Fachbereich Informatik

Wilhelm-Schickard-Institut

Cognitive Systems

Lars Rosenbaum

Sand 1

72076 Tübingen

Germany

Phone: +49 7071 29-70789

Fax: +49 7071 29-5091

lars.rosenbaum@uni-tuebingen.de

www.cogsys.cs.uni-tuebingen.de

To the editors of

Journal of Chromatography B.

April 14, 2014

**Revised manuscript “Integrated enrichment analysis and pathway-centered visualization of metabolomics, proteomics, transcriptomics, and genomics data by using the InCroMAP software”, submitted under manuscript number JCB-13-943**

Dear Prof. Theodoridis,

we thank you and Reviewer 2 for providing us with these constructive comments, which have certainly made a substantial contribution to improving the quality of our manuscript. We have now modified the paper in response to your suggestions. In the following, we would like to discuss all reviewer comments in detail. The reviewer comments are shown in red and our answers in black below.

**Guest editor comments:**

*Is Figure 2A based on NMR data? I could accept that, as at this stage software tools are fed with data matrices and the origin of the data is not the decisive point. However only one dot is colored indicating significant change of a metabolite. In text you mention four metabolites. Is this correct? Please check.*

The metabolite coloring in Figure 2A is indeed based on NMR data. We chose this dataset, since it is suited to exemplify the capability of InCroMAP to simultaneously visualize changes on various molecular levels (including mRNA, miRNA, protein expression, DNA methylation and metabolite levels) in a pathway-centered manner. In the revised manuscript, the measured metabolites shown in Figure 2A are highlighted in yellow. The figure and also its description in the main text were revised accordingly.

*Figure 2B shows changes at DNAmethylation, mRNA and protein level, but again I found only one coloured metabolite. I would ask the authors to generate new exemplary images that show more information generated at the metabolome level. I would add a sentence at end of section 2.4 to link the change at protein and metabolite level at the specific example. Finally I think that sessions 2.3 and 2.4 belong to results and not to Methods. Please consider their re-allocation.*

As done for Figure 2A, the metabolites detected in the pathways shown in Figure 2B and C are now highlighted in yellow. In each of the depicted pathways at least three metabolites could be identified and measured in a quantitative manner. Furthermore, the subsections 2.3 and 2.4 were added to the results section as suggested by the editor.

**Reviewer 2 comments:**

*The authors spent much effort into addressing all comments. But one of my main concerns about missing a good example application is addressed only in a limited way: The authors replaced Figure 2 to focus more on metabolic pathways. But if I understand right these pathway enrichment analysis is based on transcript data and not primarily on metabolite data. In the newly presented Figure 2B, I can only find one coloured metabolite, all other metabolite 'circles' are grey which means there are no data included. Since the key feature of this manuscript (and the reason to place it in this Journal) is the integration of metabolite data (InCroMAP itself was already published 2012), this feature needs to be shown at least by a simple example.*

*The difference to other tools is much better explained and it now becomes also more clear that InCroMAP is more suitable for targeted data or at least annotated data, but less appropriate for directly integrating mass/RT pairs.*

We agree with Reviewer 2, that an insufficient number of measured metabolites was shown in the previous version of Figure 2. Thus, in the current version of the manuscript 14 quantified metabolites are highlighted in Figure 2A. Furthermore, we now show the complete Glycolysis/Gluconeogenesis pathway containing 5 detected metabolites in Figure 2B and added a pathway showing the Taurine and hypotaurine metabolism (Figure 2C) in which 3 metabolites could be identified.

* TODO: Supplementary Figures erwähnen

Sincerely yours,

Lars Rosenbaum

(for the authors)