

**Welcome to the Tecan Symposium 2007 in Zürich, Switzerland**  
**Theme: Systematics Biology - moving towards automated science**

**Abstracts**

**Dr. Frank Buchholz**, Max Planck Institute of Molecular Cell Biology (CBG)  
(Germany)

***From RNAi phenotypes to molecular function:  
A pipeline to validate and characterize hits from RNAi screens in mammalian tissue culture cells***

Abstract: RNAi screens typically deliver a large number of candidate genes that play a role in a biological process. The validation of these candidates and the dissection of the molecular mechanism of action are often time consuming and cumbersome. We have developed a pipeline using BAC recombineering technology and tissue culture transgenesis to streamline the analysis of hits identified in large scale RNAi screens. Examples from this pipeline will be presented.

**Kris Gunsalus** , NYU Department of Biology (USA)

***Context-dependent molecular networks in C. elegans***

High-throughput approaches to analyze different aspects of gene function on a genomic scale are providing new perspectives on the architecture of the molecular networks underlying biological processes. Most cellular functions are carried out by specialized groups of proteins working together as a logical unit, which often correspond to physical complexes. These functional modules must then be properly coordinated to allow proper higher-order behavior of cells, such as cell division and differentiation during development. By integrating different functional genomics data for the early embryo in *C. elegans*, we have generated a first-draft global map of molecular networks underlying early embryogenesis. A further challenge is to begin to deconvolve and extend available data to generate a dynamic view of how molecular networks change during development at different times and in different tissues. A first step toward this end is to develop context-dependent views of molecular modules in terms of their components and connectivity, and we are developing approaches to begin addressing this issue. To help navigate heterogeneous functional linkages between genes and their protein products, we have also developed an interactive network browsing tool, N-Browse (<http://www.gnetbrowse.org>).

**Dr. Reinhard Hiller, CPGR (South Africa)**

**Integrated microarray solutions in research and diagnostics at the CPGR in Cape Town, South Africa**

Abstract: The Centre for Proteomic & Genomic Research (CPGR) is an integrated core technology facility, founded in South Africa in 2006 as a not-for-profit organization with a vision of establishing a modern, world-class, high throughput biology research facility that serves the needs of the life science and biotech communities in South Africa by providing state-of-the-art analytical services, technical expertise, project support and collaborative research capabilities in the genomics and proteomics sectors.

Microarray technology plays a vital role in a number of integrated workflows in the Genomics & Proteomics sector at CPGR, for research as well as diagnostic purposes. A range of commercially available DNA applications are being used in SOP-based workflows implemented on a Tecan HS 4800 in conjunction with home-brew arrays developed for the study of non-model organisms indigenous to (South) Africa. Protein microarray technology forms a major pillar in proteomic studies at the CPGR, in line with mass spectrometry based protein discovery. This presentation will highlight antibody, protein and peptide microarray applications - with a particular focus on workflow integration and automation - established or in development at CPGR with the latest biomedical and biotech research trends in (Southern) Africa.

**Joshua LaBaer, Harvard Institute of Proteomics (USA)**

**Building high quality ORF collections for functional proteomics**

Abstract: One of the most compelling steps in the post-genomic era will be learning the functional roles for all proteins. The Harvard Institute of Proteomics (HIP) has initiated a project to create a sequence-verified collections of full-length cDNAs representing human, human pathogen and model organism coding regions, in recombinational vector systems that allows the immediate in-frame transfer of all coding regions into virtually any protein expression vector. These transfers allow the addition of peptide tags to either or both end of the proteins. This repository, called the FLEXGene Repository (for **F**ull-**L**ength **E**xpression-ready), is enabling the high-throughput (HT) screening of protein function for the entire set (or any customized subset) of genes using any method of *in vitro* or *in vivo* expression. Using HT retroviral methods, these clones have been used to identify proteins capable of driving cell migration, altering the morphogenesis of normal epithelial structures, and affecting drug resistance in cells.

**Fabio Piano**, NYU Department of Biology (USA)

***Global, local and comparative networks in early nematode embryos: models for a systems view of development.***

Fertilization unites two specialized cells, egg and sperm, and activates a coordinated cascade of events to build an embryo. The global mechanisms underlying processes that guide the transition from oocyte to early embryo remain largely mysterious. What is known is derived mostly from tackling each part of the problem such as fertilization, cell cycle progression, establishing polarity, and cytokinesis as separate programs. Yet, we know these are exquisitely coordinated into a global system. Combining quantitative phenotypic analysis with other functional genomics approaches such as transcriptomics and proteomics has revealed a first-draft global map of the molecular networks underlying early embryogenesis in *C. elegans*. We have used this map as a launching point to address three areas: identifying key new regulators that bridge different embryonic processes, tackling how early embryogenesis evolves, and to build a global genetic interaction map. These studies have implications on not only to understand the function of highly conserved proteins (many of which are mutated in human diseases) but also on how a system is built and can evolve into one that is no longer working normally as in a disease state. Ultimately, it can reveal systems approaches towards treatments. We are developing tools and how to meaningfully model development using high-throughput, high content data during early embryonic development of *C. elegans* and related nematodes.

**Niroshan Ramachandran**, Harvard Medical School (USA)

***On-chip peptide synthesis***

Abstract: Developing tools for applications in functional and clinical proteomics  
We have developed a self assembling protein array called Nucleic Acid Programmable Protein Array (NAPPA), where cDNAs encoding potential antigens are printed onto glass slides and then translated into target proteins with a mammalian reticulocyte lysate. This robust method obviates the need to purify antigens, avoids stability problems during storage and provides sufficient antigen for testing. NAPPA technology is capable of producing thousands of different proteins including transmembrane proteins in a single step. Moreover proteins produced by this method have shown to be functional by maintaining the appropriate interactions and/or enzyme activity. NAPPA is also being used to display a large panel of antigens for identifying disease specific immune responses for diagnostic and therapeutic purposes.

**Nadia Rosenthal**, EMBL Monterondo (Italy)

***The automated mouse – systematic generation of disease models***

Abstract: The laboratory mouse is widely considered the model organism of choice for studying the diseases of humans, from whom they differ in only a tiny fraction of their genetic material. A distinguished history of classical genetic experimentation in the mouse has recently gathered speed with the advent of powerful new tools to manipulate the murine genome. The recent launch of several internationally sponsored initiatives for systematic mouse mutagenesis on a large scale using various genetics strategies, along with high throughput phenotyping pipelines, underscores the utility of the mouse for interpreting the mammalian genome, and for generating increasingly more accurate models of human disease.

**Alan Sawyer**, Monash University (Australia)

***The Monash Antibody Technologies Facility: A high throughput monoclonal antibody production platform for phospho-kinomics***

Abstract: Limitations in monoclonal antibody production throughput have hampered large scale microarray and multiplex assay based studies. A new hybridoma production platform comprising Tecan robotics complemented by Genetix and Arrayjet devices being established at Monash University in Australia promises throughputs of panels of monoclonal antibodies raised against five thousand novel antigenic targets per year. The core of the unit's internal research will be based around the production of monoclonal antibodies against phospho-specific antibodies raised against all of the phosphorylation states of all of the human kinases and selected substrates for use in protein microarray and other multiplex screens.

**Ian Smith**, Monash University (Australia)

**Cryptomics: Identification of Novel Bioactive Peptides from Human Tissue Extracts**

Abstract: There is increasing evidence that proteolytic cleavage gives rise to "hidden" peptides with bioactivities that are often unpredicted and totally distinct to the parent protein. The liberation of these cryptic peptides or crypteins has so far been shown to be prevalent in proteins associated with endocrine signaling, extracellular matrix, the complement cascade and milk. A broad spectrum of proteases has been implicated in the generation of natural crypteins that appear to play a role in modulating diverse biological processes such as angiogenesis, immune function and cell growth. Cryptomics is a new systematic and integrated robotically driven approach to finding crypteins in vitro. It involves a reiterative automated proteomic based process of systematic proteolytic fragmentation, chromatographic fractionation, screening for bioactivity and then proteomic identification of functional crypteins focussing on (but not limited to) the human cryptome.

Using this approach we have discovered novel crypteins with potent anti-coagulation and anti-proliferative properties derived from circulating human plasma proteins. Furthermore, we have shown that synthetic peptides based on the sequences of these crypteins also have potent in vivo activity.

We believe that the human cryptome could contain a plethora of undiscovered crypteins involved in regulating disease processes yet to be discovered and representing potential human therapeutics and/or possible biomarkers. It is also possible that additional crypteins in search of function or have not yet been subjected to the forces of natural selection reside in the cryptome.

**Dr. Markus Templin**, Natural and Medical Sciences Institute at the University of Tuebingen, NMI (Germany)

**Making it small: Quantitative Tumour Proteomics using an integrated Protein Microarray Platform**

Abstract: Limiting amounts of cell or tissue material (e.g. tumor biopsies) and multi-parametric protein analysis are key drivers for applying miniaturized microarray-based assays. We have established a throughput approach based on Reverse Phase Arrays (RPA) that is capable of screening tens to hundreds of samples in parallel for differences in abundance and activation state levels of key proteins within cellular signaling networks. The approach uses a sensitive fluorescence detection (Zeptosens waveguide technology). It provides robust and quantitative biological information for dozens of analytes, while it is at least 10-fold more economic than conventional Western Blotting with respect to cost, time, sample and reagent savings. Comprehensive and systematic studies covering efficacy testing of compounds in early discovery, mode of action studies, pathway mapping and verification of biomarkers have been performed.

**Marian Walhout**, University of Massachusetts Medical School (USA)

**Gene-centered transcription regulatory networks**

Abstract: The differential expression of each of our ~25,000 genes in different tissues or under different conditions is critical for our proper development and function. Indeed, changes in differential gene expression caused by mutations in transcription factors (TFs) or the cis-regulatory genomic DNA elements they bind to (TF binding sites, or TFBSs) can result in a variety of human diseases, including several congenital disorders and cancer. In order to fully understand both normal development and pathologies, and to design effective therapeutics it is critical to understand which TF regulates the expression of which gene, where and under which (developmental) conditions. In addition, it is essential to know the elements each TF binds to and where in the genome these TFBSs are located. However, this is a major challenge in genomic science as very little is known about the targets, binding sites, transcriptional activity and biological function for the majority of metazoan TFs. We use the nematode *C. elegans* (worm) as a model to address this challenge. The *C. elegans* genome contains ~20,000 protein-coding genes, 940 of which we predict encode regulatory TFs. Our long-term goal is to comprehensively map and characterize the protein-DNA interactions between all *C. elegans* TFs and all gene regulatory regions, and the networks that connect these interactions.

**And More from:**

**Colin Campbell**, University of Edinburgh (UK)

**Improved surface technology for protein detection**

**Robert Negm**, Gentel (USA)

**Protein biomarker screening using PATH surface technology**

**John McCafferty**, Sanger Institute (UK)

**Proteomic approaches to the cell surface**

**Gordon Robb**, AlbaBioscience (UK)

**Blood typing and immunodiagnosics**