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COMMUNICATION

An insight into the optical property of sub nano size glutathione stabilized gold cluster

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In this study, gold quantum clusters with distinct fluorescence property were developed and their structural and physical behaviour evaluated. The clusters were prepared by etching gold nanoparticles with glutathione. Three different Au₃₃ clusters with emission profile in the NIR region and one blue emitting cluster, Au₈ were developed by varying the geometrical arrangement of atoms within the cluster. These clusters having size in the range 0.7 to 2 nm were synthesized by choosing different reaction temperatures from 0°C to 70°C and pH between 1.5 and 10. In three cases, formation of self assembly of atoms within the cluster and corresponding change in optical property was observed. Detailed evaluation of the number of atoms and the core –ligand ratio using MALDI-MS and the change in binding energy as seen in the XPS study confirmed this finding. The study demonstrates that the self assembly of atoms and its arrangement is an important factor in determining the characteristics of the cluster. In this communication, we put forward a new concept where the number of atoms and their arrangement within the cluster plays a crucial role in tuning their optical property.

Thiol protected gold nanoclusters have attracted wide attention in various fields of modern science due to their unique optical property.¹ High stability and tunable fluorescence emission of these nanoclusters facilitate its use in various fields like imaging, catalysis and sensing.^{2,3} The gold – thiol ratio plays an important role in the properties of these materials, as it depends on the degree of polymerization of gold thiol system. Structural and functional properties of these systems also depend on the number of Au (I) atoms with d¹⁰ closed-shell electronic configuration which tend to form aggregation due to aurophilic interaction.⁴ The important attraction of these metallic nanoclusters is its unique optical property which originate due to the quantum confinement of

electrons compared to widely studied metallic nanoparticles.⁵ Even though the higher reactivity, size dependent optical property, catalytic property and nontoxicity of gold nanoparticle favors wide applicability in various biomedical fields including disease diagnosis and therapy, their poor fluorescence quantum yield restricts or rather complicates their use in the applications like imaging.^{6,7} As a result, imaging applications using these systems demand either multiphoton imaging facility^{8,9} or the role of additional conventional dyes^{10,11} both having their own limitations.

In contrast to other gold based nanomaterials, gold nanoclusters are unique because of their inherent fluorescence property which originate due to the extremely small size (<2nm).^{12,13} Chen et al., reported the use of thiol protected gold nanocluster for solar cell applications with a power conversion efficacy of 2.3%.¹⁴ Recently, Negishi *et al* have reported the efficiency of the catalytic activity of these clusters for water splitting property of BaLa₄Ti₄O₁₅ in comparison to nanoparticles.¹⁵ They found that photocatalytic activity for water splitting is 2.6 fold greater than that of gold nanoparticle when Au₂₅ cluster was used. Gold nanoclusters have also attracted wide acceptance in disease diagnosis and its treatment because of its non toxicity, long term stability and higher quantum yield. Recently, we have reported the use of gold cluster for the early stage diagnosis of tumor due to its optical imaging potential and treatment using photosensitizers incorporated onto it.¹⁶

Scope of researches focused on the application of these nanoclusters in various field spans from energy storage to biomedical applications. Variety in the synthesis approach allows the development of nanocluster with emission ranging from blue to red by changing the reaction condition and choosing appropriate precursor (i.e., metal to ligand) ratio.^{17,18,19} While the size dependent fluorescence property of semiconducting nanoparticles is well studied,²⁰ the actual mechanism behind the emission properties of metallic nanocluster still remains as a fantasy. It is known that the inter band and intra band transition within the conduction band is responsible for the fluorescence property in the case of

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Electronic Supplementary Information (ESI) available: UV-Visible and fluorescence spectra, TEM, XPS, theoretical representation and geometric optimization, CT and optical images of clusters and animal image. Characterization of GMSA [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

metallic nanocluster. But the key parameters for tuning these transitions still remain unknown. Efforts are underway to understand these aspects in great detail. In this connection, Negishi et al have studied the transition from the bulk structure to the cluster-specific structure in *n*-dodecanethiolate-protected gold.²¹ Similarly aggregation induced emission phenomena was studied by Gowsami et al., in thiolate protected gold clusters.²² The main aim of the present study is to understand the key factor that is responsible for the unique property of glutathione protected gold clusters. For this, a detailed study was carried out on four different gold nanoclusters developed by varying the reaction conditions like temperature and pH. The rationale for changing the reaction pH is based on the fact that at different pH, the cluster synthesised have different emission. However, effect of temperature on cluster is not yet fully established.

Different sized AuC's were synthesized by etching gold nanoparticles (GMSA) with glutathione (GSH) by varying reaction temperature and pH.¹⁻³ For the preparation of the entire sample, 1: 4 (w/w) ratio of GMSA and GSH were used. For the synthesis of gold nanoparticles, gold chloride was reduced with NaBH₄ using mercaptosuccinic acid as ligand. Briefly, 49 mg HAuCl₄ and 37 mg MSA were dissolved in 25 mL methanol and allowed to keep for 30 minutes. To this system 0.2 M sodium borohydride was added at 0°C and allowed to react for 1 h. The resulting brown colored solution was precipitated in methanol and centrifuged 4 times to get MSA gold nanoparticle (GMSA). For the preparation of AuC1, GSH was mixed with GMSA at room temperature and pH was adjusted to 1.5. The reaction was continued for 12 h at room temperature.

AuC2 was also synthesized similarly as in the case of AuC1, except the fact that the reaction of 12 h was continued at 70°C after adding GSH at room temperature. AuC3 was prepared by adding GSH at 0°C to GMSA and the reaction was continued for 12 h at a temperature 70°C. For the preparation of AuC4, GMSA and GSH were added at room temperature and the pH was adjusted to 10 and the etching process was allowed to complete for 12 h at 70°C.

All the AuC's were purified to remove heavy particles and precipitated in 1:1 ratio of methanol and isopropanol to get crude cluster. These pellets were re-dispersed in water and used for further analysis.

Absorbance spectra of the synthesized nanoparticles (GMSA) showed a broad absorbance, c.a. 530nm (Fig. S1a) and excitation dependent emission characteristics (Fig.S1b) in the visible region. GMSA had an average particle diameter of 7 nm (Fig. S1c). Upon etching GMSA with GSH at different reaction conditions, the emission profile of each resultant clusters were found to be unique. Observed fluorescence of gold nanoparticles is explained by Mooradian as due to the excitation of 5d valence electron to 6sp band, scattering in pico second time with partial energy transfer and its recombination from sp band which results in photon emission.²³ El Sayed and co workers have also explained the same as surface fluorescence in the case of gold nanorod.^{24,25} When the dimension of nanoparticle reduces to the order of

approximately Fermi wavelength of electron, it shows discrete energy level because of the quantum confinement which enhances the fluorescence efficiency of quantum cluster.

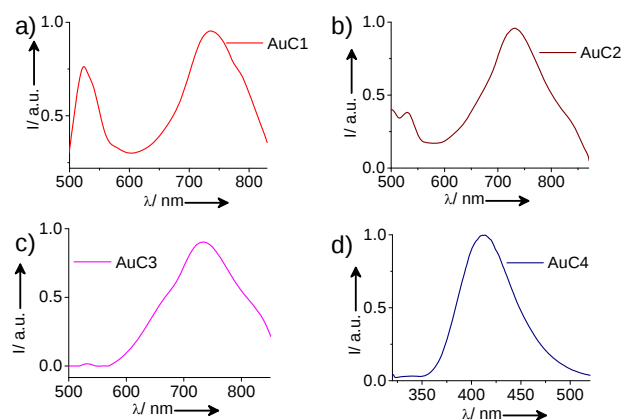


Fig. 1 Fluorescence spectra of AuC1 (a), AuC2 (b), AuC3 (c) and AuC4 (d).

Upon 450 nm excitation, AuC1, and AuC2 (Fig.1a,b) showed peculiar emission profile with two prominent emission peaks c.a. 530 nm and 750 nm with varied fluorescence intensity. To explain the appearance of the two peaks in AuC1 and AuC2, two possibilities are envisaged. First possibility is that there exists a mixture of gold nanoparticles and nanoclusters in these samples due to incomplete clustering of GMSA by GSH. If this is the case, the 530 nm emission profile can be attributed to the nanoparticle emission and 750 nm as the emission from the nanocluster. The second possibility is that the observed dual emission is from the alignment or assembly of the clusters in such a way that it shows the emission corresponding to both particles and clusters. In the case of AuC3 and AuC4, there is only one prominent emission peak, with a substantial blue shift in the case of AuC4 to 412 nm (Fig.1c,d). Shifting of excitation and emission peaks from red to blue is in accordance with the reports of GSH clusters synthesised in the acidic to basic pH.¹⁷ The ratio of 750 to 530 nm peaks is found to be 1.2, 2.5, and 50.42 respectively for AuC1, AuC2 and AuC3. Quantum yield with respect to Nile blue was found to be 0.85%, 2.5 % and 24 % respectively for AuC1, AuC2 and AuC3. The observed enhanced photoluminescence of the clusters can be explained based on the core oxidation and aggregation induced emission, a recently observed phenomenon, as explained by Gowsami et al.,²². The effect of pH and temperature on photoluminescence of different clusters are shown in Table S1. (see supporting information)

TEM analysis of the samples with same concentrations was carried out to understand size and morphology of the clusters. This technique is used to get information on specific arrangement of clusters and formation of aggregates which may shed light on the specific reason for the unique and varied fluorescence property of the different clusters.

TEM (Fig. 2) of AuC1 and AuC2 showed peculiar arrangement of the clusters with plate like and helical assembly respectively. Lower magnification images give a clear indication of the more assembled structures in these cases (fig. S2). The diameter of AuC1 ranges from 0.7 to 2 nm where as that of AuC2 ranges from 0.7 to 1.5 nm (Fig. S3). In AuC3, mono dispersed and well separated clusters with diameter of the order of 0.7nm are observed without showing any self assembly. The absence of a prominent emission peak around 530 nm in AuC3 is an indication to assume that the emission of AuC1 and AuC2 may be due to the self assembled structures. AuC4 also shows definite structural assembly in the TEM study.

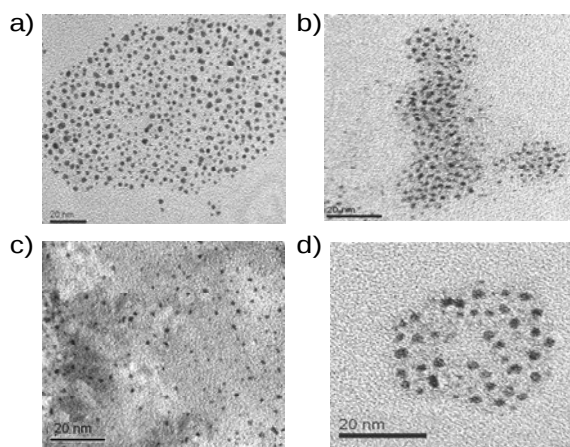


Fig. 2 TEM image of AuC1 (a), AuC2 (b), AuC3 (c) and AuC4 (d). All the scale bars represent 20 nm

Based on this observation, we propose that contribution from the combined electronic transitions of the self assembled structure has resulted in the enhancement of 530 nm emission peak while discrete energy transition within the energy level has resulted in the 750 nm peak. Since the emission profile of this cluster is different from that of others, the explanation for this cluster is given separately.

The self assembly of the cluster cannot be completely confirmed using TEM image as there is a chance for solvent evaporated assembly in TEM. To check this possibility UV-Visible absorbance spectra of the clusters were considered in detail. Broad surface plasmon peak is observed in AuC1, AuC2 and AuC4 around 500 nm to 650 nm. This may be due to the assembly of these nanoclusters in solution. Such a broad peak is absent in the case of AuC3 confirming this and the TEM data of mono-dispersed nature (Fig. S4). Slight variation in the plasmon peak among the samples and different band origin (1.79, 1.53, 1.87 and 1.69 eV) indicates the formation of different assembled structures. A mechanism similar to that of the absorbance of gold nanorods is envisaged here, where a linear assembly of gold nanoparticle results in longitudinal and transverse plasmon peaks.^{26, 27}

To further extend the knowledge on details like cluster ionization and number of atoms and ligands present in the nanocluster, MALDI -MS analysis was performed. Number of core atoms and ligands present in the material plays a crucial role in the optical property of the cluster. It is reported that 750 nm emitting cluster consists of 33 core atoms and 410 nm emitting cluster consists of 8 atoms. Assigning the core atoms accordingly, the number of ligands has been evaluated from the MALDI spectra (Fig. 3). Likewise, the compositions of the clusters were estimated as $(\text{Au})_{33}(\text{SG})_{28}$, $(\text{Au})_{33}(\text{SG})_{28}$, $(\text{Au})_{33}(\text{SG})_{11}$ and $(\text{Au})_8(\text{SG})_2$ for AuC1, AuC2, AuC3 and AuC4 respectively. These assignments were further validated theoretically using *m mass* software (Fig. 3 insets). The fragmentation in the MALDI spectra is attributed to the breakage of gold-thiol bond of the same atomic cluster on laser irradiation. Incomplete ionization was observed for AuC1 and AuC2 in the MALDI spectra. This can be due to the utilization of partial laser energy to break the assembly to individual cluster in addition to the fragmentation of Au-thiol bond. Complete fragmentation is observed in AuC3 for the same laser power (160mW), where self assembly is absent. But conversely, in AuC4, complete ionization was achieved even when there is a well established self assembled structure (Fig. 3d). This is due to the presence of comparatively lesser number of core atoms and ligands, which enabled the desorption of individual cluster and also the Au thiol-bond for the applied laser power.

Developed nanoclusters were further studied for their geometric and structural evaluation using XPS (Fig. S5). A chemical shift was observed in the $4f_{7/2}$ peak for all the clusters (85.18, 85.25, 84.83, 85.21 eV respectively for AuC1 to AuC4).

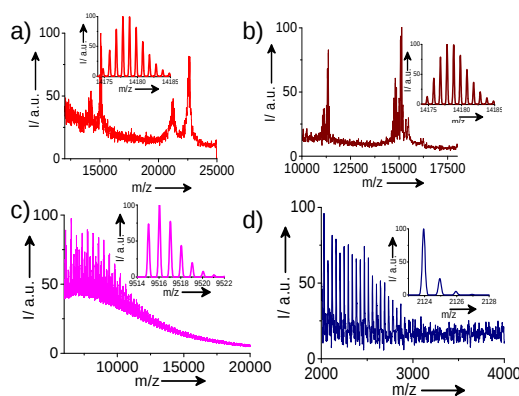


Fig. 3 MALDI spectra of AuC1 (a), AuC2 (b), AuC3(c) and AuC4 (d) Inset shows the corresponding theoretical mass spectra.

Based on the $4f_{7/2}$ peak, Negishi et al and Tanaka and co workers have explained the mechanism of photoemission by de-convoluting the $4f_{7/2}$ peak in the binding energy spectrum of gold.^{4,28,29} The peak shift has been explained based on the 'initial state effect' due to electronic transition from gold core to thiolates, which applies to the observed shift in the present study also.⁴ Slightly higher chemical shift observed for self assembled structures (AuC1, AuC2 and AuC4) is attributed to

the electronic transition from self assembled electron to gold core/ surface gold atoms. From all these studies it is inferred that assembly of individual clusters within the system play an important role in tuning the optical property. In nutshell, we can tune the cluster property by suitably modifying the reaction conditions.

Compiling the results of number of atoms per cluster, size of the cluster and observed self assembly, a plausible mechanism is explained in correlation with the reaction conditions. Accordingly, in the first case of AuC1, where the reaction takes place at room temperature, equilibrium of GSH is between two carboxylic groups, amine group and one SH group when the pH is 1.5. GSH is known to have a tendency to form linear assembly because of the presence of acid and amine moiety in it.³⁰⁻³² During this reaction, MSA gets replaced by GSH through the S- moiety forming 33 atoms to form AuC1. Further, COO⁻ will tend to form assembly with NH₃⁺ moiety of another AuC1 and the assembly continues. While balancing between the formation of stable cluster and self assembly at room temperature, uniformity is compromised which resulted in the occurrence of different sized particles in the TEM (Fig. 2a). But when GSH was added at room temperature and reaction is continued at 70°C (AuC2), etching takes place uniformly. Here, addition of GSH at room temperature results in the assembly between COO⁻ of one cluster and NH₃⁺ of another cluster, exhibiting a helical appearance to the assembly. When GSH was added at 0°C and the reaction was performed at 70°C (AuC3), the system overcomes the tendency to form self assembly accomplishing the most stable cluster of the study.

In the fourth case, at pH 10 the GSH equilibrium is between four carboxylic oxygen atom, amino group and thiol group. MALDI analysis reveals that eight gold atoms form a cluster with two GSH ligands, but with an increase in the particle size compared to other clusters. This is because the individual eight atoms of gold are slightly separated in AuC4 compared to thirty three atoms in AuC1 and AuC2 imparting bigger size to AuC4, in the TEM image. The most suitable way to explain the mismatch between number of atoms and size leads to the arrangement in such a way that 8 gold atoms interact with the negative charges of four carboxylic groups together with a centralized interaction by the thiol group so that it forms a spherical shape with spacing at the centre (Fig. 2d). Formation of different clusters is depicted schematically in fig. 4. This mechanism was confirmed with Raman spectral analysis of the samples (Fig. S6 & S6 a-d)

A theoretical representation and geometrical optimization was carried out in order to confirm the above findings. Theoretical calculations were done using *Avagadro* software (advanced molecule editor and visualizer designed for cross-platform use in computational chemistry, molecular modeling). Geometrical optimization was done using *LandI3* basic set using *GAFF* force field. For the sake of simplicity in mathematical simulation, gold atom-ligand ratio of 33:28 of AuC1 and AuC2 was reduced to 1:1, and 33:11 and 8: 2 of AuC3 and AuC4 respectively were reduced to 3:1. and 4:1. For the accuracy of mathematical simulation, same numbers of representative units of the clusters (4 numbers) were used in all the cases (Fig. S7).

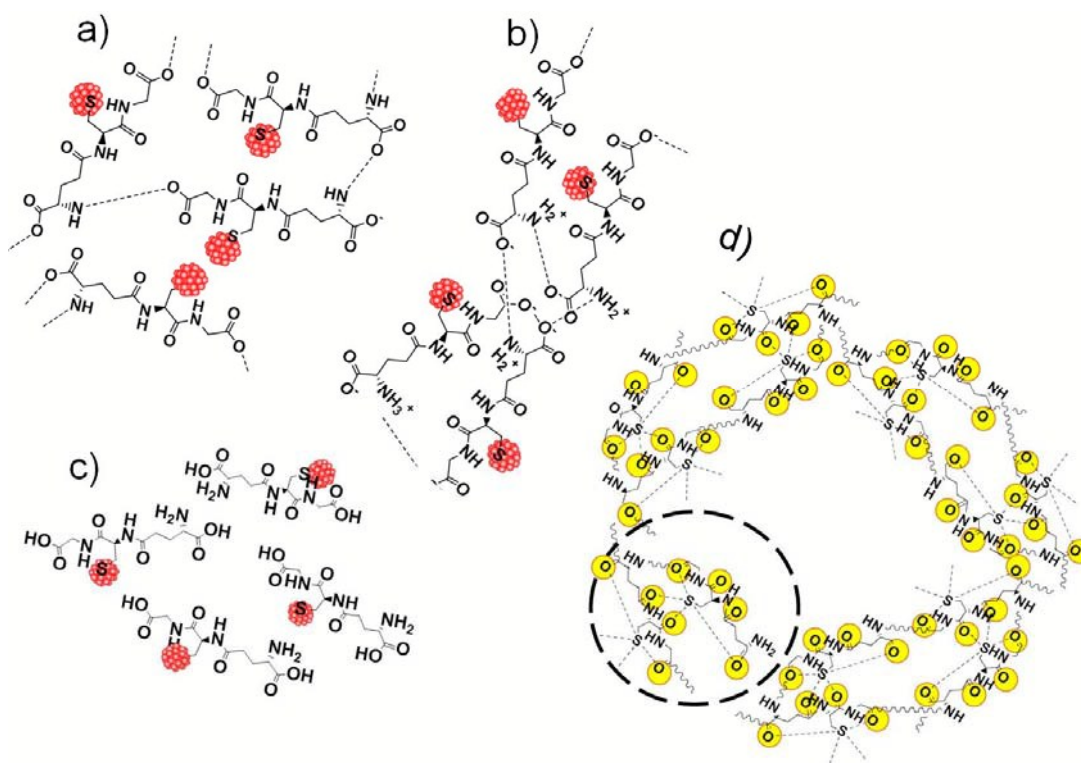


Fig. 4 Schematic representation of the formation of clusters -AuC1 (a), AuC2 (b), AuC3 (c) and AuC4 (d).

complete representation of the assembled system is shown in the typical case of AuC4 where the number of atoms and ligands involved are less (Fig. S8). The prediction of AuC4 exactly mimics the TEM image of circular patterns with a central gap. Theoretical energy calculations for the self assembled structures predicted highest energy of 1442.58 kJ/mol for AuC4. AuC1 and AuC2 have energies of 161.16 and 50.99 kJ/mol respectively. The calculated energies of the clusters justify the experimental findings in all the cases. AuC3 has the minimum energy of 9.2593 kJ/mol which accounts for its high stability among the clusters. The higher energy in the case of AuC4 is an indication of strong assembly among individual atoms and is reflected in the blue shifted fluorescence emission. Helical assembly is more stable than the non uniform assembly as is evident from the slightly different energies between AuC1 and AuC2.

The optical property of the clusters can be used for applications like sensing and imaging. In this connection the AuC3 of this study has been used as a sensor for detecting urea from whole blood and milk.³³ Moreover, the materials are found to have good optical and X ray contrast. Potential of the developed clusters for radiological CT imaging (Fig.S9) and optical imaging were evaluated (Fig. S10) using respective imaging modalities. It is clear from the CT images that AuC4 gives best image contrast with maximum hounsfield unit (HU) and AuC3, the least. Extremely small size and the monodispersed nature of AuC3 would have caused the less CT contrast compared to self assembled one. For optical imaging AuC3 leads with very good imaging potential as expected from its highest quantum yield originated due to its unique optical property. To check the *in vivo* optical imaging efficacy, AuC3 was subcutaneously injected in normal mice and imaged using Optical imaging system (IVIS Spectrum). Signal from AuC3 of subcutaneous tissue was sufficient for considering the cluster for live animal imaging applications (Fig.S11). Animal experiments were approved by the institutional animal ethics committee.

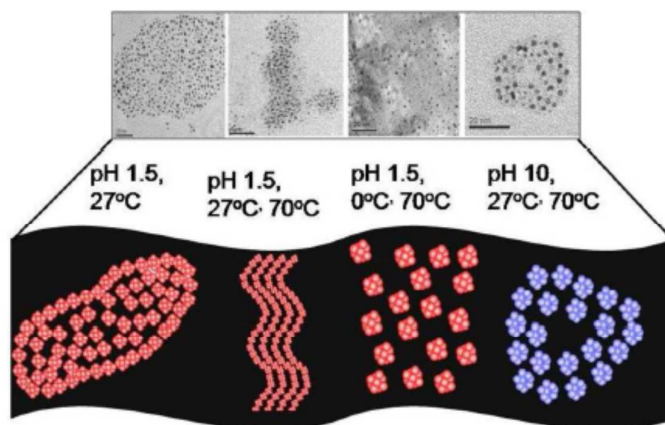
To conclude, we have developed four different gold nanoclusters and studied their properties in detail. Reaction conditions like temperature and pH have major responsibility in the cluster formation. The contribution of these factors on the size, number of atoms of the cluster, and the fluorescence properties has been explained. It is found that initial temperature and the reaction temperature plays a crucial role in the self assembly of monolayer protected cluster where as pH of the reaction determines the number of core atoms and ligands. Based on the results of absorbance, emission properties and MALDI, a probable composition was also assigned for all the clusters studied.

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Notes and references

- 1 Y. Negishi, Y. Takasugi, S. Sato, H. Yao, K. Kimura and T. Tsukuda, *J. Am. Chem. Soc.*, 2004, **126**, 6518.
- 2 L. Y. Chen, C. W. Wang, Z. Yuan and H. T. Chang, *Anal. Chem.*, 2015, **87**, 216.
- 3 X. Qu, Y. Li, L. Li, Y. Wang, Y.; J. Liang and J. Liang, *J. Nanomater.*, 2015, **2015**, 784097.
- 4 Y. Negishi, K. Nobusada and T. Tsukuda, *J. Am. Chem. Soc.*, 2005, **127**, 5261.
- 5 R. Jin, *Nanoscale*, 2010, **2**, 343.
- 6 J. Zheng, C. Zhou, M. Yu and J. Liu, *Nanoscale*, 2012, **4**, 4073.
- 7 S. Eustis and M. A. El-Sayed, *J. Phys. Chem.*, B2005, **109**, 16350.
- 8 X. Huang, I. H. El-Sayed and M. A. El-Sayed, *Methods Mol. Biol. Clifton NJ*, 2010, **624**, 343.
- 9 X. Huang, I. H. El-Sayed, W. Qian and M. A. El-Sayed, *J. Am. Chem. Soc.*, 2006, **128**, 2115–2120.
- 10 Bhattacharyya, B. S. Goldschmidt, M. Hannink, S. Alexander, *J. Viator and Clin. Lab. Med.*, 2012, **32**, 89.
- 11 Mieszawska, W. J. M. Mulder, Z. A. Fayad, D. P. Cormode, *Mol. Pharm.*, 2013, **10**, 831.
- 12 J. Zheng, P. R. Nicovich and R. M. Dickson, *Annu. Rev. Phys. Chem.*, 2007, **58**, 409.
- 13 J. Zheng, C. Zhang and R. M. Dickson, *Phys. Rev. Lett.*, 2004, **93**, 077402.
- 14 Y. S. Chen, H. Choi and P. V. Kamat, *J. Am. Chem. Soc.*, 2013, **135**, 8822.
- 15 Y. Negishi, M. Mizuno, M. Hirayama, M. Omatoi, T. Takayama, A. Iwase and A. Kudo, *Nanoscale*, 2013, **5**, 7188.
- 16 L. V. Nair, S. S. Nazeer, R. S. Jayasree and A. Ajayaghosh, *ACS Nano*, 2015, **9**, 5825.
- 17 M. A. H. Muhammed, S. Ramesh, S. S. Sinha, S. K. Pal and T. Pradeep, *Nano Res.*, 2010, **1**, 333.
- 18 A. Tlahuice-Flores, R. L. Whetten, M. Jose-Yacamán, *J. Phys. Chem. C*, 2013, **117**, 20867.
- 19 S. Wang, X. Zhu, T. Cao, M. Zhu, *Nanoscale*, 2014, **6**, 5777.
- 20 K. D. Wegner and N. Hildebrandt, *Chem. Soc. Rev.*, 2015, **44**, 4792.
- 21 Y. Negishi, T. Nakazaki, S. Malola, S. Takano, Y. Niihori, W. Kurashige, S. Yamazoe, T. Tsukuda and H. A. Häkkinen, *J. Am. Chem. Soc.*, 2015, **137**, 1206.
- 22 N. Goswami, Q. Yao, Z. Luo, J. Li, T. Chen, J. Xie, *J. Phys. Chem. Lett.* 2016, **7**, 962.
- 23 A. Mooradian, *Phys. Rev. Lett.* 1969, **22**, 185.
- 24 M. B. Mohamed, V. Volkov, S. Link, M. A. El-Sayed, *Chem. Phys. Lett.* 2000, **317**, 517.
- 25 S. Eustis, M. A. El-Sayed, *J. Phys. Chem. B* 2005, **109**, 16350.
- 26 A. Gole and C. J. Murphy, *Chem. Mater.*, 2004, **16**, 3633.
- 27 T. K. Sau and C. J. Murphy, *Langmuir*, 2004, **20**, 6414.
- 28 A. Tanaka, Y. Takeda, M. Imamura and S. Sato, *Phys. Rev. B*, 2003, **68**, 195415.
- 29 Tanaka, Y. Takeda, T. Nagasawa and K. Takahashi, *Solid State Commun.*, 2003, **126**, 191.
- 30 C. Durgadas, L. V. Nair, C. P. Sharma and K. Sreenivasan, *Sens. Actuators B Chem.*, 2011, **156**, 791–797.
- 31 S. Basu and T. Pal, *J. Nanosci. Nanotechnol.*, 2007, **7**, 1904.
- 32 P. K. Sudeep, S. T. S. Joseph and K. G. Thomas, *J. Am. Chem. Soc.*, 2005, **127**, 6516.
- 33 L. V. Nair., S.P. Divya, R. S. Jayasree, A. Ajayaghosh, *Small*, 2013, **16**, 2673.



Overall property of gold cluster depends on the number and arrangement of atoms, as tuned by the reaction conditions