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To whom it may concern,

We wish our manuscript, **“Iridium (III) Luminescent Probe for Detection of the Malarial Protein Biomarker Histidine Rich Protein-II ”** to be considered for publication in an upcoming issue of the *Journal of Visualized Experiments*.

While use of colorimetric and fluorescent probes has become a critical technique for rapid detection of biomarkers in well-equipped, first world laboratories, this need also extends to low-resource areas. Having a robust and sensitive reagent is one of the main necessities for low-resource diagnostics to succeed in the third-world disease management. In this submission, we demonstrate the synthesis and application of an emerging class of Ir(III) cyclometallated probe for detection of the malarial biomarker Histidine Rich Protein II (HRPII). Ir(ppy)2(H2O)2+ (**Ir1**) is a unique probe in that it is non-emissive in the solvento state. Only upon binding the imidazole side chains of histidine does this probe emit a long-lasting blue green signal. We demonstrated low nanomolar limits of detection of **Ir1** toward HRPII when bound to the surface of a magnetic microparticle. This ELISA-based assay takes only 1.5-2 hours, compared to 5+ hours for a traditional ELISA. By integrating the probe with particle-based separation techniques, we envision coupling this detection method with readily available diagnostic designs.

This is a highly visual technique in that simply using a black light excites the probe. With the naked eye, the user is able to visualize a concentration dependent blue-green luminescent response of this probe with histidine rich peptides. Additionally, the probe can be imaged on the surface of magnetic particles when the biomarker is bound to the particle surface. Keersten Davis synthesized the probe and conducted the in-solution and on-bead titration experiments, while Anna Bitting assisted with these experiments. Christine Markwalter and Westley Bauer assisted with the biolayer interferometry experiments. The manuscript was written by Keersten Davis, with assistance from Anna Bitting.

I would like to suggest Dr. Dik-Lung Ma (edmondma@hkbu.edu.hk) of Hong Kong Baptist University, Dr. C. Bor Fuh (cbfuh@ncnu.edu.tw) of National Chi Nan University, Dr. Blake Peterson (brpeters@ku.edu) of the University of Kansas, Dr. A. Prasanna da Silva (a.desilva@qub.ac.uk) of Queen’s University, Dr. Ola Soderberg (ola.soderberg@igp.uu.se) of Uppsala Universitet, and Dr. Ramon Martinez-Manez (rmaez@qim.upv.es) of the Univeridad Politencnica de Valencia as people well suited to review and edit this material.

Sincerely,

David Wright