Bio-functionalized Gold Nanoparticles and Their Applications in Medicine
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Colloidal gold has been known since ancient Egyptian and Roman times where it was used to stain glass. Gold suspensions were also used as a cure-all under the name Aurum Potabile by the alchemist Paracelsus. In 1842, John Herschei used colloidal gold suspensions to record images on paper. It was not until 1857 that Michael Faraday used phosphorus to reduce gold chloride to obtain the first sample of pure gold nanoparticles. Over a hundred years later, G. Frenz published a paper on the controlled nucleation of colloidal gold based on J. Turkevich’s reduction of chloroauric acid by citrate. In 1994, Brust and Schiffrin developed a two phase synthesis for colloidal gold in organic solvents which leads to smaller nanoparticles. Since then, there have been thousands of papers and patents published about gold nanoparticles and their functionalization for various applications including heterogeneous catalysis, sensitive analysis/detection, and therapeutics.

Gold nanoparticles provide a stable, chemically inert scaffold in which thiolate moieties can be bonded to the surface. It is the unique properties of these aurophilic and thiophilic interactions that are at the heart of the stability of the functionalized nanoparticles. It is through this thiophilic interaction that nanoparticles can be functionalized with pharmacologic drugs, oligonucleotides, peptides, and other SR' ligands. The gold nanoparticles also have a unique plasmon absorbance in the near-infrared region (≈700-1000 nm) of the energy spectrum which can be used for heat emission or ligand release.

Frenz, using the Turkevich method, demonstrated how monodispersed nanoparticles could be obtained from the reduction of chloroauric acid with trisodium citrate. Varying the ratio of reactants, Frenz was able to obtain nanoparticles ranging from 12 nm to 150 nm. In 1994, Brust developed a synthetic route for gold nanoparticles in organic solvents using the phase transfer agent tetraoctylammonium bromide. This new method is now the primary method for synthesizing nanoparticles smaller than 10 nm. Using the Turkevich/Frenz method (Figure 1 top), R. D. Kornberg et al. synthesized the nanoparticle Au_{102}(SC_6H_4COOH)_{44} (Figure 2). The black x-ray quality crystals thus obtained were characterized using synchrotron radiation and multi-wavelength anomalous diffraction/scattering (MAD) techniques, allowing Kornberg and coworkers to solve and refine the structure to a resolution of 1.15 Å. Thus, Kornberg. et al., obtained the first crystal structure of a gold nanoparticle (Figure 2). A year later, R. W. Murray et al. used the Brust method to synthesize [TOAB][Au_{25}(SCH_2CH_2Ph)_{18}] (Figure 1 bottom). X-ray quality crystals were grown and solved using MoKα radiation and small molecule methods. These two structures correspond to 1.6 nm and 0.9 nm nanoparticles respectively. The
structures can be combined with DFT calculations to elucidate the structures of larger nanoparticles.

As previously stated, gold nanoparticles are very thiophilic, and this property has been exploited to form very stable thiolate functionalized nanoparticles. Here, the use of thiolate-DNA ligands will be discussed in further detail. Once functionalized with DNA, also referred to as oligonucleotides, the particles can be used for detection agents, drug delivery, near-infrared radiation (NIR) induced heating, or gene therapy. The following applications will be presented:

1) Gold nanoparticles functionalized with oligonucleotide and mixed with a DNAzyme will form aggregates with a characteristic blue color. In the presence of lead (II), a cleavage reaction occurs breaking up the DNAzyme aggregates, and leads to a return of the well known red color of colloidal gold solutions. UV-Vis spectroscopy, or even the naked eye, can be used to examine the ratio of absorbance at 522 nm and 700 nm to determine the amount of free and aggregated nanoparticles in solution to quantify the amount of lead present in solution. This system was also tested on a wide range of other metallic ions and shown to only respond to lead. By altering the DNAzyme used, this detection system has since been expanded for specific detection of other ions and even biomolecules.

2) Oligonucleotide gold nanoparticles have been coupled to a platinum (IV) octahedral complex by the groups of Mirkin and Lippard through formation of a C-N bond by Steglich esterification. It was predicted that the Pt (IV) compound would undergo reduction and elimination of the axial ligands to form Cisplatin within the intracellular environment. By using gold nanoparticles as the delivery agent, an inert Pt (IV) complex can be taken up by cells, where a reduction event occurs producing the Pt (II) agent Cisplatin (Figure 3). Through this delivery method, Mirkin and Lippard have eliminated extracellular Cisplatin that can cause harmful side effects. In addition, a lower dose is needed to produce the same results as current cancer drugs.

3) J. L. West, et al., synthesized a 110 nm silica nanoparticle and functionalized the outside with alkylamine chains. To each amino chain, a 2 nm gold nanoparticle was attached followed by addition of a chloroauric acid/formaldehyde solution to reduce gold to fill in the gaps between the 2 nm nanoparticles to form a 10 nm thick gold shell on the silica nanoparticle. The thickness of the gold shell makes it possible to finely tune the absorption wavelength of the nanoshell. The Au-Si nanoshell was then reacted with thiolated PEG chains to decorate the surface. Such functionalized nanoshells were then injected into a mouse tumor and irradiated with a 820 nm laser for 6 minutes at 35 W. The site of the irradiated nanoshells showed an
average increase in temperature of 37 °C; whereas, the control site with irradiation only showed an increase of 10 °C. The induced heating that results when the nanoparticles are irradiated led to tumor cell death.

4) In 2006, Mirkin used an antisense strand of oligonucleotides to functionalize a gold nanoparticle in order to reduce expression of the gene coded for by the strand of oligonucleotides.\textsuperscript{13} The oligonucleotides were attached to the nanoparticles by two methods, the first was through an alkylthiolate to form a monothiol modified oligonucleotide and the other was through two cyclic disulfides to form a tetrathiol modified oligonucleotide. The monothiolate produced a loading on the nanoparticle of 110 strands and the tetrathioltate produced a loading of 45 strands (Figure 4). The antisense particles work by binding complimentary strands of oligonucleotides which leads to a reduction in target gene expression. The higher loading on the monothiolate nanoparticle leads to a cooperative binding effect for the complimentary strand 35 times higher than that of the tetrathioltate and free oligonucleotide strand. This study was expanded in 2008 by Mirkin and coworkers who synthesized a heterofunctionalized nanoparticle.\textsuperscript{14} The new nanoparticle was not only functionalized with strands of antisense oligonucleotides, but also peptides to aid in cellular uptake and intracellular localization. Using inductively coupled plasma mass spectrometry, Mirkin and coworkers determined that the nanoparticles functionalized with only antisense DNA were taken up by the cell in higher numbers than the heterofunctionalized nanoparticles. However, the dual functionalized nanoparticles were shown to localize in higher yield around the nuclease of the cell as determined by fluorescence microscopy. Gene expression was also examined. Whereas antisense nanoparticles knocked down 25% of gene expression, the dual functionalized nanoparticles showed about 60% gene knockdown.

These recent studies by Mirkin illustrate the future of gold nanoparticles in medicine. Nanoparticles could become specific enough to target exact cell lines or even specific genes in certain cell types, through the use of bi- or even tri- functionalized nanoparticles. The importance of this technology can be applied as a chemotherapeutic where surgery may not be an option. An injection of nanoparticles into the blood stream would localize at the site of a tumor. Once there, gene therapy or radiation therapy could be used to kill off the tumor, without surgery and long recovery times. Cancerous cells could be selectively killed off in a matter of minutes.

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\caption{Functionalization of gold nanoparticles with monothiol and tetrathiolt-modified antisense oligonucleotide.}
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References