DNA-FUNCTIONALIZED NANOPARTICLES

PREPARATION OF GOLD NANOPARTICLES by Citrate Reduction of $HAuCl_4$

-must be done under reflux or in an open flask where the volume that evaporates is replaced with distilled water

Materials

- $HAuCl_4 \bullet 3H_2O$ *Aldrich*
- N_3-citrate *Aldrich*
- Purified water (Milli Q, etc.)
- Glassware
- Filter
- Heat Plate/Stirring bar
- Reflux set-up (?)

Procedure

1. **Boil and vigorously stir:** 500mL of 1mM $HAuCl_4$ // 50mL of 0.01% $HAuCl_4$
2. **Add:** 50mL of 38.7mM sodium citrate // 1.75mL of 1% sodium citrate
3. **Boil for an additional 10 minutes**
4. **Remove from heat, continue to stir for ~15 minutes and allow to cool to room temperature**
5. **Filter contents:** 0.8μm Gelman membrane filter // 0.45μm nylon filter

PREPARATION OF OLIGONUCLEOTIDES

Materials

- 5′-Thiol modifier phosphoramidite reagent *Glen Research*
- 3′-propanethiol modifier CPG *Glen Research*
- fluorescein phosphoramidite *Glen Research*
- "other reagents" *Glen Research*
- NAP-5 Column *Pharmacia Biotech*

Procedure

Synthesis/Preparation of Oligonucleotides *assuming they are not bought*

Basic Overview:

http://www.atdbio.com/content/50/Thiol-modified-oligonucleotides

- Phosphoramidite chemistry: thiols are strong nucleophiles, interfere with the chemistry
  - thiol group must be protected // unprotected thiols spontaneously form disulphides in neutral aq. solution

1. **Disulphide protection**: protect the thiol as a disulphide
   - after oligonucleotide synthesis/deprotection, disulfide protecting group removed by reaction with dithiothreitol(DTT)
   - gel filtration removes excess DTT

Should be deprotected *just before use*

1. **treat disulphide-protected oligo** w/ 100mM solution of DTT in an aq. bugger at pH 5, for 30 min @ room temp
2. **pass through a gel filtration/desalting column** (NAP-5 or NAP-10 column)

2. Trityl protection: protect the thiol with a trityl group (low oligonucleotide yields!!) ☹
   - trityl group removed by reaction w/ silver nitrate
   - excess removed by DTT, then DTT removed by gel filtration

More In-Depth Procedure:

One-Pot Colorimetric Differentiation of Polynucleotides with Single Base Imperfections Using Gold Nanoparticle Probes
http://pubs.acs.org/doi/pdf/10.1021/ja972332i

1. 3′-alkanethiol 12-base oligomer was synthesized on a 1 µmol scale using standard phosphoramidite chemistry (w/ ThiolModifier C3 S-S CPG solid support)
   a. final dimethoxytrityl (DMT) protecting group was not removed
2. oligonucleotide placed in 1 mL of concentrated ammonium hydroxide for 16 h at 55 °C to cleave the oligonucleotide from the solid support and remove the protecting groups from the bases = mixed disulfide composed of the (mercaptopropyl)- oligonucleotide and a mercaptopropanol linker
3. After evaporation of the ammonia, the modified oligonucleotide was purified by preparative reverse-phase HPLC using an HP ODS Hypersil column (5 µm, 250 × 4 mm) with 0.03 M triethylammonium acetate (TEAA), pH 7 and a 1%/min gradient of 95% CH3CN/5% 0.03 M TEAA at a flow rate of 1 mL/min, while monitoring the UV signal of DNA at 254 nm. The retention time of the DMT protected modified 12-base oligomer was 30 min
4. DMT was subsequently cleaved by dissolving the purified oligonucleotide in an 80% acetic acid solution for 30 min, followed by evaporation
5. The oligonucleotide was redispersed in 500 µL of water, and the solution was extracted with ethyl acetate (3 × 300 µL)
6. After evaporation of the solvent, the oligonucleotide was redispersed in 400 µL of a 0.1 M DTT, 0.17 M phosphate buffer (pH 8) solution at room temperature (21 (1 °C) for 2 h to cleave the 3′ mixed disulfide
7. Aliquots of this solution (<10 ODs) were purified through a desalting NAP-5 column
   a. Purity assessed

The above procedure is solely for 3'-alkanethiol tagged oligonucleotides

Synthesis/Preparation of Oligonucleotides *assuming they are bought*

1. **Purchase thiol-tagged oligonucleotides** ☺ Both 3' and 5'-end modified oligo
   -delivered with hexanethiol protection group to reduce disulfide dimerization
2. **Cleaved by treatment** with 100mM dithiothreitol (DTT) for 30 minutes
3. **Removed by gel filtration** on a G25 NAP-10 column, DNA grade

1. **Purchase thiol-tagged oligonucleotides** with disulphide protection
2. *Follow the procedure written under "Basic Overview"*

Preparation of Oligonucleotide-functionalized Gold Nanoparticles

Materials

- NaCl
- 10mM phosphate buffer

Procedure

Basic Overview:

1. Oligonucleotides added to nanoparticles until a certain concentration is reached
2. [24 hours]
3. Buffered using NaCl and 10mM phosphate buffer (pH 7)
4. [48 hours]
5. Excess removed by centrifugation
6. Purification
7. Precipitate collected then resuspended in a mixture of NaCl and PBS

One-Pot Colorimetric Differentiation of Polynucleotides with Single Base Imperfections Using Gold Nanoparticle Probes

1. derivatizing 5 mL of an aqueous 13 nm diameter Au nanoparticle solution (=17 nM)15 with 2.5 OD of (alkanethiol)oligonucleotide (final oligonucleotide concentration is 3.61 μM)

[stand for 16 h]

2. the solution was brought to 0.1 M NaCl, 10 mM phosphate buffer (pH 7)

[stand for 40 h]

3. centrifugation for at least 25 min at 14 000
4. precipitate was washed with 5 mL of a stock 0.1 M NaCl, 10 mM phosphate buffer (pH 7) solution, recentrifuged, and redispersed in 5 mL of a 0.3 M NaCl, 10 mM phosphate buffer (pH 7), 0.01% azide solution

A real-time PCR-based method for determining the surface coverage of thiol-capped oligonucleotides bound onto gold nanoparticles

1. adding 2 nmol of DNA per ml of 30 nm diameter (0.1 nM) and 13 nm diameter gold (6 nM) nanoparticles
2. solutions were diluted to 0.15 M NaCl, 10 mM phosphate (pH 7.4)

[stand for 48 h @ ambient temperature]

3. Unconjugated oligonucleotides were removed by washing with 0.15 M NaCl, 10 mM phosphate (pH 7.4), followed by centrifugation at 14 000 r.p.m. (AM2.18 rotor) for 30 min
4. purification was accomplished by centrifugation at 9000 g for 30 min through a linear glycerol gradient of 10–30%
5. redispursed in 0.15 M NaCl, 10 mM phosphate (pH 7.4) and the optical density at 520 nm

Nonspecific and Thiol-Specific Binding of DNA to Gold Nanoparticles

1. oligonucleotides were added in different amounts to a water dispersion of gold colloids (18 nM), resulting in final concentrations between 0.45 and 9.1 μM strands (oligonucleotide samples were in a 20 mM NaCl solution before mixing) At this ionic strength, the melting temperature of the double-stranded 12-mer samples is 47 °C, and that for the 25-mer double strands is 58 °C

[After 24 h at room temperature]

2. the solution was gently brought to 10 mM phosphate buffer, 100 mM NaCl, pH 7.0 -done in steps, and the final concentration of gold particles was 9 nM

[48 h at room temperature]

Electronic absorption spectra of the oligo & NP solutions recorded w/ HP 8452a diode array spectrophotometer
TEM performed w/ Hitachi 8100 transmission electron microscope @ 200 kV

Measuring Oligonucleotide loading on NPs:

- To determine the concentration: ε (extinction coefficient at 530 nm) determined by measuring the absorbance at 530 nm of a 100μL sample of unfunctionalized Au NPs, relating it to known particle concentrations
- Liberate oligonucleotides from surface, and measure the fluorescence
- Calculate an average oligonucleotides per NP