Gold Nanoparticle-Based Immunoassay for detection of *Plasmodium falciparum* Hsp70

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Biomedical nanotechnology presents revolutionary opportunities in the detection of pathogenic microorganisms. Despite its huge burden, with forty percent of the world's population at risk of infection, the diagnosis of malaria is often not straightforward and there is an urgent need to develop rapid, sensitive, and cost-effective tests for both high- and low-resource settings. We aim to design a gold nanoparticle (**AuNP**)-based rapid detection test (**RDT**) using specific antibodies to detect *Plasmodium falciparum* (malaria parasite) antigens in clinical specimens. The characteristics of the proposed malaria RDTs include reproducibility, acceptable high sensitivity and specificity, rapidity, ease of performance and interpretation, stability when stored, and capability of species differentiation, all at an affordable price [1].

Our approach is based on the utilization of mercaptoundecanoic acid (**MUA**)-capped AuNPs conjugated with 2E6 antibodies. These antibodies specifically recognize *Plasmodium falciparum* Heat Shock Protein 70 (**PfHsp70**). Heat Shock Proteins are immunodominant antigens recognized by the host immune system in various infectious diseases. In particular, PfHsp70 which possesses chaperone and anti-apoptotic activity has recently drawn attention as a novel therapeutic target [2]. The presence of parasitic Hsp70 in the pellet of saponin treated red blood cells of infected mice (and not uninfected mice or humans) was confirmed by Western blot (Figure 1). This result suggests that this antibody-antigen set could be used in the development of an RDT for malaria in clinical samples. PfHsp70 antigens will be purified from an overexpressing *E. coli* system using His-tag or immune-affinity chromatography, and utilized as proof-of-concept for the method.

The formation of the 2E6-AuNP bionano-conjugates was assessed using a previously established method based on ζ-potential measurements ([3] and Figure 2), and conjugation stoichiometry was also verified by agarose gel electrophoresis. UV-visible spectrophotometry shows aggregation of the bionano-conjugates as a function of the solution pH, inducing a shift of the AuNP plasmon band corresponding to a red-to-blue color change of the solution. The pH at which this aggregation occurs can be related to the protonation state of superficial amino acid residues and may hence be used as an indirect evaluation of antibody-AuNP formation and surface alterations induced by antigen binding.

References:

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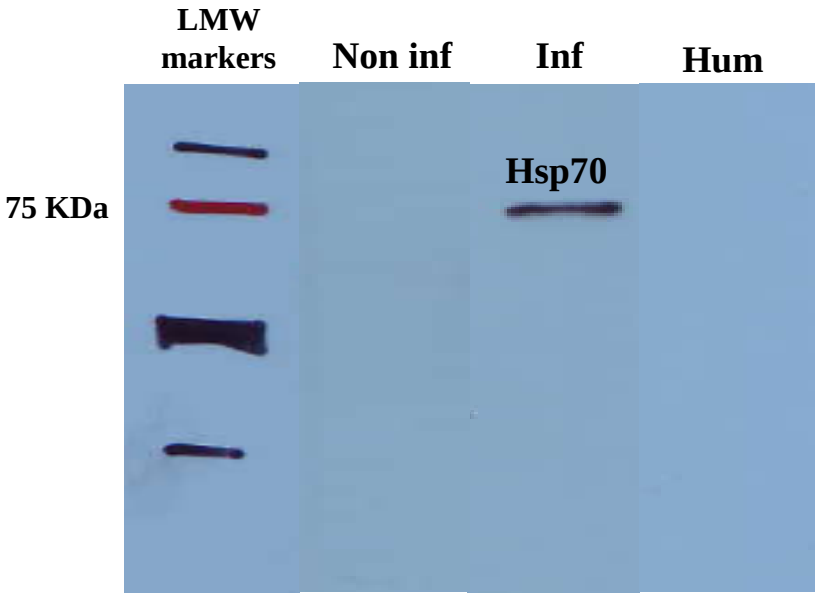
Figures:

Figure 1. Western blot analysis, using 2E6 antibody, of saponin-treated pellets of red blood cells from mice infected with *Plasmodium berghei* (Inf); non infected mice (Non inf); or a healthy human donor (Hum)



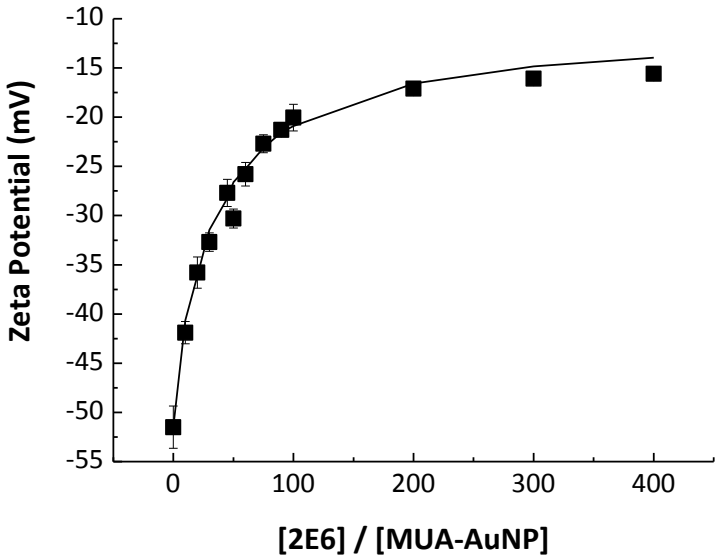


Figure 2. Zeta potential of each bionanoconjugate determined as a function of the [2E6] / [MUA-AuNP] ratio. Solid line represents fitting to a Langmuir adsorption isotherm.

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