Response to Reviewer 3 Comments

The manuscript describes the analytical and functional characterization of a conjugate of horseradish peroxidase and guinea pig anti-Vero IgG. Analyses were performed by size exclusion chromatography with multiple detectors, whereas functional characterization of the product was evaluated by ELISA.
Data are overall sound, but I recommend the publication of the manuscript after a revision of English grammar since several parts of the draft are poorly understandable and require rewording.
The major issues that have to be fixed before publication are:

Point 1: Variability (e.g., standard deviation) is missing from data reported in supporting information (table S1, S2 and S3) and in some section of the text, like from line 69 to 73 (and in some following sections). Moreover, most of the author's conclusions (e.g., line 84 "Oxidation of polysaccharide residues of the HRP didn't affect its Mw") are based on numerical considerations since statistic tests that support author's conclusions are missing.

Response 1: We would like to thank the Reviewer for the suggestions. The previous data reported in supporting information were molecular parameters for representative samples. Now, we introduced other measurements and added the variability in Table S1 and S2.
The manuscript describes a preliminary ELISA experiment where anti-Vero IgG concentration, used for coating the plate, and anti-Vero IgG-HRP conjugate dilution were varied to determine the optimal working dilution. The samples were added in monoplicate due to the numerous concentration variations of anti-Vero IgG, IgG-HRP conjugate and lysate of Vero cells.

Point 2: the sentence from line 32 to line 34 is puzzling. The authors stated that HRP is a glycoprotein with a "broad specificity that allows to be measured by absorption, fluorescence, and luminescence". This sentence is puzzling for several reasons. First, what kind of specificity are the author talking about? Second, if the so-called "specificity" allows measurements with so many different techniques it sounds more like there is no specificity at all. A rewording of the sentence would be nice to avoid misunderstandings.

Response 2: We would like to thank the Reviewer for the constructive comment. We reworded the sentence in order to avoid misunderstandings.

Point 3: What is Mn? It is mentioned for the first time at line 48 to define the parameter Mw/Mn, and many other times later on in the manuscript. However, there is no definition of what Mn is in the text. A list of abbreviations would improve manuscript reading.

Response 3: The Mn represents number-averaged molecular weight. We introduced the explanation of Mn as well as other abbreviations in the manuscript.

Point 4: statements from line 51 to 54 are not correct. RI and UV detectors do not measure the sample's concentration and composition, but instead parameters from which sample's concentration and composition can be deducted. In fact, when describing the analytical procedures, the authors indicated "Antibody concentration estimation" as title of section 4.3. Similarly, SLS detector does not reveals absolute molecular weight but can be used to measure an apparent molecular weight since protein light scattering depends on molecular weight but also conformation, pH, ionic strength and so on (see table S1 where the calculated molecular weight is changing when measured in phosphate or carbonate buffer). The same concepts are repeated multiple times in the text (e.g., in the conclusion section). I encourage the authors to be more cautious and precise about the definition of the measurements done throughout the text.

Response 4: The authors appreciate the comment. We reworded sentences and we have better described and defined roles of UV, RI and SLS detectors in the Introduction part of the revised version. We also provided additional information that could be obtained by integration of individual detectors' data, including absolute Mw determination.

Sincerely,
Adela Štimac