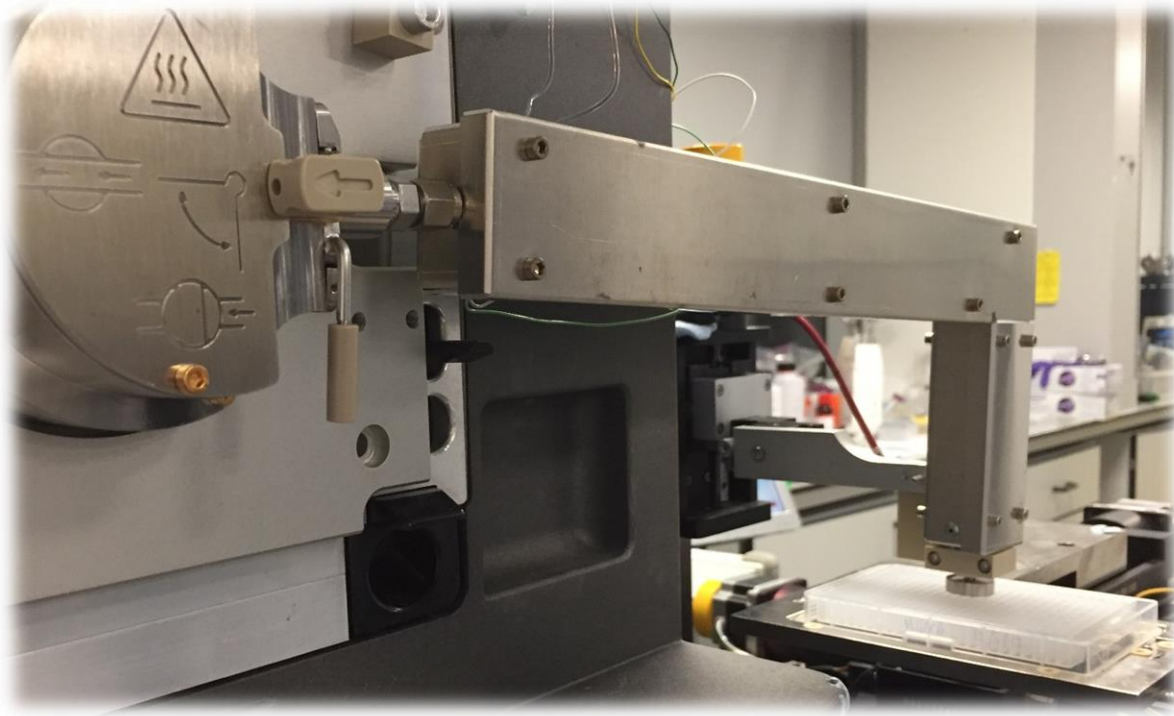
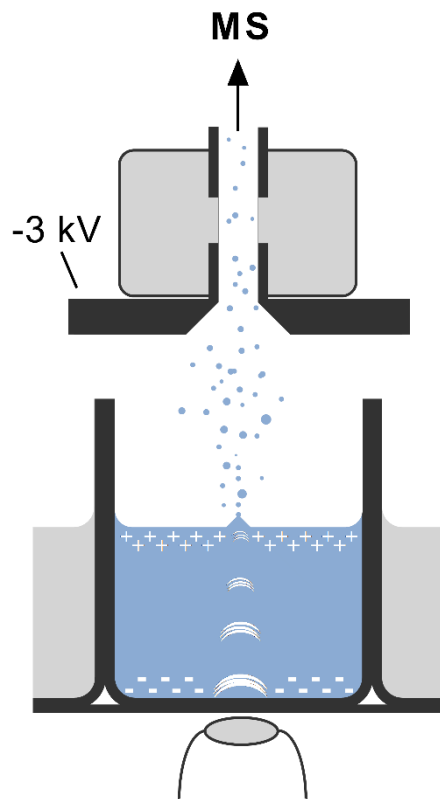


Acoustic Mist Ionisation

Enabling Ultra High Throughput Screening using Mass Spectrometry

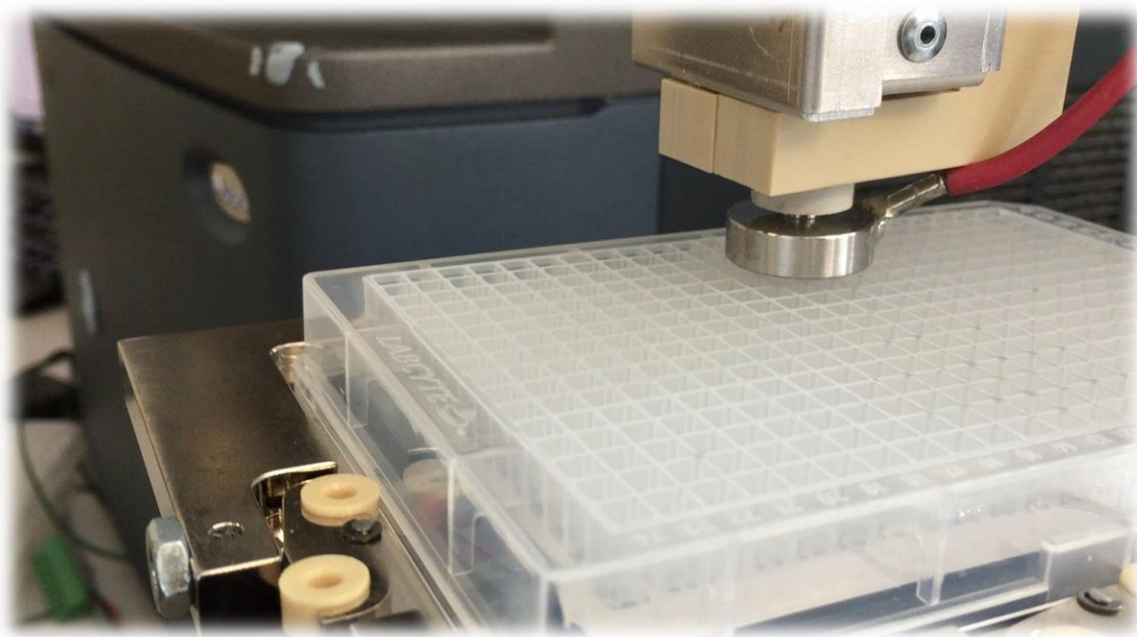
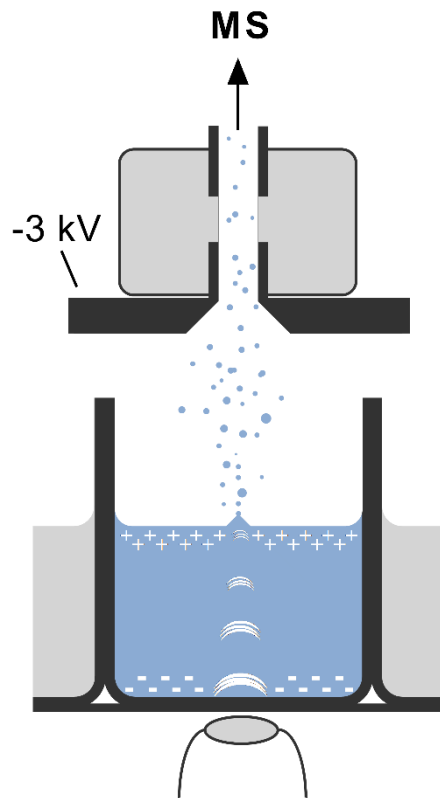
Acoustic mist ionisation – mass spectrometry (AMI-MS)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

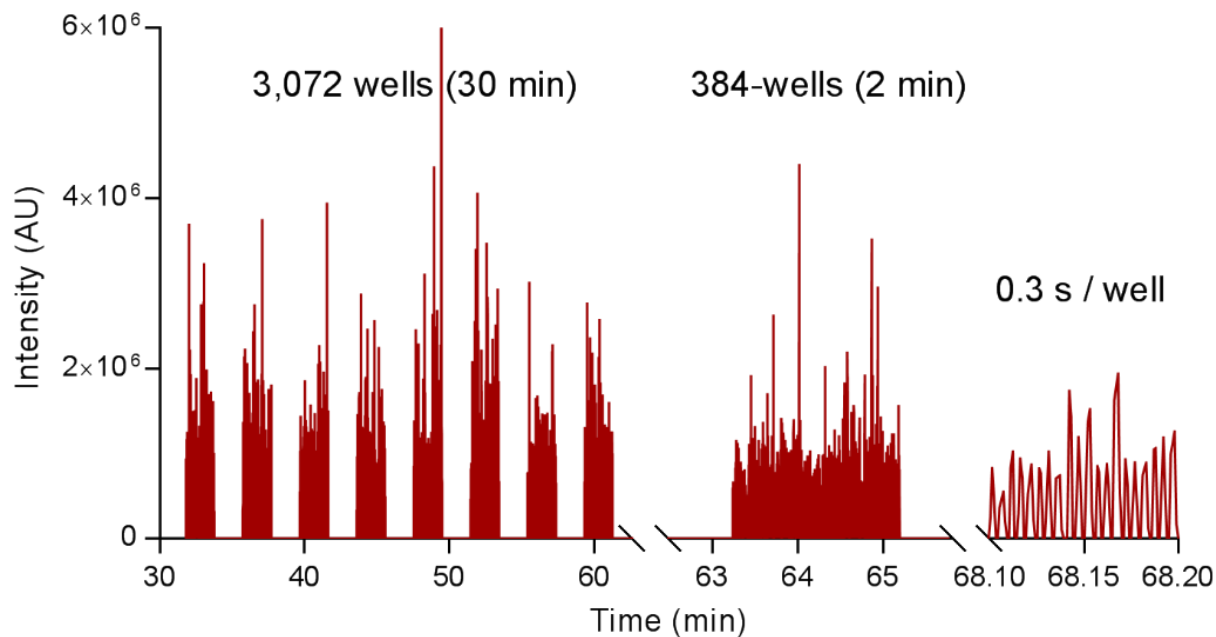
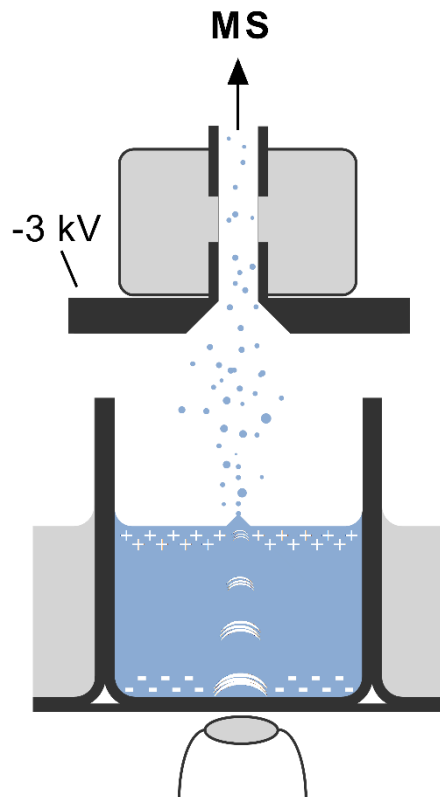


Acoustic mist ionisation – mass spectrometry (AMI-MS)

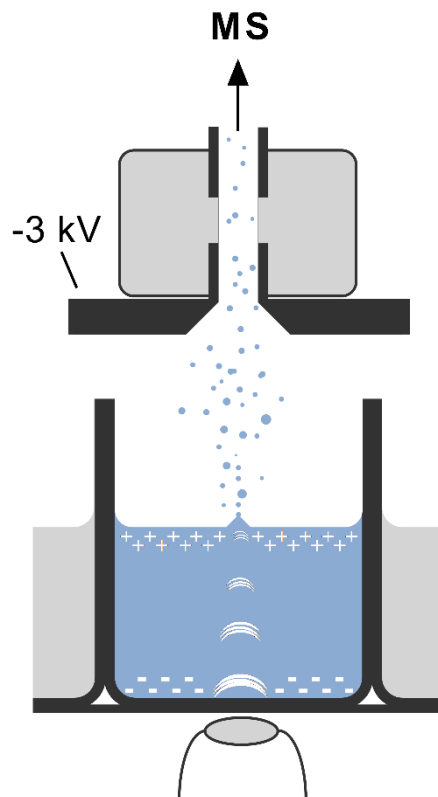
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®



Acoustic mist ionisation – mass spectrometry (AMI-MS)

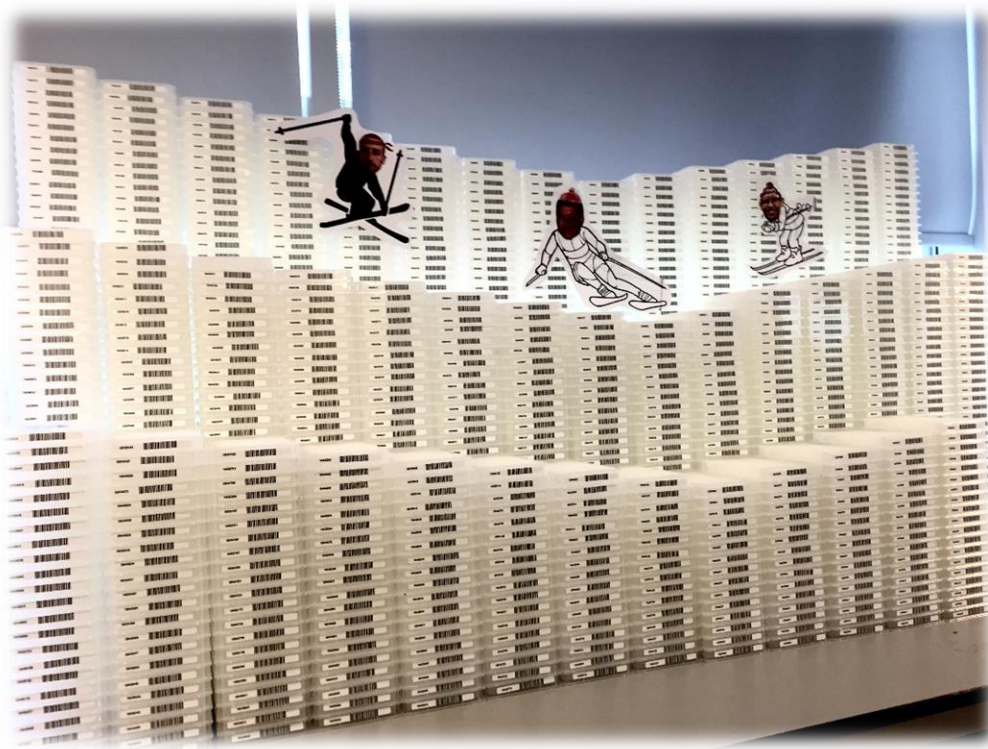
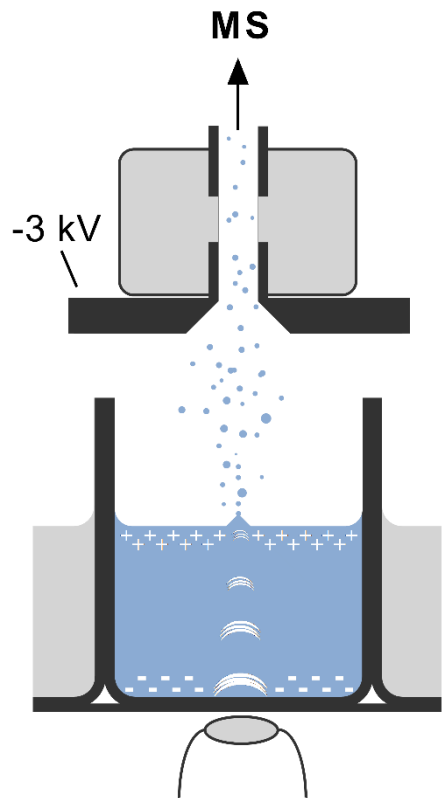


Acoustic mist ionisation – mass spectrometry (AMI-MS)



- Direct infusion electrospray-type ionisation
- CONTACTLESS. No carryover.
- Adjustable flow rates, typically 1-10 $\mu\text{l}/\text{min}$
- 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

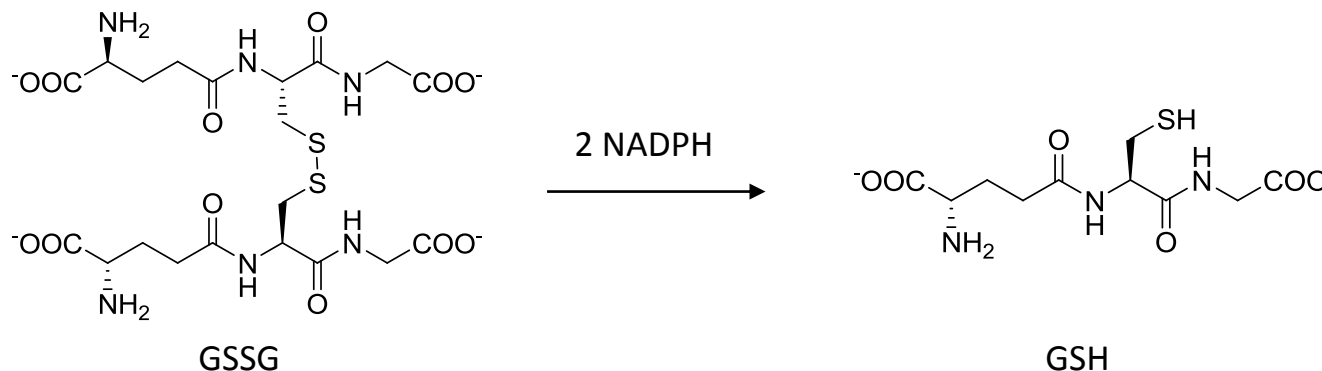
Acoustic mist ionisation – mass spectrometry (AMI-MS)



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

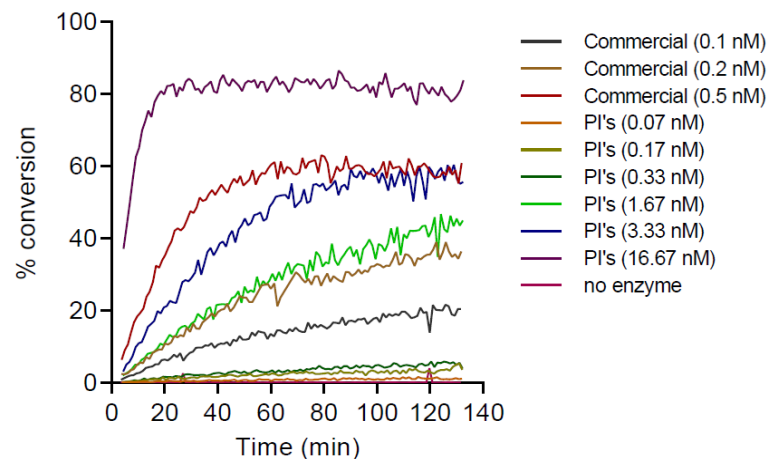
- > 6 months assay development
 - Fluorescent Markers (e.g. Thiol Green)
 - Bind to any GSH produced
- Non-specific
 - Not looking directly at the biology
- Optical readout
 - Fast

■ Acoustic MS Workflow

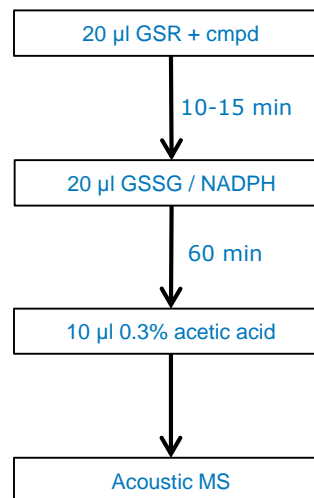
- Simplified Assay Development
 - Development time ~ 2 weeks
- Specific
 - Directly looking at the Biology
- AMI readout
 - Fast
 - Same reaction vessel

Assay Development

Commercial vs PI's enzyme

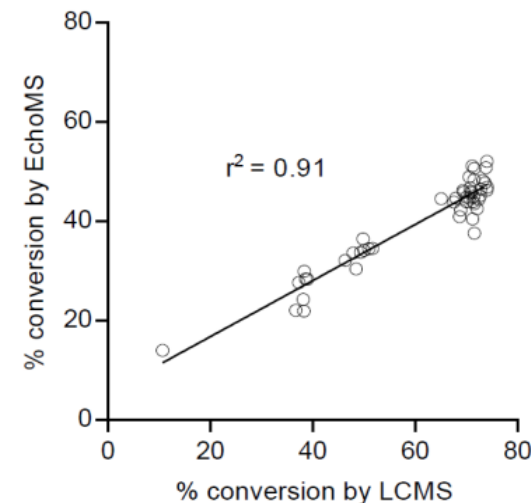


N=3 wells of multiple enzyme conditions



Pick conditions appropriate for batch processing time

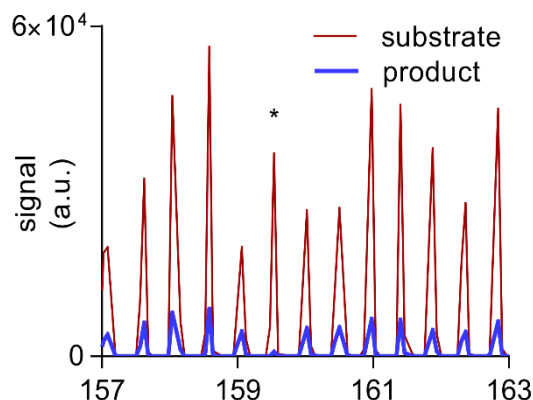
EchoMS vs. LCMS



Validate output against LCMS

Biochemical Assay

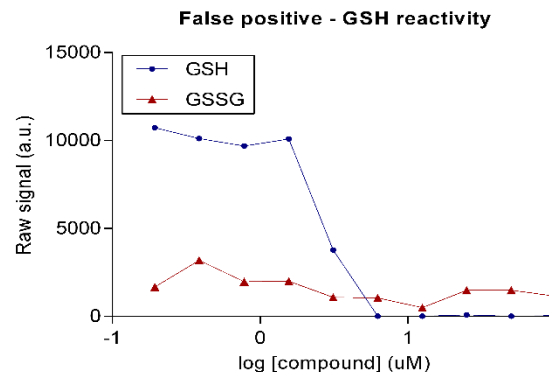
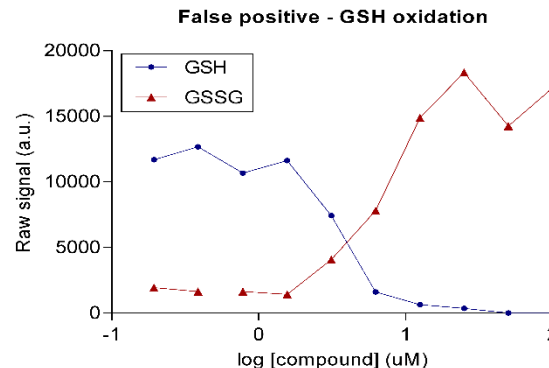
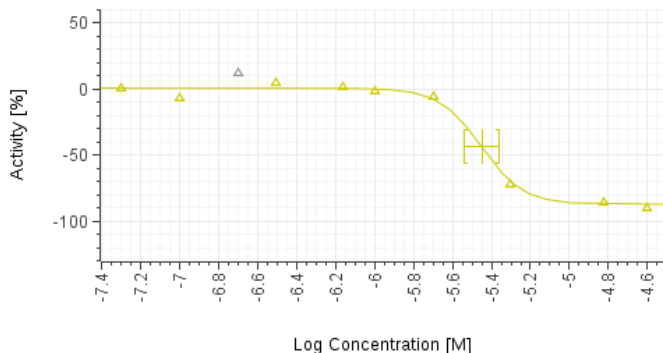
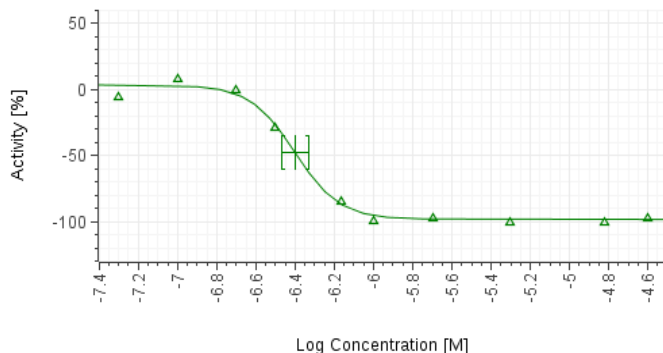
- First 50,000 samples acquired in ~28 hours
- Next 240,000 samples acquired in ~66 hours after further improvements
- Z' of >0.55 across the second batch
- **Line of sight to >200,000 samples/day**



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

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	-22	21	20	23	2	5	-6	-41	12	6	31	-100	-19	-17	1	13	-10	32	11	-24	-6	8	6	1
B	-12	12	-39	1	-5	12	14	0	-5	8	-19	-100	1	-23	-22	8	-7	-40	8	-4	14	-3	-11	-10
C	8	-24	10	-9	7	-3	18	-35	-38	2	7	-99	0	21	-20	-1	-25	-18	9	-1	16	-10	-9	-23
D	15	7	7	13	21	2	13	-6	-51	-15	13	-99	7	-27	-13	3	10	7	-3	60	22	6	-9	12
E	12	-11	-20	-10	2	2	4	-16	-46	-9	-5	-88	4	3	2	11	-14	10	-17	-15	-8	12	-32	3
F	24	18	-5	-3	-7	3	-8	-59	7	10	27	-100	9	16	21	-36	13	22	-32	26	-17	-6	-30	-9
G	-2	-16	-2	5	-9	21	2	37	-22	-33	9	-100	-11	-22	-31	-7	10	-9	-2	23	-14	-52	-100	11
H	5	30	13	24	-62	10	27	27	14	1	1	-100	-13	-20	28	-19	9	-8	11	-22	26	129	-6	-76
I	-53	28	10	36	-11	-18	22	-7	-12	5	-100	15	-10	12	-4	7	-23	19	3	3	-12	-33	-4	-7
J	-5	26	11	6	16	0	29	26	7	-37	-98	18	0	-21	-2	6	14	14	-13	9	-14	-19	-36	-6
K	-10	16	8	22	11	27	9	-6	23	35	-100	-3	-6	23	-10	21	20	16	29	0	-17	6	-7	-25
L	14	9	28	-18	9	-6	2	14	9	29	-100	9	-20	-16	19	18	-29	-8	6	12	17	16	-4	13
M	-16	18	-13	-16	-14	8	-70	-23	-11	10	-100	-19	-12	-9	8	-3	17	-19	7	-13	0	-26	-15	-5
N	22	3	-4	0	30	-29	12	6	16	5	-98	22	-29	-9	21	10	-8	-23	16	8	-26	7	-5	-4
O	5	-36	5	7	-3	19	-47	1	28	-81	-84	32	23	48	-7	10	-9	-3	-8	-63	-26	17	7	-40
P	-32	-66	-30	-14	-4	-3	-22	-51	-11	-13	-96	-5	15	-10	0	12	-2	6	6	-17	-36	-12	-6	-15

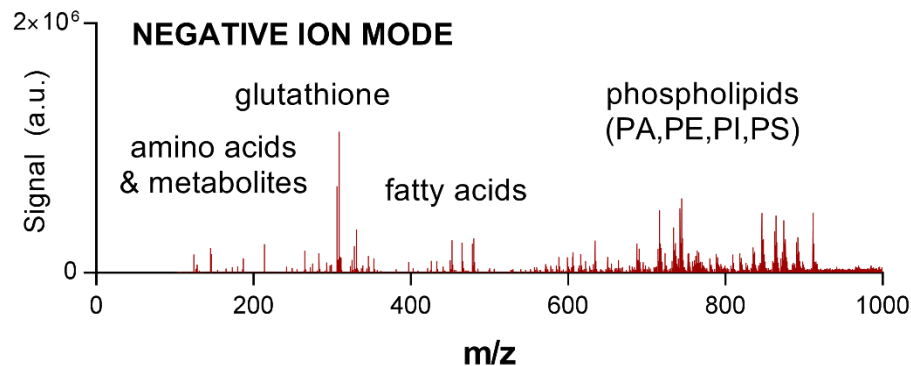
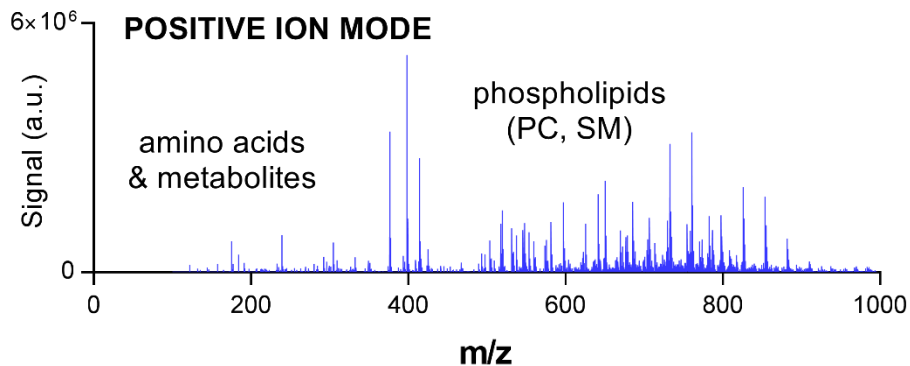
Hit Triage & Potency (IC50)



- All Hits are checked
 - Biological
 - NOT Chemical
- 4% Hits in this study
 - 11,000 compounds
 - 3,000 after initial triage
- Surviving Hits
 - Reactivity / Potency
 - IC50 Curves

Towards high-content label-free cell-based assays

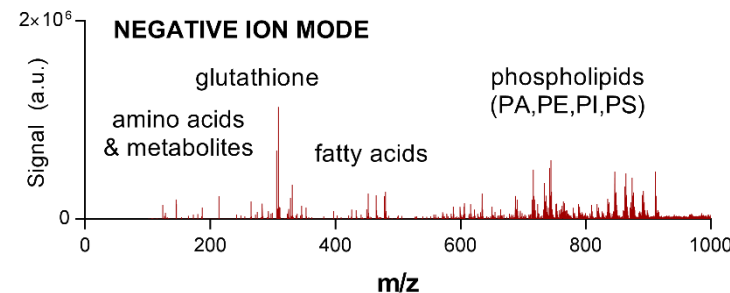
- Direct infusion ESI-MS (/MS) is known to work with crude cellular extracts (e.g. Zamboni)
- Analyte coverage depends on extraction solvents and sample preparation
- Acoustic mist can be generated from aqueous buffers and up to 100% organic solvents
- MCF7 cells lysed in 0.2% aq. AcOH – 100s of species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



Automated cellular screening

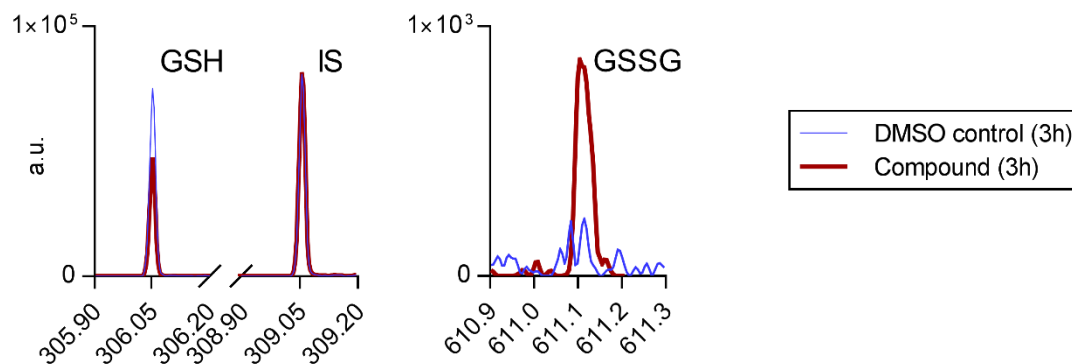


- Fully automatable process
- Tissue-culture plate coating suppresses ionisation
- Plate transfer will be removed in the future
- Estimated capacity ~20,000 compounds a day



Seeing more than cell death

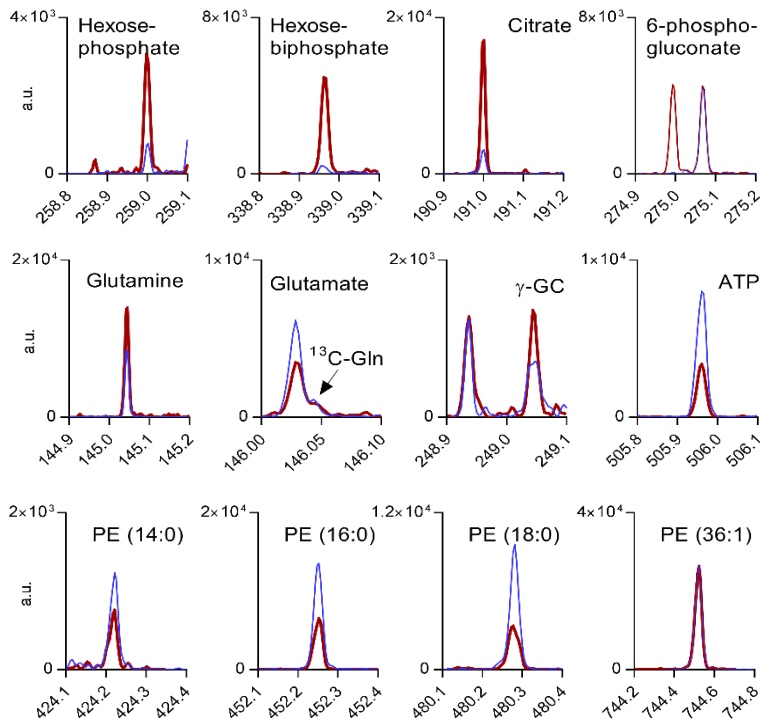
- 250,000 compounds screened in vitro against GSR using AMI-MS
- Counter-screen to remove chemically reactive compounds
- ~70 most potent in vitro actives dosed into cells
- Some compounds showed the desired profile (cell death, GSH depletion, GSSG build up)



- 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:



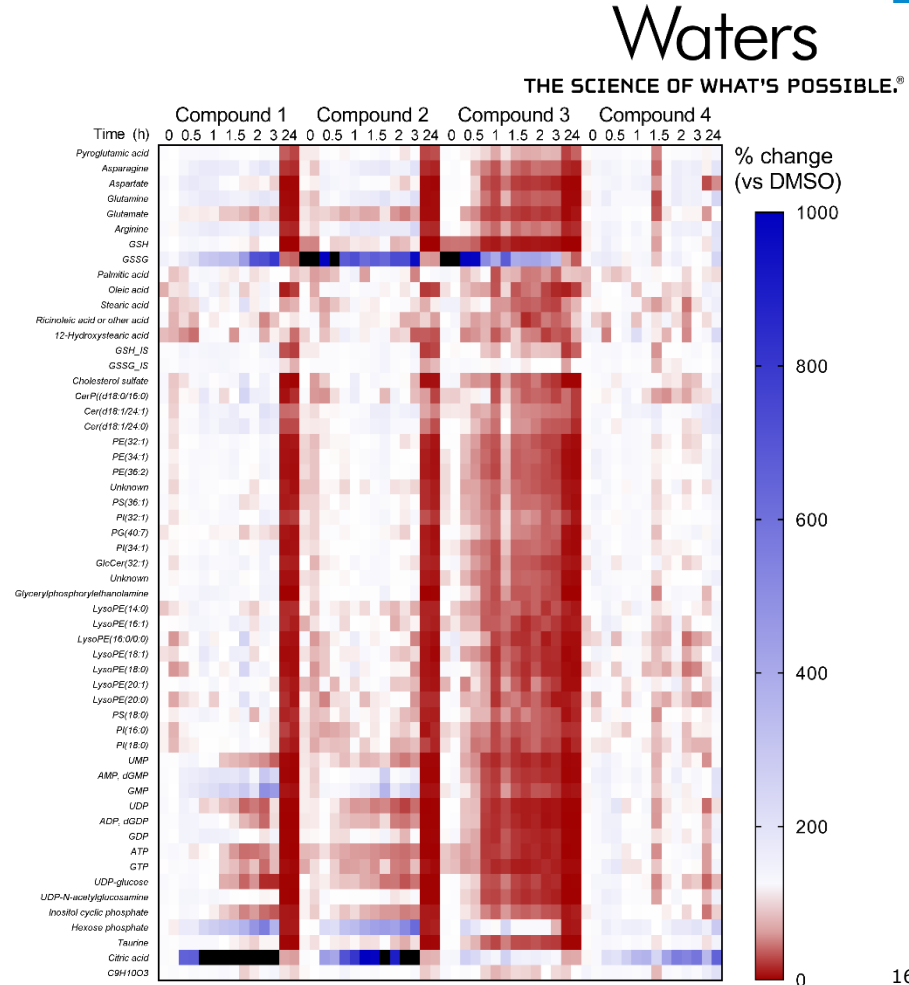
Activation of glycolysis and pentose phosphate pathway, producing NADPH

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

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

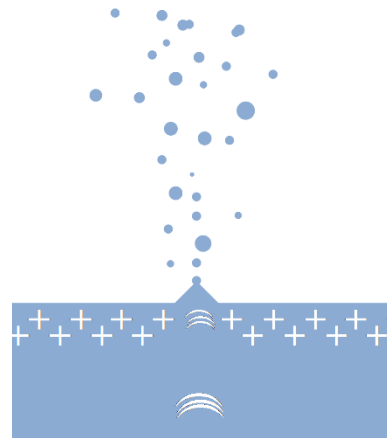
Seeing more than cell death

- 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



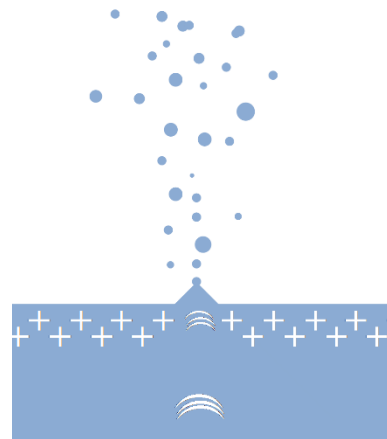
Other promising applications under development

- 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



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

Steve Pringle

Mike Morris

Rhys Jones

Richard Chapman

Emmy Hoyes

Ed Sprake

