BACTACS: A POTENTIAL STRATEGY FOR THE SELECTIVE DEGRADATION OF PROTEINS WITHIN PROKARYOTES

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Antibiotic resistance is one of the greatest threats to global health that we face in the 21st century. In order to overcome this threat, it is crucial that we find new ways to engage target proteins. It is estimated that only 30% of the bacterial genome is pharmaceutically accessible using current occupancy based drug strategies. Proteins lacking active sites such as non-enzymatic proteins, scaffolding proteins and transcription factors have remained undruggable using such strategies, thus being able to access them has great therapeutic potential.

A current strategy for accessing such "undruggable" proteins in eukaryotes involves using small bifunctional molecules (known as Protacs) to simultaneously recruit a protein of interest (POI) and recruit the natural degradation machinery of the cell. This leads to the degradation of the POI. We aim to use a simlilar strategy within prokaryotes, in which we will use small bifunctional molecules to recruit degradation mechanisms exclusive to bacterial cells and use them to degrade a protein of interest. This will potentially open the door to a vast new range of antibiotics and targets as well as allow for selective interactions with bacterial cells over human cells.

Here we show bifunctional molecules (termed *bac*terially *tar*geted *c*himeras – Bactacs) that were designed to induce the degradation of a HaloTag-RFP (Red Fluorescent Protein) fusion protein within *E. coli*, utilising various bacterial degradation machineries. (*Figure 1*)

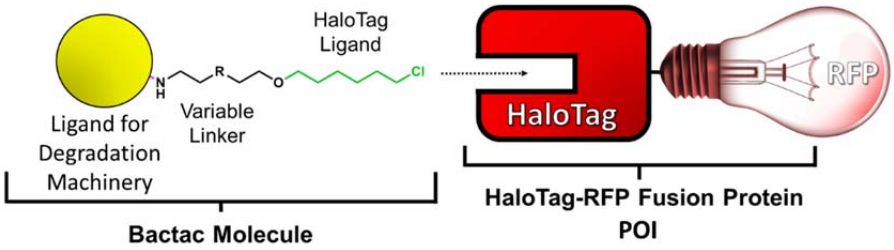

Figure 1: Bactac molecule and HRFP Protein

When cell lysate containing the HaloTag-RFP fusion protein is treated with the Bactac molecules, we observe a clear dose-responsive decrease in fluorescence over time, which is consistent with the degradation of the fusion protein. Current efforts are underway to elucidate the cause of the decrease in fluorescence; for instance, microscale thermophoresis is being used to evaluate the binding of the small molecule Bactacs to the degradation machineries and western blots are being used to determine if there is degradation of the fusion protein.