High-throughput phosphoproteomics of formalin-fixed, paraffin-embedded rat tissues using microflow Zeno SWATH

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INTRODUCTION

- Formalin-fixed paraffin-embedded (FFPE) tissues are ideal for large-scale cancer biomarker studies because they are routinely created in pathology departments, stored in long-term biorepositories, and have documented clinical data on patient diagnosis and treatment outcomes.
- The FFPE phosphoproteome is thought to play a key role in cell signalling and potentially in tumour development however, only a handful of studies have investigated this population.
- Here we explore the potential of a high throughput DIA phosphoproteomic workflow for FFPE tissues using 35-minute micro-flow trap-elute system combined with Zeno SWATH.

METHODS

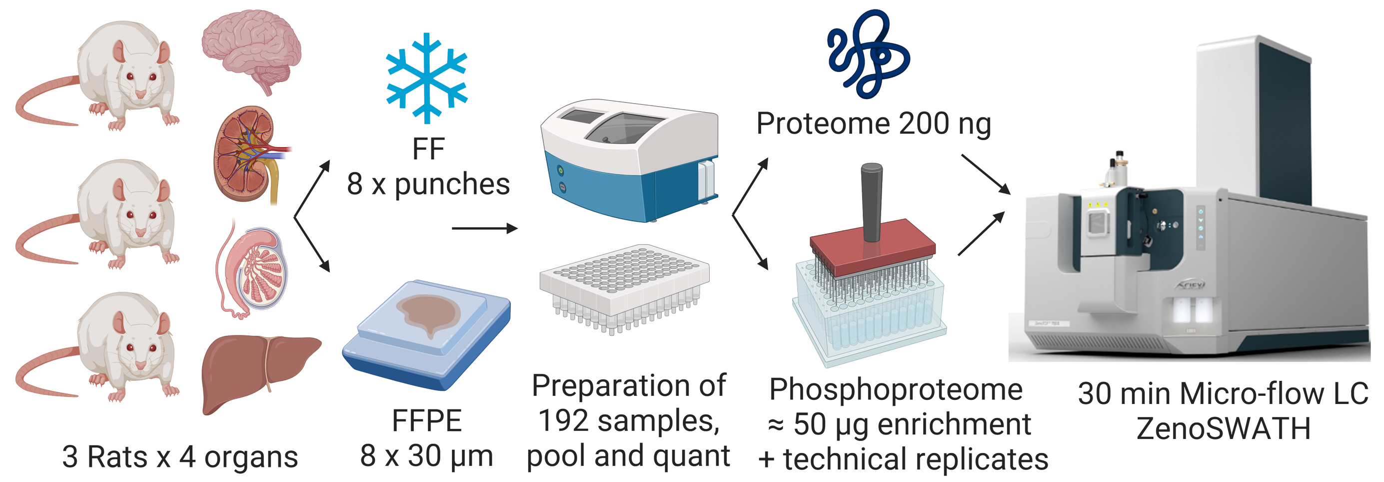

Figure 1:Workflow for analysing the proteome and phosphoproteome of both fresh frozen (FF) and FFPE tissue from 4 organs (brain, kidney, liver, testis) from 3 rats.

RESULTS - PROTEOME

Protein groups quantified using FragPipe-DIANN (7,039) and Spectronaut (6,186).

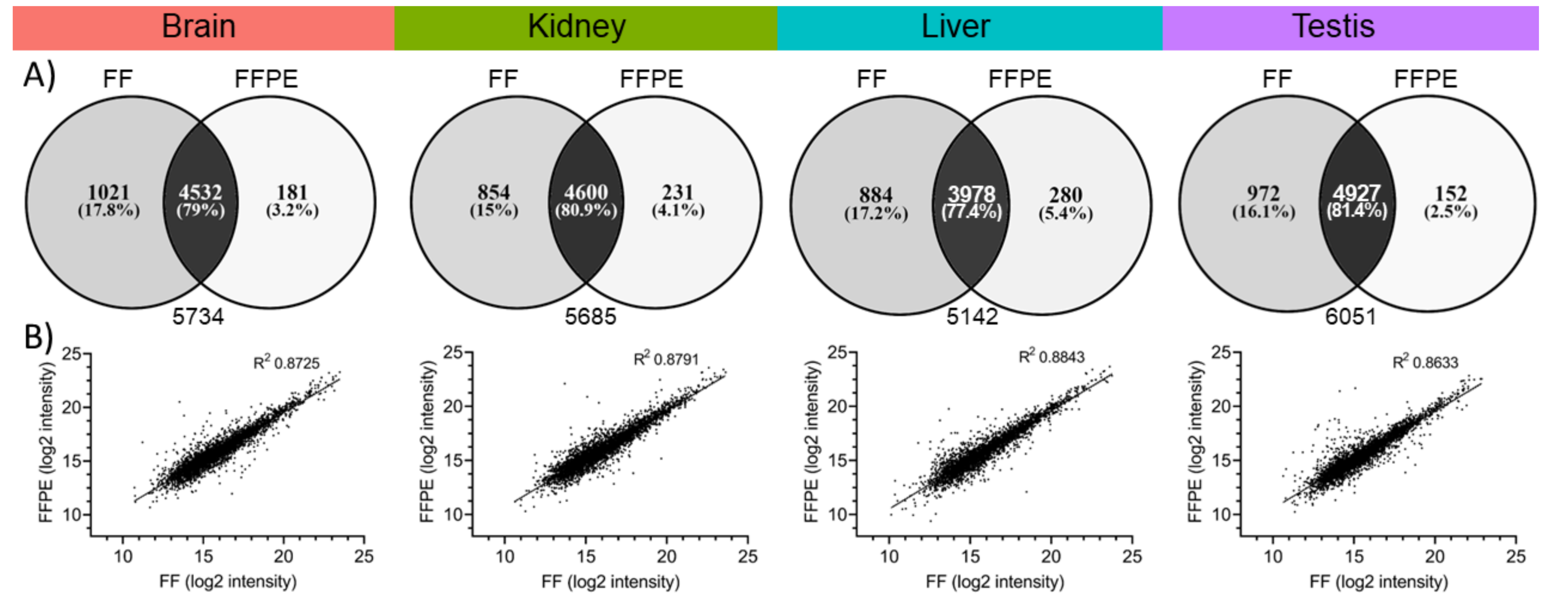

Figure 2: Venn diagrams of protein groups quantified from FragPipe-DIANN in FF and FFPE tissues across the four organs (A). Pearson correlation plots of log transformed protein groups in both FF and FFPE tissue (B).

RESULTS - PHOSPHOPROTEOME

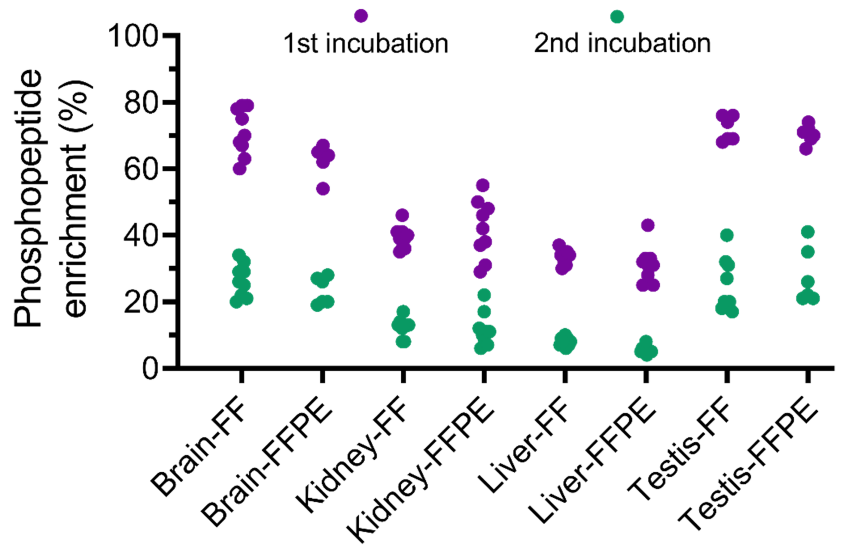

Figure 3: Phosphopeptide enrichment efficiency after first and second incubation of sample with magnetic beads.

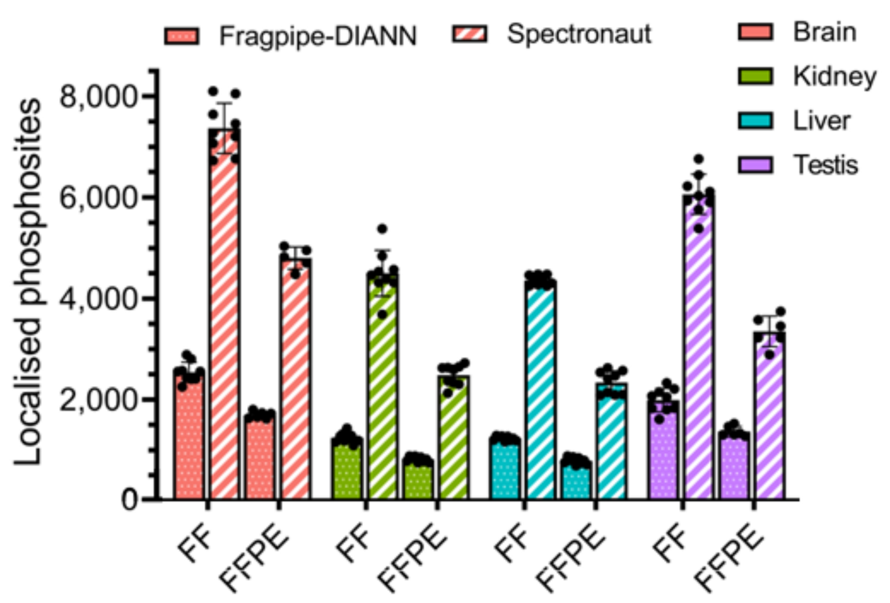

Figure 4: Number of localised phosphosites quantified.

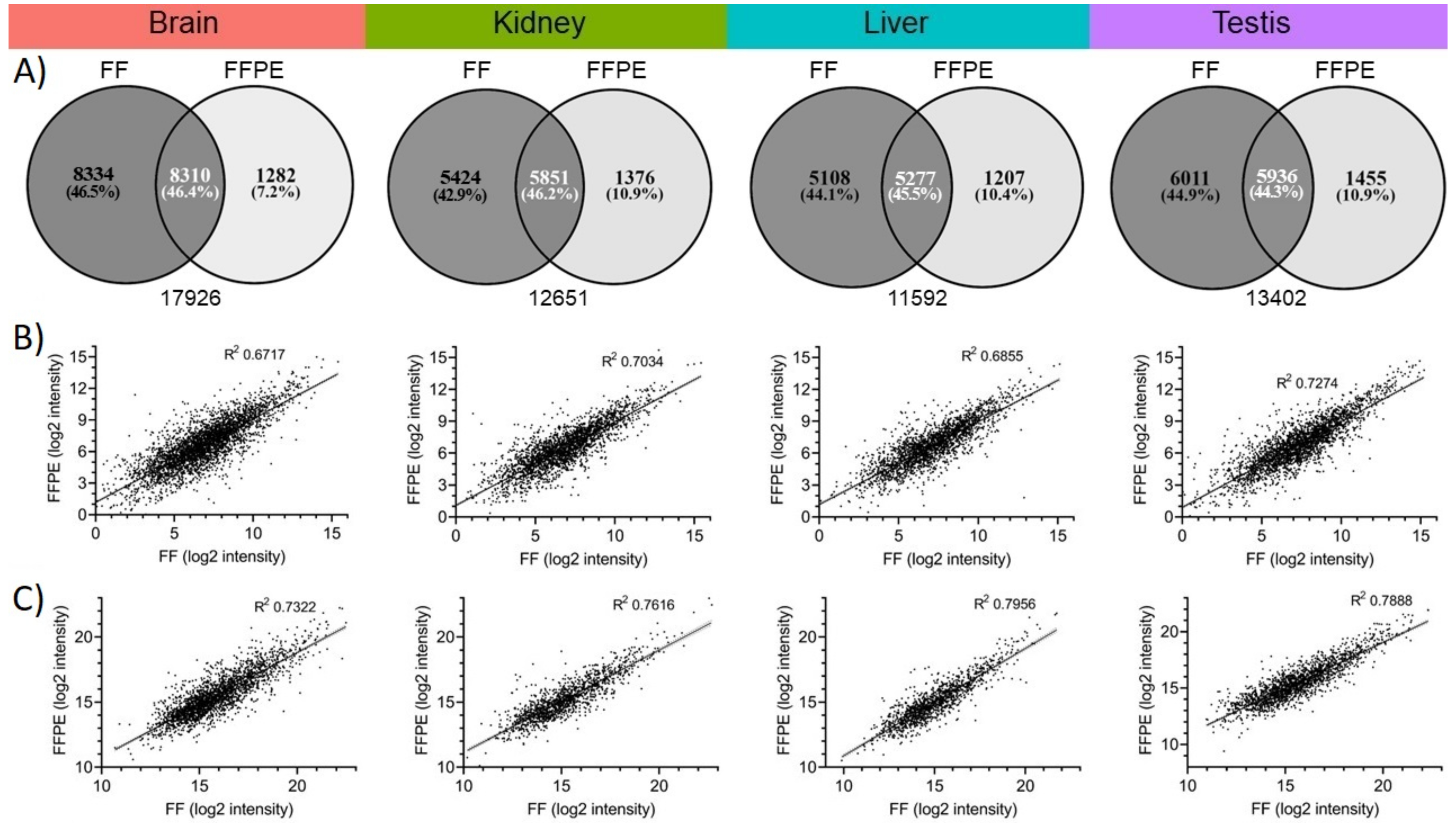

Figure 6: Venn diagrams of Spectronaut localised phosphosites in FF and FFPE tissues across the four organs (A). Pearson correlation plots of log transformed phosphosites in both FF and FFPE tissue using Spectronaut (B) and FragPipe-DIANN (C).

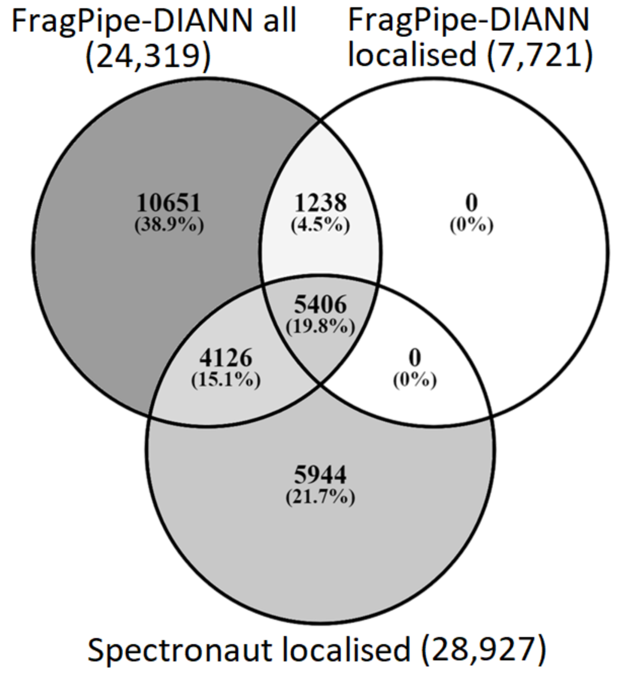

Figure 5: Overlap of Spectronaut and FragPipe-DIANN IDs.

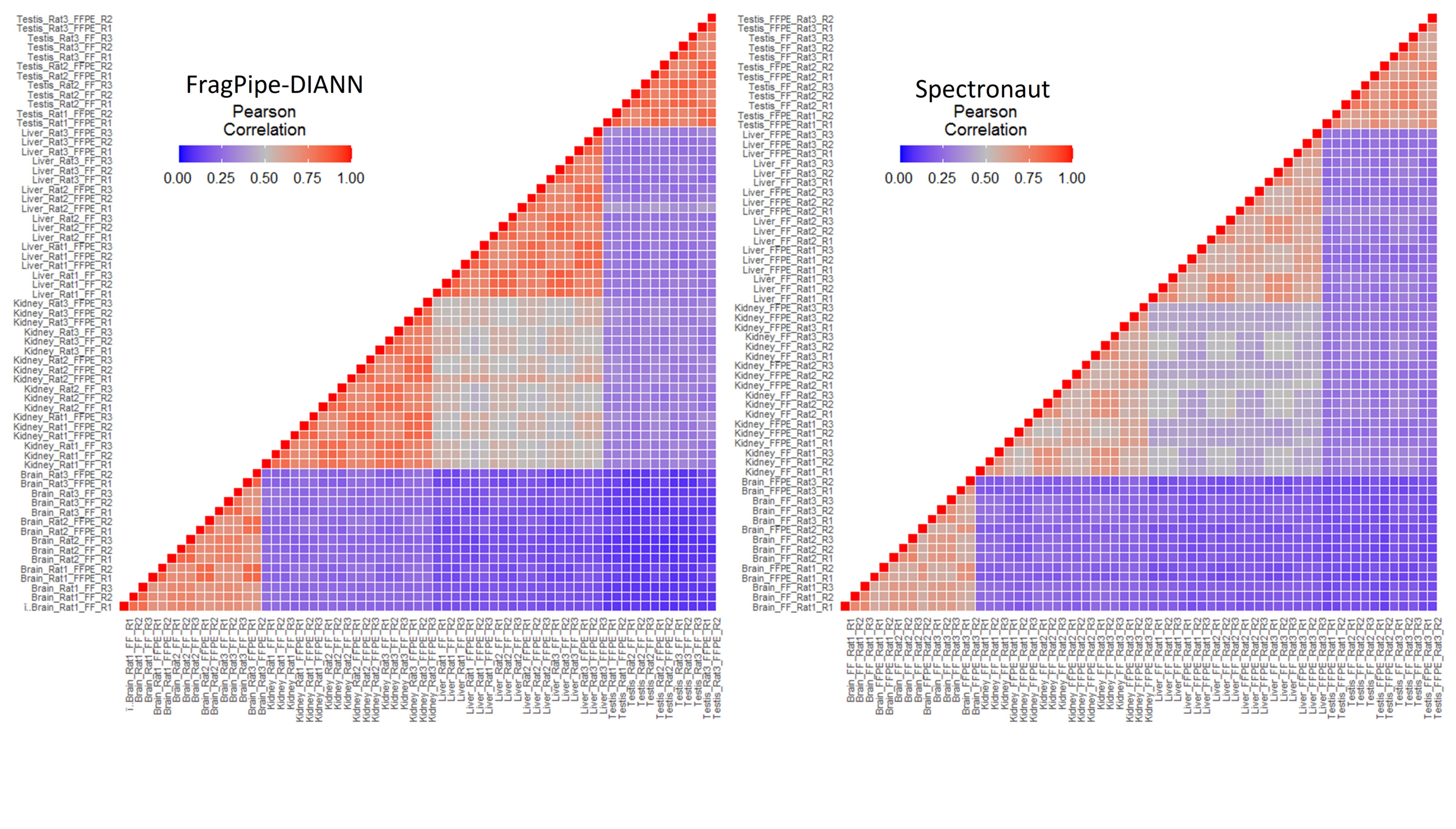

Figure 7: Pearson correlation plots of quantified phosphosites across biological replicates and enrichment replicates.

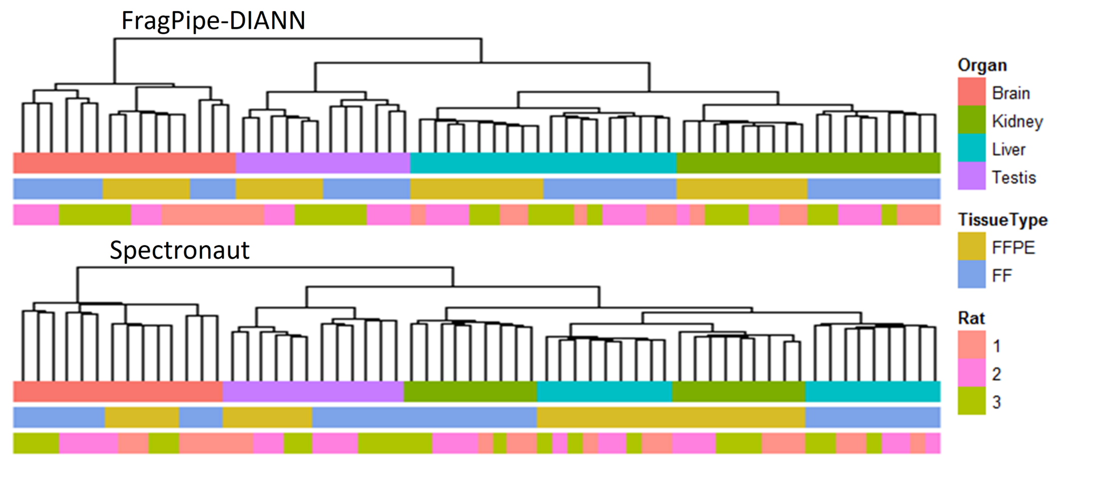

Figure 8: Unsupervised clustering of imputed matrices

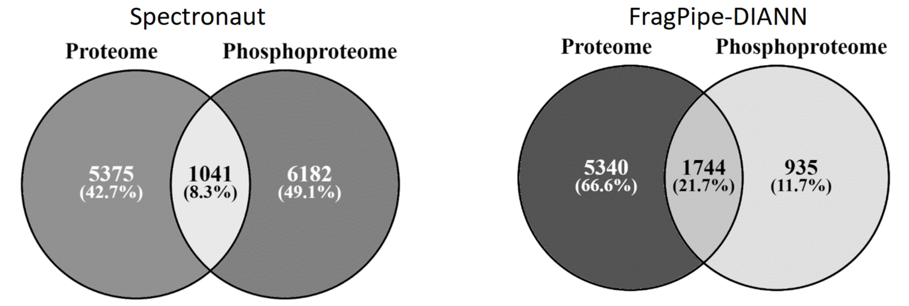

Figure 9: Overlap of protein groups quantified in proteomic and phosphoproteomic data.

PHOSPHOPROTEOMIC MINING OF PROTEOMIC DATA

We conducted a phosphoproteomic search on proteomic data to see how much of the phosphoproteome could be quantified in tissue samples without phosphopeptide enrichment.

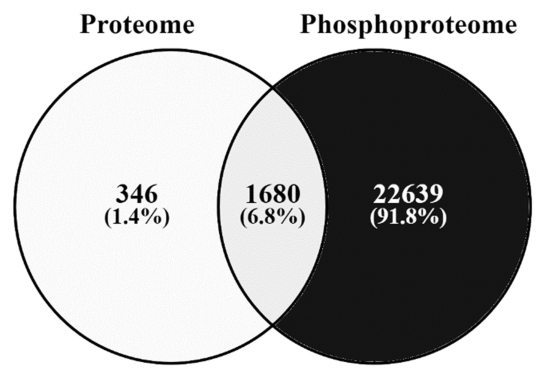

Figure 10: FragPipe-DIANN phosphosites quantified in proteomic and phosphoproteomic samples without site localisation filters

CONCLUSION

- FF and FFPE tissues are comparable for proteomic and phosphoproteomic analyses.
- A shallow phosphoproteome can be obtained from the enrichment of 50 μg of FFPE tissue using a combination of microflow and ZenoSWATH.
- Both FragPipe-DIANN and Spectronaut phosphosite matrices can distinguish between rat organs. The overlap of IDs across the two pipelines is 20%.
- FF tissues quantified more proteins and phosphosites than FFPE tissues. This may be due to inaccurate UV peptide quantitation as samples had lower MS1 and MS2 intensities.
- A large number of phosphosites are observed in both tissue types. This suggests that a portion of the phosphoproteome is preserved throughout the fixation process.
- Imputation of phosphoproteomic data can introduce new bias and negatively affect sample correlation.
- Reanalysis of proteomic data for phosphoproteomics may provide useful new biological insights.

ACKNOWLEDGEMENTS

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TOUR DE CURE

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