

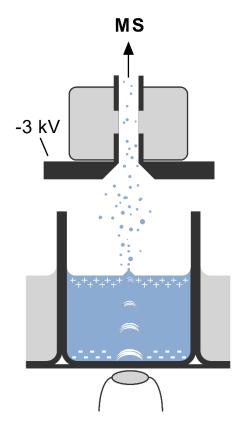


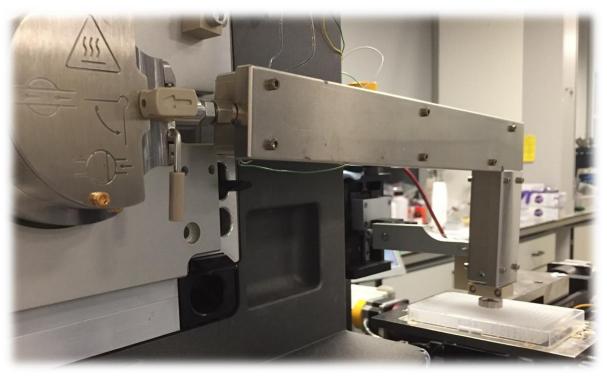


Acoustic Mist Ionisation

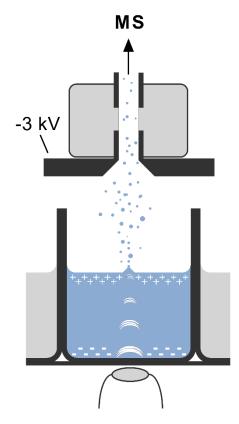
Enabling Ultra High Throughput Screening using Mass Spectrometry





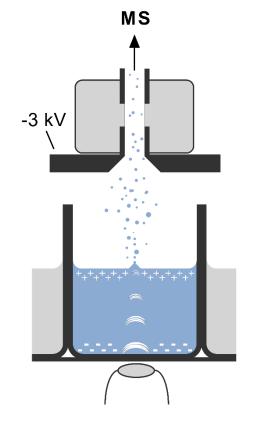


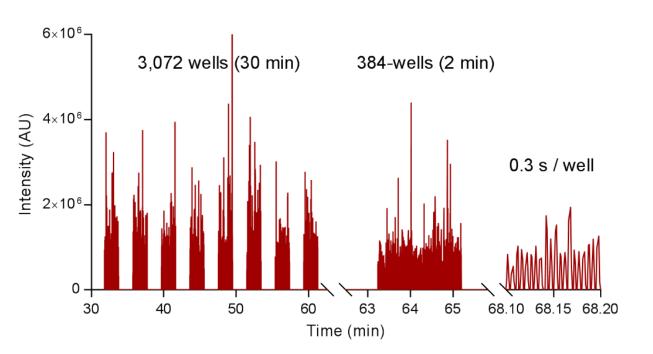




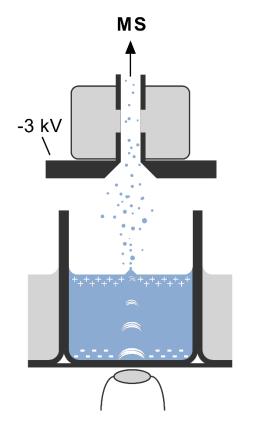


Waters



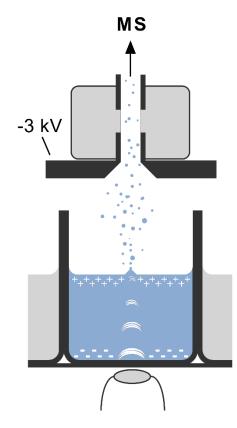






- Direct infusion electrospray-type ionisation
- CONTACTLESS. No carryover.
- Adjustable flow rates, typically 1-10 µl/min
- Flexible infusion time from 250 ms to hours
- Autosampler capacity 150 plates (57,600 samples)
- Primary use for biochemical screening
- > 2.8M samples acquired in 7 weeks





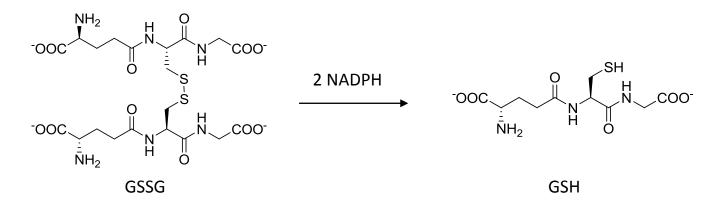
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1 week. 1,300 x 384-well plates. 500,000 samples

Case study – inhibition of glutathione reductase



- Glutathione (GSH) is an important tripeptide protecting the cells from oxidative damage
- Glutathione reductase (GSR) maintains GSH in its reduced state using NADPH



- Cancer cells can overexpress GSR to counteract increased oxidative damage
- Chased as drug target for decades but no known inhibitors active in cells

Case study – inhibition of glutathione reductase



- Current Practice
 - > 6 months assay development
 - Fluorescent Markers (e.g. Thiol Green)
 - Bind to any GSH produced

- Non-specific
 - Not looking directly at the biology

- Optical readout
 - Fast

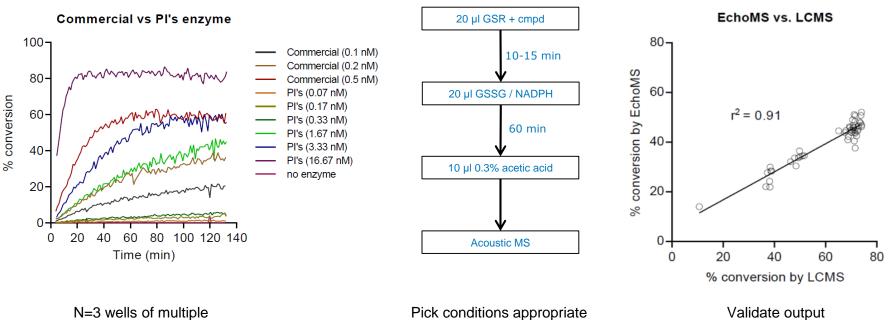
- Acoustic MS Workflow
 - Simplified Assay Development
 - Development time ~ 2 weeks

- Specific
 - Directly looking at the Biology

- AMI readout
 - Fast
 - Same reaction vessel

Assay Development

Waters



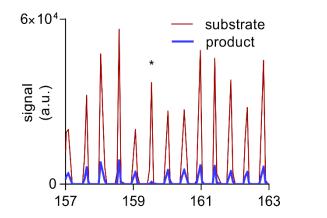
enzyme conditions

Pick conditions appropriate for batch processing time Validate output against LCMS

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Biochemical Assay

- First 50,000 samples acquired in ~28 hours
- Next 240,000 samples acquired in ~66 hours after further improvements
- Z' of >0.55 across the second batch
- Line of sight to >200,000 samples/day

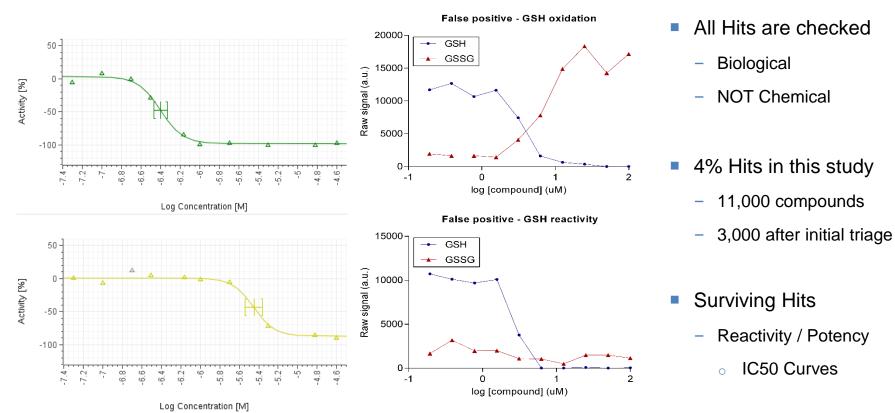


White	= inactive
Blue	= hit/partial hit
Red	= agonist

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Hit Triage & Potency (IC50)

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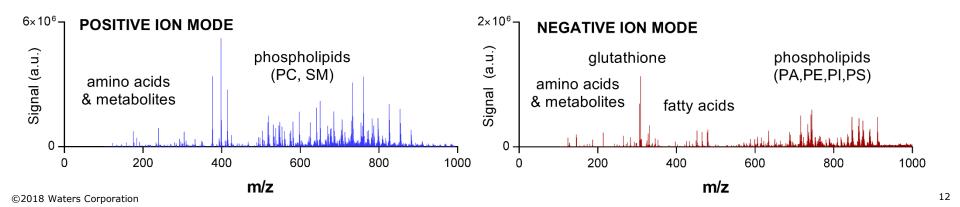
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Towards high-content label-free cell-based assays

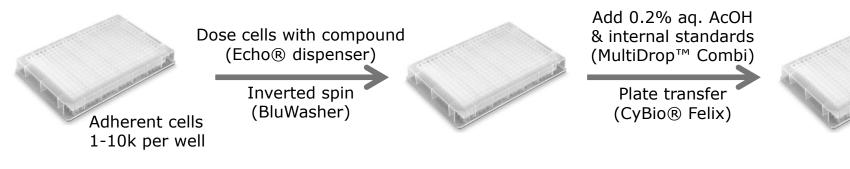


- Direct infusion ESI-MS (/MS) is known to work with crude cellular extracts (e.g. Zamboni)
- Analyte coverage depends on extraction solvents and sample preparation
- Acoustic mist can be generated from aqueous buffers and up to 100% organic solvents
- MCF7 cells lysed in 0.2% aq. AcOH 100s of species present in POS and NEG ion modes

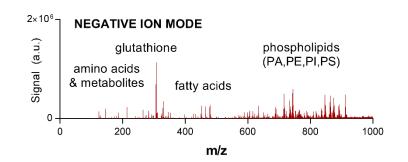


Automated cellular screening





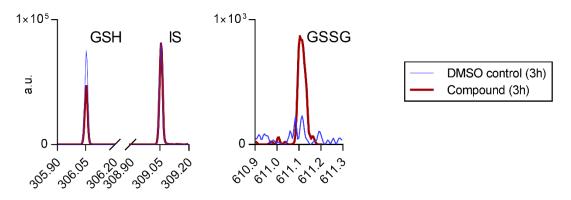
- Fully automatable process
- Tissue-culture plate coating suppresses ionisation
- Plate transfer will be removed in the future
- Estimated capacity ~20,000 compounds a day



Seeing more than cell death



- 250,000 compounds screened in vitro against GSR using AMI-MS
- Counter-screen to remove chemically reactive compounds
- ~70 most potent in vitro actives dosed into cells
- Some compounds showed the desired profile (cell death, GSH depletion, GSSG build up)

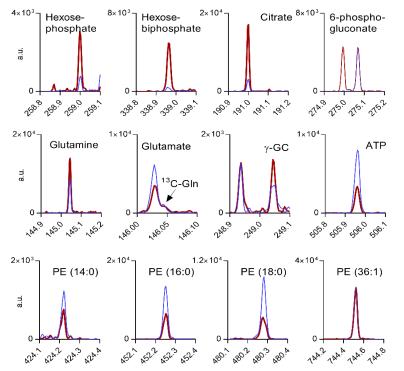


This level of information is the best one could expect from non-MS based assays

Seeing more than cell death



Metabolite and lipid profiling revealed a number of other events prior to cell death:



Activation of glycolysis and pentose phosphate pathway, producing NADPH

Higher glutamine uptake, increased de novo synthesis of glutathione, cell losing a lot of energy (ATP)

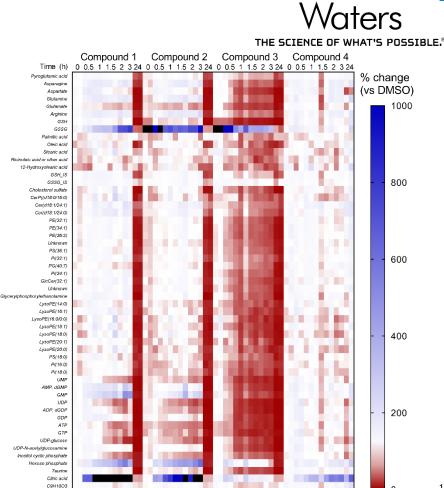
Impaired lipid synthesis, i.e. lack of NADPH!

Seeing more than cell death

 Time points and replicates possible thanks to the high throughput

 A heat map quickly visualised undesired profiles (e.g., oxidative stress at t = 0)

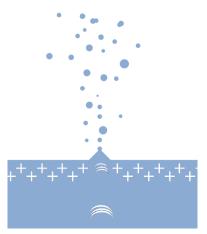
 This helped discard compounds that would otherwise be considered as hitting the target



Other promising applications under development



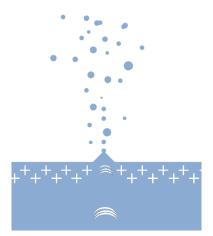
- Direct analysis of bacterial cultures
- Synthetic chemistry and biology (can monitor live reactions)
- High-throughput shotgun proteomics (collaboration with CRUK CI)
- Lipidomics of extracellular vesicles
- Direct analysis of whole blood



V VOIELS

Summary

- AMI-MS behaves like standard electrospray
- Direct infusion of up to 3 samples per second
- Nanolitres of sample are consumed, no carryover
- Suitable for untargeted profiling of a wide range of metabolites and lipids
- Suitable for other complex mixtures such as whole blood
- Targeted MS/MS possible, ion mobility to be tested
- Huge potential for early drug discovery and beyond



Development team



Ian Sinclair Jon Wingfield Martin Bachman Daniel Addison Mattias Rohman Rick Stearns

Lars Majlof

Luke Ghislain

Eric Hall

Sammy Datwani

Joe Olechno

Rich Ellson

AstraZeneca

LABCYTE 🕹

Steve Pringle Mike Morris Rhys Jones Richard Chapman Emmy Hoyes Ed Sprake

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