AFFINOMICS

Protein Binders for Characterisation of Human Proteome Function: Generation, Validation, Application

Start Date: 1st April 2010
Grant agreement number: 241481

Kickoff Meeting Cambridge 16th May 2010
Partners and PIs

1. Uppsala University (UU), Coordinator: Ulf, Fredrik
2. Royal Institute of Technology (KTH): Mathias
3. University of Zurich (UZH): Andreas
4. Lund University (ULUND): Carl
5. NNF Center for Protein Research (CPR): Michael
6. Technical Research Centre of Finland (VTT): Petri
7. EMBL Monterotondo/EBI: Alan, Henning
8. Tor Vergata University (UTOV): Gianni
11. Kassel University (UNIKASSEL): Fritz
12. Helmholtz Centre, Munich (HMGU): Marius
15. Vrije Universiteit Brussel (VUB): Serge
AFFINOMICS Essentials

• Will generate largescale resources of validated protein-binding molecules as affinity reagents for characterisation of the human proteome, applying them in structural and functional analyses of protein expression, interactions and complexes.

• Focus on 5 categories of proteins in signal transduction, cell regulation and cancer, namely: protein kinases, SH2 domain-containing proteins, protein tyrosine phosphatases, proteins somatically mutated in cancers and candidate cancer biomarkers.

• Will establish high throughput, coordinated production pipeline for antigens and binders.
• Target antigens (~1000) to include folded full-length proteins or domains, large peptide fragments (PrESTs) based on low homology to other human proteins and small peptides, including phosphorylated.

• Binder types to include monospecific polyclonals, mAbs, recombinant antibody fragments, single VH domains and non-Ig scaffolds.

• Will develop highly efficient ‘next generation’ recombinant selection methods, based on phage and ribosome display.

• Binders to be used in development of innovative and sensitive tools and technologies and to characterise protein complexes and interaction networks.
Key Objectives

• Enable systematic production of large sets of well-characterised, validated antibodies and other protein binders to meet demands to phenotype the proteome.

• Develop and implement automated methods for HT recombinant binder production.

• Develop stringent, uniform quality control methods for binders.

• Use binders to study the proteomics of signalling pathways and cancer-related proteins, particularly protein-protein interactions and complexes of protein kinases, tyrosine phosphatases and SH2-domain proteins.

• Develop application tools for characterisation of binders and proteins and to use them to analyse potential disease biomarkers in normal, clinical and biobanked samples.

• Establish procedures and logistics for access to laboratory protocols and sets of binders through databases.
Organisation

1. A binder production pipeline, with particular emphasis on novel recombinant selection systems capable of proteome wide delivery.

Workpackages and coordinators

WP1: Generation of protein antigens (SGC).
WP2: Generation of protein-binding affinity reagents (TUBS).
WP3: Characterisation, validation and quality control (KTH).
WP4: Next-generation selection systems for recombinant protein binders (UZH).
WP5: New tools and applications for characterisation of binders, proteins and biomarkers (UU).
WP7: Bioinformatics and databases (UU).
WP8: Scientific coordination (BBT).
Key Deliverables

WP1,2: Physical resources of validated binders and antigens

WP3: Standards, protocols for quality control procedures

WP4: Recombinant production facilities and systems capable of throughput required to expand to a proteomic scale

WP5: novel binder-based tools and technologies for extracellular and intracellular protein detection and functional analysis

WP6: data on molecular profiling of signalling pathways, cancer related gene products and biomarkers

WP7: publicly accessible database portals
<table>
<thead>
<tr>
<th>Year</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37-42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43-48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49-54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55-60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**WP1 Generation of antigens**

1.1 Protein antigens (SGC, CPR)

1.2 PRESTs for all targets (KTH)

1.3 Peptides and Phosphopeptides (DKFZ)

**WP2 Generation of binders**

2.1 Affinity-purified polyclonal Abs (KTH)

2.2 Monoclonal Abs (EMBL MACF, HMGU)

2.3 Recomb. scAbs (BBT, ULUND, TUBS, VIB)

2.4 Recombinant scaffolds (UZH, KTH)

**WP3 Characterisation, validation, QC**

3.1 Standard specificity tests (KTH)

3.2 Affinity, kinetics, SPR (UNIKASSEL)

3.3 Specificity on protein arrays (BBT)

3.4 Epitope mapping on peptide arrays (DKFZ)

3.5 Specificity on RNAi cell arrays (VTT)

**WP4 Next gen selection systems**

4.1: Ribosome display (BBT, UZH)

4.2: Phage display (ULUND, TUBS, VIB)

4.3: Bacterial display (KTH)
### WP5 New tools and applications

| 5.1 Epitope mapping by bacterial display (KTH) |
| 5.2 Peptide microarrays for epitope map (DKFZ) |
| 5.3 Suspension bead arrays (KTH) |
| 5.4 Protein arrays (BBT, UNIKASSEL, DKFZ) |
| 5.5 Antibody arrays (ULUND) |
| 5.6 Lysate arrays (VTT) |
| 5.7 DNA amplification proximity probes (UU) |

### WP6 Proteomics of complexes

| 6.1 Characterisation of complexes (MMBE) |
| 6.2 Growth factor pathway interactions (UTOV) |
| 6.3 Multiplexed proximity ligation (UU) |
| 6.4 Intracellular nanobodies (VIB) |
| 6.5 Combining binders with RNAi (VTT) |

### WP7 Bioinformatics

| 7.1 Antibodypedia (KTH) |
| 7.2 Software for antigen selection (VTT, UTOV) |
| 7.3 Functional proteomics data capture (EBI) |
| 7.4 Molecular Methods database (UU) |
| 7.5 PSI-PAR standard format (EBI) |

### WP8, 9 Management, Coordination

- Coordination, progress monitoring, meetings
- Website, Reporting, Financials, Contract, etc.

### WP 9: Management, Dissemination, Access

- Website, Reporting, Financials, Contract, etc.
Programme

9.00  Intro: Mike Taussig BBT, Ulf Landegren UU
9.10  Susanne Müller-Knapp SGC
9.20  Michael Sandrini CPR
9.30  Sophia Hober KTH
9.40  Joerg Hoheisel DKFZ
9.50  Stefan Dübel TUBS
10.00  Carl Borrebaeck ULUND
10.10  Andreas Plückthun UZH
10.20  Alan Sawyer EMBL-MACF
10.30  Discussion
10.45  COFFEE
11.20  Mike Taussig BBT
11.30  Serge Muyldermans VIB
11.40  Marius Ueffing HMGU
11.50  Gianni Cesareni UTOV
12.00  Ulf Landegren UU
12.10  Fritz Herberg UKASSEL
12.20  Petri Saviranta VTT
12.30  Henning Hermjakob EMBL-EBI
12.40  Discussion
13.00  LUNCH
Afternoon agenda

• Open discussion on practicalities and realistic possibilities
• How it can/cannot, should/should not be done
• KTH, SGC experience of organisation of a pipeline project
• Experience from AffinityProteome
• Planning year 1
Planning Year 1

- Target identity and priorities (avoiding duplications)
- Target format and design (antigen providers need to know what binder producers and binder users require)
- Binder types (not all binders possible for all targets, so link to applications and what users require)
- Protein production, clones, expression methods, purification (distribution between different centres; priorities; start with proteins off the shelf?)
- Peptides and PREsts (avoid overlap; PTMs for peptides)
- Binder production (distribution of targets among binder producers, according to applications)
- Binder characterisation (organisation, liaison between producers and test centres)
- LIMS and keeping track of the whole project (maintaining interaction and communication between relevant labs and groups)
Graphical presentation of the components showing their interdependencies

WP1: Generation of protein and peptide antigens

WP4: Next-generation selection systems for recombinant protein

WP5: New tools and applications for characterisation of binders, proteins and biomarkers

WP2: Generation of protein-binding affinity reagents

WP3: Characterisation, validation and quality control

WP6: Affinity-based functional proteomics of signalling protein complexes

WP7: Bioinformatics and Databases
- Antibodypedia
- ProtAffinSelect
- IntAct
- MolMeth

WP8: Scientific coordination

WP9: Project Management

Legend:
Blue: Pipeline and data / access elements
Red: Tools and applications