AstraZeneca

LABCYTE

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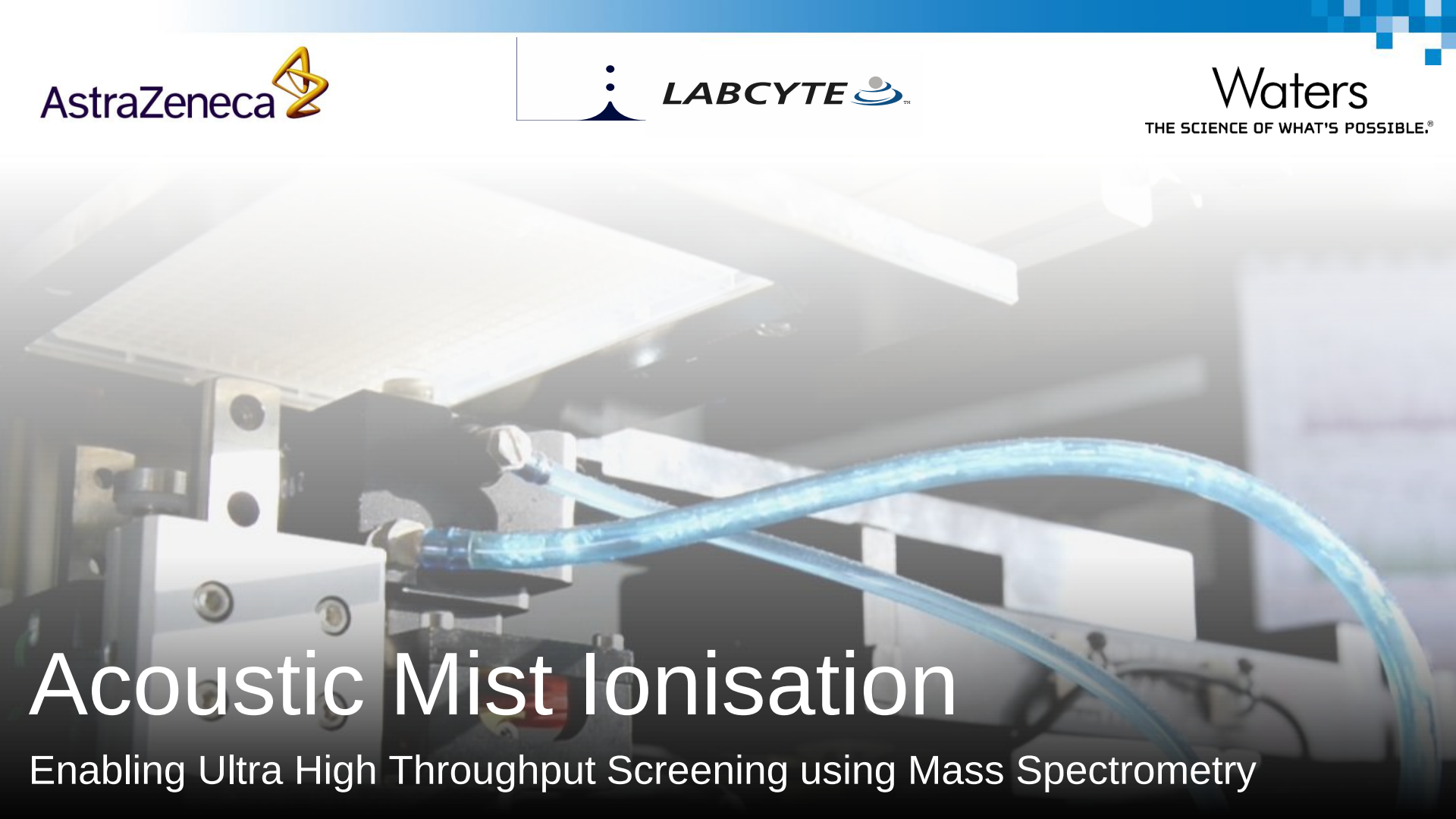
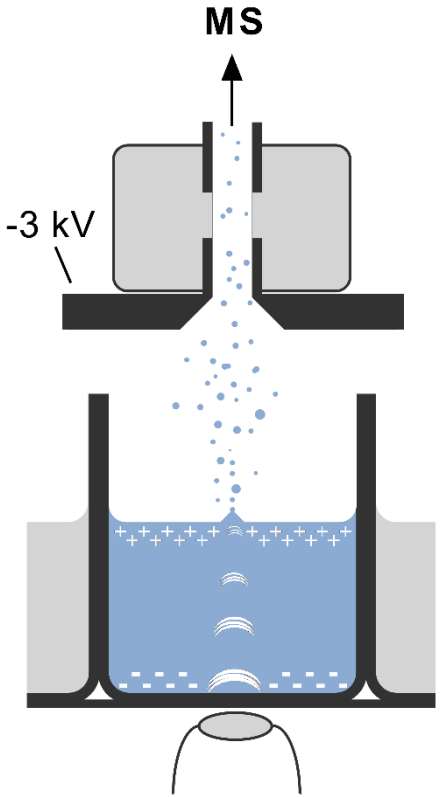
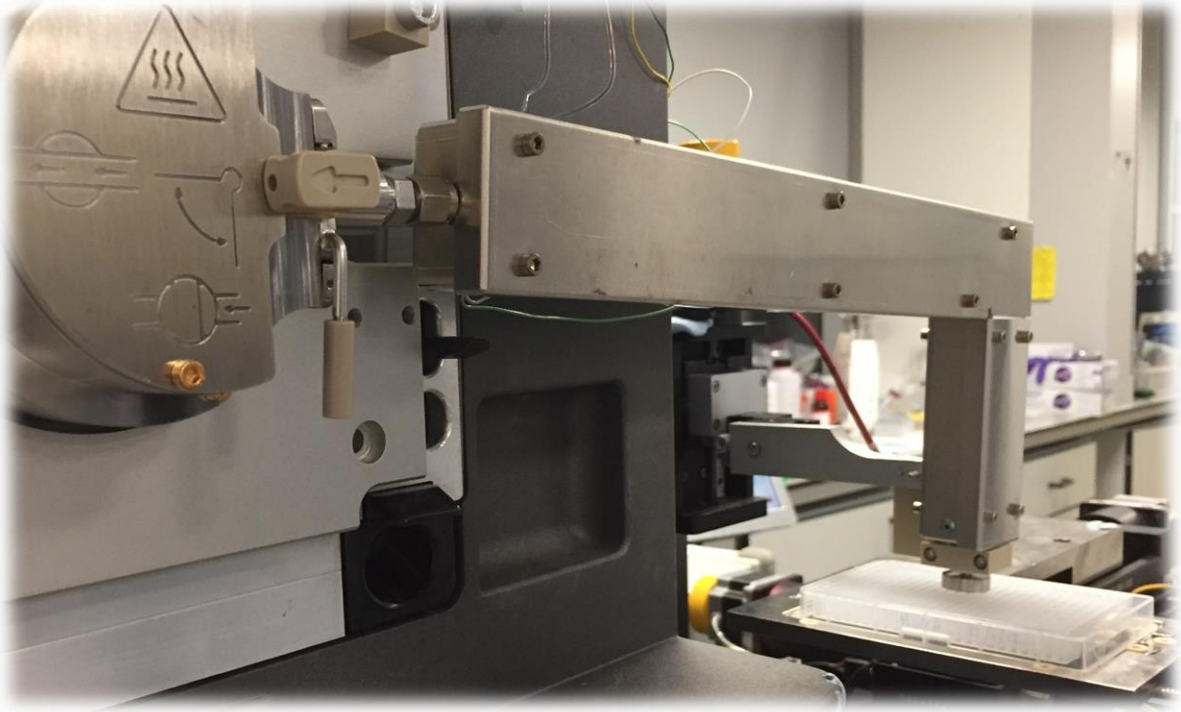


Acoustic Mist Ionisation

Enabling Ultra High Throughput Screening using Mass Spectrometry

Acoustic mist ionisation – mass spectrometry (AMI-MS)





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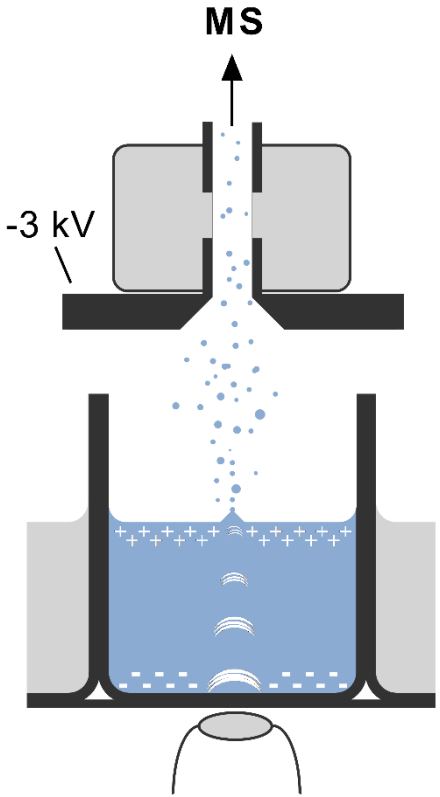

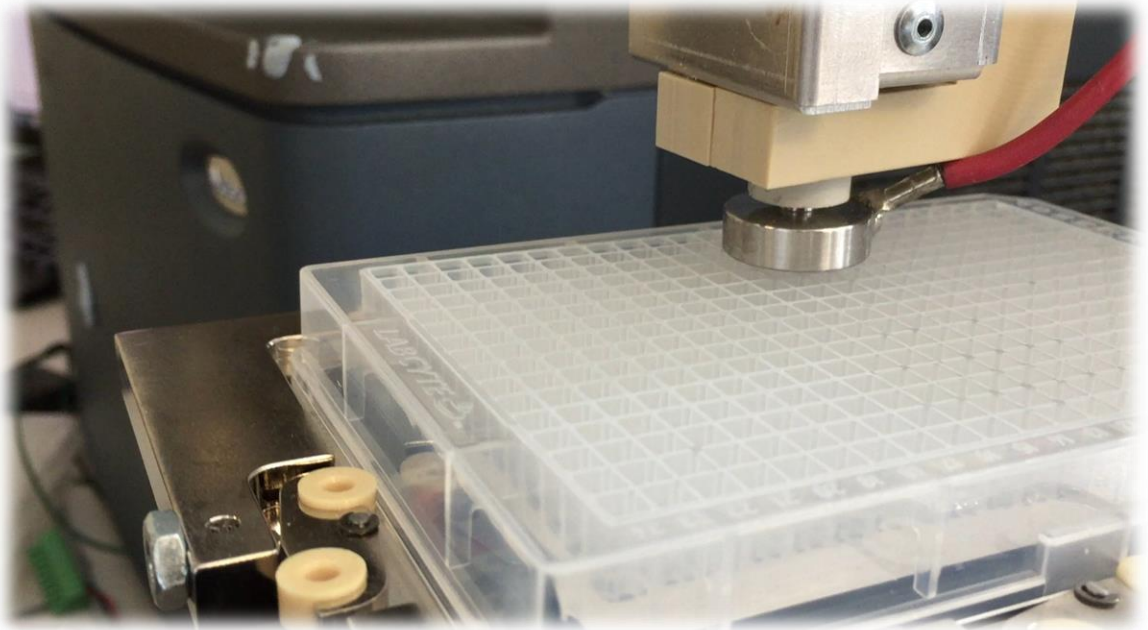

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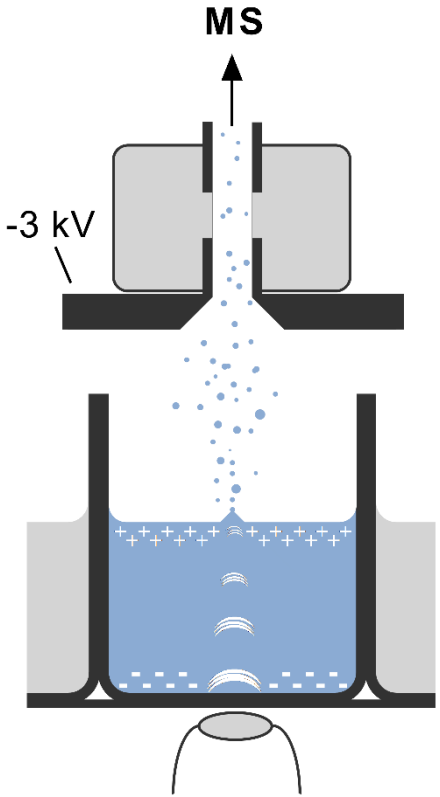

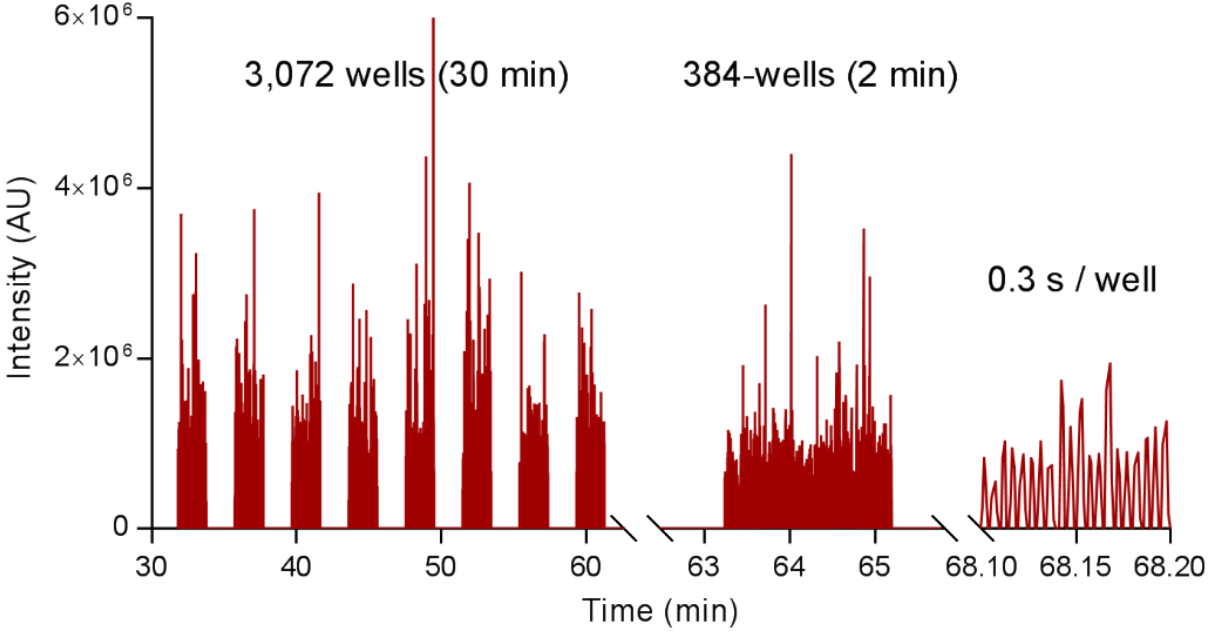

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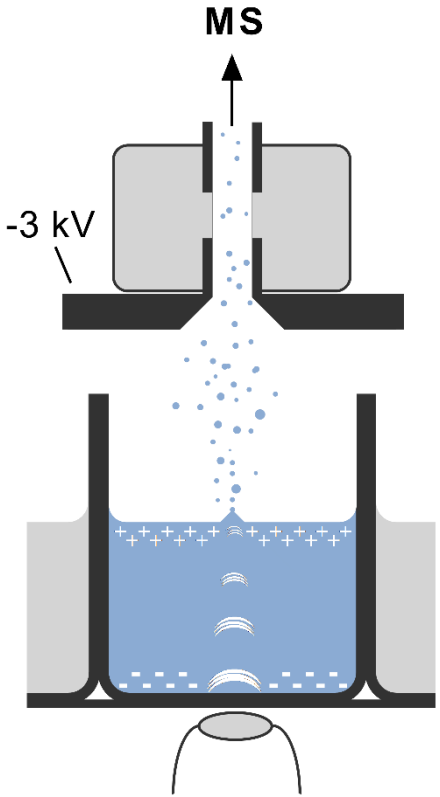
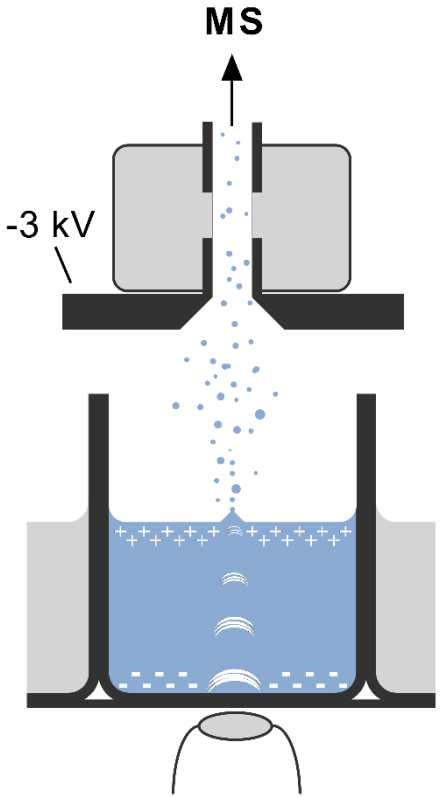
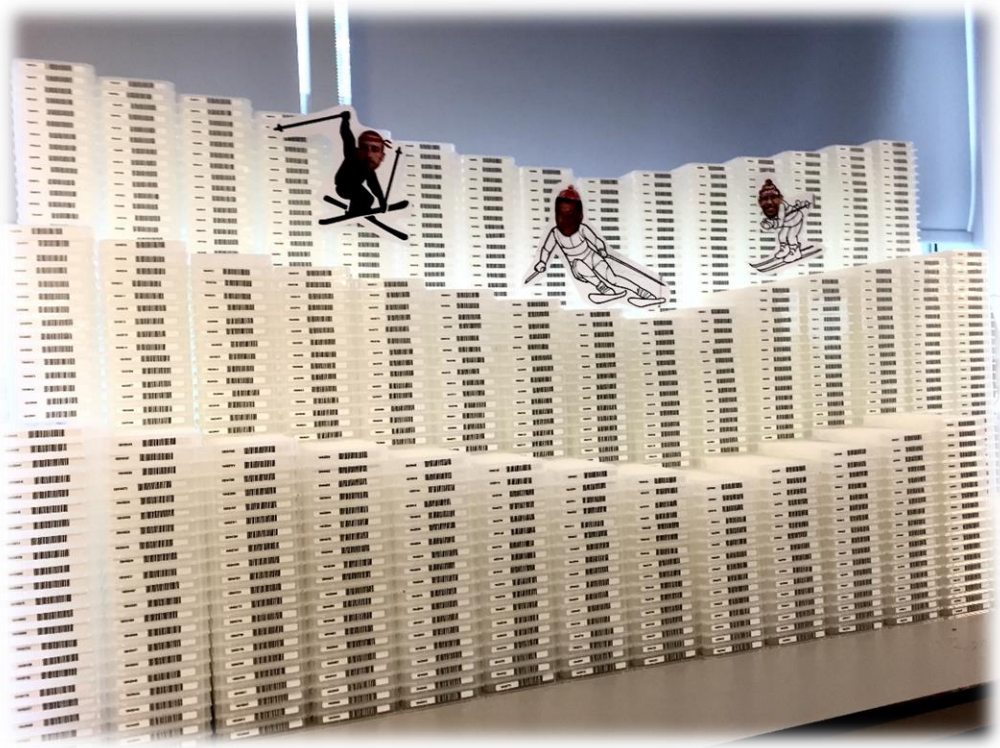


- Direct infusion electrospray-type ionisation
- CONTACTLESS. No carryover.
- Adjustable flow rates, typically 1-10 µl/min
- Flexible infusion time from 250 ms to hours
- Autosampler capacity 150 plates (57,600 samples)
- Primary use for biochemical screening
- > 2.8M samples acquired in 7 weeks

Acoustic mist ionisation – mass spectrometry (AMI-MS)

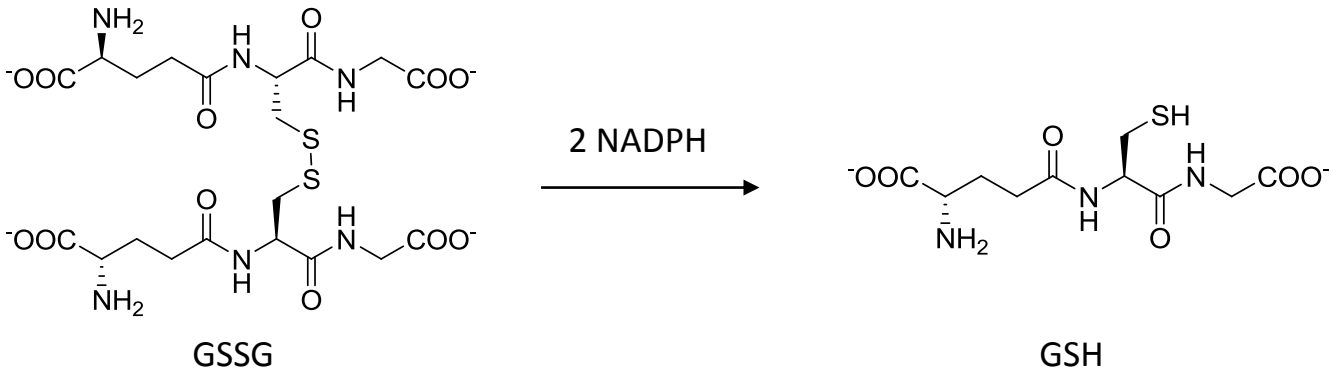




1 week. 1,300 x 384-well plates. 500,000 samples

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



GSSG GSH

- 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

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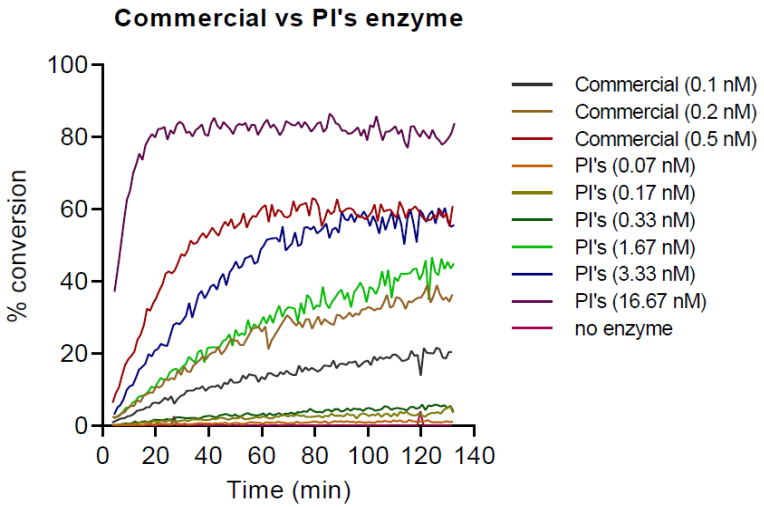
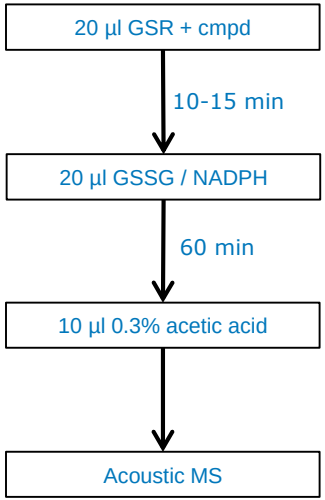
- Current Practice
  - > 6 months assay development
    - Fluorescent Markers (e.g. Thiol Green)
    - Bind to any GSH produced
  - Non-specific
    - Not looking directly at the biology
  - Optical readout
    - Fast

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

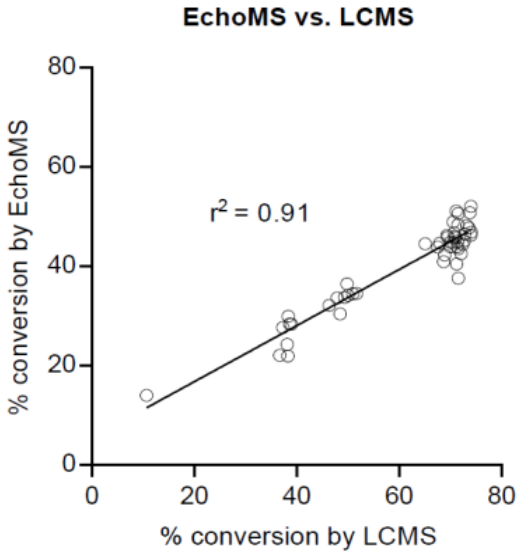
Assay Development



N=3 wells of multiple enzyme conditions



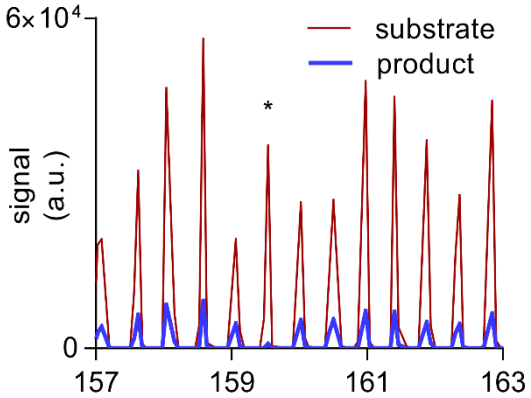
Pick conditions appropriate for batch processing time



Validate output against LCMS

Biochemical Assay

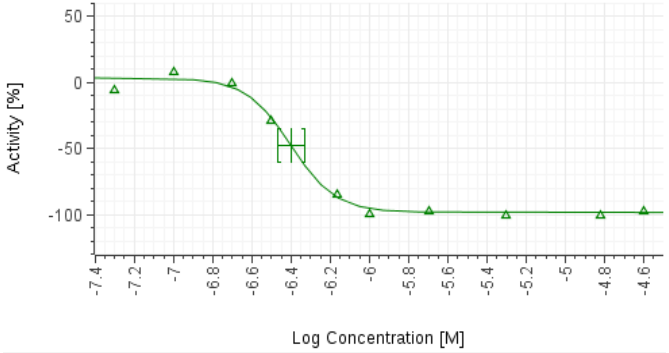
- First 50,000 samples acquired in ~28 hours
- Next 240,000 samples acquired in ~66 hours after further improvements
- Z' of >0.55 across the second batch
- **Line of sight to >200,000 samples/day**

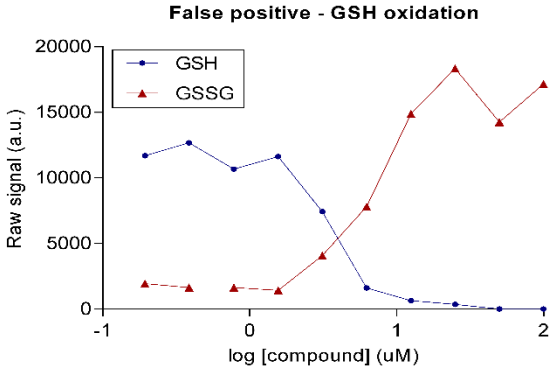


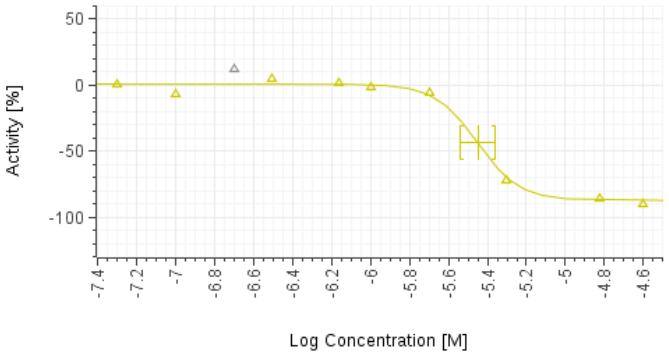
White = inactive
Blue = hit/partial hit
Red = agonist

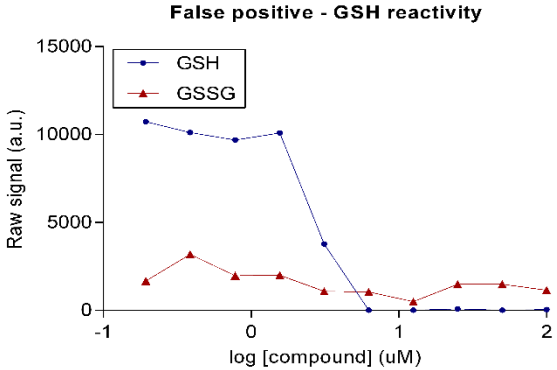
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	-22	21	20	23	2	5	-6	-41	12	6	31	-100	-19	-17	1	13	-10	32	11	-24	-6	8	6	1
B	-12	12	-39	1	-5	12	14	0	-5	8	-19	-100	1	-23	-22	8	-7	-40	8	-4	14	-3	-11	-10
C	8	-24	10	-9	7	-3	18	-35	-38	2	7	-99	0	21	-20	-1	-25	-18	9	-1	16	-10	-9	-23
D	15	7	7	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	-69	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

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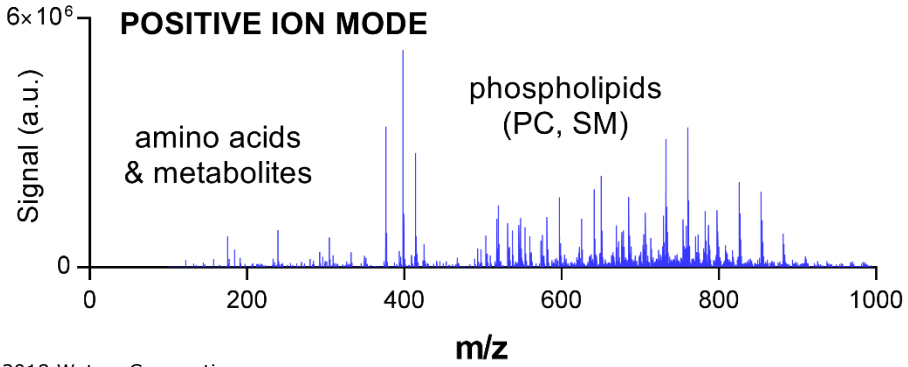
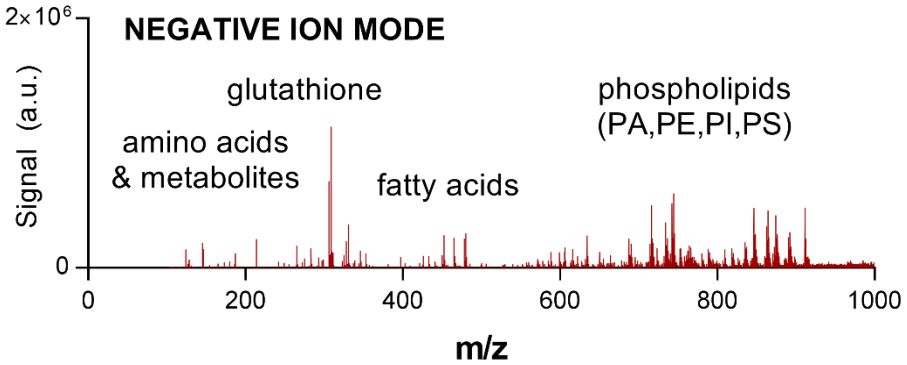
- Direct infusion ESI-MS (/MS) is known to work with crude cellular extracts (e.g. Zamboni)
- Analyte coverage depends on extraction solvents and sample preparation
- Acoustic mist can be generated from aqueous buffers and up to 100% organic solvents
- MCF7 cells lysed in 0.2% aq. AcOH – 100s of species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





Automated cellular screening

Adherent cells
1-10k per well

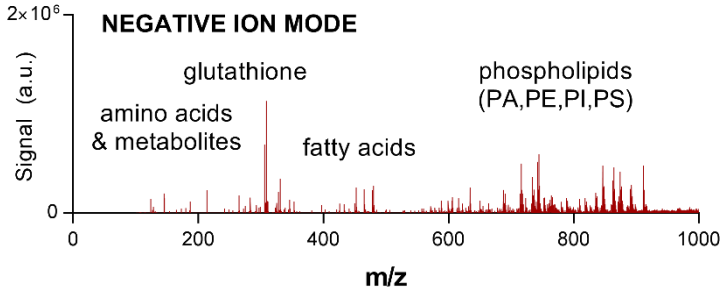
Dose cells with compound
(Echo® dispenser)

Inverted spin
(BluWasher)

Add 0.2% aq. AcOH
& internal standards
(MultiDrop™ Combi)

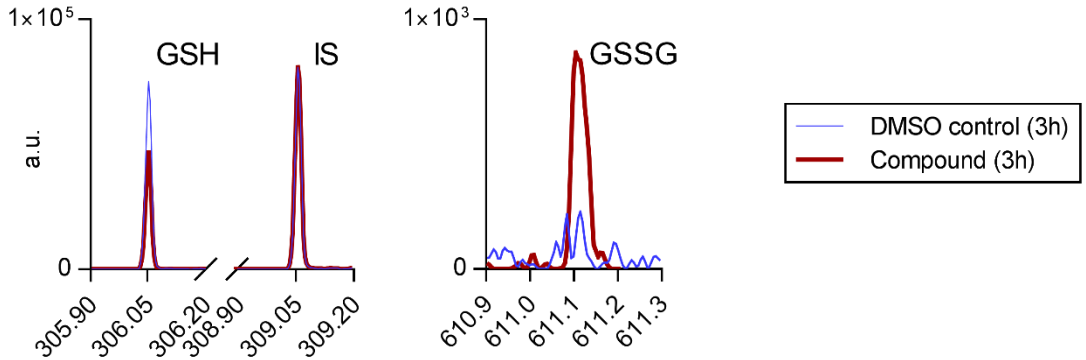
Plate transfer
(CyBio® Felix)

- 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

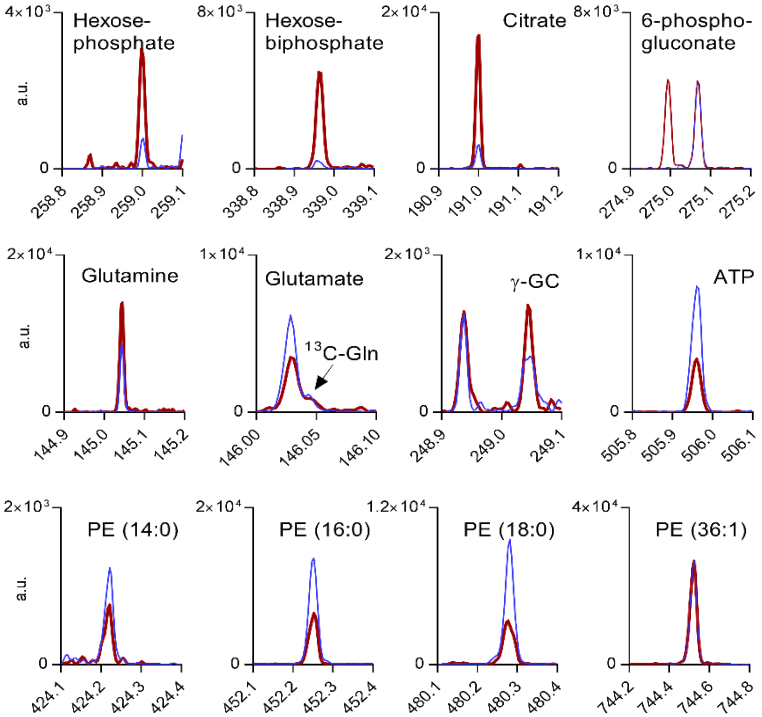
- 250,000 compounds screened in vitro against GSR using AMI-MS
- Counter-screen to remove chemically reactive compounds
- ~70 most potent in vitro actives dosed into cells
- Some compounds showed the desired profile (cell death, GSH depletion, GSSG build up)



- 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:



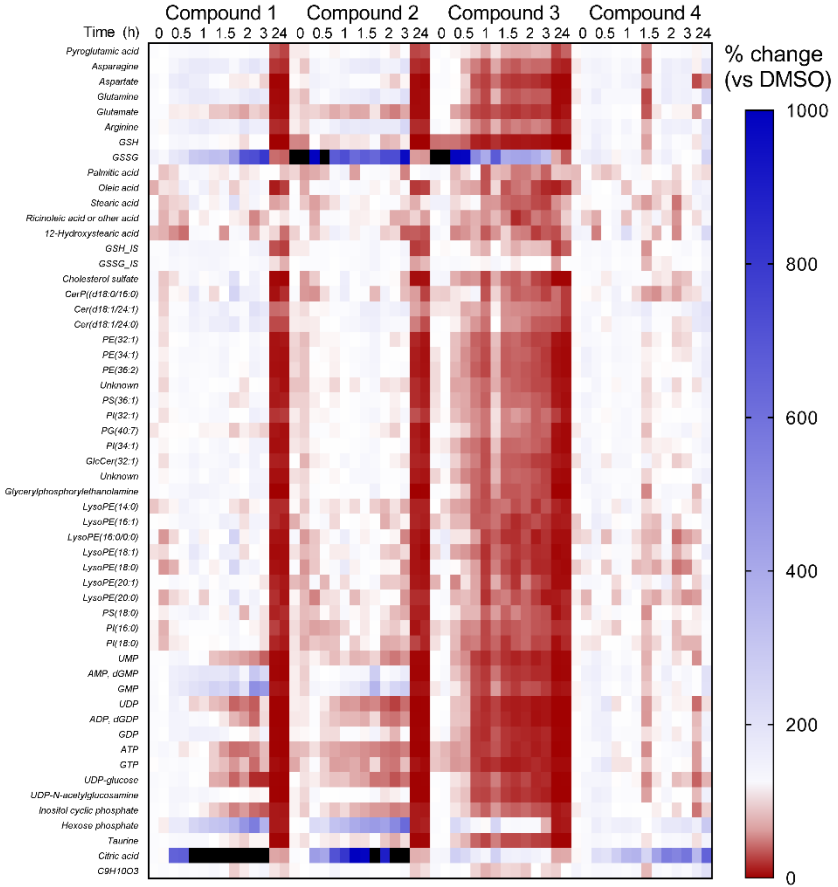
Activation of glycolysis and pentose phosphate pathway, producing NADPH

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

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

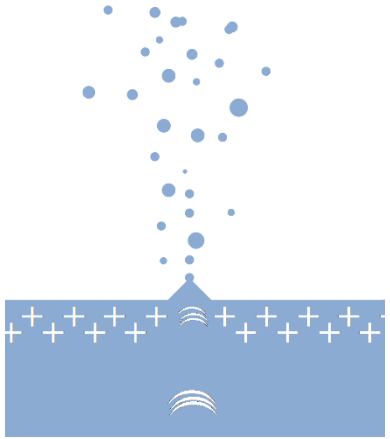
Seeing more than cell death

- 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



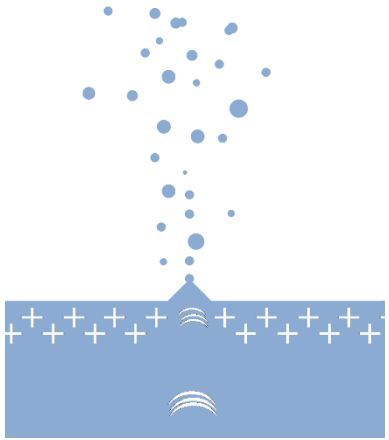
Other promising applications under development

- 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



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