Engineering Non-Immunoglobulin Binding Proteins for *In Vitro* Diagnostic Tests

by

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Technical Summary

In 2016, nearly 5.5 million deaths were attributed to infectious and parasitic diseases. Although many of these diseases are preventable and treatable, resource-constrained regions often lack access to rapid and accurate diagnostic tests to appropriately diagnose and treat these diseases. In order to improve the accessibility of diagnostics, the development of low-cost, simple, and rapid diagnostic tests is vital. Currently, antibodies are widely used as the binding reagents in diagnostic tests to detect a target biomarker from the patient sample. These tests are often designed as a sandwich assay, which requires a pair of antibodies as complementary capture and reporter reagents. However, antibodies have some limitations for use in *in vitro* applications, including variable stability from clone to clone, structural complexity, and long developmental timelines.

In this thesis, we investigated the reduced-charge Sso7d (rcSso7d) binding scaffold as an antibody replacement for use in diagnostic tests. The small, thermostable rcSso7d scaffold meets our design criteria with its intrinsic stability, inexpensive production in bacteria, and ease of genetic modification. In order to identify unique rcSso7d clones specific to the target biomarker, we use directed evolution techniques by screening through a yeast surface display library of 1.4×10^9 different rcSso7d variants. Through this process, we identified multiple high affinity clones against target biomarkers for Zika virus, malaria, inflammation and infection, and a foodborne pathogen. We also demonstrated flexibility of the *in vitro* surface-display selection process by incorporating certain selective pressures based on desired properties, e.g. complementary binding pairs, minimal off-target binding, or binding to a conserved epitope. In order to integrate the rcSso7d protein into diagnostic assays, we incorporated the scaffold into a reporter reagent format by including a fusion partner between the labeling tag and the rcSso7d scaffold. Furthermore, we demonstrated applicability and translatability of rcSso7d scaffold for use in multiple different diagnostic assay formats—including paper-based, bead-based, well plate ELISA-based, and agglutination assays—with minimal optimization required. Finally, we found that the rcSso7d scaffold had comparable functional performance to antibodies and retained full functionality when tested in 100% human serum. This work has shown that the rcSso7d binding scaffold is a promising alternative binding reagent for the development of robust, low-cost, rapid diagnostic tests that can help reduce the large global burden of infectious diseases.

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