# Introduction

From Andy’s google sheet explaining background of project briefly:

I want to see if it possible to identify sulfated peptides, based on their molecular weight (MW) profile, identified from vast amounts of tandem mass spectrometry (MS/MS) data. Fortunately for us, the data searching has already been done (I had initially planned to do lots of searches, but I think we can avoid it).

## What is the research challenge?

* Sulfation has a mass of 79.956815 Daltons <http://www.unimod.org/modifications_view.php?editid1=40>
* Phosphorylation has a mass of 79.966331 Da: <http://www.unimod.org/modifications_view.php?editid1=21>
* Mass difference = 0.009516 Da

## ppm vs Dalton accuracy

The masses of peptides with phospho or sulfo are thus very similar, and phospho is much-much more common to find. The latest MS instruments can be accurate to around 2 ppm (parts-per-million) for measuring peptidoform mass. ppm is a relative unit of measurement, it is easily understood by comparing to percentage, which is parts-per-hundred. If we measure a molecule with mass 1601.132 Daltons, with an accuracy of +/- 1%, it is mathematically straightforward to understand this. ppm is the same but with a denominator of 1 million instead of 1 hundred.

This means that the accuracy we can measure depends on the mass of a molecule. We often set our software to allow for candidates to be +/- 10ppm, so this gives rise to something like the following windows we look for:

|  |  |  |
| --- | --- | --- |
| Mass (Da) | Upper | Lower |
| 1000 | 1000.01 | 999.99 |
| 2000 | 2000.02 | 1999.98 |
| 3000 | 3000.03 | 2999.97 |

A typical peptidoform we might observe is probably say v. aprox 2000-4000 Daltons. If we set our search software to look for peptide + mass of phospho with a +/- 10ppm window, then *if it had actually be sulfated*, it would be identified positively (but wrongly, as a phosphopeptide rather than a sulfopeptide).

## Difficulties with finding positive evidence for sulfo

It is very common in proteomics to enrich for phosphopeptides, using immobilized metal ion affinity chromatography (IMAC) e.g. TiO2 (titanium dioxide). Alternative metals can also be used e.g. Fe, Zi,

It is much argued over, but we think there is evidence for around 80K human phosphosites: <https://pubs.acs.org/doi/full/10.1021/acs.jproteome.2c00131> in current databases. Mostly these are on serines, then threonines, and fewer on tyrosines.

Sulfation is much more rare, UniProt only has evidence for 50 proteins with sulfotyrosine sites: <https://www.uniprot.org/uniprotkb?query=%28proteome%3AUP000005640%29+AND+%28ft_mod_res%3Asulfotyrosine%29>

Pat and Claire Eyers won a BBSRC grant to identify lots of sulfotyrosine sites - <https://gtr.ukri.org/projects?ref=BB%2FS018514%2F1>

This has proved tough going. Leonard Daly has worked on this and found it really difficult i) to enrich for sulfopeptides instead of phosphopeptides, and ii) when you fragment a peptide by MS/MS, the sulfation side change mostly falls off, so you cannot prove that it was present on any particular amino acid in the sequence.

They recently published a protocol where they had limited success identifying sulfation sites, with a very specialised protocol: <https://pubs.acs.org/doi/10.1021/acs.jproteome.3c00425>

Sulfated proteins are usually secreted, but unknown if only on secreted proteins, and seem to be best enriched with Zirconium.

## Data we can use for analysis

Our collaborator Eric Deutsch at ISB Seattle creates large PTM builds - like human phospho 2022 build: <https://db.systemsbiology.net/sbeams/cgi/PeptideAtlas/buildDetails?atlas_build_id=537>

He has searched a huge number of public ProteomeXchange Database (PXD) MS/MS datasets that were enriched for phosphopeptides (although very few, if any, using Zirconium, and also few secretomes). So we are really looking for a needle in a haystack, and perhaps the paper will “prove” finally that you cannot identify sulfopeptides from general phospho-enriched data sets - this would be completely fine, and publishable.

The publicly available data files do not have sufficiently granular information, so Eric will package them up and send them to us in zip files - he has sent two PXD builds already in zipped pepXML format.

# Methods

An overview of the analytical pipeline is presented in Fig. 1.

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## Data collation

## Data aggregation by peptidoform

## GMMs

## Peptidoform assignment to m/z bins

## Post-GMM data exploration

## GO term ORA

## Custom terms ORA

## Peptidoform annotation

## Instrument contributions exploration

## MS2 evidence assessment

# Results

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Fig. 1. Peptidoform proportions across bins.

LEGEND PLACEHOLDER

All proportions are given as a fraction of the total number of peptidoforms that fall within each bin. The total numbers were as follows, from left to right: mz -0.0275 to 0.0225: ;

A. Serine-containing peptidoforms

B

C

D

E

F

T

E

X

T

H

E

R

E

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T

E

X

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**Cytokines known to be sulfated – we do not see that in the GO terms BUT of the known sY-containing proteins in UniProt (~51), only 6 are represented by peptidoforms that have passed all filtering stages;**

**Custom terms for known and likely novel sY might be more suitable**

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\*\*\* still need to look into last question on here \*\*\*\*

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This shows number of PSMs contributed by the different instruments used across the build across:

Row 1: all data based on number of spectra id’d – from Eric’s metadata published with the phosphobuild

Row 2: all peptidoforms in bin of interest (BOI) (\*and to the left) where we expect to get singly and doubly sulfated peptidoforms

Row3: peptidoforms assigned as likely sY candidates base don calibrated error histogram and subcellular localisation or being known sY

Row4: known sY peptidoforms (Vitronectin and **Secretogranin; ~12 peptidoforms total, to double check!)**

Probably best as supplementary

Seems to suggest that a lot of the false positives in BOI may be coming from Q Exactive (not super surprising as it’s one of the older instruments on there)

MS2 assessment figure – top PSM for each case study protein?

Not sure how we’d present this? E.g. side by side spectra of added sulfo vs no sulfo to peptidoform USI? A screenshot of a computer

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