

**Quantitative Proteomics and Flux Analysis
using ^{15}N labelling:
Studies of the Unicellular Alga
*Ostreococcus tauri***

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Overview

- Introduction: metabolic labelling
- Experimental model and considerations
- Analysis platform and validation
- A time course analysis
- Summary and future work

Overview

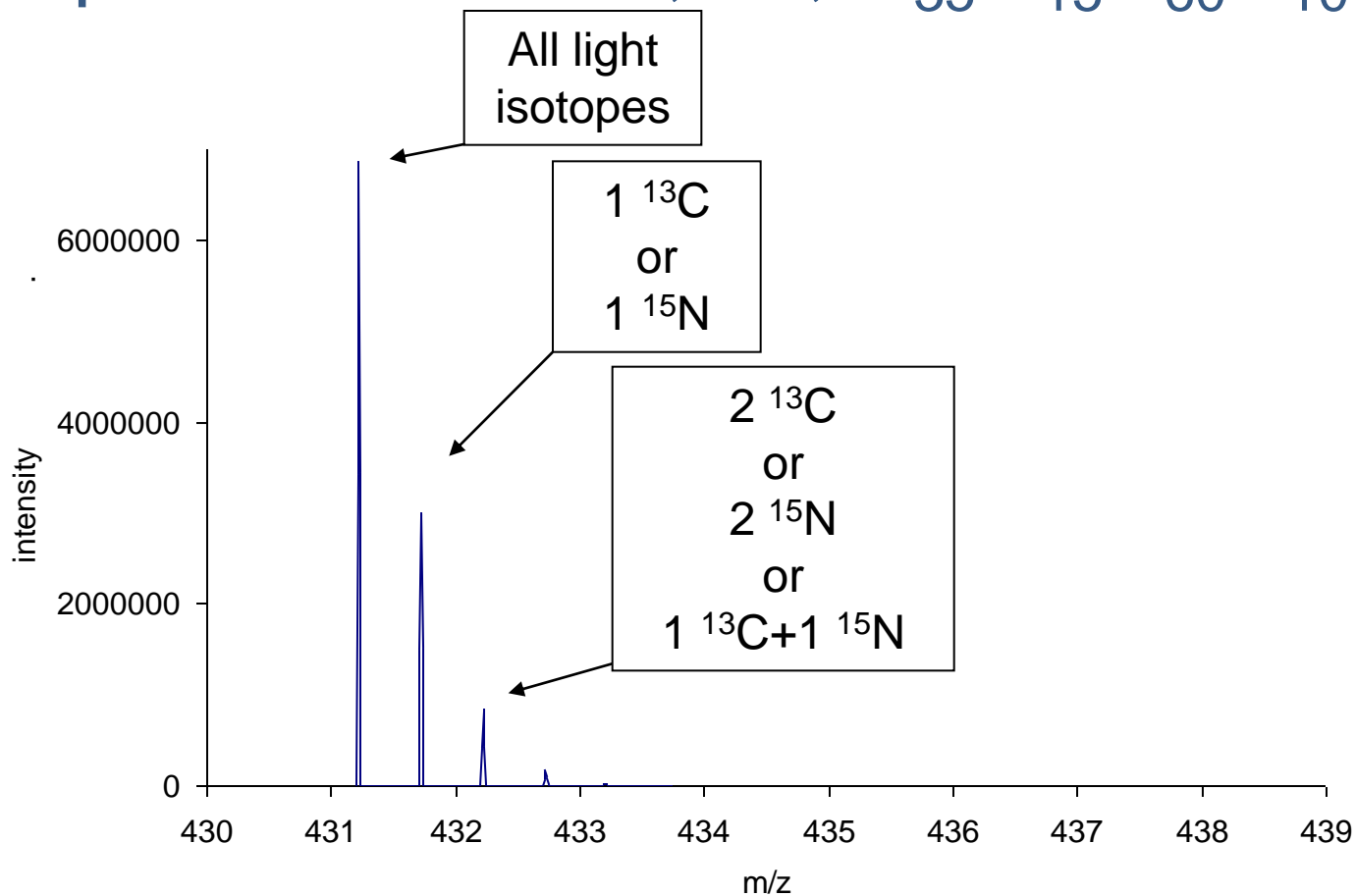
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Introduction: labelling

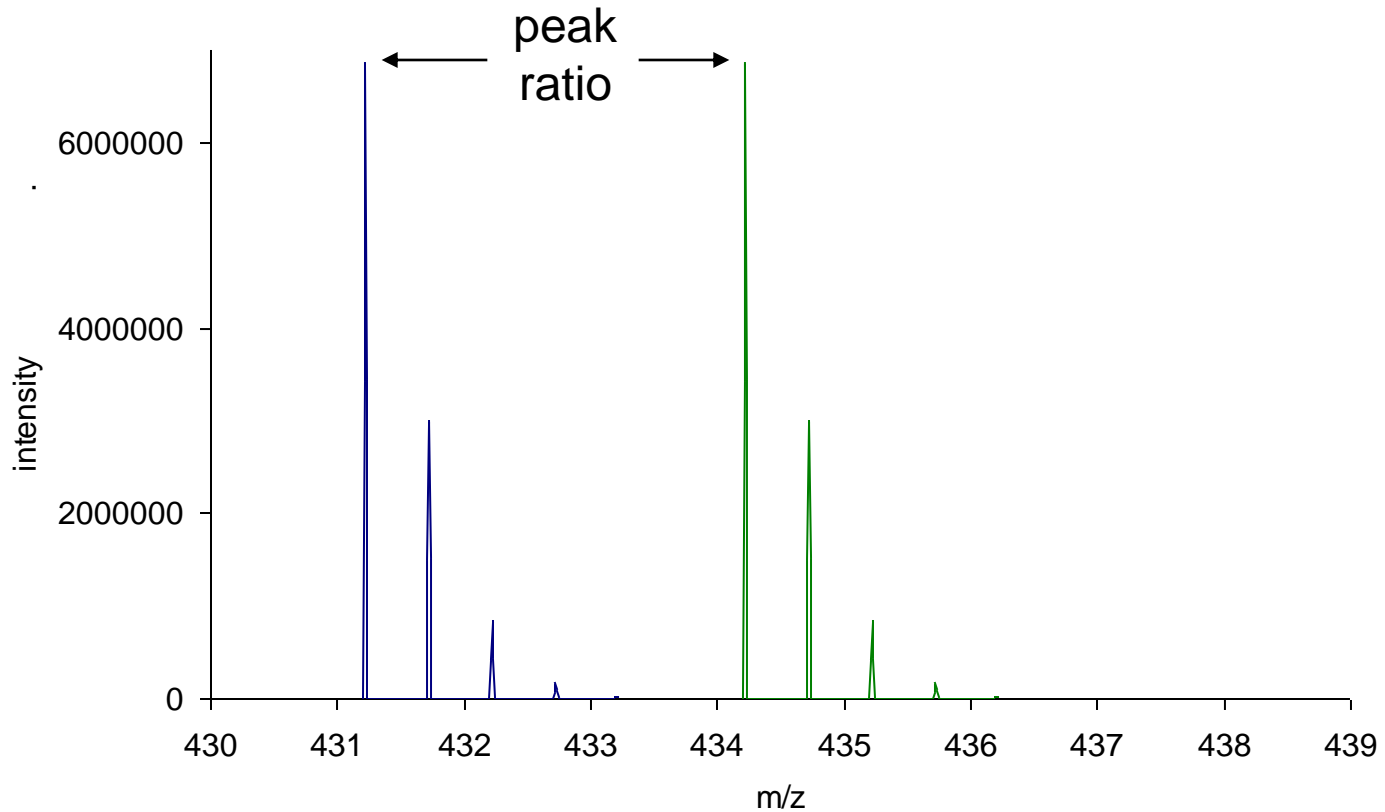
Classically used as a quantification strategy in liquid chromatography coupled mass spectrometry (LC-MS):

- Two cultures labelled *in vivo* can be pooled
- Reduces technical variability
- Enables relative quantitative comparisons in one LC-MS run

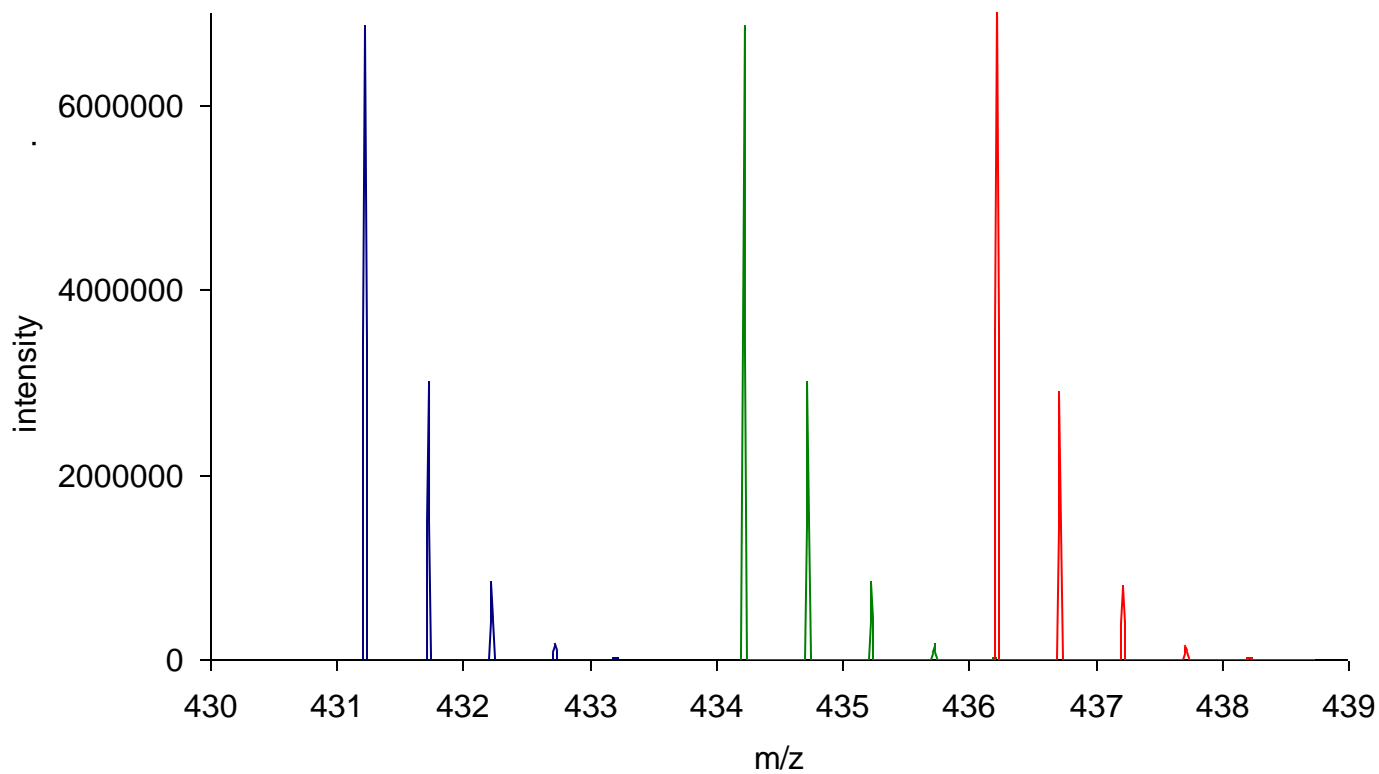
Example: NDVGDTIK, 2+, C₃₅O₁₅H₆₀N₁₀



SILAC: NDVGDTIK, (+6Da), 2+



¹⁵N: NDVGDTIK, 2+, C₃₅O₁₅H₆₀N₁₀



Introduction: labelling

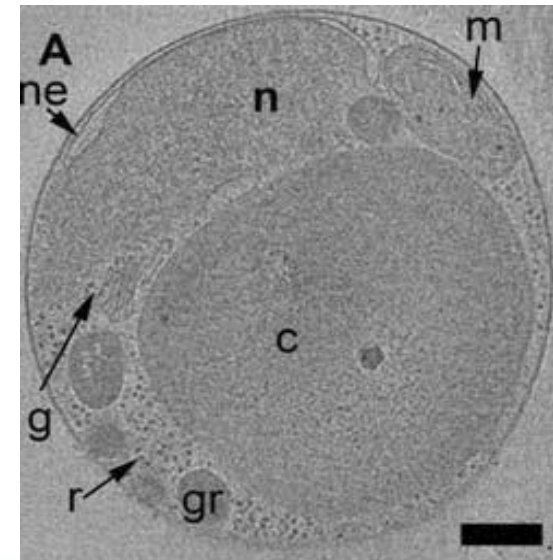
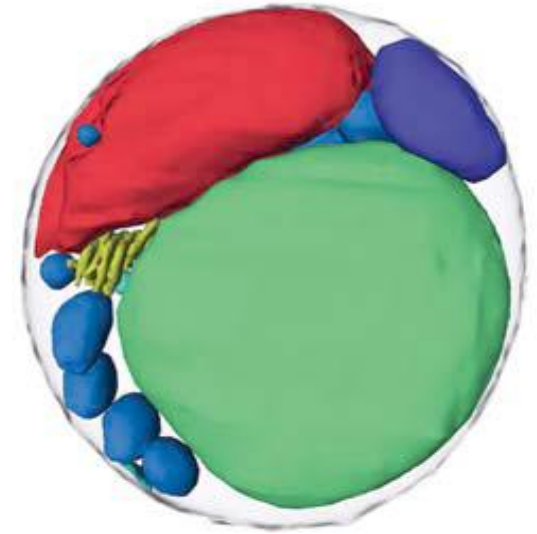
- Metabolic labelling is a powerful tool:
it introduces information on
 - amino acid synthesis and sourcing
 - protein assembly and turnover kinetics
- Nitrogen and Carbon assimilation are topical research themes:
 - nitrogen fertilizers and food security
 - carbon assimilation and bio-fuel production

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Experimental model: *Ostreococcus tauri*

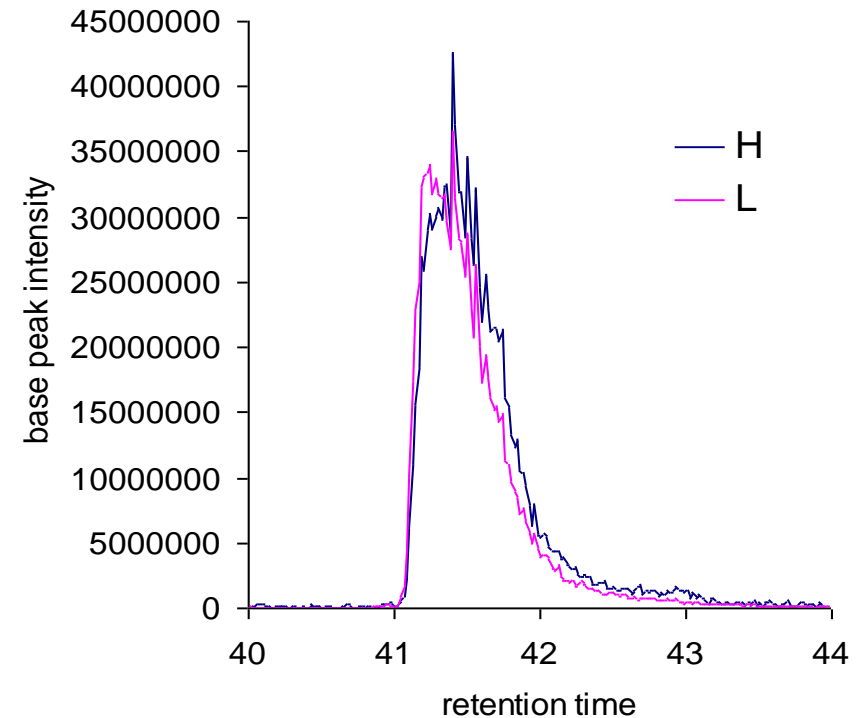
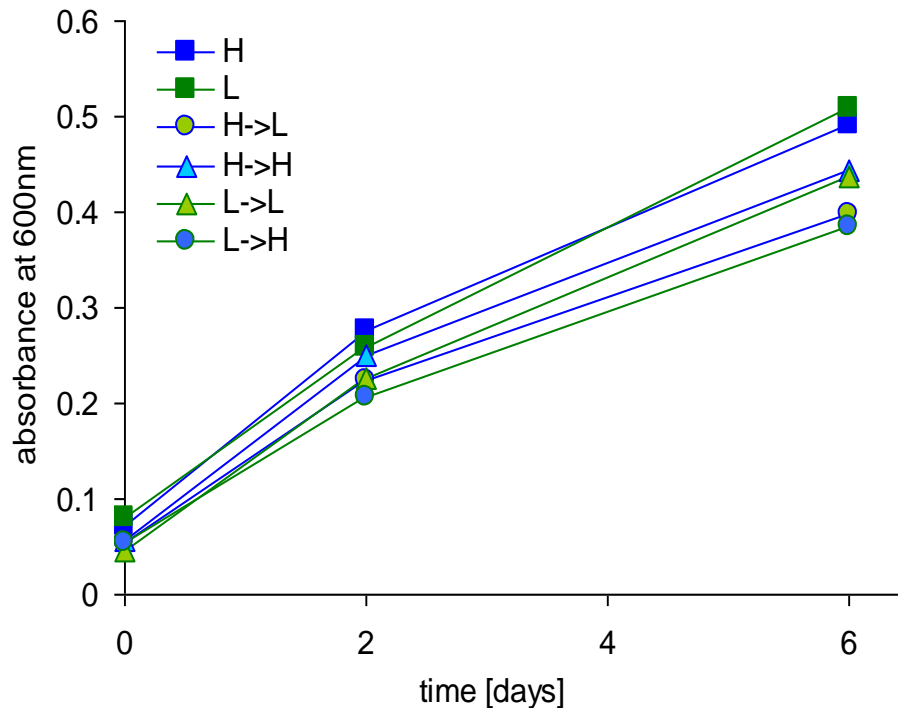
- Smallest free-living Eukaryote (Courties, *Nature* 1994)
- Ideal plant model for LC-MS analysis:
 - simple model of a very complex kingdom
 - key position at base of green lineage of plants
 - single “evening” division per day in culture (Farinas, *PlantMolBiol* 2006; Moulager, *PlantPhysiol* 2007)
 - no cellular differentiation
 - easy organelle fractionation
 - fully sequenced compact genome: 8,166 genes in 12.56Mb (Derelle, *PNAS* 2006)
- Utilized in studies of circadian rhythms, oceanic phytoplankton, photosynthesis, pesticide and industrial waste impact



Henderson, PLOS 2, 749 (2007)

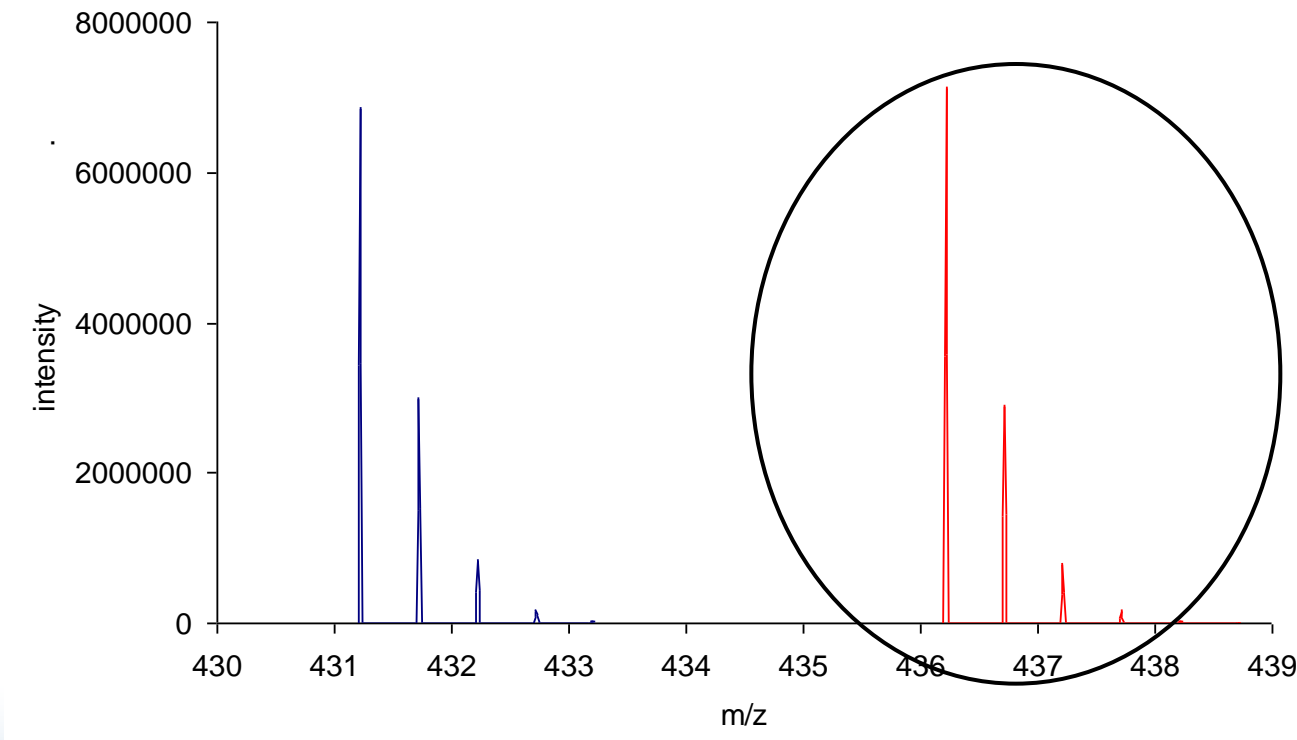
Experimental considerations

- growth in ^{14}N (L) and ^{15}N (H) media
- perturbation by centrifugation and media changes
- co-elution of labelled peptides on LC column



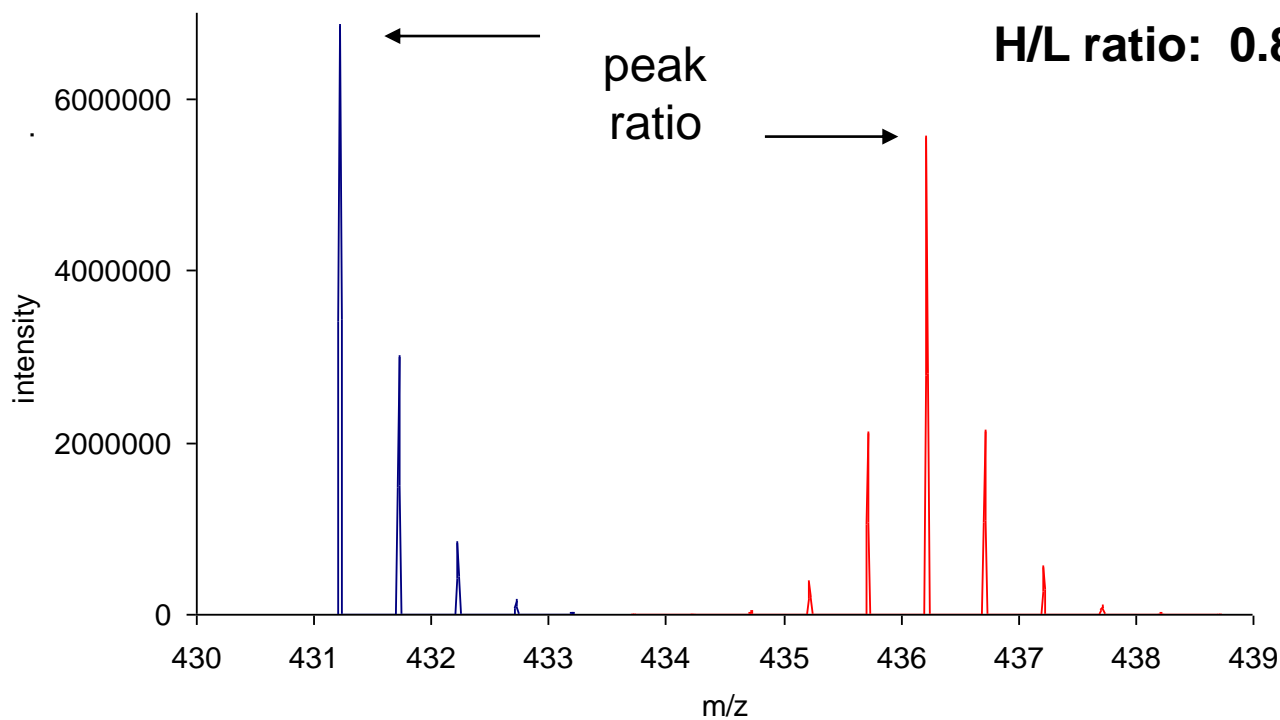
Experimental considerations

- growth in ^{14}N (L) and ^{15}N (H) media
- perturbation by centrifugation and media changes
- co-elution of labelled peptides on LC column
- partial labelling due to reagent purity



¹⁵N: NDVGDTIK, 2+, 96% labelled

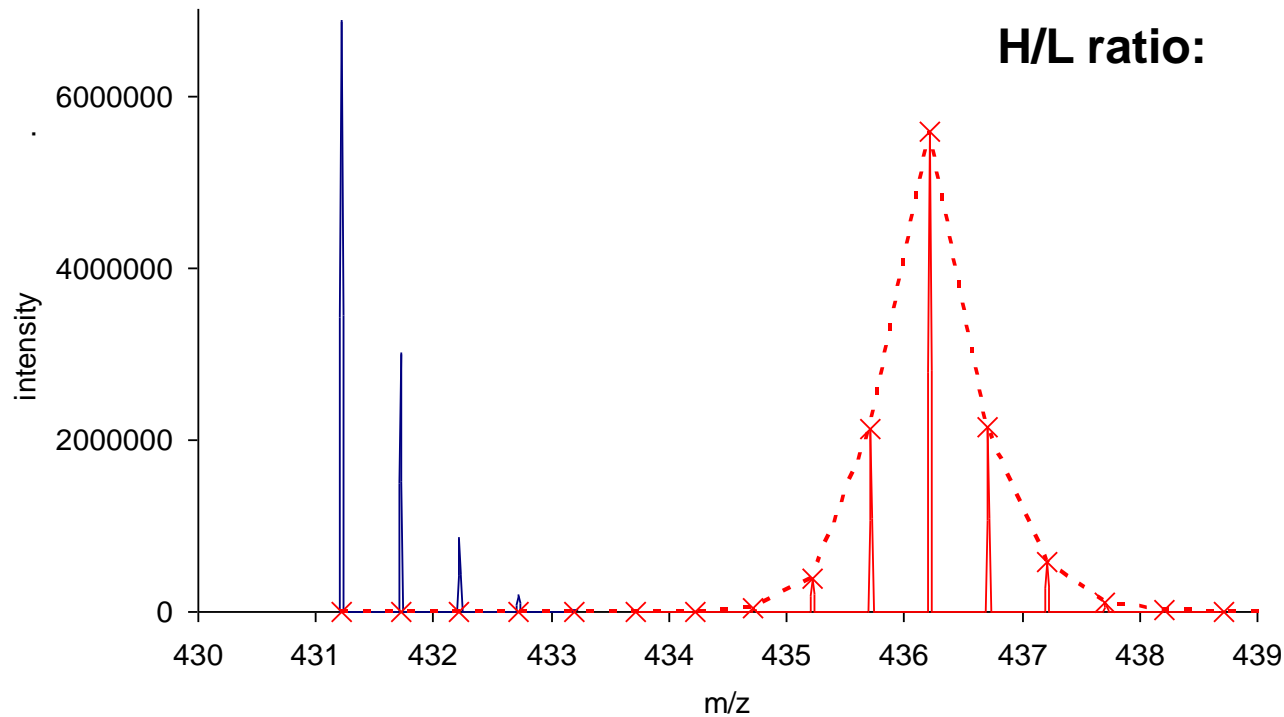
L peak: 6864706
H peak: 5578583



¹⁵N: NDVGDTIK, 2+, 96% labelled

% ¹⁵N: 96
L intensity factor: 11000000
H intensity factor: 11000000

H/L ratio: 1.0

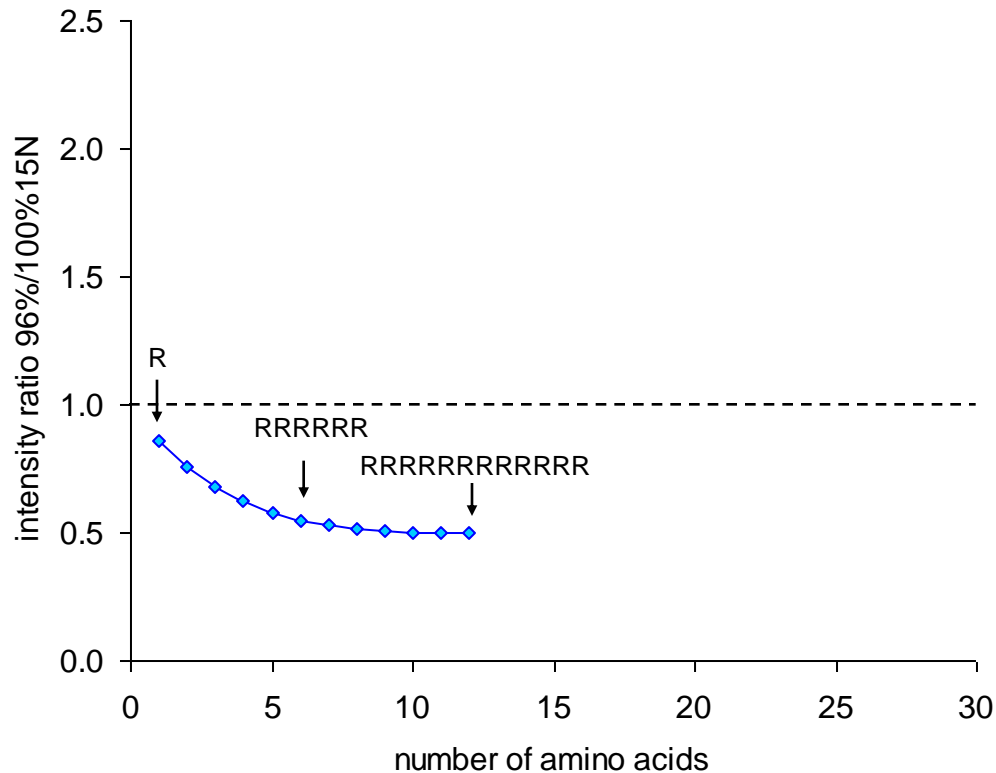


96% labelling: a little theory

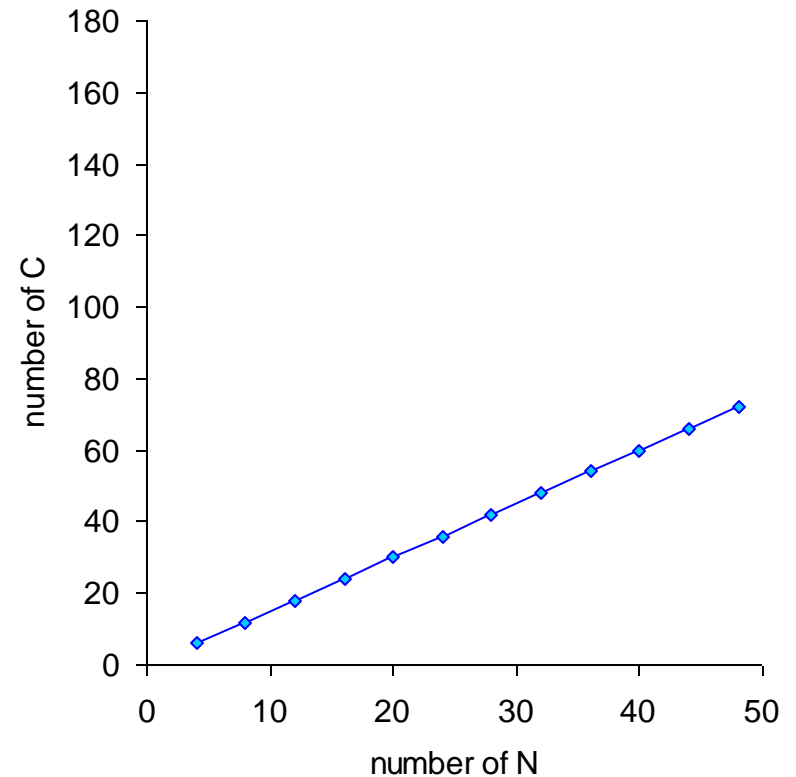
- how does the H/L peak ratio of a 1:1 mix depend on peptide length and composition?
- a closer look at selected “extreme” peptides composed of:
 - arginine R $C_6OH_{12}N_4$ $N/C = 0.67$ N rich
 - tyrosine Y $C_9O_2H_9N$ $N/C = 0.11$ N poor

96% labelling: arginine R $C_6OH_{12}N_4$

Peak ratio (96%:100%) vs peptide length

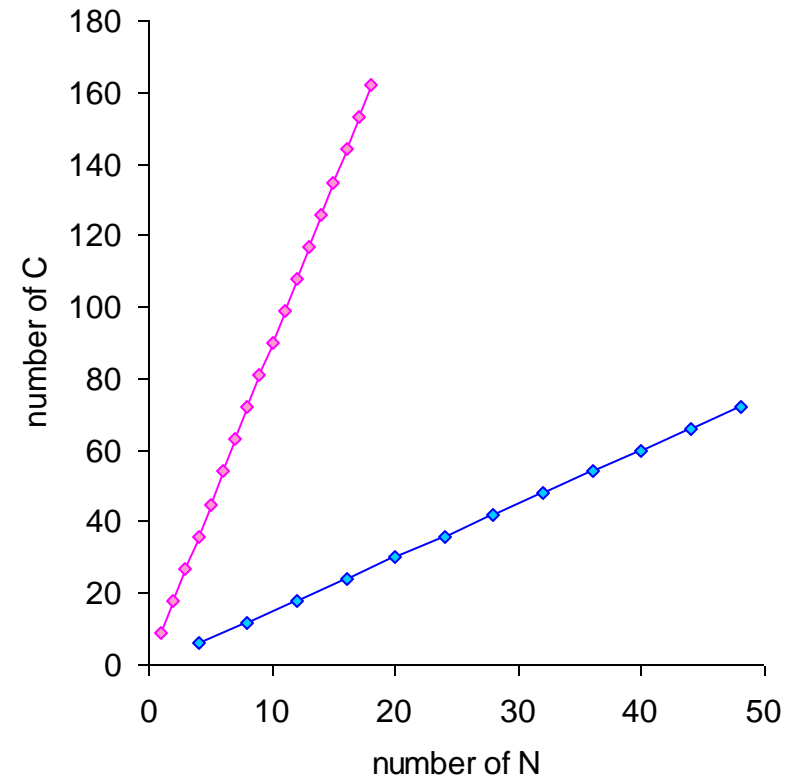
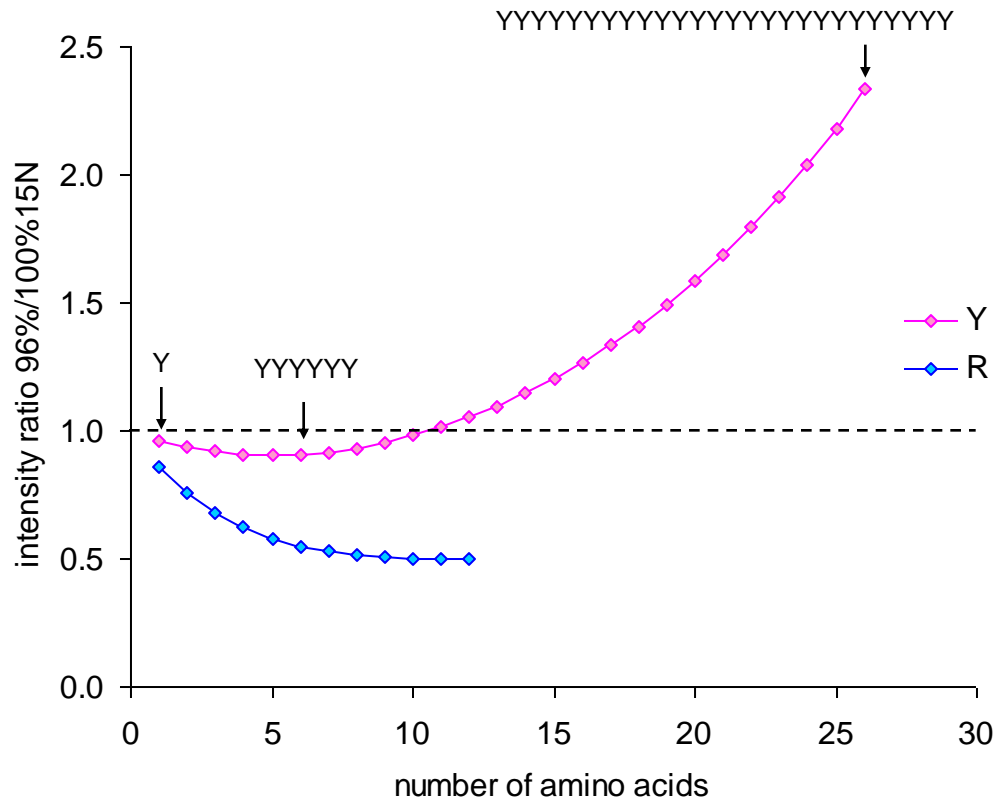


#Carbons vs #Nitrogens



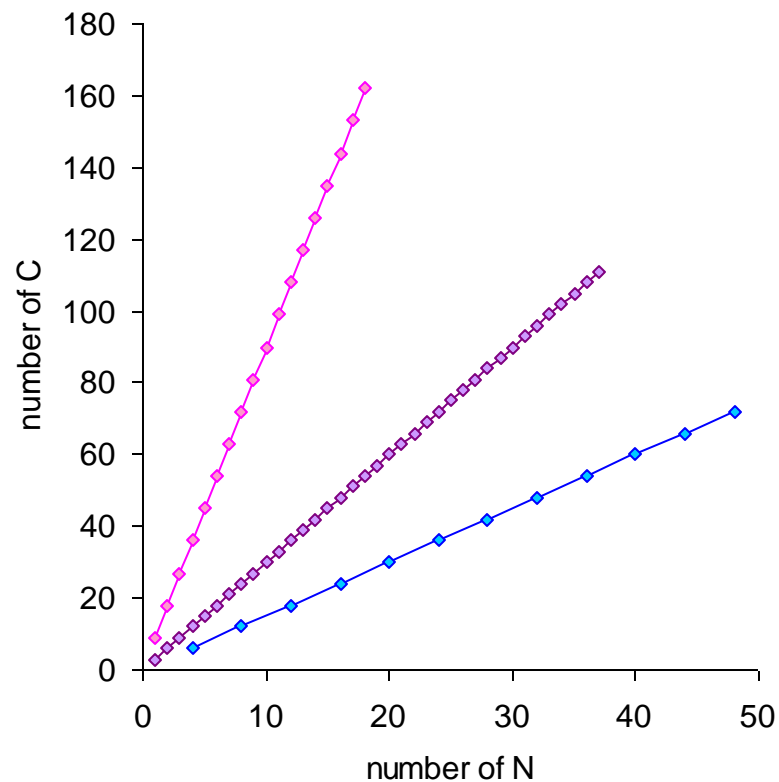
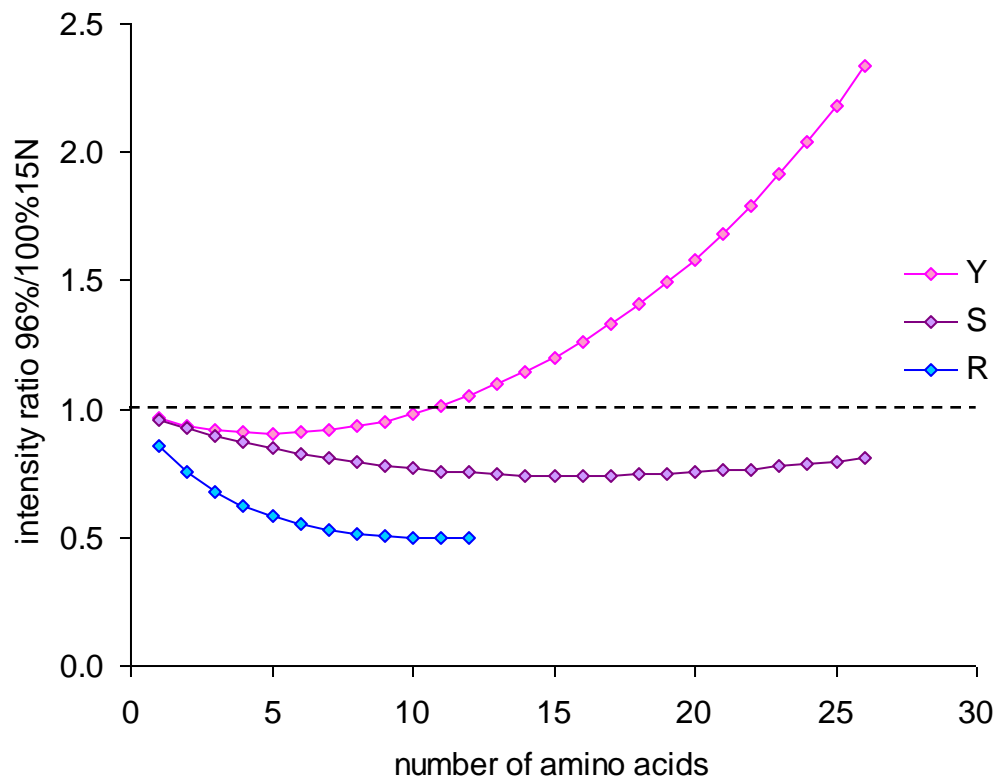
N richness leads to an underestimate of the H/L ratio

96% labelling: tyrosine Y $C_9O_2H_9N$

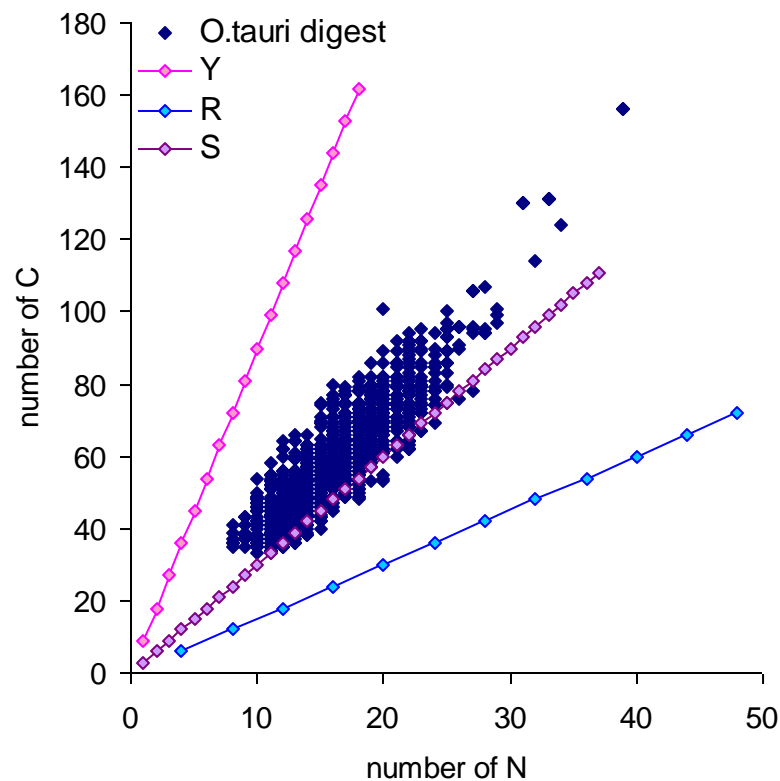
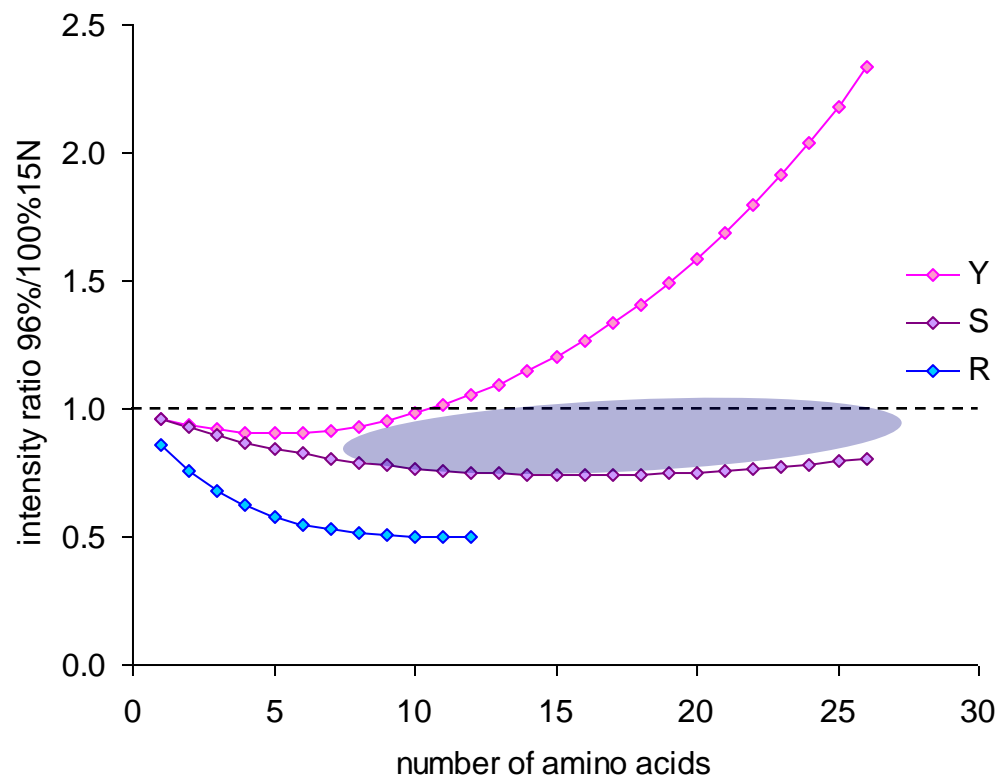


C >> N leads to an overestimate of the H/L ratio in longer peptides

96% labelling: serine S



96% labelling: what about real peptides?

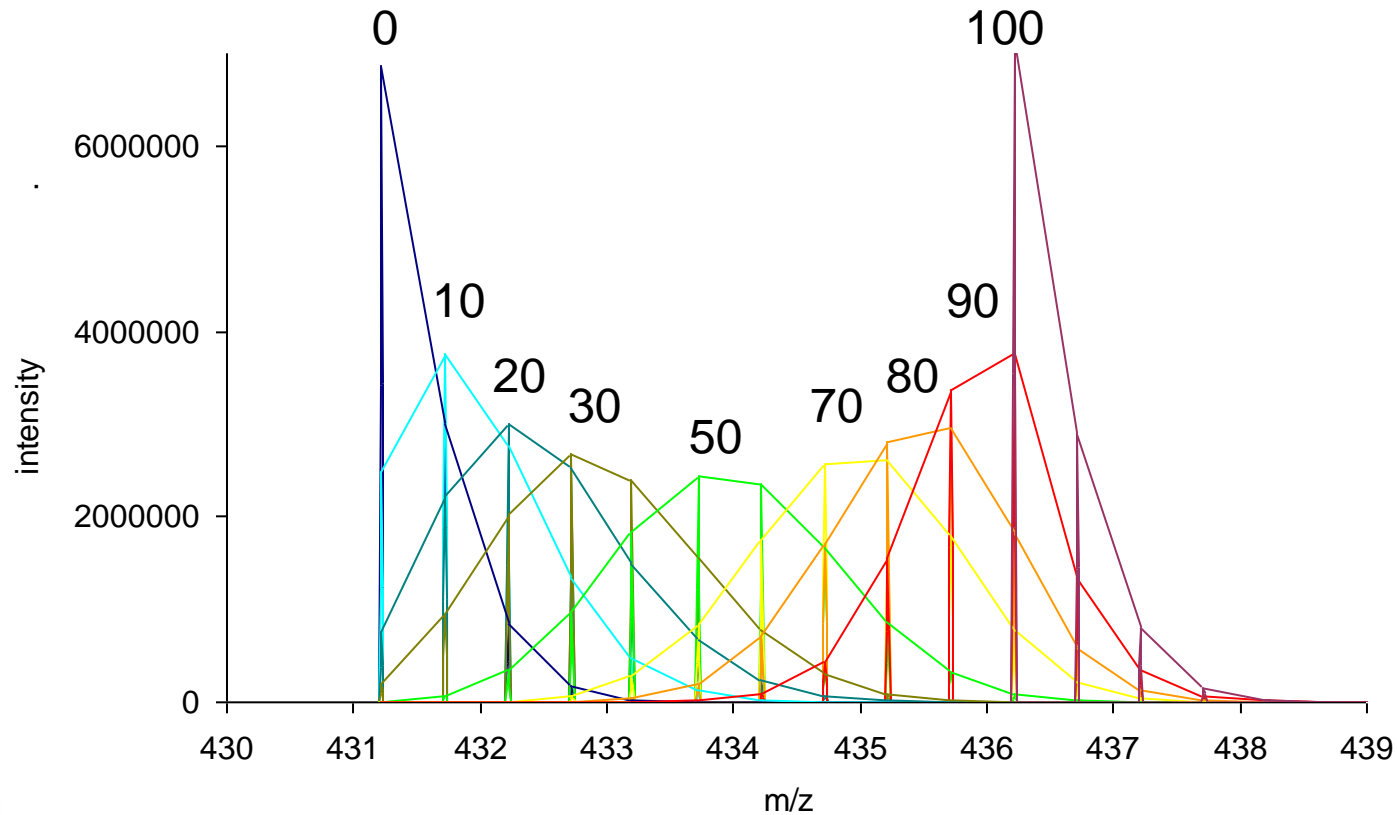


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Analysis platform

partial labelling of NDVGDTIK, 2+ : %¹⁵N varied, intensity constant



Data analysis

- batch peptide peak extraction over labelling range
- data averaging over retention time range
- fitting process returns
 - % of ^{15}N labelling \longleftrightarrow
 - intensities of L and H species \updownarrow
- enables processing at system scale

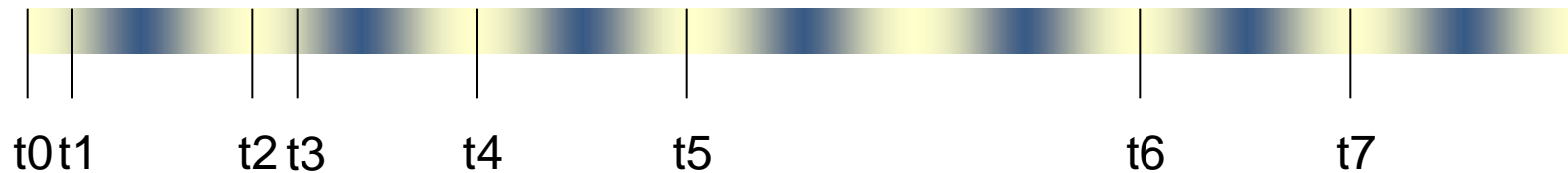
	A	B	C	D	E	F	G	H	I	J
1	Sequence	Charge	ppm	RT1	RT2	MZXMLfile	15N%	Intensity L	Intensity H	H/L
2	NDVGDTIK	2	5	1920.6	1938.6	L_H.mzxml	95.5	2214361	2454361	1.11
3	IGLFGGAGVGK	2	5	3910.2	3928.2	L_H.mzxml	94	15496041	18536041	1.20
4	FVQAGSEVSALLGR	2	5	4294.2	4312.2	L_H.mzxml	92	3828918	3908918	1.02
5	VGLTALTMAEYFR	2	5	5124	5142	L_H.mzxml	94.5	5178335	5348335	1.03
6	NHGIHFR	2	5	2094.6	2112.6	L_H.mzxml	93	1913464	1923464	1.01
7	AMHAVIDR	2	5	2216.4	2234.4	L_H.mzxml	94	4478322	4858322	1.08
8	FLFVAEAIYK	2	5	5544.6	5562.6	L_H.mzxml	93	238015	228015	0.96

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Time course analysis

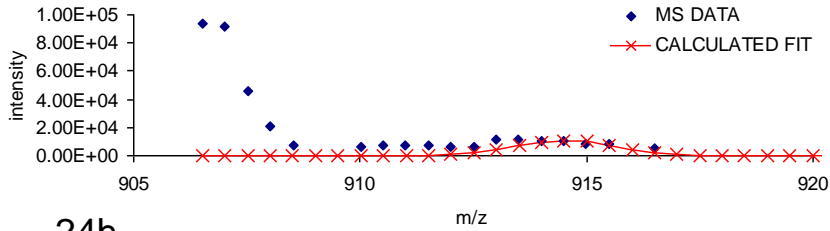
- Swap media H->L, L->H
 - spin 6-day L and H *O. tauri* cultures, filter supernatants, exchange H→L and L→H, re-suspend, replace in incubator
- Sample over 7 days
 - spin, wash, lyse and digest 50ml per time point
 - LC-MS analysis on LTQ-Orbitrap XL



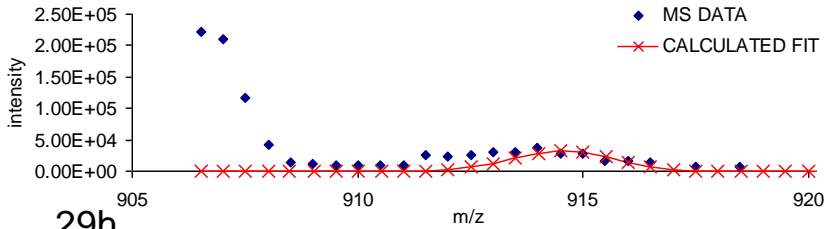
AtpB

ITQIIGPVIDAVFSPNK

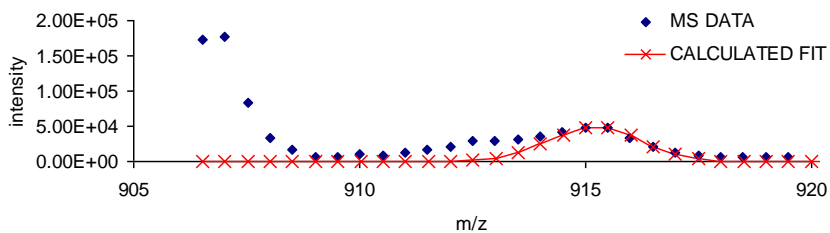
5h



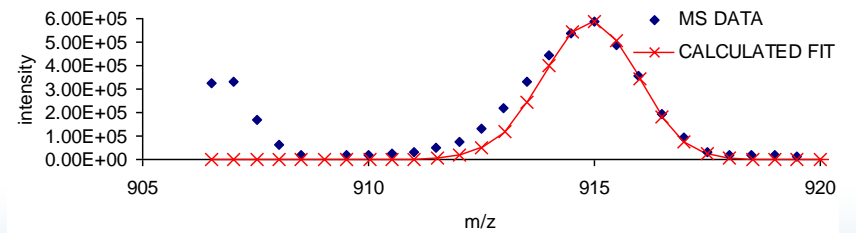
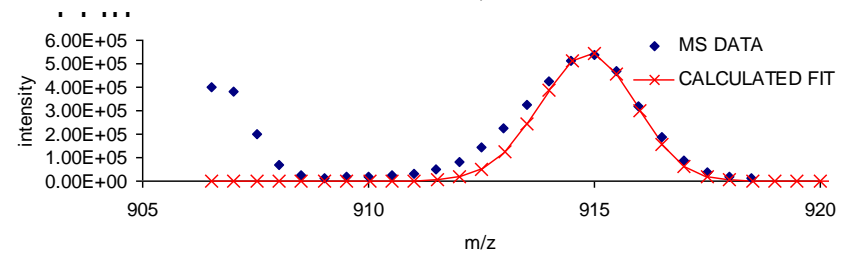
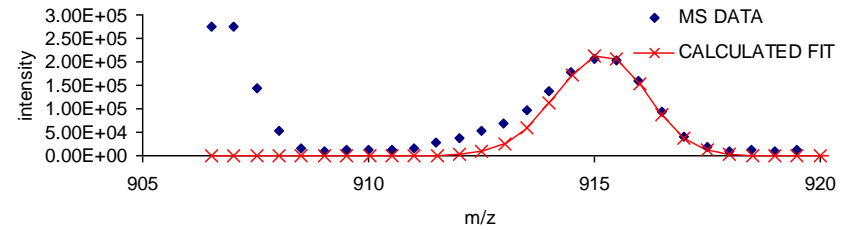
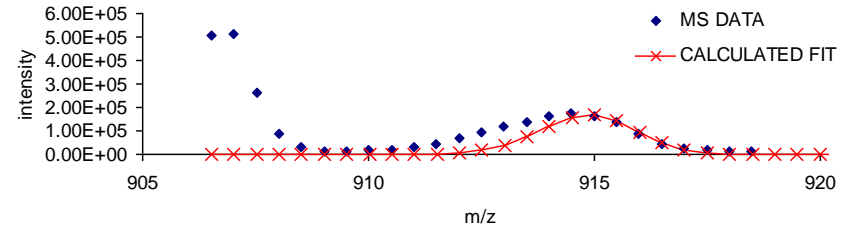
24h



29h



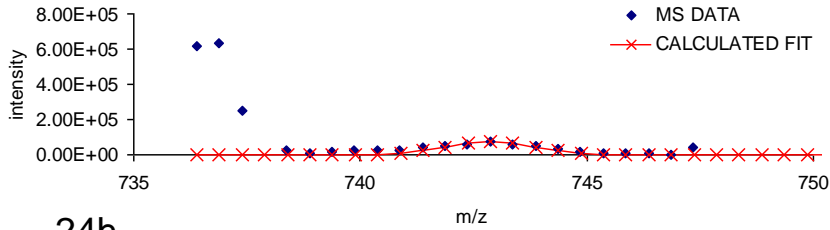
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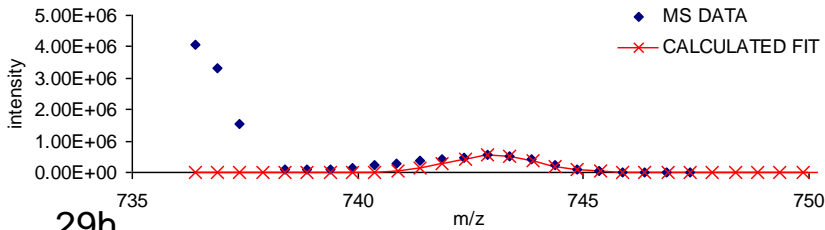
AtpB

VGLTALTMAEYFR

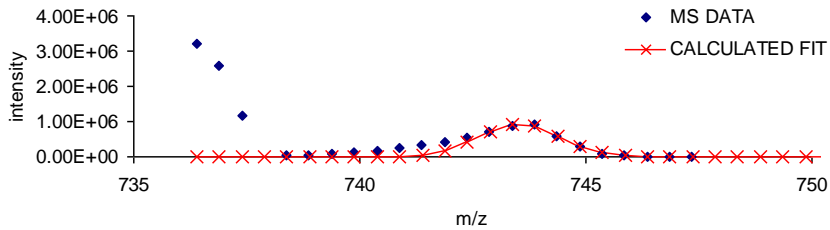
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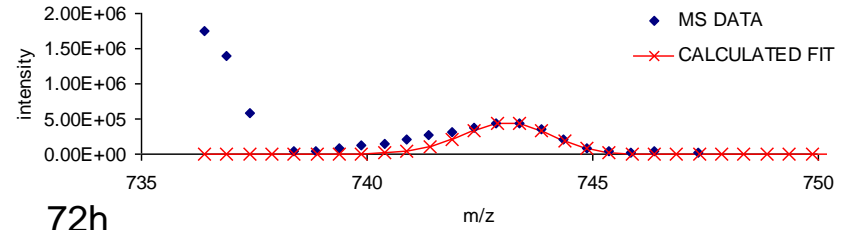
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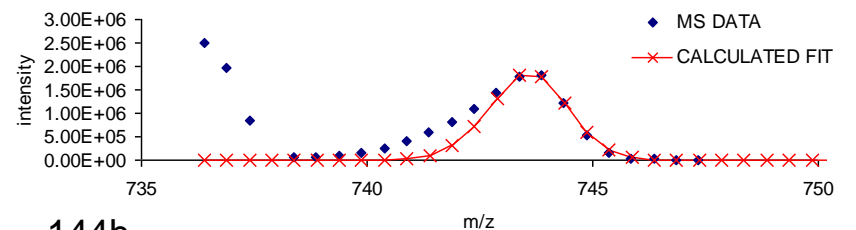
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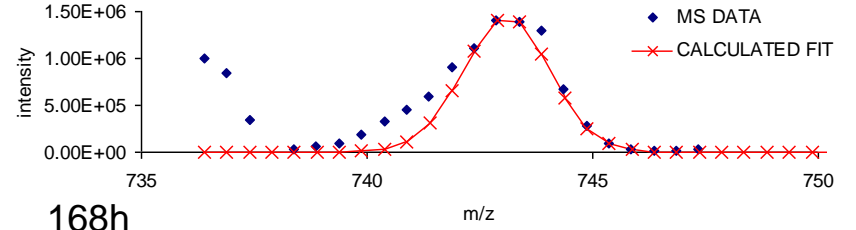
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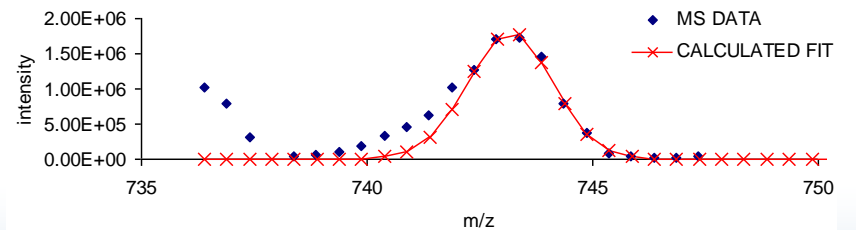
72h



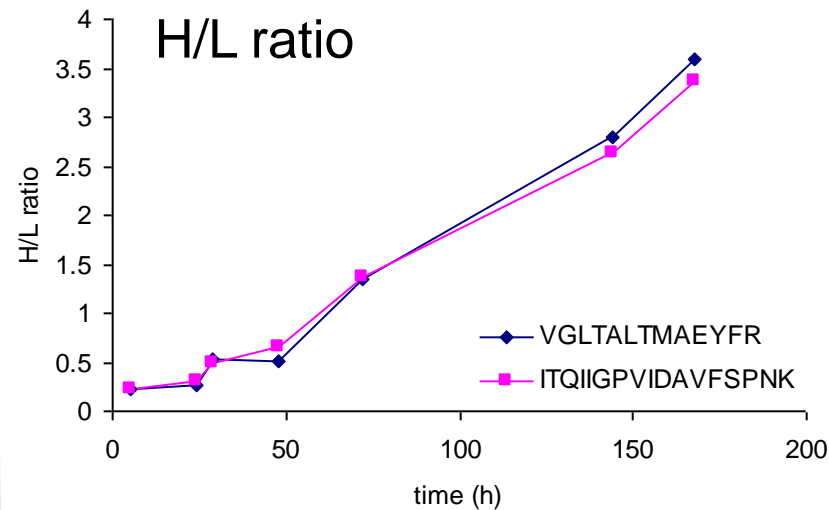
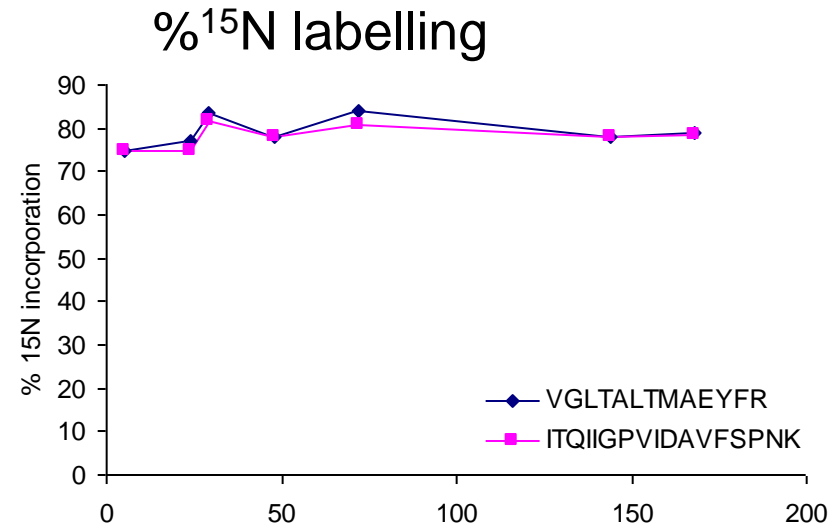
144h



168h

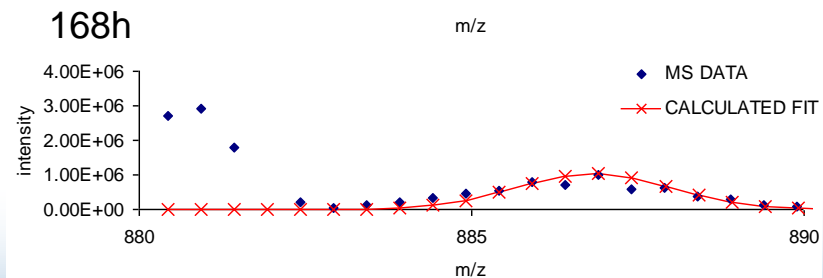
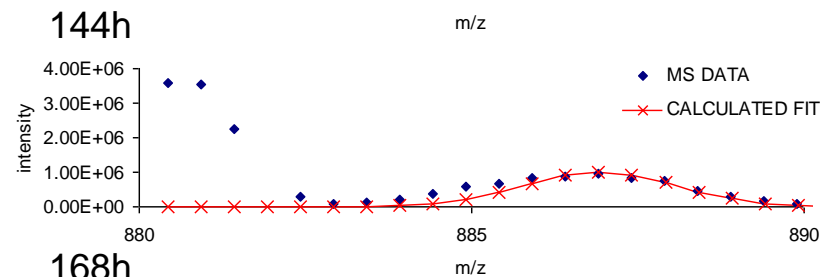
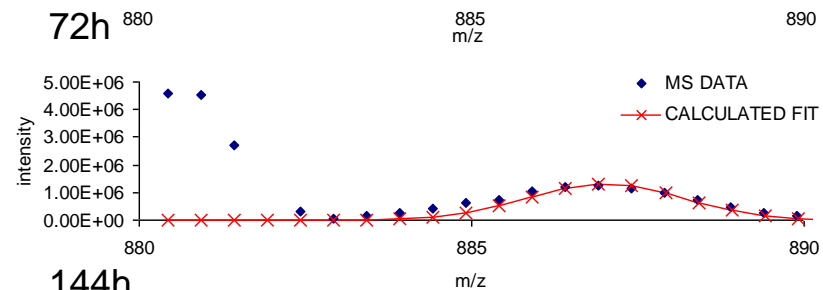
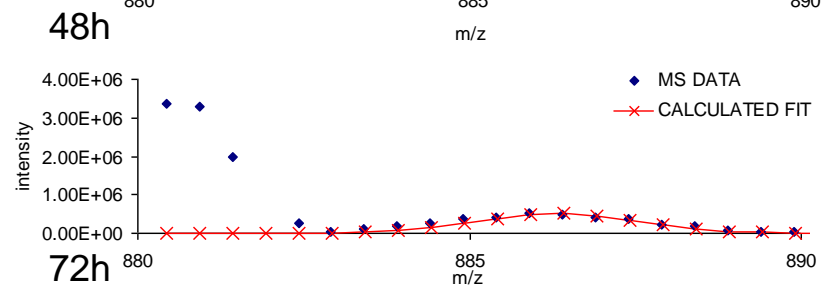
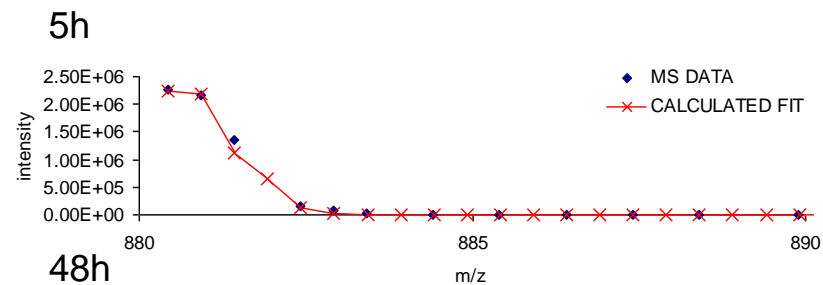
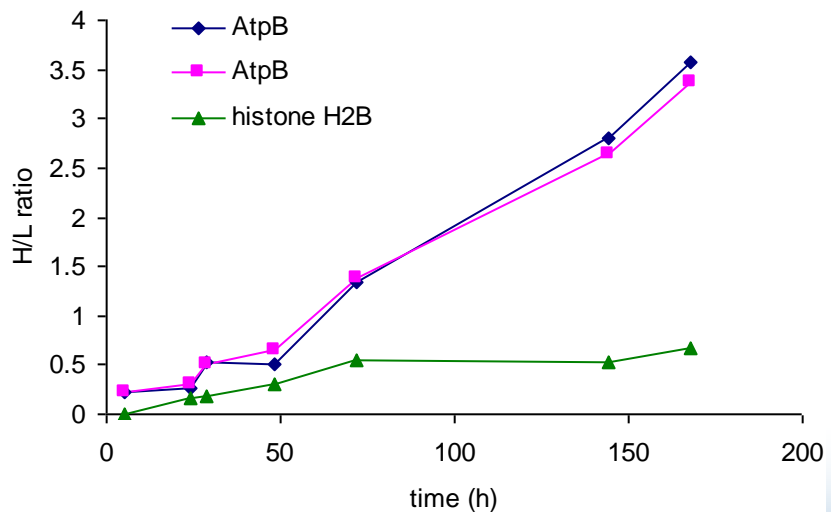
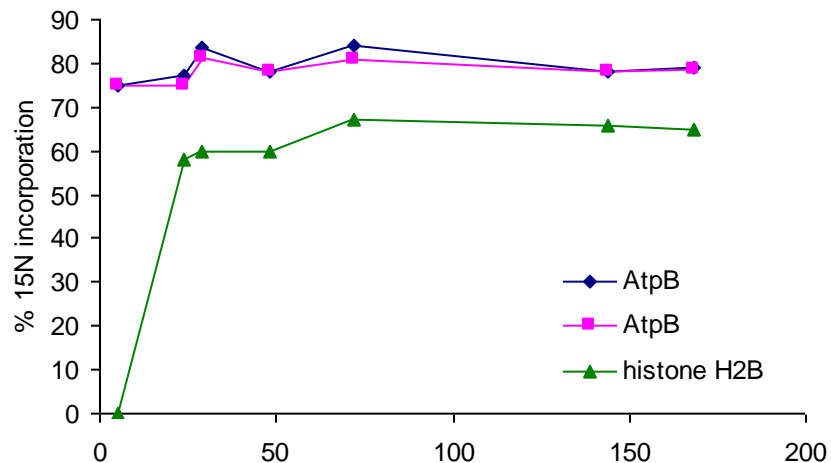


%¹⁵N labelling and H/L ratio over time



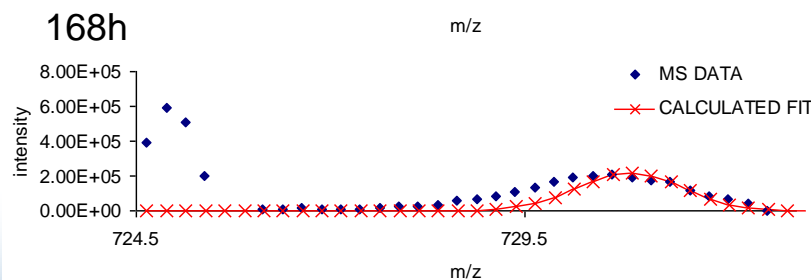
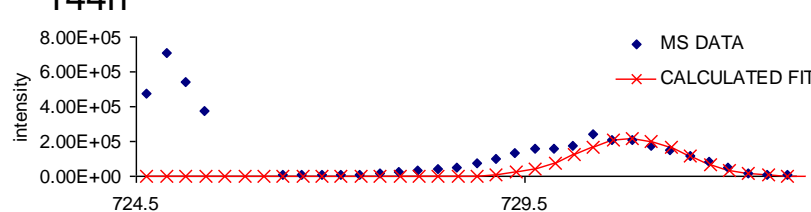
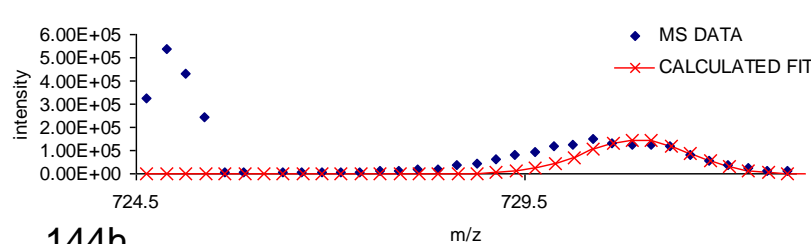
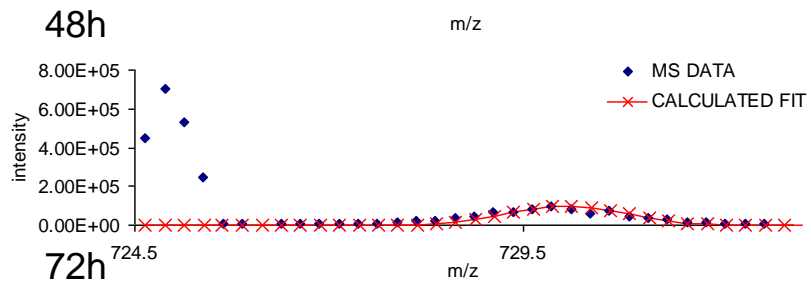
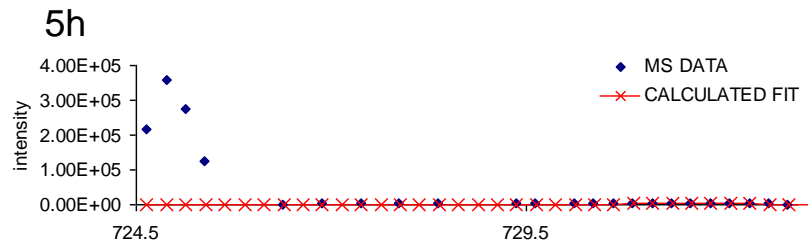
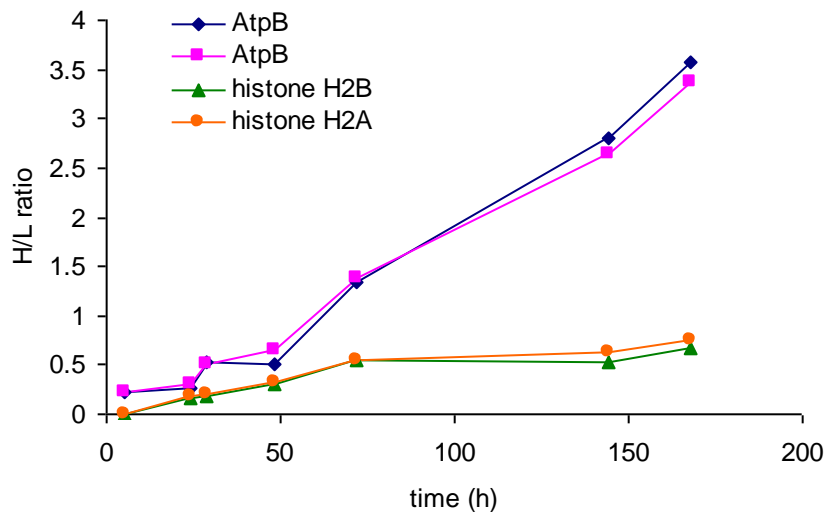
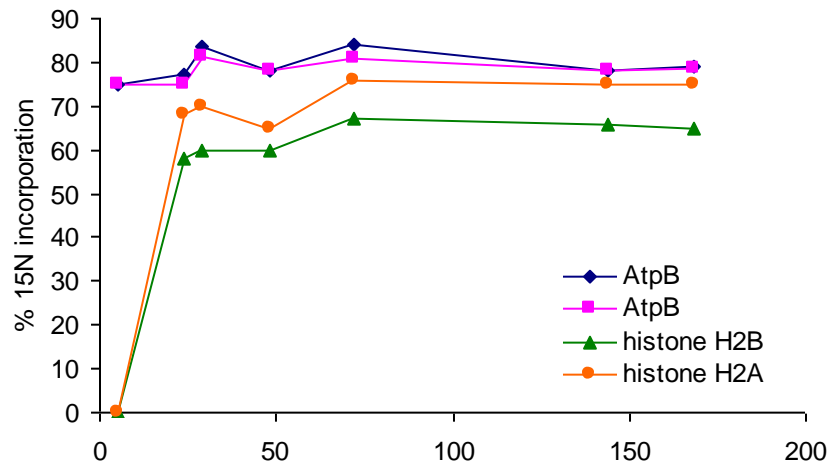
Histone H2B

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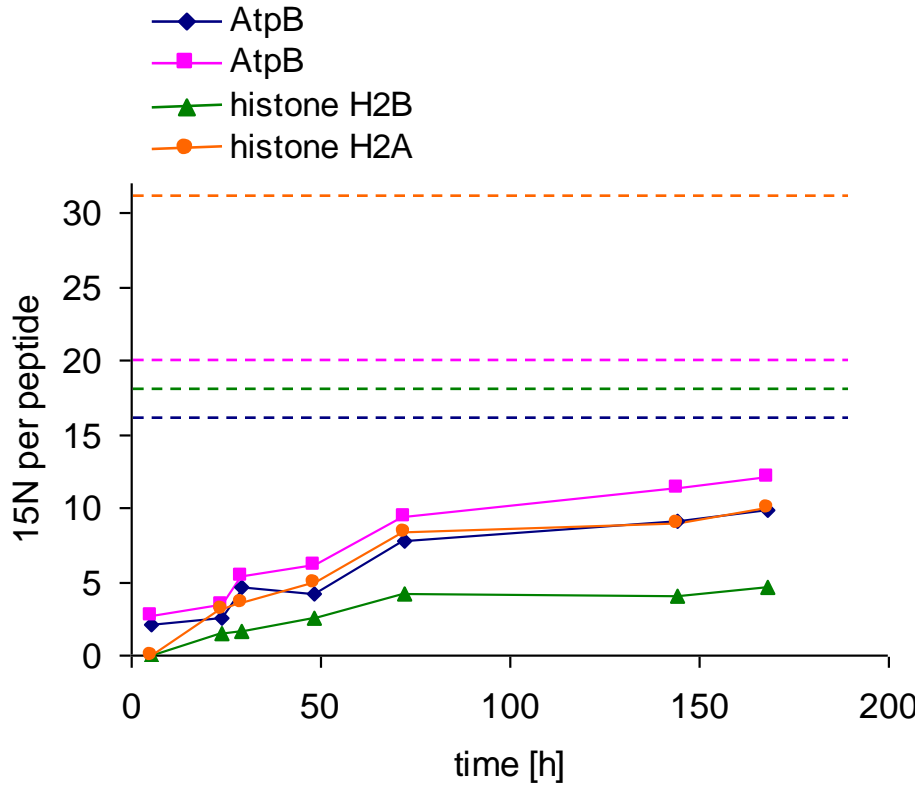


Histone H2A

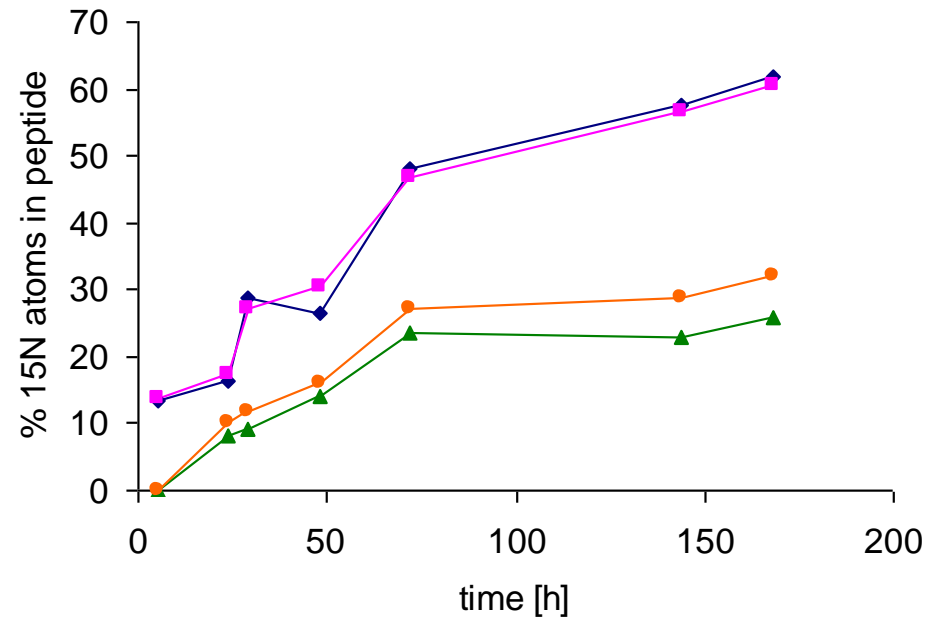
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Nitrogen incorporation



^{15}N nitrogen atoms per peptide



% ^{15}N nitrogen atoms per peptide

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Summary and future work

We have developed a novel analysis platform to quantify partial metabolic labelling of peptides

This enables the robust analysis of

- comparative experiments when the heavy state is labelled to less than 100%
- dynamics of protein turnover
- de novo synthesis of amino acids
- incorporation rates and distributions of nitrogen (and carbon) in the proteome

- Further development, expansion and de-bugging
- Software release
- Amino acid level analysis

Acknowledgements

- Shanti Munagapati – summer student, Perl programmer
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