Tandem Cancer Drug Delivery via Gold Nanoparticle Targeting Platforms

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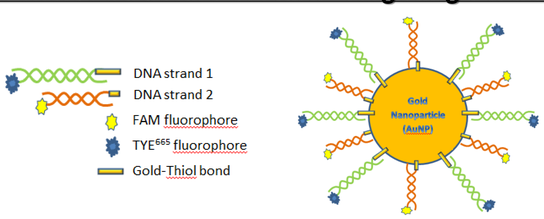
Introduction

According to the National Cancer Institute, there are several hundred drugs that have been developed with anti-cancer abilities. However, cancer is still the second leading cause of death in the United States according to the CDC. The effectiveness of most anti-cancer drugs is not limited by its potency but instead by its non-targeted delivery and unwanted side effects. Many cancer treatments such as chemotherapy can effectively kill cancer cells, but lack the selectivity and specificity to avoid wreaking havoc on the rest of the body. Thus, gold nanoparticles (AuNP) have been employed in an effort to overcome some of the downfalls of current chemotherapy options. AuNPs have the unique ability to heat upon exposure to near infrared irradiation and their nanometer sized dimensions aid in their selective delivery to solid tumors.

Using AuNPs as a platform, the ability to selectively deliver two different anti-cancer therapeutics via heat sensitive conjugations was investigated. Two DNA strands of different melting temperatures were developed separately as potential temperature-sensitive tethers for drug attachment to AuNPs. Fluorophores were utilized to tag DNA strands during simulation of drug release and track attachment to the AuNP platform. Single-stranded DNA tethers were successfully conjugated to AuNPs by gold-thiol bond formation using a 5' thiol DNA modifier, but synthesizing the full AuNP platform proved to be more challenging. After attempting several attachment sequences and methods, there were no visible indications of successful attachment of dsDNA tethers to the AuNP platform. Future work will consist of identifying what is preventing the full dsDNA tethers from attaching or if its attachment is being masked. Finally, efforts will turn to synthesizing AuNPs with both DNA tethers attached which are able to denature independently at different temperatures to achieve a timed and temperature-dependent dual-drug release.

General Structure of AuNP Targeting Platform



The AuNP tumor targeting platform consists of two dsDNA strands of different lengths and different melting temperatures (Tm) conjugated to a gold nanoparticle via gold-thiol bonds. Each non-thiolated complementary strand of DNA is tagged with either fluorescein (FAM) fluorophore or TYE665 fluorophore (a modified Cy5 substitute) for tracking temperature-sensitive release. The platform is also stabilized by Polyethylene Glycol (PEG) not shown in this diagram.

DNA Tether Sequences

Thiolseq44.2 : 5' - Thiol - ATA AGT CAT CGT ATT GTA TAG -3'

3'TYEcompseq44.2 : 5' - CTA TAC AAT ACG ATG ACT TAT - (TYE) - 3'

5'TYEcompseq44.2 : 5' - (TYE) - CTA TAC AAT ACG ATG ACT TAT - 3'

LENGTH: 21
GC CONTENT: 28.6 %
MELT TEMP: 44.2 ºC

Two complementary sequences with TYE665 attached at opposite ends were developed in order to investigate the effect of fluorescent quenching

Thiolseq60.7 : 5' - Thiol - TAT GTG GCG TAA GTC CTA AGA GTT -3'

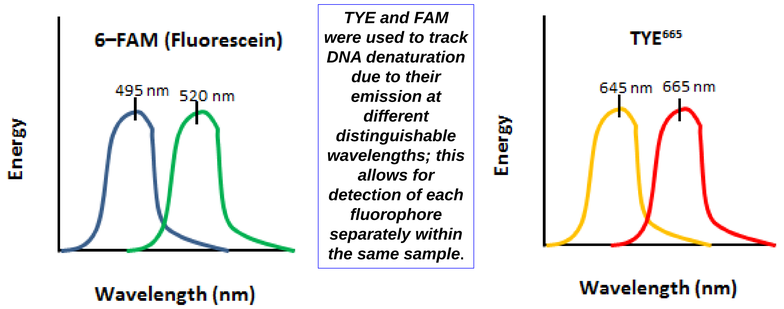
3'FAMcompseq60.7 : 5' - ATA CAC CGC ATT CAG GAT TCT CAA - (FAM) - 3'

5'FAMcompseq60.7 : 5' - (FAM) - ATA CAC CGC ATT CAG GAT TCT CAA - 3'

LENGTH: 24
GC CONTENT: 41.7 %
MELT TEMP: 60.7 ºC

The FAM tagged dsDNA sequence was developed with a melting temperature significantly higher than the TYE strand (~15ºC higher) in order to achieve timed release. This difference arises from its greater length and higher GC content.

FAM and TYE665 Emission/Excitation Spectra

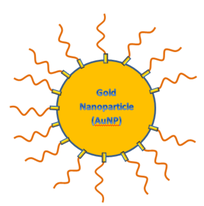


TYE and FAM were used to track DNA denaturation due to their emission at different distinguishable wavelengths; this allows for detection of each fluorophore separately within the same sample.

Conjugation and Hybridization of DNA-AuNPs

Two approaches were implemented to form the DNA-AuNP platform: 1.) Complementary thiolated and fluorescent ssDNA strands were hybridized together and then conjugated to the AuNP by a gold-thiol bond; and 2.) The thiolated ssDNA strand was conjugated to the AuNP first, followed by hybridization of its complementary fluorescent ssDNA strand.

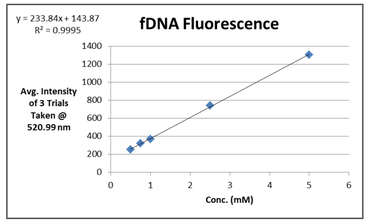
Unsuccessful hybridization of Fluorescent DNA

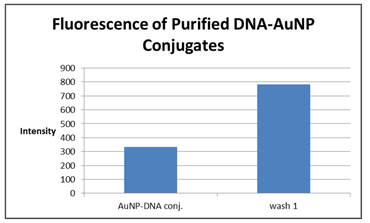


'laying down' of hybridized and conjugated DNA on the surface of the AuNP

Samples of the newly synthesized DNA-AuNP platform did not yield any fluorescence, indicating either unsuccessful hybridization or quenching of the fluorescent DNA by 'laying down' on the AuNP as shown above.

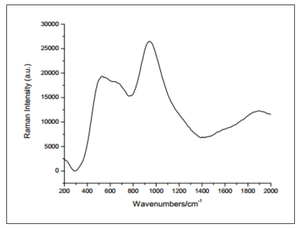
Fluorescence Intensity of DNA-AuNP platforms



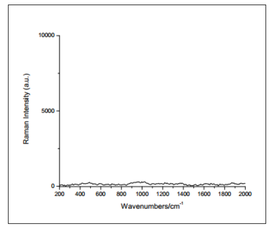


Fluorescence samples of free single-stranded TYE665 tagged DNA dilutions indicate a detection limit of approximately 0.5 mM, suggesting that dsDNA tethers could be on the AuNP surface at an undetectable concentration.

Centrifugation of newly synthesized DNA-AuNP conjugates revealed significant fluorescence in the supernatant and very little if any on the DNA-AuNP conjugates, indicating that the majority of TYE665 tagged DNA did not attach.



Surface Enhanced Raman Spectroscopy (SERS) of DNA-AuNP conjugates (left) indicates some fluorescent DNA attached to the surface of the AuNP but there was no fluorescence on pure AuNP samples (right)

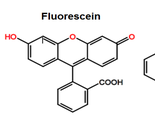


Conclusions and Ongoing Research

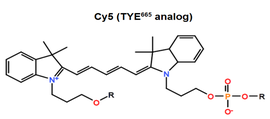
AuNP platforms were successfully synthesized and conjugated with each of the designed ssDNA strands. However, lack of fluorescence on the AuNP platform indicated that either hybridization and conjugation of either fluorescent dsDNA tether was unsuccessful or was being masked by fluorescent quenching. Fluorescence and SERS data suggest the possibility of successful DNA tether attachment at a concentration below the fluorimeter detection limit. Further experimentation will be focused on the following:

- Exploration of different DNA tether attachment techniques
- Achieving full AuNP delivery platform assembly with both DNA tethers and stabilizing PEG
- Quantification and Analysis of timed temperature-sensitive DNA release
- Loading the AuNP platform with anticancer drugs and optimizing payload delivery in living organisms to increase selectivity and efficacy of cancer treatment

Fluorescein



Cy5 (TYE665 analog)



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