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Nanostructures

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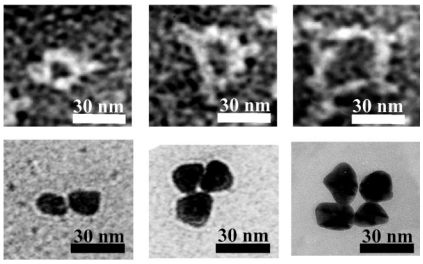

Immunoglobulin G templates: Clusters of gold nanoparticles are grown (see picture) from templates formed from the self-assembly of immunoglobulin Gs (IgGs) by using a synthetic divalent antigen. Carbohydrates inherent to IgGs create local nucleation centers for the electroless deposition of gold.

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Nanostructures

Self-Assembled Aggregates of IgGs as Templates for the Growth of Clusters of Gold Nanoparticles**

Jerry Yang, Michael Mayer, Jennah K. Kriebel, Piotr Garstecki, and George M. Whitesides**

This paper describes a procedure for generating clusters of gold nanoparticles by using as templates oligomers (dimers, trimers, and tetramers) formed by self-assembly of immunoglobulin Gs (IgGs) with bivalent antigens, and by growing the nanoparticles from redox-active centers (aldehydes) derived from the integral carbohydrates located in the F_c portion of the IgGs. In this procedure, a significant fraction ($15 \pm 5\,\%$) of these gold nanoparticles occurs in multimeric clusters (mostly dimers), with a distance of about 30 nm between particles in a cluster. This distance is consistent with the spacing between carbohydrate groups in separate antibody molecules when the antibodies are preassembled into aggregates. This method combines molecular recognition (to assemble nucleating centers for growth of nanoparticles) with redox-active functional groups on the biomolecule (to generate the nucleating centers for electroless growth of gold) to form locally ordered sets of nanoparticles.

Nanoparticles of various shapes (e.g., wires, rectangles, stars, octahedra, truncated boxes, and spirals),[1] compositions (e.g., metals or minerals),[2] and arrangements of clusters (e.g., small multimeric aggregates or large superlattices)[3] are interesting for their potential uses for the miniaturization of optical and electronic devices (e.g., optical filters, optical detectors, conducting wires, molecular electronics, MEMS).[1b,e] New techniques for the fabrication of metallic and hybrid nanostructures with defined geometry may be useful for further realization of these types of devices.

The groups of Finn, Ben–Porat, Mann, Francis, and others have used biomolecular building blocks as the basis for bottom-up fabrication of metallic nanostructures and assemblies of nanoparticles.[1d,4] Viral particles and DNA modified with thiols on their exterior surface are useful in positioning gold nanoparticles; assemblies of nanoparticles based on organization by using DNA duplexes[3b,5] show characteristic shifts in plasmon absorption in aqueous solutions upon clustering of nanoparticles.[6] Feldheim and co-workers used synthetic thiol-based molecular templates to generate multimers (dimers, trimers, and tetramers) of preformed gold and silver nanoparticles for the study of the shifts in plasmon absorption that results from the formation of small aggregates of nanoparticles.[3a,7] Several groups have grown metallic nanostructures by processes that assume coordination of metal ions from solution to biomolecular or synthetic polymeric templates; reduction of the adhering metal ions afforded metallic nanostructures that reflect the structure of the templates from which they were derived.[1c,8]

Although several strategies successfully generate metallic structures from molecular templates, so far the variety of shapes of metallic nanostructures remains limited (mostly to large arrays and lines).[1,8] To demonstrate the combination of molecular self-assembly[9] with the generation of metallic nanostructures, we produced linear and cyclic aggregates of IgGs from a divalent interaction of a compound that contained two fluorescein groups with monoclonal IgGs derived from fluorescein isothiocyanate as antigens (anti-FITC; Figure 1).[10] We subsequently grew clusters of gold nanoparticles using these aggregates as templates. We show that pairs of redox-active functional groups (carbohydrates) present in biomolecular (IgG) aggregates can (after periodate oxidation) provide nucleation sites for the electroless deposition of gold, and that the resulting pairs of gold nanoparticles reflect the geometry of these aggregates. This approach provides a new, bottom-up route to metallic nanostructures.

We incubated a divalent derivative of fluorescein (**1**, Figure 1) in a solution of monoclonal anti-FITC IgG in various relative concentrations, and determined the size of the aggregates in the resulting mixture by size-exclusion HPLC.[18] We observed high conversion of IgGs into aggregated species ($\approx 73\,\%$ of the protein in solution aggregated into $\approx 91\,\%$ dimers, $8\,\%$ trimers, and $\approx 1\,\%$ tetramers) when we incubated a 2 μM solution of **1** with a 1 μM solution of IgG at 4 °C for 3 hrs.[19] Transmission electron microscope (TEM) imaging of the aggregates of antibodies deposited on a carbon-coated Formvar substrate revealed numerous cyclic structures (Figure 2a), consistent in size with oligomers (predominantly cyclic) of antibodies, but with indistinct shape (Figure 2b). The Supporting Information summarizes the experimental details of procedures used to form the aggregates of IgGs and to prepare the samples for imaging by TEM.

We formed individual gold nanoparticles on the IgG molecules by using the carbohydrate units present on IgGs to nucleate the growth of metal particles. All IgGs contain N-linked carbohydrate groups attached to the C_H2 domains in the F_c regions.[20] These carbohydrates are used routinely to link molecules to the F_c region by initial oxidation with $NaIO_4$ to produce aldehyde groups, followed by subsequent conjugation reactions.[21] Aldehydes derived from carbohydrates have also been used to reduce silver ions for silver-enhanced staining of biological tissues.[22] We used the aldehyde moieties derived from periodate oxidation of the carbohy-

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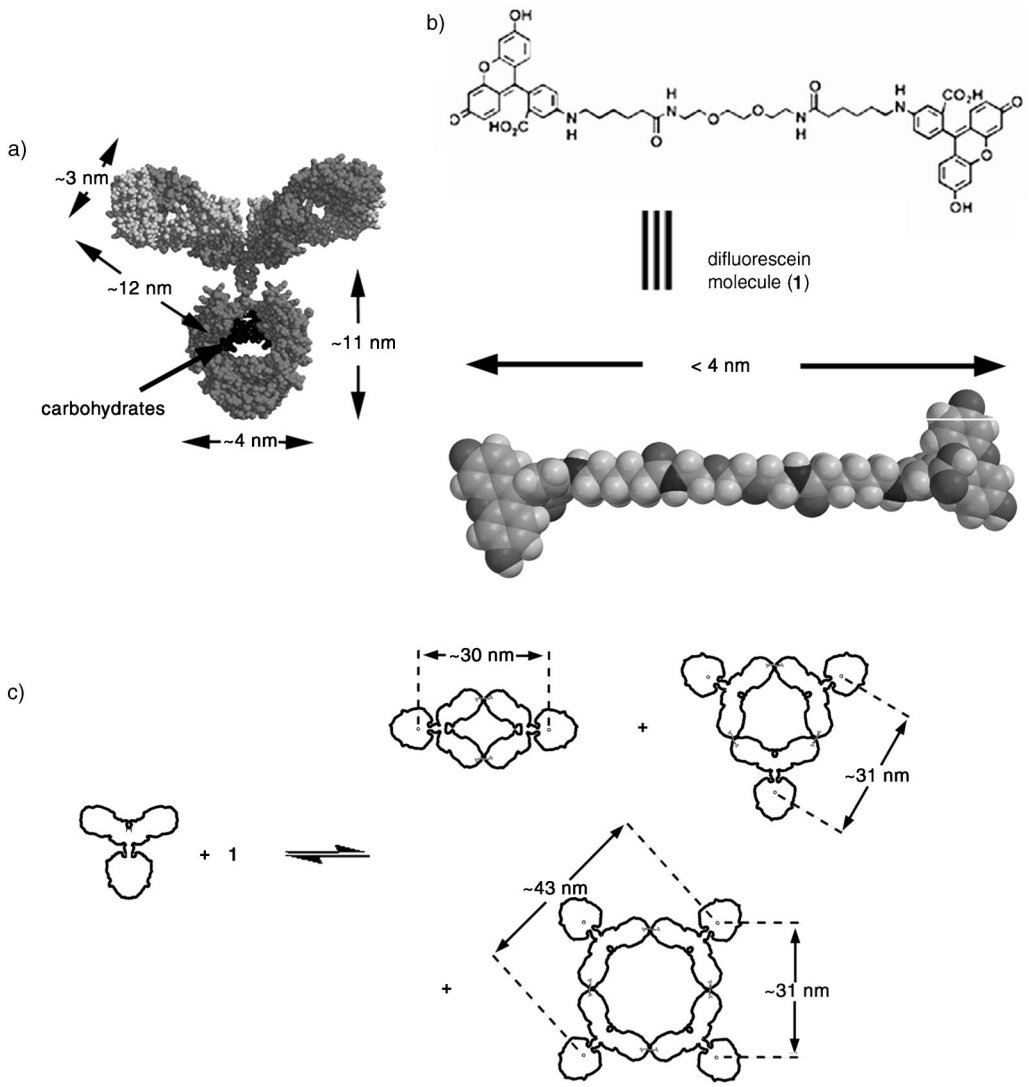

Figure 1. Schematic representation of complexes between anti-FITC IgGs and difluorescein antigen **1**: a) A reconstructed crystal structure of an IgG with the location of the carbohydrates shown in black;[25] b) schematic molecular representation of difluorescein molecule **1** (represented in an extended conformation); and c) schematic representation of cyclic oligomeric structures formed between anti-FITC IgGs and **1** (the IgGs and difluorescein antigens are drawn approximately to scale).

drates in the aggregates of IgGs to reduce a small group of silver atoms; the resulting cluster of silver atoms acted as the nucleus for electroless deposition of a gold nanoparticle.

We deposited a solution of aggregated IgG (2 μM **1** and 1 μM anti-FITC in 0.1 M phosphate buffer: pH 7.0, 0.3 M NaCl) on a Si_3N_4 membrane that had been cleaned with an air plasma. We treated the aggregates of IgGs on the surface with a solution of 10 mM $NaIO_4$ (in 0.02 M acetate buffer, pH 5.0, 0.15 M NaCl) for 60 min at 23 °C, followed by incubation of the surface with a 1 M solution of ethylene glycol for 20 min (to quench the excess periodate). We incubated the IgGs (for 90 min at 23 °C in the dark) with a 0.1 M solution of $AgNO_3$ (in 28–33 % aqueous NH_4OH, adjusted to pH 10.5) and subsequently treated the surface (for 0.5 to 2 min) with an electroless Au plating solution.[4d,23]

TEM examination revealed many clusters of nanoparticles (Figure 2e). We analyzed several large-area images (2320 × 1840 nm up to 3590 × 2843 nm) of substrates that contained these assemblies of nanoparticles by using software written to identify the distribution of nearest-neighbor distances for particles deposited on a surface (see Supporting Information for details). The image-analysis software identified a statistically significant excess in the number of particles (15 ± 5 %)[24] with the centers of the particles having a distance of nearest neighbors of 15 to 45 nm (with a maximum at ≈ 33 nm, Figure 3a). These distances are consistent with the expected distances (estimated from TEM examination of aggregates of IgGs, Figure 2a) between the carbohydrates in the F_c regions of adjacent antibodies in cyclic and linear aggregates (Figure 1). Similar analysis of substrates containing nanoparticles grown from deposited IgGs that were not preincubated with difluorescein **1** did not indicate a significant excess in the number of particles with a distance between nearest neighbors of 15 to 45 nm (Figure 3b). Instead, the analysis indicated a random distribution of distances between nearest neighbors.

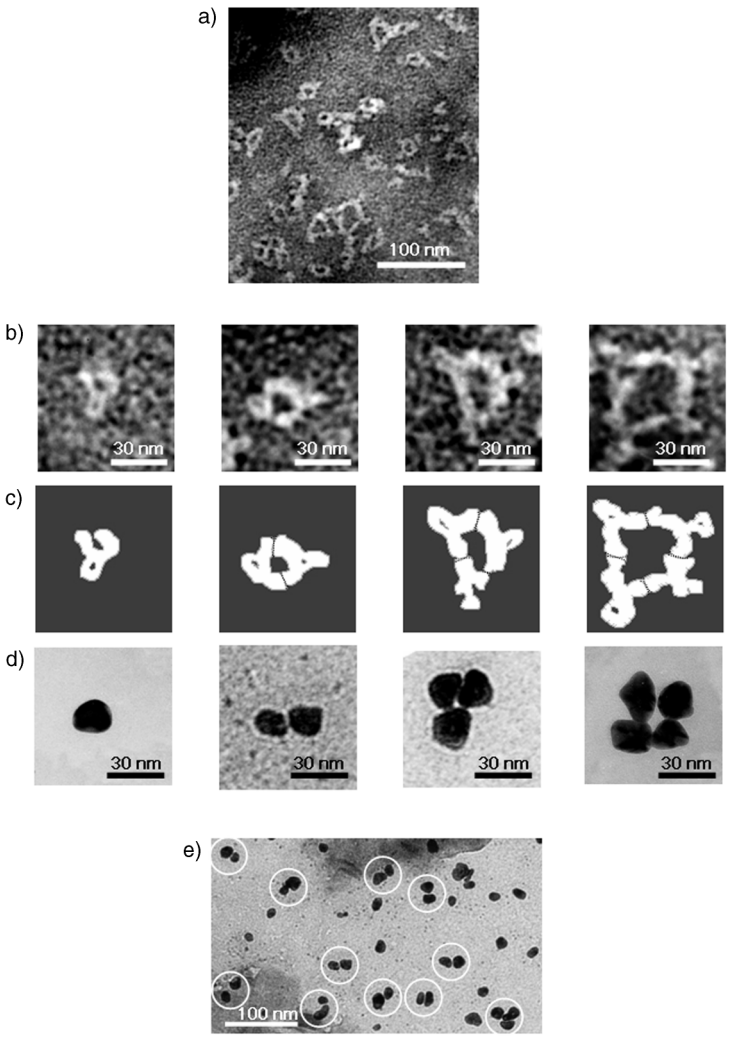

Figure 2. TEM images of aggregated antibodies. a) Image of anti-FITC IgG antibodies deposited from a solution of 1 μM protein after incubation with 2 μM solution of difluorescein antigen **1**; the substrate was negatively stained with a 2% uranyl acetate solution in water. Notice the numerous cyclic structures present. b) Magnified images of (from left to right) a single IgG molecule, cyclic dimer, cyclic trimer, and cyclic tetramer. c) Schematic representation of the corresponding IgG monomer, dimer, trimer, and tetramer in row (b) (obtained by hand-tracing threshold enhanced images of row b; the dotted lines were manually inserted to illustrate our interpretation of divisions between the individual antibody units in each aggregate structure). d) Magnified images of (from left to right) gold nanoparticles presumably grown from a single IgG molecule, a cyclic dimer, a cyclic trimer, and a cyclic tetramer after treatment with sodium periodate (60 min), ethylene glycol (20 min), silver nitrate (90 min), and electroless gold plating solution (2 min). These nanoparticles were grown to a diameter of 15–30 nm to clarify their geometric arrangement. e) Larger area of a sample of anti-FITC IgG deposited in the presence of difluorescein antigen **1** after they had been subjected to the metal-growth conditions (1 min exposure to gold electroless plating solution, clusters of nanoparticles are circled in white).

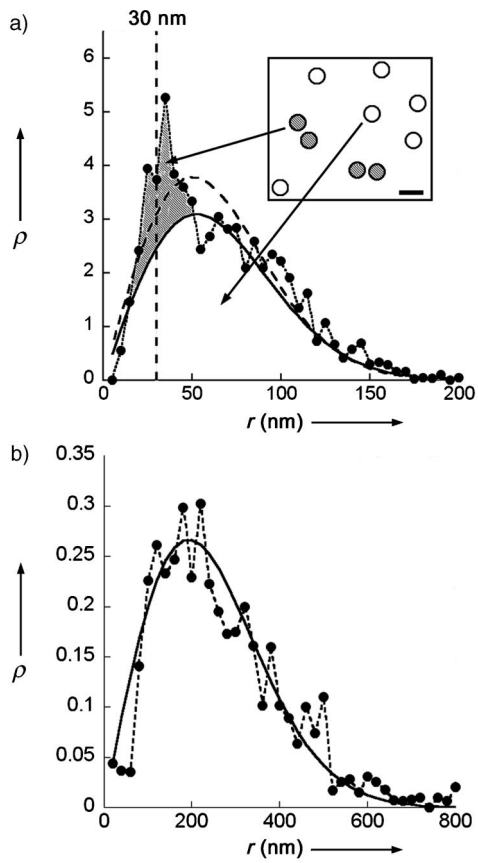

Figure 3. a) Comparison of the distribution of distances of nearest neighbors (DDNN) of particles grown from aggregated IgG templates (dotted line) with the theoretical DDNN for uniformly distributed, uncorrelated particles of the same number density (dashed line) and a density equal to 85% of the experimentally measured one (solid line). The shaded area corresponds to the excess in the density of particles incorporated into multimers compared to a random distribution of particles with a density approximated under the solid line. ρ (particles μm^{-2}) is the density of particles with nearest neighbors at a distance $r \pm \Delta r/2$. The scale bar of the inset corresponds to a distance of $\approx$30 nm. The bin size (Δr) is equal to 5 nm. b) Comparison of the DDNN of nanoparticles grown from IgGs (that were not previously incubated with difluorescein antigen **1**) (dotted line) with the reference DDNN for uniformly distributed, uncorrelated particles of the same number density (solid line). The bin size (Δr) is equal to 25 nm.

We did not observe nanoparticles when we omitted any of the steps in the metal growth procedure—that is, $NaIO_4$ oxidation of the carbohydrate, reduction of Ag^+, or electroless deposition of Au^+. We observed few nanoparticles on the surface under the metal-growth conditions in the absence of a deposited protein, or in the presence of bovine ubiquitin—a protein that does not contain a carbohydrate.

We have thus demonstrated that monoclonal anti-FITC IgGs spontaneously assemble into cyclic and linear oligomers in the presence of a divalent fluorescein antigen. These aggregated IgGs form nanostructures with linear, triangular, and square-planar geometries, and can serve as templates for the growth of clusters of nanoparticles. Further optimization is necessary to improve the disparity in shape, size, and distance between nanoparticles within an assembly.

The realization of growth of metal nanoparticles from a self-assembled molecular template, however, is a step towards generating a variety of tailored geometric metallic structures by using molecular precursors. Assemblies of nanoparticles show promise, for instance, for improved light-attenuating

pigments (owing to more efficient scattering of UV light compared to conventional dye-based inks[2]) and for sensitive molecular diagnostic devices (owing to the colorimetric change upon binding of ligands[1e,3b]).

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Keywords: antibodies · antigens · gold · nanostructures · self-assembly

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[14] IgGs are proteins that comprise four polypeptide chains joined by disulfide bonds to give a Y-shaped glycoprotein.[15] There are two antigen-binding F_{ab} fragments and one crystallizable F_c fragment, which make up the three arms of the Y in the IgG molecules. The F_{ab} arms join the F_c region at a flexible hinge region; the angle between the two F_{ab} regions can vary from 40 to over 180 degrees;[16] the flexibility at the hinge allows for the generation of several different sizes and shapes of cyclic oligomers of IgGs. The length of the F_{ab} and F_c regions varies from organism to organism, but on average the F_{ab} fragment is about 12 nm long and the F_c fragment is about 11 nm long.[10a,17]

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[18] We will report the details of these studies in solution elsewhere. The supporting information includes a representative size-exclusion HPLC chromatogram illustrating the conversion of IgGs to aggregated species.

[19] We separated these aggregates from the non-aggregated protein by FPLC (data not shown). Images of gold nanoparticles grown from a mixture of these purified aggregates appeared indistinguishable from images of particles grown from aggregates of IgGs that were not purified by FPLC.

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[23] Staining and TEM-imaging experiments of IgGs on Si_3N_4 substrates indicate that washing the surfaces in aqueous solutions does not completely remove protein from the substrates.

[24] We believe the difference in the fraction of aggregated IgGs in solution ($\approx 73\%$ of the protein) and the fraction of clusters of nanoparticles grown from the carbohydrates on the IgGs ($15 \pm 5\%$) reflects the less than quantitative yield of the chemical reactions that generate the metal nanoparticles—that is, the oxidation of the carbohydrate units, the reduction of silver ions, and the electroless growth of gold.

[25] Adapted from J. Koolman, *Color Atlas of Biochemistry* Thieme, Stuttgart, 1996.

