

Figure Legends:

Figure 1: Representative examples of analysis of the human standard

urine sample by different proteomics technologies. **A)** 1D gel used for

fractionation in the GeLC-MS enables separation by mass, and allows for ample material in the subsequent LC-MS/MS analysis of the respective tryptic

fragments. The respective molecular weight zones that were excised are

indicated on the right. **B)** 2DE-MS of the female sample, due to its high

resolution, enables separation of isoforms/different post-translational

modifications. At the same time, representation of the entire gel in the

subsequent MS/MS analysis, as in the 1DE, is generally not performed, due

to the size of the gel and the consequently high number of LC-MS/MS

analyses required. **C)** CE-MS (shown is the female sample) enables analysis

of the lower molecular weight portion of the urinary proteome that cannot be

addressed adequately by SDS-gel based approaches. However, the evidently

very high resolution can only be utilized in the analysis of molecules at lower

mass, in general < 15 kDa.

Figure 2: Comparison of the identification results from GeLC-MS and

2DE

A) Graphical representation of the results from the analysis of the 56-69kDa

band zone by GeLC-MS and 2DE referring to the number of unique protein

identifications provided by each technique; using albumin as an example, a

comparison of the number of detected isoforms, peptides, and respective

cumulative sequence coverage (%) provided by each technique is also made.

B) The sequences of the identified peptides using GeLC-MS and 2DE are

also shown. Red characters indicate the identified peptides of the protein and blue characters indicate unique identifications by one approach. For comparison, regions of the protein detected as native peptides by CE-MS and/or LC-MS are underlined.

Figure 3: Display of the low molecular weight proteome of the human urine standard. **A)** Peptides and proteins detected using CE-MS: shown are compiled patterns of the individual analyses. The molecular mass on a logarithmic scale (0.8 – 20 kDa, indicated on the left) is plotted against normalized migration time (18-48 min, indicated on the bottom). Average signal intensity is encoded by peak height and color. Upper panel: all detected signals (corresponding to **Supplementary ‘Detailed Tables’, spreadsheet: ‘CE-(ESI)-MS male, female’**). Lower panels: Distribution of the collagen alpha 1 (I), the collagen alpha 1 (III), and the uromodulin peptides, as indicated on the left. **B)** Peptides and proteins detected using LC-MS. Shown are compiled patterns of the individual analyses. The molecular mass on a logarithmic scale (0.8 – 20 kDa, indicated on the left) is plotted against retention time (18-48 min, indicated on the bottom). Average signal intensity is encoded by peak height and color. Upper panel: all detected signals (corresponding to **Supplementary ‘Detailed Tables’, spreadsheet: ‘LC-(ESI)-MS female, male’**). Lower panels: Distribution of the collagen alpha 1 (I), the collagen alpha 1 (III), and the uromodulin peptides, as indicated on the right. **C)** Inter-laboratory and inter-platform comparisons of the 40 most abundant sequenced peptides (based on ion counting) in the standard male and the female samples. These peptides account for ~40% of the total signal. The

majority of these peptides represent degradation products from collagen, uromodulin, and fibrinogen, as indicated on the bottom. As expected, a very high similarity between the data obtained on the two samples on an identical instrument can be observed, with most of these peptides being among the 40 most abundant peptides in both samples. When comparing data obtained on similar, yet different instruments (comparing upper two panels from CE-TOF with the middle 2 panels from CE-Q-TOF), good comparability can be observed, and most peptides can be detected with similar abundance. Analysis of the same samples on a completely different platform (LC-coupled Orbitrap) results in similar data. Most noticeable: the absence of very small and very large peptides in the LC-MS analysis. This is likely due to small and highly charged peptides not binding to the reversed-phase material, while large peptides likely precipitating on the column, and thereby not eluting.

Figure 4: Comparison of the identification results from the different techniques

Position of the uromodulin peptides detected by the employed techniques (2DE, GeLC-MS, CE-MS, and LC-MS) on the protein sequence. The numeric labeling is based on the protein theoretical primary sequence (grey bar). The numbers in front of the bars correspond to the spot numbers of the 2DE master gel (see **Supplementary ‘Detailed Tables’, spreadsheet: ‘2DEMaster’**) or to the gel band in the case of GeLC-MS (see **Supplementary ‘Detailed Tables’, spreadsheet: ‘GeLC-MSMS Identification List’**). The GeLC-MS female and male data are merged. In the case of the 2DE and GeLC-MS approaches the detected peptides correspond

to tryptic digests of the respective spots or bands. In the case of CE- and LC-MS analysis the naturally occurring peptides are shown, as identified following tandem mass spectrometry.

Supplementary Figure 1:

a-h: Comparison of the analysis of tryptic peptides from selected 2DE spots (depicted in the gel image on the top; spot numbers correspond to those in **Figure 1B** and **Supplementary 'Detailed Tables', spreadsheet, '2DE Master'**) by CE- and MALDI-TOF MS. Detected peptides by each technique, predicted PTMs, sequence coverage in each case and cumulative sequence coverage provided by the combination of the MS platforms are presented.