Influence of Surface Coating on the Intracellular Behaviour of Gold Nanoparticles: A Fluorescence Correlation Spectroscopy Study

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Supporting material

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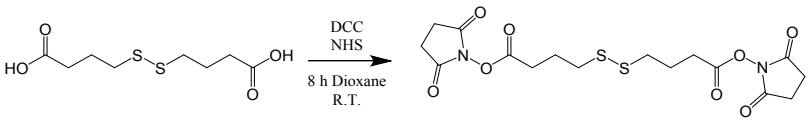
S1. Materials and methods

$HAuCl_4 \cdot 3H_2O$, $AgNO_3$, hydroquinone, 4,4'-dithiodibutyric acid, glucosamine hydrochloride, butylamine, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N,N'-Dicyclohexylcarbodiimide (DCC), N-Hydroxysuccinimide (NHS), 1,4-Dithiothreitol (DTT) and NaOH were purchased from Sigma-Aldrich, and used without further purification. $HAuCl_4 \cdot 3H_2O$ was stored at 4 °C, shielded from light, as 10 mM solution and NaOH was stored as 1 M water solution. $AgNO_3$ 10 mM and hydroquinone solutions were freshly prepared before every synthesis (avoiding the exposition to the light). HS-PEG_{5000}-OCH_3 and HS-PEG_{5000}-NH_2, purchased from Rapp Polymer GmbH, were used as received and stored under dry argon atmosphere at -20 °C. HS-Alkyl-PEG_{600}-COOH (([1-mercaptoundec-11-yl]PEG_{600})-acetic acid) was synthesized by Chorisis srl. (Varese, Italy) following a literature reported procedure[1] UV-vis spectroscopy (Spectrophotometer Bio UV-Vis V630 Jasco) was performed using a disposable cuvette with 1 cm optical path length. The experiments were performed in triplicate at 25 °C. MilliQ water was employed as baseline. Dynamic Light Scattering (DLS) measurements were performed employing a Malvern Zetasizer Nano ZS90. Specimens were filtered with a cellulose acetate syringe filter (0.22 µm) before to load the cuvette. Each sample was equilibrated for 2 min prior to measure. At least three independent measurements of 10 runs (10 s each one) were performed for each sample. A reduced volume plastic cuvette was employed for DLS experiments loaded with 450 µl of sample. A capillary zeta cell was used for ζ-potential measurements loaded with 1 ml of sample. Fluorescence spectra were registered employing Fluoromiter Fluorolog-TCSPC (Horiba-Jovin Ivon). The sample was excited at 564 nm with a 2 nm slit and averaged over 5 accumulations, to enhance the signal to noise ratio. The fluorescence signal was acquired starting at 570 nm. A disposable cuvette with 1 cm optical path length was used for the measurements. FCS data were recorded on an LSM 510 inverted microscope outfitted with the Confocor 3 FCS module (Carl Zeiss Gmbh). Acquisition and analysis of LSM image and Confocor is controlled by the Zen software. The DPSS laser at 561 nm has been employed as excitation source. The microscope objective used was a 40X water immersion objective, 1Airy Unit. Autocorrelation functions G(τ) were analyzed by Quickfit 3.0 software (DKFZ, Germany) employing in the order Simulated Annealing with box constraints and Levenberg-Marquardt Algorithm with box constraints. All the fitting were performed using a three-dimensional normal diffusion model. One or two components were used in the fitting model verifying the statistical improvement.

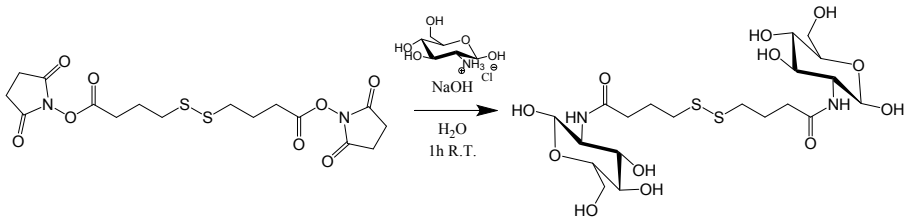
S2. Synthesis of N-4-thiobutyroil glucosamine

Synthesis 4,4'-Dithiodibutyroil NHS ester



The reaction was performed under dry conditions and Argon atmosphere. Dioxane was filtered on an alumina column to remove the peroxides and degassed under vacuum. 4,4'-Dithiobutyric acid (DTBA, 400 mg, 1.67 mmmol, 1 eq) and NHS (422 mg, 3.67 mmol, 2.2 eq) were dissolved in 12 ml of dioxane. 4 ml of a DCC (756 mg, 3.67 mmol, 2.2, eq) solution in dioxane were slowly dropped in the reaction mixture and left, under vigorous stirring, at room temperature for 8 hours. The solvent was evaporated at reduced pressure and the obtained product dissolved in Et_2O-Acetone (1:1) solution. The mixture was filtrated to remove the white insoluble solid and the 4,4'-Dithiodibutyroil NHS ester was recovered evaporating the solvent at reduced pressure. The product was directly used for the next step without any further purification.

Synthesis of 4,4'-Dithiodibutyroil glucosamine



Glucosamine hydrochloride (825 mg, 3.84 mmol, 2.3 eq) was dissolved in 4 ml NaOH 1M. 22 ml MES (0.1M) buffer were added to the solution. 4,4'-Dithiodibutyroil NHS ester (691 mg, 1.59 mmol, 1 eq) was dissolved into 42 ml anhydrous DMF and added to the glucosamine solution. After 1 h reaction the solvent was evaporated at reduced pressure. The crude was suspended in 2 ml of water, and the white insoluble solid removed by filtration. The product was purified by mean of size exclusion chromatography.

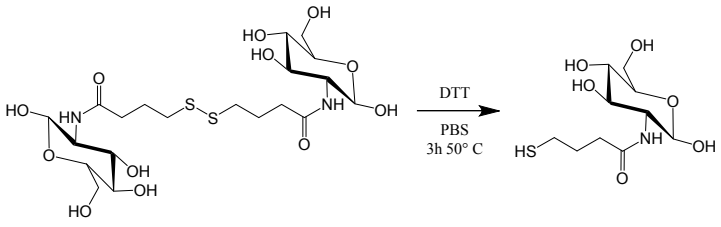
Yield = 63%

m/z= 560,17

^{1}H NMR (400 MHz, D_2O) δ= 8.41 (s, 2H, NH), 5.16 (d, *J* = 3.5 Hz, 1H, H-1α), 4.67 (d, *J* = 8.4 Hz, 1H, H-1β), 3.90 – 3.60 (m, 8H, H-2, H-3, H-5, H-6), 3.41 (m, 2H,H-4), 2.71 (t, 4H, C*H*-S), 2.39 (m, 4H, C*H*-CO), 1.97 (m, 4H,CH_2-C*H*-CH_2).

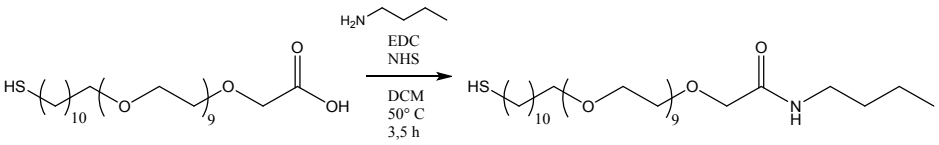
^{13}C NMR (101 MHz, D_2O) δ= 171.52 (*C*=O), 95.70-90.91 (C-1), 71.60 (H-2), 70.87 (H-5), 70.21 (H-6), 60.81 (H-4), 37.25 (H_2***C***-S), 34.35 – 34.16 (H_2***C***-C=O), 24.67 (CH_2-***C***H_2-CH_2).

Synthesis of N-4-thiobutyroil glucosamine



4,4'-Dithiodibutyroil glucosamine (40 mg, 0.07 mmol, 1 eq) was dissolved into 10 ml of PBS solution at pH 7.4. DTT (54 mg, 0.35 mmol, 5 eq) was added and the reaction mixture left under stirring at 50 °C for 3 hours, under argon atmosphere. The water was removed at reduced pressure and DTT removed from the crude by several washes with Et_2O. The obtained white solid was directly used for the coating of the Au NPs

S3. Synthesis of ([1-mercaptoundec-11-yl]PEG_{600})-acetic acid butanamide



The reaction was performed in dry conditions under argon atmosphere. HS-Alkyl-PEG_{600}-COOH (125 mg, 0.15 mmol, 1 eq) was dissolved in DCM dry. EDC (71,3 mg, 0.3 mmol, 2 eq) was added, followed , five minutes later, by NHS (43.1 mg, 0.3 mmol, 2eq). The reaction was stirred at ROOM TEMPERATURE for 30 min, then butylamine (92.5 mg, 0,75 mmol, 5 eq) were added. The reaction mixture was stirred for 3 hours at 50 °C. The solvent was evaporated at reduced pressure and the product purified by flash chromatography (DCM:MeOH=95:5).

Yield = 78 %

m/z=780.53

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.29 (s, 1H, N***H***), 4.08 (s, 2H, ***H***$_2$C-C=O), 3.67 (s, 30H, O-C***H***$_2$-C***H***$_2$-O), 3.62 – 3.57 (m, 2H, O=C-CH_2-O-C***H***$_2$), 3.47 (t, *J* = 6.8 Hz, 2H, CH_2-CH_2-C***H***$_2$-O), 3.32 (dd, *J* = 13.2, 6.9 Hz, 2H, C***H***$_2$-NH), 2.69 (t, 2H, S-S-C***H***$_2$), 2.54 (dd, *J* = 14.7, 7.4 Hz, 2H, S-C***H***$_2$), 1.74 – 1.49 (m, 6H CH_3-CH_2-C***H***$_2$, HS-CH_2-CH_2- CH_2-C***H***$_2$-CH_2-O), 1.44 – 1.34 (m, 2H, CH_3-C***H***$_2$-CH_2), 1.29 (s, 7H, CH2-C***H***2- CH2), 1.01 – 0.92 (m, 3H,C***H***$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ= 158,14 (***C***=O) 71.56 (***C***H_2-NH), 71.04 (O=C-***C***H_2-O), 70.59 (O-***C***H_2-***C***H_2-O), 70.06 (O=C-CH_2-O-***C***H_2), 38.97 (S-S-***C***H_2), 34.05 (S-CH_2-***C***H_2), 31.50 (CH_2-***C***H_2-CH_2-O), 29.50 (CH2-***C***H2- CH2), 28.38 (CH_2-CH_2-***C***H_2-O), 26.09 (***C***H_2-CH2-CH3), 24.65 (S-***C***H_2), 20.08 (***C***H_2-CH_3), 13.75 (***C***H_3).

S4. Au NPs synthesis

General procedure

A water solution of $HAuCl_4 \cdot 3H_2O$ (7.5 ml, 10 mM), sodium citrate (9 ml, 68 mM), and $AgNO_3$ (490 µl, 5.9 mM) was prepared and mixed at room temperature for 6 minutes.[2] The pre-incubated mixture was then mixed with 250 ml of water at 100 °C. The mixture was stirred at 750 rpm for 1 h. Afterwards the reaction solution was left to cool to room temperatureroom temperature and 5 ml of glycerol was added. After 10 minutes a second mixture of $HAuCl_4$ (7.5 ml, 10 mM), sodium citrate (10 ml, 34 mM) and $AgNO_3$ (426 µl, 5.9 mM) was pre-mixed for 6 minutes and then added to the reaction solution, immediately followed by a hydroquinone solution (8 ml, 91 mM). Then, the colloidal solution was left to react for 1h, stirring at 750 rpm. The obtained Au NPs were directly functionalised without any further concentration or purification.

Synthesis of Au-MSA NPs

Au-MSA NPs were obtained by adding (under argon atmosphere) 22 mg of mercapto succinic acid and 8 mg of H_2N-PEG_{5000}-SH, dissolved in 5 ml of MilliQ water, to the gold colloidal solution. The ligand proportions were calculated to obtain 10% of the NPs surface covered with PEG, considering a foot print of 5 nm^2 for H_2N-PEG_{5000}-SH and a footprint of 0,5 nm^2 for mercapto succinic acid.[3-5] The foot print was calculated by computational modelling. The reaction mixture was allowed to stir for further 48 hours at room temperature The functionalised Au NPs were purified and concentrated to a final volume of 10 ml

using Amicon centrifugal filter units. The purification of the system was completed using dialysis tubes with a cut-off of 10 kDa (48 hours, 6 changes of water).

Synthesis of Au-Glucosamine NPs

Au-Glucosamine NPs were obtained by adding (under argon atmosphere) 25 mg of 4-thiobutyl glucosamide and 5 mg of H_2N-PEG_{5000}-SH, dissolved in 5 ml of MilliQ water, to the gold colloidal solution. The ligands proportions were calculated to obtain 10% of the NPs surface covered with H_2N-PEG_{5000}-SH, considering a foot print of 5 nm^2 for H_2N-PEG_{5000}-SH and a footprint of 0.5 nm^2 for 4-thiobutyl glucosamide.[3-5] The reaction mixture was allowed to stir for further 48 hours at room temperature The functionalised Au NPs were purified and concentrated to a final volume of 10 ml using Amicon centrifugal filter units. The purification of the system was completed using dialysis tubes with a cut-off of 10 kDa (48 hours, 6 changes of water).

Synthesis of Au-PEG_{5000} NPs

Au-PEG_{5000} NPs were obtained by adding (under argon atmosphere) 27.5 mg of H_2N-PEG_{5000}-SH and 2.5 mg of H_2N-PEG_{5000}-SH, dissolved in 5 ml of MilliQ water, to the gold colloidal solution. The ligand proportions were calculated to obtain 10% of the NPs surface covered with H_2N-PEG_{5000}-SH, considering a foot print of 5 nm^2 for both the PEG molecules. [3-5] The reaction mixture was allowed to stir for further 48 hours at room temperature The functionalised Au NPs were purified and concentrated to a final volume of 10 ml using Amicon centrifugal filter units. The purification of the system was completed using dialysis tubes with a cut-off of 10 kDa (48 hours, 6 changes of water).

Synthesis of Au-Alkyl-PEG_{600} NPs

Au-Alkyl-PEG_{600} NPs were obtained by adding (under argon atmosphere) 23.5 mg of HS-Alkyl-PEG_{600}-butanamide and 6.5 mg of H_2N-PEG_{5000}-SH, dissolved in 5 ml of MilliQ water, to the gold colloidal solution. The ligands proportions were calculated to obtain 10% of the NPs surface covered with H_2N-PEG_{5000}-SH, considering a foot print of 1.5 nm^2 for HS-Alkyl-PEG_{600}-butanamide and of 5 nm^2 for H_2N-PEG_{5000}-SH. [3-5] The reaction mixture was allowed to stir for further 48 hours at ROOM TEMPERATURE The functionalised Au NPs were purified and concentrated to a final volume of 10 ml using Amicon centrifugal filter units. The purification of the system was completed using dialysis tubes with a cut-off of 10 kDa (48 hours, 6 changes of water).

S5. Au NPs characterisation

Au-MSA NPs

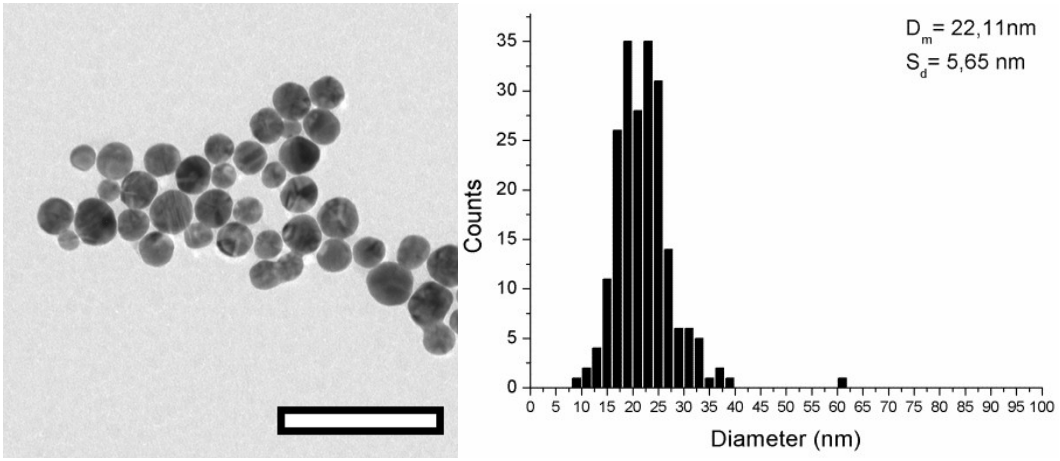


Figure S1. On the left: TEM micrograph of Au-MSA NPs (scale bar 100 nm). On the right: distribution of Au-MSA NP diameters evaluated by ImageJ.

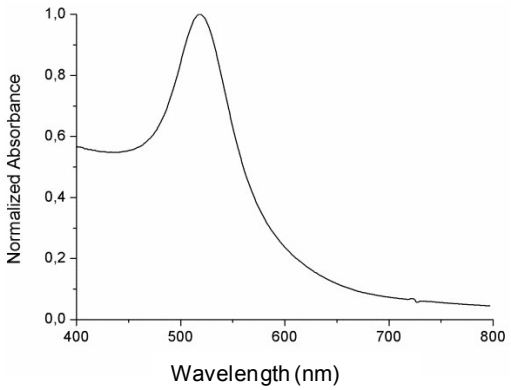


Figure S2. UV-visible spectrum of Au-MSA NPs

ζ-potential	**-24,6 ± 10,8 mV**
Hydrodynamic Diameter	32,7 ± 12,2 nm

Table S1. ζ-potential and hydrodynamic diameter of Au-MSA NPs obtained with dynamic light scattering technique.

Au-Glucosamine NPs

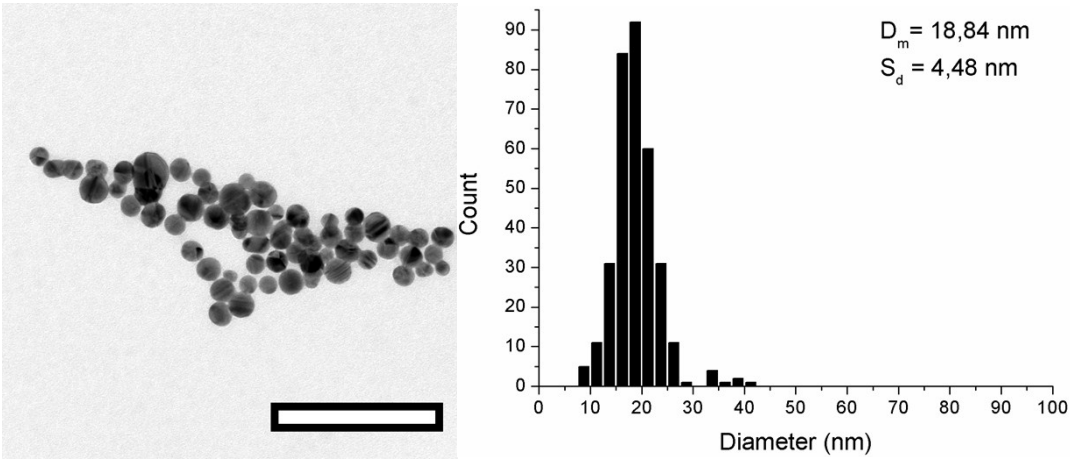


Figure S3. On the left: TEM micrograph of Au-Glucosamine NPs (scale bar 100 nm). On the right distribution of Au-Glucosamine NP diameters evaluated by ImageJ.

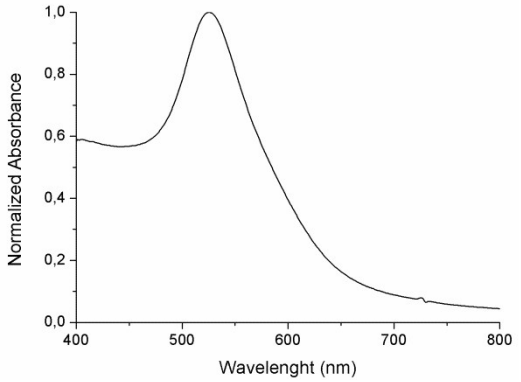


Figure S4. UV-visible spectrum of Au-Glucosamine NPs

ζ-potential	**-21,7 ± 19,0 mV**
Hydrodynamic Diameter	30,6 ± 14,0 nm

Table S2. ζ-potential and hydrodynamic diameter of Au-Glucosamine NPs from DLS measurments

Au-PEG$_{5000}$ NPs

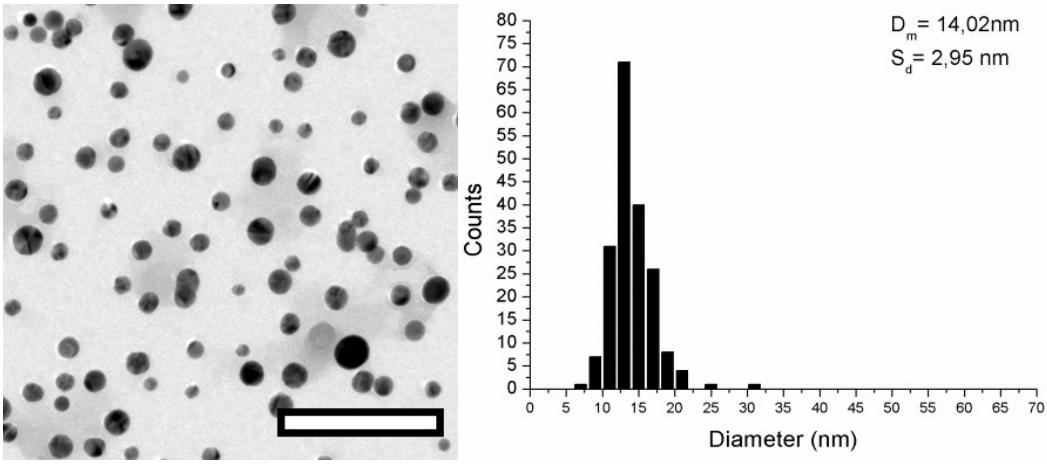


Figure S5. On the left: TEM micrograph of Au-PEG$_{5000}$ NPs (scale bar 100 nm). On the right: distribution of Au-PEG$_{5000}$ NP diameters evaluated by ImageJ.

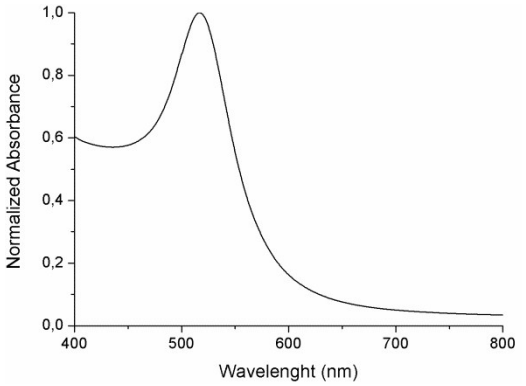


Figure S6. UV-visible spectrum of of Au-PEG$_{5000}$ NPs

ζ-potential	**-21,8 ± 9,1 mV**
Hydrodynamic Diameter	25,0± 13,1 nm

Table S3. ζ-potential and hydrodynamic diameter of Au-PEG$_{5000}$ NPs obtained with dynamic light scattering technique.

Au-Alkyl-PEG$_{600}$ NPs

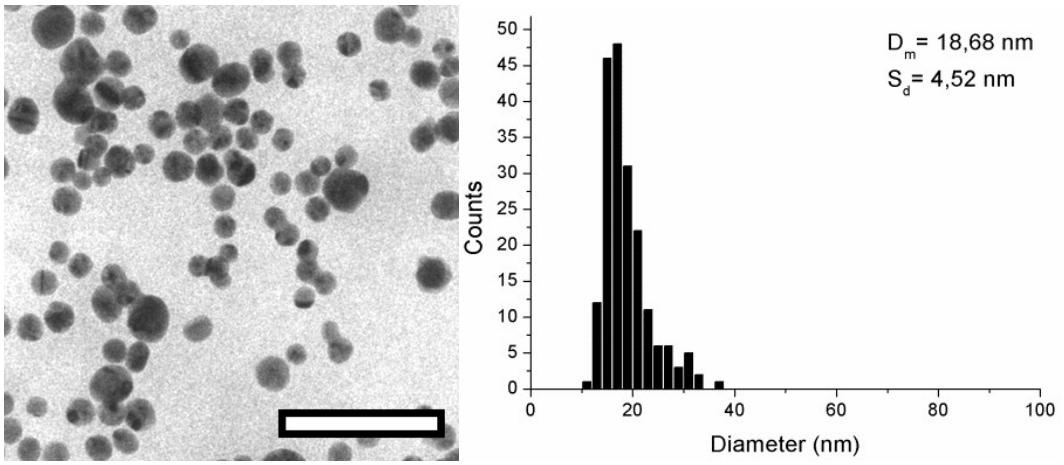


Figure S7. On the left: TEM micrograph of Au-Alkyl-PEG$_{600}$ NPs (scale bar 100 nm). On the right: distribution of Au-PEG$_{5000}$ NP diameters evaluated by ImageJ.

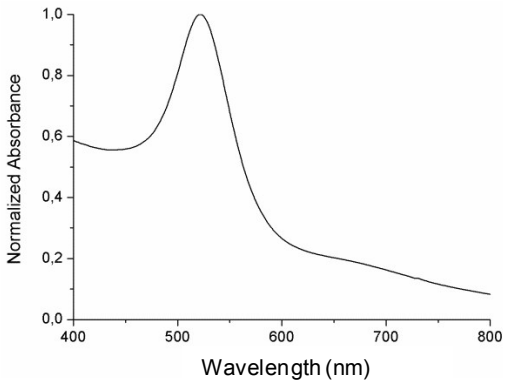


Figure S8. UV-visible spectrum of Au-Alkyl-PEG$_{600}$ NPs

ζ-potential	**-19,8 ± 11,7 mV**
Hydrodynamic Diameter	33,6± 20 nm

Table S4. ζ-potential and hydrodynamic diameter of Au-Alkyl-PEG$_{600}$ obtained with dynamic light scattering technique.

S6. Labelling of the Au NPs with ATTO550 NHS ester

The buffer employed to perform the fluorescent labelling was prepared bymixing 20 parts of a PBS buffer (Phosphate-Buffered Saline, pH 7.4) with 1 part of 0.2 M $NaHCO_3$ solution, adjusted to pH 9.0 with 2 M NaOH. The labelling buffer should have a pH of 8.3, optimal for the reaction. 3mg of Au NPs have been dissolved into 2 ml of buffer. 1.5 eq of ATTO550 NHS ester (stocked in 1 mg/ml DMSO Dry solution) was added for each free amino groups present in the reaction mixture. The mixture was sonicated for the first 10 minutes and then let to react for 1 h at ROOM TEMPERATURE under vigorous stirring. The excess of ATTO550 NHS ester was removed by centrifugal filtration on Amicon centrifugal filters (30 kDa cut-off).

The purification of the labelled Au NPs was completed using dialysis tubes with a cut-off of 100 kDa (48 hours, 6 changes of water).

S7. Characterisation of fluorescent labelled Au NPs

Au-MSA NPs*

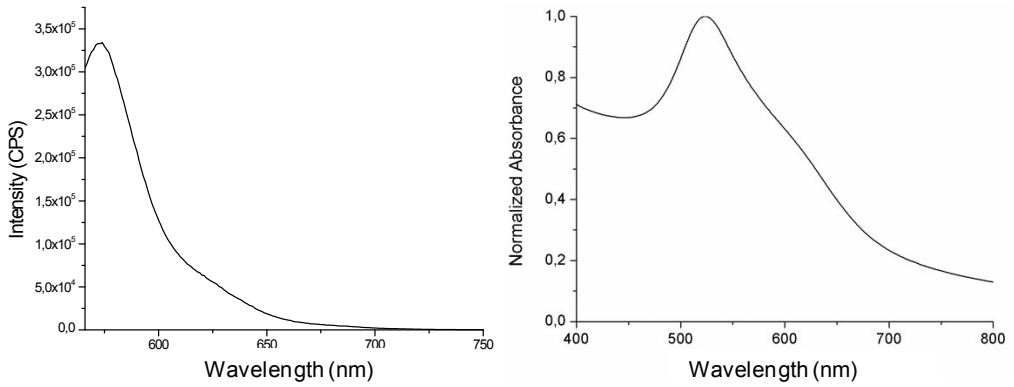


Figure S9. On the left: Fluorescence spectrum of Au-MSA NPs*. On the right: UV-visible spectrum of Au-MSA NPs*.

Au-Glucosamine NPs*

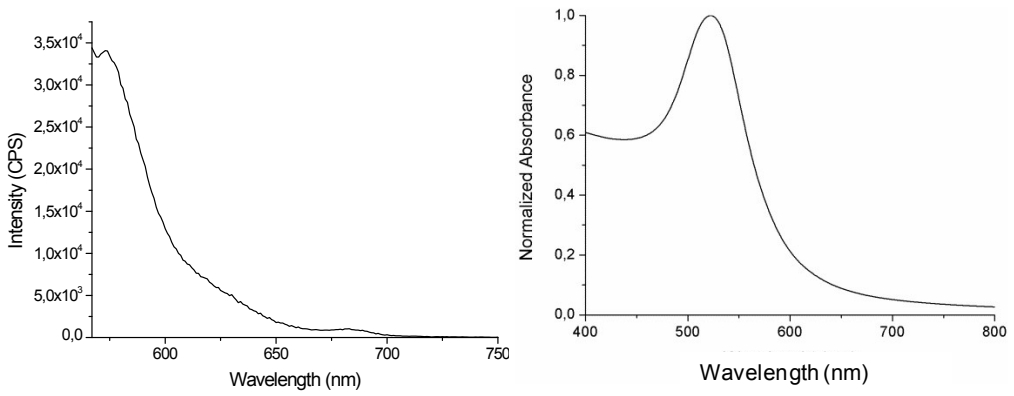


Figure S10. On the left: Fluorescence spectrum of Au-Glucosamine NPs*. On the right: UV-visible spectrum of Au-Glucosamine NPs*.

Au-PEG_{5000} NPs*

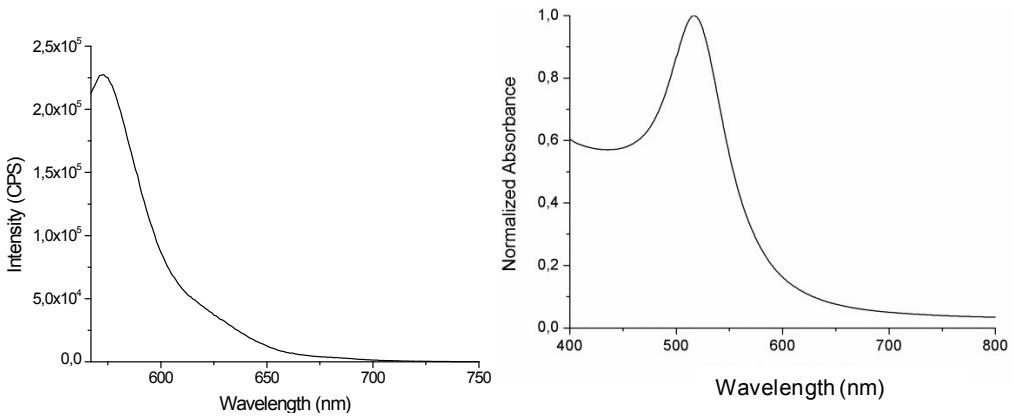


Figure S11. On the left: Fluorescence spectrum Au-PEG_{5000} NPs*. On the right: UV-visible spectrum of Au-PEG_{5000} NPs*.

Au-Alkyl-PEG_{600} NPs*

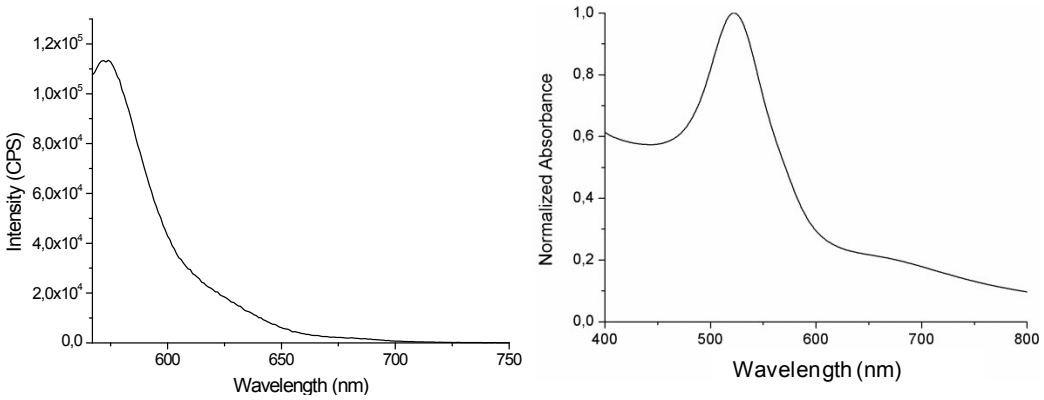
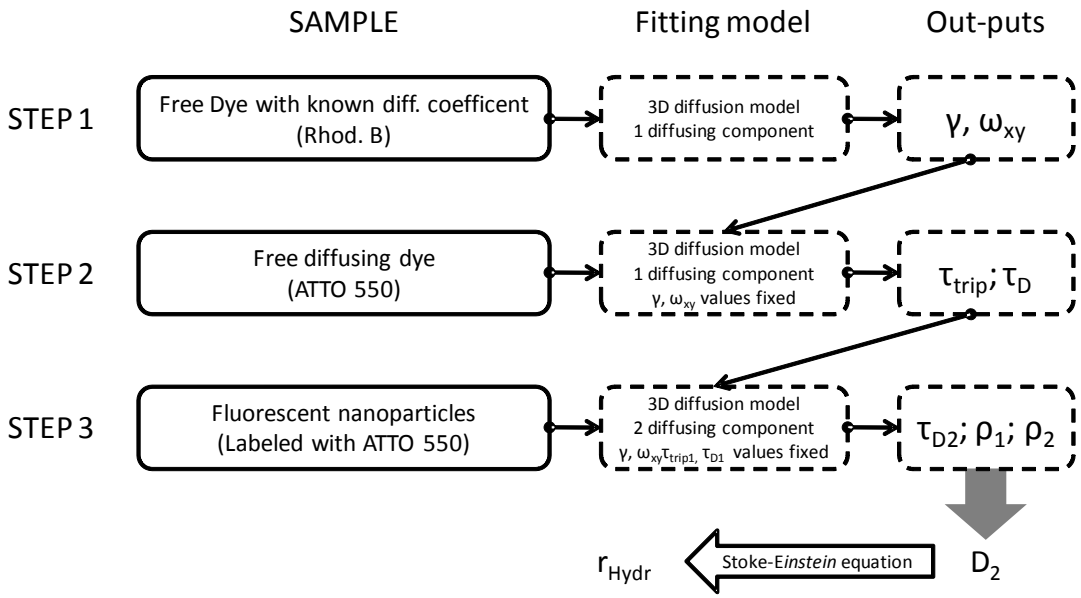


Figure S12. On the left: Fluorescence spectrum Au-Alkyl-PEG_{600} NPs*. On the right: UV-visible spectrum of Au-Alkyl-PEG_{600} NPs*.

S8. Au NPs Hydrodynamic diameter in water

Fluorescence correlation spectroscopy (FCS)

The procedure applied to determine Au NPs hydrodynamic radius in water solution is comprised of 3 steps (**Scheme S1**):



Scheme S1. Flow chart of the procedure for the FCS data elaboration

Step 1: Consist in the calibration of the instrument. Prior to each session of measurements 50 nM solution of a dye with known diffusion coefficient has been employed for the determination of the structural parameter of the system. The correlogram of the solution was registered and the autocorrelation functions G(τ) was fitted following a 3D diffusion model that can be denoted as:

$$G(\tau) = G_\infty + \frac{1}{N} X_{back} \cdot \left(\frac{1 - \theta_{non} + \theta_{non} e^{-\tau/\tau_{non}} - \theta_{trip} + \theta_{trip} e^{-\tau/\tau_{trip}}}{1 - \theta_{non} - \theta_{trip}} \right) \cdot \left[(1 - \rho_2 - \rho_3) \cdot g_1(\tau) + \rho_2 \cdot g_2(\tau) + \rho_3 \cdot g_3(\tau) \right]$$

Where the factor $g_1(\tau)$ is correspondent to:

$$g_i(\tau) = \left(1 + \frac{\tau}{\tau_{diff,i}}\right)^{-1} \cdot \left(1 + \frac{\tau}{\gamma^2 \tau_{diff,i}}\right)^{-1/2}$$

The background correction is calculated like:

$$X_{back} = \frac{(I - B)^2}{I^2}$$

I is the intensity of the signal and B is background intensity.
The parameters involved in the function are:
- **G_∞:** offset of the correlation function
- **N:** overall particle number (including currently dark particles, for example in triplet state)
- **Θ_{trip}, θ_{non}:** fractions of the particles in one of the first two non-fluorescent states
- **τ_{trip}, τ_{non}:** decay times of the first two non-fluorescent states
- **ρ_1,ρ_2,ρ_3 (ρ_1=1-ρ_2-ρ_3):** fractions of the three diffusing components
- **$\tau_{diff,i}$:** diffusion decay time of the i_{th} diffusing component
- **=:** structural parameters of the instruments that represent the ratio of the Gaussian used to approximate the focus
- **ω_{xy}:** lateral half axis of the focus
- **z_0:** longitudinal half axis of the focus
- ***cps*:** average background corrected intensity during the measurement

We chose Rhodamine B to perform the calibration, that is reported in literature to have a diffusion coefficient of 420±30 $\mu m^2/s$. [7] Knowing that the diffusion coefficient is equal to:

$$D = \frac{\omega_{xy}^2}{4\tau_D}$$

and imposing τ_D value inside a first order fitting model, we derived the values of γ and $\omega_{x,y}$ that describes the geometrical properties of the focal volume. For our system, the γ value was typically comprised between 5 and 7, $\omega_{x,y}$ between 200 and 300 nm.

Step 2: Once obtained the structural parameters of the confocal volume is possible to proceed with the measurement of the free diffusing dye (50 nM). The fitting of the auto-correlogram of free diffusing ATTO550 has been performed employing the same model and algorithm as in step 1. Fixing the previously obtained values of γ and $\omega_{X,Y}$ in the fitting model is possible to derive the values of τ_{trip}, τ_D typical of the fluorophore. Performing more than 20 measurements on free ATTO550 we obtained an average diffusion coefficient of the fluorophore of 405±23 $\mu m^2/s$ (Diffusion time 43±4 μs, detection volume 265±10 fl)

Step 3: Finally the auto-correlogram of the fluorescent nanoparticle solution (100 μg/ml) can be registered. The fitting of the auto-correlation function was performed employing a 3D diffusion model and considering 2 diffusing species: in fact despite the purification procedures a fraction of free diffusing dye was still detected in the samples. The two component fitting was chosen considering the statistical improvement. It is important to underline that this free fraction was always overestimated. In fact gold nanoparticles quench the dye molecules directly attached to their surface while enhance the fluorescence of the free dye fraction diffusing in their proximity[48]. The parameters of γ, ω_{xy}, τ_{trip} and the τ_D of the first specie were fixed to the values experimentally determined from steps 1 and 2. The fitting function will return the value of τ_{D2} ,corresponding to the diffusion time of the second specie present in solution (in our case the labelled gold nanoparticles). From the fitting function is possible to calculate also the fractions of the two populations composing the sample (residual free dye and labelled Au NPs). At the end , once derived the diffusion coefficient, employing Stoke-Einstein equation is possible to calculate the hydrodynamic radius of the NPs (Table S5).

	TEM		**FCS**		
	Core diameter (nm)	**S_d (nm)**	**D_h (nm)**	**S_d (nm)**	**Diameter increase (nm)**
Au-MSA* NPs	22.1	5.7	30.3	2.9	8.2
Au-Glucosamine* NPs	18.8	4.5	24.8	5.4	6.0
Au-PEG_{5000}* NPs	14.0	3.0	28.0	5.0	14.0
Au-Alkyl-PEG_{600}* NPs	18.7	4.5	27.8	1.7	9.1

Table S5. Summary of the diameters obtained by TEM micrographs and FCS spectroscopy with relative diameter increases.

Dynamic light scattering (DLS)

The hydrodynamic diameter of the Au NPs has been measured by mean of DLS, employing not fluorescent Au NPs. For each nanoparticle, 3 independent measurements were performed and mediated. In Table S6 are reported the diameter values for each nanoparticle, correlated with standard deviation and diameter increase in respect to the metallic core (obtained by TEM micrographs). The values are reported in terms of volume abundance and the standard deviation is referred to the half amplitude of the Gaussian dimensional distribution.

	TEM		DLS		
	Core diameter (nm)	**S_d (nm)**	**D_h (nm)**	**S_d (nm)**	**Diameter increase (nm)**
Au-MSA NPs	22.1	5.7	32.7	17.2	10.6
Au-Glucosamine NPs	18.8	4.5	31.0	14.0	12.2
Au-PEG_{5000} NPs	14.0	3.0	25.0	13.1	11.0
Au-Alkyl-PEG_{600} NPs	18.7	4.5	33.6	20.0	15.0

Table S6. Summary of the diameters obtained by TEM micrographs and DLS with relative radius increases.

UV-visible

From the UV-vis spectra is possible to determine the diameter of the NPs, by using the following formula:

$$D_H = exp(B_1 \frac{A_{spr}}{A_{450}} - B_2) \qquad \textbf{eq. 1}$$

Where A_{spr} is the absorbance of the plasmonic peak A_{450} is the absorbance at 450 nm and B_1 and B_2 are experimentally determined values (B_1= 3,55 and B_2=3,11)[6].

The diameter of the Au NPs has been measured with non fluorescent Au NPs. In Table S7 are reported the diameter values for each nanoparticle, correlated with standard deviation and diameter increase in respect to the metallic core (obtained by TEM micrographs). The reported values are the average of three independent measurements.

	TEM		UV-vis		
	Core diameter (nm)	**S_d (nm)**	**D_h (nm)**	**S_d (nm)**	**Diameter increase (nm)**
Au-MSA NPs	22.1	5.7	28.0	1.5	5.8
Au-Glucosamine NPs	18.8	4.5	28.1	1.1	9.2
Au-PEG_{5000} NPs	14.0	3.0	22.8	1.7	8.8
Au-Alkyl-PEG_{600} NPs	18.7	4.5	25.3	1.3	13.2

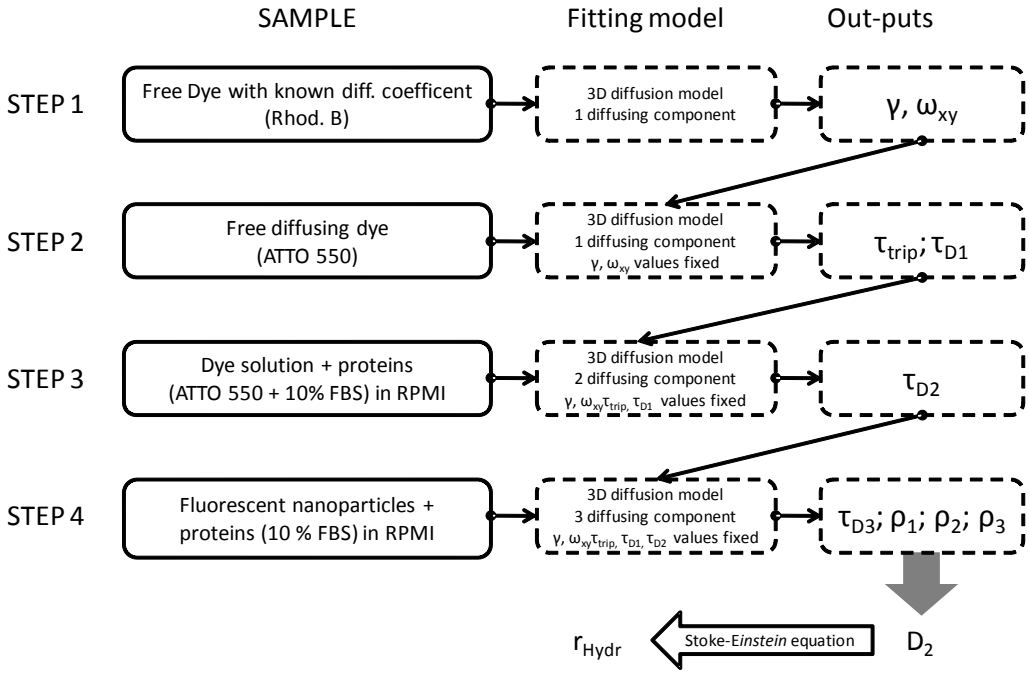
Table S7. Summary of the diameters obtained by TEM micrographs and UV-visible spectroscopy with relative radius increases.

S9. Au NPs hydrodynamic diameter in full RPMI cellular medium.

Fluorescence correlation spectroscopy (FCS)

Stock solutions of ATTO550 labelled Au NPs with a concentration of 1mg/ml were prepared with a 10% in volume of FBS. The FCS measurements were registered 10 min, 20 min, and 1h after the mixing of the solutions. Each sample of NPs was measured in 3 independent experiments, comprised by 10 runs of 10 seconds each one. As comparison, a FCS measurement was performed on bare NPs. Prior to each FCS measurement the solutions were sonicated for 5 min and double filtered on 0.22 μm regenerate cellulose syringe filters. This operation helps to remove the excess of free fluorophore that can remain electrostatically attached to the surface of the Au NPs. To perform the FCS measurements 25 μl of Au NPs were dissolved in 225 μl of a solution of phenol red free RPMI, enriched NPs in RPMI. For each measurement session 50 nM water solutions of Rhodamine B and of ATTO550 were measured to determine the structural parameter and the diffusion coefficient of the free dye. The diffusion coefficient of proteins was determined mixing 25 μg of FBS with a 50 nM solution ATTO550 in RPMI.

The procedure applied to determine Au NP hydrodynamic radius in full RPMI medium is comprised of 4 steps (1st and 2nd are identical to the procedure applied for Au NP water solutions)(Scheme S2).



Scheme S2. Flow chart of the procedure for the FCS data elaboration in protein solutions.

Step 1: Calibration of the instrument. Registration of the correlograms of a 50 nM Rhodamine B solution with known diffusion time and determination of the structural parameter of the system (γ and $\omega_{X,Y}$).

Step 2: Measurement of the free diffusing dye (ATTO550). Fixing the previously obtained values of γ and $\omega_{X,Y}$ in the fitting model, is possible to derive the values of $\tau_{trip,}$ τ_D typical of the fluorophore.

Step 3: The auto-correlogram of a mixture of ATTO550 solution in RPMI with a 10% of FBS has been registered. The fitting of the autocorrelation function is performed employing a 3D diffusion model and considering 2 diffusing species. The parameters of γ, ω_{xy}, τ_{trip} and the τ_D of the first specie were fixed to the values experimentally determined from steps 1 and 2. The fitting function will return the value of τ_{D2}, corresponding to the diffusion time of the second specie present in solution (in this case the protein mixture). From the fitting function is possible to calculate also the fractions of the two populations composing the sample (residual free dye and proteins).

Step 4: Finally the auto-correlogram of the fluorescent nanoparticle solution (100 μg/ml) in 10% of FBS (RPMI solution) can be registered at different time points. The fitting of the auto-correlation function is performed employing a 3D diffusion model and considering 3 diffusing species. The parameters of γ, ω_{xy}, τ_{trip} , $\tau_{D1,and}$ τ_{D2} of the first and second species were fixed to the values experimentally determined from steps 1, 2 and 3. The fitting function will return the value of τ_{D3} ,corresponding to the diffusion time of the third specie present in solution (corresponding to the adducts between proteins and Au NPs). From the fitting function is possible to calculate also the fractions of the three populations composing the sample (residual free dye, free proteins and AuNP-Protein adducts). Employing **eq. 12** is possible to derive, from the diffusion time, the diffusion coefficient and substituting the last one in Stoke-Einstein equation the hydrodynamic radius.

Dynamic light scattering (DLS)

To perform the DLS study of PC formation, 50 µl of not fluorescent Au NPs (1mg/ml) were dissolved a solution of phenol red free full RPMI. The NPs were incubated for 10 min, 20 min, and 1h at 37 °C. The experiments were performed registering for each NP 3 tracks at 37 °C, each one composed of 10 runs of 10 seconds. The system was allowed to equilibrate for 2 min prior to each measurement. The data were analyzed employing Zetasizer software from Malvern and reported in terms of volume abundance. The resulting correlograms are reported in Figure S13. Simply comparing the normalized correlograms of the NPs at different time points is possible to distinguish which of them are less inclined to interact with the proteins. In fact it is possible to appreciate that the correlograms of Au-MSA NPs and Au-Glucosamine NPs in FBS solutions show large shifts in the lag time during the incubation period indicating hydrodynamic dimensions increase. Instead for Au-PEG_{5000} NPs and Au-Alkyl-PEG_{600} NPs correlograms the shifts are reduced indicating a lower affinity between the coating and the proteins.

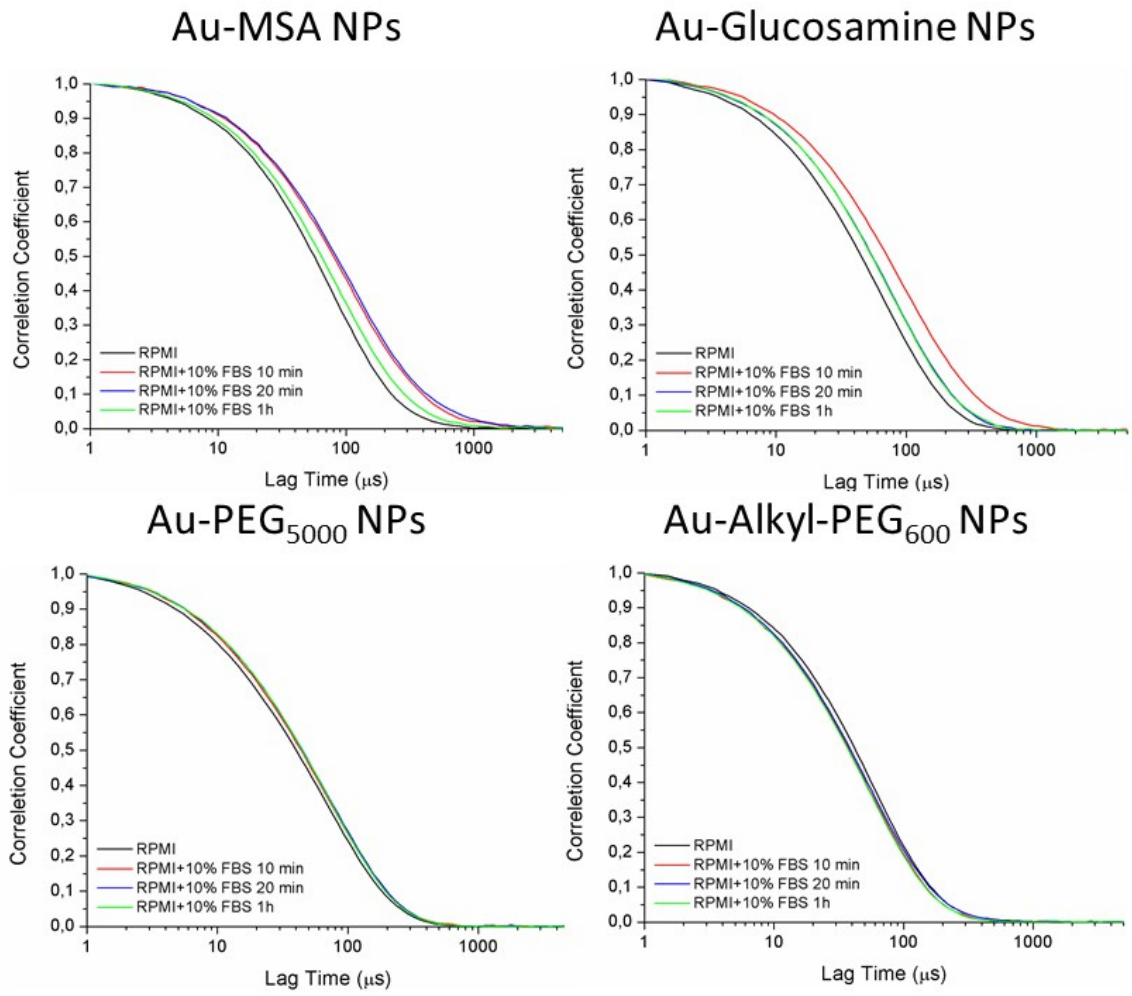


Figure S13. Correlograms of Au-MSA NPs, Au-Glucosamine NPs, Au-PEG_{5000} NPs, Au-Alkyl -PEG_{600}NPs incubated in bare and full RPMI (enriched with a 10% of FBS) at different time points.

UV-visible

To perform the UV-vis study of PC formation, 50 µl of not fluorescent Au NPs (1mg/ml) were dissolved a solution of phenol red free full RPMI (10% FBS). The NPs were incubated for 1 and 24 hours at 37 °C. The experiments were performed in triplicate at 25 °C. A UV-vis spectrum of bare NPs in RPMI has been registered. A solution of full RPMI was employed as baseline. Equation 1 was employed to derive the Au NPs diameters and the results are summarized in Figure S14.

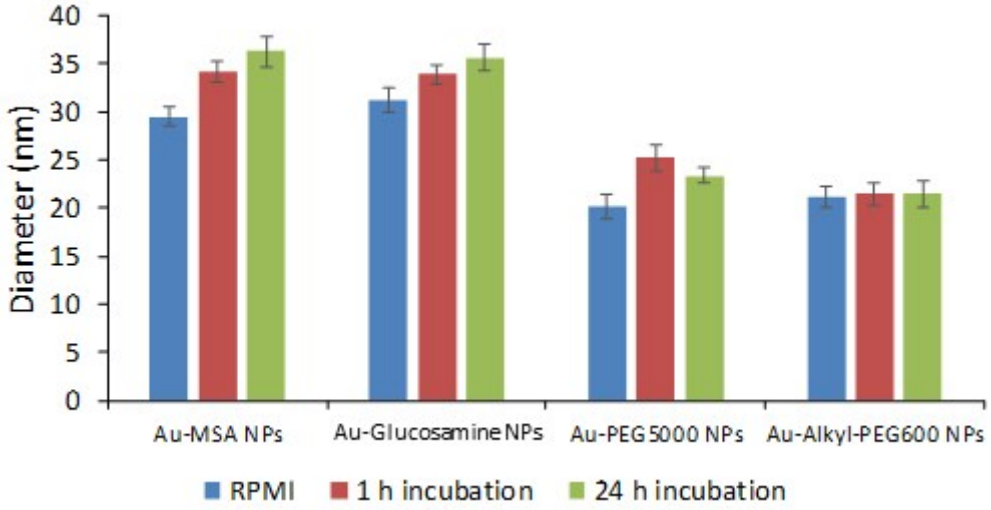


Figure S14. Hydrodynamic diameter calculated by UV-vis spectroscopy during the incubation of Au NPs with bare RPMI and full RPMI (enriched with 10% of FBS).

The plasmonic peak shift has been monitored during the observation time. The increase in the wavelength can be directly correlated with a growth in the gold structure dimensions. The smallest shift was observed for Au-Alkyl-PEG_{600} NPs (Figure S15 orange line), showing a increment of only 0.5 nm after 24 hours of incubation.

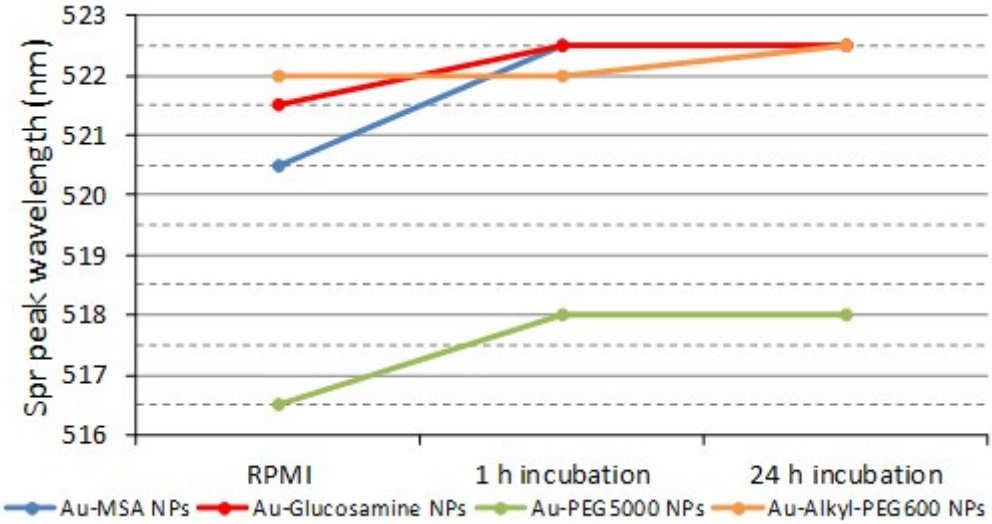


Figure S15. Plasmonic peak wavelength variation during the incubation of Au NPs with bare RPMI and full RPMI (enriched with 10% of FBS).

S10. FCS measurements in living cell

FCS measurements were done on at least 3 cells recording 20 runs of 10 second each in 8 distinct area inside the cell: 2 in the cytoplasm; 2 in the endoplasmatic and whenever visible, 2 in brighter spots near to the membrane (endocytic vesicles) and 2 in brighter spots near to the nucleus (intracellular vesicles). Moreover 1 experiment was performed on/close to the membrane and 1 on/close the nucleus. Tracks were at first screened one-by-one to remove tracks showing bleaching, low signal and visible aggregates (see Fig. S17). FCS experiments performed on/close to membrane and nucleus shown poor/no correlation and the registered diffusion times were associated to proteins (τD <500 μs) rather than NPs (Fig. S18). For these reason the nucleus and membrane were excluded from the regions of interest in the data elaboration. The remaining tracks were fitted one-by-one by QuickFit 3.0 software[7] using a 3D Normal diffusion Fit model. The fit algorithms used were in order, Simulated Annealing with box constraints and the Levenberg-Marquardt Algorithm with box constraints with one non-fluorescent state and 2(or 3) diffusing components. Considering the decay time ($\tau_{D,}$ μs) of the most abundant specie (fraction Φ>0.5), the tracks were grouped as follow: 500< τ_D (μs) < 1500; 1500< τ_D (μs) < 3000; 3000< τ_D (μs) < 6000; τ_D (μs)>6000). The average track was fitted again and the average decay time with the correspondent standard deviation calculated. The procedure was repeated for each measurement of each cell compartment. Finally decay times were averaged per compartment considering all cell replicates referring to the above-mentioned decay times grouping. The percentage of tracks representative of the species decaying with the latter diffusion times were calculated over the total of the good tracks analyzed in all cell replicates. The obtained results are summurized in Figure S19 and S20.

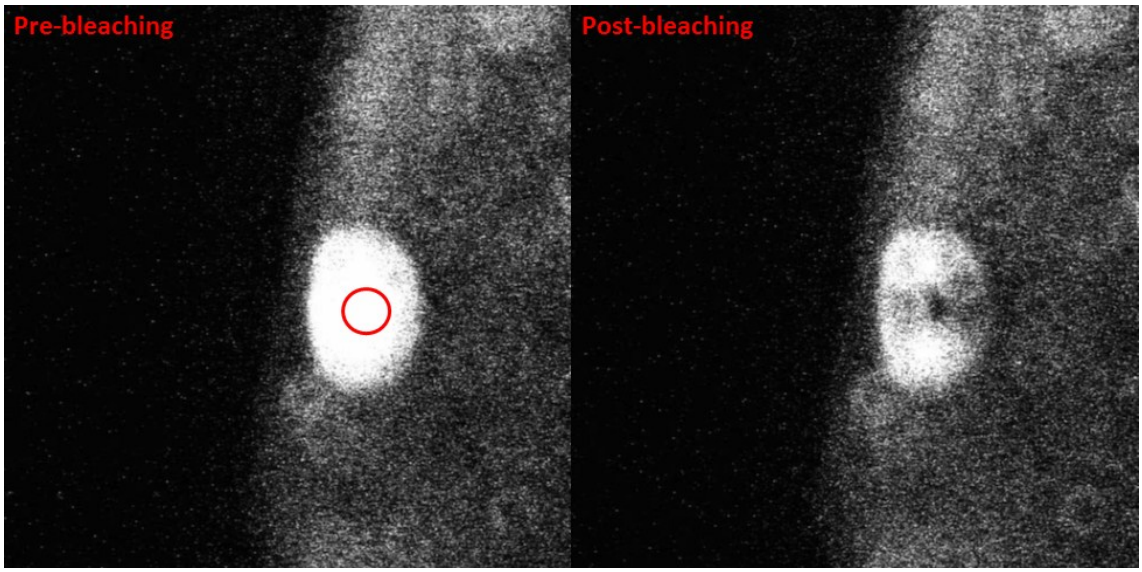


Figure S16. Confocal images pre and post bleaching, demonstrating the alignment between the FCS and confocal setups.

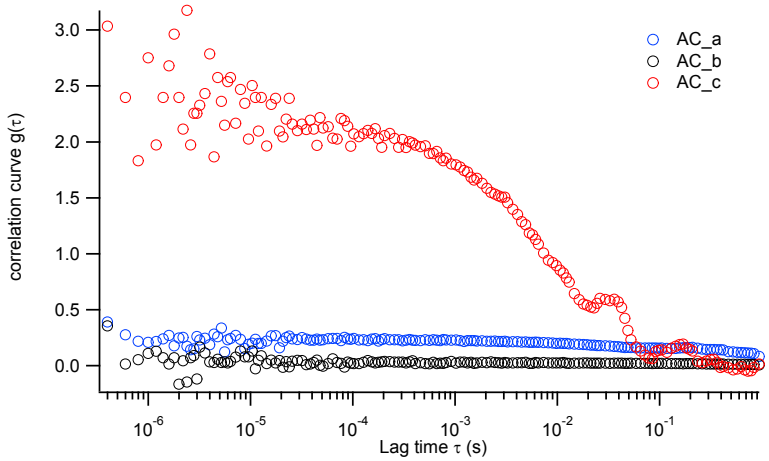


Figure S17. Representative correlation function of the excluded track showing bleaching (AC_a, blue dots), low signal (AC_b, black dots) and visible aggregates (AC_c, red dots).

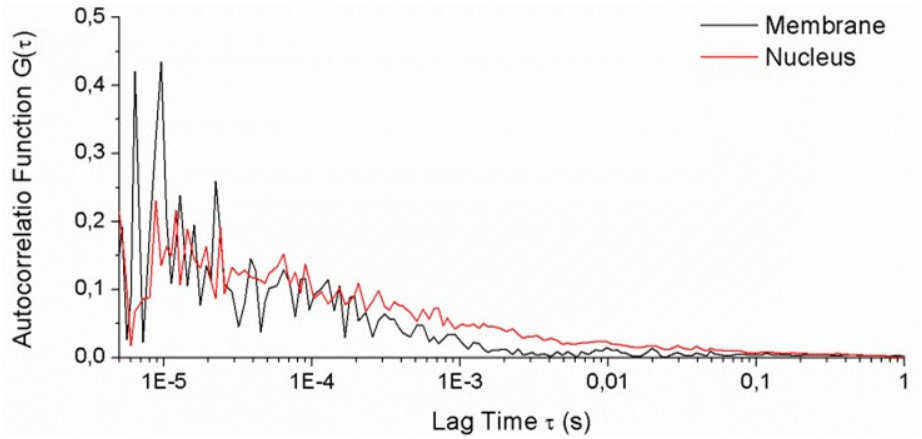


Figure S18. Representative FCS autocorrelation function for the membrane and nucleus of the cell (Au-MSA* NPs). The intensity signal registered in these regions was too low to be included in the statistical analysis.

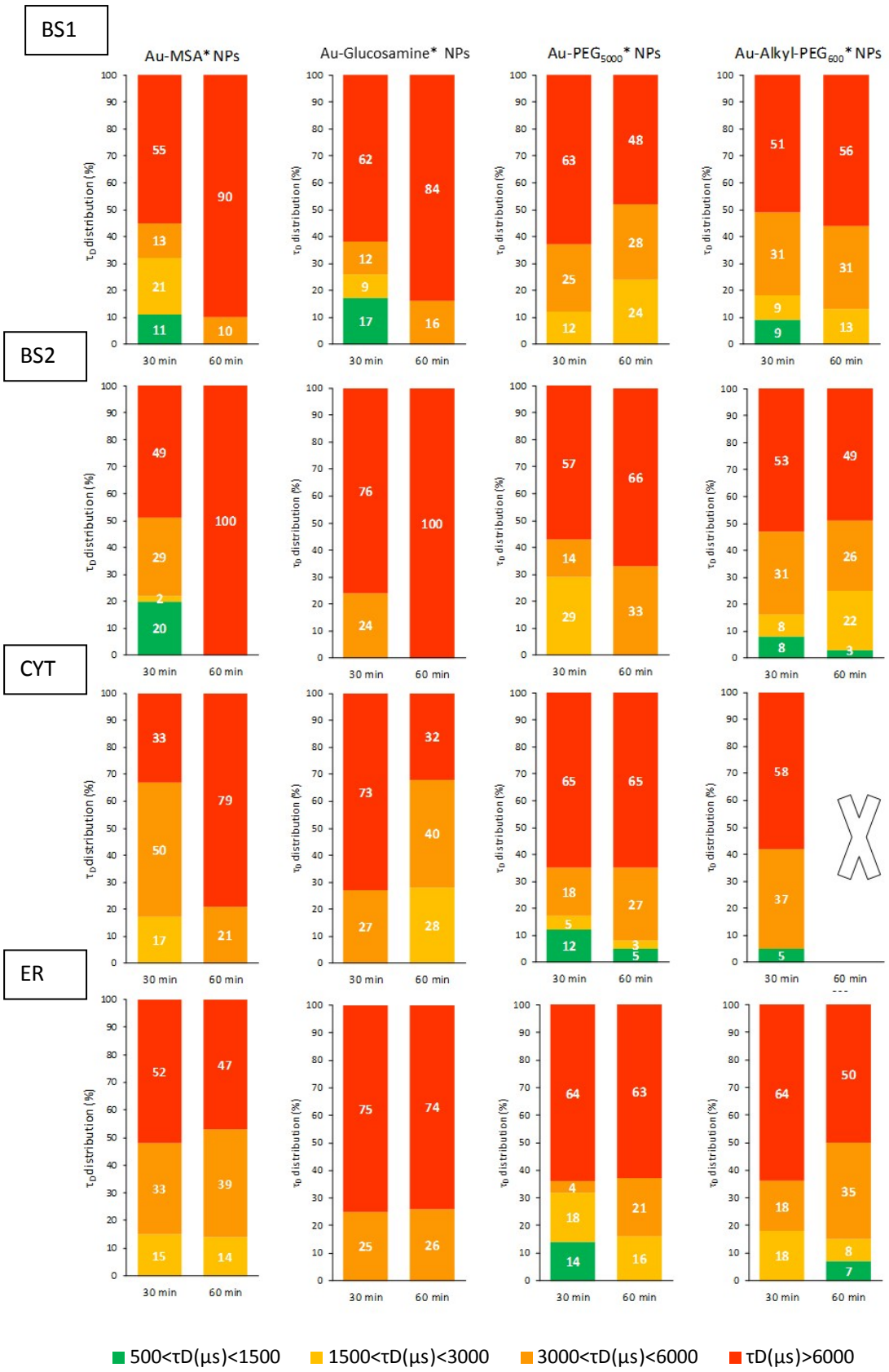


Figure S19. Percentage distribution of the D_{fast} in the different compartments of the cell grouped in four classes of diffusion times (green=500< τ_D (μs) < 1500; yellow= 1500< τ_D (μs) < 3000; orange= 3000< τ_D (μs) < 6000 and red= τ_D(μs)>6000). In each coloumn of the table are reported the results for Au-MSA* NPs, Au-Glucosamine* NPs, Au-PEG_{5000}* NPs and Au-Alkyl-PEG_{600}* NPs at 30 minutes and 1 hour of incubation.

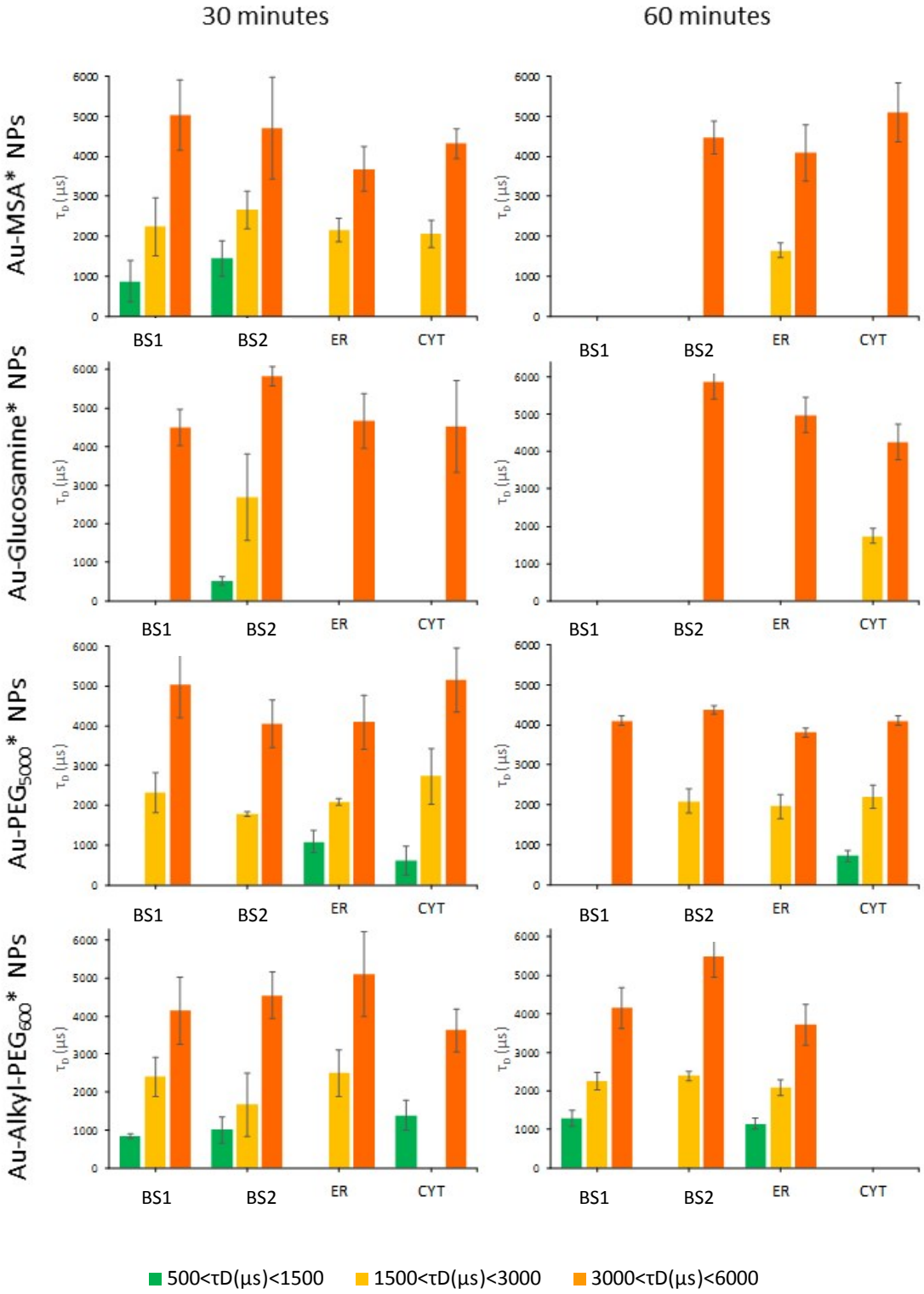


Figure S20. Histograms of the mean diffusion times for each region of interest (obtained after the grouping of the traves in the four classes of diffusion times). Each histogram reports the results for Au-MSA* NPs, Au- Glucosamine* NPs, Au-PEG_{5000}* NPs and Au-Alkyl-PEG_{600}-C4H9* NPs at 30 minutes and 1 hour of incubation. BS1=bright spots 1; BS2= bright spots 2; ER=Endoplasmatic reticulum; CYT=Cytoplasm.

A control has been performed incubating free ATTO550 with A549 cells. To perform FCS on live cells, 10.000 A549 cells were seeded on Nunc™ Lab-Tek Chambered Coverglass (purchased from Thermo Fisher Scientific) and grown in a humidified atmosphere at 37 °C with 5% CO_2 for 24 hours. The cells

were incubated with 7 nM solution of ATTO550 NHS ester for 30 and 60 minutes. The cells were washed twice with warm PBS. To perform the experiments on living cells, they were kept in Hepes 10 mM. The data were analyzed as presented above. Due to presence of several traces presenting τ_D < 500 µs, a fifth group of traces was considered. Almost the totality of the traces presents diffusion times lower than 500 µs or higher then 6000, ascribable respectively to the dye interacting with proteins or with cellular machinery (Table S8 and Table S9). The diffusion free dye showed a diffusion time distribution and intracellular trafficking completely different from the labelled Au NPs.

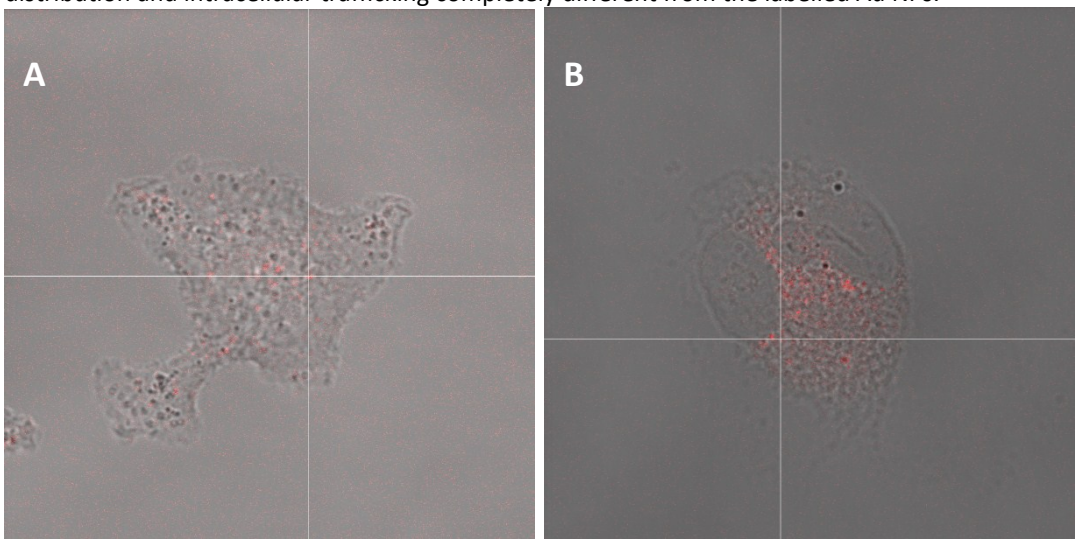


Figure S21. Confocal images of A549 cells incubated with free ATTO550 at 30 minutes (a) and 1 hour (b)

	τ_D(µs) < 500	500< τ_D(µs) <1500	1500< τ_D(µs) <3000	3000< τ_D(µs) <6000	τ_D(µs) > 6000
BS1	59%	0%	5%	6%	30%
BS2	80%	0%	10%	4%	6%
CYT	69%	0%	8%	0%	23%
ER	73%	0%	0%	7%	21%

Table S8. Free ATTO550 control after 30 minutes of incubations: percentage distribution of the diffusion times grouped in 5 classes.

	τ_D(µs) < 500	500< τD(µs) <1500	1500< τD(µs) <3000	3000< τD(µs) <6000	τD(µs) > 6000
BS1	76%	0%	4%	0%	20%
BS2	87%	0%	0%	0%	13%
CYT	85%	0%	0%	4%	11%
ER	79%	0%	0%	0%	21%

Table S9. Free ATTO550 control after 60 minutes of incubation: percentage distribution of the diffusion times grouped in 5 classes.

S11. Confocal imaging of cells

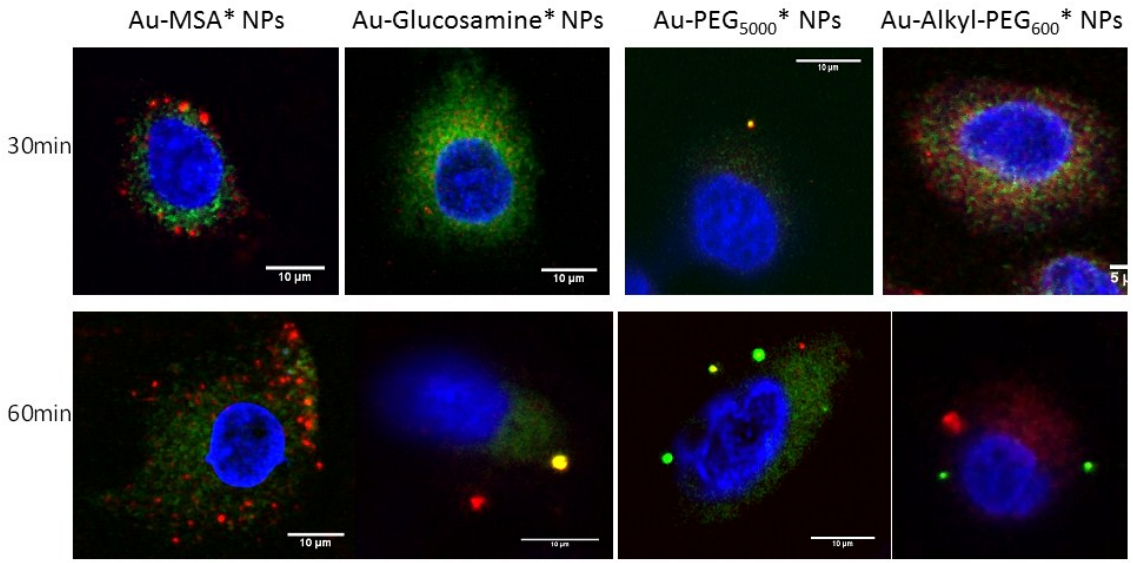
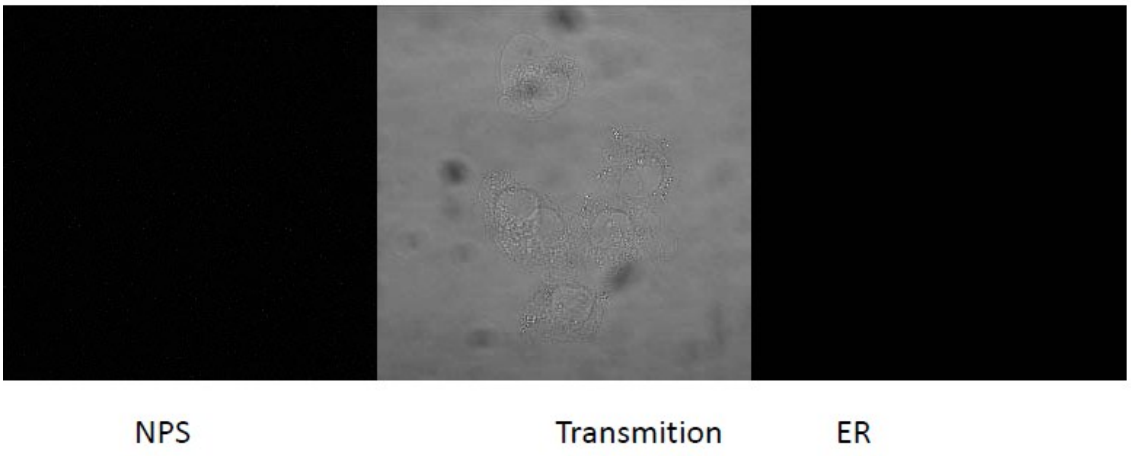


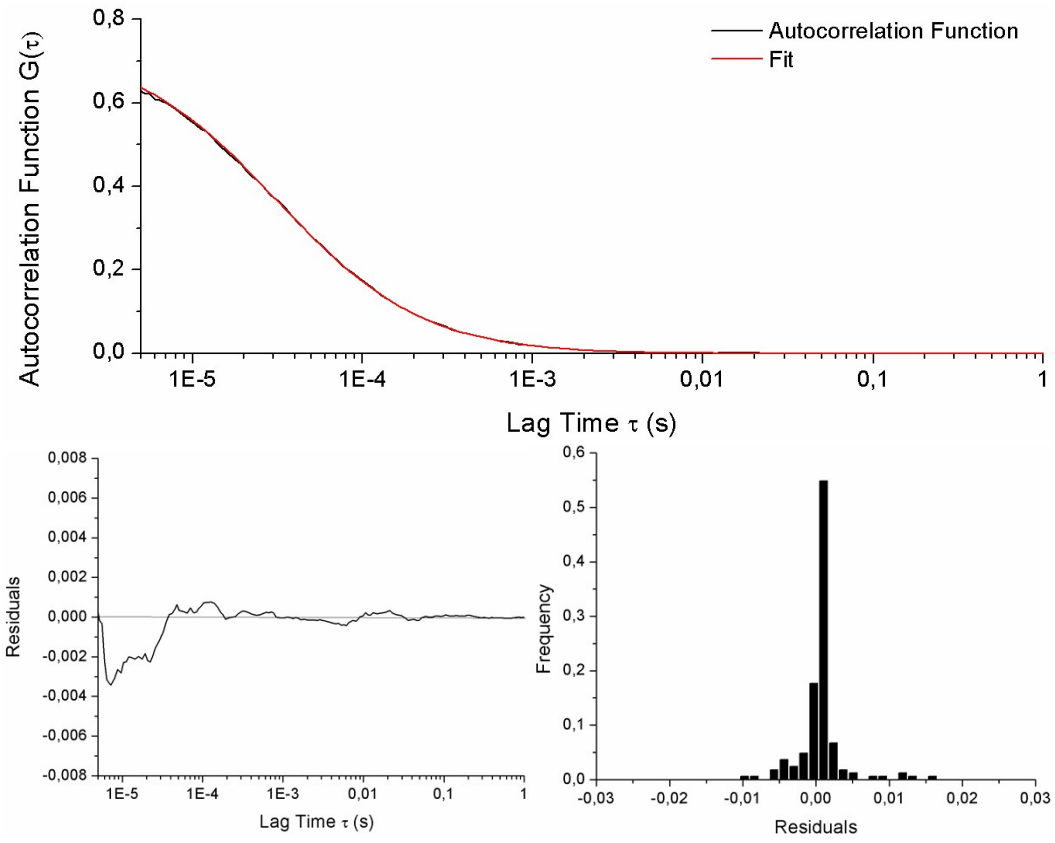
Figure S22. Confocal micrographs collected after 30 min and 1 hour of incubation. Nuclei were stained by Hoechst (blue signal), NPs were labelled by ATTO550 (red signal) and endoplasmatic reticulum stained by ER-Tracker Green (green signal). Cells were imaged in transmission mode. Excitation laser wavelengths were respectively 405 nm, 488 and 561 nm. Objective was 63x oil immersion lens (1.4 NA).



FigureS23. Confocal micrograph realized as control on cells without NPs and without staining.

S12. Fluorescence correlation spectroscopy (FCS): Fitting examples

Fitting of FCS data for ATTO550 water solution



Model Parameters (fit results):

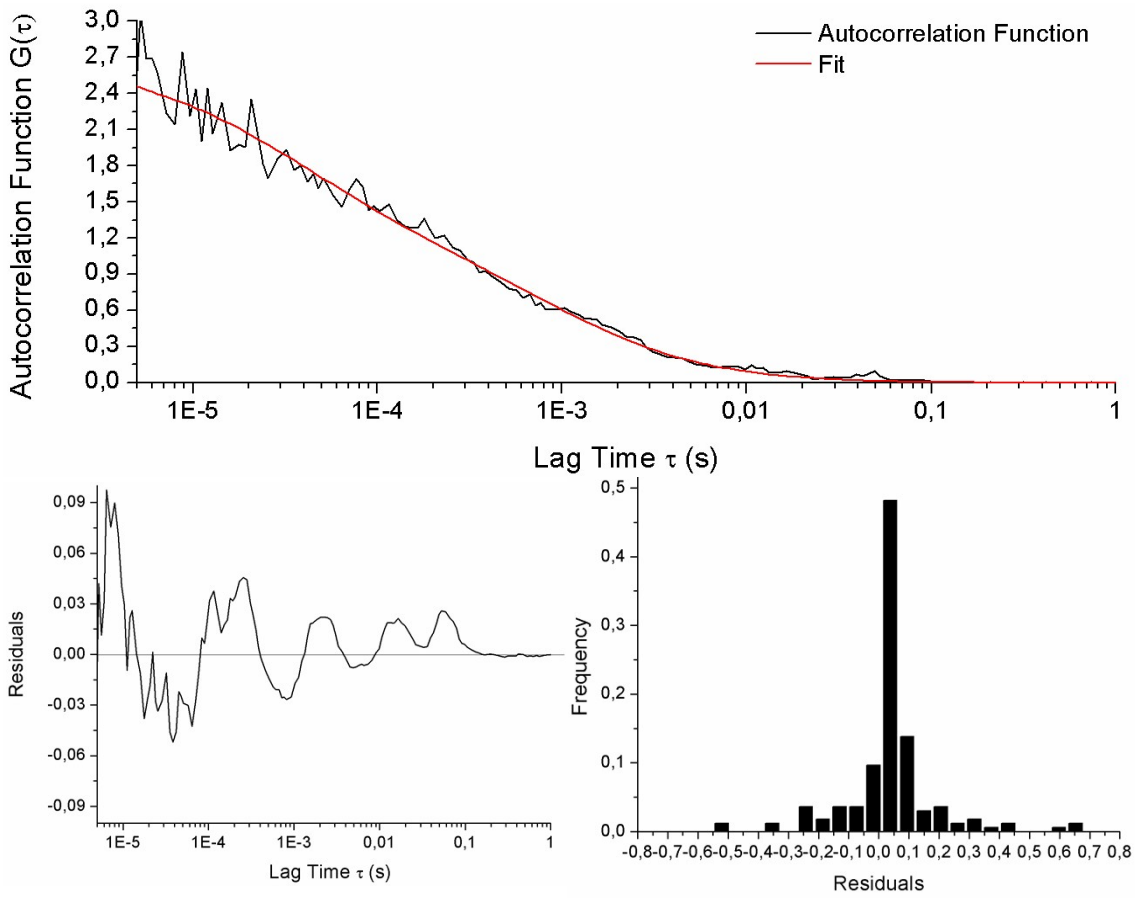
Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0...2	$w_{x,y}$ =		229.6179 ± 6.22	nm	$0...10^{4}$
components =		1	1...3	V_{eff} =	C	0.5285 ± 0.0594	fl	$0...10^{50}$
N =	F	1.35326 ± 0.00671	$10^{-10}...10^{5}$	C_{all} =	C	4.2523 ± 0.479	nM	$0...10^{50}$
1/N =	C	0.73896 ± 0.00367	$10^{-10}...10^{5}$	D_1 =	C	420.83 ± 23.5	$\mu m^2/s$	$0...10^{50}$
$\tau_{D,1}$ =	F	31.3216 ± 0.409	µs $1...10^{5}$	count rate =		0 ± nan × $10^{-2147483648}$	Hz	$0...10^{50}$
G_∞ =	FX	0 ± 0	-10...10	background =		0 ± 0	Hz	$0...10^{50}$
γ =	F	7.8391 ± 0.61	0.01...100	cnt/molec =	C	0 ± nan × $10^{-2147483648}$	Hz	$0...10^{50}$

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Statistics:

X^2 = 0.00197157	X^2 (weighted) = 35.6153
⟨E⟩ = 9.75153e-05	⟨E⟩ (weighted) = -0.0161074
$\sqrt{\langle E^2 \rangle}$ = 0.00346587	$\sqrt{\langle E^2 \rangle}$ (weighted) = 0.465733
NP = 3	NR = 164
DF = 160	TSS = 10.1577
R^2 = 0.999806	R^2 (weighted) = -2.50624
$R^2_{adjusted}$ = 0.999803	$R^2_{adjusted}$ (weighted) = -2.54979
AICc = -1851.77	AICc (weighted) = -244.293
BIC = -1842.62	BIC (weighted) = -235.143
	$max_P(\sigma_P/\|P\|)$ = 7.78151%
det(COV) = 4.00533e-07	p_{Bayes}(model|data) = 1.71655e-15 (= $10^{-14.7653}$)

Fitting of FCS data for Au-MSA* NPs water solution



Model Parameters (fit results):

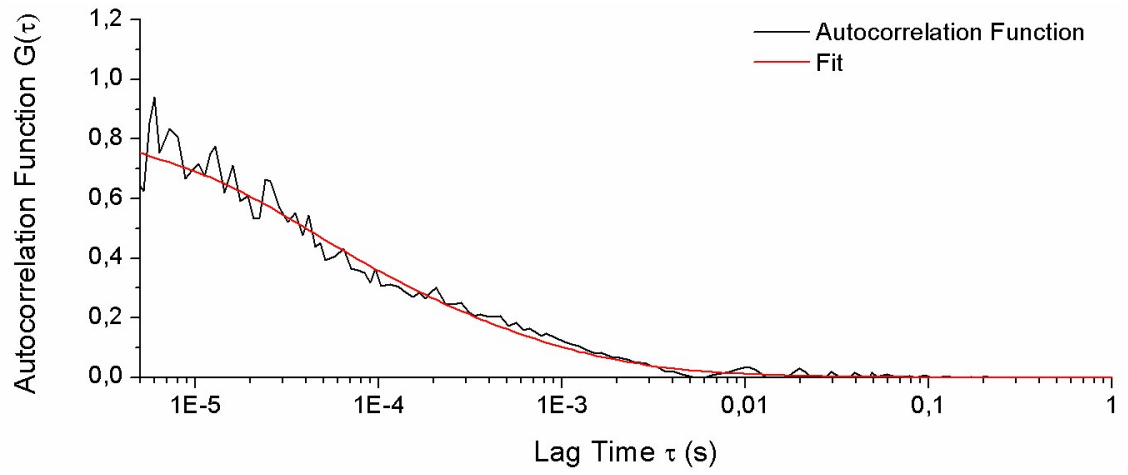
Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		1		0...2	G_∞ =	FX	0 ± 0		-10...10
components =		2		1...3	γ =	FX	6.0105 ± 1		0.01...100
τ_{trip} =	FX	1.23 ± 0	µs	0...10	$w_{x,y}$ =		240.674 ± 9.32	nm	$0...10^4$
θ_{trip} =	F	0.2952 ± 0.104		0...0.99999	V_{eff} =	C	0.4666 ± 0.0947	fl	$0...10^{50}$
N =	F	0.3806 ± 0.0118		$10^{-10}...10^5$	C_{all} =	C	1.3546 ± 0.278	nM	$0...10^{50}$
1/N =	C	2.6272 ± 0.0814		$10^{-10}...10^5$	D_1 =	C	408.3755 ± 31.6	$\mu m^2/s$	$0...10^{50}$
ρ_1 =	C	0.5576 ± 0.0299		0...0.99999	D_2 =	C	14.8396 ± 1.64	$\mu m^2/s$	$0...10^{50}$
$\tau_{D,1}$ =	FX	35.46 ± 0	µs	$1...10^5$	count rate =		$7.36867\times10^{280} \pm 2.66\times10^{284}$	Hz	$0...10^{50}$
ρ_2 =	F	0.4424 ± 0.0299		0...0.99999	background =		0 ± 0	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	975.8338 ± 77.3	µs	$1...10^8$	cnt/molec =	C	$1.93593\times10^{281} \pm 6.99\times10^{284}$	Hz	$0...10^{50}$

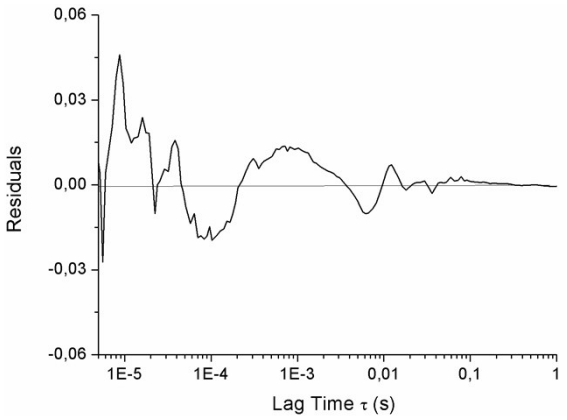
legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

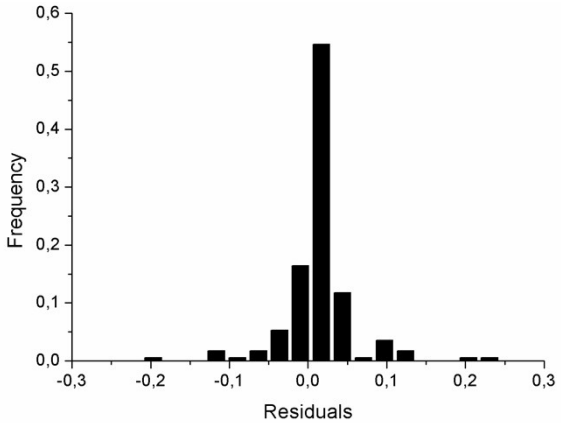
Fit Statistics:

χ^2 = 0.623219	χ^2 (weighted) = 51.1462
⟨E⟩ = 0.00604155	⟨E⟩ (weighted) = -0.0125117
$\sqrt{\langle E^2 \rangle}$ = 0.0593696	$\sqrt{\langle E^2 \rangle}$ (weighted) = 0.54047
NP = 7	NR = 175
DF = 167	TSS = 94.9918
R^2 = 0.993439	R^2 (weighted) = 0.461572
$R^2_{adjusted}$ = 0.993205	$R^2_{adjusted}$ (weighted) = 0.442343
AICc = -971.917	AICc (weighted) = -200.597
BIC = -950.434	BIC (weighted) = -179.114
	$max_P(\sigma_P/\|P\|)$ = 87.5%
det(COV) = 4.26821e-12	p_{Bayes}(model|data) = 1.32742e-30 (= $10^{-29.877}$)

Fitting of FCS data for Au-Glucosamine* NPs water solution







Model Parameters (fit results):

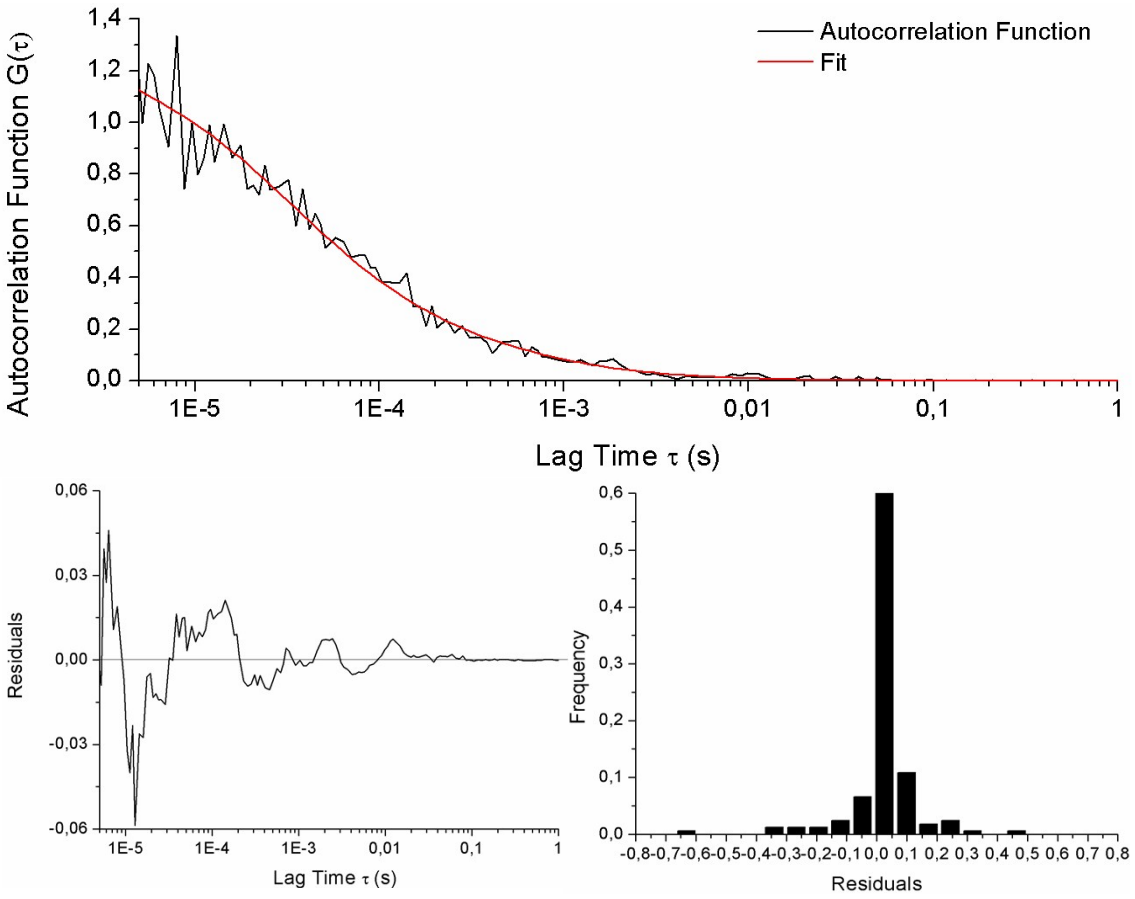
Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		1		0...2	G_∞ =	FX	0 ± 0		-10...10
components =		2		1...3	γ =	FX	7.9 ± 1		0.01...100
τ_{trip} =	FX	0.4 ± 0	µs	0...10	$w_{x,y}$ =		244 ± 6	nm	$0...10^4$
θ_{trip} =	F	0.8286 ± 0.0392		0...0.99999	V_{eff} =	C	0.639 ± 0.0936	fl	$0...10^{50}$
N =	F	1.1872 ± 0.0864		$10^{-10}...10^5$	C_{all} =	C	3.0849 ± 0.505	nM	$0...10^{50}$
1/N =	C	0.8423 ± 0.0613		$10^{-10}...10^5$	D_1 =	C	402.2703 ± 19.8	$\mu m^2/s$	$0...10^{50}$
ρ_1 =	C	0.7273 ± 0.0871		0...0.99999	D_2 =	C	25.5532 ± 8.13	$\mu m^2/s$	$0...10^{50}$
$\tau_{D,1}$ =	FX	37 ± 0	µs	$1...10^5$	count rate =		443.3933 ± 2.04×10^{236}	Hz	$0...10^{50}$
ρ_2 =	F	0.2727 ± 0.0871		0...0.99999	background =		0 ± 0	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	582.4707 ± 183	µs	$1...10^8$	cnt/molec =	C	373.4882 ± 1.72×10^{236}	Hz	$0...10^{50}$

legend: ***F**: fit parameter, **X**: fixed parameter, **C**: calculated parameter*

Fit Statistics:

X^2 = 0.843796	X^2 (weighted) = 248.023
⟨E⟩ = 0.00646012	⟨E⟩ (weighted) = 0.0409328
$\sqrt{\langle E^2\rangle}$ = 0.0693374	$\sqrt{\langle E^2\rangle}$ (weighted) = 1.19321
NP = 4	NR = 174
DF = 169	TSS = 25.2284
R^2 = 0.966554	R^2 (weighted) = -8.83108
$R^2_{adjusted}$ = 0.965964	$R^2_{adjusted}$ (weighted) = -9.00457
AICc = -918.992	AICc (weighted) = 69.9135
BIC = -906.592	BIC (weighted) = 82.313
	$max_P(\sigma_P/\|P\|)$ = 31.9399%
det(COV) = 2.53384e-05	p_{Bayes}(model|data) = 4.19732e-64 (= $10^{-63.377}$)

Fitting of FCS data for Au-PEG5000* NPs water solution



Model Parameters (fit results):

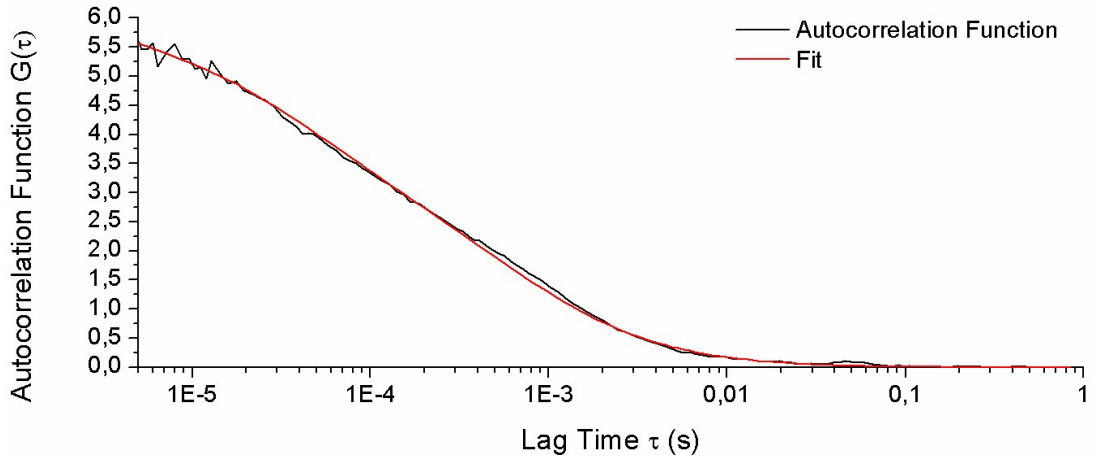
Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		1		0...2	G_∞ =	FX	0 ± 0		-10...10
components =		2		1...3	γ =	FX	6.54 ± 1		0.01...100
τ_{trip} =	FX	1.19 ± 0	µs	0...10	$w_{x,y}$ =		228.3 ± 10.8	nm	$0...10^4$
θ_{trip} =	F	0.4376 ± 0.14		0...0.99999	V_{eff} =	C	0.4333 ± 0.0904	fl	$0...10^{50}$
N =	F	0.7913 ± 0.0523		$10^{-10}...10^5$	C_{all} =	C	3.0322 ± 0.664	nM	$0...10^{50}$
1/N =	C	1.2638 ± 0.0836		$10^{-10}...10^5$	D_1 =	C	394.8552 ± 37.4	$\mu m^2/s$	$0...10^{50}$
ρ_1 =	C	0.8952 ± 0.0448		0...0.99999	D_2 =	C	17.8783 ± 9.92	$\mu m^2/s$	$0...10^{50}$
$\tau_{D,1}$ =	FX	33 ± 0	µs	$1...10^5$	count rate =		$1.58629\times10^{224} \pm 8.69\times10^{306}$	Hz	$0...10^{50}$
ρ_2 =	F	0.1048 ± 0.0448		0...0.99999	background =		0 ± 0	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	728.83 ± 398	µs	$1...10^8$	cnt/molec =	C	$2.00475\times10^{224} \pm 1.1\times10^{307}$	Hz	$0...10^{50}$

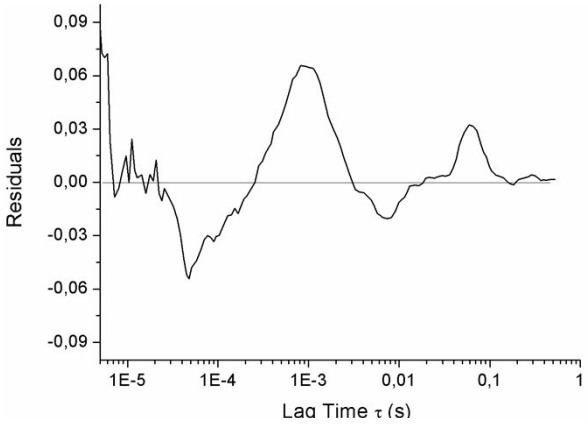
legend: ***F**: fit parameter, **X**: fixed parameter, **C**: calculated parameter*

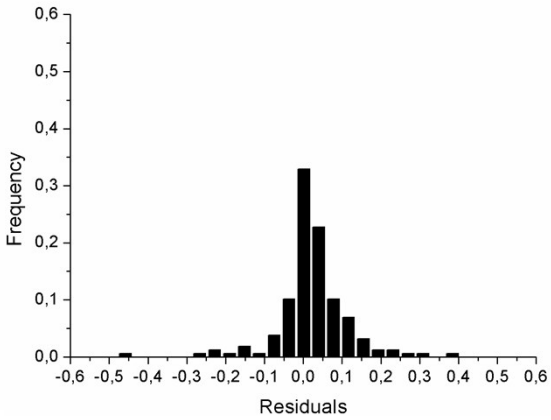
Fit Statistics:

X^2 = 3.12242	X^2 (weighted) = 48.4896
⟨E⟩ = -0.00249388	⟨E⟩ (weighted) = -0.0153988
$\sqrt{\langle E^2\rangle}$ = 0.137126	$\sqrt{\langle E^2\rangle}$ (weighted) = 0.540249
NP = 4	NR = 166
DF = 161	TSS = 44.2743
R^2 = 0.929475	R^2 (weighted) = -0.0952086
$R^2_{adjusted}$ = 0.928169	$R^2_{adjusted}$ (weighted) = -0.11549
AICc = -651.332	AICc (weighted) = -196.038
BIC = -639.133	BIC (weighted) = -183.838
	$max_P(\sigma_P/\|P\|)$ = 54.6081%
det(COV) = 0.000866215	p_{Bayes}(model|data) = 1.02336e-20 (= $10^{-19.99}$)

Fitting of FCS data for Au-Alkyl-PEG$_{600}$-C$_4$H$_9$ * NPs water solution







Model Parameters (fit results):

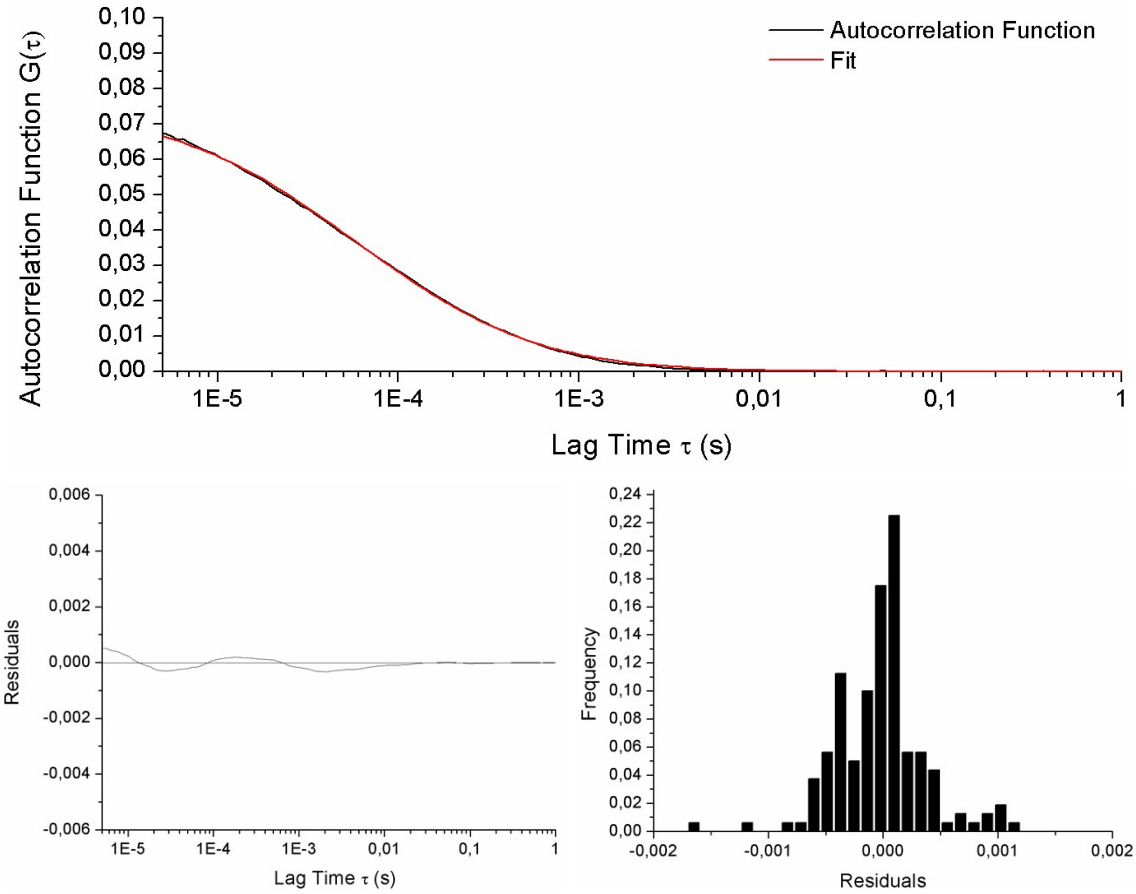
Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		1	0...2	G_∞ =	FX	0 ± 0		-10...10
components =		2	1...3	γ =	FX	7 ± 1.35		0.01...100
τ_{trip} =	FX	1.57 ± 0	µs 0...10	$w_{x,y}$ =		216 ± 10	nm	$0...10^4$
θ_{trip} =	F	0.233 ± 0.0461	0...0.99999	V_{eff} =	C	0.3928 ± 0.0934	fl	$0...10^{50}$
N =	F	0.17075 ± 0.00361	$10^{-10}...10^5$	C_{all} =	C	0.7218 ± 0.172	nM	$0...10^{50}$
1/N =	C	5.8564 ± 0.124	$10^{-10}...10^5$	D_1 =	C	302.961 ± 28.1	µm²/s	$0...10^{50}$
ρ_1 =	C	0.4925 ± 0.0425	0...0.99999	D_2 =	C	16.9701 ± 2.27	µm²/s	$0...10^{50}$
$\tau_{D,1}$ =	FX	38.5 ± 0	µs 1...10^5	count rate =		3.92711×10^{256} ± 2.55×10^{257}	Hz	$0...10^{50}$
ρ_2 =	F	0.5075 ± 0.0425	0...0.99999	background =		0 ± 0	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	687.3274 ± 66.5	µs 1...10^8	cnt/molec =	C	2.29989×10^{257} ± 1.49×10^{258}	Hz	$0...10^{50}$

legend: ***F**: fit parameter, **X**: fixed parameter, **C**: calculated parameter*

Fit Statistics:

X^2 = 1.59387	X^2 (weighted) = 25.3303
⟨E⟩ = 0.00822654	⟨E⟩ (weighted) = 0.0824572
$\sqrt{\langle E^2\rangle}$ = 0.100101	$\sqrt{\langle E^2\rangle}$ (weighted) = 0.391815
NP = 4	NR = 158
DF = 153	TSS = 843.903
R^2 = 0.998111	R^2 (weighted) = 0.969984
$R^2_{adjusted}$ = 0.998075	$R^2_{adjusted}$ (weighted) = 0.9694
AICc = -717.974	AICc (weighted) = -280.972
BIC = -705.985	BIC (weighted) = -268.983
	$max_P(\sigma_P/\|P\|)$ = 19.7854%
det(COV) = 1.03072e-08	p_{Bayes}(model|data) = 1.04948e-15 (= $10^{-14.979}$)

Fitting of FCS data for FBS labelled proteins in RPMI solution



Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		1		0...2	G_∞ =	FX	0 ± 0		-10...10
components =		2		1...3	γ =	FX	7.83 ± 0.61		0.01...100
τ_{trip} =	FX	1.35 ± 0	µs	0...10	$w_{x,y}$ =		229.62 ± 6.22	nm	$0...10^4$
θ_{trip} =	F	0.2075 ± 0.0221		0...0.99999	V_{eff} =	C	0.5279 ± 0.0594	fl	$0...10^{50}$
N =	F	13.6717 ± 0.0555		$10^{-10}...10^5$	C_{all} =	C	43.0086 ± 4.84	nM	$0...10^{50}$
1/N =	C	0.07314 ± 2.97×10^{-4}		$10^{-10}...10^5$	D_1 =	C	420.86 ± 22.8	µm²/s	$0...10^{50}$
ρ_1 =	C	0.573 ± 0.0162		0...0.99999	D_2 =	C	90.2933 ± 6.02	µm²/s	$0...10^{50}$
$\tau_{D,1}$ =	FX	31.32 ± 0	µs	$1...10^5$	count rate =		460.392 ± 620	Hz	$0...10^{50}$
ρ_2 =	F	0.427 ± 0.0162		0...0.99999	background =		0 ± 0	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	145.9835 ± 5.68	µs	$1...10^8$	cnt/molec =	C	33.6749 ± 45.3	Hz	$0...10^{50}$

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Statistics:

X^2 = 2.59463e-05	X^2 (weighted) = 141.109
⟨E⟩ = -7.40499e-05	⟨E⟩ (weighted) = -0.302123
$\sqrt{\langle E^2 \rangle}$ = 0.00039583	$\sqrt{\langle E^2 \rangle}$ (weighted) = 0.889187
NP = 4	NR = 160
DF = 155	TSS = 0.105447
R^2 = 0.999754	R^2 (weighted) = -1337.2
$R^2_{adjusted}$ = 0.999749	$R^2_{adjusted}$ (weighted) = -1362.94
AICc = -2493.29	AICc (weighted) = -11.8443
BIC = -2481.24	BIC (weighted) = 0.198333
	$max_P(\sigma_P/\|P\|)$ = 10.6506%
det(COV) = 7.06359e-11	p_{Bayes}(model|data) = 2.62982e-41 (= $10^{-40.5801}$)

Fitting of FCS data for Au-PEG$_{5000}$* NPs in living cells

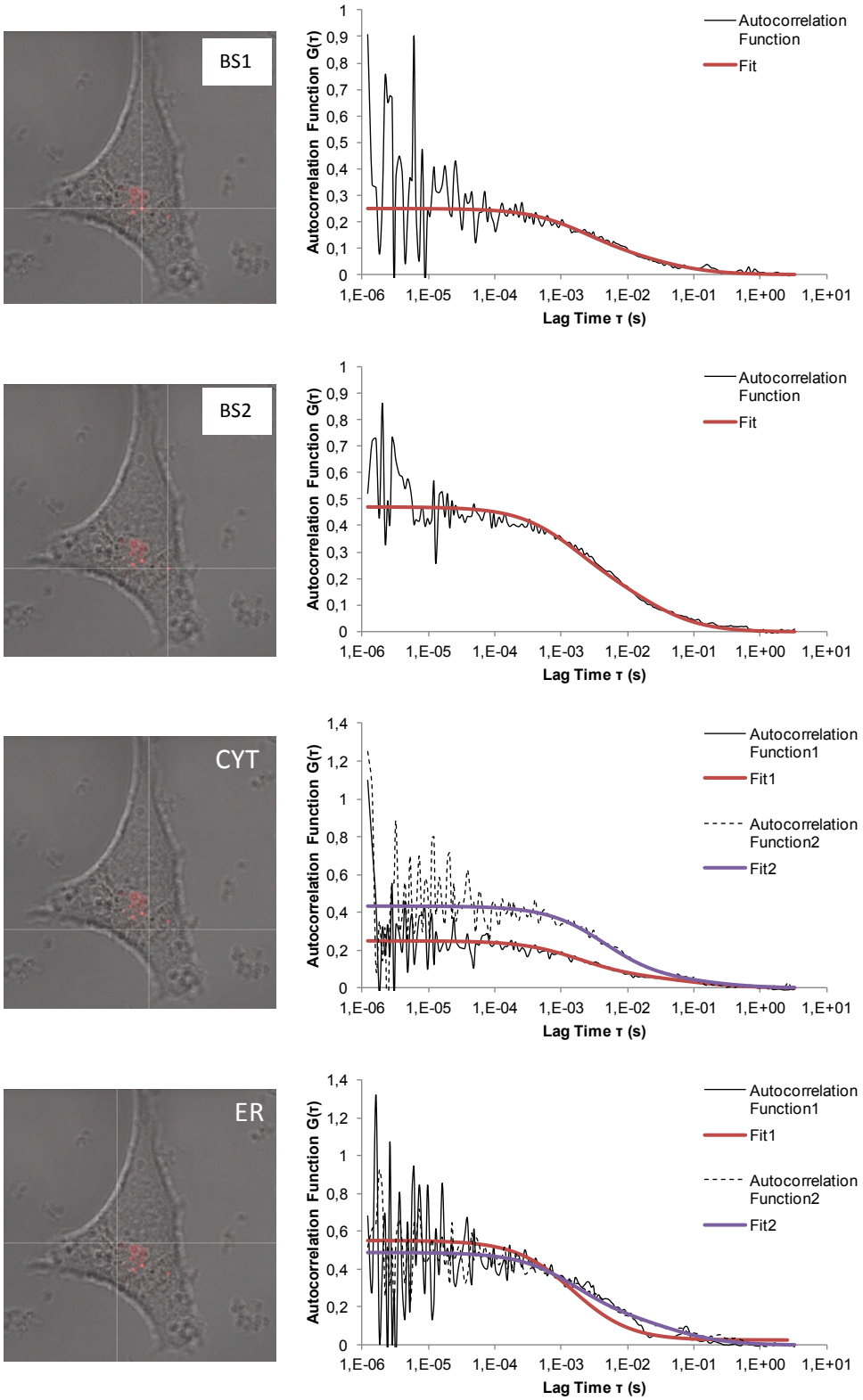


Figure S24 Confocal micrographs of A549 cell treated by Au-PEG$_{5000}$* NPs for 30 min and the correlograms related to the marked spot. For each spot 20 tracks were recorded, screened for the diffusion time and grouped according to the class of diffusion times they belonged to. The average of those tracks were fit and the fit data are reported below. Fit were done by QuickFit 3.0 by a 3D Normal Diffusion model with two diffusing components. In the statistical distributions in Fig. 8 and 9 were included only the components with fraction ρ>0.5.

BS1 fit data

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 0.6		0.01...100
components =		2		1...3	$w_{x,y}$ =		273.275 ± 8.5	nm	0...10^4
N =	F	4.0213 ± 0.171		10^{-10}...10^5	V_{eff} =	C	0.7955 ± 0.101	fl	0...10^{50}
1/N =	C	0.2487 ± 0.0106		10^{-10}...10^5	C_{all} =	C	8.3945 ± 1.12	nM	0...10^{50}
ρ_1 =	C	0.3013 ± 0.052		0...0.99999	D_1 =	C	0.4922 ± 0.0886	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	3.79328×10^4 ± 6410	µs	1...10^5	D_2 =	C	8.4528 ± 2.63	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.6987 ± 0.052		0...0.99999	count rate =		1 ± 0	Hz	0...10^{50}
$\tau_{D,2}$ =	F	2208.716 ± 675	µs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	0.2487 ± 0.0106	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 3597.49 [old_SES = 3648.2]

Fit Statistics:

χ^2 = 2.28852 χ^2 (weighted) = 3597.6

BS2 fit data

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 0.6		0.01...100
components =		2		1...3	$w_{x,y}$ =		273.275 ± 8.5	nm	0...10^4
N =	F	2.1237 ± 0		10^{-10}...10^5	V_{eff} =	C	0.7955 ± 0.101	fl	0...10^{50}
1/N =	C	0.47088 ± 0		10^{-10}...10^5	C_{all} =	C	4.4332 ± 0.562	nM	0...10^{50}
ρ_1 =	C	0.6076 ± 0		0...0.99999	D_1 =	C	12.4294 ± 0.773	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	1502.0719 ± 0	µs	1...10^5	D_2 =	C	0.779 ± 0.0485	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.3924 ± 0		0...0.99999	count rate =		1.36002×10^{272} ± nan×$10^{-2147483648}$	Hz	0...10^{50}
$\tau_{D,2}$ =	F	2.39669×10^4 ± 0	µs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	6.404×10^{271} ± nan×$10^{-2147483648}$	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

SimAneal returned after 1000 iterations and 4001 χ^2-function evaluations.
reason: stopped by abortion criterion f_{max}. SES = 24.2416 [old_SES = 24.2416]

Fit Statistics:

χ^2 = 0.648429 χ^2 (weighted) = 24.2416

Cytoplasm autocorrelation function 1 fit data

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0...2	γ =	FX	7 ± 0.6		0.01...100
components =		2	1...3	$w_{x,y}$ =		273.275 ± 8.5	nm	$0...10^{4}$
N =	F	4.05128 ± 0	$10^{-10}...10^{5}$	V_{eff} =	C	0.7955 ± 0.101	fl	$0...10^{50}$
1/N =	C	0.24684 ± 0	$10^{-10}...10^{5}$	C_{all} =	C	8.457 ± 1.07	nM	$0...10^{50}$
ρ_1 =	C	0.23171 ± 0	0...0.99999	D_1 =	C	0.1867 ± 0.0116	$\mu m^2/s$	$0...10^{50}$
$\tau_{D,1}$ =	F	9.99949×10^{4} ± 0 µs	$1...10^{5}$	D_2 =	C	9.8926 ± 0.615	$\mu m^2/s$	$0...10^{50}$
ρ_2 =	F	0.76829 ± 0	0...0.99999	count rate =		$0 \pm 7.21\times10^{307}$	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	1887.24873 ± 0 µs	$1...10^{8}$	background =		0 ± 0	Hz	$0...10^{50}$
G_∞ =	FX	0 ± 0	-10...10	cnt/molec =	C	$0 \pm 1.78\times10^{307}$	Hz	$0...10^{50}$

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

SimAneal returned after 1000 iterations and 4001 χ^2-function evaluations.
reason: stopped by abortion criterion f_{max}. SES = 143.037 [old_SES = 143.037]

Fit Statistics:

χ^2 = 1.63604 χ^2 (weighted) = 143.037

Cytoplasm autocorrelation function 2 fit data

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0...2	γ =	FX	7 ± 0.6		0.01...100
components =		2	1...3	$w_{x,y}$ =		273.275 ± 8.5	nm	$0...10^{4}$
N =	F	2.31763 ± 0	$10^{-10}...10^{5}$	V_{eff} =	C	0.7955 ± 0.101	fl	$0...10^{50}$
1/N =	C	0.43148 ± 0	$10^{-10}...10^{5}$	C_{all} =	C	4.838 ± 0.613	nM	$0...10^{50}$
ρ_1 =	C	0.89446 ± 0	0...0.99999	D_1 =	C	4.1267 ± 0.257	$\mu m^2/s$	$0...10^{50}$
$\tau_{D,1}$ =	F	4524.09614 ± 0 µs	$1...10^{5}$	D_2 =	C	0.08557 ± 0.00532	$\mu m^2/s$	$0...10^{50}$
ρ_2 =	F	0.10554 ± 0	0...0.99999	count rate =		$4.97989\times10^{297} \pm 6.06\times10^{304}$	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	2.18186×10^{5} ± 0 µs	$1...10^{8}$	background =		0 ± 0	Hz	$0...10^{50}$
G_∞ =	FX	0 ± 0	-10...10	cnt/molec =	C	$2.1487\times10^{297} \pm 2.61\times10^{304}$	Hz	$0...10^{50}$

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

SimAneal returned after 5000 iterations and 20001 χ^2-function evaluations.
reason: stopped by abortion criterion f_{max}. SES = 3382.87 [old_SES = 3382.87]

Fit Statistics:

χ^2 = 2.91795 χ^2 (weighted) = 3382.87

Endoplasmatic Reticulum autocorrelation function 1 fit data

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0...2	γ =	FX	7 ± 0.6		0.01...100
components =		2	1...3	$w_{x,y}$ =		273.275 ± 8.5	nm	0...10^4
N =	F	1.825 ± 0.0719	10^{-10}...10^5	V_{eff} =	C	0.7955 ± 0.101	fl	0...10^{50}
1/N =	C	0.5479 ± 0.0216	10^{-10}...10^5	C_{all} =	C	3.8098 ± 0.506	nM	0...10^{50}
ρ_1 =	C	0.9491 ± 0.0954	0...0.99999	D_1 =	C	12.5853 ± 2.88	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	1483.4557 ± 327	µs 1...10^5	D_2 =	C	2×10^{-4} ± 1.45	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.0509 ± 0.0954	0...0.99999	count rate =		1.12986×10^{299} ± 3.59×10^{305}	Hz	0...10^{50}
$\tau_{D,2}$ =	F	9.99999×10^7 ± 7.74×10^{11}	µs 1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0	-10...10	cnt/molec =	C	6.19087×10^{298} ± 1.97×10^{305}	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 63987.3 [old_SES = 64088.1]

Fit Statistics:

χ^2 = 4.37373 χ^2 (weighted) = 78790.8

Endoplasmatic Reticulum autocorrelation function 2 fit data

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0...2	γ =	FX	7 ± 0.6		0.01...100
components =		2	1...3	$w_{x,y}$ =		273.275 ± 8.5	nm	0...10^4
N =	F	2.0538 ± 0.0623	10^{-10}...10^5	V_{eff} =	C	0.7955 ± 0.101	fl	0...10^{50}
1/N =	C	0.4869 ± 0.0148	10^{-10}...10^5	C_{all} =	C	4.2874 ± 0.559	nM	0...10^{50}
ρ_1 =	C	0.7096 ± 0.0414	0...0.99999	D_1 =	C	11.4275 ± 2.94	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	1633.7671 ± 408	µs 1...10^5	D_2 =	C	0.3491 ± 0.0759	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.2904 ± 0.0414	0...0.99999	count rate =		1 ± 2.11×10^{306}	Hz	0...10^{50}
$\tau_{D,2}$ =	F	5.34802×10^4 ± 1.11×10^4	µs 1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0	-10...10	cnt/molec =	C	0.4869 ± 1.03×10^{306}	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 85 iterations.
reason: stopped by small δ_p. SES = 34.3491 [old_SES = 34.3491]

Fit Statistics:

χ^2 = 0.957503 χ^2 (weighted) = 34.3491

Fitting of FCS data for Au-Glucosamine* NPs in living cells

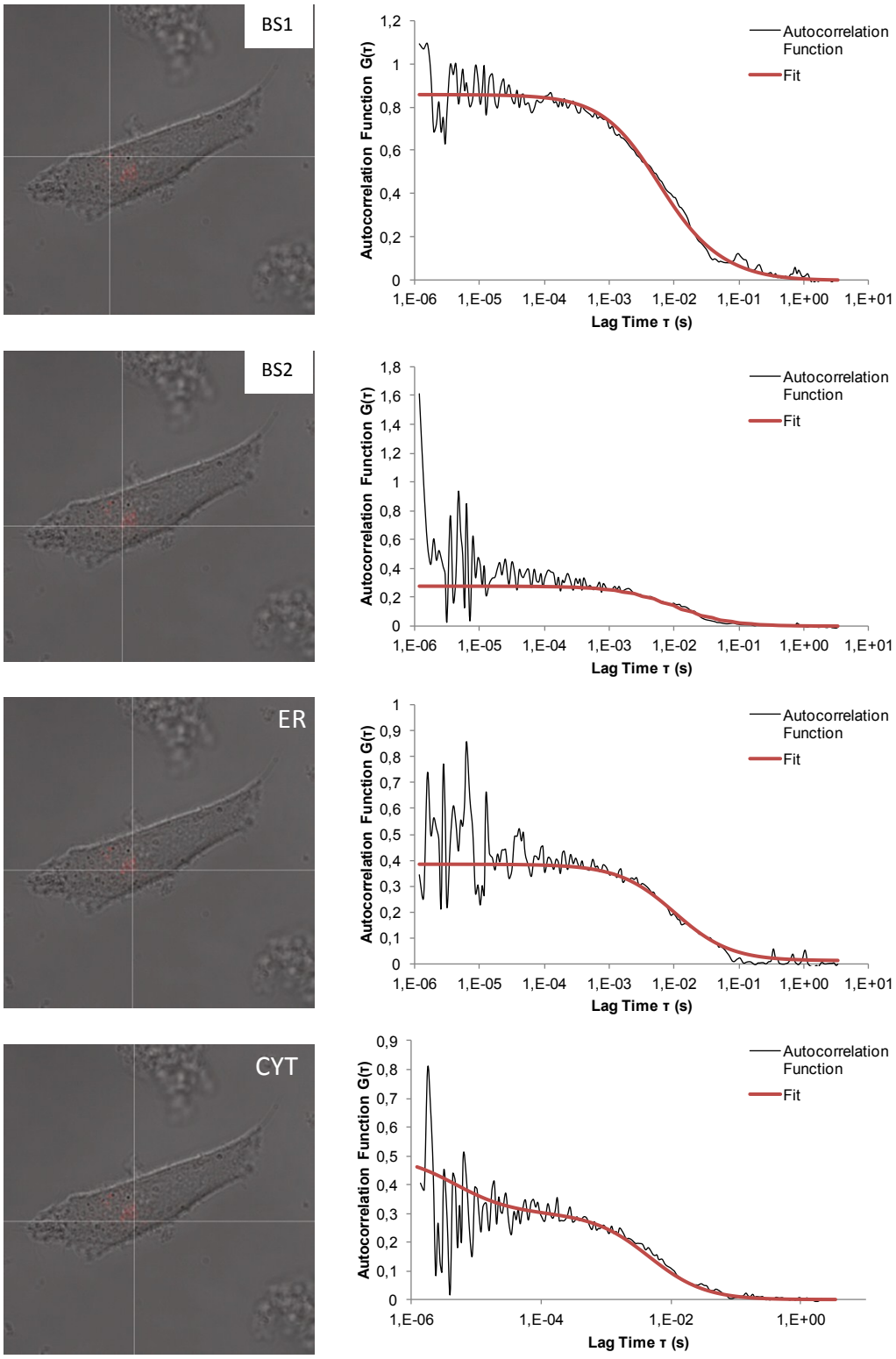


Figure S25 Confocal micrographs of A549 cell treated by Au-Glucosamine* NPs for 30 min and the correlograms related to the marked spot. For each spot 20 tracks were recorded, screened for the diffusion time and grouped according to the class of diffusion times they belonged to. The average of those tracks were fit and the fit data are reported below. Fit were done by QuickFit 3.0 by a 3D Normal Diffusion model with two diffusing components. In the statistical distributions in Fig. 8 and 9 were included only the components with fraction ρ>0.5.

BS1 fitting data

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0...2	γ =	FX	7 ± 0		0.01...100
components =		2	1...3	$w_{x,y}$ =		286.451 ± 0	nm	$0...10^{4}$
N =	F	1.1635 ± 0.0277	$10^{-10}...10^{5}$	V_{eff} =	C	0.91617 ± 0	fl	$0...10^{50}$
1/N =	C	0.8595 ± 0.0204	$10^{-10}...10^{5}$	C_{all} =	C	2.1089 ± 0.0501	nM	$0...10^{50}$
ρ_1 =	C	0.9164 ± 0.144	0...0.99999	D_1 =	C	3.6547 ± 1.29	$\mu m^2/s$	$0...10^{50}$
$\tau_{D,1}$ =	F	5612.9739 ± 1980	µs $1...10^{5}$	D_2 =	C	0.2693 ± 0.473	$\mu m^2/s$	$0...10^{50}$
ρ_2 =	F	0.0836 ± 0.144	0...0.99999	count rate =		$2.80158\times10^{270} \pm 1.09\times10^{308}$	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	$7.61829\times10^{4} \pm 1.34\times10^{5}$	µs $1...10^{8}$	background =		0 ± 0	Hz	$0...10^{50}$
G_∞ =	FX	0 ± 0	-10...10	cnt/molec =	C	$2.40783\times10^{270} \pm 9.37\times10^{307}$	Hz	$0...10^{50}$

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

converged (the relative error in the sum of squares is at most tol)

Fit Statistics:

X^2 = 0.561714 X^2 (weighted) = 62.4569

BS2 fitting data

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0...2	γ =	FX	7 ± 0		0.01...100
components =		2	1...3	$w_{x,y}$ =		286.451 ± 0	nm	$0...10^{4}$
N =	F	3.6218 ± 0.0702	$10^{-10}...10^{5}$	V_{eff} =	C	0.91617 ± 0	fl	$0...10^{50}$
1/N =	C	0.27611 ± 0.00535	$10^{-10}...10^{5}$	C_{all} =	C	6.5644 ± 0.127	nM	$0...10^{50}$
ρ_1 =	C	1 ± 0.169	0...0.99999	D_1 =	C	2.817 ± 1.49	$\mu m^2/s$	$0...10^{50}$
$\tau_{D,1}$ =	F	7282.0566 ± 3840	µs $1...10^{5}$	D_2 =	C	$3.9\times10^{-4} \pm 0.00129$	$\mu m^2/s$	$0...10^{50}$
ρ_2 =	F	0 ± 0.169	0...0.99999	count rate =		$4.42717\times10^{257} \pm 4.96\times10^{258}$	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	$5.20741\times10^{7} \pm 1.7\times10^{8}$	µs $1...10^{8}$	background =		0 ± 0	Hz	$0...10^{50}$
G_∞ =	FX	0 ± 0	-10...10	cnt/molec =	C	$1.22237\times10^{257} \pm 1.37\times10^{258}$	Hz	$0...10^{50}$

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

converged (the relative error of the parameter vector is at most tol)

Fit Statistics:

X^2 = 1.93548 X^2 (weighted) = 8437.19

Endoplasmatic Reticulum fitting data

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0...2	γ =	FX	7 ± 0		0.01...100
components =		2	1...3	$w_{x,y}$ =		286.451 ± 0	nm	$0...10^{4}$
N =	F	2.6105 ± 0.0725	$10^{-10}...10^{5}$	V_{eff} =	C	0.91617 ± 0	fl	$0...10^{50}$
1/N =	C	0.3831 ± 0.0106	$10^{-10}...10^{5}$	C_{all} =	C	4.7316 ± 0.131	nM	$0...10^{50}$
ρ_1 =	C	0.9642 ± 0.456	0...0.99999	D_1 =	C	1.981 ± 1.46	$\mu m^2/s$	$0...10^{50}$
$\tau_{D,1}$ =	F	$1.03549\times10^{4} \pm 7630$	µs $1...10^{5}$	D_2 =	C	$2\times10^{-4} \pm 3.74$	$\mu m^2/s$	$0...10^{50}$
ρ_2 =	F	0.0358 ± 0.456	0...0.99999	count rate =		$1.11113\times10^{297} \pm \text{nan}\times10^{-2147483648}$	Hz	$0...10^{50}$
$\tau_{D,2}$ =	F	$9.17511\times10^{7} \pm 1.54\times10^{12}$	µs $1...10^{8}$	background =		0 ± 0	Hz	$0...10^{50}$
G_∞ =	FX	0 ± 0	-10...10	cnt/molec =	C	$4.25632\times10^{296} \pm \text{nan}\times10^{-2147483648}$	Hz	$0...10^{50}$

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 12.5884 [old_SES = 12.5927]

Fit Statistics:

X^2 = 1.25248 X^2 (weighted) = 13914.6

Cytoplasm fitting data

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0...2	γ =	FX	7 ± 0		0.01...100
components =		2	1...3	$w_{x,y}$ =		286.451 ± 0	nm	0...10^4
N =	F	1.9839 ± 0.28	10^{-10}...10^5	V_{eff} =	C	0.91617 ± 0	fl	0...10^{50}
1/N =	C	0.5041 ± 0.0711	10^{-10}...10^5	C_{all} =	C	3.5957 ± 0.507	nM	0...10^{50}
ρ_1 =	C	0.5989 ± 0.08	0...0.99999	D_1 =	C	4.5508 ± 0.367	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	4507.6857 ± 363	µs 1...10^5	D_2 =	C	4369.4315 ± 2950	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.4011 ± 0.08	0...0.99999	count rate =		6.33259×10^{197} ± nan×$10^{-2147483648}$	Hz	0...10^{50}
$\tau_{D,2}$ =	F	4.6948 ± 3.17	µs 1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0	-10...10	cnt/molec =	C	3.19207×10^{197} ± nan×$10^{-2147483648}$	Hz	0...10^{50}

*legend: **F**: fit parameter, **X**: fixed parameter, **C**: calculated parameter*

Fit Result Message:

levmar returned after 33 iterations.
reason: stopped by small δ_p. SES = 45.4174 [old_SES = 49.6503]

Fit Statistics:

X^2 = 0.979972 X^2 (weighted) = 577.809

Fitting of FCS data for Au- Alkyl-PEG$_{600}$* NPs in living cells

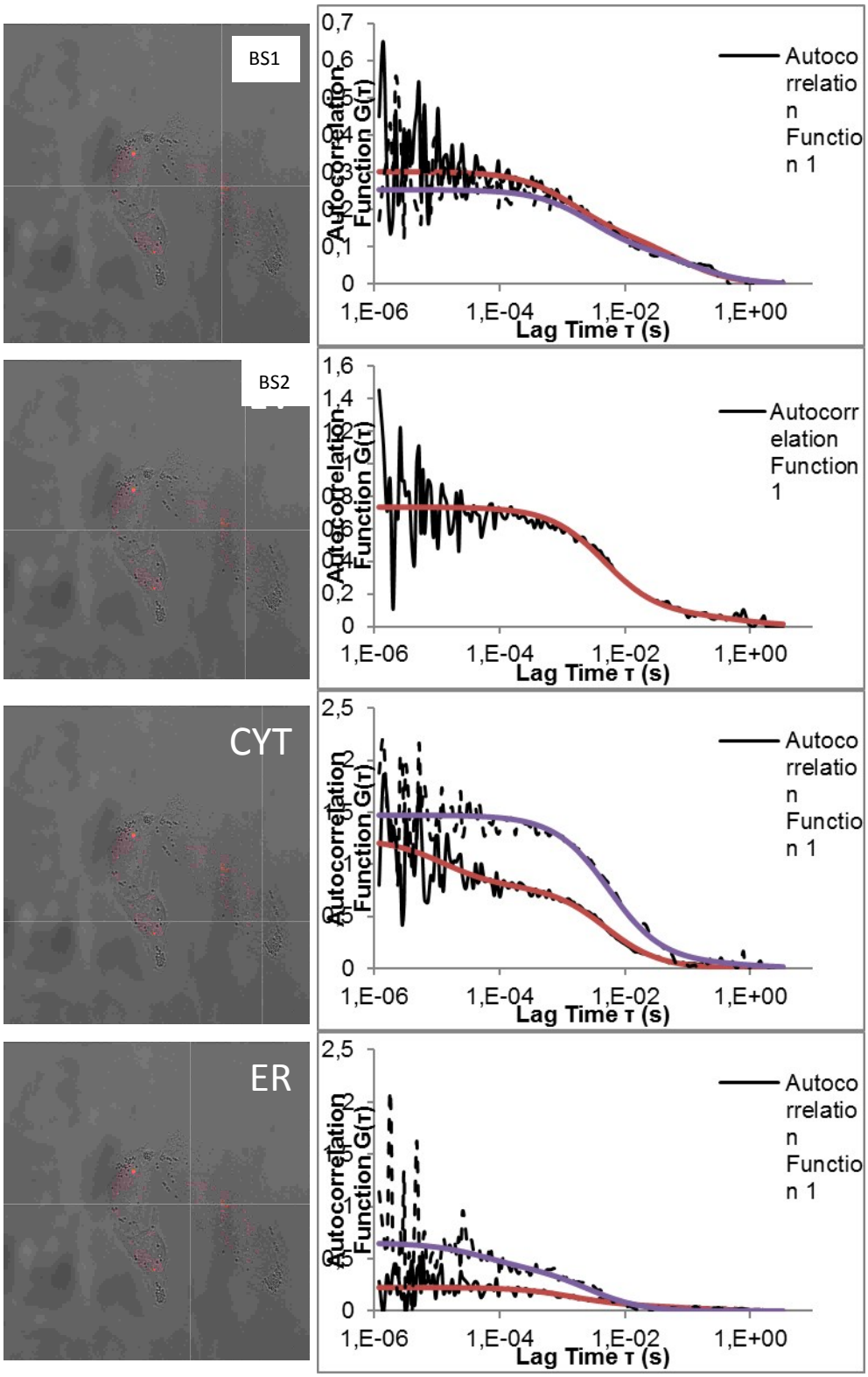


Figure S26 Confocal micrographs of A549 cell treated by Au-Alkil-PEG$_{600}$* NPs for 30 min and the correlograms related to the marked spot. For each spot 20 tracks were recorded, screened for the diffusion time and grouped according to the class of diffusion times they belonged to. The average of those tracks were fit and the fit data are reported below. Fit were done by QuickFit 3.0 by a 3D Normal Diffusion model with two diffusing components. In the statistical distributions in Fig. 8 and 9 were included only the components with fraction ρ>0.5.

BS1 fitting data 1

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0…2	γ =	FX	7 ± 0		0.01…100
components =		2	1…3	$w_{x,y}$ =		250 ± 0	nm	$0…10^{4}$
N =	F	3.3103 ± 0.04	$10^{-10}…10^{5}$	V_{eff} =	C	0.60904 ± 0	fl	$0…10^{50}$
1/N =	C	0.30209 ± 0.00365	$10^{-10}…10^{5}$	C_{all} =	C	9.0254 ± 0.109	nM	$0…10^{50}$
ρ_1 =	C	0.4317 ± 0.102	0…0.99999	D_1 =	C	0.2117 ± 0.25	$\mu m^2/s$	$0…10^{50}$
$\tau_{D,1}$ =	F	7.38022×10^{4} ± 8.71×10^{4} µs	1…10^{5}	D_2 =	C	10.4692 ± 3.24	$\mu m^2/s$	$0…10^{50}$
ρ_2 =	F	0.5683 ± 0.102	0…0.99999	count rate =		1.36755×10^{297} ± $nan\times10^{-2147483648}$	Hz	$0…10^{50}$
$\tau_{D,2}$ =	F	1492.4704 ± 462 µs	1…10^{8}	background =		0 ± 0	Hz	$0…10^{50}$
G_∞ =	FX	0 ± 0	-10…10	cnt/molec =	C	4.13125×10^{296} ± $nan\times10^{-2147483648}$	Hz	$0…10^{50}$

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 314.89 [old_SES = 315.647]

Fit Statistics:

χ^2 = 0.478372 χ^2 (weighted) = 452.912

BS1 fitting data 2

Model Parameters (fit results):

Parameter		Value	Range	Parameter		Value		Range
# non-fluorescent =		0	0…2	γ =	FX	7 ± 0		0.01…100
components =		2	1…3	$w_{x,y}$ =		250 ± 0	nm	$0…10^{4}$
N =	F	3.9471 ± 0.0343	$10^{-10}…10^{5}$	V_{eff} =	C	0.60904 ± 0	fl	$0…10^{50}$
1/N =	C	0.25335 ± 0.0022	$10^{-10}…10^{5}$	C_{all} =	C	10.7617 ± 0.0934	nM	$0…10^{50}$
ρ_1 =	C	0.6442 ± 0.0483	0…0.99999	D_1 =	C	5.5897 ± 0.715	$\mu m^2/s$	$0…10^{50}$
$\tau_{D,1}$ =	F	2795.3029 ± 357 µs	1…10^{5}	D_2 =	C	0.1107 ± 0.0304	$\mu m^2/s$	$0…10^{50}$
ρ_2 =	F	0.3558 ± 0.0483	0…0.99999	count rate =		0 ± 0	Hz	$0…10^{50}$
$\tau_{D,2}$ =	F	1.41208×10^{5} ± 3.88×10^{4} µs	1…10^{8}	background =		0 ± 0	Hz	$0…10^{50}$
G_∞ =	FX	0 ± 0	-10…10	cnt/molec =	C	0 ± 0	Hz	$0…10^{50}$

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 6443.98 [old_SES = 6443.98]

Fit Statistics:

χ^2 = 0.452642 χ^2 (weighted) = 6443.98

BS2 fitting data

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 0		0.01...100
components =		2		1...3	$w_{x,y}$ =		250 ± 0	nm	0...10^4
N =	F	1.3629 ± 0.0333		10^{-10}...10^5	V_{eff} =	C	0.60904 ± 0	fl	0...10^{50}
1/N =	C	0.7337 ± 0.018		10^{-10}...10^5	C_{all} =	C	3.7159 ± 0.0909	nM	0...10^{50}
ρ_1 =	C	0.9019 ± 0.0114		0...0.99999	D_1 =	C	3.3224 ± 0.555	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	4702.8853 ± 786	μs	1...10^5	D_2 =	C	0.02314 ± 0.00754	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.0981 ± 0.0114		0...0.99999	count rate =		0 ± 0	Hz	0...10^{50}
$\tau_{D,2}$ =	F	6.75214×10^5 ± 2.2×10^5	μs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	0 ± 0	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 45.6812 [old_SES = 45.6817]

Fit Statistics:

X^2 = 2.34138 X^2 (weighted) = 45.6813

Cytoplasm fitting data 1

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 0		0.01...100
components =		2		1...3	$w_{x,y}$ =		312.789 ± 0	nm	0...10^4
N =	F	0.8051 ± 0.0551		10^{-10}...10^5	V_{eff} =	C	1.19283 ± 0	fl	0...10^{50}
1/N =	C	1.2421 ± 0.085		10^{-10}...10^5	C_{all} =	C	1.1208 ± 0.0767	nM	0...10^{50}
ρ_1 =	C	0.3651 ± 0.0417		0...0.99999	D_1 =	C	1939.2879 ± 1170	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	12.6125 ± 7.6	μs	1...10^5	D_2 =	C	4.9235 ± 0.717	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.6349 ± 0.0417		0...0.99999	count rate =		0 ± 0	Hz	0...10^{50}
$\tau_{D,2}$ =	F	4967.8357 ± 724	μs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	0 ± 0	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 9.29021 [old_SES = 9.29023]

Fit Statistics:

X^2 = 3.45902 X^2 (weighted) = 14.6331

Cytoplasm fitting data 2

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0…2	γ =	FX	7 ± 0		0.01…100
components =		2		1…3	$w_{x,y}$ =		312.789 ± 0	nm	0…10^4
N =	F	0.6775 ± 0.0126		10^{-10}…10^5	V_{eff} =	C	1.19283 ± 0	fl	0…10^{50}
1/N =	C	1.476 ± 0.0275		10^{-10}…10^5	C_{all} =	C	0.9432 ± 0.0175	nM	0…10^{50}
ρ_1 =	C	0.9617 ± 0.013		0…0.99999	D_1 =	C	4.3798 ± 0.506	$\mu m^2/s$	0…10^{50}
$\tau_{D,1}$ =	F	5584.5975 ± 645	µs	1…10^5	D_2 =	C	0.0298 ± 0.0237	$\mu m^2/s$	0…10^{50}
ρ_2 =	F	0.0383 ± 0.013		0…0.99999	count rate =		0 ± 0	Hz	0…10^{50}
$\tau_{D,2}$ =	F	8.19941×10^5 ± 6.5×10^5	µs	1…10^8	background =		0 ± 0	Hz	0…10^{50}
G_∞ =	FX	0 ± 0		-10…10	cnt/molec =	C	0 ± 0	Hz	0…10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 33.6517 [old_SES = 33.6518]

Fit Statistics:

χ^2 = 3.78304 χ^2 (weighted) = 33.6517

Endoplasmatic Reticulum Fitting data 1

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0…2	γ =	FX	7 ± 0		0.01…100
components =		2		1…3	$w_{x,y}$ =		312.789 ± 0	nm	0…10^4
N =	F	4.3871 ± 0.0912		10^{-10}…10^5	V_{eff} =	C	1.19283 ± 0	fl	0…10^{50}
1/N =	C	0.22794 ± 0.00474		10^{-10}…10^5	C_{all} =	C	6.1073 ± 0.127	nM	0…10^{50}
ρ_1 =	C	0.7696 ± 0.0221		0…0.99999	D_1 =	C	16.3911 ± 2.92	$\mu m^2/s$	0…10^{50}
$\tau_{D,1}$ =	F	1492.23 ± 265	µs	1…10^5	D_2 =	C	0.1555 ± 0.0291	$\mu m^2/s$	0…10^{50}
ρ_2 =	F	0.2304 ± 0.0221		0…0.99999	count rate =		0 ± 0	Hz	0…10^{50}
$\tau_{D,2}$ =	F	1.57252×10^5 ± 2.95×10^4	µs	1…10^8	background =		0 ± 0	Hz	0…10^{50}
G_∞ =	FX	0 ± 0		-10…10	cnt/molec =	C	0 ± 0	Hz	0…10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 485.164 [old_SES = 487.769]

Fit Statistics:

χ^2 = 0.73685 χ^2 (weighted) = 485.164

Endoplasmatic Reticulum Fitting data 2

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 0		0.01...100
components =		2		1...3	$w_{x,y}$ =		312.789 ± 0	nm	0...10^4
N =	F	1.5398 ± 0.131		10^{-10}...10^5	V_{eff} =	C	1.19283 ± 0	fl	0...10^{50}
1/N =	C	0.6495 ± 0.0554		10^{-10}...10^5	C_{all} =	C	2.1435 ± 0.183	nM	0...10^{50}
ρ_1 =	C	0.3667 ± 0.103		0...0.99999	D_1 =	C	458.9068 ± 539	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	53.2989 ± 62.6	µs	1...10^5	D_2 =	C	8.9151 ± 3.34	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.6333 ± 0.103		0...0.99999	count rate =		4.67056×10^{198} ± 9.05×10^{198}	Hz	0...10^{50}
$\tau_{D,2}$ =	F	2743.5803 ± 1030	µs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	3.03332×10^{198} ± 5.88×10^{198}	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 34.6472 [old_SES = 34.6472]

Fit Statistics:

X^2 = 5.91977 X^2 (weighted) = 41.8104

Fitting of FCS data for Au-MSA * NPs in living cells

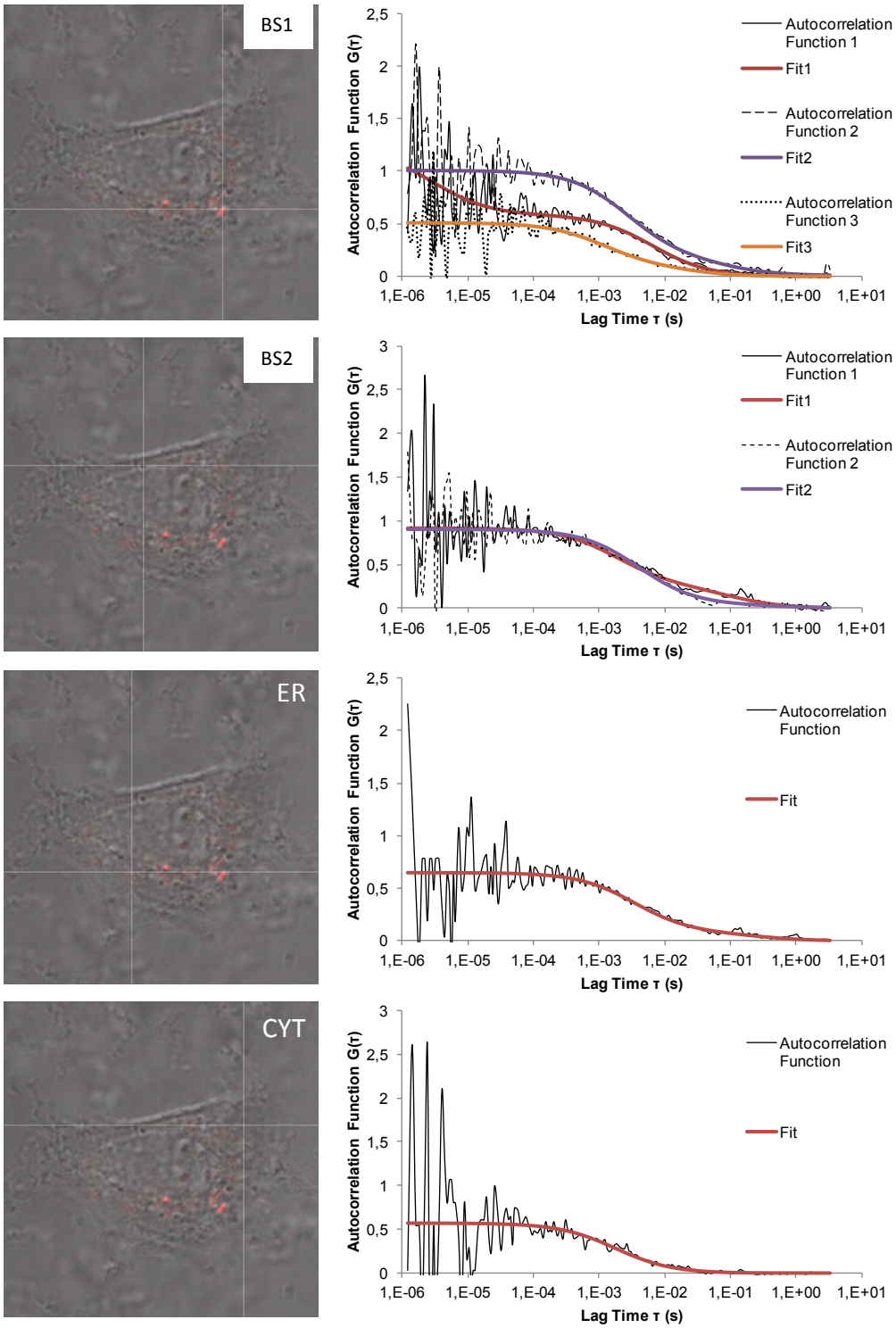


Figure S27 Confocal micrographs of A549 cell treated by Au-MSA* NPs for 30 min and the correlograms related to the marked spot. For each spot 20 tracks were recorded, screened for the diffusion time and grouped according to the class of diffusion times they belonged to. The average of those tracks were fit and the fit data are reported below. Fit were done by QuickFit 3.0 by a 3D Normal Diffusion model with two diffusing components. In the statistical distributions in Fig. 8 and 9 were included only the components with fraction ρ>0.5.

BS1 Fitting data 1

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 1.6		0.01...100
components =		2		1...3	$w_{x,y}$ =		295.47 ± 12	nm	0...10^4
N =	F	0.8265 ± 0.227		10^{-10}...10^5	V_{eff} =	C	1.0055 ± 0.26	fl	0...10^{50}
1/N =	C	1.2099 ± 0.332		10^{-10}...10^5	C_{all} =	C	1.365 ± 0.515	nM	0...10^{50}
ρ_1 =	C	0.5157 ± 0.126		0...0.99999	D_1 =	C	7451.2008 ± 7630	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	2.9291 ± 2.99	µs	1...10^5	D_2 =	C	3.3043 ± 0.445	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.4843 ± 0.126		0...0.99999	count rate =		0 ± 0	Hz	0...10^{50}
$\tau_{D,2}$ =	F	6605.269 ± 709	µs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	0 ± 0	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 19.9032 [old_SES = 19.9033]

Fit Statistics:

X^2 = 5.14181 X^2 (weighted) = 19.9032

BS1 Fitting data 2

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 1.6		0.01...100
components =		2		1...3	$w_{x,y}$ =		295.47 ± 12	nm	0...10^4
N =	F	0.9904 ± 0.0442		10^{-10}...10^5	V_{eff} =	C	1.0055 ± 0.26	fl	0...10^{50}
1/N =	C	1.0097 ± 0.045		10^{-10}...10^5	C_{all} =	C	1.6356 ± 0.43	nM	0...10^{50}
ρ_1 =	C	0.8367 ± 0.072		0...0.99999	D_1 =	C	7.8723 ± 2.87	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	2772.4479 ± 984	µs	1...10^5	D_2 =	C	0.2561 ± 0.159	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.1633 ± 0.072		0...0.99999	count rate =		0 ± 0	Hz	0...10^{50}
$\tau_{D,2}$ =	F	8.52106×10^4 ± 5.24×10^4	µs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	0 ± 0	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 68.5189 [old_SES = 68.5195]

Fit Statistics:

X^2 = 5.42513 X^2 (weighted) = 68.5189

BS1 Fitting data 3

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 1.6		0.01...100
components =		2		1...3	$w_{x,y}$ =		295.47 ± 12	nm	0...10^4
N =	F	1.9701 ± 0.0792		10^{-10}...10^5	V_{eff} =	C	1.0055 ± 0.26	fl	0...10^{50}
1/N =	C	0.5076 ± 0.0204		10^{-10}...10^5	C_{all} =	C	3.2537 ± 0.853	nM	0...10^{50}
ρ_1 =	C	0.824 ± 0.124		0...0.99999	D_1 =	C	18.2316 ± 7.85	µm²/s	0...10^{50}
$\tau_{D,1}$ =	F	1197.1351 ± 506	µs	1...10^5	D_2 =	C	0.9307 ± 0.778	µm²/s	0...10^{50}
ρ_2 =	F	0.176 ± 0.124		0...0.99999	count rate =		0 ± 0	Hz	0...10^{50}
$\tau_{D,2}$ =	F	2.34503×10^4 ± 1.95×10^4	µs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	0 ± 0	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 10.4931 [old_SES = 10.4931]

Fit Statistics:

χ^2 = 2.71288 χ^2 (weighted) = 10.4931

BS2 Fitting data 1

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 1.6		0.01...100
components =		2		1...3	$w_{x,y}$ =		295.47 ± 12	nm	0...10^4
N =	F	1.1111 ± 0.033		10^{-10}...10^5	V_{eff} =	C	1.0055 ± 0.26	fl	0...10^{50}
1/N =	C	0.9 ± 0.0267		10^{-10}...10^5	C_{all} =	C	1.835 ± 0.478	nM	0...10^{50}
ρ_1 =	C	0.9515 ± 0.0154		0...0.99999	D_1 =	C	5.3423 ± 0.855	µm²/s	0...10^{50}
$\tau_{D,1}$ =	F	4085.4731 ± 563	µs	1...10^5	D_2 =	C	0.0366 ± 0.0232	µm²/s	0...10^{50}
ρ_2 =	F	0.0485 ± 0.0154		0...0.99999	count rate =		0 ± 0	Hz	0...10^{50}
$\tau_{D,2}$ =	F	5.97098×10^5 ± 3.76×10^5	µs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	0 ± 0	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 42.8195 [old_SES = 42.8195]

Fit Statistics:

χ^2 = 5.25844 χ^2 (weighted) = 42.8195

BS2 Fitting data 2

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 1.6		0.01...100
components =		2		1...3	$w_{x,y}$ =		295.47 ± 12	nm	0...10^4
N =	F	1.0866 ± 0.0456		10^{-10}...10^5	V_{eff} =	C	1.0055 ± 0.26	fl	0...10^{50}
1/N =	C	0.9203 ± 0.0386		10^{-10}...10^5	C_{all} =	C	1.7946 ± 0.471	nM	0...10^{50}
ρ_1 =	C	0.7094 ± 0.0493		0...0.99999	D_1 =	C	11.7852 ± 4.31	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	1851.9542 ± 661	µs	1...10^5	D_2 =	C	0.2172 ± 0.0582	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.2906 ± 0.0493		0...0.99999	count rate =		0 ± 0	Hz	0...10^{50}
$\tau_{D,2}$ =	F	1.00468×10^5 ± 2.56×10^4	µs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	0 ± 0	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 67.8881 [old_SES = 68.8827]

Fit Statistics:

X^2 = 10.3734 X^2 (weighted) = 67.8881

Endoplasmatic Reticulum Fitting data 1

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	γ =	FX	7 ± 1.6		0.01...100
components =		2		1...3	$w_{x,y}$ =		295.47 ± 12	nm	0...10^4
N =	F	1.5353 ± 0.0684		10^{-10}...10^5	V_{eff} =	C	1.0055 ± 0.26	fl	0...10^{50}
1/N =	C	0.6513 ± 0.029		10^{-10}...10^5	C_{all} =	C	2.5357 ± 0.666	nM	0...10^{50}
ρ_1 =	C	0.878 ± 0.0271		0...0.99999	D_1 =	C	6.1894 ± 1.68	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	3526.3156 ± 916	µs	1...10^5	D_2 =	C	0.0765 ± 0.0272	$\mu m^2/s$	0...10^{50}
ρ_2 =	F	0.122 ± 0.0271		0...0.99999	count rate =		0 ± 0	Hz	0...10^{50}
$\tau_{D,2}$ =	F	2.85177×10^5 ± 9.85×10^4	µs	1...10^8	background =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	cnt/molec =	C	0 ± 0	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 40.2952 [old_SES = 40.2953]

Fit Statistics:

X^2 = 7.61478 X^2 (weighted) = 40.2952

Cytoplasm Fitting data 1

Model Parameters (fit results):

Parameter		Value		Range	Parameter		Value		Range
# non-fluorescent =		0		0...2	$w_{x,y}$ =		295.47 ± 12	nm	0...10^4
components =		1		1...3	V_{eff} =	C	1.0055 ± 0.26	fl	0...10^{50}
N =	F	1.7726 ± 0.118		10^{-10}...10^5	C_{all} =	C	2.9275 ± 0.783	nM	0...10^{50}
1/N =	C	0.5641 ± 0.0376		10^{-10}...10^5	D_1 =	C	12.2138 ± 2.98	$\mu m^2/s$	0...10^{50}
$\tau_{D,1}$ =	F	1786.9662 ± 411	µs	1...10^5	count rate =		0 ± 0	Hz	0...10^{50}
G_∞ =	FX	0 ± 0		-10...10	background =		0 ± 0	Hz	0...10^{50}
γ =	FX	7 ± 1.6		0.01...100	cnt/molec =	C	0 ± 0	Hz	0...10^{50}

legend: ***F****: fit parameter,* ***X****: fixed parameter,* ***C****: calculated parameter*

Fit Result Message:

levmar returned after 3000 iterations.
reason: stopped by maximum iterations. SES = 30.2462 [old_SES = 30.2462]

Fit Statistics:

X^2 = 20.1506 X^2 (weighted) = 30.2462

S13. Bibliography

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