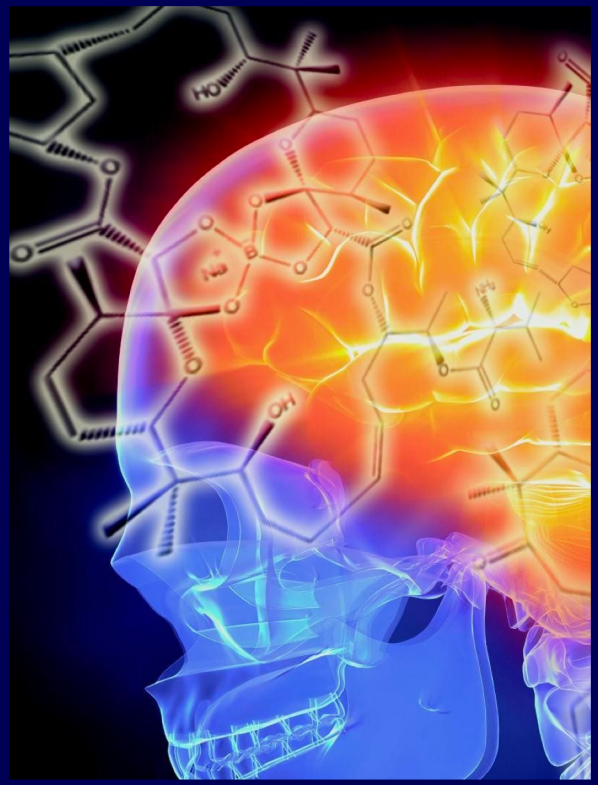


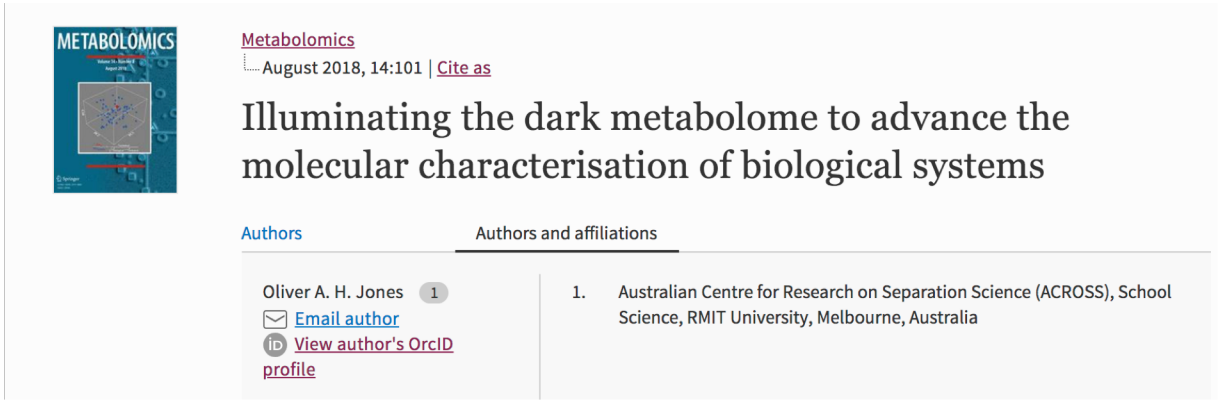
Illuminating the Dark Metabolome

Associate Professor Oliver A.H. Jones

RMIT University

RMIT UNIVERSITY

What is the Dark Metabolome



- "*all the metabolites present in a system that are either not extracted and/or not seen using standard analytical methods, or are lost/transformed during extraction*".

RMIT UNIVERSITY

Why do you think we don't see metabolites?

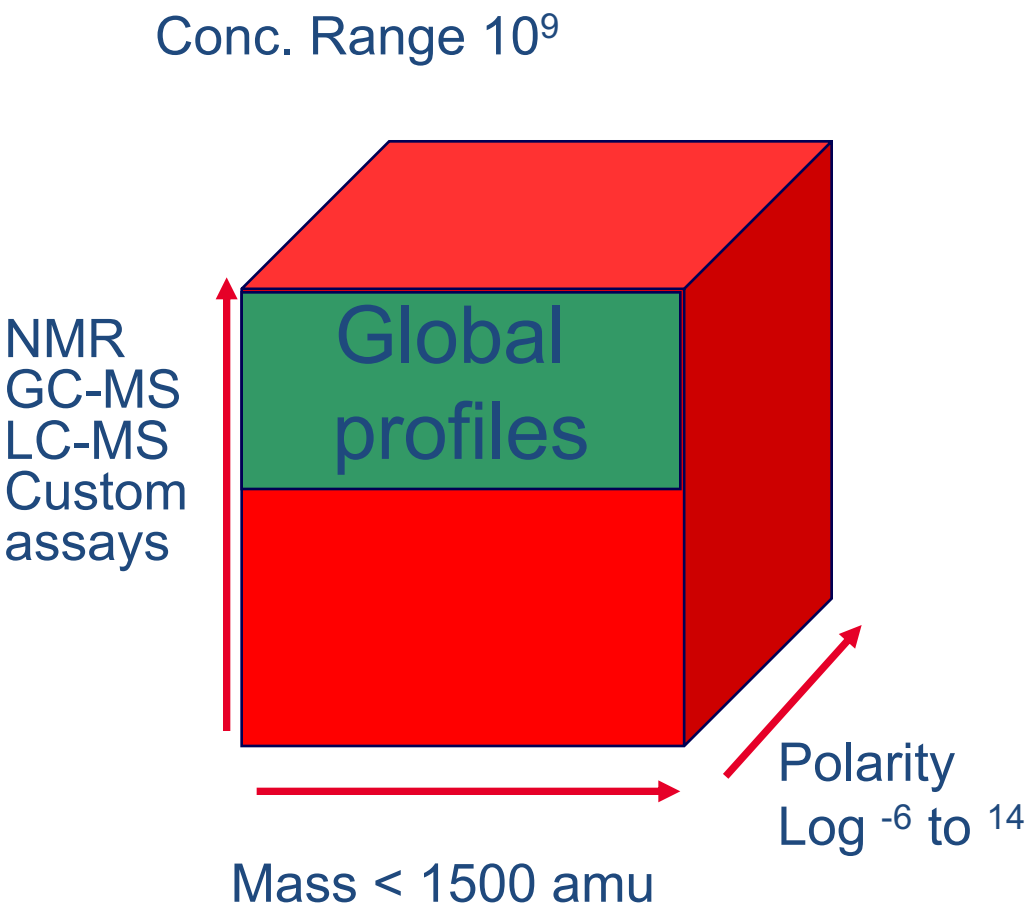
Metabolite Numbers

- The latest version of the Human Metabolome Database (version 4.0) lists 114,100 individual entries (~threefold increase from version 3.0.
- Includes large quantities of predicted MS/MS and GC-MS reference spectral data as well as predicted (physiologically feasible) metabolite structures.
- Actual number of human metabolites could be higher.
- Not counting anthropogenic compounds (e.g. pollutants)
- How many do you identify in your metabolomics studies?

RMIT UNIVERSITY

How many Metabolites?

- Just considering one class there are a huge number of permutations
  - 40 common fatty acids
  - 40 FA acyl CoA
  - 64000 TAGs
  - 120 1-, 2-, 3-MAG
  - Total = 69,000



RMIT UNIVERSITY

Why do you think we don't see metabolites?

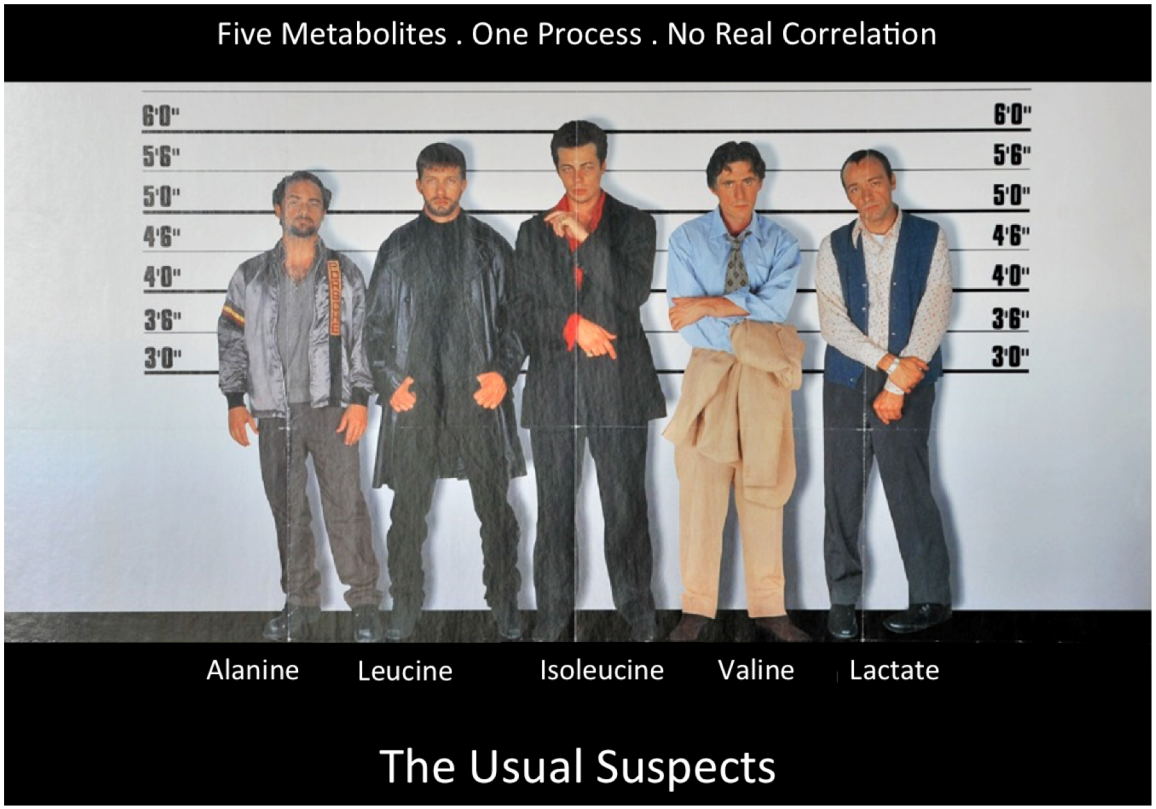
Metabolite Extraction

- Do we extract all metabolites present?
- Do we see and/or identify all the metabolites that we extract?
  - Some metabolites transformed in extraction
- "What happens to metabolites bound to proteins or lipids, or those that are sensitive to light, heat or organic solvents?"

RMIT UNIVERSITY

Why bother?

- "*Our results indicate that (insert experiment here) resulted in a change in amino acid levels and energy metabolism*".



This was a problem in 2005

RMIT UNIVERSITY

So we need to extract and identify more metabolites and we need to do it without altering them.

How?

RMIT UNIVERSITY

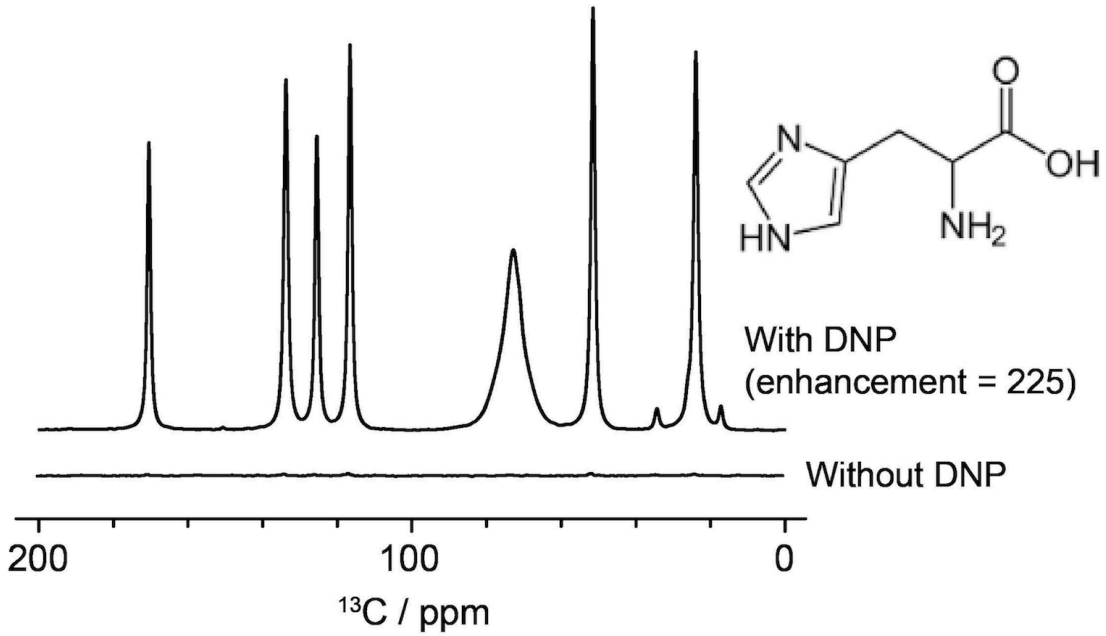
Reinvigorate old technology

- NMR is a main stay of Metabolomics but has sensitivity issues

- Dynamic Nuclear Polarization (DNP) is technique that can be used to enhance the sensitivity of NMR by combining electron paramagnetic resonance phenomena with NMR experiments

RMIT UNIVERSITY

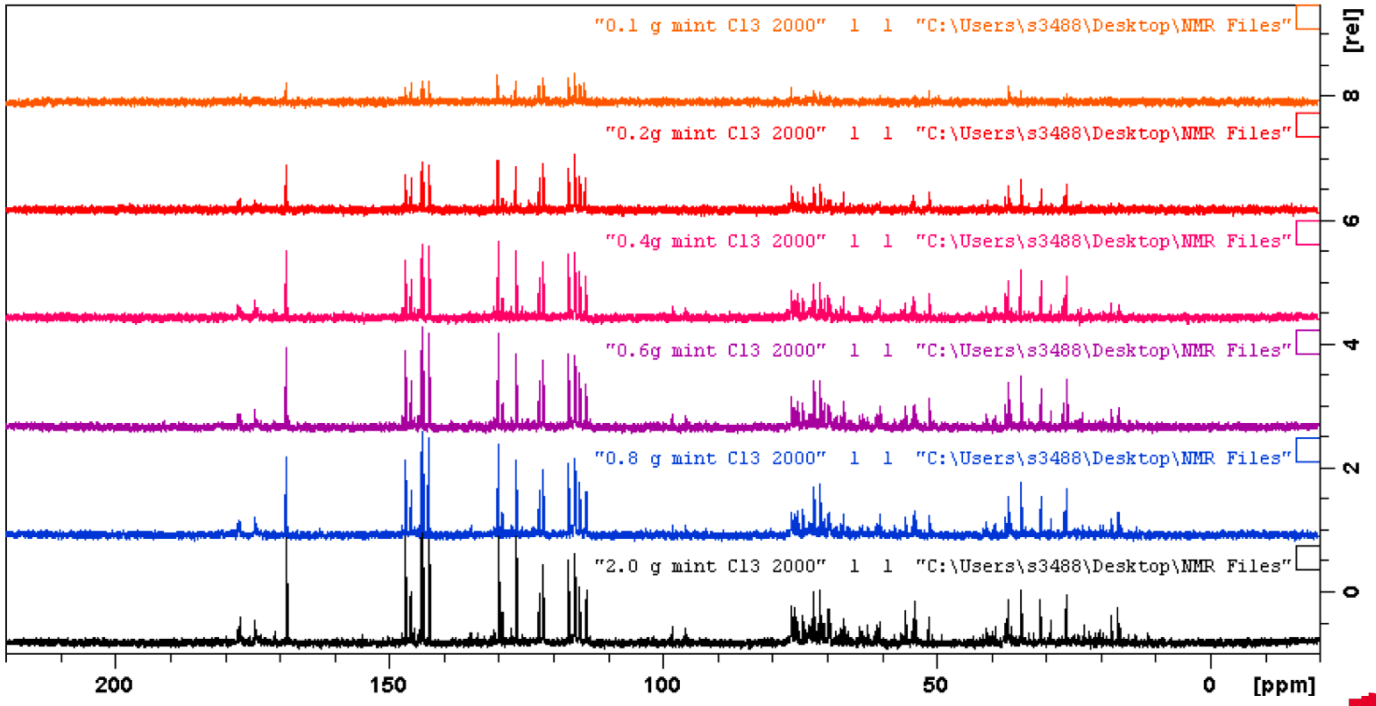
DNP



RMIT UNIVERSITY

Other Advantages – alternative nuclei

- Far less NMR sensitive than ^{1}H, ^{13}C is widely distributed in biological molecules.



RMIT UNIVERSITY

^{31}P

- Less NMR sensitive than the hydrogen and fluorine nuclei but more sensitive than ^{13}C.
- Phosphorus is well distributed in biological systems and plays a role in several important biological processes, including energy metabolism,
- ^{31}P NMR yields sharp lines and has a wide chemical shift range and thus has featured in some metabolism studies, for example environmental toxicology.

RMIT UNIVERSITY

Problems

- DNP is carried out at very low temperatures (100 K or -173.15 ^{o}C)
- Relies on the use of polarising agents such as 1-(TEMPO-4-oxy)-3-(TEMPO-4-amino)propan-2-ol (TOTAPOL) as a source of unpaired electrons
- But increasingly it is being carried out cheaply and at higher temperatures

RMIT UNIVERSITY

Problems

- Another option is to create new polarising agents.

- New lipophilic biradicals based on a cholesterol scaffold could be used, for example, to obtain homogenous DNP enhancement throughout a lipid bilayer and any metabolites attached to it *in situ*.

RMIT UNIVERSITY

What about Chromatography?

Biochem J. 1966 Dec;101(3):792-810.

A gas-liquid-chromatographic procedure for separating a wide range of metabolites occuring in urine or tissue extracts.

Dalgliesh CE[1], Horning EC, Horning MG, Knox KL, Yarger K.

Author information

Abstract

1. A gas-liquid-chromatographic procedure is described which permits separation and identification on the same chromatogram of a wide range of substances occurring in urine or tissue extracts. The method uses hydrogen flame ionization, which detects organic compounds whether free or conjugated with no requirement for specific reactive groups. 2. For chromatography, carboxyl groups are quantitatively converted into methyl esters or trimethylsilyl esters. Phenolic, alcoholic and potential enolic groups are converted into trimethylsilyl ethers. Separations are carried out on a 6ft. column of either 10% F-60 (a polysiloxane) or 1% F-60, temperature programming at 2 degrees /min. being used over such part of the temperature range 30 degrees -260 degrees as is required. Propionyl derivatives of hydroxy compounds can also be used, but only on a non-quantitative basis. Derivatives and columns have been selected for optimum range of usefulness when large numbers of samples are examined by using automated gas chromatography. 3. The method is applicable to: fatty acids above butyric acid; di- and tri-carboxylic acids; hydroxy acids and keto acids; polyhydroxy and alicyclic compounds such as glycerol, inositol, quinic acid, shikimic acid, ascorbic acid and sugar alcohols; aromatic hydroxy and acidic compounds, both benzenoid and indolic; sesquiterpenes; steroids; glycine conjugates; mercapturic acids; glucuronides. It is not satisfactory for sulphate conjugates, iminazoles or polypeptides. 4. Methylene units provide an accurate and reproducible parameter for characterizing peak position. Methylene unit values are reported for a large variety of substances occurring in, or related to those occurring in, urine and tissue extracts. 5. The nature of derivatives was confirmed by combining gas chromatography with mass spectrometry. Combined gas chromatography-mass spectrometry gives a diagnostic tool of great power in the evaluation of metabolic patterns, and various uses are discussed.

RMIT UNIVERSITY

What about HPLC

- Liquid chromatography, with the detection of over 1000 compounds being reported in certain sample types.
- The theoretical maximum peak capacity for conventional liquid chromatography is ~1500
- The use of very long columns is also required for such detailed analysis, long run times of hours or even days are often required.

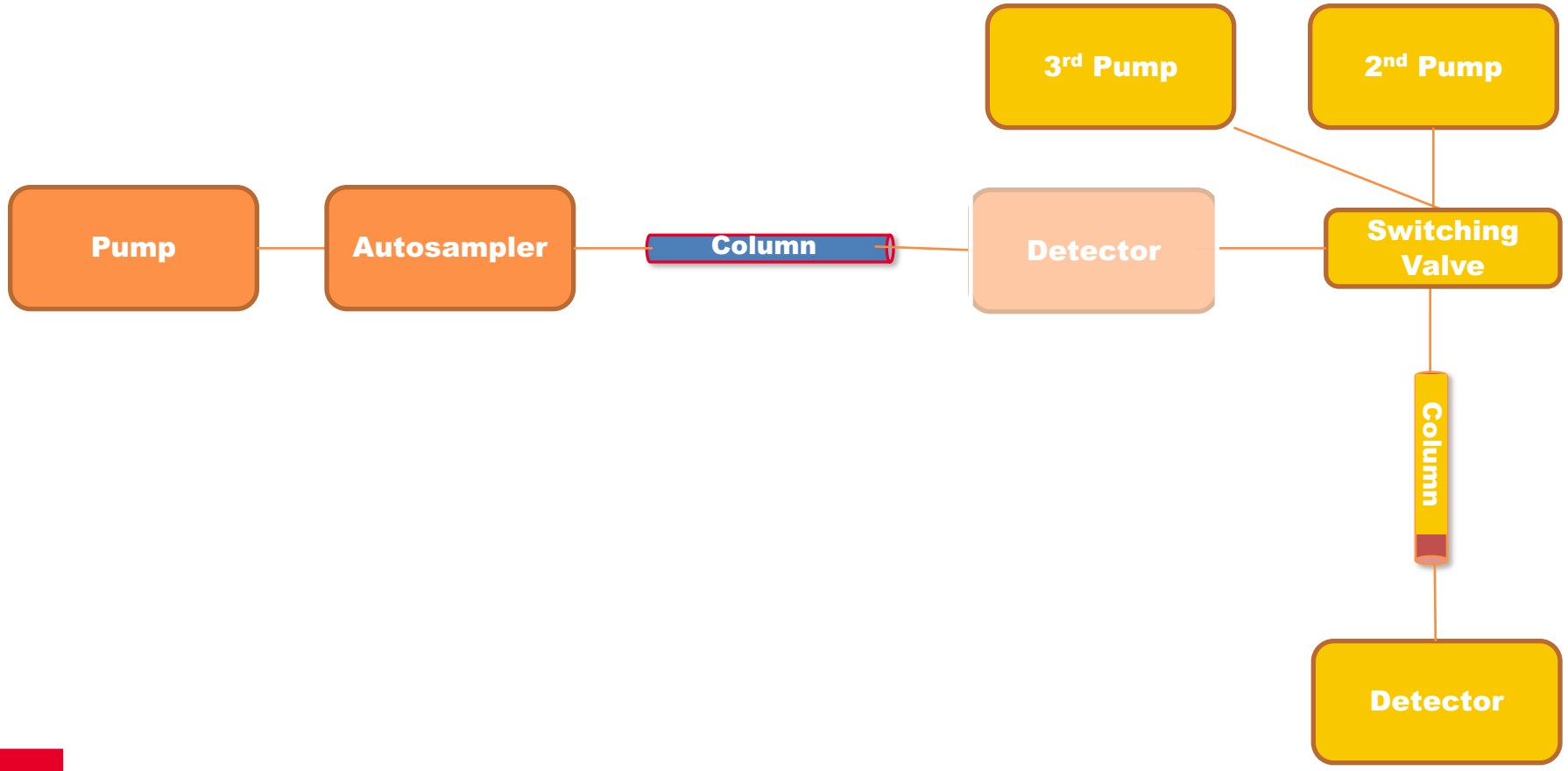
RMIT UNIVERSITY

What else could be used?

- 2D HPLC involves coupling two columns, with uncorrelated retention mechanisms (orthogonal), in series
- During the analysis fractions are collected from the first dimension and injected in the second dimension
- The total peak capacity of the system is thus the product of the peak capacity of each dimension (almost)
- Can heart-cut specific fractions of interest

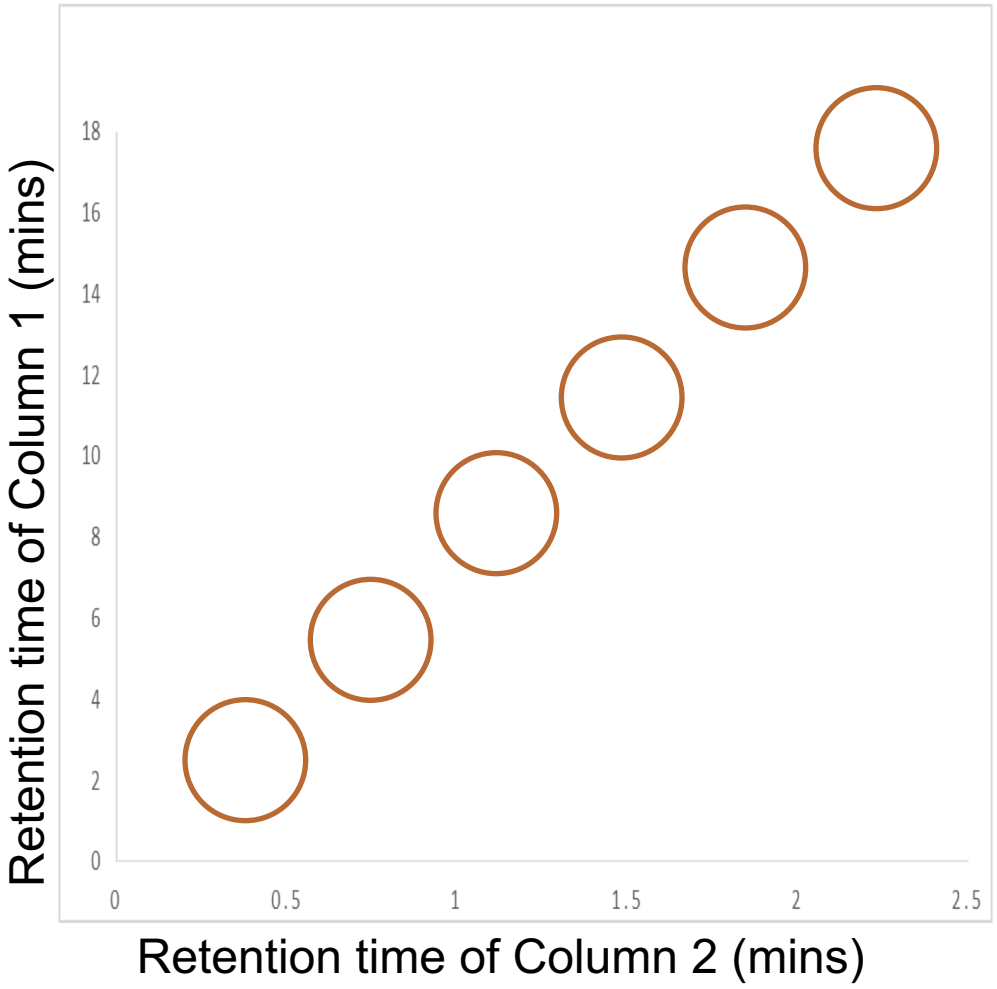
RMIT UNIVERSITY

2D HPLC



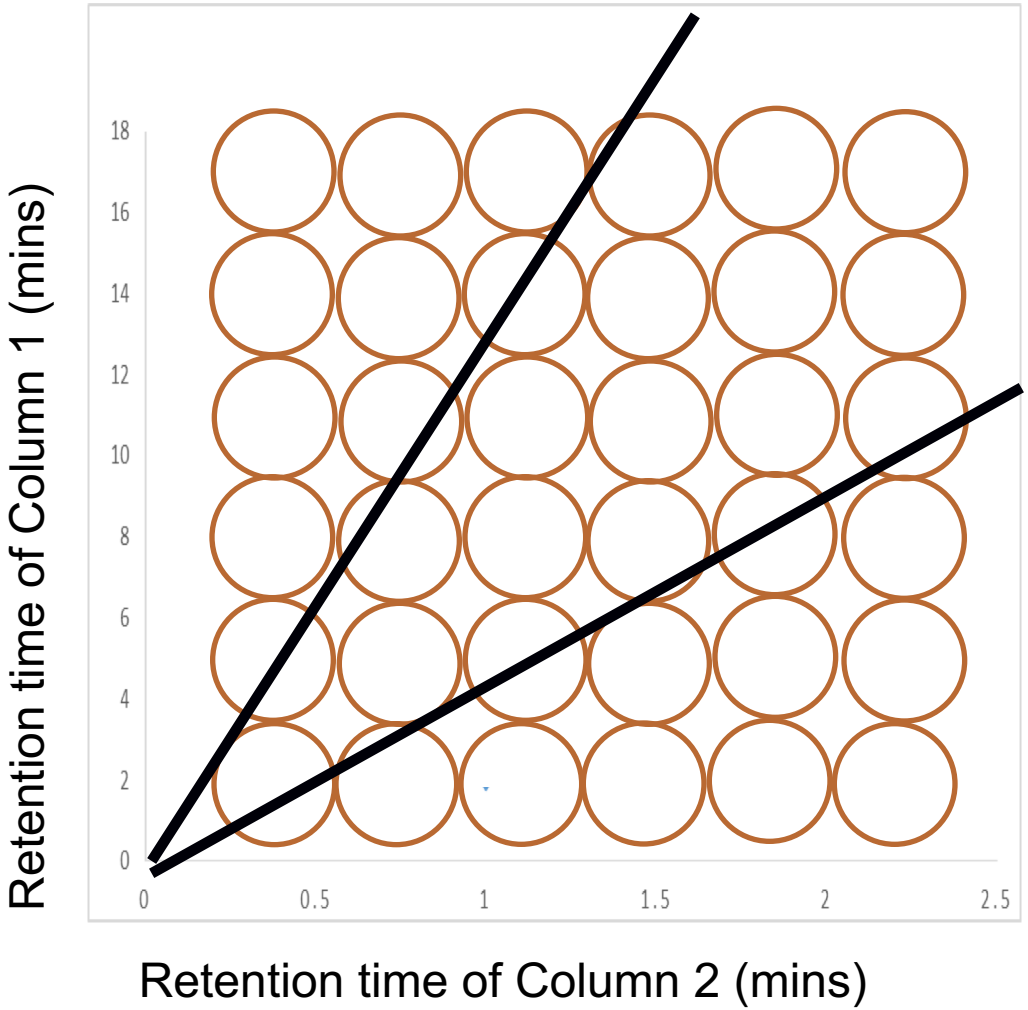
RMIT UNIVERSITY

Pick your phase



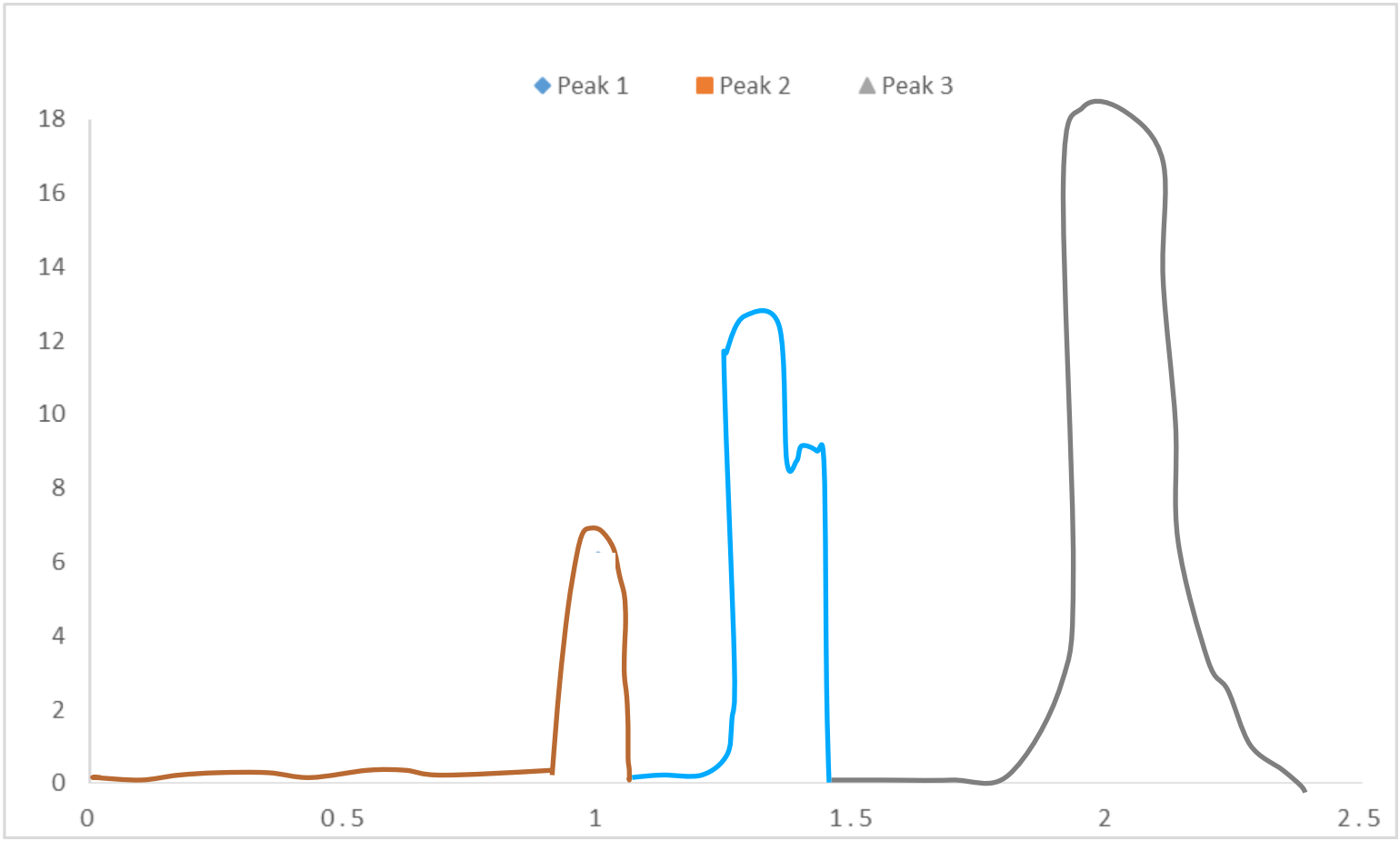
RMIT UNIVERSITY

Separation space



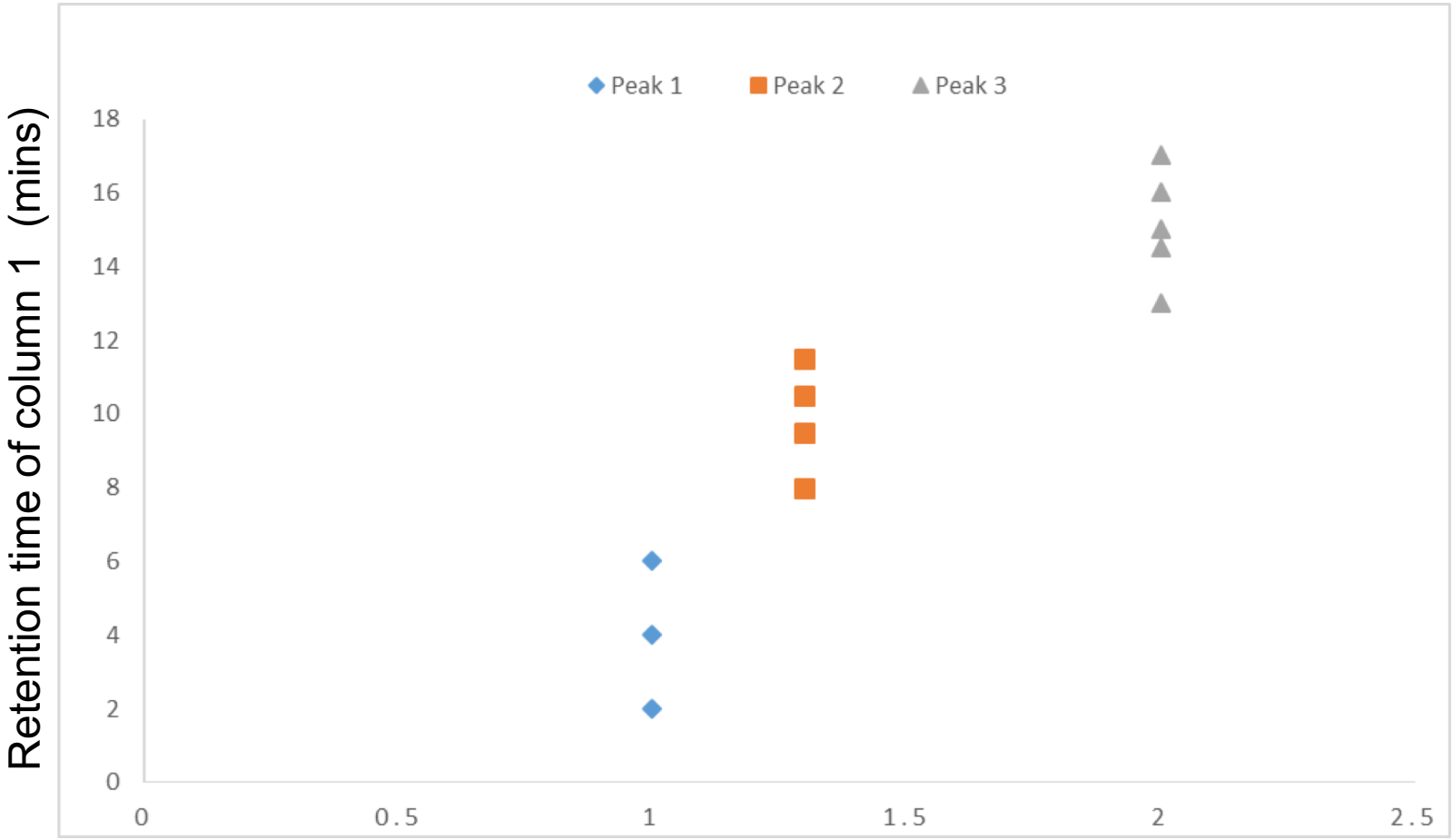
RMIT UNIVERSITY

2D vs 1D



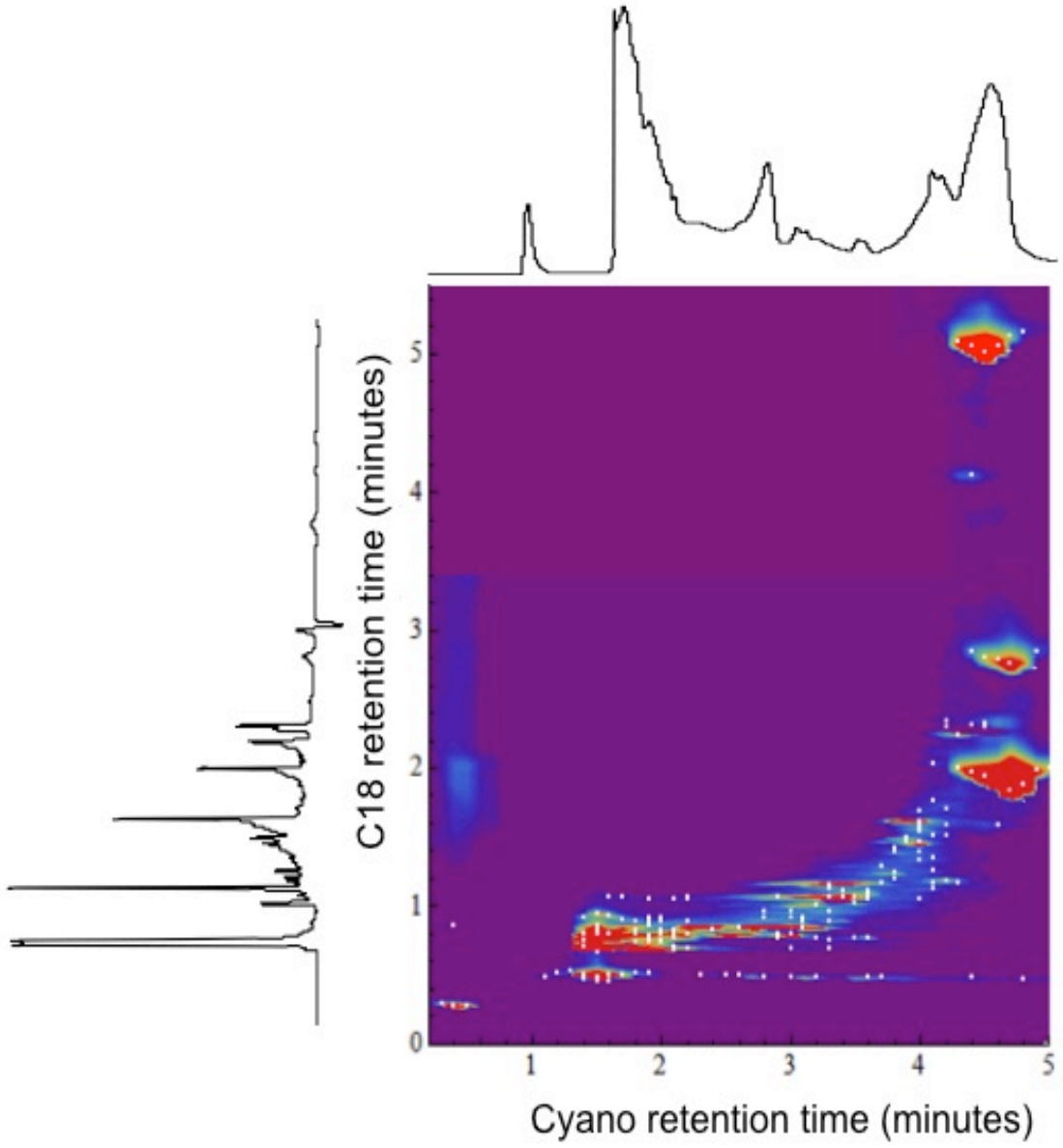
RMIT UNIVERSITY

2D vs. 1D



RMIT UNIVERSITY

2D vs. 1D



RMIT UNIVERSITY

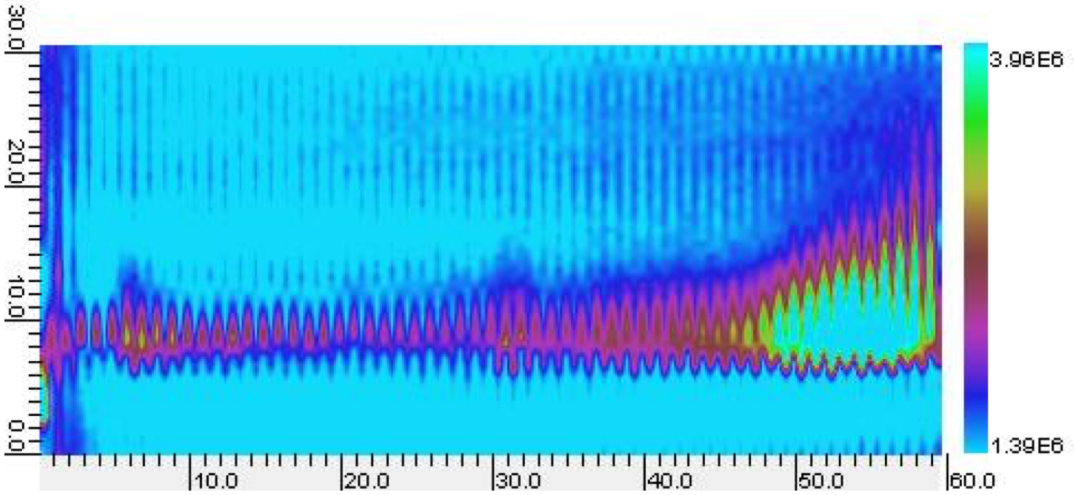
Advantages of 2D HPLC

- Increase separation space
- Increase in total peak capacity
  (1500*1500 = 225,000 - 114,100 = 110,900 'spare' capacity)
- Increase in efficiency and resolution resolution
- Can we predict structure from retention time
- Interesting chemistry to be explored

RMIT UNIVERSITY

Things to be aware of

- Sample dilution
- Column selection
- Solvent mismatch
- Column re-equilibration



RMIT UNIVERSITY

Other Options

- Commercial libraries have been complemented by extensive open-access databases, such as mzCloud and the Human Metabolome Database, containing hundreds of thousands of spectra.
- The creation of in house libraries of pure compounds may be of help but, of course, there can be no in- house libraries made of, as yet, unknown metabolites.
- *In-silico* predictive tools
- Mining existing data – e.g. MetaboLights

RMIT UNIVERSITY

Acknowledgements

PhD Students

- Lydon Alexandrou
- Christine Close
- Jake Collie
- Asal Hajnajafi
- Stuart Hombsch
- Will McCance
- Hugh McKeown
- Tim Ong
- Elvina Parlindungan
- Lada Staskova

Postdocs

- Dr YuFei Wang

Others

- Dr Jess Pandohee
- Dr Paul Stevenson

Australian Government
Australian Research Council

National Research FLAGSHIPS
Food Futures
CSIRO

The Ian Potter Foundation

PROTEOMICS & METABOLOMICS VICTORIA

VICTORIA POLICE FORENSIC SERVICES DEPARTMENT

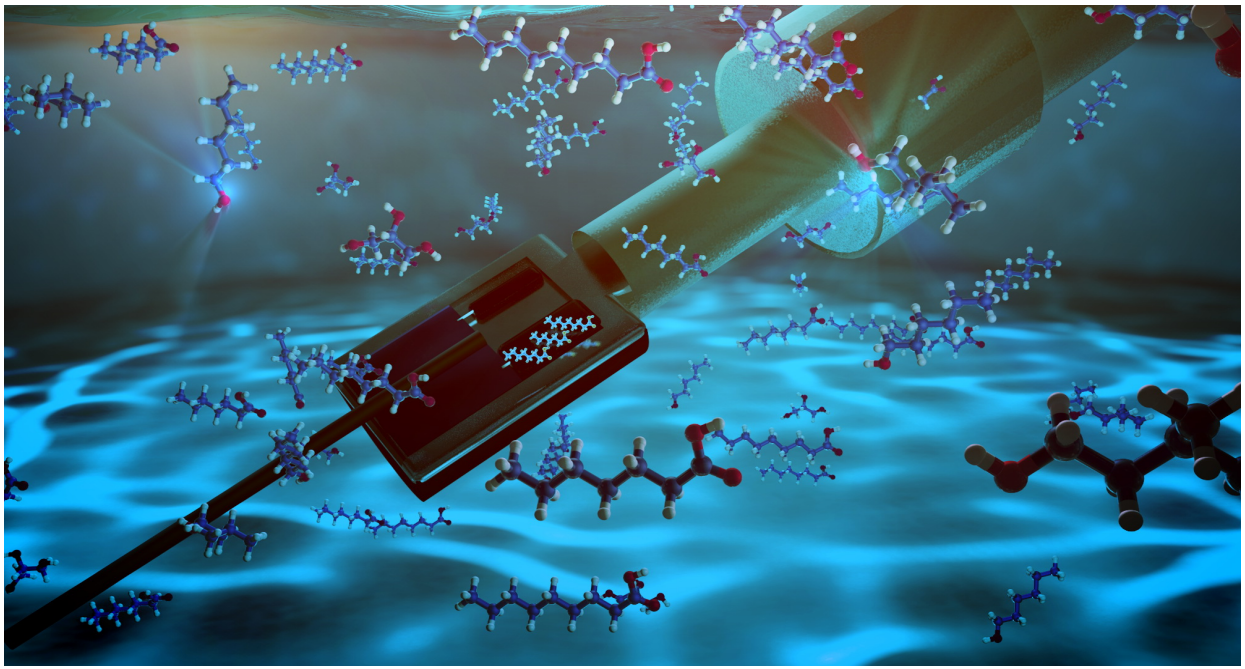
ANZMN
Australia & New Zealand Metabolomics Network

RMIT UNIVERSITY

Thank you for listening

For further information oliver.jones@rmit.edu.au

@dr_oli_jones



RMIT UNIVERSITY