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RE: External Appraiser of Kenneth Weke's thesis

Dear Maja Pega,

I have had the pleasure of reviewing Kenneth Weke's thesis, "Expanding the proteome: Advancing proteomics methodologies to uncover new insights into cancers" under the supervision of Drs. Theodore Hupp and Sachin Kote.

Critical and Analytical Commentary of the Thesis: The thesis presents an intriguing body of work with innovative proteomics approaches and a diverse array of techniques. The text maintains a good flow, and the results are generally well described, introduced with good rationales, and substantiated with nicely presented figures. In most cases, the data interpretation aligns well with the presented results. I provide below a series of critical comments. I recommend the Council of the Biotechnology Discipline to allow Kenneth Weke to the next stages of the doctoral procedure including their oral defence.

The introductory chapter offers a good overview of the research in the field. However, section 1.4 is underdeveloped. I recommend it be further developed to better frame the main aims of the thesis and how they fit together to address the overarching goal. Additionally, some additional and short explanation should be provided to explain how each thesis chapter addresses specific goals/hypotheses. This is particularly important for chapter 2, which has a slightly different focus. The last chapter, chapter 5, is also underdeveloped, and the applicant should better summarize the key points of their work, discuss in more detail the limitations of their study, and provide some additional perspective on how their work could be extended in the future.

There are several comments that may warrant some editing of the text and/or require the thesis committee to ask some follow-up questions during the thesis defense to ensure the candidate can adequately address these potential issues and demonstrate a strong understanding.

The analysis of the size and gravy scores of identified proteins in chapter 2 is interesting, but it would have been more relevant if this were also done at the peptide level in parallel and in comparison to a standard LC-MS approach to properly establish if there is any bias. The limitation should be discussed in the last chapter and/or the defense.

Another limitation of chapters 2 and 3 is that while changes in protein levels have been identified, no direct validation was performed. As well, no positive controls or standards have been used to assess the accuracy of the quantitation. The comparison of replicates is a great way to assess the robustness of the analysis but does not assess the relevance/confidence toward proteins that show changes. Could the candidate provide examples of how this could be done? The defense committee should guide some of their questions/critiques toward that point and encourage the candidate to perhaps add 1-2 related statements in the last chapter.

In chapter 3, the difference between the three assessed methods seems to have been marginal. Could the candidate comment on which one was perhaps easier to implement?

The comparison with the transcription data in chapter 3 is interesting, but given it was likely from a different set of tissues, the low correlation is perhaps not surprising. This should be clarified and briefly discussed.

Presentation of the data in chapter 4 may require some additional work, especially if this is to be used for publication.

Can the candidate identify how a mass spec method optimized for tryptic peptide identification may not always be optimum for MHC presented peptides? I more specifically want to address how the charge on tryptic peptides vs. other peptides may differ and how that might impact MS2. This may also impact results discussed below.

The idea that CPA4 extensively trims (full-length) proteins from their C-term end in the cell is rather provocative. Can the candidate identify a strong trend on numerous identified proteins to corroborate their observation on filamin-A? If not, they should consider another likely explanation for their results. I am also confused why data from immunopeptidomics was used for this analysis as stated on p78. If the CPA4 was to trim full-length proteins, then a bias against C-terminal peptides should be noted on tryptic peptides in hypoxia conditions. The rationale for using immunopeptidomics is not clear and is confusing. The usage of immunopeptidomics is then better explained in the next result section, and perhaps the second part of the result section should be rewritten before submitting the manuscript for publication.

The fact that the distribution of the peptide length seems to be altered under hypoxia in comparison to normaxia (Figure 4.6.c) is not discussed and should be better underlined.

Appraisal: In summary, the candidate has made a noteworthy contribution to knowledge using innovative proteomics approaches. I recommend acceptance of the thesis for the

Doctor of Philosophy (PhD) requirements, with some text revisions that can be done following the defence once my assessment is shared with the candidate. In our institution, these revisions would typically be considered minors and edited with the help of the supervisor without the need for re-evaluation of the thesis.

Additional minor comments: The paragraph structure of section 1.1.2 is unclear - each new paragraph should have an opening statement/sentence that introduces the main point or a short introductory paragraph should explain what each subsection is about.

Page 7 is a good example of a section that introduces too many abbreviations.

1.3.1. Sample preparation for bottom-up proteomics is biased toward new techniques developed for single-cell proteomics. It would have been more appropriate to first provide a general introduction to MS (e.g., 1.3.1 to 1.3.5), then discuss deep proteomics and the extent to which the human proteome has been analyzed before discussing the challenge of single-cell proteomics and new separate subsections.

Throughout the thesis, there are many numbers that should be either in sub- or superscript.

Chapter 2. First part of the results section. Much of the information is redundant with the introduction - new elements/citations should be moved to the introduction, and the opening sentence needs to be rewritten and shorten.

Throughout chapter 2 and especially in the figure legend of Figure 2.2, it should be made clear that the analysis was done using lysates corresponding to ~200 cells. Reading the text infers that only 200 cells were processed from the beginning, and this is misleading as lysis was done with a larger number of cells.

It should be made clearer in the methods that no filtering was done at the peptide level for protein identification/count. In chapter 2, it would be useful to also indicate the number of peptides and the average peptides per protein identified in first experiments.

It doesn't seem that technical replicates were run in the MS in chapter 2; maybe this is not possible because all the samples were loaded on the instrument. This should have been made clearer in the methods.

Some additional info should be added for the statistical analysis, especially the ones used to look at differences in protein levels, e.g., which t-test was used, how were p-values corrected.

Chapter 3. Methods 2 and 3 shows marginal benefit in terms of cleavage efficiency, and the conclusion should be rephrased to reflect this.
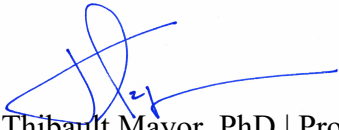
As hypoxia/normoxia is impacted by the rate of oxygen diffusion, as the amount of media in a given dish should be specified in chapter 4 methods. Confluence should also be specified as high cell density with deeper media may result in hypoxia, even when cells are grown in

a normal 5% CO_2 incubator.

The candidate indicates that their work confirms the role of HIF1alpha in hypoxia on page 71. Perhaps, it would be more important to highlight that it demonstrates that their conditions are effective to create hypoxia.

I congratulate the applicant for their work and wish them good luck for their defense.

Yours Sincerely,

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