Preparation of Gold Nanoparticle–DNA Conjugates

This unit outlines the preparation of covalent conjugates between short synthetic oligonucleotides (10 to 100 nucleotides in length) and gold nanoparticles (5 to 50 nm in diameter). These conjugates are formed readily between aqueous gold colloid solutions and synthetic oligonucleotides bearing free thiol or disulfide groups at their ends. The oligonucleotide-functionalized nanoparticles can then be isolated from starting materials and side products by centrifugation or gel electrophoresis. The two protocols presented here correspond to two distinct types of gold nanoparticle–oligonucleotide conjugates: nanoparticles functionalized with just one or a few oligonucleotide strands (Basic Protocol 1) and nanoparticles functionalized with a dense layer of many oligonucleotide strands (Basic Protocol 2). The physical and chemical properties of these two types of conjugates are different, and the relative stability and utility of the nanoparticles in different environments are discussed below (see Strategic Planning). In addition, the Support Protocol describes a simple synthesis of the aqueous gold colloid used as a starting material in the synthesis of DNA-nanoparticle conjugates.

NOTE: Use ultrapure water (e.g., Nanopure; $R > 18$ MΩ) in all solutions and protocol steps.

STRATEGIC PLANNING

Properties of Gold Nanoparticle–DNA Conjugates

Despite the thematic similarity between the two types of oligonucleotide-nanoparticle conjugates described here, their physical properties are fairly different. Gold nanoparticles with a single or a few attached oligonucleotides, originally described by Alivisatos and Schultz (Alivisatos et al., 1996), have a discrete and characterizeable number of DNA molecules attached to each particle (Zanchet et al., 2001). The remaining surface of these particles is passivated with a monolayer of anionic phosphine molecules, which protect the particles from aggregating with each other and precipitating from solution. Conjugates synthesized by the method described in Basic Protocol 1 are not stable under extended exposure to high temperatures (e.g., >60°C) or in buffers with high ionic strength (e.g., 1 M Na$^+$). In addition, fairly long oligonucleotides (e.g., >50 nucleotides) must be used in order for the different particle-DNA conjugates to be electrophoretically separated from unreacted oligonucleotide and from each other. The synthesis and electrophoretic purification of these conjugates described in Basic Protocol 1 are nearly identical to those reported by Alivisatos and Schultz (Loweth et al., 1999).

Gold nanoparticles functionalized with a layer of many attached oligonucleotides, on the other hand, are further stabilized against flocculation and precipitation at high temperature and ionic strength (Storhoff et al., 1998). Using the method described in Basic Protocol 2, reported originally by Mirkin and Letsinger (Mirkin et al., 1996), gold nanoparticles with diameters ranging from a few to tens of nanometers can be conjugated with thiol-terminated oligonucleotides containing 10 to 100 base pairs. The attached oligonucleotides still hybridize selectively to complementary DNA sequences, and the conjugates are stable under high salt concentrations (e.g., ≤2 M Na$^+$) and high temperatures (e.g., for hours at 80°C).

Disulfide- and Thiol-Containing Oligonucleotides

The starting oligonucleotide, bearing a disulfide or thiol group at the 3′ or 5′ terminus, can be purchased (e.g., Integrated DNA Technologies, Sigma-Genosys) or synthesized in the laboratory. If the oligonucleotide is purchased, it is extremely important that the product be purified from other thiols, such as dithiothreitol (DTT) or dithioerythritol (DTE), which are sometimes added as stabilizers. This can be achieved via size-exclusion chromatography, HPLC, or preparative gel electrophoresis (e.g., *CPMB UNIT 2.5A*).

If the oligonucleotide is synthesized on a DNA synthesizer (e.g., *APPENDIX 3C*) using the phosphoramidite method (*UNIT 3.3*), a disulfide functionality may be generated in a number of ways. 3′-Disulfide-containing oligonucleotides can be synthesized by beginning with a 3′-thiol modifier controlled-pore glass (CPG) or by beginning with a universal support CPG and adding a disulfide modifier phosphoramidite as the first monomer in the sequence. 5′-Disulfide-containing oligonucleotides can be synthesized by ending the synthesis with a disulfide modifier phosphoramidite. In all cases, the 4,4′-dimethoxytrityl protecting group should be removed from the 5′-hydroxy terminus under acidic conditions (80% acetic acid for 30 min; *UNIT 10.5*) before conjugation of the DNA to the particles. Oligonucleotide synthesis reagents are available from a number of suppliers (e.g., Glen Research, TriLink BioTechnologies, ChemGenes).

Oligonucleotides with a free 5′-thiol can also be generated by ending the synthesis with a 5′-tritylthiol modifier phosphoramidite, and these oligonucleotides can be conjugated to particles (Storhoff et al., 1998). However, this protocol requires that the 4,4′-dimethoxytrityl protecting group be removed from the thiol before conjugation. This deprotection procedure is described in Storhoff et al. (1998) and can be found at the Glen Research Web site (*http://www.glenresearch.com/ProductFiles/Technical/tech_questions.html#44*). In addition, because free thiol groups are not stable to prolonged storage, the strands should be used immediately after deprotection.

PREPARATION OF GOLD NANOPARTICLE–DNA CONJUGATES CONTAINING ONE TO SEVERAL DNA STRANDS PER PARTICLE

This protocol describes the synthesis and isolation of gold nanoparticles with one to several synthetic oligodeoxyribonucleotides attached. The synthetic oligonucleotide must be modified to contain a thiol or disulfide group to attach the strand to the gold surface of the particle. A strong, covalent Au-S bond is formed spontaneously between the nanoparticles and DNA by simply mixing the two components. In practice, this procedure generates particles with a statistically distributed number of oligonucleotides attached to each particle; particles with a single attached oligonucleotide can be separated from unmodified and multiply modified particles by preparative horizontal gel electrophoresis. The protocol works best for nanoparticles with diameters between 5 and 20 nm. The resulting DNA-particle conjugates can then be characterized by UV/visible spectroscopy.

Materials

Oligonucleotide: ~1 mM synthetic 5′- or 3′-disulfide-containing or thiol-containing oligonucleotide (see Strategic Planning), dissolved in water

Aqueous gold nanoparticle solution (British Biocell, Ted Pella; or see Support Protocol)

Phosphine: 4,4′-(phenylphosphinidene)bis(benzenesulfonic acid), dipotassium salt hydrate (Aldrich), solid and 0.5 M aqueous solution

NaCl, solid and 1 M aqueous solution

Methanol

5× TBE electrophoresis buffer (*APPENDIX 2A*)

30% (v/v) glycerol

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UV/vis spectrophotometer
Quartz cuvette
Razor blade, sterilized
Glass-fiber filter paper, 1.2-μm retention (e.g., GF/C; Whatman)
Dialysis membrane, MWCO 10,000 (e.g., SpectraPor Biotech RC; Spectrum
   Laboratories)
Centrifugal filter device, 0.45-μm pore size (e.g., Ultrafree-MC; Amicon)

Additional reagents and equipment for agarose gel electrophoresis (e.g., *CPMB UNIT
   2.5A*)

Quantitate oligonucleotide solution

1. Prepare a 100-fold dilution of an ~1 mM oligonucleotide solution in water.

   *Synthesized DNA, after deprotection and lyophilization, should be powdery and white and
   should readily dissolve in water. If necessary, mild heating (~60°C) and/or brief sonication
   (1 min) can be used to completely dissolve the DNA. Insoluble material may cause some
   turbidity but is not a cause for concern.*

   *Steps 1 to 3 can be skipped if the concentration of the oligonucleotide solution is already
   known (e.g., if the modified oligonucleotide was purchased).*

2. Use a properly calibrated UV/vis spectrophotometer and quartz cuvette to measure
   the absorbance at 260 nm (A_{260}) of the diluted oligonucleotide.

3. Calculate the DNA concentration (c_o in M) in the stock solution using a rearrangement
   of Beer's Law:

   $$c_o = (A_{260} \times 100)/(\varepsilon_o \times b)$$

   where 100 is the dilution factor, b is the path length of the cuvette (typically 1 cm),
   and ε_o is the extinction coefficient of the oligonucleotide at 260 nm.

   *Extinction coefficient calculators for oligonucleotide sequences are available online (see
   Internet Resources). Alternatively, extinction coefficients can be calculated using nearest-
   neighbor approximations (Breslauer et al., 1986; Sugimoto et al., 1996). See also UNIT 7.3.*

Quantitate nanoparticle solution

4. Using the spectrophotometer, measure the A_{520} of an aliquot of an aqueous gold
   nanoparticle solution. If the measured absorbance is >1, dilute the aliquot by
   increments of ten with water until the absorbance is <1.

   *If the nanoparticles are purchased, the supplier may also include a measured concentration
   of the particle solution, and steps 4 and 5 can be skipped.*

5. Estimate the concentration of the nanoparticle solution (c_n in M), again using Beer's
   Law:

   $$c_n = (A_{520} \times d)/(\varepsilon_n \times b)$$

   where ε_n is the molar extinction coefficient of the nanoparticle at 520 nm and d is the
   dilution factor used in step 4.

   *Estimated values for ε_n are sufficient for this protocol and can be obtained from Table
   12.2.1. The calculated nanoparticle concentration from this step may range from micro-
   molar to picomolar.*

Complex nanoparticles with phosphine

6. To 10 mL gold nanoparticle solution, add 2 mg phosphine (final 0.5 M). Rotate this
   solution 10 hr at room temperature on an orbital shaker at low speed.

Table 12.2.1   Estimated Extinction Coefficients for Gold Nanoparticles at 520 nm[a]

Particle diameter (nm)	Extinction coefficient (ε_{520}, M^{-1}cm^{-1})
2	8×10^5
5	1×10^7
10	1×10^8
20	1.2×10^9
50	3×10^{10}

[a]From Yguerabide and Yguerabide (1998); British Biocell International Technical Data Sheet.

The synthesis can be readily scaled up to larger volumes of nanoparticle solution. For best results, divide the total volume into several 1-mL microcentrifuge tubes.

7. Add solid NaCl until the solution turns from deep burgundy to a lighter purple color (usually at ~2 to 3 M NaCl).

8. Centrifuge the particle solution 30 min at $500 \times g$, room temperature, to pellet the particles.

9. Discard supernatant and resuspend particles in 1 mL of 0.5 mM phosphine.

10. Add 0.5 mL methanol to precipitate the particles and centrifuge again 30 min at $500 \times g$, room temperature.
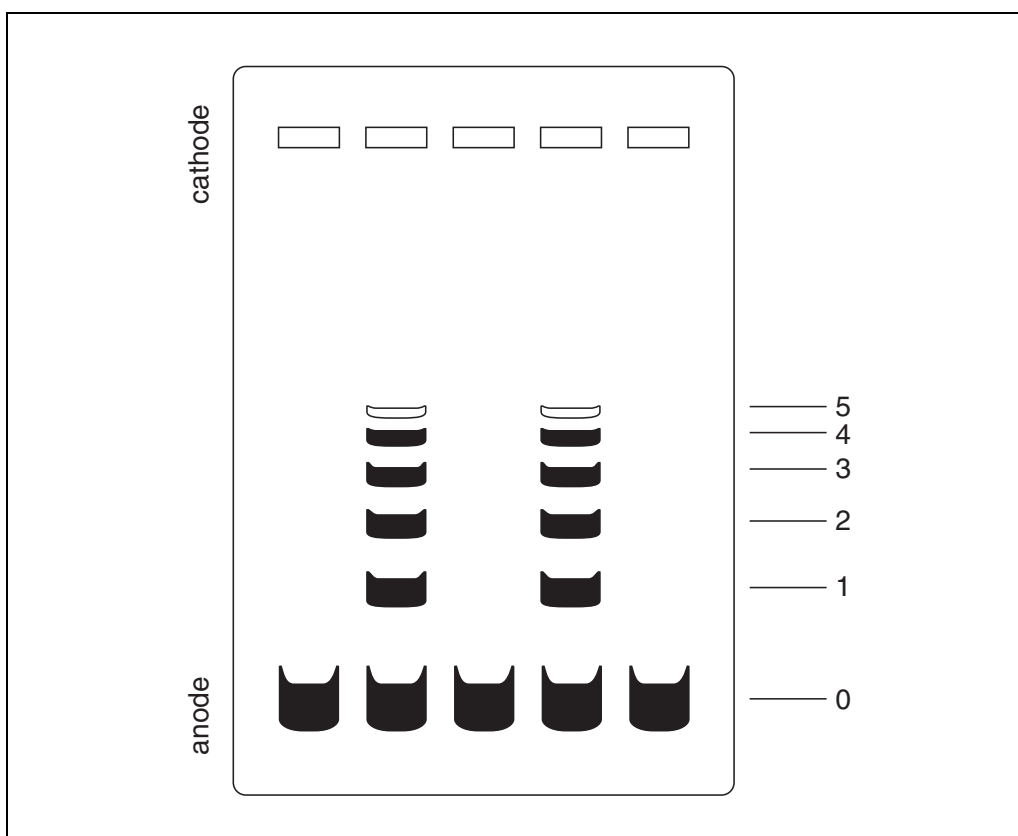

Figure 12.2.1   Schematic results for horizontal gel electrophoresis of oligonucleotide-nanoparticle conjugates. The first, third, and fifth wells were loaded with unmodified gold nanoparticle standards, and the second and fourth wells with the nanoparticle/conjugate mixture (see Basic Protocol 1, step 19). The numbers to the right of the gel indicate the number of attached oligonucleotides. The unmodified particles migrate at the zero mark. Each band of successively decreasing mobility corresponds to a nanoparticle with an additional oligonucleotide covalently attached.

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11. Discard supernatant and resuspend particles in 1 mL of 0.5 mM phosphine.

12. Add 110 µL of 5× TBE electrophoresis buffer to this solution (0.5× TBE final).

13. Repeat steps 4 and 5 to determine the concentration of this nanoparticle solution.

14. Calculate the number of moles of nanoparticles in the solution by multiplying this concentration by the solution volume (1.1 mL).

Conjugate nanoparticles and oligonucleotide

15. Dilute an aliquot of disulfide-functionalized oligonucleotide solution containing 0.9 mol eq DNA (relative to moles of nanoparticle in step 14) in enough 0.5× TBE to make the solution 50 µM in oligonucleotide.

16. Combine the oligonucleotide and nanoparticle solutions and mix well.

17. Add 0.05 vol of 1 M NaCl to bring the solution to 50 mM NaCl. Place the solution on an orbital shaker at low speed (<1 Hz) and incubate 16 hr at room temperature.

Purify conjugates

18. Add 0.2 vol of 30% glycerol (5% final) to the conjugate mixture.

19. Load aliquots of this solution into alternating wells of a horizontal agarose gel in 0.5× TBE. Also load a gel standard, prepared by diluting 20 µL unmodified particles (step 12) with 4 µL of 30% glycerol.

    A precast horizontal agarose gel (e.g., Ready Agarose Mini-Gel, Bio-Rad) can be used.

20. Electrophorese 90 min at 15 V/cm, or until the red nanoparticle bands approach the bottom of the gel.

    The resulting gel should look something like the drawing in Figure 12.2.1. Successful resolution of the oligonucleotide-nanoparticle conjugates into discrete bands in the gel is somewhat sequence dependent. See Critical Parameters and Troubleshooting for more details.

21. Disconnect the power to the gel apparatus and lift out the gel tray. With a sterilized razor blade, cut slits in the gel just beneath the bands of oligonucleotide conjugate to be isolated.

    The bands that migrate just slower than the unmodified particle standards contain particles with only one attached oligonucleotide (Fig. 12.2.1). Nanoparticles with more than one oligonucleotide attached can be specifically isolated by cutting beneath another (higher) band of the agarose gel and electroeluting appropriately.

22. Insert a piece of glass-fiber filter paper and a strip of dialysis membrane into the slit, with the filter paper facing the band.

    The pink color of the band is easily visualized as it transfers from the gel to the filter paper.

23. Return the gel tray to the electrophoresis apparatus and electrophorese at 15 V/cm until the band has electroeluted into the paper.

24. Remove the filter paper and dialysis membrane together from the gel and place them, filter paper face down, into a 0.45-µm centrifugal filter device.

25. Add 250 µL of 0.5× TBE to the device and centrifuge 3 min at $14,000 \times g$, room temperature (according to manufacturer's instructions), to elute the particles into the device receiver.

Quantitate and store conjugates

26. Repeat steps 4 and 5 to determine the concentration of particle conjugates.

    The extinction coefficient of the nanoparticles at 520 nm is not significantly affected by the attached oligonucleotide.

27. Store the conjugates at 4°C in the dark.

    Conjugates are stable for at least 1 month. Do not freeze nanoparticle conjugate solutions made by this method.

PREPARATION OF GOLD NANOPARTICLE–DNA CONJUGATES CONTAINING MANY DNA STRANDS PER PARTICLE

This protocol describes the synthesis and isolation of gold nanoparticles with a layer of many synthetic oligodeoxyribonucleotides attached. The number of oligonucleotides attached can vary widely; for a detailed discussion of this topic, see Background Information. As in Basic Protocol 1, each oligonucleotide is bound to the particle surface by a strong, covalent Au-S bond. However, the dense oligonucleotide layer formed in these conjugates obviates the need for a protective shell of anionic phosphine ligands. Also, because of the large number of oligonucleotides attached to each particle, it is not possible to separate nanoparticles bearing slightly different numbers of oligonucleotides from each other. As a result, the DNA-particle conjugates can be isolated from free, unreacted thiol-oligonucleotides by centrifugation and characterized by UV/visible spectroscopy.

Materials

Oligonucleotide: ~1 mM synthetic 5′- or 3′-disulfide-containing or thiol-containing oligonucleotide (see Strategic Planning), dissolved in water
Aqueous gold nanoparticle solution (British Biocell or see Support Protocol)
1 M NaCl
0.1 M sodium phosphate buffer, pH 7 (*APPENDIX 2A*)
0.1 M NaCl/10 mM sodium phosphate buffer, pH 7
0.3 M NaCl/0.01% (w/v) sodium azide/10 mM sodium phosphate buffer, pH 7

Additional reagents and equipment for quantitating oligonucleotide, nanoparticle, and conjugate solutions (see Basic Protocol 1)

CAUTION: Sodium azide is poisonous and explosive in solid form; wear gloves and handle with care.

Quantitate oligonucleotide and nanoparticle solutions

1. Quantitate oligonucleotide and aqueous gold nanoparticle solutions (see Basic Protocol 1, steps 1 to 5).

    Oligonucleotide sequences with a high fraction of guanosine bases form less-stable nanoparticle conjugates than do other sequences. Such conjugates may not survive exposure to the high salt concentrations or centrifugation used to isolate the particles and may frequently precipitate and fuse during preparation.

    If the concentration of the oligonucleotide and nanoparticle solutions are known (i.e., they were purchased), this step can be skipped.

Calculate amount of oligonucleotide to attach

2. Calculate the required number of moles of oligonucleotide needed to conjugate to the particles by multiplying the total nanoparticle surface area available for reaction by the expected surface density of nanoparticle-bound oligonucleotide as follows:

$$\text{mol conjugated oligonucleotide} = A_n \times c_n \times D \times V$$

$$= 4\pi r^2 \times c_n \times 35\,\text{pmol/cm}^2 \times V$$

where A_n is the surface area of the nanoparticle, c_n is the concentration of the nanoparticle solution (step 1, in nanoparticles per liter), D_o is the oligonucleotide density on each particle (~35 pmol oligonucleotide/cm^2), V is the volume of nanoparticle solution (in L), and r is the radius of the nanoparticles.

The value used for D_o is based on Demers et al. (2000); see Background Information for more details. As an example of how this equation might be used, the amount of disulfide-terminated oligonucleotide needed to functionalize 5 mL of a 17-nM solution of 13-nm-diameter gold nanoparticles would be:

conjugated oligonucleotide

$$= 4\pi(6.5\,\text{nm})^2 \times 17\,\text{nM} \times 6.02 \times 10^{23}\,\text{mol}^{-1} \times 35\,\text{pmol}\,/\,\text{cm}^2 \times 0.005\,\text{L}$$

$$= 9.5\,\text{nmol}$$

Conjugate nanoparticles and oligonucleotide

3. To the solution of gold nanoparticles, add an amount of oligonucleotide solution containing 1.5 times the moles of oligonucleotide calculated in step 2 (i.e., a 0.5-molar excess).

   The volume of nanoparticle solution is not critical; volumes from microliters to hundreds of milliliters can be used.

4. Rotate the solution 16 hr at room temperature on an orbital shaker at low speed (<1 Hz).

   This step works best if conducted in a stoppered, glass container that has been covered to prevent unnecessary exposure to light.

5. Add 0.125 vol each of 1 M NaCl and 0.1 M sodium phosphate buffer (0.1 M NaCl and 10 mM phosphate buffer final). Rotate at low speed for 24 hr at room temperature.

   For larger particles (i.e., >20 nm diameter), the synthesis may be more successful if the transition from pure water to salt solution is made gradually, with multiple small additions of NaCl and buffer solutions.

Table 12.2.2 Centrifugation Conditions for Nanoparticle-Oligonucleotide Conjugates

Particle diameter (nm)	Relative centrifugal force ($\times g$)	Duration (min)
5	64,000	60
10	20,000	30
20	10,000	20
50	4,000	15

Isolate conjugates

6. Use a benchtop or high-speed centrifuge to centrifuge the suspension to form a red oil of nanoparticles beneath a clear solution of excess oligonucleotide. Choose (or interpolate) the speed and duration of centrifugation from Table 12.2.2, based on the diameter of the nanoparticles.

   For total solution volumes >1 mL, it is best to divide the volume into several microcentrifuge tubes before centrifuging for optimal separation of the oil from the supernatant. Failure to form stable nanoparticle-DNA conjugates, for any reason, will result in the formation of a solid pellet rather than a red oil.

7. Carefully remove the clear supernatant and resuspend the oil in the same volume of 0.1 M NaCl/10 mM sodium phosphate buffer.

8. Repeat centrifugation, removal of supernatant, and resuspension twice more, but resuspend the last time in 0.3 M NaCl/0.01% sodium azide/10 mM sodium phosphate buffer.

   CAUTION*: Sodium azide is poisonous and explosive in solid form; wear gloves and handle with care.*

Quantitate and store conjugates

9. Determine the concentration of the product conjugate solution (see Basic Protocol 1, steps 4 and 5).

10. Store conjugate solution at 4°C in the dark.

    Conjugates are stable for at least 1 month. Although conjugate solution prepared in this manner can be frozen without effect, do not store the conjugates below 0°C.

SUPPORT PROTOCOL

SYNTHESIS OF AQUEOUS CITRATE-PROTECTED GOLD COLLOID

This protocol describes a simple synthesis of aqueous gold nanoparticles surrounded by shells of coordinated citrate anions. These particles can be used as starting material in Basic Protocols 1 and 2. A variety of simple protocols for synthesizing gold nanoparticles of various sizes has been reported recently (Brown et al., 2000; Jana et al., 2001). The particles in this protocol are synthesized by reducing hydrogen tetrachloroaurate ($HAuCl_4$) with a citrate salt (Frens, 1973; Grabar et al., 1995). The protocol typically yields particles with diameters of ~15 nm and narrow size distributions; however, both particle diameter and size dispersity vary from preparation to preparation. In general, both particle diameter and monodispersity are influenced by the type of reducing agent and gold precursor salt used, the concentrations of these reagents, the reaction temperature and duration, and the postreaction workup. The resulting particle diameters and dispersities can be quantified by transmission electron microscopy and statistical analysis of collections of particles. However, because of the reliability of this protocol, such characterization is not strictly necessary for conjugate synthesis.

Materials

Aqua regia: 3:1 (v/v) concentrated HCl/concentrated HNO_3
1 mM $HAuCl_4$ (Aldrich)
38.8 mM sodium citrate (Aldrich)

1-L round-bottom flask
Reflux condenser
Heating mantle
0.45-μm nylon filter

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CAUTION: Aqua regia is noxious and extremely caustic. Handle with extreme care in a well-ventilated fume hood.

1. Wash a 1-L round-bottom flask, a reflux condenser, and a large stir-bar with first aqua regia and then thoroughly with water.

2. Assemble the glassware on a heating mantle and a magnetic stirrer, and put the stir-bar in the flask.

3. Charge the flask with 500 mL of 1 mM $HAuCl_4$ and bring the solution to reflux (100°C) with vigorous stirring.

4. Add 50 mL of 38.8 mM sodium citrate all at once. Continue to reflux the solution for 20 min.

    The sodium citrate solution should be added to the mixture as rapidly as possible. If the addition is done correctly, the solution should turn from yellow to purple and then to deep red.

5. Cool the solution to room temperature and filter through a 0.45-μm nylon filter.

6. Store the solution at room temperature in a glass container in the dark.

    Gold nanoparticles synthesized and stored in this way are stable almost indefinitely. In fact, gold nanoparticles synthesized by Michael Faraday in 1857 are still on display in the British Museum.
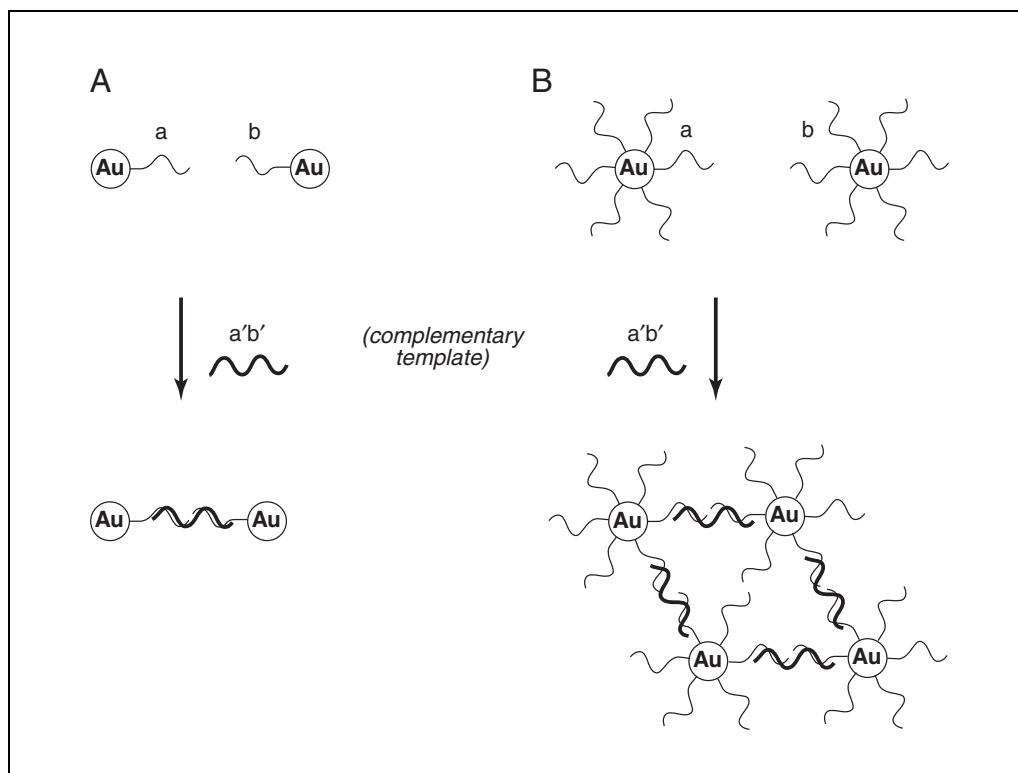

Figure 12.2.2 Assembly of gold nanoparticle conjugates, functionalized with oligonucleotide sequences a and b, onto complementary oligonucleotide templates a′b′. (**A**) Nanoparticle conjugates bearing only one oligonucleotide strand assemble selectively into dimers in the presence of the template. More complex structures can be generated from templates with additional recognition segments. (**B**) Nanoparticle conjugates bearing many oligonucleotide strands assemble into polymeric macrostructures in the presence of the complementary template. The optical changes associated with this polymeric assembly make this system particularly effective as a colorimetric DNA hybridization sensor.

COMMENTARY

Background Information

As suggested above, the structure, stability, and potential uses of the two types of DNA-nanoparticle conjugates described here are very different. Particles with a single attached oligonucleotide, synthesized by the method outlined in Basic Protocol 1, are extremely promising for building tailored nanostructures containing just a few predetermined nanoparticle components. For example, Alivisatos and co-workers have selectively synthesized clusters of two and three nanoparticles in high yields by hybridizing the attached strands to a complementary template (Loweth et al., 1999), as shown in Figure 12.2.2A. However, the instability of these conjugates to the conditions frequently encountered in DNA hybridization protocols limits their application in DNA sequence analysis and in situ hybridization. In addition, gel electrophoresis will not provide adequate separation of conjugates made with short oligonucleotide sequences (i.e., <50 nucleotides) and even some long ones.

On the other hand, conjugates with a dense layer of attached oligonucleotides, as described in Basic Protocol 2, have been used in homogeneous and heterogeneous schemes for DNA sequence analysis that require flexible control over hybridization conditions. The number of oligonucleotide strands bound to each particle depends on the surface area of the nanoparticles and the length and sequence of the DNA (Demers et al., 2000). For example, conjugates between 16-nm-diameter particles and thiol-modified 12-mer oligonucleotides were found to bear ~160 oligonucleotides per particle, but conjugates between the same particles and a 32-mer oligonucleotide bore 70 oligonucleotides per particle (Demers et al., 2000). It is important to note that not all of these oligonucleotides are available for hybridization to complementary DNA strands, due to steric compression and electrostatic repulsion at the nanoparticle surface (Levicky et al., 1998). For example, of the 160 12-mer oligonucleotides attached to each 16-nm particle described above, only 6 oligonucleotides (4%) were found to hybridize to complementary oligonucleotides under standard hybridization conditions (Demers et al., 2000). This fraction can be increased by inserting a noncomplementary "tether" sequence between the thiol group and the intended hybridization sequence; adding a 20-adenine-long tether to the 12-mer increased the hybridization efficiency from 4% to 44%.

Regardless of nanoparticle size, oligonucleotide length or sequence, the highest surface density of nanoparticle-bound oligonucleotides that has been reported is ~35 pmol/cm^2 (or ~0.2 oligonucleotides/nm^2). This highest value is used as D_o in step 2 of Basic Protocol 2 as an estimate of how much DNA will be needed to completely functionalize every particle in solution. If the oligonucleotide being used is particularly dear, the precise density of functionalization can be measured (Demers et al., 2000) and a more accurate value substituted for D_o in subsequent preparations.

Because each particle synthesized by this method bears multiple strands and thus multiple opportunities for hybridization, template strands bearing sequences complementary to different particles will typically assemble those particles into macroscopic assemblies, as shown in Figure 12.2.2B. The dramitic red-to-blue color change that accompanies this particle assembly in solution has been used to identify specific DNA target sequences in solution (Elghanian et al., 1997). In addition, using these conjugates as DNA labels has been found to increase the selectivity of DNA sequence analysis by oligonucleotide arrays (Taton et al., 2000, 2001).

Basic Protocols 1 and 2 describe the reaction of a disulfide-modified oligonucleotide with the nanoparticle surface. Oligonucleotide
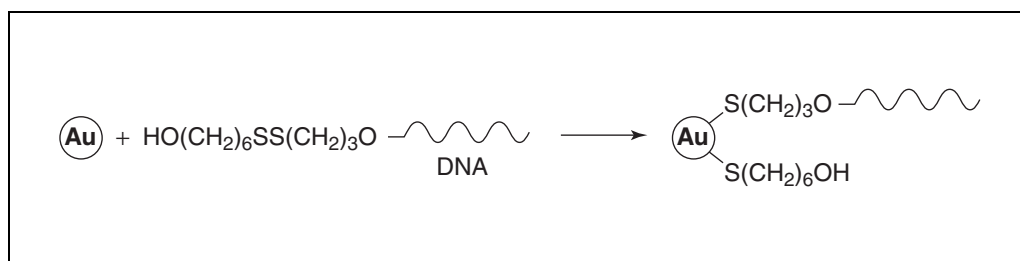

Figure 12.2.3 Scheme for conjugation of gold nanoparticles with disulfide-modified oligonucleotides. An organic mercaptoalcohol is also incorporated into the conjugate.

strands with other types of sulfur modifications, such as alkylthiol and phosphorothioate groups, would also be expected to form stable nanoparticle conjugates. Disulfide-modified starting materials have the advantage that they are stable to long-term storage and that they spontaneously react with the gold nanoparticle surface. (Alkylthiol-modified strands, on the other hand, typically bear trityl protecting groups, which must be removed before reacting with Au.) The reaction of a disulfide-terminated strand actually adds both the oligonucleotide and a mercaptoalcohol molecule to the particle (Figure 12.2.3), but this additional group does not appear to affect the stability or physical properties of the conjugates.

Critical Parameters and Troubleshooting

The success of both protocols is somewhat dependent on the sequence used. As noted above, Basic Protocol 1 provides isolatable conjugates only for sequences longer than 50 nucleotides. In addition, secondary structure caused by self-complementarity in the oligonucleotide sequence may result in broadened bands in the preparative agarose gel (and thus poorer separation). Oligonucleotides shorter than seven nucleotides will generally fail to provide stable conjugates by Basic Protocol 2. In addition, guanosine-rich sequences also fail to form stable conjugates by this method. Conjugates synthesized by Basic Protocol 2 are stable to storage over very long periods of time, but bacterial growth and degradation of hybridization activity have been observed in conjugate solutions that did not contain sodium azide as a preservative. Conjugates made from particles >10 nm may settle from solution with time, but can easily be resuspended by swirling the conjugate solution.

Anticipated Results

Basic Protocols 1 and 2 both yield red solutions of nanoparticle conjugates in which the hybridization properties of the attached oligonucleotide are fully active. This can be readily confirmed by adding a fluorescently labeled, complementary oligonucleotide to the conjugate solution; gold nanoparticles are exceptional quenchers of fluorescence (Gersten and Nitzan, 1981), and hybridization of the two components leads to a significant decrease in the solution fluorescence. The conjugates can also be analyzed by transmission electron microscopy or by dynamic light scattering to confirm particle sizes and size distributions.

Time Considerations

If the disulfide-modified oligonucleotide and nanoparticle solution are prepared or purchased in advance, then conjugates made by either protocol can easily be prepared and isolated within 3 days.

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Internet Resources

www.basic.nwu.edu/biotools/oligocalc.html

Provides an extinction coefficient calculator for oligonucleotides.

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