Anticancer Drug Delivery via Unique Properties of Gold Nanoparticles

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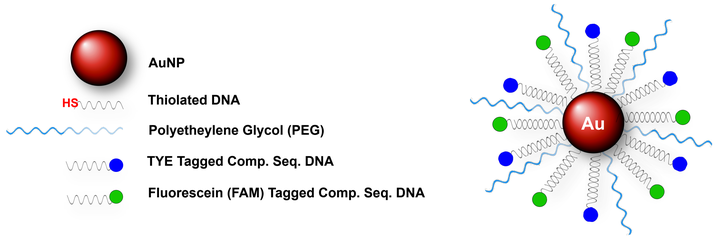
Introduction

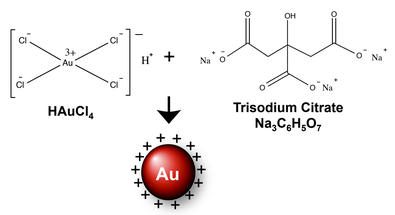
In an attempt to develop improved drug delivery systems for current anticancer drugs, gold nanoparticle (AuNP) based complexes were developed by conjugating polyethylene glycol (PEG) and two different fluorescently tagged dsDNAs (double stranded DNA) to ~20nm AuNPs. AuNPs have the ability to generate heat in the presence of near infared (NIR) irradiation. By engineering unique dsDNA sequences with distinct melting temperatures (T_M) it is envisioned the delivery of two different anticancer agents can be modulated by altering the exposure to NIR light. Fluorescent tags on the DNA are representative of the anticancer drugs being administered and will allow the visualization of release from the surface of the AuNP in addition to the characterization of ssDNAs' location and concentration. AuNPs were synthesized via citrate reduction and subsequently thiolated PEG and dsDNAs were attached to AuNPs via a Au-S bond. Selective thermal release of fluorescently tagged ssDNA was attempted by taking advantage of the unique T_M of each dsDNA pair. However, difficulties were experienced when attempting to quantify the attachment and release of the fluorescently tagged ssDNA. TCEP, a reducing agent used to reduce the disulfide bonds of the thiolated DNA to allow conjugation to the gold surface also reduced one of the fluorescent tags, fluorescein (FAM), rendering it non-fluorescent. Future work includes using alternative reducing agents and incorporating other fluorophores to enhance characterization capabilities. New methods of DNA hybridization and conjugation to AuNP surface are also being considered to increase amount of DNA attached to AuNPs.

Self-Assembled Nanoparticle Complexes

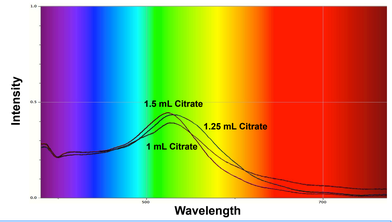


The drug delivery system seeks to eliminate some of the more prevalent side effects of chemotherapy by using a conjugated AuNP system. It is composed of a 20nm AuNP, Poly-ethyleneglycol (PEG), two different sequences of thiolated DNA, and two complimentary DNA sequences; one tagged with Fluorescein, the other with TYE.

Citrate Reduced Synthesis of Gold Nanoparticles

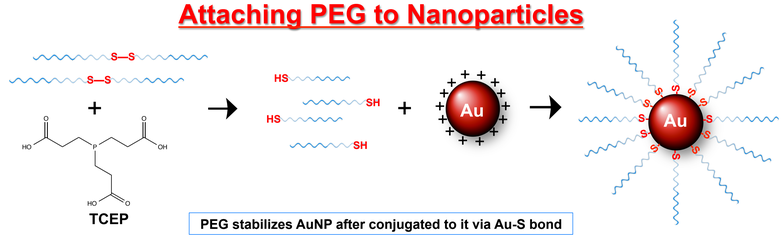


Increasing concentrations of Trisodium Citrate Dihydrate resulted in decreasing diameters of AuNPs and vice versa.



Size and concentration of AuNPs were determined using an equation corresponding peak λ and intensity to diameter and molarity of the nanoparticles.

Attaching PEG to Nanoparticles



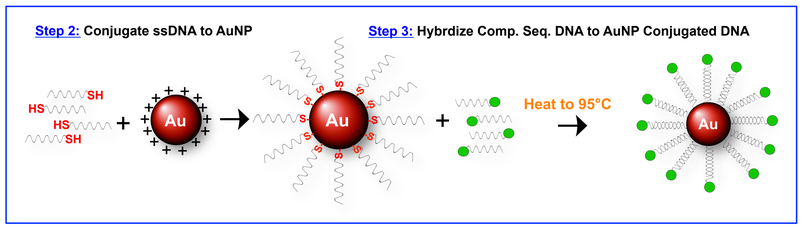
PEG stabilizes AuNP after conjugated to it via Au-S bond

Attaching DNA to Nanoparticles

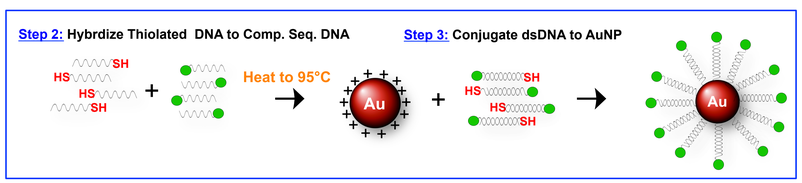
Two methods were used to attach DNA to AuNPs, both begin with cleavage of disulfidebond via TCEP

Step 1 for Both: Cleave Disulfide Bond: S—S + TCEP → SH HS

Method 1:

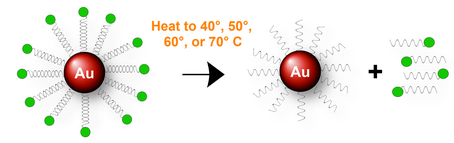


Method 2:



Both methods hybridize DNA via simulated Polymerase Chain Reaction (PCR) and attach DNAs to AuNPs using Au-S bonds, they differ only in the order in which these are done. Method 1 conjugates DNAs to AuNPs first, and hybridizes DNAs second. Method 2 hybridizes DNAs first, and attaches hybridized DNAs to AuNPs second.

Thermally Induced Release of DNA from AuNP

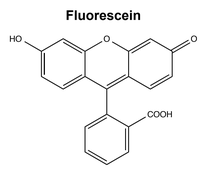


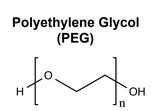
AuNPs are heated and dsDNA dehybridizes, releasing FAM-DNA. AuNPs were heated to different temperatures to determine optimum melting temperatures.

In intact DNA-AuNPs, electron transfer between FAM and AuNP causes quenching of FAM's fluorescence. Heating DNA-AuNPs dehybridizes dsDNA, releasing FAM-DNA. Upon thermal release, electron transfer between FAM and AuNP ceases and fluorescent functions return to FAM, enabling visualization of FAM-DNA.

Conclusions and Future Research

Monodisperse solutions of AuNPs were successfully synthesized and stabilized by attaching PEG, evidenced by their ability to remain suspended in solution after prolonged centrifugation. Experimentation with DNA-AuNP assembly methods will continue in order to determine the optimum protocol. This includes experimentation with different fluorophores and reducing agents, as well as new methods of quantizing the amount of thermally released DNA. Selective thermal release of different ssDNAs from the same AuNP is also the subject of future research. The full assembly of the AuNP complex, and the subsequent light-induced thermal release of DNA continue to be future goals. Distant research includes replacing FAM and TYE with different anticancer drugs, and testing in cell cultures. The end goal is to use this drug delivery system to treat cancer in living hosts and eventually humans.





DNA Sequences Used

5' CAC CGC ATT CAG GAT TCT CAA CTC GTA 3' 6-FAM

HS-6C 5' TAC GAG TTG AGA ATC CTG AAT GCG GTG 3'

6-FAM 5' CAC CGC ATT CAG GAT TCT CAA CTC GTA 3'

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