DGMS40 2007 - Workshop Quantitative Proteomics

11. März, Bremen

QconCAT – eine neue Methode zur parallelen absoluten Quantifizierung von Proteinen in der Massenspektrometrie

Entelechon
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Proteomics 2007 (1)

- Western blot
- ELISA (Enzyme Linked Immumosorbent Assay)
- RIA (Radio Immunosorbant Assay)
- FACS analysis (cell based)
- Luminex (bead based)

- DIGE (2 Dimensional Gel Electrophoresis)
- MALDI-TOF identification of proteins

Proteomics 2007 (2)

Multidimensional Liquid Chromatography

ICAT - derivatisation of cysteine residues

ITRAQ- Labeling of N-termini, multiplexing limited to six samples

SILAC

ICPL

Isotope labeled quantitation peptides – AQUA-peptides

Proteomics 2017 (1)

Multiplex parallel quantitation of proteins by MS

- **Used for diagnosis, prognosis and individual therapy control**
- **Wide applications in controlling processes in biotechnological production**
- **Important tool for systems biology (whole proteomes available)**
- **In scientific groups used to analyse changes in protein complexes, cell organelles and membrane molecules (e. g. lipid rafts)**

Proteomics 2017 (2)

Analyses will be possible using

- **Unmodified proteolytic peptides**
- **Natural peptides or protein fragments**
- **Phosphorylated peptides**
- **N- and O- glycosylated peptides**
- **Other modifications (ubiquitination, suomylation, acetylation etc.)**

QconCAT used as routine method to verify effects of RNAi therapy

AQUA – chemical synthesis (SIGMA-ALDRICH)

- enables absolute protein quantitation using stable isotope labeled peptides as an internal standard and HPLC-MS
- **Step 1:** AQUA Peptide Selection

  Select an optimal tryptic peptide and stable isotope amino acid from the sequence of your protein of interest , Order synthetic AQUA Peptide, Optimize LC-MS/MS
- **Step 2:** Implementation

  Extract protein from biological samples and add known quantity of AQUA Peptide ,
  Digest , Analyze by LC-MS/MS to quantitate protein of interest

Gerber, Scott A, et al. Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. PNAS. June 10, 2003. Vol 100. No 12. p 6940-6945

Problems

- Absolute quantification expensive
- Chemical synthesis of reference peptides not always feasible
- Sources of errors due to different treatment of reference peptide and target proteins

QconCAT – biosynthesis of multiple peptides

- Absolute Quantification
- Based on mass tagged internal standards (Q-peptides)
- One Q-peptide per analyte protein
- Multiple Q-peptides conCATenated into a synthetic gene, expressed as a heterologous QconCAT protein
- Easy construction of large sets of mass tag peptides
- Tryptic digest of reference and target peptides in the *same* tube
- Separation of reference and target peptides in the same solution

The method 1 - selection of protein targets

MS-derived peptides

DIGE-derived proteins

Western blot

ELISA

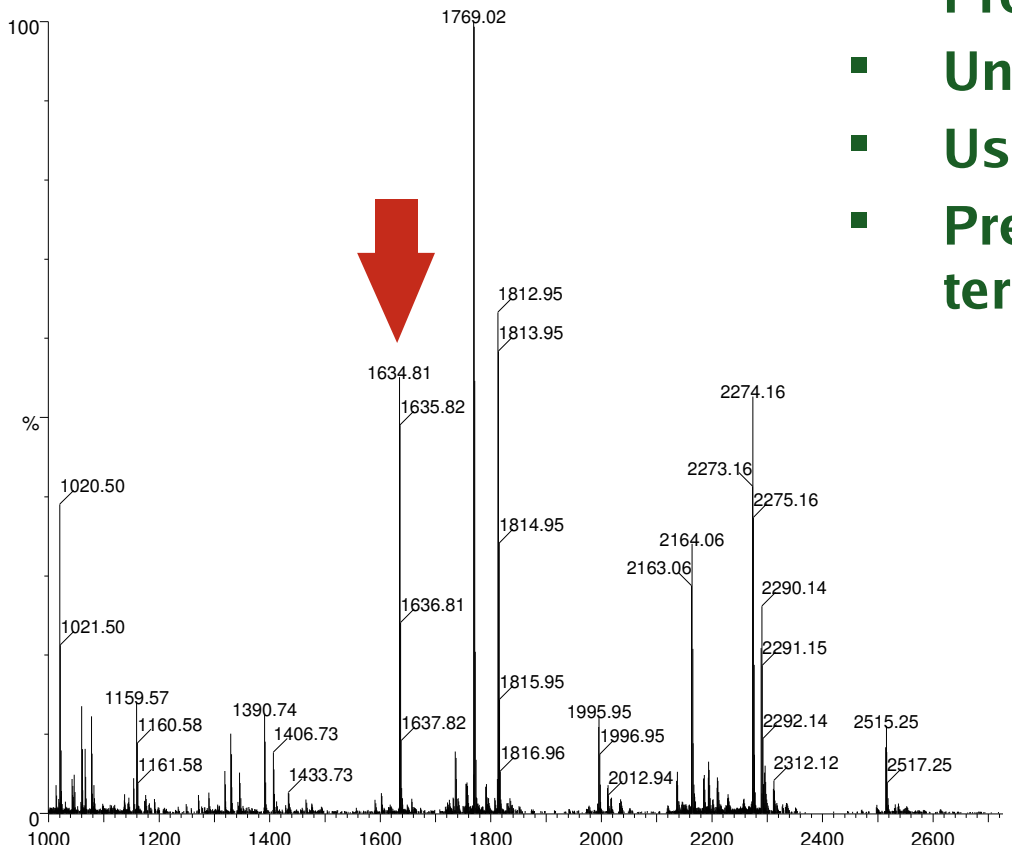
DNA-Array experiments

Genetic linkage analysis

SNP analysis(Small nucleic acid polymorphisms)

etc.

The method 2 - selection of Q-peptides (1)



- One per analyte protein
- Propensity to ionise
- Uniqueness in set
- Useable mass
- Preferably arg-terminated

The method 2 - selection of Q-peptides (2)

- **Filter the list of peptides from the discovery data**
  - Acceptable Peptides:
    - Peptides should be 6-20 amino acids (preferably 8-15)
    - Eliminate peptides containing chemically reactive residues (C, M, W)
    - Eliminate chemically unstable residues (NG, DG, QG, N-term N, N-term Q)
    - Eliminate peptides next to "ragged ends" (sequential predicted trypsin cleavage site, i.e. AGHD**RK**GLILFSDR)
    - Eliminate "LC-incompatible" peptides (high or low predicted retention times- i.e., 8-30 minutes, preferably 10-20 minutes)
    - Eliminate peptides containing an arginine adjacent to proline (potential missed cleavage)
    - Eliminate known sites of post-translational modifications (Human Protein Reference Database, www.hrpd.org)
    - The group of peptides that meet these criteria should be BLASTed to ensure that they are unique to the target protein

Introduced Mass Shifts By Heavy Amino Acid(s)

Stable Isotope Labeled Amino Acid Options

Stable Isotope Labeled Amino Acid (Fully Labeled, ^{13}C, ^{15}N > 98 atom%)	Mass Difference between Native Peptide and AQUA Peptide
L-Arginine-$^{13}C_6$,$^{15}N_4$	10 Daltons
L-Isoleucine-$^{13}C_6$,^{15}N	7 Daltons
L-Leucine-$^{13}C_6$,^{15}N	7 Daltons
L-Lysine-$^{13}C_6$,$^{15}N_2$	8 Daltons
L-Phenylalanine-$^{13}C_9$,^{15}N	10 Daltons
L-Proline-$^{13}C_5$,^{15}N	6 Daltons
L-Valine-$^{13}C_5$,^{15}N	6 Daltons

The method 3 - creation of the concatamer

PROTEINS

▼

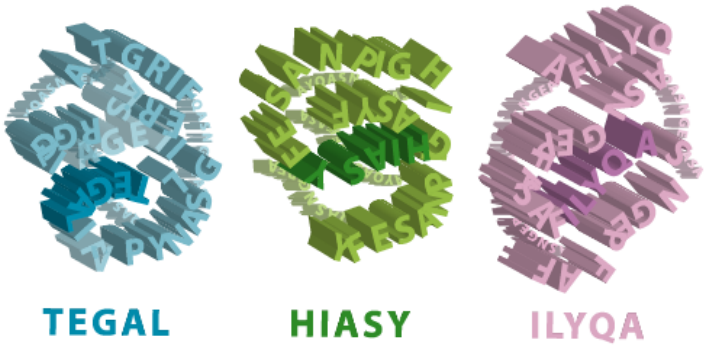
CHOSEN PEPTIDES

▼

ASSEMBLY OF PEPTIDES

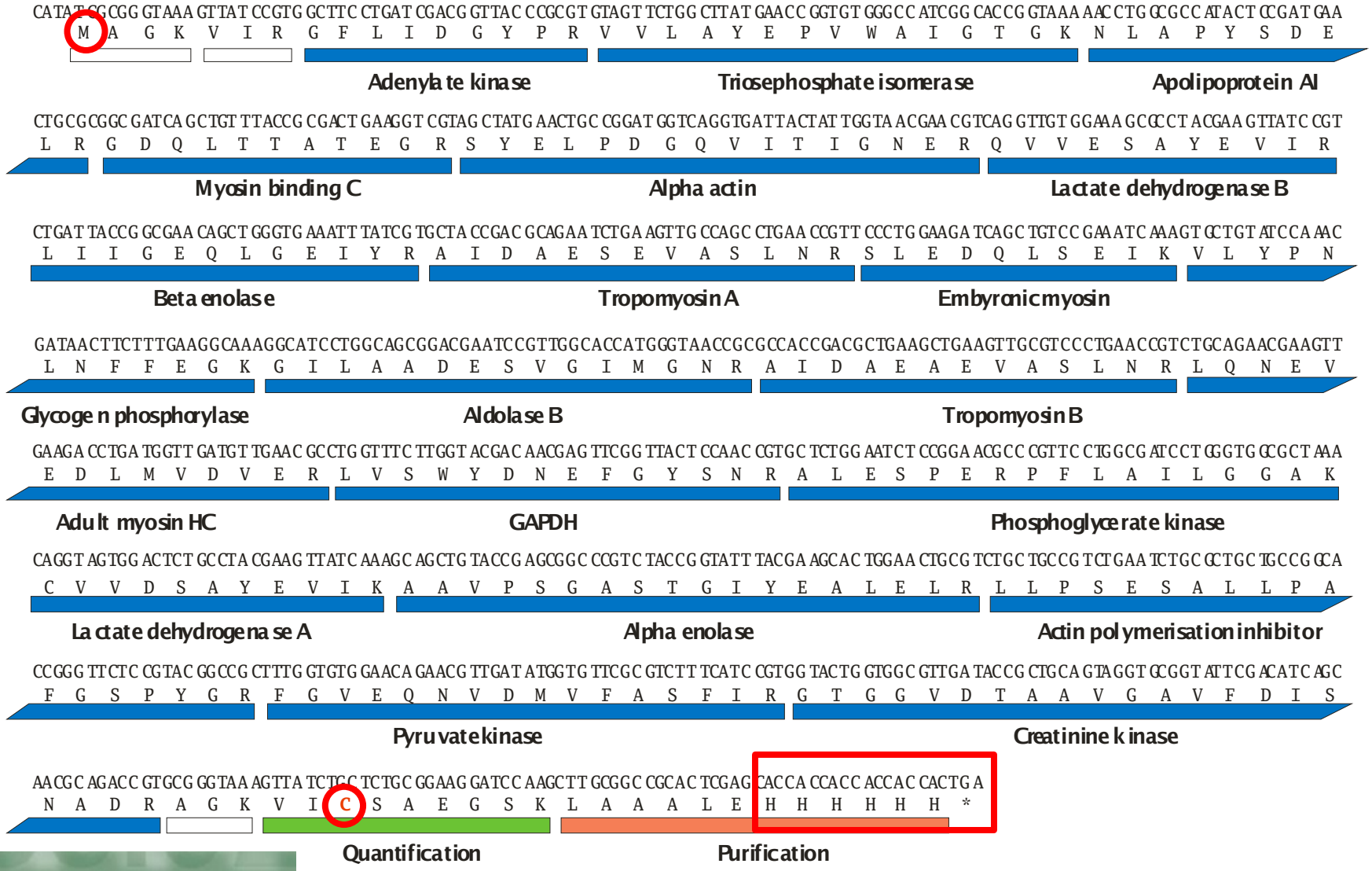
▼

BACKTRANSLATION TO DNA

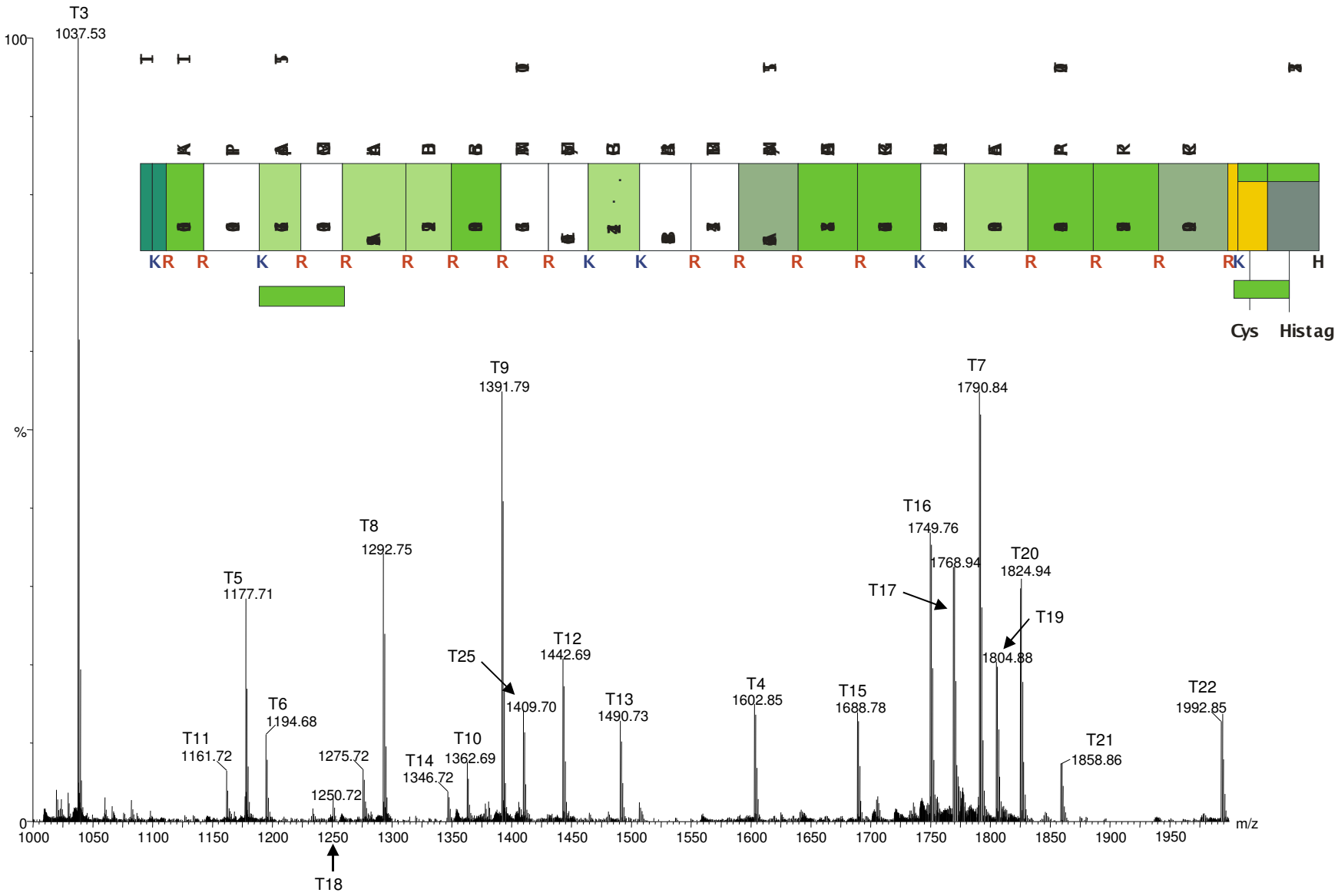


XXXXXXXXXAGAXXXXXXTGT **AGA ACA GAA GGA GCA CTT AGA CAT ATT**
GCG AGT TAC AGA ATA TTG TAT CAA GCT **AGA** XXXXXXXXXXXXXXXXXXXX

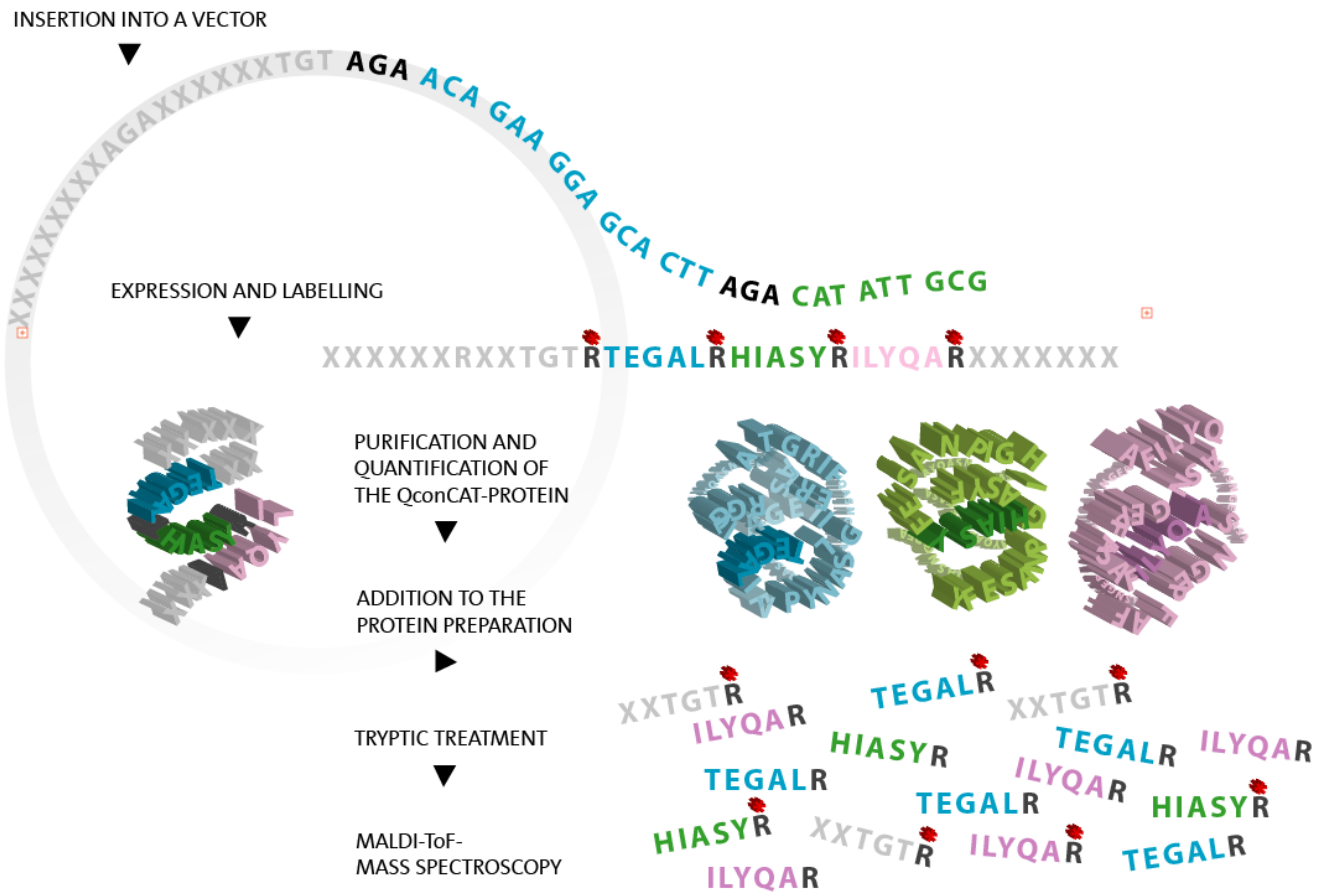
The method 4 - typical QconCAT gene



MALDI-ToF of unlabelled QconCAT

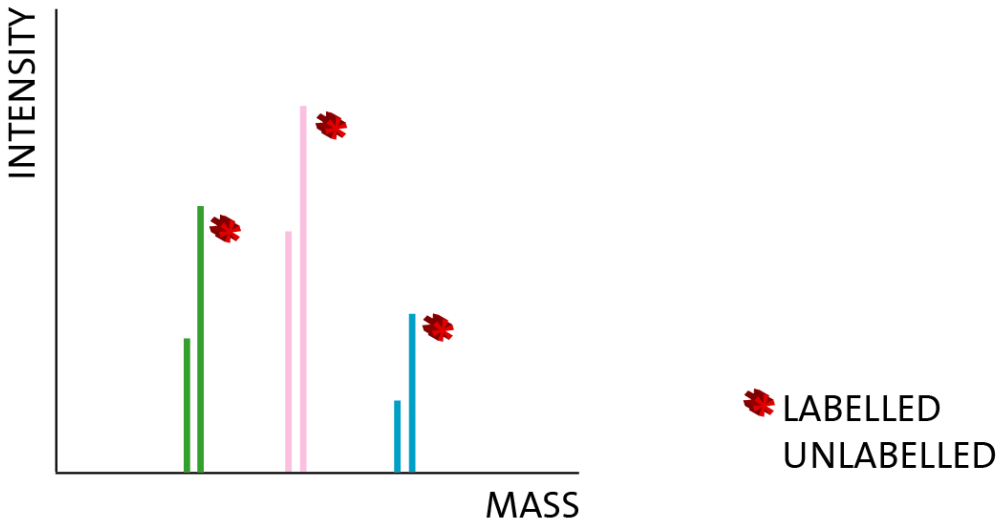


The method 5 - expression, labelling, digest

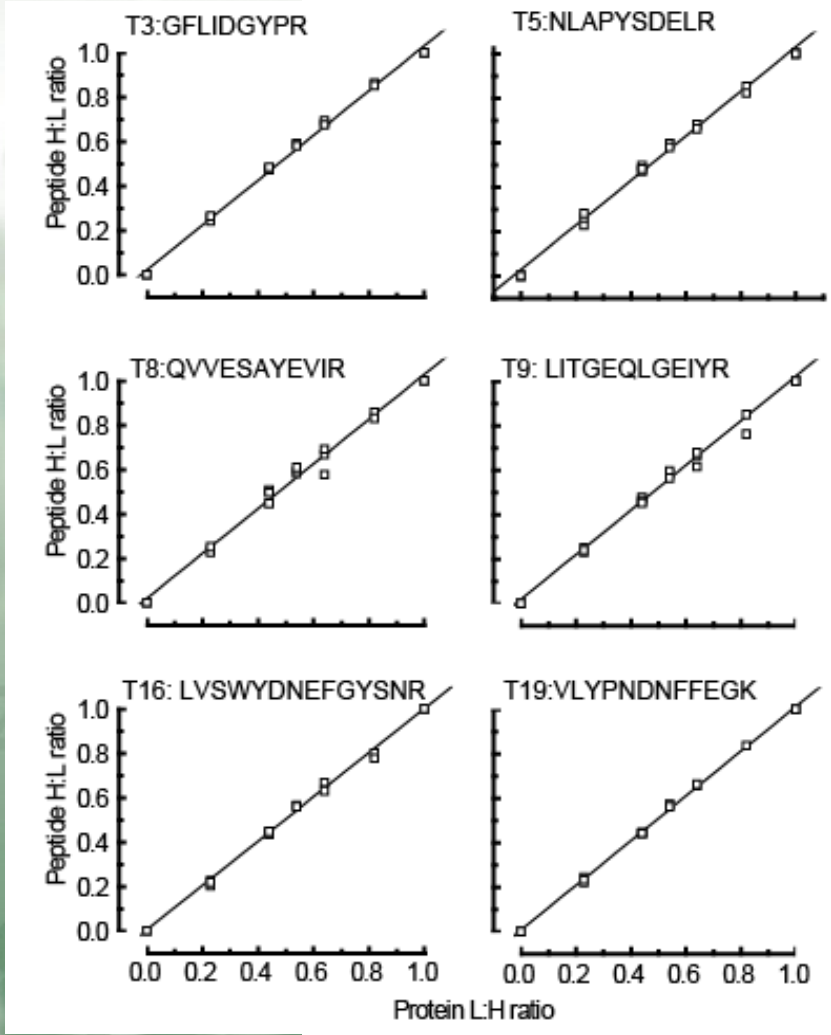


The method 6 - analysis

Mass spectrometry



The method 7 – proof of principle



- 15N-labelled and unlabelled QconCAT proteins
- Mixed in different ratios
- Proteolysed before MALDI-ToF

Advantages

- Chemically inaccessible peptides can be produced biosynthetically
- Absolute quantification at reasonable costs
- High fault tolerance, e.g. against preparation artifacts
- Low costs for isotope labelling
- Re-usability of the concatamer gene
- 'Themed' peptide sets can be provided off the shelf as quantification kits
- Realistic approach to quantify whole proteomes

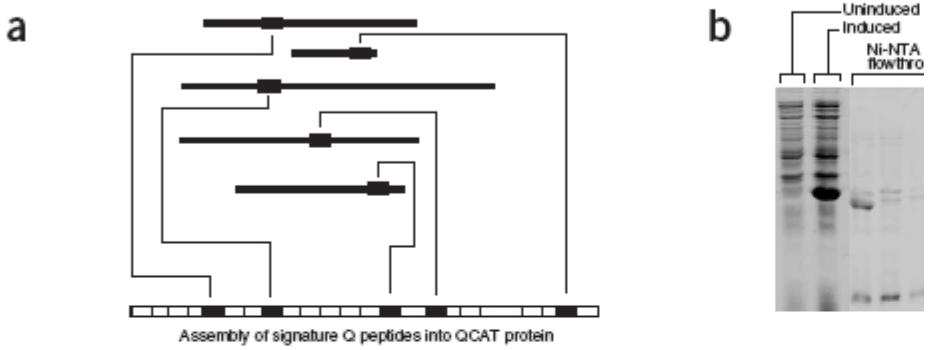
Applications

- Highly accurate quantification of large sets of analyte proteins
- Quantitative analysis of proteomes
- Comparison of results between labs
- High-precision modelling in systems biology
- Quantification of phosphorylation states and other covalent modifications (in future)
- Diagnosis
- Food quality control, environmental research

Multiplexed absolute quantification in proteomics using artificial QCAT proteins of concatenated signature peptides

Robert J Beynon[1], Mary K Doherty[1], Julie M Pratt[1] & Simon J Gaskell[2]

Absolute quantification in proteomics usually involves simultaneous determination of representative proteolytic peptides and stable isotope–labeled analogs. The principal



Inventors

- Prof. Rob Beynon, University of Liverpool
- Prof. Simon Gaskell, University of Manchester
- Dr. Julie Pratt, independent consultant

Patent application

- Entelechon GmbH

Multiplexed absolute quantification for proteomics using concatenated signature peptides encoded by QconCAT genes

Julie M Pratt[1], Deborah M Simpson[1], Mary K Doherty[1], Jenny Rivers[1], Simon J Gaskell[2] & Robert J Beynon[1]

[1]Department of Veterinary Preclinical Sciences, University of Liverpool, Crown Street, Liverpool, L69 7ZJ, UK. [2]Michael Barber Centre for Mass Spectrometry, School of Chemistry, University of Manchester, Manchester M13 9PL, UK. Correspondence should be addressed to R.B. (r.beynon@liv.ac.uk), website URL http://www.liv.ac.uk/pfg.

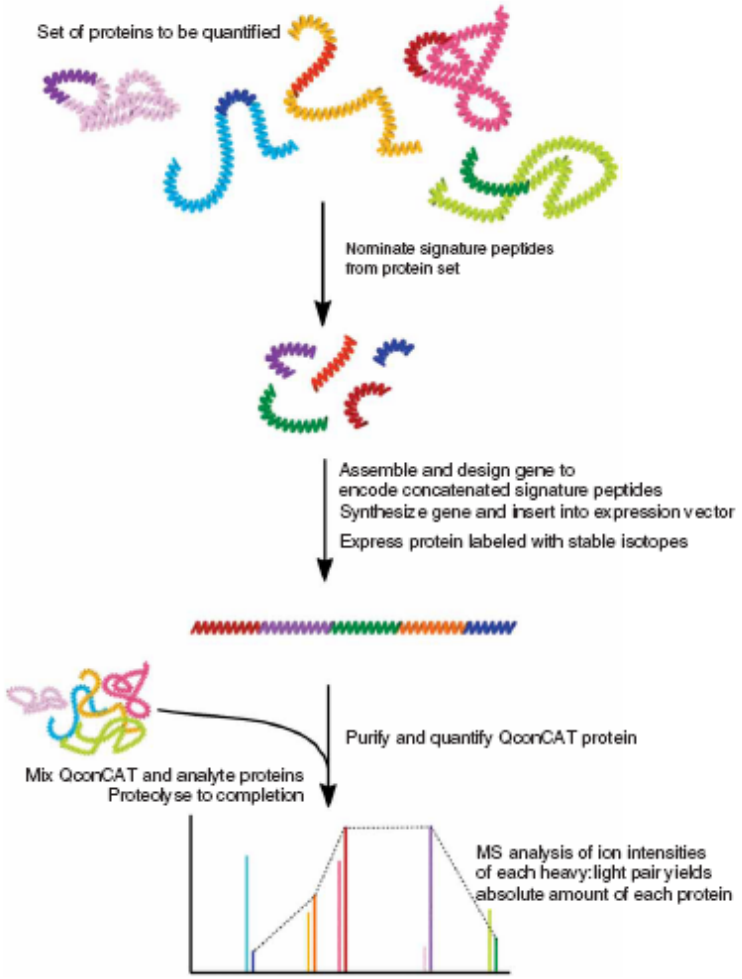


Figure 1 | General principle of a QconCAT quantification experiment.

More information

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Cooperations 2007

Rob Beynon, Julie Pratt, Liverpool

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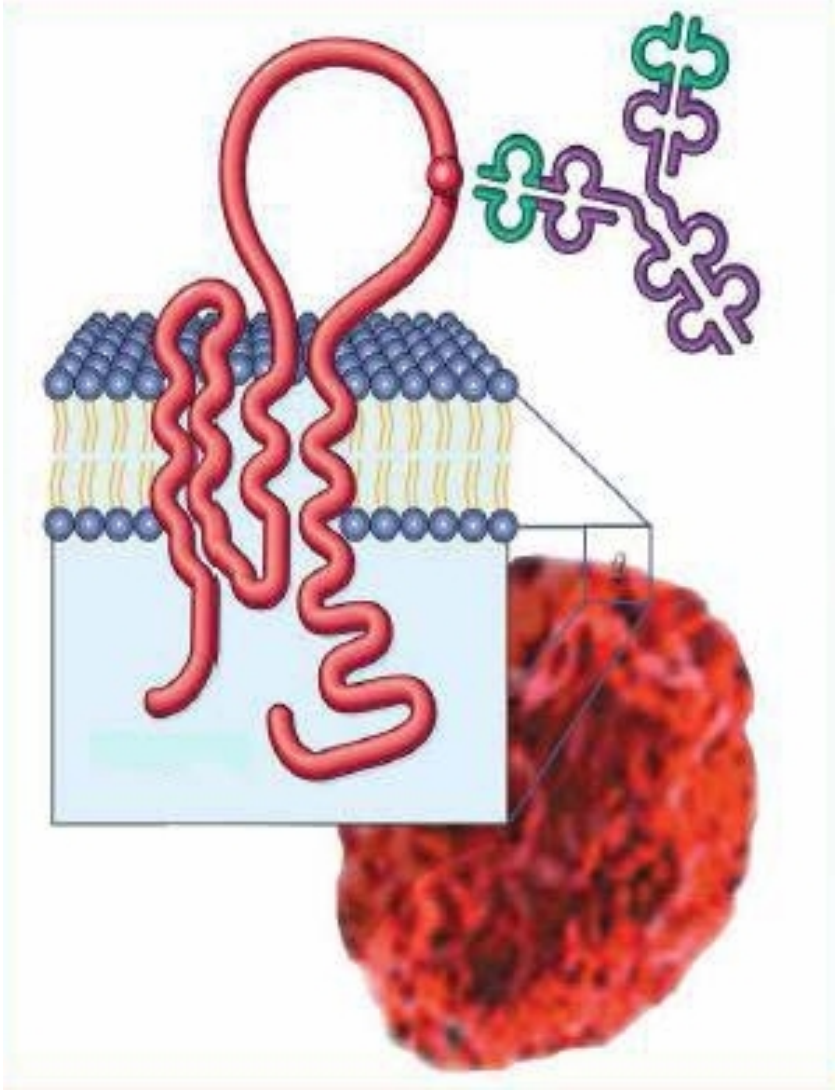
Saleh Ibrahim, Martin Eggert, Gerhard Neeck, Rostock

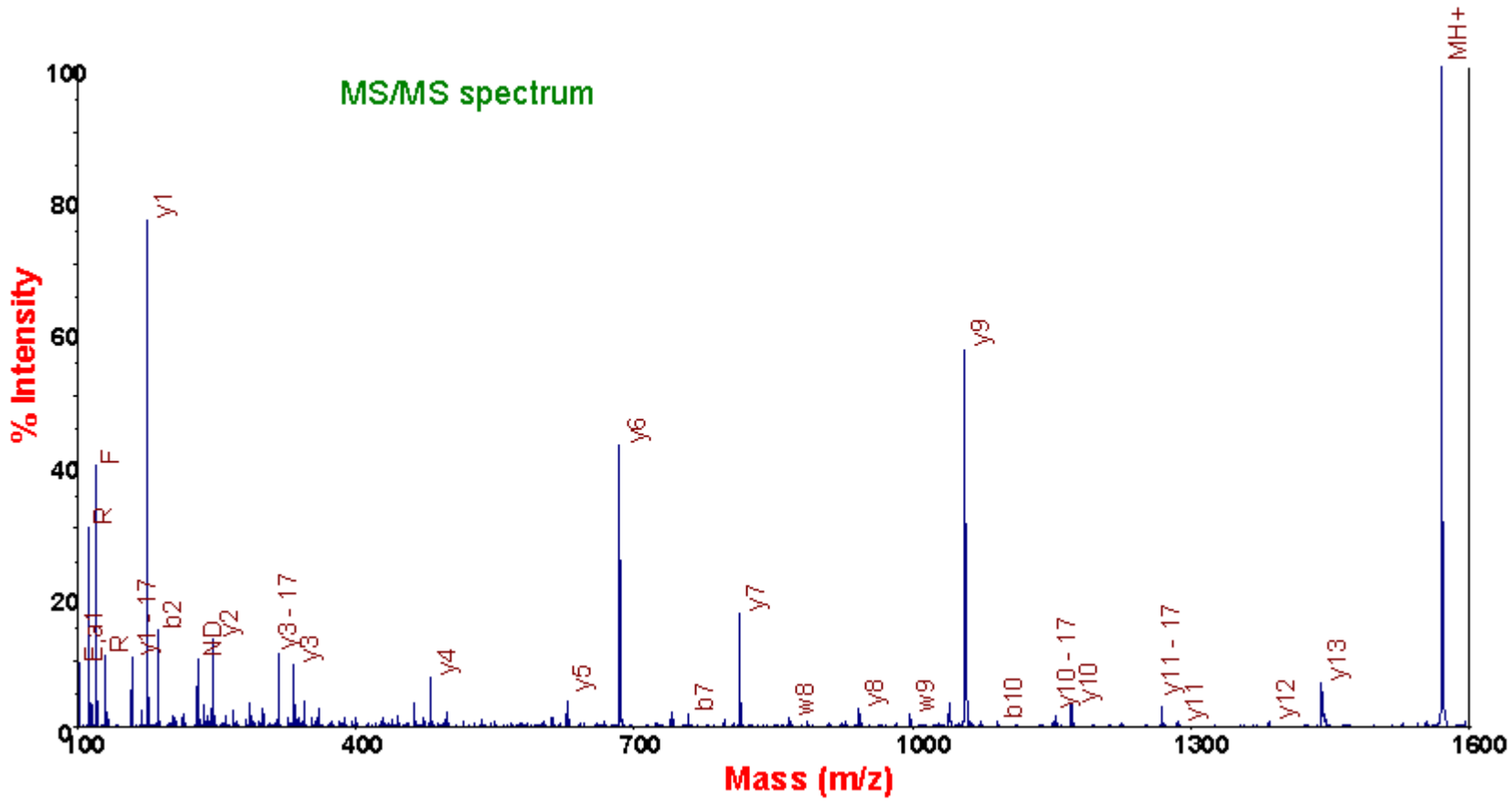
Peter Öfner, Regensburg

Panatecs, Tübingen

OMX, München

Proteome Factory, Berlin




MS/MS spectrum
% Intensity
Mass (m/z)
100
80
60
40
20
0
100
400
700
1000
1300
1600
MH+
y1
y9
y6
y7
y4
y5
y3
y3 - 17
y2
y1 - 17
b2
b7
w8
y8
w9
b10
y10 - 17
y10
y11 - 17
y11
y12
y13
R
F
E,a1
R

A new Method for the multiplexed absolute Quantification of Proteins

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Protein quantification - existing approaches

- Switch from gel-based methods to isotope-labeled derivatisation methods
- DIGE (2D differential gel electrophoresis)
- ICAT - derivatisation of cysteine residues
- iTRAQ - derivatisation of N-terminus
- Comparable accuracy
- Specific drawbacks:
  - DIGE: Complex procedure, different migration rates for isoforms
  - ICAT: Requirement for cys residues
  - iTRAQ: Multiplexing limited to four independent samples

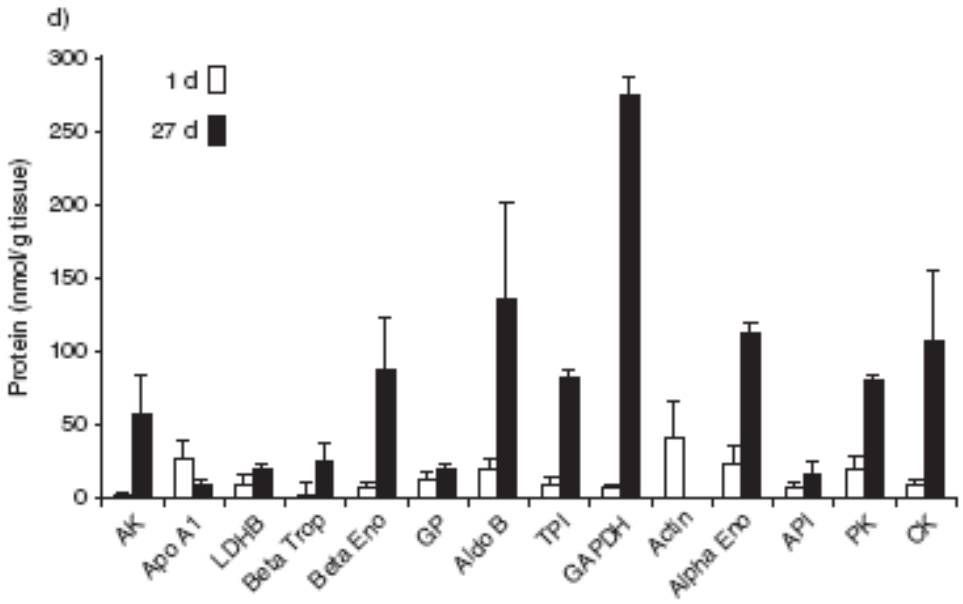


Figure 9 | Quantification of biological samples by QconCAT. A preparation of soluble proteins from 100 mg of chicken skeletal muscle at 1 d and 27 d was mixed with 290 μg ^{15}N-labeled QconCAT, digested with trypsin overnight and analyzed by MALDI-ToF. The intensity of the monoisotopic peak for the analyte peptide and the corresponding QconCAT peptide were recorded and the data converted into nanomoles of protein per gram of tissue, to give the absolute amount of each protein. Error bars, ± s.e.m.; $n = 3$. For details of the individual proteins, see reference 6. (Adapted from reference 6.)

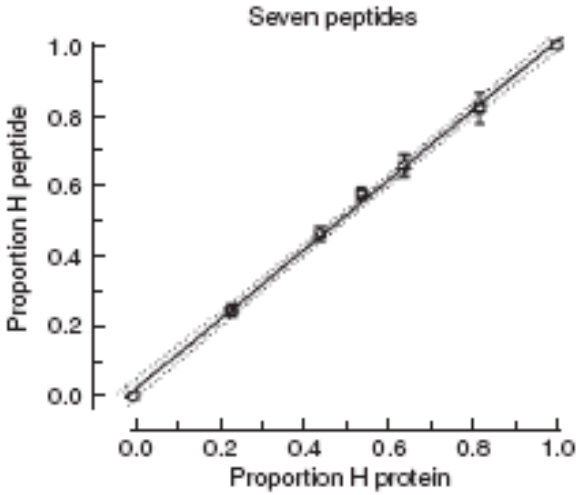


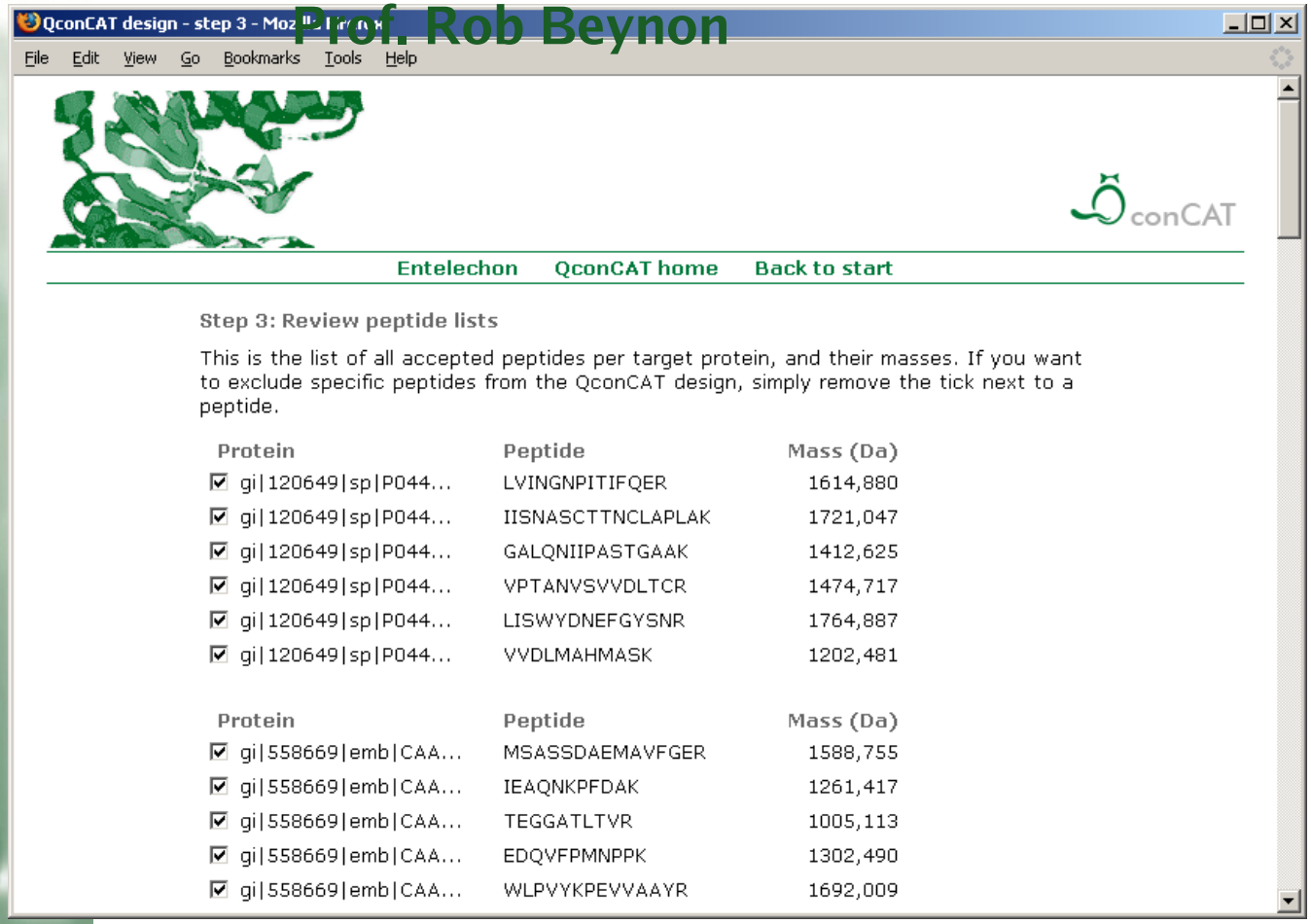
Figure 8 | Quantification by QconCATs. Unlabeled (L, light) and uniformly labeled with ^{15}N (H, heavy) QconCAT proteins were separately purified, quantified and mixed in different ratios, before tryptic digestion and measurement of peptide intensities by MALDI-ToF MS. The measured proportion of H peptide is plotted relative to the proportion of H protein in the mixture for three replicates of each of seven peptides (right); error bars ± s.e.m., $n = 7$. The dotted lines define the 95% confidence limits of the fitted line. (Adapted from reference 6.)

Quantitative Mass Spectrometry

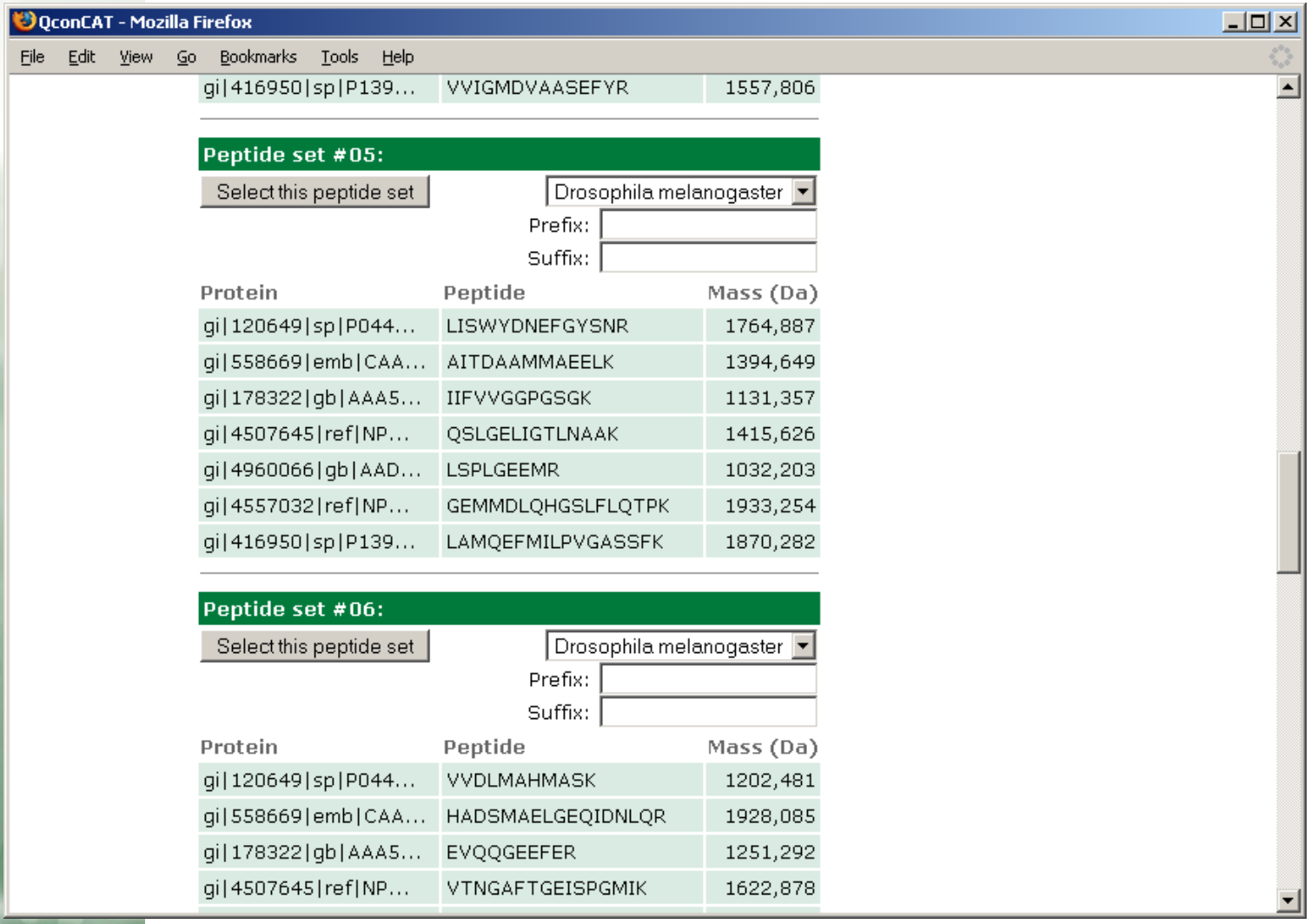
- **Due to Non-uniform Instrument Response, Mass Spectrometric Quantitation Must Be Approached Very Carefully:**
  - Only chemically identical peptides may be quantitatively compared.
  - Stable isotopes are introduced into one sample, then:
    - The same protein(s)/peptide(s) in the control and test samples are mass spectrometrically compared, i.e., one “native” and one labeled with stable isotopes.

Web application for QconCAT design

Based on the QconCAT design helper tool by Prof. Rob Beynon



Web application for QconCAT design



Frequently asked questions

- Efficiency of the tryptic digest
  - How efficient is the digest?
  - What if target proteins and QconCAT are digested at different rates?
- Quantification of the concatamer
  - How accurate is it?
- Isotope-labelling
  - Available options
  - Accuracy
- Turnaround time
- Separation of peptides

The method 4 - typical QconCAT gene