Chapter 1

Introduction

1.1 PCC AGENT COCKTAIL FOR HIV-1 DIAGNOSTICS

Accurate diagnoses are critical for effective healthcare. Misdiagnoses carry large personal and societal costs, especially in cases where the disease is transmissible or carries a large social stigma. HIV is one such disease, and HIV misdiagnoses can lead to psychosocial difficulties and have dangerous public health implications [1]. According to the World Health Organization (WHO), roughly 40 million people were living with HIV as of the end of 2004, and some regions have adult prevalence as high as 15-39%. Many countries with high incidence of HIV are resource limited and rely on easy-to-use rapid detection tests for diagnosis; however, the performance of these tests is variable. A study of the WHO HIV two-test diagnostic algorithm, where two positive rapid detection tests are used alone for diagnosis, showed an unacceptably high rate of false-positive results (10.5%), likely due to non-selective cross reactivity [2].

HIV diagnoses are often made indirectly through the detection of anti-HIV antibodies [3]. The current gold standard detection reagents are biologically-produced, and are expensive, susceptible to thermal and biological degradation, and have batch to batch performance variability; all of which can adversely affect the accuracy of diagnostic assays [4,5,6]. Typically in HIV diagnostic assays, multiple immunogenic and conserved antigens from HIV are expressed as regions of a single chimeric protein. That chimeric protein is then used to capture specific antibodies from the body fluid (e.g. blood, saliva or urine) of potentially infected patients; a positive assay result implies infection. However, the antigen has been shown to have poor signal to noise [7],

possibly related to the off-target reactivity responsible for false negative results seen in rapid detection tests.

Robust detection reagents are needed that recognize relevant biomarkers with high sensitivity and selectivity, and that perform well in resource limited areas where controlled laboratory environments are not readily available. Protein Catalyzed Capture agents (PCC agents) are peptide-based detection agents that rival the affinity and selectivity of biological reagents, and that are chemically, physically, and biologically robust. As synthetic reagents, they are also highly modular and can be easily altered for incorporation into different assay platforms. Previous work has identified a number of PCC agents that detect multiple disease relevant biomarker proteins through the process of iterative *in situ* click screening [8,9,10], many of which were engineered to be specifically epitope targeted, and some of which have inhibiting or activating functionalities.

The application of PCC agent technology to anti-HIV antibody detection is not as straightforward as targeting a single biomarker; the polyclonal diversity of antibodies across patient populations can translate into large variations in assay performance from patient to patient. To selectively capture the diversity of anti-HIV antibodies while minimizing serological cross-reactivity, a cocktail of PCC agents was developed against multiple representative anti-HIV antibodies.

Chapter 2 reports on the use of iterative *in situ* click chemistry [8,11] to prepare a cocktail of chemically synthesized PCC agents that is designed to sample the polyclonal diversity of an antibody-based immune response. Three PCC agents, derived from the

same epitope on HIV-1 envelope protein gp41, were raised against two representative anti-HIV antibodies to create a cocktail of capture agents that sample the variations between the representative antibodies, thus increasing the reagent's potential to detect an anti-HIV immune response across multiple patients.

Chapter 3 then describes a PCC agent-based assay developed to detect human antibodies that bind a conserved region of the HIV-1 envelope glycoprotein gp41, using the PCC Agent cocktail described in Chapter 2. The performance is compared against the gold standard chimeric protein antigen using sera collected from a cohort of HIV-1-positive human subjects are tested, plus controls. The assay was also used to detect anti-HIV antibodies from a validation panel of HIV-1 specific IgG containing plasma samples that were rated HIGH, MED, LOW, or NEG in accordance with the titer of anti-HIV antibodies. Additionally, the thermal stability of the capture agent cocktail is reported, with an eye towards point-of-care HIV diagnostics assays that are needed in environments where refrigeration chains may not exist. Chapters 2 and 3 were taken in part from *PloS one* **2013**, *8*, e76224 (see Appendix D).

1.2 REFERENCES

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