A Novel Real-Time Cell Target Engagement Platform for Challenging Drug Targets

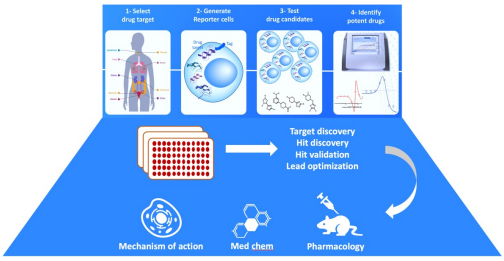
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Abstract

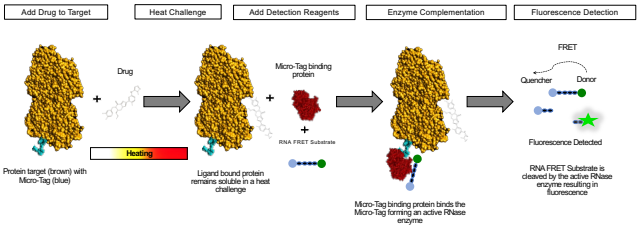
Success of drug discovery programs can be amplified by adopting emerging technologies that can interrogate drug targets within their physiological context. Cell target engagement (CTE) presents a combined biological & biophysical method, which provides quantitative evaluation of target-drug interaction within the native environment of the cell. The currently available luminescence-based CTE methods are limited to measurement of end-point signal and poor resolution, thereby limiting their mechanistic and stoichiometric insight. We have developed MICRO-TAG, a novel fluorescence-based CTE platform, which relies on complementation of split-RNAse A. It enables interrogation of target engagement without interfering with folding, localization and function of target proteins - all within the physiological milieu of the cell. Uniquely, this new platform allows for quantitation and monitoring of CTE in real- time in the cell. It enables extraction of important parameters from target-drug interaction: target thermal profile and temperature of aggregation (T_{agg}), time required to reach equilibrium (t_{eq}), apparent association rate (K_{on}) and physiologically relevant drug:target binding affinity (K_D). We provide data demonstrating potential and utility of MICRO-TAG platform on drug targets, such as KRas, MTH1, EGFR, and UBE2N. The platform demonstrates relevance for discovery of novel drug candidates targeting challenging drug targets such as transcription factors. This highly scalable and quantitative novel method for assessing drug-target interaction within cells can enhance drug discovery programs and offers potential to rapidly identify clinically relevant hit compounds for challenging drug targets.

Enabling Drug Discovery Workflow

MICRO-TAG cell target engagement platform enables drug discovery in the physiological environment of the cell. Our platform unifies the fragmented drug discovery process and de-risks early biology.



MICRO-TAG Cell Target Engagement System



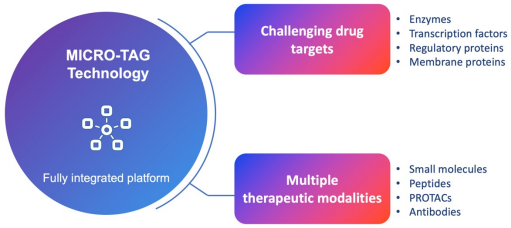
Real-Time Cell Target Engagement

MICRO-TAG Step Gradient Cell Target Engagement system utilizes thermal melting profile of target protein and monitors it in real time.

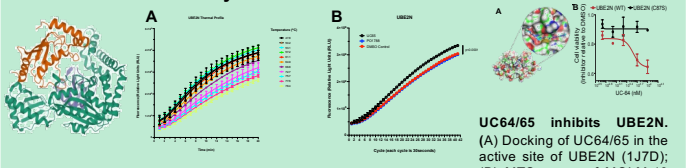


Interrogating Challenging Drug Targets

Drug discovery for challenging targets requires the intact cell biology and physiological context. Currently, >85% of human proteome is deemed undruggable with conventional tools. Our MICRO-TAG technology enables interrogation of challenging targets in the cell and discovery of novel drug candidates. Real-time cell target engagement offers a deeper insight into target behavior and abundance.



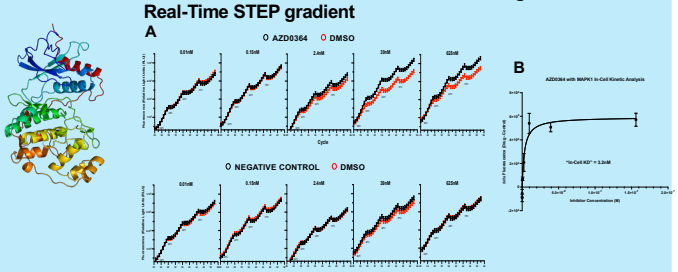
UBE2N — Discovery of UBE2N inhibitor UC64/65



Micro-Tag system assembled for UBE2N. (A) UBE2N thermal melting profile identifies Tagg(50) of 69C; (B) UC64/65 but not a structurally similar compound (PO1788) stabilizes UBE2N.

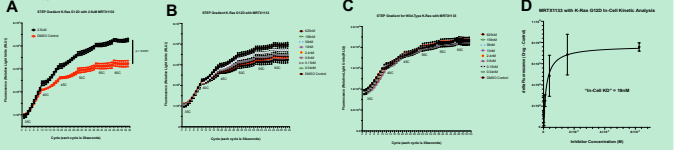
UC64/65 inhibits UBE2N. (A) Docking of UC64/65 in the active site of UBE2N (1J7D); (B) MTS assay of MOLM-13 cells transduced with UBE2N (WT) or UBE2N (C87S).

MAPK1 — Validation of MAPK1 inhibitor AZD0364 using MICRO-TAG Real-Time STEP gradient



Real-time STEP gradient results for testing ligand binding to MAPK1 Micro-Tag target.
(A) Real-time data testing AZD0364 compound and a negative control compound in real-time by STEP gradient.
(B) "In-Cell KD" determined from the real-time binding data for AZD0364 with MAPK1.

KRAS — Validation of KRAS inhibitor MRTX1133 using MICRO-TAG Real-Time STEP gradient



Real-Time Cell Target Engagement for K-Ras with MRTX1133.
(A) STEP Gradient using Micro-Tag K-Ras G12D mutant with Mirati Therapeutics compound MRTX1133 at 2.5uM.
(B) STEP gradient performed with K-Ras G12D at multiple doses of inhibitor MRTX1133.
(C) STEP gradient performed with wild-type K-Ras with multiple doses of inhibitor MRTX1133.
(D) "In-Cell KD" determination for MRTX1133 with the G12D mutant K-Ras Micro-Tag.

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Micro-Tag Platform