Master-Thesis "Applicability of Zeptosens Reverse Phase Protein Microarray (RPPM) Technology to Mouse Brain Tissue" by Oliver Tschumi

Part of a collaborative project of Roche Centre for Medical Genomics (Gaby Walker) and the Department of Biomedicine, University of Basel (PD Dr. Dieter Kunz) from January to July, 2007

In recent years the accumulation of biological information at the genomic level has massively increased the knowledge on gene functions. In parallel, new methods to study protein functions, modifications and interactions in drug discovery, drug development and disease diagnostics have been established. This technical development was mainly triggered by the demand for increased sensitivity, speed and reliability of the analytical methods.

Current high throughput proteomic technologies, most prominently two-dimensional gel electrophoresis (2-DE) in combination with mass spectrometry allow the identification of biologically relevant proteins at high resolution. However, the technology has also considerable limitations: Most spots in 2-DE observed from whole cell extracts represent soluble proteins whereas membrane proteins and low molecular weight proteins are only poorly detectable.

ELISAs have turned into a reliable and sensitive test system with many applications for the search of protein biomarkers in body fluids or tissue extracts. For instance, ELISA tests are currently used to measure expression levels of single, well-characterized cancer-associated proteins in an accurate manner and are considered to be the `golden standard` of clinical assays. A major disadvantage, however, is that ELISA applications are often laborious, time- and material-consuming with a limited number of analytes.

The RPPM-technology developed by Zeptosens is a new bioanalytical system based on the planar waveguide technology which allows performing multiplexed, quantitative biomolecular interaction analyses with highest sensitivity in a microarray format upon utilizing the specific advantages of the evanescent field fluorescence detection. The analytical system comprises an ultrasensitive fluorescence reader and microarray chips with integrated microfluidics which allows the generation of a multitude of high fidelity data in applications such as protein expression profiling or investigating protein-protein interactions. Assuming 10 µg protein extract for a single lane in SDS-PAGE, RPPM would allow the investigation of up to 10'000 spot samples using this amount of protein.

So far, no published data or knowledge about the applicability of RPPM to brain tissue exists. The major focus of the Olver Tschumi`s master thesis was to test whether RPPM could be used to study proteins and protein modifications such as phosphorylation in brain homogenates. For this purpose, the transgenic animal model on $GABA_B$-receptor knockouts was used since it is well characterized biochemically by many different research groups, Western-blot data on changes in $GABA_B$-receptor expression levels are available and, finally, the knockout animal allows the generation of clear, unambiguous data.

Hippocampus and cerebellum were chosen for analysis in order to compare expression profiles in different brain regions and to calibrate whether the antibodies would be applicable independently.

Major findings of the study are:

(i) Zeptosens RPPM data on $GABA_B$-receptor expression levels in untreated Controls as well as Controls, $GABA_{B1}$- and $GABA_{B2}$-receptor knockout mice treated with the $GABA_B$-receptor agonist Baclofen are closely correlated to data published by Western-blot analyses.

(ii) Zeptosens RPPM technology is suitable to study changes in protein modifications such as phosphorylation in brain tissue. Data on PKA expression and (p-Ser96)-PKA phosphorylation in hippocampus and cerebellum generated by RPPM are in very good correlation to published data by many different research groups using Western-blot analyses. Thus, RPPM technology is capable to reproduce the "golden standard" in $GABA_B$-receptor coupling obtained by Western-blot analyses.

(iii) A novel, very interesting result is obtained in RPPM analyses on GSK3-beta phosphorylation. In contrast to contradictory results obtained in the adjacent Western-blot analyses, RPPM analyses strongly suggest a so far unknown interaction of the $GABA_B$-receptor system with GSK3-beta phosphorylation. The data clearly show an inhibition of GSK3-beta phosphorylation in Baclofen-treated Controls and $GABA_B$-receptor knockout mice as compared to untreated Control mice. GSK3 is centrally involved in many psychiatric disorders and neurodegenerative diseases.

Taken together, the results of Oliver Tschumi`s work support, for the first time, the feasibility of RPPM in biochemical analyses of brain tissue. In the light of the enormous potential of RPPM to miniaturization of samples, the study is of particular importance and may have a strong impact on studies dealing with drug development as well as studies on the pathophysiological mechanisms involved in neurodegenerative, neurological and psychiatric diseases. Currently, Oliver Tschumi's data are summarized and will be submitted for publication.