Fusion of transcriptomic and metabolomic data to achieve deeper insights into mycotoxin effects

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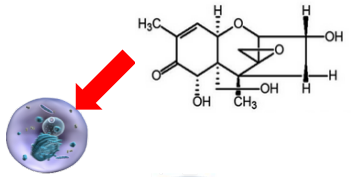
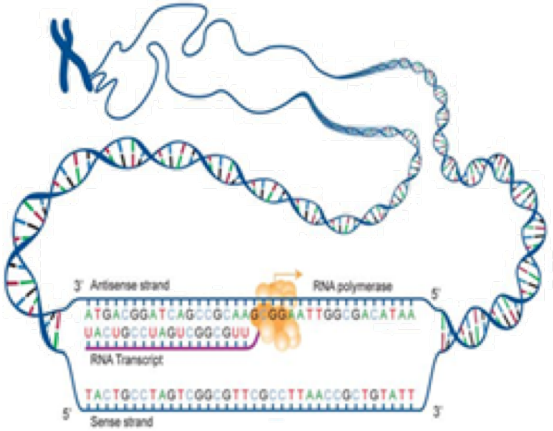
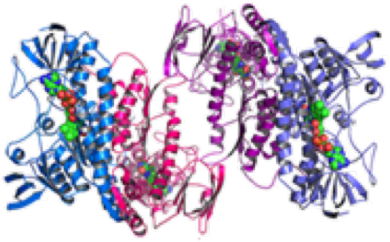
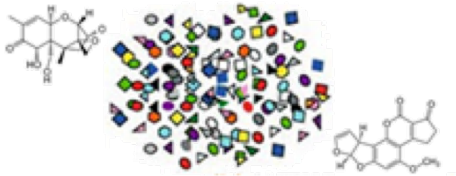
THE “OMICS” CASCADE









ADN = **GENOMICS**

Transcription

ARNm = **TRANSCRIPTOMICS**

Translation

Proteins = **PROTEOMICS**

Enzymatic reaction

Metabolites = **METABOLOMICS**

PHENOTYPE

- “omics” data = collection of data at the scale of the whole organism

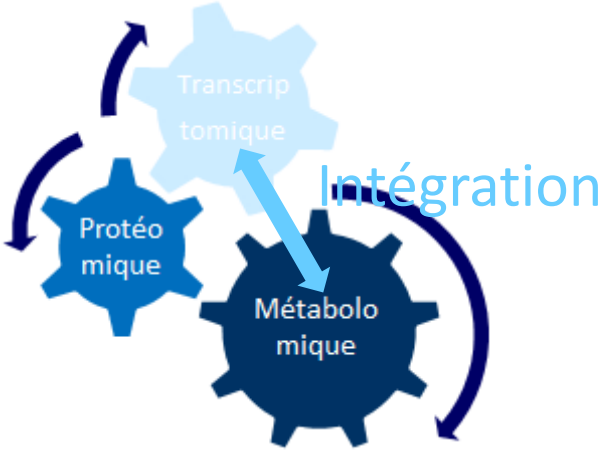
⇒ Assessment of phenotypic changes following exposure to one biological factor

DATA FUSION

- **« Combination of multiple omic datasets in order to develop multivariate models that are predictive of complex phenotypes » (Ritchie et al., 2015)**

⇒ Extraction of complementary information, on the whole biological system

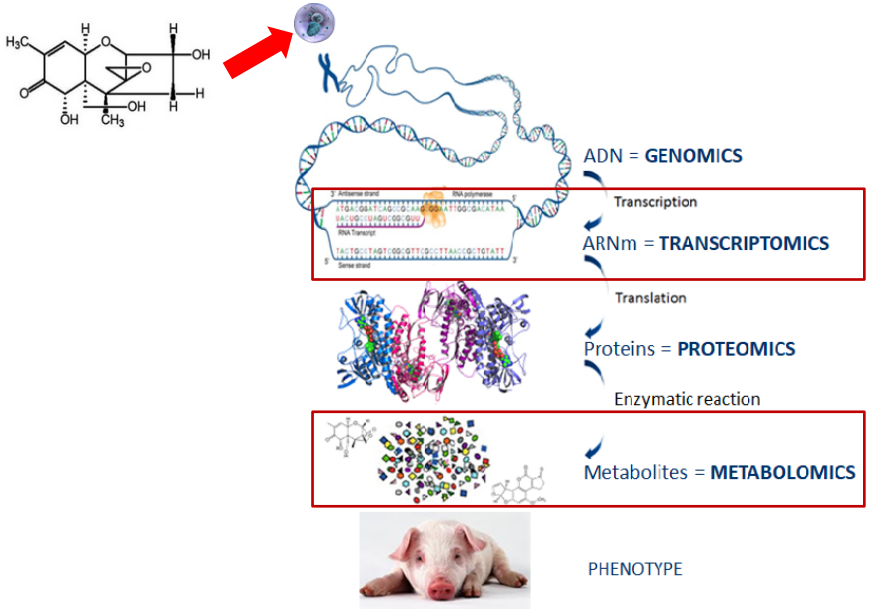
- Biological assumption (accepted): link between functional levels (Günther et al., 2014)
- *Aim: fusion of transcriptomic and metabolomic data*



⇒ Assessment of correlations between the two functional levels and identification of genes and metabolites markers of exposure to the studied factor

TRANSCRIPTOMICS / METABOLOMICS : WHY?

- **Transcriptomics** = first level of integration
  - Early response
  - Understanding of cellular activity modulations (Mele et al., 2003)
- **Metabolomics** = final level of the “omics” cascade
  - Integrated status of genetic and environmental factors = “Metabolomics is a crucial element in bridging the difference between the genotype and phenotype of an organism” (Fiehn, 2002)
  - Metabolites = final phenotypic expression of an organism



BIBLIOGRAPHY

- **Unsupervised method**: assessment of correlations between features
  - Canonical Correlation Analysis (CCA; Lê Cao et al. 2009, Wilms et Croux 2016; …)
  - Self Organized Maps (SOM; Hirai et al. 2005, Stegmayer et al. 2012)

- **Supervised method**: relationship between biological factor and "omics" features = Partial Least Squares (PLS)-based methods
  - O2-PLSDA (Bylesjö et al. 2007; Bouhaddani et al. 2016)
  - Concensus Orthogonal-PLS (Boccard et al. 2013)

AIMS

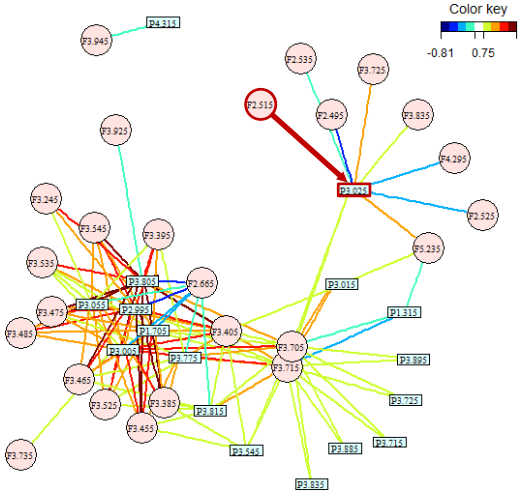
- Comparison of Canonical Correlation Analysis and Self Organized Maps to identify correlated transcriptomic and metabolomic features

- Adjust a regression model to assess relationship between factor of exposure and correlated features (as identified in the above step)

CANONICAL CORRELATION ANALYSIS (CCA)

- CCA (Hotelling, 1936) = multivariate method to assess statistical correlations between 2 datasets

⇒ **Are changes in metabolite concentrations following factor of exposure linked to changes in genes expression?**



- Maximization of the **correlation** between a latent variable from the transcriptomic block U and a latent variable from the metabolomic block *V*

$$U = \sum_{j=1}^{p} a_j X_j \; ; \; V = \sum_{k=1}^{q} b_k \, Y_k$$

⇒ Computation of weight vectors a and b such that $cor(U, V)$ $maximale$

PENALIZED CANONICAL CORRELATION ANALYSIS

- BUT…
  - High dimensionality of datasets = latent variables lack of biological interpretability
  - p >> n = computational problems

⇒ Penalization needed to select the most important features (discernable biological meaning / information)

- « **sparse** » CCA (Wilms et al., 2016)
  - Some weights equal 0 : $a_1X_1+\mathbf{0}X_2+a_3X_3+\mathbf{0}X_4+a_5X_5$

⇒ Removal of noisy features = biological interpretability of latent variables is improved

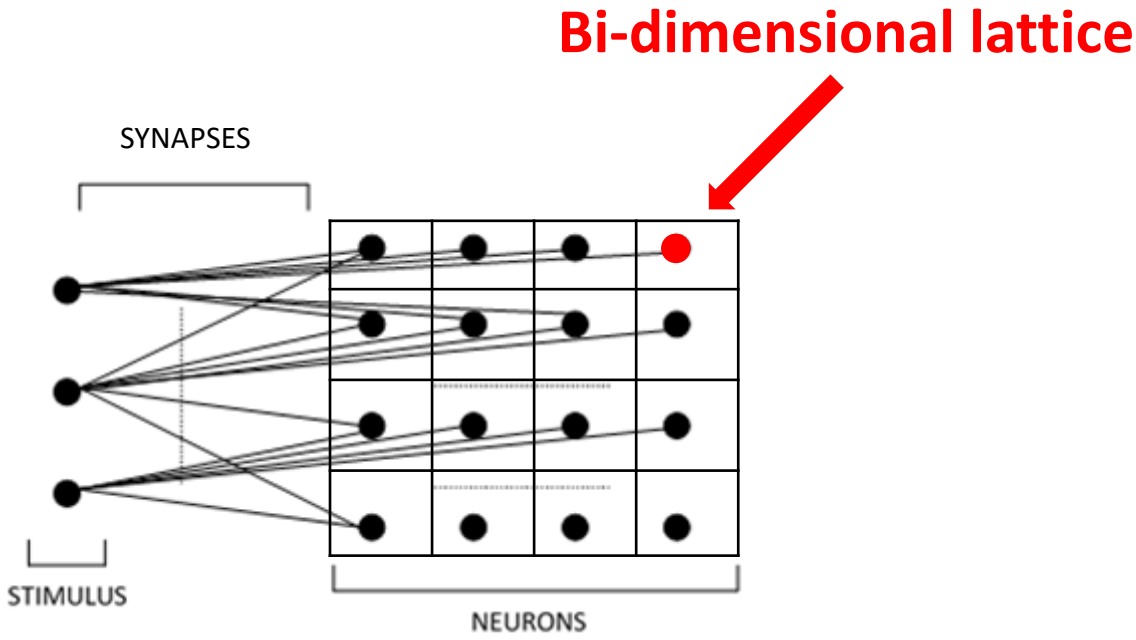
  - Computation of penalization = cross validation

- mixOmics R package

mixOmics: Exploration and Integration of Omics datasets

SELF-ORGANIZING MAPS (SOM)

- SOM (Kohonen, 1982): unsupervised method for projection and classification of objects, based on neural networks
  - **Bi-dimensional lattice** (units = neurons)



From Bessai et al. (2002)

SELF-ORGANIZING MAPS (SOM)

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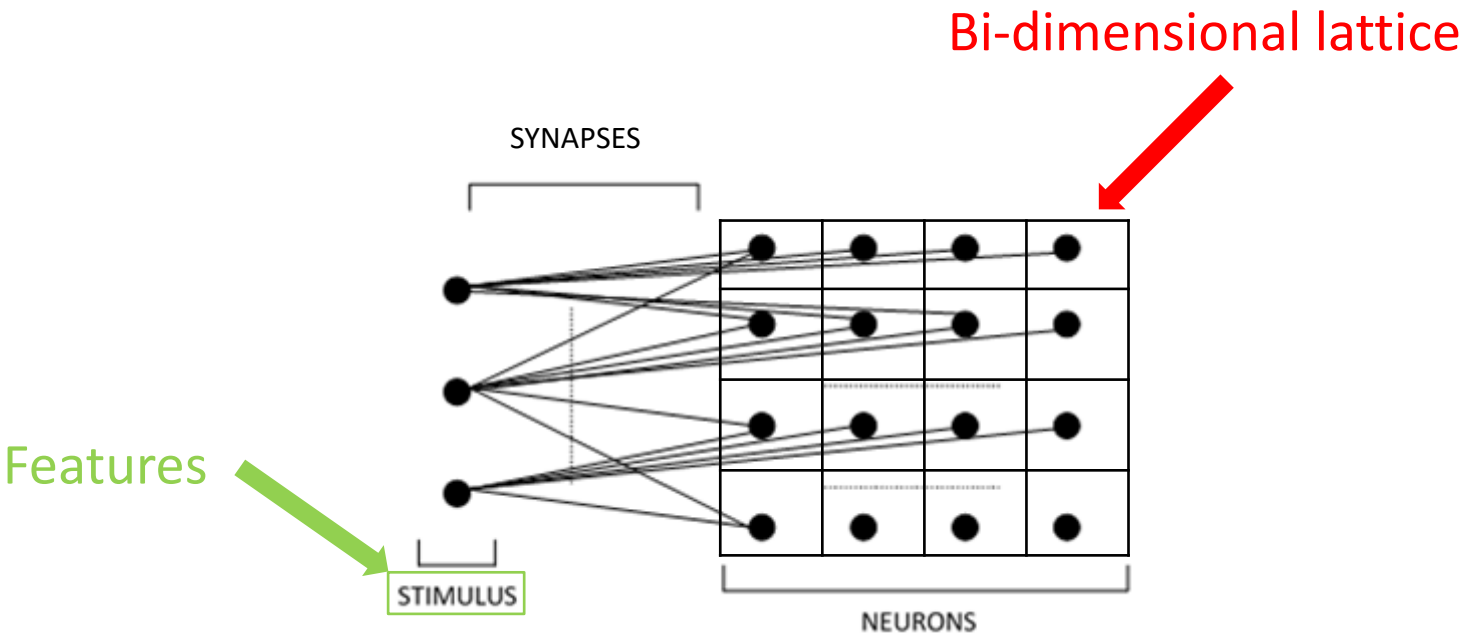
    - To each feature is associated a vector containing measured values for individuals (**stimulus**)



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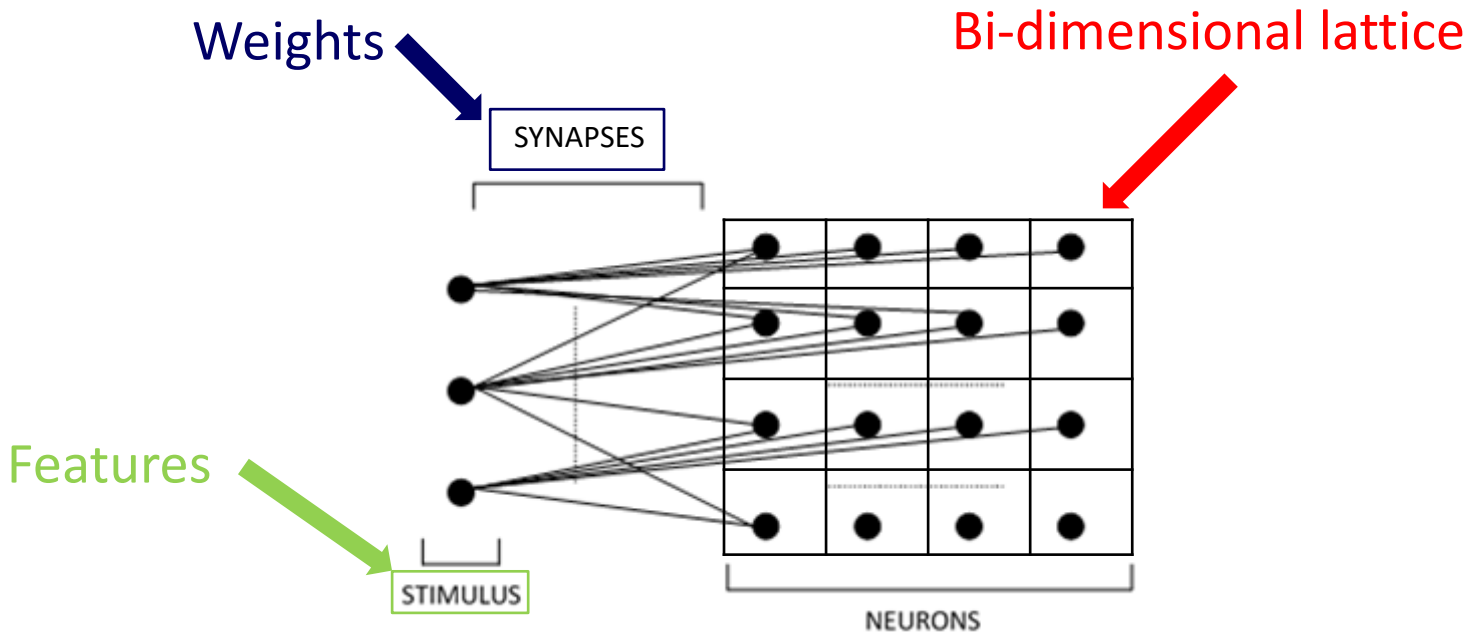
    - To each unit is associated a vector of **weights** (prototype = synapses)



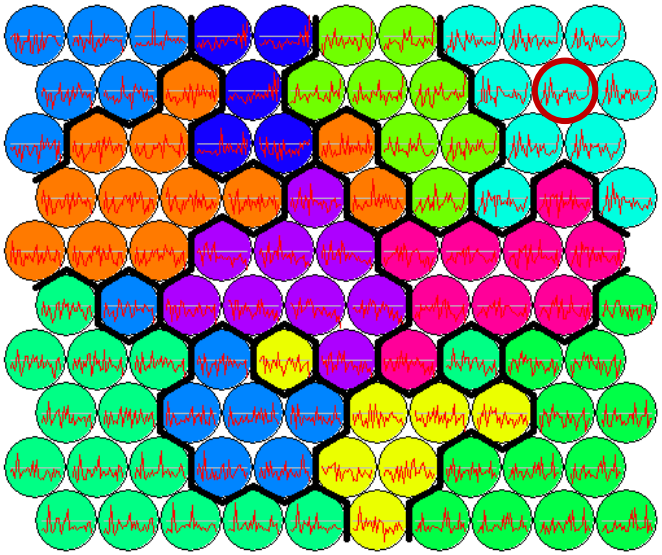
From Bessai et al. (2002)

SELF-ORGANIZING MAPS (SOM): ALGORITHM

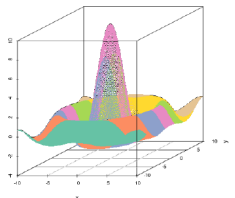
- Iterative algorithm

- Preservation of the original topology of the data: close features in the input space are clustered together into the same unit or into neighbor units on the map)

$\Rightarrow$ clustering of co-expressed genes and co-accumulated metabolites in the same unit

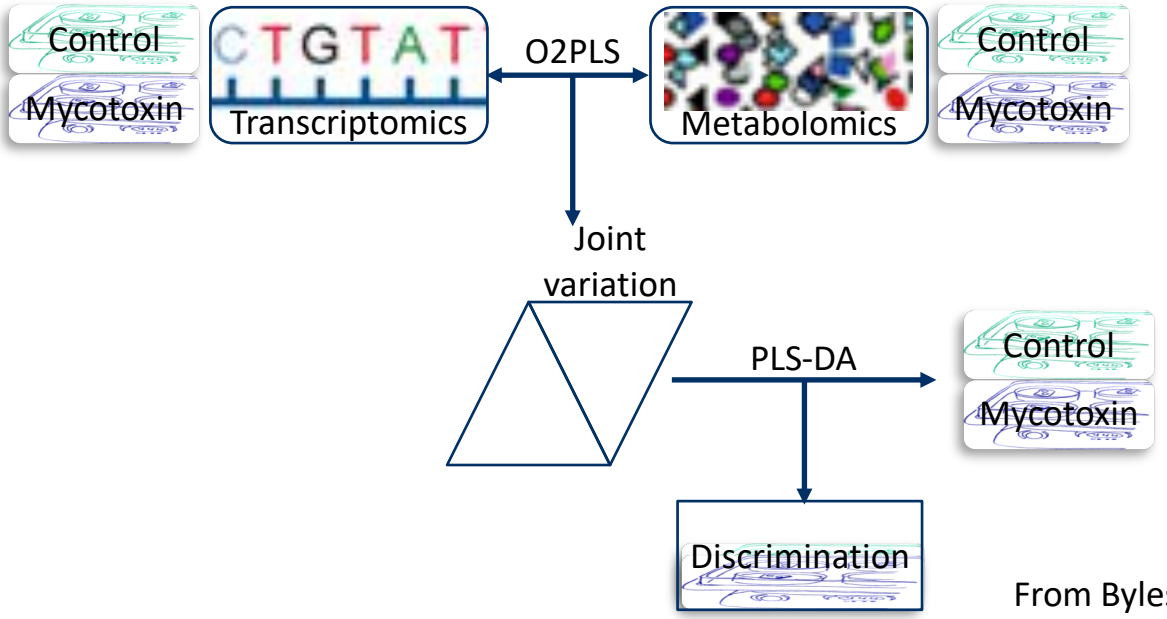


- Package R SOMbrero (Olteanu et al. 2015)



PARTIAL LEAST SQUARES – BASED METHODS

- **O2PLS**: generalization of O-PLS to two datasets
  - Separate the joint variation (e.g. used to predict metabolite levels from transcript profiles, and vice versa)
  - Orthogonal : removal of confounding variability (biological, experimental, sample collection, …)
  - PLS-DA using joint variation to model factor of exposure



From Bylesjö et al. (2007)

⇒ Discrimination of observations depending on mycotoxin exposure and list of discriminant transcripts and metabolites

MONTE CARLO SIMULATION

- Random generation of artificial data using a defined model = known structure of data

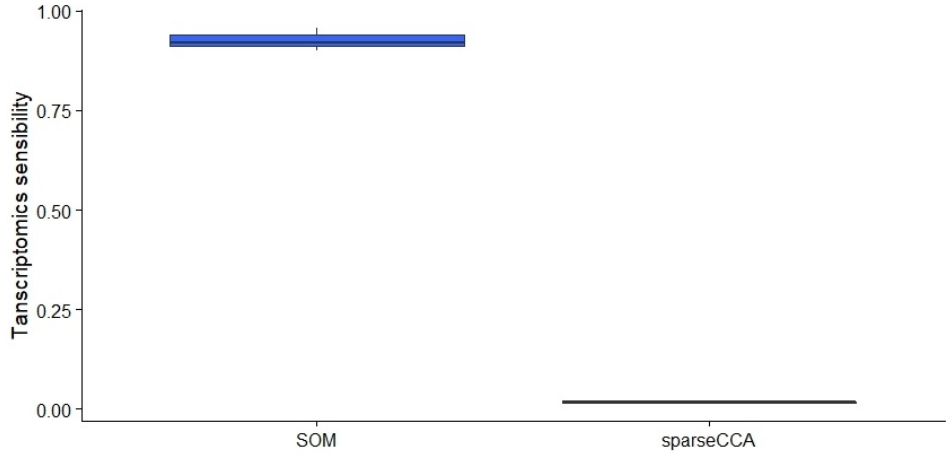
⇒ **Assessment of ability of methods to recover this structure**

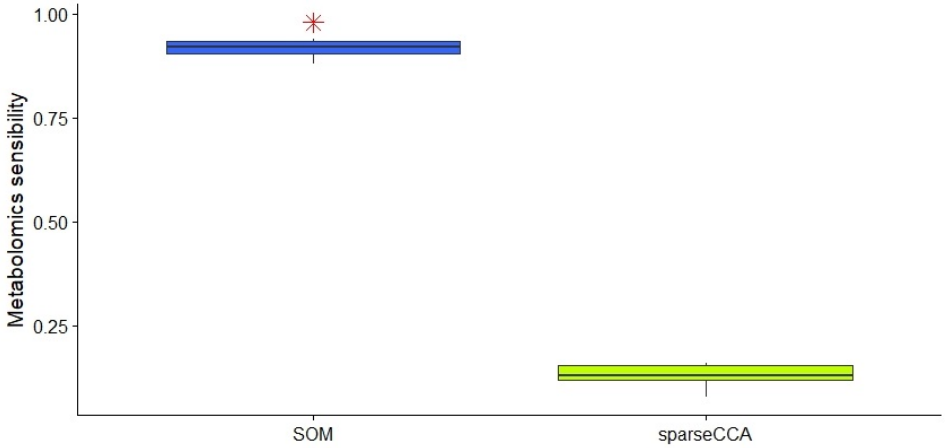
- Dataset sizes
  - n=10 observations / group

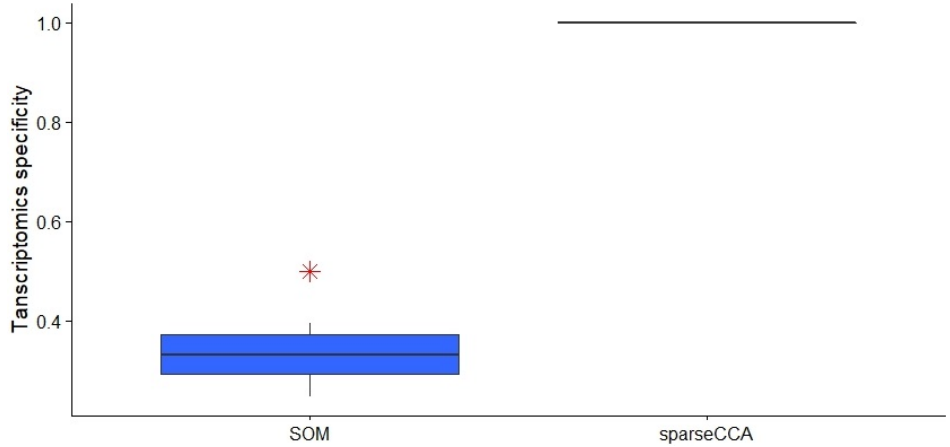
P transcripts	q NMR features
1000	100
5000	500
10000	700
12000	788

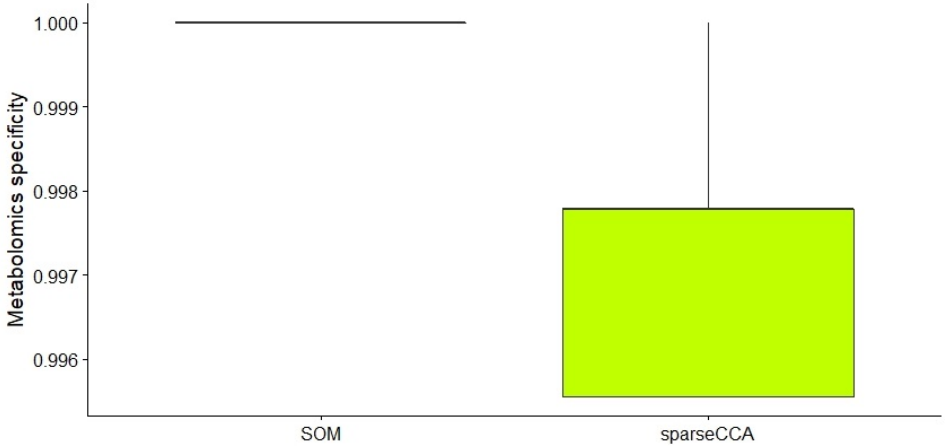
- Criteria
  - Sensitivity: ability of a test to give a positive result when an hypothesis is true (true positives)
  - Specificity: ability of a test to give a negative result when an hypothesis is false (true negatives)
  - R^2: proportion of explained variance
  - MSEP: prediction error = how well does the model classify individuals into the right group?

RESULTS : sparse CCA / SOM

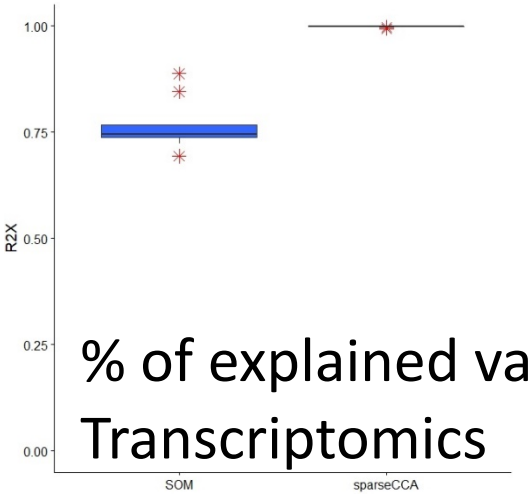




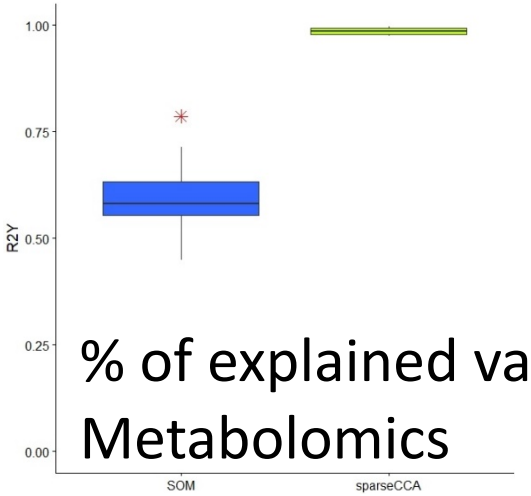




RESULTS : O2-PLSDA

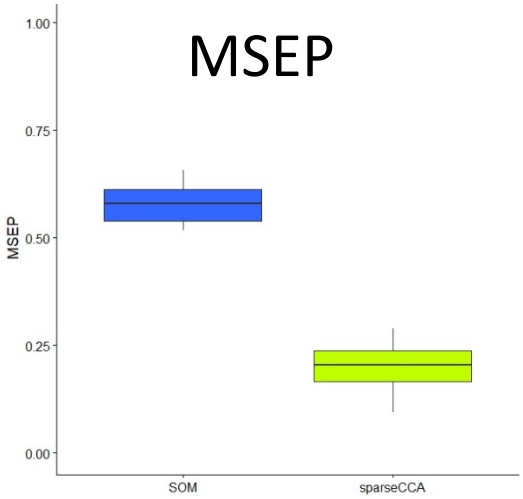


% of explained variance – Transcriptomics

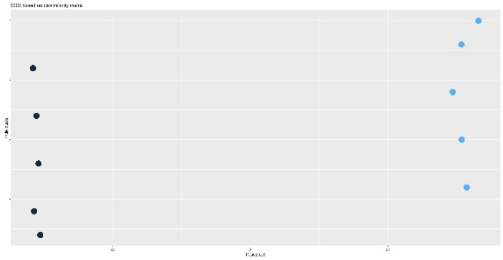


% of explained variance – Metabolomics

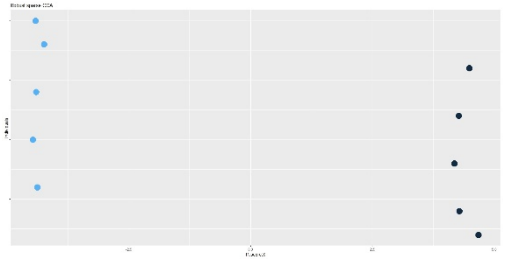
MSEP



SOM



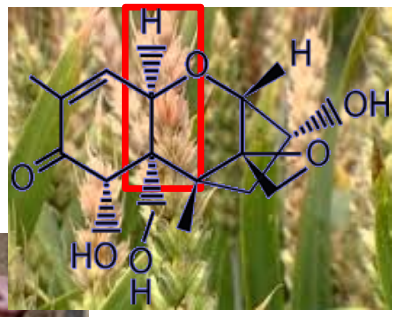
Sparse CCA



Biological application

CONTEXT

- Pig: rich-cereal food



- Fusarium: contaminant fungus of cereal

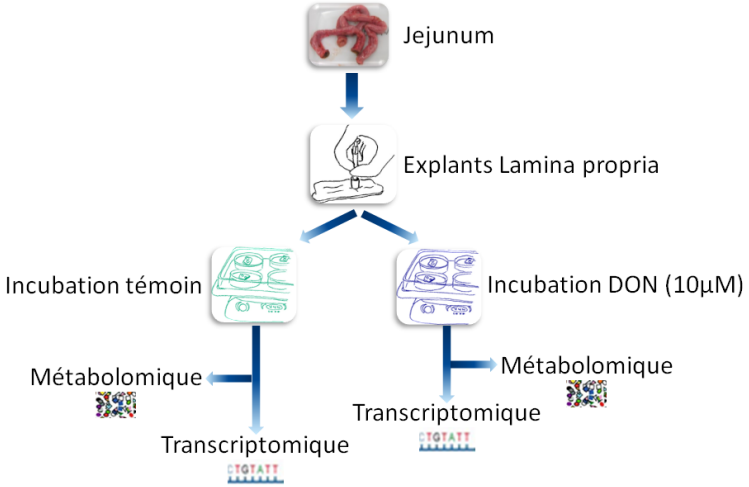
- DON: secondary metabolite of Fusarium
  - Acute and chronic disruptions on animals (gastro-intestinal tract)

⇒ Pigs are particularly exposed to DON

⇒ Identification of markers of exposure to mycotoxins is important for animal healthcare

EXPERIMENTAL DESIGN / DATA

- n=8 animals
  - Jejunal explants (ex vivo)
  - Exposition Control / Mycotoxin (10µM)



- **Transcriptomics**
  - Agilent porcine-specific microarray (60305 spots)
  - Raw data processing (signal median intensity): filtering, log2 transformation and normalization (quantiles method, Bolstad et al. 2003)

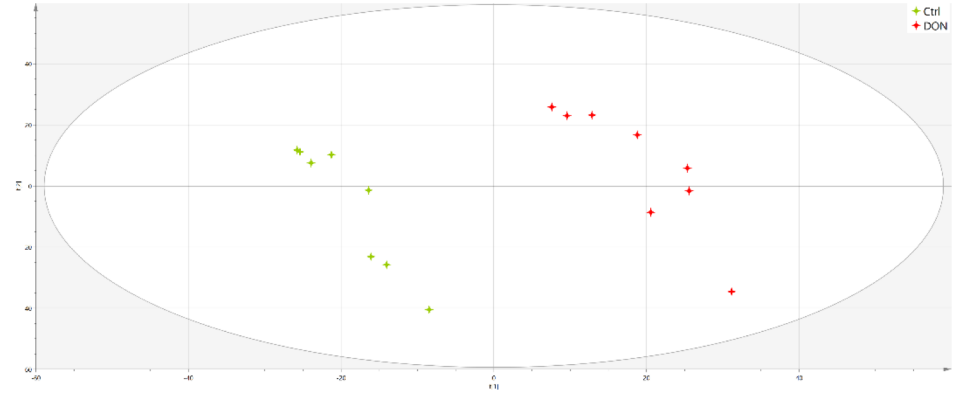
  ⇒ *p=41336 features*

- **Metabolomics**
  - ^{1}H-NMR
  - Processing: bucketing/integration et normalization (total intensity)

  ⇒ *q=751 NMR features*

INDIVIDUAL ANALYSIS

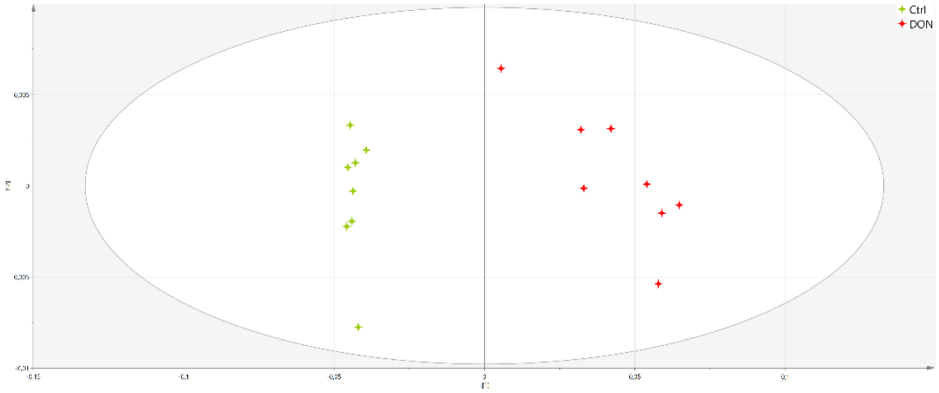
- Transcriptomics



1480 discriminant transcripts

⇒ Modulation of immunity/inflammation related genes

- Metabolomics



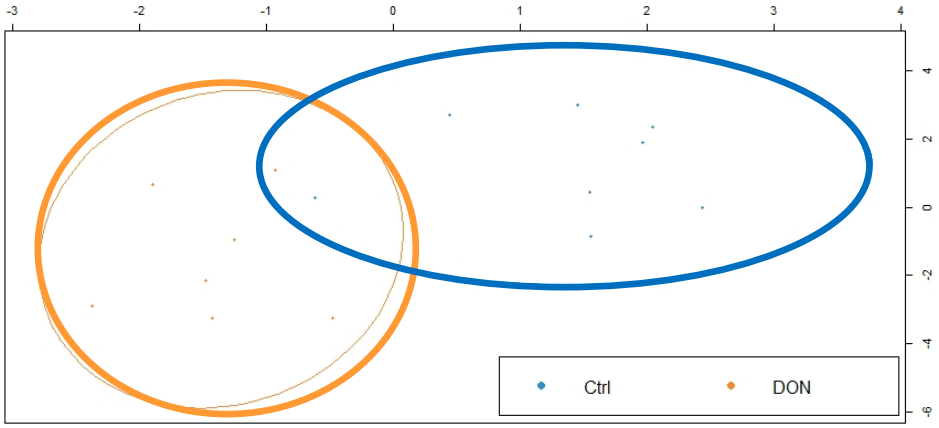
3 discriminant metabolites

⇒ Alanine et Lactic acid

Data fusion: identification of pathways linked to process changes involving metabolism of both metabolites?

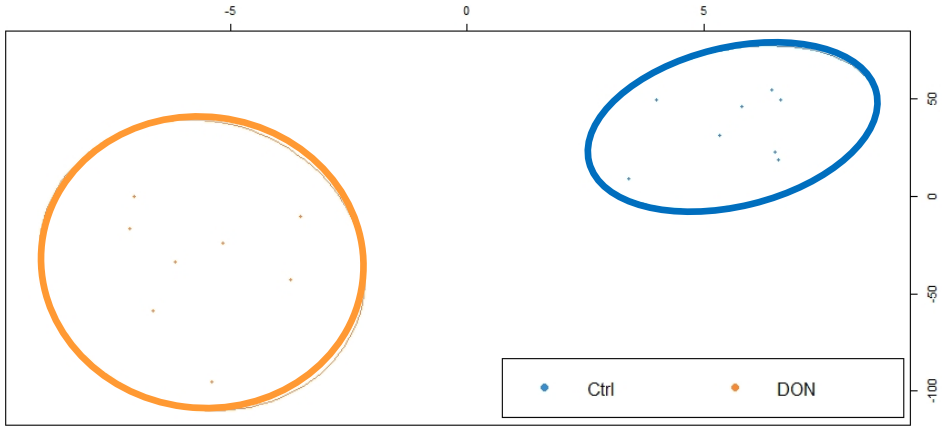
DATA FUSION (1)

- Transcripts selection: 15000 with highest standard deviation
- Sparse CCA-O2PLSDA



  - R2=60.4%
  - MSEP=0.011
  - 12 transcripts & 12 metabolites were discriminant

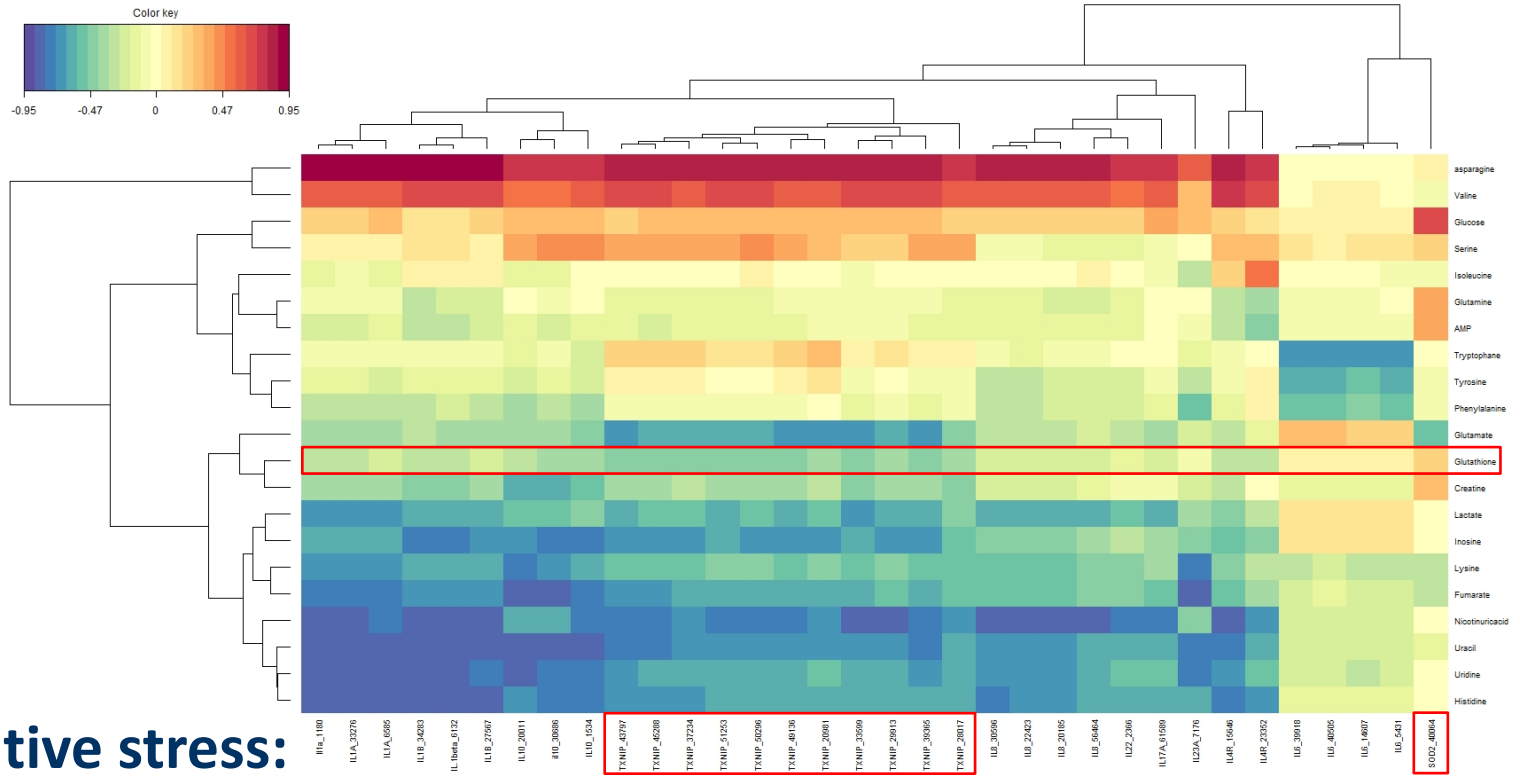
- SOM-O2PLSDA



  - R2=49.9%
  - MSEP=0.011
  - 1443 transcripts & 61 metabolites (24 identified) were discriminant

⇒ Exposed explants are better separated from Control explants with the model fitted using the SOM-selected features

DATA FUSION (2): CORRELATIONS BETWEEN DISCRIMINANT FEATURES



Oxidative stress:

- Glutathione, endogen antioxidant, correlated with:
  - TXNIP (negatively): gene encoding for a thioredoxin-binding protein. Thioredoxine (protects cells from oxidative stress): inhibition of the antioxidative function of thioredoxine $\Rightarrow$ accumulation de reactive oxygen species and cellular stress
  - SOD (superoxide dismutase, positively): antioxidant enzyme

CONCLUSION: SIMULATIONS

- **SOM**
  - High sensitivity = selection of really correlated features
  - Low specificity = selection of uncorrelated features

- **Sparse CCA**
  - High specificity but low sensitivity
  - Highly consuming-time
  - Prior selection of features

$\Rightarrow$ No universal method: combination of several methods = good alternative

CONCLUSION: BIOLOGICAL APPLICATION

- Data fusion
  - Increased number of discriminant metabolites
  - Biological link between transcripts & metabolites

- SOM
  - Best discrimination of Control observations from Mycotoxin Exposed observations
  - Biological relevance of selected features: mycotoxin exposure induces oxidative stress = reported in literature (Pierron et al. 2016) but only for the transcriptomic side

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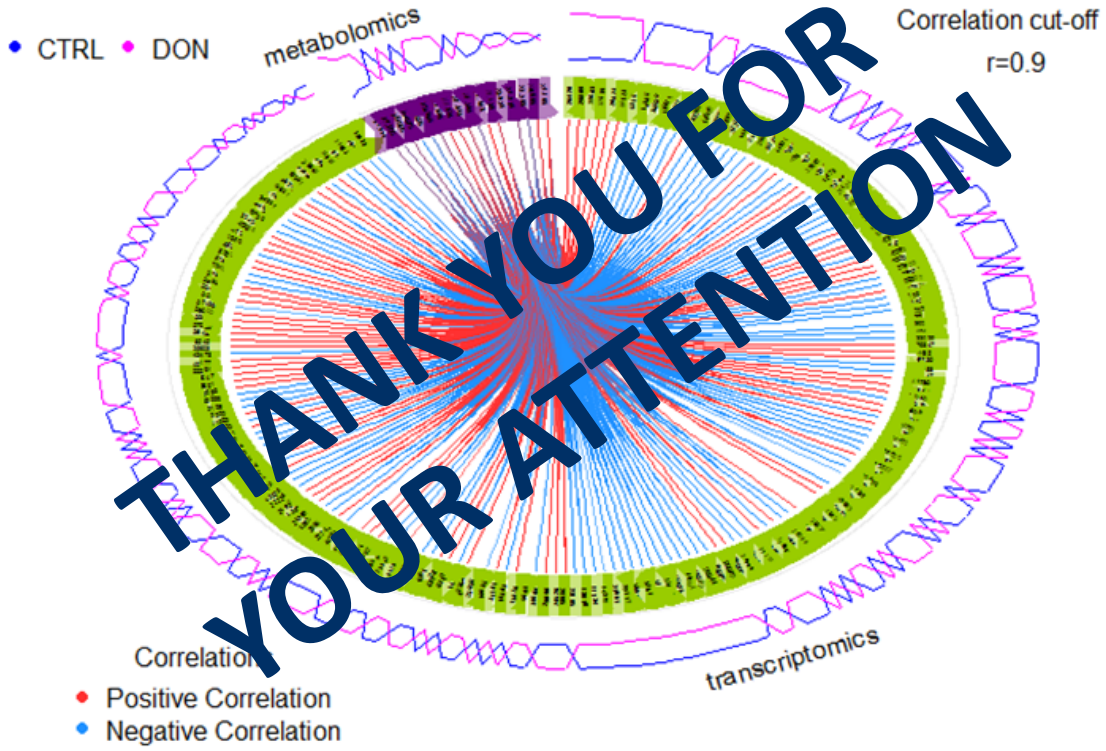
C. Canlet
R. Gautier



THANK YOU FOR YOUR ATTENTION

Y. Lippi
C. Naylies

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P. Pinton
M. Neves
I. Alassane-Kpembi