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## Article

# Dynamic Stabilization of the Ligand-Metal Interface in Atomically Precise Gold Nanoclusters Au68 and Au144 Protected by Meta-Mercaptobenzoic Acid

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# Dynamic Stabilization of the Ligand-Metal Interface in Atomically Precise Gold Nanoclusters Au<sub>68</sub> and Au<sub>144</sub> Protected by *meta*-Mercaptobenzoic Acid

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ABSTRACT Ligand-stabilized, atomically precise gold nanoclusters with a metal core of a uniform size of just 1 - 3 nm constitute an interesting class of nanomaterials with versatile possibilities for applications due to their size dependent properties and modifiable ligand layers. The key to extending the usability of the clusters in applications is to understand the chemical bonding in the ligand layer as a function of cluster size and ligand structure. Previously, it has been shown that monodispersed gold nanoclusters, stabilized by meta-mercaptobenzoic acid (m-MBA or 3-MBA) ligands and with sizes of 68 to 144 gold atoms, show ambient stability. Here we show that a combination of nuclear magnetic resonance spectroscopy, ultraviolet-visible absorption, infrared spectroscopy, molecular dynamics simulations and density functional theory calculations reveals a distinct chemistry in the ligand layer, absent in other known thiol-stabilized gold nanoclusters. Our results imply a low-symmetry  $C_1$  ligand layer of 3-MBA around the gold core of  $Au_{68}$  and  $Au_{144}$  and suggest that 3-MBA protects the metal core not only by the covalent S-Au bond formation but also via weak  $\pi$  – Au and O=C-OH...Au interactions. The  $\pi$  – Au and -OH...Au interactions have a strength of the order of a hydrogen bond, thus are dynamic in water at ambient temperature. The -OH...Au interaction was identified by a distinct carbonyl stretch frequency that is distinct for 3-MBA-protected gold clusters but is missing in the previously studied  $Au_{102}(p-MBA)_{44}$  cluster. These thiol-gold interactions can be used to explain a remarkably low ligand density on the surface of the metal core of these clusters. Our results lay a foundation to understand functionalization of atomically precise ligand-stabilized gold nanoclusters via a route where weak ligand-metal interfacial interactions are sacrificed for covalent bonding.

KEYWORDS: gold nanocluster, thiols, molecular dynamics, NMR spectroscopy, vibrational spectroscopy, carboxylic acid

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Manipulation of gold nanoparticles with atomic and molecular precision, in forms of 1-3 nanometer atom-precise clusters covered by a monolayer of organic molecules, has been made possible over the last decade by breakthroughs in synthetic chemistry and characterization techniques.<sup>1,2</sup> Properties of these versatile building blocks of nanomaterials can be tuned by controlling the size of the metal core and the chemical nature of the protecting molecular layer. Water-soluble clusters are relevant in life science and nanomedicine, for instance, for imaging, detection and theranostics. Since the clusters interact with the environment *via* the protecting ligand layer, characterizing the structure and dynamics of that layer in solution phase is key to understand the interaction to the environment.<sup>3</sup> This is an important challenge to be solved for engineering practical applications.

Among several water-soluble thiols that can stabilize nanometer-scale gold clusters,4 metamercaptobenzoic acid (3-MBA) was recently shown to produce three distinct-size clusters that are readily reactive to thiol-modified DNA and proteins with an exposed cysteine group.<sup>5</sup> The atomic structure of the gold core of the smallest cluster, Au<sub>68</sub>, was previously solved via single-particle 3D reconstruction of transmission electron microscopy (TEM) data.<sup>6</sup> The companion paper of the current work describes the atomic structure of the gold core of the largest cluster  $Au_{144}$  (ref. 7). Here, we concentrate on the structure and dynamics of the protecting ligand layer of Au<sub>68</sub> and Au<sub>144</sub> clusters in solution, and demonstrate dynamic interactions at the ligand-metal interface that contribute both to chemical protection of the gold core and opening active metal sites for conjugation reactions. These interactions are suggested from molecular dynamics (MD) simulations and density functional theory computations (DFT) and supported by infrared (IR) spectroscopy data. This observation can explain the documented reactivity of 3-MBA-protected gold nanoclusters and demonstrates a potential route for bioconjugation where covalent bonds to gold can irreversibly be added at the ligand-metal interface. The existence of gold sites at the ligand-metal interface, that are protected by weak dynamic interactions and may be available, e.g., for catalytic reactions at ambient conditions in water, creates an unexplored paradigm for the ligand-metal chemistry at the thiolate-gold interface.8

#### Results

3-MBA-protected  $Au_{144}$  and  $Au_{68}$  clusters were produced from a synthesis described earlier by using thiol-to-gold ratios of 7:1 and 2:1, respectively.<sup>5</sup> We characterized the samples by nuclear magnetic resonance (NMR) spectroscopy, diffusion ordered spectroscopy (DOSY-NMR) as well as ultraviolet-visible (UV-vis) and infrared (IR) spectroscopies.

The ligand count of the Au<sub>68</sub>(3-MBA)<sub>x</sub> cluster was previously determined to be  $x \sim 32.^{6}$  ESI-MS, XPS and TGA analyses reported in the companion paper indicate that the ligand count x in Au<sub>144</sub>(3-MBA)<sub>x</sub> may be as low as 40.<sup>7</sup> This is in stark contrast to known organo-soluble thiol-protected gold nanoclusters of a similar size, such as the Au<sub>144</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>60</sub> (refs. 9,10). Our <sup>1</sup>H NMR data of Au<sub>144</sub>(3-MBA)<sub>~40</sub> in D<sub>2</sub>O showed one broad feature without resonance fine structure in the aromatic region (5 to 9.5 ppm, Figure 1 and Figure S1, Supporting Information) and no significant correlations were seen in 2D spectra (Figures S2, S3). This differs qualitatively from the previously published <sup>1</sup>H NMR data of Au<sub>144</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>60</sub> <sup>11</sup> and Au<sub>102</sub>(*p*-MBA)<sub>44</sub> <sup>3,12</sup> as well as from Au<sub>68</sub>(3-MBA)<sub>32</sub>, measured in this work (Figure 1 and Figure S4), where a large number of resonance signals are visible in the 1D spectrum and their 2D correlations give detailed information of the local symmetry environments in the ligand layer. Previously, we have been able to make a

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successful assignment of the signals to the specific ligands in the protecting layer of  $Au_{102}(p-MBA)_{44}$  (ref. 12).

UV-vis absorption of  $Au_{144}(3-MBA)_{\sim 40}$  showed a weak plasmonic shoulder around 500 nm (Figure 2), which is absent in organo-soluble  $Au_{144}(SCH_2CH_2Ph)_{60}$ .<sup>9,13</sup> This points to unexpected, significant differences in the response of the metal electrons to light in these two systems that have exactly the same number of gold atoms.

The experimental results described above show unambiguously that the 3-MBA ligand induces very different properties of  $Au_{144}(3-MBA)_{\sim 40}$  from the ones of other gold nanoclusters of similar size. Particularly, the location of the acid group at the 3-position in the aromatic ring (Figure 1) creates a possibility for weak interactions within the ligand layer that are absent in *p*-MBA protected clusters or in organo-soluble clusters. This motivated us to consider computational models (Figure 3) to study the structure and dynamics of the 3-MBA ligand layer of  $Au_{144}$  and  $Au_{68}$ . We built hundreds of structural models (Supporting Information, Section B) of  $Au_{68}(3-MBA)_{32}$  and  $Au_{144}(3-MBA)_x$  with x = 40, 42, 44, 47, 49, 51, 53, and simulated the dynamics of the ligand layer in water and in methanol, with varying degrees of protonation between 0 and 50 %, at 300K by using GROMACS MD software.<sup>14</sup> The coordinates of the gold atoms in the metal cores of the models for  $Au_{68}(3-MBA)_{32}$  and  $Au_{144}(3-MBA)_x$  were taken from refs. 6 and 7, respectively, in addition, the coordinates of the metal core of  $Au_{144}(3-MBA)_x$  are given in Section H, Supporting Information.

Visual inspection of molecular dynamics (MD) trajectories revealed several weak interactions in the ligand layer and at the ligand-gold interface, such as formation of inter-ligand hydrogen bonds, inter-ligand  $\pi$  stacking (aromatic contacts),  $\pi$ -gold interaction where the aromatic ring lies "flat" on the gold core and hydrogen-bonding-like O=C-OH...Au interaction when the hydroxyl group is rotated towards the gold core. This interaction was dynamic, *i.e.*, was continuously formed and broken during the course of the runs. Figure 4 visualizes snapshots of the types of the weak interactions in the ligand layer. Statistical analysis of weak interactions over 50 ns trajectories is shown in Table S6.

Although two 3-MBA ligands can in principle form a hydrogen bond pair, the analysis revealed only 0 to 1.9 hydrogen bonds (Fig. 4f) in the ligand layer for  $Au_{68}(3-MBA)_{32}$  and 0.3 to 5.4 bonds in the ligand layer for  $Au_{144}(3-MBA)_{40-53}$ . The higher deprotonation state was found to promote the higher number of bonds. The low number of the inter-ligand hydrogen bonds could be explained by hydrogen bonding with the solvent molecules and by a rather strong tendency for a competing interaction, namely inter-ligand  $\pi$  stacking. On average, we found 1.4 – 1.5 aromatic contacts per ligand in  $Au_{68}(3-MBA)_{32}$  and 1.3 – 1.8 contacts per ligand in  $Au_{144}(3-MBA)_{40-53}$ , with the higher number of ligands. These numbers indicate a good degree of ligand packing, taking into account that the maximum is two contacts per ligand if all the aromatic rings in the ligand layer would be "sandwiched" between two neighboring ligands.

The analysis revealed also several other interesting and unexpected interactions at the ligand-metal interface (see Table S6 and Figure S4, Supporting Information). It is well known that carboxylic acids can adopt two spatial arrangements in the preferable planar geometry. In these conformations, the hydroxyl group is pointing either parallel or antiparallel to the carbonyl group (*syn* and *anti* conformations, respectively). In the absence of any strong external interactions, *syn* conformer (Figure 4a) has the lower energy and usually it will be the dominating species at room temperature in the gas phase, however, explicit solvent-solute interactions can reduce the *syn/anti* energy difference dramatically in solution. Our MD simulations showed that 3-MBA molecules in the ligand layer frequently transformed to *anti* conformation (Figure 4b) as well as to distinct *syn/anti* 

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 conformations where the OH group was rotated towards a close-lying Au atom at the metal core surface (Figure 4c,d). This gave rather close OH...Au interactions of 0.23 - 0.27 nm. DFT computations based on locally relaxed structures from MD simulations showed that configurations in Figures 4a,c,d are in practice isoenergetic within 0.025 eV (which is less than the inherent DFT inaccuracy of determining energies) and about 0.28 eV more favourable than the *anti* configuration in Figure 4b (note however, that no explicit solvent was considered in the DFT calculations, thus the potential stabilization effect of hydrogen bonding to solvent for the configuration shown in Figure 4b is neglected). In all MD runs we also observed  $\pi$ -gold interactions where the aromatic ring was "lying down" on the core surface (Figure 4e). DFT computations showed that the  $\pi$ -gold interaction stabilizes the "lying down" 3-MBA ligand by 0.15 eV as compared to a "standing up" configuration.

To seek experimental signals of these weak interactions we turned to vibrational spectroscopy (Figure 5). It is well known that the carbonyl stretch frequency is a sensitive measure for the local environment of the O=C-OH moiety. IR data measured for Au<sub>144</sub>(3-MBA)<sub>~40</sub> in deuterated methanol (MeOD) showed three peaks in the carbonyl region at 1695 cm<sup>-1</sup>, 1714 cm<sup>-1</sup> and 1731 cm<sup>-1</sup> <sup>1</sup> while the free 3-MBA and previously studied  $Au_{102}(p-MBA)_{44}$  cluster showed only two peaks. These two peaks belong to the syn and anti conformers of solvated molecules as explained in Supporting information (section C and Figure S9, Supporting Information). For Au<sub>68</sub>(3-MBA)<sub>32</sub> we observed the same three peaks as for the Au<sub>144</sub> cluster at 1692 cm<sup>-1</sup>, 1711 cm<sup>-1</sup> and 1732 cm<sup>-1</sup>. This result implied three distinct configurations of the O=C-OH group in the ligand layer. The two lowest frequencies matched with the signals from the free 3-MBA ligand and with the previously studied Au<sub>102</sub>(p-MBA)<sub>44</sub> cluster in MeOD <sup>15</sup> (Figure 5 and Table 1). Temperature dependence of the data showed that the relative intensities of the peaks changed with the temperature indicating dynamic equilibrium between them (Figures S10 and S11, Supporting Information). Repeating the same measurements for Au<sub>68</sub>(3-MBA)<sub>32</sub> in water under various pH conditions, we observed a broad carbonyl band centered around 1685 cm<sup>-1</sup> at lower pH values where most of the ligands are still protonated but could not resolve individual features within the band (Figure S12, Supporting Information).

We were able to assign the three IR signals measured in MeOD (Figure 5) with help of DFT computations. The computed C=O stretch frequencies of *syn* and *anti* forms of a free 3-MBA are 1727 cm<sup>-1</sup> and 1748 cm<sup>-1</sup>, respectively. For the free 3-MBA we observed frequencies of 1693 cm<sup>-1</sup> and 1715 cm<sup>-1</sup> in MeOD at room temperature, yielding the *anti-syn* difference as 22 cm<sup>-1</sup>. This matched perfectly the computed *anti-syn* frequency difference of 21 cm<sup>-1</sup> and allowed us to assign the peaks at 1695 cm<sup>-1</sup> (Au<sub>144</sub>(3-MBA)<sub>~40</sub>) and at 1692 cm<sup>-1</sup> (Au<sub>68</sub>(3-MBA)<sub>32</sub>) to the *syn* form (Figure 4a) and peaks at 1714 cm<sup>-1</sup> (Au<sub>144</sub>(3-MBA)<sub>~40</sub>) and at 1711 cm<sup>-1</sup> (Au<sub>68</sub>(3-MBA)<sub>32</sub>) to the *anti* form (Figure 4b).

Interestingly, DFT computations done on a locally relaxed configuration of the O=C-OH...Au interaction of 3-MBA in the *anti* form (Figure 4d) gave the C=O stretch of 1761cm<sup>-1</sup> which is 7 cm<sup>-1</sup> higher than the computed value for the "normal" *anti* configuration in Figure 4b and 13 cm<sup>-1</sup> higher than the computed value for the free *anti* 3-MBA. We thus assigned the highest frequency observed for both Au<sub>144</sub>(3-MBA)<sub>-40</sub> and Au<sub>68</sub>(3-MBA)<sub>32</sub> to the O=C-OH...Au interaction visualized in Figure 4d. This interaction at the ligand-metal interface has not been reported before for any thiolate-protected gold nanocluster.

Self-assembling monolayers (SAMs) of thiols on flat gold surfaces have been studied intensively from early 1980's as a convenient system for modifying physical and chemical properties of gold surfaces for applications.<sup>8</sup> A vast majority of the work has been done by using organosoluble thiols,

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which cannot have such interactions with gold as what is discussed here for 3-MBA. However, in a few cases, MBA-SAM systems have been studied.<sup>16,17</sup> Ref. 16 is particularly interesting since it presents a systematic FTIR investigation of SAMs on gold consisting of 2-MBA, 3-MBA and 4-MBA thiols. The freshly prepared 3-MBA SAM shows C=O stretch frequencies of both free (monomer) and associated (dimerized) molecules.<sup>16</sup> Upon aging in H<sub>2</sub>O and CO<sub>2</sub> –free air, the monomer peak shifts to lower frequencies (from 1750 cm<sup>-1</sup> to 1739 cm<sup>-1</sup>) indicating rearrangement of the ligand layer and possibly interaction with the gold surface. It can be noted that the lower frequency is close to our measured peak at 1731 cm<sup>-1</sup>, assigned here to the thiol-gold interaction.

## Discussion

The results reported in this work demonstrate several properties of 3-MBA-protected gold nanoclusters that set them apart from previously characterized organo-soluble or water-soluble gold clusters stabilized by other thiol ligands. Below, the distinct properties of  $Au_{68}(3-MBA)_{32}$  and  $Au_{144}(3-MBA)_{\sim 40}$  are summarized and some of their implications are discussed.

 $C_{I}$ -symmetric ligand layer and low thiol coverage. Our complementary analysis of <sup>1</sup>H-<sup>1</sup>H and <sup>1</sup>H – <sup>13</sup>C correlations using 2D TOCSY, NOESY, <sup>13</sup>C-HSQC experiments indicated 126 signals from the ligand layer of Au<sub>68</sub>(3-MBA)<sub>32</sub>. Since each 3-MBA contributes by 4 distinct signals, this result implies 31 – 32 symmetry-unique ligand environments (Tables S1-S5 and Figures S5-S8, Supporting Information). This count is remarkably close to the previously reported molecular composition of this cluster based on mass spectrometry<sup>6</sup> and shows unambiguously that the symmetry of the ligand layer is  $C_{I}$ . <sup>1</sup>H NMR spectrum for Au<sub>144</sub>(3-MBA)<sub>~40</sub> showed just one broad, unresolved feature in the aromatic region which made the more detailed analysis impossible (Figure 1). Our previously published <sup>1</sup>H NMR spectrum of compositionally precisely determined, organosoluble Au<sub>144</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>60</sub> nanocluster<sup>11</sup> was highly structured, which shows that while the ligand layer of Au<sub>144</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>60</sub> must have a higher symmetry – although still non-determined – the most likely symmetry of the ligand layer of Au<sub>144</sub>(3-MBA)<sub>~40</sub> is  $C_{I}$  just as in Au<sub>68</sub>(3MBA)<sub>32</sub>, since in such case, there are about 160 signals produced by Au<sub>144</sub>(3-MBA)<sub>~40</sub> that merge together in the aromatic region to produce the observed broad feature in Figure 1c.

 $C_1$  – symmetric ligand layer implies chirality. While we have not tried to separate the possible enantiomers of Au<sub>144</sub>(3-MBA)<sub>~40</sub> or Au<sub>68</sub>(3-MBA)<sub>32</sub> clusters, we have calculated the theoretical circular dichroism (CD) spectrum of the model cluster Au<sub>144</sub>(3-MBA)<sub>40</sub>. The spectrum (Figure S13) has strong signals in the visible and near-IR range, which confirms the chiral nature of the model cluster.

The ligand count of Au<sub>144</sub>(3MBA)<sub>~40</sub> is remarkably low as compared to its similar-size organosoluble counterparts Au<sub>144</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>60</sub>,<sup>9,13</sup> Au<sub>133</sub>(SPh<sup>t</sup>Bu)<sub>52</sub> (ref. 18) and Au<sub>130</sub>(*p*-MBT)<sub>50</sub> (*p*-MBT = para methyl benzene thiol, ref. 19). Previous work<sup>20,21</sup> has shown that bulky organosoluble ligands can lead to gold cluster structures and gold:thiol ratios that are significantly different from sterically small ligands. However, our work implies that water-soluble thiol ligands such as 3-MBA can have distinct effects on the structure of the thiol-gold interface in case where the ligand has an additional functionality (weak interaction to gold) to the conventional gold-sulfur bond. Here, our results can be contrasted to a recent work<sup>22</sup> that reports the total structure (from X-ray crystallography) of the 4-MBA protected gold cluster Au<sub>146</sub>(4-MBA)<sub>57</sub>. This cluster, despite having almost the same count of gold atoms as Au<sub>144</sub>(3-MBA)<sub>~40</sub>, has a very different number of thiols, different metal core, metal-thiol interface, and ligand layer.<sup>22</sup> The relatively low ligand density at

the thiol-gold interface found in this work for Au<sub>144</sub>(3MBA)<sub>~40</sub> and Au<sub>68</sub>(3MBA)<sub>32</sub> was found to lead to a situation where, in addition to S-Au bonds, some of the thiols interact with the gold also by intermolecular interactions *via* O=C-OH...Au and  $\pi$ -Au configurations. These interactions, while known to exist in small gold complexes,<sup>23,24</sup> have not been previously discussed in the context of large clusters.

The low ligand count in  $Au_{144}(3MBA)_{-40}$  is also supported by comparison of the computed and measured UV-vis absorption spectrum (Figure 2); the computed spectrum for the atomistic model of Au<sub>144</sub>(3MBA)<sub>40</sub> agrees qualitatively with the experimental data, indicating the experimentally observed weak plasmonic band, while the spectrum of Au<sub>144</sub>(3-MBA)<sub>53</sub> model does not. Note that it has been well established previously that organo-soluble Au<sub>144</sub>(SCH<sub>2</sub>CH<sub>2</sub>Ph)<sub>60</sub> clusters do not show the plasmon peak in the experimental or simulated UV-vis spectrum,<sup>13</sup> which makes a clear contrast to Au<sub>144</sub>(3-MBA)<sub>-40</sub>. From a theoretical viewpoint, one can understand the tendency to support the plasmon as the number of thiols is decreased and number of gold remains the same, as the density of free electrons in the same volume increases.<sup>25,26</sup> The ultimate reasons why 3-MBA thiols stabilize low-symmetry gold nanoclusters with low-density ligand layer are still to be found. Reference MD simulations for Au<sub>102</sub>(p-MBA)<sub>44-x</sub> and Au<sub>144</sub>(p-MBA)<sub>60-y</sub> models with incomplete ligand lavers (x=12, y=19) indicated that  $\pi$ -Au interactions with "lying down" p-MBA ligands are possible as well, but the -OH...Au interaction is not sterically possible (Table S6). We speculate here that the initial 3-MBA/Au<sup>1</sup> polymer phase, from which the clusters nucleate by reduction, already contains weak thiol-gold interactions in addition to the S-Au covalent bonds, which lead to the lower ligand density in the protecting monolayer.

**Reactivity to thiol-modified DNA and proteins.** The existence of the weakly protected gold sites at the thiol-gold interface gives a basis for understanding the reported reactivity of 3-MBAprotected gold nanoclusters toward thiol-modified DNA and proteins with an exposed cysteine,<sup>5</sup> thought previously to occur via thiol-to-thiol exchange reaction. Azubel and Kornberg reported recently<sup>5</sup> (Figure S14) that while reacting 4-MBA (p-MBA) protected gold clusters to thiolmodified oligonucleotides, one sees the original unreacted gold band and a few higher bands where presumably thiol-to-thiol ligand exchange reaction produces 1, 2, ... additions of the new molecule to the cluster. This is an equilibrium reaction and since the binding energies of 4-MBA and thiololigos are presumably close to each other, one sees all the time also the unreacted gold cluster band. On the other hand, in the reaction for 3-MBA protected gold, the original band disappears completely, indicating that the binding of thiol-oligos in irreversible (equilibrium fully in the product side) and all clusters bind at least one thiol-oligo. In the light of our results, this can now be understood: since (a) the weak interactions appearing at the interface of 3-MBA/gold are only of the order of 0.1-0.2 eV and thiol binding is of the order of 2 eV, and (b) the weak interactions are dynamic in water at room temperature, the gold sites protected by these interactions will be all exposed to incoming thiol-oligo, inducing the irreversible binding. Our simulations predict that there is only a low number (on average 3-4) of gold sites per cluster that are protected by the weak interactions, which agrees very well with the observed maximum number of thiol-oligos bound per cluster (Figure S14).<sup>5</sup>

**Catalytic sites at the thiol-gold interface.** Previously, several concepts have been discussed in relation to usage of ligand-stabilized gold nanoclusters as catalysts.<sup>2,27,28</sup> Our work defines a paradigm where utilizing the few weakly protected gold sites of  $Au_{68}(3-MBA)_{32}$ ,  $Au_{144}(3-MBA)_{\sim 40}$  and other nanoclusters, that could be stabilized by ligands bonding to gold *via* dynamic weak interactions, opens avenues for engineering size-controlled, ambient-stable, catalytic gold nanocatalysts that combine the concepts of heterogeneous and homogeneous catalysis. In that

 respect, the suggested  $\pi$ -gold interaction is particularly relevant since lifting the aromatic ring at the interface exposes up to three Au sites for bonding of reactant molecules.

## Conclusions

In summary, our experimental and computational characterization of  $Au_{68}(3-MBA)_{32}$  and  $Au_{144}(3-MBA)_{-40}$  shows that gold nanoclusters stabilized by 3-MBA thiols have several distinct characteristics as compared to many of the previously studied organo-soluble or water-soluble gold clusters. The protecting ligand layer has only  $C_1$  symmetry which imposes an asymmetric metal core. The acidic group in the *meta* position of the aromatic ring in 3-MBA gives rise to weak interactions within the ligand layer and at the ligand-gold interface. These interactions are missing in organo-soluble ligand layers and also in the previously well-studied *p*-MBA-stabilized clusters such as  $Au_{102}(p-MBA)_{44}$ . These interactions may also be indirectly responsible for the unusually low ligand coverage in the  $Au_{144}(3-MBA)_{-40}$ . Several currently unknown compositions and sizes of 3-MBA-protected gold nanoclusters will undoubtedly be found by variations of the known syntheses, which will open unexplored possibilities for applications of these materials in biolabeling, catalyzing biochemical reactions, imaging, detection, and theranostics.

## Methods

**NMR measurements.** For structural characterization of Au<sub>68</sub>(3-MBA)<sub>32</sub> and Au<sub>144</sub>(3-MBA)<sub>~40</sub> clusters, a set of 2D correlation experiments, *i.e.* TOCSY and NOESY for <sup>1</sup>H-<sup>1</sup>H through bond and through space connectivities, respectively, and <sup>13</sup>C-HSQC for <sup>1</sup>H-<sup>13</sup>C one-bond connectivities, were employed along with 1D <sup>1</sup>H NMR and DOSY NMR. TOCSY and NOESY spectra were collected using mixing times of 20 ms and 200 ms, respectively. All NMR spectra were measured in D<sub>2</sub>O at 303 K using Bruker Avance III HD NMR spectrometer, operating at 800 MHz of <sup>1</sup>H frequency, and equipped with a cryogenically cooled TCI <sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N probehead.

**UV-vis and IR spectroscopy.** The FT-IR measurements were done with a Nicolet Magna IR 760 spectrometer using a home-made flow-cell cuvette equipped with  $CaF_2$  windows. The optical path was set to 25 µm for the pure ligand measured in MeOD, and 500 µm for other measurements. Temperature of the sample was controlled with a MiraCool thermostat, by circulating *iso*-propanol in the aluminium cuvette-holder. The system was equilibrated for 30 minutes at every temperature. All spectra were measured with 2 cm<sup>-1</sup> resolution and processed with Happ–Genzel apodization before Fourier transform. 256 or 512 scans were averaged. All spectra presented have the solvent spectrum subtracted. The UV/vis measurements were done with a Perkin Elmer Lambda 850 spectrometer in a 1 mm quartz cuvette.

**Molecular dynamics simulations.** The initial configurations for the ligand layers of  $Au_{68}(3-MBA)_{32}$  and  $Au_{144}(3-MBA)_{40-53}$  were created by using an in-house algorithm (see details on Section B, Supporting Information). The gold core was fixed to the experimental TEM structure.<sup>6,7</sup> The ligand layer was further relaxed and dynamically simulated by using the GROMACS 5.0.4 (ref. 14) MD simulation package with an AMBER force field for thiol-gold interactions, parametrized in our previous work.<sup>29</sup> Each of the structural models were simulated with varying degrees of the protonation state. After initial equilibration phases (see technical details on Supplementary pages

21-22), the statistics was collected over 50 ns.  $Au_{102}(p-MBA)_{44}$  (ref. 1) and  $Au_{144}(p-MBA)_{60}$  (model based on ref. 10) clusters with full and defective ligand shells were also simulated for reference.

**DFT calculations.** Selected configurations from the GROMACS MD simulations were further studied by DFT calculations. We used the code package GPAW<sup>30</sup> which employs the projector augmented wave method (PAW) in the real-space grid. Scalar relativistic effects for gold were included in the PAW setup. Total energy calculation and structure optimization were carried out by using the Perdew-Burke- Ernzerhof (PBE) exchange-correlation functional<sup>31</sup> and a real space grid spacing of 0.2 Å. The structure optimization was carried out until the residual forces were below 0.1 eV/Å. During the optimization, all Au atoms were kept fixed with the positions determined from the TEM experiments.<sup>6,7</sup> The optical absorption spectrum for the optimized structures were calculated by using the linear response time-dependent DFT (LR-TDDFT) as implemented in GPAW.<sup>32</sup> The PBE functional was used for the exchange-correlation kernel, and the spacing in the real space grid was 0.25 Å. The plotted continuous absorption spectra in Fig. 2 of the main text are sums of the individual absorption lines broadened with 0.05 eV Gaussians. IR vibration analysis of selected ligands was done on structures optimized by a tighter criterion with 0.05 eV/Å residual force.

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### **Author contributions**

T.-R.T. and P.P. measured and interpreted the NMR data. B.K. and S.Mu. measured and analysed the IR and UV-vis data. E.P. and S.L. performed the MD simulations. S.Ma. devised the algorithm for creating initial conformations of the ligand layer and performed the DFT calculations on the electronic structure and IR frequencies. G.G., M.P. and H.H. advised the work and contributed to the interpretation of data. H.H. compiled the initial manuscript draft which was commented by all authors.

### Supporting Information available

Figures S1-S14, Tables S1-S6, list of Au coordinates of  $Au_{144}(3-MBA)_{\sim 40}$  from ref. 7, additional NMR analysis of  $Au_{68}$ , details of the computational methods, analysis of MD simulations, analysis of IR data. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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## **Competing financial interests**

The authors declare no competing financial interests.



**Figure 1.** One-dimensional <sup>1</sup>H NMR spectra. a, Aromatic region of <sup>1</sup>H NMR spectrum of  $Au_{102}(p-MBA)_{44}$  in  $D_2O$ -NaOH. b, the same for  $Au_{68}(3-MBA)_{30-32}$  and c,  $Au_{144}(3-MBA)_n$  in  $D_2O$ . Chemical structures of p- and 3-mercaptobenzoic acid ligands with proton labels are shown on right. The data for **a** is taken from ref. <sup>12</sup>.



**Figure 2.** UV-vis absorption spectra. Theoretical spectra obtained from LR-TDDFT computations for  $Au_{144}(3MBA)_n$  models for n=40 (red) and n=53 (blue), compared to the experimental spectrum of  $Au_{144}(3MBA)_{\sim 40}$  (inset). The arrow shows a weak plasmonic band at 510 nm computed for  $Au_{144}(3MBA)_{40}$  agreeing qualitatively well with the band around 500 nm in the experiment.





**Figure 3.** Structural models for 3MBA-protected  $Au_{68}$  and  $Au_{144}$  clusters. **a**,  $Au_{144}$ (3MBA)<sub>40</sub> **b**,  $Au_{144}$ (3MBA)<sub>53</sub> **c**,  $Au_{68}$ (3MBA)<sub>32</sub>. Au: golden, S: yellow, C: dark grey, O: red, H: white.

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**Figure 4.** Inter-ligand and ligand-metal interactions. Various conformations of 3MBA and weak interactions in the ligand layer, found in the MD simulations. **a**, *syn* pointing out, **b**, *anti* pointing out, **c**, *syn* close to gold core, **d**, *anti* close to gold core, **e**, *syn* lying flat on the core and **f**, inter-ligand hydrogen bond. DFT calculations show that structures in a, c, d are practically isoenergetic (within 25 meV).





**Figure 5.** IR spectra of gold clusters.  $Au_{144}(3MBA)_{-40}$  (purple),  $Au_{68}(3MBA)_{32}$  (blue),  $Au_{102}(p-MBA)_{44}$  (brown), free 3MBA (red). The result for  $Au_{102}(p-MBA)_{44}$  is taken from ref. **15.** The arrow indicates the distinct peak around 1730 cm<sup>-1</sup>, observable only in 3MBA-passivated clusters, and interpreted as the signal of the O=C-OH...Au interaction. All spectra were measured in MeOD.

**Table 1.** IR-active C=O modes. Exp = measured, Calc = calculated. Unit is  $cm^{-1}$ . "anti/interface" refers to the –OH...Au interaction shown in Figure 4d.

System	syn	anti	anti/interface
Exp 3-MBA in CCl <sub>4</sub>	1744		-
Exp 3-MBA in MeOD	1693	1715	-
Exp Au <sub>144</sub> (3-MBA) <sub>~40</sub>	1695	1714	1731
Exp Au <sub>68</sub> (3-MBA) <sub>32</sub>	1692	1711	1732
Calc free 3-MBA	1727	1748	-
Calc Au <sub>144</sub> (3-MBA) <sub>53</sub>	-	1754	1761

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