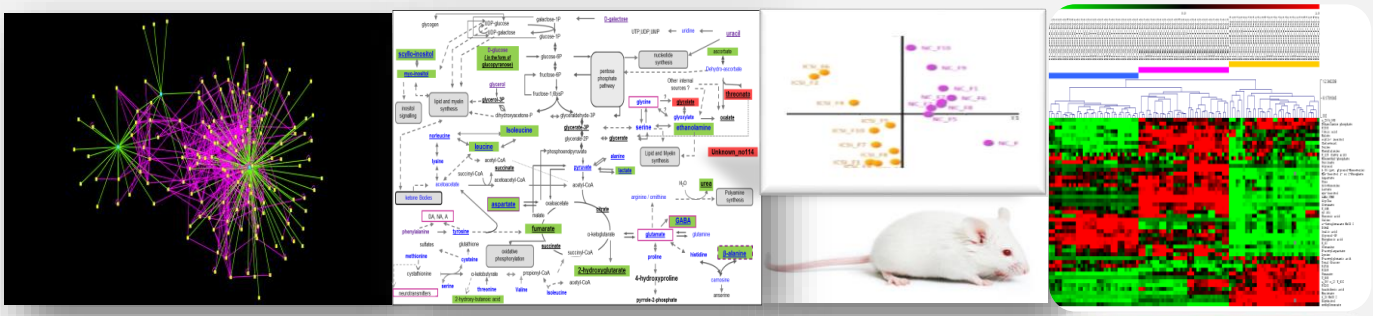
11η επιστημονική ΔΙΗΜΕΡΙΔΑ ITE 2017

FORTH
INSTITUTE OF CHEMICAL ENGINEERING SCIENCES

forth|iceht

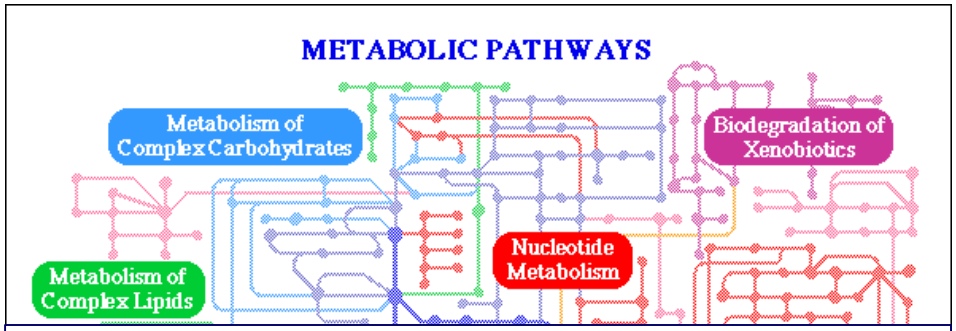
Metabolic & Protein Networks in Systems Biology



MARIA I. KLAPA

Metabolic Engineering & Systems Biology Laboratory
FORTH/ICE-HT, Patras, Greece

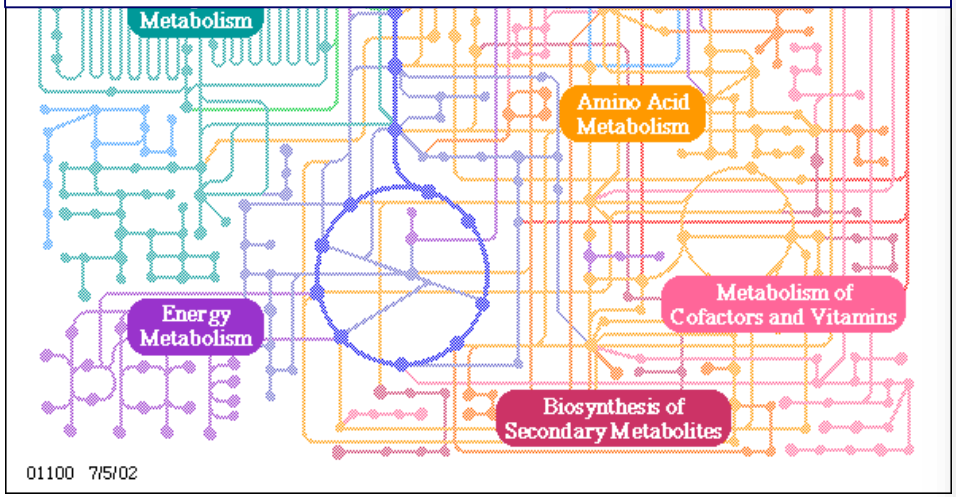
Metabolic Engineering:
Don't forget pathway connectivity and bioprocess control!



Metabolic Network Analysis

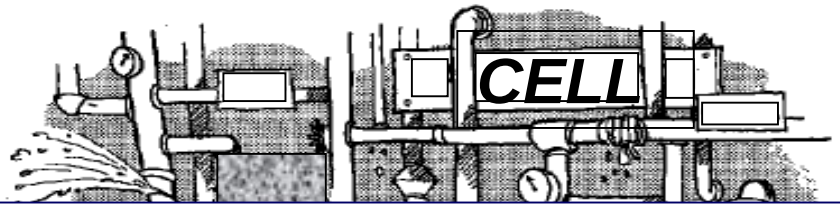
Systemic Analysis – Holism

Emerging Properties through Biomolecular Interactions



Toward a science of metabolic engineering
JE Bailey
Science 21 Jun 1991 Vol. 252 pp. 1668-1675

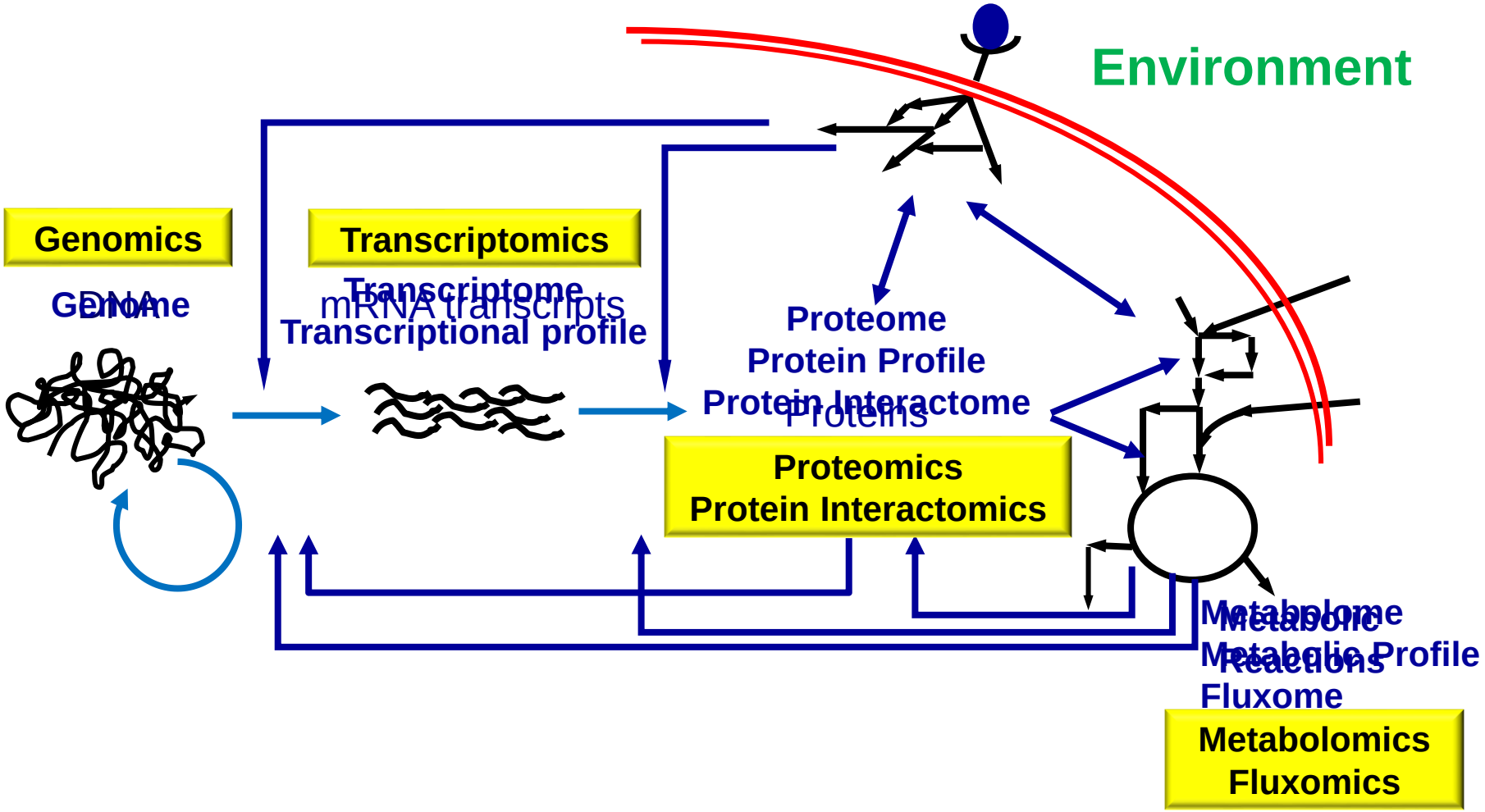
Application of recombinant DNA methods to **restructure metabolic networks** can improve production of metabolite and protein products by **altering pathway distributions and rates**. … Although some of the experimental and mathematical tools required for rational metabolic engineering are available, **complex cellular responses to genetic perturbations can complicate predictive design.**



Metabolic Pathway Rate/Flux
Process Dynamics
Flexible & Rigid Nodes
Mathematical Modeling of Biological Systems

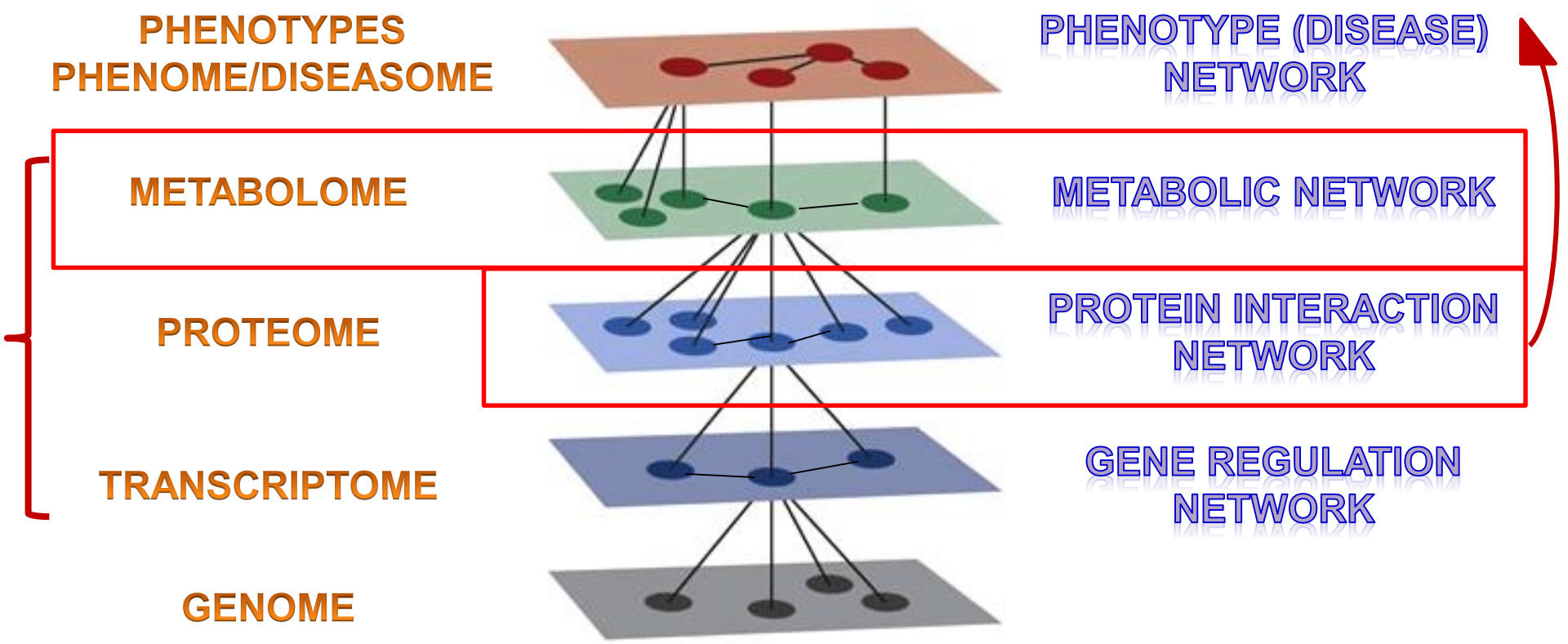
Molecular Systems Biology
From Reductionism to Holism

The revolution of high-throughput biomolecular analyses (omics)



*The regulation of gene expression and protein activity is **NEITHER** linear **NOR** unidirectional*

Cell as a network of biomolecular networks



1. Development and Application of Mass Spectrometry Metabolomic Analysis Experimental Methodologies & Computational Tools

2. Reconstruction & analysis of human protein interactome
   in silico reconstruction & analysis of bacterial PPI networks

StrepSynth

Poster F17

3. Integrated omics of stably modified for *FRA10AC1* expression *HeLa* cells
   (ARISTEIA II to N. Moschonas)

Poster F18

Metabolomic Analysis Workflow

Multistep high-throughput biomolecular analysis: ***not just chemometrics*** *!*

Experimental Design

a. Study system
b. Tissue/Cell or biofluids
c. Sex/Gender & Age

Pre-analytical part

Sample collection & Handling

↓

Quenching & Storage

Analytical part

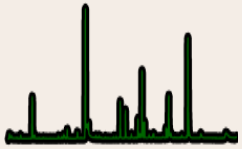
Extraction of free metabolites & Internal Standard

→

[Derivatization (GC-MS)]

→

Metabolic Profile Acquisition



Computational part

↓

Peak Identification & Quantification

↓

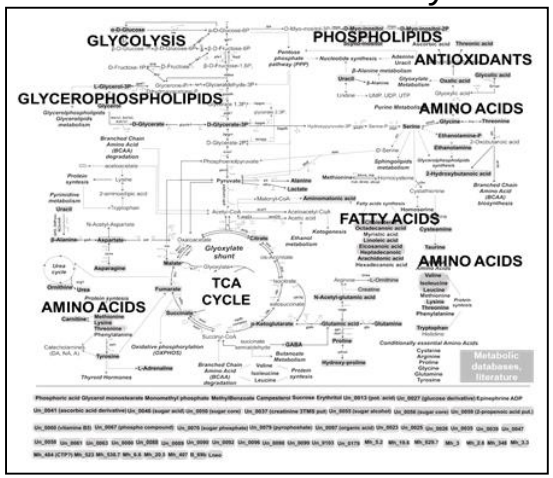
Data Normalization & Filtering

←

Multivariate Statistical Analysis & Mathematical Modeling

←

Metabolic Network Reconstruction & Analysis



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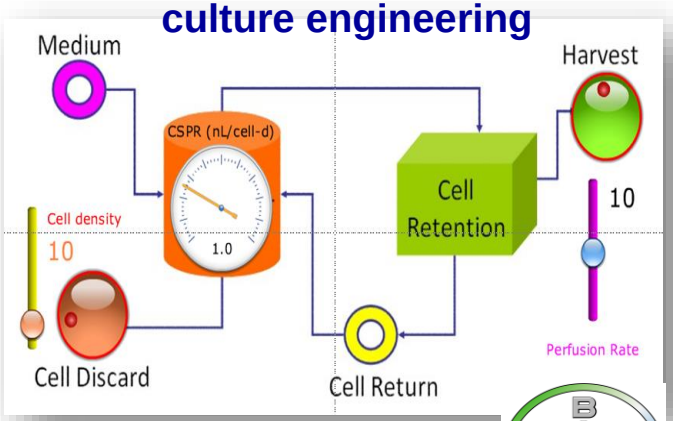
Biologically Relevant Conclusion

Metabolomics in industrial cell culture engineering



Collaboration with Bayer HealthCare, CA, USA

Chrysanthopoulos et al. Metab Eng 12: 212 (2010)
Vernardis et al. Metab Eng 19: 1-9 (2013)
Vernardis, PhD Thesis (2015);
Vernardis et. al. (2017) in prep

Tissue metabolomics in animal models

Adult Onset Hypothyroidism (AOH)

Brain

Collaboration with Human & Animal Physiology Laboratory Dept. of Biology, U. of Patras, Prof. M. Margarity

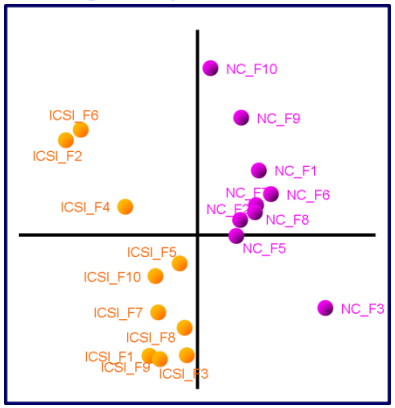
Constantinou et al. J Proteome Res 10 , 869-879 (2010)
Ioannidi Master's Thesis (2015)
Vasilopoulou and Klapa Frontiers in Physiology 7:183 (2016)
Vasilopoulou PhD Thesis (2016)
Maga-Nteve et al. J. Chrom B 1041-1042:158-166 (2017)

Dilated Cardiomyomathy (DCM)

Heart

Collaboration with BRFAA Prof. Y. Kapetanaki
NSRF Collaboration I: TREAT-HART

Metabolomics in clinical prognosis: potential predisposition of ART offspring to metabolic disorders



Collaboration with 1st Pediatrics Dept, U. of Athens, "Agia Sophia" Children's Hospital
G. Chroussos
Ch. Kanana

Telonis, Master's Thesis (2014)
Gkourogianni et al. PloS ONE 9, e94001 (2014)
Gkourogianni, PhD Thesis (2015)
Telonis et al (2017) in prep

Metabolomics of *HeLa cell lines*

Collaboration with N. Moschonas (U. of Patras & ICE-HT)

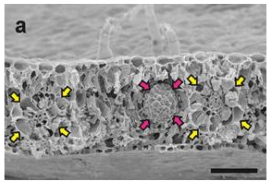
C. elegans metabolomics

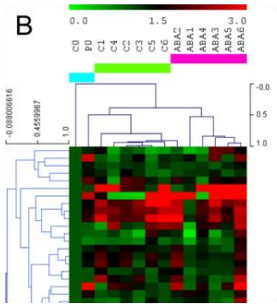
Collaboration with N. Tavernarakis (FORTH/IMBB)

Mosquito Metabolomics

Collaboration with J. Vontas (FORTH/IMBB)

Metabolomic Analysis in Plants



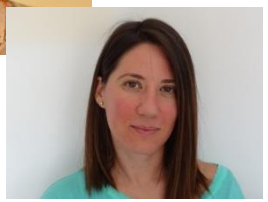


Collaboration with Agricultural U. of Athens (G. Karabourniotis) & MAI Chania (P. Kalaitzis) & U. of Thessaly (C. Kittas)
NSRF Collaboration I: PHYTOALATOTITA

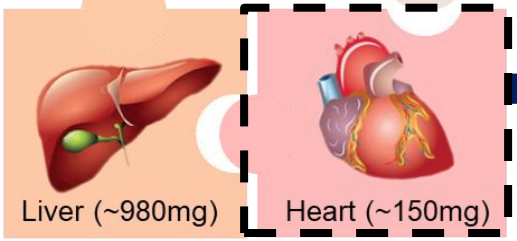
Tooulakou PhD Thesis (2014)
Tooulakou et al. Plant Physiol. 171: 2577 (2016)
Tooulakou et al. Plant Signal Behav. 11:e1215793 (2016)
Tooulakou et al. Annals of Botany(2017) submitted
Arhontakis et al., (2017) in prep

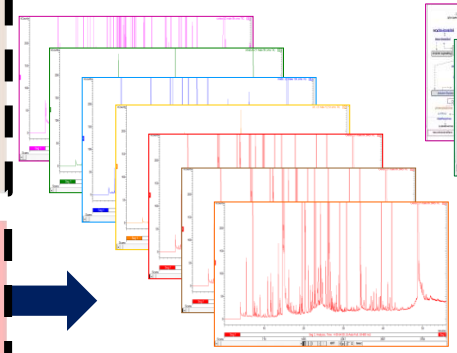
Multi-tissue metabolomic analysis in an AOH mouse model

AOH induction

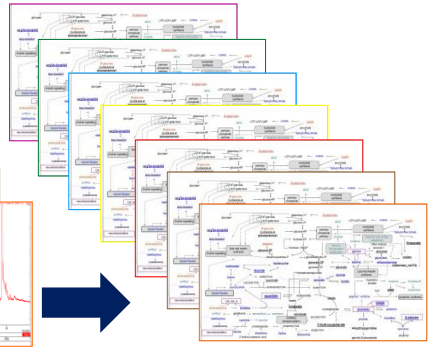


5 Brain Regions:
Cortex (~180mg)
Midbrain (~70mg)
Cerebellum (~50mg)
Hippocampus (~10mg)
Striatum (~10mg)

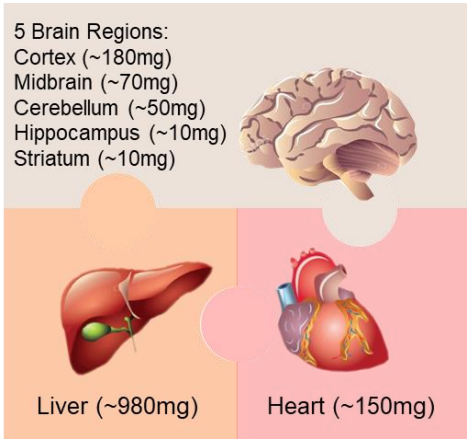




MS-Metabolic Profile



Metabolic Network Reconstruction & Analysis



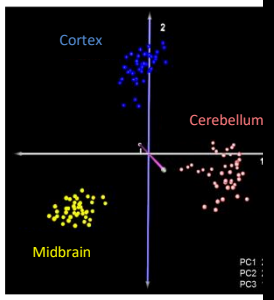
Multi-tissue Metabolic Physiology Characterization

Animal Group	$KClO_4$ Treatment duration: 45 days		$KClO_4$ Treatment duration: 60 days	
	Non-perfused tissues	Perfused tissues	Non-perfused tissues	Perfused tissues
Euthyroid Male (E_M)	6	6	6	7
Hypothyroid Male (H_M)	7	7	5	6
Euthyroid Female (E_F)	6	6	6	6
Hypothyroid Female (H_F)	5	4	4	4

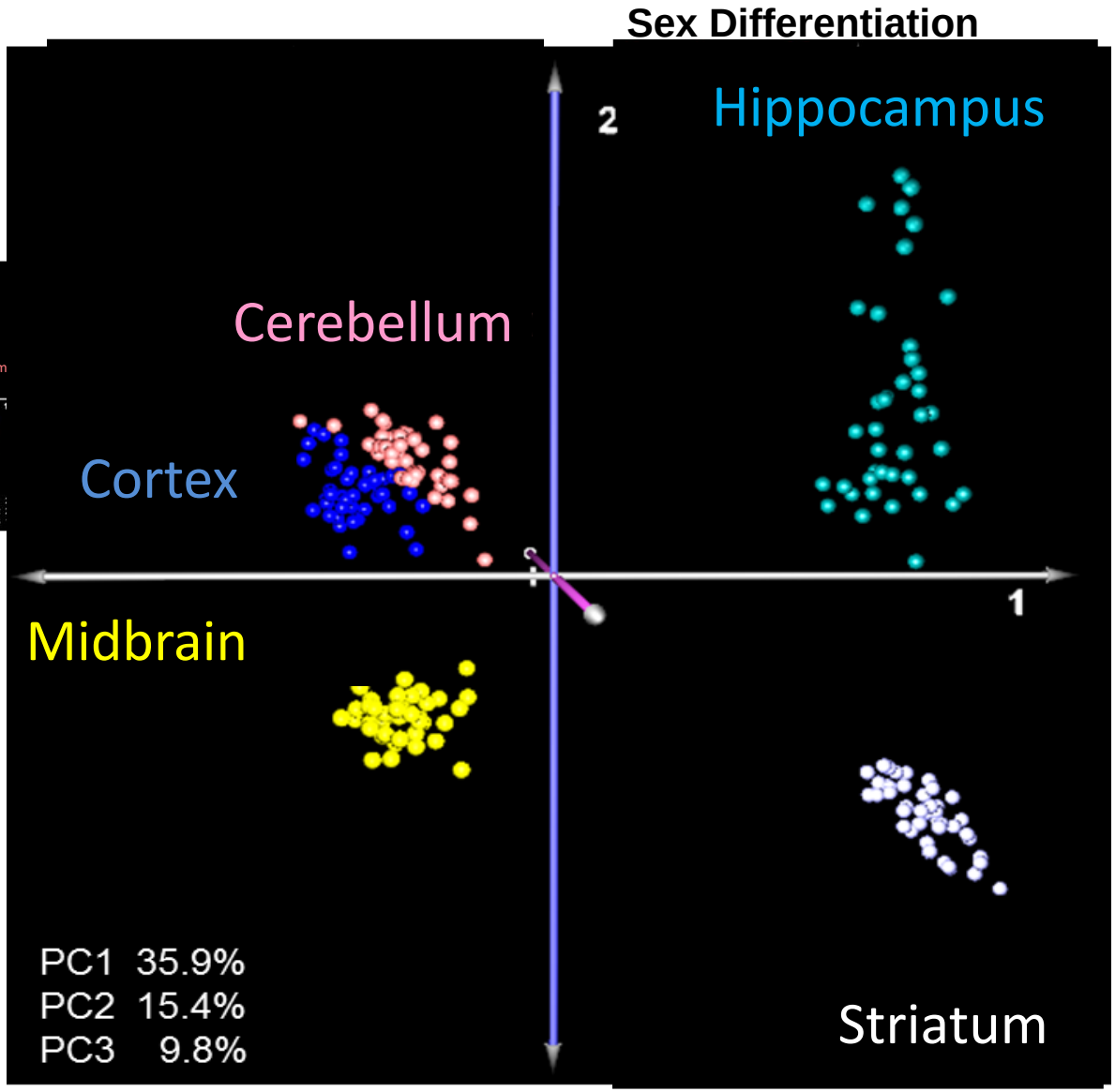
POSTER F20

Total: 91 animals

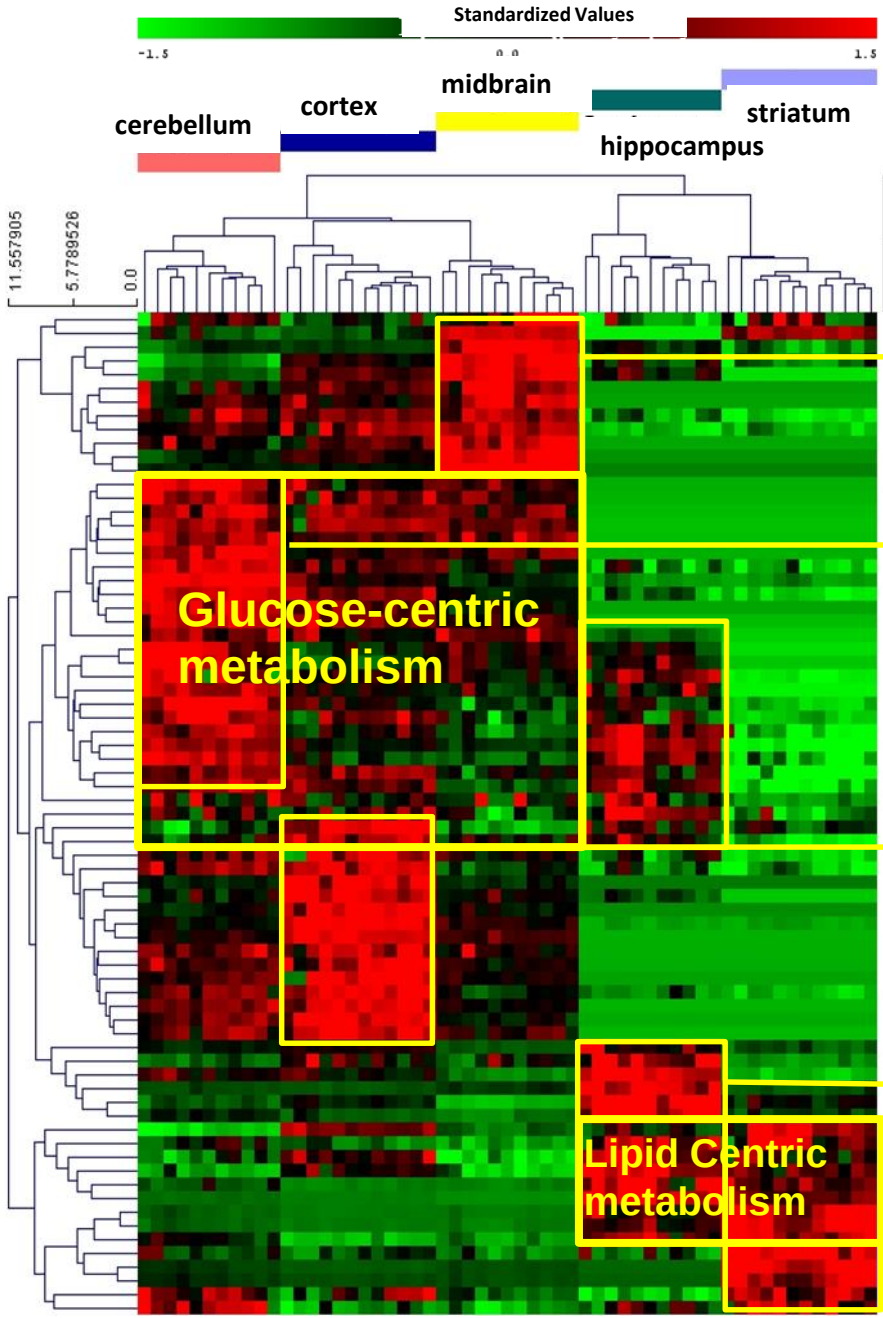
Metabolic Differentiation between brain regions (1)



PCA analysis of standardized profiles



Metabolic Differentiation between brain regions (2)



Hierarchical Clustering (Euclidean Distance Metric) of standardized metabolic profiles of euthyroid animals in both sexes in non-perfused tissues και

- Glycine, GABA, cholesterol

- Glycolysis and glycerol and inositol metabolism
- Glutamic acid and urea as nitrogen storage compounds
- Aromatic Amino acids

- Phosphoric acid and phosphates
- Oxalic and threonic acid→ antioxidant defense

- Arachidonic acid, N-acetyl-aspartate, ethanolamine

- Lipids
- Methyl-benzoate, N-acetyl-glutamate

ΕΚΘΕΜΑ 2Α

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M-IOLITE

An integrated software suite for GC-MS metabolomic analysis streamlining (*http://miolite2.iceht.forth.gr*)

1 A standardized repository for GC-MS metabolomic data based on a reviewed library of >900 metabolite peaks

2 Specialized GC-MS metabolomic data normalization & filtering methods

3 Unknown metabolite identification methods based on metabolic network reconstruction & analysis

* Tool to be supported by ELIXIR-GR

Maga-Nteve, PhD Thesis (2017)
Maga-Nteve & Klapa, IFAC-Papers Online, 49: 286-288 (2016)

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

ΕΚΘΕΜΑ 2Β

FORTH
INSTITUTE OF CHEMICAL ENGINEERING SCIENCES

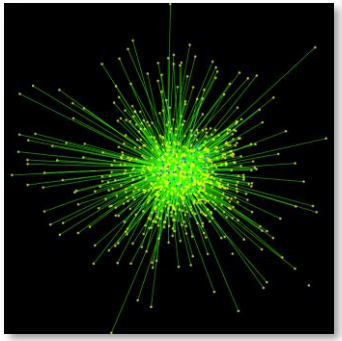
PICKLE

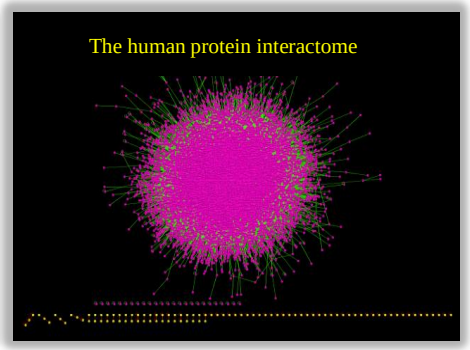
Protein **I**ntera**C**tion **K**now**L**edge bas**E**

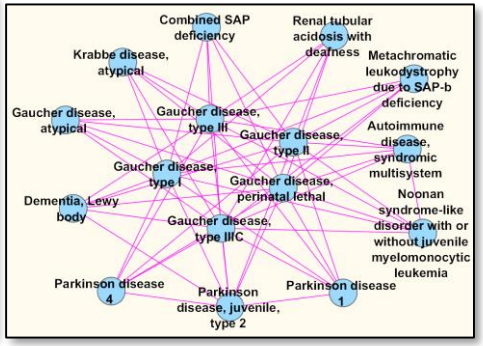
PICKLE: A human protein-protein interaction meta-database employing data integration via genetic information ontology (*www.pickle.gr*)

Aris Gioutlakis, Maria I. Klapa & Nicholas K. Moschonas

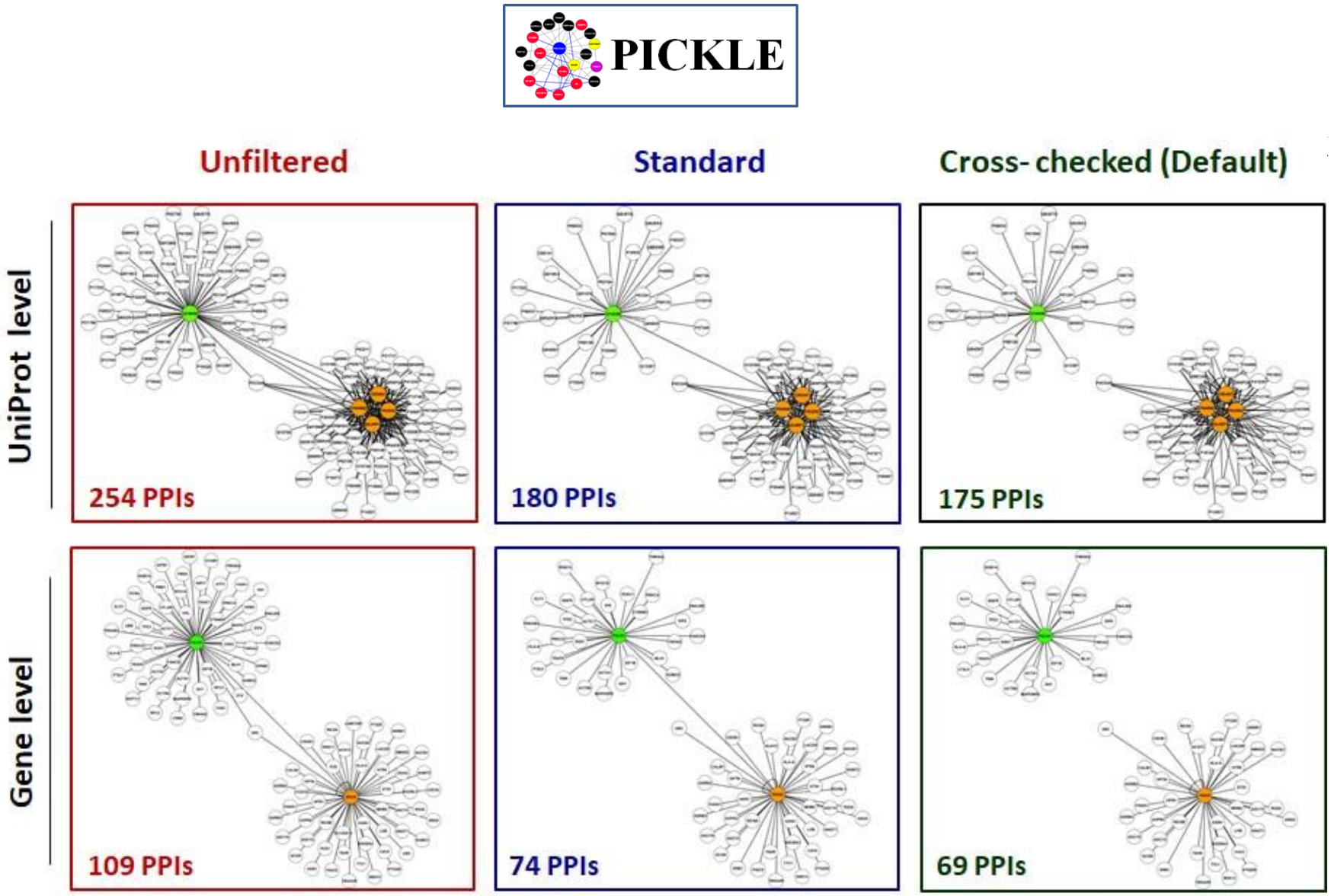
* Tool to be supported by ELIXIR-GR





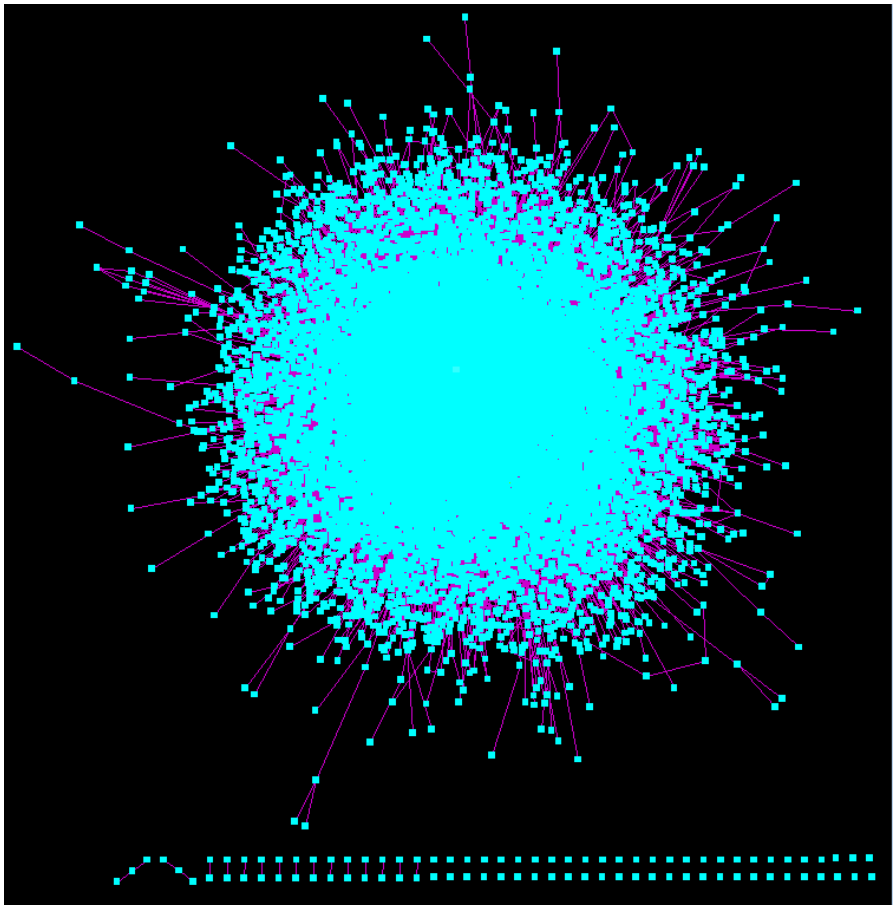


- Gioutlakis A., Klapa, M. and Moschonas N. (2017). PICKLE 2.0: A human protein-protein interaction meta-database employing data integration via genetic information ontology. *PLoS ONE* 12:e0186039 (published on October 12 2017)
- Klapa, M. I., Tsafou, K., Theodoridis, E., Tsakalidis A, and Moschonas NK (2013), Reconstruction of the experimentally supported human protein interactome: what can we learn? *BMC Syst. Biol.*, 7: 96.

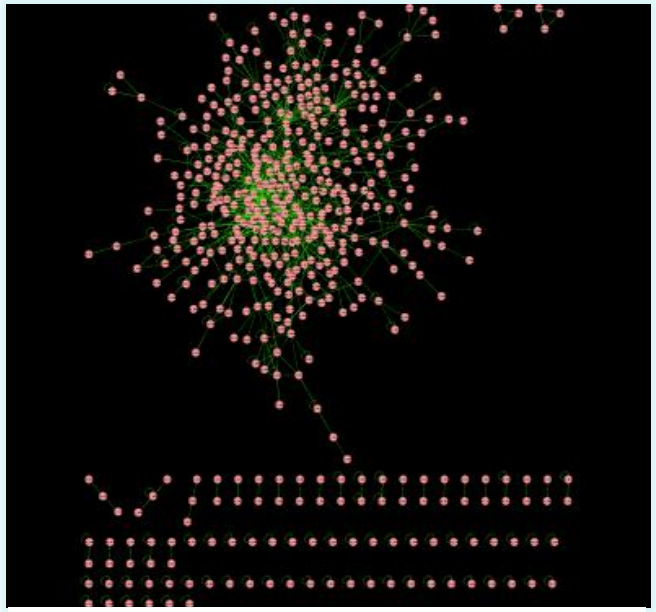


Gioutlakis A., Klapa, M. and Moschonas N. (2017). *PLoS ONE* 12:e0186039

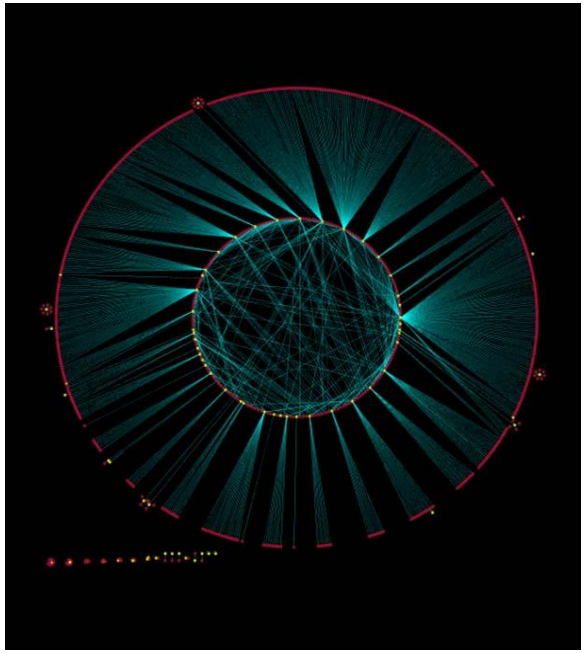
PICKLE



PICKLE 2.1
UniProt IDs: 14134
PPIs: 120882
PubMed IDs: 35752



Mitochondrial PPI network



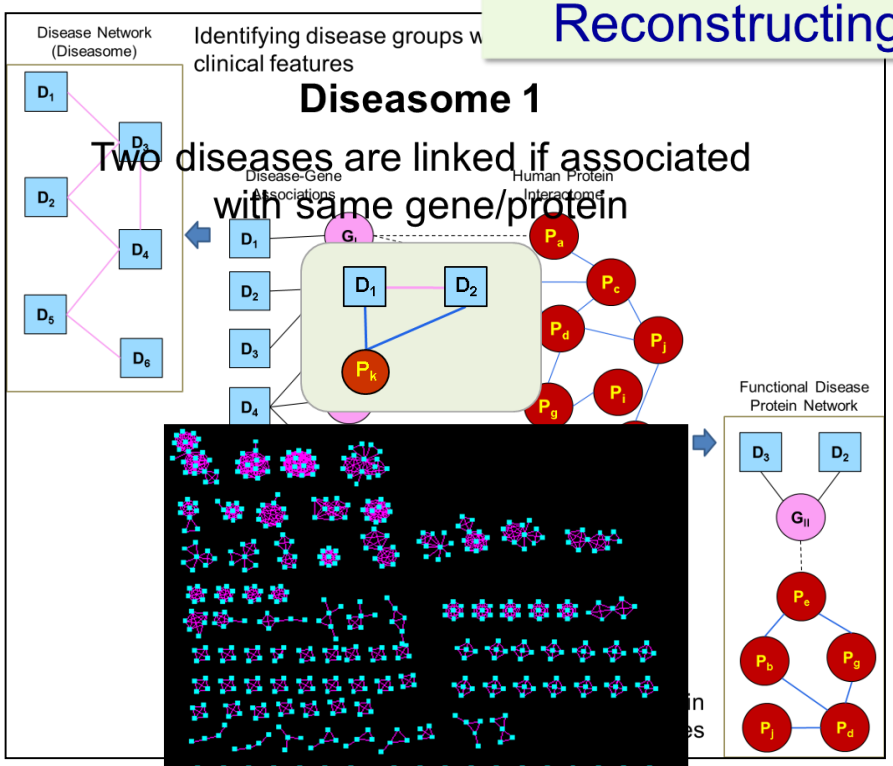
Type I Diabetes PPI Network

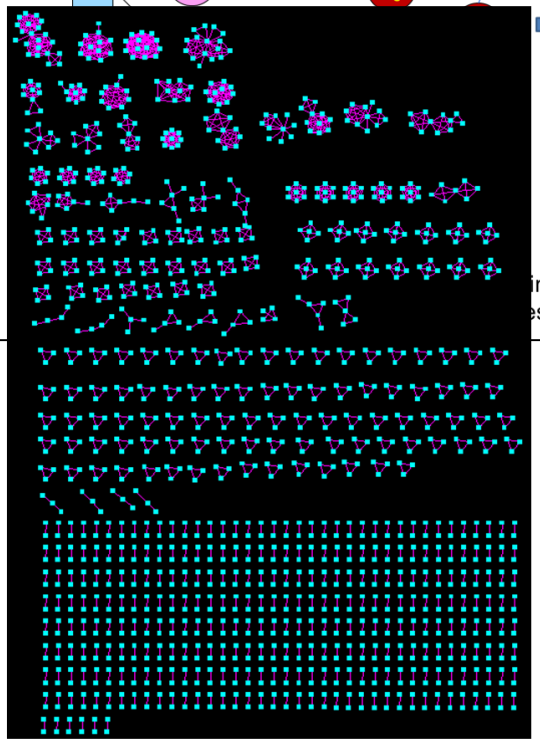
Investigating the genetic architecture of diseases

Reconstructing the diseasomes

Poster F19

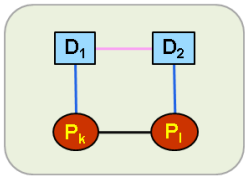
Evridiki – Pandora Tsare

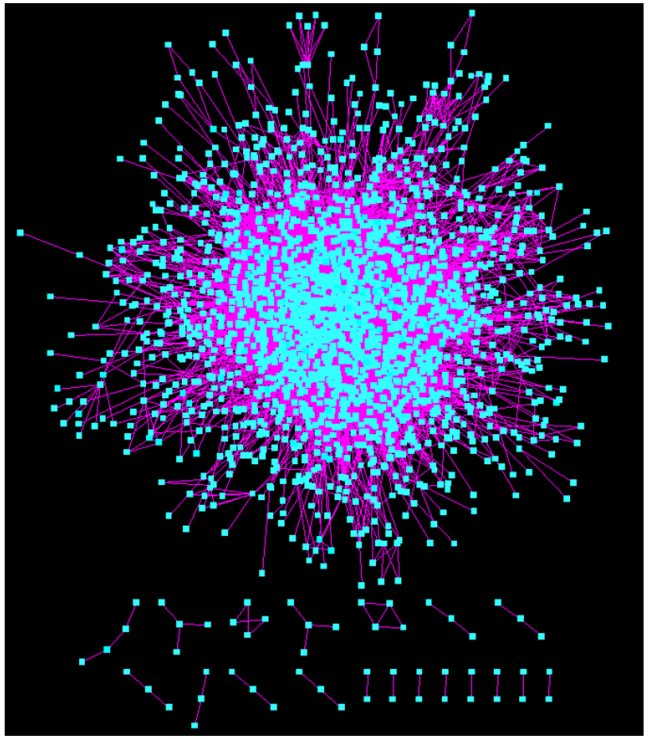




Diseasome 2

Two diseases are linked if their associated proteins interact





~98% of diseases are causally associated with proteins that are at most second neighbors in the human protein interactome

BODOSSAKI FOUNDATION

NSRF 2007-2013 programme for development

BAYER

StrepSynth

Ευχαριστώ!

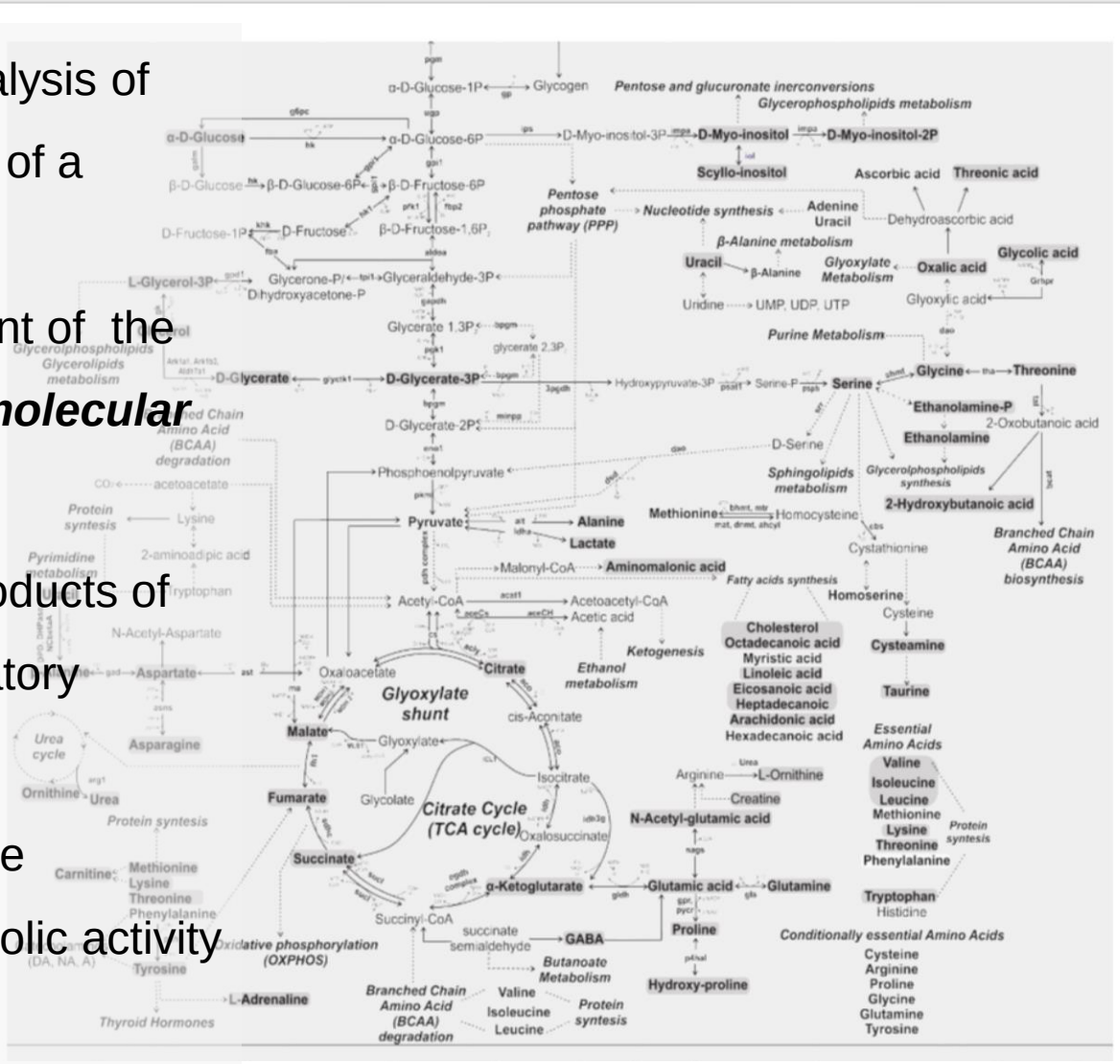
Thank you!

Untargeted metabolomics in Systems Biology…

…is the ***high-throughput*** analysis of the ***metabolic network state*** of a biological system through the simultaneous measurement of the ***concentrations*** of ***free low molecular weight metabolites***

Metabolites: Reactants & Products of Metabolic Reactions – Regulatory Molecules of Protein Activity

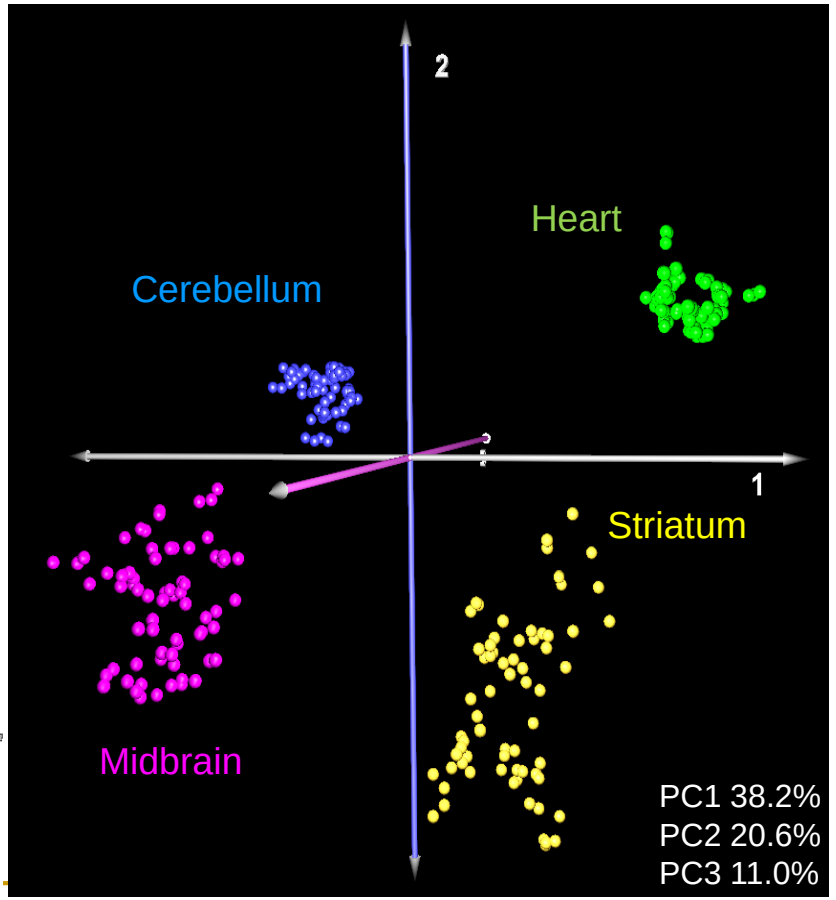
Metabolic Profile = metabolite concetration profile = a metabolic activity fingerprint



Reconstruction of the Mouse Heart Primary Metabolism Network
M.-K. Ioannidi, Master's Thesis , U. of Patras (2015)

MULTI-ORGAN ANALYSIS

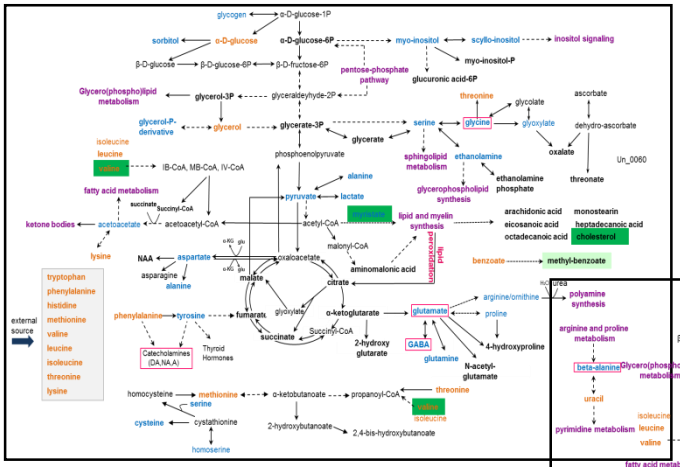
Cerebellum → 116 metabolite peaks
Midbrain → 110 metabolite peaks
Striatum → 54 metabolite peaks
Heart → 46 metabolite peaks



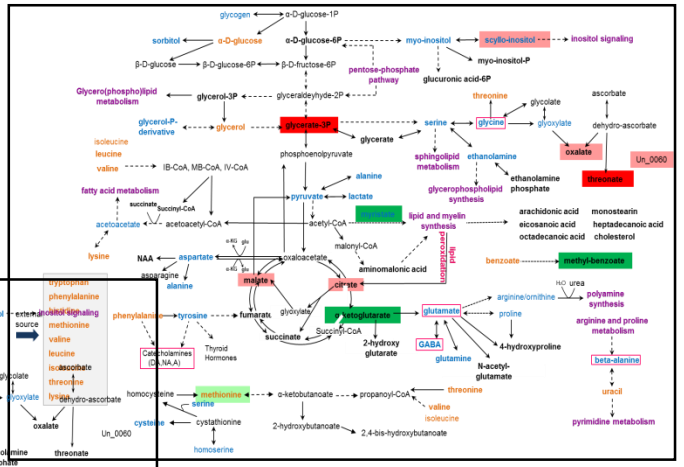
Multivariate Statistical Analysis is based on the 35 common metabolites detected in all tissues

AOH Effect on the metabolic physiology of male mice

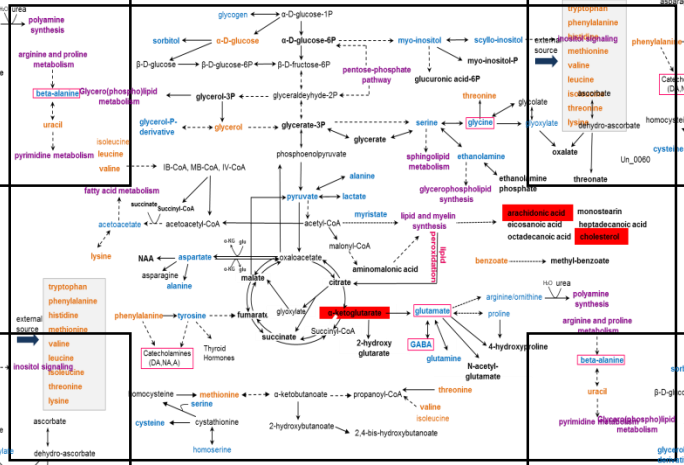
Cortex



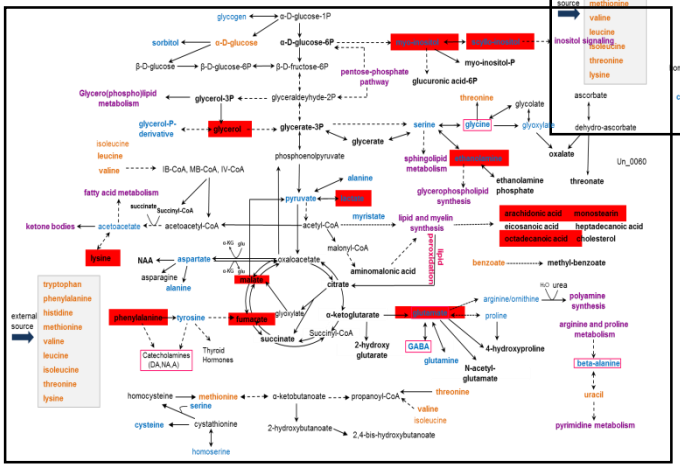
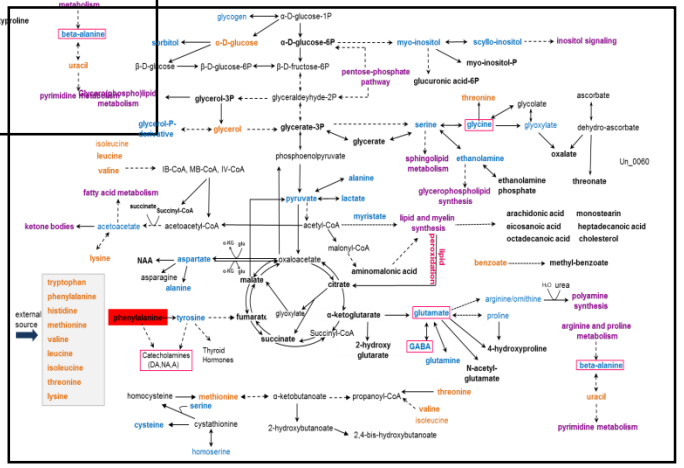
Cerebellum



Midbrain



Hippocampus



Striatum