

## An Ion Mobility-Enabled Data Independent Multi-Omics Approach to Quantitatively Characterize Urine From Children Diagnosed With Idiopathic Nephrotic Syndrome

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### Abstract

In this application note, we describe a multi-omic approach to reveal new molecular factors involved in pathogenesis of INS with potential diagnostic and therapeutic significance.

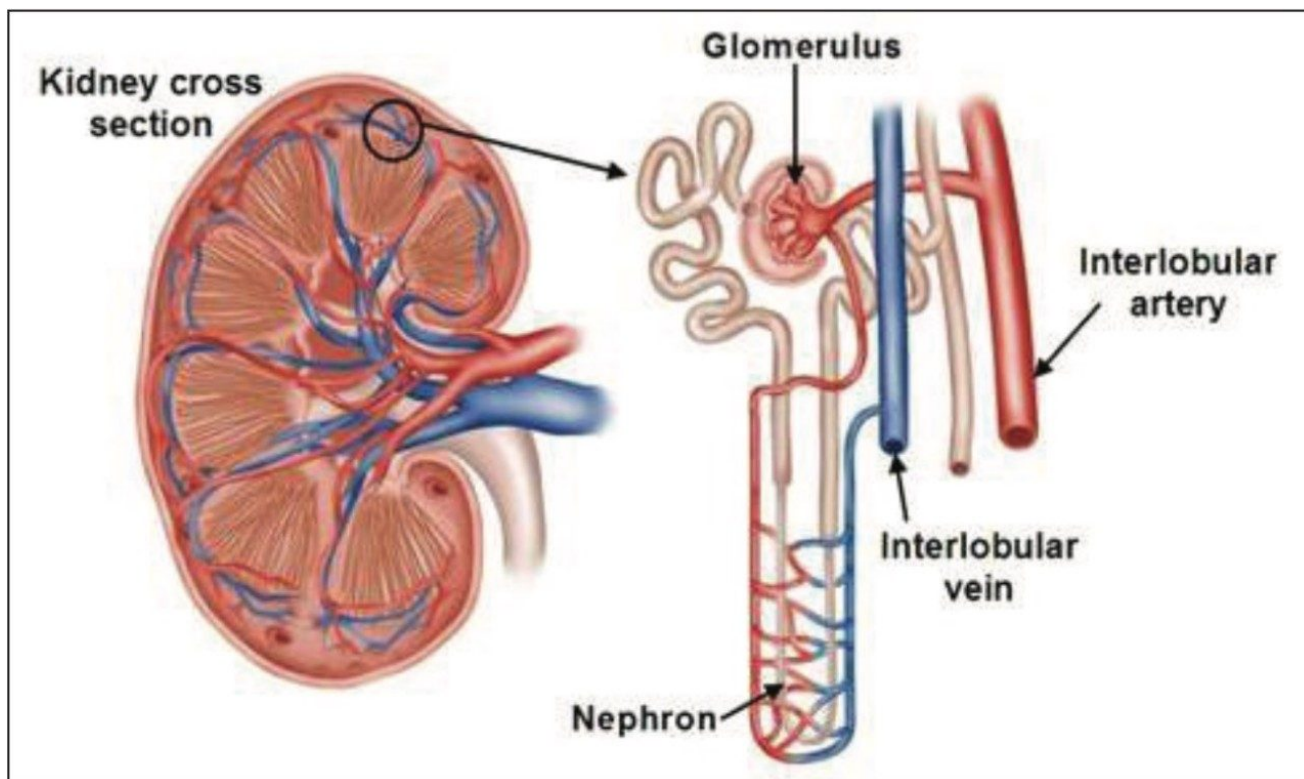
### Benefits

A label-free multi-omics approach for the analysis of the urine of Idiopathic Nephrotic Syndrome (INS) patients that provides both qualitative and quantitative information in a single experiment. This enables possible diagnostic and therapeutic solutions for patients with INS.

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## Introduction

Idiopathic Nephrotic Syndrome (INS) results from the malfunction of the glomerular filter and it is the most prevalent glomerular disease in children. In spite of some progress, its pathogenesis is still unknown and therapy options are confined to gross immune modulation. A variety of methods for diagnostic and treatment purposes are available for patients; however, the lack of understanding regarding the pathogenic mechanisms underlying INS can lead to poor therapeutic response and adverse side-effects. In this application note, we describe a multi-omic approach to reveal new molecular factors involved in pathogenesis of INS with potential diagnostic and therapeutic significance.



*Figure 1. Kidney section highlighting a single nephron. A malfunctioning glomerulus is where INS occurs (courtesy of Wellcome Images).*

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## Experimental

### Sample preparation

Pediatric urine samples intended for peptide analysis were prepared for LC-MS analysis as previously described.<sup>1</sup> Samples were treated with 1% RapiGest SF prior to reduction and alkylation. Aliquots were incubated with anti-HSA resin and centrifuged using Vivaspins 5,000 MWCO filters. A series of washes using water were implemented to ensure adequate recovery. The resulting supernatant was digested using trypsin overnight as shown in Figure 2. Metabolite analysis samples were purified using Oasis HLB Extraction Cartridges. Water/methanol (90/10) washes were performed, followed by analyte elution using methanol. The resulting residue was reconstituted in 200  $\mu$ L mobile phase and vortexed prior to LC-MS.

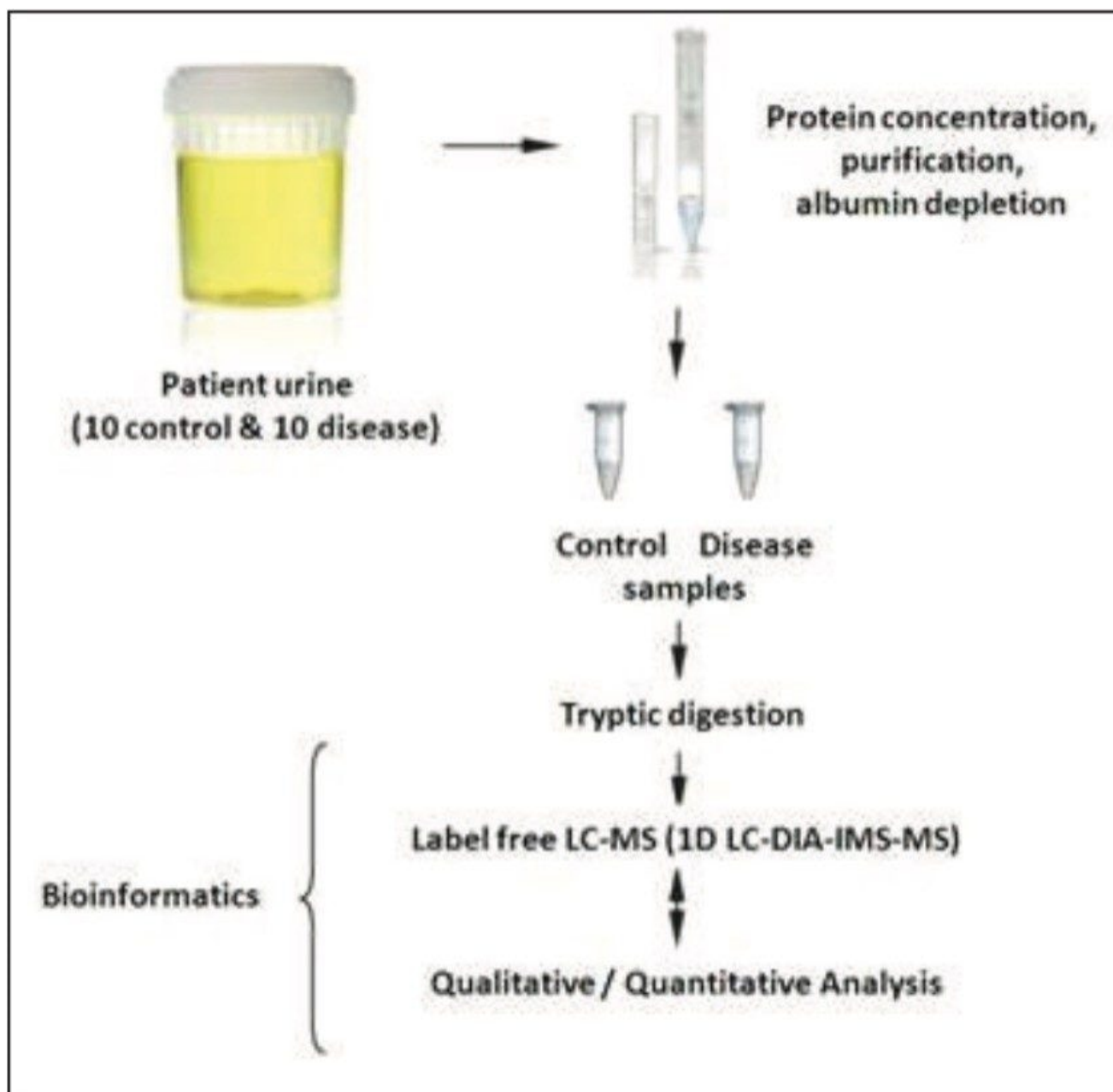


Figure 2. Experimental design study for urinary proteins.

## LC-MS conditions

Label-free LC-MS was used for qualitative and quantitative peptide analyses. Experiments were conducted using a 90 min gradient from 5% to 40% acetonitrile (0.1% formic acid) at 300 nL/min using a

nanoACQUITY UPLC System and a BEH 1.7  $\mu\text{m}$   $\text{C}_{18}$  reversed phase 75  $\mu\text{m}$  x 20 cm nanoscale LC Column.

For metabolite identification, the LC-MS experiments consisted of a 10 min gradient from 0% to 50% acetonitrile (0.1% formic acid) at 500  $\mu\text{L}/\text{min}$  using an ACQUITY UPLC System. Here, a BEH 1.7  $\mu\text{m}$   $\text{C}_{18}$  reversed phase 2.1 x 10 cm LC column was used.

Data were acquired in data independent analysis (DIA) mode that utilized a nanoACQUITY UPLC or ACQUITY UPLC system directly interfaced to a hybrid IM-*oa*Tof SYNAPT G2 Mass Spectrometer. Ion mobility (IM) was used in conjunction with both acquisition schemes, as illustrated in Figure 3.

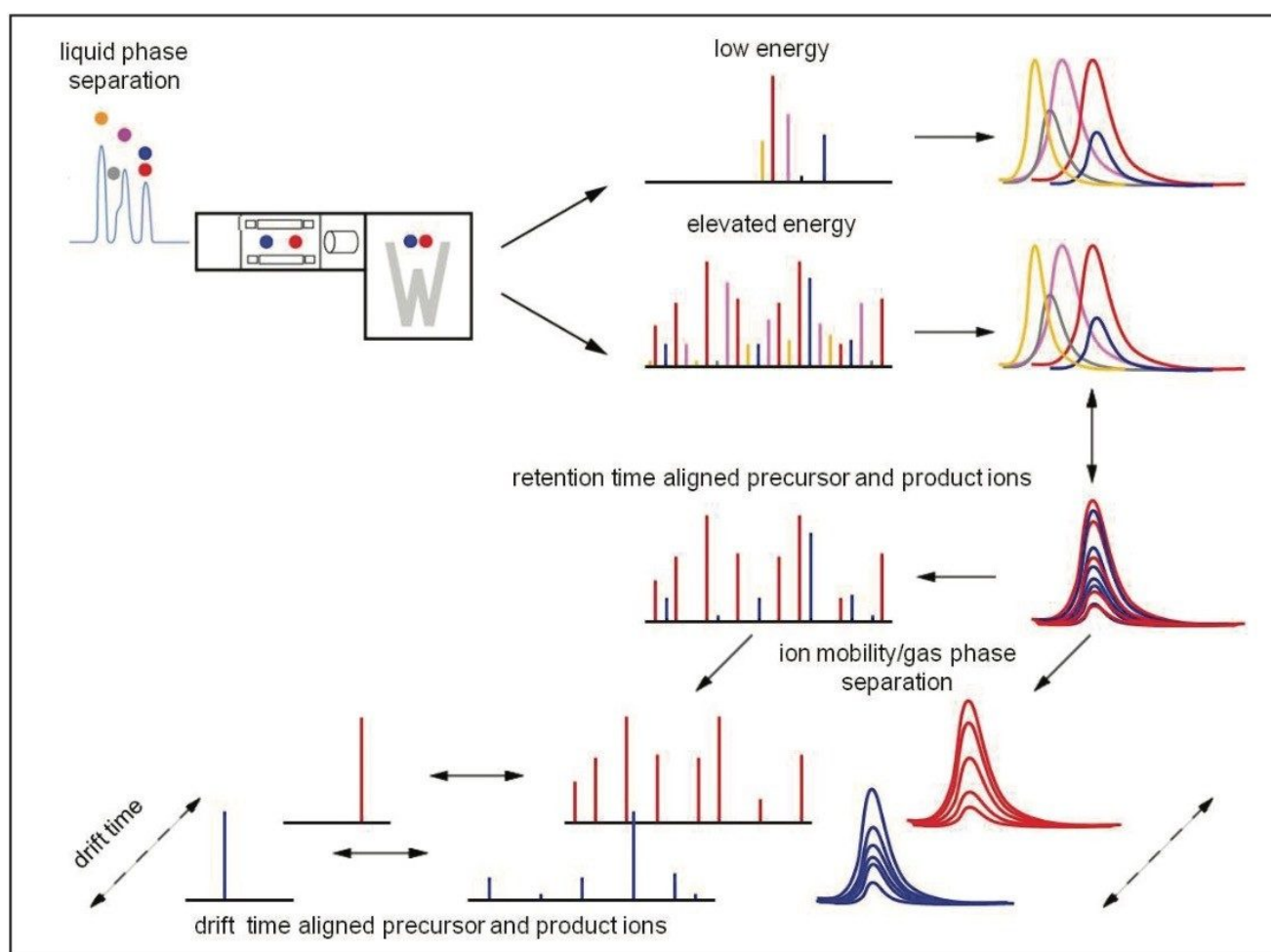


Figure 3. Retention and drift time principle ion mobility enabled data independent analysis (IM-DIA-MS/HDMS<sup>E</sup>).

## Bioinformatics

The LC-MS peptide data were processed and searched with ProteinLynx GlobalSERVER. Normalized label-free quantification was achieved using TransOmics Software. The resulting metabolomic data were processed using MassLynx Software, and additional statistical analysis conducted with EZ Info.

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## Results and Discussion

Small amounts of the purified urine were analyzed to identify, quantify, and investigate the proteomic and metabolomic variance between control and disease pre-treated subjects.

Principal Component Analysis (PCA) was used to identify and highlight significant changes between the control and disease pre-treated samples, as shown in Figure 4. Similar clustering patterns were observed for both the protein and metabolite datasets.

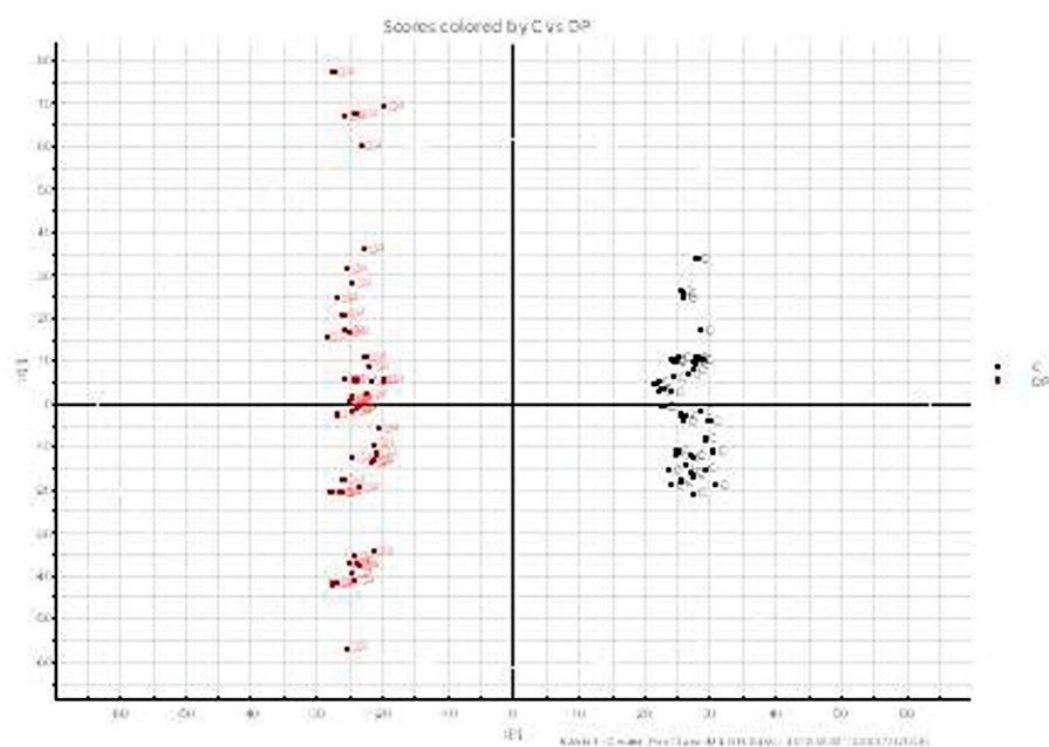
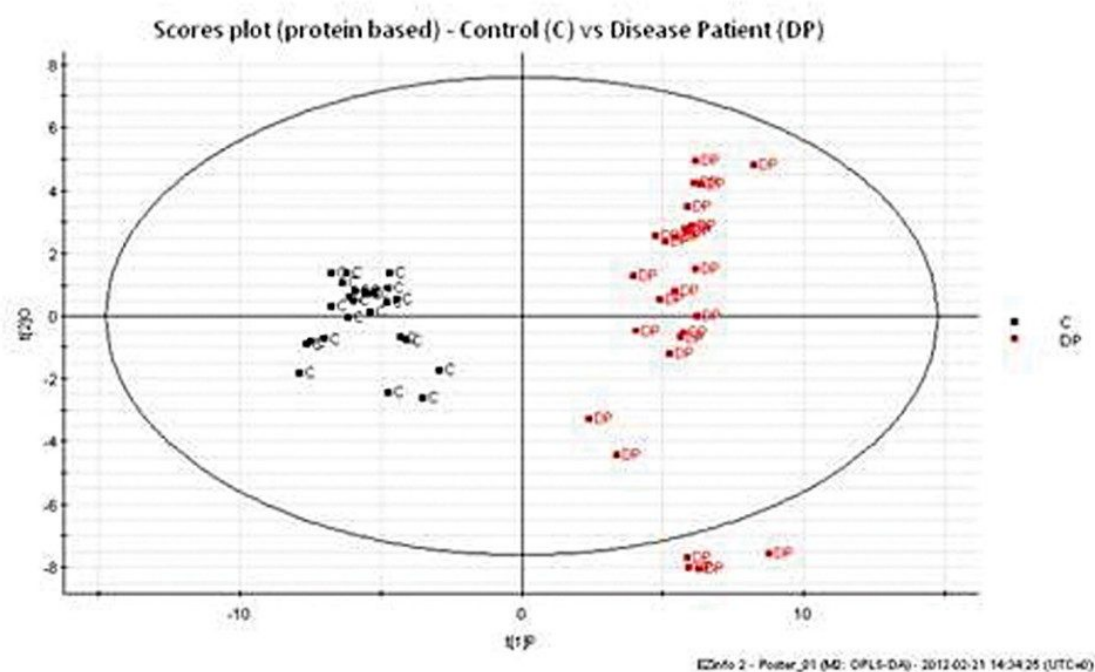


Figure 4. Scores plot from OPLS-DA analysis of disease pre-treated (red) vs. control (black).

Proteomic data were aligned, normalized, and quantified. A large proportion of the identified proteins are glycosylated and over 80% of the total number of proteins identified and exhibited a significant expression fold change. Figure 5 highlights the proteins, which have greater than a two-fold change between sample sets. The fold change at the peptide level can be visually displayed using 3D montage images. A charge state feature of one of the peptides of interest is shown in Figure 5.

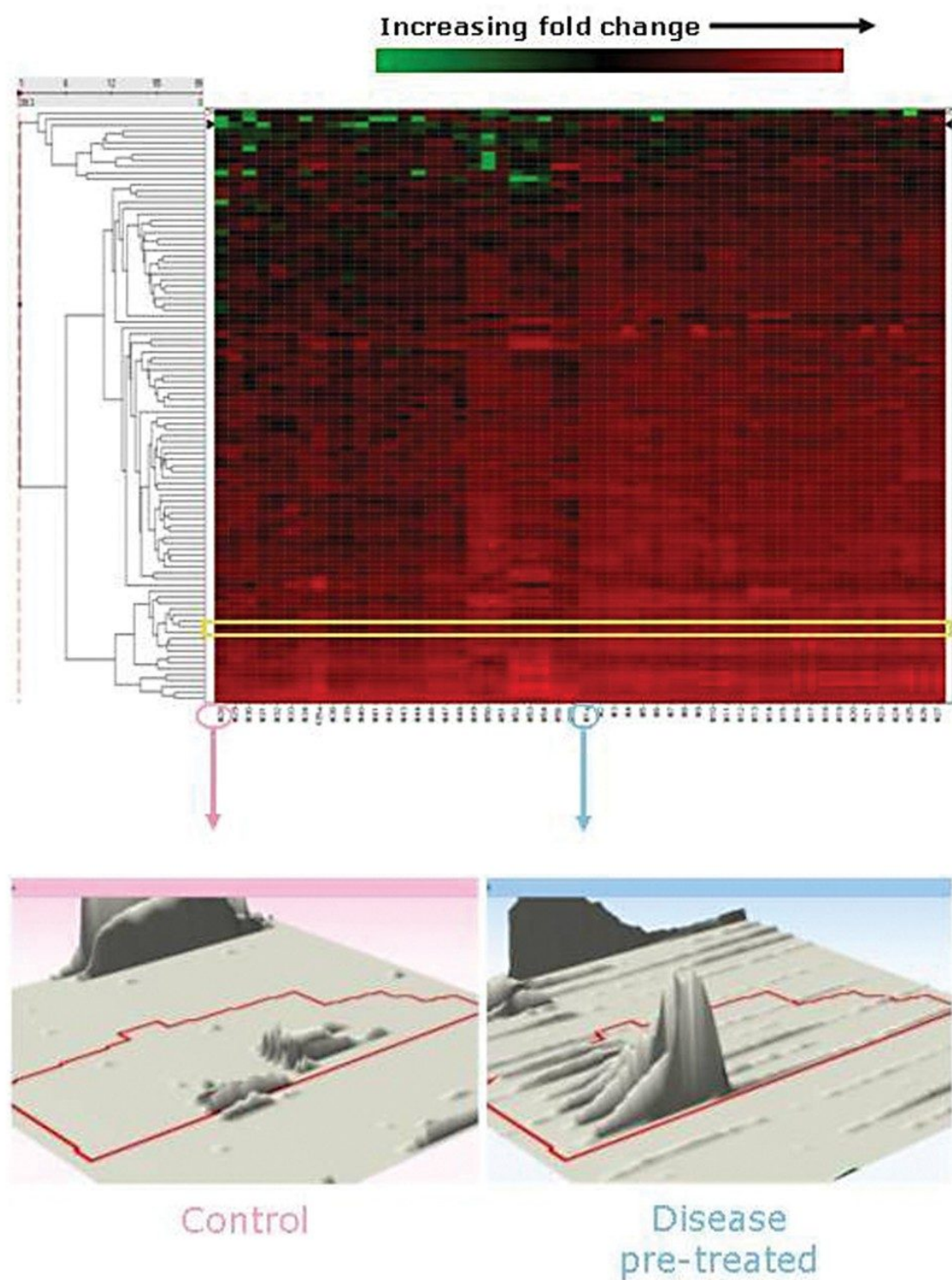


Figure 5. Hierarchical cluster analysis regulated proteins with a minimum of three identified peptides and a fold

change greater than two. The highlighted region represents prostaglandin with associated 3D montage images for TMLLQPAGSLGSYSYR.

The metabolomics workflow results are summarized in Figure 6. Using the metabolite contrasting loadings plot, significant metabolite identifications can be found at the extremes and are shaded in blue. Example compounds, which were found to contribute most significantly to the variance, are also shown.

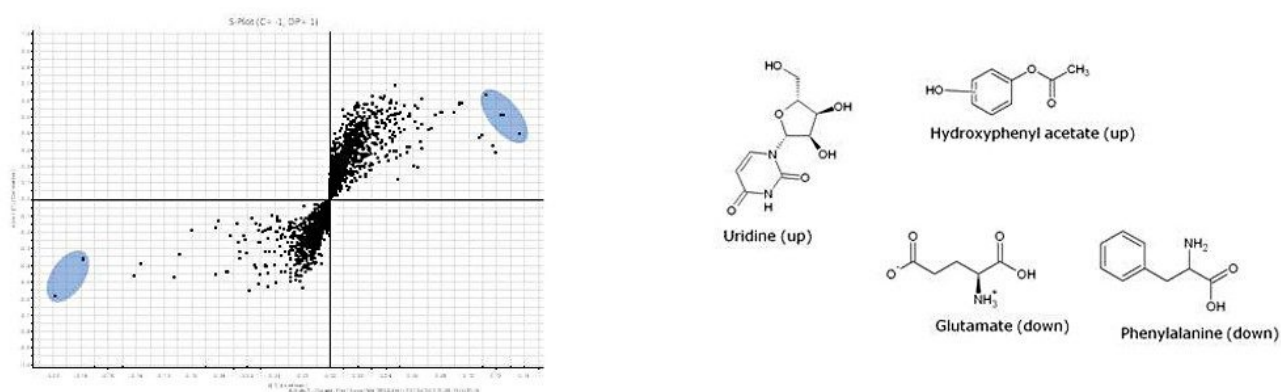


Figure 6. Metabolite loadings plot from OPLS-DA analysis of disease pre-treated versus control subjects based in positive ion mode. Metabolites contributing the greatest variance are represented within the blue shaded areas with examples provided.

A common pathway is shown in Figure 7, which illustrates a glutamate [NMDA] receptor subunit as one such example. NMDA belongs to the glutamate-gated ion channel family of proteins and is used in neuronal system pathways. Glutamate can also be located within the same pathway. Postsynaptic  $\text{Ca}^{2+}$  is thought to increase through the NMDA receptors, which activate several signal transduction pathways including Erk/MAP kinase and cAMP regulatory pathways.

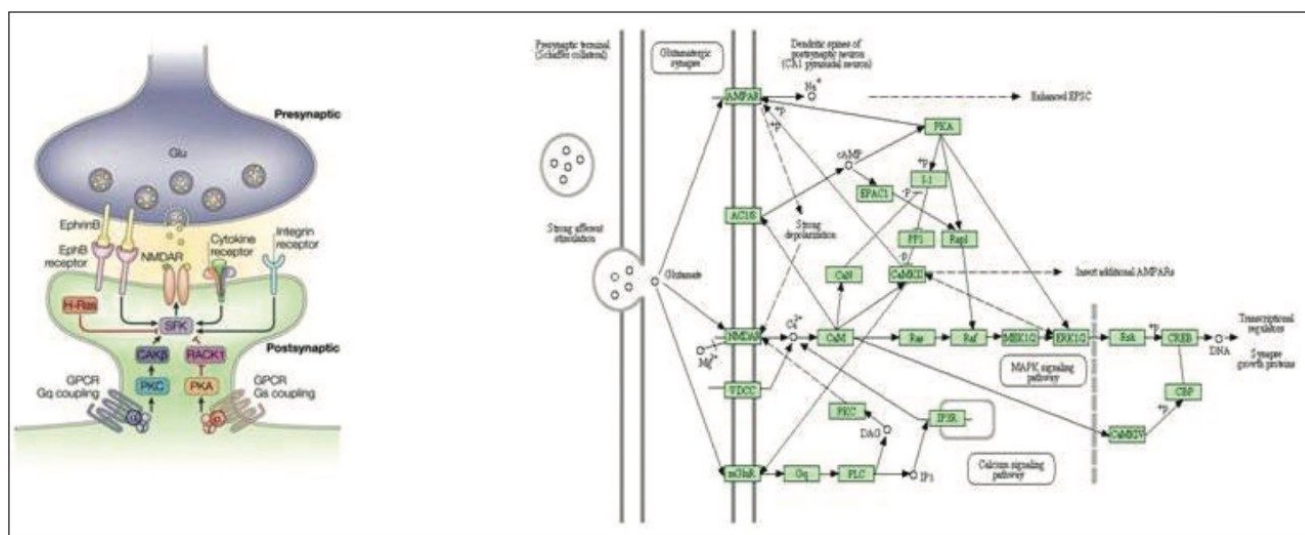


Figure 7. Neuronal system pathway, specifically highlighting the role of the glutamate [NMDA] receptor subunit and glutamate for downstream transmission in the postsynaptic cell.

## Conclusion

- 80% of the proteins identified were expressed, with 30% of proteins having a maximum fold change  $\geq 2$  and ANOVA (p) value  $\leq 0.05$ .
- The majority of identified proteins were glycosylated, of which many showed changes in relative abundance.
- PCA analysis shows both protein and metabolite data to be complimentary.
- A variety of analytes were identified as contributing towards the metabolite variance.
- Complementary information obtained from metabolite and protein analysis has been shown through the use of glutamate and NMDA within the neuronal system pathway.
- A label-free multi-omics approach has been applied for the analysis of the urine of INS patients by implementing HDMS<sup>E</sup>, which provides both qualitative and quantitative information in a single experiment.

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## References

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