The front cover shows immunofluorescent staining of U-251 MG cells with a Human Proteome Resource generated antibody (HPA006185, www.proteinatlas.org) against the protein Syndecan 1, localised in focal adhesions (green). Cell structures are further outlined by staining of the ER (yellow), tubulin cytoskeleton (red) and the nucleus (blue).

Syndecan 1 is also noteworthy because it used to make Cheryl’s life difficult – as a consequence of which she has turned to organising enjoyable meetings such as this one.

Image courtesy of the Protein Atlas (www.proteinatlas.org)
5th ESF / EU AFFINOMICS Workshop on

Affinity Proteomics

14 – 16 March 2011

Congress Centrum Alpbach, Austria

Organised by:

Cheryl Smythe
Mike Taussig

Supported by:

European Science Foundation - Frontiers of Functional Genomics network programme
European Commission - AFFINOMICS EC FP7 Collaborative Project
Welcome to the 2011 workshop on Affinity Proteomics, organised by the EU AFFINOMICS consortium and the ESF Programme of Functional Genomics.

This workshop is the latest in a series which has contributed to shaping the European landscape of Affinity Proteomics. The first two workshops (2004, 2005) led to the ProteomeBinders project (an FP6 EU Research Infrastructure Coordination Action, 2006 – 2010), recently ended. The aim of ProteomeBinders was to plan a route for the systematic generation of affinity binders against the human proteome, which included two further workshops in Alpbach (2007, 2009), and also resulted in the currently running AffinityProteome and AFFINOMICS EU FP7 Collaborative Projects.

Functional investigation of the human proteome continues to be a major ‘post-genome’ challenge with profound significance in both basic and medical research, as well as for the biotechnology and pharmaceutical industries. The affinity proteomics approach to proteome analysis calls for systematic generation of binders, in principle against all genome-encoded proteins and their variant forms; the development and impact of proteomic research in the coming years will be critically dependent on the availability of such reagents. The aim of AffinityProteome (www.affinityproteome.eu) and AFFINOMICS (www.affinomics.org) is to initiate the generation of a proteome-wide binder collection and to this end the project integrates the expertise and technologies available in leading European centres in order to create an efficient pipeline for target and binder production, validation and quality control. AffinityProteome involves both SMEs and academic groups and targets the MAPK and TGF- β signalling pathways, while AFFINOMICS is an academic partnership focused on generating comprehensive binder sets for protein kinases, protein tyrosine phosphatases, SH2-domain containing proteins, proteins with somatic or germline mutations in cancer and potential selected cancer biomarkers. As well as including the classically raised polyclonal and monoclonal antibodies, the projects are making strong efforts towards technological improvements in molecular selection systems for recombinant binder formats, particularly automation and other processes to increase throughput and reduce cost.

The present Affinity Proteomics workshop is a key element in engaging the wider scientific audience in the developments within Europe, with speakers and participants from both within and outside the projects. As in the previous years, we are looking forward to the opportunity of discussing proteomics in the alpine winter setting, and the combination of the two!

Acknowledgements

My particular appreciation and thanks go to Dr Cheryl Smythe (ESF Functional Genomics Programme administrator) for her incredibly painstaking and tireless work on every detail of the arrangements. Dr Oda Stoovesandt prepared this booklet. We thank the staff of the Alpbach Congress Centre (Eva Moser, Georg Hechenblikner) for their 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. The meeting is supported financially by the European Science Foundation through the Frontiers of Functional Genomics network programme with additional funding by the European Commission through the AFFINOMICS contract (241481). We express our appreciation to both these organisations.

Mike Taussig,
Babraham Bioscience Technologies, Cambridge, UK
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Poster Abstracts

Elizabeth A. Cook: Advances in the DNA Array to Protein Array (DAPA) Technology

Michael R. Dyson: Mapping Protein Interactions by Combining Antibody Affinity Maturation and Mass Spectrometry

Jijuan Gu & Maria Hammond: Tag-based conjugation of affinity reagents for visualization of HER2 protein via proximity ligation assay

Opher Gileadi: Expressing the human proteome: methods and output of the Structural Genomics Consortium

Natalia Golenetskaya: Assessing "last mile" tools for affinity binder databases

Mats Gullberg: Studies of dimerization and activation of receptors in native cells and tissue samples

Jonas Helma: Targeting and tracing HIV-1 virion formation with fluorescent nanobodies in living cells

Anette Jacob: Protease profiling with reporter peptides for tumor diagnosis

Brian Kay: Immunoassays to Protein Biomarkers of Laser-induced Retinal Damage

Brian Kay: Selection and Characterization of Affinity Reagents for GABA Receptors in the Retina

Brian Kay: Generation of Recombinant Antibodies to Protein Variants that Cause Neurodegenerative Disease

Brian Kay: An FN3 Monobody for Monitoring Lyn Tyrosine Kinase Activation

Brian Kay: Engineering the Forkhead-associated Domain for Phage-display and Improved Thermal Stability

Martin Lundberg: Multiplexed homogenous proximity ligation assays for high throughput protein biomarker research in serological material

Lars Molzahn: Proteomics of SH3 interaction networks during germination of the filamentous fungus Ashbya gossypii

Aurelio Nardoza: SHP-2 dimerization may offer an additional mechanism for down-regulation of SHP-2

Peter Nilsson: Systematic antigen-array based proteomic profiling

Cecilia Roque: Affinity magnetic supports for protein enrichment

Oliver Scholz: Analysis of Human Telomere Structure in vivo

Eduard Stefan: Novel insights into protein kinase A signaling circuits: Phosphoproteomic analysis of a dynamic PKA interaction network

Klaus-Peter Stengele: Profiling Antibody Recognition Patterns through High-Density Peptide Arrays

Dirk Tremmel: Design and Selection of Armadillo Repeat Proteins: A novel technology for modular peptide recognition

Johan Vänelid: Multiplexed Proximity Ligation Assays

Sophie Venet: Novel approach for the generation of antibody libraries by CDR-H3 capture
Practical Information

We are delighted to welcome you to this Affinity Proteomics workshop!
Here is some practical information to help you get started.

Registration
After checking in to your room, please come to the Conference Centre (see map page 8) to register between 14:00 and 17:00 on Monday 14/3/2011, or between 08:00 and 09:00 on Tuesday 15/3/2011.

Munich Airport Shuttle
If you are using the shuttle organised by us to get to Alpbach and/or back to Munich and are not an external invited speaker, please pay 45 € per trip in cash when registering. Please also confirm your return shuttle pickup time.

Disclaimer
In view of the wintery conditions and possible outdoor activities, please note that the organisers of the Affinity Proteomics workshop, as well as the European Commission and the European Science Foundation, shall bear no responsibility for injury to persons or damage to property during your stay in Alpbach, March 2011. We will also ask you to sign a statement to this effect on registration.

Ski rental
We have negotiated a discount of 30% on the normal price with “Sport Conny’s” (see map opposite page, weekday opening hours: 8:30 -12:00 and 15:00 - 18:00). To claim the discount, please bring the enclosed letter when hiring your equipment.

Impromptu Sessions
In case of inclement weather and/or for those not planning to enjoy Alpbach’s outdoors, informal Impromptu Sessions with presentations may be arranged in the Conference Centre at 13:30 – 15:30 on Tuesday and Wednesday. Meeting participants not presenting a talk in the main programme are welcome to contribute a presentation of up to 15 min duration. Please sign up to give a talk or register your interest to attend at the registration desk.

Taxi
Transportation in Alpbach can be arranged at your own cost with Taxi Moser. Tel. +43-(0)-5336-5616 or +43-(0)664 543 0888 (mobile).

Contacts
Conference Centre (Eva Moser): +43-(0)5336-600-100
During the conference, Mike Taussig can be reached at +44-7951 452761
Monday 14.3.2011 – Arrival and Opening of Meeting

14:00 – 17:00  Registration open at the Conference Centre Alpbach

If possible, please come along during today to avoid queues on Tuesday morning.
After registering, you might want to take out skiing equipment (See page 5).

17:00  Mike Taussig: Welcome and Introduction

17:15  Mathias Uhlén: A Human Protein Atlas for profiling cells, tissues and organs

Science for Life Laboratory and AlbaNova University Center, Royal Institute of Technology (KTH), Stockholm, Sweden

The new version 7.0 of the Human Protein Atlas (www.proteinatlas.org) contains more than 13,000 validated antibodies targeting 10,078 genes corresponding to more than 50% of the protein-encoded genes in humans. The Protein Atlas contains more than 10 million high-resolution images generated by immunohistochemistry and confocal microscopy. The long-term objective is to generate paired antibodies towards all human protein targets and use these in various diagnostic applications, such as personalized medicine based stratification of patients. We have used the human protein atlas to study the global protein expression patterns in human cells, tissues and organs as well as a discovery tool to find potential biomarkers for human diseases.

References:
A systematic effort to organize and map the entire human proteome will enhance understanding of human biology at the protein level and accelerate diagnostic, prognostic, therapeutic, and preventive medical applications. In early 2010, we proposed a gene-centric approach to generate a human proteome map with an “information backbone” about the proteins expressed from each gene locus. A working group for a “Human Proteome Project” (HPP) created in October 2009 has worked toward an international consensus and a coherent proposal for this project. We have concluded that recent substantial advances in proteomics methods specifically related to quantitative mass spectrometry, protein capture with antibodies, and global exchange of large primary datasets and databases make the generation of such a human proteome map feasible, even without further paradigm shifts in technology. As was done for the HGP, gene-centric human proteome mapping will be complemented with in-depth studies of protein variability in response to various physiological conditions and in healthy and disease states. The Chromosome-Based Consortium (C-HPP) will help map and annotate subsets of the human proteome. The future HPP consortium will encourage this “adopt-a-chromosome” strategy by implementing the same guidelines, operational approaches, data submission, and sharing of information in the portal.
18:15  General Discussion

19:00  Drinks at Hotel Alpbacherhof
      Please see map for location.

19:30  Opening Dinner at Hotel Alpbacherhof

Map for Monday 14. 3. 2011
Tuesday 15. 3. 2011 – First full day of Workshop

8:00 – 9:00 Registration open at the Conference Centre

Morning Session

8:45  Gianni Cesareni: In vitro and in vivo analysis of the phosphatase functional network

Sacco F.1, Gherardini PF.1, Tinti M.1, Palma A.1, Corallino S.1, Ragnini-Wilson, A.1, Paoluzi S.1, Neumann B.2, Heriche JK.2, Pepperkok R.2, Ellenberg J.2, Helmer-Citterich M.1, Castagnoli L.1, Cesareni G.1

1 Department of Biology, University of Rome “Tor Vergata”, Italy
2 Cell Biology and Biophysics Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
3 High-throughput Microscopy facility; Department of Translational and Cellular Pharmacology, Consorzio Mario Negri Sud, SM. Imbaro, Italy

Protein phosphorylation is a highly dynamic process tightly regulated by the opposing activities of kinases and phosphatases. These two enzyme classes, in concert with their substrates form an intricate web whose dysregulation has been implicated in several human disorders. While recent efforts have contributed to reveal many links connecting kinases to their specific substrates, the mechanisms underlying substrate selection by phosphatases have remained much fuzzier to the point that the functional role of many phosphatases is still uncertain. To advance our understanding of the network based on this enzyme family, we have developed a combined experimental and computational approach to correctly place the tyrosine phosphatases in the context of a functional human interactome. We have applied this strategy to the identification of new phosphatase substrates. To this end we have used a Bayesian approach to integrate information about substrate preference, as determined by probing high density phospho-peptide chips, and distance in the human protein interaction network to rank phospho-proteins according to likelihood of being a substrate of any member of the PTP family. In addition we have monitored the phenotypic consequences of down regulating each of the 298 human phosphatases by siRNA. The cell response to a relatively large number of perturbations was assayed by monitoring the activation of key molecular switches. Cell perturbations included inflammatory stimuli, DNA damage or incubation with growth factors while cell responses were measured by probing the activation levels of kinases such as mTOR and MAPK family members or transcription factors by automated fluorescence microscopy. The obtained dataset was used to develop a strategy for functional classification of a gene family.
Protein signaling complexes, which are organized by scaffold and adaptor proteins, in specific subcellular locations, coordinate cellular functions such as proliferation, differentiation, apoptosis and migration. We have shown previously that the Mitogen-activated protein kinase (MAPK) scaffold protein 1 (MP1) is localized to late endosomes by the adaptor protein p14 [1-5]. The other major scaffold is KSR1, regulating the formation of a MAPK signaling unit at the plasma membrane. We used tandem affinity purification (SH-TAP) coupled to mass spectrometry to identify the major scaffold signaling complexes along the EGFR/MAPK pathway, in order to obtain an interaction atlas of protein partners associating with these complexes.

All known and several novel p14/MP1 interacting proteins, including proteins of unknown function, have been identified, suggesting new functions of these proteins. We have also performed time resolved analysis of signaling/scaffold complexes upon Epidermal Growth Factor (EGF) stimulation. About ten proteins consisted p14/MP1 core interactome, several proteins were interacting with scaffold proteins in signal dependent manner. Relatively small interactome of p14/MP1 proteins suggested more specific function of the scaffold complex on late endosomes, which is opposite to rather large signaling/scaffold complexes organized by KSR1.

References:

This work was supported by the Austrian Genome Program, GEN-AU with a grant for the Austrian Proteomics Platform (APP).
Personalized health care is a topic that is attracting increasing attention by stakeholders across the health care community, from the pharmaceutical and diagnostic industry, to patient advocacy groups, physicians, regulatory authorities, and 3rd party payers. Conceptually, personalized medicine aims at drawing on a deepened understanding of inter-individual differences in drug response as well as on new insights into mechanistic aspects of disease pathobiology to design more targeted approaches to treatment. This is expected to result in more efficacious, cost-effective, and safer medical interventions. Key to achieving this targeted approach is the use of biomarkers as indicators by which patients may be stratified to such treatments. Owing, in part, to the progress made in sequencing the human genome, genetic variants have attracted much attention as potential biomarkers, and substantive research efforts have recently focused on genome-wide association studies in the search of disease-relevant markers. While many such markers have been found, the very modest magnitude of effect associated with them renders them, by and large, not clinically applicable as tools for personalized medicine. In contrast, protein biomarkers, based on their much greater qualitative differentiation and quantitative dynamic range, hold promise to be sufficiently information-rich to guide therapeutic decisions. While the initial discovery of such markers may sometimes be possible using hypothesis-free proteomic approaches, their application to actual clinical research and, ultimately, medical decision-making will require highly selective and highly sensitive affinity reagents and analysis platforms. Requirements for such affinity reagents will likely include differentiated recognition of complex conformational epitopes or even quaternary structures, as well as of post-translational modifications, and thus represent important challenges for the affinity reagent community.
To show a meaningful intracellular interaction of DARPin selected by the Plückthun group to bind JNK1 and/or JNK2, or unphosphorylated, inactive and/or phosphorylated, active ERK2, we employed Bioluminescence Resonance Energy Transfer (BRET). We cloned fusions of the proteins of interest with the RET donor *Renilla* luciferase and the acceptor GFP2 for expression in living cells. BRET is initiated by oxidation of the luciferase substrate, which results in blue light bioluminescence. If GFP2 is located within ~5-15 nm of Rluc, due to an intracellular interaction of the fusion proteins, RET occurs, resulting in green light emission. Using this ratiometric and quantitative assay, we created and optimised reporters of all DARPin/kinase pairs.

DARPins selected in vitro against JNK1 and JNK2 display significant specificity in living cells, even though the kinases are closely related. Binding to ERK2 was close to background levels. Problems with unwanted cleavage of some BRET constructs were solved by mutation of the linker region between the DARPin and GFP2.

The ERK2 targeting DARPin that were selected to bind either active, inactive or both phosphoforms of ERK2, were also found to be specific in living cells as they did not interact with either JNK. We also detected significant increase in BRET signal of the p-ERK2 specific DARPin upon activation of the kinase.

The BRET assay thus is a valuable method to correlate the intracellular interaction of DARPin with their downstream effects and with the activation state of kinases.

**Funding:** EU Affinity Proteome

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**Said Taouji : HAPIscreen, a method for high-throughput aptamer identification**

*Eric Dausse*\(^a,c\), *Said Taouji*\(^b,c\), *Laetitia Evade*\(^a,c\), *Carmelo Di Primo*\(^a,c\), *Eric Chevet*\(^b,c\)\(^2\) and *Jean-Jacques Toulme*\(^a,c\)\(^2\)

\(^a\)Inserm U869, Institut Européen de Chimie et Biologie, \(^b\)Avenir, Inserm U889, \(^c\)Université de Bordeaux, Bordeaux, France

\(^1\)Co-first authors, \(^2\)Co-corresponding authors

We describe a novel homogeneous solution-based method for screening large pools of oligonucleotide candidates generated from SELEX. This approach is carried out on the exclusive basis of oligonucleotide binding properties, thus allowing for the functional identification of aptamers. We identify high affinity binders without the needs of performing a priori sequencing of the candidates. HAPIscreen (High throughput APTamer Identification screen) is faster than the current procedures that are based on sequence comparison of selected oligonucleotides and sampling binding properties of few individuals. Moreover HAPIscreen allows for screening larger numbers of candidates. Used here for selecting anti-premiR aptamers, HAPIscreen can be adapted to any type of tagged target and is fully amenable to automation.
In recent years, the increased interest in planetary exploration and the emergence of astrobiology as a promising field of research have led to a number of programmes aiming to develop sensitive instruments for the detection of the molecular signatures of life in extreme environments. An antibody-assay based life detection instrument, the Life Marker Chip (LMC), is currently under development by a UK-lead international consortium for the European Space Agency’s (ESA) ExoMars rover – currently scheduled for launch in 2018. The organic molecules targeted for life detection by the LMC are based on an assumption of “Earth-like” life on Mars. The molecular targets for the LMC have been chosen to represent markers of extinct life (i.e. molecular targets that survive over geological timescale), extant life, abiotic chemistry (e.g. of meteoritic origin) and mission-borne Earth contamination.

The nature of a key subset of molecules currently under consideration as potential LMC targets (small hydrophobic aliphatic and aromatic hapten)s makes the generation of antibodies against them a challenging process. The production of recombinant antibodies with the use of a variety of antibody libraries (naive-native, immunised-native and synthetic) screened via phage display technology is currently being pursued as the most appropriate approach to develop the desired antibodies in terms of specificity, affinities and ability to operate in mixed solvent systems. The current progress and challenges of this approach will be discussed.
10:45   Discussion

11:00   Poster viewing and coffee break

11:30   Depart to hotel to prepare for skiing or other

**Skiing or Impromptu Session**

**Ski bus information:**
To the lift (Talstation Gondelbahn Wiedersbergerhorn):
If weather conditions are promising, our own ski bus (marked “Affinity Proteomics”) will leave from the bus stop (see map, page 18) between the Congress Centrum and Hotel Boeglerhof at **11:50**. 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 from the lift (Talstation Gondelbahn Wiedersbergerhorn) to Alpbach:
Our own ski bus (marked “Affinity Proteomics”) will leave from the Talstation Gondelbahn Wiedersbergerhorn at **15:15**. This will bring you back in time for the afternoon meeting session.
In addition, there are public ski buses – please consult the timetable.

**Impromptu Session:**
In case of inclement weather and/or for those not planning to enjoy Alpbach’s outdoors, an informal Impromptu Session with presentations may be arranged in the Conference Centre at 13:30 – 15:30.
Meeting participants not presenting a talk in the main programme are welcome to contribute a presentation of up to 15 min duration. Please sign up to give a talk or register your interest to attend at the registration desk.

16:00   Poster viewing and coffee break
Après Ski Session

16:30  Olli Kallioniemi: High-throughput cancer biology and translation towards personalized medicine

*Institute for Molecular Medicine Finland (FIMM), Nordic EMBL Molecular Medicine Partnership, Biomedicum 2U, University of Helsinki, Finland*

We are exploring cancer using genomic profiling & bioinformatics, high-throughput RNA interference and chemical biology. These are coupled with phosphoproteomics and biomarker discovery which make use of affinity reagents to probe the responses of cancer cells to drugs as well as to phenotype archival tumor specimens. The aim is not only to understand the molecular pathogenesis of cancer, but increasingly to catalogue the patterns and pathways that are active in the anti-cancer drug response. This makes it possible to develop synergistic combinatorial drug regimens that block the “escape routes” of cancer. This approach is also providing an exciting ability to generate rapidly actionable personalized medicine opportunities to impact on the clinical treatment of cancer patients, particularly in leukemias.
Andreas Plückthun: Designed Ankyrin Repeat Proteins for extracellular and intracellular targets

University of Zürich, Dept. Biochemistry, Zürich, Switzerland

Designed Ankyrin Repeat Proteins (DARPins) (1) are very stable and versatile recognition molecules. They can be selected to practically any specificity from synthetic libraries using either ribosome display (2,3) or phage display (4). DARPins can be used in applications as diverse as tumor targeting (5,6) or reprogramming viral specificity (7,8). Because of their lack of disulfide bonds they can also be expressed inside the cell. Progress will be reported in selecting kinase and phosphorylation specific DARPins and evolving them to very high affinity. Their use as sensors and intracellular inhibitors will be highlighted (L. Kummer, P. Parizek et al, unpublished). Their selectivity will be discussed in terms of the crystal structures of DARPin-kinase complexes.

References:
Classic aptamers have been around for twenty years, while natural aptamers have been around for more than a billion years. Aptamers – short single-stranded oligonucleotides – fold intra-molecularly into globular shapes that can fit the surface of target molecules and provide high-affinity, highly specific binding to both proteins and small molecules. Although thousands of aptamer papers and patents have been written, a fairly large fraction of those publications report properties of the same twenty or so aptamers.

At SomaLogic we took a different approach – we first defined a novel class of “aptamers” called SOMAmers (SOMA = Slow Off-rate Modified Aptamers), and then made 1,100 of them aimed at 1,100 different human proteins. While we plan to make many more, we have used the first batch of SOMAmers to do many of the things one might do with high quality reagents, including unbiased protein profiling of blood and tissues.

The reagents themselves will be described. Unbiased protein profiling also will be described. Protein profiling is a useful way to see complex biology through a novel lens, stunningly different from the lens of mRNA profiling. Of deeper relevance to medicine, protein profiling in plasma or serum quickly defines biomarkers associated with disease.
18:00  Andrew Bradbury: Combining Yeast and Phage display
       Biosciences Division, Los Alamos National Laboratory, USA

Yeast and phage display are in vitro display systems that offer complementary advantages and disadvantages. This talk will discuss the advantages and disadvantages of each and how they may be usefully combined.

18:30  Discussion

18:45  Departure from Conference Centre for dinner at Roßmoos

Please wear shoes suitable for walking! The walk to Roßmoos is about 30 min on a moderately ascending road (please see the map).

19:30  Dinner at Roßmoos

Map for Tuesday 15. 3. 2011
Bernhard Küster: Chemical proteomics reveals novel targets for existing drugs and identifies new drug targets in cancer

Technische Universität München, Munich, Germany

We have developed an affinity profiling tool called kinobeads that enables the capture of hundreds of in-vivo expressed kinases and other nucleotide binding proteins. Quantitative mass spectrometry (using stable isotope labelling or in label-free format) is used in conjunction to measure the amount of proteins captured from any cellular, body fluid or tissue proteome. Kinobeads can be used for a number of applications. First, kinase protein expression profiles can be generated from any cellular, body fluid or tissue proteome and these profiles compared across samples collected from different sources or over time. Kinase expression profiling using kinobeads has enabled us to identify a number of potential new drug targets and biomarkers in cancer. Second, kinobeads can be used to profile the selectivity of kinase inhibitors in an entirely label free fashion. By measuring the competition of unmodified test compounds and kinobeads for binding the kinases present in the sample, one can derive binding inhibition values that are indicative of binding strength and selectivity. This approach has allowed us to identify the receptor tyrosine kinase DDR1 as a novel target of the successful leukemia drug Imatinib. The value of systematic selectivity profiling of kinase inhibitors is the potential of the use of an existing drug in a different cancer indication which drastically shortens the time from discovery to clinical translation.
The proximity ligation assay (PLA) offers the opportunity to detect proteins with very high specificity and sensitivity, and to investigate complexes of interacting proteins. The assays are suitable for parallel analyses of large numbers of proteins, either in solution phase or by imaging the distribution of target molecules in tissues. In PLA, target molecules are being identified through binding by a set of affinity reagents, typically antibodies, modified by attaching short DNA strands to serve as PLA probes. When these are brought in proximity by binding the same target molecule/complex, the attached DNA strands can undergo ligation reactions to generate amplifiable DNA molecules. PCR is suitable when analyzing proteins in solution, while for visualization purposes a rolling circle replication reaction can give rise to a locally amplified bundle of DNA at the limit of light microscopic resolution. We have demonstrated that the technique is suitable to reveal the presence and distribution of individual proteins and protein complexes in patient tissues, forming and dissociating during cellular signal transmission. We also show that large numbers of proteins can be digitally recorded from numerous blood plasma samples with readout via next generation sequencing. Finally, we illustrate the detection of prostasomes, a multiprotein complex, as a promising new biomarker for prostate cancer, using a variant of PLA that requires coincident detection of five epitopes.
Invention and introduction of TIS robotic microscopes, resp. Multi-Epitope-Ligand-Cartographs, has enabled investigators to locate and decipher functional protein networks (toponomes) consisting of thousands of different, topologically defined and interlocked protein clusters in single cells or tissue sections (Proteomics Research Highlight “Mapping togetherness”. Nature 443, 12 Oct 2006). This is achieved by using large dye-conjugated tag libraries applied automatically by the TIS microscope to run random numbers of cycles (at least 100) of fluorescence protein tagging, imaging and bleaching in situ (on fixed cells or tissue sections on stage). This procedure leads to a power of combinatorial molecular discrimination (PCMD) per data point of at least 2100 (functional super-resolution) allowing the subcellular structure of protein networks to be visualized, and their function to be analysed. The technology proves to distinguish subcellular objects irrespective of their distance (< 1nm) and has shown to solve key problems in biology and therapy research: (i) it has uncovered a new cellular transdifferentiation mechanism of vascular cells giving rise to myogenic stem cells in situ, a finding that has led to efficient cell therapy models of muscle disorders; (ii) it has discovered a new target protein in amyotrophic lateral sclerosis by hierarchical protein network analysis, a finding that has been confirmed by a mouse KO model; (iii) it has uncovered a lead target protein in tumour cells that controls cell polarization/metastasis, and (iv) it has found a new target protein that controls chronic neuropathic pain, a finding that has been confirmed by an independent KO mouse model. Based on these proofs of principle, an initiative has been launched to systematically decipher the human protein network code across major cell types and human diseases, particularly in cancer. The presentation focusses on the subcellular resolution of protein networks in a single cell and tissue section.

References:
**10:15 Fridtjof Lund-Johansen: Highly multiplexed bead suspension arrays for high throughput testing of antibodies**

*Anders Holm, Weiwei Wu and Fridtjof Lund-Johansen*

*Department of Immunology, Oslo University Hospital, Norway*

Assays where large numbers of antibodies are used in parallel are useful both for screening during production as well as for efficient use of validated reagents. We have developed bead suspension arrays that can be used to immobilize 5000 antibodies to different particles. An important advantage compared to slide-based arrays, is that beads are processed with robotics in microwell plates. This has allowed us to develop new assays for multiplexed analysis of cellular proteins. Rather than measuring total cell lysates with a single array, the proteins are separated into 96 fractions according to size and subcellular location. Monomeric proteins and multi-protein complexes are resolved as discrete reactivity peaks. To determine the composition of protein complexes, antibodies are used to immuno-precipitate proteins from fractions corresponding to reactivity peaks. The proteins are eluted from the precipitate and analyzed with antibody arrays. We have successfully used this approach to map interactions of proteins in the cell cycle machinery and signalling networks in human T cells. With our system it is for example feasible for one person to cross-test 1000 antibodies in a few days. A thousand immuno-precipitates are analyzed with an array containing 1000 antibodies to obtain 1,000,000 individual measurements. This should provide an excellent tool for screening antibodies in any setting.

**10:25 Christoph Schröder: Profiling cancer proteomes on complex antibody microarrays**

*C. Schröder¹, A. Jacob¹, M.S.S. Alhamdani¹, A. Nieters², S. Rüffer², S. Kutschmann¹, D. Hartmann¹, A. Bauer¹, K. Fellenberg¹, J.D. Hoheisel¹*

¹*Functional Genome Analysis, ²Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany*

Antibody microarrays facilitate the simultaneous analysis of hundreds of proteins in a high-throughput manner on large sample cohorts. For such studies, only minute sample amounts (e.g. few micro-litres of plasma or serum) are needed to obtain comparable sensitivities as in ELISA, the gold standard for protein quantification in a clinical setting. Here, we present the large-scale production, quality assessment and reproducible application of antibody microarrays consisting of 1,800 features. The array represents 810 different antibodies in duplicates and appropriate controls. The antibodies are directed at 741 different cancer-related proteins, which were mainly selected based on a regulation in transcriptional studies. For highly robust measurements, a dual-colour experimental layout was implemented next to a profound quality assessment of the production and incubation steps. The performance of the established protocols demonstrated to be competitive with other proteomic techniques and comparable in reproducibility to transcriptional microarray analyses.

We apply these arrays in a variety of studies using serum, plasma, urine and tissue as well as cell culture samples for the analysis of different cancer entities. In one exemplified study, plasma samples from 100 patients diseased with the three most common B-cell lymphomas were profiled and compared with sex and age matched
controls. In another study on pancreatic cancer, we were able to distinguish healthy and diseased persons on their protein profile in urine samples. In the studies, we could demonstrate that the established antibody microarray platform can produce robust, reliable and biomedically relevant data.

10:35   Jochen M. Schwenk: Exploration of plasma protein profiles with next generation affinity arrays

   *Science for Life Laboratory, KTH - Royal Institute of Technology, Stockholm, Sweden*

There is a need for high-throughput methods for screening patient samples in the quest for potential biomarkers for diagnostics and patient care. With more than 15,000 well-characterised affinity reagents accessible through the Human Protein Atlas (www.proteinatlas.org), a systematic protein profiling of body fluids stored in biobanks is now ongoing using recently developed antibody suspension bead arrays. These arrays are performed in microtiter plates and employ magnetic beads with up to 384 colour codes. The procedure is multiplexed in the dimensions samples as well as antibodies and it was recently found that retrieving target epitopes by heat allowed promising differences in comparative studies to be identified. Protein profiles are derived from labelled, non-fractionated samples such as serum, plasma or other body fluids and with latest technological advances, up to such 150,000 immunoassays are now performed per day, consuming minimal amounts of sample with limits of detection into lower ng/ml ranges. These assay characteristics suggested that applying the method to a proteome-wide screening would allow exploring and identifying new targets for further validation studies, thus we have started to profile human serum and plasma in a systematic manner. In a hypothesis-free pilot study, more than 4,600 validated antibodies have been applied to determine profiles across 576 samples representing 24 diseases. Interesting findings are to be confirmed technically and biologically for further streamlined development of sandwich assays. Additional effort are now also focussing on generating protein profiles across ages and genders towards describing an atlas of plasma proteins.

10:45   Discussion

11:00   Poster viewing and coffee break

11:30   Depart to hotel to prepare for skiing or other

**Skiing or Impromptu Session**

Please refer to page 14.

16:00   Poster viewing and coffee break
Après Ski Session

16:30  Mike Taussig: Individualising the Proteome

Oda Stoevesandt1, Michael J. Taussig1,2
1Protein Technology Group, Babraham Bioscience Technologies, Cambridge, UK
2Cambridge Protein Arrays Ltd, Cambridge UK

The recent advances in next generation high-throughput DNA sequencing are bringing complete genome and transcriptome sequencing within reach for routine diagnosis. What is missing is a means of converting individual genomes and their expressed transcriptomes quickly into corresponding proteomes. Since cellular function is protein-driven, a technology for arraying entire cellular proteomes would represent a major breakthrough, allowing the interrogation of a large number of possible disease-related protein markers simultaneously. We aim to display sequence-specified proteomes from individuals, tissues and cells by linking genomic DNA sequences and expressed RNA with our protein array system (DAPA) (1,2). Cell free protein expression makes it possible to copy DNA into protein quickly in an array format (3), so that perturbations of biological networks can be followed through proteome-wide protein function determinations. This approach will have wide applicability, regardless of species or cell type. The combination of high throughput sequencing and cell free protein expression opens up the possibility of ‘individualised proteomics’.

References:
During their life cycle viruses hijack host networks for their own needs. Discovering the basic biochemical interactions between viral and host proteins is a crucial step towards understanding the viral life cycle. Due to the size of the host proteome high-throughput methods are required to screen for such viral-host interactions. Despite significant efforts, conventional tools such as Yeast Two Hybrid largely failed. The failure is more pronounced when it comes to membrane associated proteins. We have designed PING, a sensitive high-throughput microfluidic assay that allows the screen of viral-host protein interactions as well as screen for inhibitors of such interactions. In a proof of principle experiment we screened the two HDV proteins and HCV NS5A against a hundred human proteins. We identified 7, 4 and 6 novel partners for sHDAg, LHDAg and NS5A, respectively. The average sensitivity of a single experiment (known partners identified out of all known partners included) was 62%, similar to bacterial interaction data previously measured with PING. The cumulative sensitivity in 4 experiments was 100%. These results pave the way for a whole proteome screen, which will allow us the horizontal overview we need into the biochemical network of viruses inside their host.
A conceptually new affinity proteomics method has been designed, denoted global proteome survey. This technology opens up the possibility to probe any proteome in a species independent manner, while still using a limited set of antibodies. To this end, we have combined the premium features of antibody microarrays and MS-MS. The approach is based on context-independent-motif-specific (CIMS) antibodies, directed against short amino acid motifs, where each motif is present in up to a few hundred different proteins. First, the digested proteome is exposed to these antibodies, whereby motif-containing peptides are enriched, which then are detected and identified by mass spectrometry. Consequently, 100 CIMS antibodies have the potential to analyze >10,000 proteins. To demonstrate proof-of-concept, profiling crude extracts from human colon tissue, yeast cells lysate, and mouse liver tissue will be presented.
Crescendo is developing a single-domain antibody fragment technology using transgenic mice to combine the benefits of a human product with in vivo maturation. This technology will be coupled to a ribosome display platform which enables clone selection and analysis from immunised mice, as well as affinity maturation. The transgenic mice are being developed in a background with immunoglobulin heavy chain, κ light chain and λ light chain loci ablated, through large-scale deletions in their C regions. We have generated a colony of the ‘triple knock-out’ mice completely devoid of endogenous murine antibody expression, and believe this will be critical for efficient expression of human heavy chain antibodies in mice. These have been crossed with transgenic mice having a YAC comprising several human V genes, all the D and J genes and C\(\gamma_4\) with a C\(\gamma_1\) deletion. We have confirmed that this YAC drives VDJ rearrangement and expression of human heavy chain antibody at the RNA and protein level, and immunization studies are underway. Future versions of the platform are being developed which we believe will generate a more highly efficient system. We are using novel approaches in rapid construction of large YACs to generate a series of clones that will ultimately comprise the entire human IgH VDJ region, joined to the entire murine C region. These will be introduced into murine embryonic stem (ES) cells derived from Crescendo triple knockout mice, using the latest ES cell technology. In parallel, we have optimised our eukaryotic ribosome display technology to develop a highly robust selection/maturation system for V\(\text{H}\), that has been used to substantially increase affinity of model anti-lysozyme and anti-TNF V\(\text{H}\) fragments.
Immunoprecipitation of protein complexes to map protein interactions is limited by the availability of validated antibodies of sufficient affinity recognising available epitopes within complexes. Using populations of primary antibodies to 8 different SH2 domain-containing proteins, we created 8 new sub-libraries (10⁸ -10⁹ clones each) by combining selected V\textsubscript{H} genes with a repertoire of new V\textsubscript{L} genes. Improved binders were isolated by stringent selections from these “chain-shuffled” libraries.

A novel 96-well immunocapture screen was developed that accurately predicted the ability of an antibody to function in immunoprecipitation. This revealed a success rate of only 12%. Using antibodies of different affinities to the same epitope we show that affinity improvement was a key determinant for success and identified a clear affinity threshold value which must be breached for success in immunoprecipitation. By combining affinity capture using matured antibodies to SHC1 with mass spectrometry, we identified 7 known binding partners, and two known SHC1 phosphorylation sites in EGF stimulated human breast cancer epithelial cells. These results demonstrate that antibodies, capable of immunoprecipitation can be generated by chain shuffling providing a scalable approach to mapping protein–protein interaction networks.
18:30 Final Discussion

19:00 Buffet Dinner and Party at Conference Centre

Map for Wednesday 16. 3. 2011
Poster Abstracts

Elizabeth A. Cook: Advances in the DNA Array to Protein Array (DAPA) Technology

Elizabeth A. Cook, Michael J. Taussig, Oda Stoovesandt
Protein Technology Group, Babraham Bioscience Technologies, Cambridge, UK

We previously described a method for the rapid and economical ‘printing’ of replicate protein microarrays directly from a DNA array template using cell-free protein synthesis (termed ‘DNA array to protein array’, DAPA). In DAPA, a template DNA microarray slide of PCR-generated constructs encoding tagged proteins is assembled face-to-face with a second slide coated with a tag-capturing reagent. A membrane soaked with an in-vitro transcription and translation system is positioned between the two slide surfaces. Tagged proteins synthesised from the immobilised DNA diffuse through the membrane towards the capture slide surface, creating the protein array corresponding to the DNA array template.

We have advanced this technology by increasing the variety of DNA constructs spotted to 128 separate targets, 70 of which were expressed by DAPA to a moderate or high level. The identities of the proteins produced by DAPA are being validated using commercially-available specific antibodies, suggesting that protein arrays generated by DAPA have the potential to be used for the screening of antibodies and other binders in a high-throughput manner. Furthermore, we have increased the density of arrayed proteins to over 2000 clearly-discernable spots, with a sufficiently low intra-slide variation and spot reproducibility to allow common array applications, such as antibody cross-reactivity screening to be performed adequately.

As previously reported, DAPA template slides can be successfully re-used, and due to the clear advantages of storage and stability of DNA arrays compared to conventional protein arrays, DAPA-generated arrays have the potential to become extremely useful tools for high-throughput on-demand protein arraying.

Michael R. Dyson: Mapping Protein Interactions by Combining Antibody Affinity Maturation and Mass Spectrometry

Michael R. Dyson1, Yong Zheng2, Cunjie Zhang2, Karen Colwill2, Kritika Pershad3, Brian K. Kay2, Tony Pawson2,4 and John McCafferty1
1Department of Biochemistry, University of Cambridge, Cambridge, UK; 2Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; 3Department of Biological Sciences, University of Illinois at Chicago, IL, USA; 4Department of Molecular and Medical Genetics, University of Toronto, ON, Canada

Immunoprecipitation coupled to mass spectrometry provides a powerful means to study protein interaction networks. Widespread use of this approach however is hindered by limited availability of validated antibodies recognising native antigen complexes with sufficient affinity. Phage display has been used to generate primary antibodies in high throughput and we now demonstrate the potential for scalable generation of affinity improved antibodies for immunoprecipitation. Using populations of primary antibodies to 8 different SH2 domain-containing proteins (LYN, VAV1, NCK1, ZAP70, PTPN11, CRK, LCK and SHC1), we created 8 new sub-libraries (10^6-10^9 clones each) by combining selected V\text{H} genes with a repertoire of new V\text{L} genes. Improved binders were isolated from all libraries, encompassing a diverse set of novel light chain partners. The
availability of a panel of antibodies sharing the same heavy chain but with different affinities revealed that affinity improvement of the recombinant antibodies was a key determinant to the success of immunoprecipitation experiments. In addition there was a sharp affinity “cut-off” distinguishing between success and failure. Antibodies successful in immunoprecipitation of SHC1 possessed K_s below 60 nM and off-rates of less than 0.1 s^{-1}. By combining affinity capture using matured antibodies to SHC1 with mass spectrometry we identified 7 known binding partners, and two known SHC1 phosphorylation sites (pSer29 and pTyr313) in EGF stimulated human breast cancer epithelial cells. These results demonstrate that antibodies, capable of immunoprecipitation can be generated by phage display selection combined with simple affinity maturation, providing a scalable approach to mapping protein – protein interaction networks.

Jijuan Gu & Maria Hammond: Tag-based conjugation of affinity reagents for visualization of HER2 protein via proximity ligation assay

The Rudbeck Laboratory, Department of Genetics and Pathology, Uppsala University, Sweden

The proximity ligation assay (PLA) requires high-affinity affinity binders with attached DNA oligonucleotides. When at least two such reagents recognize the same target molecule or molecular complex, the attached oligonucleotides of the different binders are brought in close proximity and can be joined by enzymatic ligation. So far, PLA studies have mainly involved antibodies as affinity reagents. Increasingly recombinant antibody fragments or non-immunoglobulin scaffold proteins are being used for detection of biomarkers. We have developed a general approach to attach DNA strands to recombinant binders by expressing these as fusion proteins with so-called SNAP-tag domains. This protein domain has the ability to first bind a specific chemical group via an affinity interaction and then couple to it covalently. This chemical group can be conveniently introduced in oligonucleotides to be attached to the fusion protein. This means of attaching oligonucleotides to affinity reagents has the advantages that all reagents are modified in the same way and with precisely one oligonucleotide each, potentially improving performance and reproducibility. In our study, HER2-specific Designed Ankyrin Repeat Proteins (DARPins) were successfully applied to visualizing HER2 protein in cells and breast cancer tissue via in situ PLA. We aim to extend this work to other classes of small recombinant affinity proteins of potential value in PLA. We are also evaluating PLA-based strategies to validate binders for pairwise binding.
Opher Gileadi: Expressing the human proteome: methods and output of the Structural Genomics Consortium.

*Opher Gileadi¹, Susanne Gräslund², The SGC,
¹University of Oxford, UK; ²The Karolinska Institute, Stockholm, Sweden*

A large-scale affinomics project depends on the ability to express the target antigen proteins as stable, natively-folded domains. The SGC focuses on the production and structure determination of human proteins. In a 7-year period, the SGC has deposited crystal structures of 1000 human protein domains, and has additionally expressed and purified >1000 protein domains that have not yet been crystallized. The targets include a diversity of proteins, with an attempt to provide high coverage of protein families. The family approach provides an excellent basis for characterizing the selectivity of affinity reagents. We will present the methodology used to achieve high-level production of diverse human proteins in bacteria and recombinant baculovirus systems, the parameters required for success, and new challenges such as integral membrane proteins.

Natalia Golenetskaya: Assessing "last mile" tools for affinity binder databases

*Natalia Golenetskaya, David J. Sherman
Laboratoire Bordelais de Recherche en Informatique, Bordeaux, France*

The EU ProteomeBinders coordination action helped define community standards for reporting and comparing binder and binder-target properties, with an eye toward quality control. This included reporting standards, an ontology for unambiguously describing binders and their properties, and a database schema for storing the experimental molecular interaction evidence used to support claims about those properties. But it left unresolved the question of how best to enable binder producers to submit those data, and to extract meaningful intelligence from them. We report on two studies we undertook to address these issues.

The first problem we investigate is how to make the submission of experimental evidence in support of a binder easier for data producers. All data providers have data in different formats, depending on their experimental protocols and what kind of LIMS the use (if any). This is essentially a data integration problem, that ideally one could solve by leveraging existing tools. Indeed, the HUPO Proteomics Standards Initiative (PSI) defines community standards for data representation in proteomics to facilitate data comparison, exchange and verification. The MITAB25 format is part of the PSI-MI 2.5 standard and describes information about molecular interactions. To test whether this generic approach could be adapted to the specific needs of affinity proteomics, we converted a large sample of several hundred binders of different kinds (both antibodies and aptamers), and with different degrees of experimental support. This allowed us to import the data using PSICQUIC tool, developed by the MI workgroup, straightforwardly providing standard web services and search using MIQL language. We identified a number of challenges in forcing binder-target data to conform to MITAB25.

The second problem is extracting intelligence from these data. Using the approach developed by Julie Bourbeillon, we extracted RDF triples from the database and injected these relations into two difference data stores. Using this system we are able to uniformly access binders from different technologies and from different data providers, compare binders properties, and define compatible binder sets. We report on implementing these strategies using high-level queries in the SPARQL language and rule-based reasoning using SWRL.
Mats Gullberg: Studies of dimerization and activation of receptors in native cells and tissue samples

Olink Bioscience, Uppsala, Sweden

Olink Bioscience provides Duolink®, a DNA-amplifiable protein assay platform, for in situ single molecule detection of proteins, protein interactions (e.g. dimerization) and modifications (e.g. phosphorylation) based on the proprietary technology PLA™ in conjunction with rolling circle amplification (RCA) readout. Individual proteins detected by single affinity reagents are well accepted as diagnostic markers in cancer tissue samples, such as Her-2 detection for trastuzumab (Herceptin) treatment guidance. To complement these single-protein assays Duolink allows measuring protein-protein interactions within signal transduction pathways using a double affinity reagent set up. Measuring these protein-protein interactions show great promise as a new class of biomarkers and could be used to predict and monitor drug response as companion diagnostics, particularly in cancer, where signalling pathway disruption can lead to uncontrolled growth. The Duolink reagents offer novel and efficient means to study interaction-based and activated proteins as putative biomarkers at high throughput in archival tissue microarrays or to study compound effects on cells in microtiter plate based assays. The single-cell resolution aids the objective analysis of heterogeneous tissue samples. I will here present how Duolink can be used to study protein dimerizations as well as how it has been used for enhanced detection of activated proteins in both cells and archived tissue as a means of measuring relevant biomarkers.

Jonas Helma: Targeting and tracing HIV-1 virion formation with fluorescent nanobodies in living cells

J. Helma1,4, V. Lux2, K. Schmidthals 1,4, S. Igonet3, S. Nüske1, H. Oberwinkler2, F. Rey2, U. Rothbauer1,4, H.-G. Kräusslich2, H. Leonhardt1

1 Ludwig-Maximilians University, Munich, Germany; 2 University of Heidelberg, Germany; 3 Institut Pasteur, Paris, France; 4 ChromoTek GmbH, Munich, Germany

Capsid assembly is a key event in HIV-1 biogenesis and a potential target for antiviral drug developments. While the structure of the capsid is well known1, the dynamics, location and regulation of the assembly process in infected cells is in part still unknown or controversial2-3. We generated two high affinity nanobodies directed against the N-terminal domain (N-CA) and the C-terminal domain (C-CA) of the HIV-1 capsid protein (CA). We fused these nanobodies with fluorescent proteins and could show that these chromobodies4 recognize their viral target in live cells. While the N-CA specific chromobody was particularly suited for visualization of virion biogenesis, the C-CA specific chromobody functionally interfered with virion formation. To elucidate the underlying mechanism we crystallized the C-CA nanobody complex and found that the nanobody specifically blocks the C-CA/C-CA dimerization interface, which connects individual CA-hexamers and promotes capsid lattice formation. EM studies as well as cell-based assays revealed that the C-CA specific nanobody interferes with capsid assembly and infectivity. These results show that chromobodies are uniquely suited to visualize untagged viral proteins and probe defined steps of HIV-1 biogenesis in living cells, providing versatile tools for target validation and antiviral drug development.

Anette Jacob: Protease profiling with reporter peptides for tumor diagnosis

Anette Jacob1,4, Diego Yepes2, Marc Dauber1,4, J. Hoheisel1, Victor Costina2, Ralf Hofheinz3, Michael Neumaier2 and Peter Findeisen2

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Progression of many solid tumors is characterized by the release of tumor associated proteases like cancer procoagulant, MMP2 and MMP7. Consequently, detection of tumor specific proteolytic activity in serum specimens has recently been proposed as a new diagnostic tool in oncology. However, tumor associated proteases are highly diluted in serum specimens and it is quite a challenge to identify substrates that are specifically cleaved. Here, we describe the systematic optimization of a synthetic peptide substrate using a positional-scanning synthetic combinatorial library (PS-SCL) approach. The initial reporter peptide comprises the cleavage site WKPYDAAD which is part of the natural substrate of the tumor associated cysteine protease cancer procoagulant. Proteolytic fragments of reporter peptides were accumulated during prolonged incubation with serum specimens and quantified with MALDI-TOF mass spectrometry. Amino acid substitution of the first aspartatic acid (D) against asparagine (N) improved the processing of respective reporter peptides in serum specimens from patients with colorectal tumors when compared to healthy controls. Additionally, the optimized reporter peptide (WKPYNAAD) was combined with other reporter peptides VPLSLTMG and IPVSLRSG that are cleaved by the tumor associated proteases MMP2 and MMP7, respectively. Diagnostic accuracy of MS-based protease profiling was evaluated for this reporter peptide mix in a cohort of 50 serum specimens equally divided into colorectal cancer patients and healthy control individuals. Our results demonstrate that the combination of different reporter peptides with optimized sequences increase classification accuracy of functional protease profiling and thereby might lead to improved diagnosis, monitoring and prognosis of malignant disease.
Brian Kay: Immunoassays to Protein Biomarkers of Laser-induced Retinal Damage

Michael Kierny, Thomas Cunningham and Brian Kay
Department of Biological Sciences, University of Illinois at Chicago, USA

The misuse of directed energy, whether intentional or accidental, constitutes a threat to aircraft pilots. Considering the reduction in cost of high power lasers and their wide spread use, it is important to develop a simple, effective assay for detecting retinal damage to pilots as a way of assessing their ability to operate an aircraft. Currently there are no rapid methods to detect subclinical laser ablation to the retina other than visual checks by an ophthalmologist. To develop sensitive immunoassays to retinal injury biomarkers present in serum, saliva, or tear duct fluids, we are currently exploring a two-step process. In the first step, we have generated monoclonal antibodies to short peptides of four retinal proteins that have been identified by LC/MS after laser-induced retinal damage in rabbits in the laboratory of our collaborator. A phage-display library, displaying single-chain Fragments of variable regions (scFv), was used for affinity selection against synthetic peptides corresponding to the identified biomarkers. Fluorescence polarization was then used to determine the dissociation constants of the antibody clones. The best were used in western blots of rabbit retinal lysates to demonstrate recognition of the endogenous protein. These antibodies are then enhanced through mutagenic PCR or chain shuffling. We have previously generated scFvs to short peptide segments of membrane proteins, and found them to bind to the native antigen. The second step will involve the formatting of the immunoassay where engineered scFv or Fab antibodies are incorporated into a biosensor platform.

Brian Kay: Selection and Characterization of Affinity Reagents for GABA Receptors in the Retina

Sujatha P. Koduvayur¹, Adnan Memić¹, Michael Kierny¹, Hélène A. Gussin², Feng Feng², Ambarish S. Pawar², David R. Pepperberg², and Brian K. Kay¹
Departments of Biological Sciences² and Ophthalmology and Visual Sciences², University of Illinois at Chicago, USA

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the retina and other CNS neurons. GABAₐ and GABAₐ receptors, which are ligand-gated ion channels, play important roles in GABA-mediated synaptic transmission between inner retinal neurons. In age-related macular degeneration and certain other retinal diseases, the degeneration process primarily targets the rod and cone photoreceptors, but preserves the viability of inner retinal neurons. This raises the possibility that a molecular device, consisting of a light-sensitive receptor ligand anchored to the ectodomain of GABA receptors, might serve to restore visual function of the cells possessing these receptors. To generate the anchoring component of such device, we are developing affinity reagents based on single-chain Fragments of immunoglobulin variable regions (scFvs). We have screened two phage-display libraries by affinity selection, using synthetic peptides corresponding to extracellular regions of the human p1 GABA₂ and the rat α₁and β₂ GABA₂ subunits. To date, we have identified five scFv's that bind the p1 and β2 peptides each, and three that bind the α1 peptide. Preliminary analysis of some of the bacteria and yeast expressed anti-p1 and anti-α1 scFv's, by surface plasmon resonance and fluorescence polarization, indicates that their dissociation constants range from 50 nM to 12 mM. Electrophysiological recordings of Xenopus oocytes and neuroblastoma cells expressing GABA₂ receptors indicate that the anti-p1 scFv's do not alter receptor
function upon binding. Our long-term plan is to conjugate the antibody fragment with photo-regulated receptor ligands, and test for light-dependent physiological activity in retinal neurons.

Supported by NIH grant EY016094.

Brian Kay: Generation of Recombinant Antibodies to Protein Variants that Cause Neurodegenerative Disease

John D. Pavlovic1, Rachel Hilbert1, Ghanashyam Ghadge2, Ray Roos2, and Brian Kay1

1Department of Biological Sciences, University of Illinois at Chicago, 2Department of Neurology, University of Chicago, USA

A number of neurodegenerative diseases are associated with protein misfolding, including amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s disease or motor neuron disease. ALS is a devastating and progressive disease with no known treatment. Approximately 10% of ALS cases are familial, and one-fifth of familial ALS cases are caused by mutations in superoxide dismutase 1 (SOD1), an enzyme that prevents the build up of the free radical superoxide. Interestingly, mutations in SOD1 enzyme – perhaps because of its misfolding and aggregation – cause ALS because of a gain of function, not because of a loss of function. TAR DNA-Binding Protein 43 (TDP-43) also has a role in ALS. Mutations in TDP-43 cause the protein to be localized in the cytoplasm in motor neurons, instead of the nucleus (as in normal cells). In addition, all patients with sporadic ALS have aggregates of TDP-43 in the cytoplasm of motor neurons. To follow the subcellular distribution of mutant forms of SOD1 (A4V, G93A, V148G), as well as mutant TDP-43 A315T, we screened two single chain variable fragment (scFv) phage display libraries for affinity reagents. While most isolated antibodies bound equally well to wild-type and mutant proteins, a few scFvs bound to a subset of the proteins. These reagents may be useful in identifying mutant proteins in the tissues and spinal fluid of patients and also as a potential treatment of ALS in order to prevent aggregation and toxicity of TDP-43 and SOD1.

Brian Kay: An FN3 Monobody for Monitoring Lyn Tyrosine Kinase Activation

Renhua Huang, Zengping Hao, and Brian K Kay

Department of Biological Sciences, University of Illinois at Chicago, IL, USA

Lyn is a member of the Src family kinases, which, upon activation, phosphorylates target proteins and relays signals to downstream effectors that are involved in immunity and inflammation. To follow the in situ activation of Lyn kinase in eukaryotic cells, we are generating affinity reagents that selectively bind to the active, but not the inactive, form of Lyn. When activated, Lyn changes its conformation from a “closed” to an “open” state, opening up the Src Homology 3 (SH3) domain to intermolecular interactions. Thus, affinity reagents that specifically target the binding surface of the Lyn SH3 domain can be used to monitor the activation of Lyn kinase. To generate affinity reagents for such a purpose, we screened against the Lyn SH3 domain with a phage-displayed library of Fibronectin type 3 repeat (FN3) monobodies, which are randomized in their BC and FG loops with NNK codons. One isolate, TA8, selectively binds to Lyn and not 150 other human SH3 domains, and with a dissociation constant of 77 nM. The TA8 monobody is currently being evaluated for its ability to pull-down Lyn kinase in activated mast cells.

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Antibodies to monitor protein phosphorylation are conventionally generated by immunizing animals with synthetic phosphopeptides. However, with an estimated 100,000 phosphorylation sites in the human proteome, this approach would be very time consuming, expensive and laborious. An ideal alternative would be to produce affinity reagents using recombinant proteins displayed on the surface of bacteriophage M13. The Forkhead-associated domain (FHA1) is a naturally occurring phosphothreonine (pT) binding domain, which when expressed in the bacterial cytoplasm as a glutathione-S-transferase (GST) fusion, binds specific pT-containing peptides. Unfortunately, when we cloned and displayed a yeast FHA1 domain on the surface of phage, it as non-functional, presumably due to misfolding. To overcome this limitation, a FHA1 variant (FHA1 D2) with the capacity to bind was isolated from a library of variants generated by mutagenic PCR. The FHA1 D2 clone had a single residue substitution (S34F) that rescued binding to the cognate pT peptide. Further evaluation revealed that to facilitate proper folding of the FHA1 variant in the bacterial periplasm, where phage particles are assembled in E. coli, position 34 has to be occupied with a hydrophobic amino acid and not with a polar residue. Screening a second library of FHA1 variants employing high temperature as a selection pressure yielded a number of FHA1 variants (FHA1 G2 and FHA1 A1) that were more thermally stable than the wild-type form of the FHA1 domain. The FHA1 G2 variant bound to the pT peptide with a dissociation constant similar to that of the wild-type FHA1 domain (~1 µM) and the purified FHA1 variants produced yields of 55-64 mg/L (from shake flasks of E. coli). The next step will be to randomize specific residues (selected based on the results from alanine scanning) in the FHA1 G2 variant by oligonucleotide-directed mutagenesis and screen the resulting library for new anti-phosphopeptide binding properties. Lastly, these reagents will be evaluated in recognizing phosphorylated proteins in western blot, immunoprecipitation, and immunostaining experiments.

Martin Lundberg: Multiplexed homogenous proximity ligation assays for high throughput protein biomarker research in serological material

Olink Bioscience, Uppsala, Sweden

A high throughput protein biomarker discovery tool has been developed based on multiplexed proximity ligation assays (PLA) in a homogeneous format in the sense of no washing steps. The platform consists of four 24-plex panels profiling 74 putative biomarkers with sub pM sensitivity each consuming only 1 µL of human plasma sample. The system uses either matched monoclonal antibody pairs or the more readily available single batches of affinity purified polyclonal antibodies to generate the target specific reagents by covalently linking with unique nucleic acid sequences. These paired sequences are united by DNA ligation upon simultaneous target binding forming a PCR amplicon. Multiplex PLA thereby converts multiple target analytes into real-time PCR amplicons that are individually quantificatied using microfluidic high capacity qPCR in nano liter volumes. The assay shows excellent specificity, even in multiplex, by its dual recognition feature, its proximity requirement, and most importantly by using unique sequence specific reporter fragments on both antibody-based probes. To illustrate the potential of this protein detection technology, a pilot biomarker research project was performed using biobanked plasma samples for the detection of colorectal cancer (CRC) using a multivariate signature.
Lars Molzahn: Proteomics of SH3 interaction networks during germination of the filamentous fungus *Ashbya gossypii*

*Lars Molzahn, Riccarda Rischatsch, Alexander Schmidt, Peter Philippsen. Biozentrum University of Basel, Switzerland.*

Germination of *S. cerevisiae* spores leads to cells which proliferate by budding. The interaction networks controlling the localization and growth of novel buds including cell separation are well understood in *S. cerevisiae*. Germination of *A. gossypii* spores leads to germlings with continuously elongating and branching multinucleated hyphae even though both organisms carry an almost identical set of genes with highly conserved gene order (Dietrich et al. 2004). *A. gossypii* diverged from the *Saccharomyces* lineage prior to the whole genome duplication and its genome underwent only a limited number of rearrangements most likely because of the lack of mobile elements. The *S. cerevisiae* genome was shaped after its duplication by over 4000 gene deletions and over 200 translocations and inversions. The sequence identity of orthologous proteins varies from 99% to as low as 18%. In order to identify changes which contributed to the evolution of the different life styles of *S. cerevisiae* and *A. gossypii* we determined protein and mRNA expression profiles from germinating spores to fast growing hyphae. We aim to complement these studies with selected *in vivo* and *in vitro* pull-down experiments focusing on SH3 domain interactions of polarity and endocytosis factors. Our first results reveal a high background of false positives or high levels of degradation and we hope to learn new experimental approaches during the workshop to reduce this background.

Aurelio Nardozza: SHP-2 dimerization may offer an additional mechanism for down-regulation of SHP-2.

*Nardozza AP, Trapanneone R, D’Orazio M, Battistoni A, Cesareni G, Castagnoli. L Department of Biology, Department of Biology, University of Rome “Tor Vergata”, Italy*

The SHP-2 tyrosine phosphatase plays key regulatory roles in the modulation of the cell response to growth factors, hormones and cytokines. The combination of genetic, biochemical and structural data over the past decade has helped interpreting the pathological consequences of altering SHP-2 activity. However, the resulting model does not provide a complete mechanistic understanding of all the observed phenomena, implying that the current model is still patchy and that important links are missing. Here we show, by a variety of approaches, that SHP-2 can associate in a multimeric form *in vivo* and that the most abundant multimer is a dimer. Approximately 15% of SHP-2 is detected as a dimer in resting cells. In addition, two observations point to a regulatory functional role for the dimerization process. Measurement of the specific phosphatase activity in the monomeric or dimeric forms indicated that the latter is three folds less active thus pointing to the dimerization process as an additional mechanism for controlling SHP-2 activity. Consistently we observed that the proportion of SHP-2 found as a dimer is decreased by approximately 50% when cells are stimulated with growth factors after starvation. Finally we have shown that SHP-2 can dephosphorylate itself thus providing an additional mechanism of down-regulation that can be modulated by the dimerization process. Dimers formed by two SHP-2 mutants displaying distinct pathological and biochemical phenotypes respond differently to growth factor stimulation. While the E76K hyperactive leukemic mutant resolves its dimer with a kinetic comparable to wild type, the T468M catalytically inactive Leopard mutant responds by increasing the amount of dimer. Although this differential behavior cannot
be rationalized mechanistically yet, it provides a functional correlation between the different modulation of dimers in response to growth factors and the distinct biochemical/pathological phenotype.

**Peter Nilsson: Systematic antigen-array based proteomic profiling**

Dept. of Proteomics, School of Biotechnology KTH - Royal Institute of Technology, Stockholm, Sweden

A systematic and undirected biomarker discovery approach has been implemented, utilizing array-based platforms and the massive antigen and antibody production pipeline within the Human Protein Atlas. Proteomic profiling of multi-disease serum, plasma and CSF cohorts are performed with large numbers of antigens on planar microarrays and furthermore with large numbers of antibodies on highly multi-parallel suspension bead arrays which utilizes microtiter plates and magnetic color-coded beads functionalized with antibodies to generate protein profiles from labelled samples.

More than 25,000 monospecific antibodies have been generated and validated with in-house printed antigen microarrays. These protein microarrays also offer a unique resource for antigen-based profiling of body fluids to allow a systematic screening for discovery of novel autoantibodies and autoimmunity targets.

In an initial phase have 96 plasma samples from a multiple sclerosis cohort been screened for autoimmune reactivity on 11,500 antigens, which consumed 210 microarray slides but only 8 µl of plasma. A validation phase of candidate targets in a suspension bead array setup includes the 384 top ranked antigens that have given rise to distinct signals and which have been found to have a group separating power. In addition, an extended multiple sclerosis sample cohort will then be screened in this format enabling the simultaneous profiling of 384 samples on 384 proteins in a dual multiplexing fashion.

The aim is to introduce systematic antigen-based plasma profiling, where all antigens produced within the Human Protein Atlas project are utilized to screen multi-disease cohorts for the presence of autoimmunity components.

**Cecilia Roque: Affinity magnetic supports for protein enrichment**

Batalha, I.L., Hussain, A, Roque, A.C.A.

REQUIMTE - Departamento de Química, CQFB, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal

Proteomic analysis can be highly intensive and laborious with the need for low cost and high-throughput methods. Affinity-based techniques are known to be suitable for the highly selective enrichment and identification of proteins\(^1\). On the other hand, superparamagnetic nanoparticles (MNPs) represent a convenient solid support for a variety of assays and procedures based on affinity purification and with wide application in proteomics studies. MNPs can be made cheaply and easily, and coated with different polymeric materials as to increase colloidal stability, morphology and functionality. Their small size results in large surface areas per unit volume, making them ideally suited for adsorptive separations. In this work we explored the surface modification of MNPs with gum Arabic and related biopolymers by different routes\(^2\) and used these materials as a platform for the creation of affinity magnetic supports\(^3\). Polymer-coated MNPs were decorated with natural (antibodies) and synthetic (biomimetic) receptors for biological
molecules at high densities. These particles have shown to bind specifically to a model target protein, human IgG, with maximum capacities of up to 340 mg target/mg MNP and $K_v$ values of $10^8$ M$^{-1}$, while retaining the magnetization properties. When comparing with agarose, functionised particles bound six times more human IgG (in mg of protein per g of support) than the traditional chromatographic matrix agarose modified with the same ligand, which is a notable result when the manufacture costs and ease of preparation of both supports are taken into account.


Oliver Scholz: Analysis of Human Telomere Structure in vivo

Oliver Scholz and Andreas Plückthun
Biochemisches Institut der Universität Zürich, Switzerland

Human telomeres end in a 50 to 200 base long 3’-overhang with TTAGGG repeats, the G-tail. This sequence readily forms quadruplex structures in vitro, as numerous publications, most of them applying synthetic oligonucleotides, demonstrate. There is, however, only weak evidence for the formation of these structures in vivo, and their existence has remained controversial.

We want to address this question with the help of quadruplex binding proteins as probes. First, oligonucleotides with human telomeric sequence were dissolved in buffer containing 150 mM NaCl or KCl and their quadruplex conformation was confirmed with the help of CD spectroscopy. Then, quadruplex binding proteins were selected from a library of designed ankyrin repeat proteins (DARPins) with three rounds of ribosome display. DARPins were chosen for this task, because they are not known to bind DNA, minimizing unspecific background DNA binding activity and because they were successfully applied for selections against many different targets in the past. ELISA with immobilized quadruplex DNA was performed to confirm and compare the obtained binders. The best candidates exhibit binding activity to quadruplex DNA, but not telomeric duplex or unrelated DNA in ELISA, EMSA and SPR measurements. This is the first evidence for DNA binding ankyrins. Affinities reach up to 20 nM. The binders can be expressed as C-terminal GFP fusions in human cell lines, e.g. HEK293T.

The selected quadruplex DNA binding ankyrins will be used as a tool to test the structure of telomere ends inside the nucleus in co-localization experiments.
Eduard Stefan: Novel insights into protein kinase A signaling circuits: Phosphoproteomic analysis of a dynamic PKA interaction network

Eduard Stefan1, Frederico Apelt2, Ulrich Stelzl2 and Klaus Bister1
1 Institute of Biochemistry and CMBI, University of Innsbruck, Austria
2 MPI for Molecular Genetics, Berlin, Germany

The specificity of receptor initiated signaling responses is encoded by spatial and temporal dynamics of downstream signaling networks. These networks, initiating from e.g. the G protein coupled receptor (GPCR) superfamily and receptor tyrosine kinases, tightly regulate signaling pathways at critical points via feedback loops and cross talk 1,2. Signaling networks are complex and apparent cross talk between different pathways is rationalized based on complicated models. However, cross talk can often be explained by simple and occasionally surprising molecular interactions. Elucidation of the physical and dynamic connection between these pathways is therefore a key to understand aberrant signal transmission3,4.

We demonstrate that GPCR-triggered alterations of cAMP-levels affect protein:protein interactions (PPI) of the cAMP-dependent protein kinase A with distinct downstream effectors. In order to gain a more comprehensive and mechanistic understanding of cAMP-triggered changes of protein complexes we mapped PPI emanating from endogenously expressed PKA complexes using a phosphoproteomic approach. We have generated a more dimensional interaction network following activation of distinct components of the cellular cAMP-machinery for just ten minutes. In addition to drastic changes of the proteomic composition of PKA associated complexes, we have allocated novel and posttranslationally regulated connections between PKA and various GTPases, transcription factors and kinases which unveil novel intersections between the cAMP-cascade and crucial players regulating cell growth and differentiation.


Klaus-Peter Stengele: Profiling Antibody Recognition Patterns through High-Density Peptide Arrays

NimbleGen Systems GmbH (a Roche affiliate), Germany

A universal platform for efficiently mapping or profiling epitopes of a large number of antibodies will be of great use for many next generation OMICS projects in systems biology. We have successfully developed the Nimblegen Maskless Array Synthesis (MAS) technology to generate libraries of medium sized peptides (up to 18-mers) on microscope slides in a microarray format and with up to 2.1 million discrete peptides – essentially spanning the 20,300 consensus protein sequences of the “Human Proteome”. Linear combinatorial peptide synthesis was effected by light-directed and spatially resolved deprotection and coupling cycles, as it is well known for DNA array synthesis. We have applied those peptide arrays to biologically relevant applications such as:
- Epitope Mapping of polyclonal antibody sets with up to 1,000 members
Epitope Mapping and Alanine Scan of monoclonal therapeutic Ab candidates
- IgG profiling of anti-Pathogen Abs in human serum samples

We will discuss in detail results of these early feasibility experiments and scope and limitations of the technology.

Dirk Tremmel: Design and Selection of Armadillo Repeat Proteins: A novel technology for modular peptide recognition

Dirk Tremmel, Gautham Varadamsetty, Christian Reichen, Chaithanya Madhurantakam, Peer R. Mittl, Markus G. Grütter, and Andreas Plückthun
Biochemisches Institut, Universität Zürich, Switzerland

Selection from libraries represents a critical step in the generation of binding molecules. However, when many binders need to be generated (e.g. for proteomic applications), the time required for each selection and validation accumulates, making this into a daunting task. Each new binding molecule usually binds its target in a different way and, due to the different structure of the targets and the different binding modes, each selection has to be done independently and cannot take advantage of the information from the binders previously obtained. While a constant binding mode towards different protein surfaces is unlikely to be achieved, peptides and unstructured regions of proteins constitute better candidates as target for general recognition. Therefore, our aim is to generate a scaffold based on armadillo repeat proteins for peptide recognition, which can bind peptides in extended conformation and can, at least in principle, be extended to side-chain recognition of post-translational modifications of proteins.

Based on consensus design, two different well expressed armadillo repeat protein scaffolds, with high thermodynamic stability have already been developed in our lab [1]. In contrast to natural armadillo repeat proteins such designed proteins have the advantage that the size of the proteins can be easily adjusted, simply by adding or deleting one or more repeats, as all repeats are compatible.

Progress in determining the structures of the consensus armadillo repeats as well as analysis of their interaction with peptides will be presented.

Johan Vänelid: Multiplexed Proximity Ligation Assays

Johan Vänelid*, Spyros Darmanis*, Rachel Nong**, Olle Ericsson*, Sophia Hober, Jochen M. Schwenk* and Ulf Landegren*

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*Authors contributed equally to the work

We are currently developing a 36-plex assay based on the proximity ligation assay (PLA) technique. We aim to improve both the sensitivity and specificity and also the level of multiplexing over current multiplexed sandwich immunoassays. Our approach takes advantage of the potential to avoid cross-reactions among different sets of affinity reagents in PLA, and in further work we expect to reach considerably higher levels of multiplexing. A PLA probe consists of an antibody or other affinity reagent to which oligonucleotides have been coupled via linkers. Thirty-six PLA probe pairs were designed against 35 protein targets and one internal control. Target proteins were first captured by antibodies immobilized on the surface of paramagnetic microspheres. Then 36 pairs of PLA probes were added and allowed to bind to different epitopes on the antigens. Upon dual recognition by the correct pairs of PLA probes the attached oligonucleotides were joined by ligation. This formed linear oligonucleotides that could be amplified by PCR. The amplification products were then detected either in individual qPCR reactions, using PCR primers specific for amplicons that represented different detected proteins, or by hybridization capture onto Luminex MagPlex™ microspheres for a parallel readout. The signal amplification obtained through PCR in combination with the requirement for triple recognition in the PLA served to enhance both assay sensitivity and specificity over that of sandwich immunoassays, while allowing a higher degree of multiplexing without undue antibody cross-reactivity by virtue of ligation reactions that only could join cognate pairs of PLA probes.

Sophie Venet: Novel approach for the generation of antibody libraries by CDR-H3 capture

Sophie Venet, Ulla Ravn, Vanessa Buatois, Frank Gueneau, Giovanni Magistrelli,
Marie Kosco-Vilbois, Christian Heinis, Nicolas Fischer

Novimmune SA, Geneva, Switzerland

Structural and functional studies have shown that the main site of interaction of an antibody with an antigen is the CDR-H3 (Complementary Determining Region 3 of Heavy chain). CDR-H3 sequences are extremely variable but within one species, trends have been identified. Between species however, CDR-H3 characteristics can vary in length and in amino acid composition. It has been demonstrated that phage display libraries derived from immune subjects generate higher affinity antibodies compared to naive libraries. Combining those findings, we developed a new approach to exploit the central source of diversity from different species by capturing CDR-H3 sequences and inserting them into a library based on human antibody frameworks. In this context, immune animals have also been used to create libraries with CDR-H3 enriched for a defined target. Using this approach we can compare different sources of CDR-H3 diversity and also assess if CDR-H3 is sufficient to transfer the binding properties of an immune repertoire of an animal to a library of human scFv (single chain variable Fragments). The construction and evaluation of naive and immune libraries using CDR-H3 captured from mice will be presented.
SomaLogic has created a highly multiplexed proteomic assay which is continuously expanding in breadth. This assay uses SOMAmers to measure over 1000 proteins simultaneously from ~15 μl blood, with a throughput equivalent to 1 million ELISA assays per week. The average dynamic range of each protein in the assay is > 3 logs, with nearly seven logs of dynamic range achieved through multiple dilutions. The median lower limit of quantification is below 1 pM, with many low-abundance proteins being measurable at 100 fM. The median coefficient of variation for each protein is < 5% between runs. The outstanding assay performance arises from the selection of a new class of high affinity aptamers called SOMAmers. SOMAmers are ssDNA aptamers that contain chemically modified nucleotides and have slow dissociation rates from their targets.

SomaLogic has applied the SOMAmer-based multiplexed proteomic technology to quantify protein expression differences between freshly resected non-small cell lung cancer (NSCLC) tissues with healthy adjacent or distant tissues. While most analytes are unchanged, there are several proteins that are clear biomarkers of tumor tissue. The differential expression observed in tissue is compared with the differential protein levels found in serum from NSCLC patients using the same technology. The utility of the SOMAmers targeting tumor tissue-associated biomarkers as histochemical probes of tumor tissue is also demonstrated.
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PROGRAMME GLANCE

Tuesday, 15 March 2011

10:00 Poster Viewing and Coffee Break
10:45 Discussion
11:00 Poster Viewing and Coffee Break
11:30 Departure to/from Alpbacherhof
12:00 Lunch at Hotel Alpbacherhof
13:30 Impromptu Session
14:00 Discussion
15:00 Poster Viewing and Coffee Break
15:45 Discussion
16:00 Poster Viewing and Coffee Break

Wednesday, 16 March 2011

10:00 Poster Viewing and Coffee Break
10:45 Discussion
11:00 Poster Viewing and Coffee Break
11:30 Departure to/from Alpbacherhof
12:00 Lunch at Hotel Alpbacherhof
13:30 Impromptu Session
14:00 Discussion
15:00 Poster Viewing and Coffee Break
15:45 Discussion
16:00 Poster Viewing and Coffee Break

Thursday, 17 March 2011

10:00 Poster Viewing and Coffee Break
10:45 Discussion
11:00 Poster Viewing and Coffee Break
11:30 Departure to/from Alpbacherhof
12:00 Lunch at Hotel Alpbacherhof
13:30 Impromptu Session
14:00 Discussion
15:00 Poster Viewing and Coffee Break
15:45 Discussion
16:00 Poster Viewing and Coffee Break

Midday Break

Aftermath:
12:00 Amusement Park (Walt Disney World, Orlando)
13:00 Lunch at Disney's Yacht Club Resort
14:00 Afternoon Session
15:00 Discussion
16:00 Poster Viewing and Coffee Break
16:45 Discussion
17:00 Departure to/from Alpbacherhof
18:00 Dinner at Hotel Alpbacherhof
19:00 Impromptu Session
20:00 Discussion
21:00 Poster Viewing and Coffee Break
21:45 Discussion
22:00 Departure to/from Alpbacherhof
23:00 Dinner at Hotel Alpbacherhof

Who's Who:
12:00 Welcome and Introduction
13:00 Lunch at Hotel Alpbacherhof
14:00 Afternoon Session
15:00 Discussion
16:00 Poster Viewing and Coffee Break
16:45 Discussion
17:00 Departure to/from Alpbacherhof
18:00 Dinner at Hotel Alpbacherhof
19:00 Impromptu Session
20:00 Discussion
21:00 Poster Viewing and Coffee Break
21:45 Discussion
22:00 Departure to/from Alpbacherhof
23:00 Dinner at Hotel Alpbacherhof