Malaria Diagnostics based on Anti- *Plasmodium falciparum* HRPII Antibody-Functionalized Gold Nanoparticles

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Malaria is a significant health problem in many parts of the world, with an estimated 207 million cases and 627 000 deaths in 2012 mostly among African children.
Malaria is caused by five species of parasites in the genus *Plasmodium,* of which *P.falciparum* (*Pf*) is the most prevalent and deadly [1].
Rapid Diagnostic Tests (RDTs) using gold nanoparticles (AuNPs) are alternatives to conventional microscopy-based methods for malaria diagnostics.
RDTs are intended to have high reproducibility, acceptable high sensitivity and specificity, rapidity, ease of performance and interpretation, all at an affordable price.
AuNPs are ideal candidates for these tests due to their unique nanoscale properties, such as high surface areas, robustness, facile synthesis and functionalization and strong optical absorptions [2].
In this work, gold nanoparticles are functionalized with mercaptoundecanoic acid (MUA) or CALNN pentapeptide. CALNN includes a thiol group (from C) for binding to AuNPs, a hydrophobic region (AL) to promote self-assembly at the AuNP surface, and two uncharged hydrophilic asparagine residues (NN) to interact with an antibody [3]. These functionalized AuNPs are further conjugated with an anti-*Pf*HRPII monoclonal antibody.
Conjugation of the monoclonal antibody with the functionalized AuNPs was performed either by electrostatic interactions or by covalent attachment, using the cross-linking agents EDC/NHS.
The robustness and binding properties of the bionanoconjugates were evaluated by agarose gel electrophoresis and zeta potential measurements. Results showed the formation of more compact bionanoconjugates in the presence of CALNN and EDC/NHS agents. Increasing concentrations of the antigen were incubated with the bionanoconjugates for two hours. The agarose gel electrophoresis of these antigen-bionanoconjugates showed that electrophoretic mobility decreases with increasing antigen concentrations. These results indicated that it is possible to detect the *Pf*HRPII antigen up to a concentration of 700 $\mu g.mL^{-1}$ (Figure 1), opening up the possibility of implementing a simple agarose gel-based method for malaria antigen detection.
This new immunoassay will be developed to detect the antigen in malaria-infected *in vitro* blood cultures.
These bionanoconjugates are also being used in the development of a RDT on a nitrocellulose strip or on filter paper, using a competitive assay format. In this assay, the *Pf*HRPII antigen is coated on the test zone of the dipstick, capturing the AuNP-antibody conjugate and allowing the red colour to concentrate and form a spot. This study will constitute an important proof-of-concept for future tests in clinical samples.

References

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Figures

A

B

[*Pf*HRPII] / [AuNP-CALNN-anti-HRPII]

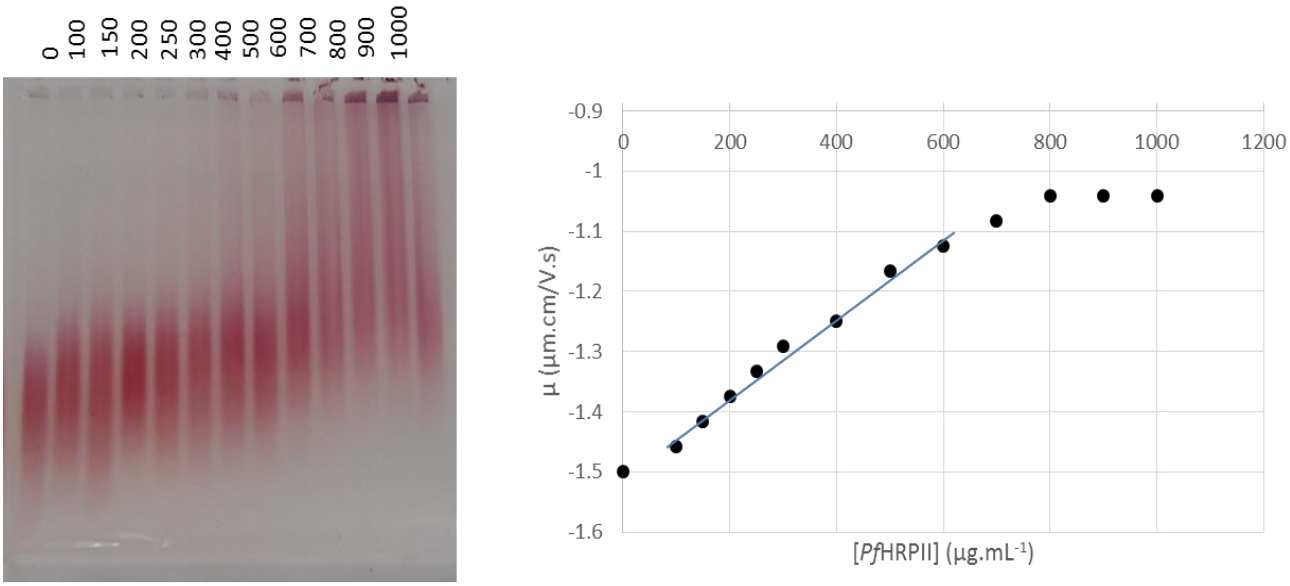

Figure 1 – **A**. Agarose gel electrophoresis of AuNP-CALNN-anti-HRPII-*Pf*HRPII, using cross-linking agents EDC/NHS. **B.** Electrophoretic mobility *vs. Pf*HRPII concentration.