About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

Clinical Metabolomics: Case study CKD. Cross-platform omics data integration in Ingenuity Systems.

Vladimir Tolstikov, Ph.D.

3rd International Conference and Exhibition on

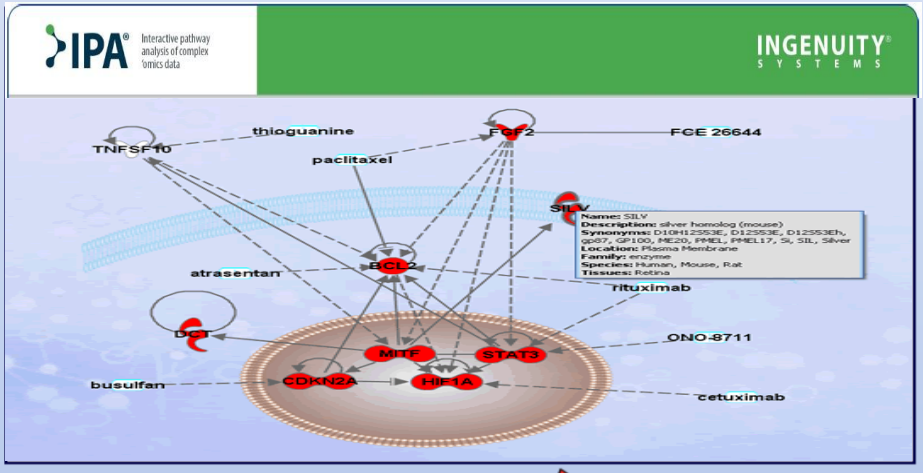
Metabolomics & Systems Biology

(March 24-26, 2014)

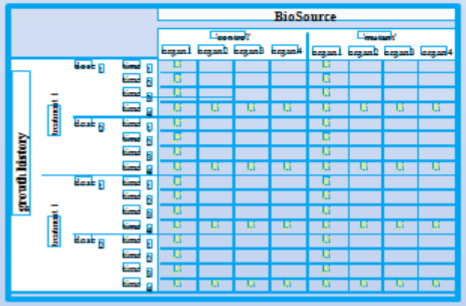
San Antonio, TX, USA

Lilly

Metabolomics workflow

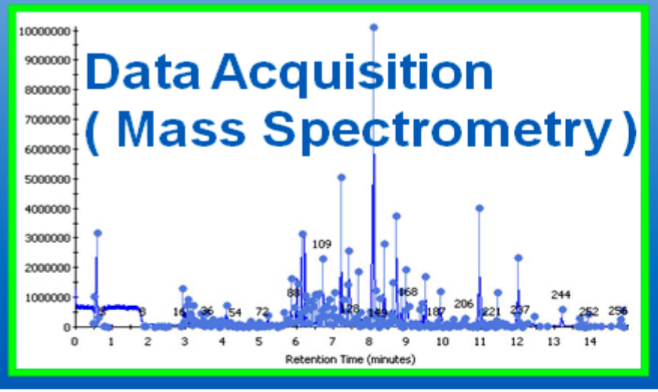


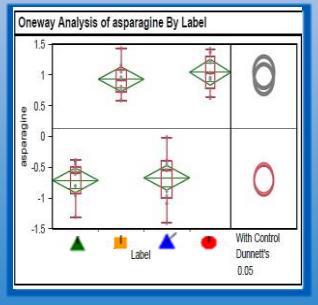
Pathway Analysis

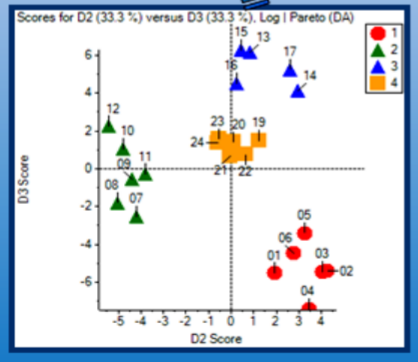


Experimental design

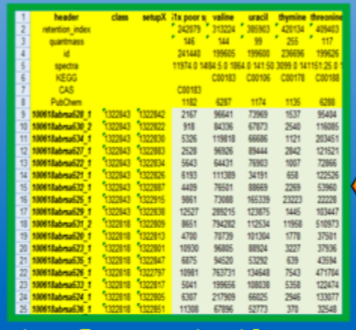
Sample Preparation
(wet chemistry)







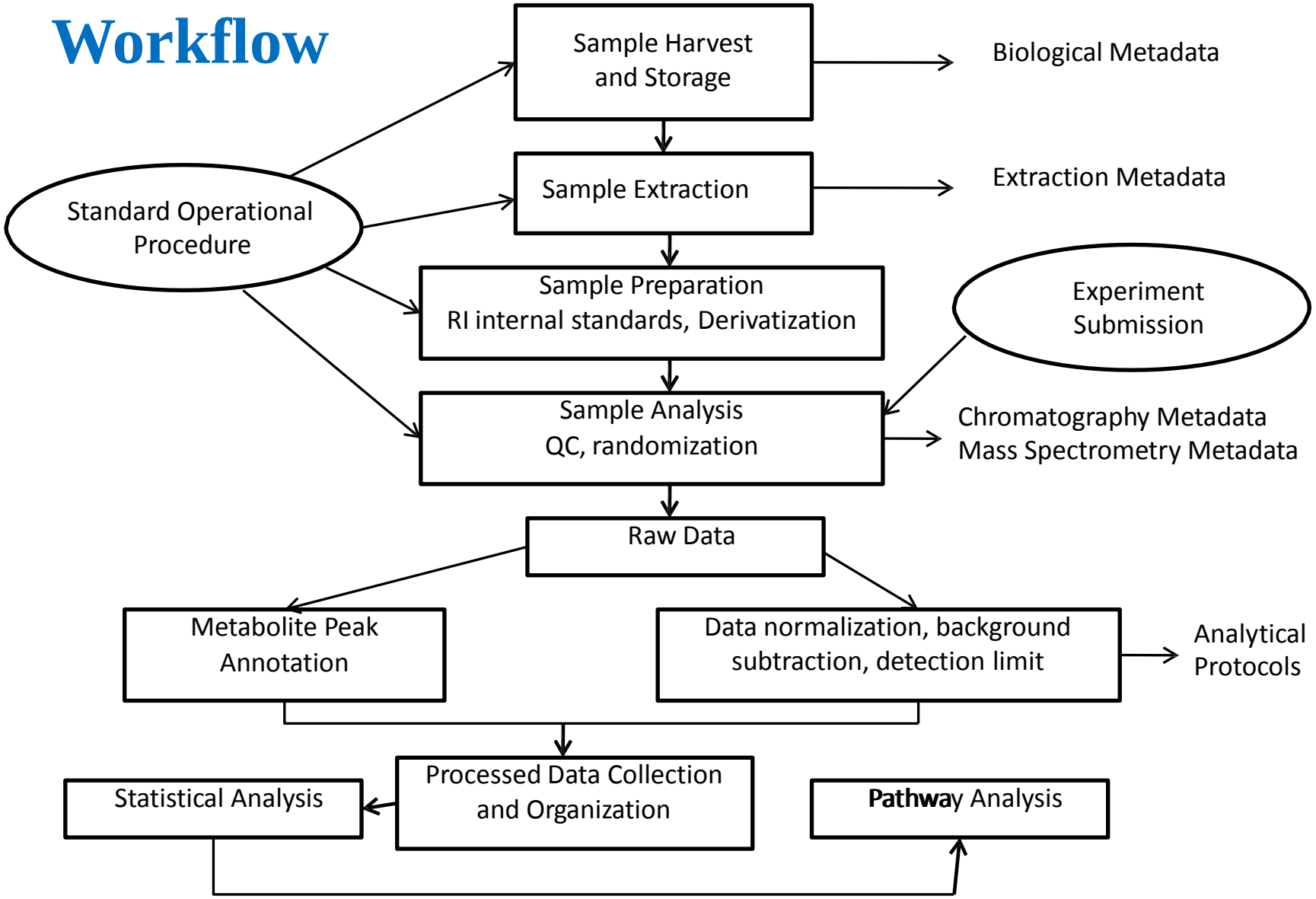
Data Analysis
(Univariate and Multivariate Statistics)



Data Annotation

Data pre-processing

Workflow


Sample Harvest and Storage
Biological Metadata
Standard Operational Procedure
Sample Extraction
Extraction Metadata
Sample Preparation
RI internal standards, Derivatization
Experiment Submission
Sample Analysis
QC, randomization
Chromatography Metadata
Mass Spectrometry Metadata
Raw Data
Metabolite Peak Annotation
Data normalization, background subtraction, detection limit
Analytical Protocols
Processed Data Collection and Organization
Statistical Analysis
Pathway Analysis

Lilly Metabolomics Platform

Ultimate combination of targeted and non-targeted approaches.

GC/MS

- Volatiles
- Essential oils
- Esters
- Perfumes
- Terpenes
- Carotenoids
- Flavanoids
- Perfumes

- Alchohols
- Amino acids
- Catecholamines
- Fatty acids
- Phenolics
- Prostanglandins
- Steroids
- Sugar phosphates

- Organic acids
- Organic amines
- Nucleosides
- Nucleotides
- Oligosaccharides

LC/MS

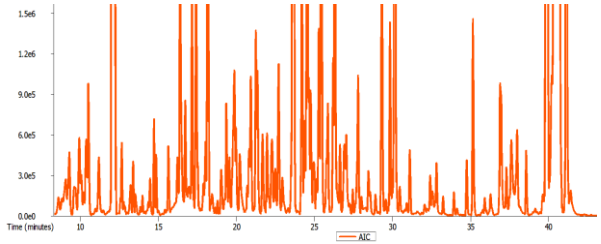
- Peptides
- Co-factors
- Polar Lipids

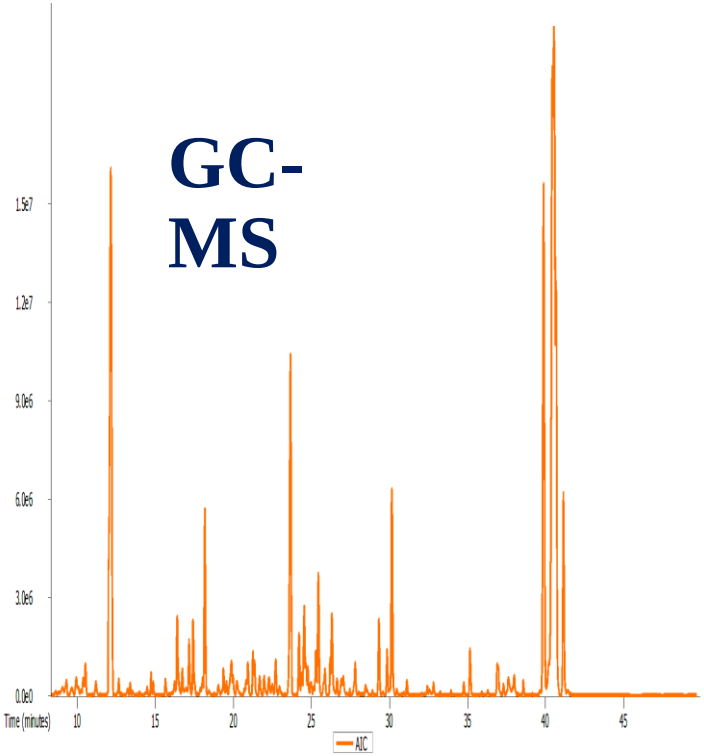
PEGASUS GC-HRT accurate mass TOF
Gerstel ALEX/CIS MultiPurpose Autosampler

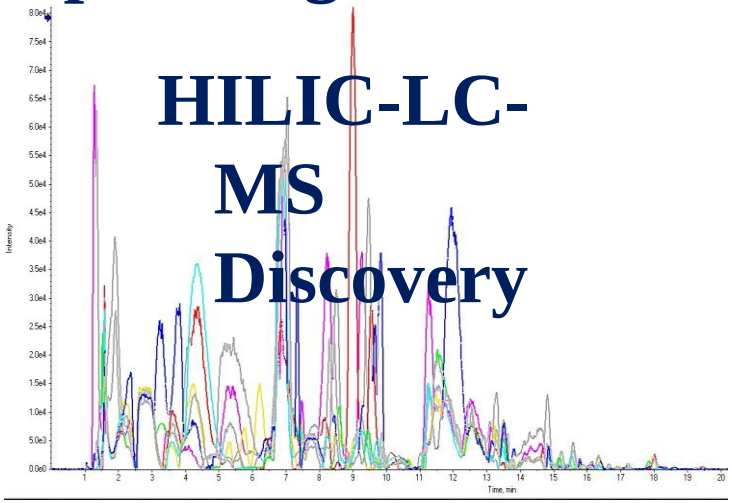
Triple quad 5500

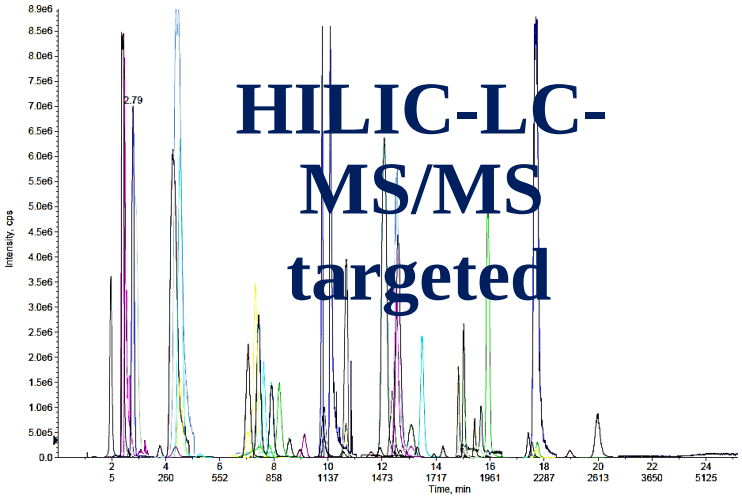
Triple TOF 5600 accurate mass

Human urine profiling





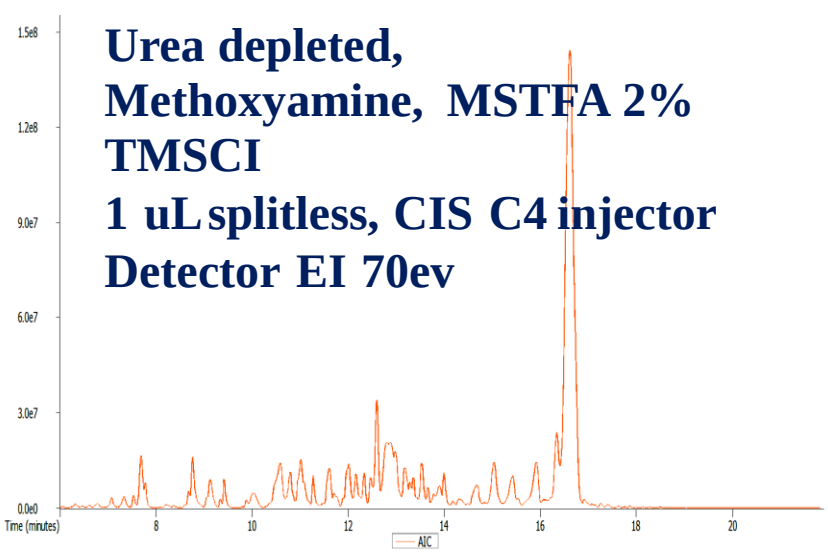


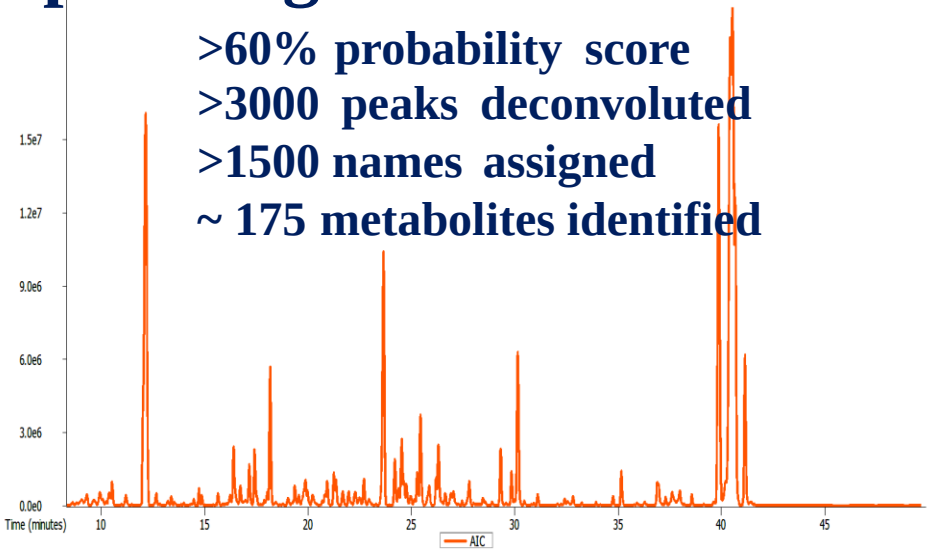


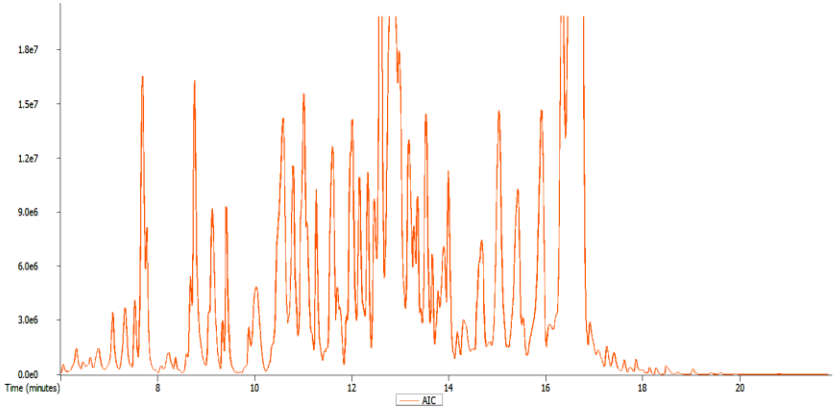
Human urine GC/MS profiling

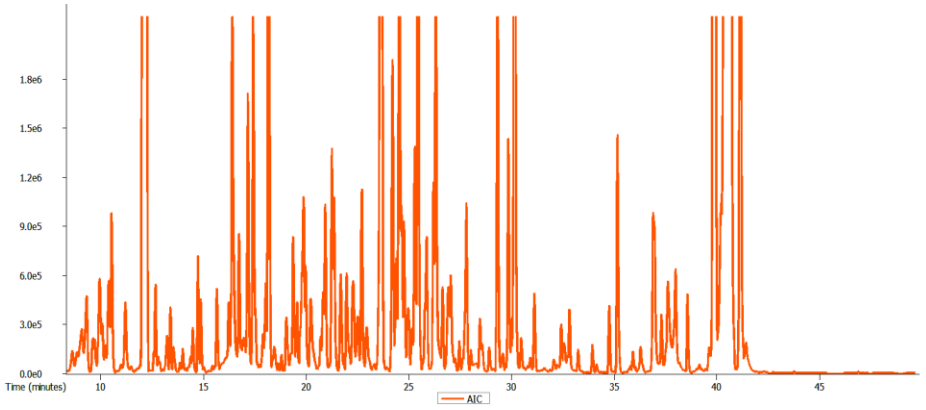
Urea depleted,
Methoxyamine, MSTFA 2% TMSCI
1 uL splitless, CIS C4 injector
Detector EI 70ev

>60% probability score
>3000 peaks deconvoluted
>1500 names assigned
~ 175 metabolites identified





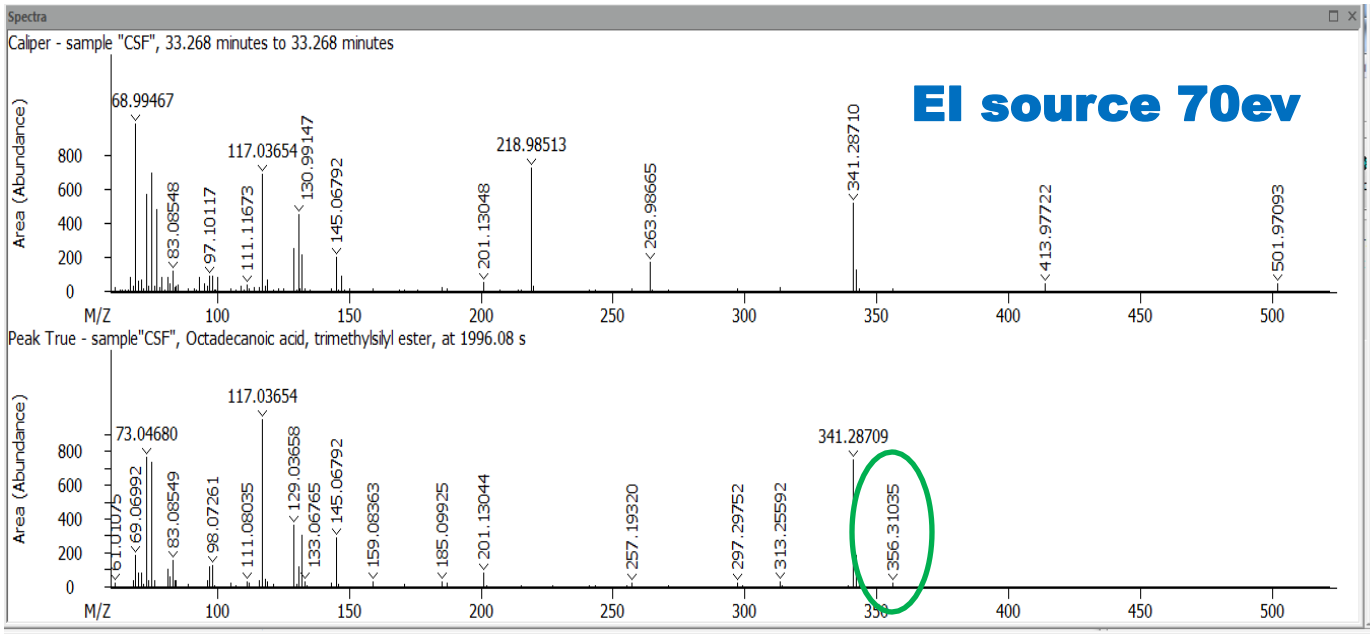


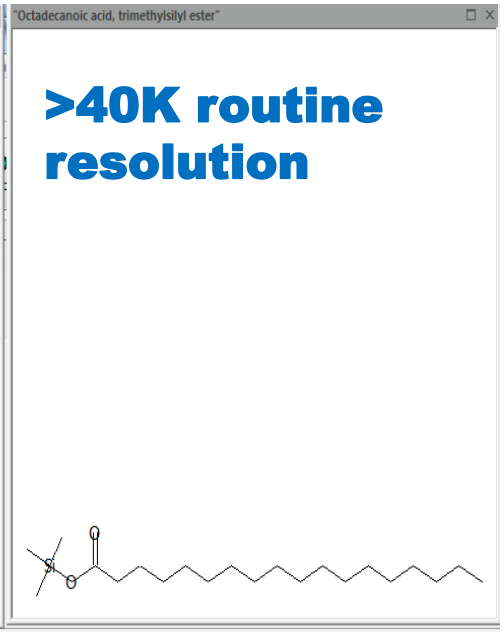


Throughput

Quality

High Resolution, High Mass Accuracy: YES or NO ID





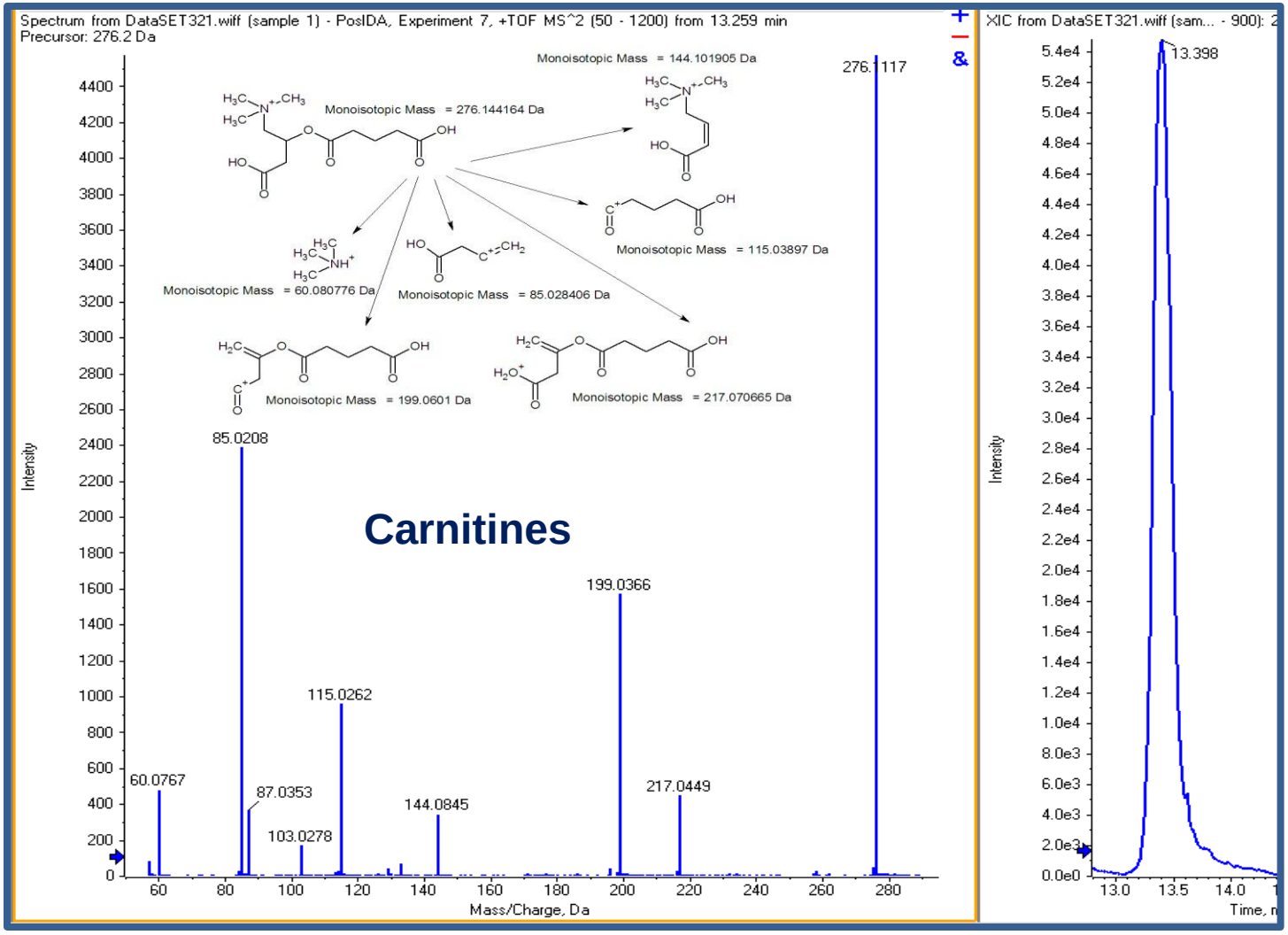
Hit Table - CSF

Hit	Name	Similarity	Mass Accuracy	Mass Differe	Probability	Reverse	Id	Formula	Expected Neutral Mass	Library
1*	Octadecanoic acid, trimethylsilyl ester	867	-0.4336	0	9620	867	86865	C21H44O2Si	356.31105718	mainlib
2	stearic acid RI 771390	727	-208.6164	0	9532	816	281	C18H36O2	284.27153039	FiehnGC
3	Octadecanoic acid, dimethyl(isopropyl)silyl ester	674	-67729.5974	0	138	742	204336	C23H48O2Si	384.34235731	mainlib
4	C18:0 TMS 877.76 s	669	-0.4336	0	9670	809	327	C21H44O2Si	356.31105718	mpimp
5	Octadecanoic acid, tert-butyldimethylsilyl ester	665	-100530.2277	0	100	675	204340	C24H50O2Si	398.35800738	mainlib
6	C18:0 TMS 877.75 s	656	-0.4336	0	9670	771	325	C21H44O2Si	356.31105718	mpimp
7	Heptadecanoic acid, trimethylsilyl ester	616	-14.1468	0	20	755	38392	C20H42O2Si	342.29540712	mainlib
8	Octadecanoic acid, benzyldimethylsilyl ester	608	-171233.3031	0	15	632	204339	C27H48O2Si	432.34235731	mainlib
9	Tetradecanoic acid, trimethylsilyl ester	597	-13.9060	0	10	752	38394	C17H36O2Si	300.24845693	mainlib
10	Tetracosanoic acid, trimethylsilyl ester	592	-186405.7547	0	8	634	38393	C27H56O2Si	440.40495757	mainlib

Peak Table - CSF

Peak #	Name	R.T. (minutes)	Height	Area	Quant Masses	Base Mass	Formula
2245	Peak 2245	33.246	233	4372	BPI(100.030695±3ppm)	100.03069473	C16H9ClN4O2
2246	Peak 2246	33.253	453	6381	BPI(247.242429±3ppm)	247.24242924	
2247	Peak 2247	33.260	515	7870	BPI(202.077798±3ppm)	202.07779794	C9F8
2248*	Octadecanoic acid, trimethylsilyl ester	33.268	292783	2630348	BPI(117.036536±3ppm)	117.03653579	C21H44O2Si
2249	(2,2-Dimethylcyclobutyl)methylamine	33.274	26164	472874	BPI(70.077754±3ppm)	70.07775352	C7H15N
2250	Peak 2250	33.282	3169	53224	BPI(99.116897±3ppm)	99.11689710	C4H5NS

LC-HRMS - Online Identification



Case study:

Chronic Kidney Disease

- Chronic kidney disease (CKD) is a progressive loss in renal function over a period of months or years. The symptoms of worsening kidney function are non-specific. Often, chronic kidney disease is diagnosed as a result of screening of people known to be at risk of kidney problems, such as those with high blood pressure or diabetes and those with a blood relative with chronic kidney disease. It is differentiated from acute kidney disease in that the reduction in kidney function must be present for over 3 months.
- The two main causes of chronic kidney disease are diabetes and high blood pressure, which are responsible for up to two-thirds of the cases
- Chronic kidney disease is identified by a blood test for creatinine. Higher levels of creatinine indicate a lower glomerular filtration rate and as a result a decreased capability of the kidneys to excrete waste products. Creatinine levels may be normal in the early stages of CKD, Recent professional guidelines classify the severity of chronic kidney disease in five stages, with stage 1 being the mildest and usually causing few symptoms and stage 5 being a severe illness with poor life expectancy if untreated. Stage 5 CKD is often called end stage renal disease (ESRD).
- There is no specific treatment unequivocally shown to slow the worsening of chronic kidney disease.

Chronic Kidney Disease

In the present exploratory cross-sectional studies, donor matched urine and serum clinical samples were obtained, extracted and analyzed. The first study was powered with 39 healthy, type II diabetic CKD (stages 3-5), and non-diabetic CKD (stages 3-5) patients. The second study was powered with 71 healthy, diabetic, diabetic CKD, and non-diabetic CKD patients.

We applied non-targeted and targeted Metabolomics Mass Spectrometry based approaches. Our in-house Lilly Metabolomics platform allowed routine detection of > 5000 features.

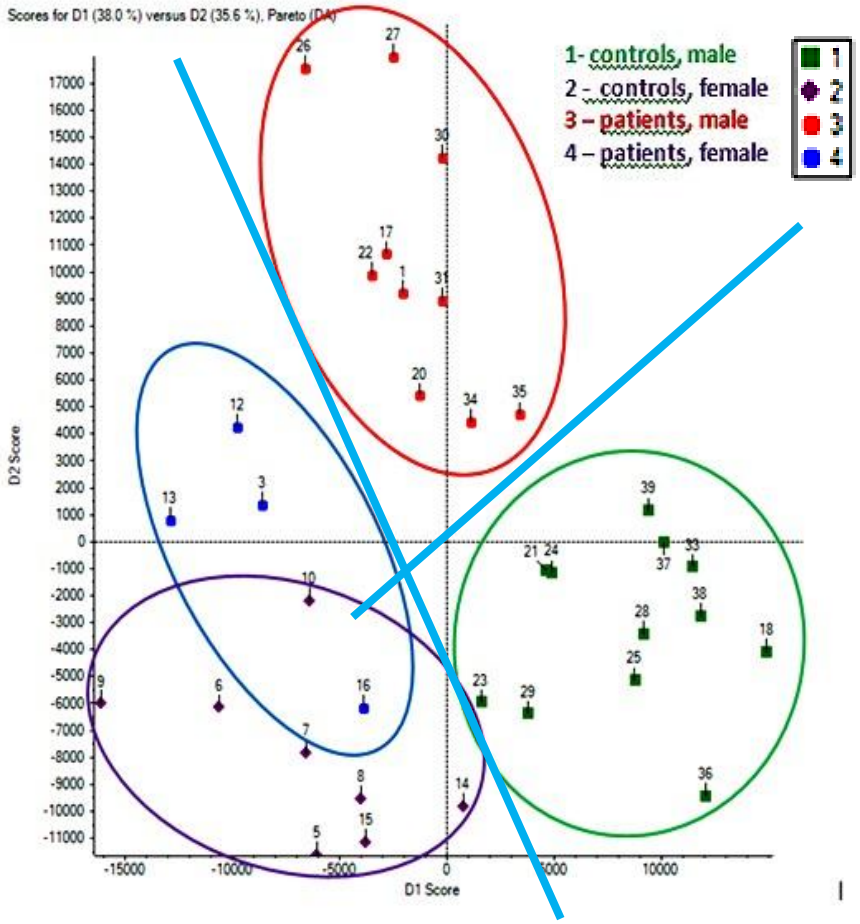
The dataset yielded several statistically significant biochemical alterations represented with >290 polar metabolites, excluding peptides, intact lipids and metabolites which levels were not changed.

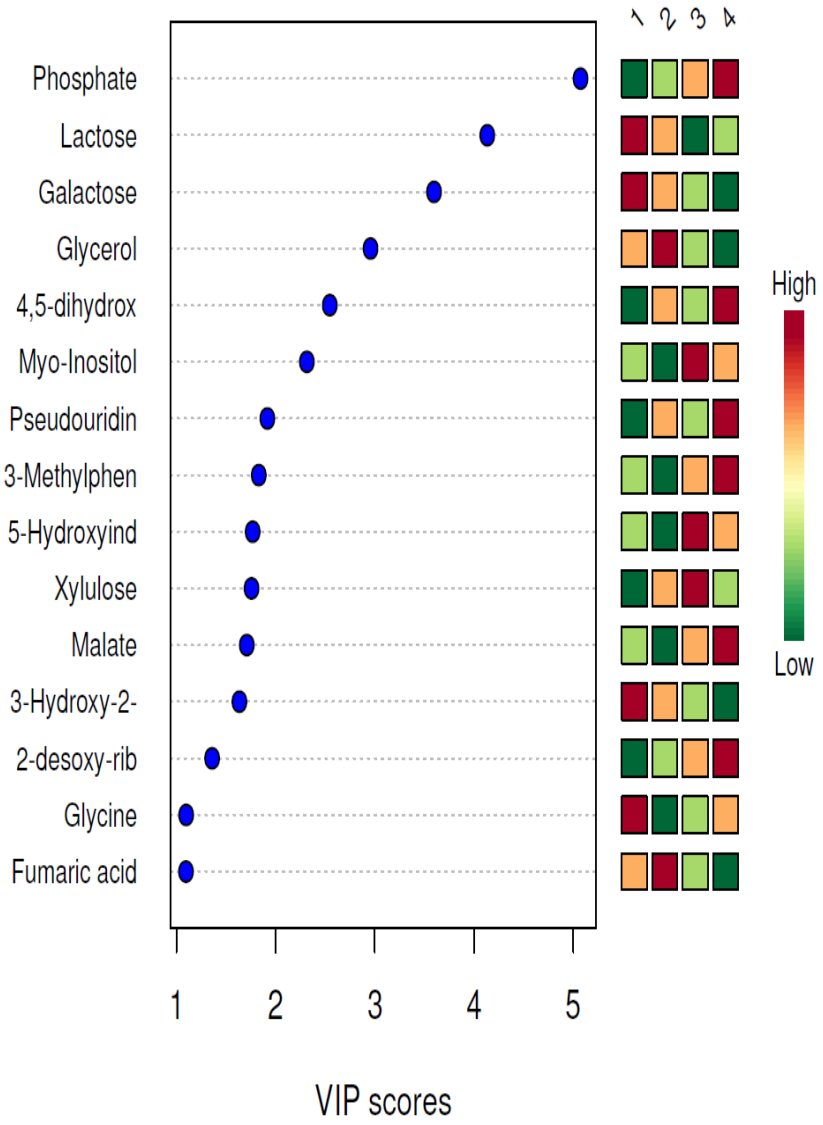
We were able to glean a variety of subtle yet distinct metabolic signatures and perform Metabolic Pathway analysis. Pathway analysis allowed pinpointing the most disturbed metabolic pathways in CKD patients and offered new hypotheses.

Multivariate Statistics

PCA DA

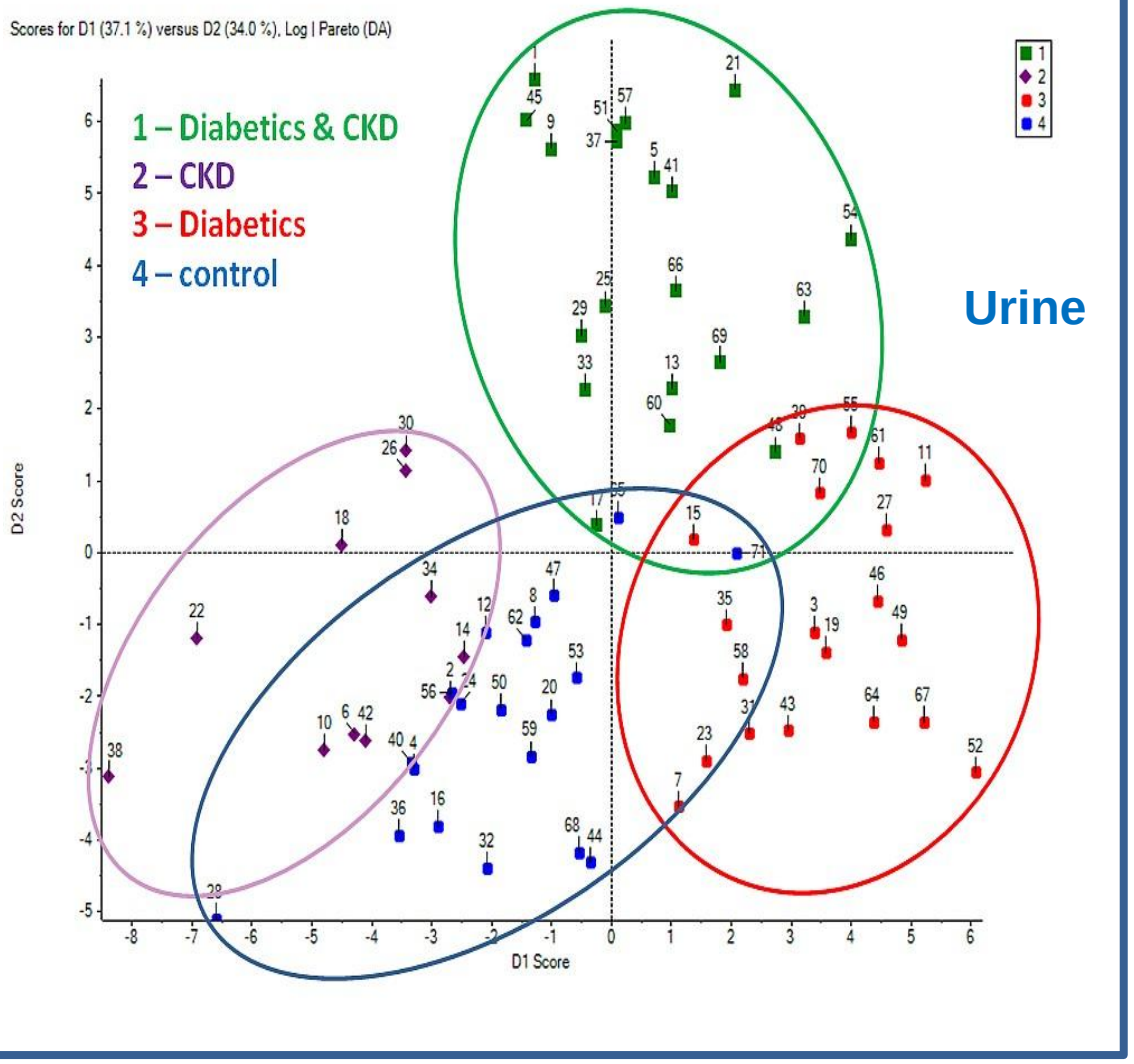
Male vs female; CKD vs control

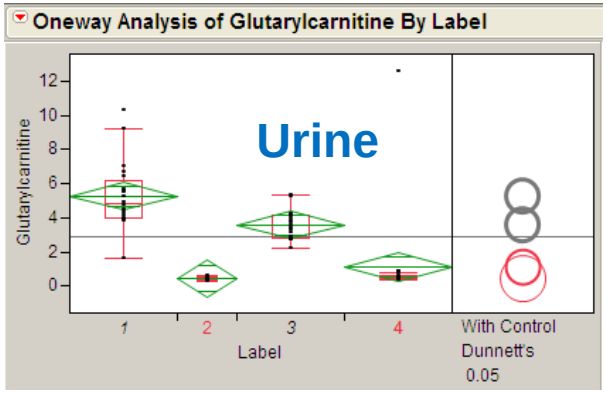


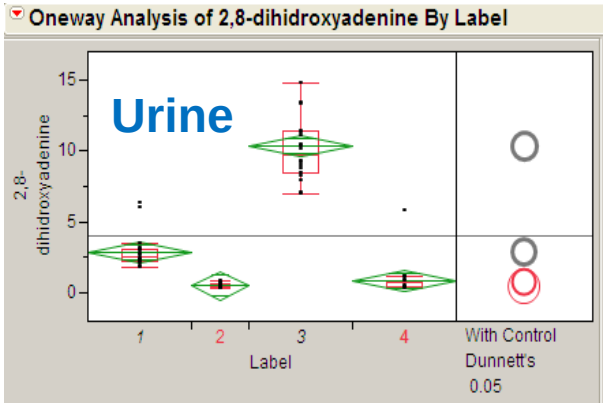


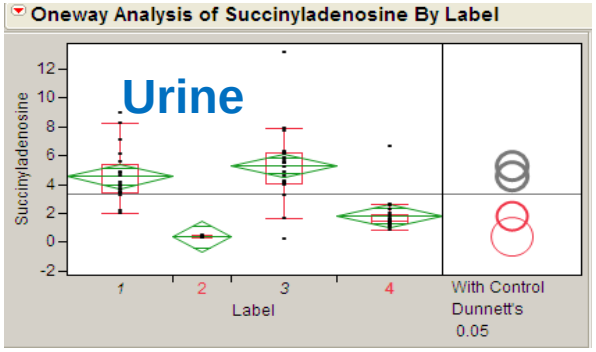
Urines

Diabetics versus non-diabetics



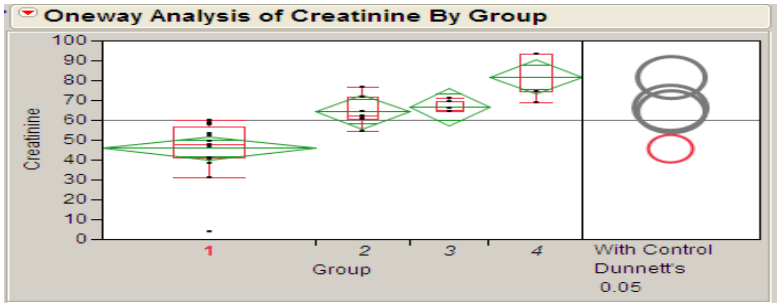


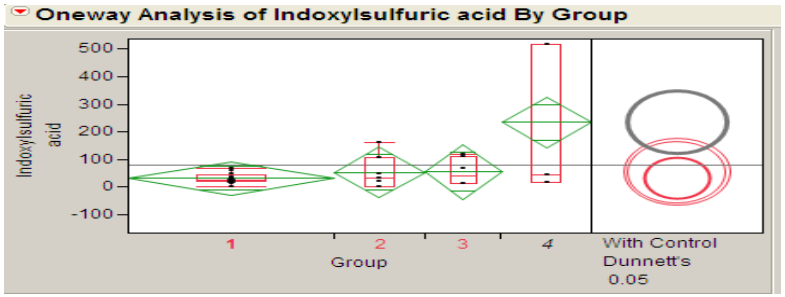


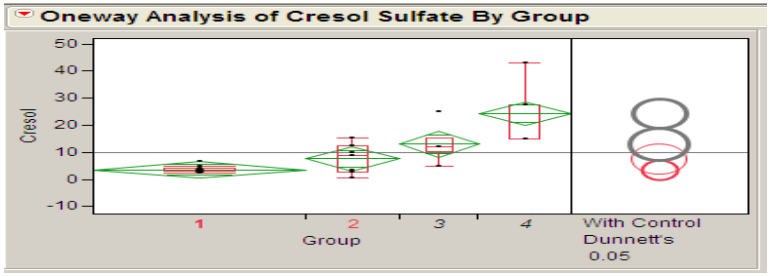


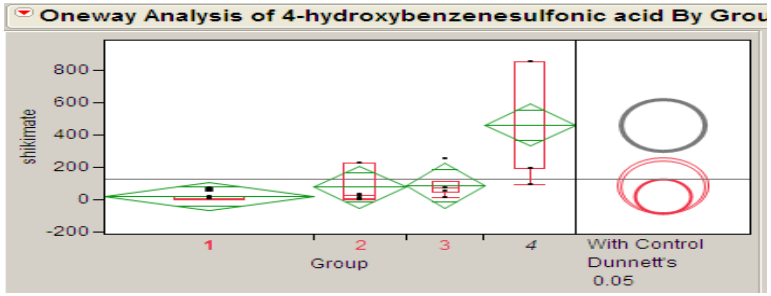
1 – Diabetics & CKD 2 – CKD
3 – Diabetics 4 – control

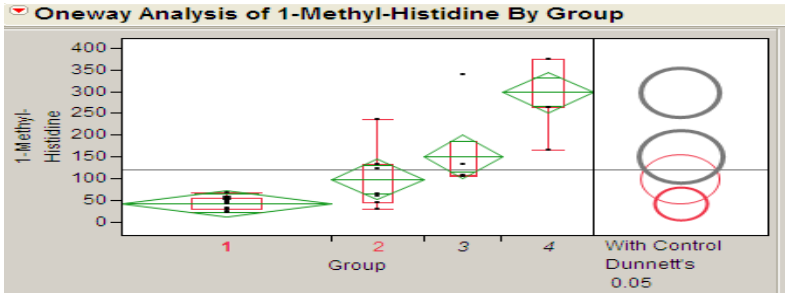
Uremic toxins accumulation in blood plasma

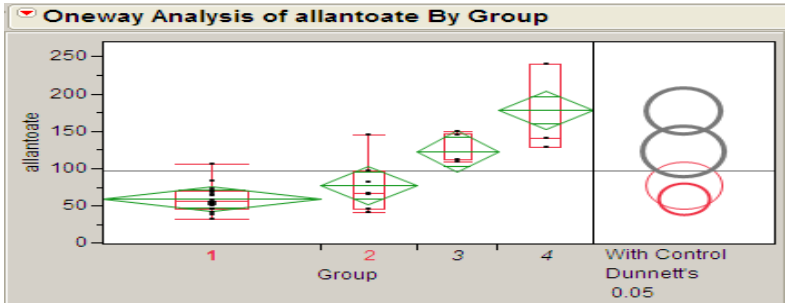












CKD stages: controls, III, IV, ESRD

Accumulation of known uremic toxins in plasma, in particular indoxylsulfate, cresol sulfate, 4-hydroxybenzenesulfonic acid, and others were observed. Uremic toxins are produced by liver and/or gastrointestinal flora metabolism and eliminated from plasma via active kidney tubular secretion.

Omics data integration characterizing CKD

Experimental Data:

Genes – 1500 (gene expression) kidney tissue, cDNA Bank

proteins - 22 (ELISA) serum/urine, in house

metabolites – 290 (GC/LC/MS) serum/urine, in house (from the same samples)

Groups:

CKD stages: controls, III, IV, ESRD

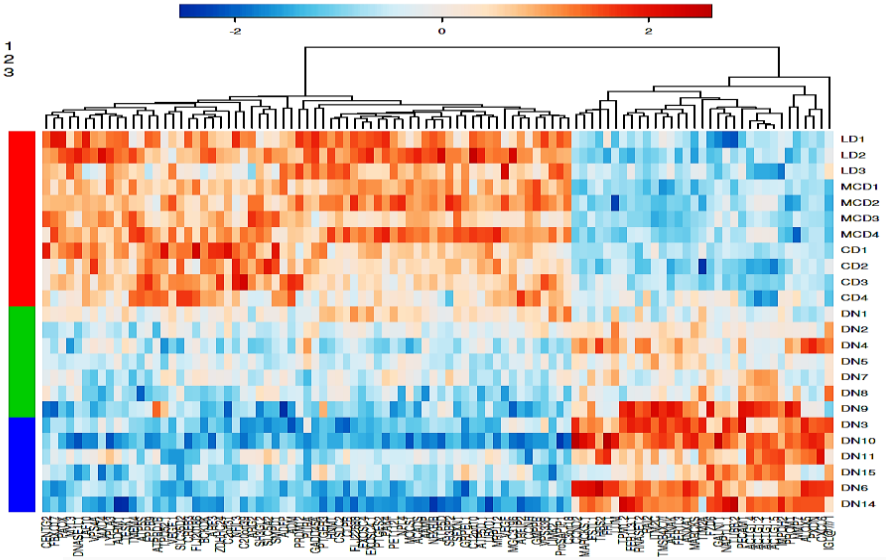
Experimental data form literature

TABLE 1
Clinical and histological characteristics of reference biopsies analyzed by oligonucleotide array–based gene expression profiling and real-time RT-PCR

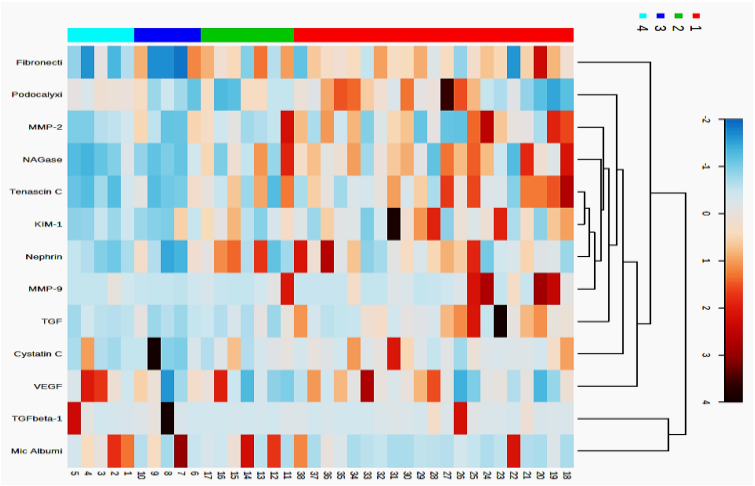
Sample name	Sex	Age (years)	Histology major diagnosis	Histology score	Creatinine (mg/dl)	Urine proteinuria (g/day)
Living donor						
Array						
LD1	F	66	LDx	NA	<1.1	<0.2
LD2	M	26	LDx w/o prev. damage	NA	0.9	<0.2
LD3	M	49	LDx w/o prev. damage	NA	<1.1	<0.2
Mean ± SEM		47 ± 9.6				<0.2
RT-PCR						
LD4	F	35	LDx	NA	<1.1	<0.2
LD5	M	39	LDx	NA	<1.1	<0.2
LD6	F	55	LDx	NA	<1.1	<0.2
LD7	M	41	LDx	NA	<1.1	<0.2
LD8	M	61	LDx	NA	<1.1	<0.2
LD9	F	58	LDx	NA	<1.1	<0.2
LD10	M	27	LDx	NA	<1.1	<0.2
LD11	F	54	LDx	NA	<1.1	<0.2
LD12	F	61	LDx	NA	<1.1	<0.2
Mean ± SEM		48 ± 14			<1.1	<0.2
Cadaveric donor						
Array						
CD1	M	50	CDx, minor int¦ fibrosis	NA	0.9	<0.2
CD2	M	54	CDx w/o prev. damage	NA	0.9	<0.2
CD3	M	61	CDx w/o prev. damage	NA	1.2	<0.2
CD4	F	51	CDx, minor int. fibrosis	NA	0.7	<0.2
Mean ± SEM		54 ± 2.1			0.9 ± 0.1	<0.2
RT-PCR						
CD5	NA	NA	CDx	NA	<1.1	<0.2
MCD/ no histological changes						
Array						
MCD1	M	32	Minimal-change GN	1	1.3	11.0
MCD2	F	32	Minimal-change GN	1	0.7	3.0
MCD3	M	16	Minimal-change GN	0	1.2	5.4
MCD4	M	20	Minimal-change GN in remission	0	0.9	0.2
Mean ± SEM		25 ± 3.6			1.0 ± 0.2	4.9 ± 2.3
RT-PCR						
MCD5	F	57	Minimal-change GN	0	1.1	10
MCD6	M	33	Minimal-change GN	1	1.4	9.1
MCD7	M	24	No histological changes	0	0.6	0.4
Mean ± SEM		38 ± 8			1.0 ± 0.2	6.5 ± 2.5

European Renal cDNA Bank (ERCB) Consortium

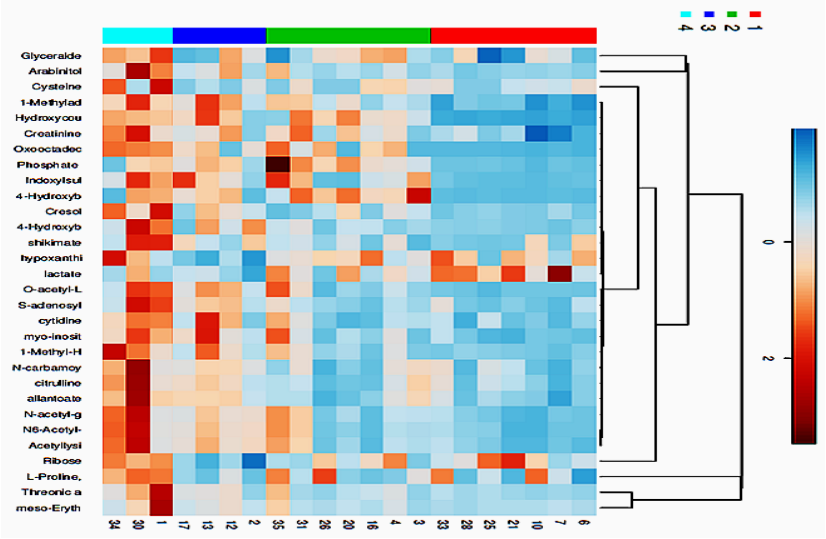
Kidney biopsies CKD levels: Control,II-IV,ESRD

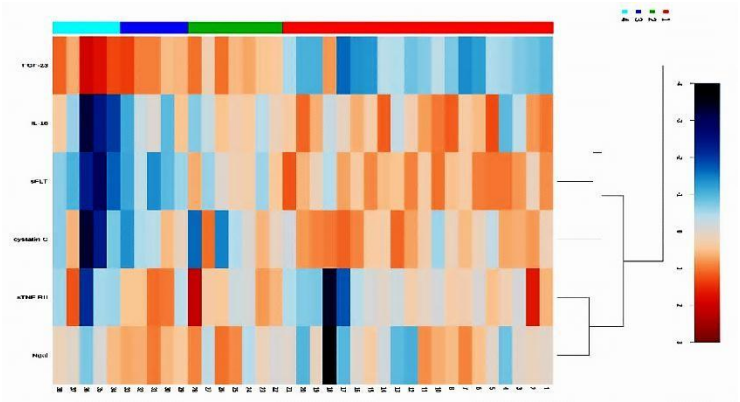


Urine



Plasma





Proteomics data: controls, III, IV, ESRD

Plasma Metabolomics data: controls, III, IV, ESRD

Red cells show increases vs. control, blue cells show decreases vs. control

Lilly

Plasma CKD ToxAnalysis

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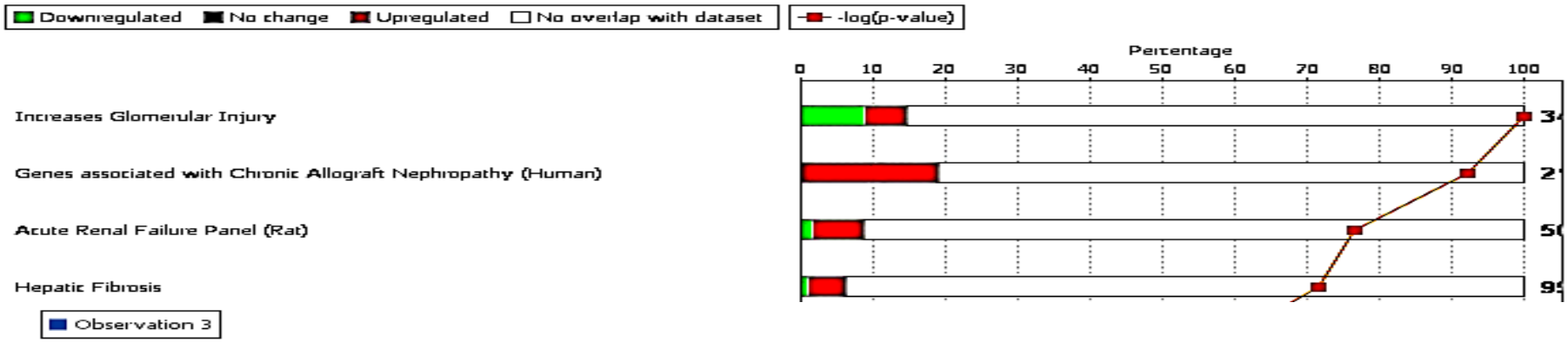
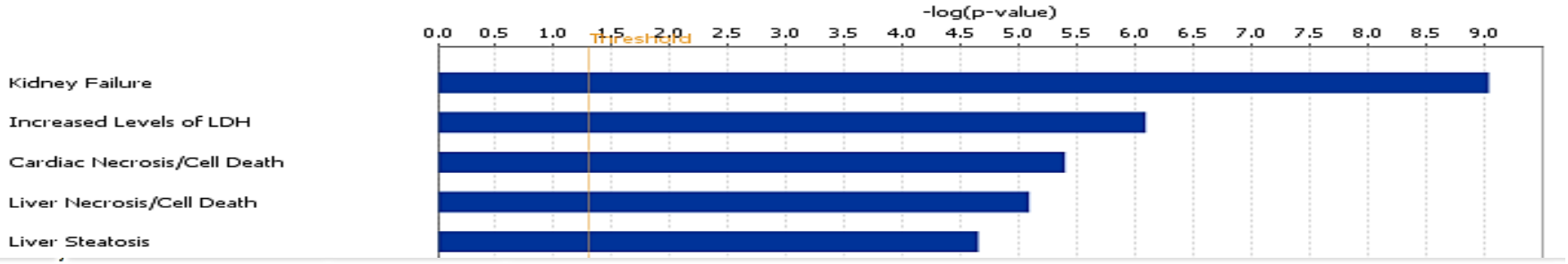
Analysis: Observation 3





Diseases or Functions Annotation	p-Value	Molecules
failure of kidney	9.01E-10	↓ADM, ↑cholesterol, ↑citric acid, ↑creatinine, ↓D-glucose, ↓DPP4*, ↓GHR, ↑indican, ↑LCN2, ↓mannitol, ↓phosphate, ↑urea, ↓... all 14
chronic renal failure	7.97E-05	↓ADM, ↑citric acid, ↓DPP4*, ↓GHR, ↑indican, ↓phosphate, ↑WFDC2 all 7
acute renal failure	1.24E-04	↑creatinine, ↑LCN2, ↓mannitol, ↓phosphate, ↑urea all 5
interstitial fibrosis of kidney	4.04E-03	↑indican, ↑WFDC2 all 2
end stage renal disease	6.44E-03	↑citric acid, ↓GHR, ↑indican, ↑WFDC2 all 4
septic acute kidney injury	1.09E-02	↑LCN2 all 1
ischemic acute renal failure	4.28E-02	↑LCN2 all 1
acute tubular necrosis	5.33E-02	↑LCN2 all 1

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Plasma CKD CoreAnalysis

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Downstream Effects Analysis: Evidence for Effects

inflammatory response (z-score 0.868). Overlap p-value **7.57E-04**

ADD TO MY PATHWAY | ADD TO MY LIST | CUSTOMIZE TABLE | CREATE DATASET

ID	Genes in dataset	Prediction (based on expression direction)	Fold Change	Findings
C00584	prostaglandin E2	Increased	↑3.240	Increases (10)
C02165	leukotriene B4	Increased	↑2.264	Increases (26)
C00575	cyclic AMP		↑1.820	Decreases (6)
C00243	lactose		1.778	Decreases (1)
C00124	D-galactose		1.327	Increases (1)
C00253	nicotinic acid	Decreased	↑1.264	Decreases (1)
C00329	glucosamine	Increased	↑1.161	Increases (2)
C00187	cholesterol	Increased	↑1.098	Increases (2)
C00037	glycine	Increased	↑1.087	Increases (1)
C00366	uric acid	Increased	↑1.069	Increases (2)
C00002	ATP	Increased	↑1.032	Increases (2)
C00008	ADP	Decreased	↓-1.290	Increases (2)
C00245	taurine	Decreased	↓-1.532	Increases (1)

leukotriene B4: Known to increase inflammatory response and is **Upregulated**↑ in the dataset, therefore predicted to increase the function.

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Canonical Pathways

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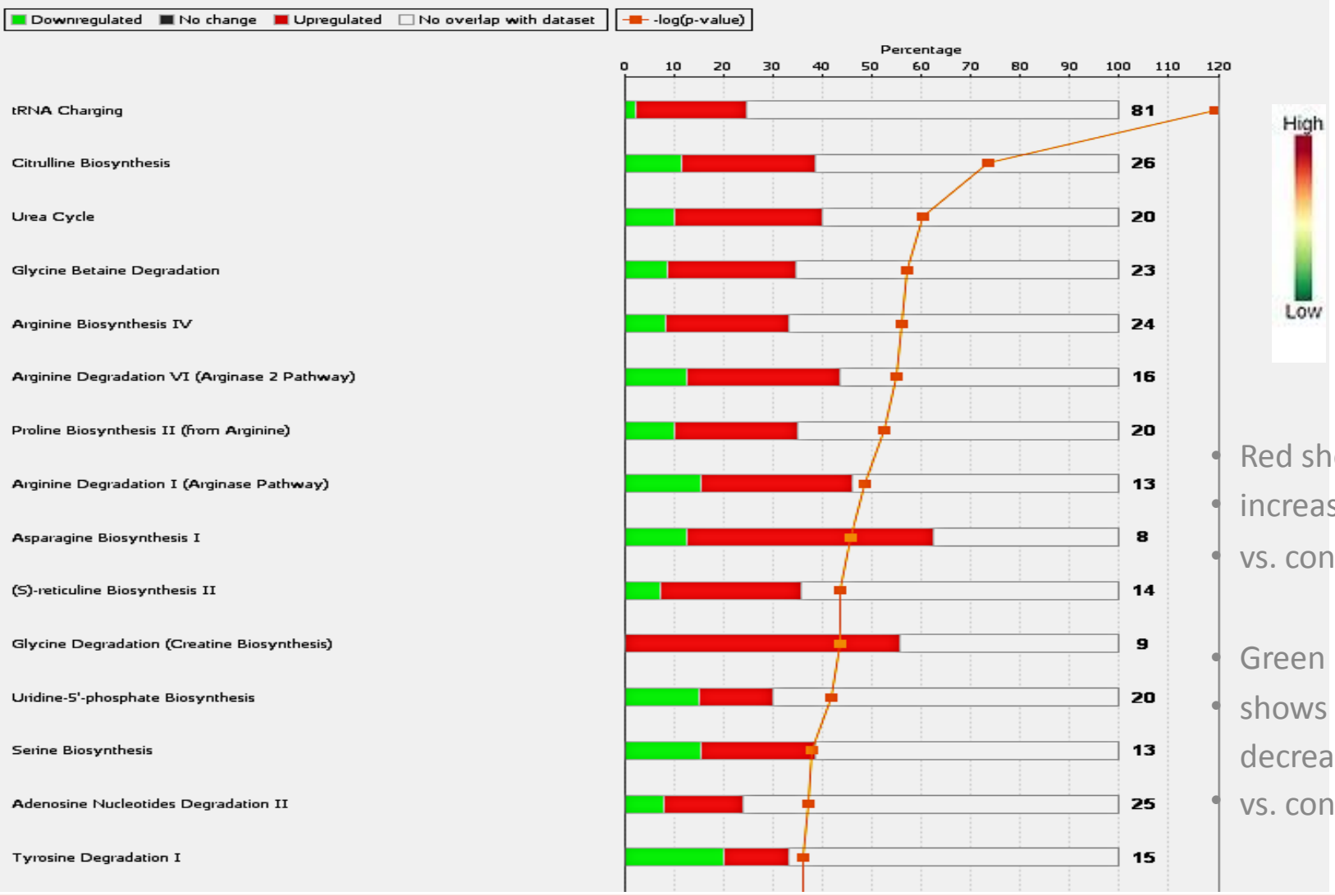

- Red shows increases vs. control
- Green shows decreases vs. control

370 pathways retrieved with uploaded data

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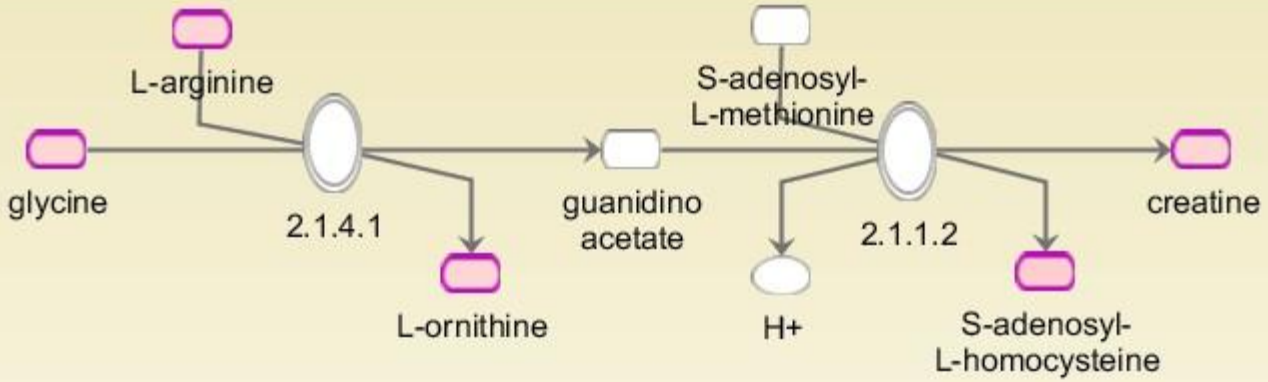
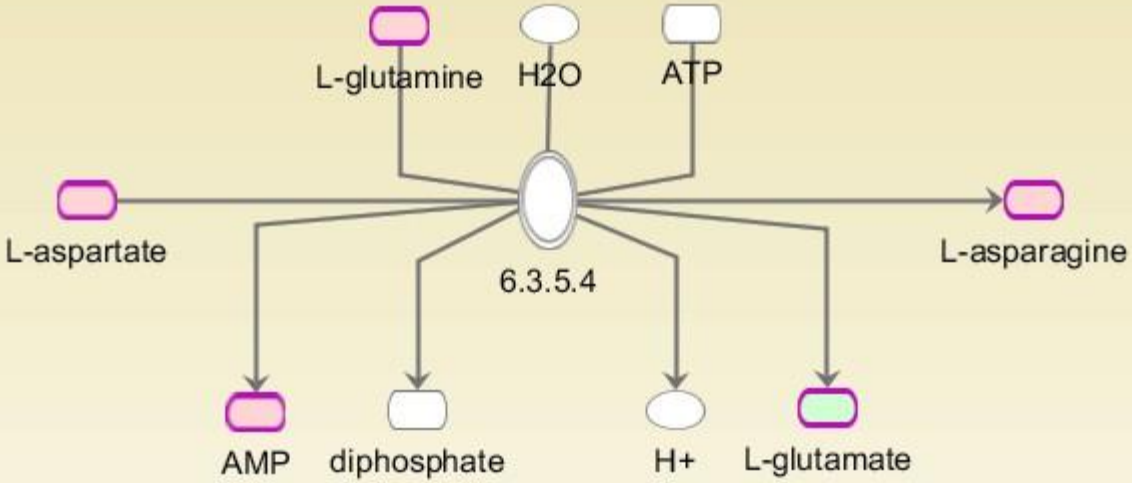
Glycine degradation

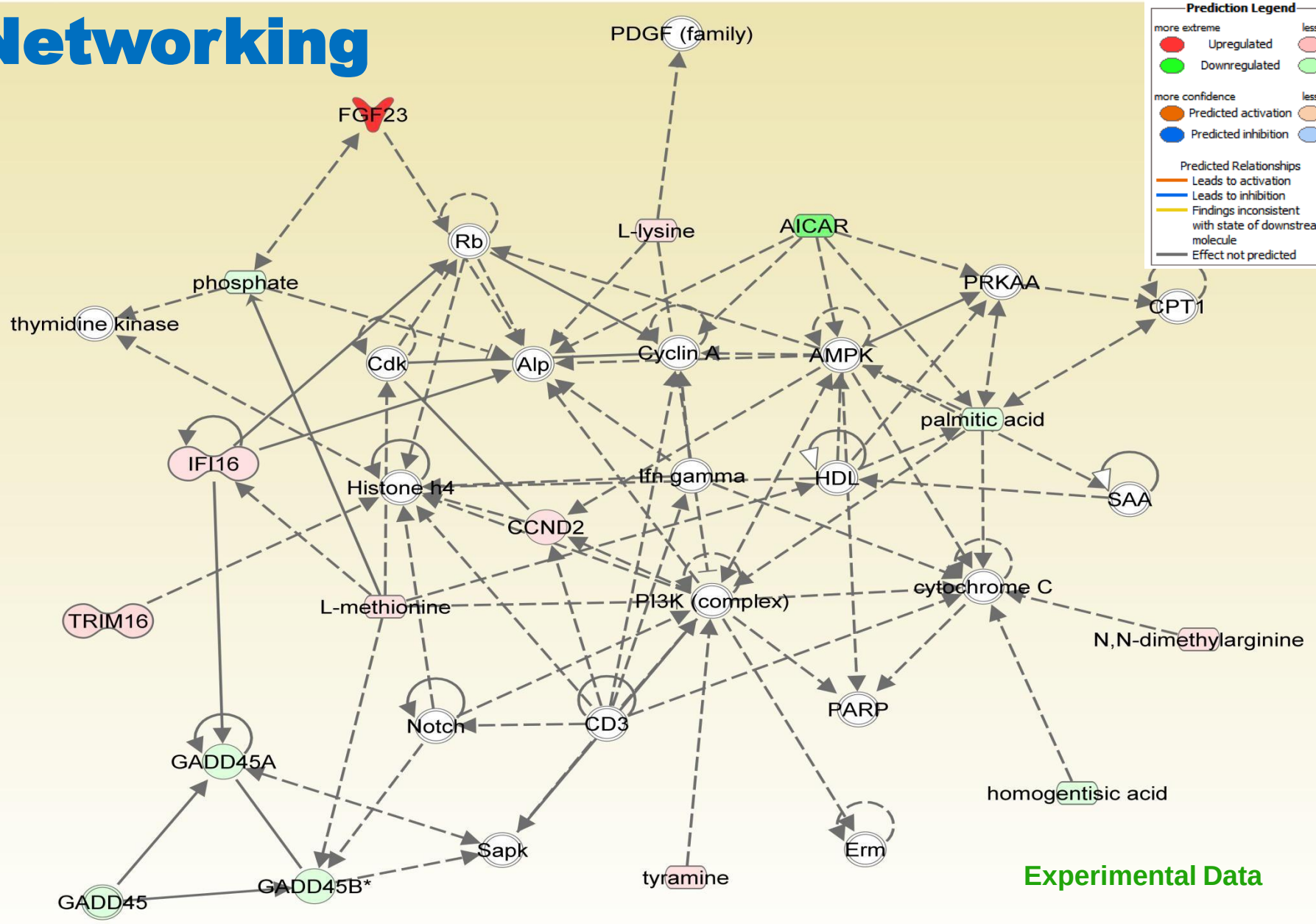


Asparagine biosynthesis



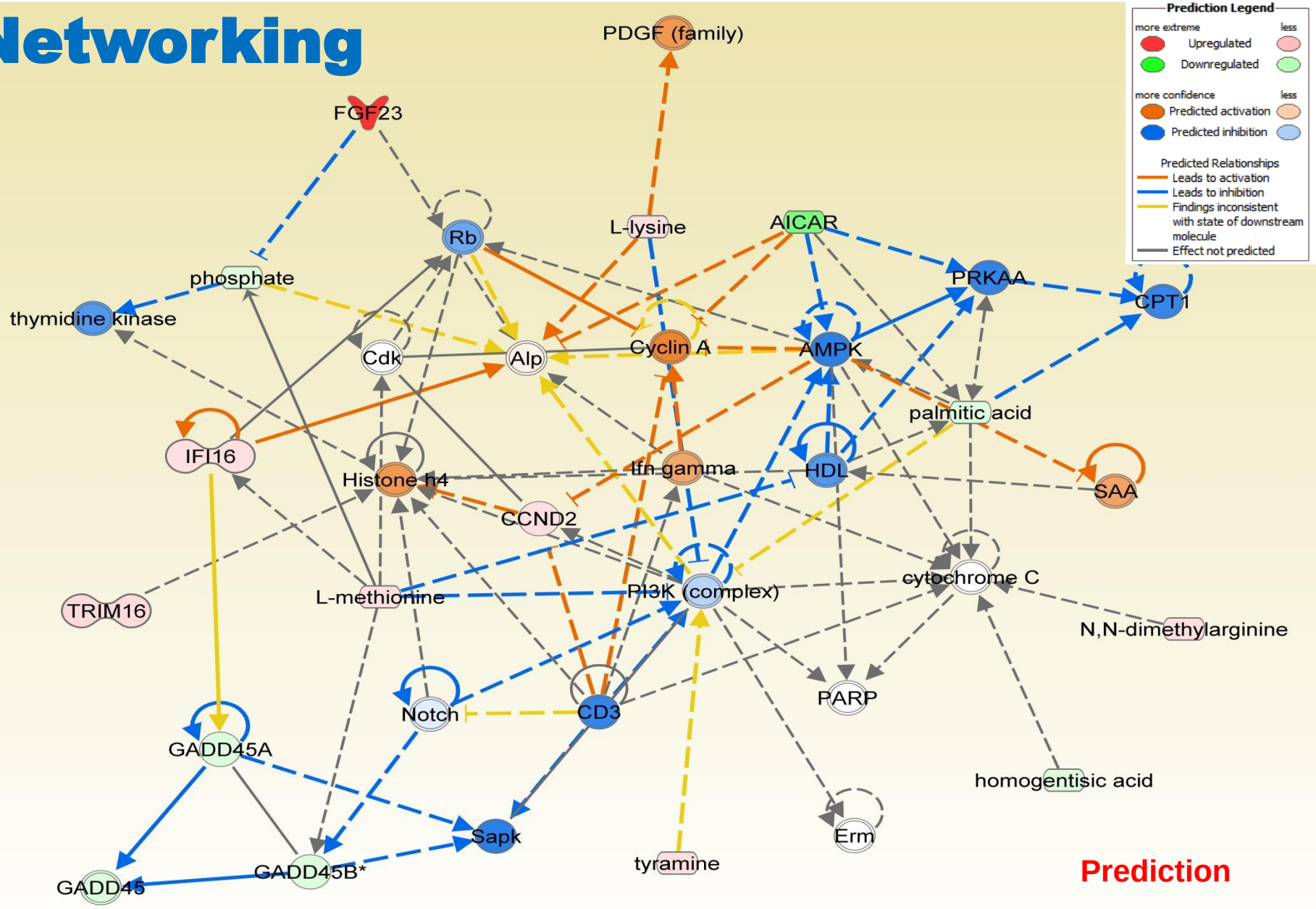
Red shows increases vs. control, green shows decreases vs. control

Networking

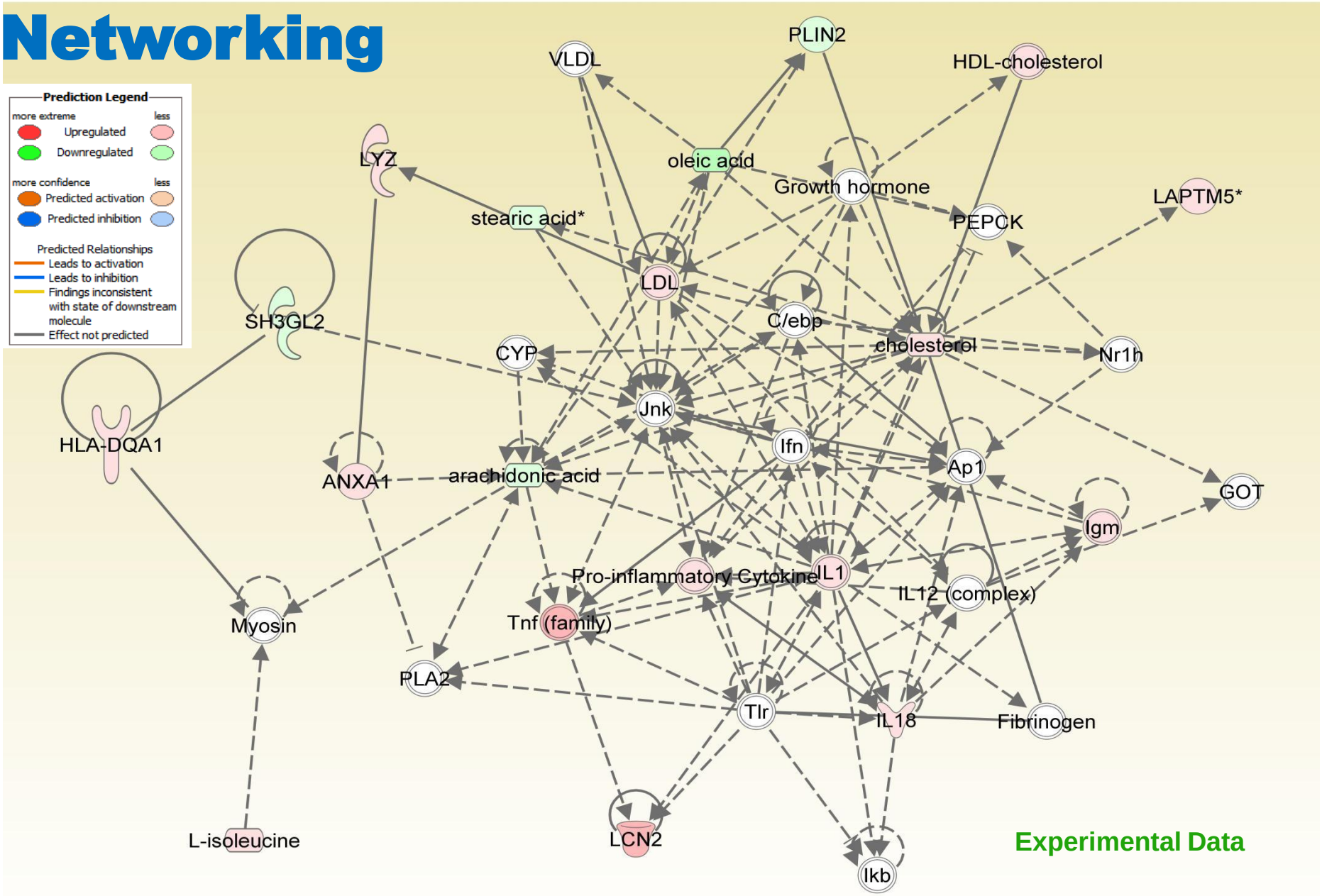


Red shows increases vs. control, green shows decreases vs. control

Networking

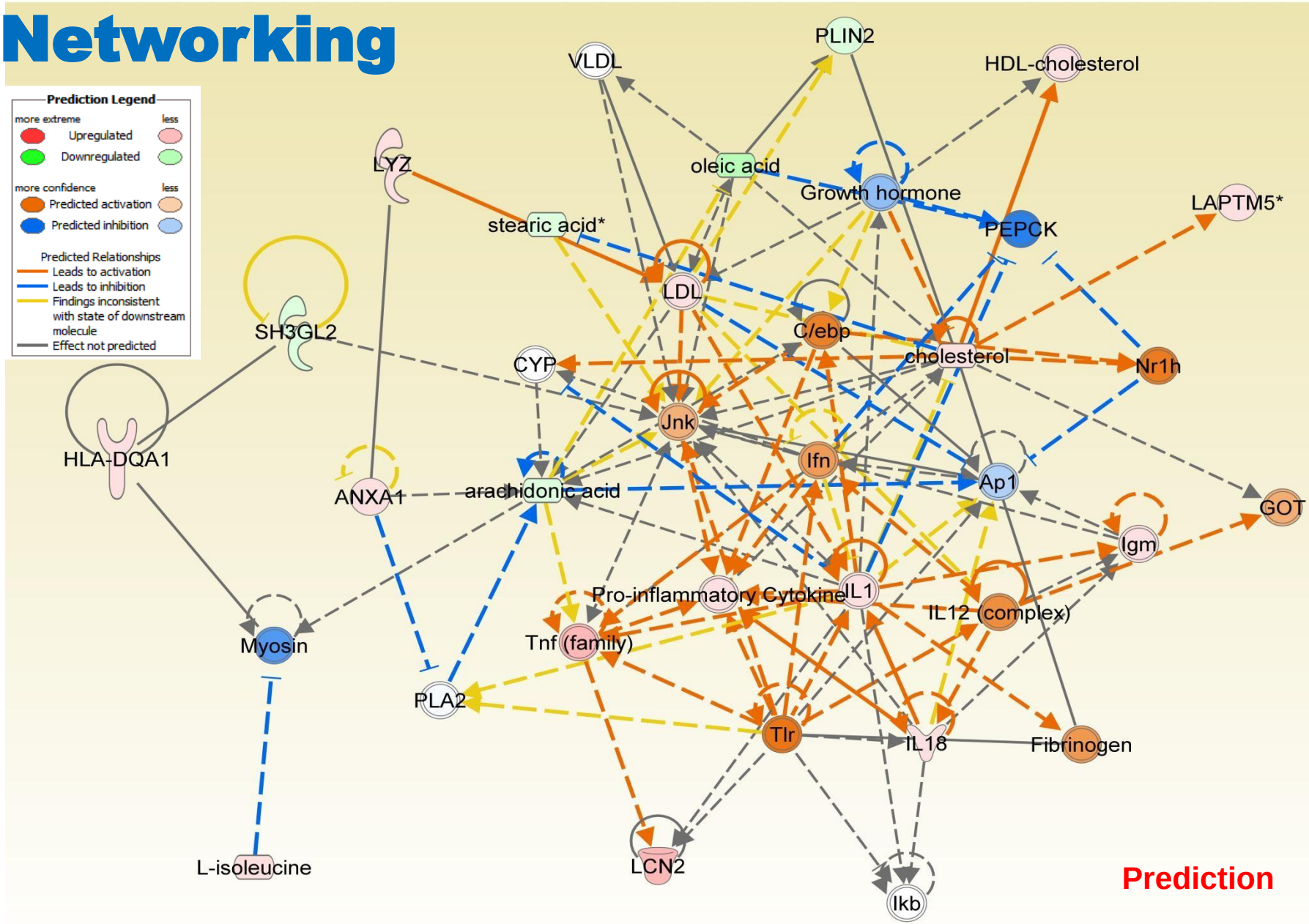

Prediction Legend
more extreme
less
Upregulated
Downregulated
more confidence
less
Predicted activation
Predicted inhibition
Predicted Relationships
Leads to activation
Leads to inhibition
Findings inconsistent with state of downstream molecule
Effect not predicted
PDGF (family)
FGF23
Rb
L-lysine
AICAR
phosphate
thymidine kinase
PRKAA
CPT1
Cdk
Alp
Cyclin A
AMPK
palmitic acid
IFI16
Histone h4
Ifn gamma
HDL
SAA
CCND2
cytochrome C
TRIM16
L-methionine
PI3K (complex)
N,N-dimethylarginine
PARP
Notch
CD3
GADD45A
homogentisic acid
Sapk
Erm
GADD45B*
tyramine
GADD45
Prediction

Networking



Red shows increases vs. control, green shows decreases vs. control

Networking



Conclusions

- Comprehensive metabolomics platform allowed to collect information on metabolic alterations for more than 290 polar metabolites excluding peptides, intact lipids and metabolites which levels were not changed.
- Statistical analysis demonstrated small molecules capable of discriminating CKD patients at different stages of disease. Diabetics were discriminated from non-diabetics based on small molecules found in patient urine and plasma.
- Omics data integration, upstream and downstream analysis offered a number of targets and hypotheses to be explored.

Acknowledgments

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Consultant Scientist

Dr. Dennis A. Laska, BS,
Consultant Biologist

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