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Editorial

European and international collaboration in affinity proteomics

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In affinity proteomics, specific protein-binding molecules (a.k.a. binders), principally antibodies, are applied as reagents in proteome analysis. In recent years, advances in binder technologies have created the potential for an unprecedented view on protein expression and distribution patterns in plasma, cells and tissues and increasingly on protein function. Particular strengths of affinity proteomics methods include detecting proteins in their natural environments of cell or tissue, high sensitivity and selectivity for detection of low abundance proteins and exploiting binding actions such as functional interference in living cells. To maximise the use and impact of affinity reagents, it will be essential to create comprehensive, standardised binder collections. With this in mind, the EU FP7 programme AFFINOMICS (http://www.affinomics.org), together with the preceding EU programmes ProteomeBinders and AffinityProteome, aims to extend affinity proteomics research by generating a large-scale resource of validated protein-binding molecules for characterisation of the human proteome. Activity is directed at producing binders to about 1000 protein targets, primarily in signal transduction and cancer, by establishing a high throughput, coordinated production pipeline. An important aspect of AFFINOMICS is the development of highly efficient recombinant selection methods, based on phage, cell and ribosome display, capable of producing high quality binders at greater throughput and lower cost than hitherto. The programme also involves development of innovative and sensitive technologies for specific detection of target proteins and their interactions, and deployment of binders in proteomics studies of clinical relevance. The need for such binder generation programmes is now recognised internationally, with parallel initiatives in the USA for cancer (NCI) and transcription factors (NIH) and within the Human Proteome Organisation (HUPO).

The papers in this volume of New Biotechnology are all contributed by participants at the 5th ESF Workshop on Affinity Proteomics organised by the AFFINOMICS consortium and held in Alpbach, Austria, in March 2011.

EU binder projects in affinity proteomics

Three major European collaborative projects have focused on the production of binders against the human proteome. The most recent, AFFINOMICS, follows on from two others. The first, ProteomeBinders (http://www.proteomebinders.org), was essentially a networking activity, which linked key groups in Europe. ProteomeBinders did not fund laboratory research but did promote bioinformatics and database development. The second, AffinityProteome (http://www.affinityproteome.eu), was a project involving SMEs as well as academic groups producing binders against components of the MAPK and TGF-β signal transduction pathways. The objective of all three projects is *to provide resources of binders to characterise the human proteome*. A 'manifesto' for the entire vision was set out in a Commentary article in Nature Methods [1], authored by the project partners.

1871-6784/$ - see front matter © 2012 Published by Elsevier B.V. http://dx.doi.org/10.1016/j.nbt.2012.05.003

This inspired an editorial in the same issue which posited that 'the European Union has a unique opportunity to positively impact research in many fields, in a concrete and substantial way, by providing generous and sustained support to ProteomeBinders. Turning this project into a full-blown, large-scale reagent-generation program can drive the field and incite additional efforts in and outside Europe . . ..' [2].

The EU binder projects follow from the realisation that binding molecules, primarily antibodies, are among the most essential and sought-after reagents in biomedical research. The premise is that to understand the proteome and to use this understanding, it is essential to create a comprehensive, standardised binder collection. Binders are an area where breakthrough achievements were made in Europe, not least the invention of monoclonal antibodies [3]. To maintain Europe's position in the field, the EU binder projects seek to establish a resource of validated, quality-controlled binding reagents for detection of all human proteins, centres of binder production and distribution, databases and sets of binder-based tools to explore protein expression and function in health and disease. The latter links to current needs in diagnostics and personalised medicine [4] (see Box 1).

The problem is the sheer potential enormity of the human proteome: to take a 'protein-centric' approach, the figure of about 23,000 genes in the human genome increases potentially by orders of magnitude for protein species, once splice variants and modifications are counted in. Of these, only a minor fraction is covered by existing binders. The implication is that new binders and, importantly, the target antigens will therefore be needed in very large numbers. Moreover, the binders must be complemented with protein detection methods capable of high sensitivity, wide dynamic range and multiplexing, for which it is often highly desirable to have more than one binder per target (e.g. for sandwich assays). One of the ongoing successful European projects in the field, the Human Protein Atlas Project, adopts a 'gene-centric' approach, where binders (polyclonal antibodies) have been made to the products of some 50% of human protein-coding genes [5].

One substantial output of the ProteomeBinders project is the Antibodypedia database (http://www.antibodypedia.com/) [6], developed at the Swedish Royal Institute of Technology (KTH). Antibodypedia lists commercially available antibodies and, where available, shows the actual results of validation experiments, which can be provided by suppliers as well as by users of the antibodies. Antibodypedia is a result of the realisation that quality control and its documentation are crucial for enabling researchers to choose the right reagent for a given application. It aims to become a complete directory of available antibodies, providing all the data to support this choice. The database has now been linked to the Nature Publishing Group and currently (May 2012) contains 186,790 reviewed antibodies, covering 17,248 genes (approximately 84% of all human genes) with primary validation data available for 31,748 experiments and evaluations for basic criteria of functionality in Western blot (WB), ELISA, immunofluorescence and immunoprecipitation.

Also in ProteomeBinders, Niall Haslam and Toby Gibson at EMBL developed an epitope selection resource called EpiC (http://bioware.ucd.ie/epic/) [7], to aid the experimental design of binders to human proteins. For a protein of interest against which a binder is to be made, EpiC collates information from multiple online resources containing up-to-date empirical annotations and bioinformatic predictions on protein structure and function, including phosphorylation, antigenicity, hydrophobicity and glycosylation, among others. EpiC also takes into account the technology in which the binder is to be used and assigns the analytical modules which will inform the summary of epitope recommendations accordingly.

A forerunner of AFFINOMICS, set up as an international proof of concept exercise without external funding, aimed to raise binders in a coordinated fashion against 20 individual SH2 domains. Collaborating groups in Sweden, UK, Germany, Canada, USA, Australia and China (the 'Renewable Protein Binder Working Group') showed that it was indeed possible to move quickly to a productive outcome, the results of which were published recently [8–10]. Over 500 renewable binders of different types were made (165 monoclonal antibodies, 340 unique recombinant scFv fragments), which were confirmed to work in ELISA, Western blot, immunohistochemistry (IHC) and on microarrays of folded SH2 domains or protein fragments (PrESTs). The binders were produced in an impressively short space of time (3 months), 25% had better than 10 nM affinities and some could discriminate very closely related targets. This project was important in demonstrating that a binder-raising project, although on a relatively small scale in regard to target number, could be carried out efficiently despite wide geographical separation of the partners.

BOX 1

Vision and aims for a European binder resource

- To provide affinity reagents against 'all' human proteins to the research community.
- To develop the required molecular resources of clones, proteins, peptides and validated paired binders.
- To establish thorough binder quality control and validation procedures.
- To develop novel tools for application of affinity reagents.
- To create public database portals for binder and protein data.
- To facilitate specific human proteome projects, for example functional annotation of the proteome; mapping of proteins in healthy and diseased tissues; plasma profiling and discovery of biomarkers.

AFFINOMICS

This EU FP7 (7th framework programme) funded collaborative project began in April 2010, with a budget of 11 million € over 5 years and now has 18 partners (http://www.affinomics.org). The objectives of AFFINOMICS are the systematic production of large sets of well-characterised, validated binders against five categories of human proteins in signal transduction, cell regulation and cancer. These are: all protein kinases (n = 503), SH2 domain proteins (n = 130) and protein tyrosine and dual specificity phosphatases (n = 45), together with selected

cancer-related proteins (e.g. somatically mutated in cancers or candidate cancer biomarkers) making up to about 1000 targets in total. The antigens are produced as folded proteins or domains, recombinant protein fragments of 50–150 amino acids (PrESTs) [11,12] and synthetic peptides, including some phosphorylated epitopes. The binders themselves range widely from monospecific rabbit polyclonal antibodies [12], to mouse hybridoma monoclonals, human scFv fragments, camelid V_HH single domain antibodies (nanobodies) [13] and the non-Ig scaffolds known as DARPins [14] and Affibodies [15]. A particular emphasis is on the improvement of 'next generation' recombinant selection methods, based on phage and ribosome display, towards automation and high throughput; recombinant methods are seen as the most practical means of tackling the numbers of binders which will be needed to cover the human proteome. The aims include not only the production of the binders themselves and their stringent quality control, but also new application tools for characterisation of proteins and for evaluation of potential biomarkers in normal, clinical and biobanked samples.

AFFINOMICS employs a distributed pipeline strategy, starting from target selection and generation of target antigens and binding reagents in the first two years, and leading to binder characterisation and quality control, with different partners performing different steps in the pipeline. Technology improvements are being developed alongside and will be implemented in the generation pipeline to increase the efficiency of this process. Ultimately, the new binders will become part of a community resource. Binders will be evaluated by the standard methods of WB, ELISA and affinity determination (surface plasmon resonance), together with epitope mapping on peptide arrays, specificity analysis on immobilised protein and PrEST arrays, and IHC combined, for validation, with RNAi cell arrays. Binder applications in AFFINOMICS include several array technologies (capture arrays, lysate arrays, protein arrays); proximity ligation, where binders are linked to DNA for ultrasensitive detection of proteins and intracellular complexes [16]; and expression as intrabody reagents for knockdown and dynamic localisation of targets in living cells [17]. The single domain nanobodies are also being used as facilitators in protein crystallisation and structure determination. As the project develops, users will gain access to a public database (ProteinBinders, currently in development) listing the binders, their evaluation data and sourcing details. The procedure for the storage and distribution of the resource will include a cost recovery element. For hybridomas it may make use of the facilities of the University of Iowa Developmental Studies Hybridoma Bank (DSHB) (http://dshb.biology.uiowa.edu/) from which supernatants can be obtained at minimal cost.

US and other binder projects

Efforts to make binder resources for proteomics are by no means limited to Europe. In the US, there are ongoing programmes funded by the National Institutes of Health (NIH). The NIH Common Fund's Protein Capture Reagents Program (http://commonfund.nih.gov/proteincapture/), initiated in 2011, has aims similar to those of the EU projects ('generating low cost, high quality, renewable affinity reagents for all human proteins'). As a test case, the NIH Common Fund is focusing on production of both monoclonal and recombinant binders against human transcription factors, with the intention to create a broadly available community resource for researchers and particularly for chromatin immunoprecipitation (ChIP) studies. There is also an emphasis on new technology development for reducing the cost and increasing the quality and throughput of present technologies. The NCI has pursued the aim of providing binding reagents for cancer studies for several years, from organising discussion workshops [18] to the current Clinical Proteomic Technologies for Cancer initiative which includes the Antibody Characterization Program (http://proteomics.cancer.gov/). This focuses on the production of mouse monoclonal antibodies against cancer associated protein targets and provides well-characterised reagents to the research community, distributed through the DSHB as well as commercial venders. The choice of targets in both the NIH Common Fund and NCI initiatives is highly complementary to those in AFFINOMICS, and indeed the NCI and AFFINOMICS consortium have recently concluded a Memorandum of Understanding for the exchange of materials.

Other activities in which sets of binders are produced and characterised are being followed through HUPO, such as the Human Antibody Initiative (http://www.hupo.org/research/hai/), which is also associated with Antibodypedia and the Human Protein Atlas, and the recently established Human Proteome Project (HPP) [19]. The HPP is adopting a chromosome-centric approach to map the entire human protein set, including the use of antibodies.

Alpbach workshops on affinity proteomics

Clearly, the importance of comprehensive binder projects for future progress in human proteomics is now widely recognised and has led to the productive and important initiatives described above. It has also led to some invigorating meetings where the issues are vigorously debated. One series is the biennial Alpbach workshops on Affinity Proteomics, organised and supported by the EU binder projects and the European Science Foundation (ESF). The articles in this volume, which are all related to the use of antibodies and other binders in proteomics projects, were part of the workshop held in Alpbach in March 2011 (http://www.functionalgenomics.org.uk/sections/activitites/2011/Taussig/info.htm). We thank the authors for their contributions and look forward to the continued success of these meetings in 2013 and 2015.

Acknowledgement

AFFINOMICS is funded by the EU 7th Framework Programme under grant agreement 241481.

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