Supporting Information

Cytosolic Diffusion and Peptide-assisted Nuclear Shuttling of Peptide-substituted circa 102 Gold Atom Nanoclusters in Living Cells

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Complement to Materials and Methods

Chemicals
5,5'-dithio-bis(2-nitrobenzoic acid)(DTNB) (Sigma-Aldrich D8130)
Hydrogen tetrachloroaurate(III) trihydrate (Sigma-Aldrich 520918-1G)
Sodium borohydride ($NaBH_4$) (Aldrich 45,288-2)
Sodium hydroxide (Fluka 71691)
4-(2-Hydroxyethyl)-piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) (Euromedex 10-110)
Trishydroxymethylaminomethane base (Tris) (Euromedex, 26-128-3094B)
Glycine (Gly)(Euromedex 26-128-6405C)
Peptides were purchased from GeneCust (Dudelange, Luxembourg)
Glutathione reduced (GSH) (Sigma G6013-5g)
Methanol (Sigma-Aldrich 179957-5L)
Water Bi-distilled
Tris(2-carboxyethyl)phosphine hydrochloride (Sigma-Aldrich BioUltra 75259)
Ammonium hydroxide 28% (EMS cat#10600 EMS group, Hatfield, PA, USA)
Glutaraldehyde 25% solution EM grade (EMS cat#16220, EMS group, Hatfield, PA, USA)
HSPEG (Code PEG1169-0005, MW 2015Da, Iris Biotech, Germany)

Materials. The pH-meter was a Hanna HI2210 instrument combined with a HI 1131B electrode. Centrifugation of 50 mL solutions was performed using an Eppendorf 5810R centrifuge and the rotor A-4-81. Centrifugation of small volumes (0.5 to 2 mL) was performed using a Sigma 1-15K Eppendorf centrifuge with a 12132-H rotor. For gold particle formation, agitation was performed on a rocking platform (Heidolph Rotamax 120). The Nuclear Magnetic Resonance (NMR) data were taken with a Bruker DPX 400 MHz spectrometer. The UV-vis absorbance spectra were recorded using a Varian Cary 100Bio spectrometer. Nanoparticles were purified and concentrated using 0.5 mL Microcon® centrifugal filters (Merck, Molsheim) with cut-off of 10 or 30 kDa according to the manufacturer's specification. The spectrofluorometer was a Xenius Safas (Monaco, France).

Devices
Small volumes of aqueous solutions were dispensed using pipettes (Gilson Pipetman® classic P10, P100, P1000 models). Large volumes were dispensed using serological pipettes (Falcon® 5 10 or 25 mL) and a single channel Omega Pipettor (Argos Technologies Inc, Elgin IL). Reactions were performed unless indicated in polypropylene tubes of different volumes (50 mL, 15 mL, 2.0 mL, 1.5 mL or 0.5 mL).

Preparation of aqueous stock solutions
- Peptides were made at 10 mM concentration in water and stored in aliquots at -20°C.
- Tris(2-carboxyethyl)phosphine (TCEP), 0.1 M solution, pH 7.4 was prepared by incremental addition of 2 M NaOH to a 0.2 M TCEP hydrochloride (286.65 mg in 5 mL H_2O) solution under stirring and by monitoring pH increase with a pH-meter. When the pH reached 7.4, the TCEP solution was set at 0.1 M and stored in aliquots at -20°C.
- 4-(2-Hydroxyethyl)-piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) (23.83 g, 0.1 mol) was dissolved in water (50 mL). The pH was adjusted to pH 7.4 with incremental addition of 1M NaOH and using a pH-meter. The solution was then adjusted to 100 mL and was filtered for sterilization using a 33 mm syringe-driven filter unit (0.22 μm Millex-GP, Millipore, Molsheim France). The 1M HEPES-Na solution pH 7.4 was stored at 4-8°C.

- Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4$ -$3H_2O$) (1g, 2.539 mmol) was dissolved in water (6.35 mL) to a final concentration of 0.4 M in aurate. This stock solution was stored at 4-6°C in a brown vial and can be stored for more than 6 months.
- The 2 M ammonium acetate solution was buffered to pH 4.7 with 2 M acetic acid solution using a pH-meter.
- 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) (198.2 mg, 50 µmol) was dissolved in 0.3 M aqueous NaOH solution (10 mL) to a final 50 mM DTNB concentration. The orange solution was used within 48h and stored at 4-6°C in the dark when not freshly used.
- Glutathione (GST) solutions were freshly prepared in water and buffered to pH 7.0 using 0.3 M NaOH. For the ESI-MS analysis, the glutathione was buffered to pH 7.8 using NH_4OH.
- The 133 mM Sorenson's buffer, pH 7.4 was prepared by combining 133 mM Na_2HPO_4 (804 mL) with 133 mM KH_2PO_4 (196 mL). After verification of the pH with the pH-meter, the solution was filtered for sterilization using a 33 mm syringe-driven filter unit (0.22 µm Millex-GP, Millipore, Molsheim France).
- The TEM storage solution was a 0.1 M Sodium cacodylate buffer, pH 7.2 containing 2% (w/v) sucrose, 1 mM $MgCl_2$ and 1 mM $CaCl_2$. The solution was filtered for sterilization using a 33 mm syringe-driven filter unit (0.22 µm Millex-GP, Millipore, Molsheim France) and stored at 4-6°C.

Estimation of the absorption coefficient of the gold nanocluster. The dried AuZ pellet (4.2 mg) was dissolved in water (350 µL) and the solution pH was adjusted to 7.0 by addition of 50 mM aqueous NH_4OH to a final concentration of 11.28 g x L^{-1}. Aliquots (1 µL volume) were withdrawn and diluted with water (final volume of 700 µL) giving a concentration of 16.114 mg/L. An optical density (OD) of 0.153 at 520 nm was measured using a 1 cm-path quartz cuvette. Using the Beer-Lambert equation ($OD = \varepsilon\ l\ c$) and an estimated molecular mass of 28382 Da ($Au_{102}TAB_{30}TNB_{14}$), extinction coefficients (ε_{520}) of 9.51 $g^{-1}L$ x cm^{-1} and 2.7 x 10^5 mol^{-1} x L x cm^{-1} were determined.

Preparation of gold nanoparticles. The citrate-stabilized gold nanoparticles were prepared according to a described procedure.[1] After preparation, the particles were purified and concentrated using the 0.5 mL Microcon® centrifugal filters (cut-off: 100 kDa). Particle concentration was estimated at 116 nM by UV/vis spectrometry using the nanoparticle absorption at 509 nm. Next, the gold particles (0.2 nmol) were reacted with the Cap peptide (32 nmol), HSPEG (32 nmol), Cap/HSNLS (9/1) mixture (32 nmol total) and Cap/HSNLS (9/1) mixture (32 nmol total) in 150 mM K_2CO_3 for 24h at room temperature. The particles were then purified and concentrated using the 0.5 mL Microcon® centrifugal filters (cut-off: 100 kDa). Concentrations of the peptide-grafteraqueous solutions were spectrophotometrically estimated and set at 4 µM.

PolyAcrylamide Gel Electrophoresis. 10% Polyacrylamide Gels (1 mm thick) were casted using the BioRad MiniProtean system in 25 mM Tris-186 mM glycine buffer pH 8.3 containing 0.1 % (w/v) Sodium Dodecyl Sulfate (SDS). For electrophoresis, the running buffer was 25 mM Tris-186 mM glycine buffer pH 8.3 containing 0.1 % SDS unless indicated otherwise. The polyacrylamide gel was pre-run for 20 min at 90V. For loading in the wells, 50% (v/v) glycerol in water was diluted to 10% into samples (5-10 µL volume; 40 nmol in AuNC). The electrophoresis was run at room temperature for 45 minutes at 90V. The gold nanoclusters were observed as black-brown bands. Peptides were visualized after Coomassie blue staining.

Thermal Gravimetric Analyses. The analysis was performed on a Q 5000IR apparatus (TA Instruments) under a flow of nitrogen on vacuum-dried particles (0.2990 mg) and at a heating rate of 10°C/min.

Electron Microscopy. Imaging of cells and peptide-modified AuNCs was performed using a FEI G2 F20 Transmission Electron Microscope operating at 200 kV. Image acquisition was performed on a 2048 x 2048 CCD camera (Ultrascan 1000, Gatan Inc., Pleasanton) by collecting 25 adjacent images using the SerialEM software (Boulder, Colorado, USA). The final image was reconstructed by stitching the adjacent images using the Montage plugin package (Jean Daraspe, Université de Lausanne, Switzerland) and the iMod software.[2]
For standard-EM imaging of isolated nanoparticles, 10 µL of a 5 µM nanocluster solution was applied onto the ultrathin carbon support film of 100 Mesh EM grids (UMC Utrecht, The Netherlands). After 1 min. adsorption, the excess liquid was removed with a filter paper.
For cryo-EM imaging, samples (3 µL) of 10 µM AuNC solution in 10 mM HEPES, pH 7.4 were applied to lacey carbon grids, blotted with filter paper from both sides for half a second in the temperature- and humidity-controlled Vitrobot Mark IV apparatus (FEI, Eindhoven, Netherlands, T = 20°C, humidity 95%, Blot Force 5, Blot time 0.5 sec) and plunge-frozen in liquid ethane pre-cooled by liquid nitrogen to embed the complex in a thin film of vitrified buffer spanning the holes of the carbon film. Images were collected at liquid nitrogen temperature with a FEI Titan Krios EM operating at 300 kV with a dose of 20 electrons per $Å^2$ using a FEI Falcon II Camera with pixel size of 0.8936 Å, integration time 1s, bining 1 and defocus of -0.5 µm.

MALDI-TOF mass spectrometry. Mass measurements were carried out on an Autoflex™ MALDI-TOF mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany). This instrument was used at a maximum accelerating potential of 20 kV in positive mode and was operated in linear mode at 19 kV. The delay extraction was fixed at 80 ns and the frequency of the laser (nitrogen 337 nm) was set at 5 Hz. The acquisition mass range was set to 5000-30000 m/z with a matrix suppression deflection (cut-off) set to 500 m/z. The equipment was externally calibrated with a standard protein calibration mixture that contained 5 proteins (Bruker Protein Standard II #8207234, Bruker Daltonics GmbH, Bremen, Germany) covering the 10,000-70,000 m/z range. Each raw spectrum was opened with flexAnalysis 2.4 build 11 (Bruker Daltonics GmbH, Bremen, Germany) software. Sample preparation was performed with the dried droplet method using a mixture of 0.5 µl of sample with 0.5 µl of matrix solution dried at room temperature. The matrix solution was prepared from a saturated solution of α-cyano-4-hydroxycinnamic acid in water/acetonitrile 50/50 diluted three fold in water/acetonitrile/trifluoroacetic acid 50/49.9/0.1.

Cell culture. The HeLa (ATCC CCL2) and U2OS (ATCC HTB-96) human cancer cell lines and the non-cancer human fibroblast HFF-1 cell line (ATCC SCRC-1041) were maintained in Dulbecco's modified Eagles tissue culture medium (DMEM) supplemented with 10% heat inactivated FCS (Life Technologies), 2 mM L-glutamine and antibiotics. Cells were grown in humidified atmosphere at 37°C and with 5% CO_2. Cells were maintained in culture for less than 12 passages.

Table S1. Sequence of peptides used for preparing Peptide-grafted AuZ gold clusters				
Abbreviation	Activity	Sequence (N->C)	Molecular mass ($g.mol^{-1}$)	Reference
GSH	Passivation	γ–E**C**G (Glutathione)	307.32	[3,4]
Cap	Protection	**C**ALNNG	590.65	[5]
NLS	Nuclear import	**C**ALNNGAGPKKKRKVED	1828.13	[6]
dNLS	Defective nuclear import	**C**ALNNGAGPK**T**KRKVED	1801.06	[6]
NES	Nuclear export	**C**ALNNGLALKLAGLDINKT$_{amide}$	1941.33	[7]

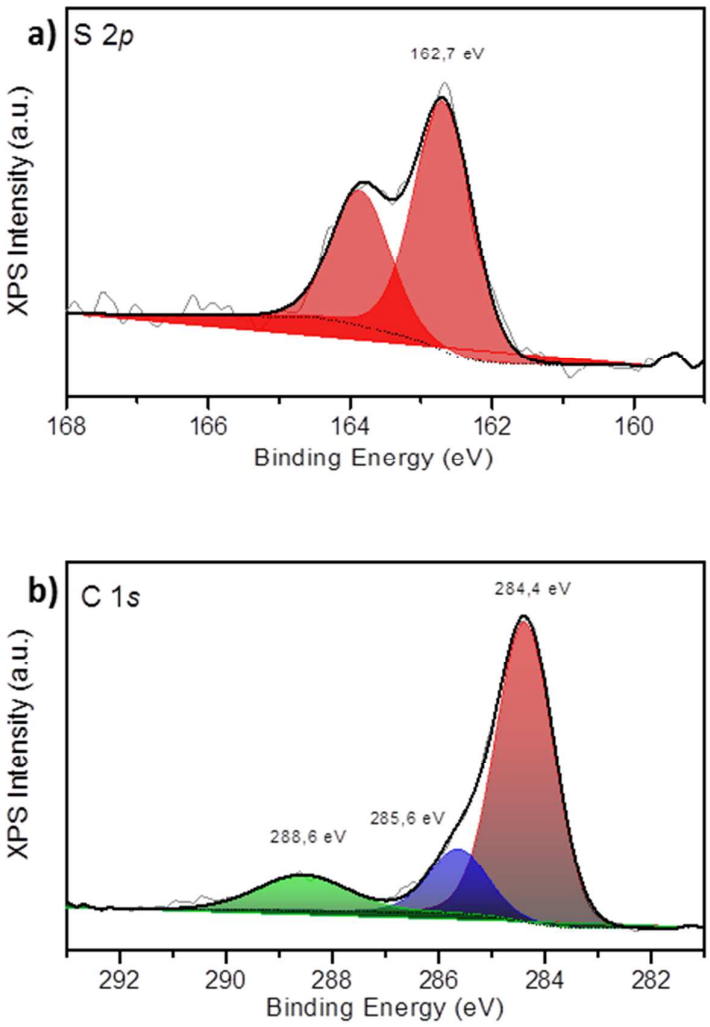

Figure S1. High resolution XPS spectra of AuZ particles **a)** S 2p and **b)** C 1s. The S2p3/2 and S2p1/2 peaks are consistent with most sulfur atoms being engaged in a coordination bond with the gold surface atoms[8] The C 1s components at 284.4, 285.6 and 288.6 eV are assigned to various carbon species C-C, C-O-C, C-N and C=O, O-C=O respectively,[9] in good agreement with the expected carbon species in the organic coating.

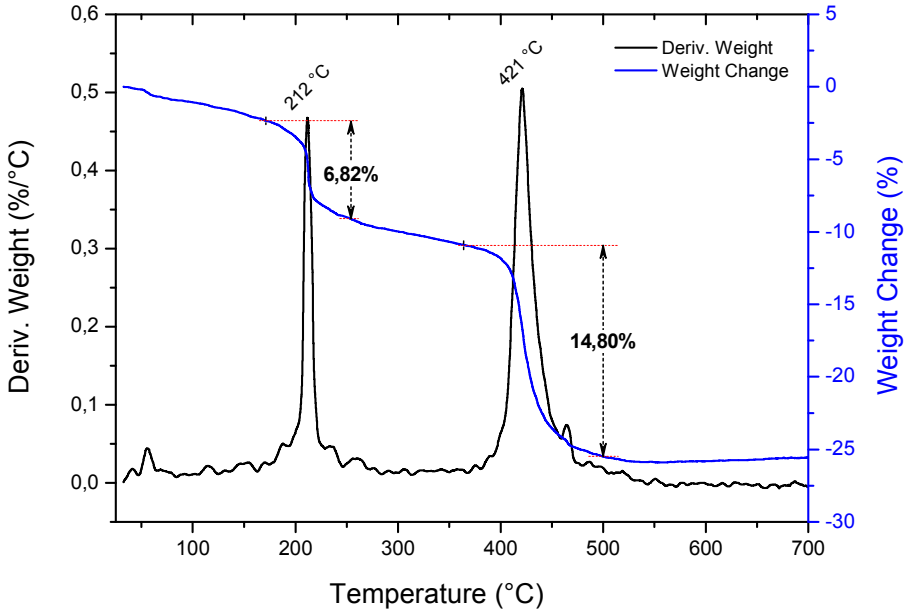

Figure S2. Thermogravimetric curve and derivative weight change curve of the AuZ.

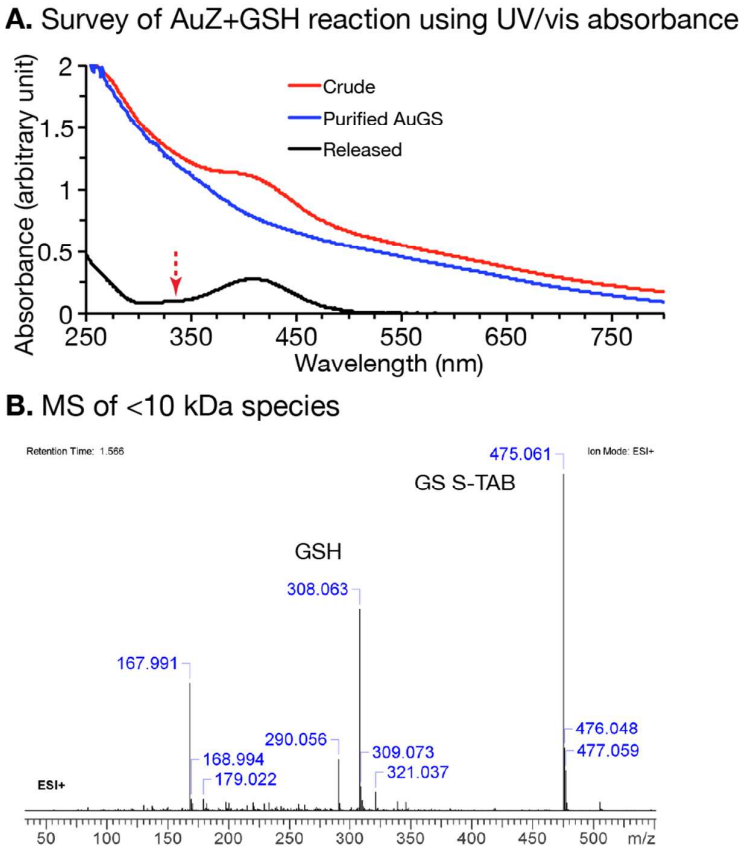

Figure S3. Analysis of reaction between AuZ and glutathione (GSH). **A.** UV/vis spectra of the crude reaction (red line), purified GSH-gold nanocluster (AuGS) (blue line) and released low molecular weight components (black line). The red dotted arrow pinpoints a novel absorption band. **B.** ElectroSpray Mass Spectrum of the released species after a 20h reaction of AuZ (45.6 nmol, 200 µL of a 228 µM solution) with Glutathione, pH 7.8 (570 nmol, 5.7 µL of a 0.1 M solution). The glutathione solution was freshly prepared and buffered by addition of NH_4OH. The gold clusters were separated from unreacted glutathione and released species using a 0.5 mL microcon-10 kDa centrifugal filter unit. The flow through contained a thionitrobenzoic acid (TNB) concentration of 848 µM using the absorption coefficient of 14,150 $M^{-1}.cm^{-1}$ at 412 nm. The mass spectrum was taken 7 days after the reaction.

Comment. The UV/vis spectra of aliquots from the crude reaction, purified AuGS and released low molecular mass species were taken into the NMR spectroscopy survey (Figure 3B). The crude reaction (red line) exhibited now an UV/vis absorption spectrum corresponding to the sum of UV/vis absorption spectra of purified AuGS (blue line) and to released aqueous-soluble ligands (black line). The spectrum of the released species was analogous to the spectrum of TNB (Figure 1B) with the exception of a light shoulder (red dot arrow), indicating release of another ligand from the AuNCs. This absorption band is likely due to TAB. Using an absorption coefficient of 14150 $M^{-1}.cm^{-1}$ at 412 nm for TNB, we estimated the concentration of released TNB at 335 µM. This value corresponds to about 50% of the overall concentration of released ligands estimated by NMR and corresponds to a release of a TNB/TAB molecular ratio of 1.

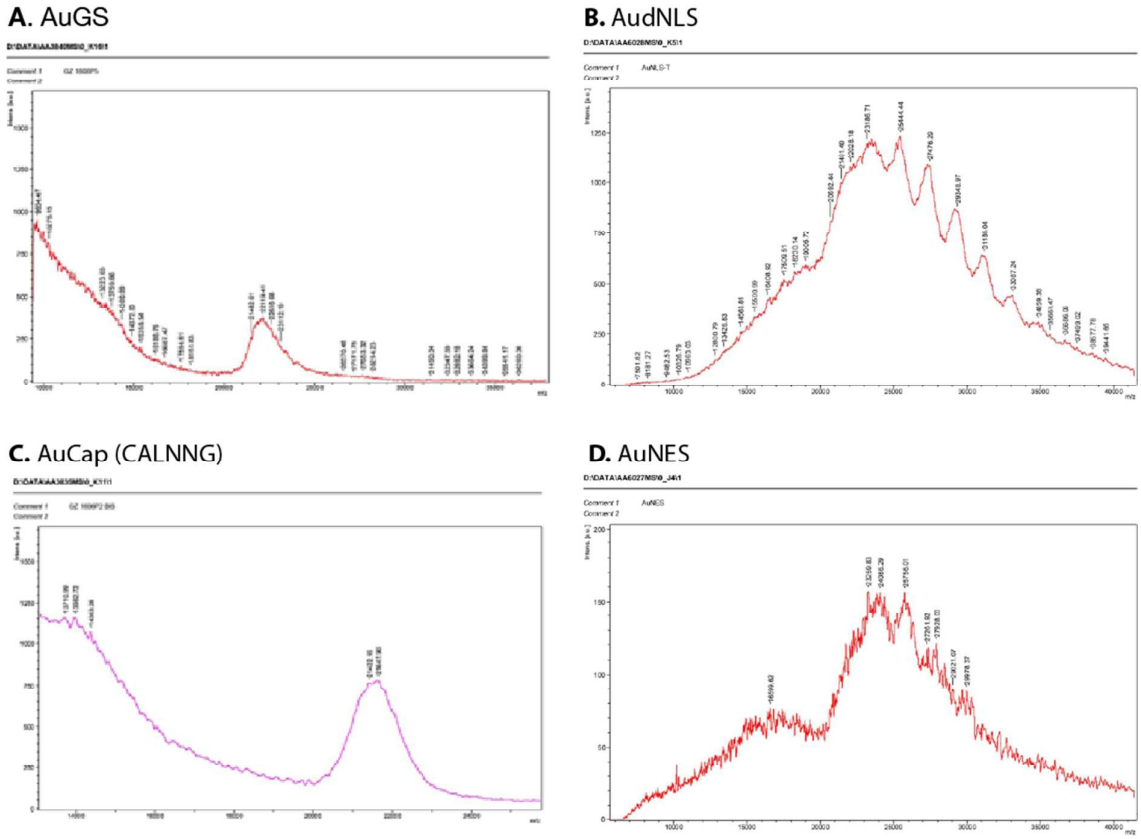

Figure S4. MALDI-TOF Mass spectra of AuGS (**A**), AudNLS (**B**), AuCap (**C**) and AuNES (**D**).

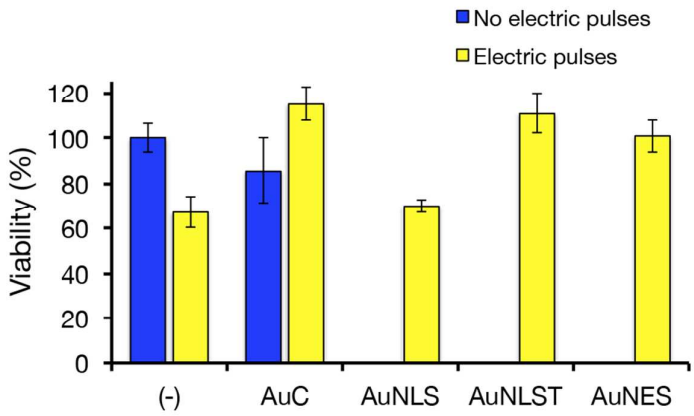

Figure S5. MTT Cell viability assay using the HFF-1 normal cell line. Freshly trypsinized HFF-1 cells (50,000 cells) in presence of the indicated nanoclusters were added in the Neon transfection device in conditions that were used for electroporation, with the electric pulses (yellow bars) or without electric pulses (blue bars). Cells were then diluted in the cell culture medium and let to growth for 24h. Values indicated the averages and standard deviations of hexaplicates.

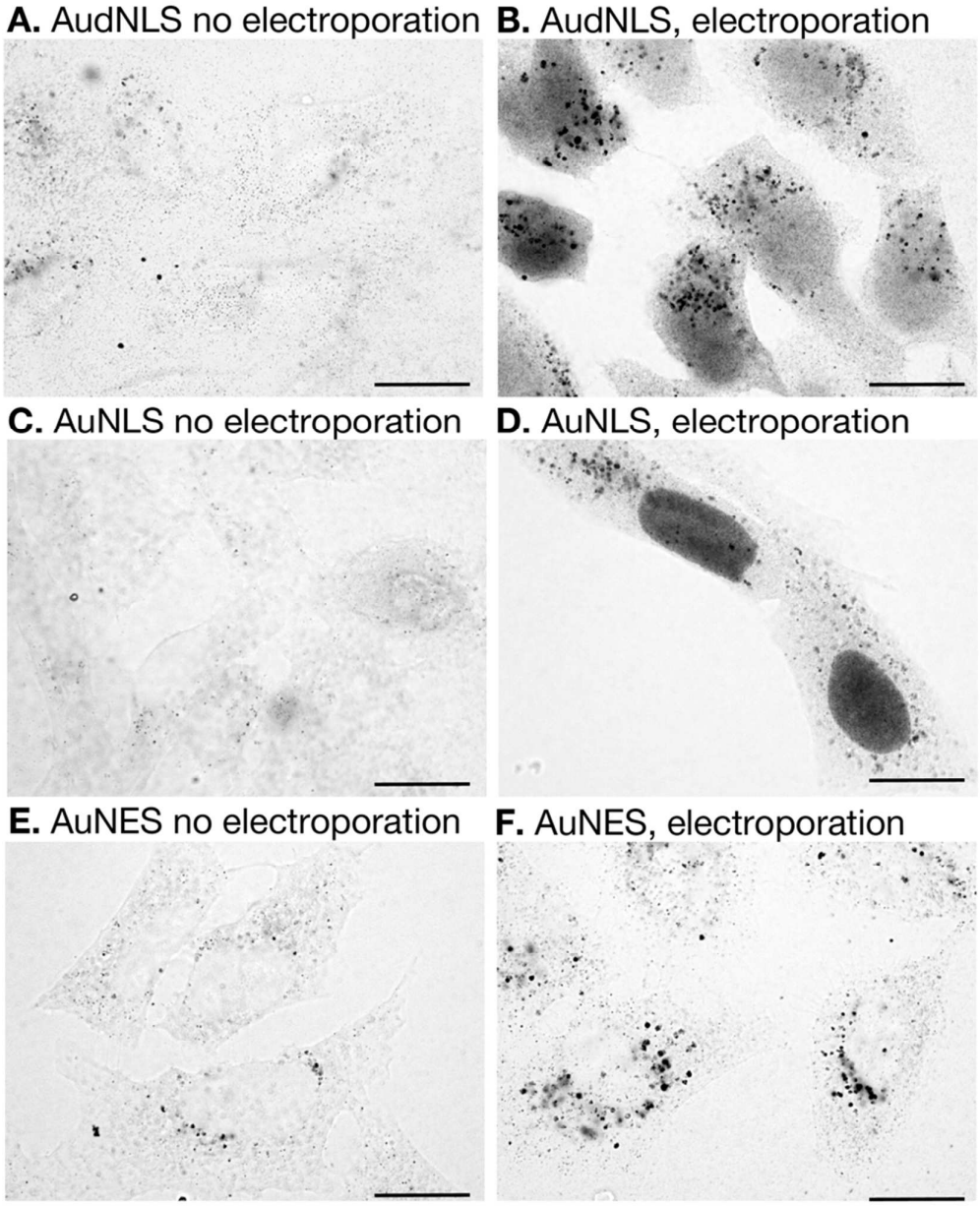

Figure S6. Optical microscopy images of U2OS cells incubated for 24h with the indicated peptide-substituted gold nanoclusters (AuNCs). **A, C and E.** The various peptide-AuNCs were added to living U2OS cells by dilution in the cell culture medium without an electroporation treatment. **B, D and F.** The U2OS cells were subjected to electroporation in presence of the indicated AuNCs. Following the 24h incubation period at 37°C, the living cells were fixed with glutaraldehyde (2.5% w/v) and AuNCs were detected by silver staining. Cell viability, estimated by density of the monolayer, was >80%. Scale bar: 20 µm.

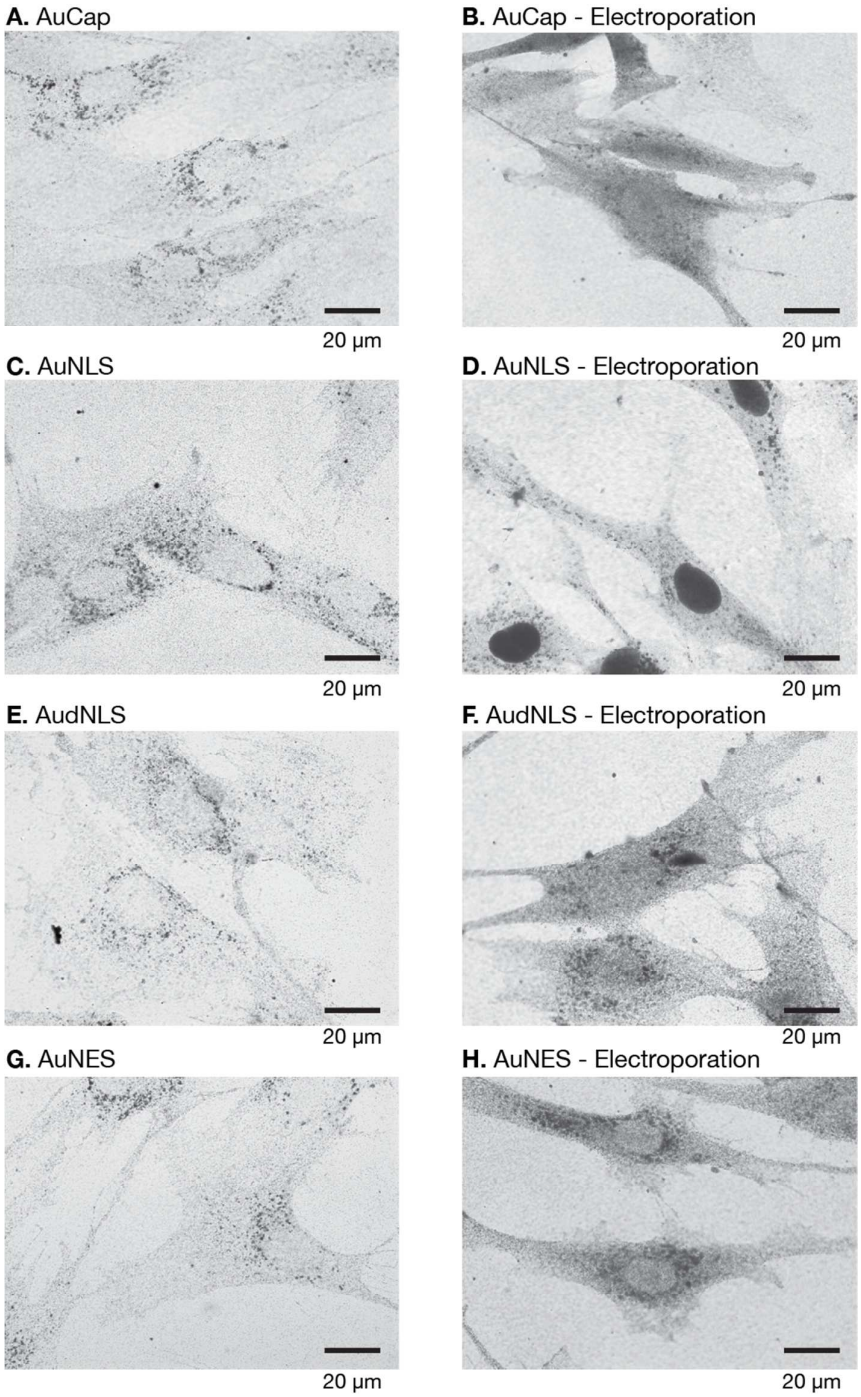

Figure S7. Optical microscopy images of HFF-1 cells incubated for 24h with the indicated peptide-substituted gold nanoclusters (AuNCs). **A, C, E and G**. The various peptide-AuNCs were added to living HFF-1 cells by dilution in the cell culture medium without an electroporation treatment. **B, D, F and H**. The HFF-1 cells were subjected to electroporation in presence of the indicated AuNCs. Following the 24h incubation period at 37°C, the living cells were fixed with glutaraldehyde (2.5% w/v) and AuNCs were detected by silver staining. Cell viability, estimated by density of the monolayer, was >80%.

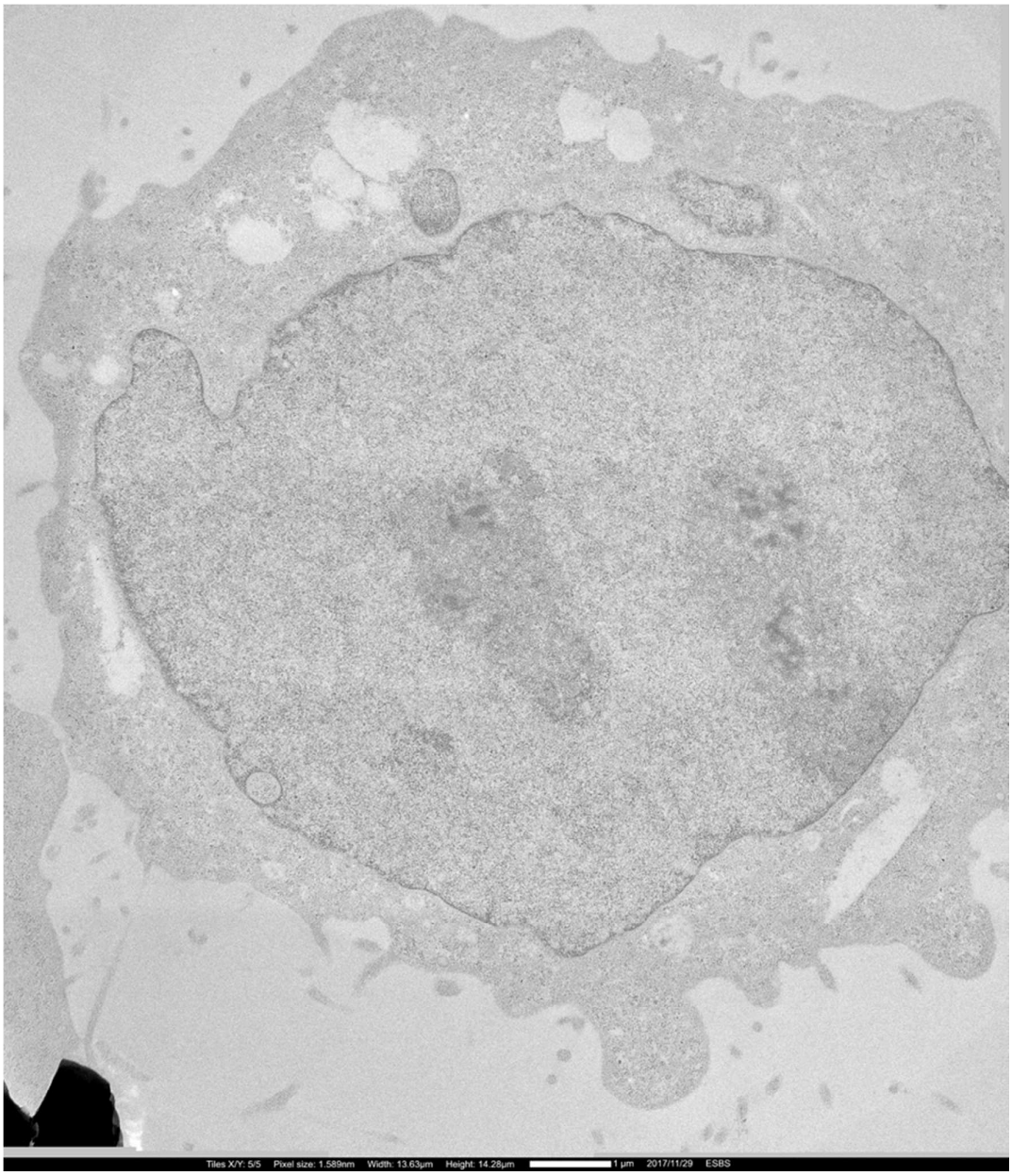

Figure S8. Transmission electron microscopy of untreated HeLa cells. After fixation with 2.5% glutaraldehyde, the specimen was treated with the Aurion Silver enhancement solution exactly as the AuNCs-containing specimens. After the silver staining, the HeLa cells were processed for resin-embedding and imaged.

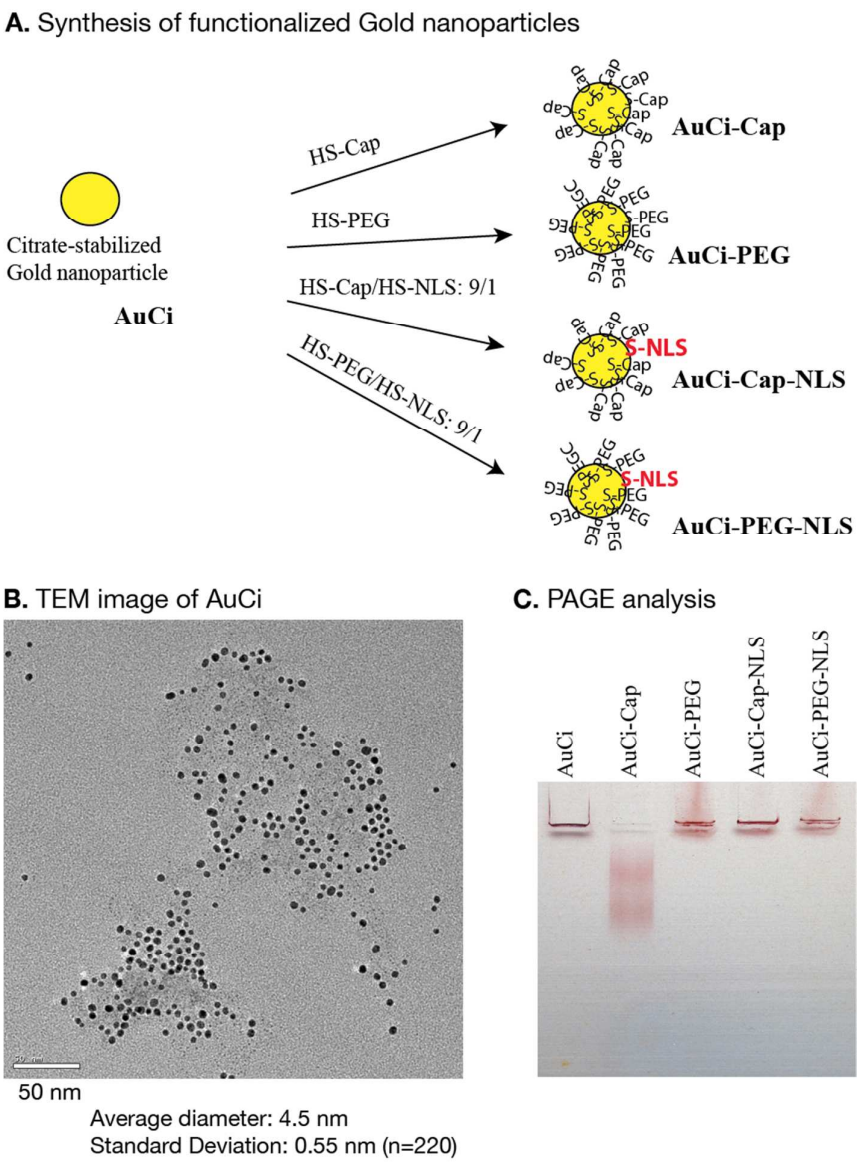

Figure S9. A. Synthesis of peptide-grafted gold nanoparticle from citrate-gold nanoparticle. **B.** TEM image of the starting gold nanoparticle showing a population of particle with average diameter of 4.5 nm. **C.** SDS-denaturing 15% Polyacrylamide gel electrophoresis of the peptide-grafted gold nanoparticles. The gold nanoparticles were red-colored and did not require any staining.

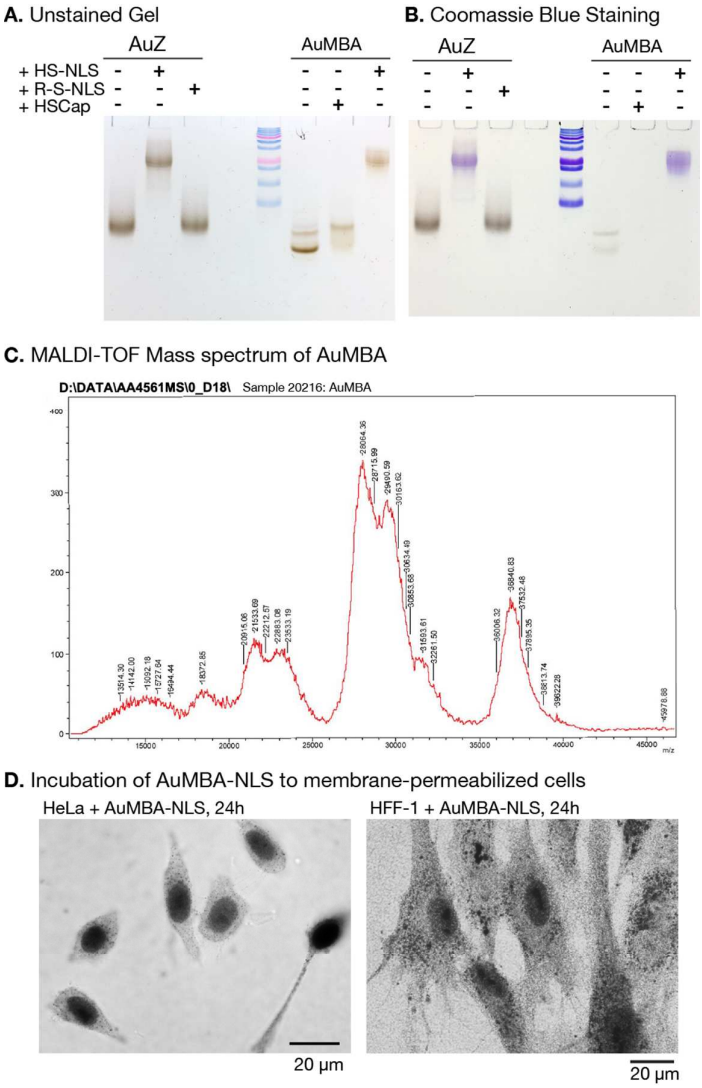

Figure S10. Reactivity and delivery of NLS-grafted gold nanoclusters from AuMBA. **A** and **B.** Comparative reactivity of the AuZ to the HS-NLS peptide or to an S-alkyl-NLS peptide (R-S-NLS) and analysis of AuMBA and subsequent reaction with HS-Cap or HS-NLS. Images are PAGE images of the same gel before and after Coomassie blue staining. The data indicated that our AuMBA batch contains several nanoclusters. We were unable to obtain a batch containing only one band. The S-Alkyl-NLS was obtained by reacting the HS-NLS to N-ethylmaleimide (1.2 molar equivalent) 30 min before addition to AuZ. Condition 1: 80 µM AuZ, 2 mM HS-NLS (or R-SNLS), 0.1 M HEPES, pH 7.4 (10 µL volume). Condition 2. 80 µM AuMBA, 4 mM HSCap or 4 mM HSNLS (100 µL volume, 16h reaction). The subsequent particles were purified using a 30 kDa UltraConcentrator device. Aliquots were analyzed and the AuMBANLS was used for the electroporation treatment (see images D). **C.** MALDI-TOF Mass spectrum of the AuMBA. The results confirmed that our preparation contains several clusters. **D.** The HeLa or the HFF-1 cells were subjected to electroporation in presence of the AuMBS-NLS. Following the 24h incubation period at 37°C, the living cells were fixed with glutaraldehyde (2.5% w/v) and AuNCs were detected by silver darkening. They accumulated as well in the cell nucleus as expected from an NLS-driven nuclear import. The AuMBA-NLS appears to be less effectively imported in the HFF-1 nuclei than the AuNLS, suggesting a benefit of the zwitterionic TAB shell. Cell viability, estimated by monolayer density was >80%.

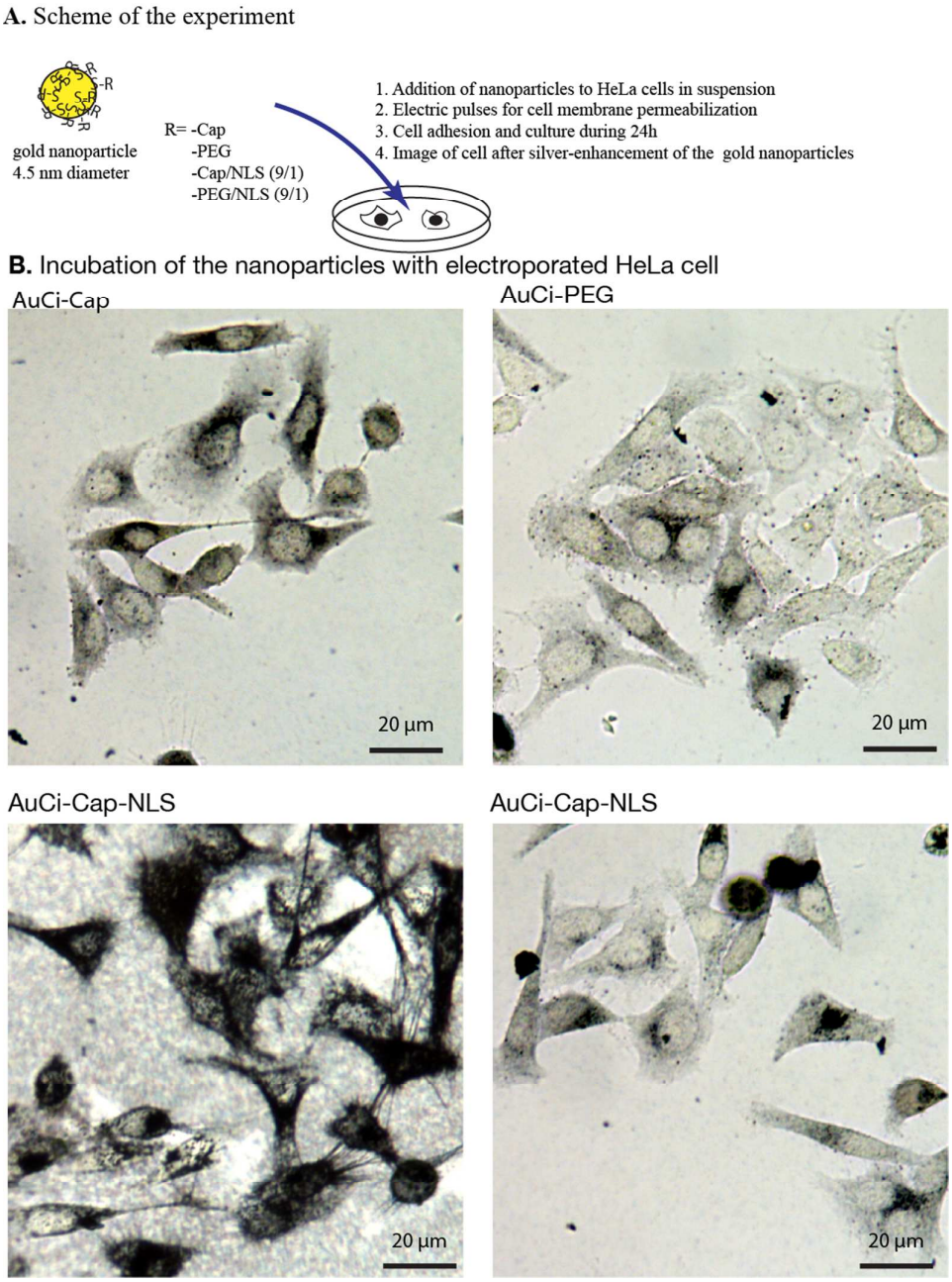

Figure S11. Evaluation of the intracellular fate of peptide-subtituted 4.5 nm nanoparticles. **A.** Scheme of the experiment. **B.** The various gold nanoparticles as indicated were mixed with HeLa cells and the mixtures s were subjected to electroporation. Following the 24h incubation period at 37°C, the living cells were fixed with glutaraldehyde (2.5% w/v) and the gold nanoparticles were detected by silver darkening. Results showed heterogeneous distribution. These NLS-equipped nanoparticles did not accumulate in the cell nuclei even when the nanoparticle's surface was equipped with PEG for minimizing unspecific association to cellular component. We did not determine whether the poor cytosolic delivery was due to poor diffusion inside he cytosol or to poor passage across the transient holes. Cell viability, estimated by monolayer density was >80%.

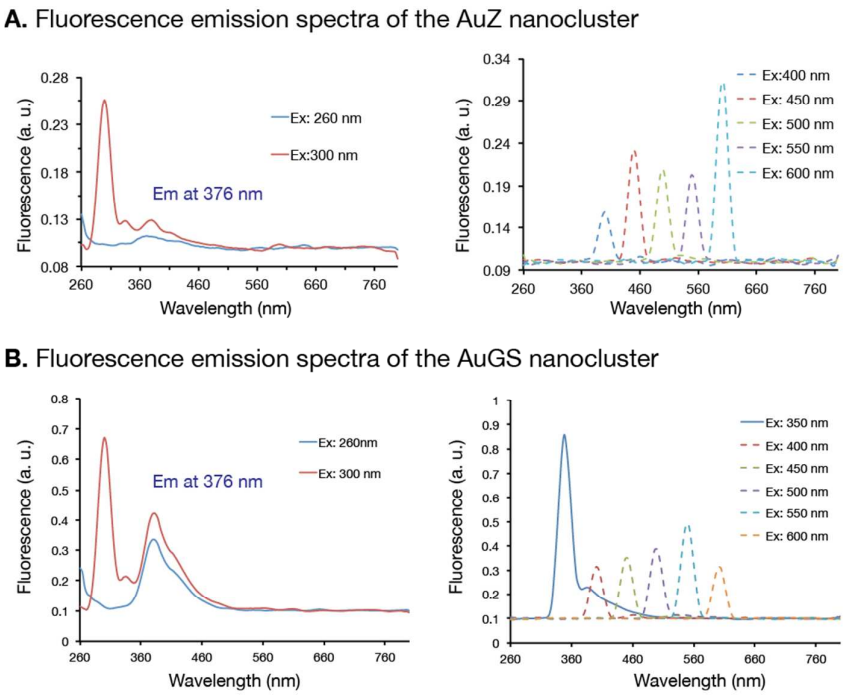

Figure S12. Fluorescence emission spectra of the AuZ (**A**) and AuGS (**B**) nanoclusters at various excitation wavelengths as indicated. Results indicated that the AuZ and the AuGS have photoluminescent abilities (Ex: 300, Em: 376 nm). The intensity of the photoluminescnet was extremely weak and practically useless for *in cellulo* imaging or nanocluster tracking. Condition: 0.6 μM AuNC in 10 mM K_2CO_3.

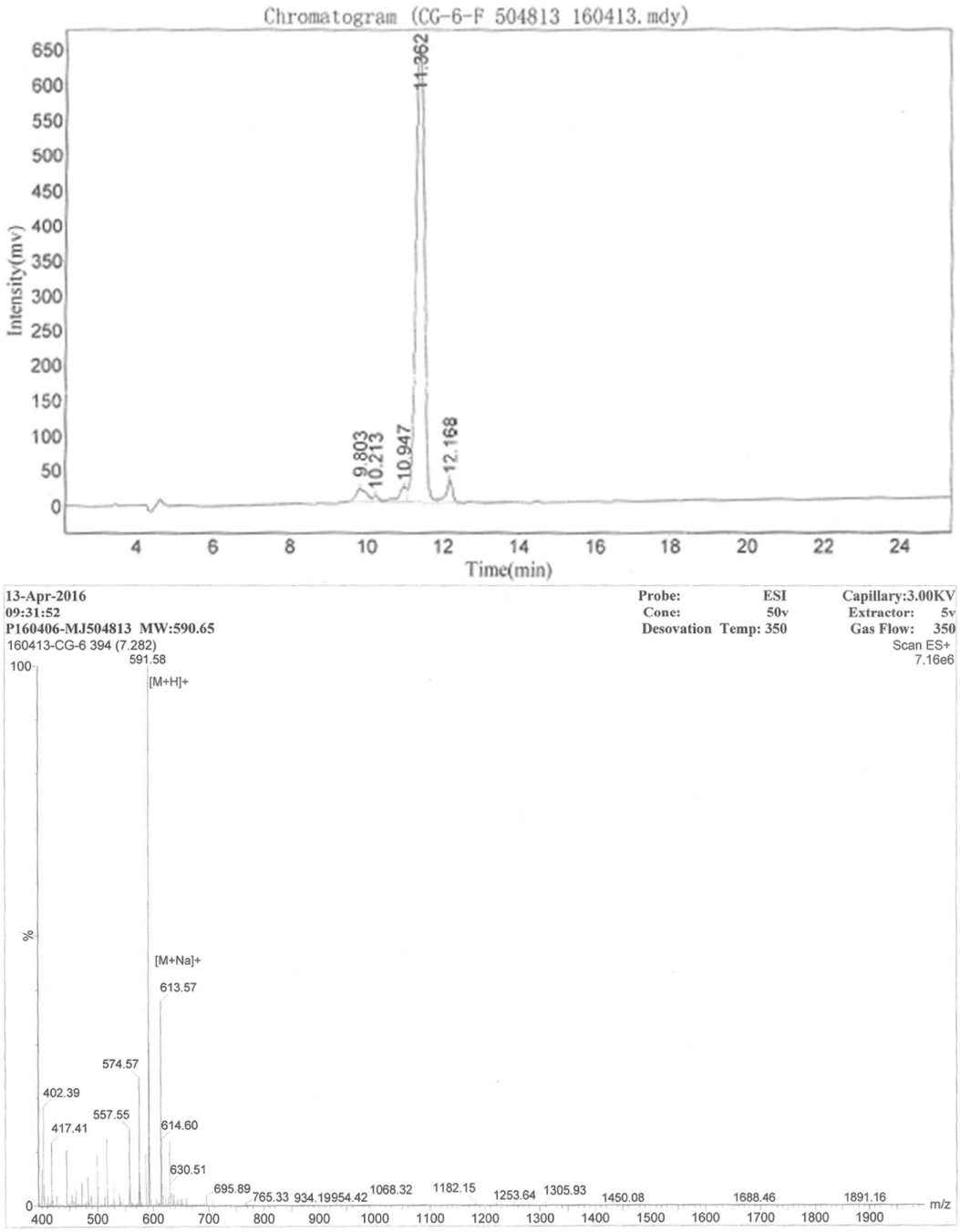

Figure S13. HPLC profile and mass spectrometry analysis of the Cap peptide (CALLNG, $C_{22}H_{38}N_8O_9S_1$; MW : 1801.06). Column: 4.6mmx250mm Diamonsil 5 µ C18. Solvent A: 0.1% TFA in 100% Acetonitrile, Solvent B : 0.1% TFA in water. Gradient: 0 to 25 min, 7 to 32% A.

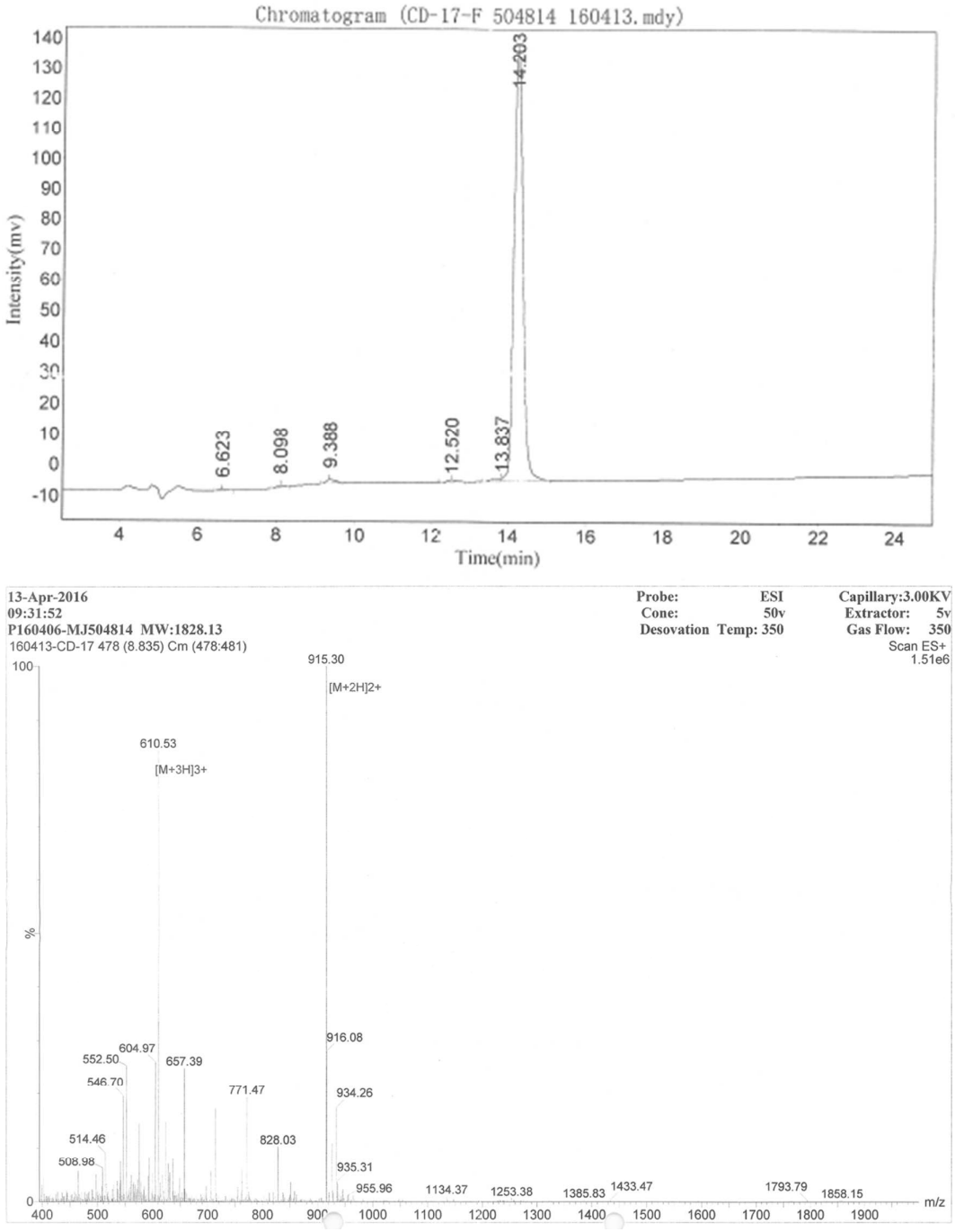

Figure S14. HPLC profile and mass spectrometry analysis of the NLS peptide (CALNNGAGPKKKRKVED, $C_{76}H_{134}N_{26}O_{25}S_1$; MW : 1828.13). Column 4.6mmx250mm Diamonsil 5 µ C18. Solvent A: 0.1% TFA in 100% Acetonitrile, Solvent B : 0.1% TFA in water. Gradient: 0 to 25 min, 7 to 32% A.

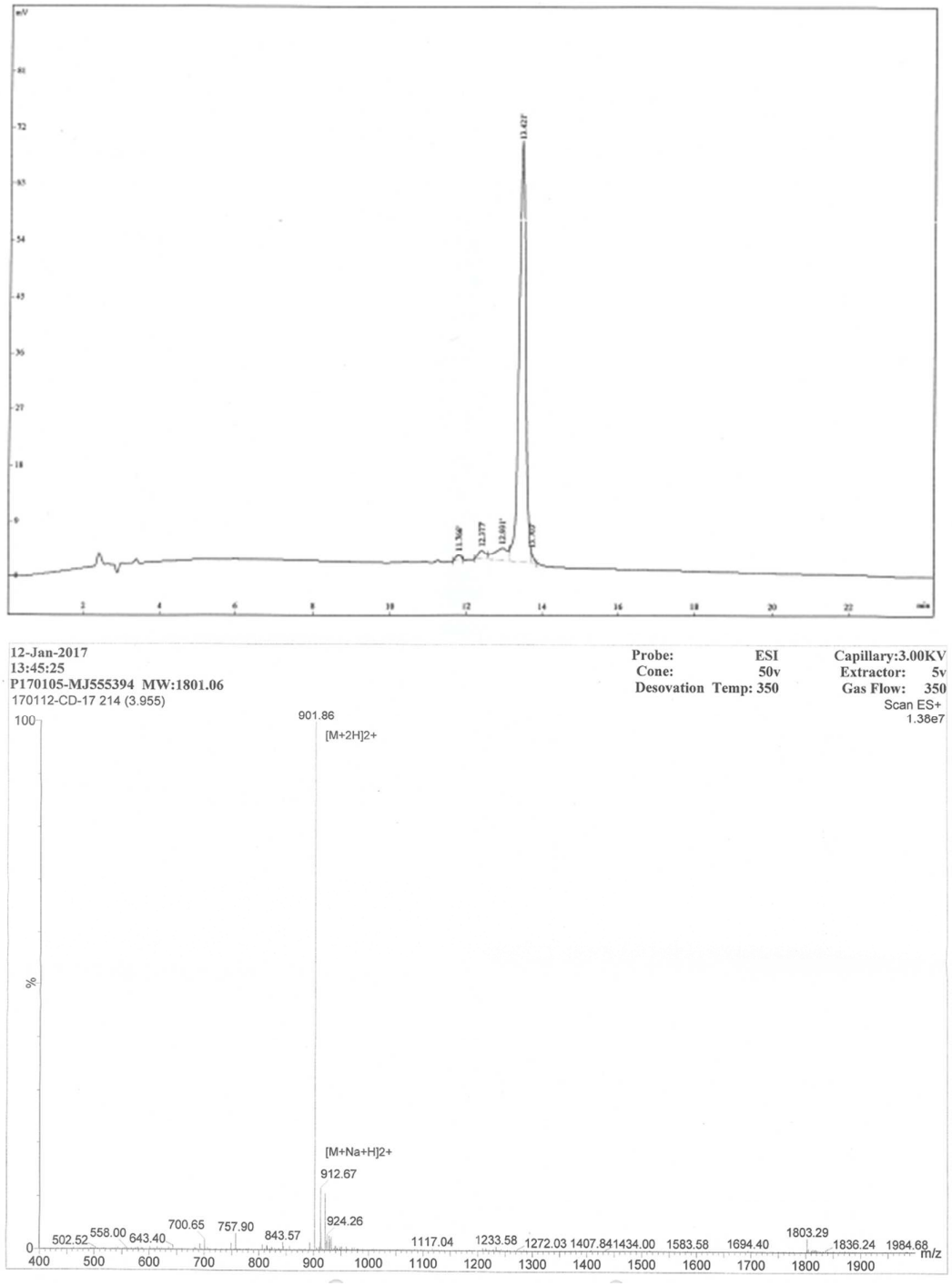

Figure S15. HPLC profile and mass spectrometry analysis of the dNLS peptide (CALNNGAGPK**T**KRKVED, $C_{74}H_{129}N_{25}O_{25}S_1$; MW : 1801.06). Column 4.6mmx250mm Inertsil ODS-SP. Solvent A: 0.1% TFA in 100% Acetonitrile, Solvent B: 0.1% TFA in water. Gradient: 0 to 25 min, 8 to 33% A.

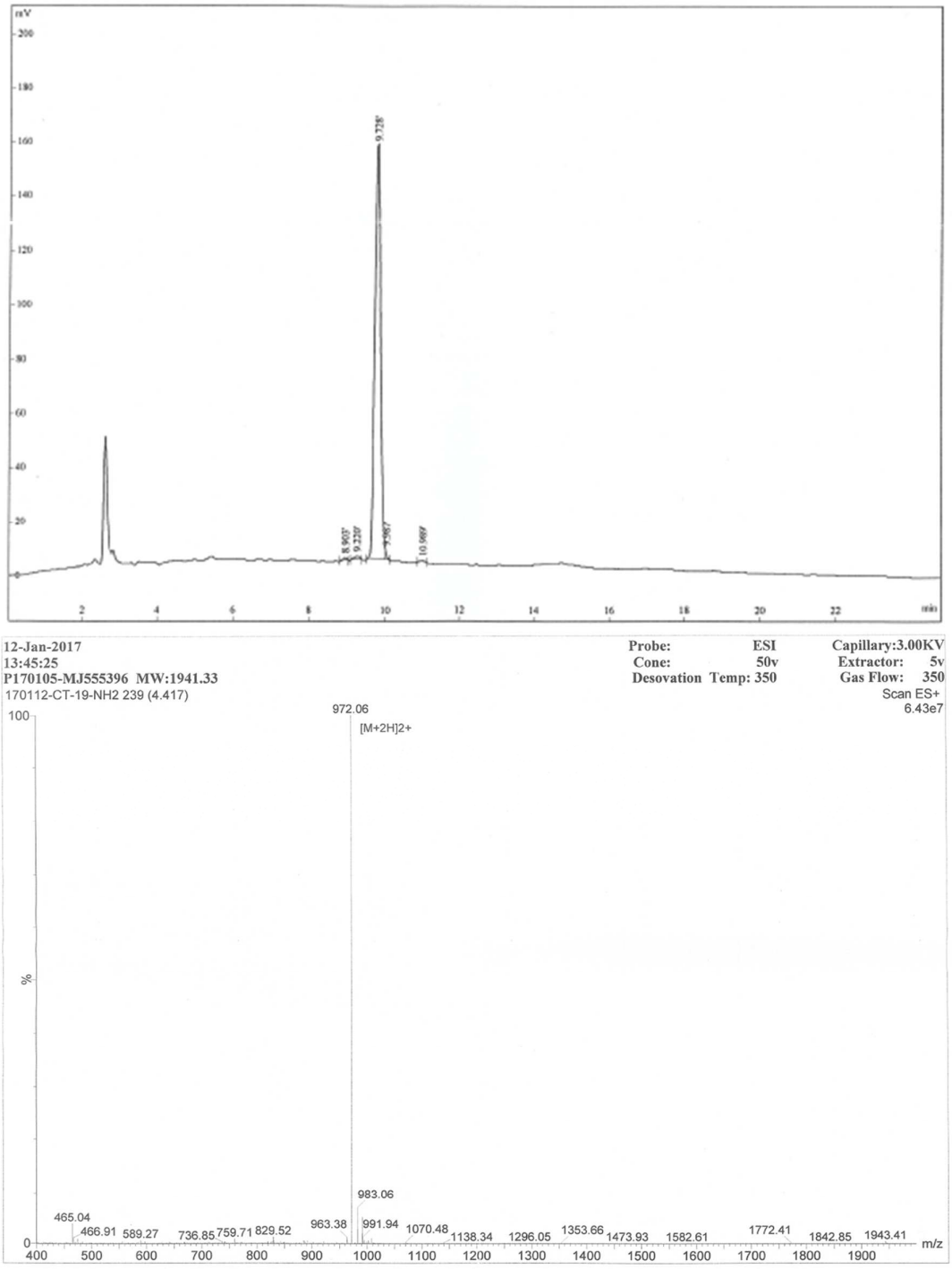

Figure S16. HPLC profile and mass spectrometry analysis of the NES peptide (CALNNGLALKLAGLDINKT$_{\text{-amide}}$, $C_{84}H_{149}N_{25}O_{25}S_1$; MW : 1941.33). Column 4.6mmx250mm Inertsil ODS-SP. Solvent A: 0.1% TFA in 100% Acetonitrile, Solvent B: 0.1% TFA in water. Gradient: 0 to 25 min, 8 to 33% A.

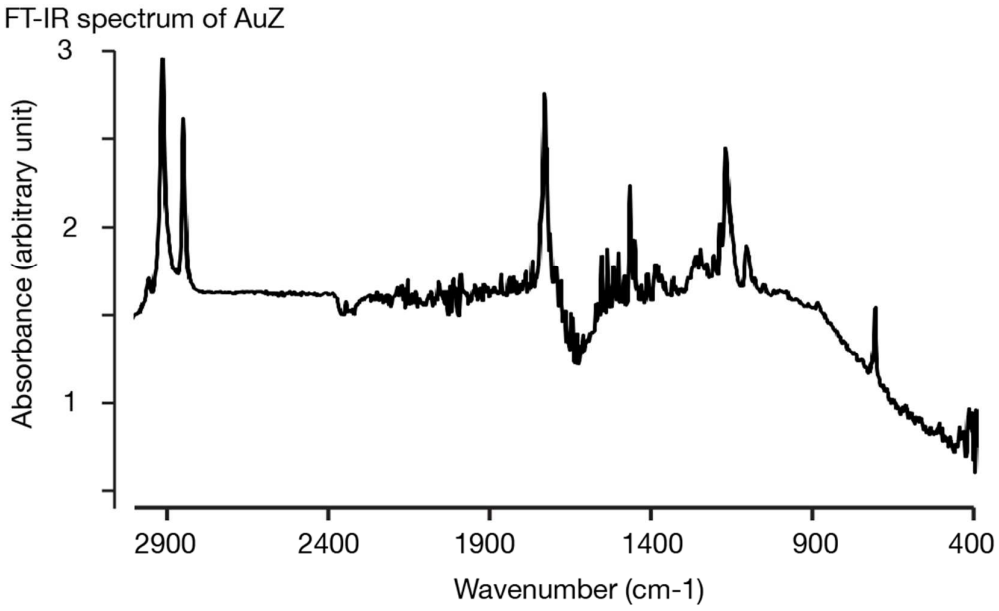

Figure S17. FT IR spectrum of the AuZ nanocluster. The spectrum was taken using a Nicolet 380 FT-IR spectrometer and a diamond ATR by ThermoFisher Scientific. Number of scans: 32.

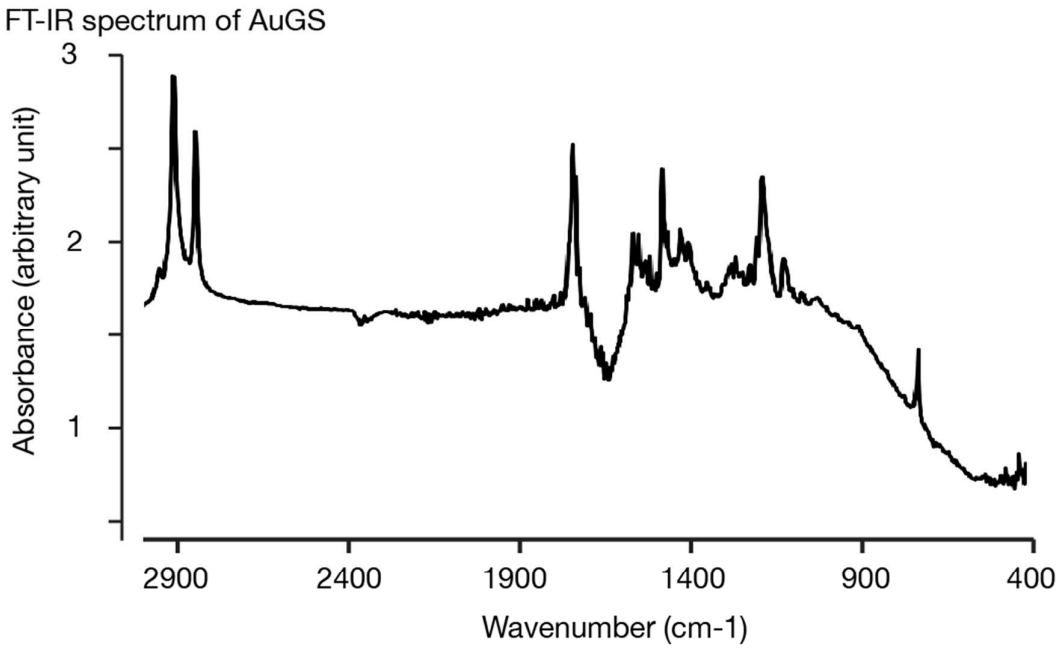

Figure S18. FT IR spectrum of the AuGS nanocluster. The spectrum was taken using a Nicolet 380 FT-IR spectrometer and a diamond ATR by ThermoFisher Scientific. Number of scans: 32.

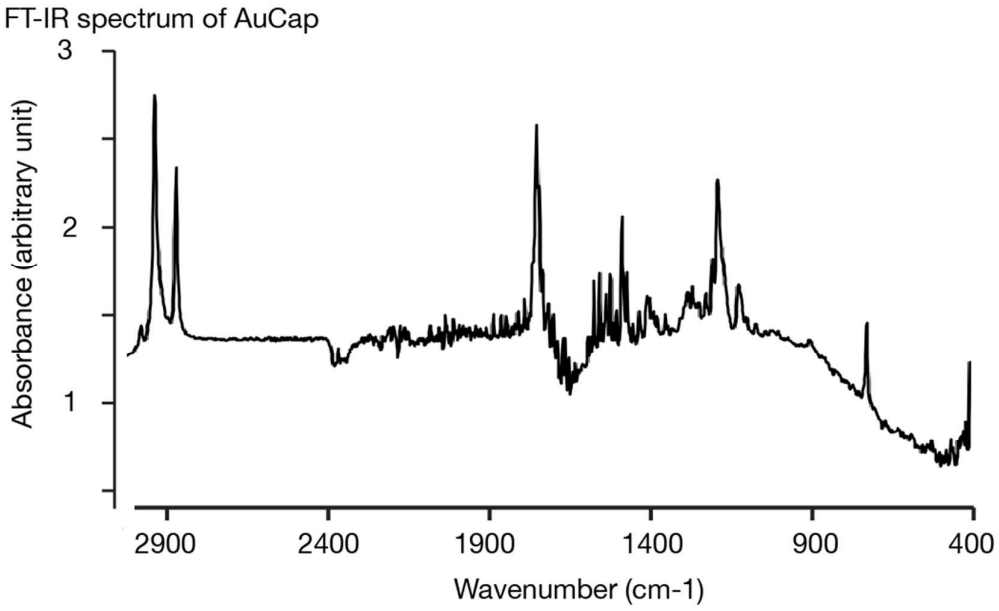

Figure S19. FT IR spectrum of the AuCap nanocluster. The spectrum was taken using a Nicolet 380 FT-IR spectrometer and a diamond ATR by ThermoFisher Scientific. Number of scans: 32.

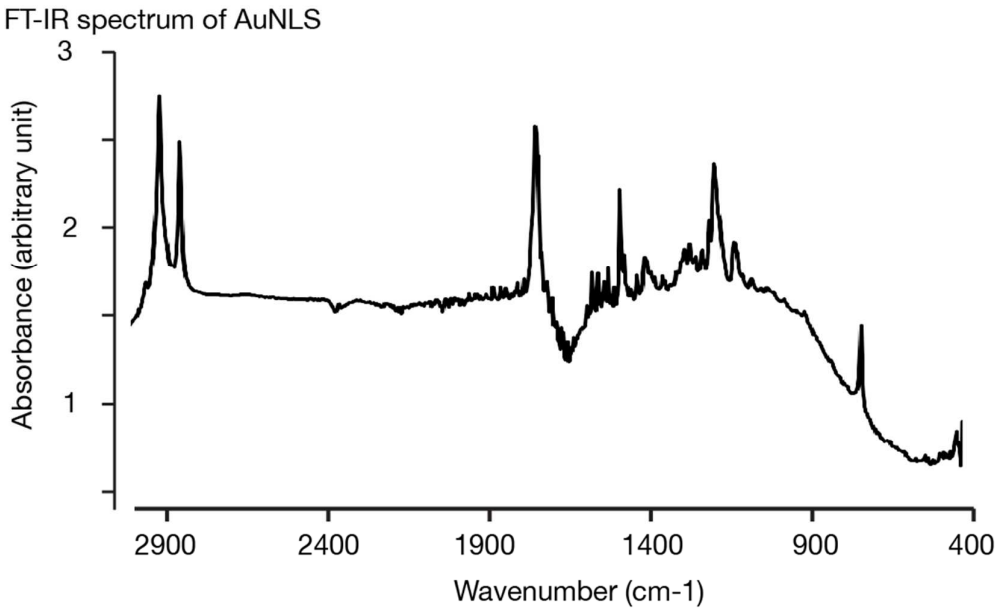

Figure S20. FT IR spectrum of the AuNLS nanocluster. The spectrum was taken using a Nicolet 380 FT-IR spectrometer and a diamond ATR by ThermoFisher Scientific. Number of scans: 32.

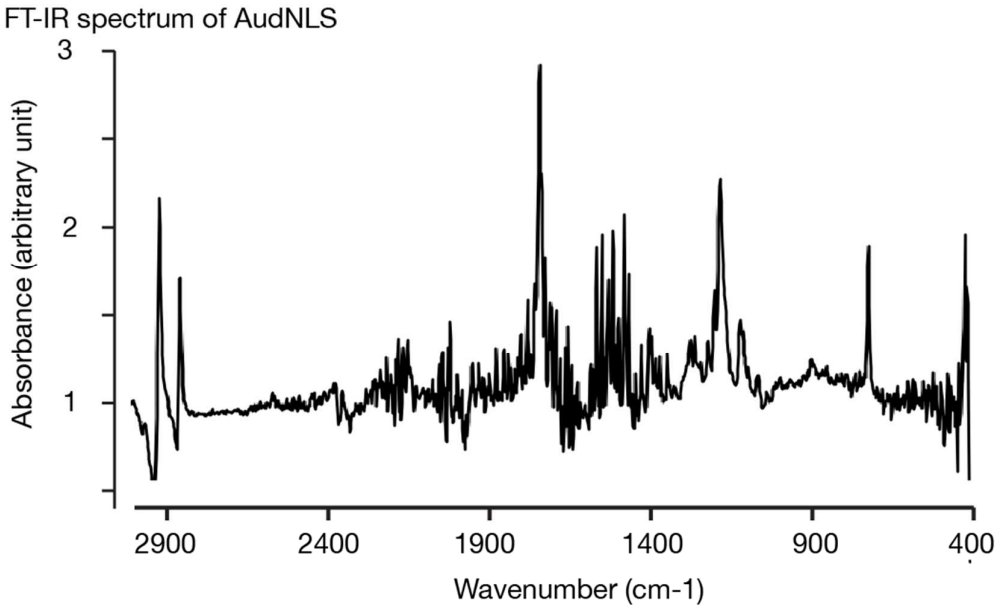

Figure S21. FT IR spectrum of the AudNLS nanocluster. The spectrum was taken using a Nicolet 380 FT-IR spectrometer and a diamond ATR by ThermoFisher Scientific. Number of scans: 32.

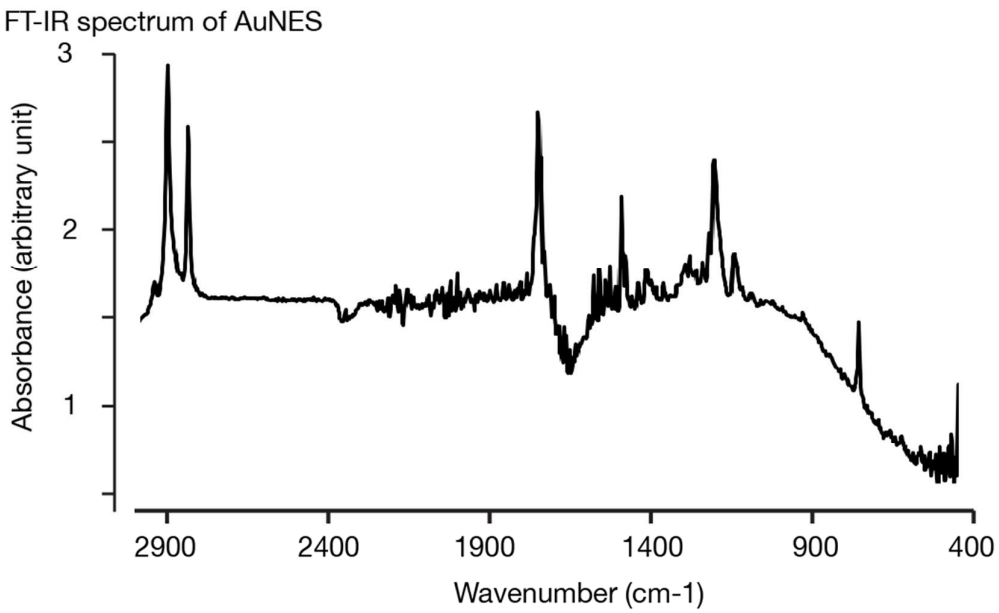

Figure S22. FT IR spectrum of the AuNES nanocluster. The spectrum was taken using a Nicolet 380 FT-IR spectrometer and a diamond ATR by ThermoFisher Scientific. Number of scans: 32.

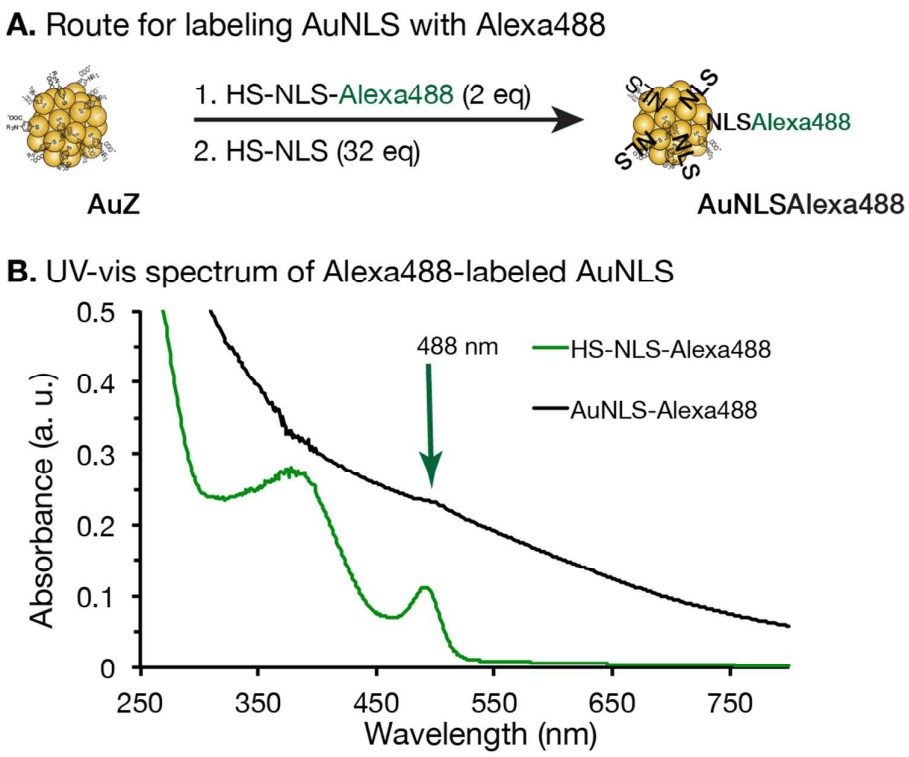

Figure S23. Synthesis of Alexa488-labeled AuNLS. **A.** Route for preparing Alexa488-labeled AuNLS. AlexaFluo488 NHS ester (ThermoFisher) (16 nmol) was mixed with the HS-NLS (16 nmol) in 150 mM K_2CO_3 and incubated for 24h for labeling the peptide on the Lysines. The reaction mixture was then treated with TCEP (32 nmol) and then AuZ (8 nmol). After a 24h reaction, unlabeled SH-NLSs (240 nmol) and TCEP (240 nmol) were added and the reaction was incubated for 24h before purification using an ultracentrifugation device with a 30 kDa cut-off. B. UV-vis spectrum of the Alexa488-labeled AuNLS (black line). The green line corresponds to the UV/vis spectrum of Alexa488-NHS ester.

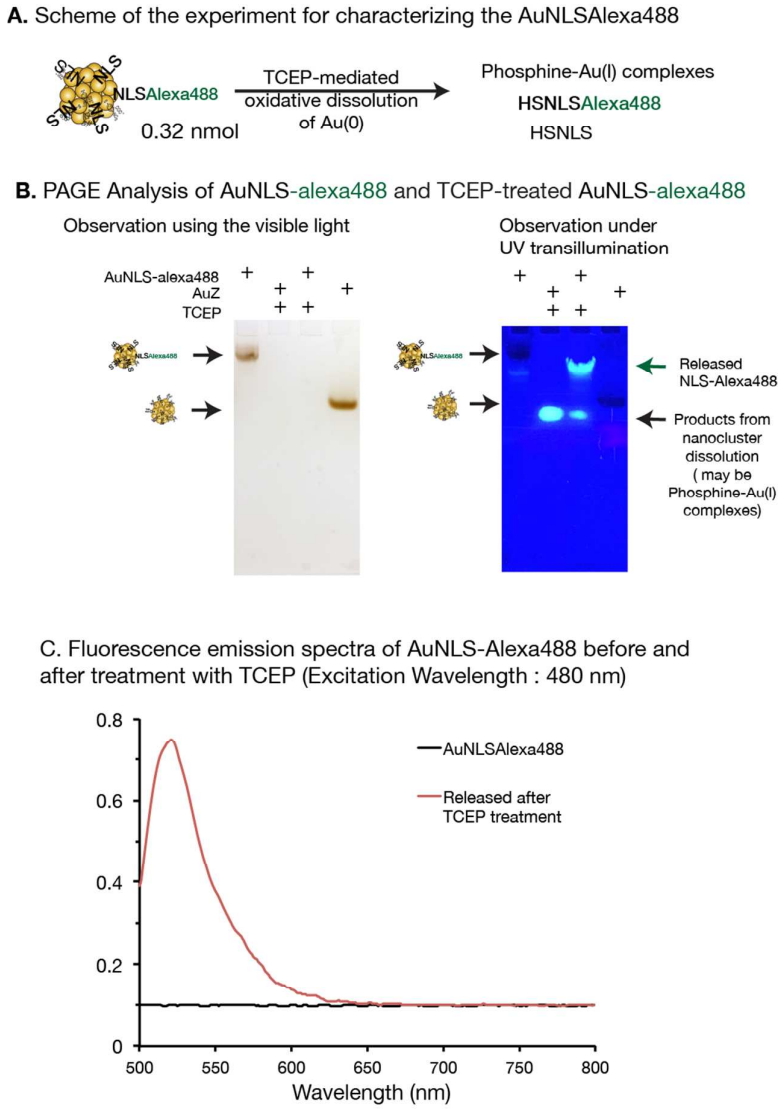

Figure S24. Characterization of Alexa488-labeled AuNLS. **A.** Scheme of experiment done for characterizing the AuNLSAlexa488. The nanocluster (0.32 nmol) was treated in water with TCEP, pH 7.0 (500 nmol) in a 6 µL volume. **B.** PAGE analysis of AuZ, AuNLSAlexa488 and the TCEP-treated corresponding nanoclusters as indicated. After analysis, the gel was visualized under visible light (left image) or under UV (right image). Data showed AuZ and AuNLSAlexa488 to be non-fluorescent (actually they absorb UV light (intense dark band). TCEP-treated samples showed release of a fluorescent species (the likely released species are indicated in the graph), suggesting that AuNC actually quenches fluorescence. **C.** Fluorescence emission spectra of the AuNLSAlexa488 and of the TCEP-treated AuNLSAlexa488.

Comment: TCEP allows an oxidative dissolution of the Au(0) and hence a release of ligands from the nanocluster.[10,11] Data indicated that the TCEP-treated AuNLSAlexa488 released fluorescent species whereas AuNLSAlexa488 did not emit the Alexa488 fluorescence. These experiment clearly demonstrated that: -1. AuNLSAlexa488 is grafted with the Alexa488, -2. The AuNC quenches fluorescence of the bound Alexa488 fluorophore, as previously observed.[4]

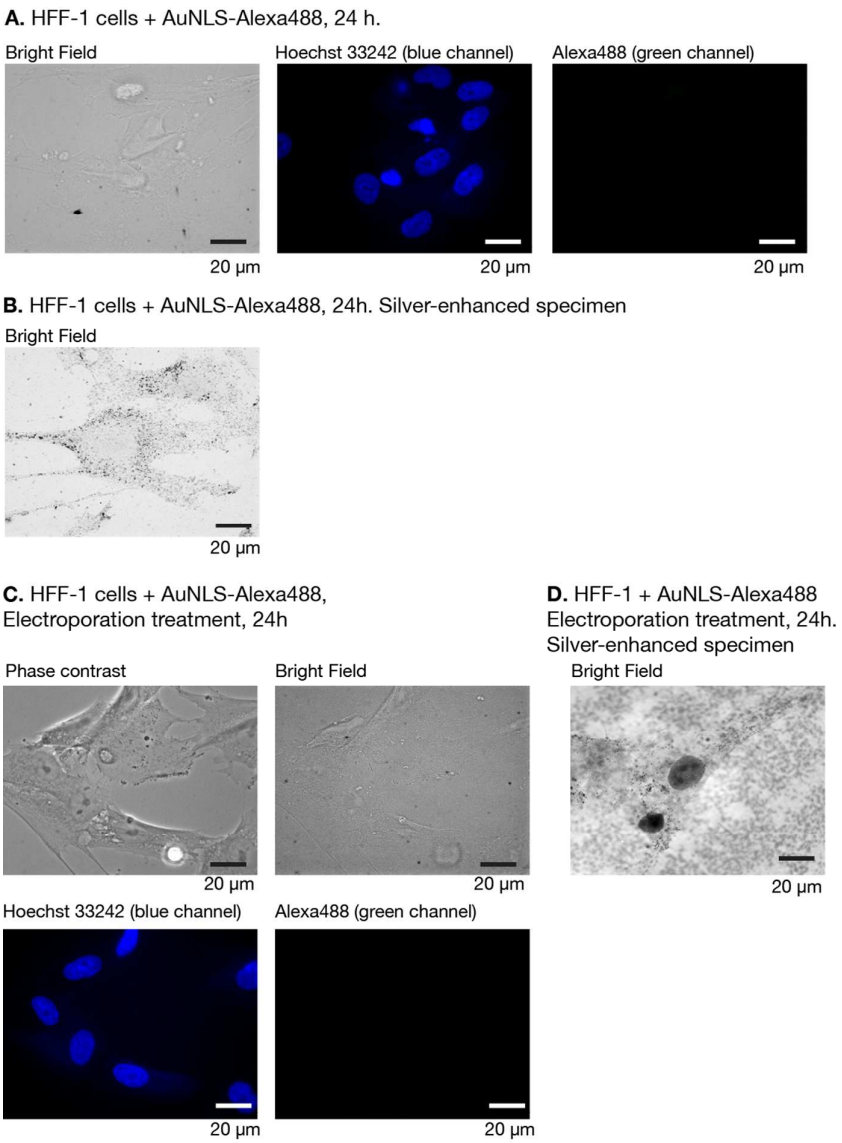

Figure S25. Analysis of fate of Alexa488-labeled AuNLS after addition to HFF-1 cells. **A.** Images of HFF-1 cells incubated with the AuNLSAlexa488 for 24h. Hoechst 33242-stained cells were observed under bright field, blue channel, for detection of Hoechst 33242-stained nuclei, and green channel for detection of the Alexa488. **B.** Images of HFF-1 cells incubated with the AuNLSAlexa488 for 24h followed by silver staining. **C.** Images of electroporated HFF-1 cells incubated with the AuNLSAlexa488 for 24h. Hoechst 33242-stained cells were observed using phase contrast, under bright field, blue channel (for detection of Hoechst 33242-stained nuclei) and green channel for detection of the Alexa488). **D.** Images of electroporated HFF-1 cells incubated with the AuNLSAlexa488 and for 24h followed by silver staining. Following the 24h incubation period at 37°C, the living cells were fixed with glutaraldehyde (2.5% w/v). Data showed absence of Alexa488 fluorescence, suggesting that the Alexa488 remained bound to the gold nanocluster (see Figure S26 for experiment showing the AuNC to quench the Alexa488 fluorescence).

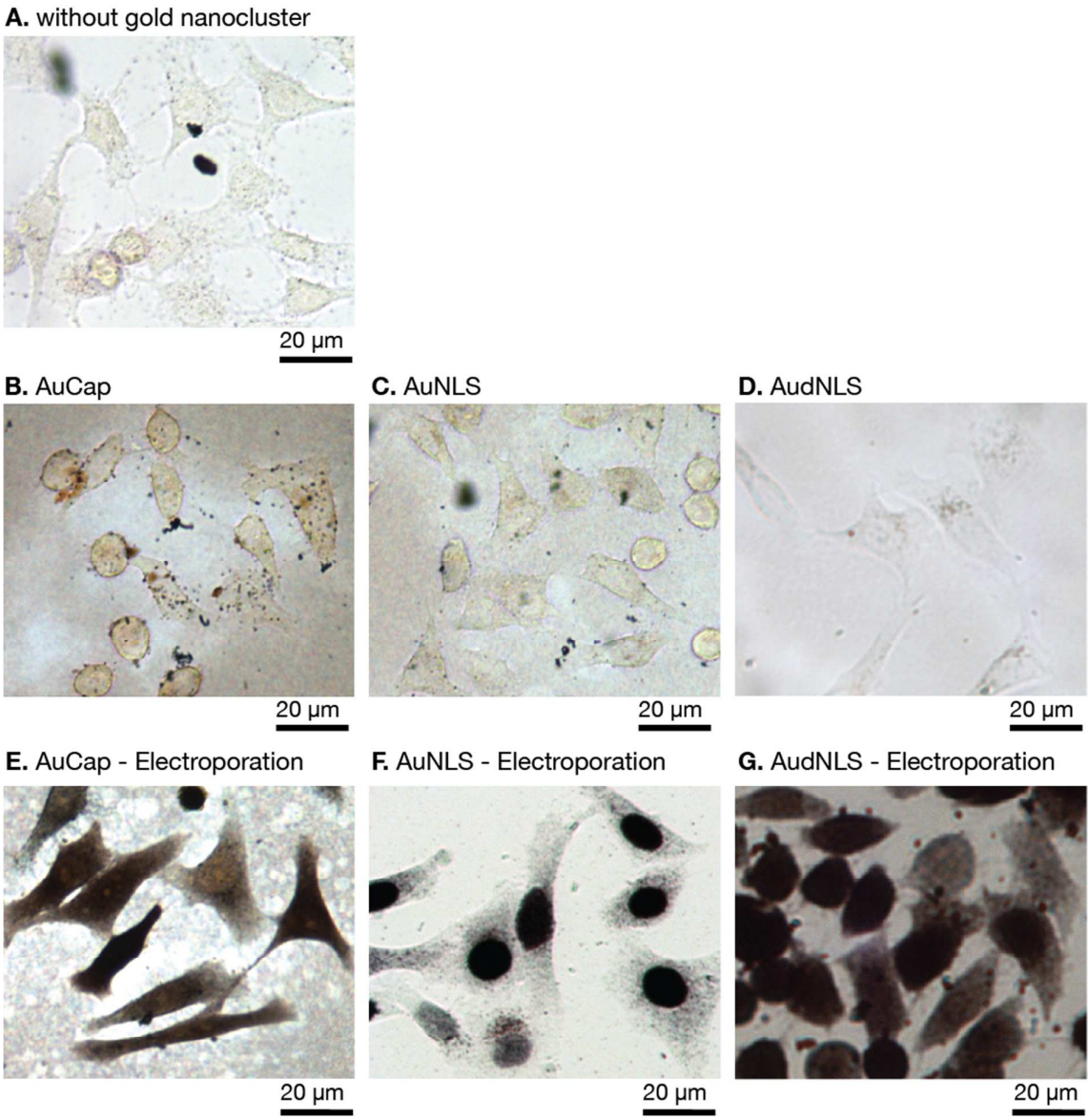

Figure S26. Color images of HeLa cells incubated with AuCap, AuNLS and AudNLS. **A.** Untreated cells**. B, C and D.** The various peptide-AuNCs were added to living HeLa cells by dilution in the cell culture medium without an electroporation treatment. **E, F and G.** The HeLa cells were subjected to electroporation in presence of the indicated AuNCs. Following the 24h incubation period at 37°C, the living cells were fixed with glutaraldehyde (2.5% w/v) and AuNCs were detected by silver staining.

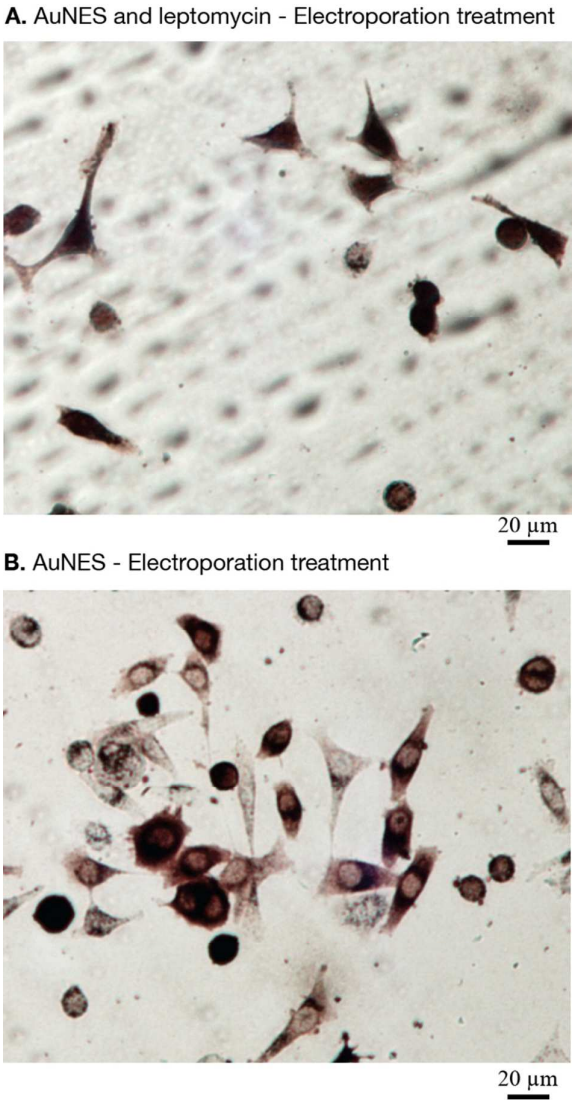

Figure S27. Color images of HeLa cells incubated or 24h with: **A**. AuNES and leptomycin, or **B.** AuNES alone. The AuNES was added to living HeLa cells by dilution in the cell culture medium with an electroporation treatment. Following the 24h incubation period at 37°C, the living cells were fixed with glutaraldehyde (2.5% w/v) and the AuNES was detected by silver staining.

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