Seminars in Cell & Developmental Biology 20 (2009) 10–26

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Antibody production, design and use for biosensor-based applications

Paul J. Conroy, Stephen Hearty, Paul Leonard, Richard J. O'Kennedy*

Biomedical Diagnostics Institute, National Centre for Sensor Research, School of Biotechnology, Dublin City University, Dublin 9, Ireland

ARTICLE INFO

Article history:
Available online 4 February 2009

Keywords:
Recombinant antibodies
Biosensor
Transduction
Ribosome display
Phage display

ABSTRACT

Currently, the reliable detection and quantification of a multitude of different analytes is crucial in many applications and settings. Biosensors have revolutionised diagnostics for use in point-of-care testing (POC), the detection of food and environmental contaminants, biological warfare agents, illicit drugs and human/animal disease markers. Antibodies continue to play a pivotal role in many sensor devices due to their exquisite specificity for their cognate antigens. In this review current biosensor platforms employing antibodies for molecular recognition are briefly described. The use of molecular biological techniques for the generation and improvement of antibodies is critically examined. Such recombinant antibodies possess improved attributes for use in biosensor development in terms of design, stability, affinity and specificity.

Contents

Abbreviations: Ab, antibody; Ag, antigen; AP, alkaline phosphatase; ARM, antibody–ribosome–mRNA; BSA, bovine serum albumin; BW, bulk wave; CDR, complementarity determining region; CH, constant heavy chain (1, 2 and 3); DNA, deoxyribonucleic acid; DTT, dithiothreitol; Fab, fragment antigen binding; FET, field effect transistor; Fv, variable fragment; Gly, glycine; HRP, horseradish peroxidase; Ig, immunogobulin; ISFET, ion-selective field effect transistor; K_d, dissociation constant; LAPS, light addressable potentiometric sensors; LED, light emitting diode; mRNA, messenger ribonucleic acid; MW, molecular weight; OWLS, optical waveguide lightmode spectroscopy; PBS, phosphate buffer saline; PDI, protein disulphide isomerase; pM, picomolar; QCM, quartz-crystal microbalance; RIfS, reflectometric interference spectroscopy; RI, Refractive index; RT-PCR, reverse transcriptase polymerase chain reaction; SAF, supercritical angle fluorescence; SAW, surface acoustic wave; scAb, single chain anitbody; scFv, single chain variable fragment; Ser, serine; SHM, somatic hypermutagenesis; SIP, selectively infective phage; SPR, surface plasmon resonance; TIR, total internal reflection; TIRE, total internal reflection ellipsometry; TIRF, total internal reflection fluorsecence; TSM, thickness shear mode.

* Corresponding author. Tel.: +353 1 700 5319; fax: +353 1 700 5412.
E-mail address: Richard.okennedy@dcu.ie (R.J. O'Kennedy).

1084-9521/$ – see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.semcdb.2009.01.010

1. Introduction

1.1. Biosensors overview

A biosensor can be described as a transducer that incorporates a biological recognition component as the key functional element. It consists of three main components as illustrated in Fig. 1: the biorecognition element, the transducer and the signal display or readout [1]. The interaction of the analyte with the biorecognition element is converted to a measurable signal by the transduction system. The signal is then converted into a readout or display.

Biosensors are powerful tools for the analysis of biomolecular interactions in clinical, biochemical and environmental analyses [2]. In the context of a medical setting, biosensors have the potential to provide rapid, real-time and accurate results in accident and emergency departments or at the physician's office. A typical example of this is the *in vitro* measurement of capillary glucose (near patient) in diabetic patients [3]. For the purposes of this review, the area of antibody-based biosensors will be evaluated with emphasis on improvements in antibody production and their implications for the design of biosensors.

1.2. Antibody overview

1.2.1. The immune system

The immune system functions as a surveillance mechanism against infectious organisms and/or their toxic products [4]. It can be categorised by two mechanisms; non-adaptive (innate) and adaptive (acquired) immunity [5]. Non-adaptive immunity is a general non-specific response to foreign molecules and includes systems such as phagocytosis (macrophages), cell lysis (natural killer cells) and a host of chemical and physical elements [4,5]. The key difference between non-adaptive and adaptive immunity is the ability of the adaptive immunity to improve following exposure to specific molecules. Adaptive immunity is mediated by lymphocytes (specifically white blood cells) that are responsible for the secretion of immunoglobulins (antibodies). B-cells are terminally differentiated to give rise to memory B-cells that immediately recognise the antigen post primary exposure, and plasma cells, which are responsible for secretion of specific antibodies in response to the antigen [5–7].

1.2.2. Antibody structure

The basic structure of an immunoglobulin is outlined in Fig. 2. The antibody molecule has four polypeptide chains, two heavy (H) chains with molecular weights of 50 kDa and two light (L) chains (25 kDa molecular weight) linked by disulphide bonds. The chains have both constant (C) and variable (V) regions. The H chain has one variable region (V_H) that is responsible for antigen binding and three constant regions (C_{H1}, C_{H2} and C_{H3}). The light chain has one variable region (V_L), which is an important part of the antigen-binding site, and one constant region (C_L) [8].

There are five classes of immunoglobulin which are distinguished by their heavy chains: IgA, IgG, IgM, IgD and IgE. Class switching of the heavy chain during gene rearrangement gives rise to the isotype of the immunoglobulin. There are two types of light chains, κ and λ, which combine with heavy chains to form a complete antibody molecule. IgG is the predominant class of antibody produced during the matured immune response and is the most widely targeted for immune library construction [8]. The Fc region consists of three domains, C_{H1}, C_{H2} and C_{H3} that confer effector functions, such as complement activation, on the antibody. Antigen binding is mediated by the variable light (V_L) and heavy (V_H) domains which bring together the hyper-variable regions of the antibody, known as the complementarity determining regions (CDRs). The antibody constant regions are generally conserved with only small differences in sequence being found in the various antibody classes. However, the CDRs exhibit a high level of sequence diversity.

1.2.3. Antibody diversity

The diversity of antibodies in the immune system is achieved by gene recombination and somatic hyper-mutagenesis of the encoding genes [9]. The encoding gene segments are outlined in Fig. 3. In vertebrate genomes, there are 11 Constant (C_H), 123–129 Variable (V_H), 27 Diverse (D_H) and 9 Joining (J_H) gene segments that combine to encode the heavy chains. During B-cell development, the immunoglobulin loci undergo rearrangements [10]. Within the heavy chain locus, the V_H-D_H-J_H rearrange and this exon becomes linked to a combination of C_H segments during transcription. Subsequently, the mRNA is translated into the immunoglobulin isotype that is specific to the lymphocyte. A similar chain of events occurs for the λ and κ loci, with the absence of D segments.

The diversity introduced into the immune system by these rearrangements is increased further by somatic hypermutation (SHM) [11]. SHM introduces errors into the genes encoding the variable regions of individual B-cells. During the narrow time frame in the proliferation of B-cells, the locus undergoes an extremely high rate of mutation, predominantly base substitution [11], approximately 1 million times higher than the spontaneous rate of mutation across the genome [12]. The introduction of random mutations gives rise to antibodies that lose their affinity for the antigen and undergo cell death, or generates those advantageous antibodies, where there is an affinity increase and subsequent proliferation of the associated antibody-producing clone of lymphocytes.

2. Biosensors

2.1. Introduction

The "*Enzyme Electrode*" was demonstrated by Clark and Lyons in 1962 as the first biosensor. It coupled glucose oxidase to an amperometric electrode for monitoring oxygen in blood [13]. In 1987, Vo-Dinh and co-workers showed that antibodies could be utilised *in situ* for the detection of a chemical carcinogen in a fibre optic-based immunosensor [14]. Antibodies have since proven their worth as powerful tools for diagnostic applications, as illustrated in Table 1. Essentially, the selectivity or specificity of the biosensor is dependant on the biorecognition element, which is capable of "sensing" the presence of an analyte [1,2]. Immunosensors utilising

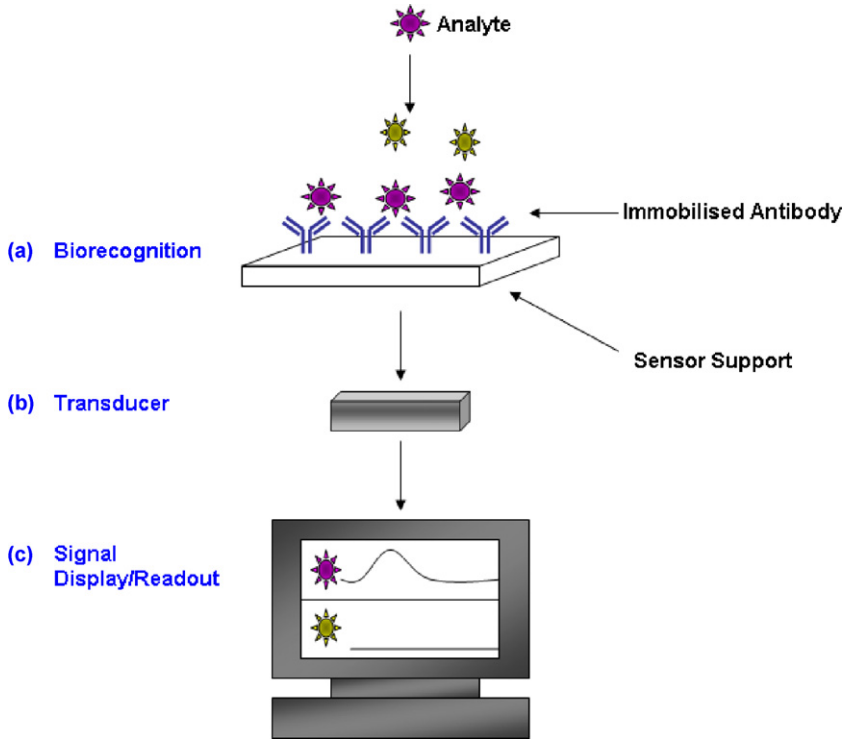

Fig. 1. Biosensor components. (a) Analyte interaction with biorecognition element: this is facilitated by the specificity of the immobilised antibody for its cognate antigen (purple). Other biorecognition elements include enzymes, lectins, receptors and microbial cells. (b) Signal transduction: converts the interaction of the analyte molecule and analyte into a quantifiable signal. (c) Readout or display: shows the specific signal generated by interaction with the analyte of interest. Yellow: non specific analyte.

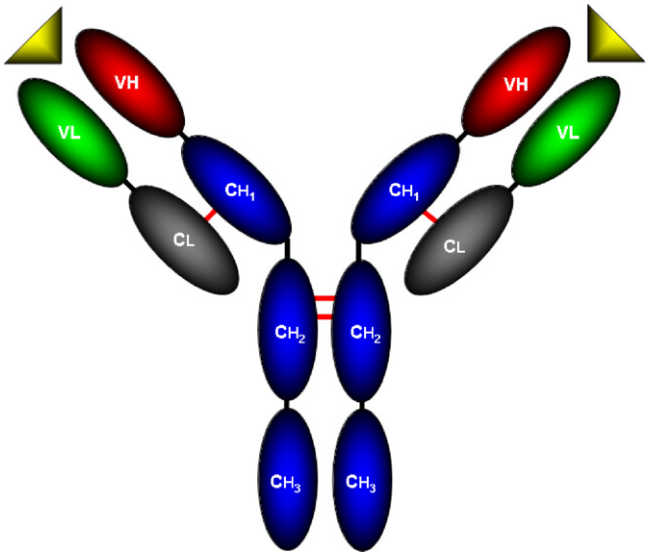

Fig. 2. Typical IgG molecule structure. Antibody molecule (~150 kDa). The antigen binding sites are indicated by the triangles and the disulphide bridges are indicated by red lines.

antibody-based recognition elements, have been developed on a wide range of transduction platforms for a multitude of analytes. The transducer element translates the selective recognition of the analyte into a quantifiable signal and thus, has major influence on sensitivity [15]. Transduction approaches include electrochemical, piezoelectric and optical systems [16].

2.2. Transduction platforms

This section briefly introduces some of the transduction platforms commonly exploited in biosensors incorporating antibody-based biorecognition. Monoclonal and polyclonal antibodies have been successfully employed and are the predominant antibody form used in biosensors. However, as discussed later, the importance of recombinant antibodies for biosensor applications is gaining increasing significance. Table 1 lists several examples of transduction elements, utilising both polyclonal and monoclonal antibodies for the detection of various analytes. The references also provide additional detailed information on the principles underpinning each of the transduction methods and are comprehensively reviewed by Jiang and co-workers [15] with respect to pesticides and both Luppa and co-workers [17] and D'Orazio [13] with respect to clinical uses.

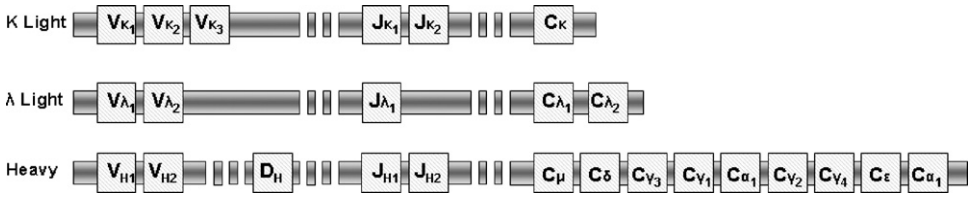

Fig. 3. Genes encoding human antibodies. Immunoglobulin loci: Heavy chain (chromosome 14), κ light chain (chromosome 2) and λ light chain (chromosome 22). Variable (V), Constant (C), Joining (J) and Diverse (D) segment genes are shown. In immature B cells V-D-J segments are rearranged and linked to Cμ to produce mRNA encoding for IgM or Cδ to give mRNA encoding for IgD. mRNA encoding IgG is produced by recombination resulting in the bringing together of $C\gamma_2$, $C\gamma_4$ Cε and $C\alpha_2$ by the deletion of the five C_H segments. This figure was adapted from [8,10].

Table 1
Examples of polyclonal and monoclonal antibody-based sensors their associated analytes and transducers.

Transducer	Analyte detected	Antibody type[a]	Ref.
Electrochemical			
Potentiometric	Terbuthylazine	Monoclonal	[39]
	Hepatitis B surface antigen	Not specified	[40]
	Diphtherotoxin	Monoclonal	[41]
Amperometric	*E. coli* 0157:H7	Polyclonal	[42]
	Carcinoembryonic antigen (CEA)	Not specified	[43]
	Aflatoxin M1	Monoclonal	[44]
	Progesterone	Monoclonal	[45]
Impedance	*Listeria monocytogenes* (Internalin B)	Polyclonal	[24]
Piezoelectric			
	E. coli 0157:H7	Polyclonal	[46]
	Canine IgG Isoforms	Monolconal	[47]
	Cocaine/derivative (BZE-DADOO)	Polyclonal	[48]
	Atrazine	Monoclonal	[49]
	Bacillus anthracis	Polyclonal	[50]
	Francisella tularensis	Polyclonal	[51]
Optical			
SPR	Urediniospores	Monoclonal	[52]
	Polychlorinated biphenyls	Monoclonal	[53]
	Vitellogenin (Carp)	Monoclonal	[54]
	Campylobacter jejuni	Polyclonal	[55]
	Listeria monocytogenes	Monoclonal	[56]
	Okadaic acid	Polyclonal	[57]
Resonant mirror	*L. monocytogenes*	Monoclonal	[58]
TIRF	Testosterone (also RIfS)	Monoclonal	[59]
	Carbohydrates (maltose and panose)	Monoclonal	[32]
RIfS	Estrone	Polyclonal	[60]
	Tuberculosis (also interferometry)	Monoclonal	[61]
	Cell adhesion	Monoclonal	[27]
OWLS	Trifluralin	Polyclonal	[62]
	Sulfamethazine	Not specified	[63]
Interferometry	Atrazine	Monoclonal	[28]
	Hepatitis B virus surface antigen	Not specified	[64]
Ellipsometry	Mycotoxin T-2 (TIRE also QCM)	Mono- and polyclonal	[65]
	Salmonella typhimurium	Monoclonal	[66]
Fibre optic	*L. monocytogenes* (Imaging)	Polyclonal	[67]
	B. anthracis (Evanescent wave FO)	Not specified	[68]
	Raptor™—biothreat (e.g. *B. anthracis*)	Various monoclonal	[69]

[a] Not specified—where the type of antibody is not stated.

2.2.1. Electrochemical methods

Electrochemical transducers are the oldest and most commonly used. They offer high specificity, low-detection limits, relative freedom from matrix interference and low cost. However, some challenges remain including high performance and cost-effectiveness [15]. Electrochemical transduction may be divided into to three types: potentiometric, amperometric and impedance.

2.2.1.1. Potentiometric. Potentiometric transduction measures the change in the potential of a system based on the Nernst equation. Changes in potential are related to specific ion activity [17]. This arises when a binding event between antigen (free) and antibody (immobilised) electrode is compared against a reference electrode [15,18]. Ion-selective electrodes use ion-selective membranes to achieve charge separation between the sample and the sensor surface. The sensor consists of a perm-selective outer layer and a bioactive material, e.g. an enzyme [18]. This perm-selective outer layer maximises the sensor selectivity by reducing interference from any electroactive species in the sample [19]. The enzyme-catalysed reaction generates or consumes a species that is detected by the ion-selective electrode and generates a logarithmic concentration-dependent signal [18]. The development of semiconductors, e.g. field effect transistors (FET) has had significant influence on potentiometric sensor design [20]. FET monitors charges at the surface of an electrode which have built up on its metal gate between the source and the drain [17]. This facilitates the detection and measurement of the potentiometric signal produced by a binding event at the gate of the FET (i.e. changes in surface charge) [18,21,22]. A further development is the ion-selective field effect transistor (ISFET) where the gate is replaced by an ion-sensitive membrane [22] and a local potential is generated by surface ions which modulate the current flow across the silicon semiconductor [20]. The so-called 'ImmunoFET' typically has the antibody immobilised at the gate. The formation of the Ag–Ab complex results in modification in the surface charge distribution (due to the electrical charge carried by molecules) and, consequently, a modulation in the drain current is observed [22,23]. This type of direct potentiometric sensor is ideal as it allows 'label-free' measurement of the analyte. However, some fundamental limitations have given rise to the indirect ImmunoFET-based immunoenzymatic assay [22]. In this case the antigen or antibody is labelled by an enzyme such as urease [21,22]. Light addressable potentiometric sensors (LAPS) involve the immuno-reaction occurring at the insulating layer resulting in potential changes at the silicon (n-type) layer, which is then detected by differential charge distribution between the two layers. LAPS measures the alternating photocurrent generated by a light source (e.g. LED) resulting in changes of potential recorded as voltages per time differentials [16,18]. Both ISFET and LAPS would facilitate potential miniaturisation of potentiometric biosensors [20,22].

2.2.1.2. Amperometric. Amperometric sensors measure the current flow generated by an electrochemical reaction. Most analytes are unable to act as redox partners in an electrochemical reaction and hence, there are few applications of direct amperometric sensors [17]. Amperometric sensors thus, employ electroactive labels [17]

to generate current. This current is generated when an electroactive species is oxidised or reduced at the working electrode to which the analyte binds specifically in comparison to the reference electrode where no specific binding should occur. The current has a linear relationship with the electro-active species concentration [21]. Reference electrodes are typically composed of Ag/AgCl whilst working electrodes commonly incorporate noble metals, graphite and modified forms of carbon or conducting polymers to which the antibody is immobilised [18]. A major advantage of this kind of sensor is its performance in complex (i.e. turbid) matrices. However, amperometric biosensors are sometimes disadvantaged by the necessity for labelling and separation of free from bound labelled antibody which may be alleviated by the use of porous membranes [13].

2.2.1.3. Impedance. Impedance (including capacitance/conductance) immunosensors detect changes in the electric field due to Ag–Ab binding and are attributed to changes in electrical conductivity or capacitance on the electrode surface [15]. Electric impedance spectroscopy (EIS) detects the electrical response generated after a periodic small amplitude AC current is applied. Variation in impedimetric transduction arises due to Ag–Ab interactions at the surface of electrically conducting polymers [24].

2.2.2. Piezoelectric methods

Piezoelectric sensors employ materials that resonate on the application of an external alternating electrical field [15,21]. Typically, quartz crystals are utilised and the frequency of oscillation in the field is a function of the crystal mass [16]. Shifts in the frequency of the oscillation occur as a result of mass changes at the crystal surface. Therefore, the interaction of an analyte with an antibody immobilised on the quartz crystal leads to a mass change and, thus, an oscillation frequency change [16]. Piezoelectric crystal devices are advantageous as they facilitate direct measurement without the need for labelling [25]. Piezoelectric sensors can be further categorised into bulk and surface acoustic wave instruments. Bulk wave (BW) instruments are applied in gravimetric devices known as quartz-crystal microbalances (QCM), which are related to mass sensitivity of the crystals, or thickness shear mode (TSM) resonators, which describe the motion of the crystals vibration [18]. In BW devices the immobilised Ab–Ag interaction occurs at the surface of the crystal, which is within an oscillating circuit, so that changes in mass lead to a decrease in resonant frequency [16,21]. Surface acoustic wave (SAW) instruments transmit an acoustic wave along a crystal face from one location to another [18]. The transducer here acts as the transmitter and the receiver. The excited wave travels across the surface of the crystal and changes due to mass loading on the acoustic path alter the phase-wave frequency [21].

For piezoelectric sensors the effects of viscoelastic and electroacoustic interference due to the sample liquid and absorbed films must be taken into account. Advances in piezoelectric sensors include the use of QCM's and micro-cantilevers [26].

2.2.3. Optical methods

2.2.3.1. Introduction. Optical immunosensors consist of a light source, components to generate light with specific characteristics, a modulating agent, a sensing area and a photodetector. Optical sensors are advantageous due to their low signal-to-noise ratio and low-reagent volume requirements [15]. Direct optical sensors are most useful for biosensor applications due to the lack of requirements for labelling of either the analyte or the antibody and the ability to work effectively in complex matrices. However, indirect optical sensors are also very valuable and are generated by incorporation of a suitable label, such as a fluorophore [13].

Techniques commonly employed in optical biosensors include reflectometric interference spectroscopy (RIfS), interferometry, optical waveguide lightmode spectroscopy (OWLS), total internal reflection fluorescence (TIRF), surface plasmon resonance (SPR), resonant mirrors, fibre optics, ellipsometry, fluorescence and ultra violet/visible (UV/vis) spectroscopy.

2.2.3.2. Principles of optical transduction. The principle of each optical transduction technique is briefly outlined in this section.

RIfS is a 'label-free' time-resolved technique which facilitates the monitoring of biomolecular interactions [27]. The technique is based upon the incidence of white light on an interface between two media of differing refractive indices. The light is reflected in part and these interacting beams produce a characteristic interference pattern. Differences in the interference pattern occur due to an increase in the optical thickness of the immobilised molecules, for example, due to an Ag–Ab-binding event [15,27]. RIfS is temperature dependant, however, it has a greater depth of penetration into the solution [27] than surface plasmon resonance.

Waveguides are typically glass, quartz or polymer films of high-refractive index (RI) imbedded in materials of lower RI. A linear laser beam is then incident and constrained within the waveguide by total internal reflection (TIR) resulting in an evanescent wave [17]. The evanescent wave generated is due to the excitation of the light itself in the dielectric layer. Some of this light penetrates the biolayer and is reflected back into the waveguide after undergoing a phase shift which interferes with the transmitted light. As a result, changes in the interference pattern can be interpreted as changes in the biolayer [17]. Interferometry is based on changes in refractive index profile within the evanescent field volume of a waveguide due to an Ag–Ab interaction. Waveguide systems such Mach-Zehnder inferometry (MZI) are exploited for this type of immunosensor [15,17,28]. OWLS is an advance in the dielectric waveguide area that exploits the science of light guided in structures smaller than its wavelength [29]. OWLS utilises linearly polarised light that is coupled by a diffraction grating into a thin waveguide [13,15]. 'Incoupling' is a resonance phenomenon that occurs at a defined angle of incidence which is defined by the RI of the medium covering the waveguide [15]. Within that waveguide the light is directed by TIR and is detected by photodiodes at the edges [15,30].

TIRF is a useful technique for the analysis of molecular interactions at an interface. Within two media of different RI, light at a specific angle incident onto the sensor surface, undergoes TIR and results in the propagation of an evanescent wave into the medium of lower RI [17]. Molecules within the sensing layer interact with this wave resulting in attenuation of the reflected light [17]. In TIRF, fluorescent molecules in the evanescent field are excited by the incident light to produce a fluorescent evanescent wave which can then be measured [13,17]. TIRF-based immunosensors have received considerable attention [31,32] and offer robustness, versatility and portability, but their main constraint is the requirement for labelled reagents [15]. TIRF is comprehensively reviewed by Dominici and co-workers [33].

Supercritical angle fluorescence (SAF) is a method for the detection of surface-localised fluorescence. Parabolic elements are currently being investigated for the efficient collection of SAF and the detection of a single fluorescent molecule has been demonstrated using a confocal detection scheme [34]. Fluorescence occurs upon the excitement of an electron, by the absorption of light, from the ground state to the excited singlet state, where upon relaxation to the lower state the molecule emits a photon with less energy at longer wavelength than the incident photon.

Fibre optic (FO) biosensors often involve the immobilisation of an antibody to the distal end of the fibre. Incident light, introduced at the proximal end, travels through the fibre by TIR causing excitation of the fluorophores [16] attached to either the antigen or an associated antibody depending on the assay configuration.

Ellipsometry employs linearly polarised light reflected at oblique incidence from a surface, resulting in elliptically polarised reflected light. Changes in the shape and orientation of the reflected light are dependant on the direction of incident light and reflective surface properties [17]. Variants include total internal reflection ellipsometry (TIRE) and imaging ellipsometry.

SPR is a phenomenon that occurs when light is incident on a metal surface. SPR was described as the charge density oscillation at the interface between two media with oppositely charge dielectric constants [18]. The resultant resonant excitation is provided by compatible light energy photons. The amplitude of the resulting evanescent wave is maximal at the metal (plasmon-generating). The emergent medium being of lower refractive index (typically aqueous) allows the penetration of an evanescent wave. This phenomenon facilitates the study of interactions at the metal surface as the evanescent wave propagates to a depth of approximately one wavelength [18]. The most notable application of SPR is in the GE Healthcare Biacore™ systems [35–37].

Resonant mirror biosensors marry the simplicity of SPR-based sensors with the sensitivity of a waveguide device. The sensor comprises of the biosensing layer, high-RI dielectric resonant layer, low-RI coupling layer and a prism [38].

3. Antibodies and their significance for biosensor development

3.1. Antibodies as biological reagents

Antibodies are ideal biorecognition elements due to their exquisite specificities and strong affinities for cognate antigens. Fig. 4 shows an overview of the generation of polyclonal [70,71], monoclonal [71,72] and recombinant antibodies [73,74] from immunised repertories.

Antibodies have numerous successful applications in the area of diagnostics with monoclonal and polyclonal antibodies being successfully exploited in many biosensors, as shown in Table 1.

Table 2
Characteristics of polyclonal, monoclonal and recombinant antibodies.

Characteristic	Polyclonal	Monoclonal	Recombinant
Ease of production	++++	+++	+++
Cost (low)	++++	+++	++
Stability[a]	+++	++	++
Commercial availability	++++	+++	+
Ease of immobilisation	++++	++++	++++
Sensitivity-affinity	+++	++++	+++++
Capacity for engineering affinity	–	– (Can be converted to rAb)	+++++

[a] Stability of rAb is dependent on the format.

Polyclonal antibodies are derived from multiple plasma cells and monoclonal antibodies are derived from a single clonal hybridoma, all of which have terminally differentiated in response to an antigen [7,70]. Recombinant antibodies, discussed at length in the following sections, are the product of genetic manipulation of antibody genes. The specific characteristics of polyclonal, monoclonal and recombinant antibodies are outlined in Table 2.

Some key parameters exist for antibodies in biosensor applications. These include sensitivity, selectivity, stability, immobilisation (hence, orientation on the surface), labelling and antibody size (impacts on the density of biolayer of the sensor). Recombinant antibodies present a viable means to optimise these factors. Primarily genetic modification facilitates improvements in selectivity, stability, size (Sections 3.2, 3.3 and 4) and, in addition, such novel antibody fragments aid effective immobilisation [75]. High-throughput screening (Section 5) and display libraries facilitate improvements in sensitivity due to the ability to screen much larger recombinant libraries (Section 3.4). The use of coupling chemistries and/or genetic insertion of tag's for immobilisation (Section 4.3) are useful methods to assist the orientation and immobilisation of novel recombinant antibodies.

Monoclonal antibody generation, using hybridoma technology, was described in 1975 [76] and, in combination with advances in our

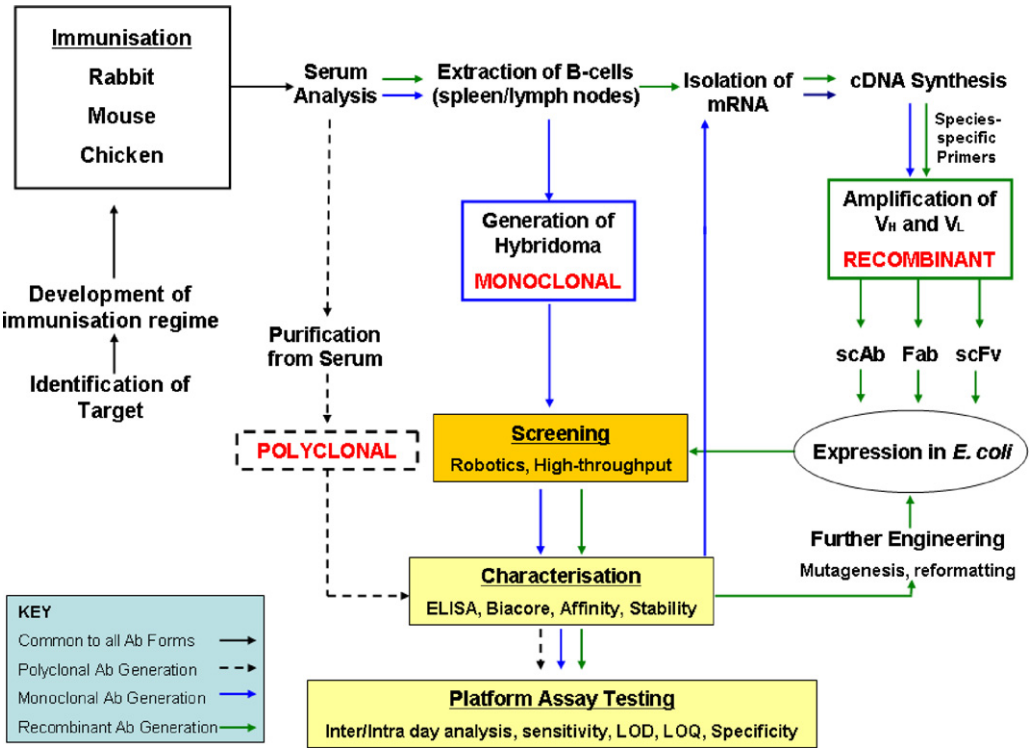

Fig. 4. Overview of antibody generation, screening and characterisation. This flow diagram illustrates the overall steps in the generation of polyclonal, monoclonal and recombinant antibodies. LOD, limit of detection; LOQ, limit of quantitation.

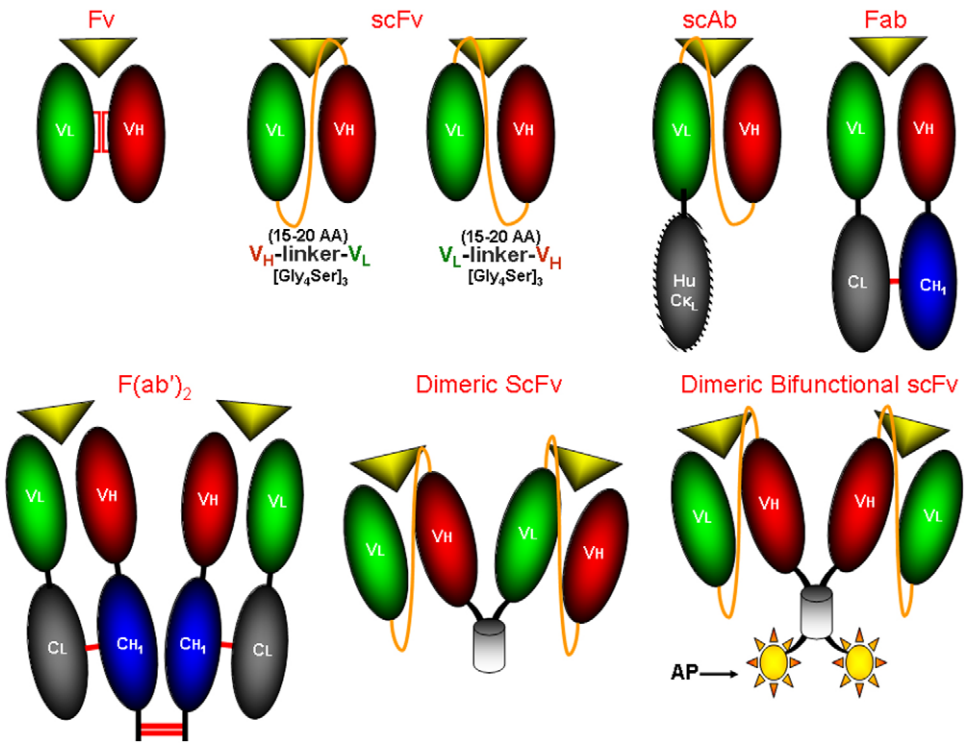

Fig. 5. Recombinant antibody formats. The Fv consists of the V_H (red) and V_L (green) chains. The scFv consists of the Fv with a flexible linker joining the terminal ends of either the V_H to V_L (or V_L to V_H). A scAb incorporates a human constant κ light chain added to the terminal of V_L. The Fab contains the Fv with both constant heavy and light chains. F(ab′)$_2$ involves linking the two Fabs by disulphide bonds (can also be achieved by proteolytic cleavage of an IgG molecule). A dimeric scFv is generated by a fusion of two scFvs via a naturally occurring dimeric protein. A dimeric bifunctional scFv incorporates alkaline phosphatase (AP) labelling to facilitate direct detection. Antigen-binding sites are indicated by the yellow triangles.

knowledge of immunoglobulin structure and DNA-based recombination [77], has facilitated the genetic manipulation of antibodies to become widely practiced. The ability to alter properties such as the size and affinity of antibodies has led to the development of novel antibodies for use in diagnostics and therapeutics. Some examples of commercially available monoclonal antibody-based biosensors for the diagnosis of cardiovascular disease include: Abbott's i-STAT® (cTnI) [78] and Roche's CARDIAC proBNP assay [79].

The specificity, and to a large degree the sensitivity, of all immunosensors is dictated by the biorecognition element [15,17] and hence, the ability to develop more sensitive and robust systems is reliant on the modification of antibodies to improve these attributes. This section comprehensively reviews the area of recombinant antibodies with respect to their selection, modification and subsequent applications in biosensors.

3.2. The emergence of recombinant antibodies

It was shown in the late 1980s that the use of vectors in bacterial expression systems could generate correctly folded antibody fragments [80,81]. Since then, recombinant antibody fragments have been produced in mammalian [82,83], insect [84,85], yeast [86], plant [87] and in 'cell-free' systems [88]. A factor limiting several of these expression systems is the inability to express large amounts of active protein and the relative advantages and disadvantages of each expression system is reviewed by Verma and co-workers [89].

There are two main sources of antibody genes: V-gene repertoires rearranged from animal or human donors and synthetic antibody V-gene repertoires constructed *de novo*, *in vitro* [73]. The cloning of antibody fragments into such systems begins with the isolation of mRNA coding for the V-genes from donor B-cells [90,91] (see Fig. 4). Such sources of mRNA include hybridomas, peripheral blood lymphocytes, spleen and bone marrow cells [92]. The mRNA is in turn reverse transcribed into cDNA [91]. The subsequent amplification of the antibody genes is then carried out by PCR-based methods. This method of recombinant antibody generation in theory clones all mRNA encoding for antibody genes present in the mRNA pool. For cloned antibodies to be useful it is imperative that the antibody genes be cloned reliably [92]. Hoogenboom and co-workers [73] and Azzazy and co-workers [91] have provided comprehensive reviews of recombinant antibody libraries and their screening.

3.3. Antibody fragments and formats

Prior to the development of recombinant antibody technologies, antibody fragments could only be generated by proteolytic cleavage yielding F(ab′)$_2$ and Fab (Fig. 5). The ability to generate fragments of antibodies that retain their stability and specificity is the basis of recombinant antibody generation. Recombinant technologies have allowed the generation of a number of distinct antibody fragments (Fig. 5) with desirable affinities and specificities for use in diagnostic applications.

The smallest fragment of the whole antibody practically used is the Fv which comprises of the V_H and V_L domains that are associated via a disulphide bond. Stability problems at lower concentrations were overcome by incorporating a flexible peptide linker into the Fv fragment resulting in a single chain Fv (scFv) [93,94]. Typically, a flexible $(Gly_4Ser)_3$ linker is used due to its tendency not to form secondary structures and the fact that it is found naturally in M13 pIII protein and, therefore, is well tolerated in phage display. However, it was demonstrated that selection based on linker mutations can influence production, stability and recognition properties of the scFv [95]. Diabody production is achieved by incorporating a shorter polypeptide linker (5–12 amino acids) forcing the association of two scFv molecules [96]. Genetically

encoding alkaline phosphatase (AP) as a fusion to the scFv have been reported [97,98] from which direct detection of bound antibody fragments can be achieved. Dimeric scFv's consist of two scFv fragments brought together via a naturally dimeric protein [92,99]. An interesting variation on the dimeric scFv, which facilitates direct detection, is a bifunctional scFv comprised of AP-labelled scFv's [91,92,100]. Once these antibody fragments are generated, an appropriate or tailored selection method is used to isolate high-affinity antibodies from a vast library.

3.4. Antibody libraries

These libraries mirror what would naturally be present in individuals allowing them to generate antibodies against antigens that might be encountered during normal everyday life. This is the basis of our immune response and allows our survival despite the prevalence of many pathogens, toxins and life-threathening infections.

The availability of combinatorial libraries is of major importance in antibody engineering. These libraries may be generated from immunised hosts (animal or human) or are available from academic or industrial sources (naïve or non-immunused libraries). The isolation of antibodies from such libraries is based on non-covalent interaction between the library member and its cognate antigen. Efficient high-throughput screening [101,102] has enabled scientists to screen large libraries thus, enhancing the probability of enriching for highly specific antibodies. This capability has also allowed engineering of antibodies with improved affinity, stability and prompted an era of 'tailor-made' antibodies for a wide range of applications.

3.4.1. Advantages of display technology

The advent of display techniques has allowed the generation of very large antibody libraries, which has introduced a viable and efficient method for the development of recombinant antibodies. The library size was demonstrated to have a major impact on the characteristics of selected antibodies, with increased library size increasing the probability of selecting antibodies with high affinity and specificity [103].

In phage display, the antibody fragment is fused to the phage coat protein which is linked to the encapsulated genetic information [104]. This physical link between the phenotype and genotype allows the selection of binders in the antibody library. In contrast, in ribosome display the mRNA, ribosome and nascent antibody form a stable, stalled antibody–ribosome–mRNA (ARM) complex allowing for RT-PCR-mediated recovery of specific binders [88,105]. Table 3 outlines the attributes of phage (plasmid library) and ribosome display (mRNA/PCR fragment) [106]. Other display technologies include yeast [107,108], bacterial [109] and mRNA display [110,111].

A major advantage of the use of combinatorial display libraries is the generation of an immunised repertoire of antibodies from practically any species compared to hybridoma technology which has been most successfully applied to rodents [116]. However, hybridoma technology have also been used with rabbit [117], cow [118], chicken [119] and human [120] lymphocytes. The ability to generate antibodies recognising human epitopes, e.g. in biomarkers of diseases, is essential for many diagnostic tests and for therapy. Generally this can be achieved in a range of species, but the antigenicity is often dependent on how phylogenetically distant the species are from humans [121]. Antibody libraries have been generated for a number of species including human [122], murine [123], chicken [121,124], rabbit [125,126], camelids [127], shark [128], bovine [129] and sheep [130]. The availability of naïve and synthetic repertoires facilitates the generation of antibodies without the need for animal use as such libraries can recognise a wide range of antigens [131]. The ability to generate recombinant antibodies to target specific antigens, for example those demonstrated in Table 4, is crucial for the development of the biorecognition element of biosensors.

Table 3
Phage display and ribosome display compared [106].

	Phage display	Ribosome display
Largest library size reported	10^{10} [112]	10^{13} [73]; Potentially 10^{14} [113]
Recovery of selected binders	Various (Fig. 7)	RT-PCR (Fig. 10)
Diversification	Mutagenesis	Mutagenesis
Transformation and cloning	Required	Not required
Highest affinity Ab reported (M)	10^{-12} [114]	10^{-15} [115]

Table 4
Examples of recombinant antibodies generated to specific targets.

Target	Analyte	Ref.
Human conditions		
Cardiac disease	Cardiac troponin	[132]
	C-reactive protein	[102]
Hormones	Thyroid stimulating hormone	[133]
Haptens		
Environmental contaminants	Atrazine	[134]
Illicit drugs	Morphine-3-glucuronide	[75,135]
Animal disease		
Foot-and-mouth	Non-structural protein 3ABC	[136]
Security		
Biological warfare pathogen	*Brucella melitensis*	[137]
Contaminants		
Foodstuffs	Aflatoxin B1	[74]

3.4.2. Phage display

3.4.2.1. Introduction. Phage display has become a widely used selection platform for antibodies since it was first described in 1985 by Smith and co-workers [138]. Use of the novel bacteriophage lambda expression system, which allows rapid identification of antibodies, was suggested as a method to supersede hybridoma technology [139]. McCafferty and co-workers isolated an antibody from a large combinatorial library [140], and, due to its robust nature, phage display has become the 'work-horse' of antibody isolation [141]. Library diversities of up to 10^{10} have been achieved [73]. Antibody selection methods based on the use of immobilised antigen [142,143], antigen-coated magnetic beads in solution [144–146], Biacore™ [147,148], surface-displayed targets [149,150], mammalian tissue culture [151] and *in vivo* approaches [151,152] have all been applied successfully in phage display.

3.4.2.2. Principle. Filamentous phage particles contain single stranded DNA (ssDNA) and are capable of infecting *Escherichia coli* cells. Filamentous phage, illustrated in Fig. 6, infect and replicate without killing the host cell [153], unlike lytic phage (e.g. T4). The phage coat contains 5 different proteins (pIII, pVI, pVII, pVIII and pIX) [141,154] where the pVIII (~50 amino acids) is the major coat protein and covers the surface of the phage cylinder. The hydrophobic 33 amino acid pVII (5 particles) and the 32 amino acid pIX proteins are located at one end of the phage. The other end contains the pVI (112 amino acids) and pIII (406 amino acids) [154].The pIII consists of the N1, N2 and CT domains. The interaction of the phage with the cell is facilitated by the pIII protein (specifically N1 and N2 domains) and phage biology is well understood and documented [141,155]. The N1 domain is required during infection of the cell and facilitates translocation of DNA into the cytoplasm [141]. The N2 region is responsible for binding to F pilus [141,154]. The

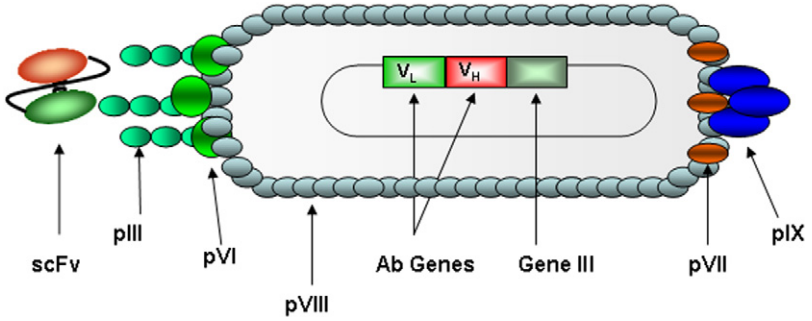

Fig. 6. Phage displaying scFv. ScFv displayed as a fusion to the pIII phage coat protein. The phage is still infective to *E. coli* via pIII-mediated attachment to F pili. pIII proteins contain N1 and N2 domains responsible for interaction with the F pilus of the cell and insertion of DNA into the cytoplasm.

CT domain is essential for the formation of a stable phage particle [154]. Antibodies consist of heterodimeric heavy and light chain variable domains that combine to form the antigen-binding site. For the phage display of scFv (Fig. 6) the two domains are linked using a polypeptide linker and integrated into the phage genome as a fusion to the gene of the pIII protein.

The development of phagemid vectors [142] has taken over from initial use of phage vectors [139] due in part to the simplistic transition to soluble expression of the antibody fragment, using an amber stop codon mutation between the geneIII and the antibody chain [91,156]. This means that the antibody fragment can be expressed without continued fusion to phage particle by use of non-suppressor strains of *E. coli* [91,156,157]. Phage systems are also limited as fusions of large polypeptides to the amino acid terminus of the coat protein, lead to compromised protein function [158]. Phagemid systems permit the display of polypeptides that could not be displayed in simple phage systems by fusion to an additional coat protein gene encoded on the phagemid vector. This attenuates effects of the fusion protein as the wild-type coat proteins are available from the helper phage [158]. Table 5 outlines considerations to be taken into account with choosing phage or phagemid methods of recombinant antibody production.

Phagemid vectors contain *E. coli* and phage origins of replication. The phagemid DNA is packaged in the phage coat and propagation is achieved by super-infection with helper phage [141]. This infection step allows the phagemid vector in the cell to be packaged into the phage particle in an identical way to phage DNA. The helper phage serves to provide the proteins and enzymes necessary for phage replication and also the structural proteins required for encapsulation. To prevent over expression of the helper phage genome, commercially available helper phage contain a defective origin of replication (M13KO7 or VCSM13) and/or packaging signal [141,153,154].

Table 5
Considerations when choosing phage or phagemid system for antibody production (this table was adapted from [154,159]).

Phage	Phagemid
Better suited to peptide display	Suited to antibody display
Large proteins effect pIII production and assembly	Carries the fusion-coat protein to be displayed (e.g. pIII)
Large proteins present on the pIII can affect infectivity	Large inserts are better maintained
Lower transformation efficiency	Higher transformation efficiency
Misfolding of large protein fusions are likely	Multiple copies of gene inserts per phage particle (valency)
No requirement for helper phage	Requires helper phage to select
Avidity effects—lower affinity antibodies	Monovalent display—higher affinity antibodies
Sub-cloning required for soluble antibody expression	Direct expression of soluble antibody
Not suited for affinity maturation	Suited for affinity maturation

Selection is achieved by multiple rounds of binding to a target which selectively enriches phage with the relevant cognate antibody fragment expressed on its surface (Fig. 7) [157]. Specific phage are eluted and re-infected into *E. coli* for subsequent sequential rounds of panning [141] or soluble expression of antibody in non-suppressor strains which facilitates downstream purification and characterisation of the selected antibody fragments [91].

3.4.2.3. Selection considerations. Despite phage display being widely practiced and mainstream in many molecular laboratories [73], the successful selection of specific antibodies remains a challenge [107]. The influence of selection conditions (Fig. 7) is a major factor determining the quality of the selected antibody fragment. Not all standard conditions lead to the selection of phage particles displaying antibody specific to a particular antigen. Selection is aimed at isolation of high-affinity antibodies, however, the level of display may preferentially recover the antibody with the highest display level (avidity) on an individual phage particle. Monovalent display has been utilised to combat this phenomenon [73,153,159].

After transformation, the phage selection cycle (steps 2–6) is sequentially reiterated in order to isolate phage displaying specific antibodies. In theory, only one round of selection from a phage library is necessary, however, in practice non-specific binding of "sticky" phage limits the enrichment that can be achieved. Typically between two to five rounds of biopanning are required to isolate specific antibody-displaying phage as the ratio of binders to non-binders before and after each round of selection varies from 5- to 1000-fold [159].

Extensive research has demonstrated the need to evaluate selection conditions for individual libraries. Conditions such as elution strategy, stringency of washing, solid or solution phase methodologies for panning, antigen form and number of cycles (increasing cycles leading to decreased diversity) have been evaluated [160,161].

Antigen presentation is of particular importance and several presentation strategies are illustrated in Fig. 7. Conformational changes due to direct immobilisation on solid supports [91] or use of peptide antigens can result in the selection of antibodies that cannot recognise native epitopes in the context of the whole target molecule. One particular publication showed that affinity-driven selections of mutated scFv on immobilised antigen preferentially selected spontaneously dimerising scFv, with higher apparent K_d values, due to avidity effects compared to selection on biotinylated antigen with streptavidin magnetic beads [143]. Lowering antigen concentration in successive rounds of selection enriches for higher affinity antibodies similar to B-cell selection *in vivo* [73,159]. Low-concentration selections are utilised to select antibodies with the highest affinity avoiding multimer formation [159]. Selection can also be tailored to favour affinity or

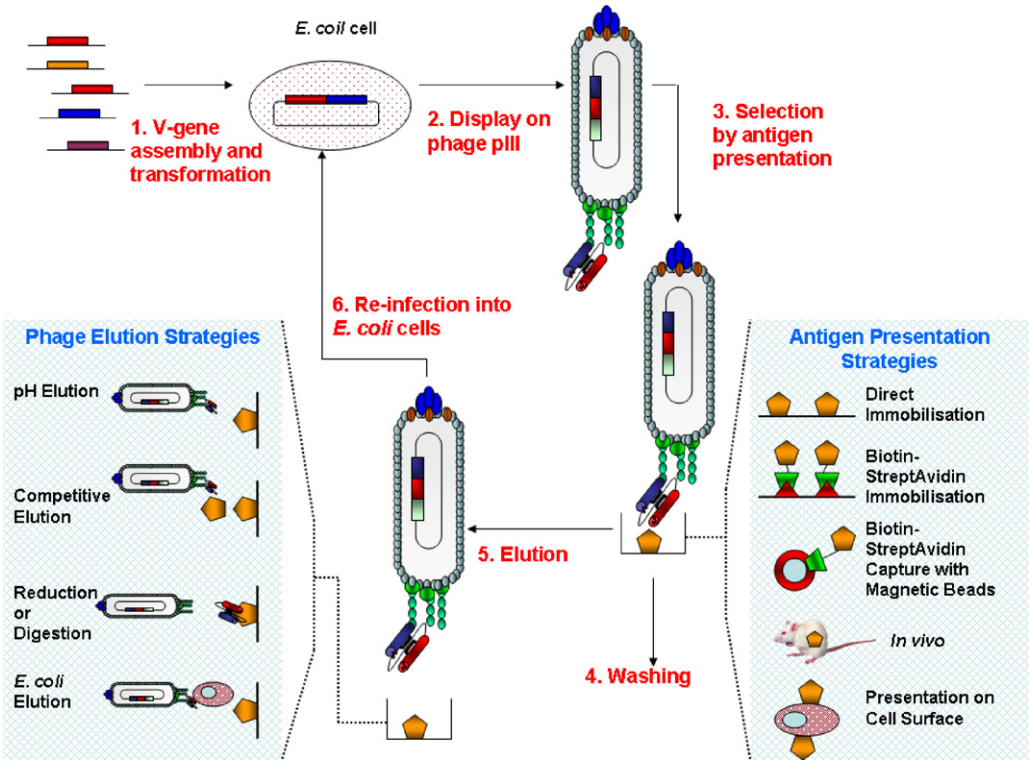

Fig. 7. Phage selection strategy. Iterative selection shown here is a multistep process. V gene assembly by PCR allows cloning of the recombinant antibody (scFv is shown) library into *E. coli* cells. Subsequent rescue by helper phage allows for the propagation of the phage antibody library. Antigen presentation is a key consideration and there are many modes of antigen-phage antibody interaction. Serially increasing the stringency of washing after antigen presentation selects those phage displaying antibodies with highly specific binding properties. Elution strategies are also illustrated. This figure was adapted from [107].

'off-rate' kinetics by gradual limitation of available antigen [157], limiting interaction time of phage with the antigen, performing solution phase selection or competitive interaction with free antigen.

The first round of selection should be regarded as an enrichment step, as excessive stringency leads to a decrease in the library diversity. The multiplicity of infection of helper phage is also of considerable importance. It is imperative to add sufficient phage (10–20:1) to infect the whole library [162]. Failure to do so in the initial amplification of the library leads to a reduction in diversity.

Suitable blocking agents to reduce non-specific binding are required and typical agents are semi-skimmed milk powder and BSA. Inclusion of detergents such as Tween-20 in washing steps and in phage preparation helps to reduce non-specific interaction also [159]. Initially, each phage is represented in low numbers and washing should be less stringent to allow recovery of all antigen-bound phage. After the initial cycle, the stringency can be increased as the phage are present in higher numbers and it is necessary to exert selective pressure to isolate strong binders [159]. When panning with immobilised antigen, washing can be performed, by rinsing the wells or the immunotube, with an increasing number of washes per round with a wash solution, e.g PBS containing Tween-20 (0.05%, v/v) [161]. Panning in solution, usually involves using magnetic beads coated with the biotinylated antigen to capture phage-displaying antibodies [159]. Subsequently, the beads are washed and then recovered using a magnet. Panning by cell surface display (e.g. surface/membrane proteins) requires centrifugation and re-suspension in wash solution [159].

Elution can be achieved in a number of ways (Fig. 7). Elution by altering pH uses acidic (e.g. glycine-HCl, pH 2.2 [163,164]) or basic (e.g triethylamine [160,165]) solutions followed by immediate neutralisation with a suitable reagent. Neutralisation is critical to prevent protein denaturation. It is also very necessary to optimise elution as increasingly stringent elution may be required as affinity increases [148]. Elution, without breaking the antigen-antibody interaction, can be facilitated by trypsin [166], cleavage of a protease-sensitive site [167], NHS-SS-biotin cleavage by DTT [168] or use of commercially available paramagnetic beads containing a nuclease-cleavable DNA linker between the bead and streptavidin [169]. These methods of specific site cleavage provide a means of decreasing background phage elution. Interaction between the phage and the *E. coli* cell can also be utilised to elute phage through the natural affinity for F pili [165].

Dual recognition panning can be useful allowing for selection against a particular peptide in solid or solution phase using biotin capture and a second selection step presenting the peptide in its natural form displayed on a protein captured by polyclonal or monoclonal antibody (Fig. 8). This re-use of the eluted phage without amplification for the next round of selection may prove useful to diminish high-background problems as described for protease inhibitors (fast screen method) [170].

Other advanced display methods include 'Pathfinder' [171] and selectively infective phage (SIP) [172]. Pathfinder is based on the *in situ* biotinylation of phage bound to target antigen using antigen-specific antibodies conjugated to HRP in the presence of biotin tyramine and results in the recovery of tagged phage [171]. SIP displays the antibody library on non-infective phage particles due to the deletion of part of the pIII protein (N1 or N2 or both regions) [172]. The infectivity of the phage is restored by interaction of the antibody with the antigen fused to the region of the pIII responsible for infection with F pili [157]. Fusions of peptide antigen to the F pilus, which abrogates wild type phage infection, have also been exploited [173]. Infectivity is then restored by interaction of scFv-displaying phage with the peptide.

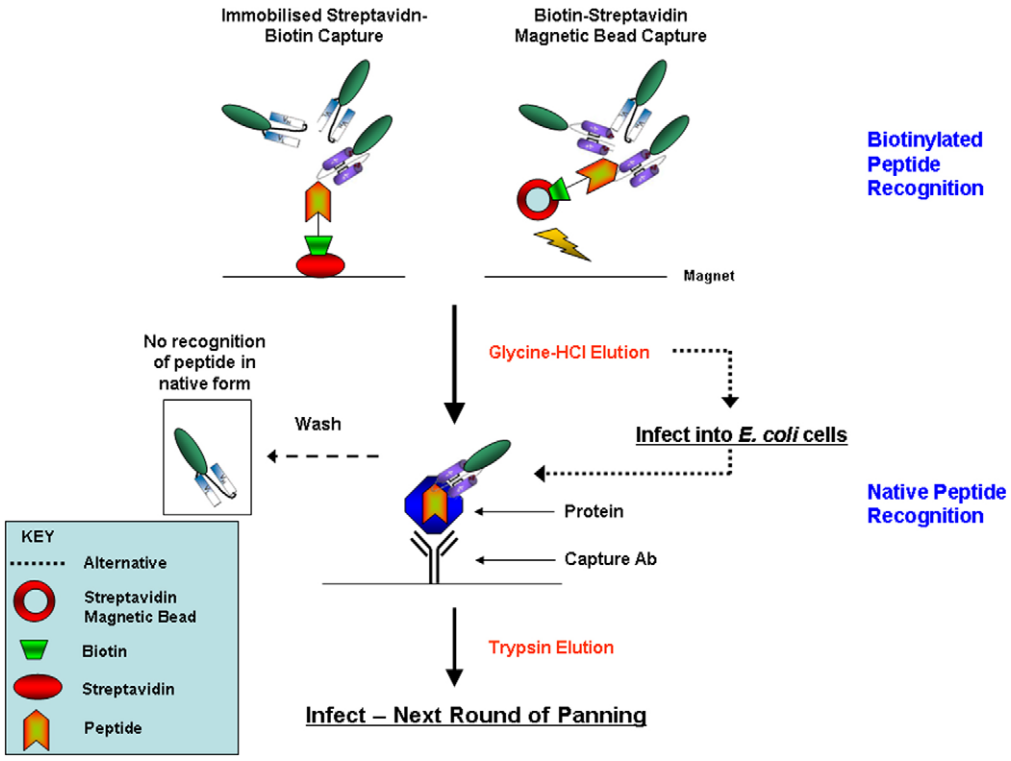

Fig. 8. Dual recognition panning. Panning against peptide and protein in native form. The strategy shown here involves dual recognition in one round of biopanning. Initially, antibody-displaying phage with specificity for the peptide are isolated and immediately challenged against the peptide in its native form on the protein. This strategy is quite stringent and may be modified by alternative rounds of peptide panning followed by panning against the peptide in its native form, thus, allowing phage to enrich before the second selection step.

4. Designing and developing recombinant antibodies

4.1. Mutagenesis

Mutagenesis introduces errors or changes in genetic material which gives rise to modified proteins, products or functions. Mutagenesis was successfully adopted as a method of directed molecular evolution. Evolution *in vitro* was proven to be a powerful tool for protein generation and refinement, with improvements in binding affinity, folding efficiency and enhanced thermodynamic stability being demonstrated [174].

Mutagenesis of genes is generally carried out using two main strategies. Site-directed mutagenesis introduces errors focused in a particular region (e.g. CDR or antibody conserved regions). Random mutagenesis causes scattered mutations across the gene in a stochastic manner and utilises error prone PCR and/or DNA shuffling. Fig. 9 outlines mutagenesis strategies for improvement in affinity of selected antibodies by manipulation of the V genes [175].

4.2. Ribosome display

4.2.1. Introduction

Ribosome display is a totally 'cell-free' method of selection and evolution of proteins that is not limited by any cell-based translation steps [175–178]. It is an *in vitro* selection method for the isolation of proteins and peptides from large libraries [179] that was successfully applied to the affinity maturation of antibodies using eukaryotic or prokaryotic systems. The use of ribosome display overcomes current limitations for protein selection technologies as diversity is not limited by transformation efficiency but by the physical number of ribosomes present and different mRNA molecules available [179] in *vitro*. Ribosome display couples the individual emerging protein (phenotypes) with its corresponding mRNA (genotype) by the formation of stable protein–ribosome–mRNA (PRM) complexes, and, after selection, the amplification of corresponding DNA for concurrent selection or diversification [180].

4.2.2. Principle

The generation of antibody–ribosome–mRNA (ARM) complexes [105] was developed based on two experimental achievements: (i) the production of single chain antibodies *in vitro* using rabbit reticulocyte lysate [181] and (ii) the experimental demonstration that nascent proteins can remain stably associated with their mRNA as a polypeptide–ribosome–mRNA complex in the absence of a stop codon [182,183] or with the inclusion of antibiotics [183]. The generation of the ARM complex is illustrated in Fig. 10, and allows for the stalled complex to be selected by interaction of the nascent protein with cognate antigen. This captured complex permits the recovery of the genetic information directly from the ribosome-bound mRNA [105].

Ribosome display (Fig. 10) has two important functions: (i) it allows selection, using proofreading polymerases and antigen-coated beads or plates [184] and (ii) evolution of antibody affinity. The combination of both of these functions allows for the selection of specific antibodies, from a combinatorial library, whilst simultaneously evolving the protein by successive diversification of the pool with each round [88,110,185,186].

The ribosome display construct generally is composed of the T7 promoter, a ribosome-binding site, a translational enhancer, such as the β-globin gene of *Xenopus laevis*, a translational initiation signal, such as Shine-Dalgarno (prokaryotic—*E. coli*) or Kozak (eukaryotic—rabbit reticulocyte) sequence [183,186,188] and an open reading frame (ORF) in which the library is fused to a C-terminal polypeptide spacer containing no stop codon sequence [177]. The absence of a stop codon is crucial as it facilitates recovery of the intact, stalled ARM complex. Spacer length is of importance

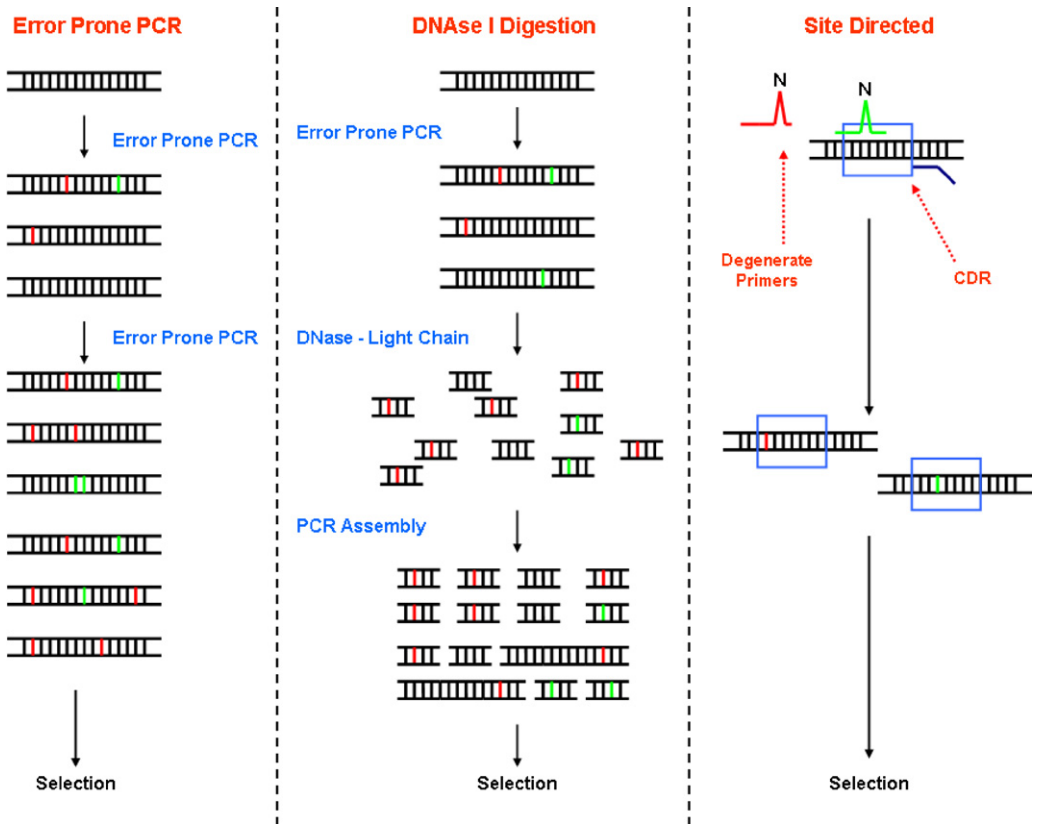

Fig. 9. Mutagenesis strategies. Strategies for the introduction of mutations for improving affinity of recombinant antibodies. Advantageous mutations (red) and deleterious mutations (green) are shown. Error prone strategies introduce mutations randomly across the gene. With increasing number of PCR cycles more mutations are introduced. The useful mutations can be masked by deleterious mutations and DNA shuffling of the light chains offers a solution by cleaving the gene with DNase I digestion and allowing the gene to reassemble randomly. Site-directed mutagenesis of the CDR regions can also be achieved using degenerate primers to introduce mutations in the antigen recognition sites. This figure was adapted from [174].

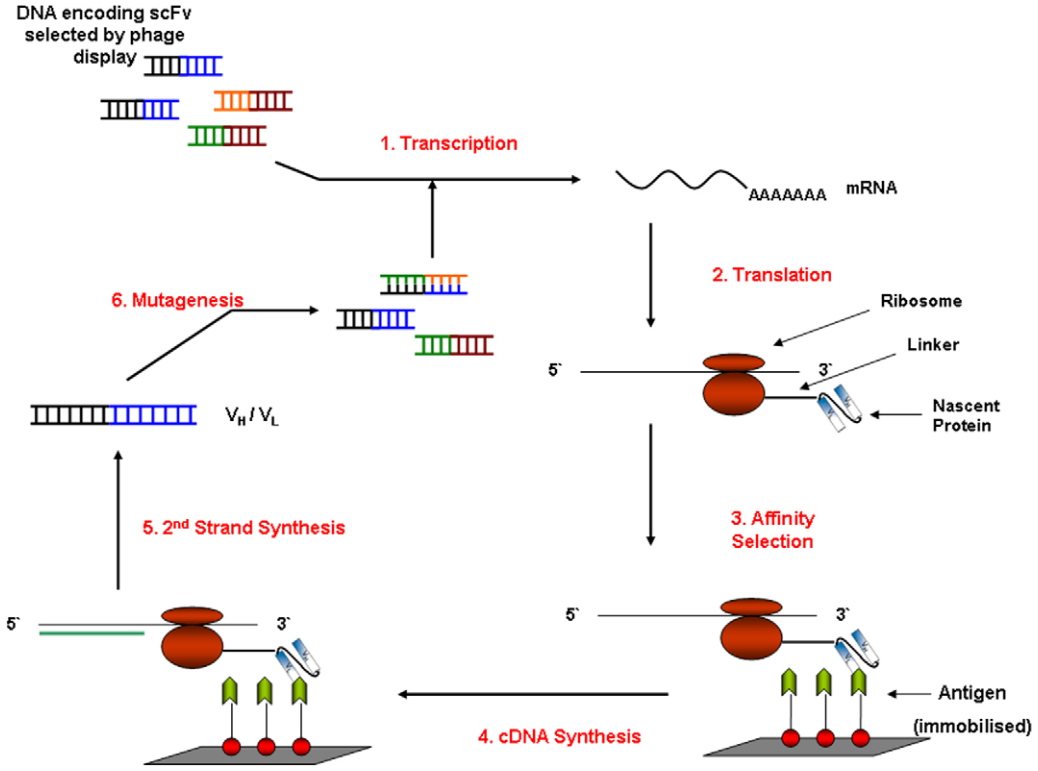

Fig. 10. Overview of eukaryotic ribosome display—selection and evolution of affinity. Selection based on ARM complex formed by stalling the ribosome. The complex can then be isolated by affinity interaction with biotinylated antigen. RT-PCR then allows for the *in situ* recovery of the mRNA encoding for specific ARM complexes. Affinity evolution can be facilitated via error prone PCR (random) light chain shuffling (random) or CDR mutagenesis (directed). This figure was adapted from [105,180,187].

Table 6
Effect of translational factors on efficiency [88,175,186,187,190].

Translational factor	Effect
Temperature	Enzymatic activity of polymerase. Degradation of mRNA by RNases. Folding efficiency of synthesised protein.
Time	Longer translational times in eukaryotic versus prokaryotic systems. Critical in uncoupled systems as mRNA is continually produced. Accumulation of small MW molecules from hydrolysis of triphosphates with longer translation times. Inactivation of α-subunit of initiation factor 2 (eIF-2) by Ca^{2+}.
Additives	PDI (Protein disulphide isomerase) catalysing formation of disulphide bonds. 10sRNA inhibition by anti-sense DNA-oligonucleotides.

as it facilitates the nascent protein folding into its correct conformation by ensuring exit from the ribosomal tunnel. Typically, at least 20–30 amino acids are required and a spacer of 116 amino acids was shown to be more efficient in displaying proteins [189]. The spacer also provides a known sequence for designing primers for the RT-PCR recovery of the selected library [180]. Commonly used spacer sequences include human Cκ and filamentous geneIII [177,189]. The inclusion of 5′ and 3′ stem loop structures was demonstrated to improve mRNA stability by protecting it against RNases [186,187].

4.2.3. Considerations

For antibody generation it was shown that folding efficiency is affected by the inclusion of transcription stabilisers, as transcription requires reducing conditions whilst translation requires oxidising conditions. Hence, the enzymatic activity of transcription (T7 RNA polymerase) requires monitoring in the absence of reducing reagents and, where reducing agents are used for transcription, oxidising conditions are required for the subsequent translational step [187,190].

Time, temperature and the inclusion of various additives for *in vitro* translation each impact the yield and efficiency of ribosome display. These effects are summarised briefly in Table 6.

4.3. Genetically coded tag's for biosensor development

The inclusion of certain peptide tags in recombinant antibody expression vectors offers a rapid and reliable method of purification and characterisation. These tags may also prove useful as capture sites in biosensor development. Oligohisitidine tagging of antibody fragments is hugely beneficial for the purification process [191] but may also be useful in non-covalent coupling of the antibody to the sensor surface using anti-histidine monoclonal antibodies [192], or potentially direct coupling to sensor surfaces, as demonstrated by Lori and co-workers [193].

FLAG (DYKDDDDK) residues incorporated into antibody fragments have also been demonstrated as a useful "capture" method for recombinant protein characterisation, as demonstrated by Mersich and co-workers, where the detection of FLAG-tag fusion proteins using anti-FLAG monoclonal antibodies in a SPR-based system was performed [194].

The AviD tag is a neutravidin- (or avidin)-specific moiety that has useful applications in the immobilisation and purification of recombinant proteins [195]. Despite the absence of examples of biosensors utilising this specific AviD tag, there have been reports of the use of avidin, streptavidin and neutravidin surfaces immobilising biotinlyated antibodies for use in biosensors [196–198].

Purification using streptavidin-binding peptide (SBP tag) has also been demonstrated as a useful approach [199]. The SBP tag interacts specifically with streptavidin but is a weaker interaction than between biotin and avidin. Hence, dissociation can be controlled and this SBP tag was utilised in surface preparation as demonstrated in both SPR- [200] and TIRF-based [201] systems.

5. High-throughput screening

The real value of these large and highly diverse recombinant libraries can only be efficiently exploited if the most judicious and discriminatory selection and enrichment regimes are employed. This is particularly true in the case of extremely large ribosome displayed libraries and especially following focused *in vitro* directed evolution campaigns that can generate large pools of closely related yet heterogeneous clones. In order to exhaustively 'mine' such libraries it is important to ensure that a suitably comprehensive number of individual clones, rather than a small representative subset, are analysed. Interestingly, some of the most useful means of accomplishing such high-throughput data-rich ranking of binding interactions is provided by the newest generation of multiplexed SPR-based biosensors. Currently the GE Healthcare Biacore®A100 instrument offers extremely high-sensitivity kinetic ranking of ligand interactions [102,202]. It can accommodate multiple 96- or 384-well plates in a temperature-controlled integrated rack housing. Four flow cells, each containing five interaction spots, employ hydrodynamic addressing to control interaction flow paths. Dedicated LIMS integration software facilitates compatibility with existing LIMS systems that are now increasingly established in high-throughput screening labs. The ProteOn XPR36 system from Bio-Rad is a unique 6 × 6 multichannel SPR platform that enables automated multiplex analysis of up to 36 biomolecular interactions in one experiment [203–205]. Such systems are anticipated to become mainstream in antibody screening laboratories in the coming years.

6. Applications of antibody-based biosensors

Whilst numerous useful diagnostic kits exist for a multitude of disease states, such as cardiac disease [206] (James and co-workers evaluate commercially available monoclonal antibody-based kits) and biological threat detection, e.g. Raptor™ [69,207,208], few biosensor devices based on recombinant antibody technology are commercially available. Biosensors have huge potential in the areas of clinical diagnosis/monitoring, environmental and food safety, biothreat analysis on the battlefield and counter terrorism [209]. In addition, POC testing can remove the need for long delays by providing relatively short testing times. However, for POC and other biosensor-based detection devices to become mainstream, current biological formats require reductions in size, sample and reagent volume requirements coupled with significant advances in reliability, ease-of use multi-analyte and high-throughput capabilities [210]. Table 7 outlines some examples of recombinant antibody-based biosensors that incorporate the platforms mentioned in Section 2.2.

A search of the literature only yields a few recombinant antibody-related biosensor publications, however, we would predict that this will change dramatically in the near future. Much of the initial investigation into biosensor transduction elements utilise commercially available antibodies (typically monoclonal or polyclonal) to demonstrate the technology and generate useful diagnostic systems. Merging the field of antibody generation/characterisation with transduction, surface and engineering sciences, nanotechnology and microfluidics will propel the applications of recombinant antibody-based sensors forward rapidly. Key factors are the ability to enhance specificity, sensitivity, stability and orientation/immobilisation for optimised antibody performance. The employment of highly specific and sensitive antibodies capable

Table 7
Recombinant antibody-based biosensors.

Analyte	Antibody form	Transducer	Ref.
Disease			
HIV-1 virion infectivity factor	scFv (VH and VHD)	Piezoelectric	[211]
L. monocytogenes	scFv	SPR	[212]
SARS virus	scFv	Imaging ellipsometry	[213]
L. monocytogenes	scFv (phage bound)	Amperometric	[214]
Biowarfare			
Venezuelan equine encephalitis virus	scFv	Potentiometric	[215]
B. anthracis S-layer protein	scFv	Resonant mirror	[216]
Haptens			
Morphine-3-glucuronide	scFv	SPR	[217]
Contaminants			
Aflatoxin B1	scFv (mono- and dimeric)	SPR	[218]
Parathion (insecticide)	scFv	Piezoelectric	[219]
Atrazine	scab	Amperometric	[220]

of deployment on nano-devices with the capacity to determine pM concentrations in nanoliter volumes, for the determination of cancer or cardiac disease-related markers, is the ultimate challenge.

Acknowledgements

All authors are supported by *Science Foundation Ireland* under CSET Grant no. 05/CE3/B754. PJC is also supported by *Irish Research Council for Science, Engineering and Technology* (IRCSET) Embark Scholarship.

References

[1] Vo-Dinh T, Cullum B. Biosensors and biochips: advances in biological and medical diagnostics. Fresen J Anal Chem 2000;366(6–7):540–51.
[2] Hock B, Seifert M, Kramer K. Engineering receptors and antibodies for biosensors. Biosens Bioelectron 2002;17(3):239–49.
[3] Turner AP, Chen B, Piletsky SA. In vitro diagnostics in diabetes: meeting the challenge. Clin Chem 1999;45(9):1596–601.
[4] Harlow E, Lane D. Antibodies: a laboratory manual. New York: Cold Spring Harbor Laboratory Press; 1988.
[5] McCullough KC, Summerfield A. Basic concepts of immune response and defence development. ILAR J 2005;46(3):230–40.
[6] Mader S. Biology. 8th ed. New York: McGraw-Hill; 2004.
[7] Harlow E, Lane D. Using antibodies: a laboratory manual. New York: Cold Spring Harbor Laboratory Press; 1999.
[8] Padoa CJ, Crowther J. Engineered antibodies: a new tool for use in diabetes research. Diab Res Clin Pract 2006;74S(2):S51–62.
[9] Ohlin M, Zouali M. The human antibody repertoire to infectious agents: implications for disease pathogenesis. Mol Immunol 2003;40(1):1–11.
[10] Brown TA. Genomes 3. 3rd ed. New York: Garland Science; 2007.
[11] Di Noia JM, Neuberger MS. Molecular mechanisms of antibody somatic hypermutation. Annu Rev Biochem 2007;76:1–22.
[12] Li Z, Woo CJ, Iglesias-Ussel MD, Ronai D, Scharff MD. The generation of antibody diversity through somatic hypermutation and class switch recombination. Genes Dev 2004;18(1):1–11.
[13] D'Orazio P. Biosensors in clinical chemistry. Clin Chim Acta 2003;334(1–2):41–69.
[14] Vo-Dinh T, Tromberg BG, Griffin GD, Ambrose KR, Sepaniak MJ, Gardenhire EM. Antibody-based fiberoptics biosensor for the carcinogen benzo(*a*)pyrene. Appl Spectrosc 1987;41(5):735–8.
[15] Jiang X, Li D, Xu X, Ying Y, Li Y, Ye Z, et al. Immunosensors for detection of pesticide residues. Biosens Bioelectron 2008;23(11):1577–87.
[16] Patel Pd. (Bio)sensors for measurement of analytes implicated in food safety: a review. Trend Anal Chem 2002;21(2):96–115.
[17] Luppa PB, Sokoll LJ, Chan DW. Immunosensors—principles and applications to clinical chemistry. Clin Chim Acta 2001;314(1–2):1–26.
[18] Leonard P, Hearty S, Brennan J, Dunne L, Quinn J, Chakraborty T, et al. Advances in biosensors for detection of pathogen in food and water. Enzyme Microbial Technol 2003;32(1):3–13.
[19] Kirwan SM, Rocchitta G, McMahon CP, Craig JD, Killoran SJ, O'Brien KB, et al. Modifications of poly(*o*-phenylenediamine) permselective layer on Pt–Ir for biosensor application in neurochemical monitoring. Sensors 2007;7(4):420–37.
[20] Pohanka M, Jun D, Kuca K. Mycotoxin assays using biosensor technology: a review. Drug Chem Toxicol 2007;30(3):253–61.
[21] Marco MP, Gee S, Hammock BB. Immunochemical techniques for environmental analysis. I. Immunosensors. Trend Anal Chem 1995;4(7):341–50.
[22] Schoning MJ, Poghossian A. Recent advances in biologically sensitive field-effect transistors (BioFETs). Analyst 2002;127(9):1137–51.
[23] Kamahori M, Ishige Y, Shimoda M. A novel enzyme immunoassay based on potentiometric measurement of molecular adsorption events by an extended-gate field-effect transistor sensor. Biosens Bioelectron 2007;22(12):3080–5.
[24] Tully E, Higson SP, O'Kennedy R. The development of a 'labeless' immunosensor for the detection of *Listeria monocytogenes* cell surface protein, Internalin B. Biosens Bioelectron 2008;23(6):906–12.
[25] Mutlu S, Cokeliler D, Shard A, Goktas H, Ozansoy B, Mutlu M. Preparation and characterization of ethylenediamine and cysteamine plasma polymerized films on piezoelectric quartz crystal surfaces for a biosensor. Thin Solid Films 2008;516(6):1249–55.
[26] McGovern JP, Shih WY, Rest R, Purohit M, Pandya Y, Shih WH. Label-free flow-enhanced specific detection of *Bacillus anthracis* using a piezoelectric microcantilever sensor. Analyst 2008;133(5):649–54.
[27] Mohrle BP, Kohler K, Jaehrling J, Brock R, Gauglitz G. Label-free characterization of cell adhesion using reflectometric interference spectroscopy (RIfS). Anal Bioanal Chem 2006;384(2):407–13.
[28] Schipper EF, Rauchalles S, Kooyman RP, Hock B, Greve J. The waveguide Mach-Zender interferometer as atrazine sensor. Anal Chem 1998;70(6):1192–7.
[29] Kim N, Kim DK, Cho YJ, Moon DK, Kim WY. Carp vitellogenin detection by an optical waveguide lightmode spectroscopy biosensor. Biosens Bioelectron 2008;24:391–6.
[30] Nemeth E, Adányi N, Halasz A, Váradi M, Szendro I. Real-time study of the effect of different stress factors on lactic acid bacteria by electrochemical optical waveguide lightmode spectroscopy. Biomol Eng 2007;24(6):631–7.
[31] Käppel ND, Pröll F, Gauglitz G. Development of a TIRF-based biosensor for sensitive detection of progesterone in bovine milk. Biosens Bioelectron 2007;22(9–10):2295–300.
[32] Engstrom HA, Andersson PO, Ohlson S. A label-free continuous total-internal-reflection-fluorescence-based immunosensor. Anal Biochem 2006;357(2):159–66.
[33] Dominici C, Schirone A, Celebre M, Ahluwalia A, De Rossi D. Development of a TIRF immunosensor: modelling the equilibrium behaviour of a competitive system. Biosens Bioelectron 1995;10(3–4):371–8.
[34] Ruckstuhl T, Rankl M, Seeger S. Highly sensitive biosensing using a supercritical angle fluorescence (SAF) instrument. Biosens Bioelectron 2003;18(9):1193–9.
[35] Homola J. Surface plasmon resonance sensors for detection of chemical and biological species. Chem Rev 2008;108(2):462–93.
[36] Homola J, Yee SS, Gauglitz G. Surface plasmon resonance sensors: review. Sens Actuat B: Chem 1999;54(1–2):3–15.
[37] GE Healthcare. Biacore; 2008. Available at: www.biacore.com.
[38] Buckle PE, Davies RJ, Kinning T, Yeung D, Edwards PR, Pollardknight D, et al. The resonant mirror—a novel optical sensor for direct sensing of biomolecular interactions. 2. Applications. Biosens Bioelectron 1993;8(7–8):355–63.
[39] Mosiello L, Laconi L, Del Gallo M, Ercole C, Lepidi A. Development of a monoclonal antibody based potentiometric biosensor for terbuthylazine detection. Sens Actuat B: Chem 2003;95(1–3):315–20.
[40] Tang D, Yuan R, Chai Y, Zhong X, Liu Y, Dai J. Electrochemical detection of hepatitis B surface antigen using colloidal gold nanoparticles modified by a sol–gel network interface. Clin Biochem 2006;39(3):309–14.
[41] Tang DP, Ren JJ. Direct and rapid detection of diphtherotoxin via potentiometric immunosensor based on nanoparticles mixture and polyvinyl butyral as matrixes. Electroanalysis 2005;17(24):2208–16.
[42] Theegala CS, Small DD, Monroe WT. Oxygen electrode-based single antibody amperometric biosensor for qualitative detection of *E. coli* and bacteria in water. J Environ Sci Health A: Tox Hazard Subst Environ Eng 2008;43(5):478–87.
[43] Zhuo Y, Yuan PX, Yuan R, Chai YQ, Hong CL. Nanostructured conductive material containing ferrocenyl for reagentless amperometric immunosensors. Biomaterials 2008;29(10):1501–8.

[44] Micheli L, Grecco R, Badea M, Moscone D, Palleschi G. An electrochemical immunosensor for aflatoxin M1 determination in milk using screen-printed electrodes. Biosens Bioelectron 2005;21(4):588–96.
[45] Carralero V, Gonzalez-Cortes A, Yanez-Sedeno P, Pingarron JM. Nanostructured progesterone immunosensor using a tyrosinase-colloidal gold-graphite-Teflon biosensor as amperometric transducer. Anal Chim Acta 2007;596(1):86–91.
[46] Su XL, Li Y. A self-assembled monolayer-based piezoelectric immunosensor for rapid detection of *Escherichia coli* O157:H7. Biosens Bioelectron 2004;19(6):563–74.
[47] Arce L, Zougagh M, Arce C, Moreno A, Rios A, Valcarcel M. Self-assembled monolayer-based piezoelectric flow immunosensor for the determination of canine immunoglobulin. Biosens Bioelectron 2007;22(12):3217–23.
[48] Halámek J, Makower A, Knosche K, Skládal P, Scheller FW. Piezoelectric affinity sensors for cocaine and cholinesterase inhibitors. Talanta 2005;65(2):337–42.
[49] Príbyl J, Hepel M, Halámek J, Skládal P. Development of piezoelectric immunosensors for competitive and direct determination of atrazine. Sens Actuat B: Chem 2003;91(1–3):333–41.
[50] Campbell GA, Delesdernier D, Mutharasan R. Detection of airborne *Bacillus anthracis* spores by an integrated system of an air sampler and a cantilever immunosensor. Sens Actuat B: Chem 2007;127(2):376–82.
[51] Pohanka M, Pavlis O, Skládal P. Diagnosis of tularemia using piezoelectric biosensor technology. Talanta 2007;71(2):981–5.
[52] Skottrup P, Hearty S, Frokiaer H, Leonard P, Hejgaard J, O'Kennedy R, et al. Detection of fungal spores using a generic surface plasmon resonance immunoassay. Biosens Bioelectron 2007;22(11):2724–9.
[53] Tsutsumi T, Miyoshi N, Sasaki K, Maitani T. Biosensor immunoassay for the screening of dioxin-like polychlorinated biphenyls in retail fish. Anal Chim Acta 2008;617(1–2):177–83.
[54] Bulukin E, Meucci V, Minunni M, Pretti C, Intorre L, Soldani G, et al. An optical immunosensor for rapid vitellogenin detection in plasma from carp (*Cyprinus carpio*). Talanta 2007;72(2):785–90.
[55] Wei D, Oyarzabal OA, Huang TS, Balasubramanian S, Sista S, Simoman AL. Development of a surface plasmon resonance biosensor for the identification of *Campylobacter jejuni*. J Microbiol Meth 2007;69(1):78–85.
[56] Hearty S, Leonard P, Quinn J, O'Kennedy R. Production, characterisation and potential application of a novel monoclonal antibody for rapid identification of virulent *Listeria monocytogenes*. J Microbiol Meth 2006;66(2):294–312.
[57] Llamas NM, Stewart L, Fodey T, Higgins HC, Velasco ML, Botana LM, et al. Development of a novel immunobiosensor method for the rapid detection of okadaic acid contamination in shellfish extracts. Anal Bioanal Chem 2007;389(2):581–7.
[58] Lathrop AA, Jaradat ZW, Haley T, Bhunia AK. Characterization and application of a *Listeria monocytogenes* reactive monoclonal antibody C11E9 in a resonant mirror biosensor. J Immunol Meth 2003;281(1–2):119–28.
[59] Tschmelak J, Kumpf M, Käppel N, Proll G, Gauglitz G. Total internal reflectance fluorescence (TIRF) biossensor for environmental monitoring of testosterone with commercially available immunochemistry: antibody characterization, assay development and real sample measurements. Talanta 2006;69(2):343–50.
[60] Tschmelak J, Proll G, Gauglitz G. Immunosensor for estrone with an equal limit of detection as common analytical methods. Anal Bioanal Chem 2004;378(3):744–5.
[61] Nagel T, Ehrentreich-Forster E, Singh M, Schmitt K, Brandenburg A, Berka A, et al. Direct detection of tuberculosis infection in blood serum using three optical label-free approaches. Sens Actuat B: Chem 2008;129(2):934–40.
[62] Szekacs A, Trummer N, Adányi N, Váradi M, Szendro I. Development of a non-labeled immunosensor for the herbicide trifluralin via optical waveguide lightmode spectroscopic detection. Anal Chim Acta 2003;487(1):31–42.
[63] Kim N, Kim DK, Kim WY. Sulfamethazine detection with direct-binding optical waveguide lightmode spectroscopy-based immunosensor. Food Chem 2008;108(2):768–73.
[64] Wang D, Jiang D, Yuan C. Affinity aspects of HBsAb-HBsAg interaction on the liquid solid interface. Colloids Surf A: Physiochem Eng Aspects 2000;175(1–2):129–34.
[65] Nabok AV, Tsargorodskaya A, Holloway A, Starodub NF, Gojster O. Registration of T-2 mycotoxin with total internal reflection ellipsometry and QCM impedance methods. Biosens Bioelectron 2007;22(6):885–90.
[66] Bae YM, Park KW, Oh BK, Lee WH, Choi JW. Immunosensor for detection of *Salmonella typhimurium* based on imaging ellipsometry. Colloids Surf A: Physicochem Eng Aspects 2005;(257–58):19–23.
[67] Geng T, Morgan MT, Bhunia AK. Detection of low levels of *Listeria monocytogenes* cells by using a fiber-optic immunosensor. Appl Environ Microbiol 2004;70(10):6138–46.
[68] Tims TB, Lim DV. Rapid detection of *Bacillus anthracis* spores directly from powders with an evanescent wave fiber-optic biosensor. J Microbiol Meth 2004;59(1):127–30.
[69] Jung CC, Saaski EW, McCrae DA, Lingerfelt BM, Anderson GP. RAPTOR: a fluoroimmunoassay-based fiber optic sensor for detection of biological threats. IEEE Sens J 2003;3(4):352–60.
[70] Kendall LV. Production of polyclonal antibodies. In: Howard GC, Kaser MR, editors. Making and using antibodies: a laboratory manual. Florida: CRC Press; 2007. p. 41–72.
[71] Leenaars M, Hendriksen CF. Critical steps in the production of polyclonal and monoclonal antibodies: evaluation and recommendations. ILAR J 2005;46(3):269–79.
[72] Sheehan KC. Production of monoclonal antibodies. In: Howard GC, Kaser MR, editors. Making and using antibodies: a laboratory manual. Florida: CRC Press; 2007. p. 73–94.
[73] Hoogenboom HR. Selecting and screening recombinant antibody libraries. Nat Biotechnol 2005;23(9):1105–16.
[74] Daly SJ, Dillon PP, Manning BM, Dunne L, Killard A, O'Kennedy R. Production and characterization of murine single chain Fv antibodies to aflatoxin B-1 derived from a pre-immunized antibody phage display library system. Food Agric Immunol 2002;14(4):255–74.
[75] Townsend S, Finlay WJ, Hearty S, O'Kennedy R. Optimizing recombinant antibody function in SPR immunosensing. The influence of antibody structural format and chip surface chemistry on assay sensitivity. Biosens Bioelectron 2006;22(2):268–74.
[76] Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256(5517):495–7.
[77] Winter G, Milstein C. Man-made antibodies. Nature 1991;349(6307):293–9.
[78] Abbott. i-STAT(R) precision; 2008. Available at: http://www.abbottpointofcare.com/istat/www/products/index.htm.
[79] Roche. Cardiac proBNP assay; 2008. Available at: http://www.roche.com/home/products/prod_diag_roche-cardiac.htm.
[80] Skerra A, Plückthun A. Assembly of a functional immunoglobulin Fv fragment in *Escherichia coli*. Science 1988;240(4855):1038–41.
[81] Plückthun A, Skerra A. Expression of functional antibody Fv and Fab fragments in *Escherichia coli*. Meth Enzymol 1989;178:497–515.
[82] Racher AJ, Moreira JL, Alves PM, Wirth M, Weidle UH, Hauser H, et al. Expression of recombinant antibody and secreted alkaline phosphatase in mammalian cells. Influence of cell line and culture system upon production kinetics. Appl Microbiol Biotechnol 1994;40(6):851–6.
[83] Dorai H, McCartney JE, Hudziak RM, Tai MS, Laminet AA, Houston LL, et al. Mammalian cell expression of single-chain Fv (sFv) antibody proteins and their C-terminal fusions with interleukin-2 and other effector domains. Biotechnology (NY) 1994;12(9):890–7.
[84] Reavy B, Ziegler A, Diplexcito J, Macintosh SM, Torrance L, Mayo M. Expression of functional recombinant antibody molecules in insect cell expression systems. Protein Expr Purif 2000;18(2):221–8.
[85] Edelman L, Margaritte C, Chaabihi H, Monchatre E, Blanchard D, Cardona A, et al. Obtaining a functional recombinant anti-rhesus (D) antibody using the baculovirus-insect cell expression system. Immunology 1997;91(1):13–9.
[86] Boder ET, Wittrup KD. Yeast surface display for screening combinatorial polypeptide libraries. Nat Biotechnol 1997;15(6):553–7.
[87] Whitelam GC, Cockburn W, Owen MR. Antibody production in transgenic plants. Biochem Soc Trans 1994;22(4):940–4.
[88] Hanes J, Plückthun A. In vitro selection and evolution of functional proteins by using ribosome display. Proc Natl Acad Sci USA 1997;94(10):4937–42.
[89] Verma R, Boleti E, George AJ. Antibody engineering: comparison of bacterial, yeast, insect and mammalian expression systems. J Immunol Meth 1998;216(1–2):165–81.
[90] Griffiths AD, Duncan AR. Strategies for selection of antibodies by phage display. Curr Opin Biotechnol 1998;9(1):102–8.
[91] Azzazy HM, Highsmith Jr WE. Phage display technology: clinical applications and recent innovations. Clin Biochem 2002;35(6):425–45.
[92] Krebber A, Bornhauser S, Burmester J, Honegger A, Willuda J, Bosshard HR, et al. Reliable cloning of functional antibody variable domains from hybridomas and spleen cell repertoires employing a reengineered phage display system. J Immunol Meth 1997;201(1):35–55.
[93] Bird RE, Hardman KD, Jacobson JW, Johnson S, Kaufman BM, Lee SM, et al. Single-chain antigen-binding proteins. Science 1988;242(4877):423–6.
[94] Glockshuber R, Malia M, Pfitzinger I, Plückthun A. A comparison of strategies to stabilize immunoglobulin Fv-fragments. Biochemistry 1990;29(6):1362–7.
[95] Tang Y, Jiang N, Parakh C, Hilvert D. Selection of linkers for a catalytic single-chain antibody using phage display technology. J Biol Chem 1996;271(26):15682–6.
[96] Atwell JL, Breheney KA, Lawrence LJ, McCoy AJ, Kortt AA, Hudson PJ. scFv multimers of the anti-neuraminidase antibody NC10: length of the linker between VH and VL domains dictates precisely the transition between diabodies and triabodies. Protein Eng 1999;12(7):597–604.
[97] Muller BH, Chevrier D, Boulain JC, Guesdon JL. Recombinant single-chain Fv antibody fragment-alkaline phosphatase conjugate for one-step immunodetection in molecular hybridization. J Immunol Meth 1999;227(1–2):177–85.
[98] Mousli M, Turki I, Kharmachi H, Saadi M, Dellagi K. Recombinant single-chain Fv antibody fragment-alkaline phosphatase conjugate: a novel in vitro tool to estimate rabies viral glycoprotein antigen in vaccine manufacture. J Virol Meth 2007;146(1–2):246–56.
[99] Liu M, Wang X, Yin C, Zhang Z, Lin Q, Zhen Y, et al. A novel bivalent single-chain variable fragment (scFV) inhibits the action of tumour necrosis factor alpha. Biotechnol Appl Biochem 2008;50(Pt 4):173–9.
[100] Lindner P, Bauer K, Krebber A, Nieba L, Kremmer E, Krebber C, et al. Specific detection of his-tagged proteins with recombinant anti-His tag scFv-phosphatase or scFv-phage fusions. Biotechniques 1997;22(1):140–9.
[101] de Wildt RM, Mundy CR, Gorick BD, Tomlinson IM. Antibody arrays for high-throughput screening of antibody–antigen interactions. Nat Biotechnol 2000;18(9):989–94.
[102] Leonard P, Safsten P, Hearty S, McDonnell B, Finlay W, O'Kennedy R. High throughput ranking of recombinant avian scFv antibody fragments from crude lysates using the Biacore A100. J Immunol Meth 2007;323(2):172–9.

[103] Ling MM. Large antibody display libraries for isolation of high-affinity antibodies. Comb Chem High Throughput Scr 2003;6(5):421–32.
[104] Rader C, Barbas III CF. Phage display of combinatorial antibody libraries. Curr Opin Biotechnol 1997;8(4):503–8.
[105] He M, Taussig MJ. Antibody-ribosome-mRNA (ARM) complexes as efficient selection particles for in vitro display and evolution of antibody combining sites. Nucl Acids Res 1997;25(24):5132–4.
[106] He M, Taussig MJ. Handbook of therapeutic antibodies. In: Dubel S, editor. Emerging developments. Wiley-VCH; 2007.
[107] 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.
[108] Razai A, Garcia-Rodriguez C, Lou J, Geren IN, Forsyth CM, Robles Y, et al. Molecular evolution of antibody affinity for sensitive detection of botulinum neurotoxin type A. J Mol Biol 2005;351(1):158–69.
[109] Stahl S, Uhlen M. Bacterial surface display: trends and progress. Trends Biotechnol 1997;15(5):185–92.
[110] Lipovsek D, Plückthun A. In-vitro protein evolution by ribosome display and mRNA display. J Immunol Meth 2004;290(1–2):51–67.
[111] Xu L, Aha P, Gu K, Kuimelis RG, Kurz M, Lam T, et al. Directed evolution of high-affinity antibody mimics using mRNA display. Chem Biol 2002;9(8): 933–42.
[112] Griffiths AD, Williams SC, Hartley O, Tomlinson IM, Waterhouse P, Crosby WL, et al. Isolation of high affinity human antibodies directly from large synthetic repertoires. EMBO J 1994;13(14):3245–60.
[113] 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 Meth 2001;248(1–2):31–45.
[114] Schier R, McCall A, Adams GP, Marshall KW, Merritt H, Yim M, et al. 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 1996;263(4):551–67.
[115] Zahnd C, Spinelli S, Luginbühl B, Amstutz P, Cambillau C, Plückthun A. Directed in vitro evolution and crystallographic analysis of a peptide-binding single chain antibody fragment (scFv) with low picomolar affinity. J Biol Chem 2004;279(18):18870–7.
[116] Rader C. Antibody libraries in drug and target discovery. Drug Discov Today 2001;6(1):36–43.
[117] Liguori MJ, Hoff-Velk JA, Ostrow DH. Recombinant human interleukin-6 enhances the immunoglobulin secretion of a rabbit–rabbit hybridoma. Hybridoma 2001;20(3):189–98.
[118] Saini SS, Kaushik A, Basrur PK, Yamashiro S. Ultrastructural and immunologic characteristics of mouse × cattle xenogeneic hybridomas originating from bovine leukemia virus-infected cattle. Vet Pathol 2003;40(4):460–4.
[119] Matsuda H, Mitsuda H, Nakamura N, Furusawa S, Mohri S, Kitamoto T. A chicken monoclonal antibody with specificity for the N-terminal of human prion protein. FEMS Immunol Med Microbiol 1999;23(3):189–94.
[120] Yu X, McGraw PA, House FS, Crowe Jr JE. An optimized electrofusion-based protocol for generating virus-specific human monoclonal antibodies. J Immunol Meth 2008;336(2):142–51.
[121] 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 Meth 2000;242(1–2):159–81.
[122] Barbas III CF, Amberg W, Simoncsits A, Jones TM, Lerner RA. Selection of human anti-hapten antibodies from semisynthetic libraries. Gene 1993;137(1):57–62.
[123] Clackson T, Hoogenboom HR, Griffiths AD, Winter G. Making antibody fragments using phage display libraries. Nature 1991;352(6336):624–8.
[124] Yamanaka HI, Inoue T, Ikeda-Tanaka O. Chicken monoclonal antibody isolated by a phage display system. J Immunol 1996;157(3):1156–62.
[125] Ridder R, Schmitz R, Legay F, Gram H. Generation of rabbit monoclonal antibody fragments from a combinatorial phage display library and their production in the yeast *Pichia pastoris*. Biotechnology (NY) 1995;13(3):255–60.
[126] Lang IM, Barbas III CF, Schleef RR. Recombinant rabbit Fab with binding activity to type-1 plasminogen activator inhibitor derived from a phage-display library against human alpha-granules. Gene 1996;172(2):295–8.
[127] Alvarez-Rueda N, Behar G, Ferre V, Pugniere M, Roquet F, Gastinel L, et al. Generation of llama single-domain antibodies against methotrexate, a prototypical hapten. Mol Immunol 2007;44(7):1680–90.
[128] Liu JL, Anderson GP, Goldman ER. Isolation of anti-toxin single domain antibodies from a semi-synthetic spiny dogfish shark display library. BMC Biotechnol 2007;7:78.
[129] Kim YJ, Lebreton F, Kaiser C, Cruciere C, Remond M. Isolation of foot-and-mouth disease virus specific bovine antibody fragments from phage display libraries. J Immunol Meth 2004;286(1–2):155–66.
[130] Li Y, Kilpatrick J, Whitelam GC. Sheep monoclonal antibody fragments generated using a phage display system. J Immunol Meth 2000;236(1–2):133–46.
[131] Park SG, Jeong YJ, Lee YY, Kim IJ, Seo SK, Kim EJ, et al. Hepatitis B virus-neutralizing anti-pre-S1 human antibody fragments from large naive antibody phage library. Antiviral Res 2005;68(3):109–15.
[132] Masson JF, Battaglia TM, Khairallah P, Beaudoin S, Booksh KS. Quantitative measurement of cardiac markers in undiluted serum. Anal Chem 2007;79(2):612–9.
[133] Ylikotila J, Valimaa L, Vehniainen M, Takalo H, Lovgren T, Pettersson K. A sensitive TSH assay in spot-coated microwells utilizing recombinant antibody fragments. J Immunol Meth 2005;306(1–2):104–14.
[134] Charlton K, Harris WJ, Porter AJ. The isolation of super-sensitive anti-hapten antibodies from combinatorial antibody libraries derived from sheep. Biosens Bioelectron 2001;16(9–12):639–46.
[135] Brennan J, Dillon P, O'Kennedy R. Production, purification and characterisation of genetically derived scFv and bifunctional antibody fragments capable of detecting illicit drug residues. J Chromatogr B: Anal Technol Biomed Life Sci 2003;786(1–2):327–42.
[136] Foord AJ, Muller JD, Yu M, Wang LF, Heine HG. Production and application of recombinant antibodies to foot-and-mouth disease virus non-structural protein 3ABC. J Immunol Meth 2007;321(1–2):142–51.
[137] 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 Meth 2003;276(1–2):185–96.
[138] Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 1985;228(4705):1315–7.
[139] Huse WD, Sastry L, Iverson SA, Kang AS, Alting-Mees M, Burton DR, et al. Generation of a large combinatorial library of the immunoglobulin repertoire in phage lambda. Science 1989;246(4935):1275–81.
[140] McCafferty J, Griffiths AD, Winter G, Chiswell DJ. Phage antibodies: filamentous phage displaying antibody variable domains. Nature 1990;348 (6301):552–4.
[141] Kehoe JW, Kay BK. Filamentous phage display in the new millennium. Chem Rev 2005;105(11):4056–72.
[142] Barbas III CF, Kang AS, Lerner RA, Benkovic SJ. Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. Proc Natl Acad Sci USA 1991;88(18):7978–82.
[143] Schier R, Bye J, Apell G, McCall A, Adams GP, Malmqvist M, et al. Isolation of high-affinity monomeric human anti-c-erbB-2 single chain Fv using affinity-driven selection. J Mol Biol 1996;255(1):28–43.
[144] Nagumo Y, Oguri H, Tsumoto K, Shindo Y, Hirama M, Tsumuraya T, et al. Phage-display selection of antibodies to the left end of CTX3C using synthetic fragments. J Immunol Meth 2004;289(1–2):137–46.
[145] Gao X, Huang Y, Zhu S. Construction of murine phage antibody library and selection of ricin-specific single-chain antibodies. IUBMB Life 1999;48(5):513–7.
[146] Wassaf D, Kuang G, Kopacz K, Wu QL, Nguyen Q, Toews M, et al. High-throughput affinity ranking of antibodies using surface plasmon resonance microarrays. Anal Biochem 2006;351(2):241–53.
[147] Malmborg AC, Duenas M, Ohlin M, Soderlind E, Borrebaeck CA. Selection of binders from phage displayed antibody libraries using the BIAcore biosensor. J Immunol Meth 1996;198(1):51–7.
[148] Schier R, Marks JD. Efficient in vitro affinity maturation of phage antibodies using BIAcore guided selections. Hum Antibodies Hybrid 1996;7(3):97–105.
[149] Figini M, Obici L, Mezzanzanica D, Griffiths A, Colnaghi MI, Winter G, et al. Panning phage antibody libraries on cells: isolation of human Fab fragments against ovarian carcinoma using guided selection. Cancer Res 1998;58(5):991–6.
[150] 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(3):774–84.
[151] Mutuberria R, Hoogenboom HR, van der Linden E, de Bruine AP, Roovers RC. Model systems to study the parameters determining the success of phage antibody selections on complex antigens. J Immunol Meth 1999;231(1–2): 65–81.
[152] Pasqualini R, Ruoslahti E. Organ targeting in vivo using phage display peptide libraries. Nature 1996;380(6572):364–6.
[153] Paschke M. Phage display systems and their applications. Appl Microbiol Biotechnol 2006;70(1):2–11.
[154] Barbas III CF, Burton DR, Scott JK, Silverman GJ. Phage display: a laboratory manual. New York: Cold Spring Harbor Laboratory Press; 2001.
[155] Iannolo G, Minenkova O, Petruzzelli R, Cesareni G. Modifying filamentous phage capsid: limits in the size of the major capsid protein. J Mol Biol 1995;248(4):835–44.
[156] Hoogenboom HR, Griffiths AD, Johnson KS, Chiswell DJ, Hudson P, Winter G. Multi-subunit proteins on the surface of filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains. Nucl Acids Res 1991;19(15):4133–7.
[157] Hoogenboom HR, de Bruine AP, Hufton SE, Hoet RM, Arends JW, Roovers RC. Antibody phage display technology and its applications. Immunotechnology 1998;4(1):1–20.
[158] Sidhu SS, Fairbrother WJ, Deshayes K. Exploring protein–protein interactions with phage display. Chembiochem 2003;4(1):14–25.
[159] Bradbury AR, Marks JD. Antibodies from phage antibody libraries. J Immunol Meth 2004;290(1–2):29–49.
[160] de Bruin R, Spelt K, Mol J, Koes R, Quattrocchio F. Selection of high-affinity phage antibodies from phage display libraries. Nat Biotechnol 1999;17(4):397–9.
[161] Lou J, Marzari R, Verzillo V, Ferrero F, Pak D, Sheng M, et al. Antibodies in haystacks: how selection strategy influences the outcome of selection from molecular diversity libraries. J Immunol Meth 2001;253(1–2):233–42.
[162] Lo BK. Antibody engineering: methods and protocols, 1st ed. Humana; 2004.
[163] Roberts BL, Markland W, Siranosian K, Saxena MJ, Guterman SK, Ladner RC. Protease inhibitor display M13 phage: selection of high-affinity neutrophil elastase inhibitors. Gene 1992;121(1):9–15.

[164] Kang AS, Barbas CF, Janda KD, Benkovic SJ, Lerner RA. Linkage of recognition and replication functions by assembling combinatorial antibody Fab libraries along phage surfaces. Proc Natl Acad Sci USA 1991;88(10):4363–6.
[165] Wind T, Stausbol-Gron B, Kjaer S, Kahns L, Jensen KH, Clark BF. Retrieval of phage displayed scFv fragments using direct bacterial elution. J Immunol Meth 1997;209(1):75–83.
[166] Engberg J, Andersen PS, Nielsen LK, Dziegiel M, Johansen LK, Albrechtsen B. Phage-display libraries of murine and human antibody Fab fragments. Mol Biotechnol 1996;6(3):287–310.
[167] Ward RL, Clark MA, Lees J, Hawkins NJ. Retrieval of human antibodies from phage-display libraries using enzymatic cleavage. J Immunol Meth 1996;189(1):73–82.
[168] Cendron AC, Wines BD, Brownlee RT, Ramsland PA, Pietersz GA, Hogarth PM. An FcgammaRIIa-binding peptide that mimics the interaction between FcgammaRIIa and IgG. Mol Immunol 2008;45(2):307–19.
[169] Santala V, Saviranta P. Affinity-independent elution of antibody-displaying phages using cleavable DNA linker containing streptavidin beads. J Immunol Meth 2004;284(1–2):159–63.
[170] Markland W, Ley AC, Lee SW, Ladner RC. Iterative optimization of high-affinity proteases inhibitors using phage display. 1. Plasmin. Biochemistry 1996;35(24):8045–57.
[171] Osbourn JK, Derbyshire EJ, Vaughan TJ, Field AW, Johnson KS. Pathfinder selection: in situ isolation of novel antibodies. Immunotechnology 1998;3(4):293–302.
[172] Jung S, Arndt KM, Muller KM, Plückthun A. Selectively infective phage (SIP) technology: scope and limitations. J Immunol Meth 1999;231(1–2):93–104.
[173] Dall'Acqua W, Carter P. Antibody engineering. Curr Opin Struct Biol 1998;8(4):443–50.
[174] Mössner E, Plückthun A. Directed evolution with fast and efficient selection technologies. Chimia 2001;55(4):324–8.
[175] Jermutus L, Ryabova LA, Plückthun A. Recent advances in producing and selecting functional proteins by using cell-free translation. Curr Opin Biotechnol 1998;9(5):534–48.
[176] Jermutus L, Honegger A, Schwesinger F, Hanes J, Plückthun A. Tailoring in vitro evolution for protein affinity or stability. Proc Natl Acad Sci USA 2001;98(1):75–80.
[177] Amstutz P, Plückthun A, Zahnd C. Ribosome display: in vitro selection of protein–protein interactions, 3rd ed. Elsevier Acedemic Press; 2006.
[178] Villemagne D, Jackson R, Douthwaite JA. Highly efficient ribosome display selection by use of purified components for in vitro translation. J Immunol Meth 2006;313(1–2):140–8.
[179] Zahnd C, Amstutz P, Plückthun A. Ribosome display: selecting and evolving proteins in vitro that specifically bind to a target. Nat Meth 2007;4(3):269–79.
[180] He M, Taussig MJ. Ribosome display: cell-free protein display technology. Brief Funct Genom Proteom 2002;1(2):204–12.
[181] Nicholls PJ, Johnson VG, Andrew SM, Hoogenboom HR, Raus JC, Youle RJ. Characterization of single-chain antibody (sFv)-toxin fusion proteins produced in vitro in rabbit reticulocyte lysate. J Biol Chem 1993;268(7):5302–8.
[182] Fedorov AN, Baldwin TO. Contribution of cotranslational folding to the rate of formation of native protein structure. Proc Natl Acad Sci USA 1995;92(4):1227–31.
[183] He M, Taussig MJ. Ribosome display of antibodies: expression, specificity and recovery in a eukaryotic system. J Immunol Meth 2005;297(1–2):73–82.
[184] Coia G, Pontes-Braz L, Nuttall SD, Hudson PJ, Irving RA. Panning and selection of proteins using ribosome display. J Immunol Meth 2001;254(1–2):191–7.
[185] 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 USA 1998;95(24):14130–5.
[186] Hanes J, Jermutus L, Plückthun A. Selecting and evolving functional proteins in vitro by ribosome display. Meth Enzymol 2000;328:404–30.
[187] Plückthun A, Schaffitzel C, Hanes J, Jermutus L. In vitro selection and evolution of proteins. Adv Protein Chem 2000;55:367–403.
[188] Schaffitzel C, Zahnd C, Amstutz P, Luginbühl B, Plückthun A. In vitro selection and evolution of protein–ligand interactions by ribosome display. 2nd ed. New York: Cold Spring Harbor Laboratory Press; 2001.
[189] Schaffitzel C, Hanes J, Jermutus L, Plückthun A. Ribosome display: an in vitro method for selection and evolution of antibodies from libraries. J Immunol Meth 1999;231(1–2):119–35.
[190] Hanes J, Jermutus L, Schaffitzel C, Plückthun A. Comparison of *Escherichia coli* and rabbit reticulocyte ribosome display systems. FEBS Lett 1999;450(1–2):105–10.
[191] Graslund S, Nordlund P, Weigelt J, Hallberg BM, Bray J, Gileadi O, et al. Protein production and purification. Nat Meth 2008;5(2):135–46.
[192] Scibek JJ, Evergren E, Zahn S, Canziani GA, Van Ryk D, Chaiken IM. Biosensor analysis of dynamics of interleukin 5 receptor subunit beta(c) interaction with IL5:IL5R(alpha) complexes. Anal Biochem 2002;307(2):258–65.
[193] Lori JA, Morrin A, Killard AJ, Smyth MR. Development and characterization of nickel-NTA-polyaniline modified electrodes. Electroanalysis 2006;18(1):77–81.
[194] Mersich C, Jungbauer A. Generic method for quantification of FLAG-tagged fusion proteins by a real time biosensor. J Biochem Biophys Meth 2007;70(4):555–63.
[195] Gaj T, Meyer SC, Ghosh I. The AviD-tag, a NeutrAvidin/avidin specific peptide affinity tag for the immobilization and purification of recombinant proteins. Protein Expr Purif 2007;56:54–61.
[196] Helali S, Fredj HB, Cherif K, Abdelghani A, Martelet C, Jaffrezic-Renault N. Surface plasmon resonance and impedance spectroscopy on gold electrode for biosensor application. Mater Sci Eng C 2008;28(5–6):588–93.
[197] Cui XQ, Pei RJ, Wang XZ, Yang F, Ma Y, Dong SJ, et al. Layer-by-layer assembly of multilayer films composed of avidin and biotin-labeled antibody for immunosensing. Biosens Bioelectron 2003;18(1):59–67.
[198] Dutra RF, Kubota LT. An SPR immunosensor for human cardiac troponin T using specific binding avidin to biotin at carboxymethyldextran-modified gold chip. Clin Chim Acta 2007;376(1–2):114–20.
[199] Keefe AD, Wilson DS, Seelig B, Szostak JW. One-step purification of recombinant proteins using a nanomolar-affinity streptavidin-binding peptide, the SBP-Tag. Protein Expr Purif 2001;23(3):440–6.
[200] Li YJ, Bi LJ, Zhang XE, Zhou YF, Zhang JB, Chen YY, et al. Reversible immobilization of proteins with streptavidin affinity tags on a surface plasmon resonance biosensor chip. Anal Bioanal Chem 2006;386(5):1321–6.
[201] Piervincenzi RT, Reichert WM, Hellinga HW. Genetic engineering of a single-chain antibody fragment for surface immobilization in an optical biosensor. Biosens Bioelectron 1998;13(3–4):305–12.
[202] Safsten P, Klakamp SL, Drake AW, Karlsson R, Myszka DG. Screening antibody–antigen interactions in parallel using Biacore A100. Anal Biochem 2006;353(2):181–90.
[203] Bravman T, Bronner V, Lavie K, Notcovich A, Papalia GA, Myszka DG. Exploring "one-shot" kinetics and small molecule analysis using the ProteOn XPR36 array biosensor. Anal Biochem 2006;358(2):281–8.
[204] Abdiche Y, Malashock D, Pinkerton A, Pons J. Determining kinetics and affinities of protein interactions using a parallel real-time label-free biosensor, the Octet. Anal Biochem 2008;377(2):209–17.
[205] Abdiche YN, Malashock DS, Pons J. Probing the binding mechanism and affinity of tanezumab, a recombinant humanized anti-NGF monoclonal antibody, using a repertoire of biosensors. Protein Sci 2008;17(8):1326–35.
[206] James S, Flodin M, Johnston N, Lindahl B, Venge P. The antibody configurations of cardiac troponin I assays may determine their clinical performance. Clin Chem 2006;52(5):832–7.
[207] Research International Inc. Raptor(TM); 2008. Available at: http://www.resrchintl.com/raptor-detection-system.html.
[208] Lim DV. Detection of microorganisms and toxins with evanescent wave fiber-optic biosensors. Proc IEEE 2003;91(6):902–7.
[209] Lim DV, Simpson JM, Kearns EA, Kramer MF. Current and developing technologies for monitoring agents of bioterrorism and biowarfare. Clin Microbiol Rev 2005;18(4):583–607.
[210] Healy DA, Hayes CJ, Leonard P, McKenna L, O'Kennedy R. Biosensor developments: application to prostate-specific antigen detection. Trends Biotechnol 2007;25(3):125–31.
[211] Encarnacao JM, Rosa L, Rodrigues R, Pedro L, da Silva FA, Goncalves J, et al. Piezoelectric biosensors for biorecognition analysis: application to the kinetic study of HIV-1 Vif protein binding to recombinant antibodies. J Biotechnol 2007;132(2):142–8.
[212] Nanduri V, Bhunia AK, Tu SI, Paoli GC, Brewster JD. SPR biosensor for the detection of *L. monocytogenes* using phage-displayed antibody. Biosens Bioelectron 2007;23(2):248–52.
[213] Qi C, Duan J, Wang Z, Chen Y, Zhang P, Zhan L, et al. Investigation of interaction between two neutralizing monoclonal antibodies and SARS virus using biosensor based on imaging ellipsometry. Biomed Microdevices 2006;8:247–53.
[214] Benhar I, Eshkenazi I, Neufeld T, Opatowsky J, Shaky S, Rishpon J. Recombinant single chain antibodies in bioelectrochemical sensors. Talanta 2001;55(5):899–907.
[215] Hu WG, Thompson HG, Alvi AZ, Nagata LP, Suresh MR, Fulton RE. Development of immunofiltration assay by light addressable potentiometric sensor with genetically biotinylated recombinant antibody for rapid identification of Venezuelan Equine Encephalitis virus. J Immunol Meth 2004;289(1–2):27–35.
[216] Love TE, Redmond C, Mayers CN. Real time detection of anthrax spores using highly specific anti-EA1 recombinant antibodies produced by competitive panning. J Immunol Meth 2008;334(1–2):1–10.
[217] Dillon PP, Manning BM, Daly SJ, Killard AJ, O'Kennedy R. Production of a recombinant anti-morphine-3-glucuronide single-chain variable fragment (scFv) antibody for the development of a "real-time" biosensor-based immunoassay. J Immunol Meth 2003;276(1–2):151–61.
[218] Dunne L, Daly S, Baxter A, Haughey S, O'Kennedy R. Surface plasmon resonance-based immunoassay for the detection of aflatoxin B1 using single-chain antibody fragments. Spectrosc Lett 2005;38(3):229–45.
[219] Horacek J, Garrett SD, Skládal P, Morgan MRA. Characterization of the interactions between immobilized parathion and the corresponding recombinant scFv antibody using a piezoelectric biosensor. Food Agric Immunol 1998;10(4):363–74.
[220] Grennan K, Strachan G, Porter AJ, Killard AJ, Smyth MR. Atrazine analysis using an amperometric immunosensor based on single-chain antibody fragments and regeneration-free multi-calibrant measurement. Anal Chim Acta 2003;500(1–2):287–98.