



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 Abolute 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

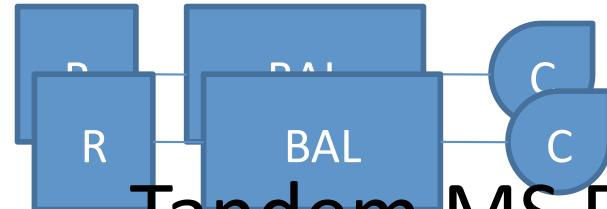
# iTRAQ Methodology

## iTRAQ Tag

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

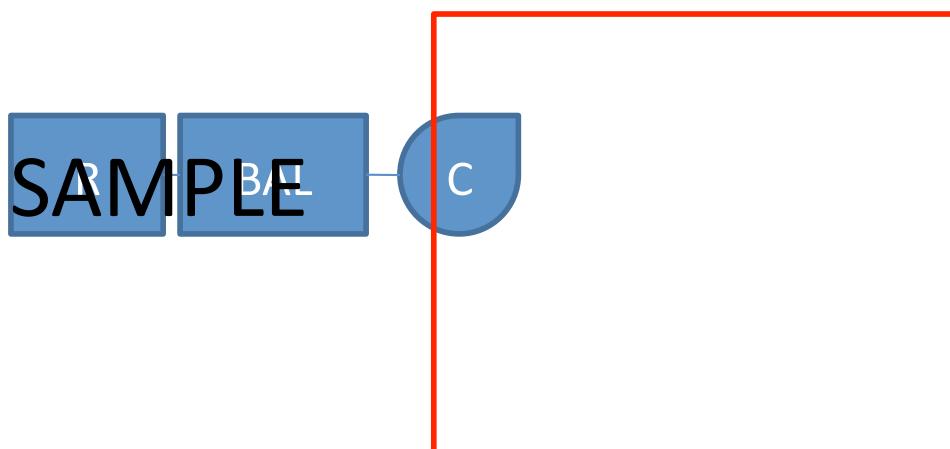
Reporter Mass	Balance Mass	Total
113	~ 192	305
114	~ 191	
115	~ 190	305
116	~ 189	
117	~ 188	
118	~ 187	
119	~ 186	305
121	~ 184	

Reporter   Balance   Rxn group

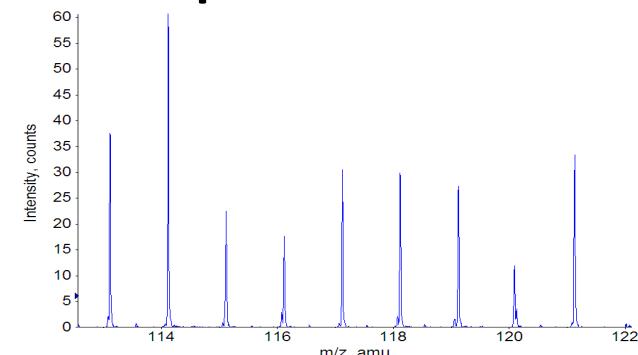


Tandem MS Fragmentation

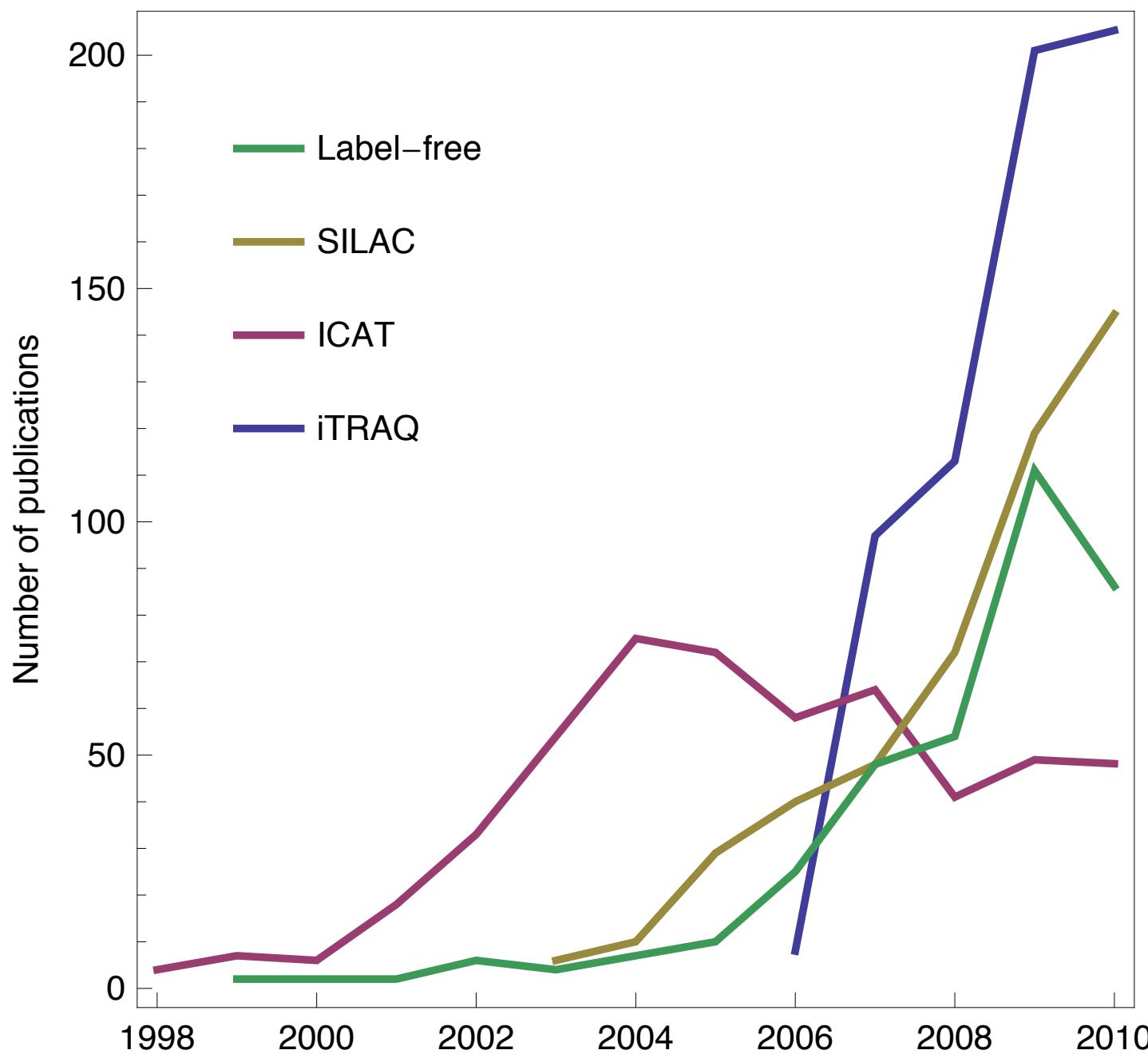
LLHSIVGGVAVSSGR  
LLHSIVGGVAVSSGR



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

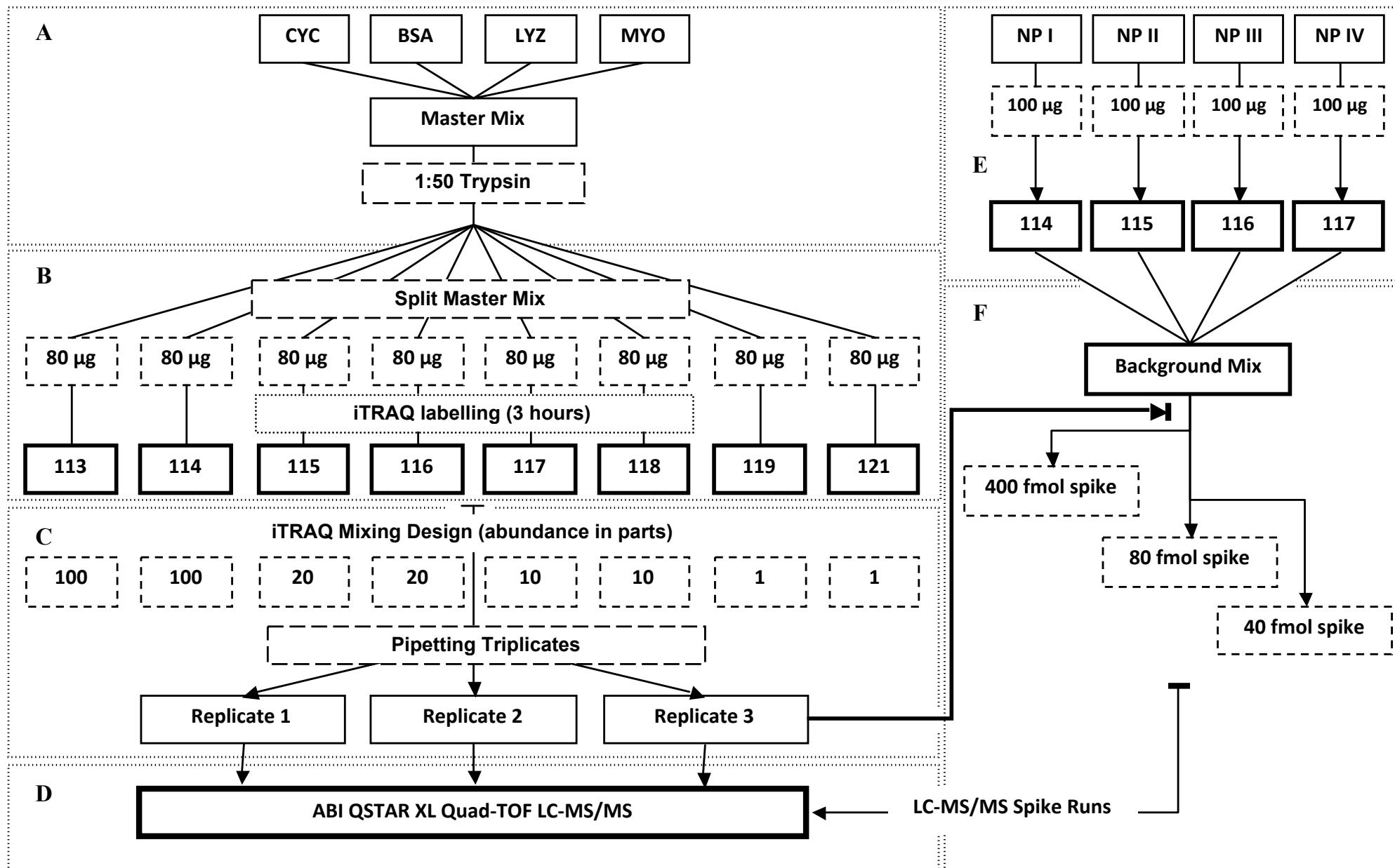
*So.....*

*Can we live with  
iTRAQ?*

*Or maybe.....*

*of iTRAQ*

# How to assess?

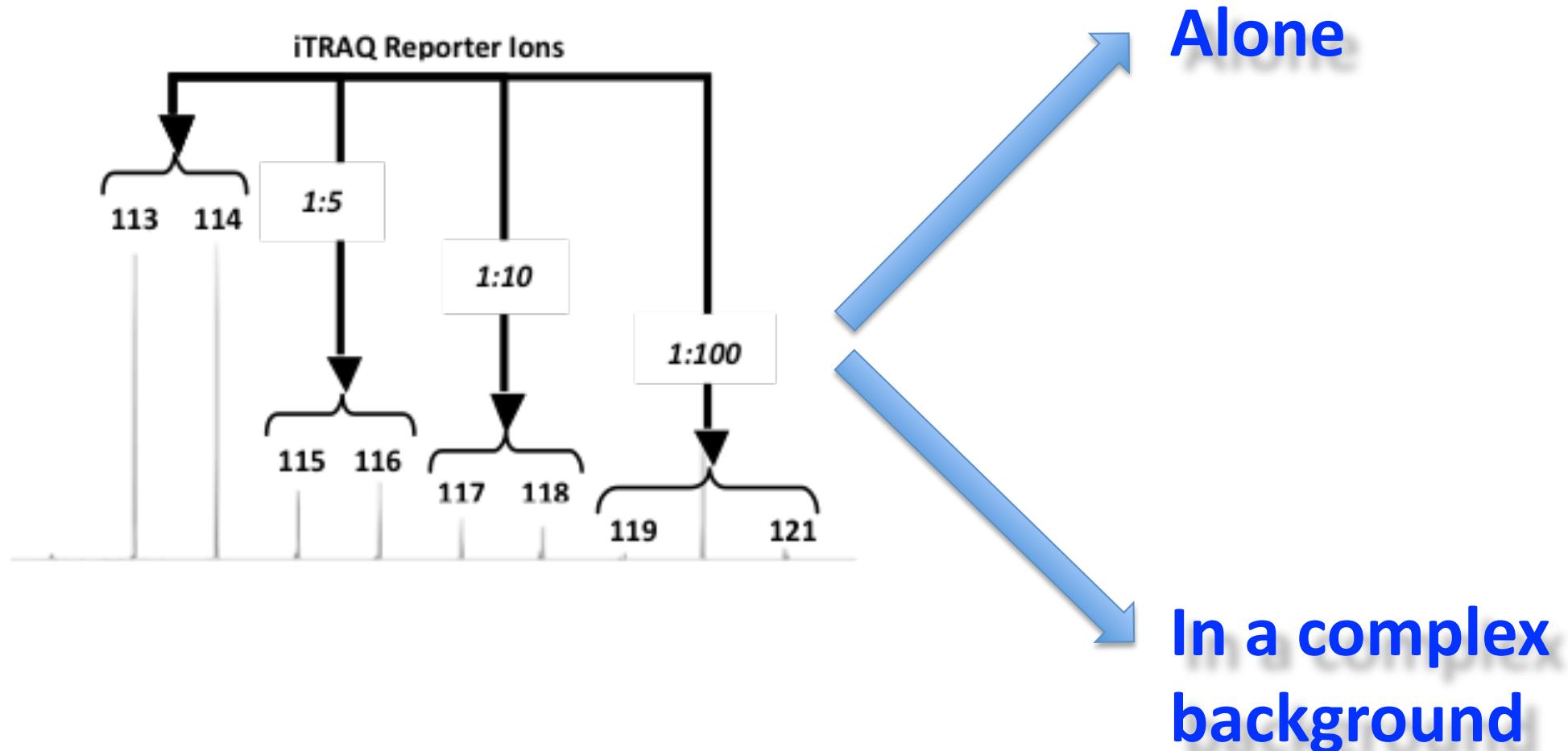


Or more simply.....

iTRAQ Underestimation in Simple and Complex Mixtures:  
“The Good, the Bad and the Ugly”

Saw Yen Ow,<sup>†</sup> Malinda Salim,<sup>†</sup> Josselin Noirel,<sup>†</sup> Caroline Evans,<sup>†,‡</sup> Ishtiaq Rehman,<sup>‡</sup> and Phillip C. Wright<sup>\*,†</sup>

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



*the good*

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

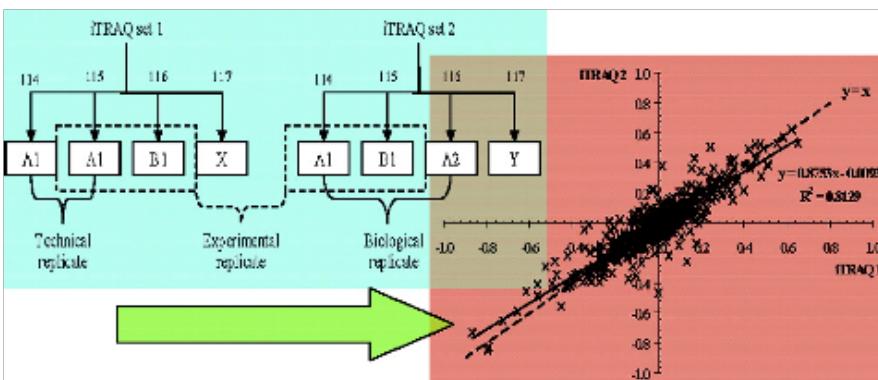
Poh Kuan Chong, Chee Sian Gan, Trong Khoa Pham, and Phillip C. Wright\*

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

Received January 18, 2006

The take home messages from this were:

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



**Table 4.** The Coefficient of Variation (CV) Calculated for the 10 Experiments Carried out<sup>a</sup>

organism	experiment	coefficient of variation (CV)			
		115:114 <sup>b</sup>	116:114 <sup>b</sup>	117:114 <sup>b</sup>	overall <sup>c</sup>
<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

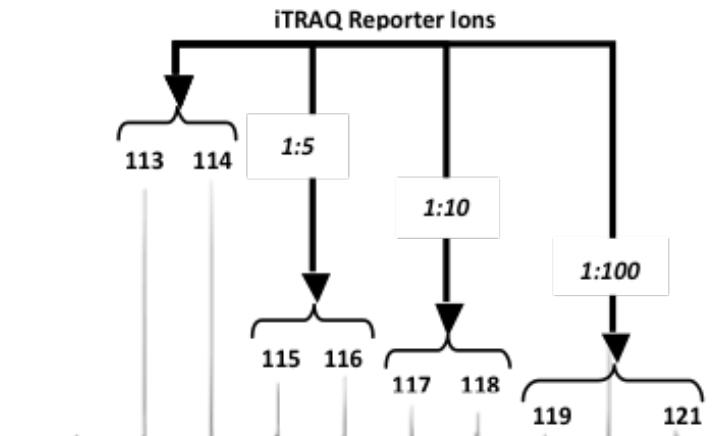
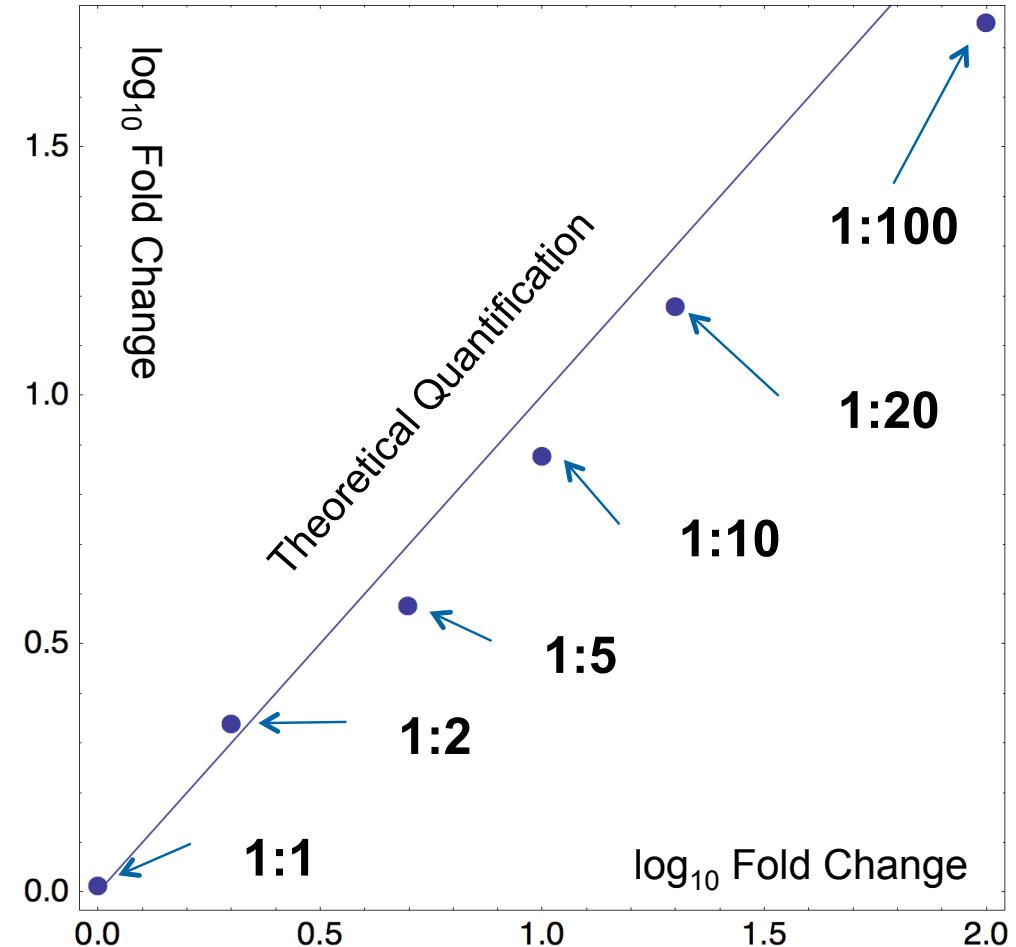
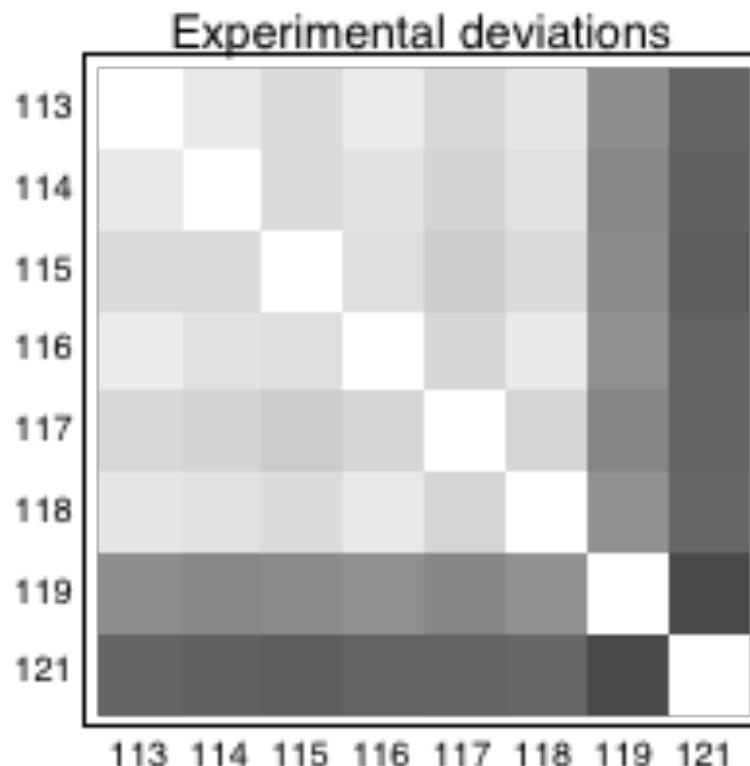
## Technical, Experimental, and Biological Variations in Isobaric Tags for Relative and Absolute Quantitation (iTRAQ)

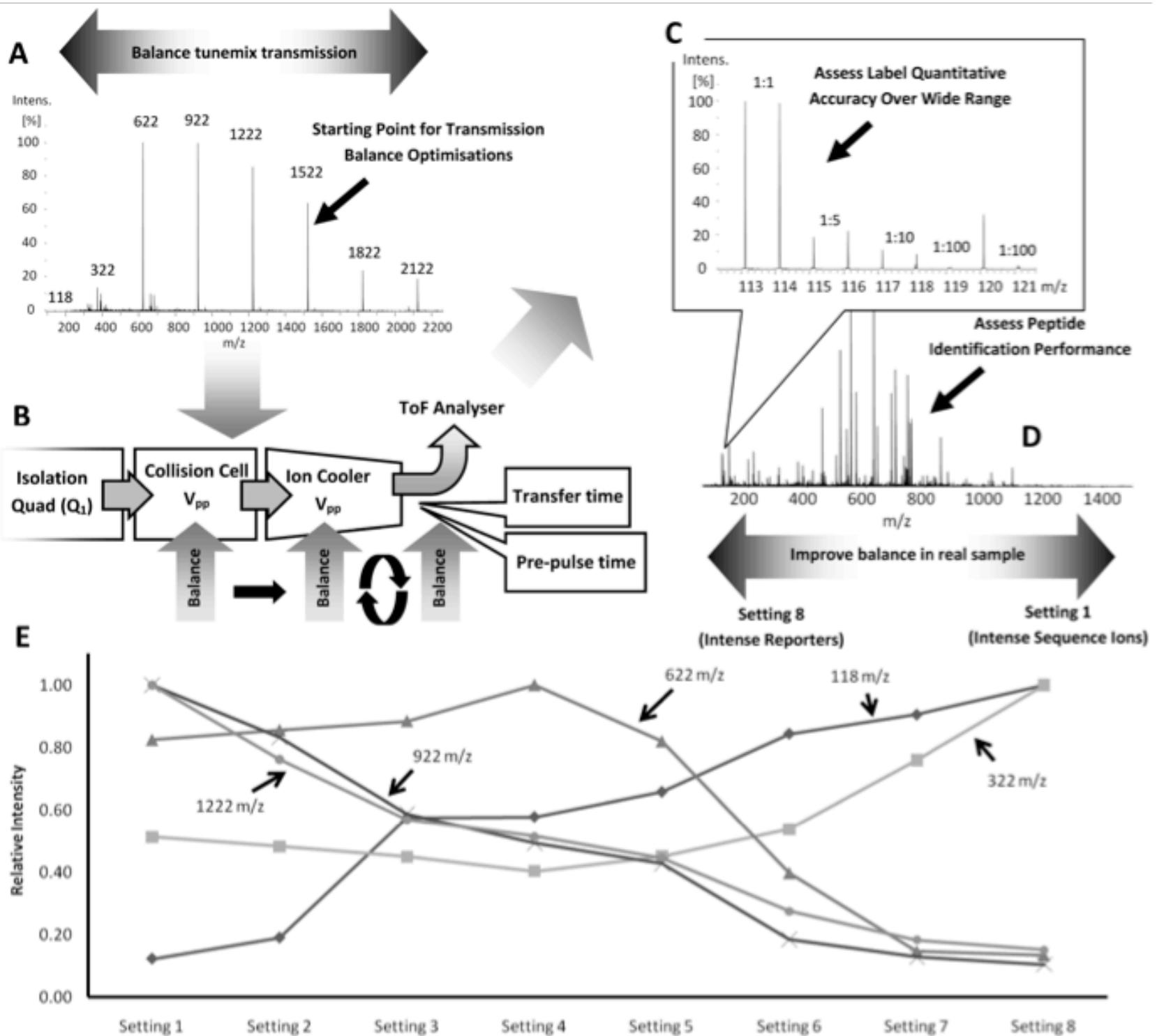
Chee Sian Gan, Poh Kuan Chong, Trong Khoa Pham, and Phillip C. Wright\*

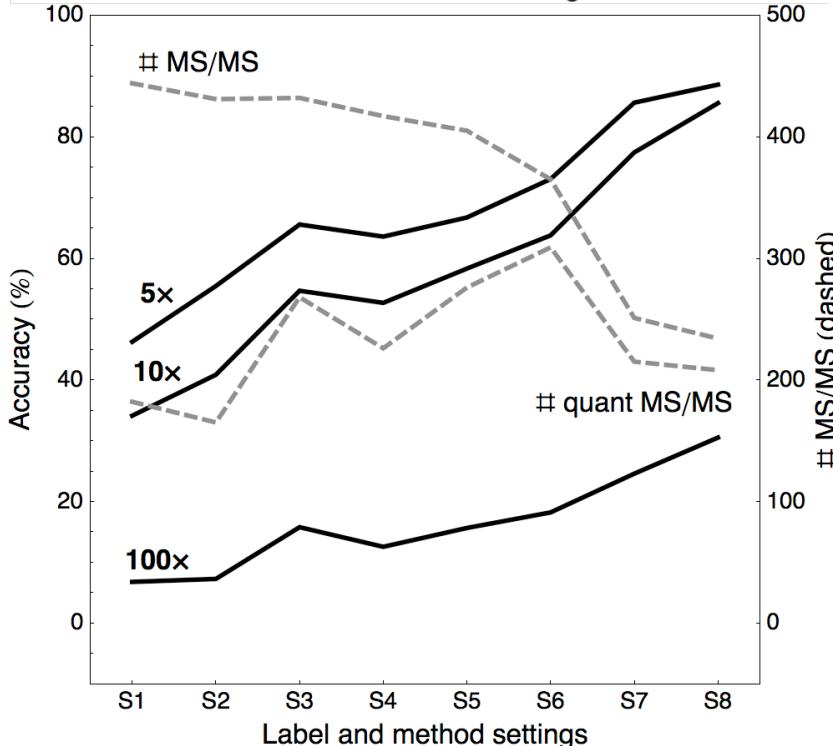
*Biological and Environmental Systems Group, Department of Chemical and Process Engineering,  
The University of Sheffield, Mappin Street, Sheffield S1 3JD, United Kingdom*

Received September 12, 2006

*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 Ow<sup>1</sup>, Josselin Noirel<sup>1</sup>, Malinda Salim<sup>1</sup>, Caroline Evans<sup>1,2</sup>, Rod Watson<sup>3</sup> and Phillip. C. Wright<sup>1</sup>



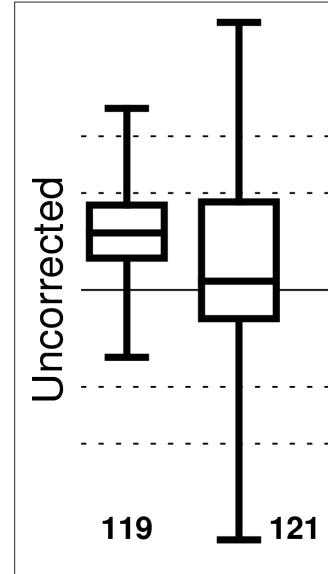
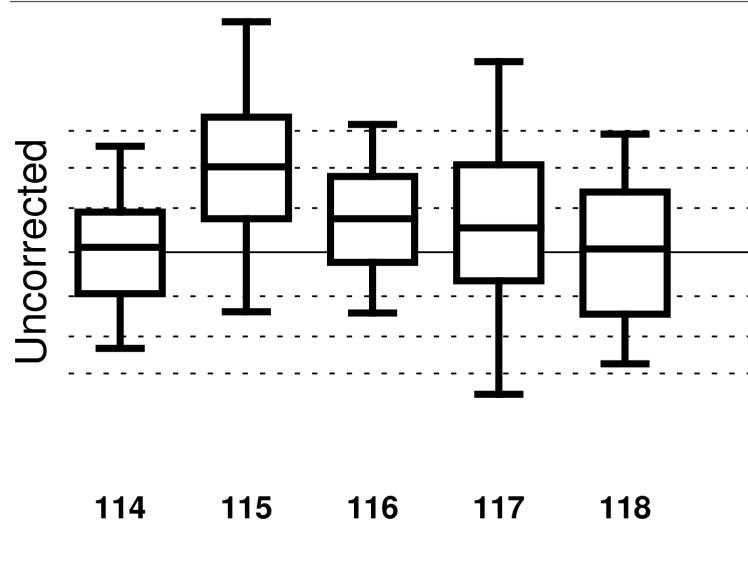
<sup>1</sup> ChELSI Institute, Department of Chemical and Process Engineering, University of Sheffield, Sheffield, UK

<sup>2</sup> Mellanby Centre for Bone Research, University of Sheffield, Medical School, Sheffield, UK

<sup>3</sup> 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’

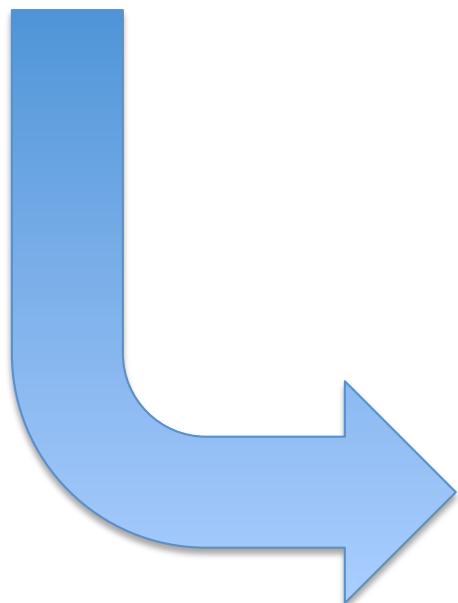
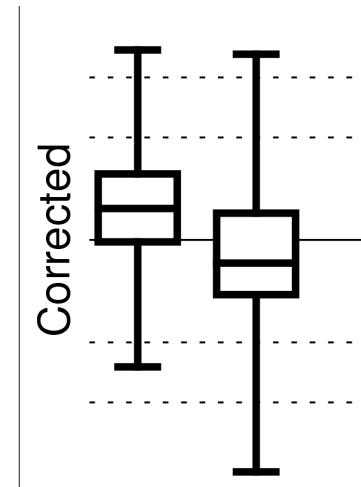
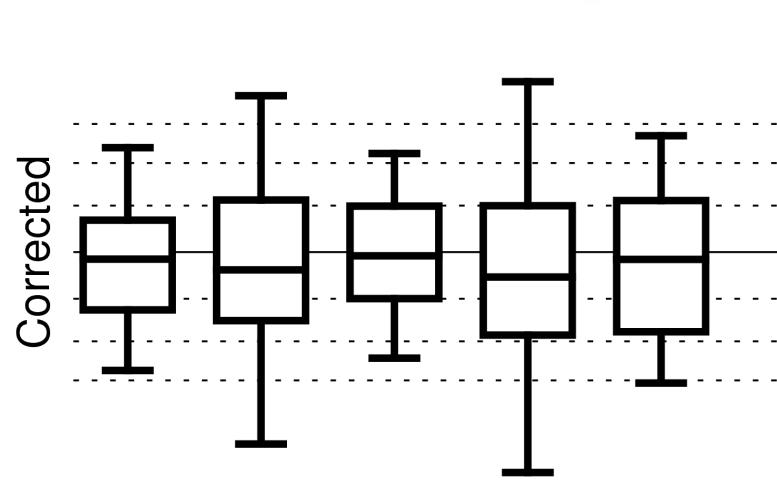
*the bad*



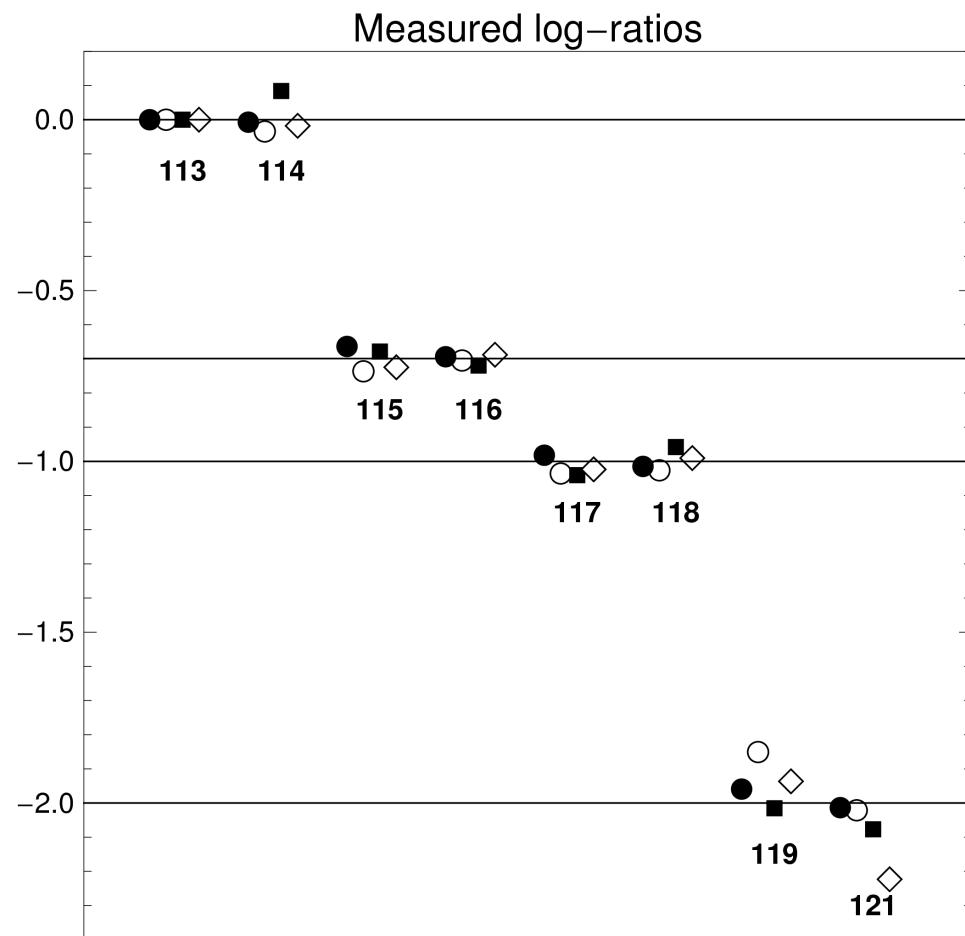
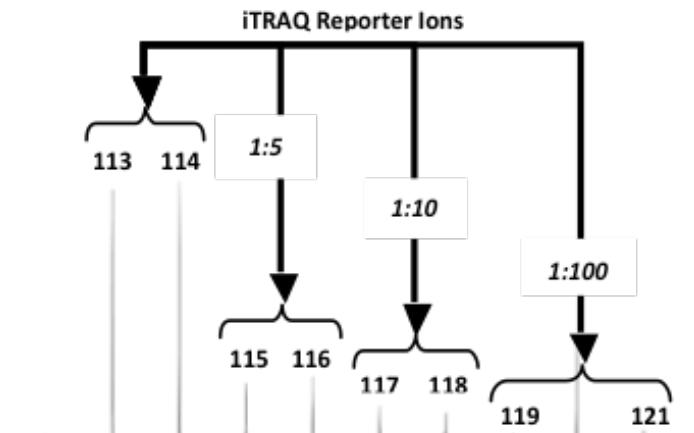
Cross-label  
isotopic impurity



Bias in certain  
reporter ions'  
intensities within  
replicates



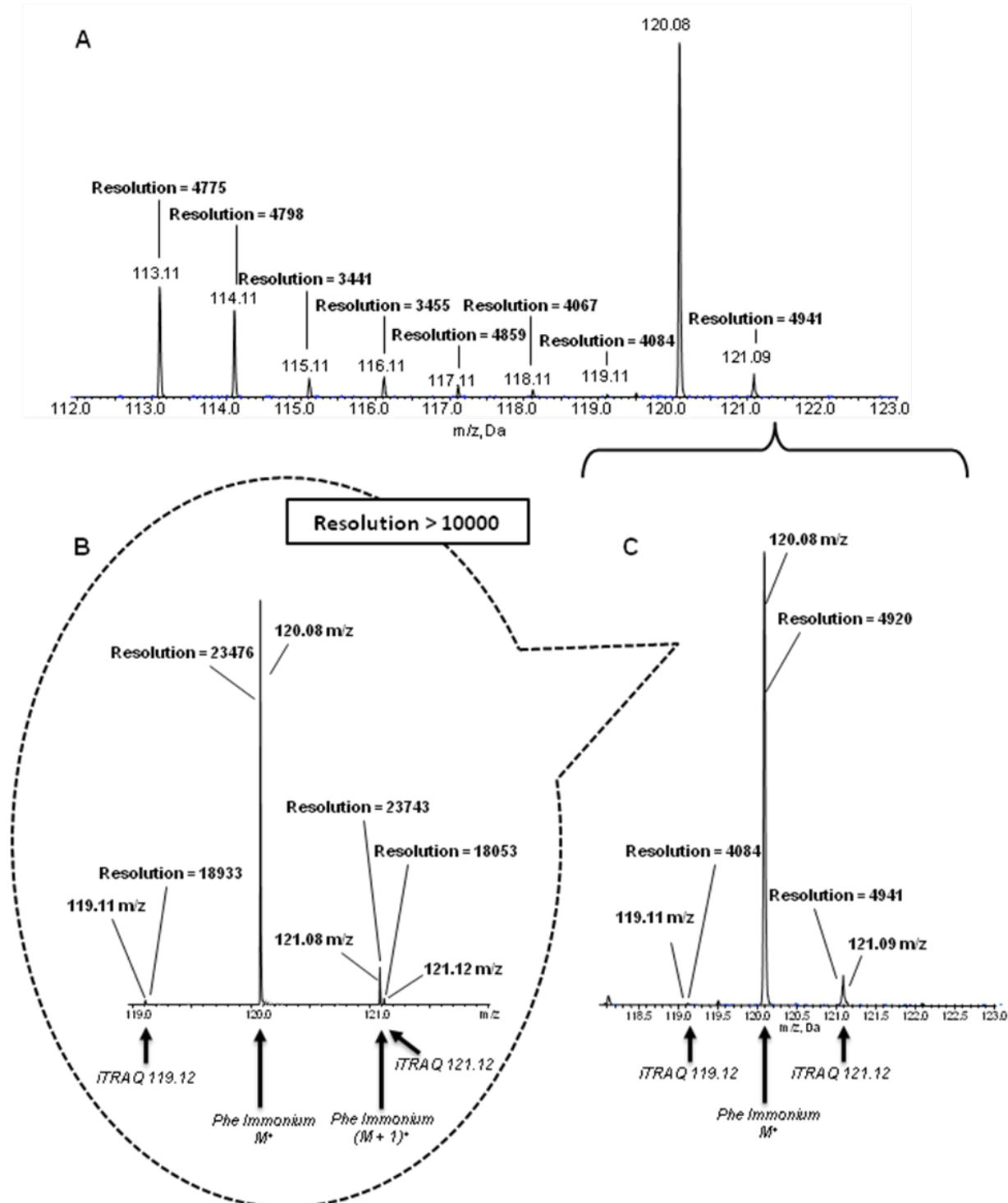
Isotopic  
correction  
improves this  
somewhat



# systematic bias:

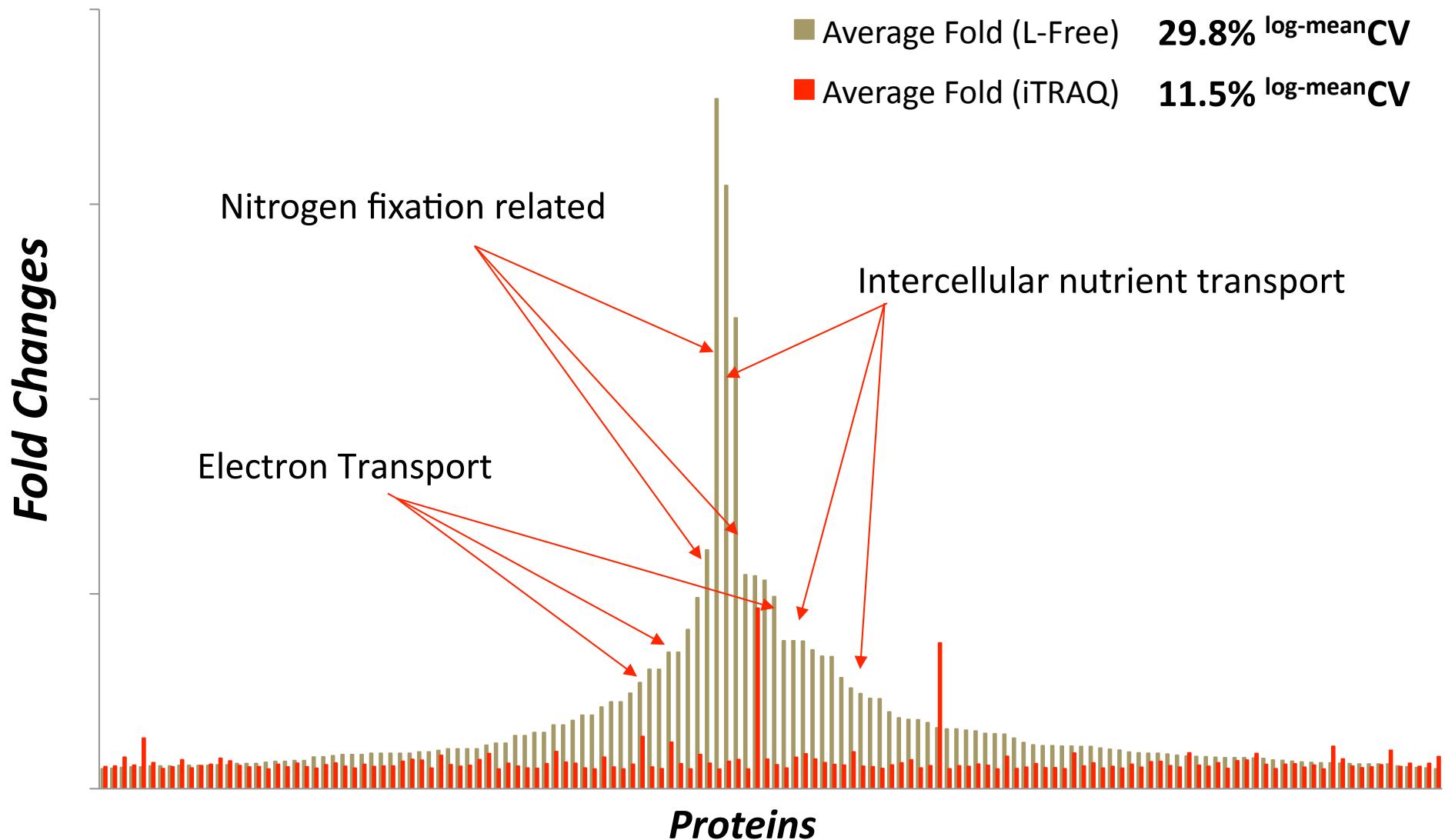
## isotopic correction

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



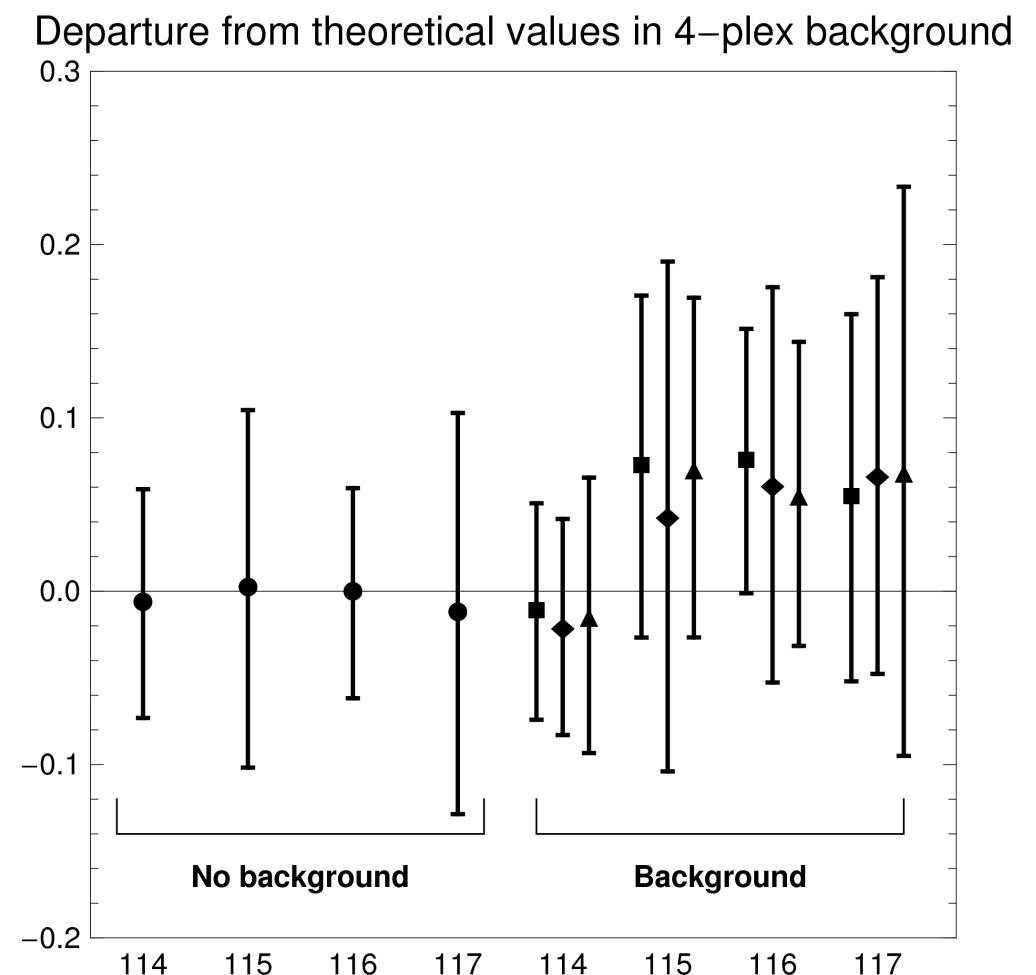
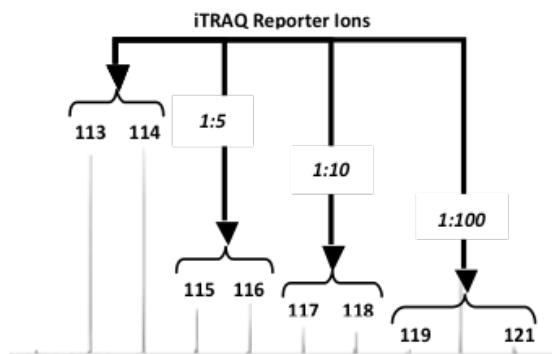
*the ugly*

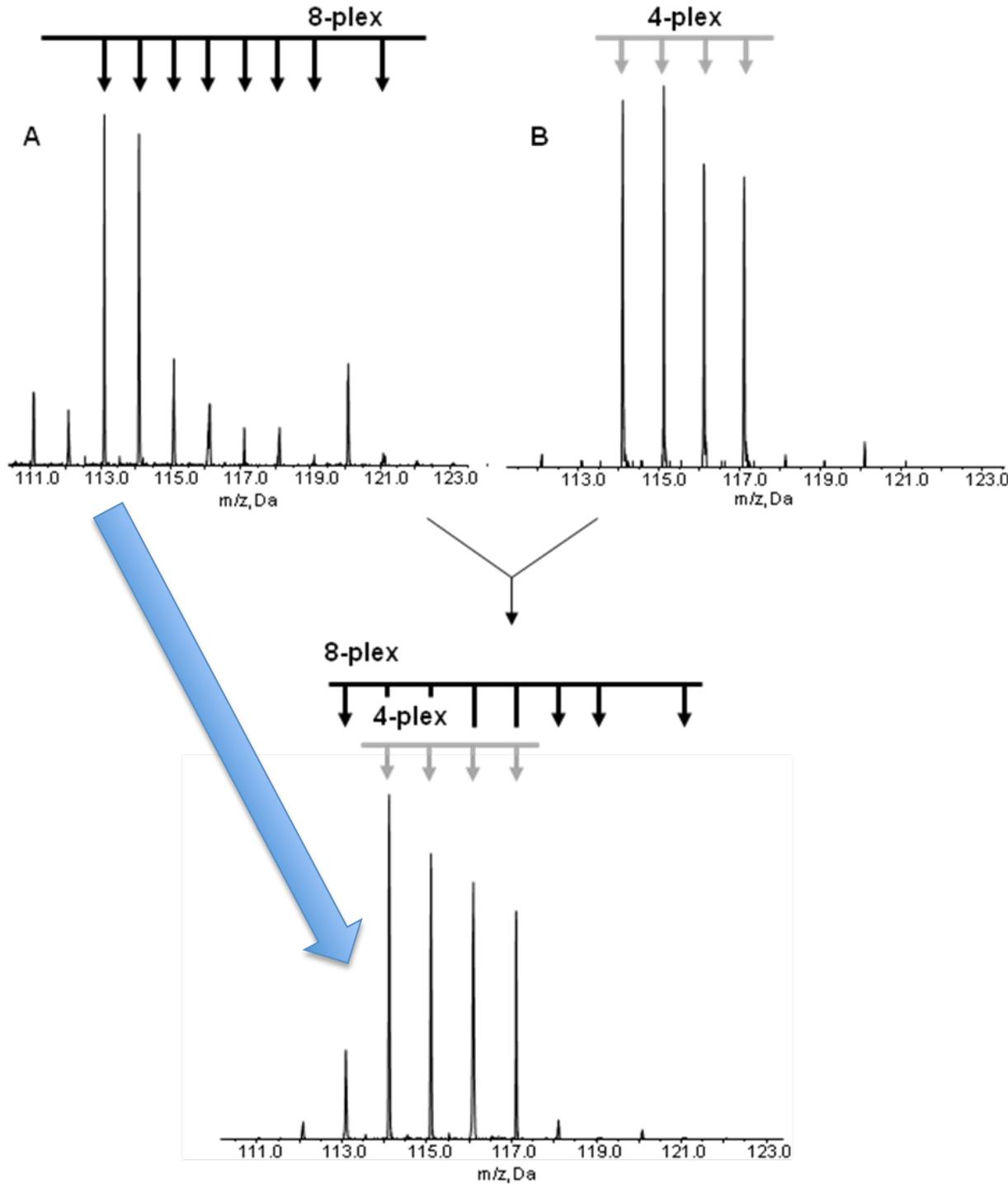
# Suppression?



# Complex background can lead to underestimations!

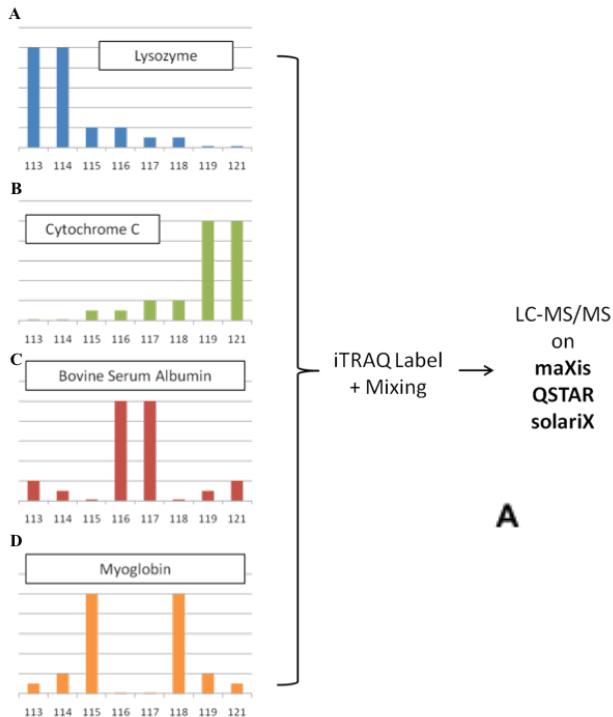
**Problem commonly observed in the iTRAQ community**





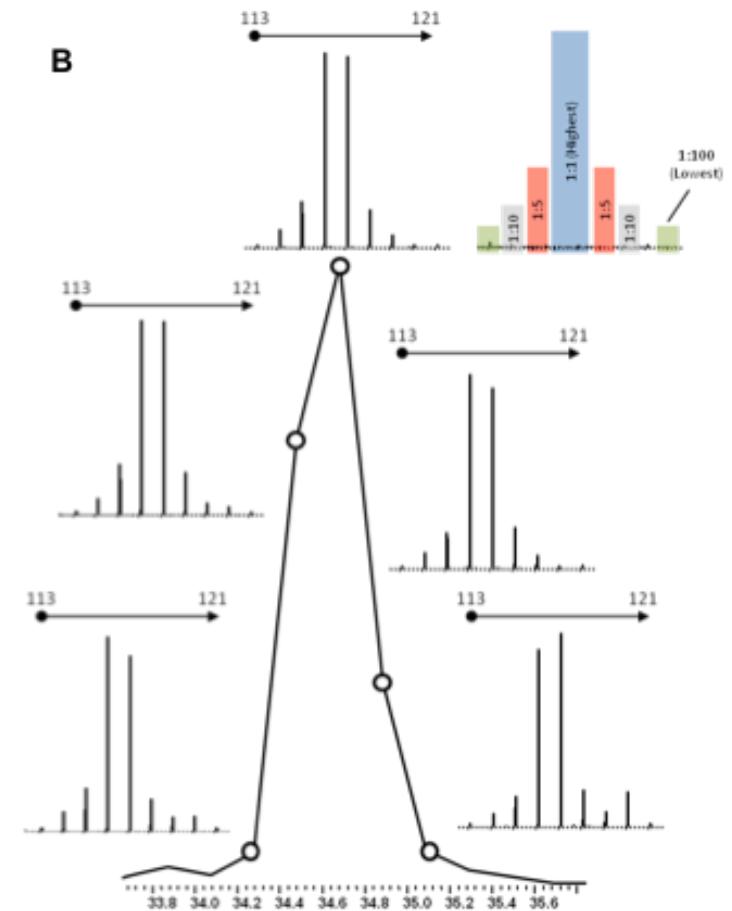
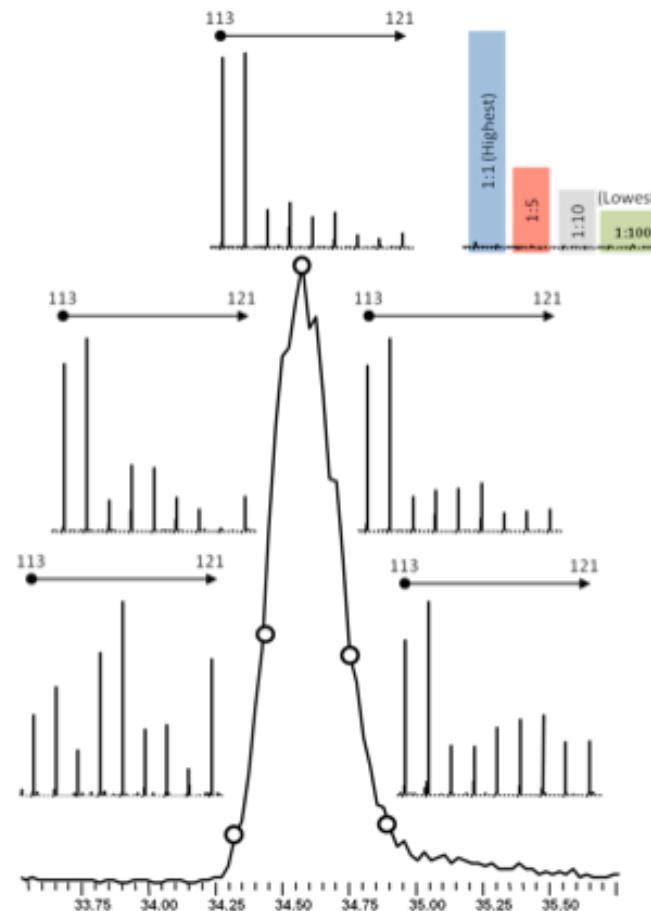
**TAKE  
important  
issue:**

**Mixed MS/  
MS**

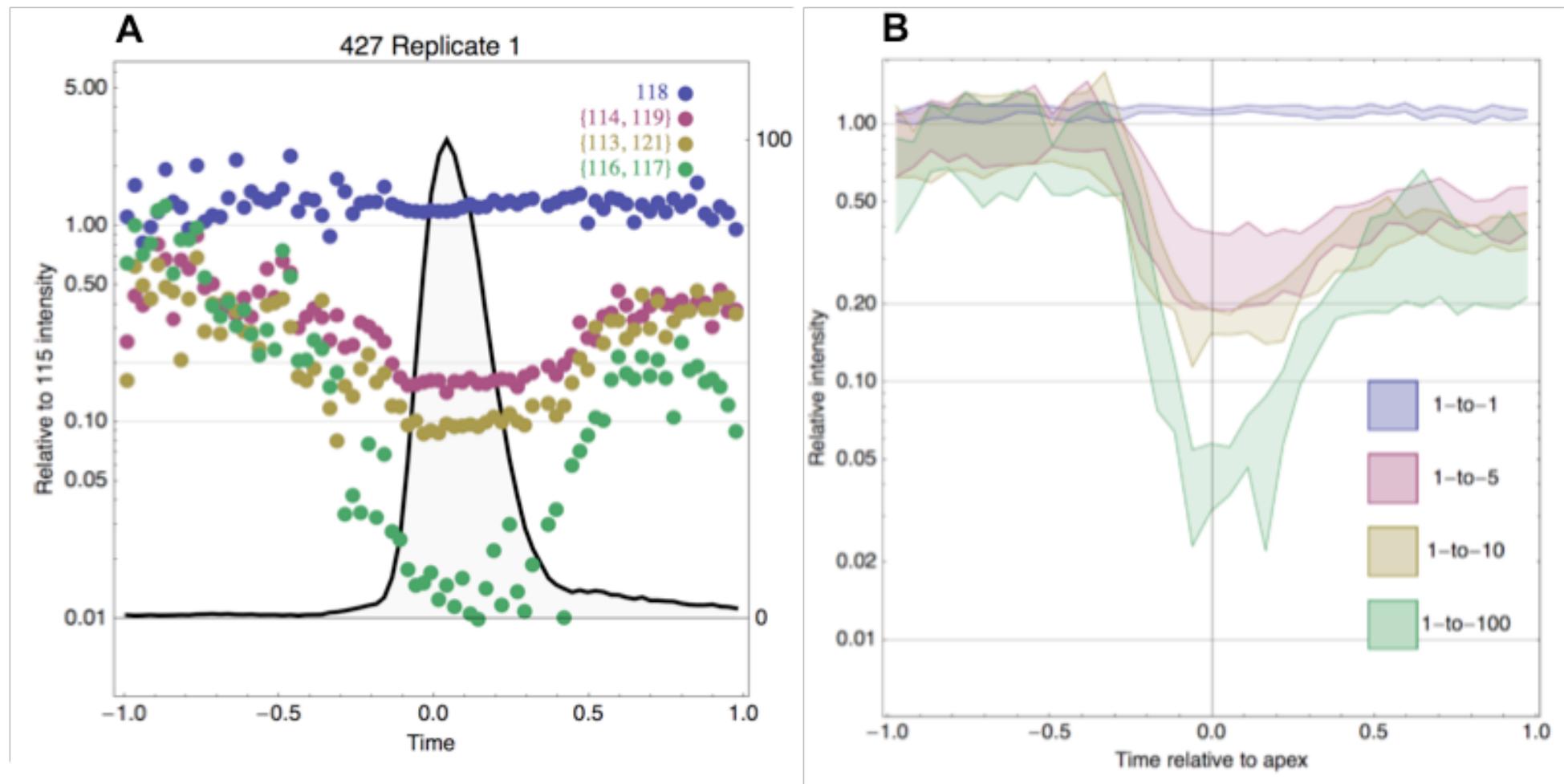


# *Walking across the peak.....*

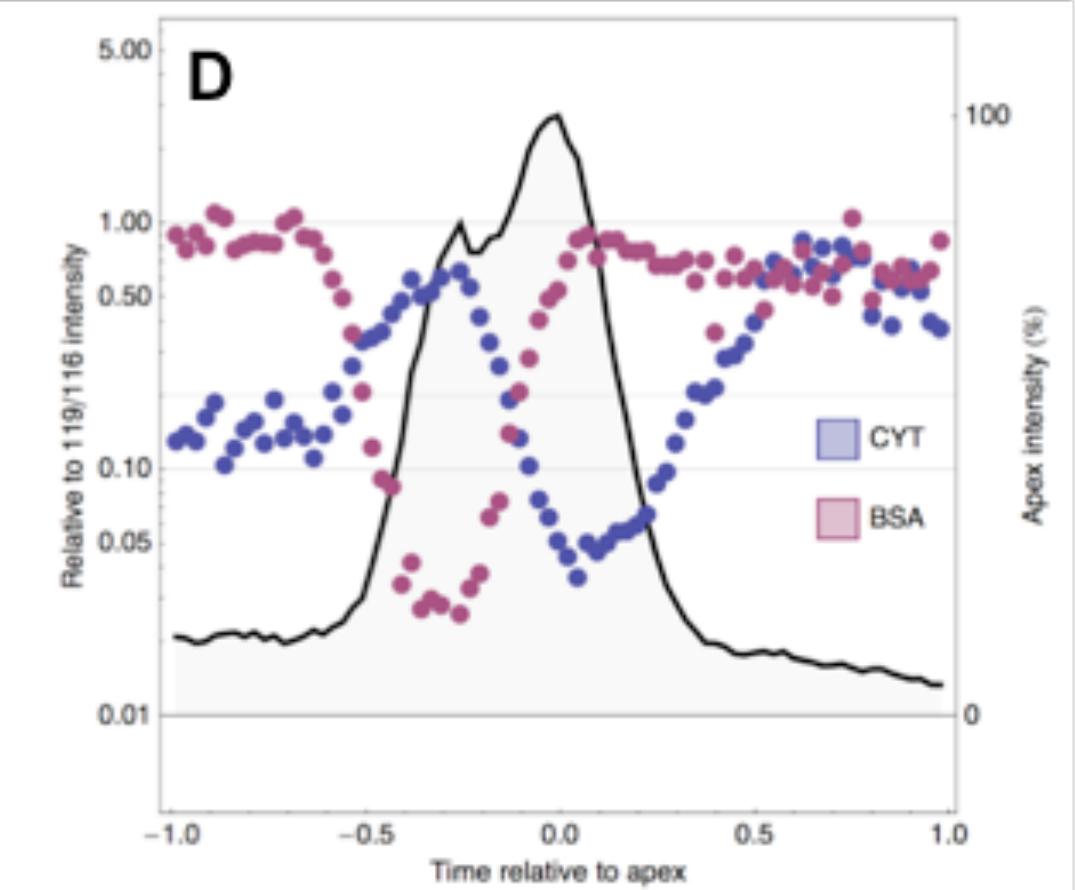
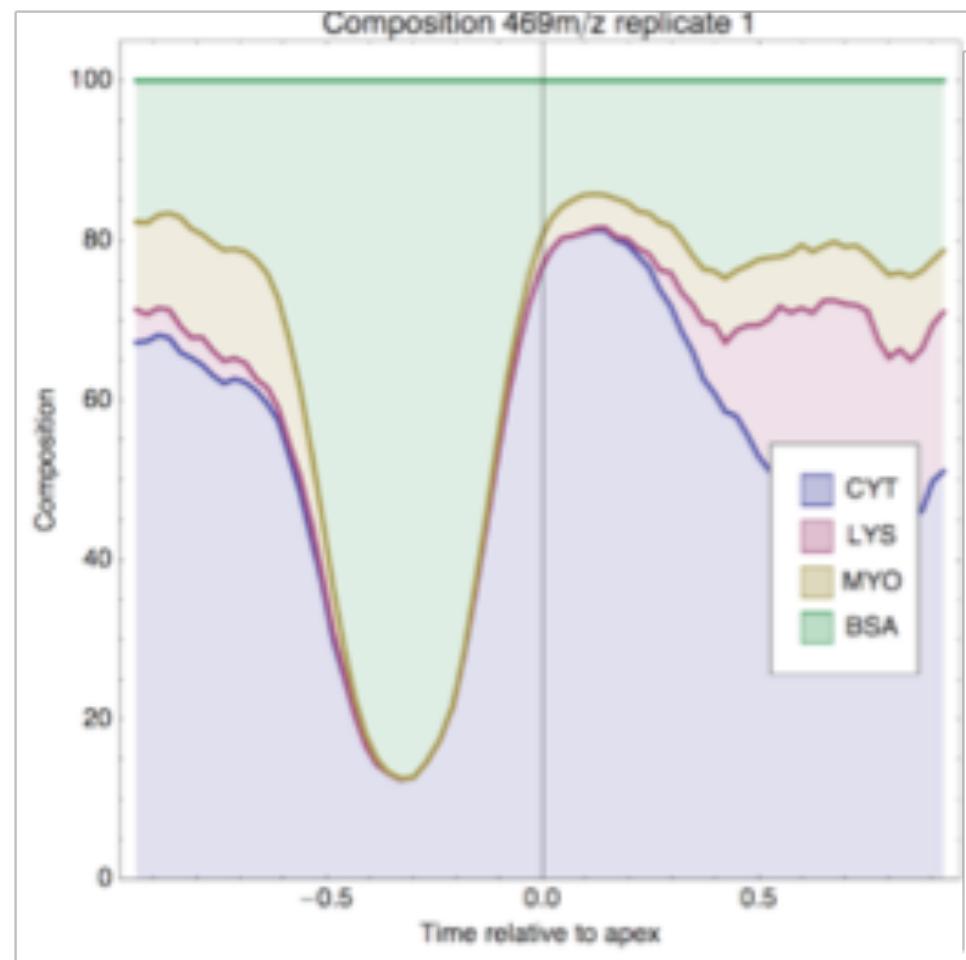
LC-MS/MS  
on  
maXis  
QSTAR  
solariX



# 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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