The front cover shows BAZ1A staining of human small intestine, image from the Human Protein Atlas (www.proteinatlas.org). Cover design by Sorcha Rabbitte.
ESF ProteomeBinders MolTools Workshop

Affinity Proteomics

13 – 15 March 2007
Congress Centrum Alpbach, Austria

Organised by:
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Supported by:

European Science Foundation / Frontiers of Functional Genomics network programme
European Commission / ProteomeBinders FP6 Research Infrastructure Coordination Action, MolTools FP6 Integrated Project.
Workshop Introduction

Mike Taussig
 Coordinator, ProteomeBinders

Welcome to this workshop on Affinity Proteomics, organised by the ProteomeBinders consortium on the occasion of our first reporting meeting, coincident with the end of year 1 of the project. This has become something of a regular series of gatherings, initially called 'ligand binders against the human proteome' workshops (Cambridge 2004, Uppsala 2005) and which led directly to the proposal for the EC Research Infrastructure Coordination Action (CA). The ProteomeBinders contract was awarded in late 2005 and the official start date of the CA was 1st March 2006.

We are all aware, of course, of the central importance of antibodies in day-to-day biomedical research of all types and increasingly in diagnostics and therapy. Our stated objective in ProteomeBinders is to lay the groundwork for a European resource of affinity reagents (a.k.a. binding molecules or simply ‘binders’) for detection of the human proteome. Apart from the focus on humans, there is in principle no restriction on the specificity or variety of the binding molecules which could be included in the resource other than that they should serve the aim of protein detection in its broadest sense. We discussed the issues involved in setting up such a resource in our recent commentary article in Nature Methods (Taussig et al., 4:13, 2007), issues which were developed further in the perceptive Editorial prefacing the same issue (Nat Methods 4:1, 2007). The latter made several important points which will be discussed during this workshop. One is the inherent complexity of the project, in some respects exceeding that of the human genome, given the possibility that the 23000 or so human genes can generate up to a million different protein species (omitting immunoglobulins) by the processes of splice variation and modification, which are essential in making the genome ‘work’ in the cell. This gives rise to many organisational questions before such a project can even start to deliver, e.g. how to prioritise the targets, how many affinity binders are needed, how to match binder properties to their applications, and so on.

The concept of affinity reagent resources has taken on wider international dimensions with the recent US initiative on proteome reagents organised from the National Cancer Institute. ProteomeBinders partners are also involved there and the prospect is for good links between the two programmes, which can avoid duplication and competition. Other initiatives, such as the Swedish Human Proteome Resource and the German Antibody Factory are already part of the ProteomeBinders consortium. Quite apart from the scientific questions, there is the whole issue of how to combine commercial and public (academic) efforts into a collaborative rather than a competitive scenario. The relationship between a publicly funded initiative such as ours, and the private sector which sees affinity reagents as excellent profit-making opportunities, could easily lead to tensions, since the EC Infrastructure will (if and when it starts functioning) operate on the principle of open, though not cost-free, access. A solution favoured in the US is to commission the biotech sector to make the reagents, which will then be distributed on a cost-recovery basis by the Infrastructure. Another possibility is for the Infrastructure to concentrate its efforts only on the targets which are so far not covered by commercial interests. But the Nature Methods editorial puts its finger on an important alternative raison d’être of ProteomeBinders, which is to ensure proper quality control of these vital reagents. The negative experience of researchers who are unable to obtain validated reagents which behave in an unambiguously correct way in their particular application is widespread. Therefore the idea that what is really needed now are quality standards and reagent certification programmes is a powerful one. The possibility that the EC and NCI programmes could institute ‘gold standard’ certification for commercial and
academically raised reagents is winning acceptance and we hope that this can be carried forward as the next phase of the ProteomeBinders project and a necessary preliminary to full scale production.

These and other issues will certainly be talked about here in Alpbach. This is our first attempt to combine molecular proteomics with winter sports, so successfully applied elsewhere on the Keystone model. We hope that the weather conditions will be kind and that we do not fall victim to global warming, even though this year has been one of the worst ever for the ski merchants, and that you will manage to enjoy the outdoor amenities as well as the serious side of the meeting. We appreciate your feedback and look forward to a splendid time discussing science of proteins in the Austrian mountains.

Acknowledgements

My particular appreciation goes to Dr Cheryl Smythe (ESF functional genomics programme administrator) and Dr Oda Stoevesandt (ProteomeBinders administrator) for their incredibly painstaking and tireless work on every detail of the arrangements. We thank the staff of the Alpbach Congress Centre (Georg Hechenblaikner, Margreth Margreiter) for their excellent cooperation both for the venue and in organising dinners and accommodation. In advance we thank the various hotels, guest houses and dining venues for what will I am sure be their excellent service.

We wish to note our sorrow at the untimely death of Dr Jane Steel, our project partner from the Beatson Institute (Glasgow) who died tragically in a diving accident in Gozo in July 2006.

The meeting is supported financially by the European Science Foundation through the Frontiers of Functional Genomics network programme, and by the European Commission through the ProteomeBinders contract 026008. We express our great appreciation to both these organisations.
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Welcome to Alpbach!

We are happy that you could join our ESF / ProteomeBinders Workshop on Affinity Proteomics!
Before the meeting begins, here is some practical information.

Registration
After moving into your room, please come to see us at the registration desk in the conference centre from 14:00 on Tuesday. We will provide you with your meeting badge and booklet. In case you were using the shuttle organised by us to get to Alpbach and/or back to Munich and are not an invited speaker, please bring 35 € per trip in cash to reimburse us. Thanks!

Ski rental
If you wish to rent skiing equipment, we have negotiated a meeting participant discount of 20% on the normal price with “Sport Conny’s” (see map opposite page). To claim the discount, please quote “ESF-meeting” when taking out your equipment. Weekday opening hours: 8:30 -12:00 and 15:00 - 18:00
We suggest that you take out equipment on Tuesday afternoon already to avoid loosing skiing time on Wednesday and Thursday.

Taxi
In case you should require transportation in Alpbach during the meeting, this can be arranged at your own cost with Taxi Moser, Tel. +43-(0)-5336-5616 or +43-(0)664 543 0888 (mobile).

Phone contact to the Congress Centrum Alpbach and to the organisers
Phone contact to the conference centre is +43-(0)5336-600-100
During the conference, Mike Taussig can be reached at +44-(0)7951 452761 or +44-(0)7768 175834.
Tuesday 13.3.2007 – Arrival and Registration

14:00 onwards: Registration open at the Conference Centre Alpbach. Please come along during the afternoon to avoid queues in the evening.

then ... take out skiing equipment? Please see page 4.

18:30 onwards: Get together at Jakober

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Map of central Alpbach with Tuesday’s areas of interest
Wednesday 14. 3. 2007 – First day of Workshop

Session 1  Overview of the human proteome
Chair: Mike Taussig

8:30  Mike Taussig: Welcome
     The Babraham Institute, Cambridge, UK

8:35  Matthias Wilm: Proteomics is chasing the time line
     EMBL, Heidelberg, Germany

Life itself is quasi by definition dynamic, always changing, developing. Mass spectrometric proteomic techniques had a massive impact into biological research by first allowing to sequence proteins in a very sensitive way, then by doing this in a high throughput fashion helping to decipher the functional relationship between proteins by analysing quasi-static protein networks. Protein complexes are at the core of networks that make up biological entities. Life building upon these networks however is inherently dynamic. Protein complexes or individual components travel through the cell or between the cell nucleus and the cytoplasm. Signals are transmitted spatially from the cell membrane to the DNA stored in the cell's centre and functionally through different classes of proteins. These changes can only be observed by a time resolved analysis. Mass spectrometric investigation will never achieve a time resolution like microscopy can but it is possible to observe changes on a truly proteomic scale. Currently the community is in the process of setting up analytical tools that will display these changes over time.

The presentation will give a short introduction to the developments at EMBL in this direction and highlight the possibilities that will become mainstream once more than one company delivers appropriate tools to the biological community.
Mathias Uhlén: A human protein atlas for normal and cancer tissues

Department of Biotechnology, AlbaNova University Center, Royal Institute of Technology, Stockholm, Sweden

Antibody-based proteomics provides a powerful approach for the functional study of the human proteome involving the systematic generation of protein-specific affinity reagents. We have used this strategy to construct a comprehensive, antibody-based protein atlas for expression and localisation profiles in 48 normal human tissues and 20 different cancers (1). The Human Protein Atlas is publicly available (www.proteinatlas.org) and contains, at present, approximately 1.2 million high-resolution images corresponding to more than 1500 antibodies towards human proteins. Each image has been annotated by certified pathologists to provide a knowledge base for functional studies and to allow queries about protein profiles in normal and disease tissues (2). We have recently (3) used the antibody reagents for validation of potential biomarkers to Mantle Cell Lymphoma (MCL) as well as high-throughput biomarker analysis of thousands of serum samples using a reverse array format (4) and multiplex assays (5). Our results suggest it should be possible to extend this analysis to the majority of all human proteins thus providing a valuable tool for medical and biological research, in particular for biomarker analysis in various patient cohorts.

References:

Andreas Plückthun: Specific binders against integral membrane proteins

Dept Biochemistry, University of Zurich, Switzerland

Monoclonal antibodies recognizing detergent-solubilised native states of integral membrane proteins (except the most robust ones) are very unlikely to be generated by the classical hybridoma route: Once injected into an animal, the structural fate of the solubilised membrane protein is uncontrollable. This is a realm where selection from libraries has an obvious advantage. Progress and results generating highly specific binders for multi-spanning membrane proteins will be discussed, based on synthetic antibodies and Designed Ankyrin Repeat Proteins, with particular emphasis on crystal formation.

Coffee
Session 2  Bioinformatics infrastructures for proteomics  
Chair: Toby Gibson

10:20  Lydie Lane: The UniProtKB-Swiss-Prot Human Proteomic Initiative (HPI) as a support for the characterisation of the human proteome in health and disease

Lydie Lane [1], Silvia Jimenez [1], Amos Bairoch [1,2], and the Swiss-Prot group.

[1] Swiss-Prot group, Swiss Institute of Bioinformatics, CMU, 1 Michel Servet, 1211 Geneva 4 [2] Department of Structural Biology and Bioinformatics, Faculty of Medicine, University of Geneva

UniProtKB/Swiss-Prot is a manually annotated protein knowledgebase, which means that data integrated into the knowledgebase, including the protein sequences, are continuously checked and updated by the Swiss-Prot expert team of biologists. In addition to sequence information, each UniProtKB/Swiss-Prot entry contains useful biological information regarding protein location, function or interactors, and cross-references for more than 100 other specialised databases. For many topics, the use of a controlled vocabulary and keywords allows creation of sets of proteins. For example, proteins from one organism, or known to be involved in a given function, or present in a particular tissue or cell compartment can be easily retrieved. This functionality can be particularly useful in design or analysis of proteomics profiling studies. Moreover, a special emphasis is laid on the annotation of biological events which generate protein diversity but which are not always predictable at the genomic level. Hence, post-translational modifications (PTMs) or alternative splicing events are extensively annotated, along with their functional consequences. One of the major goals of UniProtKB/Swiss-Prot for the next few years is to provide complete sets of annotated proteins for various model organisms. The Human Proteome Initiative (HPI) was started in 2001 in order to annotate the protein products of all known human genes and their mammalian counterparts to the standards of the UniProtKB/Swiss-Prot knowledgebase. A special effort is made to provide extensive information on human polymorphisms and disease-linked mutations. Researchers are welcome to contribute their knowledge to the scientific community by submitting relevant comments or data to Swiss-Prot at: swiss-prot@expasy.org.

10:45  Henning Hermjakob: Designing a binder database

Proteomics Services, European Bioinformatics Institute (EBI), Hinxton, UK

The primary objective of ProteomeBinders is to coordinate the establishment of a European resource infrastructure of binding molecules directed against the entire human proteome. An essential component of such a resource, in particular in a distributed European setting, is the efficient data capture, exchange and analysis of the entire pipeline, from binder production to quality assessment and research reports, within the consortium and beyond, in particular in international collaboration with related efforts. However, not all components of such a potentially complex system need to be developed from scratch. Open standards and software can be used as starting points for the overall binder data management system. We will report on the current status of the evaluation and adaptation of the HUPO Proteomics Standards Initiative framework to the representation and exchange of binder data in an international context, leveraging existing research investment and collaboration efforts.
11:05  Discussion

11:15  Depart to hotel to prepare for skiing or other

Ski bus information

To the lift:
- If weather conditions are promising, our own ski bus (marked “Affinity Proteomics”) will leave from the bus stop (see map, page 13) between the Congress Centrum and Hotel Boeglerhof at 12:00. In case the morning sessions of our workshop run over time, our bus will leave slightly later – please note announcements.

- Additional public ski buses leave from the bus stop between the Congress Centrum and Hotel Boeglerhof, and are free of charge for passengers carrying ski equipment. Please consult the timetable.

Back to Alpbach:
- For ski buses back to the village, please consult the public bus timetable. To be back in time for the afternoon session, we recommend catching the 15:30 bus at the latest.

15:30 onwards: Coffee available in the Congress Centrum
Session 3  Antibodies and alternative binders  
Chair: Marius Ueffing

16:15  Ronald Frank:  ProteomeBinders link to Chemical Biology  
Department of Chemical Biology, Helmholtz Centre for Infection Research,  
Braunschweig, Germany

ProteomeBinders aims to provide a comprehensive collection of selective binding molecules for all human proteins to be applied in various genome/proteome-wide studies. Chemical Biology uses low molecular weight compounds to probe cellular and organismal functions. The compounds act by perturbing underlying cellular processes through binding to targets such as proteins thereby interfering with their function. Compounds are largely identified by empirical screening collections of diverse molecules with assays addressing the function of interest. Biologically active compounds resulting from such screens must be considered as protein binders and many of these may fulfill future ProteomeBinders criteria. This situation was clearly highlighted by one of the reviewers of the ProteomeBinders proposal. Furthermore, the EC ESFRI “Biology and Medical Science Roadmap Working Group” merged the ProteomeBinders and the European Molecular Library Resource Centre proposals for future European infrastructures into a joint “Chemical Biology Infrastructure”, which is certainly not adequate but presumably arose because of this situation. Although quite distinct with respect to their own special goals, chemical biology infrastructures could be very supportive of the “alternative binders” activities of ProteomeBinders.

I will present our peptide/small-molecule facility at the Helmholtz Centre in Braunschweig, which is a partner of the German chemical biology network “ChemBioNet”, and outline concepts for producing small molecule ligands that may meet ProteomeBinders type criteria of quality and throughput.

16:40  Larry Gold:  SomaLogic's progress with aptamer-based proteomic arrays  
SomaLogic, Boulder, CO, USA

Proteomic arrays require "content" and "performance" - SomaLogic has reached rapid content development and high performance. I will use this talk to describe the standard operating procedures that lead to inclusion of new photoaptamers on to our array menu, and some of the modifications to our array protocol that have been made since 1997.
The use of so-called protein scaffolds for the generation of novel binding proteins via combinatorial engineering has recently emerged as a powerful alternative to natural or recombinant antibodies. This concept requires a robust protein fold which tolerates multiple substitutions or even insertions at the primary structural level. In this way, novel reagents can be generated that may be particularly useful for proteomics applications, i.e. detection and quantification of gene products via ELISA, pull-down assays, protein arrays, tissue staining, Western blotting, FACS etc. A summary of the still growing number of protein scaffold that are currently under investigation will be given. These approaches can be classified into three groups: carrier proteins for the display of single variegated loops, scaffolds providing rigid elements of secondary structure, and frameworks that support several conformationally variable loops in a fixed spatial arrangement.

Anticalins will be presented as one example for the successful development of a novel class of binding proteins that possess high affinities and specificities for prescribed target proteins. Anticalins are based on the lipocalin scaffold, which is employed by nature to yield diverse ligand-binding proteins in many organisms. Despite low mutual sequence homology they share a circularly closed eight-stranded anti-parallel \( \beta \)-sheet as their central folding motif. This \( \beta \)-barrel supports four loops at its open end, which form the entrance to the binding pocket. These loops exhibit large conformational and amino acid sequence differences between individual lipocalins and thus give rise to the variety of natural ligand specificities. Using targeted random mutagenesis of this hypervariable loop region in combination with phage display selection, colony screening as well as high throughput assays, lipocalin variants directed against a variety of molecular targets have been generated, initially starting with small hapten-like compounds (e.g. digoxigenin, fluorescein) and, more recently, extending to medically relevant protein antigens such as CTLA-4 or VEGF. Anticalins exhibit high target specificities and extraordinary affinities, reaching to the subnanomolar range. Their crystallographic analysis confirmed the high structural plasticity of the lipocalin loop region and, thus, explains the flexible molecular recognition of both low molecular weight ligands and large protein targets.

Anticalins provide several practical benefits because they are composed of a single polypeptide chain, have a small size, and their set of four loops can be easily manipulated at the genetic level. Furthermore, they do not exert immunological effector functions, which may cause side effects in many therapeutic applications. Anticalins can be generated from human lipocalin scaffolds, thus reducing the risk of immunogenicity when used for therapeutic purposes or for \textit{in vivo} imaging. They can be produced at high amounts in \textit{E. coli} and exhibit surprising robustness, with high thermal stabilities. In addition, anticalins are amenable to the production of functional fusion proteins, e.g. with reporter enzymes, offering useful reagents for proteome research.
As part of the German National Genome Research Network (NGFN) 2, the “Antibody Factory” platform aims to evaluate and establish an in vitro selection pipeline (phage display) for recombinant antibodies optimised to meet particularly the needs of proteome research. The Platform is organised in four closely cooperating projects covering the process from antigen provision to antibody selection, production and evaluation. Since 2005, improved phage display vectors and large human antibody gene libraries ($5 \times 10^9$ independent clones) have been constructed and evaluated. Significant results have been obtained already resulting in a set of monoclonal recombinant human antibodies in a prototype phase in interaction with other NGFN 2 entities and project partners in research, medicine and industry.

Many cellular activities are controlled by post-translational modifications (PTMs), the study of which is hampered by the lack of specific reagents. The small size and ubiquity of such modifications makes the use of immunisation to derive global antibodies, able to recognise them independently of context, extremely difficult. Here we demonstrate how phage display can be used to generate such specific reagents, using sulfotyrosine as an example. This modification is important in many extracellular protein-protein interaction, including the interaction of some chemokines with their receptors, and HIV infection.

We designed a number of different selection strategies, using peptides containing the sulfotyrosine modification as positive selectors in the presence of an excess of the non-modified peptide as blocking agent. We screened almost eight thousand clones after two or three rounds of selection and identified a single scFv able to recognise tyrosine sulfate in multiple sequence contexts. Further analysis shows that this scFv is also able to recognise naturally sulfated proteins in a sulfation dependent fashion, and its binding could be inhibited by soluble tyrosine sulfate, but not tyrosine or tyrosine phosphate, providing an excellent way to control for the specificity of binding. This scFv was converted into a full length IgG and into an scFv-AP fusion, both of which increased the stability. This antibody has been distributed to a number of different groups which have used it successfully, some results of which will be presented.

It has proved to be extremely difficult to generate antibodies able to recognise post-translational modifications independently of sequence context by immunisation, with antibodies against phosphotyrosine being the only well documented example. The use of phage display, as described here, provides proof of principle for the use of this technology to develop similar reagents against other post-translational modifications.
The driving force behind oncoproteomics is the search for protein signatures that are associated with a particular malignancy and that subsequently can be used for, (i) early diagnosis; (ii) predictive diagnosis, i.e. assessing the risk for tumor relapse and disease progression; and (iii) predicting treatment resistance. Recent technological development with recombinant antibody microarrays and its clinical application within solid tumors will be presented.
In the past years our group has focused on the development of new microarray concepts for protein identification. A specific goal has been the development of technology platforms that enables multimodal readout, i.e. both fluorescence and MS identification in microarray formats. Two strategies to accomplish this have been followed.

**A first approach** concerned the development of a 3-D micro- and nanoporous silicon surface developed as a carrier matrix for immobilised proteins and antibodies. Key goals were to obtain a surface that provided increased surface area for antibody antigen interaction and consequently improved detection level as compared to planar substrates. In the course of this development, surface roughness of the generated porous 3D-matrix came to play a major role in the performance of each new surface. By tuning the surface roughness to behave hydrophobically we were able to obtain the smallest reported spot size for non-contact printed microarrays, ≈ 50 µm spot size, and hence the highest array densities for non-contact printed arrays ≈ 14000 spots/cm². It should be noted that although the surface behaved hydrophobically to the spotted antibody droplets the surface was hydrophilic at a molecular scale, minimising denaturation of the dried antibodies. The 3-D porous silicon protein chip surface has been evaluated both with respect to manufacturing and assay reproducibility, showing endpoint assay data with relative spot mean intensity variations of less than 15% as measured by an IgG assay in plasma. Further work has employed the chip surface for the detection of PSA (prostate specific antigen). Several assay modes have been investigated including antibody/antigen-(labelled), reverse assays and sandwich assays, all with fluorescent readout. The protein chip surface can also be analysed directly by MALDI-TOF MS. This provides not only information on a binding event but also detailed structural information on the bound species. Proof of principle of a dual readout mode (fluorescence and MS) is shown in the renin inflammatory response pathway in blood plasma samples. To further enable this mode of operation we are now pursuing the possibility of performing localised high-speed digestion of bound species in each spot in order to obtain sufficient data for MS identification. Initial experiments on high-speed (≈30 sec) digestion of prostate cancer related antigens (PSA and human kallikrein 2, hK2) were performed and identified by MALDI TOF MS.

**A second approach** to multimodal protein microarray analysis has yielded a MALDI target with both on-target solid phase extraction and in-target fluid handling, termed ISET (Integrated Selective Enrichment Target). ISET enables enrichment of low abundance proteins on the MALDI target and direct MALDI-readout. The target plate holds a nanocolumn of microbeads for analyte enrichment. Protein and peptide enrichment, superceding the performance of any standard affinity extraction concept available, has been accomplished. The target format is currently designed in a 96-array format to meet industrial standards. Analyte handling fits directly into robotic pipette sample processing units. ISET is currently also being developed in a disposable format. Performance data and comparison to solid phase extraction pipetting will be reported. The current work now focuses on the development of protocols for both the described microarray platforms to enable screening of candidate biomarkers in large biobank repositories with a primary goal of developing a multiplexed assay approach for improved cancer diagnostics.
The biomimetic ‘bottom-up’ assembly of programmed molecular building blocks offers highly attractive strategies for the generation of functional nanomaterials. With respect to this approach, DNA oligomers are promising building blocks for the fabrication of nanostructured architecture using self-assembly strategies. Our work is focused on the DNA-functionalisation of proteins and nanoparticles. For instance, we have developed self-assembled oligomeric networks comprised of streptavidin, gold nanoparticles and DNA, which are applicable as reagents in ultra-sensitive protein detection assays.

Conjugates of DNA and proteins are also being utilised for the functionalisation of surfaces via nucleic acid hybridisation. This DNA-directed immobilisation allows one for reversible and site-selective functionalisation of solid substrates with metal and semiconductor nanoparticles, or, vice-versa, for the DNA-directed functionalisation of the nanoparticles with proteins, such as immunoglobulins and enzymes. This approach offers many applications, such as the detection of microarray-immobilised antigens. Moreover, covalent DNA-enzyme conjugates are being developed as a novel class of biocatalysts with programmable self-assembly properties. These hybrid molecules are useful for catalysis, sensing and biomaterials.

Recent References:
The ability to detect exceedingly low amounts of specific proteins could open the door to early diagnosis of a wide range of diseases. Similarly, sensitive techniques to detect *in situ* protein molecules and their patterns of interactions and covalent modifications may yield valuable insights in subcellular processes. Critical for these efforts are the development of highly specific protein detection reactions, which can then be amplified to visibility without concomitant increase of nonspecific signals. We have developed a technology, termed proximity ligation, that uses antibodies or other binding reagents coupled to oligonucleotides for proximity-dependent ligation reactions that depend on dual or triple recognition of target molecules (1-3). The assay currently permits detection of as little as a few hundred target proteins (4) or single infectious agents (5) in solution, and even single protein molecules or interacting pairs of proteins *in situ* (6). By a suitable choice of binding reagents, non-protein molecules can also be detected, and it has been used to demonstrate binding of transcription factors to specific DNA sequences (7).

In solution-phase assays, oligonucleotides conjugated to antibodies that have been brought near to each other by binding the same target protein, can be joined by ligation and later amplified by PCR with real-time detection. The assay can be performed without washes, or as sandwich assays where the proximity probes are added as secondary reagents after target molecules have been bound to immobilised affinity probes. For *in situ* detection, oligonucleotides attached to antibodies are used to template circularisation by ligation of pairs of oligonucleotides that are added after the antibodies have bound their targets. Next, the DNA circles that form are used to template rolling-circle replication reactions, primed from one of the antibody-bound oligonucleotides. Both assays for solutions-phase or *in situ* detection can be used to detect individual proteins or complexes of interacting molecules. We have used the solution-phase method to investigate cytokines and biomarker proteins, and the *in situ* assay has been applied for detection of interacting oncoproteins as well as cell surface receptors. Important future goals are to extend the methods to permit parallel analyses of large sets of target molecules, both in solution and *in situ*.

References:
Session 2: Intrabodies
Chair: Silvère van der Maarel

10:15 Terence Rabbitts: Interfering with protein-protein interactions in cancer cells with single domain antibody fragments
T.H. Rabbitts, T. Tanaka, C-H. Nam & N. Lobato
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2HN, UK
and Leeds Institute of Molecular Medicine, Section of Experimental Therapeutics, St. James's University Hospital, Leeds, LS9 7TF, UK

Most protein targets in cancer are intracellular and involved in processes that utilise protein-protein interactions. The latter is generally considered a difficult target for small molecule drugs and more suitable for macromolecular agents such as intracellular antibody fragments or peptide aptamers whose affinity and bulk represent optimal inhibitory entities. We have developed a screening approach for intracellular antibodies that has defined an optimal scaffold for CDR presentation and has shown that single immunoglobulin domains are favourable intrabodies, not requiring intramolecular disulphide bonds for interaction with high affinity to target antigen. We will illustrate this technology with an anti-RAS single VH domain that prevents tumor formation in a mouse model by interfering with activated RAS-PI3K interaction.

10:40 Silvère van der Maarel: Single domain antibody fragments for biomedical applications
Silvère van der Maarel, Sabine Baars, Rinse Klooster, Gert Jan van Ommen
Leiden University Medical Center, Department of Human Genetics

Single domain antibody fragments (VHH) are eminently suited for phage display. Over the past years we have successfully selected VHH from a non-immune library against a variety of proteins of biomedical relevance. The targets of these VHH range from nuclear, to cytosolic, vesicular or transmembrane proteins. We have demonstrated that selected VHH can be applied in a variety of immunological applications ranging from Western blotting to immunomodulation through intracellular expression of the VHH. In the context of a cellular model for ocular oropharyngeal muscular dystrophy (OPMD), widely considered a paradigm for protein aggregation disorders, we have demonstrated that intracellular expression of specific VHH could prevent or reduce nuclear protein aggregation. Current focus is on the (in vivo) use of VHH in the early diagnosis and therapy of disorders of misfolded protein aggregation. The status of the work will be presented and some current progress.
A comprehensive understanding of cellular processes and their pathophysiology requires information on abundance, localisation, post-translational modifications and dynamic interactions of cellular components. Immunofluorescence staining detects any cellular antigen exclusively in fixed cells, providing snapshots of distribution but not of mobility. In contrast, fluorescent fusion proteins reveal dynamic changes in living cells but not of endogenous proteins, their modifications nor other cellular components. Here we describe the development of fluorescent, antigen-binding proteins, termed chromobodies, to target and trace antigens in living cells. We combined the epitope-recognition fragment of Heavy-chain antibodies from Camelidae with a fluorescent protein domain, generating chromobodies against various antigens. Using chromobodies against GFP fusion proteins we could show that chromobodies can be expressed in mammalian cells and recognise antigens in different subcellular compartments and structures. Even antigens from central parts of the replication machinery as well as antigens deeply embedded in the chromatin could be traced throughout S phase and mitosis, demonstrating the suitability of chromobodies for live cell studies. Furthermore, we generated chromobodies against cytokeratin and lamin demonstrating that endogenous antigens from different subcellular compartments are equally well recognised. We anticipate that chromobodies will open up new possibilities for live cell microscopy. They will allow entirely new functional studies as potentially any antigenic structure including post-translational modifications and non-protein components can now be targeted and traced in living cells.
AlphaScreen is a bead-based non-radioactive Amplified Luminescent Proximity Homogeneous Assay. When a biological interaction brings the beads together, a cascade of chemical reactions produces a greatly amplified signal. On laser excitation, a photosensitiser in the Donor bead converts ambient oxygen to a more excited singlet state. The singlet state oxygen molecules diffuse across to react with a thioxene derivative in the Acceptor bead generating chemiluminescence at 370 nm that further activates fluorophores contained in the same bead. The fluorophores subsequently emit light at 520-620 nm thus demonstrating the interaction between two biological partners. In the absence of a specific biological interaction, the singlet state oxygen molecules produced by the Donor bead go undetected without the close proximity of the Acceptor bead. As a result, only a very low background signal is produced. AlphaScreen provides a highly versatile, sensitive, time-resolved, homogeneous and miniaturisable means to perform assay development efficiently and in HTS resulting in higher throughput at lower costs. We will describe the AlphaScreen technology and its major advantages which will then be illustrated in the context of the objectives of ProteomeBinders.
This talk will focus on two novel, counter-intuitive, and elegant approaches to deposit molecules onto surfaces and to measure binding reactions with high sensitivity. Binding experiments at the solid/liquid interface, such as DNA arrays or protein chips, require (1) surfaces with a thin layer of purified molecules, (2) a distribution or patterning of these molecules, and (3) a method to detect and quantify the binding event of the assay. The first two points are usually addressed by spotting of purified proteins or nucleic acids or small molecules onto a target surface. The third point, typically, might be realised using fluorescence labeling or surface plasmon resonance techniques.

Affinity-contact printing (αCP) is shown to extract proteins or DNA with high selectivity from crude biological sample solutions and to place them onto a surface in a functional, arbitrary pattern. αCP uses a structured elastomeric stamp derivatised with binders against the target molecules. After the target molecules have been captured (A), they are printed from the elastomer onto a variety of surfaces. The binders remain on the stamp for reuse (B). In contrast with conventional affinity chromatography, here dissociation and release of captured molecules to the substrate are achieved mechanically.

To detect binding events, it is demonstrated that inexpensive classical silver or gold staining and standard compact disc (CD) reader technology (e.g. from a laptop) can be combined into a novel binding-assay system as a substitute for the established, but expensive, signal tags and detection systems used for array-based assays. An assay reader was constructed that uses CD pickup technology to determine the concentration of an antigen in an antibody assay. As an example, the versatile inflammation marker C-reactive protein (CRP) was measured over a dynamic range of four orders of magnitude with a limit of detection (LOD) of 1 pM.
Classical immunisation strategies and novel recombinant antibody/scaffold technologies allow the isolation of binders to nearly any given target molecule. The use of high throughput methods leads to a high output generation of binding molecules, so that criteria that describe their properties have to be defined. Different anticipated applications define these quality requirements as do the methods available for the characterisation of high numbers of binding molecules. Screening systems for the characterisation of affinity, specificity and cross-reactivity will be discussed, as well as the relevant key parameters describing the properties and characteristics of binders.
European societal and health care infrastructures provide unique possibilities for postgenome era biomedical research. However, Europe has not been very efficient in the integration of competence and resources needed to maximally utilise these resources and take the studies to the next level. Since especially U.S. funding agencies are increasing tapping into these resources, the window of opportunity is rapidly closing due to inertia of European research institutes and funding agencies. Recently launched FP7 calls contain several topics relevant to such large-scale studies utilizing biological samples collected in Europe and including vast amounts of biomarker analyses.

I will discuss the critical issues for successful European efforts in this area and some developments that have taken place, partially funded by EC. For FP7, three calls for improved harmonisation and standardisation and integration of existing sample and analytical resources are:

1) HEALTH-2007-A-1.2.1.1-1: Networking major biobanking initiatives across Europe: developing standards and norms for existing and future human sample biobanks: The call aims to create standards and norms relevant to the management and coordination of existing and future human biobanks. The project should network the major biobanking initiatives across Europe with the objective of ensuring future exploitation for co-ordinated large-scale biomarker (proteomics, metabonomics, transcriptomics) discovery and validation.

2) HEALTH-2007-A-1.2.1.1-2: Molecular epidemiological studies in existing, well characterised European population cohorts: The focus is on large-scale genetic epidemiological studies on human samples from European (and/or other) population cohorts and standardised methodologies in proteomics, metabonomics and transcriptomics. This project should identify candidate susceptibility genes to multifactorial diseases via improved integration of large-scale data generated by existing initiatives to improve power and standardisation of statistical analyses.

3) HEALTH-2007-A-1.2.1.1-3: Comparative studies of genetic variation in humans: towards a reference population in Europe: The aim is to carry out genome wide SNP (or other markers)-mapping and/or resequencing from volunteers from different regions of Europe. This project should generate new knowledge about human development and evolution. The collected data should represent European population diversity, and provide a reference for ongoing and new studies on genetic epidemiology in Europe. Importantly, for all the calls, the requirement is that anonymised data must eventually be publicly released and this creates major challenges for data handling and security as well as biobank legislation in European countries.
Traditionally research infrastructures have been viewed in the 'physical sciences way': large, centralised facilities unaffordable by any member state, paid for by a collective process, e.g. CERN. Today however, biology and medical sciences have equal if not greater - and large-scale - impact on health and well-being. This calls for a 'biological adaptation' of the infrastructure concept to better serve the life sciences, with the format of a decentralised network, making existing local resources and data better accessible to others. The first steps have been taken in a section of the European Strategy Forum on Research Infrastructures (ESFRI). In one of the subcommittees, for Bio-Medical and Social infrastructures, a research infrastructure proposal of this nature has been developed to serve the Biobanking and Biological Resource Community. This has been selected, together with 37 other infrastructure proposals, for further development. In the FP7 call 140 M€ has been set aside for the preparatory phase of development of these infrastructures, corresponding to 3-5 M€ for each. Further development of this proposal will be discussed.

What exactly are research infrastructures for? The answer appears obvious in ‘big physics’ where investigators have for decades had access to space probes and atom smashers. But ‘big biology’ is fairly new - with notable successes like the Human Genome Project and the HapMap - and so we ought to think carefully about what kinds of research infrastructure it needs.

In the UK we have some experience of the rise - and the fall - of research infrastructure not only for genetics but also for genomics. The UK DNA Banking Network (UDBN) is reaching the end of its first phase of development and is beginning its second stage. Both development stages have network construction as their central theme: sample and data accrual networks; sample and data management networks; sample and data distribution networks. The first phase was a pilot with national collection consortia for 13 high impact human diseases. The second phase will involve extending the network elsewhere in the UK and abroad. This talk will identify UDBN’s problems (with some solutions) in network construction, maintenance and development and will ask why research infrastructure in biology needs to take the form of networks.
Implemented in research laboratories across the globe, proteomic technologies such as mass spectrometry and affinity-capture have provided a wealth of fundamental information on the mechanisms underlying cancer and other diseases. However, in order to complete the bridge from discovery to the patient, proteomic platforms, reagents and data analysis must meet rigorous performance criteria to assure their acceptability for clinical application. This requires the development of laboratory techniques that are rapid, accurate, reproducible, robust and reasonably economical. Yet many challenges exist in achieving this goal, such as pervasive problems with research design, data analysis, reproducibility, and comparability of research results, a lack of common reagents and highly qualified public data sets, and the inability to manage and interpret large quantities of pre-processed data.

In an effort to accelerate the development of clinical protein detection systems, the NCI has recently established the Clinical Proteomic Technologies Initiative for Cancer. This program is designed to accelerate the translation of proteomics from a research tool into a reliable and robust clinical application by improving protein measurement capabilities, evaluating promising technologies for applicability in both analytical and clinical validation studies, and providing a reagent resource composed of well characterised peptides, proteins, and antibodies to the scientific community. The CPTI program will identify major sources of experimental variability and optimise existing proteomic platforms in order to enable laboratories to compare data and results; develope innovative and advanced proteomic technologies capable of identifying rare cancer-related proteins circulating in body fluids such as blood or urine; and develope a much needed clinical reagents and resources core of well-characterised biological samples, reagents, reference sets, and standards available to the scientific community. Information on this program and how the NCI is addressing these issues of reproducible and reliable metrology will be presented. In addition, other background material including educational tutorials are located at the program's Web site (http://proteomics.cancer.gov).
ChemBioNet is a national resource network to support chemical biology in academia (www.chembionet.de). The ChemBioNet was initiated by chemists and biologists from academia who realised the need for an interdisciplinary platform to enhance research projects exploiting the systematic usage of small molecules to study biological systems. ChemBioNet established and maintains an appropriate infrastructure necessary to perform HTS/HCS screening projects. Three partner institutes (GBF, MDC and FMP) co-financed a shared central compound collection, located at the FMP. This repository is continuously complemented by compound collections donated from chemists of the network. Screening of compounds will be carried out on individual conditions defined by the donors and on the basis of academic collaboration agreements. ChemBioNet provides an online database for all generated screening results with regulated access ensuring both IP rights and maximum free academic use. The mission of ChemBioNet is to ensure that every chemist should be able to know about the biological activity profile of their compounds and every biologist should be able to get a small molecule tool to manipulate biological systems in a dosage, time and spatially dependent manner.

Classical antibodies and recombinant binders play an ever-increasing role as research tools as well as in biomedicine. Given their vast diversity and multiple applications, ranging from classical research reagents to protein therapeutics, and the huge investments undertaken in the private sector, how can coordinated activities undertaken by consortia like Proteome Binders make a difference? The talk will attempt to discuss pros and cons of current strategies inside and outside ProteomeBinders.
19:30  Set out to dinner location
Please wear suitable shoes!
The walk to Rossloos is about 35 min on a gently ascending road.

20:00  Dinner at Roosmoos

Map for Thursday
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**Thursday, 15 March 07**

**Session 1: Affinity and methods Chair: Dorothée Chillu**

08:30 Thomas Tirkkonen: On-chip affinity probing by fluorescence and MALDI MS

08:55 Christof Niemeyer: Semi-synthetic DNA-Protein conjugates for diagnostic applications

09:20 Ulf Landegren: Assay formats for enhanced protein analyses

10:15 Martin Yuille: SomaLogic’s progress with aptamer-based proteomic arrays

10:40 Larry Gold: SomaLogic’s progress with aptamer-based proteomic arrays

11:15 Eric Chevet: Alphascreen: technology and applications

12:00 André Bernard: Affinity contact printing and CD readers for protein patterning and affinity sensing

**Session 2: Bioinformatics infrastructure Chair: Toby Gibson**

10:05 Discussion

10:20 Anusha Arora: Tracking protein interactions in living cells with fluorescent nanobodies

10:45 Andrew Reading: Recombinant post-translational modifications: the sulfotyrosine example

11:05 Hans Straub: Human proteome database by cross-referencing with bioinformatic tools from the UK

11:30 Henry Luan: Building a human protein atlas using EpitopeDB: any new opportunities provided by EpitopeDB

12:00 Depart to prepare for skiing or other

12:30 Dinner at Rossmoos

13:00 Conference at Congress Centrum

15:30 Coffee available at Congress Centrum

15:45 Hans Straub: Data mining strategies to build a drug database

16:10 Kousuke Matsumoto: Recombinant protein technology and plant-based expression systems for the production of recombinant proteins

16:35 Gert-Jan van Ommen: The ESFRI process and research infrastructures: a journey from the UK to the future

17:15 Tomoaki Tanaka: The human plasma proteinome: a targeted approach

17:45 Andrew Reading: Recombinant post-translational modifications: the sulfotyrosine example

18:15 Marius Ueffing: Anticalins and other alternative scaffolds

18:45 Carl Borrebaeck: Detecting cancer with recombinant antibody microarrays

19:00 …-final discussion

19:30 Set out to dinner location

19:30 Dinner at Reblaus