Supporting Information for

A Zwitterion-DNA Coating Stabilizes Nanoparticles Against Mg^{2+} Driven Aggregation Enabling Attachment to DNA Nanoassemblies

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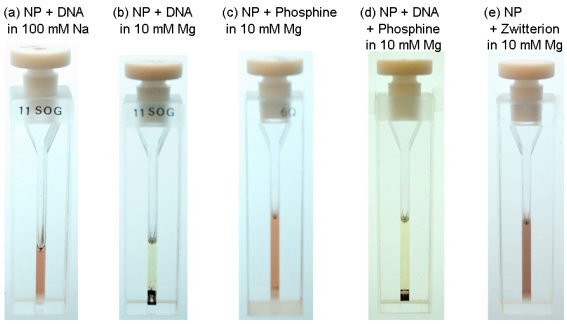
1.Demonstration of ligand stabilization of 5nm AuNPs



Photographs of 100 nM concentrations of 5 nm diameter AuNPs with various surface treatments or ligands in borate buffers with sodium chloride or magnesium acetate added as indicated to provide ions. Reddish tints indicate well-dispersed NPs, while yellowish tints with black precipitates indicate aggregated NPs.

Figure 1 shows photographs of a series of cuvettes each with 100 nM AuNPs but with different surface treatments and ionic environments. When unaggregated, the NPs give a reddish tint to the solution that is easily apparent to the eye. When the larger nanoparticles aggregate, however, they form a blackish precipitate on the bottom of the cuvette, leaving behind a residue of smaller NPs that give the bulk of the solution a yellowish tint.

Figures 1 a and b illustrate the significance of the need for divalent cation-stable NPs. The DNA-coated NPs are stable in a buffer with 100 mM Na^{+} ions, but aggregate in just 10 mM Mg^{2+} ions. Figures 1 c and d show another complication: phosphine coating can protect the NPs from aggregation in Mg^{2+} ions, but when DNA is ligated onto the NPs in addition to the phosphine, the NPs aggregate in the same buffer. Presumably the divalent cations draw together the negative charges of the DNA phosphate backbones with enough strength to bring the NPs together. This aggregation can be reversed, temporarily, if the particles are diluted and sonicated. As Figure 1 e shows, the zwitterionic ligand shell also protects the NPs from aggregating in the presence of Mg^{2+} ions.

2. Experimental Methods

2.1 Zwitterion Disulfide Synthesis

Synthesis of zwitterion disulfides followed as per Rouhana *et al.*[1] Briefly, 2-dimethylaminoethane thiol was oxidized to bis(2-dimethylaminoethyl)disulfide in aqueous acetic acid with sodium perborate. The reaction mixture was neutralized with sodium hydroxide pellets and the resulting oil was extracted with ether. The ether was evaporated via rotovap and treated with propane sultone in dry acetone to form disulfide zwitterions. When exposed to AuNPs, the disulfide bonds would be disrupted as each sulfur preferentially bound to the Au, leaving individual mono-sulfide zwitterions ligated to the NPs.

2.2 NP Surface Treatments

Citrate capped, AuNPs 5 nm in diameter were purchased from Ted Pella, Inc. at a concentration of $5x10^{13}$ NPs/mL. Surface treatments were done on with 300-500 μL of the NPs at a time.

2.2.1 Preparation of Phosphine-Coated NPs

The citrate-coated NPs were mixed with 10mM phosphine (4,4'-(Phenylphosphinidene) bis(benzenesulfonic acid) dipotassium salt hydrate 97%, Aldrich) in 10 mM phosphate buffer (pH 7). Excess phosphine was removed with a 50 kDa cut-off filter (Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-50 membrane). The resulting phosphine-coated AuNPs were resuspended in water or buffer as needed.

2.2.2 Attachment of Thiolated DNA to NPs

3' thiolated DNA oligo-nucleotides were purchased from Integrated DNA Technologies, Inc. The DNA was treated with DTT (DL-Dithiothreeitol ≥ 98% (TLC), ≥ 99%(titration), Sigma-Aldrich) to reduce the dithiol bonds and leave the sulfurs available for attachment to the AuNPs. DTT was removed using an Illustra NAP-5 sephadex desalting column , the DNA was suspended in water, and quantitated using UV absorption at 260 nm. The DNA was then added to the NPs in 5 steps, up to a final DNA to NP ratio of 50 to 1. After each addition of DNA, the solution was vortexed strongly for at least a minute. After the final addition of DNA, the NPs would be rotated on a rotisserie at about 0.1 Hz over night at room temperature.

2.2.3 Attachment of Zwitterions to NPs

The zwitterions were added either to the citrate capped AuNPs, or the AuNPs with thiolated DNA already on it. The zwitterion-disulfides were dissolved at 10 mg/mL in a 10 mM phosphate buffer (pH 7). 100 μL were then combined with the NPs, suspended in the same buffer. The solution was then mixed in a rotisserie at about 0.1 Hz over night at room temperature. After zwitterion attachment the unbound zwitterions were removed from the solution by filtration with a 50 kDa cut-off filter (Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-50 membrane). The NPs were rinsed three times with water, and then resuspended in water or buffer as needed. The particles can be stored at 4ºC.

2.3 DNA Scaffold Preparations

2.3.1 Geometrical and Sequence Design

The Mao triangles for the scaffold were initially modeled geometrically using TriangleAnglestoGideon10.xls, and GIDEON.[2] The distances between various binding sites were calculated based on coordinates exported from GIDEON. The sequences for the triangles were generated using the program SEQUIN, subject to the restrictions that no GGG, CCC, or self-complementary GC sequences longer than three bases were allowed.[3] Two bases about each junction use the J1 sequences.[3] The sticky ends were designed with unique 4-mers, and in such a way that they could not fold on themselves with more than 1 consecutive proper base pairing.

2.3.2 Scaffold Assembly

The strands of the scaffold were all purchased from Integrated DNA Technologies, Inc. either with Poly-Acrylamide Gel Electrophoresis (PAGE) purification, or with desalting, and subsequent in-house PAGE purification and quantitation via UV_{260} absorption.[4]

The unfunctionalized arrays were formed by mixing together stoichiometric quantities of all the strands for triangles 1 and 2 at 0.750 μM concentration in a 0.6 μL tube. A borate buffer was used (45 mM borate, 10 mM $Mg(CH_3COO)_2$, pH 8). The 0.6 μL tube was then placed at the bottom of an air-filled 50 mL tube, which, in turn, was placed in a 1 L bottle of 95 ºC water. This arrangement holds the small tube at a relatively uniform temperature, and prevents condensation of water at the top of the tube. The system was annealed via slow cooling over 12 hours down to room temperature.

The functionalized arrays were formed by taking the 5 nm diameter AuNPs with zwitterions and suitable DNA and mixing into the solution yielding a final concentration for each triangle in the array of 0.37 μM, and a NP concentration of 1.9 μM. The NPs were given 30 minutes at room temperature to bind to the arrays, and then the solution was deposited onto freshly cleaved mica. After 3 minutes, the system was gently rinsed with 200 μL of 200 μM aqueous magnesium acetate. The residual liquid was wicked off and the sample was blown dry with a dust gun.

2.4 Analytical methodology.

2.4.1 Dynamic Light Scattering

Samples were prepared immediately before starting DLS observations. DLS measurements were performed using a glass micro-cuvette at concentrations of 500 nM The observations were made using a Malvern Zetasizer Nano.

2.4.2 Atomic Force Microscopy

5 μL of annealed arrays were dropped onto freshly cleaved mica. After 3 minutes, 600 μL of buffer was added. AFM micrographs were taken in liquid, in tapping mode using an Asylum Research MFP-3D. Small NPS tips with resonance frequency at 13.6 KHz in liquid were used. Vertical scales for the micrographs were approximately 4 nm.

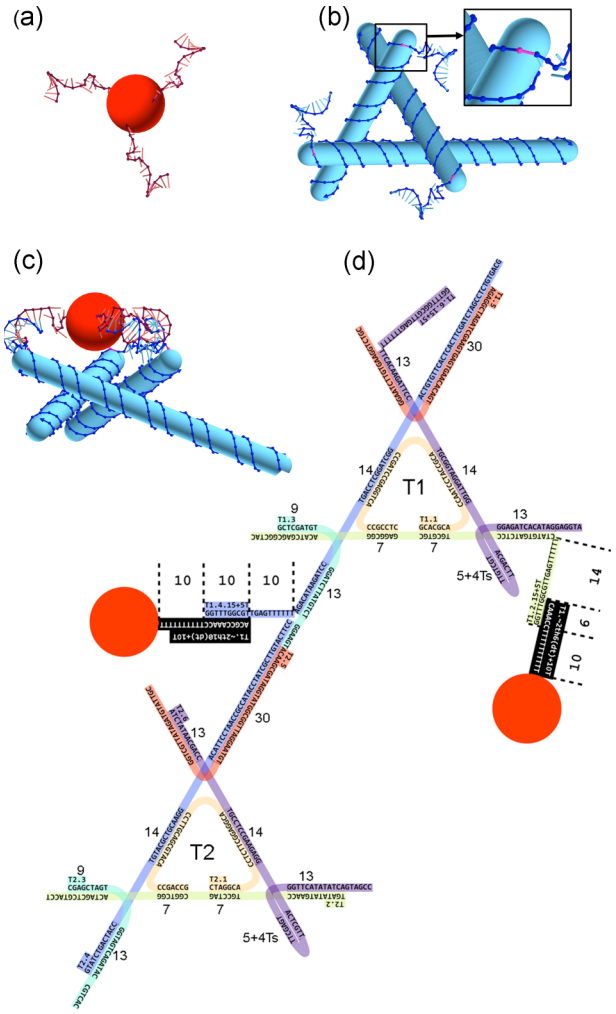
2.4.3 Scanning Electron Microscopy

The samples studied via SEM were prepared as specified in the text. Imaging on an insulator such as mica is challenging, but possible. Typical key settings for the Hitachi-4800 SEM we used are as follows. Accelerating Voltage was 2.5 KV. Deceleration Voltage was 1.5 KV. Working Distance was 2.5 mm. Emission current was 2300 nA. Backscatter detection was used. The SEM scales were calibrated by setting the center-to-center spacing between aggregated, un-scaffolded DNA-coated NPs to that observed for a similar sample via TEM.

2.4.4 Micrograph Post-Processing

Images were processed using Scanning Probe Image Processor, SPIP™ 5.0.5. Images were flattened using line-wise correction, LMS fit of degree 0 or 1 as needed for clarity. SEM images were subjected to a Gaussian smoothing with a kernel size of 13x13 and a standard deviation of 2.6 (or less, as needed), to reduce noise, and scales were selected to optimize contrast.

3. Sequence of triangles and other projections of triangle design



(a) Scale drawing of a 5 nm diameter AuNP with three DNA ligands positioned in a possible configuration to attach to a DNA scaffold.

(b and c) show drawings of DNA nanostructures where base stacks are indicated by colored cylinders with lines around the cylinders indicating phosphate backbones. The DNAs linking the NP to the scaffold are shown with bases represented by rods.

(b) shows the triangle without NP attached, with inset showing that the DNA attaches on the upward facing side of the triangle.

(c) shows front view of a NP bound to a triangle. Because the double helices that make up the edges of T1 are so short, their effective width is almost half their length, and the structure is significantly non-planar.

(d) shows a sequence-structure diagram of the array tiles with two different NP attachment schemes. Strands are named near their 5' ends.

References

1. L. L. Rouhana, J. A. Jaber and J. B. Schlenoff, *Langmuir*, 2007, **23**, 12799-12801.
2. J. J. Birac, W. B. Sherman, J. Kopatsch, P. E. Constantinou and N. C. Seeman, *J Mol Graph Model*, 2006, **25**, 470-480.
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4. M. T. Kumara, D. Nykypanchuk and W. B. Sherman, *Nano Lett*, 2008, **8**, 1971-1977.