Enabling *Cellular DEL* Discovery with MICRO-TAG® Cell Target Engagement

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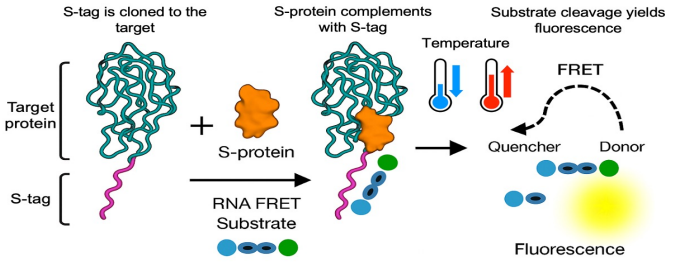
Abstract

Discovery of small-molecule modulators for intracellular protein complexes is constrained by biochemical and domain-based screening methods that lack cellular relevance and scalability. To address this gap, we developed a cell-first discovery platform integrating Cellular DNA-Encoded Library (**Cellular DEL**) screening with **MICRO-TAG®** real-time cellular target engagement (CTE). Parallel Cellular DEL selections are performed under controlled thermal stress to enrich for compounds exhibiting persistent intracellular engagement, rather than transient binding. DEL selection is monitored using **MICRO-TAG®** real-time system, and hits are called through sequencing and data analysis. Hits are subsequently prioritized using real-time MICRO-TAG® CTE, which provides quantitative and kinetic measurements of target engagement in cells without reliance on purified recombinant proteins.

Application of this integrated platform to the chromatin remodeler CHD4 enabled the discovery of **CH41**, a covalent inhibitor demonstrating durable cellular engagement of CHD4. Real-time CTE profiling facilitated rapid discrimination between reversible and persistent binders, streamlining probe triage prior to downstream characterization. The Cellular DEL–MICRO-TAG® CTE workflow is scalable, automatable and broadly applicable to other targets. This platform provides a generalizable framework for discovering novel therapeutic candidates for **challenging drug targets,** such as transcriptions factors and GPCRs.

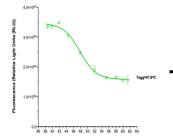
MICRO-TAG® Enzyme Complementation

The MICRO-TAG® platform is built on a novel fluorescence-based cell target engagement system. It enables interrogation of drug targets without interfering with folding, localization and function - all within the physiological milieu of the cell.



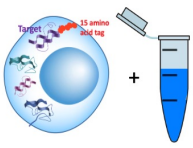
MICRO-TAG® Cellular DEL Workflow

1. Thermal Profile



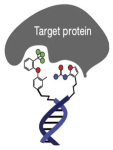
Identify thermal melting profile of target: Tagg-0-25-50-75

2. Cellular DEL



MICRO-TAG® reporter cells + DEL

3. Parallel DEL Selection



Thermal pressure to perturb target

4. Real-time Monitoring



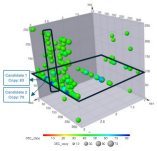
Monitor target thermal stabilization profile in real time with DEL

5. Sequencing & Analysis



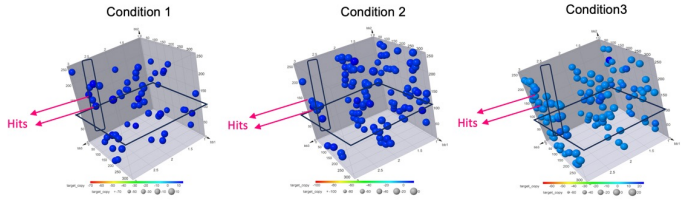
Sequencing analysis with sufficient depth and controls

6. Data Analysis



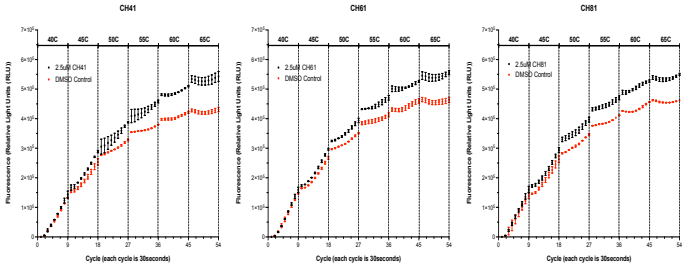
Subtractive analysis to identify selective, ir/reversible binders

Cellular DEL Enriches Selectively Target Binders



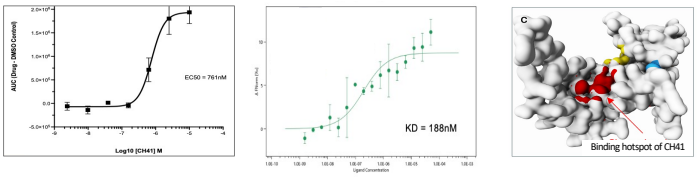
Parallel cellular DEL selection has identified a set of ligands that engaged the target at low temperatures (Tagg-25) and selectively enriched at higher temperatures (Tagg-50 and T-agg75), suggesting a profile of a covalent allosteric binder.

Real-Time Interrogation of Selected Candidates using MICRO-TAG®



Candidate CH41 and closely related analogs (CH61 and CH81) were tested at a concentration of 2.5uM for cell target engagement using real-time MICRO-TAG® technology employing real-time monitoring of binding with programmed steps as previously described (see below). All three compounds demonstrated direct binding to the CHD4 target. The binding was detected as a stabilization of the CHD4 at temperatures above 50°C, consistent with the temperature of aggregation (Tagg) of 50°C. The data demonstrates CH41 and related analogs and able to interact directly with the target CHD4.

CH41 – Novel Inhibitor of CHD4



We have identified CH41 - a novel stabilizing inhibitor of CHD4. MICRO-TAG® Real-time cell target engagement was instrumental in discovery of CH41. CH41 engages with CHD4 with EC50 of 761 in MICRO-TAG® cell target engagement system. CH41 has KD/affinity of 188nM to purified CHD4 protein domain. CH41 maps to a druggable hotspot of CHD4.

References

Ivan Babic, ... Daniel Starczynowski, **Elmar Nurmemmedov**. MICRO-TAG enzyme complementation enables quantification of cellular drug-target engagement in temperature series. 2026 Feb:38:100291. PMID: 41285198.

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