Generation of spectral libraries for murine cardiac proteomic studies using SWATH-MS

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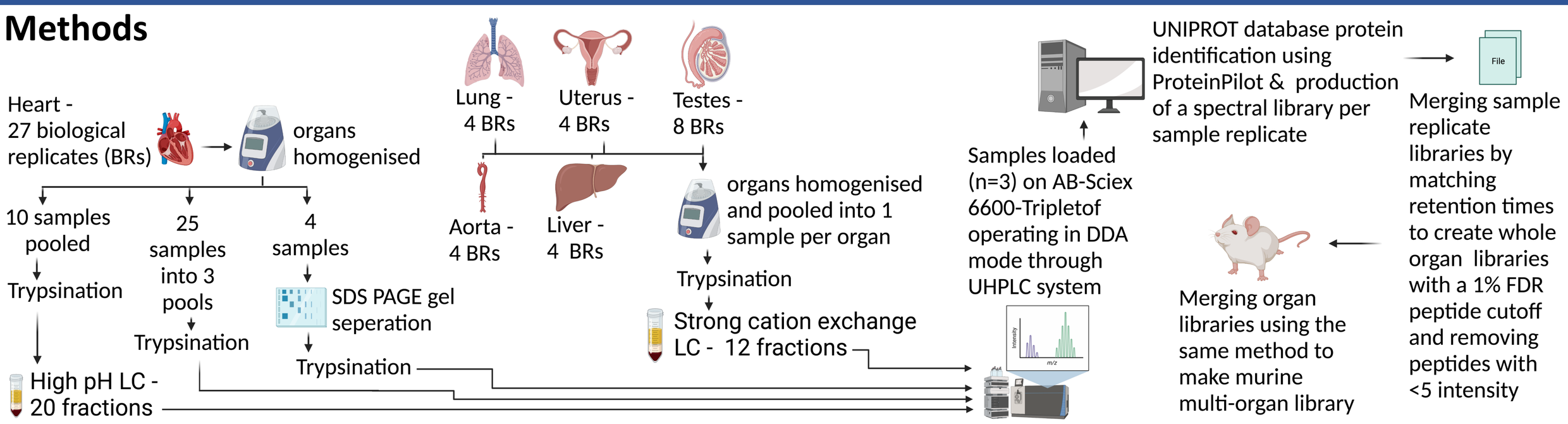
Introduction

Changes in the proteome can lead to phenotypic alterations; thus the study of proteins can help answer biological questions. Sequential window acquisition of all theoretical mass spectra (SWATH-MS) quantifies a broader range of peptides compared to data-dependent analysis (DDA) but requires a DDA spectral library for reference. It is unknown if an organ library or a multi-organ library that contains additional peptide/protein information is more sensitive in quantifying peptides, presenting a need for both libraries when analysing an organ sample. A lack of murine cardiac and multi-organ DDA libraries has limited SWATH-MS use in murine heart proteomic studies.

Aims

- Generate a heart spectral library representing a range of physiological conditions.
- Create a multi-organ murine library by generating extra organ libraries and merging libraries.
- Compare in-house multi-organ library with available public multi-organ libraries.

Methods



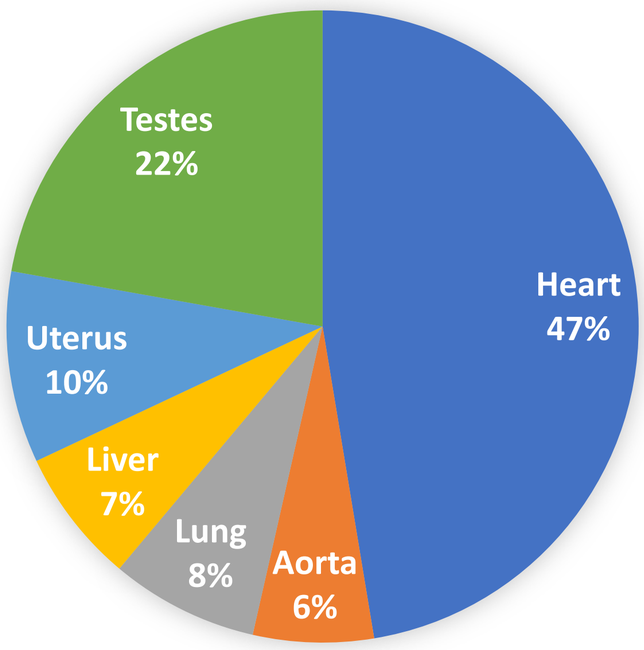
Results and discussion

Organ spectral library outcomes

	Heart	Aorta	Lung	Liver	Uterus	Testes
Number of unique peptides	65955	36764	36612	37397	54056	52464
Number of unique proteins	6574	3795	5117	4478	5927	6977

Murine heart, testes and uterus spectral libraries were generated for the first time. The heart library contained the most peptides, whereas the testes library contained the most proteins.

Distribution of tissue-specific proteins uniquely found in each tissue



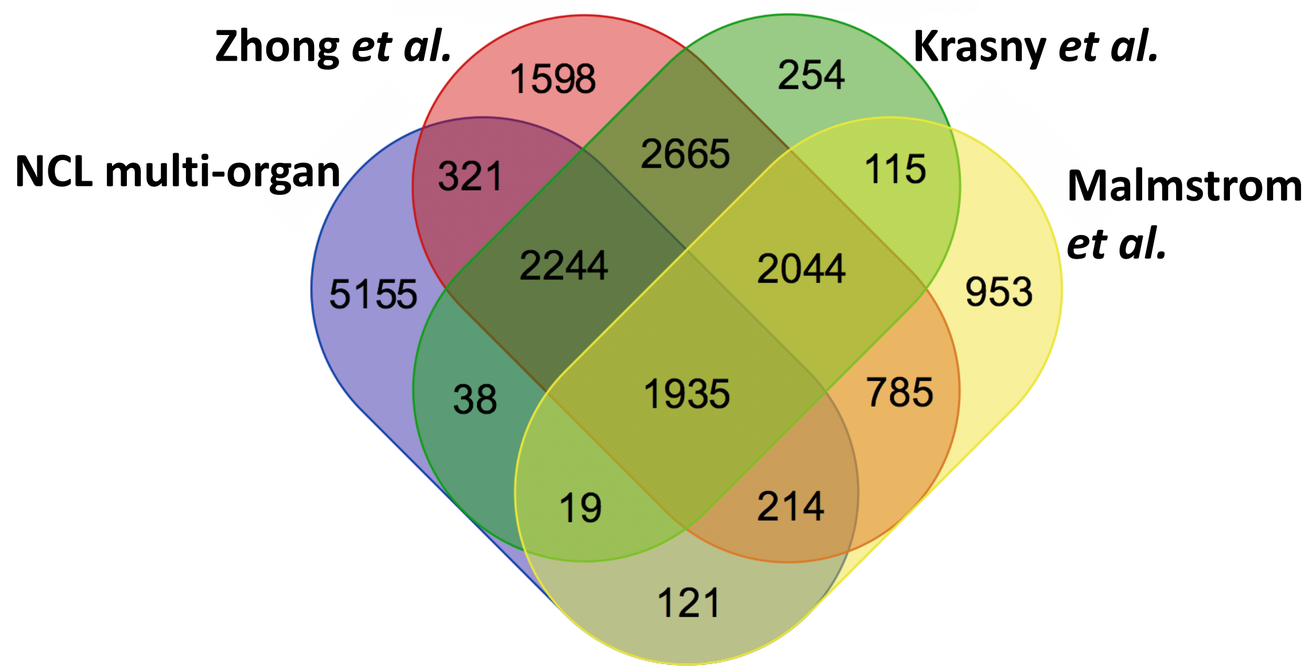
The heart library contributes the highest number of unique proteins to the multi-organ library likely due to more biological and technical replicates used compared to other organs. Conversely, the aorta library contributes the least unique proteins, likely due to large smooth muscle and cardiovascular proteomes shared by other organs. Additionally, while the testes contains the most proteins, many of these are also found within the other libraries.

In-house multi-organ library outcomes and comparison with external multi-organ libraries.[1][2][3]

	NCL murine multi-organ library	Krasny *et al.*	Zhong *et al.*	Malmstrom *et al.*
Number of unique peptides	134528	102500	236449	66857
Number of unique proteins	10046	9312	11804	6185
Proteome coverage	59%	55%	69%	39%

After merging organ libraries, the NCL multi-organ library covered 59% of the murine proteome, making it the 2nd largest library. Zhong *et al.* is the largest library, likely due to using ~12 biological replicates per organ more than the NCL library.

Comparison of all murine multi-organ libraries.[1][2][3]



The in-house multi-organ library contains the most proteins not present within other multi-organ murine libraries. The next step would be to merge these libraries using the raw data that is available in the Zhong *et al.* and Krasny *et al.* library repositories to create a multi-organ library with a higher proteome coverage.

Conclusion

The NCL spectral library dataset enables a researcher to investigate the murine heart proteome using SWATH-MS with both; a comprehensive cardiac-specific library and the 2nd largest multi-organ library.

References

1) Malmström E, Kilsgård O, Hauri S, Smeds E, Herwald H, Malmström L, *et al.* Large-scale inference of protein tissue origin in gram-positive sepsis plasma using quantitative targeted proteomics. **Nat Commun**. 2016;**7**.

2) Krasny L, Bland P, Burns J, Lima NC, Harrison PT, Pacini L, *et al.* A mouse SWATH-mass spectrometry reference spectral library enables deconvolution of species-specific proteomic alterations in human tumour xenografts. **DMM Dis Model Mech**. 2020;13(**7**).

3) Zhong CQ, Wu J, Qiu X, Chen X, Xie C, Han J. Generation of a murine SWATH-MS spectral library to quantify more than 11,000 proteins. **Sci Data**. 2020;7(**1**):1–9.