Supporting Information

Entrapment of Hydrophobic Drugs in Nanoparticle Monolayers with Efficient Release into Cancer Cell

Chae Kyu Kim,[†] Partha Ghosh,[†] Chiara Pagliuca,[‡] Zheng-Jiang Zhu,[†]
Stefano Menichetti,[‡] and Vincent M. Rotello[†,*]

[†]*Department of Chemistry, University of Massachusetts at Amherst, USA, 01003,*

[‡]*Dipartimento di Chimica Organica 'U. Schiff'Università di Firenze, Fiorentino, Italy, 50019*

To whom correspondence should be addressed, E-mail: rotello@chem.umass.edu

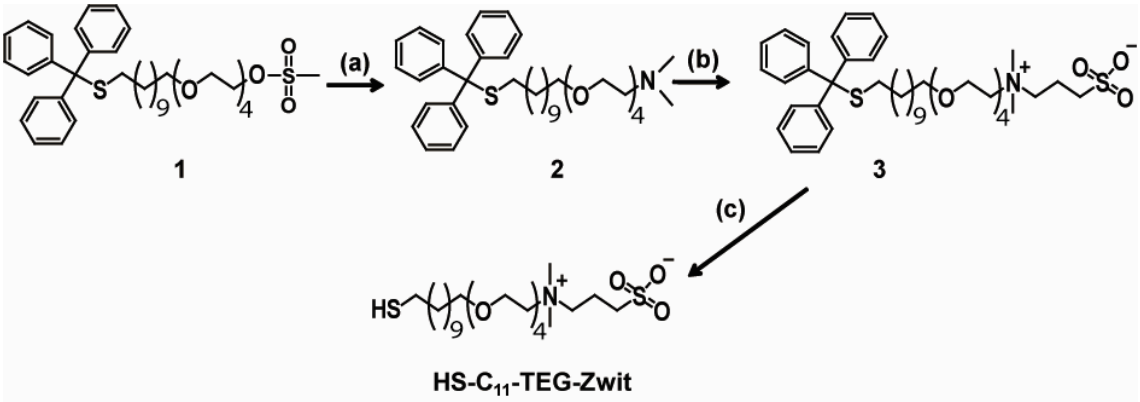
Materials and Instruments

Dichloromethane (DCM) used after distillation under $CaCl_2$ and other chemicals from Aldrich were used as received. Dimethylamine, 1,3-Propanesulfone, trifluroacetic acid (**TFA**), triisopropylsilane (**TIPS**), and tamoxifen (**TAF**) were purchased from Aldrich. 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**Bodipy**), _-lapachone (**LAP**), and **AuNPTTMA** were prepared by the literature procedures.[1-3] Theramanox® coverslips, uranyl acetate, osmium tetroxide, glutaraldehyde, lead citrate, epoxy embedding kit (low viscosity, as reported by Dr. Spurr) and 300 mesh copper grids with carbon film were purchased from Electron Microscopy Sciences.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 at 400 and 100 MHz, respectively. ESI-MS spectra were obtained using JEOL MStation JMS700. UV-vis spectra were recorded on Hewlett-Packard 8452A spectrophotometer. Photoluminescence spectrum was measured using Photon Technology International

fluorescence spectrometer. Dynamic light scattering (DLS) was measured by Zetasizer Nano ZS. Fixed cell sections for TEM were cut by using Reichert Ultracut E Ultramicrotome and imaged using JEOL 100S electron microscopy. Cell viability using Alarmar blue assay was assessed by SpectraMax M5 microplate spectrophotometer and plotted by Origin 8. Fluorescence images was obtained using Zeiss LSM510 meta confocal microscope.

Synthesis of HS-C11-TEG-Zwit



Scheme 1. Synthesis of **HS-C_{11}-TEG-Zwit**. (a) Dimethylamine, DCM, RT, overnight (b) 1,3-Propanesulfone, acetone, RT, overnight, (c) **TFA**, **TIPS**, RT, 4h

Compound **2**

Compound **1**[3] (1 g, 1.4 mmol) was added to a solution of dimethylamine (0.6 g, 14 mmol in dichloromethane (20 mL) and the reaction mixture was stirred at room temperature for overnight. The reaction mixture was poured into a mixture of dichloromethane and distilled water. Organic layer was separated and concentrated at reduced pressure. The crud product was purified by column chromatography over silica gel using hexane/ethyl acetate (1:4, v/v) as an eluent. Sovent was removed in vacuo to afford compound **2** as colorless oil (Yield 0.6 g, 65 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 6H, *Ph*-), 7.27 (m, 6H, *Ph*-), 7.20 (m, 3H, *Ph*-), 3.65-3.56 (m, 14H, -C*H_2*-*TEG*-

), 3.44 (t, J = 6.8 Hz, 2H -OC*H*$_2$-CH$_2$-N(CH$_3$)$_2$), 2.53 (t, J = 5.8 Hz, 2H, -OCH$_2$-C*H*$_2$-N(CH$_3$)$_2$), 2.84 (s, 6H, -N(C*H*$_3$)$_2$), 2.14 (t, J = 7.3 Hz, 2H, -S-C*H*$_2$-), 1.40 (m, 18H, -S-CH$_2$-(C*H*$_2$)$_9$-); ^{13}C NMR (100.64 MHz, CDCl$_3$) δ 26.10, 28.60, 29.02, 29.19, 29.41, 29.48, 29.56, 29.65, 32.04, 45.81, 58.77, 66.36, 69.23, 70.07, 70.40, 70.61, 71.55, 126.49, 127.79, 129.62, 145.10.

Compound **3**

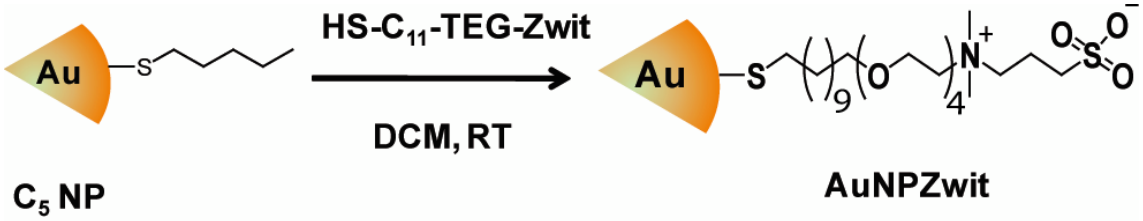
A anhydrous acetone solution (20 mL) of compound **2** (0.5 g, 0.77 mmol) and 1,3-propanesultone (0.14 g, 1.15 mmol) in anhydrous acetone was stirred at room temperature for overnight. The reaction mixture was filtered, and the resulting solid was washed with ethyl acetate/hexane (1:4, v/v) and dried in vacuum to afford compound **3** as a white solid (Yield 0.5 g, 84 %). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.41 (m, 6H, *Ph*-), 7.27 (m, 6H, *Ph*-), 7.20 (m, 3H, *Ph*-), 3.94 (m, 2H, -OC*H*$_2$-CH$_2$-N(CH$_3$)$_2$-), 3.78 (m, 2H, -OCH$_2$-C*H*$_2$-N(CH$_3$)$_2$-), 3.71-3.56 (m, 14H, -C*H*$_2$-*TEG*- and -CH$_2$-CH$_2$-C*H*$_2$-sulfonate), 3.42 (t, J = 6.8 Hz, 2H, -OC*H*$_2$-CH$_2$-N(CH$_3$)$_2$-), 3.23 (s, 6H, -N(C*H*$_3$)$_2$-), 2.88 (t, J = 6.7 Hz, 2H, -C*H*$_2$-CH$_2$-CH$_2$-sulfonate), 2.26 (m, 2H, -CH$_2$-C*H*$_2$-CH$_2$-sulfonate), 2.12 (t, J = 7.3 Hz, 2H, -S-C*H*$_2$-), 1.40 (m, 18H, -S-CH$_2$-(C*H*$_2$)$_9$-); MS (ESI-MS) calcd for C$_{43}$H$_{66}$NO$_7$S$_2$ 773.12, found 794.6 [M+Na]$^+$

HS-C$_{11}$-TEG-Zwit

A dichloromethane solution of compound **3** (0.6 g, 0.77 mmol), trifluoroacetic acid (1.78 g, 16 mmol), and triisopropylsilane (0.18 g, 1.1mmol) was stirred at room temperature for 6 h under argon. After removal of the solvent at reduced pressure, the residue was purified by washing with diethyl ether (20 ml x 5). After drying residue

under high vacuum, white solid of product was obtained. (Yield 0.36 g,87 %) ^{1}H NMR (400 MHz, $CDCl_3$) 3.91 (m, 2H, -OC*H*$_2$-CH$_2$-N(CH$_3$)$_2$-), 3.79-3.52 (m, 16H, OCH$_2$-C*H*$_2$-N(CH$_3$)$_2$-, -C*H*$_2$-*TEG*-, and -CH$_2$-CH$_2$-C*H*$_2$-sulfonate), 3.42 (t, J = 6.8 Hz, 2H, -OC*H*$_2$-CH$_2$-N(CH$_3$)$_2$-), 3.41 (t, J = 6.8 Hz, 2H, -OC*H*$_2$-CH$_2$-N(CH$_3$)$_2$-), 3.04 (s, 6H, -N(C*H*$_3$)$_2$-), 2.90 (brs, 2H, -C*H*$_2$-CH$_2$-CH$_2$-sulfonate), 2.49 (q, J=7.2 Hz, 2H, HS-C*H*$_2$-), 2.26 (brs, 2H, -CH$_2$-C*H*$_2$-CH$_2$-sulfonate), 1.40 (m, 18H, -S-CH$_2$-(C*H*$_2$)$_9$-); MS (ESI-MS) calcd for $C_{24}H_{52}NO_7S_2$ 530.32, found 552.4 $[M+Na]^+$.

Preparation of AuNPZwit



Scheme 2. Synthetic pathway of **AuNPZwit**

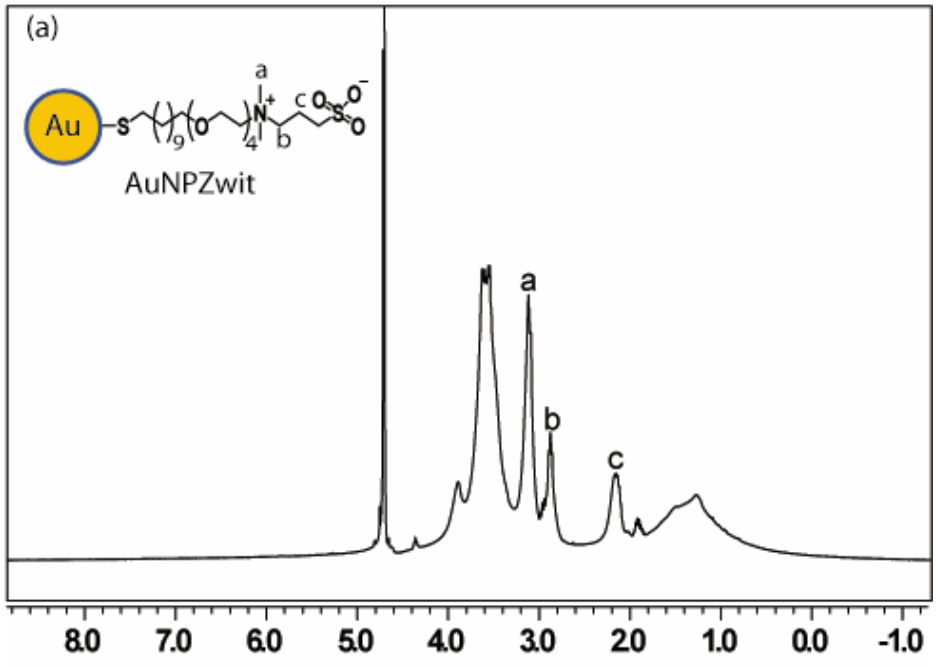
Gold nanoparticles, **C$_5$ NP** were prepared by following the Brust-Schiffrin two-phase method using 1-pentanethiol as capping ligands. To fabricate AuNPZwit via ligand place exchange reaction, 30 mg of C_5 NP were mixed with 100 mg of **HS-C$_{11}$-TEG-Zwit** in dichloromethane (10 mL). The solution was stirred at room temperature for 24 hours. Solvent was removed by rotary evaporator and the nanoparticles were washed with diethyl ether (20 mL x 5). The nanoparticles were further purified by dialysis in MiliQ water using SnakeSkin pleated dialysis tubing (10,000 MWCO) for 1days. ^{1}H NMR spectrum is shown in Figure S1 (a).

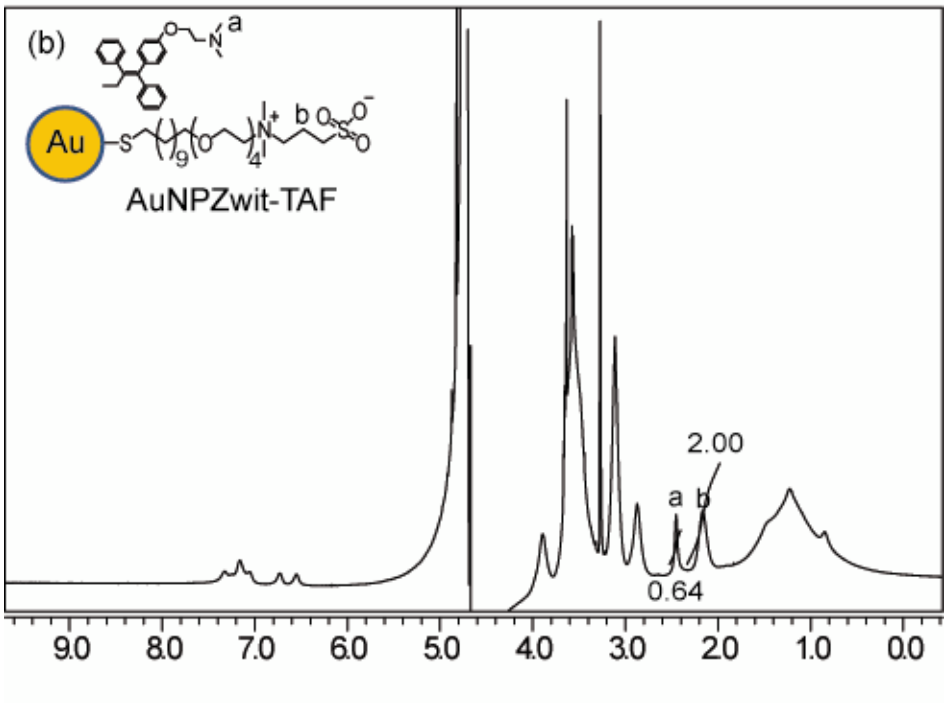
Preparation and characterization of AuNPZwit complexes

In order to prepare **AuNPZwit** complexes, solvent displacement method was carried out using water and acetone. An acetone solution of each guest compound (10 mg) and aqueous solution of **AuNPZwit** (80 μM) were well mixed. Acetone was then removed by rotary evaporator or slow evaporation at room temperature. During the evaporation of acetone, some guest compounds were entrapped in interior of **AuNPZwit** due to hydrophobic interaction and the rest of guest compounds were precipitated out. The mixture was then filtrated using filter (0.2 μm pore) to remove the precipitate. Any residue of free guest compounds in the aqueous solution may affect to result of cytotoxicity and fluorescence related experiments. For further purification, the **AuNPZwit** complexes solution was washed with distilled water and centrifuged with Amicon Ultra-4 tube (10,000 MWCO) several times (2 mL x ~5 times) until no absorbance of guest molecules in filtrate was detected by spectrophotometer. **AuNPTTMA-Bodipy** was also prepared by the same procedure.

AuNPZwit complexes conjugates were characterized by nuclear magnetic resonance (NMR). ^{1}H NMR spectrum of **AuNPZwit** complexes are shown in Figure S1 (b)-(d). The broadened ^{1}H NMR Peak of the guest compounds indicates that guest compounds were entrapped in hydrophobic pocket of **AuNPZwit**. It is also noted that the H^{1} NMR peak of guest compounds (**TAF** and **Bodipy**) in D_2O was not present until **AuNPZwit** complex was formed. The stoichiometry of the **AuNPZwit** complexes was calculated by measuring integral of **AuNPZwit** and guest compounds. From TGA and TEM analysis, it is estimated that 100 ligands are placed on each **AuNPZwit** (*vide infra*).[4] Around 10~11 **TAF**s were entrapped in **AuNPZwit**. The number of entrapped guest compounds for **AuNPZwit-LAP** and **AuNPZwit-Bodipy** were calculated from the

NaCN-induced decomposition experiment due to weak ^{1}H NMR signal of guest compound.





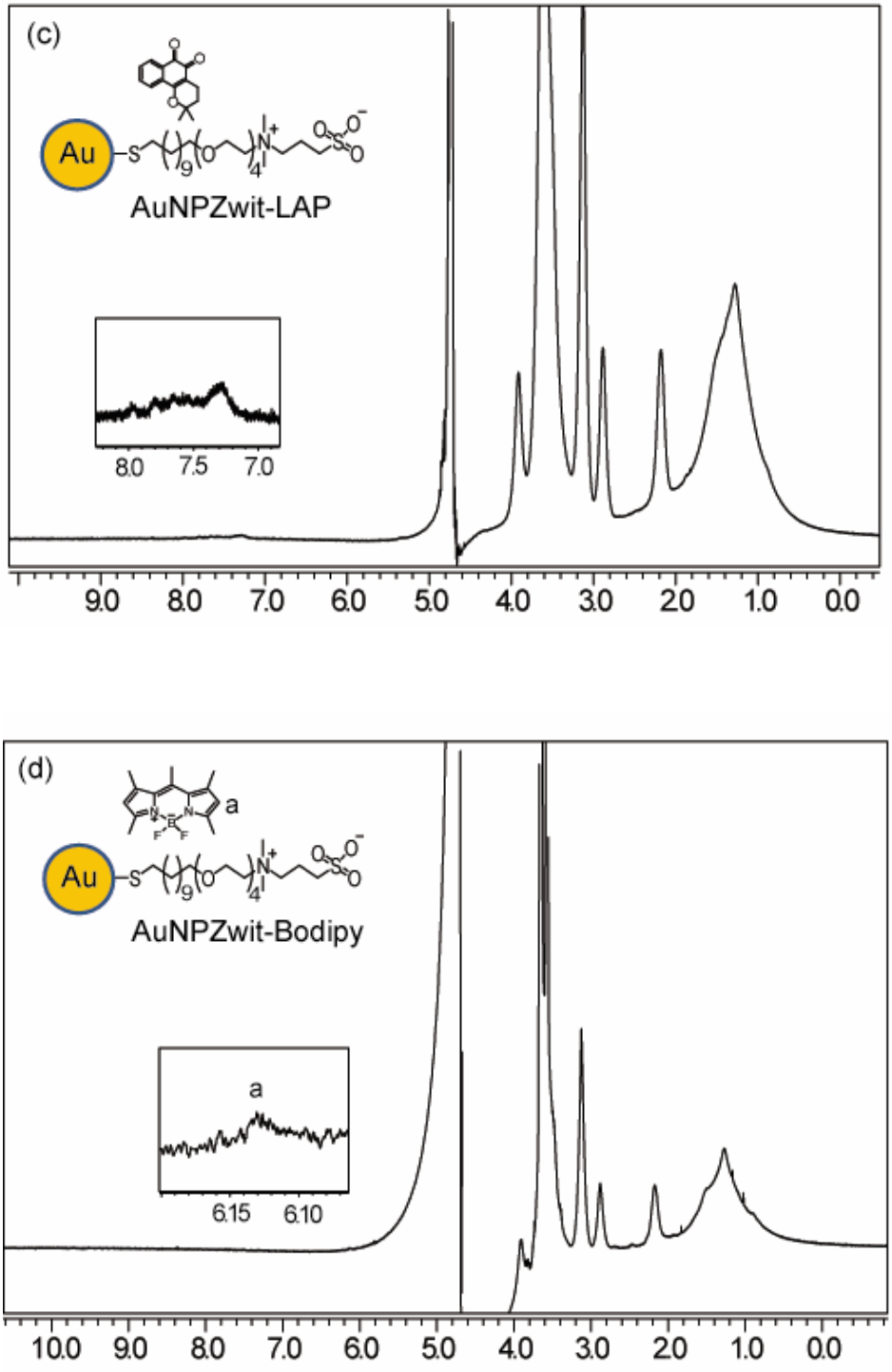


Figure S1. ^{1}H NMR spectrum of AuNPZwit and AuNPZwit complexes (a) AuNPZwit (b) AuNPZwit-TAF, (c) AuNPZwti-LAP, (d) AuNPZwit-Bodipy.

NaCN-induced decomposition of AuNPZwit complexes

To 1 ml of a solution of the desired **AuNPZwit** complexes in THF (final concentration of 0.5 ~ 4 μM) was added 1 mL of an aqueous NaCN solution (final concentration 0.1 M) followed by briefly agitating the mixture. After 2 h, plasomon absorption band of AuNP at 520 nm was completely decayed while absorbance of guest compounds remained. Concentration of guest compounds was then calculated based on Beer-Lambert law (λ_{max} = 496 nm for **Bodipy**, λ_{max} = 290 nm for **LAP**, and λ_{max} = 280 nm for **TAF**). By comparing with the concentration of **AuNPZwit** before etching, the number of guest compounds entrapped in **AuNPZwit** was estimated. The molar extinction coefficient of free guest compounds in THF/H_2O (1:1) was obtained for this estimation (Bodipy ε = 7.07 x 10^3 at 496 nm, LAP ε = 1.2 x 10^4 at 290 nm, and TAF ε = 1.1 x 10^4 at 280 nm).

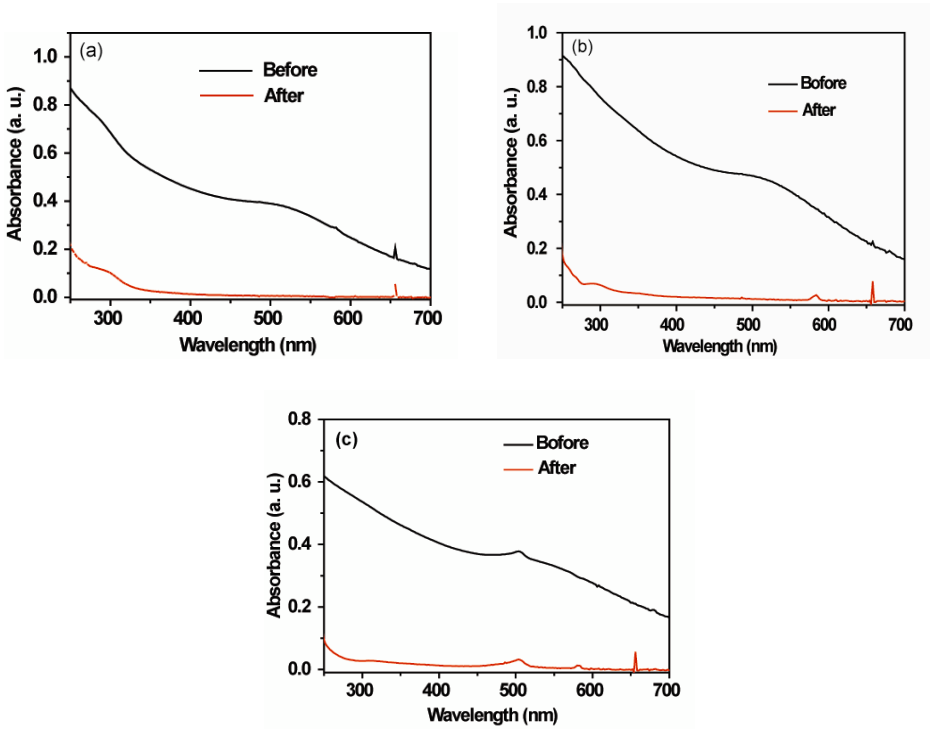


Figure S2. Uv-vis spectrum of AuNPZwit complexes before and after NaCN-induced decomposition experiment. (a) AuNPZwit-TAF, (b) AuNPZwit-LAP, and (c) AuNPZwit-Bodipy.

Stability test of AuNPZwit complexes in PBS

1 mL of **AuNPZwit** complexes (5 μM) in PBS was centrifuged with Amicon Ultra-4 tube (10,000 MWCO) at 8,000 rpm for 30 min. Stability of **AuNPZwit** complexes was examined by measuring the absorbance of the filtrate. Any absorbance of guest compound in filtrate was not observed at least for one month.

Partition Coefficient logP

The partition coefficient, logP was obtained by UV-Vis of the octanol and water phases according to our previous study. [5] The logP for the hydrophobic compounds as followings: LAP: 2.68, TAF: 3.64, and Bodipy: 4.0.

Cell culture

MCF-7 cells were grown in a cell culture flask in low glucose Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (FBS) and 1 % of antibiotics at 37 °C in a humidified atmosphere of 5 % CO_2.

Cytotoxicity test of AuNPZwit complexes

MCF-7 cells were seeded at 20,000 cells in 0.2 ml per well in 96-well plates 24 h prior to the experiment. The old medium were replaced by different concentrations of **TAF**, **LAP**, **AuNPZwit-TAF**, **AuNPZwit-LAP**, and **AuNPZwit** in serum containing medium and incubated for 24 h at 37 °C in a humidified atmosphere of 5 % CO_2. The cells were then completely washed with PBS buffer three times and 10 % Alamar blue of serum containing medium was added to each well and further incubated at 37 ºC for 3 h. The cell viability was then determined by measuring the fluorescence intensity at 570 nm using a SpectraMax M5 microplate spectrophotometer. Curves were fitted by DoseRep function in Origin 8.

Confocal laser scanning microscopy

MCF-7 cells were seeded in 35 mm Petri dish (Mat Tek Corporation, MA) at 50,000 cells in 1 mL of serum containing medium 24 h prior to the experiment. The medium was replaced by serum containing medium and **AuNPZwit-Bodipy** to a final concentration of 1 μM and were incubated for 2 h. Cells were washed with PBS three times before being imaged under a confocal microscope (Zeiss LSM510 meta confocal microscope equipped with an argon-HeNe laser, λ_{ex} = 488 nm).

ICP-MS Instrumentation

All ICP-MS measurements were performed on a Perkin Elmer Elan 6100. Operating conditions of the ICP-MS are listed below: RF power: 1200 W; plasma Ar flow rate: 15 L/min; nebulizer Ar flow rate: 0.96 L/min; isotopes monitored: ^{197}Au; dwell time: 50 ms; nebulizer: cross flow; spray chamber: Scott.

ICP-MS sample preparation and measurements[6]

AuNPZwit-Boidpy and **AuNPTTMA-Bodipy** (1 μM) were incubated with pre seeded MCF-7 cell line in 24 well plates (200,000 cells/well) for 4h and 24 h (Figure S3). After incubation and lysing the cells, the resulting cell lysate was digested overnight using 3 mL of HNO_3 and 1 mL of H_2O_2. On the next day, 3 mL of aqua regia, **which is highly corrosive and must be use with extreme caution**, was added, and then the sample was allowed to react for another 2-3 h. The sample solution was then diluted to 100 mL with de-ionized water, and aqua regia. The final AuNP sample solution contained 5% aqua regia. The AuNPs sample solution was measured by ICP-MS under the operating conditions described above. Cell uptake experiments with each AuNP were repeated 3 times, and each replicate was measured 5 times by ICP-MS. A series of gold standard solutions (20, 10, 5, 2, 1, 0.5, 0.2, 0 ppb) were prepared before

each experiment. Each gold standard solution contained 5% aqua regia. Each standard solution was also measured 5 times by ICP-MS using the operating conditions described above. The resulting calibration line was used to determine the gold amount in taken up by the cells in each sample. A ~100 ppm solution of dithiothreitol was used to wash the instrument between analyses to facilitate gold removal.

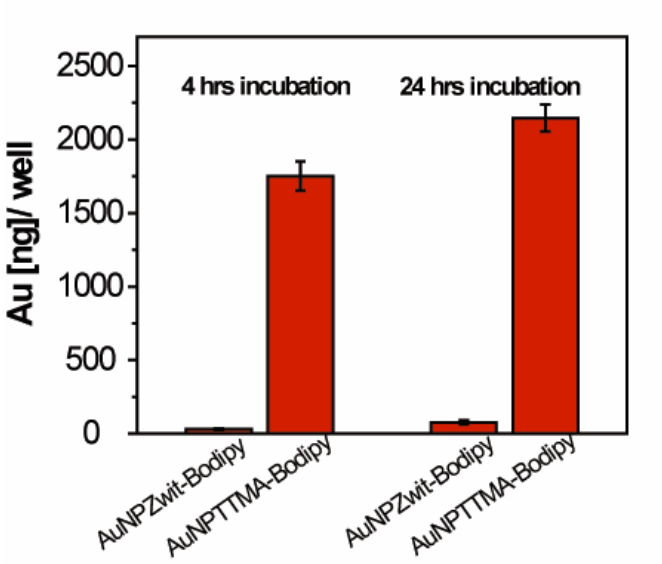


Figure S3. ICP-MS measurement of AuNPZwit-Boidpy and AuNPTTMA-Bodipy for 4 and 24 h.

TEM preparation for AuNPZwit complexes and fixed cell treated with AuNPs

MCF-7 cells were seeded and incubated on 15 mm diameter Theramanox® coverslips (Nalge Nunc International, NY) placed in 24 well plates at amount of 100,000 cells in 1 ml of serum containing medium for 24 h prior to the experiment. The medium was replaced by 1 ml of serum containing medium and **AuNPZwit-Bodipy** or **AuNPTTMA** to a final concentration of 1 μM and incubated for 4 h.[7] The medium containing the gold nanoparticles not taken up by the cells was discarded, and the cells were completely washed with PBS buffer three times. The cells were then fixed in 2 % glutaraldehyde with 3.75 % sucrose in 0.1 M sodium phosphate buffer (pH 7.0) for 30 min and then

washed with 0.1 M PBS containing 3.75% sucrose three times over 30 min. They were postfixed in 1 % osmium tetroxide with 5 % sucrose in 0.05 M sodium phosphate buffer solution (pH 7.0) for 1 hr and the rinsed with distilled water three times. They were dehydrated in a graded series of acetone (10 % step), and embedded in epoxy resin. The resin was polymerized at 60 °C for 48 h. Ultrathin sections (50_70 nm) obtained with a Reichert Ultracut E Ultramicrotome were stained with 2% aqueous uranyl acetate and 2% aqueous lead citrate and imaged under a JEOL 100S electron microscopy.

TEM samples of **AuNPZwit** and **AuNPZwit** complexes were prepared by placing one drop of the desired AuNP solution (3 μM) on to a 300-mesh Cu grid coated with carbon film. These samples were analyzed and photographed using the same instrument. TEM image of **AuNPZwit** is shown in Figure S4 (a). The average diameter of Au core is 2.5 ± 0.4 nm.

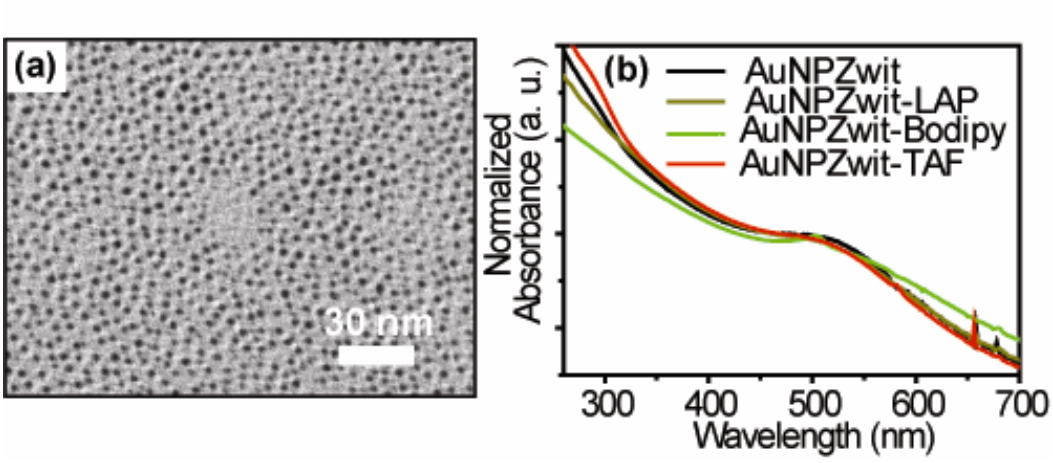


Figure S4. (a) TEM images of AuNPZwit. (b) UV-vis spectrum of AuNPZwit and AuNPZwit complexes. No red shift of plasmon absorption band was observed.

Thermogravimetric analysis (TGA)

TGA was performed using a TGA 2950 high-resolution thermo-gravimetric analyzer (TA Instruments, Inc., New Castle, DE), which was equipped with an open platinum pan and an automatically programmed temperature controller. The TGA data were obtained as follows: about 3.0 mg of **AuNPZwit** was placed in the TGA pan and heated in a nitrogen atmosphere at a rate of 10 °C / min up to 600 °C. TGA curve of **AuNPZwit** is shown in Figure S5. The TGA data showed that the weight percentage of organic ligands and Au core of **AuNPZwit** is 35 % and 65 %, respectively. Accordingly, the average number of the lignads is estimated as ~100,[4] which is comparable to the literature.[8]

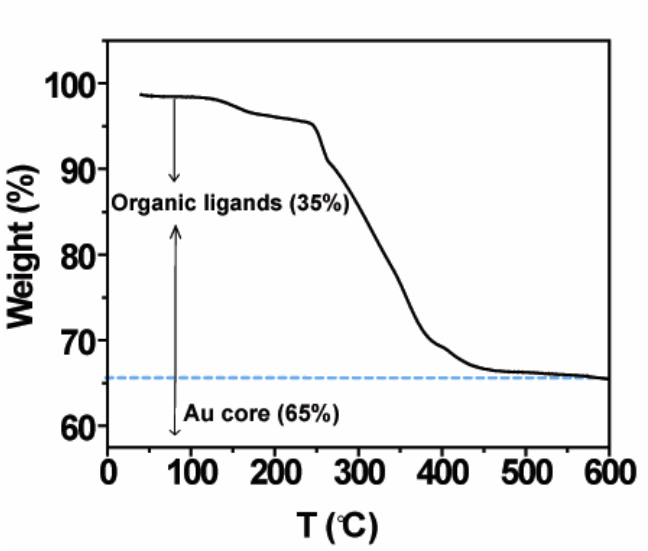


Figure S5. Thermal gravimetric analysis (TGA) data of AuNPZwit.

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