Developing a Toolkit for Conditional Targeted Protein Degradation with Protease and Sortase A Responsiveness for Cellular Reprogramming

Hopen Yang
Advisor: Dr. Wilfred Chen
Committee Members: Dr. April Kloxin, Dr. Karl Schmitz, Dr. Millicent Sullivan

Friday, February 16th, 2024 at 3:00 PM (EST)
CLB 366 | https://udel.zoom.us/j/93456765286 | Password: TPD

Small molecule inhibitors have been a mainstay of pharmaceutical products since the inception of the industry. Breakout examples include penicillin, aspirin, and naproxen sodium. However, the successful development of small molecule programs hinges on targets having well-defined binding pockets. In contrast, over 70% of cellular proteins are structurally intractable: "undruggable".

Targeted protein degradation (TPD) offers a promising avenue for developing new modalities. TPD platforms deputize native ubiquitin protease system (UPS) machinery to mediate proteasomal degradation against a protein of interest (POI). Importantly, TPD approaches do not require a well-defined binding pocket; it is no longer necessary to *block* a protein's function when you are simply *removing* it.

The pioneering approach to TPD is the small molecule proteolysis-targeting chimeric molecule (PROTAC). These have demonstrated tremendous success with *in vivo* target knockouts but continue to be plagued by other inherent shortcomings of ligand-based therapeutics: poor specificity, low potency, extended discovery timeframes.

Recently, biological analogs to PROTACs have taken center-stage as potential therapies or tools for program discovery. These exhibit remarkable specificity and full target ablation. Moreover, modular construction enables rapid target acquisition when interrogating new modalities. However, a lack of spatial-temporal control over point-and-shoot TPD hinders application to diseases where a restorative effect is desired rather than full depletion of the target.

To address this limitation, we have engineered **L**ogic-gated **A**dPROM deploying **S**rtA-mediated **E**lement **R**ecombination (LASER): a novel select-fire bioPROTAC system that can toggle between multiple intracellular protein targets, using Boolean logic and an integrated safety.

We first used Tobacco Etch Virus (TEV) protease-mediated signal transduction to demonstrate conditional TPD via ligand induction. Moreover, we developed a palette of photoactivatable proteases to elicit optogenetically targeted protein degradation. We then wished to integrate expanded functionality for modulating conditional TPD at its basic level. As well, we sought to evince switchable targeting intracellularly and logic-gated input responsiveness. Sortase A, a transpeptidase that can both cleave and ligate proteins, was selected as the control mechanism.

After evaluating the feasibility of Ca^{2+}-independent hepta-mutant Sortase A ($SrtA_{7+}$) for directing turn-OFF and turn-ON control via cleavage and ligation, we leveraged the full functionality of SrtA to elicit switchable TPD between orthogonal POIs. Building upon this foundation, we then integrated the SrtA and TEV protease mechanisms, enhancing our toolkit's versatility by introducing NOR- and AND-gate input responsiveness. Finally, by combining the AND-gate and target-toggle, we enabled selectable targeting with a safety switch. These cumulative advancements constitute LASER: a modular programmable targeted protein degradation toolkit, for applications in interrogating protein function and facilitating the discovery of therapeutic targets.

Expanding the scope of Sortase A bioconjugation as a control mechanism for intracellular reprogramming, we developed a split Cre-*loxP* gene expression system that efficiently directed SrtA-induced transgene expression. Validation of this system was conducted through both a firefly luciferase reporter, and an mCherry fluorescent reporter. Notably, split Cre_{SrtA} demonstrates minimal background reconstitution and activity compared to existing conditional Cre systems. Lastly, we applied SrtA-induction to direct split flavoprotein reconstitution, illustrating that a mitochondrially-localized split SOPP3 could induce apoptosis under blue light illumination only after SrtA ligation.

These advancements establish the promising applications of LASER as a platform for eliciting well-regulated TPD, and $SrtA_{7+}$ as a powerful control mechanism for intracellular synthetic biology tools.