FOOD QUALITY CONTROL USING PEPTIDE BASED GAS SENSOR ARRAYS

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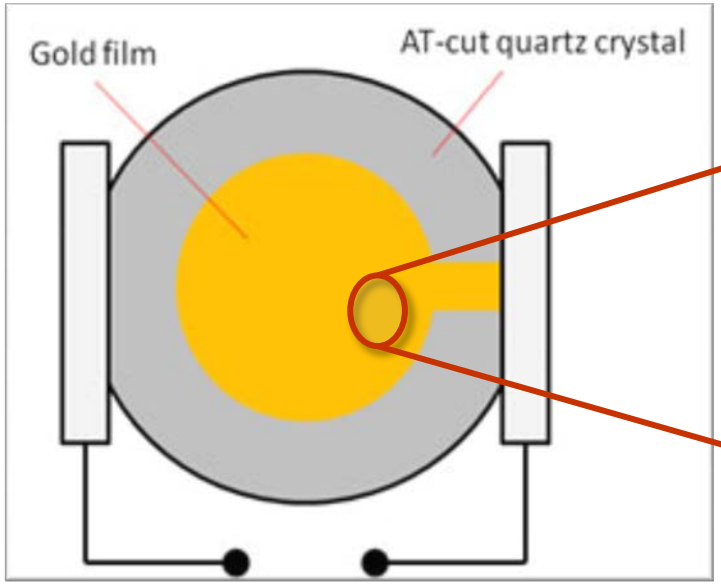
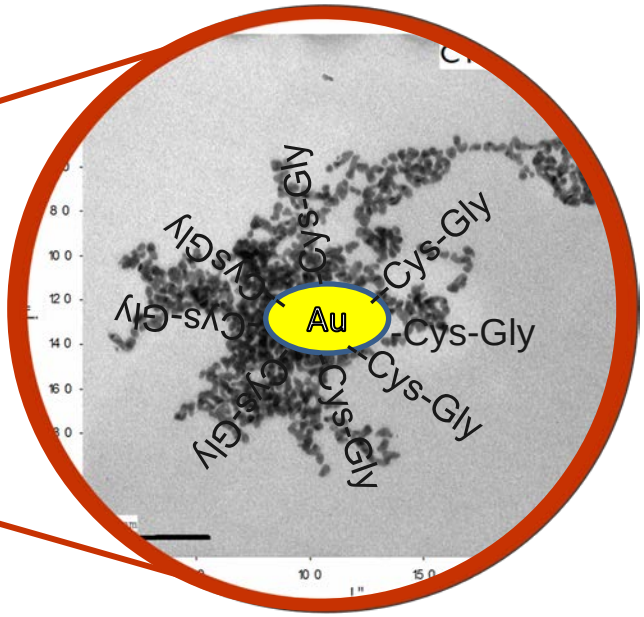
UNIVERSITÀ DEGLI STUDI DI TERAMO

3° International ISEKI Food Conference, Athens, May 2014, 21-23

Quartz crystal micro-balance





14 mm AT quartz

7 mm gold

Resonant frequency 20 MHz

Why peptides?

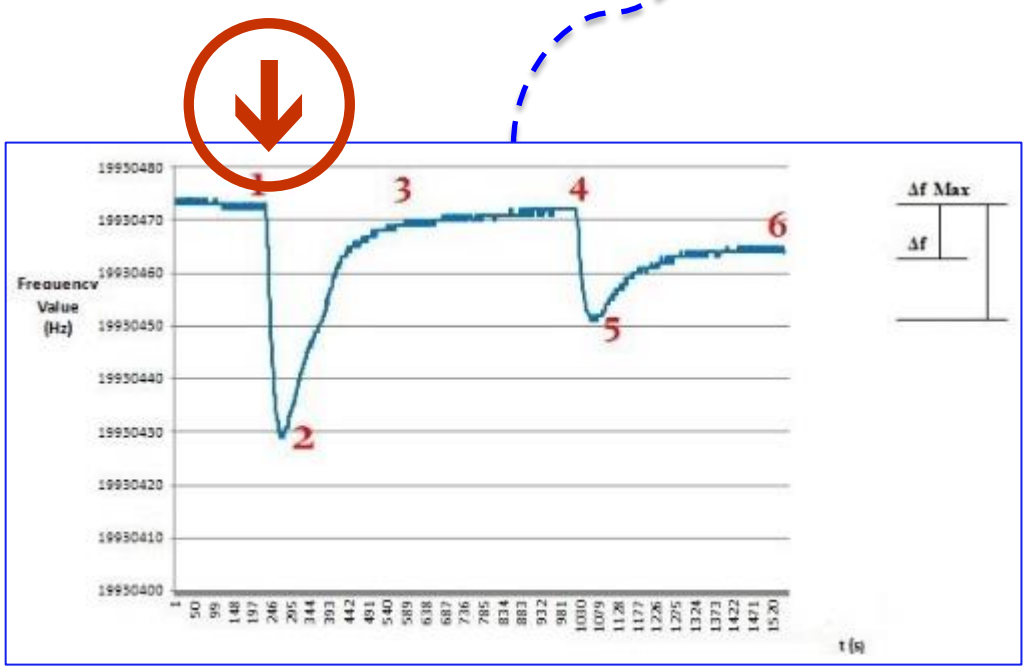
- Easy synthesis
- Easy and fast support derivatization
- Large number of combination
- Possibility of biomimetic approach and virtual design

- GNP to increase sensor surface

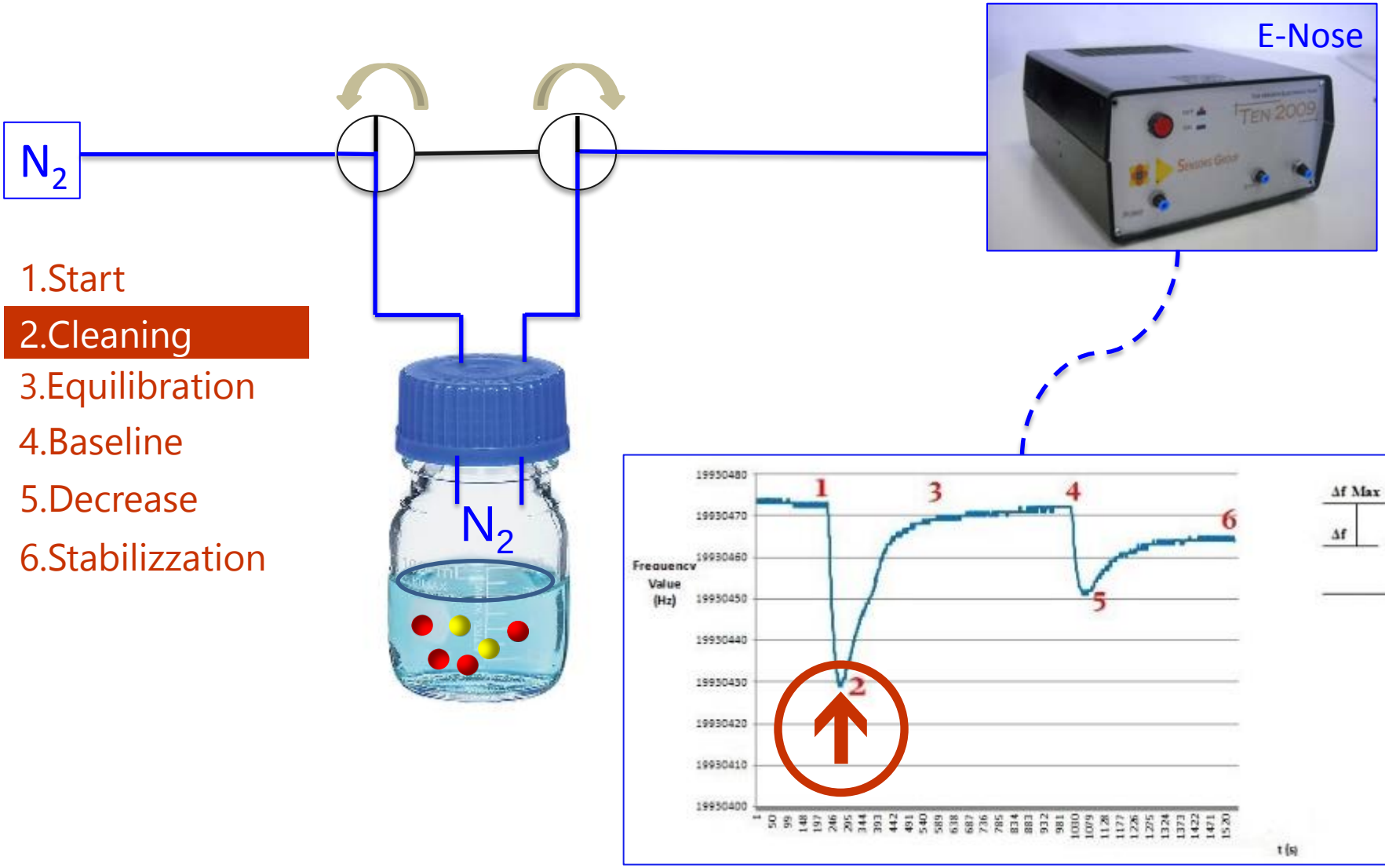
Measurement system

N_2

1.Start
2.Cleaning
3.Equilibration
4.Baseline
5.Decrease
6.Stabilizzation



Measurement system



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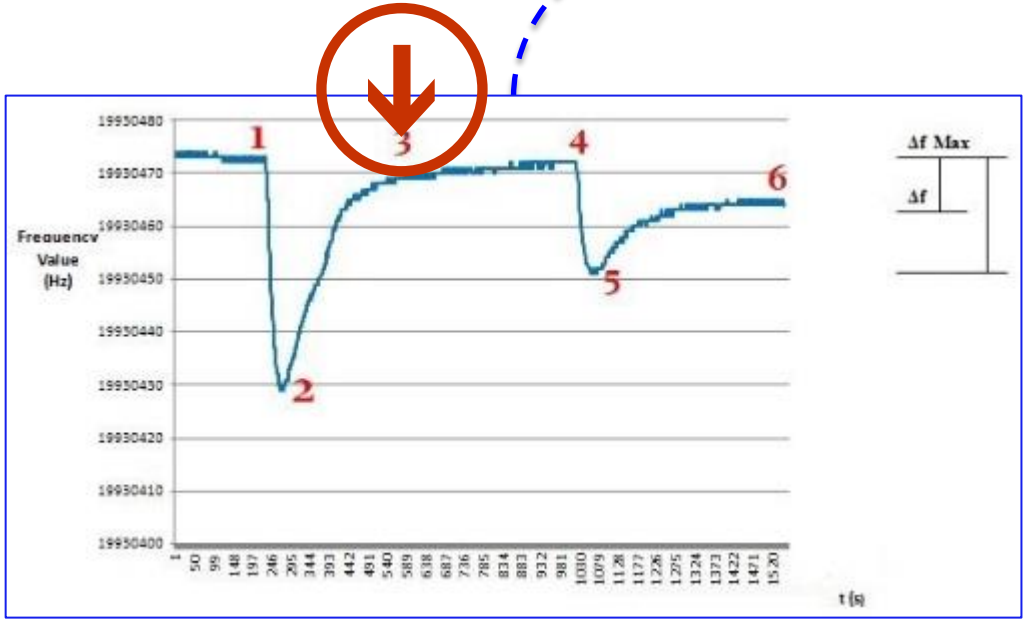

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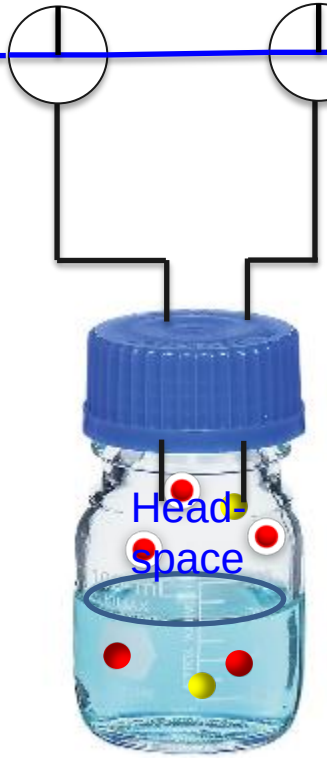

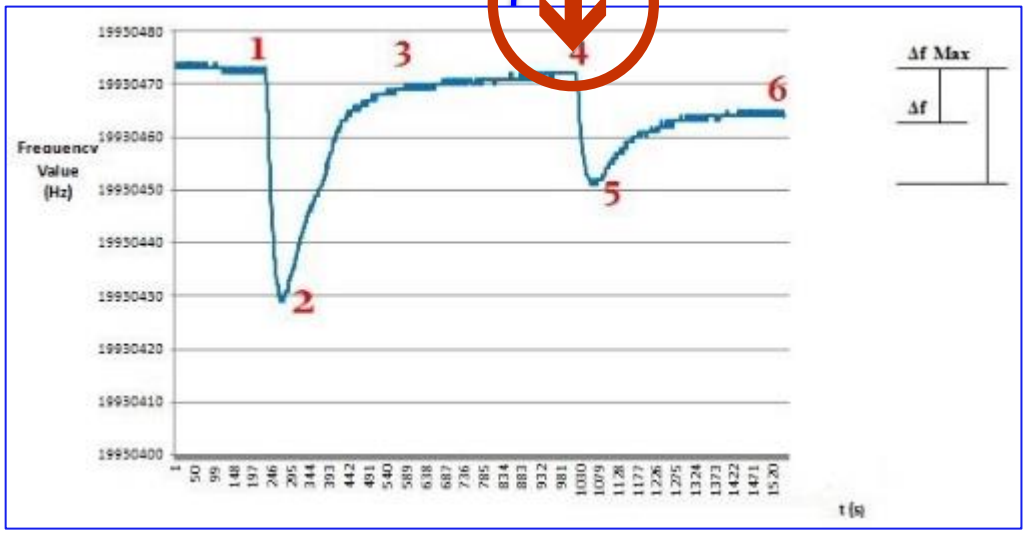

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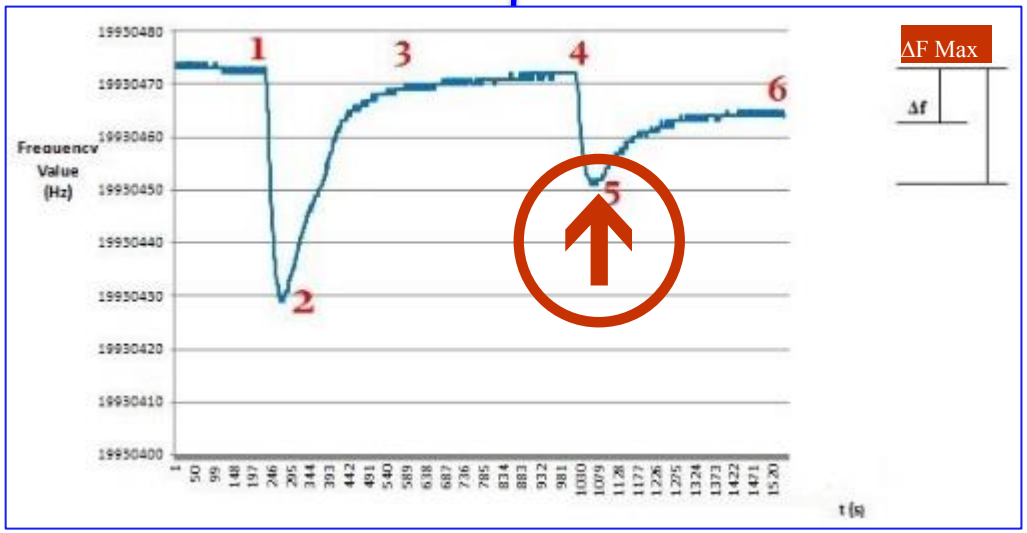

Measurement system

N_2

1.Start
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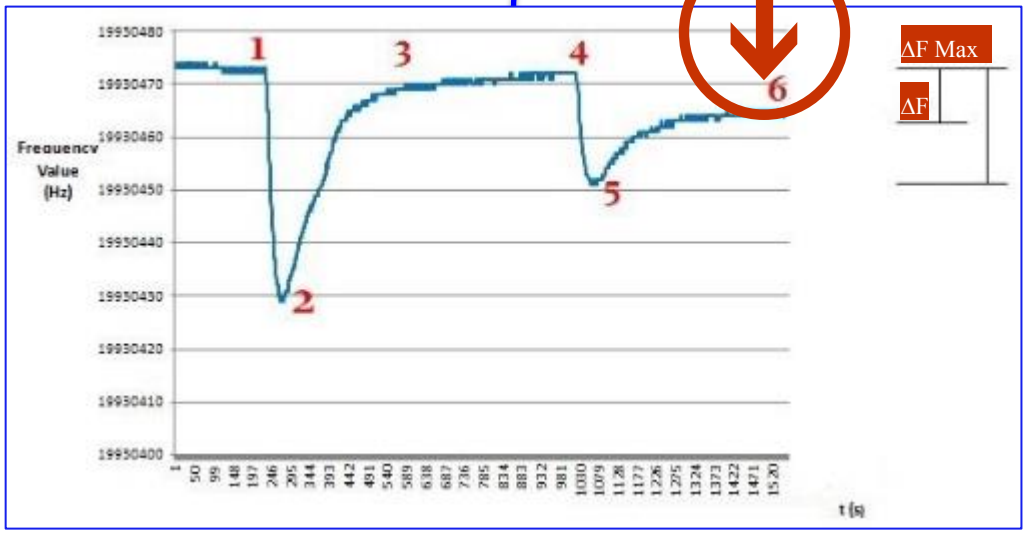
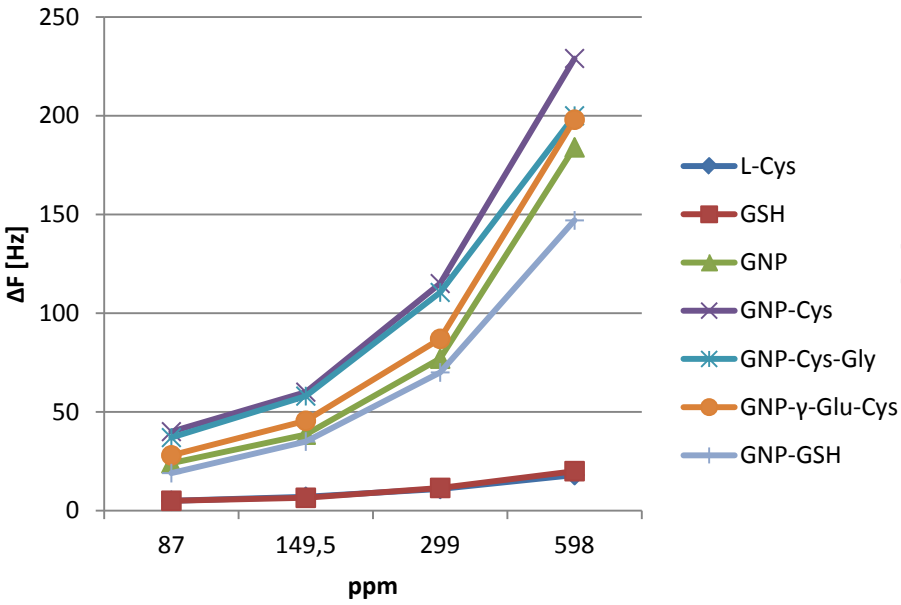
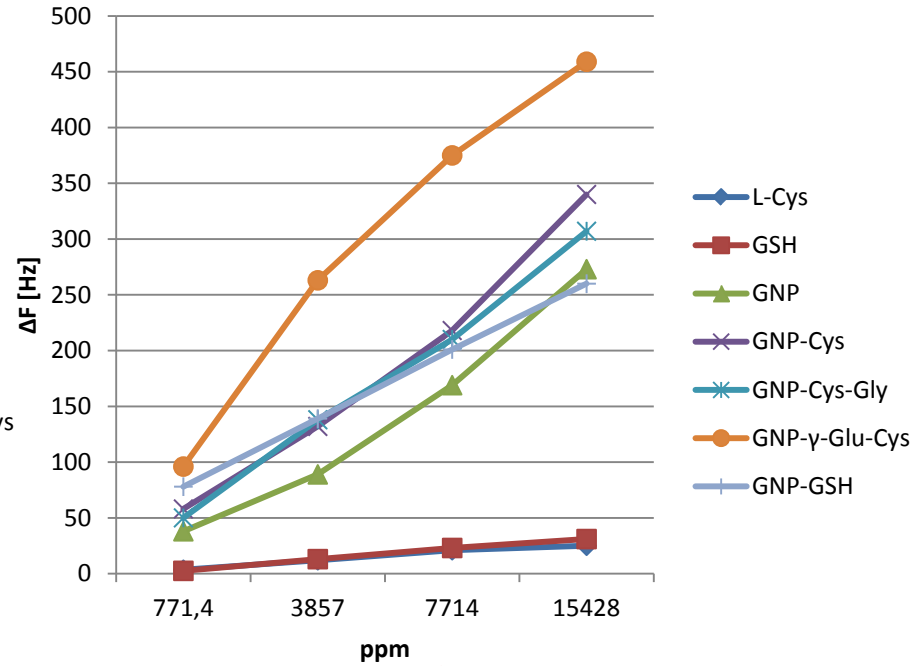
3.Equilibration
4.Baseline
5.Decrease
6.Stabilization



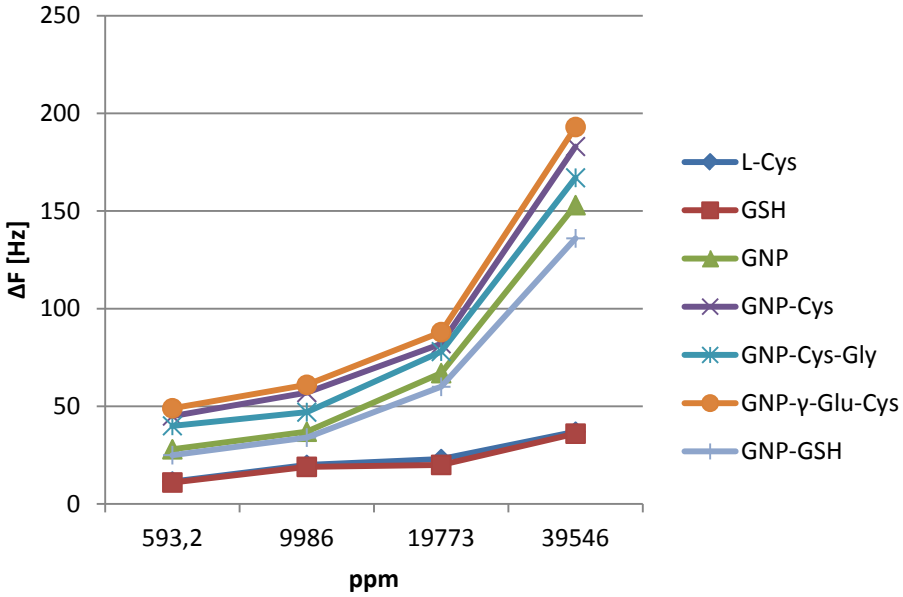
Trimethylammine



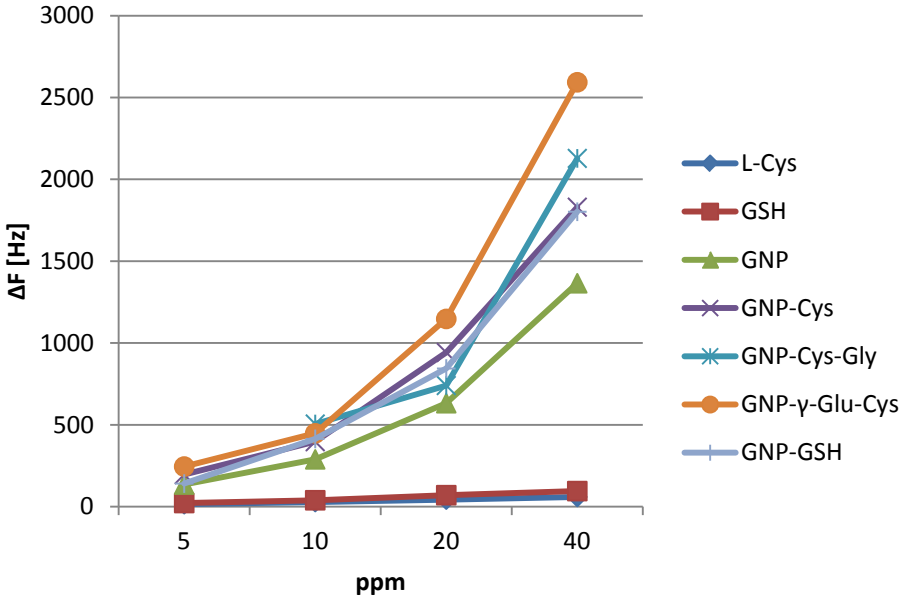
Ethanol



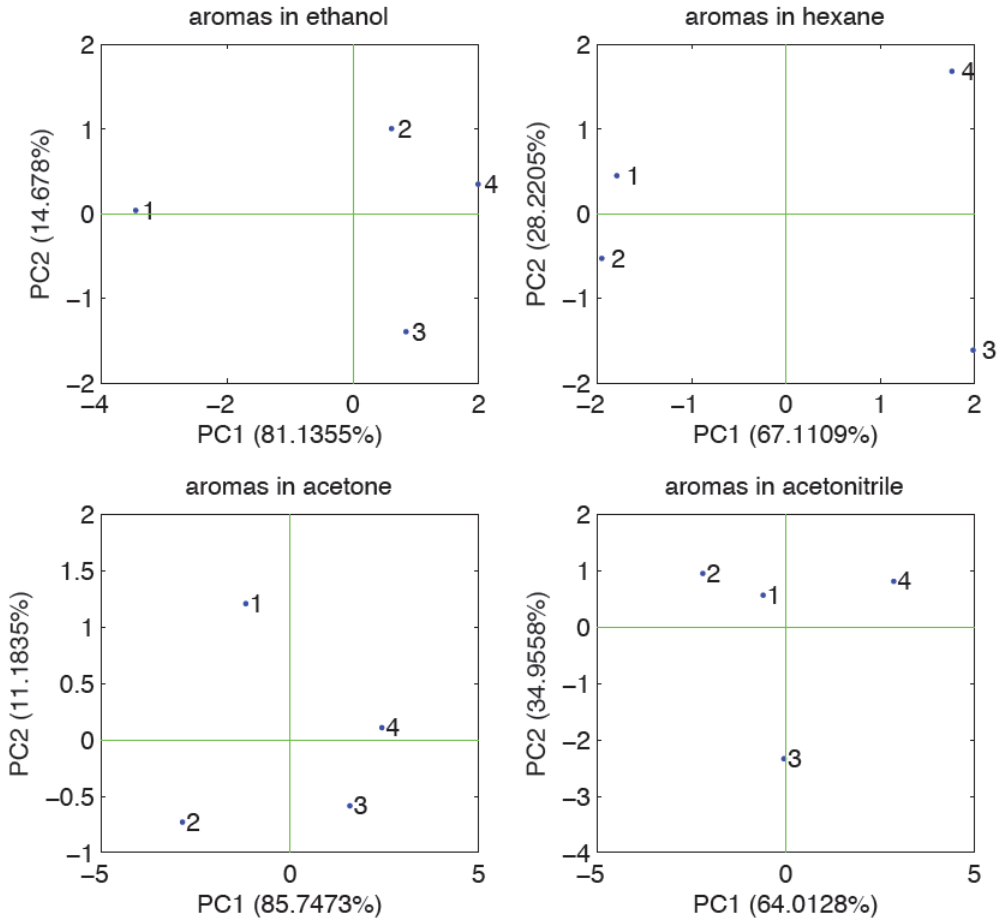
Hexan

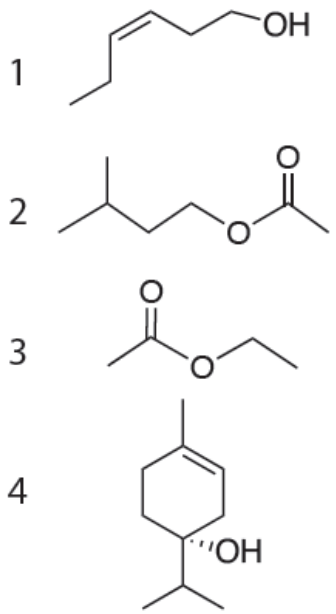


%RH



Model solutions
(o.1 % aromas in solvent)

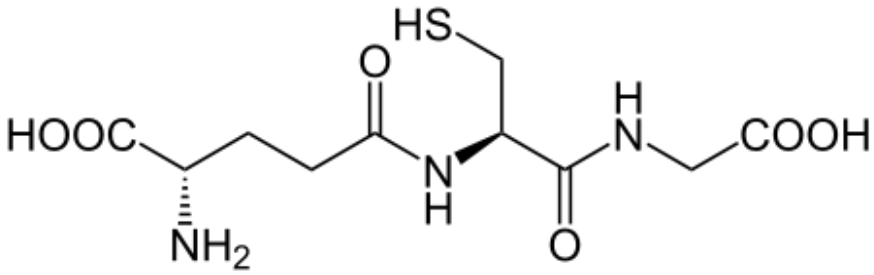




D. Compagnone, G.C. Fusella, M. Del Carlo et al *Biosensors and Bioelectronics* 42, 618–625, 2013

Modified GNPs

- GNP-Glutathione
- GNP-Cys-Gly [CG]
- GNP-Cys
- GNP-Thioglicolic Acid
- GNP-Cys-Arg-Gln-Val-Phe [CRQVF]
- GNP-Cys-Ile-His-Asn-Pro [CIHNP]
- GNP-Cys-Ile-Gln-Pro-Val [CIQPV]
- GNP



	CRQVF	CIHNP	CIQPV
Molecular Weight	651.79	582.68	558.7
Iso-electric point	9.01	7.15	5.33
Net charge at pH 7	1	0	0
Estimated solubility	Good	Poor	Poor

Real samples

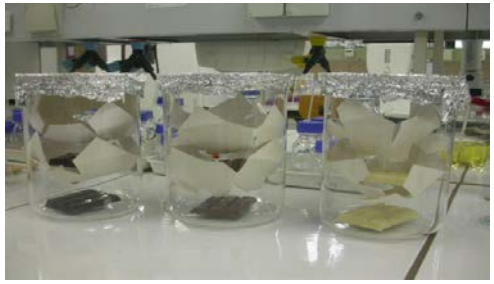
Chocolate

- Temperature: 40°C
- Equilibration time: 10 min
- 15g in 100 mL lab bottle grated and melted
- 4 L/h

Standard Samples
VS
Off-flavoured samples

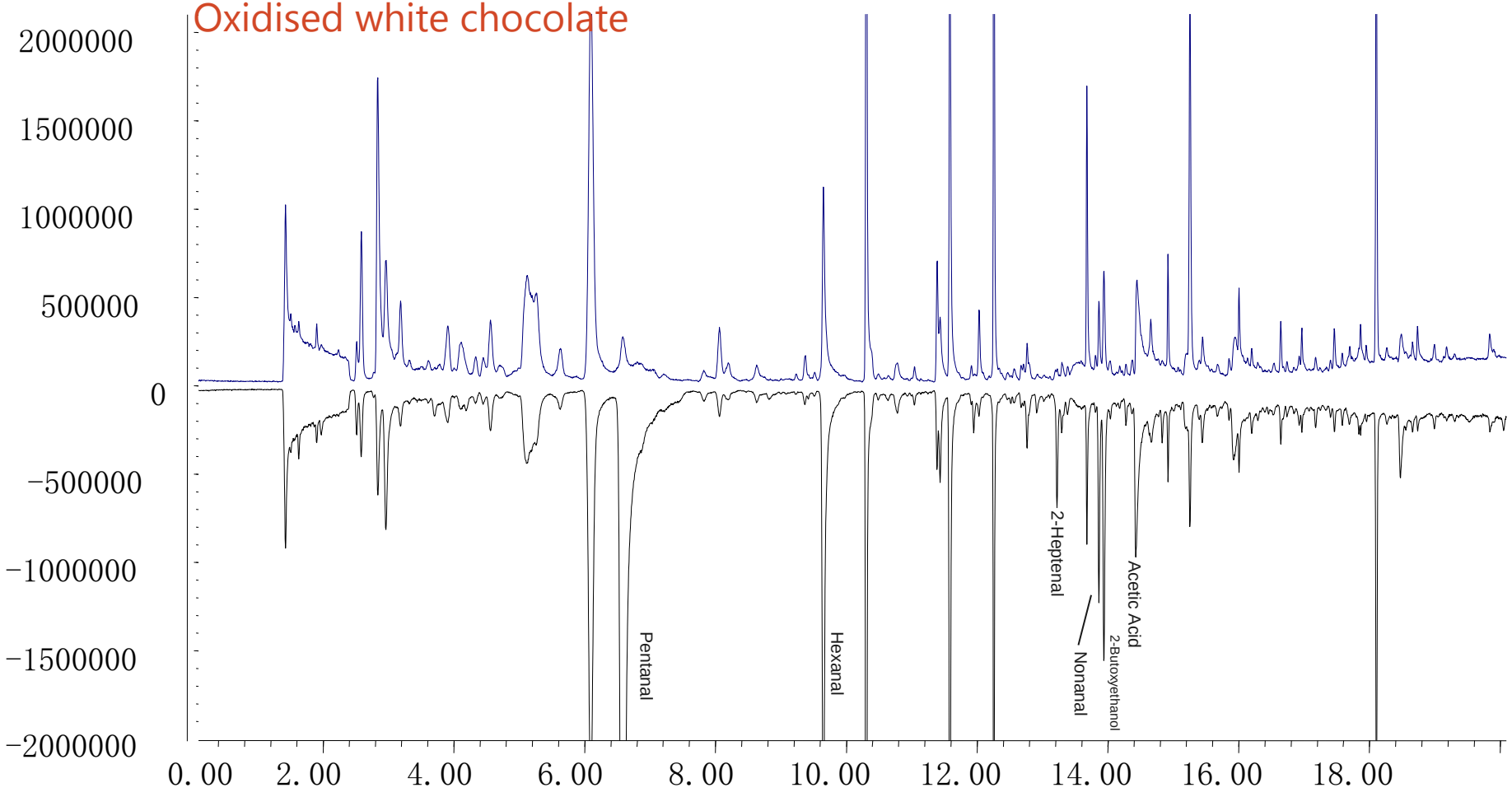
PLS-DA analysis

Off-Flavour	Process
3 methylbutanal	Fermentation volatiles
Phenylacetaldehyde	
Acetic Acid	Conching process
Tetramethylpyrazine	Roasting Process
2-acetylpyrrole	
2-nonenal	Fat related (oxidation)
2,4-decadienal (t,t)	



Off-flavours were preliminarily added in the cocoa butter to achieve the concentration of 125 ppm. One tea spoon of contaminated cocoa butter was then added to 400 g of chocolate to obtain an estimated final concentration in the sample of ~ 6ppm.

Oxidised white chocolate



Electronic nose sensor arrays

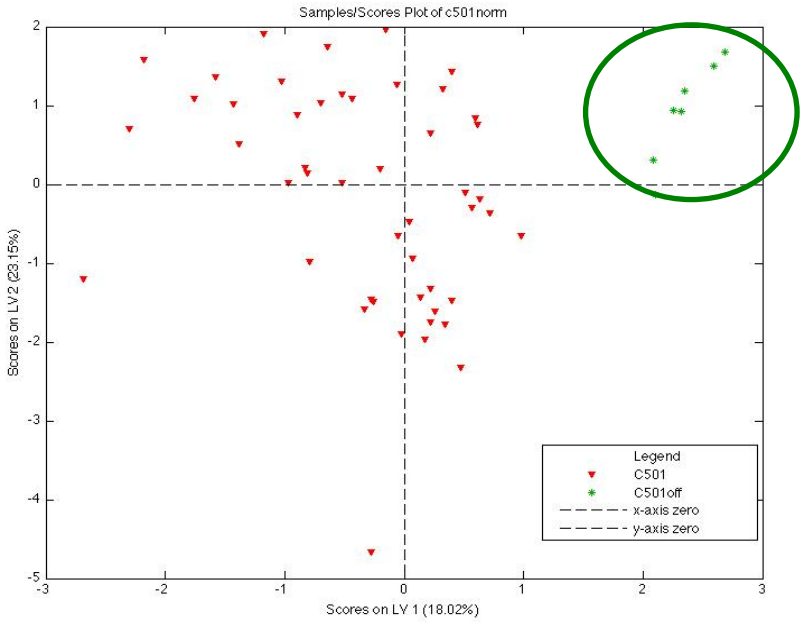
GNP-Peptide based

- GNP-Glutathione
- GNP-Cys-Gly
- GNP-Cys
- GNP-Thioglicolic Acid
- GNP-Cys-Arg-Gln-Val-Phe
- GNP-Cys-Ile-His-Asn-Pro
- GNP-Cys-Ile-Gln-Pro-Val
- GNP

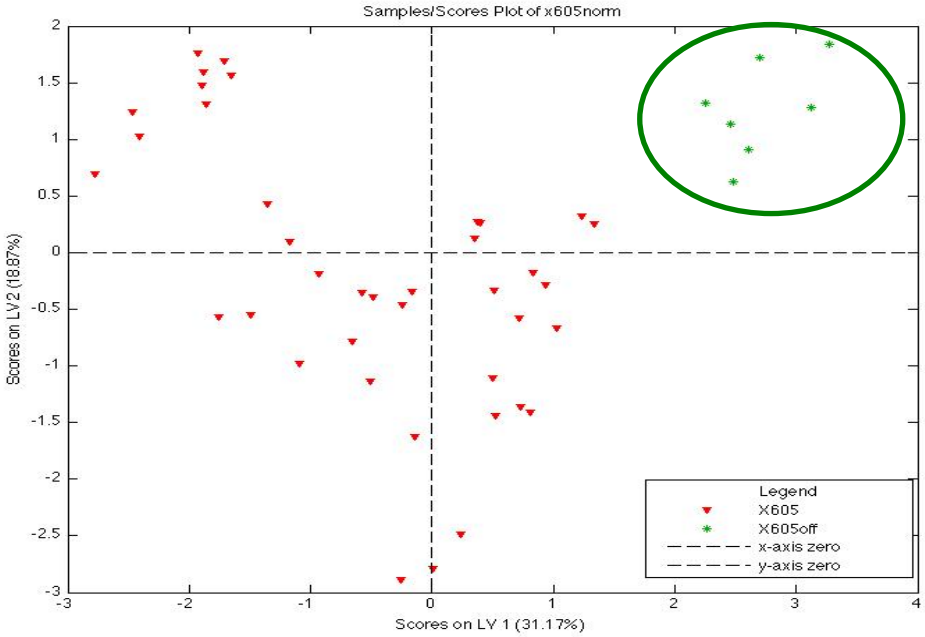
Porphyrin based

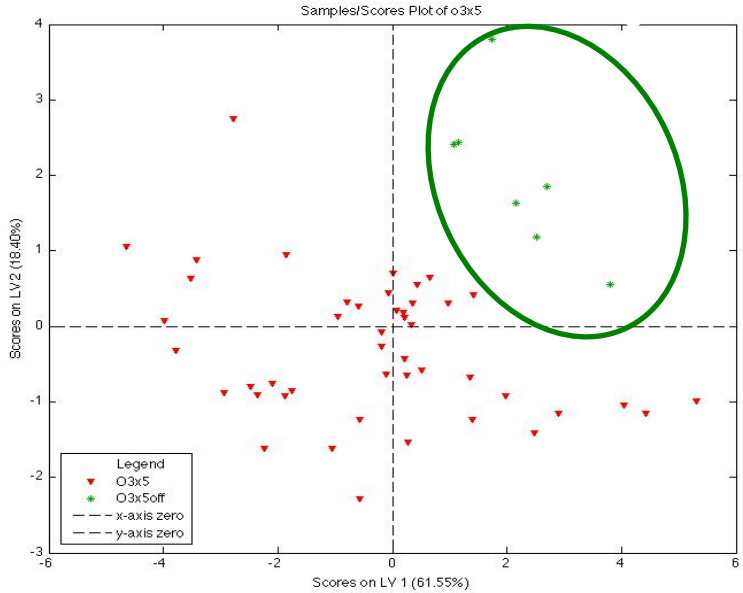
- Cu-Buti-TPP
- Co-Buti-TPP
- Zn-Buti-TPP
- Mn-Buti-TPP
- Fe-Buti-TPP
- Sn-Buti-TPP
- H_2-Buti-TPP
- Mg-Buti-TPP

Dark Chocolate



White Chocolate





Milk Chocolate

GNP-Peptide vs. Porphyrin

GNP-Peptide based

	Regular	Off Flavours	% Correct
Regular	48	0	100
Off flavours	0	7	100

Tot. Correct: 100%

	Regular	Off Flavours	% Correct
Regular	39	0	100
Off flavours	0	7	100

Tot. Correct: 100%

	Regular	Off Flavours	% Correct
Regular	51	1	98
Off flavours	0	7	100

Tot. Correct: 98%

Porphyrin based

	Regular	Off Flavours	% Correct
Regular	14	1	93
Off flavours	1	9	90

Tot. Correct: 92%

	Regular	Off Flavours	% Correct
Regular	13	1	92
Off flavours	4	8	67

Tot. Correct: 81%

	Regular	Off Flavours	% Correct
Regular	15	1	94
Off flavours	4	8	67

Tot. Correct: 82%

D. Compagnone, M. Faieta, D. Pizzoni, C. Di Natale, T. Van Caelemberg, B. Behyedtc, P. Pittia, Sensors and Actuator B, submitted

Candies

- **3 structuring agents**
  - Gelatine [Gel]
  - Pectin [Pec]
  - Gum Arabic [G.Ar.]
- **2 aromas**
  - Natural [A]
  - Synthetic[B]
- **2 concentrations**
  - 0.15% [1]
  - 0.30% [2]

GC-MS

Pure Aromas

- **29 major compounds** (25 identified with MS-spectra database)
- **4 compounds only in Natural aroma**
- **4 compounds only in Synthethic aroma**
- **Strong differences in concentration for most compounds**

Volatile compunds	GC Peak area (UA)	
	Natural aroma	Natural-identic aroma
Ethanol	13.354.000	324.689.000
α-methyl-butanal	32.196.000	16.823.000
Ethyl-acetate	188.210.000	2.467.000
Not identified	36.083.000	9.974.000
Not identified	9.171.000	1.790.000
Etihyl-propanoate	nd	2.534.000
Not identified	4.185.000	1.457.000
1,2-propandiol	5.231.000	15.770.000
Ethyl-isobutirrate	3.897.000	5.877.000
Not identified	1.702.000	nd
Ethyl-butirrate	3.839.248.000	5.257.073.000
Ethyl-α-methyl-butirrate	265.613.000	2.299.000
Ethyl-β-methyl-butirrate	178.392.000	1.062.000
Cis-3-hexenol	6.422.000	24.803.000
α-Pinene	9.462.000	4.191.000
β-Pinene	10.198.000	6.219.000
β-Myrcene	nd	2.724.000
Ethyl-hexanoate	217.670.000	nd
Octanal	nd	705.000
1,4-cineole	nd	457.000
O-Cymene	4.432.000	2.020.000
Limonene	167.835.000	108.208.000
Eucalyptol	399.000	nd
γ-Terpinene	6.603.000	2.795.000
α-terpinolene	2.207.000	1.117.000
Nonanal	6.971.000	2.158.000
cis-3-hexenil-isobutanoate	13.056.000	3.514.000
Ethyl-octanoate	23.971.000	nd
Decanal	6.682.000	4.545.000

GC-MS

Candies

Compounds	G.ar A1	G.Ar A2	Pec A1	Pec A2	Gel A2	Gel A2
Ethanol	30	94	15	27	16	30
ethyl-acetate	1	3	3	3	2	2
ethyl-butanoate	0,07	0,23	2	3	2	4
ethyl-α-methyl-butanoate	1	3	3	4	3	5
ethyl-β-methyl-butanoate	1	4	3	5	3	6
Cis-3-hexenol	87	506	224	343	151	476
Ethyl-hexanoate	Nd	Nd	7	9	6	12
Limonene	0,48	1	2	3	2	3
cis-3-hexenyl-iso-butanoate	Nd	Nd	13	18	14	22
Decanal	Nd	Nd	17	24	37	53

Natural aroma

$$\text{Values} = \frac{\text{Compound peak area in candy}}{\text{Compound peak area in pure aroma}}$$

10 compounds confirmed with analytical standard and monitored in candies as significant

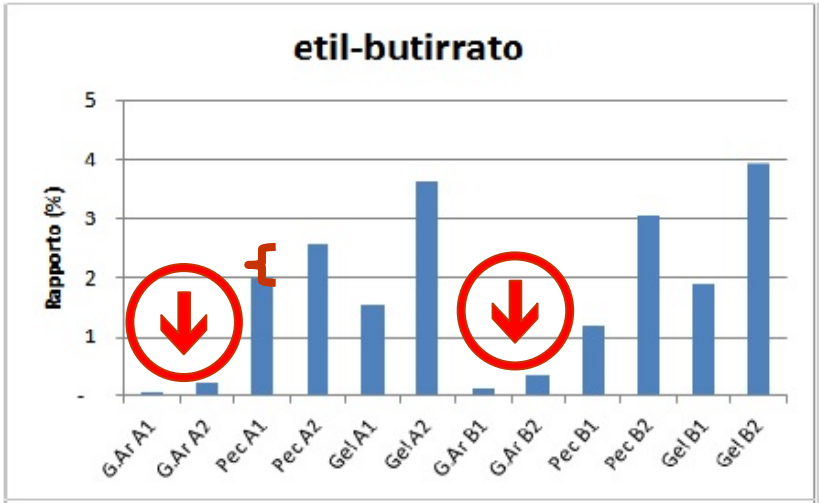
Synthetic aroma

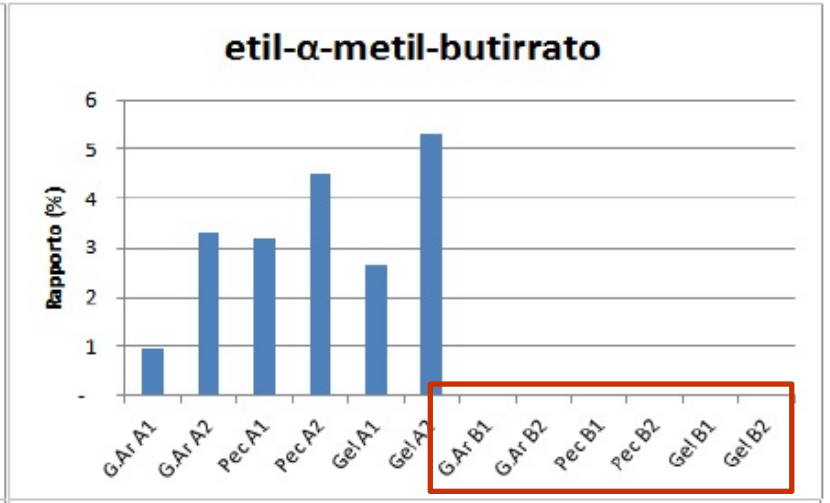
Compounds	G.Ar B1	G.Ar B2	Pec B1	Pec B2	Gel B1	Gel B2
Ethanol	5	13	3	14	5	11
ethyl-acetate	170	90	164	196	120	109
ethyl-butanoate	0,14	0,36	1	3	2	4
ethyl-α-methyl-butanoate	Nd	Nd	Nd	Nd	Nd	Nd
ethyl-β-methyl-butanoate	Nd	Nd	Nd	Nd	Nd	Nd
Cis-3-hexenol	68	297	66	256	92	243
Ethyl-hexanoate	Np	Np	Np	Np	Np	Np
Limonene	1	2	1	7	2	5
cis-3-hexenyl-iso-butanoate	Nd	Nd	40	227	96	221
Decanal	Nd	Nd	Nd	Nd	Nd	Nd

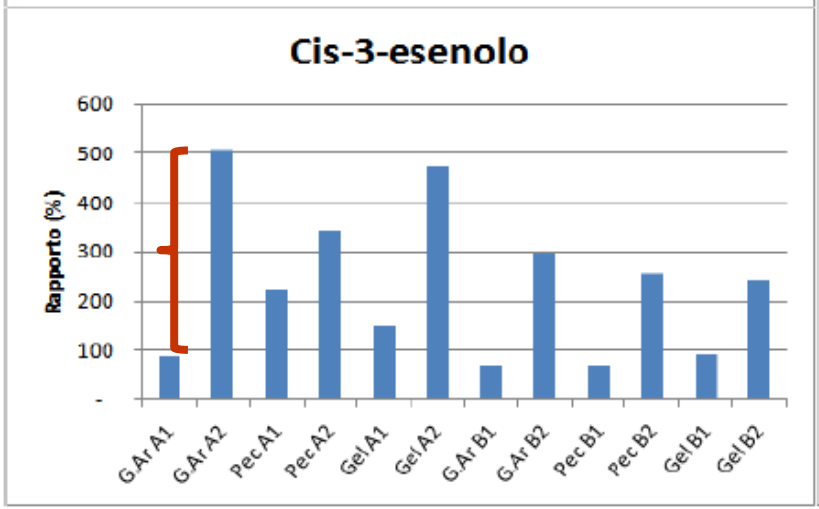
Nd=not detected; Np=not present in pure aroma

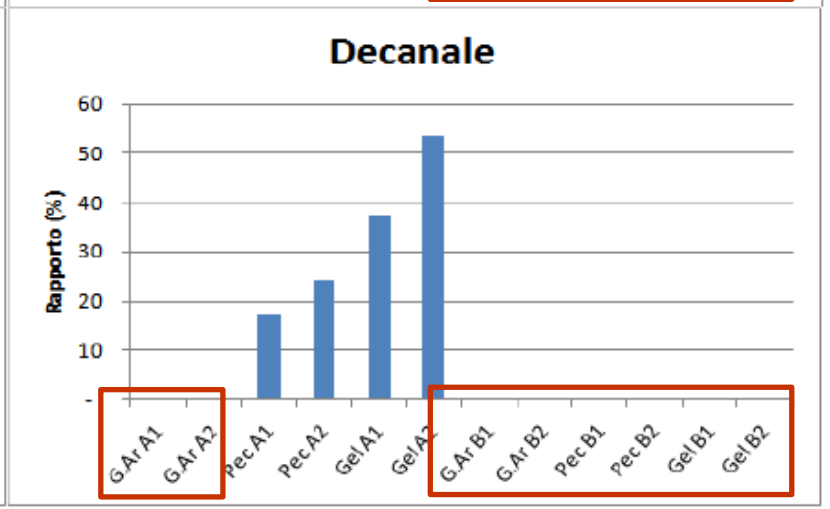
GC-MS

Candies



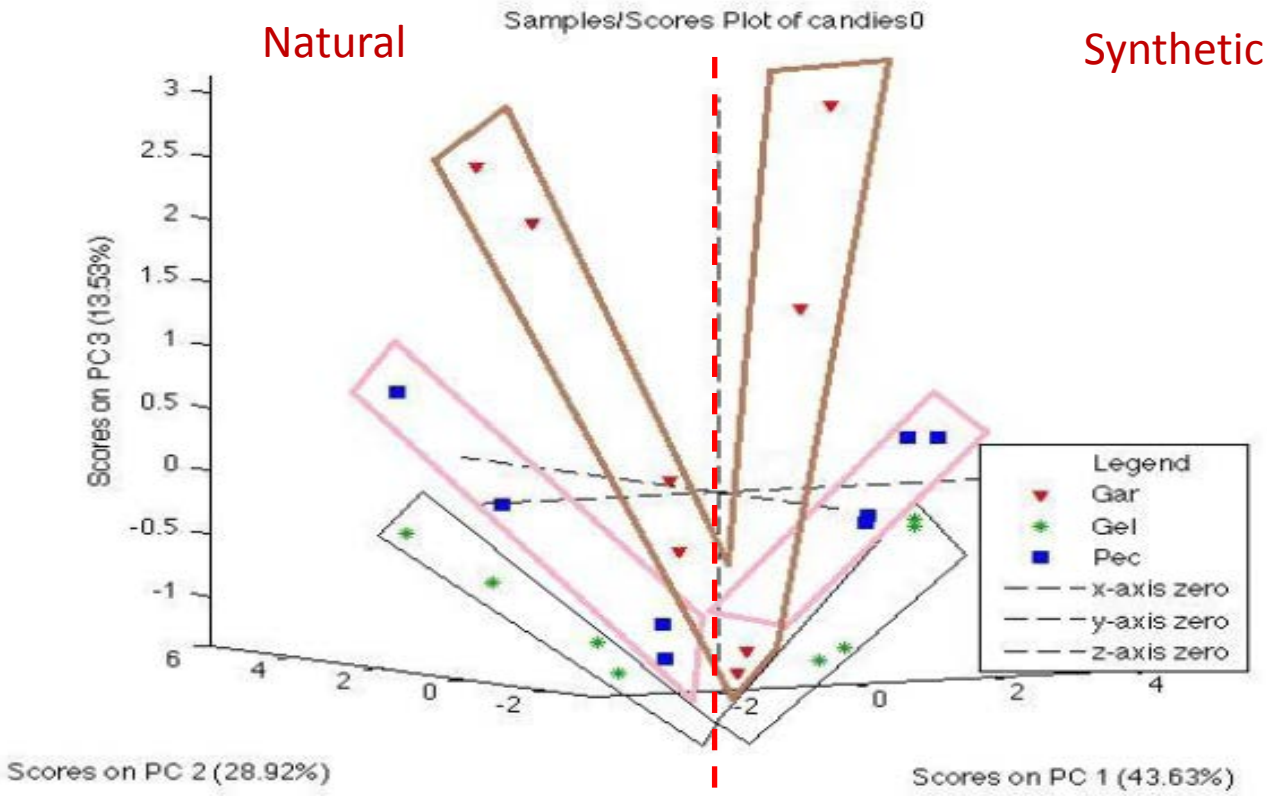






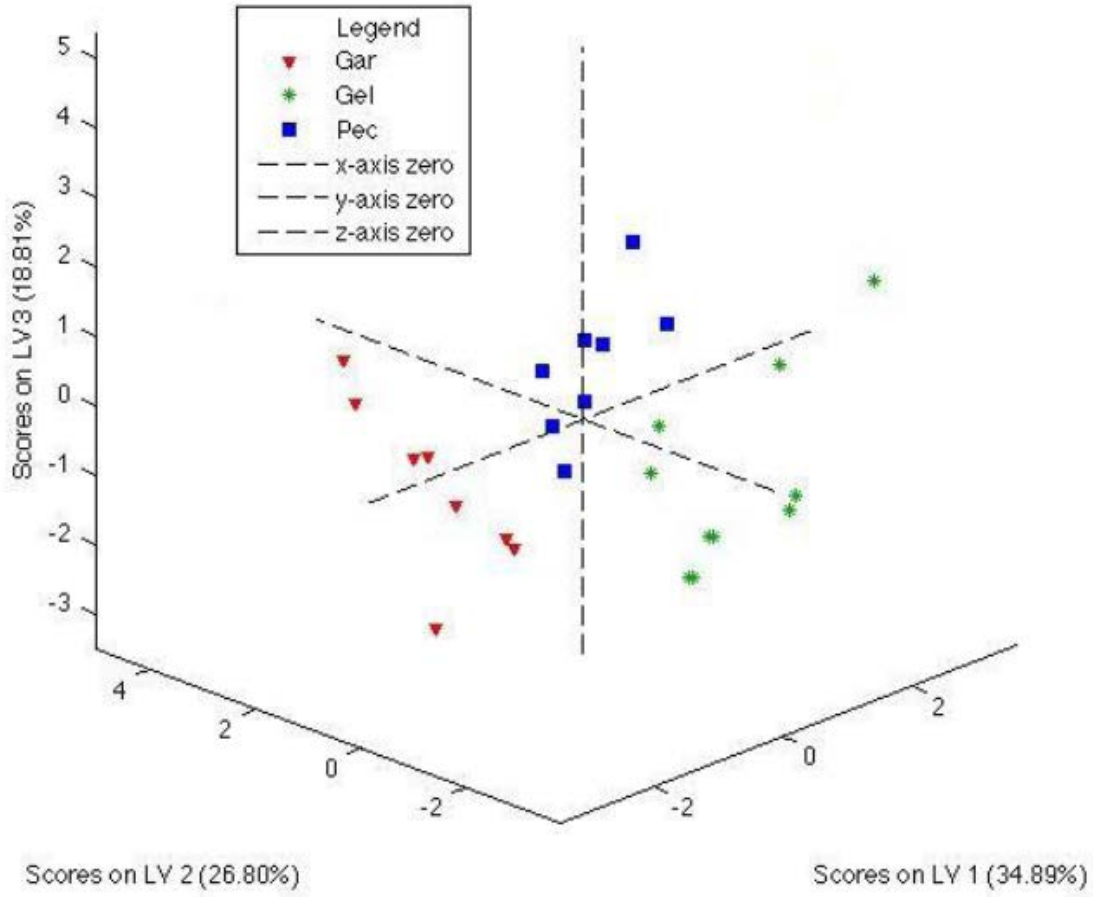
GC-MS

Candies



GC-MS

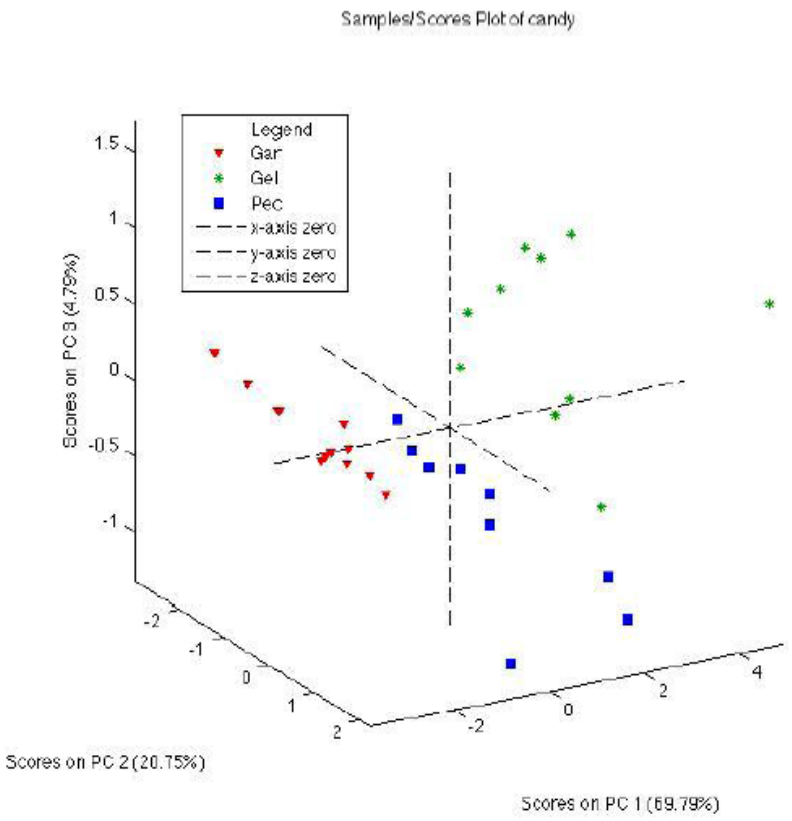
Candies structuring PLS-DA



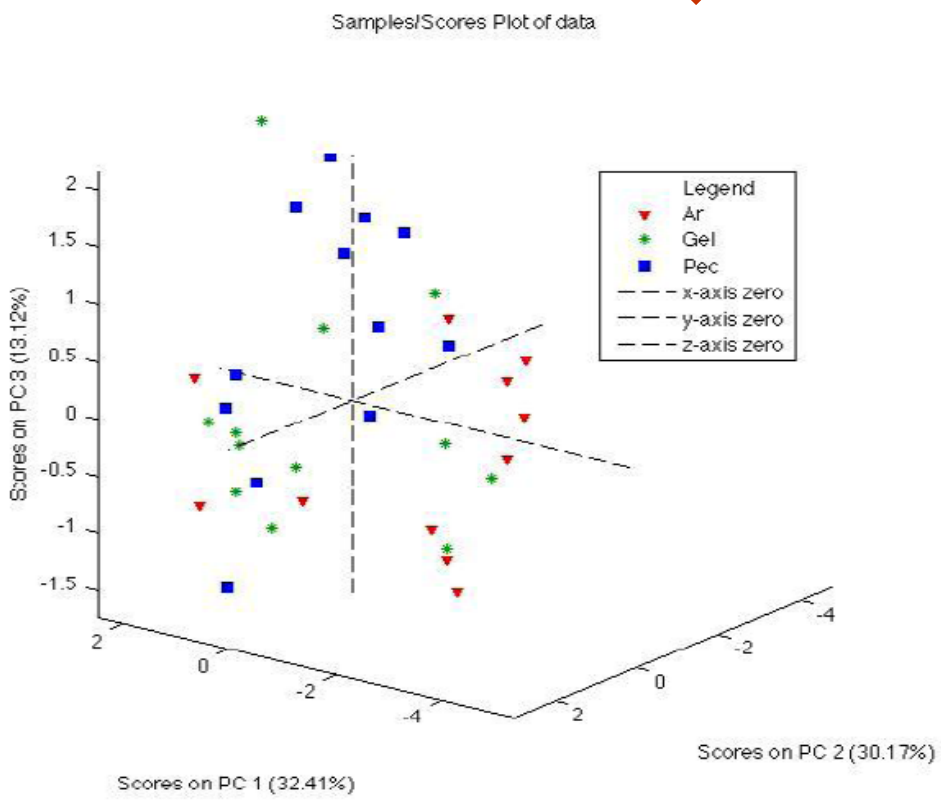
Electronic nose

Structuring

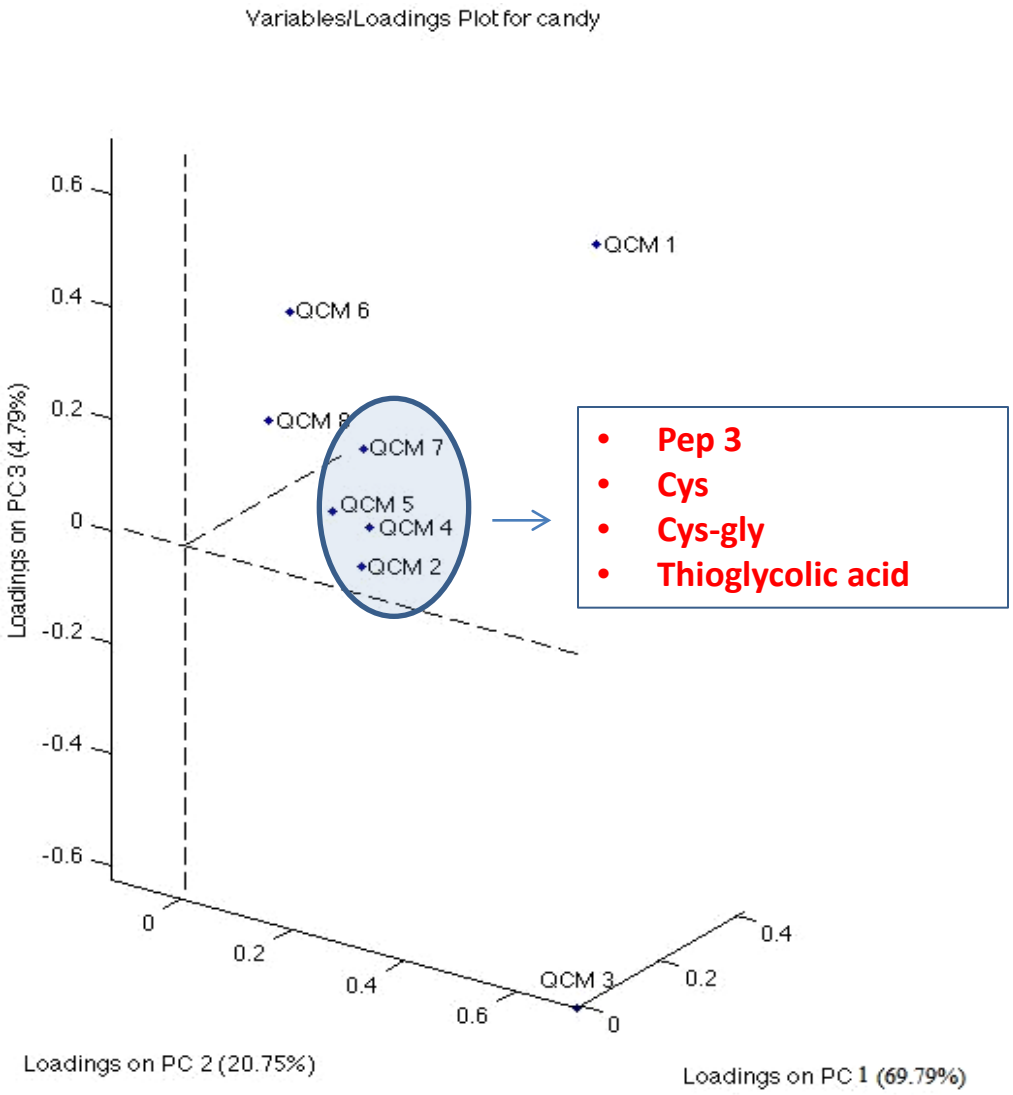
GNP-Peptide based



Porphyrin based



GNP-Peptide based (aroma)



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Ongoing work

- **Olive oil**
- **Gummy candies (synthethic vs. naturally extracted dyes)**
- ***Halal salami***
- **Coffee mixtures**
- **Cheese process**

Peptide design

Virtual screening

5 peptides studied
(CG, Glutathione, CIHNP, CIQPV, CRQVF)

VS

14 volatile compounds
(different chemical classes, shapes, dimensions, hydrophobicity)

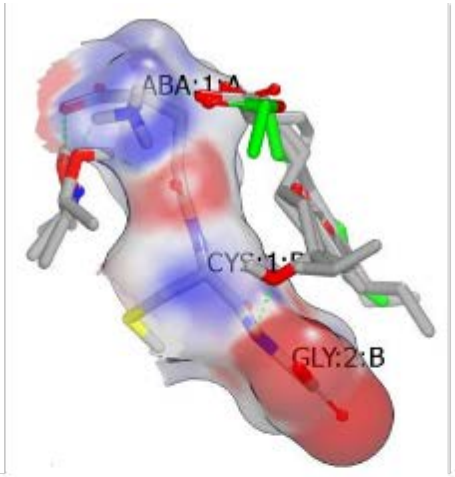
- **Docking box generation**
- **10 conformers for each peptide**
- **From 1 to 200 conformers for each volatile compound**
- **Binding score: average of all conformers**

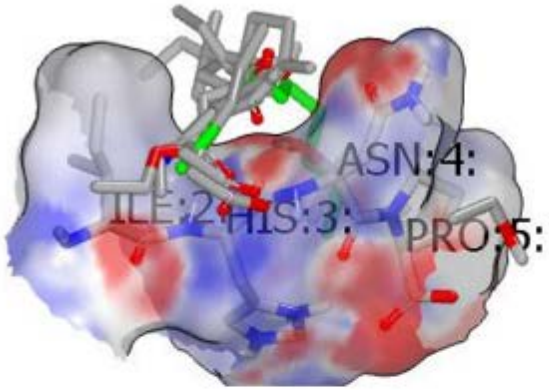
- **Free software**
- **Common laptop**
- **"Fast" elaboration** (~3min/peptide conformer)

Peptide design

Virtual screening

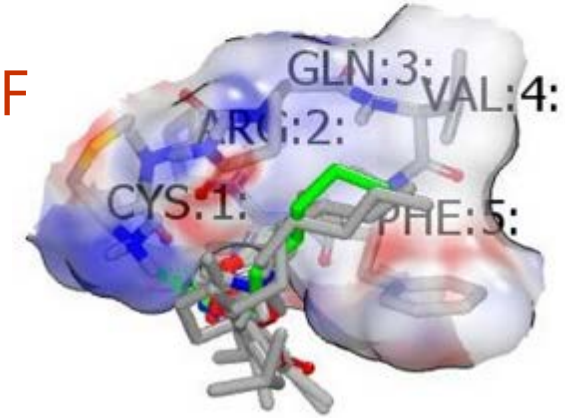
Glutathione

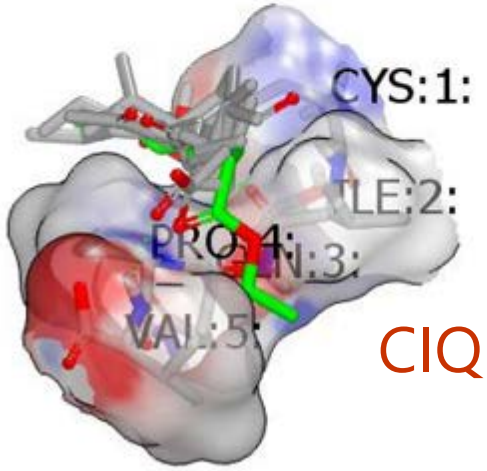




CIHNP

CRQVF





CIQPV

Peptide design

Virtual VS Experimental data

- **Binding scores compared with real samples ΔF data (T-test), after normalization**
- **Data having p-value>0.05 were considered statistically equivalent as positive match (marked with "+")**

	CG	Glutathione	ClHNP	ClQPV	CRQVF
2-Propanol	-	-	+	+	-
Acetone	-	+	-	+	+
Acetonitrile	-	+	+	+	+
Butane-2,3-dione	+	-	+	+	+
Ethanol	-	-	+	+	-
Ethyl acetate	+	+	+	+	+
Ethyl butanoate	+	+	+	+	+
Ethyl octanoate	-	+	-	-	+
Hex-3-en-1-ol	-	+	+	+	-
Hexane	+	+	+	+	+
Isopentyl acetate	-	+	+	+	-
Nonanal	-	+	+	+	+
Octanal	-	+	+	+	+
Terpinen-4-ol	-	-	+	+	+

78% good matching

Peptide design

Virtual VS Experimental data

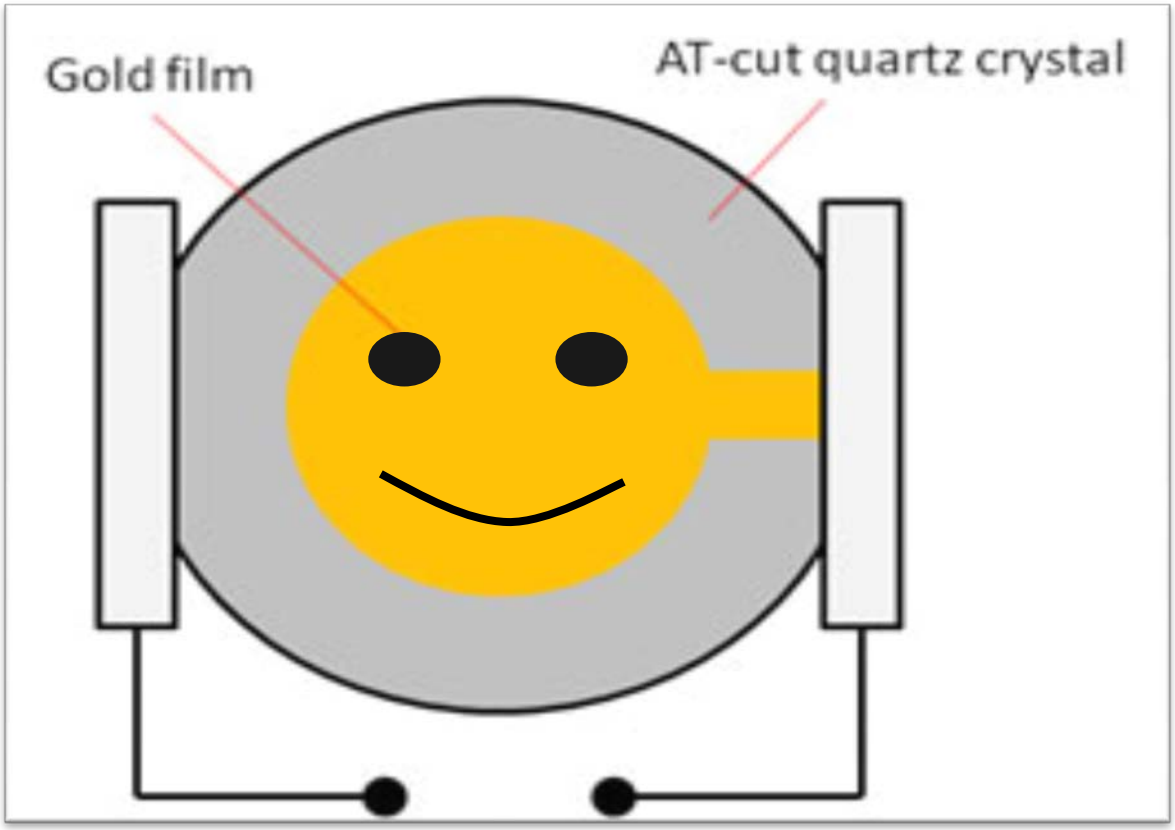
	CG	Glutathione	ClHNP	ClQPV	CRQVF
2-Propanol	-	-	+	+	-
Acetone	-	+	-	+	+
Acetonitrile	-	+	+	+	+
Butane-2,3-dione	+	-	+	+	+
Ethanol	-	-	+	+	-
Ethyl acetate	+	+	+	+	+
Ethyl butanoate	+	+	+	+	+
Ethyl octanoate	-	+	-	-	+
Hex-3-en-1-ol	-	+	+	+	-
Hexane	+	+	+	+	+
Isopentyl acetate	-	+	+	+	-
Nonanal	-	+	+	+	+
Octanal	-	+	+	+	+
Terpinen-4-ol	-	-	+	+	+

- CG only 29% positive match
- Glut.: 71%. CRQVF: 71% ClHNP: 86% ClQPV: 93%
- Very bad matching with ethanol and 2-propanol
- Very good matching with Esters and Aldehydes
- Best matching with compounds with MW>60g/mol

D. Pizzoni, M. Mascini,V. Lanzone, M. Del Carlo, C. Di Natale, Biosensors and Bioelectronics, 52, 247-254, 2014

Conclusions

- Development of GNP-peptide-based piezoelectric sensors
- Application to real samples for discrimination and sensing approach
- Development of a computational method with good correspondence between virtual and real data

- Creation of new sensors with peptides designed on specific purposes

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