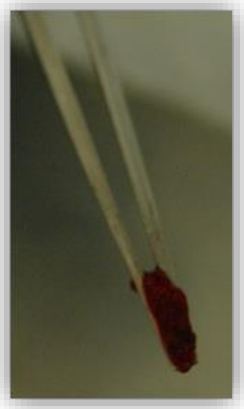
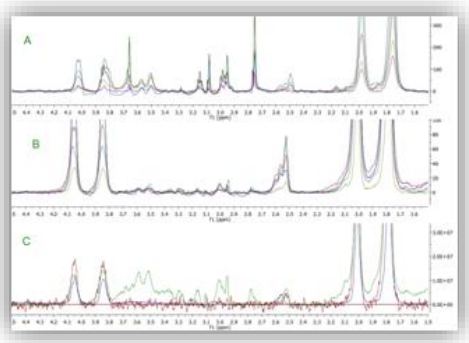
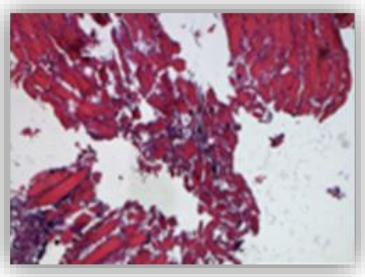
Metabolomics of Intact Tissues: Discrimination Between Different Regions of Osteolytic Lesions in Multiple Myeloma Patient using 1H HRMAS NMR spectra







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Metabolomics of Intact Tissues: Discrimination Between Different Regions of Osteolytic Lesions in Multiple Myeloma Patient using 1H HRMAS NMR spectra

- CLINICAL CONTEXT
- DESIGN OF THE EXPERIMENT and SAMPLING
- HR-MAS SPECTRA ACQUISITION and PROCESSING
- STATISTICAL ANALYSIS
- RESULTS and FUTURE PERSPECTIVES

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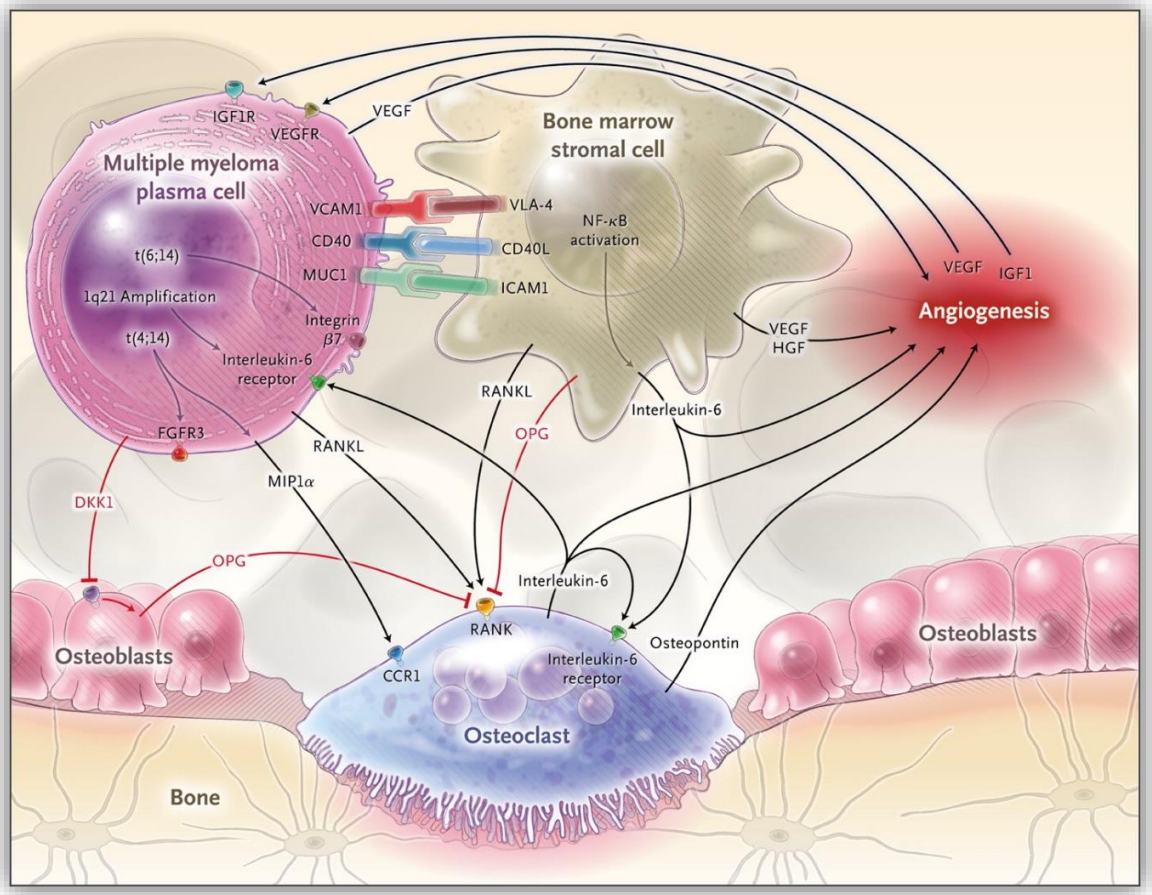
Osteolytic Bone Diseases



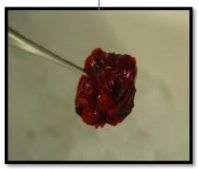
Breast cancer
Lung cancer
Renal cancer
Multiple myeloma
Adult T cell leukaemia

- **pathological bone fractures**
- **pain**
- **hypercalcaemia**
- **spinal cord and**
- **nerve-compression syndromes**

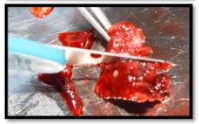
Lindon JC PNAS 55 (2009) 79–100
Beckonert Nature Protocols 5 (2010) 1019-1032
Weilbaecher KN Nature Review Cancer (2011) 411-425
Edwards CM Bone 42 (2008) 1007–1013

A 68-years old female, with no previous history of malignancy, transferred to San Raffaele Orthopaedics Unit for pathological fracture of undetermined origin

SAMPLING DURING PATIENT SURGERY



Feamale, age 68, newly diagnosed with IgG-secreting MM



STORED @ -80°C

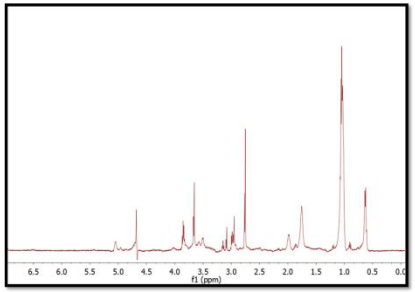
HR-MAS INSERT PREPARATION

FROM -80°C TO COLD ROOM

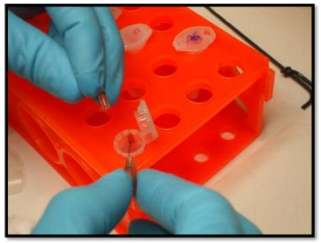
AGAIN STORED @ -80°C

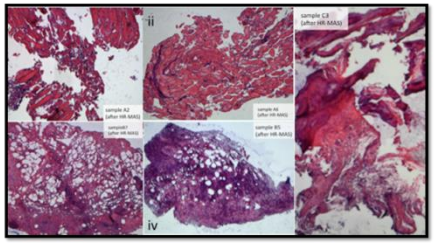
NMR SPECTRA ACQUISITION AND SAMPLE RECOVERY

@ 10°C



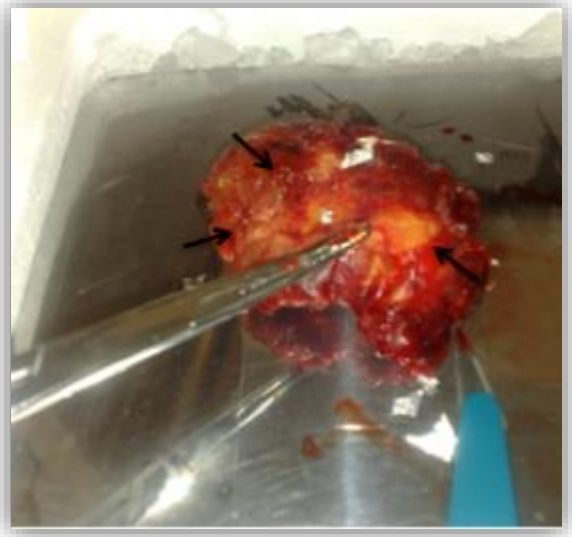
After approximately 20 minutes of experiment sample were remove from insert and place into formalin for further histopathological analysis

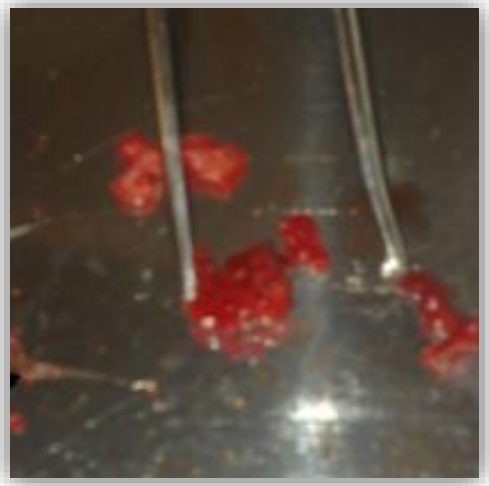




HR-MAS spectra
Standard 1dnoesypr and 1dcpmgpr
Rotation 4kHz, T 10°C
NS 120; size 16k
(jres experiments acquired also on selected samples)

Bioptical tumor specimen lying on a metal plate on an ice-bed (in a 4°C room) for sample preparation





Muscle fragments classified as **"A"**, retaining normal gross morphology and consistency as suggested by macroscopic fibers

Result of longitudinal cut of the specimen.

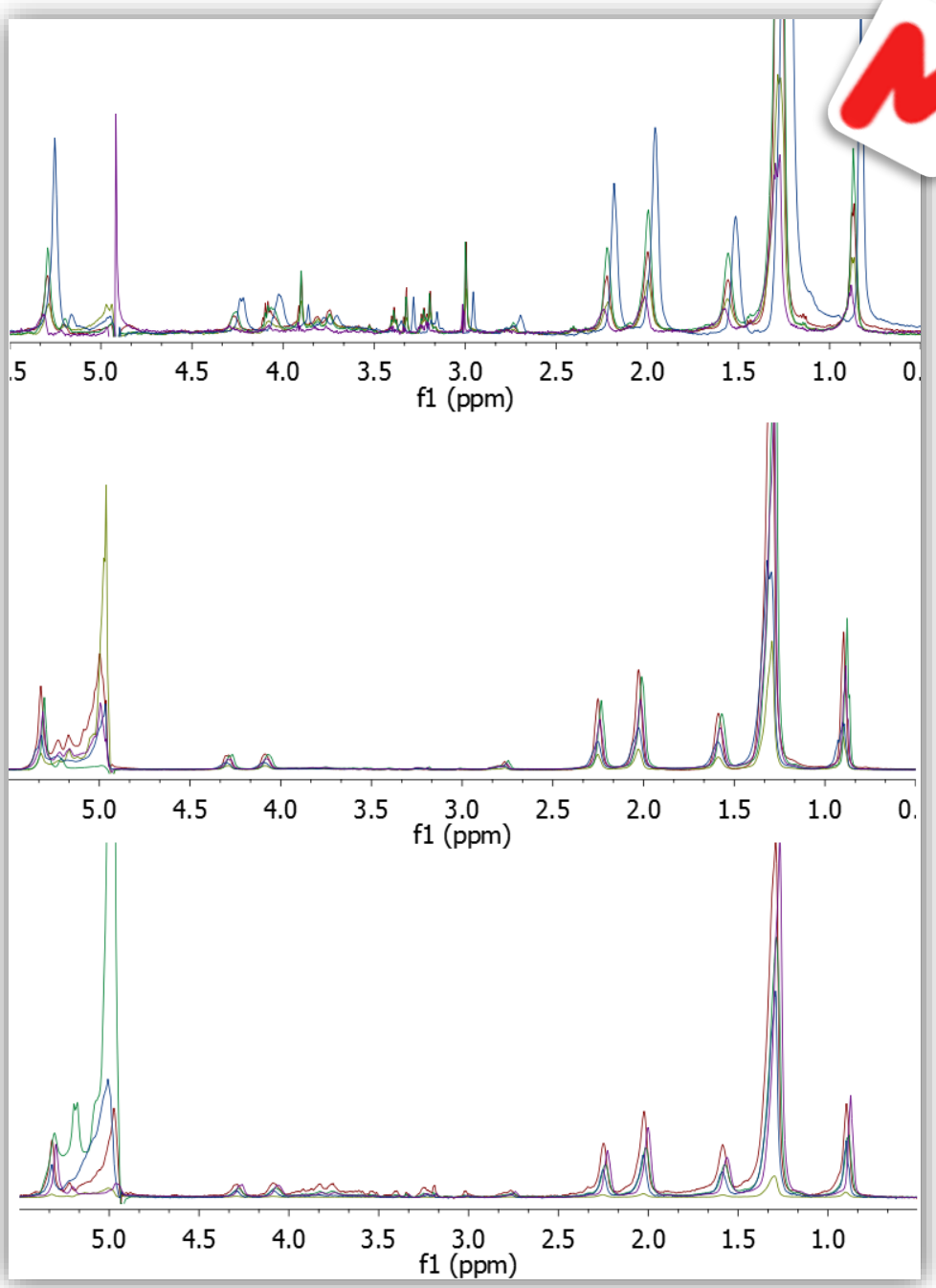
A more lardaceous region was labeled as **"B"**.

A harder area was identified with calcified appearance, which was classified as **"C"**.

Samples A
muscle

Samples B
bone lesion (lipid part)

Samples C
bone lesion (calcified part)



- GLOBAL ALIGNMENT
- GLOBAL SPECTRAL DECONVOLUTION (GSD)
- SYNTHETIC SPECTRA GENERATION
- MATRIX GENERATION FOR STATISTICAL ANALYSIS

- **GLOBAL ALIGNMENT**
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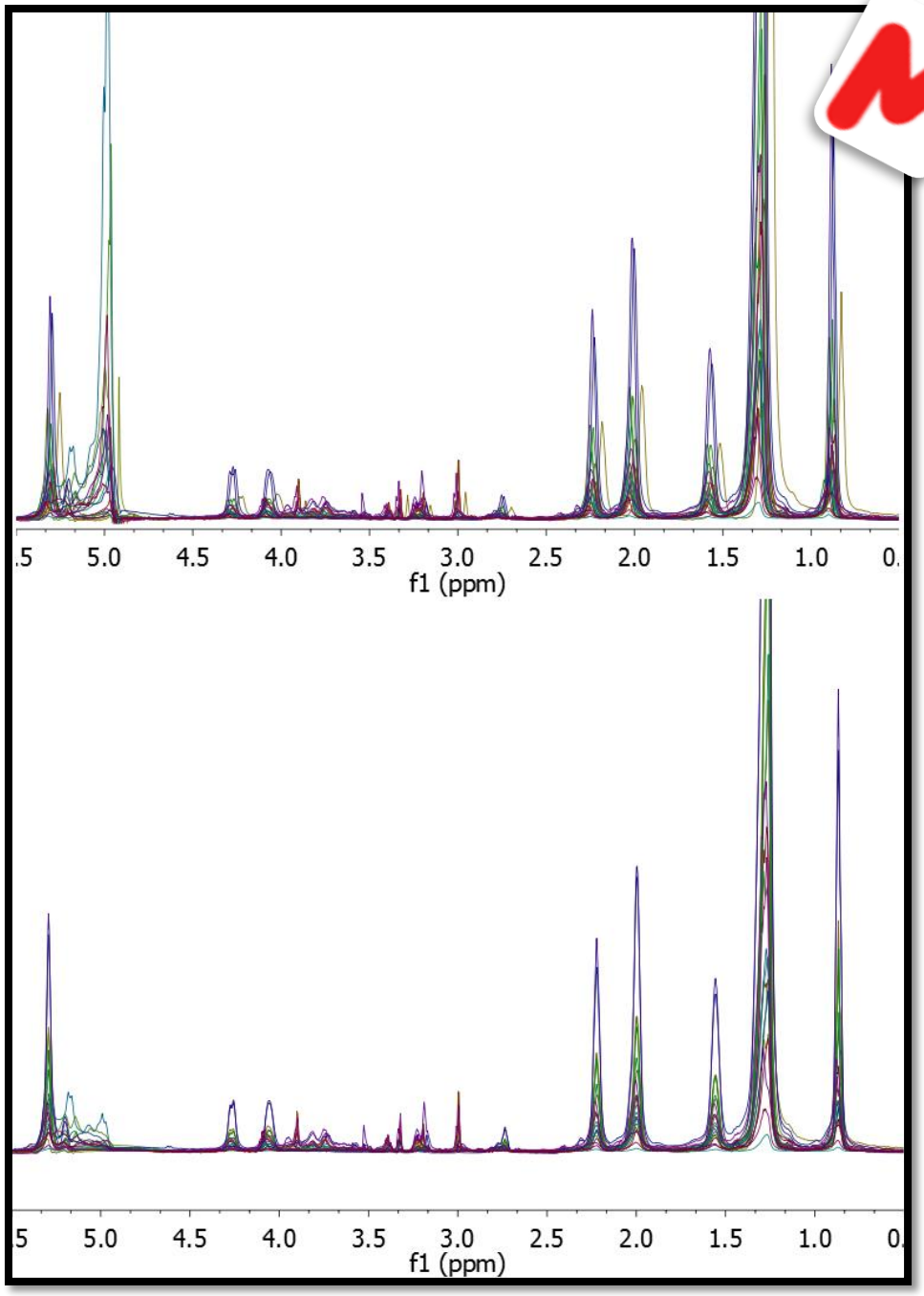

- GLOBAL ALIGNMENT
- **GLOBAL SPECTRAL DECONVOLUTION (GSD)**
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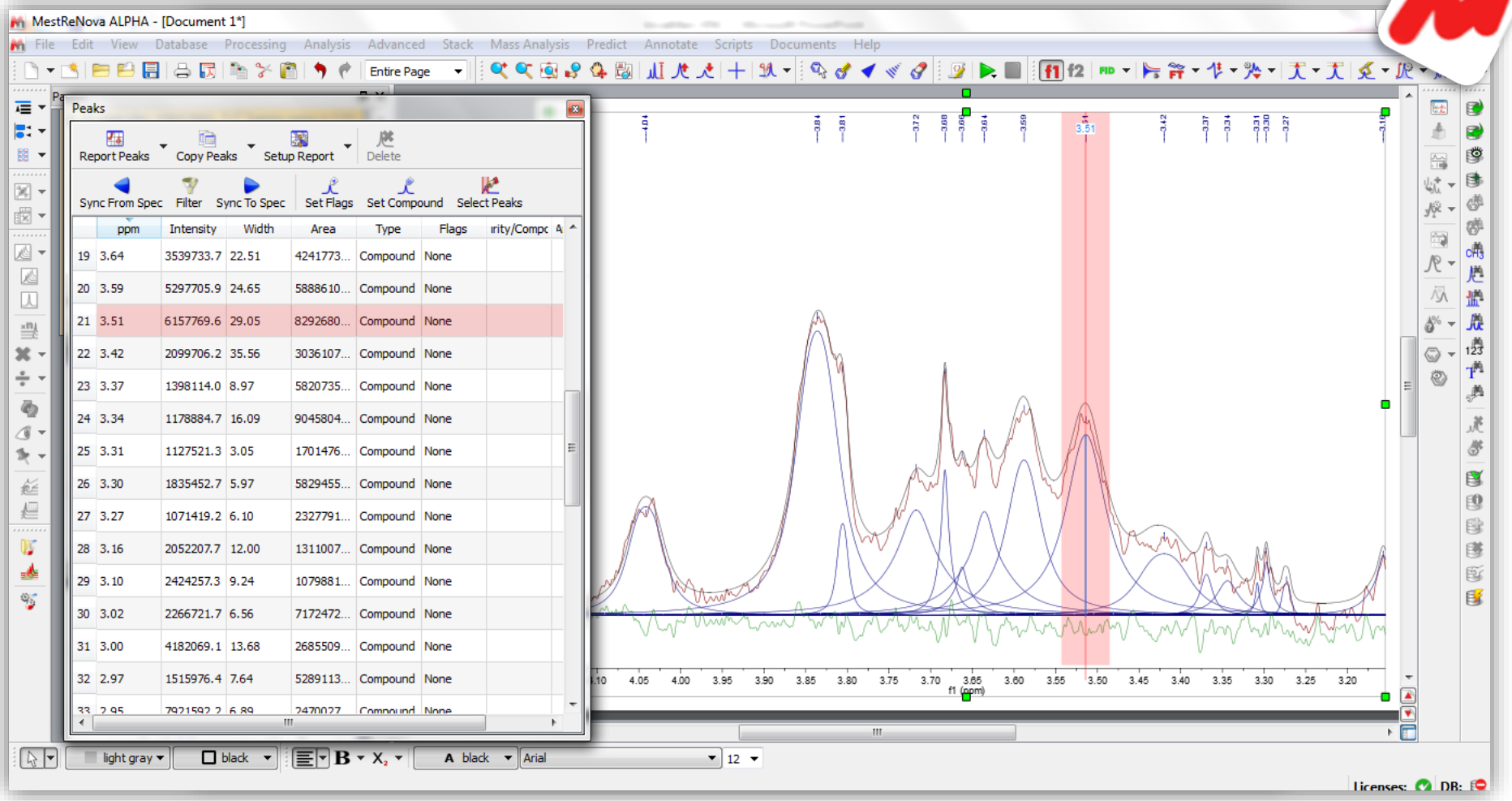
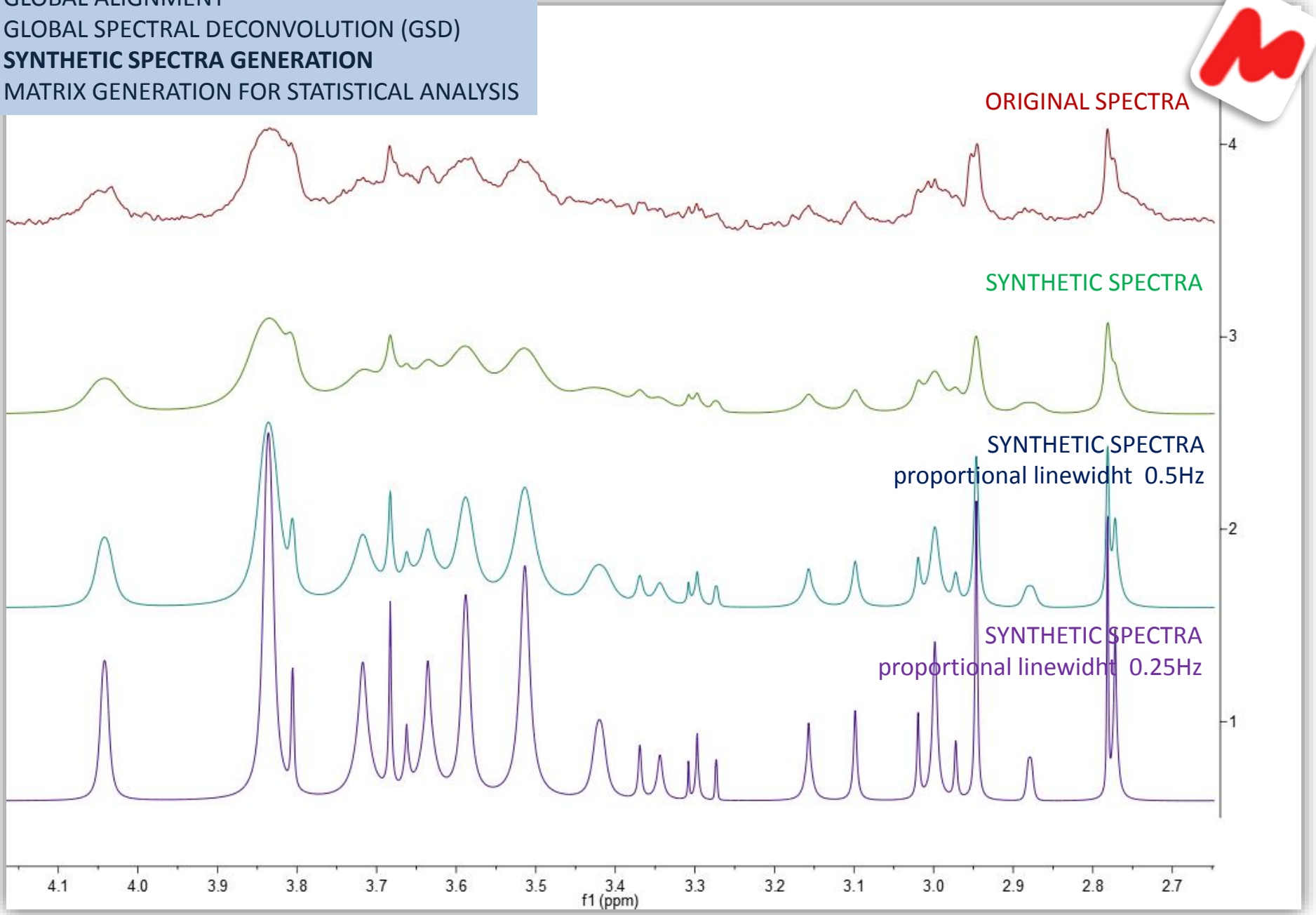


- GLOBAL ALIGNMENT
- GLOBAL SPECTRAL DECONVOLUTION (GSD)
- **SYNTHETIC SPECTRA GENERATION**
- MATRIX GENERATION FOR STATISTICAL ANALYSIS



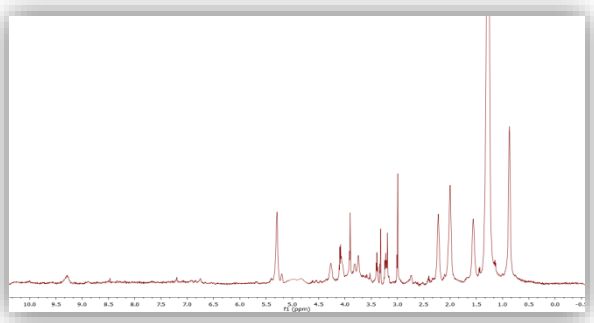
MATRIX GENERATION:

1. Regular binning

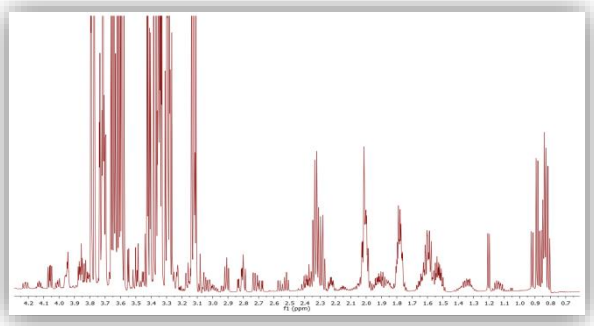
2. Variable size binning

3. GSD peak table binning

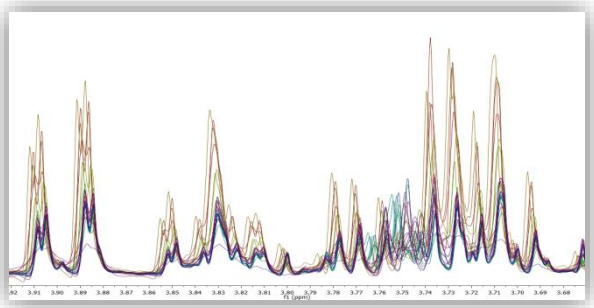
TYPICAL APPLICATION:



•Mixture with few components (typically less than 10);
•Suitable to be implemented in automatic statistical analysis (no prior information of peak positions) and for model construction and use



•Complex Mixture (typically more than 50 components);
•Suitable when dealing with similar spectra and small variation have to be detected (prior information of peak positions is an advantage).

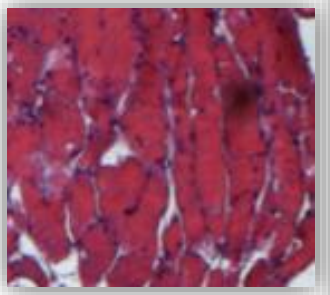


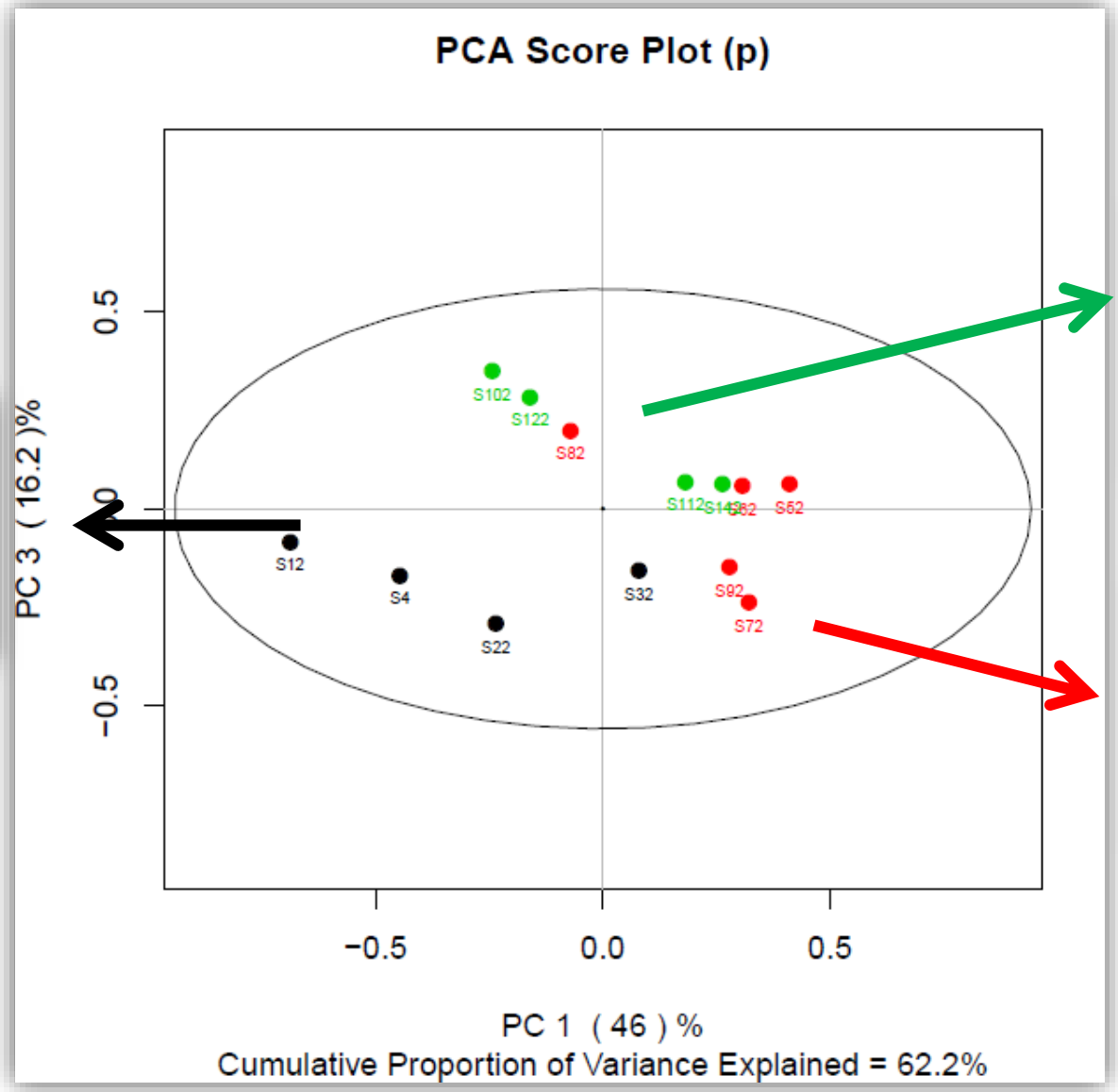
•Suitable as regular binning when dealing with complex mixtures;
•Particular useful when dealing with peak shifts difficult to be aligned with standard tools.

MUMA package (**M**ultivariate and **U**nivariate **M**etabolomic **A**nalysis)

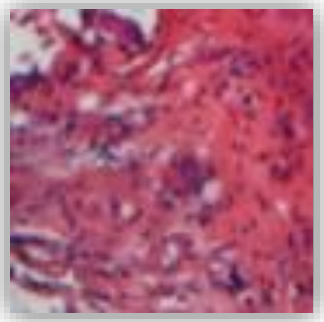
- Prepocessing Data *(Pareto ,Auto, Vast, Range)*
- Principal Components Analysis (PCA)
- Univariate Analysis *(Shapiro Wilk, Welch and Mann Whitney-Wilcoxon test)*
- Merge Univariate and Multivariate information
- Partial Least Square Discriminant Analysis (PLS-DA)
- OPLS-DA analysis
- STOCSY and OSTOCSY analysis
- RANSY analysis

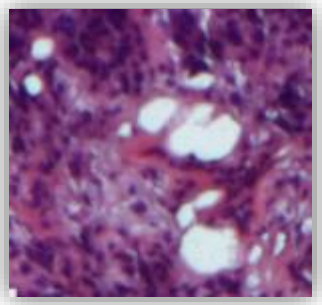
A: muscle



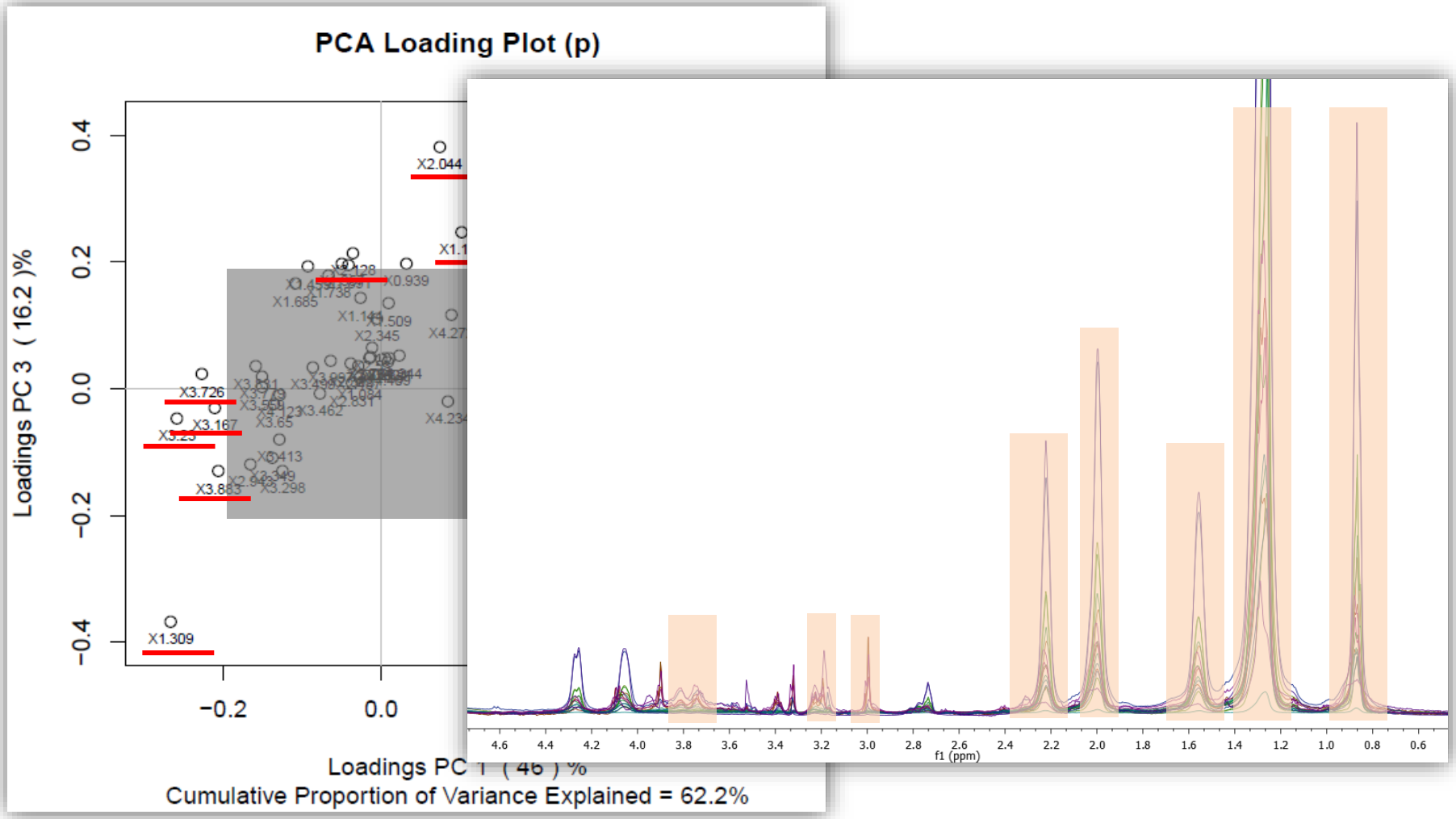


C: increase ECM





B: anaplastic growth


PCA Loading Plot (p)
Loadings PC 3 (16.2)%
Loadings PC 1 (46) %
Cumulative Proportion of Variance Explained = 62.2%
X2.044
X1.309
X3.726
X3.167
X3.883
X0.939
X1.685
X2.345
X4.27
X4.234
X3.462
X3.65
X3.298
f1 (ppm)

CONCLUSIONS and PERSPECTIVES:

- Reproducible 1H-NMR spectra can be obtained by HR-MAS NMR on samples from bone lesions in multiple myeloma.

- Histopathological analysis could be performed on the very same biopsy and nicely correlate with HR-MAS NMR data.

- From the translational point of view, metabolomics analysis of bioptical samples may be used in the future to improve prognostic stratification or differential diagnosis.

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Stanislav Sykora

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