Polyoxometalate-Engineered Building Blocks with Gold Cores for the Self-Assembly of Responsive Water-Soluble Nanostructures

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Abstract: The controlled assembly of gold nanoparticles (AuNPs) with the size of quantum dots into predictable structures is extremely challenging as it requires the quantitative and topologically precise placement of anisotropic domains on their small, approximately spherical surfaces. We herein address this problem by using polyoxometalate leaving groups to transform 2 nm diameter gold cores into reactive building blocks with hydrophilic and hydrophobic surface domains whose relative sizes can be precisely tuned to give dimers, clusters, and larger micelle-like organizations. Using cryo-TEM imaging and 2H DOSY NMR spectroscopy, we then provide an unprecedented “solution-state” picture of how the micelle-like structures respond to hydrophobic guests by encapsulating them within 250 nm diameter vesicles whose walls are comprised of amphiphilic AuNP membranes. These findings provide a versatile new option for transforming very small AuNPs into precisely tailored building blocks for the rational design of functional water-soluble assemblies.

Supramolecular structures comprising gold nanoparticles (AuNPs) have potential applications in medical imaging, photodynamic therapy, drug delivery, catalysis, and optical or electronic devices.[1] Fundamental to the rational design and synthesis of these superstructures is the development of AuNP building blocks with anisotropic ligand shells programmed to react in a predictable fashion. This, in turn, requires precise control over the topological organization of diverse ligand-shell domains on the surfaces of the AuNPs.[2] While considerable progress has been made in modifying the surfaces of relatively large or anisotropic gold cores,[3] this is not the case for very small, approximately spherical AuNPs, for which precise control over the formation of ligand-shell domains remains exceedingly difficult.[4] This is particularly true in water, the solvent of choice for numerous applications of AuNP superstructures in green chemistry and medicine.

Important advances in this direction include the surface modification of AuNPs to produce amphiphilic Janus particles[5] on solid templates[6] and at liquid–air[7] or liquid–liquid[8] interfaces. To overcome the low yields associated with the limited surface areas of these interfaces, methods have been developed to combine mixtures of different ligands at the particle surface,[9] with spontaneous phase separation[10] leading to anisotropic ligand domains. Nevertheless, the synthesis of amphiphilic AuNPs in which both the relative sizes and locations of different ligand-shell domains are quantitatively controlled remains a serious obstacle to the design of well-defined AuNP building blocks.

In this regard, polyoxometalate (POM) ligand shells provide finely tunable control over the reactions of thiolates with spherical AuNPs in water.[11] For example, POM ligands were recently used to direct the assembly of 200 nm AuNP supraspheres, which served as soluble hosts for over two million hydrophobic guests, an uptake capacity rivalling those of zeolites and metal–organic frameworks.[12] We now use POM ligands to direct the synthesis of quantitatively well-defined anisotropic ligand shells on 2 nm AuNPs, thereby controlling their self-assembly into responsive nanostructures. This is achieved using 1 nm large POM cluster anions, AlW₁₁O₃₉⁻ (I), as structure-directing and “self-reporting” leaving groups (Scheme 1).

This method takes advantage of two critically important attributes of the POM ligands. First, the POM monolayer shells are robust enough to direct the placement of incoming thiolate ligands (Scheme 1, step 1)[13,11] yet labile enough to be entirely removed by the subsequent addition of a second type of thiolate ligand (Scheme 1, step 2). Second, the POM leaving groups are self-reporting in that changes in the surface...
plasmon resonance (SPR) absorbance in response to their displacement, which can be monitored by UV/Vis spectroscopic analysis, precisely correlate with the growth and extent of thiolate monolayer domains. Together, these features enable the fine-tuning of the relative sizes of the hydrophilic and hydrophobic thiolate ligand domains, which in turn controls the self-assembly of dimers, small aggregates, and eventually of responsive micelle-like AuNP aggregates (Scheme 1, far right). These structures are documented in detail below, and in situ cryo-TEM images show how the micelle-like nanostructures rearrange to encapsulate hydrophobic guests.

Borohydride reduction of HAuCl₄ in the presence of K₂CO₃ in water gives 2 nm gold-core NPs that are protected by 1 (see the Supporting Information for details; Figures S1 and S2). The protecting ligands 1 were then incrementally displaced by water-soluble mercaptocarboxylates, namely 3-mercaptopropionate (MPA) or 11-mercaptoundecanoate (MUA). As shown in Figure 1, displacement of the metal oxide cluster anions (1) by the thiolate ligands results in a linear decrease in the SPR absorbance, leading to a titration endpoint at 51 μM MPA, which corresponds to complete removal of the POM ligands (the same endpoint is obtained using MUA; see Figures S3–S5).

Importantly, this endpoint served as a calibration standard for the quantitative formation of 90, 50, and 30% MPA or MUA domains (step 1 in Scheme 2). This step leaves 10, 50, and 70%, respectively, of the NP surfaces still covered by POM domains. Subsequent displacement of the remaining POM anions by hexanethiol (HSR; step 2 in Scheme 2) gives amphiphilic particles with ligand shells featuring quantitatively precise coverage by hydrophilic (MPA or MUA) and hydrophobic (SR) domains. Importantly, experimental and theoretical studies show that for small (2 nm) gold cores, the presence of separate hydrophilic and hydrophobic domains is the lowest-energy (most thermodynamically stable) ligand organization.

Control over the size of the hydrophobic domains on the amphiphilic AuNP surfaces (as well as the size of the hydrophilic ligands) led to the spontaneous self-assembly of the tailored building blocks into dimers, small clusters, and micelles (step 3 in Scheme 2), which were characterized by TEM, cryo-TEM, and dynamic light scattering (DLS; Figure 2).

DLS analysis of aqueous solutions containing particles with small (10%) coverage by hydrophobic SR domains and deliberately short (i.e., sterically unobtrusive) MPA ligands
revealed a small increase in the average hydrodynamic diameter ($D_H$) from 3.5 to 4.1 nm, indicative of association between particles (the $D_H$ values clearly increase with the size of the SR domain; see Figures S2, S7, and S8). TEM images (Figure 2a) suggest that the small increase in diameter is due to a mixture of individual particles and dimeric structures (see additional images in Figure S9). The hydrophobic interactions induced by the relatively small (10%) SR ligand domains may be weak such that, while difficult to assess, the individual particles and dimeric structures may be in equilibrium. As the SR coverage is increased to 50% and then to 70%, small clusters and larger micelle-like aggregates form, with respective hydrodynamic diameters of 7.9 and 14 nm. This growth in size results from hydrophobic effects acting to minimize exposure of the incrementally larger hydrophobic domains to water (see Figures S7 and S8). Cryo-TEM images of these assemblies are shown in Figure 2b,c (see additional images in Figures S10–S13).

The spherical shape of the micelle-like structures (Figure 2c) was confirmed by variable-angle DLS as the $D_H$ values remained constant as the detector angle was increased from 30 to 150° (Figure S14). The amphiphilic character of the nanoparticles with 70% SR coverage (Figure 2c) was further demonstrated by spontaneous assembly at a water/organic-solvent interface. For this, CH$_2$Cl$_2$ was added to an aqueous solution of the micelle-like structures; after stirring for 2 h, the AuNPs had migrated nearly quantitatively to the water/CH$_2$Cl$_2$ interface (95% by UV/Vis analysis of the liquid phases). This film was carefully removed and imaged by TEM (Figures 2d and S15). By contrast, no interfacial film was formed when the same procedure was carried out using entirely hydrophilic 100% MUA-capped AuNPs (Figure S16). Moreover, for AuNPs with 90% SR coverage, the hydrophobic domain was large enough to promote clean transfer into CH$_2$Cl$_2$ (Figure S16).

Janus AuNPs containing both hydrophobic and hydrophilic ligand shells are known to behave as conventional molecular surfactants, forming micelle-like (oil-in-water Pickering emulsions) or reverse-micelle-like (water-in-oil) supraparticulate structures. This surfactant-like behavior has been used to transfer hydrophobic nanoparticles from CH$_2$Cl$_2$ to water, and water-soluble nanoparticles from water to CH$_2$Cl$_2$. Notably, however, finely tuned POM-facilitated control over incremental increases in the relative extent of the hydrophobic ligand-shell domains now makes it possible to reveal, for the first time, an (effectively) stepwise process of aggregation, from dimers to small clusters to micelle-like structures. Furthermore, cryo-TEM imaging (below), in which the objects remain in their native (vitrified) solution states, now provides an unprecedented picture of how micelle-like structures of amphiphilic gold-core building blocks reorganize into spherical assemblies with diameters of about 250 nm in response to organic solvents or hydrophobic nanoparticle guests.

First, p-xylene was reacted (by vortex mixing) with an aqueous solution of the micelle-like structures (70% SR-covered AuNPs) for which a cryo-TEM image is shown in Figure 2c. DLS measurements of the optically clear solution revealed new structures with an average hydrodynamic diameter of 260 nm (Figure 3a and inset). Cryo-TEM images revealed similarly sized structures (Figure 3b, inset; see also Figures S17–S19). Their spherical shape was confirmed by a statistically definitive DLS experiment in which the $R_H$ values remained constant as the detector angle was varied from 30 to 150° (Figure 3b; some distortion, however, is seen in the cryogenically trapped structure in the inset; see also Figure S20). Consistent with this, their approximately spherical shape in the cryo-TEM images remained the same upon tilting the specimen grid over a range of nearly 90° (Figure S19).

Next, the presence of encapsulated p-xylene was confirmed by $^1$H DOSY NMR spectroscopy (Figure 3c; see also Figures S21). The methyl and aromatic proton resonances at 2.19 ppm and 7.08 ppm ($H_{out}$ and $H_{out}$, respectively), with a corresponding diffusion coefficient of approximately $1 \times 10^{-9} \text{m}^2\text{s}^{-1}$, arise from p-xylene dissolved in bulk D$_2$O, while the signals with a much smaller average diffusion coefficient of about $2 \times 10^{-11} \text{m}^2\text{s}^{-1}$, which are located farther upfield at approximately 1.68 and 6.43 ppm ($H_{in}$ and $H_{out}$, respectively), were assigned to encapsulated p-xylene. These findings are fully consistent with emulsified p-xylene nano-droplets encapsulated within amphiphilic AuNP membranes.
Additional evidence for these vesicular structures was obtained by dissolving a blue organic dye, azulene, in the p-xylene prior to its encapsulation. After agitation, an optically clear solution of larger (ca. 260 nm) structures was again obtained, and the UV/Vis spectra were diagnostic for azulene in p-xylene (see Figure S22 and the quantitative analysis provided in the Supporting Information). Furthermore, utilizing the photoluminescence properties of azulene, the p-xylene vesicles were detected by fluorescence microscopy (Figure S23).

The vesicular nature of these assemblies was further confirmed by SEM images (obtained under vacuum).Collapsed membranes were observed, which is consistent with evaporation of the encapsulated p-xylene (Figure 3d and Figure S24).

Next, the micelle-like structures (Figure 2c) were reacted with a p-xylene solution of (hydrophobic) SR-capped 2 nm AuNPs (Figure 4a). After agitation, a clear solution was obtained (Figure 4b), and clustering of the encapsulated hydrophobic NP guests was confirmed by a characteristic increase in the SPR absorbance at about 700–800 nm (Figure S25). DLS data revealed the presence of structures with a diameter of 250 nm, which were subsequently imaged by cryo-TEM (Figure 4c, inset; see also Figure S26).

Now, however, in contrast to vesicles containing only p-xylene (Figure 3d), the SEM images (under vacuum) showed no evidence of collapse (Figure 4c). Rather, solid spherical structures were observed, confirming the formation of densely packed spherical clusters of encapsulated SR-capped AuNPs.

In summary, self-reporting POM leaving groups have been shown to provide fine control over the size of hydrophilic and hydrophobic thiolate ligand domains on small, approximately spherical gold cores. Depending on the extent of these domains, the self-assembly of dimers, small aggregates, or micelle-like structures was observed whose solution-state reorganization in response to p-xylene and hydrophobic AuNPs was documented by cryo-TEM, DLS, 1H DOSY NMR spectroscopy, and SEM. Moreover, POM ligands were shown to provide a versatile new option for transforming very small AuNPs into precisely tailored building blocks for the rational design of functional water-soluble assemblies.

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Conflict of interest
The authors declare no conflict of interest.

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replacement of the POM shell by thiolates leads to Janus particles (Ref. [11]). In accord with computational (Ref. [16]) and experimental data (Refs. [9c, 15]), the presence of two discrete domains at the surfaces of these relatively small particles is thermodynamically favored. An analogous situation applies to reactions of the 2 nm gold cores used here.

While the DLS data show an increase in average size, the dimeric structures are difficult to image by electron microscopy. Under cryo-TEM, the dimers are too small to be located and imaged properly, and during drying for traditional TEM, some of the particles appear to fuse together. When more than 10% hexanethiol is used, small clusters comprising more than two AuNPs begin to form.

Consistent with co-encapsulation of these hydrophobic AuNPs within the $p$-xylene-filled vesicles, addition of CH$_2$Cl$_2$ to the aqueous solution led to phase transfer and disassembly, giving a CH$_2$Cl$_2$ solution of individual SR-capped AuNPs (see Ref. [12]). This was demonstrated by UV/Vis spectroscopy and TEM (see Figure S27).