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Biosensor Development Using CRISPR to Quantify Endogenous Protein Expression Modulated by PROTACs

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An assay platform that robustly and sensitively quantifies the kinetics of endogenous protein turnover is crucial for discovery of disease-relevant therapeutic agents. This need is particularly relevant for a new class of therapeutics known as PROTACs, chimeric molecules that target specific disease-relevant proteins for degradation by the cellular ubiquitin-proteasome system. Using advanced CRISPR technology and our well-established Enzyme Fragment Complementation (EFC) system, we introduced a small β-galactosidase fragment (ED) into the BRD4 locus in a physiologically relevant blood cancer cell line. The homogeneous format and high sensitivity of the EFC assay allows direct and rapid quantitation of drug-induced changes in endogenous BRD4-ED protein levels, making this assay suitable for medium to high throughput screens. We tested a panel of PROTACs targeting BRD4 in this system and observed differential kinetics for BRD4-ePL degradation with individual PROTACs that were consistent with previous reports using other physiological endpoints, such as proliferation. This suggests that discovery of new molecular entities that modulate BRD4 protein levels should be feasible using this assay format. We are currently expanding our biosensor platform to additional protein targets and disease cell models where sensitive detection of endogenous protein modulation will be critical.