Proteome analysis is a powerful methodology to investigate protein expression in tissues involved in diseases not linked to particular genetic defects such as Alzheimer’s and Parkinson’s disease (AD and PD). Direct measurement of protein expression is essential for the analysis of these diseases, since posttranscriptional and translational control such as splicing of mRNA, posttranslational modification of proteins (e.g. phosphorylation, ubiquitinylation, oxidation etc.) cause a non-predictive correlation between mRNA and protein levels. Differential proteome analyses comparing diseased and non-diseased brain tissue will result in the identification of target proteins associated with AD and PD. In collaboration with diseased brain tissue will result in the identification of potential biomarkers for AD and PD. For the separation and detection of differential proteins the 2D-DIGE™ system (minimal labelling technique) is used. Proteins of interest are excised from the gels and digested using different proteases such as trypsin. Resulting peptides are analysed by mass spectrometry. The obtained data are automatically interpreted using different search algorithms (Sequest™, Mascot) and software tools (Proteinscape™, DTASelect™).

Differential proteome analysis of age-related mouse models - The α-synuclein PD model and UBB+1 AD model

In both studies the protein patterns of brain tissue of diseased and non-diseased control mice are compared resulting in the identification of potential biomarkers for AD and PD. For the separation and detection of differential proteins the 2D-DIGE™ system (minimal labelling technique) is used. Proteins of interest are excised from the gels and digested using different proteases such as trypsin. Resulting peptides are analysed by mass spectrometry. The obtained data are automatically interpreted using different search algorithms (Sequest™, Mascot) and software tools (Proteinscape™, DTASelect™).

Differential proteome analysis of PD mouse models

In our differential proteome study, striatum, brain stem and cerebellum from A53T α-synuclein transgenic mice and FVB/N (control) mice are dissected at different stages of age. The differences in the brain proteome between these mouse lines will be determined to elucidate the role of α-synuclein at different stages of PD.

First experiments have shown that our preparation protocol extracts sufficient proteins out of small tissues like striatum or brain stem to run several 2D-gels. Three different age stages (6, 12 and 22 months of age) of each model – the α-synuclein transgenic and the knock-out mouse model – have to be differentially compared with the respective controls. To date the differential analysis of the brains of the α-synuclein transgenic mouse model (stages 6 & 22 months) were completed. In total 22 differential proteins were detected and 12 of these could be identified up to now. The stages of 12 months will follow and data will be validated soon. In the next step the knock-out mice of the stages 6 & 22 months will be analysed as well as cerebellum of transgenic, knock-out and control mice.

Differential proteome analysis of an AD mouse model

Our specific aim is to elucidate a number of downstream targets in transgenic mice with UBB+1-mediated UPS inhibition, such as phosphorylated, oxidised and ubiquitinated...
proteins. For this purpose protein patterns of cortices from the UBB+1 transgenic and control mice are differentially analysed at different stages of age (3, 9, 15 months). Post-translationally modified proteins are detected after 2D-gel electrophoresis using different methods, e.g. antibodies and a phosphoprotein specific fluorescence stain (ProQDiamond).

Different proteins are identified so far using a hybrid triple quadrupole/linear ion trap mass spectrometer – the 4000 Q Trap®. The first results demonstrate that all techniques provide a good access to the differential phosphoproteome analysis of the UBB+1 transgenic mice: Several differentially phosphorylated proteins are already detected after ProQDiamond staining and mass spectrometric analysis of one spot successfully resulted in localisation of a phosphorylation site in HSP 90b.

Proteins from brain lysates are first fractionated by strong anion exchange (SAX) or strong cation exchange (SCX), respectively. We will start with about 100 mg of protein for the separation in the first dimension (SAX or SCX, respectively). Resulting fractions are collected and transferred to a second LC-based protein separation step (RP-HPLC). In the third step the proteins are separated by 1D-SDS-PAGE. Quantification will be first done optically by fluorimetric detection. These experiments will be repeated 3 to 5 times to prove the reproducibility of the quantitative results. Thereafter, differentially detected protein bands will be proteolytically digested and the resulting peptide mixtures will be analyzed using nano-liquid chromatography/mass spectrometry (LC/MS).

In first experiments we established a separation technique including SAX chromatography in the first and RP chromatography in the second dimension using pig brain proteins lysates. Fractionated proteins are separated using large high resolution SDS-PAGE (20 x 20 cm). Reiterations of the experiments exhibit a high reproducibility of both chromatographic (first and second dimension) and gel electrophoretic level, emphasizing the applicability of the designed prefractionation technique.

Results obtained from the mouse model studies will be validated in human material, i.e. preparations of selected human brain regions of donors who suffered from AD or PD, or of neurologically healthy control donors. We hope to link changes in AD and PD patients to the molecular pathways disturbed by the transgenesis in the analysed mouse model, and to better understand these multifatorial diseases. New avenues for therapy will be discovered: a number of proteins, e.g. modified and trapped in aggregates at different age stages will be known, and mouse models based on this knowledge will have shown their individual contribution to neurodegeneration.

After optimization of the multidimensional HPLC with samples of pig brain protein lysates the method is usable for the analysis of high complex protein mixtures. The next step is the implementation of the DIGE™ technique to this chromatographic approach allowing differential quantitative analysis. These new high-sensitivity methodic developments will explicitly advance the performance of current proteomic analyses. The aim is the identification of differential low abundant proteins serving as target molecules for the diagnosis and therapy of diseases - such as PD and AD - not detectable by other analysis techniques.