PROTEIN DIGESTION

Nota: En rojo parámetros específicos para cada trabajo. El usuario contactará con el servicio para "personalizarlo".

In solution digestion:

After denaturation of protein with 8 M urea in 50 mM ammonium bicarbonate pH 8.8, the sample was reduced and alkylated: disulfide bonds from cysteinyl residues were reduced with 10 mM DTT for 1 h at 37 ºC, and then thiol groups were alkylated with 10 mM iodoacetamide for 30 min at room temperature in darkness. The sample was diluted to reduce urea concentration below 1.4 M and digested using sequencing grade trypsin (Promega, Madison, WI) or chymotrypsin (Roche, Mannheim, Germany) or Glu-C (Thermo Scientific, Waltham, MA) overnight at 37º C (trypsin and Glu-C) or 25ºC (chymotrypsin) using a 1:20 (w/w) enzyme:protein ratio. Digestion was stopped by the addition of 1% TFA. Whole supernatants were dried down and then desalted onto ZipTip C18 Pipette tips (Millipore) or OMIX Pipette tips C18 (Agilent Technologies) or OASIS C18 columns (Waters) until the mass spectrometric analysis.

Torres, L.L., Cantero, A., Del Valle, M., Marina, A., Gallego, F.L., Guisán, J.M., Berenguer, J., Hidalgo, A. "Engineering the Sustrate Specificity of a Thermophilic Penicillin Acylase from Thermus thermophilus". *Applied and Environmental Microbiology* 2012, 79(5):1555-1562.

In-Gel Digestion (Bands or Spots):

After drying, gel bands or spots were destained in acetonitrile:water (ACN:H_2O, 1:1), were reduced and alkylated (disulfide bonds from cysteinyl residues were reduced with 10 mM DTT for 1 h at 56 ºC, and then thiol groups were alkylated with 10 mM iodoacetamide for 30 min at room temperature in darkness) and digested in situ with sequencing grade trypsin (Promega, Madison, WI) or chymotrypsin (Roche, Mannheim, Germany) or Glu-C (Thermo Scientific, Waltham, MA) as described by Shevchenko et al. [1] with minor modifications [2]. The gel pieces were shrunk by removing all liquid using sufficient ACN. Acetonitrile was pipetted out and the gel pieces were dried in a speedvac. The dried gel pieces were re-swollen in 100 mM Tris-HCl pH 8, 10mM $CaCl_2$ with 12.5 ng/µl trypsin or chymotrypsin or Glu-C for 1 hr in an ice-bath. The digestion buffer was removed and gels were covered again with 100 mM Tris-HCl pH 8, 10mM $CaCl_2$ and incubated at 37°C (trypsin and Glu-C) or 25ºC (chymotrypsin) for 12 hr. Digestion was stopped by the addition of 1% TFA. Whole supernatants were dried down and then desalted onto ZipTip C18 Pipette tips (Millipore) until the mass spectrometric analysis.

1. Shevchenko A, Wilm M, Vorm O, Mann M: Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem 1996, 68:850-858.

2. Pérez, M., García-Limones, C., Zapico, I., Marina, A., Lienhard Schmitz, M., Muñoz, E., Calzado, Marco. A. "Mutual regulation between SIAH2 and DYRK2 controls hypoxic and genotoxic signaling pathways", *Journal of Molecular Cell Biology 2012*, 4:316-330.

In-Gel Digestion (Stacking gel):

The protein extracts, were suspended in a volume up to 50 µl of sample buffer, and then applied onto 1.2-cm wide wells of a conventional SDS-PAGE gel (0.75 mm-thick, 4% stacking, and 10% resolving). Then run was stopped as soon as the front entered 3 mm into the resolving gel, so that the whole proteome became concentrated in the stacking/resolving gel interface. The unseparated protein bands were visualized by Coomassie staining, excised, cut into cubes (2 x 2 mm), and placed in 0.5 ml microcentrifuge tubes [1]. The gel pieces were destained in acetonitrile:water (ACN:H_2O, 1:1), were reduced and alkylated (disulfide bonds from cysteinyl residues were reduced with 10 mM DTT for 1 h at 56 ºC, and then thiol groups were alkylated with 10 mM iodoacetamide for 30 min at room temperature in darkness) and digested in situ with sequencing grade trypsin (Promega, Madison, WI) or chymotrypsin (Roche, Mannheim, Germany) or Glu-C (Thermo Scientific, Waltham, MA) as described by Shevchenko et al. [2] with minor modifications. The gel pieces were shrunk by removing all liquid using sufficient ACN. Acetonitrile was pipetted out and the gel pieces were dried in a speedvac. The dried gel pieces were re-swollen in 100 mM Tris-HCl pH 8, 10mM $CaCl_2$ with 60 ng/µl trypsin or chymotrypsin or Glu-C at 5:1 protein:enzyme (w/w) ratio. The tubes were kept in ice for 2 h and incubated at 37°C (trypsin and Glu-C) or 25ºC (chymotrypsin) for 12 h. Digestion was stopped by the addition of 1% TFA. Whole supernatants were dried down and then desalted onto ZipTip C18 Pipette tips (Millipore) or OMIX Pipette tips C18 (Agilent Technologies) or OASIS C18 columns (Waters) until the mass spectrometric analysis.

1. Moreno, M.L., Escobar, J., Izquierdo-Álvarez, A., Gil, A., Pérez, S., Pereda, J., Zapico, I., Vento, M., Sabater, L., Marina, A., Martínez-Ruiz, A., Sastre, J. "Disulfide stress: a novel type of oxidative stress in acute inflammation". *Free Radical Biology and Medicine*. 2014, 70:265-277.

2. Shevchenko A, Wilm M, Vorm O, Mann M: Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem 1996, 68:850-858.

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis

Peptide mass fingerprinting was conducted as previously described [1] using an Autoflex™(Bruker Daltonics, Bremen, Germany) mass spectrometer in a positive ion reflector mode employing 2,5-dihydroxybenzoic acid as matrix and an AnchorChip™surface target (Bruker Daltonics). Peak identification and monoisotopic peptide mass assignation were performed automatically using Flexanalysis™software, version 2.2 (Bruker Daltonics). Database searches were performed using MASCOT http://matrixscience.com [2] against the NCBI non-redundant protein sequence database http://www.ncbi.nih.gov. The selected search parameters were as follows: tolerance of two missed cleavages, carbamidomethylation (Cys) and oxidation (Met) as fixed and variable modifications, respectively, and setting peptide tolerance to 100 ppm after close-external calibration. A significant MASCOT probability score ($p < 0.05$) was considered as condition for successful protein identification.

1. Villar, M., Torina, A., Nuñez, Y., Zivkovic, Z., Marina, A., Alongi, A., Scimeca, S., La Barbera, G., Caracappa, S., Vázquez, J., De la Fuente, J. "Application of highly sensitive saturation labeling to the analysis of differential protein expression in infected ticks from limited samples" *Proteome Science* 2010, 8:43.

2. Perkins DN, Pappin DJ, Creasy DM, Cottrell JS: Probability-based proteinidentification by searching sequence databases using mass spectrometry data. Electrophoresis 1999, 20:3551-3367.

Reverse phase-liquid chromatography RP-LC-MS/MS analysis (Dynamic Exclusion Mode+ SMIM Mode)

The desalted protein digest was dried, resuspended in 10 µl of 0.1% formic acid and analyzed by RP-LC-MS/MS in an Easy-nLC II system coupled to an ion trap LTQ-Orbitrap-Velos-Pro hybrid mass spectrometer (Thermo Scientific). The peptides were concentrated (on-line) by reverse phase chromatography using a 0.1mm × 20 mm C18 RP precolumn (Thermo Scientific), and then separated using a 0.075mm x 250 mm C18 RP column (Thermo Scientific) operating at 0.3 µl/min. Peptides were eluted using a 180-min dual gradient. The gradient profile was set as follows: 5−25% solvent B for 135 min, 25−40% solvent B for 45min, 40−100% solvent B for 2min and 100% solvent B for 18 min (Solvent A: 0,1% formic acid in water, solvent B: 0,1% formic acid, 80% acetonitrile in water). ESI ionization was done using a Nano-bore emitters Stainless Steel ID 30 µm (Proxeon) interface at 2.1 kV spray voltage with S-Lens of 60%. The Orbitrap resolution was set at 30.000. [1]

Peptides were detected in survey scans from 400 to 1600 amu (1 µscan), followed by twenty data dependent MS/MS scans (Top 20), using an isolation width of 2 u (in mass-to-charge ratio units), normalized collision energy of 35%, and dynamic exclusion applied during 60 seconds periods. Charge-state screening was enabled to reject unassigned and singly charged protonated ions.

If the sequence of any peptide of interest is known, the mass spectrometer was further operated in the selected MS/MS ion monitoring mode (SMIM mode) [2]. In this mode, the LTQ-Orbitrap-Velos-Pro detector was programmed to perform, along the same entire gradient, a continuous sequential operation in the MS/MS mode on the doubly or triply charged ions corresponding to the peptide/s selected previously from the theoretical prediction.

1. Ruth Alonso, Diana Pisa, Ana Isabel Marina, Esperanza Morato, Alberto Rábano, Izaskun Rodal and Luis Carrasco: Evidence for Fungal Infection in Cerebrospinal Fluid and Brain Tissue from Patients with Amyotrophic Lateral Sclerosis. International Journal of Biological Sciences 2015; 11(5): 546-558.

2. I. Jorge, E.M. Casas, M. Villar, I. Ortega-Pérez, D. López-Ferrer, A. Martínez-Ruiz, M. Carrera, A. Marina, P. Martínez, H. Serrano, Benito-Cañas, F. Were, J. M. Gallardo, S. Lamas, J. M. Redondo, D. García-Dorado and J. Vázquez: "High-sensitivity analysis of specific peptides in complex samples by selected MS/MS ion monitoring and linear ion trap mass spectrometry: application to biological studies". *Journal of Mass Spectrometry* 2007, 42(11):1391-403.

Data processing:

Proteome Discoverer 2.2:

Peptide identification from raw data was carried out using the SEQUEST algorithm (Proteome Discoverer 2.2, Thermo Scientific). Database search was performed against uniprot-mus-musculus.fasta (55153 entries; UniProt release 08/2019). The following constraints were used for the searches: tryptic cleavage after Arg and Lys or chymotryptic cleavage after Tyr, Trip, Phe and Leu or Glu-C cleavage after Glu, up to two missed cleavage sites, and tolerances of 20 ppm for precursor ions and 0.6 Da for MS/MS fragment ions and the searches were performed allowing optional Met oxidation and Cys carbamidomethylation. Search against decoy database (integrated decoy approach). Identified peptides were filtered using the Percolator algorithm with a q-value threshold of 0.01 (High Confidence Filter settings, FDR < 1%). Only those proteins with at least two distinct peptides being discovered from LC/MS/MS analyses were considered reliably identified.
The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD002703. (http://www.ebi.ac.uk/pride/archive/projects/PXD002703)

Ruth Alonso, Diana Pisa, Ana Isabel Marina, Esperanza Morato, Alberto Rábano, Izaskun Rodal and Luis Carrasco: Evidence for Fungal Infection in Cerebrospinal Fluid and Brain Tissue from Patients with Amyotrophic Lateral Sclerosis. International Journal of Biological Sciences 2015; 11(5): 546-558.

PEAKS Studio X+:

Peptide identification from raw data was carried out using PEAKS Studio X+ search engine (Bioinformatics Solutions Inc., Waterloo, Ontario, Canada). Database search was performed against uniprot-mus-musculus.fasta (55153 entries; UniProt release 08/2019) (decoy-fusion database). The following constraints were used for the searches: tryptic cleavage after Arg and Lys or chymotryptic cleavage after Tyr, Trip, Phe and Leu or Glu-C cleavage after Glu (semispecific), up to two missed cleavage sites, and tolerances of 20 ppm for precursor ions and 0.6 Da for MS/MS fragment ions and the searches were performed allowing optional Met oxidation and Cys carbamidomethylation. False discovery rates (FDR) for peptide spectrum matches (PSM) was limited to 0.01. Only those proteins with at least two distinct peptides and at least one unique peptides being discovered from LC/MS/MS analyses were considered reliably identified.
The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD002703. (http://www.ebi.ac.uk/pride/archive/projects/PXD002703)

Tran NH, Qiao R, Xin L, Chen X, Liu C, Zhang X, Shan B, Ghodsi A, Li M. Deep learning enables de novo peptide sequencing from data-independent-acquisition mass spectrometry. Nature Methods. 16(1), 63-66. 20/12/2018

Tran NH, Zhang X, Xin L, Shan B, Li M. De novo peptide sequencing by deep learning. Proceedings of the National Academy of Sciences of the United States of America. 114(29). 18/7/2017

Tran NH, Rahman MZ, He L, Xin L, Shan B, Li M. Complete De Novo Assembly of Monoclonal Antibody Sequences Scientific Reports. 6(31730). 26/08/2016.

iTRAQ/TMT LABELING AND HIGH PH FRACTIONATION

iTRAQ:

The resultant peptide mixture from desalted proteins tryptic digest (100 µg) was labeled using chemicals from the iTRAQ Reagents 4plex Multiplex Kit (Applied Biosystems, MA, USA). (Reagents 114 and 115 for WT samples and 116 and 117 for KO samples) essentially as described [1]. Briefly, peptides were dissolved in 30 μL of 0.5 M triethylammonium bicarbonate (TEAB), adjusted to pH 8. For labeling, each iTRAQ reagent was dissolved in 70 μL of ethanol and added to the respective peptide mixture and then incubated at room temperature for one hour. Labelling was stopped by the addition of 0.1% formic acid. Whole supernatants were dried down and the four samples were mixed to obtain the "4plex-labeled mixture". The mixture was analysed by RP-LC-MS/MS to check the efficiency of the labelling.

Yichun Zou, Pian Gong, Wenyuan Zhao, Jianjian Zhang, Xiaolin Wu, Can Xin, Zhongwei Xiong, Zhengwei Li, Xiaohui Wu, Qi Wan, Xiang Li, Jincao Chen. Quantitative iTRAQ-based proteomic analysis of piperine protected cerebral ischemia/reperfusion injury in rat brain. Neurochemistry International 124 (2019), 51–61

TMT:

The resultant peptide mixture from desalted proteins tryptic digest (50 µg) was labeled using chemicals from the TMT sixplex Isobaric Mass Tagging Kit (Thermo Fisher Scientific, MA, USA). (Reagents 126, 127 and128 for WT samples and 129, 130 and 121 for KO samples) essentially as described by manufacturer. Briefly, peptides were dissolved in 50 μL of 100 mM triethylammonium bicarbonate (TEAB), adjusted to pH 8. For labeling, each TMT reagent was dissolved in 41 μL of acetonitrile and added to the respective peptide mixture and then incubated at room temperature for one hour. Labelling was stopped by the addition of 8 μL 5% hidroxilamine. Whole supernatants were dried down and the six samples were mixed to obtain the "6plex-labeled mixture". The mixture was analysed by RP-LC-MS/MS to check the efficiency of the labelling.

Xinhua Zhou, Wei Xiao, Zhiyang Su, Jiehong Cheng, Chengyou Zheng, Zaijun Zhang, YuqiangWang, LiangWang, Benhong Xu, Shupen Li, Xifei Yang, and Maggie Pui Man Hoi. Hippocampal proteomic alteration in triple transgenic mouse model of Alzheimer´s disease and implication of PINK 1 regulation in donezepil treatment. Journal of Proteome Research (2019) 18(4):1542-1552.

FRACTIONATION:

The sample was then fractionated using the Pierce High pH Reversed-Phase Peptide Fractionation Kitt (Thermo Fisher Scientific, MA, USA) as described with minor modifications. The sample re-swollen in 0.1%TFA and then, loaded onto an equilibrated, high-pH, reversed-phase fractionation spin column. A step gradient of increasing acetonitrile concentrations (5-80%) in a volatile high-pH (Triethylamine (0.1%)) is then applied to the columns to elute bound peptides into nine different fractions collected by centrifugation. The fractions obtained from high-pH, reversed-phase 6plex/4plex-labeled mixture were dried and stored until analysis by mass spectrometry for quantification.

QUANTITATIVE ANALYSIS BY REVERSE PHASE-LIQUID CHROMATOGRAPHY RP-LC-MS/MS
(Para i-TRAQ o TMT)

The fractions were resuspended in 10 µl of 0.1% formic acid and analysed by RP-LC-MS/MS in an Easy-nLC II system coupled to an ion trap LTQ-Orbitrap-Velos-Pro hybrid mass spectrometer (Thermo Scientific). The peptides were concentrated (on-line) by reverse phase chromatography using a 0.1mm × 20 mm C18 RP precolumn (Proxeon), and then separated using a 0.075mm x 250 mm C18 RP column (Proxeon) operating at 0.3 µl/min. Peptides were eluted using a 90-min dual gradient. The gradient profile was set as follows: 5−25% solvent B for 68 min, 25−40% solvent B for 22min, 40−100% solvent B for 2min and 100% solvent B for 18 min (Solvent A: 0,1% formic acid in water, solvent B: 0,1% formic acid, 80% acetonitrile in water). ESI ionization was done using a Nano-bore emitters Stainless Steel ID 30 µm (Proxeon) interface at 2.1 kV spray voltage with S-Lens of 60%.
The instrument method consisted of a data-dependent top-20 experiment with an Orbitrap MS1 scan at a resolution ($m/\Delta m$) of 30,000 followed by either twenty high energy collision dissociation (HCD) MS/MS mass-analyzed in the Orbitrap at 7,500 ($\Delta m/m$) resolution. MS2 experiments were performed using HCD to generate high resolution and high mass accuracy MS2 spectra.
The minimum MS signal for triggering MS/MS was set to 500. The lock mass option was enabled for both MS and MS/MS mode and the polydimethylcyclosiloxane ions (protonated (Si(CH3)2O))6; m/z 445.120025) were used for internal recalibration of the mass spectra.
Peptides were detected in survey scans from 400 to 1600 amu (1 µscan) using an isolation width of 1.3 u (in mass-to-charge ratio units), normalized collision energy of 40% for HCD fragmentation, and dynamic exclusion applied during 60 seconds periods. Charge-state screening was enabled to reject unassigned and singly charged protonated ions.

Cristina C. Clement, Wei Wang, Monika Dzieciatkowska, Marco Cortese, Kirk C. Hansen, Aniuska Becerra, Sangeetha Thangaswamy, Irina Nizamutdinova, Jee-Young Moon, Lawrence J. Stern , Anatoliy A. Gashev, David Zawieja & Laura Santambrogio. Quantitative Profiling of the Lymph Node Clearance Capacity. Scientific Reports| (2018) 8:11253

QUANTITATIVE DATA ANALYSIS

i-TRAQ / TMT: (el ORBI en modo HCD)

Peptide identification from raw data (a single search was performed with all nine raws from the fractionation) was carried out using PEAKS Studio X+ search engine (Bioinformatics Solutions Inc., Waterloo, Ontario, Canada). Database search was performed against uniprot-mus-musculus.fasta (55153 entries; UniProt release 08/2019) (decoy-fusion database). The following constraints were used for the searches: tryptic cleavage after Arg and Lys (semispecific), up to two missed cleavage sites, and tolerances of 20 ppm for precursor ions and 0.05 Da for MS/MS fragment ions and the

searches were performed allowing optional Met oxidation and Cys carbamidomethylation and fixed iTRAQ 4plex/TMT 6plex reagent labeling at the N-terminus and lysine residues. False discovery rates (FDR) for peptide spectrum matches (PSM) was limited to 0.01. Only those proteins with at least two distinct peptides and at least one unique peptides being discovered from LC/MS/MS analyses were considered reliably identified and sent to be quantified.
Quantitation of iTRAQ/TMT labeled peptides was performed with PEAKS Studio X+ search engine, selected "Reporter Ion Quantification iTRAQ/TMT" under the "Quantifications" options. We use Auto normalization mode that calculate a global ratio from the total intensity of all labels in all quantifiable peptides. The -10LgP, Quality (12) and Reporter Ion Intensity (1e5) were used for Spectrum filter and Significance (20, PEAKSQ or ANOVA method) was used for peptide and protein abundance calculation. For the Protein quantification we consider protein groups for peptide uniqueness, use only unique peptides for protein quantification and the modified peptides were excluded.

Yichun Zou, Pian Gong, Wenyuan Zhao, Jianjian Zhang, Xiaolin Wu, Can Xin, Zhongwei Xiong, Zhengwei Li, Xiaohui Wu, Qi Wan, Xiang Li, Jincao Chen. Quantitative iTRAQ-based proteomic analysis of piperine protected cerebral ischemia/reperfusion injury in rat brain. Neurochemistry International 124 (2019), 51–61 (I-TRAQ)

Xinhua Zhou, Wei Xiao, Zhiyang Su, Jiehong Cheng, Chengyou Zheng, Zaijun Zhang, YuqiangWang, LiangWang, Benhong Xu, Shupen Li, Xifei Yang, and Maggie Pui Man Hoi. Hippocampal proteomic alteration in triple transgenic mouse model of Alzheimer´s disease and implication of PINK 1 regulation in donezepil treatment. Journal of Proteome Research (2019) 18(4):1542-1552. (TMT)

Label-Free: (el ORBI en modo CID)

Peptide identification from raw data (Technical triplicates) was carried out using PEAKS Studio X+ search engine (Bioinformatics Solutions Inc., Waterloo, Ontario, Canada). Database search was performed against uniprot-mus-musculus.fasta (55153 entries; UniProt release 08/2019) (decoy-fusion database). The following constraints were used for the searches: tryptic cleavage after Arg and Lys (semispecific), up to two missed cleavage sites, and tolerances of 20 ppm for precursor ions and 0.6 Da for MS/MS fragment ions and the searches were performed allowing optional Met oxidation and Cys carbamidomethylation. False discovery rates (FDR) for peptide spectrum matches (PSM) was limited to 0.01. Only those proteins with at least two distinct peptides and at least one unique peptides being discovered from LC/MS/MS analyses were considered reliably identified and sent to be quantified.
Quantitation of peptides was performed with PEAKS Studio X+ search engine, selected "Label Free Quantification" under the "Quantifications" options using 20 ppm for mass error tolerance and 2 min for retention time shift tolerance. We use the total ion current (TIC) of the samples to calculate the normalization factors. Normalized abundance is calculated from the raw abundance divided by the normalization factor. The Quality (11) and Avg. Intensity (5e5) were used for Spectrum filter and Significance (20, PEAKSQ or ANOVA method) was used for peptide and protein abundance calculation. For the Protein quantification we consider protein groups for peptide uniqueness, use only unique peptides for protein quantification and the modified peptides were excluded.

Cristina C. Clement, Wei Wang, Monika Dzieciatkowska, Marco Cortese, Kirk C. Hansen, Aniuska Becerra, Sangeetha Thangaswamy, Irina Nizamutdinova, Jee-Young Moon, Lawrence J. Stern , Anatoliy A. Gashev, David Zawieja & Laura Santambrogio. Quantitative Profiling of the Lymph Node Clearance Capacity. Scientific Reports (2018) 8:11253

SILAC: (el ORBI en modo CID)

Peptide identification from raw data was carried out using PEAKS Studio X+ search engine (Bioinformatics Solutions Inc., Waterloo, Ontario, Canada). Database search was performed against uniprot-mus-musculus.fasta (55153 entries; UniProt release 08/2019) (decoy-fusion database). The following constraints were used for the searches: tryptic cleavage after Arg and Lys (semispecific), up to two missed cleavage sites, and tolerances of 20 ppm for precursor ions and 0.6 Da for MS/MS fragment ions and the searches were performed allowing optional Met oxidation, Cys carbamidomethylation and SILAC reagent labelling (Arg 8, Lys 6). False discovery rates (FDR) for peptide spectrum matches (PSM) was limited to 0.01. Only those proteins with at least two distinct peptides and at least one unique peptides being discovered from LC/MS/MS analyses were considered reliably identified and sent to be quantified.
Quantitation of SILAC labeled peptides was performed with PEAKS Studio X+ search engine, selected "Precursor Ion Quantification eg SILAC" under the "Quantifications" options with 1 min for retention time range and R to P Convert enable. We use No normalization mode. The -10LgP, Quality and Avg. Area were used for Spectrum filter and Significance (PEAKSQ or ANOVA method) was used for peptide and protein abundance calculation. For the Protein quantification we consider protein groups for peptide uniqueness, use only unique peptides for protein quantification and the modified peptides were excluded.

Christoph U. Schräder, Shaun Moore, Aaron A. Goodarzi, and David C. Schriemer. Lysine Propionylation To Boost Sequence Coverage and Enable a "Silent SILAC" Strategy for Relative Protein Quantification. Anal. Chem. (2018) 90, 9077−9084