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

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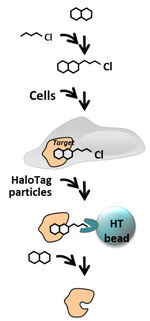
1. Introduction

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.

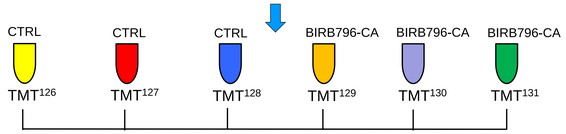
2. Technology Overview



- Generate chloroalkyl (CA) derivative of compound
- Verify phenotype or activity of CA compound derivative
- Add CA labeled compound to cells for binding to intracellular targets
- Cellular lysis and capture onto HaloTag particles
- Wash and elute using excess unconjugated compound
- Analyze by mass spectrometry

3. Analytical Strategy

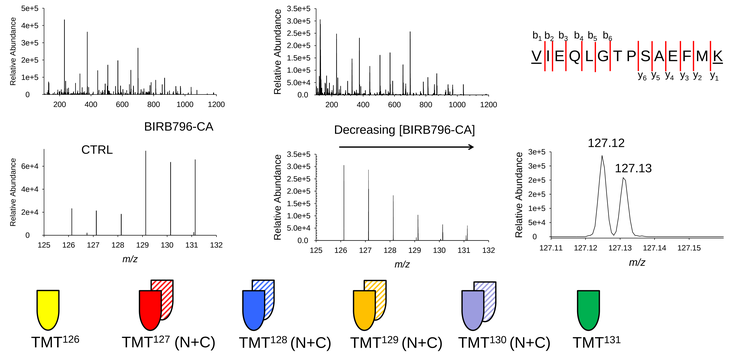
- Treatment of cells with 20µM compound-CA while control cells remain untreated (biological triplicates)
- Detergent cell lysis
- Capture of BIRB796-CA and interacting targets on HaloTag particles
- Competitive drug elution or SDS elution
- SDS-PAGE,
- In-gel trypsin digestion using ProGest Robot (DigiLab).
- Pool digests, dry and StageTip to desalt.



- Chemical labelling with Tandem Mass Tag (TMT)
- Pool labeled samples.

- 2hr or 4hr LC-MS/MS analysis.
- Data processing with MaxQuant and Perseus (Max Planck Inst.).

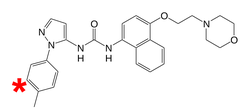
4. Protein Quantitation



- Representative target protein peptide fragmentation spectra of a MAPK9 peptide.
- The reporter ions can be used to measure the abundance of samples in the multiplex.

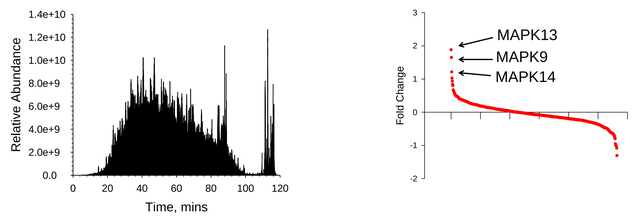
5.0 BIRB Case Study

- 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 proceeding panels.



BIRB-796 with the modified site indicated with a red asterisks.

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.

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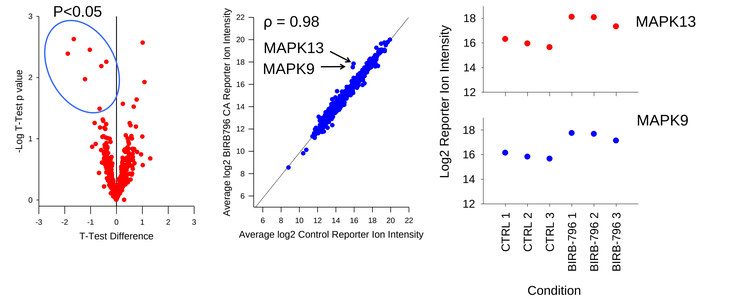
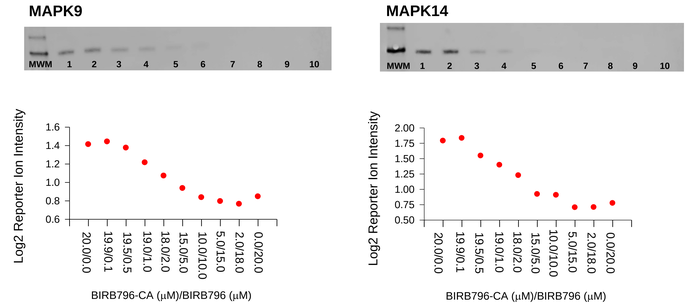
5.2 Target Identification



- 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 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.