

# Generating recombinant antibodies to the complete human proteome

Stefan Dübel<sup>1</sup>, Oda Stoevesandt<sup>2</sup>, Michael J. Taussig<sup>2</sup> and Michael Hust<sup>1</sup>

<sup>1</sup>Technische Universität Braunschweig, Institute of Biochemistry and Biotechnology, D-38106 Braunschweig, Germany

<sup>2</sup>Protein Technology Group, Babraham Bioscience Technologies Ltd, Babraham Research Campus, Cambridge CB22 3AT, UK

***In vitro* antibody generation technologies have now been available for two decades. Research reagents prepared via phage display are becoming available and several recent studies have demonstrated that these technologies are now sufficiently advanced to facilitate generation of a comprehensive renewable resource of antibodies for any protein encoded by the approximately 22,500 human protein-coding genes. Antibody selection *in vitro* offers properties not available in animal-based antibody generation methods. By adjusting the biochemical milieu during selection, it is possible to control the antigen conformation recognized, the antibody affinity or unwanted cross-reactivity. For larger-scale antibody generation projects, the handling, transport and storage logistics and bacterial production offer cost benefits. Because the DNA sequence encoding the antibody is available, modifications, such as site-specific *in vivo* biotinylation and multimerization, are only a cloning step away. This opinion article summarizes opportunities for the generation of antibodies for proteome research using *in vitro* technologies.**

## Are antibodies needed for every human protein?

The structural and functional individuality of cells as diverse as neurons, myocytes and hepatocytes – all of which share an identical genome – reflect the differential expression, localization and modification of proteins. For the majority of the 22,500 protein-coding genes currently predicted from the human genome ([www.ensembl.org](http://www.ensembl.org)), characterization of the expressed protein products and their functions is still lacking. Antibodies are essential reagents for many protein analyses, including localization, isolation, interaction and quantification, and have helped to define completely new areas of research, as evidenced by antibody translation to the clinic for routine diagnostics and therapies (Figure 1). For example, the current wealth of knowledge in immunology and hematology would not exist without antibodies available to define the numerous CD antigens present on cells of the hematopoietic lineage. Powerful antibody-based methods, such as proximity ligation [1] and correlation spectroscopy [2] for detection of protein interactions with molecules that can likewise be specifically recognized by antibodies, are continuously added to the laboratory repertoire.

Current powerful proteomic approaches based on mass spectrometry would be enhanced by the availability of

antibodies to any given human protein. In their visionary paper in 2003 on the future of interaction proteomics, Aebersold and Mann [3] stated that ‘...ideally, endogenous proteins can serve as bait if an antibody or other reagent exists that allows specific isolation of the protein with its bound partners. Unfortunately, there are currently no comprehensive antibody collections and many current antibodies do not immunoprecipitate well or lack sufficient specificity.’ Overexpression of tagged bait proteins and co-immunoprecipitation of their bound interaction partners via anti-tag antibodies [4] only provide a partial solution because introduction of the overexpressed heterologous protein can skew cellular interaction equilibria. This problem still exists, but significant efforts have been made toward systematic generation of a resource of antibodies to the entire proteome at reasonable cost, such as the Human Protein Atlas initiative ([www.proteinatlas.org](http://www.proteinatlas.org)) [5], the Sanger Institute Antibody Atlas Database, the German Antibody Factory project, the NCI Clinical Proteomics initiative [6], the HUPO human antibody initiative ([www.hupo.org/research/hai/](http://www.hupo.org/research/hai/)), and an increasing list of EC-funded initiatives (ProteomeBinders, AffinityProteome, Affinomics; [www.proteomebinders.org](http://www.proteomebinders.org)).

## Recombinant antibody fragments versus intact IgG antibodies

Evolution has shaped the antibody molecule as well as the underlying mechanisms that lead to binding site diversity such that a matching structural solution for every protein can always be found, even for antigens that do not occur naturally [7]. Antibody diversity is achieved in precursor B cells by combinatorial DNA rearrangements using a gene fragment lottery to assemble the antigen-binding surface of antibody variable (V) domains. The antibody is completed by combining heavy- and light chain V domains to form the final binding site [8]. For recombinant selection technologies, intact antibody molecules as generated by the immune system have been reengineered into simpler, smaller entities, known as antibody fragments. Intact IgG molecules consist of 12 structural domains, of which only four provide the paired antigen-binding sites: the V regions of the heavy and light chains combining with the Fv fragments. In *in vitro* selection systems, these fragments are typically covalently connected either by an oligopeptide linker to form a single-chain Fv fragment (scFv) or by the two adjacent constant domains, which interact and are connected by a disulfide bridge (the Fab fragment) (Figure 2).

Corresponding author: Dübel, S. ([s.duebel@tu-bs.de](mailto:s.duebel@tu-bs.de)).

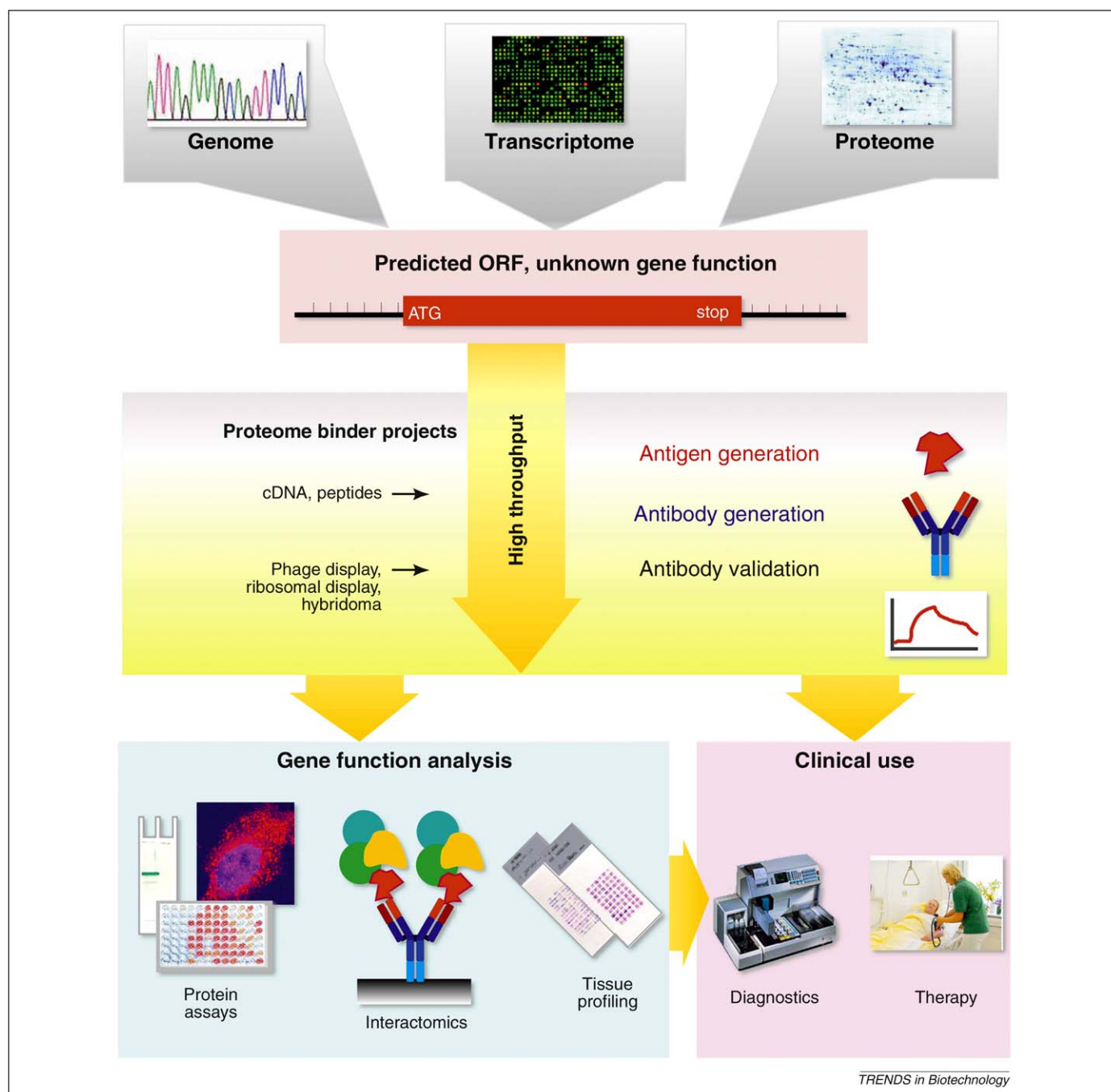


**Figure 1.** Schematic depicting why antibodies to the entire human proteome are needed. Most of the bioinformatically predicted open reading frames (ORFs) in the genome have not been verified to date. An antibody pipeline to generate binders to all of them would significantly speed up proof of their existence in a spatial- and time-dependent manner, and further provide functional analysis tools (i.e. protein assays, interactomics and tissue profiling). When human antibodies are used as a resource, a direct development pathway into a therapeutic lead compound is available in cases for which appropriate *in vivo* activity is found.

Genetic modification facilitates the formation of fusions to antibody Fc regions (e.g. scFv-Fc), enzymes or multimerization domains, or the addition of tags for detection, purification or biotinylation. Antigen-binding properties are retained in all of these recombinant molecular derivatives. Recombinant methods for antibody generation, such as phage display [9–14], and ribosome and mRNA display [15–19] rely on *in vitro* enrichment of an antibody gene physically linked to the corresponding gene product using the antigen-binding function of the latter for selection (Figure 2). Extremely diverse molecular repertoires of binder molecules can be generated in a volume of a few hundred microliters and binders can be selected in a multiplex

manner [20,21]. A large number of vectors, libraries and robust protocols for antibody phage display have been developed [13], making it the most widely used *in vitro* selection system. Antibody libraries have been developed beyond the scope offered by nature, for example using synthetic complementarity-determining regions (CDRs) composed of only a few amino acids, which can nevertheless form high-affinity binding sites [22,23]. The affinity of antibodies resulting from recombinant *in vitro* selection is at least on a par with that of classical antibodies, with typical  $K_d$  values in the double- to single-digit nanomolar range before affinity maturation. Typically, antibodies with the highest observed affinity are of recombinant origin and selected by *in vitro*

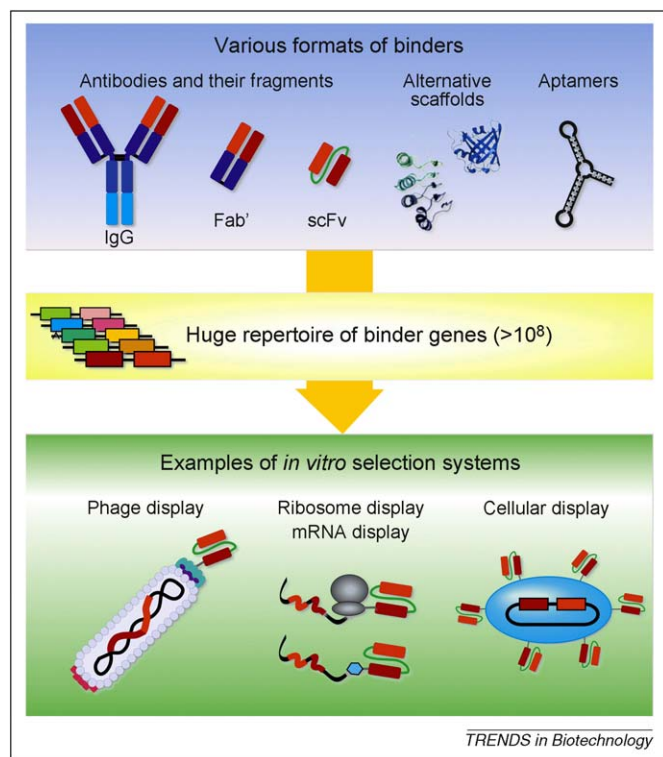


**Figure 2.** Formats and sources of binding molecules. To generate binders *in vitro*, many different systems and molecules have already been developed. For example, scFv antibody fragments or alternative scaffolds can be selected by phage, ribosome or yeast display. The choice of the molecular format and the selection system can therefore be chosen to best fit the experimental goal.

methods [24,25]. Concerning fine specificity, antibodies selected *in vitro* are also hard to surpass: a different location of two, or very few, atoms can be discriminated [26,27].

### Recombinant binders based on non-antibody scaffolds

Non-antibody molecular frameworks have been developed, initially driven primarily by efforts to avoid the restrictions imposed by antibody engineering patents. These frameworks generally carry two or more randomized stretches of amino acids that mimic antibody CDRs. Examples of these alternative scaffolds include Adnectins, Affibodies, Anticalins and DARPins, among others [28]. By combining parts of existing binding sites with randomized repertoires, molecular clamps with high affinity can be selected [29,30]. Even non-peptide molecules have been developed based on the 'framework + randomized loops' principle to generate repertoires of recombinant binders. The most prominent examples of this group are nucleic acid aptamers [31,32], which also can be selected in a high-throughput manner [33] and used in highly parallelized assays [34]. This entire group of alternative scaffolds shares important advantages with the engineered antibodies: their recombinant nature and compatibility with *in vitro* selection procedures. As recombinant entities, these binders are characterized by a known and accessible gene sequence, which renders them immediately amenable to further targeted engineering.

### Adopting *in vitro* methods as the new gold standard for antibody generation

To date, traditional generation of monoclonal antibodies (mAbs) using hybridoma technology has set the perform-

ance standard to which emerging recombinant antibody methods and alternative scaffolds are compared. However, with respect to throughput and despite becoming streamlined and automated to a considerable extent [35], hybridoma technology has approached its limits after 35 years of optimization. Hybridoma generation requires immunization of an individual animal, which has several disadvantages. First, even during multiplex immunizations, in which one animal is injected with several antigens concurrently, the potential for reducing the space and cost of the infrastructure required for animal care is limited. Moreover, the immune system defines the minimum time required to obtain an immune response and its possible range; for example, antigens or epitopes that are sufficiently similar to those of the host animal might fail to provoke an *in vivo* response through tolerance, whereas toxic antigens can kill the animal before an immune reaction can take place and particular classes of substances might not be immunogenic at all.

By contrast, *in vitro* library display systems can bypass these restrictions, providing tremendous potential for miniaturization and acceleration of the selection process. Theoretically, the speed achievable for *in vitro* techniques is limited only by the diffusion time of the antigen-antibody binding event and the reactions used to propagate and amplify the binder-encoding DNA. Further benefits of repeated use of the same recombinant library repertoire of binding molecules are in substituting a single multi-well plate for an animal facility housing hundreds of cages, with the potential to reduce the antibody selection procedure to just a series of liquid handling steps. The cost per binder decreases in proportion. Key features of *in vitro*- and immunization-based antibody generation methods are compared in Table 1.

### Streamlining and integration of antigen production and antibody characterization

It is already evident that the future limiting factor in antibody generation will be the supply of high-quality native antigens, both for initial selection and for subsequent characterization. The foreseeable further reduction in the quantities of antigen required for miniaturized *in vitro* antibody selection in multi-well plates might render recombinant antigen production in bacteria or mammalian cells obsolete. We have achieved high rates of success for panning on synthetic peptides, resulting in antibodies that, although not always reacting with the folded protein, are useful in many assays relying on denatured protein or peptide fragments (immunoblots, fixed tissue arrays, MS, etc.). Of particular interest here is the general capability of *in vitro* selection to generate phosphorylation-specific antibodies by competing with a soluble unphosphorylated variant of the phosphopeptide used in the selection. For assays requiring antibodies to folded proteins, the future of high-throughput *in vitro* antibody selection is likely to rely on the use of relatively low quantities of antigen obtained from *in vitro* transcription or translation systems. Significant miniaturization and parallelization of cell-free protein expression has been demonstrated in the systematic conversion of >13,000 human transcripts into the encoded proteins [36]. With

**Table 1. Comparison of capabilities and cost factors for antibody generation technologies**

| Feature                                      | Polyclonal sera                                        | Hybridoma mAb                                                                                       | <i>In vitro</i> -selected mAb                                                                                            |
|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| Antigen for selection                        | Amount for immunization: 1–200 µg to immunize a rabbit | Amount for immunization: ~30 µg                                                                     | Amount for <i>in vitro</i> selection: µg                                                                                 |
| Setup for selection                          | Animal facility for immunization and care              | Animal facility for immunization and care                                                           | <i>In vitro</i> liquid handling                                                                                          |
| Setup for clonal characterization            | Not applicable                                         | Mammalian cell culture                                                                              | <i>E. coli</i> cultivation                                                                                               |
| Predetermination of Ab properties            | Not possible                                           | Not possible                                                                                        | By adjusting biochemical milieu during selection                                                                         |
| Predetermination of epitope structure        | Not possible                                           | Not possible                                                                                        | By adjusting selection conditions (antigen presentation)                                                                 |
| Predetermination of conformation specificity | Not possible                                           | Not possible                                                                                        | By adjusting selection conditions to provide correct antigen conformation                                                |
| Avoidance of cross-reactivity                | Not possible                                           | Not possible                                                                                        | By addition of competitor during selection                                                                               |
| Toxic antigen use                            | Difficult                                              | Difficult                                                                                           | Possible                                                                                                                 |
| Minimal time for selection                   | Months                                                 | Months                                                                                              | Weeks                                                                                                                    |
| Miniaturization potential for selection      | No                                                     | No                                                                                                  | Yes                                                                                                                      |
| Application in assays                        | IHC, western blot, ELISA, FACS, IP                     | IHC, western blot, ELISA, FACS, IP                                                                  | IHC, western blot, ELISA, FACS, IP                                                                                       |
| Ab size (kDa)                                | Not defined (mixture)                                  | 150 (IgG) or 900 (IgM)                                                                              | 29 (scFv), 55 (Fab) or 150, can be adjusted after selection                                                              |
| Affinity                                     | Not defined (mixture)                                  | nM to µM                                                                                            | nM to µM                                                                                                                 |
| Affinity improvement                         | Not possible                                           | Tedious; requires subcloning from hybridoma cell followed by mutation and <i>in vitro</i> selection | Easy; mutation and <i>in vitro</i> selection                                                                             |
| Avidity                                      | Not defined (mixture)                                  | 1, 2 or 10 antigen-binding sites (Fab after proteolysis, IgG, IgM)                                  | 1, 2, 4 or more antigen-binding sites (adjustable)                                                                       |
| Detection system                             | Anti-Fc reagents                                       | Anti-Fc reagents                                                                                    | Anti-tag antibodies, streptavidin, anti-Fc reagents (various species); can be changed after selection                    |
| Use as intrabody                             | Not possible                                           | Tedious; requires subcloning from hybridoma                                                         | One-step cloning                                                                                                         |
| Therapeutic use                              | Not possible for chronic diseases                      | Requires humanization (to avoid HAMA)                                                               | Yes (human origin)                                                                                                       |
| Stability maturation                         | Not possible                                           | Difficult                                                                                           | Easy; mutation and <i>in vitro</i> selection                                                                             |
| Production system                            | Rabbit, mouse, other animals                           | Mammalian cell culture                                                                              | <i>E. coli</i> , Gram-positive bacteria, yeasts, fungi, insect cells, plants, mammalian cell culture, transgenic animals |
| Direct use of culture supernatant            | Not applicable                                         | Yes                                                                                                 | Yes                                                                                                                      |
| Typical purification                         | Affinity chromatography; precipitation                 | Protein A column                                                                                    | Nickel chelate column                                                                                                    |
| Conjugation                                  | Chemical                                               | Chemical                                                                                            | <i>In vivo</i> during production (alkaline phosphatase, biotin, streptavidin, etc.)                                      |
| Ab amount                                    | Limited by animal blood volume                         | Unlimited                                                                                           | Unlimited                                                                                                                |
| Ab storage temperature (°C)                  | 4 or –80                                               | 4 or –80                                                                                            | 4 or –80                                                                                                                 |
| Clone storage                                | No clone available                                     | Liquid nitrogen                                                                                     | Freezer or computer hard drive                                                                                           |
| Clone transport logistics                    | No clone available                                     | Dry ice                                                                                             | Room-temperature stab culture, DNA or email (sequence)                                                                   |
| Patent protection                            | None                                                   | None for mouse hybridoma                                                                            | Last patent expires 2011 (discussed in [65,66])                                                                          |

<sup>a</sup>Abbreviations: Ab, antibody; FACS, fluorescence-activated cell sorting; HAMA, human anti-mouse response; IHC, immunohistochemistry; IP, immunoprecipitation.

reported average yields of 2.6 µg of soluble protein per 150 µl of expression volume in a multi-well format, the antigen requirement of *in vitro* antibody panning could be met. Consequently, integration of multi-well-format cell-free antigen generation with *in vitro* panning for antibodies is an obvious aim. Of similar importance for the

overall efficiency and throughput of binder generation is the miniaturization of single-clone characterization assays (i.e. testing of individual molecular species resulting from the selection process, classically by ELISA). Here, various array formats offer either a significant reduction in the amount of antigen, time and cost required [37–40] or

improved data quality by assessing parameters such as epitope structure and cross-reactivity patterns in high-throughput systems [41–43]. Thus, continued optimization towards higher throughput will lead to *in vitro* selection methods that far exceed the capabilities of those currently in existence.

After their selection, recombinant antibodies have to be produced. Numerous production systems for recombinant antibodies that provide up to g/l yields have been developed in various Gram-negative and -positive bacteria, yeasts, fungi, insect cells, plants, mammalian cell cultures and even transgenic animals [44]. For most research applications, however, the amounts obtained from cheap and robust *Escherichia coli* cultures are sufficient, ranging from 10 mg/l to 1 g/l for scFv or Fab fragments [44]. Culture supernatants can be used directly or an affinity chromatography step on a nickel chelate column provides a typical purification and concentration step.

#### Improve your antibody

From the moment of *in vitro* selection, the gene encoding the antibody is available and can be modified easily to add novel functionality to the antigen-binding site, thus setting this technology apart from hybridoma-generated mAbs. For example, a mouse, rat, rabbit or human Fc region can be fused with the same antigen-binding fragment, facilitating adaptation to different animal models or detection procedures [45]. Similarly, multimerization domains or enzymes can be fused with the antigen recognition domains and *in vivo* biotinylated antibodies can be readily produced in *E. coli* [46]. Various binder genes have been used for intracellular functional analysis of the role of their respective targets. By intracellular expression in mammalian cells, knockdown of the target protein function can be achieved [47–49], as demonstrated by the abolition of phosphorylation events in viral IL6 (vIL6) signalling by an anti-vIL6 intrabody or inhibition of cell adhesion via an anti-VCAM intrabody. As an alternative to intracellular expression, intrabodies have been targeted to the cytoplasm from the outside as cargo in receptor-mediated delivery of the short neuropeptide substance P, followed by escape from the endosome [50]. This approach provides a generic route for delivery of binders for targeted interference with intracellular functions. In a longer-term perspective, this might even be applied therapeutically, extending the scope of antibody therapies to intracellular targets. In basic research, functional analysis of predicted gene products will strongly benefit from the combination of these methods with the high-throughput generation of recombinant antibodies, requiring little more than subcloning of the antibody gene cassette into the knockdown vector.

#### Complete control with *in vitro* selection

A final distinct advantage of *in vitro* selection systems compared with hybridomas is the ability to control antibody–antigen reactivity. The key step in phage or ribosome display is the direct molecular interaction of the antibody binding site and the antigen. *In vitro*, the biochemical milieu during this selection can be completely controlled. This includes the use of competitors in the selection to

avoid cross-reactions [26,51], or even making the selection specific for an elusive allosteric variant that is only stable in the presence of a cofactor [52]. Moreover, it enables the user to select antibodies under relevant assay conditions from the outset, and panning conditions or antigen presentation can be varied to steer selection towards binders that recognize conformational or linear epitopes [53]. None of this can be achieved with animal-based antibody generation methods. To harness this potential, researchers might have to change their way of thinking about antibody generation: immunization can be likened to a lottery, because there is little control over how the animal's immune system will process the administered antigen (e.g. with respect to conservation of structural epitopes). Thus, the resulting antibodies have to be extensively tested *a posteriori* for cross-reactivity and suitability in each particular assay method. By contrast, *in vitro* selection enables researchers to decisively influence *a priori* in which application the antibody will work (western blotting, pull-down immunoprecipitation or other assays) by adjusting the biochemistry of the selection reaction accordingly from the start. Although this does not avoid the necessity of validating recombinant antibody clones, it does facilitate targeted development of binders for highly specialized applications [51–53].

#### Systematic recombinant binder generation by phage display

Phage display is an *in vitro* technique for antibody selection that uses bacteriophage DNA. A very diverse ( $>10^9$ ) library of rearranged immunoglobulin genes, typically antigen-binding fragments such as scFv antibody constructs, is obtained either from human B cells or synthetically. The library is cloned into a vector linking the antibody genes in frame to a gene that codes for a phage surface protein such that the antibody fragments are expressed on the outer surface of the phage. This mixture of billions of different functional antibody fragments, each linked to corresponding encoding genes, is exposed to immobilized antigen in the panning process. The vast majority of antibody phages do not interact with the antigen and are subsequently washed away. After elution from the antigen, the affinity-enriched phage population is used to re-infect *E. coli* for multiplication and propagation, leading to monoclonal *E. coli* producing the encoded antibody fragment.

During its first decade of use, antibody phage display was mainly applied to the generation of human antibodies for therapy, yielding a steadily increasing list of clinically important products ([www.landesbioscience.com/html/journals/mabs/table-1.htm](http://www.landesbioscience.com/html/journals/mabs/table-1.htm)) [54,55]. Adaptation to the needs of basic research took longer because the requirements for almost all factors influencing the selection protocol design differ significantly [56,57]. Initial comparisons demonstrated that recombinant antibody generation for research suffers from no major disadvantages, in contrast to technology based on animal immunization [58]. In an extensive systematic study [20], successful high-throughput generation of antibodies was achieved using phage display. In 2008, the Structural Genomics Consortium [59] initiated a pilot study for the generation of binders to their fast-growing list of available human proteins, focusing on

SH2 domains; as a result, eight groups provided hundreds of antibodies to 20 SH2 domains. Significantly, five of the groups used phage display and *in vitro* selection (<http://www.proteomebinders.org/>) [21,60]. Recent wet laboratory successes have boosted concurrent development of bioinformatic tools, databases and an approved quality standard [61–63], all of which will facilitate the envisaged open access to the antibody resource. The results of these studies demonstrate that high-throughput *in vitro* recombinant antibody generation has arrived at a point at which systematic generation of binders against larger numbers of antigens, sub-proteomes, entire gene families or even entire proteomes is possible at reasonable cost. Recombinant antibodies can already be generated at a similar cost to monoclonal hybridoma antibodies. Handling, logistics, production and storage of a large biobank of *in vitro*-generated antibodies can be expected to be significantly cheaper and more robust because it is based solely on *E. coli* instead of mammalian cell lines. Moreover, all the DNA sequences of the antibodies are known from the selection process (they are sequenced to identify individual binders among the primary ELISA hits); this biobank can therefore have a bioinformatic backup, making it “indestructible” because the same specificity can always be reconstituted by gene synthesis. With the enormous potential for miniaturization and automation, there is ample room for further cost reductions for the *in vitro* methods, rendering them the preferred solution for any future high-throughput antibody generation project.

### Concluding remarks and future challenges

It has been proven that phage display can deliver valuable research tools that have started to appear in the antibody catalogues alongside the classical polyclonal and hybridoma antibodies ([www.axxora.com](http://www.axxora.com)). But there is still more to come. In less than 10 years from now, we all might have a desktop robot in our laboratory that is capable of generating a recombinant antibody within a couple of days. Challenges towards this aim include the integration of antigen production and purification into a fully automated antibody panning pipeline. For systematic high-throughput binder generation projects, integration and automation of panning and screening will be of equal importance. These bottlenecks are already being actively tackled [64]. Forces that are expected to continue driving research in the field of proteomics are the hunger for specific, reliable and affordable reagents and the spread of functional proteomics applications (such as intrabodies), which rely on recombinant antibody formats. By using *in vitro* selection methods available today, individual antibodies or alternative binders can already be generated in a little over a week, based on the observation that two selection rounds are sufficient when using Hyperphage, and identified hits can go directly to shaker flask-scale production [21]. In the future, using systems that can completely avoid expression in bacteria, such as ribosome display or other molecular display methods, this time might be even further reduced.

Given an appropriate funding decision, the generation of a renewable resource of monoclonal binder reagents to the entire human proteome could be achieved in a few years at a fraction of the cost invested in the sequencing of

the human genome, while offering similar scientific value to the life science community and the biotechnological and pharmaceutical industries. With the evident miniaturization potential of recombinant antibody selection tools – which have not been exhaustively explored – we can anticipate that further method development will continue to decrease the time and cost for generating individual antibodies.

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### References

- Söderberg, O. *et al.* (2008) Characterizing proteins and their interactions in cells and tissues using the *in situ* proximity ligation assay. *Methods* 45, 227–232
- Stoevesandt, O. *et al.* (2005) One-step analysis of protein complexes in microliters of cell lysate. *Nat. Methods* 2, 833–835
- Aebbersold, R. and Mann, M. (2003) Mass spectrometry-based proteomics. *Nature* 422, 198–207
- Rigaut, G. *et al.* (1999) A generic protein purification method for protein complex characterization and proteome exploration. *Nat. Biotechnol.* 17, 1030–1032
- Ponten, F. *et al.* (2009) A global view of protein expression in human cells, tissues, and organs. *Mol. Syst. Biol.* 5, 337
- Haab, B. *et al.* (2006) A reagent resource to identify proteins and peptides of interest for the cancer community. *Mol. Cell Proteomics* 5, 1996–2007
- Braden, B.C. *et al.* (2000) X-Ray crystal structure of an anti-Buckminsterfullerene antibody Fab fragment: biomolecular recognition of C<sub>60</sub>. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12193–12197
- Neuberger, M.S. (2008) Antibody diversification by somatic mutation: from Burnet onwards. *Immunol. Cell Biol.* 86, 124–132
- McCafferty, J. *et al.* (1990) Phage antibodies: filamentous phage displaying antibody variable domain. *Nature* 348, 552–554
- Breitling, F. *et al.* (1991) A surface expression vector for antibody screening. *Gene* 104, 1047–1153
- Barbas, C.F. *et al.* (1991) Assembly of combinatorial antibody libraries on phages surfaces: the gene III site. *Proc. Natl. Acad. Sci. U. S. A.* 88, 7982–7987
- Hoogenboom, H.R. *et al.* (1991) Multi-subunit proteins on the surface of filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains. *Nucleic Acids Res.* 19, 4133–4137
- Hust, M. and Dübel, S. (2004) Phage display vectors for the *in vitro* generation of human antibody fragments. *Methods Mol. Biol.* 295, 71–95
- Hust, M. *et al.* (2009) Antibody phage display. In *Therapeutic Monoclonal Antibodies* (An, Z., ed.), pp. 191–211, Wiley-VCH
- He, M. and Taussig, M.J. (2007) Eukaryotic ribosome display with *in situ* DNA recovery. *Nat. Methods* 4, 281–288
- Zahnd, C. *et al.* (2007) Ribosome display: selecting and evolving proteins *in vitro* that specifically bind to a target. *Nat. Methods* 4, 269–279
- Roberts, R.W. and Szostak, J.W. (1997) RNA–peptide fusions for the *in vitro* selection of peptides and proteins. *Proc. Natl. Acad. Sci. U. S. A.* 94, 12297–12302
- Lipovsek, D. and Plückthun, A. (2004) *In-vitro* protein evolution by ribosome display and mRNA display. *J. Immunol. Methods* 290, 51–67
- Odegrip, R. *et al.* (2004) CIS display: *in vitro* selection of peptides from libraries of protein-DNA complexes. *Proc. Natl. Acad. Sci. U. S. A.* 101, 2806–2810
- Schofield, D.J. *et al.* (2007) Application of phage display to high throughput antibody generation and characterization. *Genome Biol.* 8, R254
- Mersmann, M. *et al.* (2010) Towards proteome scale antibody selections using phage display. *N. Biotechnol.* 27, 118–128
- Birtalan, S. *et al.* (2008) The intrinsic contributions of tyrosine, serine, glycine and arginine to the affinity and specificity of antibodies. *J. Mol. Biol.* 377, 1518–1528

- 23 Koide, S. and Sidhu, S.S. (2009) The importance of being tyrosine: lessons in molecular recognition from minimalist synthetic binding proteins. *ACS Chem. Biol.* 4, 325–334
- 24 Schier, R. *et al.* (1996) Isolation of picomolar affinity anti-c-erbB-2 single-chain Fv by molecular evolution of the complementarity determining regions in the center of the antibody binding site. *J. Mol. Biol.* 263, 551–567
- 25 Boder, E.T. *et al.* (2000) Directed evolution of antibody fragments with monovalent femtomolar antigen-binding affinity. *Proc. Natl. Acad. Sci. U. S. A.* 97, 10701–10705
- 26 Moghaddam, A. *et al.* (2003) Identification of scFv antibody fragments that specifically recognise the heroin metabolite 6-monoacetylmorphine but not morphine. *J. Immunol. Methods* 280, 139–155
- 27 Dörsam, H. *et al.* (1997) Antibodies to steroids from a small human naive IgM library. *FEBS Lett.* 414, 7–13
- 28 Nygren, P.A. and Skerra, A. (2004) Binding proteins from alternative scaffolds. *J. Immunol. Methods* 290, 3–28
- 29 Koide, S. (2009) Generation of new protein functions by nonhomologous combinations and rearrangements of domains and modules. *Curr. Opin. Biotechnol.* 20, 398–404
- 30 Huang, J. *et al.* (2009) Structural basis for exquisite specificity of affinity clamps, synthetic binding proteins generated through directed domain-interface evolution. *J. Mol. Biol.* 392, 1221–1231
- 31 Famulok, M. and Mayer, G. (1999) Aptamers as tools in molecular biology and immunology. *Curr. Top. Microbiol. Immunol.* 243, 123–136
- 32 Brody, E.N. and Gold, L. (2000) Aptamers as therapeutic and diagnostic agents. *J. Biotechnol.* 74, 5–13
- 33 Keeney, T.R. *et al.* (2009) Automation of the SomaLogic proteomics assay: a platform for biomarker discovery. *J. Assoc. Lab. Autom.* 14, 360–366
- 34 Ostroff, R. (2010) The stability of the circulating human proteome to variations in sample collection and handling procedures measured with an aptamer-based proteomics array. *J. Proteomics* 73, 649–666
- 35 De Masi, F. *et al.* (2005) High throughput production of mouse monoclonal antibodies using antigen microarrays. *Proteomics* 5, 4070–4081
- 36 Goshima, N. *et al.* (2008) Human protein factory for converting the transcriptome into an *in vitro*-expressed proteome. *Nat. Methods* 5, 1011–1017
- 37 Wong, J. *et al.* (2009) Rapid detection of antibodies in sera using multiplexed self-assembling bead arrays. *J. Immunol. Methods* 350, 171–182
- 38 Angenendt, P. *et al.* (2004) Seeing better through a MIST: evaluation of monoclonal recombinant antibody fragments on microarrays. *Anal. Chem.* 76, 2916–2921
- 39 Lee, K.B. *et al.* (2002) Protein nanoarrays generated by dip-pen nanolithography. *Science* 295, 1702–1705
- 40 He, M. *et al.* (2008) *In situ* synthesis of protein arrays. *Curr. Opin. Biotechnol.* 19, 4–9
- 41 Dikmans, A. *et al.* (2006) SC<sup>2</sup>: a novel process for manufacturing multi-purpose high-density chemical microarrays. *QSAR Comb. Sci.* 25, 1069–1080
- 42 Beyer, M. *et al.* (2007) Combinatorial synthesis of peptide arrays onto a microchip. *Science* 318, 1888
- 43 Stadler, V. *et al.* (2008) Combinatorial synthesis of peptide arrays with a laser printer. *Angew. Chem. Int. Ed.* 47, 7132–7135
- 44 Schirrmann, T. *et al.* (2008) Production systems for recombinant antibodies. *Front. Biosci.* 13, 4576–4594
- 45 Moutel, S. *et al.* (2009) A multi-Fc-species system for recombinant antibody production. *BMC Biotechnol.* 9, 14
- 46 Thie, H. *et al.* (2009) Multimerization domains for antibody phage display and antibody production. *N. Biotechnol.* 26, 314–321
- 47 Kovaleva, M. (2006) Abrogation of viral interleukin-6 (vIL-6)-induced signaling by intracellular retention and neutralization of vIL-6 with an anti-vIL-6 single-chain antibody selected by phage display. *J. Virol.* 80, 8510–8520
- 48 Strebe, N. *et al.* (2009) Functional knockdown of VCAM1 at the posttranslational level with ER retained antibodies. *J. Immunol. Methods* 341, 30–40
- 49 Böldicke, T. (2007) Blocking translocation of cell surface molecules from the ER to the cell surface by intracellular antibodies targeted to the ER. *J. Cell Mol. Med.* 11, 54–70
- 50 Rizk, S.S. *et al.* (2009) An engineered substance P variant for receptor-mediated delivery of synthetic antibodies into tumor cells. *Proc. Natl. Acad. Sci. U. S. A.* 106, 11011–11015
- 51 Kirsch, M.I. *et al.* (2008) Development of human antibody fragments using antibody phage display for the detection and diagnosis of Venezuelan equine encephalitis virus (VEEV). *BMC Biotechnol.* 8, 66
- 52 Nizak, C. *et al.* (2003) Recombinant antibodies to the small GTPase Rab6 as conformation sensors. *Science* 300, 984–987
- 53 Schütte, M. *et al.* (2009) Identification of a putative Crf splice variant and generation of recombinant antibodies for the specific detection of *Aspergillus fumigatus*. *PLoS ONE* 4, e6625
- 54 Reichert, J.M. (2001) Monoclonal antibodies in the clinic. *Nat. Biotechnol.* 19, 819–822
- 55 Reichert, J.M. (2009) Global antibody development trends. *mAbs* 1, 86–87
- 56 Hust, M and Dübel, S. (2004) Mating antibody phage display to proteomics. *Trends Biotechnol.* 22, 8–14
- 57 Tauszig, M.J. *et al.* (2007) ProteomeBinders: planning a European resource of affinity reagents for analysis of the human proteome. *Nat. Methods* 4, 13–17
- 58 Ohara, R. *et al.* (2006) Antibodies for proteomic research: comparison of traditional immunization with recombinant antibody technology. *Proteomics* 6, 2638–2646
- 59 Edwards, A. (2009) Large-scale structural biology of the human proteome. *Annu. Rev. Biochem.* 78, 541–568
- 60 Pershad, K. *et al.* (2010) Generating a panel of highly specific antibodies to 20 human SH2 domains by phage display. *Protein Eng. Des. Sel.* 23, 279–288
- 61 Björling, E. and Uhlén, M. (2008) Antibodypedia – a portal for sharing antibody and antigen validation data. *Mol. Cell Proteomics* 7, 2028–2037
- 62 Nilsson, P. *et al.* (2005) Towards a human proteome atlas: high-throughput generation of mono-specific antibodies for tissue profiling. *Proteomics* 5, 4327–4337
- 63 Gloriam, D.E. *et al.* (2010) A community standard format for the representation of protein affinity reagents. *Mol. Cell Proteomics* 9, 1–10
- 64 Turunen, L. *et al.* (2009) Automated panning and screening procedure on microplates for antibody generation from phage display libraries. *J. Biomol. Screen.* 14, 282–293
- 65 Braunagel, M. and Das, R. (2007) Intellectual property issues. In *Handbook of Therapeutic Antibodies* (Vol. 1) (Dübel, S., ed.), In pp. 301–322, Wiley-VCH
- 66 Storz, U. (2010) IP issues in the therapeutic antibody industry. In *Antibody Engineering* (Vol. 1) Kontermann, R. and Dübel, S., eds In pp. 517–581, Springer Verlag