Implementation of a High Throughput Screening Platform using MALDI – MS as a Readout

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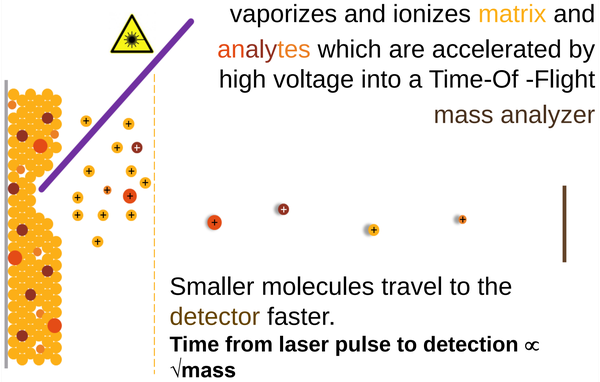
In high throughput screening there are still a number of biochemical assays where detection of reactant consumption or production formation can not be tracked with fluorescent or colorimetric reagents.

Mass spectrometry is a label free readout technology with the ability to detect a broad range of analytes with high sensitivity. Typically for drug discovery liquid chromatography (LC) is employed, the time between injections for a single column can be as low as 6 seconds per sample but could be 10 times as long. This limits the throughput to less than 15,000 samples in a 24 day.

We have recently established a high throughput screening platform using matrix assisted laser desorption and ionization (MALDI) as the mechanism to introduce ions into the mass spectrometer, the system is running at approximately 1s per sample giving a throughput of up to 80,000 samples a day, enabling true HTS.

Principles of MALDI – time of flight (TOF)

A laser beam focused on a stainless steel target, vaporizes and ionizes matrix and analytes which are accelerated by high voltage into a Time-Of -Flight mass analyzer



Smaller molecules travel to the detector faster.

Time from laser pulse to detection ∝ √mass

MALDI Detection Considerations

Biochemical Assays should be run under optimal conditions
Assays would be stopped prior to transfer to MALDI plate

MALDI plates are typically expensive
MALDI matrix (α-Cyano-4-hydroxycinnamic acid ,CHCA) is prepared in 50% Acetonitrile and evaporates quickly
MALDI matrix can clog pipette tips after repeated pipetting

# of ions generated in one laser pulse train is variable
Numerous factors can affect ionization efficiency

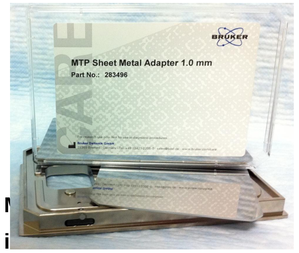
Plate handling needs to be automated
Data handling needs to be automated
Require throughput of >50K data points / day

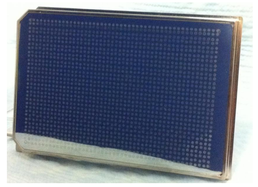
Innovations

MALDI Plates

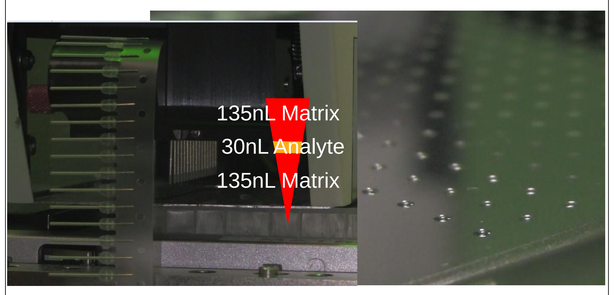
We developed a disposable magnetic stainless steel sheet to serve as a MALDI target

Bruker developed with us a low mass magnetic plate holder to accommodate the magnetic sheet



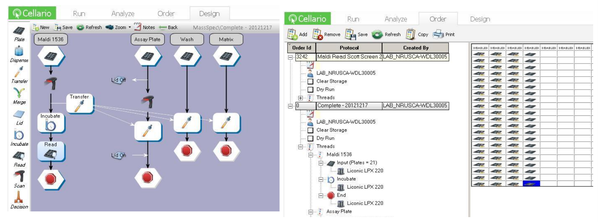


We developed with TTP LabTech a protocol using their Mosquito HTS to enabling spotting of matrix mixed analyte with high spatial resolution in a 1536 format. The system pierces Foil Sealed 384-well plates containing volatile MALDI matrix in 50% acetonitrile, or methanol wash solution.
Sample deposition involves: aspirating a 300nL "Sandwich" of 135nL matrix / 30nL analyte / 135nL matrix and depositing them precisely onto the MALDI target.

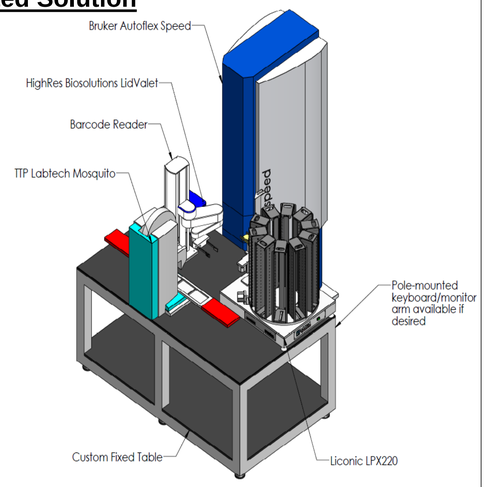


High Throughput Application of the Bruker AutoFlex

We collaborated with HiRes Biosolutions and Bruker to enable full automation of the Bruker Autoflex Speed MALDI-TOF MS. This included writing Cellario drivers, modifying the Bruker protocols and batch data parser. This resulted in a robust platform with automated data handling.



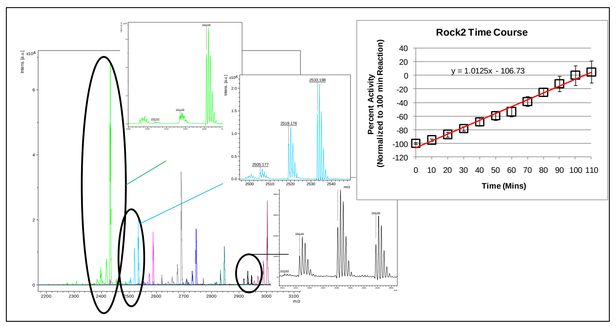
Integrated Solution



The assay runs on an HTS system in 1536 format and is stopped when complete. Plates are transferred to the Liconic LPX220 which has been preloaded with MALDI targets, 384-well MALDI matrix plates and 384-well methanol wash plates.
The robot moves the assay plate (de-lids), matrix, wash and MALDI targets to the Mosquito and links barcodes.
The Mosquito transfers the assay plate to the MALDI target with matrix (28 min / 1536 well plate)
The robot moves MALDI targets back to the Liconic LPX220 to complete matrix drying and then moves dry targets to the Autoflex Speed. The targets are read (38 min / 1536 well plate @ 1000 shots / spot) and the data is associated with the barcode. The targets are then returned to the Liconic LPX220.

Assay Development

The power of a TOF instrument can be leveraged to accelerate assay development. For initial identification of histone demethylase substrates we mixed a number of peptides together, with discrete masses and then examined the kinetics of product formation for all substrates concurrently. Since we were extracting 30nL of analyte at one time we could repeatedly sample from a 5µL assay volume

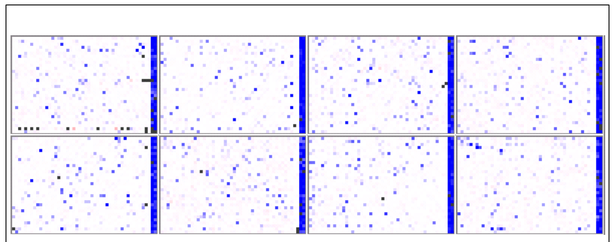


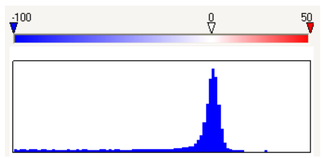
Screening

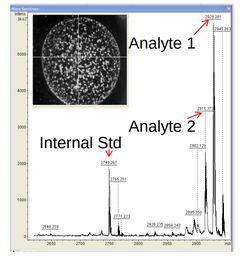
The enzyme assays we have developed so far typically rely on the generation of >1µM product. This gives robust counts and sufficient dynamic range to detect >90% inhibition of the enzyme.

Using an internal standard we can lower our control CVs to <10%, and in the case of strong signals to <5%.

Screening Z's by MALDI tend to be a little lower than for equivalent LC-MS assays, but with 6 enzymes tested to date we have always managed to generate Z's above 0.5 – typically by increasing product concentration.

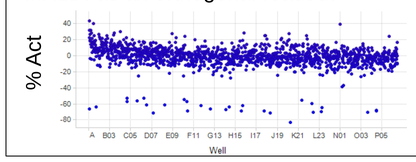






Typical Data (Z' 0.6 +/- 0.05)
Hit rate of 0.7%
Few "missing wells"
Good correlation with data from orthogonal assay

MALDI as an orthogonal readout



1408 compounds with <-80% activity in TR-FRET counterscreen

Summary

We have developed a high throughput screening platform utilizing MALDI-TOF MS readout.

By working closely with vendors – Bruker, TTP LabTech and HiRes Biosolutions - we were able to integrate a number of different functions to produce a cost effective, robust, reliable read out platform for screening.

Throughput for plate production is < 30 minutes per 1536 well plate and read times vary but we can read a 1536 MALDI plate in <40 minutes leading to a throughput of 36 plates / 24 hours, equivalent to 50,000 compounds tested per day.

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