Novel Fluorescence High-Throughput Drug Assay to Identify Promiscuous Inhibitors among Screening Compounds (OTT1368)

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Problems with High-Throughput Screening

Problems:

- High-Throughput Screening (HTS) can lead to hundreds or thousands of hit molecules that include a significant number of false positive hits
- Many electrophilic compounds are among these false positive hit compounds due their ability to interact non-specifically with protein targets.
- Currently, HTS assays that identify electrophilic properties of small molecules and the ability to react with naturally occurring thiols have yet to be fully developed

Solutions:

- A new fluorescence-based assay that enables the identification of thiol-reactive small molecules in a high-throughput manner (up to 1536 well plate)
- Less interference from molecule due to use of a far-red probe
- Stable acetylated precursor allows for storage of the assay probe and its reliable generation *in situ*

Market, Intellectual Property, & Partnering

Market

- Global Information, Inc. reported the global market for drug discovery technologies and products at $41.4B in 2012 and predicted to $79B 2017
- The HTS market is expected to grow from $11.5 B in 2012 to $20B in 2017
- Drug development lead time has not followed the rapid development of these tools showing the gap between the initial introduction of new platforms and their further application and commercial success

Intellectual Property

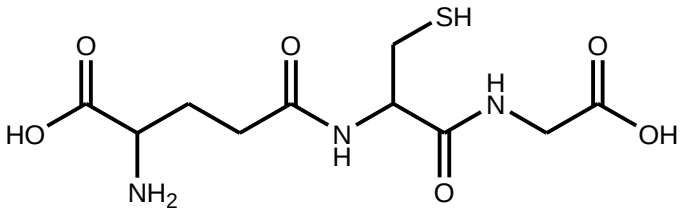
- A U.S. Provisional Patent Application has been filed for the novel compounds and methods of use

Licensing

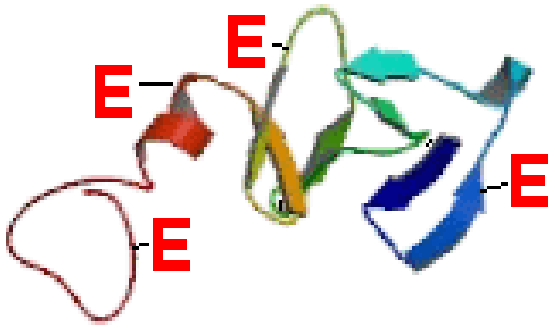
- Offering non-exclusive research licenses to companies interested in the use of the assay for HTS and drug development
- Looking for up front fee and annual license fee for use of the material

Importance of Thiol Groups

- **Reduced thiol groups are essential in many biochemical and physiological reactions**
  - Cysteine for 3D structure of proteins
  - Dimerization of proteins
  - Enzymatic active site (cysteine protease)
  - Glutathione (conjugation, reduction)

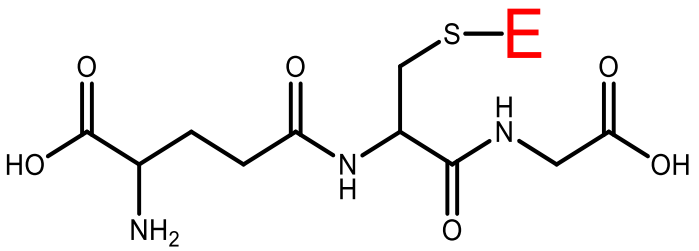


Drug Compounds Often Electrophilic



Electrophile binding to a cysteine-rich protein

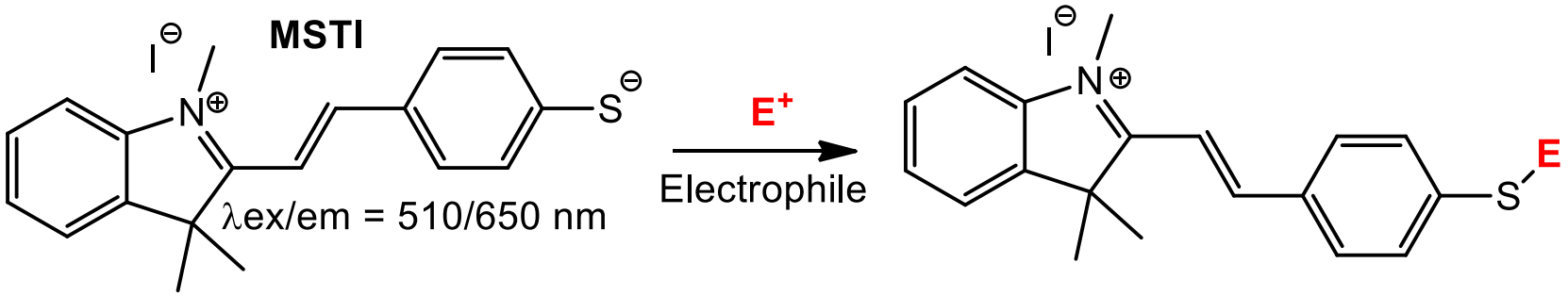
Modulating protein function



Electrophile binding to glutathione

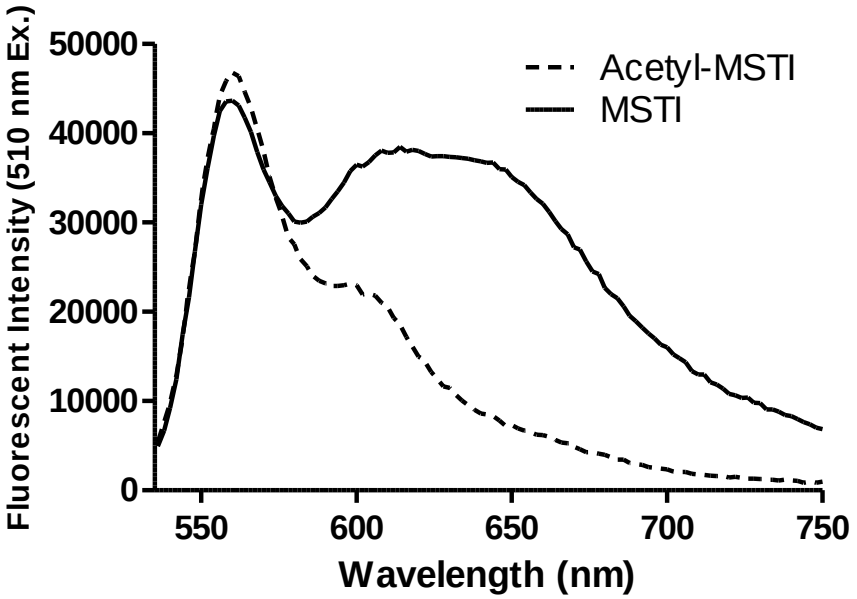
Modulating metabolic functions

A New Assay: MSTI

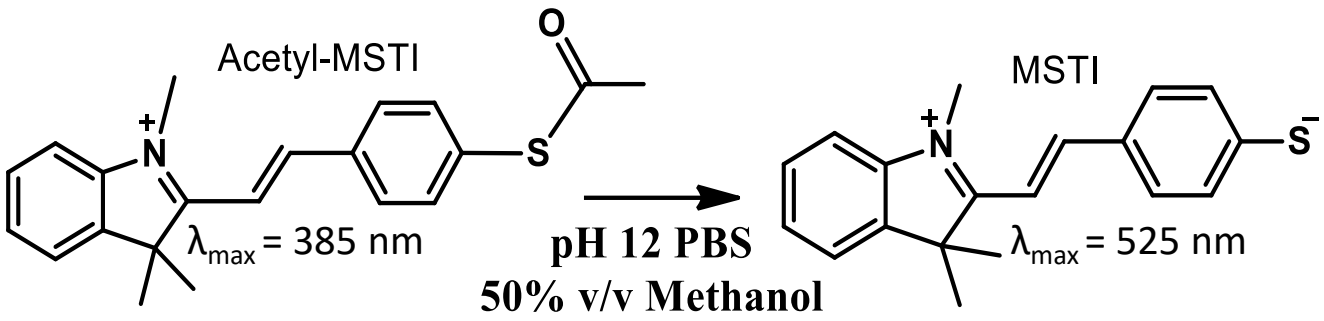


(E)-2-(4-**M**ercapto**s**tyryl)-1,3,3-**t**rimethyl-3H-1-**i**ndolium)

- 20 µL per well
- 384 well black, flat bottom plate
- 100 µM compound concentration
- 30 µM MSTI and Acetyl-MSTI
- Reactive compounds yield lower fluorescence

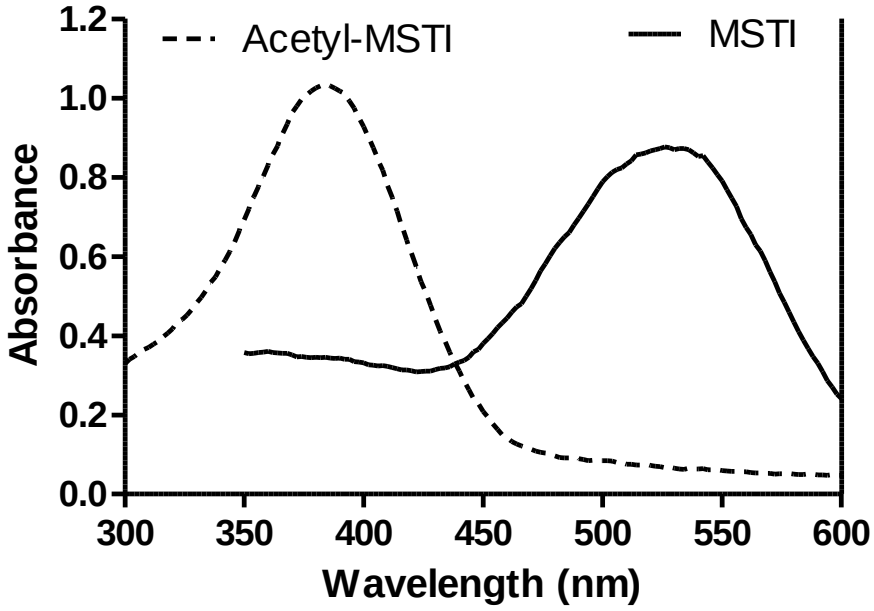


Acetyl-MSTI is Converted *In Situ*



70-80% conversion to MSTI

Acetyl-MSTI MSTI

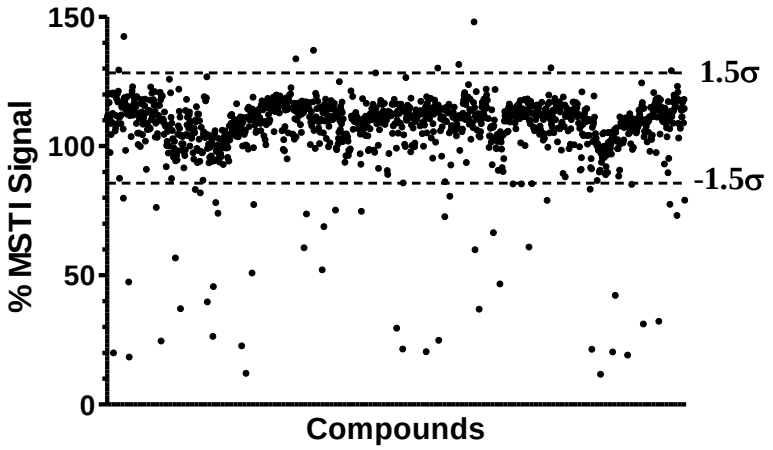


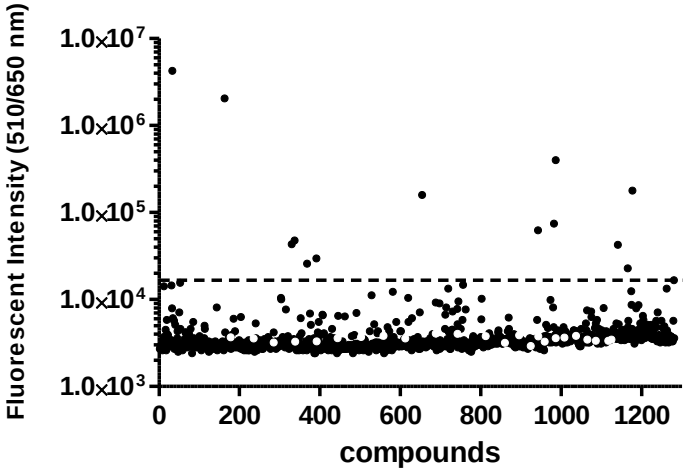
Assay Optimization

- **Buffer Compositions Tested**
  - Ionic vs. Non-Ionic
  - Ionic Strength
  - Buffer pH
- **Buffer Co-Solvents Tested**
  - 0.01% NP40, 2% DMSO, 5% Methanol
- **Incubation Time**
  - Immediate, 30 min, or 1hr
- **Compound Concentration**
  - 50µM, 100µM, 150µM

Test Screening

- LOPAC-1280 Screen
  - 5% Hit Rate for compounds screened in triplet
  - Identification of different reactive compounds including electrophilies, radicals, and redox-active compounds
  - < 1% compound interference





Conclusions

- First HTS assay to detect small compounds electrophiles
- Optimized for a 384 well format, it is easily convertible to 1536 well format
- *In situ* conversion to MSTI circumventing any challenging storage regimes
- Far-red detection of MSTI limits the number of molecules interfering
- Excellent reproducibility (Z' > 0.6 and RSD < 5%)
- Dose dependent reaction between MSTI and electrophilic compounds

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