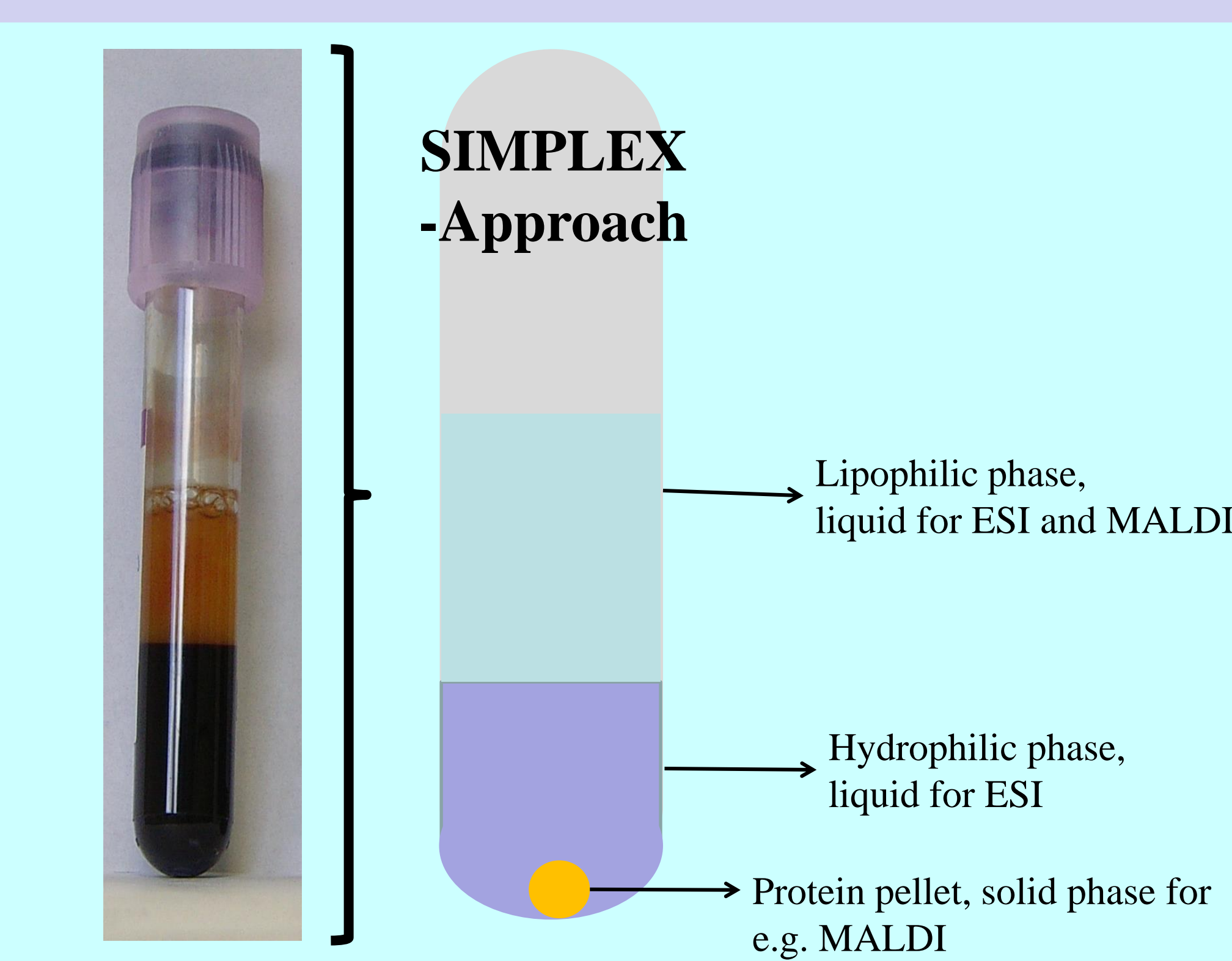


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Introduction

Since **May 2016** a state-of-the-art ultra-high resolution mass spectrometry system is installed at the division of Food Technology. Based on this system, the lipid- and metabolomics approach will be applied to the SH-community. The system allows the analyses of complex samples without a chromatographic separation ("shotgun"). This analyses have a high precision, accuracy and used the isotopic fine structure for sum formula generation and validation.



SIMPLEX (Simultaneous Metabolite, Protein, Lipid Extraction)

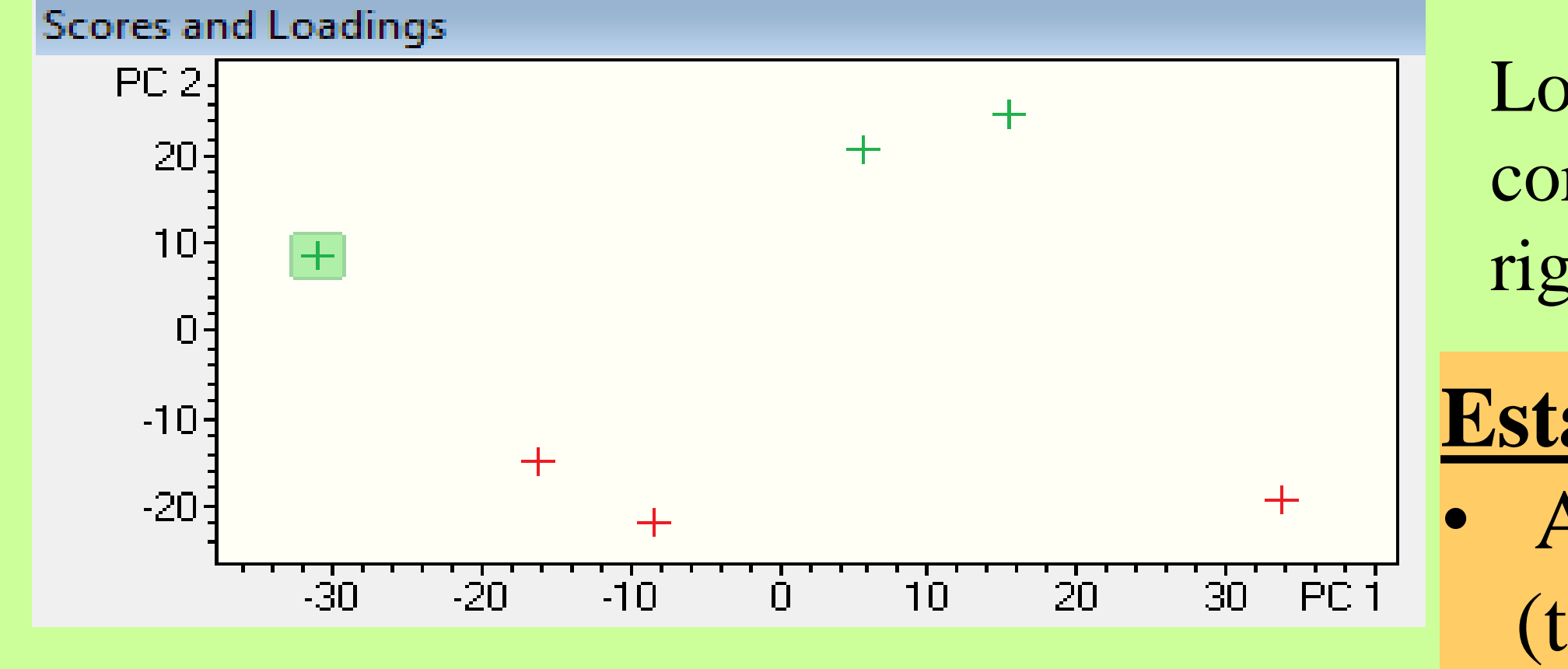
- liquid-liquid extraction
- low needed sample volume (> 50 µl)
- applicable for solid or liquid particles
- clearly defined phase boundaries (compared to e.g. Folch for lipid extraction)
- rapid and useful for high-throughput
- solvents: tert-methyl-butyl ester (MTBE, non-polar), water, methanol & formic acid (polar)

Omics of cell fluids [Application example]

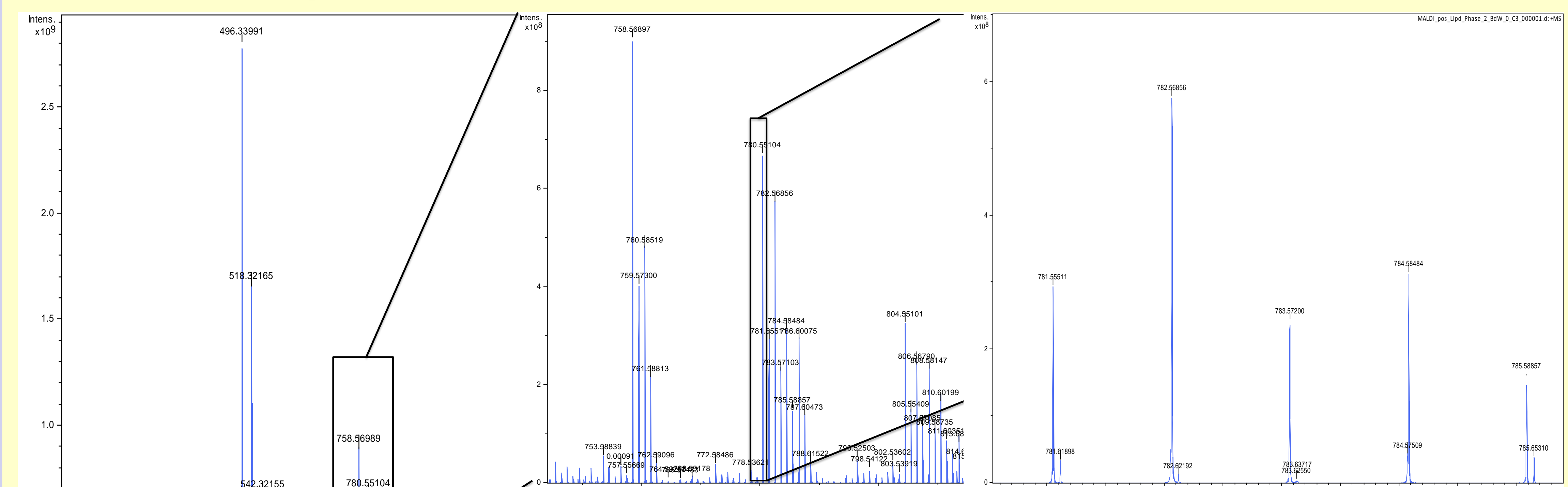
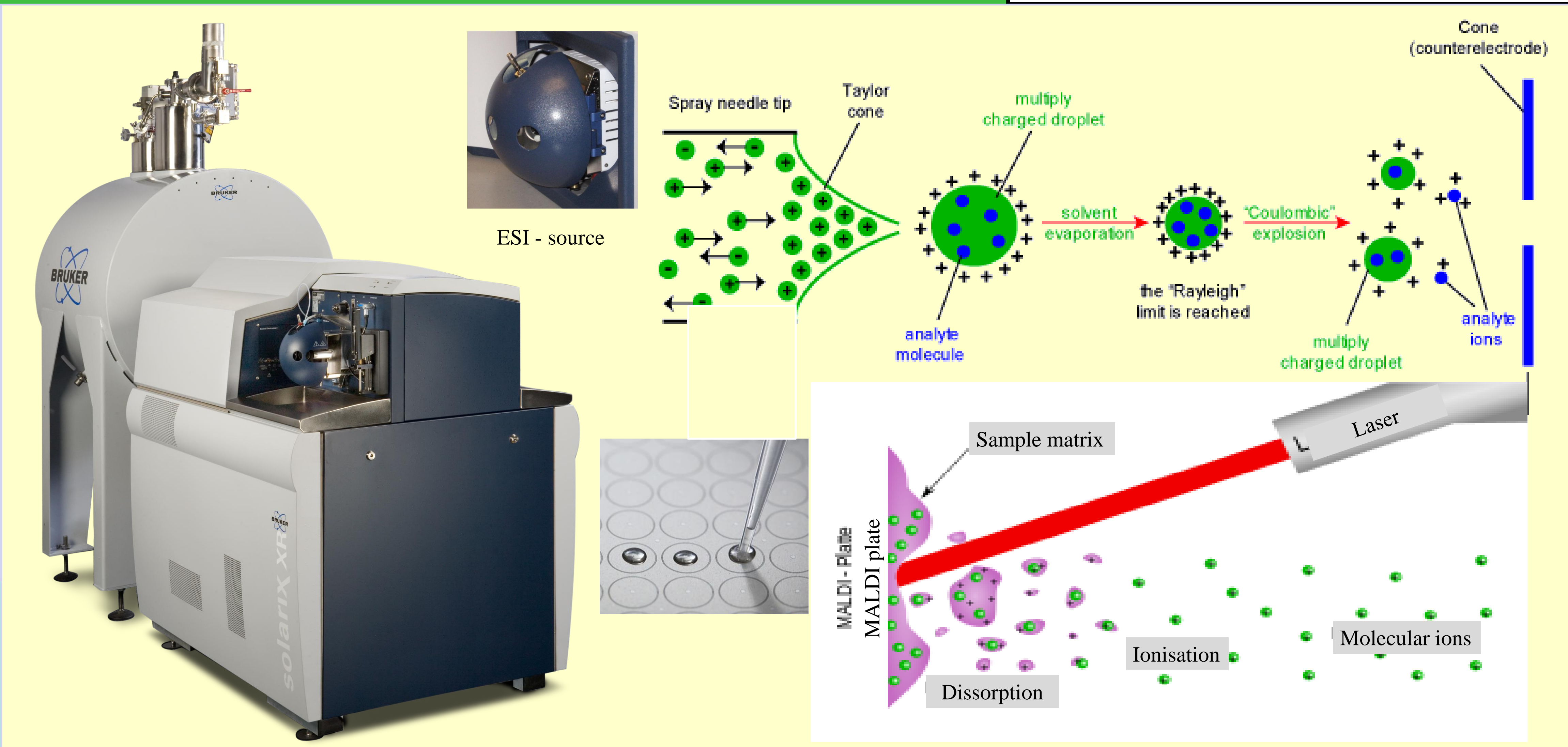
- Human cell lines
- Cultivated at standardized conditions
- treated vs. non-treatment [Control]
- Treatment: milk-oligopeptides
- Expected effects: reduced inflammatory values within the treated cells

| Bucket | Name / Formula | Include | 9_U_pos_P1 | 10_U_pos_P1 | 11_U_pos_P1 | 12_U_pos_P1 |
|--------|----------------|---------|------------|-------------|-------------|-------------|
| 28132 | 2896.52m/z | ✓ | 2722610.33 | 3780114.99 | 3838477.68 | 3262075.35 |
| 28133 | 2897.32m/z | ✓ | 2863252.11 | 3109770.96 | 2909674.99 | 3280647.79 |
| 28134 | 2898.50m/z | ✓ | 3858527.02 | 5383205.90 | 2986921.05 | 3091604.61 |
| 28135 | 2899.41m/z | ✓ | 4115373.74 | 2783660.31 | 3033520.42 | 3298126.58 |
| 28136 | 2900.69m/z | ✓ | 4216761.87 | 3166143.86 | 4402484.31 | 4126164.54 |
| 28137 | 2901.07m/z | ✓ | 4165679.75 | 3930766.15 | 1875630.10 | 4088505.71 |
| 28138 | 2902.12m/z | ✓ | 2771042.56 | 2706782.58 | 2978632.93 | 4426076.98 |
| 28139 | 2904.23m/z | ✓ | 2251108.88 | 3547851.32 | 3222137.30 | 2618149.29 |
| 28140 | 2905.44m/z | ✓ | 3367033.37 | 3246423.87 | 3541412.21 | 4120276.17 |
| 28141 | 2906.60m/z | ✓ | 3459084.95 | 4585007.42 | 3801024.90 | 3046230.30 |
| 28142 | 2908.61m/z | ✓ | 2447838.12 | 3167862.77 | 3061824.01 | 3884389.33 |
| 28143 | 2909.40m/z | ✓ | 3081824.01 | 2731212.24 | 3359211.20 | 4194488.54 |
| 28144 | 2910.68m/z | ✓ | 2706782.58 | 4042628.07 | 4042628.07 | 2106910.35 |
| 28145 | 2911.04m/z | ✓ | 2390067.07 | 2517212.17 | 2757548.04 | 2681073.06 |
| 28146 | 2912.11m/z | ✓ | 2491930.44 | 2460158.89 | 2854240.90 | 2168365.18 |
| 28147 | 2913.81m/z | ✓ | 3759274.75 | 2681073.06 | 2476786.49 | 3895451.73 |
| 28148 | 2916.00m/z | ✓ | 1965953.75 | 2667260.57 | 3430925.70 | 2917746.86 |
| 28149 | 2917.00m/z | ✓ | 1380460.12 | 2643171.46 | 3091604.61 | 2603854.76 |
| 28150 | 2918.45m/z | ✓ | 3547851.32 | 3124319.15 | 3759274.75 | 2667260.57 |
| 28151 | 2919.56m/z | ✓ | 2986921.05 | 3336446.00 | 2220314.83 | 2771042.56 |
| 28152 | 2919.97m/z | ✓ | 5193439.12 | 4132159.55 | 2667260.57 | 4549913.04 |
| 28153 | 2920.36m/z | ✓ | 2592636.61 | 1645819.80 | 2505750.71 | 2630678.89 |
| 28154 | 2920.94m/z | ✓ | 3515193.21 | 3438291.11 | 2357154.81 | 4453602.37 |
| 28155 | 2924.40m/z | ✓ | 3831422.02 | 3425317.54 | 3280647.79 | 3553983.20 |

Bucket table of six samples (3x treated & 3x controls) => more than 28,000 metabolites

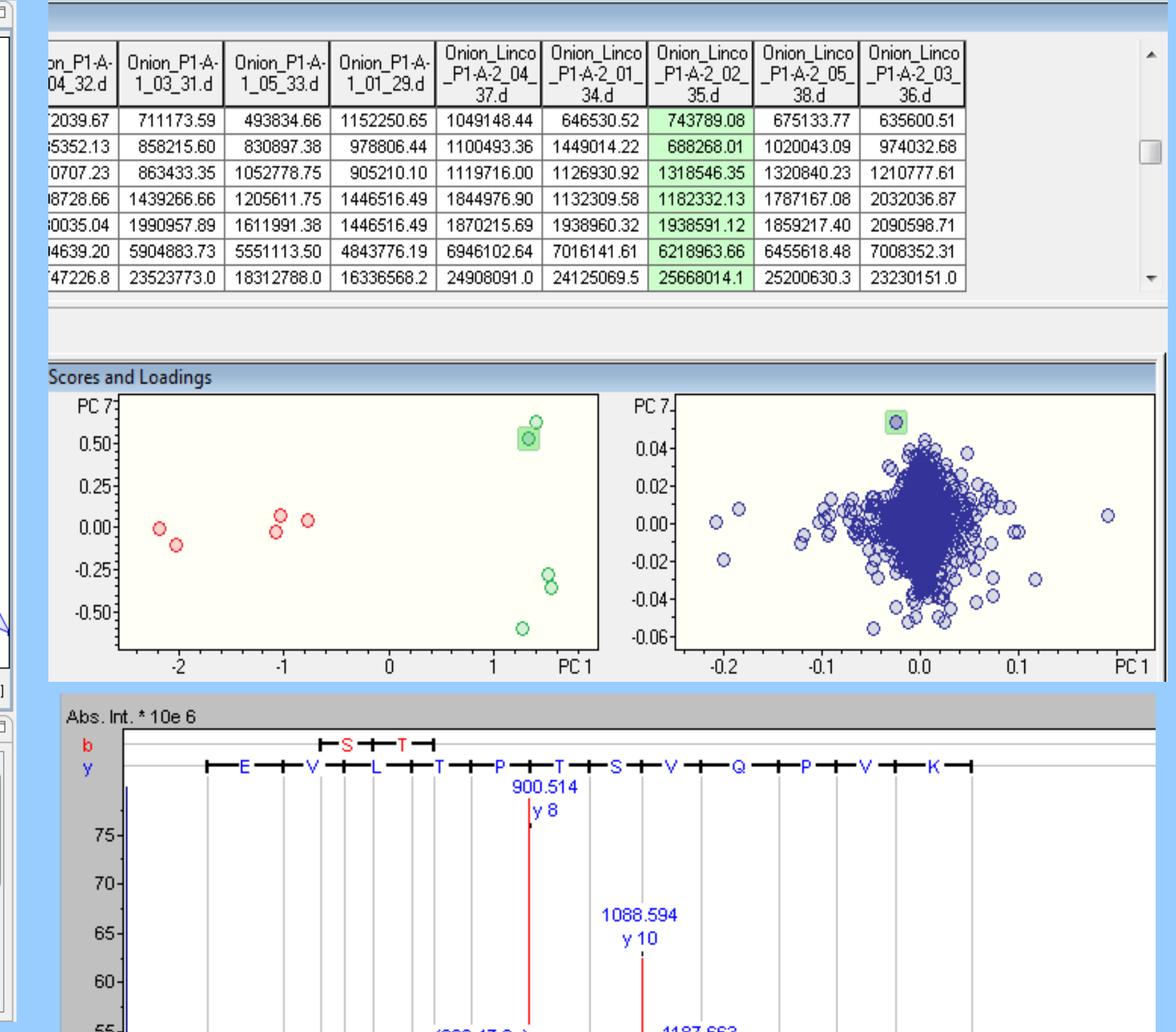
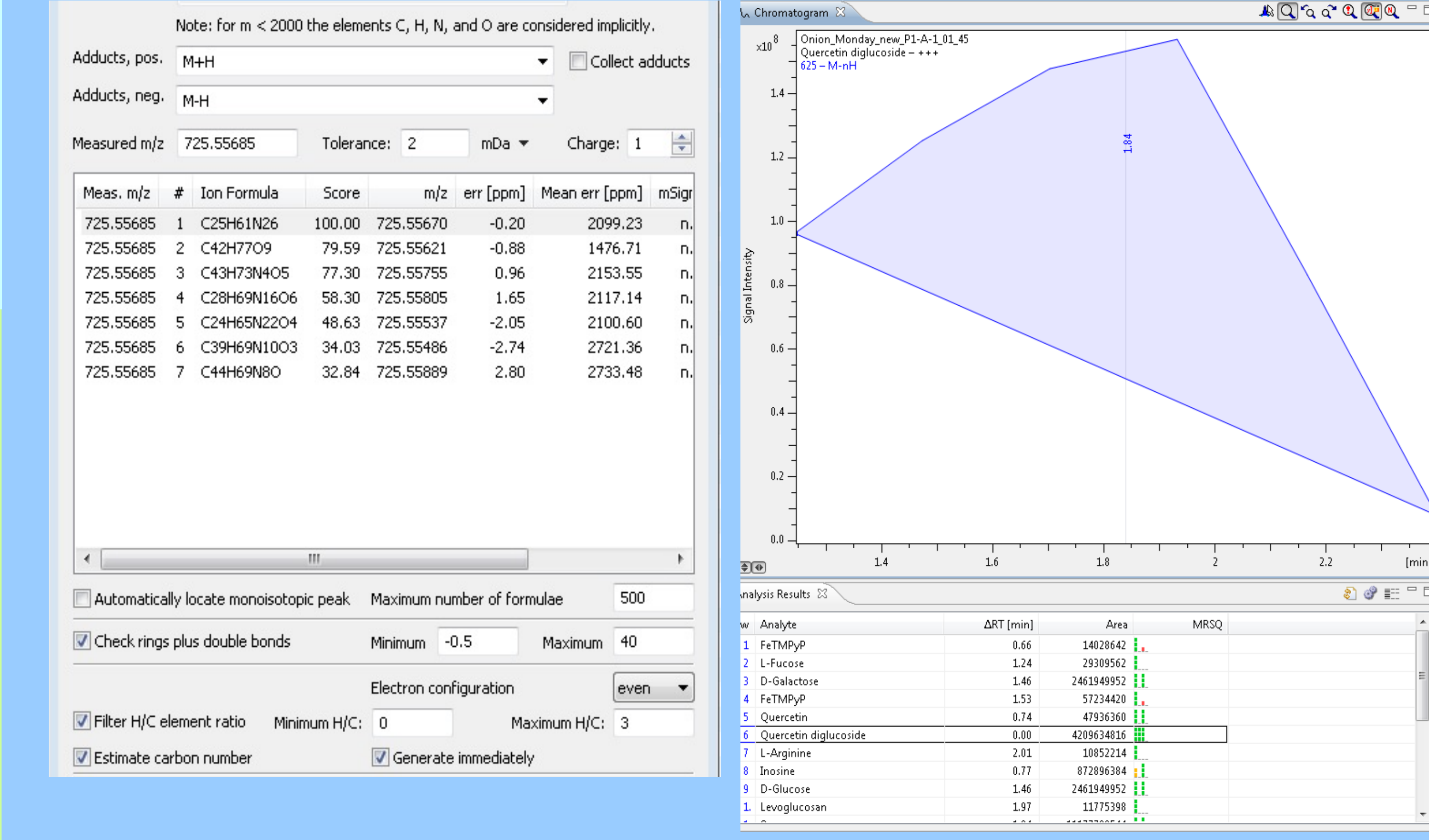


Score-plot (PCA model) of the cell lines; green pluses = treated, red = treated



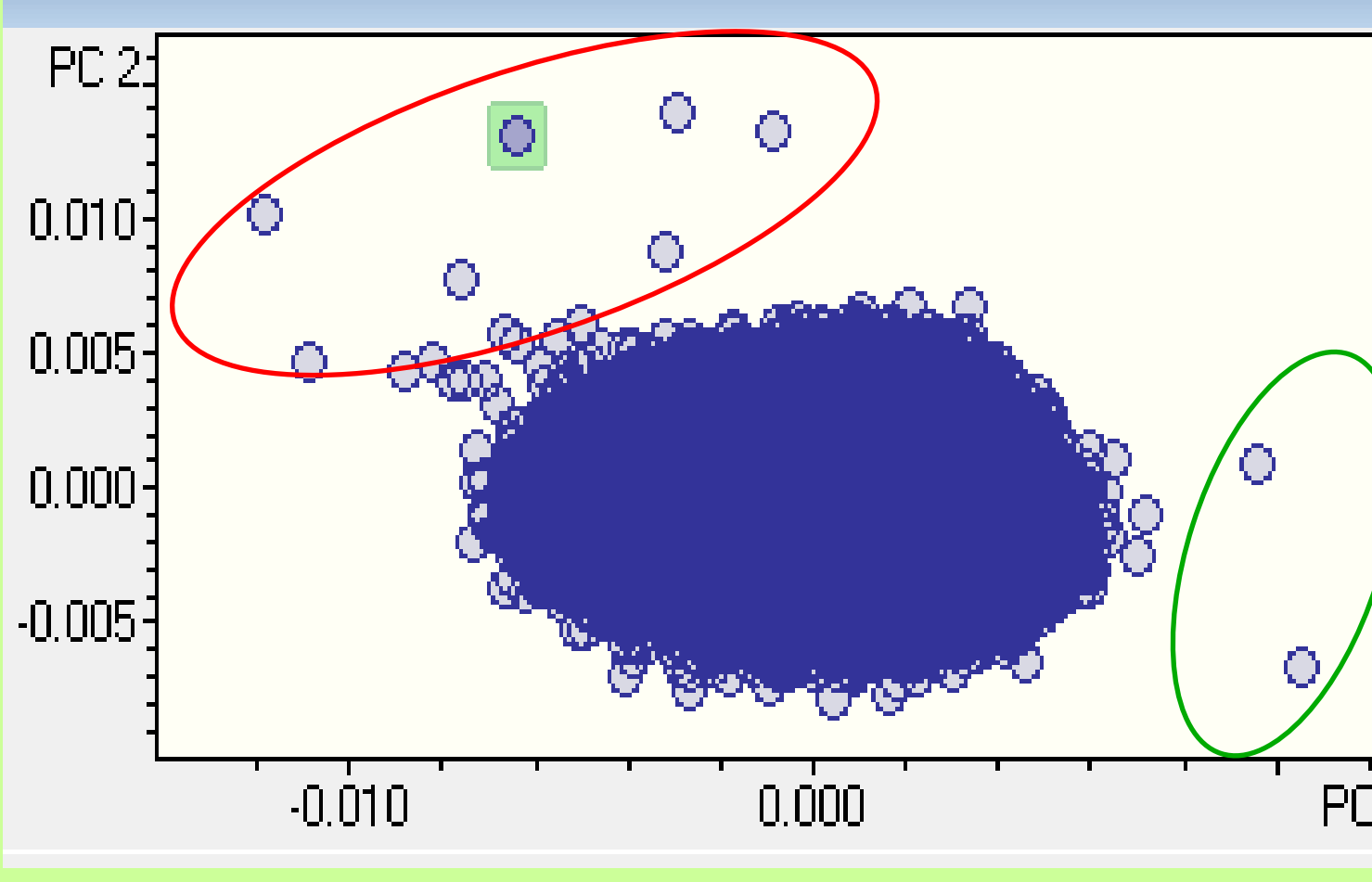
Isotopic fine structure:

- Resolution of > 2 Mio. (depends on settings)
- 4 million data-points
- Identification of isotopes (sulfur, phosphate, carbon etc.) per peak

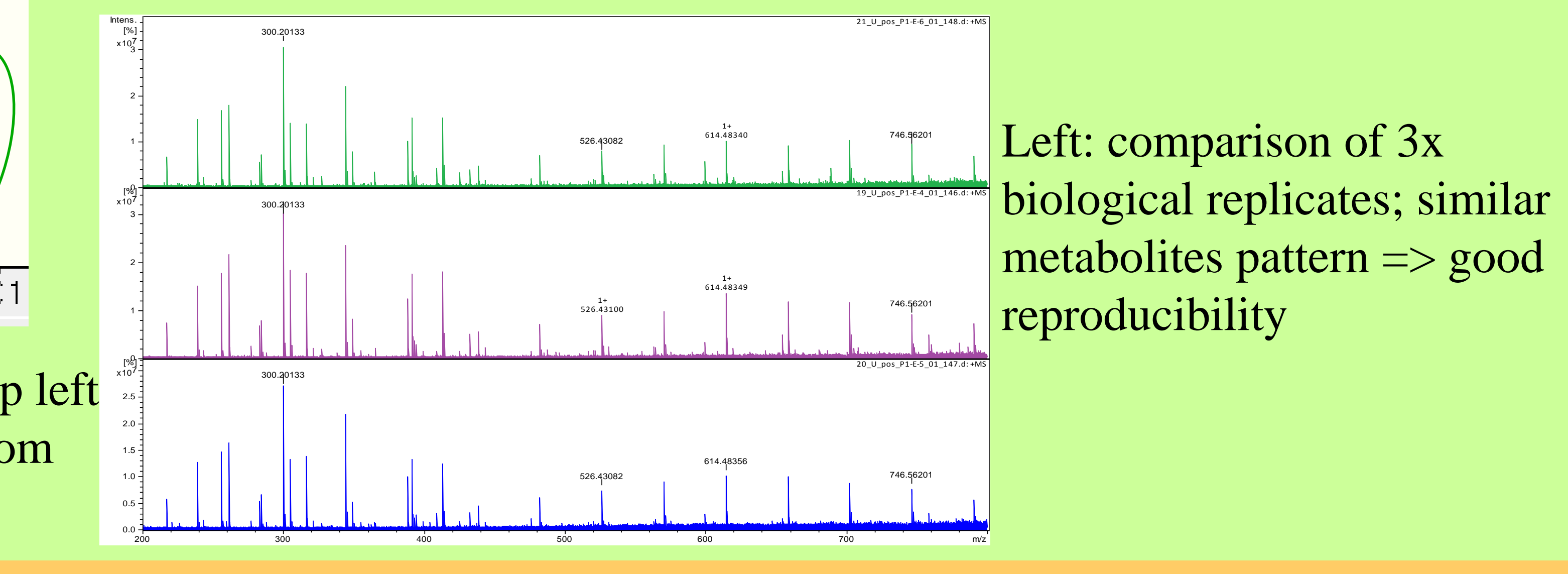


Actual used software (Bruker)

- Sum formula generation
- Statistical data evaluation with PCA & PLS
- Database queries (Metlin, HMDB & ChemSpider)
- Automatically peak-picking (Pathway Screener)
- Protein identification (Protein Scope & Mascot)



Loadings-plot of all 28,000 metabolites; top left correlates with the treated (red cycle), bottom right with the non-treated (green cycle)



Established data evaluation:

- A local data base with > 800 human metabolites (targeted approach)
- Validation protocol for large studies by means of QC approach
- Validation of identified compounds with LC-QToF-system

Open questions for the platform:

- Predication model (e.g. random forest)
- More multivariate statistical tests

