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SPLIT-LUCIFERASE TAG WITH MULTIFACETED UTILITY IN THE PRODUCTION AND INTERROGATION OF PURIFIED PROTEINS

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Producing, isolating, and characterizing proteins is central to many downstream biological research applications. Affinity tags are commonly used to mediate the processes of detection and isolation of a protein of interest from a complex biological milieu. We are developing a novel affinity tag that can serve a variety of roles in the processes of expression, purification, and downstream experiments. This method is based on a split-luciferase and offers a unique value proposition as a multipurpose tool in the preparation and interrogation of proteins. Our affinity tag is an 11-amino acid sequence (β10) that comprises the smaller component of the split-luciferase. When the larger component (11S) of the enzyme is present, the small and large pieces together form a fully-active luciferase that is capable of generating bioluminescence.

In this proof of concept study, our aim is to show the breadth of applications that can be addressed by this affinity tag. Moreover, we seek to demonstrate the applicability of this tag by evaluating its utility when fused to various proteins. Our initial objective is to demonstrate the tag's utility in monitoring protein expression, affinity chromatography, and downstream assays. Whereas other affinity tags may require combination with separate tags in order to facilitate the breadth of a researcher's objectives, our tag is designed as a standalone tool that does not necessitate such a combination.

Figure 1:

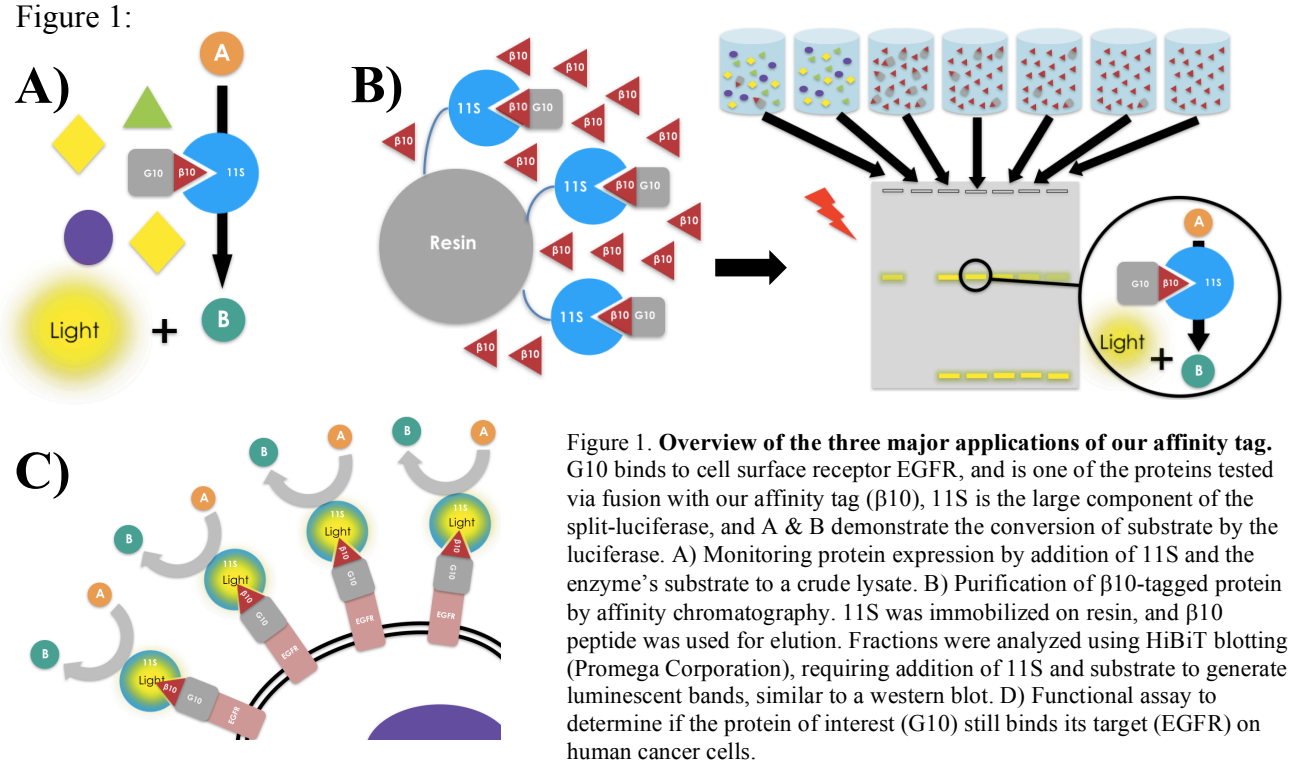

Figure 1. **Overview of the three major applications of our affinity tag.** G10 binds to cell surface receptor EGFR, and is one of the proteins tested via fusion with our affinity tag (β10), 11S is the large component of the split-luciferase, and A & B demonstrate the conversion of substrate by the luciferase. A) Monitoring protein expression by addition of 11S and the enzyme's substrate to a crude lysate. B) Purification of β10-tagged protein by affinity chromatography. 11S was immobilized on resin, and β10 peptide was used for elution. Fractions were analyzed using HiBiT blotting (Promega Corporation), requiring addition of 11S and substrate to generate luminescent bands, similar to a western blot. D) Functional assay to determine if the protein of interest (G10) still binds its target (EGFR) on human cancer cells.