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Technical Notes

Purification of Molecularly Bridged Metal Nanoparticle Arrays by Centrifugation and Size Exclusion Chromatography

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Size exclusion chromatography and centrifugation separation protocols were developed and compared for isolating enriched fractions of phenylethynyl-bridged metal nanoparticle dimers and trimers from the monomeric particle starting material. Both methods enabled the isolation of enriched fractions of a desired array without causing significant sample aggregation or replacement of the phenylethynyl bridge. Solutions containing ca. 70% bridged gold dimers were obtained using either method. The further development of methods for separating discrete arrays of covalently bridged nanoparticle homo and hetero structures is expected to help advance our understanding of collective metal particle electronic structure−function relationships.

In this Technical Note, two methods are compared for collecting enriched fractions of molecularly bridged metal nanoparticle arrays: centrifugation and size exclusion chromatography. Metal nanoparticle arrays consist of gold or silver nanoparticles covalently linked by phenylethynyl di-, tri-, and tetrathiols (Scheme 1). These thiols have been shown previously to "template" the formation of particle arrays with $D_{\infty h}$, D_{3h}, D_{4h}, and T_d symmetries and with variable interparticle distances (Figure 1).[1] The arrays are being employed to assess electronic and electromagnetic communication between metal particles as a function of array symmetry and interparticle distance. Indeed, distance and symmetry have proven to affect nanoparticle linear and nonlinear optical properties significantly.[2] For example, large optical first hyperpolarizabilities (β) were measured recently for solutions containing gold particles arranged in a noncentrosymmetric trimer geometry using molecule **IIIa** relative to centrosymmetric dimers formed using molecule **Ia** or monomeric particles.[2b] Moreover, β decreased as the distance between particles in the trimer was increased from that dictated by the dimensions of **IIIa** to that of **IIIb**.

Our current efforts are focused on measuring the electron transport characteristics of organic molecules bridging metal particles. This is being accomplished by electrochemical techniques (e.g., differential pulse voltammetry) and by wiring the arrays directly to electrical contacts on silicon chips.[3] These techniques will require highly enriched fractions of a desired array separated from excess monomeric particles that result from the assembly process (vide infra). Our initial attempts at isolating enriched suspensions of nanoparticle arrays are shown here.

EXPERIMENTAL SECTION

Molecularly bridged nanoparticle arrays were assembled as described previously.[1−3a,b] A typical gold dimer assembly was formed by first diluting 2.0 mL of 3 nM citrate-capped gold nanoparticles (10 nm diameter) with 2.5 mL of 3 mM citrate solution. A stoichiometric amount of linker (**I**) in 500 μL of 2:3 tetrahydrofuran/ethanol was added in 50-μL aliquots at 10 min intervals while stirring. The solution was stirred for an additional 2 h to complete the linking procedure.

Separation by Centrifugation. Purifying citrate-capped nanoparticle arrays by centrifugation is complicated by (i) particle aggregation upon sample concentration and (ii) instability when combined with high ionic strength or nonaqueous solvents. To prevent particle aggregation during centrifugation, the original citrate capping layer bound to the nanoparticle arrays was exchanged with bis(*p*-sulfonatophenyl)phenylphosphine (BSPP). This ligand is used commonly to stabilize metal and semiconductor nanoparticles.[4] It was chosen because its adsorption energy on gold is intermediate between that of citrate and thiol ligands. Thus, it was anticipated that the BSPP ligand would replace citrate ligands but not the phenylethynyl bridge, a thesis confirmed

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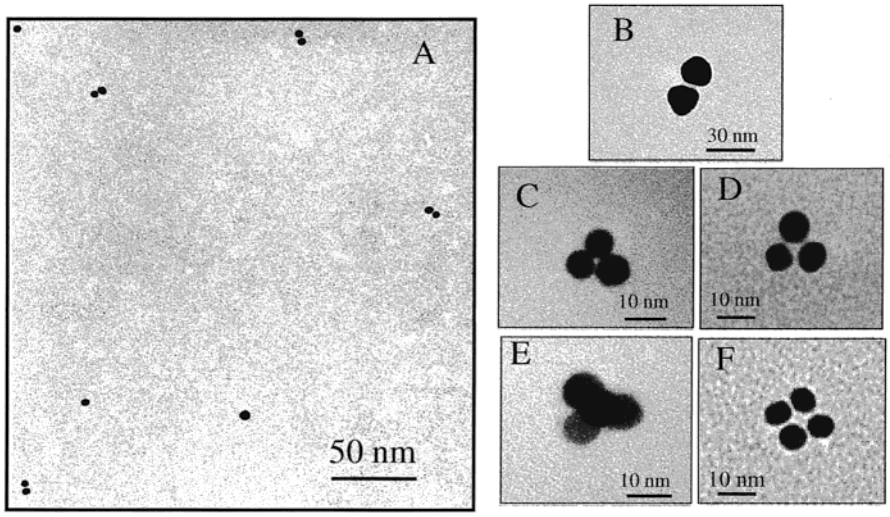
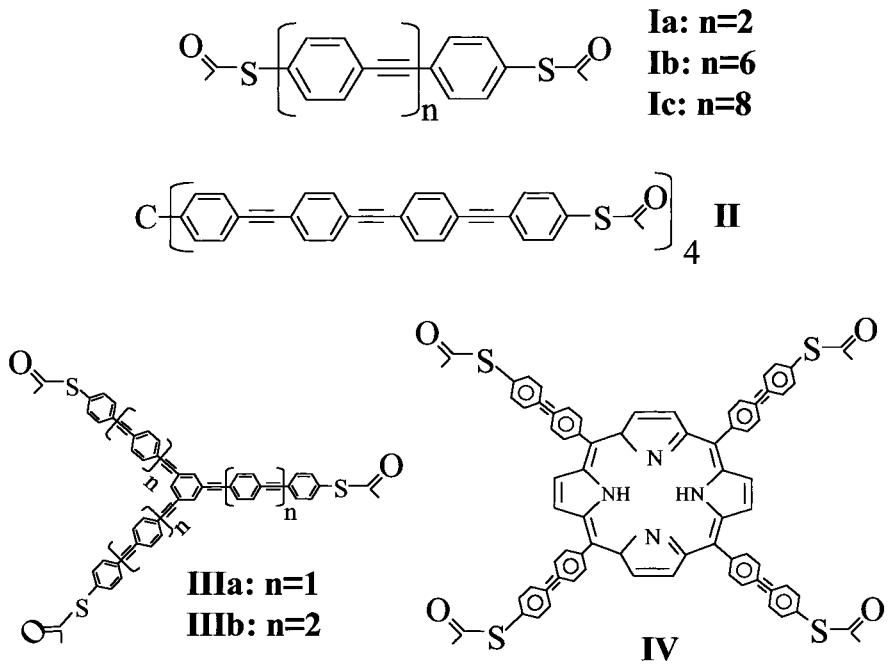


Figure 1. Transmission electron micrographs of gold nanoparticle arrays formed using structures **IC** (Figure 1A,B), **IIIa**, and **IIIb** (Figure 1C,D), and **II** and **IV** (Figure 1E,F).

Scheme 1



experimentally (vide infra). Importantly, exchanging BSPP for citrate does not cause particle aggregation; in fact, nanoparticle monomers capped with BSPP may be collected as highly viscous slurries by centrifugation and subsequently resuspended in water without aggregation (i.e., no change in the gold plasmon resonance).[4b]

Ligand exchange was accomplished by adding 1 mg/mL of the disodium BSPP salt to the nanoparticle array suspension and stirring for 4 h. The water-soluble BSPP-capped nanoparticle array mixture was centrifuged in 1 M aqueous sucrose solution. Density gradient centrifugation was used to separate particles with different densities. For biomolecules, a sucrose density gradient is commonly used.[5] In our approach, a uniform density was used, and the centrifugation speed was increased systematically to increase the *g* force. Thus, the method used here is a stepwise *g*-force density centrifugation. The sample was rotated at an initial rate of 2500 rpm for 30 min. The rotation rate was then increased by 500 rpm every 30 min until a final rate of 6000 rpm was reached. Fractions were collected from the bottom of the centrifuge tube after each 30 min. increment.

Separation by Size Exclusion Chromatography. Purifying gold nanoparticles by size exclusion chromatography has the same complications as centrifugation plus the additional problem of irreversible adsorption to the stationary phase. This was prevented by using an aqueous mobile phase containing 40 mM sodium dodecyl sulfate (SDS) buffer, as described by Wang and co-workers for purifying metal nanorods.[6] The size exclusion chro-

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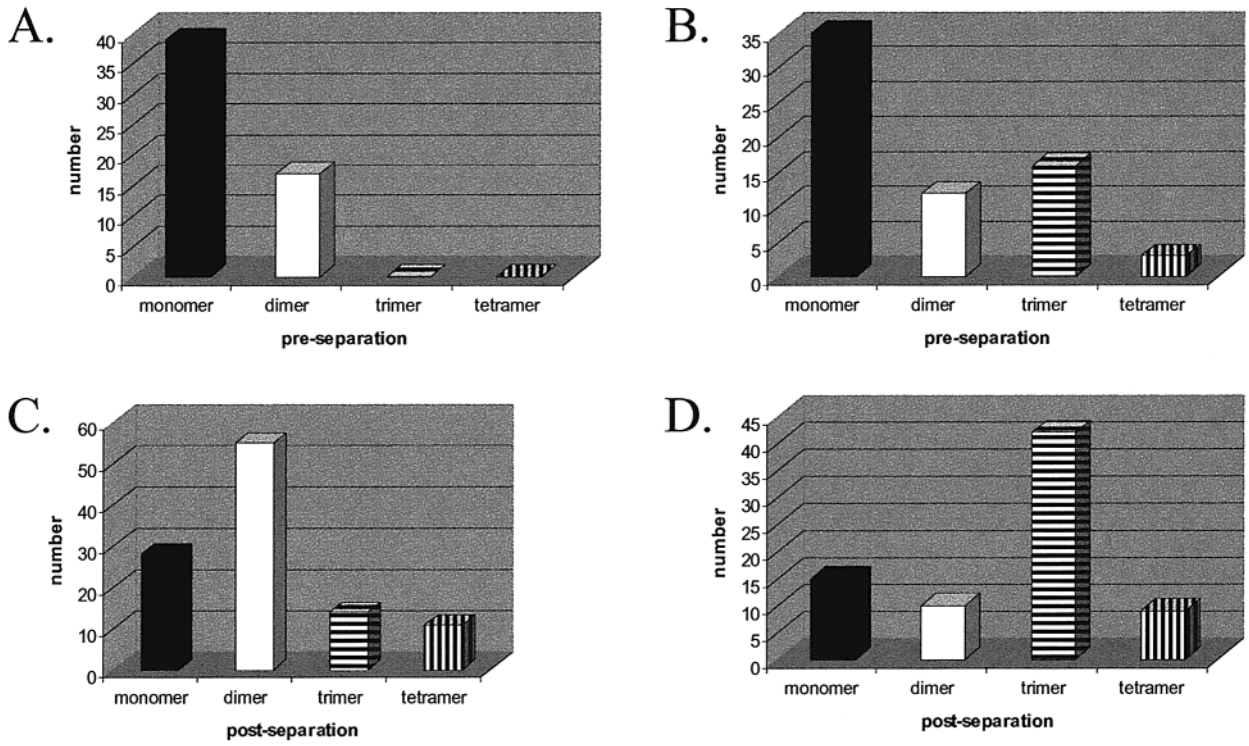

Figure 2. Number of nanoparticle dimer and trimer arrays located by TEM prior to (panels A and B, respectively) and following separation by centrifugation (panels C and D, respectively).

matography contained a Waters UK6 injector, 590 programmable solvent pump, and Rainin Dynomax UV−C single wavelength UV−vis detector set at 525 nm (λ_{max} for the single particle plasmon absorbance). A 500- and a 350-Å pore diameter silica microsphere GPC column were mounted in series (Alltech, Inc.). Injection volumes were 25 μL for all samples studied. Larger pore diameter microspheres were used previously by Kelley and co-workers to separate 5-nm-diameter gold particles from 15-nm-diameter particles.[7] Our attempts at separating nanoparticle arrays with larger pore materials failed, however, and will not be discussed further.

Samples for transmission electron microscopy (TEM) were prepared by placing one drop of nanoparticle suspension on a Formvar-coated Cu grid in contact with filter paper. The solvent was quickly wicked away by the paper, a procedure that has been shown by our group and others to prevent the formation of aggregates on the grid.[1−3a,b,4a] Figure 1A, for example, shows a wide view TEM image of a sample of gold nanoparticle dimers prior to purification. Numerous dimers can be counted in this image. Note that the particles in each dimer are not fused, as would be expected by uncontrolled particle aggregation.

RESULTS

Separation by Centrifugation. Purification of molecularly bridged gold particle dimers and trimers using centrifugation was characterized by TEM. One representative example is shown in Figure 2. In this example, TEM revealed 15 dimers and 36 monomers before centrifugation (Figure 2A). No higher-number aggregates were found on the grid, in agreement with previous work by our group that demonstrated that the linking protocol does not cause particle cross-linking.[1−3a,b,8] Raman spectroscopy of a single dimer fixed to poly(lysine)-coated quartz confirmed the presence of the phenylacetylene bridge between particles.[8] The as-assembled trimer solution contained ca. 15 trimers, 10 dimers, and 34 monomers (Figure 2B). No higher-number aggregates were found on the grid.

Figure 2C,D shows that enriched fractions of dimers and trimers were isolable by centrifugation. Postcentrifugation, the compositions of the dimer and trimer samples by TEM were, respectively, 51 dimers and 25 monomers (dimer sample; Figure 2C), and 40 trimers, 8 dimers, and 12 monomers (trimer sample, Figure 2D). A wide view TEM of an enriched fraction of trimer arrays is shown in Figure 3. These data represent enrichments from 30 to 53% for the dimers, and from 25 to 62% for trimers.

Separation by Size Exclusion Chromatography. Purification of nanoparticle arrays by size exclusion chromatography was characterized by UV−vis spectroscopy (fixed wavelength, 525 nm) and TEM. Separate solutions containing pure 10-, 20-, and 30-nm-diameter gold monomers were first analyzed to determine their respective retention times. A mixture of 10- and 30-nm gold monomers was then injected; the 30-nm-diameter particles were intended as crude mimics of trimers of 10-nm particles. Figure 4 shows the results of these test systems. At the optimum flow rate of 0.25 mL/min., the 10- and 30-nm-diameter gold monomers elute with a resolution R_s of 0.8, indicating good separation (R_s is 2× the peak separation distance divided by the sum of the peak widths).[9] Note that higher-number aggregates, if present in the sample, elute at lower retention times than do the monomeric particles (Figure 4a).

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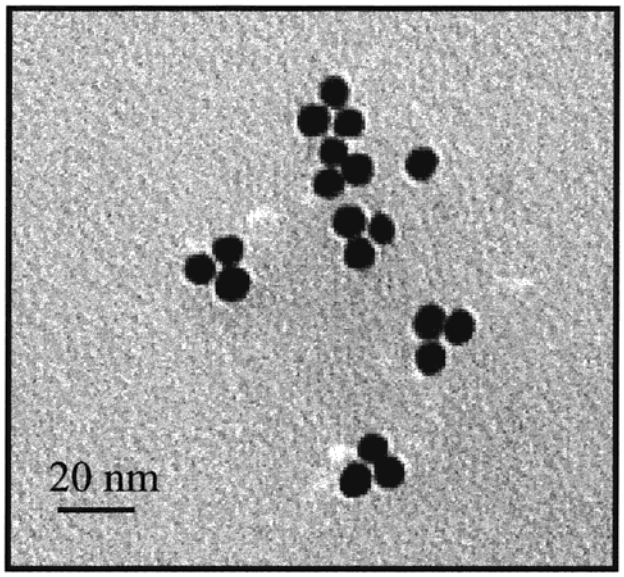

Figure 3. Transmission electron microscope image of gold nanoparticle trimer arrays following separation by centrifugation.

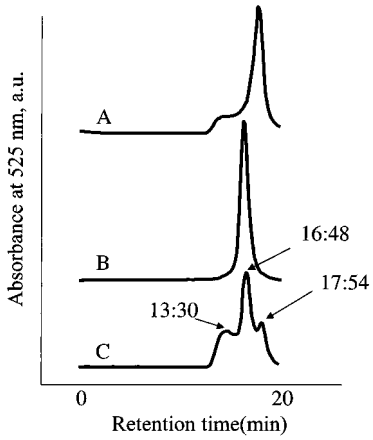

Figure 4. Chromatogram showing retention times of (a) 10-nm-diameter gold monomers, (b) 30-nm-diameter gold monomers, and (c) a 1:1 mixture of 10- and 30-nm-diameter gold monomers.

The optimized flow rate was used subsequently to separate nanoparticle dimer and trimer arrays from excess monomer particles (Figure 5). Separate peaks are discernible in the chromatograms, although the R_s values dropped to 0.25 and 0.5 for trimers and dimers, respectively. Selected fractions of a dimer sample were collected at 17:20 min (fraction 2) and 18:50 min (fraction 1) and analyzed by TEM (Figure 6). Fraction 1 was enriched to 70% dimers, as compared to 21% found in fraction 2.

CONCLUSIONS

The purification of metal nanostructures and assemblies built from nanoscale building blocks is an important challenge in materials science. The results presented above suggest that centrifugation and size exclusion chromatography are viable options for the collection of enriched fractions of nanometer-sized

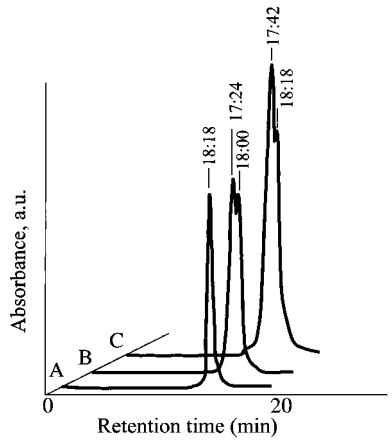

Figure 5. Chromatograms for 10-nm-diameter gold monomers (A), a gold nanoparticle trimer array suspension (B), and a gold nanoparticle dimer array suspension (C).

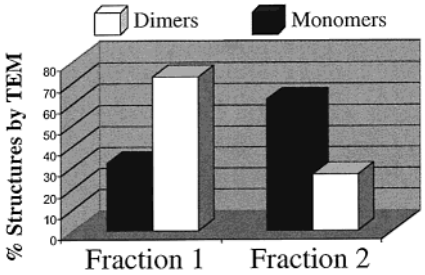

Figure 6. Percent composition of gold particle dimer arrays and gold monomers located by TEM in fractions collected at retention times of ca. 18:50 min (fraction 1) and 17:20 min (fraction 2).

hybrid organothiol/metal particle architectures when care is taken to avoid (i) particle aggregation induced by the composition of the mobile phase and (ii) irreversible binding to the stationary phase. Centrifugation is clearly the simpler and less expensive of the two, but size exclusion chromatography has the potential advantage of postcolumn analysis of nanoparticle optical[7] and electrochemical properties. Importantly, these methods do not cause significant particle aggregation or desorption of the phenylethynyl bridge. Among the challenges for the future include the isolation of nanoparticle structures of similar size and shape but differering composition (e.g., gold−silver heterodimers from gold−gold homodimers). The protocols exploited above are expected to facilitate the elucidation of collective nanoparticle structure−optical and −electronic property relationships.

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