

Supplementary Material (ESI) for Chemical Communications

Enhanced Cellular Uptake of Amphiphilic Gold Nanoparticles with Ester Functionality

Kenya Kobayashi, Kenichi Niikura,* Chie Takeuchi, Shota Sekiguchi,
Kyoji Hagiwara, Hideyuki Mitomo, Takafumi Ninomiya, Yoshihiro Ito,
Yoshihito Osada, Kuniharu Ijiri
RIKEN Hirosawa 2-1, Wako (Japan)
Hokkaido University, N21W10, Sapporo (Japan)

Experimental Section

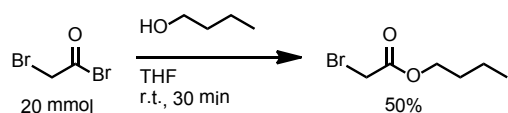
General information

Citric-acid-coated gold nanoparticles (10 nm in diameter) were purchased from BBI (UK). All commercially available reagents were purchased from Wako Pure Chemical Industries (Japan), Tokyo Chemical Industry (Japan) or Sigma-Aldrich (USA) and used without further purification. Thin-layer Chromatography (TLC) was performed on glass-backed precoated silica gel plate (60F254, Merck & Co., Inc., USA). Molecules were visualized by iodine/silica gel. Products were isolated by column chromatography on silica-gel (Kanto Chemical, 60N, spherical, neutral, 40-50 μm). MALDI-TOF-MS spectra were measured with a microflex (Bruker Daltonics). NMR spectra were recorded on a 400 MHz JEOL spectrometer. Au concentrations were measured with a ICPE-9000 (SHIMADZU). Ultrathin sections of samples were examined with a JEM-1230 or JEM-1400 transmission electron microscope (JEOL, Japan).

Synthesis of PEG derivative ligands

C2-Ether and C4-Ether was synthesized according to our previous report.¹

Synthesis of *n*-butyl bromoacetate



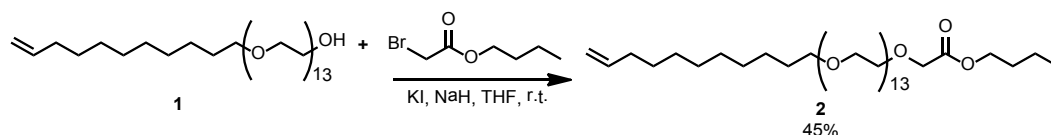
n-Butanol (1.8 mL, 1 equiv.) and *N*-ethyldiisopropylamine (3.4 mL, 1 equiv.) was dissolved in THF (12 mL). The solution was cooled on ice bath and bromo acetyl bromide (1.7 mL, 20 mmol) was slowly added and stirred for 30 min at room temperature. Ethyl acetate was added and washed with HCl. Organic layer was dried with Na_2SO_4 and evaporated in vacuum. The residue was chromatographed on a silica-gel column using hexane/ethyl acetate to yield clear syrup (1.97 g,

50%).

^1H NMR (400 MHz, CDCl_3) δ 4.18 (t, $J = 6.6$ Hz, 2H), 3.84 (s, 2H), 1.69–1.61 (m, 2 H), 1.44–1.35 (m, 2 H), 0.94 (t, $J = 7.4$ Hz, 3H)

^{13}C NMR (100 MHz, CDCl_3) δ 137.34, 66.15, 30.46, 25.98, 19.01, 13.66

Synthesis of compound 2

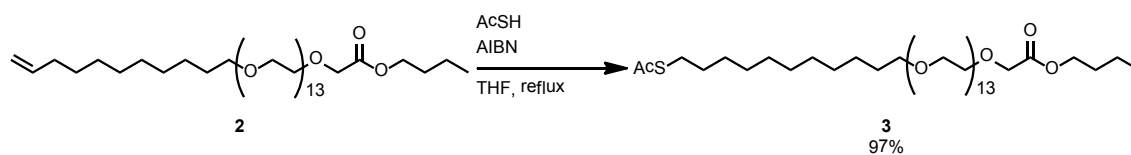


Compound 1 was synthesized according to our previous report.¹ Compound 1 (760 mg, 0.9 mmol) and potassium iodide (30 mg, 0.2 equiv.) was dissolved in THF (9 mL). The solution was cooled on ice bath and sodium hydride (7.2 mg, 2.5 equiv.) was slowly added and stirred for 10 min and *n*-butyl bromoacetate (355 mg, 2 equiv.) was added and stirred for overnight at room temperature. Water was added and extracted with chloroform. The combined organic phase was dried over Na_2SO_4 and concentrated in vacuum. The residue was chromatographed on a silica-gel column using dichloromethane to dichloromethane/methanol (30:1) to yield clear syrup (393 mg, 45%).

^1H NMR (400 MHz, CDCl_3) δ 5.87–5.75 (m, 1H), 5.04–4.91 (m, 2H), 4.16 (t, $J = 6.8$ Hz, 2H), 4.15 (s, 2H), 3.74–3.55 (m, 48H), 3.44 (t, $J = 7.0$ Hz, 2H), 2.08–2.00 (m, 2H), 1.67–1.55 (m, 4H), 1.45–1.21 (m, 12H), 0.94 (t, $J = 7.6$ Hz, 3H)

MS (MALDI-TOFMS): calcd for $\text{C}_{37}\text{H}_{72}\text{O}_{13}\text{Na}$ $[\text{M}+\text{Na}]^+$ 747.49, $\text{C}_{39}\text{H}_{76}\text{O}_{14}\text{Na}$ $[\text{M}+\text{Na}]^+$ 791.51, $\text{C}_{41}\text{H}_{80}\text{O}_{15}\text{Na}$ $[\text{M}+\text{Na}]^+$ 835.54, $\text{C}_{43}\text{H}_{84}\text{O}_{16}\text{Na}$ $[\text{M}+\text{Na}]^+$ 879.57, $\text{C}_{45}\text{H}_{88}\text{O}_{17}\text{Na}$ $[\text{M}+\text{Na}]^+$ 923.59, found 747.39, 767.36, 791.41, 835.43, 879.45, 923.47.

Synthesis of compound 3

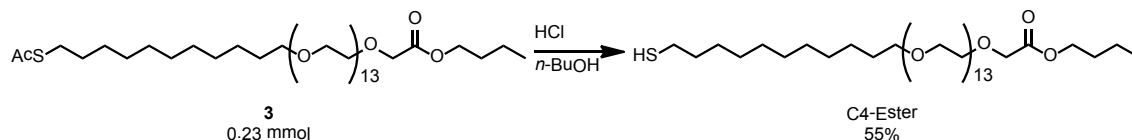


Compound 2 (207 mg, 0.24 mmol), thioacetic acid (79 μL , 5 equiv.) and AIBN (36 mg, 1 equiv.) were dissolved in THF (4 mL) and refluxed for overnight. The solvent was removed in vacuum and the residue was chromatographed on a silica-gel column using dichloromethane to dichloromethane/methanol (30:1) to yield clear syrup (219 mg, 97%).

^1H NMR (400 MHz, CDCl_3) δ 4.16 (t, $J = 6.8$ Hz, 2H), 4.15 (s, 2H), 3.74–3.56 (m, 58H), 3.44 (t, $J = 6.8$ Hz, 2H), 2.86 (t, $J = 7.6$ Hz, 2H), 2.32 (s, 3H), 1.68–1.51 (m, 6H), 1.43–1.25 (m, 16H), 0.94 (t, $J = 7.4$ Hz, 3H)

MS (MALDI-TOFMS): calcd for $C_{39}H_{76}O_{14}SNa [M+Na]^+$ 823.49, $C_{41}H_{80}O_{15}SNa [M+Na]^+$ 867.51, $C_{43}H_{84}O_{16}SNa [M+Na]^+$ 911.54, $C_{45}H_{88}O_{17}SNa [M+Na]^+$ 955.56, $C_{47}H_{92}O_{18}SNa [M+Na]^+$ 999.59, found 823.47, 867.49, 911.52, 955.55, 999.57.

Synthesis of C4-Ester

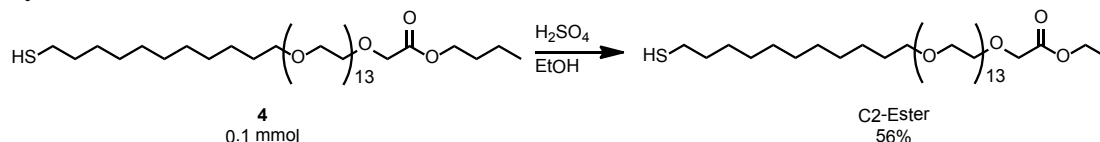


Compound **3** (218 mg, 0.23 mmol) and hydrochloric acid (0.23 mL) was dissolved in *n*-BuOH (2.3 mL) and stirred at 70 °C for overnight. The solvent was removed in vacuum and the residue was chromatographed on a silica-gel column using dichloromethane to dichloromethane/methanol (20:1) to yield clear syrup (113 mg, 55%).

1H NMR (400 MHz, $CDCl_3$) δ 4.16 (t, $J = 6.8$ Hz, 2H), 4.15 (s, 2H), 3.73–2.53 (m, 51H), 3.44 (t, $J = 6.8$ Hz, 2H), 2.52 (m, 2H), 1.68–1.51 (m, 6H), 1.42–1.21 (m, 16H), 0.94 (t, $J = 7.2$ Hz, 3H)

MS (MALDI-TOFMS): calcd for $C_{33}H_{66}O_{12}SNa [M+Na]^+$ 693.42, $C_{35}H_{70}O_{12}SNa [M+Na]^+$ 737.45, $C_{37}H_{74}O_{13}SNa [M+Na]^+$ 781.47, $C_{39}H_{78}O_{14}SNa [M+Na]^+$ 825.50, $C_{41}H_{82}O_{15}SNa [M+Na]^+$ 869.53, found 693.39, 737.41, 781.43, 825.46, 869.49.

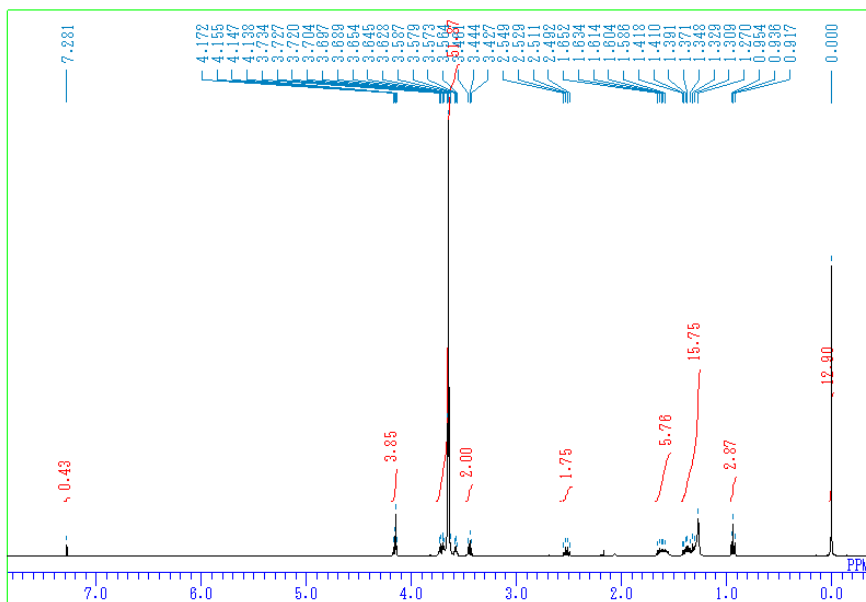
Synthesis of C2-Ester



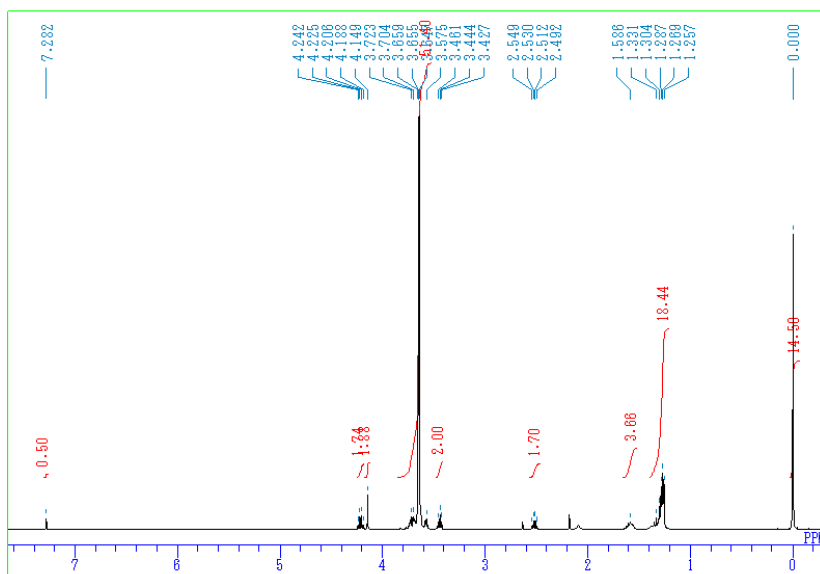
C4-Ester (86 mg, 0.1 mmol) and sulfuric acid (0.1 mL) was dissolved in ethanol (2 mL) and heated at 80 °C for overnight. The reaction mixture was allowed to cool to room temperature, saturated sodium bicarbonate was added and extracted with chloroform. The combined organic phase was dried over Na_2SO_4 and concentrated in vacuum. The residue was chromatographed on a silica-gel column using dichloromethane to dichloromethane/methanol (30:1) to yield clear syrup (49 mg, 56%).

1H NMR (400 MHz, $CDCl_3$) δ 4.22 (t, $J = 7.6$ Hz, 2H), 4.15 (s, 2H), 3.88–3.45 (m, 51H), 3.44 (t, $J = 6.8$ Hz, 2H), 2.52 (m, 2H), 1.63–1.52 (m, 4H), 1.39–1.21 (m, 18H)

MS (MALDI-TOFMS): calcd for $C_{35}H_{70}O_{13}SNa [M+Na]^+$ 753.44, $C_{37}H_{74}O_{14}SNa [M+Na]^+$ 797.47, $C_{39}H_{78}O_{15}SNa [M+Na]^+$ 841.50, $C_{41}H_{82}O_{16}SNa [M+Na]^+$ 885.52, $C_{43}H_{86}O_{17}SNa [M+Na]^+$ 929.55, found 753.36, 797.37, 841.39, 885.41, 929.41.



$^1\text{H-NMR}$ spectrum (CDCl_3) of C4-Ester



$^1\text{H-NMR}$ spectrum (CDCl_3) of C2-Ester

Preparation of BSPP-AuNPs

BSPP (26.7 mg) was added to an aqueous solution of 10-nm citric acid-coated AuNPs (9.5 nM, 10 mL) and let stand for 24 hours. The excess ligands were removed by centrifugation using an Amicon Ultra 100K filter (6000 G, 10 min, three times) and diluted by MiliQ water to 100 nM.

Preparation of Ester-AuNPs

To an aqueous solution of BSPP-AuNPs (100 nM, 300 μ L), an ethanol solution of C2-ester or C4-ester ligand (10 mM, 7.5 μ L) was added and let stand for 2 hrs at room temperature. The excess ligands were removed by centrifugation (3000G, 3 min, three times) using an Amicone Ultra 100 K filter and then redispersed in 300 μ L of MiliQ water. AuNPs coated with ether PEG-derivative ligands were also prepared according to above method.

AuNP phase transfer

PBS solutions of AuNPs were added to a small glass tube containing dichloromethane (200 μ L) and let stand for 2 days. After complete phase transfer, a solution of NaOH (1 M, 200 μ L) was further added and stand for further 1 day.

Incubation of AuNPs with esterase

BSPP-, C2-Ester- and C2-Ether-AuNP (200 μ L, 100 nM) in water were added to PBS (200 μ L) and 5 μ L of esterase solution (from porcine liver, 0.4 mg/mL, Sigma), respectively and incubated at 37°C for 6 hrs. Ten μ L of esterase-treated AuNPs and untreated AuNPs were then subjected to electrophoresis on 1.5% agarose gel at 50 mV for 40 min.

ICP Analysis

HeLa cells were seeded onto 6-well plates in DMEM containing 10% FBS at a density of 100,000 cells/well for 48 hrs before exposure to AuNPs. The culture medium was exchanged to Opti-MEM (life technologies) and AuNPs were added at a concentration of 10 nM and then incubated for 3 or 24 hrs. After incubation, Opti-MEM was removed and the cells were washed three times with PBS. Cells were then treated with trypsin, collected and 1 mL of aqua regia was added. The cells were then let stand for 1 day for complete ionization of the AuNPs. Ten mL of MQ was then added to each sample and they were then subjected to ICP analysis. The values of the Au concentrations were converted to the number of AuNPs according to reported literature.²

ICP Analysis in the presence of endocytosis inhibitors

HeLa cells were seeded onto 6-well plates in DMEM containing 10% FBS at a density of 200,000 cells/well for 24 hrs before exposure to AuNPs. The culture medium was exchanged to Opti-MEM

and Chlorpromazine hydrochloride, 5-(*N*-ethyl-*N*-isopropyl)amiloride, or Genistein³ were added at a final concentration of 3, 4, or 40 μ M, respectively and then incubated for 1 hr. The culture medium was washed out and AuNPs were added at a concentration of 10 nM and then incubated for 3 hrs in Opti-MEM. After incubation, Opti-MEM was removed and cells were washed three times with PBS. Cells were treated with trypsin, collected and 1 mL of aqua regia was added. The cells were then let stand for 1 day for complete ionization of AuNPs. Ten mL of MQ was added to each sample and they were then subjected to ICP analysis. The values of the Au concentrations were converted to the number of AuNPs according to reported literature.²

Preparation of TEM samples.

HeLa cells were incubated with the AuNPs (final concentration: 50 nM) for 3 hr in Opti-MEM on BioCoat™ Poly-D-Lysine 8-well CultureSlides (BD). After washing with PBS buffer, the cells were fixed in 2.5% glutaraldehyde/0.1 M phosphate buffer (pH 7.4) overnight at 4°C, post-fixed in a mixed aqueous solution of 1% osmium tetroxide and 1.5% potassium ferrocyanide for 2hr at room temperature, dehydrated in a graded ethanol series, and embedded in Epon 812 (TAAB). Ultrathin sections of samples were examined at 80 kV with a JEM-1230 or JEM-1400 transmission electron microscope (JEOL, Japan).

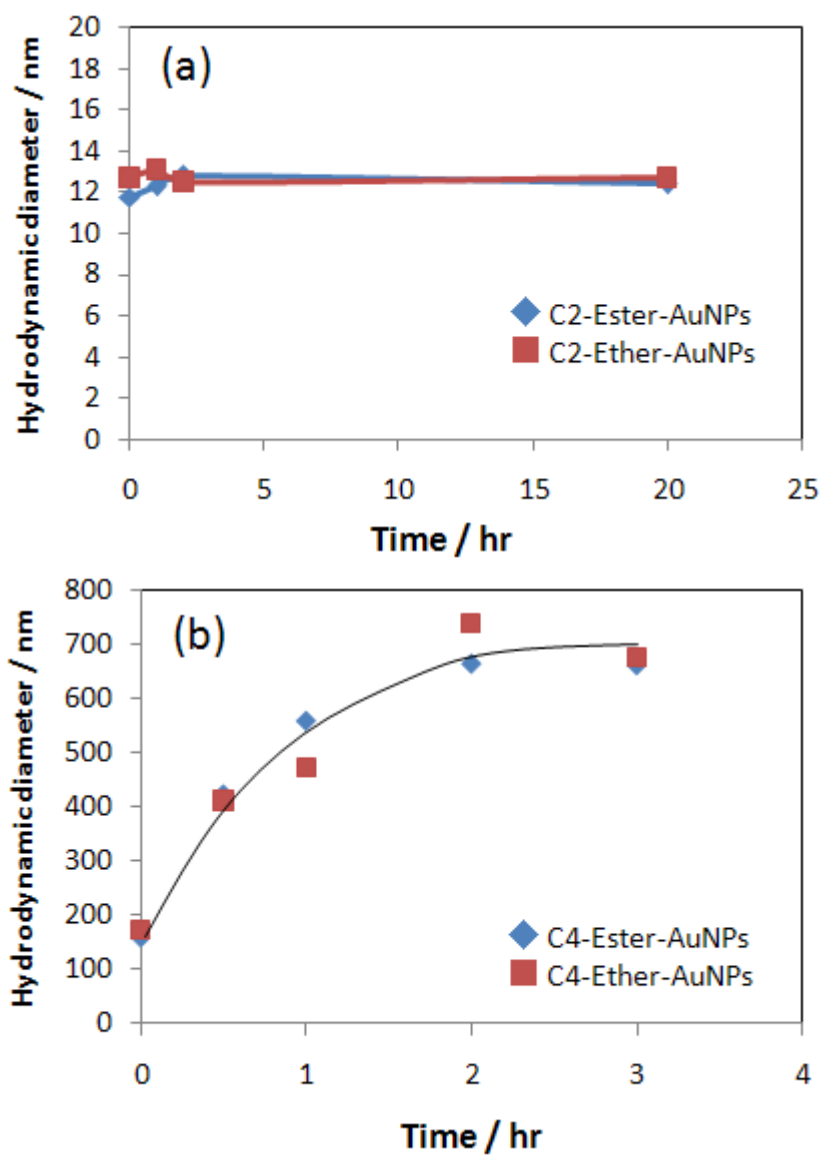


Fig. S1 Time course of hydrodynamic diameters determined by DLS in Opti-MEM. (a) : C2-Ester- and C2-Ether-AuNPs, (b) : C4-Ester and C4-Ether-AuNPs.

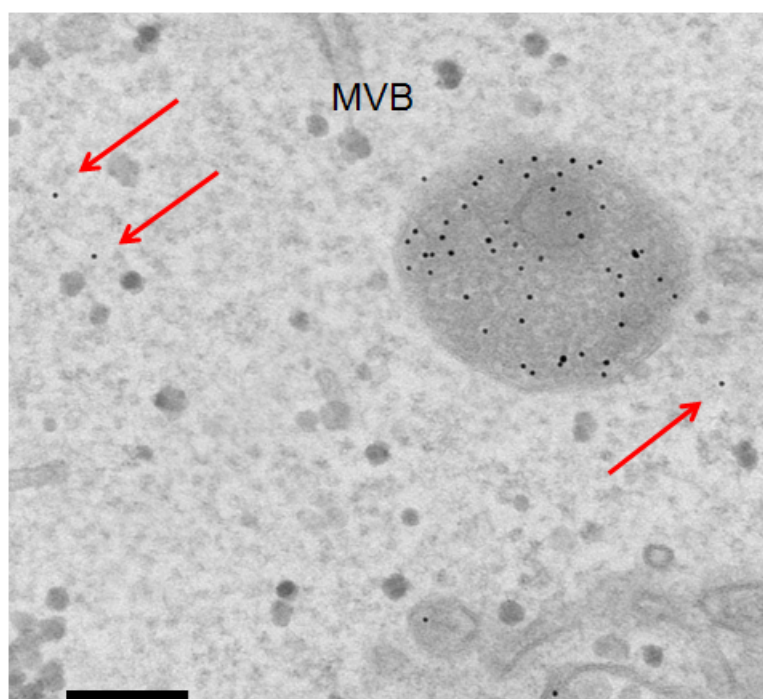
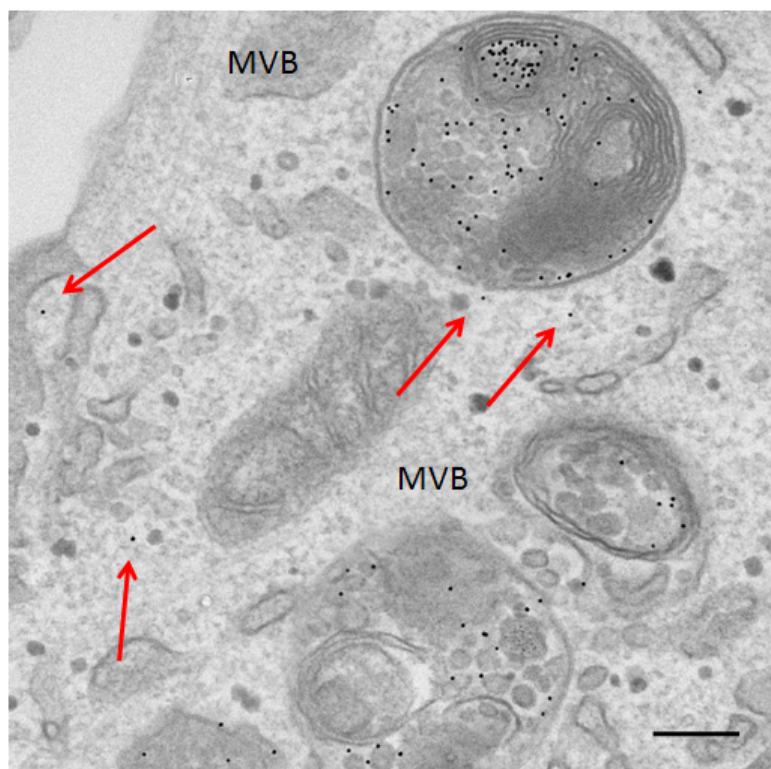
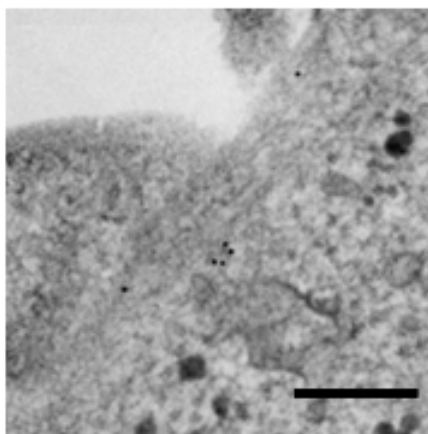
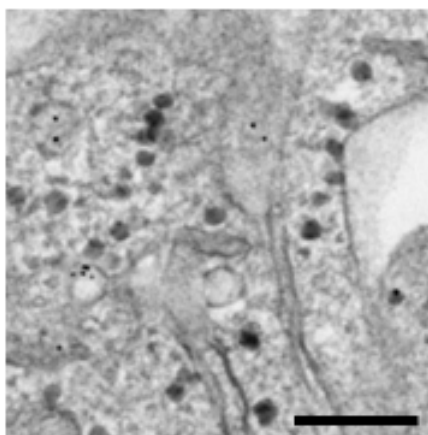


Fig. S2 TEM images of HeLa cells after incubation for 3hrs in the presence of C2-Ester-AuNPs. Red arrows indicate nanoparticles within the cytosol. Scale bar: 200 nm. Red arrows indicate AuNPs in the cytosol.

5 min



30 min



60 min

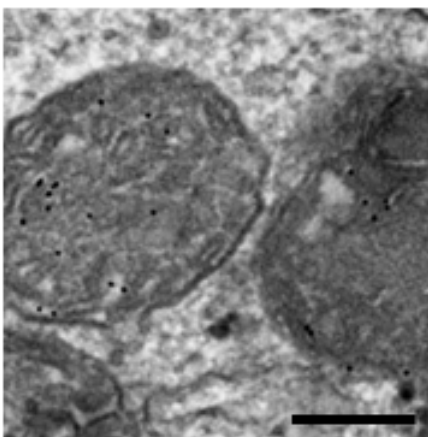


Fig. S3 Time courses of TEM images of HeLa after addition of C2-Ester-AuNPs. Scale bar: 100 nm.

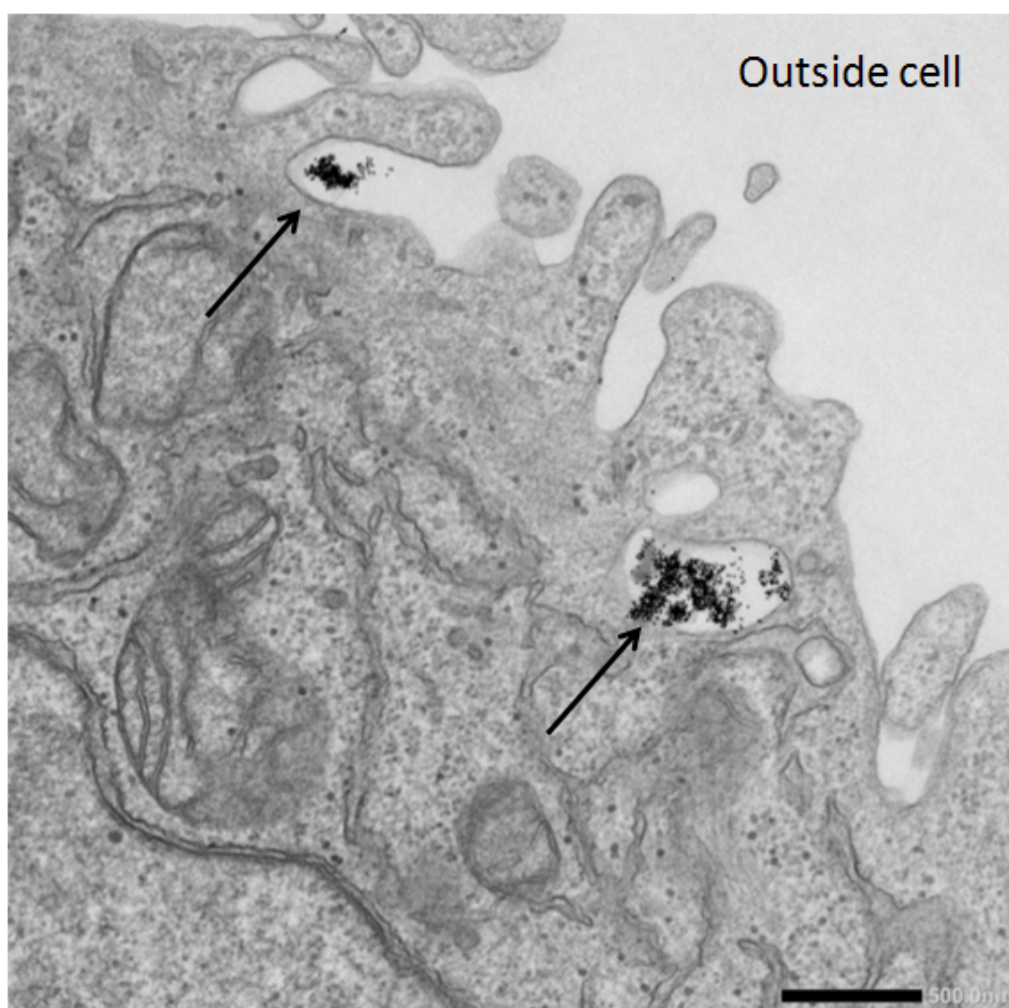


Fig. S4 TEM images of HeLa cells after incubation for 3hrs in the presence of C4-Ester-AuNPs. Scale bar: 500 nm. Scale bar: 500 nm. Black arrows show the aggregates of C4-Ester-AuNPs.

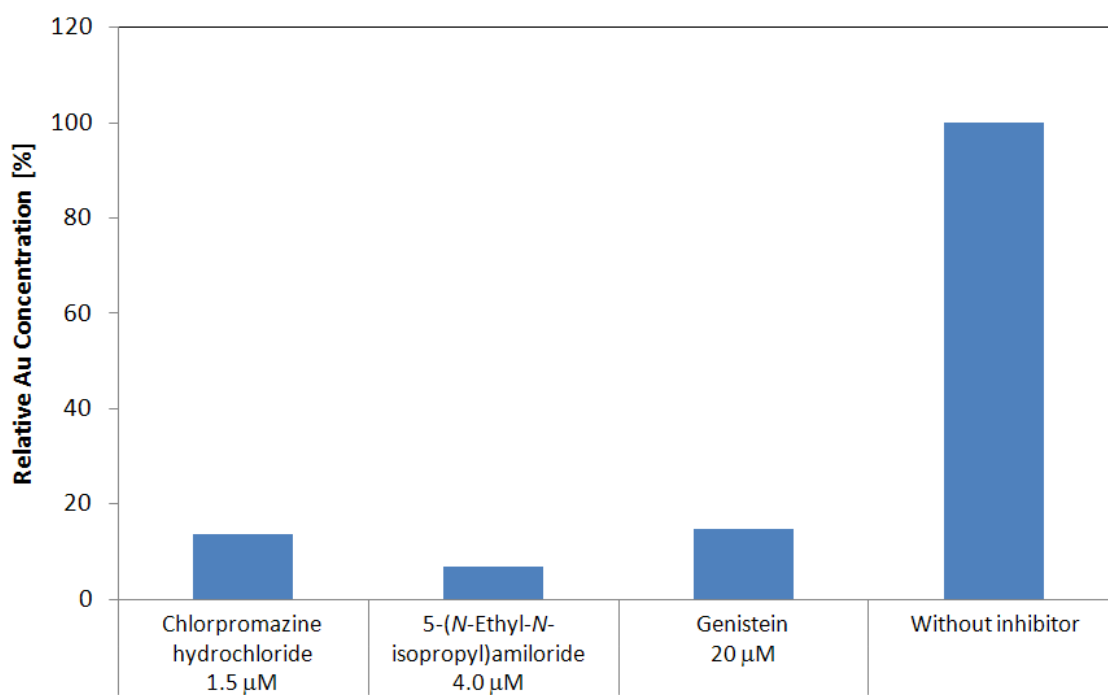


Fig. S5 The relative Au concentration in the presence of endocytosis inhibitors determined by ICP. The values are percentages of the negative control (Without Inhibitor) normalized as 100%.

References

- 1 S. Sekiguchi, K. Niikura, Y. Matsuo and K. Ijio, *Langmuir*, 2012, **28**, 5503.
- 2 E. Oh, J. B. Delehanty, K. E. Sapsford, K. Susumu, R. Goswami, J. B. Blanco-Canosa, P. E. Dawson, J. Granek, M. Shoff, Q. Zhang, P. L. Goering, A. Huston and I. L. Medintz, *ACS Nano*, 2011, **5**, 6434.
- 3 E. A. Untener, K. K. Comfort, E. I. Maurer, C. M. Grabinski, D. A. Comfort and S. M. Hussain, *ACS Appl. Mater. Interfaces*, 2013, **5**, 8366.