Palomo et al. apply various proteomic searching tools to experimentally degraded beta-lactoglobulin. This is a paper that has important implications in paleoproteomics. A couple of things that I would like to see included is a closed MSFragger search to compare with the other closed searches and a detailed supplementary list of parameters for each algorithm. I realize they are included on Zenodo, but having them with the paper will be beneficial.

With Metamorpheus, what search type was used? Specifically was classic search used for tryptic, non-tryptic, and nonspecific or the modern indexed search versions?

For denovo/any search, especially on the 128 day samples, is there a correlation to the level of amino acid identification to the isoelectric point of the peptides? It seems like there is a bias toward certain sections of coverage for the tryptic peptides compared to the non-specific searches. Can one of these approaches help find different types of peptides/protein fragments based on the composition of the peptides?

Can you include a comparison of the number of PSMs detected per algorithm as well? Also, I'd be interested to see Figure 4 with PSM identifications instead/in addition to the unique peptide counts, so the unit of comparison between the algorithms is the same.

Line 126: Should 156 long be 156 amino acid long?

Line 148: What aqueous solution was used?

Figure 1: For the schematic, change the LC-MS/MS picture to an Exploris.

Line 160-161: Include a short form of the Cappellini et al. 2019 extraction protocol beyond the various basic summary included here.

Table 1. List the versions of pFind 3, Metamorpheus, Mascot, Novor, DirecTag, and PepNovo+.

Line 194-196: Why was Fragpipe only run on a cluster instead of also on the MiniMax workstation? Fragpipe can natively run Thermo RAW file format as well. Conversely, why wasn't the same peak picked mzML file used with pFind3, Metamorpheus, and Maxquant.

Line 321: Figure 2 should be Figure 3.

Figure 3: What do the 2 dashed blue lines represent? Additionally, make sure that the color choices are colorblind accessible. With a simulator Tryptic DB2 is very similar to Semi DB2 and Tryptic DB1 is very similar to NS DB1.

Figure 4: A series of Upset plots may be easier to understand/compare than these 3 circle Venn diagrams.

Figure S2 was not included in this preprint.

Figure 5 A, B, D: Make sure to use a colorblind palette. The importance of the amino acid colors here is completely lost with this palette.