# Development of a high-throughput MALDI-TOF MS drug discovery assay for ERAP1

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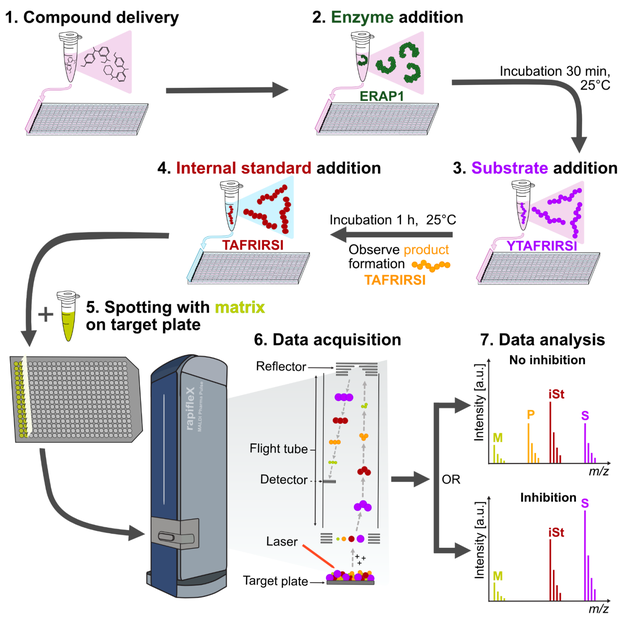
## Introduction

MALDI-TOF MS has been exploited in high-throughput screening (HTS) campaigns to provide fast and label-free readout for *in vitro* assays.[1] Here, we describe the development and validation of a MALDI-TOF MS based drug discovery assay for the endoplasmic reticulum aminopeptidase 1 (ERAP1). ERAP1 can influence the peptide repertoire displayed on the cell surface for immune cell recognition and is therefore a target in immuno-oncology, and for auto-immune diseases.[2] ERAP1 activity is mediated by substrate properties, and thus screening with a label-free technique is vital.

## MALDI-TOF MS assay

- The *in vitro* enzyme assay is stopped by addition of an acidic solution containing an internal standard.
- The matrix is then mixed with the sample to aide analyte ionisation. Analyte ions are separated according to their mass-to-charge ratio (*m/z*).
- The detected ions and their intensities are used to determine enzymatic activity.

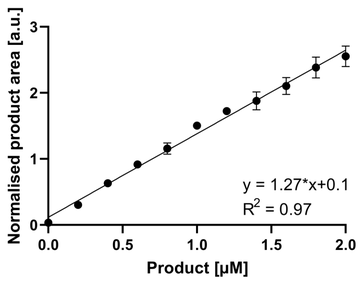



## Assay optimisation

### Limit of detection

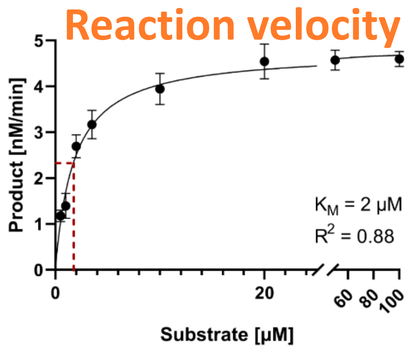
| Peptides | mass-to-charge ratio (*m/z*) | | | LOD (fmol) |
|---|---|---|---|---|
| | $[M+H]^+$ | $[M+Na]^+$ | $[M+2Na-H]^+$ | |
| Substrate (YTAFRIRSI) | 1126.6 | 1148.6 | 1170.6 | 0.5 |
| Product (TAFRIRSI) | 963.6 | 985.6 | 1007.5 | 1 |

### Linearity of detection

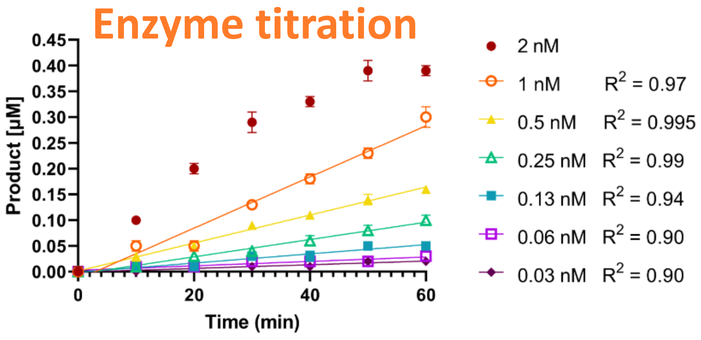



- The peptides can be detected with sufficient (fmol) sensitivity.
- A heavy labelled internal standard (TAFRIRSI($^{13}C^{15}N$)) was used to reduce the signal variability and ensure linear detection.

### Reaction velocity

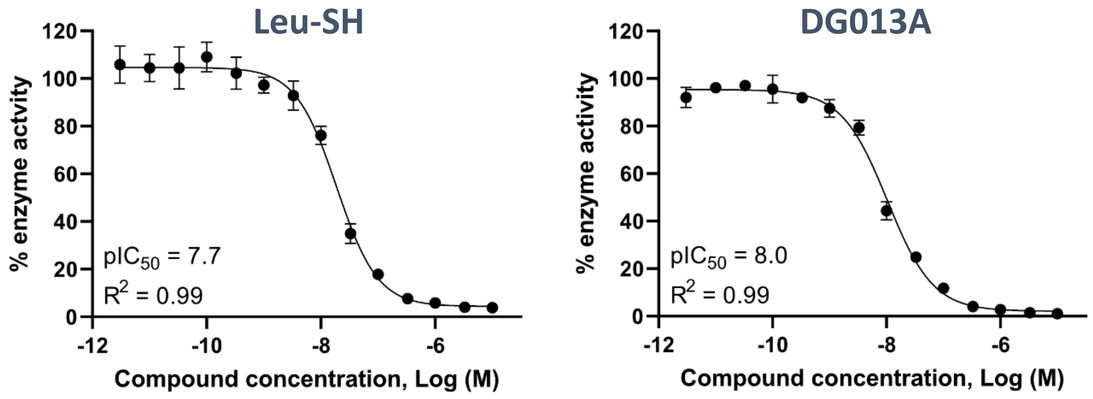



### Enzyme titration




- Screening for inhibitors was carried out with a substrate concentration around $K_M$.
- Enzyme titration was conducted to ensure linear reaction progression.

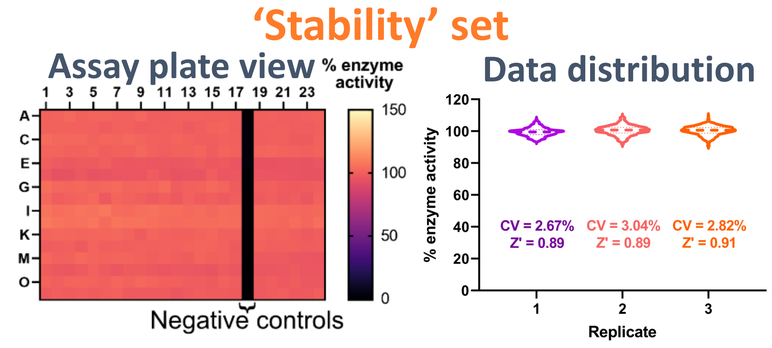
## Assay validation



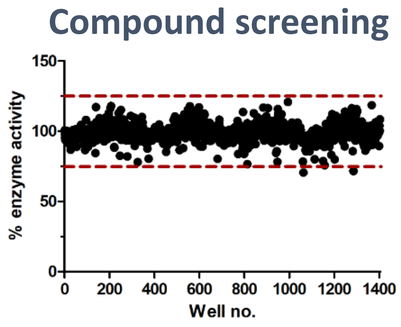

- $pIC_{50}$ values were determined for two known ERAP1 inhibitors (L-Leucinethiol and DG013A) to validate the assay.

## Assay automation

- Stable intra-plate and inter-day assay performance was observed in the absence of compounds.

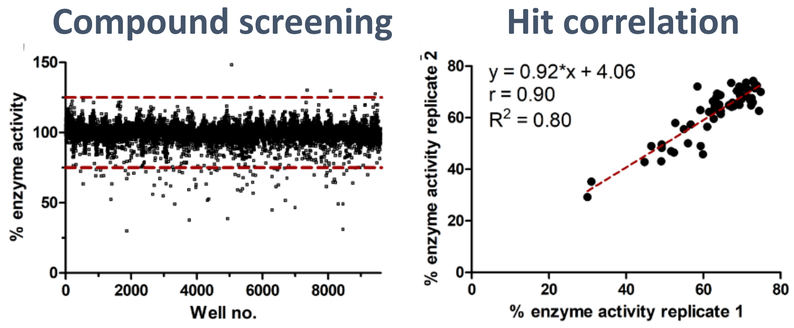



### 'Robustness' set



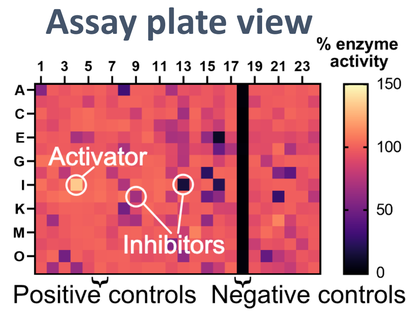

- No interferences, with conjugating or redox compounds, were identified.

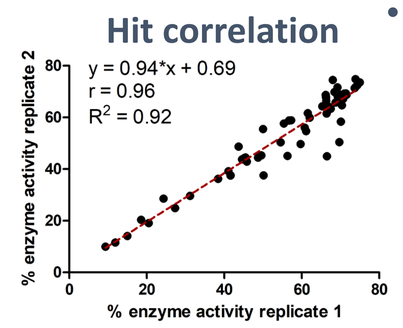
### 'Validation' set




- Duplicate screen of a ~9,600 compound set showed:
  - Hit rates of ~1%.
  - High reproducibility for hit compounds.

### 'ERAP1 binders' set

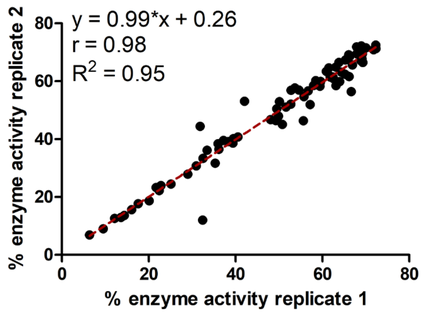



- From a set of experimentally determined ERAP1 binders, activators and inhibitors were successfully, and reliably identified.

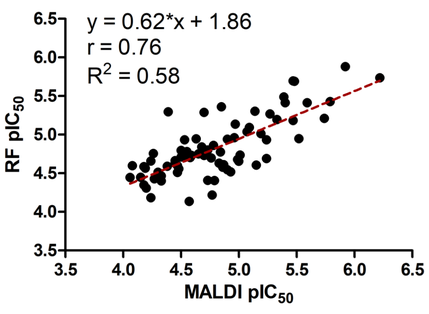
## Assay platform comparison

### Single concentration screening




- Screening of the 'ERAP1 binders' with an established RapidFire MS assay showed:
  - Similar hit reproducibility.
  - Hit matches with the MALDI-TOF MS assay.

### Dose-response screening




- Correlation of the $pIC_{50}$ values from the hits underlined the comparable platform performance.

## Conclusion

- We successfully developed and validated a novel MALDI-TOF MS assay for the identification of ERAP1 inhibitors.
- The assay showed sufficient stability, reproducibility and throughput to enable label-free HTS.
- Comparison with an established RapidFire MS assay showed comparable performance by providing higher speed and reduced assay volumes.

## Ongoing/ Future work

- The activity of some hit compounds will be evaluated in cellular assays.

## Acknowledgements

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References

1. De Cesare V, Moran J, Traynor R, et al. High-throughput matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry-based deubiquitylating enzyme assay for drug discovery. *Nat Protoc.* 2020;15(12):4034-4057
2. Liddle J, Hutchinson JP, Kitchen S, et al. Targeting the Regulatory Site of ER Aminopeptidase 1 Leads to the Discovery of a Natural Product Modulator of Antigen Presentation. *J Med Chem.* 2020;63(6):3348-3358