Dr. Kim-Anh Lê Cao

Senior Lecturer, Statistical Genomics

NHMRC Career Development Fellow

School of Mathematics and Statistics

Centre for Systems Genomics Bld 184

The University of Melbourne | VIC 3010

T: +61 (0)3834 43971 | kimanh.lecao@unimelb.edu.au

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Dear Editor of Genome Biology,

We wish to submit our manuscript “**DIABLO: identifying key molecular drivers from multi-omic assays, an integrative approach**” for consideration as a research article in your journal.

In the omics era, computational solutions to integrate different types of biological data measured on the same specimens or samples are trailing behind data generation. Our manuscript aims to feel this gap by proposing an efficient, flexible and easy-to-use computational framework to integrate multiple omics data generated from emerging high-throughput technologies.

The main challenge facing multi-omics data integration is the large heterogeneity and difference in scales between omics platforms. Statistical integrative methods for biomarker discovery are still at their infancy and provide limited insight into complex biological processes. They are built on existing multi-steps methods that either concatenate or combine the analyses from each data set separately, and do not model the correlation structure between the different molecular levels. This is highly problematic as important information can be missed, leading to incorrect conclusions. DIABLO maximises the correlation between data sets whilst identifying the key molecular features that explain and reliably classify a phenotype of interest. The dimension reduction process enables intuitive visualisations of the samples and selected multi-omics signatures. We benchmarked and demonstrated the ability of DIABLO to select relevant correlated and discriminative biomarkers in a comprehensive simulation studies and in six multi-omics studies including two case studies in human breast cancer and asthma. In each of those studies we integrated various omic data sets ranging from transcriptomics (mRNA, miRNA), epigenomics (CpGs), proteomics and cell-type frequencies.

DIABLO facilitates the integration of large and heterogeneous data sets to identify relevant biomarker candidates in a wide range of biological settings. The method will be of significant interest to the scientifically diverse readership of *Genome Biology* to capitalise on multi omics data currently being generated and push novel biological discoveries of an unprecedented level.

We are fervent advocates of open data and open science. All analyses are available in R markdown format as supplementary material, and the method is implemented in the open source R package mixOmics, along with detailed tutorials on the companion website <http://www.mixOmics.org/mixDIABLO>.

The submitted manuscript has been approved by all authors and has not been submitted to any other journal. A previous submission to *Genome Biology*, **GBIO-D-16-01112** was rejected from Genome Biology after revisions. We have carefully considered the reviews of the reviewers and have considerably revised the current version of the manuscript. We provide a point-by-point response to reviewers in the next section. We look forward to your reply.

Yours sincerely,

Dr. Kim-Anh LÊ CAO

**Reviewer #1:**

*The article has several strengths:*

*a) The article is very well written and provides a good overview of various statistical methods for analyzing*

*genomic data.*

*b) I think it presents an honest analysis of the data. The authors resist the temptation to oversell their method.*

*They acknowledge that their method does not outperform existing methods when it comes to accuracy.*

*c) The authors have implemented the method in an R package*

*d) This is a multi-omic method that integrates data.*

*e) The authors apply their method to both empirical data and to simulated data.*

We appreciate the positive comments from the reviewer and the careful review. In the previous iteration of the manuscript we had focused specifically on the classification performance which failed to showed superior classification performance using a single breast cancer case study. In the current iteration of the manuscript, we have instead focused on whether our methods can elucidate **superior biological enrichment** compared to other integrative strategies using four different multi-omics cancer datasets. However, we demonstrate using four real world cancers (lung, kidney, colon and glioblastoma) with multi-omics datasets (mRNA, miRNA and CpGs) that our method elucidates significantly more biological enrichment compared to other integrative strategies for multi-omics data integration.

*There are a few weaknesses.*

*The method is quite complicated and involves several parameter choices surrounding the underlying*

*correlation structure. Why use a complicated method when simpler methods have similar predictive accuracy?*

We agree with the review that the depiction of the method was lacking sufficient details which made its interpretation confusing. Therefore, in the revised manuscript we have incorporated other multi-omics methods and demonstrate that indeed our method does not require many parameters settings as compared to other existing multi-omics data integration methods (**see Supplement**). Briefly, our method requires 3 parameters, 1) number of variables to select from each omic dataset, 2) number of components to select from each omic dataset and 3) whether the correlation between certain omics datasets should be maximised (e.g. mRNA and miRNA). We demonstrate that explaining the correlation structure across omic datasets while discriminating between phenotypic groups is the key to identifying superior biological enrichment across various collections of gene set databases.

*Why measures different types of data when a single data source (e.g. mRNA) already leads to good accuracy?*

We completely agree with the reviewer, that if the focus is on biomarker discovery, why not use the simplest, and cheapest strategy to identify biomarkers? Therefore, in the revised manuscript we discussion an important consideration between discrimination and correlation, that is, whether the focus is to identify strong predictive biomarkers or generate novel biological hypothesis for underlying disease mechanisms. This shift in focus is depicted in our manuscript title, “**DIABLO: identifying key molecular drivers from multi-omic assays, an integrative approach**”, changing the focus from biomarkers to key molecular drivers.

*I am not convinced that the method helps to elucidate the underlying biology. I understand that the latent structure might uncover interesting biology but I would never use this method to learn biology. Rather, I would use cluster analysis or unsupervised learning methods.*

In the previous version of the manuscript we did not compare the biological enrichment of the various methods that were used. However, in the revised version of the manuscript, we extensive explored this area, using multiple cancer multi-omics dataset, multiple gene-set databases and both unsupervised and supervised integrative methods for data-integration which perform variable selection (select important variable). Since the reviewer suggested that we include unsupervised methods, we performed a literature search for integrative methods for multi-omics data integration and categorized these methods into supervised and unsupervised and whether they performed variable selection or not (**see Supplementary Fig. 1**). In the revised version of the manuscript we have included unsupervised methods used for multi-omics data integration and demonstrate that our method out-performs these methods with respect to biological enrichment thus elucidating more known biology. We demonstrate using a case study on human breast cancer, that our method also implicates novel biomarkers that have not been previously associated with breast cancer.

*Overall, I am not sure how much biology can be learnt by applying this method. Bottom line: this predictive method does not seem to improve predictive accuracy.*

The focus in the revised version is not the prediction accuracy which we would like to point out was based on a single multi-omic dataset. In the revised manuscript with have extending our analyses to include comprehensive simulation studies and six multi-omics studies including two case studies in human breast cancer and asthma. In each of those studies we integrated various omic data sets ranging from transcriptomics (mRNA, miRNA), epigenomics (CpGs), proteomics and cell-type frequencies. We believe this manuscript to provide an important contribution to data integration for multi-omics studies, because it demonstrated the trade-off between explaining the correlation structure across multiple biological domains and discrimination between multiple phenotypic groups. Importantly we show that considering these two factors leads to improve biological enrichment and thus provides novel hypotheses to be further tested.

In the revised implementation of our method, we have incorporated a weighed majority vote scheme which gives more important to stronger datasets over datasets with a weaker signal, thereby improving the classification performance. The implementation shows that our method performance is comparable with existing multi-step classification scheme in simulated datasets.

**Reviewer #2:**

*This is a well written article which addresses an important need in the field. 1) In the introduction, the longer intro to sparse CCA should be provided. In the methods the actual method is more clearly stated "DIABLO extends sparse gCCA to a classification framework".*

In the revised version of the manuscript we provide a clear explanation of our method DIABLO (see lines 99-116).

*“DIABLO (****D****ata* ***I****ntegration* ***A****nalysis for* ***B****iomarker discovery using* ***L****atent c****O****mponents) maximizes the common or correlated information between multiple omics (multi-omics) datasets while identifying the key omics variables (mRNA, miRNA, CpGs, proteins, metabolites, etc.) and characterizing the disease sub-groups or phenotypes of interest. DIABLO uses Projection to Latent Structure models (PLS) [1], and extends both sparse PLS-Discriminant Analysis [2] to multi-omics analyses and sparse Generalized Canonical Correlation Analysis [3] to a supervised analysis framework. In contrast to existing penalized matrix decomposition methods [4], DIABLO is a component-based method (or a dimension reduction technique) that transforms each omic dataset into latent components and maximizes the sum of pairwise correlations between latent components (user-defined) and a phenotype of interest [5]. DIABLO is, therefore, an integrative classification method that builds predictive multi-omics models that can be applied to multi-omics data from new samples to determine their phenotype. Users can specify the number of variables to select from each dataset and visualize the omics data and the multi-omics panel into a reduced data. The method is highly flexible in the type of experimental design it can handle, ranging from classical single time point to cross-over and repeated measures studies. Modular-based analysis can also be incorporated using pathway-based module matrices [6] instead of the original omics matrices, as illustrated in one of our case studies.”*

The mathematical formulas such as the sGCCA algorithm, and its extension to a discriminant framework can be found in the Methods section.

*2) Can the approach can handle missing data, that is missing row or column observations or is it only missing datasets. I presume, the later, as the intersection of tumors with complete data was used in training real data. This is important and should be made clear in the intro, abstract and discussion.*

Our method cannot account for complete observations or variables that are missing, because each dataset is projected using loading vectors to construct latent components (linear combination of the original variables). However, missing values are allowed in the dataset matrices since their values are merely skipped when computing the values for the latent components.

*3) Can PLS DA be applied to multi class classification. Was this tested?*

Yes, the revised version of the manuscript uses sparse Partial Least Squares Discriminant Analysis (sPLS-DA), in various multi-step classification schemes such as concatenation and ensemble-based schemes.

*4) The order of pair comparisons appears important. (discussion page 17, 18 and methods).*

*Those unfamiliar with their data may specify a suboptimal Design Matrix. Could there be some tools that provide guidance? For example, multiple factorial analysis or one of many tensor decompositions could be used to compute an RV coefficient. Alternative, can datasets be weighted in the analyses? In multi dataset approaches, data are often weighted by quality/size, the first eignenvector etc (reviewed by Meng et al., Brief Bioinform (2016) doi: 10.1093/bib/bbv108). If data has a batch effect, and this data were used to seed the analysis (aka in the first pair of data analyzed) , would that skew the results ? Could this please be tested.*

First and foremost, there is no concept of an “order” to the pairwise comparisons in the DIABLO framework, as it is the **sum of pairwise correlations** that is maximized, **see Methods**. Therefore the pairwise correlations are considered simultaneously in one step of the SGCCA algorithm[3].

We have opted to borrow some techniques from the multiblock literature such as multiblock partial least squares (MBPLS[7]) which datasets (also called blocks) are weights based on their correlation with the response variable. Therefore, in the current implementation of DIABLO we have used a weighted majority vote scheme based on the correlation between the latent component of each omic dataset with the latent component of the response matrix. This has significantly improved our classification error rates, as the strongest discriminatory datasets is given a higher weight in the overall class prediction for a new sample. Further, the use of weighted majority vote overcome the case when an equal number of voting classifiers and no consensus can be achieved.

In the discussion, we state the influence of batch effects on the multivariate modelling performed by our method (see lines 322-327).

*“Finally, DIABLO, like other methods we benchmarked, will be affected by technical artifacts of the data, such as batch effects and presence of confounding variables that may affect downstream integrative analyses. Therefore, we recommend exploratory analyses be carried out in each single omics dataset to assess the effect, if any, of technical factors and use of batch removal methods prior to the integration analysis* [8–10]*.”*

*5) On page 8, "validation of the Diablo methods on synthetic data" . Three different criteria are explored 1) CorNonDis 2) CorDis 3) NonCorDis. Please explain the rational behind nonCorDis should be explained. In a 2 class system, methods such as CCA or PLS extract eigenvectors of correlated variables. Therefore a discriminate eigenvector will represent a set of correlated variables. Gene expression and 'omics data, measure genes which work in pathways, and therefore data has considerable correlation structure. Discriminatory non-correlated vectors, may reflect system noise.*

The rationale for including four-types of variables was to determine the influence of the correlation structure between datasets and discrimination between phenotypic groups. Therefore different combination of discrimination (discriminatory, non-discriminatory) and correlation (correlated, uncorreled) were used to create four types of variabes; 30 correlated-discriminatory (corDis) variables, 30 uncorrelated-discriminatory (unCorDis) variables, 100 correlated-nondiscriminatory (corNonDis) variables, and 100 uncorrelated-nondiscriminatory (unCorNonDis) variables (**Supplementary Note, Supplementary Fig. 2**).

*6) please provide a discussion on filtering data. In each case, data were filtered and reduced. Is this to reduce "noise" or for computational efficiency. Please discuss and comments on the computational cost of larger datasets.*

We provide a discussion about filtering data in the revised version of the manuscript (lines 319-320). *“…we advise using a broad filtering strategy to alleviate computational time when dealing with extremely large datasets (e.g. > 50,000 features each).” We also provide additional guidance on filtering in our mixOmics manuscript [11].*

*7) On page 11 the acronym BER (balanced error rate) is used before it is defined.*

Since we do not use the acronym BER many times in the manuscript, we have removed it altogether and explicitly state balanced error rate.

*8) on Page 13, please describe "eigengene summarizaton" in more detail. Please describe how to interpret the results, saying it is "common approach" on page 29 is insufficient;*

We provide a description of eigengene summarization in the revised manuscript (lines 504-509).

*“****Modular analysis:*** *Eigengene summarization is a common approach to decompose a n x p dataset (where n is the number of samples and p is the number of variables in a module), to a component (linear combination of all p variables) that represents the summarized expression of genes in the module [6]. For the asthma study, 15,683 genes were reduced to 229 KEGG pathways and 292 metabolites were reduced to 60 metabolic pathways using eigengene summarization.”*

*9) page 16. Why were 9 variables (36 in total) selected in analysis of the BRCA data? Is there any guidance as to how many variables should be selected. For example, mRNA and protein were more informative in gsea, therefore it might be better to select more variables from these datasets?*

Yes, we provide a tune function in the mixOmics R-package [11], which uses a grid approach to select an optimal number of variables to select from each omic dataset. A section on parameter tuning discusses the grid approach to identifying the optimal number of variables and components to select (lines 438-456).

*“Finally, the third set of parameters to tune is the number of variables to select per dataset and per component. Such tuning can rapidly become cumbersome, as there might be numerous combinations of selection sizes to evaluate across all K datasets. For the breast cancer study, we used 5-fold cross-validation repeated 50 times to evaluate the performance of the model over a grid of different possible values of variables to select (****Supplementary Fig. 8****). The performance of the model for a given set of parameters (including number of component and number of variables to select) was based on the balanced classification error rate using majority vote or average prediction schemes with centroids distance. The balanced classification error rate is useful in the case of imbalanced class sizes, where the majority classes can have strong influence on the overall error rate. The balanced error rate measure calculates the weighted average of the individual class error rates with respect to their class sample size. In our experience, the number of variables to select in each dataset provided less of an improvement on the error rate compared to tuning the number of components. Therefore, even a grid composed of a small number of variables (<50 with steps of 5 or 10) may suffice as it does not substantially change the classification performance. This is because of the use of regularization constraints which reduces the variability in the variable coefficients and thus maintains the predictive ability of the model. Further, the variable selection size can also be guided according to the downstream biological interpretation to be performed. For example, a gene-set enrichment analysis may require a larger set of features than a literature-search interpretation.”*

*10) page 17, 3rd line from top. "known cell-types and pathwaysin the" typo. Insert space*

The above error has been corrected in the revised version of the manuscript.

*11) The statement on p 18, "To our knowledge DIABLO is the only integrative classification methods that models the correlation structure between omics data" is inaccurate. See the Meng et al., review. There are many many methods that use dimensions reduction to extract correlated structure in multiple 'omics data. Also Jeffrey et al., 2007 Bioinformatics. 2007 Feb 1;23(3):298P305. Epub 2006 Nov 24. used discriminative analysis with coinertia analysis and described a supervised integrative latent variable approach, which is related to this work but did not employ sparse methods.*

We have removed the statement from the revised manuscript.

*12) In the methods, p21. In the abstract/intro, the method is describes as a supervised PLS/DA approach, but on p21 it appears to be cluster to partitioning around centroids, with majority voting. Please describe the approach clearly and consistently.*

*13) is the analysis effective by the number of variables. For example if dataset A has several thousand variables and dataset B has less than 50, would this impact the analysis?*

Each dataset is scaled by the number of variable such that the total variability for each dataset is the same. Therefore, the difference in the number of variables in each dataset does not impact the analysis as the total amount of variation is the same for each dataset.

*14) p25, The de-duplication effort in GSEA is important and should be clear to users, If a more stringent assignment were used, would this impact results?*

GSEA is impacted by the number of features that are input into the analysis and the types of gene sets that are used to determine biological enrichment. In the revised manuscript we include a benchmarking experiment where we constructed multi-omic biomarker panels of equivalent number of features, a total of 180 features (60 features of each omics type across 2 components). Further we tested 10 different gene set databases, from Molecular signature database[12], blood transcriptional modules[13] and cell-specific expression from Benita *et al*. [14].

*15) p 27. Data Processing. Were 3,073 BRCA clinical variables used in this study? The PAM50 assignments for tumors (obtained from TCGA staff) should be made available together with the filtered TCGA data, such that others can reproduce this work.*

The 3,073 variables listed describe the data that were obtained from TGCA. From the clinical data, only the PAM50 labels and sample-type variables were used. The complete code and data files can be found with the github repository (https://github.com/singha53).

*16) p28. Terms in the Voom equation are not fully defined. Filtering removed "genes with counts less than 0". Does this mean the sum of the gene across all tumors was zero, or that any gene which has a zero tumor in any 1 tumor was excluded?*

We have clarified this following in the revised manuscript (**see Supplementary Data file**). The count data for the mRNA dataset, Xcounts was normalized to log2-counts per million (logCPM), Xnorm, similar to limma voom [15]:

**

After library size (lib.size = total number of reads per sample) normalization, genes with counts less than 0 in more than 70% of samples were removed. The PAM50 genes were also removed from the mRNA dataset prior to analyses. Similarly, the miRNA count data was normalized to logCPM and miRNA transcripts with counts less than 0 in more than 70% of the samples were also removed.

*17) p28 Asthma study. Genes were reduced 229 KEGG pathways and metabolites were reduced to 60 pathways. Why were variables reduced to GeneSet. The rational and need for this is not explained. Was it simply to aid biological interpretation of the data or was it for computational reasons?*

In the current version of the manuscript, the original numbers of features were retained for all analyses for the benchmarking experiment and human breast cancer case study. The datasets consists of hundreds of variables in some datasets (e.g. miRNA) and greater than 20,000 variables in other datasets (e.g. CpGs). For the asthma case-study we wanted to incorporate modular-based analyses within the DIABLO framework to focus in pathways that span a common biological mechanism.

*18) Figure 1 A) not clear if concatenation is performed on genes or tumors (rows/cols). C) The DIABLO diagram is confusing. it is not clear that DIABLO is a pairPwise approach.*

The figure has been updated to clarify the integration and classification aspects of the DIABLO framework (**see Supplementary Figure 3**). Each dataset is a n x p*j* matrix, where p*j* is the number of variables (columns) for the *j*th dataset. For the concatenation-based analysis, the datasets are combined row-wise since the number of samples are the same for each omics dataset, that is, the multi-omics data is obtained for the same set of samples.

Although DIABLO computes the pairwise correlation between latent components of pairs of omic datasets, similar to PLS and CCA, its objective is to maximize the sum of pairwise correlation between different omics datasets (see objective function in **Methods**).

*19) Figure 4 legend. DIABLO 1P12 are not defined, What was the difference between these models.*

In the previous version of the manuscript, the concatenation and ensemble biomarker panels were tuned such that each panel consisted of a specific number of variables. We had created DIABLO panels with equivalent numbers of variables in order to keep the comparisons consistent. However, given this extra confusion, we have gone back and instead equal number of variables of each omic-type for the different multi-omics classification methods.

*20) Figure 5. There is no scale on the ciros plot (gene level) which makes its interpretation difficult. Also please add Gene Names to the heatmap (E)*

The purpose of the circosplot is to depict the inter-correlations between omic datasets. These can be observed from the red (positive) and blue (negative) lines between omics datasets (different colors). The scale for the lines surrounded the ideogram is not depicted as it is centered at zero, therefore the line height represent the average expression levels of a given variable in a given phenotypic grous compared to others. Gene names have not been added to the heatmap, due to size limitation of the figure. However, the feature plot in Figure 3a lists all the features selected by the multi-omic biomarker panel.

*21). Reference 38 Gauvreau et al., is in upper case*

All references have been checked and the capitalization has now been fixed.

*22) Please provide more details on the computational complexity of the method as 1) the number of variables increases 2) the number of datasets increased 3) the impact of correlated datasets (eg microarray and RNAseq)*

The purpose of the revised analysis was to determine the effect of the design matrix on the types of variables selected, its impact on the error rate and whether this lead to superior biological enrichment as compared to other integrative strategies. Instead of using more stimulation studies, we decided to focus more on real world datasets and determine other attributes of the multi-omic biomarker panels such as network connectivity and biological enrichment.

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