



SCIENTIFIC NOTEBOOK  
Summer School  
12-15 September 2022



**Scientific Notebook of  
the 3<sup>rd</sup> Summer School organized by INRAE**

**“Innovative approaches and tools to  
substantiate the link between dietary  
polyphenols and Health  
- From clinical to mechanistic research”**

**Coordinators:** Dr. Christine MORAND, Dr. Laurent-Emmanuel MONFOULET

**Place:** INRAE, 147 rue de l'université, 75077 PARIS -France

**Dates:** 12 – 15 september 2022 (3 days)



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 951994.

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## AGENDA

Monday 12 September 2022	
14h – 14h45	Welcome and registration of attendees
14h45 – 15h	Introduction by Dr. Christine Morand (INRAE, France)
15h – 17h30	<p><b>Session 1: Meta-analysis on the health effects of polyphenols</b></p> <p><i>Prof. Emilie COMBET (Univ. of Glasgow – UK)</i> <i>Dr. Laurent-Em. MONFOULET (INRAE, HNU, France)</i></p> <ul style="list-style-type: none"> <li>• Contribution of meta-analysis in strengthening the clinical evidence of the cardiometabolic effects of polyphenols</li> <li>• Assessment of meta-analysis quality and practical examples</li> </ul>
Tuesday 13 September 2022	
9h30 – 13h	<p><b>Session 2: Microbiome analysis to investigate relationship between polyphenols and gut microbiota</b></p> <p>The (poly)phenol/gut microbiota conundrum: towards new models to explain how (poly)phenols affect health? - <i>Prof. Yves DESJARDINS (Institut Nutrition et Aliments Fonctionnels, Univ. of Laval – Québec, Canada)</i></p> <p>Current practices applied to gut microbiome data analysis, their advantages and limitations with a particular focus on 16S and shotgun metagenomics approaches - <i>Dr. Stanislas MONDOT (INRAE, Micalis, Jouy en Josas - France)</i></p>
13h -14h30	Lunch
14h30 – 17h	<p><b>Session 2: Microbiome analysis to investigate relationship between polyphenols and gut microbiota</b></p> <p>Practical training dedicated to the analysis of a 16S data set (<i>with the support of the INRAE-bioinformatic Platform-Migale</i>) <i>Dr. Stanislas MONDOT (INRAE, Micalis, Jouy en Josas - France)</i></p>
Wednesday 14 September 2022	
9h30 – 13h	<p><b>Session 3 : Use of nutrigenomics to elucidate the cellular and molecular mechanisms of polyphenols</b></p> <p>Multi-genomic molecular mechanisms of action of polyphenols underlying cardiometabolic and neurovascular health effects <i>Dr. Dragan MILENKOVIC (Univ. of California, Davis, CA – USA)</i> <i>Prof. Tatjana RUSKOVSKA (Goce Delcev University - Stip, North Macedonia)</i></p>
13h -14h30	Lunch
14h30 – 17h	<p>Presentation of bioinformatic tools to analyze nutrigenomic data: practical data set analyses by attendees <i>Dr. Dragan MILENKOVIC (Univ. of California, Davis, CA – USA)</i> <i>Prof. Tatjana RUSKOVSKA (Goce Delcev University - Stip, North Macedonia)</i></p>



Thursday 15 september 2022	
9h30 – 11h 30	<p><b><u>Session 4: In-silico docking to identify new molecular targets of polyphenol metabolites</u></b></p> <p><i>Assist. Prof. Christos KONTOGIORGIS (Democritus University of Thrace – Greece)</i> &amp; <i>Assist. Prof. Eleni Pontiki, (Aristotle University of Thessaloniki, Greece)</i></p> <ul style="list-style-type: none"><li>• Introduction to molecular docking to predict molecule-protein interactions</li><li>• Application for the identification of the molecular targets of polyphenol metabolites</li></ul>

## INTRODUCTION

This Summer school is organized by INRAE within the framework of European Twinning Project PhenolAcTwin coordinated by the Tubitak Institute (Turkey) and in which INRAE is partner. PhenolAcTwin aims to Enhance Research and Innovation Capacity of TUBITAK MRC Food Institute in the field of Dietary Polyphenols and Bioavailability / Bioefficacy (<https://phenolactwin.eu/en/#>).

The Human Nutrition Unit of INRAE (Clermont-Ferrand, France) has built this training school with the aim to enhance the research capacity of young scientists interested in research on Polyphenols & Health and to help bridging opportunities for further collaborations between delegates.

In this training, a diversity of innovative approaches to develop research on polyphenols and health will be addressed by international experts, including meta-analysis, microbiome analysis, nutrigenomics and *in silico* docking. This training aims to maintain tight connections between trainees and trainers in order to provide an appropriate support to young scientists in their future research and favor the development of collaborative projects.

This training is dedicated to Master Students, PhD students and Early Career Scientists\*, from European Universities and Institutes with a priority given to partners of the PhenolAcTwin Consortium.







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## SESSION 3: Use of nutrigenomics to elucidate the cellular and molecular mechanisms of polyphenols

### Multi-genomic molecular mechanisms of action of polyphenols underlying cardiometabolic and neurovascular health effects

**Dr. Dragan MILENKOVIC (Univ. of California, Davis, CA – USA).** He is associate Faculty Researcher at the Department of Nutrition at University of California Davis, Davis, CA, USA. He received his master's degree in genetics and molecular modelling from University of Paris, France, and his PhD from University of Versailles, France, in molecular genetics. His research aims to demonstrate effects of macronutrients and micronutrients, particularly polyphenols, on vascular function and development and/or prevention of cardiovascular and neurodegenerative diseases. The projects also aim to identify variability in responsiveness to intake of these nutrients and identify factors involved, such as gender, age, health status or genetic polymorphism. His projects use translational research approach involving in-vitro studies, animal models and clinical trials. Dr Milenkovic's second significant axis of research aims to decipher detailed molecular and cellular mechanisms of actions underlying observed effects using multi-genomic and bioinformatic approaches. He uses integrated and systems biology to incorporate results from genomics studies, including modifications in expression of genes (protein coding and noncoding like microRNAs, long non-coding RNAs, short non-coding RNAs) and proteins, epigenetics as well as in-silico docking studies to evaluate interaction between polyphenol metabolites and cell signalling proteins

**Prof. Tatjana RUSKOVSKA (Goce Delcev University - Stip, North Macedonia).** She obtained her BSc (1990), MSc (1997), and Ph.D. (2002) degrees at the Faculty of Natural Sciences and Mathematics, Institute of Biology, Biochemistry and Physiology Group, Ss. Cyril and Methodius University, Skopje, North Macedonia. Positions held: biochemist and head of the laboratory at the Central Clinical Laboratory, Military Hospital in Skopje (1994 to 2010); joined the Faculty of Medical Sciences Goce Delcev University in Stip, North Macedonia (2010), where she was vice-dean from 2011 to 2014 and head of the school for doctoral studies (2018-2020). In the 2014/2015 academic year, she was appointed visiting professor in the Department of Biochemistry, Molecular Biology, and Biophysics at the University of Minnesota, USA, as a Fulbright Scholar. Currently, she works as a full professor of Clinical Chemistry and biochemistry at the Faculty of Medical Sciences, Goce Delcev University in Stip, North Macedonia.

(Presentations in the ANNEX)



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**Presentation of bioinformatic tools to analyze nutrigenomic data: practical data set analyses by attendees**

**Pipeline for bioinformatics analyses of nutrigenomics data**

- 1) PCA and heatmap of global gene expression profiles  
Tools: *MetaboAnalyst, ClustVis*  
<https://www.metaboanalyst.ca/>  
<https://biit.cs.ut.ee/clustvis/>
- 2) Identification of different types of RNA  
Tool: *ShinyGO*  
<http://bioinformatics.sdstate.edu/go/>
- 3) Pathways of mRNA  
Tool: *GeneTrail*  
<https://qenetrail.bioinf.uni-sb.de/>
- 4) Clustering of pathways into groups  
Tool: *Manual grouping*
- 5) Protein-protein interactions (PPIs)  
Tools: *STRING, OmicsNet*  
<https://string-db.org/>  
<https://www.omicsnet.ca/>
- 6) Transcription factors  
Tools: *Enrichr, OmicsNet*  
<https://maayanlab.cloud/Enrichr/>
- 7) miRNA targets  
Tools: *MIENTURNET, miRWalk, miRNA diseases database*  
<http://userver.bio.uniroma1.it/apps/mienturnet/>  
<http://mirwalk.umm.uni-heidelberg.de/>  
<https://www.cuilab.cn/hmdd>
- 8) lncRNAs targets  
Tools: *LncRRlsearch*  
<http://rtools.cbrc.jp/LncRRlsearch/index.cgi>
- 9) Integration analysis  
Tools: *Venny*  
<https://bioinfoq.cnb.csic.es/tools/venny/>
- 10) Associated diseases  
Tool: *Enrichr, Comparative Toxicogenomics Database (CTD)*  
<https://ctdbase.org/>





# Molecular Bioinformatic Analyses for Systematic Reviews



Prof. Tatjana Ruskovska  
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# Systematic Reviews



**GAINING COMPLETE INSIGHT INTO  
THE CURRENT STATUS FOR THE  
SCIENTIFIC TOPIC OF INTEREST**



**POSSIBILITY TO EXTRACT  
RELEVANT EXPERIMENTAL DATA**



**POSSIBILITY TO INTEGRATE AND  
ANALYZE THE EXTRACTED DATA**

# Strategy

## Keywords



Literature searches performed on PubMed and Web of Science



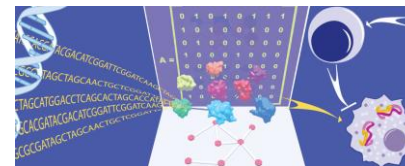
Screening of selected papers



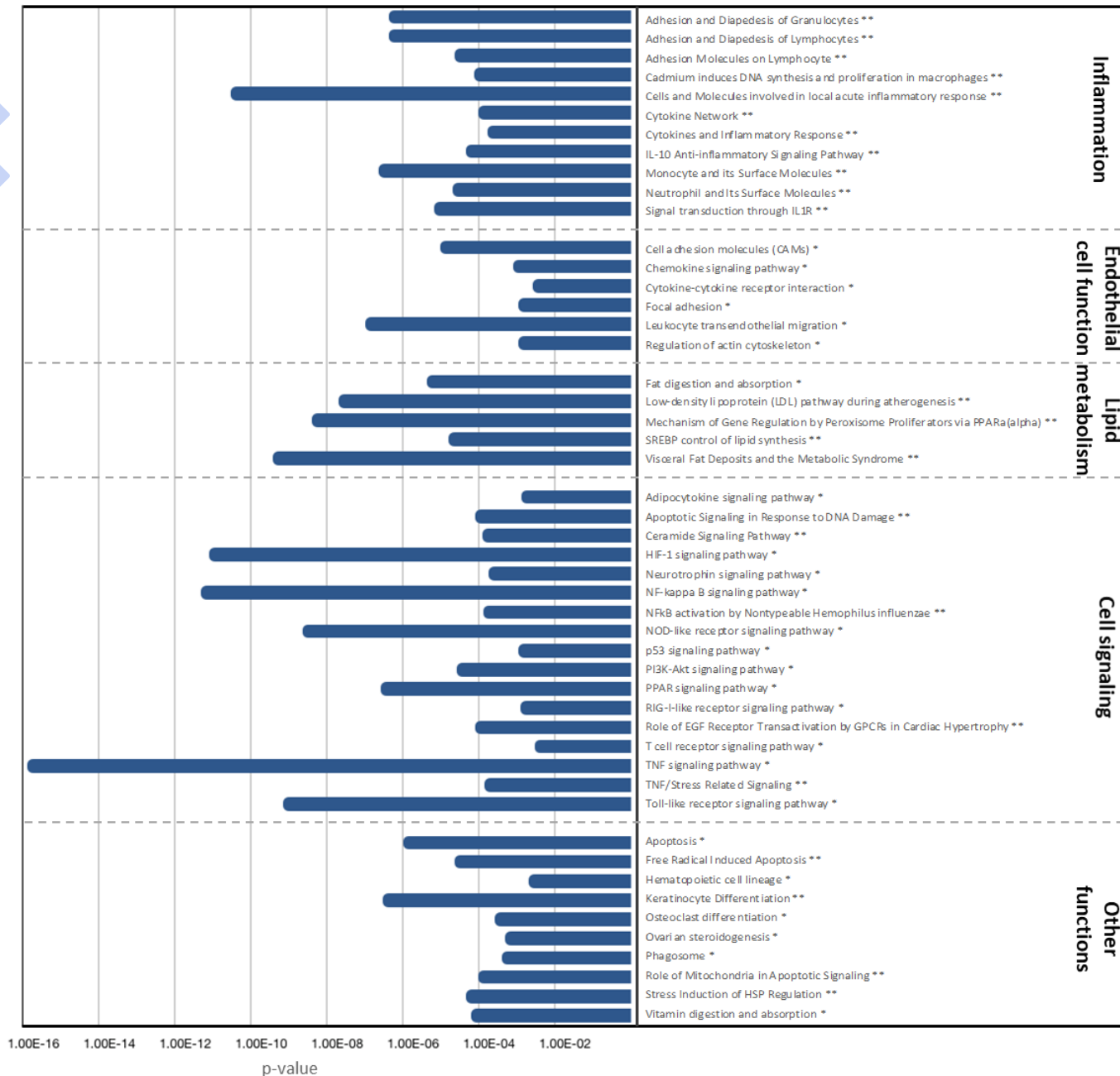
Data extraction



Preparation of Template for the data extraction



Data integration

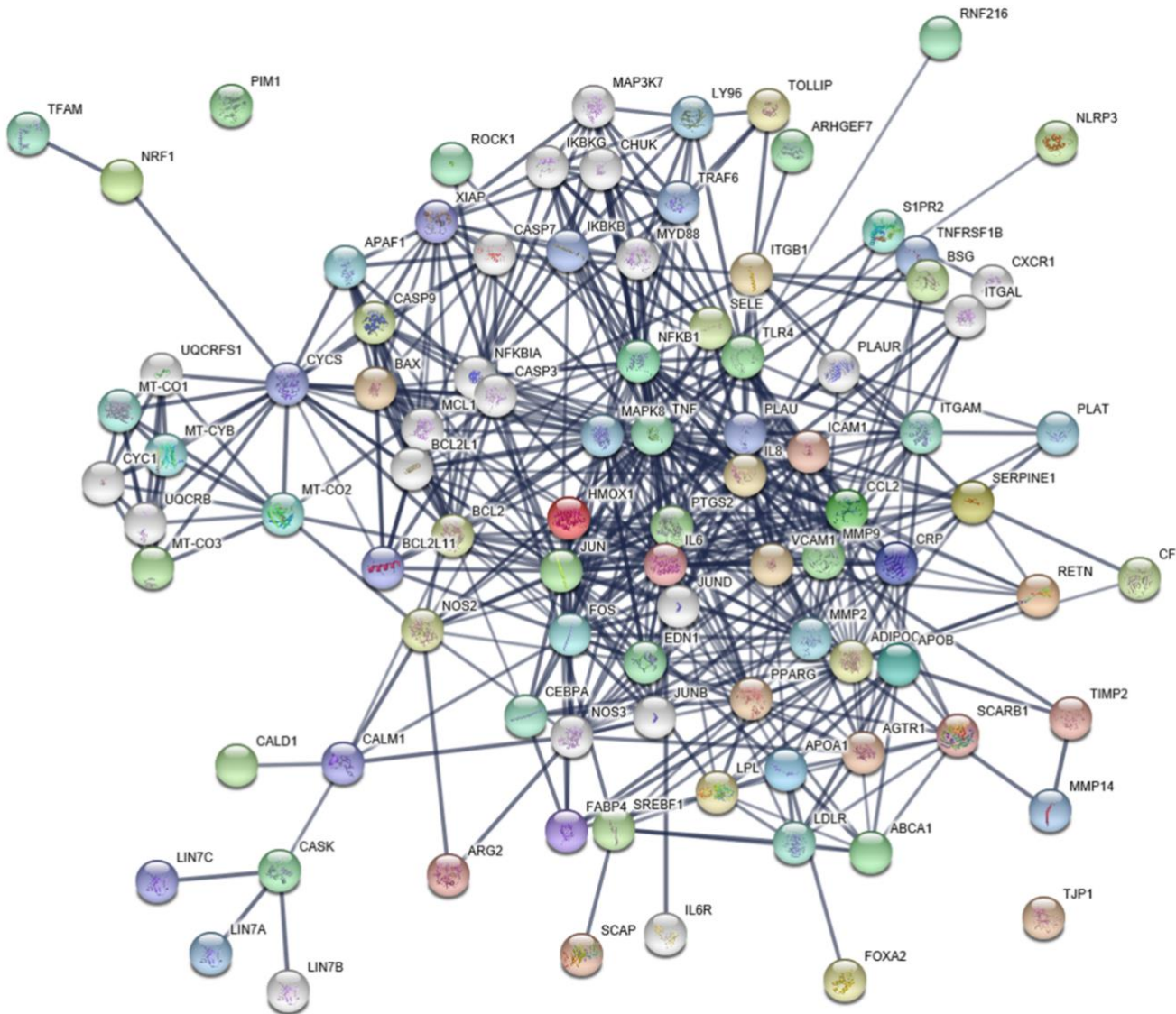


- Pathway enrichment analysis

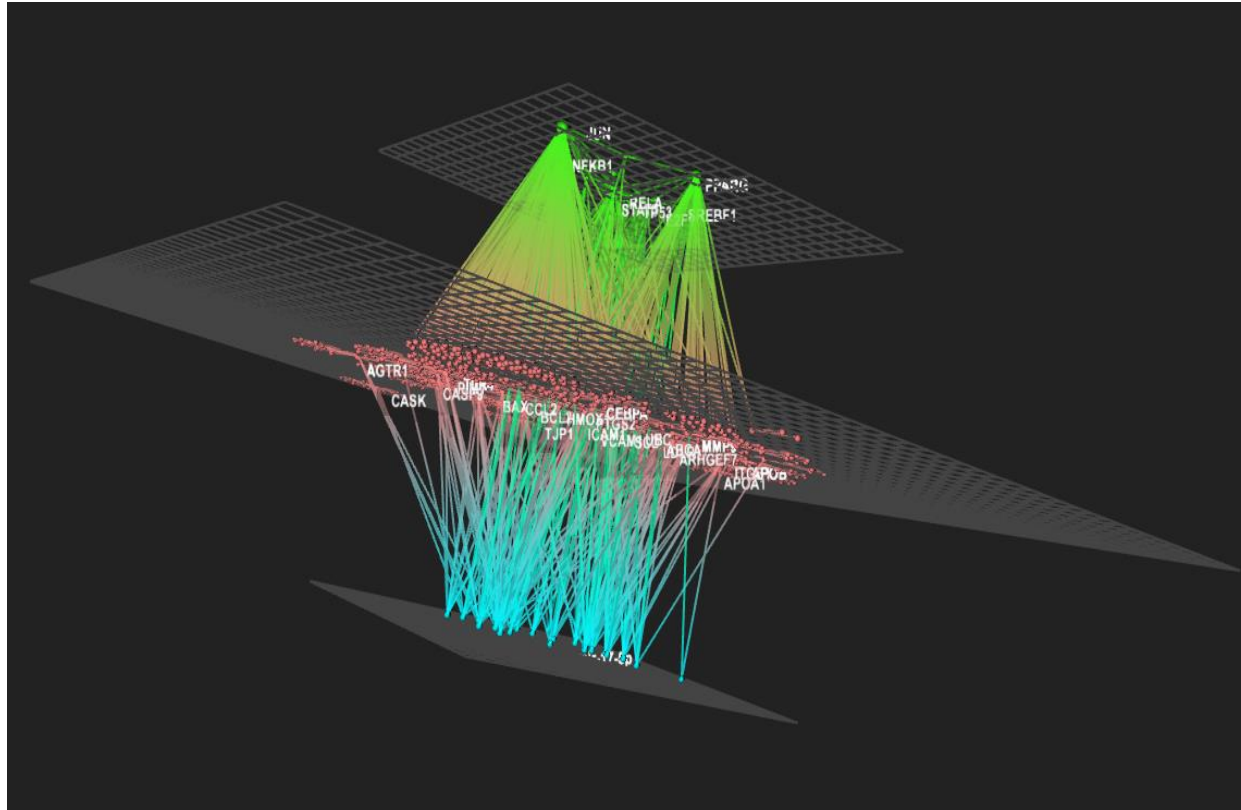
- GeneTrail2 (<https://genetrail2.bioinf.uni-sb.de/>), as a platform to access KEGG and BioCarta databases

- \*KEGG
- \*\*BioCarta





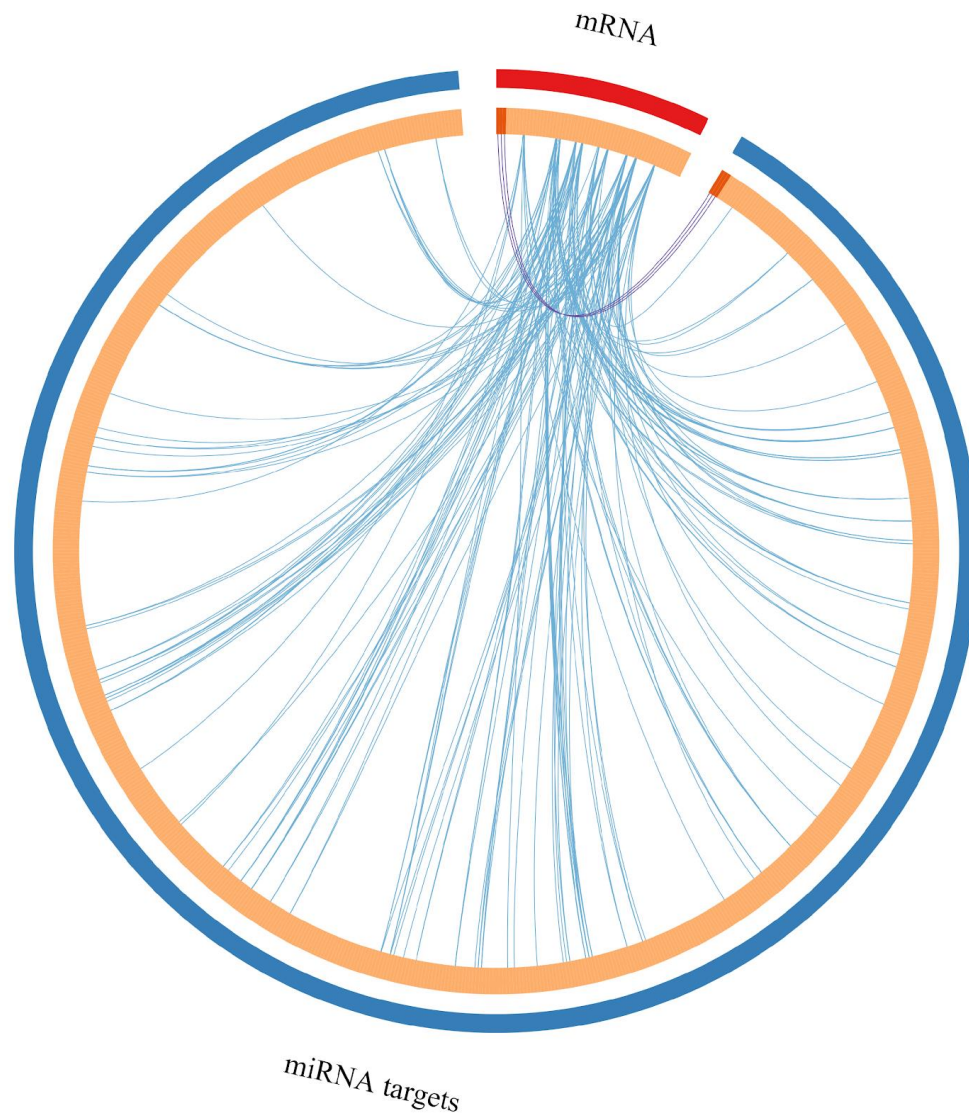
- Protein-protein interactions (PPIs)
  - Proteins coded by the DEGs extracted from the literature and
  - Their neighboring proteins
  - TNF, IL6, JUN, TLR, NFKB1, and MAPK8  $\geq 30$  interactions
  - STRING (<https://string-db.org/>)



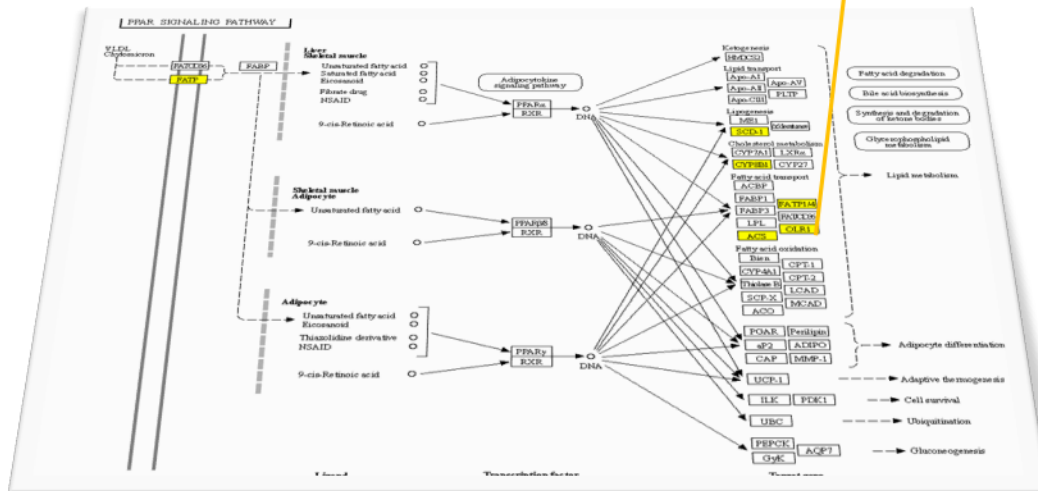
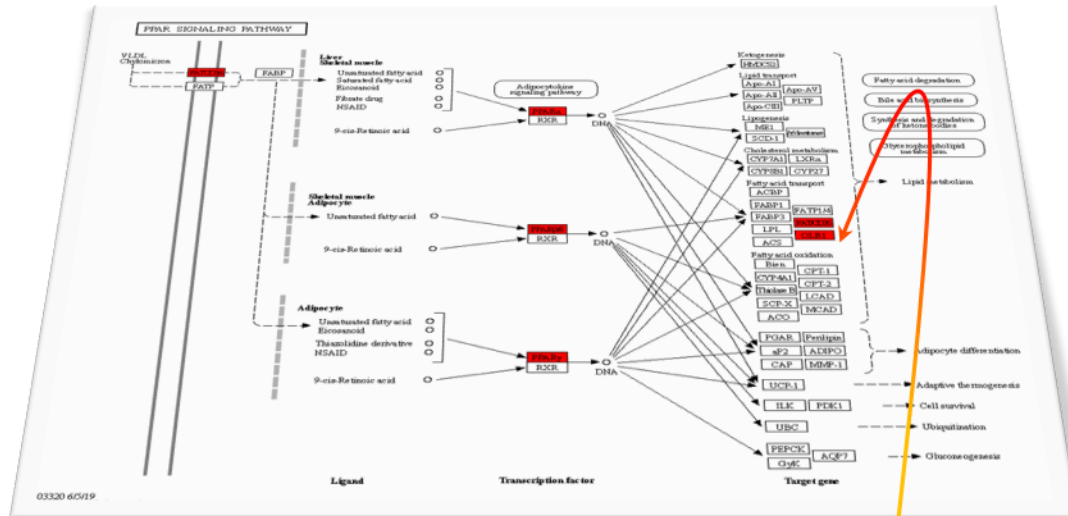
- Regulation of the network of PPI by TFs and miRNAs

- OmicsNet (<https://www.omicsnet.ca/faces/home.xhtml>)



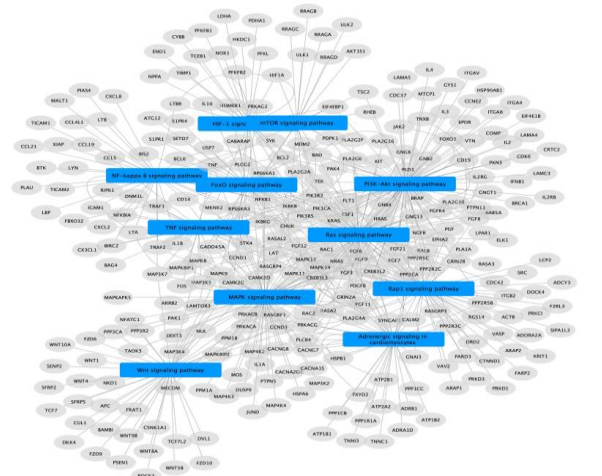
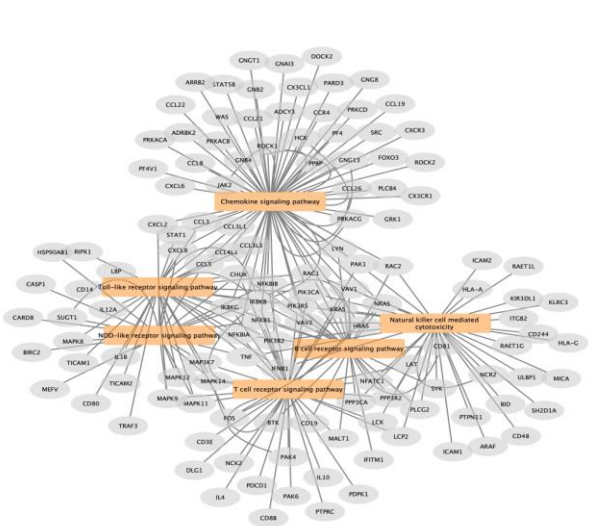


- Integration analyses
  - Integration of mRNAs (DEGs) and miRNA targets
    - 3 overlapping genes: *FASN*, *ADIPOR2*, and *OLR1*
    - Numerous functional interactions
  - Metascape (<http://metascape.org>)

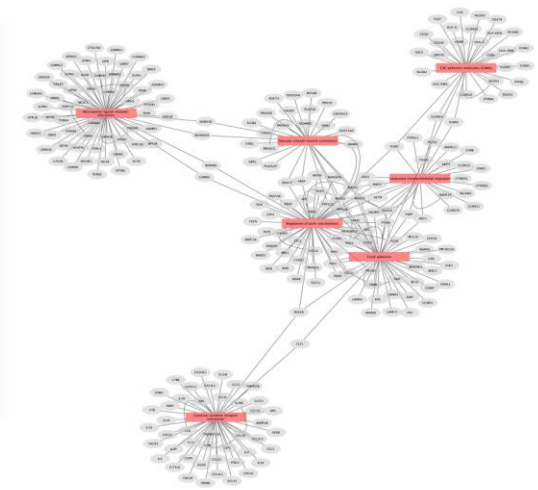


## Integration analyses

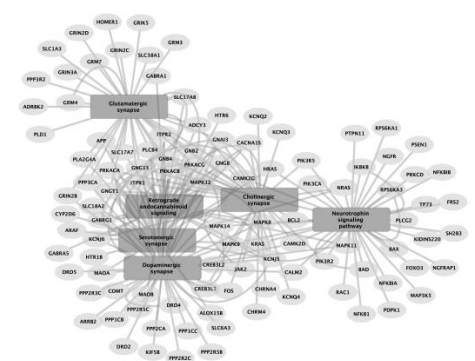
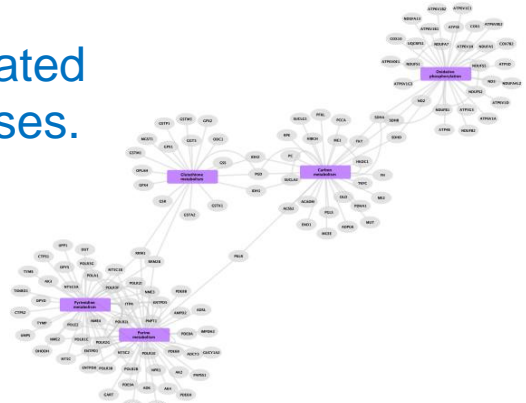
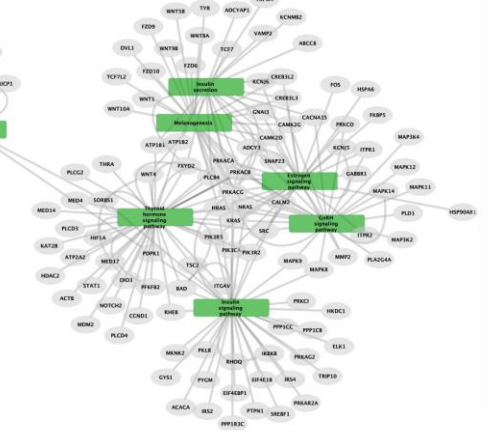
This integrated analysis shows complex mode of action of polyphenols, including both protein coding and non-coding genes involved in pathways that form a complex network that allows regulation of cellular functions.



Functions and systems affected	Pathways in common
Cell signaling	<i>PI3K-Akt signaling pathway</i> <i>MAPK signaling pathway</i> <i>Ras signaling pathway</i> <i>NF-kappa B signaling pathway</i> <i>TNF signaling pathway</i> <i>FoxO signaling pathway</i> <i>Rap1 signaling pathway</i> <i>Wnt signaling pathway</i> <i>HIF-1 signaling pathway</i> <i>mTOR signaling pathway</i> <i>Adrenergic signaling in cardiomyocytes</i>
Immune system	<i>Chemokine signaling pathway</i> <i>Natural killer cell mediated cytotoxicity</i> <i>B cell receptor signaling pathway</i> <i>T cell receptor signaling pathway</i> <i>Toll-like receptor signaling pathway</i> <i>NOD-like receptor signaling pathway</i>
Cell motility and interaction	<i>Focal adhesion</i> <i>Cell adhesion molecules (CAMs)</i> <i>Cytokine-cytokine receptor interaction</i> <i>Leukocyte transendothelial migration</i> <i>Regulation of actin cytoskeleton</i> <i>Vascular smooth muscle contraction</i> <i>Neuroactive ligand-receptor interaction</i>
Endocrine system	<i>Estrogen signaling pathway</i> <i>Thyroid hormone signaling pathway</i> <i>PPAR signaling pathway</i> <i>Insulin signaling pathway</i> <i>Insulin secretion</i> <i>Melanogenesis</i> <i>GnRH signaling pathway</i>
Nervous system	<i>Dopaminergic synapse</i> <i>Cholinergic synapse</i> <i>Serotonergic synapse</i> <i>Neurotrophin signaling pathway</i> <i>Retrograde endocannabinoid signaling</i> <i>Glutamatergic synapse</i>
Metabolism	<i>Glutathione metabolism</i> <i>Purine metabolism</i> <i>Pyrimidine metabolism</i> <i>Carbon metabolism</i> <i>Oxidative phosphorylation</i>



Networks of pathways related to specific cellular processes. (Cytoscape)



**Thank you for your attention!**



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