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Determination of the Intracellular Stability of Gold Nanoparticle Monolayers using Mass Spectrometry

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Abstract

Monolayer stability of core-shell nanoparticles is a key determinant of their utility in biological studies such as imaging and drug delivery. Intracellular thiols (e.g., cysteine, cysteamine, and glutathione) can trigger the release of thiolate-bound monolayers from nanoparticles, a favorable outcome for controllable drug release applications but an unfavorable outcome for imaging agents. Here, we describe a method to quantify the monolayer release of gold nanoparticles (AuNPs) in living cells using parallel measurements by laser desorption/ionization (LDI) and inductively coupled plasma (ICP) mass spectrometry. This combination of methods is tested using AuNPs with structural features known to influence monolayer stability and on cells types with varying concentrations of glutathione. Based on our results, we predict that this approach should help efforts to engineer nanoparticle surface monolayers with tunable stability, providing stable platforms for imaging agents and controlled release of therapeutic monolayer payloads.

Keywords

Mass spectrometry; gold nanoparticle; monolayer release; glutathione; drug delivery

Introduction

Monolayer-protected nanoparticles (NPs) consist of an inorganic core and an organic monolayer shell.¹ The wide variety of core materials and monolayer tunability make NPs promising materials for a broad range of biological and biomedical applications.² Monolayer-protected gold NPs (AuNPs) have been widely used in drug delivery and therapeutics, bioimaging and disease diagnostics.^{2a,3} The introduction of organic monolayers onto AuNP surface stabilizes them against aggregation and agglomeration.⁴ Moreover, monolayer engineering provides control over the physical and chemical properties of the particle surface, enabling selective interactions with biomolecules⁵ and biological systems.⁶ For example, recent studies have shown that AuNP with the appropriate surface monolayers can disrupt protein-protein interactions,⁷ regulate DNA transcription,^{5c} and control NP cellular uptake⁸ and toxicity.⁹

The structure of the monolayer is of crucial importance for AuNP applications. However, exchange and release of ligands from the AuNP surface have to be taken into consideration when using AuNPs in biological and biomedical applications. Biogenic thiols such as

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Supporting Information. Description of the synthesis of the AuNP ligands, the calibration curves, additional LDI-MS data, and measurements of the AuNP stability under different pH conditions are found in the supporting information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

cysteine, cysteamine and glutathione (GSH), with intracellular concentrations as high as tens of mM,¹⁰ provide the potential for exchanging and/or releasing alkanethiolate-bound monolayers of AuNPs (Figure 1).¹¹ This monolayer release is a “double-edged sword” for the application of AuNPs. On one hand, a tunable labile monolayer is required to control the release of therapeutic monolayer payloads in drug delivery and therapy.^{11–12} In contrast, NPs (e.g., AuNPs and quantum dots (QDs)) used as bioimaging agents require monolayer stability to preserve their optical imaging properties and prevent their aggregation and degradation.¹³ Moreover, the transformation of NP surfaces caused by the release of the protective monolayer may also change the *in vivo* fate (biodistribution, uptake, and excretion) and toxicity of the NPs.¹⁴ Therefore, engineering the monolayer to control monolayer stability is important to fully realize the potential of NPs in biological applications. Moreover, accurate analytical tools and methods for determining the stability of these particles in biological systems are required.

Several techniques have been used to characterize monolayer exchange and release of AuNPs, including nuclear magnetic resonance (NMR)¹⁵ and fluorescence spectroscopy.^{12,16} These studies have shown that the dynamics of monolayer displacement depend on the structures of the alkanethiol monolayers and incoming exchange thiols.^{12,16b,17} However, NMR is not applicable for measuring AuNP monolayer release in live cells and *in vivo*, while fluorescence spectroscopy can only monitor the intracellular release of fluorophore-labeled monolayers, requiring a label and making quantification challenging.¹¹

For AuNPs having intracellular and/or *in vivo* diagnostic and therapeutic potential, correlations between monolayer structure and biogenic thiol-mediated monolayer release have not been fully explored due to inadequate measurement tools. For example, Mattoussi *et al.* showed that dithiol-capped AuNP are more resistant to dithiothreitol (DTT) displacement than monothiol capped NPs *in vitro*, but no evidence has been provided that these dithiol-capped AuNPs are also more stable in cells.^{13c,18} Here, we describe the use of a laser desorption/ionization mass spectrometry (LDI-MS) method to quantitatively measure AuNP monolayer stability in a label-free fashion. Monolayer ions are generated during the LDI process and act as “mass barcodes” that can be used for monolayer quantitation.^{5a,8a,19} We then combine LDI-MS with inductively coupled plasma-MS (ICP-MS) to quantitatively measure AuNP monolayer stability in living cells (Figure 2a). Using this combination of methods, we find a correlation between monolayer structure and AuNP stability in various cellular environments, demonstrating that controlled release or essentially complete stability can be obtained through monolayer choice.

Experimental Section

AuNP synthesis

The Brust-Schiffrin two-phase method²⁰ was used to synthesize pentanethiol capped AuNPs with core diameters around 2 nm (Figure S1 in Supporting Information). After that, ligand place-exchange was used to obtain the AuNPs shown in Figure 2b (see reference 8a and its Supporting Information for details). The syntheses of the ligands for AuNPs **1**, **2**, and **5** have been reported in our previous work.^{8a,9c,21} The syntheses of the ligands for AuNP **3** and AuNP **4** are described in the Supporting Information.

Cell culture and cellular uptake of AuNPs

All of the cell lines used in this study were purchased from and certified by ATCC (Manassas, VA, USA). Extra care was taken to avoid contaminating the different cell lines. Fortunately, the Hep G2 cells have very different cellular morphologies than HeLa cells, which helped us to easily differentiate the two cell lines. HeLa cells (30,000 cells/well) were

grown on a 24-well plate in low-glucose Dulbecco's modified Eagle's medium (DMEM; glucose (1.0 g L^{-1})) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 I.U./ml penicillin and 100 $\mu\text{g/ml}$ streptomycin). Hep G2 cells (30,000 cells/well) were grown in high-glucose DMEM (glucose (4.5 g L^{-1})) with 10% fetal bovine serum (FBS) and 1% antibiotics. Cultures were maintained at 37°C under constant saturated humidity with 5% CO_2 . After 24 h of plating, the cells were washed three times with cold phosphate buffer saline (PBS). Then, 500 μL of media containing 100 nM of AuNP were added. Following a certain culture time, the cells were washed five times with cold PBS to remove extra AuNPs and lysed for 10 min with a lysis buffer (400 μL ; Genlantis, USA). Each sample was prepared in six replicates, which were split with half being separately analyzed by ICP-MS and LDI-MS. To prepare cell lysates without AuNP, HeLa cells (30,000 cells/well) were grown on a 24-well plate for 24 h, and lysed with 400 μL of lysis buffer. To increase the intracellular concentration of GSH, media containing 10 mM glutathione ethyl ester (Sigma, USA) was incubated with HeLa cells for 1 h, and then replaced by media containing AuNP for a further 2 or 6 h incubation. The intracellular concentration of total GSH was measured using the glutathione detection kit (BioVision, USA). All cell experiments at each incubation time were repeated at least six times.

ICP-MS sample preparation and measurements

After cellular uptake, the lysed cells were digested with 0.5 mL of fresh *aqua regia* (*highly corrosive and must be use with extreme caution!*) for 1 h. The digested samples were mixed with an internal standard ^{103}Rh (10 ppb) and diluted into 10 mL with de-ionized water. 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 also contained 5% *aqua regia* and 10 ppb ^{103}Rh . The gold standard solutions and AuNP sample solutions were measured on a Perkin-Elmer Elan 6100 ICP mass spectrometer. The instrument was operated with a 1500 W RF power and the nebulizer Ar flow rate was optimized around 0.9 – 1.1 L/min. A ~100 ppm solution of dithiothreitol was used to wash the instrument between analyses to facilitate gold removal.

LDI-MS sample preparation and measurements

After cellular uptake, the lysed cells were mixed for 15 min with a certain amount (typically, 5 pmol) of AuNP 5 as the internal standard. Following centrifugation at 14,000 rpm (~20,000 g) for 15 min, the AuNPs taken up by cells and the internal standard were collected as part of precipitate and washed with 60% acetonitrile/40% water. Later, the samples were transferred onto a MALDI target for LDI-MS analysis without adding any organic matrix. External calibration curves were generated before sample analyses. Each AuNP with the internal standard at different ratios (0.1, 0.25, 0.5, 1, 2, 3, and 4) were spiked into cell lysate and vortexed for 15 min. The AuNP mixtures were collected by the centrifugation, washed, and analyzed by LDI-MS. The intensity ratios of the molecular ions for each AuNP and the internal standard AuNP were determined and plotted against the AuNP concentration ratios (Figure S2 in the Supporting Information) to generate a calibration curve. The AuNP amounts taken up by the cells were then determined by using of the internal standard and comparing to the calibration curve. All of the LDI-MS measurements were carried on a Bruker Autoflex III MALDI-TOF mass spectrometer (Billerica, MA, USA). All mass spectra were acquired in the reflectron mode and represent an average of 500 laser shots at a repetition frequency at 100 Hz. The accelerating voltage was set to 19 kV. The laser power was optimized in the range of 40–65 % for each sample. The Bruker software (flexAnalysis Version 3.3) was used for data analysis. Each sample was measured 10 times by LDI-MS.

Results and Discussion

Four different AuNPs (Figure 2b, AuNPs **1–4**) were used to investigate the ability of LDI/MS and ICP/MS to determine monolayer release, with AuNP **5** used as an internal standard for quantitation. The AuNPs used in this study feature the same 2-nm cores (Figure S1 in the Supporting Information, TEM imaging of AuNPs) but different surface monolayers (Figure 2b). We have designed these AuNP monolayers with four functional layers, including an anchor group, a hydrophobic alkane group, a tetra(ethylene glycol) (TEG) group, and a terminal functional group, that make AuNP stable, biocompatible,²² soluble in water, and chemically diverse.^{8a} The tetra(ethylene glycol) layer in the monolayer, which has proven to prevent nonspecific protein binding and avoid denaturation of the proteins, provides these AuNPs with good biocompatibility. While not the focus of the current studies, this nanoparticle design has shown to be biocompatible in mice and fish.^{6d,6e} As is clear from Figure 2b, the ligands on AuNP **1–4** have structural differences amongst these layers, and these differences influence their stability. For example, dithiol linking groups are known to provide greater stability than monothiol groups.^{13c,18} We have used these differences to explore the ability of our approach to measure monolayer release in different cellular environments, including cells with different intracellular glutathione concentrations.

To determine the intracellular stability of the AuNPs, the AuNPs were first delivered into the cells. In our previous work^{8a} and in the work by others,²³ it has been demonstrated that the cellular uptake of these cationic nanoparticles is most likely through an endocytosis mechanism. The positively-charged surfaces of AuNPs **1–4** were designed to facilitate the cellular uptake of these AuNPs and improve the desorption/ionization efficiency of the ligands during the LDI-MS measurements.^{8a} AuNP amounts taken up by cells were quantified using ICP-MS to measure total gold, then converted to particle amounts.²⁴ In a parallel experiment, the LDI-MS method was used to determine the monolayer amounts remaining on the AuNP surface. The difference between the AuNP amount determined by ICP-MS and that determined by LDI-MS then provides the amount of monolayer released from the AuNP (Figure 2a).

To test the combined LDI/ICP-MS approach, 100 nM of AuNP **1** was added to HeLa cell culture for varying time points, ranging from 1 to 24 hours. The longer time points (> 4 h) were chosen to allow us to focus on any ligand release from the nanoparticles once they were in the cells, rather than on uptake kinetics. After a given time, the cells were lysed, and the sample was split, with half being analyzed for Au using ICP-MS and the other half analyzed for the monolayer by LDI-MS. The uptake of AuNP as determined by ICP-MS increases with time and reaches a maximum after 9 h (Figure 3c [black curve]). To determine monolayer amount by LDI-MS measurement, the cells were lysed and AuNP **5** (5.0 pmol) was added as an internal standard into cell lysate, with the resulting lysate mixture immediately centrifuged. The AuNPs were then collected as part of the precipitate and subjected to LDI-MS analysis. In the LDI mass spectrum (Figure 3a and 3b), ions at m/z 422 and 464 that correspond to the molecular ions (M^+) of the intact alkanethiol monolayers of AuNP **1** and AuNP **5**, respectively, are observed. Fragment ions of the monolayers are also observed at lower m/z ratios; the identities of these fragment ions have been previously reported.^{8a,19} The ion intensity ratios of AuNP **1** relative to the internal standard AuNP **5** are 1.48 ± 0.06 and 1.88 ± 0.04 for 4 h and 24 h, respectively (Figure 3a and b). An external calibration curve (Figure S2a in the Supporting Information) was used to determine the AuNP **1** monolayer amount from this ratio (see Experimental Section). As a result, the LDI-MS reveals that the monolayer amounts on AuNP **1**, after normalizing to AuNP amount, were 6.4 ± 0.3 pmol and 8.3 ± 0.2 pmol at 4 h and 24 h, respectively. The LDI-MS results also increase over time (Figure 3c [red curve]). The curves obtained from the ICP-MS results (gold nanoparticle amount) and the LDI-MS results (monolayer amount) match quite

well (Figure 3c). Indeed, a t-test indicates that AuNP amounts at each time point are not statistically different ($P>0.05$). The conclusion from this experiment is that AuNP **1** has a very stable monolayer of ligands that is not released upon uptake into HeLa cells for up to 24 h (Figure 3d).

We further tested the LDI/ICP-MS approach using AuNPs with ligands that were expected to have different stability than AuNP **1**. AuNP **3**, for example, was expected to have a lower stability than AuNP **1** because of its shorter alkane chain,^{9d} while AuNP **4** was expected to have greater stability than AuNP **3** because of its dithiol anchoring group. The results for AuNPs **2** – **4** are summarized in Figure 4. From the data in Figure 4, it is clear that the LDI/ICP-MS results are as predicted. Shortening the alkane chain length decreases monolayer stability, while a dithiolate functional group attached to the Au core enhances stability relative to a monothiolate group.

For AuNP **3** (Figure 4b and e), the monolayer is quickly released with about 22% of it gone after only 2 h of incubation with HeLa cells. After 24 h, about 63% of its monolayer is released. This observation highlights the critical importance of the alkane chain on AuNP stabilization.^{9d} Presumably, the five-carbon alkane chain of AuNP **3** provides significantly less hydrophobic packing around the gold core than the 11-carbon chains of AuNP **1**, resulting in lower monolayer stability. Shortening the alkane chain length of the monolayer effectively switches the AuNP from stable to unstable against biogenic thiol displacement in cellular environments. Upon comparing the data for AuNP **3** and **4** (Figure 4b and c, respectively), it is clear that the lost stability of the shorter alkane chain can be recovered by using a dithiolate group to anchor the ligand to the gold core instead of a monothiolate group. No significant monolayer release is observed for AuNP **4** for incubation times up to 24 h (Figure 4f).

The lower stability of AuNP **2** is interesting. The tetra(ethylene glycol) group is designed to minimize nonspecific protein binding to the AuNP surface. Without the tetra(ethylene glycol) group, the hydrodynamic diameter of AuNP **2** is reduced to 7.1 nm compared to 9.0 nm for AuNP **1** based on dynamic light scattering (DLS) data. In order to prove that such a relatively short monolayer structure provides enough colloidal stability for AuNP **2** in cell culture media and under acidic conditions, like that found in endolysosomes after endocytosis, we incubated AuNP **2** both in cell culture media for up to 24 h at 37 °C (Figure S3 in the Supporting Information) and in phosphate buffers at pH 5, 7, and 9 (Figure S4 in the Supporting Information). The results for AuNP **2** (and indeed for all the AuNPs studied here) clearly demonstrate that the monolayer provides enough protection for AuNP **2** to allow it to maintain colloidal stability for up to 24 h in both media and low pH conditions. Once in the cells, however, the monolayer is gradually released so that only 45% of the ligands remain after 24 h (Figure 4a and d; LDI mass spectra in the Supporting Information (Figure S5)). Presumably, the absence of ethylene glycol functionality allows intracellular proteins to bind more avidly to AuNP **2**,²⁵ thereby disrupting the ordered structure of the monolayer and facilitating displacement by incoming thiols. Alternatively, the shorter ligand length of AuNP **2** might present less steric hindrance than AuNP **1** to incoming biogenic thiols, enabling more facile monolayer release.

To further verify the ability of the LDI/ICP-MS approach to accurately monitor AuNP stability in cells, the intracellular concentration of GSH was increased. The expectation is that increased intracellular concentrations of this thiol-containing peptide will decrease the stability of the AuNPs. Two different scenarios were explored. First, 10 mM glutathione monoethyl ester (GSH-OEt) was used to increase the GSH concentration by 150% (Figure S6 in the Supporting Information) in HeLa cells. As a neutral molecule, GSH-OEt efficiently penetrates the cell membrane and rapidly hydrolyzes to generate GSH, thus

offering a simple method to increase intracellular GSH concentrations.²⁶ Second, human liver cells (Hep G2) were also investigated. As shown in Figure S6 in the Supporting Information these cells contain 50% more GSH than HeLa cells. The LDI/ICP-MS approach was then used to determine AuNP monolayer stability in these different cellular environments containing higher intracellular GSH concentrations (Figure 5). Upon comparing the untreated HeLa cells with the treated HeLa and Hep G2 cells, it is clear that increased cellular concentrations of GSH lead to increased monolayer release (Figure 5). For example, the monolayer on AuNP **2** decreases from $70 \pm 10\%$ in the untreated HeLa cells to $41 \pm 5\%$ in the GSH-OEt treated cells and $52 \pm 11\%$ in the Hep G2 cells after 6 h of incubation. Similar behavior is also observed for AuNP **1** and **3**. In contrast, the dithiolate anchor group of AuNP **4** continues to provide excellent monolayer stability for up to 6 h in both GSH-OEt treated HeLa cells and Hep G2 cells. While we predict that GSH displaced the ligands on the AuNPs, we could find no evidence in the LDI mass spectrum for peaks corresponding to GSH ions. It is very likely that the positively-charged ligand ions on the AuNPs effectively suppress the ion signal from negatively-charged GSH. Future studies will attempt to improve the LDI-MS approach so that the displacing thiols can also be detected.

Conclusions

We report here the use of a label-free LDI/ICP-MS method for measuring the monolayer stability of AuNPs in cells. In this approach LDI-MS determines the monolayer amount on the AuNPs in cells, and ICP-MS determines the gold core amount. The difference between the two measurements provides a quantitative indication of the stability of the AuNPs in cells. While no other measurement approach currently exists to validate this approach, our results are internally consistent because changes to ligand structure and increases in intracellular GSH concentrations give the expected changes in AuNP stability. AuNPs with shorter alkanethiol chain lengths are found to have low monolayer stability as expected, and AuNPs with a dithiolate group attached to the gold core are found to have excellent monolayer stability as expected. Moreover, increasing the intracellular concentrations of a biogenic thiol (i.e. GSH) gives the expected decrease in AuNP monolayer stability. Overall, this LDI/ICP-MS method should be valuable in the development of NPs with tunable stability. Development of semi-stable monolayers, for example, can facilitate drug release in drug delivery agents. In contrast, NPs with highly stable monolayers would be useful as bioimaging and diagnostic agents. Our approach enables quantitative screening of intracellular stability, thereby providing important insight for the design of NPs for a variety of biomedical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. De M, Ghosh PS, Rotello VM. *Adv. Mater.* 2008; 20:4225–4241.
2. (a) Boisselier E, Astruc D. *Chem. Soc. Rev.* 2009; 38:1759–1782. [PubMed: 19587967] (b) Hao R, Xing R, Xu Z, Hou Y, Gao S, Sun S. *Adv. Mater.* 2010; 22:2729–2742. [PubMed: 20473985] (c) Smith AM, Duan HW, Mohs AM, Nie SM. *Adv. Drug Delivery. Rev.* 2008; 60:1226–1240.(d) Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. *Nat. Mater.* 2005; 4:435–446. [PubMed:

- 15928695] (e) Gill R, Zayats M, Willner I. *Angew. Chem. Int. Ed.* 2008; 47:7602–7625. (f) Liu W, Howarth M, Greytak AB, Zheng Y, Nocera DG, Ting AY, Bawendi MG. *J. Am. Chem. Soc.* 2008; 130:1274–1284. [PubMed: 18177042]
3. (a) Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. *Angew. Chem. Int. Ed.* 2010; 49:3280–3294. (b) Murphy CJ, Gole AM, Stone JW, Sisco PN, Alkilany AM, Goldsmith EC, Baxter SC. *Acc. Chem. Res.* 2008; 41:1721–1730. [PubMed: 18712884]
4. Templeton AC, Wuelfing MP, Murray RW. *Acc. Chem. Res.* 2000; 33:27–36. [PubMed: 10639073]
5. (a) Zhu ZJ, Rotello VM, Vachet RW. *Analyst.* 2009; 134:2183–2188. [PubMed: 19838403] (b) You CC, De M, Rotello VM. *Curr. Opin. Chem. Biol.* 2005; 9:639–646. [PubMed: 16226485] (c) McIntosh CM, Esposito EA, Boal AK, Simard JM, Martin CT, Rotello VM. *J. Am. Chem. Soc.* 2001; 123:7626–7629. [PubMed: 11480984]
6. (a) Leroueil P, Hong S, Mecke A, Baker J, Orr B, Banaszak Holl M. *Acc. Chem. Res.* 2007; 40:335–342. [PubMed: 17474708] (b) Verma A, Stellacci F. *Small.* 2010; 6:12–21. [PubMed: 19844908] (c) Lin J, Zhang H, Chen Z, Zheng Y. *ACS Nano.* 2010; 4:5421–5429. [PubMed: 20799717] (d) Zhu ZJ, Carboni R, Quercio MJ, Yan B, Miranda OR, Anderton DL, Arcaro KF, Rotello VM, Vachet RW. *Small.* 2010; 6:2261–2265. [PubMed: 20842664] (e) Arvizo RR, Miranda OR, Moyano DF, Walden CA, Giri K, Bhattacharya R, Robertson JD, Rotello VM, Reid JM, Mukherjee P. "Modulating Pharmacokinetics, Tumor Uptake and Biodistribution by Engineered Nanoparticles". *PLoS One.* 2011; 6:e24374. [PubMed: 21931696]
7. Bayraktar H, Ghosh PS, Rotello VM, Knapp MJ. *Chem. Commun.* 2006:1390–1392.
8. (a) Zhu ZJ, Ghosh PS, Miranda OR, Vachet RW, Rotello VM. *J. Am. Chem. Soc.* 2008; 130:14139–14143. [PubMed: 18826222] (b) Giljohann DA, Seferos DS, Patel PC, Millstone JE, Rosi NL, Mirkin CA. *Nano Lett.* 2007; 7:3818–3821. [PubMed: 17997588] (c) Liang M, Lin IC, Whittaker MR, Minchin RF, Monteiro MJ, Toth I. *ACS Nano.* 2009; 4:403–413. [PubMed: 19947583] (d) Nativo P, Prior IA, Brust M. *ACS Nano.* 2008; 2:1639–1644. [PubMed: 19206367]
9. (a) Lewinski N, Colvin V, Drezek R. *Small.* 2008; 4:26–49. [PubMed: 18165959] (b) Chompoosor A, Saha K, Ghosh PS, Macarthy DJ, Miranda OR, Zhu ZJ, Arcaro KF, Rotello VM. *Small.* 2010; 6:2246–2249. [PubMed: 20818619] (c) Goodman CM, McCusker CD, Yilmaz T, Rotello VM. *Bioconjugate Chem.* 2004; 15:897–900. (d) Harper SL, Carriere JL, Miller JM, Hutchison JE, Maddux BLS, Tanguay RL. *ACS Nano.* 2011; 5:4688–4697. [PubMed: 21609003]
10. (a) Krepela E, Prochazka J, Karova B. *Biol. Chem.* 1999; 380:541–551. [PubMed: 10384960] (b) Meister A, Anderson ME. *Annu. Rev. Biochem.* 1983; 52:711–760. [PubMed: 6137189] (c) Hassan SSM, Rechnitz GA. *Anal. Chem.* 1982; 54:1972–1976.
11. Hong R, Han G, Fernandez JM, Kim BJ, Forbes NS, Rotello VM. *J. Am. Chem. Soc.* 2006; 128:1078–1079. [PubMed: 16433515]
12. Chompoosor A, Han G, Rotello VM. *Bioconjugate Chem.* 2008; 19:1342–1345.
13. (a) Mei BC, Susumu K, Medintz IL, Delehanty JB, Mountziaris TJ, Mattoussi H. *J. Mater. Chem.* 2008; 18:4949–4958. (b) Mei BC, Susumu K, Medintz IL, Mattoussi H. *Nat. Protoc.* 2009; 4:412–423. [PubMed: 19265800] (c) Stewart MH, Susumu K, Mei BC, Medintz IL, Delehanty JB, Blanco-Canosa JB, Dawson PE, Mattoussi H. *J. Am. Chem. Soc.* 2010; 132:9804–9813. [PubMed: 20578776]
14. (a) Soo Choi H, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, Bawendi MG, Frangioni JV. *Nat. Biotechnol.* 2007; 25:1165–1170. [PubMed: 17891134] (b) Gao X, Cui Y, Levenson RM, Chung LWK, Nie S. *Nat. Biotechnol.* 2004; 22:969–976. [PubMed: 15258594]
15. (a) Hostetler MJ, Templeton AC, Murray RW. *Langmuir.* 1999; 15:3782–3789. (b) Donkers RL, Song Y, Murray RW. *Langmuir.* 2004; 20:4703–4707. [PubMed: 15969185]
16. (a) Montalti M, Prodi L, Zaccheroni N, Baxter R, Teobaldi G, Zerbetto F. *Langmuir.* 2003; 19:5172–5174. (b) Hong R, Fernandez JM, Nakade H, Arvizo R, Emrick T, Rotello VM. *Chem. Commun.* 2006:2347–2349.
17. Caragheorghieopol A, Chechik V. *Phys. Chem. Chem. Phys.* 2008; 10:5029–5041. [PubMed: 18701949]
18. Mei BC, Oh E, Susumu K, Farrell D, Mountziaris TJ, Mattoussi H. *Langmuir.* 2009; 25:10604–10611. [PubMed: 19588955]

19. Yan B, Zhu ZJ, Miranda OR, Chompoosor A, Rotello VM, Vachet RW. *Anal. Bioanal. Chem.* 2010; 396:1025–1035. [PubMed: 19911174]
20. Brust M, Walker M, Bethell D, Schiffrin DJ, Whyman R. *J. Chem. Soc. Chem. Commun.* 1994:801–802.
21. Phillips RL, Miranda OR, Mortenson DE, Subramani C, Rotello VM, Bunz UHF. *Soft Matter.* 2009; 5:607–612.
22. (a) You CC, De M, Rotello VM. *Org. Lett.* 2005; 7:5685–5688. [PubMed: 16321022] (b) Hong R, Fischer NO, Emrick T, Rotello VM. *Chem. Mater.* 2005; 17:4617–4621.
23. Al-Hajaj NA, Moquin A, Neibert KD, Soliman GM, Winnik FoM, Maysinger D. *ACS Nano.* 2011; 5:4909–4918. [PubMed: 21612298]
24. Chithrani BD, Ghazani AA, Chan WCW. *Nano Lett.* 2006; 6:662–668. [PubMed: 16608261]
25. Hong R, Fischer NO, Verma A, Goodman CM, Emrick T, Rotello VM. *J. Am. Chem. Soc.* 2004; 126:739–743. [PubMed: 14733547]
26. (a) Puri RN, Meister A. *Proc. Natl. Acad. Sci. U. S. A.* 1983; 80:5258–5260. [PubMed: 6577420] (b) Campbell EB, Griffith OW. *Anal. Biochem.* 1989; 183:21–25. [PubMed: 2619045] (c) Anderson ME, Meister A. *Anal. Biochem.* 1989; 183:16–20. [PubMed: 2619041]

Figure 1.
Intracellular biogenic thiols mediated monolayer release from gold nanoparticles (AuNP).

Figure 3.

LDI mass spectra of AuNP 1 (100 nM) taken up by HeLa cells after (a) 4 h and (b) 24 h of incubation. 5.0 pmol of the internal standard AuNP 5 is spiked into the sample containing the lysed cells just prior to analysis. Ions at m/z 422 and 464 that correspond to the molecular ions (M^+) of the intact alkanethiol monolayers of AuNP 1 and AuNP 5, respectively. (c) ICP-MS and LDI-MS measurements of AuNP 1 after uptake by HeLa cells. (d) Percentages of the monolayer retained on AuNP 1 when taken up into HeLa cells, as calculated through comparison of LDI-MS and ICP-MS results.

Figure 4. ICP-MS and LDI-MS measurements of (a) AuNP 2, (b) AuNP 3, and (c) AuNP 4 after uptake by HeLa cells. 100 nM of each AuNP was incubated with cells for varying time points. Percentages of the monolayer retained on (d) AuNP 2, (e) AuNP 3, and (f) AuNP 4 when taken up into cells.

Figure 5.

Percentages of the monolayer retained on AuNPs 1–4 when taken up into HeLa, Hep G2 cells, and GSH-OEt treated HeLa: (a) uptake time 2 h; (b) uptake time 6 h. GSH-OEt treated HeLa refers to HeLa cells treated with 10 mM glutathione monoethyl ester (GSH-OEt) for 1 h prior to incubation with the AuNPs.