

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 'Proteomics and Functional Genomics Group, Liverpool' website. The URL in the address bar is <http://www.liv.ac.uk/pfg/>. The page features the University of Liverpool logo and navigation links for About PFG, Research, Facilities, Services, Links, and Intranet. A sidebar on the left lists 'Breaking News' from various months in 2010 and 2009, along with a section for 2008 and 2007. The main content area includes a photo gallery and a 'What you will find on this website:' summary.

Visited 36,022 times

UNIVERSITY OF LIVERPOOL PROTEOMICS AND FUNCTIONAL GENOMICS RESEARCH GROUP

About PFG Research Facilities Services Links [Intranet]

Welcome

Contents ►

Breaking News

Mar 2010 ► Yvonne Woolerton joins us as a new PhD student. [Welcome Yvonne!](#)
Dec 2009 ► Welcome Baby Lupe Gomez-Baena!
Dec 2009 ► Amy successfully defended her thesis. [Congratulations, Dr Claydon!](#)
Nov 2009 ► Many Peffers joins us on her Wellcome Trust Fellowship. [Welcome Mandy!](#)
Oct 2009 ► Stu successfully defended his thesis [Congratulations, Dr Armstrong!](#)
Sept 2009 ► Installation of our Waters Synapt and a Waters Xevo QqQ for quantitative studies
Aug 2009 ► Jenny and John create an unbreakable covalent bond. [Congratulations!](#)
June 2009 ► Phil Brownridge and Vicky Harman join us for the big proteome project [Welcome both!](#)
April 2009 ► Lupe Gomez-Baena joins us from Cordoba as a Marie Curie Research fellow for biomarker studies. [Welcome Lupe!](#)
Feb 2009 ► Amy rejoins us to work on ribosome proteomics. [Welcome back, Amy](#)
Feb 2009 ► Rob starts his sabbatical in earnest, and is working as a BBSRC Industrial Exchange Visitor at Waters in Manchester
Jan 2009 ► We received formal notification of our large grant on global quantitative proteomics [\[LINK\]](#)

2008 (all) ►

- We've been joined by a new PhD student, Andie Pennington. [Welcome Andie!](#)
- We've ordered our third Dionex Ultimate 3000, this one will supply the maxIS
- At last, the MaxIS is coming! Photo diary to follow.
- At 3m tall, it will be major installation challenge, on the first floor of a building with no lifts!
- We presented two posters at the Siena Proteomics meeting
- Rosie successfully defended her PhD thesis. [Congratulations, Dr Moore!](#)
- We presented four posters at the HUPO meeting in Amsterdam
- We have been awarded a new BBSRC grant to work on supramolecular assembly and dynamics, with colleagues in Manchester
- Jenny successfully defended her PhD thesis. [Congratulations, Dr Rivers!](#)
- We ordered our Bruker MaxIS QToFsystem (resolution=50,000, accuracy=<1ppm, 20Hz acquisition)

2007 (all) ►

- We took delivery of our second ThermoElectron LTQ system, coupled to a Dionex Ultimate 3000
- Ian successfully defended his PhD thesis. [Congratulations, Dr Edwards!](#)
- We presented posters at the 4th joint BSPR/EBI meeting in Hinxton
- We were awarded a new BBSRC grant with Simon Hubbard (Manchester) to develop our positional proteomics approach to rational proteome simplification
- We were awarded a new BBSRC grant with Daniel Coca (Sheffield) to extend our studies on FPGA computing to MS/MS and Quantification data

Acknowledgments

QconCAT (Liverpool)

Jenny Rivers
Mary Doherty
Amy Claydon
Deborah Simpson

QconCAT (Manchester)

Claire Eyers
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

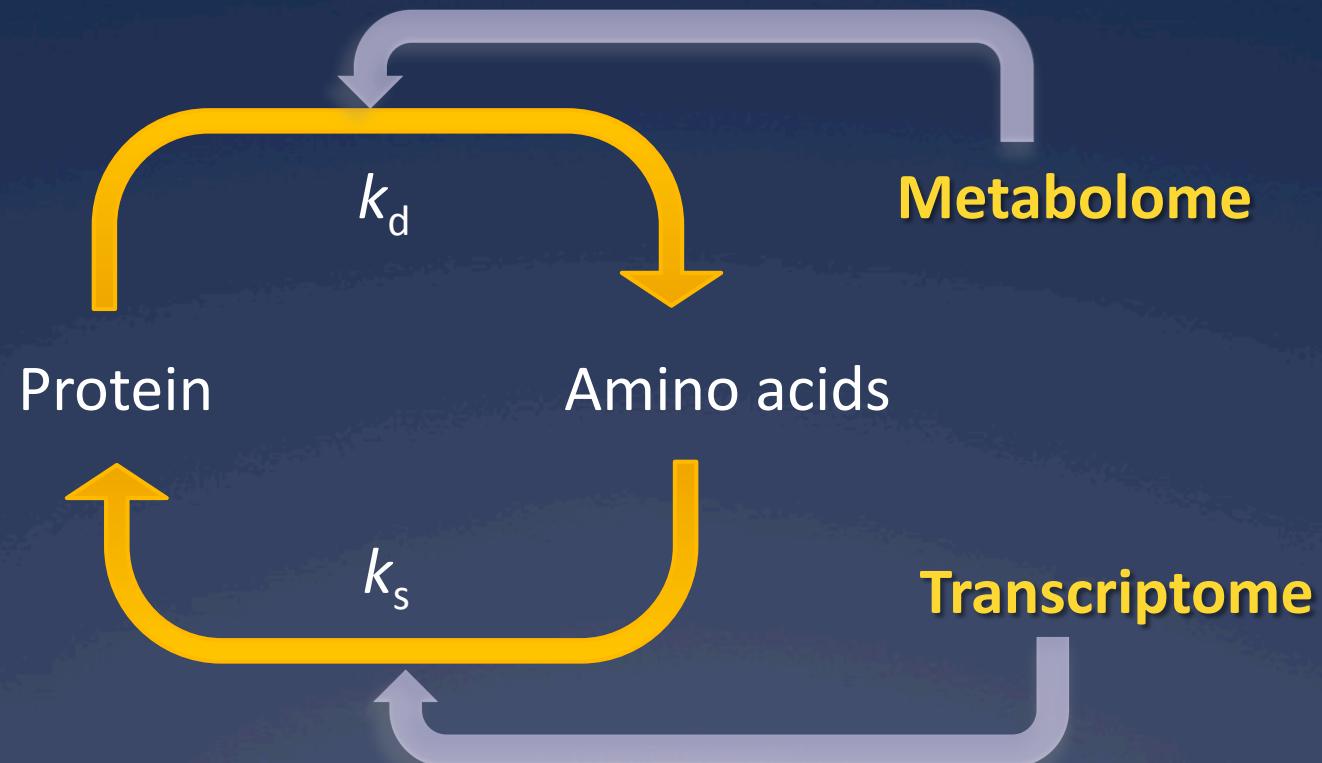
Post-translational modifications
Intracellular stability

Quantitative

Relative quantification "*Protein Z is 2.5 ± 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

Surrogacy

Are peptides suitable for protein quantification?

Proteolysis

The essentiality of complete digestion to limit peptides

The problem of protein ‘species’

Post-translational space

Proteome dynamics

Post-translational space

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

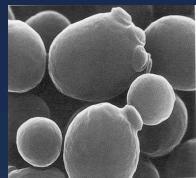
Absolute
AQUA, QconCAT

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 100 μ g/200 μ L digest
(17 million cells in digest)

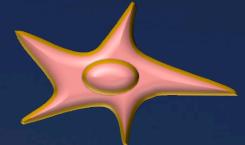


Apply 1000ng 'protein' (1 μ L)
~170,000 cells on column



Lowest sensitivity=
360 copies/cell

QqQ @ 100attomol
=60,000,000 molecules



HeLa

~2.5mg protein = 250pg/cell



Digest 100 μ g/200 μ L digest
(0.5 million cells in digest)



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

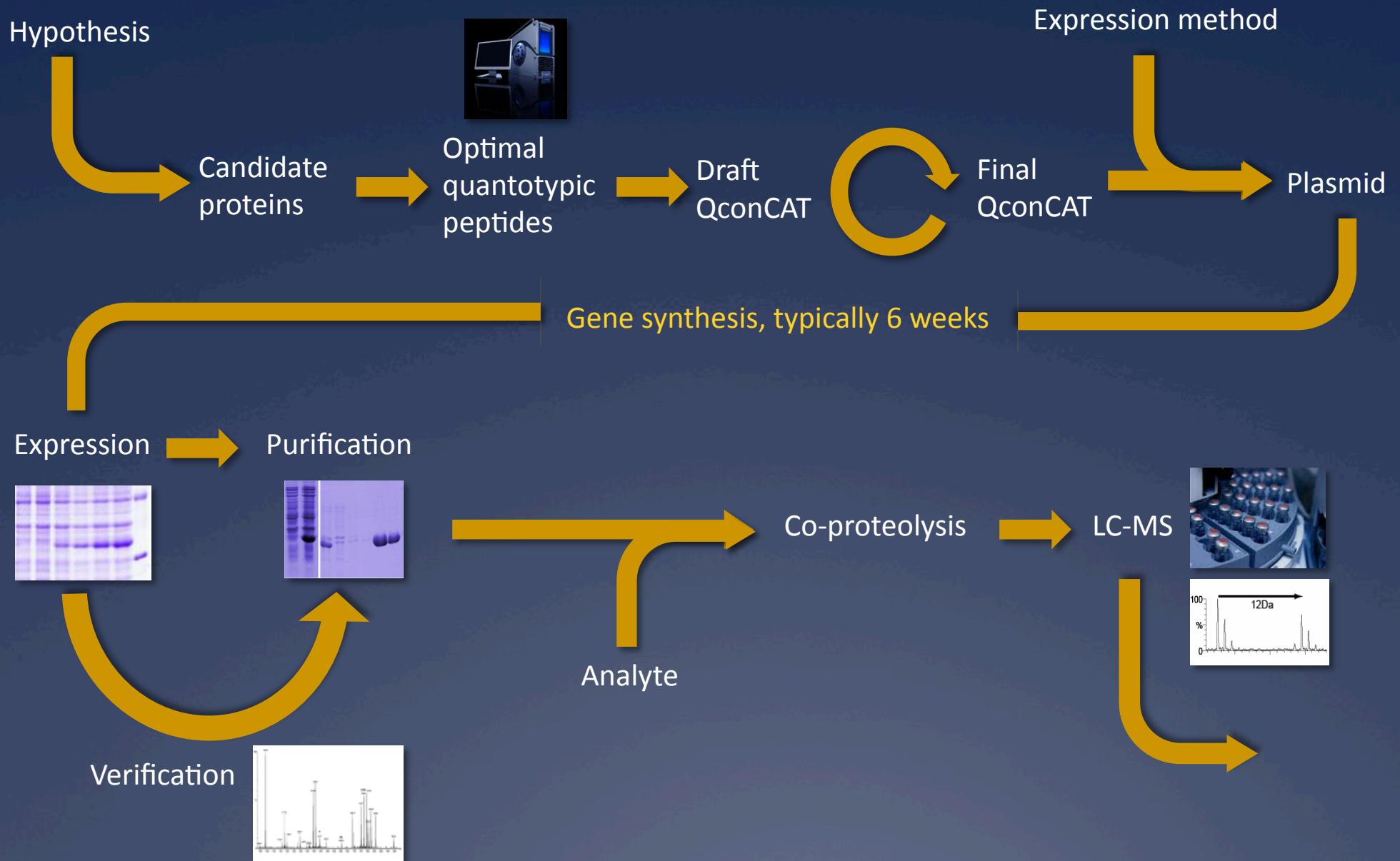


Lowest sensitivity=
15,000 copies/cell

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

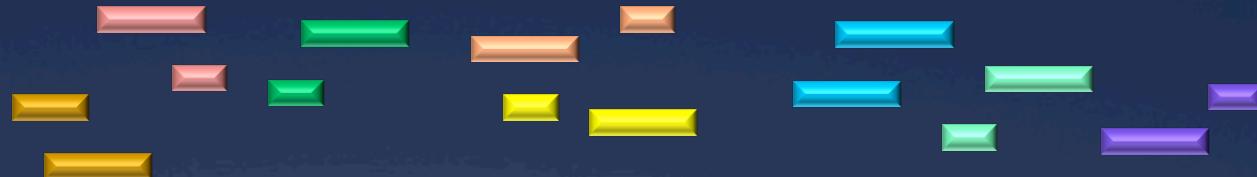
Designer genes for multiplexed quantification

Nominate target protein set
(n=20-50)

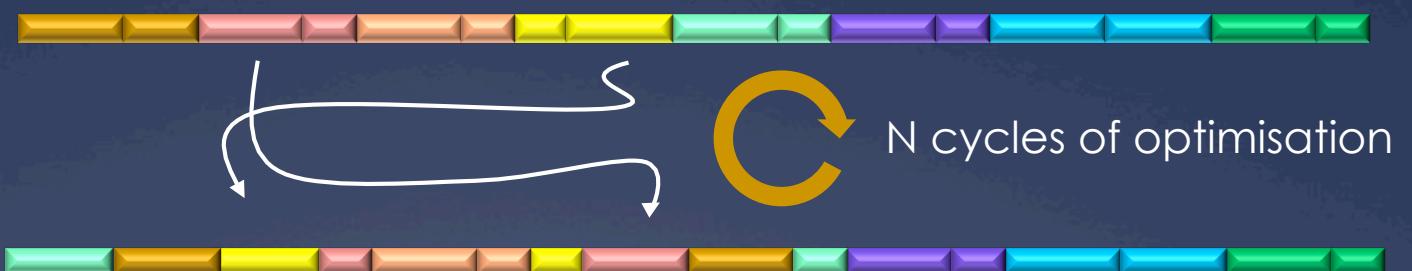


Designer genes for multiplexed quantification

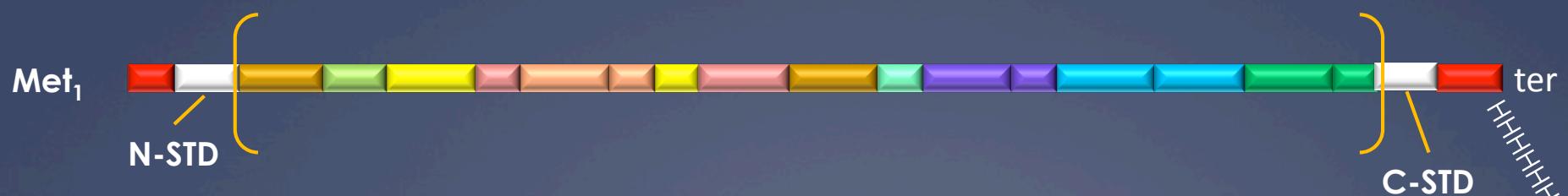
Selected Q-peptides



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



Add features:

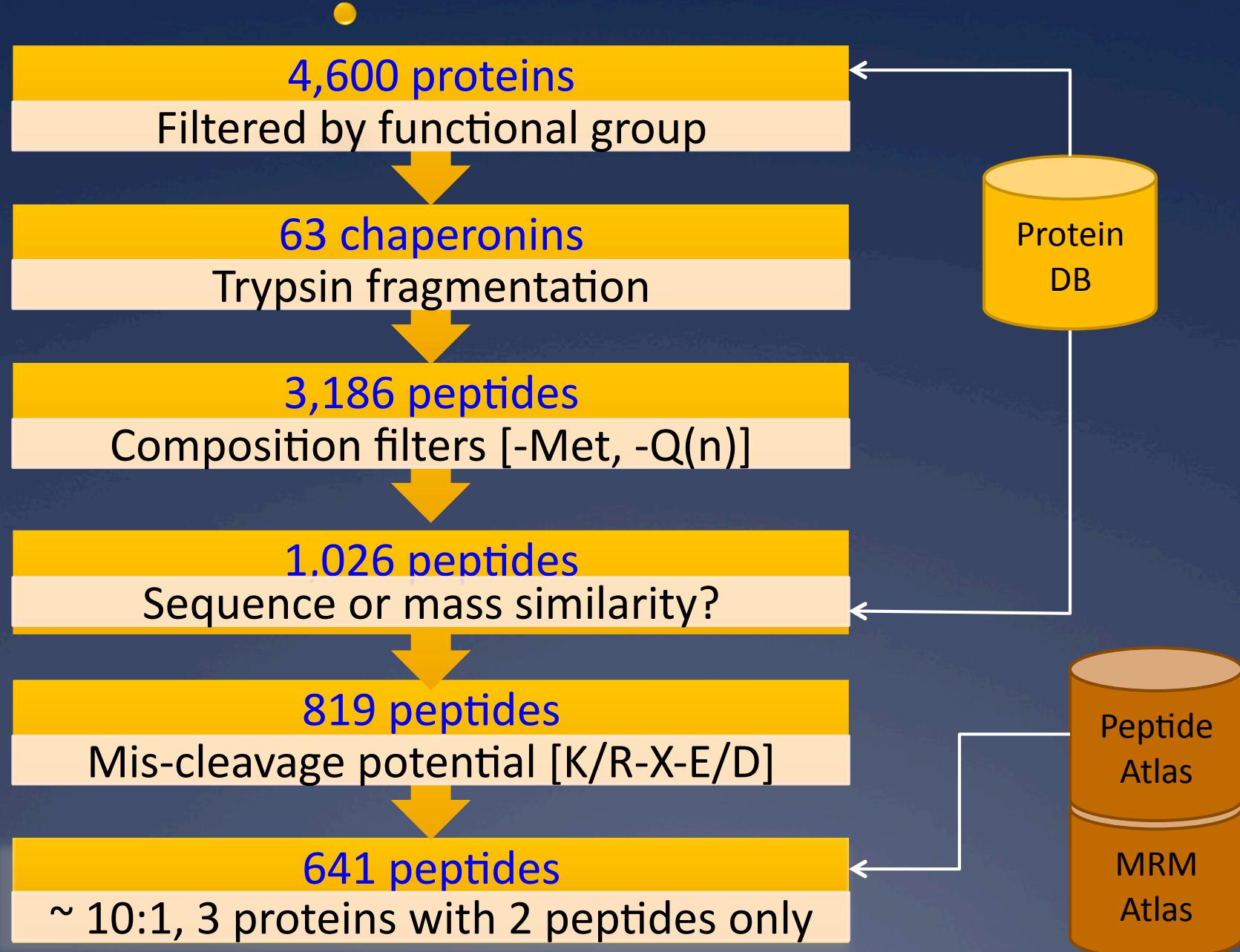


Beynon et al., Nature Methods. (2005) 2:587

Pratt et al., Nature Protocols (2006) 1:1029

Rivers et al., MCP (2007) 6:1416

Design Phase 1: peptides in silico



Design Phase 2: gene optimisation and synthesis



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

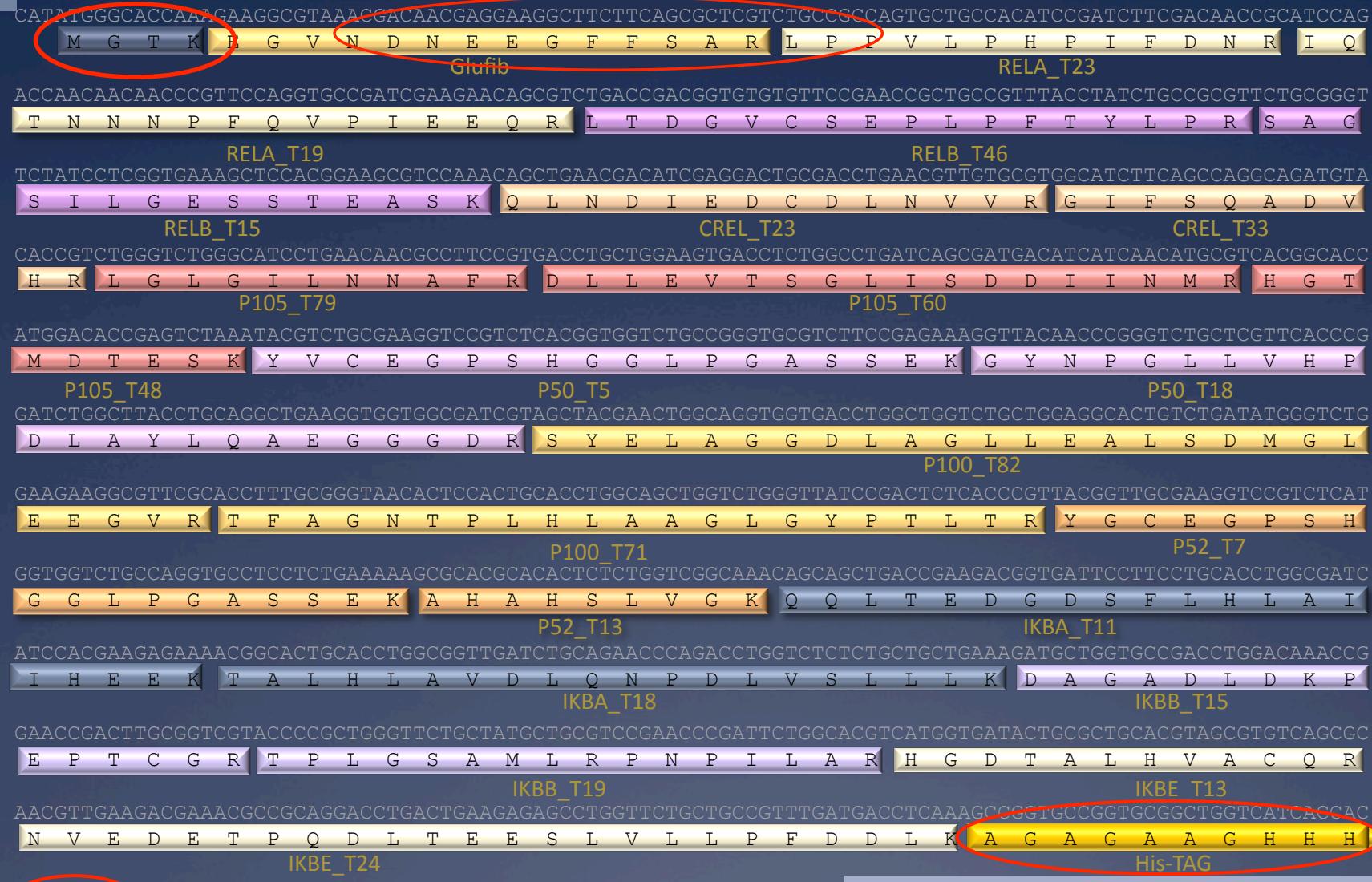
NFkappaB QconCAT



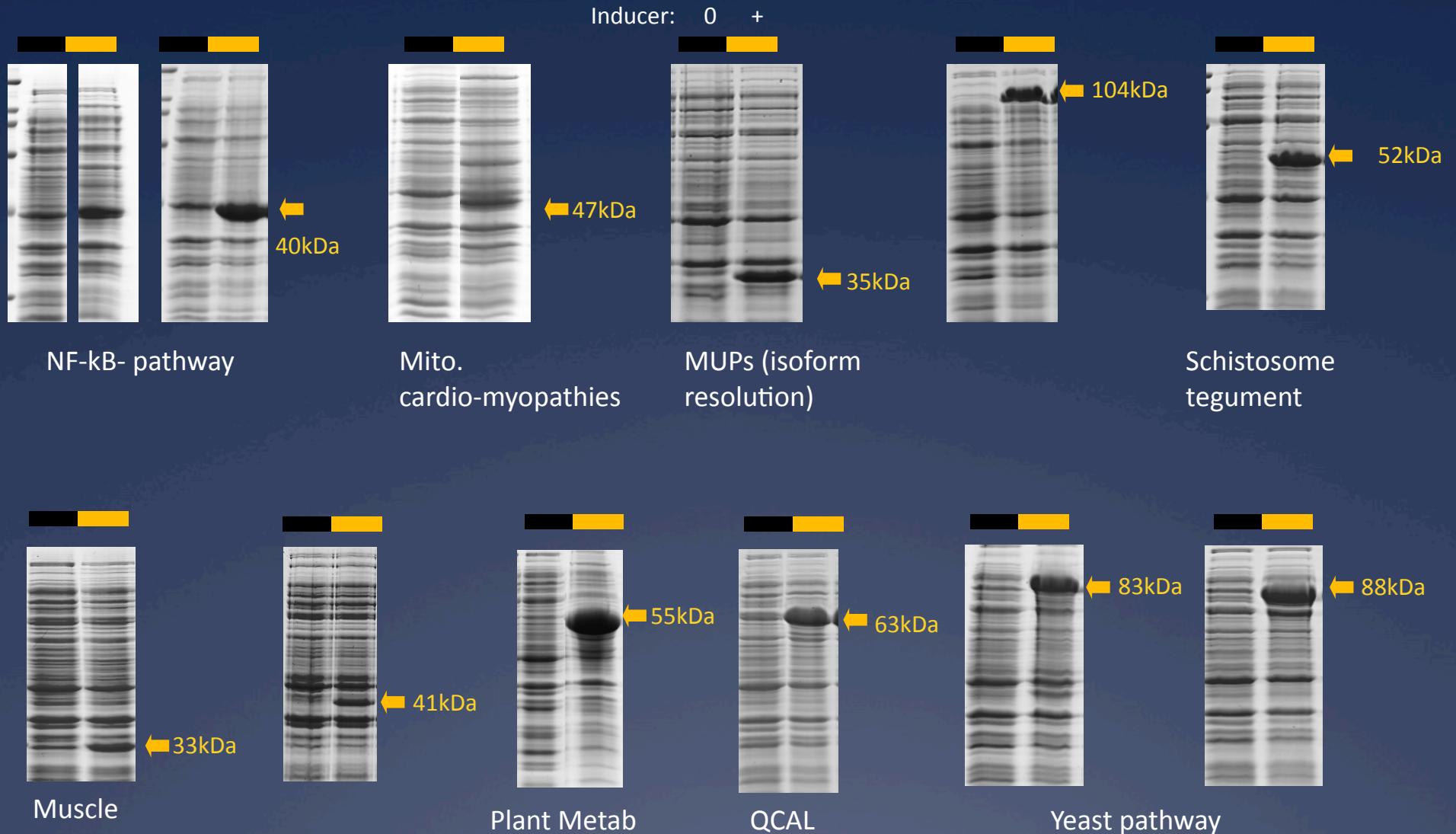
UNIVERSITY OF
LIVERPOOL

Met₁

Glufib (Quantification/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 ($\sim 60\text{kDa}$ 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
KYTVSFIEGDGIGPEISKSGLNNEAEIFEVIDFFLSEECKALLEGDTYTGTIQLHPDRGIDLINESLVAAYKHGIFVGPTIDKNAGAIH
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GGGIKVLPLVESVPEASILAKNFQLIDSTLRNTAFLGLYEPCHGSAPDLPKYILVSGITPTPLGEKGKDIAEIASAVDKNSYLIIVGDEEVQL
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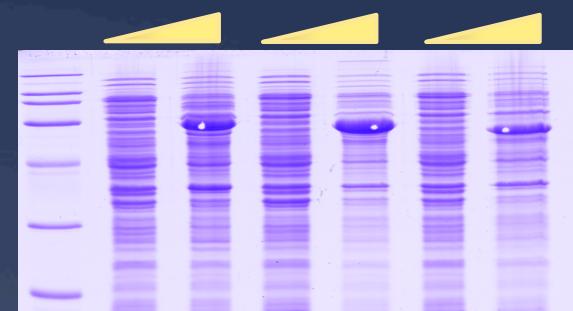
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ESQGIDVEYLTLQLDNWSTGPYKYINEGNCSVNFPEVALKFADAVNNALSGFSNYSFEKDNVVPWSEKVLGIDGGPGKNNFGQ
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ITKGYDAGENTYQAPPADRFVLSDDAHGVAQVGVCYDKNFHAEVSTPQVLSAKFIYPEEQSLGVVGLSSLPEIYEKIYTSETPEALPI
LLEDASRAISLDVTDDSLADKLYIAQEEIPDADLKLVNLSPSFNWSAHGFDDKSLNIDDVDSIICKYTDQPEGPIYPLDVLRANVALIDCG
VKEVIDTILALVKEVAVANNWPLDVRYTNEFLIHAADVEGLCGGIDELLVSKELSIVLAPFSGGQGKGNVVFLDTILDEARVPANFISEG
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DKAQYNEIQGWDHLSLLPTFGAKDQTNDQTVDSATATLKTATYDGEEGILAAKLTDSSPIPYPEQPPIKLLGAPSEGGSSVFEVTYF
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LEWLK

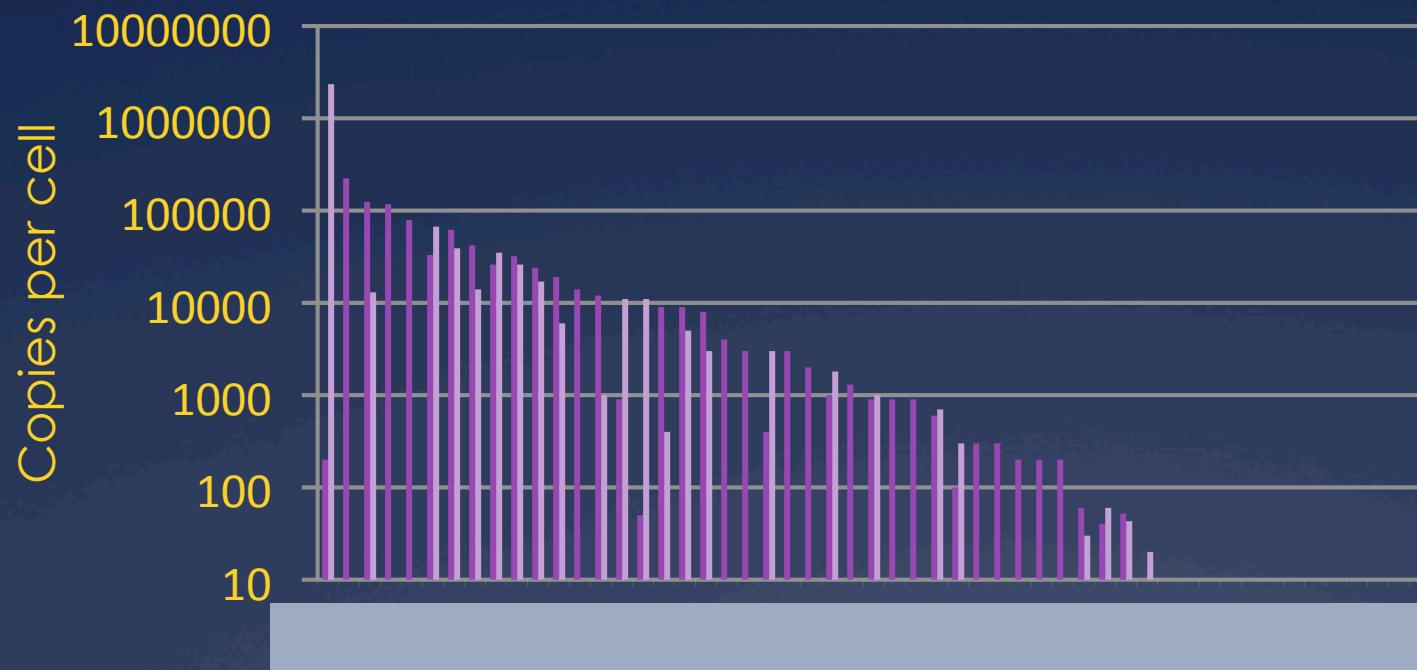
COPYCATs Phase 1

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
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CopyCat030	Proteolysis	20
CopyCat031	Proteolysis	21
Total		1257

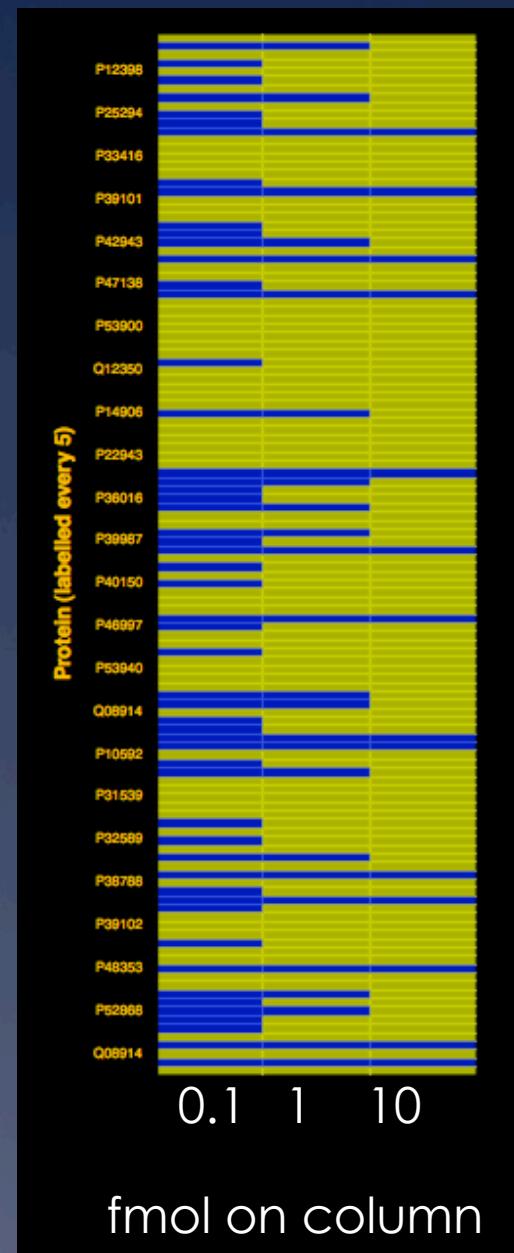
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CopyCat038	Proteolysis	21
CopyCat039	Proteolysis	21
CopyCat040	Proteolysis	21
CopyCat041	Amino Acid Metabolism	23
CopyCat042	Amino Acid Metabolism	23
CopyCat043	Amino Acid Metabolism	23
CopyCat044	Amino Acid Metabolism	23
CopyCat045	Amino Acid Metabolism	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
Total		1257



Chaperonins: absolute quantification



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



Absolute quantification by MS^E



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

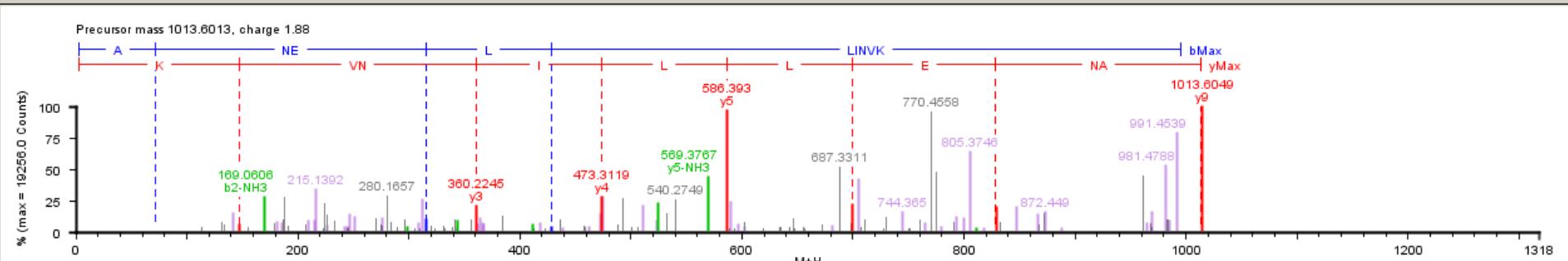
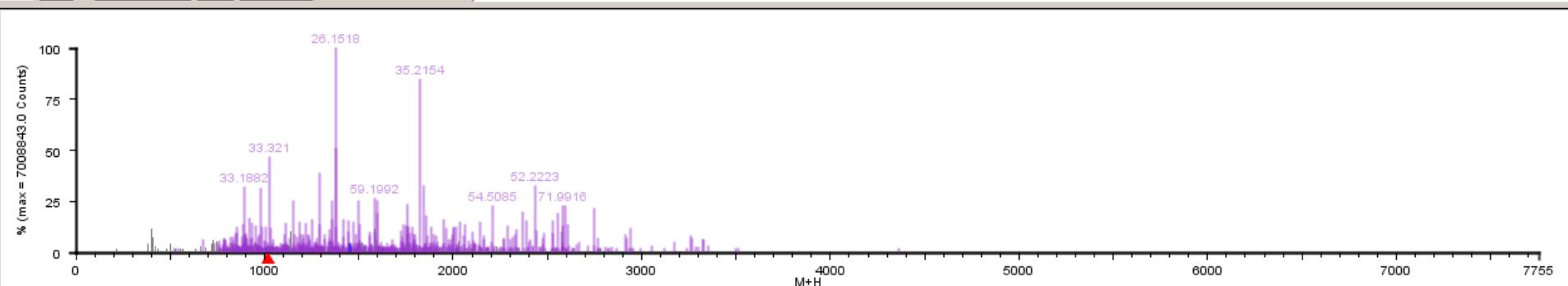
*Silva et al., (2006) MCP 5, 144

YeastManCam24 (ManCamYeast:2,c)

 448 hits, 487 proteins / ESTs (Showing All)

+■■■c	Enolase 2 OS Saccharomyces cerevisiae GN ENO2 PE 1 SV 2	P38720	6PGD1_YEAST	6 phosphogluconate dehydrogenase decarboxylating 1 OS Saccharomyces cerevisiae GN GND1 PE 1 SV 1	90.0	58.00
+■■■c	Glyceraldehyde 3 phosphate dehydrogenase 3 OS Saccharomyces cerevisiae GN GAP3 PE 1 SV 1	P53319	6PGD2_YEAST	6 phosphogluconate dehydrogenase decarboxylating 2 OS Saccharomyces cerevisiae GN GND2 PE 1 SV 1	57.5	5.17
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+■■■c	Pyruvate decarboxylase isozyme 1 OS Saccharomyces cerevisiae GN PDC1 PE 1 SV 1	P52910	ACS2_YEAST	Acetyl coenzyme A synthetase 2 OS Saccharomyces cerevisiae GN ACS2 PE 1 SV 1	41.9	16.10
+■■■c	Fructose bisphosphate aldolase OS Saccharomyces cerevisiae GN FBA1 PE 1 SV 1	P60010	ACT_YEAST	Actin 0S Saccharomyces cerevisiae GN ACT1 PE 1 SV 1	93.1	163.96
+■■■c	Glyceraldehyde 3 phosphate dehydrogenase 2 OS Saccharomyces cerevisiae GN GAP2 PE 1 SV 1	P00330	ADH1_YEAST	Alcohol dehydrogenase 1 OS Saccharomyces cerevisiae GN ADH1 PE 1 SV 4	75.3	100.31
+■■■c		P00331	ADH2_YEAST	Alcohol dehydrogenase 2 OS Saccharomyces cerevisiae GN ADH2 PE 1 SV 3	56.3	2.35
+■■■c		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)YVVVDTSK(-)
✓	1251.6783	1.93	1251.6692	0.0072	5.7305	7.96	288	299	(K)SISIVGSYVGVRN(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)ATDGGAHGVINVSVSEAAIEASTR(Y)
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YeastManCam24 (ManCamYeast2.c)

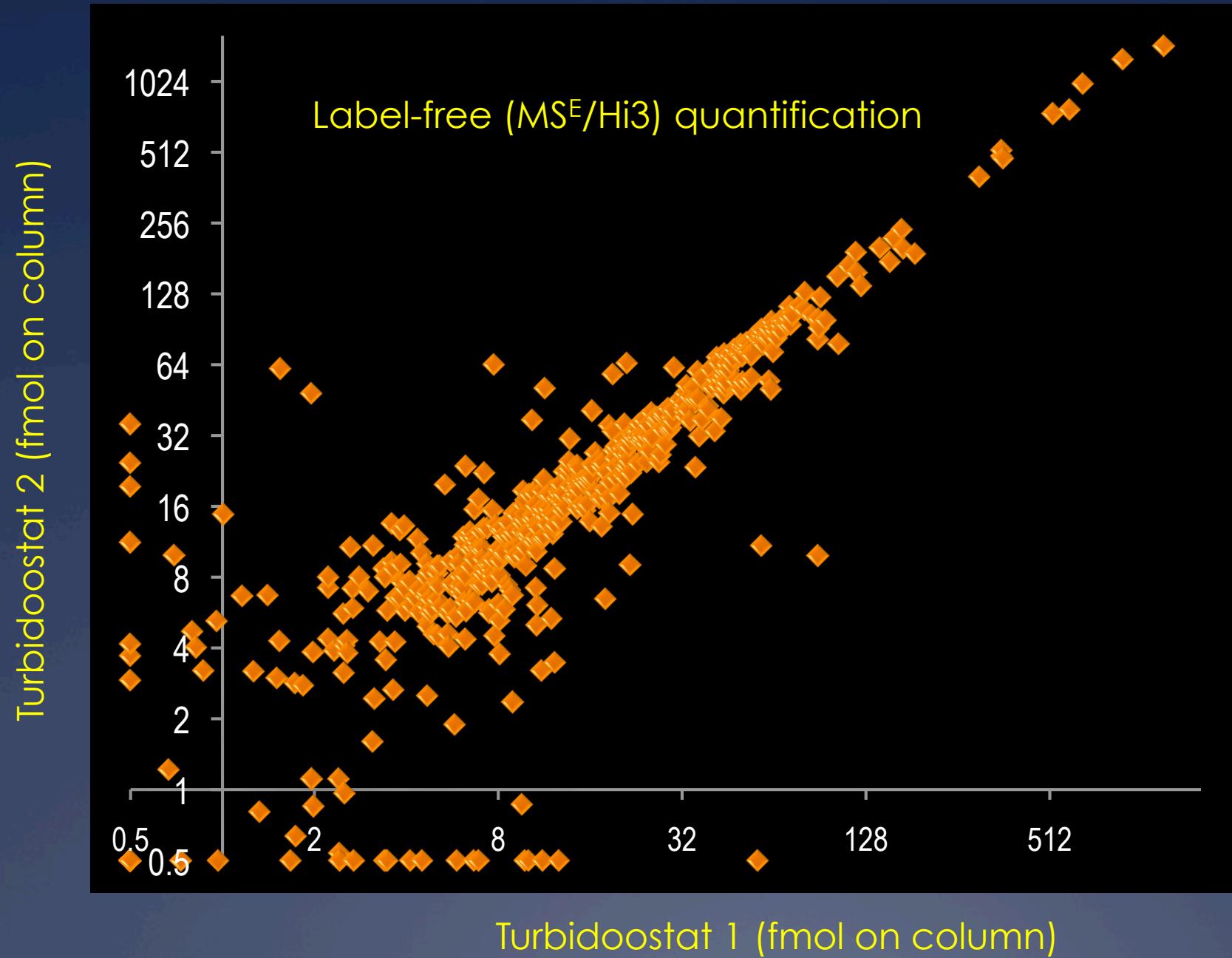
 148 hits, 487 proteins / ESTIs (Showing All)

c Endolase 2 OS Saccharomyces cerevisiae GN ENO2 PE 1 SV 2	P38720	6PGD1_YEAST	6 phosphogluconate dehydrogenase decarboxylating 1 OS Saccharomyces cerevisiae GN GND1 PE 1 SV 1	90.0	58.00
c Glyceraldehyde 3 phosphate dehydrogenase 3 OS Saccharomyces cerevisiae GN GAP1 PE 1 SV 1	P53319	6PGD2_YEAST	6 phosphogluconate dehydrogenase decarboxylating 2 OS Saccharomyces cerevisiae GN GND2 PE 1 SV 1	57.5	5.17
c Pyruvate kinase 1 OS Saccharomyces cerevisiae GN PYK1 PE 1 SV 1	P15891	ABP1_YEAST	Actin binding protein 0S Saccharomyces cerevisiae GN ABP1 PE 1 SV 4	56.9	19.17
c Endolase 1 OS Saccharomyces cerevisiae GN ENO1 PE 1 SV 2	P31787	ACBP_YEAST	Acyl CoA binding protein 0S Saccharomyces cerevisiae GN ACB1 PE 1 SV 3	94.3	40.61
c Glyceraldehyde 3 phosphate dehydrogenase 1 OS Saccharomyces cerevisiae GN GAP2 PE 1 SV 1	P32316	ACH1_YEAST	Acetyl CoA hydrolase 0S Saccharomyces cerevisiae GN ACH1 PE 1 SV 2	42.0	4.38
c Phosphoglycerate kinase 0S Saccharomyces cerevisiae GN PGK1 PE 1 SV 1	P19414	ACON_YEAST	Aconitase hydratase mitochondrial 0S Saccharomyces cerevisiae GN ACO1 PE 1 SV 2	39.7	26.09
c Pyruvate decarboxylase isozyme 1 OS Saccharomyces cerevisiae GN PDC1 PE 1 SV 1	P52910	ACS2_YEAST	Acetyl coenzyme A synthetase 2 OS Saccharomyces cerevisiae GN ACS2 PE 1 SV 1	41.9	16.10
c Fructose bisphosphate aldolase 0S Saccharomyces cerevisiae GN FBA1 PE 1 SV 1	P60010	ACT_YEAST	Actin 0S Saccharomyces cerevisiae GN ACT1 PE 1 SV 1	82.1	162.06
c Glyceraldehyde 3 phosphate dehydrogenase 2 OS Saccharomyces cerevisiae GN GAP2 PE 1 SV 1	P00330	ADH1_YEAST	Alcohol dehydrogenase 1 OS Saccharomyces cerevisiae GN ADH1 PE 1 SV 4	75.3	100.31
c Elongation factor 1 alpha 0S Saccharomyces cerevisiae GN TEF1 PE 1 SV 1	P00331	ADH2_YEAST	Alcohol dehydrogenase 2 OS Saccharomyces cerevisiae GN ADH2 PE 1 SV 3	56.3	2.35
c Peroxiredoxin type 2 OS Saccharomyces cerevisiae GN AHP1 PE 1 SV 1	P38113	ADH5_YEAST	Alcohol dehydrogenase 5 OS Saccharomyces cerevisiae GN ADH5 PE 1 SV 1	36.8	0.83

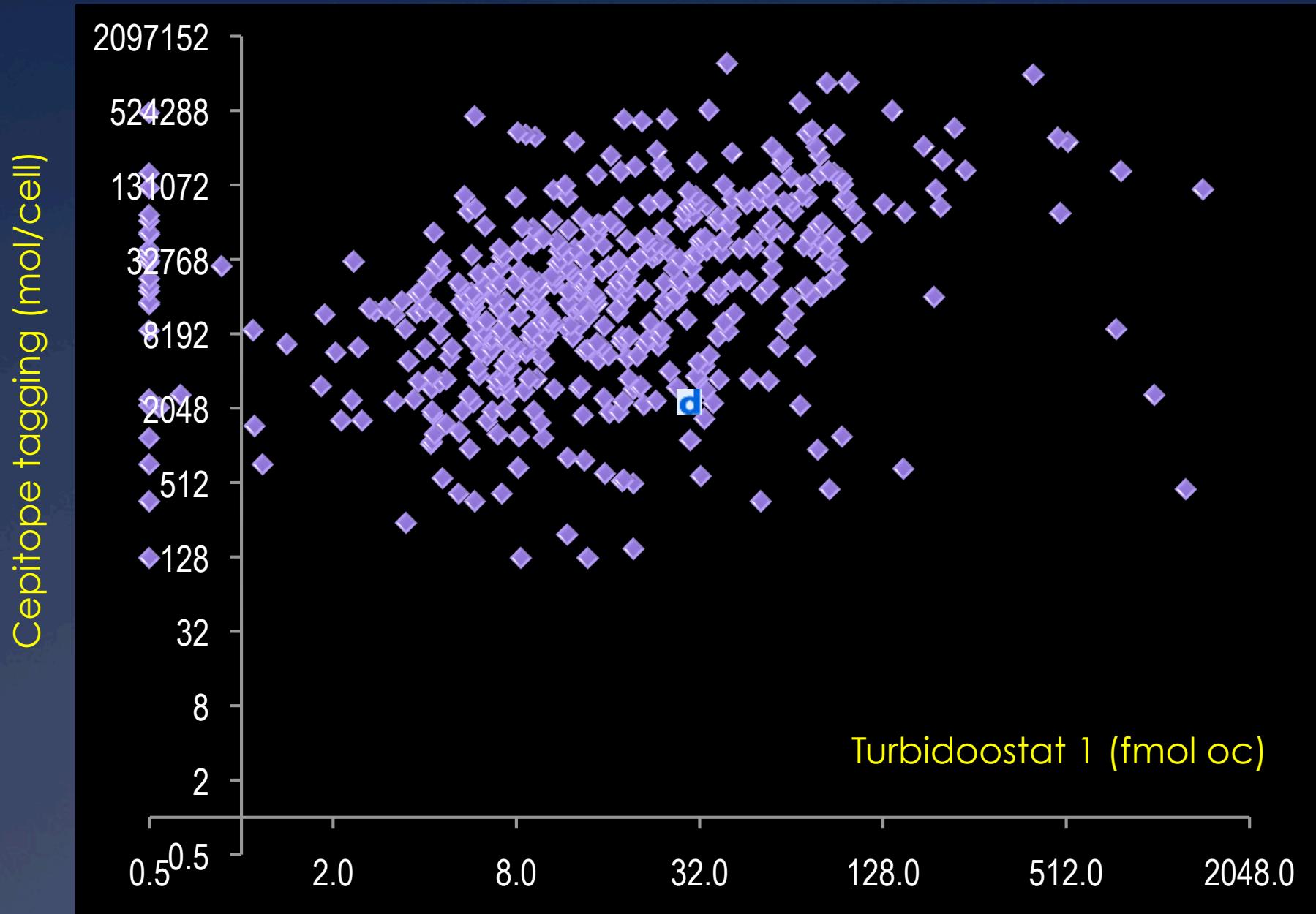
Description	Coverage (%)	Amount (fmol)
Phase 1 OS Saccharomyces cerevisiae GN ADH1 PE 1 SV 4	75.3	100.3184
Phase 2 OS Saccharomyces cerevisiae GN ADH2 PE 1 SV 3	56.3	2.3570
Phase 5 OS Saccharomyces cerevisiae GN ADH5 PE 1 SV 1	36.8	0.8314
OS Saccharomyces cerevisiae GN ADO1 PE 1 SV 1	55.6	35.8954
rotein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



Label free biological replication



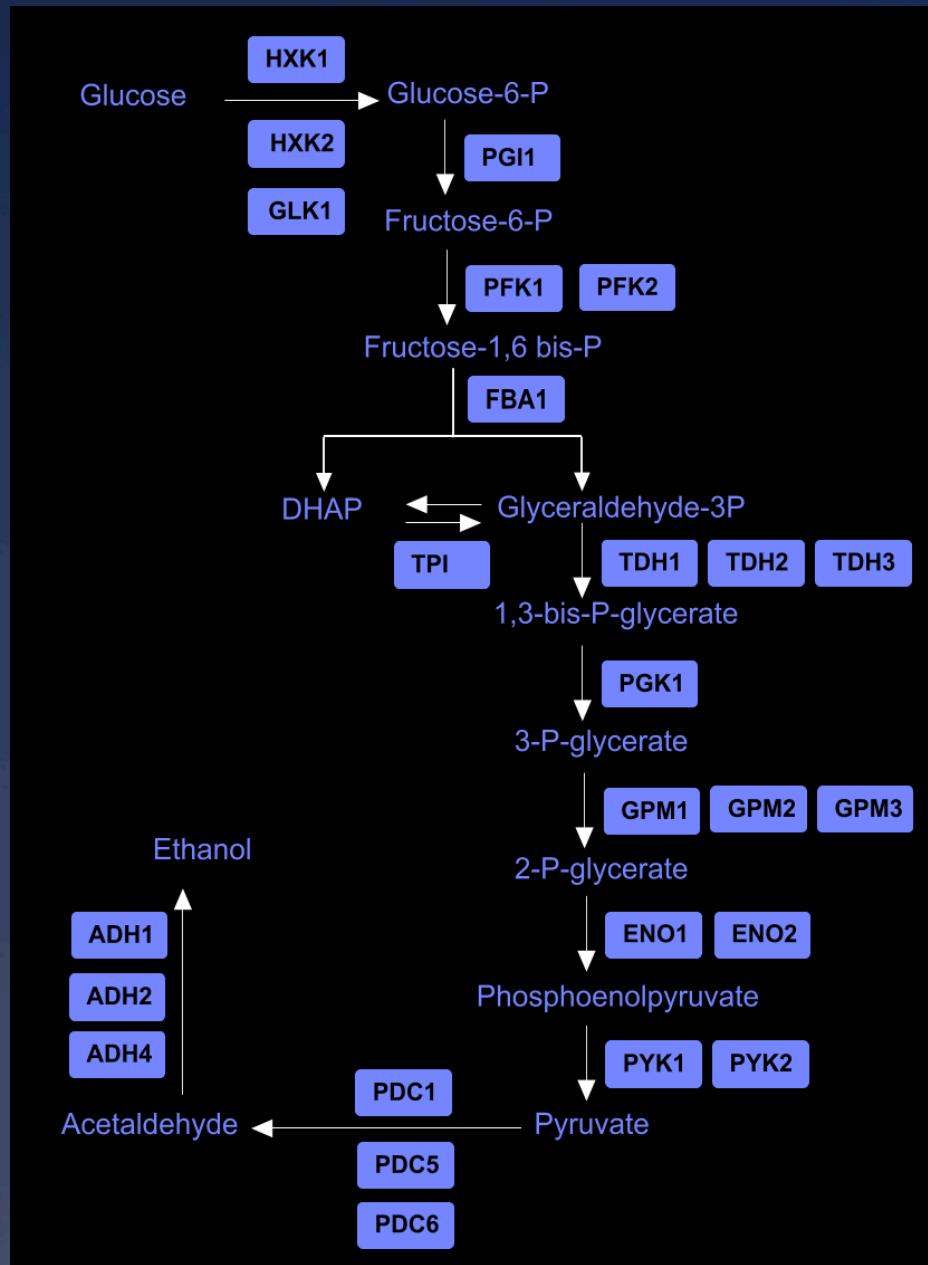
Label free vs epitope tagging



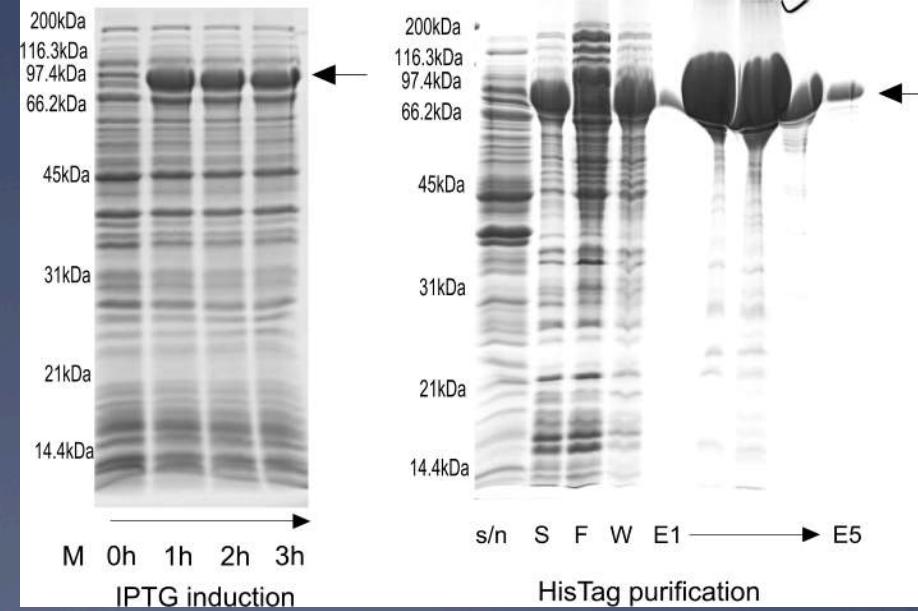
Quantification of a glycolytic pathway



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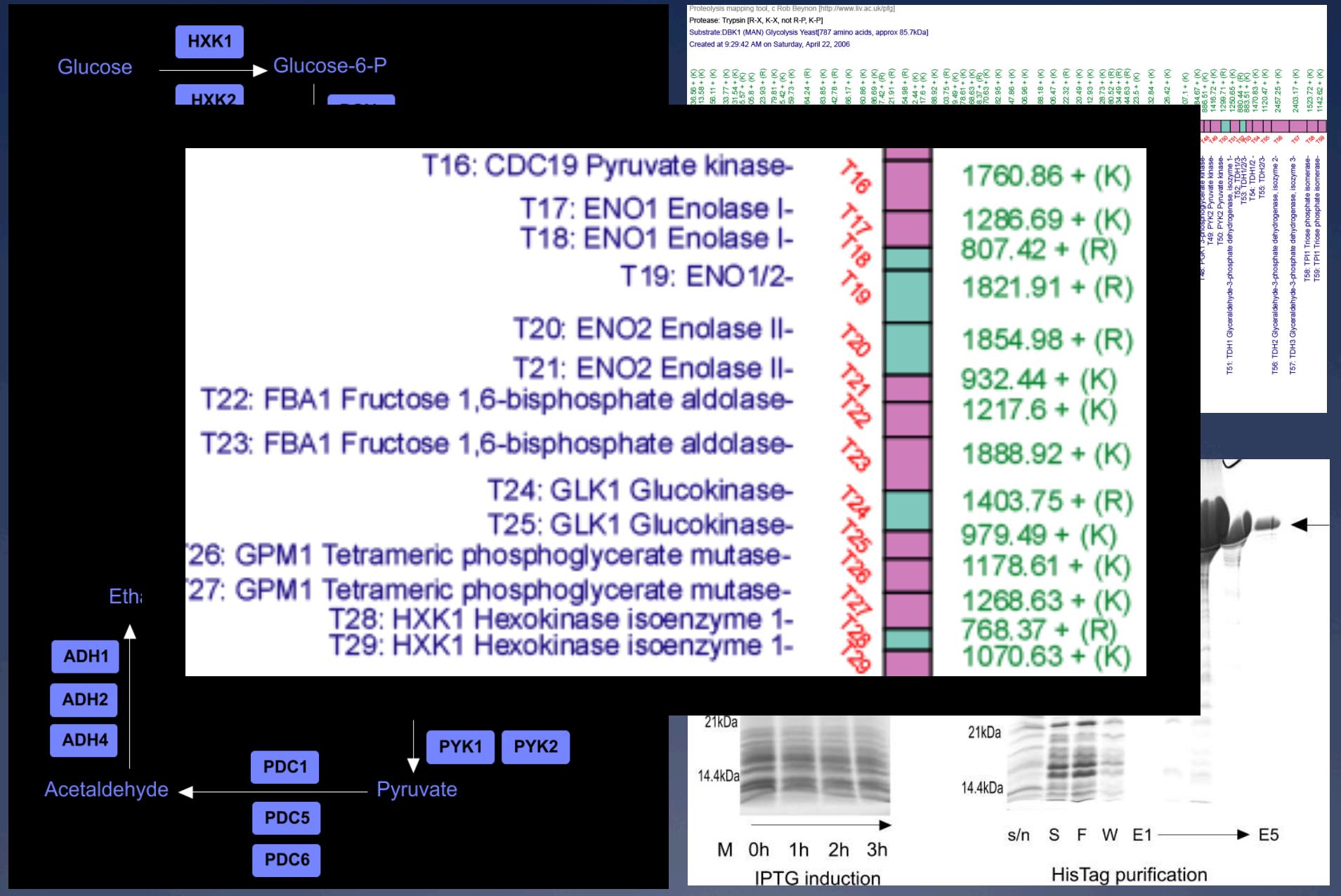
Proteolysis mapping tool, c Rob Beynon [http://www.liv.ac.uk/pfg]																																																																																																																																																																														
Protease: Trypsin [R-X, K-X, not R-P, K-P]																																																																																																																																																																														
Substrate: DBK1 (MAN) Glycolysis Yeast 787 amino acids, approx 85.7kDa]																																																																																																																																																																														
Created at 9:29:42 AM on Saturday, April 22, 2006																																																																																																																																																																														
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alcohol dehydrogenase family member</td><td>2764.24 + (R)</td><td></td></tr> <tr><td>T12: ADH6 NADPH-dependent ornithine alcohol dehydrogenase family member</td><td>1580.88 + (K)</td><td></td></tr> <tr><td>T13: ADH7 NADPH-dependent ornithine alcohol dehydrogenase family member</td><td>1542.78 + (R)</td><td></td></tr> <tr><td>T14: ADH7 NADPH-dependent ornithine alcohol dehydrogenase family member</td><td>2206.17 + (K)</td><td></td></tr> <tr><td>T15: CCD19 Pyruvate kinase</td><td>178</td><td></td></tr> <tr><td>T16: CCD19 Pyruvate kinase</td><td>178</td><td></td></tr> <tr><td>T17: ENO1 Endonuclease L</td><td>1760.86 + (K)</td><td></td></tr> <tr><td>T18: ENO1 Endonuclease L</td><td>1295.55 + (K)</td><td></td></tr> <tr><td>T19: ENO2</td><td>801.42 + (R)</td><td></td></tr> <tr><td>T20: ENO2 Endonuclease II</td><td>1821.98 + (R)</td><td></td></tr> <tr><td>T21: ENO2 Endonuclease II</td><td>1854.98 + (R)</td><td></td></tr> <tr><td>T22: FBA1 Fructose 1,6-bisphosphate aldolase</td><td>932.44 + (K)</td><td></td></tr> <tr><td>T23: FBA1 Fructose 1,6-bisphosphate aldolase</td><td>1277 + (K)</td><td></td></tr> <tr><td>T24: GLK1 Glucokinase</td><td>1888.92 + (K)</td><td></td></tr> <tr><td>T25: G3P-G3P kinase</td><td>1403.75 + (R)</td><td></td></tr> <tr><td>T26: GPM1 Tetrameric phosphoglycerate mutase</td><td>979.48 + (K)</td><td></td></tr> <tr><td>T27: HK1 Hexokinase isozyme I</td><td>1285.61 + (K)</td><td></td></tr> <tr><td>T28: HK1 Hexokinase isozyme I</td><td>1298.57 + (K)</td><td></td></tr> <tr><td>T29: HK1 Hexokinase isozyme I</td><td>1070.63 + (K)</td><td></td></tr> <tr><td>T30: HK1 Hexokinase isozyme I</td><td>2162.98 + (K)</td><td></td></tr> <tr><td>T31: HKX2 Hexokinase isozyme 2</td><td>1547.86 + (K)</td><td></td></tr> <tr><td>T32: HKX2 Hexokinase isozyme 2</td><td>2000.96 + (K)</td><td></td></tr> <tr><td>T33: PDC1 Major of three pyruvate decarboxylase isozymes</td><td>2388.18 + (K)</td><td></td></tr> <tr><td>T34: PDC1 Major of three pyruvate decarboxylase isozymes</td><td>1005.47 + (K)</td><td></td></tr> <tr><td>T35: PDC5 Minor isozyme of pyruvate decarboxylase</td><td>2322.32 + (R)</td><td></td></tr> <tr><td>T36: PDC5 Minor isozyme of pyruvate decarboxylase</td><td>1020.48 + (K)</td><td></td></tr> <tr><td>T37: PDC5 Minor isozyme of pyruvate decarboxylase</td><td>2012.93 + (K)</td><td></td></tr> <tr><td>T38: PFK1 Alpha subunit of heterocotrimeric phosphofructokinase</td><td>1288.73 + (K)</td><td></td></tr> <tr><td>T40: PFK1 Alpha subunit of heterocotrimeric phosphofructokinase</td><td>1058.55 + (K)</td><td></td></tr> <tr><td>T41: PFK2 Beta subunit of heterotrimeric phosphofructokinase</td><td>1024.63 + (K)</td><td></td></tr> <tr><td>T42: PFK2 Beta subunit of heterotrimeric phosphofructokinase</td><td>1223.5 + (K)</td><td></td></tr> <tr><td>T43: PYK1 Glycolytic enzyme phosphoglucomutase</td><td>1522.84 + (K)</td><td></td></tr> <tr><td>T44: PYK2 Glycolytic enzyme phosphoglucomutase</td><td>1298.71 + (K)</td><td></td></tr> <tr><td>T45: 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dehydrogenase isozyme 2</td><td></td><td></td></tr> <tr><td>T56: TDH2 Glyceraldehyde-3-phosphate dehydrogenase isozyme 2</td><td></td><td></td></tr> <tr><td>T57: TDH3 Glyceraldehyde-3-phosphate dehydrogenase isozyme 2</td><td></td><td></td></tr> <tr><td>T58: TPI1 Triose phosphate isomerase</td><td></td><td></td></tr> <tr><td>T59: TPI1 Triose phosphate isomerase</td><td></td><td></td></tr> </table>	T1: ADH1 Alcohol dehydrogenase I	1136.55 + (K)		T2: ADH1 Alcohol dehydrogenase II	1013.58 + (K)		T3: ADH2 Glucose-repressible alcohol dehydrogenase I	2155.11 + (K)		T4: ADH2 Glucose-repressible alcohol dehydrogenase II	1483.77 + (K)		T5: ADH3 Mitochondrial alcohol dehydrogenase isozyme I	1035.54 + (K)		T6: ADH4 Mitochondrial alcohol dehydrogenase isozyme II	985.57 + (K)		T7: ADH4 Alcohol dehydrogenase type IV	1055.8 + (K)		T8: ADH4 Alcohol dehydrogenase type V	1470.91 + (K)		T9: ADH5 Alcohol dehydrogenase isozyme V	825.42 + (K)		T10: ADH5 Alcohol dehydrogenase isozyme VI	1559.73 + (K)		T11: 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Quantification of a glycolytic pathway



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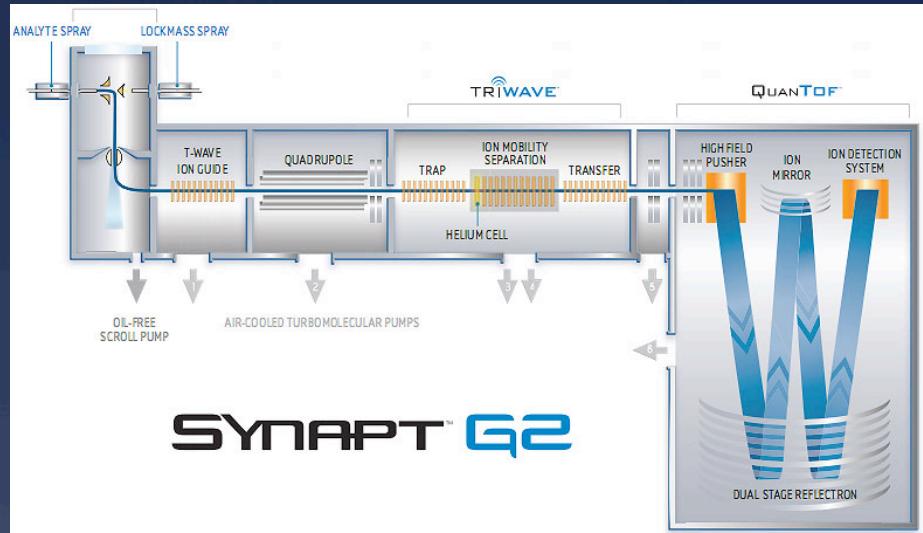
Instrument-based enhancements

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



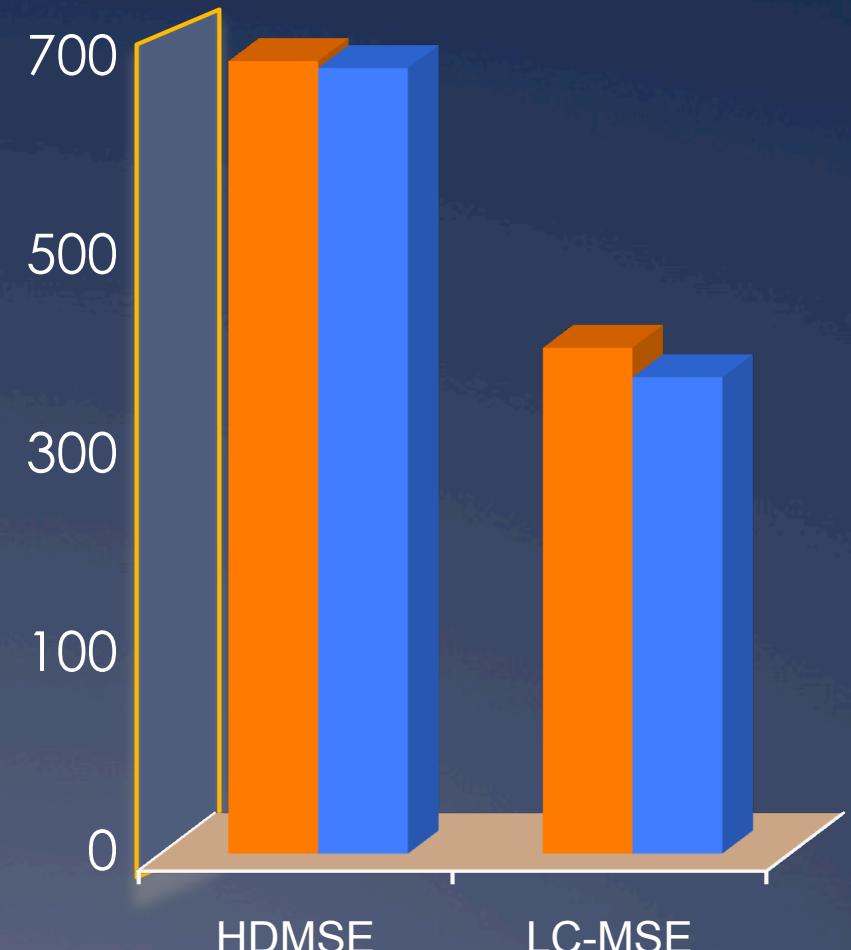
- Batch grown cultures, full aeration
- 4 biological replicates: control + stress

- 5X extraction (98%+)

- 500ng protein on column
- 50fmol internal standard

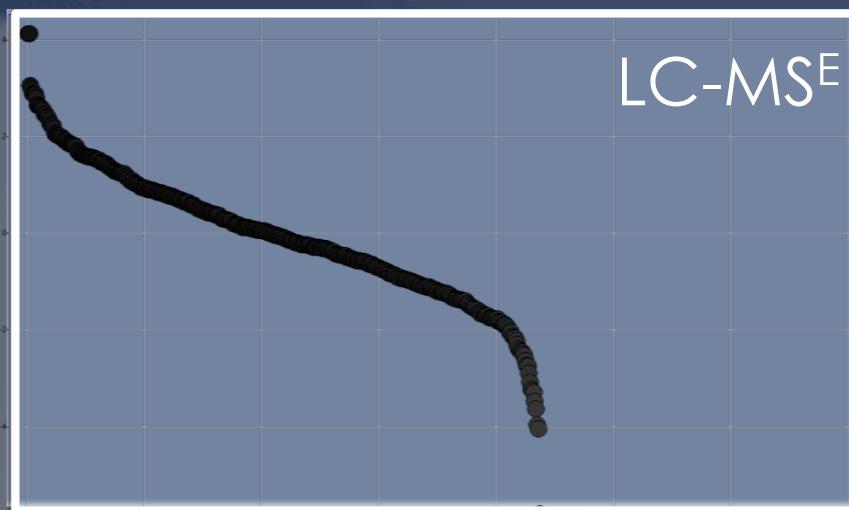
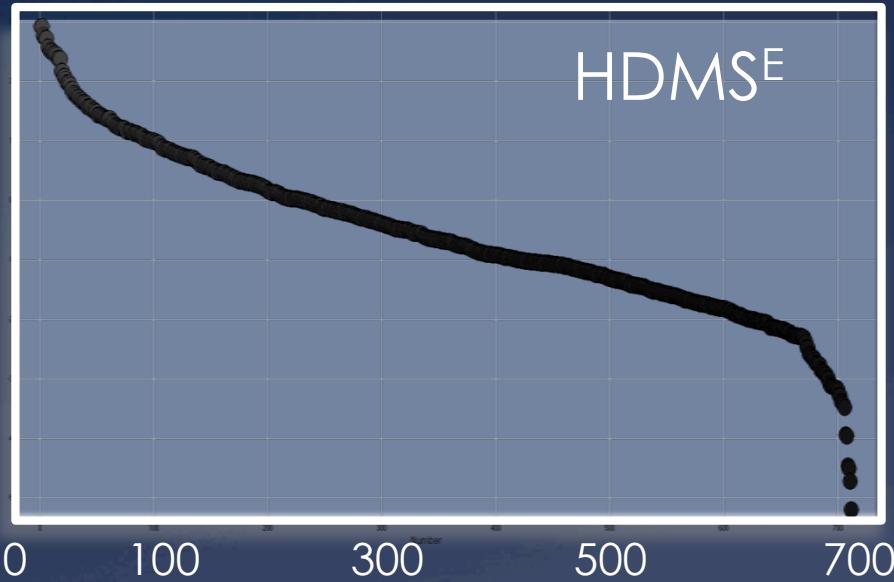
- 120 min 1D RP gradient,
- MS^E acquisition, Synapt G2
- HDMS^E acquisition, Synapt G2

- PLGS 2.4, Identity and Hi3 Quantification

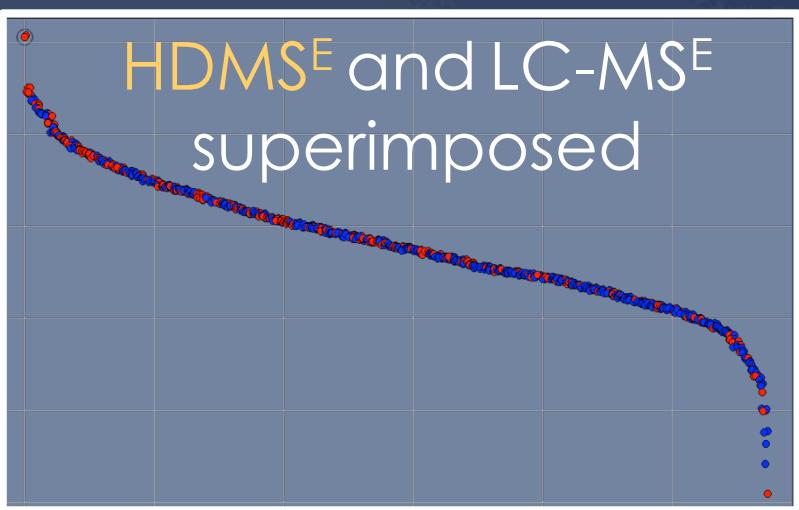


Identification/quantification gain with HDMS^E

Abundance (In scale)



Protein index



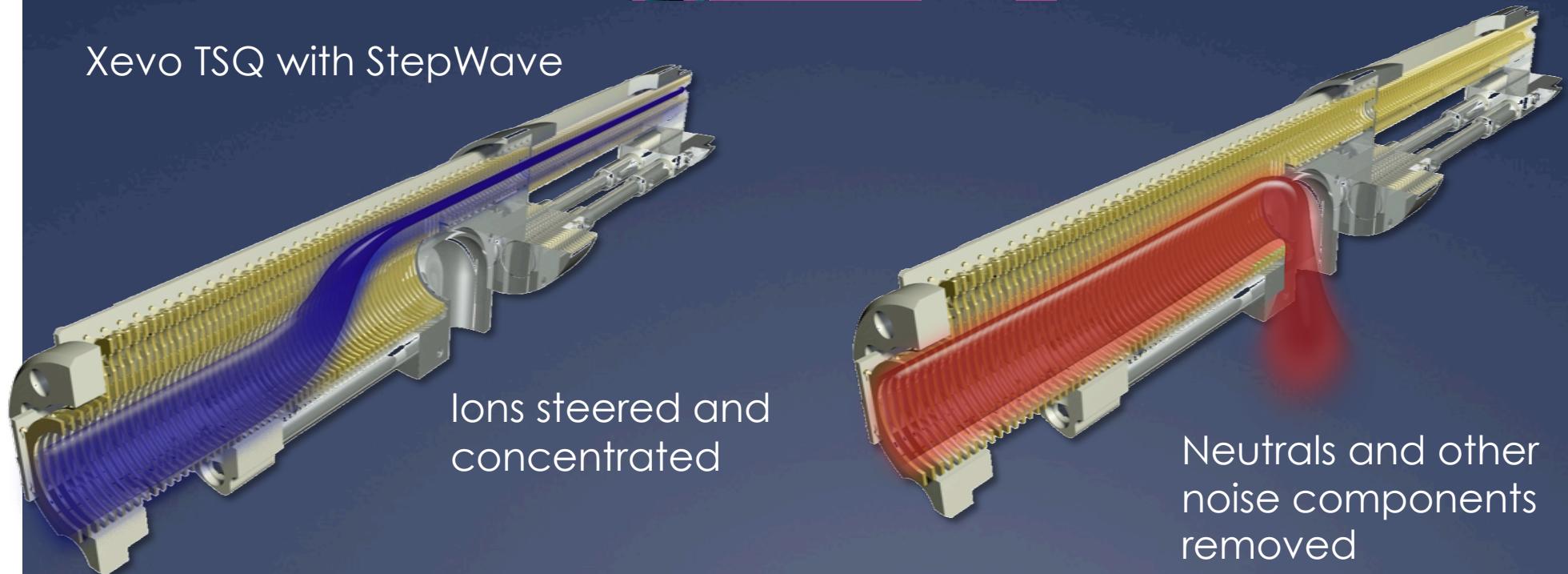
Protein index

Gain through source enhancements



STEP WAVE™

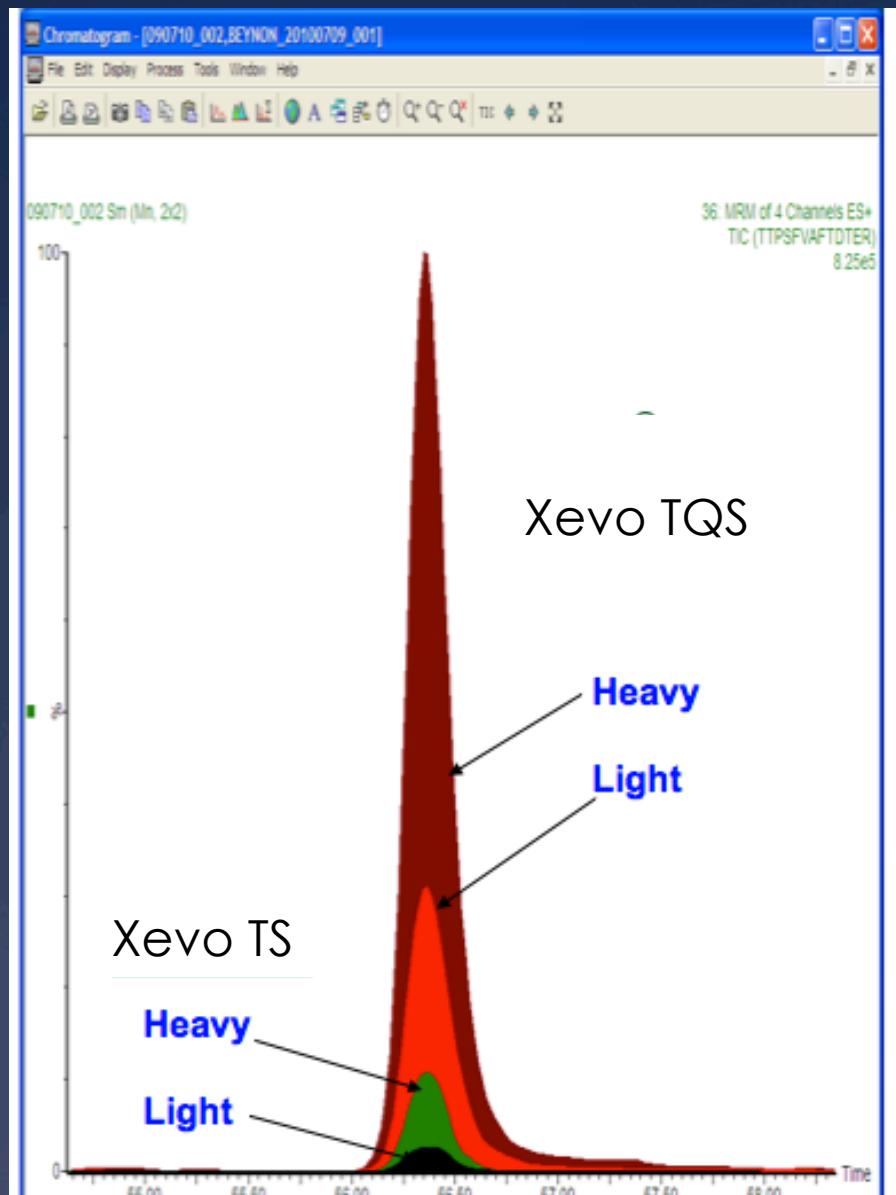
Xevo TSQ with StepWave



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

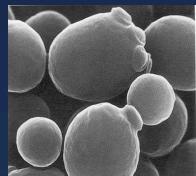
QCONCAT Peptide :
[¹³C₆]TTPSFVAFDTDTER

Yeast Peptide TTPSFVAFDTDTER

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 100 μ g/200 μ L digest
(17 million cells in digest)

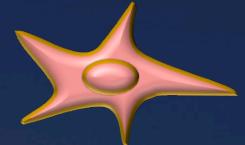


Apply 1000ng 'protein' (1 μ L)
~170,000 cells on column



Lowest sensitivity=
~4 copies/cell

QqQ LOQ=1attomol
=600,000 molecules



HeLa

~2.5mg protein = 250pg/cell



Digest 100 μ g/200 μ L digest
(0.5 million cells in digest)



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



Lowest sensitivity=
150 copies/cell