Jamie Ware, Discovery Biology, Discovery Sciences, R&D, AstraZeneca, Cambridge, UK

Title

Development of a high-throughput quantitative biochemical ubiquitination assay platform to support E3 ligase recruitment and modulation drug discovery programmes

Abstract

E3 ligases are of great interest across drug discovery as both a target for inhibition and also through their recruitment via Proteolysis-targeting chimeras (PROTACS). E3 ligase X is highly expressed in murine and human CD4+ and CD8+ T cells responsible for the negative regulation of T cell activation, and as such it is an attractive immuno-oncology target. Herein, we describe the development and application of a high-throughput homogeneous biochemical ubiquitination assay to monitor substrate specific E3 ligase activity. Using Förster resonance energy transfer (FRET) technology we are able to monitor both the auto-ubiquitination of E3 ligase X and the covalent transfer of fluorescently labelled ubiquitin onto specific substrates labelled with a cognate FRET donor. E3 ligase inhibitors can be profiled in this functional assay platform to determine IC_{50} potencies, thereby linking direct binding affinities of E3 ligase X engagers with phenotypic cell-based data. Furthermore, we have demonstrated the capability of this platform to characterise E3 ligase recruiters (e.g. PROTACs) and determine catalytic parameters for ubiquitin transfer from active ternary complexes. These critical kinetic parameters support modelling and key decision making in the lead generation of PROTACs in development at AstraZeneca.