

BSPR: July 2010

# Quantitative proteomics: what's the point?

Rob Beynon

**MERCUTIO:**

*Nay, if our wits run the wild-goose chase*

Romeo And Juliet Act II, scene IV

# Overview

- \* Strategies quantification, challenges and limits
- \* Global quantification of a proteome
- \* Label-free quantification or label-mediated quantification?



The screenshot shows a web browser window displaying the website for the Proteomics and Functional Genomics Group at the University of Liverpool. The browser address bar shows the URL <http://www.liv.ac.uk/pfg/>. The website header includes the University of Liverpool logo and the text "UNIVERSITY OF LIVERPOOL" and "PROTEOMICS AND FUNCTIONAL GENOMICS RESEARCH GROUP". Below the header is a navigation menu with links for "About PFG", "Research", "Facilities", "Services", "Links", and "[Intranet]". The main content area is titled "Welcome" and features a banner image showing laboratory equipment and a person. Below the banner, there is a "Contents" section with a "What you will find on this website:" section, followed by a "Breaking News" section with a list of news items dated from January 2009 to March 2010. The news items include announcements about new PhD students, thesis defenses, and research achievements.

# Acknowledgments

## QconCAT (Liverpool)

Jenny Rivers  
Mary Doherty  
Amy Claydon  
Deborah Simpson

## QconCAT (Manchester)

Claire Evers  
Simon Gaskell  
Hannah Johnson

## Waters (Label-free)

Jim Langridge  
Therese McKenna  
Chris Hughes  
Hans Vissers



## COPYCAT (Liverpool)

Phil Brownridge  
Vicky Harman  
Lynn McLean  
Duncan Robertson

## COPYCAT(Manchester)

Simon Hubbard  
Paul Sims  
Chris Grant  
Craig Lawless  
Rae Watkins  
Steve Holman  
Karin Linthaler

# Phases of proteomics

Identification

What is/are the identities of the proteins in this preparation?

Characterisation

Post-translational modifications  
Intracellular stability

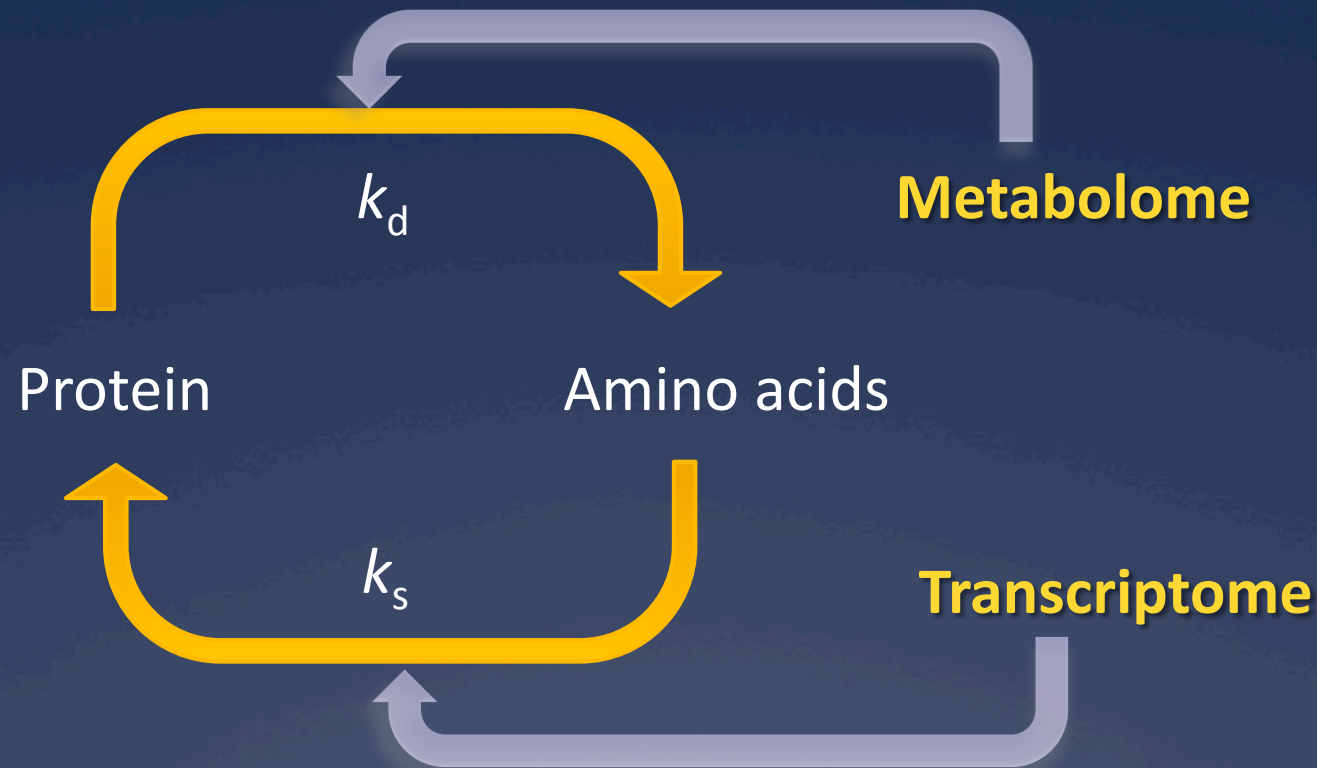
Quantitative

Relative quantification “*Protein Z is  $2.5 \pm 0.2$  times more abundant in Cell A than Cell B*”

Absolute quantification “*Protein Z present at 0.1 amol/cell A, 2.5 amol/cell B*”



# Quantitative proteomics & proteome dynamics



$$\sum_{i=1}^n \frac{dP_i}{dt} = k_{Ii} \cdot R \cdot [mRNA] - k_{di} \cdot P_i - \mu P_i$$

# The many hurdles in quantitative proteomics

## Dynamic range

Proteome depth and ion statistics

## The problem of protein 'species'

Post-translational space

## Surrogacy

Are peptides suitable for protein quantification?

## Proteome dynamics

Post-translational space

## Proteolysis

The essentiality of complete digestion to limit peptides

## Protein dynamics

Proteome turnover and intracellular protein dynamics

# Label free and label-mediated methods

Label-mediated  
(differential isotope labelling)

Relative (*in vitro*)

ITRAQ, ICAT, etc

Relative (*in vivo*)

SILAC, etc

Absolute

AQUA, QconCAT

Label-free  
(Absolute & relative)

Precursors (MS)

counting

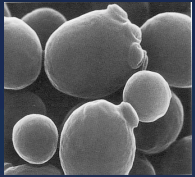
intensity

Fragments (MS/MS)

counting

intensity

# The scale of the challenge



Yeast

[ 10 million cells ]

0.06mg protein = 6pg/cell



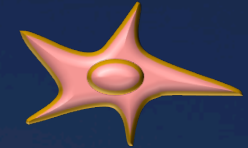
Digest 100µg/200µL digest  
(17 million cells in digest)



Apply 1000ng 'protein' (1µL)  
~170,000 cells on column



Lowest sensitivity=  
360 copies/cell



HeLa

~2.5mg protein = 250pg/cell



Digest 100µg/200µL digest  
(0.5 million cells in digest)



Apply 1000ng 'protein' (1µL)  
~4,000 cells on column



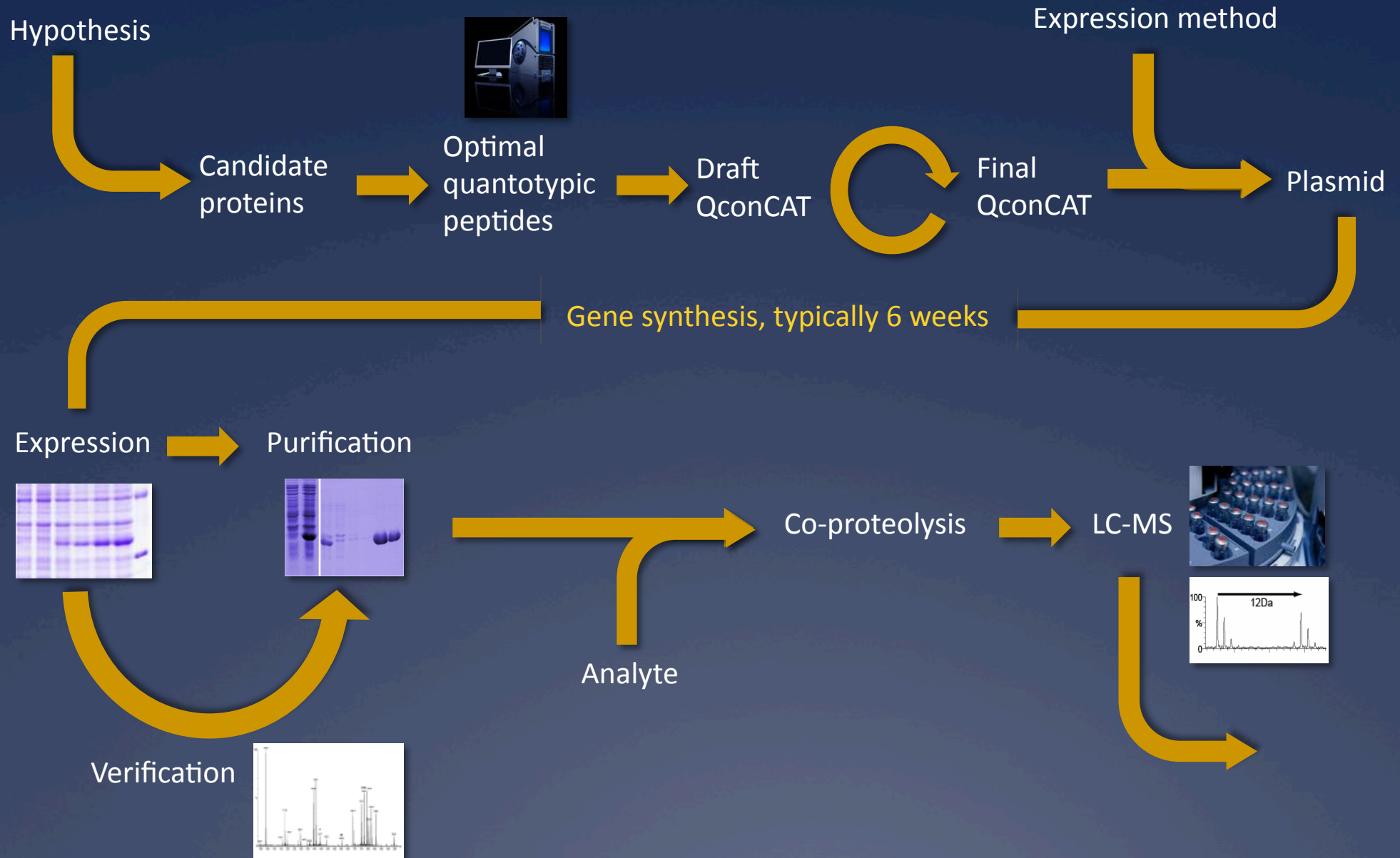
Lowest sensitivity=  
15,000 copies/cell

QqQ @ 100attomol  
=60,000,000 molecules

# Absolute quantification: QconCATs

- Mass tagged internal standards (quantotypic peptides, Q-peptides)
- Synthesised as a concatamer by *de novo* gene synthesis
- Expressed as a heterologous QconCAT artificial protein in bacteria, labelled with stable isotopes in the medium
- Co-digested with analyte, leading to heavy:light peptide ions for quantification
- All peptides stoichiometrically 1:1

# QconCAT workflow





# Absolute quantification: QconCATs

- Mass tagged internal standards (Q-peptides)
- At least one Q-peptide per analyte protein
- Multiple Q-peptides conCATenated *in silico*
  - Addition of features
  - Reverse translated, codon optimised to synthetic gene
  - Optimisation of mRNA secondary structure
- Expressed as a heterologous QconCAT artificial protein, labelled with stable isotopes in the media

# Designer genes for multiplexed quantification

Nominate target protein set  
(n=20-50)



Select Q-peptides on the  
basis of prior knowledge,  
prediction or experience  
(Replication=2)



# Designer genes for multiplexed quantification

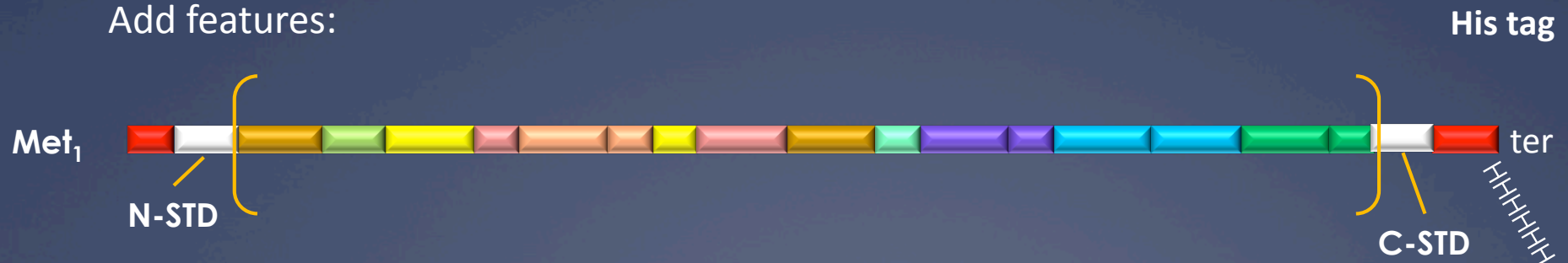
Selected Q-peptides



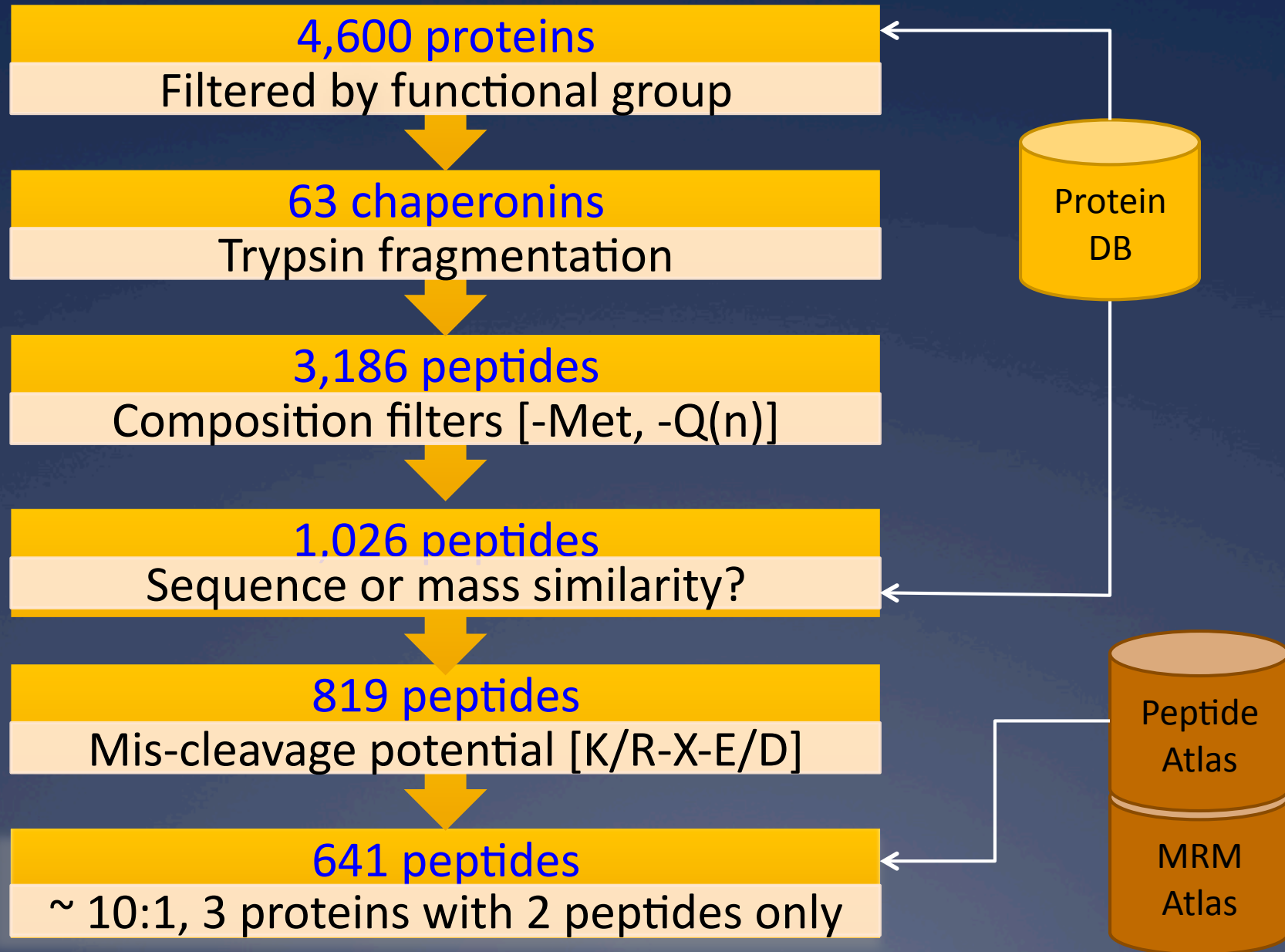
Concatenate and reverse translate *in silico* for QconCAT gene design / expression



Add features:



# Design Phase 1: peptides in silico



# Design Phase 2: gene optimisation and synthesis



[www.polyquant.com](http://www.polyquant.com)

- Optimisation
  - Codon optimisation,
  - GA 'directed random walk' to assess 'fitness' of gene variants, based on codon usage, mRNA 2° structure, GC content, repetitive motifs
- Synthesis of oligonucleotides
  - Overlapping 50mers, 5' phosphorylation,
- Ligation (highT ligase)
- PCR amplification → vector → sequence

# NFkappaB QconCAT



## Glufib (Quantification/Intactness)

Met<sub>1</sub>

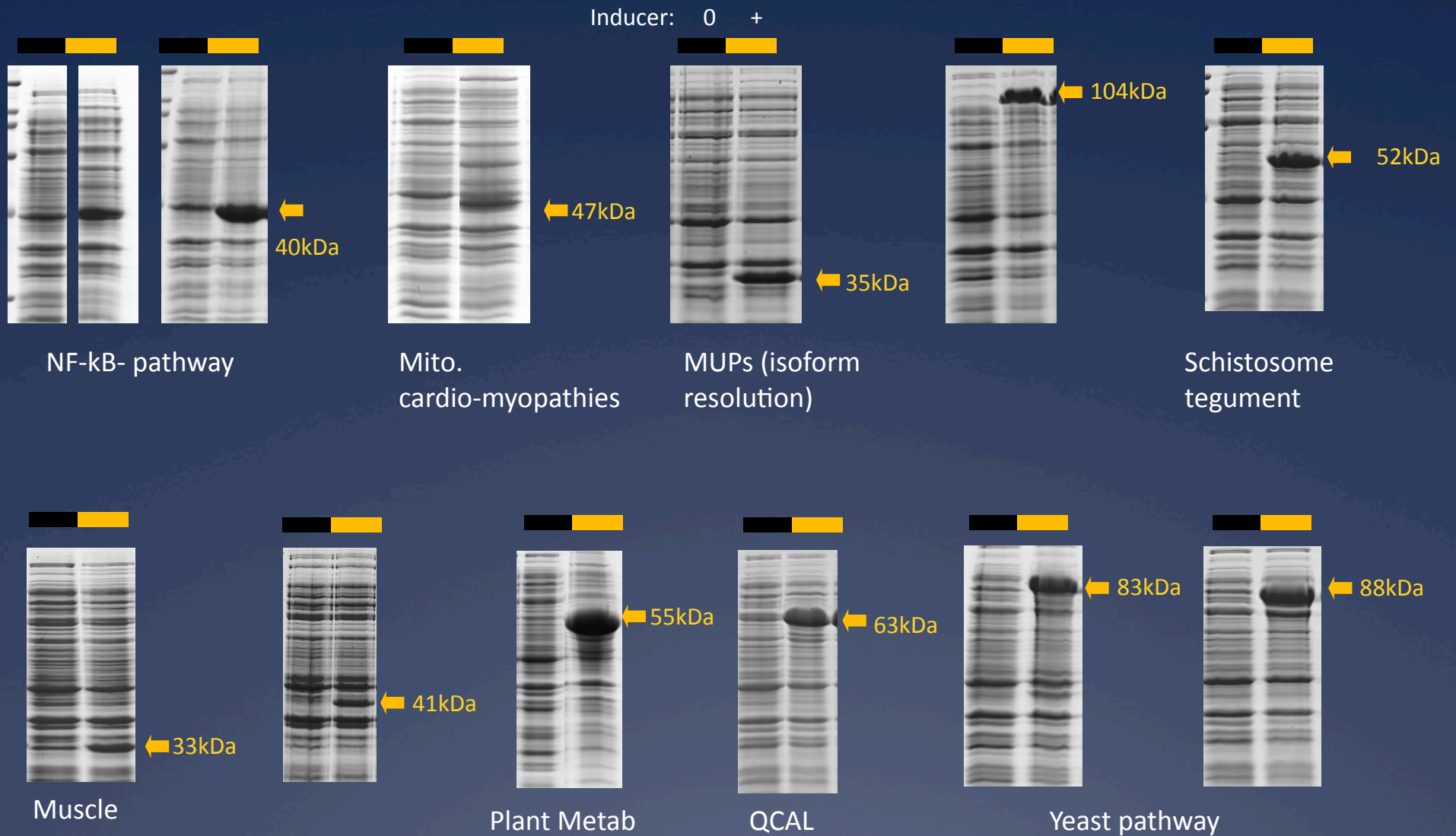
CATATGGGCACCAAA GAAGGCGTAAACGACAACGAGGAAGGCTTCTTCAGCGCTCGTCTGCCCCAGTGTGCCACATCCGATCTTCGACAACCGCATCCAG  
M G T K E G V N D N E E G F F S A R L P P V L P H P I F D N R I Q  
Glufib RELA\_T23  
ACCAACAACAACCCGTTCCAGGTGCCGATCGAAGAACAGCGTCTGACCGACGGTGTGTGTTCCGAACCGCTGCCGTTTACCTATCTGCCGCGTTCTGCGGGT  
T N N N P F Q V P I E E Q R L T D G V C S E P L P F T Y L P R S A G  
RELA\_T19 RELB\_T46  
TCTATCCTCGGTGAAAGCTCCACGGAAGCGTCCAAAACAGCTGAACGACATCGAGGACTGCGACCTGAACGTTGTGCGTGGCATCTTCAGCCAGGCAGATGTA  
S I L G E S S T E A S K Q L N D I E D C D L N V V R G I F S Q A D V  
RELB\_T15 CREL\_T23 CREL\_T33  
CACCGTCTGGGTCTGGGCATCCTGAACAACGCCTTCCGTGACCTGCTGGAAGTGACCTCTGGCCTGATCAGCGATGACATCATCAACATGCGTACACGGCACC  
H R L G L G I L N N A F R D L L E V T S G L I S D D I I N M R H G T  
P105\_T79 P105\_T60  
ATGGACACCGAGTCTAAATACGTCTGCGAAGGTCCGTCTCACGGTGGTCTGCCGGGTGCGTCTTCCGAGAAAAGTTACAACCCGGGTCTGCTCGTTACCCCG  
M D T E S K Y V C E G P S H G G L P G A S S E K G Y N P G L L V H P  
P105\_T48 P50\_T5 P50\_T18  
GATCTGGCTTACCTGCAGGCTGAAGGTGGTGGCGATCGTAGCTACGAACTGGCAGGTGGTGCCTGGTCTGCTGGAGGCACTGTCTGATATGGGTCTG  
D L A Y L Q A E G G G D R S Y E L A G G D L A G L L E A L S D M G L  
P100\_T82  
GAAGAAGGCGTTGCGACCTTTGCGGGTAACACTCCACTGCACCTGGCAGCTGGTCTGGGTTATCCGACTCTACCCGTTACGGTTGCGAAGGTCCGTCTCAT  
E E G V R T F A G N T P L H L A A G L G Y P T L T R Y G C E G P S H  
P100\_T71 P52\_T7  
GGTGGTCTGCCAGGTGCCTCCTCTGAAAAAGCGCACGCACACTCTCTGGTCCGCAAACAGCAGCTGACCGAAGACGGTGATTCCCTTCCCTGCACCTGGCGATC  
G G L P G A S S E K A H A H S L V G K Q Q L T E D G D S F L H L A I  
P52\_T13 IKBA\_T11  
ATCCACGAAGAGAAAACGGCACTGCACCTGGCGGTTGATCTGCAGAACCAGACCTGGTCTCTCTGCTGCTGAAAGATGCTGGTGCCGACCTGGACAAAACCG  
I H E E K T A L H L A V D L Q N P D L V S L L L K D A G A D L D K P  
IKBA\_T18 IKBB\_T15  
GAACCGACTTGGGTGCTACCCCGCTGGGTTCTGCTATGCTGCGTCCGAACCCGATTCTGGCAGTCATGGTGATACTGCGCTGCACGTAGCGTGTGACGGC  
E P T C G R T P L G S A M L R P N P I L A R H G D T A L H V A C Q R  
IKBB\_T19 IKBE\_T13  
AACGTTGAAGACGAAACGCCGAGGACCTGACTGAAGAGAGCTGGTCTGCTGCCGTTTGGATGACCTCAAAGCCGGTGGCGGTGCGGCTGGTTCATCAGCAC  
N V E D E T P Q D L T E E S L V L L P F D D L K A G A G A A G H H H  
IKBE\_T24 His-TAG

CATCACCATTAATGA  
H H H

HisTAG (Purification/Intactness)



# Exemplar QconCATs (from over 50, 25kDa to 160kDa)



# Global quantification by QconCATs



Global QconCAT quantification strategy  
Objective: 4000 proteins

Absolute quantification & turnover analysis  
 $\Sigma P=4000$ , QconCAT=50 Qpeptides (~60kDa each)

	Q peptide defaulter rate			
QRL	0	1%	5%	10%
1	80	81	84	88
1.5	120	122	126	132
2	160	162	168	176
3	240	243	252	264

QRL: Number of Qpeptides per protein

Requires <200 QconCATs = 10,000 peptides for 4,000 proteins

# Typical COPYCATS

>Copycat041 | P32785 | P10127 | P38986 | P53960 | P13188 | P07245 | P46672 | P28241 | P38197 | P32785 | P10127 | P47095 | P47095 | Q04728 | P46672 | P07236 | P11325 | P13188 | P53960 | P39692 | P16467 | Q04728 | Q04066 | P11325 | Q12525 | P07246 | Q07648 | P07246 | P33734 | Q12122 | P38986 | P28241 | P16467 | P04173 | Q07648 | P33734 | P39692 | Q12122 | P04173 | P07245 | Q12525 | P07236 | Q04066 | P38197 | Liverpool

LQFEGFAVERTQPNPNIANVTAGLKVCDIEYEQLCNVDSKIGEETVDEVSTSKDINPESEVVYKLLTPVPSDIDISRAPAGGAADAAA  
KYTVSFIEGDGIGPEISKSGLNNEAEIFEVIDFFLSEECKALLEGDTYTGVTIQTLHPDRGIDLINESLVAAYKHGIFVWGPTIDKNAGAIHT  
HSQNAVICSLFGDEFRTALYGQDANWGREQSAQAAQWESVLKLNPGDGAFYGPKIIDTVPFTSTDGVLTKITYSSDYFDELYRNLLS  
CSETQISEKVIAPDAAFDDVLDKILLETPIDLSLKPNDAAEAEVVRINVSFIATDNSEPRVFLDGIYSLKNWIGESVGAELVFKVLILDGG  
QGTELENRIQQGTDLAEVAPILCAGVTYKVSQASVVVDSKLPVGGHEGAGVVVKVSIQTDVAVYAAEKLGDVRPLNIDDVDSIIK  
AIDSSQTAGYHVDLTIQDLLDAIPDISKEYPDLTLETELIDNSVLKNPVILADACASRIVVLPGDHVGQEITAEAIKELYEEFLKTVFVPLTV  
GGGIKTVLPLVESVPEASILAKNFQLIDSTLRNTAFGLYEPCHGSAPDLPKYILVSGITPTPLGEGKDIAEIASAVDKNYSYLIIVGDEEVQL  
QKELLVEYPEYDCFTREFGENYVQELIEK

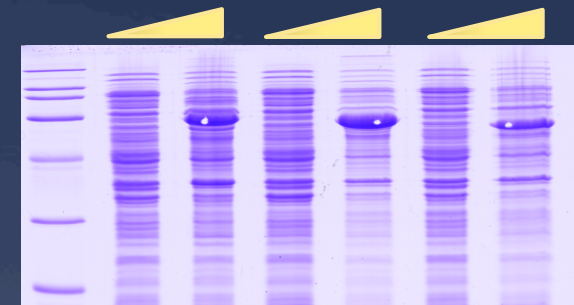
>Copycat042 | P38840 | P40054 | P16120 | Q12031 | P00331 | P39965 | P38999 | P38840 | P09436 | P40054 | P38690 | P19414 | P38635 | P48570 | P38635 | P00331 | P04802 | P38999 | P16120 | Q12031 | P48570 | P38714 | P07258 | P06169 | P19414 | P40545 | P00812 | P38714 | P09436 | P00927 | P00812 | P00899 | P41939 | P28777 | P06169 | P41939 | P28777 | P00899 | P04802 | P39965 | P00927 | P07258 | P38690 | P40545 | Liverpool

ESQGIDVEYLTQLLDNWSTGPYKYINEGNSVGSVNFPEVALKFADAVNNALS GFSNYSFEKDNVVPWSEKVLGIDGGPGKNNFGQ  
ISDVSIVDAQENEICGKSFLSYCGGLPAPEDSDNPLGYKAPSGTSQAIVYSTIKGLPESAVQAGQETRSVAELVIAEIIISLAREFWILHGL  
ITKGYDAGENTYQAPPADRFVLSDDAHGVAQVGVCYDKNFHAEVSTPQVLSAKFIYPEEQSLGKVVGLSSLPEIYEKIYISETPEALPI  
LLEDASRAISLDVTDDSAIDKLYIAQEEIPDADLKL VYNLSPSFNWSAHGFDDKSLNIDDVDSIIKYTDQPEGPIYPLDVL RANVALIDCG  
VKEVIDTILALVKEVAVANNWPLDVRYTNEFLIHAADVEGLCGGIDELLVSKELSIVLAPFSGGQKGNVFLDTILDEARVPANFISEG  
LDQTRVAQQVVDEVVLVNTDEICAAVKFPLTLGGDHSIAIGTVSAVLDKGATTEEDDALADQLRATDVIVPEEGELRNLP TGLGEP CF  
DKAQYNEIQGWDHLSLLPTFGAKDQTNDQVTVD SATATLKTATYDGEEGILAAKLTDDSSPIPYPEQPPIKLLGAPSEGGSSVFEV TYF  
KIPFVSAWADSGDIGGEFSKLG SQVVLYGNDFDEAKEGVAAITGVDTRIHDV LGYSVSEYVAHREALQESPQFLQVGGGINDTNC  
LEWLK

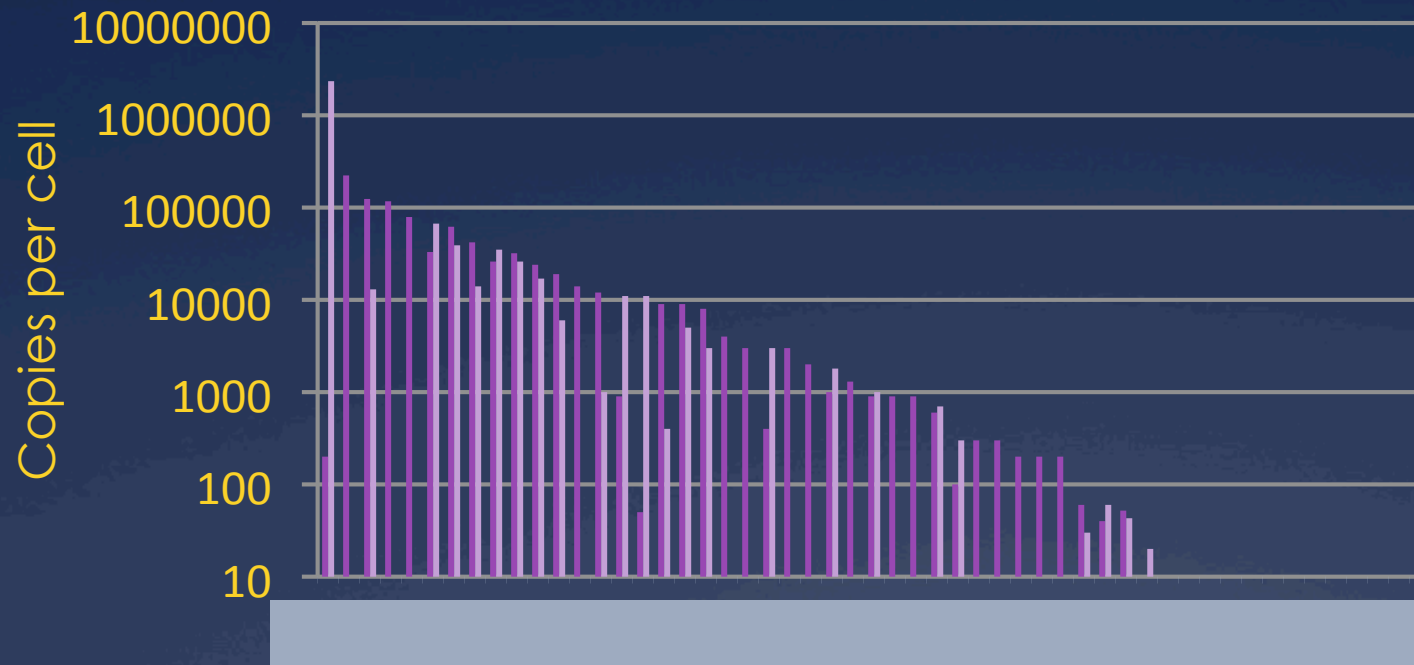
# COPYCATs Phase 1

CopyCat	Family	Proteins
CopyCat001	Chaperone	21
CopyCat002	Chaperone	21
CopyCat003	Chaperone	21
CopyCat004	Transcription Factor	23
CopyCat005	Transcription Factor	22
CopyCat006	Transcription Factor	22
CopyCat007	Transcription Factor	23
CopyCat008	Transcription Factor	22
CopyCat009	Transcription Factor	22
CopyCat010	Signalling	20
CopyCat011	Signalling	20
CopyCat012	Signalling	20
CopyCat013	Signalling	20
CopyCat014	Signalling	20
CopyCat015	Signalling	20
CopyCat016	Signalling	21
CopyCat017	Signalling	20
CopyCat018	Signalling	20
CopyCat019	Signalling	20
CopyCat020	Signalling	20
CopyCat021	Signalling	20
CopyCat022	Signalling	15
CopyCat023	Proteolysis	20
CopyCat024	Proteolysis	20
CopyCat025	Proteolysis	20
CopyCat026	Proteolysis	20
CopyCat027	Proteolysis	20
CopyCat028	Proteolysis	21
CopyCat029	Proteolysis	21
CopyCat030	Proteolysis	20
CopyCat031	Proteolysis	21

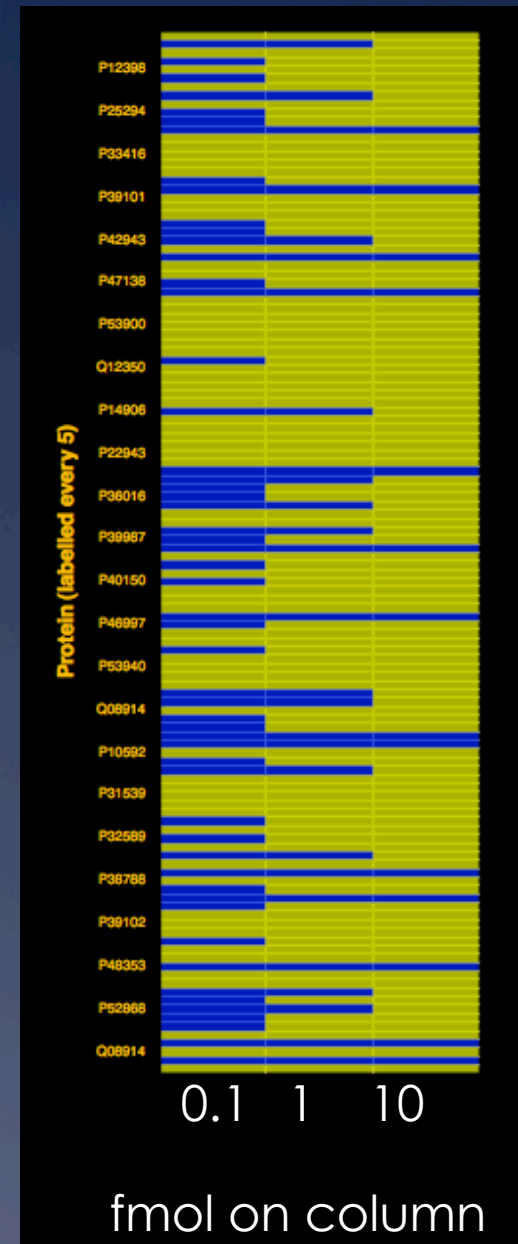
CopyCat	Family	Proteins
CopyCat032	Proteolysis	20
CopyCat033	Proteolysis	21
CopyCat034	Proteolysis	21
CopyCat035	Proteolysis	21
CopyCat036	Proteolysis	21
CopyCat037	Proteolysis	21
CopyCat038	Proteolysis	21
CopyCat039	Proteolysis	21
CopyCat040	Proteolysis	21
CopyCat041	Amino Acid Metabolism	23
CopyCat042	Amino Acid Metabolism	23
CopyCat043	Amino Acid Metabolism	23
CopyCat044	Amino Acid Metabolism	23
CopyCat045	Amino Acid Metabolism	22
CopyCat046	Amino Acid Metabolism	22
CopyCat047	Amino Acid Metabolism	22
CopyCat048	Amino Acid Metabolism	22
CopyCat049	Amino Acid Metabolism	22
CopyCat050	Amino Acid Metabolism	22
CopyCat051	Nitrogen Metabolism	17
CopyCat052	Nitrogen Metabolism	17
CopyCat053	Nitrogen Metabolism	17
CopyCat054	Kinases	20
CopyCat055	Kinases	20
CopyCat056	Kinases	20
CopyCat057	Kinases	20
CopyCat058	Kinases	19
CopyCat059	Kinases	19
CopyCat060	Kinases	20
CopyCat061	Kinases	20
<b>Total</b>		<b>1257</b>



# Chaperonins: absolute quantification



Signal:noise ratio >10 for each peptide in chaperonin QconCATs (SRM)





# Absolute quantification by MS<sup>E</sup>



LC-MSMS based approach (developed by Waters/Micromass)\*

Data independent acquisition

Alternating low energy and high energy scans

Collect all precursor and product ion data

Deconvolute and build precursor/product relationships through retention time, peak shape and fragmentation knowledge, leading to identification

Accumulate abundance data by summing three most intense precursors (peptide ions): Hi3 strategy

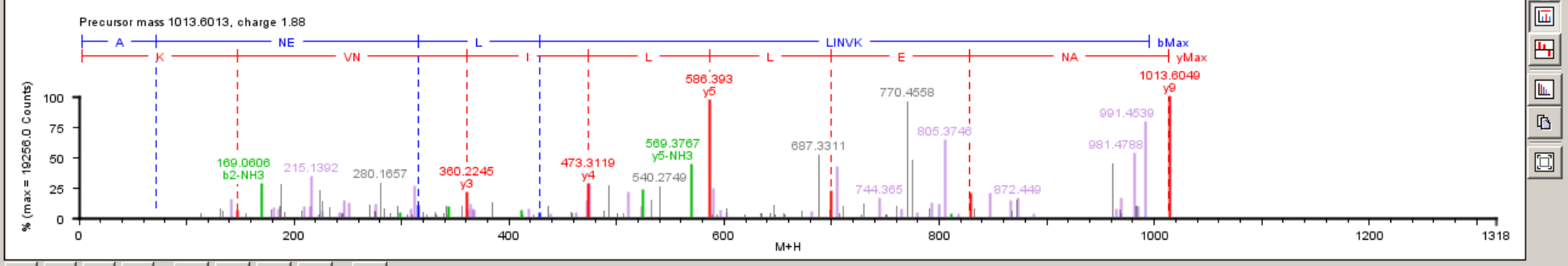
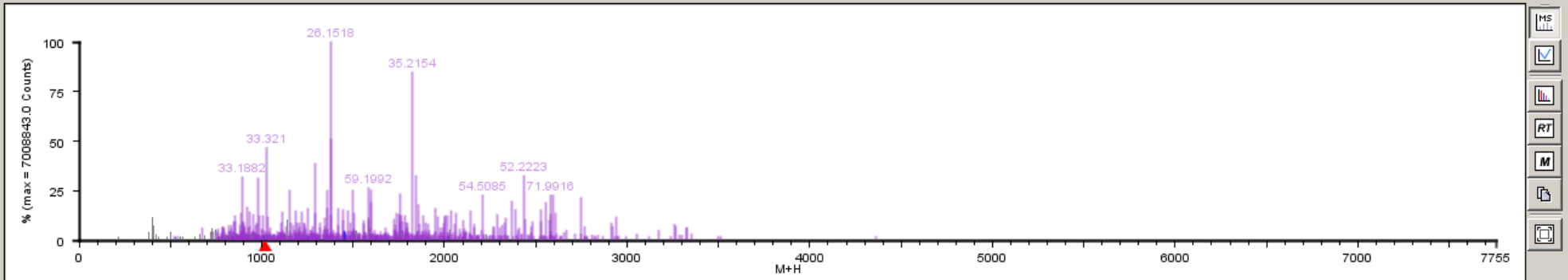
*\*Silva et al., (2006) MCP 5, 144*



- 448 hits, 487 proteins / ESTs (Showing All)
- Enolase 2 OS Saccharomyces cerevisiae GN ENO2 PE 1 SV 2
  - Glyceraldehyde 3 phosphate dehydrogenase 3 OS Saccharomyces cerevisiae GN GND3 PE 1 SV 1
  - Pyruvate kinase 1 OS Saccharomyces cerevisiae GN PYK1 PE 1 SV 1
  - Enolase 1 OS Saccharomyces cerevisiae GN ENO1 PE 1 SV 2
  - Glyceraldehyde 3 phosphate dehydrogenase 1 OS Saccharomyces cerevisiae GN GND1 PE 1 SV 1
  - Phosphoglycerate kinase OS Saccharomyces cerevisiae GN PGK1 PE 1 SV 1
  - Pyruvate decarboxylase isozyme 1 OS Saccharomyces cerevisiae GN PDC1 PE 1 SV 1
  - Fructose biphosphate aldolase OS Saccharomyces cerevisiae GN FBA1 PE 1 SV 1
  - Glyceraldehyde 3 phosphate dehydrogenase 2 OS Saccharomyces cerevisiae GN GND2 PE 1 SV 1
  - Elongation factor 1 alpha OS Saccharomyces cerevisiae GN TEF1 PE 1 SV 1
  - Peroxiredoxin type 2 OS Saccharomyces cerevisiae GN AHP1 PE 1 SV 1
  - 40S ribosomal protein S7 A OS Saccharomyces cerevisiae GN RPS7A PE 1 SV 1
  - Protein BMH1 OS Saccharomyces cerevisiae GN BMH1 PE 1 SV 4
  - Phosphoglycerate mutase 1 OS Saccharomyces cerevisiae GN GPM1 PE 1 SV 1
  - 40S ribosomal protein S0 A OS Saccharomyces cerevisiae GN RPS0A PE 1 SV 1
  - Heat shock protein SSA1 OS Saccharomyces cerevisiae GN SSA1 PE 1 SV 1
  - Inositol 3 phosphate synthase OS Saccharomyces cerevisiae GN IIS1 PE 1 SV 1
  - 60S ribosomal protein L14 B OS Saccharomyces cerevisiae GN RPL14B PE 1 SV 1
  - Peptidyl prolyl cis trans isomerase OS Saccharomyces cerevisiae GN PPI1 PE 1 SV 1
  - 40S ribosomal protein S2 OS Saccharomyces cerevisiae GN RPS2A PE 1 SV 1

Accession	Entry	Description	Coverage (%)	Amount (fmol)
P38720	6PGD1_YEAST	6 phosphogluconate dehydrogenase decarboxylating 1 OS Saccharomyces cerevisiae GN GND1 PE 1 SV 1	90.0	58.00
P53319	6PGD2_YEAST	6 phosphogluconate dehydrogenase decarboxylating 2 OS Saccharomyces cerevisiae GN GND2 PE 1 SV 1	57.5	5.17
P15891	ABP1_YEAST	Actin binding protein OS Saccharomyces cerevisiae GN ABP1 PE 1 SV 4	56.9	19.17
P31787	ACBP_YEAST	Acyl CoA binding protein OS Saccharomyces cerevisiae GN ACB1 PE 1 SV 3	94.3	40.61
P32316	ACH1_YEAST	Acetyl CoA hydrolase OS Saccharomyces cerevisiae GN ACH1 PE 1 SV 2	42.0	4.38
P19414	ACON_YEAST	Aconitate hydratase mitochondrial OS Saccharomyces cerevisiae GN ACO1 PE 1 SV 2	39.7	26.09
P52910	ACS2_YEAST	Acetyl coenzyme A synthetase 2 OS Saccharomyces cerevisiae GN ACS2 PE 1 SV 1	41.9	16.10
P60010	ACT_YEAST	Actin OS Saccharomyces cerevisiae GN ACT1 PE 1 SV 1	93.1	163.96
P00330	ADH1_YEAST	Alcohol dehydrogenase 1 OS Saccharomyces cerevisiae GN ADH1 PE 1 SV 4	75.3	100.31
P00331	ADH2_YEAST	Alcohol dehydrogenase 2 OS Saccharomyces cerevisiae GN ADH2 PE 1 SV 3	56.3	2.35
P38113	ADH5_YEAST	Alcohol dehydrogenase 5 OS Saccharomyces cerevisiae GN ADH5 PE 1 SV 1	36.8	0.83

OK	Precursor MH+ (Da)	z	Peptide MH+ (Da)	MH+ Error (Da)	MH+ Error (ppm)	Score	Start	End	Sequence
✓	1013.6013	1.88	1013.5990	0.0009	0.8945	8.33	31	39	(K) ANELLINVK (Y)
✓	1447.8008	1.95	1447.8043	-0.0059	-4.0860	8.27	320	332	(K) VVGLSTLPEIYEK (M)
✓	811.4197	1.81	811.4196	-0.0009	-1.1601	7.98	342	348	(R) YVVDTSK (-)
✓	1251.6783	1.93	1251.6692	0.0072	5.7305	7.96	288	299	(K) SISIVGSYVGNR (A)
✓	1618.8416	2.75	1618.8435	-0.0007	-0.4028	7.82	198	212	(R) VLGIDGGEGKEELFR (S)
✓	2312.1494	2.88	2312.1477	0.0044	1.8952	7.78	235	258	(K) ATDGGAGHGVINVSVEAAIEASTR (Y)
✓	836.4511	1.88	836.4512	-0.0012	-1.4620	7.70	85	92	(K) IGDYAGIK (W)
✓	1355.6219	1.95	1355.6083	0.0115	8.4770	7.60	277	287	(K) CCSDFVNOVVK (S)
✓	1071.6492	2.06	1071.6407	0.0068	6.3627	7.53	225	234	(K) EKDIVGAVLK (A)
✓	2019.0669	2.86	2019.0692	-0.0003	-0.1282	7.50	61	81	(K) LPLVGGHEGAGVVVGMGENVK (G)
✓	1136.5693	3.00	1136.5735	-0.0034	-3.0320	7.35	9	18	(K) GVIFYESHGK (L)
✓	2589.2378	3.00	2589.2437	-0.0028	-1.0711	7.18	277	299	(K) CCSDFVNOVVKISIVGSYVGNR (A)
✓	1804.9724	2.73	1804.9738	0.0002	0.1293	7.07	259	276	(R) YVRANGITVLVGPAGAK (C)



YeastManCam24 (ManCamYeast2,c)

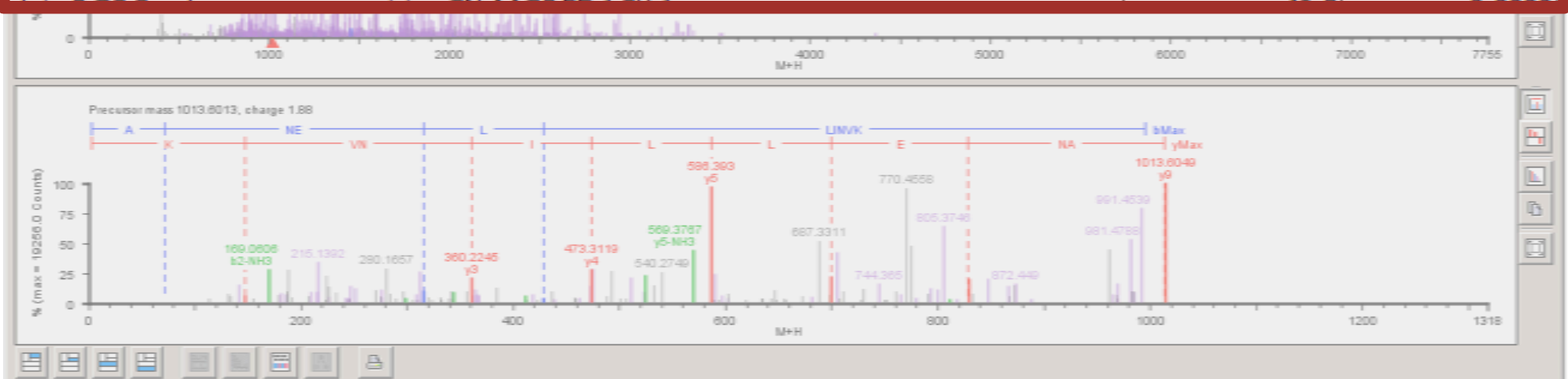
448 hits, 487 proteins / ESTs (Showing All)

Accession	Entry	Description	Coverage (%)	Amount (fmol)
P38720	6PGD1_YEAST	6-phosphogluconate dehydrogenase decarboxylating 1 OS Saccharomyces cerevisiae GN GND1 PE 1 SV 1	90.0	58.00
P53319	6PGD2_YEAST	6-phosphogluconate dehydrogenase decarboxylating 2 OS Saccharomyces cerevisiae GN GND2 PE 1 SV 1	57.5	5.17
P15891	ABP1_YEAST	Actin binding protein OS Saccharomyces cerevisiae GN ABP1 PE 1 SV 4	56.9	19.17
P31787	ACBP_YEAST	Acyl CoA binding protein OS Saccharomyces cerevisiae GN ACBP1 PE 1 SV 3	94.3	40.61
P32316	ACH1_YEAST	Acetyl CoA hydrolase OS Saccharomyces cerevisiae GN ACH1 PE 1 SV 2	42.0	4.38
P19414	ACON_YEAST	Aconitate hydratase mitochondrial OS Saccharomyces cerevisiae GN ACO1 PE 1 SV 2	39.7	26.09
P52910	ACS2_YEAST	Acetyl coenzyme A synthetase 2 OS Saccharomyces cerevisiae GN ACS2 PE 1 SV 1	41.9	16.10
P60010	ACT_YEAST	Actin OS Saccharomyces cerevisiae GN ACT1 PE 1 SV 1	99.1	162.86
P00330	ADH1_YEAST	Alcohol dehydrogenase 1 OS Saccharomyces cerevisiae GN ADH1 PE 1 SV 4	75.3	100.31
P00331	ADH2_YEAST	Alcohol dehydrogenase 2 OS Saccharomyces cerevisiae GN ADH2 PE 1 SV 3	56.3	2.35
P38113	ADH5_YEAST	Alcohol dehydrogenase 5 OS Saccharomyces cerevisiae GN ADH5 PE 1 SV 1	36.8	0.83

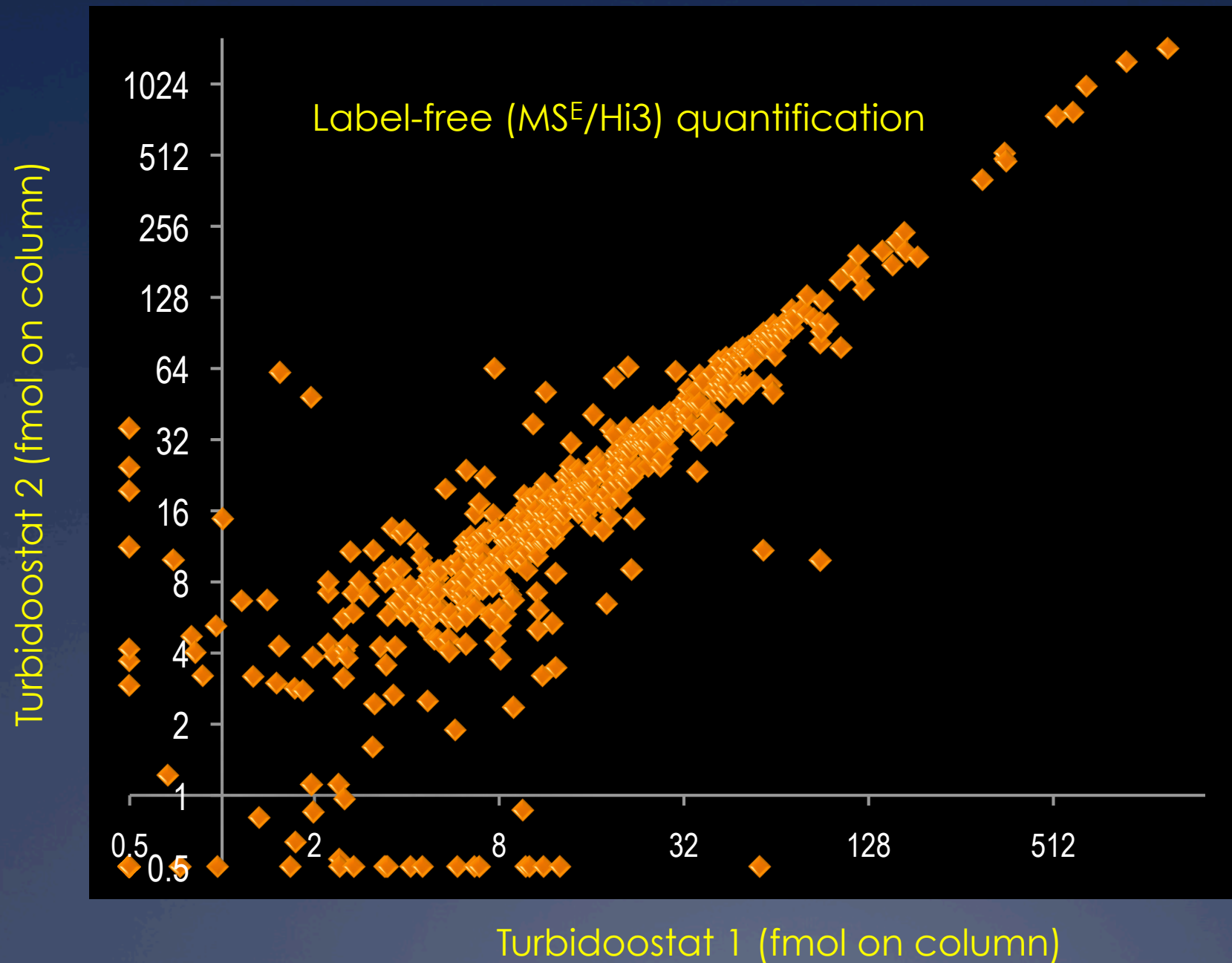
  

OK	Precursor MH+ (Da)	z	Peptide MH+ (Da)	MH+ Error (Da)	MH+ Error (ppm)	Score	Start	End	Sequence
✓	1013.6013	1.88	1013.5990	0.0009	0.8945	8.33	31	39	(K) INPELLINVK (Y)
✓	1447.8008	1.95	1447.8043	-0.0059	-4.0860	8.27	320	332	(K) VVGLSTLPRITPK (M)
✓	811.4197	1.81	811.4196	-0.0009	-1.1601	7.98	342	348	(R) YVDVTSK (-)
✓	1251.6783	1.93	1251.6692	0.0072	5.7305	7.96	288	299	(K) SIVSVGSYVQNR (A)
✓	3518.8416	2.75	3518.8435	-0.0007	-0.4028	7.82	198	212	(R) VLGIDGGGGREELFR (S)
✓	2312.1494	2.88	2312.1477	0.0044	1.8952	7.78	235	258	(K) ATDGGARHGVINVSVEAAIEASTR (Y)
✓	836.4511	1.88	836.4512	-0.0012	-1.4620	7.70	85	92	(K) IGDYAGIK (W)
✓	1355.6219	1.95	1355.6083	0.0115	8.4770	7.60	277	287	(K) CCSDFVEMQVVK (S)
✓	3071.6492	2.06	3071.6407	0.0068	6.3627	7.53	225	234	(K) EKDIVGAVLK (A)
✓	2019.0669	2.86	2019.0692	-0.0003	-0.1282	7.50	61	81	(K) LPLVGGEGGAGVVGMCSEVK (G)
✓	1136.5693	3.00	1136.5735	-0.0034	-3.0320	7.35	9	18	(K) GVIFYESHGK (L)

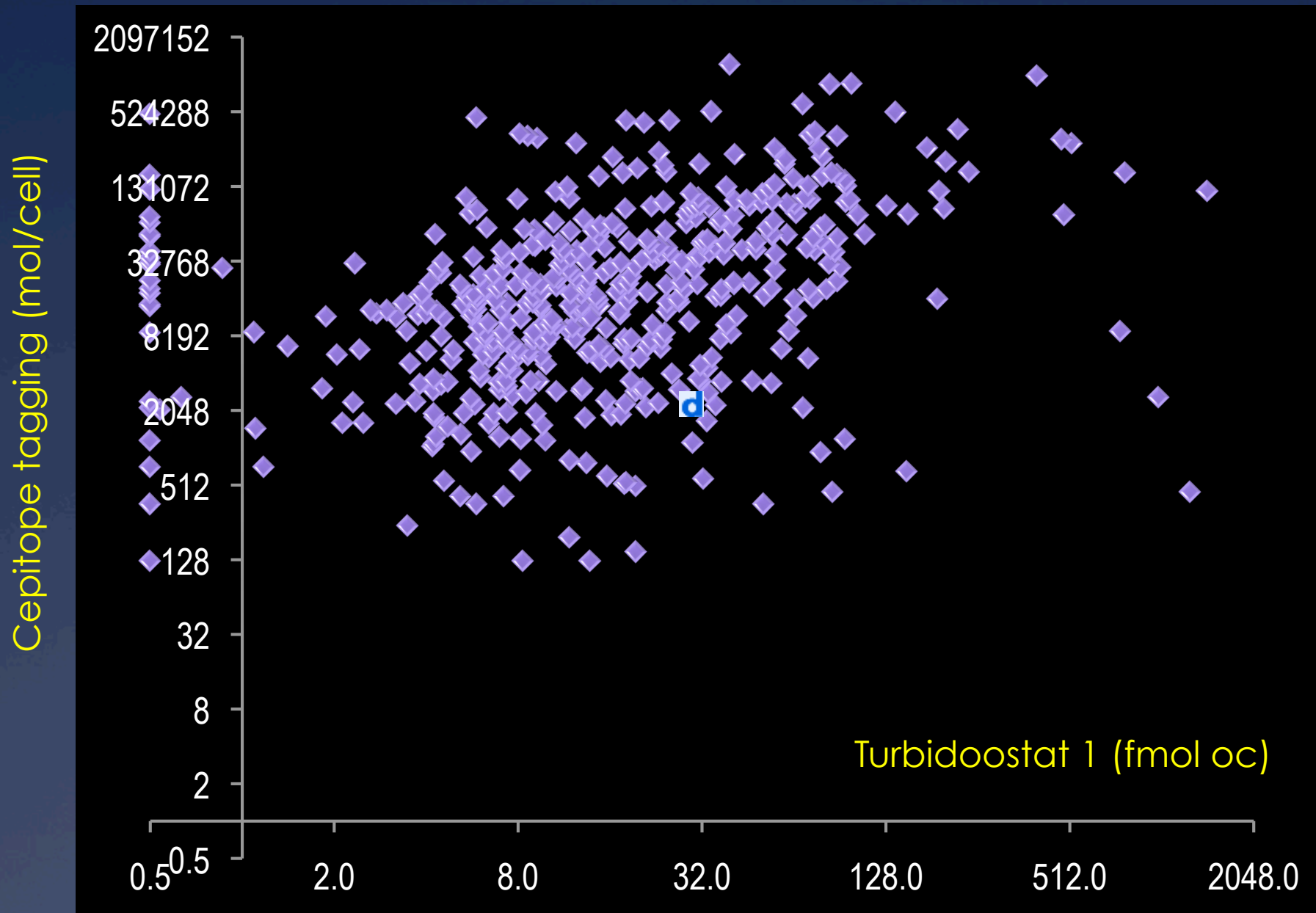
Description	Coverage (%)	Amount (fmol)
Alcohol dehydrogenase 1 OS Saccharomyces cerevisiae GN ADH1 PE 1 SV 4	75.3	100.3184
Alcohol dehydrogenase 2 OS Saccharomyces cerevisiae GN ADH2 PE 1 SV 3	56.3	2.3570
Alcohol dehydrogenase 5 OS Saccharomyces cerevisiae GN ADH5 PE 1 SV 1	36.8	0.8314
Alcohol dehydrogenase 1 OS Saccharomyces cerevisiae GN ADO1 PE 1 SV 1	55.6	35.8954
Acetyl coenzyme A synthetase 2 OS Saccharomyces cerevisiae GN AAC2 PE 1 SV 2	49.7	68.1344



# Label free biological replication

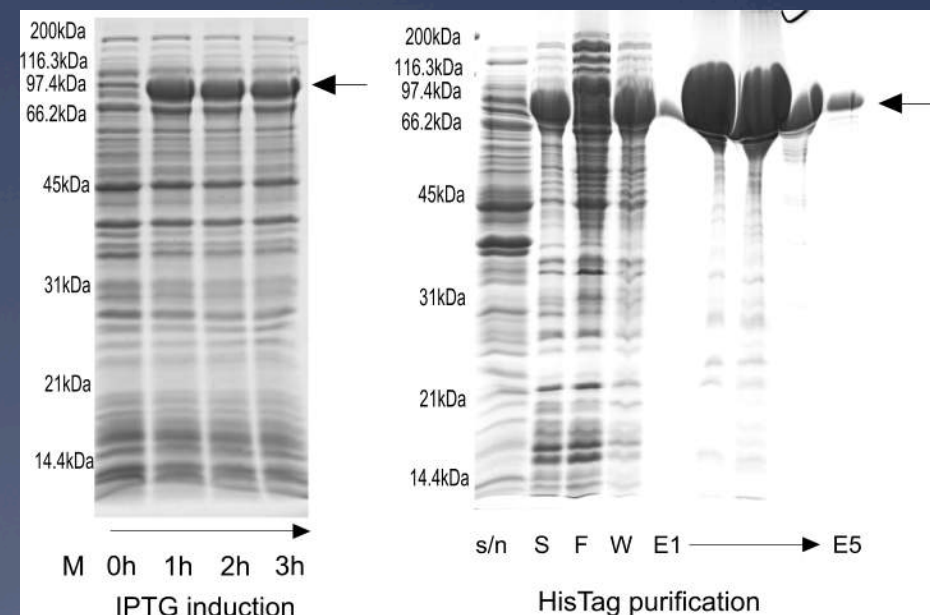
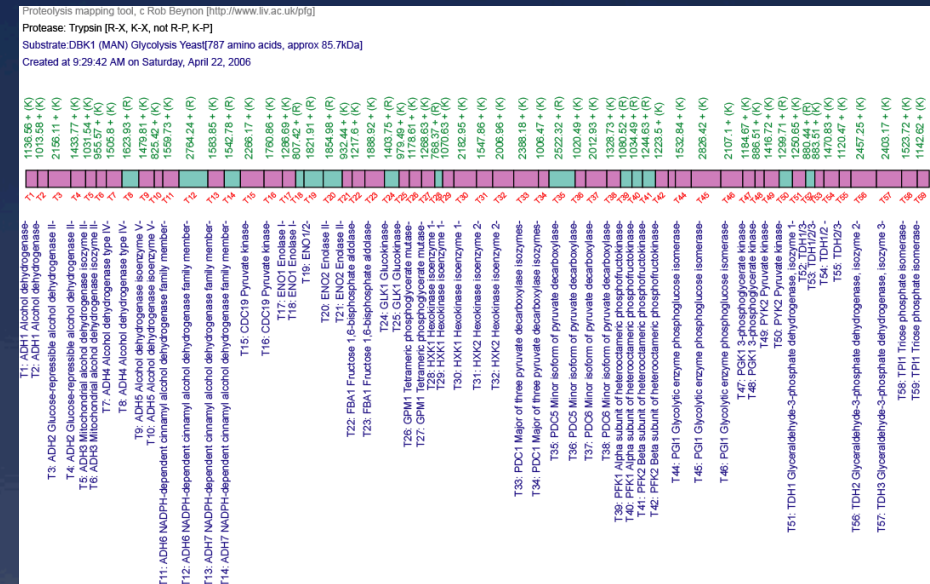
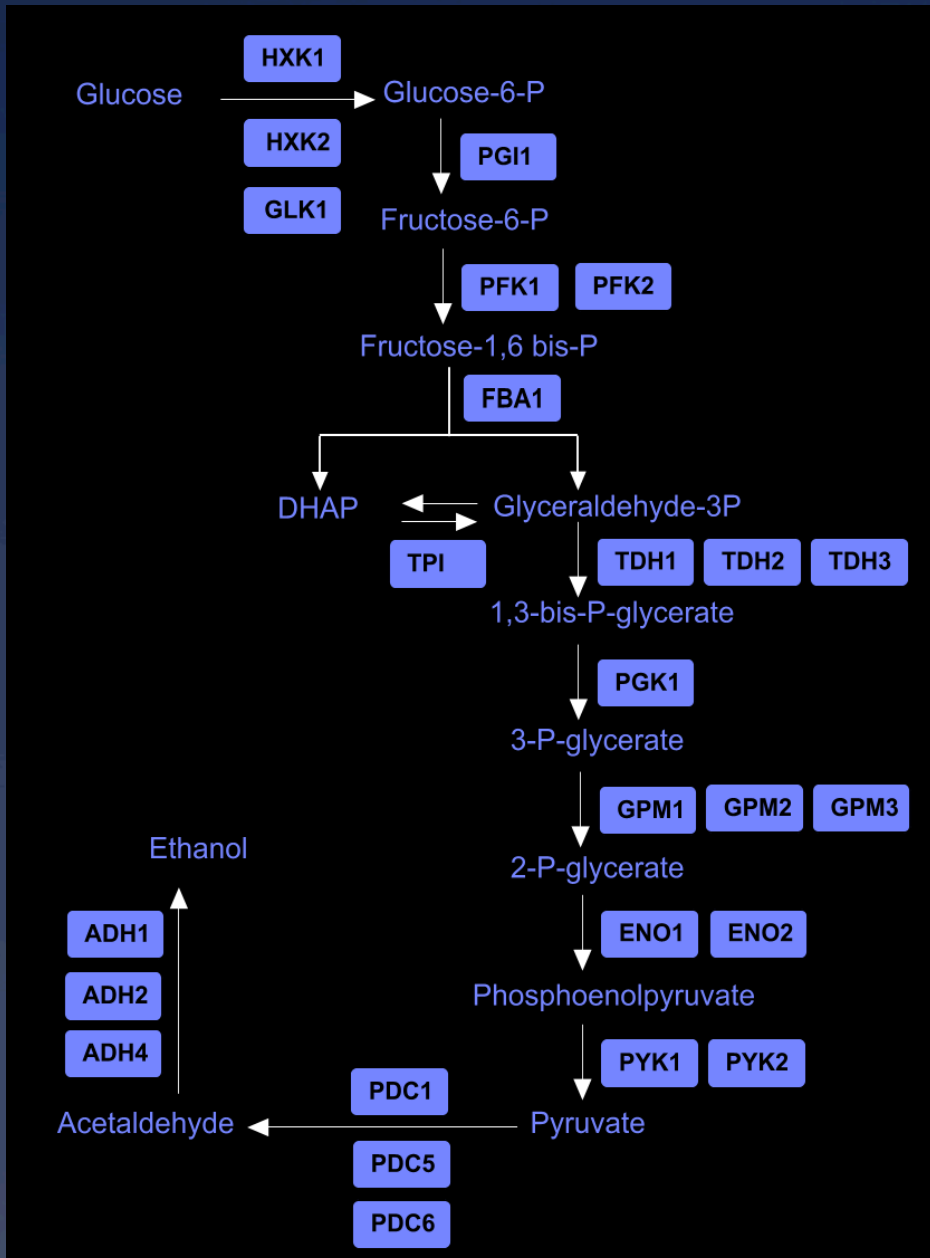


# Label free vs epitope tagging

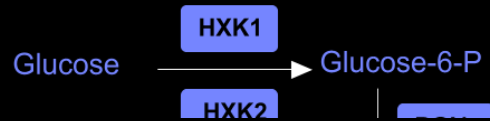




# Quantification of a glycolytic pathway



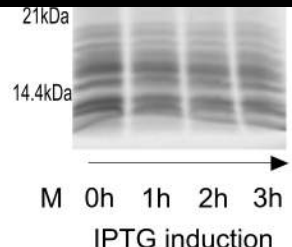
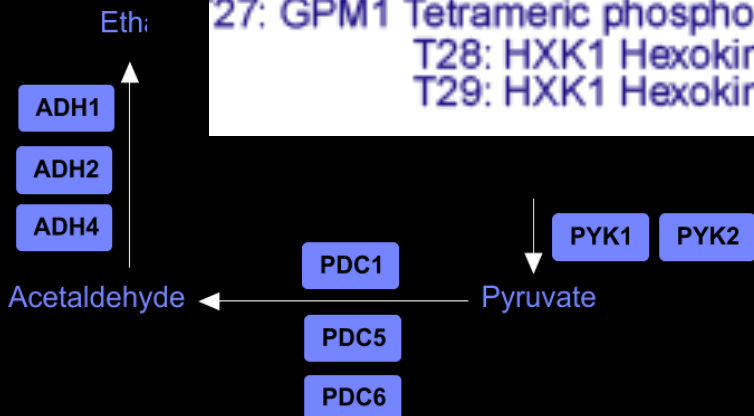
# Quantification of a glycolytic pathway



Proteolysis mapping tool, c Rob Beynon [http://www.liv.ac.uk/ptg]  
 Protease: Trypsin [R-X, K-X, not R-P, K-P]  
 Substrate: DBK1 (MAN) Glycolysis Yeast [787 amino acids, approx 85.7kDa]  
 Created at 9:29:42 AM on Saturday, April 22, 2006

T16: CDC19 Pyruvate kinase-	1760.86 + (K)
T17: ENO1 Enolase I-	1286.69 + (K)
T18: ENO1 Enolase I-	807.42 + (R)
T19: ENO1/2-	1821.91 + (R)
T20: ENO2 Enolase II-	1854.98 + (R)
T21: ENO2 Enolase II-	932.44 + (K)
T22: FBA1 Fructose 1,6-bisphosphate aldolase-	1217.6 + (K)
T23: FBA1 Fructose 1,6-bisphosphate aldolase-	1888.92 + (K)
T24: GLK1 Glucokinase-	1403.75 + (R)
T25: GLK1 Glucokinase-	979.49 + (K)
T26: GPM1 Tetrameric phosphoglycerate mutase-	1178.61 + (K)
T27: GPM1 Tetrameric phosphoglycerate mutase-	1268.63 + (K)
T28: HXK1 Hexokinase isoenzyme 1-	768.37 + (R)
T29: HXK1 Hexokinase isoenzyme 1-	1070.63 + (K)

T28: HXK1 Hexokinase isoenzyme 1-	768.37 + (R)
T29: HXK1 Hexokinase isoenzyme 1-	1070.63 + (K)
T26: GPM1 Tetrameric phosphoglycerate mutase-	1178.61 + (K)
T27: GPM1 Tetrameric phosphoglycerate mutase-	1268.63 + (K)
T25: GLK1 Glucokinase-	979.49 + (K)
T24: GLK1 Glucokinase-	1403.75 + (R)
T23: FBA1 Fructose 1,6-bisphosphate aldolase-	1888.92 + (K)
T22: FBA1 Fructose 1,6-bisphosphate aldolase-	1217.6 + (K)
T21: ENO2 Enolase II-	932.44 + (K)
T20: ENO2 Enolase II-	1854.98 + (R)
T19: ENO1/2-	1821.91 + (R)
T18: ENO1 Enolase I-	807.42 + (R)
T17: ENO1 Enolase I-	1286.69 + (K)
T16: CDC19 Pyruvate kinase-	1760.86 + (K)





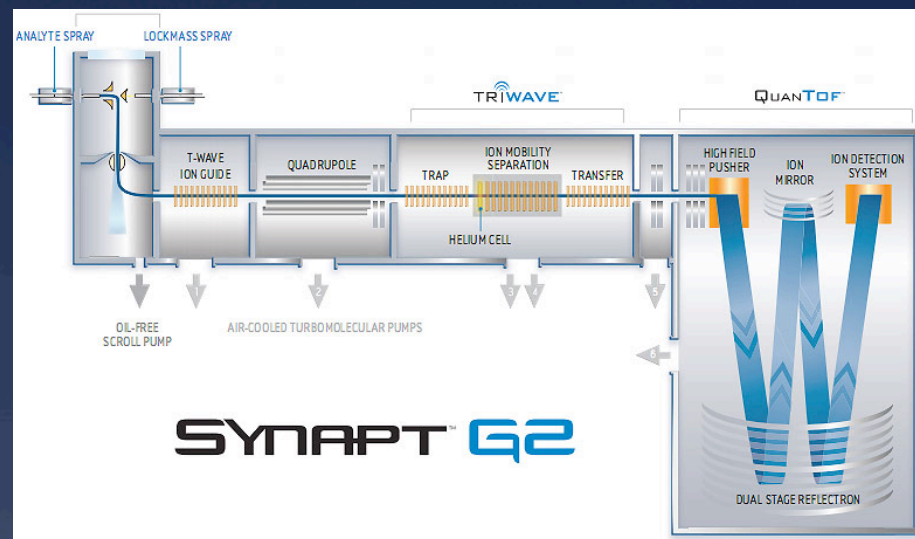
# Instrument-based enhancements

Synapt G2 has an ion mobility cell

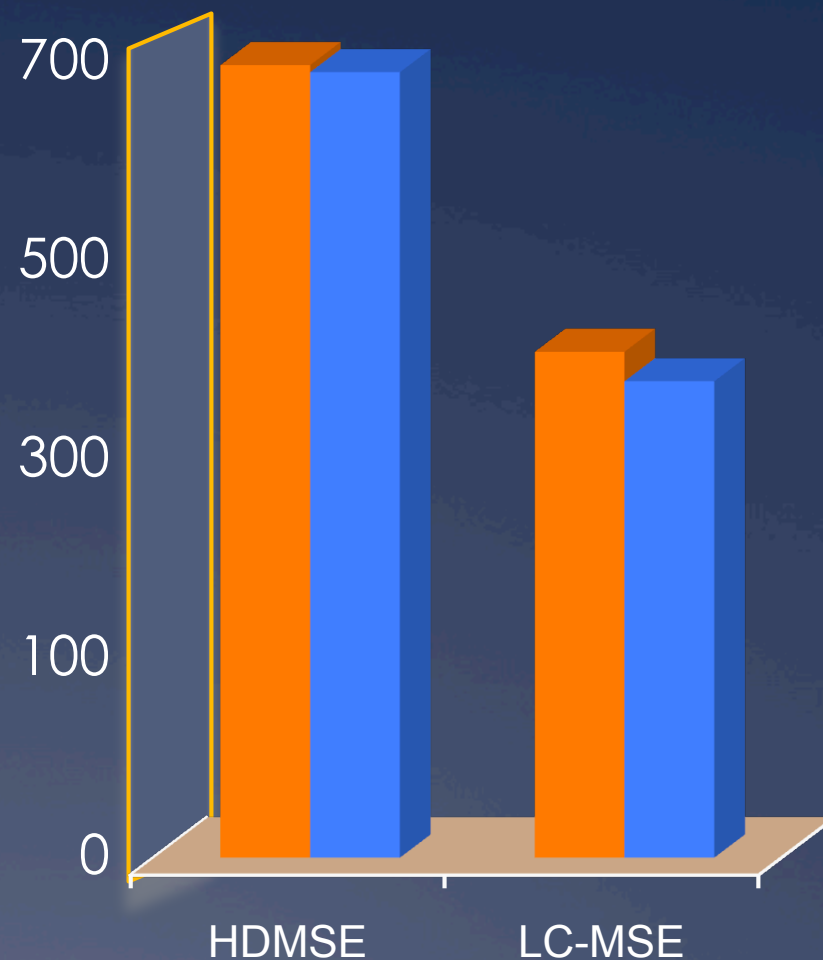
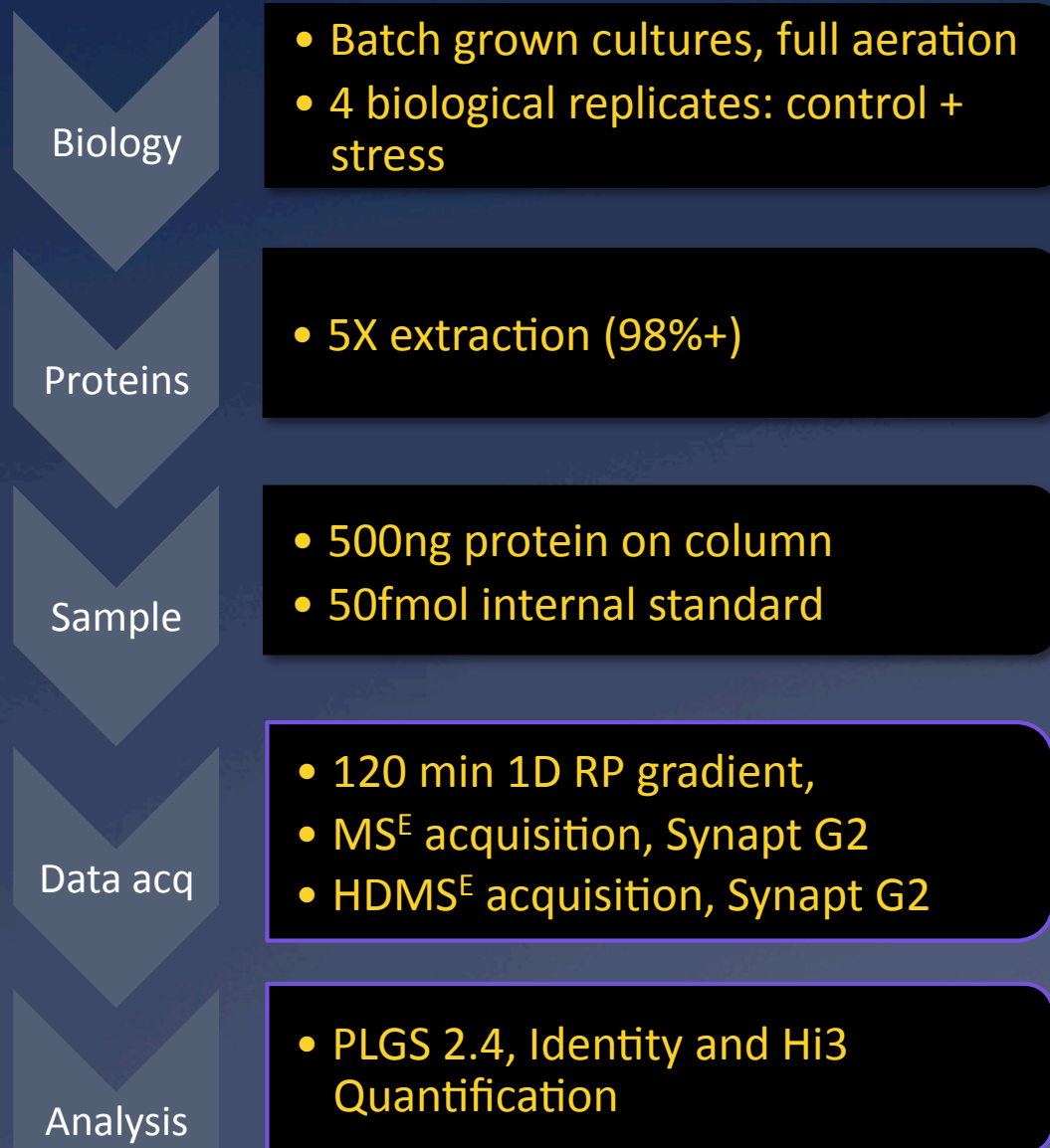
This can be used for precursor fractionation in the gas phase

Drift time increases the number of precursor/product associations

Greater identifications, all quantified

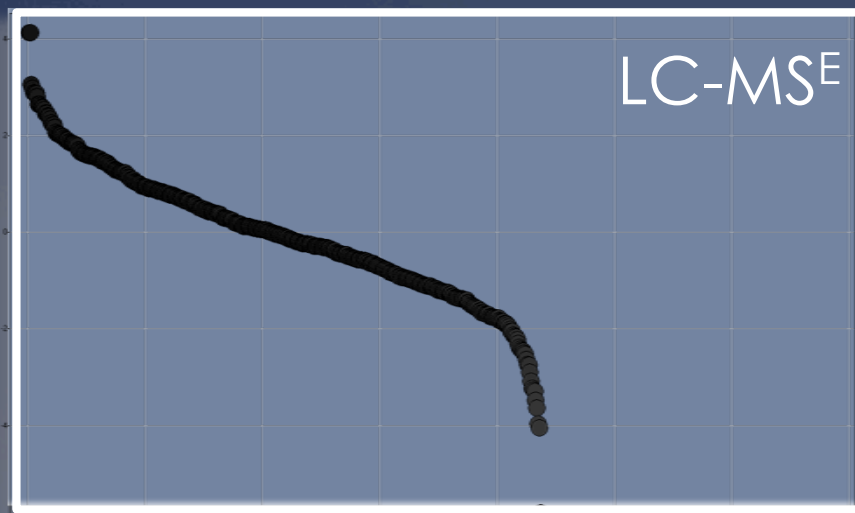
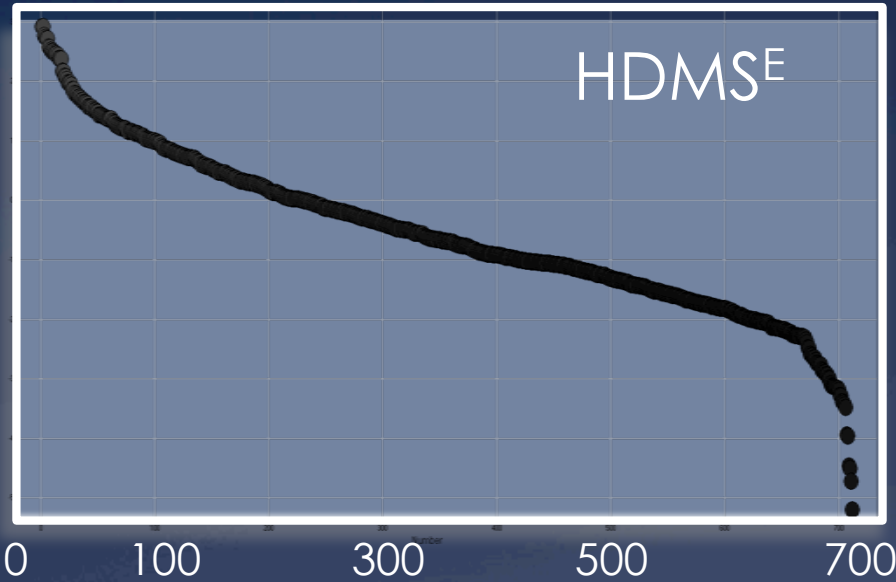


# Label free analysis, Xxxx stress response

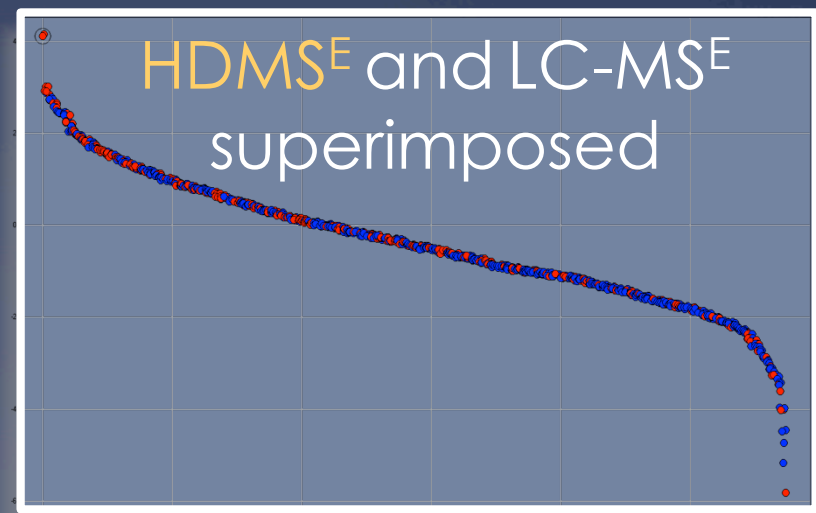
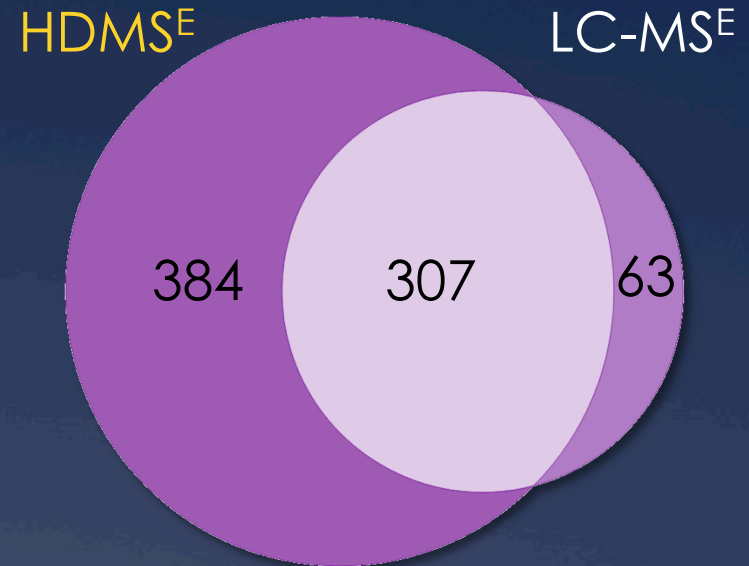


# Identification/quantification gain with HDMS<sup>E</sup>

Abundance (In scale)

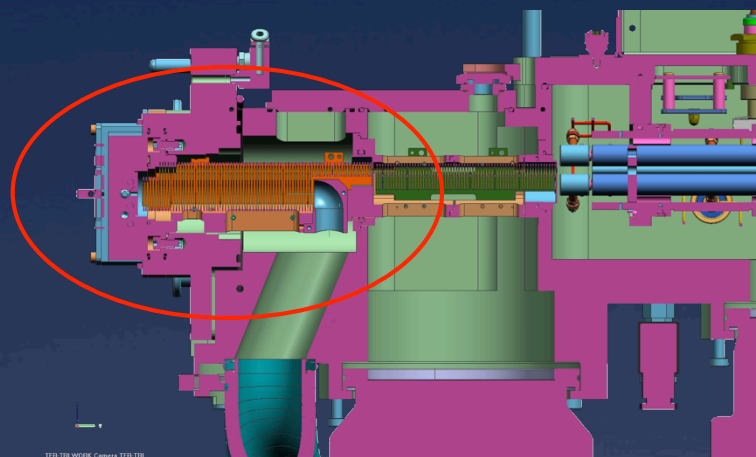


Protein index

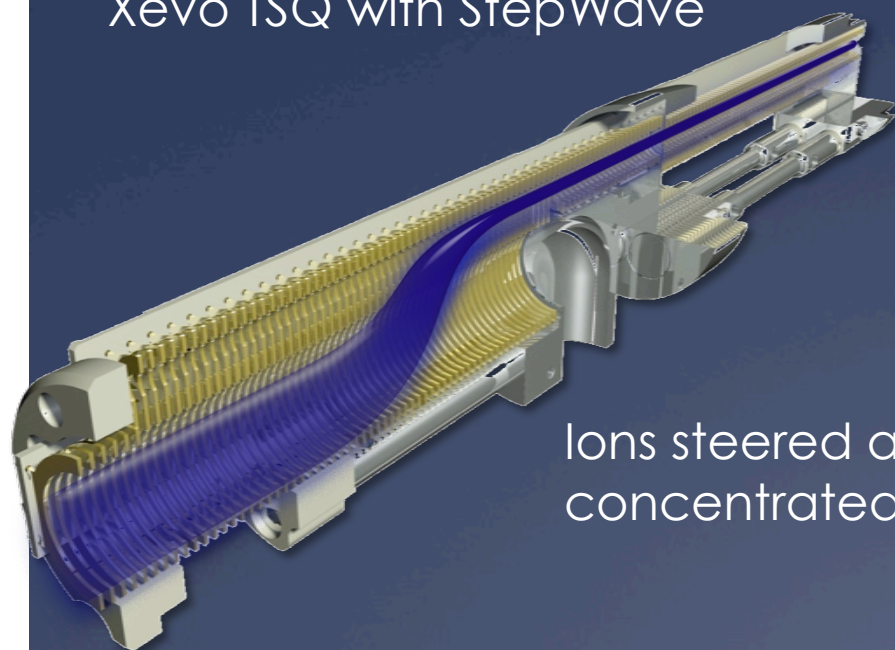


Protein index

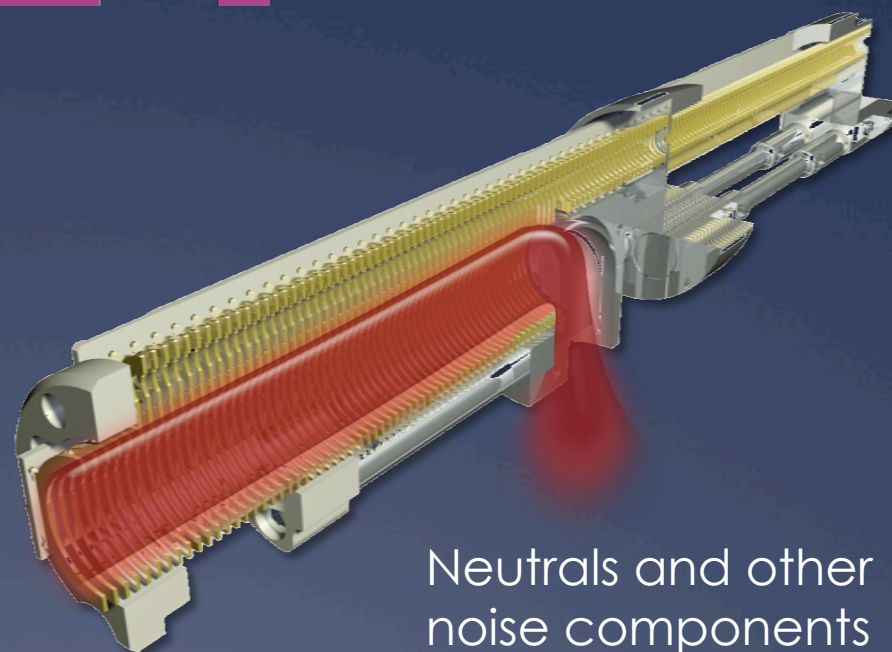
# Gain through source enhancements



Xevo TSQ with StepWave

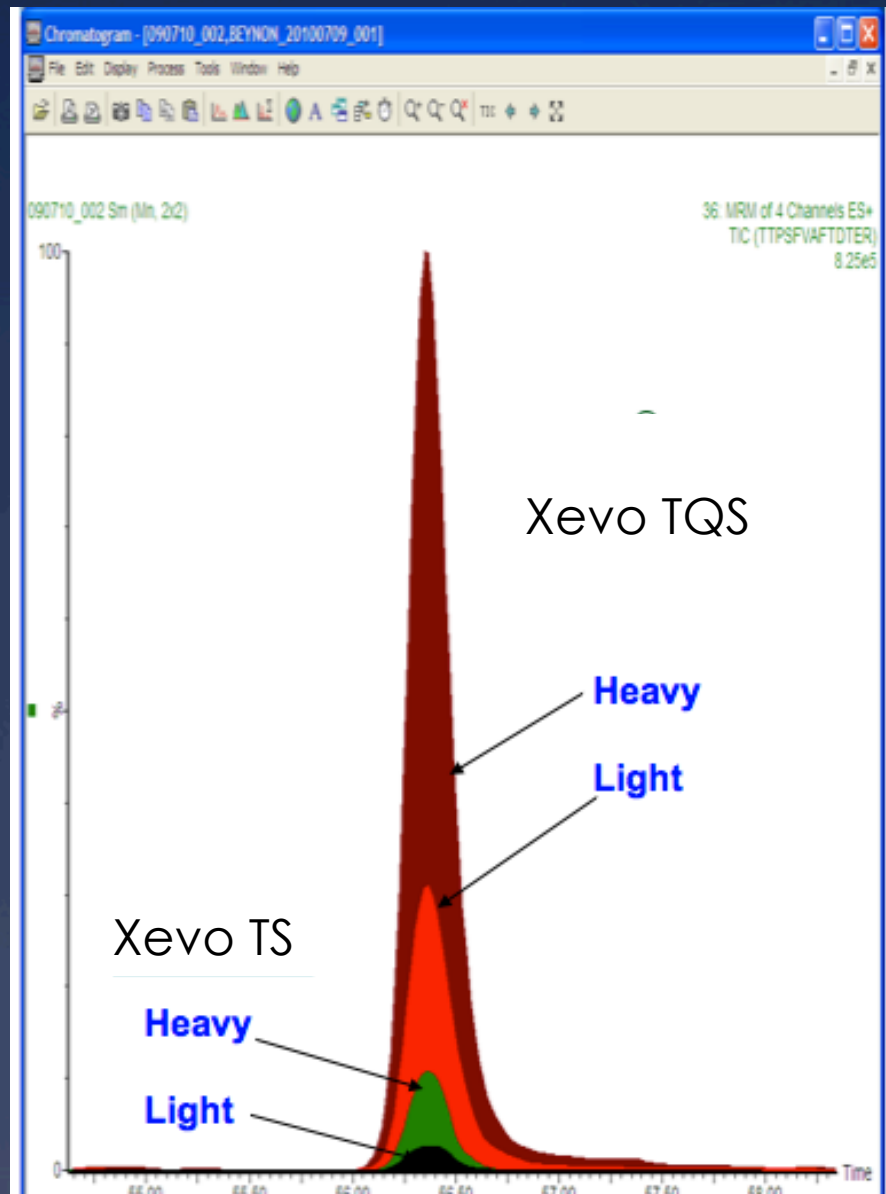


Ions steered and concentrated



Neutrals and other noise components removed

# Source enhancements



Xevo TQ and Xevo TQ-S analysis of COPYCAT 1 in yeast tryptic digest

QCONCAT Peptide :  
[<sup>13</sup>C<sub>6</sub>]TTPSFVAFTDTER

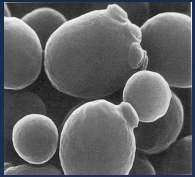
Yeast Peptide TTPSFVAFTDTER

TIC from four MRM channels overlaid and axis linked

Sensitivity increase 10 fold



# The scale of the challenge



Yeast

[ 10 million cells ]

0.06mg protein = 6pg/cell



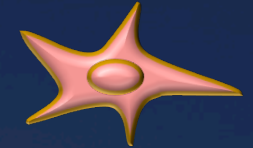
Digest 100µg/200µL digest  
(17 million cells in digest)



Apply 1000ng 'protein' (1µL)  
~170,000 cells on column



Lowest sensitivity=  
~4 copies/cell



HeLa

~2.5mg protein = 250pg/cell



Digest 100µg/200µL digest  
(0.5 million cells in digest)



Apply 1000ng 'protein' (1µL)  
~4,000 cells on column



Lowest sensitivity=  
150 copies/cell

QqQ LOQ=1 attomol  
=600,000 molecules