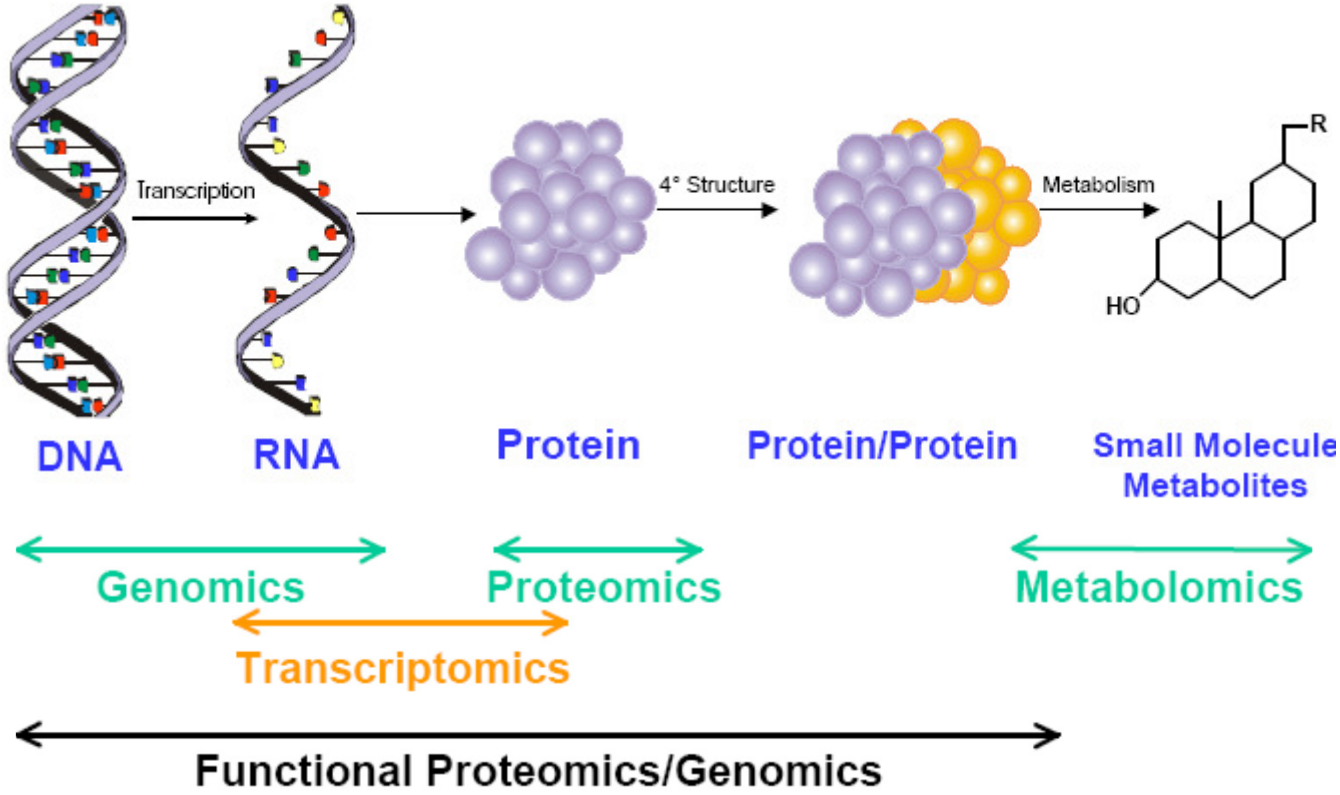
Definitions and Background

Metabolomics = high-throughput analysis of metabolites

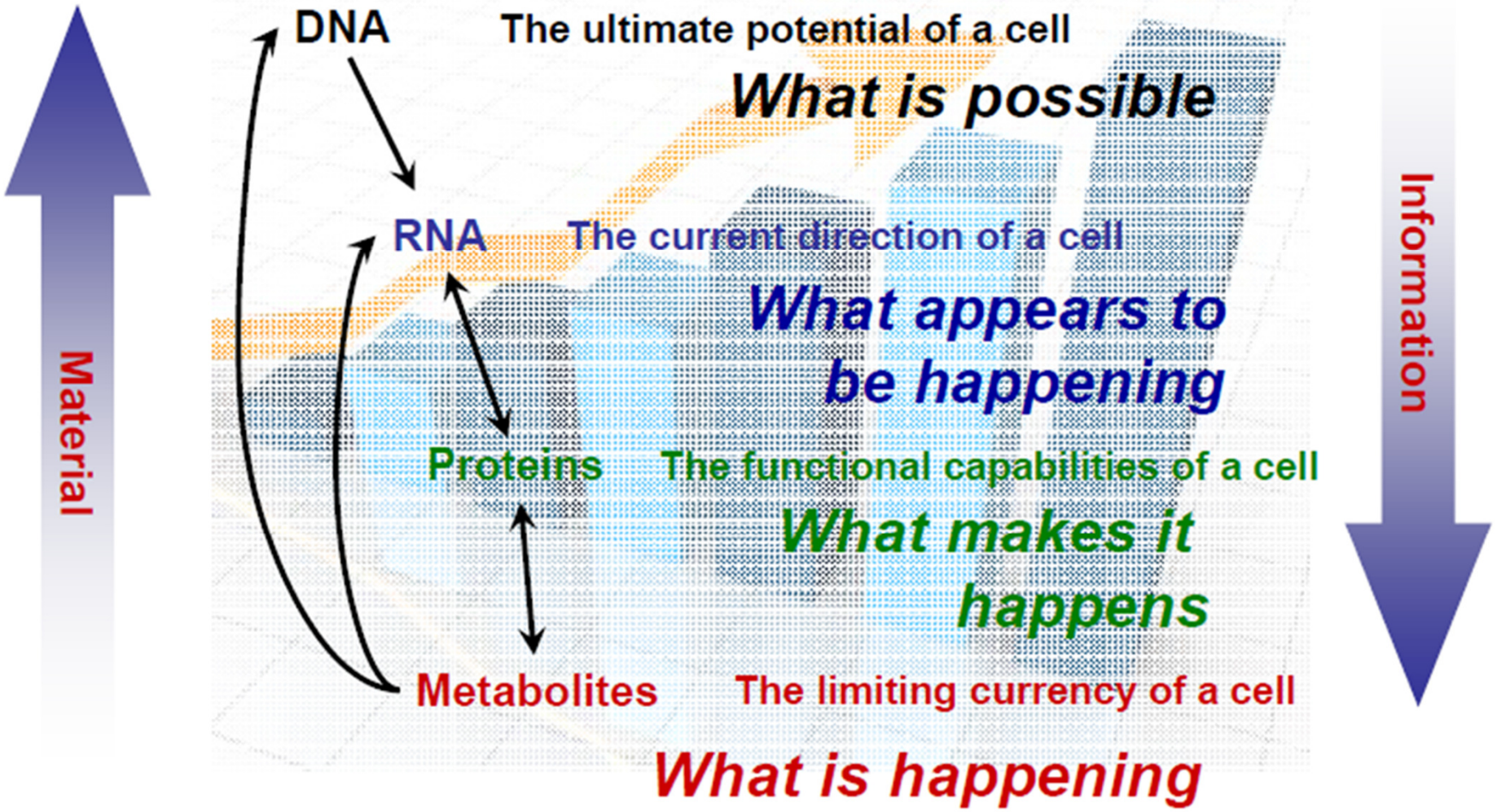
Metabolomics is the simultaneous ('multiparallel') measurement of the levels of a large number of cellular metabolites (typically several hundred). Many of these are not identified (i.e. are just peaks in a profile).



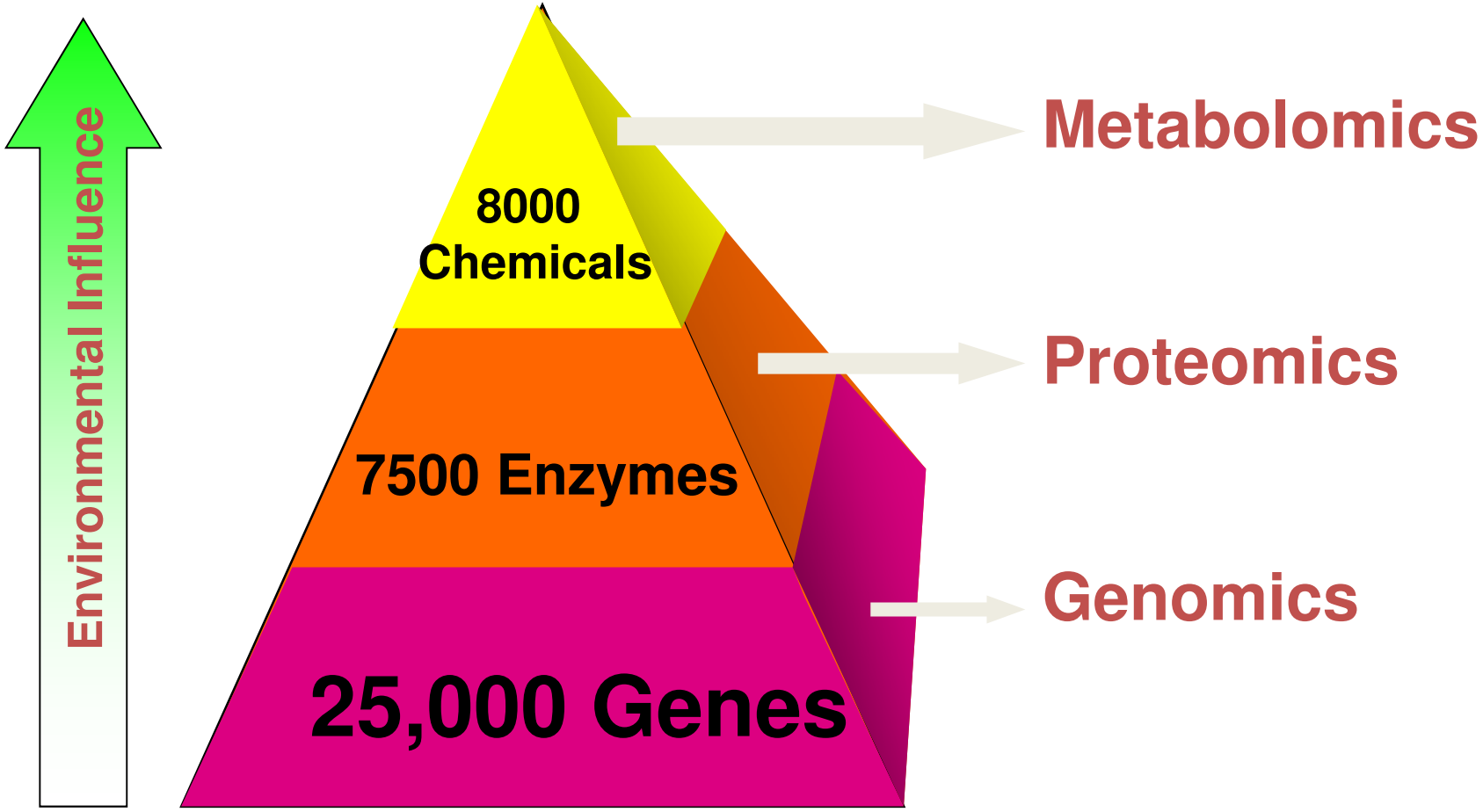
Not hypothesis driven

snapshot

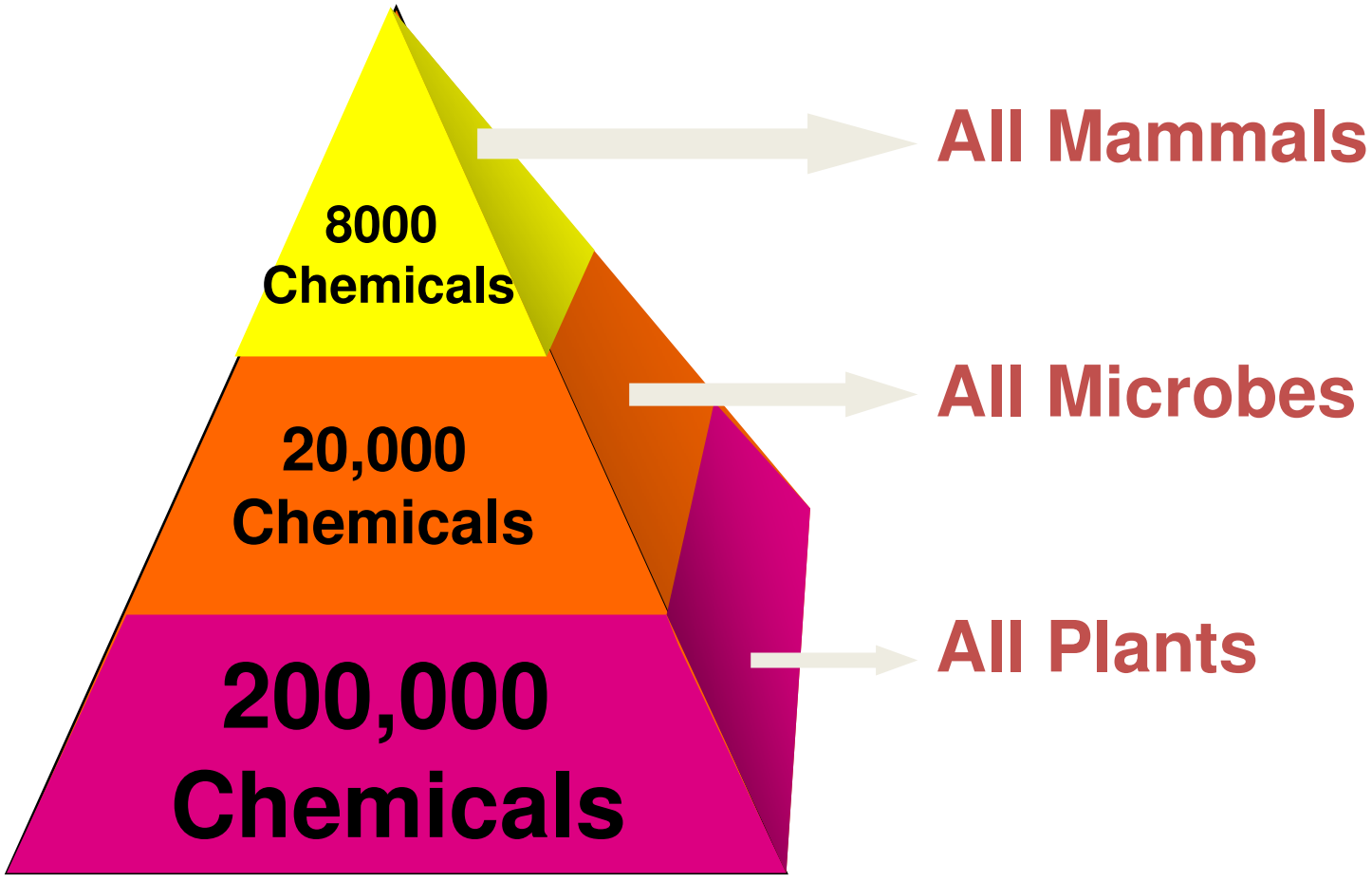
Definitions and Background



The Pyramid of Life



Different Metabolomes

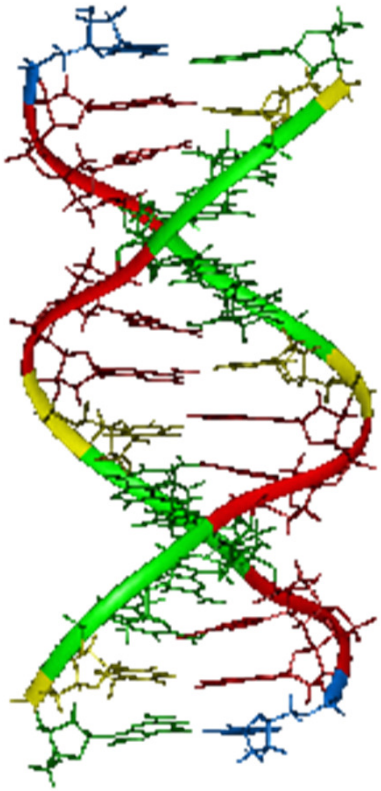


The Pyramid of Life

Small Molecules Count…

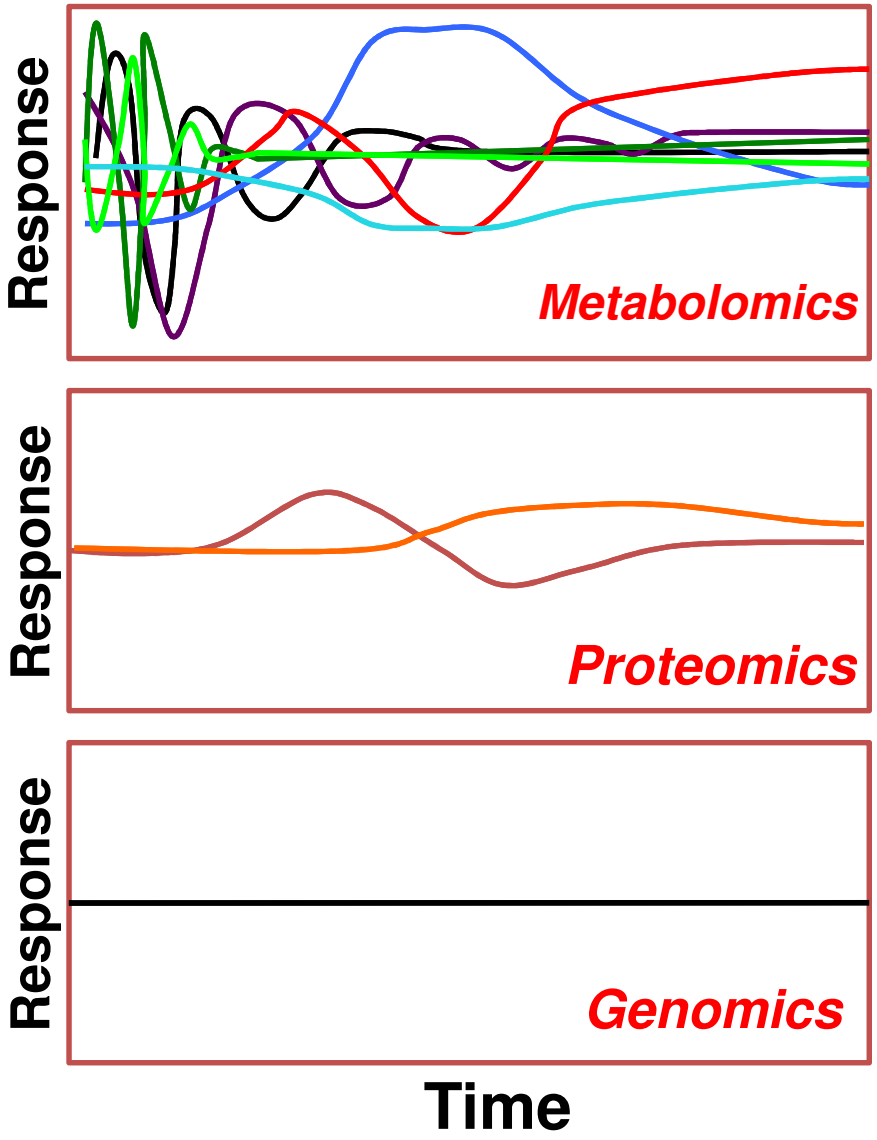
- >95% of all diagnostic clinical assays test for small molecules
- 89% of all known drugs are small molecules
- 50% of all drugs are derived from pre-existing metabolites
- 30% of identified genetic disorders involve diseases of small molecule metabolism
- Small molecules serve as cofactors and signaling molecules to 1000’s of proteins

Metabolites Are the Canaries of the Genome

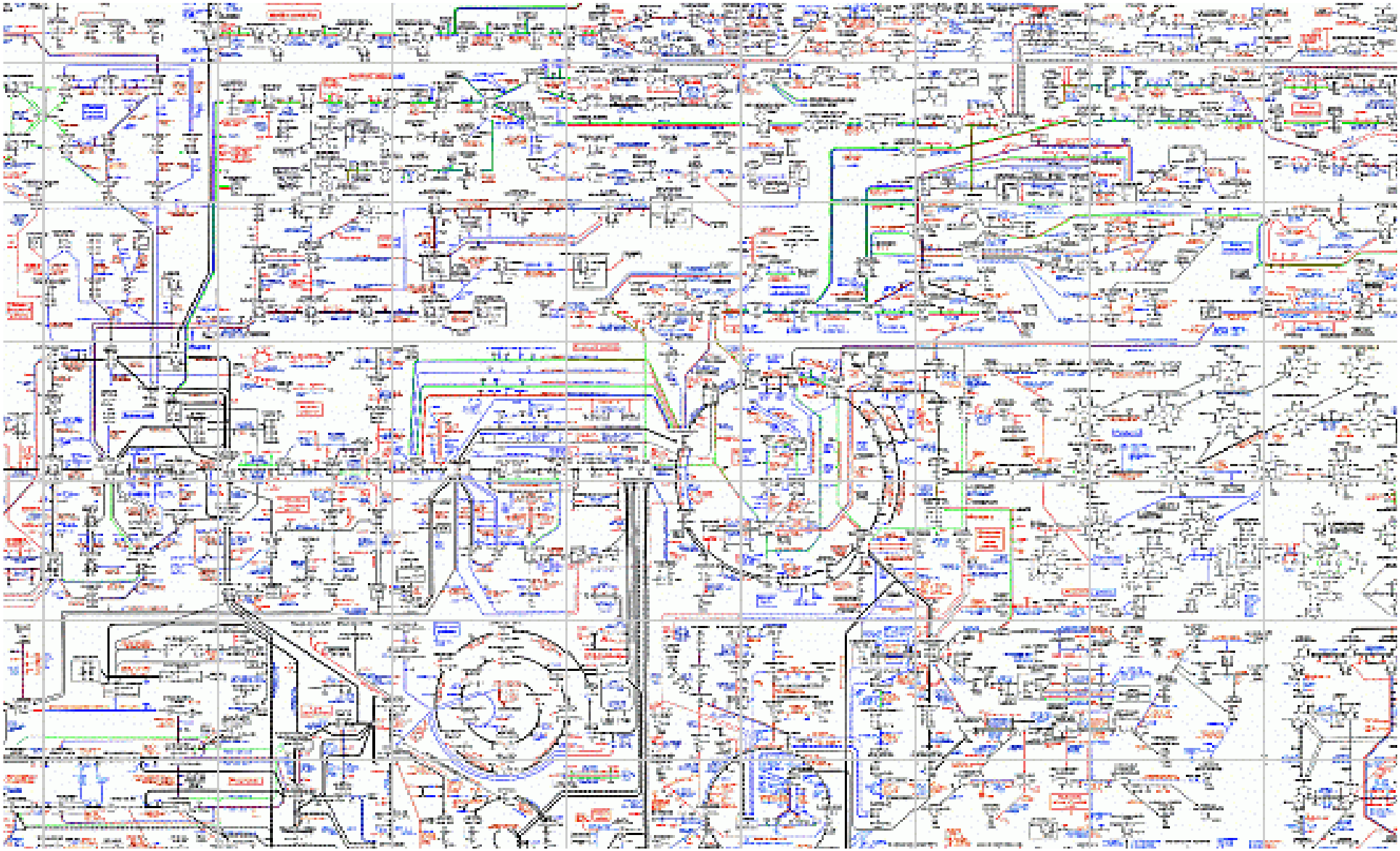


A single base change can lead to a 10,000X change in metabolite levels

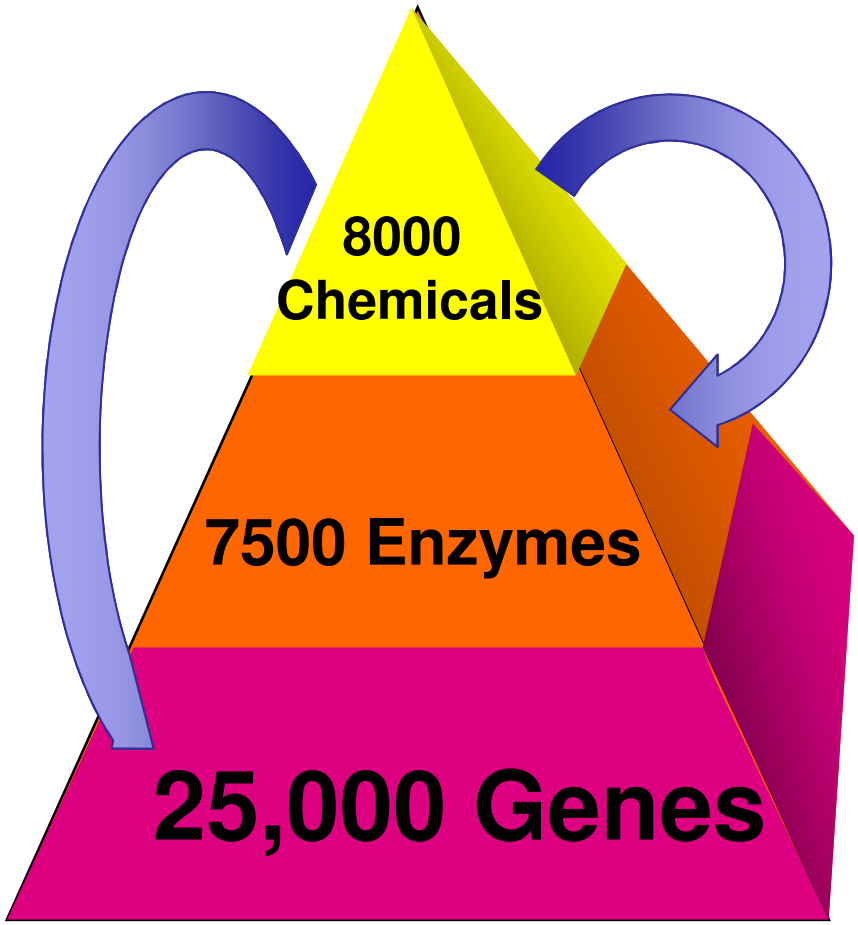
Metabolomics is More Time Sensitive Than Other "Omics"



Metabolism is “Understood”

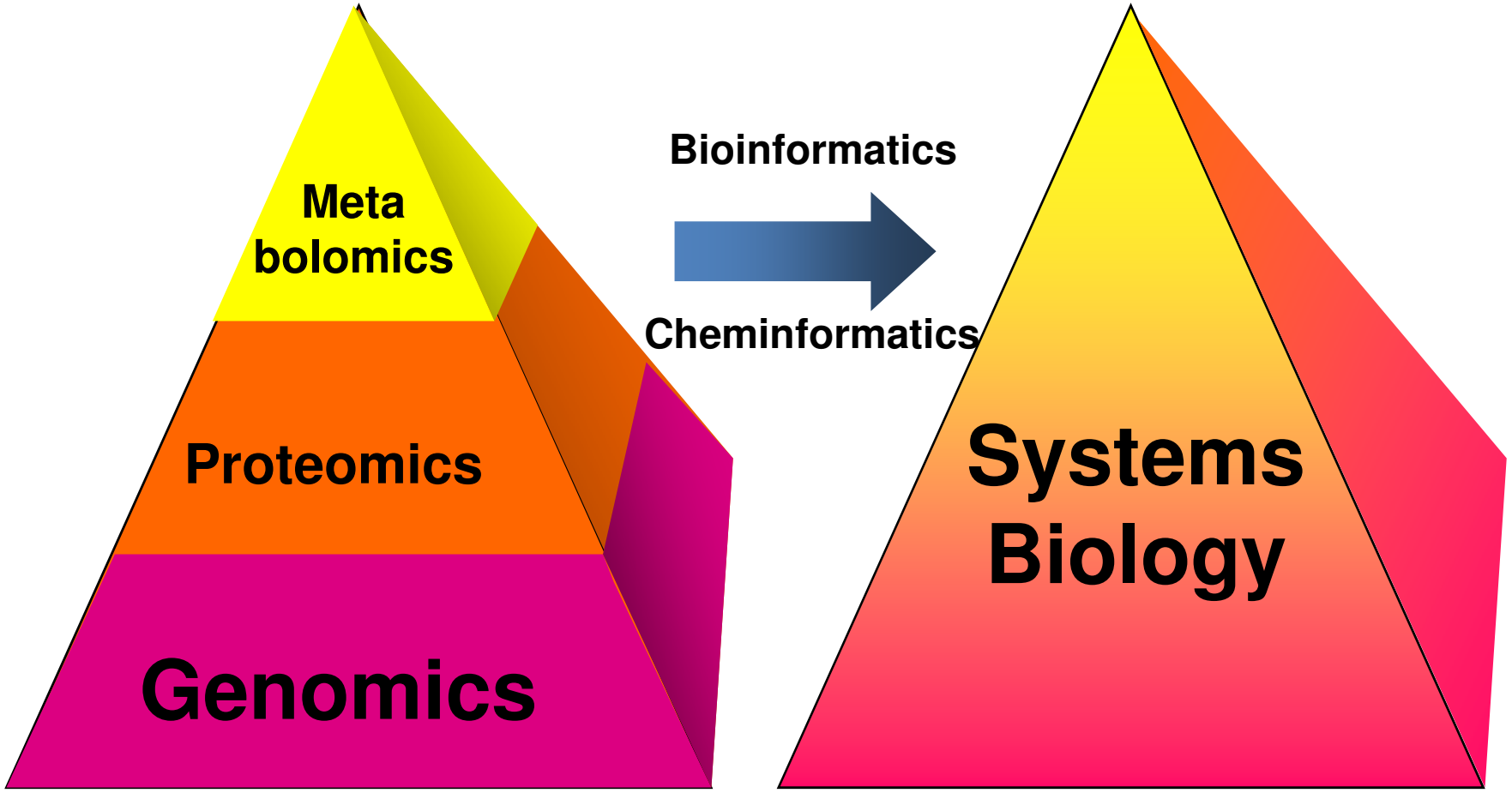


The Metabolome is Connected to all other “Omes”



The Pyramid of Life

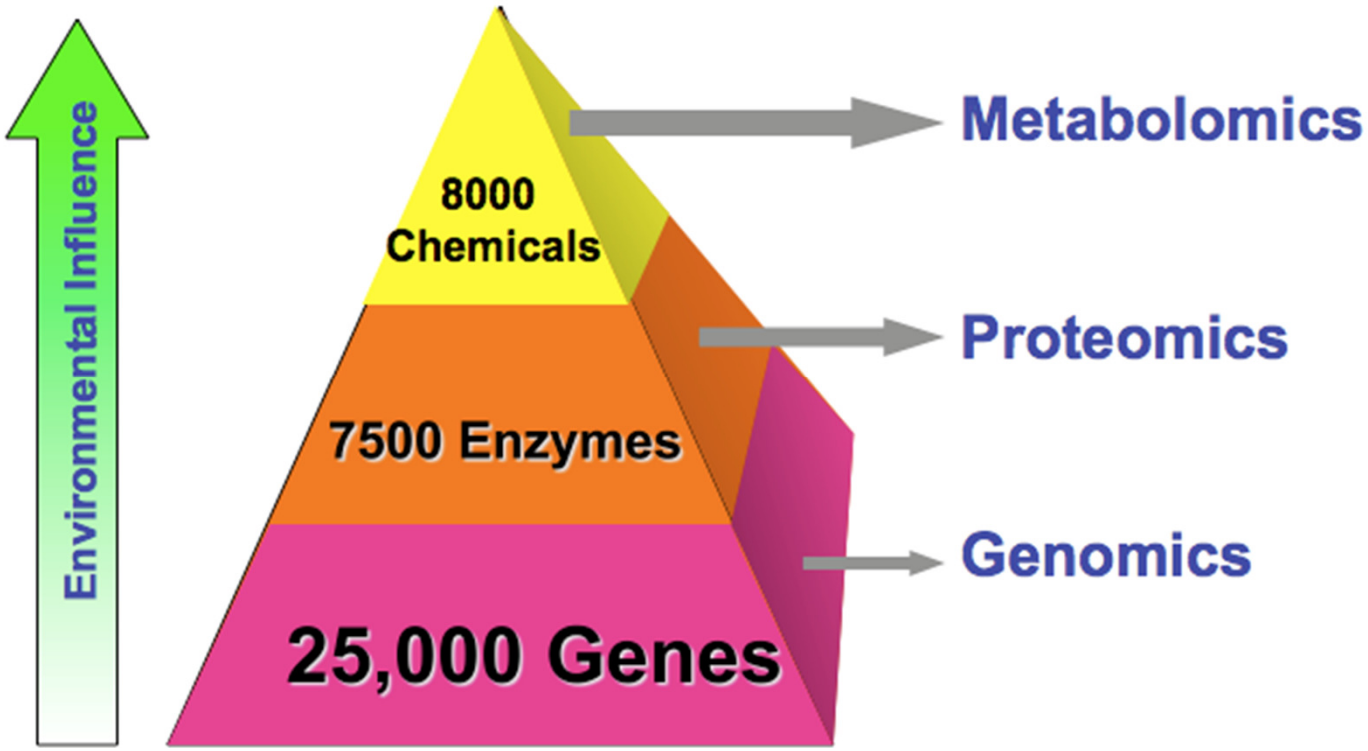
Metabolomics Enables Systems Biology



Metabolomics Applications

- Toxicology Testing
- Clinical Trial Testing
- Fermentation Monitoring
- Food & Beverage Tests
- Nutraceutical Analysis
- Drug Phenotyping
- Water Quality Testing
- Petrochemical Analysis
- Genetic Disease Tests
- Nutritional Analysis
- Clinical Blood Analysis
- Clinical Urinalysis
- Cholesterol Testing
- Drug Compliance
- Transplant Monitoring
- MRS and CS imaging

Metabolomics Methods

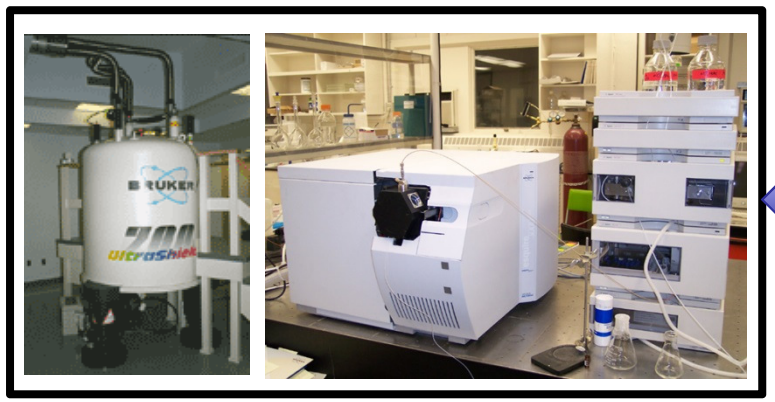


Metabolomics Workflow

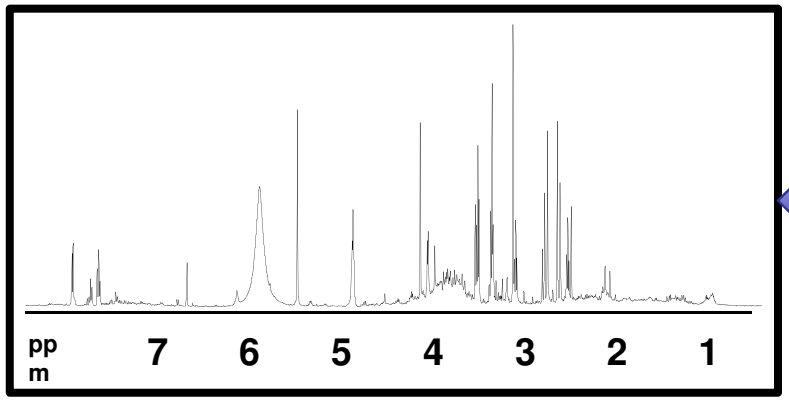
Biological or Tissue Samples

Extraction

Biofluids or Extracts

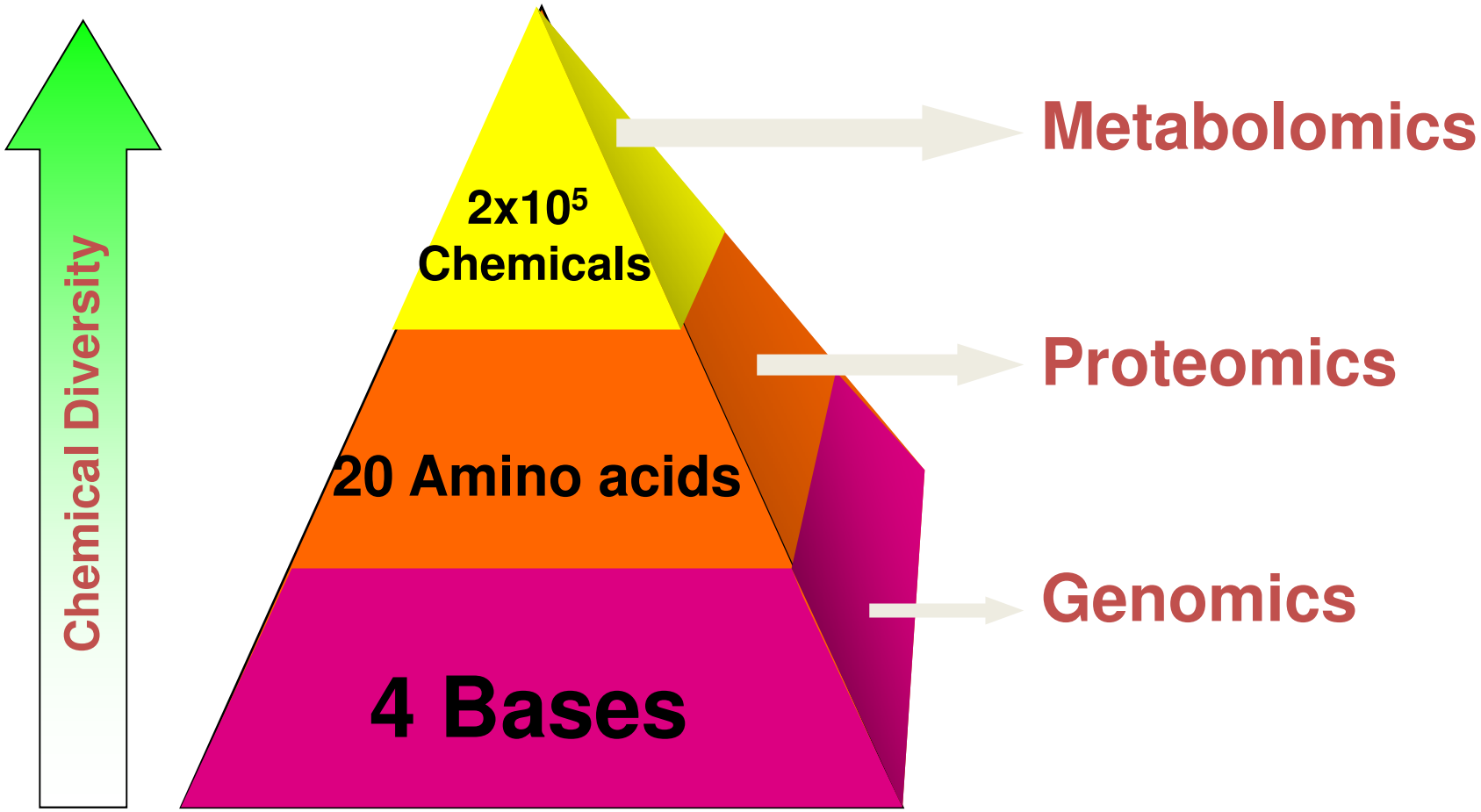


Chemical Analysis



Data Analysis

Why Metabolomics is Difficult

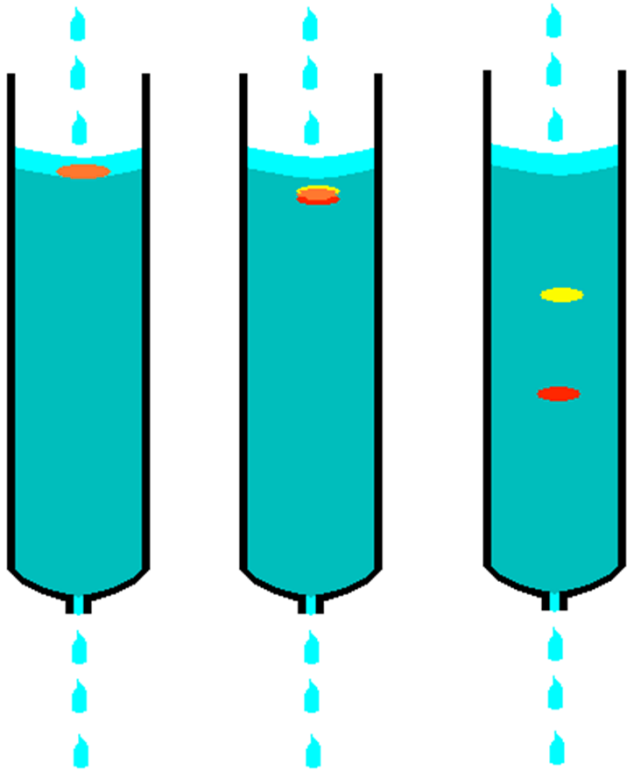


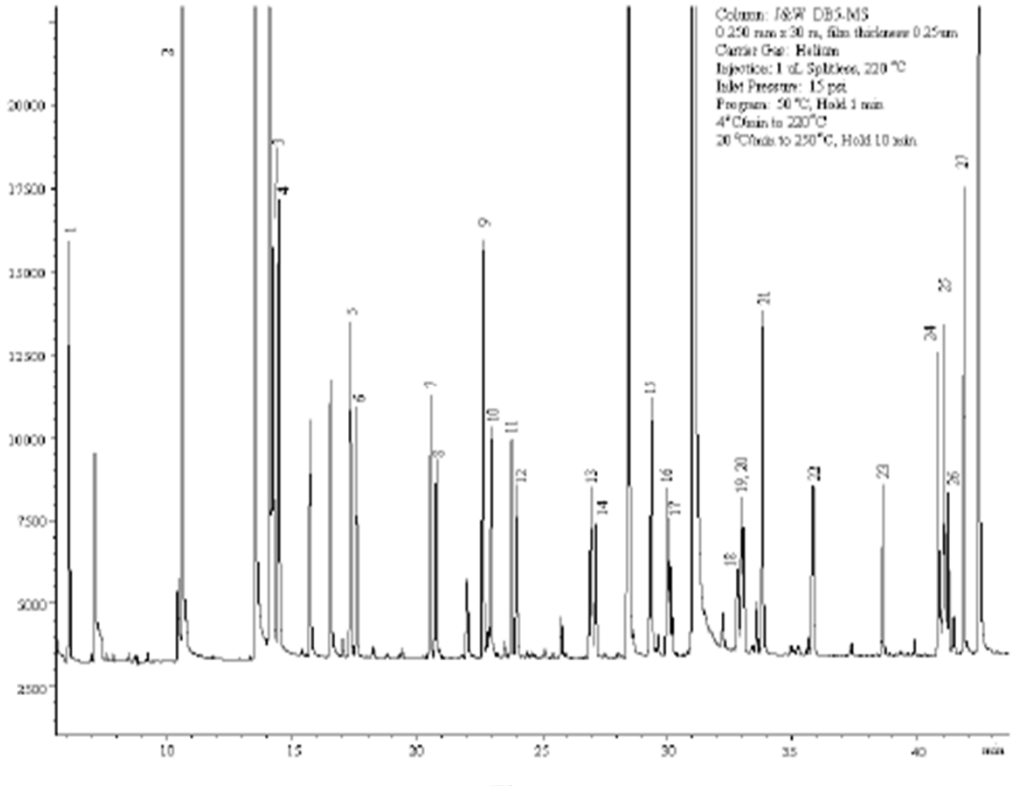
The Pyramid of Life

Metabolomics Technologies

- UPLC, HPLC
- CE/microfluidics
- LC-MS
- FT-MS
- QqQ-MS
- NMR spectroscopy
- X-ray crystallography
- GC-MS
- LIF detection

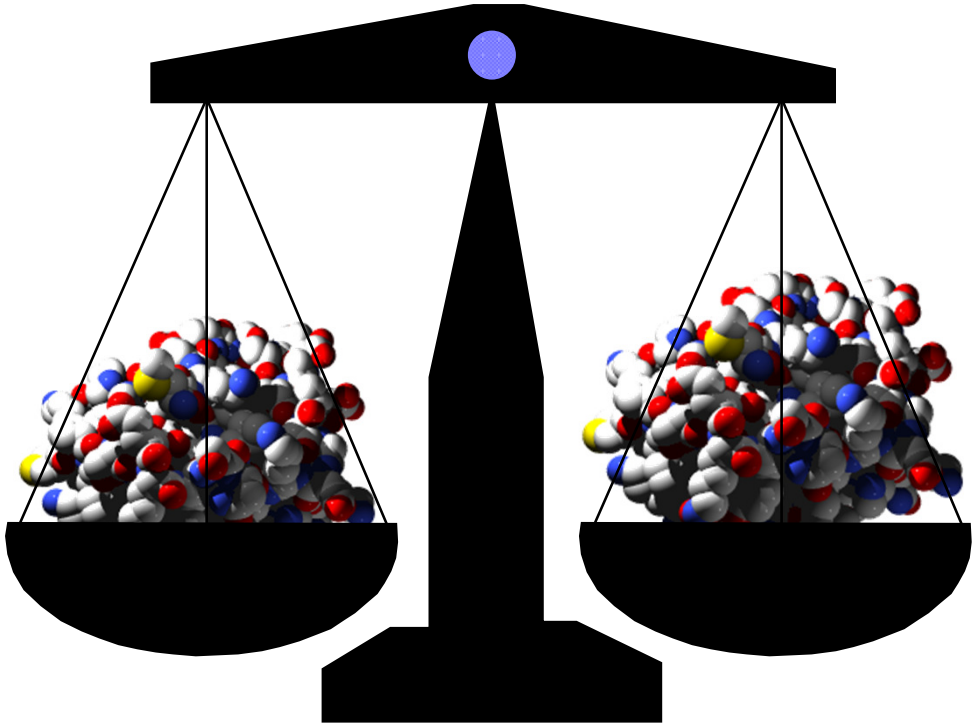
Chromatography



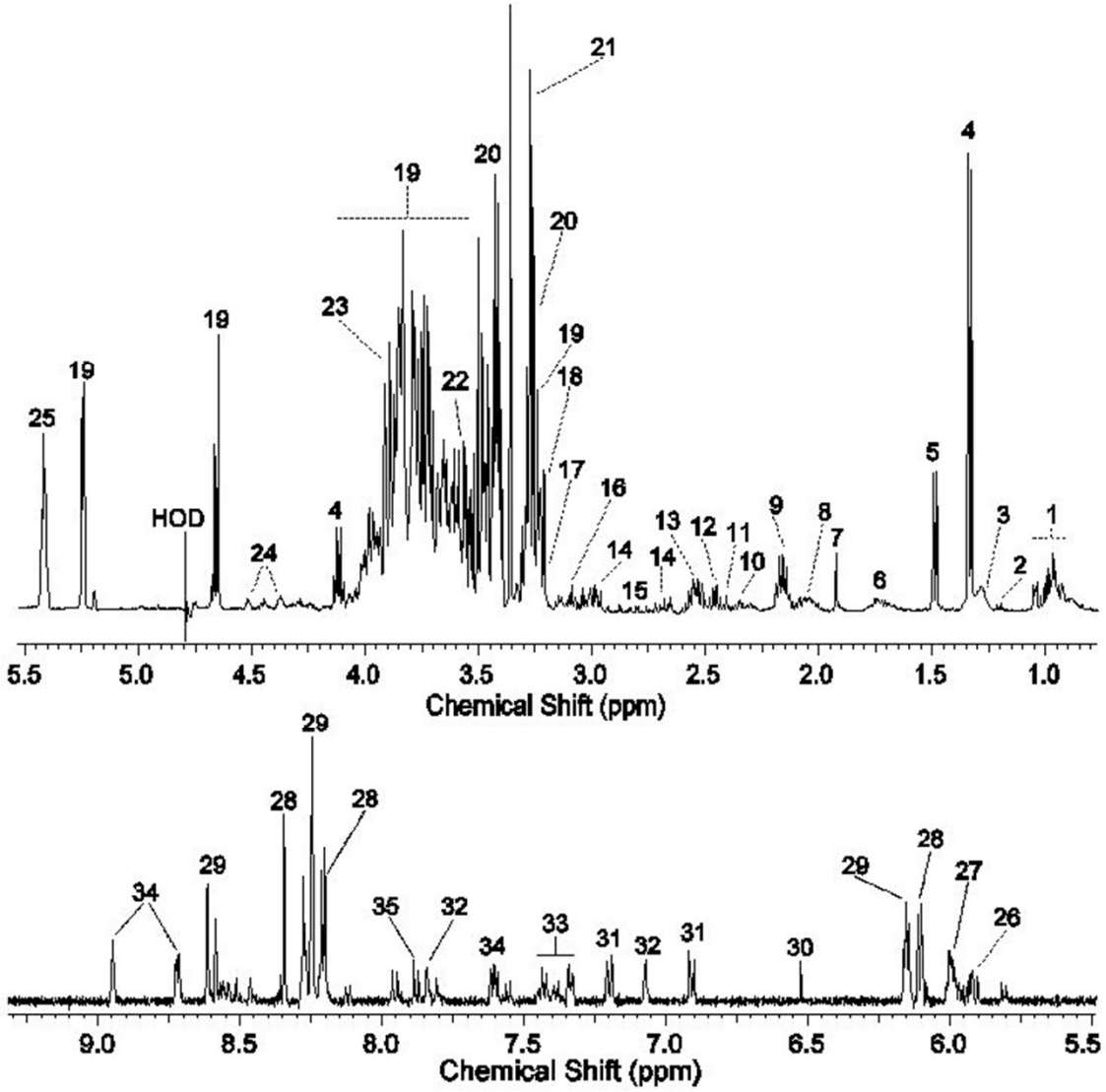


Mass Spectrometry

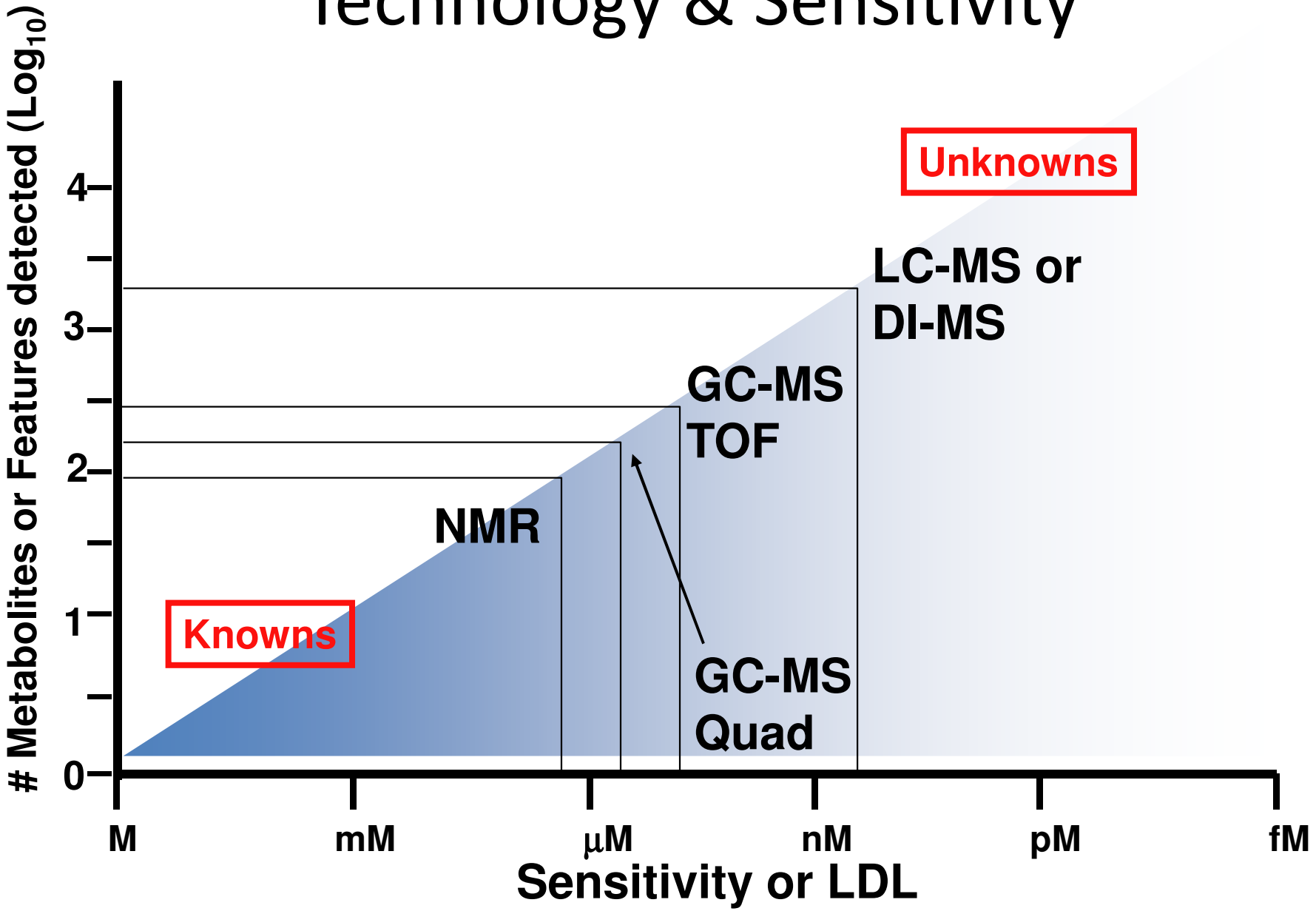
- Analytical method to measure the molecular or atomic weight of samples



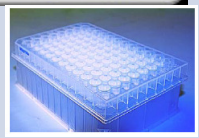
NMR Spectrum of a Biological Mixture



Technology & Sensitivity



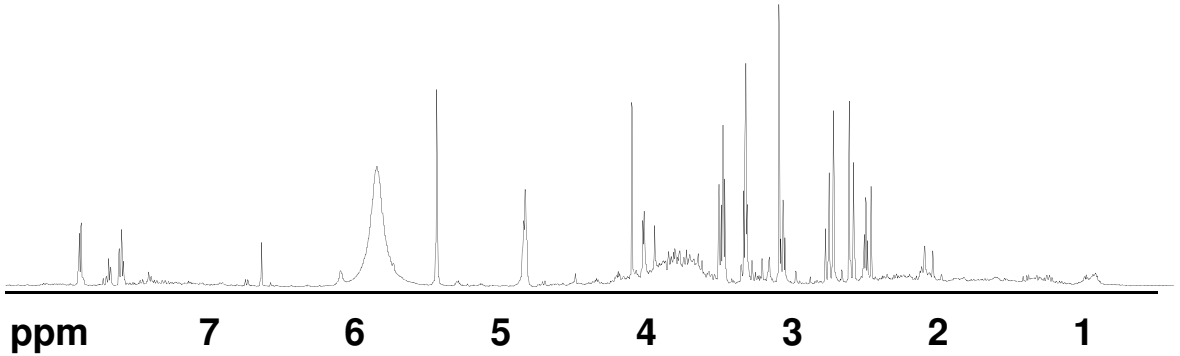
Comparison

	NMR (with cold probe)	GC-MS	DI-MS
Techniques			 
Metabolites	Water-soluble (amino acids, organic acids, sugars)	mainly water-soluble (some hydrophobic)	Mainly hydrophobic (some water-soluble)
Types of samples	Biofluids, plant, bacterial, animal tissue extracts, Food	Biofluids, plant, bacterial, animal tissue extracts, Food	Mainly biofluids
Sample Volume	100 µL (min)	30-50 µL (min)	10 µL

Comparison

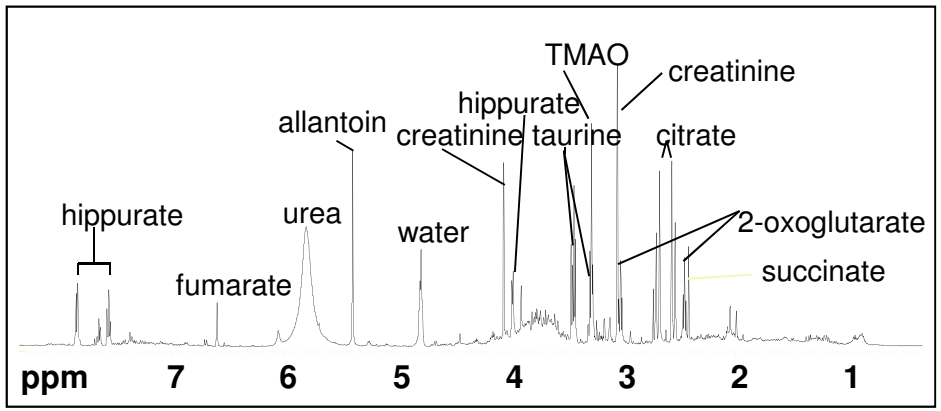
	NMR	GC-MS	DI-MS
Sample prep time	30 -120 min/20 samples	30 -120 min/20 samples	3-4 h for 96 samples
Run time	20 -90 min/sample	30-60 min/sample	7 min/sample
Data Analysis	30-60 min / sample	30-60 min / sample	1-2 h for 96 samples
Limit of Detection	~ 5 µM	~ 100 nM	~ 5 nM
No. of metabolites	~ 20 - 50	~20 -50	~ 100-180
Overlapping Metabolites	10-15	10-15	10-15
Cross-checking	10-30 %	10-30 %	10-30 %

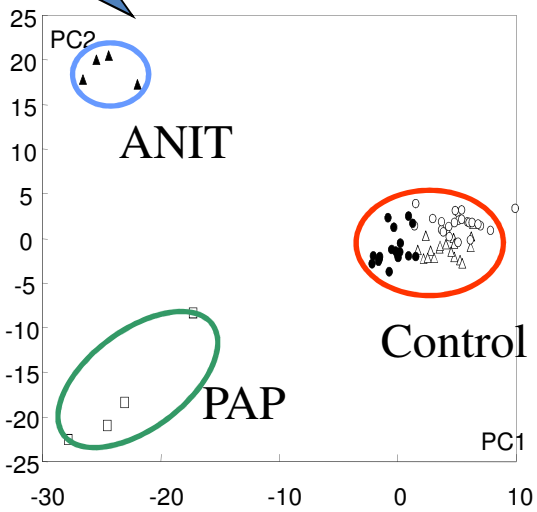
2 Routes to Metabolomics



Quantitative (Targeted) Methods

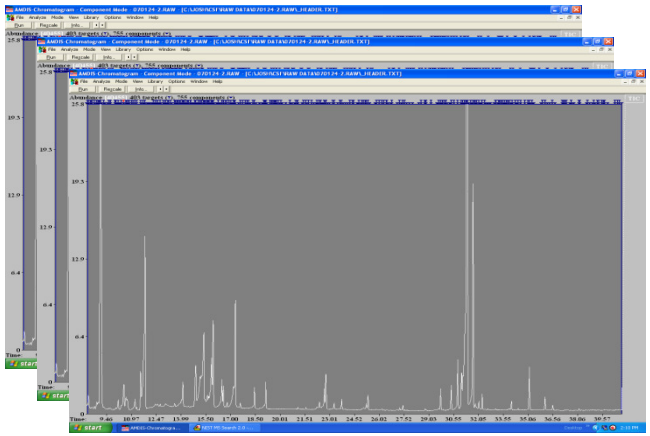
Chemometric (Profiling) Methods



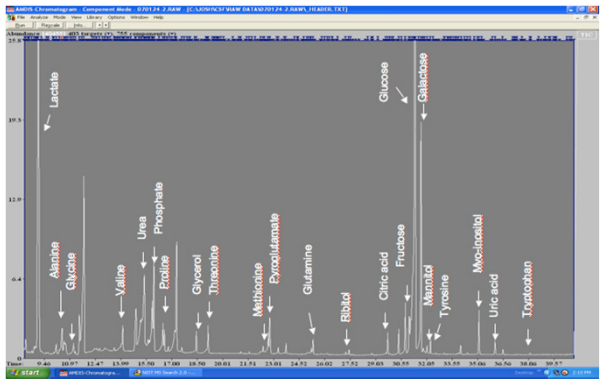


Profiling (Untargeted)

Sample Prep

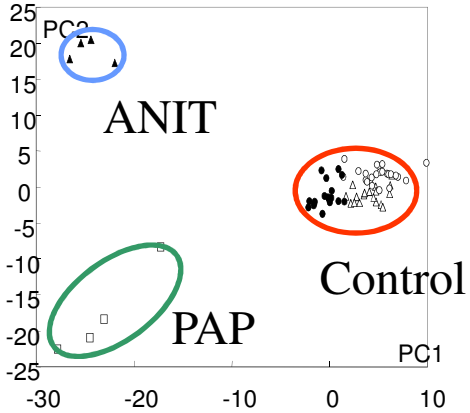


Data Collection



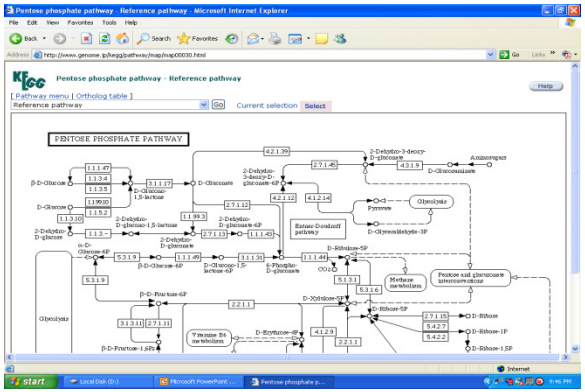
Metabolite Identification

Data Reduction

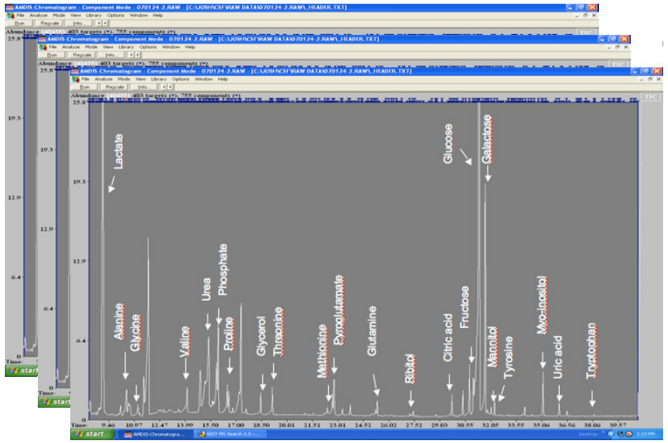


Quantitative (Targeted)

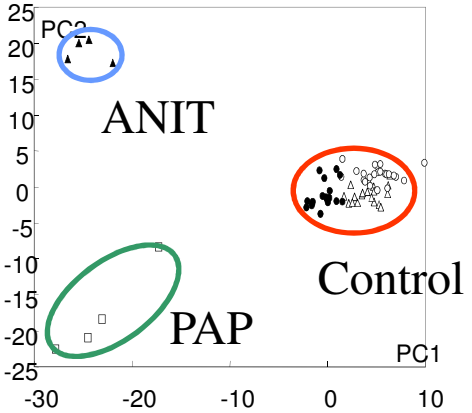
Sample Prep



Biological Interpretation

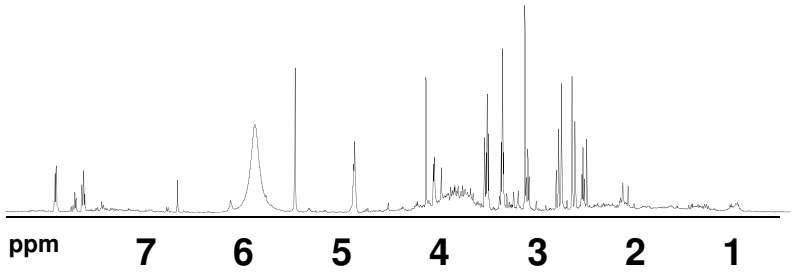


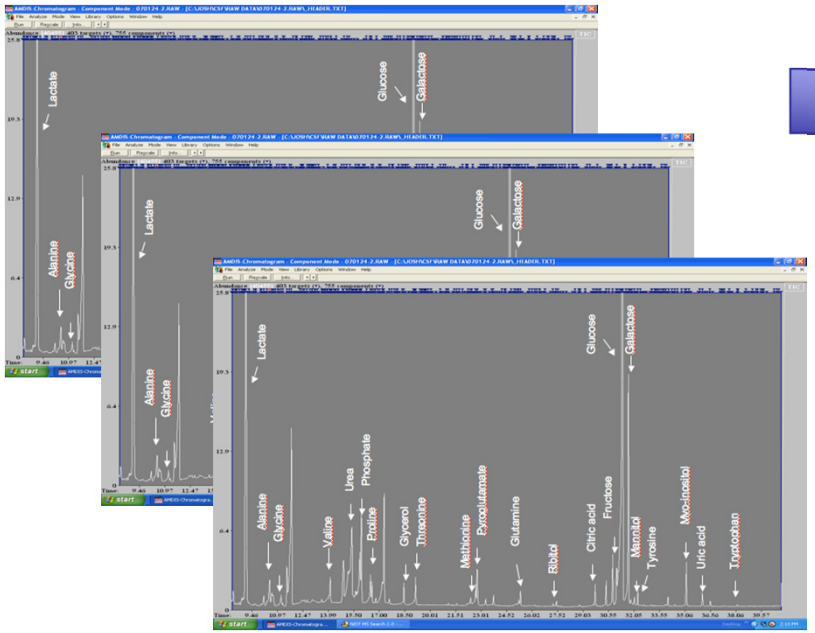
Metabolite Identification & Quantification



Data Reduction

From Spectra to Lists

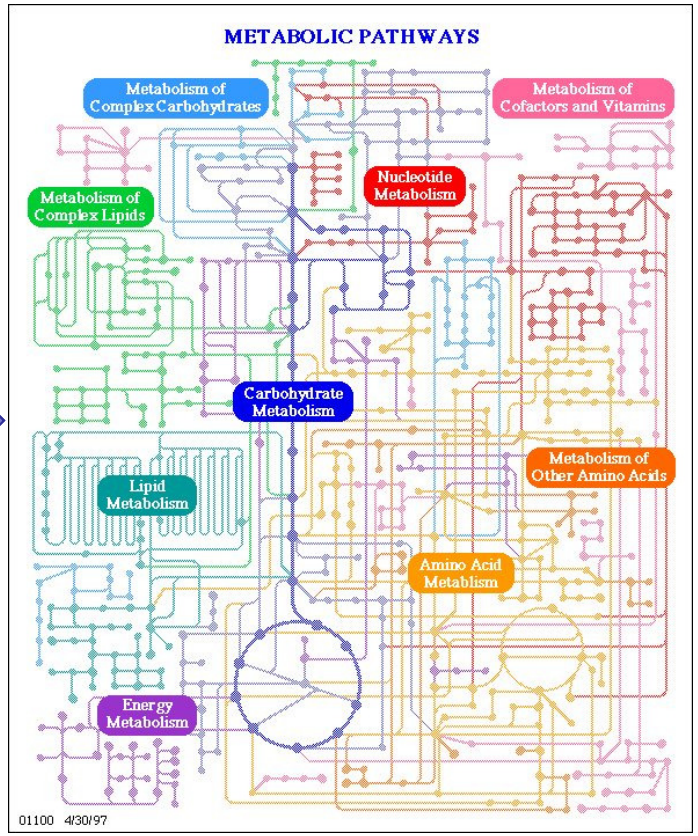




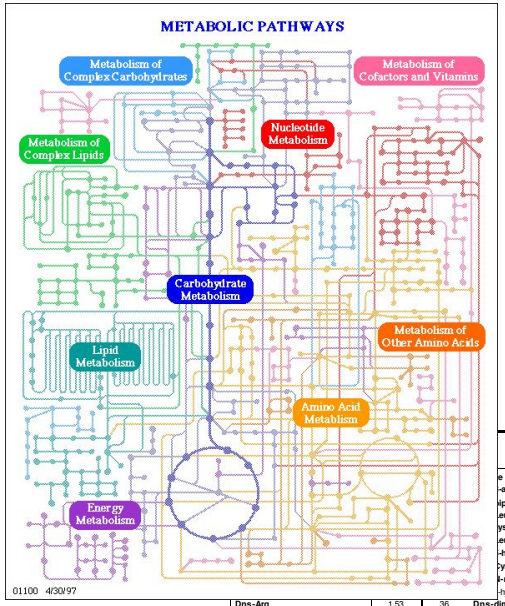
Compound	Retention Time (min)	Conc. in Urine (µM)	Compound	Retention Time (min)	Conc. in Urine (µM)
Dns-o-phospho -L-serine	0.92	<D.L. *	**Dns-Ile**	6.35	25
Dns-o-phospho -L-tyrosine	0.95	<D.L.	**Dns-3-aminosalicylic acid**	6.44	0.5
Dns-adnosine monophosphate	0.99	<D.L.	**Dns-pipecolic acid**	6.50	0.5
Dns-o-phosphoethanolamine	1.06	16	**Dns-Leu**	6.54	54
Dns-glucosamine	1.06	22	**Dns-cystathionine**	6.54	0.3
Dns-o-phospho -L-threonine	1.09	<D.L.	**Dns-Leu-Pro**	6.60	0.4
Dns-6-dimet hylamine purine	1.20	<D.L.	**Dns-5-hydroxylysine**	6.65	1.6
Dns-3-methyl -histidine	1.22	80	**Dns-Cystine**	6.73	160
Dns-taurine	1.25	834	**Dns-N-norleucine**	6.81	0.1
Dns-carnosine	1.34	28	Dns-5-hydroxydopamine	7.17	<D.L.
Dns-Arg	1.53	36	**Dns-dimethylamine**	7.33	293
Dns-Asn	1.55	133	**Dns-5-HIAA**	7.46	18
Dns-hypotaurine	1.58	10	**Dns-umbelliferone**	7.47	1.9
Dns-homocarnosine	1.61	3.9	Dns-2,3-diaminoproprionic acid	7.63	<D.L.
Dns-guanidine	1.62	<D.L.	**Dns-L-ornithine**	7.70	15
Dns-Gln	1.72	633	**Dns-4-acetyamidophenol**	7.73	51
Dns-allantoin	1.83	3.8	**Dns-procaine**	7.73	8.9
Dns-L-citrulline	1.87	2.9	**Dns-homocystine**	7.76	3.3
Dns-1 (or 3 -)-methylhistamine	1.94	1.9	**Dns-acetaminophen**	7.97	82
Dns-adenosine	2.06	2.6	**Dns-Phe-Phe**	8.03	0.4
Dns-methylguanidine	2.20	<D.L.	**Dns-5-methyo xysalicylic acid**	8.04	2.1
Dns-Ser	2.24	511	**Dns-Lys**	8.16	184
Dns-aspartic acid amide	2.44	26	Dns-aniline	8.17	<D.L.
Dns-4-hydroxy -proline	2.56	2.3	**Dns-leu-Phe**	8.22	0.3
Dns-Glu	2.57	21	**Dns-His**	8.35	1550
Dns-Asp	2.60	90	Dns-4-thialysine	8.37	<D.L.
Dns-Thr	3.03	157	Dns-benzylamine	8.38	<D.L.
Dns-epinephrine	3.05	<D.L.	**Dns-1-ephedrine**	8.50	0.6
Dns-ethanolamine	3.11	471	**Dns-tryptamine**	8.63	0.4
Dns-aminoadipic acid	3.17	70	Dns-pyrydoxamine	8.94	<D.L.
Dns-Gly	3.43	2510	Dns-2-methyl -benzylamine	9.24	<D.L.
Dns-Ala	3.88	593	**Dns-5-hydroxytrptophan**	9.25	0.12
Dns-aminolevulinic acid	3.97	30	**Dns-1,3-diaminopropane**	9.44	0.23
Dns-r-amino-butyric acid	3.98	4.6	**Dns-putrescine**	9.60	0.5
Dns-p-amino-hippuric acid	3.98	2.9	**Dns-1,2-diaminopropane**	9.66	0.1
Dns-5-hydro xymethyluricil	4.58	1.9	**Dns-tyrosinamide**	9.79	29
Dns-tryptophanamide	4.70	5.5	**Dns-dopamine**	10.08	140
Dns-isoguanine	4.75	<D.L.	**Dns-cadaverine**	10.08	0.08
Dns-5-aminopentanoic acid	4.79	1.6	**Dns-histamine**	10.19	0.4
Dns-sarcosine	4.81	7.2	**Dns-3-methoxy -tyramine**	10.19	9.2
Dns-3-amino-isobutyrate	4.81	85	**Dns-Tyr**	10.28	321
Dns-2-aminobutyric acid	4.91	17	Dns-cysteamine	10.44	<D.L.

From Lists to Pathways

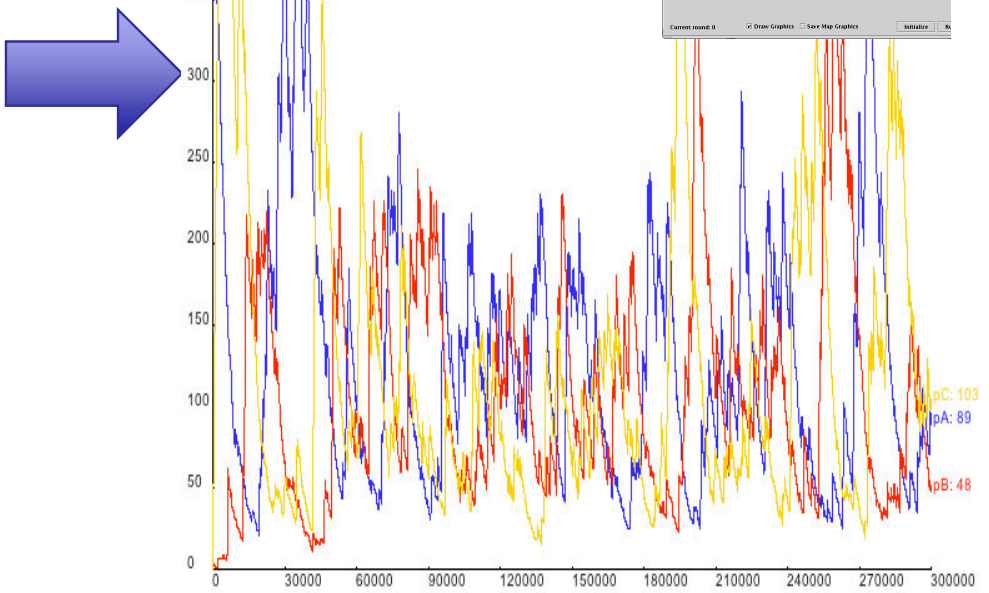
Compound	Retention Time (min)	Conc. in Urine (µM)	Compound	Retention Time (min)	Conc. in Urine (µM)
Dns-o-phospho -L-serine	0.92	<D.L.*	**Dns-Ile**	6.35	25
Dns-o-phospho -L-tyrosine	0.95	<D.L.	**Dns-3-aminosalicylic acid**	6.44	0.5
Dns-adnosine monophosphate	0.99	<D.L.	**Dns-pipecolic acid**	6.50	0.5
Dns-o-phosphoethanolamine	1.06	16	**Dns-Leu**	6.54	54
Dns-glucosamine	1.06	22	**Dns-cystathionine**	6.54	0.3
Dns-o-phospho -L-threonine	1.09	<D.L.	**Dns-Leu-Pro**	6.60	0.4
Dns-6-dimet hylamine purine	1.20	<D.L.	**Dns-5-hydroxylysine**	6.65	1.6
Dns-3-methyl -histidine	1.22	80	**Dns-Cystine**	6.73	160
Dns-taurine	1.25	834	**Dns-N-norleucine**	6.81	0.1
Dns-carnosine	1.34	28	Dns-5-hydroxydopamine	7.17	<D.L.
Dns-Arg	1.53	36	**Dns-dimethylamine**	7.33	293
Dns-Asn	1.55	133	**Dns-5-HIAA**	7.46	18
Dns-hypotaurine	1.58	10	**Dns-umbelliferone**	7.47	1.9
Dns-homocarnosine	1.61	3.9	Dns-2,3-diaminoproprionic acid	7.63	<D.L.
Dns-guanidine	1.62	<D.L.	**Dns-L-ornithine**	7.70	15
Dns-Gln	1.72	633	**Dns-4-acetyamidophenol**	7.73	51
Dns-allantoin	1.83	3.8	**Dns-procaine**	7.73	8.9
Dns-L-citrulline	1.87	2.9	**Dns-homocystine**	7.76	3.3
Dns-1 (or 3 -)-methylhistamine	1.94	1.9	**Dns-acetaminophen**	7.97	82
Dns-adenosine	2.06	2.6	**Dns-Phe-Phe**	8.03	0.4
Dns-methylguanidine	2.20	<D.L.	**Dns-5-methyo xysalicylic acid**	8.04	2.1
Dns-Ser	2.24	511	**Dns-Lys**	8.16	184
Dns-aspartic acid amide	2.44	26	Dns-aniline	8.17	<D.L.
Dns-4-hydroxy -proline	2.56	2.3	**Dns-leu-Phe**	8.22	0.3
Dns-Glu	2.57	21	**Dns-His**	8.35	1550
Dns-Asp	2.60	90	Dns-4-thialysine	8.37	<D.L.
Dns-Thr	3.03	157	Dns-benzylamine	8.38	<D.L.
Dns-epinephrine	3.05	<D.L.	**Dns-1-ephedrine**	8.50	0.6
Dns-ethanolamine	3.11	471	**Dns-tryptamine**	8.63	0.4
Dns-aminoadipic acid	3.17	70	Dns-pyrydoxamine	8.94	<D.L.
Dns-Gly	3.43	2510	Dns-2-methyl -benzylamine	9.24	<D.L.
Dns-Ala	3.88	593	**Dns-5-hydroxytrptophan**	9.25	0.12
Dns-aminolevulinic acid	3.97	30	**Dns-1,3-diaminopropane**	9.44	0.23
Dns-r-amino-butyric acid	3.98	4.6	**Dns-putrescine**	9.60	0.5
Dns-p-amino-hippuric acid	3.98	2.9	**Dns-1,2-diaminopropane**	9.66	0.1
Dns-5-hydro xymethyluricil	4.58	1.9	**Dns-tyrosinamide**	9.79	29
Dns-tryptophanamide	4.70	5.5	**Dns-dopamine**	10.08	140
Dns-isoguanine	4.75	<D.L.	**Dns-cadaverine**	10.08	0.08
Dns-5-aminopentanoic acid	4.79	1.6	**Dns-histamine**	10.19	0.4
Dns-sarcosine	4.81	7.2	**Dns-3-methoxy -tyramine**	10.19	9.2
Dns-3-amino -isobutyrate	4.81	85	**Dns-Tyr**	10.28	321
Dns-2-aminobutyric acid	4.91	17	Dns-cysteamine	10.44	<D.L.



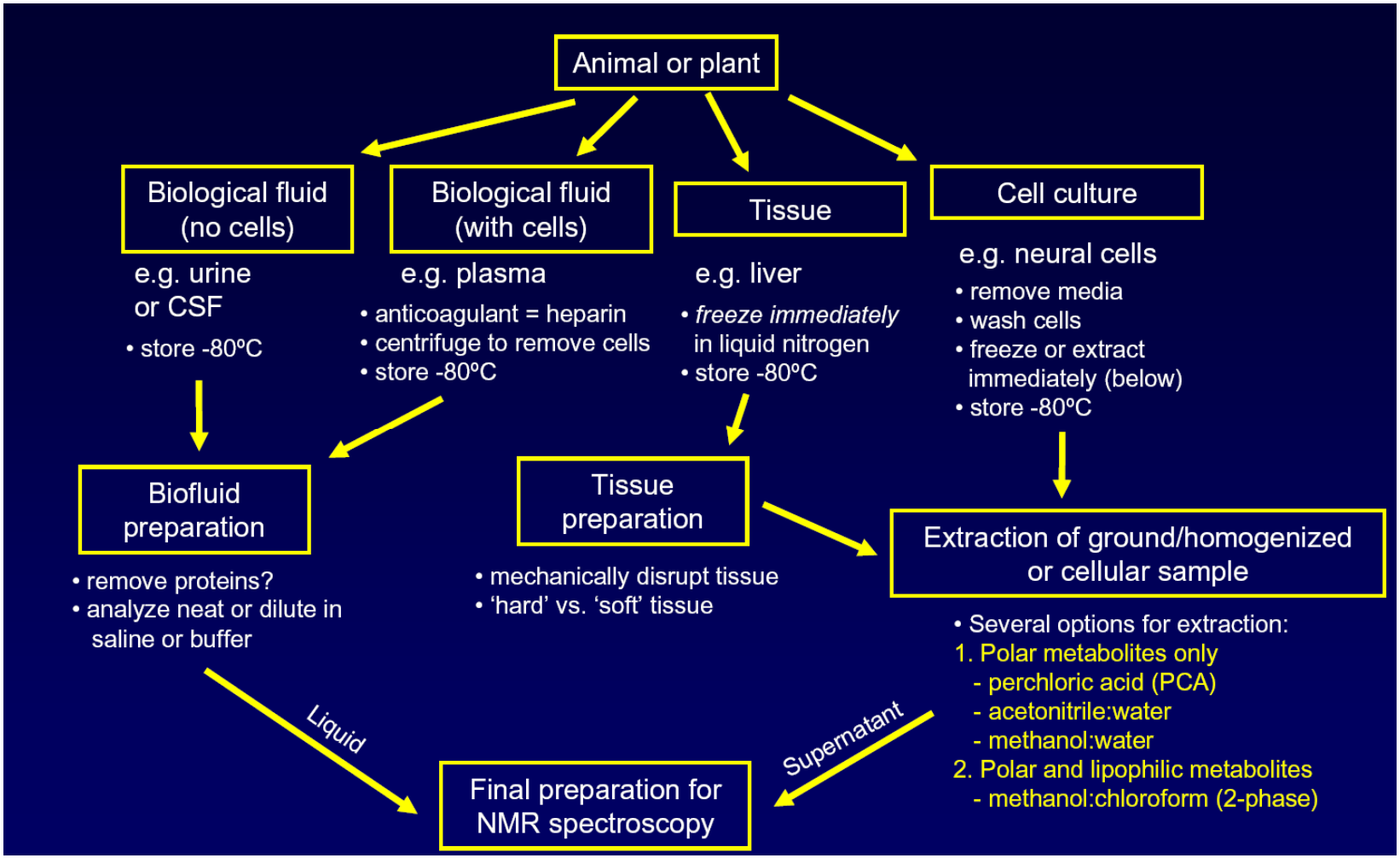
From Pathways & Lists to Models & Biomarkers



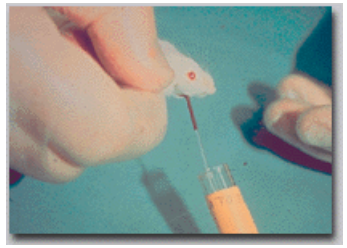
Compound	Retention Time (min)	Conc. in Urine (µM)
e	6.35	25
-aminosalicylic acid	6.44	0.5
ipecolic acid	6.50	0.5
eu	6.54	54
ystathionine	6.54	0.3
eu-Pro	6.60	0.4
-hydroxylysine	6.65	1.6
Cystine	6.73	160
l-norleucine	6.81	0.1
-hydroxydopamine	7.17	<D.L.
Dns-Arg	1.53	36
Dns-Asn	1.55	133
Dns-hypotaurine	1.58	10
Dns-homocarnosine	1.61	3.9
Dns-guanidine	1.62	<D.L.
Dns-Gln	1.72	633
Dns-allantoin	1.83	3.8
Dns-L-citrulline	1.87	2.9
Dns-1 (or 3 -)-methylhistamine	1.94	1.9
Dns-adenosine	2.06	2.6
Dns-methylguanidine	2.20	<D.L.
Dns-Ser	2.24	511
Dns-aspartic acid amide	2.44	26
Dns-4-hydroxy-proline	2.56	2.3
Dns-Glu	2.57	21
Dns-Asp	2.60	90
Dns-Thr	3.03	157
Dns-epinephrine	3.05	<D.L.
Dns-ethanolamine	3.11	471
Dns-aminoadipic acid	3.17	70
Dns-Gly	3.43	2510
Dns-Ala	3.88	593
Dns-aminolevulinic acid	3.97	30
Dns-r-amino-butyric acid	3.98	4.6
Dns-p-amino-hippuric acid	3.98	2.9
Dns-5-hydroxymethyluricil	4.58	1.9
Dns-tryptophanamide	4.70	5.5
Dns-isoguanine	4.75	<D.L.
Dns-5-aminopentanoic acid	4.79	1.6
Dns-sarcosine	4.81	7.2
Dns-3-amino-isobutyrate	4.81	85
Dns-2-aminobutyric acid	4.91	17
Dns-dimethylamine	7.33	293
Dns-5-HIAA	7.46	18
Dns-umbelliferone	7.47	1.9
Dns-2,3-diaminopropionic acid	7.63	<D.L.
Dns-L-ornithine	7.70	15
Dns-4-acetyamidophenol	7.73	51
Dns-procaine	7.73	8.9
Dns-homocystine	7.76	3.3
Dns-acetaminophen	7.97	82
Dns-Phe-Phe	8.03	0.4
Dns-5-methyo xysalicylic acid	8.04	2.1
Dns-Lys	8.16	184
Dns-aniline	8.17	<D.L.
Dns-leu-Phe	8.22	0.3
Dns-His	8.35	1550
Dns-4-thalysine	8.37	<D.L.
Dns-benzylamine	8.38	<D.L.
Dns-1-ephedrine	8.50	0.6
Dns-tryptamine	8.63	0.4
Dns-pyrydoxamine	8.94	<D.L.
Dns-2-methyl -benzylamine	9.24	<D.L.
Dns-5-hydroxytrptophan	9.25	0.12
Dns-1,3-diaminopropane	9.44	0.23
Dns-putrescine	9.60	0.5
Dns-1,2-diaminopropane	9.66	0.1
Dns-tyrosinamide	9.79	29
Dns-dopamine	10.08	140
Dns-cadaverine	10.08	0.08
Dns-histamine	10.19	0.4
Dns-3-methoxy -tyramine	10.19	9.2
Dns-Tyr	10.28	321
Dns-cysteamine	10.44	<D.L.



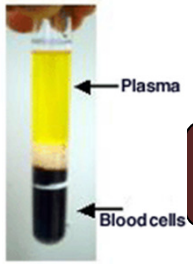
Sample preparation for NMR-based metabolomics



Plasma sample preparation



Blood collection

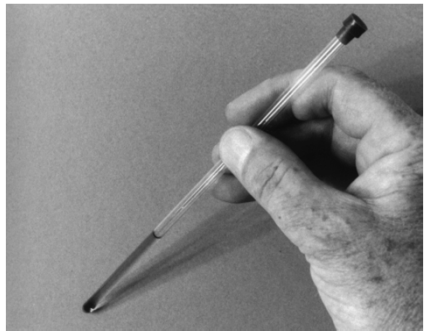


Plasma separation

Filtration

Sample volume and pH adjustment

Ready for NMR spectroscopy



Manual vs automated sample preparation

• Manual prep

Gloves, coat, precision pipettes

• Automation mode

Safety, Reproducibility, high throughput

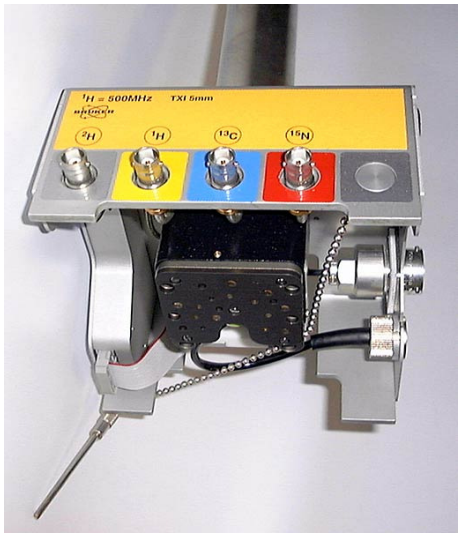
Barcode system

Instrumentation setup

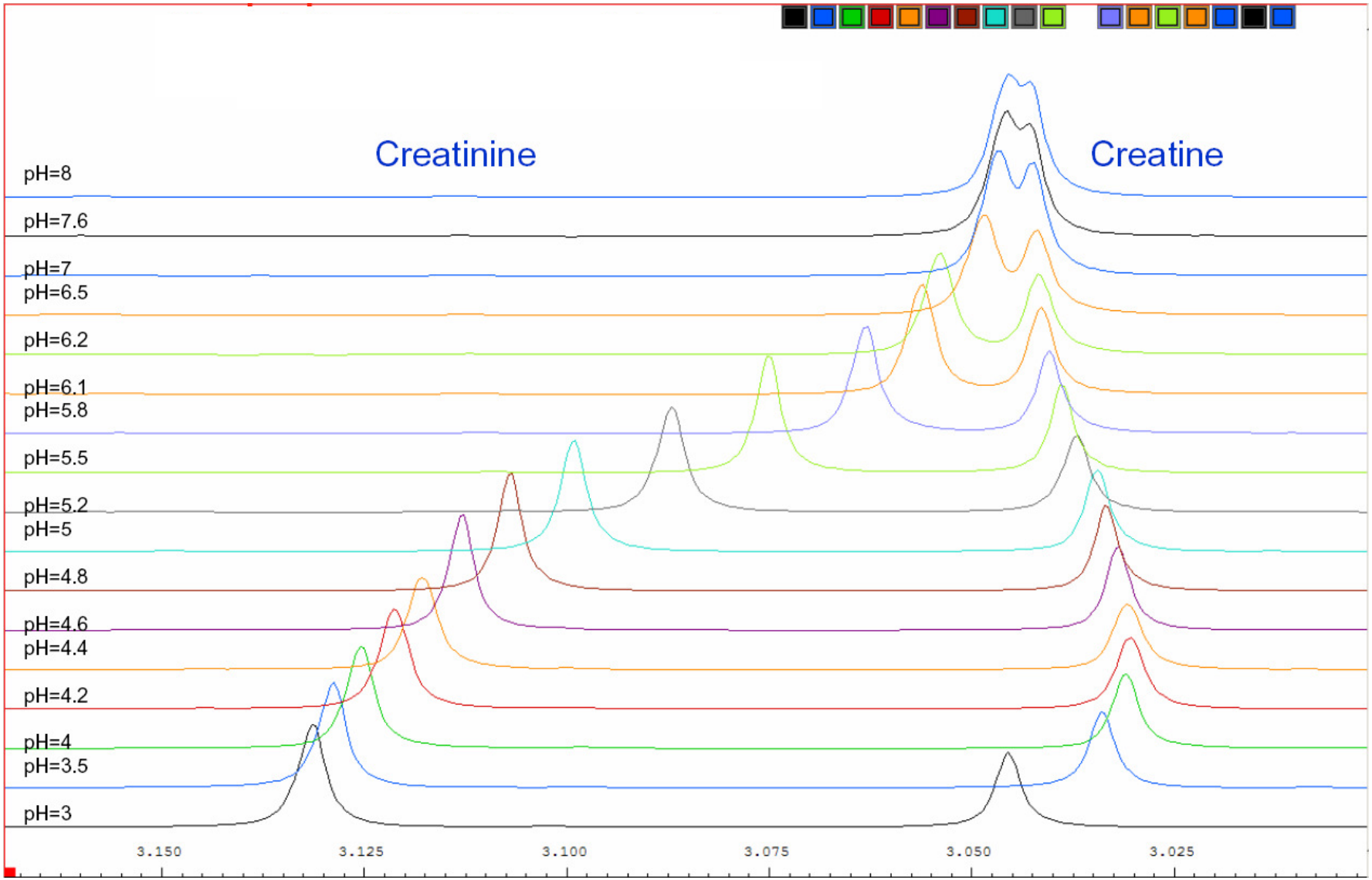
- Manual prep

- Automation mode

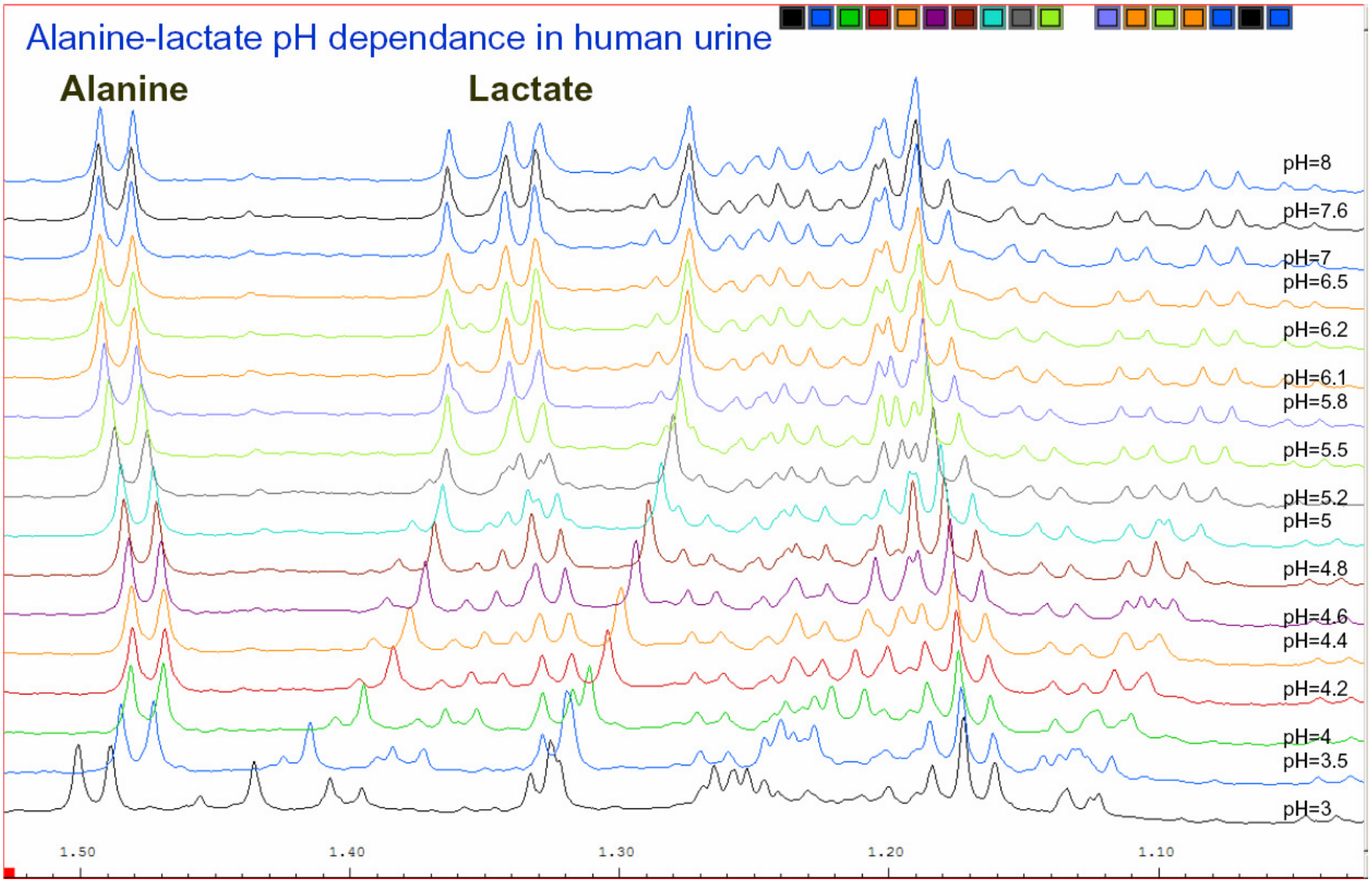
Sample changer, auto T/M



pH changes


Creatinine
Creatine
pH=8
pH=7.6
pH=7
pH=6.5
pH=6.2
pH=6.1
pH=5.8
pH=5.5
pH=5.2
pH=5
pH=4.8
pH=4.6
pH=4.4
pH=4.2
pH=4
pH=3.5
pH=3
3.150
3.125
3.100
3.075
3.050
3.025

pH changes (…)

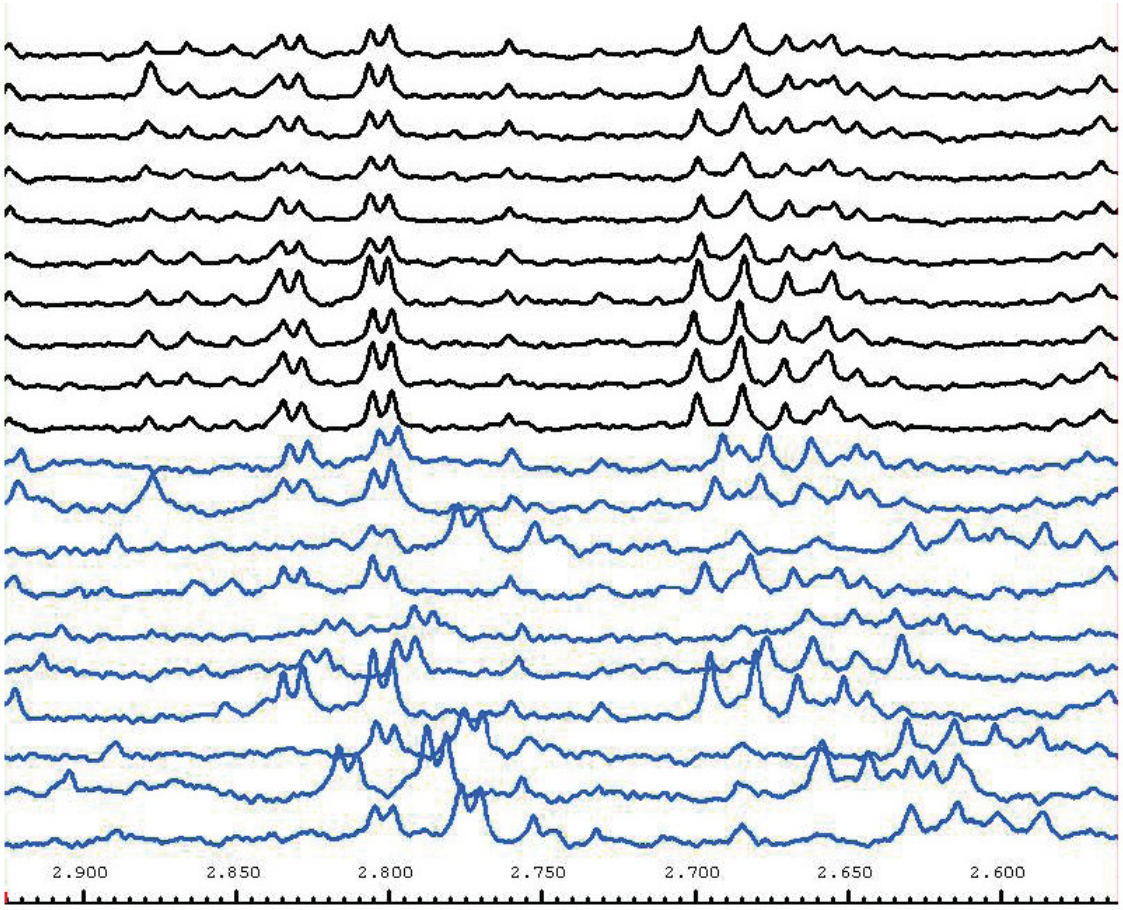

Alanine-lactate pH dependance in human urine
Alanine
Lactate
pH=8
pH=7.6
pH=7
pH=6.5
pH=6.2
pH=6.1
pH=5.8
pH=5.5
pH=5.2
pH=5
pH=4.8
pH=4.6
pH=4.4
pH=4.2
pH=4
pH=3.5
pH=3
1.50
1.40
1.30
1.20
1.10

pH adjustment

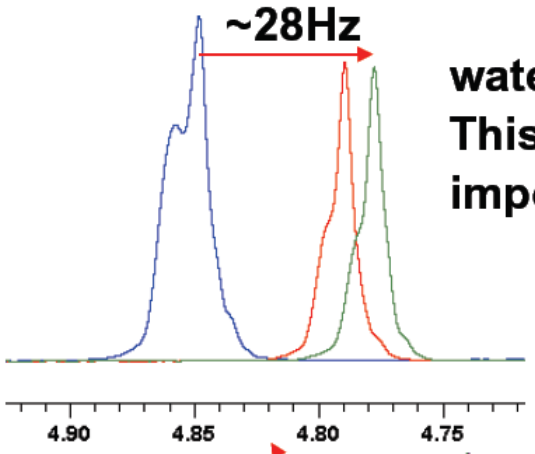
Buffer + pH adjustment
(pH = 7)

Buffer:
1.5M phosphat buffer
(KH2P04) in D20.
~0.01% NaN3 and
0.1% TSP is added.

extreme cases most affected region



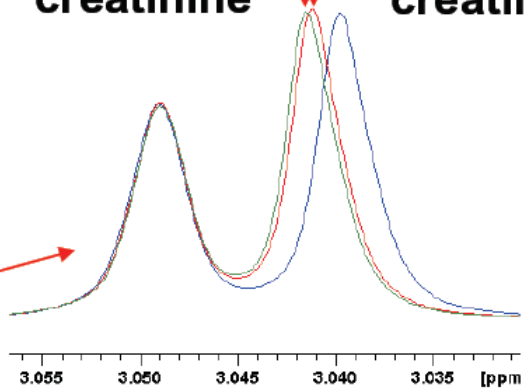
Temperature effects



water: ~4.5 Hz / K!
This is the most important effect!!!

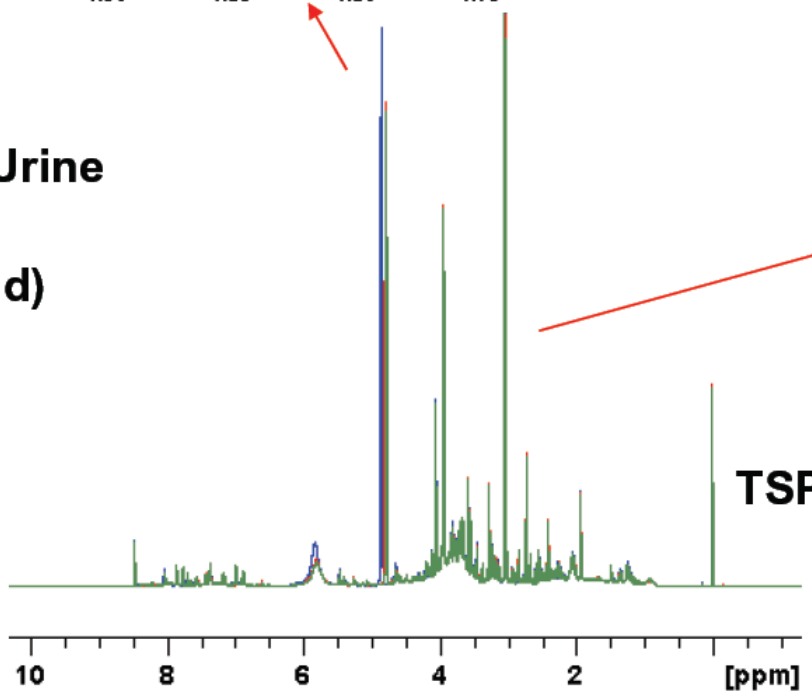
~0.1Hz / K

creatinine creatine

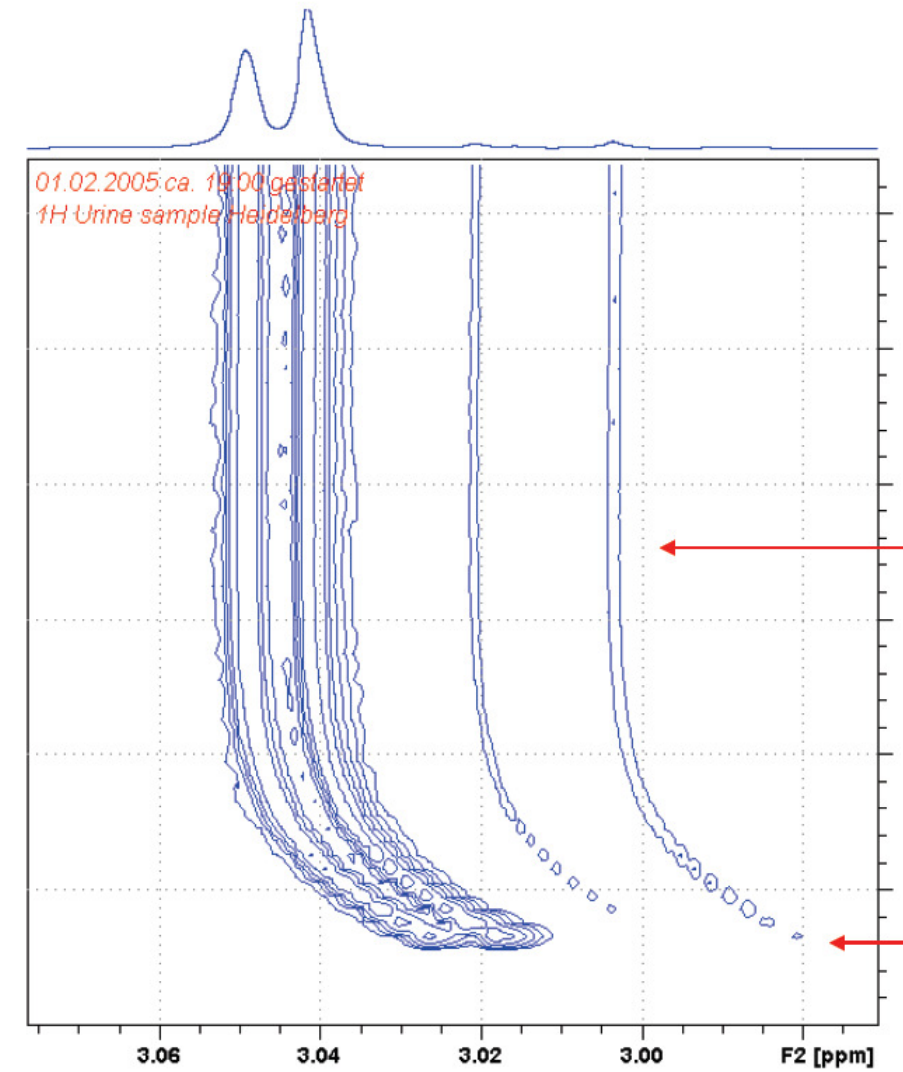


1H NMR on Urine
@ 400 MHz
(noesygppr1d)

295K
300K
301K



Temperature equilibration

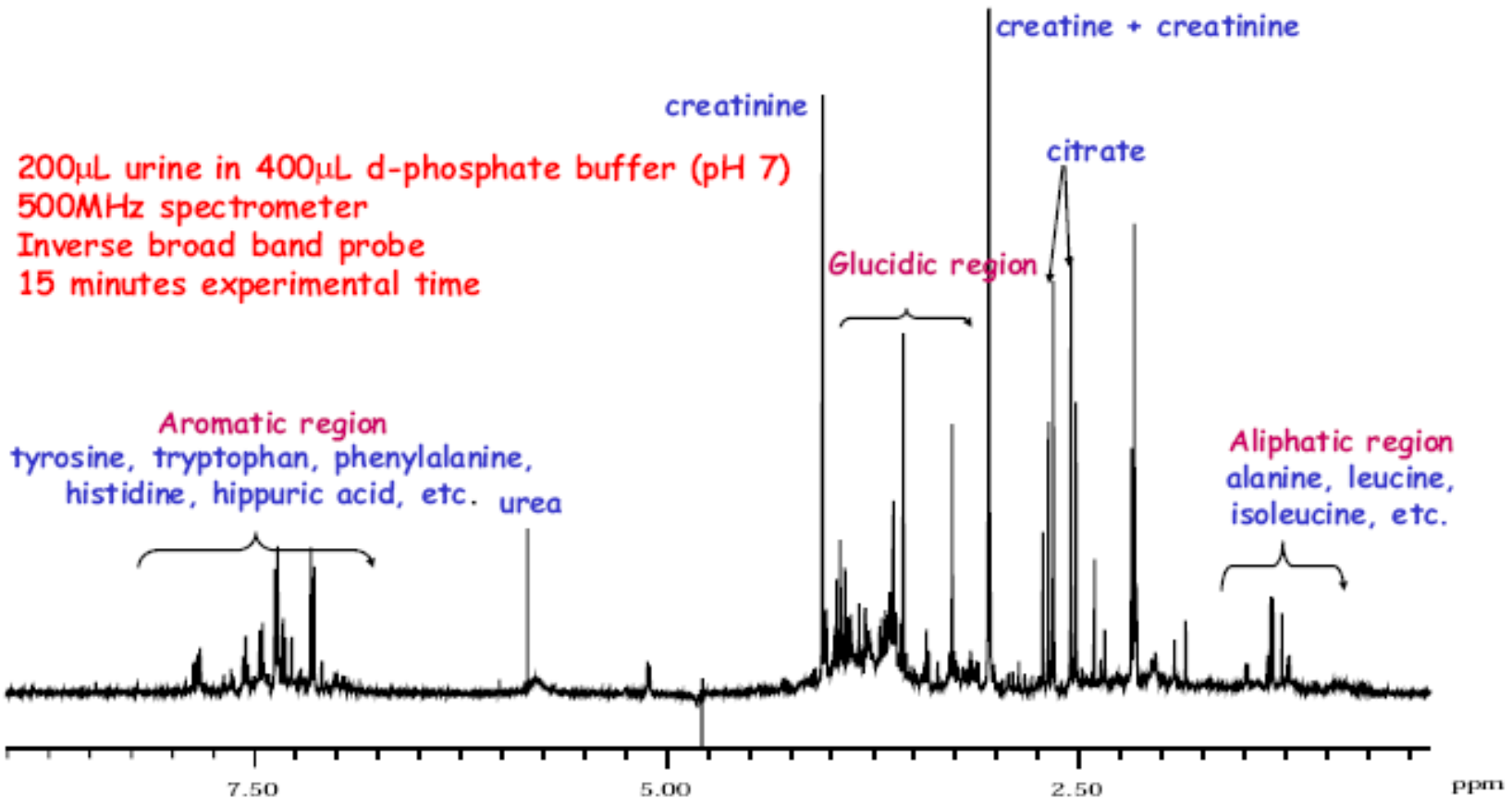


temperature equilibrated

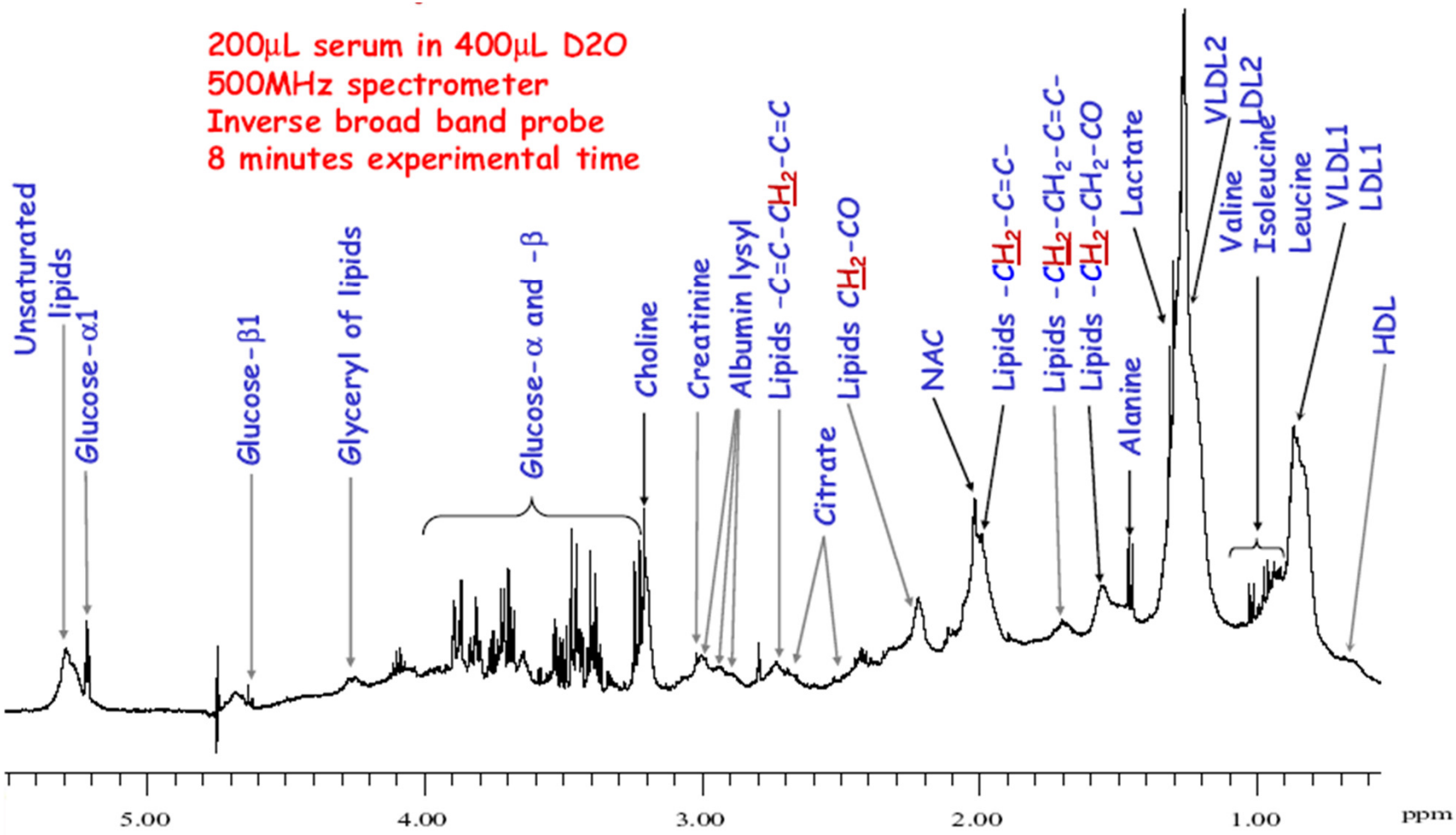
5 min

sample dropped Into magnet

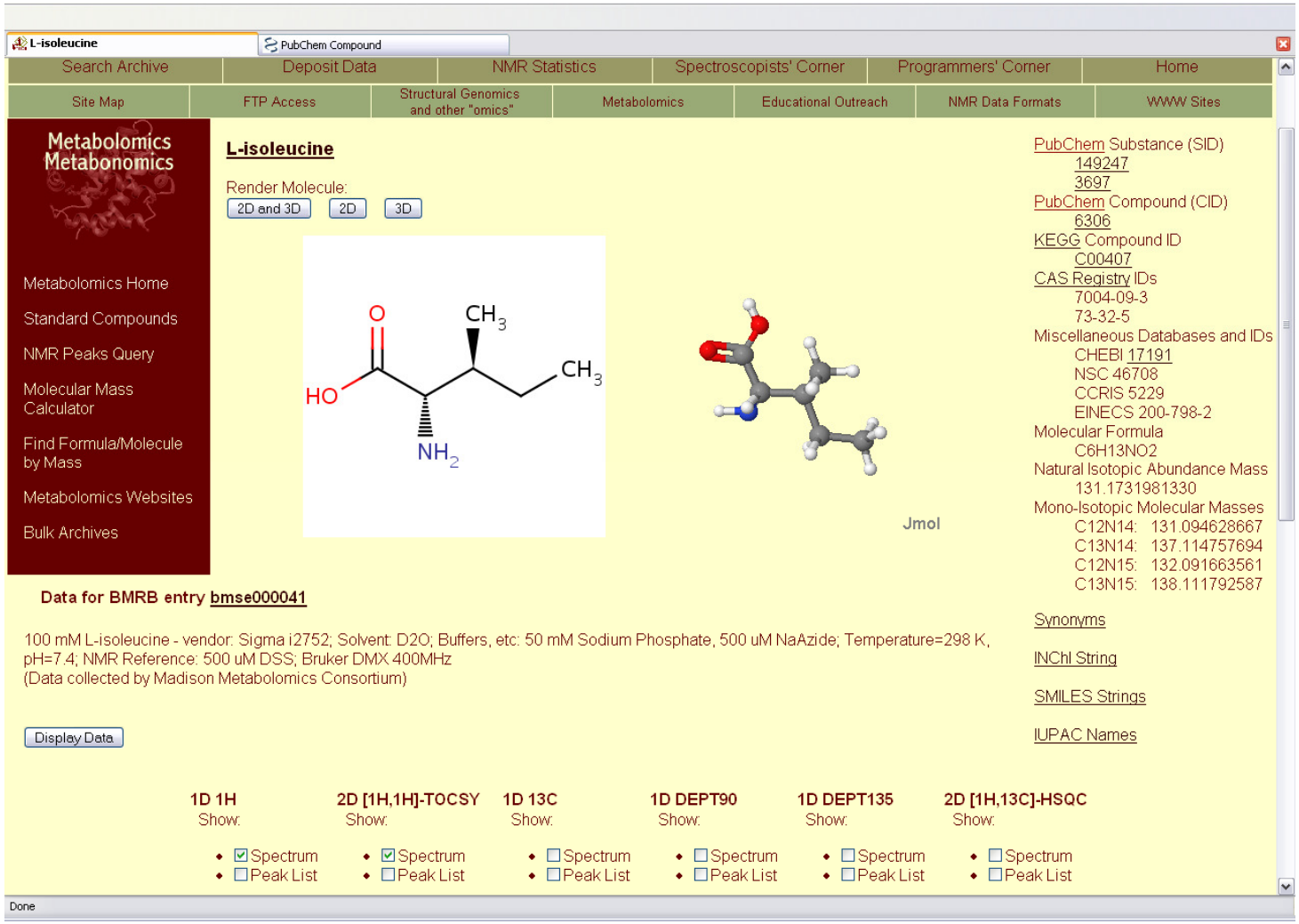
Assignment of major peaks/regions



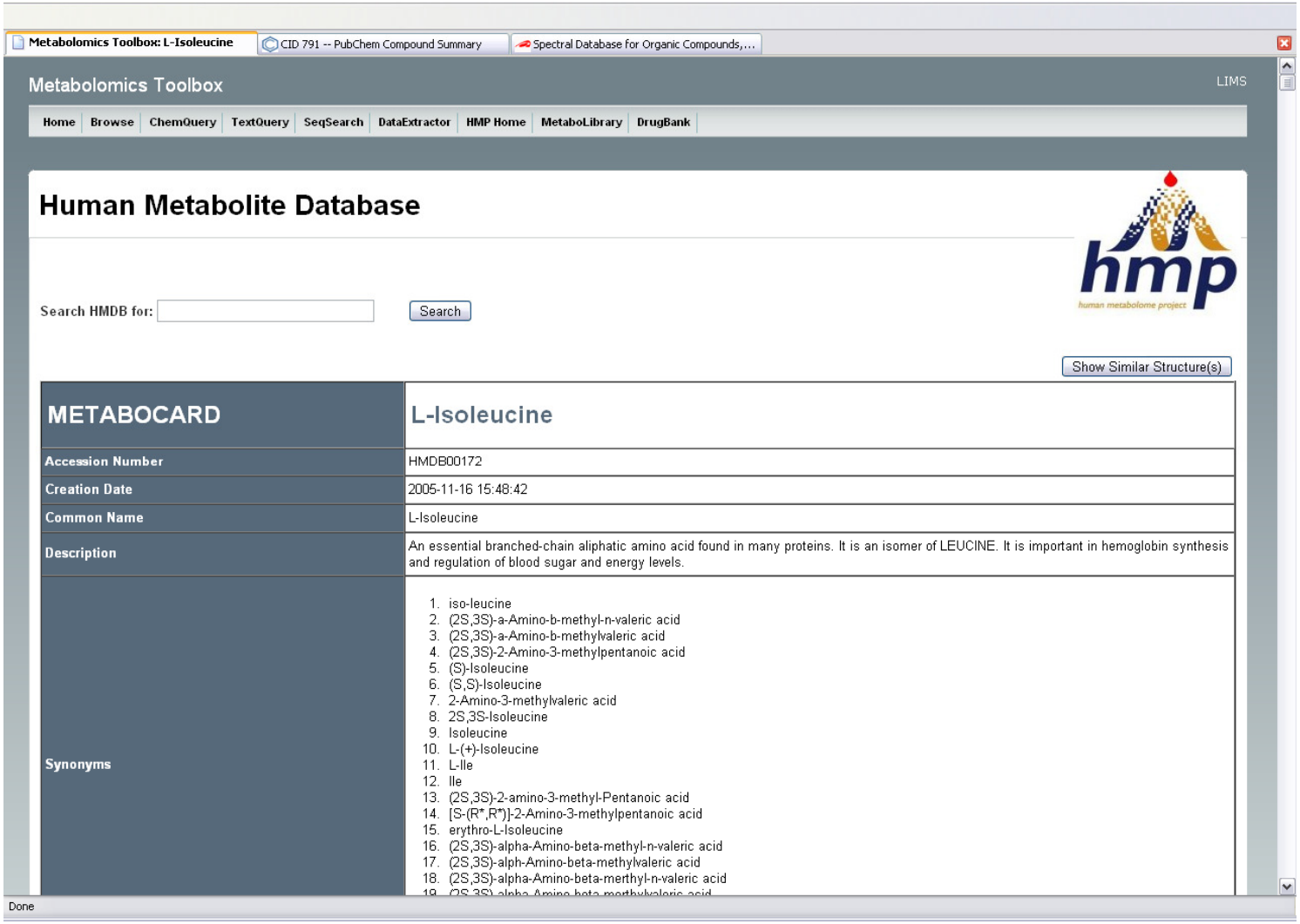
1H NMR of human blood serum

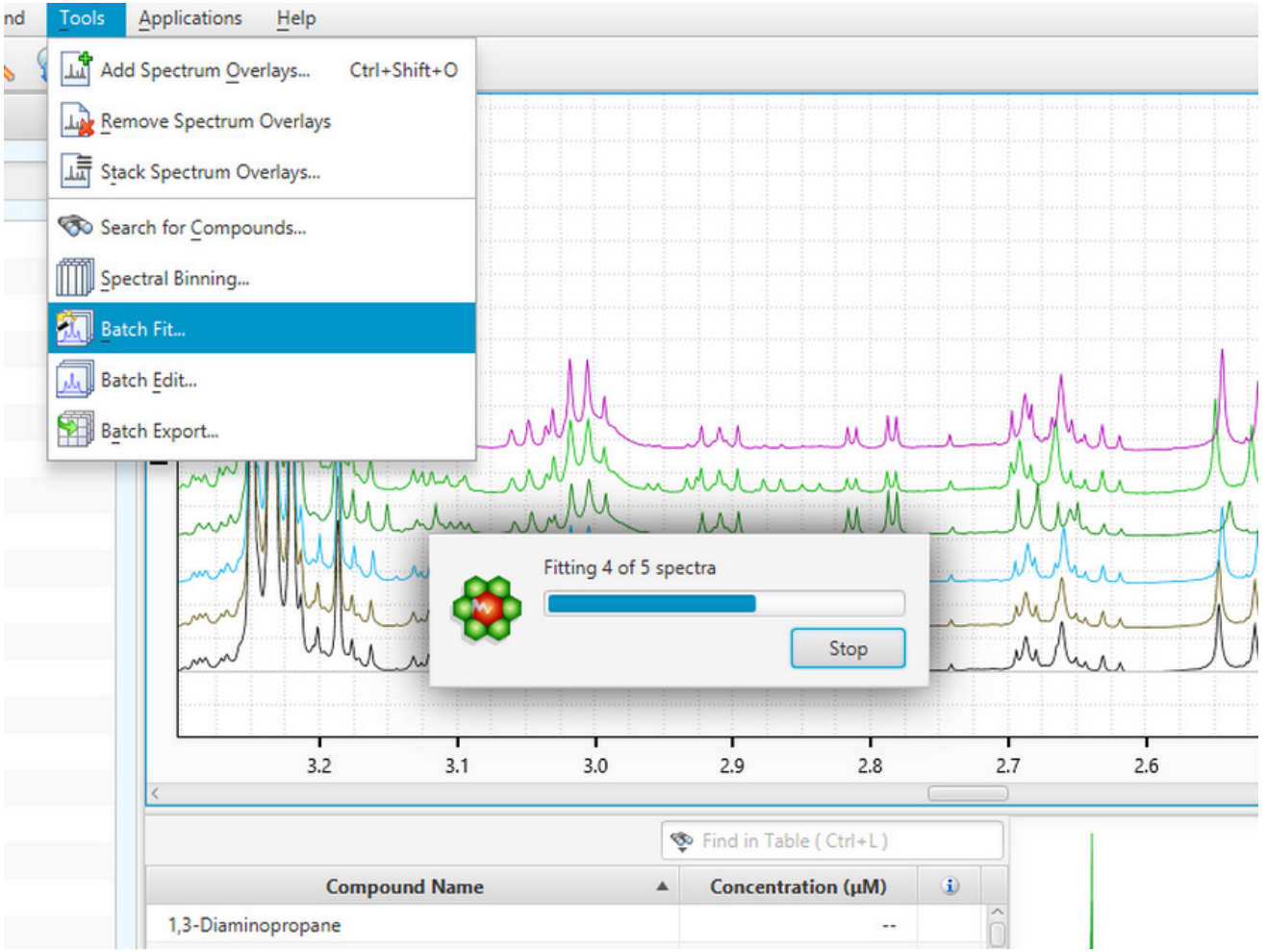


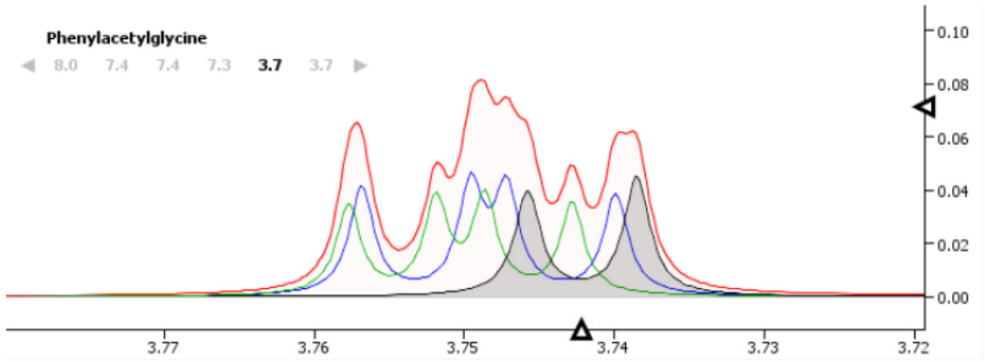
… BMRB: metabolite entries, isoleucine



THE HUMAN METABOLITE DATABASE
(www.hmdb.ca)

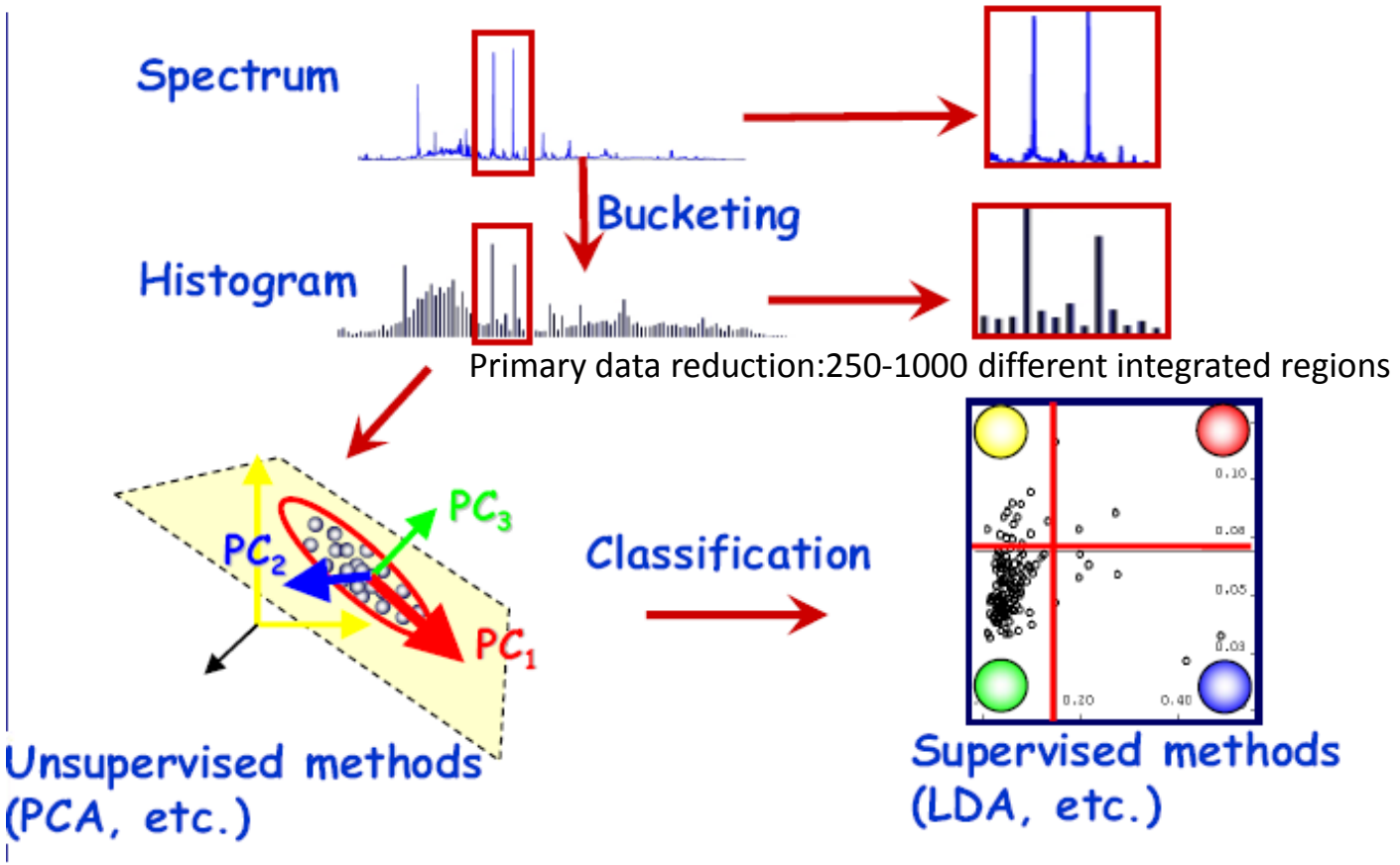






Identificazione di metaboliti

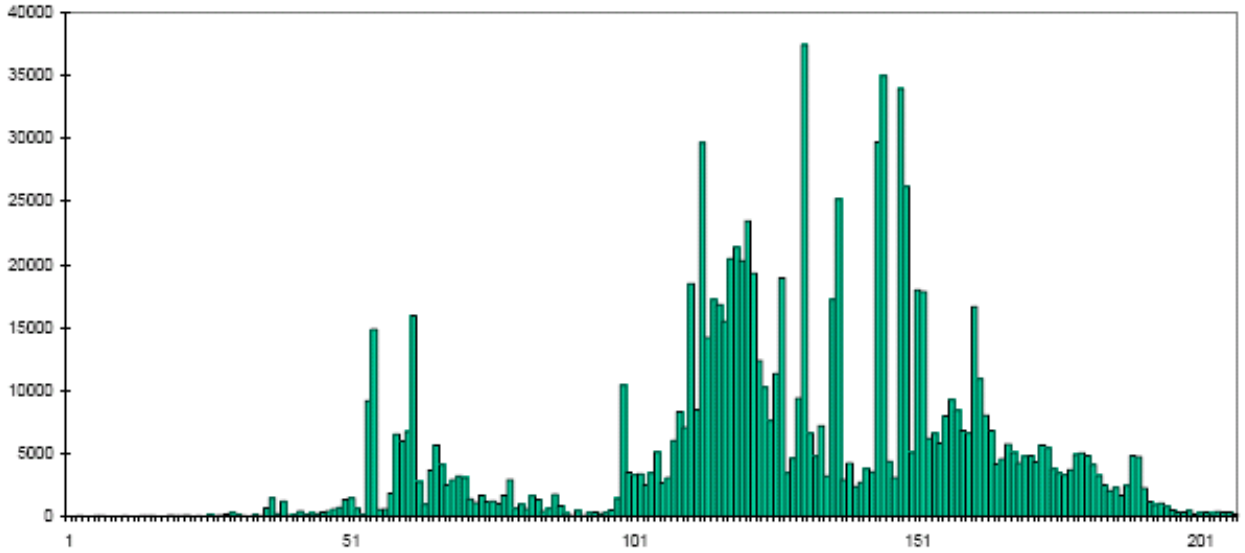
NMR-based metabolomics: the concept



No *a priori* knowledge of the class of samples

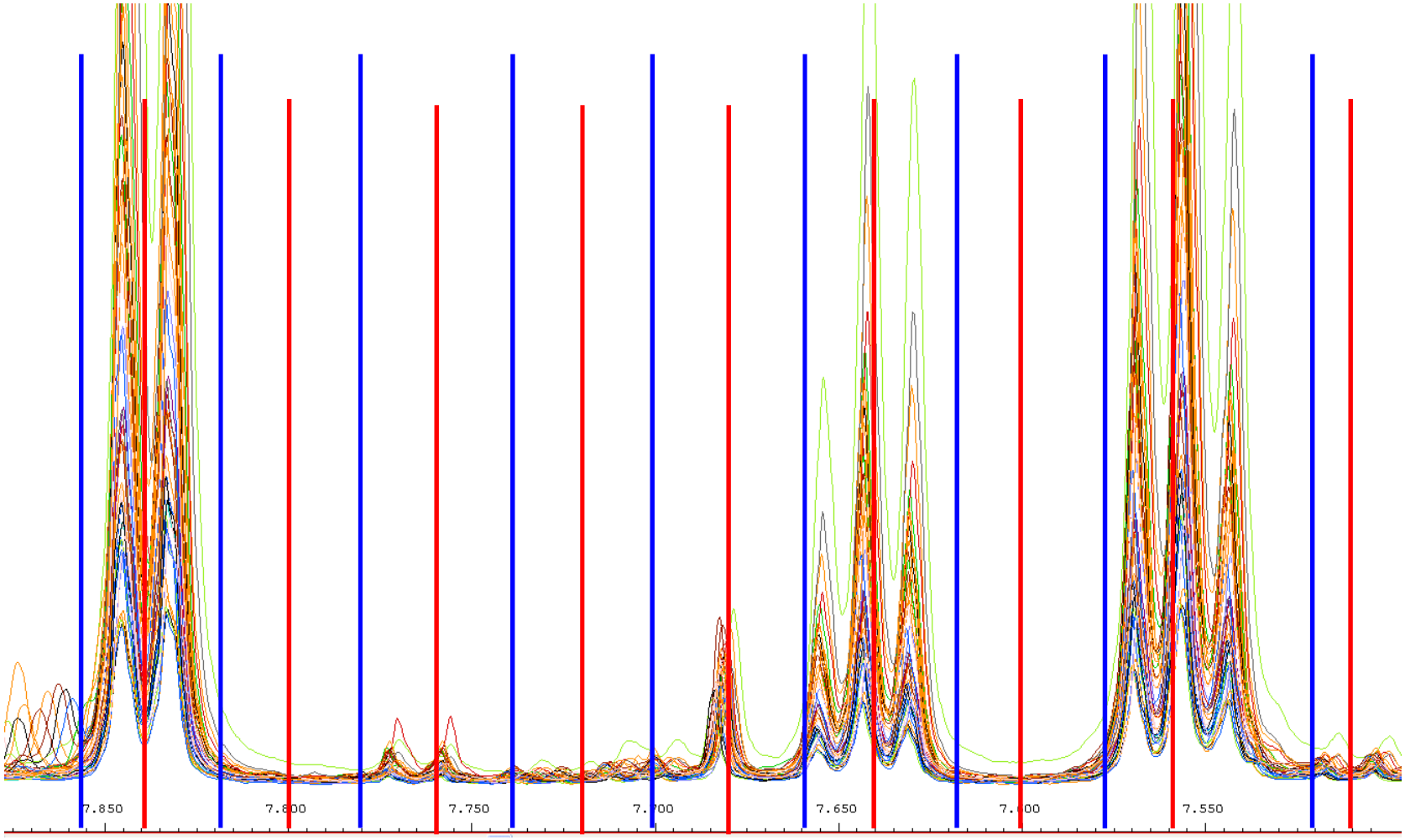
Model for the prediction of independent data
Use class information to maximise separation among classes

Data pre-processing (NMR)

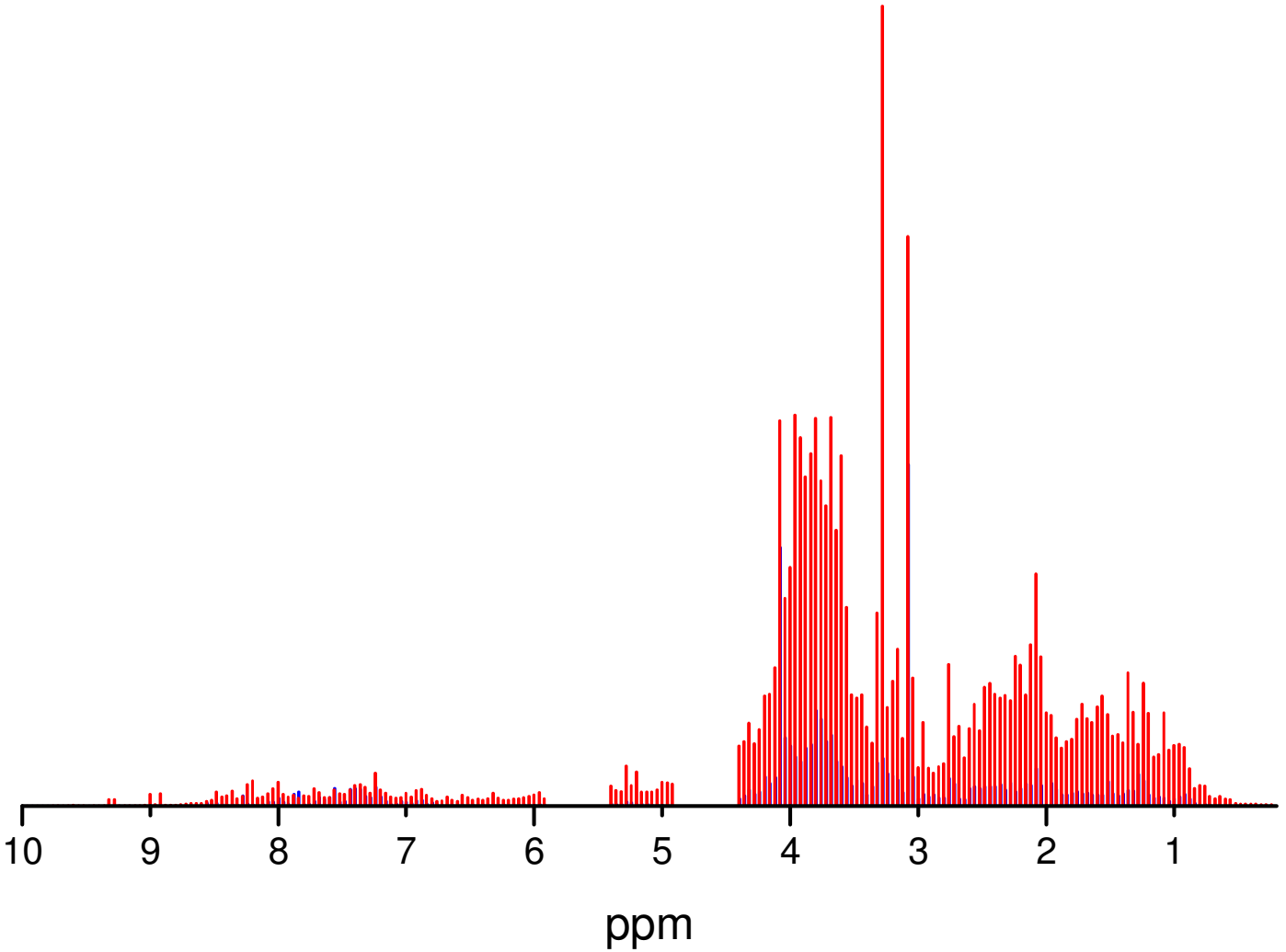


- Discretise x-axis into *n* equal sized bins, height = area under intensity (reduces impact of small variations in chemical shift e.g. due to pH)
- Normalise bars for constant total area (removes effect of differences in concentration across samples)
- Remove insignificant regions (e.g. water and urea resonances in urine spectra)

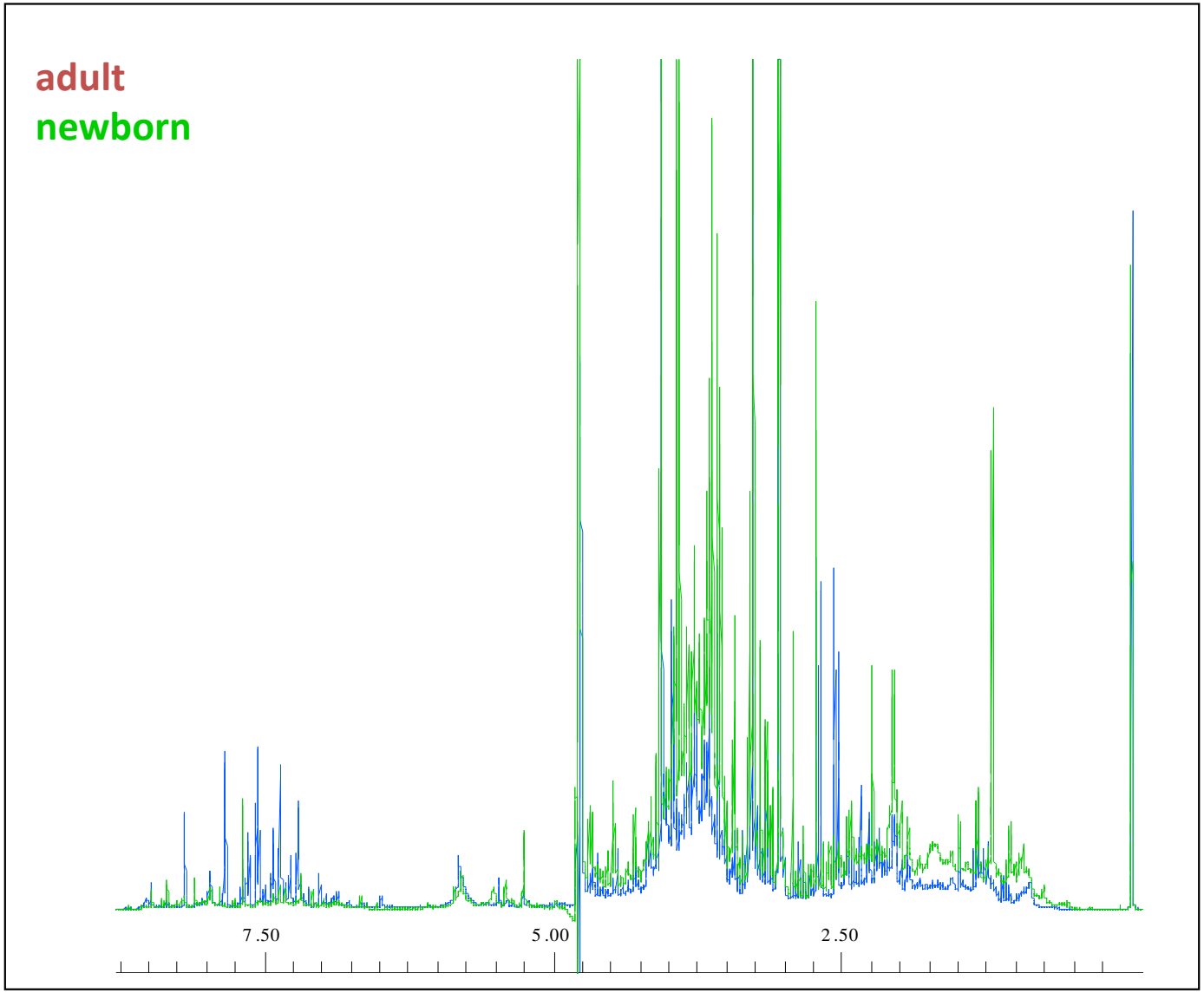
Fixed vs variable bucketing



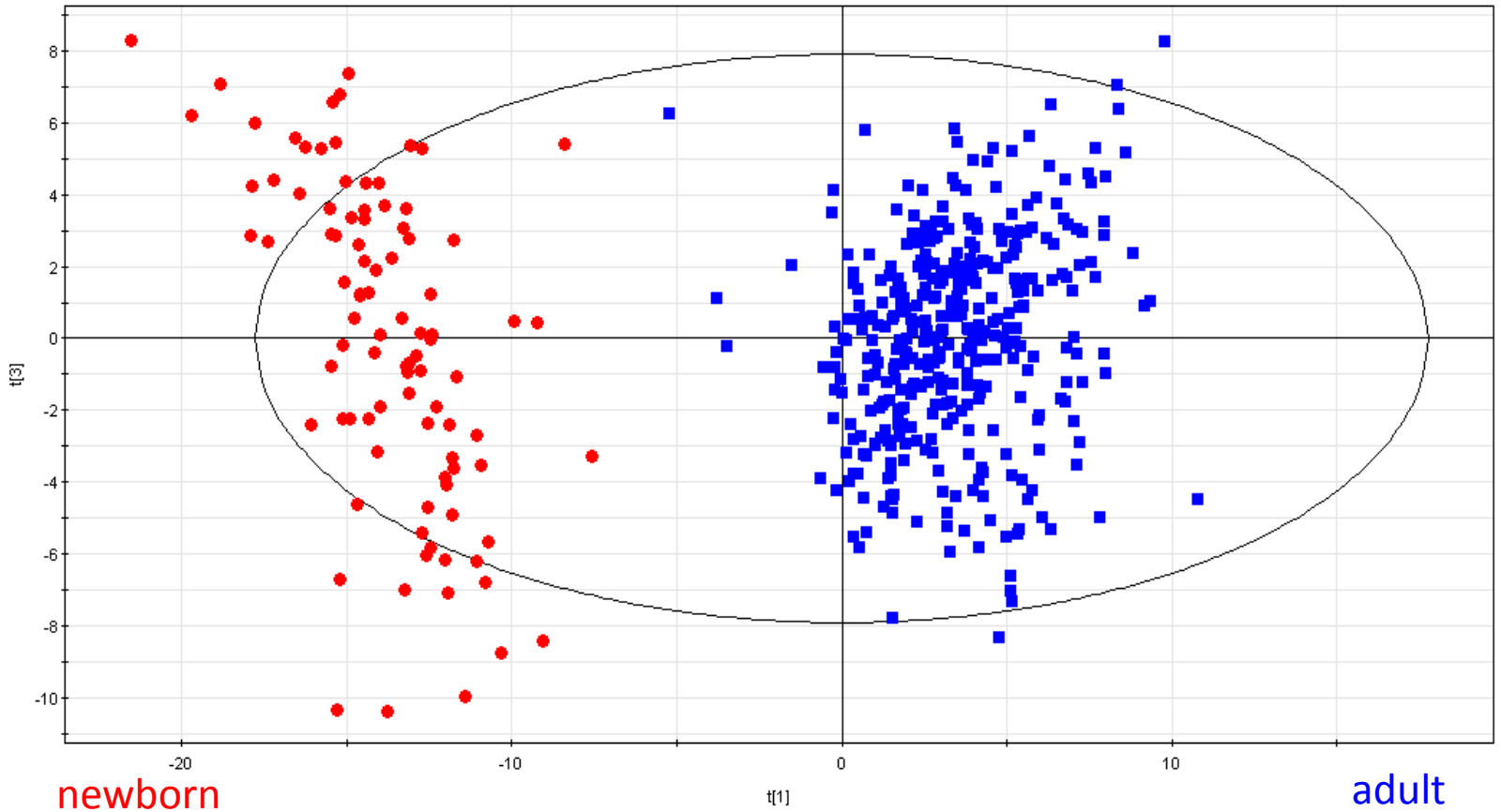
Normalization



Visualizing age-related differences



PCA newborns vs adults



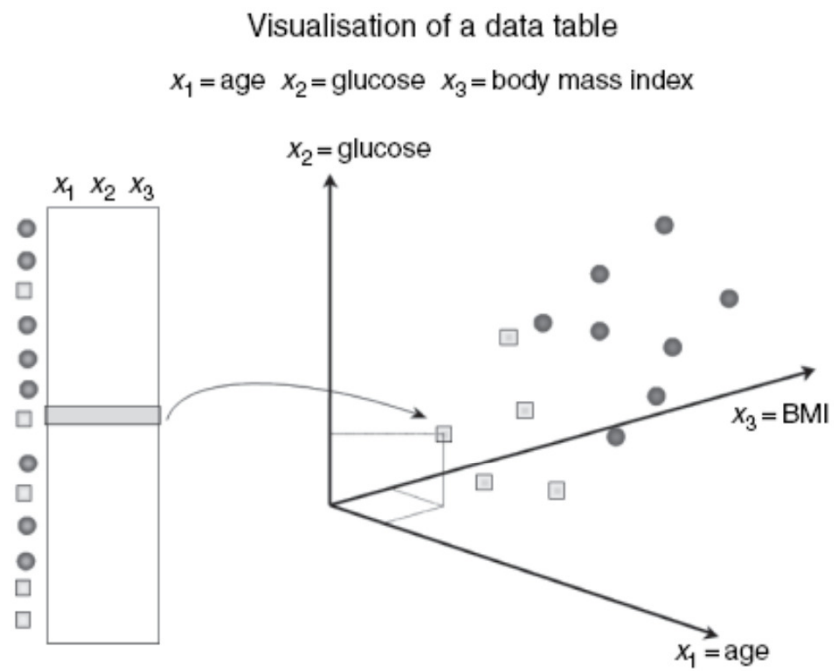


Figure 6.1. Each row (e.g. object or observation) in a K-dimensional data table (here with $K = 3$ variables, designated x_1, x_2, x_3) can be represented as a point in a K-dimensional space (here one point in a three-dimensional space). The coordinates for each object in this multi-dimensional space are given by its three variables, that is a multivariate profile. A data table with N rows then corresponds to a swarm of points. Points that are close to each other have more similar properties than points that lie far apart.

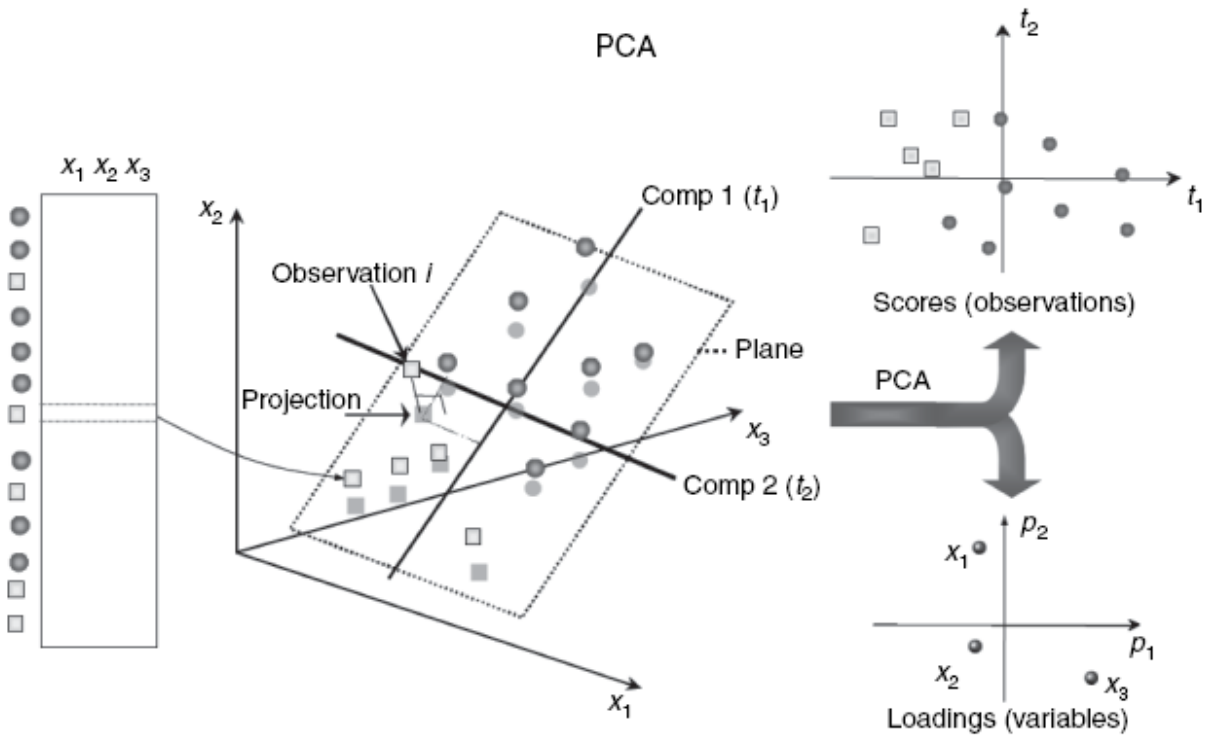


Figure 6.2. A principal component analysis (PCA) model approximates the variation in a data table by a low dimensional model plane. This model plane represents a two-dimensional projection of the multi-dimensional data and provides a score plot, where the relation among the observations or samples in the data table is visualized, for example if there are any groupings, trends or outliers. The loadings plot describes the influence of the variables and the relation among them. An important feature is that directions in the score plot correspond to directions in the loading plot, and vice versa.

… KEGG: metabolic pathways