Engineered proteins are very versatile tools that have been applied in assay development for various purposes. They have been made into genetically encoded biosensors/probes or affinity agents for biomarker detection.

This thesis explored a few topics using assays developed with engineered proteins. The genetically encoded hydrogen peroxide generator, D-amino acid oxidase (DAAO) was used to understand the hours-long intracellular hydrogen peroxide (H_2O_2) generation. This study elucidated that the primary respondent of cytosolic H_2O_2 is peroxiredoxin 1 and the H_2O_2 induced apoptosis initiates before the collapse of Prx/Trx/TR antioxidant network. Then, a genetically encoded FRET sensor was used to design a high-throughput screening assay that identified three small-molecule drugs from over 600 compounds that can mediate toxicity through H_2O_2.

This thesis also explored the applications of engineered proteins in diagnostic assay development. I engineered binders against various targets for gram-positive and gram-negative pathogenic bacteria, and two of them that have been tested and showed binding to *Salmonella* whole cells. The engineered binders were also used to develop a SARS-CoV-2 rapid tests. In this project, sikes lab members developed a paper-based assay to detect the SARS-CoV-2 nucleocapsid protein as a team and successfully validated the assay with patient samples. Subsequently, I improved the thermo-stability of the reporter binder protein used in the assay by switching the fusion partner of the binder to a thermally stable protein. I also identified the bottleneck of an epigentotyping assay development and provided insight for future direction.

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