Reviewer: 1

Comments to the Author

The authors performed additional work, impoved the manuscript and addressed most of the reviewers' comments.

Remaining minor issues are:

>>Although the article focuses at the algorithm, a brief description of the software in which this algorithm

was implemented, would be very useful for chemists and biologists. And a pack of web-services

providing the functionality of GlycReSoft would be even better.

>Response: We have provided references to full descriptions of the software in the revised manuscript

I could not find this reference in the manuscript, neither it is marked in the delta-file. I only see links to software itself and to its source code.

Response: We added a description at the end of the introduction to the manuscript on revised page 2.

>>What is the proof that they cover the diversity of samples, which the users of your algorithm may face

(especially taking into account that most of samples came from a single publication)?

>Response: These samples were chosen because they span different instrument types, such as the

noisiness of Time-of-Flight (TOF) mass spectra which leads to more spurious low-signal-to-noise ratio

peaks as well as Orbitrap data which are much cleaner. Orbitrap instruments clean and fast, but they

produce truncated isotopic patterns on the low end of abundance scale and need an isotopic pattern

fitting algorithm that is forgiving of missing peaks.

These samples also span different sample preparation and data acquisition modes. We demonstrate our

method works on native samples acquired in negative mode, and demonstrate that our method is able

to handle the associated formate adduction on neutral and partially neutral glycans. We demonstrate

our method works on permethylated and multiple types of reduction treatments, and can tolerate

ammonium adduction.

The manuscript itself lacks this explanation, at least I could not find the corresponding text among the marked changes.

Response: We have explicitly stated these properties on revised page 2.

>>What is the proof that the number (and kind) of samples is statistically sufficient for testing purposes?

>Response: As described on revised p. 2, we analyzed published data from the two most widely used high

resolution mass spectrometry instrument types.

But it does not mean that sampling is statistically credible.

Please provide a proof, (at least) that the selected number of samples is enough to count the whole test credible.

Response: We are sorry, but we are confused by the reviewer's request. The methodology we propose does not conduct any statistical tests, so there is no need to perform a power-like calculation to identify a minimal number of samples to correctly reject the test. We recall that we showed that the new method performs as well or better than existing methods, based upon unambiguous numbers of identified glycan composition chromatograms, building on publicly available data.

>>line 40: what was the background for these magic numbers 10 ppm and 5 ppm?

>Response: These numbers are commonly used mass error tolerances for these types of instruments

based upon information provided by their manufacturers. The mass accuracy parameters are adjustable

by the user.

please, add this information to the manuscript

Response: We have done so on revised page 3.

Reviewer: 2

Comments to the Author

(There are no comments.)

Reviewer: 3

Comments to the Author

The authors have answered my concerns in this revision and the manuscript reads well. However, parts of the manuscript appear to be rushed especially the supplementary material, and the authors are recommended to improve the quality of figures. Also missing text for one section (last point below).

1) Section 2.6 missing reference "Kronwitter et al. "

Response: We could not find a citation of “Kronwitter et al”. We cited “Kronewitter et al 2014”, referencing Kronewitter, S. R., Slysz, G. W., Marginean, I., Hagler, C. D., LaMarche, B. L., Zhao, R., Harris, M. Y., Monroe, M. E., Polyukh, C. A., Crowell, K. L., Fillmore, T. L., Carlson, T. S., Camp, D. G., Moore, R. J., Payne, S. H., Anderson, G. a., and Smith, R. D. (2014). GlyQ-IQ: Glycomics quintavariate-informed quantification with high-performance computing and glycogrid 4D visualization. Analytical Chemistry, 86(13), 6268– 6276.

2) Page 3 Section 2.6.2 "For example, For example, " repeated text

Response: We have removed the repeat

3) Table 2 "... and the number of Combinatorial N-glycan..." lower case C

Response: We have altered the text as suggested

4) Page 4 "For the simplicity, we chose to include all of Hybrid in Asialo-Bi-Antennary and permit up to one NeuAc in it." rephrase 'in it'.

Response: We have rephrased “in it” to “in its members”

5) Page 5 needs to be formatted. Text in column 2 should be top of page.

6) Fig 1 needs improvement. EIC part includes overlapping text. Total abundance x-axis is not readable.

Response: We’ve reduced the text overlap and increased the scale of the total abundance plot to increase the size of the x axis text. We note that all of the figures we submit are vector graphics and will resize without degradation when viewed in PDF format.

7) Page 8 both table legends need to be correctly justified, also space between commas in first sentence e.g. "20141103-02-Phil-BS ,".

Response: We have centered the table captions and removed the trailing spaces.

8) Fig 4 page 9 EIC values are overlapping and difficult to read (last line of values above x-axis).

Response: We’ve removed the number of superfluous labels so that just the abundant ones are marked to make the plot more read-able.

8) Supplementary Fig 4 part C difficult to read text. Part D x-axis text again needs to be readable.

Response: We have reduced the overlap of the labels in Supplementary Figure 4C. We have rearranged the figure to allow the x-axis text to scale up.

9) Supplementary Section 9 page 10. Add PREFIXs to SPARQL query

Response: We have added namespace prefix declarations for all namespaces used in the query.

10) Supplementary Section 9 page 10. Figures in between text. Please improve formatting in this section. This is the same problem for Section 3.1.

Response: We’ve insisted to the LaTeX layout engine to place the figures before the next subsection here.

11) Supplementary Fig 1. Please add more content to the Figure legend and positioning it sits in a paragraph.

Response: We’ve added more content describing the figure, and the layout has been adjusted.

11) Supplementary 7.3 Results for IGG no text in this section?

Response: We have added content describing the results from this section in more detail.