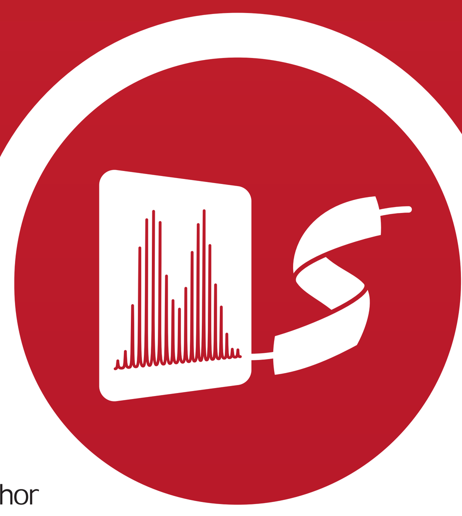
Proteolytic MALDI compatible chips for structural proteomics

Michal Rosulek[1,2], P. Darebna[1,2], M. Volny[3], P. Man[1,2], P. Pompach[1,2] and P. Novak[1,2]

1 BIOCEV, Institute of Microbiology CAS, Prague, CZECH REPUBLIC 2 Charles University in Prague, CZECH REPUBLIC
3 Affipro, s.r.o., Mratin, CZECH REPUBLIC

mailto: author



Introduction

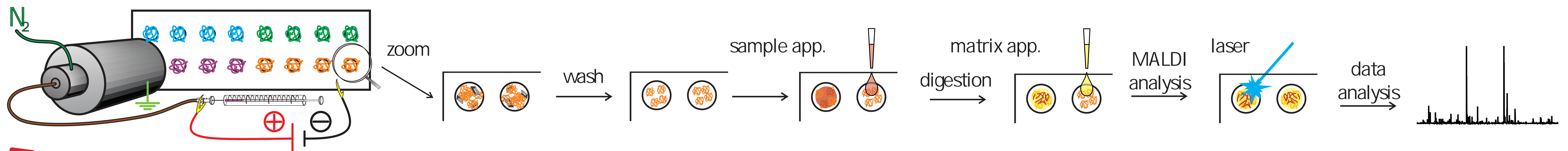
Protein digestion represents one of the most important steps in all bottom up proteomic studies. A wide range of proteases is currently used because of their diverse digestion specifities. Due to many reasons, proteases are also immobilized on solid substrates, when covalent bond is most often mediated with chemical cross linker. However, another techniques for native protein immobilization have been recently introduced. One of these techniques ambient soft landing enables noncovalent and nondestructive protein ion immobilization to conductive surfaces[1][2]. In this case, protein ions are generated in electrospray, transfered through an ion optic and deposited to the conductive surface at atmospheric pressure. In this study, different types of serine and aspartic proteases have been immobilized and thus functionalized surfaces have been tested in several proteomic applications.

Methods

functionalized surface: ITO (Indium Tin Oxide) glass slide
depositing conditions: 5µM protease solution, flow 60µl/h, gas temp. 50°C
immobilized proteases:
- pepsin from *Sus scrofa* (Fig. 1)
- protease type XIII (Aspergilopepsin) from *Aspergillus niger* (Fig. 1)
- trypsin from *Bos taurus* (Fig. 2, 3 and 4)
- protease type VIII Subtilisn A *Bacillus licheniformis* (Fig. 4)

protein sample: 2µM protein in 20mM ammonium bicarbonate solution, 1 µl per spot (acidified for HDX experiments)
digestion conditions: various, depends on the purpose of digestion
digestion quench: HCCA matrix solutions addition, vacuum drying
reaction products analysis: MALDI TOF and FT ICR mass spectrometer
data interpretation: DataAnalysis 4.4, Xlinks, DeutEx, webapps MSTools[3]

Workflow



Applications

1 Hydrogen/deuterium exchange

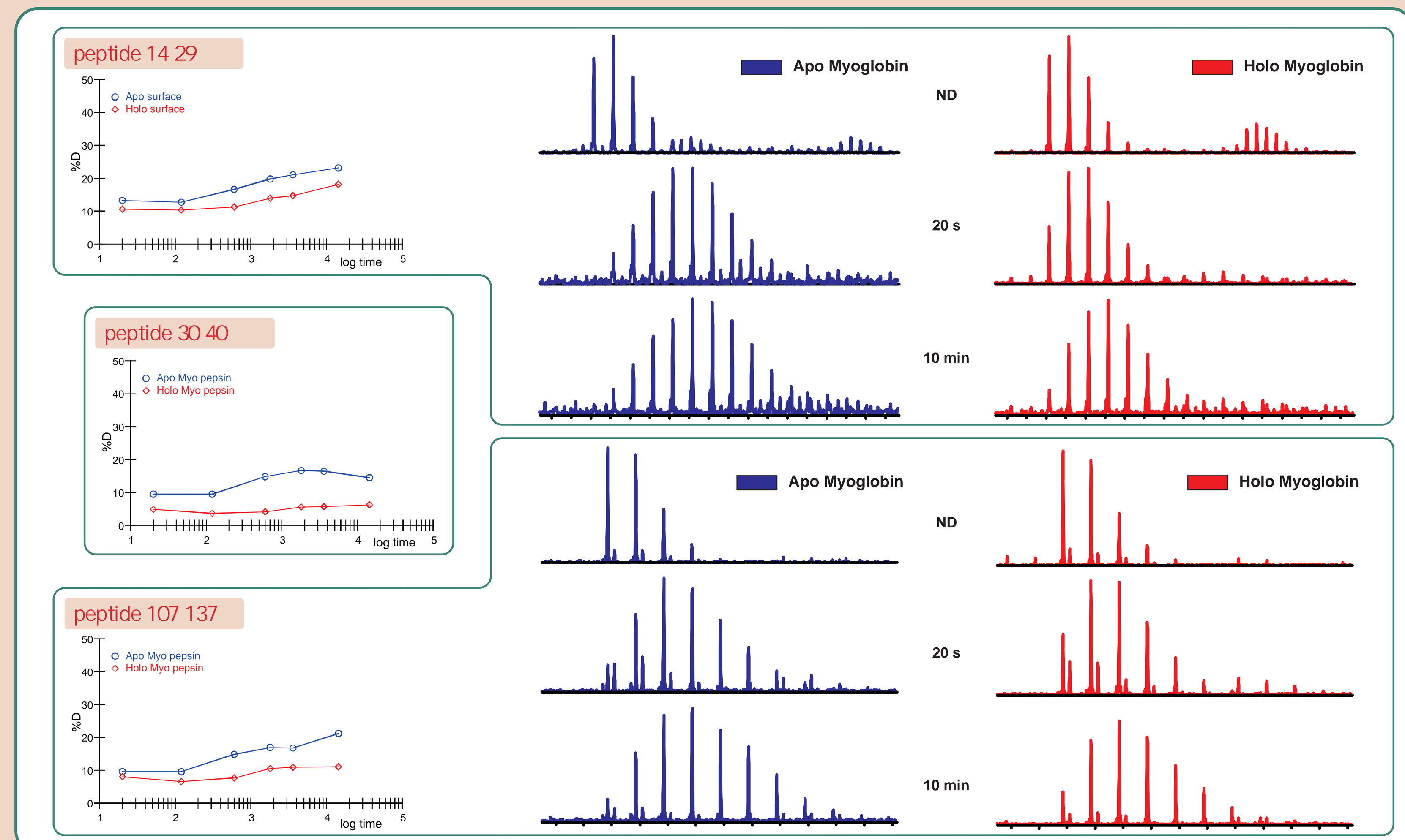


Fig. 1: Application of immobilized aspartic proteases Aspartic proteases pepsin and XIII were deposited for HDX products analysis. To protect sample against deuterium backexchange the digestion was performed at pH 2,3 for 5 minutes at 5°C. Data obtained from model study on Apo and Holo myoglobin forms demonstrate ability of functionalized surfaces for HDX experiments.

2 Digestion of protein DNA complex

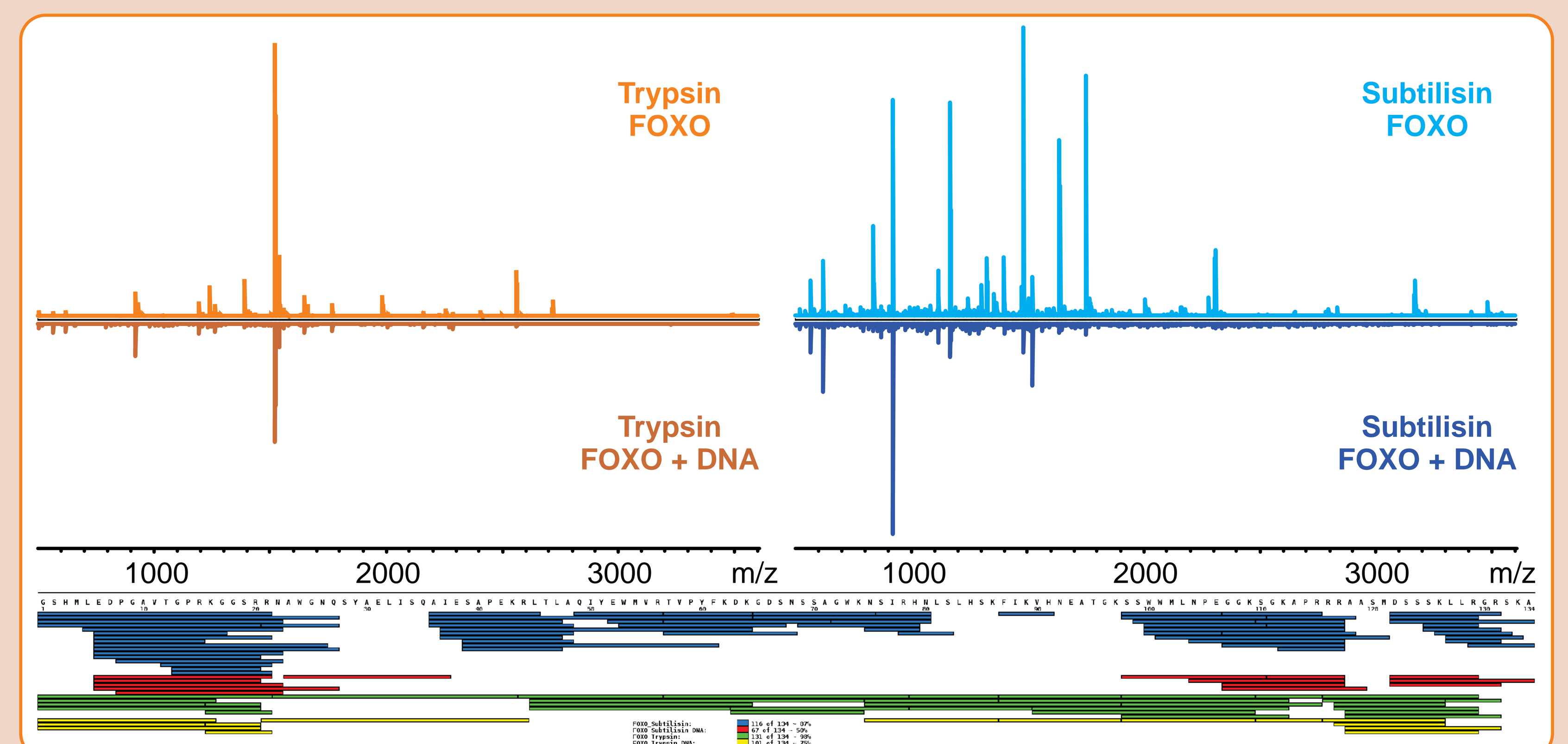


Fig. 2: Digestion of FOXO and FOXO DNA complex Differences in FOXO transcription factor digestion caused by the presence of DAF16 response element. Digestion was performed for one hour on 37°C. Peptide map represents all peptides obtained from each used protease. Nucleic acid in +DNA samples strongly interacts with protein core (missing regions on the digest map) and protects protein against effective digestion.

3 Disulfide bond identification

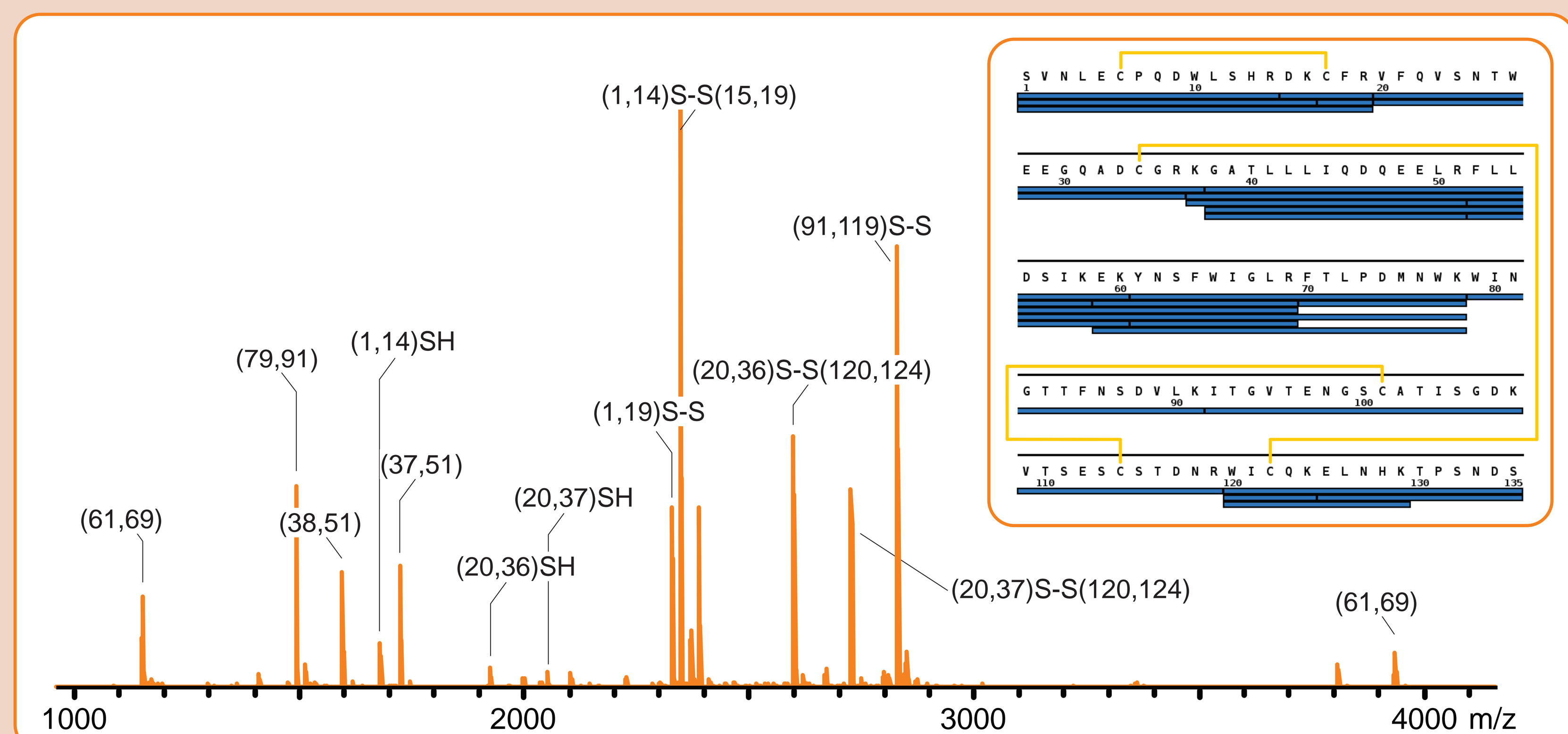


Fig. 3: Nkrp1B trypsin digest Ligand binding domain of protein Nkrp1B digested *in situ* at 37°C for 1 hour. Disulfide bridges distribution through the sequence is shown on the peptide map. Disulfide linked peptides generates the most abundant peaks in the spectrum. Even though reduced single peptides also present, functionalized trypsin surface is a useful tool for very fast disulfide bond identifications.

4 Limited proteolysis

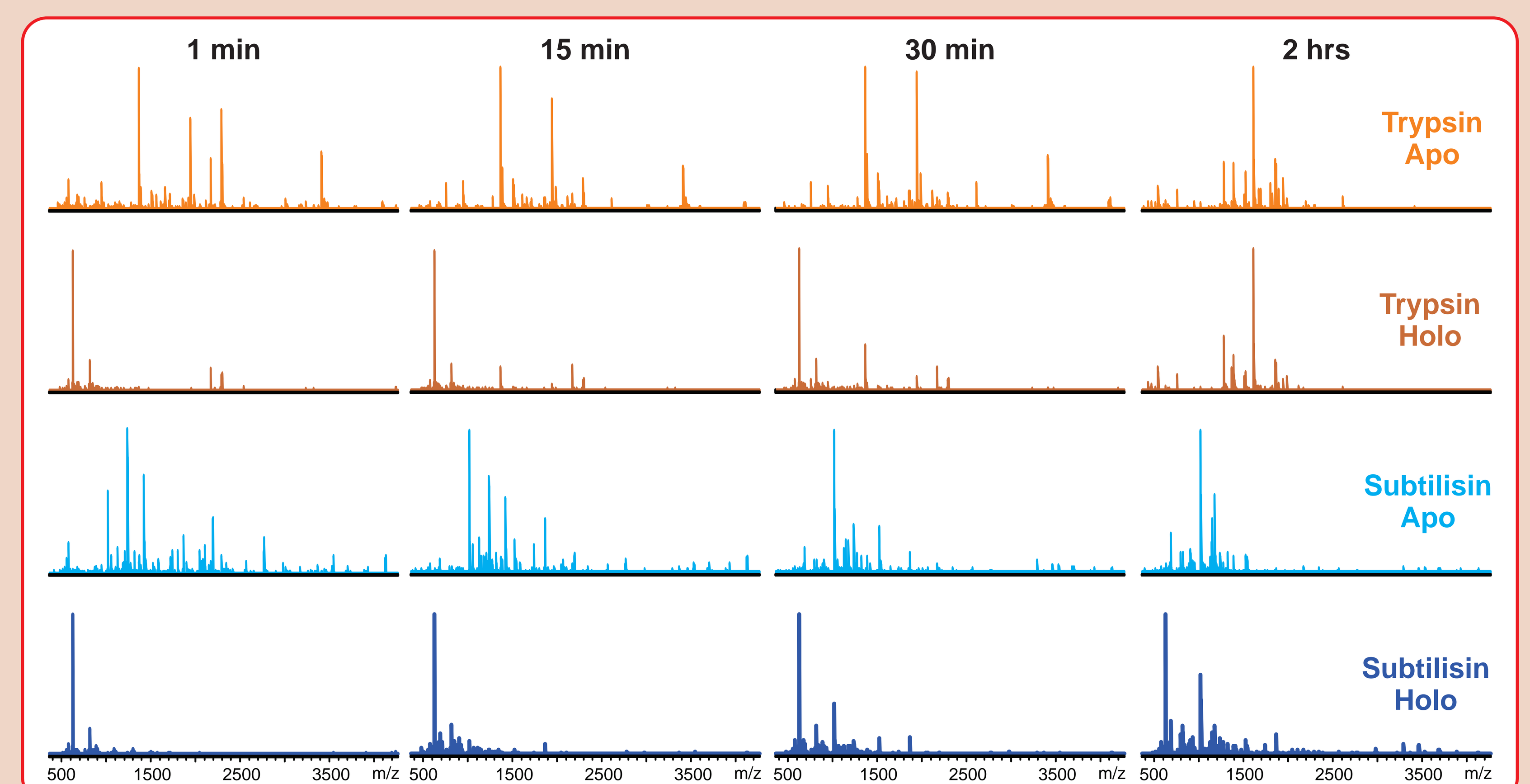


Fig. 4: Limited proteolysis spectra Spectra demonstrate different behavior of Apo and Holo Myoglobin forms during digestion. Serine proteases trypsin and subtilisin A were used for this experiments. The complete digestion of holoprotein wasn't achieved until several hours, because of structure protection and stabilization by heme cofactor. Digest conditions: 37°C, higher humidity, pH 7,5. Quench: matrix adition, rapid drying.

Summary

Different proteases were immobilized onto MALDI MS compatible ITO glass. Thus prepared proteolytical surfaces shows great potential to serve as a simple, but very effective tool in various proteomic experiments. Fig. 1 4

References

[1] Cooks *et al.*, Anal Chem, 83(7), 2648-54 (2011)
[2] Novak *et al.*, Anal Chem, 88(17), 8526-34 (2016)
[3] Kavan and Man, Int J Mass Spectrom, 302, 53-58 (2011)

BIOCEV LABORATORY OF STRUCTURAL BIOLOGY AND CELL SIGNALING

This work has been supported by the Grant Agency of Charles University (932316), grants from the Ministry of Education, Youth and Sports of the Czech Republic (LH15010, LD15089), the Czech Science Foundation (16 24309S) and European Regional Development Funds (CZ.1.05/1.1.00/02.0109).