

PhD-project available:

Discovery of novel biocatalysts for industrial biotechnology

Background:

In proteomics, mass spectrometry has become the dominant technique because of its unparalleled ability to acquire high-content quantitative information about biological samples of enormous complexity. However, the high sample complexity in combination with a dynamic range of up to 10 orders of magnitude, hinder the identification and quantification of less prevalent enzymes. Depletion of highly abundant matrix proteins and enrichment of proteins of interest are therefore key challenges in proteomics. Activity-based proteomics makes use of molecular probes specifically targeting specific classes of enzymes, allowing a detailed view on sub-proteomes and is therefore an excellent front end technique for mass spectrometry.

Essentially, based on existing knowledge and existing enzymes, activity-based probes (which are suicide inhibitors mimicking model substrates) are developed, which irreversibly and specifically bind to enzymes of interest. These probes can be fluorescence or affinity labeled allowing isolation of the novel enzymes by electrophoretic or chromatographic separations and identification by mass spectrometry and database search (**Fig. 1**).

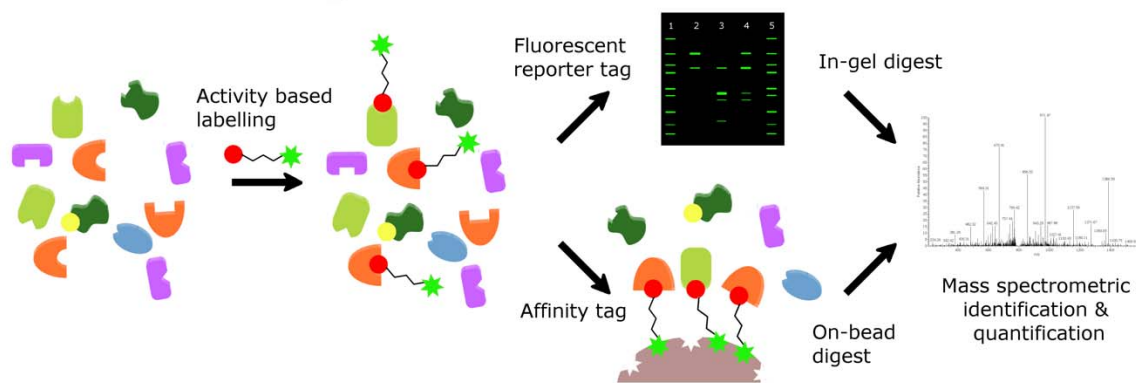


Figure 1: Activity-based proteomics: Discovery of unknown enzymes in complex proteomes

In contrast to other modern screening approaches such as based on metagenomics, this technology approaches from the substrate architecture rather than from the architecture of existing enzymes. Thus, this approach allows the exploitation of nature towards totally novel enzymes and novel more efficient enzymes can be expected as screening results.

Aim:

We have successfully developed and applied an activity-based proteomic platform to discover, characterize, profile and localize mammalian lipases in health and disease over the last decade (see references given below). In this project, we would like to translate our platform from medical research to biotechnology and extend our activity-based probe portfolio to enzymes which are of special interest to industrial biotechnology. As a first example, you will set up an activity-based platform for discovery of polyesterases (polyethylene terephthalate (PET) modifying enzymes) since this class of enzymes is of special interest to industrial biotechnology. In the last few years polymer functionalization and processing with enzymes has gained a lot of importance. Applications range from surface hydrophilization of synthetic materials to complete recycling of synthetic polymers. The platform can later be translated to other enzyme classes, e.g. polyamidases, PLA-esterases, PHA-depolymerases, etc. These enzyme classes all belong to the family of serine hydrolases, which can be specifically targeted in their active site by p-nitrophenyl or fluorophosphonates. These phosphonates resemble the tetrahedral transition state of the active site nucleophile attacking the carboxylate carbon of carboxyl ester substrates. But while carboxyl esters are hydrolyzed, phosphonates will remain covalently bound to the active site after losing their leaving group (p-nitrophenyl or fluoro). In addition, the released p-nitrophenol can be quantified by its absorption allowing quantitative measurement of captured enzymes. By adding appropriate binding groups to the probes their selectivity can be tuned towards specific enzyme classes within one family. E.g. for lipases we have extended the chain length or

added dialkylglycerol or cholesterol groups to mimic triacylglycerols or cholesterol esters more closely to produce specific probes for these enzyme classes. For PET hydrolases we will design polyester mimicking phosphonate probes and functionally test them on known polyesterases before we apply them to screen preselected microbes with known polyesterase activities.

Techniques: Organic synthetic chemistry, activity-based proteomics (gel electrophoresis, liquid chromatography and mass spectrometry), enzymatic activity assays, fermentation, cell fractionation

Laboratory environment: We are a young and very motivated laboratory of currently six group members with scientific experience in functional proteomics, biocatalysis and lipid metabolism located at the Center for Medical Research (ZMF). We are excellently equipped with proteomics technology and work in a highly interactive and fun environment. You will participate in the PhD program DK-MCD and the Austrian Center of Industrial Biotechnology.

Your experience: Ideally, you have a thorough background in organic chemistry, biotechnology, protein biochemistry and experience in protein analytical techniques.

Further information:

Homepages: <http://www.medunigraz.at/zmf/ms/proteomics.html>, http://www.medunigraz.at/DK_MCD, <http://lamp3.tugraz.at/~acib/index.php/wbindex/start>

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Application forms: http://www.medunigraz.at/DK_MCD

Application deadline: December 4, 2011

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