Malaria Diagnostics based on Antibody-Functionalized Gold Nanoparticles and *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 (*Pf*Hsp70). Heat Shock Proteins are immunodominant antigens recognized by the host immune system in various infectious diseases. In particular, *Pf*Hsp70 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. *Pf*Hsp70 antigens purified from an overexpressing *E. coli* system using His-tag chromatography [3], were targeted by the 2E6 antibodies, as proven by Western blotting analysis. Such antibody-antigen system was used as proof-of-concept for the detection method.

The formation of the 2E6-MUA-AuNP bionano-conjugates was assessed using a previously established method based on ζ-potential measurements [4], indicating that stable bionano-conjugates can be obtained with *ca*. 150 molecules of antibody per each MUA-AuNP (Figure 2). The bionano-conjugates were produced by simple incubation of the MUA functionalized AuNPs with the 2E6 antibody, suggesting electrostatic and van der Walls forces are involved in formation of the bionano-conjugates. On the other hand, agarose gel electrophoresis showed the formation of more compact 2E6-MUA-AuNP bionano-conjugates in the presence of the cross-linking agents EDC/NHS. We propose cross-linking of the antibody to the MUA-AuNP allows bionano-conjugates that are more robust and appropriate for detection in comparison with their non-crosslinked counterparts.

References

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Figures

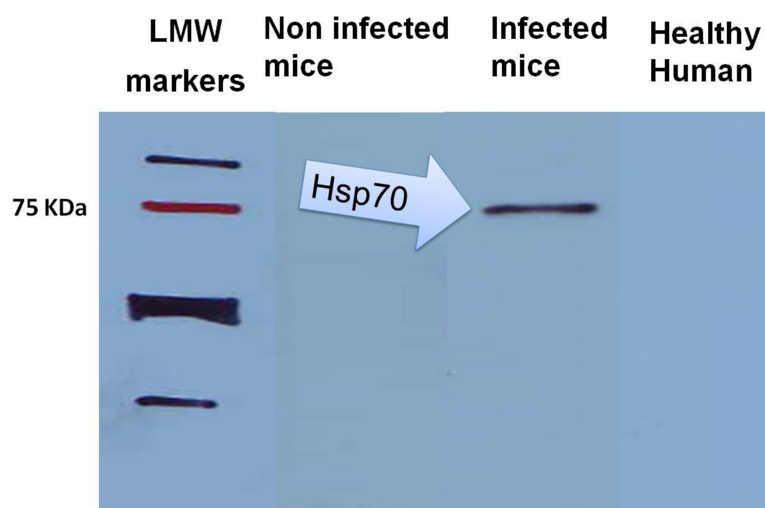

Figure 1. Western blot analysis, using 2E6 antibody, of saponin-treated pellets of red blood cells from mice infected with *Plasmodium berghei*; non infected mice; or a healthy human donor.

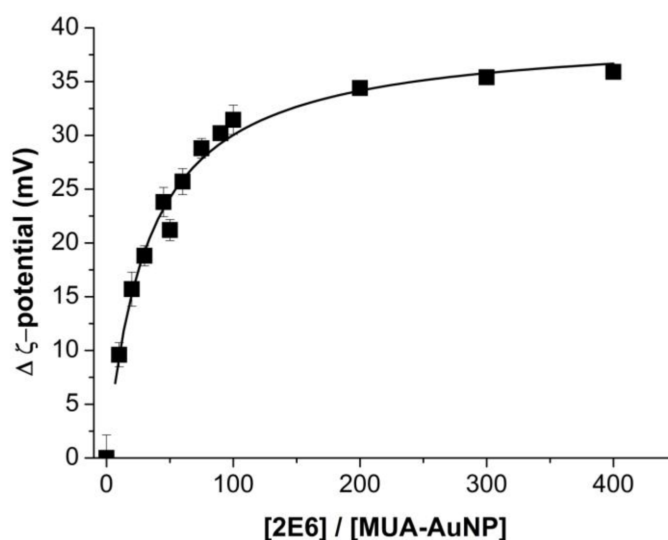

Figure 2. Variation of ζ-potential for each bionano-conjugate in relation to the ζ-potential value for MUA-AuNP alone, determined as a function of the [2E6] / [MUA-AuNP] ratio. The solid line represents the fitting to a Langmuir adsorption isotherm.

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