Newsletter

3-2023

About this edition:

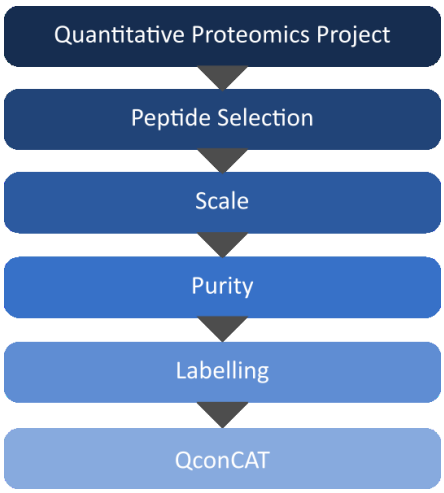
In this edition of our quarterly Newsletter, we discuss labelling methods for QconCATs (Part 2 of our series on QconCAT design) and present a whitepaper where we compare sample preparation methods and their influence on data quality.
We also highlight a current publication involving one of our custom-made QconCAT reference standards.

You can meet and talk to us at the Symposium on "New concepts in Prokaryotic Virus-host Interactions" in Berlin (October 2nd-4th) and at the Bio Europe in Munich (November 6th-8th).

How to design the most appropriate QconCAT for your research project

Part 2: Labelling

In our previous newsletter, we explained strategies for selecting suitable peptides to obtain the optimal QconCAT for your research project.
To be able to distinguish the reference peptides from endogenous peptides, the QconCAT needs to be labelled. QconCATs can be labelled after production (tandem mass tag, TMT) or during production (stable isotope labelling by/with amino acids in cell culture, SILAC).
SILAC is the most widespread method for producing heavy isotope labelled proteins. As proteins released by tryptic digest terminate with Arginine or Lysine, heavy isotope-labelled Arginine and Lysine are used. The resulting QconCAT releases peptides with a mass shift of 6 Da when using ^{13}C Arg and ^{13}C Lys. Alternatively, using ^{13}C,^{15}N Arg and ^{13}C,^{15}N Lys results in mass shifts of 10 Da and 8 Da, respectively. Although these mass shifts can be easily measured, the labelling degree achieved with SILAC is normally around 96-98%. The remaining unlabelled portion cannot be distinguished from endogeneous protein and may those pose a problem for the quantification of low expressing proteins. A higher labelling rate can be achieved by increasing the excess of the labelled precursors, but increases the production costs of the QconCAT.
A more cost efficient method for producing heavy isotope labelled QconCATs is ^{15}N labelling, routinely enabling a labelling degree of 99.9%-100%. These ultrapure QconCATs show no signal in the light trace and can thus be used even for very low abundant targets.



Unsure which method to use for your quantitative proteomics project? We will be happy to consult you finding the most suitable labelling for your QconCAT!

Publication Alert:

Quantification of drug metabolising enzymes and transporter proteins in the paediatric duodenum via LC-MS/MS proteomics using a QconCAT technique

Goelen J, Farrell G, McGeehan J, Titman CM, J W Rattray N, Johnson TN, Horniblow RD, Batchelor HK
Eur J Pharm Biopharm. 2023 Aug 23:S0939-6411(23)00214-X

Goelen et al. developed a simplified method for studying intestinal proteins using pinch biopsies from the paediatric duodenum. Due to changes in small intestinal physiology with age, pharmacokinetic (PK) profiles observed in adults may not always directly extrapolate to pediatric populations and there is currently not enough data for physiologically-based pharmacokinetic (PBPK) modelling in pediatric populations.
In this study, Goelen et al. employed mass spectrometry to study intestinal proteins from gut and used a QconCAT reference standard to simultaneously quantify 21 proteins of the three key intestinal Drug Metabolising Enzymes and Transporter (DMET) protein families (transporters, CYP and UGT-enzymes). Their research demonstrated the feasibility of this novel method and provides the basis for future research to develop appropriate predictive models.

Personalised modelling of clinical heterogeneity between medium-chain acyl-CoA dehydrogenase patients

Odendaal C, Jager EA, Martines AMF, Vieira-Lara MA, Huijkman NCA, Kiyuna LA, Gerding A, Wolters JC, Heiner-Fokkema R, van Eunen K, Derks TGJ, Bakker BM
BMC Biol. 2023 Sep 4;21(1):184.

Patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD) show a wide phenotypic heterogeneity even when carrying the same genetic variant and within families. Some develop, if untreated, severe metabolic decompensations while others remain asymptomatic for their whole life.
In this study, Odendaal et al built and validated, a kinetic model of the human liver mitochondrial fatty acid oxidation (mFAO). As proteome remodeling can adapt for enzyme deficiencies, they performed targeted proteomics in HepG2 MCAD knockout cell lines and in patient fibroblasts. Using QconCATs from PolyQuant as reference standard to determine the absolute quantities of 9 key proteins, they were able to observe a clear distinct pattern of the SCAD protein in the asymptomatic patient. Their data underlines that kinetic models are powerful tools, complementing models based on genomic data.

Meet us:

Bio Europe

We are looking forward to attending Europe's largest gathering of biopharma professionals. The Bio Europe 2023 partnering event will take place in Munich from November 6th-8th 2023.
You can find us in Hall 6B, Booth 70.

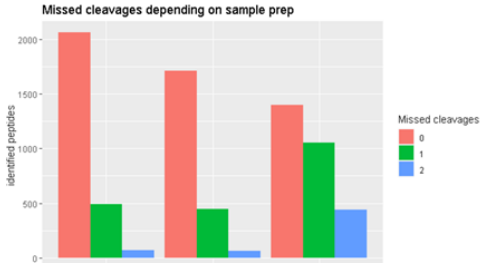
Symposium: New Concepts in Prokaryotic Virus-host Interactions:

The 2023 Symposium on **"New Concepts in Prokaryotic Virus-host Interactions"** will be held from October 2nd-4th in Berlin. The Symposium will connect researchers from all over the world who are part of the DFG-funded priority programme 2330 "New concepts in prokaryotic virus-host interactions – from single cells to microbial communities", an international research consortium studying fundamentally new concepts and mechanisms in phage biology.
We are looking forward to lots of interesting presentations and fruitful discussions and are especially proud to be sponsoring the award for the best poster.

Whitepaper:

The impact of sample preparation on data quality in plasma proteomics

Generation of reliable data is highly important for medical research, especially molecular diagnostics. As sample preparation can significantly impact reproducibility we tested three methods for processing of plasma samples with respect to reproducibility of quality values like overall signal intensity, number of identified peptides/proteins, missed cleavages etc. We summarized our findings in this Whitepaper.



PolyQuant GmbH, founded in 2007, provides DIN ISO 9001:2015 certified products, services and bioinformatics support focusing on mass spectrometry-based absolute protein quantification using our proprietary QconCAT technology. QconCATs by PolyQuant have been successfully applied to numerous projects in academia and industry worldwide and have been used for projects such as biomarker identification and validation, quality control and life science research.

Subscribe here to continue receiving our Newsletter
If you do not wish to receive our Newsletter please send „unsubscribe" to marketing@polyquant.com

Polyquant GmbH

Industriestr. 1
93077 Bad Abbach
Germany
Tel. +49 (0) 9405 96999 10
Fax. +49 (0) 9405 96999 28
www.polyquant.com

Commercial register of the local courts: Amtsgericht Regensburg, HRB 10564
Managing director: Dr. Florian Christoph Sigloch, Dr. Werner Deininger

---

PolyQuant GmbH – your service provider in targeted proteomics