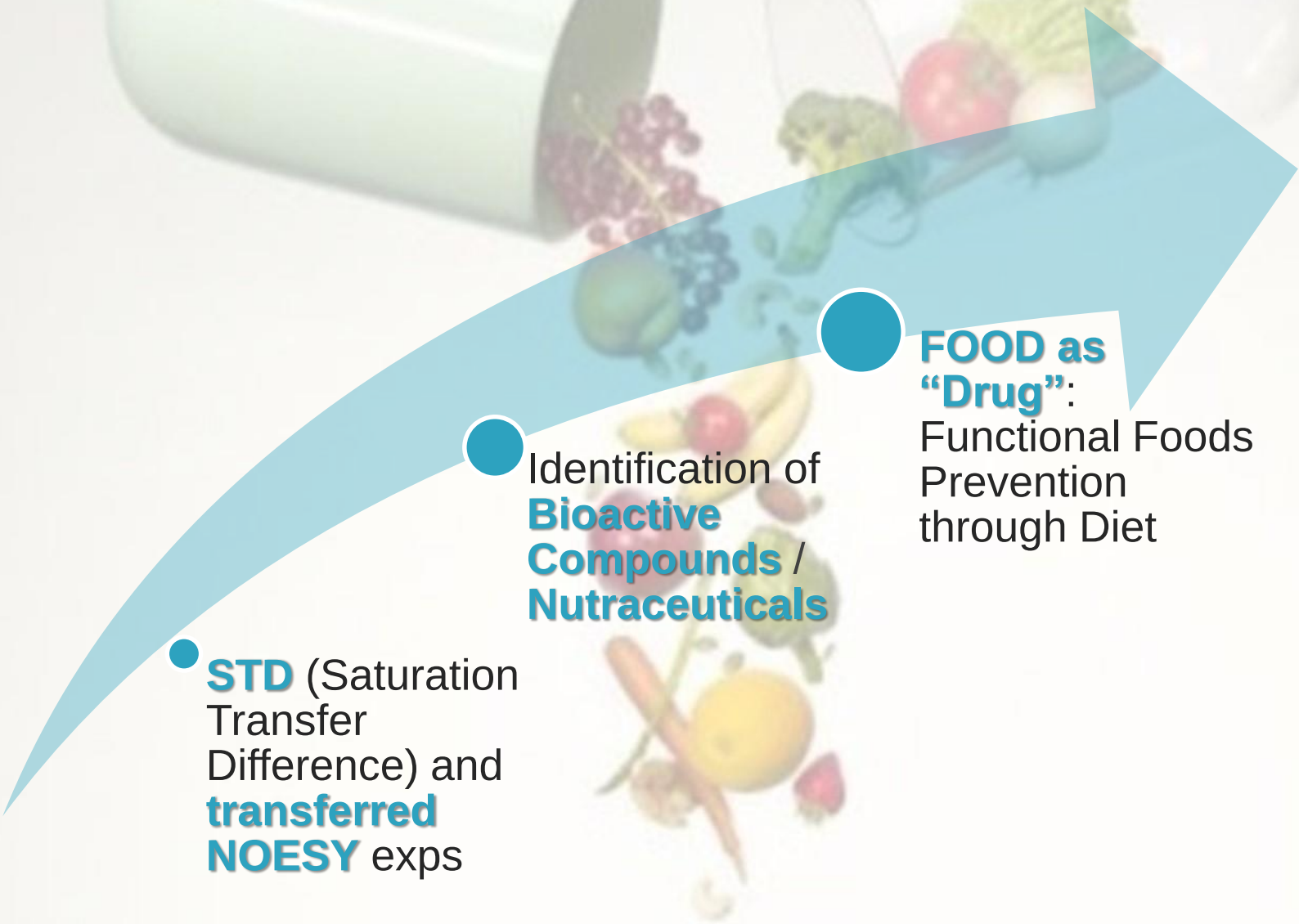
NMR Molecular Recognition studies on natural extracts from edible plants

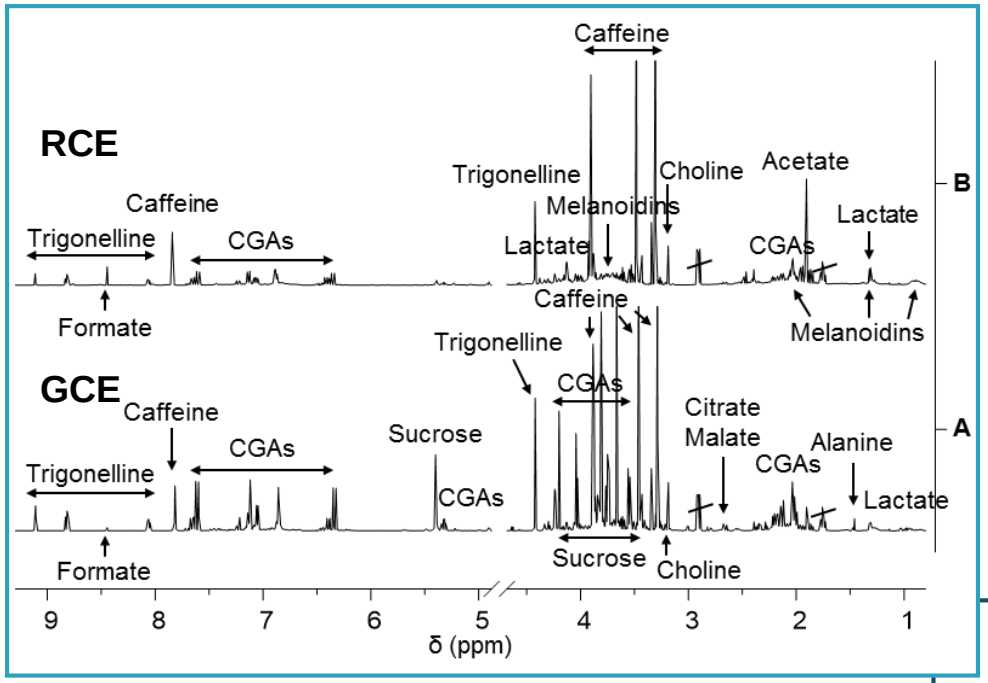


STD (Saturation Transfer Difference) and **transferred NOESY** exps

Identification of **Bioactive Compounds** / **Nutraceuticals**

FOOD as "Drug": Functional Foods Prevention through Diet

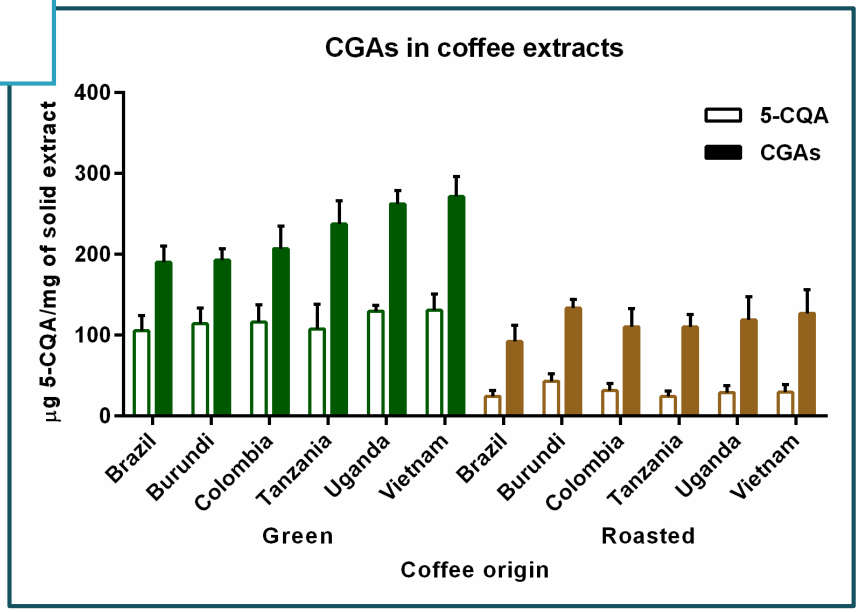
NMR-BASED IDENTIFICATION OF Aβ LIGANDS IN GREEN AND ROASTED COFFEE EXTRACTS



^{1}H-NMR metabolic profiling of six coffee varieties (3 Robusta, 3 Arabica)

Roasted coffee extracts (RCEs)

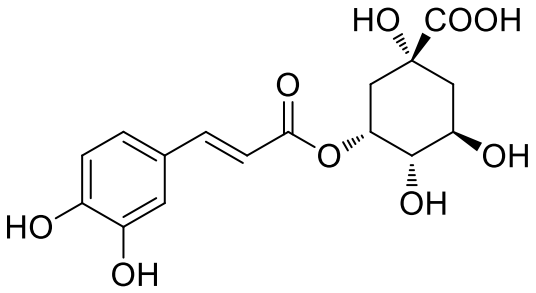
Green coffee extracts (GCEs)



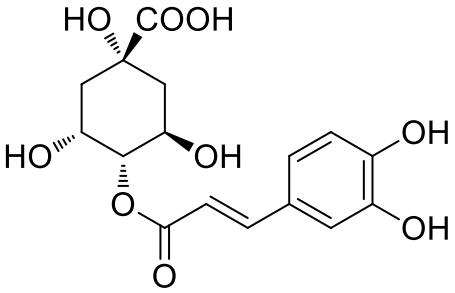
GCE polyphenols content, in particular chlorogenic acids (CGAs), is higher

Ciaramelli C., Palmioli A., De Luigi A., Colombo L., Sala G., Riva C., Zoia C. P., Salmona M., Airoldi C., Food Chemistry ***2018****, 252, 171-180.*

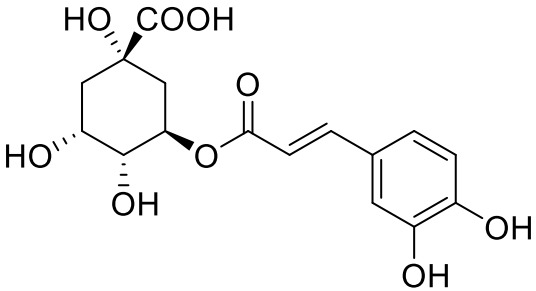
CGAS PRESENT IN GREEN AND ROASTED COFFEE BIND Aβ OLIGOMERS



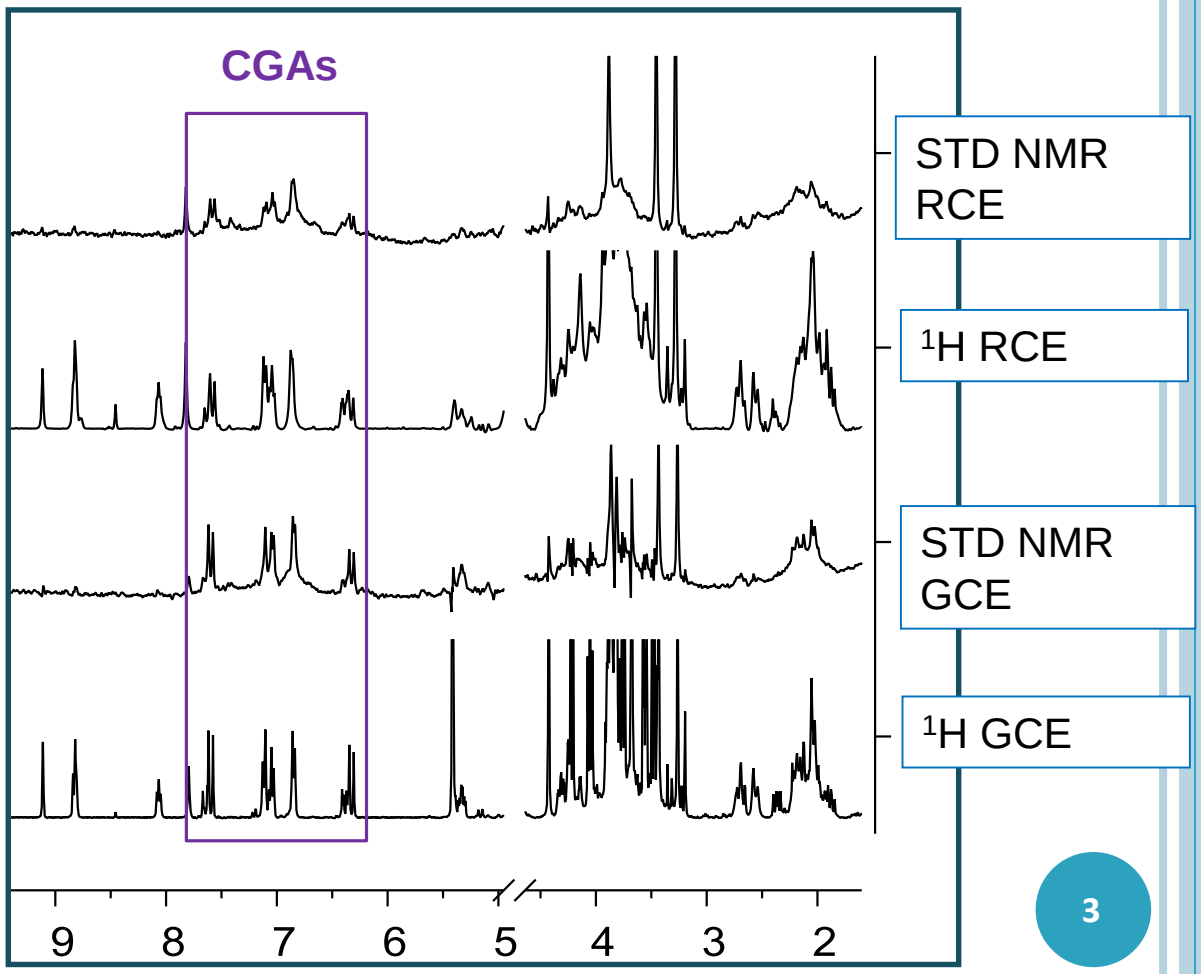
Neochlorogenic acid (3-CQA)



Criptochlorogenic acid (4-CQA)

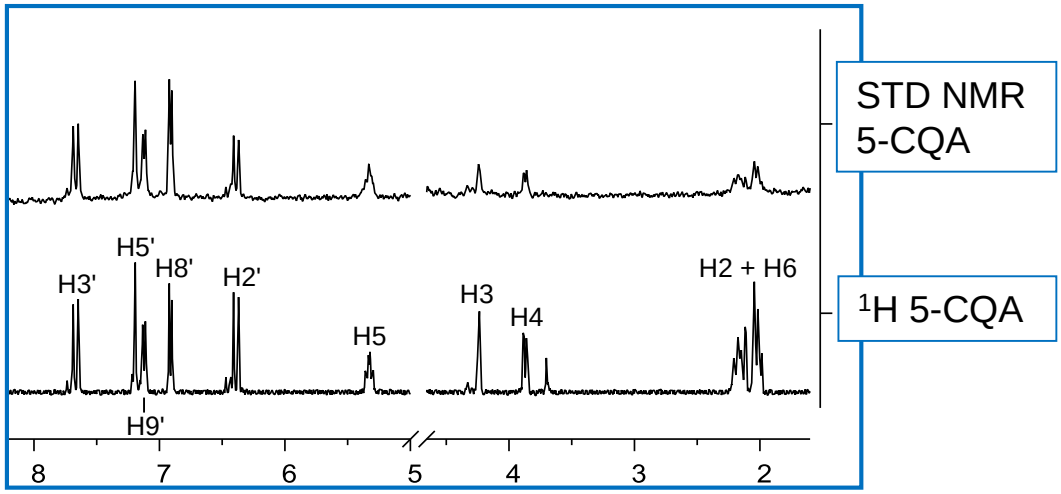


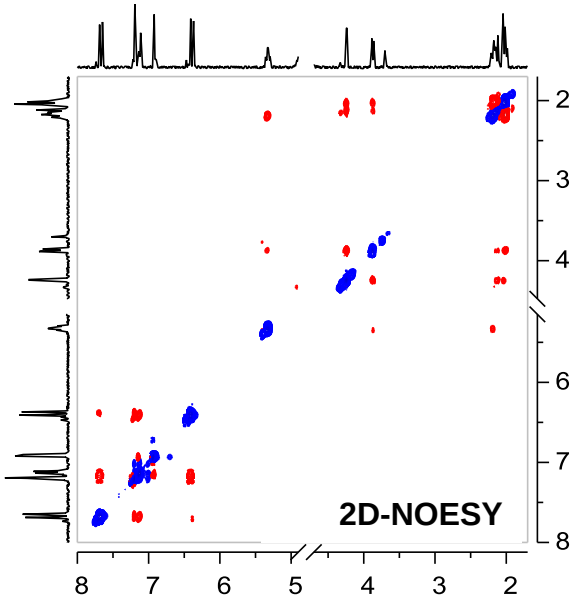
Chlorogenic acid (5-CQA)

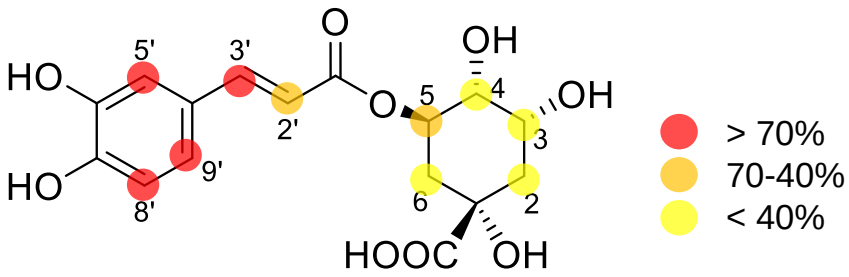


*Ciaramelli C., Palmioli A., De Luigi A., Colombo L., Sala G., Riva C., Zoia C. P., Salmona M., Airoldi C., Food Chemistry **2018**, 252, 171-180.*

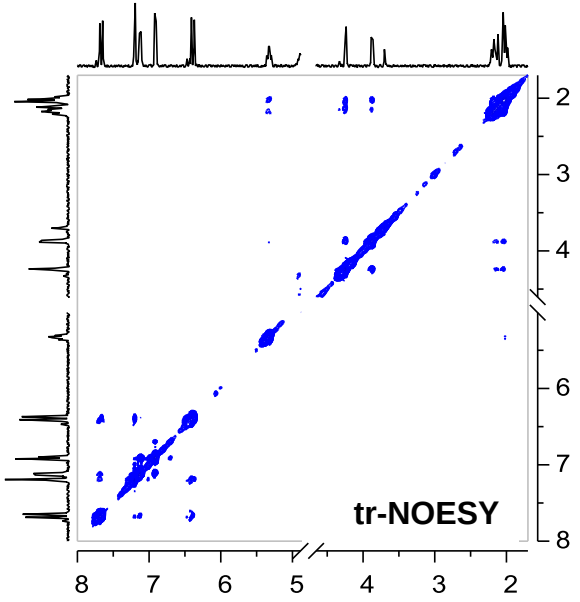
NMR CHARACTERIZATION OF 5-CQA BINDING TO Aβ OLIGOMERS





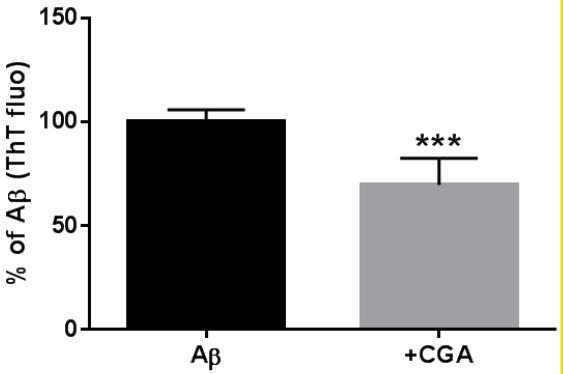


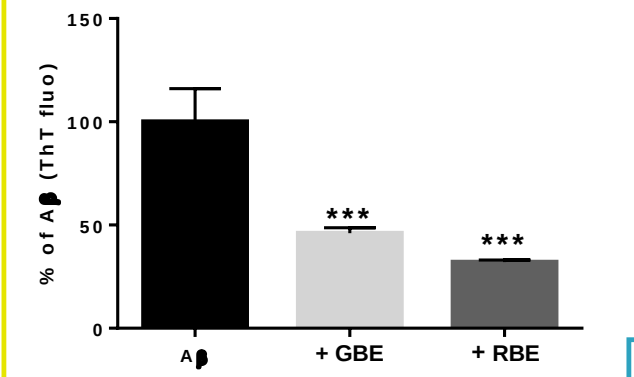
STD relative intensities



Ciaramelli C., Palmioli A., De Luigi A., Colombo L., Sala G., Riva C., Zoia C. P., Salmona M., Airoldi C., Food Chemistry ***2018****, 252, 171-180.*

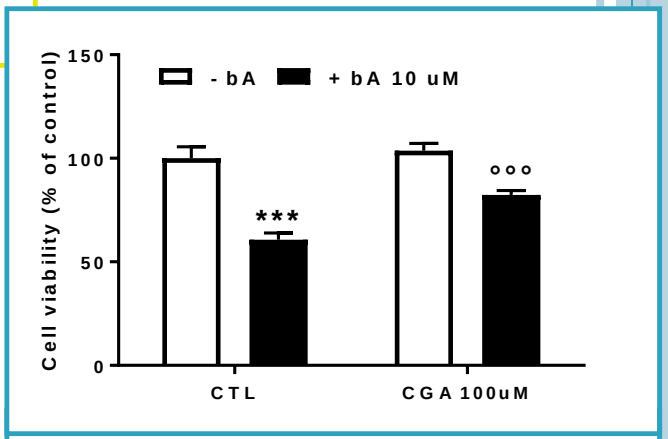
5-CQA and Coffee Extracts Inhibit Aβ peptide Aggregation and Aβ-induced Neurotoxicity

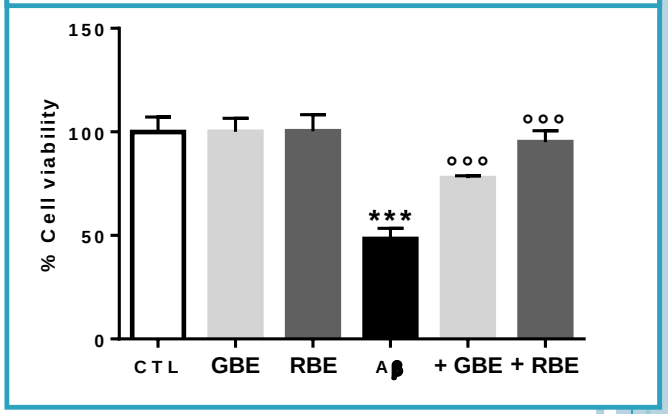




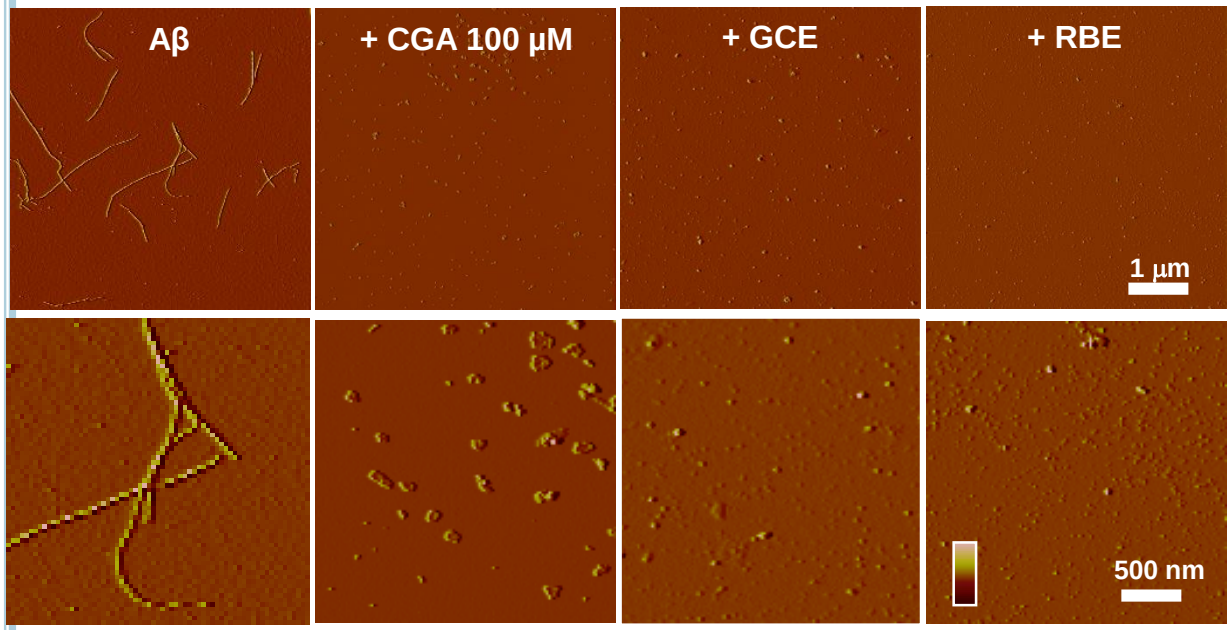
ThT assay

Citotoxicity assay in human SH-SY5Y neuroblastoma cells

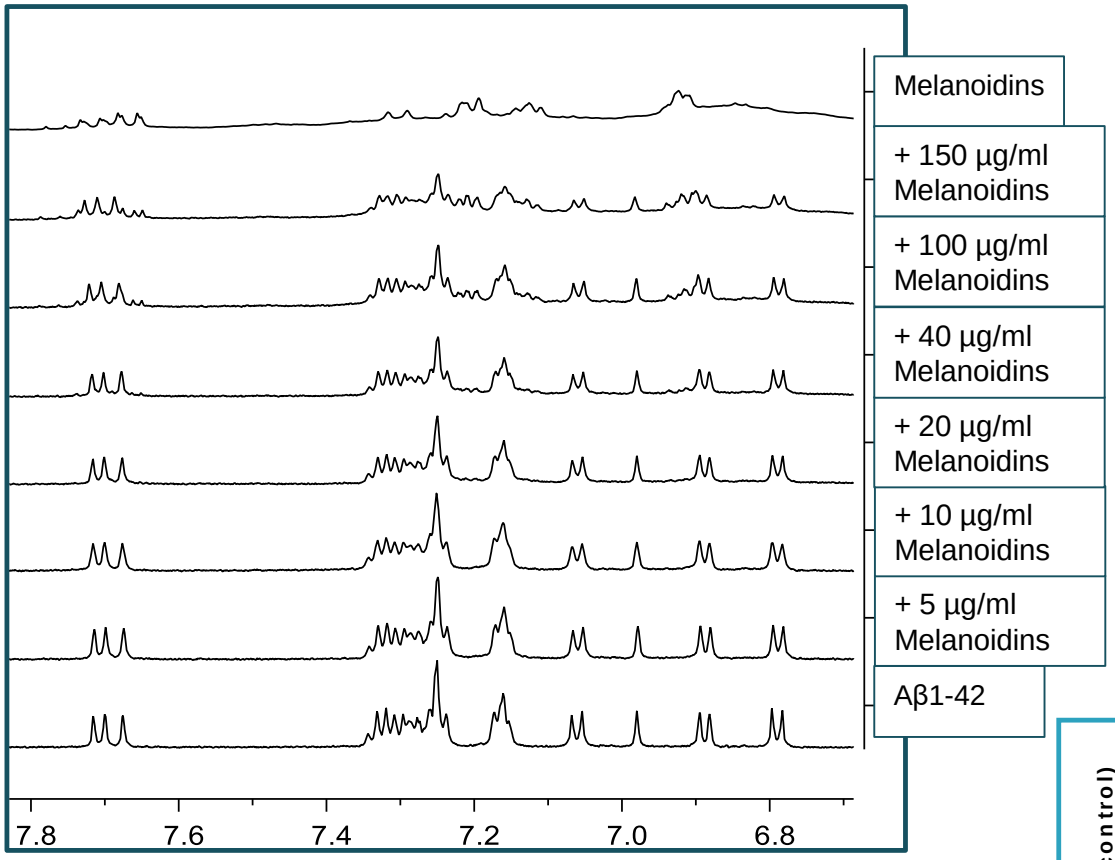




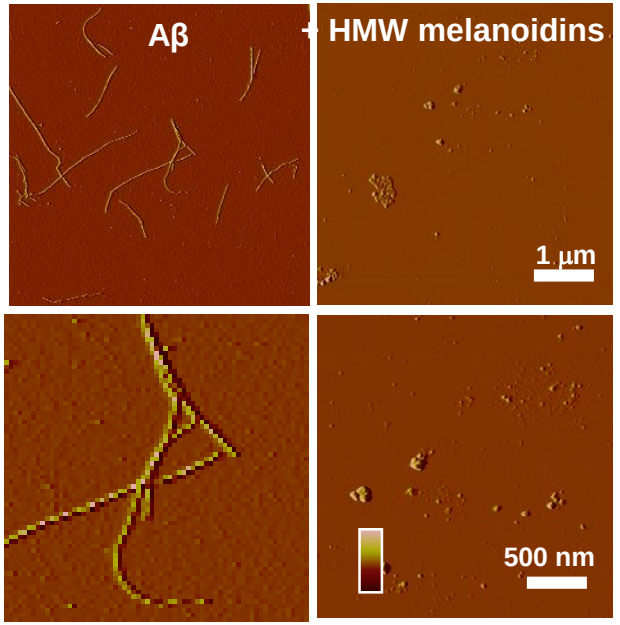
AFM morphological analysis

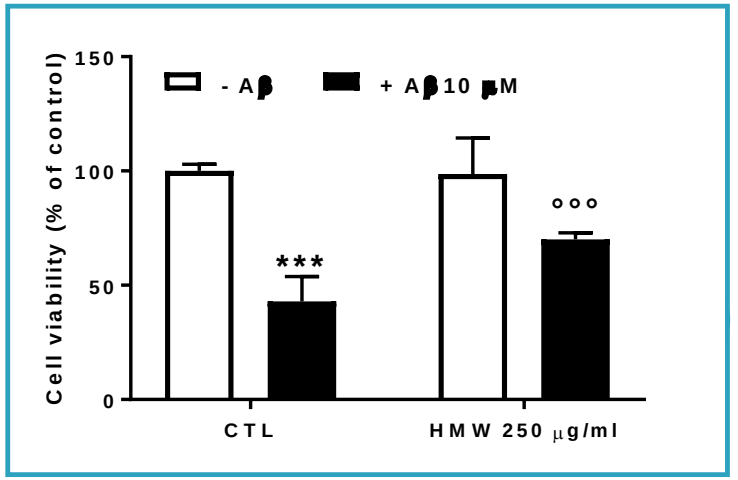


RCEs contain another anti-amyloidogenic species: Melanoidins



AFM morphological analysis



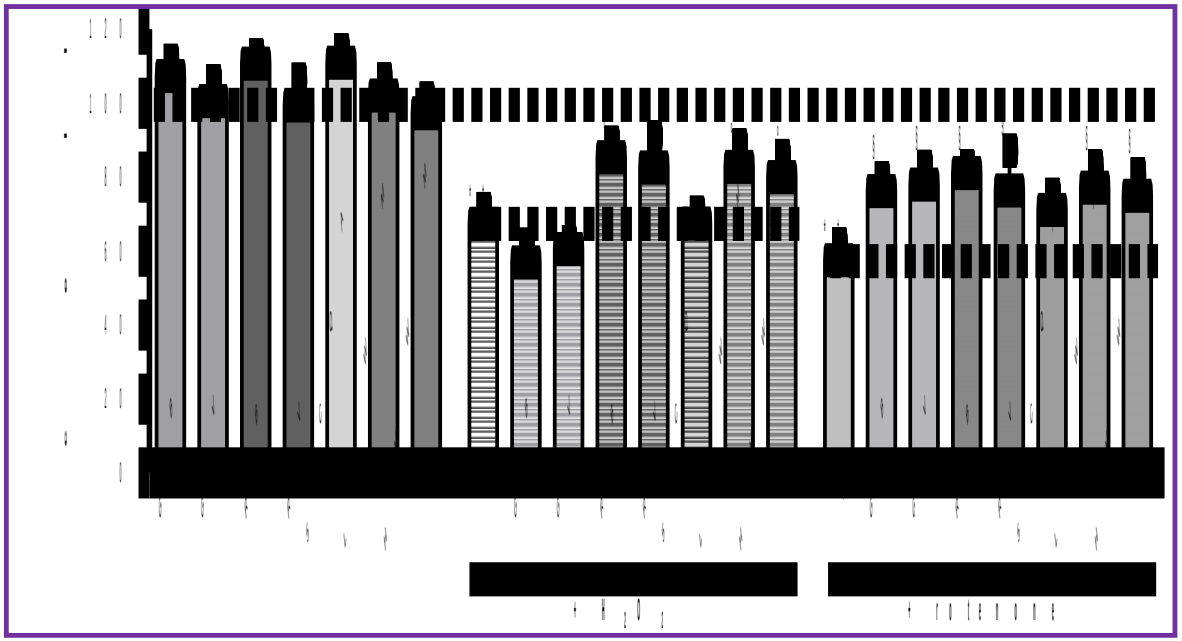


Melanoidins interact with Aβ1-42 preventing its aggregation and toxicity

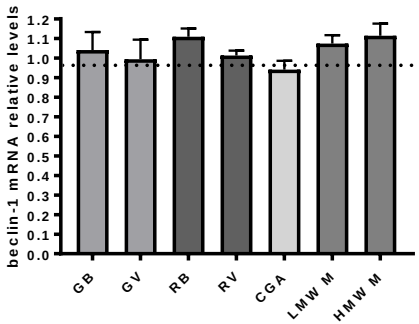
Ciaramelli C., Palmioli A., De Luigi A., Colombo L., Sala G., Riva C., Zoia C. P., Salmona M., Airoldi C., Food Chemistry ***2018****, 252, 171-180.*

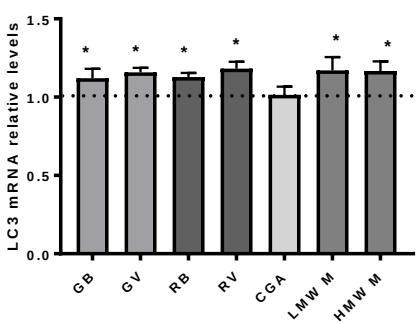
Coffee Extracts and Melanoidins counteract Oxidative Stress and modulate some Autophagic Pathway

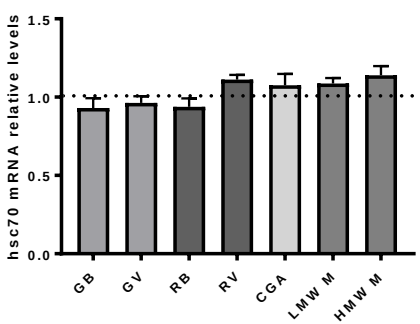
Coffee extracts and melanoidins prevent H_2O_2 and rotenone induced citotoxicity

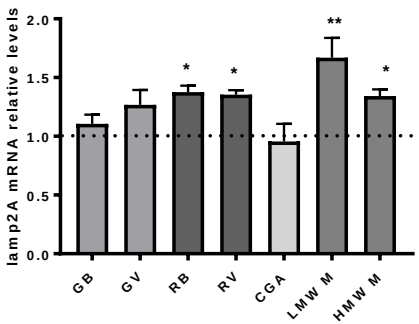


Coffee extracts and melanoidins modulate LC3 and lamp2A mRNA levels

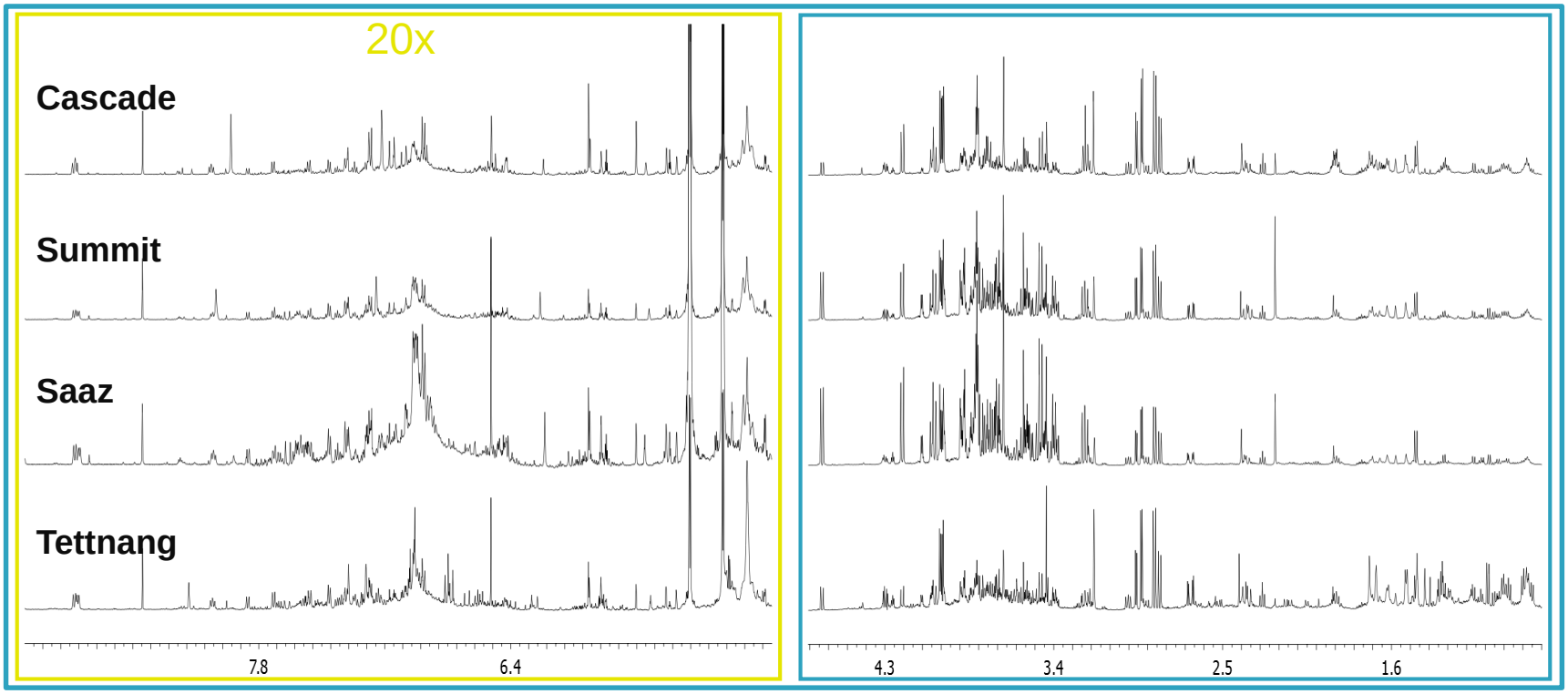








Identification of Ligands and Inhibitors of Aβ1-42 oligomerization in Hop extracts

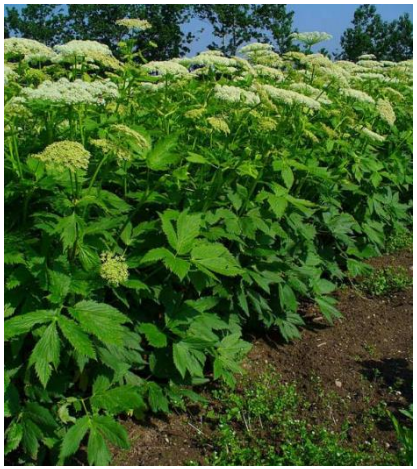


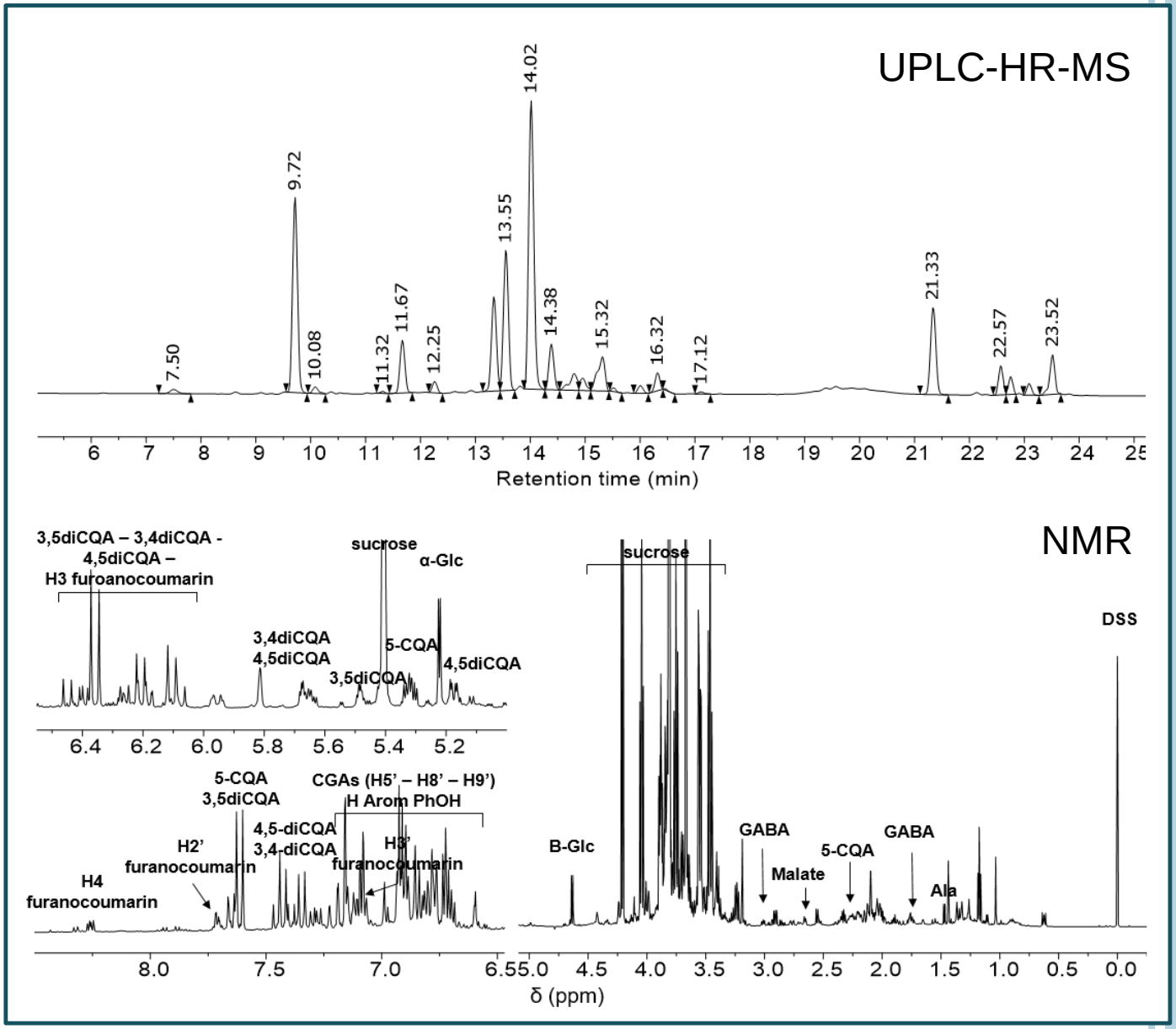
^{1}H NMR spectra of 15 mg of the extract in boiling water of four hop varieties. All the samples were dissolved in deuterated PBS, pH 7.5, 25°C.

Palmioli A., Bertuzzi S., De Luigi A., Colombo L., La Ferla B., Salmona M., De Noni I., Airoldi C., ***2019****, 83, 76-86*

… TRYING TO DEMONSTRATE THE HEALING ACTIVITY OF IMPERATORIA EXTRACTS…

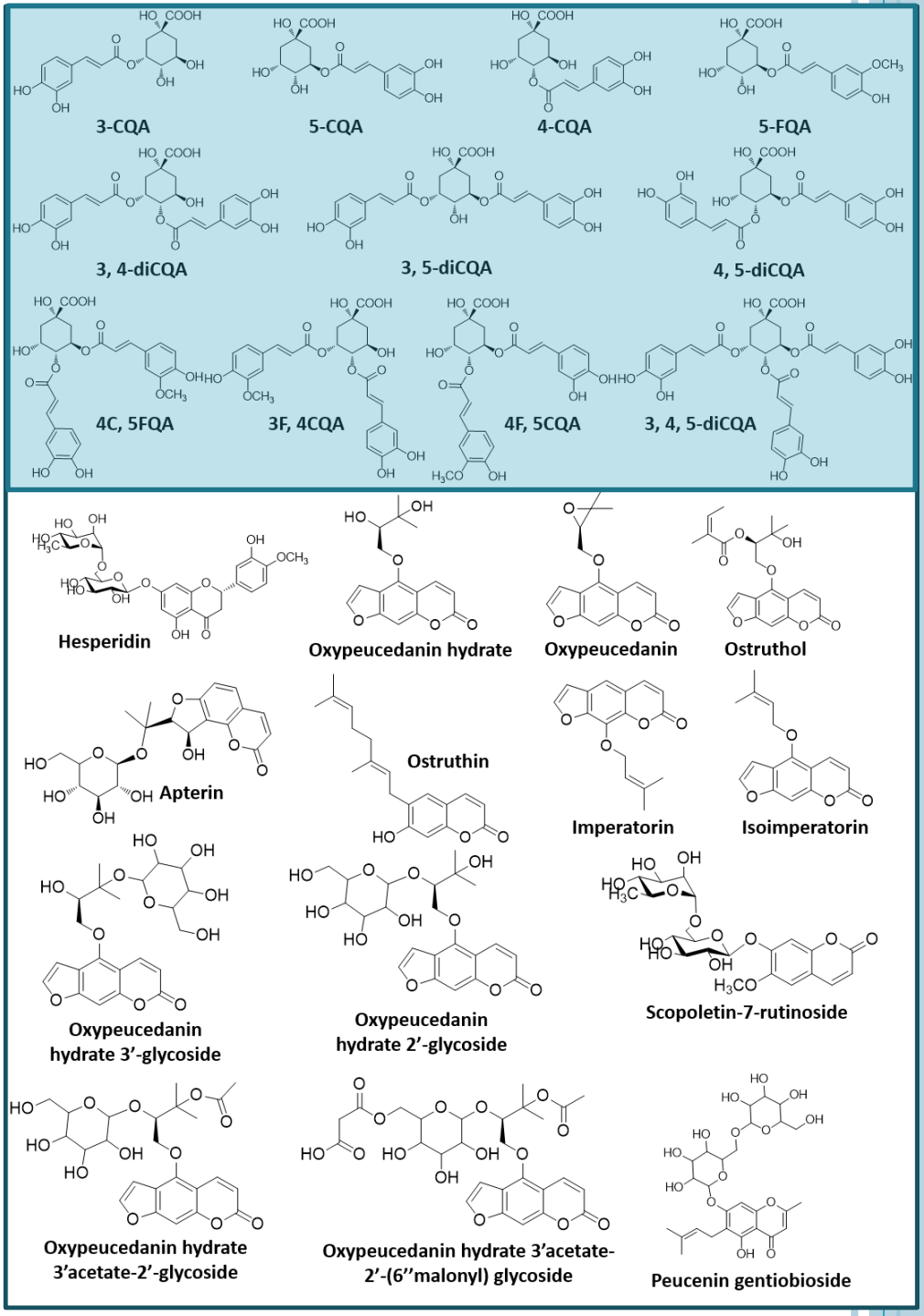
Imperatoria *(Peucedanum ostruthium)* is a medicinal plant traditionally employed in Austria and Italy.



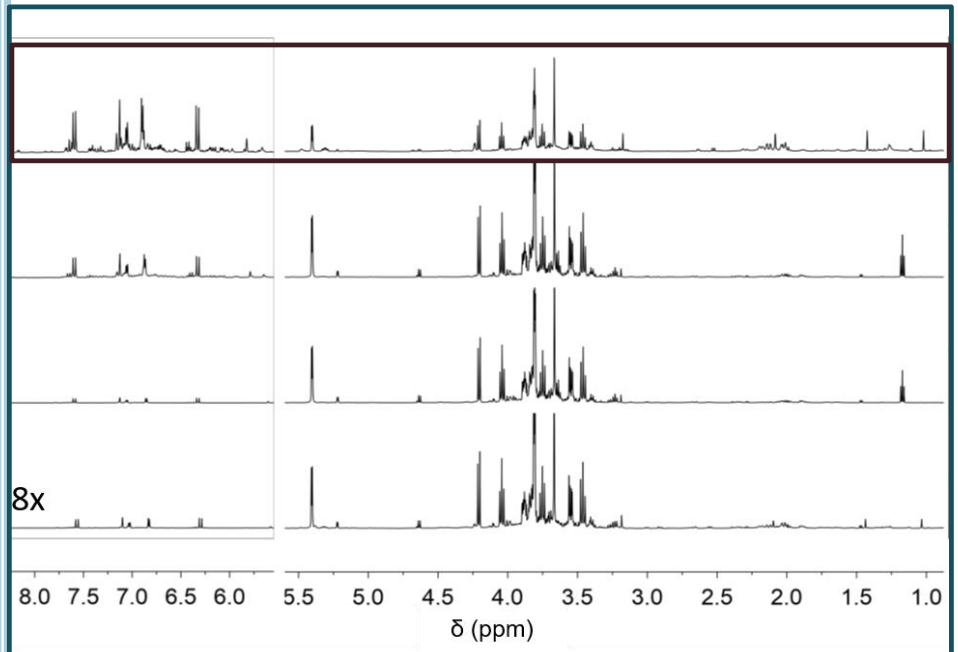


Palmioli A., Bertuzzi S., De Luigi A., Colombo L., La Ferla B., Salmona M., De Noni I., Airoldi C., Bioorg. Chem, ***2019****, 83, 76-86*

Imperatoria extracts from leaves and rhizome show a significant content of polyphenols, among which chlorogenic acids (CGAs)



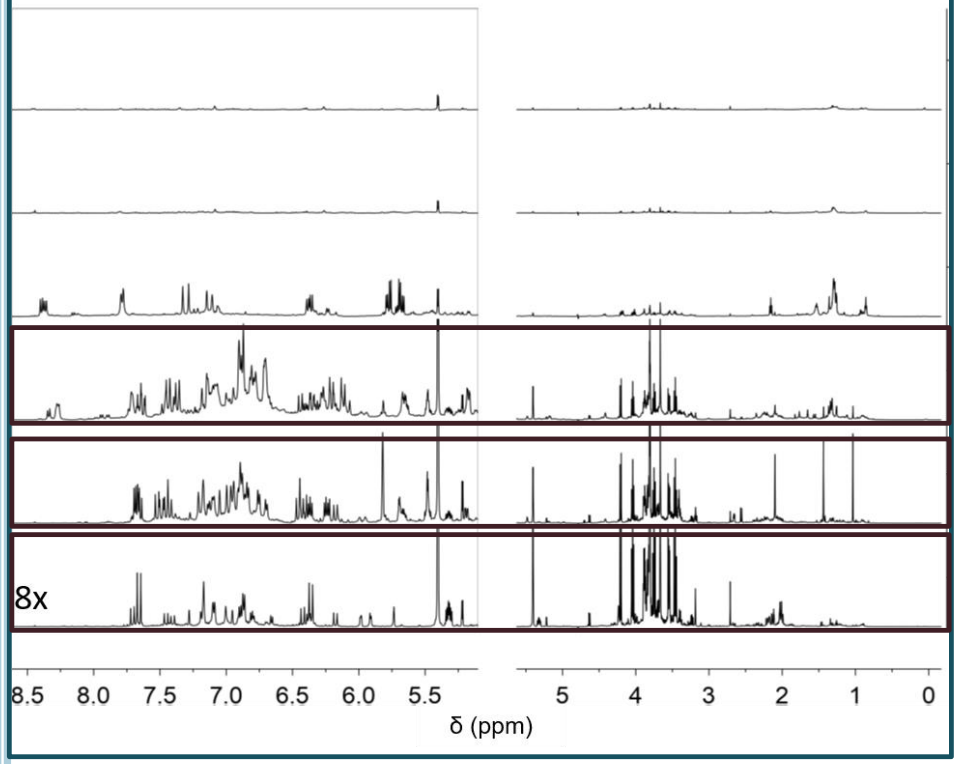
Palmioli A., Bertuzzi S., De Luigi A., Colombo L., La Ferla B., Salmona M., De Noni I., Airoldi C., ***2019****, 83, 76-86*



Fraction XAD

Solid Phase Extraction on XAD-4

Fractions enriched in polyphenols



Fraction C

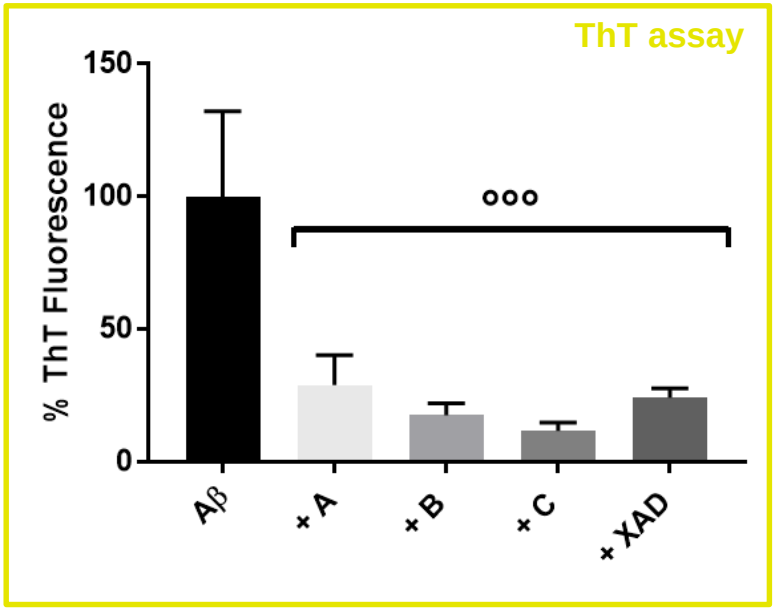
Fraction B

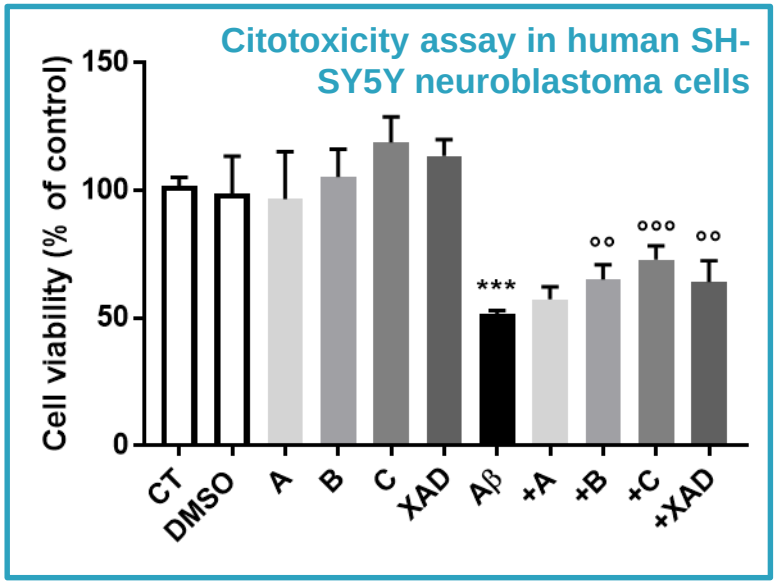
Reverse Phase chromatography

Fraction A

Palmioli A., Bertuzzi S., De Luigi A., Colombo L., La Ferla B., Salmona M., De Noni I., Airoldi C., ***2019****, 83, 76-86*

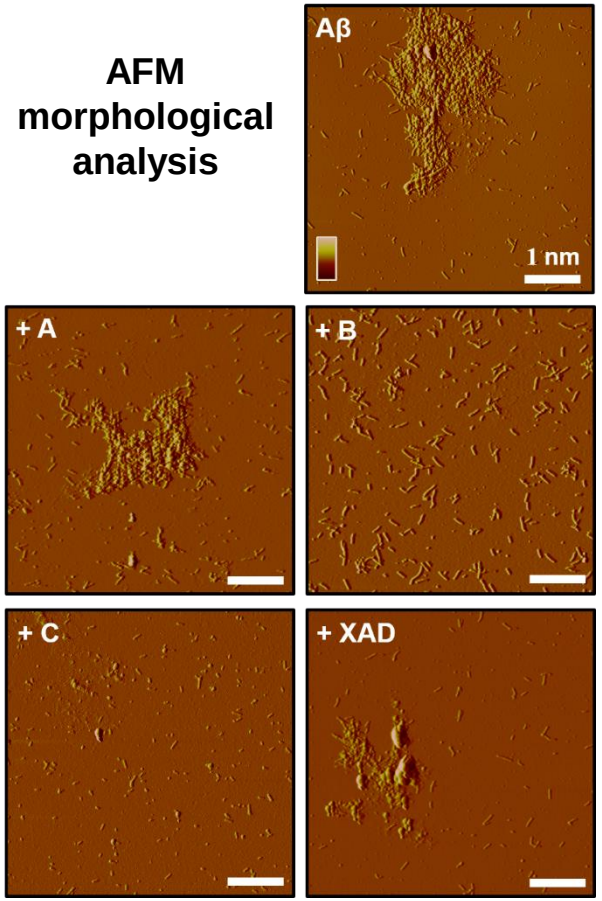
… WE DISCOVERED THEIR ANTI-AMYLOIDOGENIC ACTIVITY…





"potency" following the order
XAD < B < C

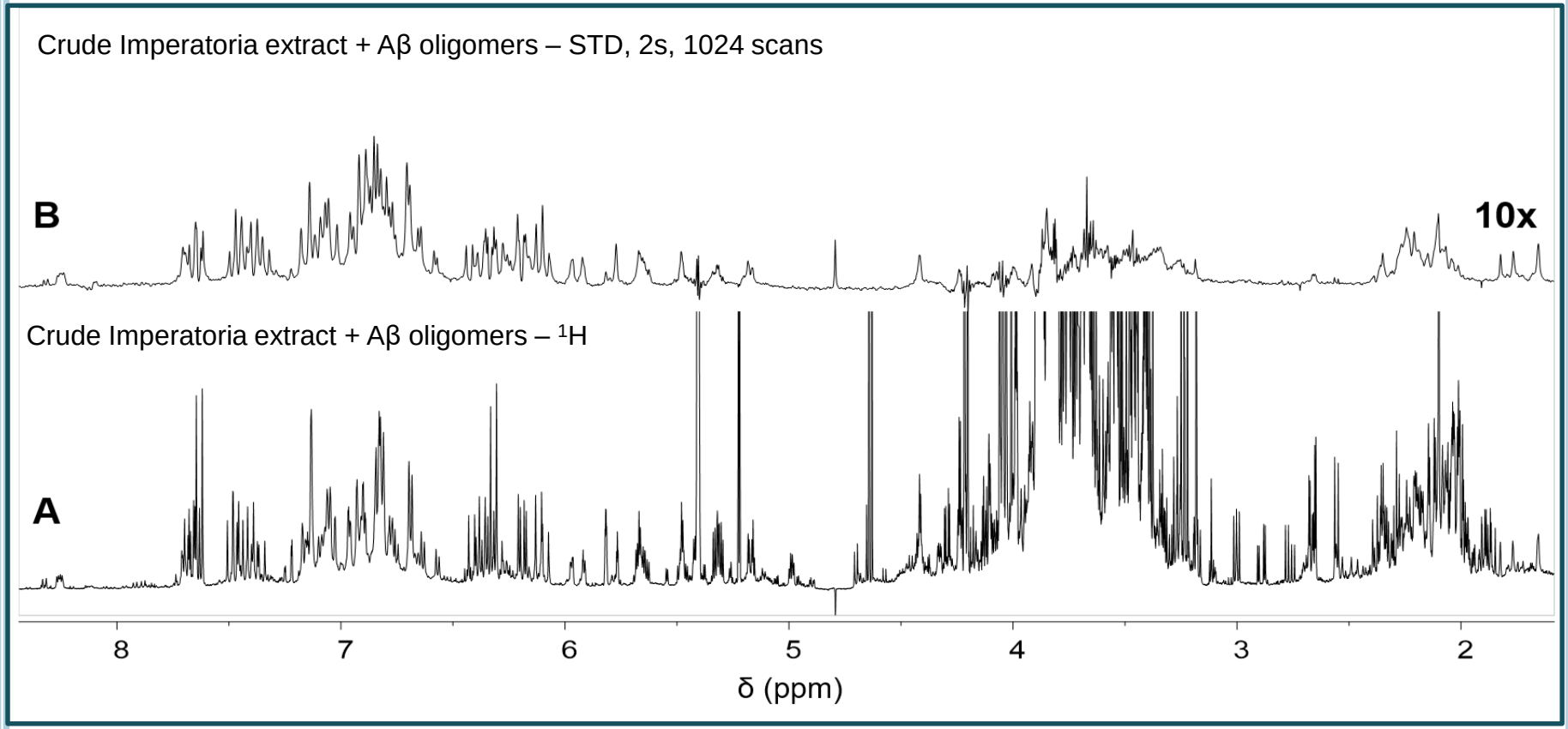
AFM morphological analysis



Fraction anti-amyloidogenic activity correlates with their contents of CGAs, furanocoumarins and flavonoid glycosides

Palmioli A., Bertuzzi S., De Luigi A., Colombo L., La Ferla B., Salmona M., De Noni I., Airoldi C., ***2019****, 83, 76-86*

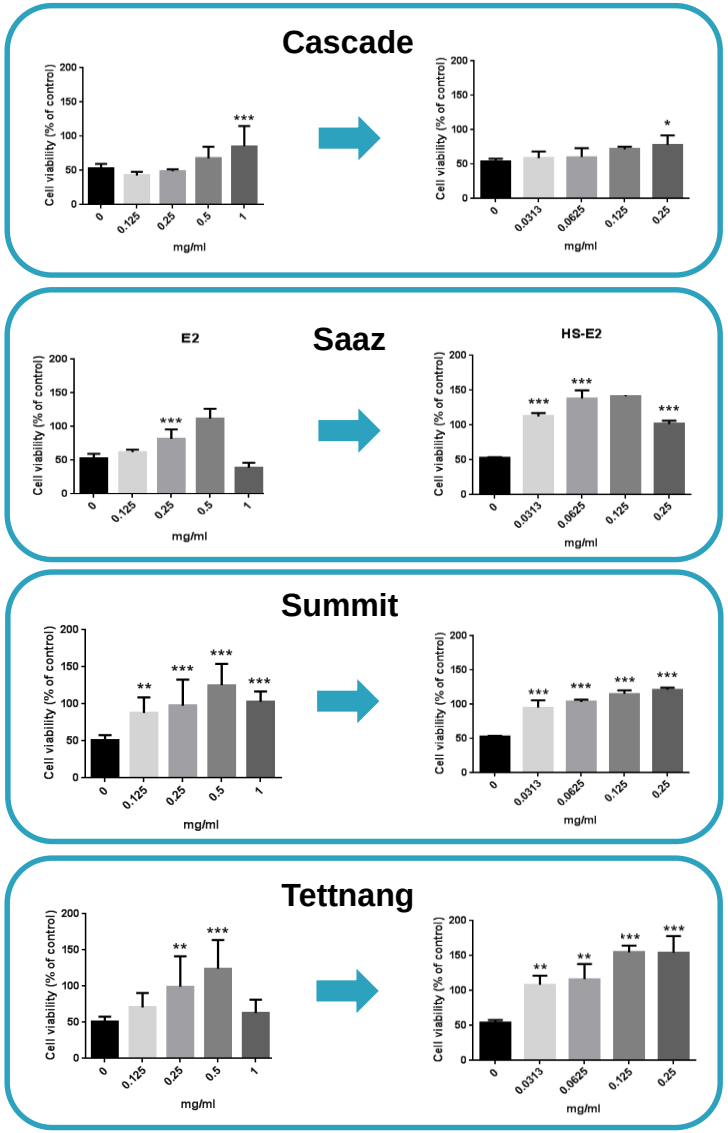
Di-substituted CGAs are the Aβ ligands showing the highest affinity



- **Di-substituted CQAs are the best ligands of Aβ1-42 oligomers, as they showed relative STD intensities higher than mono-substituted CQAs and thus higher affinities for the target.**
- **Also glycosylated flavonoids and furanocoumarins bind Aβ1-42 oligomers.**
- **The co-presence of these compounds in the same extract allows obtaining a significant biological activity.**

Hop Extracts Anti-amyloidogenic Activity: Prevention of Aβ-induced Citotoxicity

boiling water extracts phenol-enriched extracts



Hop extract effect on the viability of neuronal cells treated with Aβ1-42 protein of increasing concentrations.

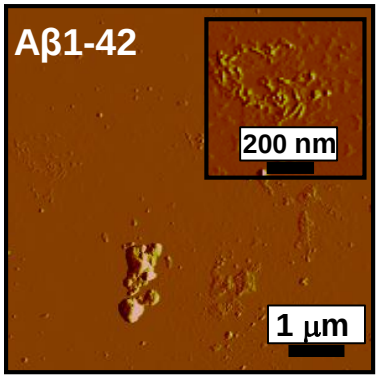
Comparison between boiling water hop extracts and hop phenol-enriched extracts.

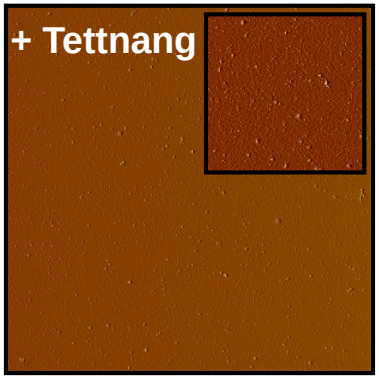
All extracts protect cell against Aβ oligomers-induced citotoxicity

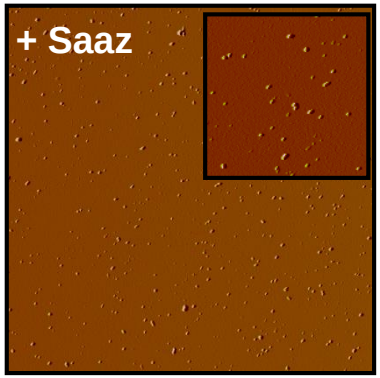
Extract enrichment (XAD-4 or flash chromatography) in polyphenols compounds increases the biological activity

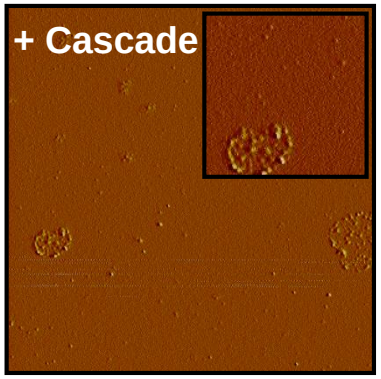
Hop Extracts Anti-amyloidogenic Activity: Inhibition of Aβ oligomerization

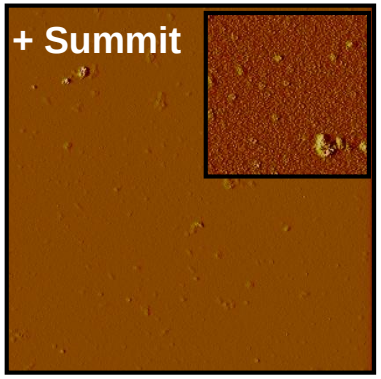
AFM

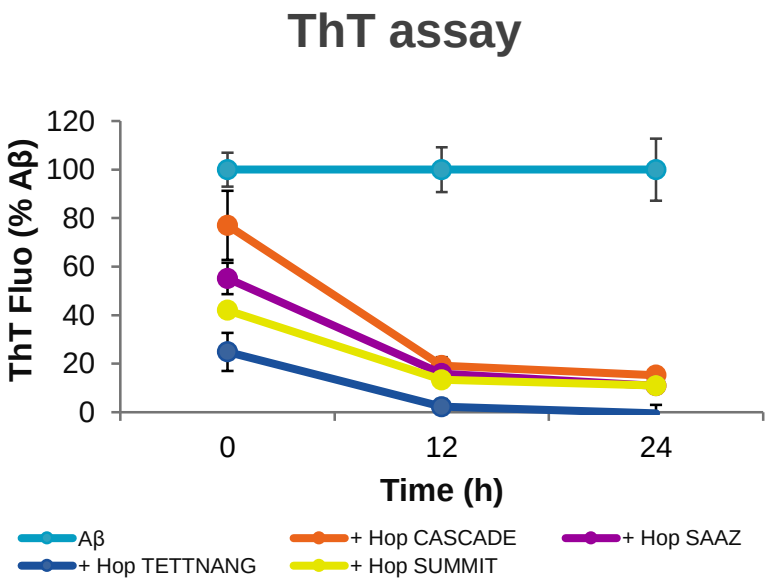






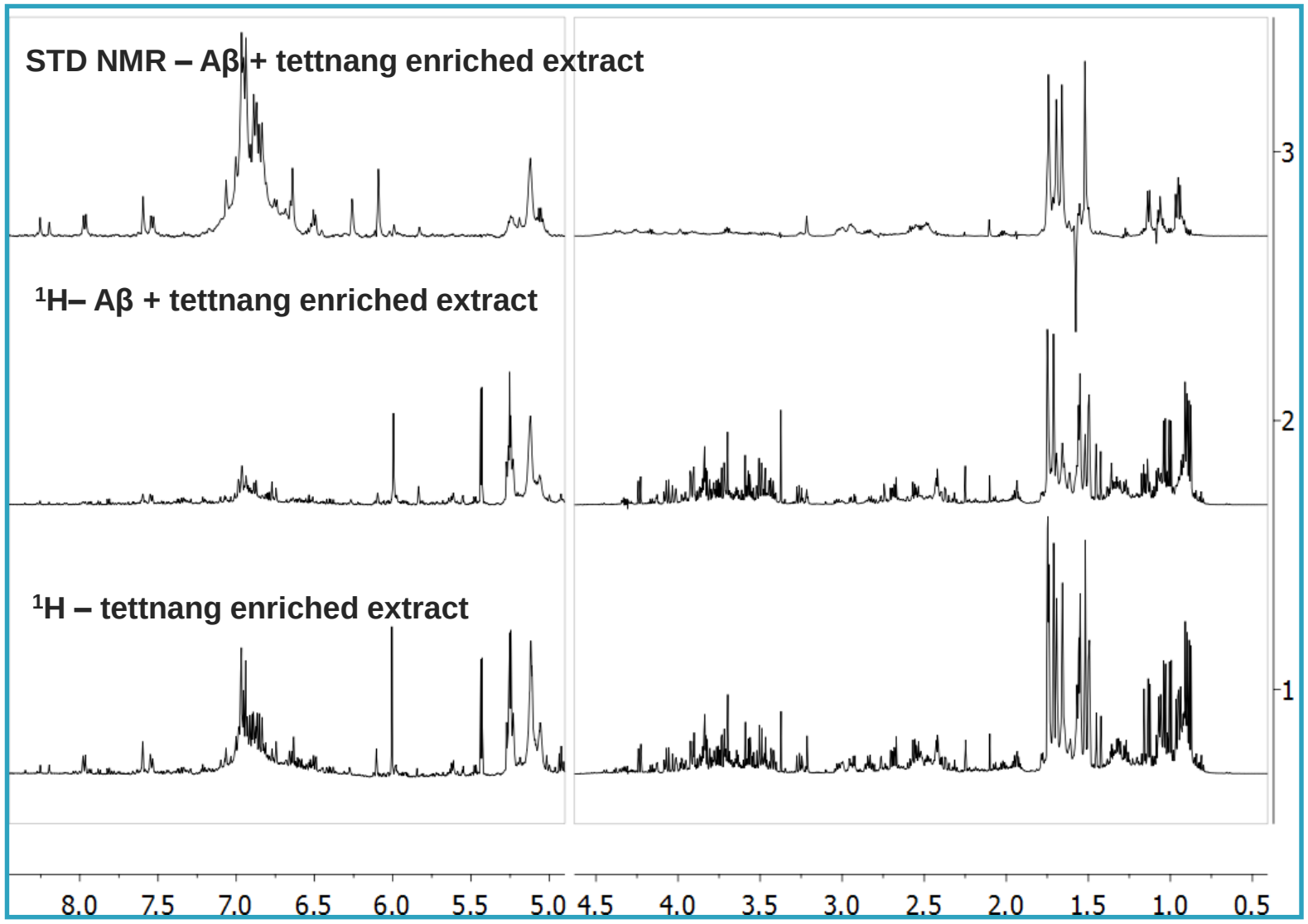






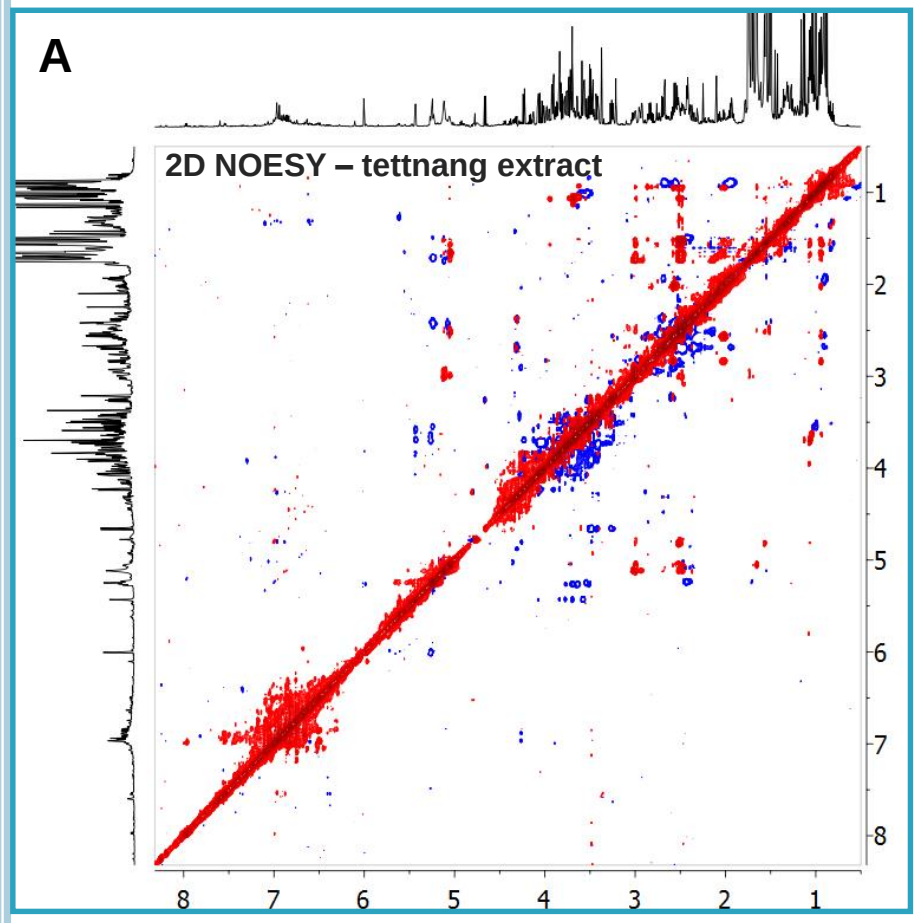
All extracts inhibit Aβ oligomerization

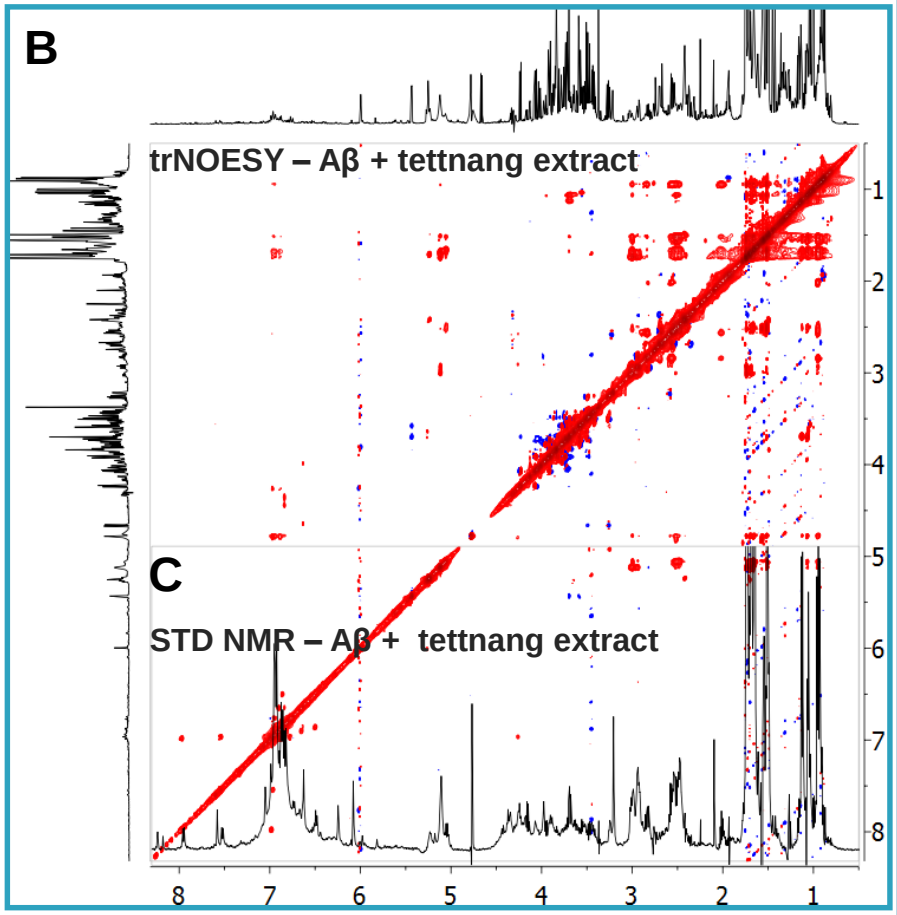
Molecular recognition of Hop extract with Aβ1-42 peptide



1) 1H NMR spectrum of the 20 mg of the AcOEt extract of *Hop tettnang*; 2) 1H NMR spectrum of the mixture containing Aβ1-42 (160 uM) and 20 mg of the AcOEt extract of *Hop tettnang*; D) STD-NMR spectrum of this mixture at 2s saturation time All the samples were dissolved in deuterated PBS, pH 7.5, 25 °C.

Molecular recognition of Hop extract with Aβ1-42 peptide

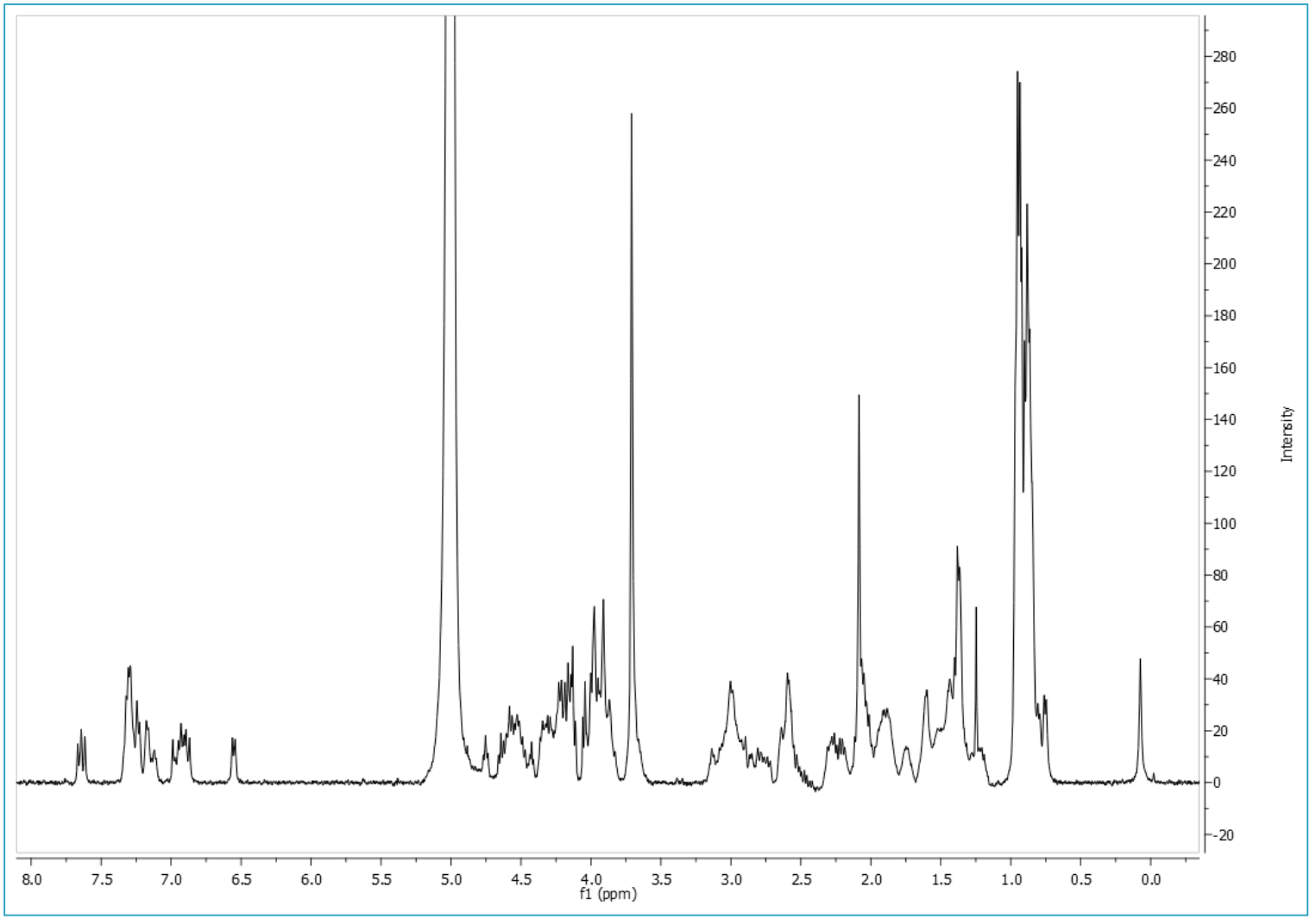




A) 600 MHz 2D-NOESY of 20 mg AcOEt extract of Hop, with a mixing time of 0.9 s. B) trNOESY of the mixture containing Aβ1-42 (160 uM) and 20 mg of AcOEt extract of Hop, with a mixing time 0.3 s. Both samples were dissolved in deuterated PBS, at pH 7.5 and 25°C. Positive cross-peak are in blue; negative, in red. C) STD NMR spectrum of the mixture containing Aβ1-42 (80 uM) and 15 mg of AcOEt extract of Hop

Experiments Based on Complex Observation – Resolution of Protein 3D-Structure

^{1}H Spectra of Proteins

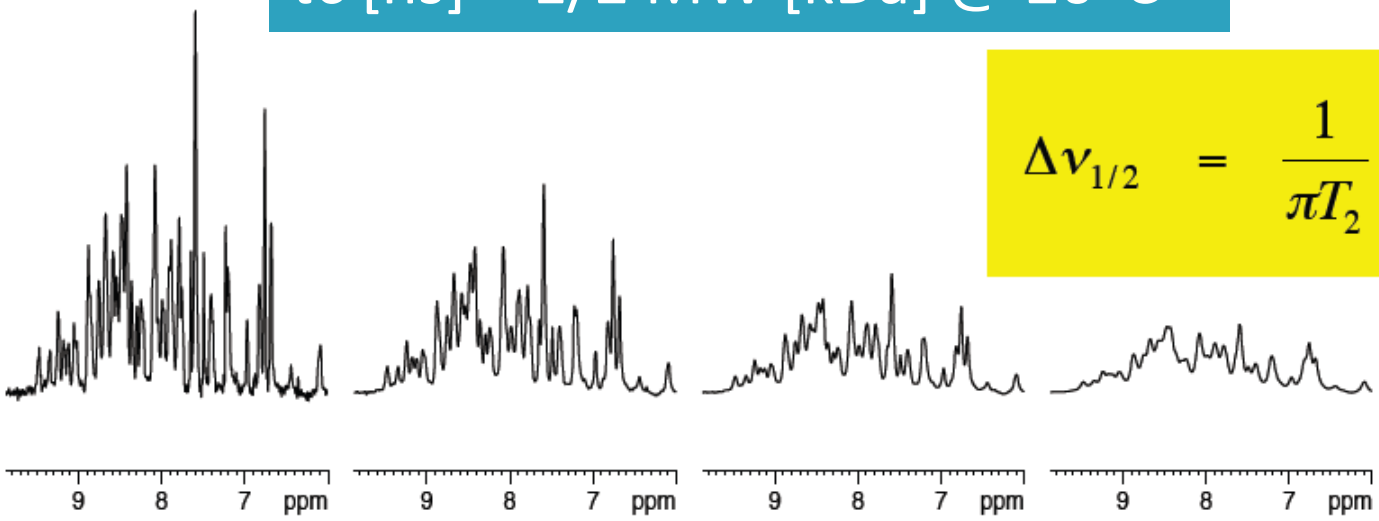


^{1}H spectrum of Aβ 1-40, D_2O, 128 scans

Efficient Relaxation results in Broad Lines: The effect of increasing Correlation Times

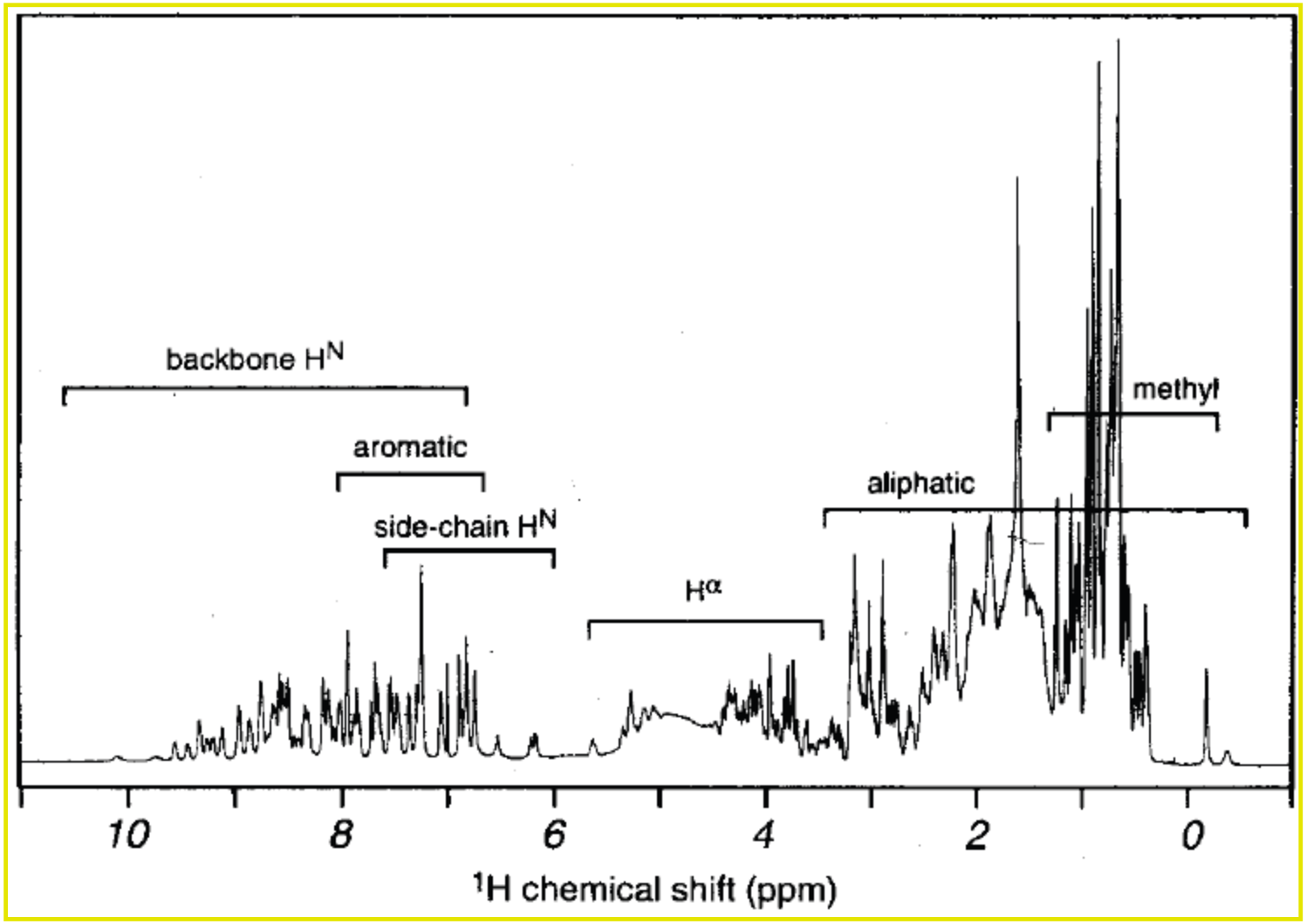
τc [ns] ~ 1/2 MW [kDa] @ 20°C

$$\Delta\nu_{1/2} = \frac{1}{\pi T_2}$$



τ_c	5ns	10ns	15ns	25ns
MW	10kDa	20kDa	30kDa	50kDa

^{1}H Spectra of Proteins : Which info can we obtain?



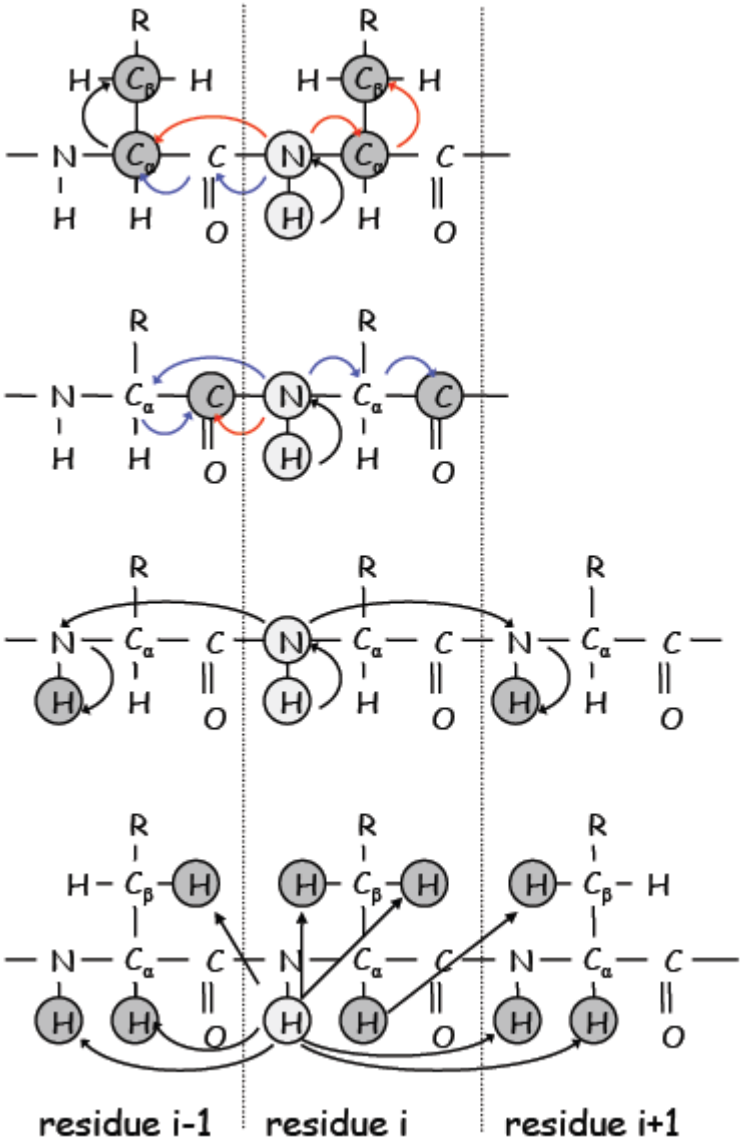
Backbone Assignment – 3D Experiments

HNCACB /
HN(CO)CACB

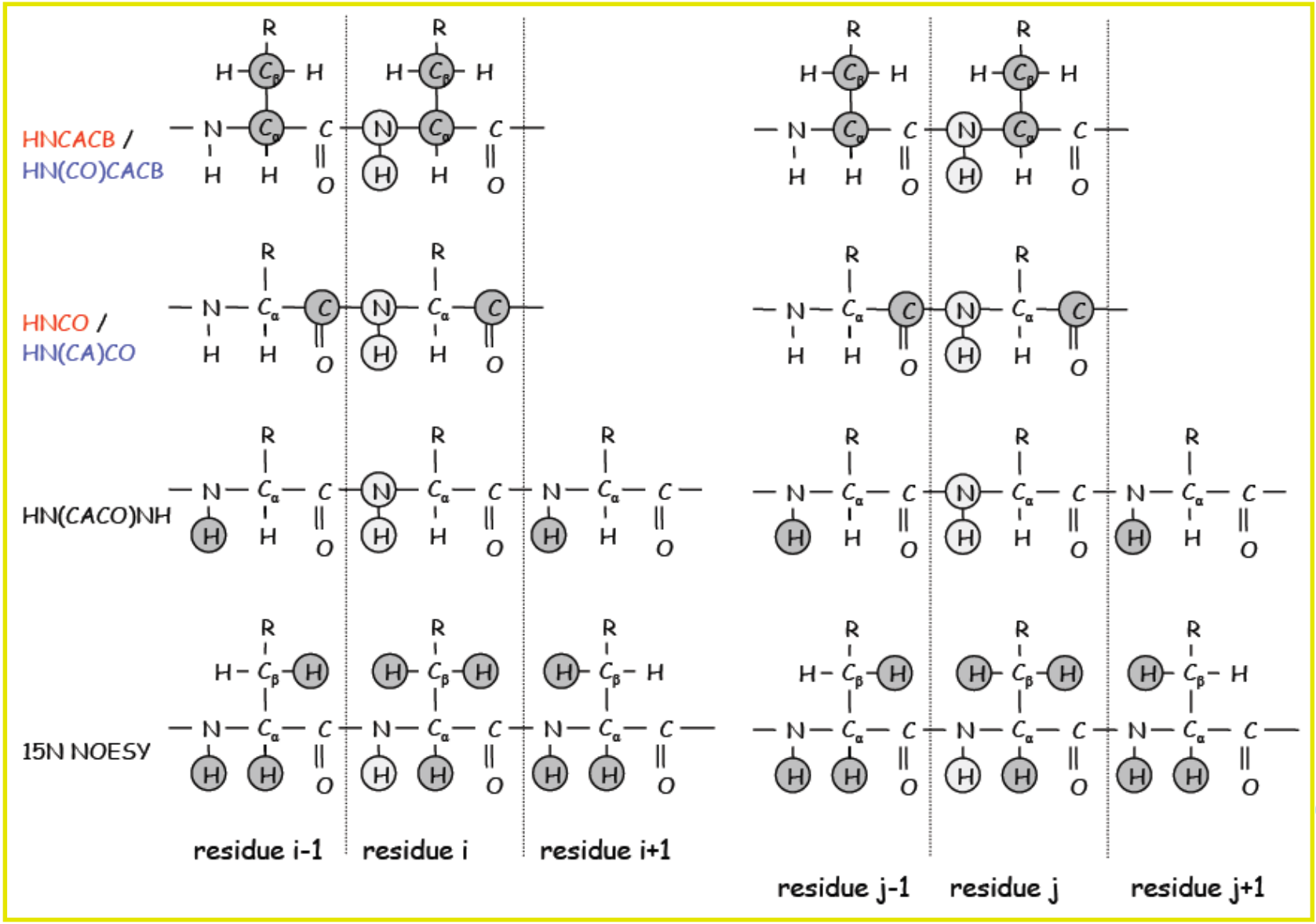
HNCO /
HN(CA)CO

HN(CACO)NH

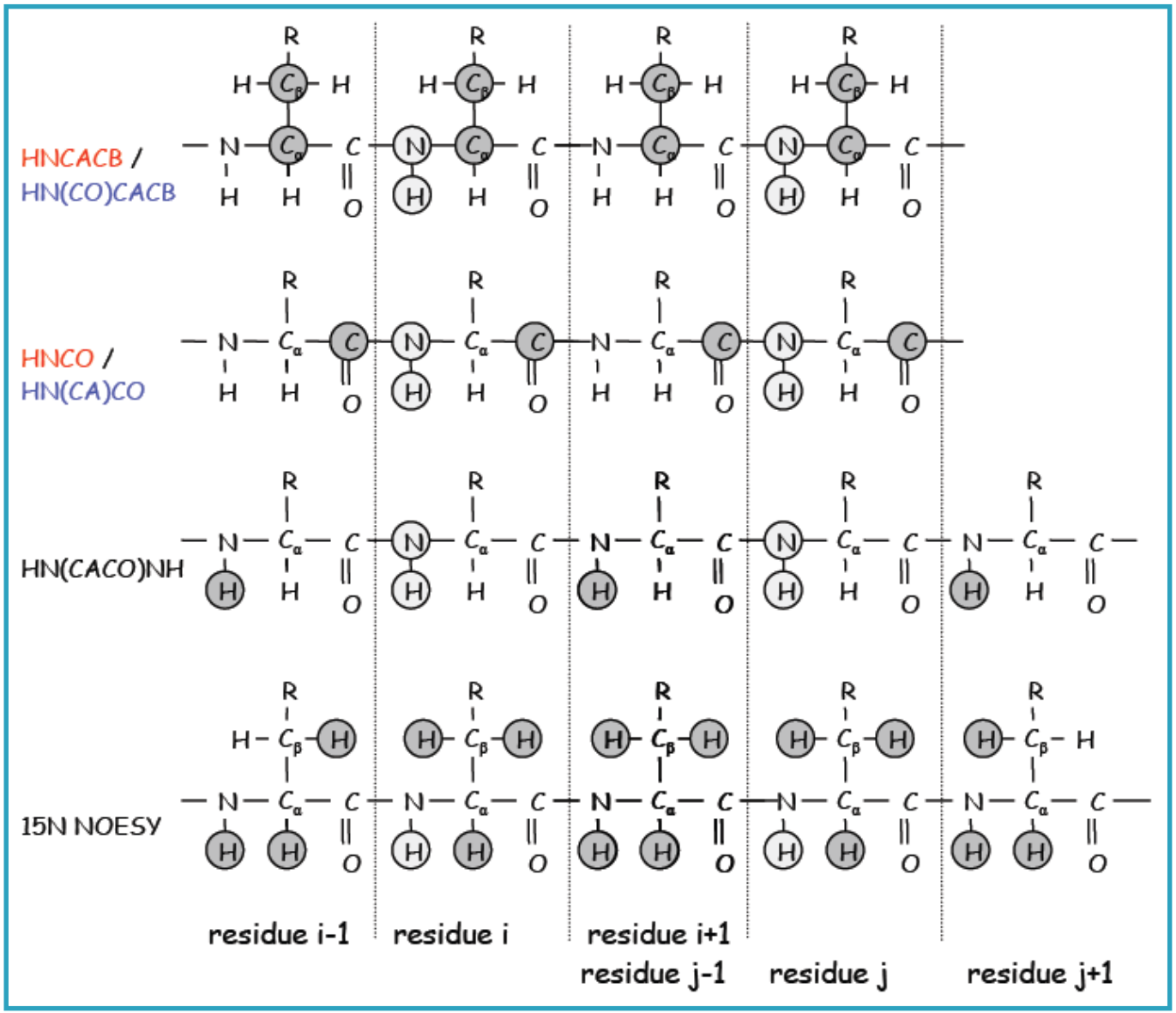
15N NOESY



Backbone Assignment – 3D Experiments



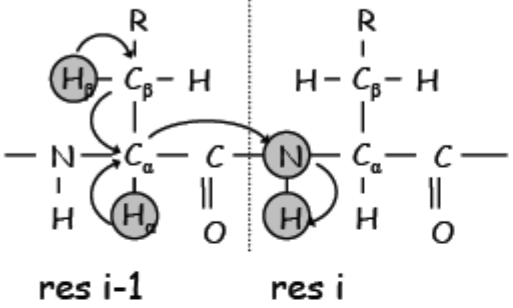
Backbone Assignment – 3D Experiments



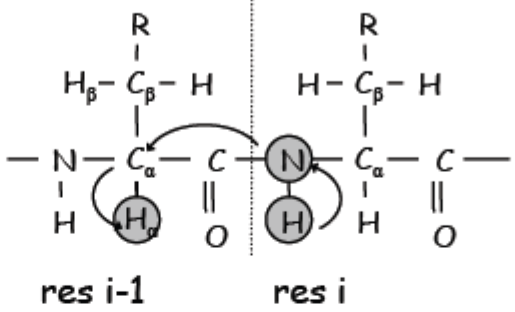
Side Chain Assignment Strategies

Identification of backbone protons:

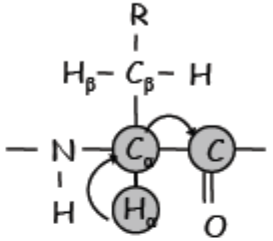
HBHA(CACBCO)NH



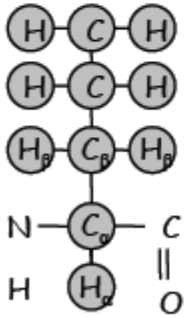
HN(COCA)HA

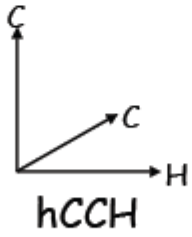


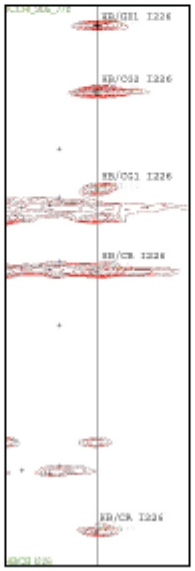
HACACO

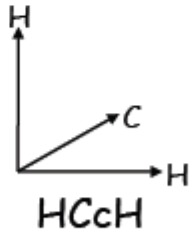


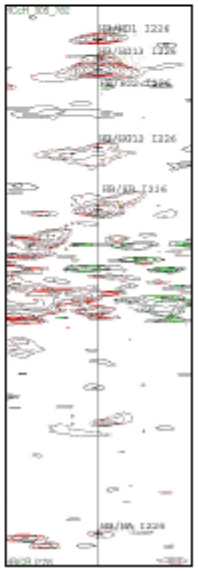
Side chain assignment:







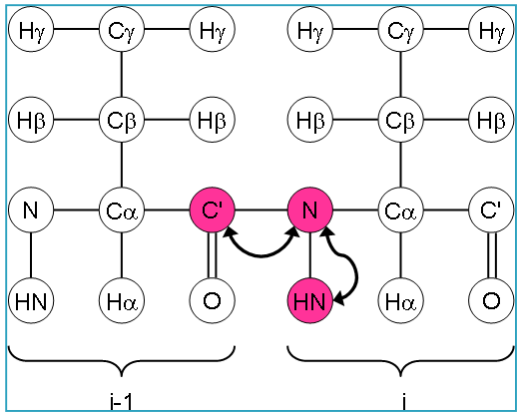




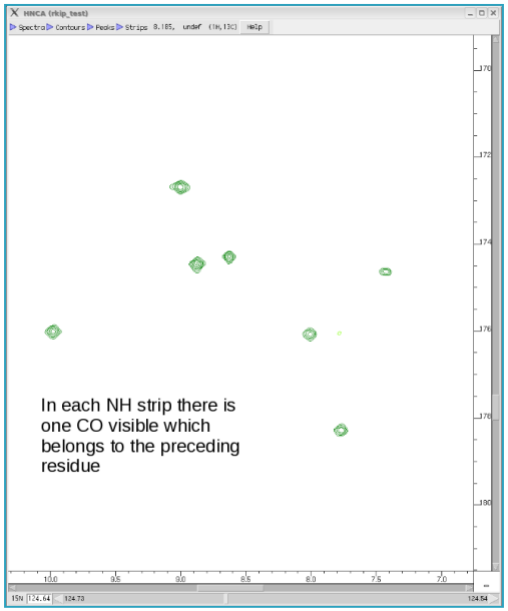
^{15}N, ^{13}C, ^{1}H HNCO Spectra

^{15}N and ^{13}C labelling are required

Magnetisation is passed from ^{1}H to ^{15}N and then selectively to the carbonyl ^{13}C via the $^{15}N^{H}$-^{13}CO J-coupling. Magnetisation is then passed back via ^{15}N to ^{1}H for detection. The chemical shift is evolved on all three nuclei resulting in a three-dimensional spectrum.



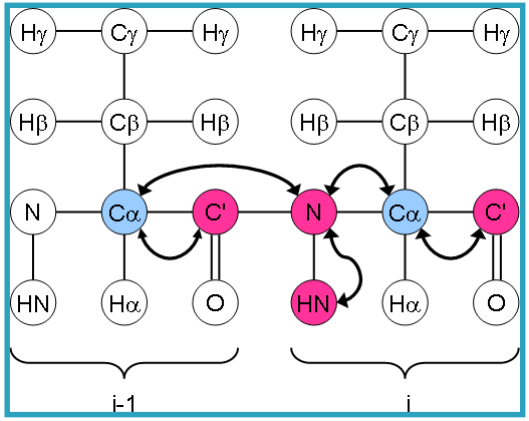
This is the most sensitive triple-resonance experiment. In addition to the backbone CO-N-HN correlations, Asn and Gln side-chain correlations are also visible. It is mainly used to obtain CO chemical shifts which can be used in a program like TALOS to helppredict secondary structure. The HNCO can also be useful for backbone assignment in conjunction with the HN(CA)CO, if the CBCANNH and CBCA(CO)NNH spectra are of bad quality.



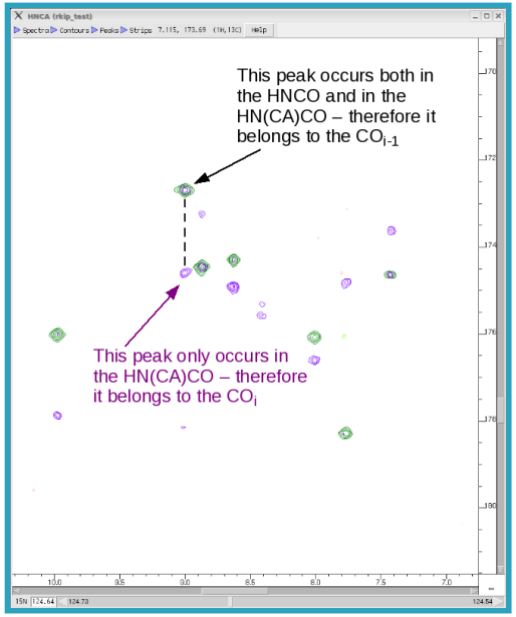
^{15}N, ^{13}C, ^{1}H HN(CA)CO Spectra

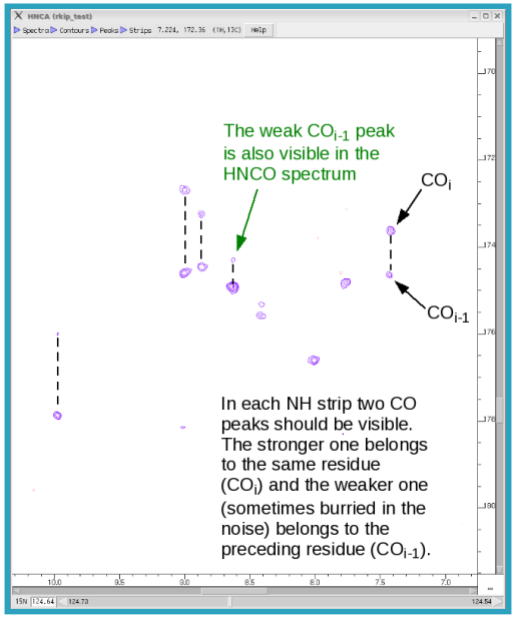
^{15}N and ^{13}C labelling are required

The magnetisation is transferred from ^{1}H to ^{15}N and then via the N-Cα J-coupling to the $^{13}C\alpha$. From there it is transferred to the ^{13}CO via the $^{13}C\alpha$-^{13}CO J-coupling. For detection the magnetisation is transferred back the same way: from ^{13}CO to $^{13}C\alpha$, ^{15}N and finally ^{1}H. The chemical shift is only evolved on ^{1}H, ^{15}N and ^{13}CO and not on the $^{13}C\alpha$. The result is a three-dimensional spectrum. Because the amide nitrogen is coupled both to the Cα of its own residue and that of the preceding residue, both these transfers occur and transfer to both ^{13}CO nuclei occurs. Thus for each NH group, two carbonyl groups are observed in the spectrum. But because the coupling between N_i and $C\alpha_i$ is stronger than that between N_i and $C\alpha_{i-1}$, the H_i-N_i-CO_i peak generally ends up being more intense than the H_i-N_i-CO_{i-1} peak.



This experiment can be useful for backbone assignment when used in conjunction with the HNCA, HN(CO)CA and HNCO if the CBCANNH and CBCA(CO)NNH spectra are of bad quality.



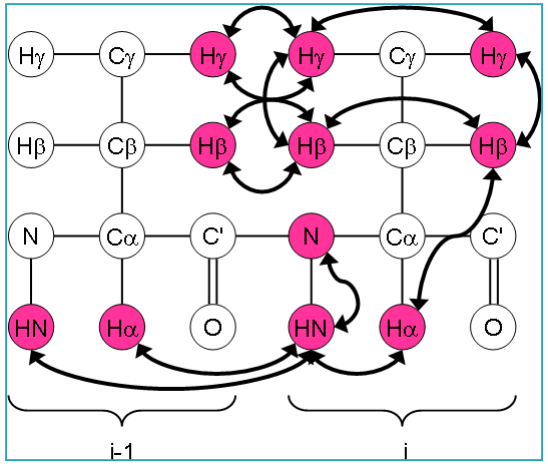


An overlay of the HNCO and HN(A)CO spectra makes it very easy to distinguish between CO_i and CO_{i-1} for each NH group.

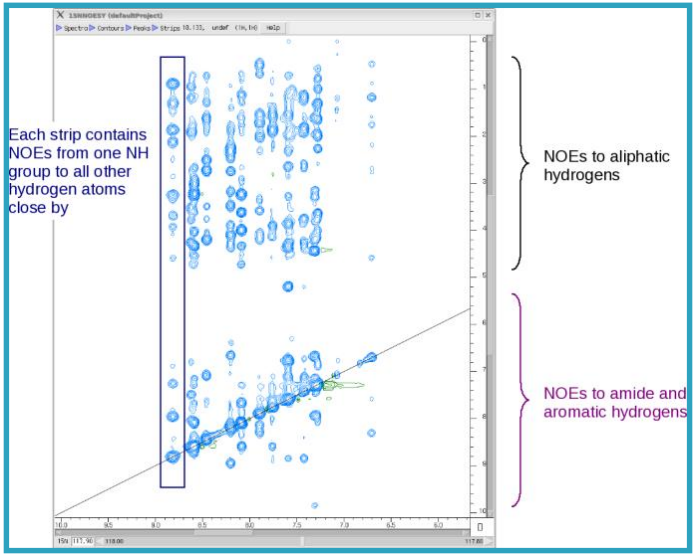
^{15}N, ^{1}H NOESY-HSQC Spectra

^{15}N labelling is required

To start with, magnetisation is exchanged between all hydrogens using the NOE. Then the magnetisation is transferred to neighbouring ^{15}N nuclei and back to ^{1}H for detection.



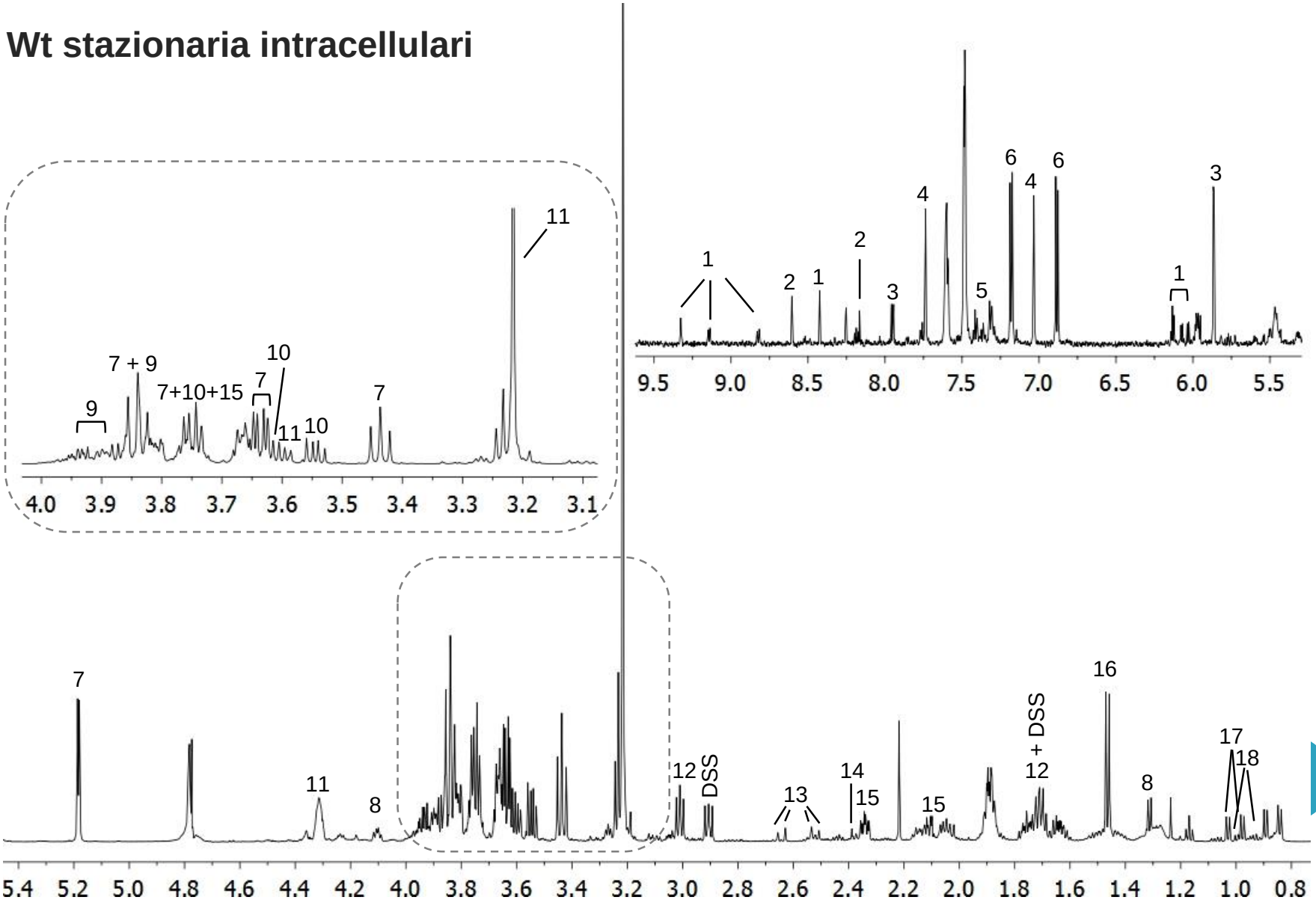
This spectrum can be used to obtain restraint for structure calculations. In this case the NOESY mixing time should probably be around 80ms. It can also be used to help assignment, and for small to medium-sized proteins, assignment using this and 15N-TOCSY-HSQC only is possible. In this case it may be useful to use a slightly longer NOESY mixing time.



NMR and Metabolomics

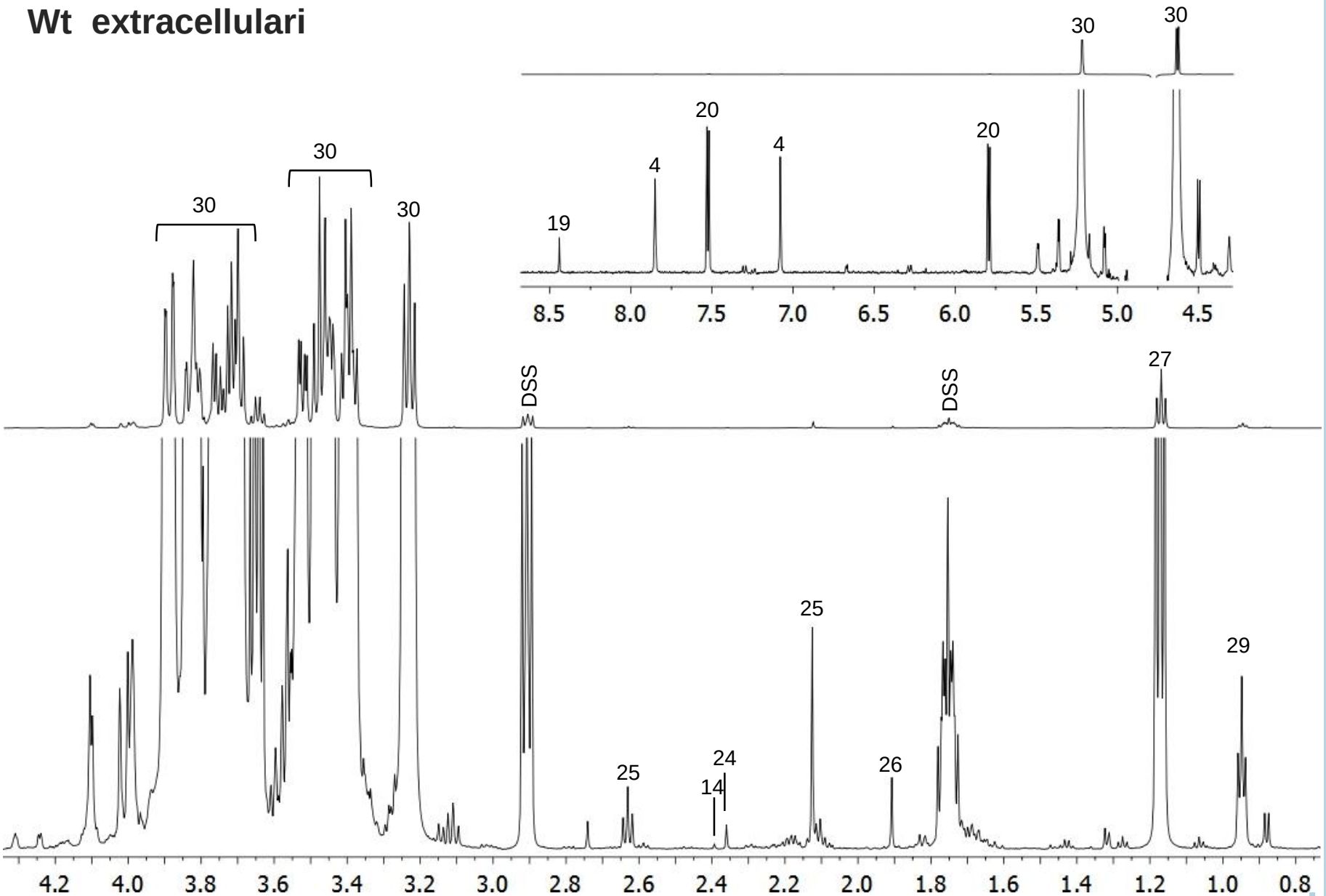
YEAST CELL METABONOMIC

Wt stazionaria intracellulari



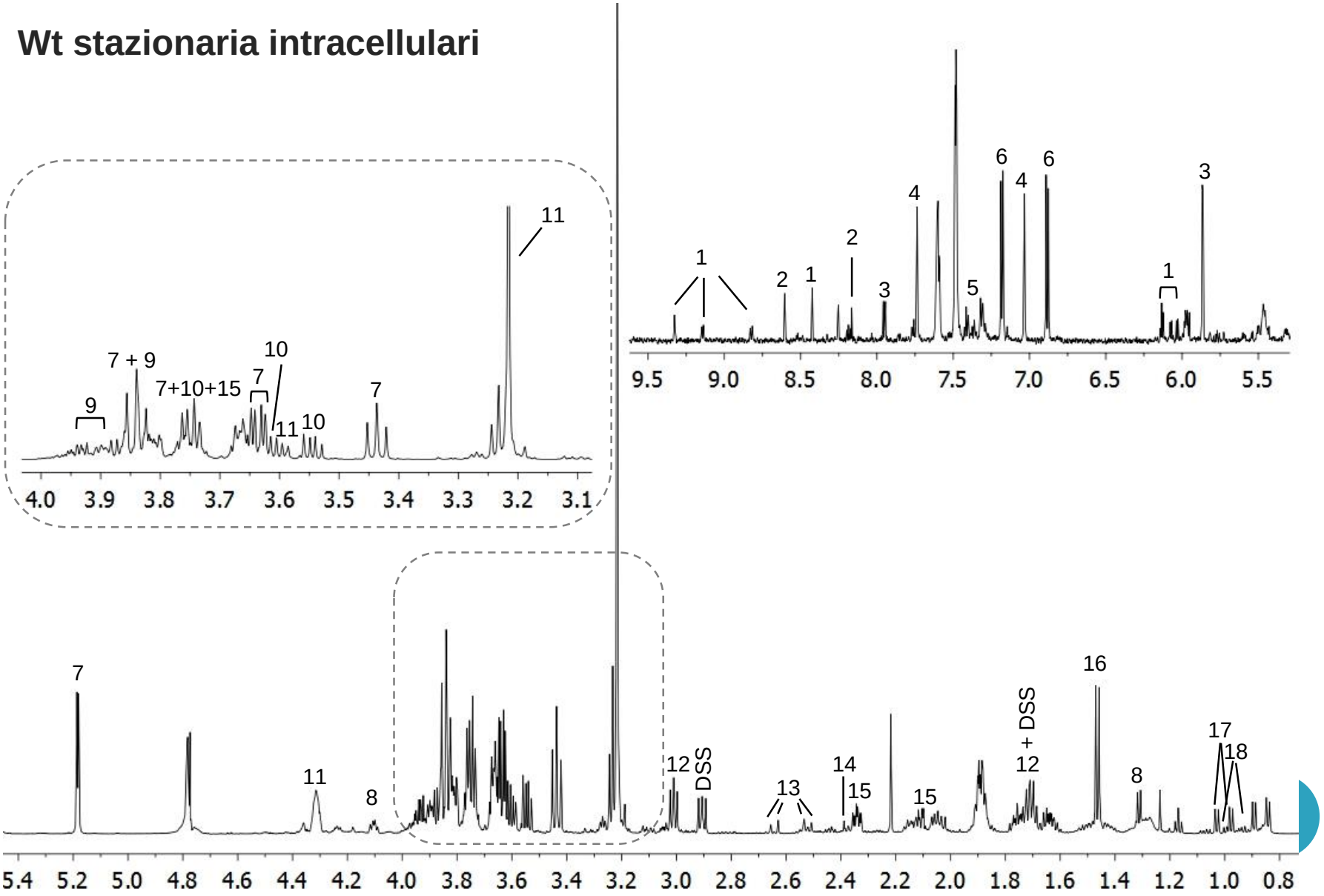
YEAST CELL METABONOMIC

Wt extracellulari



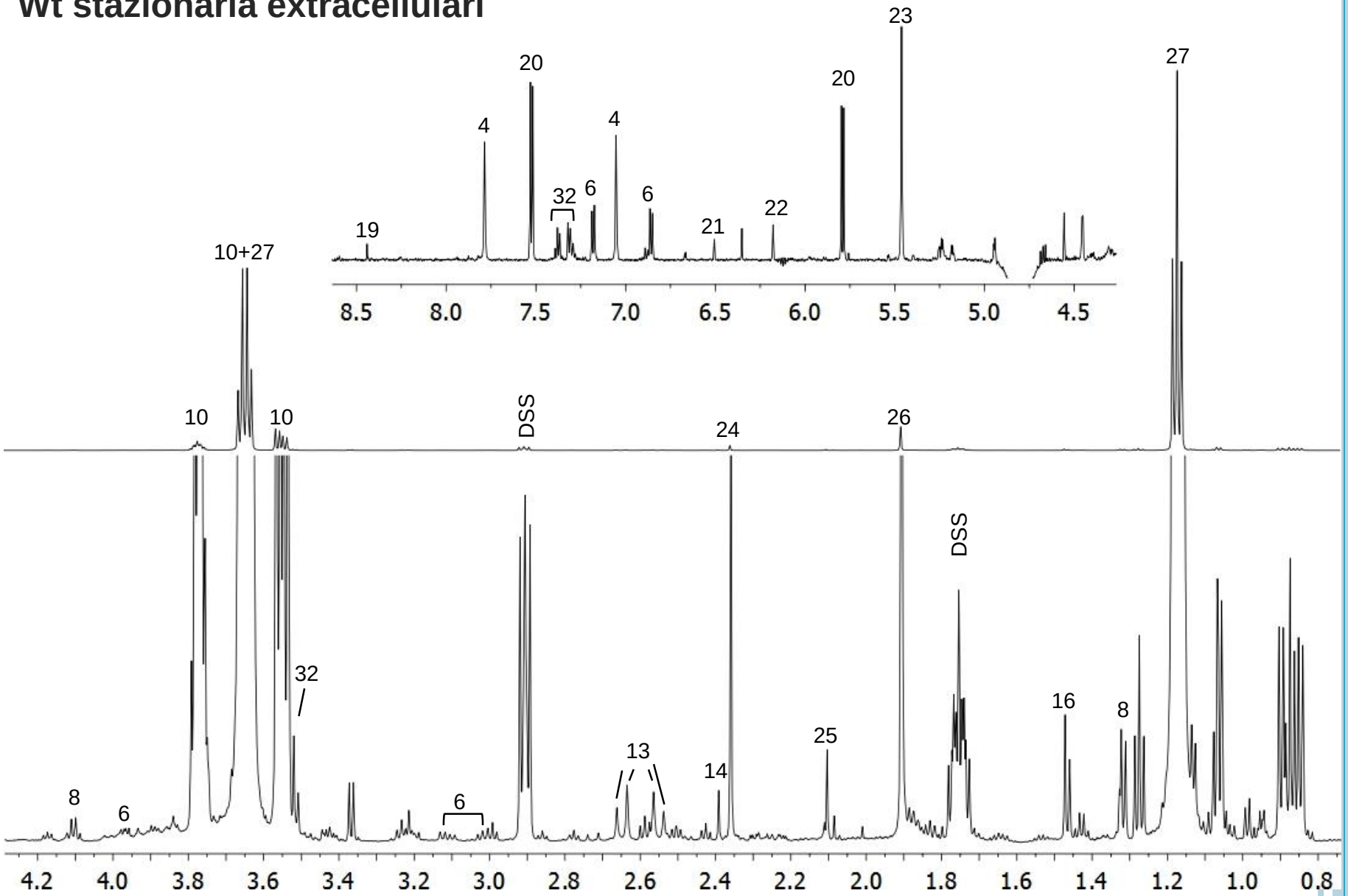
YEAST CELL METABONOMIC

Wt stazionaria intracellulari



YEAST CELL METABONOMIC

Wt stazionaria extracellulari



YEAST CELL METABONOMIC

Assigned number	Metabolite	chemical shift (ppm)[a]
1	NAD	9.33 (s) 9.15 (d) 8.83 (d) 8.42 (s) 8.19 (m) 6.13 (d) 6.08 (d) 6.02 (d)
2	AMP derivate	8.6 (s) 8.17 (s)
3	UDP derivate	7.95 (d)
4	Histidine	7.8 (s) 7.05 (s) 3.96 (dd) 3.22 (dd) 3.12 (dd)
5	Phenylalanine	7.42 (m) 7.36 (m) 7.32 (d) 3.97 (dd) 3.29 (dd) 3.12 (dd)
6	Tyrosine	7.18 (d) 6.89 (d) 3.97 dd 3.13 (dd) 3.02 (dd)
7	Trehalose	5.18 (d) 3.85 (m) 3.75 (dd) 3.64 (dd) 3.44 (t)
8	Lactate	4,11 (dd) 1.32 (d)
9	Serine	3.94 (m) 3.83 (dd)
10	Glycerol	3.77 (m) 3.65 (dd) 3.55 (dd)
11	Glycerophosphocholine	4.31 (m) 3.6 (dd) 3.22 (s)
12	Lysine	3.7 (m) 3.00 1.87 (m) (t) 1.71 (m) 1.45 (m)
13	Citrate	2.64 (d) 2.52 (d)
14	Succinate	2.39 (s)
15	Glutamate	3.74 (dd) 2.34 (dt) 2.05 (m)
16	Alanine	1.47 (d)
17	Valine	1.03 (d) 0.98 (d)
18	Isoleucine	1.00 (d) 0.94 (t)
19	Formate	8.44 (s)
20	Uracil	7.53 (d) 5.79 (d)
21	Fumarate	6.5 (s)
22	Uracil-6-carboxylate	6.18 (s)
23	Thiamine derivate	5.46 (s)
24	Pyruvate	2.36 (s)
25	Methionine	2.63 (t) 2.12 (s)
26	Acetate	1.91 (s)
27	Ethanol	3.65 (q) 1.71 (t)
28	Aspartate	3.88 (dd) 2.80 (dd)
29	Leucine	3.71 (m) 1.69 (m) 0.95 (t)
30	Glucose	5.22 (d) 4.64 (d) 3.89 (dd) 3.83 (m) 3.73 (m) 3.52 (dd) 3.46 (m) 3.40 (td) 3.23 (dd)
31	Threonine	1.31 (d) 4.24 (m)
32	Phenylacetate	3.52 (s) 7.38 (m) 7.30 (m)
33	Glutathione ox	3.30 ppm (dd) 2.96 ppm (dd)

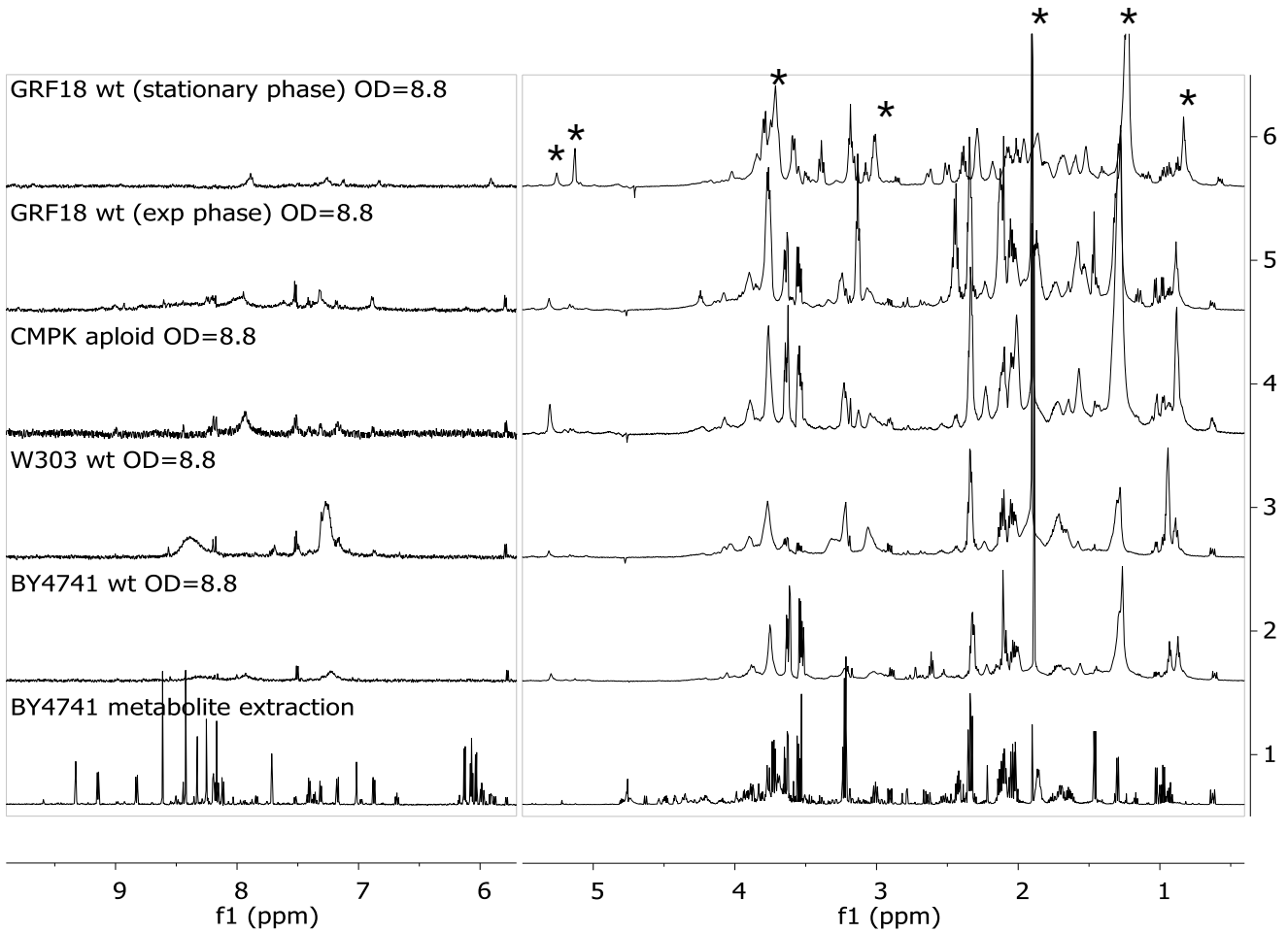
[a] chemichal shifs are referred to DSS and multiplicities showed in brackets. Abbreviation: (s) singlet, (d) doublet, (t) triplet, (m) multiplet , (dd) double doublet, (td) triple doublet.

Budding Yeast Metabolic Profiling working on Intact Cells

Farida Tripodi

Luca Brambilla

Paola Coccetti



- Metabolite extraction avoided
- Much faster sample preparation
- Presence of lipid resonances (*)

Manuscript in preparation

Clinical Metabolomics Studies

NMR metabolic profiling performed on **biofluids from patients**:

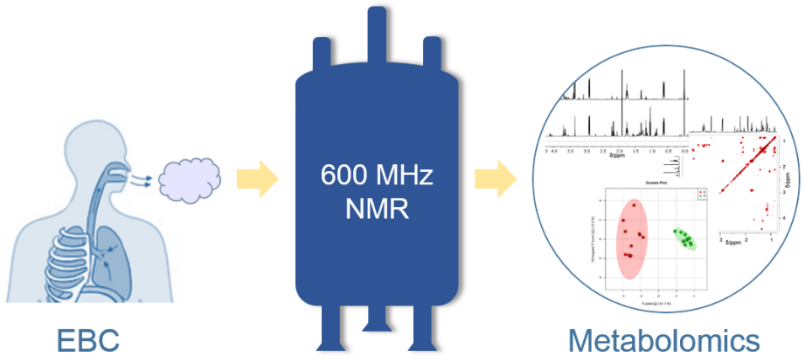
- **Serum** – collaboration with Prof. Stefano Aliberti (UNIMI and Policlinico) – Project VIBRO project VIBRO (12/2014-12/2017): Ruolo della colonizzazione ed infezione virale sulle riacutizzazioni ed ospedalizzazioni in pazienti affetti da bronchiectasie

- **Urine** – collaboration with Prof. Stefano Aliberti (UNIMI and Policlinico)

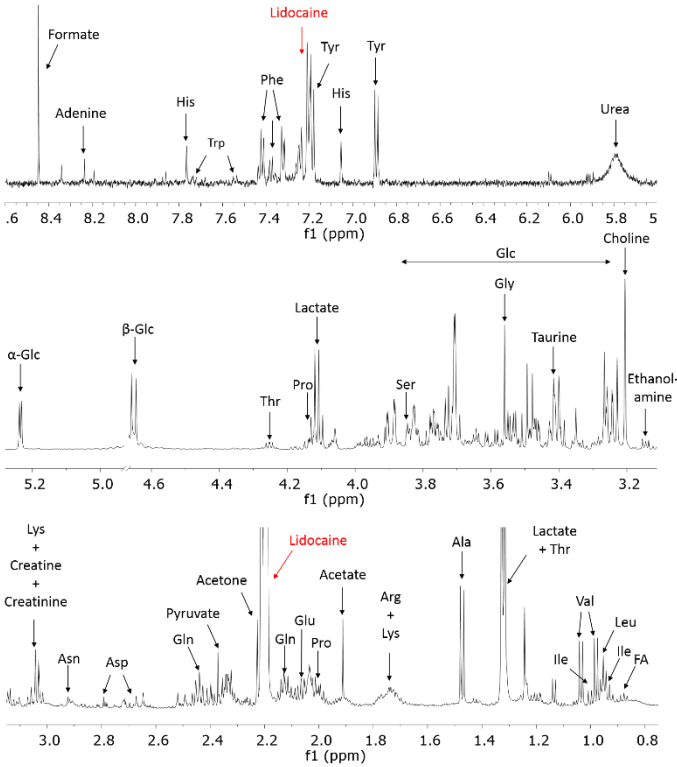
- **EBC** (Exhaled Breath Condensate) – collaboration with Prof. Jan Stolk, Leiden University Medical Center

- **BALf** (Broncho Alveolar Lavage fluid) – collaboration with Prof. Iadarola and Meloni, UNIPV and San Matteo Hospital) - Fondazione CARIPLO, project 2013-0820 (3/2014-12/2016): BALf metabolomics in chronic lung rejection: an innovative approach to identify predictive markers and sub-phenotypes.

Airoldi C., Ciaramelli C., Fumagalli M., Bussei R., Mazzoni V., Viglio S., Iadarola P., Stolk J., 1H-NMR to explore the metabolome of exhaled breath condensate in α1-antitrypsin deficient patients: a pilot study, J. Prot. Res., **2016**, 10.1021/acs.jproteome.6b00648



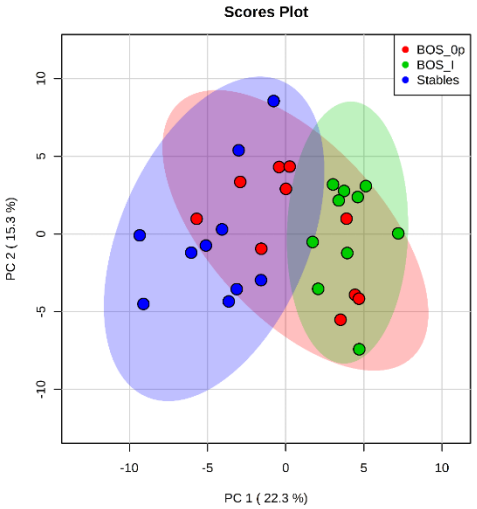
^{1}H-NMR TO EVALUATE THE METABOLOME OF BRONCHOALVEOLAR LAVAGE FLUID (BALF) IN BRONCHIOLITIS OBLITERANS SYNDROME (BOS)



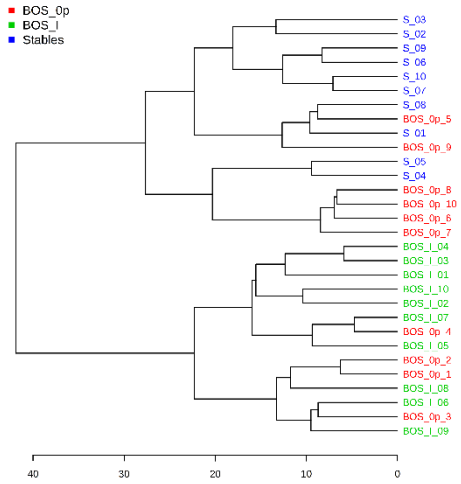
Discrimination of different pathological stages through BALf sample metabolic profiling

Ciaramelli C., Fumagalli M., Viglio S., Bardoni A. M., Piloni D., Meloni F., Iadarola P., Airoldi C., J. Proteome Res., ***2017****, 16, 4, 1669–1682*

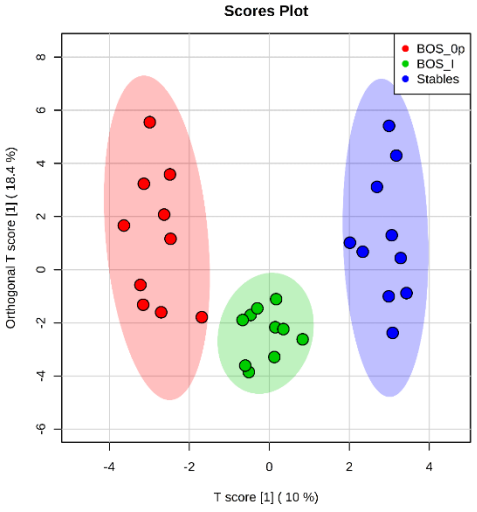
PCA Analysis



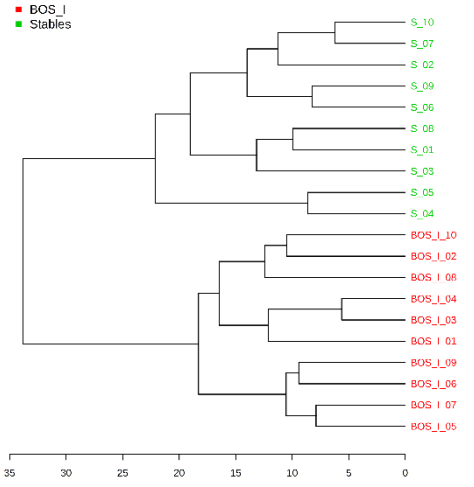
Cluster Analysis S vs BOS 0p vs BOS I



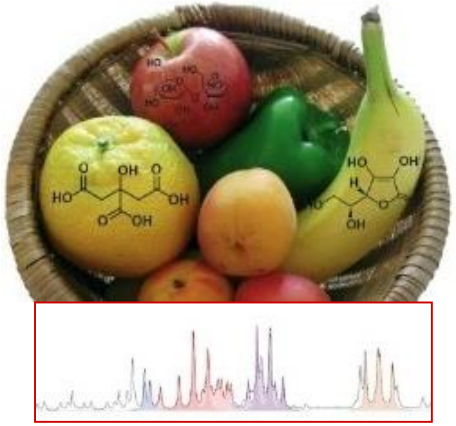
PLS-DA Analysis



Cluster Analysis S vs BOS I



Spettroscopia NMR nella scienza degli alimenti e della nutrizione



Applicazioni relative alla caratterizzazione di matrici alimentari, alla loro lavorazione e alla loro stabilità

- Struttura e Funzione
- Compositione
- **Qualità e sicurezza degli alimenti**
- **Identificazione di composti bioattivi tramite STD-NMR**