

Multiplexed chemoproteomic profiling as a tool to decipher the intracellular interactions between proteins and small molecules

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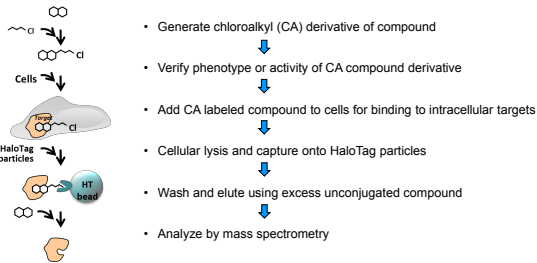
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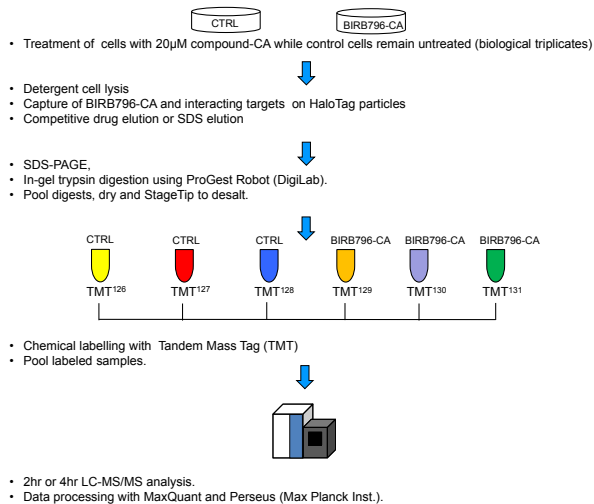
1. Introduction

Chemoproteomic technologies enable the quantitative and qualitative profiling of small molecule protein interactions. Here we report a new approach utilizing a chloroalkane (CA) capture tag which can be chemically attached to small molecules to enable the isolation of their respective protein targets through selective capture onto an immobilized HaloTag protein. In general, derivatization of small molecules with the CA tag has minimal impact on their cell permeability and potency. The retention of cell permeability allows validation of the pharmacological activity of the modified compound as well as target engagement in living cells. We have combined our chemoproteomics workflow with multiplexed chemical labeling methods to enable the analysis of dose dependent target engagement studies in a single LC-MS/MS experiment.

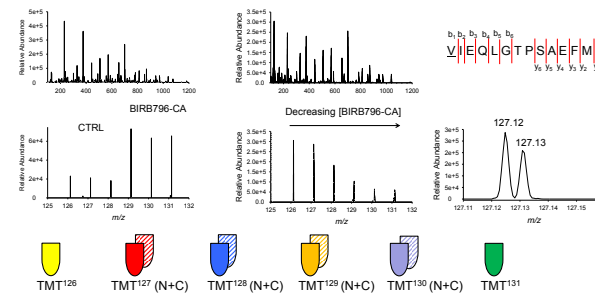
2. Technology Overview



3. Analytical Strategy

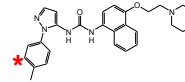


4. Protein Quantitation

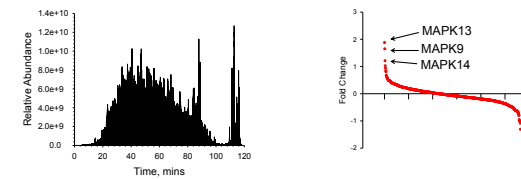


5.0 BIRB Case Study

- We tested our multiplexed chemoproteomics workflow using the interaction of MAP kinases (MAPK) with BIRB796, an allosteric kinase inhibitor.
- A target discovery experiment was simulated by treating HepG2 cells with 20µM BIRB796-CA. A parallel control experiment was carried out using BIRB-796.
- As a means of validating putative target proteins we titrated BIRB0796-CA with BIRB-796 in HEK293 cells to generate a 6 point curve.
- Data from these experiments are presented in the following preceding panels.

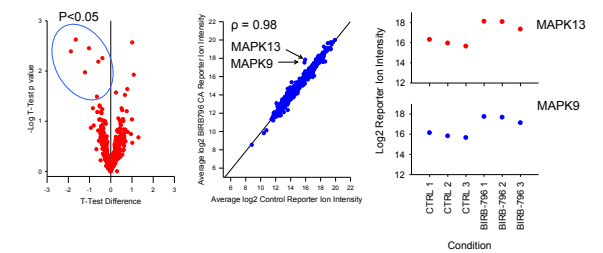


5.1 Data Acquisition



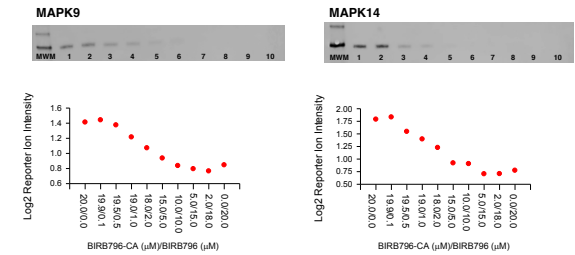
- A typical 2hr LC-MS/MS chromatogram from a TMT labeled target enriched sample is shown here.
- Data were analyzed using MaxQuant Version (1.5.0.25) and Perseus (Version 1.5.0.5) from the Max Planck Inst.
 - MaxQuant performs the following tasks:
 - Recalibration of MS data
 - Protein/peptide identification using the Andromeda database search engine
 - Filtering of database search results at the 1% protein and peptide False discovery rate (FDR)
 - Calculation of reporter ion intensity values
- Fold change data for 565 proteins identified with a minimum of three reporter ion intensity values in at least the control or test group is presented.

5.2 Target Identification



- T-Test analysis of multiplex reporter ion data enables a simple way to visualize putative BIRB796-CA interacting proteins.
- Average log₂ reporter ion intensity of the control and BIRB796-CA groups were used to assess linearity.
- Log₂ reporter ion intensity values are extracted and plotted to enable visualization of measurement precision of putative targets.

5.3 Target Validation



- The relative amounts of BIRB796-CA and BIRB796 were titrated and the capture of putative targets monitored.
- A clear relationship can be observed between the target abundance and the concentration of the BIRB796-CA.
- Western blot data provides an orthogonal measurement of target abundance/BIRB796-CA relationship.

6. Summary

- We have demonstrated target capture from living cells using an affinity tagged small molecule.
- We have shown our target enrichment workflow is compatible with chemical labeling quantitative proteomics.
- Using the BIRB796 model system we reaffirmed previous observations on the target specificity of this molecule.
- Varying the concentration of the capture molecule showed target engagement was drug dependent.

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