Chapter 2

Ligand Exchange and ^{1}H NMR Quantification of Single- and Mixed-Moiety Thiolated Ligand Shells on Gold Nanoparticles

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Abstract

The use of nanoparticles in biomedicine critically depends on their surface chemistry. For metal nanoparticles, a common way to tune this surface chemistry is through mass action ligand exchange, where ligand exchange can be used to expand the functionality of the resulting nanoparticle conjugates. Specifically, the quantity, identity, and arrangement of the molecules in the resulting ligand shell each can be tuned significantly. Here, we describe methods to exchange and quantify thiolated and non-thiolated ligands on gold nanoparticle surfaces. Importantly, these strategies allow the quantification of multiple ligand types within a single ligand shell, simultaneously providing ligand composition and ligand density information. These results are crucial for both designing and assigning structure-function relationships in bio-functionalized nanoparticles, and these methods can be applied to a broad range of nanoparticle cores and ligand types including peptides, small molecule drugs, and oligonucleotides.

Key words Gold nanoparticles, Nanoparticle functionalization, Ligand exchange, Ligand quantification, ^{1}H NMR

1 Introduction

Gold nanoparticles (AuNPs) are versatile materials that display ever-growing potential in fields that range from bioimaging [1, 2] to drug delivery [3, 4]. As with all NPs, their surface chemistry has a strong impact on their behavior in these applications and dictates many of their interactions in biological environments. Therefore, it is important to be able to understand and tailor this surface chemistry (e.g., control the quantity and composition of the appended ligands). Ligand quantification has been achieved in various ways, including thermogravimetric analysis [5–7], optical spectroscopy methods [8–10], and nuclear magnetic resonance spectroscopy (NMR) [11–13]. Yet, only NMR has demonstrated the necessary chemical resolution to yield information about ligand identity [12, 14], quantity [15–17], and arrangement [18, 19], even within a

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single experiment. Moreover, postsynthetic modification of the ligand shell is typically not necessary for ^{1}H NMR quantification analysis. Therefore, one can analyze the *active* molecule, peptide, or protein of interest without modifications such as fluorophore labeling, which can alter the behavior of the conjugate within the system of interest and prevent correlation with the un-labeled conjugate. Here, we will describe the quantification of 1 kDa poly(ethylene glycol) methyl ether thiol (PEGSH) and 8-mercaptooctanoic acid (MOA) ligands, both as single-ligand and mixed-ligand shells. PEG-based ligands are of particular interest because they are widely used in nanobiomedicine for solubilization, stability, particle size control, and anti-biofouling. [20–23]. This method is remarkably general and may be applied to any ligand shell on a NP of interest, provided that the ligands composing the shell each have at least one spectroscopically distinct chemical shift.

2 Materials

Prepare all solutions using ultrapure water (resistivity 18.2 MΩ·cm), unless otherwise noted. Before use, wash all glassware and Teflon-coated stir bars with aqua regia (3:1 ratio of concentrated hydrochloric acid (HCl) and nitric acid (HNO_3) by volume) and subsequently rinse thoroughly with water. *Caution: Aqua regia is highly toxic and corrosive and requires proper personal protective equipment. Aqua regia should be handled in a fume hood only.*

2.1 Gold Nanoparticle (AuNP) Synthesis [24]

1. $HAuCl_4$ solution: 1.0 mM. Weigh 0.197 grams of hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4{\cdot}3\ H_2O$) (*see* **Note 1**, chemical purity), and dissolve it in 500 mL of water in a 1-L three-neck round-bottom flask to form a pale yellow solution. Add a 1-inch Teflon-coated stir bar.
2. Trisodium citrate solution: 33.0 mM. Weigh 0.493 grams of sodium citrate tribasic dihydrate (citrate) (*see* **Note 2**, NP size control), and dissolve it in 50 mL of water to form a clear solution.
3. Reflux condenser.
4. 500 mL media bottle.

2.2 Citrate to Thiol Ligand Exchanges

1. 0.45 μm disposable poly(vinylidene fluoride) (PVDF) filters (25 mm GD/XP filters, Whatman, Inc.).
2. 1.5 mL centrifuge tubes.
3. Poly(ethylene glycol) methyl ether thiol (PEGSH), average MW = 1000 Da (Laysan Bio, Inc., Arab, AL, USA) solution: 5.0 mM in water (*see* **Note 3**, potential ligands). Store at 4 °C.

4. 8-mercaptooctanoic acid (MOA) (>95%, Sigma-Aldrich, St. Louis, MO, USA) solution: 1.0 mM in water. Store at 4 °C.
5. Base solution: 10 mM sodium hydroxide in water (*see* **Note 4**, role of base).
6. Deuterium oxide (D_2O): D, 99.9%.

2.3 Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Au Concentration Determination

1. Ultrapure aqua regia solution: In a 3:1 ratio, combine HCl (37 wt.% in H_2O, 99.999% trace metal basis) and HNO_3 (70%, purified by redistillation, ≥99.999% trace metal basis) to form a red, fuming solution (*see* **Note 5**, aqua regia cautionary statement).
2. 5% aqua regia matrix: Add 25 mL of concentrated ultrapure aqua regia to 475 mL of water.
3. 200 ppb Au stock solution: Dilute 2 μL of a gold standard for ICP (Fluka, TraceCERT 1001 ± 2 mg/L Au in HCl) with the 5% aqua regia matrix in a 10 mL volumetric flask.
4. 15 mL centrifuge tubes.
5. 10 mL volumetric flasks.

2.4 ^{1}H NMR for Ligand Concentration Determination

1. 5 mm borosilicate glass NMR tubes.
2. Acetonitrile (ACN) standard solution: 0.25% v/v, 15 μL of ACN in 6 mL of D_2O.
3. PEGSH solution: 1 mM in D_2O. Store at 4 °C.
4. MOA solution: 1 mM in D_2O. Store at 4 °C.

3 Methods

3.1 Synthesis of 13 nm AuNPs

1. While stirring at a rate of at least 800 rpm, bring the $HAuCl_4$ solution to a rapid reflux, with a drip rate of approximately 1 drop/second.
2. Rapidly add the citrate solution to the refluxing solution. Allow to mix for 5 min before removing from heat. Within these 5 min, the solution will change from yellow to clear to black to purple to ruby red ($\lambda_{max} = 519$ nm, *see* **Note 6**, NP colors). Cool the NP solution to room temperature before transferring to a clean 500-mL media bottle. Store at 4 °C.

3.2 Preliminary Controls for Citrate to Thiol Ligand Exchanges

3.2.1 Time of Ligand Exchange

1. Before use, filter 50 mL of the citrate-capped AuNPs through a PVDF filter. After filtration, concentrate the NPs by transferring 1.5 mL of NPs into a 1.5 mL centrifuge tube and centrifuging the solution at 20,000 rcf for 5 min. Remove the supernatant and add another 1.5 mL of NPs to the same centrifuge tube. Centrifuge again, and remove the supernatant to yield a concentrated pellet of citrate-capped NPs (*see* **Note 7**,

washing citrate-capped NPs). This yields a single tube with a concentrated particle pellet that will be used for subsequent ligand exchange and analysis. Prepare a total of 16 tubes with concentrated pellets (*see* **Note 8**, tube count).

2. Sequentially add 900 μL of water, 50 μL of PEGSH solution, and 50 μL of base solution to resuspend the concentrated pellets. Place the resulting mixtures on a temperature-controlled mixer at 1000 rpm and 25 °C. To test the time necessary for ligand exchange to proceed to completion while at a high excess (*see* **Note 9**, ligand excess), remove two tubes at each time point (here, 1, 2, 3, 4, 6, 8, 20, and 24 h after initiating ligand exchange) (*see* **Note 10**, time controls).
3. After the determined incubation time, wash the NPs by centrifuging the tubes for 5 min at 20,000 rcf. Remove the supernatant, and resuspend the pellet in 1 mL of water. Repeat this washing process for a total of two washes in water and two washes in D_2O. After the final centrifuge cycle in the second wash with D_2O, remove the supernatant to yield the concentrated pellet of PEGSH-capped AuNPs.
4. These experiments establish the necessary time for ligand exchange to proceed to a state consistent with equilibrium (Fig. 1a). Once the plot of ligand density vs. time reaches a plateau, it can be assumed that the ligand exchange has proceeded to completion. Here, a steady state is reached within 1 h, and all further experiments are conducted with an incubation time of 4 h to ensure that time is not a limiting factor in the ligand exchanges (*see* **Note 11**, completion of ligand exchange).

3.2.2 Determining Necessary Excess Ligand Concentrations

1. Repeat **step 1** from Subheading 3.2.1 for ten tubes with concentrated pellets of citrate-capped AuNPs.
2. After the necessary time for ligand exchange to reach a steady state is confirmed, the ligand excess with respect to NP surface area must be tested so that experiments are conducted where the ligand concentration is not the limiting factor for ligand coverage on the NP surface. Resuspend the concentrated pellets in various amounts of PEGSH to test different ligand excess amounts, all with 50 μL of base solution, added sequentially after the water and PEGSH. Here, for example, we will use an excess of 5× (944.75 μL water/5.25 μL PEGSH), 10× (939.5 μL water/10.5 μL PEGSH), 20× (929.0 μL water/21.0 μL PEGSH), 30× (918.5 μL water/31.5 μL PEGSH), and 50× (897.5 μL water/52.5 μL PEGSH). Place these tubes on a temperature-controlled mixer for 4 h.
3. After 4 h, wash the NPs as described in **step 3** in Subheading 3.2.1. After the final centrifuge cycle in the second wash with

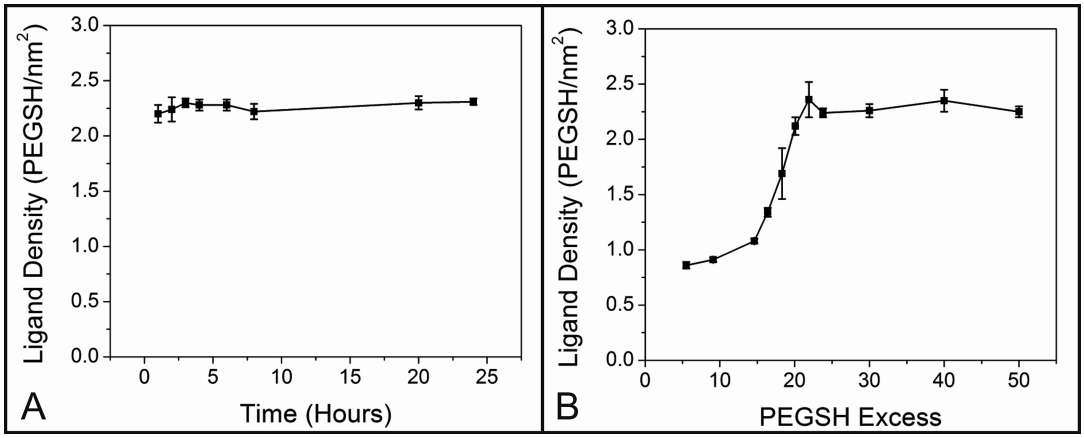

Fig. 1 Plots of PEGSH ligand density on the AuNP as a function of time in excess (50×) PEGSH (**a**) and as a function of PEGSH excess after an incubation time of 4 h (**b**). Results indicate that maximum loading reaches a steady state on the timescale of minutes and at an excess of at least 20×. Adapted with permission from ref. 11. Copyright 2015 American Chemical Society

D_2O, remove the supernatant to yield the concentrated pellet of PEGSH-capped AuNPs from the various ligand excesses.

4. These samples will establish the necessary ligand excess with respect to surface area for ligand exchange to proceed to a steady state (Fig. 1b). Once the plot of ligand density vs. ligand excess reaches a plateau, a steady state of ligand density can be assumed. Here, a steady state is reached at approximately a 20× excess, and all further experiments are conducted with an excess of 50× to ensure ligand excess is not a limiting factor in the ligand exchanges (*see* **Note 12**, ligand exchange completion).
5. These experiments were completed to establish the necessary time and ligand excess for ligand exchange for the MOA ligand as well. These controls yielded results consistent with those obtained with PEGSH, and all subsequent experiments for the MOA ligand will also be conducted at a 50× excess for 4 h.

3.3 Thiol-To-Thiol Backfilling Procedure

1. Working with the pellet of PEGSH-capped AuNPs generated after **step 3** of Subheading 3.2.1, sequentially add 687.5 μL of water, 50 μL of base solution, and 262.5 μL of MOA solution to resuspend each pellet (*see* **Note 13**, MOA concentration). Place these tubes on a temperature-controlled mixer for 4 h (*see* **Note 14**, ligand exchange time).
2. After this time, wash the NPs as described in **step 3** in Subheading 3.2.1. After the final centrifuge cycle in the second wash with D_2O, remove the supernatant to yield the concentrated pellet of AuNP conjugates (*see* **Note 15**, extent of ligand exchange).

3. Comparable experiments can be conducted for MOA-capped AuNPs to be backfilled with PEGSH. In this situation, working with ten tubes with a concentrated pellet of MOA-capped AuNPs, sequentially add 897.5 μL water, 52.5 μL PEGSH, and 50 μL of base solution to resuspend each pellet.

3.4 Thiol Co-Loading Procedure

1. Filter and concentrate citrate-capped NPs as described in **step 1** of Subheading 3.2.1 to obtain five tubes with concentrated citrate-capped AuNPs.
2. To these concentrated pellets, sequentially add 792.5 μL of water, 50 μL of base solution, 26.25 μL of PEGSH solution, and 131.25 μL of MOA solution (*see* **Note 16**, premixing ligand solutions). Place these tubes on a temperature-controlled mixer for 4 h.
3. After this time, wash the NPs as described in **step 3** in Subheading 3.2.1. After the final centrifuge cycle in the second wash with D_2O, remove the supernatant to yield the concentrated pellet of mixed-moiety AuNPs capped with a mixture of PEGSH and MOA ligands (*see* **Note 17**, ligand stoichiometry).

3.5 ICP-MS Preparations and Method

1. Prepare 5 Au standards from the 200 ppb Au stock solution by diluting in the 5% aqua regia matrix (*see* **Note 18**, matrix considerations). Specifically, prepare the five different standards of 1, 5, 10, 20, and 30 ppb by diluting 50, 250, 500, 1000, and 1500 μL of the 200 ppb Au stock to 10 mL with the 5% aqua regia matrix in 10 mL volumetric flasks. Transfer the standards to 15 mL centrifuge tubes for storage (*see* **Note 19**, storage of standards).
2. Digest the washed pellets of thiol-capped AuNPs formed above in the ligand exchange steps with ~5 μL of fresh, concentrated aqua regia (*see* **Note 20**, digestion considerations). Allow digestion to proceed overnight (*see* **Note 21**, extent of digestion). After digestion, dilute the digested pellet to a volume of 500 μL. Then, remove 1 μL of this solution and dilute in 10 mL of the 5% aqua regia matrix.
3. Analyze the five standards by ICP-MS, measuring each standard five times and averaging to build a 5-point calibration curve (*see* **Note 22**, multi-element calibration standards). Next, analyze the unknown, digested thiol-functionalized AuNP samples, measuring each in triplicate and averaging. Use a five-minute flush time with the 5% aqua regia matrix between each run, and analyze a blank sample consisting of only 5% aqua regia between samples to confirm residual Au has been removed (*see* **Note 23**, "sticky" elements).
4. In conjunction with the AuNP size (which can be determined using transmission electron microscopy), the concentration of

Au reported by the ICP-MS will allow for calculation of the number of AuNPs in the sample. First, calculate the number of Au atoms in the particle size used (e.g., 13 nm AuNPs contain ~71,970 atoms; *see* **Note 24**, number of atoms calculation). Then, divide the number of Au atoms determined by ICP-MS by the number of Au atoms in the AuNP to find the number of AuNPs in the sample, taking into account any dilution factors.

3.6 ^{1}H NMR Preparations and Method

1. Prepare five PEGSH standards from the 1 mM PEGSH stock solution by diluting in D_2O. Specifically, dilute this stock for the five different standards of 0.1, 0.25, 0.50, 0.75, and 1.0 mM by diluting 50, 125, 250, 375, and 500 μL of the 1 mM PEGSH stock to 500 μL with D_2O. To each standard, add 5 μL of the ACN standard solution. Mix well. Repeat these dilutions for the MOA ligand to prepare five additional MOA standards. Transfer each standard to an NMR tube.
2. Use the remainder of the digested and diluted AuNP samples from **step 2** in Subheading 3.5. Add 5 μL of the ACN standard solution to each sample and mix thoroughly (*see* **Note 25**, standard concentration). Transfer each sample to an NMR tube.
3. For all ^{1}H NMR spectra to be obtained, it is recommended to apply water suppression (*see* **Note 26**, water suppression). Run the first standard sample on an NMR to obtain the ^{1}H spectrum (*see* **Note 27**, number of scans and signal-to-noise ratio). Integrate the ACN standard peak and the most prominent ligand peak (Fig. 2a). Repeat for the remaining standards to generate a calibration curve for both PEGSH and MOA, where ligand concentration is plotted against the integrated ratio of ligand/ACN (Fig. 2b). From this plot, a linear equation can be

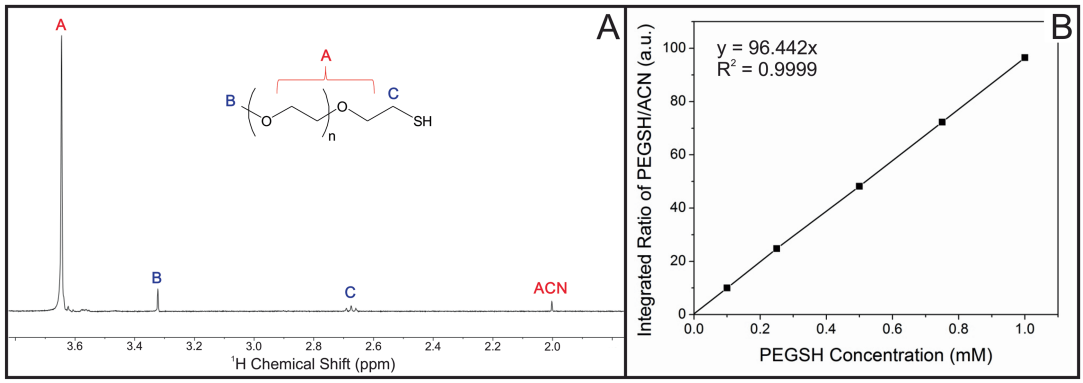

Fig. 2 Sample PEGSH ^{1}H NMR spectrum displaying integrated peaks labeled in *red* (peak A from the PEGSH and ACN) (**a**), and a sample calibration curve for PEGSH generated by plotting the PEGSH concentration against the ratio of PEGSH to ACN integrated peak intensities (where "peak" refers to the selected peaks shown in (**a**)) (**b**). The equation of the line obtained from the calibration curve allows for the calculation of unknown ligand concentrations. Adapted with permission from ref. 11. Copyright 2015 American Chemical Society

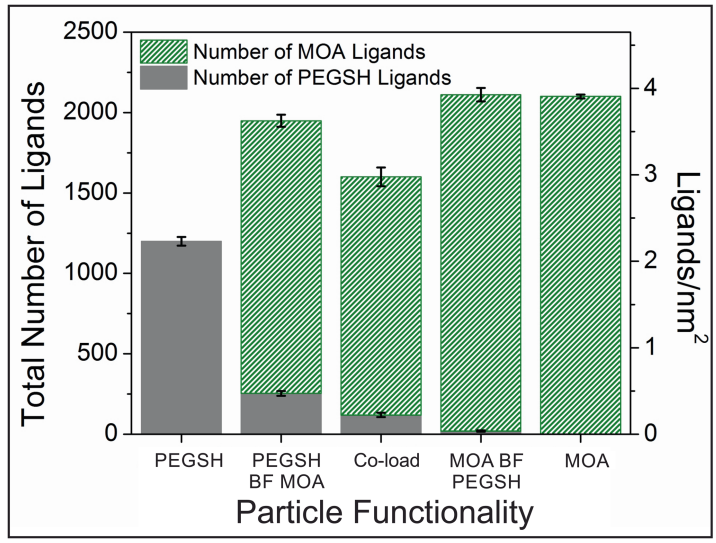

Fig. 3 Comparison of the amount of ligands appended to a AuNP and ligand density for both single moiety (PEGSH and MOA) and mixed moiety (backfilled (BF) and co-loaded) ligand exchanges. In both backfilling and co-loading, the MOA dominates the ligand shell. Adapted with permission from ref. 11. Copyright 2015 American Chemical Society

obtained that will allow for the evaluation of the ligand concentration in the unknown samples.

4. After obtaining the ^{1}H spectrum of each unknown, thiol-functionalized AuNP sample, integrate the ACN standard peak and the same ligand peak as was integrated for the relevant standards above. Using the equation from the calibration curve, insert the ratio of ligand/ACN integrated peak intensities to solve for the unknown ligand concentration in the sample.
5. Calculate ligand/particle numbers by dividing the number of ligands found using NMR by the number of particles found using ICP-MS. Ligand density in units of ligands/nm^2 can be calculated by dividing the number of ligands per particle found using NMR by the surface area of a single NP (Fig. 3). Ligand density can also be calculated by dividing the total number of ligands found using NMR by the total amount of NP surface area in the sample as determined by NP concentration. These two density calculations give similar values.

4 Notes

1. Using pure reagents can help to improve both the yield and monodispersity of the resulting NPs. Thus, it is recommended to use the highest purity reagents available for both ligand

exchange and NP synthesis. Polydispersity in the ligand MW (chain length) can impact both ligand exchange and NP synthesis [25].

2. The AuNP size can be controlled by altering the Au:citrate molar ratio used in the particle synthesis. For 13 nm AuNPs, a 1:3.35 Au:citrate molar ratio was used. By increasing this ratio, larger NPs can be synthesized. Mole ratios of Au:citrate higher than 1 are not recommended as they do not produce stable, monodisperse NPs.
3. Here, 1 kDa PEGSH and MOA are used in the ligand exchanges. Other water-soluble thiolated ligands can also be appended to the NPs by following the same procedures described here.
4. The base solution is added to the ligand exchanges to prevent multilayer formation due to hydrogen bonding between the terminal carboxylic acids on the MOA. While it is not necessary to use the base with the PEGSH samples, it is added to all ligand exchanges for consistency.
5. Aqua regia is highly toxic and corrosive. Wear proper personal protective equipment and handle only in a fume hood. Do not mix with organic solvents and always allow the solution to vent—gasses evolve continuously upon mixing the HCl and HNO_3 solutions.
6. While not an absolute indicator, visual color inspection can be useful in monitoring the AuNPs. Citrate-capped 13 nm AuNPs will appear ruby red; as the particle size increases, the red will darken to purple. Additionally, if at any time during subsequent ligand exchanges the color dramatically changes (e.g., from red to purple or black), the NPs have irreversibly aggregated and will not be suitable for either further use or robust analysis.
7. Avoid over-washing the citrate-capped AuNPs. Because citrate is weakly bound to the AuNPs, washing more than three times will cause the NPs to begin to aggregate.
8. The number of tubes necessary for each sample will vary depending on NP concentration, NP size, and ligand identity. The number of tubes contained herein is appropriate for the PEGSH and MOA system; the number can be increased or decreased as necessary.
9. These concentrations of PEGSH are based on the ligand excess with respect to NP surface area. See ref. 11 for a detailed explanation and sample calculations.
10. The time necessary for ligand exchange can vary depending on the ligand identity. It is therefore recommended to complete a time analysis for all ligand types studied.

11. If the ligand exchanges are not given enough time to proceed to completion, the final ligand shell will be more variable in terms of both composition and ligand quantity from batch to batch. To ensure accurate and consistent particle preparation, allow enough time such that the plot of ligand density vs. time of ligand exchange has reached a plateau before continuing with further ligand studies.
12. Similar to time of exchange being a limiting factor, a limiting ligand excess will also produce variable particle conjugates with inconsistent numbers of ligands.
13. Using this concentration of MOA will yield roughly a 50× ligand excess with respect to particle surface area (depending on exact NP concentrations). Here, we used a 50× excess out of an abundance of caution to remain on the plateau region in Fig. 1b, however a 50× excess is not necessary to achieve full ligand loading as indicated by the surface coverage plot in Fig. 1b. The ligand concentration mentioned in this step can be adjusted depending on desired excess.
14. The time for ligand exchange can and should be altered, depending upon the necessary time for the ligand exchange to reach completion. As shown in Fig. 1a, the ligand loading using the ligands described here reaches a steady state within an hour, so any time frame longer than this period can be used. We use 4 h as a compromise between experiment expediency and an abundance of caution.
15. The ligand exchange of PEGSH for MOA is unlikely to completely displace all of the original ligand present on the NP surface (here, PEGSH). This incomplete exchange is exceptionally likely in cases where the particle binding moiety is the same for both the ligand on the particle and the ligand that is being added (as is the case here). In this case, where we seek to exchange PEGSH appended to the particle with a new ligand, MOA, we have shown that the displacement is not complete, and under the conditions listed produced a particle with a ligand shell that is 13% PEGSH and 87% MOA. The degree of ligand displacement depends strongly on the particle binding moiety of the two ligands, as well as the total ligand architecture. The degree of displacement must be determined for every ligand combination studied. Luckily, the composition of the ligand shell is readily extracted from the NMR experiment described herein and does not require any additional analytical steps or procedures. Indeed, there is no intrinsic limit on the number of different ligands that can be identified in a single NMR analysis, provided each ligand has at least one spectroscopically distinct chemical shift and is present in a quantity above the detection limit of NMR.

16. It is recommended to premix the ligand solutions before addition to the AuNPs to ensure that neither one has additional time for ligand exchange with respect to the other. Additionally, these concentrations are to co-load the ligands at a 50× total ligand excess, with a 25× excess for each ligand and at a 50:50 ratio. This ratio can be altered to yield final ligand shells with different compositions [26] (*see* also **Note 17**).
17. When attempting to functionalization a Au NP with two ligands simultaneously (i.e., "co-loading"), it is natural to expect that the stoichiometry of the ligands added will be reflected in their final composition ratios on the NP. Unfortunately, the relationship is much more complex and depends on both ligand–ligand interactions in solution (e.g., ligands may form small aggregates or "rafts," as in the case of lipids), as well as the affinity of each ligand for the NP surface. The relationship between the stoichiometry of ligands added and the stoichiometry appended to the particle after ligand introduction must be determined for every new ligand combination to predict the relationship between the amount of ligand added and the amount of ligand that ultimately binds to the particle. However, two points are important to note, despite these challenges. First, on-particle ligand compositions *can* be tuned by changing the stoichiometry of the ligands added, it just may not directly match the ratio added. Second, the NMR analysis method can be used, in all cases, to elucidate what the relationship is between ligand composition added and ligand composition appended.
18. An aqua regia matrix is used since it is effective for digesting Au. However, some metals (e.g., silver) are incompatible with this matrix (since silver chloride will precipitate out in the presence of the chloride ions). In these situations, the matrix can be altered. For example, in the case of silver, a 5% nitric acid matrix can be used.
19. For best results, prepare fresh standards for ICP analysis every day, as any matrix evaporation or metal adsorption on containment vessels will alter the standard concentrations.
20. Use only a small (<10 μL) amount of aqua regia in the digestion, as samples with a high ionic strength are difficult to tune on the NMR.
21. After digestion, the Au samples will be a pale yellow color. Black or purple specks indicate that the digestion is not complete. Sonicating the tubes or placing them on the temperature-controlled mixer at ~35 °C can aid in the digestion.

22. Calibration curves will be needed for all elements being analyzed on the ICP-MS. As long as there are no inter-element interferences, multi-element standards can be used.
23. Certain elements, including Au and to lesser degree Ag, can be "sticky" in the ICP-MS, leaving residual Au in the tubing and internal components that will be reflected in subsequent measurements. Long flush times and analyzing a blank will help to confirm removal of the residual metals. Analyzing samples at lower concentrations will also help to reduce the problem of sticking, but if longer (>5 min) flush times do not yield clean blank samples, a flush with 2% Triton detergent solution can aid in the removal of sticky metals.
24. The number of atoms in a pseudospherical AuNP can be estimated by using the diameter of the NP to find the NP volume and dividing this volume by the volume of a Au unit cell [11]. Then, knowing that there are 4 Au atoms/unit cell, the number of atoms/particle is obtained.
25. While here we add 5 μL of ACN standard solution to both each ligand standard as well as each ligand sample, this concentration can be adjusted so that the internal standard peak is proportional in intensity to ligand peaks of interest.
26. Water suppression for the ^{1}H NMR spectra is useful when the AuNPs are synthesized and undergo ligand exchange in water. Even after multiple washes in D_2O, residual water will remain; this water will contribute to a peak at 4.7 ppm. Depending on the amount of water, the peak can be large, obscuring the baseline for the relevant ligand peaks. A water suppression pulse sequence minimizes this peak, allowing for a smoother baseline and more accurate integration of the ligand peaks.
27. Depending on sample concentrations, more or less scans can be run on each sample. For the concentrations contained herein, 64 scans should give sufficient resolution for a minimum signal-to-noise ratio of 10.

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References

1. Huang X, Jain PK, El-Sayed IH, El-Sayed MA (2007) Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy. Nanomedicine 2:681–693
2. Murphy CJ, Gole AM, Stone JW, Sisco PN, Alkilany AM, Goldsmith EC, Baxter SC (2008) Gold nanoparticles in biology: beyond toxicity to cellular imaging. Acc Chem Res 41:1721–1730

3. Ghosh P, Han G, De M, Kim CK, Rotello VM (2008) Gold nanoparticles in delivery applications. Adv Drug Deliv Rev 60:1307–1315
4. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA (2010) Gold nanoparticles for biology and medicine. Angew Chem Int Ed 49:3280–3294
5. Hostetler MJ, Wingate JE, Zhong C-J, Harris JE, Vachet RW, Clark MR, Londono JD, Green SJ, Stokes JJ, Wignall GD (1998) Alkanethiolate gold cluster molecules with core diameters from 1.5 to 5.2 nm: core and monolayer properties as a function of core size. Langmuir 14:17–30
6. Takae S, Akiyama Y, Otsuka H, Nakamura T, Nagasaki Y, Kataoka K (2005) Ligand density effect on biorecognition by PEGylated gold nanoparticles: regulated interaction of RCA120 lectin with lactose installed to the distal end of tethered PEG strands on gold surface. Biomacromolecules 6:818–824
7. Corbierre MK, Cameron NS, Sutton M, Laaziri K, Lennox RB (2005) Gold nanoparticle/polymer nanocomposites: dispersion of nanoparticles as a function of capping agent molecular weight and grafting density. Langmuir 21:6063–6072
8. Demers LM, Mirkin CA, Mucic RC, Reynolds RA, Letsinger RL, Elghanian R, Viswanadham G (2000) A fluorescence-based method for determining the surface coverage and hybridization efficiency of thiol-capped oligonucleotides bound to gold thin films and nanoparticles. Anal Chem 72:5535–5541
9. Xia X, Yang M, Wang Y, Zheng Y, Li Q, Chen J, Xia Y (2012) Quantifying the coverage density of poly(ethylene glycol) chains on the surface of gold nanostructures. ACS Nano 6:512–522
10. Hurst SJ, Lytton-Jean AK, Mirkin CA (2006) Maximizing DNA loading on a range of gold nanoparticle sizes. Anal Chem 78:8313–8318
11. Smith AM, Marbella LE, Johnston KA, Hartmann MJ, Crawford SE, Kozycz LM, Seferos DS, Millstone JE (2015) Quantitative analysis of thiolated ligand exchange on gold nanoparticles monitored by 1H NMR spectroscopy. Anal Chem 87:2771–2778
12. Anderson NC, Hendricks MP, Choi JJ, Owen JS (2013) Ligand exchange and the stoichiometry of metal chalcogenide nanocrystals: Spectroscopic observation of facile metal-carboxylate displacement and binding. J Am Chem Soc 135:18536–18548
13. Valdez CN, Schimpf AM, Gamelin DR, Mayer JM (2014) Low capping group surface density on zinc oxide nanocrystals. ACS Nano 8:9463–9470
14. Hens Z, Martins JC (2013) A solution NMR toolbox for characterizing the surface chemistry of colloidal nanocrystals. Chem Mater 25:1211–1221
15. Crawford SE, Andolina CM, Smith AM, Marbella LE, Johnston KA, Straney PJ, Hartmann MJ, Millstone JE (2015) Ligand-mediated "turn on," high quantum yield near-infrared emission in small gold nanoparticles. J Am Chem Soc 137:14423–14429
16. Becerra L, Murray C, Griffin R, Bawendi M (1994) Investigation of the surface morphology of capped CdSe nanocrystallites by 31P nuclear magnetic resonance. J Chem Phys 100:3297–3300
17. Sachleben JR, Wooten EW, Emsley L, Pines A, Colvin VL, Alivisatos AP (1992) NMR studies of the surface structure and dynamics of semiconductor nanocrystals. Chem Phys Lett 198:431–436
18. Liu X, Yu M, Kim H, Mameli M, Stellacci F (2012) Determination of monolayer-protected gold nanoparticle ligand–shell morphology using NMR. Nat Commun 3:1182
19. Guarino G, Rastrelli F, Scrimin P, Mancin F (2012) Lanthanide-based NMR: A tool to investigate component distribution in mixed-monolayer-protected nanoparticles. J Am Chem Soc 134:7200–7203
20. Jokerst JV, Lobovkina T, Zare RN, Gambhir SS (2011) Nanoparticle PEGylation for imaging and therapy. Nanomedicine 6:715–728
21. De Jong WH, Borm PJ (2008) Drug delivery and nanoparticles: applications and hazards. Int J Nanomedicine 3:133
22. Boisselier E, Astruc D (2009) Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem Soc Rev 38:1759–1782
23. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE (2001) Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release 70:1–20
24. Frens G (1973) Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. Nature 241:20–22
25. Marbella LE, Andolina CM, Smith AM, Hartmann MJ, Dewar AC, Johnston KA, Daly OH, Millstone JE (2014) Gold-cobalt nanoparticle alloys exhibiting tunable compositions, near-infrared emission, and high T2 relaxivity. Adv Funct Mater 24:6532–6539
26. Smith, A.M., Johnston, K.A., Marbella, L.E., Millstone, J.E. (2016) unpublished results

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