Light-Triggered Reversible Supracolloidal Self-Assembly of Precision **Gold Nanoclusters**

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and formation of disc-like colloidal superstructures ($d \sim 100-1000$ nm). The superstructures readily disassemble into individual nanoclusters upon irradiating with visible light at 435 nm. Systematic changes in both the electronic absorption bands and nuclear magnetic resonance spectra of chromophores in solution



suggest that the photoisomerization of surface ligands drives the self-assembly. High-resolution transmission electron microscopy, electron tomographic reconstruction, dynamic light scattering, and small-angle X-ray powder diffraction show that the disc-like superstructures contain densely packed nanoclusters. Long-range self-assembly and disassembly under ultraviolet and visible light, respectively, demonstrate reversible photoswitching in nanoclusters.

KEYWORDS: photoisomerization, switchable Au₂₅ nanoclusters, light-stimulated reversible assembly, electron tomography, superstructures

■ INTRODUCTION

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Atomically precise noble metal nanoclusters (NCs) show diverse applications in many fields owing to their quantum confinement, nanometric dimensions, larger surface area, diverse functionalization, and stability toward chemicals and light.^{1–10} NCs with a varying number of metal atoms in the core and covalently bound functional ligands have been reported in the literature.¹¹⁻²⁶ Among them, functional thiolate-tethered NCs display important features, particularly Förster resonance energy transfer,²⁷ optical chirality,^{28,29} and biocompatibility useful for the construction of bioconjugated protein machinery for extracellular labeling and intracellular delivery.³⁰ Smart molecular imprinting on NC surfaces can also be extended to build self-assembled superstructures. Such novel materials can find applications in advanced optical materials due to their collective and enhanced optical properties that arise from the periodic organization. Recently, NC self-assemblies have been reported via dipole-induced van der Waals attraction,³¹ hydrogen bonding,³² C-H··· π interactions,³³ metal chelation,³⁴ and aurophilic interactions.³⁵ Importantly, self-assembled gold NC superstructures have been shown to alter the luminescence, catalytic activity, and bioavailability.³⁴ Since NCs in such self-assembled structures interact with one another with local forces, the efficient reversibility of such static assemblies will be difficult. In this

context, photoactive molecules offer a unique opportunity to achieve reversible molecular self-assembly and even for plasmonic nanoparticles.³⁶ Therefore, it is relevant to investigate whether this approach can be implemented to NC self-assembly by developing photoisomerizing ligand³⁷⁻⁴²tethered NCs. As the photostability of NCs and their interactions with photons are significantly different from those of the corresponding plasmonic nanoparticles, photonassisted reversible self-assembly of NCs in solution at room temperature is still challenging. Furthermore, to achieve NC self-assembly in solution, it is crucial to overcome the thermal fluctuation of the surroundings, which is close to the inter-NC interaction.⁴³ In the literature, Au₂₅ NCs functionalized with two types of photoswitchable chromophores, viz., azobenzene⁴⁴ and spiropyran,⁴⁵ are known. However, the reported studies are limited to their spectroscopic investigations. A detailed understanding of the morphological changes and potential colloidal-level self-assembly is still lacking. To address

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Figure 1. Synthesis and photophysical properties. (A) Schematic representation showing the synthesis, energy-minimized structures, and photoisomerization of $[Au_{25}(C_3-AMT)_{18}]^-$ NCs. (B) Absorption spectra of C_3 -AMT under (a) visible light and (b) UV light. Energy-minimized structures of the two geometrical isomers of C_3 -AMT are shown in the inset (B1). (C) Absorption spectra of (d) $[Au_{25}(C_3-AMT)_{18}]^-$ and (c) $[Au_{25}(PET)_{18}]^-$ NCs. ESI-MS of both NCs are shown in the inset (C1). (D) Temporal absorption spectra of $[Au_{25}(C_3-AMT)_{18}]^-$ at 345 nm illumination. A plot of OD (ca. 345 nm) *vs* illumination time under ultraviolet and visible light is shown in the inset (D1).

this challenge, we demonstrate the synthesis and photoninduced reversible self-assembly of azobenzene-alkyl monothiol (C₃-AMT)-tethered Au₂₅ NCs $[Au_{25}(C_3-AMT)_{18}]^-$. We show that when a solution of $[Au_{25}(C_3-AMT)_{18}]^-$ was irradiated with ultraviolet light (345 nm) disc-like superstructures ($d \sim 100-1000$ nm) were formed. The stable structure obtained readily disassembled upon irradiating with visible light (435 nm), demonstrating the light-triggered assembly and disassembly. The temporal evolution of the self-assembled superstructures was studied extensively using various spectroscopic and microscopic techniques followed by their correlation with electron tomography reconstruction.

First, we discuss the synthesis and characterization of C3-AMT ligand and $[Au_{25}(C_3-AMT)_{18}]^-$ NCs. AMT with a C_3 spacer (Figure 1A) was synthesized according to a reported literature procedure.46,47 Details of reactions involved in the preparation of C3-AMT and its characterization (Scheme S1 and Figures S1-S3 in the Supporting Information (SI)) are provided in the Experimental Section. Absorption spectra of C₃-AMT in dichloromethane (DCM) illuminated under ultraviolet (345 nm) and visible (435 nm) light are shown in Figure 1B. The trans isomer of C_3 -AMT (a) has an absorption maximum in the UV region ($\lambda_{max} = 345$ nm), whereas its cis isomer (b) absorbs more in the visible ($\lambda_{max} = 435$ nm) domain. Energy-minimized structures of the two geometrical isomers of C₃-AMT optimized using density functional theory (DFT) are shown in inset B1. The time-dependent absorption spectra of C₃-AMT illuminated separately at 345 and 435 nm are given in the SI (Figure S4A,B). When illuminated at 345 nm, the absorption spectra of C3-AMT show a gradual decrease in the optical density (OD) ca. 345 nm and an increased OD ca. 435 nm, whereas the opposite trend was observed when illuminated at 435 nm wavelength (inset in Figure S4B).

The synthesis of $[Au_{25}(C_3-AMT)_{18}]^-$ NCs were achieved using a modified Brust–Schiffrin single-phase method.⁴⁸ To

compare the optical absorption features of the newly synthesized $[Au_{25}(C_3-AMT)_{18}]^-$ NCs, a phenylethanethiol (PET) analogue, $[Au_{25}(PET)_{18}]^-$, was also synthesized and characterized.48 Details are provided in the Experimental Section. Figure 1C shows the absorption spectra of (d) $[Au_{25}(C_3-AMT)_{18}]^-$ and its comparison with that of (c) [Au₂₅(PET)₁₈]⁻. The peak ca. 680 nm (highest occupied molecular orbital-lowest unoccupied molecular orbital (HOMO-LUMO)) in the absorption spectra of $[Au_{25}(C_3 AMT)_{18}$ suggests the presence of monodisperse NCs. However, the peaks corresponding to HOMO-sp (ca. 450 nm) and d-sp transitions (ca. 400 nm) were merged with optical features of the azobenzene chromophore. The molecular composition of $[Au_{25}(C_3-AMT)_{18}]^-$ was analyzed using electrospray ionization-mass spectrometry (ESI-MS; negative ion mode), and the spectral patterns were compared with those of the PET analogue. The peaks (inset C1) at m/z7391 and 9808 represent $[Au_{25}(PET)_{18}]^-$ and $[Au_{25}(C_3 - C_3 - C_3)^-]$ $AMT)_{18}$]⁻ NCs, respectively.

The appearance of a single peak ca. m/z 9808 in $[Au_{25}(C_3-AMT)_{18}]^-$ in ESI-MS confirms the purity of the NCs. The metal core of NCs and the nature of binding were evaluated using X-ray photoelectron spectroscopy. Binding energies of Au 4f levels in $[Au_{25}(C_3-AMT)_{18}]^-$ were in agreement with the reported values,⁴⁹ with Au 4f_{7/2} having ca. 84.06 eV and Au 4f_{5/2} ca. 87.75 eV (Figure S5). A slight change in the BE of these clusters is attributed to the different electron density of ligands. The S 2p binding energy was in accordance with thiolate binding.

We then studied the photoisomerization of Au_{25} NCs in DCM. Temporal absorption spectra of $[Au_{25}(C_3-AMT)_{18}]^-$ NCs illuminated at 345 nm light (0.2 mW/cm²; Figure 1D) exhibits a gradual decrease in the OD ca. 345 nm and an increased OD ca. 435 nm (inset D1). Similar, but opposite, photoswitching behavior was seen at 435 nm (0.5 mW/cm²) excitation (Figure S6). The change in absorption spectra under

UV and visible light is attributed to the cis-trans isomerization of the surface azobenzene ligands, as shown using DFT energy-minimized structures of $[Au_{25}(C_3-AMT)_{18}]^-$ in Figure 1A.

Photoswitching aspects of C_3 -AMT and $[Au_{25}(C_3-AMT)_{18}]^-$ NCs were further evaluated by analyzing their



Figure 2. (A) Geometrical isomers of C₃-AMT. ¹H NMR spectra of (B) trans, (C) trans/cis, and (D) cis isomers of C₃-AMT in CDCl₃. (E, F)¹H NMR spectra of trans and cis isomers of $[Au_{25}(C_3-AMT)_{18}]^-$ in acetone- d_6 , respectively. Resonance peaks are assigned and labeled above the traces.

nuclear magnetic resonance (NMR) spectra (Figure 2). The ¹H NMR spectrum of trans C₃-AMT (Figure 2B) shows three sets of peaks in the aromatic region, ca. 7.97–7.84 (dd, 4H), 7.54–7.4 (m, 3H), and 7.05–6.96 ppm (d, 2H). The NMR spectrum of the cis isomer (Figure 2D) shows an upfield shift due to a larger shielding effect compared to its trans counterpart. Figure 2C represents the NMR spectrum of a mixture of cis and trans isomers, recorded in between illumination. A similar photoisomerization was also observed for the surface C₃-AMT ligands in Au₂₅ NCs (Figure 2E,F). However, due to their different possible conformations as well as the chemical and magnetic nonequivalent nature of ligands,⁵⁰ the ¹H NMR spectrum of C₃-AMT in [Au₂₅(C₃-AMT)₁₈]⁻ displays complex overlapping resonance peaks.

Reversible photoswitching in NCs was further investigated by transmission electron microscopy (TEM) imaging. First, Au_{25} NCs were characterized using TEM by drop-casting and drying ~100 μ M solution of $[Au_{25}(C_3-AMT)_{18}]^-$ in DCM on a copper grid with a carbon support film. However, due to electron beam damage, Au_{25} NCs underwent rapid coalescence; therefore, high-resolution imaging of individual NCs remained a challenge.^{45,49,51} Nevertheless, the nonaggregated nature of Au₂₅ NCs is evident from the TEM micrographs (Figure 3A). To monitor the light-induced self-assembly, ~ 100 μ M solution of NC in DCM at room temperature was illuminated at 345 nm light (0.2 mW/cm^2) for 120 minutes in a temperature-controlled bath. The solution was continuously stirred during the photoisomerization process to avoid the possibility of thermally induced aggregation. The illuminated NC solution was drop-casted onto TEM grid and dried in the dark. Remarkably, TEM images of [Au₂₅(C₃-AMT)₁₈]⁻ NCs show the formation of circular superstructures (Figure 3B) with an average diameter of ~100-200 nm. The evolution of the self-assembled superstructures was investigated in detail by illuminating the same NC solution with 345 nm light for a longer illumination time. Similar trends were observed in the TEM analysis of samples illuminated for 240 min (Figure 3C). The overall diameter of the superstructures was increased from ~200-1000 nm. The increase in width and thickness suggests extended self-assembly of Au₂₅ NCs. Large-area TEM micrographs of the self-assembled NC superstructures obtained by illuminating the NC solution for 120 and 240 min are shown in Figure S7. High-resolution TEM (HRTEM) images (Figure 3E,G) clearly show the presence of individual NCs. The above results suggest that the superstructure formation is facilitated by inter-NC interactions through the surface ligands.

Attractive dipole-dipole interactions³⁹ between nearby NCs in the cis arrangement facilitate the self-assembly in NCs. A cartoon representation of the dipole-induced attraction of NCs (Figure 3F), self-assembly of NCs, and their possible arrangements within the circular superstructure is shown in the SI (Scheme S2). Based on the observations from the timedependent TEM micrographs, we found that the formation of circular monolayer aggregates of NCs is the early stage of the assembly. Accumulation of more cis NCs on such monolayer aggregates under continuous UV illumination helps to enhance the thickness and diameter of the superstructures. However, there is no regular order of NCs within the layer or between the layers. More interestingly, photoactivation of such selfassembled superstructures in solution at room temperature under 435 nm light (0.5 mW/cm²; 300 min) shows the formation of well-separated NCs by disassembly (Figure 3D). Large-area TEM images representing the stepwise disassembly of superstructures are given in the SI (Figure S8). A decrease in the diameter of the superstructures with illumination time and the presence of individual NCs suggest controlled disassembly.

Photographs of NC solutions used for reversible switching are given in the SI (Figure S9). Notably, there was no precipitation of NCs during assembly and disassembly. To further check the stability of the self-assembled NCs under dark conditions, we have recorded and analyzed the timedependent absorption spectra of superstructures stored in a dark room. Absorption spectra (Figure S10) of assembled NCs do not show any changes in their optical absorption features, suggesting the stability of the superstructures in the dark. In other words, disassembly of superstructures can be triggered only in the presence of visible (435 nm) light.

To check the importance of C₃-AMT in the light-induced assembly, the PET analogue, $[Au_{25}(PET)_{18}]^-$ NC solution was illuminated at 345 nm light for 240 min. TEM images of $[Au_{25}(PET)_{18}]^-$ NCs before and after the illumination are similar (Figure S11), which shows the inability of $[Au_{25}(PET)_{18}]^-$ NCs for light-induced self-assembly. Addi-



Figure 3. TEM micrographs of (A) trans $[Au_{25}(C_3-AMT)_{18}]^-$ NCs and (B, C) cis $[Au_{25}(C_3-AMT)_{18}]^-$ NC assembly captured during (B) 120 min and (C) 240 min of light illumination (345 nm). The TEM image of self-assembled $[Au_{25}(C_3-AMT)_{18}]^-$ NC superstructures after 435 nm illumination is shown in (D), clearly showing their disassembly. Zoom-in and focused HRTEM images taken from the edges are shown in (E) and (G), respectively. A cartoon representation of dipole-induced self-assembly in $[Au_{25}(C_3-AMT)_{18}]^-$ NCs is shown in (F).

tionally, we have analyzed the time-dependent absorption spectra of trans $[Au_{25}(C_3-AMT)_{18}]^-$ NC solution under dark conditions. As there were no changes in the optical absorption features of NCs, the possibility of NC self-assembly in the dark was ruled out (Figure S12). These two control experiments validate the role of C₃-AMT and light for the switchable assembly and disassembly in $[Au_{25}(C_3-AMT)_{18}]^-$ NCs. Apart from the circular structures, dimers were also formed during self-assembly (Figure S13A). Electron microscopic analysis at the interface of dimers shows well-organized NCs (Figure S13B).

The morphology and thickness of the superstructures were further evaluated by atomic force microscopy (AFM). A solution of self-assembled NCs drop-casted and dried on a silicon substrate was used for AFM measurements. The tapping mode AFM image (topography) of a single selfassembled superstructure (240 min illumination) shows an average thickness and diameter of 50 and 800 nm (Figure 4A,C), respectively. The morphology of the superstructures shown in the AFM image is similar to that obtained from the TEM analysis. The above results suggest that self-assembly occurs in solution in the presence of light, which helps us to rule out the possibility of electron-beam-induced selforganization of NCs on the TEM grid. The elemental



Figure 4. (A, C) AFM images of ((A) topography and (C) amplitude) a *cis* $[Au_{25}(C_3-AMT)_{18}]^-$ self-assembled single superstructure captured after 240 min of illumination. The height profile is shown in (B).

composition analysis of the self-assembled superstructures using energy-dispersive X-ray analysis (EDS) shows the presence of gold, sulfur, nitrogen, oxygen, and carbon. Elemental mapping and the corresponding spectrum are shown in Figure S14.

To further evaluate the light-induced assembly of $[Au_{25}(C_3-AMT)_{18}]^-$, time-dependent dynamic light scattering (DLS) spectra of NC solutions illuminated at 345 nm light were measured. DLS measurements showed an increase in the size of the superstructures with illumination time. The average particle size within 120 min of illumination (345 nm) was ~250 nm, which was increased to ~550 nm upon continuous illumination, and finally to ~1.5 μ m (Figure S15A–C). On the other hand, a gradual decrease in the diameter of the superstructures was observed at 435 nm illumination (Figure S15D,E). An increase in the size of superstructures at 345 nm illumination and a decrease in the size of the superstructures at 435 nm illumination, respectively, confirm the switchable assembly in NCs. DLS data are in good agreement with TEM micrographs.

Transmission electron tomography (ET) reconstruction was carried out to gain information on the internal structural details of the self-assembled superstructures. Accordingly, the specimen prepared from a solution of NCs after irradiating for 240 min was used to acquire a series of two-dimensional (2D) projections between $\pm 69^{\circ}$ with an increment angle of 2° (details are provided in the Experimental Section). Figure 5A-C shows the TEM images of a superstructure tilted at -60, 0, 0and +60°. Tilt series and corresponding three-dimensional (3D) reconstruction (Figure 5D-F) suggest that the superstructures are disc-like colloidal particles. It is important to note that the disc-like structures can also be partly due to the deformation resulting from solvent evaporation upon drying. However, these 3D superstructures are composed of densely packed NCs (Video S1). The results obtained from electron tomography reconstruction are in agreement with the AFM analysis results.

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Figure 5. (A–C) TEM micrographs of a tilted self-assembled $[Au_{25}(C_3-AMT)_{18}]^-$ disc-like superstructure collected at -60, 0, and +60°. (D) TEM micrograph of a superstructure, (E) electron tomography reconstruction image, and (F) cross-sectional view of a self-assembled superstructure.



Figure 6. (A) Small-angle powder X-ray diffraction pattern of disc-like superstructures. (B) TEM micrograph of a disc-like superstructure. Zoom-in and focused HRTEM images (C, D) from areas 1 and 2 from TEM micrograph in (B) and corresponding line profiles showing inter-NC distances (E, F).

The self-assembled superstructures were further characterized using small-angle powder X-ray diffraction (SAXRD). Dried self-assembled NC powders were used to measure SAXRD. Figure 6A shows the SAXRD pattern collected from the disc-like colloidal superstructures. The calculated *d*-spacing value suggests an interparticle distance of 3.27 nm, which is in good agreement with the inter-NC distance measured from TEM micrographs $(3.3 \pm 0.5 \text{ nm})$ shown in Figure 6B–F. The cross-sectional view of the 3D reconstructed disc-like structure also supports the above observation (Figure S16). Finally, dark-field scattering microscopy was utilized to image the selfassembled structures in the solution state. Accordingly, an ~100 nM solution of self-assembled superstructures in DCM was drop-casted on a 1 mm thick glass slide and sandwiched with a 0.14 mm thick glass coverslip. Golden-yellow scattering spots represent the individual self-assembled superstructures of

NCs (Figure S17A). However, no such scattering spots were observed in the control sample (Figure S17B).

CONCLUSIONS

In summary, we have successfully demonstrated reversible selfassembly in atomically precise Au_{25} NCs. Using light as an external stimulus, we introduced a simple strategy to selfassemble NCs. To achieve this goal, photoswitchable NCs were designed and synthesized using a thiol derivative of the azobenzene chromophore with a suitable spacer length. Photoisomerization of the chromophore-functionalized and PET analogue NCs under ultraviolet and visible light was studied through systematic changes in the electronic absorption bands and chemical shifts in NMR spectra. Microscopic techniques were employed to understand the reversible photoswitching in NCs. The time-dependent

evolution of light-mediated self-assembly was monitored using DLS, AFM, TEM, and subsequent electron tomography reconstruction. Detailed investigations of self-assembled superstructures have shown the long-range periodic organization of individual NCs. The stacking of NCs in the self-assembly was evaluated using SAXRD. The calculated *d*-spacing value was in good agreement with TEM micrograph values. The development of such smart self-assembled architectures will create new materials for electrochemistry, biology, photocatalysis, and light-harvesting technology owing to their collective and enhanced optical and electronic properties. In particular, switchable NCs with appropriate molecular functionalities will be a potential cargo system to load and unload drug molecules by a light-triggered mechanism. Also, such self-assembled superstructures will be a suitable probe for the fabrication of electrochemical and photochemical devices.

EXPERIMENTAL SECTION

Reagents and Materials. All chemicals and solvents were of analytical grade and used without any further purification. Phenol, aniline, potassium nitrite (KNO₂), 1,3-dibromopropane, hexamethyl-disilathiane (HMDST), tetrabutylammonium fluoride (TBAF), tetraoctylammonium bromide (TOAB), ammonium chloride (NH₄Cl), ammonia solution (25%), potassium carbonate (K₂CO₃), potassium iodide (KI), 2-phenylethanethiol (PET), gold(III) chloride trihydrate (HAuCl₄·3H₂O), and sodium borohydride (NaBH₄) were purchased from Sigma-Aldrich.

Characterization. ¹H and ¹³C NMR measurements were carried out using Bruker AVANCE III HD 400/500 MHz spectrometers. UV/Vis absorbance spectra were recorded on a VARIAN Cary 500 Scan spectrometer. Fourier transform infrared (FT-IR) measurements (attenuated total reflection (ATR) mode) were carried out using a Bruker Tensor 27 spectrometer. Liquid chromatography-mass spectrometry (LC-MS) was performed using a Waters Xevo TQD triple quadrupole mass spectrometer. Electrospray ionization-mass spectrometric (ESI-MS) measurements were carried out on a Waters Synapt G2-Si high-definition mass spectrometer. Typical experimental parameters of ESI-MS were as follows: capillary voltage: 3 kV, cone voltage: 150 V, source offset: 150 V, source temperature: 100 °C, desolvation temperature: 100 °C, and desolvation gas flow: 400 L/h. HRTEM images were recorded on a JEOL 3010 (300 kV) transmission electron microscope. Scanning transmission electron microscopy (STEM) images with EDS and elemental mapping were recorded using a FEI Talos F200S (200 kV) transmission electron microscope. Transmission electron tomographic reconstruction and tilt series of 2D projections were acquired with the Serial EM-software package.^{52,53} The specimen was tilted between $\pm 69^{\circ}$ with $2-3^{\circ}$ increment steps in low-dose mode.⁵⁴ The acquired raw stack of images was first subjected to a series of preprocessing, coarse alignment, and final alignment and was further aligned using the IMOD software package.⁵⁵ The final aligned file was then utilized for 3D reconstruction with a custom-made maximum entropy method (MEM) program with a regularization parameter value of $\lambda = 1.0 \times$ 10⁻³ on MacPro.^{56,57} The 3D isosurface and solid-colored images were produced using UCSF Chimera. High-resolution tapping mode (AC mode) AFM measurements were performed on an Oxford Instruments Asylum Cypher ES. X-ray photoelectron spectra were recorded using a Thermo Scientific ESCALAB 250Xi. Photoisomerization of chromophores and NCs was performed using a Newport Mercury-Xenon light source (500 W) equipped with a monochromator (Oriel). DLS measurements were performed using a Nanotrac Ultra NPA 253 from Microtrac. Particle size and distribution of self-assembled NC colloid solutions were obtained using the Microtrac Flex software. Dark-field scatting imaging was performed on a Cytoviva hyperspectral imaging system (HSI) equipped with a Cytoviva high-resolution dark-field condenser (oil immersion). Here, a sample slide kept in an optical path was illuminated using a halogen lamp after passing through an oilwww.acsami.org

immersion dark-field condenser. Scattering light was focused through a microscope objective (40×), and signals were collected with a colored CCD camera. SAXRD measurement was performed using a third-generation Empyrean Malvern PANalytical X-ray diffractometer. The X-ray source was Cu K α (1.5418 Å). Dried powders of NC superstructures were used to measure the diffraction. Energyminimized structures of NCs were optimized using density functional theory (DFT) as implemented in the grid-based projector-augmented wave (GPAW) software package.^{58,59} The following PAW setups were considered for Au(5d¹⁰6s¹), S(3s²3p⁴), O(2s²2p⁴), N(2s²2p³), C(2s²2p²), and H(1s¹), with scalar-relativistic effects included for Au. The geometry optimizations were carried out using the PBE⁶⁰ functional and DZP (double-zeta plus polarization) basis set in LCAO mode⁶⁰ with a grid spacing of 0.2 Å and the convergence condition that the residual forces be 0.05 eV/Å, without imposing any symmetry constraints. All optimized structures were visualized using the visual molecular dynamics (VMD) software.⁶¹

Different steps involved in the synthesis of molecules 1-3 are shown in Scheme S1 (SI).

Synthesis of Molecule 1. Molecule 1 was synthesized using a reported protocol.⁴⁶ Aniline (4.6 mL; 50 mmol) was dissolved in 15 mL of conc. HCl and cooled to 0 °C. An aqueous solution (15 mL) of KNO₂ (4.4 g; 52 mmol) was added dropwise to the above solution under constant stirring. A clear solution of freshly prepared diazonium salt was added dropwise into phenol (4.7 g; 50 mmol) dissolved in 400 mL of NH₃-NH₄Cl buffer solution (pH ~ 9) at 0 °C. The reaction was carried out at ice-cold temperature for another 3 h followed by addition of excess con. HCl. The precipitated product was washed and collected by vacuum filtration. Washing was repeated multiple times to remove excess reagents and starting materials. ¹H NMR (400 MHz, DMDO- d_6); $\delta = 10.3$ (s, 1H), 7.85-7.78 (broad-d, 4H), 7.59-7.45 (m, 3H), and 6.98-6.93 (broad-d, 2H); ¹³C NMR (100 MHz, DMDO- d_6); δ = 161.4, 152.6, 145.7, 130.9, 129.8, 125.3, 122.6, and 116.4; LC-MS ($C_{12}H_{10}N_2O$), m/z + 1 = 199; FT-IR $(\nu_{\text{max}}) = 3117, 1587, 1500-1370, 1275, 1227, 1139, 831, and 762 \text{ cm}^{-1}.$ ¹H and ¹³C NMR, LC–MS, and FT-IR spectra of molecule 1 are shown in SI (Figure S1).

Synthesis of Molecule 2. A suspension of molecule 1 (1.98 g; 10 mmol), 1,3-dibromopropane (~10 mL; 100 mmol), K₂CO₃ (1.66 g; 12 mmol), and a catalytic amount of KI was refluxed in acetone (50 mL) for 5 h at 80 °C.⁴⁷ The reaction mixture was cooled and filtered. Excess 1,3-dibromopropane was removed by pumping in a rotary evaporator. The crude product was chromatographed over silica gel and eluted with a 2% ethyl acetate/hexane mixture. ¹H NMR (400 MHz, CDCl₃); δ = 7.95–7.85 (m, 4H), 7.53–7.4 (m, 3H), 7.04–6.99 (dd, 2H), 4.2–4.17 (t, 2H), 3.65–3.60 (t, 2H), and 2.4–2.32 (m, 2H); ¹³C NMR (100 MHz, CDCl₃); δ = 161.1, 152.7, 147.1, 130.4, 129.0, 124.8, 122.6, 114.7, 65.6, 32.2, and 29.8; LC–MS (C₁₅H₁₅N₂OBr), *m/z* + 1 = 319 and 321; FT-IR (ν_{max}) = 2960–2870, 1588, 1500–1370, 1237, 1100–1020, 916, 825–760, and 678 cm⁻¹. ¹H and ¹³C NMR, LC–MS, and FT-IR spectra of molecule 2 are shown in SI (Figure S2).

Synthesis of Molecule 3. A stirred solution of molecule 2 (0.319 g; 1 mmol) in freshly distilled tetrahydrofuran (THF) (4 mL) was cooled to -10 °C followed by addition of a mixture of HMDST (255 μ L; 1.2 mmol) and TBAF (0.35 g; 1.1 mmol) dissolved in THF (1 mL).47 The mixture was warmed to room temperature while being stirred. After 1 h, the reaction mixture was diluted with dichloromethane and washed with saturated NH4Cl solution. The crude product was chromatographed over silica gel and eluted with a 2% ethyl acetate/hexane mixture. ¹H NMR (500 MHz, CDCl₃); δ = 7.95-7.85 (m, 4H), 7.53-7.4 (m, 3H), 7.04-6.99 (dd, 2H), 4.2-4.15 (t, 2H), 2.8-2.73 (q, 2H), 2.17-2.08 (m, 2H), and 1.44-1.39 (t, 1H); ¹³C NMR (125 MHz, CDCl₃); δ = 161.3, 152.8, 147.1, 130.4, 129, 124.8, 122.6, 114.7, 66, 33.2, and 21.2; LC-MS $(C_{15}H_{16}N_2OS), m/z + 1 = 273;$ FT-IR $(\nu_{max}) = 2930-2875, 2563,$ 1589, 1495-1380, 1298, 1237, 1137, 1044, 949, and 827 cm⁻¹. ¹H and ¹³C NMR, LC-MS, and FT-IR spectra of molecule 3 are shown in SI (Figure S3).

Synthesis of $[Au_{25}(PET)_{18}]^-$. $[Au_{25}(PET)_{18}]^-$ NCs were synthesized using a reported protocol.⁴⁸ To a solution of HAuCl₄· $3H_2O$ (20 mg/2 mL THF), TOAB (33 mg/1.75 mL THF) was added and stirred until the color turned dark red. Then, PET (34 μ L; 5 mol equivalents w.r.t. gold) was added and stirred at 400 rpm for 1 h. The Au-thiolate solution formed was further reduced by injecting a freshly prepared ice-cold aqueous NaBH₄ solution (19 mg/1.25 mL; 10 mol equivalents w.r.t. gold). The mixture was stirred at room temperature for another 8-9 h to complete the reaction. Using a separating funnel, the organic and aqueous layers were separated (toluene was added for good separation), and the organic layer was washed several times with Millipore water. Excess thiol and other impurities were removed by precipitating NCs by adding methanol. This washing step was repeated three times. Insoluble Au–S polymers were removed from the crude mixture by extracting NCs in DCM. Finally, NCs were re-dispersed in acetone to remove byproducts (Au₁₄₄ NCs). Dried $[Au_{25}(PET)_{18}]^-$ NCs were stored at 4 °C for further characterizations.

Synthesis of [Au₂₅(C₃-AMT)₁₈]⁻. A modified Brust-Schiffrin single-phase procedure was used for the synthesis of $[Au_{25}(C_3 - C_3 - C_$ AMT)₁₈]^{-.48} To a solution of HAuCl₄·3H₂O (20 mg/2 mL THF), TOAB (33 mg/1.75 mL THF) was added and stirred until the color turned dark red. Then, C3-AMT (69 mg; 5 mol equivalents w.r.t. gold) was added and stirred at 400 rpm for 1 h. The Au-thiolate solution formed was further reduced by injecting a freshly prepared ice-cold aqueous NaBH₄ solution (19 mg/1.25 mL; 10 mol equivalents w.r.t. gold). The mixture was stirred at room temperature for another 8–9 h to complete the reaction. Using a separating funnel, the organic and aqueous layers were separated (toluene was added for good separation), and the organic layer was washed several times with Millipore water. Excess thiol and other impurities were removed by precipitating NCs by adding methanol. This washing step was repeated three times. Insoluble Au-S polymers were removed from the crude mixture by extracting NCs in DCM. Finally, NCs were redispersed in acetone to remove byproducts (Au144 NCs). Dried [Au₂₅(C₃-AMT)₁₈]⁻ NCs were stored at 4 °C for further characterizations.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.0c00328.

Scheme for the synthesis of molecules 1-3 (C₃-AMT); ¹H and ¹³C NMR, LC–MS, and FT-IR spectra of molecules; temporal absorption spectra of C₃-AMT and $[Au_{25}(C_3-AMT)_{18}]^-$; XPS spectra of NCs; mechanism for the dipole-induced self-assembly; photographs of NC solution during the course of assembly and disassembly; large-area TEM micrographs of assembly and disassembly; remporal absorption spectra of self-assembled superstructures and trans NCs under dark; TEM images of $[Au_{25}(PET)_{18}]^-$ before and after light illumination; TEM images of self-assembled dimer; elemental maps and EDS spectum of superstructure; temporal DLS spectra of assembly and disassembly; dark-field scattering image of superstructures; cross-sectional views of 3D-reconstructed disc-like superstructures (PDF)

Three-dimensional superstructures are composed of densely packed NCs (MOV)

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Author Contributions

The project was designed and supervised by E.S.S. J.V.R. synthesized the chromophore and nanoclusters. J.V.R. and E.S.S. conducted experiments. Nonappa performed electron tomography data acquisition and reconstruction. The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

NC, atomically precise noble metal nanocluster C₃-AMT, azobenzene-alkyl monothiol

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