



Review

Phage display antibodies for diagnostic applications



Nur Hidayah Hairul Bahara^a, Gee Jun Tye^a, Yee Siew Choong^a, Eugene Boon Beng Ong^a, Asma Ismail^{a,b}, Theam Soon Lim^{a,*}

^a Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

^b Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia

ARTICLE INFO

Article history:

Received 14 February 2013

Received in revised form

29 March 2013

Accepted 2 April 2013

Keywords:

Phage display

Antibodies

Diagnostics

scFv

ABSTRACT

With major developments in molecular biology, numerous display technologies have been successfully introduced for recombinant antibody production. Even so, phage display still remains the gold standard for recombinant antibody production. Its success is mainly attributed to the robust nature of phage particles allowing for automation and adaptation to modifications. The generation of monospecific binders provides a vital tool for diagnostics at a lower cost and higher efficiency. The flexibility to modify recombinant antibodies allows great applicability to various platforms for use. This review presents phage display technology, application and modifications of recombinant antibodies for diagnostics.

© 2013 The International Alliance for Biological Standardization. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Hybridoma technology was the main corner stone for mono-specific antibody production. The production of monoclonal antibodies using the hybridoma technology involves the fusion of an immortal myeloma cell with an antibody-producing spleen cell [1]. The resulting hybrid will contain the features of both cells maintaining immortality and antibody production. However, the use of hybridoma technology to produce monoclonal antibodies has several setbacks (Table 1) [2]. The advent of recombinant DNA technology set about the evolution of antibody generation methods. Recombinant antibody generation was realized with the introduction of *in vitro* display methods.

In vitro display methods such as phage display were introduced as a major alternative for the generation of recombinant human monoclonal antibodies [3,4]. It is an *in vitro* process that is independent of any regulation by the immune system. The most common phage display method used employs the use of filamentous bacteriophage M13 [5]. The ability of a bacteriophage to present a recombinant target on its surface was evident with the pioneering work by George Smith with peptides. In his work, genes encoding the peptides were fused to the minor coat protein pIII. After successful packaging of phage particles harboring the peptide gene sequence, the peptide will be expressed as a fusion peptide to the

pIII coat protein [6]. Uniquely, each phage particle will present a single peptide based on the genes encoded in the system and this information is easily retrieved via a physical linkage between phenotype and genotype. Antibody presentation on phage surface is carried out in similar fashion with the antibody gene fused to the pIII gene of the filamentous phage for a phenotypic representation of the antibody protein as a fusion to the coat protein III [7,8].

Traditionally antibodies are represented graphically as a Y-shape structure (Fig. 1) with two identical ends [9]. At each end, a heavy chain is linked via interchain disulphide bonds to a light chain to generate the Fragment Antigen Binding (Fab) [10]. Within the Fab structure, the binding pockets of the antibody are made up of the variable light and variable heavy domains [11]. The introduction of recombinant antibodies via phage display has also introduced a plethora of new antibody formats [12]. Newer formats such as human domain antibodies, camelid domain antibodies [13], single domain shark antibodies [14], single chain fragment variable (scFv) [15], tandem scFv, diabody, tetrabody, minibody and single chain fragment antigen binding have been introduced [16–20]. The introduction of newer formats allowed researchers to overcome certain limitations imposed by the folding machinery of *Escherichia coli* [21]. It is this reason why smaller fragments such as domain antibodies, scFv and Fab have been routinely used for phage display [18,22].

Other than phage display, a number of new display technologies have also been introduced to circumvent the challenge of antibody generation. Display technologies such as yeast, ribosome, puromycin-based plasmid, bacterial, baculovirus and CIS display

* Corresponding author. Tel.: +604 653 4801; fax: +604 653 4803.

E-mail address: theamsoon@usm.my (T.S. Lim).

Table 1

Comparison of monoclonal antibody production using conventional hybridoma technology and phage display technology.

Features	Hybridoma technology	Phage display technology
Animal host	Required	Not required
Up-scaling	By intraperitoneal method	Bacterial expression system
Attachments-tags, enzymes, etc.	Chemicals	Biochemicals and chemical means
Antibodies against toxins	Not possible	Possible
Antibodies against conserved antigens	Not possible	Possible
Affinity maturation	<i>In vivo</i>	<i>In vitro</i>
Therapeutic application limitation	Human anti-mouse antibody reaction	None
Selection conditions	Little control over antibody produced	Precisely control under manipulation
Engineering	Not possible	Gene encoding antibody is cloned simultaneously with selection

have been used successfully to generate recombinant antibodies [23–33]. The three main display methods commonly used for antibody generation are phage, yeast and ribosome display. The fundamental principle of all these display technologies remains the same whereby the physical linkage between genotype and phenotype is imperative.

In ribosome display, the antibody is transcribed and translated *in vitro* using prokaryotic [34,35] or eukaryotic cell-free expression systems [36,37]. A ribosome display system requires an environment whereby magnesium ion concentration is elevated, temperature is low and the design of the plasmid is without a stop codon to function optimally. The combination of this allows the ribosome to slow down at the end of the mRNA while the emerging processed antibody is folded and presents itself outside of the ribosome tunnel for selection. A major advantage of ribosome display is the *in vitro* phenotype–genotype coupling and amplification that allows easier handling of larger libraries as opposed to phage display [38]. Large libraries can be generated rapidly in ribosome display because the transformation of huge numbers of plasmids into a host is no longer required [34]. This system allows easy mimicry of the affinity maturation process by permitting mutations to be introduced at every round of selection because a polymerase chain reaction (PCR) step is required rather than *in vivo* amplification [39–41]. Although ribosome display could circumvent the drawbacks of library generation associated to *in vitro* display methods, it also has some drawbacks [34]. Some problems associated with ribosome display are due to the meticulous handling that is required as mRNA in the ribosomal complexes is easily degradable by RNase. Premature termination of protein translation as a consequence possibly of ribosome stalling prior to the end of the

mRNA is another setback of the method [34,40]. In ribosome display, dissociation of the synthesized polypeptide to the ribosomal complex reduces the experimental success rate because of the subsequent loss of genotype and phenotype coupling. This makes ribosome display rather challenging for the general experimenter.

Yeast display technology displays antibody formats on *Saccharomyces cerevisiae* by presenting the antibody as a fusion protein to the agglutinin adhesion receptor complex (Aga1 and Aga2) [42]. The Aga2p protein functions to mediate cell–cell interaction during yeast cell mating. Presentation of antibody proteins as Aga2p fusions allows proper presentation of the antibodies away from the cell surface and minimizes potential interaction with other molecules on the yeast cell wall [65]. The main advantage of this platform lies in the high eukaryotic expression and processing mechanism, the assurance of affinity and stability maturation from secretion, minimal avidity effects and the gap repair ability of yeast. *S. cerevisiae* gap repair is an endogenous recombination process that allows the insertion of genes in plasmids at exact sites without the need of restriction enzymes. This allows easy library generation and affinity enrichment of CDR regions [43]. The major advantage is the coupling of Fluorescent-activated Cell Sorting (FACS) for yeast display panning to monitor antibody expression or antibody–antigen-binding efficiency with fluorescent labeled antigens. The main pitfall of this technology is the smaller size of antibody libraries used due to the confines of yeast transformation efficiencies [24,44]. Another problem worth noting is the loss of protein function after expression in soluble form as was reported with the selection of mammalian calmodulin (CaM) protein antibodies. The loss of the binding ability was suggested to be due to the contribution that Aga2 has to the binding when fused with the antibody molecule. Therefore when the antibody is expressed in soluble form without Aga2, the antibody would not be able to bind to the antigen with similar efficiency [45].

In comparison to *in vitro* display methods, the conventional hybridoma method has some disadvantages which makes it unattractive for antibody generation [46]. The time required to generate recombinant antibodies using display methods is relatively shorter than the conventional hybridoma method that could span up to months. The choice for display methods as an alternative is further enhanced by the ability to precisely control and manipulate selection conditions for antibodies-specific traits. The flexibility of display methods also helps to overcome the difficulty of generating anti-self antibodies due to tolerance. This is possible because the library generation process allows combinations of heavy and light chain that normally does not exist in nature to be presented as well [47]. Another attractive feature of recombinant antibodies is the introduction of selectivity during the cloning process. Affinity maturation of selected antibodies is possible with *in vitro* methods to generate antibodies ranging from picomolar to femtomolar affinity [30,48–50]. The degree of freedom on offer for researchers by phage display antibody technology has led to it gaining popularity

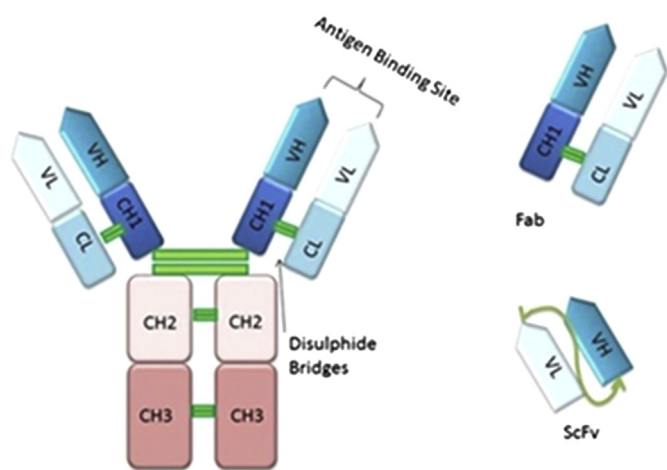


Fig. 1. Illustrations depicting the basic Y shape of an immunoglobulin and smaller antibody derivatives commonly used for phage display, Fab (Fragment antigen binding) and scFv (single chain fragment variable).

for diagnostic applications [51–54]. Although with various technological platforms being introduced, phage display still remains the preferred choice for recombinant antibody generation due to the robustness and stability of the method coupled with the flexibility to incorporate conditions favored by the user [55].

2. Phage display of antibody libraries

The success of any phage display experiment to yield good quality antibodies is heavily dependent on the collection of antibodies presented. The collection or library of antibodies is a collection of antibody variable genes that are cloned as a fusion to the phage coat protein with library sizes ranging from 10^6 to 10^{10} depending on the type of library generated [56]. Antibody libraries are constructed by obtaining genes encoding antibodies from B-cells via PCR amplification with a set of specific primers to obtain substantial amount of DNA for cloning [57,58]. The diversity of these genes is derived from natural *in vivo* processes such as V(D)J recombination. The source of antibody genes can be categorized based on the origin of antibody sequences being either natural or synthetic. Natural source repertoire libraries can yield either naïve or immunized libraries [59–61]. The selection of which antibody isotype to harvest is dependent on the application of the library. Synthetic source repertoires are constructed entirely *in vitro* and is devoid of natural biases and redundancies of *in vivo* antibody repertoires that allows the control of genetic information and introduction of diversity [61–63].

An immunized library derives the antibody genes from patients that had prior exposure to a certain disease or infection and recovered. The repertoire is from activated B-cells where the diversity has undergone *in vivo* maturation where the natural antibodies that have reacted towards the infection are harvested from the B-cells [62]. The Ig isotypes normally used for immune library generation is IgG [64] but other isotype libraries like IgA and IgE have been generated with reference to disease pathogenesis such as coeliac disease [65,66] and latex allergy [67] patients respectively. Therefore this increases the possibility to obtain antibodies against antigens of these conditions, as the repertoire is bias towards the particular disease antigen. The size of an immunized library need not be as high as a naïve library due to the natural selection of antibodies for the given condition by the immune system [62]. Therefore, two main characteristics of an immunized library are the enriched population of antigen-specific antibodies and high affinity antibodies as some of these antibodies would have underwent affinity maturation [62,68]. The obvious complication to generate such libraries is the availability of B-cells as it is impossible to immunize humans. Some other setbacks are such as the time required for recovery after exposure, tolerance to toxicity of certain antigens, lack of immune response to self-antigens and most of all the need to generate a new library for a new disease [64].

For the construction of naïve antibody libraries, IgM mRNA of B-cells from healthy donors is normally used [62]. There have been cases whereby IgD, a subset of Ig family expressed jointly with IgM on B-cells has been used for naïve antibody library generation [69–71]. The B-cells are normally isolated from peripheral blood lymphocytes [51], bone marrow or tonsils [72]. Due to the non-specificity of the antibodies in naïve populations, a higher library size is commonly required for an increase chance of obtaining higher affinity antibodies and improves success rates of selection against a multitude of antigens [73,74]. The main advantage of libraries with a naïve repertoire is the ability to screen for antibodies against any antigen [75]. Several naïve libraries with an average diversity of 10^{10} have been reported to successfully generate antibodies against various targets ranging from self-, non-immunogenic, toxic antigens and chemical haptens [72,76]. Antibodies generated from naïve

libraries have a tendency to exhibit lower affinities than the ones obtained from immunized libraries. This can be circumvented by diversification of the V-genes through randomization of the CDRs. The resulting library would have a single framework with variations occurring only in the CDRs. This library is called a semi-synthetic library because the main framework was derived from a naïve library that is subsequently modified *in vitro* to yield antibodies of different affinities [77–79].

The final class of antibody library is the synthetic library. Synthetic antibody libraries are designed bottom up from information-generated bioinformatically regarding antibody epitopes [80], antibody–protein interactions [81], affinity maturation design [82], V-gene recombination patterns [83,84] and structural predictions of variable regions [85] to design a synthetic repertoire to be used. Studies on the variable gene repertoires have helped designers to determine amino acid preferences and variability patterns in hypervariable loops [86–88]. Normally a stable antibody framework is used and randomization is carried out at specific CDR regions to generate diversity [89]. Of the three CDRs available, CDR3 of the heavy chain is commonly used for diversification as it has the highest surface interaction with antigens [90]. A lot of effort has been invested in studying the role CDR3 of the heavy chain plays in antigen recognition [91–93]. This brought about the introduction of the H3-Rules, where it governs the substitution of amino acid at specified positions within the CDR3 to produce an enhanced identification of the tertiary structure [94]. Beyond amino acid propensities, the length distribution of CDRs also underwrites the diversity that can be generated [92]. This is not confined to only CDR3 but also other CDRs for the identification of specificity-determining residues [95]. The difference in binding structures of antibodies correlates to the library diversity due to the different folds generated by varying amino acid sequences and length of both chains [96,97]. As antibody antigen interactions are also based on the collective binding of the light and heavy chain, work on light and heavy chain interactions has also assisted designers in designing better antibody structures [98–100]. With all this information coupled with the innovations in mutagenesis and cloning technology, an entire antibody scaffold can be built with a high degree of specificity and accuracy [59,62,101,102].

The obvious advantage of such libraries is the independence from a natural immune repertoire that would allow easy generation of higher diversity libraries for *in vitro* technologies. Synthetic libraries also function as naïve libraries thus allowing the user to screen for multiple targets without the limitations of the immune libraries. Frameworks can be selected based on stability, high expression yield or lack of immunogenicity that is vital for therapeutics [96]. With all the promise in hand, the use of synthetic antibody libraries has not really taken off in comparison to naïve libraries. This can be attributed to the high frequency of unnatural amber stops and glycosylation sites being introduced by randomization, which could hamper the conversion to full IgG molecules at the same time losing specificity after conversion [103]. The cost to generate such libraries is enormous which makes it an inapt choice of library for most researchers. This is evident with the existing synthetic libraries and research originating from the Scripps Research Institute, Medical Research Council, Morphosys AG and Genentech [96].

3. Antibody selection by panning

The *in vitro* selection process of antibodies from antibody libraries based on target affinity is termed as “panning” [104]. The process is an iterative process whereby the population of target-specific antibodies is enriched relative to the number of panning rounds [105]. For the panning process, target antigens are coated on

many different solid phases. Common solid phase used are nitrocellulose, magnetic beads, agarose columns, monolithic columns, polystyrene tubes and 96-well microtitre plates [106].

Fig. 2 shows the illustration of the complete phage display panning process. In general, the target antigens are coated on the surface of the solid phase for presentation and will be incubated with antibody-bearing phage particles from the library. This will be followed by an incubation step to allow binding of antibodies to the antigen. During this time, parameters such as physical, chemical or biological can be regulated [107]. To remove unbound phage particles from bound phage particles, a wash step is required by which stringency can be controlled. Variations in wash strategies can result in the enrichment of different characteristic clones. Finally an elution step is carried out either by enzymatic digestion or pH shift. The eluted phage particles are then reamplified by infection of *E. coli*. At this time, the phage particles can either be repackaged for use in the subsequent panning round or for final analysis.

The identification of bound phage particles will then be carried out after panning. This is usually so after 4–6 rounds of panning. Panning experiments can also be carried out in a high-throughput manner using robotic handlers [108]. Evaluation can either be in soluble form or antibodies or antibody presenting phages. The most convenient method for evaluation is by ELISA [109]. The positive clones will then be sequenced to obtain the genotypic information pertaining to the positive clone. As the genetic information of the clone is now available, modifications to the antibody can be done and produced in different hosts depending on the platform the antibodies will be used in. The availability of the genetic information of the antibodies would also facilitate additional modifications in terms of stability and affinity maturation [110].

The adaptability of the panning process to different solutions, conditions and stress factors allows the generation of antibodies

against a plethora of antigens otherwise impossible to obtain from conventional hybridoma method. The hybridoma method would require the introduction of an infectious agent or toxin to the host animal in order to generate antibodies against them. This is not practical, as the host animal will not survive the infection or toxin to yield the desired antibodies. Using phage display, antibodies against a variety of infectious agents like dengue [111], influenza [112], Ebola [113], anthrax [114] and others [115] have been successfully generated. In some instances, the whole virus particles are used for panning hence generating antibodies against the virus envelope proteins rather than the host cell proteins. The recombinant method also allows the generation of antibodies against membrane proteins that requires the presence of detergents or a membrane to maintain its native conformation. This is not likely in animals, as the denaturing effects of the serum environment will destroy the native conformations of the proteins. The instability of protein conformations from immunized animals makes generating antibodies against specific protein conformations a challenge. Modifications to panning strategies such as negative selections or panning with active cell membrane receptors have been used to generate conformation-specific antibodies [116,117]. The ability to control and regulate the panning process is very useful to generate highly specific antibodies against proteins that are highly similar in sequence with little variation that is not possible by immunization [118–121]. Post-translational modification such as sulfotyrosine is a common modification that effects about 30% of all secretory and membrane proteins [122]. As such a modification is rather typical in nature making antibodies against sulfotyrosine using immunization very challenging if not an impossible task. However, phage display was successfully used to generate antibodies against sulfotyrosine-modified proteins [123,124].

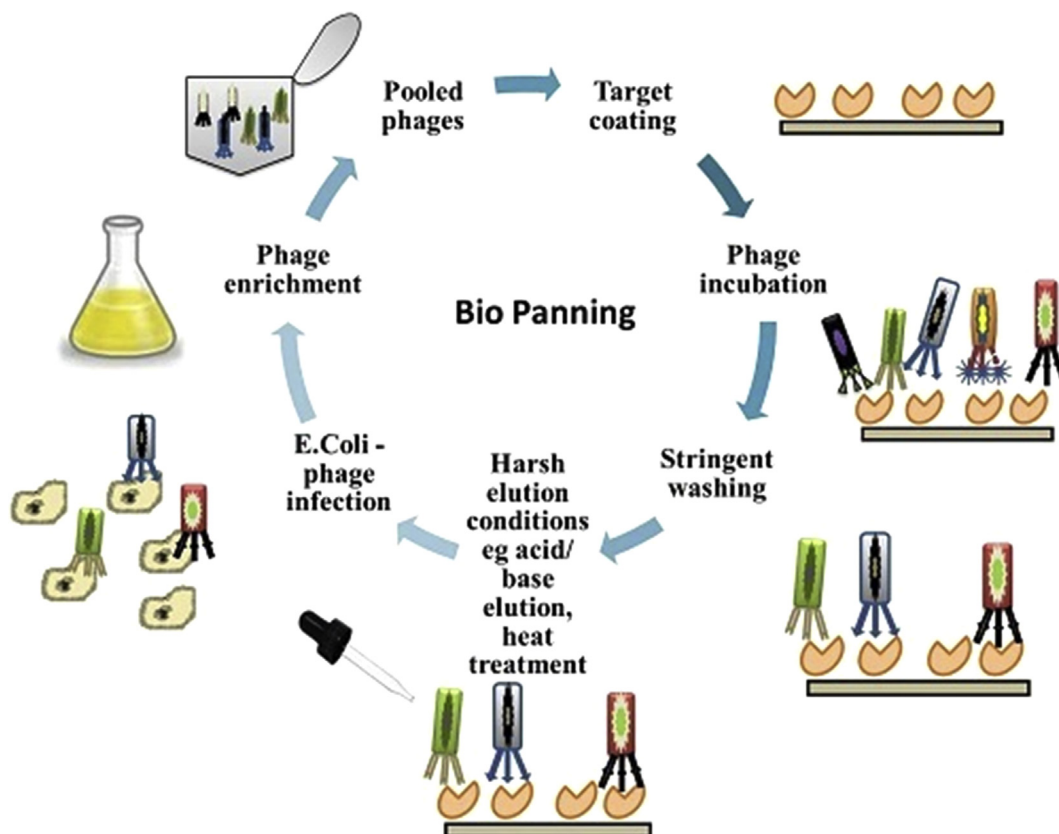


Fig. 2. Illustration showing an overview of the processes involved in selection of antibodies by phage display panning.

4. Modifications of antibodies for diagnostics

The application of phage display-derived antibodies for diagnostics will allow the design of different forms of diagnostics. Depending on the need, test kits are designed according to different mechanisms of interaction. The main benefit of using recombinant antibodies for diagnostics is the freedom to modify the antibodies. The vigorous nature of recombinant antibodies has seen an influx of applications applying them. There are many different platforms that have successfully applied recombinant antibodies for diagnostics. Diagnostic platforms such as lateral flow strips [125], bead-based assays such as Luminex [126], flow cytometry [127], proximity ligation assay [128], molecular imaging [129], resonance technology [130–132], microfluidics [133,134] and more recently DNAzyme [135,136] have benefited from phage display-derived antibodies. With the advent of molecular biology, many new modifications can be carried out for diagnostic applications.

4.1. Affinity maturation

Naturally, antibodies produced by B lymphocytes normally have undergone an affinity maturation process. The affinity maturation process *in vivo* can be carried out via two mechanisms. Somatic hypermutation occurs in the CDR genes of the immunoglobulin [137]. Second is clonal selection in which B-cells that have undergone SHM will compete for limited availability of antigen for expansion [138]. In recombinant antibody technology, affinity maturation is carried out *in vitro*. The two main principle strategy for *in vitro* affinity maturation of either engineered or phage selected antibodies lies in the site-directed mutagenesis in selected CDR and framework regions as well as selection process wherein, error prone PCR, chemical mutagens and radiation are the main source for random mutation [139,140]. This method is especially being utilized in the optimization of antibody fragment. Depending on the design of the developed assay, antibodies of different affinities could be preferred. Therefore, the ability to modify antibody affinities via *in vitro* methods would allow freedom for antibody customization for diagnostic applications.

4.2. Protein and enzyme fusions

One of the key success factors for the application of antibody phage display is the synergy with high-throughput screening methods. Molecular modifications of recombinant antibodies allow the generation of antibody–protein and antibody–enzyme fusion. These fusion constructs offer the advantage of signal amplification [12]. Some common conversions of selected antibody clones are fusion with enzymes such as alkaline phosphatase and horseradish peroxidase to facilitate the signal development [141]. This method allows direct detection with substrate without the need of chemical conjugation processes that will be more cost effective to produce with higher yield and reproducibility. Besides enzyme fusion, proteins are also efficiently fused to antibody. The most common are fluorescent protein fusions such as the fluorobodies that has been widely used for recombinant antibody detection, as it is a direct and rapid without the need for invasive chemical imaging probe. These antibodies can also be applied for fluorescent microscopy [142,143], cell sorting [144,145] and even for immunohisto-pathology [146].

4.3. Peptide tags

Peptide tags are constantly used in molecular biology especially in recombinant protein expression systems mainly for purification [147]. The same can be said for its use in phage display antibodies

whereby peptide tags such as the 6xhistidine-tag is used to allow easy purification and detection of antibodies [148,149]. This is vital for the production process of diagnostic kits as antibodies of good yield and purity are required. Even so, other peptide tags such as the Myc-tag have been extensively used as a method for signal development [150,151]. Another application related to diagnostic is the use of SNAP-tag antibodies [152–154] for covalent attachment of antibodies to solid phase. It is also possible to introduce a specific peptide sequence such as the AVI-tag to initiate *in vitro* enzymatic biotinylation of Fab fragment *in vitro* using biotin ligase, biotin, and ATP [155–157]. The construct exhibited uniform antigen-binding affinity which was used in a competitive immunoassay [158]. The introduction of various peptide tags for purification, attachment or signal development is only possible using recombinant antibodies.

4.4. DNA functionalization

DNA directed functionalization has been recently applied to fabricate the protein DNA hybrids. Such technology has been extensively study to serve in the development of antibody arrays. Single stranded DNA-tagged immunoglobulin can be immobilized onto the gold surface that carries complementary oligonucleotides. This work utilizes the concept of oligonucleotide complementary captures by means of highly specific Watson–Crick base pairing onto the gold surface [159]. The application of DNA functionalized antibodies lies in the detection method assay such as dot-blot assay wherein, generation of antibody–DNA hybrid molecules to target specific antigens for easy detection [160]. On the same note, microspot array is also another method, which combines protein biomarker detection with nucleic acid diagnostics. The advantage of combining both methods is that probes can be designed base on target due to hybridization of DNA [161]. The attachment of DNA molecules would also aid in terms of signal amplification. For higher sensitivity innovative methods such as immuno-PCR and immuno-RCA have been successfully applied for diagnostics [162–164]. Both methods apply the same concept whereby amplification of the attached DNA molecules to increase the amount of signal generation by DNA chelating dyes. Immuno-PCR uses the conventional polymerase chain reaction for DNA multiplication whereby Immuno-RCA uses isothermal rolling circle amplification for DNA increment [165].

5. Conclusion

The current diagnostic landscape is becoming more challenging especially for the diagnostics of infectious diseases with the occurrence of emerging and re-emerging infectious diseases. This increases the urgency to produce quality antibodies for diagnostics as a startling percentage of commercial antibodies show poor specificity, fail to recognize their targets or are unavailable all together [46]. The speed at which new genomic and proteomic technologies are churning out new biomarkers has made it a tall order for antibody producers to play catch up. This increase has basically put hybridoma methods out of contention to fulfill the required demand. Therefore the emergence of *in vitro* methods such as phage display provides an avenue to fulfill the demands of the diagnostic community for new and better antibodies. Together with the introduction of new technologies like next generation sequencing, robotics and nanotechnology, high-throughput screening of phage display libraries for rapid antibody generation is now a reality. Moreover, new molecular-based techniques for library generation and panning strategies will set the tone for the constant improvement of phage display in antibody generation for diagnostics. With the increasing discoveries of genomics and proteomics derived biomarkers, phage display antibodies are

undoubtedly positioned to play a bigger role in the future of diagnostics.

Acknowledgments

The authors would like to acknowledge support by the Malaysian Ministry of Higher Education through the Higher Institution Centre of Excellence (HiCoE) Grant (Grant No. 311/CIPPM/44001005) and Research University Cluster Grant (Grant No. 1001/PSKBP/8630015).

References

- [1] Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495–7.
- [2] Schirrmann T, Meyer T, Schütte M, Frenzel A, Hust M. Phage display for the generation of antibodies for proteome research, diagnostics and therapy. *Molecules* 2011;16:412–26.
- [3] Barbas CF, Kang AS, Lerner RA, Benkovic SJ. Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. *Proc Natl Acad Sci* 1991;88:7978–82.
- [4] Silacci M, Brack S, Schirru G, Märlind J, Ettorre A, Merlo A, et al. Design, construction, and characterization of a large synthetic human antibody phage display library. *Proteomics* 2005;5:2340–50.
- [5] Barbas SM, Barbas CF. Filamentous phage display. *Fibrinolysis* 1994;8(Suppl. 1):245–52.
- [6] Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science (New York, NY)* 1985;228:1315.
- [7] Winter G, Griffiths AD, Hawkins RE, Hoogenboom HR. Making antibodies by phage display technology. *Annu Rev Immunol* 1994;12:433–55.
- [8] Kay BK, Kasanov J, Yamabhai M. Screening phage-displayed combinatorial peptide libraries. *Methods* 2001;24:240–6.
- [9] Wood PJ. Understanding immunology. Prentice Hall; 2006.
- [10] Rader C, Barbas CF. Phage display of combinatorial antibody libraries. *Curr Opin Biotechnol* 1997;8:503–8.
- [11] Huston JS, Margolies MN, Haber E. Antibody binding sites. *Adv Protein Chem* 1996;49:329–450.
- [12] Hudson PJ. Recombinant antibody fragments. *Curr Opin Biotechnol* 1998;9:395–402.
- [13] Harmsen M, De Haard H. Properties, production, and applications of camelid single-domain antibody fragments. *Appl Microbiol Biotechnol* 2007;77:13–22.
- [14] Dooley H, Flajnik MF, Porter AJ. Selection and characterization of naturally occurring single-domain (IgNAR) antibody fragments from immunized sharks by phage display. *Mol Immunol* 2003;40:25–33.
- [15] Boel E, Verlaan S, Poppelier MJG, Westerdal NAC, Van Strijp JAG, Logtenberg T. Functional human monoclonal antibodies of all isotypes constructed from phage display library-derived single-chain Fv antibody fragments. *J Immunol Methods* 2000;239:153–66.
- [16] Little M, Kipriyanov S, Le Gall F, Moldenhauer G. Of mice and men: hybridoma and recombinant antibodies. *Immunol Today* 2000;21:364–70.
- [17] Andris-Widhopf J, Rader C, Steinberger P, Fuller R, Barbas III CF. Methods for the generation of chicken monoclonal antibody fragments by phage display. *J Immunol Methods* 2000;242:159–81.
- [18] Holt LJ, Herring C, Jespers LS, Woolven BP, Tomlinson IM. Domain antibodies: proteins for therapy. *Trends Biotechnol* 2003;21:484–90.
- [19] Hussack G, Keklikian A, Alsughayyir J, Hanifi-Moghaddam P, Arbabi-Gahroudi M, van Faassen H, et al. A VL single-domain antibody library shows a high-propensity to yield non-aggregating binders. *Protein Eng Des Sel* 2012.
- [20] Hust M, Jostock T, Menzel C, Voedisch B, Mohr A, Brenneis M, et al. Single chain Fab (scFab) fragment. *BMC Biotechnol* 2007;7:14.
- [21] Holliger P, Hudson PJ. Engineered antibody fragments and the rise of single domains. *Nat Biotechnol* 2005;23:1126–36.
- [22] Dudgeon K, Famm K, Christ D. Sequence determinants of protein aggregation in human VH domains. *Protein Eng Des Sel* 2009;22:217–20.
- [23] Chao G, Lau WL, Hackel BJ, Szazinsky SL, Lippow SM, Wittup KD. Isolating and engineering human antibodies using yeast surface display. *Nat Protoc* 2006;1:755–68.
- [24] Feldhaus MJ, Siegel RW. Yeast display of antibody fragments: a discovery and characterization platform. *J Immunol Methods* 2004;290:69–80.
- [25] Figueiredo P, Roberts RL, Nester EW. DARTs: a DNA-based in vitro polypeptide display technology. *Proteomics* 2004;4:3128–40.
- [26] Matsumura N, Yanagawa H. Recent progress and future prospects in protein display technologies as tools for proteomics. *Curr Proteomics* 2006;3:199–215.
- [27] Grabherr R, Ernst W, Oker-Blom C, Jones I. Developments in the use of baculoviruses for the surface display of complex eukaryotic proteins. *Trends Biotechnol* 2001;19:231–6.
- [28] Ojala K, Koski J, Ernst W, Grabherr R, Jones I, Oker-Blom C. Improved display of synthetic IgG-binding domains on the baculovirus surface. *Technol Cancer Res Treat* 2004;3:77.
- [29] Rothe A, Hosse RJ, Power BE. In vitro display technologies reveal novel biopharmaceuticals. *FASEB J* 2006;20:1599–610.
- [30] Hudson PJ, Souriau C. Engineered antibodies. *Nat Med* 2003;9:129–34.
- [31] Daugherty PS. Protein engineering with bacterial display. *Curr Opin Struct Biol* 2007;17:474–80.
- [32] Samuelson P, Gunneriusson E, Nygren P-Å, Ståhl S. Display of proteins on bacteria. *J Biotechnol* 2002;96:129–54.
- [33] Zahnd C, Amstutz P, Plückthun A. Ribosome display: selecting and evolving proteins in vitro that specifically bind to a target. *Nat Methods* 2007;4:269–79.
- [34] Schaffitzel C, Hanes J, Jermutus L, Plückthun A. Ribosome display: an in vitro method for selection and evolution of antibodies from libraries. *J Immunol Methods* 1999;231:119–35.
- [35] Amstutz P, Forrer P, Zahnd C, Plückthun A. In vitro display technologies: novel developments and applications. *Curr Opin Biotechnol* 2001;12:400–5.
- [36] Katzen F, Chang G, Kudlicki W. The past, present and future of cell-free protein synthesis. *Trends Biotechnol* 2005;23:150–6.
- [37] He M, Taussig MJ. Ribosome display of antibodies: expression, specificity and recovery in a eukaryotic system. *J Immunol Methods* 2005;297:73–82.
- [38] Dufner P, Jermutus L, Minter RR. Harnessing phage and ribosome display for antibody optimisation. *Trends Biotechnol* 2006;24:523–9.
- [39] Yan X, Xu Z. Ribosome-display technology: applications for directed evolution of functional proteins. *Drug Discov Today* 2006;11:911–6.
- [40] Hanes J, Jermutus L, Weber-Bornhauser S, Bosshard HR, Plückthun A. Ribosome display efficiently selects and evolves high-affinity antibodies in vitro from immune libraries. *Proc Natl Acad Sci* 1998;95:14130–5.
- [41] Irving RA, Coia G, Roberts A, Nuttall SD, Hudson PJ. Ribosome display and affinity maturation: from antibodies to single V-domains and steps towards cancer therapeutics. *J Immunol Methods* 2001;248:31–45.
- [42] Feldhaus MJ, Siegel RW, Opreko LK, Coleman JR, Feldhaus JMW, Yeung YA, et al. Flow-cytometric isolation of human antibodies from a nonimmune *Saccharomyces cerevisiae* surface display library. *Nat Biotechnol* 2003;21:163–70.
- [43] Weaver-Feldhaus JM, Lou J, Coleman JR, Siegel RW, Marks JD, Feldhaus MJ. Yeast mating for combinatorial Fab library generation and surface display. *FEBS Lett* 2004;564:24–34.
- [44] Boder ET, Wittup KD. Yeast surface display for screening combinatorial polypeptide libraries. *Nat Biotechnol* 1997;15:553–7.
- [45] Weaver-Feldhaus JM, Miller KD, Feldhaus MJ, Siegel RW. Directed evolution for the development of conformation-specific affinity reagents using yeast display. *Protein Eng Des Sel* 2005;18:527–36.
- [46] Geyer CR, McCafferty J, Dübel S, Bradbury A, Sidhu SS. Recombinant antibodies and in vitro selection technologies. *Methods Mol Biol (Clifton, NJ)* 2012;901:11.
- [47] Sblattero D, Lou J, Marzari R, Bradbury A. In vivo recombination as a tool to generate molecular diversity in phage antibody libraries. *Rev Mol Biotechnol* 2001;74:303–15.
- [48] Pansri P, Jarusarane N, Rangnoi K, Kristensen P, Yamabhai M. A compact phage display human scFv library for selection of antibodies to a wide variety of antigens. *BMC Biotechnol* 2009;9:6.
- [49] Shi L, Wheeler JC, Sweet RW, Lu J, Luo J, Tornetta M, et al. De novo selection of high-affinity antibodies from synthetic Fab libraries displayed on phage as pIX fusion proteins. *J Mol Biol* 2010;397:385–96.
- [50] Yang W-P, Green K, Pinz-Sweeney S, Briones AT, Burton DR, Barbas CF. CDR walking mutagenesis for the affinity maturation of a potent human anti-HIV-1 antibody into the picomolar range. *J Mol Biol* 1995;254:392–403.
- [51] Marks JD, Hoogenboom HR, Bonnett TP, McCafferty J, Griffiths AD, Winter G. By-passing immunization: human antibodies from V-gene libraries displayed on phage. *J Mol Biol* 1991;222:581–97.
- [52] Siegel D. Recombinant monoclonal antibody technology. *Transfus Clin Biol* 2002;9:15–22.
- [53] Ohara R, Knappik A, Shimada K, Frisch C, Ylera F, Koga H. Antibodies for proteomic research: comparison of traditional immunization with recombinant antibody technology. *Proteomics* 2006;6:2638–46.
- [54] Holt LJ, Enever C, de Wildt RMT, Tomlinson IM. The use of recombinant antibodies in proteomics. *Curr Opin Biotechnol* 2000;11:445–9.
- [55] Smothers JF, Henikoff S, Carter P. Affinity selection from biological libraries. *Science* 2002;298:621–2.
- [56] Christ D, Famm K, Winter G. Tapping diversity lost in transformations — in vitro amplification of ligation reactions. *Nucleic Acids Res* 2006;34:e108.
- [57] Sidhu SS, Fellouse FA. Synthetic therapeutic antibodies. *Nat Chem Biol* 2006;2:682–8.
- [58] Marks C, Marks JD. Phage libraries — a new route to clinically useful antibodies. *N Engl J Med* 1996;335:730–3.
- [59] Mondon P, Dubreuil O, Bouayadi K, Kharrat H. Human antibody libraries: a race to engineer and explore a larger diversity. *Front Biosci* 2008;13:1117–29.
- [60] Hoogenboom HR. Selecting and screening recombinant antibody libraries. *Nat Biotechnol* 2005;23:1105–16.
- [61] Watkins NA, Ouweland WH. Introduction to antibody engineering and phage display. *Vox Sang* 2000;78:72–9.
- [62] Sidhu SS. Phage display in biotechnology and drug discovery. *CRC*; 2005.
- [63] Iba Y, Kurosawa Y. Comparison of strategies for the construction of libraries of artificial antibodies. *Immunol Cell Biol* 1997;75:217–21.

- [64] Azzazy HME, Highsmith Jr WE. Phage display technology: clinical applications and recent innovations. *Clin Biochem* 2002;35:425–45.
- [65] Sblattero D, Florian F, Azzoni E, Ziberna F, Tommasini A, Not T, et al. One-step cloning of anti tissue transglutaminase scFv from subjects with celiac disease. *J Autoimmun* 2004;22:65–72.
- [66] Rhyner C, Weichel M, Hübner P, Achatz G, Blaser K, Cramer R. Phage display of human antibodies from a patient suffering from coeliac disease and selection of isotype-specific scFv against gliadin. *Immunology* 2003;110:269–74.
- [67] Laukkanen M-L, Mäkinen-Kiljunen S, Isoherranen K, Haahtela T, Söderlund H, Takkinen K. Hevein-specific recombinant IgE antibodies from human single-chain antibody phage display libraries. *J Immunol Methods* 2003;278:271–81.
- [68] Clackson T, Hoogenboom HR, Griffiths AD, Winter G. Making antibody fragments using phage display libraries. *Nature* 1991;352:624–8.
- [69] Geisberger R, Lamers M, Achatz G. The riddle of the dual expression of IgM and IgD. *Immunology* 2006;118:429–37.
- [70] Schroeder Jr HW, Cavacini L. Structure and function of immunoglobulins. *J Allergy Clin Immunol* 2010;125:S41–52.
- [71] Koelsch K, Zheng N, Zhang Q, Duty A, Helms C, Mathias MD, et al. Mature B cells class switched to IgD are autoreactive in healthy individuals. *J Clin Invest* 2007;117:1558.
- [72] Vaughan TJ, Williams AJ, Pritchard K, Osbourn JK, Pope AR, Earnshaw JC, et al. Human antibodies with sub-nanomolar affinities isolated from a large non-immunized phage display library. *Nat Biotechnol* 1996;14:309–14.
- [73] Benhar I. Biotechnological applications of phage and cell display. *Biotechnol Adv* 2001;19:1–33.
- [74] Marks JD, Bradbury A. Selection of human antibodies from phage display libraries. *Methods Mol Biol* 2004;248:161–76.
- [75] Holt LJ, Büssov K, Walter G, Tomlinson IM. By-passing selection: direct screening for antibody–antigen interactions using protein arrays. *Nucleic Acids Res* 2000;28:e72.
- [76] Brichta J, Hnilova M, Viskovic T. Generation of hapten-specific recombinant antibodies: antibody phage display technology: a review. *Vet Med* 2005;50:231–52.
- [77] Soderlind E, Strandberg L, Jirholt P, Kobayashi N, Alexeiva V, Aberg A-M, et al. Recombining germline-derived CDR sequences for creating diverse single-framework antibody libraries. *Nat Biotechnol* 2000;18:852–6.
- [78] Söderlind E, Ohlin M, Carlsson R. Complementarity-determining region (CDR) implantation: a theme of recombination. *Immunotechnology* 1999;4:279.
- [79] Strachan G, McElhiney J, Drever M, McIntosh F, Lawton L, Porter A. Rapid selection of anti-hapten antibodies isolated from synthetic and semi-synthetic antibody phage display libraries expressed in *Escherichia coli*. *FEMS Microbiol Lett* 2006;210:257–61.
- [80] Ponomarenko J, Bui H-H, Li W, Fusseder N, Bourne PE, Sette A, et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. *BMC Bioinformatics* 2008;9:514.
- [81] Ponomarenko JV, Bourne PE. Antibody–protein interactions: benchmark datasets and prediction tools evaluation. *BMC Struct Biol* 2007;7:64.
- [82] Barderas R, Desmet J, Timmerman P, Meloen R, Casal JI. Affinity maturation of antibodies assisted by in silico modeling. *Proc Natl Acad Sci* 2008;105:9029–34.
- [83] Toseland CP, Clayton DJ, McSparron H, Hemsley SL, Blythe MJ, Paine K, et al. Antigen: a quantitative immunology database integrating functional, thermodynamic, kinetic, biophysical, and cellular data. *Immunome Res* 2005;1:4.
- [84] Volpe JM, Kepler TB. Large-scale analysis of human heavy chain V(D)J recombination patterns. *Immunome Res* 2008;4:3.
- [85] Sircar A, Kim ET, Gray JJ. Rosetta antibody: antibody variable region homology modeling server. *Nucleic Acids Res* 2009;37:W474–9.
- [86] Chothia C, Lesk AM, Gherardi E, Tomlinson IM, Walter G, Marks JD, et al. Structural repertoire of the human VH segments. *J Mol Biol* 1992;227:799–817.
- [87] Tomlinson IM, Walter G, Marks JD, Llewellyn MB, Winter G. The repertoire of human germline VH sequences reveals about fifty groups of VH segments with different hypervariable loops. *J Mol Biol* 1992;227:776–98.
- [88] Tomlinson IM, Cox J, Gherardi E, Lesk A, Chothia C. The structural repertoire of the human V kappa domain. *EMBO J* 1995;14:4628.
- [89] Goletz S, Christensen PA, Kristensen P, Blohm D, Tomlinson I, Winter G, et al. Selection of large diversities of antiidiotypic antibody fragments by phage display. *J Mol Biol* 2002;315:1087–97.
- [90] Tomlinson IM, Walter G, Jones PT, Dear PH, Sonnhammer EL, Winter G. The imprint of somatic hypermutation on the repertoire of human germline V genes. *J Mol Biol* 1996;256:813–7.
- [91] Morea V, Tramontano A, Rustici M, Chothia C, Lesk AM. Conformations of the third hypervariable region in the VH domain of immunoglobulins. *J Mol Biol* 1998;275:269–94.
- [92] Oliva B, Bates PA, Querol E, Avilés FX, Sternberg M. Automated classification of antibody complementarity determining region 3 of the heavy chain (H3) loops into canonical forms and its application to protein structure prediction. *J Mol Biol* 1998;279:1193.
- [93] Bajorath J, Harris L, Novotny J. Conformational similarity and systematic displacement of complementarity determining region loops in high resolution antibody X-ray structures. *J Biol Chem* 1995;270:22081–4.
- [94] Kuroda D, Shirai H, Kobori M, Nakamura H. Structural classification of CDR-H3 revisited: a lesson in antibody modeling. *Proteins* 2008;73:608–20.
- [95] Almagro JC. Identification of differences in the specificity-determining residues of antibodies that recognize antigens of different size: implications for the rational design of antibody repertoires. *J Mol Recognit* 2004;17:132–43.
- [96] Potter M. Structural correlates of immunoglobulin diversity. *Immunol Res* 1983;2:27–42.
- [97] Abergel C, Tipper J, Padlan E. Structural significance of sequence variability in antibody complementarity-determining regions. *Res Immunol* 1994;145:49.
- [98] de Wildt RM, Hoet R, van Venrooij WJ, Tomlinson IM, Winter G. Analysis of heavy and light chain pairings indicates that receptor editing shapes the human antibody repertoire. *J Mol Biol* 1999;285:895–901.
- [99] Kuroda D, Shirai H, Kobori M, Nakamura H. Systematic classification of CDR-L3 in antibodies: implications of the light chain subtypes and the VL–VH interface. *Proteins* 2009;75:139–46.
- [100] Wang N, Smith WF, Miller BR, Aivazian D, Lugovskoy AA, Reff ME, et al. Conserved amino acid networks involved in antibody variable domain interactions. *Proteins* 2009;76:99–114.
- [101] Lim BN, Choong YS, Ismail A, Glöckler J, Konthur Z, Lim TS. Directed evolution of nucleotide-based libraries using lambda exonuclease. *BioTechniques* 2012;53:357.
- [102] Benhar I. Design of synthetic antibody libraries 2007.
- [103] Clementi N, Mancini N, Solfrosi L, Castelli M, Clementi M, Burioni R. Phage display-based strategies for cloning and optimization of monoclonal antibodies directed against human pathogens. *Int J Mol Sci* 2012;13:8273–92.
- [104] Kretzschmar T, von Rüden T. Antibody discovery: phage display. *Curr Opin Biotechnol* 2002;13:598–602.
- [105] Mullen LM, Nair SP, Ward JM, Rycroft AN, Henderson B. Phage display in the study of infectious diseases. *Trends Microbiol* 2006;14:141–7.
- [106] Kontermann RE, Dübel S. Antibody engineering. Springer; 2010.
- [107] Lee CMY, Iorno N, Sierro F, Christ D. Selection of human antibody fragments by phage display. *Nat Protoc* 2007;2:3001–8.
- [108] Konthur Z, Walter G. Automation of phage display for high-throughput antibody development. *Targets* 2002;1:30–6.
- [109] Walter G, Konthur Z, Lehrach H. High-throughput screening of surface displayed gene products. *Chem High T Scr* 2001;4:193–205.
- [110] Pini A, Bracci L. Phage display of antibody fragments. *Curr Protein Pept Sci* 2000;1:155–69.
- [111] Cabezas S, Rojas G, Pavan A, Alvarez M, Pupo M, Guillen G, et al. Selection of phage-displayed human antibody fragments on *Dengue virus* particles captured by a monoclonal antibody: application to the four serotypes. *J Virol Methods* 2008;147:235–43.
- [112] Sui J, Hwang WC, Perez S, Wei G, Aird D, Chen L-M, et al. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol* 2009;16:265–73.
- [113] Maruyama T, Parren PW, Sanchez A, Rensink I, Rodriguez LL, Khan AS, et al. Recombinant human monoclonal antibodies to Ebola virus. *J Infect Dis* 1999;179:S235–9.
- [114] Zhou B, Wirsching P, Janda KD. Human antibodies against spores of the genus *Bacillus*: a model study for detection of and protection against anthrax and the bioterrorist threat. *Proc Natl Acad Sci* 2002;99:5241.
- [115] Zhang M-Y, Shu Y, Phogat S, Xiao X, Cham F, Bouma P, et al. Broadly cross-reactive HIV neutralizing human monoclonal antibody Fab selected by sequential antigen panning of a phage display library. *J Immunol Methods* 2003;283:17–26.
- [116] Heitner T, Moor A, Garrison JL, Marks C, Hasan T, Marks JD. Selection of cell binding and internalizing epidermal growth factor receptor antibodies from a phage display library. *J Immunol Methods* 2001;248:17–30.
- [117] Hoogenboom HR, Lutgerink JT, Pelsers MM, Rousch MJ, Coote J, van Neer N, et al. Selection-dominant and nonaccessible epitopes on cell-surface receptors revealed by cell-panning with a large phage antibody library. *Eur J Biochem* 1999;260:774–84.
- [118] Willats WG. Phage display: practicalities and prospects. *Plant Mol Biol* 2002;50:837–54.
- [119] Saggy I, Wine Y, Shefet-Carasso L, Nahary L, Georgiou G, Benhar I. Antibody isolation from immunized animals: comparison of phage display and antibody discovery via V gene repertoire mining. *Protein Eng Des Sel* 2012;25:539–49.
- [120] Carmen S, Jermutus L. Concepts in antibody phage display. *Brief Funct Genomics* 2002;1:189–203.
- [121] Bradbury AR, Sidhu S, Dübel S, McCafferty J. Beyond natural antibodies: the power of in vitro display technologies. *Nat Biotechnol* 2011;29:245–54.
- [122] Monigatti F, Gasteiger E, Bairoch A, Jung E. The sulfinator: predicting tyrosine sulfation sites in protein sequences. *Bioinformatics* 2002;18:769–70.
- [123] Kehoe JW, Velappan N, Walbolt M, Rasmussen J, King D, Lou J, et al. Using phage display to select antibodies recognizing post-translational modifications independently of sequence context. *Mol Cell Proteomics* 2006;5:2350–63.
- [124] Hoffhines AJ, Damoc E, Bridges KG, Leary JA, Moore KL. Detection and purification of tyrosine-sulfated proteins using a novel anti-sulfotyrosine monoclonal antibody. *J Biol Chem* 2006;281:37877–87.
- [125] Schweers BA, Old J, Boonlayangoor P, Reich KA. Developmental validation of a novel lateral flow strip test for rapid identification of human blood (Rapid Stain Identification™-Blood). *Forensic Sci Int Genet* 2008;2:243–7.
- [126] Vignali D. Multiplexed particle-based flow cytometric assays. *J Immunol Methods* 2000;243:243.
- [127] Sashihara J, Burello PD, Savoldo B, Pierson TC, Cohen JL. Human antibody titers to Epstein–Barr Virus (EBV) gp350 correlate with neutralization of infectivity better than antibody titers to EBV gp42 using a rapid flow cytometry-based EBV neutralization assay. *Virology* 2009;391:249–56.
- [128] Schlingemann J, Leijon M, Yacoub A, Schlingemann H, Zohari S, Matyi-Tóth A, et al. Novel means of viral antigen identification: improved detection

- of avian influenza viruses by proximity ligation. *J Virol Methods* 2010;163:116–22.
- [129] Cai J, Liu Z, Wang F, Li F. Phage display applications for molecular imaging. *Curr Pharm Biotechnol* 2010;11:603–9.
- [130] Norbert S, Arminder G, Viatcheslav M, Jos R, Christian M, Yilmaz A, et al. Phage display antibody-based proteomic device using resonance-enhanced detection. *J Nanosci Nanotechnol* 2002;2:375–81.
- [131] Saha K, Bender F, Gizeli E. Comparative study of IgG binding to proteins G and A: nonequilibrium kinetic and binding constant determination with the acoustic waveguide device. *Anal Chem* 2003;75:835–42.
- [132] Malmqvist M. Surface plasmon resonance for detection and measurement of antibody–antigen affinity and kinetics. *Curr Opin Immunol* 1993;5:282–6.
- [133] Bange A, Halsall HB, Heineman WR. Microfluidic immunosensor systems. *Biosens Bioelectron* 2005;20:2488–503.
- [134] Glöckler J, Angenendt P. Protein and antibody microarray technology. *J Chromatogr B* 2003;797:229–40.
- [135] Leemhuis H, Stein V, Griffiths AD, Hollfelder F. New genotype–phenotype linkages for directed evolution of functional proteins. *Curr Opin Struct Biol* 2005;15:472–8.
- [136] Niazov T, Pavlov V, Xiao Y, Gill R, Willner I. DNAzyme-functionalized Au nanoparticles for the amplified detection of DNA or telomerase activity. *Nano Lett* 2004;4:1683–7.
- [137] Low NM, Holliger P, Winter G. Mimicking somatic hypermutation: affinity maturation of antibodies displayed on bacteriophage using a bacterial mutator strain. *J Mol Biol* 1996;260:359–68.
- [138] Teng G, Papavasiliou FN. Immunoglobulin somatic hypermutation. *Annu Rev Genet* 2007;41:107–20.
- [139] Thie H. Affinity maturation by random mutagenesis and phage display. *Antibody Eng* 2010;397–409.
- [140] Irving RA, Kortt AA, Hudson PJ. Affinity maturation of recombinant antibodies using *E. coli* mutator cells. *Immunotechnology* 1996;2:127–43.
- [141] Wels W, Harwerth IM, Zwickl M, Hardman N, Groner B, Hynes NE. Construction, bacterial expression and characterization of a bifunctional single-chain antibody-phosphatase fusion protein targeted to the human ERBB-2 receptor. *Nat Biotechnol* 1992;10:1128–32.
- [142] Weissleder R, Tung CH, Mahmood U, Bogdanov A. In vivo imaging of tumors with protease-activated near-infrared fluorescent probes. *Nat Biotechnol* 1999;17:375–8.
- [143] Demeule B, Gurny R, Arvinte T. Detection and characterization of protein aggregates by fluorescence microscopy. *Int J Pharm* 2007;329:37–45.
- [144] Bäckström BT, Brockelbank JA, Rehm BHA. Recombinant *Escherichia coli* produces tailor-made biopolyester granules for applications in fluorescence activated cell sorting: functional display of the mouse interleukin-2 and myelin oligodendrocyte glycoprotein. *BMC Biotechnol* 2007;7:3.
- [145] Georgiou G, Stathopoulos C, Daugherty PS, Nayak AR, Iverson BL, Curtiss III R. Display of heterologous proteins on the surface of microorganisms: from the screening of combinatorial libraries to live recombinant vaccines. *Nat Biotechnol* 1997;15:29–34.
- [146] Uhlen M, Ponten F. Antibody-based proteomics for human tissue profiling. *Mol Cell Proteomics* 2005;4:384–93.
- [147] Terpe K. Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. *Appl Microbiol Biotechnol* 2003;60:523–33.
- [148] Zentgraf H, Frey M, Schwinn S, Tessmer C, Willemann B, Samstag Y, et al. Detection of histidine-tagged fusion proteins by using a high-specific mouse monoclonal anti-histidine tag antibody. *Nucleic Acids Res* 1995;23:3347–8.
- [149] von Strandmann P. A highly specific and sensitive monoclonal antibody detecting histidine-tagged recombinant proteins. *Protein Eng* 1995;8:733–5.
- [150] Hayhurst A, Happe S, Mabry R, Koch Z, Iverson BL, Georgiou G. Isolation and expression of recombinant antibody fragments to the biological warfare pathogen *Brucella melitensis*. *J Immunol Methods* 2003;276:185–96.
- [151] Artsaenko O, Kettig B, Fiedler U, Conrad U, Düring K. Potato tubers as a biofactory for recombinant antibodies. *Mol Breed* 1998;4:313–9.
- [152] Puettmann C, Kolberg K, Hagen S, Schmies S, Fischer R, Naehring J, et al. A monoclonal antibody for the detection of SNAP/CLIP-tagged proteins. *Immunol Lett* 2012.
- [153] Provost CR, Sun L. Fluorescent labeling of COS-7 expressing SNAP-tag fusion proteins for live cell imaging. *J Vis Exp* 2010.
- [154] Veggiani G, de Marco A. Improved quantitative and qualitative production of single-domain intrabodies mediated by the co-expression of Erv1p sulfhydryl oxidase. *Protein Expr Purif* 2011;79:111–4.
- [155] Trilling AK, Beekwilder J, Zuilhof H. Antibody orientation on biosensor surfaces: a minireview. *Analyst* 2013;138:1619–27.
- [156] Paduch M, Koide A, Uysal S, Rizk SS, Koide S, Kossiakoff AA. Generating conformation-specific synthetic antibodies to trap proteins in selected functional states. *Methods* 2012.
- [157] Saviranta P, Haavisto T, Rappu P, Karp M, Lövgren T. In vitro enzymatic biotinylation of recombinant Fab fragments through a peptide acceptor tail. *Bioconjug Chem* 1998;9:725–35.
- [158] Aburatani T, Sakamoto K, Masuda K, Nishi K, Ohkawa H, Nagamune T, et al. A general method to select antibody fragments suitable for noncompetitive detection of monovalent antigens. *Anal Chem* 2003;75:4057–64.
- [159] Niemeyer CM, Ceyhan B. DNA-directed functionalization of colloidal gold with proteins. *Angew Chem Int Ed* 2001;40:3685–8.
- [160] Hansen MH, Sode LL, Hyldig-Nielsen JJ, Engberg J. Detection of PNA/DNA hybrid molecules by antibody Fab fragments isolated from a phage display library. *J Immunol Methods* 1997;203:199–207.
- [161] Ling MM, Ricks C, Lea P. Multiplexing molecular diagnostics and immunoassays using emerging microarray technologies. *Expert Rev Mol Diagn* 2007;7:87–98.
- [162] Niemeyer CM, Adler M, Wacker R. Immuno-PCR: high sensitivity detection of proteins by nucleic acid amplification. *Trends Biotechnol* 2005;23:208–16.
- [163] Kakizaki E, Yoshida T, Kawakami H, Oseto M, Sakai T, Sakai M. Detection of bacterial antigens using immuno-PCR. *Lett Appl Microbiol* 2008;23:101–3.
- [164] Van Dessel W, Vandenbussche F, Staes M, Goris N, De Clercq K. Assessment of the diagnostic potential of immuno-RCA in 96-well ELISA plates for foot-and-mouth disease virus. *J Virol Methods* 2008;147:151–6.
- [165] Schweitzer B, Wiltshire S, Lambert J, O'Malley S, Kukanskis K, Zhu Z, et al. Immunoassays with rolling circle DNA amplification: a versatile platform for ultrasensitive antigen detection. *Proc Natl Acad Sci* 2000;97:10113–9.