**TRIPTI: Harvest / Lysis / Preparation of beads (mounting with antibody)/ incubation with beads/ Load on columns /wash. Check out Priyankas paper, but double check if it is the same. Do not copy the methods!**

**Methods**

**On column digestion.**

Proteins bound on beads were digested as previously described with some modifications 1,2. Briefly, proteins were incubated using a 30µl of a buffer containing 5ng/µL Trypsin dissolved in 2M urea, 12.5mM Ammoniumbicarbonate and 5mM Tris HCl, pH=8.0. After 30 minutes on room temperature, peptides and proteins were eluted using 100µL of elution buffer containing 2M Urea, 10mM Iodoacetamide and 12.5mM Ammoniumbicarbonate and 5mM Tris, pH=8.0. Peptides were digested over night at room temperature in the dark. Peptides were purified using StageTips as previously described 3.

**LC-MS/MS**

Peptides were analyzed using data-dependent nLC-MS/MS on a QExactive plus tandem mass spectrometer. A 100 min gradient was used as previously described with few modifications 4. Peptides were separated on a 15cm C18 column (Dr. Maisch). During spectrum acquisition process, MS1 spectra were acquired in the range of 300-1750 m/z at a resolution of 70000. The Top 10 peaks were selected for MS/MS fragmentation, with dynamic exclusion option enabled. (20s). MS2 spectra were acquired in the range of 200 to 2000 m/z at a resolution of 35000.

**Data analysis**

Thermo raw files were analyzed using MaxQUant Version 1.4.1.2 5. Briefly, raw files were searched using default settings against the uniprot reference proteome database of February 2014 including common contaminants. Mass accuracy for MS precursor identification was set to 20 and 4.5 ppm for the first and second search, respectively. Protein, peptide and site FDR was set to 0.01 (revert database), and minimal peptide length was 7 amino acids. Fixed modification were carbamidomethylation of cysteins, and variable modifications were N-terminal Acetylation and Methionine oxidation. 5 modifications were allowed. Label-free quantification (LFQ) 6 (fast LFQ) and the “match between runs” option was enabled. 2 peptides were required for a ratio. Protein group output file was then generically uploaded into the Perseus software (version 1.4.11) and analyzed as previously described with some modifications 1. Proteins only identified by site, as reverse or contaminant were removed. LFQ intensities were logarithmized or clustered using correlational analysis. At least 3 valid values were required for undergoing imputation of missing invalid values. Imputation was performed to mimick low-expressed proteins. P-Values were visualized using a volcano plot. FDR corrected (s0=1) cutoff was visualized and significant interactors were exported. For stringent cutoff, a FDR of 0.05 was used (s0=1) and for non-stringent cutoff, a FDR of 0.35 was used (s0=1). GO-terms, KEGG pathways and Corum terms were imported based on uniprot identifiers and tested for significant enrichment against all other detected proteins in the sample using a Fisher’s exact test. A cutoff of 0.1 Bonferroni corrected pvalue was chosen. Stoichiometry of complex was determined using iBAQ values as previously described 1,7 .

1. Kohli, P. *et al.* Label-free quantitative proteomic analysis of the YAP and TAZ interactome. *Am. J. Physiol. Cell Physiol.* (2014). doi:10.1152/ajpcell.00339.2013

2. Hubner, N. C. & Mann, M. Extracting gene function from protein-protein interactions using Quantitative BAC InteraCtomics (QUBIC). *Methods San Diego Calif* **53,** 453–459 (2011).

3. Rappsilber, J., Ishihama, Y. & Mann, M. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. *Anal. Chem.* **75,** 663–670 (2003).

4. Rinschen, M. M. *et al.* Phosphoproteomic Analysis Reveals Regulatory Mechanisms at the Kidney Filtration Barrier. *J. Am. Soc. Nephrol. JASN* (2014). doi:10.1681/ASN.2013070760

5. Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.* **26,** 1367–1372 (2008).

6. Cox, J. *et al.* MaxLFQ allows accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction. *Mol. Cell. Proteomics MCP* (2014). doi:10.1074/mcp.M113.031591

7. Smits, A. H., Jansen, P. W. T. C., Poser, I., Hyman, A. A. & Vermeulen, M. Stoichiometry of chromatin-associated protein complexes revealed by label-free quantitative mass spectrometry-based proteomics. *Nucleic Acids Res.* **41,** e28 (2013).

**RAW Parameters**

Method of Q Exactive Plus

OVERALL METHOD SETTINGS

Global Settings

use lock masses best

Lock mass injection ―

Chrom. peak width (FWHM) 15 s

Time

Method duration 100.00 min

Customized Tolerances (+/-)

Lock Masses ―

Inclusion ―

Exclusion ―

Neutral Loss ―

Mass Tags ―

Dynamic Exclusion ―

Experiment

FULL MS / DD-MS² (TOPN)

General

Runtime 0 to 100 min

Polarity Positive

In-source CID 0.0 eV

Default charge state 2

Inclusion ―

Exclusion ―

Tags ―

Full MS

Microscans 1

Resolution 70,000

AGC target 3e6

Maximum IT 20 ms

Number of scan ranges 1

Scan range 300 to 1750 m/z

Spectrum data type Profile

dd-MS² / dd-SIM

Microscans 1

Resolution 35,000

AGC target 5e5

Maximum IT 120 ms

Loop count 10

MSX count 1

TopN 10

Isolation window 1.8 m/z

Isolation offset 0.0 m/z

Scan range 200 to 2000 m/z

Fixed first mass ―

NCE / stepped NCE 25

Spectrum data type Profile

dd Settings

Underfill ratio 0.1 %

Intensity threshold 4.2e3

Apex trigger ―

Charge exclusion unassigned, 1

Peptide match On

Exclude isotopes on

Dynamic exclusion 20.0 s

If idle .. do not pick others

Setup

TUNEFILES

General

Switch Count 0

Base Tunefile C:\Xcalibur\methods\methods\_bn\nano\_fixed\_tune.mstune

CONTACT CLOSURE

General

Used False

Start in Closed True

Switch Count 0

SYRINGE

General

Used False

Start in OFF True

Stop at end of run False

Switch Count 0

Pump setup

Syringe type Hamilton

Flow rate 3.000 µL/min

Inner diameter 2.303 mm

Volume 250 µL

DIVERT VALVE A

General

Used False

Start in 1-2 True

Switch Count 0

DIVERT VALVE B

General

Used False

Start in 1-2 True

Switch Count 0

LOCK MASSES

(no entries)

INCLUSION LIST

(no entries)

EXCLUSION LIST

(no entries)

NEUTRAL LOSSES

(no entries)

MASS TAGS

(no entries)

**Parameters MQ**

|  |  |
| --- | --- |
| Parameter | Value |
| Version | 1.4.1.2 |
| Fixed modifications | Carbamidomethyl (C) |
| Decoy mode | revert |
| Special AAs | KR |
| Include contaminants | True |
| MS/MS tol. (FTMS) | 20 ppm |
| Top MS/MS peaks per 100 Da. (FTMS) | 12 |
| MS/MS deisotoping (FTMS) | True |
| MS/MS tol. (ITMS) | 0.5 Da |
| Top MS/MS peaks per 100 Da. (ITMS) | 8 |
| MS/MS deisotoping (ITMS) | False |
| MS/MS tol. (TOF) | 0.1 Da |
| Top MS/MS peaks per 100 Da. (TOF) | 10 |
| MS/MS deisotoping (TOF) | False |
| MS/MS tol. (Unknown) | 0.5 Da |
| Top MS/MS peaks per 100 Da. (Unknown) | 10 |
| MS/MS deisotoping (Unknown) | False |
| PSM FDR | 0.01 |
| Protein FDR | 0.01 |
| Site FDR | 0.01 |
| Use Normalized Ratios For Occupancy | True |
| Min. peptide Length | 7 |
| Min. score for unmodified peptides | 0 |
| Min. score for modified peptides | 40 |
| Min. delta score for unmodified peptides | 0 |
| Min. delta score for modified peptides | 17 |
| Min. unique peptides | 0 |
| Min. razor peptides | 1 |
| Min. peptides | 1 |
| Use only unmodified peptides and | True |
| Modifications included in protein quantification | Acetyl (Protein N-term);Oxidation (M) |
| Peptides used for protein quantification | Razor |
| Discard unmodified counterpart peptides | True |
| Min. ratio count | 2 |
| Site quantification | Use least modified peptide |
| Re-quantify | True |
| Use delta score | False |
| iBAQ | False |
| iBAQ log fit | False |
| MS/MS recalibration | False |
| Match between runs | True |
| Matching time window [min] | 1 |
| Alignment time window [min] | 20 |
| Find dependent peptides | False |
| Fasta file | E:\Users\MarkusRinschen\databases\Homo\_sapiens\_Ref\_Uniprot\_140223.fasta |
| Labeled amino acid filtering | True |
| Site tables | Oxidation (M)Sites.txt |
| Cut peaks | True |
| Decoy mode | revert |
| Special AAs | KR |
| Include contaminants | True |
| RT shift | False |
| Advanced ratios | False |
| AIF correlation | 0.47 |
| First pass AIF correlation | 0.8 |
| AIF topx | 20 |
| AIF min mass | 0 |
| AIF SIL weight | 4 |
| AIF ISO weight | 2 |
| AIF iterative | True |
| AIF threshold FDR | 0.01 |