Institute of Molecular Biology

IMB TechTalk

Prof. Dr. Mike Schutkowski

Martin-Luther-University Halle-Wittenberg,
Institute of Biochemistry & Biotechnology,
Department Enzymology

"Profiling of Epigenetic Targets using Peptide Microarrays"

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Seminar room 2nd floor

Institute of Molecular Biology (IMB)

Johannes Gutenberg University Campus Mainz

All are welcome to attend

Host: Dr. Vijay Tiwari, IMB

For further information, please contact: Bianca Steinbach, E-mail: b.steinbach@imb-mainz.de, tel.: +49-(6131)-39-21510

Abstract:

Profiling of Epigenetic Targets using Peptide Microarrays

Acetylation of lysine residues in proteins is one of the most frequently occurring post-translational modification playing a major role in protein-protein-interaction as well as in regulating DNA transcription and metabolic processes.
Using acetyl coenzyme A as donor for the acetyl group, lysine acetyltransferases are transferring the acetyl group onto the ε-amino-group of lysine residues in proteins. The positive charge of the side chains is neutralized, thus remodeling biological activity of enzymes and protein-protein-interaction. Bromodomains as protein interaction modules are recognizing specifically the acetylated lysine side chains.
To understand substrate specificity of lysine acetyltransferases and binding specificity of bromodomains, we created a high-density peptide microarray displaying 6800 peptides derived from all known acetylation sites in human proteins in both unmodified and acetylated form resulting in more than 13.600 different features per microarray pair (acetylome microarray). The acetylome microarrays were treated with lysine acetyltransferases in the presence of acetyl-CoA followed by an optimized mixture of anti-acetyl-lysine-antibodies.
Additionally, treatment of the acetylome microarray with Sirtuins, NAD^+-dependent protein lysine-deacetylases involved in regulation of central physiological functions, such as energy metabolism, cell cycle progression, and aging processes, resulted in decrease of signals for several of the acetylated peptides in comparism to the control experiment in the absence of NAD^+. We were able to identify subsite specificities and substrate sequences for all of the seven human Sirtuin isoforms. Besides known substrates our results provide novel substrate candidates for the different Sirtuin isoforms which could be confirmed in subsequent solution phase experiments.
Principally, each enzyme transfering residues to the side chains of lysines could be analysed with the acetylome microarray using either radioisotopic labeling (lysine-methyltransferases and labelled AdoMet, poly-ADP-ribosyltransferases and labelled NAD^+) or antibody-based readout (poly-ADP-ribosyltransferases and ethenoNAD$^+$ followed by anti-ethenoNAD$^+$-antibody, ubiquitin/SUMO transfering enzymes followed by anti-ubiquitin/SUMO-antibodies).