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Nanostructured DNA Templates

Jeffery L. Coffer, Russell F. Pinizzotto, and Young Gyu Rho

Summary

We have developed methods for nanostructure fabrication relying on the size and shape of a polynucleotide to dictate the overall structure of an assemblage of individual semiconductor nanoparticles. Use of the circular plasmids pUCLeu4 and $\phi\chi$ 174 when anchored to a suitably derivatized substrate yields arrays of semiconductor nanoparticles matching the shapes of the biopolymer stabilizer. The viability of the methodology was confirmed using high-resolution transmission electron microscopy and selected-area electron diffraction.

Key Words

pUCLeu4; $\phi\chi$ 174; plasmid; semiconductor nanoparticles; cadmium sulfide; templates.

1. Introduction

The use of polynucleotides for nanostructure fabrication ***(1–3)*** is a useful development in a rapidly expanding area of interdisciplinary nanoscale science in which biological systems are used to control the size, shape, orientation, assembly, and so on of the material ***(4)***. The final nanostructures also indirectly mirror DNA structure without the necessity of shadowing or staining techniques ***(5)***. Ideally, if semiconducting materials are fabricated using this strategy, the resulting composite also provides an interface for future hybrid device platforms applicable to DNA-based computation ***(6)***. In principle, an almost unlimited number of nanostructures can be formed using DNA molecules of various sizes, shapes, and composition as structural templates for additional synthesis ***(7)***. The simplest choices include quantum wires, assembled using nucleotides 2–50 kb in length; quantum rings, analogous to quantum corrals, assembled using covalently closed circular polynucleotides known as plasmids; and quantum dots formed directly from small DNA fragments of 30 bp or less (or, alternatively, condensed larger DNA molecules) ***(8–11)***. Based on some of the more

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complex DNA topologies described in the literature (e.g., knots, crosses) ***(12)***, other more elaborate motifs can also be envisioned.

In this chapter, we provide protocols for the formation of networks of quantum-confined cadmium sulfide nanoparticles (Q-CdS) formed using circular plasmid DNA molecules anchored to a solid substrate. By varying the deposition sequence and/or reactant concentrations, two extreme morphologies are illustrated: isolated rings of Q-CdS/plasmid DNA and weblike arrays of Q-CdS/plasmid DNA. Overall, the topology of the DNA bound to the solid substrate acts as a template to control the overall shape of the self-assembled nanoparticle structure.

2. Materials

1. Carbon rods, spectroscopic grade (less than 2-ppm impurities).
2. Copper grids for transmission electron microscopy (TEM), 400 mesh.
3. Cadmium perchlorate, $Cd(ClO_4)_2 \bullet 6H_2O$.
4. Hydrogen sulfide, H_2S (99.5%).
5. Plasmid DNA, pUCLeu4 and $\phi\chi$ 174 RF II.
6. Ultrapure water, 18 MΩ quality.

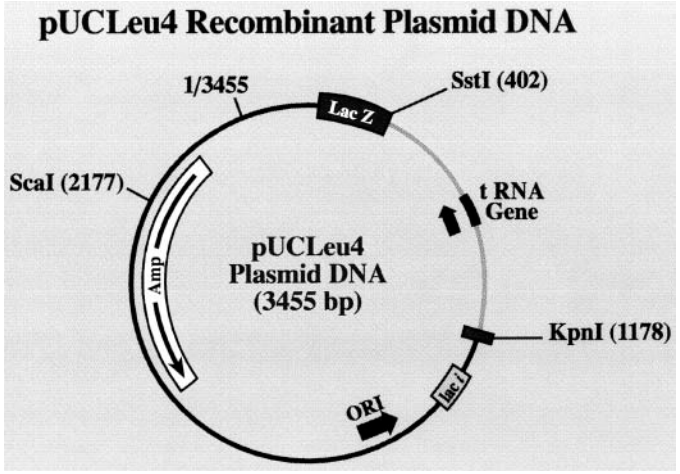
3. Methods

The methods outlined next describe the properties of the specific plasmids used as templates, proper preparation of the carbon-coated copper grids necessary for TEM analysis, and fabrication of the CdS nanostructures on the plasmid templates. In addition, typical results for some of the procedures, as determined in our laboratories, are presented.

3.1. Synthesis and Purification of Plasmid Templates

pUCLeu4 DNA was generously provided by Dr. Robert M. Pirtle of the University of North Texas. pUCLeu4 DNA was generated by cloning a human DNA fragment (781-bp *Kpn*I-*Sst*I) encompassing a leucine $tRNA_{AAG}$ gene as a foreign DNA into pUC19 vector DNA (**Fig. 1A**). A large amount of pUCLeu4 plasmid DNA, produced by *Escherichia coli*, was extracted from the bacterial cells by an alkaline procedure and treated with RNases A and T_1 to degrade the bacterial RNA. The bacterial RNA was extracted with phenol:chloroform (1:1) and chloroform:isoamyl alcohol (24:1) and precipitated with cold ethanol. The chromosomal bacterial DNA and degraded RNA were removed from the pUCLeu4 plasmid DNA by high-performance liquid chromatography on an anion-exchange column using an NaCl gradient ***(13)***.

According to polyagarose gel electrophoresis, approx 70% of the undigested pUCLeu4 plasmid DNA synthesized with this method was supercoiled. The supercoiled pUCLeu4 plasmid DNA molecules were treated to generate the



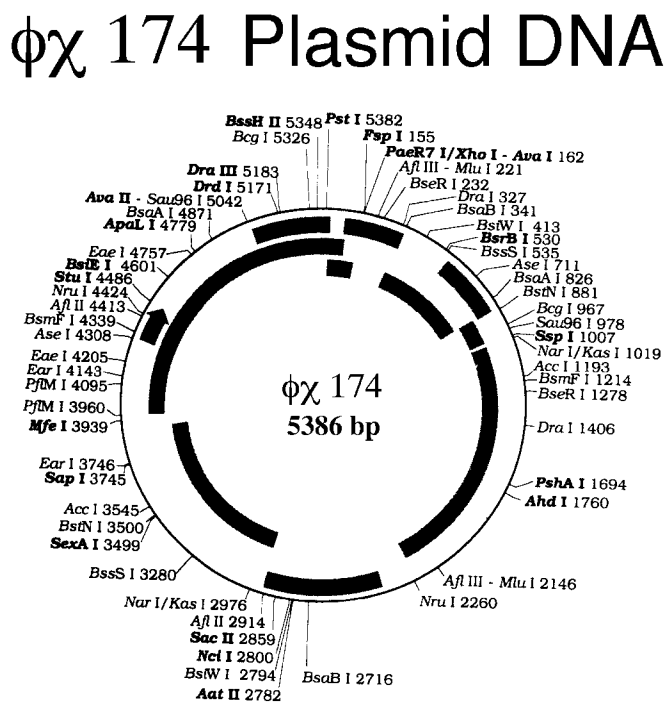


Fig. 1. Composition maps for plasmids (top) pUCLeu4 and (bottom) X 174 RF II utilized as templates.

relaxed circular form for use as templates. The population of relaxed circular DNA present can be enhanced by cutting and rejoining one of the 3′ or 5′ phosphodiester bonds using DNA topoisomerase I enzyme.

The plasmid $\phi\chi$ 174 RF II was obtained commercially from New England Biolabs (Beverly, MA). It is a larger plasmid vector containing 5386 nucleotides and is approx 1.83 μm in circumference (for the relaxed, open circular conformation).

3.2. Fabrication and Preparation of Ultrathin Carbon-Coated TEM Grids

3.2.1. Fabrication of Carbon Substrate

Since the scale of the Q-CdS/DNA semiconductor structures is in the nanometer regime, very fine and flat substrate surfaces are necessary for TEM analysis. Microstructural characterization of the carbon films was started because commercial carbon films have many artifacts that can make TEM analysis difficult, and potentially nondiagnostic of the resulting nanostructures. In particular, some of these microstructures, formed during film fabrication, are similar in size and shape to the microstructure of Q-CdS/linear DNA. In the initial stages of our experiments, these artifacts were misleading. Therefore, the carbon films used for TEM substrates were fabricated in our laboratory and always examined before use as substrates.

The thin carbon films that were the primary substrates used for these experiments were fabricated using a JEOL JEE-4X evaporator operating at 2×10^{-6} torr. As a carbon source for the evaporator, one side of a carbon rod was sharpened to approx 1 mm in diameter for a length of 6–8 mm using a fine file (*see* **Note 1**). Carbon evaporation is achieved by resistively heating the contact area of two sharpened carbon rods, so the thin carbon tip makes it easier to control the evaporation process. The carbon rods were positioned about 10–15 cm above the specimen stage. Freshly cleaved mica, with the fresh side facing upward toward the carbon source, was placed on the specimen stage (underneath the carbon rods) using tweezers. When preparations were complete, the work chamber was evacuated for 1 h to reduce the vacuum level to lower than 2×10^{-6} torr. When high vacuum was achieved, the mica substrate was covered with a shutter and the heating current increased slowly until the tip of the carbon rod began to glow red. After a few seconds, the particles that were generated during the sharpening of the carbon rod were removed. The shutter was then opened and the heating current increased to evaporate the carbon. The current was increased as slowly as possible until the tips of the carbon rods glowed white so that the carbon

films were deposited by evaporation and not by arcing, which produces many carbon fragments. Depending on the height of the carbon rods, but after two or three evaporation cycles with each approx 20–30 s long, carbon film deposition was complete.

3.2.2. Preparation of Carbon Substrates on Cu TEM Grids

The carbon films were placed on acetone-cleaned 400-mesh Cu TEM grids. The grids were placed shiny side up on a wire mesh that was in steam-sterilized distilled water contained in a Buchner funnel or a dish that could be drained. The carbon film was separated from the mica substrate by dipping the mica into water and floating off the carbon film. The TEM grids were coated by aligning the separated carbon film on top of the TEM grids and then draining the water. The wire mesh was picked up with tweezers and placed on filter paper to remove the water remaining on both the wire mesh and the TEM grids. The carbon-coated grids were used within 30 min, before they were completely dry, or after complete drying, depending on the type of experiment performed (*see* **Note 2**).

3.3. Preparation of Q-CdS/pUCLeu4 or Q-CdS/ϕχ 174 RF II Samples

The range of fabrication strategies for quantum-confined cadmium sulfide nanoparticles on DNA templates is outlined in **Fig. 2**. All routes entail four basic steps: (1) preparation of both DNA and Cd ion solutions at the requisite concentrations, (2) formation of a plasmid–cadmium ion complex, (3) adsorption onto a solid support, and (4) reaction with a gaseous sulfide source. These steps are typically followed in the order listed, but the option exists for switching steps 2 and 3 if desired (*see* **Subheading 3.3.5.**).

3.3.1. Preparation of DNA and Cd^{2+} Solutions

In a typical experiment, approx 2 mL of 2 m*M* DNA (the amount of DNA is defined on a per-nucleotide basis) was prepared from the original concentration of about 2 mg/mL. To prepare this target molar nucleotide concentration of 2 m*M*, a 10-µL aliquot of 2 mg/mL DNA solution was taken via microliter syringe and diluted with 10 mL of distilled deionized water in a 50-mL flask. The DNA solution was thoroughly mixed by shaking it manually for approx 10 min. The concentration of the diluted solution was measured spectrophotometrically using an ε value of 6600 M^{-1} cm^{-1} at an absorption wavelength of 260 nm. In a separate beaker, 100 m*M* $Cd(ClO_4)_2 \cdot 6H_2O$ solution was freshly prepared from the solid, which was dissolved slowly by shaking for approx 10 min. To make 2 mL of 2 m*M* cadmium ion solution from this stock 100 m*M* Cd^{2+} solution, 40 µL of the 100 m*M* solution was diluted in 1.96 mL of distilled deionized water.

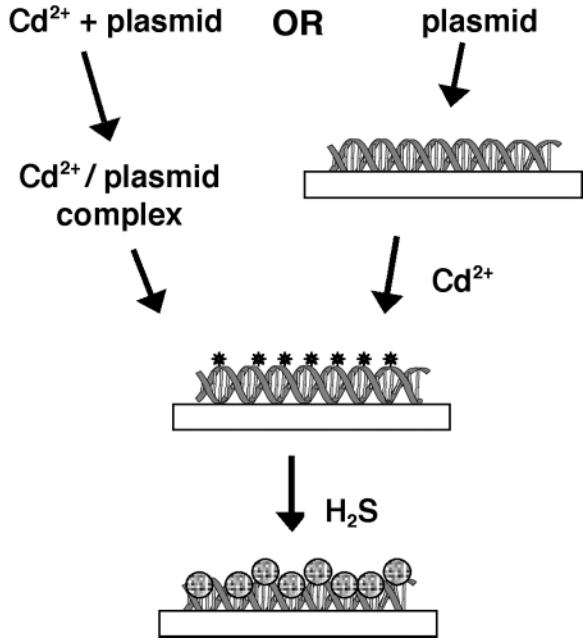


Fig. 2. Routes to semiconductor nanostructure formation using DNA templates.

Some experiments entail the variation of cadmium ion concentration in nanostructure formation. In these experiments, two strategies were explored: (1) an overall lower loading of nanostructures on a given support, and (2) in one of these diluted nanostructures a large excess of cadmium ion to nucleotide. For the former, 2 mL of a 2 m*M* Cd^{2+} solution is mixed with 2 mL of 5 μg/mL (approx 15 μ*M* in nucleotide) pUCLeu4 plasmid DNA or, alternatively, 2 mL of a 1 μg/mL solution (approx 3 μ*M* in nucleotide) for the case of the φχ 174 RF II. In the latter example, 2 mL of 2 μ*M* Cd^{2+} is mixed with 2 mL of 5 μg/mL pUCLeu4 plasmid DNA or 1 μg/mL of 2 mL φχ 174 RF II DNA, close to a target ratio of 1:1.

3.3.2. Mixing of Cd^{2+} and DNA in Solution

Formation of cadmium metal ion/DNA complexes was carried out by mixing the two previously prepared solutions of Cd^{2+} and plasmid DNA in a 1:1 ratio in a 10-mL beaker. The mixed solution was stirred with a pipet by squeezing the solution in and out of it for 2 to 3 min to mix the Cd^{2+} and DNA homogeneously. The beaker was shaken slowly for approx 10 min to maximize the interaction between the cadmium ions and the DNA.

3.3.3. Reactant Adsorption Onto Solid Supports and Reaction With Sulfide

Samples were prepared on the previously described thin amorphous carbon films to make characterization straightforward. One to two microliters of 2 m*M* Cd^{2+}/DNA (at a 1:1 DNA to Cd^{2+} ratio) was dropped onto carbon film/copper TEM grids using a microliter syringe. Excess solution was blotted from the side or bottom of the grid with a sharp corner of a piece of filter paper (*see* **Note 3**). The carbon-coated TEM grids were prepared as described in **Subheading 3.2.2.** and used within 30 min, but before the carbon films were completely dry. After air-drying, the samples were placed on antistatic polyshield paper and placed in a vial that could be sealed with a rubber septum. The samples were exposed to H_2S by adding this gaseous reagent into the vial using a gas tight microliter syringe. The reaction was allowed to proceed for approx 30 min to 1 h to form Q-CdS particles on DNA.

3.3.4. Cd^{2+}/DNA Deposition by Floating Grids in Solutions

To alter the relatively high concentration of supercoiled DNA and the high density of netlike microstructures found over large, localized areas, in these experiments, the carbon-coated TEM grid was placed on the Cd^{2+}/DNA complex instead of dropping the mixture onto the TEM grid. In this way, the Cd^{2+}/DNA complexes can be anchored uniformly over the carbon film by adsorption.

The concentrations and the Cd^{2+}/DNA ratio (2 μ*M* and 2 m*M* cadmium solutions with either 5 μg/mL of pUCLeu4 plasmid DNA or 1 μg/mL of ϕχ 174 RF II DNA) were the same as described in **Subheading 3.3.1.** Preparation and mixing of these solutions were the same as described previously (*see* **Note 4**).

One or two drops of the well-mixed Cd^{2+}/DNA complexes were dropped on a clean glass slide using a Pasteur pipet. Using tweezers, carbon-coated TEM grids were placed on the complex solution with the carbon-coated side down. Approximately 1 or 2 min later, the grid was picked up and placed on filter paper with the carbon film side up to remove the excess water. After air-drying for approx 30 min, the grids were exposed to H_2S gas as described in **Subheading 3.3.3.** Either wet or dry carbon films can be used with this deposition method.

3.3.5. Sequential Addition of DNA and Cd^{2+} to Carbon Surfaces

To prevent extensive entanglement of the CdS/DNA structures in some experiments, DNA molecules were anchored onto the carbon film first, and then the grids with the attached DNA were dipped in Cd^{2+} solution to allow the Cd^{2+} ions to interact with the DNA molecules directly (*see* **Note 5**).

Two sets of samples with two different DNA concentrations, 5 and 0.5 µg/mL, were prepared by dipping them into a 2 m*M* cadmium ion solution. The DNA and cadmium solutions were prepared as described in **Subheading 3.3.** One or two drops of DNA solution was dropped onto a clean glass slide with a Pasteur pipet. Carbon-coated TEM grids were placed on the DNA solution, and after 1 or 2 min the grids were picked up and placed on filter paper to remove the excess water. The samples were air-dried for 6–10 min, then dipped very slowly into cadmium solution one by one, while trying not to disturb the solution, to reduce washing away of the DNA from the grid. After approx 3–5 min, the grids were placed on filter paper with the carbon film side up and air-dried for approx 30 min. The grids were then exposed to H_2S gas for approx 30 min, as described in **Subheading 3.3.3.**

3.4. Characterization

3.4.1. Instrumentation

Both analytical transmission electron microscopy (AEM) and high-resolution election microscopy (HREM) can be used for microstructural characterization of the Q-CdS/DNA mesostructures. The mesoscale semiconductor structures are imaged using a combination of bright-field, dark-field, and high-resolution electron microscopy. In our previous experiments, analytical electron microscopy, X-ray energy dispersive spectroscopy for elemental chemical analysis, and selected-area electron diffraction for phase identification were performed using both a JEOL 100 CX and a JEOL 200 CX operating at 100 and 200 kV, respectively. Lattice imaging of Q-CdS particles on DNA was performed using a Hitachi H-9000 high-resolution electron microscope with a demonstrated lattice resolution of 1 Å operating at an accelerating voltage of 300 kV. Q-CdS nanoparticle sizes and size distributions were determined using HREM micrographs. Some samples were examined both with and without metal shadowing.

3.4.2 Results

Figure 3 illustrates the type of structure observed when the procedure in **Subheading 3.2.2.** is followed, i.e., adding microliter amounts of 2 m*M* Cd^{2+}/nucleotide complex onto carbon film/copper TEM grids using a microliter syringe. The dark ring observed is an assembly of quantum confined cadmium sulfide nanoparticles on a circular pUCLeu4 template. Since DNA is a biopolymer with relatively low electron density, plasmids are not observed alone in the TEM. With this particular variant of the method, CdS nanoparticles are observed not only on the plasmid, but also on the carbon surface as well, since excess Cd^{2+} is not completely removed. This particular Q-CdS ring structure is

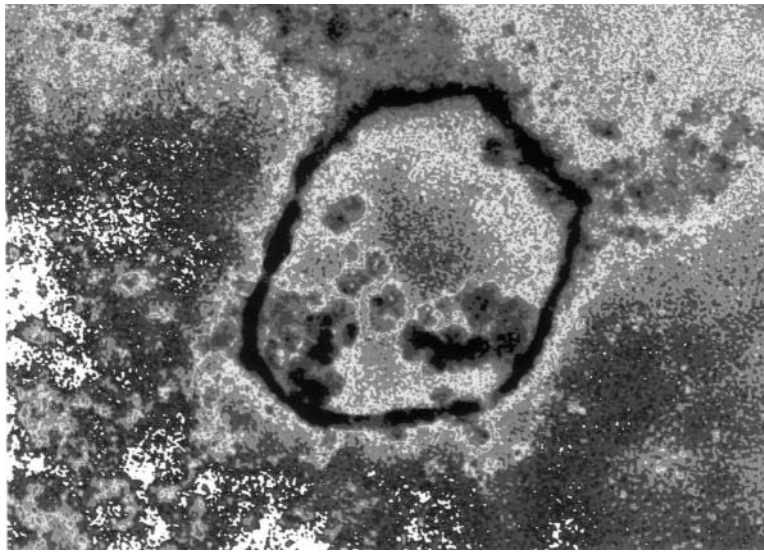


Fig. 3. Bright-field TEM micrograph of circular plasmid pUCLeu4 DNA molecule that has served as nucleation site for ring of Q-CdS nanoparticles. The measured circumference of the ring (1.2 μm) closely matches the predicted value of the relaxed circular conformation of the plasmid DNA molecule.

approx 10 nm thick and 700 nm long. HREM confirms that the dark ring comprises an assembly of randomly oriented, closely packed CdS nanoparticles, with an average size of approx 5 nm (**Fig. 4**). The elemental composition is confirmed by concomitant energy dispersive X-ray analysis (showing the expected presence of cadmium and sulfur), and selected-area electron diffraction measurements verify that the phase of the material is the cubic form of cadmium sulfide.

To circumvent nonselective Cd^{2+} binding, as well as to reduce the number of CdS structures templating on supercoiled DNA, two specific strategies have proven useful. One involves the flotation of the carbon surfaces in an aqueous solution containing the plasmid and cadmium ions; if a large excess of Cd^{2+} (say, 1000-fold) is present, then extensive aggregation of the CdS/plasmid occurs, resulting in numerous "weblike" structures across the surface of the film (**Fig. 5**). The other option is to decouple the Cd^{2+}- and plasmid-binding events and add them individually to the carbon surface, rather than collectively as a complex (*see* **Subheading 3.3.5.**). This method produces a relatively more dispersed series of Q-CdS rings across the surface (**Fig. 6**).

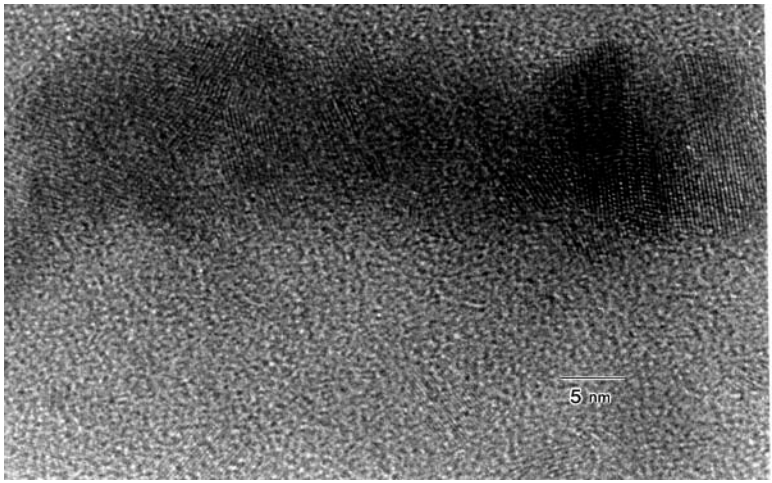


Fig. 4. HREM image of one section of Q-CdS/DNA ring. The ring consists of numerous nanoparticles with different orientations.

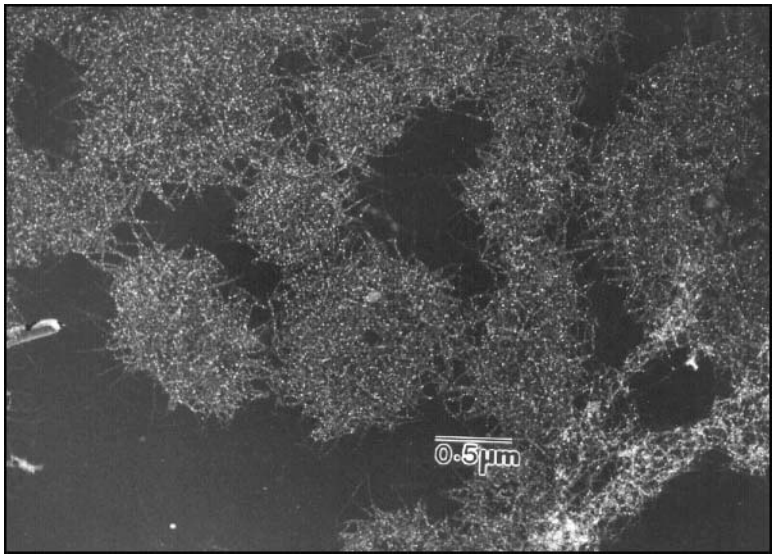


Fig. 5. Bright-field TEM image of weblike pattern of CdS nanostructures induced by cadmium ion-induced plasmid aggregation. Control experiments using Cd^{2+} bound to this plasmid under comparable conditions (and imaged with the assistance of metal shadowing) confirm that this is the case. Note the presence of the individual CdS nanocrystals (showing up as small white spots in the image).

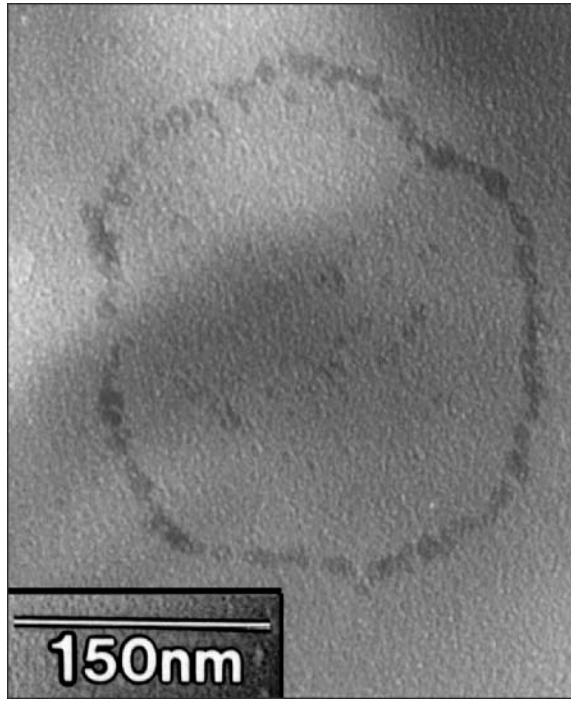


Fig. 6. Discrete "necklace" of CdS nanocrystals formed by separate addition of plasmid to a carbon surface, followed by cadmium ion addition and H_2S exposure.

4. Notes

1. If the diameter of the tip is too large or the tip is sharpened into a conical shape without making it long enough, the thin tip area may be burnt off before achieving the required film thickness
2. For example, wet carbon films were used when the DNA was deposited using the dropping method (*see* **Subheading 3.3.3.**), and dry carbon substrates were used when the DNA was deposited by floating the grids on the DNA or Cd^{2+}/DNA solutions (as described in **Subheading 3.3.4.**).
3. Because of the hydrophobic nature of the carbon film and the surface tension of the water drop, the deposition of the Cd^{2+}/DNA complexes onto the carbon-coated TEM grids was extremely difficult. The carbon films usually broke owing to these effects, but breakage can be reduced by using wet carbon films.
4. In the method that employs floating the TEM grids on the complex solution, it is imperative that freshly mixed Cd^{2+} solutions be used for satisfactory results.
5. The amount 5 μg/mL of DNA was used because we anticipated that many of the DNA molecules attached to the carbon film would be washed away when the grids were dipped into the cadmium solution. A high cadmium concentration,

2 m*M*, was used to reduce the interaction time to reduce the removal of DNA from the grids.

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