Simultaneous Non-Negative Matrix Factorization for Multiple Large Scale Gene Expression Datasets in Toxicology

Clare M. Lee¹, Manikhandan A. V. Mudalair², D. R. Haggart³, C. Roland Wolf³,⁴, Gino Miele⁵, J. Keith Vass¹, Desmond J. Higham¹*, Daniel Crowther⁶

Department of Mathematics and Statistics, University of Strathclyde, Glasgow, United Kingdom, 1 College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom, 2 University of Dundee Medical Research Institute, Ninewells Hospital & Medical School, Dundee, United Kingdom, 3 CXR Biosciences Ltd, Dundee, United Kingdom, 4 Epsistem Ltd., Manchester, United Kingdom, 5 Sanofi, Frankfurt am Main, Germany

Introduction

The aim of this work is to highlight the usefulness of a recently proposed extension to the technique of non-negative matrix factorization (NMF) by demonstrating its promise for early detection of toxicity in the drug discovery process. In particular, we (a) show that any number of related datasets can be treated simultaneously with this approach, (b) deal with practical issues that arise when the algorithm is applied to real datasets, (c) demonstrate its use with a new large scale microarray dataset, and (d) interpret the results from a biological perspective.

Computational Background

NMF seeks to represent a large complex dataset in terms of smaller factors. The name covers many algorithms. Each approximates a non-negative matrix as the product of two or more smaller non-negative matrices, by attempting to minimise some objective function. Lee and Seung [1] showed that when applying multiplicative non-negative factorization to images of faces, each row/column pair of the factors expresses a recognizable facial feature. These techniques have since been used in many settings to learn parts of the data as well as to factorize and cluster datasets. For example, when applied to text data in [1] the algorithm can differentiate multiple meanings of the same word by context. On microarray data, NMF has been used to find patterns in genes or samples, typically bi-clustering both groups in a similar manner to two-way hierarchical clustering [2–7]. The review article [8] shows how NMF has also been successful in other areas of computational biology, including molecular pattern discovery, class comparison and biomedical informatics. The new challenge that we address in this work is to apply the NMF methodology to multiple, related, large scale, data sets simultaneously. We use the

Abstract

Non-negative matrix factorization is a useful tool for reducing the dimension of large datasets. This work considers simultaneous non-negative matrix factorization of multiple sources of data. In particular, we perform the first study that involves more than two datasets. We discuss the algorithmic issues required to convert the approach into a practical computational tool and apply the technique to new gene expression data quantifying the molecular changes in four tissue types due to different dosages of an experimental panPPAR agonist in mouse. This study is of interest in toxicology because, whilst PPARs form potential therapeutic targets for diabetes, it is known that they can induce serious side-effects. Our results show that the practical simultaneous non-negative matrix factorization developed here can add value to the data analysis. In particular, we find that factorizing the data as a single object allows us to distinguish between the four tissue types, but does not correctly reproduce the known dosage level groups. Applying our new approach, which treats the four tissue types as providing distinct, but related, datasets, we find that the dosage level groups are respected. The new algorithm then provides separate gene list orderings that can be studied for each tissue type, and compared with the ordering arising from the single factorization. We find that many of our conclusions can be corroborated with known biological behaviour, and others offer new insights into the toxicological effects. Overall, the algorithm shows promise for early detection of toxicity in the drug discovery process.

Citation: Lee CM, Mudalair M, Haggart DR, Wolf CR, Miele G, et al. (2012) Simultaneous Non-Negative Matrix Factorization for Multiple Large Scale Gene Expression Datasets in Toxicology. PLoS ONE 7(12): e48238. doi:10.1371/journal.pone.0048238

Editor: Ramin Homayouni, University of Memphis, United States of America

Received February 14, 2012; Accepted September 25, 2012; Published December 14, 2012

Copyright: © 2012 Lee et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by the Translational Medicine Research Collaboration—a consortium made up of the Universities of Aberdeen, Dundee, Edinburgh and Glasgow, the four associated NHS Health Boards (Grampian, Tayside, Lothian and Greater Glasgow & Clyde), Scottish Enterprise and Pfizer, by EPSRC Grant EP/E49370/1, by the Knowledge Transfer Account of the University of Strathclyde and by the 2007 DTI grant “New serum Biomarkers for Preclinical and Clinical Drug Safety Assessment”. CML and DJH were supported by the Engineering and Physical Sciences Research Council of the UK, under their Fundamentals of Complexity Science call. DJH was funded by a Fellowship from the Leverhulme Trust. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: DC was employed by Pfizer and legacy company Wyeth during the course of this work and is now employed by Epistem. CRW is employed by CXR. Pfizer agreed to the publication of this manuscript. MM, DRH and JKV were funded through the TMRC. This does not alter the authors’ adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: d.j.higham@strath.ac.uk

Introduction

The aim of this work is to highlight the usefulness of a recently proposed extension to the technique of non-negative matrix factorization (NMF) by demonstrating its promise for early detection of toxicity in the drug discovery process. In particular, we (a) show that any number of related datasets can be treated simultaneously with this approach, (b) deal with practical issues that arise when the algorithm is applied to real datasets, (c) demonstrate its use with a new large scale microarray dataset, and (d) interpret the results from a biological perspective.

Computational Background

NMF seeks to represent a large complex dataset in terms of smaller factors. The name covers many algorithms. Each approximates a non-negative matrix as the product of two or more smaller non-negative matrices, by attempting to minimise some objective function. Lee and Seung [1] showed that when applying multiplicative non-negative factorization to images of faces, each row/column pair of the factors expresses a recognizable facial feature. These techniques have since been used in many settings to learn parts of the data as well as to factorize and cluster datasets. For example, when applied to text data in [1] the algorithm can differentiate multiple meanings of the same word by context. On microarray data, NMF has been used to find patterns in genes or samples, typically bi-clustering both groups in a similar manner to two-way hierarchical clustering [2–7]. The review article [8] shows how NMF has also been successful in other areas of computational biology, including molecular pattern discovery, class comparison and biomedical informatics. The new challenge that we address in this work is to apply the NMF methodology to multiple, related, large scale, data sets simultaneously. We use the
The mice were randomly divided into three groups and treated with either Vehicle or two concentrations of PPM201 (6 or 20 mg/kg body weight). The response to the “therapeutic dose”, 6 mg/kg, was found to vary widely for ALT (alanine aminotransferase), AST (aspartate aminotransferase), LDH (lactate dehydrogenase) and CK (creatine kinase). AST is raised in PPM201 treated animals, with mouse E (6 mg/kg) seeming to be especially raised; AST is known to be variable between animals, but mouse E also shows a higher level of ALT, indicating that there may be a shared mechanism for the two enzymes. Creatinine is decreased in liver and possibly kidney disease; the contrasts observed here are inconclusive. BUN (Blood, Urea and Nitrogen) is raised in kidney disease; results are again inconclusive. Following cardiac infarction LDH is increased after 12 hours, possibly also caused by liver toxicity; mouse E is markedly lower than the other PPM201 treated animals and it may be that its heart muscle profile might be more similar to the untreated mice. CK is, like LDH, increased in myocardial infarction and this supports the LDH findings for mouse E.

doi:10.1371/journal.pone.0048238.t001

Table 1. Blood clinical chemistry analysis for each mouse.

| Group | Mouse ID | Dose (mg/kg b.wt) | ALT (U/L) | AST (U/L) | Creatinine (µmol/L) | BUN (U/L) | LDH (U/L) | CK (U/L) |
|-------|---------|-------------------|----------|----------|---------------------|----------|----------|----------|
| I     | A       | Vehicle           | 42       | 188      | 12                  | 4        | 348      | 484      |
| I     | B       | Vehicle           | 41       | 92       | 9                   | 6        | 364      | 258      |
| I     | C       | Vehicle           | 29       | 75       | 9                   | 6        | 278      | 166      |
| II    | D       | 6                 | 95       | 441      | 11                  | 8        | 1218     | 4930     |
| II    | E       | 6                 | 692      | 981      | 8                   | 7        | 2126     | 1130     |
| II    | F       | 6                 | 52       | 83       | 9                   | 8        | 294      | 152      |
| III   | G       | 20                | 312      | 1300     | 6                   | 8        | 3172     | 2544     |
| III   | H       | 20                | 462      | 937      | 8                   | 6        | 1760     | 1182     |
| III   | I       | 20                | 698      | 1090     | 6                   | 7        | 2616     | 1592     |

The value of the objective function for $k = 2, \ldots, 16$. (a) The area under consensus cumulative density, $[3,14]$. (b) The cophenetic correlation coefficient, $[3]$. doi:10.1371/journal.pone.0048238.t001

Figure 1. Three measures of the performance versus specified cluster size, $k$, when the data set is factorised as a single entity. (a) The value of the objective function for $k = 2, \ldots, 16$. (b) The area under consensus cumulative density, $[3,14]$. (c) The cophenetic correlation coefficient, $[3]$. doi:10.1371/journal.pone.0048238.g001
Figure 2. Factorising as a single dataset; reordering using the NMF for \( k = 4 \). The columns show the samples and the rows the gene expression for each of the 45037 genes. Genes and samples are organised by cluster number. Elements within each cluster are ordered, with the largest value at the bottom/right. Each tissue is characterised by a group of highly expressed genes; from the top left to bottom right these are heart, skeletal muscle, liver and kidney. For comparison purposes, the characteristic 100 “best” genes in the four columns are names heart1, skeletal muscle1, liver1, and kidney1.

doi:10.1371/journal.pone.0048238.g002
method on more than two. We also consider various practical
issues that must be tackled in order to produce a useful
computational tool. To minimize the number of algorithmic
parameters, make the results straightforward to interpret, and
exploit the natural sparsity in the algorithm [9, section 3],
we focus on hard clustering. The interesting issue of
allowing clusters to overlap in this context is therefore left as
future work.

**Biological Background**

We analyse gene expression data describing the molecular
changes in four tissue types due to different dosages of an
experimental pan-peroxisome proliferator-activated receptor
(pan-PPAR) agonist PPM-201, provided by Plexxikon. PPARs have
attracted great interest as potential therapeutic targets for diabetes
[11], but major concerns have arisen due to clinically observed
side-effects [12]. Hence, there are compelling reasons for
toxicological studies at the gene expression level.

The material is organised as follows. In Section we describe the
simultaneous NMF algorithm and outline our approach for using
the output to order and cluster a dataset. Section describes the
mouse microarray data, and the NMF results that arise when we
treat it as a single dataset are given in Section . This is followed in
Section by the analysis of the data split into four datasets
corresponding to the known tissue types; liver, kidney, heart and
skeletal muscle. In Section we compare the gene clusters from
Sections and , and Section discusses the results. Conclusions are
given in Section .

**Methods**

**Algorithms**

Given $d$ non-negative data matrices $A(i)$ of size $m(i) \times n$ for
$i = 1, \ldots, d$, our aim is to simultaneously factorize all matrices so that

$$A(i) \approx W(i)H$$

with the additional constraints that $W(i)$ is a non-negative matrix
of size $m(i) \times k$ for $i = 1, \ldots, d$, and $H$ is a non-negative matrix of
size $k \times n$. Generalising naturally from the $d=2$ case in [9], we
seek to minimise the objective function

$$f(A(i), W(i), H) = \sum_{i=1}^{d} \beta(i) \|A(i) - W(i)H\|^2$$

where $\beta(i) = \frac{\lambda(i)}{\lambda(1) + \lambda(2)}$. Here $\| \cdot \|$ denotes the Frobenius norm. As in
[9] the $\beta$ coefficients are designed to give equal weight to the
different error terms. Based on the multiplicative update rules
developed in [13], an iterative algorithm that attempts to solve the
optimisation problem can be derived using a gradient descent
method $d+1$ times. This gives us the following sequence of approximations for $j=1,2,\ldots$, given initial choices $W(i)^0$ and
$H^0$,

$$W(i)^{j+1} = W(i)^j + \eta_W^j \cdot \left( A(i)H^jT - W(i)^jH^jH^jT \right)$$

$$H^{j+1} = H^j + \eta_H^j \cdot \left( \sum_{i=1}^{d} \beta(i) \left( W(i)^jT A(i) - W(i)^jT W(i)^j H^j \right) \right)$$

work of Badea [9,10], who considered an extension of NMF that
deals with two data matrices. Simultaneous NMF is used in [9]
to study pancreatic cancer microarray data alongside extra
information concerning transcription regulatory factors. In [10]
microarray datasets for pancreatic ductal adenocarcinoma and
sporadic colon adenocarcinoma are simultaneously factorized in
order to discover expression patterns common to both data sets.
This simultaneous NMF approach readily extends to the case of
an arbitrary number of data matrices and here, for what we
believe to be the first time, we implement and evaluate the
method on more than two. We also consider various practical
for some small positive matrices $\eta_{W(i)}^j$ and $\eta_H^j$, with $\ast$ representing element-wise multiplication. The iteration may be motivated through the intuition that when $\eta_{W(i)}^j$ and $\eta_H^j$ are sufficiently small and positive each of these equations should reduce the objective function. This allows us to set

$$
\eta_{W(i)}^j = \frac{W(i)^{\[j\]} W(i)^{\[j\]T} H^{\[j\]} H^{\[j\]T}}{\sum_{i=1}^d \beta(i) W(i)^{\[j\]T} W(i)^{\[j\]T} H^{\[j\]} H^{\[j\]T}} \quad \text{and} \quad \eta_H^j = \frac{H^{\[j\]} \ast \sum_{i=1}^d \beta(i) W(i)^{\[j\]T} A(i)}{\sum_{i=1}^d \beta(i) W(i)^{\[j\]T} W(i)^{\[j\]T} H^{\[j\]} H^{\[j\]T}},
$$

again with the division being performed element-wise. Hence the overall iteration has the form

$$
W^{[j+1]}(i) = W^{[j]}(i) \ast \frac{A(i) H^{[j]T}}{W(i)^{[j]T} H^{[j]} H^{[j]T}}
$$

The values in $\eta_{W(i)}^j$ and $\eta_H^j$ are non-negative due to the constraints on the matrices, however they are not necessarily small. The iteration decreases the objective function (1), so this leads to a locally optimum solution, but we cannot guarantee convergence to a global optimum. In particular, different initial conditions can lead to different factorizations of different quality.

Having iterated up to some stopping criterion and produced the factorizations, we use them to bi-cluster the data. Each sample is assigned to the cluster for which it has the largest value in the gene

**Figure 3. Factorising as a single dataset.** The clustering of the mouse samples for $k=1, \ldots, 16$. Within each column the samples in the same colour are clustered together. No value of $k$ reveals the known tissue/dosage subgroups, or places different tissues in the same cluster.
doi:10.1371/journal.pone.0048238.g003
cluster and vice versa. In reordering the data for easy visualisation we organise the rows and columns by cluster number (assigned arbitrarily) and sort the elements within each cluster from the appropriate sample/gene set, with the largest value at the bottom/right of that cluster. Given that the second factor is common to all the factorizations, it produces a matching ordering of the columns of the data.

Because the result depends on the choice of initial condition, and because the choice of $k$ is not automatic, further information is needed in order to specify a practical algorithm. To deal with the lack of uniqueness, we try several initial conditions and pick a realisation that minimises the objective function (1). We then continue until further runs do not significantly alter the results. The objective function value is also one of the criteria we use in order to decide which rank/clustering is the most “appropriate” for the data. By regarding the objective function as a function of $k$, we identify values of $k$ where the decay in the objective function begins to diminish. In addition we also form a consensus matrix as in [3, 14] for the clustering of the objects. This is the average of the connectivity matrices $C$ where for each initialisation $C_{ij}=1$ if objects $i$ and $j$ are clustered together and 0 otherwise. So the consensus matrix contains values between 0 and 1 with the $(i,j)$th element being the likelihood that objects $i$ and $j$ cluster together. The cumulative density of these values is constructed, by summing the appropriate probabilities, and the area under this curve is the second measure we look at when considering choices for $k$. The third measure is the Pearson correlation of the cophenetic distances, as explained in [3].

**Mouse data**

We apply these techniques to mouse gene expression data quantifying changes in four different tissue types following administration of different dosages (vehicle, therapeutic and toxic) of an experimental pan-PPAR agonist. The study design and clinical chemistry results are summarised in Table 1. ALT and AST are known markers in rodents for liver toxicity [15] and from this criterion mouse E may be showing a toxic response to...
PPM201, despite it being administered at a supposedly therapeutic dose level. This conditions our expectation of the gene-expression pattern for mouse E and suggests that it may be similar to the toxic level group III for liver.

Nine wild type mice (strain: C57BL/6J) were randomly divided into three groups; - Group-I, II and III. PPM-201 in the vehicle base was administered daily for 14 days at 6 mg/kg body weight dose rate to each mouse in Group-II and at 20 mg/kg body weight dose rate to each mouse in Group-III while the mice in Group-I received only the vehicle base. On 15th day, the mice were sacrificed to harvest blood, heart, skeletal muscle, liver and kidney tissues for clinical chemistry, microarray and histopathology.

Figure 5. Factorisation of the four separate tissue types using simultaneous NMF with $k = 3$. Top left, kidney; top right, liver; lower left, heart; lower right, skeletal muscle. The four tissue types are treated as separate sources of information across a common set of mice. Genes are therefore ordered differently in each of the four tissues, but the mice ordering is global. The resulting mouse ordering and mouse clusters are detailed in Table 3.

doi:10.1371/journal.pone.0048238.g005
In the clinical chemistry analysis, alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), creatinine kinase (CK, U/L), blood urea nitrogen (BUN, mmol/L), creatinine (µmol/L) and lactate dehydrogenase (LDH, U/L) were measured from the blood of each mouse. Two sections of liver, two sections of kidney, one or two sections of skeletal muscle, and one section of heart were prepared from each mouse, stained with hematoxylin and eosin (H&E), and examined by a veterinary pathologist. Total RNA was isolated from murine tissues using Qiazol-based homogenization and subsequent column-based purification (Qiagen) with on-column DNase-treatment. DNase-free RNA was assessed for quality using Agilent Bioanalyser electrophoresis and acceptance criteria of RNA Integrity Number (RIN) greater than seven. 50 ng of total RNA was subsequently utilized as input to cDNA-based amplification and biotin-labelling using single-primer isothermal amplification according to the manufacturer’s instructions (Ovation System, NuGEN Technologies). Unlabelled and biotin-labelled cDNA was qualitatively assessed by Agilent Bioanalyser electrophoresis to ensure identical size distributions of all samples pre- and post-fragmentation. Fragmented, biotin-labelled cDNA were hybridized to MOE430 2.0 GeneChip arrays (Affymetrix) with subsequent scanning and feature extraction according to the manufacturer’s instructions.

The dataset has been approved by the GEO curators and assigned the accession number GSE31561.

**Ethics Statement**

The in vivo procedures undertaken during the course of this study (Ref: CXR0631) were subject to the provisions of the United Kingdom Animals (Scientific Procedures) Act 1986. The study was approved by the CXR Biosciences Local Ethics Committee and complied with all applicable sections of the Act and the associated Codes of Practice for the Housing and Care of Animals used in Scientific Procedures and the Humane Killing of Animals under Schedule 1 to the Act, issued under section of the Act.

| Cluster | Mouse | Dosage |
|---------|-------|--------|
| 1       | E     | 6 mg/kg |
| 1       | G     | 20 mg/kg |
| 1       | I     | 20 mg/kg |
| 1       | H     | 20 mg/kg |
| 2       | F     | 6 mg/kg |
| 2       | D     | 6 mg/kg |
| 3       | B     | Vehicle |
| 3       | A     | Vehicle |
| 3       | C     | Vehicle |

The mouse clusters when split by tissue type and reordered using the 4-way simultaneous factorization for \( k = 3 \).

doi:10.1371/journal.pone.0048238.g006

doi:10.1371/journal.pone.0048238.t003
Results

Single dataset

First, the samples are treated as a single dataset, with thirty six samples and 45037 genes, hence the data matrix $A$ is 45037 x 36. This corresponds to the case where $d = 1$ in Section . The factorizations were performed twenty times for each $k = 2, 3, ..., 16$, with a consensus matrix formed from the clustering of the samples. All gene clusters associated with this analysis are labelled with a subscript 1, e.g., clustering of the samples. All gene clusters associated with this analysis are labelled with a subscript 1, e.g., clustering of the samples.

Figure 1(a) shows the minimum size of the objective function that we observed for each value of $k$. We see that this value decreases monotonically, with a slower rate starting at around $k = 4$. Figure 1(b) shows the area under the cumulative density curves for the same values of $k$. This subfigure clearly points to $k = 4$, as does subfigure (c) showing the cophenetic correlation.

Based on Figure 1, we conclude that when the data is factorized as a single entity, $k = 4$ clusters is the most appropriate choice. Reordering the dataset using the ordering for $k = 4$ in the manner described in Section gives the images shown in Figure 2. This figure shows the samples in the columns and samples with cluster one at the top. To aid visualisation, the sample clusters are split by white lines, as are the gene clusters. This reordered data matrix shows a distinctive “ramp” effect in the blocks on the diagonal, placing genes that are most influential in identifying each tissue type to the bottom of the block. This figure also shows some of the differences in expression behaviour between the tissue types, particularly for the most influential genes.

Because we know the origin of the samples, we can confirm that the algorithm has put the heart samples in cluster one, the skeletal muscle samples in cluster two, the liver samples in cluster three, and the kidney samples in cluster four. The exact ordering of the samples is shown in Table 2. This table also shows the mouse identification information for each sample, and we see that the mice are not ordered in the same way within each cluster. It is the liver and skeletal muscle samples that most closely respect the dosage levels within the clusters. Both these clusters only have one sample mis-ordered.

Given that the factorization has been performed for $k = 2, ..., 16$ we know what the clustering would be from all these rank factorizations. This information is displayed in Figure 3. Here the rows representing the samples are ordered in tissue then dosage subgroups. For each rank $k$, samples with the same colour are assigned to the same cluster. As we have seen before, for $k = 4$ the samples are split into tissue types. The figure shows that this split persists at $k = 5$ with an empty cluster forming. In fact, for this range of $k$ there are at most twelve clusters of samples. We also see from this figure that for no value of $k$ are the twelve tissue/dosage subgroups found.

Multiple datasets

The test in Section indicates that the basic NMF factorization approach can deliver biologically meaningful results—separating the twelve samples by tissue type. But the failure to order correctly within tissue type according to dosage motivates the use of the multiple dataset generalization introduced in Section , where the four tissue types are treated as separate sources of information across a common set of mice. Intuitively, we would expect to add value to the data analysis by building known biology into the algorithm in this way. In this section, we therefore factorize the four new datasets simultaneously. This is similar to the test in Section in the sense that it produces a single ordering for the mice, but it has the potential to add extra information by providing four different, tissue-level, gene orderings. We thus have $d = 4$ matrices...

| Table 4. Gene cluster comparison for individual tissues in the single matrix, “tissue1,” with the four separate tissue matrices “tissue2,…” |
|---|---|---|---|---|---|---|---|
| H1 | SM1 | L1 | K1 |
| **Cluster** | **No.** | **Probability**<sup>1</sup> | **No.** | **Probability**<sup>1</sup> | **No.** | **Probability**<sup>1</sup> | **No.** | **Probability**<sup>1</sup> |
| Heart<sub>1</sub> | Clust.1 | 22 | 2.2188e-38 | 0 | 0.8005 | 0 | 0.8005 | 0 | 0.8005 |
| Clust.2 | 1 | 0.1785 | 2 | 0.0195 | 49 | 5.444e-108 | 0 | 0.8005 |
| Clust.3 | 11 | 4.3469e-16 | 7 | 2.8360e-09 | 1 | 0.1785 | 5 | 3.0037e-06 |
| total | 34 | 5.496e-67 | 9 | 1.4338e-12 | 50 | 6.309e-11 | 5 | 3.0037e-06 |
| S. Muscle<sub>1</sub> | Clust.1 | 4 | 7.3075e-05 | 15 | 1.1260e-12 | 8 | 6.8371e-11 | 0 | 0.8005 |
| Clust.2 | 0 | 0.8005 | 0 | 0.8005 | 0 | 0.8005 | 0 | 0.8005 |
| Clust.3 | 4 | 7.3075e-05 | 14 | 1.0243e-21 | 0 | 0.8005 | 0 | 0.8005 |
| total | 8 | 6.8371e-11 | 29 | 1.3672e-54 | 8 | 6.8371e-11 | 0 | 0.8005 |
| Liver<sub>1</sub> | Clust.1 | 1 | 0.1785 | 0 | 0.8005 | 13 | 8.4974e-11 | 1 | 0.1785 |
| Clust.2 | 0 | 0.8005 | 0 | 0.8005 | 0 | 0.8005 | 0 | 0.8005 |
| Clust.3 | 1 | 0.1785 | 2 | 0.0195 | 16 | 1.1336e-25 | 2 | 0.0195 |
| total | 2 | 0.0195 | 2 | 0.0195 | 29 | 1.3672e-54 | 3 | 0.0014 |
| Kidney<sub>1</sub> | Clust.1 | 0 | 0.8005 | 0 | 0.8005 | 1 | 0.1785 | 0 | 0.8005 |
| Clust.2 | 2 | 0.0195 | 1 | 0.1785 | 1 | 0.1785 | 0 | 0.8005 |
| Clust.3 | 0 | 0.8005 | 0 | 0.8005 | 2 | 0.0195 | 18 | 8.9507e-30 |
| total | 2 | 0.0195 | 1 | 0.1785 | 4 | 7.3075e-05 | 18 | 8.9507e-30 |

H1, SM1, L1 and K1 are the gene clusters most characteristic for the heart, skeletal muscle, liver and kidney, respectively, in the single (combined) data set, as in Figure 2. Clust.1, 2, or 3 denotes the 100 genes most securely placed within the clusters of the differently ordered genes in the 4-way factorization shown in Figure 5. The order of the clusters is 1–3, from the top of the figure, for each tissue. We refer to these clusters as “heart1, heart2,” etc. The overlap of the heart1 from the one-way factorization to heart1 is referred to as heart1, heart2 cluster 1.

<sup>1</sup> doi:10.1371/journal.pone.0048238.t004
We again performed 20 factorizations, this time for $k = 2, \ldots, 10$ and these have been used to generate a consensus for clustering the mice.

The objective function and the consensus measurements are shown in Figure 4. The objective function in subfigure (a) does not show much decrease in convergence rate until we get to nine clusters. This is the point where each mouse is put into a cluster on its own. The area under the cumulative density curve in Figure 4(b) suggests using either rank $k = 3$, or $k = 5$ factorizations for the clustering. The correlation coefficients shown in subfigure (c) give the same two values as peaks, as well as $k = 8$, though the $k = 3$ peak is the highest.

Given these measurements we consider the four-way simultaneous factorization for $k = 3$ in Figure 5. The reordered datasets are shown separately with the kidney dataset in the top left, the liver dataset in the top right, the heart dataset in the bottom left and the skeletal muscle in the bottom right. The mouse ordering and mouse clusters that arise are shown in Table 3. The four subfigures in Figure 5 also illustrate that the gene clusters are different for each dataset. The three clusters for each tissue in this 4-way factorization are subsequently referred to in the form “tissue$_i$, cluster 1, 2 or 3.” Table 3 shows that the simultaneous NMF approach has recovered the known mouse treatments except for one misplacement. Figure 6 shows the clustering for the four-way simultaneous factorizations for $k = 1, \ldots, 9$. This indicates that this mouse does not cluster with all those of the same dosage for any rank of factorization greater than two, instead it associates with the higher more toxic dosage. This is borne out by the known blood chemistry, as summarised in Table 1; the mouse that is mis-

![Figure 7. Enrichment of canonical pathways in the four tissue specific gene clusters.](image)

The top one hundred most influential probe-sets in the four tissue specific gene clusters obtained in the first factorization were subjected to signalling and metabolic pathways analysis in the IPA software. This graph shows the comparison of canonical pathways enriched in the four tissue specific gene clusters, heart$_1$, muscle$_1$, kidney$_1$ and liver$_1$. The coloured bars show the significance of the enrichment for a particular pathway in the cluster computed by Fisher’s exact test. doi:10.1371/journal.pone.0048238.g007
classified exhibits a toxic response and is therefore classified with the mice that received the higher dose.

Comparing Gene clusters

Our aim now is to test the results from the novel multi-way NMF algorithm used in Section in order to see whether they (a) show consistency and (b) add value to the results in Section from standard NMF. We know that the four simultaneously factorized datasets correspond to the four clusters of samples that were discovered in an unsupervised manner from the single factorization of the full dataset. It could therefore be conjectured that the most influential genes in the first factorization will appear as influential genes in the four-way simultaneous factorization for that dataset, but less so for the other datasets.

Our comparisons involve four reference sets. For illustration, we chose an arbitrary threshold of one hundred; that is, we consider the top one hundred most influential genes from the four clusters in the first factorization shown in Figure 2. For easy reference these sets are referred to using the known tissue type. This means that the genes from cluster one are the heart1 genes, those from cluster two are the muscle1 genes, those from cluster three are the liver1 genes and those from cluster four are the kidney1 genes. The 4-way factorization shown in Figure 5 identifies differently ordered gene clusters for each tissue, which we will refer to as “kidney4, cluster 1, 2 or 3, etc.” Table 4 shows the total number of co-incident genes between the top 100 lists arising from the one-way and four-way factorisations. The table also shows the probability of the two lists having that number of genes in common if the second list were randomly selected; hence these values come from the hypergeometric distribution. We see that the important genes for each tissue type appear significantly highly in the clusters from that tissue’s data type. In addition, all the tissue type genes also appear significantly within the reordering of the heart dataset. This link is reciprocated, with the heart genes appearing significantly frequently within the skeletal muscle dataset. Surprisingly, the greatest overlap arose between liver1 and heart4 cluster 2. One of these genes, Apolipoprotein A1, is being considered as a marker for cardiac toxicity [16].

We would like to demonstrate the utility of the factorization method by using the gene clusters obtained in our analysis to understand tissue specific effects of the experimental drug, PPM-201. Of course, we are not claiming that this is an exhaustive analysis of the effects of PPM-201. We analysed the gene clusters...
Table 5. Enrichment of KEGG pathways in the four tissue specific gene clusters.

| Kegg Pathways                                      | Heart | Muscle | Kidney | Liver |
|----------------------------------------------------|-------|--------|--------|-------|
| 1 mmu05412: Arrhythmogenic right ventricular cardiomyopathy | *    |       |        |       |
| 2 mmu04020: Calcium signalling pathway              | *    | *      |        |       |
| 3 mmu04260: Cardiac muscle contraction              | *    | *      |        |       |
| 4 mmu05414: Dilated cardiomyopathy                  | *    | *      |        |       |
| 5 mmu05410: Hypertrophic cardiomyopathy (HCM)       | *    | *      |        |       |
| 6 mmu04530: Tight junction                          |       | *      |        |       |
| 7 mmu00590: Arachidonic acid metabolism             |       |        | *      |       |
| 8 mmu00983: Drug metabolism                         |       |        |        | *    |
| 9 mmu04610: Complement and coagulation cascades     |       |        |        |       |
| 10 mmu00980: Metabolism of xenobiotics by cytochrome P450 |       |        |        |       |
| 11 mmu03320: PPAR signalling pathway                |       |        |        |       |
| 12 mmu00830: Retinol metabolism                     |       |        |        |       |
| 13 mmu00040: Pentose and glucoronate interconversions |       |        |        |       |
| 14 mmu00971: Linoleic acid metabolism               |       |        |        |       |
| 15 mmu00053: Ascorbate and aldarate metabolism      |       |        |        |       |
| 16 mmu00860: Porphyrin and chlorophyll metabolism   |       |        |        |       |
| 17 mmu00500: Starch and sucrose metabolism          |       |        |        |       |
| 18 mmu00150: Androgen and estrogen metabolism       |       |        |        |       |
| 19 mmu00140: Steroid hormone biosynthesis           |       |        |        |       |

The top one hundred most influential probesets in the four tissue specific gene clusters were analysed using DAVID functional annotation tool. This table shows the comparison of KEGG pathways enriched in the four tissue specific gene clusters. The * icon indicates a p-value < 0.001 and the ◆ a 0.001 < p-value < 0.05 showing the significance of the enrichment.

doi:10.1371/journal.pone.0048238.t005

Table 6. Enrichment of KEGG pathways in the common genes between the clusters found by the two ways of factorization.

| Kegg Pathways                                      | Heart1 | Heart1 | Muscle1 | Muscle1 | Liver1 | Liver1 | Liver1 | Liver1 |
|----------------------------------------------------|--------|--------|---------|---------|--------|--------|--------|--------|
|                                                   | Heart4 | Muscle4 | Heart4  | Muscle4 | Liver4 | Liver4 | Liver4 | Liver4 |
|                                                   | clust. 1 | clust. 3 | clust. 1 | clust. 1 | clust. 1 | clust. 3 | clust. 2 | clust. 1 |
| 1 mmu04020: Calcium signalling pathway             |        |        | *       |         |        |        |        |        |
| 2 mmu04260: Cardiac muscle contraction             |        |        | *       |         |        |        |        |        |
| 3 mmu04610: Complement and coagulation cascades    |        |        | *       |         |        |        |        |        |
| 4 mmu05414: Dilated cardiomyopathy                 |        |        | *       |         |        |        |        |        |
| 5 mmu00983: Drug metabolism                        |        |        |         |         |        |        |        |        |
| 6 mmu05410: Hypertrophic cardiomyopathy (HCM)      |        |        | *       |         |        |        |        |        |
| 7 mmu03320: PPAR signalling pathway                |        |        |         |         |        |        |        |        |
| 8 mmu04530: Tight junction                         |        |        |         |         |        |        |        |        |

The probesets common to clusters formed by the 4-way simultaneous factorization and the top one hundred most influential probesets in the four tissue specific clusters were analysed for enrichment of KEGG pathways using DAVID functional annotation tool. Fishers’ exact p-values for pathway enrichment in the clusters are shown graphically in this table. The * icon indicates a p-value < 0.001 and the ◆ a 0.001 < p-value < 0.05.

doi:10.1371/journal.pone.0048238.t006
### Table 7. Muscle genes present in the calcium signalling pathway.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene ID | Entrez Gene Name |
|-----|-------------|-------------|----------------|-----------------|
| 1   | 1427735 a at | ACTA1       | 11459          | Actin, alpha 1, skeletal muscle |
| 2   | 1419312 at  | ATP2A1      | 11937          | ATPase, Ca++ transporting, cardiac muscle, fast twitch 1 |
| 3   | 1422598 at  | CASQ1       | 12372          | Calsequestrin 1 (fast-twitch, skeletal muscle) |
| 4   | 1427520 a at| MYH1        | 17879          | Myosin, heavy chain 1, skeletal muscle, adult |
| 5   | 1425153 at  | MYH2        | 17882          | Myosin, heavy chain 2, skeletal muscle, adult |
| 6   | 1458368 at  | MYH4        | 17884          | Myosin, heavy chain 4, skeletal muscle |
| 7   | 1452651 a at| MYL1        | 17901          | Myosin, light chain 1, alkali; skeletal, fast |
| 8   | 1457347 at  | RYR1        | 20190          | Ryanodine receptor 1 (skeletal) |
| 9   | 1440962 at  | SLC8A3      | 110893         | Solute carrier family 8, member 3 |
| 10  | 1417464 at  | ACTC1       | 11464          | Actin, alpha, cardiac muscle 1 |
| 11  | 1416551 at  | ATP2A2      | 11938          | ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 |
| 12  | 1422529 s at| CASQ2       | 12373          | Calsequestrin 2 (cardiac muscle) |
| 13  | 1448827 s at| MYH6        | 17888          | Myosin, heavy chain 6, cardiac muscle, alpha |
| 14  | 1448394 at  | MYL2        | 17906          | Myosin, light chain 2, regulatory, cardiac, slow |
| 15  | 1427769 x at| MYL3        | 17897          | Myosin, light chain 3, alkali; ventricular, skeletal, slow |
| 16  | 1421126 at  | RYR2        | 20191          | Ryanodine receptor 2 (cardiac) |
| 17  | 1418370 at  | TNNT2       | 21954          | Troponin T 1 type 3 (cardiac) |
| 18  | 1440244 at  | TRDN        | 76757          | Triadin |

Table shows the probe-sets enriched for calcium signalling among the top 100 probe-sets from the muscle1 gene cluster.
doi:10.1371/journal.pone.0048238.t007

### Table 8. Heart genes present in the calcium signalling pathway.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene ID | Entrez Gene Name |
|-----|-------------|-------------|----------------|-----------------|
| 1   | 1415927 at  | ACTC1       | 11464          | Actin, alpha, cardiac muscle 1 |
| 2   | 1416551 at  | ATP2A2      | 11938          | ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 |
| 3   | 1422529 s at| CASQ2       | 12373          | Calsequestrin 2 (cardiac muscle) |
| 4   | 1448827 s at| MYH6        | 17888          | Myosin, heavy chain 6, cardiac muscle, alpha |
| 5   | 1448394 at  | MYL2        | 17906          | Myosin, light chain 2, regulatory, cardiac, slow |
| 6   | 1427769 x at| MYL3        | 17897          | Myosin, light chain 3, alkali; ventricular, skeletal, slow |
| 7   | 1421126 at  | RYR2        | 20191          | Ryanodine receptor 2 (cardiac) |
| 8   | 1418370 at  | TNNT2       | 21954          | Troponin T 1 type 3 (cardiac) |
| 9   | 1440244 at  | TRDN        | 76757          | Triadin |

Table shows the probe-sets enriched for calcium signalling among the top 100 probe-sets from the heart1 gene cluster.
doi:10.1371/journal.pone.0048238.t008

### Table 9. Liver genes present in the calcium signalling pathway.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene ID | Entrez Gene Name |
|-----|-------------|-------------|----------------|-----------------|
| 1   | 1449817 at  | ABCB11      | 27413          | ATP-binding cassette, sub-family B (MDR/TAP), member 11 |
| 2   | 1419393 at  | ABCG5       | 27409          | ATP-binding cassette, sub-family G (WHITE), member 5 |
| 3   | 1419232 a at| APOA1       | 11806          | Apolipoprotein A-I |
| 4   | 1418278 at  | APOC3       | 11814          | Apolipoprotein C-III |
| 5   | 1448309 at  | CYP2B1      | 13124          | Cytochrome P450, family 2B, subfamily B, polypeptide 1 |
| 6   | 1418190 at  | PON1        | 18979          | Paraoxonase 1 |
| 7   | 1450261 a at| SLC10A1     | 20493          | Solute carrier family 10, member 1 |
| 8   | 1449112 at  | SLC27A5     | 26459          | Solute carrier family 27, member 5 |
| 9   | 1449394 at  | SLC01B3     | 28253          | Solute carrier organic anion transporter family, member 1B3 |
| 10  | 1424934 at  | UGT2B4      | 71773          | UDP glucuronosyltransferase 2 family, polypeptide B4 |

Table shows the probe-sets enriched for calcium signalling among the top 100 probe-sets from the liver1 gene cluster.
doi:10.1371/journal.pone.0048238.t009
Analysis of the same sets of genes for enrichment of toxicity functions in the IPA shows, in Figure 8, cardiac hypertrophy in heart1 genes, increased level of creatinine and hydronephrosis in kidney1 genes, and increased levels of lactate dehydrogenase (LDH) and steatosis in liver1 genes.

The common genes between the top one hundred most influential probe-sets in the four tissue specific clusters and the top one hundred most influential probe-sets in the clusters formed by 4-way simultaneous factorization of the split dataset were also analysed for enrichment of pathways, gene ontology and toxicity functions using DAVID and IPA. Tables 6, 7, 8, 9, 10, 11, 12, 13,

Table 10. Heart1:heart4 cluster 1. Common probesets between the top one hundred most influential probesets in the heart1 cluster and 20 mg/kg dosage cluster (cluster 1) of the heart4 dataset.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene Name |
|-----|-------------|-------------|-----------------|
| 1   | 1415927 at  | ACTC1       | actin, alpha, cardiac muscle 1 |
| 2   | 1416551 at  | ATP2A2      | ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 |
| 3   | 1452363 a at| ATP2A2      | ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 |
| 4   | 1417607 at  | COX6A2      | cytochrome c oxidase subunit Vla polypeptide 2 |
| 5   | 1460318 at  | CSRP3       | cysteine and glycine-rich protein 3 (cardiac LIM protein) |
| 6   | 1416023 at  | FABP3       | fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor) |
| 7   | 1453628 s at| LRRC2       | leucine rich repeat containing 2 |
| 8   | 1451203 at  | MB          | myoglobin |
| 9   | 1418551 at  | MYBPC3      | myosin binding protein C, cardiac |
| 10  | 1448554 s at| MYH6        | myosin, heavy chain 6, cardiac muscle, alpha |
| 11  | 1448826 at  | MYH6        | myosin, heavy chain 6, cardiac muscle, alpha |
| 12  | 1448394 at  | MYL2        | myosin, light chain 2, regulatory, cardiac, slow |
| 13  | 1427768 s at| MYL3        | myosin, light chain 3, alkal; ventricular, skeletal, slow |
| 14  | 1428266 at  | MYL3        | myosin, light chain 3, alkal; ventricular, skeletal, slow |
| 15  | 1418769 at  | MYOZ2       | myozin 2 |
| 16  | 1450952 at  | PLN         | phospholamban |
| 17  | 1423859 a at| PTGD5       | prostaglandin D2 synthase 21 kDa (brain) |
| 18  | 1418370 at  | TNNC1       | troponin C type 1 (slow) |
| 19  | 1422536 at  | TNNI3       | troponin I type 3 (cardiac) |
| 20  | 1418726 a at| TNNT2       | troponin T type 2 (cardiac) |
| 21  | 1424967 x at| TTNNT2      | troponin T type 2 (cardiac) |
| 22  | 1423049 a at| TPM1        | tropomyosin 1 (alpha) |

doi:10.1371/journal.pone.0048238.t010

Table 11. Heart1:heart4 cluster 3.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene Name |
|-----|-------------|-------------|-----------------|
| 1   | 1422529 s at| CASQ2       | calsequestrin 2 (cardiac muscle) |
| 2   | 1444429 at  | LRMT1       | leucine-rich repeats and transmembrane domains 1 |
| 3   | 1439101 at  | MYLK3       | myosin light chain kinase 3 |
| 4   | 1426615 s at| NDRG4       | NDRG family member 4 |
| 5   | 1436188 a at| NDRG4       | NDRG family member 4 |
| 6   | 1438452 at  | NEBL        | nebulette |
| 7   | 1437442 at  | PCDH7       | proteocadherin 7 |
| 8   | 1436277 at  | RNF207      | ring finger protein 207 |
| 9   | 1423145 a at| TCAP        | titin-cap (teletinon) |
| 10  | 1436833 x at| TLL1        | tubulin tyrosine ligase-like family, member 1 |
| 11  | 1444638 at  | TTN         | titin |

doi:10.1371/journal.pone.0048238.t011
summarise the results of this analysis, which are discussed further in the next section.

**Discussion**

The factorization and reordering of the dataset as a whole set (Figure 2 and Table 2) successfully clustered samples from the same tissue and further investigation showed that it simultaneously identified genes with a known relevance to those tissues. It was therefore reasonable to study the genes that were responsible for this differentiation. In the one-way clustering, the top 100 probe-sets from each of the four tissue specific clusters show remarkable coherence for tissue specific pathways. The calcium signalling pathway is highly enriched in both heart1 and muscle1 clusters; these genes are linked to muscle contraction function. Muscle contraction is the prime function of cardiac and skeletal muscles. A deeper look at the probe-sets (Tables 7 and 8) from the heart and skeletal muscle clusters shows a successful identification of differences in the tissue types for this pathway; see Figure 9. MYH1, MYH2, MYH4 and MYL1 of the myosin family, which are specific to skeletal muscle, are found in the muscle1 cluster while cardiac muscle specific myosin family members MYH6, MYL2 and MYL3 are found in the heart1 cluster [19]. This pattern is also true for tropinin, calsequestrin, ryanodine and actin.

**Table 12. Muscle1,muscle4 cluster 1.**

| Sr. | Probeset ID | Gene Symbol | Entrez Gene Name |
|-----|-------------|-------------|-----------------|
| 1   | 1427735 a at | ACTA1 | actin, alpha 1, skeletal muscle |
| 2   | 1418677 at  | ACTN3 | actinin, alpha 3 |
| 3   | 1419312 at  | ATP2A1 | ATPase, Ca++ transporting, cardiac muscle, fast twitch 1 |
| 4   | 1417614 at  | CKM | creatine kinase, muscle |
| 5   | 1438059 at  | CTXN3 (includes EGd629147) | cortexin 3 |
| 6   | 1455736 at  | MYBPC2 | myosin binding protein C, fast type |
| 7   | 1427868 x at | MYH1 | myosin, heavy chain 1, skeletal muscle, adult |
| 8   | 1427026 at  | MYH4 | myosin, heavy chain 4, skeletal muscle |
| 9   | 1448371 at  | MYLF | myosin light chain, phosphorylatable, fast skeletal muscle |
| 10  | 1418155 at  | MYOT | myotilin |
| 11  | 1427306 at  | RYR1 | ryanodine receptor 1 (skeletal) |
| 12  | 1417464 at  | TNNC2 | troponin C type 2 (fast) |
| 13  | 1416889 at  | TNN2 | troponin I type 2 (skeletal, fast) |
| 14  | 1450118 a at | TNNT3 | troponin T type 3 (skeletal, fast) |
| 15  | 1426142 a at | TRDN | triadin |

Common probesets between the top one hundred most influential probesets in the muscle1 cluster and 20 mg/kg dosage cluster (cluster 1) of the muscle4 dataset.

doi:10.1371/journal.pone.0048238.t012

**Table 13. Muscle1,muscle4 cluster 3.**

| Sr. | Probeset ID | Gene Symbol | Entrez Gene Name |
|-----|-------------|-------------|-----------------|
| 1   | 1453657 at  | 2310065F04RIK | RIKEN cDNA 2310065F04 gene |
| 2   | 1434722 at  | AMPD1 | adenosine monophosphate deaminase 1 |
| 3   | 1460256 at  | CA3 | carbonic anhydrase III, muscle specific |
| 4   | 1422598 at  | CASQ1 | calsequestrin 1 (fast-twitch, skeletal muscle) |
| 5   | 1439332 at  | DDIT4L | DNA-damage-inducible transcript 4-like |
| 6   | 1427400 at  | LBX1 | ladybird homeobox 1 |
| 7   | 1419487 at  | MYBPH | myosin binding protein H |
| 8   | 1458368 at  | MYH4 | myosin, heavy chain 4, skeletal muscle |
| 9   | 14441111 at| MYLK4 | myosin light chain kinase family, member 4 |
| 10  | 1418373 at  | PGAM2 | phosphoglycerate mutase 2 (muscle) |
| 11  | 1444480 at  | PRKAG3 | protein kinase, AMP-activated, gamma 3 non-catalytic subunit |
| 12  | 1417653 at  | PVRL8 | parvalbumin |
| 13  | 1422644 at  | SH3BG1R | SH3 domain binding glutamic acid-rich protein |
| 14  | 1449206 at  | SYPL2 | synaptophysin-like 2 |

Common probesets between the top one hundred most influential probesets in the muscle1 cluster and vehicle dose cluster (cluster 3) of the muscle4 dataset.

doi:10.1371/journal.pone.0048238.t013
Table 14. Liver-heart cluster 2.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene Name |
|-----|-------------|-------------|-----------------|
| 1   | 1449817     | ABCB11      | ATP-binding cassette, sub-family B (MDR/TAP), member 11 |
| 2   | 1425260     | ALB         | albumin         |
| 3   | 1416649     | AMBP        | alpha-1-microglobulin/bikunin precursor |
| 4   | 1419233     | APOA1       | apolipoprotein A-I |
| 5   | 1438840     | APOA1       | apolipoprotein A-I |
| 6   | 1455201     | APOA1       | apolipoprotein A-I |
| 7   | 1419232     | APOA1       | apolipoprotein A-I |
| 8   | 1417950     | APOA2       | apolipoprotein A-II |
| 9   | 1417610     | APOA5       | apolipoprotein A-V |
| 10  | 1417561     | APOC1       | apolipoprotein C-I |
| 11  | 1418278     | APOC3       | apolipoprotein C-III |
| 12  | 1418708     | APOC4       | apolipoprotein C-IV |
| 13  | 1416677     | APOH        | apolipoprotein H (beta-2-glycoprotein I) |
| 14  | 1424011     | AQP9        | aquaporin 9 |
| 15  | 1419549     | ARG1        | arginase, liver |
| 16  | 1421944     | ASGR1       | asialoglycoprotein receptor 1 |
| 17  | 1450624     | BHMT        | betaine–homocysteine S-methyltransferase |
| 18  | 1451600     | CES3        | carboxylesterase 3 |
| 19  | 1455540     | CPS1        | carbamoyl-phosphate synthase 1, mitochondrial |
| 20  | 1418133     | CYP2D10     | cytochrome P450, family 2, subfamily d, polypeptide 10 |
| 21  | 1416913     | ES1         | (includes EG13884) esterase 1 |
| 22  | 1418897     | F2          | coagulation factor II (thrombin) |
| 23  | 1417556     | FABP1       | fatty acid binding protein 1, liver |
| 24  | 1418438     | FABP2       | fatty acid binding protein 2, intestinal |
| 25  | 1424279     | FGA         | fibrinogen alpha chain |
| 26  | 1428079     | FGB         | fibrinogen beta chain |
| 27  | 1416025     | FGG         | fibrinogen gamma chain |
| 28  | 1426547     | GC          | group-specific component (vitamin D binding protein) |
| 29  | 1419196     | HAMP        | hepcidin antimicrobial peptide |
| 30  | 1419197     | HAMP        | hepcidin antimicrobial peptide |
| 31  | 1436643     | HAMP        | hepcidin antimicrobial peptide |
| 32  | 1425137     | HLA-A       | major histocompatibility complex, class I, A |
| 33  | 1448881     | HP          | haptoglobin |
| 34  | 1423994     | HXP         | hemopexin |
| 35  | 1434110     | LOC100129193| major urinary protein pseudogene |
| 36  | 1428005     | MOSC1       | MOCO sulphurase C-terminal domain containing 1 |
| 37  | 1417835     | MUG1        | murinoglobulin 1 |
| 38  | 1451054     | ORM1        | orosomucoid 1 |
| 39  | 1418190     | PON1        | paraoxonase 1 |
| 40  | 1417246     | PZP         | pregnancy-zone protein |
| 41  | 1426225     | RBP4        | retinol binding protein 4, plasma |
| 42  | 1451513     | SERPINA1    | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 |
| 43  | 1418282     | SERPINA1    | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 |
| 44  | 1423866     | SERPINA3K   | serine (or cysteine) peptidase inhibitor, clade A, member 3K |
| 45  | 1417909     | SERPINC1    | serpin peptidase inhibitor, clade C (antithrombin), member 1 |
| 46  | 1449112     | SLC27A5     | solute carrier family 27 (fatty acid transporter), member 5 |
| 47  | 1449394     | SLC01B3     | solute carrier organic anion transporter family, member 1B3 |
| 48  | 1419093     | TDO2        | tryptophan 2,3-dioxygenase |
### Table 14. Cont.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene Name |
|-----|-------------|-------------|------------------|
| 49  | 1422604 at  | UOX         | urate oxidase, pseudogene |

Common probesets between the top one hundred most influential probesets in the liver1 cluster and 6 mg/kg dosage cluster (cluster 2) of the heart4 dataset.

doi:10.1371/journal.pone.0048238.t014

### Table 15. Liver1-liver4 cluster 1.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene Name |
|-----|-------------|-------------|------------------|
| 1   | 1425260 at  | ALB         | albumin          |
| 2   | 1419059 at  | APCS        | amyloid P component, serum |
| 3   | 1419232 a at| APOA1       | apolipoprotein A-I |
| 4   | 1419233 x at| APOA1       | apolipoprotein A-I |
| 5   | 1438840 x at| APOA1       | apolipoprotein A-I |
| 6   | 1455201 x at| APOA1       | apolipoprotein A-I |
| 7   | 1417950 a at| APOA2       | apolipoprotein A-II|
| 8   | 1416677 at  | APOH        | apolipoprotein H (beta-2-glycoprotein I) |
| 9   | 1419549 at  | ARG1        | arginase, liver |
| 10  | 1417556 at  | FABP1       | fatty acid binding protein 1, liver |
| 11  | 1428079 at  | FGB         | fibrinogen beta chain |
| 12  | 1426547 at  | GC          | group-specific component (vitamin D binding protein) |
| 13  | 1448881 at  | HP          | haptoglobin |

Common probesets between the top one hundred most influential probesets in the liver1 cluster and 20 mg/kg dosage cluster (cluster 1) of the liver4 dataset.

doi:10.1371/journal.pone.0048238.t015

### Table 16. Liver1-liver4 cluster 3.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene Name |
|-----|-------------|-------------|------------------|
| 1   | 1428981 at  | 2810007J24RK| RIKEN cDNA 2810007J24 gene |
| 2   | 1449817 at  | ABCB11      | ATP-binding cassette, sub-family B (MDR/TAP), member 11 |
| 3   | 1417085 at  | AKR1C4      | aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxy steroid dehydrogenase, type I; dihydrodiol dehydrogenase 4) |
| 4   | 1451600 s at| CES3        | carboxylesterase 3 |
| 5   | 1449242 s at| HRG         | histidine-rich glycoprotein |
| 6   | 1431808 a at| ITH4        | inter-alpha (globulin) inhibitor H4 (plasma Kallikrein-sensitive glycoprotein) |
| 7   | 1434110 x at| LOC100129193| major urinary protein pseudogene |
| 8   | 1420465 s at| LOC100129193| major urinary protein pseudogene |
| 9   | 1426154 s at| LOC100129193| major urinary protein pseudogene |
| 10  | 1420525 a at| OTC         | ornithine carbamoyltransferase |
| 11  | 1436615 a at| OTC         | ornithine carbamoyltransferase |
| 12  | 1446860 at  | SERPINA1    | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 |
| 13  | 1448506 at  | SERPINA6    | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6 |
| 14  | 1449394 at  | SLC01B3     | solute carrier organic anion transporter family, member 1B3 |
| 15  | 1424934 at  | UGT2B4      | UDP glucuronosyltransferase 2 family, polypeptide B4 |
| 16  | 1422604 at  | UOX         | urate oxidase, pseudogene |

Common probesets between the top one hundred most influential probesets in the liver1 cluster and vehicle dose cluster (cluster 3) of the liver4 dataset.

doi:10.1371/journal.pone.0048238.t016
Family members [20–25] (Tables 7 and 8). FXR/RXR activation pathway genes are significantly enriched in liver cluster (Figure 7) with most of the enriched genes present in the bile acid synthesis and regulation (Figure 10) pathway, which is one of the core functions of liver [26–28]. FXR/RXR activation is also found in the kidney cluster, albeit with moderate significance; FBP1 and HNF4A are the two genes present in this pathway and they may be involved in gluconeogenesis in kidney [29].

Splitting the dataset into four on the basis of tissue types and simultaneous non-negative factorization of them gave us the added reassurance of clustering the samples according to the dosage groups (Figure 5 and Table 3). The clustering of one mouse (Mouse E) from the lower dosage group (Group-II) with the higher dosage group (Group-III) can be explained by the higher PPM201 drug sensitivity of that mouse, indicated by the elevated levels of the toxocology markers ALT, AST, LDH and CK, compared with the rest of its group (Table 1). Comparisons of top probe-sets in tissue specific clusters with dosage specific clusters also show very high overlap of tissue specific genes in the four tissue types. Heart cluster has 22 probe-sets that are common between the top 100 probe-sets of heart cluster and 20 mg/kg dosage cluster of heart4 dataset, and are highly enriched for cardiac muscle contraction and hypertrophic cardiomyopathy pathways (Table 6). ACTC1, ATP2A2, MYH6, MYL2, MYL3, TNNC1, TNNT2, TNNI3, TPM1 are the genes enriched for these two pathways and shared between these two clusters. However, heart cluster 3, with 11 probe-sets in common between the top 100 probe-sets of heart cluster and vehicle dose cluster of heart4 dataset, does not show enrichment for cardiac muscle contraction and hypertrophic cardiomyopathy pathways. From this we may assume that perturbation of cardiac muscle contraction and hypertrophic cardiomyopathy pathways by 20 mg/kg dosage may indicate toxic responses. We also see a similar pattern in skeletal muscle. Between the top 100 probe-sets

Table 17. Kidney1_kidney4_cluster_3.

| Sr. | Probeset ID | Gene Symbol | Entrez Gene Name |
|-----|-------------|-------------|-----------------|
| 1   | 1456190     | ACSM2A      | acyl-CoA synthetase medium-chain family member 2A |
| 2   | 1427223     | ACSM2A      | acyl-CoA synthetase medium-chain family member 2A |
| 3   | 1425207     | BC026439    | cDNA sequence BC026439 |
| 4   | 1424713     | CALML4      | calmodulin-like 4 |
| 5   | 1424592     | DNAE1       | deoxynribonuclease I |
| 6   | 1448485     | GGT1        | gamma-glutamyltransferase 1 |
| 7   | 1460233     | GUCA2B      | guanylate cyclase activator 2B (uroguanylin) |
| 8   | 1415969     | KAP         | kidney androgen regulated protein |
| 9   | 1415968     | KAP         | kidney androgen regulated protein |
| 10  | 1435094     | KCNJ16      | potassium inwardly-rectifying channel, subfamily J, member 16 |
| 11  | 1450719     | MEP1A       | meprin A, alpha (PABA peptide hydrolase) |
| 12  | 1418923     | SLC17A3     | solute carrier family 17 (sodium phosphate), member 3 |
| 13  | 1417072     | SLC22A6     | solute carrier family 22 (organic anion transporter), member 6 |
| 14  | 1423279     | SLC34A1     | solute carrier family 34 (sodium phosphate), member 1 |
| 15  | 1425606     | SLC5A8      | solute carrier family 5 (iodide transporter), member 8 |
| 16  | 1449301     | SLC7A13     | solute carrier family 7, (cationic amino acid transporter, y+ system) member 13 |
| 17  | 1435064     | TMEM27      | transmembrane protein 27 |
| 18  | 1423397     | UGT2B17     | UDP glucuronosyltransferase 2 family, polypeptide B17 |

Common probesets between the top one hundred most influential probesets in the kidney1, cluster and vehicle dose cluster (cluster 3) of the kidney4 dataset. doi:10.1371/journal.pone.0048238.t017

Figure 9. Heart and muscle genes enriched in calcium signalling – muscle contraction pathway. IPA analysis of the top 100 probe-sets from heart and muscle gene clusters (Figure 7) showed the enrichment of calcium signalling pathway. In this figure, we have highlighted the genes present in this pathway in orange. Though this pathway is generalised for skeletal muscle contraction and cardiac muscle contraction, they differ in the members of the same gene family. The heart and muscle genes present in this pathway are given in Tables 7 and 8. Pathway diagram was drawn using Path Designer function of IPA [18].

doi:10.1371/journal.pone.0048238.g009
of muscle1 cluster and 20 mg/kg dosage cluster of muscle4, and between the top 100 probe-sets of muscle1 and vehicle dose cluster of muscle4, 15 and 14 probe-sets were in common and are named as muscle1:muscle4 cluster 1 and muscle1:muscle4 cluster 3, respectively. The calcium signalling–skeletal muscle contraction pathway is enriched in muscle1:muscle4 cluster 1 with the presence of ACTA1, ATP2A1, MYH1, MYH4, RYR1, TNNC2, TNNI2, TNNT3 and TRDN genes, whereas muscle1:muscle4 cluster 3 does not show any significant enrichment for signalling or metabolic pathways.

Interestingly, 49 probe-sets in the liver1:heart4 cluster 2 are common between the top 100 probe-sets of liver1 cluster cluster and 6 mg/kg dosage cluster of liver4 and highly enriched for acute phase response signalling, prothrombin activation and FXR/RXR activation pathways with the presence of ALB, ABCB11, AMBP, APOA1, APOA2, APOC3, APOH, F2, FGA, FGB, FGG, HAMP, HP, HPX, ORM1, PON1, RBP4, SERPINA1, SERPINC1, SLC27A5 and SLC01B3 genes (Figure 11). This suggests alterations in lipid metabolism in liver along with tissue injury in heart induced by PPM-201 at 6 mg/kg dosage [30–33], which becomes more plausible when we look at the genes in liver1:heart4 cluster 1 that are common between the top liver4 genes and 20 mg/kg dosage cluster of liver4 dataset. Enrichment of toxicity functions in liver1:heart4 cluster 2 using IPA shows increased level of LDH as one of the toxicity functions (Figure 12) which has been validated with the increased level of LDH in the clinical chemistry results.

Conclusions

We have demonstrated that multi-way simultaneous nonnegative matrix factorization can be usefully applied to the case of multiple datasets—here, for what we believe to be the first time, more than two large scale matrices were treated. The results were shown to be consistent with, and to add value to, standard nonnegative matrix factorization of the whole dataset.

In summarizing our biological findings, we first note that the roles of the three different isoforms of PPARs - PPAR-α, PPAR-β (also known as PPAR-δ) and PPAR-γ in metabolism and their difference in expression in different tissues and different species are well known [34–36]. In mouse, PPAR-α is highly expressed in liver and to a lesser degree in kidney, heart and skeletal muscle; PPAR-β is expressed in many tissues but peaks in kidney, heart and intestine whereas PPAR-γ is mostly expressed in adipose tissue
Figure 11. Enrichment of canonical pathways in the liver heart gene cluster no. 2. This gene cluster has 49 common probe-sets between the top one hundred most influential probe-sets in the liver gene cluster and top one hundred probe-sets in cluster number 2 (6 mg/kg dose rate) of the heart dataset reordered by 4-way simultaneous factorization. Canonical pathways enrichment for these 49 probe-sets analysed using the IPA software is shown in this figure. The length of the bars shows the Fisher’s exact test p-value for enrichment for a particular pathway in the cluster. doi:10.1371/journal.pone.0048238.g011

Figure 12. Enrichment of toxicity functions in liver heart cluster 2. This gene cluster has 49 common probe-sets between the top one hundred most influential probe-sets in liver heart cluster 2 (6 mg/kg dose rate). Toxicity functions enrichment for these 49 probe-sets analysed using the IPA software is shown in this figure. The length of the bars shows the Fisher’s exact test p-value for enrichment for a particular pathway in the cluster. doi:10.1371/journal.pone.0048238.g012
Pan-PPAR agonists activate two or all of the pan-PPAR isoforms and differ in their pharmacological actions. Factorisation of the dataset after splitting it on the tissue basis appears to be beneficial in identifying tissue specific and dosage effects of the experimental pan-PPAR agonist PPM-201 in this study. This approach could be useful in understanding molecular mechanisms and identifying potential tissue specific toxicological effects before they are apparent in histopathology studies. In this study, histopathology examination of heart did not show any defect though our method of gene expression analysis could identify enrichment of acute phase response signalling genes in heart that may point towards building up of toxic responses in heart. Given the fact that many PPAR agonist drugs have been shown to cause cardiac toxicity on prolonged usage and FDA’s requirement of one year toxicity study for PPAR agonist drugs, our results show promising early detection of toxicity in the drug discovery process. Overall, our aim here is to establish a proof of principle for the approach of simultaneously analysing multiple, related large datasets. We therefore focused on a dataset where clear-cut validation is possible. However, we note that the technique is very general, and therefore opens up many new opportunities in data-driven computational biology. In particular, it can be applied to heterogeneous sources of data; for example, generated by different laboratories or experimental methodologies. We are currently pursuing this approach in the study of colon cancer.

Acknowledgments

The computational work reported here made extensive use of the High Performance Computer Facilities of the Faculty of Engineering and Institute of Complex Systems at the University of Strathclyde. The authors also acknowledge Flexxonix for use of the compound.

Author Contributions

Conceived and designed the experiments: CML MAVM DRH CRW GM JKV DJH DC. Performed the experiments: CML MAVM DRH CRW GM JKV DJH DC. Analyzed the data: CML MAVM JKV DJH DC. Contributed reagents/materials/analysis tools: CML MAVM DRH CRW GM JKV DJH DC. Wrote the paper: CML MAVM JKV DJH DC.

References

1. Lee DD, Seung HS (1999) Learning the parts of objects by non-negative matrix factorization. Nature 401: 788–791.
2. Carmona-Saez P, Pascual-Marqui R, Tirado F, Carazo J, Pascual-Montano A (2006) Bioclustering of gene expression data by non-smooth non-negative matrix factorization. BMC Bioinformatics 7: 78.
3. Brunet JP, Tamayo P, Golub TR, Mesirov JP (2004) Metagenes and molecular pattern discovery using matrix factorisation. Proc Nat Acad Sci 101: 4164–4169.
4. Fogel P, Young SS, Hawkins DM, Ledirac N (2007) Inferential, robust non-negative matrix factorization analysis of microarray data. Bioinformatics 23: 44–49.
5. Kim H, Park H (2007) Sparse non-negative matrix factorizations via alternating non-negativity-constrained least squares for microarray data analysis. Bioinformatics 23: 1495–1502.
6. Pascual-Montano AD, Carmona-Saez P, Chagoyen M, Tirado F, Carazo JM, et al. (2006) bioNMF: a versatile tool for non-negative matrix factorization in biology. BMC Bioinformatics 7: 366.
7. Gao Y, Church G (2003) Improving molecular cancer class discovery through sparse non-negative matrix factorization. Bioinformatics 21: 3970–3975.
8. Devarajan K (2008) Nonnegative matrix factorization: An analytical and interpretive tool in computational biology. PLoS Comput Biol 4: e1000029.
9. Badea L (2007) Combining gene expression and transcription factor regulation data using simultaneous nonnegative matrix factorization. In: Proc. BIOSPCon 2007, pp. 127–131.
10. Badea L (2008) Extracting gene expression profiles common to colon and pancreatic adenocarcinoma using simultaneous nonnegative matrix factorization. In: Proc. Pacific Symposium on Biocomputing PSB-2008. pp. 267–278.
11. Artis DR, Lin JJ, Zhang C, Wang W, Mehra U, et al. (2009) Scaffold-based discovery of meditrazep, a ppa pan-anti-angiogenic agent. Proceedings of the National Academy of Sciences 106: 262–267.
12. Jones D (2010) Potential remains for PPAR-targeted drugs. Nature Reviews Drug Discovery 9: 668–669.
13. Lee DD, Seung HS (2001) Algorithms for non-negative matrix factorization. Adv Neural Info Proc Syst 13: 556–562.
14. Monit S, Tamayo P, Mesirov J, Golub T (2003) Consensus clustering: a resampling-based method for class discovery and visualization of gene expression microarray data. Machine Learning 52: 91–116.
15. Hall R (2003) Principles of clinical pathology for toxicology studies. In Principles and methods of toxicology. Taylor and Francis, Philadelphia, 5th edition.
16. Florvall G, Basu S, Larson A (2006) Apolipoprotein A1 is a stronger prognostic marker than are HDL and LDL cholesterol for cardiovascular disease and mortality in elderly men. The Journals of Gerontology Series A, Biological Sciences and Medical Sciences 61: 1262–1266.
17. Huang JW, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protoc 4: 44–57.
18. Redwood City CISI (2011). Ingenuity: Ingenuity pathways analysis; version 8.8.
19. Weiss A, McDonough D, Wettman B, Acappo-Satchivi L, Montgomery K, et al. (1999) Organization of human and mouse skeletal myosin heavy chain gene clusters is highly conserved. Proc Natl Acad Sci USA 96: 2958–63.
20. Zhang L, Kelley J, Schmeisser G, Kobayashi YM, Jones LR (1997) Complex formation between junctin, triadin, calsequestrin, and the ryanodine receptor.