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Supporting Information

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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*

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138

Supporting Information

6-(5-aminofluoresceinyl)hexanoic acid succinimidyl ester was purchased from Molecular Probes, Inc. All chemicals, reagents, and buffers used were obtained from Sigma-Aldrich Co. Monoclonal anti-fluorescein IgG (subclass IgG_1, clone ZSF1.1) was purchased from Zymed, Inc. Si_3N_4 membranes (50 nm thick) were purchased from Structure Probes, Inc. Prior to use, the Si_3N_4 membranes were rendered hydrophilic with an air plasma treatment for 15 sec.

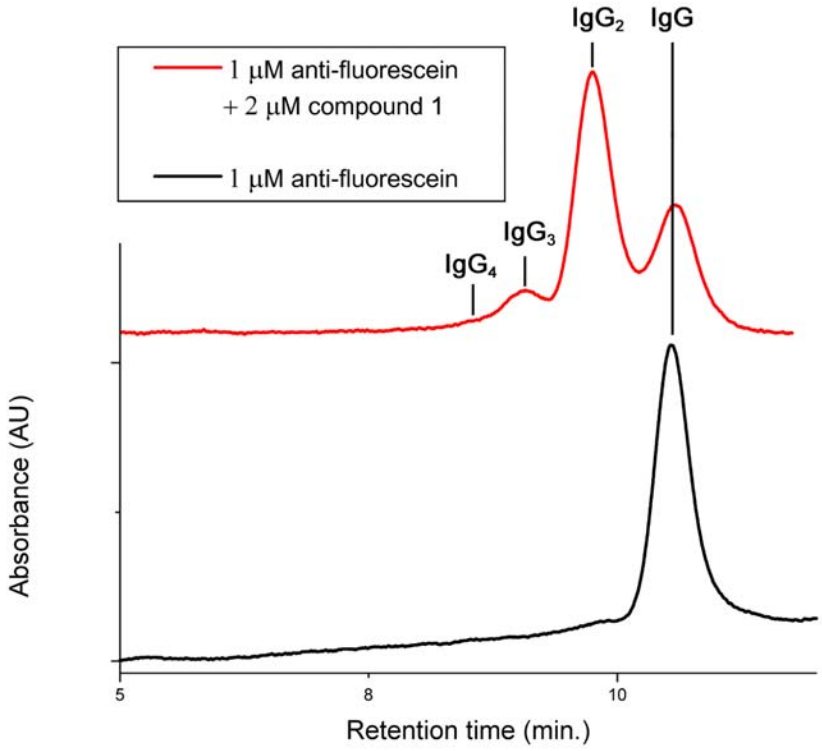
*Synthesis of difluorescein molecule **1***

Compound **1** was synthesized in a single step by adding 10 mg (17 μmols) of 6-(5-aminofluoresceinyl)hexanoic acid succinimidyl ester to 0.62 μL (4 μmols) of 2,2'-(ethylenedioxy)diethylamine in 1 mL dry DMF. The product was isolated by column chromatography, eluting with 90:10:1 CH_2Cl_2:methanol:acetic acid to give 4.3 mg of **1** (96% isolated yield). ^{1}H NMR (500MHz, CD_3OD): δ = 8.42 (s, 2H), 8.02 (d,2H), 7.31 (d, 2H), 6.97 (s, 2H), 6.95 (s, 2H), 6.63 (m, 4H), 6.59 (d, 2H), 6.57 (d, 2H) 3.59 (s, 4H), 3.52 (m, 4H), 3.42 (m, 4H), 3.38 (m, 4H), 2.21 (t, 4H), 1.65 (m, 8H), 1.45 (m, 4H). ESI-MS: 517 (M^{2+})

HPLC analysis of aggregates of antibodies

HPLC measurements were performed on a Dynamax SD-300 (Varian, Inc.) solvent delivery system using a Tosohaas TSK-GEL G4000SWXL size exclusion column. The crude protein solutions were eluted with a 0.1 M phosphate buffer (pH=7.0) containing

0.3 M NaCl, using a 1 mL min^{-1} flow rate. The products were detected by UV-Vis detector, monitoring at λ=214 and 280 nm. The concentration of proteins used in all experiments was kept constant at 1 μM. Unless stated otherwise, all samples analyzed by HPLC were incubated at 4 ºC for 12 hrs prior to injection. Supplemental Figure A presents representative chromatograms of the commercial anti-fluorescein IgG (bottom trace) and a mixture of aggregated anti-fluorescein IgGs with unaggregated protein after incubation with 2 mol. equivalents of compound **1** (top trace). We will report the details of our HPLC studies elsewhere.



Supplemental Figure A Representative size-exclusion HPLC chromatograms (λ=214 nm) of the anti-fluorescein IgG (bottom, black trace) and a mixture of aggregated IgGs and unaggregated protein in the presence of 2 mol. equiv. of compound **1** (top, red trace).

Preparation of samples of antibodies for TEM imaging

TEM samples of nanostructures of IgGs were prepared by floating freshly cleaned (by air plasma oxidation for 30 sec.) carbon-coated Formvar grids onto a 10 μL drop of 1 μM solutions of proteins for 1 min. The excess protein solution was drawn off the grid with filter paper. The grids with deposited proteins were then floated on a 10 μL drop of 2% uranyl acetate staining solution for 1 min. Excess staining liquid was partially drawn off, leaving the grids wet. The grids were dried in air prior to imaging. TEM images were obtained using a Philips EM 210 electron microscope (80 keV).

Procedure for templated growth of gold nanoparticles

Aggregates of protein (formed from a solution of 2 μM **1** and 1 μM anti-fluorescein in pH=7.0, 0.1 M phosphate, 0.3 M NaCl buffer) were deposited on a Si_3N_4 membrane for 1 min. by floating the membrane over a ~20 μL droplet of the protein solution on Parafilm®. The membrane was washed three times by floating over ~100 μL distilled water droplets deposited on Parafilm® (1 min. per wash). The membrane was floated over a ~100 μL droplet of a freshly prepared solution of 10 mM sodium periodate (in pH=5.0, 0.02 M, 0.15 M NaCl acetate buffer) for 1 hr. The membrane was immediately transferred to a droplet of 1 M ethylene glycol solution for 20 min. to quench excess periodate and then washed three times with distilled water (1 min. per wash). The membrane was incubated for 90 min. with a 0.1 M $AgNO_3$ solution (in 28-33% NH_4OH adjusted to pH=10.5 with conc. HNO_3); *Caution:* the addition of nitric acid to the ammonium hydroxide solution is violent, and should be carried out dropwise at 0°C under vigorous stirring. After three 1 min. washes over ~100 μL

distilled water droplets deposited on parafilm, the membrane was incubated with freshly prepared gold electroless plating solution.[1] The size of the resulting nanoparticles on the deposited IgGs varied from 5-30 nm depending on the time of exposure to the plating solution (0.5-2 min.).

Description of image analysis to determine the distribution of distances between nearest neighbors (DDNN) of particles deposited on a 2D surface

We examined six TEM images of the substrates containing gold nanoparticles grown from aggregated antibody templates. The area covered by a single microscopy image ranged from 2320 × 1840 nm to 3590 × 2843 nm. All of the images were represented as 1300 × 1030 pixels grayscale matrixes and further analyzed with image recognition software developed in our lab. In brief, the software locates the nanoparticles and outputs a list of the coordinates of the center of mass of the particles. Due to limited resolution of the images, the program could not always resolve the particles incorporated into multimers; that is, the particles incorporated into multimers sometimes merged on the image and were identified as a single object. Also, we excluded particles > 50 nm or < 1 nm in size for this analysis. Thus the results using this procedure most likely underestimate the number of multimeric assemblies of nanoparticles in a given image.

[1] $AuCl_3$ (19 mg) was dissolved in 1 mL deionized water and added to a solution of KSCN (60 mg, in 1 mL deionized water) to afford an orange precipitate. The precipitate was centrifuged to a pellet (1 min. at 4000*g*). The excess liquid was removed and the precipitate was dissolved in 8 mL of phosphate buffer (1 M, pH = 5.5). The gold solution was incubated with hydroquinone (5.5 mg in 1 mL deionized water) and used immediately for electroless deposition of gold.

Within the set of six images examined the average number density of identified particles was $\rho_{total} = <\rho> = 62.8\ \mu m^{-2}$ with the standard deviation $\sigma_\rho = 4.5\ \mu m^{-2}$.

We used the list of coordinates of the nanoparticles to generate a graph of the number of particles versus the distance to their nearest neighbor (Figure 3a of the main text). We compared these histograms with the theoretical DDNN for a random (uncorrelated) distribution of particles deposited with a uniform distribution of density on a 2D surface. This reference DDNN is constructed as follows—the probability of finding the nearest neighbor $dP(\rho,r,dr)$ at a distance $d \in (r, r+dr)$ is equal to the probability $Q_1(\rho,r)$ that there is no particle at a distance smaller than r multiplied by the probability $dQ_2(\rho,r,dr)$ of finding a particle at a distance $d \in (r, r+dr)$. If the particles are deposited with a uniform distribution of density, the probability Q_1 that there is no particle in a circle of radius r is given by the Poisson distribution as $Q_1(\rho,r) = \exp[-\rho\pi r^2]$. The probability $dQ_2(\rho,r,dr)$ of finding a particle within a ring with an inner radius r and outer radius r+dr is $dQ_2(\rho,r,dr) = \rho 2\pi r dr$. Combining these two terms together gives

$$dP(\rho,r,dr) = \rho 2\pi r \exp[-\rho\pi r^2]dr \qquad (1)$$

with the property that the integral of P over the whole range of distances is equal to unity. The expected number of particles $dW(\rho,r,dr)$ having their nearest neighbors at distance $d \in (r, r+dr)$ is equal to the probability $dP(\rho,r,dr)$ times the density of particles, that is $dW(\rho,r,dr) = \rho\, dP(\rho,r,dr)$.

For the sake of simplicity we assume that the particles are associated only into single particles and dimers of particles; that is, we exclude the trimmers and tetramers from the analysis. Then the total density of particles is

$$\rho_T = \rho_1 + \rho_2 \qquad (2)$$

where ρ_1 and ρ_2 are the densities of single particles and particles incorporated into dimers respectively. The particles incorporated into dimers are separated by a distance $d \in (15, 45)$ nm. Therefore they cannot have their nearest neighbors further than 45 nm. The single particles, however, are affected by the presence of dimers—as particles incorporated into dimers might happen to be the nearest neighbors of the random, uncorrelated particles. Positions of the particles within a dimer are not independent and only half of the density ρ_2 will add up to the density of uncorrelated particles ρ_U. The density ρ_U of uncorrelated particles is equal to:

$$\rho_U = \rho_1 + \rho_2/2 \qquad (3)$$

The density of uncorrelated particles will affect the distribution of distances to the nearest neighbors. The expected number of nearest neighbors for the random, uncorrelated particles is:

$$dW_1(\rho_U, \rho_T, r, dr) = \rho_1 \, dP(\rho_U, r, dr) = (2\rho_U - \rho_T)\, \rho_U \, 2\pi r \exp[-\rho_U \pi r^2] \, dr \qquad (4)$$

In order to determine the density of uncorrelated particles ρ_U, we fit equation 4 to the experimentally determined histogram for $r > 45$ nm. This procedure yielded $\rho_U = 0.925\ \rho_T \pm 0.025\ \rho_T$. We represent this fit by the solid line in Figure 3a (in the main text). For comparison, we plotted a distribution for $\rho_U = \rho_T$ (dashed line). This curve should coincide with the experimental histogram if there were no correlations between the positions of the particles. Having an estimate of ρ_U we can calculate the densities of single particles $\rho_1 = 0.85\ \rho_T$ and the density of particles incorporated into dimers $\rho_2 = 0.15 \pm 0.05\ \rho_T$. We represent the contribution of dimers to the experimentally determined histogram of the distances to the nearest neighbors by the shaded area in Figure 3a.

Based on the analysis described above we estimate that 15 ± 5% of the particles localized by the image recognition software are in multimers.

In order to check the validity of the analysis, we performed the same procedure for analysis (four TEM images) on control images of substrates containing nanoparticles grown from IgGs that were not previously incubated with difluorescein antigen **1**. Figure 3b (in the main text) presents a comparison of the graph of the experimental DDNN (dotted line) of these control images and the theoretical DDNN for a random (uncorrelated) distribution of particles (solid line). The average number density of identified particles was $\rho_{control}=<\rho>=4.3\ \mu m^{-2}$ with the standard deviation $\sigma_\rho=0.6\ \mu m^{-2}$. We did not find a statistically significant excess of particles having their nearest neighbor at a distance $d \in (15, 45)$ nm. The experimental DDNN correlates well with the theoretical DDNN for a random distribution for the same density of particles $\rho_{control}$.

Supplemental Figure B presents one (small area) example of an image of nanoparticles grown from templates of aggregated IgGs: a) unaltered image; b) same image with particles identified by the imaging software highlighted with red circles; and c) same image as (b) with a line drawn to indicate what the program identifies as a particles nearest neighbor. We converted a digital jpeg image of particles grown from a substrate to a bitmap form for the image analysis; this conversion to bitmap from a jpeg format makes Supplemental Figure B differ in quality from the image in Figure 2E of the manuscript.

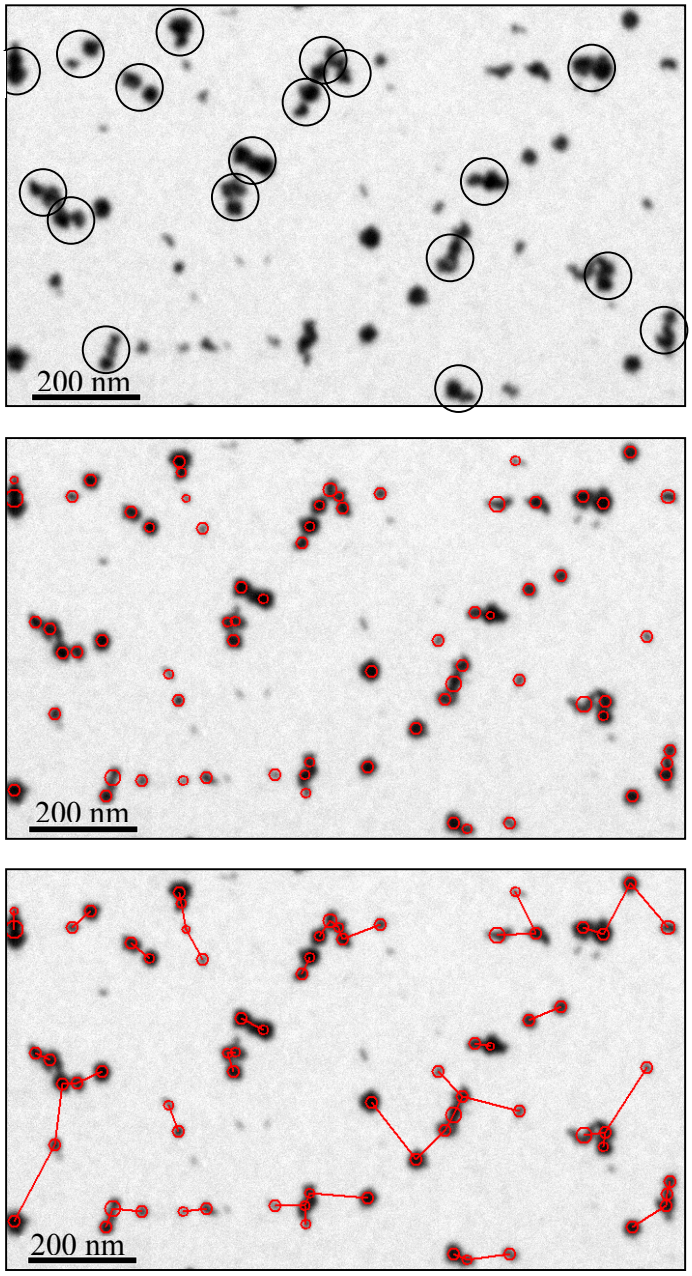
Supplemental Figure C presents one example of an image of nanoparticles grown from templates of IgGs (without prior incubation with difluorescein antigen **1**): a) unaltered image; b) same image with particles identified by the imaging software

highlighted with red circles; and c) same image as (b) with a line drawn to indicate what the program identifies as a particles nearest neighbor.

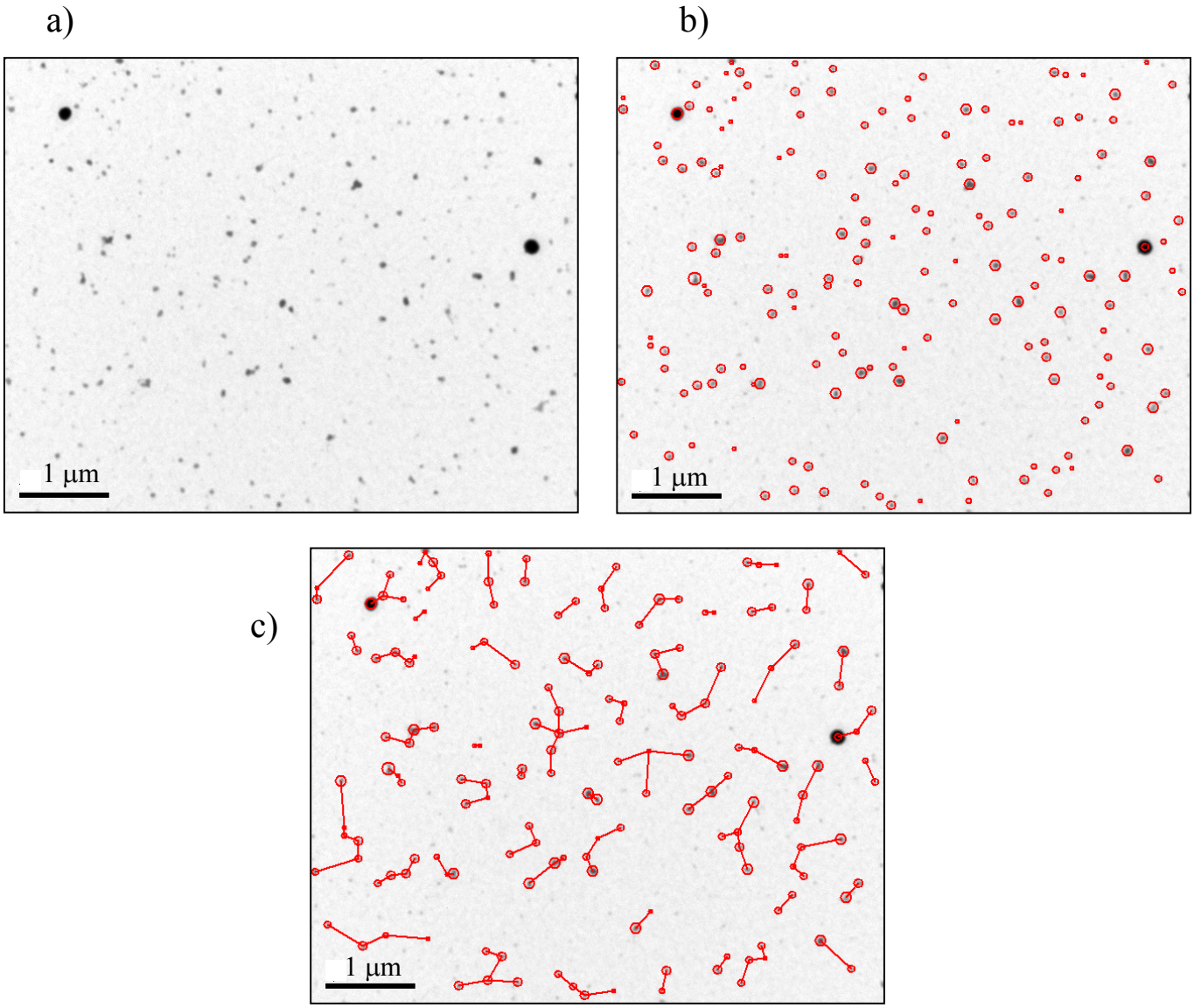
a)

b)

c)



Supplemental Figure B. Example of the TEM image of the substrate with nanoparticles grown from aggregates of IgGs; a) unaltered image (with clusters of particles determined by the imaging software circled in black), b) image with the nanoparticles identified by the image recognition software marked with small red circles, c) image with lines indicating the nearest neighbor of each particle.



Supplemental Figure C. Example of the control TEM image of the substrate with nanoparticles grown from single IgGs (without preincubation with difluorescein antigen **1**); a) unaltered image, b) image with the nanoparticles identified by the image recognition software marked with red circles, c) image with lines indicating the nearest neighbor of each particle.