based stationary phase was used in hydrophilic-interaction-chromatography (HILIC) mode to purify APTS labeled glycans. The whole sample preparation procedure can be performed at the 96-well-plate level with a hands-on time of less than 2.5 h. Purified APTS-labeled N-glycans were analyzed using a standard capillary DNA sequencer. The optimized sample preparation, combined with robust, HR, high-sensitive multiplexed CGE-LIF based measurement and fully automated data evaluation, enables highly reproducible "real" HTP analysis of protein N-glycosylation.

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347: Tailored methods for CGE-LIF based multiplexed high-throughput glycoanalysis with respect to sample characteristics

M. Borowiak, R. Hennig, R. Kottler, U. Reichl, E. Rapp; Max-Planck-Institute for Dynamics of Complex Technical Systems, Magdeburg/DE
borowiak@mpi-magdeburg.mpg.de

Glycomics is a rapidly emerging field, that can be viewed as a complement to other „omics" approaches including proteomics and genomics. Hence, there is a dramatic increase in the demand for sophisticated databases and analytical tools in glycobiology, respectively glycobiotechnology. In order to enhance and improve the comparatively small set of existing glyco-analytical methods and toolboxes, a system and method for automated straightforward, sensitive high-throughput (HTP) and high-resolution glycoanalysis was developed [1,2]. This glycoanalysis approach is based on multiplexed capillary gelelectrophoresis with laser induced fluorescence detection (CGE-LIF), using a capillary DNA-Sequencer (Applied Biosystems). Data is evaluated in conjunction with a novel modular software-tool for data-processing and automated structural elucidation by interfacing a corresponding oligosaccharide-database. The aim of the project presented was to develop tailor-made workflows for multiplexed CGE-LIF based glycoanalysis, optimized for different types of samples, each with its specific characteristics. Exemplarily, three different sample types were chosen: first, a broad variety of N-glycans released from citrate plasma proteins (a complex sample of medium to large, charged and uncharged glycostructures); second, a relative small set of N-glycans released from IgG (a modest sample with medium sized mainly uncharged glycostructures); and third, the complete pool of oligosaccharides from human breast milk (complex sample of small charged and uncharged glycostuctures). The whole workflow starting from sample clean-up until CGE-LIF analysis using a standard capillary DNA Sequencer was optimized individually to each of the three representative samples. For sample preparation, different chromatographic techniques and materials were tested and different polymers and capillary lengths were investigated for final CGE-LIF analysis.

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348: Trypsin is not enough - Glycoproteomic analysis of recombinantly expressed proteins by mass spectrometric approaches

J. Grass[1], M. Pabst[1], L. Neumann[1], M. Chang[2], F. Altmann[1]; [1]Department of Chemistry, University of Natural Resources and Life Sciences (BOKU), Vienna/AT, [2]Polymun Scientific, Vienna/AT
josephine.grass@boku.ac.at

For the efficient and safe use of glycoprotein pharmaceuticals, it is necessary to analyse the structures of the carbohydrate moieties of those glycosylated drugs. Analysis of the consistency of protein glycosylation at the glycopeptide level allows the use of standard proteomic methods, which take advantage of the favourable ionisation properties of the peptide moiety that exceeds the lower proton affinity of sugars. As a consequence, the glycopeptide spectra approximate the true glycosylation pattern at a given site. For this reason we used this approach to analyse the glycosylation status of the recombinantly expressed glycoprotein drugs human erythropoietin (r-hEPO) and human follicle stimulating hormone (r-hFSH). Trypsin digest of r-hEPO resulted in one O-glycopeptide and two N-glycopeptides, one of them comprising two glycosylation sites. Since each of those sites carried various glycan forms, the obtained spectra were highly complex. An additional digest with endoproteinase GluC led to clear spectra of the separated glycosylation sites. In the case of r-hFSH, only three of the four expected tryptic glycopeptides were found, whereas after chymotryptic digestion, all four glycopeptides could be identified. Three sites were occupied by mainly di-antennary N-glycans, whereas the glycopeptide three on the FSH-unique ß-chain carried larger N-glycans with lactosamine repeats. Glycopeptides were finally quantified by the software MassMap®, which determines the intensity of an entire mass peak including the relevant isotopes, adducts and different charge states. Thus, a reproducible quantification of glycoforms in complex data sets can be achieved. The glycopeptide data were corroborated by total oligosaccharide analysis performed by LC on porous graphitic carbon with mass spectrometric detection.