THE PLANT METABOLOME

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מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

THE PLANT METABOLOME

- Code: 20073152

- Credits: 1.0 (including a mini review)

- Wednesday 14:00-16:00, Katzir Hall, Ulmann Building

- 15 classes

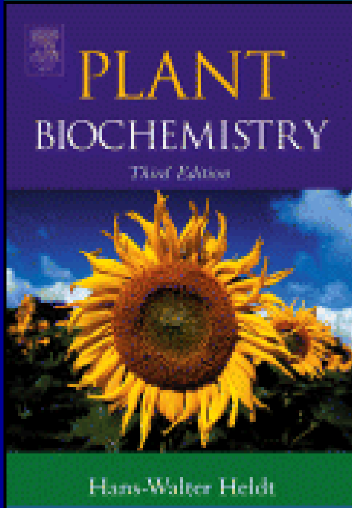
What will we hear and talk about?

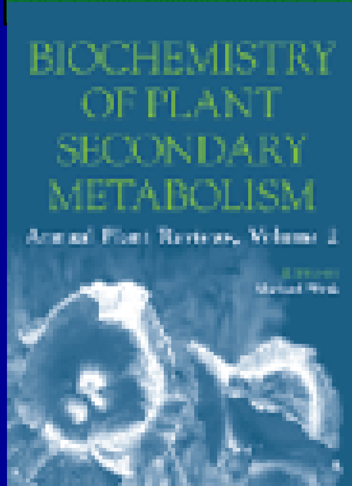
The PLANT Metabolme:

- CORE PATHWAYS (BOTH PRIMARY AND SECONDARY)
- PATHWAY REGULATION
- ANALYSIS / MEASURMNET
- EVOLUTION
- APPLICATIONS
- ENGINEERING

Textbooks and Readings

- Biochemistry & Molecular Biology of Plants, ASPB http://www.aspb.org/publications/biotext/biochemorderinfo.cfm
- Plant Biochemistry and Molecular Biology by Hans-Walter Heldt
- Biochemistry of Plant Secondary Metabolism: Edited by Michael Wink
- Readings from original literature

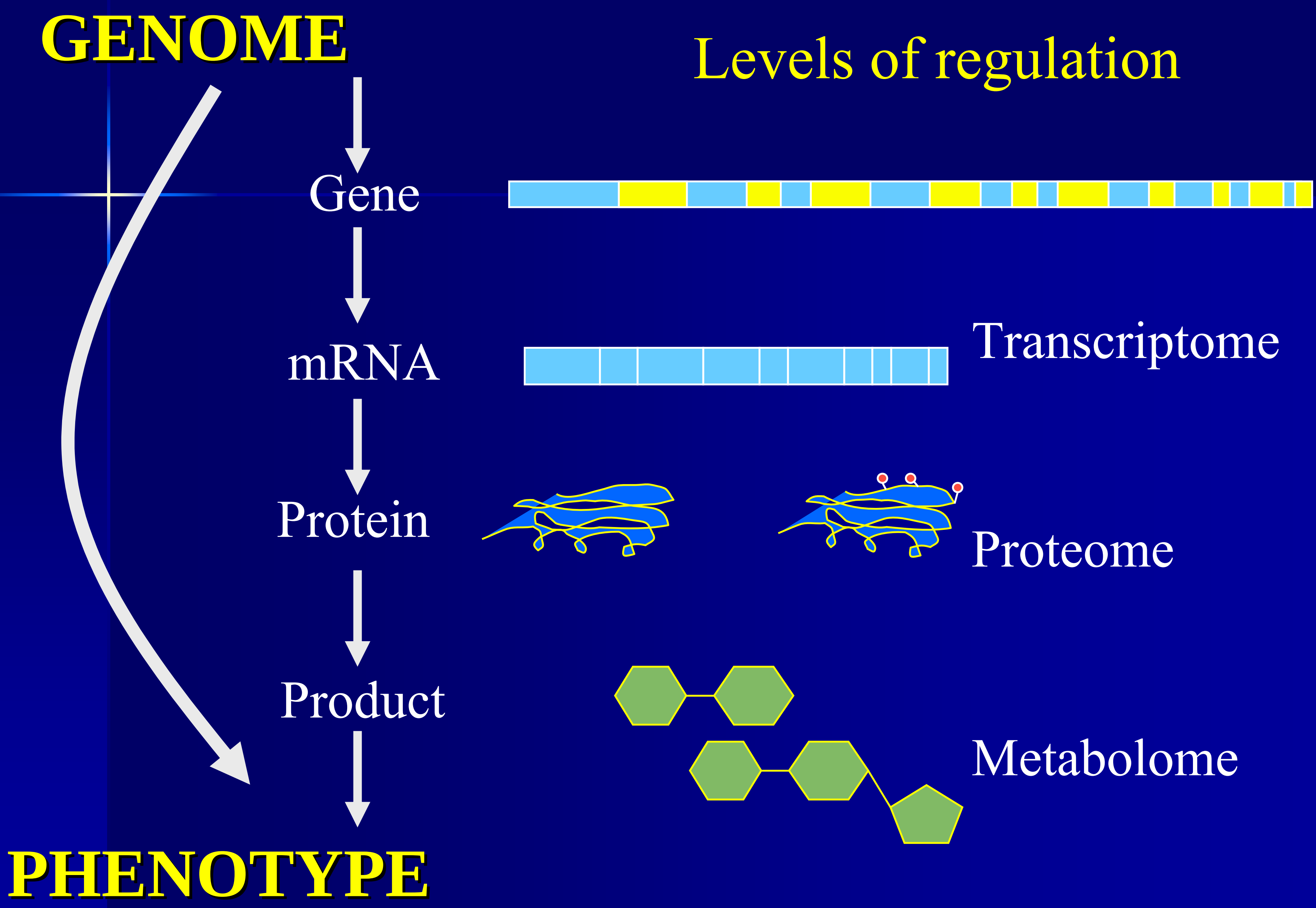




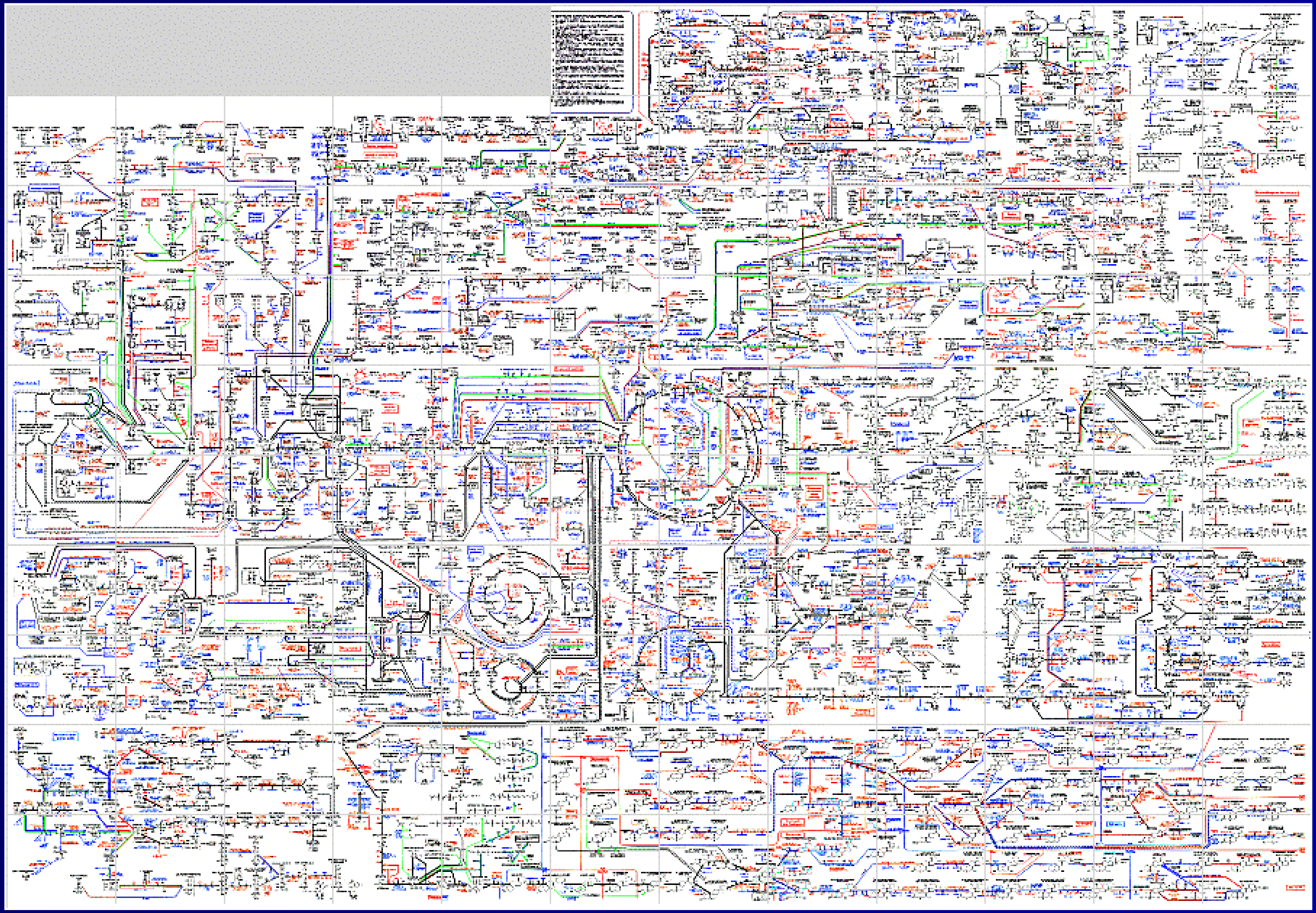
The Metabolome:

Is a comprehensive profile of all the low molecular weight compounds of an organism, a tissue, or a cell

Oliver et al., (1998)


GENOME
Levels of regulation
Gene
mRNA
Transcriptome
Protein
Proteome
Product
Metabolome
PHENOTYPE

Network of Metabolic Pathways in a Single Cell



Network of Metabolic Pathways in a Single Cell

- All reactions occur in a cell that is less than 0.1 mm in diameter

- Nearly every reaction requires a different enzyme

- The same compound can be part of many different pathways

- Pyruvate, a substrate for more than six enzymes, each one modify it in a different way

Network of Metabolic Pathways in a Single Cell

- In multicellular organisms most complex regulation:

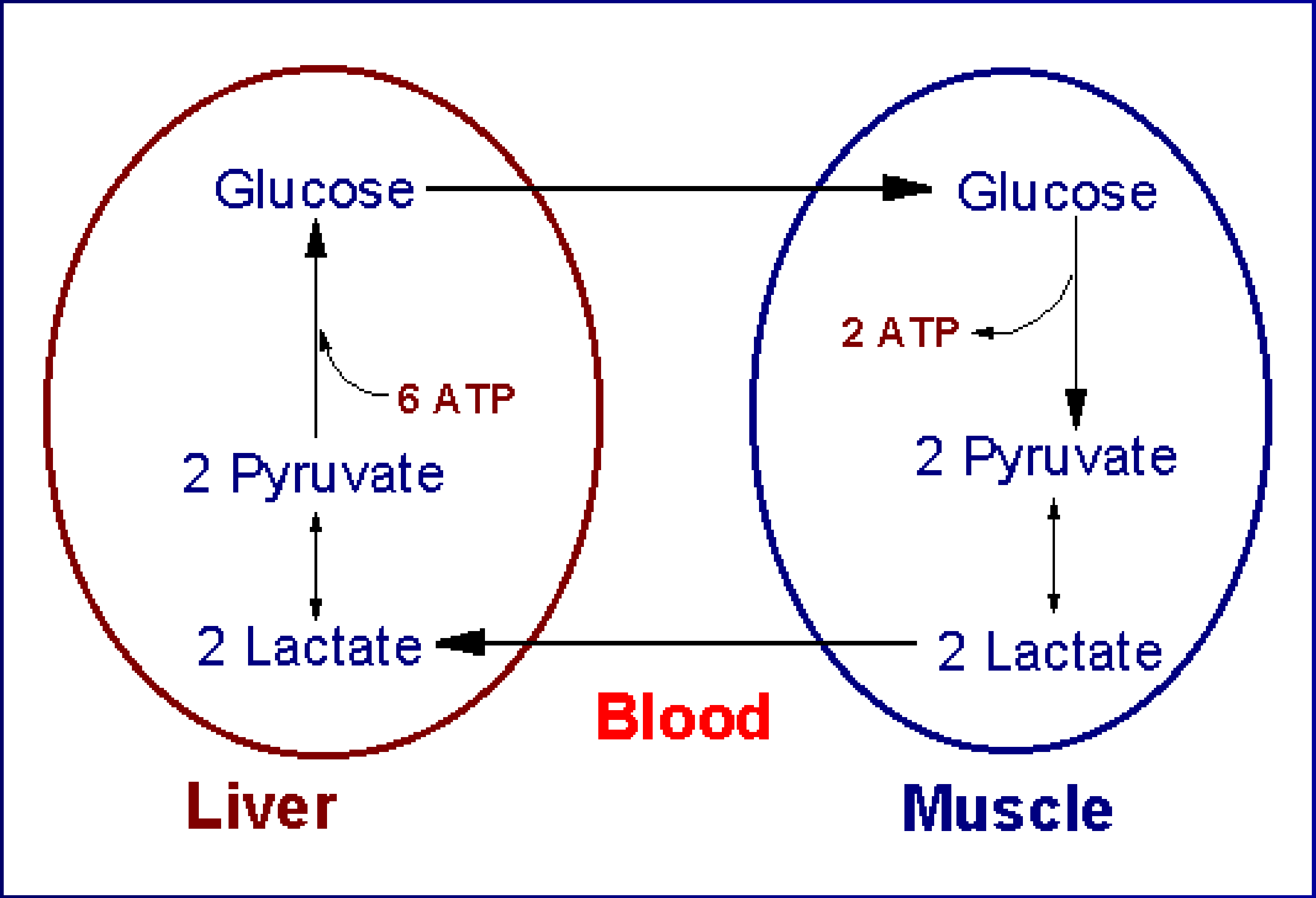
  - Different cell types require different sets of enzymes

  - Different tissues/organs make distinct contributions to the chemistry of the organism as a whole

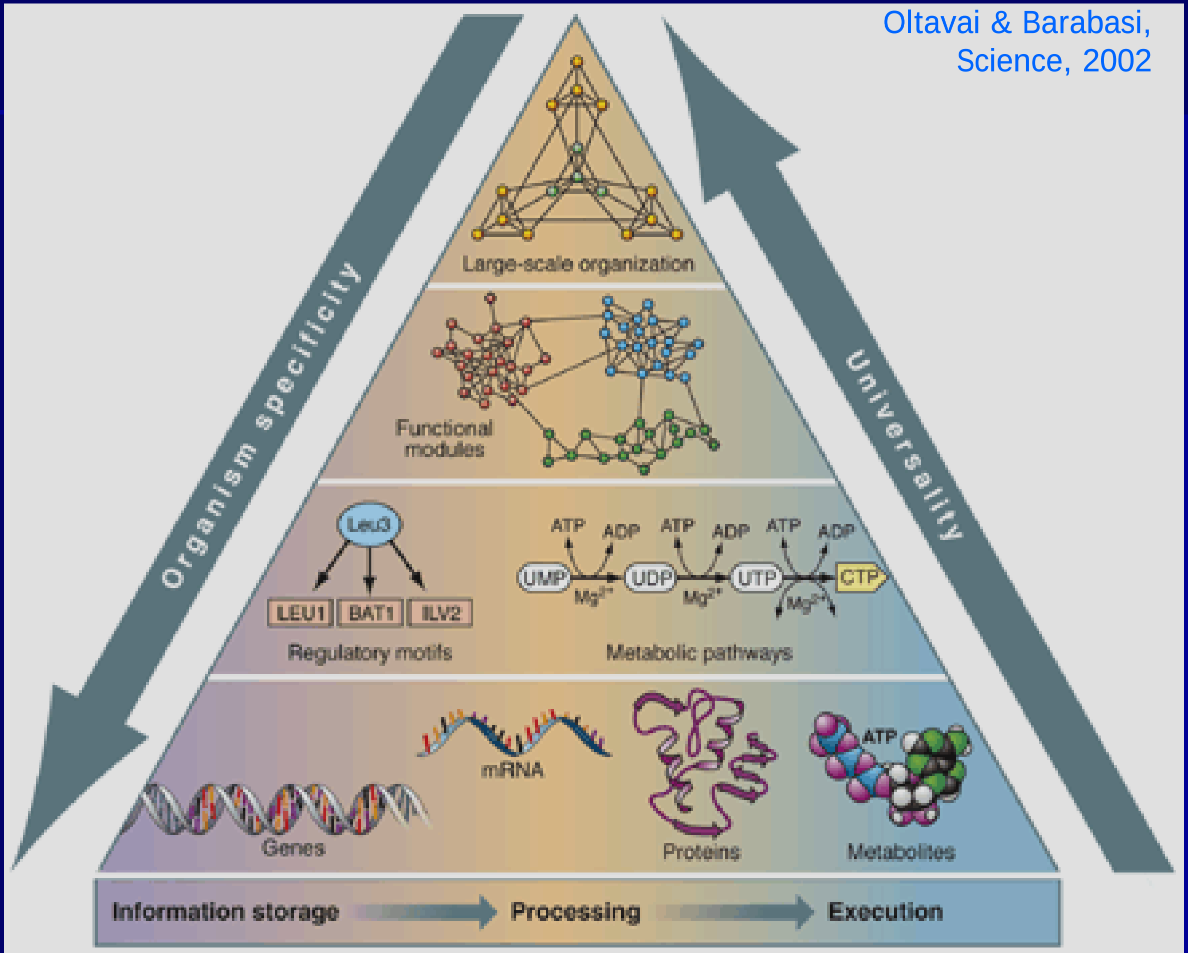
  - Levels of metabolites required differ between tissues

  - Cell types & tissue organs cooperate by exchange of metabolites in normal and stress states

The Cori Cycle: Liver-Muscle Interaction



Life's Complexity Pyramid

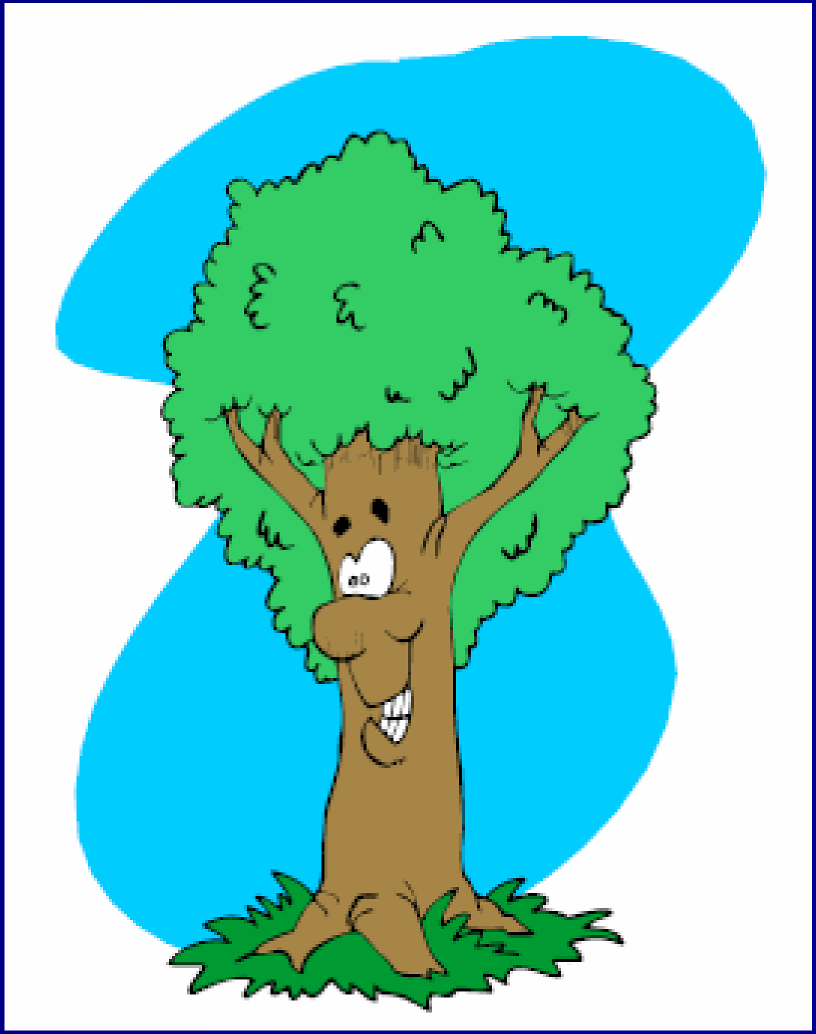


The cell is a complex network in which the components are connected by functional links

PLANT METABOLISM

For an organism to be alive it must:

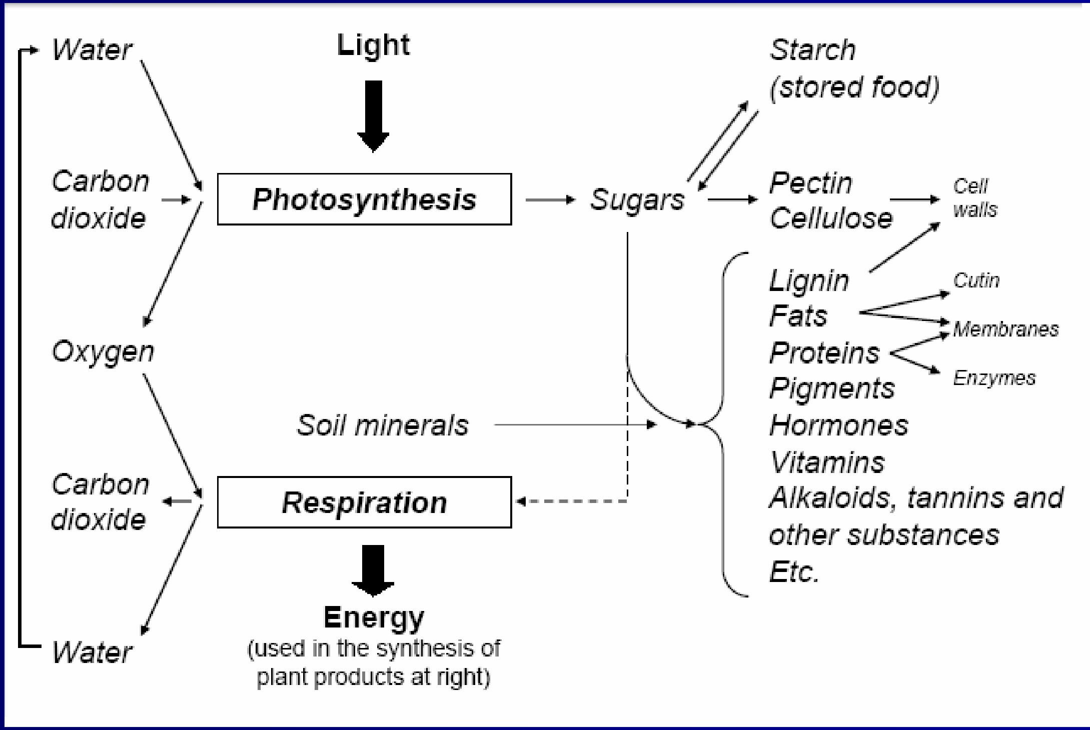
A. Respond to stimulus
B. Reproduce
C. Grow
D. METABOLIZE



PLANT METABOLISM

The complex of physical and chemical events of photosynthesis, respiration, and the synthesis and degradation of organic compounds

PLANT METABOLISM



Photosynthesis produces the substrates for respiration and the starting organic compounds used as building blocks for subsequent biosyntheses of nucleic acids, amino acids, and proteins, carbohydrates and organic acids, lipids, and natural products.

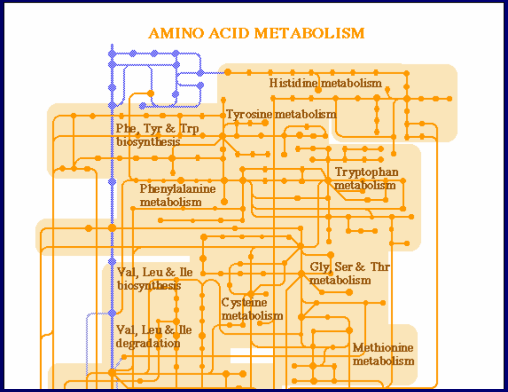
Estimated Size of the Metabolome

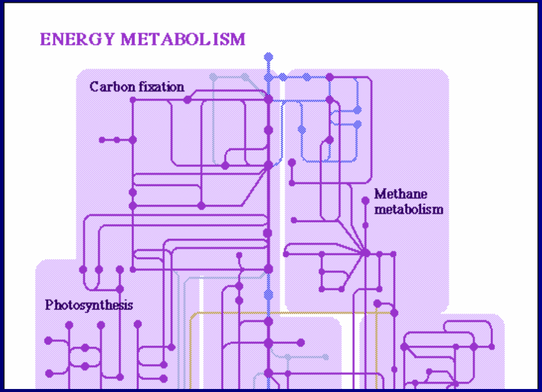
- In yeast 550 compounds in a cell

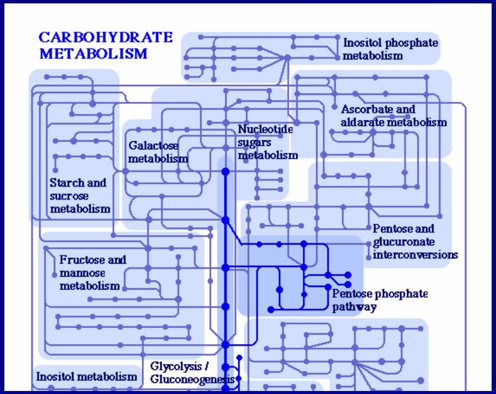
- In plants 4,000 to 20,000 compounds in a cell

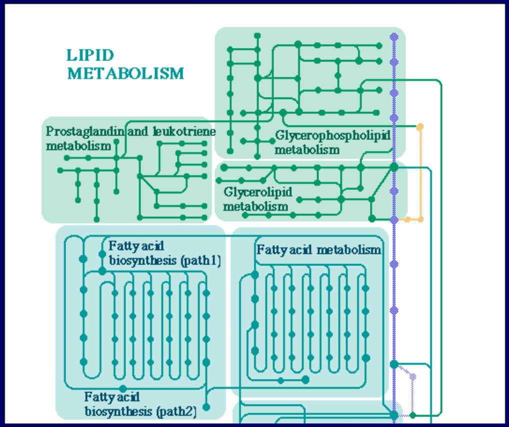
- To date, a total of 200,000 structures identified from plants

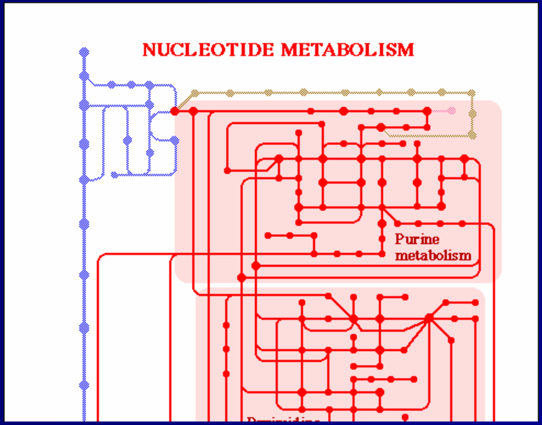
KEGG: 13,803 compounds, 6579 reactions

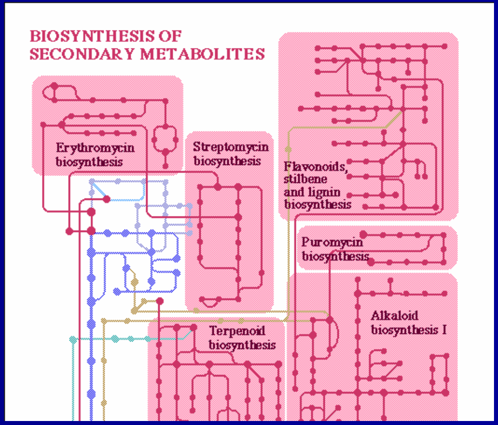












Enzyme Classification

1. Oxidoreductase	Transfer of electrons
2. Transferase	Transfer of functional groups
3. Hydrolase	Bond cleavage using water
4. Lyase	Bond cleavage by elimination
5. Isomerase	Intramolec. rearrangement
6. Ligase	Bond formation (ATP dep.)

The Metabolome

1. The Metabolome is organism, tissue and cell specific
2. Influenced by the environment

The Metabolome

1. The metabolome is tissue and cell specific
2. Influenced by the environment

Primary Metabolites

- Produced by all organisms

- Needed for cell viability and proliferation and organism growth and developemnt

- Example- amino acids, phytosterols, acyl lipids, and nucleotides

Secondary Metabolites

- Also termed <u>specialized metabolites</u> (in certain plant taxonomic groups) or <u>natural plant products</u>

- Plants will survive without most of them but will be damaged

- Play an important role in the interaction between plants and the environment (biotic and abiotic stresses)

- Important since plants are sessile

Secondary Metabolites

- Approx. 50,000 structures known

- Belong to ditinct classes that are often linked to each other

- Often complicated chemistry

- Utilty as dyes, polymers, fibers, glues, oils, waxes, flavouring agents, perfumes, and drugs

Main Groups of Secondary Metabolites in Plants

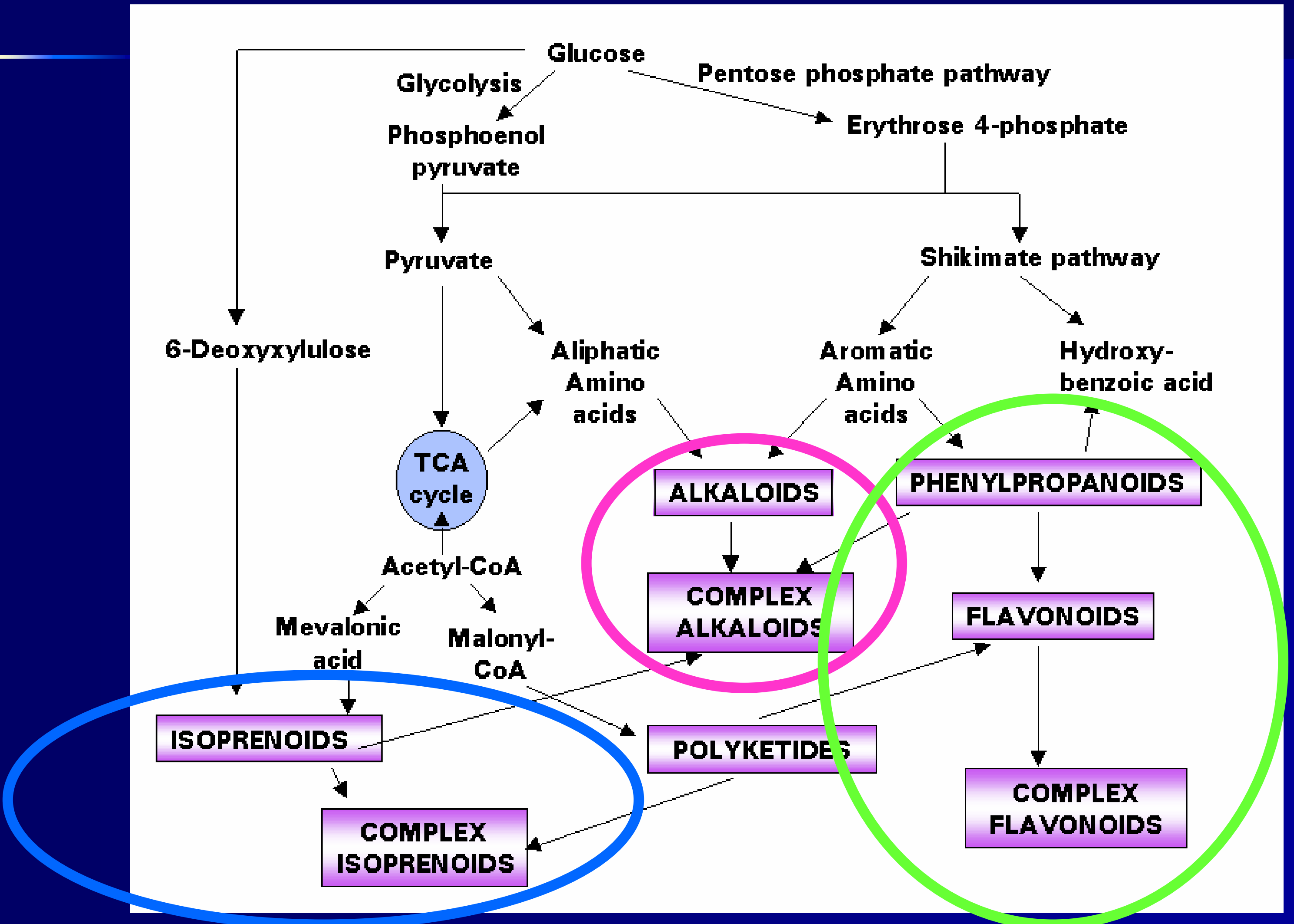
29,000 terpenes

12,000 alkaloids

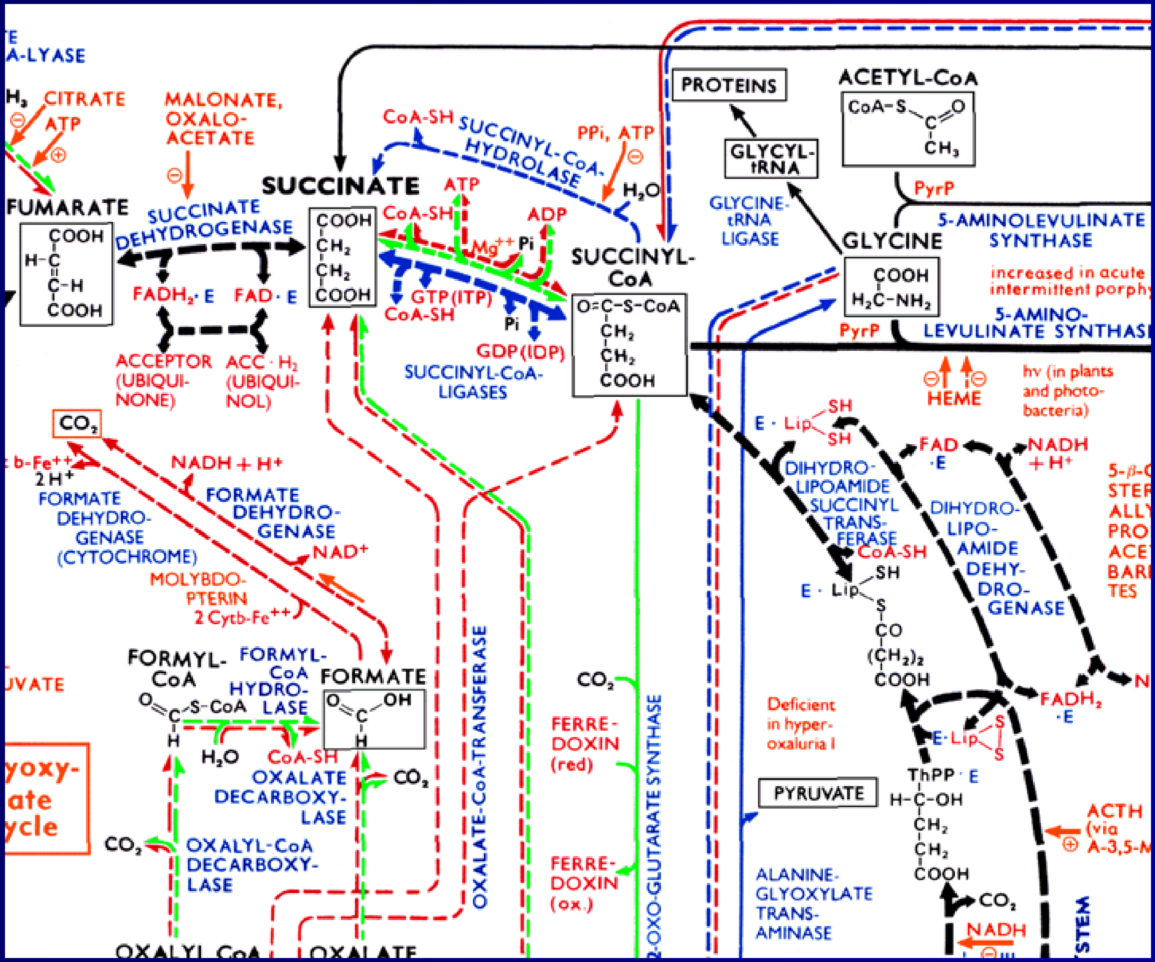
8,000 phenolics

Croteau et al., 2000

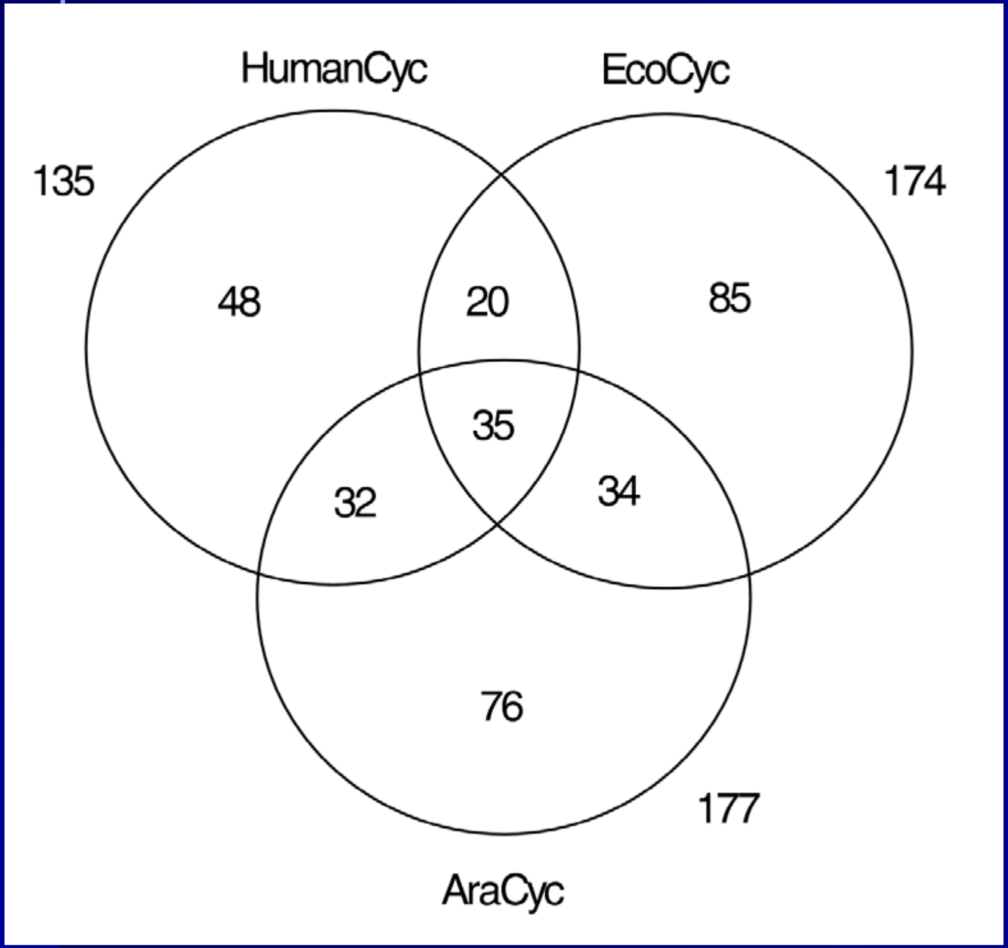
Secondary Metabolites are Derived from Primary Metabolites



Plant, unicellular and animal biochemistry sometimes differ



The Metabolome of Human, E.coli & Arabidopsis



According to HumanCyc (http://HumanCyc.org/):

35 metabolic pathways are common to all three organisms

Regulation of Metabolism- Characteristics

- Stoichiometric requirements (quantative relationship between reactants and products in a reaction)
- Avoid waste (energy that is needed when it is needed)
- Directionality of metabolism
  - Most reactions are reversible
  - The cytoplasm as a soup (how does anything get done?)

Methods of Metabolic Regulation

1. Properties of enzymes
2. Compartmentation
3. Transcriptional and post-transcriptional

Properties of Enzymes

- Affinity for substrate, inherent catalytic capacity
- Feed-back regulation / feed-forward / loop-gain
- Allosteric effects, competitive versus non-competitive inhibition
- Redox control of enzymes
- pH and Mg regulation (especially chloroplast enzymes)

Promiscuous Activity

Promiscuous activity in a protein is also termed:

substrate ambiguity
cross reactivity
moonlighting activity

Too few genes, too many metabolites?

- In plants more than 200,000 structures have been identified
- Every plant estimated to contain- 4k-20k
- Diversity in modification of the same backbone structure
- Example: 300 different glycosides of the flavonol quercitin
- In Arabidopsis according to AraCyc:

1900 genes encoding enzymes with defined function

Too few genes, too many metabolites?

How to explain METABOLOME size/diversity?

a. DNA level: Alternative reading frames and gene fusion
b. mRNA level: Alternative splicing
c. Protein: Post translational modification and Hetrodimer formation

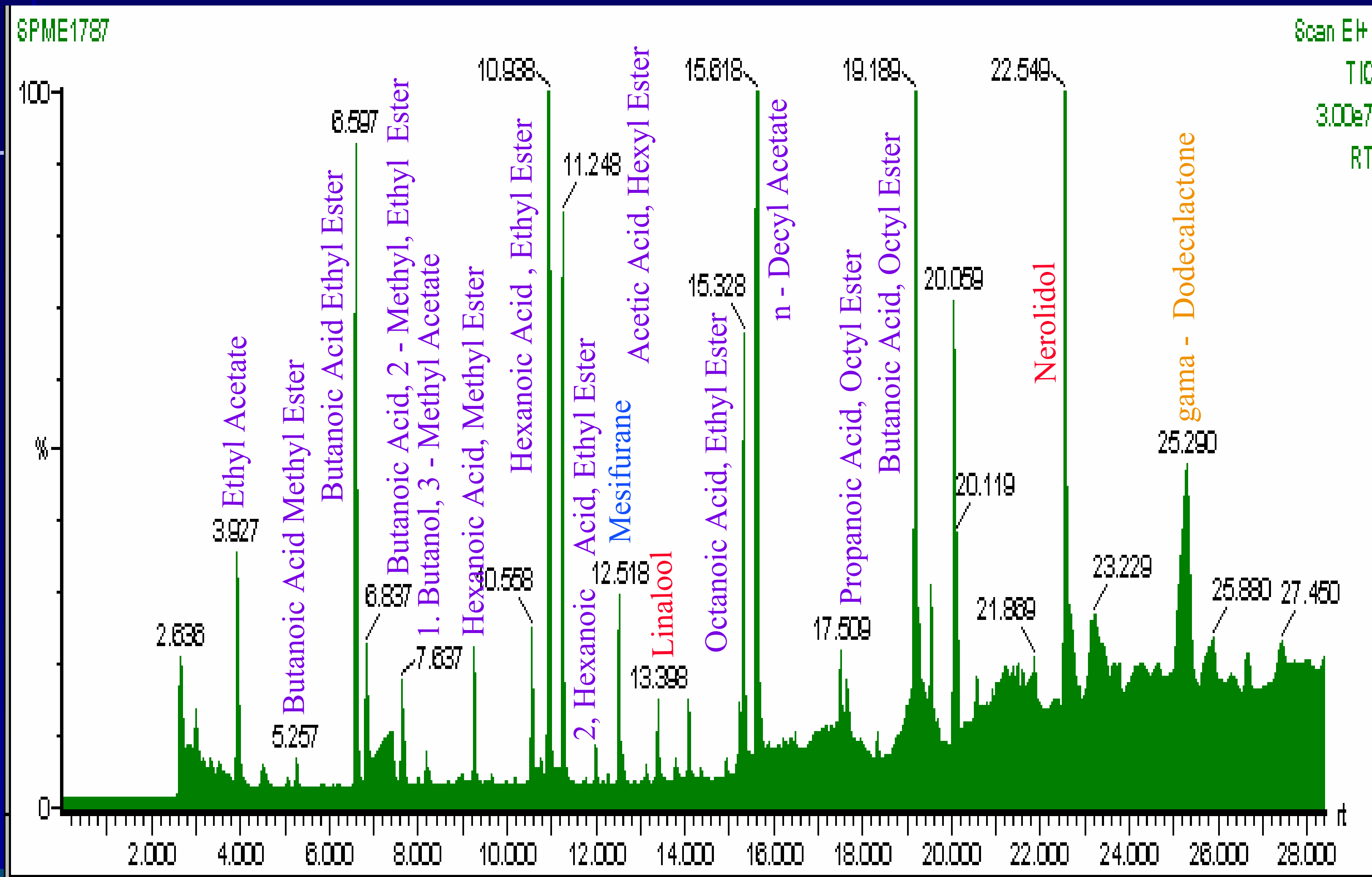
And…… Enzyme Specificity!

Plant Secondary Metabolism: An Excellent Example

Alcohol acyl-CoA transferases

One enzyme…..Multiple substrates…..Multiple products

Volatile Composition of Cultivated Strawberry



Ester | Furan | Terpene | Lactone

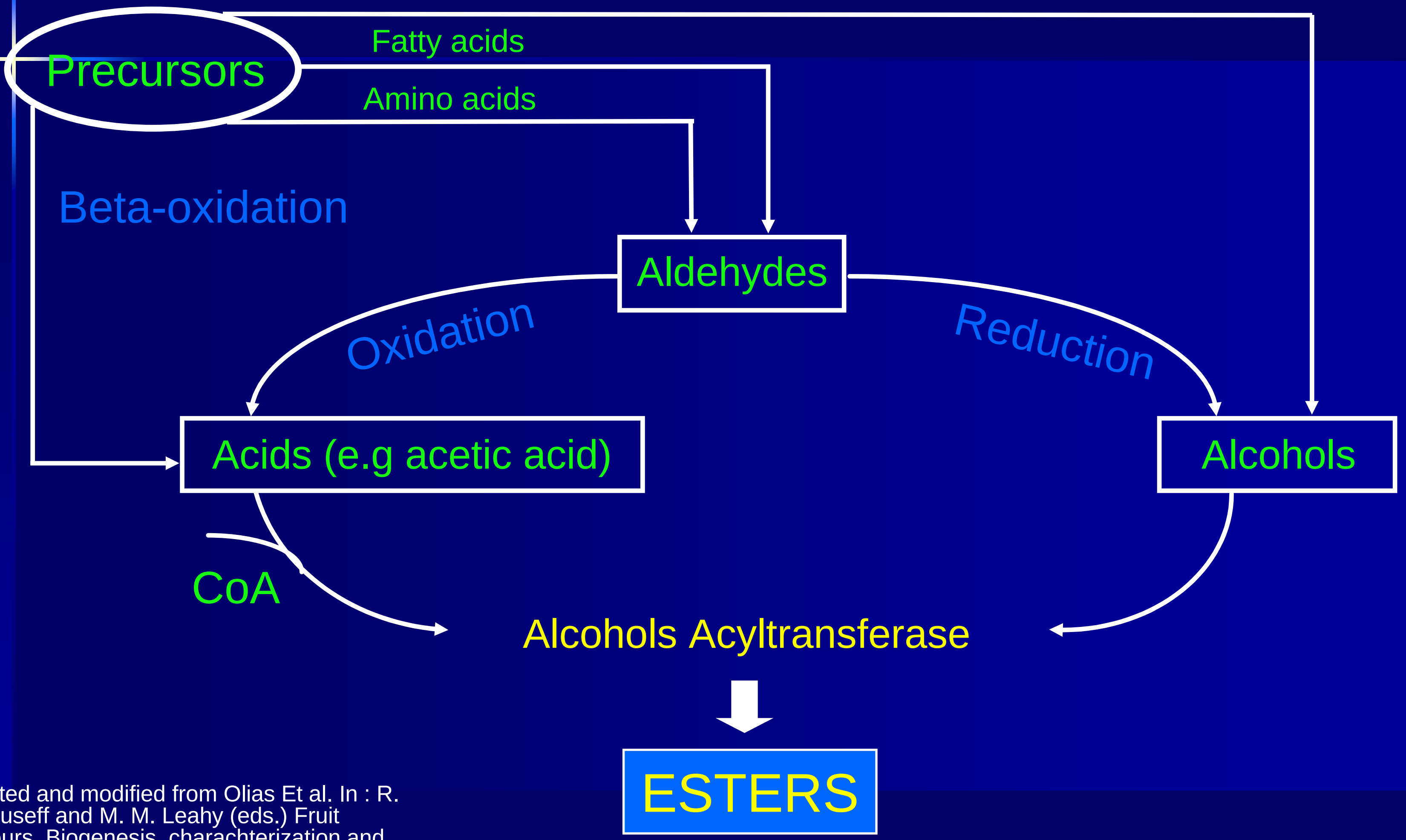
Volatile Esters Contribute to the Aroma of Most Fruit

92 esters

>100 esters

- Some are responsible for a particular fruit aroma (e.g. banana)

Metabolic Route for the Formation of Esters in Fruit



Adapted and modified from Olias Et al. In : R. L. Rouseff and M. M. Leahy (eds.) Fruit Flavours. Biogenesis, charachterization and authentication (1995).

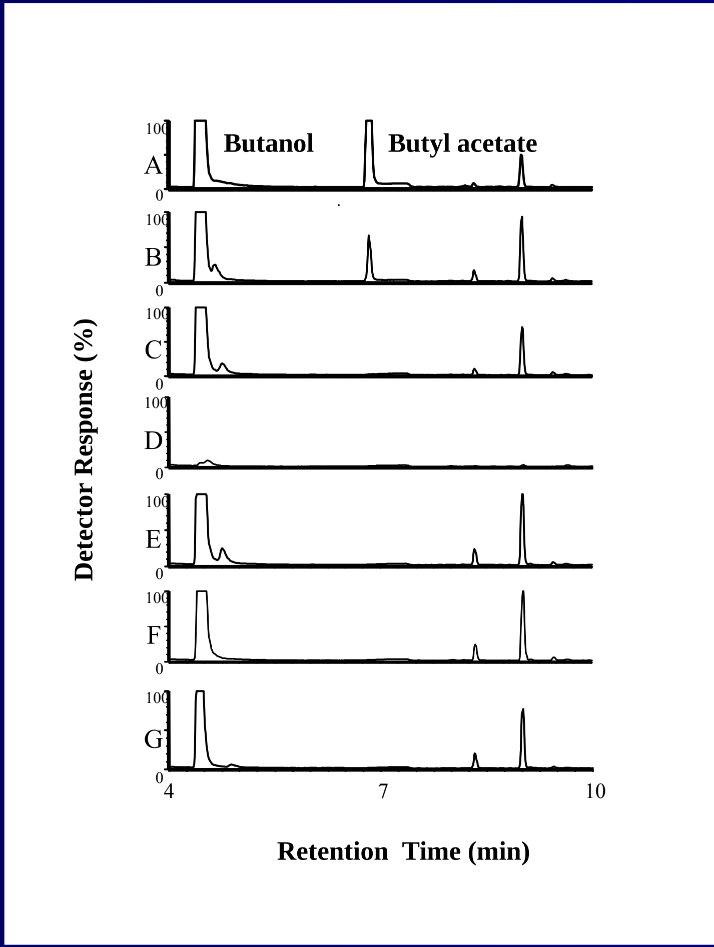
Ester Formation by Alcohol Acyltransferase (AAT)

Ethylalcohol → Ethylacetate

Acetyl-CoA + AAT

Isoamylalcohol → Isoamylacetate

Ester Formation by *SAAT* Expressed in *E.coli* Cells



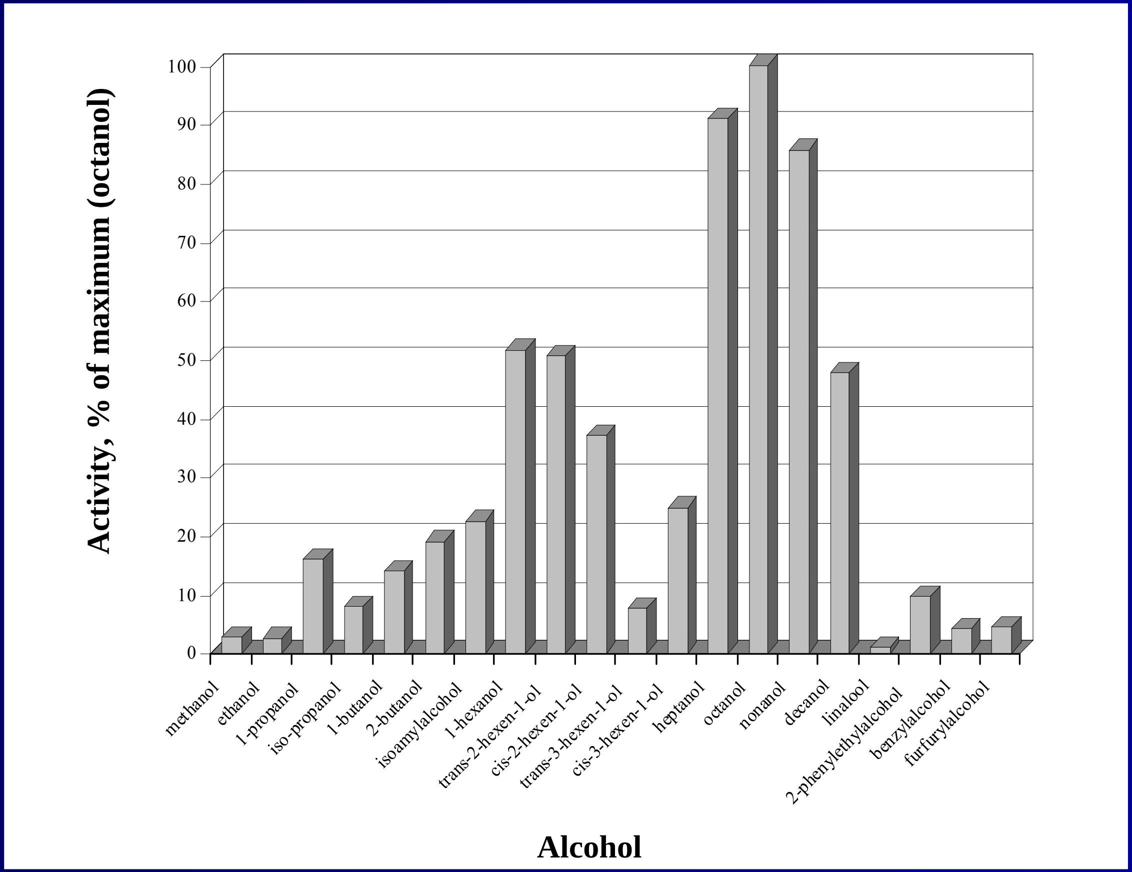
In B:

Butanol + Acetyl CoA

↓

Butylacetate

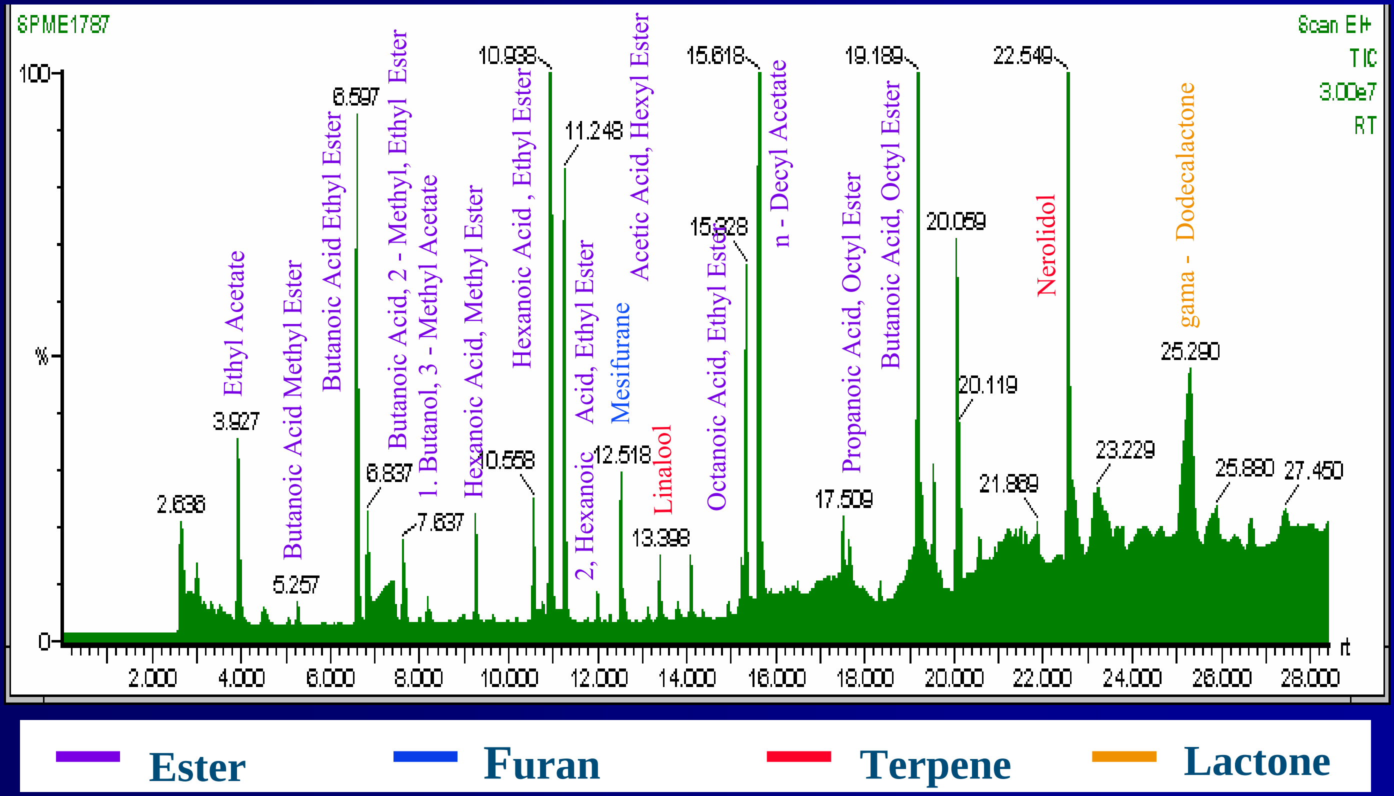
Broad substrate specificity (alcohols)



Broad Substrate Specificity (acyl CoAs)

Acyl CoAs (Carbon no.)	Alcohol	Ester formed	Ester properties
n-propionyl CoA (C3:0)	1-butanol	1-butyl propanoate	ethereal, banana
n-butyryl CoA (C4:0)	1-propanol	1-propyl butyrate	sharp, pungent, rancid, sweaty, sickening
n-butyryl CoA (C4:0)	1-butanol	1-butyl butyrate	fruity, pineapple
isobutyryl CoA (C4:0)	1-butanol	1-butyl isobutyrate	fruity, apple, banana and pineapple
n-crotonoyl CoA (C4:1)	1-butanol	1-butyl crotoate	not described
n-hexanoyl CoA (C6:0)	1-propanol	1-propyl hexanoate	wine-like, cheese
n-decanoyl CoA (C10:0)	1-butanol	1-butyl decanoate	Brandy (Whisky-Cognac)-like odor
benzoyl CoA (C7:0)	1-butanol	1-butyl benzoate	mild floral-balsamic odor

SAAT is a member of a small gene family in strawberry responsible for generating more than 100 different type of esters in the ripe fruit



Plant Secondary Metabolism: An Excellent Example

Terpene Cyclases

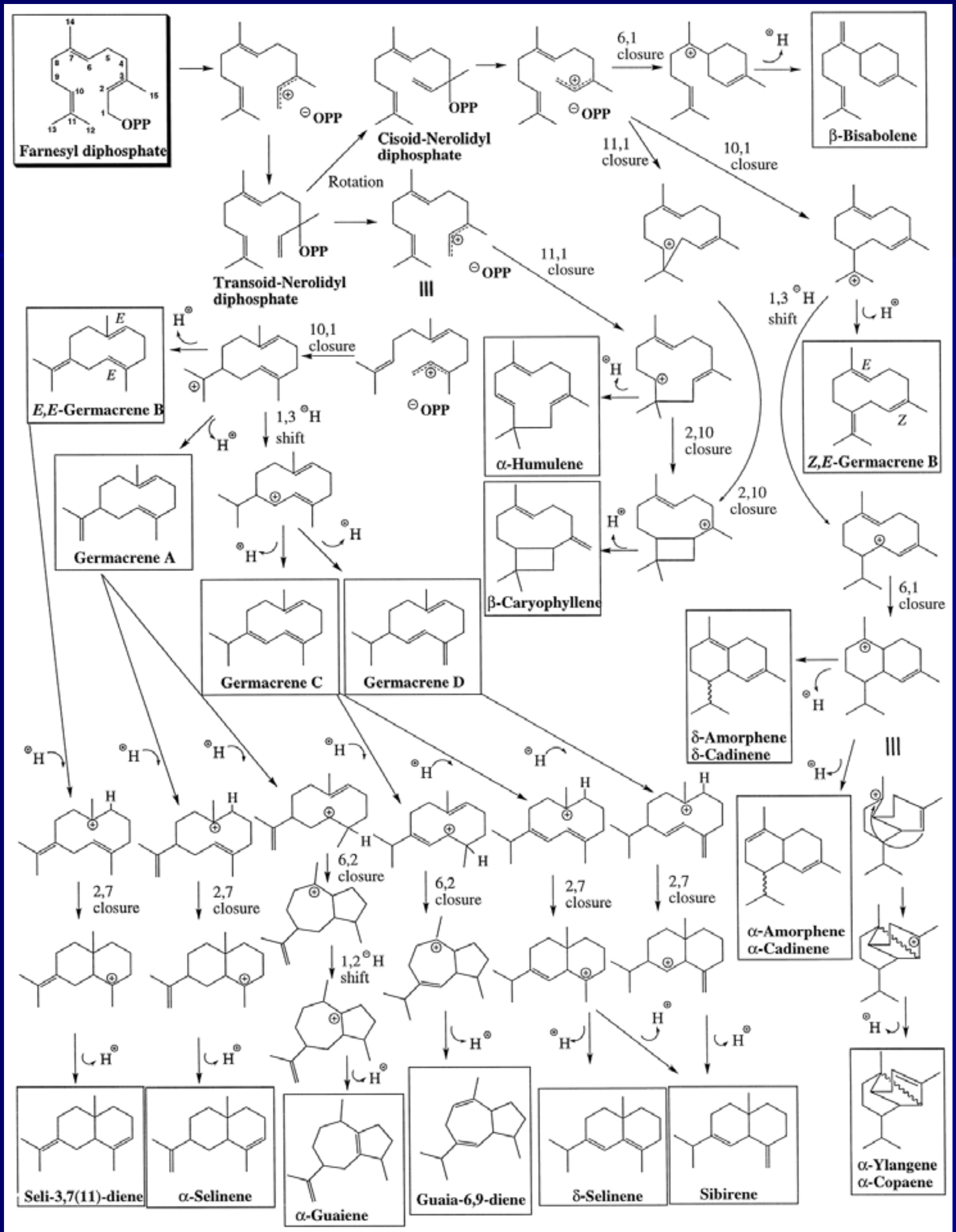
One enzyme…..One substrate…..Multiple products

Plant Secondary Metabolism: An Excellent Example

Trees such as Grand Fir (אשוח):

- Produce oleoresin in response to stem wounding and insect attack
- The turpentine fraction of the oleoresin contains terpenes, mainly mono, sesqui and diterpenes
- In Grand Fir, 38 sesquiterpenes (12.5% of turpentine) and the remaining monoterpenes
- Two terpene synthases expressed in E.coli could synthesize three major products but in total 35 and 53 total sesquiterpenes.

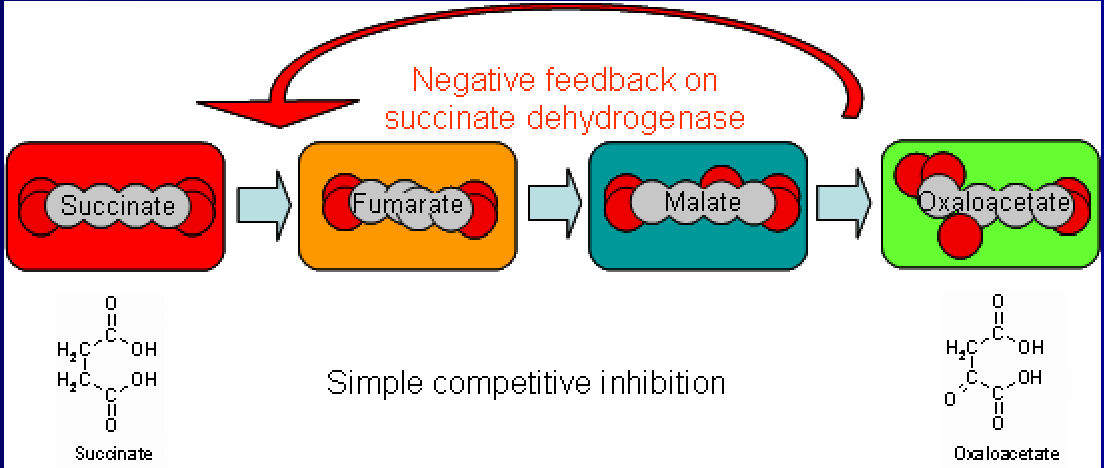
Steele et al., 1998

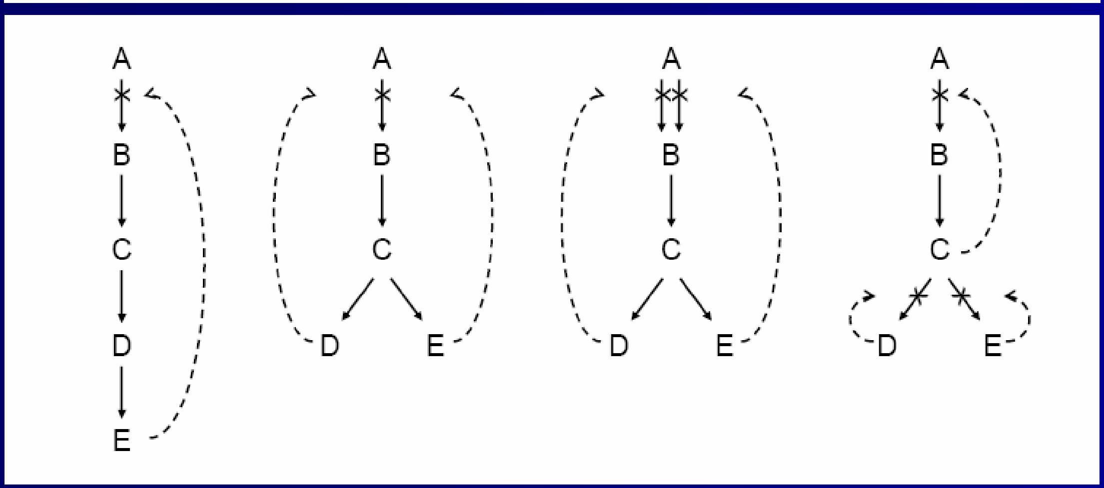


Farnesyl diphosphate
OPP
Cisoid-Nerolidyl diphosphate
Transoid-Nerolidyl diphosphate
Rotation
6,1 closure
11,1 closure
10,1 closure
1,3 H shift
2,10 closure
6,2 closure
2,7 closure
1,2 H shift
β-Bisabolene
E,E-Germacrene B
Z,E-Germacrene B
α-Humulene
β-Caryophyllene
Germacrene A
Germacrene C
Germacrene D
δ-Amorphene
δ-Cadinene
α-Amorphene
α-Cadinene
α-Ylangene
α-Copaene
Seli-3,7(11)-diene
α-Selinene
α-Guaiene
Guaia-6,9-diene
δ-Selinene
Sibirene

Properties of Enzymes

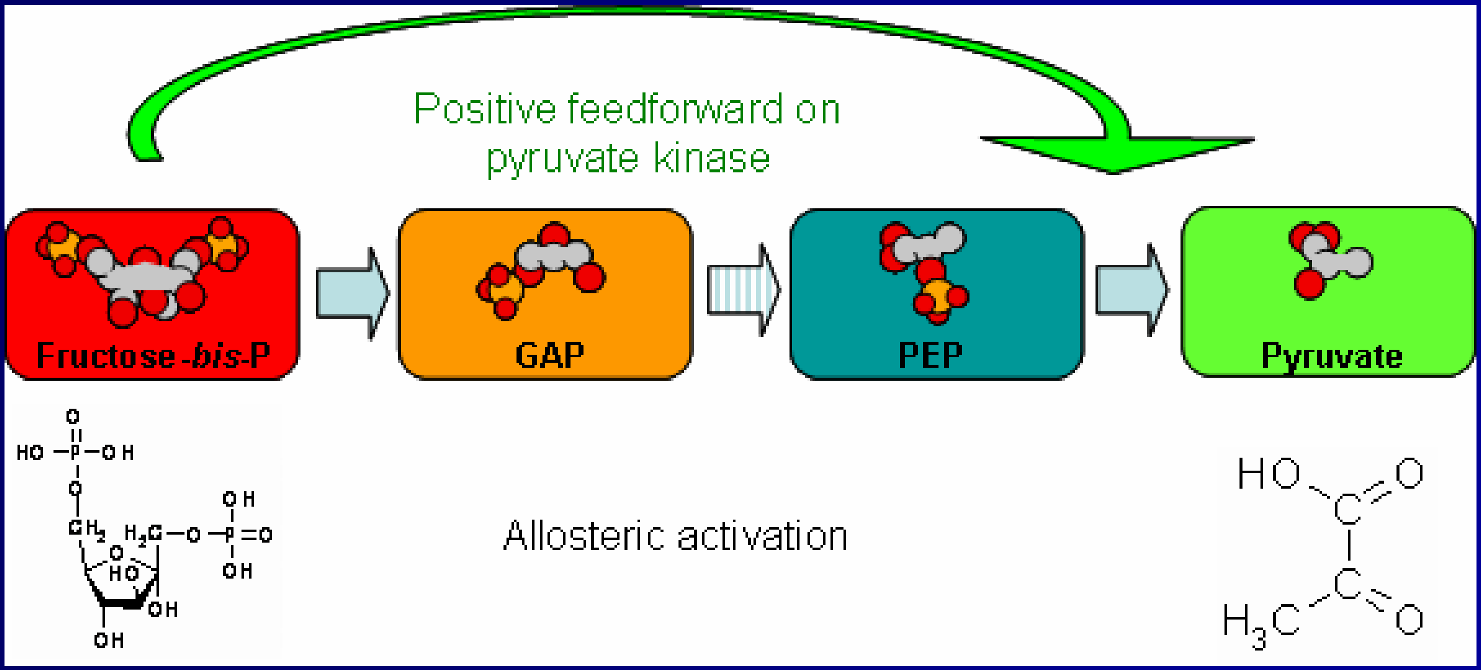
End Product Inhibition or Feed-back Regulation





Properties of Enzymes

Feed-forward Regulation

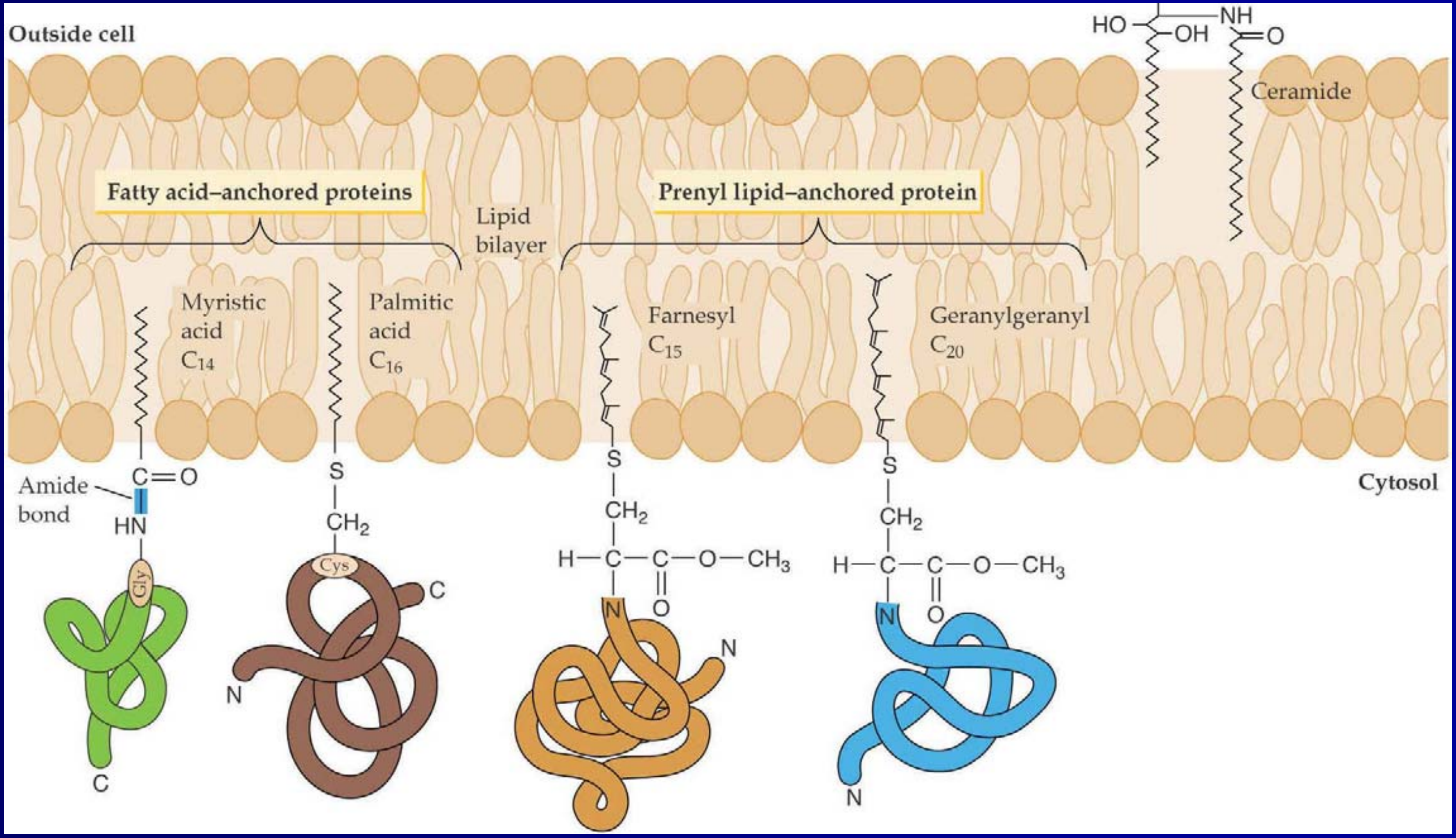


Properties of Enzymes

Post-translational Regulation

- Phosphorylation
  - Protein kinases and phosphatases
  - Turns enzymes on or off, can affect sensitivity to effectors
- Fatty acids
  - Palmitic acid in a regulatory way, myristic acid is non-regulatory
- Prenylation
  - Fanesylation (3 isoprenoids, 15 C) CaaX C-terminus
  - Geranylgeranylation (20 carbons) CaaL C-terminus
- Fatty acids and prenylation anchors proteins to membranes or to other proteins

Anchoring Proteins to Membranes



Buchannan et al. (ASPB book)

Methods of Regulation

1. Properties of enzymes
2. Compartmentation
3. Transcriptional and post-transcriptional

Cellular compartmentation

- Hallmark of eukaryotic cells
- Oxygen reactions mostly in mitochondria and chloroplasts
- Chloroplasts – more generally plastids – are what make plants unique
  - Cell walls, vacuoles also distinctive but not unique
  - Plastids are biochemical powerhouses

Biochemistry Inside Plastids

- Photosynthesis – reduction of C, N, and S
- Amino acids, essential amino acid synthesis restricted to plastids
  - Phenylpropanoid amino acids and secondary compounds start in the plastids (shikimic acid pathway)
  - Site of action of several herbicides, including glyphosate
  - Branched-chain amino acids
  - Sulfur amino acids
- Fatty acids – all fatty acids in plants made in plastids

Biochemistry Inside Plastids

- Carotenoids – source of vitamin A
- Thiamin and pyridoxal, B vitamins
- Ascorbic acid – vitamin C
- Tocopherol – vitamin E
- Phylloquinone (an electron acceptor in PS I – vitamin K)

Methods of Metabolic Regulation

1. Properties of enzymes

2. Compartmentation

3. Transcriptional and post-transcriptional

Transcriptional and Post-transcriptional Regulation

- Normally slow relative to metabolic control
- Allows metabolism to be changed in response to environmental factors
- Transcriptional control most common
  - Sometimes variation in transcription rate not reflected in enzyme amount
- Translational control also found
  - No change in mRNA levels but changes in protein amounts

Time Out- 15 min.

ANALYSING the METABOLOME

- More complicated compared to protein and nucleic acids:

- Unknown pathways
- Often difficult to purify
- Can be impossible to synthesize
- No amplification

ANALYSING the METABOLOME

Different metabolites, different characteristics:

- volatile
- non volatile: polar, semi polar and apolar

Analysing the METABOLOME

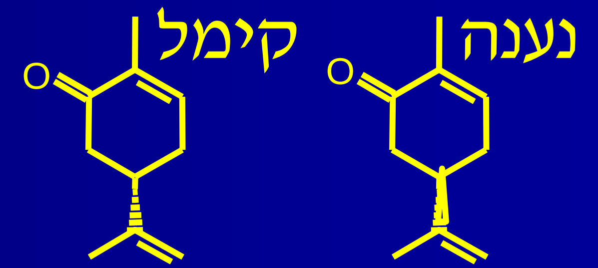
Metabolites :

Elemental composition

Order of the atoms

Type of bonds

Stereochemical orientation



(+)-Carvone (-)-Carvone

ANALYSING the METABOLOME, WHY?

- Assessing gene function and relationship to phenotypes

- Understanding metabolism and predicting novel pathways

- To increase metabolite fluxes into valuable biochemical pathways using metabolic engineering

- To compare genetically modified organisms to non-modified

- To measure flux of carbon under varying conditions

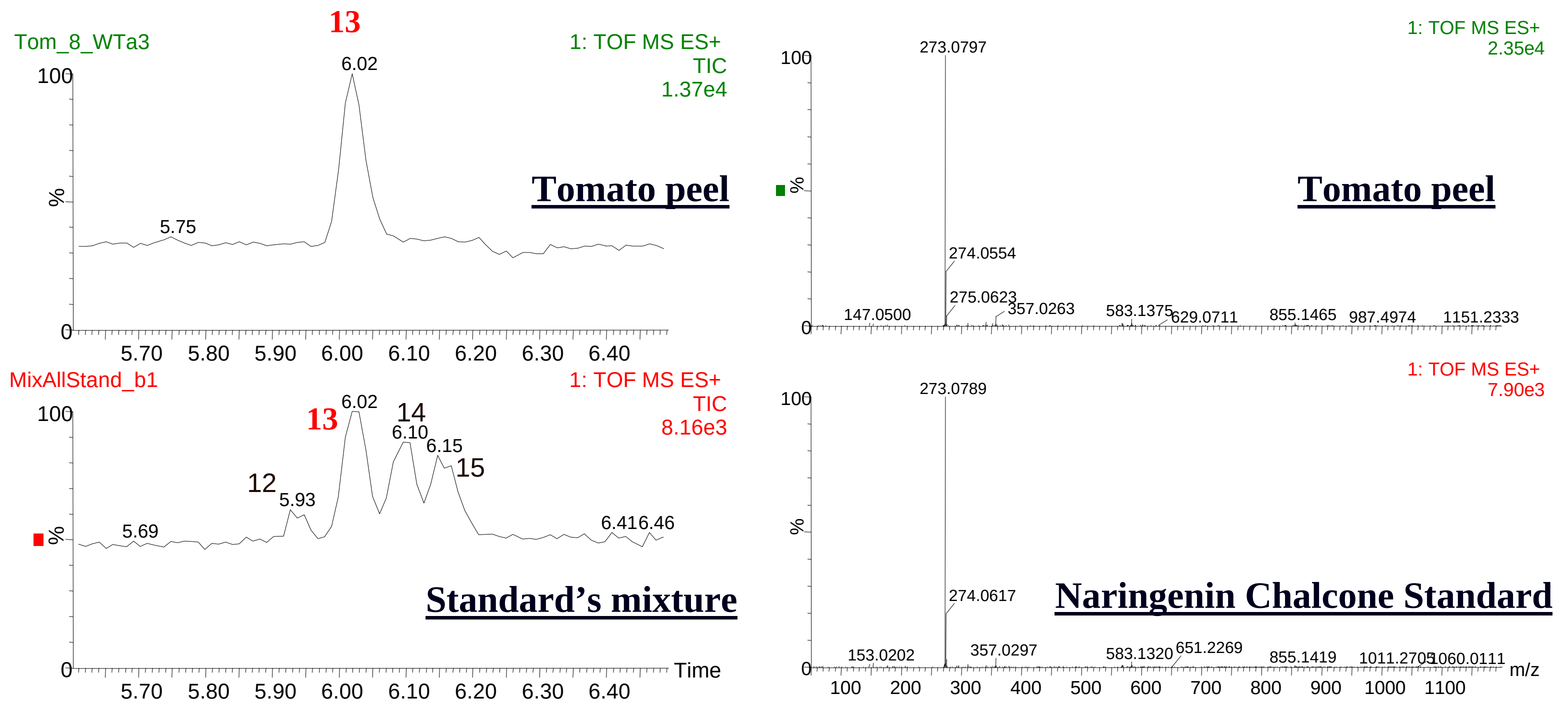
- To asses the effect of environmental changes

Analysing the METABOLOME

- Metabolite target analysis: just a few specific compounds
- Metabolic profiling: classes of compounds
- Metabolic fingerprinting: with pattern recognition
- Metabolomics: as comprehensive as possible ***

Fiehn, Comp. Func. Genomics 2,155-168 (2001)

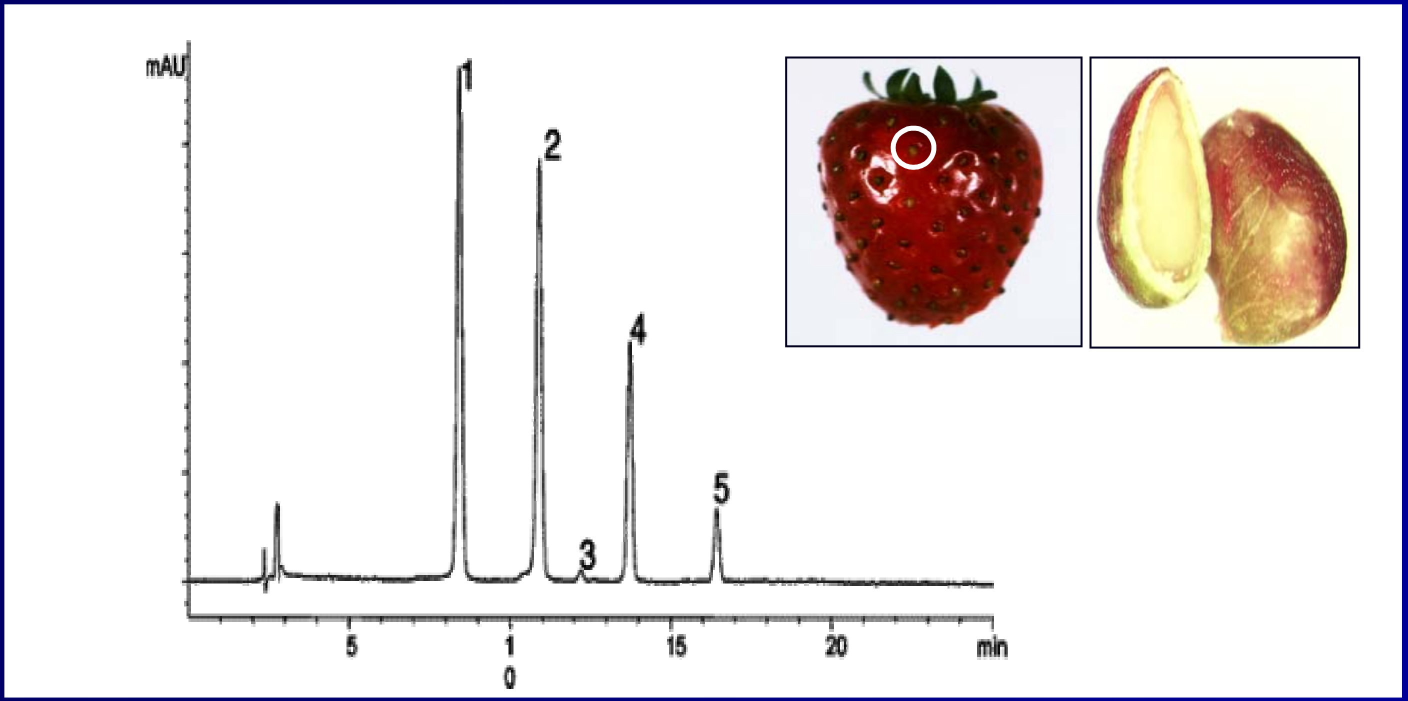
Metabolite Target Analysis



12 – Cinnamic acid
13 – Naringenin Chalcone
14 – Naringenin
15 – Kaempferol

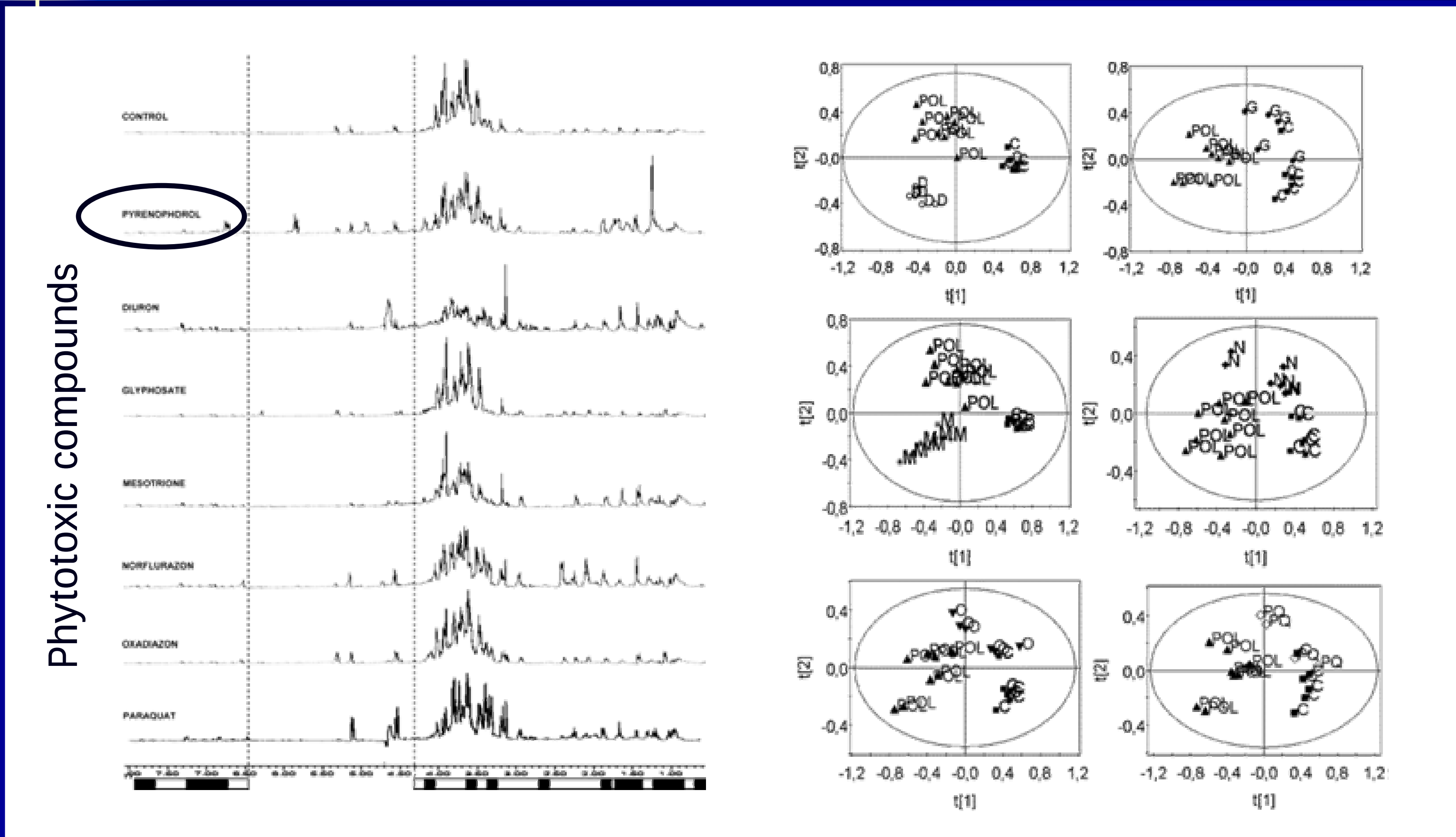
Metabolic profiling

HPLC chromatograms (recorded at 520 nm) of anthocyanins of red ripe strawberries ACHENES: 1 = cyanidin-3-glucoside; 2 = pelargonidin-3-glucoside; 3 = pelargonidin-3-rutinoside; 4 = cyanidin-3-glucoside-malonate; 5 = pelargonidin-3-glucoside-malonate .



Metabolic Fingerprinting

Investigation of the Mode of Action of the Phytotoxin (5S,8R,13S,16R)-(-)-Pyrenophorol Using (1)H Nuclear Magnetic Resonance Fingerprinting (Konstantinos A. Aliferis and Maria Chrysayi-Tokousbalides, JAFC, 2006)



Metabolic FOOTprinting or Exometabolome

"A Strategy for analyzing the properties of cells or tissues by looking in a high-throughput manner at the metabolites that they excrete or fail to take up from their surroundings (Kell et al, 2005)."

- Similar to fingerprinting, based on pattern recognition
- In Fingerprinting intracellular metabolites analyzed while in Footprinting the culture media (as in the case of yeast).
- Stimulating metabolic changes by adding various carbon compounds or inhibitors

Metabolomics and Other OMICS Approaches

- Transcriptomics: Monitor the expression levels of tens of thousands of genes
- Proteomics: Monitor abundance patterns of thousands of proteins
- Metabolomics: Monitor thousands of low molecular weight metabolites simultaneously
- Bio-informatics: Processing data and extracting biological meaning

METABOLOMICS

- The comprehensive, quantitative and qualitative analysis of all metabolites within a cell, tissue or an organism is far from reality in any system

-Multiple technologies are required

-The field is developing rapidly

Metabo-l-omics and Metabo-n-omics

Nicholson et al. 1999

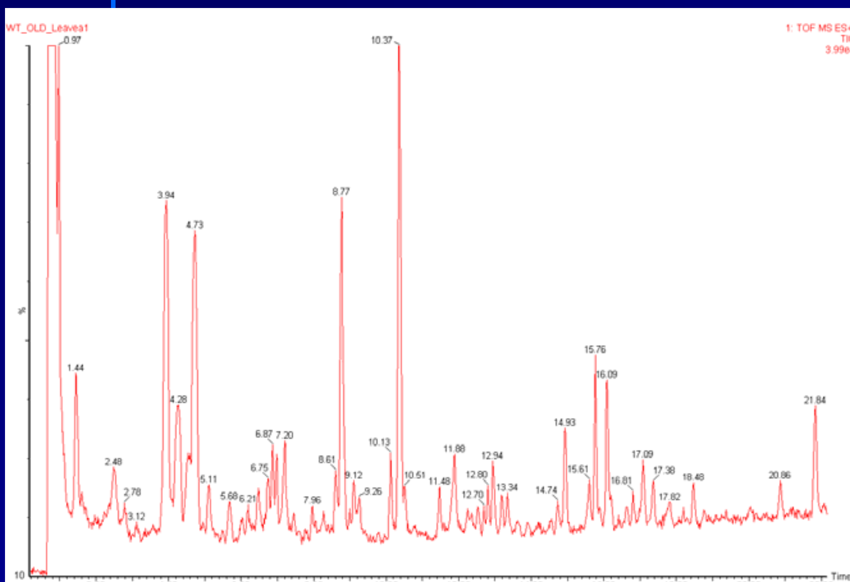
- Metabonomics: “the quantitative measurement of the time – related multi-parametric response of living systems to pathophysioloical stimuli or genetic modification”

Fiehn,

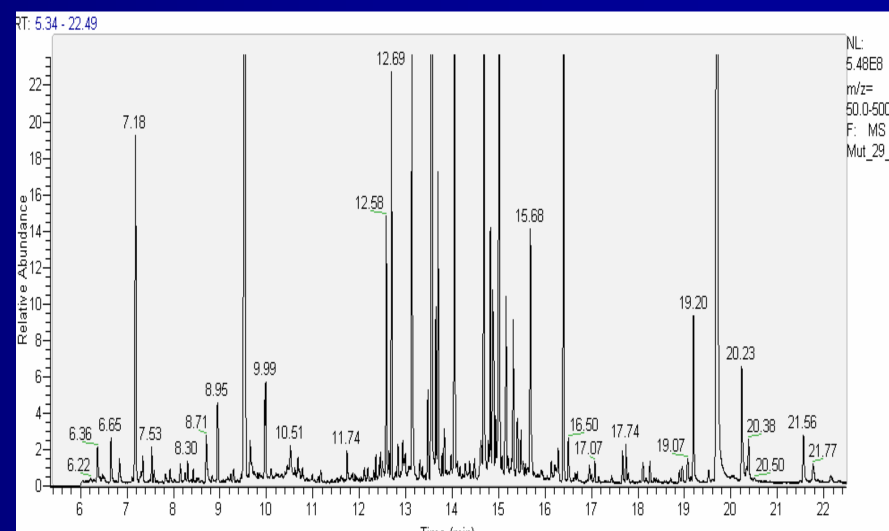
- Metabolomics: “the comprehensive and quantitative analysis of all metabolites”

Approaches are the same, Nomics more metabolic response to drugs and diseases (animal systems and with NMR) while Lomics more bacterial/plants (with MS (GC, LC)

Metabolomics:
Detecting Multiple Metabolites



QTOF-MS



GC-MS

Analysing the METABOLOME

1. Metabolite Extraction
2. Metabolite (separation not always) detection
3. Data analysis:

- From raw data to information which is ready for mining
- Extraction of biological relevance
- Data visualization (maps, tables, charts etc..)

Analysing the METABOLOME

1. **Metabolite Extraction**
2. Metabolite detection (with or without separation)
3. Data analysis

EXTRACTION

Each group of metabolites will have an optimal extraction method
(no single solution)

Stopping the enzymatic activity!!

General Metabolite Extraction Methods

- Liquid phase extraction

  Grind sample, extract with solvent
- Liquid : Liquid extraction

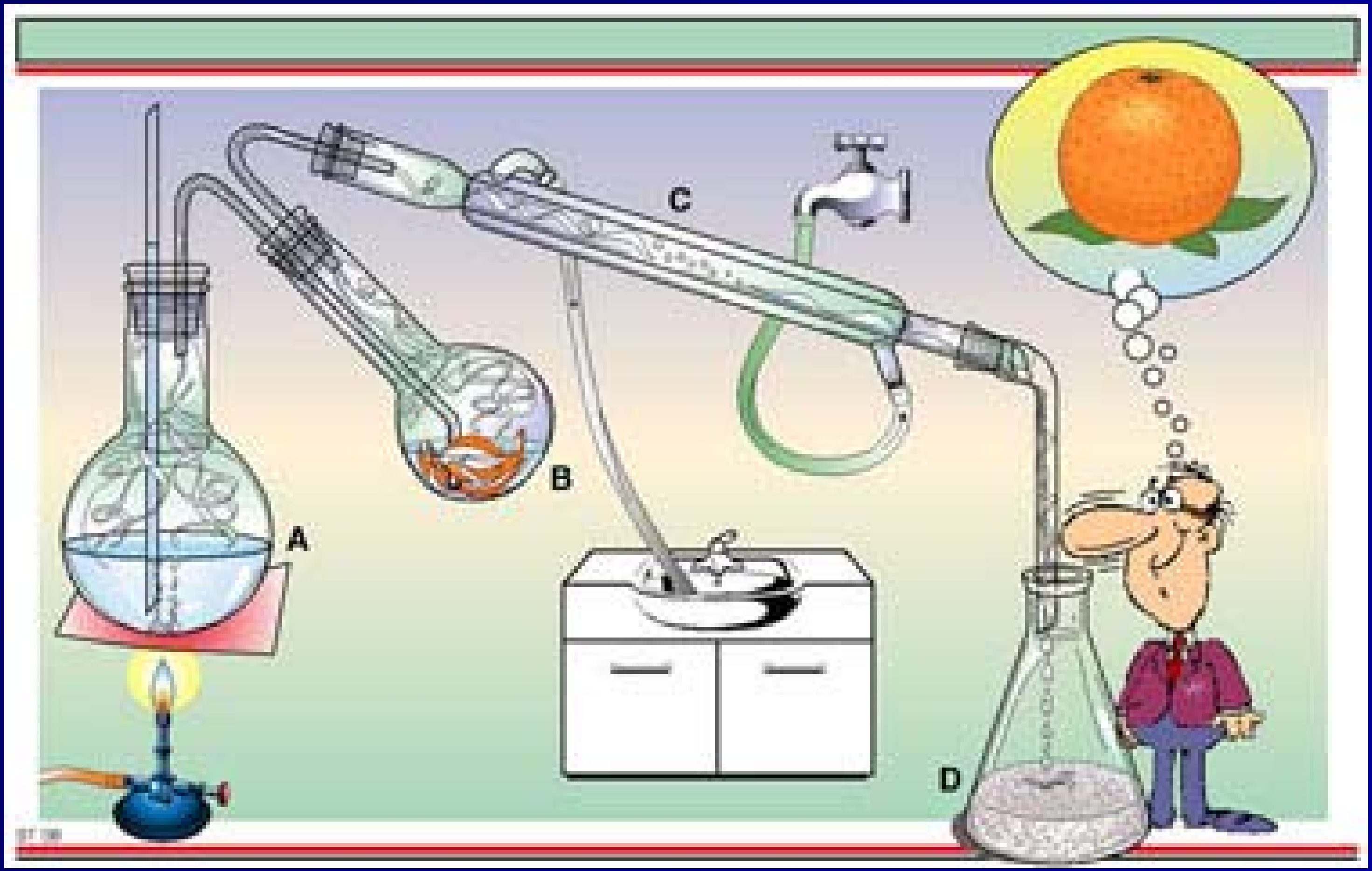
  Take liquid extract, extract with another solvent
- Solid : Liquid Extraction

  Take liquid extract, extract with solid phase material

Volatile Metabolite Extraction

- Steam distillation
- Headspace
- Headspace & solid phase extraction (Trapping)
- Solid phase micro-extraction (SPME)

Volatiles (Essential oils) Steam Distillation



Headspace & solid phase extraction (Trapping)

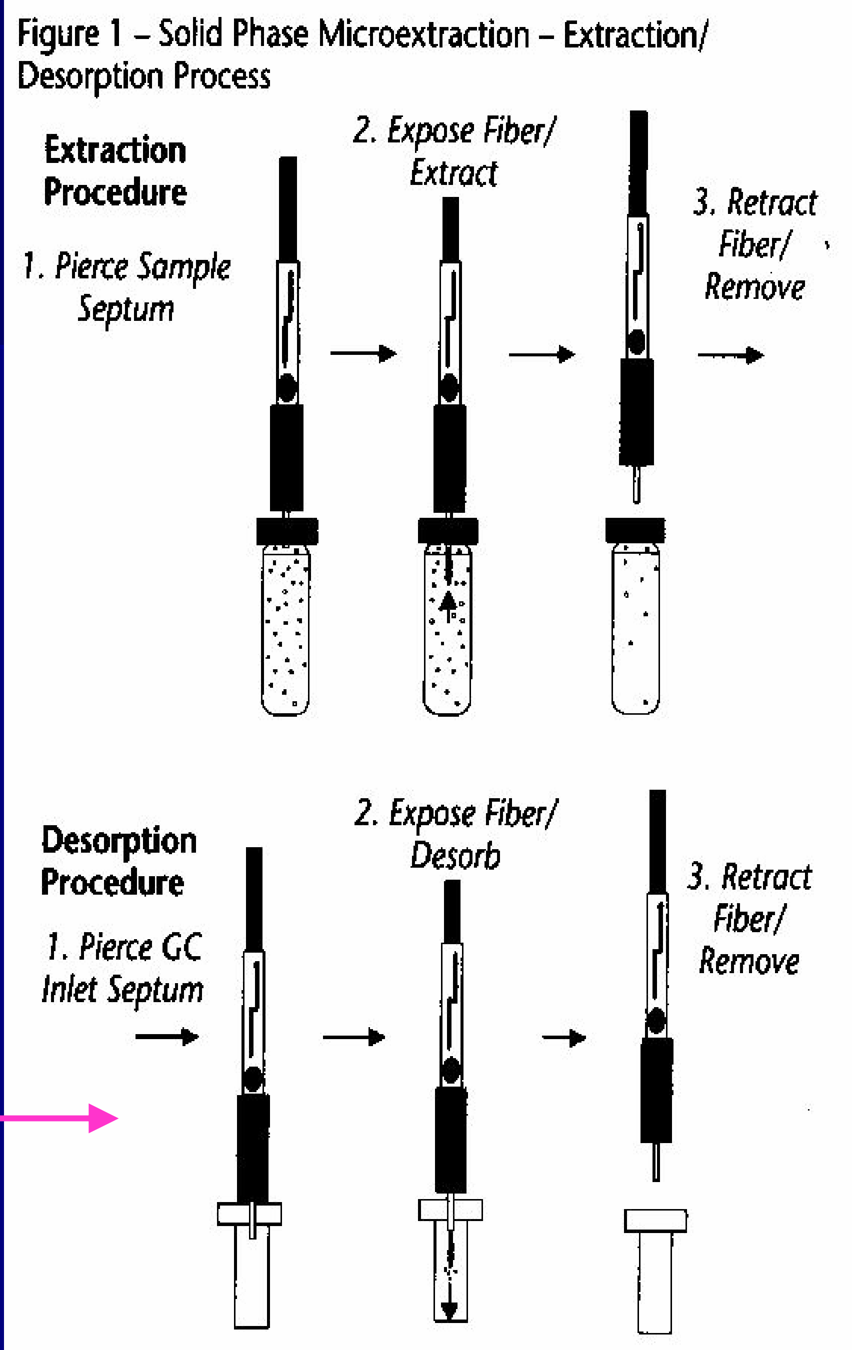
Measuring Headspace Volatiles Emitted by Arabidopsis

Headspace & solid phase extraction (Trapping)

Measuring Headspace Volatiles Emitted by Roses

Solid Phase Micro Extraction (SPME)

To GC-MS injection port



Analysing the METABOLOME

1. Metabolite Extraction

2. Metabolite detection:
with or without prior separation

3. Data analysis

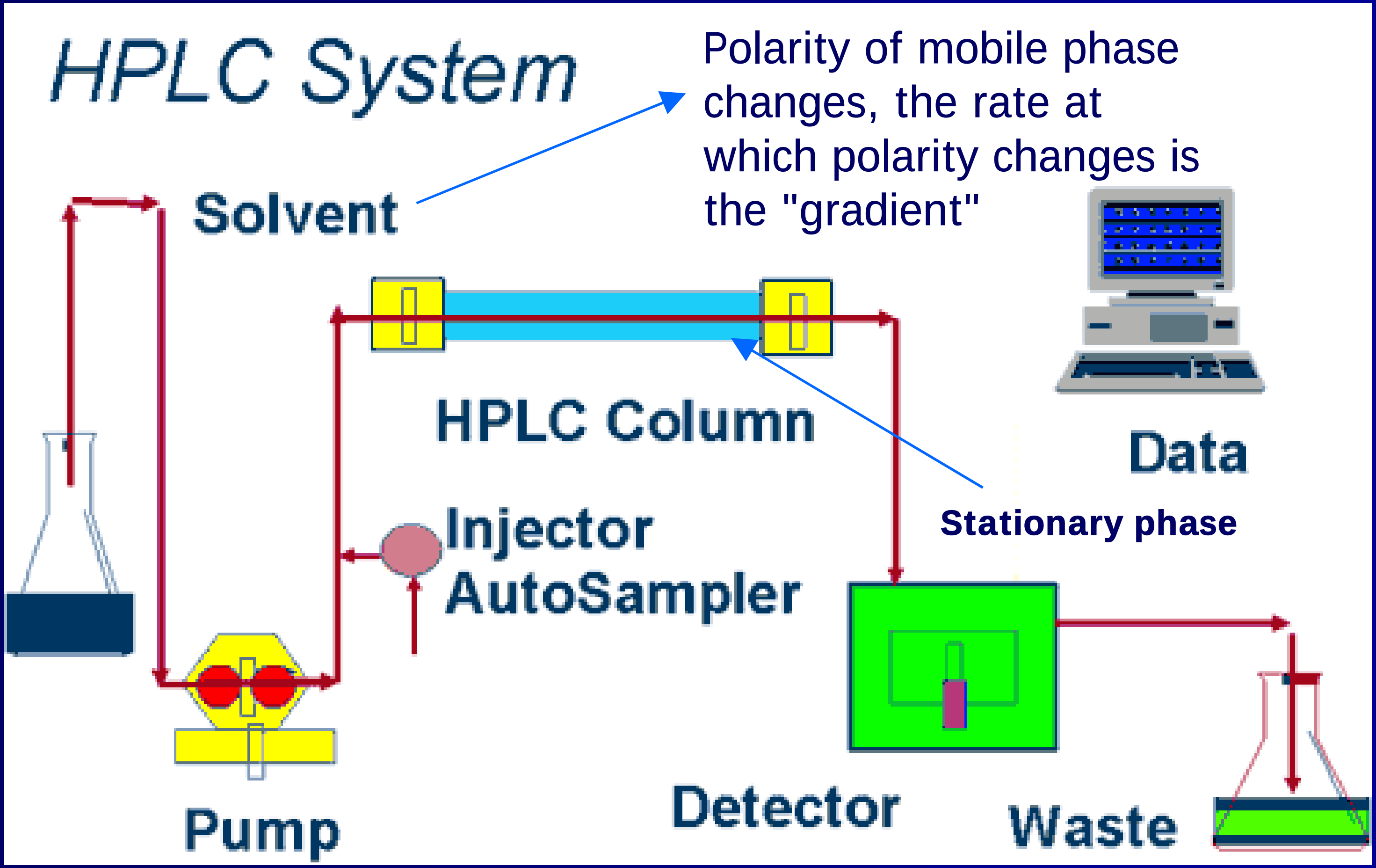
Metabolome Analyses Technologies

- Infrared spectroscopy (IR)
- Nuclear magnetic resonance (NMR)
- Mass spectrometry (MS)
- Thin layer chromatography (TLC)
- High performance liquid chromatography (HPLC) equipped with different kinds of detectors: UV or photodiode array (PDA), fluorescent, electrochemical, etc.
- Capillary electrophoresis (CE) coupled to different detectors: UV, laser induced fluorescent (LIF), mass spectrometer (MS or MSMS), etc.
- Gas chromatography (GC) coupled to different detectors: MS or MSMS, FID
- Liquid chromatography tandem mass spectrometry (LC/MS or LC/MS/MS)
- Fourier transform ion cyclotron mass spectrometry (FTMS)
- HPLC coupled to NMR detection (LC/NMR)
- HPLC coupled to NMR and MS detectors (LC/NMR/MS)

Separation Methods

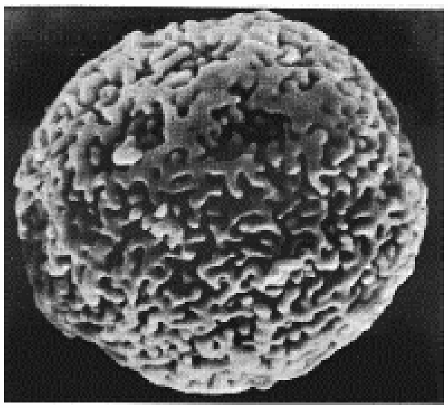
1. Thin layer chromatography (TLC)
2. High Performance Liquid Chromatography (HPLC)
3. Gas chromatography (GC)
4. Capillary electrophoresis (CE)

HPLC Separation



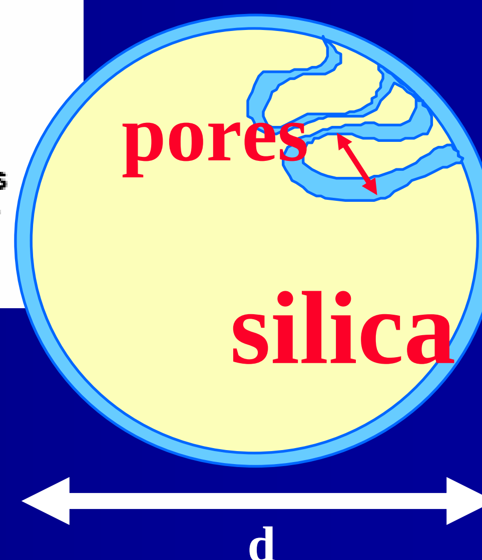
Stationary Phase in the HPLC Column

Pore size, shape and distribution



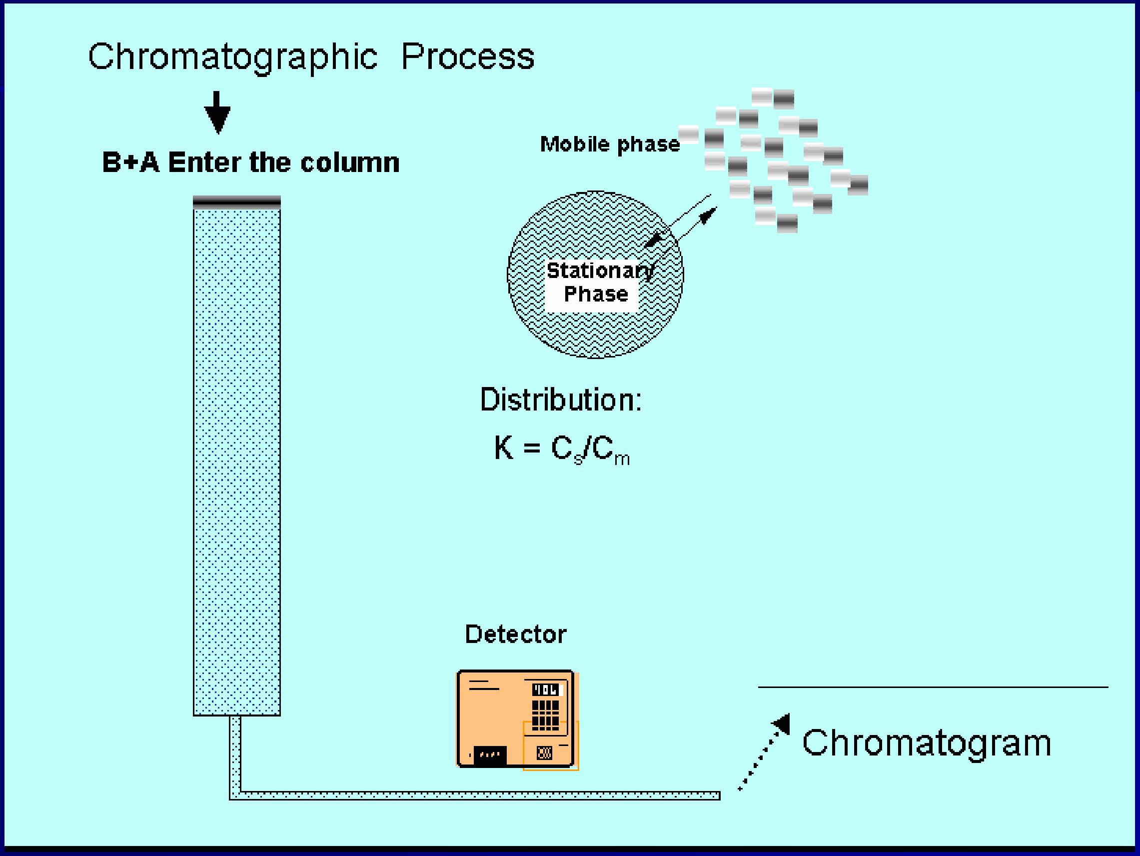
- Macroporous spherical silica particle. [K.K.Unger, Porous silica, Elsevier, 1979]

Pore size defines an ability of the analyte molecules to penetrate inside the particle and interact with its inner surface. This is especially important because the ratio of the outer particle surface to its inner one is about 1:1000. The surface molecular interaction mainly occurs on the inner particle surface.



Analytical HPLC –
3, 5, 10 μm particle size

Stationary Phase and Mobile Phase in HPLC



As the analytes pass through the column they interact between the two phases--mobile and stationary--at different rates.

The difference in rates is primarily due to different polarities for the analytes.

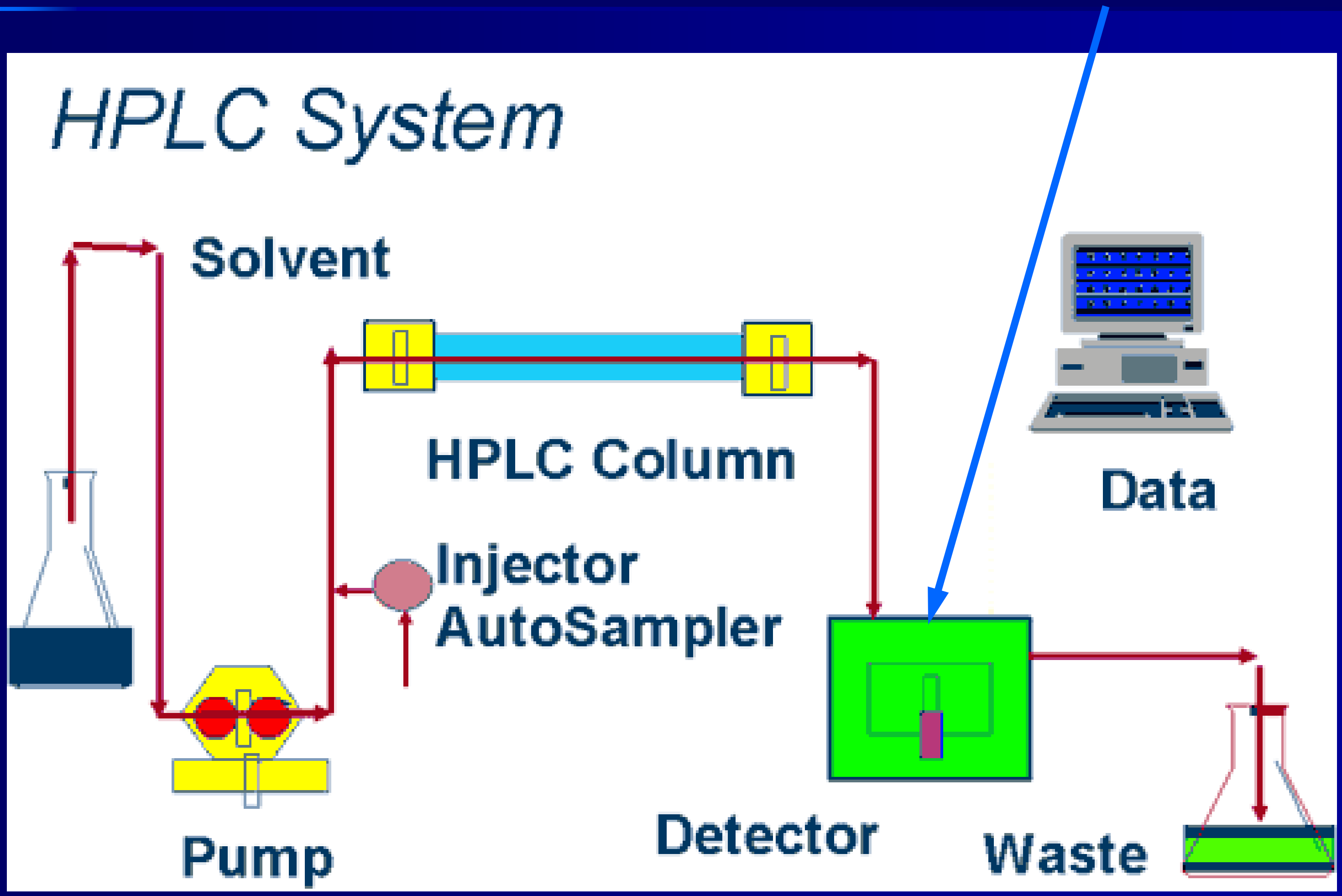
HPLC- Normal & Reverse Phase

Reverse Phase Chromatography:

Stationary Phase- non-polar and Mobile phase-polar

Most polar analyte elutes first

The HPLC Instrument & Detectors



Detectors for HPLC

1. UV/VIS: Fixed wavelength; Photo Diode array
2. Refractive index
3. Fluorescence
4. Conductivity
5. Antioxidant
6. Evaporative light scattering
7. Electrochemical
8. NMR
8. Mass Spectrometer

Fixed Wavelength Absorbance (320nm)

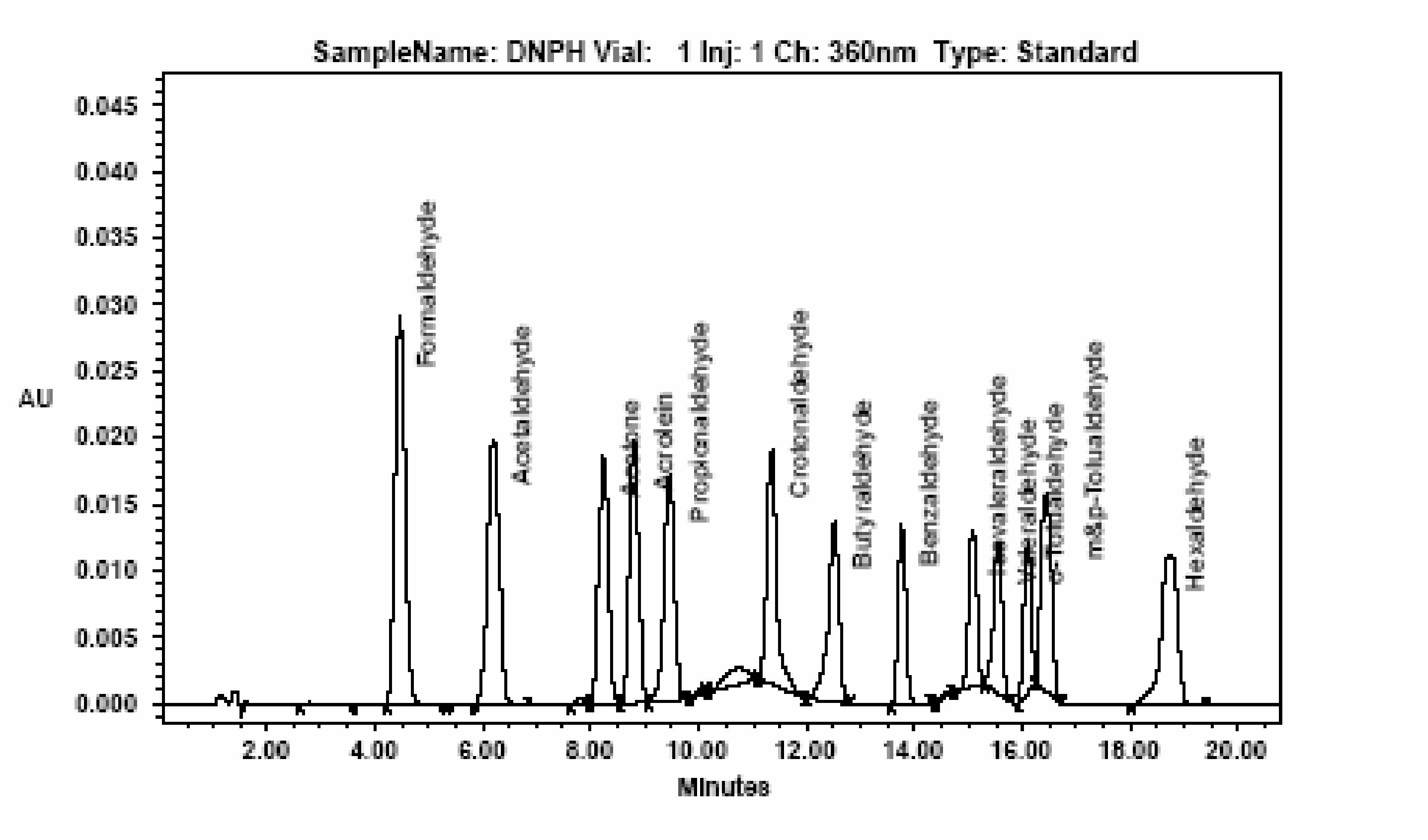
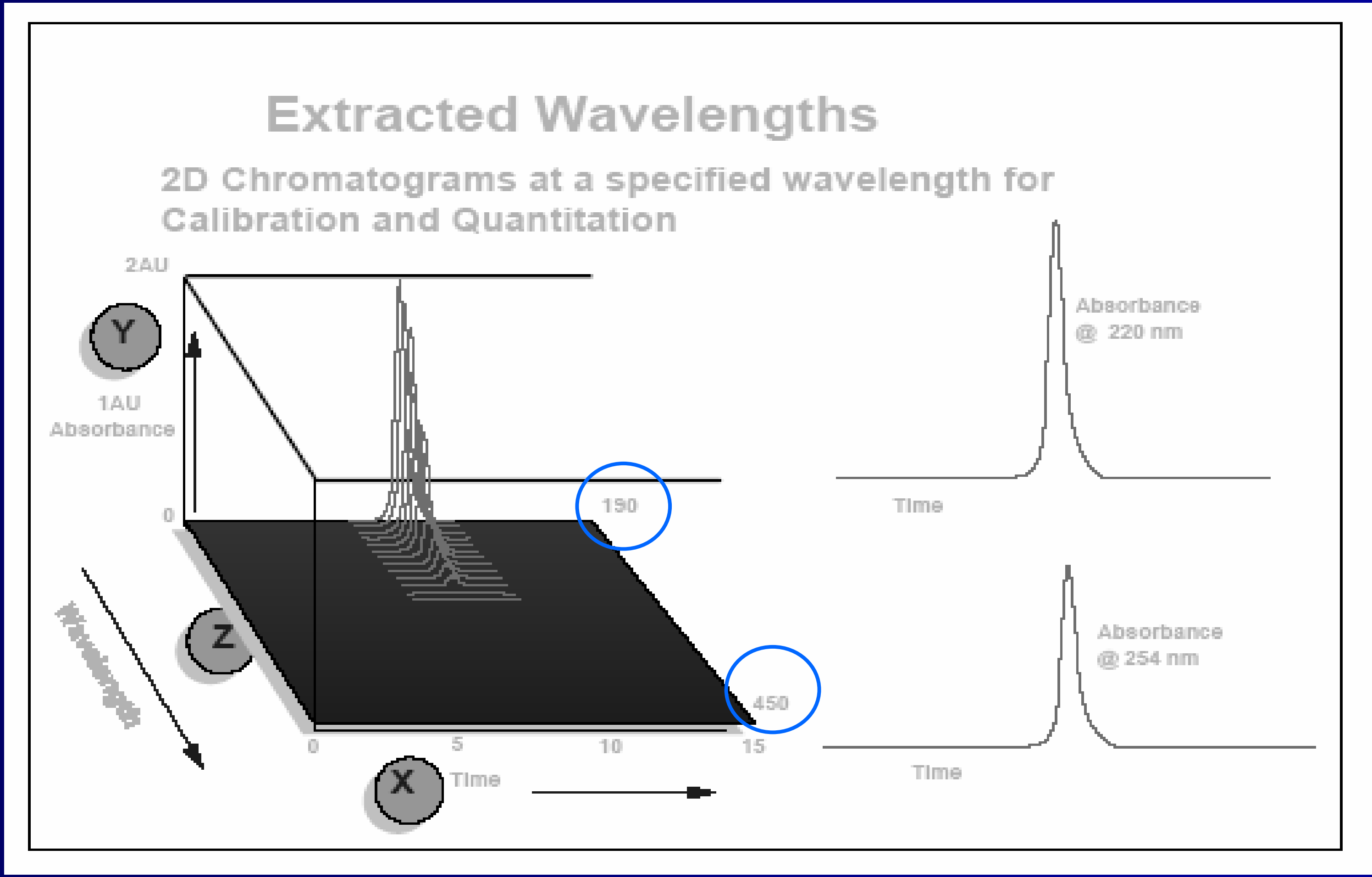
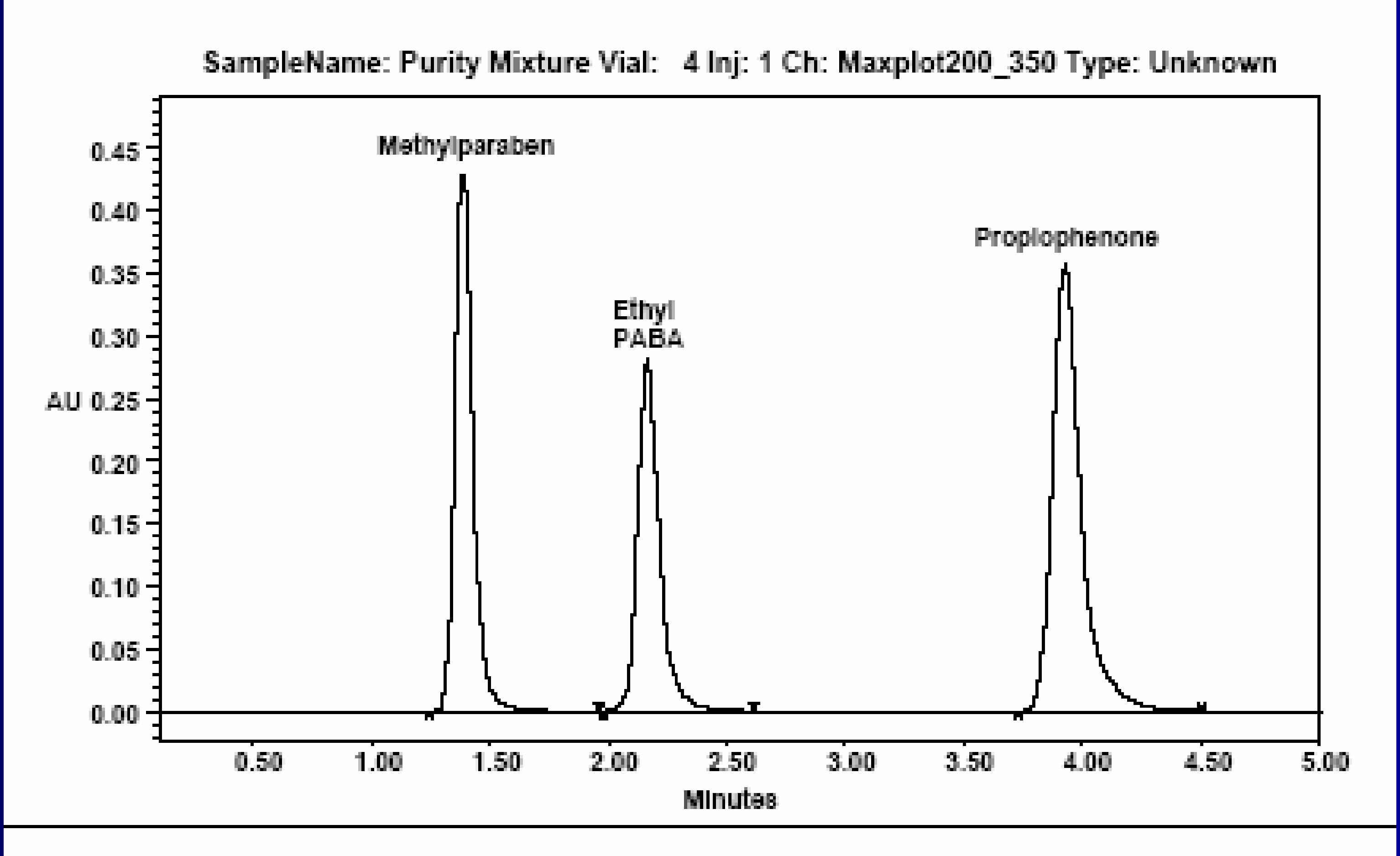


Photo Diode Array (PDA) Detector



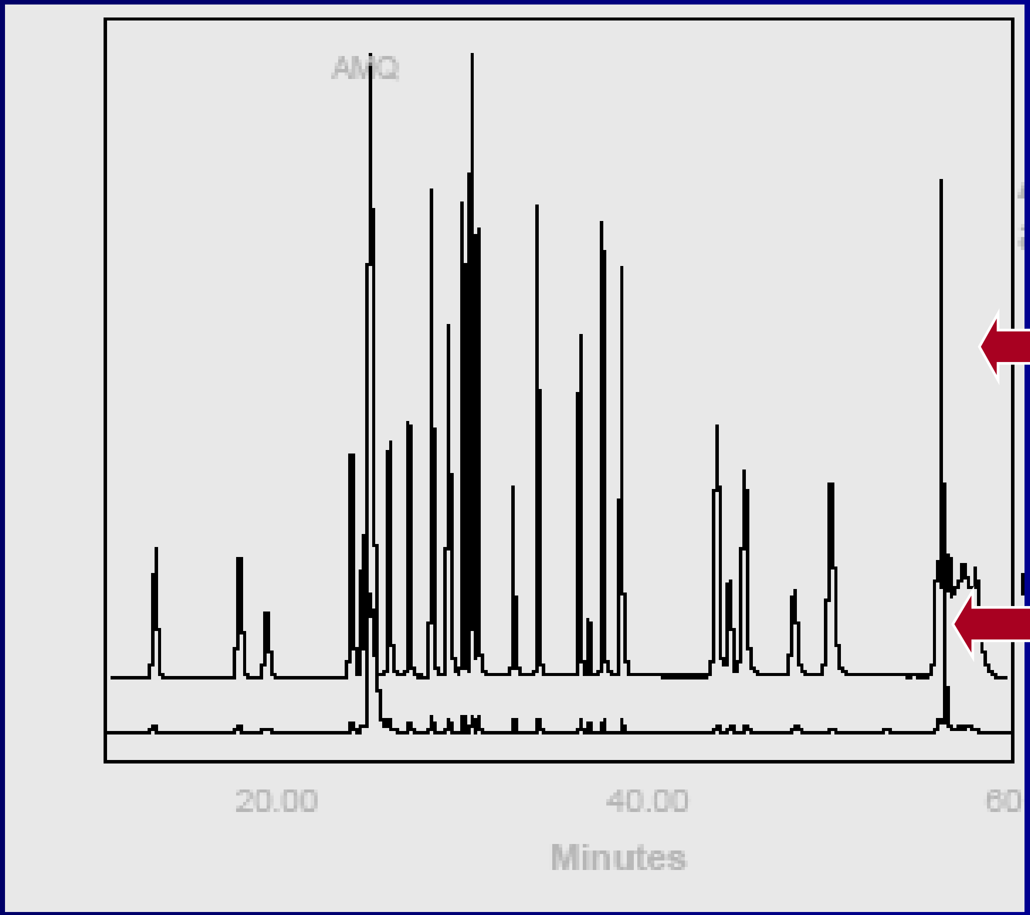
PDA Detector and Visualization with the MaxPlot Option



Detectors

1. *UV/VIS: Fixed wavelength; Variable wavelength; Diode array*
2. Refractive index
3. Fluorescence
4. Conductivity
5. Antioxidant
6. Evaporative light scattering
7. Electrochemical
8. NMR
9. Mass Spectrometer

Fluorescence vs. UV Detection

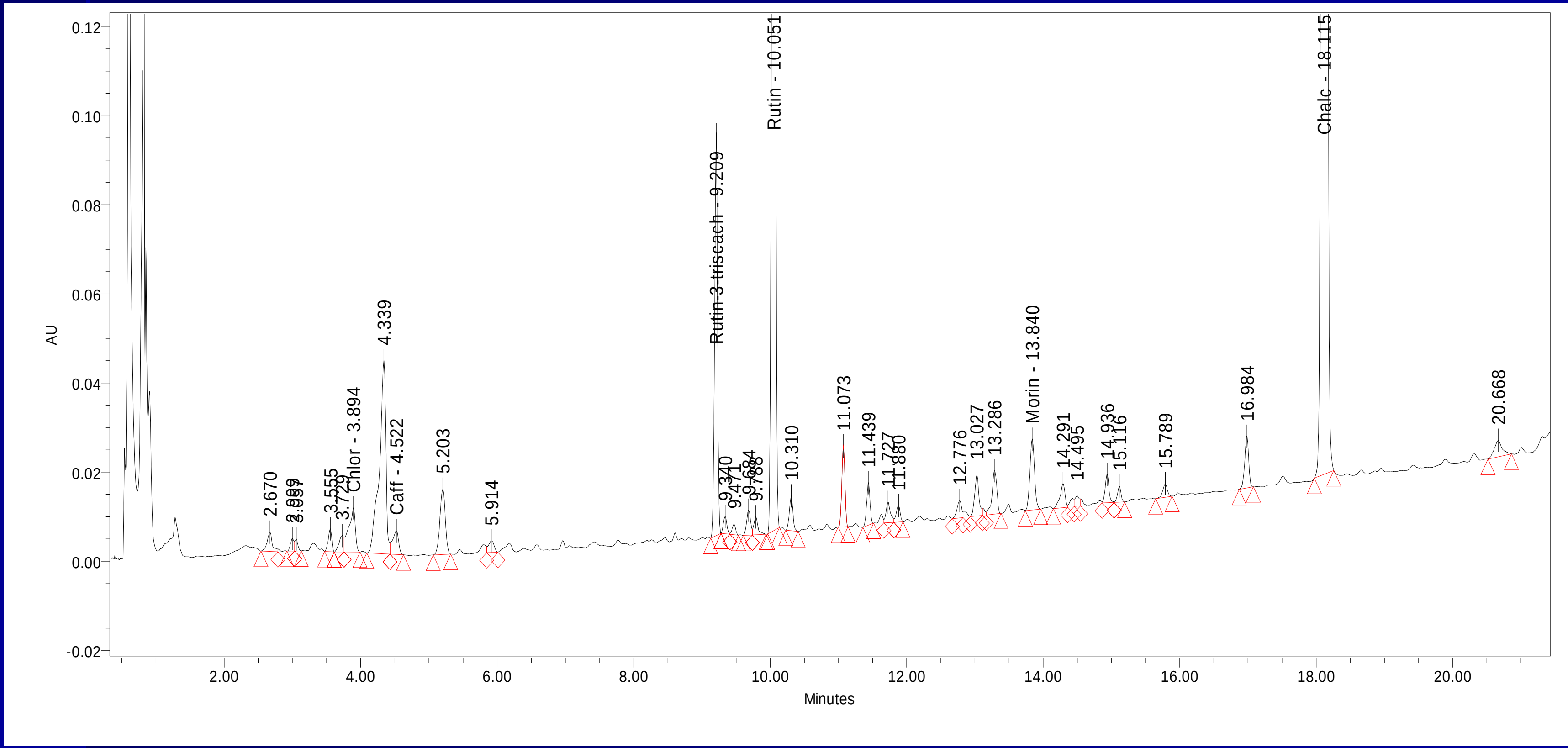


Fluorescence
Excitation at 250 nm
Emission at 395 nm

UV
Absorbance 254nm

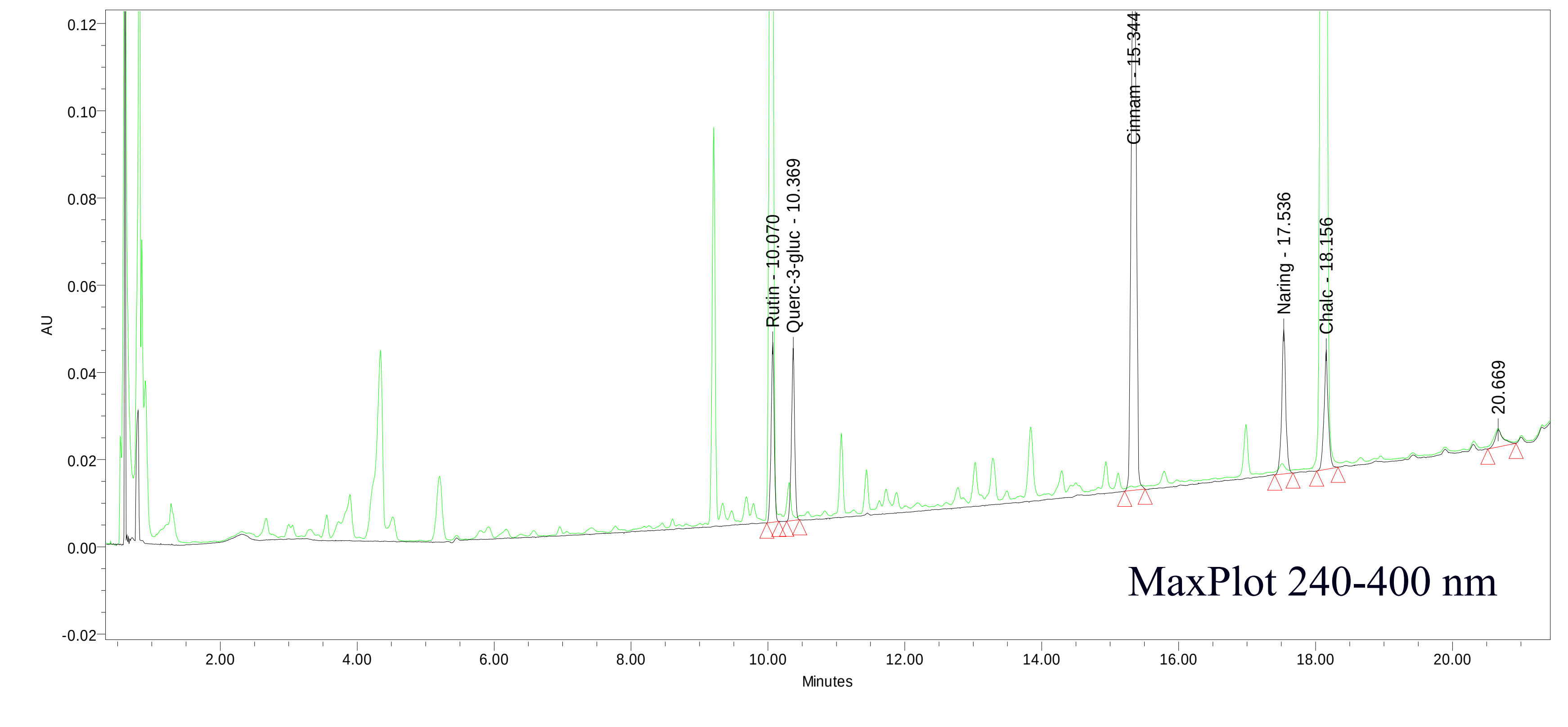
HPLC Chromatogram of a Tomato Sample

Tomato, WT, peel, MaxPlot 240-400 nm

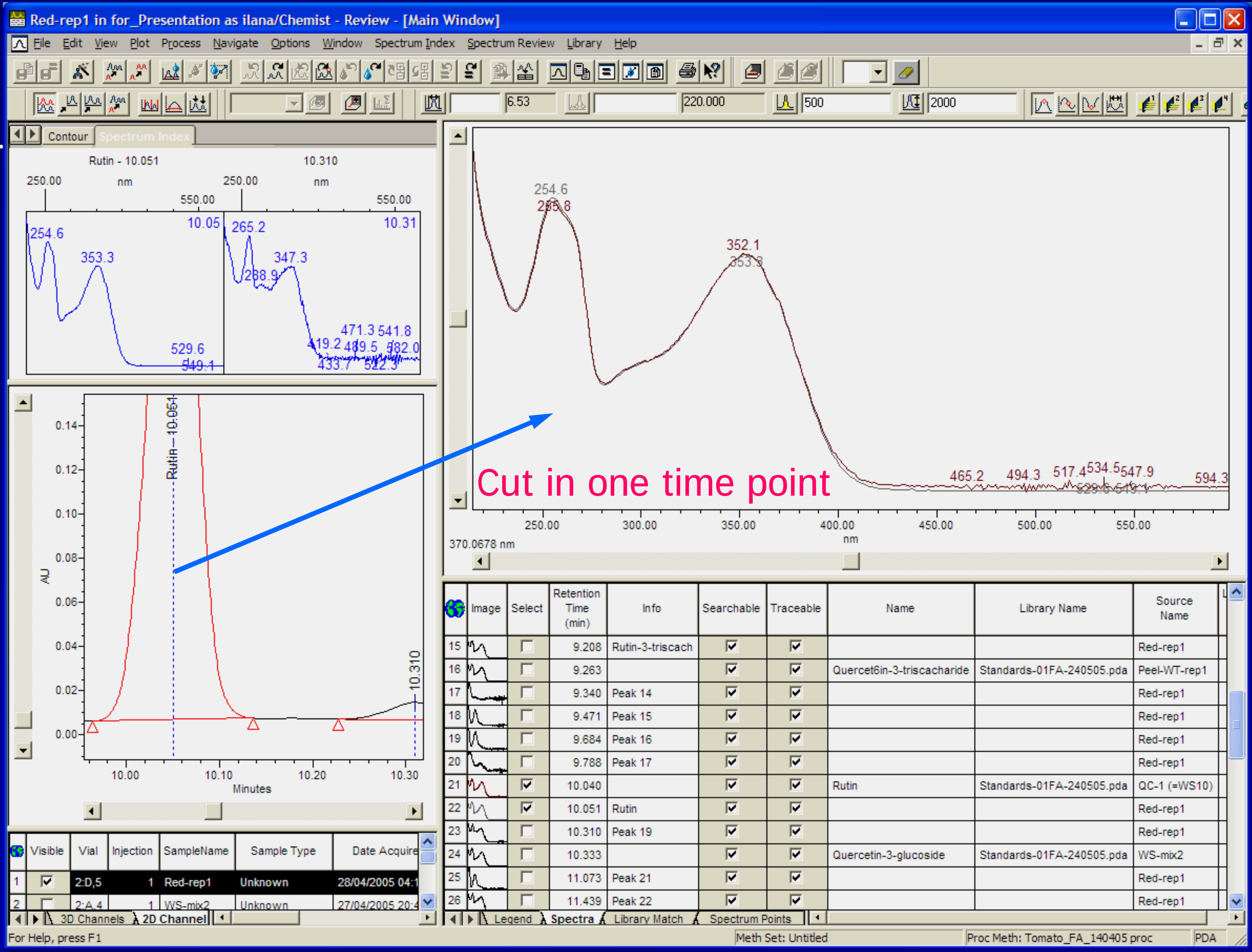


Peaks assignment: Comparison of sample chromatogram with the known standards

1. Comparison of Retention times (RT)



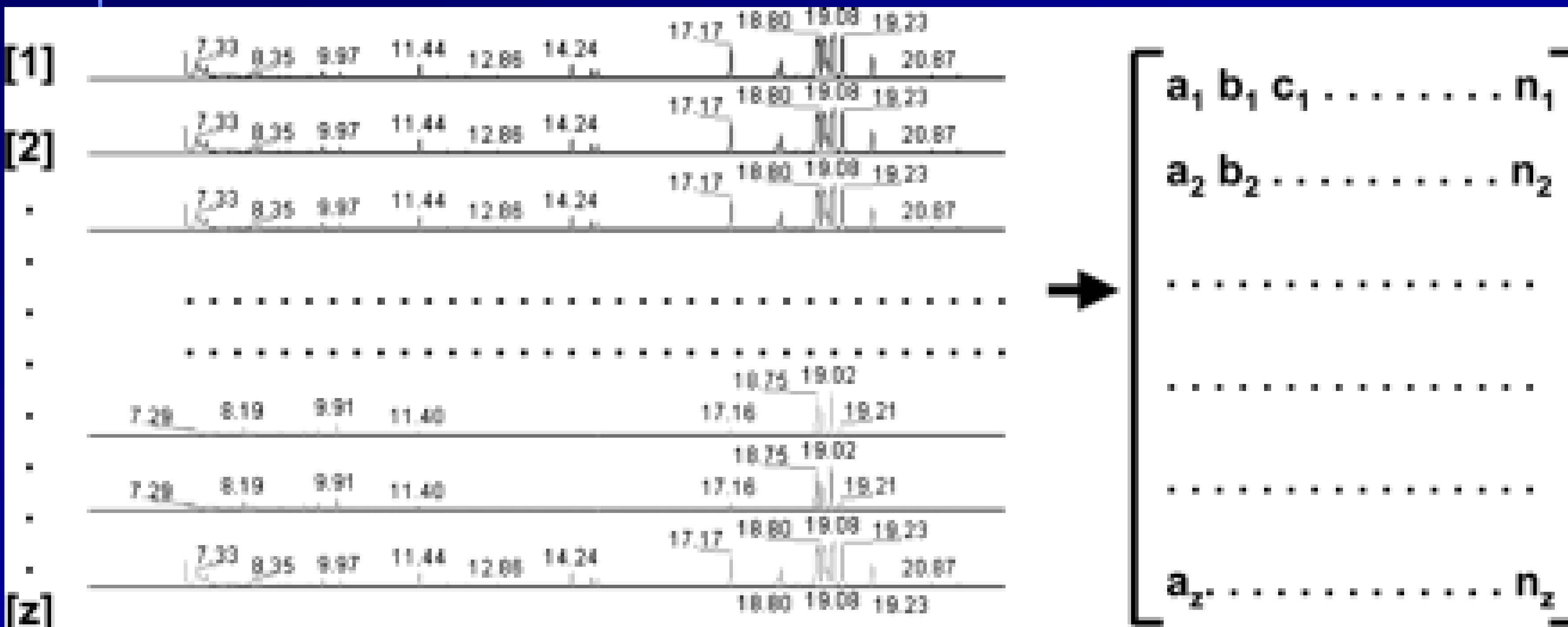
2. Comparison of UV Spectra



Analysing the METABOLOME

1. Metabolite Extraction
2. Metabolite detection (with or without separation)
3. **Data analysis (HPLC-PDA only)**

Data Analysis (HPLC-UV)



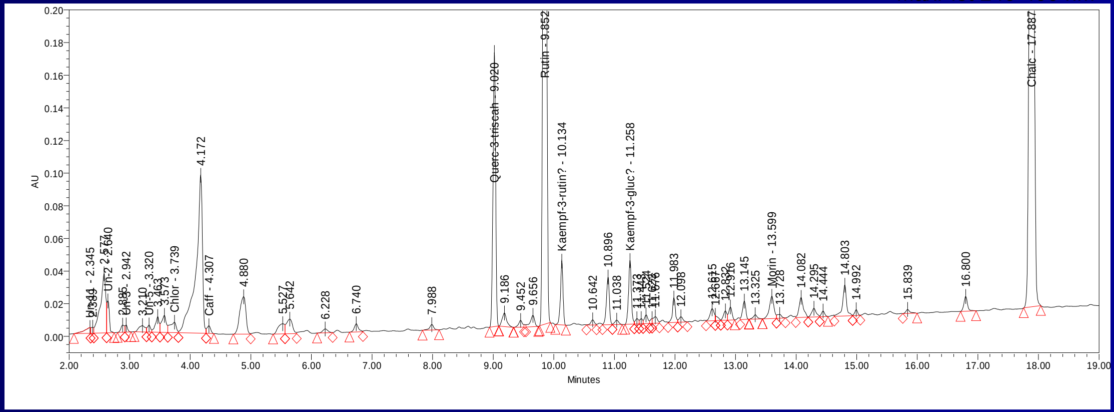
Areas of mass chromatographic peaks corresponding to components (a,b,c...n) are entered into a peak table for each sample chromatogram (1,2,3...z).

Data Analysis HPLC-UV

Metabolite profiling of Tomato samples

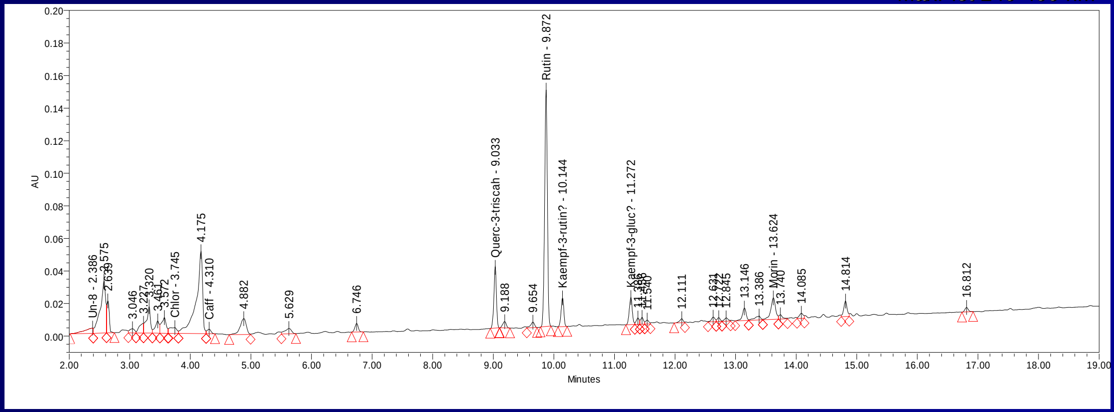
Tomato, Ailsa Craig, WT, peel

MaxPlot 240-400 nm



Tomato, mutant LA 3189, peel

MaxPlot 240-400 nm

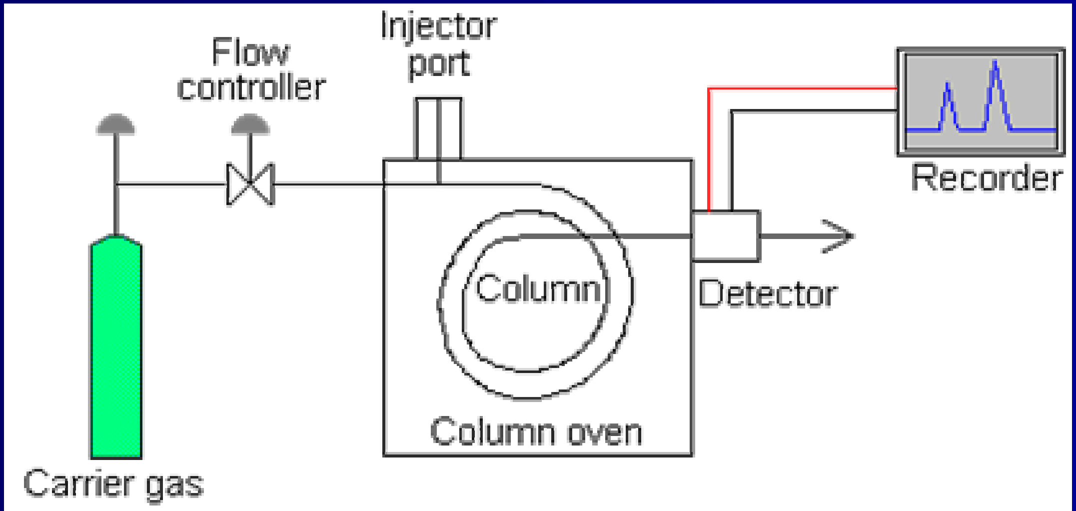


Data Analysis HPLC-UV

Peak table for WT and mutant samples, replicate injections

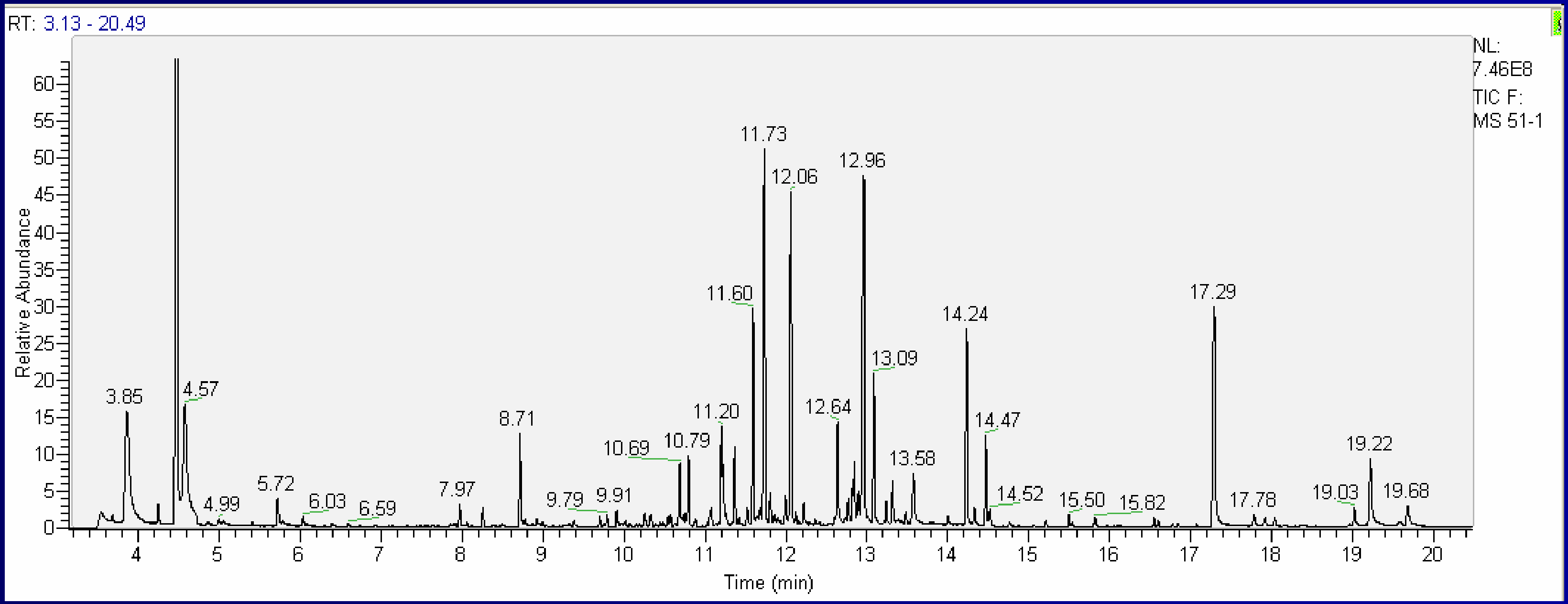
SampleName	Peel-WT-rep1	Peel-WT-rep2	Peel-LA#3-rep2	Peel-LA#3-rep1	Peel-LA#1-rep2	Peel-LA#1-rep1	Peel-WT	Peel-LA#7-rep2	Peel-LA#7-rep1	Peel-LA#4-rep2
Un-1	241419	206868	127397	127465	283709	288794	223706	135812	143342	198087
Un-2	58658	49553	44465	45298	102208	96992	56025	35551	41020	55688
Un-3a		11983	11980	11853	33604	28021	17871			
Un-3	15697	13955			16356	13766	12349			
Un-4a			45896	51075	46460	41609		48528	62768	14663
Un-4	23317	21423	9951	14101	22116		27942	39957	32262	28838
Un-5	20978	18301	110211	106722	91241	81591	15828	122351	148823	74525
Un-6	40369	34817	44531	45493	34699	31952	41978	32308	36773	35221
Un-7	59121	52742	54175	56498	62357	62883	56481	43456	51682	52655
Chlor	60635	51580	50031	47574	55521	56685	46114	25864	32777	29494
Un-8a			39871	42790	26326	25153		57171	58947	
Un-8	797014	750225	431987	435713	550682	570399	739248	422014	431370	374172
Caff	31757	28552	18032	20774	27292	28168	20320	10940	16075	12492
Un-9	154463	130710	118495	117701	96152	103453	160836	88943	87544	69428
Un-10	46934	44525	14103	12741	18646	13982	46447			
Un-11	58382	51794	32383	33504	42987	46055	63893	36171	37916	24898
Un-12	24969	22427	28086	24816	23448	29831	24283	21412	21020	20592
Un-12a			10784	10907						
Un-13	15962	14911					16454			
Querc-3-triscah	548496	490093	142452	143017	231519	247238	506375	138289	132110	108476
Un-14	40676	33057	34559	27633	18420	19195	43531	25382	23390	16342
Un-15	19185	18774	10124	9608			18593			
Un-16	32835	30307	26264	26374	21456	22488	34726	23371	22987	15531
Rutin	2165740	1855442	586954	584920	1023246	1112867	2161457	500042	483482	459848
Kaempf-3-rutin?	115876	100613	54579	54070	70633	75203	114859	48936	46941	55266
Un-17			19119	16196						
Un-18	11641	12457					12891			
Un-19	100923	94039					98629			
Un-20	12273	11041					12377			
Kaempf-3-gluc?	123120	101469	62389	60959	96890	108029	125141	54871	53133	57656
Un-21	12150	10519			14970	15960	12123	13275	12882	13679
Un-22	11670	11517	14896	15341	12646	13589	10048	13895	13273	14338
Un-23	23697	21827			14099	14825	21655	9729	9304	9320
Un-24	19321	17837			8832	9591	20225			
Un-25	56180	48832					50890			
Un-26	17586	16376	11359	11052		11554	15299	9623	11407	9931
Un-27	36367	31995	21220	21936	10728	11290	35381	12254	10414	11770
Un-28	12688	11200	13503	14015	6914	6982	9336	9234	8182	8315
Un-29	26325	23456	15359	14310	23423	24290	22341			8964
Un-30	35669	30426					30896			
Un-31	45809	39438	63621	63139	41221	43591	41085	40248	37798	32968
Un-32	17508	12101	13800	13874	18755	18933	11717	17368	16111	16724
Un-32a			12553	12805			12095	11454	11099	11572
Morin	78144	73707	75296	76038	78599	78383	71043	77880	76128	72394
Un-33	63320	54971	29735	29592	16903	18412	61414	20785	23672	14215
Un-34	30660	25598	15932	13710			28092	11134		
Un-35	15954	13386	10221	9951	11424	12546	12983	11196	11283	
Un- 36	92129	84365	51525	52151	46150	47945	86399	41251	41093	37133
Un-37	14063	12546		7681			12198			
Un-38	42542	38626	15852	15838	17312	18458	37353	9360	10311	10419
Chalc	2535919	2230976					2370837	21394	20105	

Gas Chromatography (GC)



- The sample is vaporized in the injection port
- Sample injected to the head of the chromatographic column
- The sample transported through the column (in a heated oven) by the flow of inert, gaseous mobile phase
- Separation according to boiling points of compounds

A Typical Gas Chromatography (GC) Output Data



GC Detectors (except MS)

Detector	Selectivity
Flame ionization (FID)	Most organic compounds
Thermal conductivity (TCD)	Universal
Electron capture (ECD)	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics
Nitrogen-phosphorus	Nitrogen, phosphorus
Flame photometric (FPD)	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium
Photo-ionization (PID)	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics
Hall electrolytic conductivity	Halide, nitrogen, nitrosamine, sulphur

Next Week

First hour: Mass spectrometry in metabolite analyses

Second hour: A visit to the lab (Asaph & Chemical services WIS), HPLC-PDA, HPLC-FLOURESCENT, UPLC-PDA, LC-MS