We would like to thank the reviewers for their comments. The resulting changes will provide a clearer discussion of the spectral networks paradigm.

# Reviewer 1

Comments to the Author:

This paper provides a comprehensive review of the spectral network approach for sequencing and identification of proteins. The manuscript was well written by a group who pioneered the spectral network approach, and will provide a very useful reference to the proteomics community. The only suggestion I have is for the spectral library matching section. In its current form, this section is not well integrated into the rest of the paper.

The choice of including a section on spectral library matching is motivated by this being the most common current application of spectral matching for peptide identification – a precursor to the spectral networks approach which expands this concept to matching between spectra of unidentified peptides for determination of consensus interpretations (e.g., as in Shotgun Protein Sequencing). In addition, the current developments on using spectral library search to identify multiplex spectra from more than one peptide is also worth mentioning since it could become a precursor to future spectral networks algorithms.

Furthermore, the application of NeuroPedia to improve the peptide identification up to 10 fold seems an arguable statement, because the false discovery rate of spectra matching may not be well estimated. If the authors decide to keep this section, they may consider to review the spectra prediction approaches that may provide more flexibility for spectra matching.

We note that the claim that NeuroPedia improves peptide identification up to 10 fold is qualified by the use of decoy spectral libraries to calculate FDR:

“… As a result, peptide identification by matching spectra of unidentified peptides against *spectral* *libraries* of identified peptide spectra14 has recently gained new relevance, especially since the introduction of decoy spectral libraries26 for calculation of false discovery rates10,27… (Page 2, column 2)”

Remaining text in the next paragraph of page 2, column 2 details how the reduction in search space, identification of short peptides, and advanced knowledge of expected peptide fragmentation patterns also support the claim.

If the authors decide to keep this section, they may consider to review the spectra prediction approaches that may provide more flexibility for spectra matching.

We thank the reviewer for this suggestion and have added the following sentence to page 2, column 2:

“… Spectral prediction is a promising emerging approach through recent results still indicate that there is ample room for improvement in this direction28,29 ”

# Reviewer 2

Comments to the Author

Dear Editor and Authors,

This reviewer found the manuscript titled “The Spectral Networks Paradigm in High Throughput Mass Spectrometry” to be a well-written and highly informative description of a novel paradigm shift in analysis of tandem mass spectra. This use of spectral networks offers significant advantages in analyzing peptide data, especially for endogenous peptides, which often contain properties not otherwise easily studied using mass spectral analysis. The strength of the spectral network approach lies in the high number of matches required for aligned peaks, while also allowing for any type, number, and location of peptide modifications. The spectral network methods translate well to other types of molecules such as glycans. The spectral network offers an entire order of magnitude improvement in reducing the number of false identifications when compared to de novo sequencing analysis methods, and the improvements are impressive. This paper will fit well with the audience.

Major Issues

1. A good deal of text is dedicated to describing the metabolic molecules of microbes, but not a single reference is present in the first paragraph of Section 6. I suggest adding a reference such as: Interspecies Chemical Communication in Bacterial Development by Paul D Straight and Roberto Kolter, in Annual Review of Microbiology, Vol. 63: 99-118, October 2009.

We thank the reviewer for this suggestion and have added multiple references (including the suggested reference) describing metabolic molecules of microbes (ref # 93 – 100) to the first paragraph of Section 6.

Minor Issues

1. Terminology. The abbreviation for tandem mass spectra used for the majority of the paper is MS/MS, and then MS2 is introduced later, which could give the impression that the two abbreviations represent different experiments to someone not overtly trained in mass spectrometry. I suggest including both abbreviations, such as “(MS/MS or MS2)” in the very first sentence.

All terminology for tandem mass spectrometry is now “MS2”

2. Page 4. The term “branch- and-bound” is used in describing how M-SPLIT works. An additional sentence may be warranted to describe the technique and how it is achieving the six order of magnitude reduction in search area.

We added two sentences briefly describing M-SPLIT’s search strategy to the last paragraph of section 2:

“…In brief, M-SPLIT uses single-peptide matches to prune the search space for mixture peptides. It iteratively considers mixtures of single-peptide matches where adding another match could possibly exceed the best current score… (Page 3, Column 1)”

3. Page 8. The sentence “In particular, Cyanopeptide X was an unknown bioactive molecular whose identity was elucidated using the very time intensive workflow os isolating..” might need to read “ In particular, Cyanopeptide X was an unknown bioactive molecule whose identity was elucidated using the very time intensive workflow of isolating…”

The sentence was revised to read:

“In particular, Cyanopeptide X was an unknown bioactive molecular whose identity was elucidated using the very time intensive workflow of isolating, purifying and collecting 2D NMR data to obtain the structure91 … (Page 7, Column 2)”

4. Figure 3, part e. MS4 could read MS4.

This has been fixed.

5. Page 9, last sentence. “Using on a combination of new…” where “on” could be deleted.

The sentence now reads: “Using a combination of new …”