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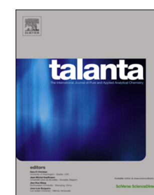

Ni(II)NTA AuNPs as a low-resource malarial diagnostic platform for the rapid colorimetric detection of *Plasmodium falciparum* Histidine-Rich Protein-2

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ABSTRACT

Diagnosing infectious diseases remains a challenge in the developing world where there is a lack of dependable electricity, running water, and skilled technicians. Although rapid immunochromatographic tests (RDTs) have been deployed to diagnose diseases such as malaria, the extreme climate conditions encountered in these regions compounded with the discrepancies in test manufacturing have yielded varying results, so that more innovative and robust technologies are sought. Devoid of antibodies and thermally sensitive materials, we present a robust, colorimetric diagnostic platform for the detection of a malarial biomarker, *Plasmodium falciparum* Histidine-Rich Protein 2 (*Pf*HRP-II). The assay exploits the optical properties of gold nanoparticles, covalently coupling them to a Ni(II)NTA recognition element specific for *Pf*HRP-II. In the presence of the recombinant malarial biomarker (rcHRP-II), the Ni(II)NTA AuNPs begin to crosslink and aggregate in as little as one minute, triggering a red-to-purple color change in solution. To increase assay sensitivity and platform stability suitable for low-resource regions, the Ni(II)NTA AuNPs were assembled with varying spacer ligands in a mixed monolayer presentation. When assembled with a negatively charged Peg_4-thiol ligand, the Ni(II)NTA AuNPs demonstrate low nanomolar limits of rcHRP-II detection in physiological concentrations of human serum albumin and maintain excellent stability at 37°C when stored for four weeks. Detection of the malaria biomarker is also measured by capturing and processing images of aggregated gold nanoparticles with a smartphone camera. By utilizing a smartphone camera and image processing application, there is no significant difference in assay sensitivity and rcHRP-II limit of detection in comparison to a spectrophotometer, further making this diagnostic platform applicable for use in low-resource regions.

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1. Introduction

In the developing world, early, rapid, and accurate diagnosis is essential to control epidemics and curb resistance for diseases such as tuberculosis, HIV/AIDS, and malaria. Unfortunately, high healthcare costs in these regions compounded with a lack of reliable electricity, a lack of skilled technicians, and a minimal infrastructure burdens disease diagnosis. For malaria, a parasitic disease that threatens most of the developing world each year, rapid point-of-care diagnostic devices are necessitated. In order to combat diagnosis issues presented in the developing world, immunochromatographic rapid diagnostic strip tests (RDTs) have been distributed to detect malarial infection in as little as 15–30 min by capturing *Plasmodium falciparum* malarial parasitic antigens in patient blood samples [1]. In 2011, the World Health Organization (WHO) implemented an assessment of these strip tests and determined that only 20% retained the 200–2000 parasites/μL threshold range in patient blood samples recommended by the WHO [2]. One of the primary explanations for test failure included antibody degradation from long-term storage in the extreme climates encountered in low-resource regions. As a result, innovative, sensitive, and robust approaches for antigen recognition are necessitated for accurate malaria diagnosis.

Upon infection, the *Plasmodium falciparum* parasite secretes 97% of its synthetic biomarker protein, Histidine-Rich Protein-2 (*Pf*HRP-II), into the host's blood, offering an attractive biomarker for disease diagnosis [3]. However, recent discoveries have revealed that *P. falciparum* strains in Senegal and India have exhibited genetic diversity, and polymorphisms in the *Pf*HRP-II antigen have been discovered, further complicating diagnosis via immunoassay platforms [4,5]. Approximately ~85% of the primary structure of *Pf*HRP-II is comprised of AHH and AHHAAD repeats, so that targeting these histidine subunit repeats offers an alternative approach for molecular recognition [6]. Ni(II)Nitrilotriacetic acid

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(NTA) chelation has been well established as a strategy to extract and isolate His-tagged proteins, and this octahedral organometallic complex retains micromolar affinity to histidine by coordinating two amino acid subunits per molecule [7–10]. Considering *Pf*HRP-II is a naturally occurring His-tagged protein, Ni(II)NTA complexes have the potential to coordinate multiple repeats per protein.

The coupling of a Ni(II)NTA recognition component to a more robust and sensitive platform would alleviate many of the aforementioned issues with existing RDTs. Colloidal gold nanoparticles offer a desirable signal transduction approach because of their unique optical properties and biologically inert surface. In the presence of visible light, the electrons at the AuNP particle surface oscillate in-phase with the incident radiation, so that monodisperse colloids exhibit a narrow localized surface plasmon resonance (LSPR) band in the visible spectrum, thus transmitting bright red colors in solution. The LSPR signal is sensitive to changes in the dielectric environment encompassing the particles, inspiring applications in both sensor and diagnostic development. Shifting the LSPR band can be induced by the physical aggregation of as little as 2–10 particles, which ultimately triggers a color change in solution [11–13]. Although the gold surface is understood to be biologically inert, colloidal gold nanoparticles are well known for their facile ligand coupling via covalent Au–thiol chemistry, providing a robust and stable interface capable of enduring the extreme climates typically found in South America, sub-Saharan Africa, and southern Asia where malaria is endemic [14]. By exploiting these properties, colloidal gold nanoparticles have been well established as aggregation-induced colorimetric sensors for detection of nucleic acids, proteins, small molecules, and metal ions [15–19].

In this investigation, we aim to create a robust, low-resource colorimetric indicator for malaria diagnosis by selectively and rapidly aggregating Ni(II)NTA AuNPs only in the presence of a recombinant *Pf*HRP-II (rcHRP-II) biomarker, triggering a spectrophotometric redshift and subsequently, a red-to-purple color change (Fig. 1). A custom, thiolated Ni(II)NTA recognition ligand has been previously synthesized and validated in our lab as a proof-of-concept design with Ni(II)NTA Au and AgNPs by detecting histidine-rich peptide mimics [20]. However, the activity of Ni(II) NTA AuNPs has never been assessed against *Pf*HRP-II and the limits of detection for the peptide mimics are outside of the clinically relevant range for malaria diagnosis. We also aspire to optimize the AuNP platform with varying spacer ligands for enhanced signal and stability, along with validating the diagnostic utility of these particles in a complex physiological matrix, mimicking patient samples. In addition, smartphones are emerging as rapid detection technologies in the medical industry, especially through the use of high dynamic range cameras and image processing applications. The rapid colorimetric detection of explosives, pH, and disease biomarkers has been previously detected and analyzed using smartphone technology and processing [21–24]. The ability to capture a color image of aggregated nanoparticles with a smartphone and subsequently analyze the respective signal intensity using an application would reduce user bias and aid in low-resource diagnosis of infectious diseases.

2. Experimental

2.1. Materials and reagents

Citrate-stabilized gold nanoparticles (15 nm) were purchased from Ted Pella, Inc. Thiol-dPeg$_4$-acid (Peg$_4$) was purchased from Quanta Biodesign, Ltd. Recombinant *P. falciparum* Histidine-Rich Protein-II (rcHRP-II) was purchased from CTK Biotech. The thiolated NTA recognition ligand was synthesized previously in our lab. 11-Mercaptoundecanoic acid (MUA) and 6-mercaptohexanol (MHOL) were both purchased from Sigma-Aldrich. The confounding proteins, human serum albumin (HSA) and glutathione-S-transferase (GST), were purchased from Sigma-Aldrich. All other materials/buffers were purchased from either Sigma-Aldrich or Fisher Scientific. All deionized water was used with a resistivity greater than 18 MΩ cm^2.

2.2. Instrumentation and equipment

UV–vis spectra were taken with an Agilent 8453 spectrophotometer with a photodiode array detector. Spectrophotometric assays were performed in Corning 384 well plates and measured with a Biotek Synergy H4 plate reader. Electron microscopy images were taken with a CM 20 transmission electron microscope at 200 kV. A Malvern Zetasizer was utilized for in-solution dynamic laser light scattering size distribution measurements. An Eppendorf 5415D microcentrifuge was used to centrifuge AuNPs in 1.5 mL Eppendorf tubes. A Thermo Reciprocal Shaking Bath was utilized to store the particles at 37°C. Photographs of suspended Ni (II)NTA AuNPs were taken with an iPhone 4S smartphone (Apple, CA).

2.3. Synthesis of Ni(II)NTA AuNPs

A 2 nM aliquot of 15 nm citrate-stabilized AuNPs was incubated with 5 μM thiolated NTA ligand and 5 μM spacer ligand and allowed to assemble on the particle surface overnight. For synthesis of the 100% Ni(II)NTA AuNPs, 10 μM thiolated NTA ligand was incubated with the particles and assembled overnight. The next morning 1 mL aliquots of NTA-functionalized AuNPs were washed by centrifugation at 7200g for 45 min. The supernatant was removed and the particles were resuspended in

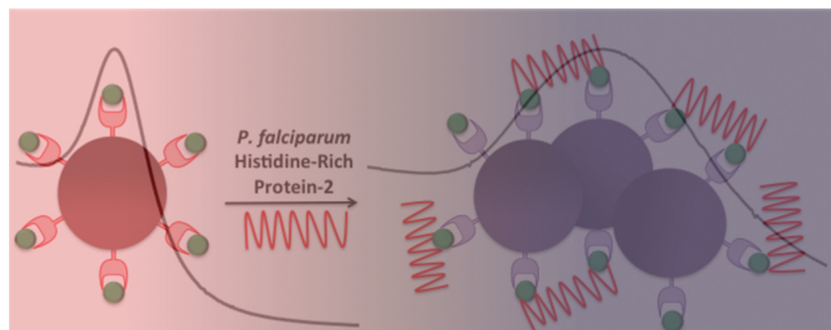

Fig. 1. Color change of Ni(II)NTA AuNPs upon aggregation induced by rcHRP-II. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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