

XV°  
Corso Nazionale



RESPONSABILI SCIENTIFICI:  
**Massimiliano Corneli**  
**Silvia Conforti**

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**2023**



**FABRIANO**

## METABOLOMICA APPLICATA AL TERRENO PER ORGANOCOLTURA CORNEALE

Massimo Ricciutelli

Università degli studi di Camerino

# Metabolomica

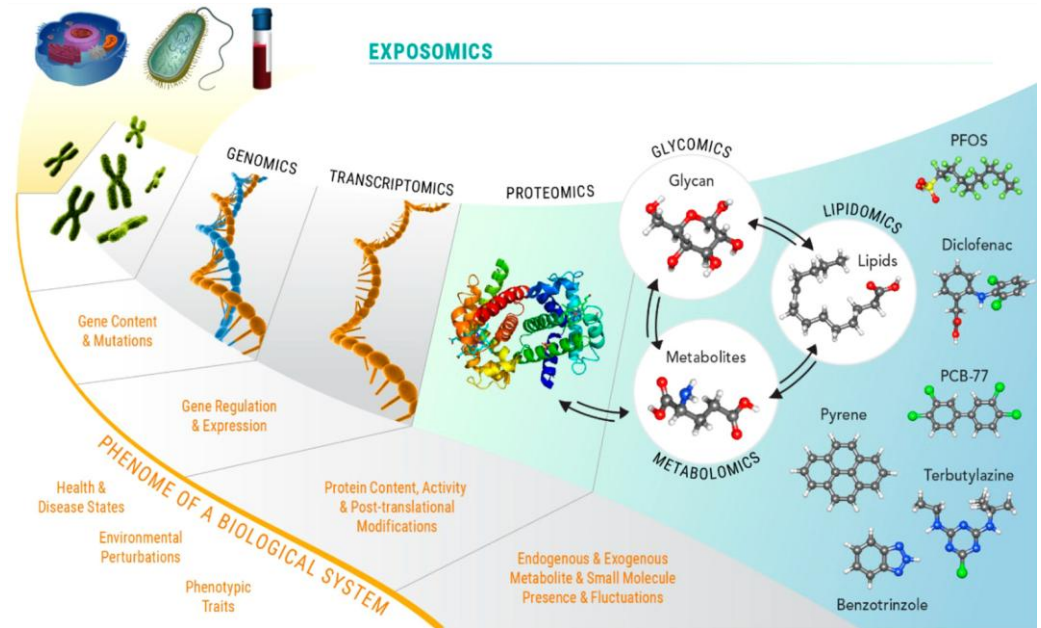
- La metabolomica è la disciplina scientifica che si occupa di identificare gli specifici metabolomi che descrivono i processi biologici.

# Metaboloma

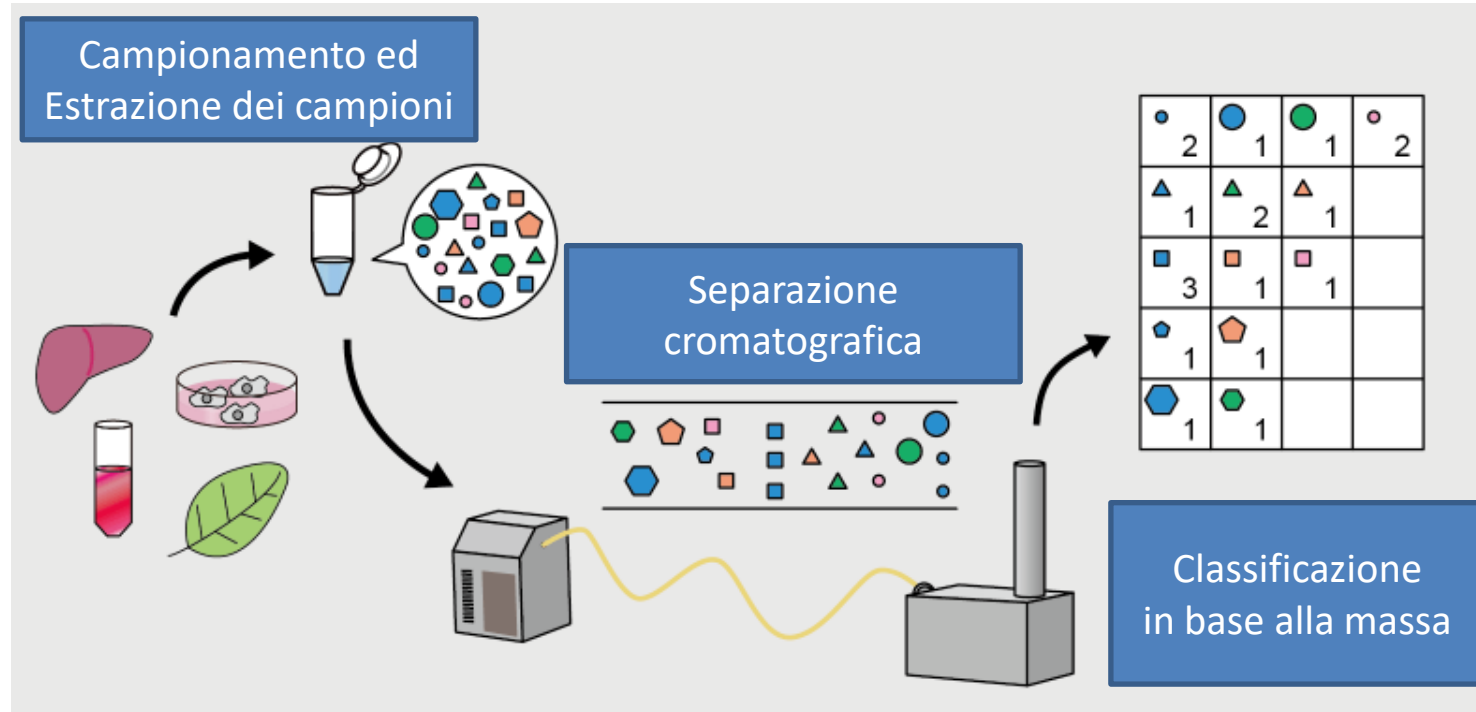
- La collezione univoca dell'insieme di metaboliti (molecole a basso peso molecolare) prodotti durante un determinato metabolismo a livello cellulare, tissutale o sistemico.

# Scienze Omiche

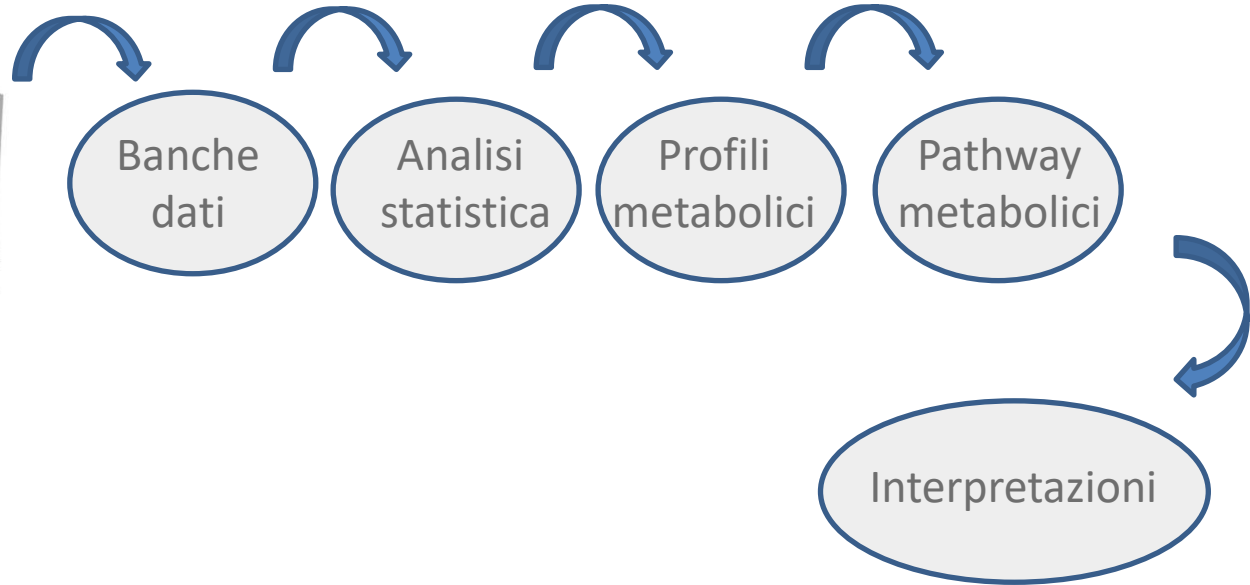
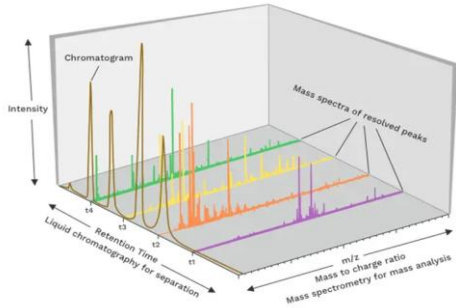
- La metabolomica si posiziona alla base di una piramide.
- Al vertice troviamo la genomica.
- Poi a seguire abbiamo la trascrittomica o analisi dell'espressione genica attraverso la collezione di mRNAs.
- Scendendo nella piramide, passiamo all'analisi di funzionalità genica con la proteomica che studia l'insieme delle proteine tradotte da specifiche sequenze geniche.



# Analisi con liquido-massa



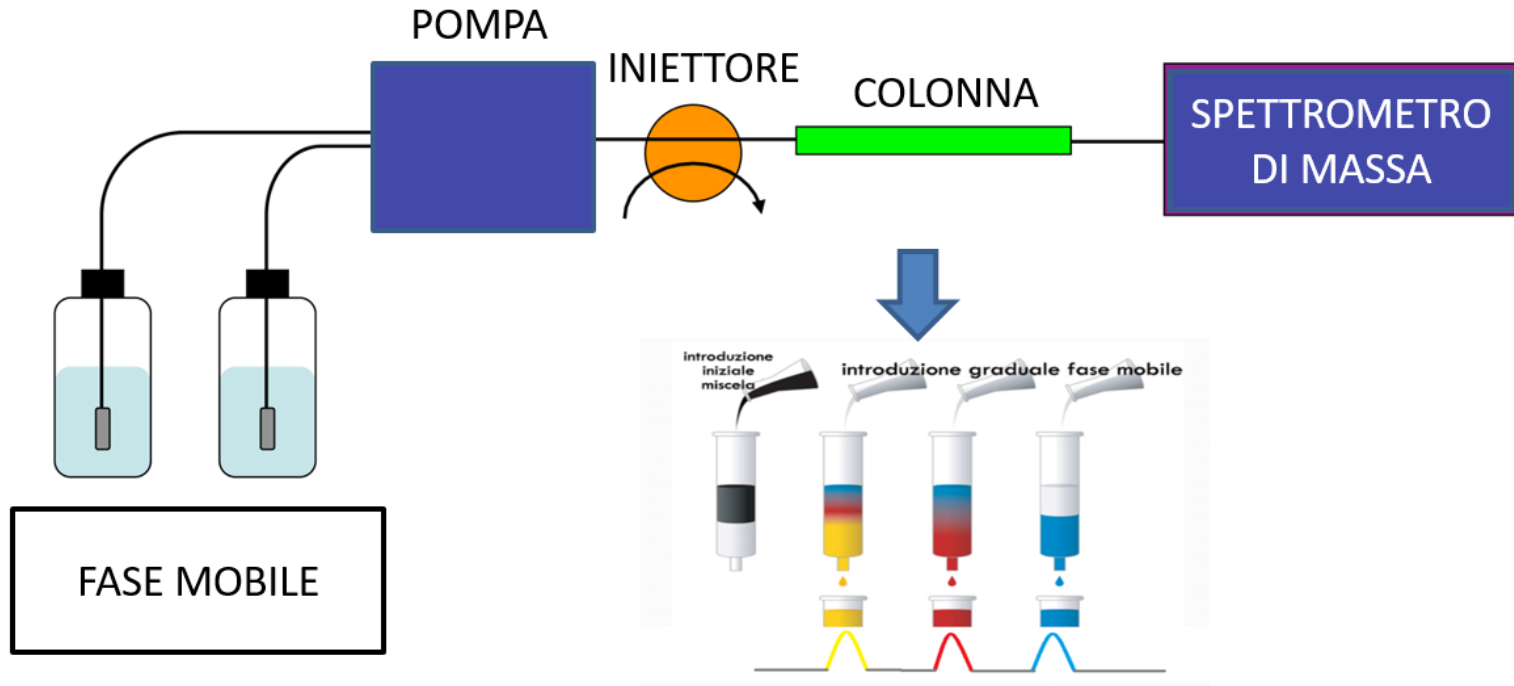
# Analisi bioinformatica



# Cromatografia liquida (LC) abbinata alla spettrometria di massa (MS)

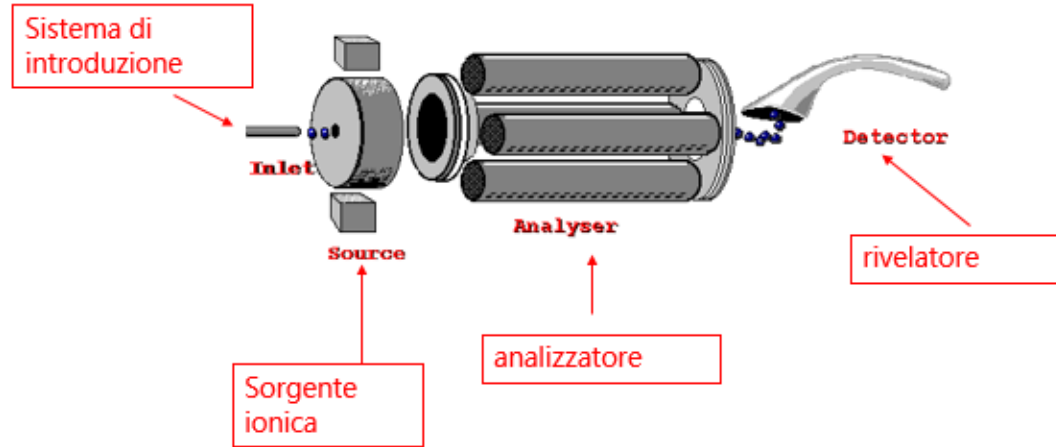
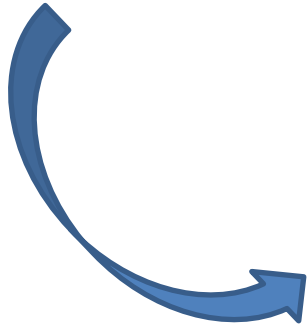
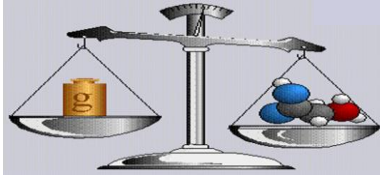
- La cromatografia è una tecnica in grado di separare i componenti di una miscela di composti organici.
- La spettrometria di massa è una tecnica di caratterizzazione molecolare in grado di misurare il peso molecolare di un composto ed eventualmente dei suoi gruppi costituenti dopo frammentazione.

# Cromatografo liquido

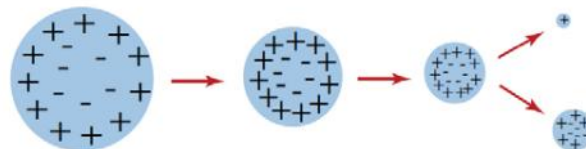
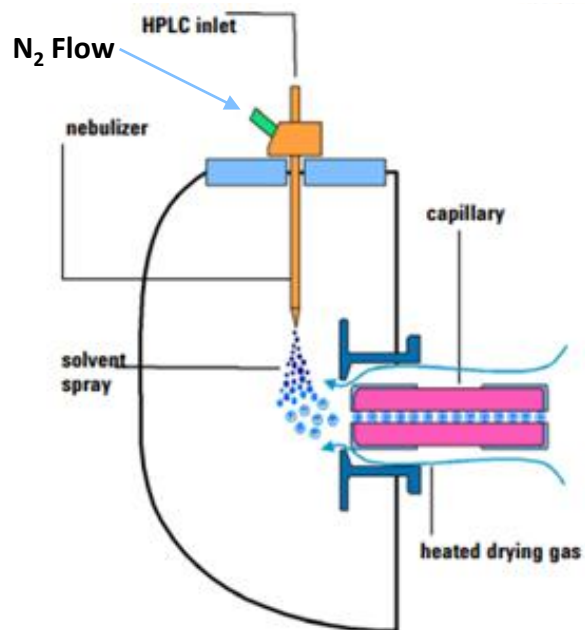




# Spettrometro di massa



# Electrospray Ionization (ESI)

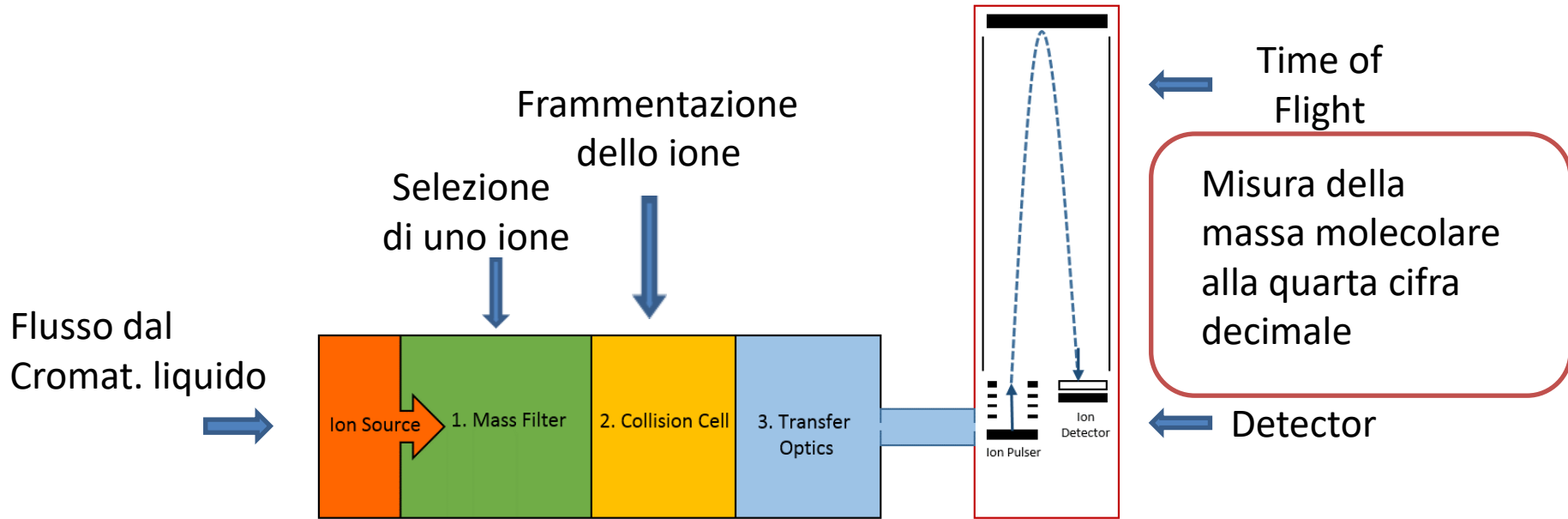


The Nobel Prize in  
Chemistry 2002

"For the development of methods  
for identification and structures  
analysis of biomolecules"



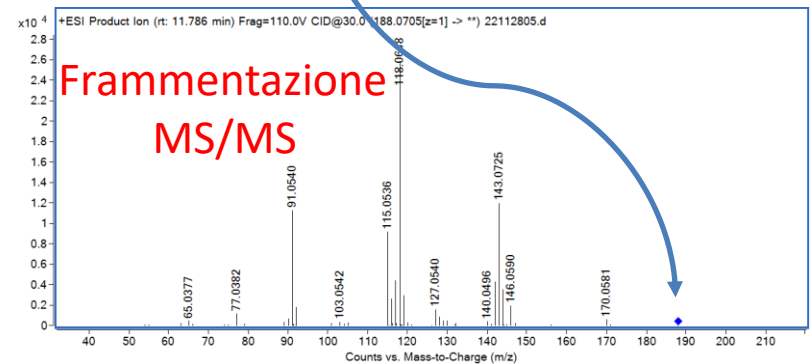
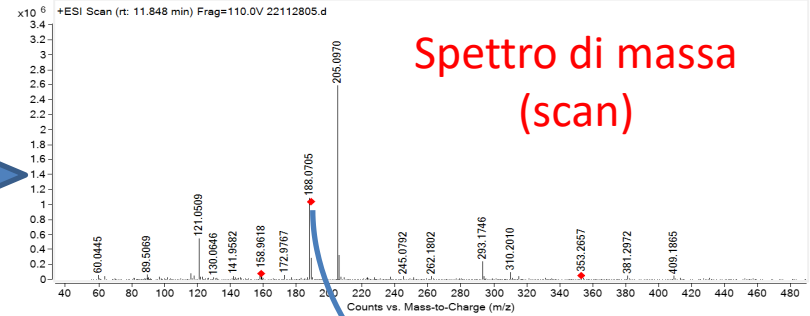
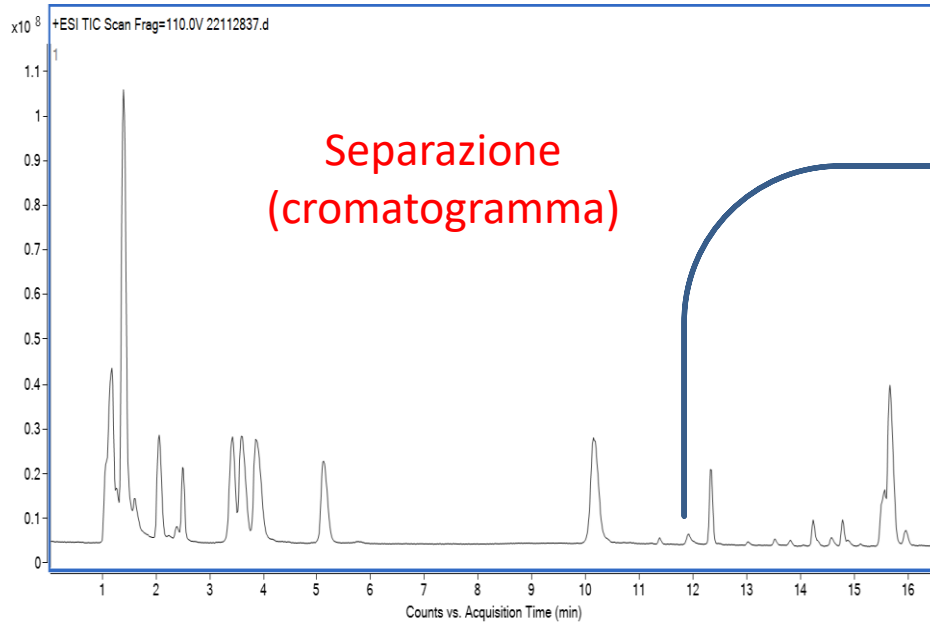
# Spettrometro di massa ESI-Q-TOF



# UHPLC-ESI-Q-TOF



# Dati liquido-massa



# Caratteristiche del tessuto

- Donatore

- M, 54 anni, emorragia cerebrale, microbiologia negativa (BH), sierologia negativa, IDP=1:25.

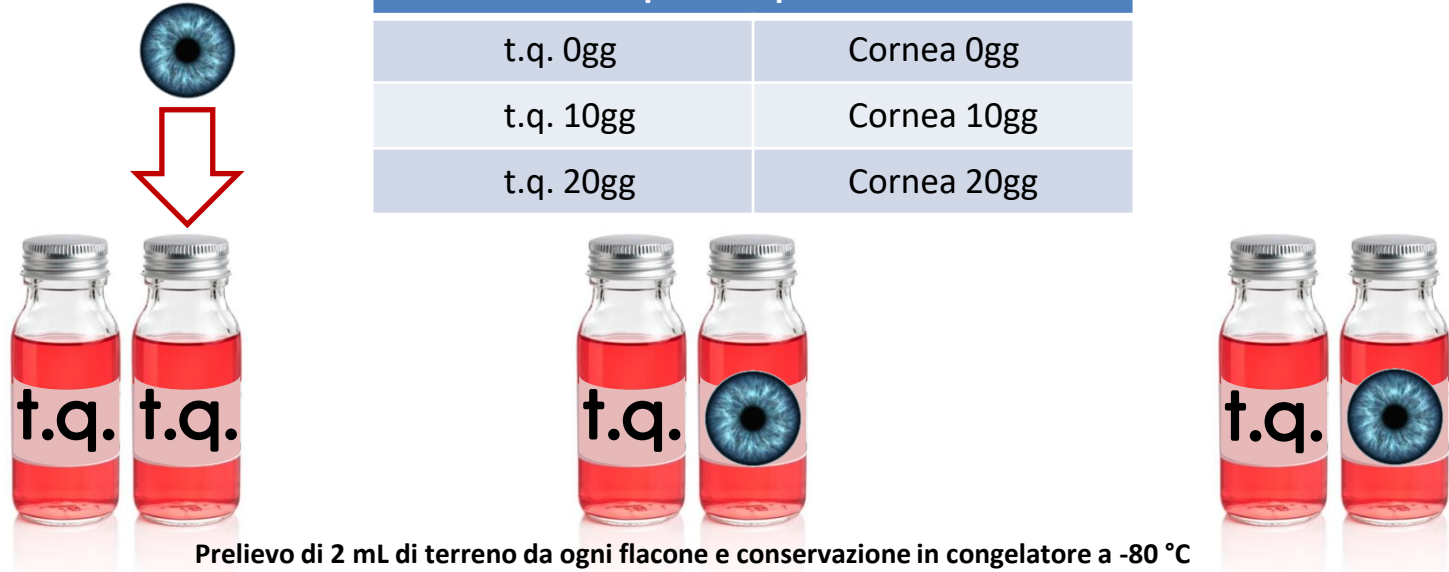
- Cornea

- 4 °C: polimorfismo lieve, margini omogenei, mortalità su pieghe 6-10%, 2400 cells/mm<sup>2</sup>.
- 31 °C: morfologia alterata, margini irregolari, mortalità su pieghe >10%, 1600 cells/mm<sup>2</sup>.

# Procedura di campionamento

## 6 campioni: t.q. vs Cornea

t.q. 0gg	Cornea 0gg
t.q. 10gg	Cornea 10gg
t.q. 20gg	Cornea 20gg



Prelievo di 2 mL di terreno da ogni flacone e conservazione in congelatore a -80 °C

T.A.  
0gg

31 °C  
10gg

31 °C  
20gg

# Misure in LC-MS-ESI-Q-TOF

Ioni Pos  
e Neg

Acquisizione  
spettri di massa  
(scan)

Acquisizione  
spettri in MS/MS

BIANCO
TQ T0
TQ T0
TQ T0
BIANCO
TQ T10
TQ T10
TQ T10
BIANCO
TQ T20
TQ T20
TQ T20

BIANCO
CORNEA T0
CORNEA T0
CORNEA T0
BIANCO
CORNEA T10
CORNEA T10
CORNEA T10
BIANCO
CORNEA T20
CORNEA T20
CORNEA T20

BIANCO
Pool DDA
Pool DDA
Pool DDA
Pool DDA
Pool DDA
Pool DDA
Pool DDA
Pool DDA
Pool DDA
Pool DDA



# Dati processati con MS-DIAL



# Lista di molecole e loro andamento

Alignment Table

Num of rows: 144 Metabolite Name Filter Comment Filter 50.00 Mz Range 1696.41 0.2 RT Range 31.8

ID	RT(min)	m/z	Type	Fill %	Metabolite name	Comment	Correlation	S/N	ANOVA P-value	Fold change (Max/Min)	BarChart
3625	10.50	279.1009	[M+H] <sup>+</sup>	0.80	Glu-Met		-0.04	132.9	1.15E-25	82.64	
4083	15.38	309.1274	[M+H] <sup>+</sup>	0.97	5'-(N-Ethylcarboxamido)adenosine		0.73	8917.6	5.15E-23	4.85	
2055	13.59	180.1019	[M+H] <sup>+</sup>	0.46	Benzyl 3-aminopropanoate		0.91	515.7	1.01E-21	1612.42	
1192	1.21	133.0970	[M+H] <sup>+</sup>	0.57	D-Ornithine		0.93	83.1	2.04E-21	22.75	
1760	13.41	160.0427	[M+H-C2H3NO] <sup>+</sup>	0.80	(+)-6-Aminopenicillanic acid		0.32	59.9	3.45E-21	53.08	
1483	1.45	148.0602	[M+H-C7H11NO3] <sup>+</sup>	0.91	(2S)-2-[[[(1S)-1-Carboxy-3-methylbutyl]carbamoylamino]...		0.11	1497.8	5.86E-21	19.52	
1239	1.62	137.0711	[Cat] <sup>+</sup>	0.46	1-Methylnicotinamide cation		0.92	777.0	1.20E-20	3067.54	
1121	3.49	130.0509	[M+H] <sup>+</sup>	0.80	L-Pyroglutamic acid		0.82	6764.0	1.70E-20	3.43	
852	1.61	116.0705	[M+H] <sup>+</sup>	0.94	L-Proline		1.00	253.9	5.93E-20	4.19	
4415	13.82	335.1061	[M+H] <sup>+</sup>	0.69	N-Phthalyl-L-tryptophan		-0.11	726.6	7.11E-20	344.29	
3581	1.80	276.1190	[M+H] <sup>+</sup>	0.80	Glu-Gln		-0.04	2093.8	1.00E-19	52.53	
1236	4.56	137.0458	[M+H-C5H8O4] <sup>+</sup>	0.54	Inosine		0.55	272.9	1.49E-19	256.22	
2348	15.16	198.1276	[M+H-C8H4O3] <sup>+</sup>	1.00	2-[[[Di(2-benzylamino)carbonyl]benzoic acid		0.93	52.9	2.15E-19	26.81	
1572	5.86	153.0404	[M+H] <sup>+</sup>	0.54	Xanthine		0.72	154.7	6.02E-19	290.32	
1472	14.57	147.1125	[M+NH4] <sup>+</sup>	0.71	D-Pipecolic acid		0.67	146.3	6.51E-19	29.76	
2038	13.12	178.0861	[M+H-H2O] <sup>+</sup>	0.80	O-Benzyl-D-serine		0.25	93.0	9.33E-19	9.33	
4525	1.46	347.0938	[M+Na-H2O] <sup>+</sup>	0.71	2.alpha.-Mannobiose		-0.06	107.4	9.50E-19	307.50	
3319	3.49	259.0925	[2M+H] <sup>+</sup>	0.80	D-Pyroglutamic acid		0.83	13652.4	1.43E-18	6.62	
2897	11.40	232.1547	[M+H] <sup>+</sup>	0.66	(R)-Butyrylcarnitine		0.77	40.9	1.83E-18	7.92	
3194	2.14	249.1079	[M+H] <sup>+</sup>	0.63	Glu-Thr		-0.03	56.2	2.04E-18	69.41	
2542	1.48	210.1335	[M+H] <sup>+</sup>	0.54	2,2-Bis[hydroxymethyl]-2,2',2''-nitriolotriethanol		0.57	34.4	2.94E-18	910.69	
3162	10.28	247.1287	[M+H] <sup>+</sup>	0.80	Glu-Val		0.02	116.9	4.97E-18	9.25	

# Totale composti

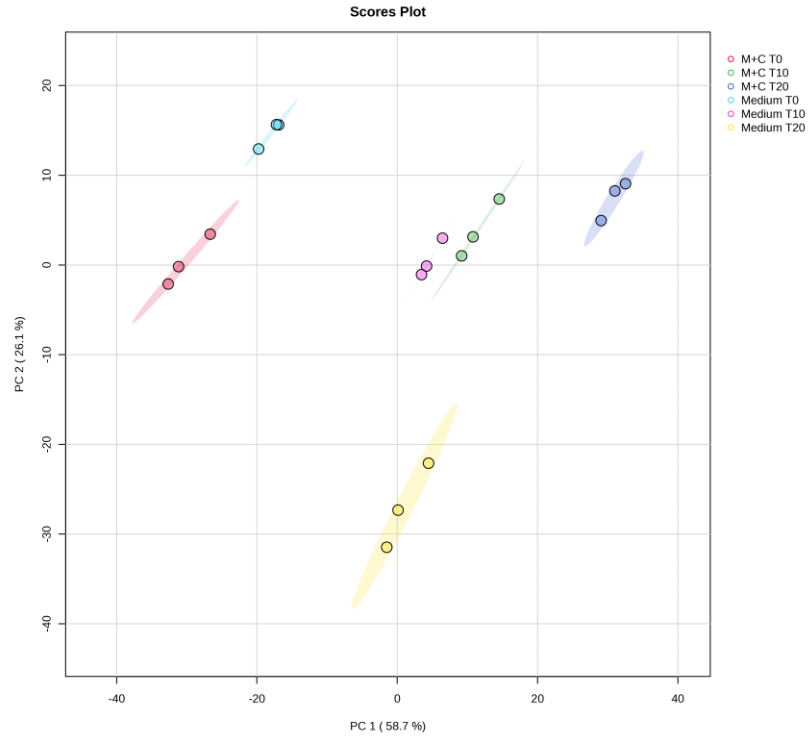
Tot annotati in pos.....	123
Tot annotati in neg.....	66
Trovati in entrambe le polarità.....	10
Eliminati perché non presenti in tutti i campioni...5	
Tot finale annotati MS/MS NIST.....	<b>174</b>

# Elaborazione MetaboAnalyst

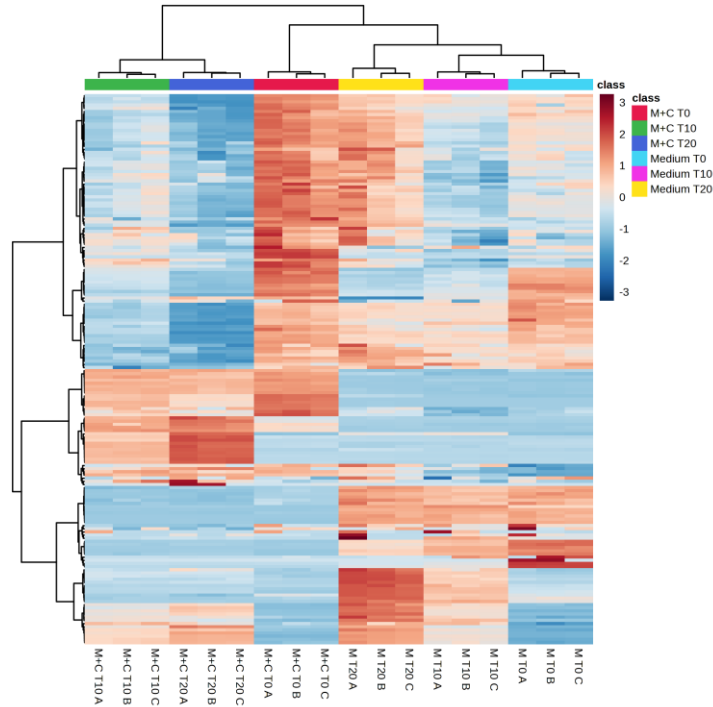


- Esportazione dati da MS-DIAL a MetaboAnalyst
- Analisi statistica PCA
- Analisi statistica Heat Map
- Analisi dei Pathway

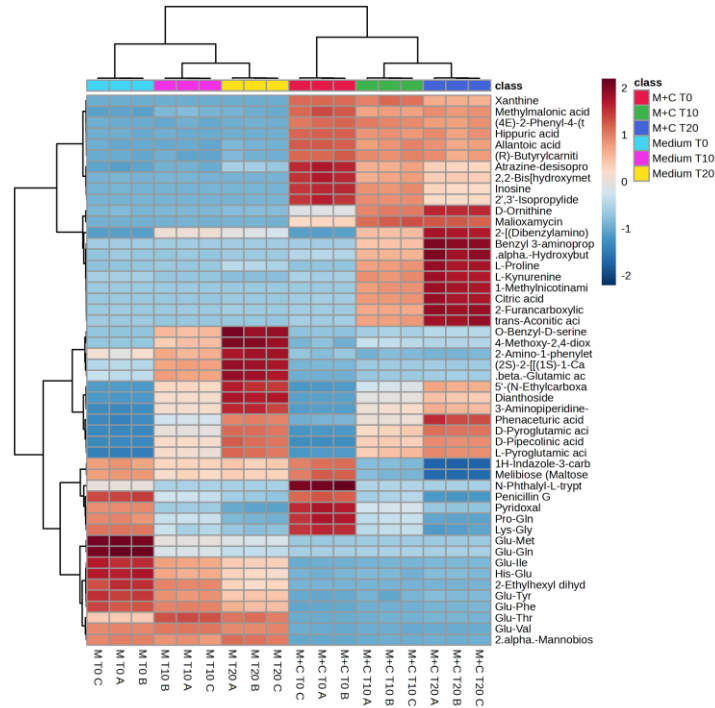
# PCA



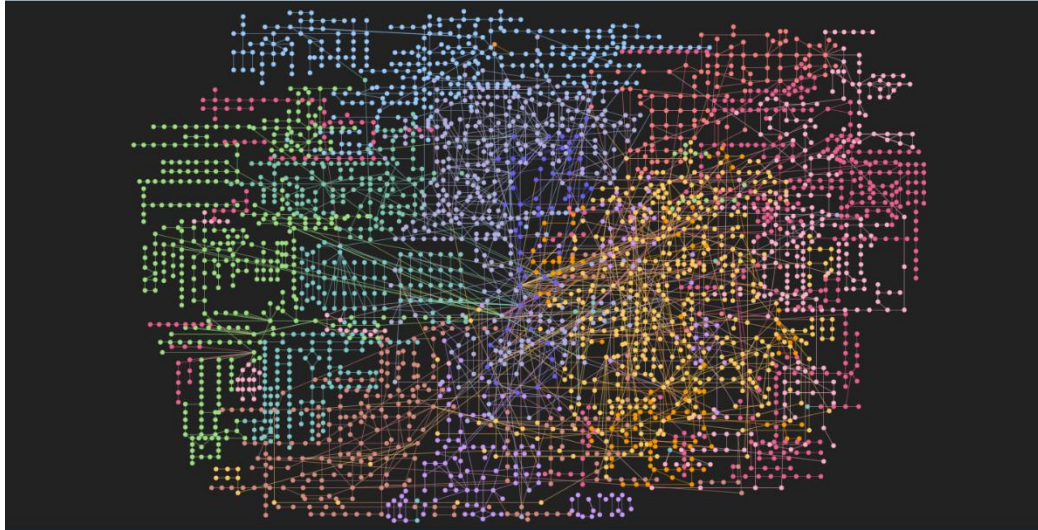
# Analisi dei Cluster (Heat Map)



# Heat Map dei TOP 50



# Network metabolico umano



105 molecole

36 pathway

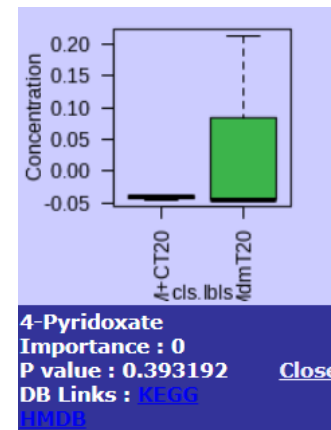
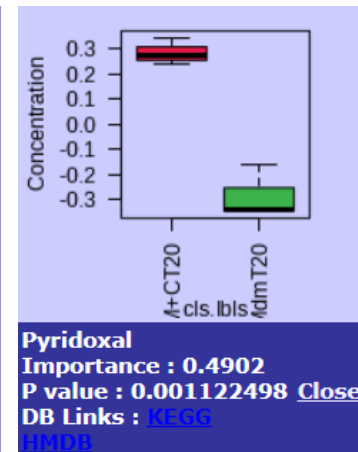
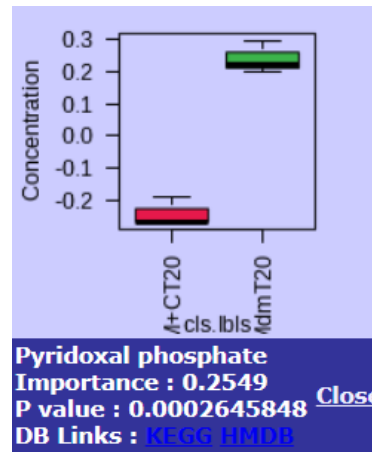
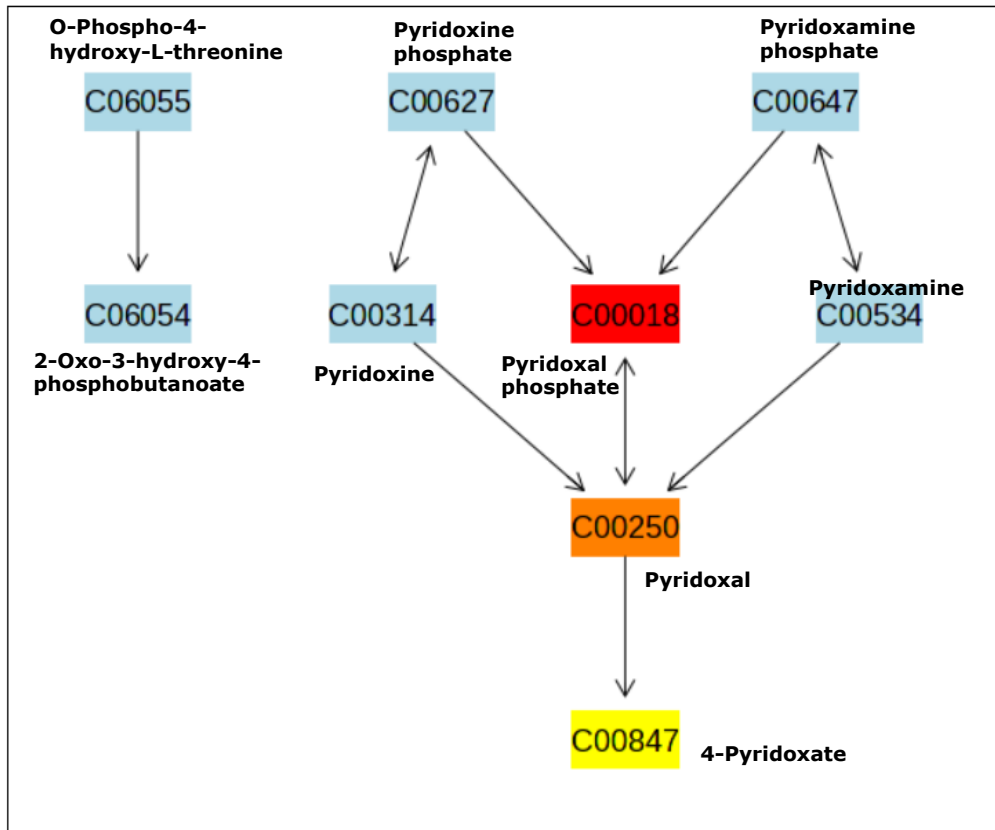


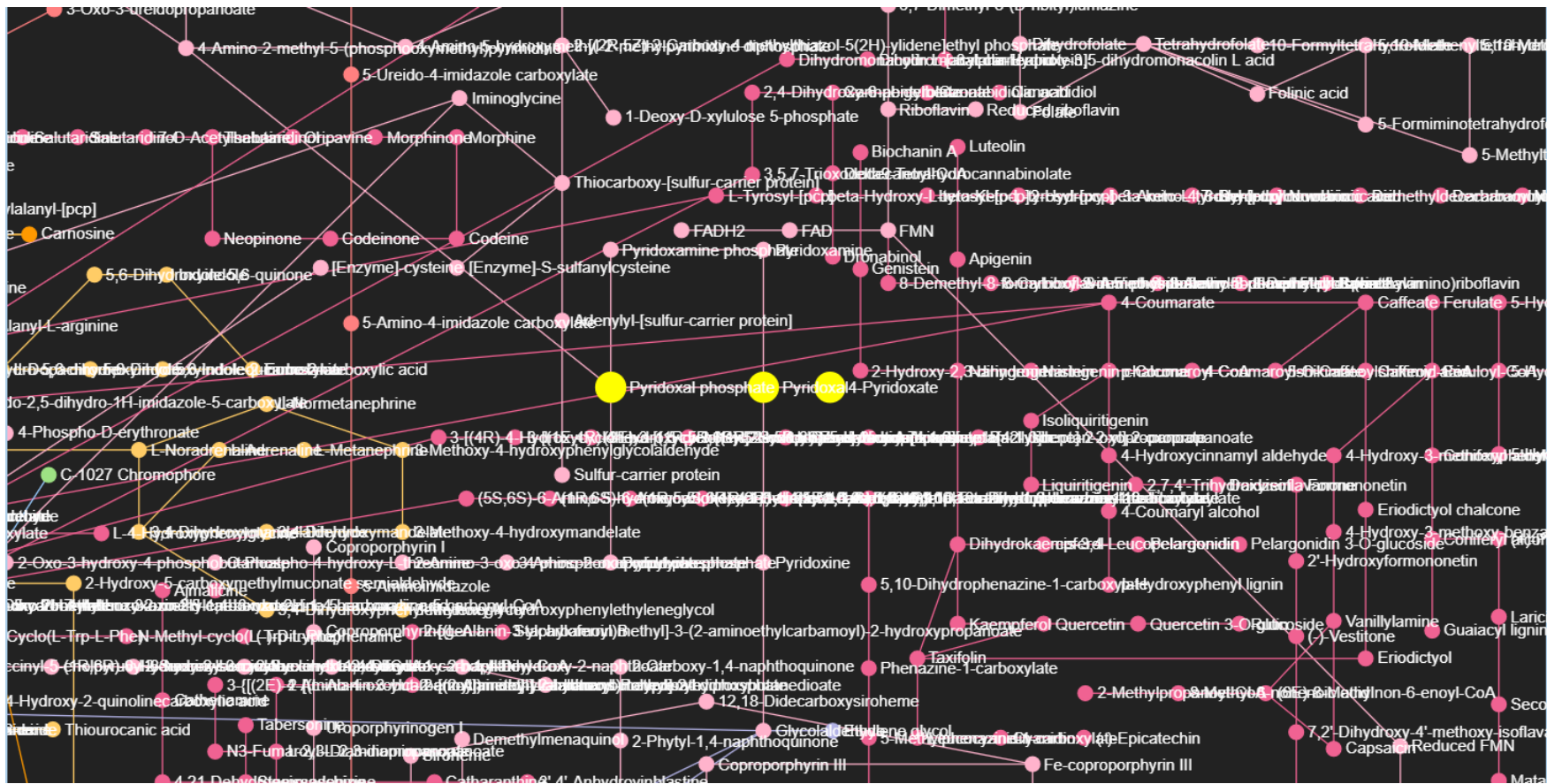
# Analisi dei pathway

1	D-Arginine and D-ornithine metabolism
2	Starch and sucrose metabolism
3	Purine metabolism
4	Primary bile acid biosynthesis
5	Vitamin B6 metabolism
6	Amino sugar and nucleotide sugar metabolism
7	Citrate cycle (TCA cycle)
8	D-Glutamine and D-glutamate metabolism
9	Glycine, serine and threonine metabolism
10	Glutathione metabolism (meccanismo antiossidante)
11	Riboflavin metabolism B2
12	Alanine, aspartate and glutamate metabolism
13	Glyoxylate and dicarboxylate metabolism
14	Propanoate metabolism
15	Glycerophospholipid metabolism
16	Pyrimidine metabolism
17	Nitrogen metabolism
18	Tryptophan metabolism

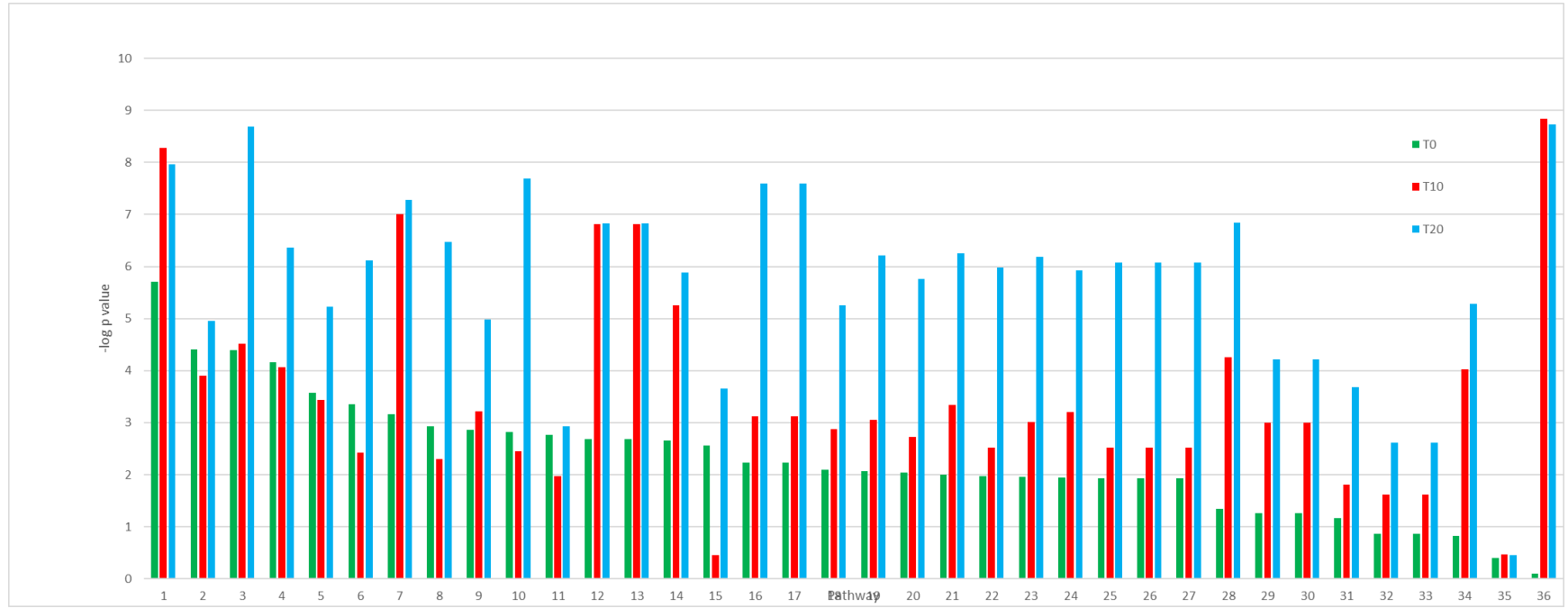
19	Valine, leucine and isoleucine degradation
20	Pantothenate B5 and CoA biosynthesis
21	Arginine biosynthesis
22	Phenylalanine metabolism
23	Valine, leucine and isoleucine biosynthesis
24	Aminoacyl-tRNA biosynthesis
25	Phenylalanine, tyrosine and tryptophan biosynthesis
26	Tyrosine metabolism
27	Ubiquinone and other terpenoid-quinone biosynthesis
28	Arginine and proline metabolism
29	One carbon pool by folate
30	Folate biosynthesis B9
31	Cysteine and methionine metabolism
32	Lysine degradation
33	Biotin metabolism B8
34	Thiamine metabolism B1
35	Drug metabolism - cytochrome P450
36	Nicotinate and nicotinamide metabolism B3

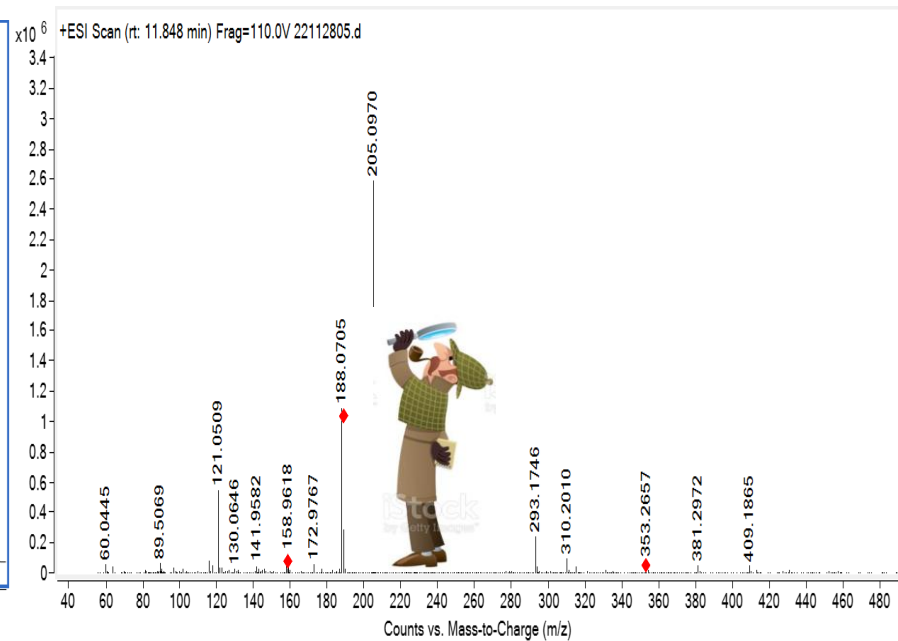
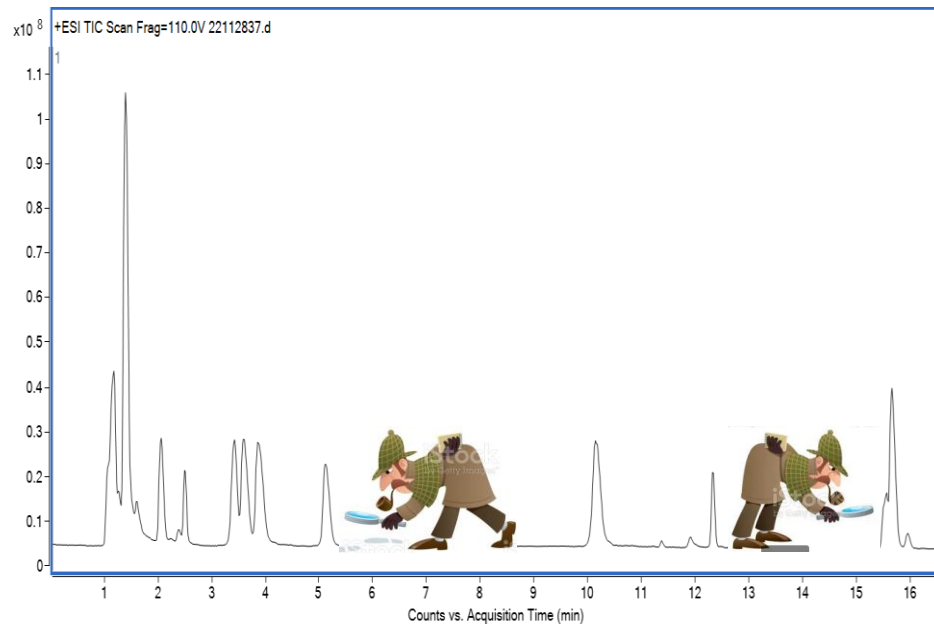
## Vitamin B6 metabolism





# Confronto pathway ai 3 tempi





# *Un sincero grazie a tutti....*

.....voi per la  
Gentile attenzione

alla **SIBO**

alla **Banca degli  
Occhi di Fabriano**



Simone Angeloni

Massimiliano Corneli

Silvia Conforti

Roberto Pellegrino

Husam Alabed