Advanced affinity tools and technologies for high throughput studies of the human proteome

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Final Report Summary - AFFINITYPROTEOME (Advanced affinity tools and technologies for high throughput studies of the human proteome)

Executive Summary:
EXECUTIVE SUMMARY
Specific affinity reagents (binders) are essential probes for proteomics research, being used to detect multiple proteins in tissues and fluids through technologies which apply their properties of specificity and

target affinity. Consequently this field is known as 'Affinity Proteomics' and is complementary to the use of mass spectrometry for protein detection and identification in complex mixtures. The AffinityProteome project has focused on production of recombinant binders (antibody fragments, DARPins, aptamers) and advanced detection methodologies (microarrays, proximity ligation, intrabodies) for analysis of proteins in two critical signal transduction pathways, namely the MAP kinase and TGF-beta pathways. These are implicated in many cellular responses and diseases, such as cancers, and are an area where high quality binding reagents are particularly needed. The partners were 5 enterprises (4 of which are SMEs) and 5 academic groups, which collectively provided wide experience of binder production, analytical tools and cell signalling. The project design was that of a pipeline in which protein or peptide targets were produced as target antigens, followed by selection of binders in recombinant technologies such as phage display, ribosome display and SELEX; binders then underwent characterisation for affinity and specificity and finally were applied in sensitive protein detection assays. During the first 18 month period, the objectives were to define and produce targets, by recombinant expression in bacteria or peptide synthesis, and then to select the binders and establish methods for their characterisation and adaptation to application systems. In the second and concluding period, characterisation was completed and protocols were developed for specific technical applications, which in turn were used in protein expression studies and investigations of protein interactions in fixed and living cells. A number of commercial opportunities have been progressed by the SMEs, including improved reagents for sale, technical advances and product accessories. A new company specialising in protein arrays was founded by the coordinator. 37 original papers and reviews have been published and a number of others are in preparation. Since the pathways investigated in AffinityProteome are frequently disturbed in major diseases, such as cancers, the availability of the binders generated in the project will be of benefit in medical research and potentially development of new diagnostic assays.

Project Context and Objectives:
SUMMARY OF CONTEXT AND OBJECTIVES
For the large-scale analysis of the human proteome there is a widely acknowledged need to expand both the number and type of specific binding reagents in production and to tackle issues of throughput and cost. Ensuring the validation and quality control of the reagents is also a priority. In parallel, there is a requirement to increase the throughput and sensitivity of affinity-based applications and technologies. In this project we have aimed to develop technical solutions for affinity proteomics (methods, reagents, processes and applications) to a level where they can be exploited as commercial products, and to demonstrate how they can be enabling in the functional protein context using signal transduction in human cells as a model. The aim of this project was therefore to facilitate and accelerate developments in affinity proteomics for the biotechnology sector by linking producers of recombinant binding molecules with the developers of novel advanced technologies for their application.

The SME and academic groups combine technological innovation in binder selection, characterisation and proteomics research with the ability to bring about the commercial realisation of new concepts. The project aimed to provide answers to key practical problems associated with the very large number of reagents and test samples involved in tackling the proteome, including cost, efficiency, quality and throughput. The binding molecules themselves encompassed both established antibody fragments - single chain (sc)Fv - and alternative binders of particular promise (Designed Ankyrin Repeat Proteins or DARPins and nucleic acid aptamers called SOMAmers). Appropriate stringent quality control of the reagents is clearly a

necessary prerequisite for their application. The project provided a platform for the comparison of different binder types both in quality attributes and in specific applications, where sensitivity and specificity are essential. The innovative technologies of protein microarrays, proximity ligation and intrabodies address different areas of binder application. The binders arising from this project have the potential to make an important contribution to elucidation of functional kinase interactions, the role of molecular modifications, and cross-talk between pathways. This also serves to demonstrate the applicability of different binder types to pathway and network analysis and protein-protein interactions, critical areas for practical applications in biomarker discovery, diagnostics and drug discovery.

The workplan was designed to exploit and develop synergies between binder producers, quality control facilities and applications providers, both from SMEs and basic research institutions. The aim was to select binding molecules against component proteins of the complete TGF-beta and MAPK signal transduction pathways and to provide recognition of multiple epitopes on each protein, where appropriate in both phosphorylated and unphosphorylated forms. Selection of a panel of binders against each protein would provide reagents for assays such as proximity ligation studies, where two epitopes must be recognised on each target protein.

The pipeline to produce and characterise binders comprised tasks within workpackages (WP) 1-3, while WP4 was concerned with applications and WP5 with project management. The intention was to use about 30 proteins as targets. They included the SMAD proteins involved in TGF-beta pathway signalling , TGF-beta receptors and transcription factors known to interact with SMAD proteins. For the MAPK pathway, the targets included MAPKs, notably ERKs, JNKs and p38s, MEKs, MEKKs, RAF, scaffold proteins such as beta-arrestin and some small GTPases such as Ras and Rac. BIAFFIN had the task of expression and purification of biologically active, human protein kinases of the MAPK pathway in sufficient amounts for binder production and benchmarking activities, while UU provided SMADs and other proteins of the TGF-beta pathway. As well as full length proteins, for which binders against conformational epitopes are expected and where cross reactions with family members are likely, linear peptide epitopes for specific regions unique for each target, including phosphorylation sequences, were synthesised. Peptide epitopes, of circa 20 amino acids, were designed at UU and UZH from sequence comparisons and structural information on known functional regions, and synthesised at DKFZ, where appropriate in both phosphorylated and nonphosphorylated forms. The expressed proteins and synthesised peptides were distributed to the consortium for selections and analytical procedures.

A number of different selection methods were used to select binders from recombinant libraries. ScFv fragments initially generated from TUBS were established by a streamlined selection procedure from a validated universal clone phage display library. Steps towards higher throughput were that clones can be selected, produced and tested in 96 well format throughout the procedure and no subcloning or strain changes are necessary, which substantially decreases the effort invested per clone compared to classical phage display panning pipelines. scFv fragments were also selected by BBT using their ribosome display method, employing this technique in particular to improve the binding characteristics of candidates selected by phage display, using mutagenesis and selection. DARPins were also selected by ribosome display from libraries containing different numbers of randomised repeats (UZH). For this, a streamlined procedure was developed in which the target protein is biotinylated using an Avi Tag and selection is carried out in solution, with capturing using magnetic beads containing Neutravidin or Streptavidin. This

method is amenable to high level automation. Production of ssDNA aptamers against proteins provided in WP1 was carried out by SOMAlogic, who use their SELEX technology, in which a library comprising a pool of up to 10e15 random ssDNA oligonucleotides is exposed to the target of interest and selected sequences recovered by PCR. Reiteration of the process with error-prone PCR over several cycles leads to selection of improved binders and high specificity and affinity. SOMA focused particularly on production of aptamers with very slow dissociation rates, so-called SOMAmers.

The objectives of WP2 were to characterise and quality control binders by determination of affinity and reaction kinetics, coupled with detailed target specificity and cross reactivity studies. This allows ranking of binders for further applications and selection of pairs for diverse assays. Technologies with high throughput and multiplexing capacity were implemented, including protein and peptide microarray analysis. Binder characterisation by BIAFFIN was for affinity and kinetic parameters, using state of the art biosensors to carry out binder screening and validation by real time kinetic analysis based on surface plasmon resonance (SPR). This yields both equilibrium binding constants and separate association and dissociation rate constants, providing a complete kinetic profile. Microarray systems of various types are a highly effective means of high throughput screening of specificity and cross-reactivity of large numbers of affinity binders against multiple proteins and peptides. BBT and DKFZ have used their expertise in producing protein microarrays by cell free protein expression to analyse the specificity of protein-binder interactions. BBT have developed the prototype of a system called DAPA, which enables ‘printing’ of multiple copies of a protein array directly from a single, re-usable DNA array. Cell-free protein synthesis is performed in a membrane held between the DNA array surface and the protein tag capture surface; tagged proteins synthesised from the arrayed DNA are immobilised on the capture surface. DAPA arrays show good reproducibility and spot quality and in principle can be scaled to any requirement. In addition to in situ arrays, DKFZ and BBT analysed binder specificity on spotted protein arrays, where either the protein target or its binder is immobilised and exposed to a labelled binder or protein target respectively. Selected binders, showing high specificity in the above screen were further characterised by epitope mapping (DKFZ). High density microarrays comprising peptides which represent the sequence of the binder with an overlap of e.g. six amino acids are produced. In order to guarantee high array quality and density, peptides are synthesised in 96- or 384-microwell plates, on a small, yet flexible scale (0.05 – 0.4? mol) allowing for purification and quality control by MALDI-mass spectrometry.

The task in WP3 was to develop and refine advanced affinity-based technologies for proteomics. Before conducting experiments to detect signal transduction proteins in cells and extracts (WP4), standard conditions for the use of the different binders had to be established. Novel affinity binder-based application methods were optimised using the binders selected and characterised in WP1 and WP2 and adapted for high throughput studies. The methods were capture arrays of immobilised binders for protein expression profiling in extracts; in situ proximity ligation assay (PLA) to demonstrate individual proteins and complexes in fixed cells and tissues; and functional intracellular expression in living cells as intrabodies. The specific binder probes make it possible to detect where and when specific molecules and molecular events appear in a cell. The aim of PLA on fixed cells and tissues is to provide quantitative information about what single molecules are present, in which cellular substructures and in what interactions. Several steps of in situ PLA were investigated for optimal performance. A critical feature is the preparation of reagents and the selection of paired combinations of binders for efficient detection of single molecules and protein complexes. Two further hurdles to overcome for high-throughput applications were converting primary

binders to an applicable format and reducing reagent consumption. Efforts were made to reduce cost and time for assay completion by optimising all steps, from pre-treatment of the samples, probe incubation, ligation, rolling circle amplification (RCA) and detection. The efficacy of intracellular application of binders in living cells depends on intrinsic features of the intrabody and its expression level. One objective was therefore to optimise and validate stable and transient expression protocols for expression of protein intrabodies - scFv fragments (TUBS) or DARPins (UKASSEL, UZH) - in mammalian cells and to compare binders against consortium targets. At UKASSEL, high throughput in vivo analyses in a 96 well format were based on Bioluminescence Resonance Energy Transfer (BRET) using fusion proteins with GFP and luciferase or split GFP to quantify binder-target interactions. This has been used successfully by UKASSEL for interaction patterns of protein kinase isoforms in several cellular systems.

In WP4, novel affinity-based technologies were applied in functional proteomics of signal transduction pathways. Cell signal transduction pathways can be analysed using the binders and assay systems developed in WP1-3, to explore their use in comprehensively studying a sub-proteomic biological system. The aim was the systematic analysis of signal transduction systems, potentially in normal and diseased tissues, in a rapid and parallel fashion. The focus was on SMAD interactions for the TGF-beta pathway and on ERK1/2, JNK1/2 and other proteins in the MAPK pathways. The uses of the application tools established in WP3 included (i) quantitation of expression levels and activation state of the kinases and other pathway proteins using cell extracts and binder arrays, (ii) determination of their cellular localisation and physical interactions within the TGF-beta and MAPK pathways, and across the two pathways, using fixed cells and tissues by in situ PLA, and (iii) examination of functional disruption of signalling pathways in living cells with intrabodies. Based on the prototype specific protocols delivered in WP3, OLINK examined interactions between the proteins of interest using in situ PLA on cell lines. Evidence for cross talk between these pathways has recently begun to emerge. By looking at the interactions of members of these pathways, data can be provided on the subcellular localisation of the interactions as well as changes under variable experimental conditions.

At UZH, DARPin binders were characterised both in vitro and in vivo. Blocking enzymatic activity of the kinase in question in vitro was shown using an ELISA to detect substrate phosphorylation. DARPins also selectively precipitated the cognate form of the kinases from cell lysates. Intracellular activity of specific binders (DARPins and scFv) was studied by transfection into cell lines and pathway activity and disruption monitored by enzymatic assays, phosphorylated protein detection on binder arrays and transcriptional reporter assays; the latter could be modified for live cell imaging (UZH, UKASSEL). In a collaboration between UZH and UKASSEL, the BRET system was designed to monitor the intracellular action of binders against kinases and quantify functional interference in cellular signal transduction pathways. By fusing the kinase with Renilla luciferase and the DARPin to GFP, an energy transfer from luciferase to GFP could be observed in COS-7 cells upon intracellular complex formation. BRET showed that DARPins specifically recognise the modification status of the kinase inside the cell; moreover, the anti-ERK2 DARPin was seen to inhibit ERK phosphorylation as it blocks the target inside the cell. Disruption of kinase activity in vivo can be due not only to inhibition of the kinase activity itself, but also to steric blocking of substrate binding, blocking of kinase activation by the upstream kinase, or blocking of binding to the scaffolding protein. The phosphostatus of the specific phosphorylation sites was also analysed quantitatively using mass spectrometry analysis (UNIKASSEL). The strategy of creating activation-state specific sensors and kinase-specific inhibitors may add to the repertoire to investigate intracellular signaling in real time.

Final data integration was carried out at BBT. The application of different binder-based technologies to cell signalling resulted in raw and processed data of diverse formats, as developed by the individual partners under WP3 and 4. Conclusions on the analysed biological systems drawn by the individual participants were ‘translated’ into easily understandable figures and tables. Together with the raw and processed data, this enables efficient understanding of the implications of data from various methods.

Management and Dissemination were carried out at BBT (Dr Mike Taussig, coordinator).

Project Results:
MAIN S & T RESULTS AND FOREGROUNDS

PRODUCTION / DISTRIBUTION OF TARGET PROTEINS AND PEPTIDES
To allow for greater coverage of proteins and domains of interest, the original list of 30 proteins was expanded to 51 full length proteins and 24 domains (54 different molecular targets in total). In order to accommodate considerations of expression efficiency (mainly in E. coli) and the requirements of binder selection protocols, constructs were designed to have one or more of three sequence tags. These are the hexa-histidine tag, for purification on Nickel or Cobalt chelation columns, the AviTag, for biotinylation in vivo or in vitro, and Protein D from phage ? to enhance soluble expression and folding in E. coli. Clones and constructs were produced by SBS and BIAFFIN.

A list of peptides representing epitopes of interest, some of which were modified by phosphorylation or other groups, was assembled by UU with contributions from UNIKASSEL and UZH. Peptides to be used for binder selections were N-terminally biotinylated and two different linker sequences were used to avoid selection of anti-linker scFv in phage display; for phosphopeptides and other modifications, both the modified and nonmodified forms were produced. A total of 168 peptides, corresponding to 84 unique epitopes, were designed, covering the TGF-beta pathway in depth, and including all currently known phosphorylations of SMAD2, SMAD3, and SMAD4 and acetylation of SMAD2 and SMAD7. In all, extensive sets totalling 203 peptide epitopes for the MAPK and TGF-beta pathways were defined for synthesis, including PTMs responsible for protein interactions within and between pathways, where there is evidence of cross-talk, and in formats appropriate for recombinant binder selections.

At partner BIAFFIN, the first 18 months of the project were used to perform protein expression, purification and quality control (QC) of selected target proteins of the MAPK pathway to be delivered, in high amounts and high quality, to the binder-producing partners. For QC, SDS-PAGE (purity), Western blots using 6xHis specific antibodies (degradation), mass spectrometry (MS, identification) and size exclusion chromatography (aggregation) were applied in order to obtain comprehensive data about the proteins produced. All were identified by MS with sequence coverage in a range of 33 to 89%. In some cases modified amino acids, i.e. phosphothreonines, could be identified, indicating autophosphorylation activity. Expression constructs yielding only low amounts of proteins in expression profiling were tested in a number of different bacterial cell lines (BL21.DE3 RIL, RP, AI, LysS) in order to overcome problems with solubility, codon usage and degradation. About 25% were expressible as soluble products in E. coli. Where appropriate, kinase activity was tested for several target proteins including the four bacterially expressed and purified p38 MAPK isoforms (MAPK11-14) using Caliper mobility shift assay. By the project midpoint

(August 2010), 11 highly purified, QC validated, full length MAPK pathway proteins, each in biotinylated and non-biotinylated forms, had been produced on a large scale and provided to partners UZH, TUBS, BBT and SOMA for binder production and validation testing, with a further three in late stage of preparation. During the second project period, all protein production was carried out at BIAFFIN, where larger scale facilities were available, and production increased to 22 proteins of the MAP kinase and TGF-beta pathway in a total amount of 555 mg. All proteins were highly purified (>90%) and many were made available as both non-biotinylated and biotinylated variants.
Production of TGF-beta pathway proteins was carried out in the first period at UU and in the second period at BIAFFIN. At UU, expression was performed for 6 full length proteins, including SMADs, and 13 domains, including TGF-betaR2 ectodomain, of which 11 proteins gave yields sufficient to send to binder producers; 7 were biotinylated and distributed to TUBS for phage display selections. Together with the 22 proteins from BIAFFIN, a total of 29 full length proteins, biotinylated and nonbiotinylated were delivered to partners out of the project target of 30.

At DKFZ, 110 peptides were synthesised, corresponding to 40 different epitope regions, including several with post-translational modificiations (PTMs) (phospho/dephospho and acetylated/deacetylated pairs), and distributed to appropriate binder producers.

OPTIMISATION OF DISPLAY TECHNOLOGIES FOR HIGH THROUGHPUT SELECTION OF RECOMBINANT ANTIBODY FRAGMENTS, DARPINS AND APTAMERS
Both BBT and UZH have developed ribosome display methods for selection of binding fragments from large libraries. During the first period, BBT designed and constructed a novel naïve human flexible library for ribosome display and selection of antibody fragments, in which the scFv was fused with a spacer C? domain at the C-terminus (scFv-C?) for both display of the protein on the surface of ribosome and to provide a common priming site for RT-PCR recovery. Selections were carried out on JNK2, MAPKK4 and MAPKK6 proteins. However, expression turned out to be poor which limited selection efficiency and required a high number of cycle iterations. At the recommendation of the reviewer and after discussion among the partners, it was agreed that the primary focus at BBT should be shifted to improvement of binders for which the specificity or affinity was less than desired, using a strategy of focused mutagenesis, screening or selection by ribosome display, and specificity evaluation on protein arrays. This was implemented successfully in the second period of the project and a strategy for improving cross-reactive binders by rational mutation library design, extensive screening and differential ribosome display selection was implemented (see below). A number of improvements to the ribosome display panning protocol were introduced which will be available for future applications, including use of an uncoupled transcription and translation system and an additional DNA digestion step during the RNA processing which eliminated the issue of input DNA contamination.

UZH analysed the ribosome display protocol and identified bottlenecks for higher throughput. Adaptation of the the workflow and procedures for automated selections for (a) targets immobilised on plates and (b) with magnetic beads was performed. A robotic magnetic bead handling system (KingFisher Flex, Thermo Scientific) was purchased and produced encouraging results. The throughput achieved was about 20 selections in parallel and the system works with either selection strategy. After selections, binder fractions are cloned into an E. coli expression vector and single clones are obtained to express and analyse the binders. The standard test procedure is ELISA with crude extract from E. coli cultures expressing the

binders. Thus, a protocol and infrastructure for growing many cultures in parallel and handling many ELISA samples had to be established. To inoculate the cultures, a colony picking robot (K62 BiOcto-Pik, KBiosystems) was purchased and integrated into the workflow. UZH added a manual 96-well pipette, a 384-well capable ELISA washer and an electronic multiwell dispenser to their equipment. As a result, they can now handle about 2200 cultures for growth (in 96 well plates) and ELISA (384-well plates) in parallel. Data quality is equal to that of the low throughput procedure performed before.

DARPins binding to the kinases received from BIAFFIN were selected at UZH. The proteins were obtained chemically biotinylated and selections alternated between plates and in solution. To further generate very high affinity DARPins useful for applications, affinity maturation was established in HT format. Binders were selected by off-rate selections. In general, ca. 90 successful binders were analysed per target and semi-quantitatively tested for affinity. In parallel, peptides corresponding to the activation loops of kinases, in both phosphorylated and non-phosphorylated forms, were obtained from DKFZ, and DARPins were successfully selected against them. The binders gave rise to strong ELISA signals with good specificity against control peptides and most discriminated between the phosphorylated and non-phosphorylated forms. However, while it appears that DARPin binders against peptides discriminate well between the peptides themselves, they may not be generally useful if the goal is to detect proteins on Western blots. Structures of DARPin/ MAPK complexes were determined by X-ray crystallography, namely the DARPin/ERK2 complex, which was solved at a resolution of 1.9 Å, and that of the phospho-specific DARPin/pERK2 solved at 2.7 Å. Both DARPins recognise the phosphorylation loop and, simultaneously, the C-terminal lobe, and the structures of the complexes explain how DARPins discriminate the two states of ERK2 and in addition are specific for ERK2. Importantly, these structures are invaluable for the future design of specific kinase-sensors based on these DARPins. Altogether, DARPins were selected against 8 MAPK proteins and 4 pairs (phospho/nonphospho) of peptides.

At TUBS, the aims in the first period were the establishment and optimisation of an efficient antibody phage display selection pipeline and its application in selecting scFv binders against cell signalling proteins. A "two step cloning" strategy was applied for the generation of the naive antibody gene libraries designated HAL4, HAL7, HAL8; the scFv antibody format was chosen because of its superior display on phage and the ease with which scFvs can be engineered to construct other formats, such as scFv-Fc fusions. The application of recombinant scFv binders can be facilitated by fusion with tags, enzymes or multimerisation domains as well as by changing the expression system. For this purpose, a set of E. coli and mammalian expression vectors was constructed, allowing one step cassette subcloning from the phage display libraries, but also from other single chain antibody vectors carrying the appropriate NcoI and NotI sites. At the same time, for optimisation of the selection pipeline, all steps in the selection procedure were analysed and, where necessary, modified to establish a standardised antibody generation pipeline.

During the first period, TUBS selected and identified 38 scFv fragments against 13 protein targets, including MAPKs, MAPKKs and cAMP dependent kinase subunits, and 53 scFv against 9 SMAD and TGF-betaR2 peptides, a total of 91 binders. The yield of phospho/ nonphospho-specific scFv could be increased by using the appropriate soluble (nonbiotinylated) peptides for competition. In the second period, selections against project targets increased from 91 to 122 scFv, of which 76 were cloned and expressed as scFv-Fc fusions for use in partner assay systems. Altogether, 63 scFv were obtained against 26 MAPK and cAMP-dependent kinase subunits, together with 53 scFv against 14 SMAD and TGF-

betaR2 peptides. 76 scFv were cloned as Fc fusions and provided to 6 partners for immunoassays.

SOMAmers (Slow Off-rate Modified Aptamers) are a special class of aptamer with unique chemical and kinetic properties; while they are made of nucleic acids (like classic aptamers), they utilise modified nucleotides that provide entirely different binding to their target proteins and unprecedented intramolecular nucleic acid structures that have not been observed in natural nucleic acid structures. SOMA received 18 proteins from BIAFFIN for the production of ssDNA SOMAmers and used a modified SELEX technology to select binders binders with high specificity and affinity, and slow dissociation rate constants. Active SOMAmers were identified to 13 of these targets, and active pools were identified for the remaining 5. Both amine and biotinylated SOMAmers were synthesised and provided for screening on microarrays. Affinities were in the nM range or better, the highest being against MAPK13 (8x10e-11M) and RAC3 (4x10e-11M). Pull-down experiments were performed with the 10 SOMAmers that were synthesised for distribution in order to verify that the major band in the target protein preparation was recognised; 7 of the SOMAmers showed clear binding of the major band present.

CHARTING BINDER PRODUCTION BY SPREADSHEET COMMUNICATION SYSTEM
Information exchange regarding production of targets and binders and general progress in the project was through the versatile online Google docs system of spreadsheets (BBT). This provided ready access for all partners with the ability to insert and edit data online and proved to be a highly efficient communication medium for the project. A series of such spreadsheets was generated by BBT in collaboration with other partners, covering distinct areas of the project. For example, the Cloning and Expression progress spreadsheet was used to define the IMAGE clones required for each protein or domain target, and to follow progress in cloning into entry and acceptor vectors, their distribution to protein producers and further progress in expression. Separate spreadsheets identified and described all the protein and domain targets, with UniProt accession numbers, details of the producers and how they will be expressed, together with progress. The peptide spreadsheets gave the sequences of all peptide epitopes to be synthesised, the formats to be adopted and followed their production. Another collected all the data on scFvs, including full listing of clones, assays carried out, genetic regions (VH, D, JH, VL, JL) determined by sequencing, and so on.

QUALITY CONTROL AND CHARACTERISATION OF BINDER AFFINITY AND SPECIFICITY
A new label-free technology for biomolecular interaction analysis, Fortebio OctetRed, was evaluated by BIAFFIN in year 1. This system is based on Bio-Layer Interferometry (BLI) and detects biomolecular interactions in real time. In order to test the applicability of the OctetRed system for binder characterisation within the AffinityProteome project, binding of different anti-ERK2 (MAPK1) scFv fragments provided by TUBS were tested. Compared with BIAcore, data quality of protein-protein interactions analysed using BLI technology was rather poor, most likely due to increased nonspecific binding to the sensor tips, instability of baselines and inaccurate data evaluation procedures. Due to these limitations BLI technology was classified as unsuitable for further binder characterisation in a higher throughput at the current stage.

In the second project period, all binders were initially tested for their binding characteristics and specificity with their respective or related targets, and promising candidates were kinetically characterised further in detail (BIAFFIN) to quantitatively determine their kinetic constants including association and dissociation rates (kass and kdiss) and affinities (KD values). 41 scFv fragments were characterised, in some cases

using two experimental setups to validate the interactions in reverse orientations, by either coupling the scFv-Fc fusions to anti-Fc capturing surfaces or by coupling the biotinylated target proteins to a biotin capture chip. The highest affinities determined were 75pM for an anti?PKA RIa and 0.5nM for an anti-ERK2. A total number of 16 DARPins from UZH (against ERK2, pERK2 and unphosphorylated and phosphorylated peptides of JNK1, JNK2, p38 and ERK2) were tested and kinetically characterised. Several could discriminate the phosphorylated target (pERK2) from the nonphospho form, with up to a 63-fold difference in affinity. Besides SPR analysis, the ERK2 specific DARPins were also tested by mobility shift analysis for their potential to either inhibit enzymatic activity by blocking ATP or substrate binding or to inhibit ERK2 activation by specifically blocking the phosphorylation site for the activating upstream kinase MEK1. Seven SOMAmers were characterised and, in contrast to the antibody fragments and the DARPins, they showed very slow binding kinetics and lower KD values than expected.

BBT progressed production of protein arrays of MAPK and TGF-beta pathway proteins by the DAPA (DNA array to protein array) method in period 1. In DAPA, a DNA array is used to template a protein array by performing cell-free protein synthesis in a membrane held between the DNA array surface and the protein tag capture surface. Tagged proteins synthesised from the arrayed DNA are immobilised on the capture surface as the protein array. In order to demonstrate the applicability of DAPA to a wide range of arrayed proteins, an extensive collection of 100 different protein constructs was systematically assembled, including 56 from project targets. In addition to the coding sequences of interest, DAPA constructs contained an upstream triple c-myc tag for protein detection and a downstream fragment encoding a C-terminal double (His)6-tag for immobilisation of the resulting protein on Ni-NTA surfaces. The constructs were generated by assembly PCR, labelled for immobilisation and detection of DNA and spotted on epoxysilane coated slides. To create the protein array the DNA template slide was assembled with a Ni-NTA functionalised slide, separated by a porous membrane soaked with E. coli cell free protein expression system. On the resulting protein array, the vast majority of proteins could be detected clearly by immunofluorescence staining against the c-myc tag. The AffinityProteome targets were all expressed, with 40 of 56 (71%) rated as moderate, highly or very highly expressed. Expression efficiency is an individual property of each construct, as found in other large scale studies of cell free protein expression. However, the detection of binders using DAPA arrays showed some unexpected variability, with some antibodies detecting the protein strongly (e.g. anti-p53), while other binders were inconsistent. This may be due to different folded states of the proteins on the arrays. As a result, it was decided to use classically spotted protein microarrays for specificity screening with project binders while working to eliminate the variability of binder detection on DAPA arrays.

Full cross-reactivity and specificity screening of the binders was carried out (BBT) using spotted protein microarrays. Altogether, 34 proteins were obtained from BIAFFIN, UU and UZH for arraying. 45 scFv-Fc binders were received for protein array assessment from TUBS. As an overview, 26 of the scFv-Fc binders showed clear recognition of their own targets on the protein arrays. Of these, 9 binders (20%) were free of cross-reactivity (anti-p38beta, -p38?, -MKK4, -ERK2, -SMAD4, -PKAR2B) and a further 10 (22%) showed cross-reactivity only against closely-related proteins (anti-JNK1, -JNK2, - PKAR1A, - PKAR1B, - PKAR2A). In many cases, clear differences were seen between the reactivity and specificity of different binders against the same target. Nine DARPins were received from UZH; since a number of these (predominantly against ERK2) showed no significant binding to their targets on protein arrays, DARPin arrays were generated by immobilising the DARPins on an epoxysilane surface. Of 9 DARPins tested, 5

showed positive binding to their target, and examples of specificity against ERK2 and JNK1. Six SOMAmers were received from SOMA, all of which were provided with a a photo-cleavable biotin group and a directly-conjugated Cy3 fluorophore, and screened on protein arrays. SOMAmer arrays were also generated, with the SOMamers spotted at a variety of concentrations on either aminosilane or streptavidin slides. SOMAmers targeted against Rac1, ERK2, MKK6 and p38beta performed best on the protein arrays. SOMAmer binding was particularly sensitive to changes in temperature and buffer composition.

Complementary to the protein array assay by BBT, scFv-Fcs were analysed as protein capture microarrays (DKFZ). A total of 55 scFv-Fc fusions was delivered by TUBS, of which 52 against 24 targets were used for microarray production, resulting in identification of 44 specific binders; 19 had a good or very good performance, implying high affinities along with generally good specificities. The scFv-Fc fusions performed extremely well in the microarray assays and yielded signal intensities and specificities that were as good as or even superior to those obtained with native human antibodies. In several cases there was good agreement with the BBT protein array screens, e.g. for binders against ERK2, p38beta, MKK4, SMAD4 and PRKAR1A. However, protein arrays appear to be a more sensitive method for detection of low level cross reactions. Similar screening was performed on 24 DARPins delivered by UZH; however, due to the relatively small molecule size, DARPins appear to be sensitive to the attachment chemistry and other environmental factors. Seven SOMAmers were delivered by SOMA and used for microarray production, but essentially no specific binding was obtained for any targets, which may be due to particularly stringent conditions required for the ssDNA binders. Hence neither the DARPins nor the SOMAmers gave comparable results to the scFv-Fc fusions on capture arrays. Direct binding to protein arrays was therefore a more satisfactory analytical method for these alternative binders.

DKFZ also developed a method of synthesising in situ up to 10,000 peptides of entirely free sequence. For the attachment of synthesised peptides and the concomitant purification of full-length molecules, a new process was established and filed for patent protection: “A new dimedon derivative and a method for the purification of PNA and peptide oligomers.”

Since some binders showed quite extensive cross reactivity, e.g. due to sequence similarities among kinases, a strategy for improving cross-reactive binders by rational mutation library design, screening and differential ribosome display selection was implemented by BBT. An anti-ERK2 scFv binder (SH510-IIB7, from TUBS) showing high cross-reactivity with ERK1, was chosen as the starting template on which diversity was introduced after computational modelling by the Rosetta antibody modelling program. SH510-IIB7 had a ratio of binding for ERK2:ERK1 of 1.6 on protein arrays, explained by the 85% sequence identity between these proteins. Hence improvement of such a cross-reactive antibody to recognise specifically only one target, and reduce or abolish the recognition of the highly similar one, was challenging but potentially of significant value. On the basis of computational modelling of the H-CDR3 loop of SH510-IIB7, six residues located on the extremities of the loops extending outwards and considered as potentially contributing to loop flexibility, were chosen for site directed mutagenesis. The mutant library was expressed in E. coli and differentially screened on ERK2 and ERK1 proteins by ELISA and on protein arrays. A number of mutants displaying improved binding activity when compared with the wild-type were identified, for which specificity for ERK2 versus ERK1 was improved by up to 5-fold, the best ratio being a 9-fold discrimination of ERK2 over ERK1 compared with the starting ratio of 1.6. In a second example, for a highly cross-reactive binder against MKK4, a mutant ribosome display library was

designed targeting specific residues in all three HCDRs and selections carried out by differential panning on a mixture of proteins exhibiting high cross-reactivity and then on the specific target. Target-specific recovery by ribosome display was demonstrated.

DEVELOPMENT OF BINDER-BASED APPLICATION METHODS

SPR based methods for quantitative protein expression analyses (calibration free concentration analysis, CFCA) were developed by BIAFFIN. The target protein (cAMP-dependent protein kinase A) was specifically captured onto an immobilised ligand surface (cAMP) and two different scFv-Fc binders previously validated against the target were injected onto these surfaces at varying flow rates under mass transfer limited conditions. By using the diffusion parameters of the binder molecules, it was possible to determine active concentrations of the binder and to correct protein concentrations determined by classical methods. In a second step the CFCA method was applied to determine accurately active concentrations of target proteins by specifically capturing anti-ERK2 scFv-Fc binders onto anti-Fc capture surfaces and monitoring ERK2 binding at varying flow rates. The prerequisites for applicability of the CFCA assay were defined and a general protocol for the method applicable to varying binder formats was prepared. This method can be applied for QC and batch-to-batch comparison of recombinant proteins during the manufacturing process and for the detection and quantification of target proteins in crude cell lysates. A protocol for SPR based calibration routines complementary to CFCA was established and is suitable for stability analyses and for sensitive detection of even low concentrations of target proteins in inhomogeneous mixtures using different binder formats.

Two different approaches were evaluated by OLINK for high-throughput conjugation of different sets of binders, making them suitable for use with in situ proximity ligation (PLA). The first approach was to use anti-hapten PLA probes. Hapten-conjugation to proteins is a well established method likely to work with all proteinaceous binders; a method using haptens as targets for PLA probes should thus be generally applicable. The concept was tested on full length antibodies, since project binders could not be available at the start. The results were so encouraging that the work was transferred early on to a separate product development project within OLINK. Besides allowing any binder to be used together with in situ PLA, an advantage was that it made it possible to study homodimeric complexes using a secondary antibody approach. Anti-hapten PLA probes were released as a company product during Q3 2009, the first product based on results obtained within the AffinityProteome project.

Besides being able to perform high throughput conjugation of binders, the in situ PLA protocol needed simplification to allow high throughput evaluation of the binders produced within AffinityProteome. The protocol available on the market as the Duolink product consisted of five reaction steps and a total of 12 washing steps with an assay time of approximately 360 minutes (after the primary antibody). OLINK set out to shorten this, mainly by reducing the number of steps and washings that needed to be performed. It was found that it is possible to combine 4 of the steps into 2, thereby reducing the number of steps to 3 reaction steps plus 7 washes, and an assay time of 240 minutes (after the primary antibody). This will significantly enhance the throughput and ease of automation adoption. The findings were transferred to a separate product development project where the prototype reagents were further optimised. In May 2010, a product based on the findings was launched by OLINK called ‘Duolink II’.

Once novel project binders became available, optimised protocols for their use in in situ (PLA) were

developed by OLINK and UU. The scFv-Fc binders provided by TUBS were straightforward to convert to the format for in situ PLA, being essentially antibodies, which allowed for rapid and efficient evaluation. A set of DARPin scaffolds (UZH) was also chosen for evaluation of the SNAP/CLIP tag conjugation protocol. In both cases the binders showed equal performance and worked under similar conditions to those for classical antibodies. The results clearly show that recombinant binders, whether scFv or DARPins, selected under appropriate conditions and against appropriate targets, can be directly implemented in immunoassays such as in situ PLA. The anti-ERK2 scFv-Fc fusion SH-IIB1 was especially interesting as commercial antibodies against human ERK2 showed undesirable staining on the slide surface outside cells, while the scFv-Fc ERK2 binders did not experience this problem. In collaboration with UU, an efficient and convenient approach to functionalise recombinant affinity reagents for in situ PLA was developed by expressing the reagents as fusion partners with SNAP protein tags. This allowed conjugation of oligonucleotides in a site-specific fashion, yielding precisely one oligonucleotide per affinity reagent. This method was demonstrated with DARPins recognising the tumour antigen HER2. Before applying binders for use in in situ PLA, they were investigated for function against PFA-fixed targets in a standard immunofluorescence format. Several binders were found to be functional, i.e. gave rise to a signal, in a standard system utilising the SK-BR-3 cell line.

OLINK also progressed prototype kits for new assays. Of major interest was an scFv-Fc binder SH544-IIC4 (produced by TUBS) against a phosphorylated epitope (designed by UU and synthesised by DKFZ) within the linker region of SMAD3. This is a site against which no commercial antibodies are available, but is of great biological interest. It was shown that this binder does indeed report the activation of SMAD3. Furthermore the scFv-Fc anti-ERK2 binder SH-IIB1 has been utilised in a prototype kit. The latter binder is of special interest as it exhibits a clear and distinct staining pattern, as opposed to commercial binders evaluated by OLINK. SH-IIB1 was used together with others in a multiplexed prototype kit. Multiplexed formats were researched as part of the extra funding allocated to OLINK for the second project period. SH-IIB1 was evaluated in an assay of potential clinical importance and OLINK and TUBS have initiated discussions about potential commercialisation. A license is required from the company holding commercial rights on the HAL4/7/8 libraries. OLINK is also in contact with a potential customer for the assay and the decision to use SH-IIB1 will be made by OLINK’s development partner. Potential future diagnostic use of the binder and the promise it shows over existing commercial binders is an exciting outcome of the project.

OLINK also evaluated and developed protocols for the use of one semi-automated, and accessories for fully automated, in situ PLA in microtiterplates. In order to evaluate and compare different binders they focused on finding a format for increased throughput, maintaining the reproducibility of the manual assay. The results obtained were sufficiently interesting for OLINK to evaluate the release of a commercial protocol utilising the Shandon system. After further development and evaluation this was commercially released in February 2011.

In in situ PLA, the signal is a special fluorescent object in that it contains a very high density of fluorophores in a very confined volume. This requires that mounting media contain fluorescent bleaching quenchers, normally used in microscope mounting media, but not normally in microtiter plate based assays. To be able to use microtiter plates within the project, different formulations were investigated and one was found that worked unexpectedly well. This was taken forward within OLINK and launched with accessories to the Duolink product line in February 2011 as a Heat Transfer Block, Nuclear Stain and Anti-

Fade reagents for use with microtiter plates.

Conditions for intracellular function of binders were investigated by UZH, UKASSEL and TUBS. Using specific anti-JNK DARPins, ELISA-based enzyme assays were first performed (UZH) to investigate whether they inhibit kinase activation and/or kinase activity in vitro. The phosphorylation of JNK itself (JNK activation) was monitored by a JNK-phospho-specific antibody, while that of the JNK substrate c-Jun (JNK activity) was measured using a c-Jun phospho-specific antibody. No significant inhibition of JNK activity by the DARPins was observed. This implies that the selected DARPins cannot directly prevent activated JNK from phosphorylating its substrate, indicating that they do not interfere with substrate binding or catalysis. In contrast, at the level of JNK activation by MKK4, the upstream kinase of JNK1 and JNK2, two DARPins were potent inhibitors and caused almost complete inhibition of both JNK isoforms (0.6-3.5% residual activity). It was conclusively shown that the specific inhibitors all prevent the respective JNK isozyme from being phosphorylated itself. The inhibition of JNK activation by the DARPins also prevented JNK from phosphorylating its substrate c-Jun. Thus, the overall inhibition mode of all inhibiting DARPins seems to be at the level of JNK activation, which results in a downstream inhibitory effect towards its substrate c-Jun. Interestingly, all inhibitory DARPins show the same mode of action, protecting the kinase from being phosphorylated itself, independent of their isoform-specificity. For the best inhibitory DARPins, the inhibition observed at the protein level was comparable with gene knockouts. These findings suggest that the DARPins act as inhibitors at the level of JNK activation by either preventing binding of the upstream kinase(s) MKK4/7 to JNK or by interfering with the activation process itself, e.g. by an allosteric effect.

Pull-down experiments were performed to analyse the binding of selected DARPins to endogenous JNK isoforms directly from human cells. Endogenous JNK isoforms were immunoprecipitated from HEK 293T cell lysate by individual DARPins coupled to Ni-NTA beads and analysed by Western blotting (WB). UZH and UKASSEL chose the most promising DARPins to investigate binding specificities in living cells using BRET technology in COS-7 cells (96 well format). For BRET, all DARPins were expressed as GFP2 fusions and the kinases JNK1?1, JNK2?2 and the highly similar MAPK ERK2 were Renilla luciferase (Rluc) fusions (Rluc-kinase). All selected DARPins showed significantly higher specific binding signals for their corresponding JNK isoforms, implying that the interactions of the individual DARPins are specific within these cells. To identify JNK isoform-specific intracellular inhibitors, the DARPins were further tested for their inhibitory effect on JNK activation in eukaryotic cells. A transient transfection method was used to deliver DARPin genes into HEK293T cells to achieve constitutive and high intracellular expression levels of the inhibitors and, after expression for 24h, the JNK pathway was activated with anisomycin for 1 hour and the cell lysate analysed by WB. Over-expression of DARPins resulted in almost complete inhibition of JNK activation in the sorted cells. Thus, it was demonstrated that the generated DARPins can be very potent intracellular inhibitors of the JNK pathway when expressed at high levels in all cells, acting at the level of JNK activation. Furthermore, there is compelling evidence that these DARPins are indeed capable of JNK isoform-specific inhibition in living cells.

The application of scFv fragments as intrabodies was investigated by TUBS. For this purpose, scFv binding to cell surface receptors with single to double digit nM affinities were isolated from the phage display pipeline. Using them as model antibodies, many parameters were compared. A first interesting result was that the best knockdown effect in one case was obtained with the scFv showing the lowest

affinity, while two single digit nM candidates were less effective, despite superior FACS results, indicating that epitope accessibility should not be a major issue, at least for the mature receptors. Secondly, various designs of the antibody fragments and different vector systems were compared. As well as the fate of the target antigen, that of the scFv intrabody was also extensively analysed. A significant observation was achieved by confocal microscopy after visualisation of the scFv intrabodies inside the cell. Interestingly, the overproduction of KDEL-tagged intrabodies did not lead to an accumulation of precipitates in the cell, indicating a smooth removal of any overproduced materials. This observation is underlined by the lack of stress protein responses in cells overproducing intrabodies. This was analysed with the unfolded protein response, measured by expression changes of GRP94 against a GAPDH standard. The results strongly argue against effects mainly induced by unspecific alterations of cell physiology resulting from fragment overproduction, a prerequisite for the use of scFv intrabodies in the project.

APPLICATIONS OF ADVANCED AFFINITY-BASED TECHNOLOGIES IN FUNCTIONAL PROTEOMICS OF SIGNAL TRANSDUCTION PATHWAYS

Binder arrays were used to monitor expression of signalling proteins in cell extracts (DKFZ), with the focus on the identification of cellular variations that could be utilised as molecular evidence for disease characteristics in cancer cell lines. Advantage was taken of an antibody microarray of 810 antibodies that permits the analysis of the expression levels of 741 distinct proteins, including those of the TGF-beta and MAPK pathways. With this resource, the cellular proteomes of 24 pancreatic cancer cell lines and two control cells were studied. Several pancreatic ductal adenocarcinoma (PDAC) cell lines were collected so as to represent different cellular characteristics repeatedly, and as controls the Human Umbilical Endothelial Cell (HUVEC) and the Human Pancreatic Duct Epithelial (HPDE) cell line were utilised. Cellular proteins were extracted using an optimised extraction process developed at DKFZ, labelled using the fluorescent dyes DY-549 or DY-649 and incubated with the antibody microarrays. This microarray assay meets the quality requirements of the FDA for DNA-microarrays and could therefore be used in principle even in a clinical setting. (Note: Applying a sandwich assay would have reduced the performance of the arrays drastically in comparison to directly labelling the protein extracts prior to incubation on the binder arrays. In particular the multiplexing factor, which is critical for the analysis of patient samples, would have been reduced by at least two orders of magnitude without any gain in overall quality. Therefore, sandwich assays were not pursued.) In the analysis, 132 significantly regulated proteins were observed (49 up- and 83 down-regulated) of which 60 (45%) had been reported as biomarkers at the transcript or protein level before, while more than half had not been defined before as markers for pancreatic cancer. An analysis of the functional aspects of these PDAC-specific proteins revealed strong connections to cell death, cellular development, movement, growth and differentiation. Cell lines originating from primary tumours and metastatic tumour cells were compared in order to find metastasis-specific changes. In total, 187 proteins were found to be differentially expressed (106 down, 81 up) between the two groups, of which 39% are involved in regulating cellular movement, migration, invasion and chemotaxis. In the TGF-beta pathway, SMAD4 was shown to be expressed at an elevated level in several cancer cell lines, which is in agreement with other findings. Well-established key players in the events leading to PDAC, such as p53, NF?B, ERBB2, p38 MAPK, CDK4 and SMAD4 were all found to be overexpressed in the cancer cells. TGF-beta2 was also among the proteins over-expressed in cells from liver metastases. This growth factor is known to affect negatively the immune response to cancer cells and promote cancer progression through proliferation, metastasis, and angiogenesis.

To carry out quantitative estimates of protein expression levels using aptamers, UU prepared and supplied SOMA with a set of cell extracts from HaCat cells and HCT116 colorectal cancer cells treated with either TGF-beta or GDF15 for varying times. GDF15 is a distant member of the TGF-beta superfamily, expressed in large amounts by several different tumour types. The receptor is unknown, and some reports in the literature suggest either TGF-beta or BMP-regulated SMADs are downstream of GDF15 signaling. The aptamer technology provided by SOMA offers a possibility to detect proteins regulated by this important biomarker. SOMAmers to 12 AffinityProteome analytes as well as the other >1000 SOMAmers in the standard SOMAscan, were used in the aptamer array. Only one SOMAmer ligand met the 2x threshold after one hour stimulation with TGF-beta, namely MAPK11 (p38-beta). At the 24-hour time point, however, there were many SOMAmers whose signals were increased or decreased at least 2x when comparing with and without stimulation. MAPK11 overexpression lessened by 24 hours, but additional measurements would be needed to determine significance. Complement C3 and several kinases appeared to be underexpressed at 24 hours post-stimulation, while fibronectin, a number of other cell-matrix associated proteins, some other kinases, and several additional proteins appeared to be overexpressed. The results suggest many interesting changes that would warrant a more thorough study with additional samples.

Analysis of protein interactions in the TGF-beta pathway by proximity ligation was carried out by OLINK and UU. Based on initial IF results, UU investigated selected binders with in situ PLA in cells treated or not treated with TGF-beta stimulation. Increased numbers of interactions could be detected after 1 hr stimulation with TGF-beta; an increased number of signals due to SMAD2-SMAD4 complexes could be detected in the cell nucleus after 1 hr stimulation, using the anti-SMAD4 scFv-Fc (PaS7-C7) in combination with a SMAD2/3 antibody, in line with literature data on the kinetics of TGF-beta signaling. Phosphorylated (p179)SMAD3-SMAD4 complexes, and SMAD7 and TGF-betaRI interaction were both demonstrated in the cytoplasm. The result with the TUBS scFv-Fc SH544-IIC4 anti-(p179)SMAD3 demonstrated well the presence of SMAD3 phosphorylated in the linker as the cells go through mitosis. The results demonstrate that AffinityProteome has generated a full coverage of the TGF-beta regulated SMADs with highly specific scFv-Fc fusions. The field of TGF-beta signalling has hitherto been hampered by insufficient antibody coverage and the reagents generated within this project have the potential to provide enabling technologies for further research and drug development. This set of reagents can be a very useful tool for the development of assays in order to screen the effects of compounds that target mitotic cells.

An interesting format for multiplexing is to be able to measure whether protein A or protein B of two interactants is modified. This is of special interest in the MAPK and TGF-beta pathways as many proteins do present very similar phosphorylation sites and there are few or no protein-specific, phospho-specific antibodies available. Using in situ PLA it is possible to use dual recognition with one generic or multi-target phospho-specific antibody and one protein-specific binder in combination. OLINK used this method to set up multiplexed assays in the ERK-pathway as well as for the SMADs, taking advantage of the fact that the scFv fusion proteins in the project contained human IgG Fcs. This allowed them to use secondary PLA probes against rabbit, mouse or goat and combine them with an anti-human PLA probe to facilitate setting up assays with three binders and still use generic secondary reagents. The possibility to perform the assay in multiplex would be highly beneficial and therefore the multiplexed format researched within the project represent a significant commercial opportunity for OLINK.

Functional intracellular activity and pathway disruption by intrabodies was studied by UKASSEL, UZH and TUBS. TUBS tested the stability of scFv antibody fragments under reducing conditions in order to develop a simple screening test for antibodies that are potentially useful as cytoplasmic intrabodies. ELISA tests in the presence of high concentrations (up to 12 mM) of reducing agents such as DTT, DTE and 2-ME, revealed that all 7 ERK2- specific scFvs tested were still able to bind their antigen and some were even resistant to 200 mM of reducing agent without losing antigen binding properties. In cooperation between TUBS and UKASSEL, kinase specific scFvs against ERK2, JNK2 and PKAC-? were tested as cytoplasmic intrabodies by BRET assay. All combinations of N- and C-terminal Rluc and GFP scFv intrabody constructs were cytoplasmically co-expressed with the corresponding GFP- and Rluc-ERK2 antigen variants in HEK293 cells. A positive BRET signal could be detected only for ERK2 scFv, either N or C-terminally fused to Rluc.

Functional effects of intracellular DARPins were explored in depth by UZH and UKASSEL. Dramatic effects of DARPins in living cells could be detected, again based on BRET. First it was demonstrated that a phospho(p)ERK2 specific DARPin selectively bound to pERK2 in HEK293T and COS-7 cells. Secondly, and even more impressively, fetal bovine serum (FBS)-mediated phosphorylation of ERK2 could be inhibited by expression of an ERK2-specific DARPin which blocked the target inside the cell. All 3 tested JNK DARPins bound with high specificity to their respective JNK isoform and no JNK2 phosphorylation could be detected in the presence of 3 different anti-JNK DARPins. The results emphasise the great potential of DARPins as a novel class of highly specific intracellular inhibitors of distinct enzyme isoforms for use in biological studies and as possible therapeutic leads. This strategy of creating activation-state specific sensors and kinase-specific inhibitors may add to the repertoire to investigate intracellular signaling in real time.

A complete study employing mass spectrometry (MS) top-down and bottom-up proteomics was performed (UKASSEL) focusing on the phophostatus of cAMP-dependent protein kinase catalytic subunit PKA-C. Several novel phosphosites were identified from protein recombinantly produced in E. coli. Large changes in the (auto)-phosphorylation patterns could be observed with variations in phosphostatus from 1 phosphorylation site (protein expressed in cell free expression), 2 sites (protein derived from porcine heart, skeletal muscle and murine heart tissue), up to 7 sites (murine heart overexpressed in E. coli), demonstrating the importance of phosphostatus analysis in quality control of proteins.

Also at UKASSEL, the database software tool KinetXbase was expanded to implement BRET data. KinetXBase is a practical data handling system for molecular interaction data of any kind, allowing for distinct data formats and providing a synopsis of data derived from different technologies. This novel SQL programmed database is well suited to generating a unified view on data developed in different areas of binder analysis, including high resolution kinetic characterisation by SPR, and interactions with protein targets. Quantitative and qualitative in vivo interaction data from live cell assays such as BRET can be implemented. While avoiding the intricacies of a LIMS, it can represent all relevant information for a consistent interpretation of interaction studies. It allows for formulation of simple and complex queries on all important aspects of an interaction experiment. Data can be exported to spreadsheet applications for further analysis and the software package is free for download by academic users.

DATA INTEGRATION
Integration of the data collected during the project was carried out by BBT as a series of 7 flow chart figures. These summarise: full length target proteins produced in the MAPK and TGF-beta pathways; target peptides produced in the MAPK and TGF-beta pathways; scFv binders made for the MAPK and TGF-beta pathways; DARPins and SOMAmers made for the MAPK pathway; Assessment of binder specificity for the MAPK and TGF-beta pathways on protein arrays; Selected binder affinities (KD) determined by SPR (Biacore); and Applications of binders as intrabodies. Tables were also provided summarising the specificity and cross-reactivity of scFv-Fc, DARPins and SOMAmer binders by 3 different methods, with the most promising binders in terms of unique specificity and low cross-reactivity highlighted.

Potential Impact:
POTENTIAL IMPACT
Affinity binding reagents, particularly antibodies, and their application systems are among the most commercially important of all biological products and important drivers of progress in the life and health sciences. They are universal and powerful analytical tools for biomedical research, diagnostics and, increasingly, therapeutics for cancer and autoimmune disease. Affinity binders serve not only as tools for detection and discovery, but more and more in multiplexed research applications and, economically even more important, as diagnostics for clinical assays as well as biological drugs. Given their exceptional importance in the life sciences, this fast growing area will in the future rely on high quality, well characterised protein binding reagents and the technologies to exploit them in high throughput applications. These priorities are reflected in the central objectives of the AffiintyProteome project, namely to enhance commercial and academic efforts for production of binding reagents against human proteome targets, to quality control them in relation to technologies of utility to the biotechnology and biomedical research communities, and to develop specific applications in an area of cellular proteomics with a major impact on human health. The impacts of this project will be apparent in commercial, exploitation and research opportunities for the individual SMEs and academic groups concerned.

The development of strategies for high throughput production, quality control and application of affinity binding reagents in proteomics as carried out in AffinityProteome will have a major impact both on basic biological research and the biomedical, pharmaceutical and biotechnology industries in general. Ever since the development of proteome scale research, principally by application of mass spectrometry, the unmet need for comprehensive panels of affinity reagents has become strikingly apparent. The discovery of novel targets reaches a major bottleneck when there are no reagents for further investigations including hypothesis driven and highly focused applications. AffinityProtome has set in place technical procedures for production of comprehensive panels of recombinant binders, by providing validated methods of high throughput production, identifying criteria against which the reagents can be assessed, and comparing different possible reagent and application formats in advanced applications. This will allow choices to be made of molecules and tests which will be applicable in large scale proteomics challenges in the future.

An area of biomedical impact which can be directly linked to the use of affinity reagents to analyse human normal and diseased sample collections is the discovery and validation of biomarkers. In particular, high-throughput capture microarrays of affinity reagents enable rapid, global comparative studies on proteomes in health and disease. This gives a unique atlas of biomarkers associated with disease progression,

diagnostics, stratification and therapy, not available by other approaches, with clear potential medical applications in diagnostics, e.g. monitoring of plasma, urine, etc. It is exemplified in AffinityProteome by the studies on extracts of pancreatic cancer cell lines using binder arrays in WP4. The standardised reagents and procedures to be developed in the project and beyond will be of enormous benefit both for applications in monitoring the proteome and in plasma diagnostics and classification of complex disease states, such as cancers. Thus in the longer perspective, some of the well validated reagents provided by the resource may be adapted for clinical diagnostics.

While binders are normally conceived to act at cell surfaces or in solution, the demonstration of novel intracellular actions of DARPins and scFv fragments in WP3 and WP4 opens up a whole new potential for these binders both in research and even as therapeutic agents within living cells. Intrabody technology is a cost-effective and relatively straightforward approach to study endogenous proteins in cells as it is based on conventional transient and stable expression technology. This is a domain of recombinant reagents, and the use of conventional mAbs and polyclonal antibodies is not an option. The potential of intrabodies has been highlighted in this project with the DARPins which are able to block cell signalling actions in living cells. Given a set of reagents that would work in the cell, a number of completely new research programs, most obviously in the field of target discovery for the pharmaceutical industry, could be launched. Altogether, the ready availability of comprehensive sets of reliable reagents will underpin progress in intracellular systems biology.

The project will also be of particular benefit for the SMEs involved, which on their own would not have the resources to encompass all the technologies required to exploit the commercial potential of the binders made in this project. Their participation alongside academic groups has also generated important biological insights, clinical relevance and potential exploitation opportunities for the reagents being developed within the project covering major cell signalling pathways. The SMEs in this project will benefit in different ways, from improved robustness and throughput of their technologies, to the availability of binders to exemplify and test their systems and ultimately commercially exploitable quality assured reagents. These are evident from the examples of exploitation of foreground results listed below.

Finally, the use of recombinant human antibody fragments (scFvs) or alternatives such as DARPins and SOMAmers, facilitates the development of binders, selected within this project, into therapeutic agents. Recombinant antibodies currently represent the fastest-growing and most abundant group of biomedical therapeutics, with over 400 clinical studies ongoing. These developments are relevant to disease areas as diverse as infectious and autoimmune diseases, transplantation, wound healing and various types of cancer. Recombinant antibodies further hold the record for federal approval and are “blockbuster” products of several major European pharma companies. They may soon be joined by the ‘alternative’ binder types developed in AffinityProteome.

MAIN DISSEMINATION ACTIVITIES

• Data generated in AffinityProteome has been disseminated in the form of publications in international peer-reviewed journals and contributions to meetings and symposia. Partners have published 37 papers with AffinityProteome results (listed below) and have been very active in making presentation at conferences and workshop; a list of over 80 such presentations can be found below.

• The partners participated at the 3rd and 4th workshops on Affinity Proteomics held in Alpbach, Austria, in

March 2009 (immediately following the kickoff meeting) and March 2011, organised by the two other EU binder projects, ProteomeBinders (2009) and Affinomics (2011).
• The consortium organised a symposium titled ‘Probing the Proteome with Binders’ at the ESF conference on Functional Genomics and Disease in Dresden (April 16th 2010). The session was chaired by Mike Taussig with presentations by Drs Taussig (BBT), Plückthun (UZH), Hoheisel (DKFZ), and Gullberg (OLINK).
• Partners have maintained close contact with similar initiatives in the USA, namely the National Cancer Institute (NCI) Clinical Proteomics Reagent Initiative and the National Institutes of Health (NIH) Common Fund Program on Protein Capture Reagents, which have complementary aims to the EU binder projects. Partners Taussig, Dübel and Gold attended the NIH Capture Reagents Workshop in Washington, DC (October 2010). The meeting brought together the US and EU project leaders for the first time and they were given the opportunity to present EU projects to a US audience. Dr Taussig also gave a presentation on EU binder projects at the NIH Protein Capture Reagents Consortium Kick-Off Meeting in Washington in December 2011. NIH and NCI representatives reciprocated by attending the EU/ESF affinity proteomics workshop in Alpbach in 2011.

PROJECT PUBLICATIONS
The following 37 publications appeared during the period of the project and acknowledged project funding:
• Alhamdani, M.S. Schröder, C. & Hoheisel, J.D. (2010). Analysis conditions for proteomic profiling of mammalian tissue and cell extracts with antibody microarrays. Proteomics 10, 3203-3207
• Alhamdani MS, Youns M, Buchholz M, Gress TM, Beckers MC, Maréchal D, Bauer A, Schröder C, Hoheisel JD (2012) Immunoassay-based proteome profiling of 24 pancreatic cancer cell lines. J Proteomics. 2012 May 8. [Epub ahead of print]
• Blokzijl A, Friedman M, Pontén F, Landegren U. (2010) Profiling protein expression and interactions: proximity ligation as a tool for personalized medicine. J Intern Med. 268:232-45
• Bradbury AR, Sidhu S, Dübel S, McCafferty J. (2011) Beyond natural antibodies: the power of in vitro display technologies. Nat Biotechnol. 29:245-54.
• Darmanis S, Nong RY, Hammond M, Gu J, Alderborn A, Vänelid J, Siegbahn A, Gustafsdottir S, Ericsson O, Landegren U, Kamali-Moghaddam M. (2010) Sensitive plasma protein analysis by microparticle-based proximity ligation assays. Mol Cell Proteomics 9:327-35
• Dübel S, Stoevesandt O, Taussig MJ, Hust M. (2010) Generating recombinant antibodies to the complete human proteome. Trends Biotechnol. 28:333-9.
• Düvel J, Bertinetti D, Möller S, Schwede F, Morr M, Wissing J, Radamm L, Zimmermann B, Genieser HG, Jänsch L, Herberg FW, Häussler S. (2012) A chemical proteomics approach to identify c-di-GMP binding proteins in Pseudomonas aeruginosa. J Microbiol Methods. 88:229-36.
• Erlbruch A, Hung CW, Seidler J, Borrmann K, Gesellchen F, König N, Kübler D, Herberg FW, Lehmann WD, Bossemeyer D. (2010) Uncoupling of bait-protein expression from the prey protein environment adds versatility for cell and tissue interaction proteomics and reveals a complex of CARP-1 and the PKA Cbeta1 subunit. Proteomics. 10:2890-900.
• Friedrich A, Hoheisel JD, Knemeyer JP, Marmé N. (2011) A universally applicable process for preparing stoichiometrically 1:1 labelled functional proteins Proteomics. 11:3757-60.
• Gold L et al. (2010) Aptamer-based multiplexed proteomic technology for biomarker discovery. PloS One 2010 5:e15004.
• Gu G, Friedman M, Jost C, Johnsson K, Kamali- Moghaddam M, Plückthun A, Landegren U, Söderberg

O (2012) Protein tag-mediated conjugation of oligonucleotides to recombinant affinity binders for proximity ligation. New Biotechnology (in press)
• Hanke SE, Bertinetti D, Badel A, Schweinsberg S, Genieser HG, Herberg FW. (2011) Cyclic nucleotides as affinity tools: phosphorothioate cAMP analogues address specific PKA subproteomes. N Biotechnol. 28:294-301.
• Hust M, Meyer T, Voedisch B, Rülker T, Thie H, El-Ghezal A, Kirsch MI, Schütte M, Helmsing S, Meier D, Schirrmann T, Dübel S. (2011) A human scFv antibody generation pipeline for proteome research. J Biotechnol. 152:159-70
• Hust M, Steinwand M, Al-Halabi L, Helmsing S, Schirrmann T, Dübel S. (2009) Improved microtitre plate production of single chain Fv fragments in Escherichia coli. N Biotechnol. 25:424-8.
• Hust M, et al (2012) Generating recombinant antibodies for research, diagnostics and therapy using phage display. Current Biotechnology 1: 33-41
• Kummer L, Parizek P, Rube P, Millgramm B, Prinz A, Mittl PRE, Kaufholz M, Zimmermann B, Herberg FW and Plückthun A (2012) Phosphorylation-specific ERK kinase-binders from designed ankyrin repeat protein libraries, Proc. Natl. Acad. Sci. USA, in press.
• Landegren U, Vänelid J, Hammond M, Nong RY, Wu D, Ulleros E, Kamali-Moghaddam M. (2012) Opportunities for sensitive plasma proteome analysis. Anal Chem. 84:1824-30.
• Liu Y, Gu J, Hagner-McWhirter A, Sathiyanarayanan P, Gullberg M, Söderberg O, Johansson J, Hammond M, Ivansson D, Landegren U. (2011) Western blotting via proximity ligation for high performance protein analysis. Mol Cell Proteomics. 10:O111.011031.
• Lolicato M, Nardini M, Gazzarrini S, Möller S, Bertinetti D, Herberg FW, Bolognesi M, Martin H, Fasolini M, Bertrand JA, Arrigoni C, Thiel G, Moroni A. (2011) Tetramerization dynamics of C-terminal domain underlies isoform-specific cAMP gating in hyperpolarization-activated cyclic nucleotide-gated channels. J Biol Chem. 286:44811-20.
• Mustafa SA, Hoheisel JD, Alhamdani MS. (2011) Secretome profiling with antibody microarrays. Mol Biosyst. 7:1795-801.
• Parizek P, Kummer L, Rube P, Prinz A, Herberg FW, Plückthun A. (2012) Designed Ankyrin Repeat Proteins (DARPins) as Novel Isoform-specific Intracellular Inhibitors of c-Jun N-terminal Kinases. ACS Chem Biol. 2012 May 8. [Epub ahead of print]
• Marschall, A., Frenzel, A., Schirrmann, T., Schüngel, M. and Dübel, S. (2011) Targeting antibodies to the cytoplasm. MAbs. 2011 3:3-16. Review.
• Schirrmann T, Meyer T, Schütte M, Frenzel A, Hust M. (2011) Phage display for the generation of antibodies for proteome research, diagnostics and therapy. Molecules. 16:412-26. Review.
• Schmidt J, Müsken M, Becker T, Magnowska Z, Bertinetti D, Möller S, Zimmermann B, Herberg FW, Jänsch L, Häussler S. (2011) The Pseudomonas aeruginosa chemotaxis methyltransferase CheR1 impacts on bacterial surface sampling. PLoS One. 6:e18184.
• Schmidt, R., Jacak, J., Schirwitz, C., Stadler, V., Michel, G., Marmé, N., Schütz, G.J. Hoheisel, J.D. & Knemeyer, J.-P. (2010). Single-molecule detection on a protein-array assay platform for the exposure of a tuberculosis antigen. J. Proteome Res., 10:1316-1322
• Schröder C et al. (2010) Antibody microarrays for expression analysis. Antibody Engineering 2: 429-445
• Schröder C, Alhamdani MS, Fellenberg K, Bauer A, Jacob A, Hoheisel JD. (2011) Robust protein profiling with complex antibody microarrays in a dual-colour mode Methods Mol Biol. 785:203-21.
• Sill M, Schröder C, Hoheisel JD, Benner A, Zucknick M (2010) Assessment and optimisation of normalisation methods for dual-colour antibody microarrays. BMC Bioinformatics. 11:556.

• Søberg K, Larsen AC, Diskar M, Backe PH, Bjørås M, Jahnsen T, Laerdahl JK, Rognes T, Herberg FW, Skålhegg BS. (2012) Identification and Characterization of Novel Mutations in the Human Gene Encoding the Catalytic Subunit Calpha of Protein Kinase A (PKA). PLoS One. 7:e34838
• Stoevesandt O, Vetter M, Kastelic D, Palmer EA, He M, Taussig MJ. (2011) Cell free expression put on the spot: advances in repeatable protein arraying from DNA (DAPA). New Biotechnol. 28:282-90.
• Stoevesandt O (2012) Protein arraying by cell-free expression and diffusion across a fluid-filled gap. New Biotechnol. Apr 24. [Epub ahead of print]
• Stoevesandt O , Taussig M (2012) Affinity Proteomics: The Role of Specific Binding Reagents in Human Proteome Analysis. Expert Revs Proteomics, in press.
• Stoevesandt O,He M, Taussig MJ. (2011) Protein microarrays printed from DNA microarrays. Methods Mol Biol. 671:95-106.
• Tavoosidana G, Ronquist G, Darmanis S, Yan J, Carlsson L, Wu D, Conze T, Ek P, Semjonow A, Eltze E, Larsson A, Landegren UD, Kamali-Moghaddam M. (2011) Multiple recognition assay reveals prostasomes as promising plasma biomarkers for prostate cancer. Proc Natl Acad Sci U S A. 108:8809-14.
• Yepes D, Jacob A, Dauber M, Costina V, Hofheinz R, Neumaier M, Findeisen P (2011) Substrate optimization and clinical validation of reporter peptides for MS-based protease profiling in serum specimens: a new approach for diagnosis of malignant disease. Int J Oncol. 39:145-54
• Zieba A, Grannas K, Söderberg O, Nilsson M, Landegren U (2012) Molecular tools for companion diagnostics. New Biotechnology (in press)
• Zieba A, Pardali K, Soderberg O, Lindbom L, Nystrom E, Moustakas A, Heldin CH, Landegren U. (2012) Intercellular variation in signaling through the TGF-beta pathway and its relation to cell density and cell cycle phase. Mol Cell Proteomics. [Epub ahead of print]

PRESENTATIONS AT CONFERENCES AND WORKSHOPS
BBT:
• 14th European Congress on Biotechnology: Symbiosis: Science, Industry & Society; Sep 15, 2009, Barcelona, Spain; Audience: Scientific community (higher education, Research) – Industry; Size: 75; Countries: EU
• Affinity Mass Spectrometry Workshop; Dec 15, 2009, Konstanz, Germany; Audience: Scientific community (higher education, Research); Size: 50; Countries: Germany
• 4D Biology for Health and Disease Workshop; Mar 16, 2010; Brussels, Belgium; Audience: Scientific community (higher education, Research) - Policy makers; Size: 25; Countries: EU
• Analytica workshop, Bioanalytical Tools for Protein-Protein-Interactions; Mar 25, 2010; Munich, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 60; Countries: Germany
• 4th ESF Conference on Functional Genomics and Disease; Apr 16, 2010; Dresden, Germany; Audience: Scientific community (higher education, Research) - Industry - Policy makers; Size: 200; Countries: EU
• NIH Common Fund Workshop: Renewable Protein Capture Reagents; Oct 20, 2010; Washington DC, USA; Audience: Scientific community (higher education, Research) - Policy makers; Size: 40; Countries: USA
• 5th Workshop on Affinity Proteomics; Mar 15, 2011; Alpbach, Austria; Audience: Scientific community (higher education, Research) – Industry; Size: 120; Countries: EU
• Conference: Proteomic Forum 2011 Apr 4, 2011; Berlin, Germany; Audience: Scientific community

(higher education, Research) - Industry - Policy makers; Size: 250; Countries: EU
• Discusssion meeting: British involvement in the Human Proteome Project; Apr 19, 2011; London, UK; Audience: Scientific community (higher education, Research) - Policy makers; Size: 20; Countries: EU
• Joint BSPR/EBI Conference 2011, From the Visible to the Hidden Proteome; Jul 12, 2011; Hinxton, Cambridge, UK; Audience: Scientific community (higher education, Research) – Industry; Size: 250; Countries: EU
• 5th Central Eastern European Proteomic Conference; Sep 22, 2011; Prague, Czech Republic; Audience: Scientific community (higher education, Research); Size: 100; Countries: Czech Republic
• Workshop: Protein Capture Reagents Consortium Kick-Off Meeting; Dec 15, 2011; Washington DC, USA; Audience: Scientific community (higher education, Research) - Policy makers; Size: 50; Countries: USA

TUBS
• BIT's 3rd Annual Congress of Antibodies (ICA-2011); Mar 25, 2011;, Beijing, China; Audience: Scientific community (higher education, Research) – Industry; Size: 200; Countries: all
• Conference: IMMUNOPHARMACOLOGY 2011; DateJun 29, 2011; Varadero, Cuba; Audience: Scientific community (higher education, Research) – Industry; Size: 150; Countries: all
• PEGS Europe Conference - Protein & Antibody Engineering Summit; Oct 10, 2011: Hannover, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 250; Countries: all
• Conference: Drug Discovery and Therapy; Feb 13, 2012; Dubai; Audience: Scientific community (higher education, Research) – Industry; Size: 200; Countries: all
• Cell Line Development and Engineering 2012; Feb 15, 2012; Cologne, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 100; Countries: all
• 3rd CAPRI2010 Advanced Laboratory Workshop; Feb 13, 2012; Rijeka, Croatia; Audience: Scientific community (higher education, Research); Size of audience: 60; Countries: Croatia, Slovenia, Bosnia, Serbia
• PEGS Europe Conference - Protein & Antibody Engineering Summit; Oct 11, 2012; Hanover, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 120; Countries: EU
• Bioprocessing, Biologics & Biotherapeutics Conference; Jul 20, 2011; Edinburgh, Scotland; Audience: Scientific community (higher education, Research) – Industry; Size: 40; Countries: all
• 9th International Workshop IMMUNOTHERAPY 2010; Nov 18, 2010; Havana, Cuba; Audience: Scientific community (higher education, Research); Size: 60; Countries:: all
• 3rd Annual Proteins Congress, Berlin; Oct 28, 2010; Berlin, Germany; Audience: Scientific community (higher education, Research) – Industry: Size: 75; Countries: all
• 8th RNase Conference; Oct 21, 2010; Naples, Italy; Audience: Scientific community (higher education, Research); Size: 100; Countries: all
• Conference: 2nd bioprocessing summit; Aug 25, 2010; Boston, USA; Audience: Scientific community (higher education, Research) – Industry; Size: 80; Countries: all
• 6th PEGS Protein Engineering Summit; May 20, 2010; Boston, USA; Audience: Scientific community (higher education, Research) – Industry; Size: 120; Countries: all

UZH
• 4th Workshop on Affinity Proteomics; Mar 24, 2009; Alpbach, Austria; Audience: Scientific community (higher education, Research) – Industry; Size: 120; Countries: EU

• Keystone Symposium on Molecular and Cellular Biology; Mar 28, 2009; Whistler, British Columbia, Canada; Audience: Scientific community (higher education, Research) – Industry; Size: 300; Countries: USA
• Conference: Biofine 2009; Apr 16, 2009; Freiburg, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 100; Countries: EU
• 3rd European Conference on Chemistry for Life Sciences ECCLS; Sep 3, 2009; Frankfurt, Germany; Audience: Scientific community (higher education, Research) – Industry: Size: 100; Countries: EU
• Conference: SPP Tagung, Directed Evolution to Optimize and Understand Molecular Biocatalysts; Sep 17, 2009; Regensburg, Germany; Audience: Scientific community (higher education, Research); Size: 150; Countries: EU
• Workshop: School of Biophysics 2009; Sep 26, 2009; Rovinj, Croatia; Audience: Scientific community (higher education, Research); Size: 100; Countries: EU
• PEGS Europe Conference - Protein & Antibody Engineering Summit; Oct 6, 2009; Hannover, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 200; Countries: EU
• Wyatt Technology's International Light Scattering Colloquium 2009; Oct 20, 2009; PlaceSanta Barbara, CA, USA; Audience: Scientific community (higher education, Research) – Industry; Size: 150; Countries: USA
• Conference: Physical Principles of Protein Behavior in the Cell (PHPPBC09); Oct 29, 2009; Dresden, Germany; Audience: Scientific community (higher education, Research); Size:150; Countries: EU
• Conference: 25 Jahre Genzentrum München 'Past, presend, and future'; Nov 5, 2009; Munich, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 150; Countries: EU
• 4th ESF Conference on Functional Genomics and Disease; Apr 16, 2010; Dresden, Germany; Audience: Scientific community (higher education, Research); Size: 100; Countries: EU
• Course: EMBO practical course 'Structural Characterization of Macromolecular Complexes; Jun 1, 2010; Grenoble, France; AudienceScientific community (higher education, Research); Size: 100; Countries: EU
• IBC's 5th Annual Beyond Antibodies ' Novel Scaffolds and Preclinical-Clinical Progress'; Jun 21, 2010; San Francisco, CA, USA; Audience: Scientific community (higher education, Research) – Industry; Size: 300; Countries: all
• MPSA 2010, 18th International Conference of Methods in Protein Structure Analysis; Aug 28, 2010; Uppsala, Sweden; Audience: Scientific community (higher education, Research) – Industry; Size: 200; Countries: EU
• LSS 2010 Life Science Symposium on "Engineering Life"; Sep 4, 2010: Lausanne, Switzerland; Audience: Scientific community (higher education, Research) – Industry; Size: 150; Cuntries: EU
• EMBO Conference Series 'Chemical Biology 2010'; Sep 23, 2010: Heidelberg, Germany; Audience: Scientific community (higher education, Research); Size: 250; Countries: EU
• 3rd IMPRS-CB student symposim 'Chemical Biology - Exploring the Interface'; Sep 27, 2010; Dortmund, Germany; Audience: Scientific community (higher education, Research); Size: 100; Countries: EU
• 3rd Annual Proteins Congress ' Outlook on Antibody Therapeutics'; Oct 27, 2010; Berlin, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 200; Countries: EU
• NVBMB Fall Symposium on Synthetic Biology; Dec 12, 2010; Groningen, The Netherlands; Audience: Scientific community (higher education, Research); Size: 100; Countries: EU
• SCNAT: Rigi-Workshop "From biological machines to molecular devices of the future"; Jan 23, 2011: Rigi Kulm, Switzerland; AudienceScientific community (higher education, Research): Size: 100; Countries: EU

• Conference: GDCh Lecture Series; Feb 3, 2011; Bielefeld, Germany; Audience: Scientific community (higher education, Research); Size: 100; Countries: EU
• GRK 1026 'Conformational Transitions in Macromolecular Interactions', 2nd Meeting; Mar 3, 2011; Halle-Saale, Germany; Audience: Scientific community (higher education, Research); Size: 200; Countries: EU
• London Birkbeck College Lecture Series; May 11, 2011; London, UK; Audience: Scientific community (higher education, Research); Size: 100; Countries: EU
• Affinity 2011, 19th Biennial Meeting of the International Society for Molecular Recognition; Jun 17, 2011; Tavira, Portugal; Audience: Scientific community (higher education, Research) – Industry; Size: 150; Countries: EU
• Conference: Biochemical and Molecular Engineering XVII 'Emerging Frontiers'; Jun 28, 2011; Seattle, WA, USA; Audience: Scientific community (higher education, Research) – Industry; Size: 100; Countries: all
• Conference: PEGS Europe 2011; Oct 12, 2011; Hannover, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 700; Countries: EU
• IBC's 22nd Annual International Conference on Antibody Engineering; Dec 5, 2011; San Diego, CA, USA; Audience: Scientific community (higher education, Research) – Industry; Size: 700; Countries: all

OLINK
• Workshop on single cell analysis; Oct 26, 2011; Shinjuku, Tokyo, Japan; Audience: Scientific community (higher education, Research) – Industry; Size: 50; Countries: Japan
• Conference: 7th International Forum on Post-Genome Technologies; Oct 27, 2011; Chongqing, China; Audience: Scientific community (higher education, Research) – Industry; Size: 250; Countries: Japan, China, East Asia
• 4th ESF Conference on Functional Genomics and Disease; Apr 16, 2010; Dresden, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 200; Countries: EU

BIAFFIN
• MIPTEC Conference, Caliper User Meeting; Oct 12, 2009; Basel, Switzerland; Audience: Scientific community (higher education, Research) – Industry; Size: 45; Countries: EU
• Conference: FIGON Dutch Medicines Days; Oct 12, 2009; Lunteren, The Netherlands; Audience: Scientific community (higher education, Research) – Industry; Size: 80; Countries: EU

DKFZ
• 28th Annual Convention of Indian Association for Cancer Research; Aug 1, 2009; Bangalore, India; AudienceScientific community (higher education, Research); Size: 400; Countries: India
• Symposium on Medical Proteome Analysis; Sep 10, 2009; Bochum, Germany; Audience: Scientific community (higher education, Research); Size: 200; Countries: all
• EMERALD Workshop on Enhancing Data Quality; Sep 1, 2009; Stockholm, Sweden; Audience: Scientific community (higher education, Research); Size: 30; Countries: all
• Workshop on Functional Genome Analysis; Nov 1, 2009; Montepellier, France; Audience: Scientific community (higher education, Research); Size: 40; Countries: all
• 2nd German-Japanese Workshop on Cancer Research; Aug 1, 2009; Tokyo, Japan; Audience: Scientific community (higher education, Research): Size: 40: Countries: all

• Spetses Summer School on Proteins and their Networks - from Specific to Global Analysis; Jul 20, 2009; Spetses, Greece; Audience: Scientific community (higher education, Research); Size: 50; Countries: all
• GEN-AU Workshop on Non-Coding RNA; Oct 1, 2009; Seefeld, Austria; Audience: Scientific community (higher education, Research); Size: 50; Countries: all
• 1st Biomarker Discovery Center Workshop; Nov 15, 2009; Heidelberg, Germany; Audience: Scientific community (higher education, Research) - Industry – Medias: Size: 35; Countries: Germany
• NCRI Cancer Conference 2009; Sep 15, 2009; Birmingham, UK; Audience: Scientific community (higher education, Research) - Industry - Policy makers – Medias; Size: 400; Countries: all
• Workshop: Valencian Genomic & Proteomic Network; Oct 15, 2009; Valencia, Spain; Audience: Scientific community (higher education, Research) - Policy makers; Size: 100; Countries: Spain
• 12th Dechema Status Seminar on Chip Technologies, Sequencing and Functional Genomics; Feb 2, 2010; Frankfurt, Germany; Audience: Scientific community (higher education, Research) - Industry – Medias; Size: 250; Countries: Germany
• Analytica Conference 2010; Apr 1, 2010; Munich, Germany; Audience: Scientific community (higher education, Research) - Industry – Medias; Size: 500; Countries: all
• 76th Annual Conference of the German Cardiac Society; May 1, 2010; Mannheim, Germany; Audience: Scientific community (higher education, Research) - Industry - Policy makers – Medias; Size: 700; Countries: Germany
• 4th ESF Conference on Functional Genomics and Disease; Apr 16, 2010; Dresden, Germany; Audience: Scientific community (higher education, Research) – Industry; Size: 200; Countries: EU
• m3: microelectronics meets medicine; Jul 15, 2010; Munich, Germany; Audience: Scientific community (higher education, Research) - Industry - Policy makers; Size: 30; Countries: Germany
• CNIO Frontiers Meeting Cancer Pharmacogenetics: Personalizing Medicine; Sep 1, 2010; Madrid, Spain; Audience: Scientific community (higher education, Research); Size: 80; Countries: all
• United European Gastroenterology Federation Professional Trainee Course on Colorectal Cancer; Mar 1, 2011: Heidelberg, Germany; AudienceScientific community (higher education, Research); Size: 80; Countries: all
• Workshop: Treffpunkt in vitro Diagnostik Onkologie; May 5, 2011; Berlin, Germany; Audience: Civil society - Policy makers – Medias; Size: 30; Countries: Germany
• Workshop: 1st CAGEKID Symposium; Jun 10, 2011; Hinxton, UK; Audience: Scientific community (higher education, Research); Size: 30; Countries: all
• 2nd OpenGENE Workshop; Jul 1, 2011; Tartu, Estonia; Audience: Scientific community (higher education, Research) - Industry – Medias; Size: 100; Countries: all
• 10th HUPO World Congress; Sep 1, 2011; Geneva, Switzerland; Audience: Scientific community (higher education, Research) - Industry – Medias; Size: 500; Countries: all
• 4th NGFN Meeting; Oct 4, 2011; Berlin, Germany; Audience: Scientific community (higher education, Research) - Industry – Medias; Size: 120; Countries: all
• 7th Workshop Molecular Interactions; Oct 15, 2011; Berlin, Germany; Audience: Scientific community (higher education, Research); Size: 70; Countries: all
• 4th German-Israeli Cancer Research School; Nov 1, 2011; Kfar Giladi, Israel; AudienceScientific community (higher education, Research): Size: 80; Countries: Germany, Israel
• Workshop: Perspectives in Clinical Proteomics; Mar 15, 2012; Hinxton, UK; Audience: Scientific community (higher education, Research) - Industry - Policy makers; Size: 60; Countries: all

Media briefings
• DKFZ: Bundeswettbewerb Jugend Forscht; May 10, 2010; Heidelberg, Germany;
• UKASSEL: Press release: Proteinbinder geben neue Einblicke in Krankheiten; Apr 4, 2011; Kassel, Germany
• UKASSEL: Press release: Nanotechnologisches Kooperationsprojekt: VDI-Preis 2012 für Matthias Joseph Knape; Feb 9, 2012; Kassel, Germany; Audience: Scientific community (higher education, Research) - Civil society; Countries: Germany

Broadcast
• BBT: Mike Taussig was interviewed about affinity proteomics on the BBC World Service, November 8th , 2010. The recorded interview was broadcast on several days thereafter.

School presentations
• DKFZ: Schülerforum, Personalised Medicine; Oct 20, 2010;Heidelberg, Germany; Audience: Civil society; Size 120; Countries: Germany
• DKFZ: Seminar at Johann-Philipp-Bronner-Schule; Nov 14, 2010; Wiesloch, Germany; Audience: Civil society; Size: 40; Countries: Germany

EXPLOITATION OF RESULTS

INTELLECTUAL PROPERTY
European Patent Application 10 187 366.9. A new dimedon derivative and a method for the purification of PNA and peptide oligomers. Deutsches Krebs Forschungs Zentrum; Jacob, Anette; Hoheisel, Jörg; Dauber, Marc; Wiessler, Manfred; Lorenz, Peter; Fleischhacker, Heinz; Kliem, Hans-Christian

SPINOUT COMPANY
Cambridge Protein Arrays Ltd., established by Dr Mike Taussig, 14th May 2010
Company number 7254575
Address: Babraham Research Campus, Cambridge CB22 3AT, UK
Website: www.cambridgeproteinarrays.com

EXPLOITABLE FOREGROUND: COMMERCIAL EXPLOITATION OF RESULTS

OLINK AB
• Duolink Heat transfer block and Duolink Anti-Fade and Nuclear Stain, for Immunohistochemical assays; accessories to Duolink product family improving the functionality and performance for high-throughput use of Duolink products using micro-titer plates; launched as a product 2011.
• Duolink In Situ, for Immunohistochemical assays; improved protocol yielding a faster and more robust assay; launched 2010
• Duolink Probemaker, for Immunohistochemical assays; Novel means of making reagents for use in Olink’s Duolink product; launched 2010
• Duolink Detection Green; for immunohistochemical assays; Improved detection reagent for Duolink In situ found when exploring multiplexed formats within the project; launched 2011

BIAFFIN GmbH
• Kinascreen SPR assays for real-time analysis of kinase inhibitor interactions; improved protocols for real-time kinetic characterization of small molecules binding to a number of MAP kinase target proteins using surface plasmon resonance biosensorsfor pharmaceutical drug development; launched as a service 2012
• Semi-quantitative SPR analysis of high affinity antibody antigen interactions; Improved protocols for faster kinetic characterization of high affinity binders using Biacore SPR biosensors applying single cycle kinetics measurements; biotechnology sector; launched as a service 2012.
• SPR assays using Biotin capture chips; Improved protocols for reversible coupling of biotinylated proteins on biotin capture chips for real-time analysis of high affinity antibody antigen interactions using surface plasmon resonance biosensors; biotechnology sector; launched 2012
• Recombinant proteins (MAP kinases) as research reagents (enzymes, substrates) for exploring cellular signaling cascades and interaction networks; launched as products 2011
• SPR-based CFCA assay; Improved protocols for determination of active concentrations of recombinant proteins and antibodies applying calibration-free concentration analysis on SPR biosensors; quality control sector; launched as a service 2012

BBT
• Improved DAPA (DNA array to protein array) technology; to be developed further by spinout Cambridge Protein Arrays Ltd. with licence from BBT; biotechnology sector.

List of Websites:
The website address is www.affinityproteome.eu.
Contact: Dr Oda Stoevesandt, Babraham Bioscience Technologies, Cambridge CB22 3AT (oda.stoevesandt@babraham.ac.uk).

Powiązane dokumenty

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