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

METHODS
Sample preparation
Frogfish urine samples were diluted and stored for peptide analysis were performed for LC-MS as previously described [1]. Samples were treated with diode Re-competition, re-competition, and acetonitrile (0.1% formic acid) at 5 µL/min using a Waters ACQUITY UPLC® system with a 2.1 x 10 cm C18 column was used.

Metabolite analysis samples were purified using Oasis HLB extraction cartridges. Water/methanol (30:70) washes were performed, followed by a methanol wash using the resulting reconstituted in 200 µL mobile phase and vials prior to LC-MS.

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% acetonitrile (0.1% formic acid) at 300 nL/min using a nanoACQUITY system and a 380,000 MWCO filters. A series of washes with water were implemented to ensure adequate recovery. The resulting supernatant was digested using trypsin overnight as shown in Figure 2.

Results from the malfunction of the
Idiopathic nephrotic syndrome (INS) with a minimum of 3 identified peptides and a fold change greater than 2. The highlighted region represents an annotated and fold change images. A change state feature of one of the peptides of interest is shown in Figure 5.

CONCLUSIONS
- 80% of the proteins identified were expressed, with 31% of proteins having a maximum fold change ≥ 2 and ANOVA (p) ≤ 0.05
- The majority of identified proteins are glycosylated of which many of which also show changes in relative abundance.
- PCA analysis shows both protein and metabolite data to be complimentary.
- Variety of compounds are identified as contributing towards the metabolite variance.

A label-free multi-omics approach has been applied for the analysis of the urine of INS patients by implementing IM-DIA-MS, providing both qualitative and quantitative information in a single experiment.

REFERENCES
1. Lee A Getting, Johannes PC Vissers, John P Shockcor, Stephen McDonald, Robert Topley, Sandra Kraljevic Pavlici, Mirela Sodic, Maja Lemač, Danica Batinić, Olga Vasiljeva, and Jadrank Ljandrgjeri
2. Waters Corporation, Manchester, UK; 3. Waters Corporation, Milford, MA, USA; 4. Department of Biotechnology, University of Rijeka, Rijeka, Croatia; 5. Department of Pediatrics, Zagreb University Hospital Centre, Zagreb, Croatia; 6. Institute of Integrative Biology, University of Liverpool, UK

Figures
Figure 1. Kidney section highlighting a single nephron. A normal glomerulus function was in 350 patients.

Figure 2. Experimental design study for urinary proteins.

Figure 3. Retention and drift time principle ion mobility mass spectrometry.

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

Figure 5. Hierarchical cluster analysis regulated proteins with a minimum of 3 identified peptides and a fold change greater than 2. The highlighted region represents an annotated and fold change images. A change state feature of one of the peptides of interest is shown in Figure 5.

Figure 6. Metabolite loadings plot from OPLS-DA analysis of disease pre-treated vs. control subjects based in conjunction with both acquisition schemes, illustrated in Figure 3.

Figure 7. Identified functions/disorders based on the metabolites identified from the loadings distribution. Only metabolites with a significant p-value were included. Dark blue columns represent metabolites which are shown to be down regulated, whilst light blue columns indicate up regulated metabolites.

Figure 8. Ingenuity pathway analysis resulting from the combined protein and metabolite datasets. These include up (red) and down (green) regulated functions with the intensities depicting fold change variations.

Figure 9. Paternity diagnosis and absorption pathway with specific reference to apolipoprotein functionally highlighting in red text.