Project title: Aptamer-mediated DNA nanocage encapsulated gold nanoparticles for malaria diagnosis medical devices

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Abstract

Aptamers are oligonucleotides (short DNA or RNA molecules) that are created by selection from pools of variant sequences and bind to molecular targets including proteins with high specificity and affinity [1, 2]. Aptamers are capable of molecular recognition, and therefore, DNA aptamers have been used in various biomedical applications including both therapeutics and diagnostics [3]. Malaria is a life-threatening disease that kills over 1 million children per year in Africa [4]. Drug resistance, ineffective diagnostics, and cost of treatment are major concerns regarding malaria in today's developing world, leading to the need of novel and accessible diagnostics of malaria as ways to save lives and reduce the effects of these concerns [4]. Conventionally, point-of-care diagnostic tests for malaria are based on the use of antibodies; however, these tests are rather costly and face the challenge of the instability of protein antibodies in tropical climate [3]. Aptamers are capable of molecular recognition, which is similar to antibodies, but can be synthesized at a lower cost and comparatively more stable than antibodies [5]. Cheung et al. (2013) have applied a unique DNA aptamer against *Plasmodium falciparum* lactate dehydrogenase (PfLDH), which is a known molecular target for antimalarials. Their results indicated the high affinity and specificity of the selected DNA aptamers for PfLDH and revealed the unique structure formed by the binding of aptamers to PfLDH. This has shown the potential application of aptamers in developing rapid diagnostic test for malaria disease, and lateral flow point-of-care test is a promising medical test that can make use of aptamers.

Lateral flow assay (LFA) is a test for detecting analytes in mixtures, where samples can be placed in the device and results can be obtained after a short period of time [6]. Gold nanoparticles (AuNPs), which can be modified with chemical and biological molecules, have been used in therapeutics treatment, drug delivery, and diagnostics including LFA [7]. In LFA, the changes of colometric signals derive from the aggregation of colloidal AuNPs [8]. In aptamer-mediated LFA, AuNP's function of detecting the targets is associated with aptamers. However, there are

challenges of controlling the ratios between aptamers, the AuNP's cap made up of alkylthiol-capped oligonucleotides which can be reduced by biological buffers, and the instability of aptamer decorated AuNP in certain temperatures and environments [9]. Solving these challenges will result in the development of a stable aptamers functionalized AuNP and eventually aptamer-mediated LFA as a point-of-care medical device for rapid diagnostics test, which can be applied for malaria because an aptamer for the disease has been well-studied.

Methodology

1. Construction of aptamer-mediated DNA nanocage

DNA nanocages are designed to have an octahedron shape. Two methods of nanocage construction are tested: (1) mixture of all single strands to form octahedrons and (2) forming 2 semi-spheres then incubate them at RT to form octahedron.

Chemically synthesized DNA oligonucleotides are mixed together. The samples are put into thermal cycler for annealing: 95^{o}C in 5 minutes and cool down to RT in 4h.

The samples are then run on PAGE to verify the structure. Controls of mixtures of 1 to 7 strands are used. A gradually increasing in size of different samples are expected on the gel.

2. Encapsulation of gold nanoparticles using aptamer-mediated DNA nanocage

After successful construction of DNA nanocage, the structure will be used to encapsulate gold nanoparticles. Similarly, two methods are tested: (1) mixture of all single strands with AuNPs and annealing in thermal cycler, (2) mixture of 2 semi-spheres with AuNPs at RT for encapsulation.

The samples are verified based on the following observation:

- Since aggregation of AuNPs will cause the color change from red to blue, DNA nanocage encapsulation of AuNPs should prevent aggregation and the color should be red.
- The samples are also verified on gel.
- Transmission Electron Microscopy Imaging is used to observe and compare the aggregation of AuNPs between AuNPs sample and DNA nanocage-encapsulated AuNPs.

After successful encapsulation of AuNPs, the structure will be further tested for binding affinity to PfLDH.

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