

## **NON-TECHNICAL ABSTRACT:**

The emergence of HIV-resistant strains together with the terrible side effects of existing drugs highlights the need for novel therapeutic drugs as well as innovative methods to facilitate their discovery. FDA-approved drugs include drugs against Protease (PR), a viral protein absolutely required for infection. Resistance to existing FDA approved drugs is becoming a world-wide concern. Here, we intend to search for novel inhibitors of clinically prevalent HIV-1 PR mutants, which are resistant to FDA approved drugs. In order to facilitate their discovery, we have developed a technique in mammalian cells to distinguish between active or inactive PR. In the assay, the cells become green when PR is inactive or inhibited. The assay was originally developed with PR from a wild-type virus, which is sensitive to FDA approved PIs. The goals of this proposal are to: a) adapt the assay to resistant PRs, and b) engineer the assay in a way that allows analysis of several mutant PRs in the same sample (referred to as multiplexing). This will be done by mixing fluorescent cells of different colors (blue, red, and blue and red), each of them also becoming green fluorescent when their own PR is inhibited. This process will drastically enhance the power of the assay for drug discovery in a very efficient and fast manner. As such, it will provide the basis for future NIH funding, to screen for novel PR inhibitors.