Proteomic platform for comprehensive and quantitative urinary proteomes

Garwin Pichler[1], Nils A. Kulak[1], Marta Murgia[1] and Matthias Mann[1,2]

[1] Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, D-82152 Martinsried, Germany

[2] NNF Center for Protein Research, Faculty of Health Sciences, University of Copenhagen

Urine is a desirable body fluid for clinical research as it can be obtained non-invasively in large quantities from every patient. Proteins in urine are mainly derived from plasma or are secreted from kidney and the urinary tract, providing a potential read-out for various diseases and representing an attractive source for biomarkers.

Mass spectrometry (MS)-based proteomics could in principle be an ideal technology to discover and detect protein biomarkers in this clinically accessible and relevant body fluid. However, analysis of urine is a challenging task due to the high levels of salt and other interfering compounds, low protein concentrations and a high degree of intra- and inter-individual variability.

Here we build on recent developments in our group in the sample-preparation workflow[1] to enable reproducible, parallelized and sensitive processing of urine proteins. We collected urine samples from 6 healthy male donors on 3 consecutive days. We identified a total of 3284 proteins in urine and calculated the label-free quantification values[2] of 2200 proteins in average per sample, with MS-signals spanning 6 orders of magnitude. Remarkably, we identified 1354 proteins, which have not been reported in urine before and these are enriched for terms such as "humoral immune response". Encouragingly, a common set of 1112 urine proteins ('core proteome') were detectable in all studied individuals. Median coefficient of variation (CV) was 27% for technical replicates, which is excellent for label-free shotgun proteomics. The core proteome had a CV of 42% between the 6 individuals.

Our developments contribute to a robust and sensitive high-throughput urine proteomics platform which we hope will open urine proteomics to routine, quantitative analysis of patient samples in clinical settings.

1) Kulak NA, Pichler G, Paron I, Nagaraj N, Mann M. Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. Nat Methods. 2014 Mar;11(3):319-24. doi: 10.1038/nmeth.2834. Epub 2014 Feb 2.

2) Cox J, Hein MY, Luber CA, Paron I, Nagaraj N, Mann M (2014) Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. *Mol Cell Proteomics* **13:** 2513-2526