AI/ML AND VIRTUAL HUMAN PLATFORMS FOR THREAT AGENT HAZARD ASSESSMENT AND MEDICAL COUNTERMEASURE DISCOVERY AND DRUG DEVELOPMENT

High Throughput Test Tools For Expedited And MI-assisted Enzyme Design

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Enzymes are powerful catalysts that enhance the rate of reactions by several orders of magnitude and perform function with utmost specificity and finesse. The biocatalytic power of enzymes can be utilized to build sensitive diagnostic, therapeutic and prophylactic agents against chemical threats providing on demand countermeasures to the warfighters. In this work, we show development of novel high throughput screening for the Paraoxon (PXN) hydrolyzing enzyme, such as PON1. A sensitive whole cell biosensor for the PXN degradation product, p-nitrophenol (PNP) was established by using a heterologous transcription factor scaffold (native inducer p-hydroxybenzoate) and optimizing the binding pocket to respond to PNP. The sensor allowed detection of PNP as a degradation product from PXN hydrolysis. A single round of rational design of PON1 enzyme and high throughput screening using PNP whole cell biosensor, that allowed quick evaluation of over 40,000 PON1 variants on barely four petri dishes, showed several variants with improved catalytic efficiency when compared to the starting PON1 sequence. Whole microbial cell consisting of genes for PXN hydrolyzing enzyme and for sensor-reporter can be applied for sensing PXN pesticide in the war zones. The improved enzymes with high homology to human proteins are promising candidates for therapeutic and prophylactic use against PXN poisoning. Further the technology enhances the speed of design-build-test, by relieving the bottleneck in the test step and by providing rich datasets for machine learning adapted for expedited and accurate design of enzymes.

Financial support was provided by the Defense Threat Reduction Agency (CBCALL12-LS6-1-0622). The author would like to thank Dr. Charlie Strauss for co-leading the research. The author thank Late Prof. Dan Tawfik (Weizmann Institute of Science) for the kind gift of Pon1 gene.