Improved Protein Extraction and Identification from Archival Formalin-fixed Paraffin-embedded Human Aortas using High Pressure and Heat

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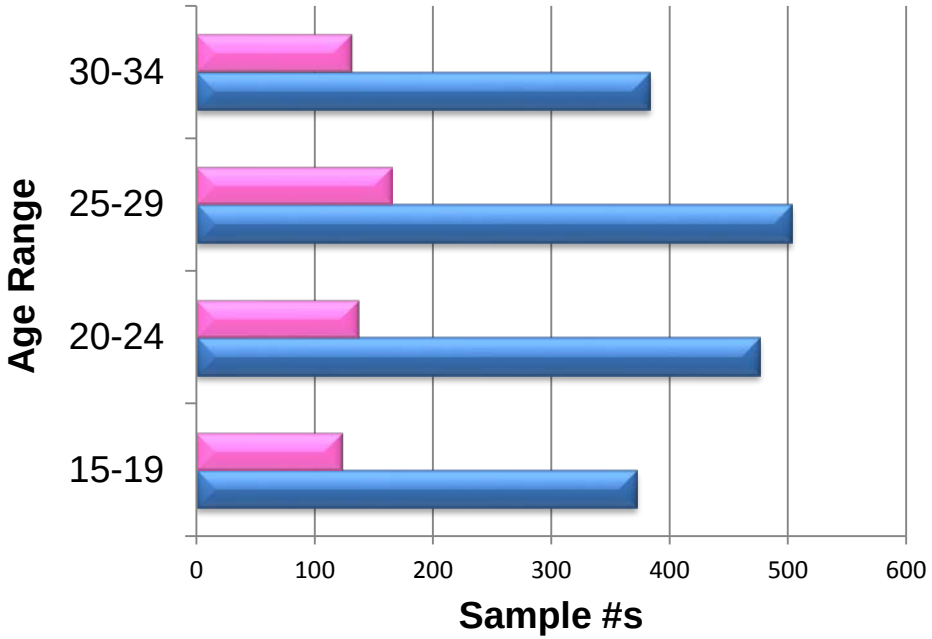
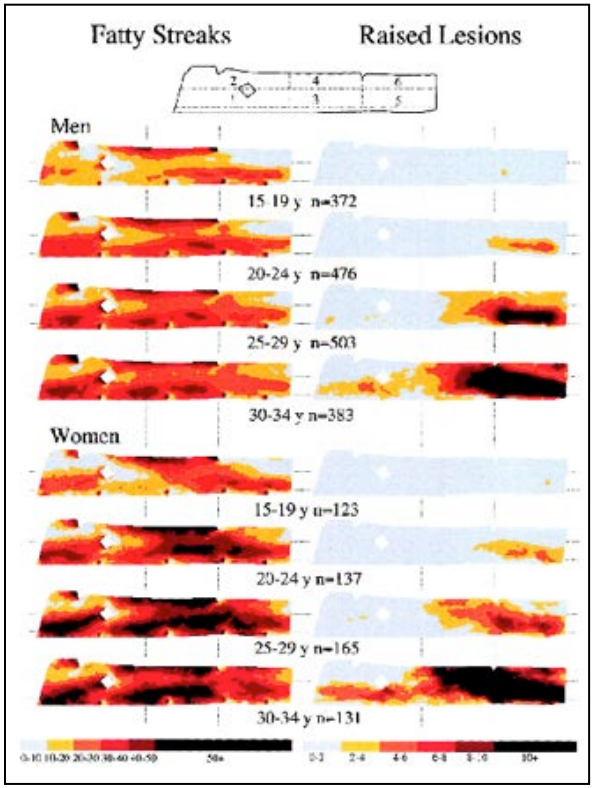
Relationship of Atherosclerosis in Young Men to Serum Lipoprotein Cholesterol Concentrations and Smoking

Cohort: The Pathobiological Determinants of Atherosclerosis in Youth (PDAY)



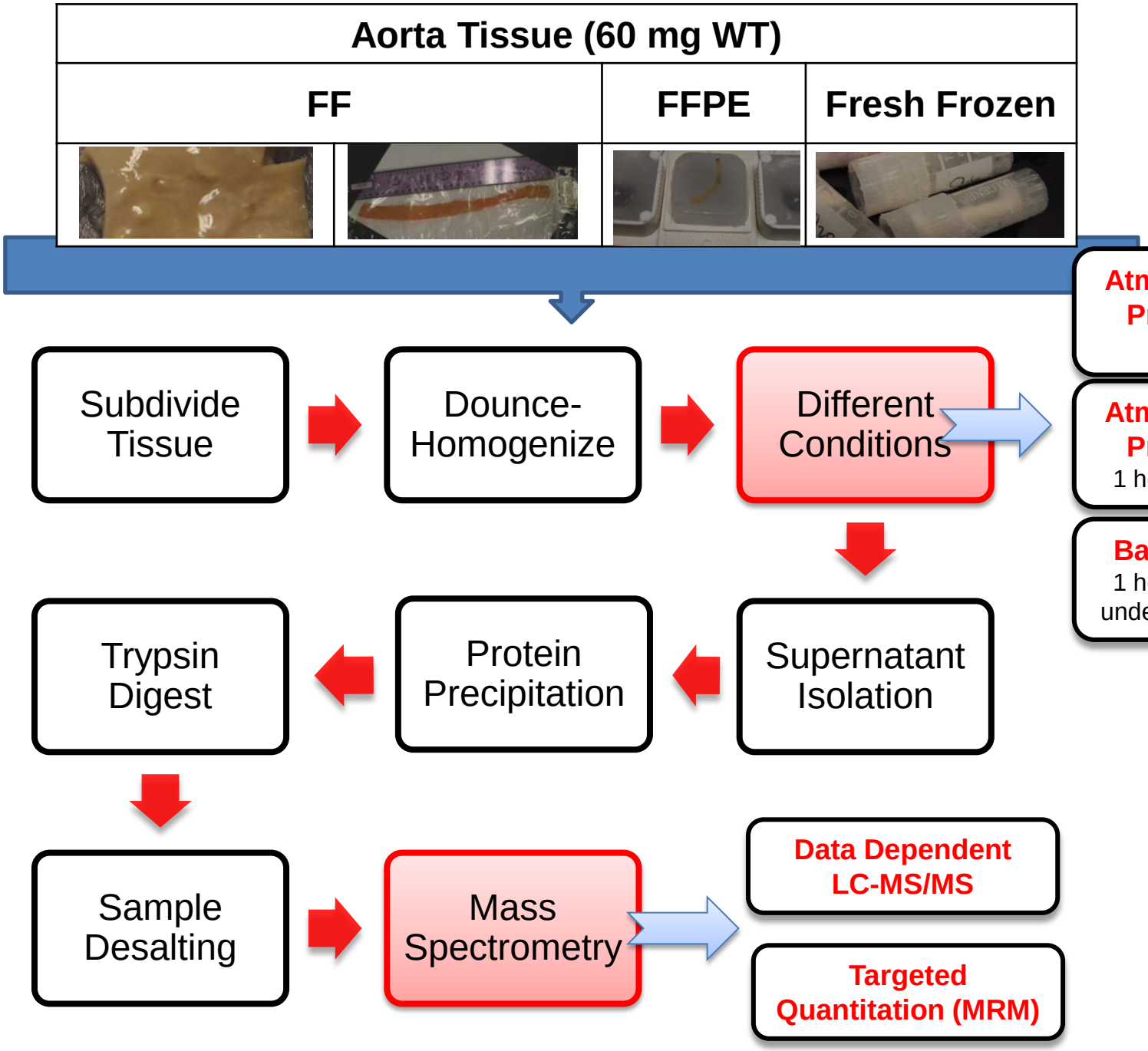


JAMA. 1990, Dec.; 264(23)

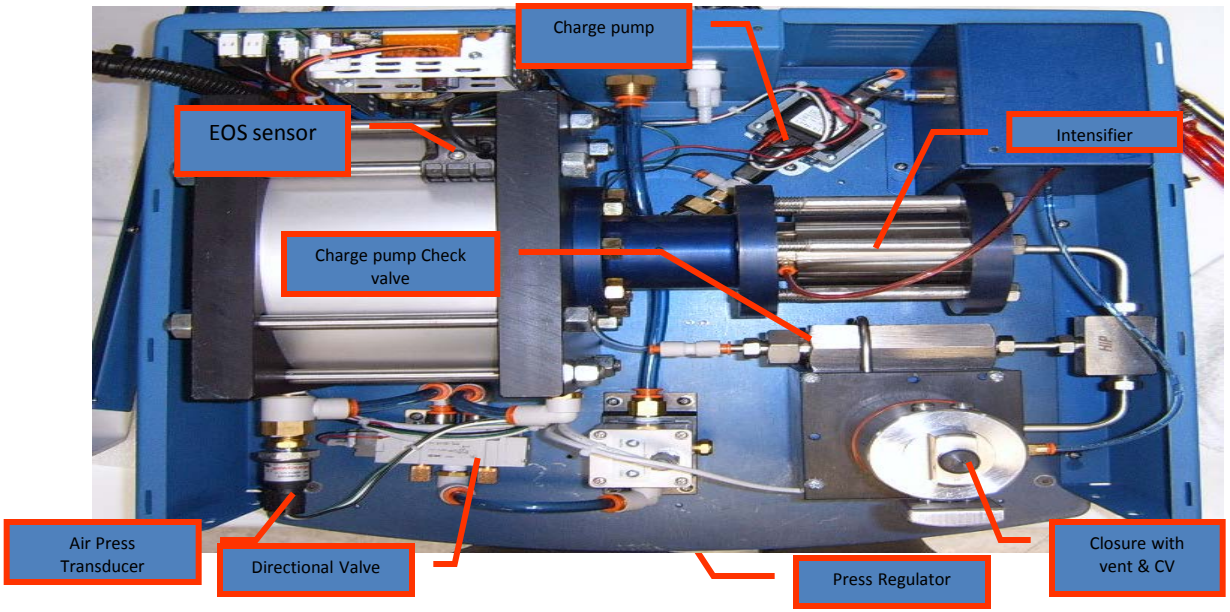
Study Objectives

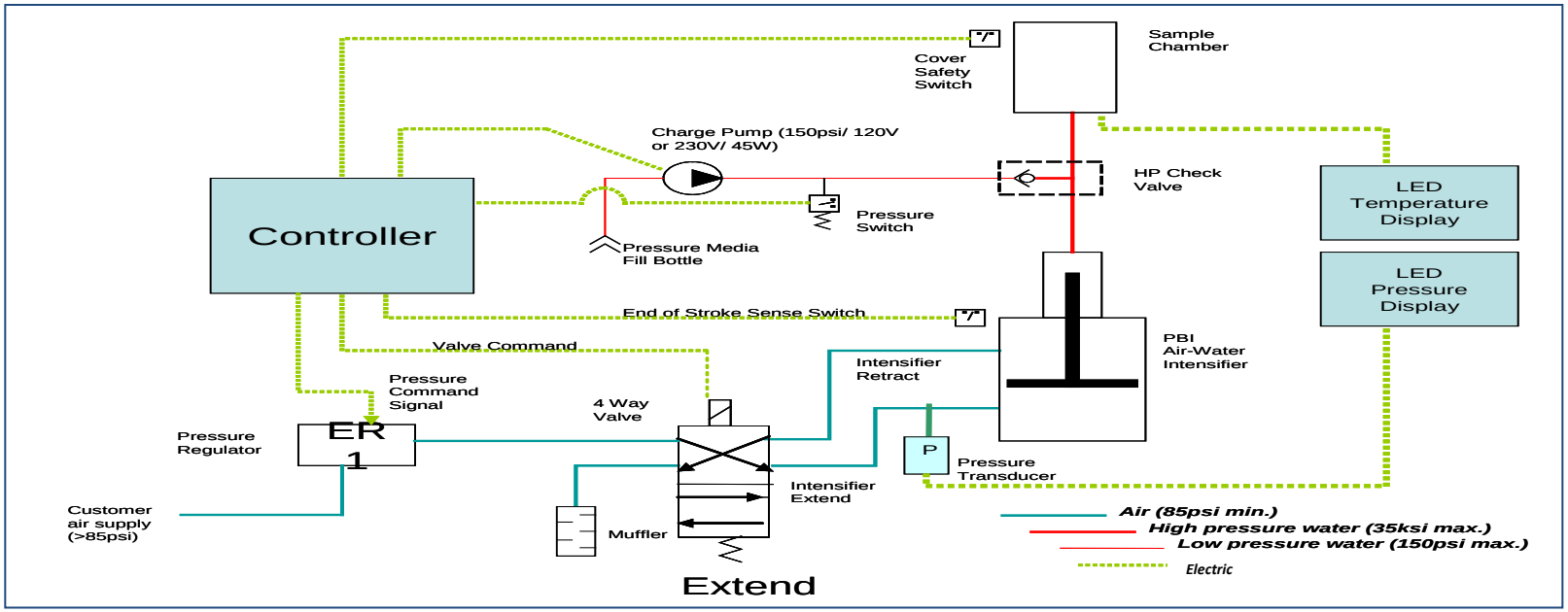
- To optimize PROTEIN EXTRACTION from FFPE Aorta for biomarker discovery
- To evaluate QUALITY of PDAY aorta samples

Sample Type	Sample #	Harvest condition	Storage Time	Origin
unfixed	3	**Fresh Frozen** (-80ºC)	- 3 months - 2 years	Johns Hopkins University Hospital
FF	3	**Formalin-fixed**	3 months	
	3		15 years	PDAY
FFPE	2	**Formalin-fixed, paraffin-embedded**	15 years	


Aorta Tissue (60 mg WT)
FF
FFPE
Fresh Frozen
Subdivide Tissue
Dounce-Homogenize
Different Conditions
Atmospheric Pressure: 25°C
Atmospheric Pressure: 1 hour at 95°C
Barocycler: 1 hour at 95°C under 40,000 psi
Supernatant Isolation
Protein Precipitation
Trypsin Digest
Sample Desalting
Mass Spectrometry
Data Dependent LC-MS/MS
Targeted Quantitation (MRM)

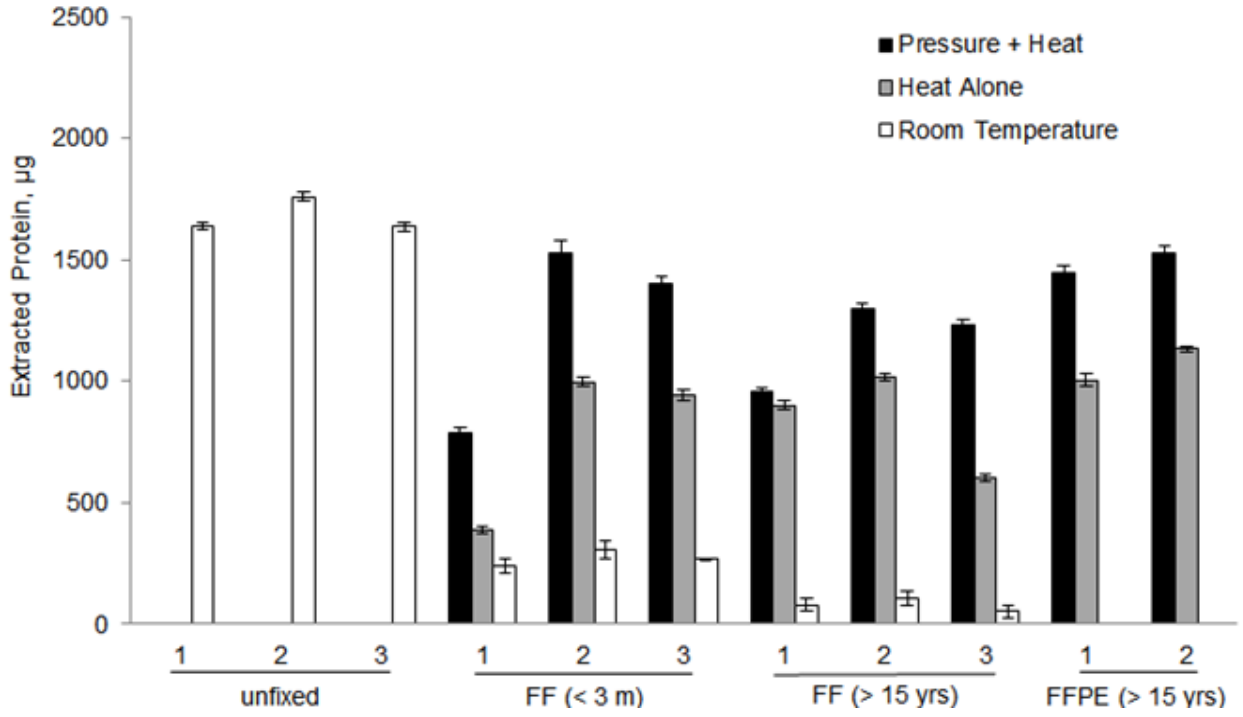
Pressure Generation





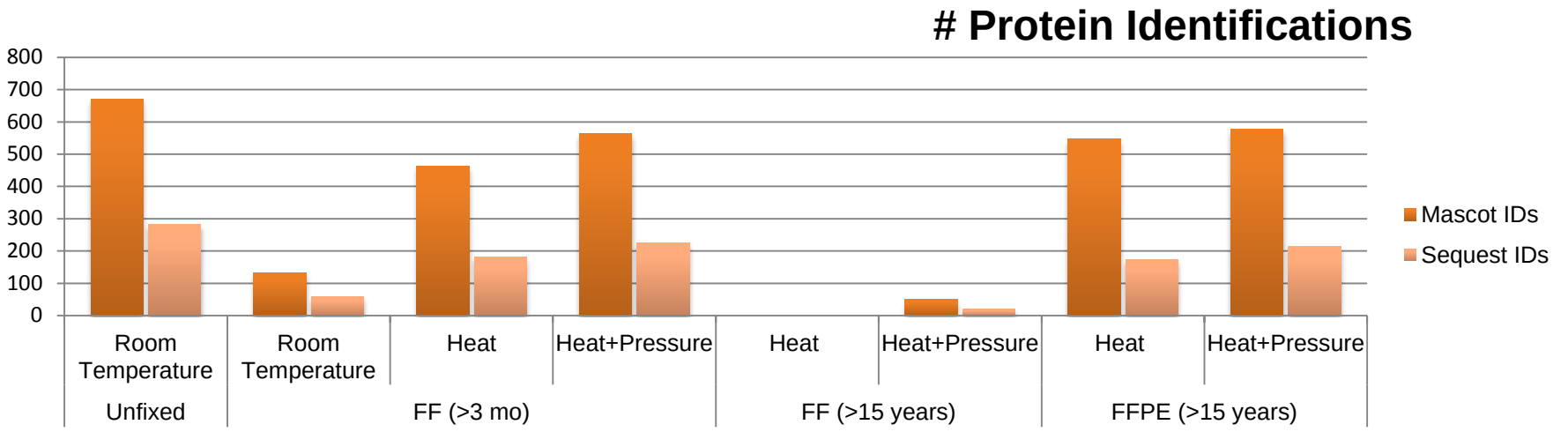
Experimental Impact
FFPE Sample Preparation Conditions

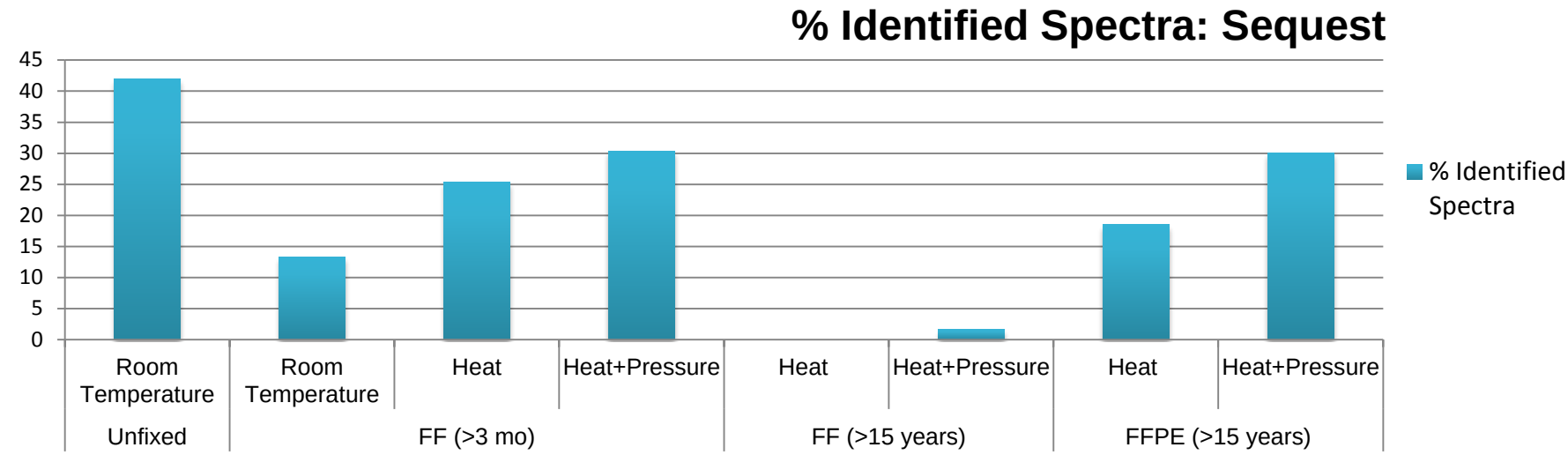
PRESSURE and HEAT improve Extraction Yield



***All proteins were extracted from 60 mg of wet tissues except the FFPE aortas were from 20, and 31mg and normalized to 60 mg for comparison.**

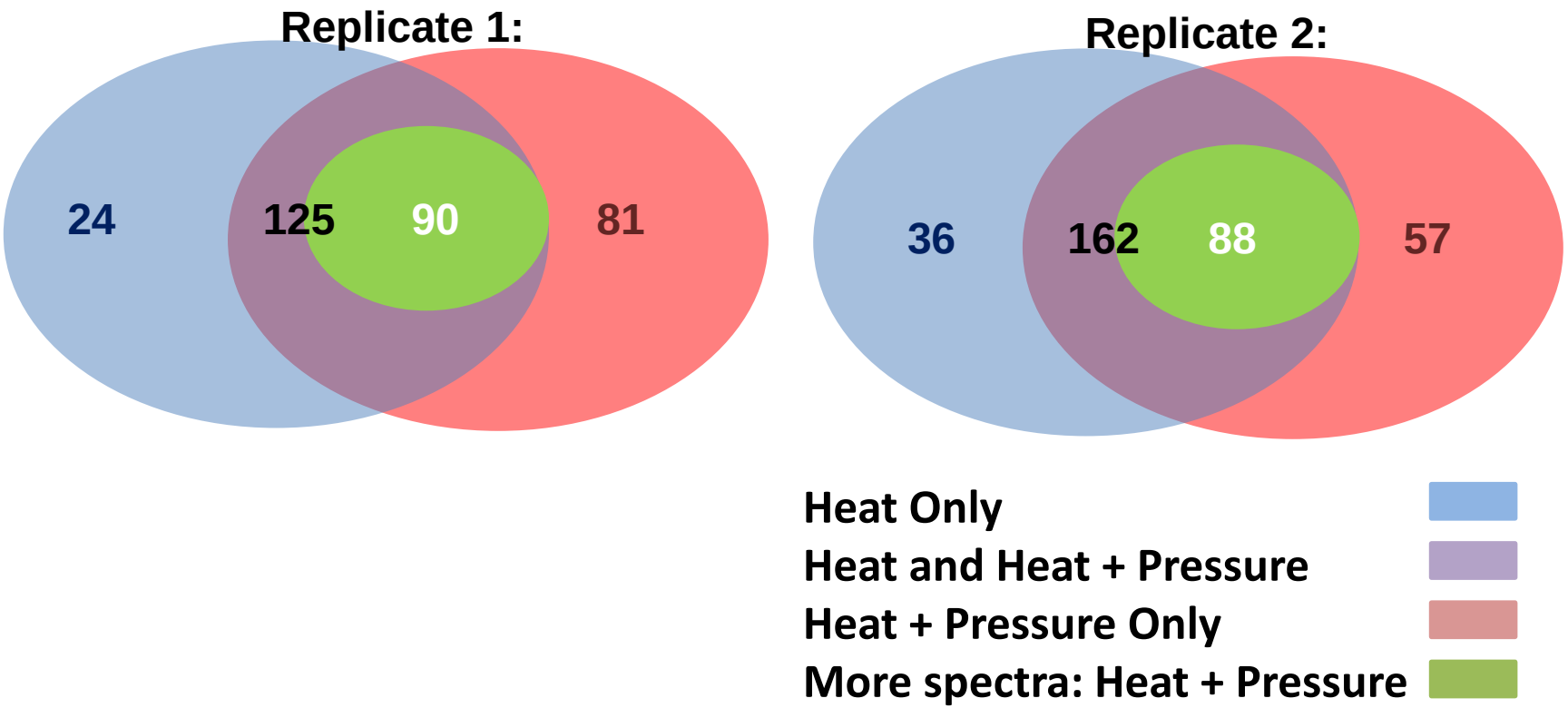
Experimental Impact
FFPE Sample Preparation Conditions





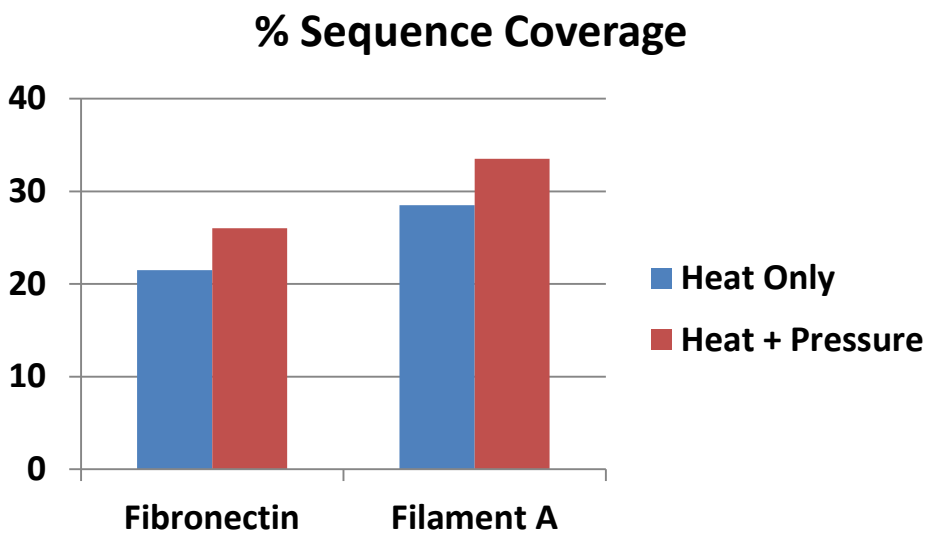
Experimental Impact
FFPE Sample Preparation Conditions

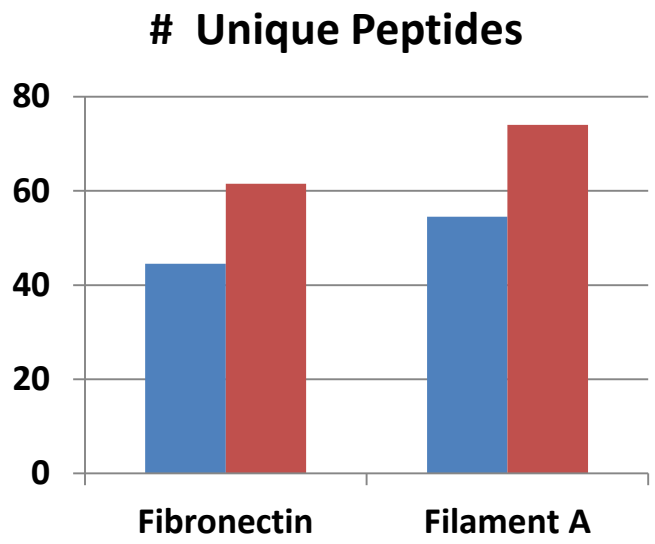
PRESSURE improves protein identification number and yield more spectra for some proteins in FFPE samples



Experimental Impact
FFPE Sample Preparation Conditions

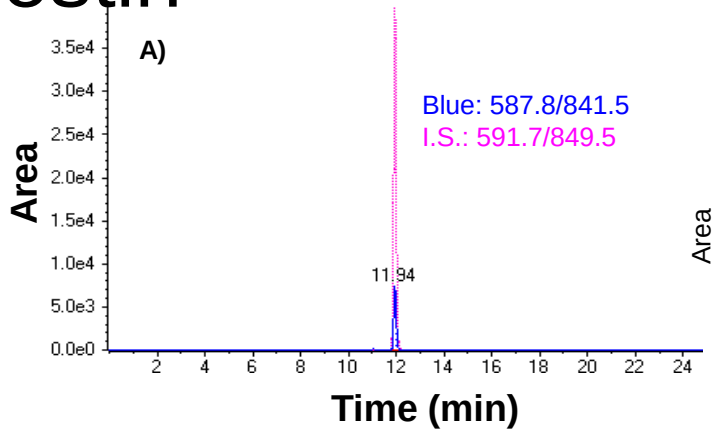
PRESSURE and HEAT improve Sequence Coverage

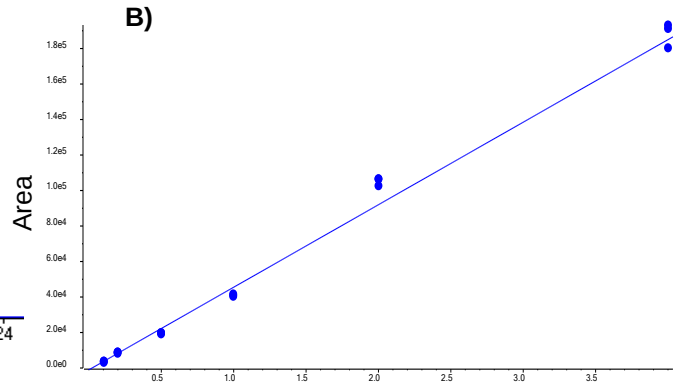


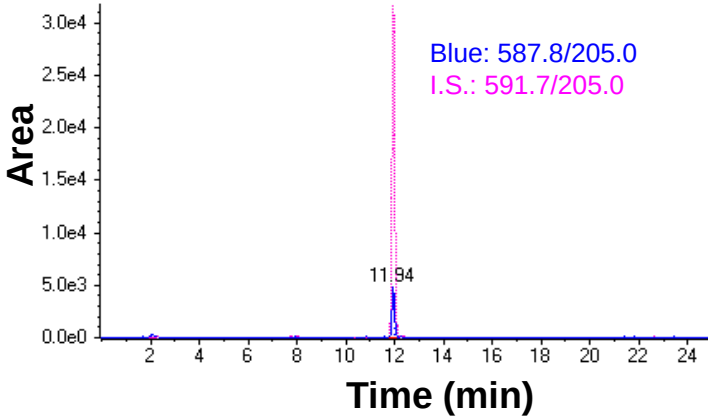


Quantitative SRM Assay

Periostin







C)

	S/N	In Buffer *	In Aorta Extract *
LLOD	3	34.84 pg	41.03 pg
LLOQ	10	64.21 pg	91.05 pg

***Amount of periostin on column**

D)

No.	Peptides	Transition	Ion	R.T. (min)	CE
1	GFEPGVTNILK	587.83 / 205.0	2b2	12.1	31
		587.83 / 841.51	2y8		26
		587.83 / 970.56	2y9		26
2	IIDGVPVEITEK	656.87 / 815.45	2y7	11.5	29
		656.87 / 971.54	2y9		29
		656.87 / 1086.57	2y10		29

Conclusions

- High PRESSURE and HEAT improves total protein extraction and identification from FF and FFPE aortic tissue
- FFPE tissue is better preserved than FF long term

Acknowledgements

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New Model of PBI Instrument

HUB 440