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NMR-based quantitative metabolomics of biological tissues

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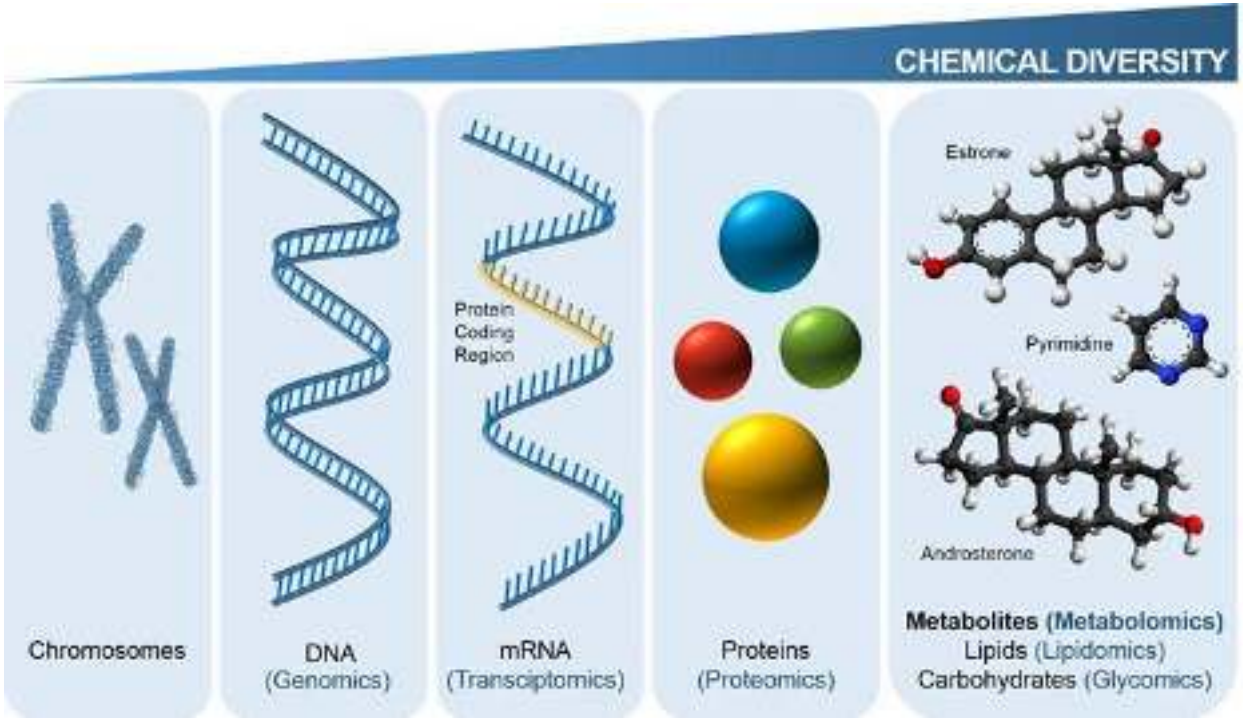
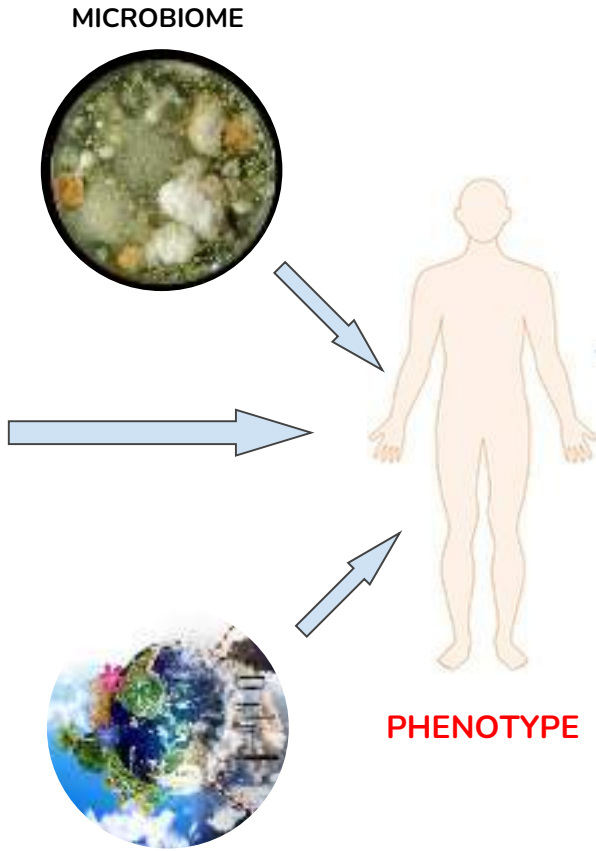
International Tomography Center SB RAS

Post-genomic sciences



https://www.vanderbilt.edu/cit/introduction-metabolomics-research/



Metabolomics and metabolites

What "metabolites" are?

- ★ Amino acids
- ★ Antioxidants
- ★ Nucleotides and nucleosides
- ★ Organic acids
- ★ Lipids
- ★ Sugars
- ★ Osmolites

Metabolomics

Qualitative	Semi-quantitative	Quantitative
Sample A contains alanine, while sample B does not	Concentration of alanine in sample A is threefold higher than in sample B	Concentration of alanine in sample A is 25.1 nmol/g

Metabolome: sample preparation

Metabolite extraction

Tissue homogenization and metabolism quenching

Protein removal

extract

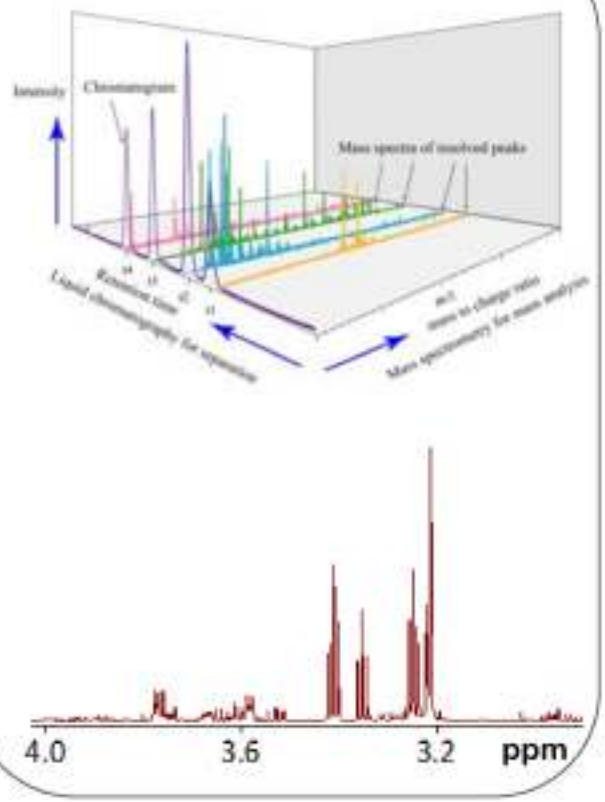
Tissue

LC-MS

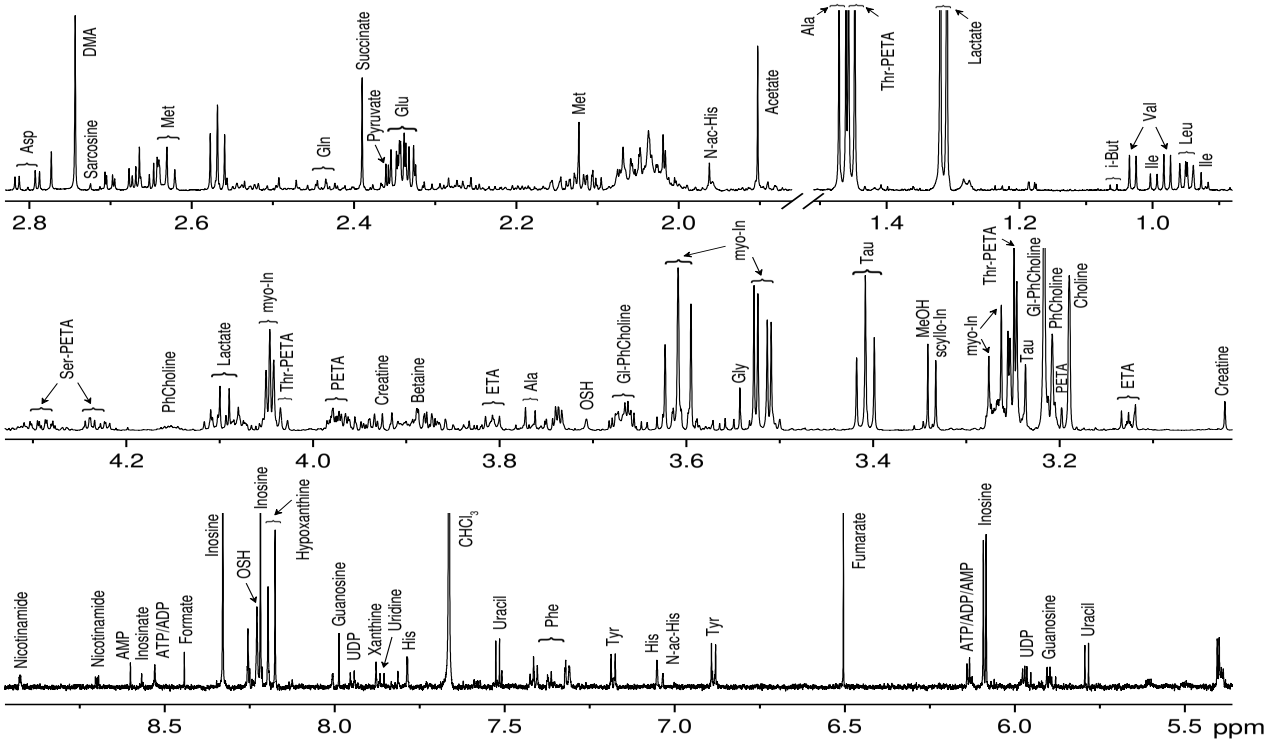
Dionex UltiMate3000RS +
+ DAD flow cell +
Bruker ESI-q-TOF MaxIs 4G

NMR

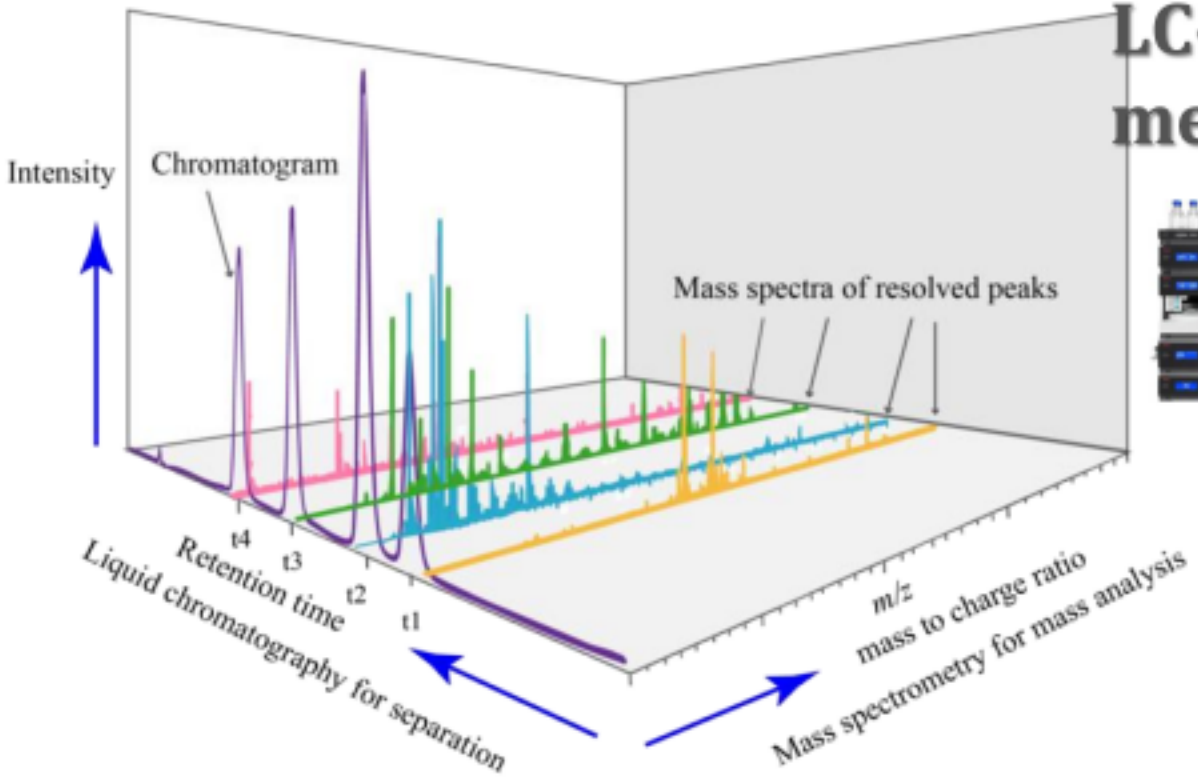
Bruker Avance III HD 700 MHz

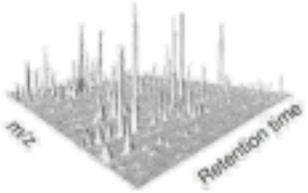


NMR spectrum of the fish gills

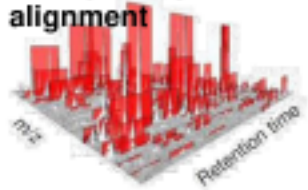


LC-MS for metabolomics





Peak picking & RT alignment



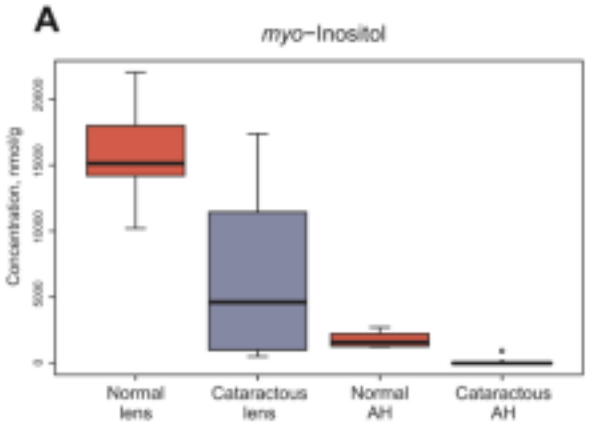
Every LC-MS peak is characterized by RT, m/z, and integral

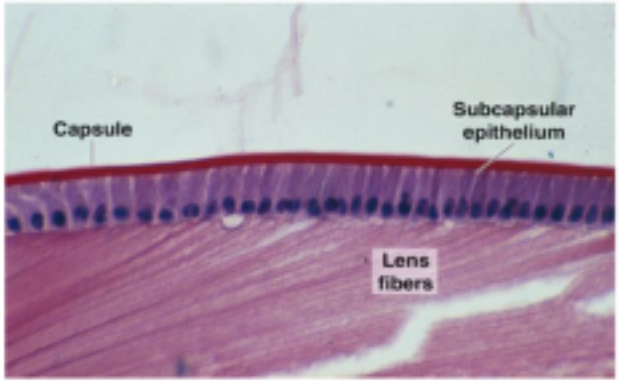
Typically, hundreds and thousands of peaks are detected – "Big data"

Compound	Concentration (mean±SD), nmol/g			
	Cataract (n = 12)		Normal post-mortem (n = 7)	
	Lens	AH	Lens	AH
Amino acids and peptides				
Alanine	590±360	300±80	600±130	780±240
Arginine	28±9	90±30	44±10	340±90
Creatine	660±660	120±50	1900±500	510±200
Glutamate	770±620	5.1±3.1	1600±300	620±200
Glutamine	680±360	310±260	860±210	830±280
Glycine	160±90	15±22	230±70	350±110
GSH	690±740	-	1200±400	35±64
GSSG	66±54	0.2±0.1	220±70	35±50
Isoleucine	100±50	53±25	110±30	120±30
Leucine	200±90	120±70	290±70	320±110
Lysine	30±12	100±30	140±40	250±100
Phenylalanine	230±110	110±70	250±70	180±50
Proline	62±55	41±29	77±18	390±210
Pyroglutamate	480±350	25±13	490±110	110±60
Serine	210±80	140±20	-	-
Taurine	170±130	36±25	1100±300	2700±800
Threonine	180±170	57±58	300±80	250±150
Tyrosine	250±140	110±40	260±100	130±40
Valine	390±170	270±90	350±90	310±70
Organic acids and alcohols				
2-Hydroxybutyrate	6.0±5.4	55±96	120±170	310±380
2-Hydroxy-3-methylbutyrate	23±42	3.5±4.8	120±240	190±310
3-Hydroxybutyrate	3.4±3.7	31±36	410±890	680±1200
Acetate	1200±900	120±50	320±180	770±750
Formate	88±24	30±26	170±110	57±40
Glycerol	290±200	60±64	440±210	1000±500
Lactate	4000±1200	5300±1800	11600±2700	32000±7300
myo-Inositol	6500±6100	80±270	16000±4000	1800±700
Propyleneglycol	15±12	0.4±1.3	140±160	200±200
Pyruvate	25±17	160±290	21±16	35±31
scyllo-Inositol	94±84	3.6±4.8	210±110	50±7
Succinate	-	-	-	260±100
Nucleotides and nucleosides				
Hypoxanthine	21±11	0.2±0.5	55±24	150±60
Inosine	16±10	1.3±0.7	47±11	160±110
UV filters				
3OHKG	160±240	0.3±0.5	400±140	42±57
AHBG	37±52	-	86±38	4.6±6.2
GSH-3OHKG	19±23	-	14±13	-
KN	8.6±6.1	1.4±0.9	22±11	5.1±3.8
Others				
Choline	8.4±4.7	8.6±4.3	44±17	190±70
Creatinine	56±37	47±20	110±30	110±90
Glucose	480±49	2700±1100	900±740	1500±1600

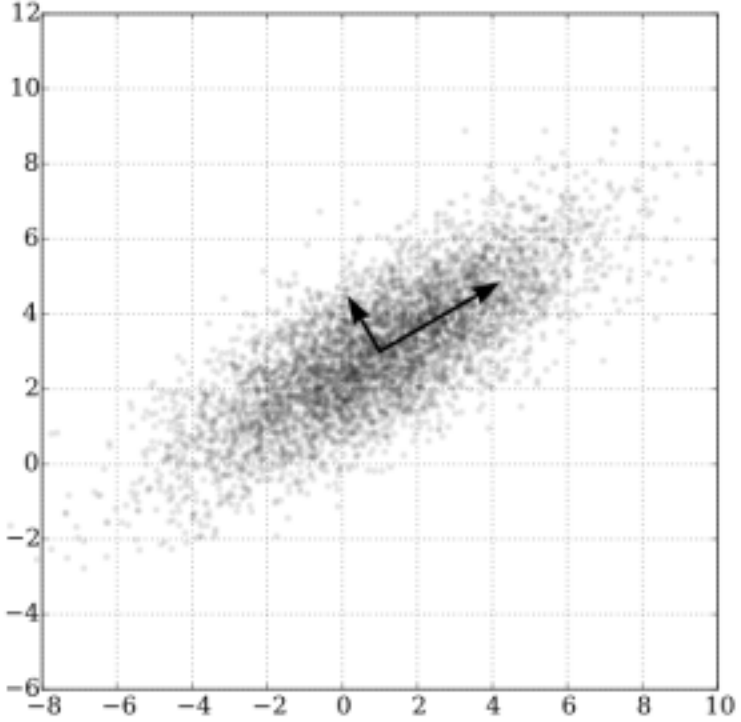
(Part)

Example: metabolomic analysis of the eye lens

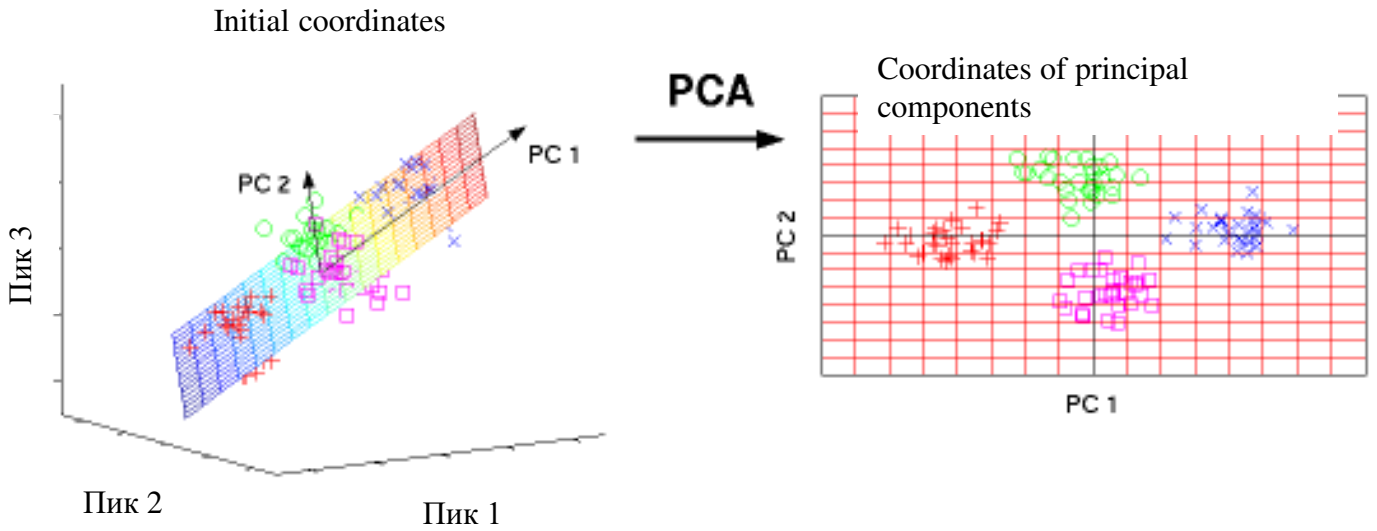




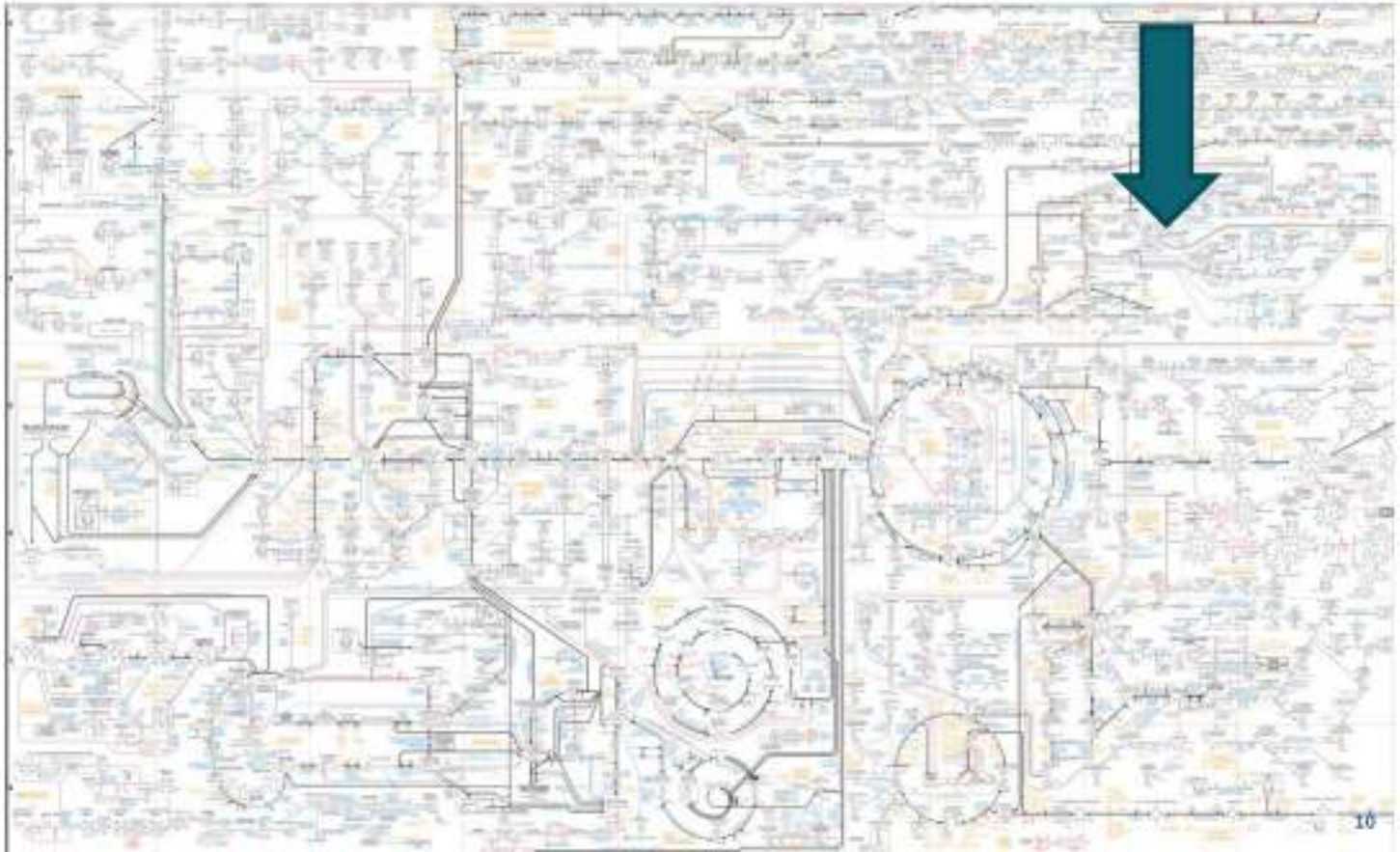
Principal Component Analysis (PCA)



Principal Component Analysis (PCA)

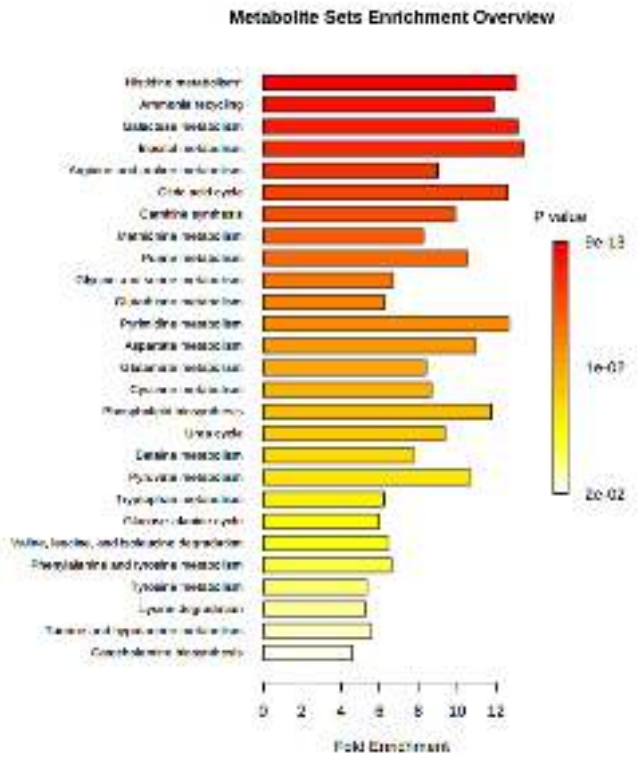


Metabolic pathways



Analysis of metabolic pathways

Seasonal variations (Autumn-Spring) of metabolomic composition of pike-perch lens



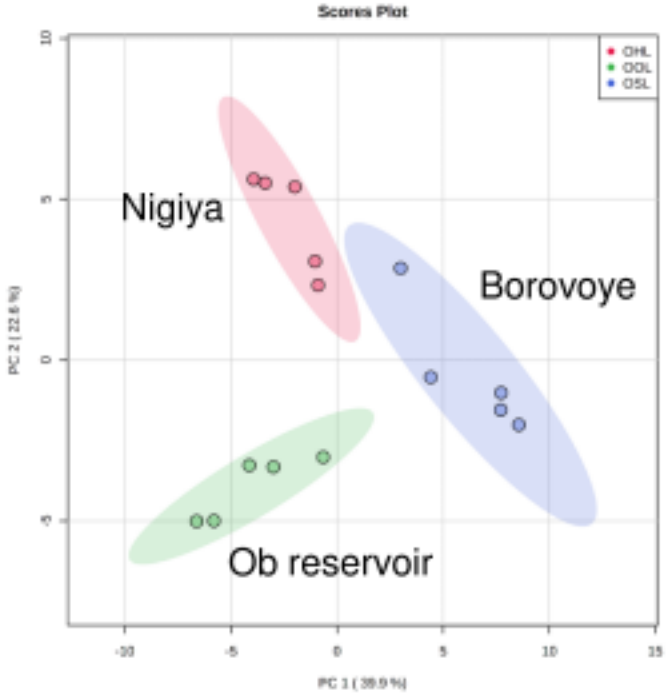
Examples of metabolomics application

- Influence of water purity and oxygen level on fish metabolome
- Mechanisms of cataractogenesis
- Metabolomic biomarkers of Parkinson’s disease
- Evaluation of the post-mortem interval
- Comparison of metabolomic profiles of embryonic stem cells, fibroblasts, and induced pluripotent stem sells

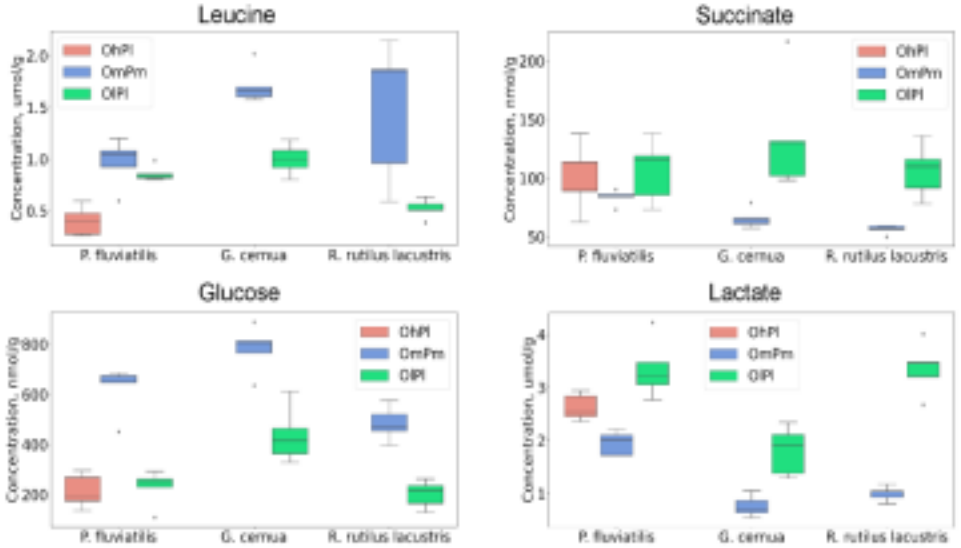
Metabolomic analysis of fish tissues

	River Nigiya	Lake Borovoye	Ob Reservoir
O_2 level, mg/L	6.8	13.1	9.7
pH	6.8	7.5	5.3

Metabolomic analysis of fish tissues



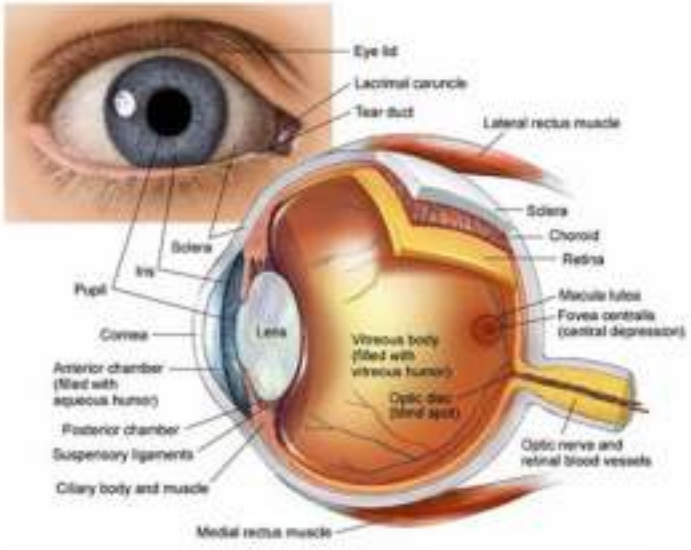
PCA of perch lens from Ob reservoir, river Nigiya, and lake Borovoye

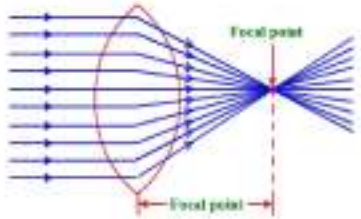


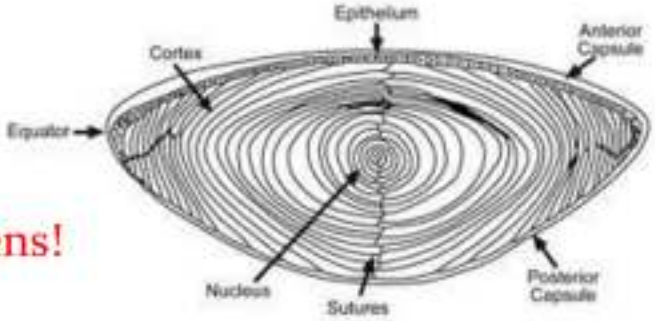
Boxplots for concentrations of leucine, succinate, glucose, and lactate in fish lenses

STRUCTURE OF THE LENS

and its physical properties

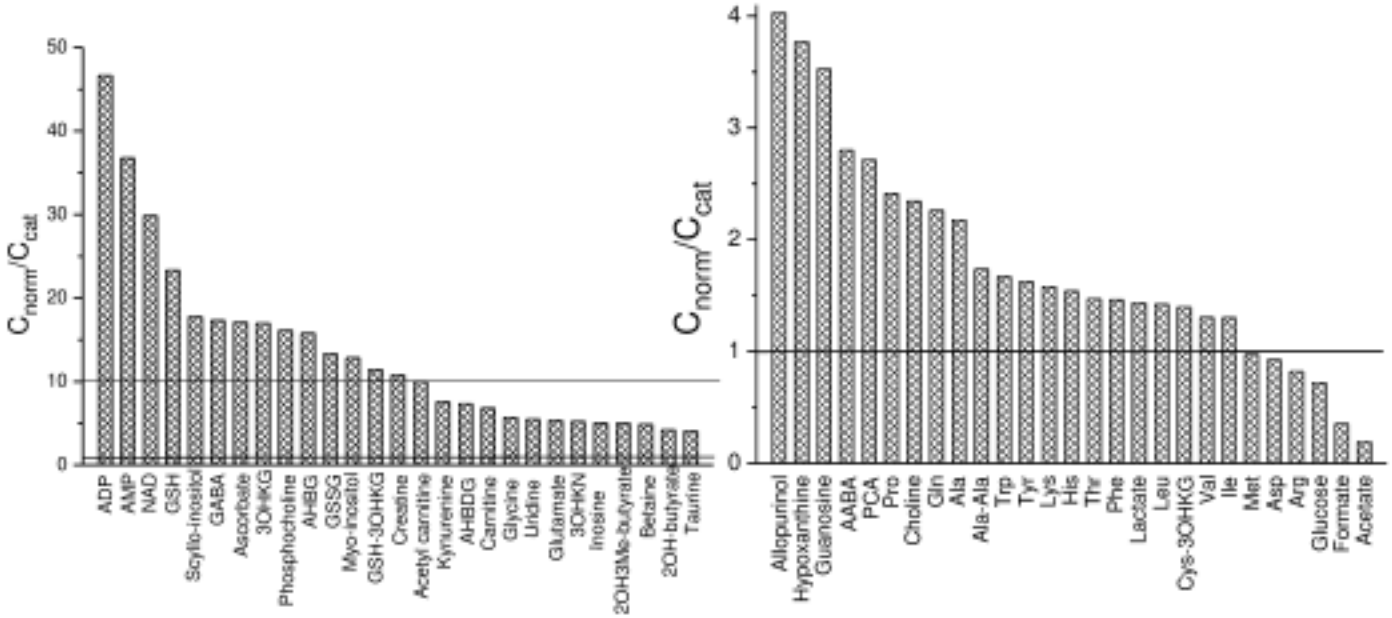






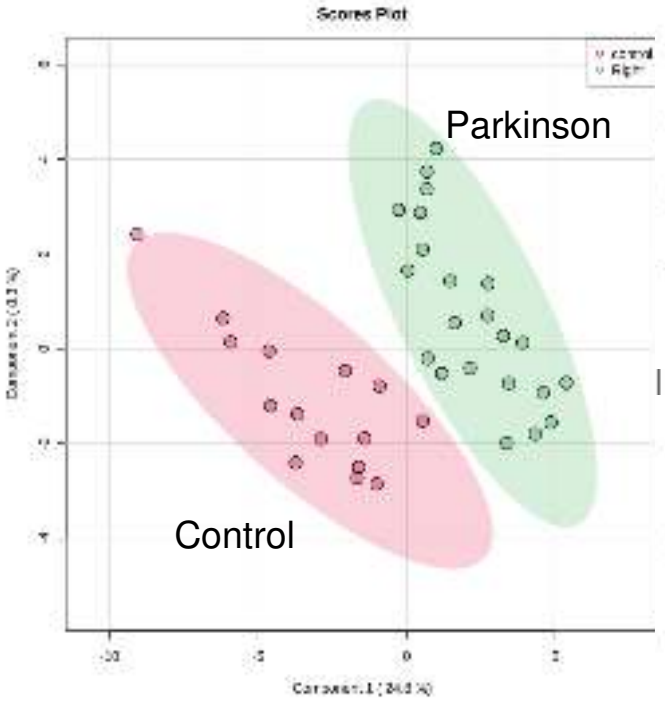
No vascular system in the lens!

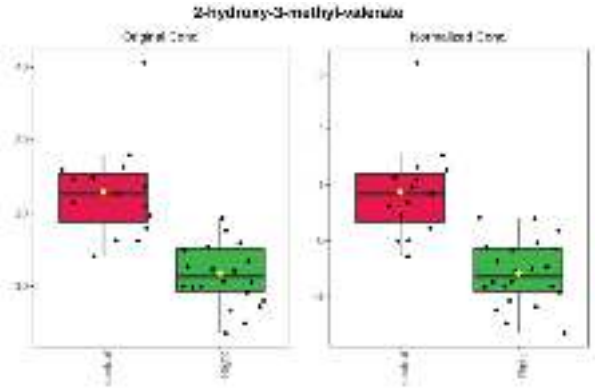
RATIOS NORMAL/CATARACTOUS LENS

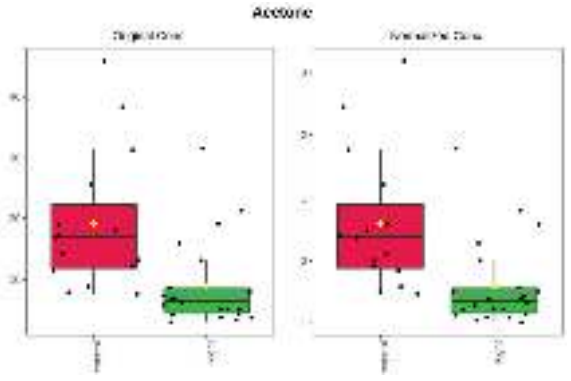


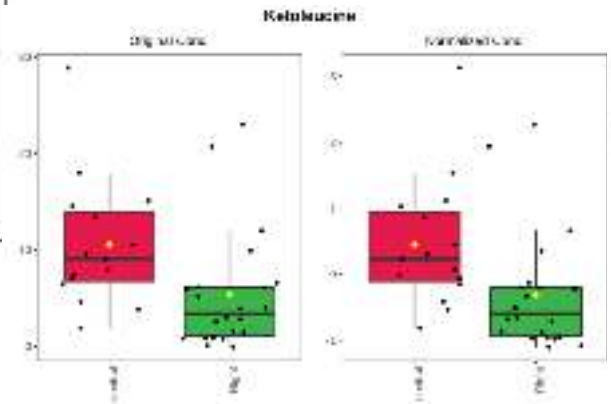
Level of almost all metabolites is higher in the normal lens. For most important metabolites, the difference exceeds the factor of ten!

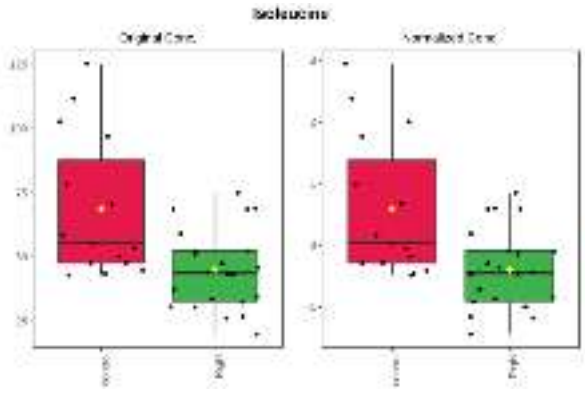
Biomarkers of Parkinson's disease



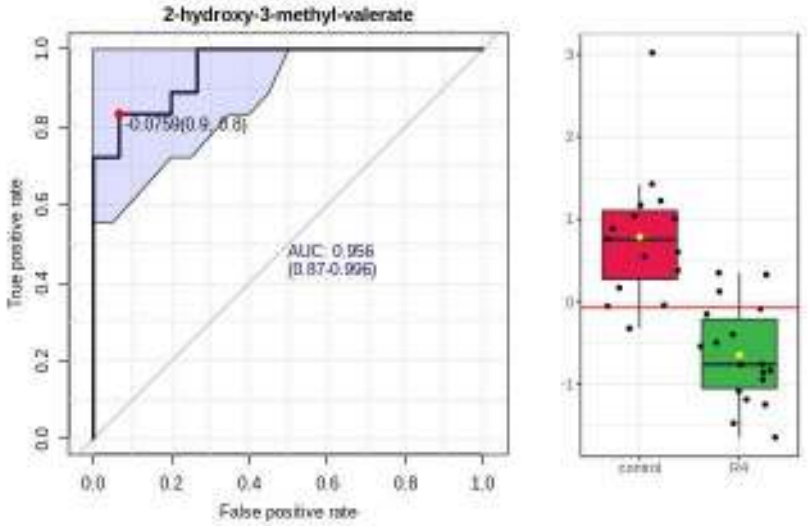


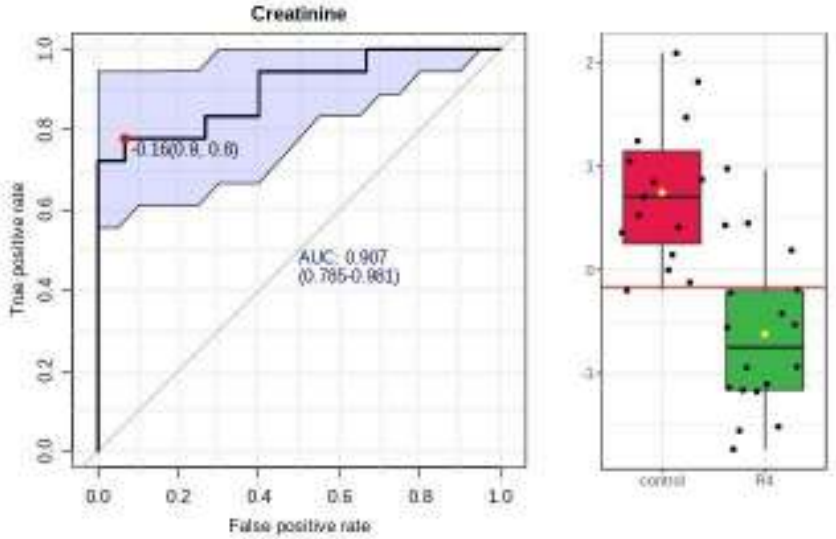






Biomarkers of Parkinson’s disease

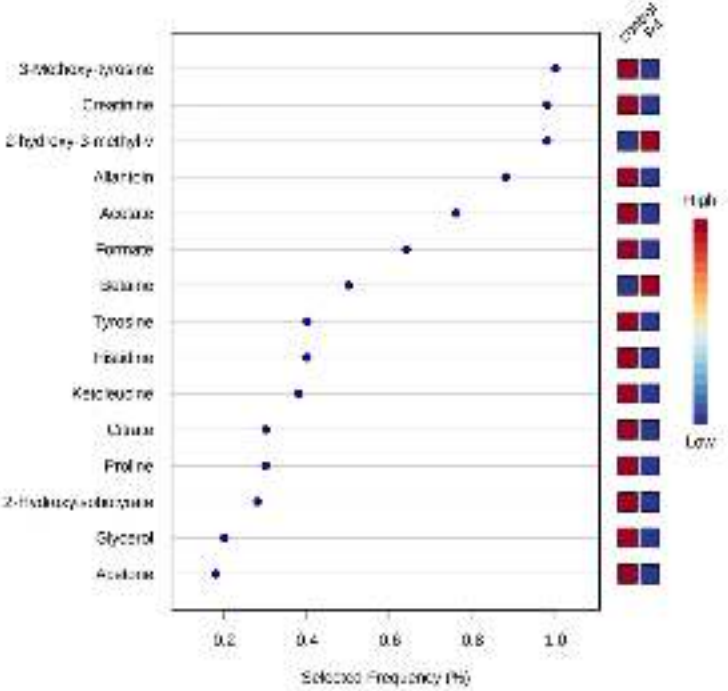


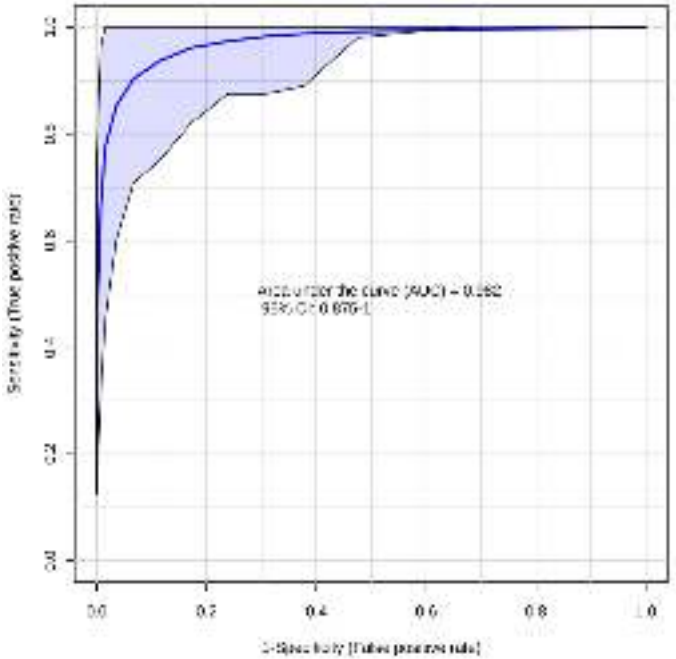


2-hydroxy-3-methyl-valerate is the best potential biomarker of Parkinson’s disease

Creatinine is the second best candidate

Biomarkers of Parkinson’s disease





Multicomponent analysis: 10 compounds

Applications of metabolomics to Thanatochemistry

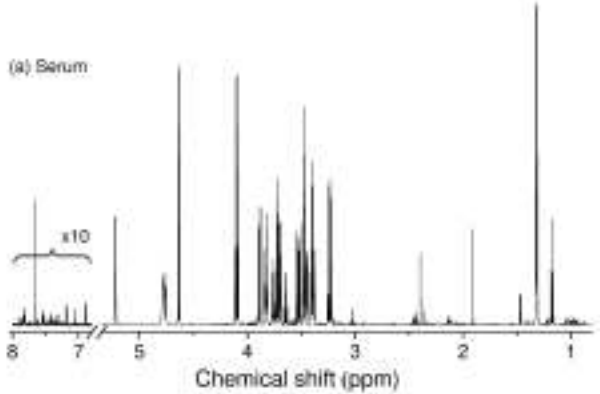
Animal model

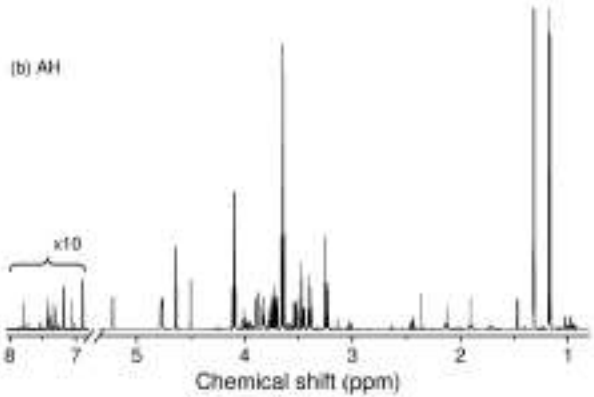
rabbit

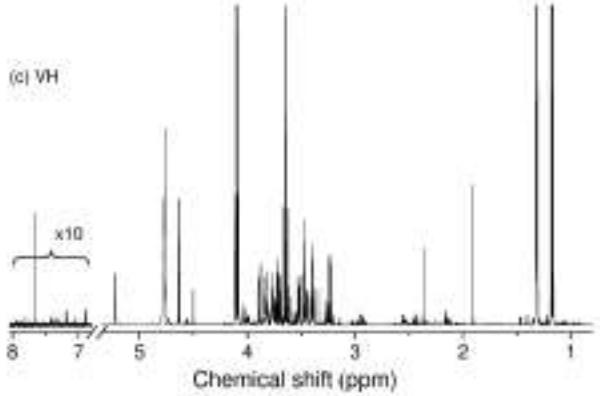
Cardiac blood
Aqueous humor
Vitreous humor

→ Levels of **61** metabolites

→ Ante-mortem
Post-mortem (daily dynamics)







Representative ^{1}H NMR spectra of the protein-free lipid-free extracts from the rabbit serum (a), AH (b) and VH (c) taken 10 min post-mortem.

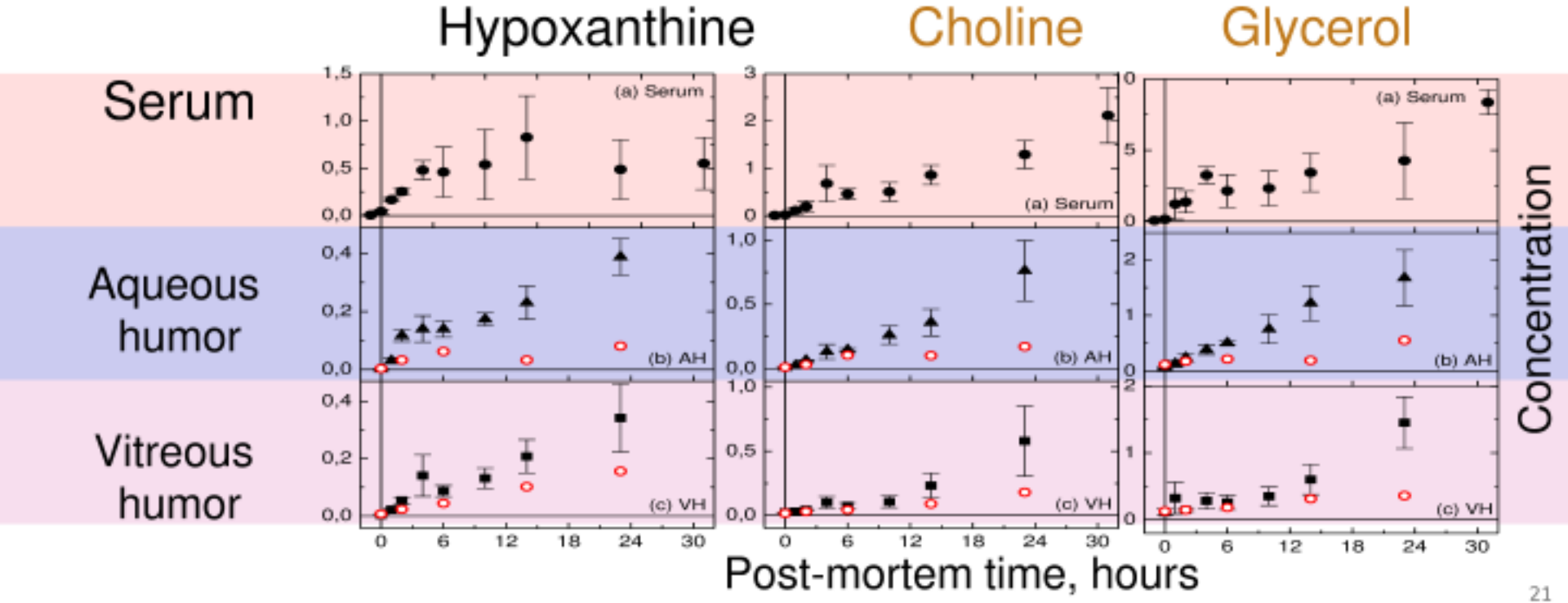
Post-mortem changes in metabolomic composition of biological fluids

Metabolomics (2016)12:132
DOI 10.1007/s11306-016-1110-2
ORIGINAL ARTICLE

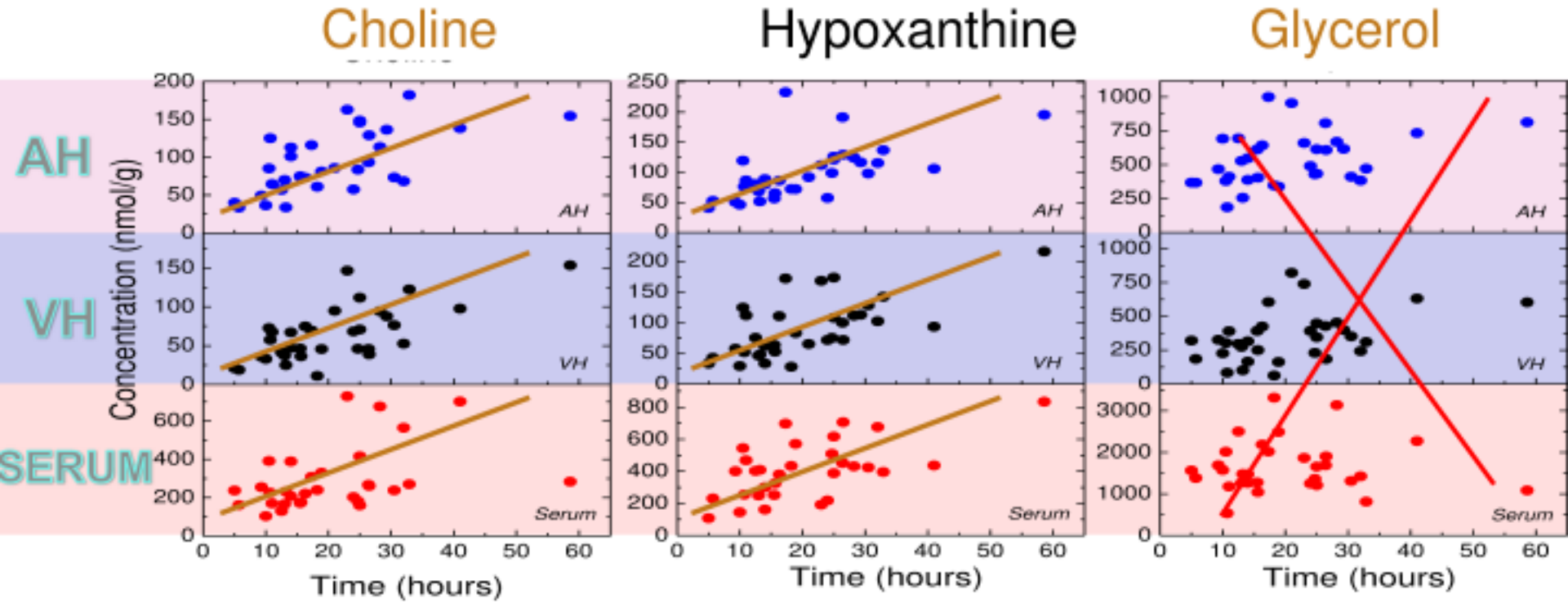
Post-mortem changes in the metabolomic compositions of rabbit blood, aqueous and vitreous humors

Ekaterina A. Zelentsova[1,2] · Lyudmila Y. Yanshole[1,2] · Olga A. Savenkova[1,2] · Vadim Y. Yanshole[1,2] · Yuri P. Tsentalovich[1,2] · Rinad Z. Sagdeev[1]

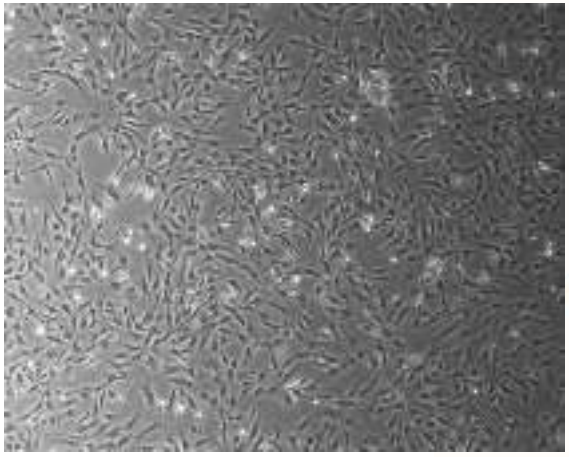
Potential metabolomic markers of post-mortem time

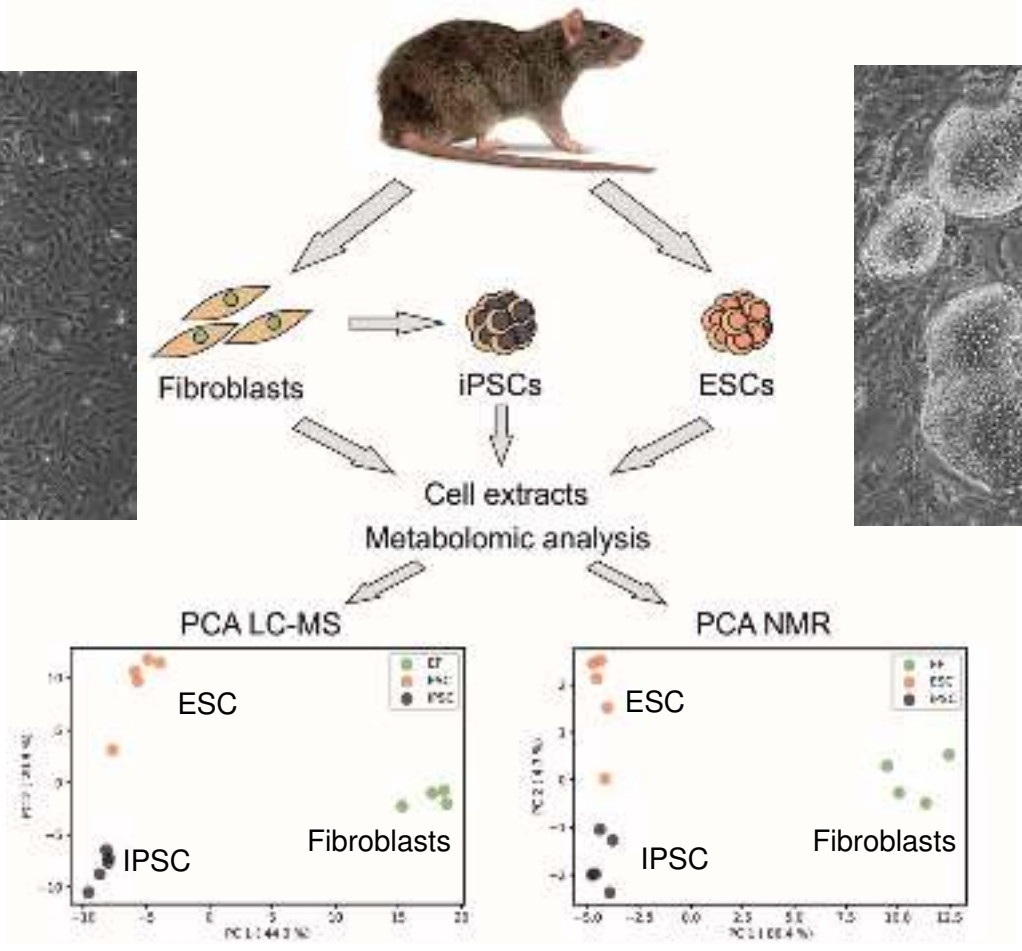


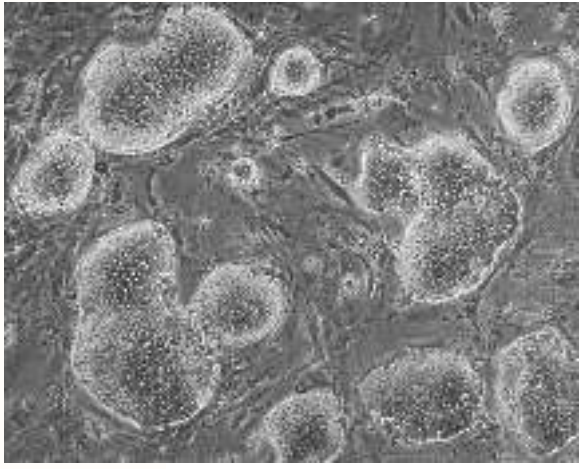
Results: human



Metabolomics of cell cultures







Summary

Методы	Объекты	Ткани	Стресс-факторы
ЯМР **ВЭЖХ-МС** **ВЭЖХ-ОД** **Статистический анализ** **Хемометрика: метод главных компонент, графики Вулкано, дендрограммы, тепловые карты**	Человек Млекопитающие Птицы Рыбы Насекомые Культуры клеток	Сыворотка крови Цельная кровь Хрусталик Водянистая влага Стекловидное тело Роговица Мышца Печень Мозг Селезенка Кишечник Почка	Катаракта Кератоконус Колит Болезнь Крона Онкологические заболевания Болезнь Альцгеймера Болезнь Паркинсона Болезнь Шарко Голодание Гипоксия Замерзание Загрязнение воды









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