

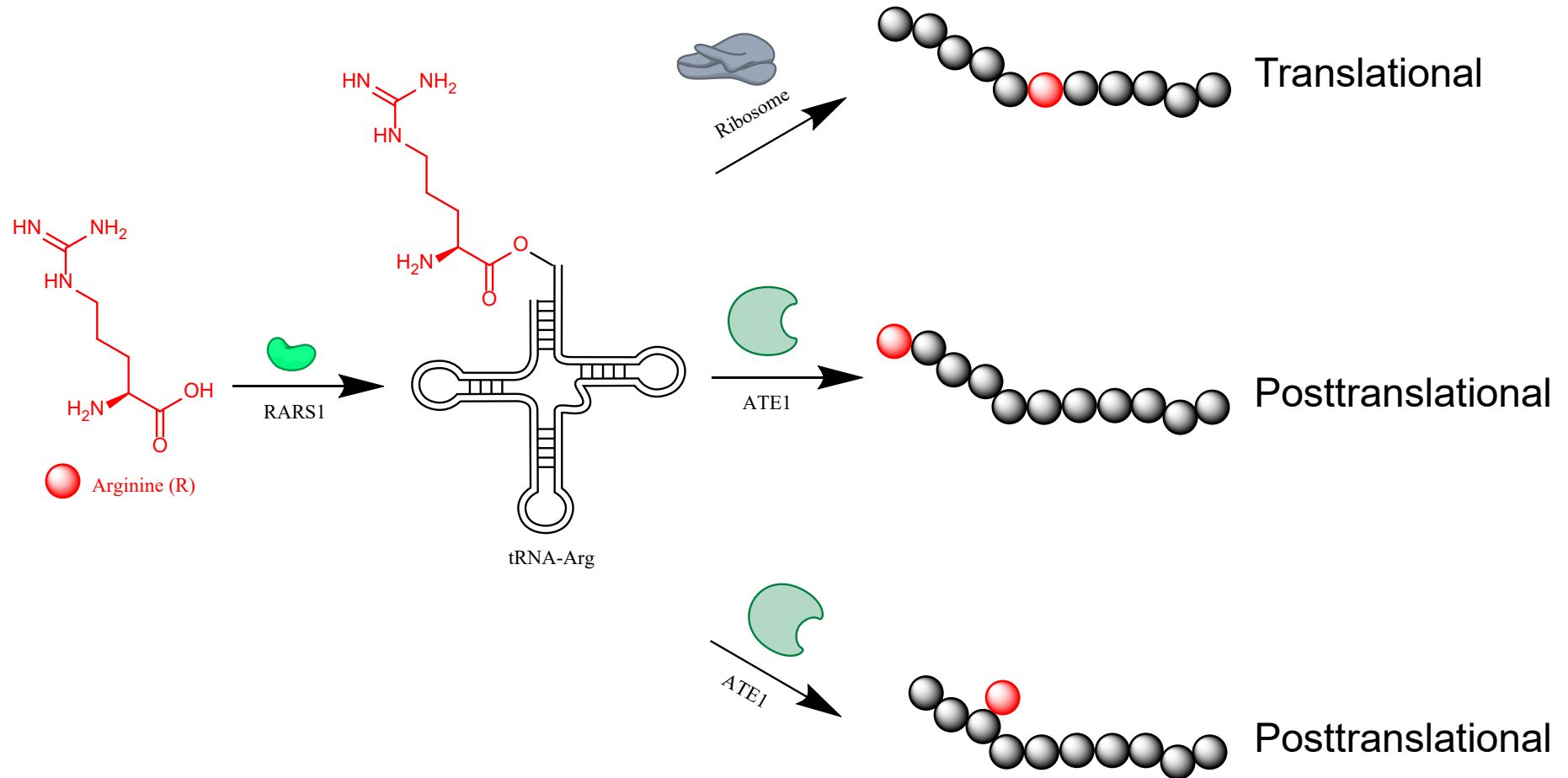
# An Unbiased Proteomics Method to Discover Posttranslational Arginylation Sites from Whole Proteomes

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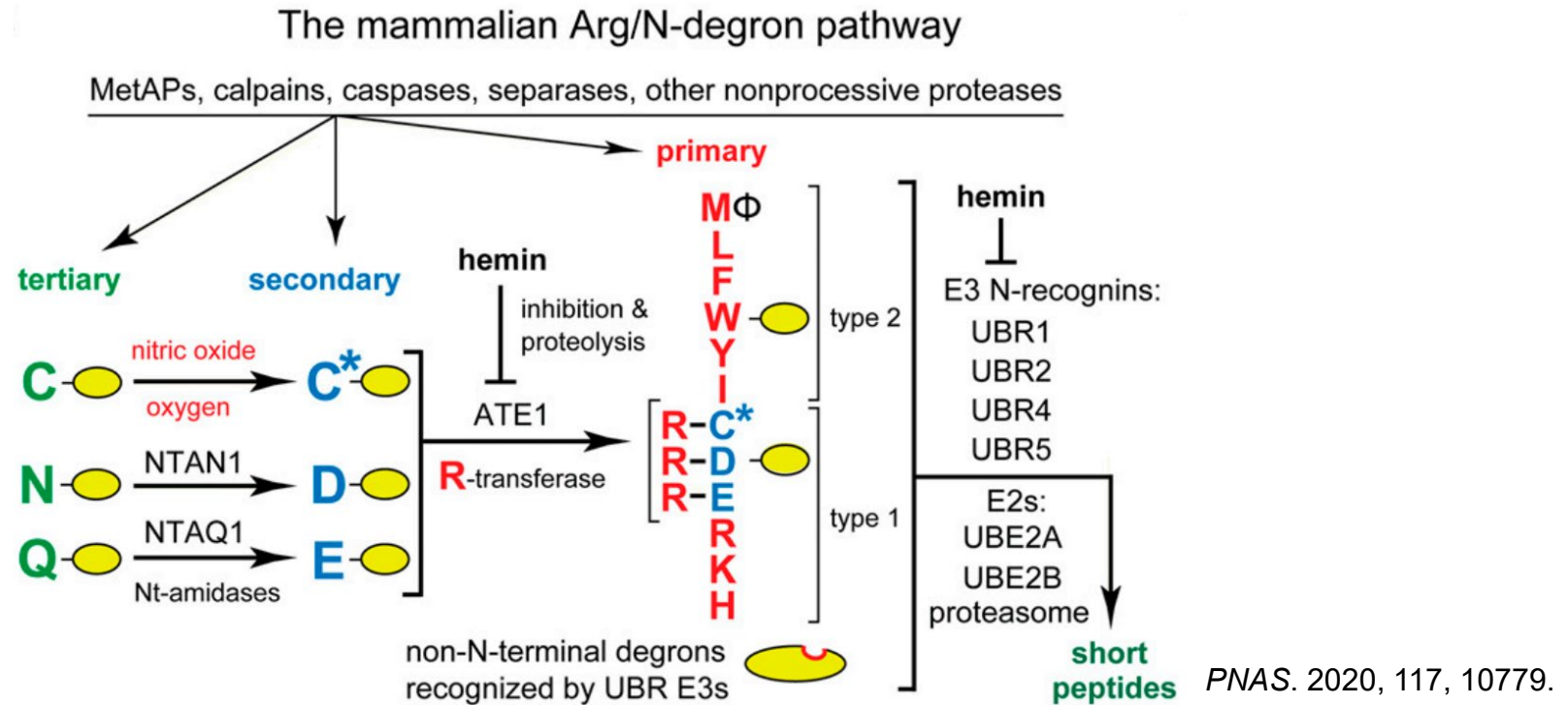
Skyline User Meeting @ ASMS 2023  
2023. 06. 04

# Posttranslational Arginylation and Its Functions



ATE1 KO causes embryonic lethality in mice.

# Canonical and Non-canonical Biology of Arginylation

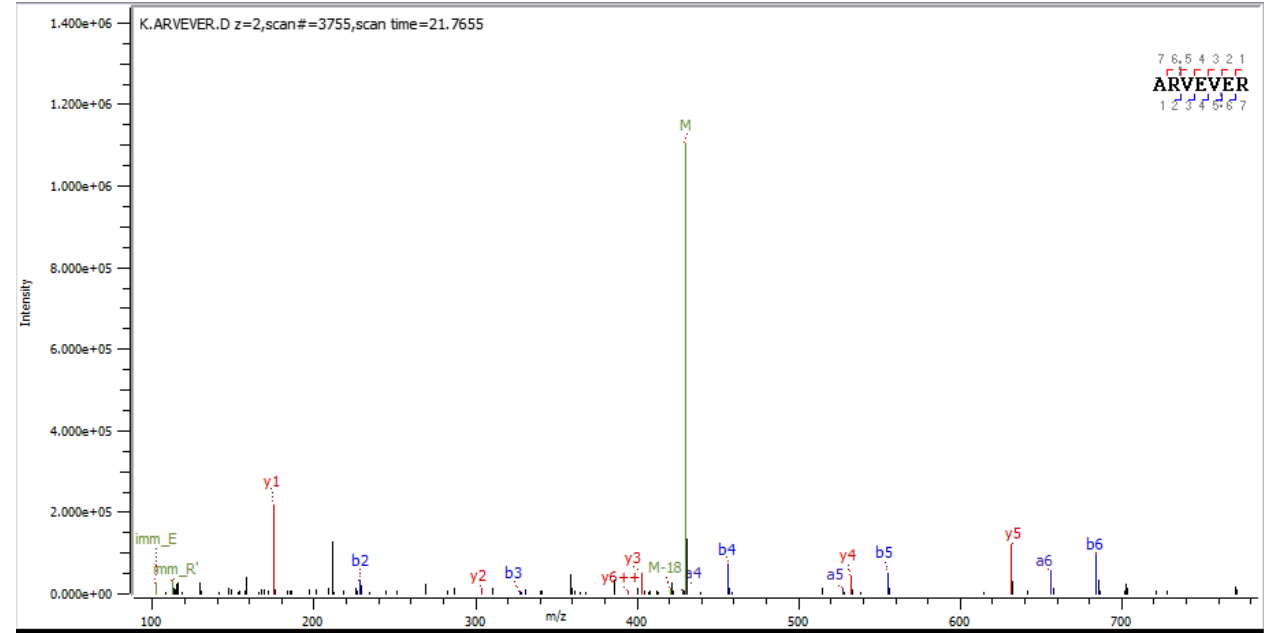
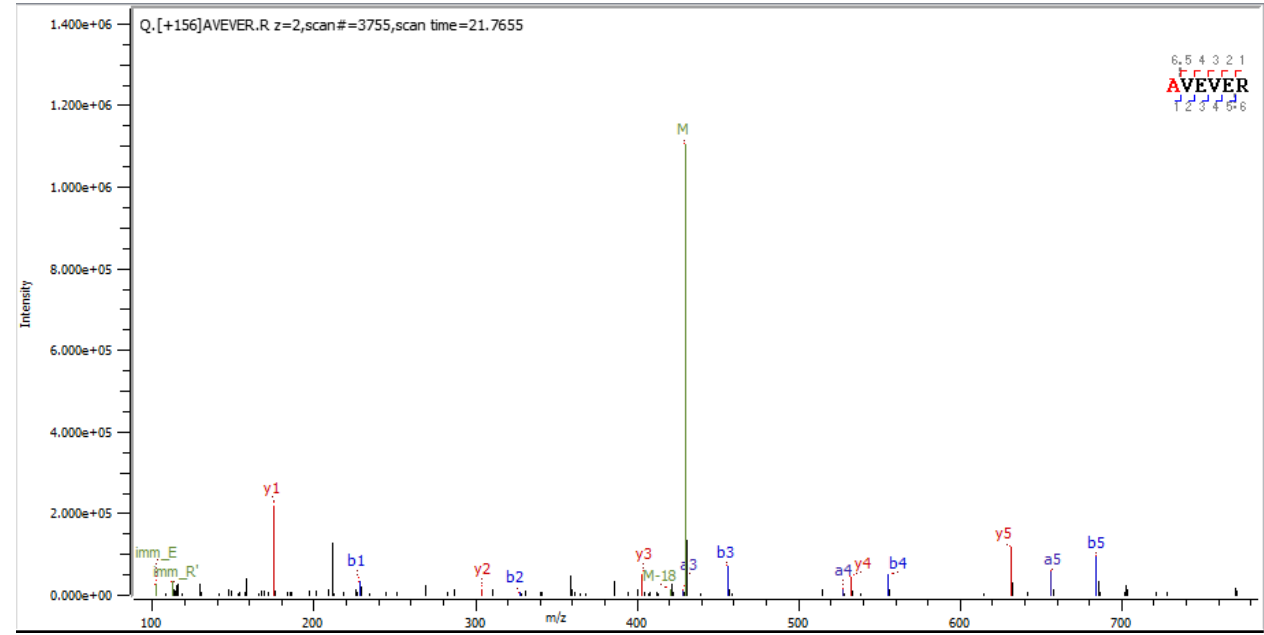


Protein	Site	Function	Reference
CALR	R-D18---	Regulate stress granules	<i>Nat Cell Biol.</i> 2015, 17, 917.
RGS4/5/16	R-C2(O <sub>3</sub> )---	Sense NO and O <sub>2</sub>	<i>Science.</i> 2002, 297, 96.
SNCA	---E46 <sup>R</sup> --- ---E83 <sup>R</sup> ---	Prevent neurodegeneration	<i>Sci Rep.</i> 2017, 7, 11323.
HSPA5 (BiP)	R-E19---	Degrade AKT	<i>Nat Cell Biol.</i> 2015, 17, 917.
BRCA1	R-D1156---		
CDC6	R-D101---	Modulate autophagy proteolysis	<i>PNAS.</i> 2018, 115, E2716.
PDI	R-D18---		
ACTB	R-D3---	Regulate cell cytoskeleton and migration	<i>Science.</i> 2006, 313, 192.

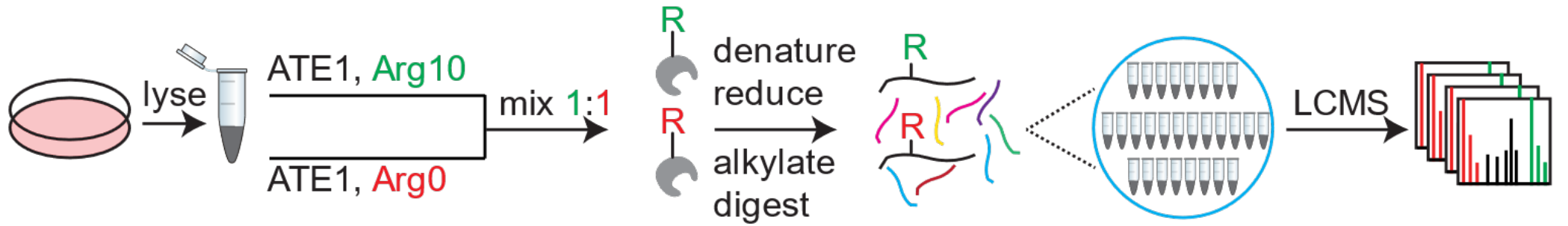
# Challenges to Discover Arginylation

PTM? [156]YNIPHGPVVGSTR  
or  
Miss cleavage? RYNIPHGPVVGSTR [156] = R

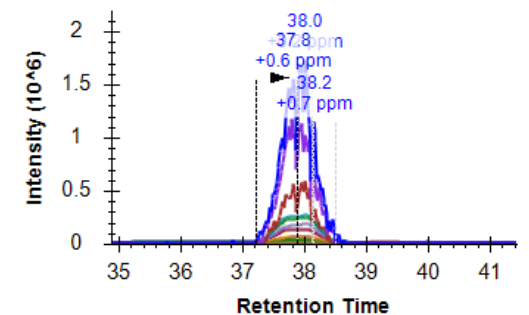
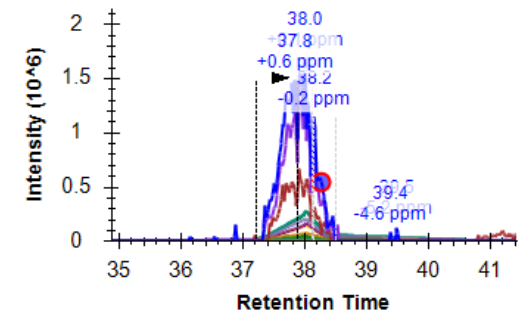
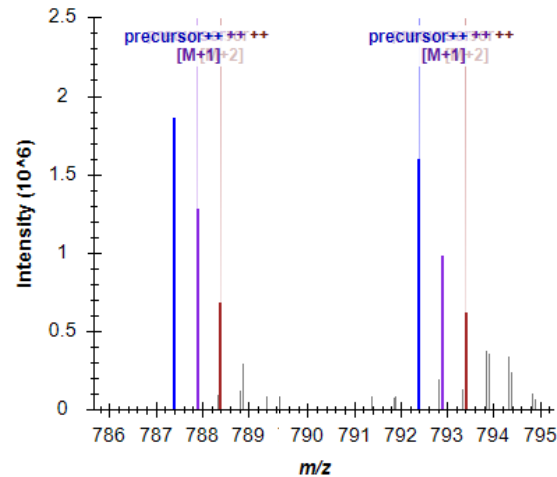
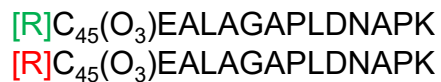
PTM? [156]AVEVER  
or  
Miss cleavage? ARVEVER  
Proteomics bias



# Unbiased Arginylome Profiling Using Isotopic Arg

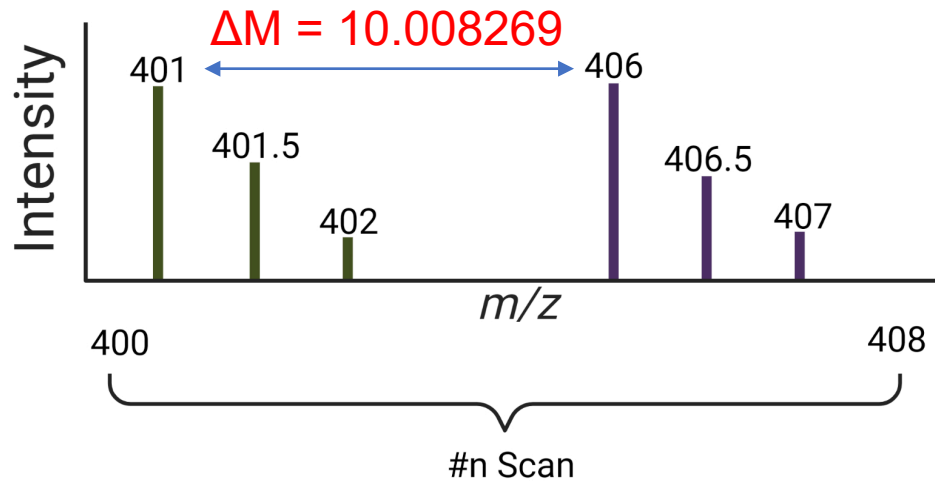


## RM12 (example)

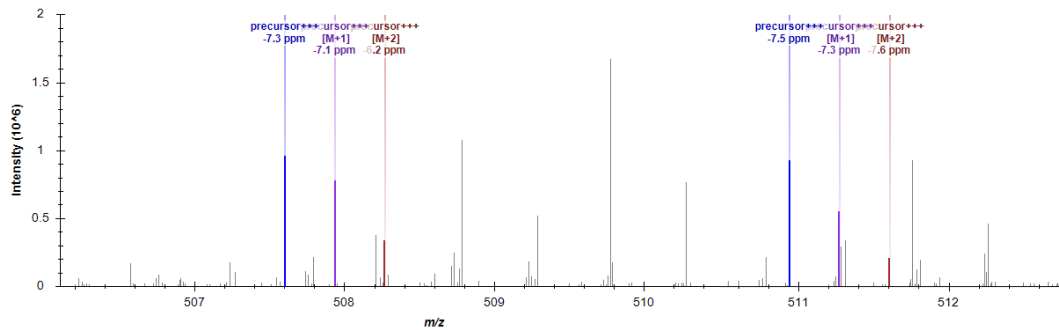




# DIA-Skyline Strategy for Arginylation Discovery

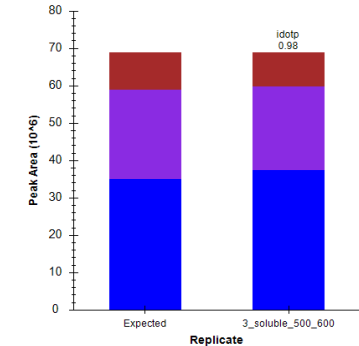
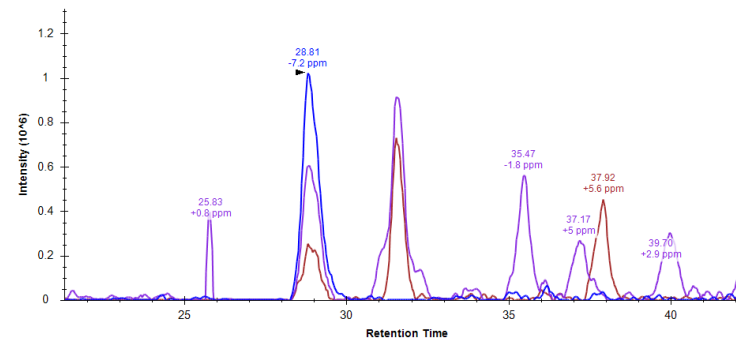
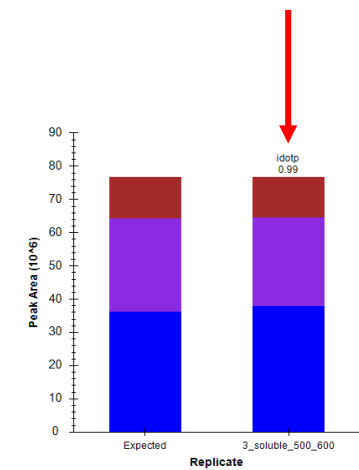
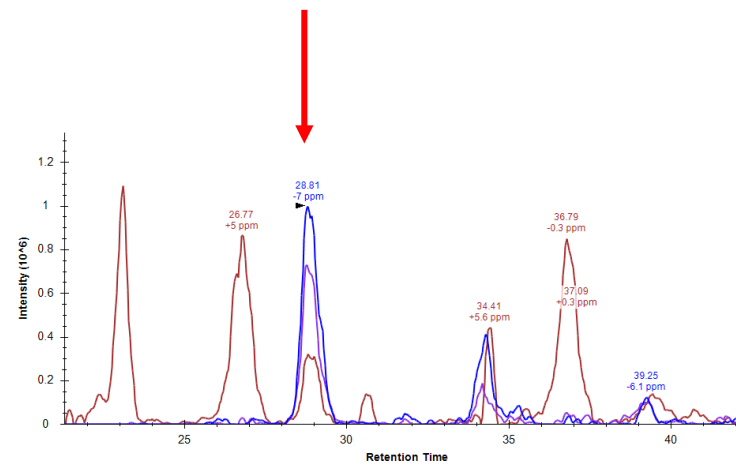
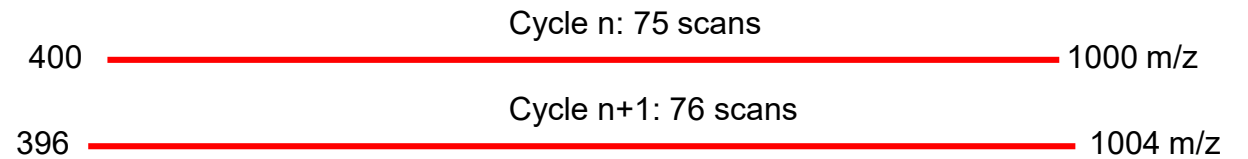


SSBP1 (example) [R]ESETTSLVLER  
[R]ESETTSLVLER



$\Delta M = 10.008269$

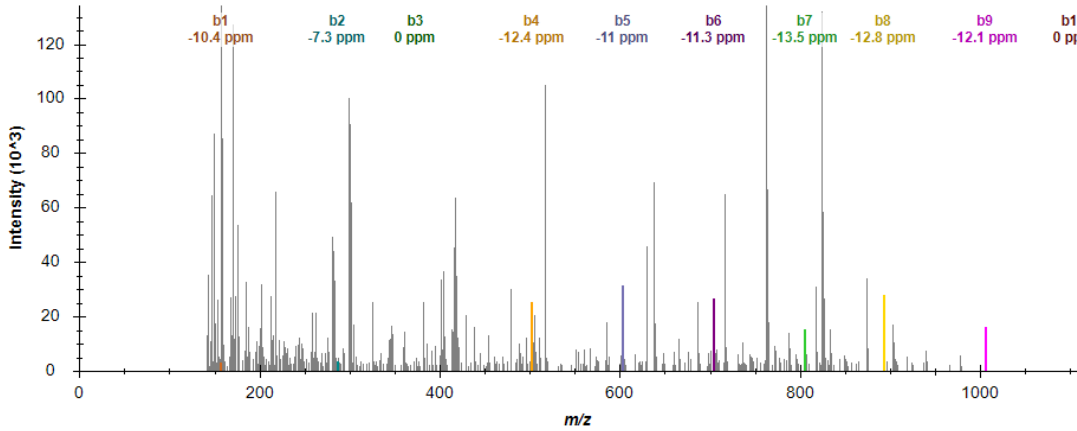
Stagger DIA Strategy, Window 8 m/z



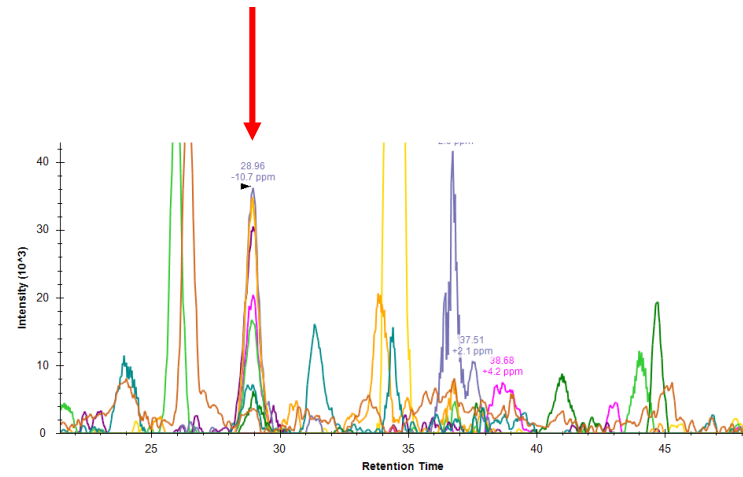
# DIA-Skyline Strategy for Arginylation Discovery

[R]E|S|E|T|T|T|S|L|V|L|R  
 b1 b2 ... b12

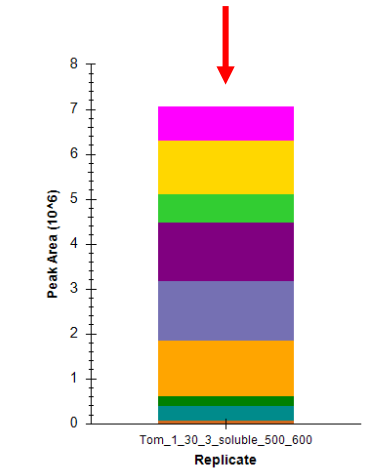
20230517\_Tom\_1\_30\_3\_soluble\_500\_600.raw (28.90 min) Scan Number: 0.1.20355



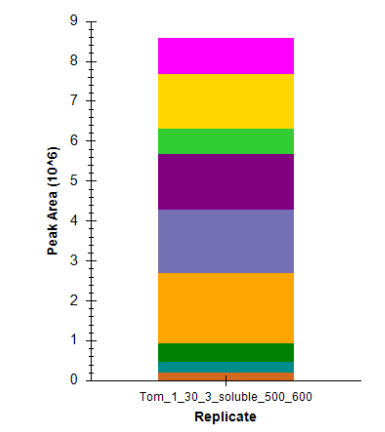
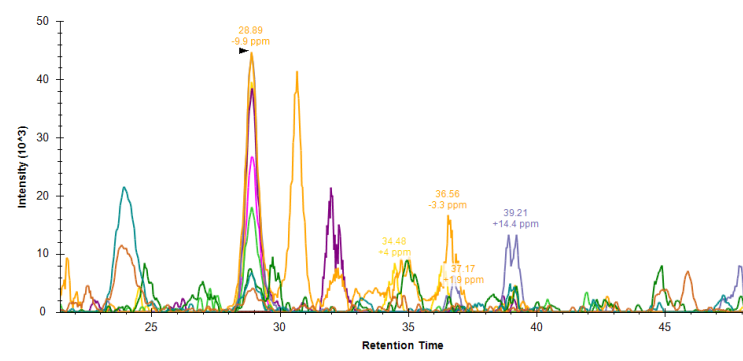
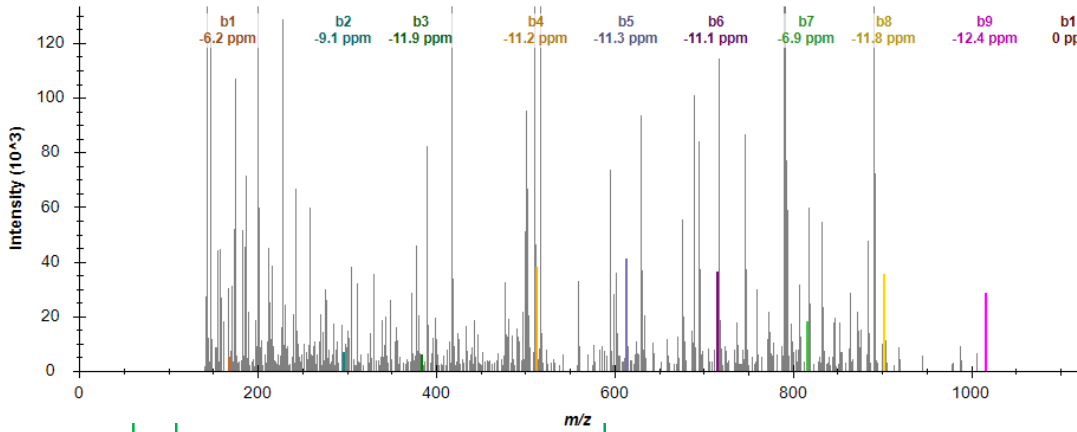
$\Delta M = 10.008269$   
 b ions ← → b ions



H/L ratio: 1.2



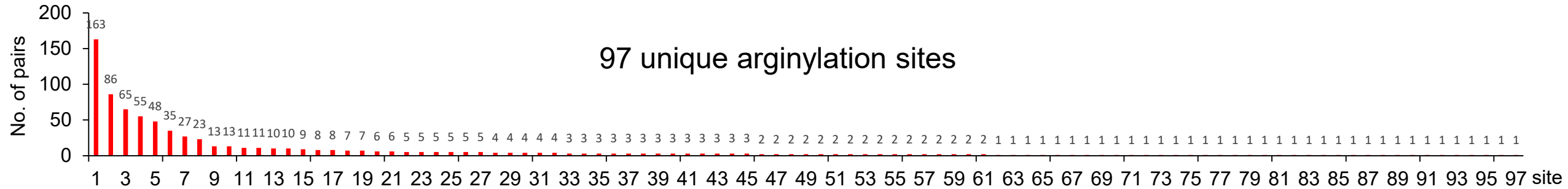
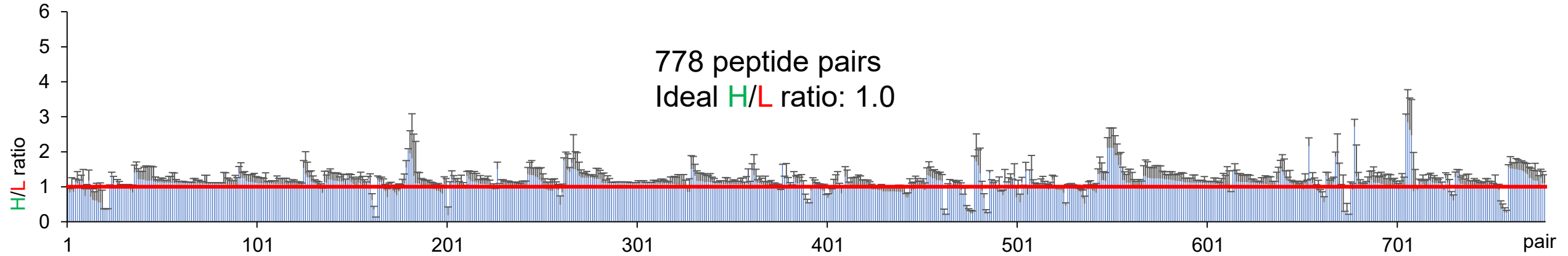
20230517\_Tom\_1\_30\_3\_soluble\_500\_600.raw (28.90 min) Scan Number: 0.1.20356



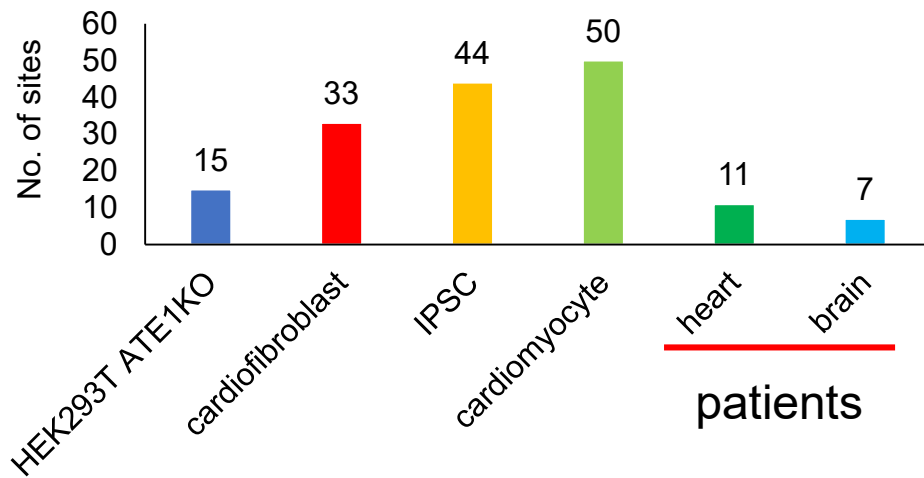
[R]E|S|E|T|T|T|S|L|V|L|R  
 b1 b2 ... b12



# Arginylation Sites from Cells and Patient Samples



Total Sites: 97



## Next Steps

- Form an arginylation consortium
- Apply to common cells, mouse tissues, patient samples
- Establish an online arginylation database

# Acknowledgements

- **Current Members**

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Axe Xie, PhD  
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Emily Zahn, PhD  
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Joanna Gongora  
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Rick Searfoss  
Kirill Miachin  
Blake Sells

- **Past Lab Members**

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Faith Robison  
Bhanu Natarajan, PhD  
Libby Porter, PhD  
Marianna Lund, PhD  
Peder Lund, PhD



**ArginylomePlot**

<https://github.com/BeckyHan/Garcia-Lab>

- **Collaborators**

Michael Greenberg, PhD  
Anna Kashina, PhD  
Ron Dolle, PhD  
Mingzhou Zhou, PhD  
Dongwen Lyu, PhD

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Thank you for your time!  
&  
Questions?

**Poster:** **MP 663**, Monday, Jun 05, 10:30-14:30.  
Title: Enzyme-based Arginylome Discovery in Whole Proteomes Using Isotopic Labeling  
Presenter: Zongtao Tom Lin

**Poster:** **ThP 605**, Thursday, June 08, 10:30-2:30.  
Title: Arginylome Discovery Using an Unbiased Bottom-Up Proteomic Technique  
Presenter: Joanna Gongora