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The Production and Purification of Split-β-Lactamase Fusion Constructs

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Targeted drug delivery enables the localization of an increased concentration of drugs to a disease cell population. Importantly, directed delivery lowers the levels of drugs in off-target sites and can minimize toxicity. This is advantageous when it comes to treatments for diseases like cancer as the side effects for patients undergoing these treatments can be severe. While clinical success has been achieved with several platforms, premature release of drug before reaching the target site continues to be a major challenge in the development of new targeted therapeutics.

Through an approach called Target Engaged Complementation (TEC), the Owen lab proposes that an enzyme can be split into two inactive components, fused to antibody fragments, and separately delivered to a tumor cell (Figure 1). Once in proximity, the enzyme will refold and only then can activate a prodrug to its toxic form. Critically, in this strategy the enzyme fragments remain inactive in circulation, which should eliminate systemic toxicity.

The goal of this project is to design and produce split-β-lactamase fusion proteins and evaluate their binding parameters and activities. If successful, this project could unlock the potential of targeted drug delivery systems and provide a baseline for building new platforms to treat a variety of diseases.

The split-β-lactamase fusion proteins (βN-9.29 and G3-βC) were expressed in genetically engineered SHuffle T7 *E. coli*. These proteins were then purified using Immobilized Metal Affinity Chromatography (IMAC). Upon purification, the functionality of the proteins was evaluated using chromogenic substrates CENTA and nitrocefin which allow for fast β-lactamase characterization. βN-9.29 and G3-βC were put in solution with either CENTA or nitrocefin, with complete substrate turnover occurring within 20 minutes.

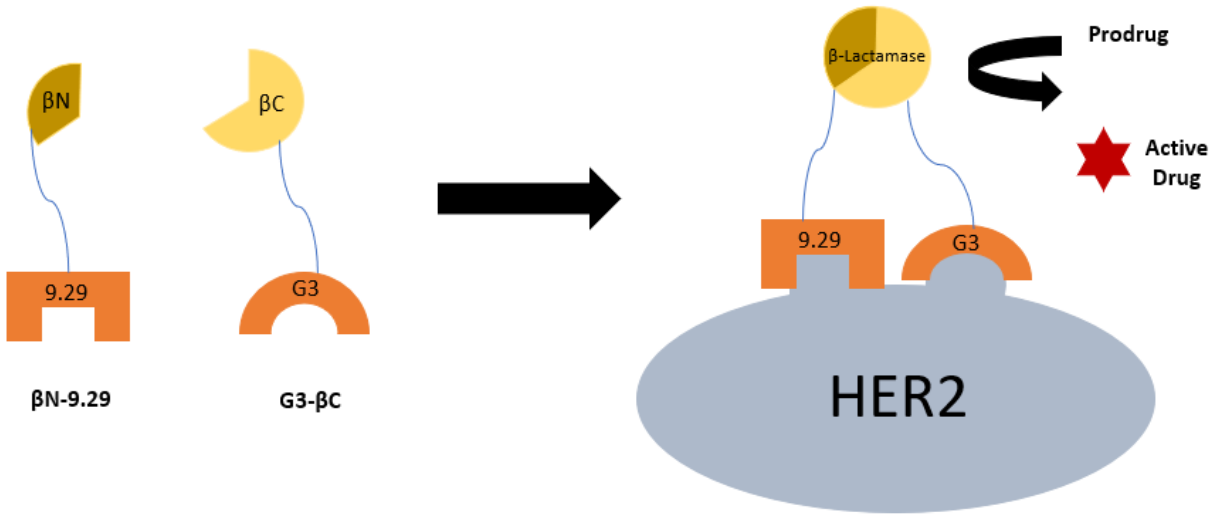

Figure 1. The mechanism of action for Target Engaged Complementation (TEC).