INTRODUCTION

Typically, tens to hundreds of Au atoms constitute gold nanoclusters (Au NCs) with diameters between 2 and 3 nm. Au NCs are generally synthesized by accumulation of Au ions aided by ligands or protein that protect the Au core.1,2 The widely used two-phase synthetic strategy in the Brust–Shiffrin method is often used to obtain \([\text{Au}_n(\text{SR})_m]\) type thiolate-protected Au NCs of uniform size distribution, where \(n\) is the number of Au atoms, \(m\) is the number of thiolate ligands (SR), and \(q\) is the total valence charge on the cluster (if any).3,4 As a result of the ultrasmall size, these nanoclusters are considered as quantum particles where discrete valence electronic states are found for Au, instead of a quasi-continuous 5d band, and hence they adopt molecule-like properties including fluorescence emission due to transition between highest occupied molecular orbital and lowest unoccupied molecular orbital.5–7

Although various types of protecting ligands are used to synthesize Au NCs, among all, the thiolate ligands show excellent stability and versatility in surface functionalization.8 Gold-thiolate protecting units are created by the thiolate group (–SR) by strongly disturbing the surface structure of the Au core.8 Expectedly, due to their great potential, the thiolate-protected Au NCs have become inevitable components for applications in the field of catalysis, sensing, fluorescence labeling, biomedicine, and so on.9–12

In this regard, small naturally occurring peptides, such as glutathione or GSH, have proved to be good surface ligands for Au NCs, which help them escape the reticulo-endothelial system absorption and hence resulting ease of deposition in tumors.13,14 Stress-induced cytotoxicity of thiolate-protected chiral Au NCs has been critically explained by Zhang et al.15 They have also shown that these Au NCs can be utilized in targeted photodynamic therapy as they possess superior penetration and retention in tumors.16 Such Au NCs can even reprogram DNA epigenetic patterns.17 Reports have shown that among the glutathione-protected (SG) Au NCs, Au25(SG)18 is particularly stable compared to the stability in the other sizes.18,19 Wu et al. have clarified the structural stability of Au25(SG)18 with two types of Au–S binding.20 They have explained the uniqueness of Au25(SR)18 clusters where, except the central gold atom in the Au-icosahedron, all of the 24 gold atoms in the two shells are bonded to SG.

Cyclodextrin (CD)-capped Au–SG NCs have been used to probe Ag(I) in water in the concentration range between 0.5 nM and 0.1 μM, with a detection limit at 0.3 nM.21 In another report, Mathew et al. have presented interaction between an atomically precise 25-atom Au NC anchored to a ligand and β-CD.22 They have showed that the CD moieties present on the cluster surface provide enhanced stability. They have also mentioned their potency in biology and toward supramolecular functionalization. Similar study on the interaction of adamantine-terminated Au NCs with α-, β-, and γ-CDs has been reported by Yan et al.22 They found β-CDs to be better...
candidates compared with \( \alpha \)- and \( \gamma \)-CDs to efficiently chemisorb onto the Au NCs.

The Au25(SG)18 clusters display strong one- and two-photon excitation emission and are applied as fluorescence contrast agents for live cell imaging.\(^{24}\) They are photostable and biocompatible and have very low toxicity to live cells even at high concentrations. These Au NCs typically emit at 650 nm due to one-photon excitation.\(^{24}\) Considering the significance, stability, and biocompatibility of the Au25(SG)18 clusters, herein we have demonstrated that on interaction with CDs of various cavity space dimensions, these clusters form suprastructures that are either spherical or cubic. The structural modifications induce extensive effects on the photophysics of the Au NCs, which has been ascribed to collisional quenching of energy.

**RESULTS AND DISCUSSION**

The ultrasmall GSH-coated Au NCs were synthesized following a reported protocol.\(^{25}\) It is also suggested that these Au NCs are excellent candidates for radiotherapy, causing negligible damage to normal tissues, and can be efficiently cleared by the kidney.\(^{13}\) The synthesized GSH-protected Au NCs were characterized by transmission electron microscopy (TEM), energy dispersive spectroscopy (EDS), and absorption and emission spectroscopies (Figure 1a–d). The characterization data show that the Au NCs have around 2 nm diameter, and the EDS data confirm the presence of Au in the clusters. The absorption and emission spectra typically match with Au29–43(SG)27–37 clusters.\(^{13}\)

In a previous report, we showed that l-cysteine can form a double layer while protecting the Au NCs with the cysteinyi thiol groups projecting outward. As thiol group is hydrophobic, the hydrophobic CD cavities could encapsulate them to accumulate around the Au NCs.\(^{26}\) The CD sizes developed spherical to cubic suprastructures with embedded Au NCs. This work was performed with eight-atom blue-emitting Au NCs. On the basis of a similar principle, we have developed the present work, where the Au NCs have around 29 Au atoms and hydrophobicity was induced tactically so that the different stages of formation of the suprastructure can be clearly understood.

The GSH-coated Au NCs \((\text{Au}_{n}(\text{SG})_{m})\), where the GSH molecules attach to the Au atoms through the S atom, have two projected arms on both sides of the S atom that contain two carboxyl groups. The initial acidic solution of the Au NCs (pH 3–4) was made alkaline (pH 8–9) to convert the \(-\text{COOH}\) groups to \(-\text{COO}^-\) keeping the Au NCs intact, which was checked spectroscopically, and the \(\zeta\)-potential was calculated (Figure S1), which confirmed the negative surface charge of the Au NCs. At this pH, we added cetyltrimethylammonium bromide (CTAB) (a cationic surfactant) to the solution. Owing to the electrostatic interaction between the cationic head group of CTAB and the anionic \(-\text{COO}^-\) groups of the protecting thiolate, the Au NCs became hydrophobic as the hydrocarbon tails of the CTAB molecules protrude outward. This process gradually reduces the fluorescence of the Au NCs as they separate out from the aqueous environment due to hydrophobicity (Figure 2). To avoid formation of CTAB micelles, the surfactant concentration was kept sufficiently low.

As mentioned earlier, CDs are excellent candidates for host–guest chemistry as their hydrophobic cavities readily encapsulate hydrophobic guests. On addition of \(\alpha\), \(\beta\), and \(\gamma\)-CDs (inner cavity diameters are approximately 5.7, 7.8, and 9.5 Å, respectively),\(^{27}\) we observed modification of the spectroscopic characteristics of the Au NCs. On addition of CDs to the slightly alkaline solution of GSH–CTAB-coated Au NCs, the pH became neutral and hence the guest–host chemistry took place at pH 7. To avoid unwanted aggregation of the CDs, comparable concentrations were added to the NCs.
spheres. This is possible as the rims of the CDs contain minute spherical units have assembled together to form larger dimension and texture of the aggregates indicate that multiple phobic tails of the surrounding CTAB molecules. The suprastructures grew up due to incorporation of the hydrophobic tails of the CTAB molecules. The concentration of the Au NCs was 0.1 mM in water (pH 8–9).

Figure 2. Fluorescence intensity of GSH-coated Au NC (black) decreases on addition of 0.01–0.04 mM CTAB ($\lambda_{\text{ex}} = 365$ nm). Concentration of the Au NCs was 0.1 mM in water (pH 8–9).

CD could approximately double the quenched emission of the Au NCs, and $\beta$-CDs tripled it. The unusual behavior of $\gamma$-CD attracts attention and hence creates avenues for further studies.

The scanning electron microscopy (SEM) micrographs (Figure 4) show that on addition of the three types of CDs, different self-assembled suprastructures were developed incorporating the GSH–CTAB-coated Au NCs. Existence of Au NCs inside the CD suprastructures has been confirmed by collecting EDS data, as shown in Figure S3. Enhancement of fluorescence emission from the Au NCs resulted due to solubilization of the insoluble hydrophobic GSH–CTAB–Au NCs by $\alpha$- and $\beta$-CDs. In both these cases, spherical suprastructures grew up due to incorporation of the hydrophobic tails of the surrounding CTAB molecules. The dimension and texture of the aggregates indicate that multiple minute spherical units have assembled together to form larger spheres. This is possible as the rims of the CDs contain hydroxyl groups that are capable of forming hydrogen bonds and connect similar units. However, we found cubes on addition of $\gamma$-CDs to the GSH–CTAB-coated Au NCs. The difference in the texture of the aggregates is, thus, presumed to be due to the difference in the dimension of the CDs. The probable nature of arrangement of the CDs around the GSH–CTAB-coated Au NCs is presented in Figure S5. Although the sizes of the $\alpha$- and $\beta$-CDs allow approaching in multiple numbers around the Au NCs, the bigger size of the $\gamma$-CDs can ideally accommodate six of them. The number (six) of $\gamma$-CDs could be proposed by considering the size of the Au NCs and the CD. The diameter of the Au NCs is $\sim 2$ nm, and the outer rim size of $\gamma$-CD is approximately 1 nm. Hence, the estimated number of $\gamma$-CDs that can surround one Au NC could not be more than six due to space limitation. This initiates the formation of a cubic unit cell that multiplies through intermolecular hydrogen bonding between the $\gamma$-CDs to create the bigger cubic suprastructures.

Typically, the $\alpha$, $\beta$, and $\gamma$-CDs have 13.7, 15.3, and 16.9 Å outer diameters, respectively. The loosely arranged $\alpha$-CDs around the GSH–CTAB-coated Au NCs due to their smallest size could double the quenched fluorescence emission of the GSH–CTAB-coated Au NCs by solubilization, whereas the bigger $\beta$-CDs could enhance the emission further due to more compact arrangement. However, the fluorescence from the Au NCs was further quenched on interaction with the $\gamma$-CDs, which is the largest within the CD family. The results indicate better solubilization of the Au NCs with $\alpha$- and $\beta$-CDs than that with $\gamma$-CDs. Moreover, there can be collisions between the fluorescing suprastructures, which may result in partial quenching of fluorescence due to energy transfer. Although for $\alpha$- and $\beta$-CDs the latter are subdued by solubilization, it is overwhelming for $\gamma$-CD. The reason could be the shape of the suprastructures. The area of contact between colliding spheres is much less compared to that of cubes, which leads to better energy dissipation for the cubic Au NC-embedded $\gamma$-CD suprastructures.

Time-resolved fluorescence measurements on the Au NCs and composites support our proposition, as given in Table 1. The pristine GSH-protected Au NCs decay data take triexponential fit due to the present components, which are Au(I) ($\tau_1$), Au(0) ($\tau_2$), and electron transfer between Au(I) and Au(0) ($\tau_3$). The data show that the contribution of $\tau_3$ increases considerably on addition of CDs to the GSH–CTAB-protected Au NCs. This probably happens due to the aggregation of Au NCs into larger entities where intracluster electron transfer between Au(I) and Au(0) can occur along with the intracluster process. The other excited state lifetimes are comparable and indicate that the difference in texture of the aggregates could be the principle cause for the variation in photophysics of the Au NCs on interaction with the different CDs. The collisional energy transfer between the suprastructures generally takes place at a very fast time scale (few picoseconds) and hence could not be monitored by the present setup.

**CONCLUSIONS**

GSH-protected Au NCs have been developed in acidic medium, followed by dehydrogenation of the thiolates under alkaline condition to electrostatically interact with the cationic heads of CTAB, resulting in conversion of the hydrophilic Au NCs into hydrophobic ones and hence eliminating their fluorescence due to separation from water. The fluorescence was differentially revived by $\alpha$- and $\beta$-CDs through spherical suprastructure formation that was seeded by spherical unit cells through hydrogen bonding between the surrounding CDs. Accumulation of six $\gamma$-CDs around one GSH–CTAB-coated Au NC, due to size restriction, developed cubic unit cells that grew into microcubes and collided to shed energy to the environment. Hence, no increase in fluorescence emission was
observed in this case. The results, on the one hand, provide important information on guest–host interaction between CDs of various sizes and Au NCs and, on the other hand, provide interesting mechanisms for suprastructure formation in solution through self-accumulation via hydrogen bonding. Moreover, the ingredients are biocompatible, and hence the outcome from the present work has biological relevance as CDs are often used as carriers for biomarkers. The work opens new methods of applications in the fields of constructing logic gates or on/off molecular switches.

## MATERIALS AND METHODS

All the chemicals were procured from Sigma and used as received without further purification. The solutions were prepared in triple distilled water. The absorption spectra were recorded by a Hitachi U-2900 spectrophotometer. The steady-state fluorescence measurements were carried out using a QM 40 spectrophuorimeter from PTI Inc. The time-resolved fluorescence experiments were carried out using a Horiba Jobin Yvon Fluorocube instrument with a 377 nm diode laser excitation source (with a temporal resolution of 70 ps) using time correlated single photon counting method. Scanning

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**Table 1. Time-Resolved Fluorescence Data for the GSH-Protected Au NCs, and the Resulting Composites with the Different CDs**

| Sample Description | \(\tau_1\) (ns) | \(\tau_2\) (ns) | \(\tau_3\) (ps) | \(\chi^2\) | \(\tau_{avg}\) (ns) |
|--------------------|-----------------|-----------------|-----------------|-----------|--------------------|
| Au GSH             | 7.19 (5)        | 124 (88)        | 150 (7)         | 1.02      | 109.56             |
| Au GSH + 0.04 mM CTAB + 0.4 mM \(\alpha\)-CD | 1.31 (2)        | 86.48 (23)      | 70 (75)        | 1.18      | 19.57              |
| Au GSH + 0.04 mM CTAB + 0.4 mM \(\beta\)-CD | 3.71 (3)        | 93.48 (31)      | 160 (67)       | 1.10      | 28.77              |
| Au GSH + 0.04 mM CTAB + 0.4 mM \(\gamma\)-CD | 4.04 (2)        | 95.10 (27)      | 190 (71)       | 1.20      | 26.20              |

*Values in parentheses are the percentage contributions to the fit for the respective components. The \(\chi^2\) values indicate goodness of fits to the raw data.*

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electron microscopic (SEM) images were taken from Zeiss (supra) instrument. Electron dispersive spectroscopy (EDS) was performed using a JEM-2100F field emission gun electron microscope with EDS, diffraction pattern software, and high-angle annular dark-field scanning transmission electron microscopy detector. Transmission electron microscopy (TEM) images were recorded with a JEOL, JEM-2100F microscope using a 200 kV electron source at the DST-FIST facility, IISER, Kolkata. ζ-Potential measurements were performed in Nano Particle Analyzer SZ-100 from Horiba Scientific.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01914.

Methods of synthesis, characterization, instrumental methods, and additional supporting data (PDF)

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Notes

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