



### Proteomics Overview

This study tested several recent (2024) digestion methods to determine the sensitivity for small starting total protein amounts as low as 1ug: we used suspension-trapping (S-trap), magnetic bead protein aggregation capture (PAC) and surfactant-assisted one pot (DDM) Digestion of all samples was done either manually or using recent automated devices like Opentrons-2 and King Fisher APEX. This study compared consistency and sensitivity between manual vs automated preparation strategies.

### METHODS

**Proteomics Digestion:**  
Tryptic digestions were done to assess the lowest amount of total protein we can start with and still obtain good number of IDs. Mouse liver homogenates were subjected to reduction / alkylation / tryptic proteolysis, except the MagReSyn™ hydroxyl beads PAC digestion. For complete methods see Posters Section of the Proteomics core website.

### LCMS

timsTOF HT (Bruker Daltronics) / Evosep nanoLC (Evosep )  
-data-independent analysis-Parallel Accumulation Serial Fragmentation (DIA-PASEF)

### Data Analysis

DIA files were analyzed with Spectronaut v.19 software (Biognosis), using reviewed FASTA database for Mus Musculus, UP0000000589.

### Results of Digestion technique evaluation, Fig.1

- 1) surfactant-assisted one-pot digestion, using a non-ionic detergent n-dodecyl-b-D-maltoside (DDM).
- 2) PAC using Hydroxyl magnetic beads (ReSynBiosciences) and processed on a KingFisher Apex (Thermo Fisher) sample prep device
- 3) PAC using Hydroxyl magnetic beads (ReSynBiosciences), processed manually
- 4) suspension-trap spin columns (ProtiFi, Farmingdale, NY)

### Results of Sample Handler comparison, Fig.2

- 1) Students who took part in the 2023 Proteomics Summer Short Course
- 2) Students who took part in the 2024 Proteomics Summer Short Course
- 3) The KingFisher Apex (Thermo Fisher) sample prep device
- 4) Staff of the UC Davis Proteomics Core Facility

### Conclusion

When possible, you should use automated sample preparation methods to reduce variation in the results. Or have Lauren in the core prepare your samples.

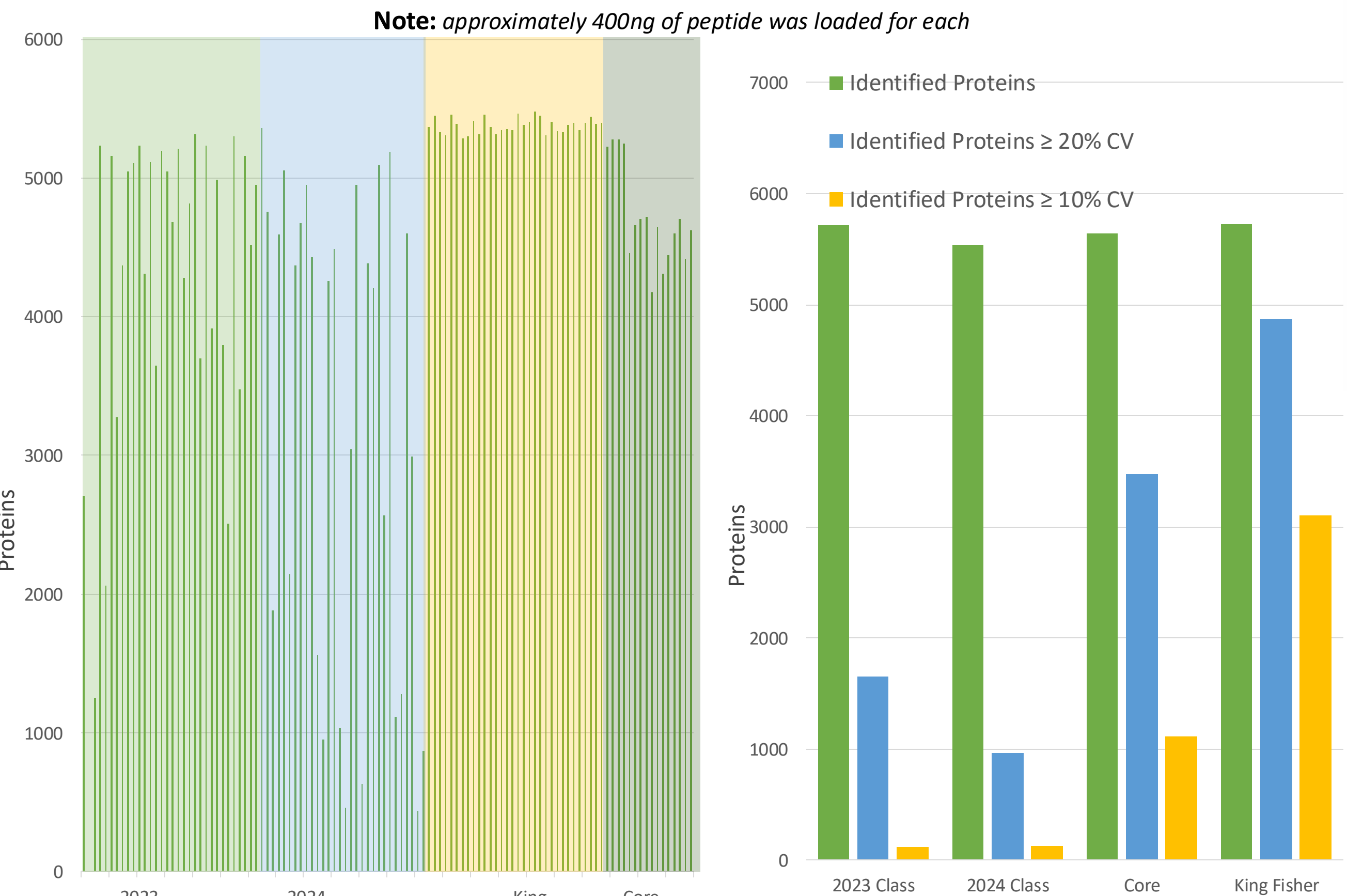
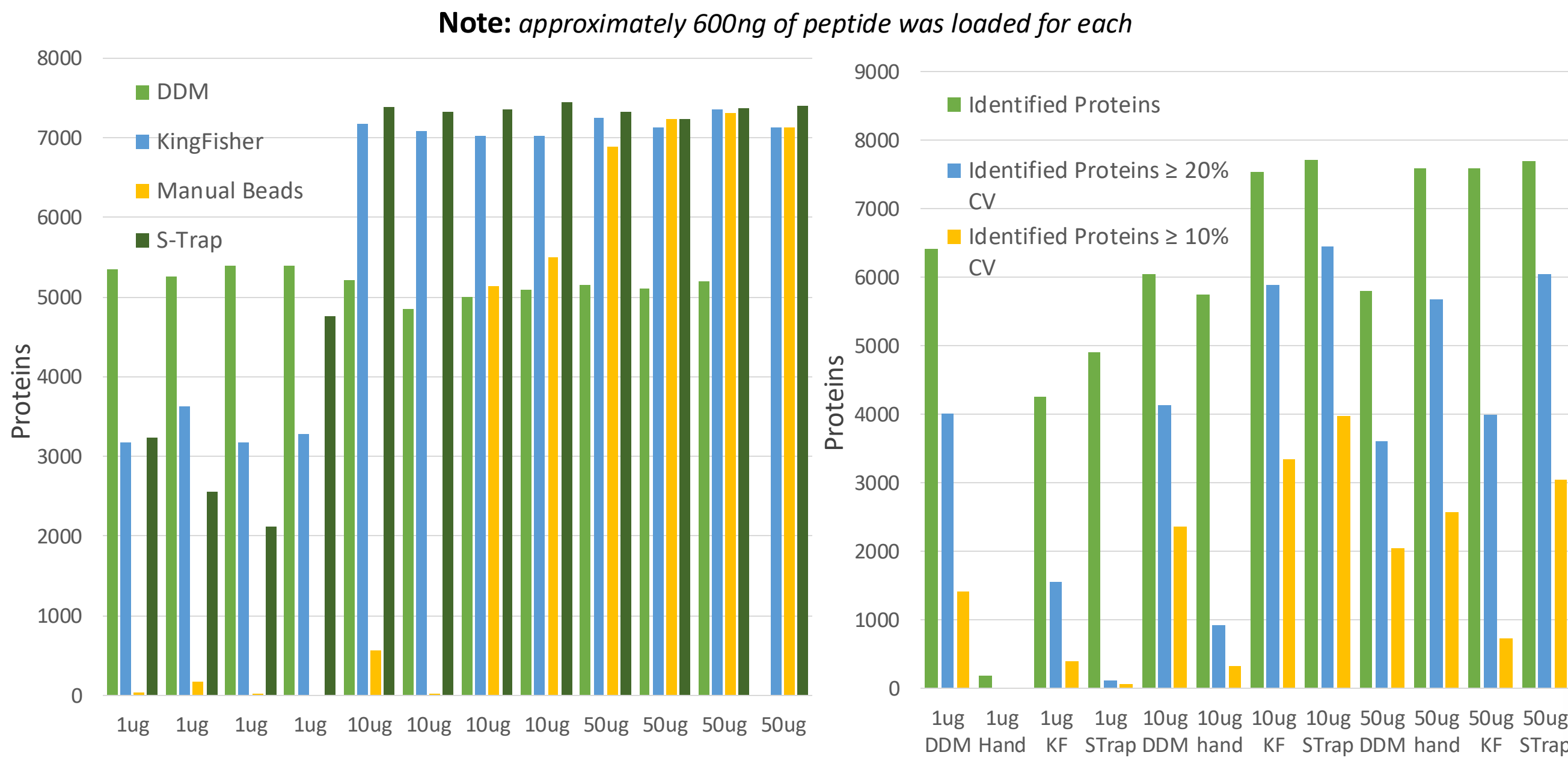
And bring chocolate...

# Popular Proteomics Sample Preparation Methods & Amino Acid Analysis

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### Amino Acid Analysis

Anything that contains protein can be measured with AAA. For example, most any food substance can be analyzed by amino acid analysis here in the proteomics core. Our technique is much more informative that the either the Dumas or Kjeldahl methods which measure total nitrogen and figures the protein content from this value (much nitrogen comes from amino acids) as AAA will quantify the individual amino acids.

### Amino Acid Analysis

Liquid phase hydrolysis was performed on a noted volume of the sample beer using 6N HCL, 1% Phenol at 110C for 24hr in vacuo. After drying the sample was taken up in sample solution buffer and Sodium Diluent (Pickering, 40nmol/mL) and NorLeucine internal standard. 50μL of the sample was injected onto the ion-exchange column on Hitachi 8800 Amino Acid Analyzer.

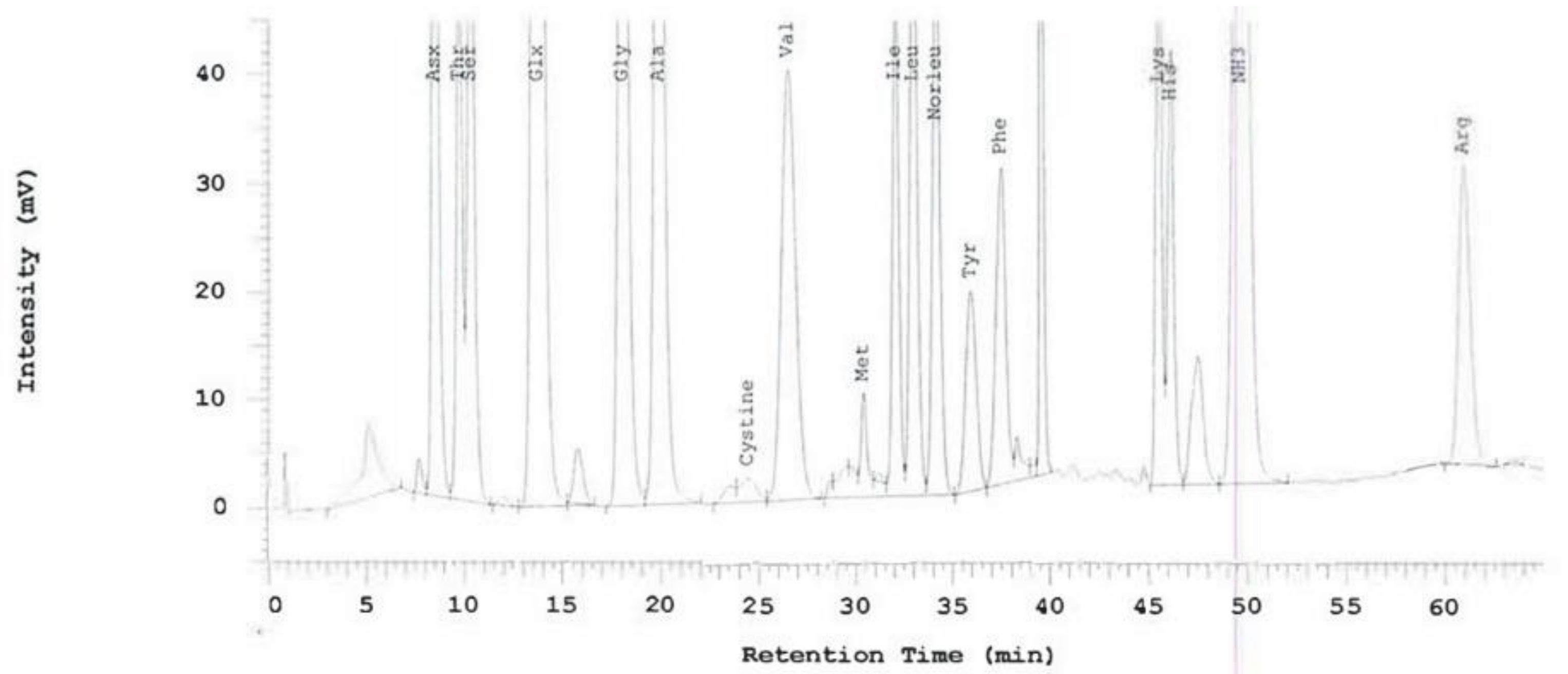


Fig.3 Amino Acid Trace generated by Hitachi L-8800 AAA System

### Results:

Recently we performed amino acid analysis on Heineken. We found the sample was 2.7mg/mL protein! Most probably don't think of beer as a high protein substance, though 12oz provides nearly a gram of protein per a serving! Something to think about the next time you are enjoying this popular adult beverage.

Amino Acid	nm/inj	nm/50ul	ugr/50ul	mole %	weight %
Asx	3.432	3.358	0.387	6.57	7.17
Thr	1.655	1.619	0.164	3.17	3.04
Ser	2.274	2.225	0.194	4.35	3.59
Glx	10.115	9.897	1.278	19.36	23.7
Pro	10.208	9.988	0.97	19.54	17.99
Gly	5.314	5.2	0.297	10.17	5.51
Ala	5.258	5.145	0.366	10.06	6.78
Val	3.034	2.969	0.294	5.81	5.46
Ile	1.507	1.475	0.167	2.88	3.1
Leu	2.268	2.219	0.251	4.34	4.66
Tyr	1.138	1.114	0.182	2.18	3.37
Phe	1.493	1.461	0.215	2.86	3.99
His	1.218	1.192	0.163	2.33	3.03
Lys	1.583	1.549	0.199	3.03	3.68
Arg	1.745	1.707	0.267	3.34	4.95
Cystic acid	0.000	0	0	0	0
MetSO2	0.000	0	0	0	0
Trp	0.000	0	0	0	0
TOTAL		51.12	5.39		
Total ug			539.2		
Conc. (ug/uL)			2.7		

Table.1 (right) Calculation table for Amino Acid Analysis

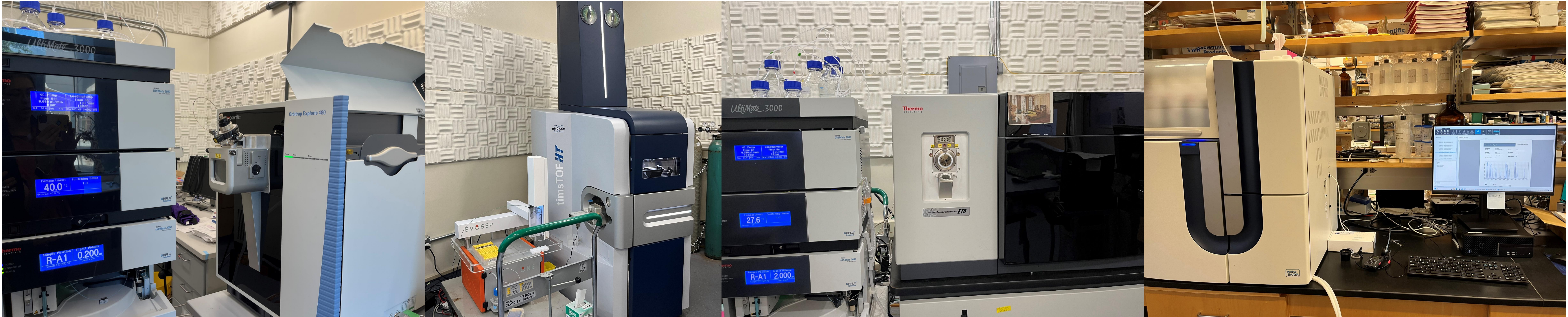


Image 1: ThermoFisher Orbitrap Exploris 480

Image 2: Bruker timsTOF HT

Image 3: ThermoFisher Fusion Lumos

Image 4: Hitachi LA8080