Sensing and Sensomics

the chemistry behind olfactory pleasure

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Overview

DSTF

Foreword

Sensory Quality - Food Selection - Quality of life

The Olfactory Sense

- ✓Olfactory Receptors and Receptor Code
- ✓The human Odorome

Modern “*omics*” in food research: *sensomics*

Analytical strategies designed to exploit the complexity

Advanced *sensomics* platforms

- ✓ Multidimensionality as a key-factor
- ✓ Unrevealing the Chemical Odor Code of Milk

Pharmaceutical Biology
DSTF-UNITO

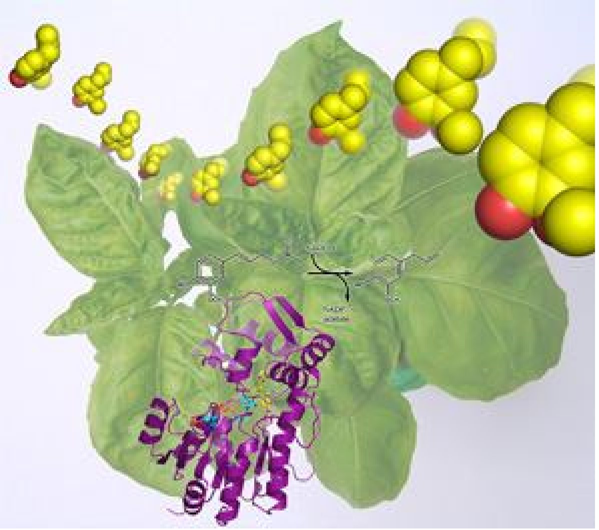
Food Chemistry
DSTF-UNITO

Concluding remarks

Behind pleasure, a further key for well-being

Foreword

Plant volatilome

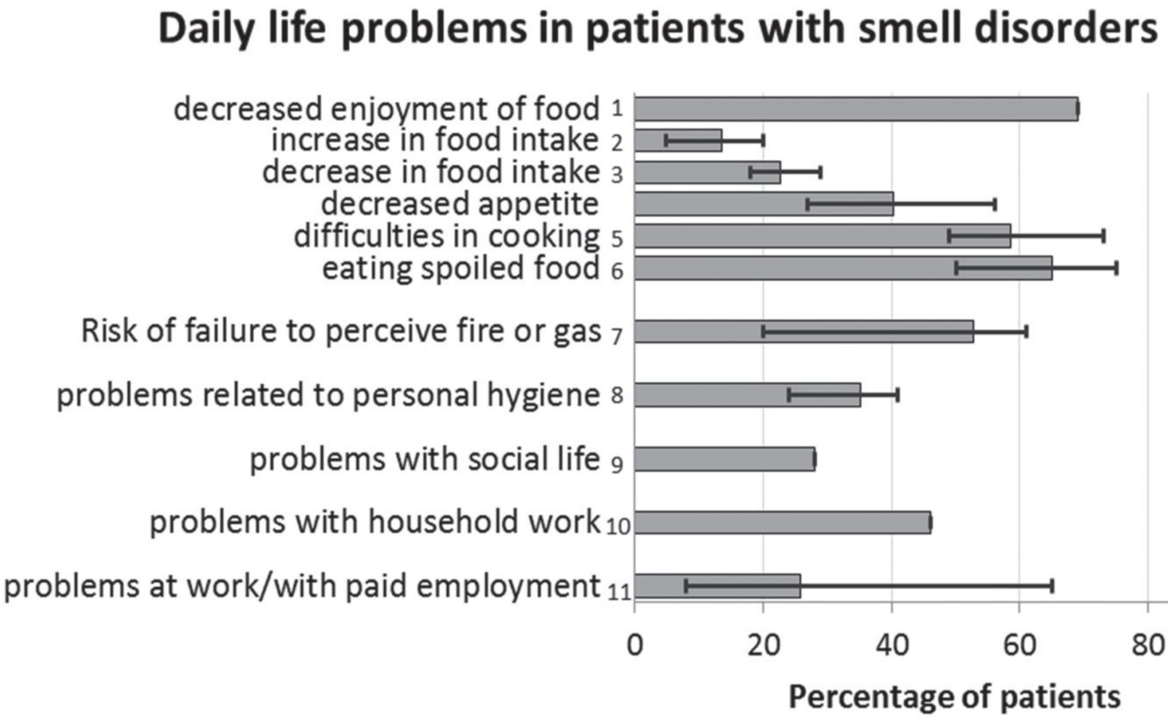


Food odorome

> “…it has become possible to include the chemical space in system biology, i.e. systems chemical biology.”

Oprea TI, Tropsha A, Faulon JL, Rintoul MD (2007) Systems chemical biology. Nat Chem Biol 3: 447–450

Sensory Quality and Food Selection and much more

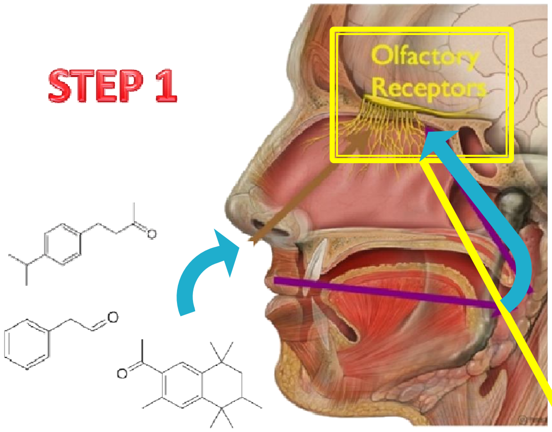


Complex and intriguing task:

1. Native flavor compounds trace level
2. Olfactory perception is triggered by ligand-receptor interactions
3. One ligand interacts with more than one receptor
4. Receptors activation generates a complex patter of signals

[1] Schieberle, P., Hofmann, T. (2003) Chemie in Unserer Zeit, 37 (6), pp. 388-401

The Olfactory Sense


STEP 1
Olfactory
Receptors

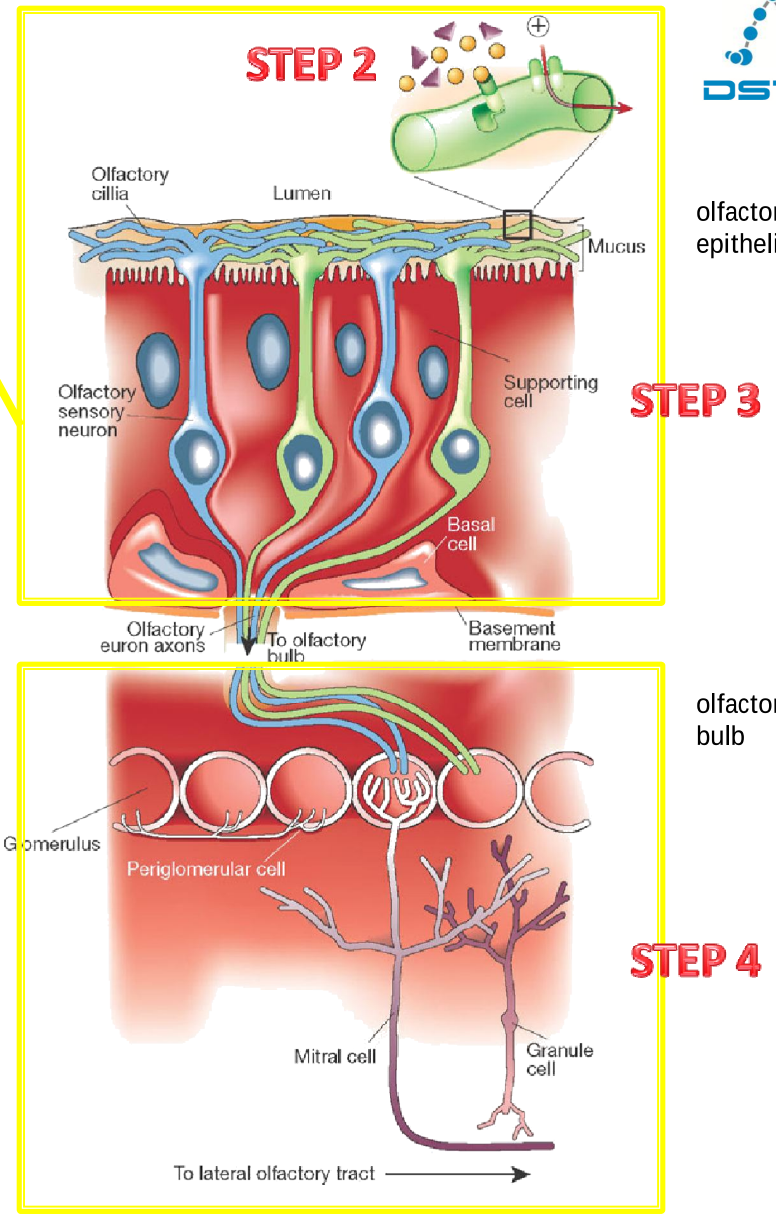
Chemistry behind olfaction:

✓Identification of ligands for olfactory receptors (pharmacological space)

✓Identification of chemical features related odor quality and odor threshold
(SAR studies – medicinal chemistry space)

✓Characterization of perireceptor events that modulate odorant-receptor interaction (enzymatic activity)

✓Identification of chemical patterns related with the combinatorial code of olfaction


STEP 2
Olfactory
cillia
Lumen
Mucus
Olfactory
sensory
neuron
Supporting
cell
STEP 3
Basal
cell
Olfactory
euron axons
To olfactory
bulb
Basement
membrane
Glomerulus
Periglomerular cell
STEP 4
Mitral cell
Granule
cell
To lateral olfactory tract

olfactory
epithelium

olfactory
bulb

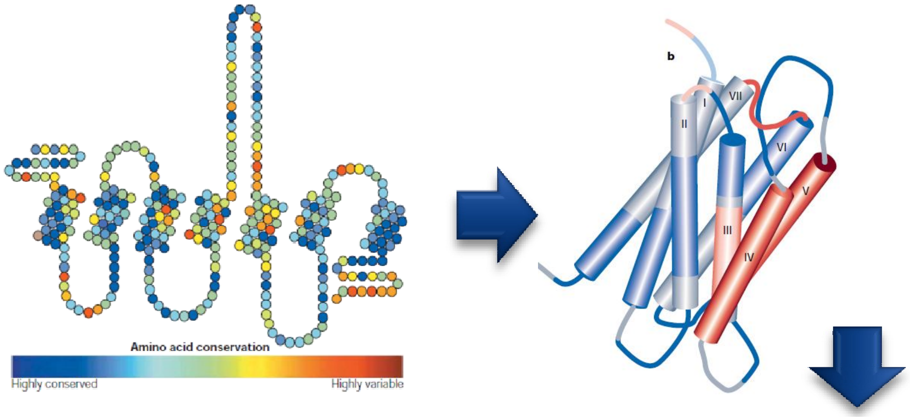
Olfactory receptors

The Nobel Prize Medal for Physiology or Medicine Year 2004

Richard Axel

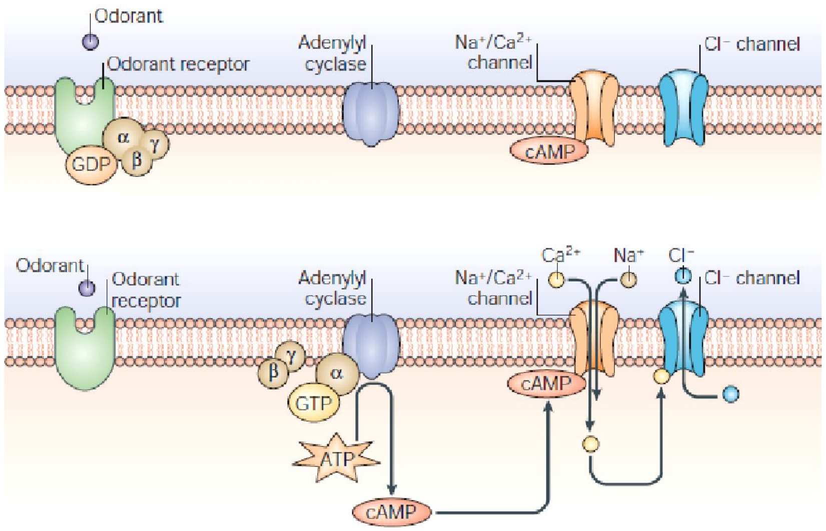
Linda B. Buck

"for their discoveries of odorant receptors and the organization of the olfactory system"


Amino acid conservation
Highly conserved
Highly variable
b
I
II
III
IV
V
VI
VII

ORs are members of a superfamily of up to 1000 different **G-protein coupled receptors** (**G_{olf}**) with seven trans-membrane (7TM) domains.
The location of odorant binding is thought to be a hydrophobic pocket in trans-membrane regions 3, 4, and 5 of the seven-membrane-spanning receptor.

Canonical pathway of signal transduction in olfactory sensory neurons (OSN)


Odorant
Odorant receptor
Adenylyl cyclase
Na⁺/Ca²⁺ channel
Cl⁻ channel
GDP
α
β
γ
cAMP
Odorant
Odorant receptor
Adenylyl cyclase
Ca²⁺
Na⁺
Cl⁻
Na⁺/Ca²⁺ channel
Cl⁻ channel
β
γ
α
GTP
ATP
cAMP
cAMP

Ligand-Receptor interaction

Dose response curves

Functional expression of ORs in heterologous expression systems, i.e., human embryonic kidney cells (HEK293).

Acetophenone (Acp) and **Benzaldehyde** (Bnz)
Agonists (different sensitivity -10 fold dose shift) at M71 OR

M71-expressing olfactory sensory neurons (OSNs) are evidenced by green fluorescence.

Molecular Tuning of Odorant Receptors and Its Implication for Odor Signal Processing

Johannes Reisert[1] **and Diego Restrepo**[2]

[1]Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, USA and
[2]Department of Cell and Developmental Biology and Neuroscience Program, University of Colorado Denver, Anschutz Medical Campus, 12801 East 17th Avenue, Aurora, CO 80045, USA

Chem. Senses 34: 535–545, 2009

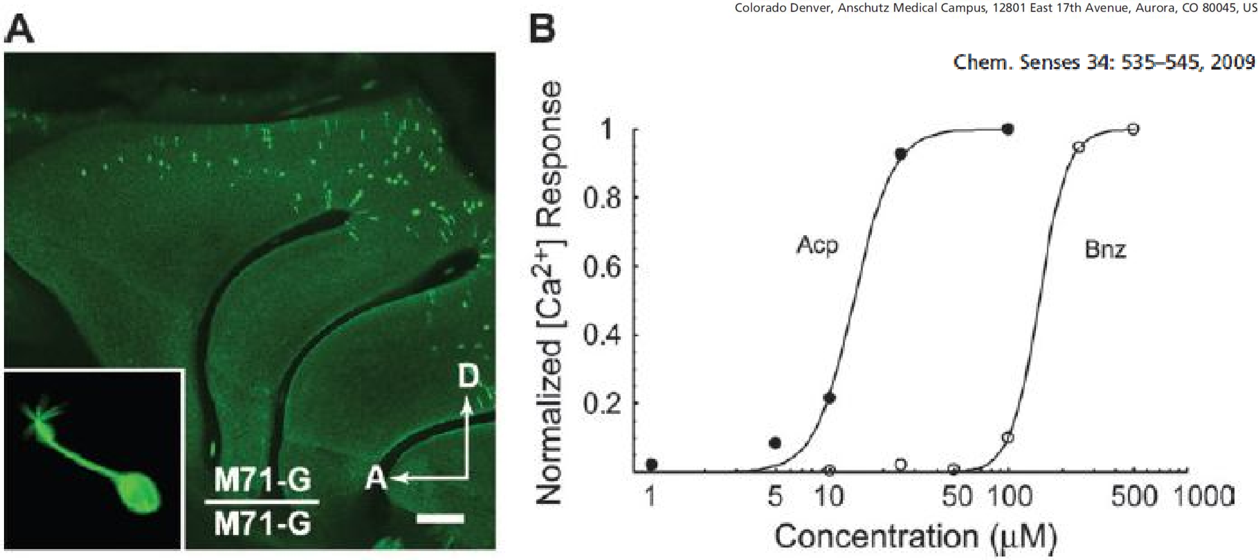
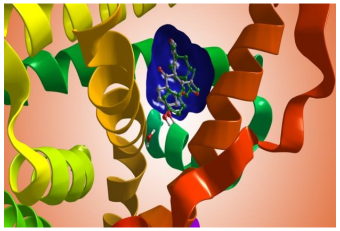
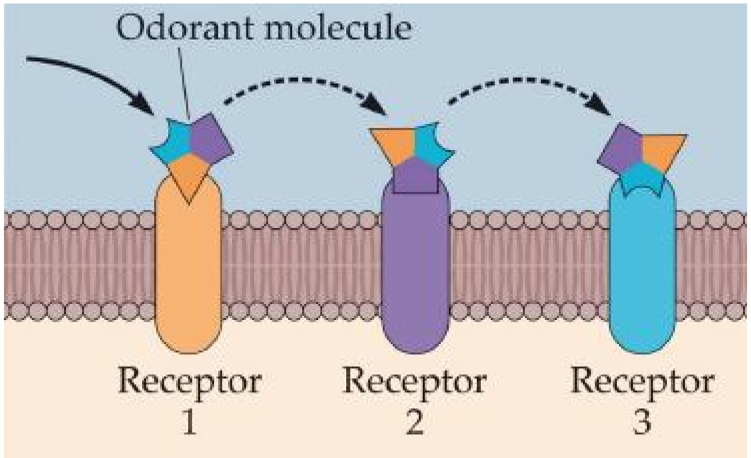


Figure 3 Recording odorant-induced responses from olfactory sensory neurons expressing the M71 OR. **(A)** The introduction of IRES-tauGFP into the mouse genome following the coding sequence for the M71 OR allowed the identification and visualization of all M71-expressing olfactory sensory neurons, which are found in the most dorsal zone on the olfactory turbinates. Scalebar, 250 μm. **(B)** Screening of GFP-positive olfactory sensory neurons using Ca^{2+} imaging and a variety of odorants revealed the M71 agonists acetophenone (Acp) and benzaldehyde (Bnz). Note the ~10-fold shift difference in sensitivity. Modified from Bozza et al. (2002) with permission from the Society of Neuroscience.

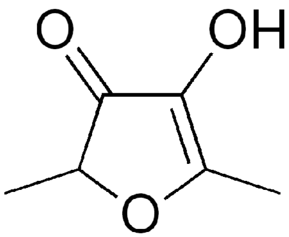
Ligand-Receptor interaction “Odotope theory “

A combination of activated receptors is responsible for any one smell – **Receptor Code**
Receptors **recognize small structural features on each molecule** and the brain is responsible for processing the combined signal into an interpreted smell

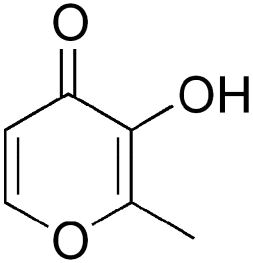



Odorant molecule
Receptor 1
Receptor 2
Receptor 3

Caramel-like

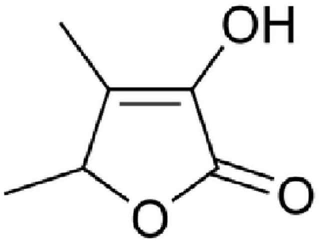


Furaneol

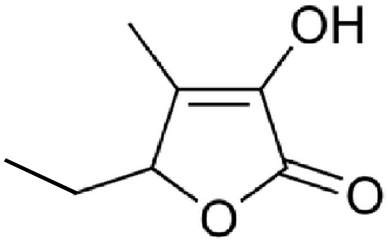


Maltol

Spicy flavor

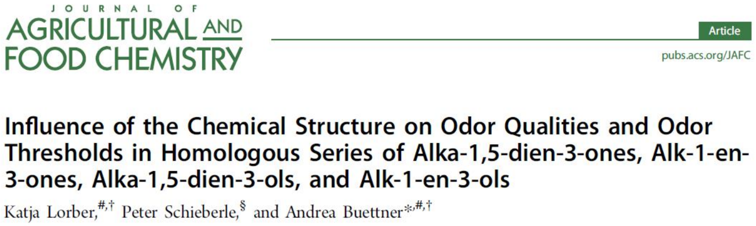


Sotolon



Ethyl-Sotolon

Ligand-Receptor interaction “Odor potency”



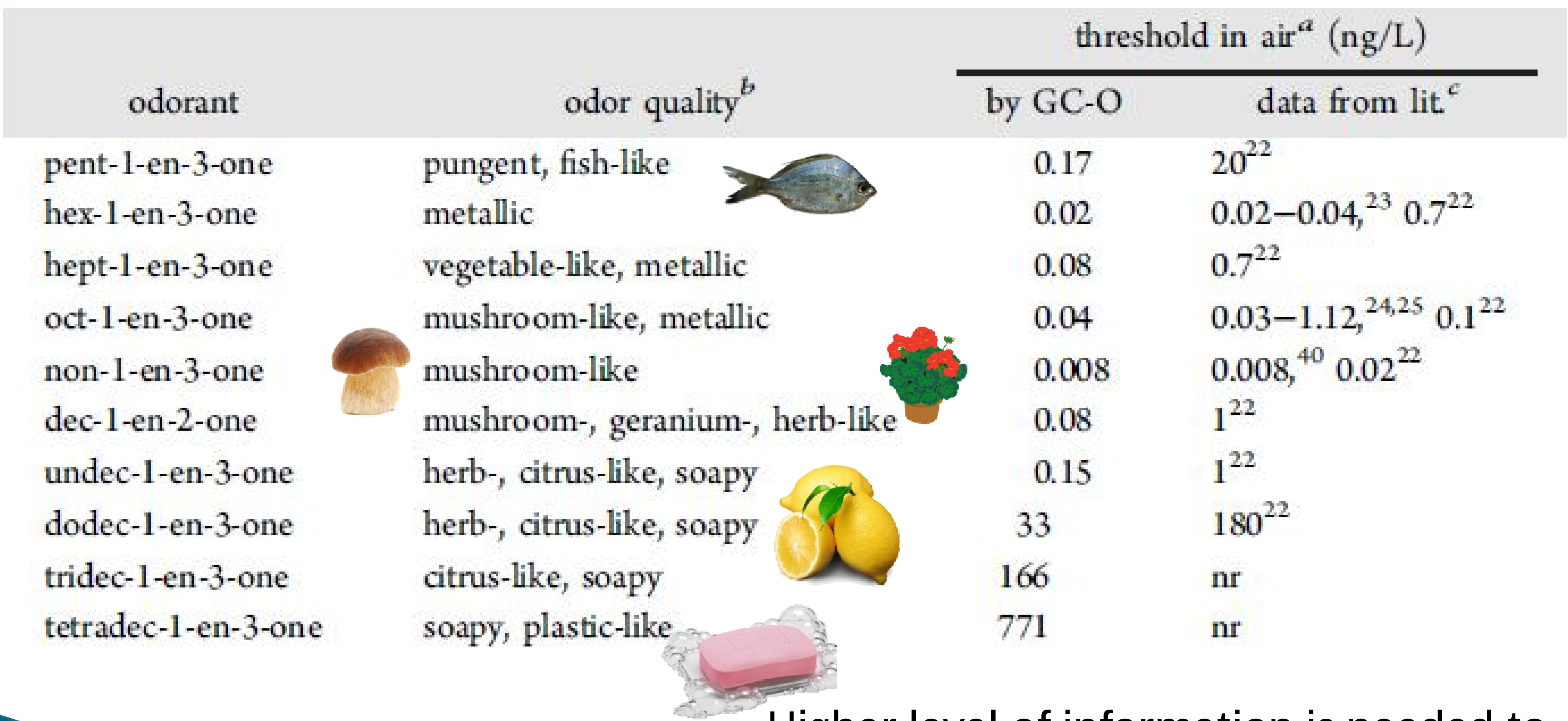
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Influence of the Chemical Structure on Odor Qualities and Odor Thresholds in Homologous Series of Alka-1,5-dien-3-ones, Alk-1-en-3-ones, Alka-1,5-dien-3-ols, and Alk-1-en-3-ols

Katja Lorber,[#,†] Peter Schieberle,[§] and Andrea Buettner[*,#,†]

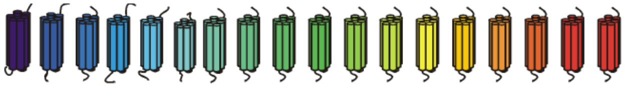


odorant	odor quality[b]	threshold in air[a] (ng/L) by GC-O	threshold in air[a] (ng/L) data from lit.[c]
pent-1-en-3-one	pungent, fish-like	0.17	20[22]
hex-1-en-3-one	metallic	0.02	0.02–0.04,[23] 0.7[22]
hept-1-en-3-one	vegetable-like, metallic	0.08	0.7[22]
oct-1-en-3-one	mushroom-like, metallic	0.04	0.03–1.12,[24,25] 0.1[22]
non-1-en-3-one	mushroom-like	0.008	0.008,[40] 0.02[22]
dec-1-en-2-one	mushroom-, geranium-, herb-like	0.08	1[22]
undec-1-en-3-one	herb-, citrus-like, soapy	0.15	1[22]
dodec-1-en-3-one	herb-, citrus-like, soapy	33	180[22]
tridec-1-en-3-one	citrus-like, soapy	166	nr
tetradec-1-en-3-one	soapy, plastic-like	771	nr

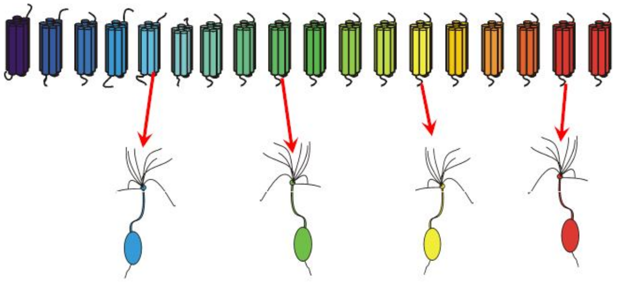
Higher level of information is needed to correctly interpret the complex phenomena behind olfactory perception.

The Olfactory Sense
Receptor Code

1. Humans ≈ 400 different ORs

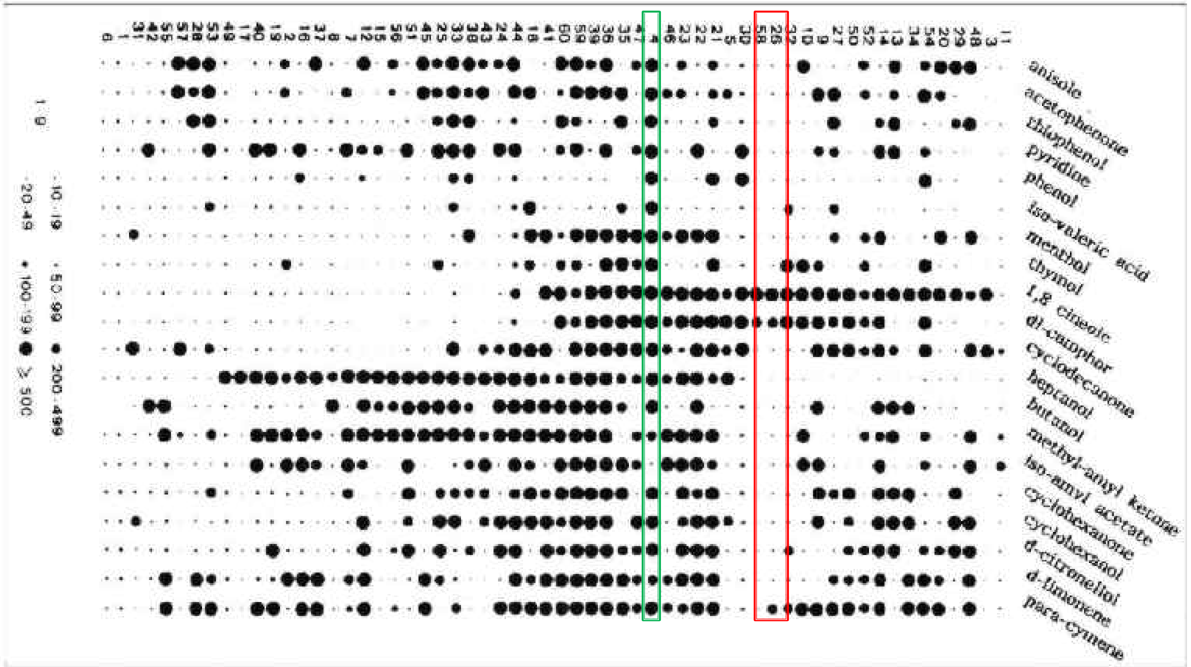


2. Each OSN expresses only one kind ORs

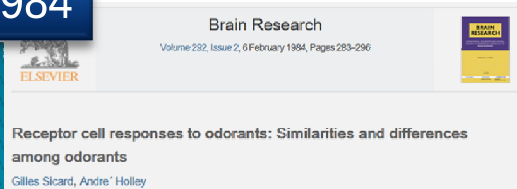


Selectivity study on 60 olfactory neurons tested with 20 different odorants.
Each neuron is equivalent to one column, each odorant to one row. The size of the dots symbolizes the number of action potentials.
Neurons 26 and 58 react specifically on cineole and camphor.
Neuron 4 reacts on all odorants except isoamyl acetate.

3. ORs are not highly specific
multiple activation/modulation



1984


Brain Research
Volume 292, Issue 2, 6 February 1984, Pages 283-296

Receptor cell responses to odorants: Similarities and differences among odorants
Gilles Sicard, Andre´ Holley

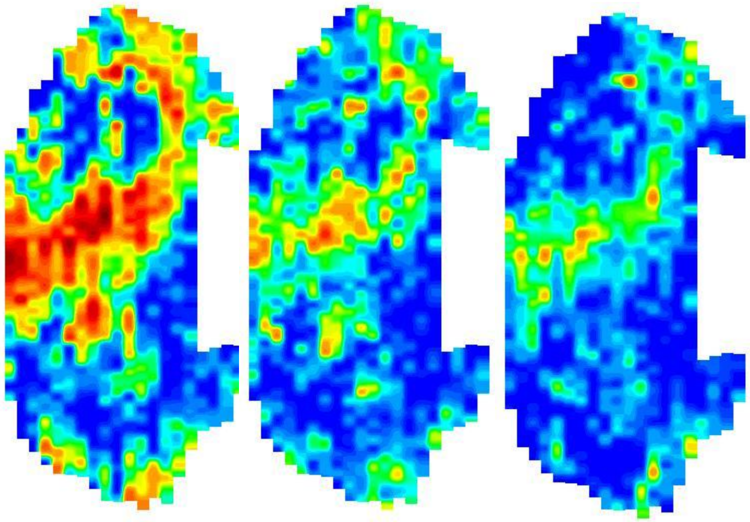
Receptor Code
Complex activity pattern of many differently tuned olfactory neurons that project in specific regions of the olfactory bulb

The Olfactory Sense
Receptor Code

Olfactory Bulb Odor Map

Ligand: **Isoamyl acetate**
Species: Rat – **Strain**: Sprague-Dawley
Exposure time: 128 sec
Brain Region: Olfactory bulb
Sub Region: Glomerular layer
Side: Right
View: Lateral

Topography of the activation pattern



To lateral olfactory tract CNS integration

Functional MRI images revealing the olfactory bulb activation pattern for ISAc at different concentrations (4500 – 520 – 41 ppm)

Modeling of the odorant human combinatorial coding

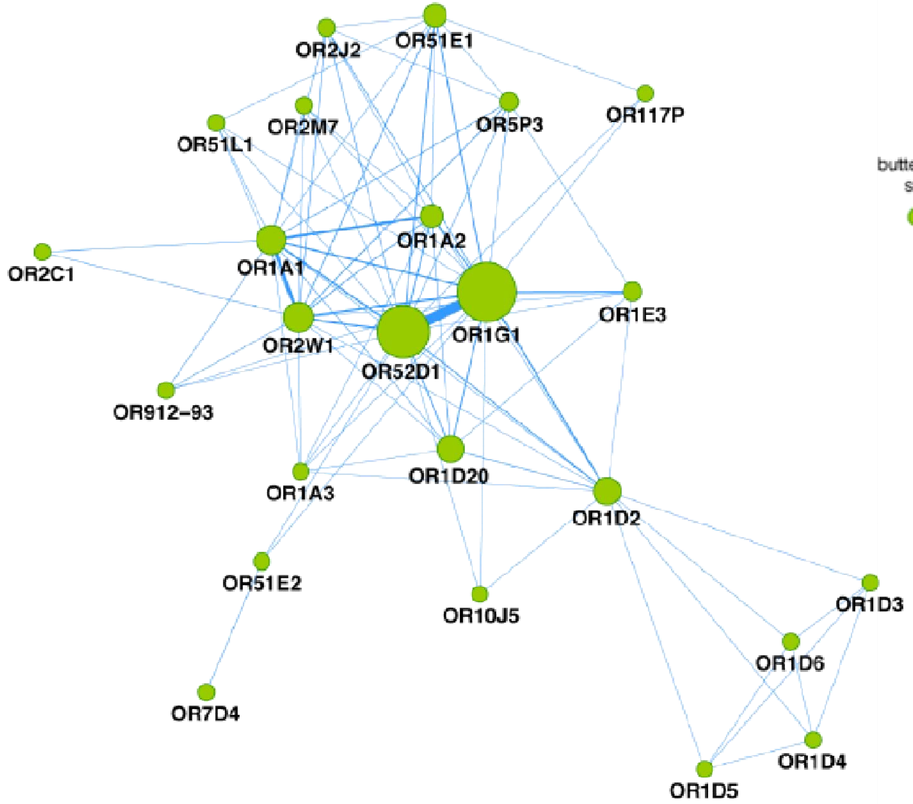
The Olfactory Sense
Bioinformatics

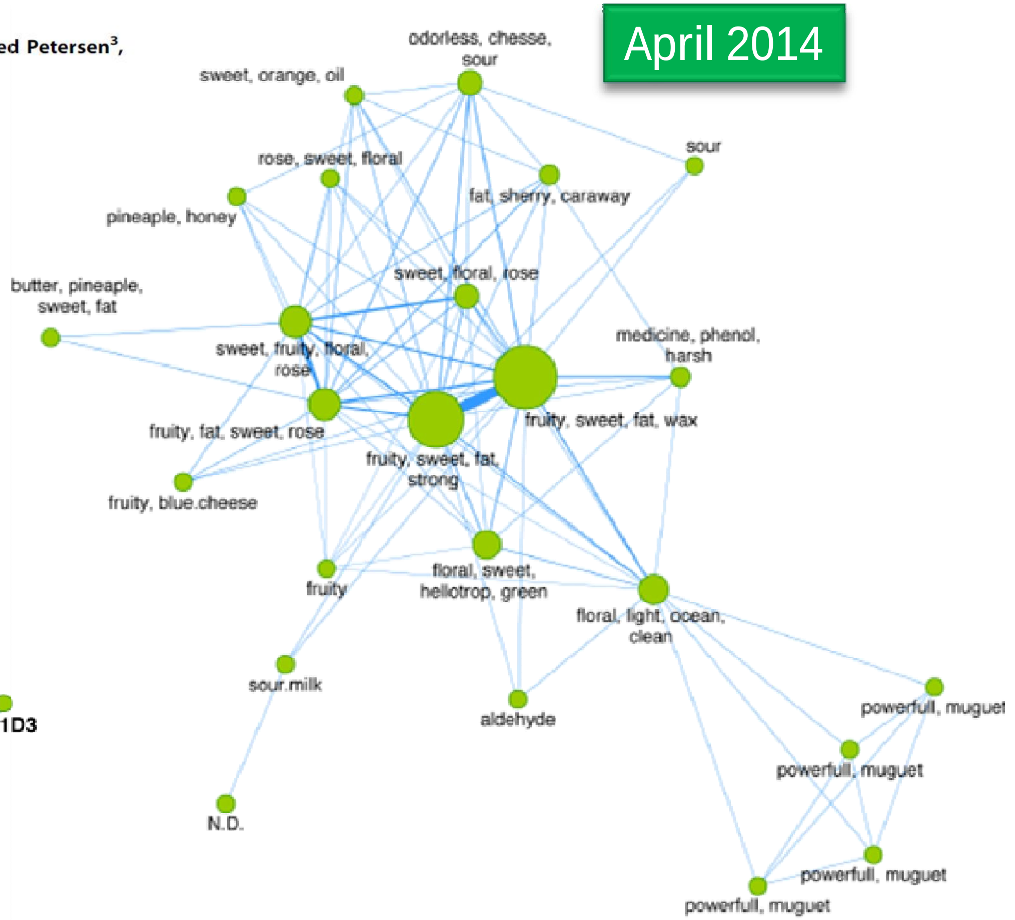
April 2014

OPEN ACCESS Freely available online
PLOS | ONE

Identification of Odorant-Receptor Interactions by Global Mapping of the Human Odorome

Karine Audouze[1], Anne Tromelin[2], Anne Marie Le Bon[2], Christine Belloir[2], Rasmus Koefoed Petersen[3], Karsten Kristiansen[3], Søren Brunak[1], Olivier Taboureau[1,4]*


OR2J2
OR51E1
OR2M7
OR5P3
OR117P
OR51L1
OR1A2
OR2C1
OR1A1
OR1E3
OR2W1
OR1G1
OR52D1
OR912-93
OR1A3
OR1D20
OR1D2
OR51E2
OR10J5
OR1D3
OR1D6
OR7D4
OR1D4
OR1D5


odorless, chesse, sour
sweet, orange, oil
sour
rose, sweet, floral
fat, sherry, caraway
pineaple, honey
butter, pineaple, sweet, fat
sweet, floral, rose
sweet, fruity, floral, rose
medicine, phenol, harsh
fruity, fat, sweet, rose
fruity, sweet, fat, wax
fruity, sweet, fat, strong
fruity, blue.cheese
floral, sweet, heliotrop, green
fruity
floral, light, ocean, clean
sour.milk
aldehyde
powerfull, muguet
powerfull, muguet
N.D.
powerfull, muguet
powerfull, muguet

Node size: number of odorants known to bind to the OR (83 ORs and 938 associations).
Width of the edges: weighted score is the strength of the link between two ORs based on the number of shared compounds.

Mapping of odor descriptions on the human odorome by an association score based on the number of compounds associated to an odor.

Q. Starting from food composition, how can we tackle (at a molecular basis) these complex phenomena, how can we objectify odor perception?
A. Comprehensive chemical investigation at multiple level of information

Foodomics[1]

investigates food and nutrition domains (food science and nutrition) combining information from emerging working areas, advanced analytical techniques and bioinformatics

Sensomics[2] - Flavoromics [3,4] - Flavour metabolomics[5]

correlate analytical data to perception extend investigation to all possible *stimuli* of a multimodal perception collecting data on volatiles, semi-volatiles and non-volatiles in an efficient manner

[1] Herrero M. et al (2012) Mass Spectrom Rev. 31(1):49-69
[2] Schieberle, P., Hofmann, T. (2003) Chemie in Unserer Zeit, 37 (6), pp. 388-401.
[3] G. A. Reineccius. (2008) In Abstracts of Papers, 235th American Chemical Society, New Orleans, LA, USA, pp. AGFD–061.
[4] Charve J et al (2011) Flavour Fragrance J. 26(6):429-440.
[5] de Vos C. H. R. et al (2008) Proceedings of the 12th Weurman Symposium, Interlaken, Switzerland pp. 573–580

Collaboration with Prof. Peter Schieberle
Food Chemistry Department
Technical University of Munich

Targeted analysis

Chemical features are just known-markers and analytical efforts are directed to few informative analytes

informative potential limited to known attributes

Information from unknowns is lost and untreated for routine practice

Classic *Sensomics*
Focus on few relevant odor active compounds

Untargeted analysis

Each and every sample component is a potential *informative chemical feature* all detectable chemical information is considered

informative potential is extended to the whole chemical pattern

Exploits the hidden information enveloped in the sample fingerprint

Deep insights into the complex phenomena of sensory perception
Detailed chemical characterization

Advanced *Sensomics* approaches aims at merging discrete and time-consuming operations in one step by adopting multidimensional analytical platforms

1. extraction/isolation of volatile fraction compounds
2. concentration of extracts
3. pre-separation of volatile compounds
4. main separation of volatile compounds
5. localization of intense aroma compounds
6. identification of aroma compounds
7. quantification of aroma compounds
8. evaluation of their aroma contribution
9. aroma recombination

Sensu lato multidimensional techniques:

Liquid Chromatography coupled with high/low resolution Mass Spectrometry (LC-MS^n)
Gas Chromatography coupled with high/low resolution Mass Spectrometry (GC-MS)

Sensu stricto multidimensional techniques:

Two-Dimensional Comprehensive GC (GC×GC) coupled with MS and Tandem MS
Two-Dimensional Comprehensive LC (LC×LC) coupled with MS and Tandem MS

Omics Analytical Platforms

LC-MS LC-MS^n LC×LC-MS LC×LC-MS^n
HS-MS GC-MS GC×GC-MS GC×GC-MS^n

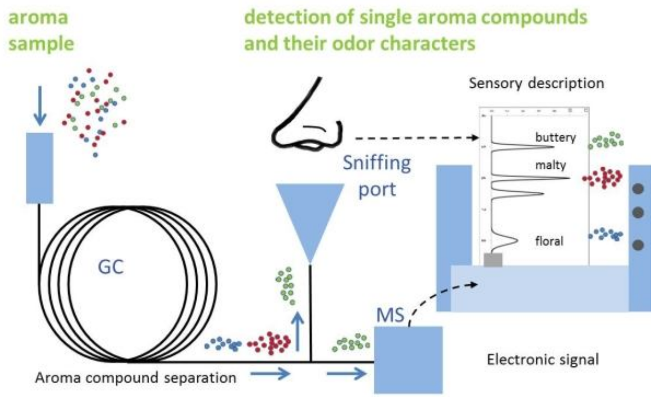
Bio-guided screening
Olfactometry & Sensory analysis

Chemical information

Identity
exact mass assignment
fragmentation pattern
multiple reaction monitoring

Physicochemical behavior
volatility, polarity, partition coefficient, solubility

Mass quantitation
absolute concentration and relative abundance


aroma sample
detection of single aroma compounds and their odor characters
Sensory description
buttery
malty
floral
Sniffing port
GC
MS
Aroma compound separation
Electronic signal

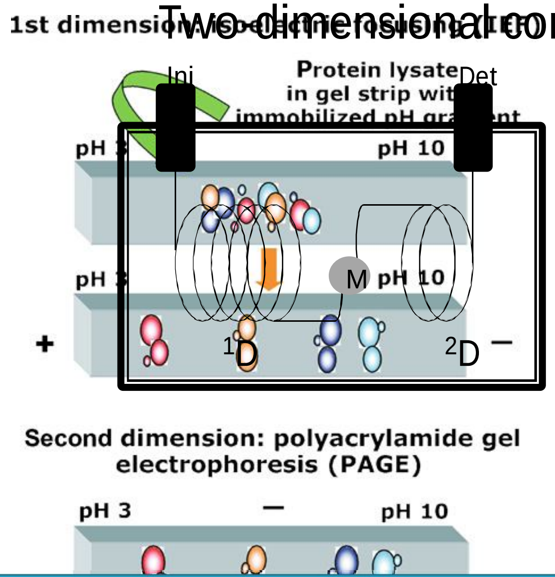
Aroma, taste, texture, aspect, etc…
orthogonal and **complementary**
treated together with chemical data

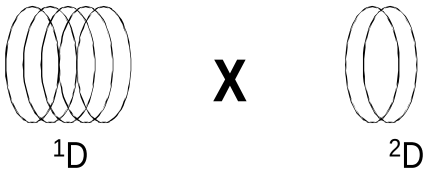
interpret chemical speciation
Identifying the Chemical Odor Code

Omics Analytical Platforms

LC-MS LC-MS^n LC×LC-MS LC×LC-MS^n
HS-MS GC-MS GC×GC-MS GC×GC-MS^n

Two-dimensional comprehensive gas chromatography

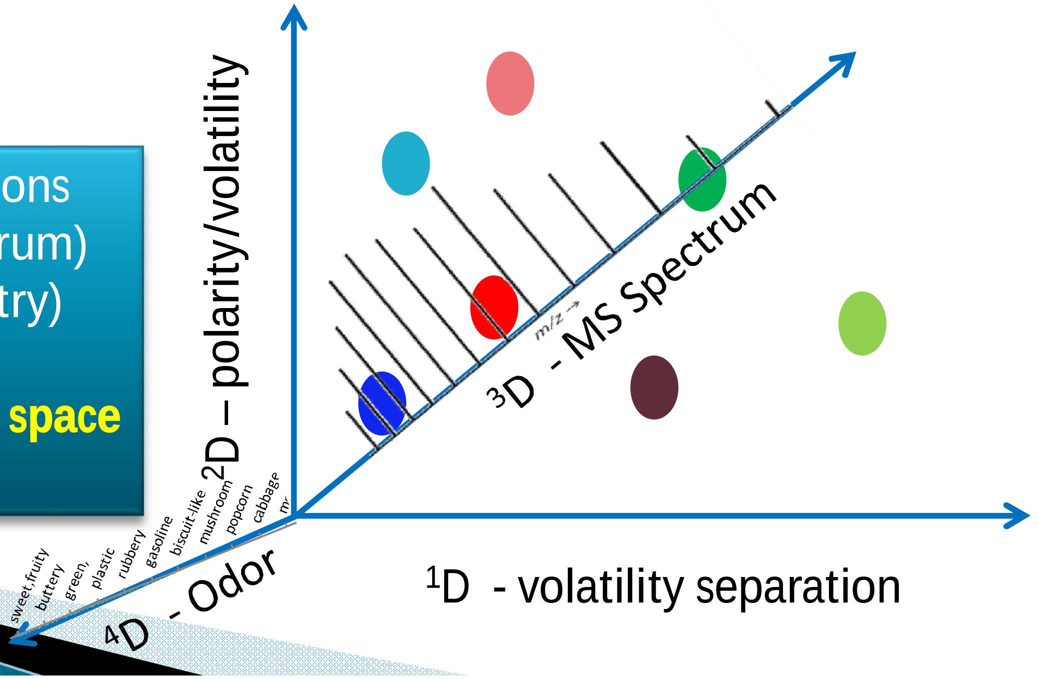

1st dimension: isoelectric focusing (IEF)
Inj
Protein lysate in gel strip with immobilized pH gradient
Det
pH 3
pH 10
pH 3
M
pH 10
+
^{1}D
^{2}D
–
Second dimension: polyacrylamide gel electrophoresis (PAGE)
pH 3
–
pH 10



volatility   polarity/volatility

Further information dimensions
Molecular identity (MS spectrum)
Odor descriptor (Olfactometry)

Multidimensional information space

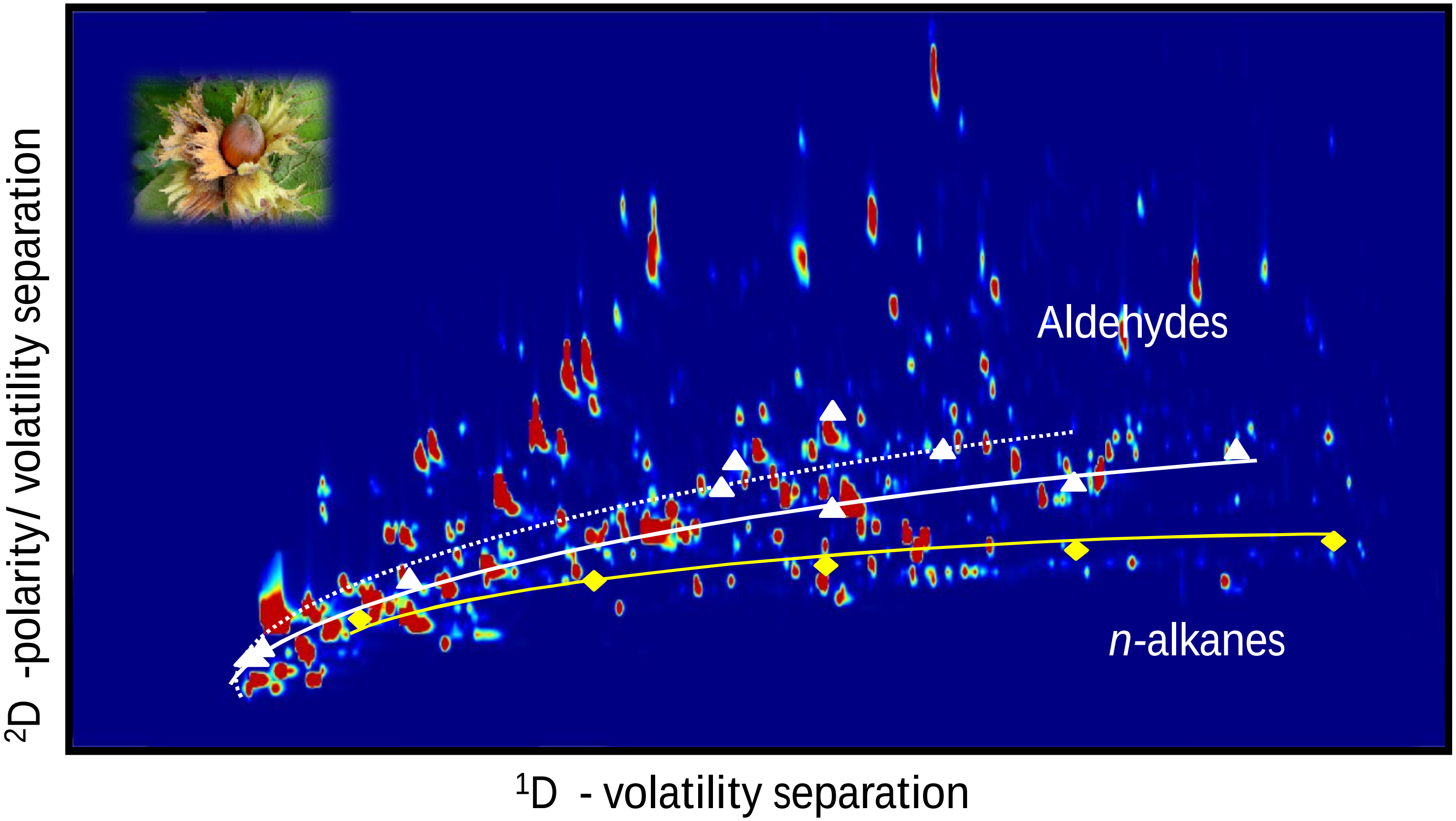

^{2}D – polarity/volatility
^{3}D - MS Spectrum
m/z →
^{1}D - volatility separation
^{4}D - Odor
sweet,fruity
buttery
green,
plastic
rubbery
gasoline
biscuit-like
mushroom
popcorn
cabbage

Omics Analytical Platforms

LC-MS LC-MS^n LC×LC-MS LC×LC-MS^n

HS-MS GC-MS **GC×GC-MS GC×GC-MS^n**

Modern omics disciplines
Advanced Sensomics



Aldehydes

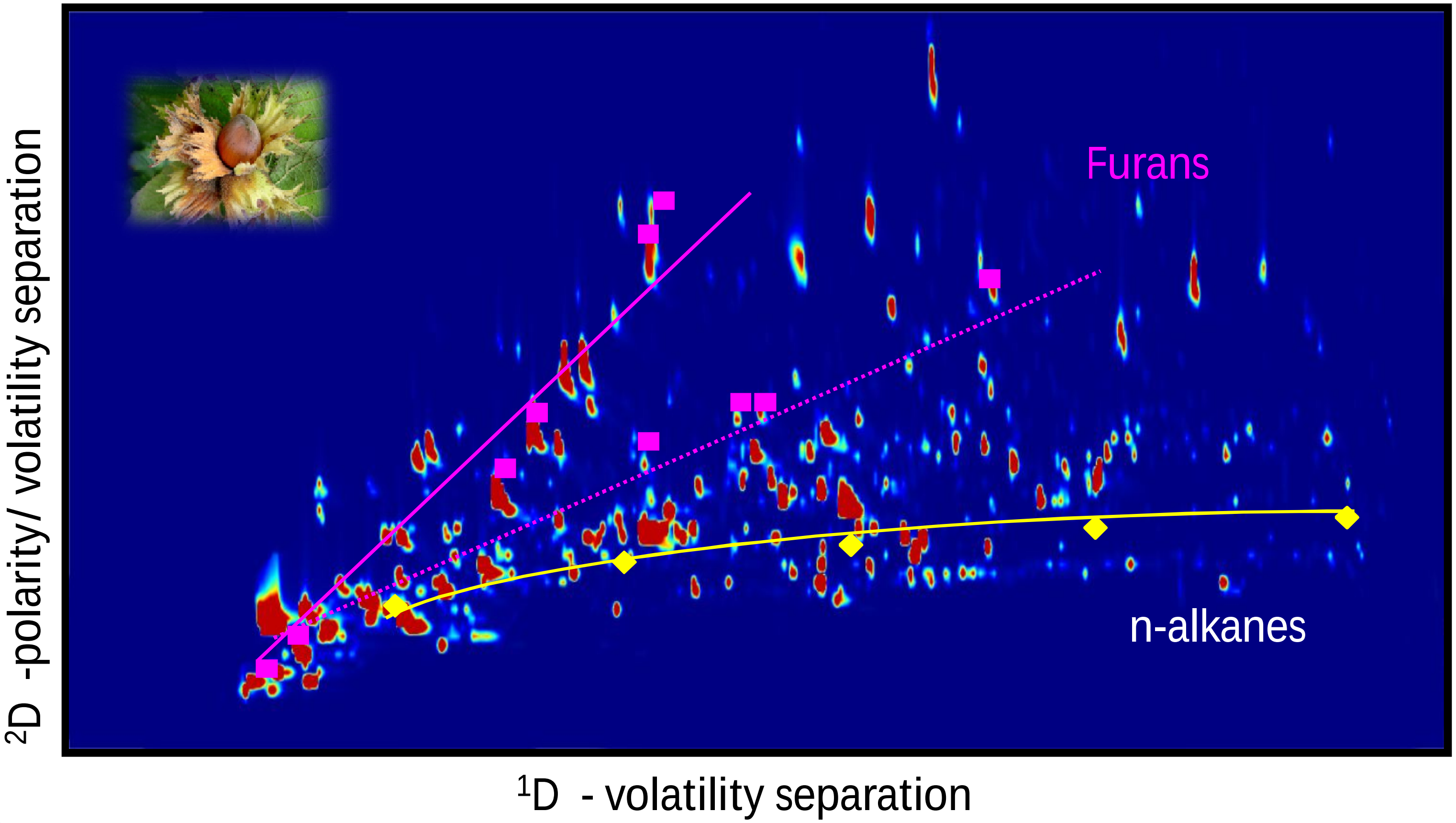
2-Methyl Butanal
Crotonaldehyde
Capronaldehyde
2E-Heptenal
Benzaldehyde
3-Carboxaldehyde Thiophene
Caprylaldehyde
Phenyl Acetaldehyde
Nonanal
2-Nonenal

Omics Analytical Platforms

LC-MS LC-MS^n LC×LC-MS LC×LC-MS^n

HS-MS GC-MS **GC×GC-MS GC×GC-MS^n**

Modern omics disciplines
Advanced Sensomics



Furans

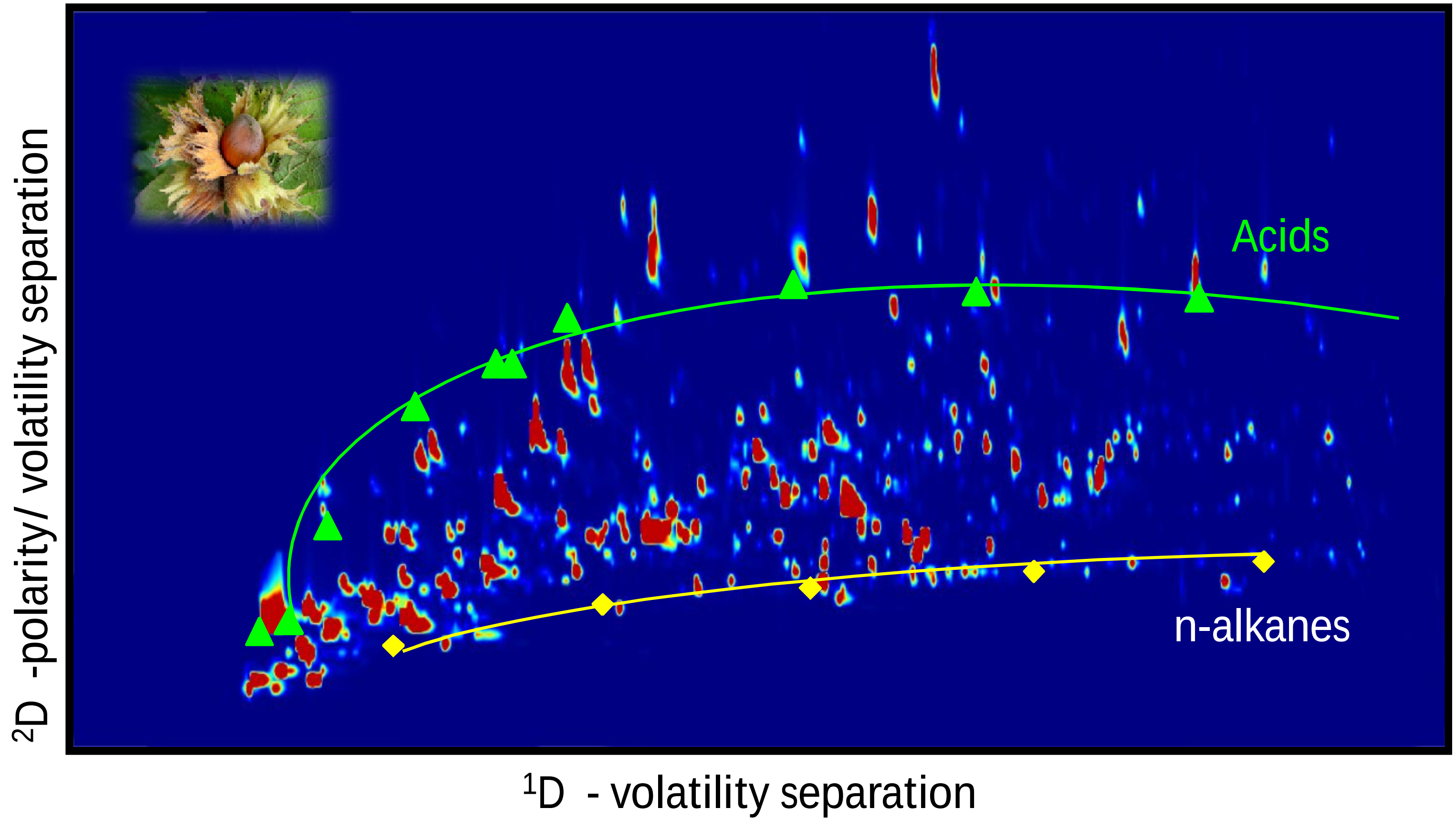
2-Methylfurane
THF
Dihydro-2(3H)-Furanone
2-Furancarboxaldehyde
2-Furanemethanol
2-Acetylfurane
2(5H)-FURANONE
5-Methyl-2-Furanemethanol
5-Methyl-2-Furanecarboxaldehyde
2,5-Dimethyl-3(2H)Furanone

Omics Analytical Platforms

LC-MS LC-MS^n LC×LC-MS LC×LC-MS^n

HS-MS GC-MS **GC×GC-MS GC×GC-MS^n**

Modern omics disciplines
Advanced Sensomics



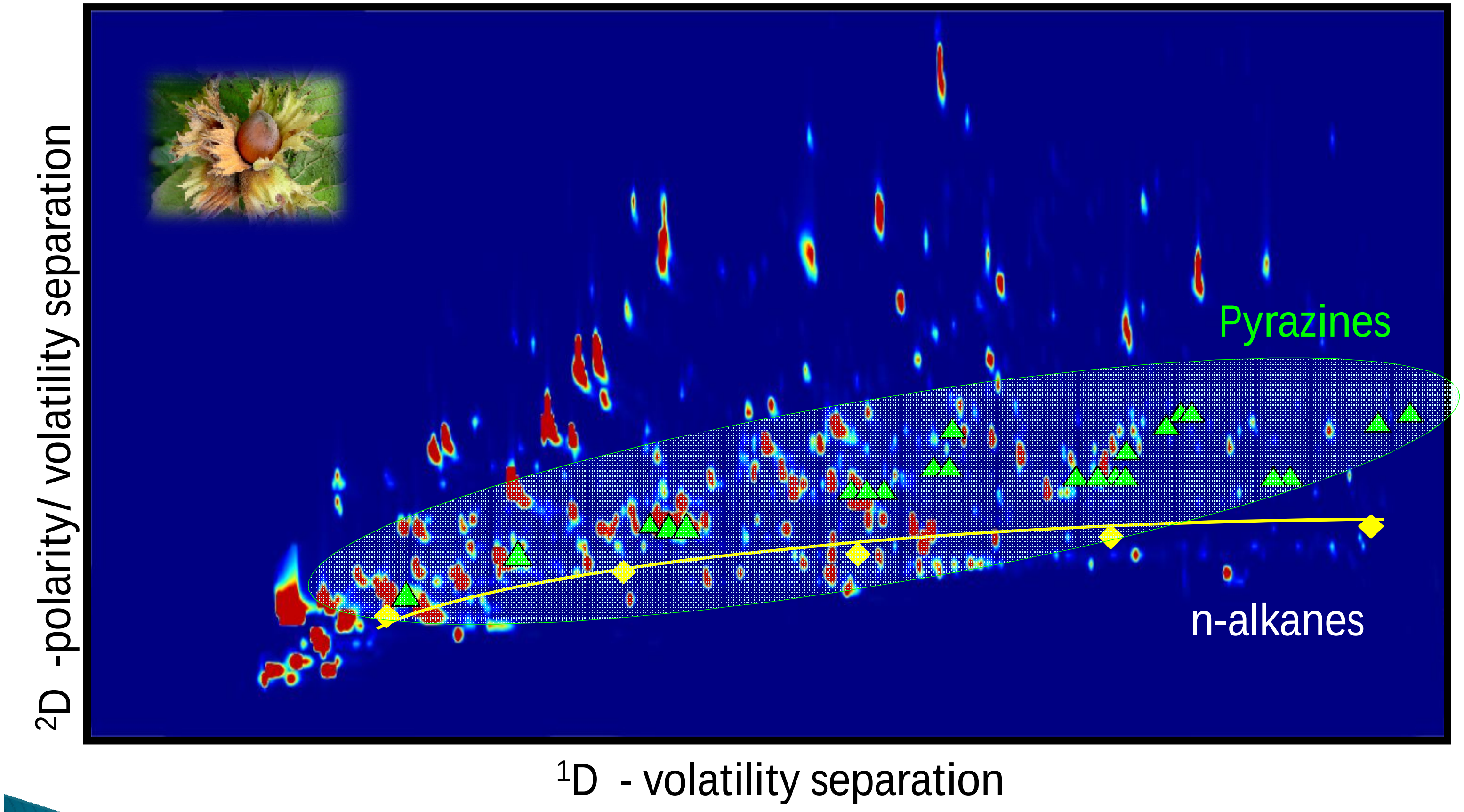
Acids

Formic acid
Acetic acid
Propionic acid
Butyric acid
Valeric acid
Isovaleric acid
2-methyl butanoic acid
Capronic acid
Enanthic acid
Caprylic acid

Omics Analytical Platforms

LC-MS LC-MS^n LC×LC-MS LC×LC-MS^n
HS-MS GC-MS **GC×GC-MS GC×GC-MS^n**

Modern omics disciplines
Advanced Sensomics

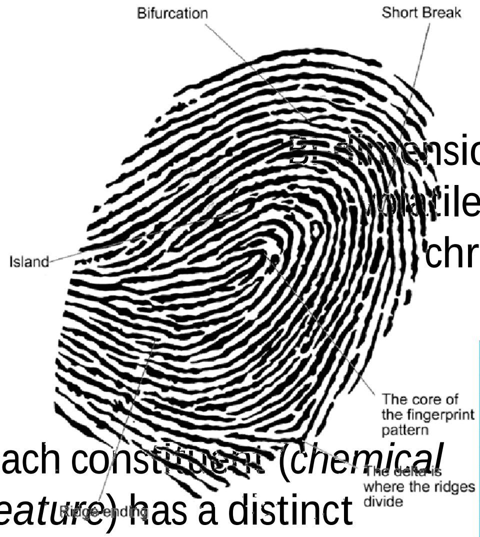


Pyrazines

Pyrazine
Methylpyrazine
2,5-Dimethylpyrazine
2,6-Dimethylpyrazine
2-Ethylpyrazine
2,3-Dimethylpyrazine
2-Ethyl-6-Methylpyrazine
2,3,5-Trimethylpyrazine
2-Ethyl-5-Methylpyrazine
2-Ethyl-3-Methylpyrazine
2-Ethenyl-5-methylpyrazine
2-Ethenyl-6-methylpyrazine
2-Acetyl pyrazine
2-Ethyl-3,6-dimethylpyrazine
2,3-Diethylpyrazine
3-Ethyl-2,5-diMethyl pyrazine
2-Isopropenylpyrazine
2-Acetyl-6-methylpyrazine
2-Acetyl-5-methylpyrazine
2,3-Diethyl-5-methylpyrazine
(E)-2-Methyl-5-(1-propenyl)pyr
2-Acetyl-3-ethylpyrazine

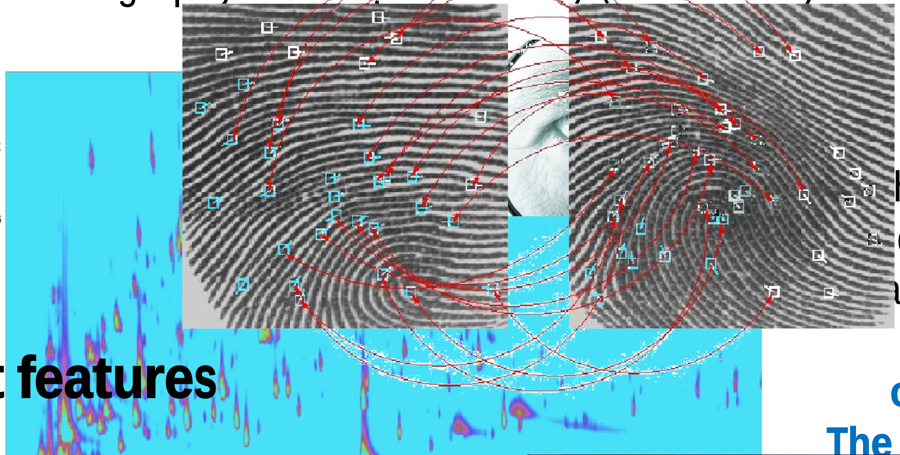
Biometric Fingerprinting[1]

Modern omics disciplines
Advanced Sensomics


Bifurcation
Short Break
Island
The core of the fingerprint pattern
The delta is where the ridges divide
Ridge ending

Bi-dimensional separation pattern (2D chromatogram) of hazelnut volatiles obtained by two-dimensional comprehensive gas chromatography- mass spectrometry (GC×GC-MS)

Comparison within stored templates



Each constituent (*chemical feature*) has a distinct position over the 2D plane and a unique information pattern (MS spectrum) that enables **identification and quantitation**

Image Acquisition

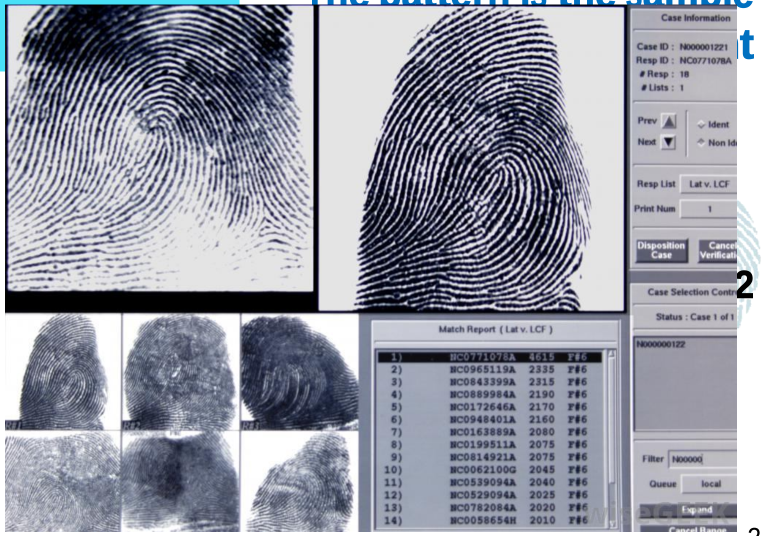
Profiling of relevant features

h 2D pattern is unique d informative, collects ta on multiple sample properties, including **odorants distribution.**

The pattern is the sample t

(Detailed) Profiling[2]

Identification


Case Information
Prev
Next
Ident
Non Id
Disposition Case
Cancel Verificati
Case Selection Contr
Expand
Cancel Range
wiseGEEK

Case ID : N000001221
Resp ID : NC0771078A
Resp : 18
Lists : 1
Resp List Lat v. LCF
Print Num 1
Status : Case 1 of 1
N000000122
Filter N00000
Queue local

Match Report (Lat v. LCF)

```
 1)   NC0771078A  4615  F#6
 2)   NC0965119A  2335  F#6
 3)   NC0843399A  2315  F#6
 4)   NC0889984A  2190  F#6
 5)   NC0172646A  2170  F#6
 6)   NC0948401A  2160  F#6
 7)   NC0163889A  2080  F#6
 8)   NC0199511A  2075  F#6
 9)   NC0814921A  2075  F#6
10)   NC0062100G  2045  F#6
11)   NC0539094A  2040  F#6
12)   NC0529094A  2025  F#6
13)   NC0782084A  2020  F#6
14)   NC0058654H  2010  F#6
```

2

Sensomics advanced Platform
Sample preparation Separation-Detection
Sensory screening Data elaboration

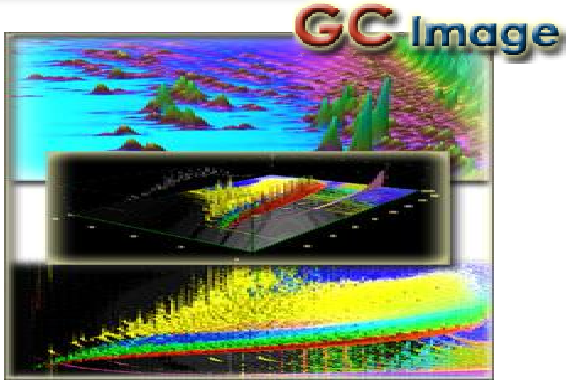
Modern omics disciplines
Advanced Sensomics

Agilent 6890-5975C MS operating in EI mode (70 eV) Scan range 35/240 m/z, Scan rate 10,000 amu/s

Zoex KT2004 loop modulator Cryogenic: liquid nitrogen, Massflow ctr. Optimode™ by SRA Italy

GC-O sniffing port: design by Grosh et al at FID outlet before ^{2}D column.

GC×GC-MS: injector temperature: 250°C, injection mode: split, ratio: 1/10; carrier gas: helium, initial head pressure 260 KPa;
Column set: ^{1}D Sol-GelWax® (30 m, 0.25 mm ID, 0.25 um d_f) (SGE Analytical Science, Ringwood, Victoria, Austalia) and ^{2}D OV1701 (1 m, 0.1 mm ID, 0.1 um d_f) (Mega, Legnano (Milan), Italy); loop capillary 1 m, 0.1 mm ID, 0.1 um d_f
GC oven: 40°C (1 min) to 170°C at 3.0°C/min and to 260°C at 50°C/min ([illegible] min)



Data Acquisition: Agilent Chem Station ver D.02
Data elaboration : GC-Image ver 2.1b4

Collaboration with
Prof. S.E. Reichenbach
Computer Science and Engineering
Department University of Nebraska

Cordero, C., et al. "Targeted and Non-Targeted Approaches for Complex Natural Sample Profiling by GC×GC-qMS." Journal of chromatographic science 48.4 (2010): 251-61.

Cordero, C., et al. "Profiling Food Volatiles by Comprehensive Two-Dimensional Gas chromatography Coupled with Mass Spectrometry: Advanced Fingerprinting Approaches for Comparative Analysis of the Volatile Fraction of Roasted Hazelnuts (*Corylus Avellana* L.) from Different Origins." Journal of Chromatography A 1217.37 (2010): 5848-58.

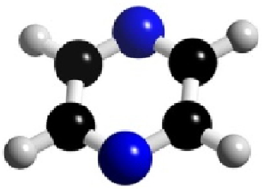
Kiefl, J., et al. "Performance Evaluation of Non-Targeted Peak-Based Cross-Sample Analysis for Comprehensive Two-Dimensional Gas Chromatography-Mass Spectrometry Data and Application to Processed Hazelnut Profiling." Journal of Chromatography A 1243 (2012): 81-90.

Reichenbach, S. E., et al. "Features for Non-Targeted Cross-Sample Analysis with Comprehensive Two-Dimensional Chromatography." Journal of Chromatography A 1226 (2012): 140-8.

Reichenbach, S. E., et al. "Reliable Peak Selection for Multisample Analysis with Comprehensive Two-Dimensional Chromatography." Analytical Chemistry 85.10 (2013): 4974-81.

Whole and nonfat dry milk: a case study

Maillard reaction products (including sulphur derivatives)
Strecker aldehydes
Lipid oxidation products (enzymatic and ozonolysis during spray-drying)
Products of TG hydrolysis (free fatty acids, including short chain fatty acids)
Lactones and furanones
Aromatic AA degradation (phenolic derivatives responsible of cowy and fecal flavors)
Terpenoids and norisoprenoids



Target chemical features

Volatility range (25°C) from 49.3 of 3-methylbutanal to 1.72E-5 Torr of (*E*)-2-octadecenal
Log P values range: 0.32 of acetic acid to 8.05 of octadecanal
Water solubility (pH 6 – 25°C) range: from 16.6 of acetic acid to 5.6E-06 mol/L of 2-octadecanone
Odor thresholds (orthonasal in water at 25°C) 0.15 ng/g for the (*E*)-2-nonenal

Sensory features

odor descriptors
off-flavors (shelf life)
positive attributes responsible of the aroma

Highly volatiles - Orthonasal perception

Static Headspace
Temperature - Time - Phase ratio (β)

Dynamic Headspace
breakthrough volume

S-HS

HS-SPME
Selective extraction

Sorption
PDMS
PA

Adsorption
CW

Mixed
mode

HSSE
Larger volumes

Sorption
PDMS

Mixed
mode
PDMS/
Carbopack

HS-
MMSE
High
surface

Adsorption
ODS
Carbopack

D-HS
Larger sampling capacity

Sorption PDMS
(foams and particles)

Adsorption
(Tenax TA)

SPME

SBSE

MMSE

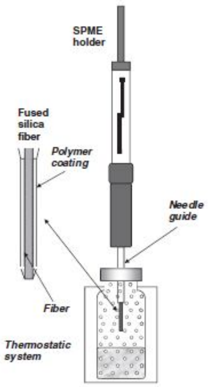
In-solution sampling
Temperature - Time - Phase ratio (β)

Evaporation in oral cavity - Retronasal perception

S-HS

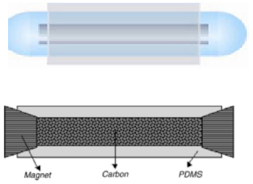
Sampling: 50 (°C)
Syringe temperature: 60 (°C) Incubation: 40 min Fill speed: 40 (mL/min)
Sample volume: 1 (mL) Fill volume: 2 (ml) Injection speed: 100 (mL/min)
Flush time: 2 (min)
System HT2100H (HTA srl - Brescia Italy)

HS-SPME Selective extraction


SPME holder
Fused silica fiber
Polymer coating
Needle guide
Fiber
Thermostatic system

Sampling temperature: 50°C
Sampling time: 40 min
Vial volume: 20 mL
Fiber PDMS 100 µm
Polyacrylate 85 µm
Carbowax 60 µm
DVB/CAR/PDMS 50/30 µm 2 cm
Supelco Bellefonte

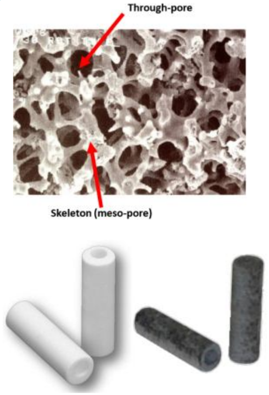
HSSE Larger volumes


Magnet
Carbon
PDMS

Sampling temperature: 50°C
Sampling time: 40 min
Vial volume: 20 mL

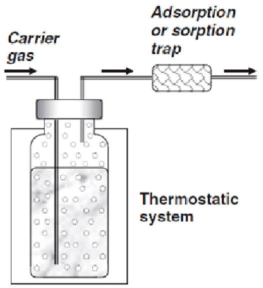
Conventional PDMS Twister (length: 20 mm, thickness: 0.5 mm).
Dual phase Twisters PDMS-Carbopack B (length: 20 mm, thickness: 0.5 mm)
Gerstel Mulheim DE.

HS-MMSE High surface


Through-pore
Skeleton (meso-pore)

Sampling temperature: 50°C
Sampling time: 40 min
Vial volume: 20 mL
Monotrap Rod type: 2.9 mmOD x 5 mm height
Octadecylsilica-Active Carbon Black GL Sciences Inc. Tokyo Japan

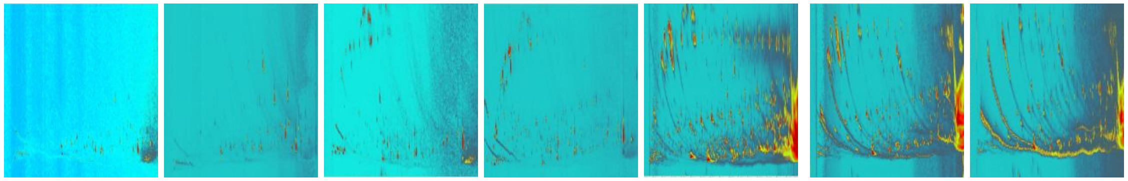
D-HS Larger sampling capacity


Carrier gas
Adsorption or sorption trap
Thermostatic system

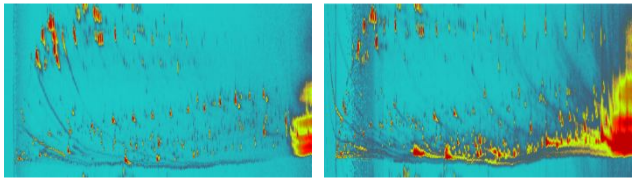
Sampling temperature: 40°C
Trap temperature: 25°C Time: 20 min
Stripping gas: N_2 at 10 mL/min
Vial volume: 20 mL
PDMS particles and foam by Gerstel Mulheim DE
Tenax TA (60/80 mesh) Supelco Bellefonte

Modern omics disciplines
Advanced Sensomics

Static Headspace (8)



Dynamic Headspace (2)



SPME
Sorption

SPME
Adsorption

SPME
Mixed mode

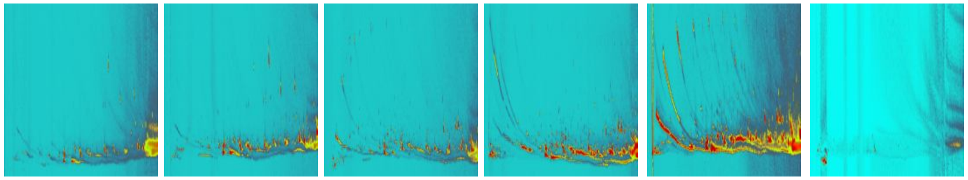
HSSE
Sorption

HSSE
Mixed mode

MMSE
Adsorption

Sorption PDMS

Adsorption

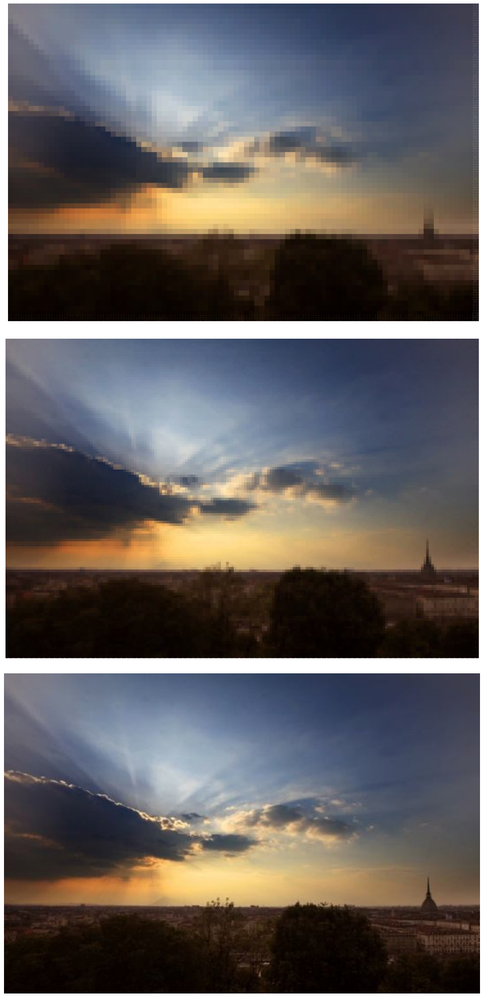


In-solution sampling (6)

Whole and nonfat milk (2)
Sampling replicates (3)
8×2×6×3=96 2D patterns

Bio-guided Chromatographic fingerprinting
Detailed untargeted Profiling

Chemical Odor Code of Milk



Bio-guided fingerprinting
Bio-guided screening of samples pattern

Low resolution fingerprinting
based on *region features*[1]

specific regions of the chromatographic plane where detector (single or multi channel) intensities are summed together
Information is extracted , aligned and compared across samples

these regions can be fixed *a priori* (grids)
enables bio-guided screening of the most informative regions of the chromatographic retention space

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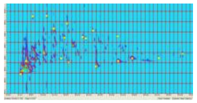
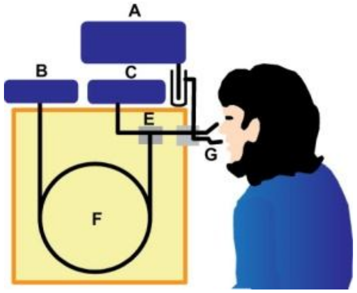
[1] Reichenbach S.E., Tian X., Cordero C., Tao T. (2012) J. Chromat. A, 1226 :140– 148



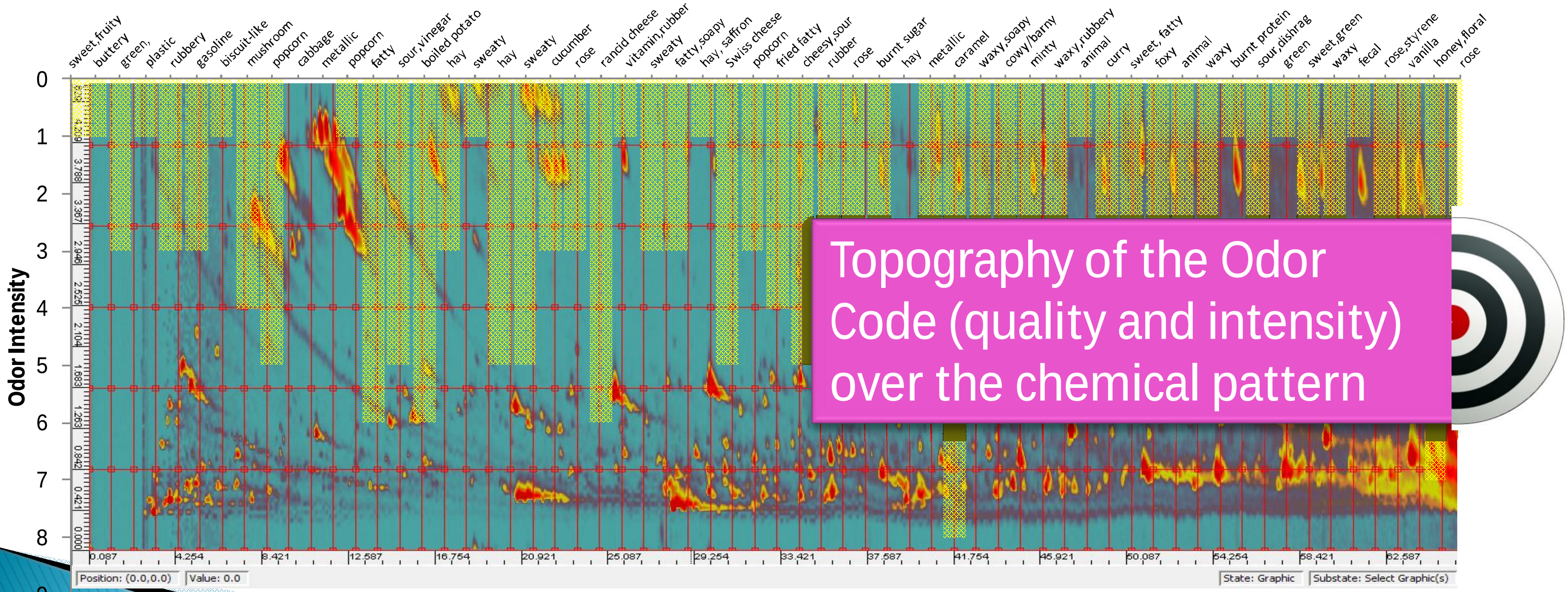
Bio-guided Chromatographic Fingerprinting[1]

Region features

1. comprehensive subdivision of the chromatographic plane

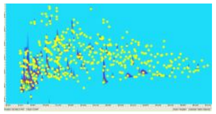


- ✓ uniform grid with a fixed number of contiguous and non-overlapping panels corresponding to ^{1}D LRI
- ✓ GC(O)×GC-MS screening



Topography of the Odor Code (quality and intensity) over the chemical pattern

Picture from:
TrAC (2011) 30(11) :1756–1770

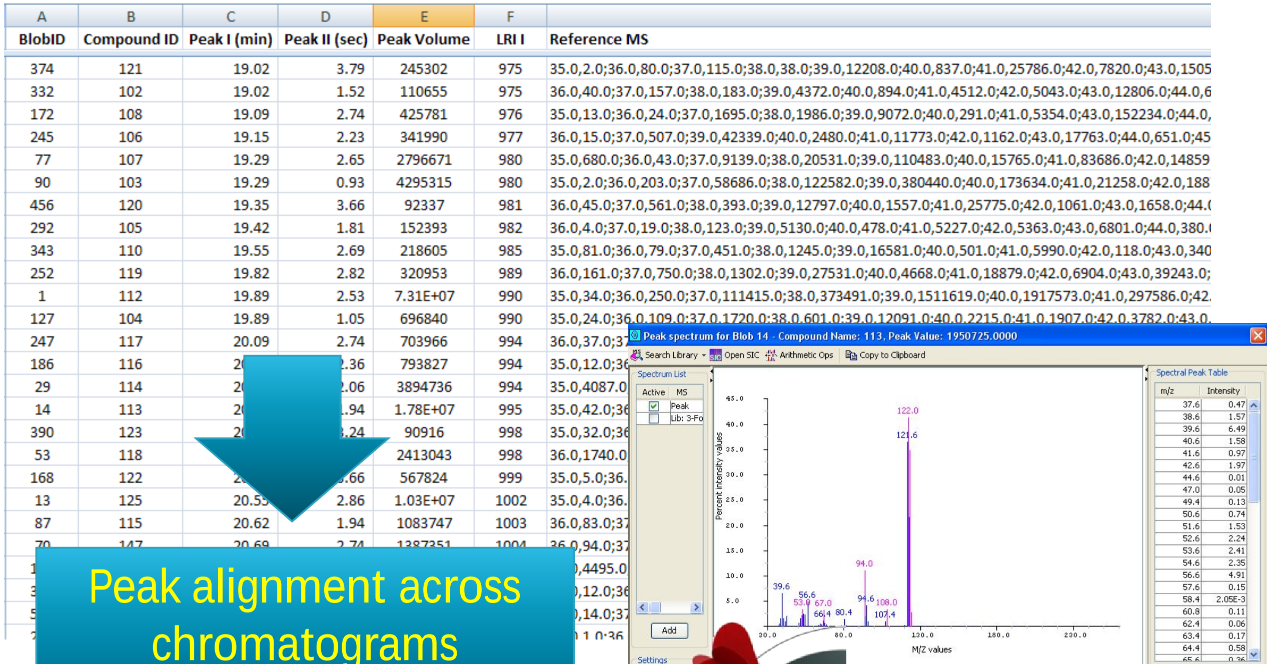


Detailed untargeted Profiling
Comprehensive Template Matching Fingerprinting[1]

Modern omics disciplines
Advanced Sensomics

Peak feature

To improve the specificity of the process one has to limit correspondences to peaks resulting from the same analyte



A	B	C	D	E	F	
BlobID	Compound ID	Peak I (min)	Peak II (sec)	Peak Volume	LRI I	Reference MS
374	121	19.02	3.79	245302	975	35.0,2.0;36.0,80.0;37.0,115.0;38.0,38.0;39.0,12208.0;40.0,837.0;41.0,25786.0;42.0,7820.0;43.0,1505
332	102	19.02	1.52	110655	975	36.0,40.0;37.0,157.0;38.0,183.0;39.0,4372.0;40.0,894.0;41.0,4512.0;42.0,5043.0;43.0,12806.0;44.0,6
172	108	19.09	2.74	425781	976	35.0,13.0;36.0,24.0;37.0,1695.0;38.0,1986.0;39.0,9072.0;40.0,291.0;41.0,5354.0;43.0,152234.0;44.0,
245	106	19.15	2.23	341990	977	36.0,15.0;37.0,507.0;39.0,42339.0;40.0,2480.0;41.0,11773.0;42.0,1162.0;43.0,17763.0;44.0,651.0;45
77	107	19.29	2.65	2796671	980	35.0,680.0;36.0,43.0;37.0,9139.0;38.0,20531.0;39.0,110483.0;40.0,15765.0;41.0,83686.0;42.0,14859
90	103	19.29	0.93	4295315	980	35.0,2.0;36.0,203.0;37.0,58686.0;38.0,122582.0;39.0,380440.0;40.0,173634.0;41.0,21258.0;42.0,188
456	120	19.35	3.66	92337	981	36.0,45.0;37.0,561.0;38.0,393.0;39.0,12797.0;40.0,1557.0;41.0,25775.0;42.0,1061.0;43.0,1658.0;44.0
292	105	19.42	1.81	152393	982	36.0,4.0;37.0,19.0;38.0,123.0;39.0,5130.0;40.0,478.0;41.0,5227.0;42.0,5363.0;43.0,6801.0;44.0,380.
343	110	19.55	2.69	218605	985	35.0,81.0;36.0,79.0;37.0,451.0;38.0,1245.0;39.0,16581.0;40.0,501.0;41.0,5990.0;42.0,118.0;43.0,340
252	119	19.82	2.82	320953	989	36.0,161.0;37.0,750.0;38.0,1302.0;39.0,27531.0;40.0,4668.0;41.0,18879.0;42.0,6904.0;43.0,39243.0;
1	112	19.89	2.53	7.31E+07	990	35.0,34.0;36.0,250.0;37.0,111415.0;38.0,373491.0;39.0,1511619.0;40.0,1917573.0;41.0,297586.0;42.
127	104	19.89	1.05	696840	990	35.0,24.0;36.0,109.0;37.0,1720.0;38.0,601.0;39.0,12091.0;40.0,2215.0;41.0,1907.0;42.0,3782.0;43.0,
247	117	20.09	2.74	703966	994	36.0,37.0;37
186	116	20	.36	793827	994	35.0,12.0;36
29	114	20	.06	3894736	994	35.0,4087.0;
14	113	20	.94	1.78E+07	995	35.0,42.0;36
390	123	20	.24	90916	998	35.0,32.0;36
53	118			2413043	998	36.0,1740.0;
168	122	2	.66	567824	999	35.0,5.0;36.
13	125	20.5	2.86	1.03E+07	1002	35.0,4.0;36.
87	115	20.62	1.94	1083747	1003	36.0,83.0;37
70	147	20.69	2.74	1387351	1004	36.0,94.0;37

Peak spectrum for Blob 14 - Compound Name: 113, Peak Value: 1950725.0000

Active	MS
☑	Peak
☐	Lib: 3-Fo

Spectral Peak Table

m/z	Intensity
37.6	0.47
38.6	1.57
39.6	6.49
40.6	1.58
41.6	0.97
42.6	1.97
44.6	0.01
47.0	0.05
49.4	0.13
50.6	0.74
51.6	1.53
52.6	2.24
53.6	2.41
54.6	2.35
56.6	4.91
57.6	0.15
58.4	2.05E-3
60.8	0.11
62.4	0.06
63.4	0.17
64.4	0.58

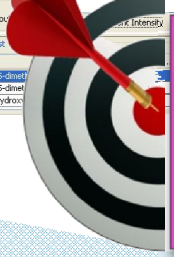
Active Hit List

Name
3-Formyl-4,5-dimet
2-Formyl-4,5-dimet
4-Amino-2-hydrox

Peak alignment across chromatograms
232 reliable analytes

Peak feature

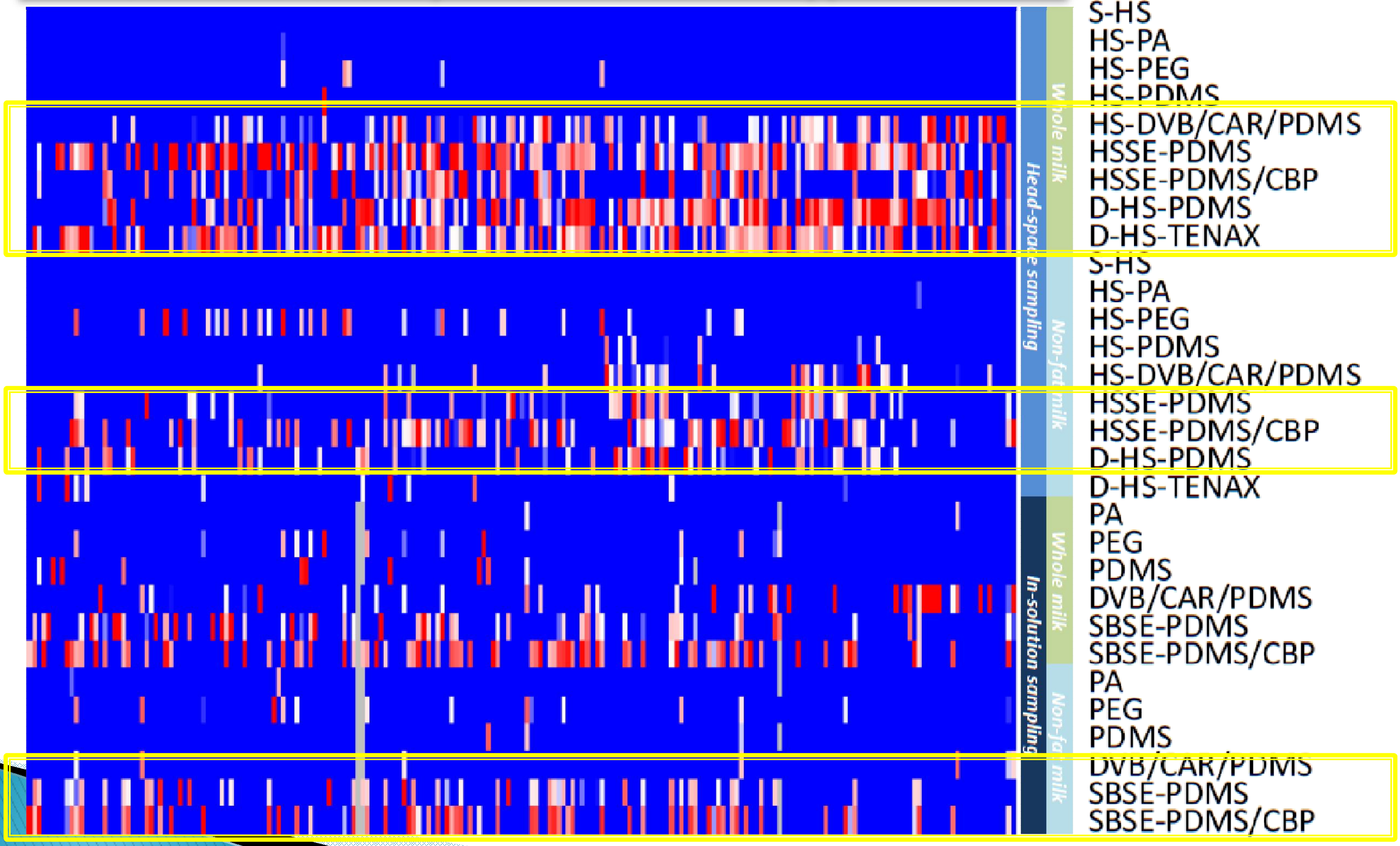
Retention times
Detector response
MS spectum



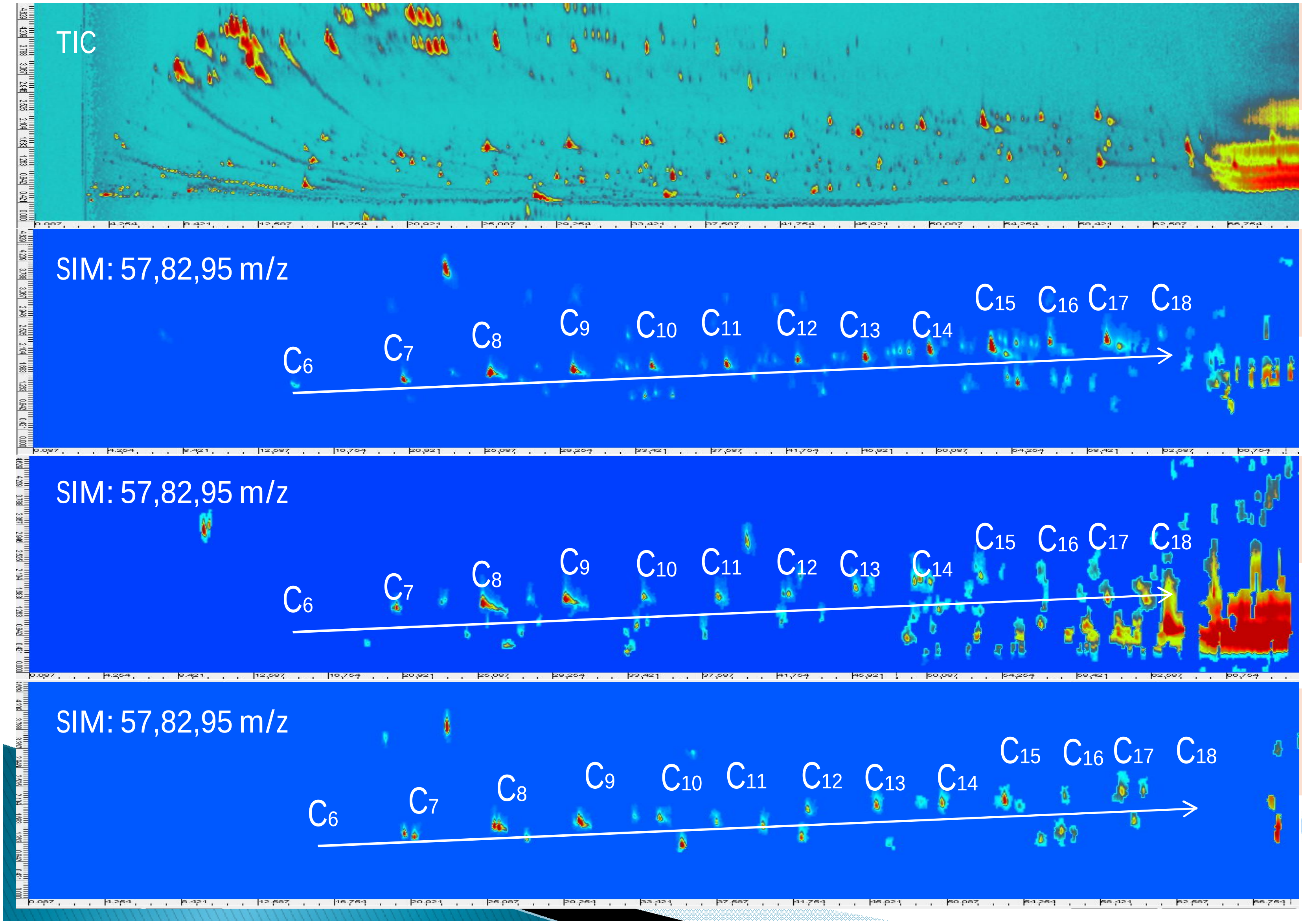
Topography of the Chemical Code – Analytes identity and concentration in the matrix

Q. Which is the most informative pattern?
Q. Which technique provides it?

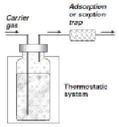
Quantitative distribution of 232 untargeted chemical features revealed by different extraction approaches



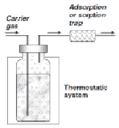
Fingerprinting on most relevant chemical classes - Aldehydes



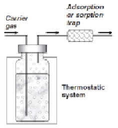
TIC
D-HS-PDMS


Carrier gas
Adsorption or sorption trap
Thermostatic system

SIM
D-HS-PDMS


Carrier gas
Adsorption or sorption trap
Thermostatic system

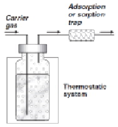
SIM
D-HS-TENAX


Carrier gas
Adsorption or sorption trap
Thermostatic system

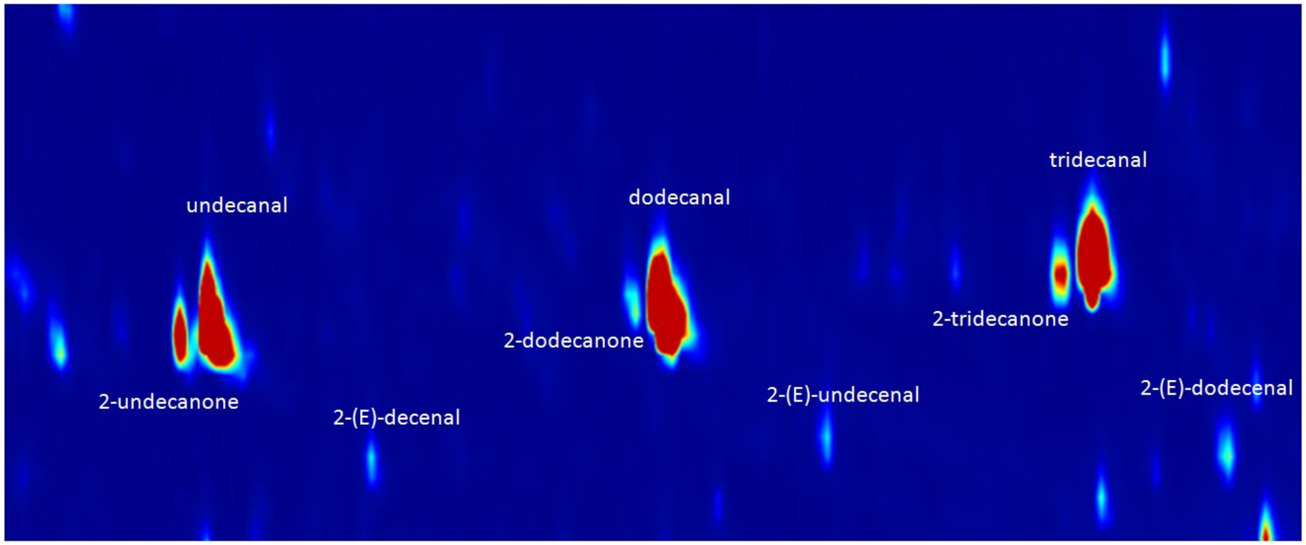
SIM
HSSE-PDMS

Homologue series fingerprinting – structured patterns

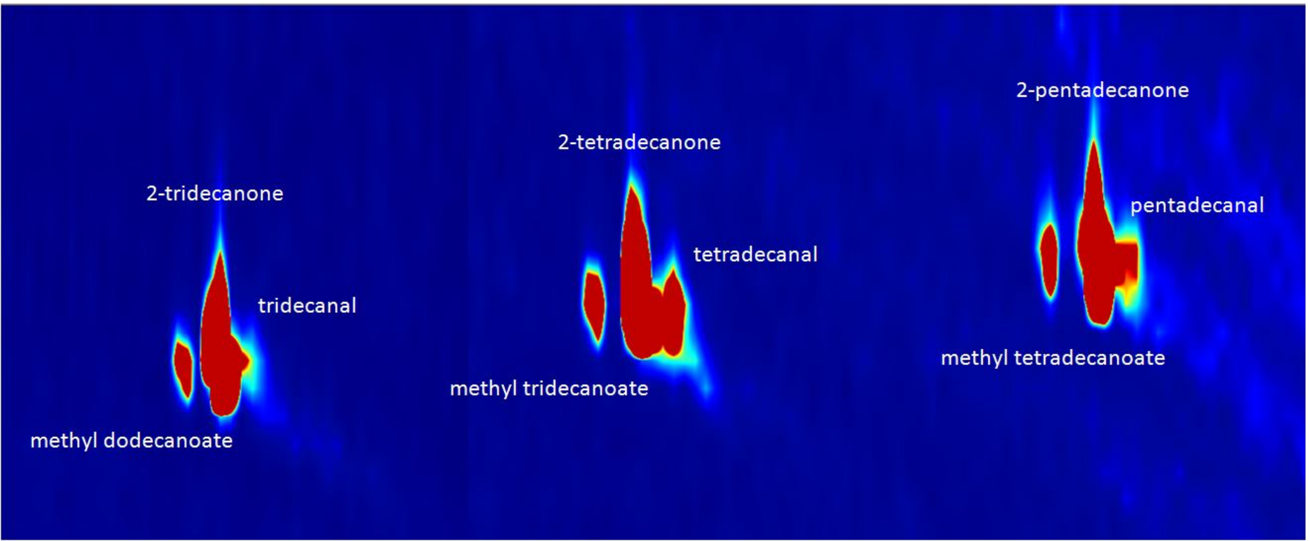
TIC
D-HS-PDMS
Headspace


Carrier gas
Adsorption or sorption trap
Thermostatic system

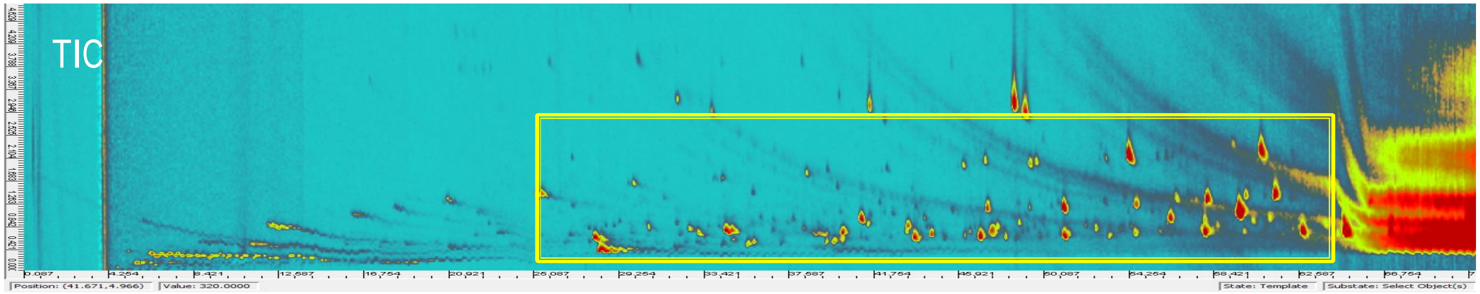
Odor thresholds
Undecanal: 5 µg/L
2-Undecanone: 7 µg/L
(*E*)-2-Decenal: 0.3-0.4 µg/L



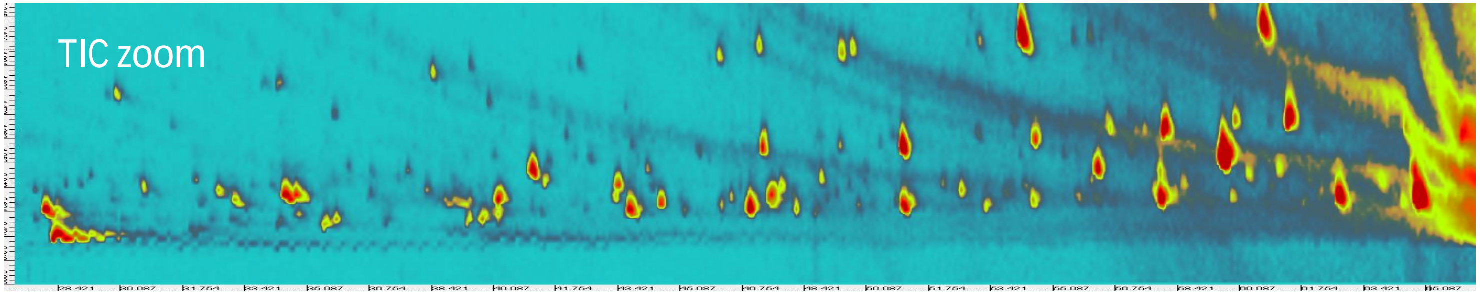
TIC
MMSE
Adsorption
In-solution



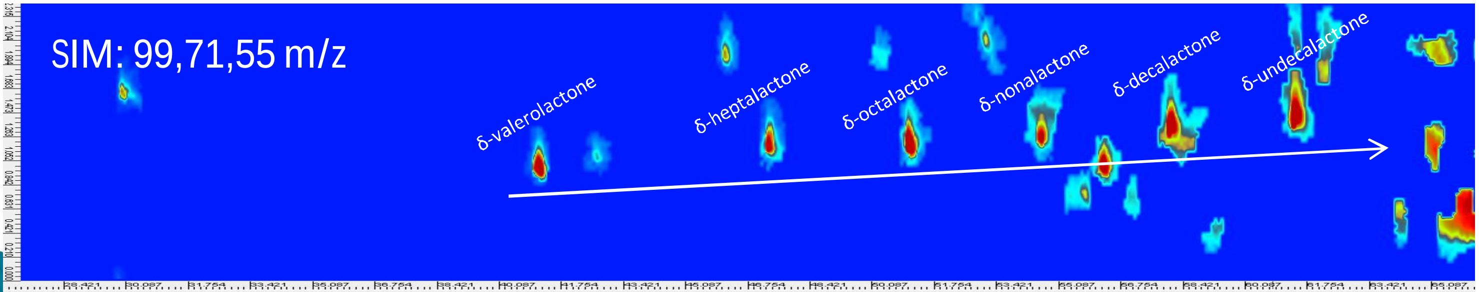
Fingerprinting on most relevant chemical classes
Lactones



TIC
HSSE-PDMS



TIC zoom
HSSE-PDMS

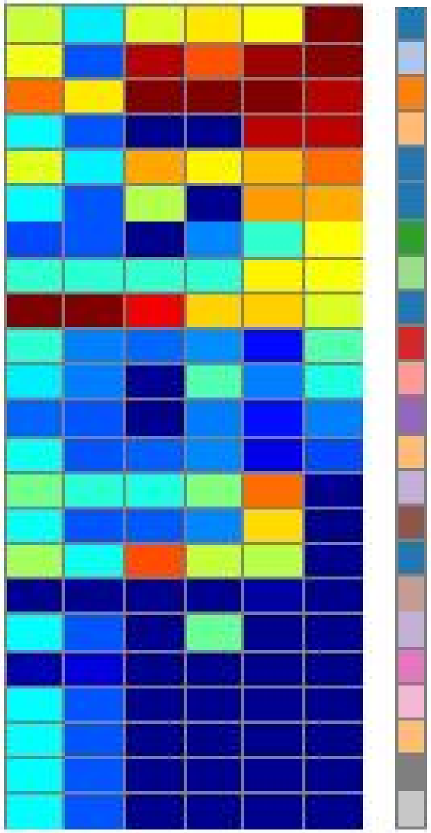


SIM
HSSE-PDMS

Q. Which are analytes that represent the Chemical Odor Code of milk?

^{4}D Odor **^{1}D-^{2}D-^{3}D Chemical Code** **ORs Code**

Odor descriptor
- 🟪 cabbage
- 🟪 cheesy
- 🟩 fatty
- 🟥 green
- 🟪 hay-cucumber
- 🟩 rancid
- 🟪 rubber
- 🟧 sour-vinegar
- 🟫 stale, hay
- 🟫 sweaty
- 🟧 sweet
- ⬛ sweet-fatty
- ⬜ sweet-green
- 🟦 vitamin-rubber
- 🟦 waxy
- 🟥 waxy-green



decanoic acid
2-furanmethanol
acetic acid
maltol
octanoic acid
decenoic acid
(E,E)-2,4-decadienal
butanoic acid
dodecanoic acid
hexanal
(E)-2-undecenal
(E)-2-nonenal
3-methylbutanal
hexanoic acid
3-methylbutanoic acid
nonanoic acid
β-ionone
pentanoic acid
benzothiazole
dimethyl trisulfide
δ-undecalactone
δ-decalactone
γ-dodecalactone

CeCR472
ORL1138
ORL1500
ORL1520
ORL1549
ORL1580
ORL1582
ORL1629
ORL2156
ORL2162
ORL3370
ORL461
ORL464
ORL465
ORL467
ORL468
ORL470
ORL471
ORL472
ORL52
ORL533
ORL876

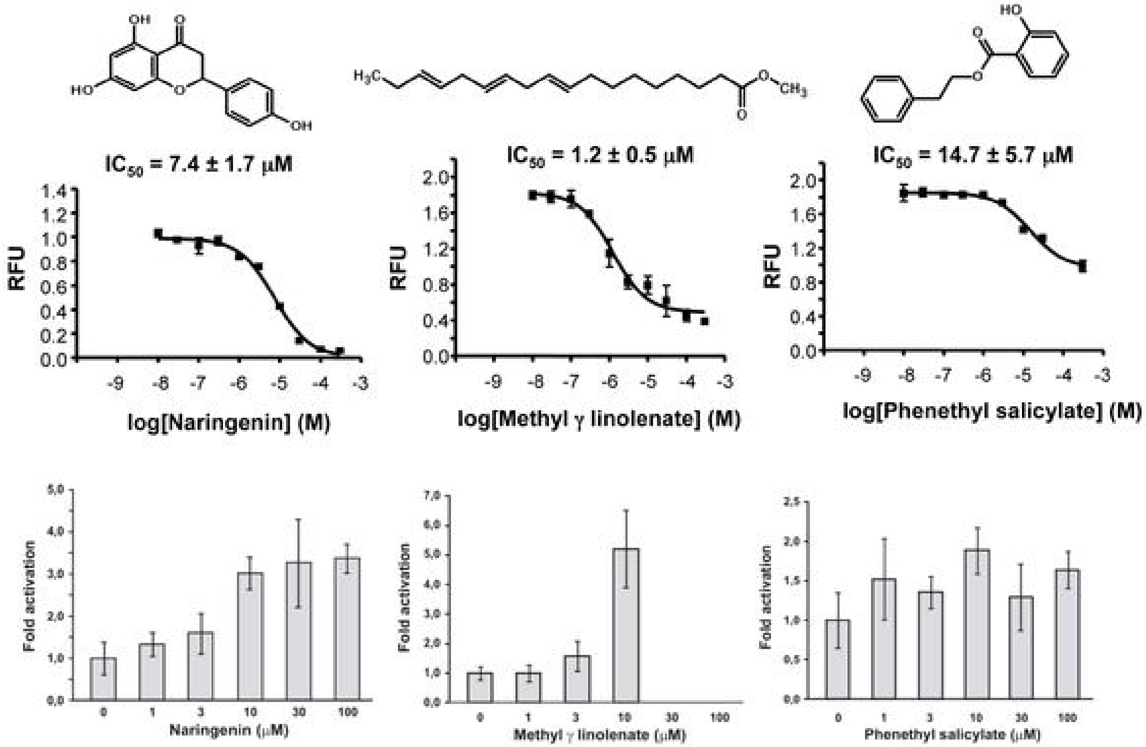
Experimental data **Prediction**

Concluding Remarks

- **Advanced *Sensomics*** and its peculiar investigation strategies are **fundamental to identify triggering factors** (ligands-odorants) **of odor perception**
- Our contribution relay on the **ability to consider all possible informative dimensions** and **giving** them the **appropriate priority** during investigation
- Advanced analytical **complex** platforms could effectively contribute to unlock closed doors giving **new perspectives in biological phenomena interpretation**

Odorants play also a role in human metabolism regulation
Behind pleasure we can find a further key for well-being

Results of bio-activation of three flavor active compounds on the PPARγ receptor.



Audouze K, Tromelin A, Le Bon AM, Belloir C, et al. (2014) Identification of Odorant-Receptor Interactions by Global Mapping of the Human Odorome. PLoS ONE 9(4): e93037. doi:10.1371/journal.pone.0093037
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0093037

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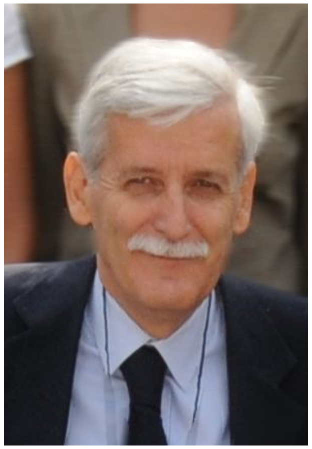
Grazie per l’attenzione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