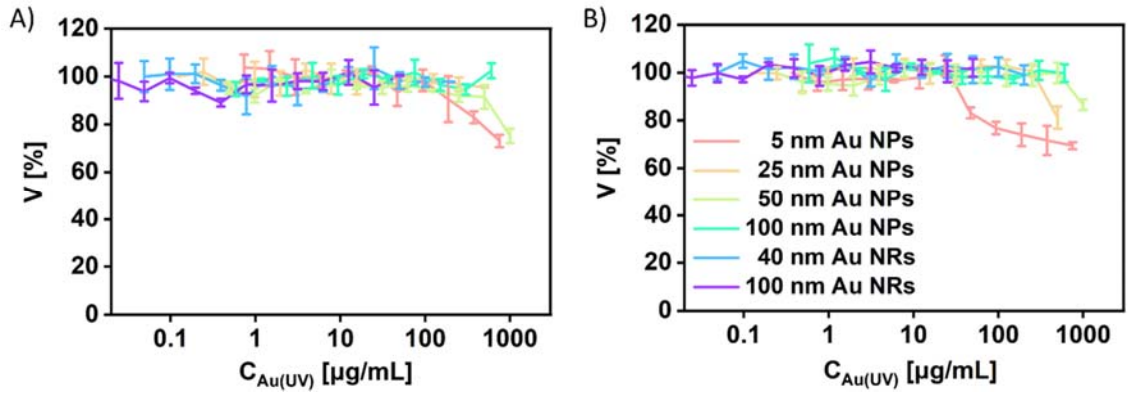

Figure SI III-1. Cell viability V of HeLa cells after having been exposed to 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs for A) 24 h, and B) 48 h in "static" exposure conditions. The

data in the figure correspond to mean values ± standard deviations (SD) from three independent experiments.

The data show, that for all of the NP/NR samples there is reasonable cell viability at $C_{Au(UV)}$ = 10 µg/mL and 20 µg/mL, which were the exposure concentrations as used for the uptake experiments.

III.2 Exposure condition-dependence of cell viability of cells exposed to NPs/NRs based on the resazurin assay

As next step the influence of the different exposure conditions on the cell viability were carried out using the standard resazurin assay. This was done at a NP/NR exposure concentration of $C_{Au(UV)}$ = 20 µg/mL, which was the highest used NP concentrations used for the uptake experiments. Cells were cultured following the same procedures as in the uptake study and then the resazurin assay was conducted. Briefly, $3 \cdot 10^5$ cells in 3 mL complete cell media were seeded in T12.5 flasks for the "static" and "dynamic" exposure geometry (seeding area per flask = 12.5 cm^2). $1 \cdot 10^5$ cells in 100 µL complete cell media were seeded in microfluidic channels (µ-slides I 0.2 Luer, Ibidi GmbH, Martinsried, Germany) for the "fluid" exposure geometry (seeding area in the flow channel = 2.5 cm^2). These cells were incubated in a cell incubator overnight, allowing for attachment of the cells to the substrate. The next day, the old cell medium was aspirated, and the cells were incubated in 3 mL ("static", "dynamic" geometry) or 6 mL ("fluid" geometry) of Au NP/NR containing complete culture medium at the concentration of $C_{Au(UV)}$ = 20 µg/mL for 24 h. "No NPs" samples are HeLa cells of the same passage number that were exposed to complete cell media instead of Au NP/NR solution, i.e. $C_{Au(UV)}$ = 0. After 24 h of incubation, the cells were washed and trypsinated as described in section II.1. After addition of fresh medium to quench the effect of trypsin, the detached cells were centrifuged at 300 rcf for 5 min and the supernatant was discarded. Then cell pellets were redispersed in 1 mL complete culture media and counted. The cell density was adjusted by additional addition of complete cell medium to $1.5 \cdot 10^5$ cell/mL. 100 µL of this cell suspension (which contained 15,000 cells) were seeded per well in 96 well plates with triplicate wells and were incubated for several hours until the cells had attached to the wells (usually this takes about 5 h). Then the medium was discarded by aspiration and 100 µL of 0.025 mg/mL resazurin solution were added per well. After 4 h of incubation, the fluorescence of the plates was read determined by a fluorescence meter and the cell viability was calculated, as described in section III.1. All cell viability data was obtained from three independent experiments with cells of three passage numbers. The results are presented in Figure SI III.2 and show, that under the used conditions there was no reduction of cell viability due to exposure to NPs/NRs.

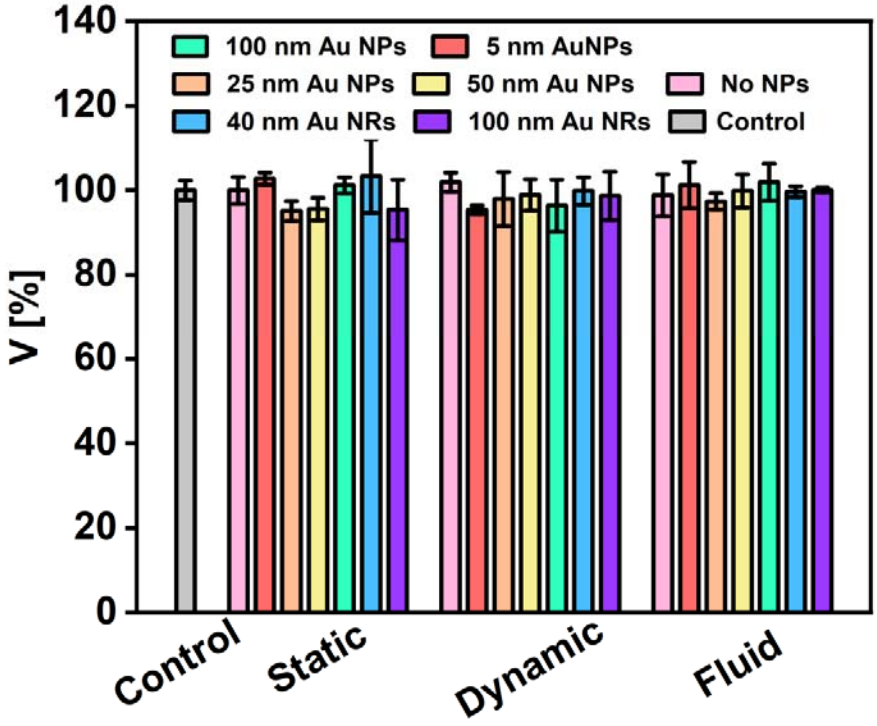

Figure SI III-2. Cell viability V of HeLa cells after exposure to 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs at $C_{Au(UV)}$=20 µg/mL for 24 h in "static", "dynamic", and "fluid" geometry. The data are mean values ± SDs from three independent experiments.

III.3 Exposure condition-dependence of cell viability of cells exposed to NPs/NRs based on a life/dead assay

As additional probe for possible cytotoxic effects of the NPs/NRs on cells a commercial life/dead assay (FisherScientific, L3224) was used [5]. In the case of the "static" and "dynamic" exposure geometry, HeLa cells in complete cell medium were seeded in Ibidi µ-slide 8 well plates at the density of 10000 cells/well with a volume of 300 µL/well and were kept in a cell incubator overnight for cell attachment. The next day, cells of three triplicate wells were exposed to 300 µL Au NP/NR containing complete culture medium per well at the concentration of $C_{Au(UV)}$ = 20 µg/mL and were cultured under "static" and "dynamic" exposure conditions for 24 h. For the 2 controls, "no NP" and "ethanol" cells were just exposed to complete cell media without Au NPs/NRs. After 24 h exposure, for all but for the "ethanol" control sample sample, the medium was removed by aspiration and cells were washed three times with 100 µL PBS. In the case of the "ethanol" sample, which acted as positive control (i.e. all cells are dead), after 24 h the medium was discarded by aspiration and then the cells were killed by exposure to 100 µL 75% ethanol for

20 min, after which the ethanol was removed and cells were washed twice with 100 µL PBS. After the washing all samples were exposed to 100 µL of live/dead assay reagents (2 µM Calcein AM and 4 µM Ethidium homodimer-1 in PBS) per well for 45 min at room temperature (RT). Afterwards, images of the cells and their fluorescence in the greed and red were recorded with a confocal microscope (Karl Zeiss LSM 510, Germany), see Figure SI III-3.

In the case of the "suspension" exposure geometry, $3 \cdot 10^5$ cells were suspended in 6 mL complete media in 15 mL Falcon tubes. Then an appropriate amount of Au NP/NR solution was added to reach a final exposure concentration of $C_{Au(UV)}$ = 20 µg/mL. For the 2 controls, "no NP" and "ethanol" cells were just exposed to complete cell media without Au NPs/NRs. The Falcon tubes were installed on a tube revolver (Thermo ScientificTM) in a 37 °C incubator and were rotated at a speed of 10 rpm for 24 h as described in section II.1. Afterward, for all samples except the "ethanol" control samples, cells were firstly centrifuged at 300 rcf for 5 min and then the cell pellet was washed twice by addition of 1 mL PBS and centrifugation at 300 rcf for 5 mins followed by discarding the supernatant. In case of the "ethanol" control sample the cells were firstly centrifuged at 300 rcf for 5 min, the supernatant was discarded, and cells were redispersed in 1 mL of 75% ethanol for 20 min. Cells were then again centrifuged at 300 rcf for 5 min, the supernatant was discarded and the cell pellet was redispersed in 1 mL PBS, again centrifuged at 300 rcf for 5 min and supernatant was discarded. In all cases cells were redispersed in 0.5 mL live/dead assay solution. Cells were counted and the cell density in solution was adjusted to $3 \cdot 10^5$ cells/mL by further addition of live/dead assay reagent. 100 µL of the cell suspension in the live/dead assay reagent (i.e. $3 \cdot 10^4$ cells) were seeded per well of Ibidi µ-slide 8 wells and were incubated for 45 min at room temperature. Afterwards, images of the cells and their fluorescence in the green and red were recorded with a confocal microscope (Karl Zeiss LSM 510, Germany), see Figure SI III-3.

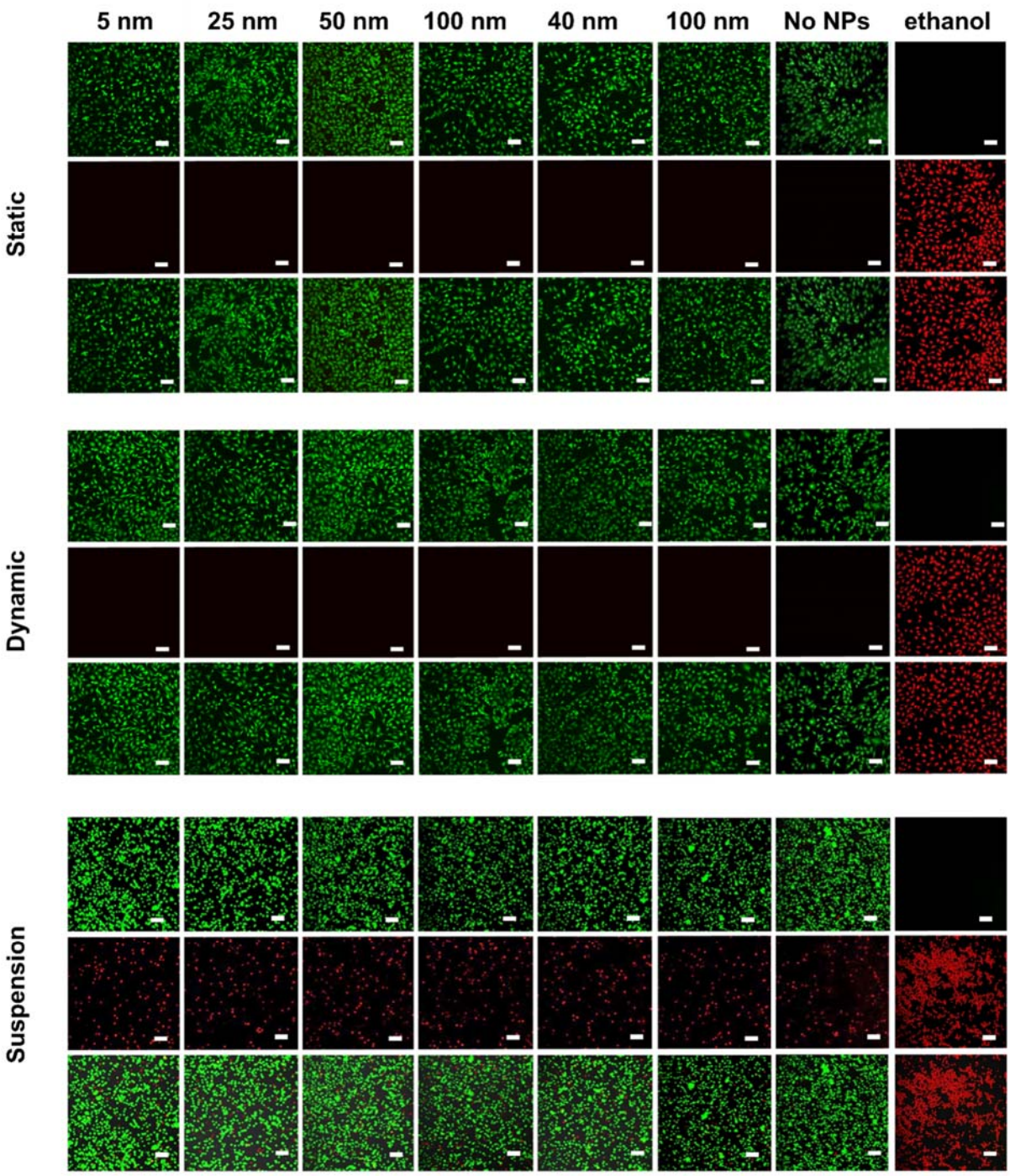

Figure SI III-3. Live/dead staining images of cells which had been incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs at $C_{Au(UV)}$ = 20 μg/mL for 24 h. The scale bars correspond to 50 μm. For the green and red channels of fluorescence the following settings were used: λ_{ex} = 488 nm, λ_{em} = 517 nm, and λ_{ex} = 535 nm λ_{em} = 617 nm.

The cell viability V was calculated according to Equation SI III-2.

$$V = \frac{N_{cell(g)}}{N_{cell(g)} + N_{cell(r)}} \qquad \text{Equation SI III-2}$$

$N_{cell(g)}$ is the number of live cells as marked by their green color of fluorescence in each image, and $N_{cell(r)}$ is the number of dead cells as marked with red color in each image. Recorded data was from three independent experiments with cells of three passage numbers, see Figure SI III-4. Data confirm that under the used NP exposure conditions the cells remained fully viable.

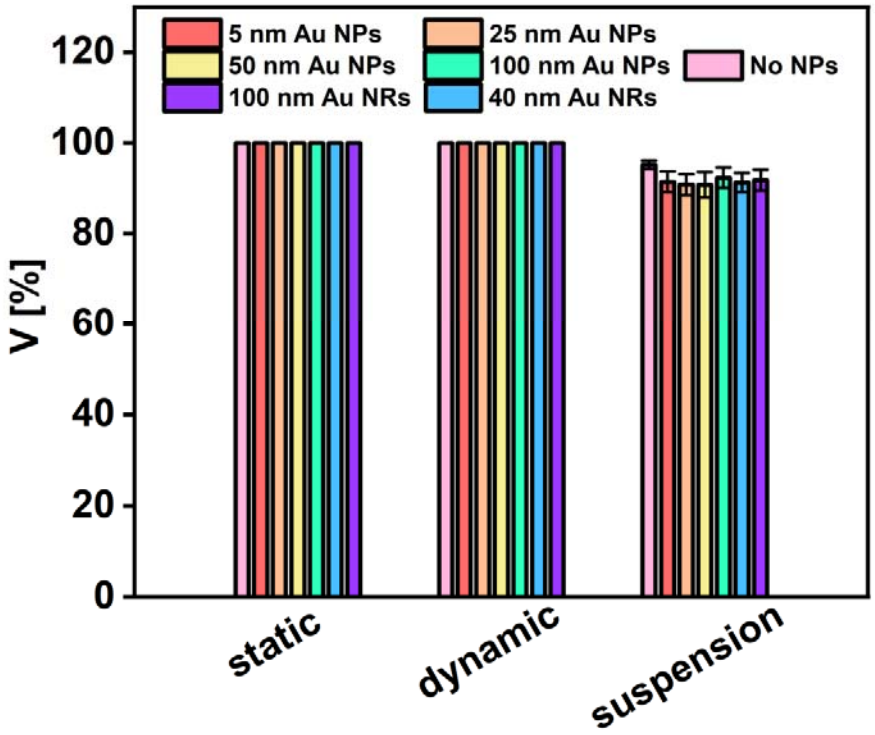

Figure SI III-4. Cell viability V of cells which had been exposed for 24 h to $C_{Au(UV)}$ = 20 µg/mL NPs/NRs. The data correspond to mean values ± SDs from three independent experiments.

IV Cellular uptake of NPs and NRs

IV.1 "Static" culture conditions

HeLa cells were seeded in T12.5 cm^2 flask (12.5 cm^2 seeding area) with the density of $3 \cdot 10^5$ cells in 3 mL complete cell culture media, resulting in a seeding density of 24,000 cells/cm^2. After having had enough time to attach to the surface of the flask, the old medium was removed and the cells were exposed to either V_{exp} = 3 mL or 6 mL of Au NPs/NRs dispersed in complete culture medium with the concentration $C_{Au(UV)}$ = 10 µg/mL and 20 µg/mL. Cells were incubated with the Au NPs/NRs for t_{exp} = 4, 8, and 24 h. After the respective incubation, the cells were washed twice with 1 mL Dulbecco's phosphate-buffered saline (DPBS, i.e.PBS containing Ca^{2+} and Mg^{2+}) and 1 time with 1 mL PBS, then cells were detached by adding 0.3 mL of trypsin (0.05%).

Then, 1 mL of DPBS was added to disperse the cells. Afterwards, cells were counted (number of cells after exposure time t_{exp}: $N_{cell}(t_{exp})$). Cells were then centrifuged at 300 rcf for 5 min and the supernatant above the cell pellet was discarded. The mass of Au in the cell pellet $m_{Au/pellet}$ was measured by ICP-MS. To prepare the ICP-MS samples, the cell pellet was firstly digested with 50 µL of HNO_3 (aq, 67 wt%) overnight, and then 100 µL of HCl (aq, 35 wt%) was added, to further dissolve the Au NPs/NRs. Then the digested sample was diluted with 2 mL of 2% V/V HCl (prepared by mixing 2 mL 35 wt% HCl aq. with 98 mL Milli-Q water). This diluted sample was used for the IPC-MS measurements. By dividing the mass of Au found in the pellet by the number of cells in the pellet, the amount of Au per cell was determined:

$$m_{Au/cell} = m_{Au/pellet} / N_{cell(pellet)} \qquad \text{Equation SI IV-1}$$

The results for the different NPs/NRs and exposure times are given in Table SI IV-1 and Figure SI IV.1 and Figure SI IV.2.

Sample			5 nm Au NPs	25 nm Au NPs	50 nm Au NPs	100 nm Au NPs	40 nm Au NRs	100 nm Au NRs
$C_{Au(UV)}$ [µg/mL]	V_{exp} [mL]	t_{exp} [h]						
10	3	4	0.12 ± 0.03	0.13 ± 0.04	0.75 ± 0.23	3.48 ± 0.88	0.22 ± 0.00	0.23 ± 0.04
10	3	8	0.16 ± 0.01	0.27 ± 0.10	2.00 ± 0.34	6.02 ± 1.28	0.24 ± 0.03	0.38 ± 0.12
10	3	24	0.22 ± 0.02	0.58 ± 0.05	5.35 ± 1.47	7.63 ± 2.10	0.49 ± 0.14	2.15 ± 0.01
10	6	4	0.11 ± 0.05	0.11 ± 0.05	0.87 ± 0.30	3.48 ± 0.73	0.23 ± 0.02	0.27 ± 0.06

10	6	8	0.17 ± 0.03	0.22 ± 0.07	2.16 ± 0.51	6.46 ± 0.63	0.21 ± 0.06	0.67 ± 0.06
10	6	24	0.22 ± 0.03	0.59 ± 0.07	6.67 ± 1.65	10.47 ± 1.33	0.52 ± 0.14	2.73 ± 0.02
20	3	4	0.25 ± 0.07	0.21 ± 0.09	1.75 ± 0.38	5.16 ± 0.72	0.26 ± 0.05	0.48 ± 0.04
20	3	8	0.25 ± 0.09	0.32 ± 0.05	2.81 ± 0.23	8.13 ± 0.63	0.34 ± 0.11	1.13 ± 0.14
20	3	24	0.51 ± 0.07	0.87 ± 0.01	9.99 ± 1.80	11.54 ± 2.16	0.78 ± 0.19	3.30 ± 0.24
20	6	4	0.24 ± 0.03	0.18 ± 0.05	2.10 ± 0.28	6.36 ± 0.64	0.19 ± 0.04	0.58 ± 0.03
20	6	8	0.25 ± 0.07	0.33 ± 0.09	2.97 ± 0.26	10.78 ± 2.38	0.33 ± 0.05	1.17 ± 0.01
20	6	24	0.50 ± 0.12	0.89 ± 0.11	11.70 ± 1.26	16.7 ± 3.65	0.83 ± 0.14	4.69 ± 0.59

Table SI IV-1. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "static" exposure geometry.

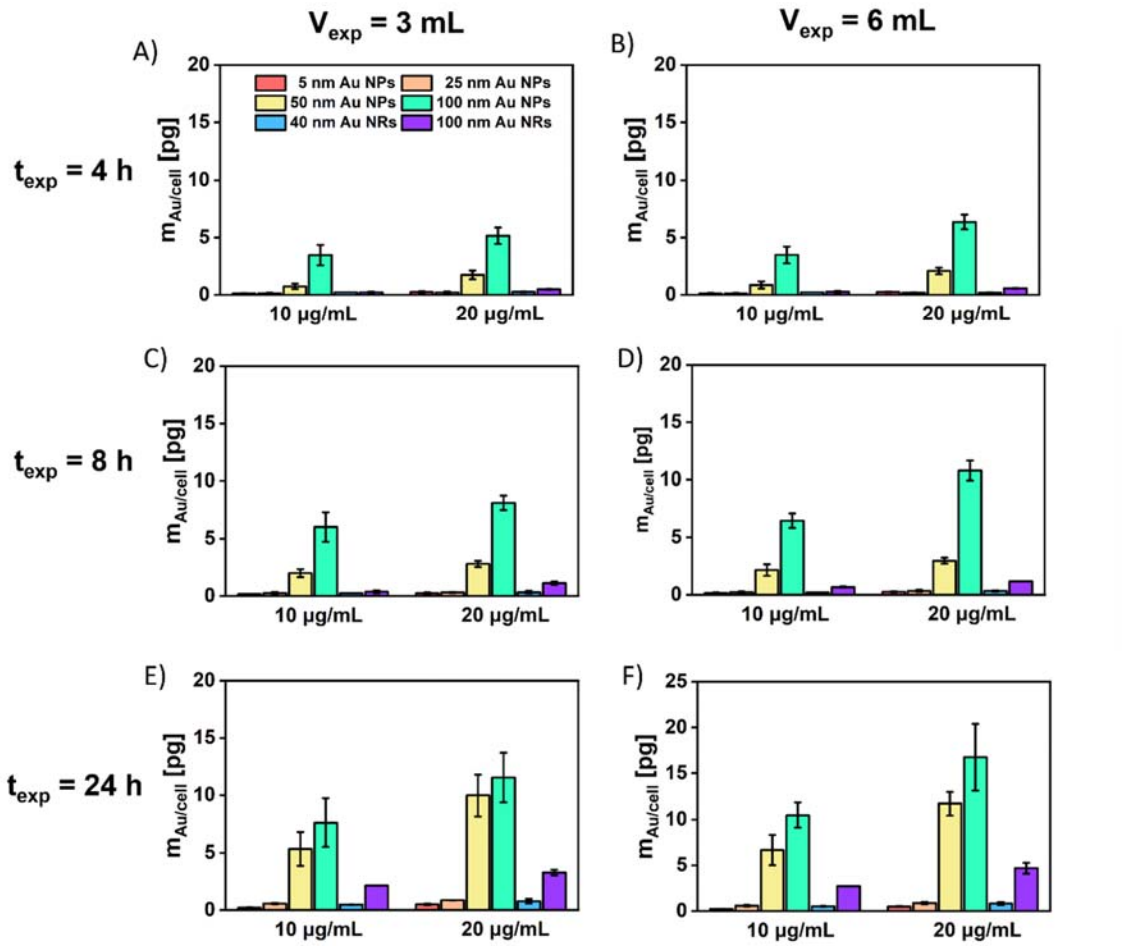

Figure SI IV-1. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "static" exposure geometry. (A, C, E) V_{exp} = 3 mL, (B, D, F) V_{exp} = 6 mL. (A, B) t_{exp} = 4 h, (C, D) t_{exp} = 8 h, (E, F) t_{exp} = 24 h. The data correspond to mean values ± SDs from three independent experiments.

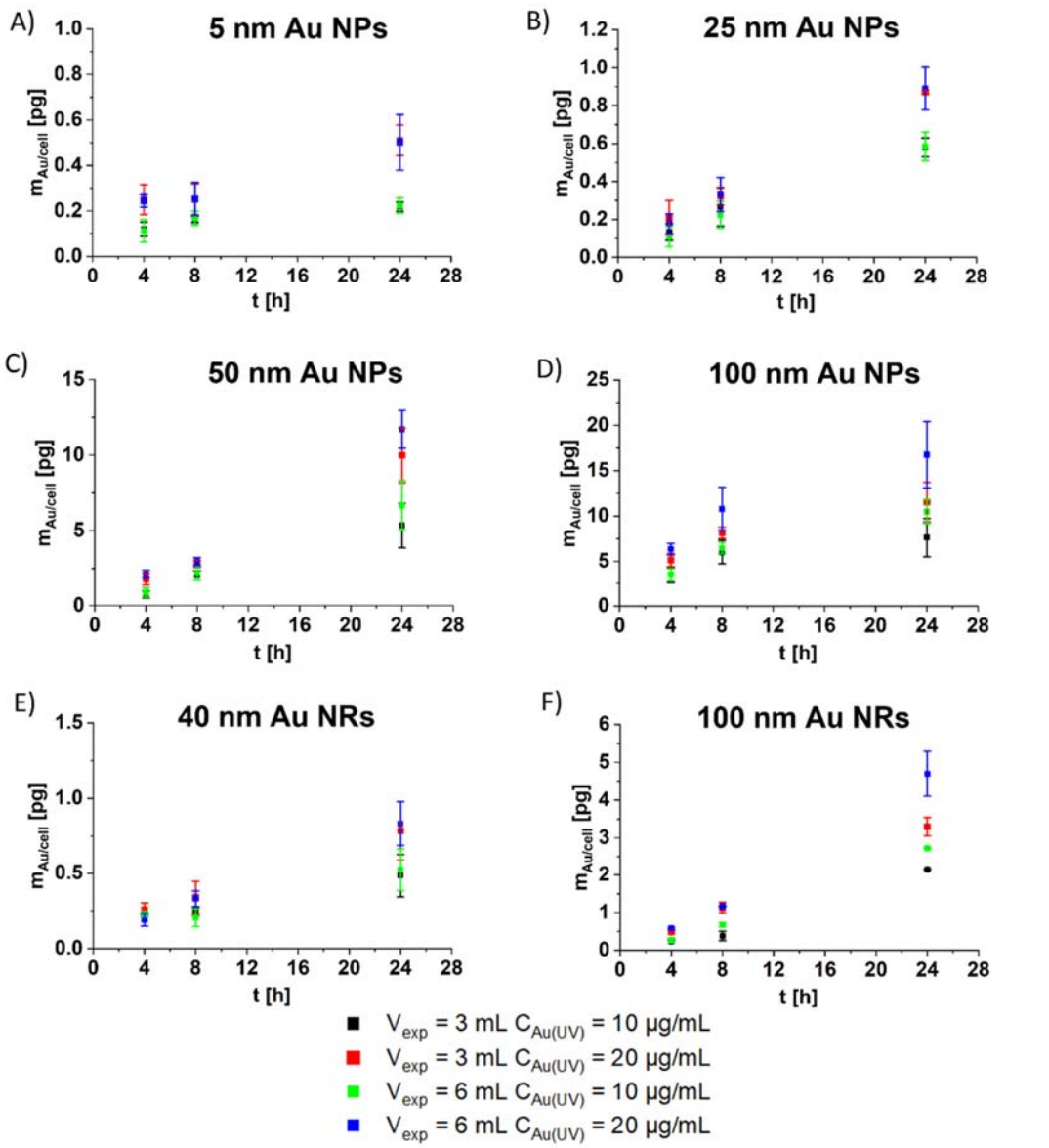

Figure SI IV-2. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "static" exposure geometry. The data correspond to mean values ± SDs from three independent experiments. These are the same data as shown in Figure SI IV-1, but displayed in another format. If in the graphs not all points for the four colors are visible this is because the black dots are covered by the red dots and the green dots are covered by the yellow dots.

IV.2 "Dynamic" culture conditions

The exposure scenario was the same as described in section IV.1 with the exception that during NP/NR exposure of cells the flasks were rotated at a speed of 10 rpm. The results for the different NPs/NRs and exposure times are given in Table SI IV-2 and Figure SI IV.3 and Figure SI IV.4.

Sample			5 nm Au NPs	25 nm Au NPs	50 nm Au NPs	100 nm Au NPs	40 nm Au NRs	100 nm Au NRs
$C_{Au(UV)}$ [µg/mL]	V_{exp} [mL]	t_{exp} [h]						
10	3	4	0.13 ± 0.02	0.14 ± 0.03	0.62 ± 0.16	1.49 ± 0.11	0.19 ± 0.03	0.52 ± 0.05
10	3	8	0.15 ± 0.01	0.32 ± 0.00	0.98 ± 0.11	1.67 ± 0.32	0.15 ± 0.02	0.83 ± 0.09
10	3	24	0.30 ± 0.10	0.48 ± 0.10	0.80 ± 0.07	2.35 ± 0.47	0.42 ± 0.02	1.77 ± 0.33
10	6	4	0.11 ± 0.00	0.15 ± 0.03	0.69 ± 0.03	1.53 ± 0.29	0.12 ± 0.02	0.48 ± 0.01
10	6	8	0.12 ± 0.02	0.46 ± 0.03	1.07 ± 0.02	1.71 ± 0.07	0.16 ± 0.01	0.88 ± 0.03
10	6	24	0.33 ± 0.08	0.56 ± 0.18	0.89 ± 0.06	2.26 ± 0.55	0.44 ± 0.00	2.24 ± 0.30
20	3	4	0.21 ± 0.05	0.24 ± 0.05	1.88 ± 1.04	3.31 ± 0.16	0.22 ± 0.01	1.18 ± 0.28
20	3	8	0.30 ± 0.06	0.63 ± 0.07	3.33 ± 0.05	4.02 ± 0.10	0.12 ± 0.03	1.94 ± 0.01
20	3	24	0.61 ± 0.08	0.63 ± 0.10	3.09 ± 0.89	4.81 ± 0.84	0.36 ± 0.01	2.58 ± 0.37
20	6	4	0.21 ± 0.01	0.23 ± 0.02	2.25 ± 0.87	3.43 ± 0.47	0.18 ± 0.01	1.14 ± 0.40
20	6	8	0.31 ± 0.10	0.76 ± 0.07	3.48 ± 0.09	3.92 ± 0.42	0.17 ± 0.01	1.66 ± 0.11
20	6	24	0.60 ± 0.13	0.95 ± 0.06	3.26 ± 0.19	5.22 ± 0.79	0.50 ± 0.01	3.00 ± 0.45

Table SI IV 2. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "dynamic" exposure geometry.

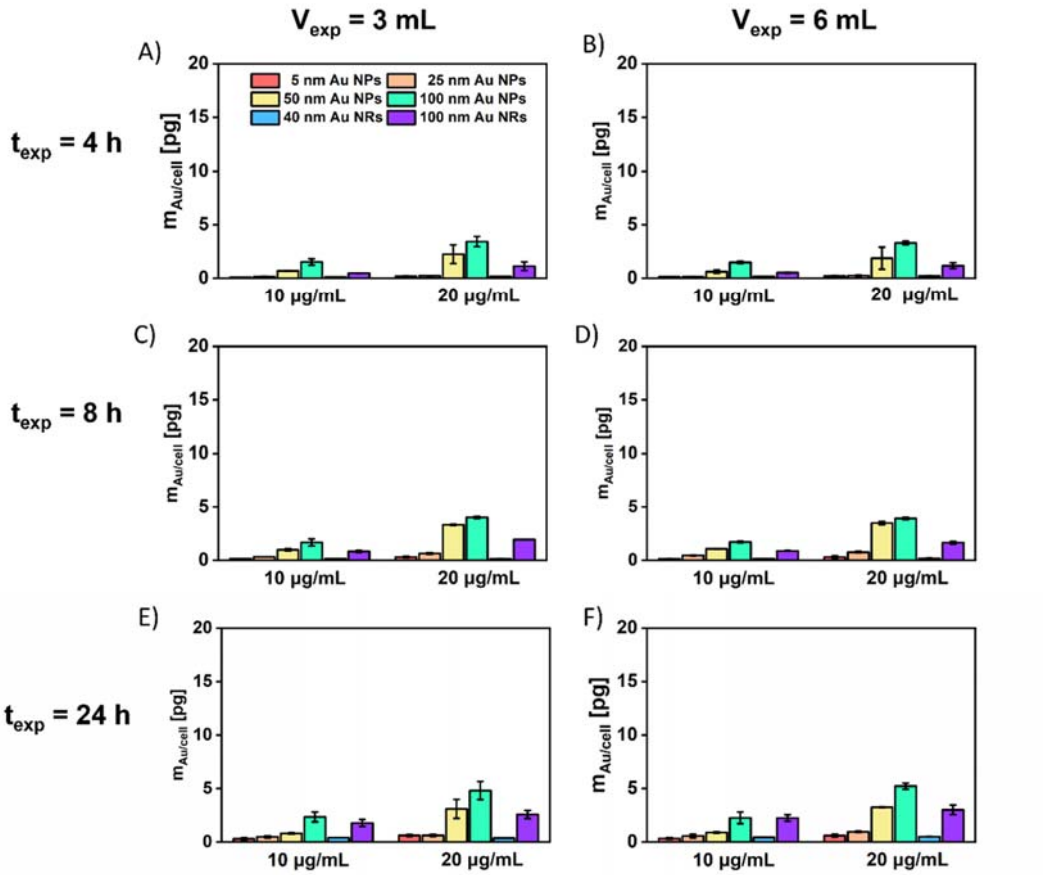

Figure SI IV-3. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "dynamic" exposure geometry. (A, C, E) V_{exp} = 3 mL, (B, D, F) V_{exp} = 6 mL. (A, B) t_{epx} = 4 h, (C, D) t_{epx} = 8 h, (E, F) t_{epx} = 24 h. The data correspond to mean values ± SDs from three independent experiments.

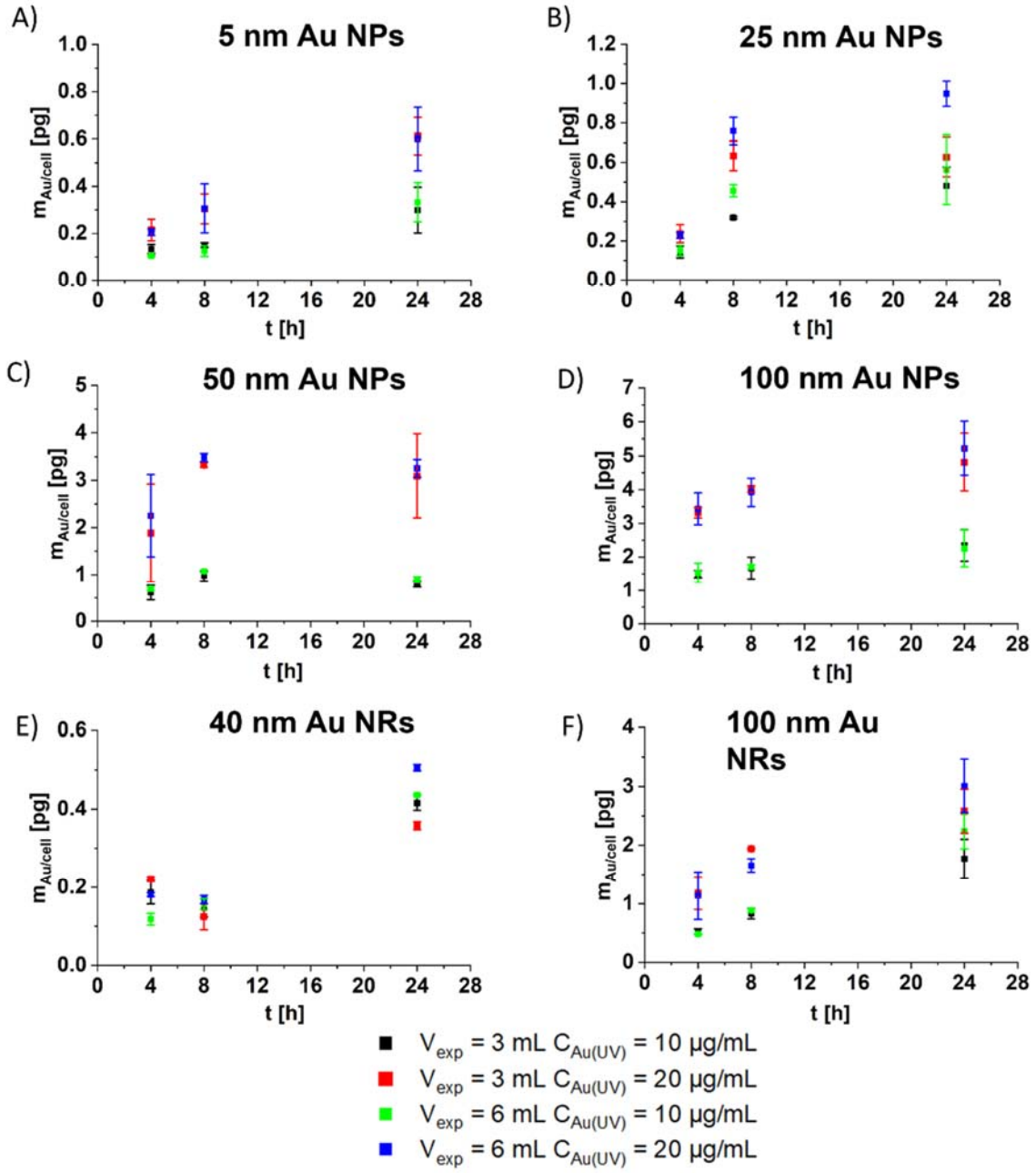

Figure SI IV-4. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "dynamic" exposure geometry. The data correspond to mean values ± SDs from three independent experiments. These are the same data as shown in Figure SI IV-3, here displayed in another format. If in the graphs not all points for the four colors are visible this is because the black dots are covered by the red dots and the green dots are covered by the yellow dots.

IV.3 "Fluid" culture conditions

100 µL HeLa cells with a density of 10^6 cells/mL were cultured in µ-slides I 0.2 Luer (Ibidi GmbH, Martinsried, Germany, 2.5 cm^2 seeding area in each slide, resulting in a seeding density of 40,000 cells/cm^2). After about 7 h of culture, in which the cells had enough time to attach to the surface of the flow channel, the slides were connected to the Ibidi pump system. The cells were then subject to homogeneous unidirectional laminar flow of 6 mL DMEM complete cell medium and 0.498 Pa shear stress overnight. The flow rate was 1.43 mL/min corresponding to a flow speed of 1.43 mm/min (the channel volume was 0.05 mL and the channel length was 50 mm). Afterward, the cells were exposed to V_{exp} = 6 mL unidirectional laminar flow of DMEM complete cell media containing Au NPs/NRs with the concentration of $C_{Au(UV)}$ = 10 µg/mL and 20 µg/mL for 4, 8, and 24 h. During incubation it is important to avoid bubbles in the µ-slides. After the desired exposure times t_{exp}, experiments were stopped and cells were washed three times with 100 µL PBS, after that cells were detached with 100 µL trypsin (0.05%) and then 500 µL complete medium was used to collect the cells. After counting the cells ($N_{cell}(t_{exp})$) with a Neubauer chamber (Celeromics Technologies, València, Spain), cells were centrifuged at 300 rcf for 5 min, the supernatant was discarded and the cell pellet was collected for ICP-MS measurement. The ICP-MS measurements were carried out as described in chapter IV.1. The results for the different NPs/NRs and exposure times are given in Table SI IV-3 and Figure SI IV.5 and Figure SI IV.6.

Sample			5 nm Au NPs	25 nm Au NPs	50 nm Au NPs	100 nm Au NPs	40 nm Au NRs	100 nm Au NRs
$C_{Au(UV)}$ [µg/mL]	V_{exp} [mL]	t_{exp} [h]						
10	6	4	0.04 ± 0.01	0.09 ± 0.03	0.06 ± 0.02	0.14 ± 0.04	0.15 ± 0.04	0.19 ± 0.02
10	6	8	0.19 ± 0.06	0.24 ± 0.04	0.43 ± 0.06	0.47 ± 0.01	0.33 ± 0.06	0.41 ± 0.11
10	6	24	0.28 ± 0.06	0.29 ± 0.04	0.98 ± 0.08	1.38 ± 0.27	0.47 ± 0.06	0.61 ± 0.02
20	6	4	0.09 ± 0.01	0.07 ± 0.03	0.13 ± 0.03	0.19 ± 0.05	0.15 ± 0.03	0.20 ± 0.01
20	6	8	0.24 ± 0.08	0.33 ± 0.01	0.68 ± 0.14	0.68 ± 0.18	0.42 ± 0.04	0.60 ± 0.16
20	6	24	0.41 ± 0.03	0.42 ± 0.03	1.63 ± 0.16	1.97 ± 0.14	0.66 ± 0.06	0.83 ± 0.06

Table SI IV-3. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "fluid" exposure geometry.

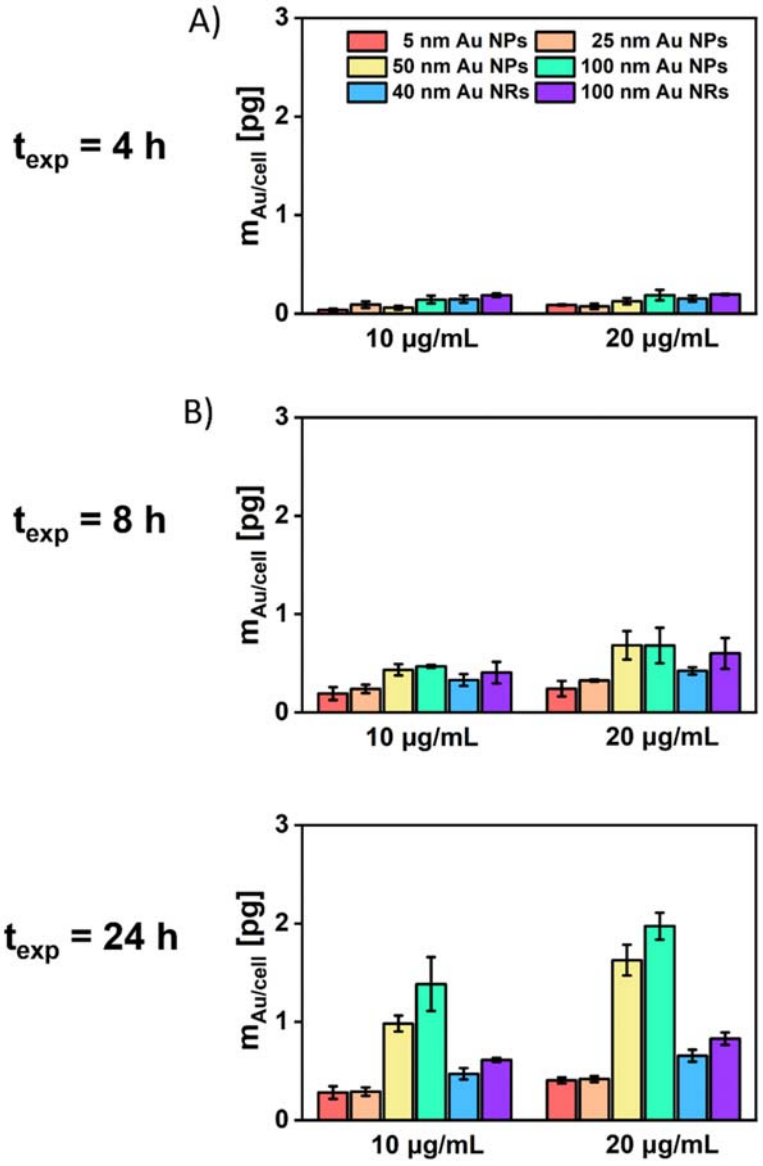

Figure SI IV-5. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "fluid" exposure geometry. V_{exp} = 6 mL. (A) t_{epx} = 4 h, (B) t_{epx} = 8 h, (C) t_{epx} = 24 h. The data correspond to mean values ± SDs from three independent experiments.

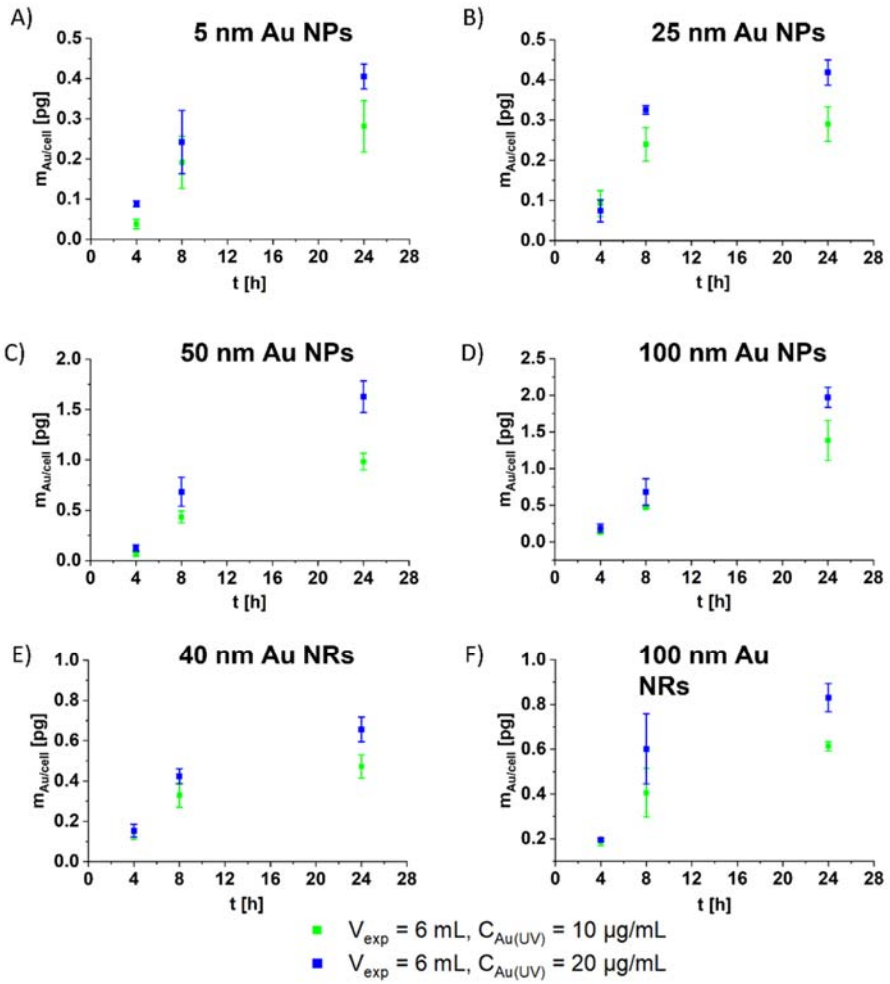

Figure SI IV-6. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "fluid" exposure geometry. The data correspond to mean values ± SDs from three independent experiments. These are the same data as shown in Figure SI IV-5, here displayed in another format.

IV.4 "Suspension" culture conditions

For determining the amount of internalized Au per cell ($m_{Au/cell}$) residual Au NPs/NRs from the cell medium have to be removed before ICP-MS quantification. In the case of adherent cell cultures ("static", "dynamic", "fluid" exposure conditions) this can be simply done by aspiration of the culture medium. In the case of "suspension" culture conditions it is more complicated to separated NPs/NRs in the medium from the suspended cells. To separate cells from free NPs/NRs in the "suspension" exposure conditions thus a purification protocol had to be developed. This protocol is based on the observation, that suspended cells sediment faster than

the here used NPs/NRs. Therefore, a low centrifugation speed was chosen, in which the cells pelleted, but the Au NPs/NRs remained in solution. According to our knowledge, Au NPs/NRs especially the "big" 100 nm Au NPs settle down to the bottom of tubes at a centrifugation speed of 130 rcf (1000 rpm, rotator radius 120 mm). Therefore, a lower centrifugation speed and more washing steps were used to separate the free NPs and cells. Here, 34 rcf (500 rpm, rotator radius 120 mm) 5 min centrifugation was used to separate cells and free Au NPs/NRs. To test this, a test mixture of 300000 cells suspended in 6 mL cell culture media containing $C_{Au(UV)}$ = 20 µg/mL NPs/NRs in 15 mL falcon tubes was prepared. No time was given for uptake of the NPs/NRs by cells. Immediately after mixing, the first washing cycle was carried out. For this centrifugation at 34 rcf (500 rpm, rotator radius 120 mm, minimal speed of the centrifugation rotor) was used for 5 min. The supernatant was collected and 50 µL of it were used for ICP-MS measurements. The cell pellet was collected and resuspended in 1.2 mL PBS, and the number of cells N_{cell} was determined. We found that after the 1st time of centrifugation, more than 90% of the cells could be collected in the pellet. The washing cycle was repeated, i.e. the centrifugation, cell counting, and NP/NR quantification steps were repeated three times (washing cycles #2, #3, and #4). 50 µL of the supernatants from each step were used for ICP-MS measurement. For ICP-MS measurement, the 50 µL supernatant were digested overnight with 200 µL fresh aqua regia and then diluted with 1.8 mL 2% (V/V) HCl (prepared by mixing 2 mL 35 wt% HCl aq. with 98 mL Milli-Q water), leading to a total sample volume of 0.05 mL + 1.8 mL + 0.2 mL = 2.05 mL (i.e. the supernatant had been diluted by a factor of 2.05 mL/0.05 mL = 41). The concentration of Au NPs/NRs $C_{Au(ICP)}$ found in each sample after the respective washing cycles, as determined with ICP-MS, is shown in Figure SI IV.7. The Au concentration in the supernatant was 41 times the concentration measured in each sample (due to the 41 times dilution).

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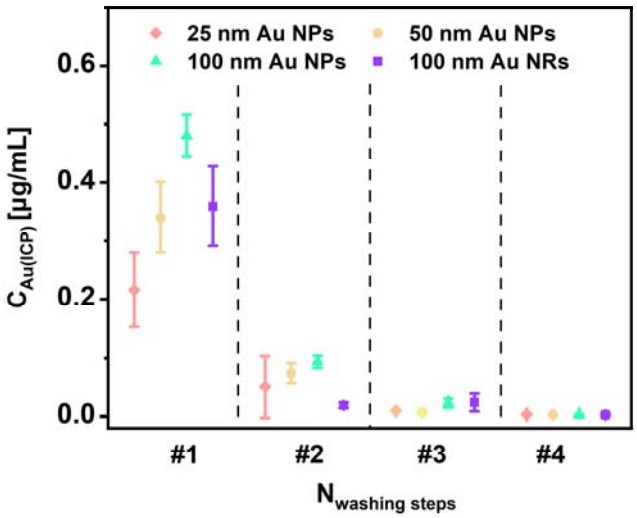

Figure SI IV-7. Concentration of residual Au in the supernatant after the respective washing cycles. The data shown correspond to mean values ± SDs from three independent experiments.

We can see that in the case of 25 and 50 nm Au NPs, three washing cycles were enough for NP-cell separation, while for the 100 nm Au NPs a fourth cycle was needed for the separation. After the 4th washing cycle also the amount of Au in the cell pellet was determined by ICP-MS and by dividing it by the number of cells in the pellet the residual mass $m_{Au/cell}$ of Au per cell in the cell pellet was calculated, see Figure SI IV.8. Thus value imposed the experimental limit for the determination of $m_{Au/cell}$ in the following Au NP/NR uptake studies. Data shown in Figure SI IV.9 show that the amount of internalized Au is higher than this experimental limit.

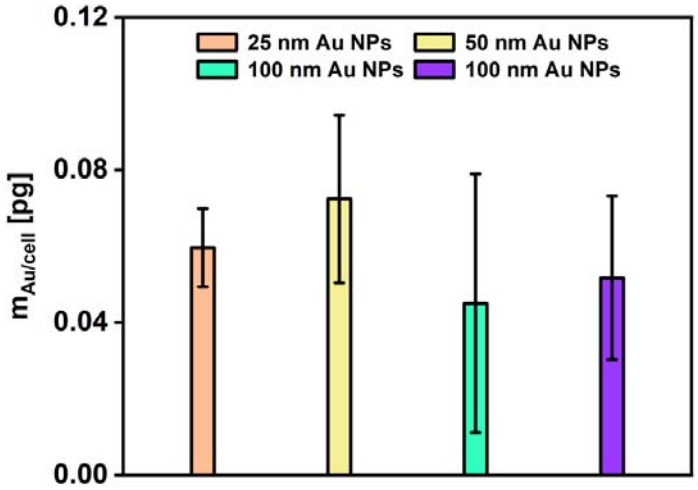

Figure SI IV-8. Mass of Au NPs $m_{Au/cell}$ sticking to cells after 4 times washing in the NP-cell separation test.

For the uptake studies, $3 \cdot 10^5$ HeLa cells were dispersed in V_{exp} = 3 mL and 6 mL complete cell media in 15 mL Falcon tubes supplemented by Au NPs/NRs with a final concentration of $C_{Au(UV)}$. The Falcon tubes were afterwards mounted in a tube revolver (Thermo ScientificTM). The tubes were rotated with 10 rpm for the exposure times t_{exp} of 4, 8, and 24 h. After this the mixture of cells and Au NPs/NRs was centrifuged at 500 rpm for 5 min, and the supernatant was discarded. 4 washing cycles were carried out, involving dispersion of the cell pellet in 1 mL PBS in a 2 mL Eppendorf tube, centrifugation at 500 rpm for 5 min, and discarding of the supernatant. After the last washing cycles, the cell in the pellet were counted and the amount of Au in the cell pellet was measured by ICP-MS, leading to the determination of $m_{Au/cell}$, see Table SI IV.4 and Figure SI IV-9 and Figure SI IV-10.

Sample			5 nm Au NPs	25 nm Au NPs	50 nm Au NPs	100 nm Au NPs	40 nm Au NRs	100 nm Au NRs
$C_{Au(UV)}$ [μg/mL]	V_{exp} [mL]	t_{exp} [h]						
10	3	4	0.17 ± 0.04	0.26 ± 0.09	0.13 ± 0.02	0.08 ± 0.02	0.14 ± 0.05	0.05 ± 0.02
10	3	8	0.18 ± 0.06	0.23 ± 0.03	0.22 ± 0.04	0.32 ± 0.03	0.23 ± 0.01	0.09 ± 0.01
10	3	24	0.59 ± 0.20	0.51 ± 0.13	0.41 ± 0.06	0.64 ± 0.18	0.45 ± 0.03	0.17 ± 0.05
10	6	4	0.12 ± 0.01	0.22 ± 0.05	0.13 ± 0.03	0.11 ± 0.03	0.18 ± 0.05	0.05 ± 0.02
10	6	8	0.20 ± 0.06	0.22 ± 0.02	0.22 ± 0.04	0.32 ± 0.04	0.23 ± 0.01	0.08 ± 0.01
10	6	24	0.64 ± 0.24	0.66 ± 0.09	0.40 ± 0.01	0.59 ± 0.15	0.46 ± 0.04	0.17 ± 0.02
20	3	4	0.27 ± 0.01	0.35 ± 0.05	0.22 ± 0.06	0.22 ± 0.04	0.25 ± 0.03	0.08 ± 0.02
20	3	8	0.31 ± 0.12	0.39 ± 0.03	0.45 ± 0.01	0.59 ± 0.03	0.47 ± 0.08	0.15 ± 0.02
20	3	24	1.25 ± 0.26	1.16 ± 0.13	0.70 ± 0.11	0.92 ± 0.12	0.75 ± 0.03	0.31 ± 0.06
20	6	4	0.29 ± 0.03	0.35 ± 0.08	0.22 ± 0.01	0.24 ± 0.08	0.25 ± 0.06	0.11 ± 0.02
20	6	8	0.27 ± 0.07	0.41 ± 0.03	0.40 ± 0.02	0.57 ± 0.02	0.46 ± 0.07	0.13 ± 0.05
20	6	24	1.29 ± 0.27	1.25 ± 0.40	0.59 ± 0.06	0.95 ± 0.05	0.73 ± 0.11	0.29 ± 0.04

Table SI IV-4. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "suspension" exposure geometry.

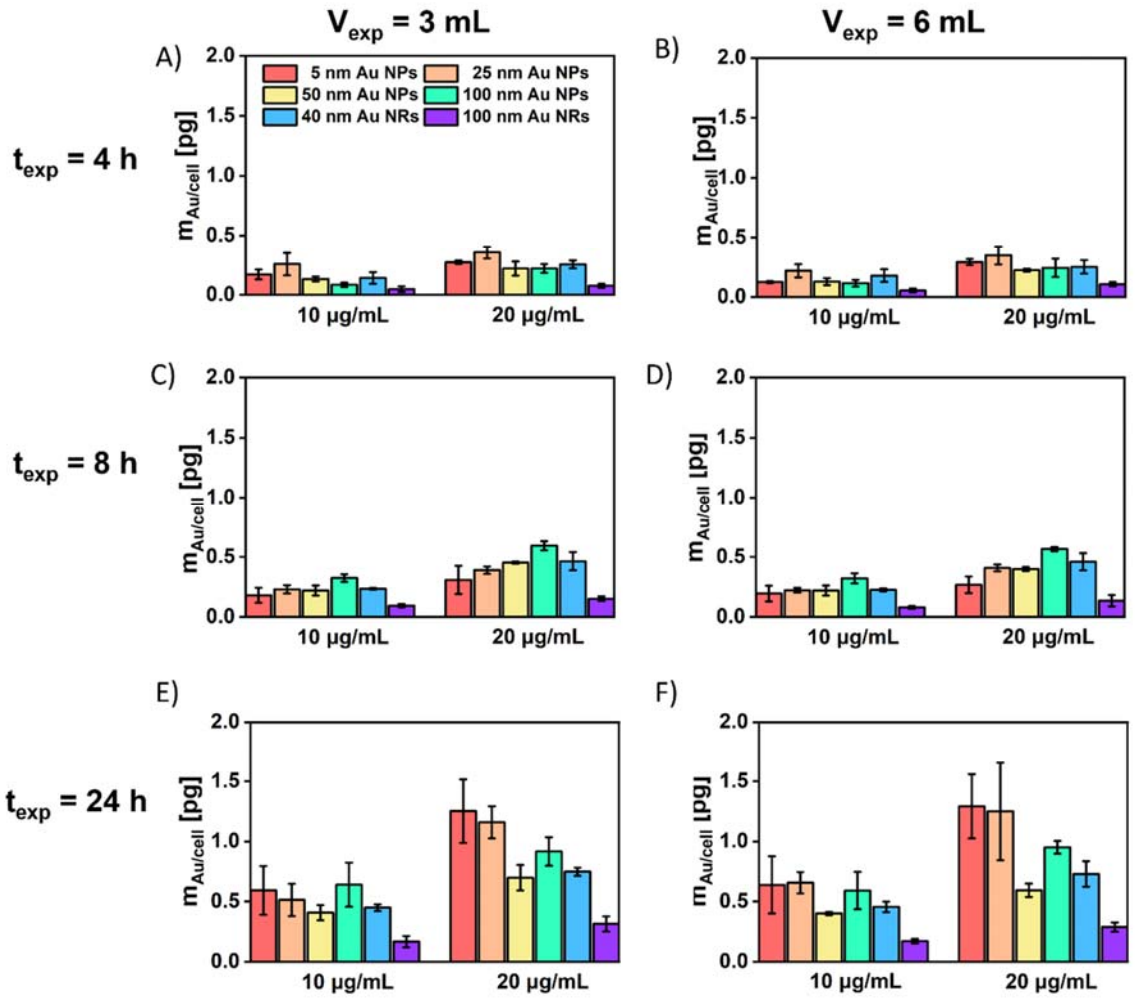

Figure SI IV-9. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "suspension" exposure geometry. (A, C, E) V_{exp} = 3 mL, (B, D, F) V_{exp} = 6 mL. (A, B) t_{epx} = 4 h, (C, D) t_{epx} = 8 h, (E, F) t_{epx} = 24 h. The data correspond to mean values ± SDs from three independent experiments.

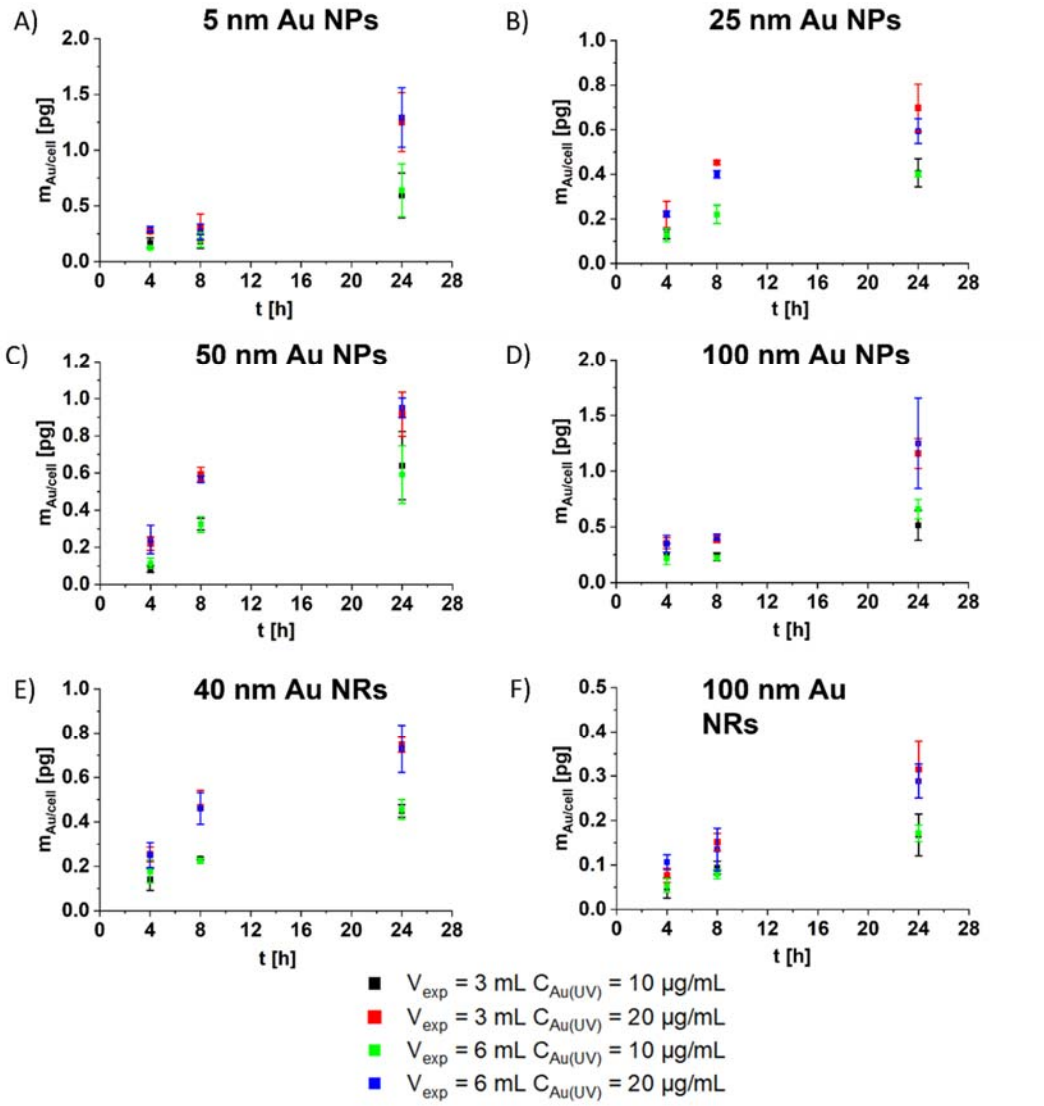

Figure SI IV-10. Mass of internalized Au per cell $m_{Au/cell}$ for HeLa cells incubated with 5, 25, 50, and 100 nm Au NPs and 40 and 100 nm Au NRs in the volume of V_{exp} of NP/NR containing medium of concentration $C_{Au(UV)}$ for the time t_{exp} in "suspension" exposure geometry. The data correspond to mean values ± SDs from three independent experiments. These are the same data as shown in Figure SI IV-9, now displayed in another format. If in the graphs not all points for the four colors are visible this is because the black dots are covered by the red dots and the green dots are covered by the yellow dots.

IV.5 Estimation of the fraction of NPs/NRs that has been incorporated by cells

When cells take up NPs/NRs and accumulate them in endosomes/lysosomes, then the Au concentration inside cells will become bigger than the Au concentration in the extracellular

medium [6]. Using the $m_{Au/cell}$ values and by assuming a volume of $V_{cell} \approx 5000\ \mu m^3 = 5 \cdot 10^{-9}$ mL [7] the intracellular Au concentration $C_{Au(ICP)intracellular}$ can be calculated as

$$C_{Au(ICP)intracellular} = m_{Au/cell} / V_{cell} \qquad \text{Equation SI IV-3}$$

Data are presented in Table SI IV-5, Table SI IV-6, Table SI IV-7, and Table SI IV-8. They demonstrate that the NP/NR concentration inside cells $C_{Au(ICP)intracellular}$ is much higher than the concentration $C_{Au(UV)}$ to which the cells are exposed to NPs/NRs in the cell medium. Actually internalized NPs/NRs are not distributed in the entire cell volume, but are confined to endosomes/lysosomes, where their local concentration would be even higher than the here calculated values.

Sample	$m_{Au/cell}$ [pg]	$C_{Au(ICP)intracellular}$ [µg/mL]
5 nm Au NPs	0.51 ± 0.07	102 ± 14
25 nm Au NPs	0.87 ± 0.01	174 ± 2
50 nm Au NPs	9.99 ± 1.80	1998 ± 360
100 nm Au NPs	11.54 ± 2.16	2308 ± 432
40 nm Au NRs	0.78 ± 0.19	156 ± 38
100 nm Au NRs	3.30 ± 0.24	660 ± 48

Table SI IV-5. Intracellular concentration of Au NPs/NRs in HeLa cells after incubation for t_{exp} = 24 h at $C_{Au(UV)}$ = 20 µg/mL and V_{exp} = 3 mL under "static" incubation conditions. The $m_{Au/cell}$ data are from Table SI IV-1.

Sample	$m_{Au/cell}$ [pg]	$C_{Au(ICP)intracellular}$ [µg/mL]
5 nm Au NPs	0.61 ± 0.08	122 ± 16
25 nm Au NPs	0.63 ± 0.10	126 ± 20
50 nm Au NPs	3.09 ± 0.89	618 ± 178
100 nm Au NPs	4.81 ± 0.84	962 ± 168
40 nm Au NRs	0.36 ± 0.01	72 ± 2
100 nm Au NRs	2.58 ± 0.37	516 ± 74

Table SI IV-6. Intracellular concentration of Au NPs/NRs in HeLa cells after incubation for t_{exp} = 24 h at $C_{Au(UV)}$ = 20 µg/mL and V_{exp} = 3 mL under "dynamic" incubation conditions. The $m_{Au/cell}$ data are from Table SI IV-2.

Sample	$m_{Au/cell}$ [pg]	$C_{Au(ICP)intracellular}$ [µg/mL]
5 nm Au NPs	0.41 ± 0.03	82 ± 6
25 nm Au NPs	0.42 ± 0.03	84 ± 6
50 nm Au NPs	1.63 ± 0.16	326 ± 32
100 nm Au NPs	1.97 ± 0.14	394 ± 28
40 nm Au NRs	0.66 ± 0.06	132 ± 12
100 nm Au NRs	0.83 ± 0.06	166 ± 12

Table SI IV-7. Intracellular concentration of Au NPs/NRs in HeLa cells after incubation for t_{exp} = 24 h at $C_{Au(UV)}$ = 20 µg/mL and V_{exp} = 6 mL under "fluid" incubation conditions. The $m_{Au/cell}$ data are from Table SI IV-3.

Sample	$m_{Au/cell}$ [pg]	$C_{Au(ICP)intracellular}$ [µg/mL]
5 nm Au NPs	1.25 ± 0.26	250 ± 50
25 nm Au NPs	1.16 ± 0.13	232 ± 26
50 nm Au NPs	0.70 ± 0.11	140 ± 22
100 nm Au NPs	0.92 ± 0.12	184 ± 24
40 nm Au NRs	0.75 ± 0.03	150 ± 6
100 nm Au NRs	0.31 ± 0.06	62 ± 12

Table SI IV-8. Intracellular concentration of Au NPs/NRs in HeLa cells after incubation for t_{exp} = 24 h at $C_{Au(UV)}$ = 20 µg/mL and V_{exp} = 3 mL under "suspension" incubation conditions. The $m_{Au/cell}$ data are from Table SI IV-4.

IV.6 Correction for the error in exposure concentration determination

As mentioned in section I.4 the concentration $C_{Au(UV)}$ as determined via UV/vis absorption measurements relates differently to the concentration $C_{Au(ICP)}$ as determined with ICP-MS for the different NP/NP species. We assume in the following that uptake of the NPs/NRs is in the linear range, e.g. doubling of the NP/NR exposure concentration $C_{Au(UV)}$ would lead to a double amount of Au found per cell $m_{Au/cell}$. This assumption can be verified by looking at the $m_{Au/cell}$ values for $C_{Au(UV)}$ = 10 µg/mL and 20 µg/mL in Table SI IV-1, Table SI IV-2, Table SI IV-3, and Table SI IV-4. In this way, if the percentual deviation between $C_{Au(ICP)}$ and $C_{Au(UV)}$ is know, than, in order to obtain the amount of Au per cells $m_{Au/cell(corr)}$ that should be internalized for a concentration $C_{Au(ICP)}$ can obtained by scaling by this percentage:

$$m_{Au/cell(corr)} = m_{Au/cell} \cdot C_{Au(ICP)}/C_{Au(UV)} \qquad \text{Equation SI IV-4}$$

In Table SI IV-9 the $C_{Au(ICP)}/C_{Au(UV)}$ data from Table SI I-2 were used to calculate $C_{Au(ICP)}$ corresponding to $C_{Au(UV)}$ = 20 µg/mL: $C_{Au(ICP)} = 20\ \mu g/mL \cdot (C_{Au(ICP)}/C_{Au(UV)})$ Using the $m_{Au/cell}$ data from Table SI IV-1, Table SI IV-2, Table SI IV-3, and Table SI IV-4 then the concentration corrected values $m_{Au/cell(corr)}$ were calculated according to Equation SI IV-4. In Figure SI IV-11 the $m_{Au/cell}$ and $m_{Au/cell(corr)}$ data are compared. They show that the dependency is the same and that thus error in concentration determination had no influence on the main tendencies.

Exposure condition	Sample			5 nm Au NPs		25 nm Au NPs		50 nm Au NPs	
	$C_{Au(UV)}$ [µg/mL]	V_{exp} [mL]	t_{exp} [h]	$C_{Au(ICP)}$ [µg/mL]	$m_{Au/cell(corr)}$ [pg]	$C_{Au(ICP)}$ [µg/mL]	$m_{Au/cell(corr)}$ [pg]	$C_{Au(ICP)}$ [µg/mL]	$m_{Au/cell(corr)}$ [pg]
"static"	20	6	24	11.56	0.29 ± 0.04	10.76	0.46 ± 0.05	15	8.77 ± 0.95
"dynamic"	20	6	24	11.56	0.36 ± 0.07	10.76	0.51 ± 0.03	15	2.44 ± 0.14
"fluid"	20	6	24	11.56	0.24 ± 0.02	10.76	0.23 ± 0.02	15	1.22 ± 0.12
"suspension"	20	6	24	11.56	0.74 ± 0.15	10.76	0.67 ± 0.21	15	0.44 ± 0.04

Exposure condition	Sample			100 nm Au NPs		40 nm Au NRs		100 nm Au NRs	
	$C_{Au(UV)}$ [µg/mL]	V_{exp} [mL]	t_{exp} [h]	$C_{Au(ICP)}$ [µg/mL]	$m_{Au/cell(corr)}$ [pg]	$C_{Au(ICP)}$ [µg/mL]	$m_{Au/cell(corr)}$ [pg]	$C_{Au(ICP)}$ [µg/mL]	$m_{Au/cell(corr)}$ [pg]
"static"	20	6	24	21.6	18.04 ± 3.94	13.32	0.55 ± 0.09	10.2	2.39 ± 0.30
"dynamic"	20	6	24	21.6	5.63 ± 0.85	13.32	0.33 ± 0.01	10.2	1.53 ± 0.22
"fluid"	20	6	24	21.6	2.13 ± 0.15	13.32	0.44 ± 0.04	10.2	0.42 ± 0.03
"suspension"	20	6	24	21.6	1.03 ± 0.05	13.32	0.48 ± 0.07	10.2	0.15 ± 0.02

Table SI IV-9. Concentration corrected values $m_{Au/cell(corr)}$.

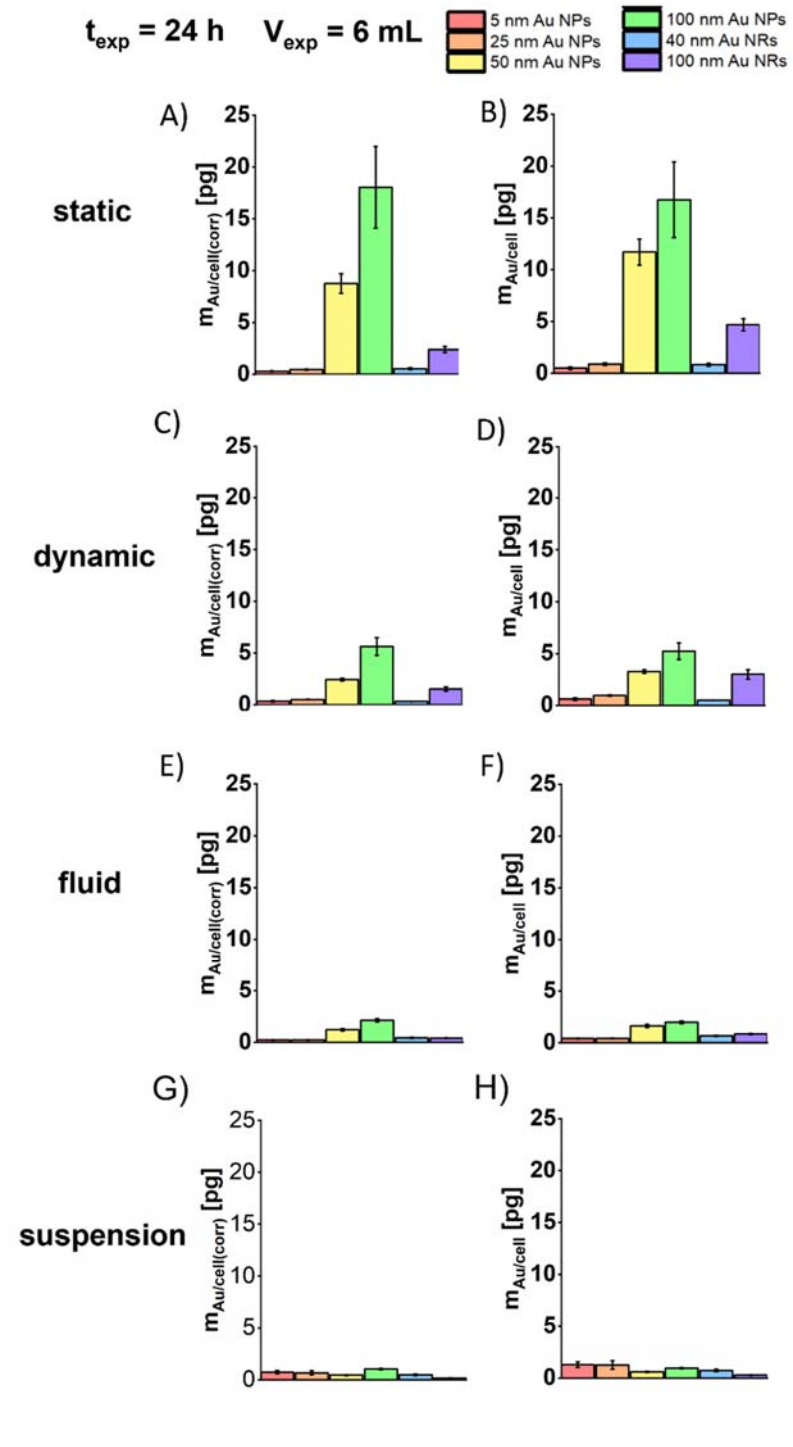

Figure SI IV-11. Concentration corrected values $m_{Au/cell(corr)}$ versus the original values $m_{Au/cell}$. $C_{Au(UV)}$ = 20 μg/mL, V_{exp} = 6 mL, t_{exp} = 24 h.

V References

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