## IMPERIAL



# Brain Tissue Profiling: A Multi-Omics Mapping Approach using DESI & iTRAQ

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## Introduction

- Glioblastoma multiforme (GBM) is a highly diverse tumour with metabolically distinct, heterogeneous regions.(1)
- Mapping the spatial distribution of analytes in GBM allows for the identification of molecularly homogeneous tumour regions.

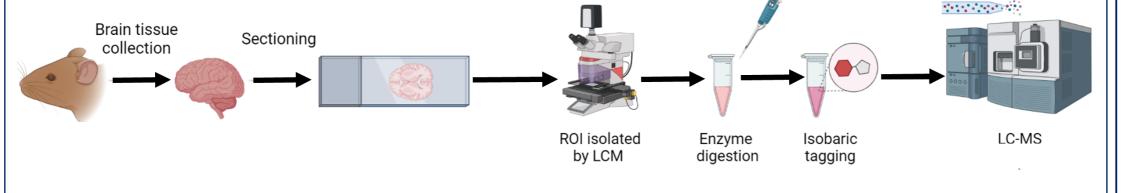
#### **Research Focus:**

- 1. Identify proteomic markers associated with GBM heterogeneity and metabolic behaviour.
- 2. Explore molecular signalling pathways, particularly in response to metabolic therapies.

#### **Study Aims:**

- 1. Evaluate the efficiency of protein extraction across lysis methods
- 2. Determine the minimum sample size for iTRAQ proteomic analysis
- 3. Evaluate the destruction of proteins by DESI imaging
- 4. Combine technologies to create a workflow incorporating DESI and LC-MS.

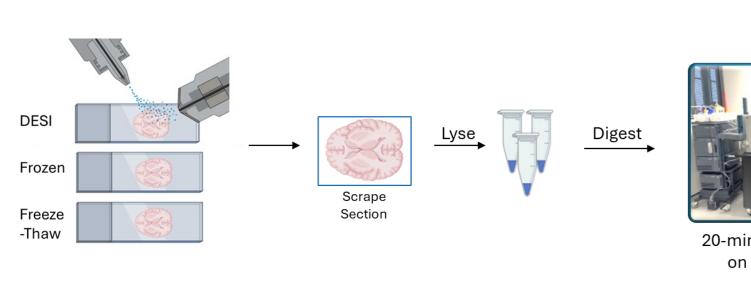
#### **Overall Workflow:**



### Methods 1. Protein Extraction Optimisation 6% SDS, 500 mM Lysis buffer Sonicfitation (denaturant) 2% SDC, 100 mM TrisHCl, 10 mM TCEP, 40 mM CAA Mouse Brain 5 x 5

2. Minimum Tissue Size Optimisation >B< A= 1000 μm x 1000 μm B= 500 μm x 500 μm C= 100 μm x 100 μm

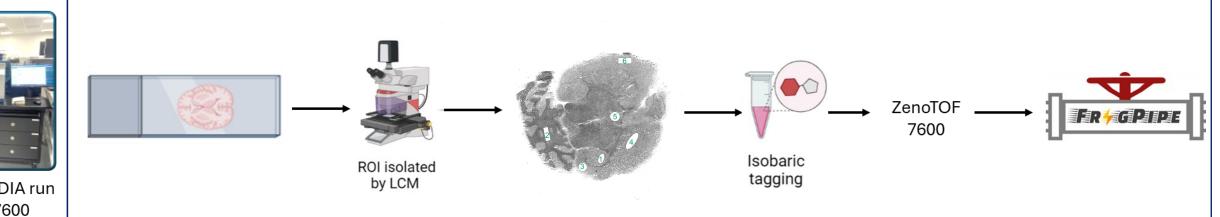
3. Effects of DESI on Proteomics Optimisation



4. Regions of Interest Proteomics Optimisation

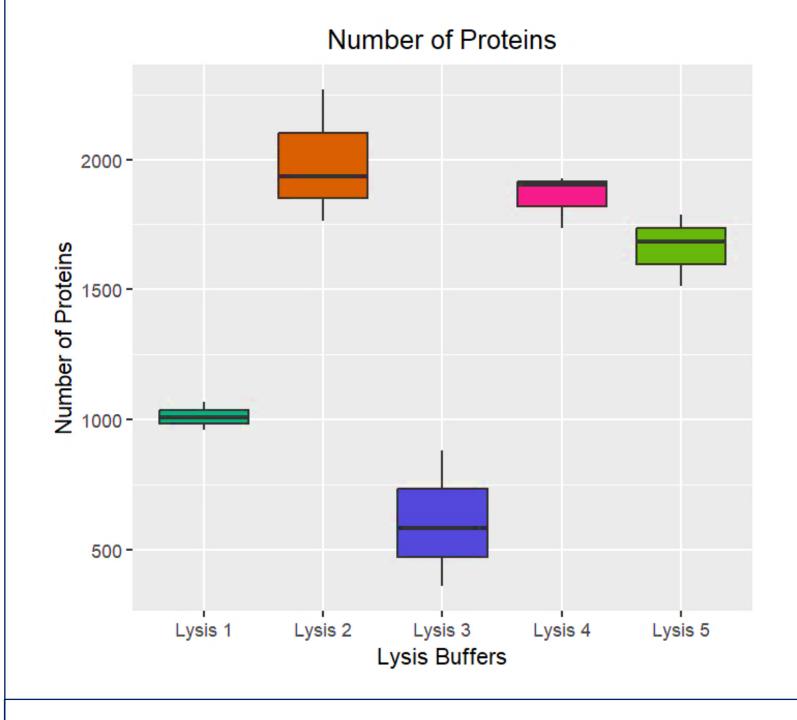
 $D = 10 \mu m \times 10 \mu m$ 

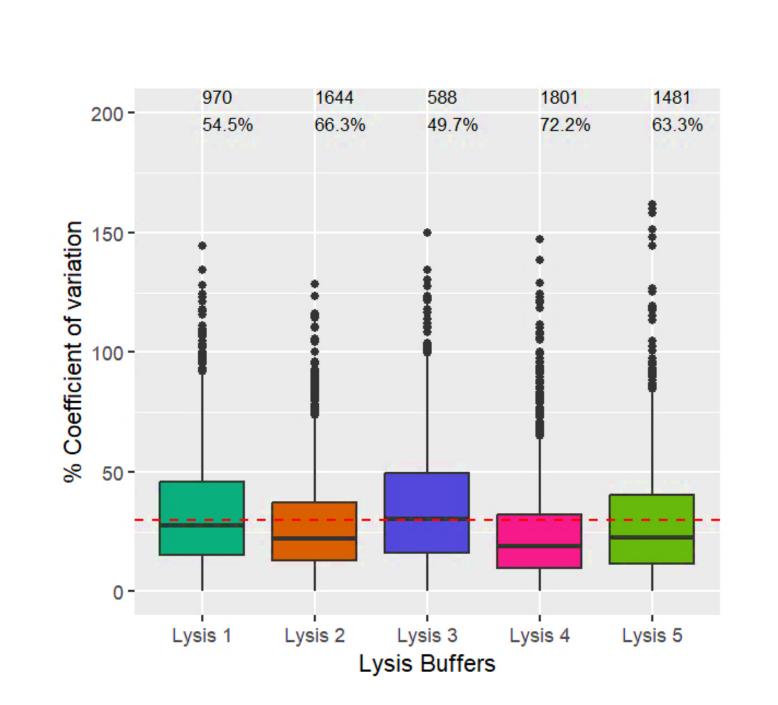
E= Outer brain (for normalisation)



## Results

#### 1. Protein Extraction

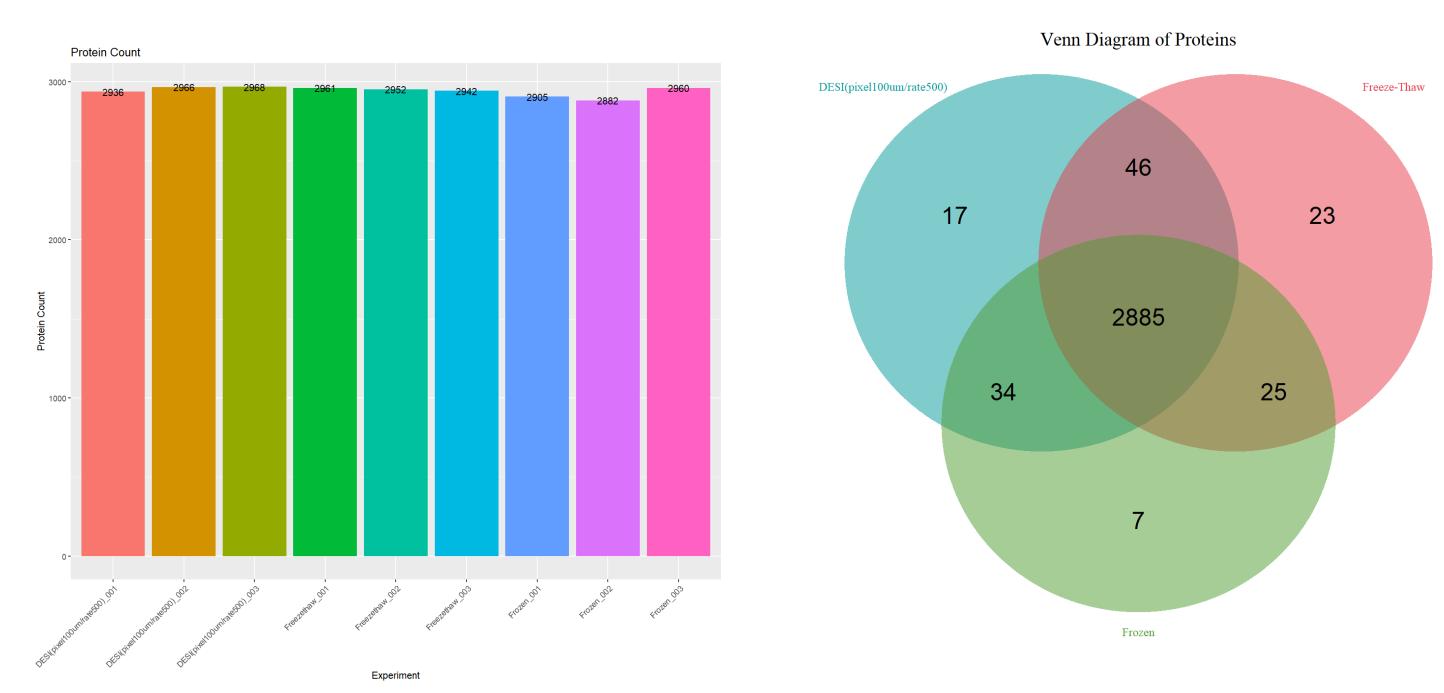




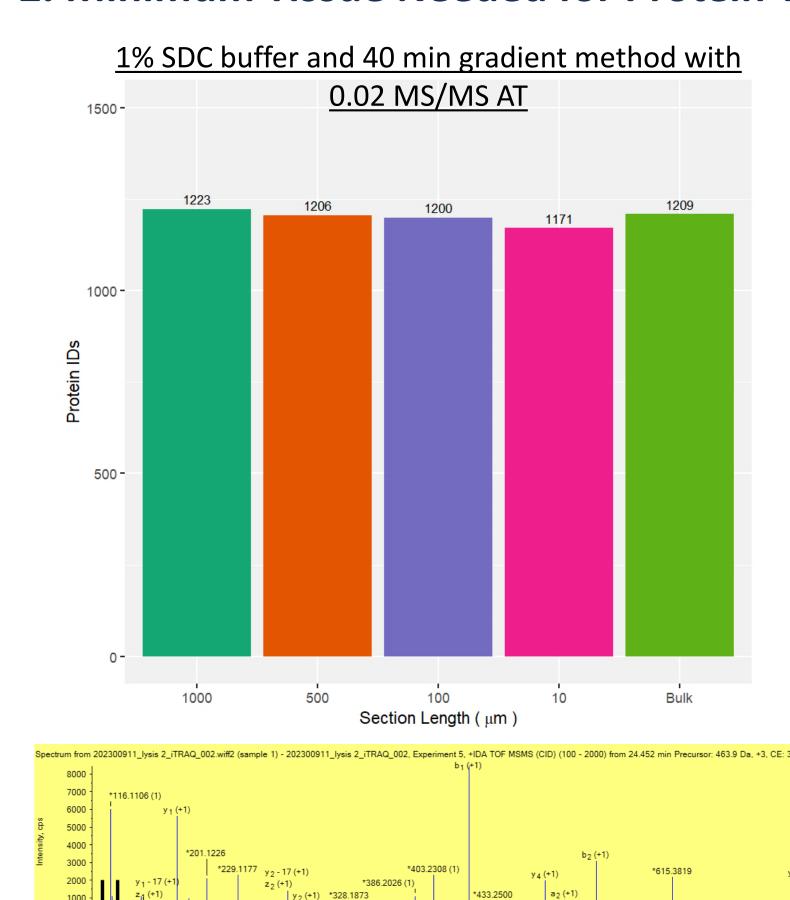
8 µm thick

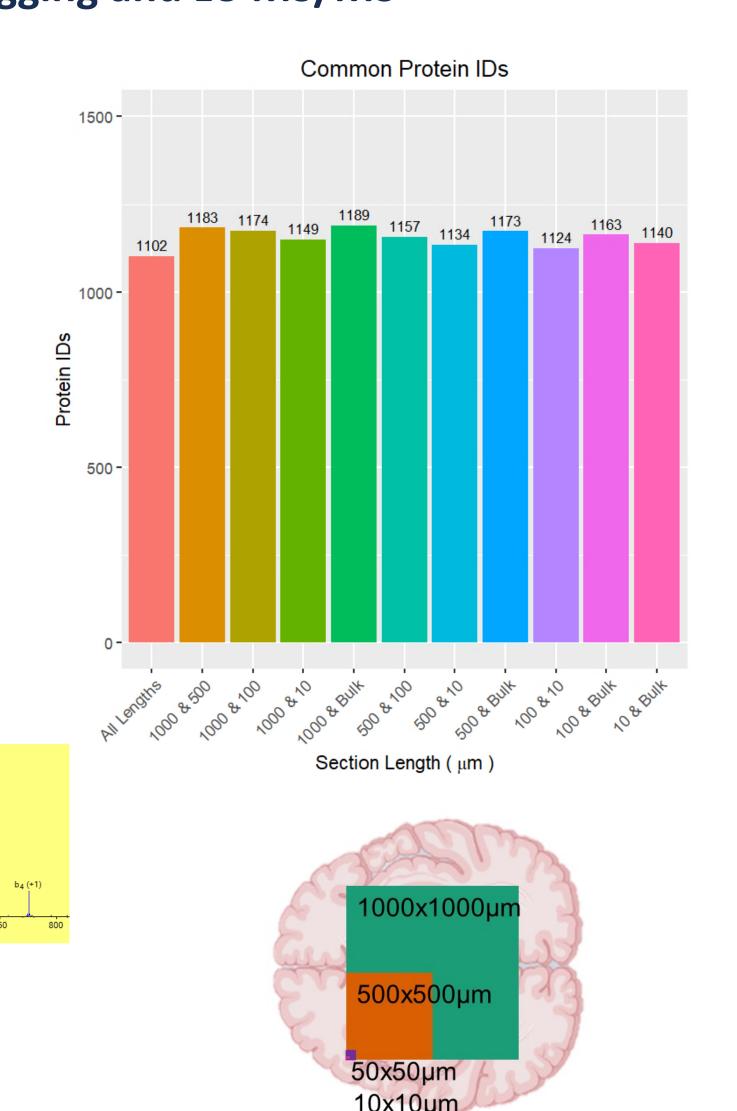
#### 3. DESI Spray Effects on Proteins

with DIA-NN

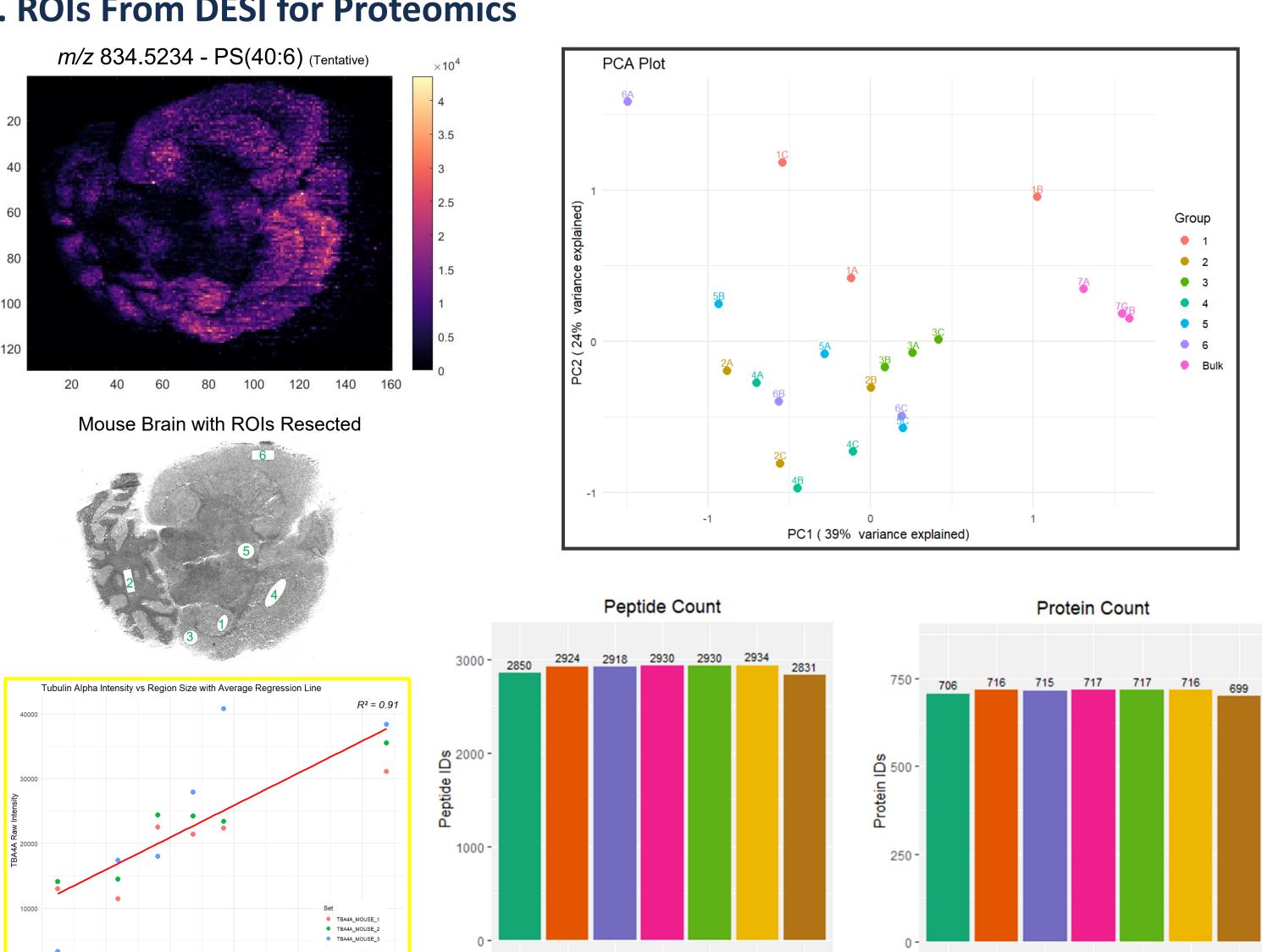


#### 2. Minimum Tissue Needed for Protein Tagging and LC-MS/MS





#### 4. ROIs From DESI for Proteomics



### Conclusion

1% sodium deoxycholate (SDC) was the optimal lysis buffer for efficient protein extraction in moues brain

Tubulin-α

- 2. Peptide identification was maximised with a 40-minute gradient, 0.02s MS/MS AT, and 50°C column temperature.
- 3. This combination gives the best results for analysing 10 µm x 10 µm (single cell) areas in mouse brain, helping to facilitate the identification of molecular signalling pathways associated with GBM in future work.
- DESI-MS is a soft ionisation technique used for MS1 analysis, with minimal impact on protein extraction.
- DESI-MS can be used to guide ROI selection, and regions can be precisely cut using LCM for targeted proteomics using iTRAQ

#### **Future work**

- Implement the optimised methods on GBM models.
- Enhance protein identification by leveraging nanoflow LC-MS to further increase sensitivity.

### Reference

1. Randall EC, Lopez BGC, Peng S, Regan MS, Abdelmoula WM, Basu SS, et al. Localized Metabolomic Gradients in Patient-Derived Xenograft Models of Glioblastoma. Cancer research. 2020;80(6):1258-67.