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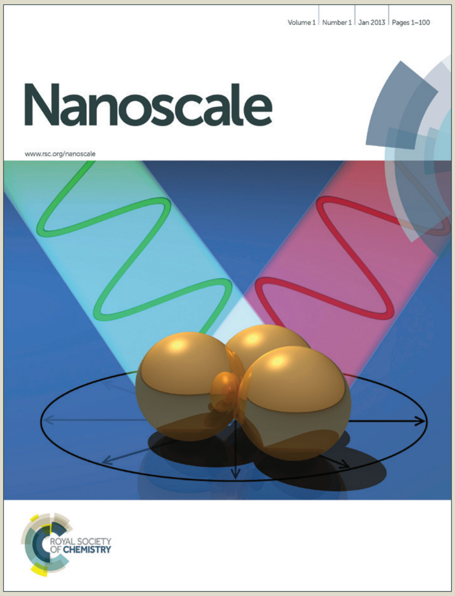

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Solvation Chemistry of Water-Soluble Thiol-Protected Gold Nanocluster Au_{102} from DOSY NMR Spectroscopy and DFT Calculations

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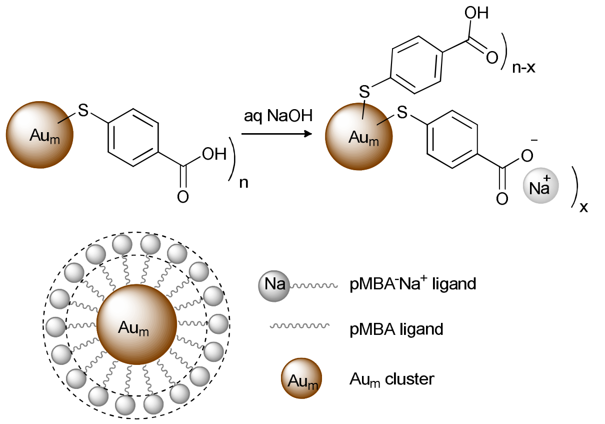
Kirsi Salorinne,[a] Tanja Lahtinen,[a] Sami Malola,[b] Jaakko Koivisto[a] and Hannu Häkkinen*[ab]

Hydrodynamic diameter of $Au_m(pMBA)_n$ [(m,n) = (102,44) and (144,60)] clusters in aqueous media was determined via DOSY NMR spectroscopy. The apparent size of the same (n,m) cluster depends on the counter ion of the deprotonated $pMBA^-$ ligand as explained by the competing ion-pair strength and hydrogen bonding interactions studied in DFT calculations. The choice of the counter ion affects the surface chemistry and molecular structure at the organic/water interface, which is relevant for biological applications.

Water-soluble, thiolate monolayer-protected gold nanoclusters have several biological applications in microscopy and imaging of biomolecules and bionanoparticles, in drug delivery and in disease targeting.[1] As the interface between the gold core and the biomolecules or tissues, the structural properties of the protecting ligand layer become essential when considering the prevailing interactions in a complex biological environment. The well-known and characterized water-soluble $Au_{102}(pMBA)_{44}$ and $Au_{144}(pMBA)_{60}$ clusters are composed of a metal core and a protecting *p*-mercaptobenzoic acid (pMBA) ligand layer (Scheme 1). However, the clusters become water-soluble only when the carboxylic acid group of the pMBA ligand is deprotonated. Therefore, in aqueous solution the $Au_m(pMBA)_n$ clusters in fact exist in a salt form ($pMBA^- X^+$) and depending on the counter ion, the nature and properties of the gold cluster, particularly the solvent/organic interface may change accordingly. Detailed studies of these effects are lacking in the literature.

Here we report DOSY NMR studies of $Au_m(pMBA)_n$ [(m,n) = (102,44) and (144,60)] clusters in aqueous sodium hydroxide and ammonium acetate solutions. It was observed that the diffusion coefficient of the cluster with the same (m,n) depends significantly on the solution, which indicates that the hydrodynamic diameters are different. Density functional theory (DFT) calculations shed light onto this unexpected result. While sodium acts as a simple counter cation with ionic binding to the deprotonated pMBA ligand, the interaction to ammonium cation and residual acetic acid is mediated by intervening water molecules, increasing the hydrodynamic diameter of the cluster significantly in agreement with the experimental observations. Counter ions and solvent molecules thus affect the molecular structure at the interface between the solvent and the organic surface of the cluster.



Scheme 1. Schematic presentation of the $Au_m(pMBA)_n$ clusters as their sodium salts in aqueous NaOH solution highlighting the effect of the sodium counter cation on the hydrodynamic diameter (----) of the cluster. pMBA stands for *p*-mercaptobenzoic acid. Figure is not in scale.

Table 1. Average diffusion coefficients D (m^2s^{-1}) and measured and computed hydrodynamic diameters d_{exp} and d_{calc} (nm) of the $Au_m(pMBA)_n$ clusters in D_2O at 300–302 K.[a].

	D[b]	d_{exp}	Solvent	$D_{solvent}$[c]	d_{calc} (cluster)	d_{calc}[d] (cluster + X)	d_{calc}[d] (cluster + X + nH_2O)
$Au_{102}(pMBA)_{44}$	$1.380 \cdot 10^{-10}$	3.28	D_2O-NaOH	$2.178 \cdot 10^{-9}$	3.11	3.29 (1a)	3.83 (1d)
$Au_{102}(pMBA)_{44}$	$1.135 \cdot 10^{-10}$	4.00	D_2O-NH_4OAc	$2.197 \cdot 10^{-9}$	3.11	3.57 (1b)	3.89 (1h) 4.11 (1e) 4.38 (1g)
$Au_{102}(pMBA)_{44}$	$1.045 \cdot 10^{-10}$	4.34	D_2O[e]	$2.139 \cdot 10^{-9}$	3.11	3.87 (1c)	4.36 (1i) 4.50 (1f)
$Au_{144}(pMBA)_{60}$	$1.142 \cdot 10^{-10}$	3.66	D_2O-NaOH	$2.023 \cdot 10^{-9}$	3.32	3.56 (1a)	4.11 (1d)
pMBA	$5.935 \cdot 10^{-10}$	–	D_2O-NaOH	$1.985 \cdot 10^{-9}$	–	–	–

Diffusion coefficients of *p*-mercaptobenzoic acid (pMBA) and solvent are reported for comparison and for reference, respectively. [a] $Au_{102}(pMBA)_{44}$ clusters were measured at 302 K and $Au_{144}(pMBA)_{60}$ and pMBA ligand were measured at 300 K. [b] Standard deviation of less than 5 %. [c] Literature value for the diffusion coefficient of D_2O at 298 K is $1.872 \cdot 10^{-9}$ m^2s^{-1}.[2] [d] X = Na^+, NH_4^+ or AcOH, see the structures in Figures 1a-i for parenthesis description. [e] NMR sample in D_2O contains residual acetic acid (or acetate).

Materials and Methods

Synthesis

$Au_{102}(pMBA)_{44}$ was synthesized by a modified method (see details in ESI) from a previously published synthesis protocol using *p*-mercaptobenzoic acid (pMBA) as the protecting ligand.[3,4] Larger clusters, commonly referred to as $Au_{144}(pMBA)_{60}$, were prepared as previously reported.[3] All materials were commercial and used as such without further purification.

Analytical Methods

The purity and monodispersity of the $Au_m(pMBA)_n$ clusters were analysed by gel electrophoresis and ^1H NMR and UV-Vis spectroscopy (Figure S1-S4), see details in the ESI. Hydrodynamic size of the $Au_m(pMBA)_n$ clusters were obtained by diffusion ordered NMR spectroscopy (Figure S5-S7), see details in the ESI.

Computational Methods

Isolated pMBA + ion + nH_2O complexes (shown in Figure 1) were studied by using DFT as implemented in the real-space grid projector augmented wave code package GPAW.[5] For structural relaxation we used PBE exchange-correlation functional, 0.2 Å grid spacing and 0.05 eV/Å criteria for the maximum force acting on atoms. Using the relaxed coordinates, model clusters were constructed using the crystal structure of $Au_{102}(pMBA)_{44}$ [6] and the previously published theoretical structure of $Au_{144}(SR)_{60}$.[7] Hydrodynamic diameters of the model clusters were estimated from the radial distribution of all the atoms by doubling the maximal radial value. Molecular dynamics (MD) simulations with DFT forces were done with Langevin dynamics at 300 K using atomic orbital basis set (LCAO, dzp). An assembly of $pMBA^- + NH_4^+ + 4H_2O$, shown in Figure 1g), was surrounded by 196 randomly oriented water molecules, making the total number of water molecules in the system to be 200. Initial density of the water molecules was built to be close to the true expected density. The system was simulated with 1 fs time step for about 4 ps.

Results and discussion

As depicted in Scheme 1, the surface chemistry and the hydrodynamic size of the $Au_m(pMBA)_n$ clusters are expected to depend on the counter ion and the strength of the ionic bond. For this purpose, diffusion ordered NMR spectroscopy (DOSY) is an applicable tool as it allows determination of the hydrodynamic size based on the diffusion coefficient of the cluster and the species bound to it at a given temperature and media. [8,9]

The synthesis protocol of the $Au_m(pMBA)_n$ clusters uses ammonium acetate as the base in the purification step by fractional precipitation, which is convenient as the ammonium counter ion is easily removed under vacuum when necessary leaving the $Au_m(pMBA)_n$ clusters mostly in the protonated form with acetic acid (or acetate) in the residue. Therefore, DOSY NMR experiments were carried out in the presence of Na^+ and NH_4^+ counter cations and residual acetic acid (AcOH) by preparing the NMR samples in NaOH-D_2O, NH_4OAc-D_2O and D_2O solutions, respectively. In order to avoid possible aggregation of the gold clusters due to too high pH or concentration, the sample and the salt concentration was kept as low as possible to maintain water-solubility of the clusters and to ensure enough counter ions to see the effect on the hydrodynamic radius. It was anticipated that in the basic sodium hydroxide and ammonium acetate solutions, the pMBA ligand would be in a salt form, whereas, in the presence of residual acetic acid a hydrogen bond stabilized acid dimer would preferentially form (Figure 1). It was furthermore expected that the hydrodynamic diameter of the $Au_m(pMBA)_n$ cluster would change accordingly, depending on the size of the counter ion or the hydrogen bonded assembly.

The DOSY NMR experiment gave indeed different solution-dependent diffusion coefficients and hydrodynamic diameters for the $Au_{102}(pMBA)_{44}$ and $Au_{144}(pMBA)_{60}$ clusters as shown in Table 1. The expected hydrodynamic diameter for a fully protonated $Au_{102}(pMBA)_{44}$ cluster is 3.11 nm as taken from the crystal structure.[6] When considering the effect of the

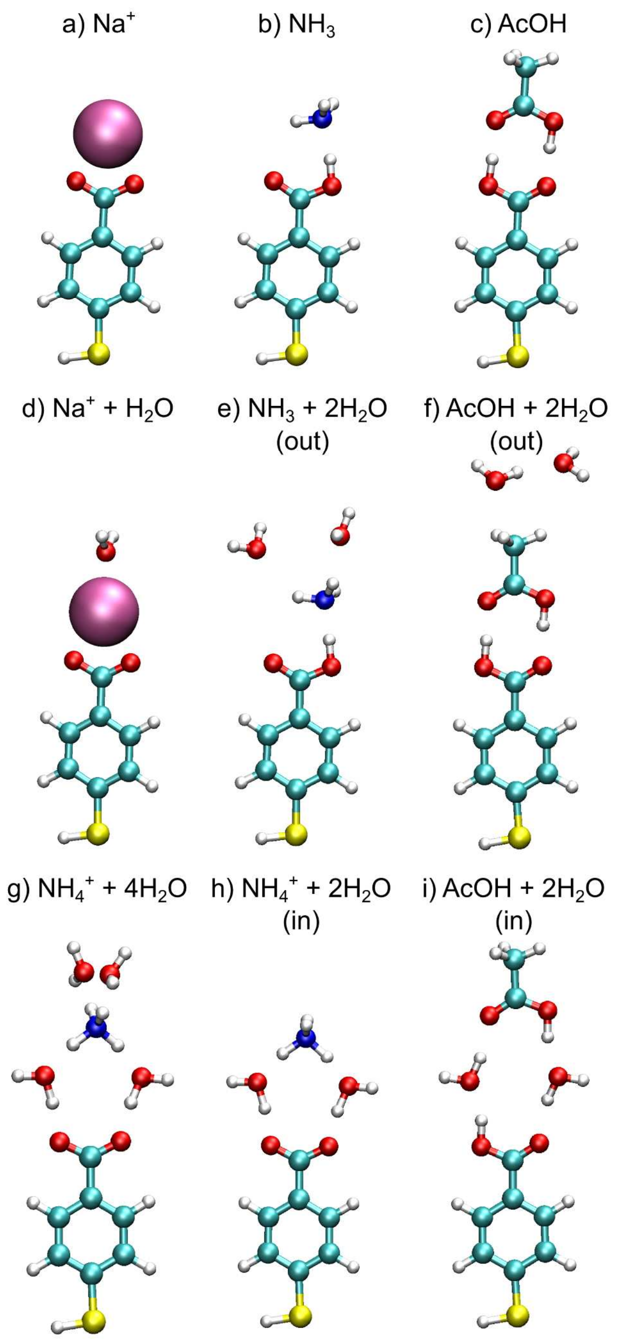


Figure 1. Relaxed structures of pMBA ligand with sodium and ammonium ions and acetic acid are shown in panels a)-b). Relaxed structures of the same complexes with water molecules are shown in d)-f) for the outside configurations of H_2O and in g)-i) for the inside configurations of H_2O. During structural relaxation $pMBA^-$ NH_4^+ pair reconstructed spontaneously to pMBA NH_3 pair regarding configurations b) and e). Coloring: hydrogen white, carbon light blue, oxygen red, sulfur yellow, nitrogen blue, sodium pink.

counter ion or the hydrogen bonded assembly, the calculated hydrodynamic diameter for the $Au_{102}(pMBA)_{44}$ cluster becomes 3.29, 3.57 and 3.87 nm with sodium, ammonium and acetic acid counter parts, respectively (Table 1). In the case of the bigger $Au_{144}(pMBA)_{60}$ cluster the hydrodynamic diameter was determined only in NaOH-D_2O solution. The calculated hydrodynamic diameter for the fully protonated $Au_{144}(pMBA)_{60}$ cluster is 3.32 nm and in a deprotonated form with sodium as the counter ion the hydrodynamic diameter increases to 3.56 nm. Comparison of the calculated hydrodynamic diameters of the $Au_m(pMBA)_n$ clusters with the experimental values obtained by the DOSY NMR experiment showed good agreement for both cluster sizes – Au_{102} and Au_{144} – with sodium as the counter ion (Table 1). In the case of the ammonium ion and acetic acid, however, the experimental hydrodynamic diameters were approximately 0.4 nm larger than expected.

The reason for the discrepancy between the experimental and the calculated hydrodynamic diameters became evident when the ionic strength of the formed $pMBA^-$ X^+ ion pair (X = Na or NH_4), the stability of the hydrogen bonded pMBA···AcOH acid dimer and the surrounding water molecules were considered using DFT calculations. When surrounded by sodium ions, the deprotonated $pMBA^-$ ligand forms a strong $pMBA^-$ Na^+ ion pair shown in Figure 1a and the hydrodynamic diameter is expected to increase from 3.11 nm to 3.29 nm. Surrounding water molecules can bind to the sodium atom outside the cluster as is shown in Figure 1g but because of the non-directional characteristics of Na···H_2O interaction, and the competing directional interactions of water molecules in solution, the effective radius is not expected to increase further.

Structure optimization shows that the $pMBA^-$···NH_4^+ ion pair reconstructs spontaneously to a pMBA···NH_3 pair and the hydrodynamic diameter increases from 3.11 nm to 3.57 nm. This value is not large enough to explain the rather high experimental diameter of 4.00 nm, for which reason water molecules were introduced. Water molecules can bind either directly to pMBA ligand or next to NH_4^+ ion. From the two possible configurations, the energy of the calculated $pMBA^-$···$2H_2O$···NH_4^+ complex (Figure 1h) is 0.516 eV lower compared to the pMBA ···NH_3···$2H_2O$ complex (Figure 1e). The binding energy per water molecule is 0.258 eV for $pMBA^-$···$2H_2O$···NH_4^+ complex as it is only 0.089 eV for the pMBA···NH_3···$2H_2O$ complex. Remarkably, calculated diameters of the water-containing model clusters are in a very good agreement with the experimental results. $pMBA^-$···$2H_2O$···NH_4^+ complex gives a diameter of 3.89 nm, whereas, the pMBA···NH_3···$2H_2O$ complex gives a diameter of 4.11 nm. A complete model cluster of the $Au_{102}(SR)_{44}$ with the most favorable $pMBA^-$···$2H_2O$···NH_4^+ complex is shown in Figure 2.

We further tested the effects of the solvent interactions on the structure of one ligand-counterion $pMBA^-$ / NH_4^+ pair, by immersing the pair in a “water droplet” containing 200 water molecules. DFT-MD simulation at 300 K for a few picoseconds showed that the counter ion-ligand distance fluctuate around two well-defined values corresponding to different configurations of the intermediate water molecules transmitting the ion-pair interactions (Figure S8). These simulations confirm the expectations from the static optimization calculations and show that the water molecules that transmit the ion-pair interactions remain as an integral, but dynamic, part of the organic surface of the $Au_{102}(pMBA)_{44}$ surface. pMBA ligand

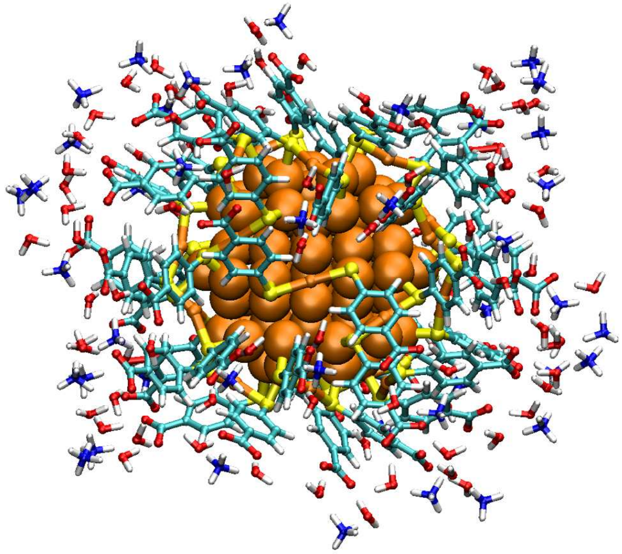


Figure 2. Model cluster of $Au_{102}(SR)_{44}$ build using computationally relaxed $pMBA^-$---$2H_2O$---NH_4^+ complex as a ligand, with an estimated hydrodynamical diameter of 3.89 nm.

and acetic acid behave similarly as pMBA ligand and NH_4^+ cation. The diameter of the cluster modeled with the pMBA···AcOH pair is only 3.87 nm, which is again considerably lower compared to the experimentally measured diameter of 4.34 nm. If water molecules are added, the pMBA···$2H_2O$···AcOH complex (Figure 1i) gives a diameter of 4.36 nm whereas pMBA···AcOH···$2H_2O$ complex (Figure 1f) gives a diameter of 4.50 nm. Similarly to the NH_4^+ ion case, water molecules bind directly to the pMBA ligand rather than outside the acetic acid by an energy gain of 0.590 eV. Binding energies of the water molecules are 0.128 eV/molecule and 0.295 eV/molecule respectively in Figure 1f and 1i, respectively. For the pMBA···AcOH···$2H_2O$ complex (Figure 1f) there is a formation of a H_2O dimer that is weakly bound to acetic acid.

The clearest difference in binding of ammonium ion and acetic acid to pMBA ligand compared to the binding of sodium ion to pMBA ligand is in the support for directional hydrogen bonds together with surrounding water molecules. Directionality of the weak interactions is expected to make the $pMBA^-$ ···$2H_2O$···NH_4^+ and pMBA···$2H_2O$···AcOH complexes more stable and rigid compared to non-directional interactions in sodium complexes.

Conclusions

The hydrodynamic size of water-soluble $Au_m(pMBA)_n$ clusters in aqueous media was studied by DOSY NMR spectroscopy. It was discovered that the hydrodynamic diameter of the *p*-mercaptobenzoic acid (pMBA) protected gold clusters was affected to a great extent by the size and nature of the counter ion of the deprotonated $pMBA^-$ ligand. DFT calculations furthermore showed that competing ion-pair strength and stabilizing hydrogen bonding interactions between the $pMBA^-$ ligand, sodium and ammonium counter ions, residual acetic acid and surrounding water molecules played a role by affecting the nature and the hydrodynamic size of the $Au_m(pMBA)_n$ clusters. In certain cases the solvent molecules may become an "integral" part of the ligand layer. Thus, the surface chemistry of the $Au_m(pMBA^- X^+)_n$ clusters is altered depending on the counter ions, which is an important aspect when considering chemical modifications or biological applications of water-soluble $Au_m(pMBA)_n$ clusters.

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Notes and references

[a] Department of Chemistry, Nanoscience Center, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland. Email: hannu.hakkinen@jyu.fi

[b] Department of Physics, Nanoscience Center, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland.

Electronic Supplementary Information (ESI) available: Synthesis scheme and details, analytical methods and details, UV-Vis spectra, NMR spectra (^{1}H and 2D DOSY) and snapshots from MD simulations of a $pMBA^-$ / NH_4^+ ion pair in water. See DOI: 10.1039/c000000x/

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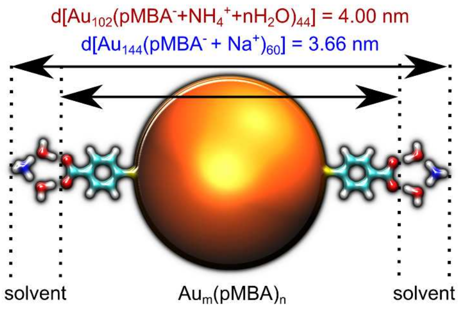

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