**Guest editor comments:**

*1) Please consider proof reading of the manuscript by a metabolomics expert in order to improve weak points in descriptions and terminology (as suggested by referee 1)****.***

As suggested by Reviewer 1 the manuscript was reviewed by an expert in the field of metabolomics. The corrections specifically included the revision of sentences where an inappropriate terminology was used in the previous version of the manuscript. Furthermore, we included detailed descriptions of the algorithms (e.g., for pathway enrichment analysis based on cross-omics data) implemented in the InCroMAP software.

“Then, the deregulated metabolites, genes and proteins are determined for each platform, based on appropriate cutoffs. Next, relevant pathways related to the biological background of the experiments are inferred. For the detection of relevant pathways, InCroMAP employs a special pathway enrichment algorithm, which integrates deregulated metabolites, genes, and proteins across multiple platforms.”

*2) Please provide some additional results (preferably from metabolomics studies) and comparison with the performance of other software tools.*

In order to emphasize the increasing importance of cross-omics studies, which particularly involve the profiling of the metabolome, we cited two pioneering studies in this field, which have recently been conducted by Gruden *et al.* and Armiour *et al.*, respectively. Furthermore, Figure 2 is now based on real data from a study focused on the analysis of metabolic alterations in mouse liver tumors. The corresponding manuscript has recently been accepted for publication by the International Journal of Cancer, which is briefly described in the revised manuscript. For the sake of completeness, we also included the final version of this manuscript into the submission.

TODO: Lars. Comparison with other visualization tools for metabolomics data

“Furthermore, modern mass spectrometry platforms are able to detect and measure the relative intensity of thousands of metabolites with high accuracy. In recent years, increasing attention has been drawn to the integration of metabolomics with molecular profiling on the transcriptional and protein level [PMID: 22253695, 22936829].”

*3) The way that data is imported could be described more eloquently.*

The subsection 2.1 in which we describe the import of heterogeneous types of omics data into the software was revised accordingly.

“Siehe Abschnitt 2.1”

TODO: Lars. Über den Import werde ich noch etwas mehr schreiben. Habe da auch noch paar Bugs gefixt.

“Mögliche Änderungen im Text…”

*4) Comments from referee 2 on targeted metabolomics data should be given consideration.*

The reader is referred to the revision notes for Reviewer 2.

**Reviewer 1 comments:**

*1) Also the implementation of the metabolomics part is very simple: only differentially expressed metabolites are considered. First of all, this terminology is strange: metabolites are not espressed but formed.*

We agree with Reviewer 1 that the algorithm used for pathway enrichment analysis is simple in the sense that only differentially formed metabolites are considered. If more sophisticated algorithms (e.g., single sample profiling or quantitative enrichment analysis) that account for metabolite concentrations are of interest, metabolite set enrichments can also be calculated externally by using the MSEA (<http://www.msea.ca>) or IMPaLA ([http://impala.molgen.mpg.de](http://impala.molgen.mpg.de/)) software. The pathways of interest may then be automatically loaded from KEGG or imported into InCroMAP in the BioPAX format. Please note that we do not intend to replicate these features in our software. Nevertheless, we set a high value on ensuring the compatibility to related tools in order to overcome potential limitations of our software.

The issue concerning the terminology was addressed by writing “deregulated” instead of “differentially expressed”, which should be more appropriate.

“Siehe Punkt 1 vom Editor”

*2) Secondly, which kind of metabolomics is considered here? The authors talk about pathways but if one measures in blood, then the concept of pathway is very difficult.*

We now mention in the introduction that InCroMAP was primarily designed for the detection of metabolic pathway alterations in tissue samples and cell cultures. Furthermore, we now included illustrations obtained from a concrete example application, where metabolomic changes were monitored in mouse liver tumors, based on NMR-based metabolomics data as well as mRNA, miRNA, protein and DNA methylation data. For metabolic pathway analysis of blood sample data the algorithm recently proposed by Krumsiek *et al.* (<http://www.ncbi.nlm.nih.gov/pubmed/22713116>) may be more appropriate than the statistics implemented in InCroMAP.

“InCroMAP is a stand-alone Java software originally developed for the enrichment analysis and pathway-based visualizations of genomic and proteomic data, where multiple biological layers were monitored in the same set of tissue samples or cell cultures.”

*3) The authors are clearly not from the field of metabolomics (they do not mention the proper and much used tools for data (pre)m processing in metabolomics. They do not refer to existing tools or software for metabolite enrichment analysis.*

TODO: Vergleich mit existierenden Tools machen.   
TODO: Rainer/Lars. Was gibt es noch, was für die Vorprozessierung der Daten in Metabolomics verwendet wird? Wir haben R und MayDay (für Transcriptomics/Proteomics bisher) zitiert. In den Sinn käme mir noch kommerzielle Statistiksoftware.

Mit was werden sonst die Rohdaten prozessiert (mal die Software der Gerätehersteller ausgeschlossen)? Hier fällt mir noch XCMS (von Ralf Tautenhahn am Scripps) ein, welches in R läuft und wohl auch relativ bekannt ist.

“Umsetzung im Text…”

**Reviewer 2 comments:**

**Major Revisions**

*1) The multi-level integration is clearly an advantage of the proposed tool. But this could be demonstrated by a powerful application to show which new biological insights can be provided by integrating more than two levels. Since the first software version was published in 2012, are there experimental studies using this tool? This should be cited. Also unpublished collaboration work could be shortly explained.*

*Since the main purpose of the manuscript is the integration of metabolomics data, an example application from the viewpoint of metabolomics would be important.*

In the EU-funded project MARCAR ([http://www.imi-marcar.eu](http://www.imi-marcar.eu/)), we perform extensive cross-omics profiling based on liver samples from rodent in-vivo studies. Most notably, one of our manuscripts entitled „Metabolic programs orchestrated by the activated Ha-ras and β-catenin oncoproteins in mouse liver tumors” was recently accepted for publication by Int. J. Cancer. In this study the InCroMAP software was extensively applied to the pathway-based, integrated analysis of transcriptomics, proteomics and metabolomics data obtained from mouse liver tumors differing in their mutation status. As proposed by Reviewer 2 this work is now cited and shortly explained in the revised version of the manuscript. Additionally, we updated Figure 2, which is now based on real data from the mouse tumor study. However, since we would like to remain the focus on the presentation of the novel features of the InCroMAP software (e.g., enhanced support for metabolomics data), an in-depth discussion of the gained biological insights is beyond the scope of this article.

“Umsetzung im Text“

*2) How does the proposed tool outperform existing tools?*

*The authors avoid a detailed comparison with existing tools like MassTRIX and Paintomics by using the argument that those tools consider only two omics levels. Even though the proposed tool has the advantage of integrating several omics levels, typical practical applications consider only two levels, e.g., metabolomics and transcriptomics. A more detailed comparison of the proposed software tool with existing tools would therefore be necessary.*

TODO: Tabelle, die die Fähigkeiten vergleicht. Da natürlich insbesondere auf Interaktive Analyse eingehen. (Java standalone tool). Des Weiteren auf gradielle Farbdarstellung im Vergleich zu MassTRIX. Nachteil wäre die Annotation, aber ich denke hier kann man durchaus argumentieren, dass man dies ja professionellen Tools wie z.B. OpenMS/XCMS überlassen kann. Tools die für einen Vergleich in Frage kommen: (MetaMapp (O. Fiehn), MassTRIX (Philippe), Paintomics , Cytoscape)

“Umsetzung im Text“

Furthermore, InCroMAP provides unique features for data analysis and visualization which are not offered by any published software. Most notably, a structured, global view of the changes in cellular metabolism can be generated by using the metabolic overview feature provided by InCroMAP (shown in Figure 2B). While InCroMAP facilitates the integrated analysis of metabolomics, mRNA, miRNA, protein and DNA methylation data, other pathway analysis tools are limited to either solely metabolomics data (e.g., MSEA, MBRole, MPEA) or facilitate the integration with only one other platform (e.g., IMPaLA).

Können ja mal ne Tabelle reinsetzen und dann immer mal wieder paar Häkchen hinzufügen.

*3) The authors emphasize their strong effort in accepting and merging different kinds of gene or metabolite identifiers. But it remains unclear which of those tasks are new compared to similar software.*

We thank Reviewer 2 for making us aware of this important point which was insufficiently addressed in the old version of the manuscript. In this context we would like to point out that InCroMAP automatically recognizes gene identifiers from diverse databases (e.g., EntrezGene IDs, HGNC gene symbols, etc.) as well as probeset identifiers from the most common microarray platforms (e.g., Affymetrix, Agilent, etc.). While different types of identifiers are also accepted by the IMPaLA software, which also facilitates the calculation of integrated pathway enrichments, IMPaLA does not support probeset IDs and enable the mapping of these identifiers to gene symbols. Furthermore, InCroMAP is currently the only pathway enrichment analysis tool which is capable of integrating miRNA expression data by modeling the impact on pathways based on confirmed miRNA-mRNA interactions.

TODO: Lars: bezüglich Metabolite Identifier? Mega neues gibt es da wohl nicht, ich habe nur versucht möglichst sorgfältig zu sein beim mergen der Datenbanken und immer wieder kontrolliert. Es gab ja auch eine Studie die Überlappungen gesucht hat und dann auch Strukturen verglichen hat. Ich gucke mal ob ich da was finde. Zusätzlich versuche ich noch was wegen InChIKeys zu finden.

“Umsetzung im Text“

*4) To identify pathways affected by an experiment, a "special pathway enrichment" algorithms is used, but a detailed description of how metabolomics data are included in the algorithm is missing as well as a comparison to pathway enrichment of other tools. Also, what does it mean "pathway enrichment is limited to targeted metabolomics data"? Is the visualisation in general limited to targeted data?*

We thank Reviewer 2 for this critical remark. To the best of our knowledge only one other software (IMPaLA) exists which is capable of the integrating both transcriptomics and metabolomics data for the purpose of pathway enrichment analysis. In short, IMPaLA calculates the enrichment p-values independently for each platform using either a hypergeometric test or Wilcoxon enrichment analysis. Then, as the experiments are considered independent, the joint p-value is computed based on the product of the p-values calculated for the individual platforms. On the contrary, InCroMAP performs only one statistical test in which all genes and metabolites showing significant changes are combined to one list. Analogously, the gene and metabolite universe are pooled in the hypergeometric test statistic. As suggested by Reviewer 2, the conceptual differences between the InCroMAP and IMPaLA algorithm for cross-platform enrichments are now explained comprehensively in the revised version of the manuscript.

“Umsetzung im Text: siehe Punkt 1 von Reviewer 3“

TODO: Lars: Natürlich auch untargeted daten möglich, aber Problem der Signifikanz bei Enrichment wegen many-to-many Mappings beschreiben. Ebenso werden keine möglichen Metabolitannotationen generiert. Tools zitieren und vergleich anstreben. Zeigen, dass analyse mit many-2-many mappings noch problematisch ist.

TODO: Rainer. Gibt es noch andere Tools, die du kennst, die zur Analyse verwendet wird. Womit macht ihr z.B. eure Heatmap plots?

“Umsetzung im Text“

*5) The presented tool requires input data in form of significant values (p-value/ log fold change). This means the complete data pre-processing remains by the user. A big user friendly improvement could be done by integrating some parts of data processing or even accepting raw data as input, as partly done by other tools.*

We thank Reviewer 2 for this suggestion. However, we would like to point out that the use of tabular processed data corresponding to fold changes and/or p-values is one of the central design concepts underlying the InCroMAP software. All core developers agreed on this concept as firstly, the central aim of InCroMAP is not the replication of features (e.g., for the pre-processing of raw data) offered by existing tools and secondly, as the ease-of-use is ensured by a largely uniform input format which is suitable for the import of heterogeneous omics datasets. Thus, beyond elementary convenience functions (e.g., for the detection and conversion of IDs) no support in data preprocessing is provided.

“Umsetzung im Text: Im Text (Methodenteil) wird auf diese Einschränkung der Software klar hingewiesen. Ich würde daher im Text gar nichts ändern.”

**Minor Revisions**

*1) What is the meaning of the acronym InCroMAP? I could not find.*

As suggested by Reviewer 2 the meaning of the acronym InCroMAP (Integrated analysis of Cross-platform MicroArray and Pathway data) is now explained in the introduction.

“In this contribution we present an extended version of the tool InCroMAP (Integrated analysis of Cross-platform MicroArray and Pathway data).“

*2) The title "Straightforward interpretation… " might be misleading since there is no data interpretation in the manuscript.*

We agree with Reviewer 2 that the title should indicate more clearly that the focus of this work lays on the software and not on its application to biological data. Thus, the title of the manuscript was changed to “Integrated enrichment analysis and pathway-centered visualization of metabolomics, proteomics, transcriptomics and genomics data by using the InCroMAP software”.

*3) Figure 2B shows a pathway, but metabolite data (colored circles) seems not to be included in the visualization.*

Since the focus of the manuscript is on metabolomics data visualization, we agree with Reviewer 2 that it is more appropriate to show a metabolic pathway in Figure 2B. The Figure was now updated and depicts the KEGG pathway “Glycolysis / Gluconeogenesis”. Since Reviewer 1 also suggested to mention experimental studies using InCroMAP in this article, the pathway was overlaid with real data from a study of the intermediary metabolism of Ras-mutated mouse liver tumors. As stated previously the corresponding manuscript was recently accepted by the International Journal of Cancer and was added to the submission for the sake of completeness.

**Reviewer 3 comments:**

**Major Revisions**

*1) I´m missing more detailed explanations on the computation, which is important in the field of such tools. E.g. good background set for genes, proteins and metabolites are needed for calculation of enrichment and p-values. Furthermore, the authors do not explain in detail how combined enrichment works and how values are calculated.*

We fully agree with Reviewer 3 that a more detailed description of the algorithm used for the pathway enrichment is required. Thus, we added a comprehensive explanation of the adopted methods to the methods section (see subsection 2.2). Furthermore, as suggested by Reviewer 2 we also explain how the algorithm implemented in InCroMAP differs from the approaches used in related software.

„siehe Abschnitt 2.2.”

*2) I would also like to know how they implemented the metabolite database for mapping between the different identifiers. This is important, because a lot of people are struggling with harmonizing different metabolomics databases*

TODO: Lars. Nochmal genau beschreiben, wie die Integration gelaufen ist.

“Umsetzung im Text“

**Minor Revisions**

*1) Page4 line 2-3. Sentence "A main goal..." can be improved.*

In response to the suggestion made by Reviewer 3 the corresponding sentence was revised accordingly.

“The application was specifically designed to provide a high ease of use for experimental biologists.“