Possible pitfalls in urinary proteomics: evaluating strengths and weaknesses in the sample pretreatment

Håvard Loftheim[1, 2], Thien Duc Nguyen[1], Helle Malerød[3], Elsa Lundanes[3], Anders Åsberg[2] and Léon Reubsaet[1]

[1] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo.
[2]Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo.
[3]Department of Chemistry, University of Oslo.

In the field of kidney transplantation, acute rejection is one of the major risk factors for developing chronic allograft nephropathy which again is an important cause of late graft loss. Suspicion of acute rejection episodes following renal transplantation is currently based on serum creatinine changes. This is not an ideal marker for acute rejection due to its lack of selectivity and the fact that it consequently needs to be confirmed by a renal allograft biopsy. The objective of the present project is to identify a urine protein biomarker using differential proteomics allowing earlier detection as well as a non invasive procedure for acute allograft rejection diagnosis

In this work, optimization of every step in a bottom-up urinary proteomics approach was studied with respect to maximize the protein recovery and making the downstream steps in the workflow fully compatible without compromising robustness. Sample enrichment and desalting using centrifugal filtration (5 kDa cut-off) yielded protein recoveries up to 97 % when 8 M urea was used. Although lower recoveries (88 %), addition of TrisHCl/NaCl was considered a better choice due to better compatibility with the next step in the sample preparation. The next step was depletion of human serum albumin (HSA), using an immuno-affinity column, which was successfully adapted for use in urine. Separation of the trypsin generated peptides in an off-line two-dimensional chromatographic system consisting of a Hydrophilic Interaction Chromatography (HILIC) column followed by a reversed phase chromatography (RP) column showed a high peak capacity and good repeatability of the chromatography. All operations were modified in order to keep sample handling between every step to a minimum, reducing the variability of each process. In order to test the suitability of the full method in an extensive proteomic experiment, a urine sample from a kidney transplanted patient was analyzed. The total variability of the method was satisfactory which is important to know in a biomarker search. Eventually, we identified a total of 1668 peptides and 438 proteins from a single urine sample despite the use of low-resolution MS/MS equipment. The optimized and "streamlined" complex method was shown to have a great applicability and potential for future urinary proteomic studies.