Chemical Proteomic Analysis of BTK and PI3K Using Chloroalkane-Derivatized Small Molecule Inhibitors

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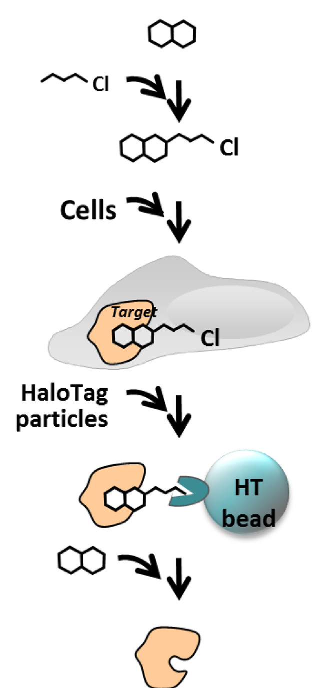
1. Introduction

Chemical proteomics is a rapidly evolving technology that allows for drug target identification for small molecule inhibitors. Here we present an approach utilizing a chloroalkane (CA) moiety capture handle to pull down protein binding partners using the HaloTag technology. CA-modified compounds are cell permeable and have minimal impact on potency, allowing for phenotypic assays of the derivatized compound to be recapitulated.

Here we present a study looking at the targets of two compounds - a Brutons Tyrosine Kinase (BTK) small molecule inhibitor, G02599124, and a Phospho-Inositol 3 Kinase (PI3K) small molecule inhibitor, Taselisib. BTK is a non-receptor tyrosine kinase expressed in most hematopoietic cells except T cells, it is essential for B cell receptor (BCR) signaling in B cells and as such is the therapeutic focus in many autoimmune diseases, including rheumatoid arthritis (RA) and lupus. Upon binding to growth factor receptor, PI3K is activated which initiates a signaling cascade involving AKT and mTOR that promotes cell cycle progression and cell proliferation. Mutations of the catalytic subunit of PI3KA are observed in several types of cancers. Inhibition of PI3K is therefore an important therapeutic focus.

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. Experimental Methods

CTRL | PI3K-CA

- Treatment of T cells (Jurkat) and B cells (Ramos) with 20μM G02599124-CA for 2.5h.
- Control cells treated with DMSO.
- Biological triplicates.

- Detergent cell lysis.
- Capture of G02599124-CA and interacting targets on HaloTag particles for 30min.
- Competitive drug elution with 400μM parental compound.

- SDS-PAGE, excise into 20 segments per lane.
- In-gel trypsin digestion.
- 20 x 1h nano LC/MS/MS Orbitrap Velos Pro per sample.

- Database searching using Mascot.
- Data compilation using Scaffold at 1% protein and peptide FDR..
- Label-free quantitation using Normalized Spectral Abundance Factors (NSAF).

- Treatment of HCC-1954 cells with 20μM Taselisib-CA for 1h.
- Control cells treated with DMSO.
- Biological triplicates.

- Detergent cell lysis.
- Capture of Taselisib-CA and interacting targets on HaloTag particles for 30min.
- Competitive drug elution with 400μM parental compound.

- SDS-PAGE, excise into 20 segments per lane.
- In-gel trypsin digestion.
- 20 x 1h nano LC/MS/MS Orbitrap Velos Pro per sample.

- Database searching using Mascot.
- Data compilation using Scaffold at 1% protein and peptide FDR..
- Label-free quantitation using Normalized Spectral Abundance Factors (NSAF).

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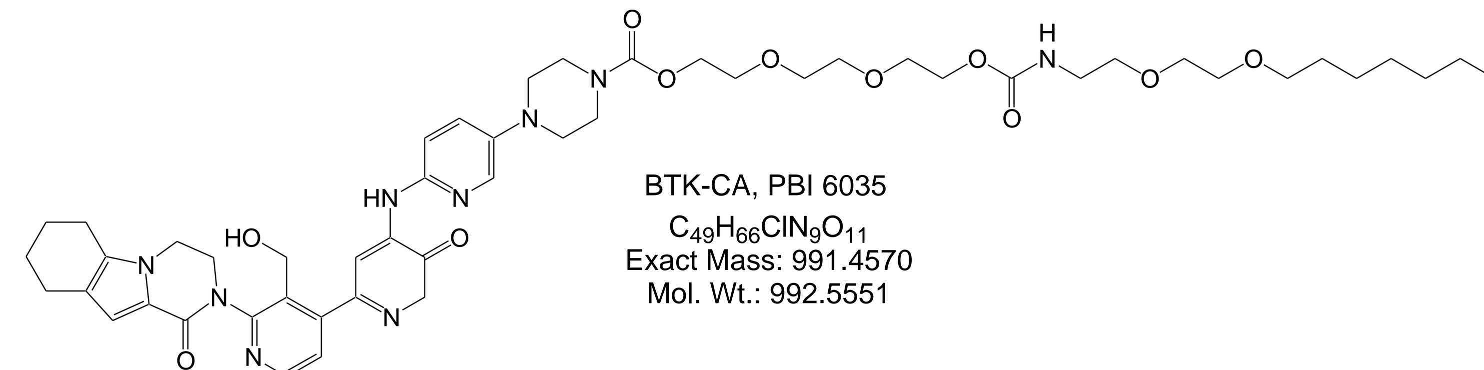
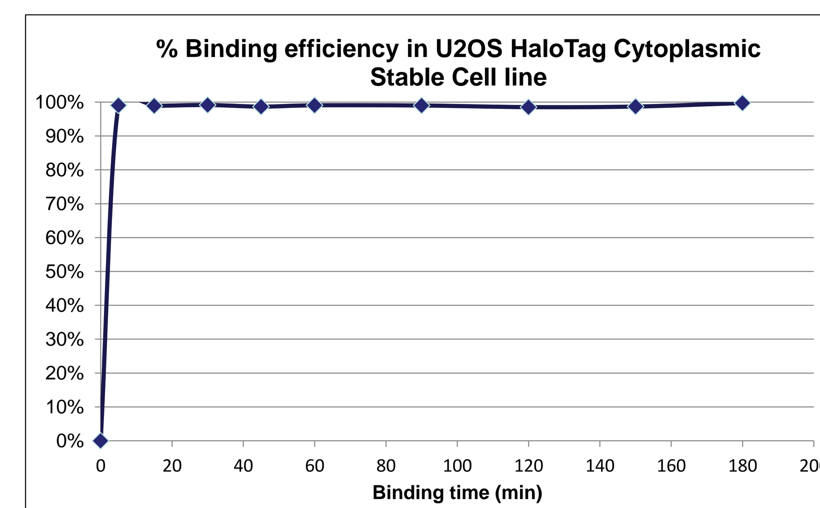
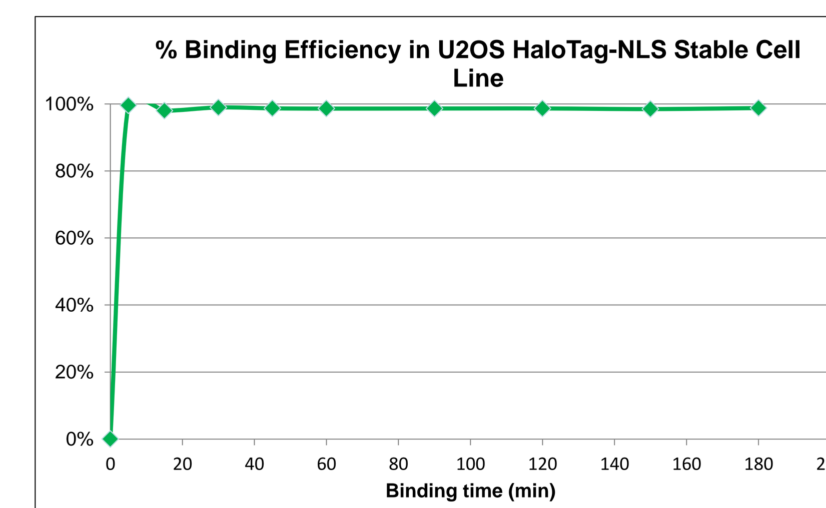
4. BTK Study

- The G02599124-CA chemical structure is shown below:

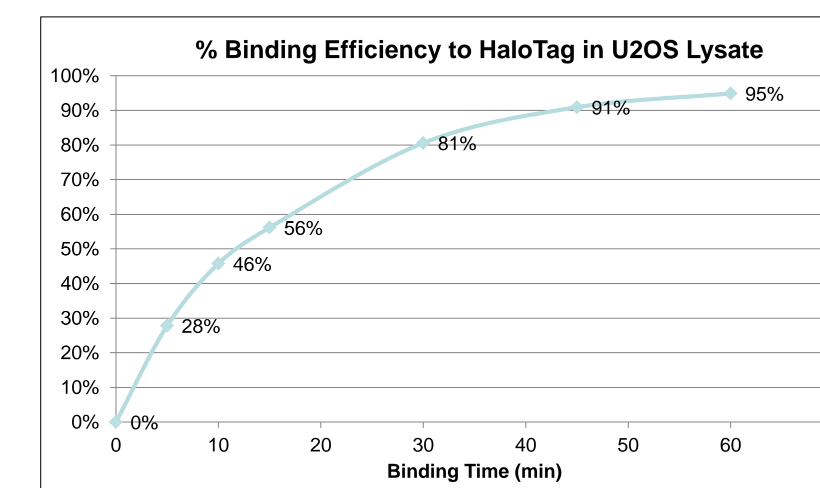


- Cytoplasmic and nuclear permeability of G02599124-CA in U2OS cells:

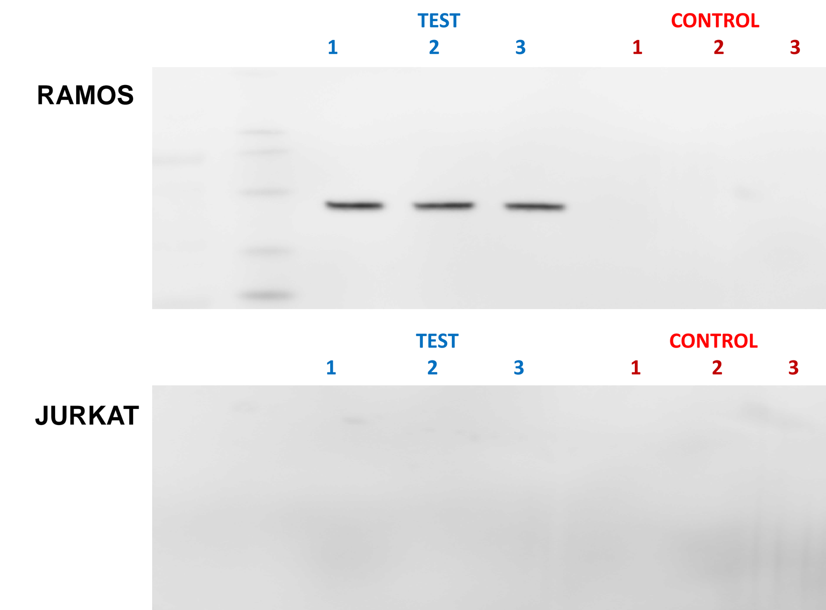




- Binding of G02599124-CA to HaloTag in U2OS lysate. Based on the data a 30min capture time was chosen:



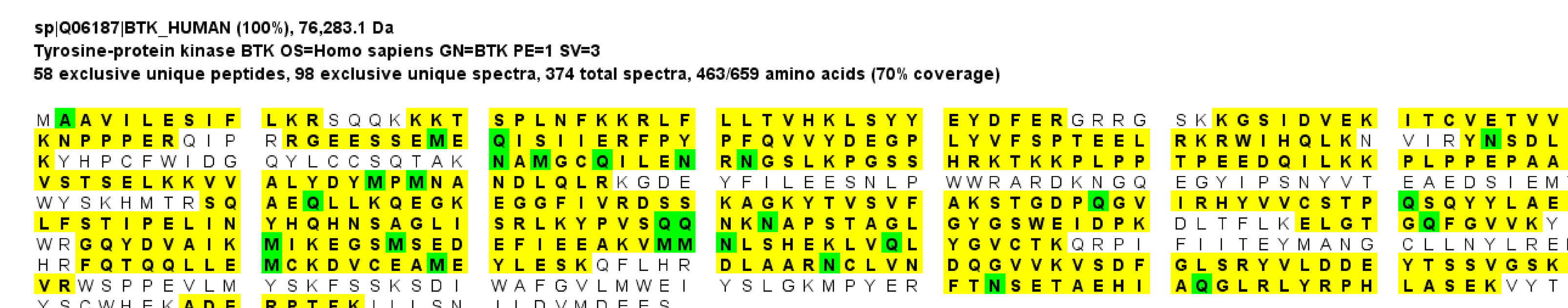
- Western blot for BTK in both cell lines:



- Mass spectrometry results for Ramos cell line. Data were filtered for proteins present in all three test samples with at least 5 spectral counts and unique or 4x enrichment based on mean NSAF values.

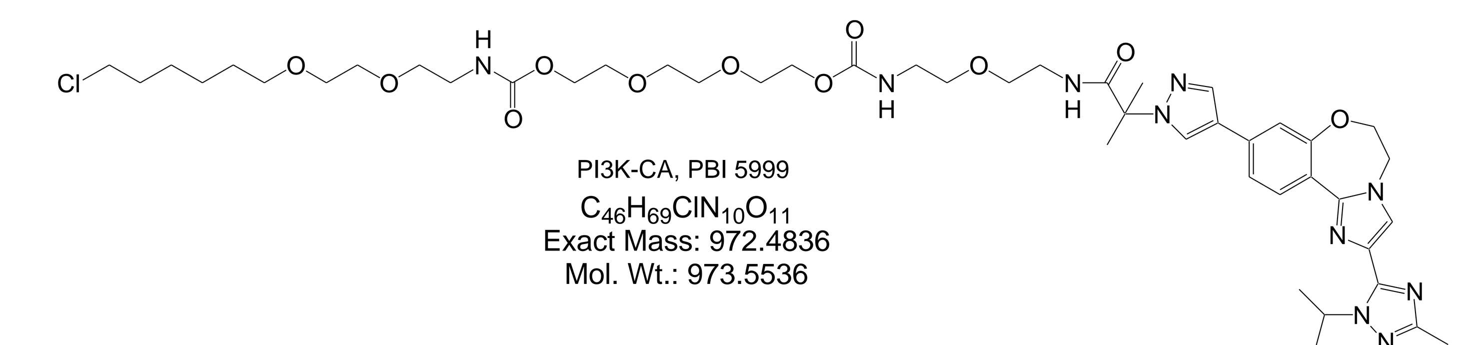
Proteins (4)	Description	Mean NSAF Control	Mean NSAF Test
BTK	Tyrosine-protein kinase BTK	1.1E-03	3.6E-02
TCEB2	Transcription elongation factor B polypeptide 2	1.7E-03	7.2E-03
PPIL3	Peptidyl-prolyl cis-trans isomerase-like 3	0.0E+00	3.2E-03
YME1L1	ATP-dependent zinc metalloprotease YME1L1	9.7E-05	4.7E-04

- Only 4 proteins passed the filtering criteria, the top protein was BTK and was not observed in the Jurkat cell line. The coverage map from one of the Ramos replicates is shown below; we observed 70% and 374 spectral counts. Matched peptides are highlighted in yellow:

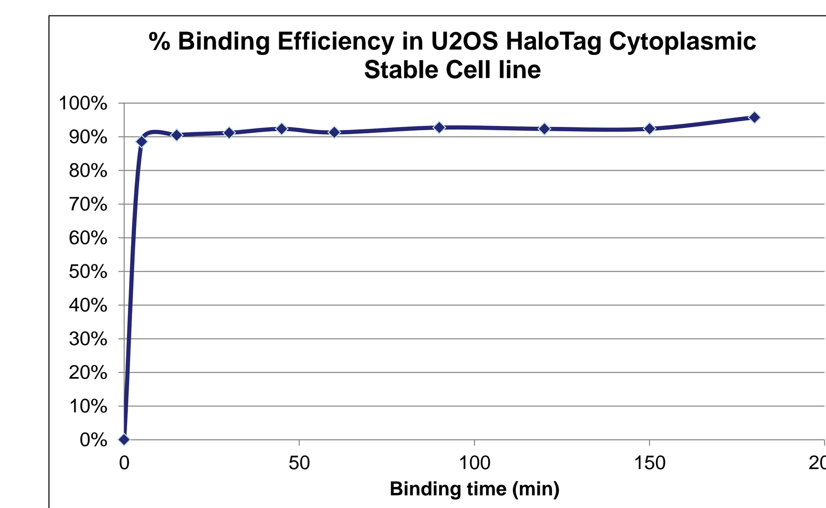


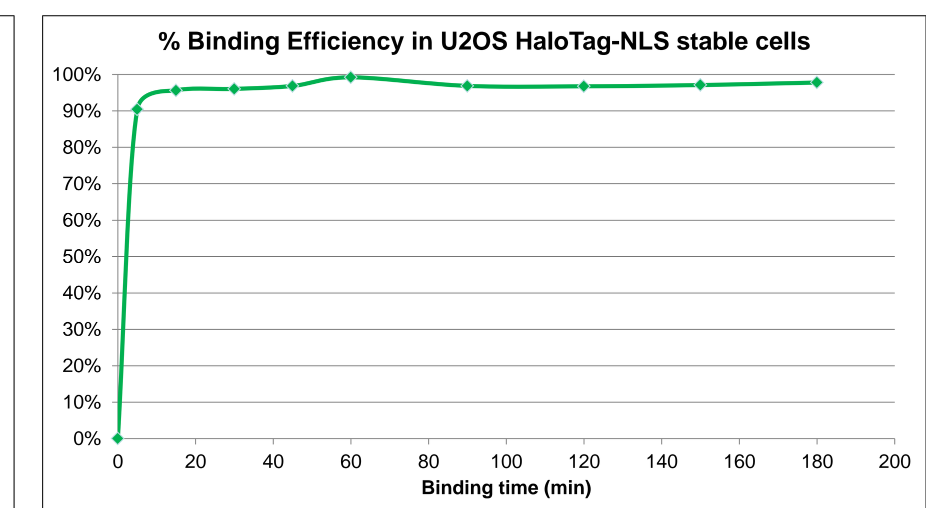
5. PI3K Study

- The Taselisib-CA chemical structure is shown below:

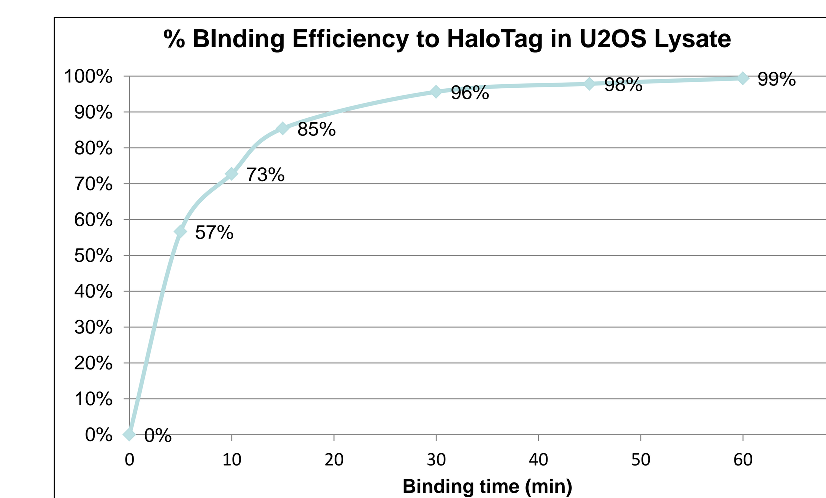


- Cytoplasmic and nuclear permeability of Taselisib-CA in U2OS cells:

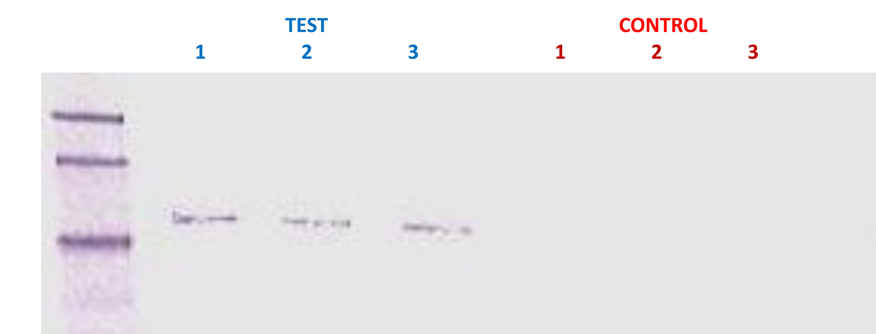




- Binding of Taselisib-CA to HaloTag in U2OS lysate. Based on the data a 30min capture time was chosen:



- Western blot for PI3K p85:



- Mass spectrometry results at 1h treatment. Data were filtered for proteins present in all three test samples with at least 5 spectral counts and unique or 4x enrichment based on mean NSAF values. Two PI3K regulatory subunits R1 and R2, and one catalytic, CA, were observed as the most abundant proteins:

Proteins (3)	Description	Mean NSAF Test	Mean NSAF Control
PIK3R1	Phosphatidylinositol 3-kinase regulatory alpha	4.2E-03	0
PIK3CA	Phosphatidylinositol 3-kinase catalytic alpha	2.2E-03	0
PIK3R2	Phosphatidylinositol 3-kinase regulatory beta	1.6E-03	0

- The coverage map for PI3KCA from one of the replicates is shown below; we observed 33% and 147 spectral counts. Matched peptides are highlighted in yellow: