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Probing DNA Structure With Nanoparticles

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Summary

Semiconductor nanoparticles, also known as quantum dots, are receiving increasing attention for their biological applications. These nanomaterials are photoluminescent and are being developed both as dyes and as sensors. Here we describe our "sensor" use of quantum dots to detect different intrinsic DNA structures. Structural polymorphism in DNA may serve as a biological signal in vivo, highlighting the need for recognition of DNA structure in addition to DNA sequence in biotechnology assays.

Key Words

DNA; oligonucleotides; quantum dots; semiconductor; nanoparticles.

1. Introduction

The overall double helical structure of DNA has been known since the 1950s ***(1,2)***, but since the 1980s it has become increasingly recognized that local DNA structure and dynamics can affect its function ***(3–7)***. The DNA duplex, as a result of chemical damage or by virtue of its intrinsic sequence, may exhibit bends, loops, bulges, kinks, and other unusual structures on a few-base-pair-length scale ***(5,8–15)***.

Characterization of local DNA structure can be done, of course, by traditional nuclear magnetic resonance or X-ray crystallographic methods (***[14]***; This article describes the features of the Protein Data Bank, the standard database to search and download crystal structures, NMR structures, and some theoretical structures of biological macromolecules, including DNA: http://www.rcsb.org/pdb/. Rutgers University also maintains the Nucleic Acid Database for DNA/RNA sequence and structure searching: http://ndbserver.rutgers.edu/NDB/ndb.html.). A powerful alternative approach—should the DNA samples not be amenable to characterization by these methods—is to bind a probe to the DNA target, and use the optical

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properties of the probe to infer the nature of local DNA structure or dynamics ***(15)***. Common examples of such probes include fluorescent intercalators ***(15)***.

A more unusual example of an optical probe for DNA is an inorganic nanoparticle ***(16–20)***. Nanoparticles are materials that have diameters on the 1- to 100-nm scale and are therefore in the size range of proteins. Nanoparticles made of inorganic materials such as semiconductors and metals have unusual, size-dependent optical properties in this size range ***(16–20)***. In particular, semiconductor nanoparticles are known as "quantum dots" because they exhibit "particle-in-a-box" quantum confinement effects at the nanoscale ***(16–20)***. CdSe and CdS nanoparticles can be made by a number of routes in the 2- to 8-nm size range, and these nanoparticles are photoluminescent in the visible, with wavelength maxima that depend on size and surface group ***(16–20)***. Some researchers have used CdSe quantum dots as inorganic dyes for biological labeling assays ***(21–28)***. We have used CdS nanoparticles as protein-sized probes of local DNA structure ***(29–34)***. In this chapter, we describe how we make our nanoparticles and how titration experiments are performed with unusual DNAs, and we give examples of the typical data and results.

2. Materials

1. Anhydrous Na_2S (Alfa), NaOH (Aldrich), sodium polyphosphate (Aldrich), 2-mercaptoethanol (Aldrich), and $Cd(NO_3)_2 \bullet 4H_2O$ (Aldrich) for CdS nanoparticle synthesis: these are used as received. Sodium nitrate is obtained from either Fisher or Aldrich. Deionized and purified water (Continental Water Systems) is used for all solutions.
2. Tris buffer (5 m*M* tris hydrochloride; 5 m*M* NaCl, pH 7.2) and tris-EDTA buffer (10 m*M* tris hydrochloride; 1 m*M* EDTA dipotassium salt; 200 m*M* KCl, pH 8.0): prepare from Sigma-Aldrich or Fisher compounds for DNA solutions.
3. Oligonucleotides. These can be obtained commercially from many different companies; we have used Operon, MWG Biotech, and Midland Certified Reagents.
4. Stir plate.
5. pH meter.
6. Ultraviolet (UV) blacklight.
7. Spectrofluorometer, for acquiring data.
8. High-performance liquid chromatograph, for purifying DNA (unnecessary if one purchases purified DNA).
9. Heat block or hot water bath, to anneal DNA.

3. Methods

3.1. Synthesis of "Unactivated" CdS Nanoparticles Stabilized by Polyphosphate

CdS nanoparticles are prepared with reagent weights based on a final concentration of 2×10^{-4} *M* (*see* **Note 1**).

1. In a three-necked flask held over a stir/heat plate, place a stir bar and 100 mL of deionized water, and degas the water by bubbling nitrogen gas through it for 15 min.
2. Maintain a blanket flow of nitrogen through the rest of this procedure.
3. Add 6.2 mg of cadmium nitrate tetrahydrate, $Cd[NO_3]_2 \bullet 4H_2O$ to the flask, with stirring.
4. Add 12.1 mg of sodium polyphosphate to the flask, with stirring.
5. Insert a pH meter into the flask and check the pH of the aqueous solution. Adjust the pH to 9.8 by adding small amounts of 0.1 *M* NaOH via a pipet.
6. Stir the solution vigorously.
7. Weigh out 1.6 mg of anhydrous sodium sulfide (Na_2S) and dissolve it in 2 mL of water. Immediately add this solution dropwise to the three-necked flask. The reaction mixture should become light yellow.
8. Continue stirring for 20 min.
9. Adjust the pH of the solution to 10.3 with 0.1 *M* NaOH. The reaction has produced approx 4-nm CdS nanoparticles, surface capped with polyphosphate. Size can be checked by transmission electron microscopy. The solution should be stored under nitrogen in the dark. If one irradiates the solution with a UV blacklight, dull pinkish yellow emission will be observed. If one desires to "activate" the nanoparticles with Cd(II) (*see* **Subheading 3.2.**), the solution should remain undisturbed for 24 h.

3.2. Activation of CdS Nanoparticles With Cd(II)

The solution of CdS made in **Subheading 3.1.** should remain undisturbed for 24 h after its synthesis. Prepare a solution of 0.01 *M* $Cd(NO_3)_2$ tetrahydrate (*see* **Note 2**). Then monitor the photoluminescence of the unactivated CdS solution while adding drops of 0.01 *M* $Cd(NO_3)_2$ tetrahydrate solution to it. The CdS solution before activation has an emission peak maximum at approx 550 nm. As the activation progresses, a new peak appears at approx 440 nm and the intensity of this peak increases. Addition of Cd(II) is continued until this new peak at 440 nm has reached its maximum intensity. Toward the end, addition of Cd(II) is done very carefully, and the emission spectrum is checked after each addition of 1 or 2 drops, because if more Cd(II) is added than what is required for maximum activation, the photoluminescence of the activated CdS solution will decrease.

During activation the pH of the solution decreases. After activation is complete, the pH of the solution is brought back to 10.3 with 0.1 *M* NaOH. The "activated" CdS solution has a very bright pinkish white glow under UV light. The nanoparticles should not have changed in size and now are coated with a loose "web" of Cd(II). This activation procedure can be performed with other divalent metal salt solutions as well.

3.3. Synthesis of 2-Mercaptoethanol-Capped CdS

The synthesis of 2-mercaptoethanol-capped CdS produces nanoparticles that bear —SCH_2CH_2OH groups that are ionically neutral compared with the

activated ones from **Subheading 3.2.** As before, CdS nanoparticles are prepared with reagent weights based on a final concentration of 2×10^{-4} *M*.

1. In a three-necked flask held over a stir/heat plate, place a stir bar and 100 mL of deionized water, and degas the water by bubbling nitrogen gas through it for 15 min.
2. Maintain a blanket flow of nitrogen through the rest of this procedure.
3. Add 6.2 mg of $Cd(NO_3)_2 \bullet 4H_2O$ and 1.4 µL of mercaptoethanol to the water, with stirring.
4. Insert a pH meter into the flask and check the pH of the aqueous solution. Adjust the pH to 9.8 by adding small amounts of 0.1 *M* NaOH via a pipet.
5. Stir the solution vigorously.
6. Weigh out 1.6 mg of Na_2S and dissolve it in 2 mL of water. Immediately add this solution dropwise to the three-necked flask. The reaction mixture should remain colorless.
7. Continue stirring for 20 min.
8. Adjust the pH of the solution to 10.3 with 0.1 *M* NaOH. The colorless solution, which glows yellow-green when viewed under UV light, contains CdS nanoparticles surface coated with mercaptoethanol. The nanoparticle diameter should be approx 4 nm. The solution should be stored in the dark under nitrogen (*see* **Note 3**).

3.4. DNA Purification by High-Performance Liquid Chromatography and Annealing

One must decide what sequences of DNA one wishes to probe and choose control DNAs that are allegedly "normal" compared with the "unusual" one that is of interest. DNAs can be commercially obtained and purified; here we describe our procedures for purification and annealing.

In our experiments, we choose the sequences 5′-GGGTCCTCAGCTTGCC-3′ and complement as a "straight" duplex, 5′-GGTCCAAAAAATTGCC-3′ and complement as a "bent" duplex, the self-complementary 5′-GGTCATGGCC ATGACC-3′ as a "kinked" duplex. We have also examined single-stranded DNAs (ssDNAs) that fold up into unusual structures, such as 5′-CGGCGGCGGCG GCGGCGGCGG-3′, $d(CGG)_7$, and 5′-CCGCCGCCGCCGCCGCCGCCG-3′, $d(CCG)_7$. These are commercially purchased and received as a dried-down pellet ("trityl off") on a 1-µmol scale.

Oligonucleotides are purified by high-performance liquid chromatography (HPLC) on a C_8 or C_{18} reverse-phase column with a triethylammonium acetate/acetonitrile gradient, collected and dried down, and redissolved in tris buffer (5 m*M* tris hydrochloride; 5 m*M* NaCl, pH 7.2) for duplex DNAs (*see* **Note 4**). The exact nature of the HPLC gradient can vary, depending on the user's DNA and HPLC.

For annealing duplex DNAs, equal amounts of the complementary single strands are mixed together in Eppendorf tubes and placed in a boiling water

bath or a heat block. The heat is turned off and the mixture is allowed to cool down to room temperature. Ultraviolet (UV)-visible melting temperature experiments confirm that the duplexes are double stranded (T_m approx 50°C in the stated buffer at approx 1 m*M* nucleotide concentration).

For annealing unusual ssDNAs into their folded states, the literature must be consulted closely for the proper ionic strength conditions. In the case of the d(CGG) and d(CCG) triplet repeats, which are similarly purified by reverse-phase HPLC, the proper buffer is tris-EDTA. These oligonucleotides are annealed at 90°C in a heating block for 10 min, allowed to cool gradually to room temperature, and then allowed to cool down further to 4°C for 48 h to obtain the properly folded structures, which can be confirmed by circular dichroism spectroscopy and melting temperature experiments.

3.5. Titrations of Cd(II)-Activated CdS With Duplex DNAs

Binding of DNA to cationic Cd(II)-activated quantum dots is monitored by photoluminescence spectroscopy. The DNA quenches the emission of the nanoparticle solution, and from such data equilibrium binding constants can be obtained.

1. Place 200 µL of a 2×10^{-4} *M* activated CdS solution into a quartz cuvet, and place the cuvet in a spectrofluorometer. Ensure that the size of the excitation light beam in the fluorometer matches the sample size; we have used small 400-µL cuvets instead of the standard 1-cm, 3-mL cuvets.
2. Using an excitation wavelength of 350 nm, which is absorbed by the CdS nanoparticles but not by the DNA, acquire an emission spectrum from 400–800 nm. Slit widths and detector settings will vary by instrument, but for our spectrofluorometer (SLM-AMINCO 8100) we use 4-nm slits.
3. Take a 5-µL aliquot of an approximately millimolar (nucleotide) DNA solution in tris buffer (*see* **Note 4**) and add it to the 200 µL of CdS solution. Mix by inverting the stoppered cuvet several times, and record the emission spectrum.
4. Wait 30 min, and repeat **step 3** until the photoluminescence of the CdS is completely quenched (*see* **Note 5**).
5. Repeat **steps 3** and **4** for different DNAs. Do all of these photoluminescence titrations in a single day, and without changing the spectrofluorometer parameters.
6. Repeat **steps 3** and **4** for a buffer solution without DNA, as a control for any loss of photoluminescence intrinsic to the CdS sample. Typically, this is a minor effect. DNA titration experiments with other DNAs in other buffers (e.g., the triplet repeat sequences mentioned in **Subheading 3.4.**) with the proper buffer control can be performed analogously.

To calculate binding constants of the DNA to the nanoparticles, first integrate the areas under the photoluminescence spectra data curves. From **Fig. 1**, one could integrate the entire area under the curves from 400 to 800 nm, or one

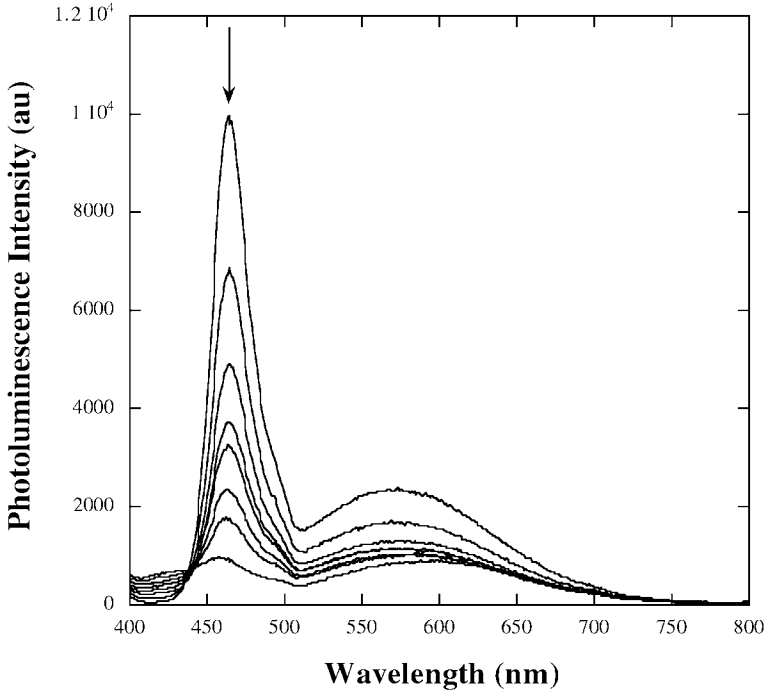

Fig. 1. Raw photoluminescence titration data for Cd(II)-activated CdS nanoparticles, made as described in the text, with a 32mer duplex DNA in tris buffer. The top spectrum is the original Cd(II)-activated CdS data; subsequent addition of DNA decreases the intensity. The peak at approx 570 nm corresponds to the "unactivated" CdS, and the approx 460 nm peak corresponds to the Cd(II)-CdS "activated" species.

could use only the "surface-sensitive" peak at 460 nm. In the latter case, integrating the area from approx 440–510 nm would be appropriate.

Should buffer alone quench the emission of the nanoparticles more than approx 5% of the amount of DNA quenching, the DNA data must be corrected. To do this, I(corrected) = I(DNA) – I(buffer), in which I(corrected) is the corrected integrated photoluminescence intensity for a given concentration of DNA; I(DNA) is the raw integrated intensity for a given concentration of DNA, as in **Fig. 1**; and I(buffer) is the raw integrated intensity for the same volume of buffer that goes with the given DNA concentration.

We have used the Frisch-Simha-Eirich (FSE) adsorption isotherm, which physically corresponds to a long polymer adsorbing to a flat surface in short segments, to extract binding constants from our data ***(35)***. Other models, of course, may be used. The FSE isotherm takes the form

$$[\theta \exp(2K_1\theta)]/(1 - \theta) = (KC)^{1/\upsilon} \quad (1)$$

in which θ, the fractional surface coverage of DNA on the nanoparticles, is equated to fractional change in photoluminescence (i.e., $\theta = [I(\text{DNA}) - I_0]/[I(\text{DNAfinal}) - I_0]$, in which I(DNA) is the corrected integrated intensity of the CdS emission at a particular DNA concentration; I_0 is the initial CdS photoluminescence integrated intensity without DNA; and I(DNAfinal) is the corrected integrated intensity of the CdS emission at the highest [most quenched] DNA concentration); C is the DNA concentration; K_1 is a constant that is a function of the interaction of adsorbed polymer segments, which we manually fit; K is the equilibrium constant for the binding of DNA to the nanoparticle; and υ corresponds to the average number of segments attached to the surface, which has no physical meaning in our system, and is allowed to mathematically float.

Plots of $[\theta \exp(2K_1\theta)]/(1 - \theta)$, with varying values for K_1, vs C are constructed, and fits to a "power" function in the software (Kaleidagraph or Excel) yield values for K and υ. We have generally found that $K_1 = 0.5$ gives the best fits to our data, and υ does not generally vary much.

With the oligonucleotides referred to in **Subheading 3.4.** as "straight," "bent," or "kinked," the binding constants we obtained to 4 nm Cd(II)-CdS nanoparticles were 1000, 4200, and 7200 M^{-1}, respectively. In this case, the kinked DNA was thought to have a larger bend angle than the bent DNA, and the trend from our data suggests that the more bent the DNA, the tighter it binds to a curved surface. In the case of triplet repeat DNAs of unknown structures, we observed that they bound to our particles in much higher salt buffers, under conditions in which normal duplex DNA did not bind to the particles. This suggests that these unknown structures either were more able to wrap about a curved surface than duplex DNA, or perhaps were less sensitive to electrostatics in binding to the nanoparticles, possibly because of increased van der Waals interactions.

3.6. Time Trace Experiments

In this experiment, one can examine the kinetics of DNA binding to nanoparticles. The amount of DNA needed to quench all of the CdS emission in a 200-µL sample is determined in the titration experiments in **Subheading 3.5.**

In a fresh cuvet is placed 200 µL of the colloidal CdS solution, and the cuvet is placed in a fluorometer. The excitation wavelength is set to 350 nm, and the emission monochromator is set to the CdS emission maximum wavelength, approx 460 nm in **Fig. 1**. Then the amount of DNA calculated to quench all of the CdS emission in a 200-µL sample is added all at once. The emission intensity at 460 nm is recorded every 10 s over a period of 300 s. Control experiments are run by adding the appropriate volume of buffer without the DNA.

The kinetics of adsorption can be fit by many models, none of which are really representative of the physical system (small DNA, large "molecule" binding). In general, one must assume that the DNA is either free or bound to CdS, and if bound to CdS will quench its emission. To estimate association rate constants, we have fit data to von Hippel's formulation for protein-DNA binding kinetics for very long DNAs ***(36)***. The integrated rate equation is

$$\left[\frac{1}{(R)-(O)}\right] \ln\left\{\frac{(O)\,[(R)-(RO)]}{(R)\,[(O)-(RO)]}\right\} = k_a t \qquad (2)$$

in which R is the concentration of free particle, O is the concentration of the free DNA, RO is the concentration of bound DNA (i.e., the concentration of the CdS–DNA complex), t is the time, and k_a is the association rate constant. In this treatment, binding is viewed as a simple second-order association reaction that has a negligible dissociation rate, which might not be the case in our system. This model does not fit our data that well, likely because its assumptions of infinitely long DNA and tight binding constants are not appropriate for our system. We retained the assumptions we made for fitting our luminescence data to the FSE adsorption isotherm: namely, that all DNA is either free or bound, and that fractional change in luminescence is proportional to the fraction of DNA that is bound. We have also assumed in the von Hippel treatment that one bound duplex is sufficient to quench the emission for a given particle. Readers may choose other kinetics models for their own situations.

3.7. Reverse Salt Titrations

In this experiment, we add salt to a premade DNA–nanoparticle complex and dissociate the complex. From the data we can extract the electrostatic contribution of binding between the two species ***(31,37–39)***.

1. To the standard 200 µL of Cd(II)-activated CdS solution in a cuvet, add sufficient DNA to quench approx 75% of the emission (from **Subheading 3.5.**).
2. Prepare a stock solution of 0.25 *M* $NaNO_3$.
3. Add known amounts of the $NaNO_3$ stock solution, in microliter aliquots, to the CdS-DNA solution, and record the photoluminescence spectrum after each aliquot addition, using the same parameters as before. The intensity of CdS emission increases on salt addition, reversing the quenching observed with DNA. Sodium nitrate is preferred to sodium chloride because of the tendency of Cd(II) to make complex ions with Cl^- (e.g., $[CdCl_4]^{2-}$). Sufficiently high salt concentrations cause precipitation of the nanoparticles.
4. From the data, construct a plot of equilibrium constant K as a function of salt concentration, and extract from that the contribution that electrostatics makes to the overall DNA-nanoparticle binding. To do this, make the same assumptions

about free and bound DNA as in the time trace experiments, at each salt concentration; calculate the free DNA and bound DNA; and estimate the "free" particle concentration from the photoluminescence integrated intensities. Once that is obtained, a plot of log K vs log [Na^+] should produce a straight line with a slope corresponding to the number of counterions released as the nanoparticle and DNA bind ***(31,37–39)***. Once the number of counterions released is known, calculate the contribution of this electrostatic component to the overall binding according to

$$\Delta G_{electro} = (\text{no. of counterions released})(RT)\ \ln[Na^+] \quad (3)$$

in which $\Delta G_{electro}$ is the portion of the free-energy change that is owing to electrostatics, at a given sodium ion concentration [Na^+]; R is the gas constant; and T is the temperature.

4. Notes

1. The quantum dots prepared as described here are not "passivated," meaning that they are not coated with a material (ZnS, or another higher-band-gap semiconductor, or silica) that will insulate the quantum dot from its environment and render its emission stable and bright regardless of the surrounding medium. In the kind of experiments described here, the intensity of the photoluminescence of the quantum dots should be sensitive to the environment. Hence, control experiments with buffer alone, and standard DNAs, are required.
2. We have activated with Zn(II) and Mg(II) as well; the resulting CdS nanoparticles act similarly, but not identically, with DNA.
3. In the synthesis of thiol-capped CdS, as in **Subheading 3.3.**, other thiols may give different optical properties. For example, we have used L-cysteine as a thiol, and the resulting CdS solution appears pale yellow and glows bright yellow under UV light.
4. The ionic strength of the buffer is lower than that of a typical tris buffer, because the nanoparticles associate electrostatically with the DNA, and a salt concentration that is too high will screen the binding.
5. It is important during the course of the titration to make sure that equilibrium has been reached at each DNA concentration along the way; hence, the 30-min waiting period between spectra.

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