Towards Directed & Streamlined Rapid Diagnostics Engineering

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Our grueling encounter with the COVID-19 pandemic has helped to accelerate various incubating biotechnology innovations, paving the way for their widescale adoption. However, it has also simultaneously revealed the shortcomings in both timeliness and quality of our healthcare response. Rapid diagnostic tests (RDTs) are one such technology that is both a beneficiary and victim – while its benefit for fast diagnosis and isolation of infectious individuals is undeniable, it has been frequently associated with reduced sensitivity. Furthermore, despite being already well-established, it was still outpaced by the virus in the early months of the pandemic. Together, these highlight that while RDTs have immense potential, there is a disjoint between its development and the needs of healthcare. To better prepare ourselves for the next healthcare challenge, there must be a more efficient and targeted approach to RDT engineering.

In response, this thesis seeks to enable a generalizable, yet directed approach to RDT development. Recognizing the gap between assay and binding reagent development, this thesis holistically evaluates the needs dictated by RDTs and develops methods to enforce them both in assay optimization and upstream protein engineering. Embracing the design-focused philosophy of engineering, rational theoretical models lie at the heart of these methods, unifying the disconnected fields of assay development and binder development.

To provide structure for targeted optimization, physically rational models for two relevant RDTs, electrochemical and paper-based assays were developed and validated. Workflows that incorporate predictions of relevant performance metrics, such as limit-of-detection, sensitivity and signal-to-noise ratios for facile optimization were proposed to holistically evaluate and fast-track RDT performance. These RDT models subsequently revealed inadequacies in the presently generic processes for binder selection, motivating the selection criteria more suited for RDTs in upstream protein engineering. Specifically, they find an unmet need for faster binders, as well as a means to engineer them, that can attain higher sensitivities within the short assay times typical of RDTs. To address this, this thesis integrates the use of microfluidics into the binder engineering process to enact temporally precise kinetic screening for fast binders. A unique approach in binder selection, biophysical models were introduced to theoretically predict binder selection performance and rigorously validated. Model findings were then applied to optimize the novel kinetic selection scheme, paving the way for its implementation. Finally, the proposed kinetic screen was successfully demonstrated to preferentially enrich fast binders and proved to be applicable across multiple antigen targets. The work presented here lays important and promising groundwork in facilitating *fit-for-purpose* binder engineering and acceleration of RDT development. Ultimately, these advancements strive to enhance performance and sensitivities of RDTs and empower them to attain their untapped potential in healthcare.

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