Researchers propose a facile strategy for comprehensive proteome analysis of urine

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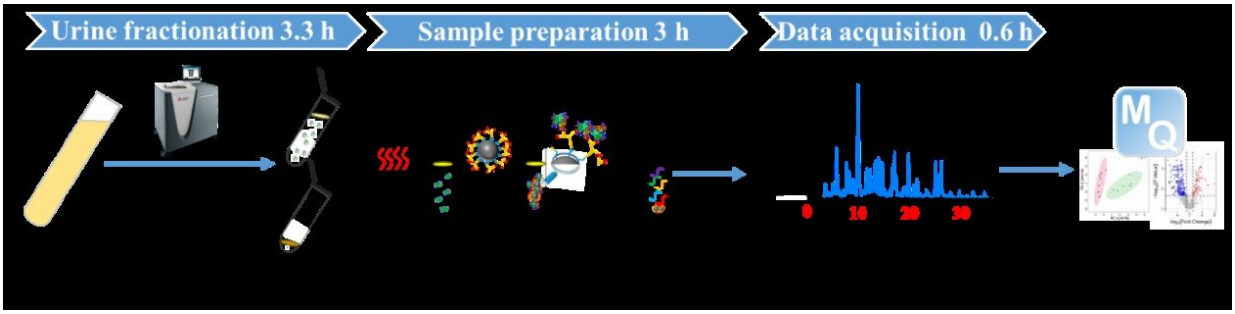

Strategy For Achieving Comprehensive Proteome Analysis Of Urine (Cpu). Credit: *URINE* (2023). DOI: 10.1016/j.urine.2023.05.002

Urine is one of the attractive sources for early and sensitive biomarker discovery since it can accumulate and reflect changes in the human body while being collected non-invasively. However, analysis of the urine proteome presents challenges due to its wide dynamic range, spanning approximately 10 orders of magnitude in protein concentrations.

The presence of high-abundance proteins in urine can overshadow potential disease biomarkers, making their identification difficult. Fractionation and depletion strategies are commonly employed before performing mass spectrometry (MS) analysis in order to enhance the detection and identification of these elusive biomarkers.

As a promising alternative, urine fractionation through ultracentrifugation (UC) can be employed to deplete high-abundance proteins, allowing for the isolation of exosomes with high purity while retaining most of the high-abundance proteins in the supernatant. Nonetheless, the strong centrifugal shearing force during UC can unavoidably disrupt the intact structure of exosomes, resulting in the isolation of only trace amounts from a small urine volume.

With all the existing limitations in mind, a team of researchers in China proposed a novel solid-phase alkylation (SPA)-based sample preparation method for low-loss, anti-interference processing of sub-microgram proteomic samples. The team reported their study in the journal *Urine*.

"Our method combines UC fractionation, solid-phase extraction (SPA) sample preparation, and liquid chromatography-mass spectrometry (LC-MS) to enable comprehensive proteome profiling of urine, known as CPU, or comprehensive proteome profiling of urine," explained corresponding author Huiming Yuan, a professor at the Dalian Institute of Chemical Physics, Chinese Academy of Sciences. "This facile strategy resulted in the identification of a total of 1,659 proteins using a short LC gradient of approximately one hour, which is 2.3 times more than the 730 proteins identified from raw urine without fractionation."

Notably, in comparison to existing urine sample preparation methods, CPU offers significant advantages. Not only does it drastically reduce the analysis time by 3–4 times, but it also enhances the identification coverage of the urine proteome by 130%–160%.

"The method was further employed in combination with label-free quantification to conduct comparative proteome analysis of urine from both IgA nephropathy (IgAN) patients and healthy donors," said lead author Xinxin Liu, a technician at Dalian Institute of Chemical Physics, Chinese Academy of Sciences. "As a result, 227 differentially expressed

proteins were identified, shedding light on potential biomarkers associated with IgAN."

Several members of the solute carrier family 22 (SLC22) were found to be up-regulated in IgAN patients, suggesting a potential link to the disruption of renal metabolic function in these individuals.

"Our results demonstrated that our developed method holds promise as a valuable tool for the discovery of disease-related biomarkers in urine," Liu concluded.

More information: Xinxin Liu et al, A facile strategy for comprehensive proteome analysis of urine using ultracentrifugation fractionation, solid-phase alkylation based sample preparation and liquid chromatography-mass spectrometry, *Urine* (2023). DOI: 10.1016/j.urine.2023.05.002

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