



The
University
Of
Sheffield.

Can we live with iTRAQ?

Phillip C Wright

ChELSI Institute

Department of Chemical and Process Engineering,
The University of Sheffield

- Quantitation is obviously very important
- There are numerous methods for this
- Keys for our lab are
 - Speed
 - Reproducibility / precision
 - Accuracy
 - And, yes, cost

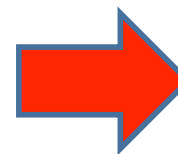
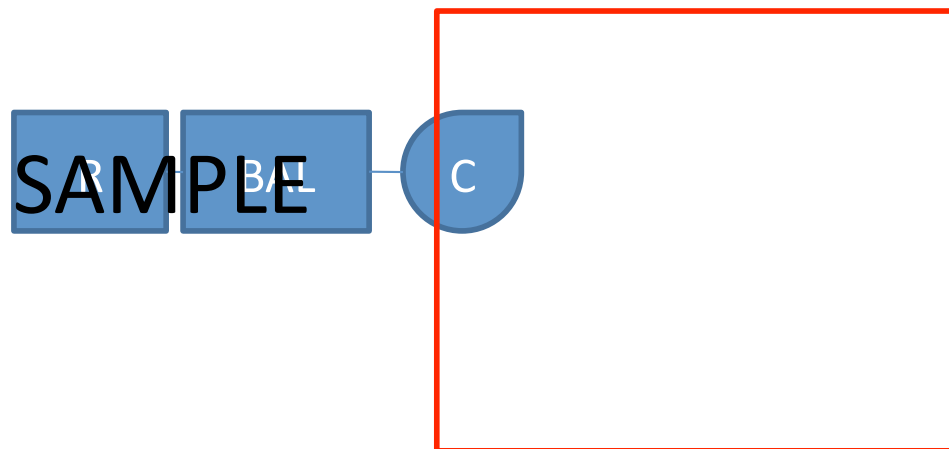
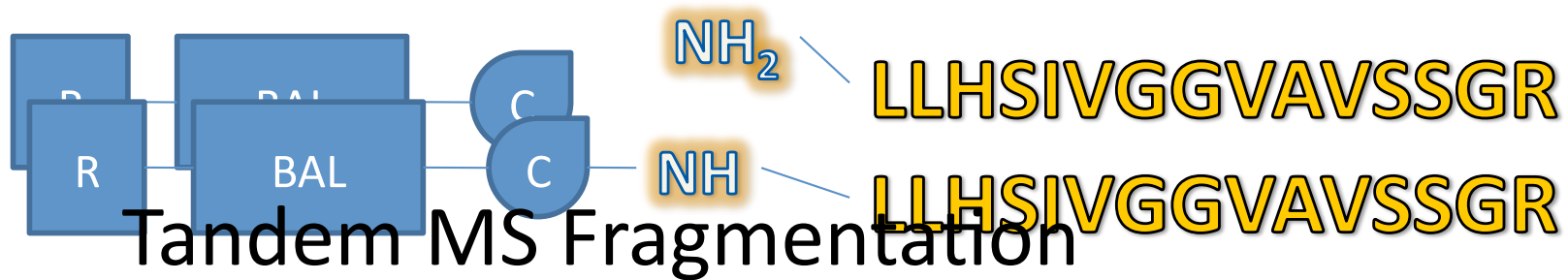
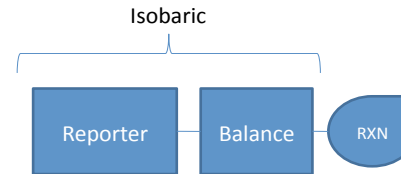
iTRAQ

- iTRAQ - Isobaric Tag for Relative and Absolute Quantification
- Protein/Peptide level isobaric tags
- Highly scalable multiplexing
 - Original reagents were **4 plex**
 - **8 plex** has been around for some time now
- It is NOT a black box

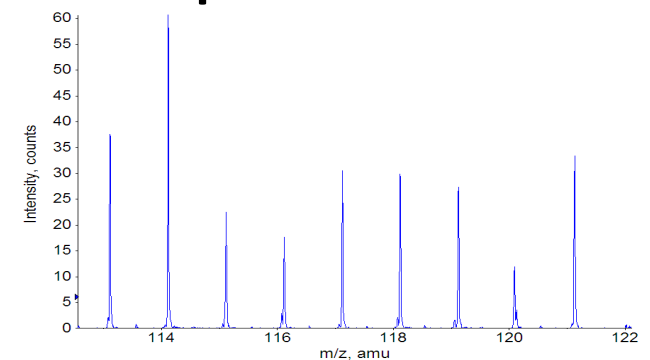
iTRAQ Methodology

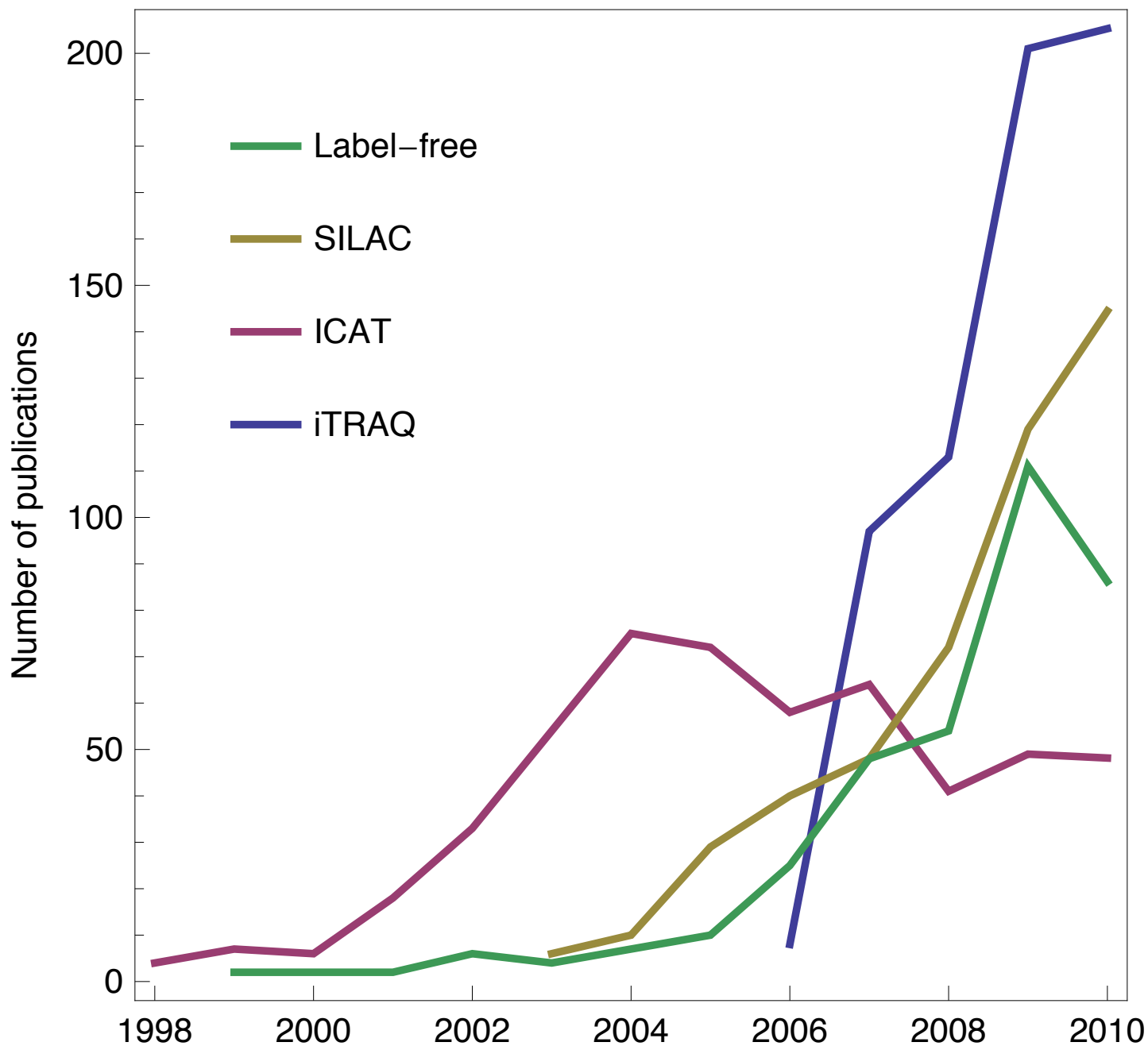
- iTRAQ uses isobaric labels
- Varies only between the mass of 'Reporter' and 'Balance'

Reporter Mass	Balance Mass	Total
113	~ 192	305
114	~ 191	
115	~ 190	
116	~ 189	305
117	~ 188	
118	~ 187	
119	~ 186	305
121	~ 184	



Reporter Ions





**iTRAQ is
very
popular!**

*Overwhelmingly
in the 4 plex
flavour*

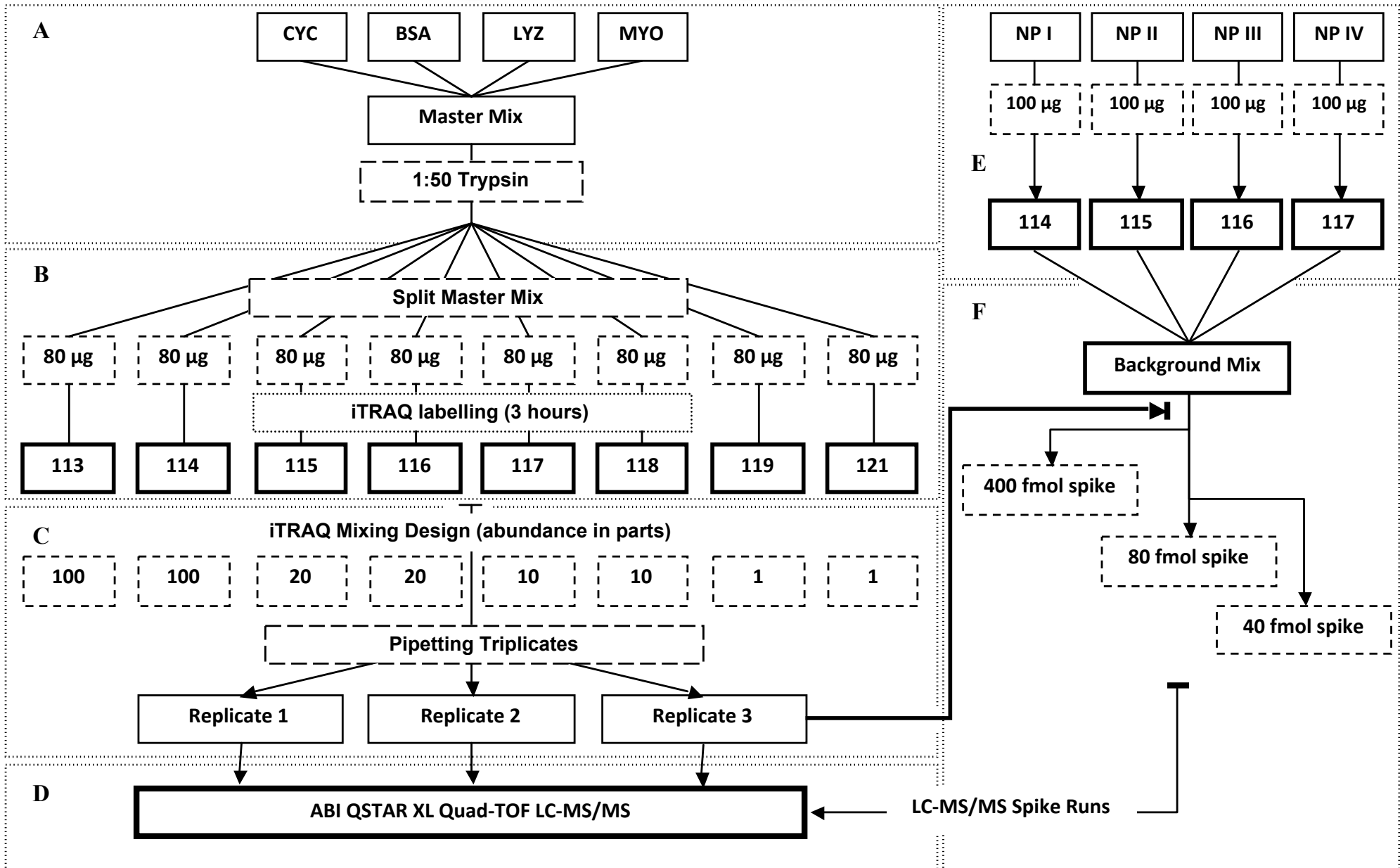
J Noirel, C Evans, M Salim, J Mukherjee, SY Ow, J Pandhal, TK Pham, CA Biggs and PC Wright, *Methods in quantitative proteomics: setting iTRAQ on the right track, Current Proteomics, submitted*

So.....

*Can we live with
ITRAQ?*

Or maybe..... of iTRAQ

How to assess?

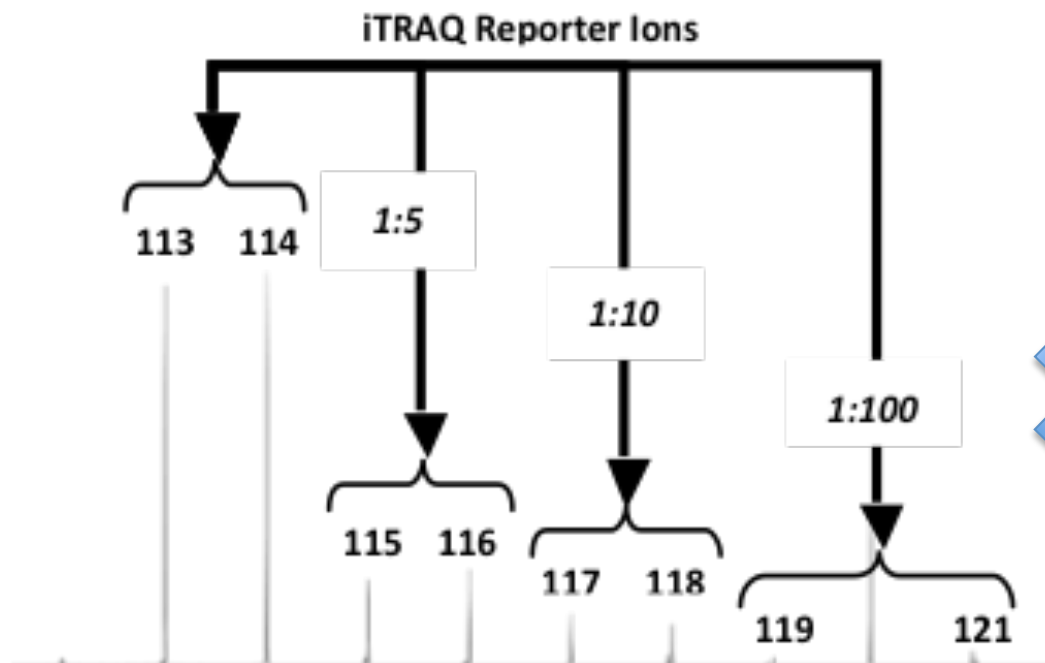


Or more simply.....

**iTRAQ Underestimation in Simple and Complex Mixtures:
"The Good, the Bad and the Ugly"**

Saw Yen Ow,[†] Malinda Salim,[†] Josselin Noirel,[†] Caroline Evans,^{†,‡} Ishtiaq Rehman,[‡] and Phillip C. Wright^{*,†}

ChELSI Institute, Chemical and Process Engineering, University of Sheffield, Mappin Street, S1 3JD Sheffield, United Kingdom, and Mellanby Centre for Bone Research, University of Sheffield, Medical School, Sheffield, S10 2RX, United Kingdom



Alone

In a complex background

the good

Table 4. The Coefficient of Variation (CV) Calculated for the 10 Experiments Carried out^a

organism	experiment	coefficient of variation (CV)				
		115:114 ^b	116:114 ^b	117:114 ^b	overall ^c	
<i>S. cerevisiae</i>	1	0.04	0.08	0.09	0.07	
	<i>S. solfataricus</i>	2	0.06	0.06	0.05	0.05
		3	0.06	0.05	0.06	0.06
		4	0.13	0.09	0.11	0.11
		5	0.10	0.08	0.09	0.09
	Average	0.09	0.07	0.07	0.08	
<i>Synechocystis</i> sp.	6	0.05	0.12	0.12	0.10	
	7	0.11	0.08	0.10	0.10	
	8	0.09	0.06	0.06	0.07	
	9	0.13	0.05	0.14	0.11	
	10	0.10	0.10	0.08	0.09	
		Average	0.10	0.08	0.10	0.09

Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) Reproducibility: Implication of Multiple Injections

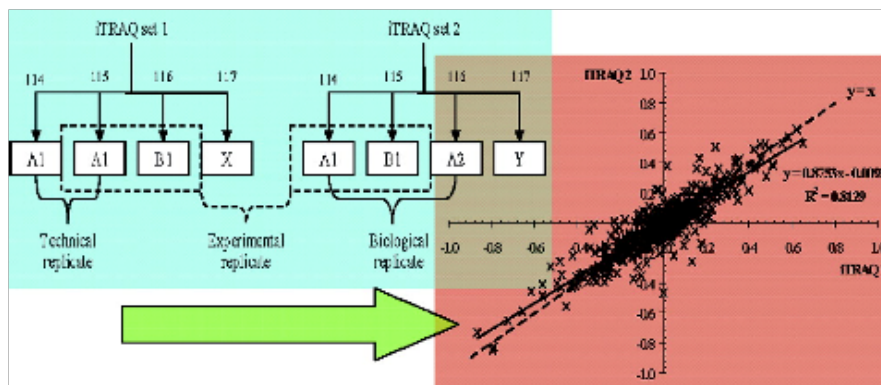
Poh Kuan Chong, Chee Sian Gan, Trong Khoa Pham, and Phillip C. Wright*

Biological and Environmental Systems Group, Department of Chemical and Process Engineering,
University of Sheffield, Mappin Street, Sheffield S1 3JD, United Kingdom

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The take home messages from this were:

1. iTRAQ can be reproducible
2. Technical variation is usually pretty low (biological variation matters much more!)



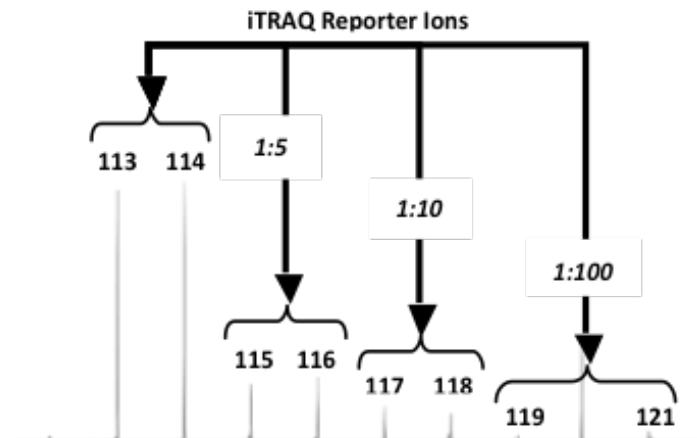
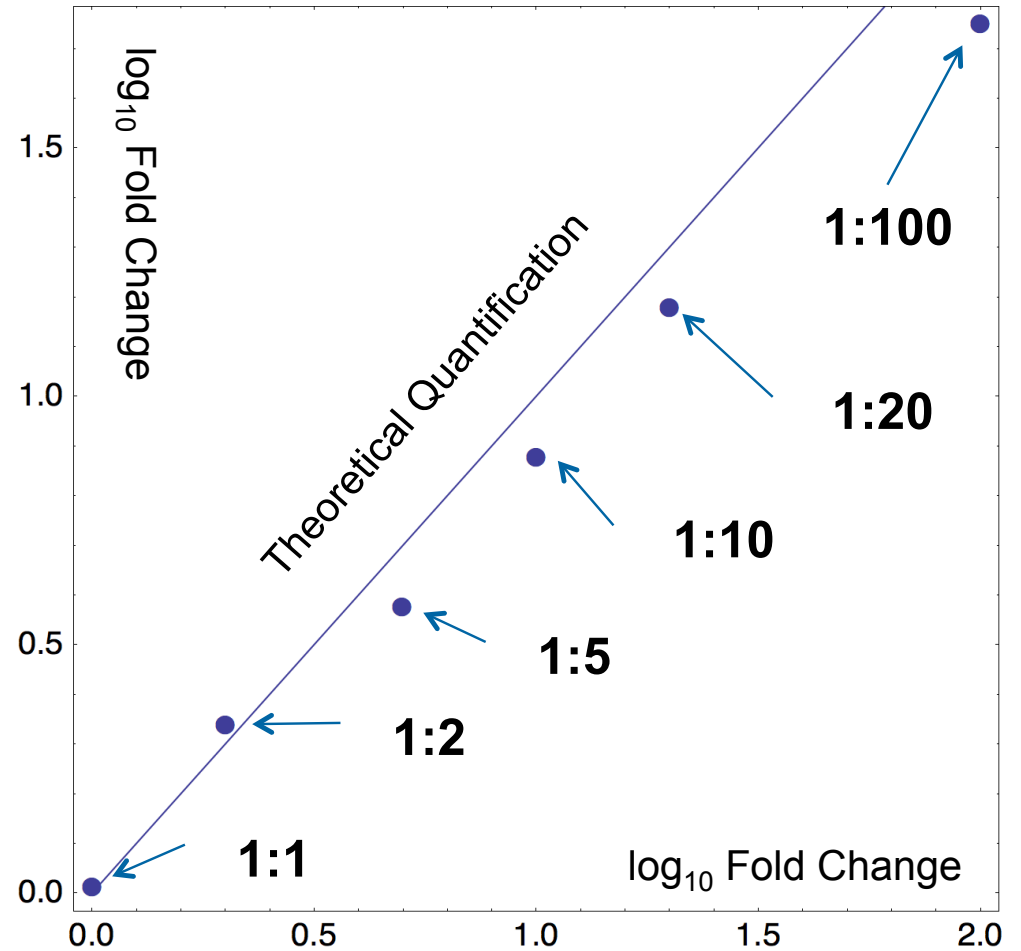
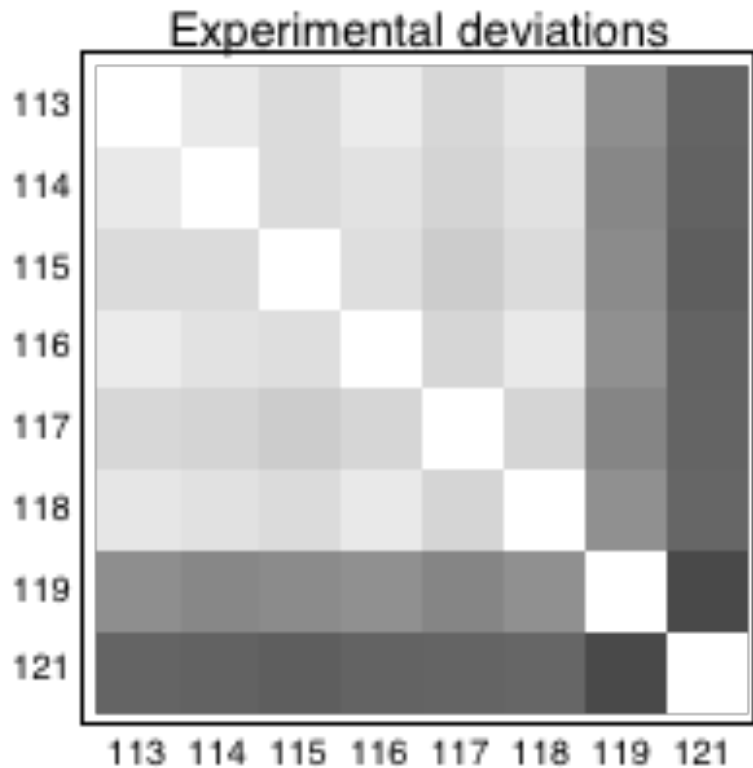
Technical, Experimental, and Biological Variations in Isobaric Tags for Relative and Absolute Quantitation (iTRAQ)

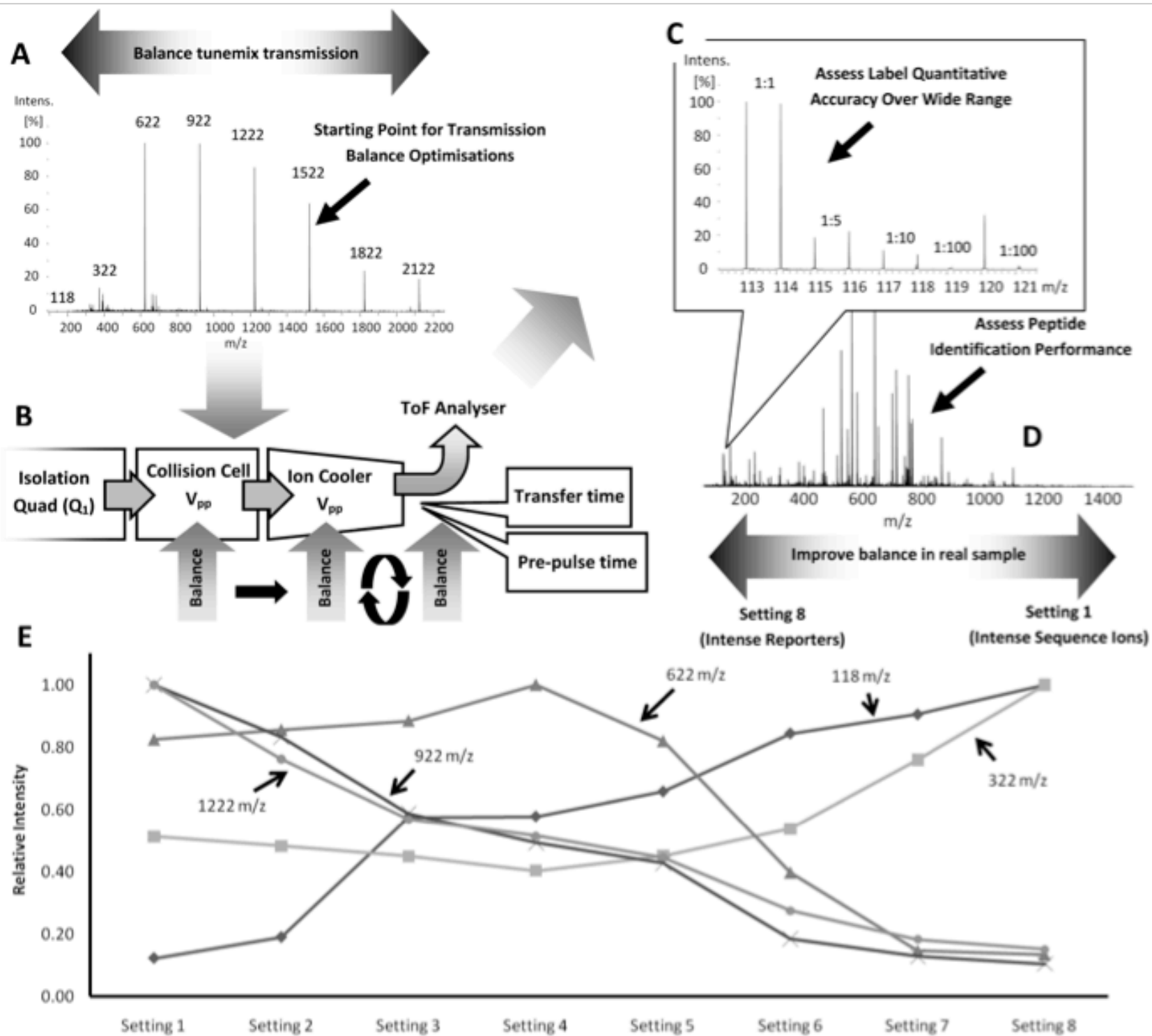
Chee Sian Gan, Poh Kuan Chong, Trong Khoa Pham, and Phillip C. Wright*

Biological and Environmental Systems Group, Department of Chemical and Process Engineering,
The University of Sheffield, Mappin Street, Sheffield S1 3JD, United Kingdom

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Can quantify up to 2 orders of magnitude





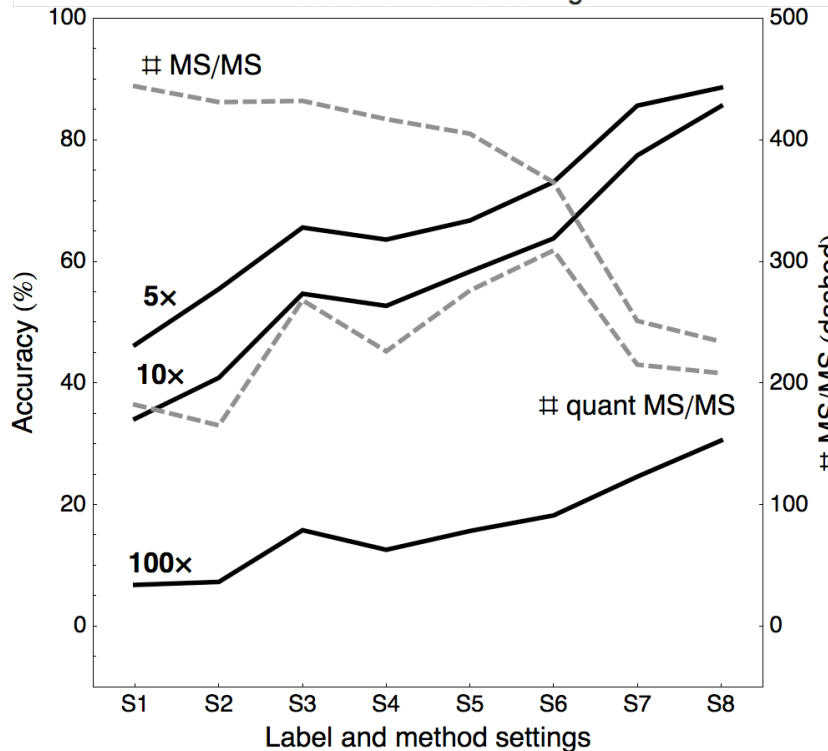
Balancing robust quantification and identification for isobaric tags for relative and absolute quantification: Application of UHR-ToF mass spectrometry

Saw Yen Ow¹, Josselin Noirel¹, Malinda Salim¹, Caroline Evans^{1,2}, Rod Watson³ and Phillip. C. Wright¹

¹ ChELSI Institute, Department of Chemical and Process Engineering, University of Sheffield, Sheffield, UK

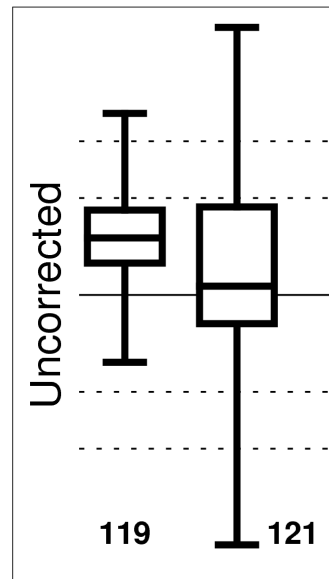
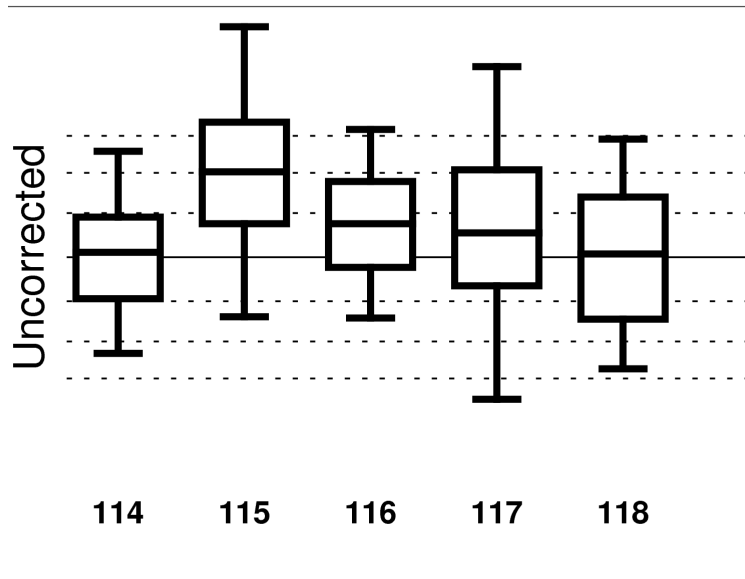
² Mellanby Centre for Bone Research, University of Sheffield, Medical School, Sheffield, UK

³ Bruker Daltonics Limited, Coventry, UK



At this stage, as is known in the community, there seems to be a low chance of a global 'sweetspot'

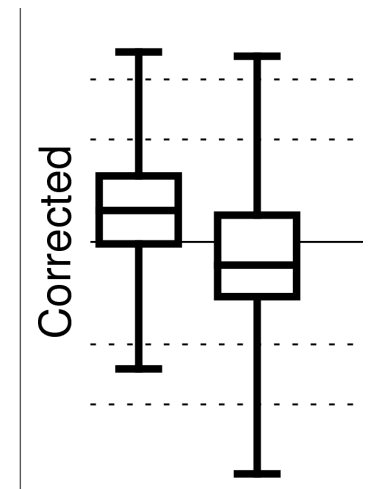
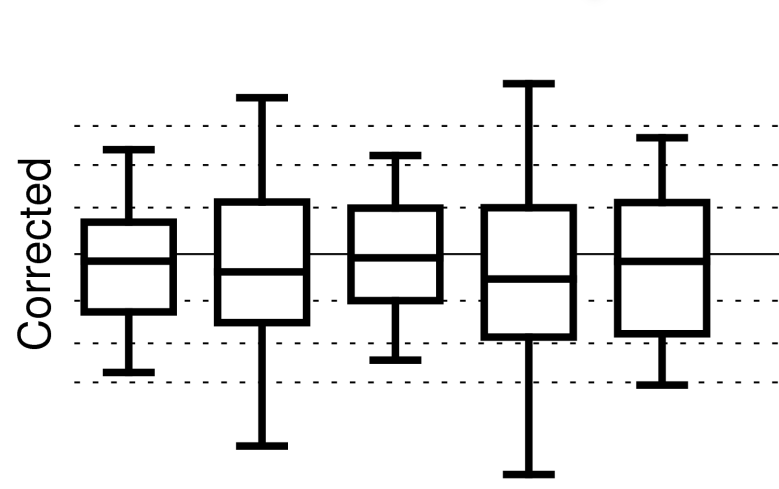
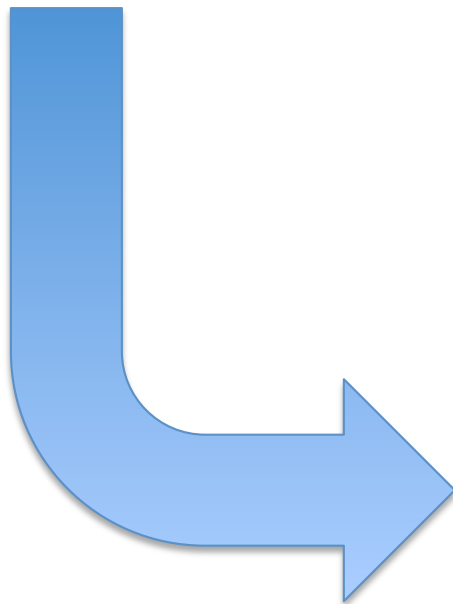
the bad



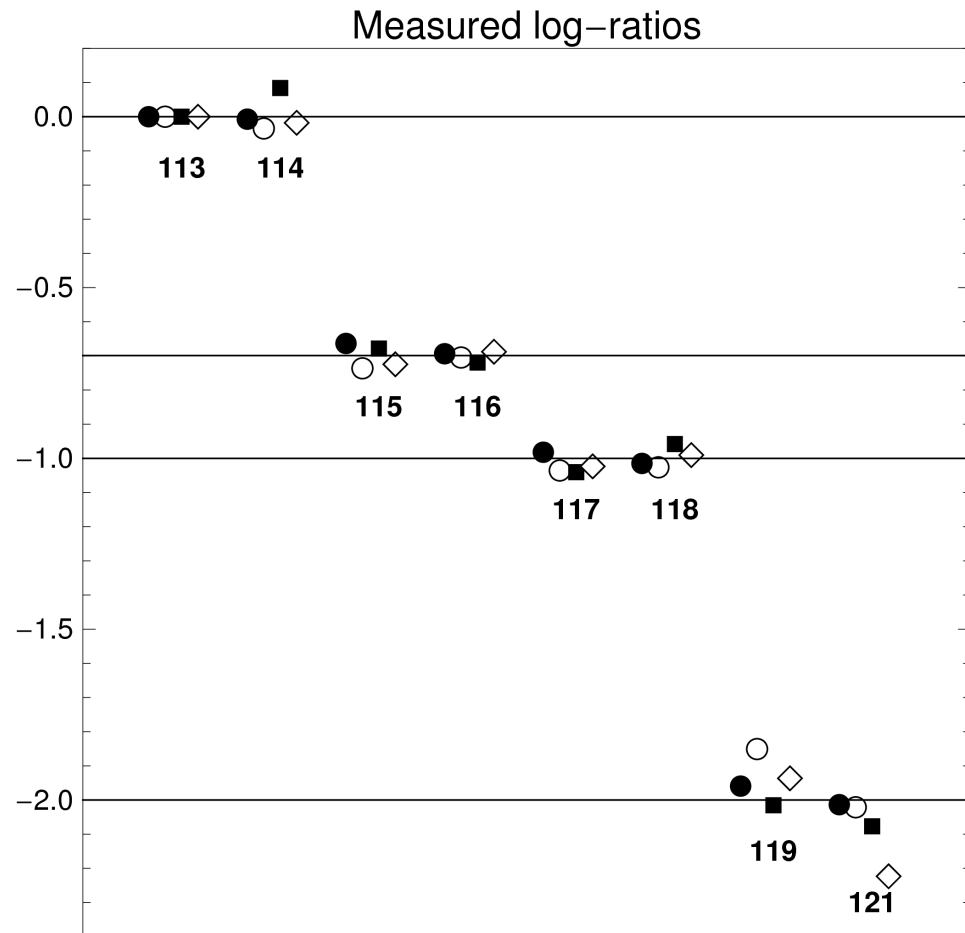
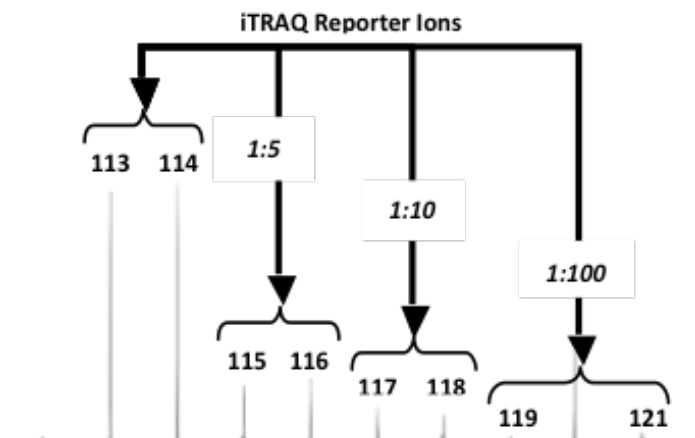
**Cross-label
isotopic impurity**



**Bias in certain
reporter ions'
intensities within
replicates**



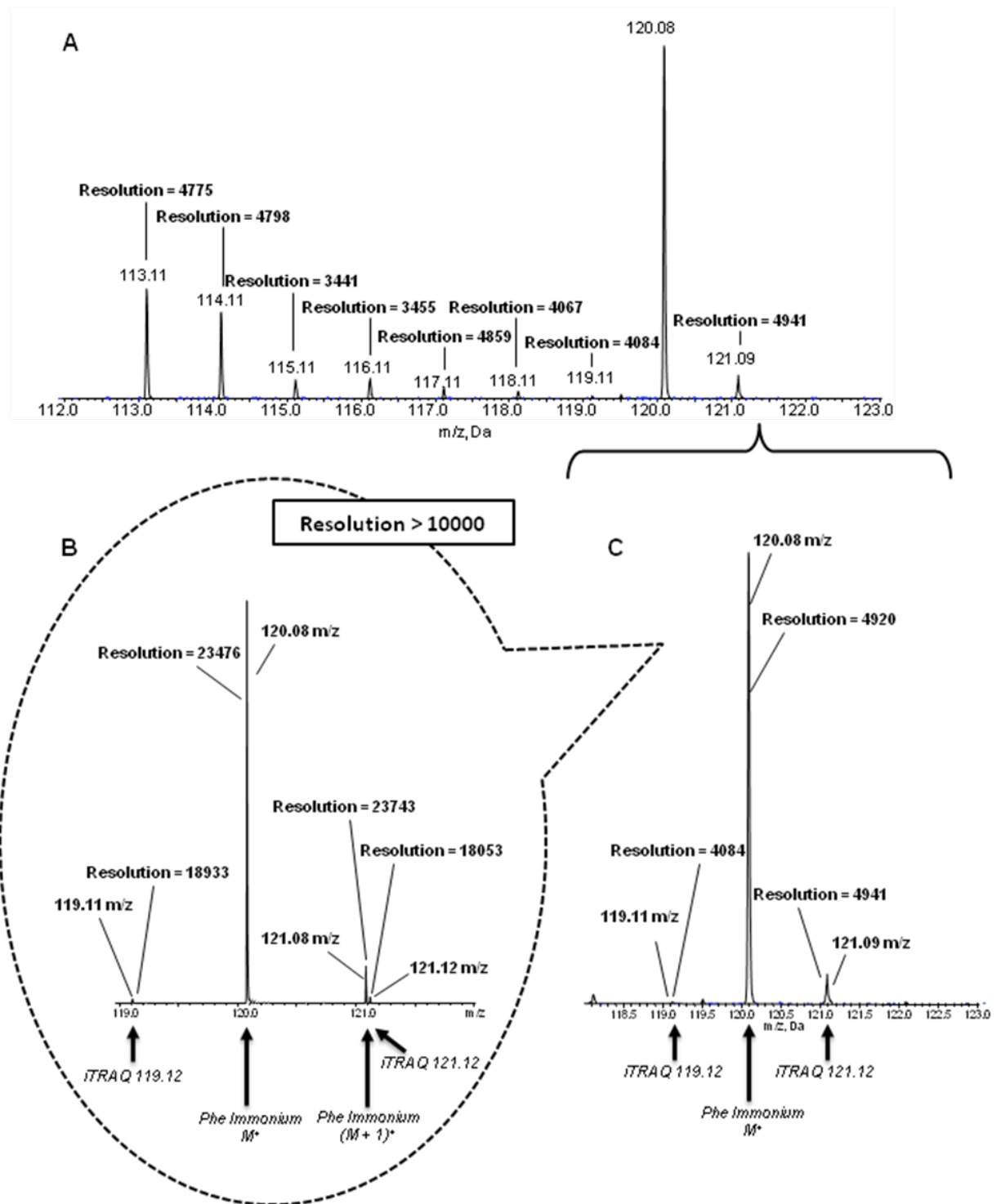
Isotopic
correction
improves this
somewhat



systematic
bias:

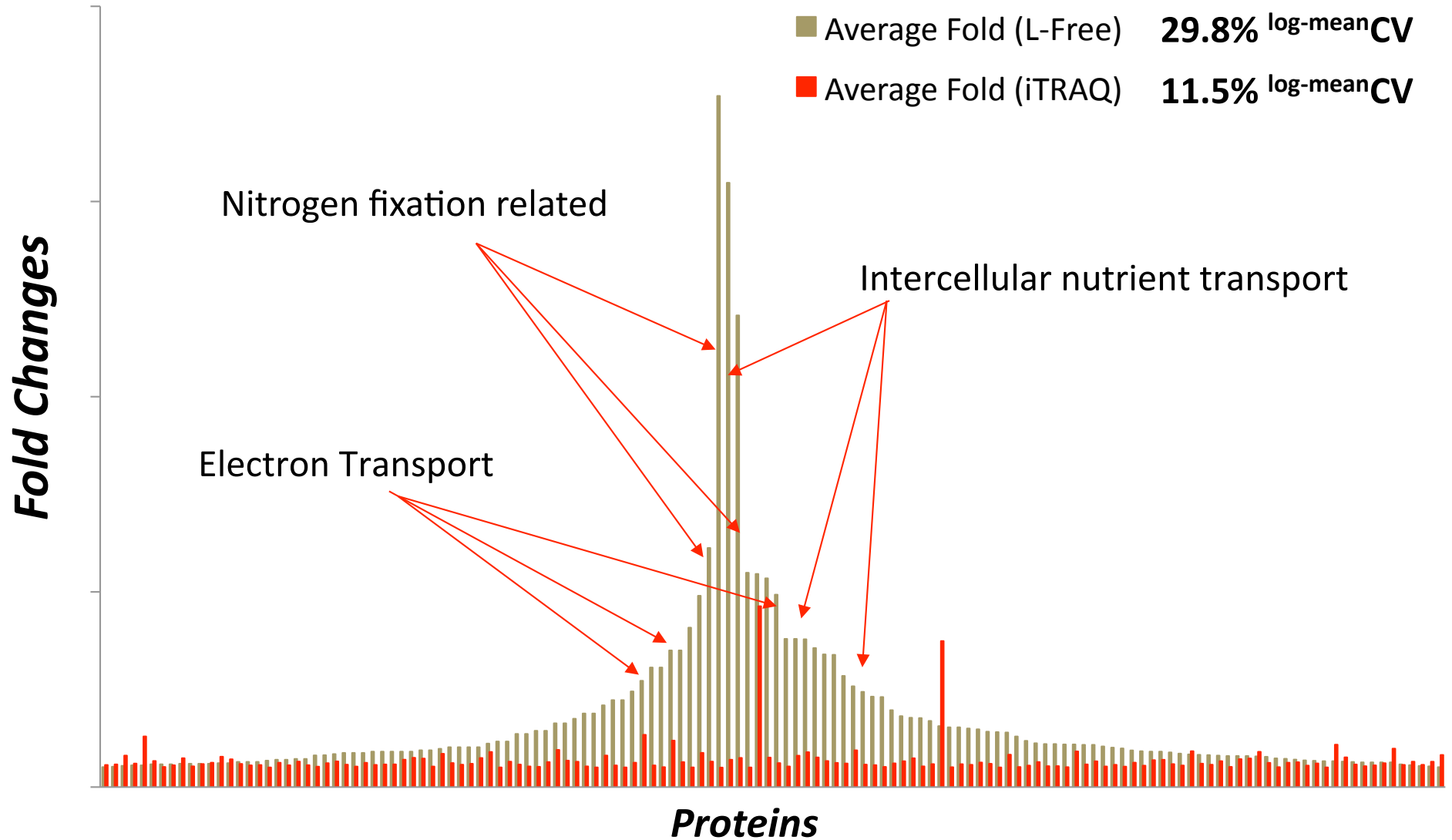
isotopic
correction

In the case of the
121 reporter ion,
the phenylalanine
immonium-ion
isotope may
dampen accuracy



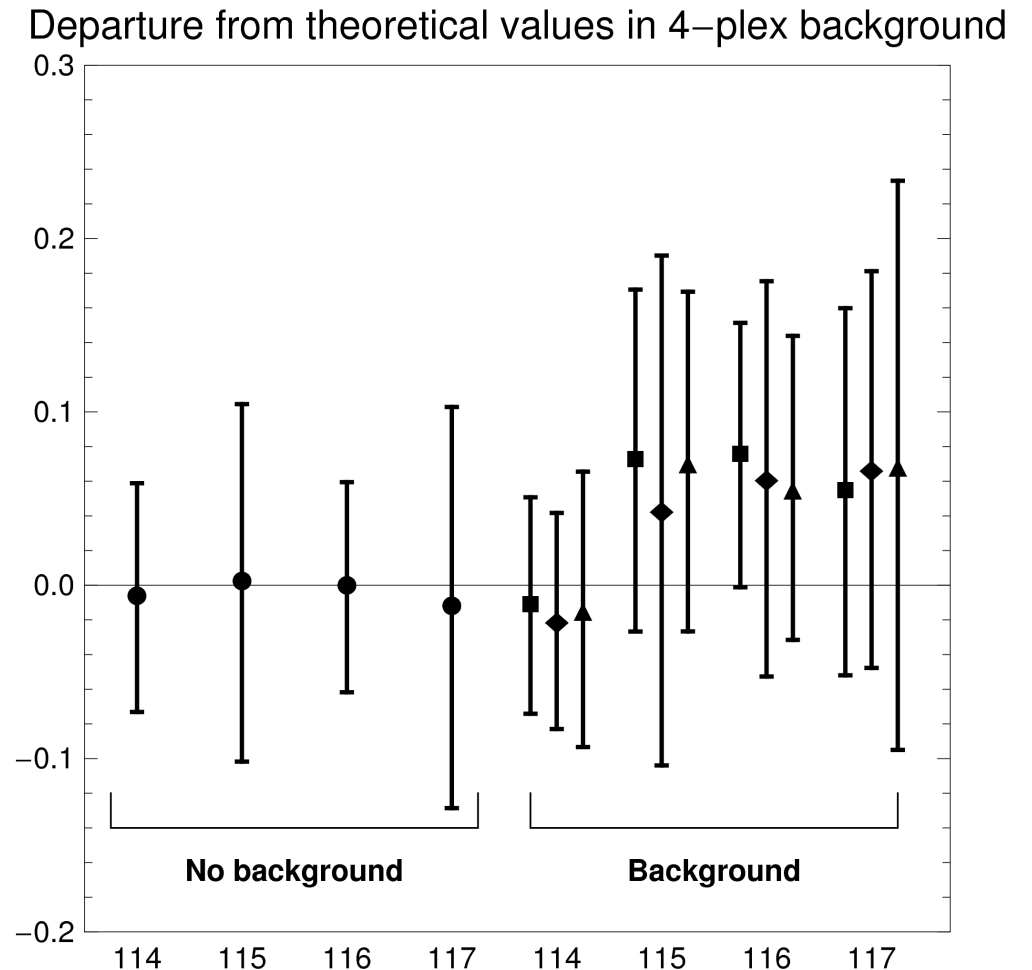
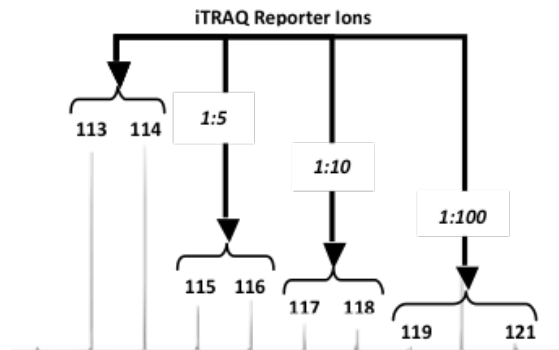
the ugly

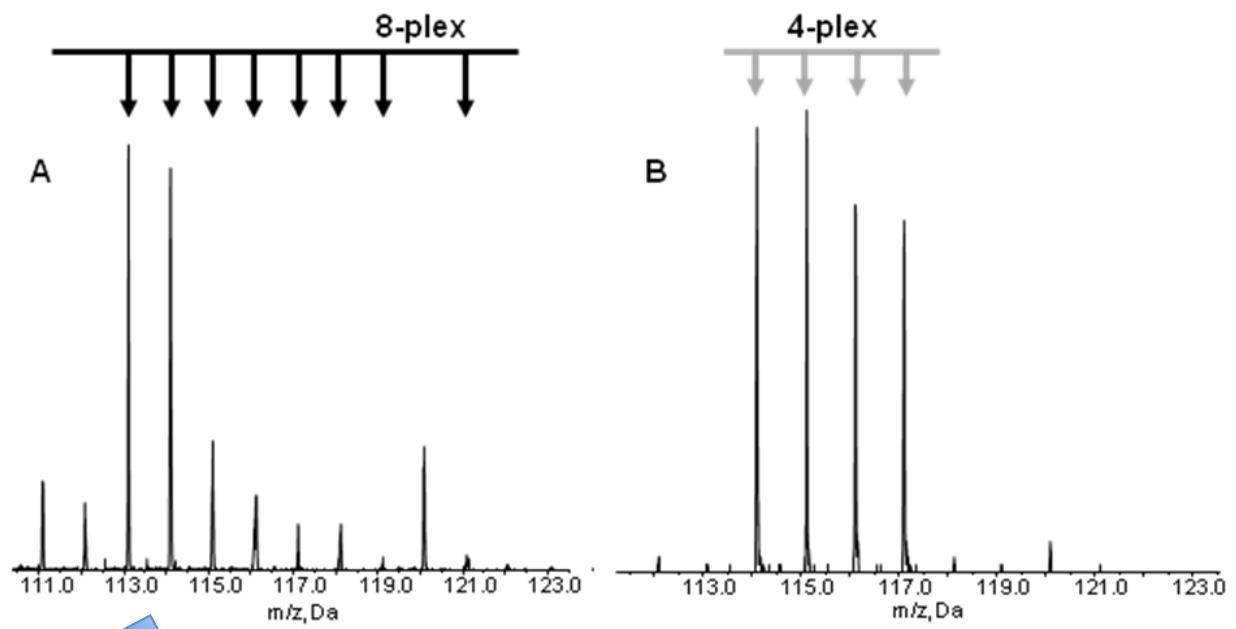
Suppression?



Complex background can lead to underestimations!

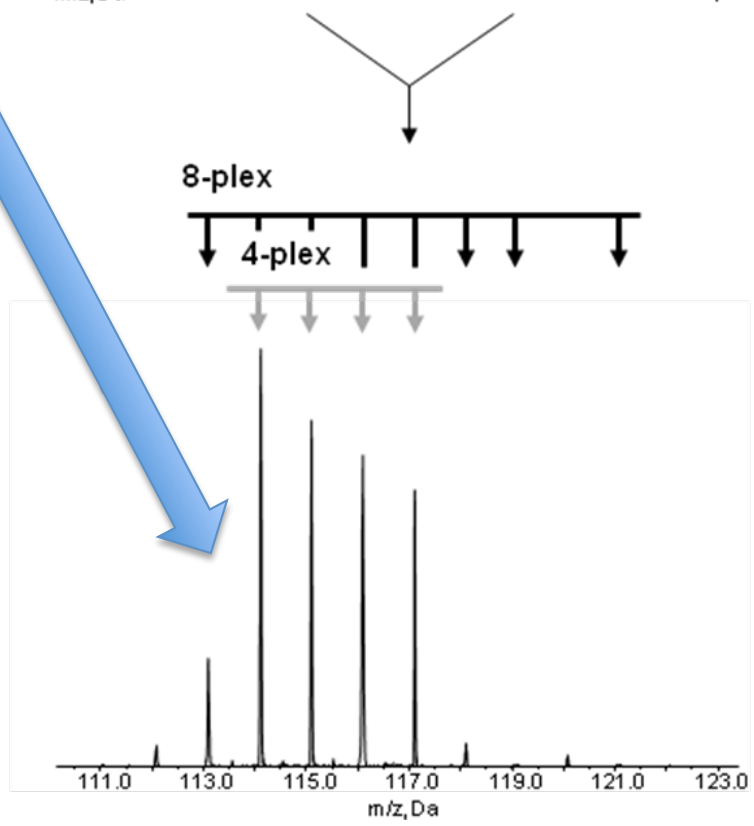
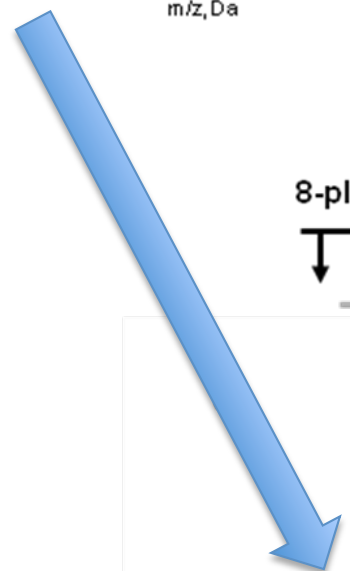
Problem commonly observed in the iTRAQ community



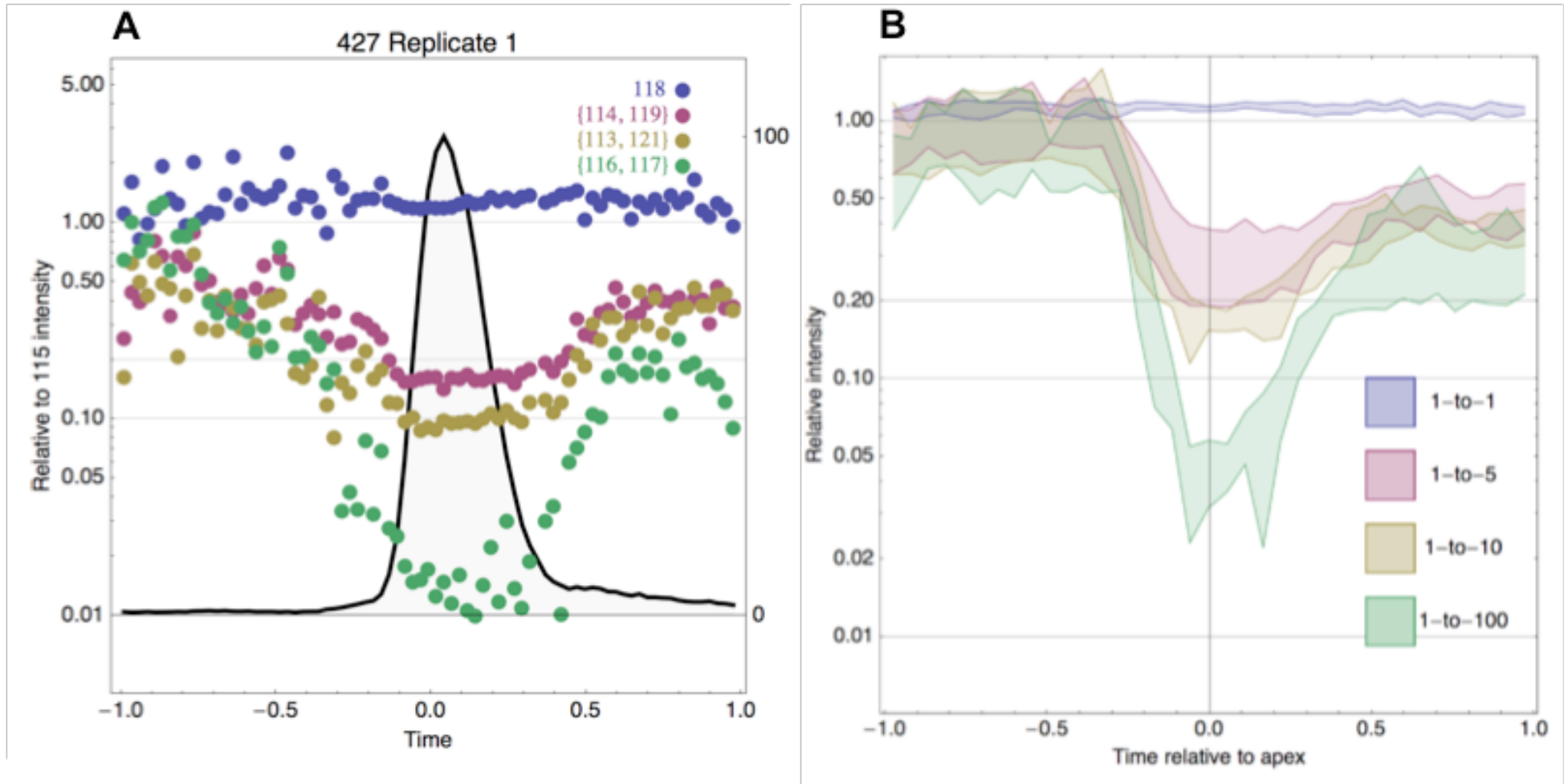


*THE
important
issue:*

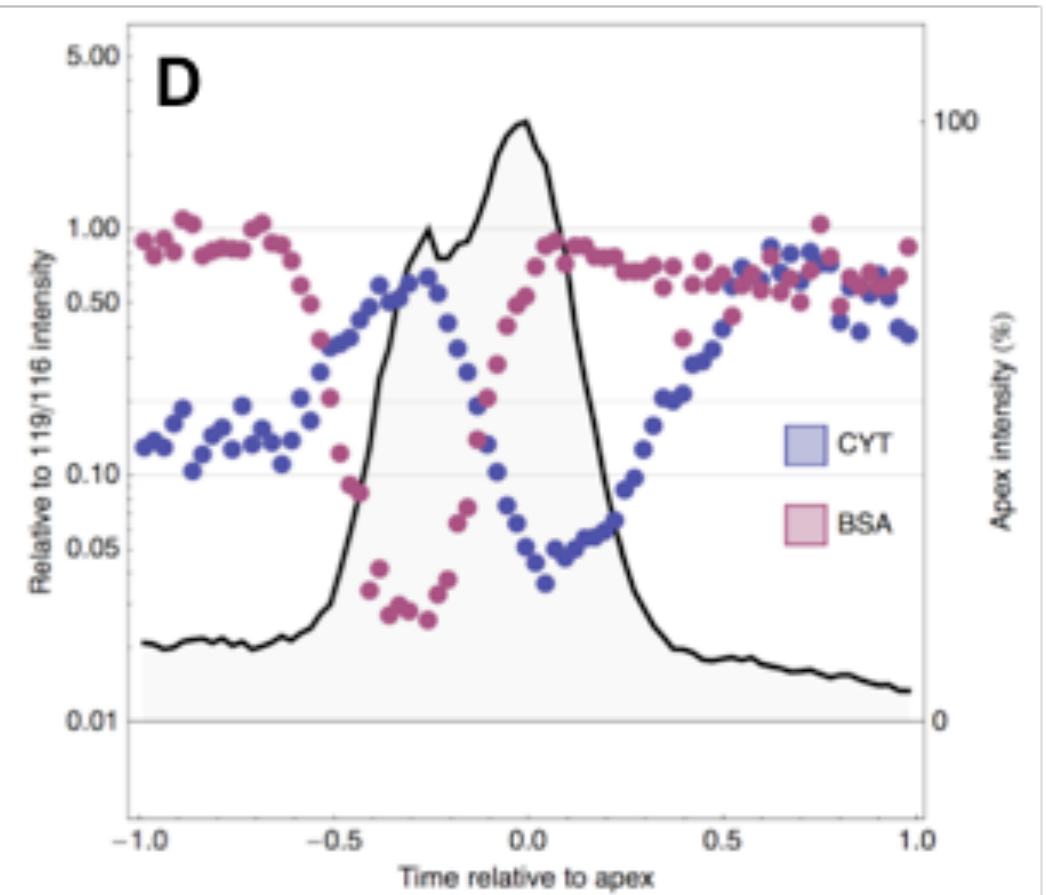
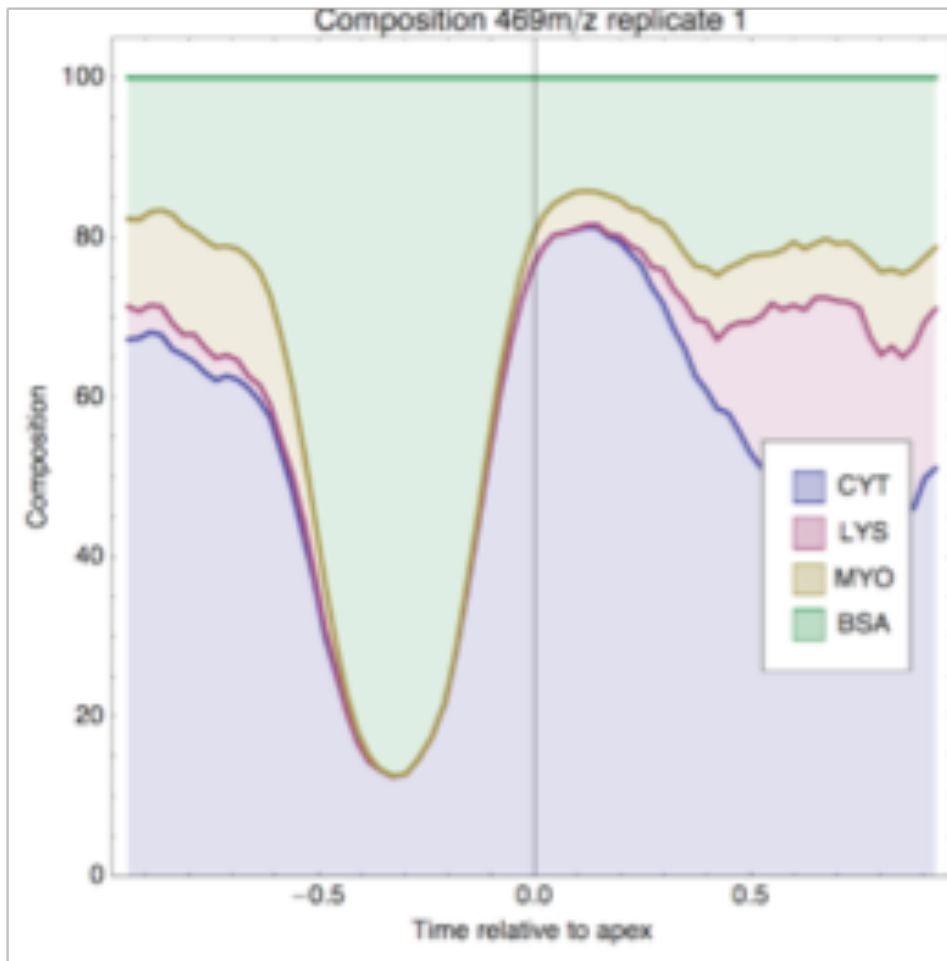
*Mixed MS/
MS*



Mixed MS/MS: Early and late triggers



Mixed MS/MS



So, can we live with iTRAQ?

Yes.....

- Can quantify up to 2 orders of magnitude
- Direction of change is fine so far
- In our hands – robust and easy to use
- Good multiplexing....

but.....

What is the 'but'?

- iTRAQ is **not** a black box
- There are some issues on isotopic contamination that can be solved/minimised
- Adjusting instrument parameters can improve accuracy, but with a trade-off
- There are some 'ugly' problems to solve – **esp mixed MS/MS** (and probably others)

Acknowledgements.....



**Dr Saw Yen
Ow**



Dr Josselin Noirel



Dr Caroline Evans

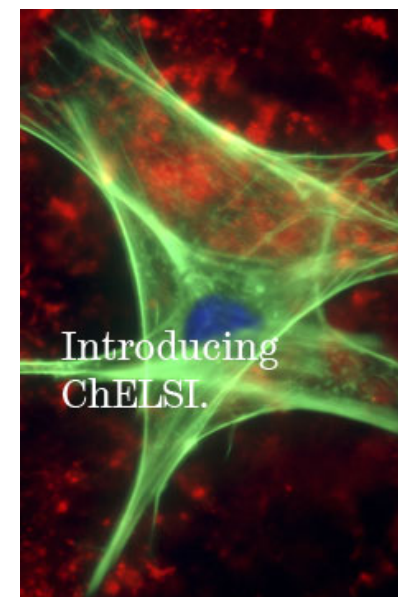
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