142P fMRI IN ANIMALS TO STUDY NEURAL PATHWAYS AND DRUG ACTION

C A Marsden, P Morris*, V Chapman, M Prior & Y Shah*
Schools of Biomedical Sciences and *Physics and Astronomy, University of Nottingham, University Park, Nottingham NG7 2RD UK

Since 1991 the emphasis on MRI for human studies has shifted from structural analysis to measurement of changes in regional cerebral blood flow in response to specific stimuli or tasks, as a measure of brain neuronal activation under non-invasive conditions. Functional MRI (fMRI) signal intensity can be measured with the T2*-weighted blood oxygenation level-dependent (BOLD) contrast method. With improvement in the speed of image collection and the resolution attainable, conditions used in humans can be applied to animal studies of fMRI. This talk considers the application of fMRI methods to the identification of changes in regional brain function in response to peripheral noxious stimulation and cannabinoid drug administration in rats.

The studies described were made using a 2.35T Bruker Biospec Avance imaging system with anaesthetised rats (core temperature maintained with a customised water bed within the imaging system). Basal scans were obtained before physiological or pharmacological intervention; evoked BOLD responses were compared to vehicle-injected controls.

Intraplantar injection of formalin (5%) into the hind paw was associated with significant increases in BOLD response in specific supraspinal brain regions associated with somatosensory processing, including the periaqueductal grey (PAG), thalamus, amygdala and areas of the somatosensory cortex. The peak increase in response was observed 40 min after formalin administration. In contrast, saline produced no effects in these regions apart from a small increase in the thalamus.

These data demonstrate that fMRI in the rat can be used to monitor CNS processing of noxious stimuli and offer the opportunity to investigate drug-induced alterations. The BOLD method has also been used to detect drug-induced changes (pharmacological MRI) in regional activation, as demonstrated with the results using both amphetamine and the CB receptor agonist HU210. The cannabinoid agonist increased BOLD response in the ventral tegmental area (VTA), PGA and the dentate gyrus, but not other areas of the hippocampal region, while BOLD response in the somatosensory cortex was reduced. These data help to provide anatomical explanations for some of the varied behavioural effects observed with cannabinoids.

fMRI and phMRI should lead to an improvement of our understanding of brain mechanisms and allow closer comparison between rodent and human brain in relation to the use of animal models for the study of human brain disorders.

143P GENERATION AND APPLICATIONS OF HIGH DENSITY PROTEIN ARRAYS

Dolores J. Cahill, Max-Planck-Institut of Molecular Genetics, Dept. of Prof. Hans Lehrach, Ihnestraße 73, D-14195 Berlin, Germany and PROT@GEN AG, Im Lottental 36, D-44801 Bochum, Germany.

A full understanding of the expression profile of a tissue or organism requires the screening of many genetic and or protein samples in parallel as rapidly as possible. Those steps which have been automated and miniaturised in our laboratory to enable a high-throughput and highly parallel approach to large-scale cDNA and protein analysis will be described. Specifically the generation and picking of cDNA expression libraries, and arraying of clones into microtitre-plates.

A technique known as oligonucleotide fingerprinting which has been developed to characterise cDNA libraries, which allows the generation of a non-redundant, human Unigene-Uniprotein set, will be described. The hEx1 library has been oligo-nucleotide fingerprinted to generate a non-redundant set, which contains over 10 000 non-redundant genes and proteins obtained from a human brain cDNA expression library. We have clonally expressed proteins from this library and produced high density protein arrays on filters and glass (chips).

These protein arrays have been screened with antibodies, which detected specific protein products. This approach makes translated gene products directly amenable to high-throughput experimentation, allowing a link between expressed protein and sequence.

Initial results of characterising antibody specificity and profiling auto-immune sera on protein arrays will be presented. Applications of high density protein and antibody arrays in proteomics will be discussed.

144P PROfusion: A BROAD-BASED PROTEOMICS PLATFORM

Ashley Lawton, Phylos, Inc, 128 Spring Street, Lexington, MA 02421, USA

Phylos is developing a broad based proteomic platform based on novel *in vitro* combinatorial biology technology. The PROfusion™ technology is being used in a variety of applications (e.g. protein arrays, research reagents, protein interactions and pathway analysis) that will be used by research groups in pharmaceutical and biotechnology companies to identify and validate the role of proteins in disease development and progression. Phylos anticipates that these activities will generate significant near-term revenue flows that will be used to develop protein-based biopharmaceutical products.

Long-term value and revenues will be generated through the development of a pipeline of therapeutic products (TRINECTIN™ binding proteins) based on an abundant human protein structure that possesses the ability to be manipulated to produce high-affinity and highly target-specific binding proteins. This new class of binding proteins will position Phylos to compete strongly with the current industry leaders that are developing antibody-based therapeutic products.

Phylos' PROfusion™ technology facilitates the selection of small, high-affinity, and robust binding proteins to a variety of targets (e.g. proteins, small molecules) in a high-throughput, low-cost process. PROfusion is an *in vitro* method that covalently links each protein, in a library of 100 trillion different proteins, to its own messenger RNA. With complete control over the selection conditions, Phylos' scientists are able to rapidly select proteins from these libraries on the basis. of their specific characteristics (e.g. high-affinity), and amplify ("enrich") them through PCR of the attached genetic material. After a number of rounds of selection and enrichment Phylos is able to consistently select binding proteins with single-digit nanomolar and picomolar binding affinities.

The PROfusion technology has proved to be a robust system that has been used successfully with libraries of peptides, proteins (e.g. specific scaffolds) and cellular proteins. All major applications have been enabled. The system has also been automated and current efforts are focused on optimization of the automated system.

145P PHAGE ANTIBODIES AS DISCOVERY TOOLS AND DRUGS

John McCafferty Cambridge Antibody Technology, The Science Park, Melbourn, Cambs SG8 6JJ

Phage display is a powerful method for the generation of antibodies as research reagents and clinical candidates. Over the years, the use of this technology has been developed to the point where 4 different phage derived human antibodies are in clinical trial.

More recently, the use of automation and high throughput screening has been integrated into the antibody selection and screening process, initially in binding assays such as ELISA and immunocytochemistry. A wider range of biological and signaling assays are now being employed earlier in the drug discovery process. Thus phage display provides an important addition to the repertoire of protein based tools applicable to pharmacology.

The presentation will review the use of phage display across the spectrum of activities, from the creation of therapeutic drugs to the generation of research and target validation reagents.

146P FUNCTIONAL PROTEIN ARRAYS

Michelle Mulder*, Mitali Samaddar*, Joe Boutell*, Darren Hart*, Ben Godber*, Jens Koopman*, Roland Z. Kozlowski*# & Jonathan M. Blackburn*+ *Sense Proteomic Limited, Babraham Hall, Cambridge CB2 4AT, UK, #Department of Pharmacology, The Medical School, University of Bristol, University Walk, Bristol, BS8 1TD, UK & +Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1GA, UK.

Recently, considerable interest has focused on the development of protein microarrays. To date, published findings have revolved around the non-specific attachment of proteins to solid substrates or the creation of antibody arrays to produce "chips" specifically for the purpose of capturing novel proteins. However, many of the potential applications of protein arrays rely on the ability to create arrays of many different types of protein for functional interrogation.

We have developed sequence-independent approaches towards this goal which involve modifying each individual gene at the cDNA library level in a single pot such that, following clonal separation and expression of the library in an appropriate host, each recombinant protein has an affinity tag appended at either the C or N terminus. We will show data on the output of our COVET process and will show that the tag can then be used to impart a commonality to downstream purification and immobilisation of the proteins in an automation-compatible format.

Clearly, the value of our method relies on proteins retaining their function once they have been oriented and tethered to a surface, irrespective of whether the format is microwell plate- or chip-based. We have performed a range of assays to demonstrate the viability and function of N and C terminally tagged proteins tethered to different substrates. Examples include: (i) enzymatic activity of glutathione S transferase, luciferase, alanine racemase, horseraddish peroxidase, β-galactosidase; (ii) DNA-binding activity of the transcription factors NF-κB, NFAT and p53; (iii) on-chip phosphorylation of P53 mutants; (iv) binding of include small molecules; and (v) protein-protein interactions. In addition, we have successfully used mass spectrometry to identify proteins specifically immobilised directly on a MALDI target.

In conclusion, we have gained substantial experimental evidence to support the idea that proteins can retain their function when specifically immobilised onto surfaces and that the resultant arrays can be probed using a number of detection methods for novel protein-protein, protein-ligand and protein-DNA interactions. We therefore argue that our tagging methodology provides access to large protein arrays which will prove to be of particular use in the processes of target: identification and candidate compound validation.

147P PROTEIN BIOCHIPS AS NEW TOOLS IN PROTEOMICS

Steffen Nock, Zyomyx, 26101 Research Road, Hayward, CA 94545, USA

Novel high-throughput biological applications in the drug discovery process and disease diagnosis require highly parallel, miniaturized device technology applied to proteins and their biochemical pathways. While technological innovation has adapted the analysis of genetic material to a miniaturized format, the more delicate nature of protein structures has precluded the development of analogous devices for proteins. Protein microarrays have started to emerge recently based on new developments and integration efforts in advanced materials, protein engineering, and detection physics.

We have developed high-density protein microarrays for quantification of multiple proteins in complex mixtures. The implementation of new surface chemistries allows the immobilization of exactly defined quantities of proteins on each spot while retaining the full activity of the protein. Using this platform we have developed a multiplexed, microchip-based immunoassay to analyze expression levels of serum proteins. Detection limits on this microassay are equal to or lower than commercial ELISA tests and reduce the sample volume by many orders of magnitude.

148P *IN SILICO* PROTEOMICS – A NOVEL APPROACH

William D.O. Hamilton Proteom Limited, Babraham Hall, Babraham, Cambridge CB2 4AT, UK.

One of the most significant disciplines to develop in the post-genomic era is that of proteomics. With the number of genes now more or less defined for the human genome and the number of potential drug targets apparently smaller that originally expected, the focus is now changing to the identification, analysis and cataloguing of all the different protein variants in every conceivable cell type under a multitude of conditions. The huge amount of genomics data now currently available will soon be dwarfed by a deluge of proteomics data.

The proteomics field is dominated by laboratory-based technologies with associated bioinformatics tools enabling projects to be run faster and on a much larger scale. However, while such efforts with provide a wealth of new data, alternative approaches will also be required in order to understand the complex interactions that exist within the proteome. One such approach is the emerging field of *in silico* proteomics. This concerns, the prediction of aspects of protein structure, interaction or function.

By way of introduction, a number of studies that seek to determine predictive rules for protein functionality from biological data will be reviewed. The approach taken by Proteom will be described with reference to its proprietary informatics platform and progress in the design of peptide ligands, the prediction of binding sites on proteins and the prediction of protein-protein interactions.

149P WHAT CAN TEACHING AND LEARNING RESOURCE PACKS DO FOR YOU?

Michael Hollingsworth, School of Biological Sciences, G38 Stopford Building, Manchester University, Oxford Road, Manchester M13 9PT.

Funding under the Teaching and Learning Technology Programme (TLTP) 2 enabled the development of 45 computer-assisted (CAL) packages and videoclips dealing with a wide range of topics in pharmacology. More than 1750 copies have been supplied worldwide. However, staff reported that there were constraints to the implementation of such technology based teaching materials, such as lack of time to develop support materials and use these methods, and a culture which did not promote non-traditional approaches (Markham, Jones & Sutcliffe, 1997). Student usage of CAL increased if it was integrated into the course and if it was assessed (Dewhurst & Hughes, 1999).

The main aim of the pharmacology TLTP3 project was to assist staff to embed CAL packages in the curricula. This process was to be achieved by the development of Teaching and Learning Resource Packs (TLRPs). These TLRPs contain already prepared teaching material for staff that give students tasks to do, based around one or more CAL packages. TLRPs may contain workbooks, exercises, multiple choice questions, problem-solving exercises, problem-based learning exercises, cases and assessments. Most come with Tutor notes and, where appropriate, model answers. The TLRPs are designed to be customisable for local needs and so are in electronic format (Word and PowerPoint). They are useful for a wide range of students (science, medical and para-medical).

To ensure that the TLRPs were of a high standard and could meet varied needs, there was a defined development cycle. In the first year a small team of pharmacologists (2-5) from different Universities developed the material and trialled it in their own institutions. Other members of the consortium formally evaluated these TLRPs in the second year. Evaluation was overseen by people not involved in the development process (Norris, 2001).

Seven TLRPs were completed by September 1999 (Hollingsworth *et al.*, 1999) and copyright invested in the British Pharmacological Society to make them readily available. A further 12 TLRPs can now be obtained on the following topics: Enzymes as Drug Targets; Ligand-Gated Ion Channels as Drug Targets; Drug Metabolism I and II, Haemostasis; Clinical Trials and Drug Development; Synaptic Transmission in the CNS: Dopaminergic Transmission I and II; Pharmacokinetic Simulations; Calculations for Pharmacologists; Blood Pressure Simulation. For full details go to http://www.bps.ac.uk, Educational Resources.

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