# Experimental Strategies to Improve Drug-target Identification in Mass Spectrometry-based Thermal Stability Assays

Clifford Phaneuf[1,2], Amanda M. Figueroa-Navedo[1], Konstantin Aizikov[3], Dmitry Grinfeld[3], Arne Kreutzmann[3], Daniel Mourad[3], Oliver Lange[3], Daniel Dai[2], Bailin Zhang[2], Alexander A. Makarov[3], Alexander R. Ivanov[1]

1 - Barnett Institute for Chemical and Biological Analysis, Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA, USA.
2 – Sanofi, Cambridge, MA, USA. 3 – Thermo Fisher Scientific, Bremen, Germany

## Overview

**Purpose:** Mass-spectrometry-based thermal stability assays (MS-TSA) enable the on and off-target profiling of drugs[2,3], which is a key bottleneck in drug development[1]. We investigated a combination of experimental MS-based approaches for the improved qualitative and quantitative performance of MS-TSA.

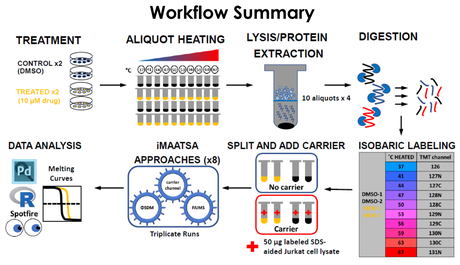
**Methods:** Jurkat cells were treated with either DMSO (control) or a MEK inhibitor. Melt curves were prepared by heating aliquots to different temperatures, digesting supernatant, and labelling with TMT10 plex. Half of the samples were spiked with an isobarically labelled whole cell digest. Unfractionated samples were evaluated with eight experimental data acquisition approaches.

**Preliminary Data:** Individually, each of the evaluated experimental approaches demonstrated benefits compared to the control analysis, while the approach implementing all three technologies (ΦSDM, FAIMS, and a carrier channel) produced the most unique high-quality protein melt curve comparisons.

## Introduction

Global and targeted mass spectrometry-based thermal stability assays (MS-TSAs) have recently emerged as one of the most promising solutions for the identification of protein-drug interactions[2,3]. MS-TSAs exploit the phenomenon of ligand-induced thermal stabilization of proteins, whereby modulated protein melting temperatures in drug-treated samples compared to a control set indicate protein-drug binding. We have investigated Phased-constrained Spectral Deconvolution Method[4,7] (ΦSDM), Field Asymmetric Ion Mobility Spectrometry[5] (FAIMS), and the implementation of an isobaric carrier channel[6] individually and in combination as improved MS-based acquisition approaches for thermal stability assays (iMAATSA) for the improved qualitative and quantitative performance of MS-TSA.

## Methods




**iMAATSA Combinations**

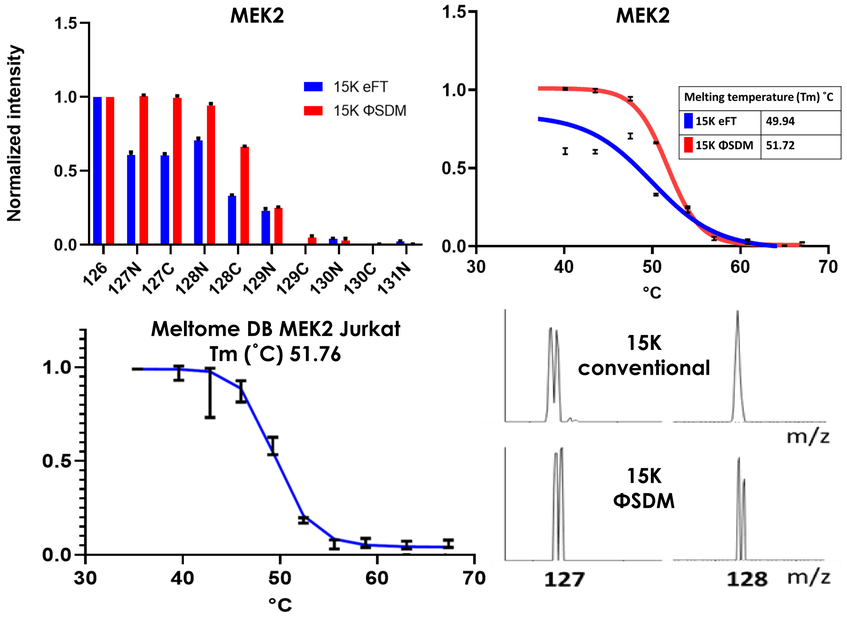
| abbreviation | carrier channel | FAIMS | ΦSDM |
|---|---|---|---|
| C_F_Φ | + | + | + |
| C_F_E | + | + | - |
| C_nF_Φ | + | - | + |
| C_nF_E | + | - | - |
| nC_F_Φ | - | + | + |
| nC_F_E | - | + | - |
| nC_nF_Φ | - | - | + |
| nC_nF_E | - | - | - |

Easy Spray ES803
90-minute gradient
FAIMS CV: -35,-50, -65
eFT: 54 ms max IT
64 ms transient length
30K resolution setting
ΦSDM: 22 ms max IT
32 ms transient length
15K resolution setting

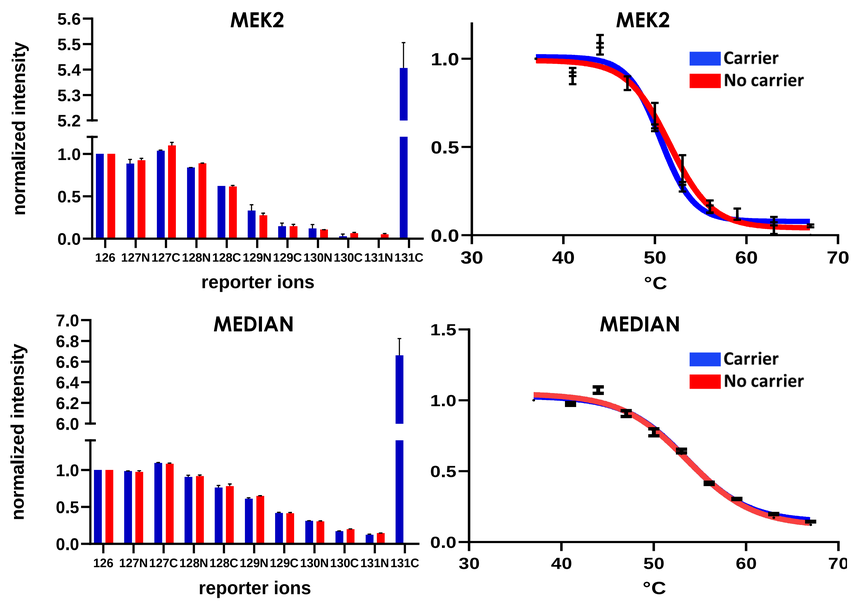
ΦSDM ~ Fourier transform algorithm implemented for increased resolution and speed
FAIMS ~ Separates ions based on their interaction with a charged field and reduces isolation interference
Isobaric carrier channel ~ Detergent-aided isobarically labeled whole-cell digest used to increase the signal of low intensity peptides

## Results

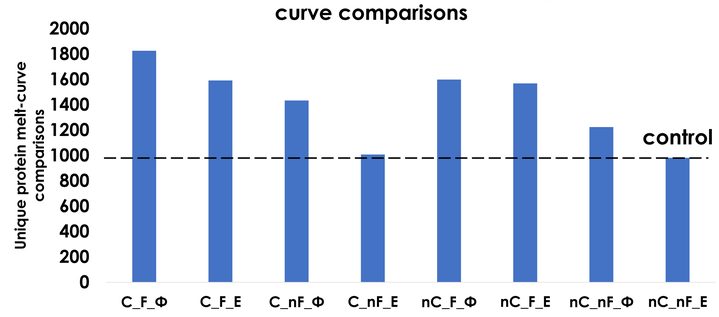
**ΦSDM provides sufficient resolving power to separate 15N and 13C TMT reporter ions at a resolution setting of 15K, which resulted in accurate melting curves at high scan speed**




**The inclusion of a whole-cell labelled digest as a carrier channel (131C) did not significantly alter melt-curve profiles**




**The addition of each iMAATSA technology appears to have a synergistic effect, with the approach incorporating all three leading to the highest melt-curve comparisons**




## Conclusions

- Accurate melting curves can be produced using a resolution setting of 15K with ΦSDM
- The use of an isobaric carrier channel does not alter melting-curve profiles or Tm determination
- The use of iMAATSA allowed for significantly more comparisons of high-quality melt-curves
- The use of iMAATSA allowed for significantly more comparisons of high-quality melt-curves

## References

1. Seashore-Ludlow, B., Axelsson, H. & Lundbäck, T. Perspective on CETSA literature: Toward more quantitative data interpretation. SLAS DISCOVERY: Advancing the Science of Drug Discovery 25, 118-126 (2020)
2. Savitski, M. M. et al. Tracking cancer drugs in living cells by thermal profiling of the proteome. Science (New York, N.Y.) 346, 1255784, doi:10.1126/science.1255784 (2014).
3. Molina, D. M. et al. Monitoring Drug Target Engagement in Cells and Tissues Using the Cellular Thermal Shift Assay. Science 341, 84, doi:10.1126/science.1233606 (2013)
4. Kelstrup, C.; Aizikov, K.; Batth, T.; Kreutzman, A.; Grinfeld, D.; Lange, O.; Mourad, D.; Makarov, A.; Olsen, J., Limits for Resolving Isobaric Tandem Mass Tag Reporter Ions Using Phase-Constrained Spectrum Deconvolution. Journal of Proteome Research. 2018, 1535-3893
5. Schweppe, D. K. *et al.* Characterization and Optimization of Multiplexed Quantitative Analyses Using High-Field Asymmetric-Waveform Ion Mobility Mass Spectrometry. *Analytical Chemistry* **91**, 4010-4016, doi:10.1021/acs.analchem.8b05399 (2019)
6. Yi, L. *et al.* Boosting to Amplify Signal with Isobaric Labeling (BASIL) Strategy for Comprehensive Quantitative Phosphoproteomic Characterization of Small Populations of Cells. *Analytical chemistry* **91**, 5794-5801, doi:10.1021/acs.analchem.9b00024 (2019)
7. D. Grinfeld, K.; Aizikov, A. Kreutzmann, E.; Damoc, A. Makarov., Phase-Constrained Spectrum Deconvolution for Fourier Transform Mass Spectrometry . Anal. Chem. 2017 89 (2), 1202-1211
8. Jarzab, A., Kurzawa, N., Hopf, T. et al. Meltome atlas—thermal proteome stability across the tree of life. Nat Methods 17, 495–503 (2020). https://doi.org/10.1038/s41592-020-0801-4

## Funding

Sanofi
Thermo Fisher Scientific
NIH NIGMI 1R01GM120272
NIH NCI R01CA218500
NIH NIGMI R35GM136421