Simple and Efficient Separation of Atomically Precise Noble Metal Clusters

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ABSTRACT: There is an urgent need for accessible purification and separation strategies of atomically precise metal clusters in order to promote the study of their fundamental properties. Although the separation of mixtures of atomically precise gold clusters \( \text{Au}_{25} \) where \( L \) are thiylates, has been demonstrated by advanced separation techniques, we present here the first separation of metal clusters by thin-layer chromatography (TLC), which is simple yet surprisingly efficient. This method was successfully applied to a binary mixture of \( \text{Au}_{25} \) with different ligands, as well as to a binary mixture of different cluster cores, \( \text{Au}_{25} \) and \( \text{Au}_{144} \), protected with the same ligand. Importantly, TLC even enabled the challenging separation of a multicomponent mixture of mixed-monolayer-protected \( \text{Au}_{25} \) clusters with closely similar chemical ligand compositions. We anticipate that the realization of such simple yet efficient separation technique will progress the detailed investigation of cluster properties.

Atomically precise clusters of noble metals protected with monolayers are some of the most fascinating molecules of contemporary chemical science. Most of the reports on such clusters are concerned with gold, though a few analogues of silver and copper have also appeared in the literature. Molecules such as \( \text{Au}_{25} \), \( \text{Au}_{38} \), and \( \text{Au}_{144} \) are some of the most stable species in this family of materials. Catalytic and biological applications of such materials are fast evolving. Along with this development, we have begun to explore the complex chemistry of these systems. The different chemically nonequivalent environments at their surfaces offer different possibilities for ligand exchange. The possibility of core rearrangement, reduction in size and chirality of the core contribute to the diversity of their chemistry. Whereas some synthesis protocols may produce directly atomically precise metal clusters without the need for separation, other synthesis protocols always yield a mixture of slightly different atomically precise metal clusters and their separation often is a challenge. Efficient methods to isolate the chemically varying species would enhance the growth of science in this area. In this article, we present a simple yet effective way of separating atomically precise clusters, which helps to expand the exploration of their diverse properties.

Although high-pressure liquid chromatography (HPLC) and gel electrophoresis (PAGE), and solvent extraction are used extensively in separating clusters, the simplest of chromatographic techniques, namely, thin-layer chromatography (TLC) has not been attempted for the separation of such clusters. In the following, we show that differently functionalized clusters of the same core, varying cores with the same chemical functionality and even mixed-monolayer-protected clusters of the same core having only slight structural differences are well separable by a simple TLC methodology. Even though TLC has been used in organic chemistry for a long time, the realization of its applicability to metal clusters adds a new tool in the toolbox of cluster science and further emphasizes the analogous nature of metal clusters and small organic molecules.

**EXPERIMENTAL SECTION.** Gold(III) chloride trihydrate (≥99.9%), butanethiol (99%), hexanethiol (99%), phenyl-ethanethiol (≥99%), sodium borohydride (95%), tetroctylammonium bromide (TOABr, 98%), and trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB, > 98%) were purchased from Sigma-Aldrich. Tetrathiolated calix[4]arene (25,26,27,28-tetakis(4-mercapto-n-butoxy)calix-...
Au25PET18 and Au144PET60 was dissolved in DCM and washed with methanol 5 times. The crude product containing precipitated clusters were collected by centrifugation and after that, 115 mg of NaBH4 dissolved in 6 mL of ice-cold H2O ethanethiol was added, and the mixture was stirred for 15 min. μ orange-red in color. To this solution, 216 (120 mg) was dissolved in 15 mL of methanol, and 193 mg of TOABr was added to this vigorously stirred solution. The stirring was continued for 15 min after which the solution was vigorous stirring (1200 rpm). The stirring was continued for 2 h during which the solution turned colorless. After that, 78 mg of NaBH4 dissolved in 5 mL of ice-cold H2O was rapidly added to the reaction solution under vigorous stirring, and the stirring was continued for 5 h in the case of Au25BT18 and 15 h in the case of Au25Calix0 phenylethanethiolate, PhCH2CH2S-) and Au25HT18 (HT-THF (in case of Au25Calix0 C4H9S-) and the calixarene-functionalized Au25 clusters were synthesized by a reported method.32 Briefly, H2AuCl6·3H2O (80 mg) was dissolved in 15 mL of THF and 130 mg of TOABr was added to this vigorously stirred solution. The reaction mixture by rotary vacuum evaporation, and clusters were purified by centrifugal washing with methanol (4 times, 3000 RCF). In the case of Au25BT18, methanol/water mixture (3:1 v/v) was used as the matrix. The measurements were performed in positive ion mode. For each measurement, typically 500 scans were acquired.

RESULTS AND DISCUSSION

Separation of Binary Mixture of Au25L18, L = BT/HT/PET.
In Figure 1, we show the separation of Au25HT18 (HT-hexanethiolate, C9H17S-) and Au25BT18 (BT- butanethiolate, C4H9S-) from a mixture of two, even though the polarity difference of BT and HT is very small. A mixture of clusters was spotted on the TLC plate and then eluted using DCM/hexane mixtures. For this system, the best separation occurred at a DCM/hexane ratio of 40:60 (unless otherwise noted, the solvent ratios are expressed as volume ratios throughout the manuscript). A photograph of the two separated bands is shown in Figure 1A. After optimization of the solvent mixture, preparative scale separation was performed by simultaneously eluting multiple spots of this mixture. The separated bands 1 and 2 were cut off from the plate and individually extracted using DCM. The UV–vis spectra of band 1 (red) and band 2 (blue) show characteristic absorption features originating from the Au25 core (Figure 1).

To verify the identity of these bands, we performed MALDI MS using DCTB as the matrix which is known to enhance intact ionization for this system at threshold laser powers.33 MALDI MS data of bands 1 (red) and 2 (blue) are shown in Supporting Information (Table S-1).
Also separated a mixture of two different cluster cores, Au$_{25}$ and Au$_{144}$, protected by the same ligand, PET. The mixture of clusters on the TLC plate (Figure S-2). The mixture of oxidation, even separation of Au$_{25}$L$_{18}$ is realized with TLC, since Au$_{25}$L$_{18}$ oxidize to Au$_{25}$L$_{18}^0$. If drying and Figure 1, the charge of the separated clusters is neutral. Au$_{25}$L$_{18}^0$ is likely due to the attached TOA$^+$ (Figure S-3).

To further explore the potential of TLC separation, we continued our study with mixed-monolayer-protected Au$_{25}$ clusters, in which the monolayer consisted of BT and tetrathiolate of 25,26,27,28-tetrakis (4-mercapto-n-butoxy)calix-[4]arene (Calix, Figure 3 inset). MALDI MS measurements confirmed that the clusters contained 0–3 Calix moieties (Figure 3), which is in agreement with our earlier electrospray ionization mass spectrometric study. The MALDI MS data shows that the crude product contains both Au$_{25}$PET$_{18}$ and Au$_{144}$PET$_{60}$ (Figure 2B). As ionization efficiency of Au$_{25}$ is considerably higher than that of the bigger cluster Au$_{144}$, the intensity of MALDI MS peak for the former is higher. Expanded spectrum shows the presence of Au$_{144}$. Blue and red traces confirm that bands 1 and 2 contain pure Au$_{25}$ and Au$_{144}$ respectively. Due to its reduced size, Au$_{25}$ elutes faster on the TLC plate. As differently sized clusters can be separated by TLC, we foresee this method to be applicable in monitoring cluster synthesis (see further discussion below).

Separation of Di↵erent Cluster Nuclearities. We have also separated a mixture of two different cluster cores, Au$_{25}$ and Au$_{144}$, protected by the same ligand, PET. The mixture of clusters was dissolved in a minimum amount of DCM and spotted on a TLC plate. The sample was eluted with a DCM/hexane mixture (60:40), and two separate bands were observed (Figure 2A, inset). The UV–vis spectra of those two isolated bands were measured after extracting them in DCM (Figure 2A). Blue and red traces correspond to bands 1 and 2, respectively, and these traces match with those of Au$_{25}$PET$_{18}$ and Au$_{144}$PET$_{60}$, respectively. To confirm the purity of each band, we performed MALDI MS measurements of the crude and isolated bands using DCTB as the matrix. The mass spectrum of the crude product contains both Au$_{25}$PET$_{18}$ and Au$_{144}$PET$_{60}$ (Figure 2B). As ionization efficiency of Au$_{25}$ is considerably higher than that of the bigger cluster Au$_{144}$, the intensity of MALDI MS peak for the former is higher. Expanded spectrum shows the presence of Au$_{144}$. Blue and red traces confirm that bands 1 and 2 contain pure Au$_{25}$ and Au$_{144}$ respectively. Due to its reduced size, Au$_{25}$ elutes faster on the TLC plate. As differently sized clusters can be separated by TLC, we foresee this method to be applicable in monitoring cluster synthesis (see further discussion below).

Separation of Mixed-Monolayer-Protected Clusters. To further explore the potential of TLC separation, we continued our study with mixed-monolayer-protected Au$_{25}$ clusters, in which the monolayer consisted of BT and tetrathiolate of 25,26,27,28-tetrakis (4-mercapto-n-butoxy)calix-[4]arene (Calix, Figure 3 inset). MALDI MS measurements confirmed that the clusters contained 0–3 Calix moieties (Figure 3), which is in agreement with our earlier electrospray ionization mass spectrometric study. The MALDI MS data shows that the crude product is composed of several different clusters varying slightly in their monolayer composition (Au$_{25}$Calix$_{0-3}$BT$_{0-18}$).

Notably, the tetrathiolated Calix ligands are bound to Au$_{25}$ surface predominantly in tetradentate or bidentate manner leading to the absence of odd numbers of BT ligands on the clusters. Peak positions and molecular compositions are discussed in Supporting Information (Tables S-2 and S-3). This cluster mixture was subjected to TLC with DCM/hexane mixture as the eluent. For this system, the optimal DCM/hexane ratio was found to be 30:70. Even though the clusters elute more slowly in such low polarity media, we observed undesired smearing of bands with higher DCM content. In

Figure 2. (A) UV–vis spectra of the two bands separated by TLC. Red trace matches well with the previously reported UV–vis spectrum of Au$_{144}$PET$_{60}$ cluster, and blue trace matches with the UV–vis spectrum of Au$_{25}$PET$_{18}$ cluster. The red trace has been vertically shifted for clarity. Inset: photograph of the TLC plate used for cluster separation (soret front marked as a dashed line). (B) MALDI MS data of crude (black trace) and isolated clusters (blue and red trace) confirming that bands 1 and 2 consist of isolated Au$_{25}$PET$_{18}$ and Au$_{144}$PET$_{60}$, respectively. The fragmented product, Au$_{25}$L$_{18}^0$, is shown with an asterisk (*).
order to achieve a greater separation between the bands, the same TLC plate was eluted several times. Four distinct bands could be separated in this manner, in addition to the immobile base band (Figure 4A). To confirm the identity of these bands, they were extracted and analyzed by MALDI MS using DCTB as the matrix (Figure 4B).

From the MALDI MS data of bands 1–5, it is evident that the fastest eluting clusters are Au25BT18 (band 1, blue), followed by Au25Calix1BT16 (band 2, green) and Au25Calix1BT14 (band 3, magenta). Surprisingly, even clusters having such minor differences in composition could be separated by TLC. Au25Calix1BT16 is less polar than Au25Calix1BT14 based on the difference in conformation and binding of Calix on the cluster surface, and therefore, it elutes faster (see further discussion below). Furthermore, band 4 is composed of clusters having exclusively two Calix moieties (Au25Calix2BT10−16), whereas the majority of clusters in the immobile base band 5 have three Calix units attached. It is worthwhile to mention that no separation of Au25Calix0−3BT6−18 clusters could be achieved by size-exclusion chromatography.

Bands 4 and 5 from the TLC run still contain multiple mixed-monolayer compositions. These bands were extracted, combined, and subjected to another TLC run with a slightly higher polarity eluent (DCM/hexane 35:65). Three bands could be extracted from the second run, containing two bands composed of Au25CalixBT clusters (band 1: n = 12−14, band 2: n = 10−12) and one band composed of various Au25Calix2−4BT6−12 clusters (Figure 5). Thus, Au25Calix2BT10−16 clusters could be separated into two fractions (bands 1 and 2), which were not separable in the first TLC run because they were retained near the base band. Thus, it is possible to separate more products by running the TLC of third band again by tuning the polarity of the eluent. It is also noteworthy that separation can reveal new cluster compositions. Note that clusters having four Calix moieties were not observed in MALDI MS of the crude sample.
that the synthesis of Au25PET18 was completed after 8 h. The sample shows a single band (Figure S-5A, right). It implies decreases as the reaction proceeds and after 8 h, and TLC of 5A after 1 and 3 h of reaction. The fraction of larger clusters DCM/hexane 60:40. Remaining thiol. Subsequently, the clusters were eluted with most of the excess thiol. Thereafter, the clusters were spotted to of water and further washed with methanol to quickly remove aliquots of the raw cluster mixture were precipitated by addition excess thiol typically present after the synthesis. Initially, the TLC method can be used to remove this small amount of thiolate-protected cluster samples despite rigorous washing of clusters after synthesis. In Figure 6, we show that TLC of synthesis of Au25PET18. In this experiment, Information (Figure S-5), we have presented the time-dependent TLC separation, this rule seems to hold also for clusters with two and three Calix moieties. Clusters having more BT ligands have more Calix ligands bound with two thiocarbonyls. On the other hand, clusters with a smaller amount of BT have more Calix ligands bound with four thiocarbonyls. These two binding modes explain the polarity differences of the clusters: Binding with four thiocarbonyls exposes the polar ether groups of Calix, whereas two thiocarbonyl binding causes pinching of the calixarene cone, thus shielding the polar oxygen atoms.

**Purification of Clusters from Excess Thiol and Monitoring Cluster Synthesis.** Excess thiol is a common impurity in thiolate-protected cluster samples despite rigorous washing of clusters after synthesis. In Figure 6, we show that TLC method can be used to remove this small amount of excess thiol typically present after the synthesis. Initially, the sample was eluted with DCM/hexane mixture (70:30) to get a band. Then the sample was further eluted with 100% hexane three times. The TLC of a four-times-methanol-washed Au25PET18 cluster revealed a fast moving colorless band due to excess phenylethananethiol, which is visible under UV light (Figure 6).

We have also shown that it is possible to monitor the progress of cluster synthesis by TLC. In Supporting Information (Figure S-S), we have presented the time-dependent TLC of synthesis of Au25PET18. In this experiment, aliquots of the raw cluster mixture were precipitated by addition of water and further washed with methanol to quickly remove most of the excess thiol. Thereafter, the clusters were spotted to a TLC plate and eluted with hexane to completely remove the remaining thiol. Subsequently, the clusters were eluted with DCM/hexane 60:40.

Initially, a mixture of clusters is formed as shown in Figure S-5A after 1 and 3 h of reaction. The fraction of larger clusters decreases as the reaction proceeds and after 8 h, and TLC of the sample shows a single band (Figure S-5A, right). It implies that the synthesis of Au25PET18 was completed after 8 h. The bands after 1 h of reaction were individually extracted with DCM and further purified by another TLC run. The bands were analyzed by MALDI MS (Figure S-SB). These data show that the top band (band 1) consists of Au38PET24 and Au40PET24 clusters. Moreover, bands 3 and 4 were found to contain multiple larger clusters in the size range of 12 000–15 000 m/2 and 14 000–21 000 m/2, respectively. In addition, band 3 produced a strong MALDI MS signal of Au53PET35 clusters. This experiment further validates that clusters of different nucleicities can be separated by TLC. We foresee TLC as a highly applicable tool in monitoring cluster synthesis that advances the understanding of reaction pathways leading to specific clusters and provides a straightforward way for optimization of synthetic methods.

**CONCLUSIONS**

In summary, we have shown the surprisingly efficient TLC separation of atomically precise clusters of gold varying in ligand structure, core size, and mixed-monolayer composition. The data presented show that simple, inexpensive chromatographic tools can be used for the isolation of monolayer-protected clusters, although they are chemically complex. We anticipate that such a simple, broadly applicable methodology will enhance the detailed investigation and understanding of chemical and photophysical properties of well-defined cluster systems.

**ASSOCIATED CONTENT**

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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