

Approaches to allergy detection using aptasensors

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1. Immunoglobulins and IgE

Antibodies are glycoprotein molecules which are produced by plasma cells in response to an immunogen (1). They belong to a class of spherical proteins called globulins and are hence known as immunoglobulins (Igs). Their primary function is to mediate the host immune response by binding to antigens.

Igs have been divided, on the basis of physical and functional properties, into the five following classes: IgG, IgM, IgA, IgD, and IgE. They have a Y-shaped structure and are composed of two identical light chains and two identical heavy chains, which are held together by disulfide bonds (2), as illustrated in Figure 1. Each heavy chain and each light chain has a variable (V) domain and a constant (C) domain. The V domain is responsible for binding to the antigen and the C domain mediates the antibody's function (2).

Figure 1

1.1. IgE

Human IgE is a monomeric immunoglobulin of approximately 190 000 Da (3). Initially termed IgND after its discovery by Johansson in 1967 (4), it was soon confirmed to be identical to the γ E discovered by Ishizaka and Ishizaka (5), and determined to be central in the immediate hypersensitivity reaction pathway (6). Of similar structure to the other immunoglobulins (IgG, IgM, IgD and IgA), it is composed of two light chains and two heavy chains, as shown in Figure 2, and constitutes about 0.0005% of total serum immunoglobulins in adults (3).

Figure 2

1.2. The hypersensitivity reaction

The term “allergy” was originally introduced by von Pirquet in 1906, meaning “changed reactivity” of the host after the second or subsequent contact with an allergenic agent (7). The first evidence of a transferable / soluble factor as the mediator of an allergic reaction was published in 1919 by Ramirez (8), where the author reported on an incident during which a man entering a horse-drawn carriage experienced an acute asthmatic episode two weeks after having received a blood transfusion from a man with a known horse allergy (7). It was not until 1921, however, that Prausnitz and Küster gave the first scientific description of the mechanism of the allergic reaction, showing that hypersensitivity could be transferred from an allergic patient to that of a non-allergic patient through a serum factor (7). Type I hypersensitivity reactions, in which IgE plays a central role, include wheal and flare eruptions of the skin, sneezing, rhinorrhoea and conjunctival irritation (9). More serious conditions include asthma and anaphylaxis, which are believed to share a similar pathogenesis (9).

The production of IgE is mainly under control of T cells and T cell cytokines. T cells can undergo two separate differentiation pathways, namely Th1 and Th2, in response to distinct stimuli, and characterised by the type of cytokines predominantly produced (7). Th1 cells secrete, amongst others, interleukin (IL)-2, interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α) and lymphotoxin (LT). These cytokines mobilise the cellular and humoral defence mechanisms against intracellular pathogens and antagonise IgE responses (9). The cytokines secreted by Th2 cells include IL-4, IL-5, IL-6, IL-9, and IL-13. These cytokines coordinate the host defence against large, extra-cellular pathogens (9).

The hypersensitivity cascade pathway, depicted in Figure 3, begins with the presentation of an antigen / allergen to a T helper cell by an antigen presenting cell (APC) and, in this case, native T cells undergo differentiation along the Th2 pathway, defined by the production of Th2 cytokines (7), as described above. IL-4 and IL-13 then direct the differentiation of B cells to IgE-producing plasma cells (10). This differentiation also requires interaction between the CD40 antigen, a cell surface receptor expressed on B cells, and its ligand (CD154) on the T cell surface (9). The biological activities of the free circulating IgE are then mediated through specific receptors (7): the high-affinity receptor (FcεRI) (see Figure 2) is mainly expressed on mast cells and basophiles, whereas the low affinity receptor (FcεRII) is expressed on B cells. Following binding of IgE to the high affinity receptors, mast cells remain sensitised for up to 12 weeks, thereby greatly increasing the half life of free-circulating serum IgE, which is of approximately two and a half days (7). Re-exposure of sensitized patients to the allergen leads to specific binding of the allergen to the IgE-FcεRI complexes on the mast cells. Cross-linking of the receptors then triggers the release of inflammatory cytokines, chemokines and mediators, such as histamine and heparin (7). It is these molecules that cause the symptoms observed in the allergic response. IgE is thus the “gatekeeper” of immediate type I hypersensitivity.

Figure 3

1.3. Importance of IgE

Allergies represent a very serious health problem, with the prevalence of asthma, hay fever and other IgE-mediated diseases dramatically increasing over the past two to three decades in industrialised countries (7; 11). In the United States, for example, asthma prevalence increased by

75% between 1980 and 1994 (12), with an estimated 15 million individuals affected and more than 5000 asthmatics dying annually (11). In Britain, 23% of children aged six to seven years, and 21% of children aged 12 to 14 years, suffer from asthma (13). Furthermore, in Sweden, the number of children with allergic rhinitis, asthma or eczema doubled during the years 1979 – 1991 (14). In addition, the high degree of morbidity associated with allergic disorders requires large quantities of costly medication to relieve symptoms. In the United States for example, the total annual costs for treating asthma were estimated at \$6 billion in 1997 (15).

A number of theories attempting to explain the observed increase in allergic disease have been put forward, many of them centring on the role of the western lifestyle. Reasons put forth include the increase in air pollution as well as a lack of exposure of the immune system to infections due to increased sanitisation and vaccinations, resulting in the failure of the immune system to “learn” optimally (10). Although there is much evidence to support these hypotheses, the causes remain complex and many unknowns still remain.

The above examples expose the severity and scope of allergic diseases and consequently highlight the importance of IgE as a medical analyte.

1.4. Current diagnosis methods

The primary aim of allergy testing is to determine whether an individual presenting with symptoms of an allergic hypersensitivity has demonstrable allergen-specific IgE. The first step in the diagnostic procedure is an assessment of symptom history and a physical examination. Once the clinician has concluded that there is a high degree of suspicion of an allergic disorder, *in vivo* and *in vitro* analyses for the presence of IgE can be performed to strengthen the likelihood of the

diagnosis being correct (3). *In vivo* testing refers to skin testing, which has become the standard to which other detection methods are compared, and is the primary method for detection of specific IgE (6). *In vitro* tests, examined in more detail below, refer to IgE immunoassays which are used as an alternative to skin tests when these are not practical or possible, or when equivocal results are obtained (6). This could be the case, for example, when an allergenic substance is not available as a licensed extract for skin testing (e.g. latex, industrial chemicals), or when a patient is taking medication that would preclude a skin test (6). Generally, results of skin testing performed under optimal conditions agree with those obtained using specific IgE immunoassays, but experts agree that these two methods are not interchangeable (6). When discrepancies in results exist, the skin test is usually positive and the immunoassay negative, probably due to the limitations of the immunoassays (6). On the other hand, in the case of a positive immunoassay test and a negative skin test, one should question whether the skin test was performed correctly (6). Most studies, however, show a sensitivity of approximately 70 – 90% when immunoassays are compared to skin testing (16; 17).

The first commercial *in vitro* assay designed for the detection of IgE was the Phadebas RAST (Radioallergosorbent Test) (Pharmacia, Uppsala, Sweden). In this format, an allergosorbent was prepared by covalently coupling an allergen of a particular specificity onto cyanogen bromide activated cellulose paper disks. Allergen specific antibody of all isotypes in the serum could then bind after addition of the sample. After washing, radio-iodinated anti-human IgE was used to detect bound IgE. After a second wash, radioactivity relating to the amount of IgE initially present in the sample was measured using a gamma-counter (3). Although the term “RAST” is still commonly used to refer to an immunoassay for allergen specific IgE, it is in fact a trademark name and refers to an assay which is now very rarely used (6). So called “second generation” allergen-specific IgE antibody assays have almost all been based on the RAST chemistry, but use

a larger number and higher quality of allergen extract for the preparation of allergosorbents. Furthermore, the shelf-life, ease of use and safety of these tests have improved due to the use of non-radioactive, enzyme-labelled antibodies. Automation has also improved precision and reproducibility of results (3). Consequently, these assays have become a lot more competitive with the *in vivo* skin tests.

There are, however, a number of problems associated with immunoassays, the greatest of which is the lack of standardisation. This is in most part due to the varying sources of raw allergenic materials, different methods for binding allergen to the detection matrix and different detection systems (6). Although no official test standard exists, the Pharmacia CAP system is in worldwide use and is a *de facto* standard to which other tests are compared (6).

It is important to bear in mind that although these assays are often promoted as allergy diagnosis tests, they should best be regarded as tests indicating the presence or absence of detectable specific IgE (6). Furthermore, specific IgE can be found in patients with allergic diseases as well as in about 15% of normal asymptomatic individuals (6). Immunoassays or skin tests therefore cannot be used to determine whether the patient will exhibit symptoms of IgE mediated hypersensitivity upon exposure to an allergen. Assays for total serum IgE, based on the same format as specific IgE immunoassays, are also available. High serum levels of IgE may correlate to an atopic (i.e. allergic) state in the individual, although the limitations of using total serum IgE as a diagnostic analyte include its age-related concentration and the wide overlap in concentrations between atopic and non-atopic individuals (6). The detection of total IgE therefore cannot result in a definite clinical diagnosis, but rather, would serve as a first-line test to guide clinicians in their decision making process.

1.5. Affinity biosensors

Affinity based biosensors are devices incorporating a Molecular Recognition Element (MRE) such as an antibody, receptor protein, nucleic acid, molecular imprinted polymer (MIP) or aptamer. The use of affinity-based biosensors has increased significantly over the past decade (18). This is due to the progress made in signal transduction technologies, as well as to the fact that MREs, especially antibodies, have become easier to isolate and purify and hence have become more widely available. When an affinity biosensor includes an antibody or antibody-related substance as the MRE, it is known as an immunosensor (19). Affinity biosensors can be further classified based on the MRE incorporated into the device and on the type of transducer used. The following is a brief overview of affinity biosensors and their applications, in particular as applied to the detection of IgE.

1.5.a. Types of affinity biosensor

1.5.a.i. Immunosensors

Although immunosensors have been designed in a variety of different ways, incorporating different transduction technologies, they generally fit in to one of three basic formats, namely direct non-competitive, competitive (direct or indirect) or sandwich (20) (see Figure 4). In the direct non-competitive format, the binding of the antigen to the antibody is directly detected based on the electrochemical or optical properties of the analyte (Figure 4(A)). Surface Plasmon Resonance (SPR), discussed in Section 1.2, is a pertinent example of direct optical detection of the binding event. Competitive assay formats are generally used when the analyte is of a low molecular weight and the binding event cannot be detected directly. In these formats, the signal is

inversely related to the analyte concentration (18) (Figure 4B). The sandwich format is based on the Enzyme Linked Immunosorbent Assay (ELISA) principle, where the analyte tracer is conjugated to an enzyme, such as horseradish peroxidase, and is detected after addition of a substrate (Figure 4C). Most electrochemical transducers are based on this format, where the secondary antibody is usually conjugated to an enzyme capable of generating an electro-active product after addition of a substrate (21).

Although electrochemical transducers are the most well characterised of the transducer technologies (18), they do not lend themselves well to affinity based sensors. This is because most affinity-based reactions are not electrochemically active and therefore cannot participate directly in redox reactions (21). In the case of amperometric immunosensors, coupling of an immuno-reaction to the transducer usually requires a labelled immuno-agent to facilitate the production of electro-active species, which in turn can be detected electrochemically (21). The first report of an amperometric immunosensor was from Aizawa (1979) (22), who used catalase as a label to detect human chorionic gonadotrophin (hCG). Major advantages of this type of electrochemical immunosensor include the ability to detect the label in turbid media, which is not possible with optical sensors, and the ability to increase the surface area in order to increase sensitivity (21). Potentiometric immunosensors, based on charge differences between antibody-antigen complexes and the antibody or antigen alone, ion-selective or gas-sensing electrodes, have also been reported (21). They have, however, been less successful due to lack of sensitivity caused by non-specific binding and other background interferences with the transducer (21). They also require additional time due to the indirect nature of the configurations.

Figure 4

Piezoelectric immunosensors, the most common of which is the quartz crystal microbalance (QCM), have also been widely applied to detect antibody binding to an immobilised antigen (18). This technique is able to measure small changes in surface properties, such as bound surface mass and surface viscosity, which can then be related to the concentration of the analyte in the sample (18; 20). The major advantages of these devices include their small size, high sensitivity and stability, simplicity of construction and operation, and low power requirement (20).

A recent development in the field of immunosensors is the use of antibodies labelled with paramagnetic particles (23; 24). With the antigen immobilised, the binding event can be detected by measuring the magnetic field induced by the magnetic particles. A major advantage of this method is the ability to permanently record the signal, which could, in principle, be re-measured at any time.

The last category is optical immunosensors, for which direct and indirect detection methods exist. Direct methods, requiring no labelling of the antibody or antigen, include internal reflectance spectroscopy, evanescent wave sensing and surface plasmon resonance, which will be examined in detail in Section 1.3.

The above-mentioned methods have also been applied to indirect immunosensing, in which a label, usually fluorescent, is used to monitor the binding event (21). This approach improves the sensitivity and selectivity of the assay, given that the quantitative signal arises only from the presence of the label, thereby eliminating non-specific binding interference.

1.5.a.ii. Other types of affinity biosensors

Other types of affinity based biosensors include DNA biosensors, also known as gene chips. These biosensors are used for recognition and quantitation of target DNA sequences, based on hybridisation of complementary strands to immobilised single-stranded (ss) DNA (20; 21). Electrochemical and optical transduction methods have been applied, using electro-active or fluorescent labels specific for binding to hybridised sequences. Label-free electrochemical detection is also possible through the direct oxidation of guanine bases (25; 26). Fluorescent tags are still the labelling method of choice for DNA biosensors and were first introduced by Affymetrix with the GeneChip, in 1996 (21). Piezoelectric DNA biosensors, capable of detecting the mass change associated with hybridisation, have also been reported (27), as have electrochemical sensors, such as those from Nanogen.

Molecularly imprinted polymers (MIPs) are artificial ligands which possess steric and chemical memory for a template, which they can bind with specificity similar to that of an antibody-antigen interaction (28). They have been employed as non-biological alternatives to antibodies in competitive binding assays, and applications for analytes such as cortisol, theophylline and morphine have been investigated, suggesting that MIPs are a promising technique for use in affinity sensors (19).

Lastly, aptamers are also a very promising technology for the use in immunosensors, and will be discussed in detail in Section 1.3.

1.6. Recent work on IgE biosensors

Early work performed by Su *et al.* (1999) (29) made use of anti-human IgE antibodies immobilised on a piezoelectric sensor for the detection of total IgE in serum samples. The

immunosensor proved to have a sensitivity of 12 μ g/l and could be regenerated five times without appreciable loss of activity. Further work was performed by Kreuzer *et al.* (2001) (30), who described an amperometric immunosensor for the detection of IgE. The sensor used a disposable screen-printed carbon electrode and had a detection limit of 90ng/l of IgE in whole blood, with an analysis time of 30 minutes.

More recently, Liss *et al.* (2002) (31) reported on work performed using a quartz crystal biosensor and compared the performance of a specifically designed aptamer and an antibody for the detection of IgE. The authors compared a well-established, published aptamer, which they subsequently improved on during experimentation, with a commercially available monoclonal anti-IgE antibody. Their work demonstrated an equal specificity and sensitivity (100 μ g/l) of the aptamer with respect to the antibody but with an extended linear detection range to 10-fold higher concentrations of IgE. In addition to this, the study demonstrated for the first time that an aptamer-based biosensor could specifically detect an analyte in a complex protein matrix. Lastly, regeneration of the aptamer receptor layer was shown to be possible.

The work used to illustrate this chapter is based on surface plasmon resonance aptasensors, and as such the theory of surface plasmon resonance and aptamers is expanded on in the Sections 1.2 and 1.3, respectively.

2. Surface Plasmon Resonance

SPR, as applied to biosensors, is a relatively new technology which has been used in a number of fields to determine a desired quantity by measuring the change in refractive index occurring due to the interaction between, for example, a receptor molecule and its ligand (32). The development

of SPR-sensing configurations and applications has been described for the measurement of physical, chemical and biological quantities. It is, however, in the field of affinity biosensors that SPR has shown the greatest potential, allowing real-time (i.e. under continuous flow conditions) analysis of bio-specific interactions without the use of labelled molecules (33).

Much of the success of SPR is due to the fact that it is a fast, reliable and sensitive method that can be used to answer a number of fundamental questions about the interactions of the molecules being investigated. The information that can be gained includes the following (34):

- Concentration determination of the interacting molecules.
- Determination of the association and dissociation rate constants and affinity.
- Determination of active binding regions and relative binding patterns (e.g. epitope mapping).
- Specificity of the interaction (for a particular molecule or a class of molecules).

The potential of SPR for the characterisation of thin molecular films and monitoring processes at metal interfaces was recognised in the late seventies (35; 36). Currently, several companies have commercialised SPR sensor technology, which has become a leading method for the direct real-time observation of biomolecular interactions (37).

2.1. Theory

A surface plasmon (SP) is a charge-density oscillation (wave) that may exist at the interface of two media with dielectric constants of opposite signs, such as a metal and a dielectric (e.g. water)

(32). The wave is strongly localised and propagates along the interface between the metal and the ambient medium (34).

The basic principle behind using SPR as a biospecific-interaction analysis (BIA) technique is as follows. Incident light is used to excite an SP. At the surface plasmon resonance angle, where the energy and momentum of the incident light coincide with that of the charge density wave, the photon energy is transferred to the charge density wave (38). This phenomenon is observed by a sharp dip in intensity of the reflected incident light. The resulting wave propagates along the interface between the metal and the ambient medium and is extremely sensitive to changes in refractive index near the metal surface, for example due to receptor-ligand interactions (38). Changes in the SPR angle, for small angular shifts, are proportional to changes in the refractive index and consequently to the mass concentration of the biomolecules at the surface of the metal, which is the basis of the use of SPR for bio-sensing purposes (34).

The most common setup for SPR applications is the Kretschmann configuration, shown in Figure 5. In this configuration, SPs are excited at the surface of a gold film deposited on a prism. The thickness (d) of the gold film is crucial, as the efficiency of conversion of bulk waves into SPs decreases with increasing thickness and decreasing transparency of the gold film (39). On the other hand, if the film is too thin, SPs are rapidly reconverted into bulk waves, significantly changing the width of the resonance curve and reducing the sensitivity of the SPR measurements (39). The optimal thickness for a gold film using, for example, a wavelength of 790nm, is 45nm (39).

As mentioned above, SPs occur at the interface between two media of dielectric permeabilities of opposite sign, such as between free electron-like metals including silver, aluminium and gold,

and water. Given that the dielectric permeability of any material is a function of the wavelength of the incident light, it follows that for any given interface, the excitation of SPs will not be possible at all wavelengths (39). For metals, the dielectric constant (ϵ_m) is negative in the infrared-visible range of the spectrum, whereas it is positive for water (ϵ_b) within the same range. This therefore allows the existence of SPs at metal-water interfaces (39). Due to its optical and chemical properties, gold is the most commonly used metal for SPR applications, a thin film of which is typically deposited onto a glass substrate (39).

Figure 5

SP waves are distinguished by the fact that they have maximal intensity at the interface, decaying exponentially in both the metal and the ambient medium, with distance from the surface. The SP wave is thus specifically associated with the metal-dielectric interface and is therefore different to a bulk wave. The propagation of electromagnetic waves is characterised by a wave, or propagation, vector. The propagation vectors for the SP wave (k_{sp}) and the bulk wave (k_b) are defined below (39) (see Figure 5):

$$k_{sp} = k_0 [\epsilon_m \epsilon_b / (\epsilon_m + \epsilon_b)]^{1/2} \quad (\text{Equation 1})$$

$$k_b = k_0 \epsilon_b^{1/2} \quad (\text{Equation 2})$$

where k_0 is the propagation vector in a vacuum.

The condition required for resonance, that is to say the condition required for the bulk wave to be transformed to the SP wave, is that the bulk propagation vector be equal to the SP propagation vector (39):

$$k_{sp} = k_b \quad (\text{Equation 3})$$

Furthermore, resonance depends on the angle of incidence, ϕ , in the following way (39):

$$k_{sp} = n_p k_b \sin\phi \quad (\text{Equation 4})$$

where n_p is the refractive index of the prism.

At a particular angle of incidence, k_{sp} will equal k_b and the conditions for resonance will be fulfilled (see Equation 3). At this angle there is maximal coupling of incident light into SPs, which results in maximal absorption of the incident light by the gold film and a resultant sharp decrease (minimum) in the reflection coefficient of the incident light is observed (see Figure 9 (A)). The angle at which the SPR minimum occurs is greatly dependent on all the refractive indices of all the boundary media, including the gold film, the bulk solution in contact with the gold-coated prism and additional layers such as molecules deposited on the gold surface (39).

Generally, k_{sp} increases proportionately with increasing bio layer thickness, resulting in increases in the resonance angle (see Equation 4) (39). Therefore, the thicker the bio layer at the surface, the greater the SPR-minimum shifts to higher angles will be. These shifts can be measured in real time during the adsorption of biomolecules at the gold film surface.

The SPR technique is extremely sensitive to optical thickness changes, and it has been shown that a change in protein surface concentration of 1ng/mm^2 will generate a change of SPR coupling angle of 0.1° (40).

2.2. Concept

Generally, an SPR device consists of an optical system, a transducing medium and an electronic system supporting the optoelectronic components of the sensor and allowing data processing (32). The optical component of the SPR device contains a source of optical radiation and an optical structure in which the SP wave is excited and interrogated. The transducing medium interrelates the optical and bio(chemical) domains, relating changes in the refractive index to changes in the quantity of interest. Its properties also determine the selectivity and response time of the sensor. The sensitivity, stability and resolution of the sensor, on the other hand, depend on the properties of both the optical system and the transducing medium (32). Most modern SPR devices use light emitting diodes (LEDs) as light sources and linear arrays of charge-coupled devices (CCDs) to detect reflected light from the surface. The use of array detectors allows reflected light to be measured at a wide range of angles, thereby avoiding the need to mechanically control the angular position of the detector (39).

2.3. Applications

SPR has been applied to a number of fields, including physics, chemistry and molecular biology. In the field of physics, SPR sensing devices have been developed to exploit certain physical phenomena that occur in various optical transducing materials, such as humidity induced

refractive index changes in porous thin layers and polymers; or temperature sensors based on the thermo-optic effect in hydrogenated amorphous silicon (32).

Most chemical SPR sensors are based on the measurement of SPR variations due to adsorption or a chemical reaction of an analyte with a transducing medium which results in changes in its optical properties (32). Applications of these sensors include, amongst others, monitoring of the concentration of vapours of hydrocarbons, aldehydes and alcohols by adsorption in polyethylene glycol films, and the detection of vapours of aromatic hydrocarbons by their adsorption in Teflon films (32).

It is, however, in the field of molecular biology that SPR has been most successful, with applications including immunological analysis, studies of protein-protein interactions, molecular-biological studies on the mechanisms of gene expression, signal transduction and cell-cell interactions, screening of new ligands, quantification of protein adsorption and immobilisation, the evaluation of surfaces for biocompatibility, epitope mapping, determining affinity constants, and the examination of binding kinetics (39). The first application of SPR to biosensing was demonstrated in 1983 (41). Since then, the technology and consequent breadth of applications has continued to develop. This is due in major part to the fact that it is possible to measure the kinetics of biomolecular interactions in real time with a high degree of sensitivity; and that no labelling of the biomolecules is necessary for their detection (39). Furthermore, analysis of receptor-ligand interactions with a wide range of molecular weights, affinities, binding rates and in numerous different chemical environments is possible (42). Indeed, analyses of analytes with masses ranging from hundreds of Daltons to whole cell binding have been reported (42).

Detection methods are most often direct, where analyte quantification is carried out by direct detection of the binding of the analyte to the immobilised receptor. In cases where the analyte is small and its binding to the receptor does not produce a measurable increase in the refractive index, indirect sandwich or competitive assay methods may be used (32).

Table 1 lists the most common molecular biological applications of SPR biosensors. Examples of the *italicised* applications are given below.

Table 1

As mentioned previously, the use of SPR for the determination of kinetic parameters is a major contributor to its success as an analytical technology. Early work using SPR for the determination of reaction kinetics was performed to determine the effect of single base pair mismatches on DNA hybridisation kinetics. In work performed by Gotoh *et al.* (1995) (44), it was shown that the association kinetics for DNA hybridisation were inversely proportional to the number of mismatched base pairs, and that the dissociation kinetics were even more strongly influenced by the number of mismatches.

In more recent work, the determination of kinetic parameters has proven to be extremely valuable. Indeed, numerous examples exist in which interaction rates are more descriptive of a given biological process than the equilibrium binding affinities (42). For example, Leferink *et al.* (2000) (45) described growth factor interactions with ErbB-1 in which the dynamic rate constants correlated better with mitogenic activity than did the equilibrium constants (42). A more pertinent example, however, is that of McDonnell *et al.* (2001) (46), who identified a role for the Cε2 domain for IgE (see Figure 2) in allergic responses. Removal of this domain from IgE

has little effect on the overall affinity of IgE for its receptor FcεRI, but has a clear effect on the off rate. It was thus demonstrated that Cε2 contributes to the slow off rate of IgE-mediated mast cell sensitisation during the allergic response.

Insights into binding reaction mechanisms can be gained by analysing binding kinetics and thermodynamics over a range of conditions, such as temperature, ionic strength and pH. Measurements over a range of ionic strengths, for example, can help describe the role of electrostatic interactions for a particular binding reaction. Thus one can, for example, discriminate between specific and non-specific protein-DNA binding, due to their difference in ionic strength dependence. Indeed, non-specific protein-DNA binding interactions are highly dependent on ionic strength due to the significant electrostatic contribution of the negatively charged phosphates of the DNA backbone (42).

SPR has also been used for investigating biological membrane events. This has been made possible by the recent introduction of sensor surfaces specifically designed for this purpose, such as the HPA (hydrophobic) or L1 (lipophilic) surfaces from BIAcore (43). These surfaces offer the ability to specifically orient immobilised ligands and have been shown to be a successful membrane mimic (42). Danelian *et al.* (2000) (47), for example, used a liposome-covered sensor surface for an assay for lipid absorption for a panel of 27 drugs and showed a strong correlation with passive intestinal absorption. Future advances in this field might include inserting ion channels, transmembrane receptors and cell-signalling molecules within the immobilised lipid surface in an attempt to closer mimic *in vivo* systems (43).

Lastly, the general versatility, ease of automation, lack of labelling requirements and low sample consumption of SPR analysis make it a promising means for large scale screening for binding

events, both for small molecules in drug discovery and for macromolecules in large scale ligand fishing experiments (42). It is able to perform functional characterisation of “hits” from primary screens, providing information not obtainable from traditional screening methods and making it possible to rank ligands based on affinity as well as association and dissociation kinetics.

Additionally, some of the more advanced instruments (BIAcore 2000 and 3000) have a throughput of 100 – 300 samples per day, depending on assay conditions (43), thus making this method capable of high throughput analysis. SPR has in fact been used to monitor binding of thyroxin analogues to an immobilised antibody (48), as well as by the La Jolla Pharmaceutical Company for the development of a new drug for the treatment of systemic lupus erythromatosis (42).

2.4. Future developments

Despite the many advantages offered by SPR, there are a number of advances that will need to be made in order for this analytical technique to become as widespread as, for example, immunoassays, which are the major competitor of SPR within the field of analysis and detection of biochemical substances, and which offer low cost and high sensitivity and specificity testing. These advances are likely to include (32):

- Improvement of detection limits. Current detection limits stand at about 1pg/mm^2 , which is not sufficient for detecting low concentrations of low molecular weight analytes. Although optimisation of SPR optical instruments and refining of data processing methods may lower the current detection limits, no approach currently exists that will lower this limit by the necessary orders of magnitude. Progress will therefore need to be made in this area.

- Multi-channel performance. This would be required for high throughput screening and detection for new pharmaceuticals. Advances have already been made in this area, with the introduction of a four-channel chip which can be rotated by 90°, effectively providing 16 channels. Myszkka and Rich (2000) (49) have more recently described a prototype micro array chip with 64 individual immobilisation sites in a single flow cell.
- Development of advanced recognition elements for applications involving complex realistic samples (e.g. blood). Stable receptor matrices which allow sensor responses and non-specific background effects to be resolved will also have to be developed.

All these developments may eventually lead to miniaturised, integrated, compact and rugged sensing elements which would fulfil a number of the requirements of an ideal biosensor.

3. Aptamers

Aptamers have been defined by James (2000) (50) in the Encyclopaedia of Analytical Chemistry as: "...artificial nucleic acid ligands that can be generated against amino acids, drugs, proteins and other molecules. They are isolated from complex libraries of synthetic nucleic acid by an iterative process of adsorption, recovery and re-amplification. They have potential applications in analytical devices, including biosensors, and as therapeutic agents." In essence, they are synthetic oligonucleotide sequences which are able to bind a wide array of molecules (ligands) with high affinity and specificity (51; 52). Their name is derived from the Latin word "*aptus*", meaning "to fit" (52).

Importantly, the ligand binding capacity of aptamers is based on their three dimensional conformation and not on nucleotide base pair complementarity (53). Indeed, upon association

with their molecular targets, aptamers fold into molecular structures in which the target becomes an intrinsic part of the nucleic acid structure (54). They are therefore able to bind proteins and other molecules that would not normally interact with deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), making them extremely powerful as screening tools (53). They were initially reported on in 1990 quasi simultaneously by three independent laboratories: those of Joyce (55), Szostak (56) and Gold (57), and are obtained using an *in vitro* selection and purification technique, now known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment), as described in Section 1.4.2 (51).

3.1. Aptamer libraries

Before the SELEX process is undertaken, an aptamer library has first to be created. Part of the enormous potential of aptamers lies precisely in the fact that libraries with vast numbers of potential ligands can be created and screened within a few days. Typically, aptamer libraries consist of $10^{13} - 10^{18}$ random oligonucleotide sequences (51) and can be screened within a number of days. This is all the more impressive when compared to conventional libraries of potential drugs which consist generally of no more than 10^6 different molecules and may take months to screen (53).

When creating a library however, the following factors need to be taken into account (53):

The aptamer library complexity: given random manufacture of aptamers, the library complexity can be calculated relatively easily. For example, a library of oligonucleotides of N nucleotides in length generated from y different nucleotides (not solely limited to the four naturally occurring nucleotides) will have a complexity of y^N . However, for practical purposes, and as mentioned

above, the maximum number of unique sequences that can be screened in a library is limited to $10^{13} - 10^{18}$. It is interesting to note, however, that further diversity may be introduced during the SELEX process itself (see Section 1.4.2), in particular due to the infidelity of the DNA polymerase used in the polymerase chain reaction (PCR) step, which may not be 100% accurate.

The nucleotide chemistry: this is important because, firstly, it defines the range of possible three-dimensional structures into which the aptamer can fold, and secondly, the nucleotide chemistry plays an extremely important role in relation to the aptamer's stability to degradation. Indeed, the susceptibility of single stranded oligonucleotides to enzymatic or chemical cleavage is a severe practical handicap to the use of aptamers that needs to be overcome for the molecules to be stable in biological fluids. Three different approaches have been adopted to overcome this problem:

- Modification of nucleotide bases, which has been the most commonly used method. The modification of pyrimidines at the 5' position with I, Br, Cl, NH₃ and N₃ and at the 2' position with NH₂, F and OCH₃, for example, has been described by Pieken *et al.* (1997) (58). These modifications can increase the RNA oligonucleotide half-life up to 15 hours (54). It should be noted, however, that any modified nucleotides should still be compatible with the enzymes used in the SELEX protocol (for example DNA polymerase). Interestingly, some problems associated with modified nucleotides have been overcome by a modification of the SELEX protocol known as "Transcription Free SELEX", in which random RNA fragments bind random DNA templates, after which the fragments are ligated either enzymatically or using a standard chemical condensation reaction. The RNA aptamer can then be recovered by melting the duplex.

- Modification of the phosphodiester backbone, for example through the use of α -thio substituted deoxynucleotide triphosphates, although this technique has been more successful with DNA as opposed to RNA aptamers.
- The use of enantiomeric aptamers, known as spiegelmers (from the German word meaning “mirror”). This technique consists in creating a mirror image of the target and selecting an aptamer for this mirror image. A stereo isomer of the selected aptamer is then created (i.e. the spiegelmer), which will be specific for the target but will not be susceptible to normal enzymatic degradation due to the substitution of the natural D-ribose with L-ribose (54).

The constant region primer design: the random aptamer sequence has to be flanked by 5' and 3' constant sequences, usually 20 – 25 base pairs in length, which provide hybridisation sites during a number of steps of the SELEX process (51). The 3' flanking sequence generally acts as an attachment site for the reverse transcriptase primer; and the 5' flanking sequence acts as the attachment site for the PCR primers during the amplification step of the SELEX protocol. The design of the constant region for the SELEX protocol is even more important than for normal PCR given that a complete SELEX protocol may include up to 200 cycles of PCR. Any artefacts would thus be drastically amplified in the final aptamer population.

3.2. SELEX

After a suitable aptamer library has been prepared, it can undergo the SELEX protocol, as shown in Figure 6. This technique essentially consists of the repeated binding, selection and amplification of aptamers from the initial library until one (or more) aptamer(s) displaying the desired characteristic(s) has (have) been isolated (53).

Referring to Figure 6, Steps 1 and/or 2 involve the preparation of the aptamer library/candidate mixture, as detailed above. Note that the library would generally consist of double-stranded DNA, which either needs to be transcribed (for RNA selection) or strand-separated (for single-stranded DNA selection), in order to be in a suitable form for selection (54).

In Step 3, the target and the aptamers are brought together under favourable binding conditions, where the aptamers with the highest affinity will bind the target. These aptamers are then partitioned from the aptamers with lower affinity in Step 4. This step can be performed by attaching the aptamers to a solid phase support, such as sepharose, and specifically eluting the desired aptamers after binding has taken place. Immobilising the aptamers, however, is likely to affect the binding interactions between the aptamer and the target. The same would be true if the target were immobilised instead of the aptamer (53). Alternatively, the aptamer and target could be allowed to interact freely in solution, after which the target-aptamer complex could be recovered by filtration through nitrocellulose (54). This method is commonly used, although it is important to note that it is only applicable when the target molecule is a protein. A negative selection step is also frequently used at this stage, in which the aptamers are passed over a cellulose filter in the absence of the target. This is to eliminate aptamers that bind the filter in a target-independent manner (54). Counter selection is also sometimes used, where aptamers that bind structures similar to that of the target are removed (51). Affinity chromatography is generally used to isolate aptamers for small molecular targets (54).

The high affinity aptamers are then amplified by reverse transcription PCR (RT-PCR) (for RNA aptamers) (Step 5) or by PCR for DNA aptamers (Step 6), in order to create a new aptamer library enriched with the aptamers of high affinity. The entire process is then repeated (Step 7),

resulting in fewer and fewer unique sequences, with higher and higher affinity to the target, being retained. Note that during each round of selection, the binding conditions for the aptamer and the target are generally made more stringent in order to increase the selective pressure on the remaining aptamers. Generally, a complete SELEX process (between 8 – 15 cycles) will yield a final mixture of no more than ten aptamers (53; 54). The aptamers can then be cloned and sequenced, allowing further identical aptamers to be generated by chemical synthesis.

Figure 6

The SELEX procedure is clearly involved and can take weeks to months to produce a suitable aptamer when performed manually. Cox *et al.* (1998) (59) first reported on the automation of the procedure. A more recent report (60), however, details the automation of the procedure where approximately 12 rounds of selection can be carried out in two days. Based on these figures, it has been estimated that one robot could produce aptamers against 120 targets in one month, exceeding manual throughput by 10 – 100 fold. It has also been suggested that further development of the automated procedure could lead to the production of aptamers to upwards of 1000 targets per month. Several robots working in parallel could therefore generate aptamers to an entire proteome within a relatively short period of time, further highlighting the immense potential of aptamers. An additional advantage of an automated procedure is the consistency of the repetitive tasks that it offers, creating a bench mark to allow comparisons between different laboratories and eliminating variations due to manual selection techniques (59).

3.3 Aptamers and antibodies

Given the characteristics of aptamers described above, it is clear that they could be of great use for therapeutic, analytical and diagnostic procedures. Currently, antibodies are most frequently used in procedures where high affinity and specificity for a particular target are required, such as ELISAs. The following is a discussion of the advantages and disadvantages of using aptamers or antibodies for these techniques.

The use of antibodies to detect analytes became widespread in the 1970s, when polyclonal sera from immunized animals were the most popular choice (52). It was not long thereafter till the discovery of monoclonal antibody technology which allowed the production of a unique antibody in large quantities (52). This technology allowed affinity based assays to be further refined and optimised and was embraced throughout the scientific community. The many advantages of antibodies include their high affinity and specificity for their particular antigen, typically with very low dissociation constants. Furthermore, selected clones producing the antibody of choice can be cultured continuously, hence providing a limitless supply of a particular antibody (theoretically). Lastly, the immunogen used for the identification of a monoclonal antibody does not have to be pure (52).

There are, however, a number of disadvantages and limitations associated with the use of antibodies. These are listed below (52):

- The production of antibodies requires animals. The generation of antibodies against molecules that are not well tolerated by the animal (e.g. toxins) or against molecules that are not inherently immunogenic can therefore be problematic.

- The production and identification of monoclonal antibodies are laborious procedures and are likely to be costly in searches for rare antibodies requiring the screening of large numbers of colonies.
- High yields of antibodies may be problematic to obtain due to difficulties associated with growing certain hybridomas *in vivo*, the manner in which high yields are typically achieved.
- The performance of the same antibody may vary between batches, requiring the immunoassay to be re-optimised with each new batch of antibody.
- It is not feasible to identify antibodies that could detect targets under non-physiological conditions.
- There is no control over the selection of the target protein to which the antibody binds (this is “determined” by the immune system of the animal) (54).
- The kinetic parameters of antibody-target interactions cannot be manipulated on demand.
- Antibodies are heat-labile, undergoing irreversible denaturation.
- They have a limited shelf-life.

It is important to note that various approaches addressing the shortcomings listed above are being investigated, including humanisation of antibodies, antibody engineering and *in vitro* immunisation (52).

Listed below are the various advantages aptamers offer over antibodies for the use in analytical and diagnostic devices (52):

- Aptamers do not require the use of animal systems for their production.

- The properties of aptamers can be changed on demand due to the fact that they are produced through an *in vitro* process that does not depend on animals or on *in vivo* conditions.
- Selection conditions can be manipulated to obtain aptamers that bind the target under non-physiological conditions, for example. Similarly, the kinetic parameters of the aptamer can also be manipulated on demand.
- Aptamers can be generated against targets that are toxic or that are not inherently immunogenic.
- Batch to batch variation is eliminated in aptamer production, given the fact that they are chemically synthesised.
- Aptamers can be easily engineered to include reporter molecules at a precise location, specified by the user, without affecting their binding characteristics.
- Small aptamers can be used to generate dense receptor layers, thus allowing increased sensitivity for a given affinity (31).
- The heat denaturation process in aptamers is reversible, allowing them to be easily regenerated.
- Aptamers are stable in long-term storage.

The main advantages of aptamers over antibodies are summarised in Table 2.

Table 2

The main drawbacks of aptamers include the time required for selection using a manual SELEX process, although this is slowly being overcome by the introduction of automated SELEX processes. Additionally, their affinity constants are generally lower than those of antibodies and

their structural stability is still questionable (19). Furthermore, the fact that the science of aptamers is relatively young and much of the work and knowledge-base in the field is still highly experimental is an additional concern hindering their common use.

3.4. Applications

3.4.a. Two site binding assays

ELISA sandwich assays, using antibodies, are a very common diagnostic assay. The term ELONA (Enzyme Linked Oligonucleotide Assay) is applied to the same assay format using aptamers (51). Various ELONA formats can be adopted, using for example (51):

- Aptamers as capture molecules and antibodies as reporter molecules;
- Antibodies as capture molecules and labelled aptamers against the antibody-antigen complex as reporter molecules;
- Aptamers as capture molecules and labelled aptamers against the aptamer-analyte complex as reporter molecules.

ELONAs have been demonstrated to be successful for the detection of VEGF (61) and CD4⁺ cells (62).

3.4.b. Flow cytometry

Flow cytometry is a powerful tool that is used both analytically and diagnostically for the multi-parameter analysis of cells in suspension. Generally, this method is performed using labelled

monoclonal antibodies. Recently however, aptamers have been used to perform the role of monoclonal antibodies in this process, with promising results. Indeed, it has been shown that aptamers can be relatively easily conjugated to small fluorophores, such as fluorescein, or even to larger proteins such as phycoerythrin, and still retain their binding characteristics, demonstrating their ability to be used with a wide variety of reporter molecules that are generally used in diagnostics (52). Generally, aptamers have been shown to perform as well as antibodies or can even be used in combination with them for flow cytometry analyses (52).

3.4.c. Biosensors

Affinity sensors should ideally fulfil at least three basic criteria (52), namely:

- The ability to transduce the binding event without adding extra reagent;
- The ability to detect and quantify the target within the desired concentration range and time period;
- The ability to make repeated measurements on the same transducer.

The use of aptamer-based biosensors would offer a number of advantages in this respect. Indeed, the function of immobilised aptamers could be regenerated using extremes of heat, salt concentration or chelating agents, without loss of activity, resulting in a reusable transducer (52). Aptamers can also be easily modified for immobilisation without affecting their function (52). Furthermore, they can be selected-for in conditions resembling those of the real matrix, which is particularly useful for environmental and food applications (51). Lastly, aptamers can be easily labelled with a wide range of reporter molecules, allowing the design of a variety of detection formats (52).

Recent work on aptamers in affinity sensors includes the use of an RNA aptamer specific for L-adenosine (63) and a DNA aptamer for human thrombin (64). Results in both cases were promising, with the aptamers showing high specificity and the ability to be regenerated (52).

3.4.d. Molecular beacons

Molecular beacons are a class of fluorogenic probe in which a fluorophore is attached to one terminus and a quencher is attached to the other. In the absence of the target molecule, the probe forms a hairpin loop, bringing the fluorophore and the quencher into close proximity, resulting in the absence of a fluorescent signal. In the presence of a target molecule, however, the hairpin loop structure is opened (the target is complementary to the loop region), the fluorophore and quencher are distanced from each other and a fluorescent signal is produced, as illustrated in Figure 7. Until recently, these probes have only been available for the detection of nucleic acid targets, due to the inability of these probes to interact with other classes of targets, such as proteins (52).

Figure 7

Aptamers, however, have the ability to interact with a very wide range of targets and have therefore been exploited to extend this technique to include targets other than nucleic acids. For this purpose, molecular beacons, termed ligand beacons when used in this way, are designed to be complementary to a nucleotide region in the aptamer. In the absence of the target (Figure 8(A)), the ligand beacon binds the aptamer, resulting in the separation of the fluorophore and the quencher and the production of a fluorescent signal. In the presence of the target (Figure 8(B)),

however, the binding site on the aptamer is no longer available to the ligand beacon because it is bound by the target. This results in the ligand beacon adopting an internal hairpin loop structure and in the absence of a fluorescent signal (52).

Figure 8

This format is also applicable to a multiplex system, in which a number of different analytes are detected simultaneously with the use of ligand beacons conjugated to fluorophores emitting at different wavelengths (52).

This type of assay once again clearly shows the potential of aptamers for analytical and diagnostic procedures, which cannot be fulfilled by antibodies.

3.4.e. Capillary electrophoresis

Capillary electrophoresis (CE) is a separation technique based on the same principle as conventional electrophoresis. It offers the advantages of speed, small sample volumes, suitability for automation, sensitivity and the possibility of performing multiplex assays (52). CE is now being adapted for immunoassays, in which antibody-antigen complexes are separated on a fluidic stream under an applied electric field (52). However, there are many practical difficulties associated with this method, such as poor separation between antibody-target complexes and free, labelled, antibodies when the targets are small and uncharged (52).

Recently, German *et al.* (1998) (65) adapted the technique to use a fluorescently labelled DNA aptamer for the detection and quantification of human IgE. The technique was able to separate

free aptamer from the aptamer-IgE complex in the presence and in the absence of serum, indicating that the aptamer-target interaction was not affected by the complex medium.

3.4.f. Molecular switches

A very relevant example, as described by Jayasena (1999) (52), of where aptamers can be used as molecular switches is in PCR. In order to avoid non specific amplification, which is often due to non-specific binding of primers at sub-optimum temperatures and to the ambient temperature activity of the DNA polymerase enzyme, an essential amplification ingredient can be withheld until the temperature is sufficient to ensure specific binding of the primers. This has been achieved, for example, by using a monoclonal antibody which neutralises the activity of the DNA polymerase. When the temperature of the reaction mixture is increased to begin the amplification reaction, the antibody is denatured and the DNA polymerase regains its activity. One of the shortcomings of this method is the high temperature at which the antibody is denatured ($>75^{\circ}\text{C}$). Although suitable for DNA amplification, these high temperatures would denature RNA targets, making this method of preventing non-specific amplification unsuitable for RT-PCR.

For this purpose, aptamers able to inhibit the activity polymerase enzymes and which are “switched off” above 40°C have been selected using high temperature selection conditions (52), demonstrating how aptamers can be used as temperature-sensitive molecular switches.

It is clear, however, that aptamers could be selected as molecular switches sensitive to other criteria such as pH, salt or the presence of metal chelating agents (52).

3.4.g. Aptamer arrays

Although antibody-based microarrays for the use in proteomics are currently being developed, aptamer-based arrays would be attractive for the following reasons (52):

- Aptamer identification can be performed on an automated platform;
- Aptamers can be immobilised at defined densities and at precise locations using existing deposition technology;
- Homogenous preparations of aptamers are readily available from chemical synthesis;
- Aptamer-based arrays would be robust and have a long shelf life;
- Proteins could be bound irreversibly using specifically modified aptamers.

These arrays will certainly play an increasingly important role in the study of proteomics as the wealth of information at the DNA level continues to increase with the multitude of genome screening projects.

3.4.h. Aptazymes

Aptazymes are aptamers linked to ribozymes (catalytic RNA molecules) possessing catalytic activity. They are able to directly transduce molecular recognition into a quantifiable catalytic event and can be generated using two different strategies (54):

- Pre-existing aptamers and pre-existing catalytic RNAs, with known specificity and catalytic properties, can be integrated. In this way, the resulting molecule will possess the desired affinity for the target as well as the expected catalytic effect.

- The aptazyme with the desired binding specificity is selected from a pool of aptazymes containing a random sequence receptor site and a catalytic region with established properties.

Aptazymes have been applied to the monitoring of post-translational modification (66) and have also been adapted to a chip array able to distinguish between various metabolites (67).

4. Implementation and Illustration of Instrumentation

The Biacore X (Biacore, Uppsala, Sweden) SPR analytical instrument is a typical device with which this type of work can be performed. The following is a brief description of the methods involved in this work.

4.1. Immobilisation

The IgE-specific aptamer was immobilised to a dextran sensor chip (CM5) obtained from Biacore (Uppsala, Sweden) via a streptavidin-biotin reaction. The dextran chip allows for a more “biocompatible” environment, increased receptor activity, increased receptor density, as well as reduction in non-specific sample interactions. Once immobilisation was complete, binding experiments using IgE could then be performed by running appropriate sample concentrations and volumes over the sensor surface. After binding of the target molecule, the sensor surface could be regenerated using a solution of EtOH/NaOH. A typical result obtained from an SPR experiment, known as a sensogram, is shown in Figure 9.

Figure 9

4.2. Calibration

Using a range of concentrations, it is possible to obtain a calibration curve, which can be used to extrapolate the concentration of target analyte in solutions of unknown concentrations. Such a calibration curve is shown in Figure 10.

Figure 10

Although this work has been carried out successfully, there remain a number of areas where vast improvements can still be made, particularly with respect to the sensitivity of the measurements. Indeed, in this particular case, the sensitivity of the aptasensor has not yet been optimised and is not sufficient for the detection of IgE in real samples. For measurements of very low concentrations of target analyte to be feasible, a high association constant of the aptamer and IgE is necessary. This constant is very much dependent upon the three dimensional stability of the aptamer and further developments in the design and selection of these aptamers needs to be made.

5. Future Prospects

If an aptasensor for the detection of IgE were to be developed for routine clinical use, the aptamer selected would need to form a very stable complex with the target analyte in order to withstand the numerous washing steps which would be necessary for its detection in a complex solution such as blood. Selection of an aptamer known to form a stable complex with IgE when immobilised to a solid support would therefore appear to be necessary.

Further work would also be needed to test the aptamer in complex protein solutions which would undoubtedly be necessary to develop an aptasensor capable of clinical application.

The ultimate aim would be to develop allergen-specific IgE aptamers and incorporate them into a commercially viable aptasensor which could be used by an individual to diagnose precisely to which allergen they are allergic.

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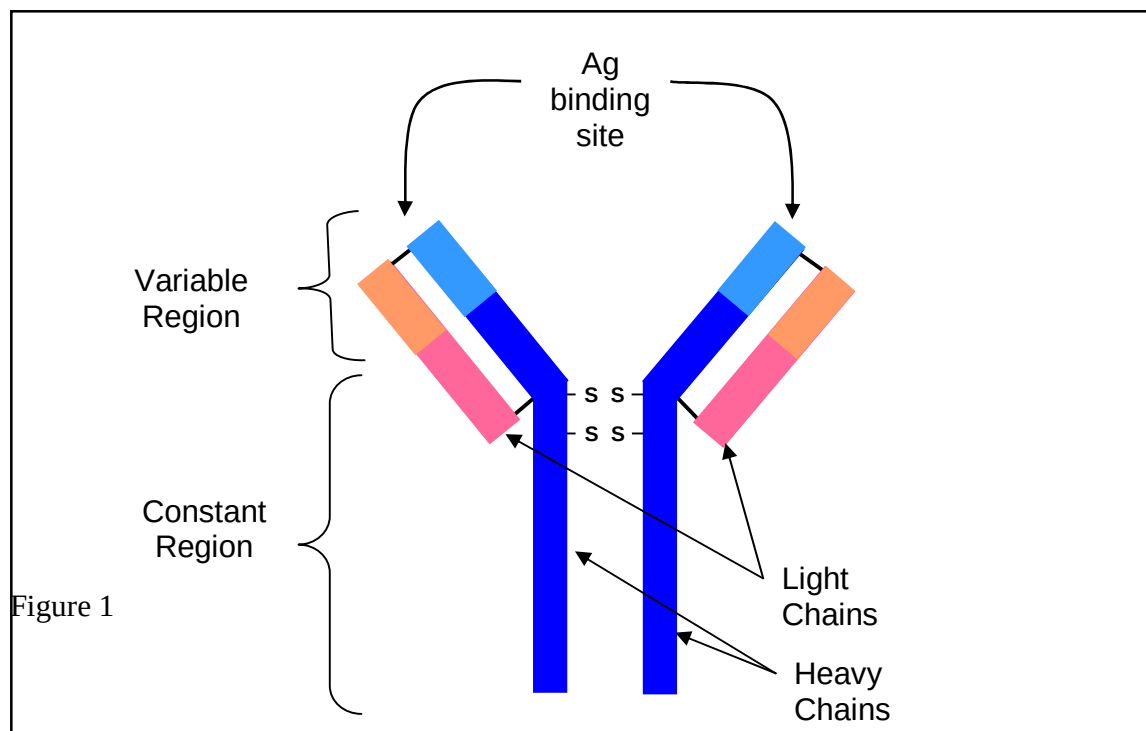
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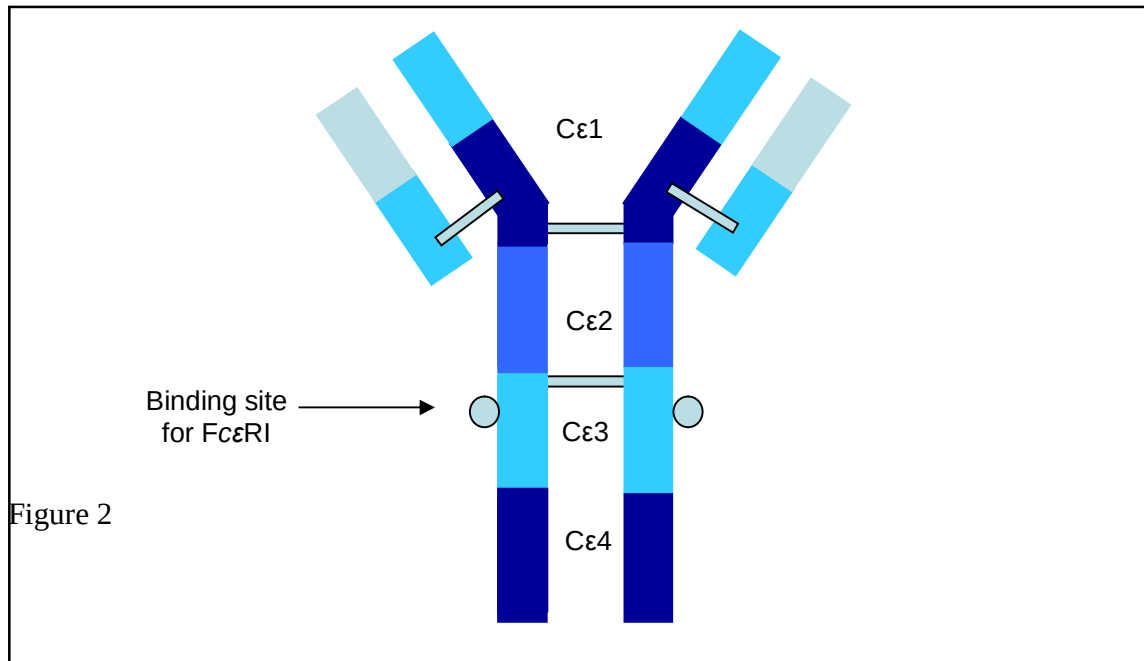


Figure 2

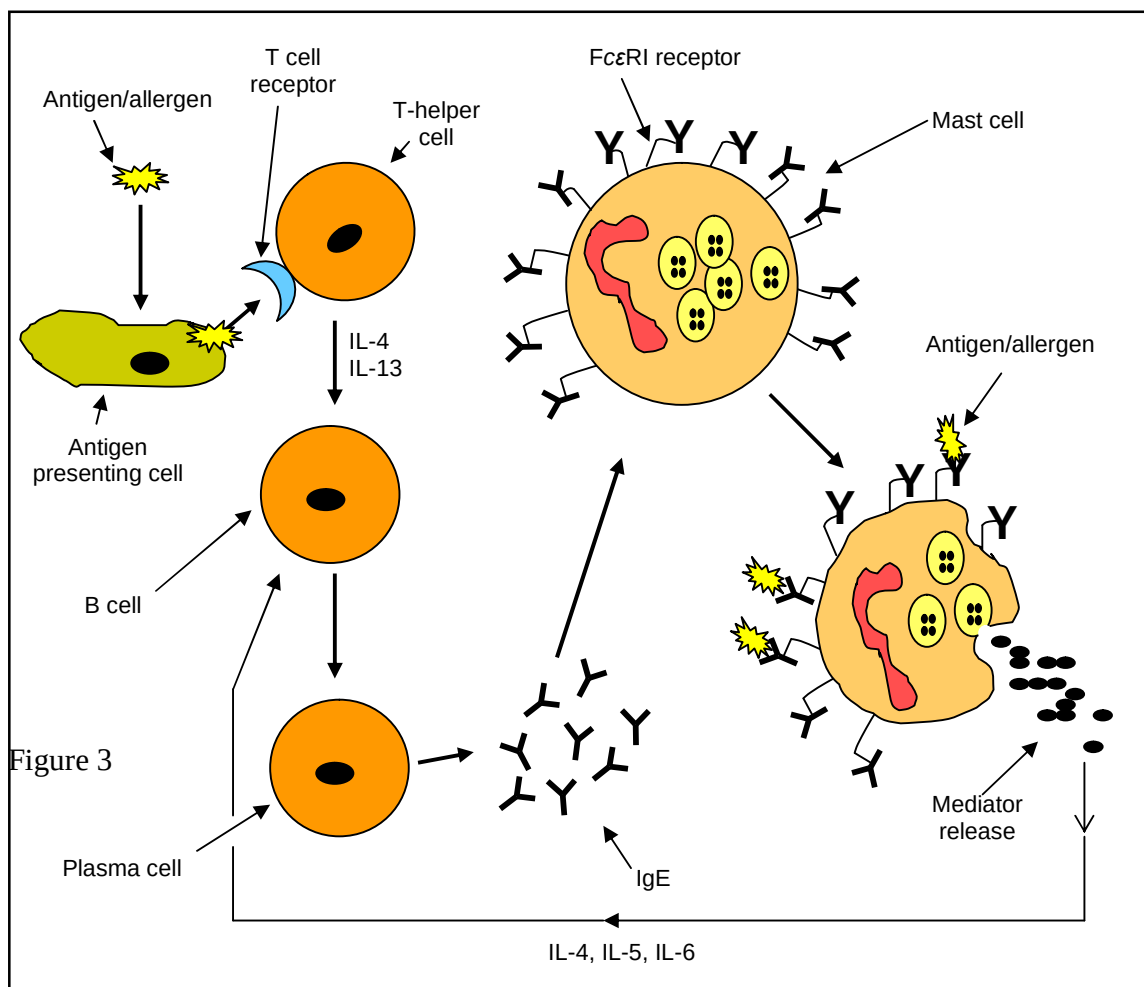
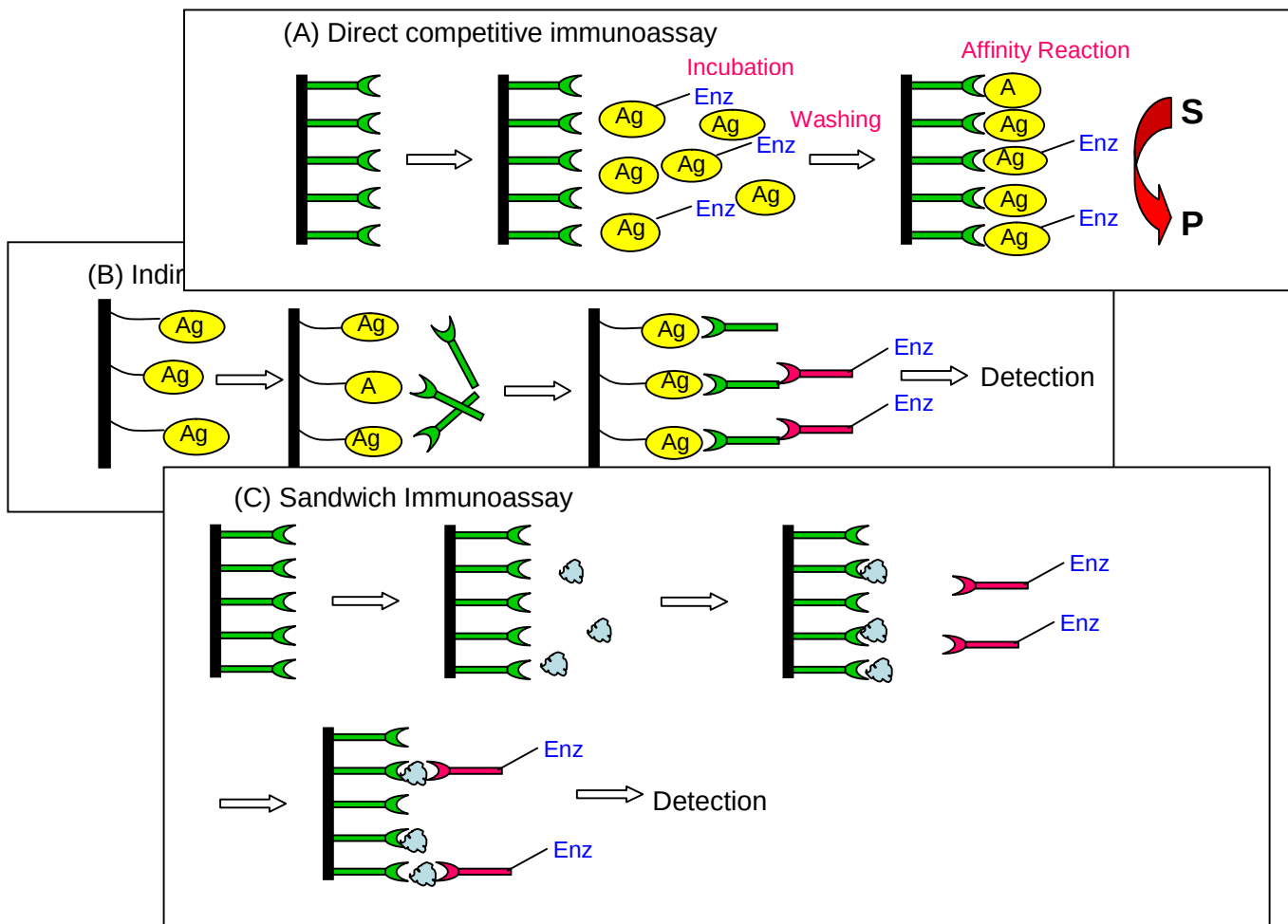


Figure 3



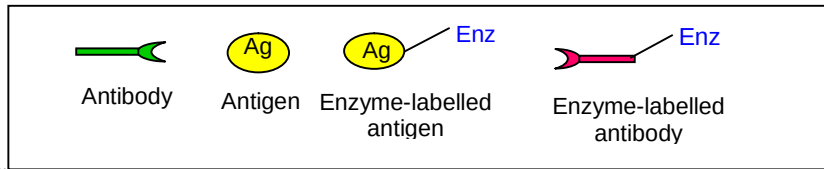


Figure 4

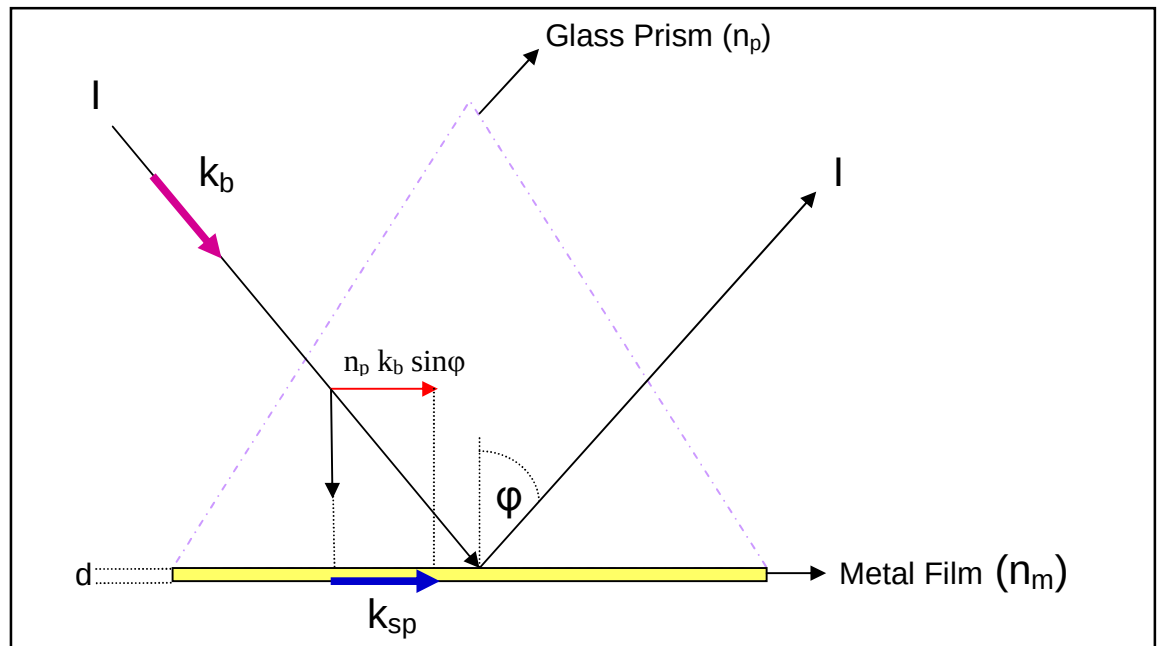


Figure 5

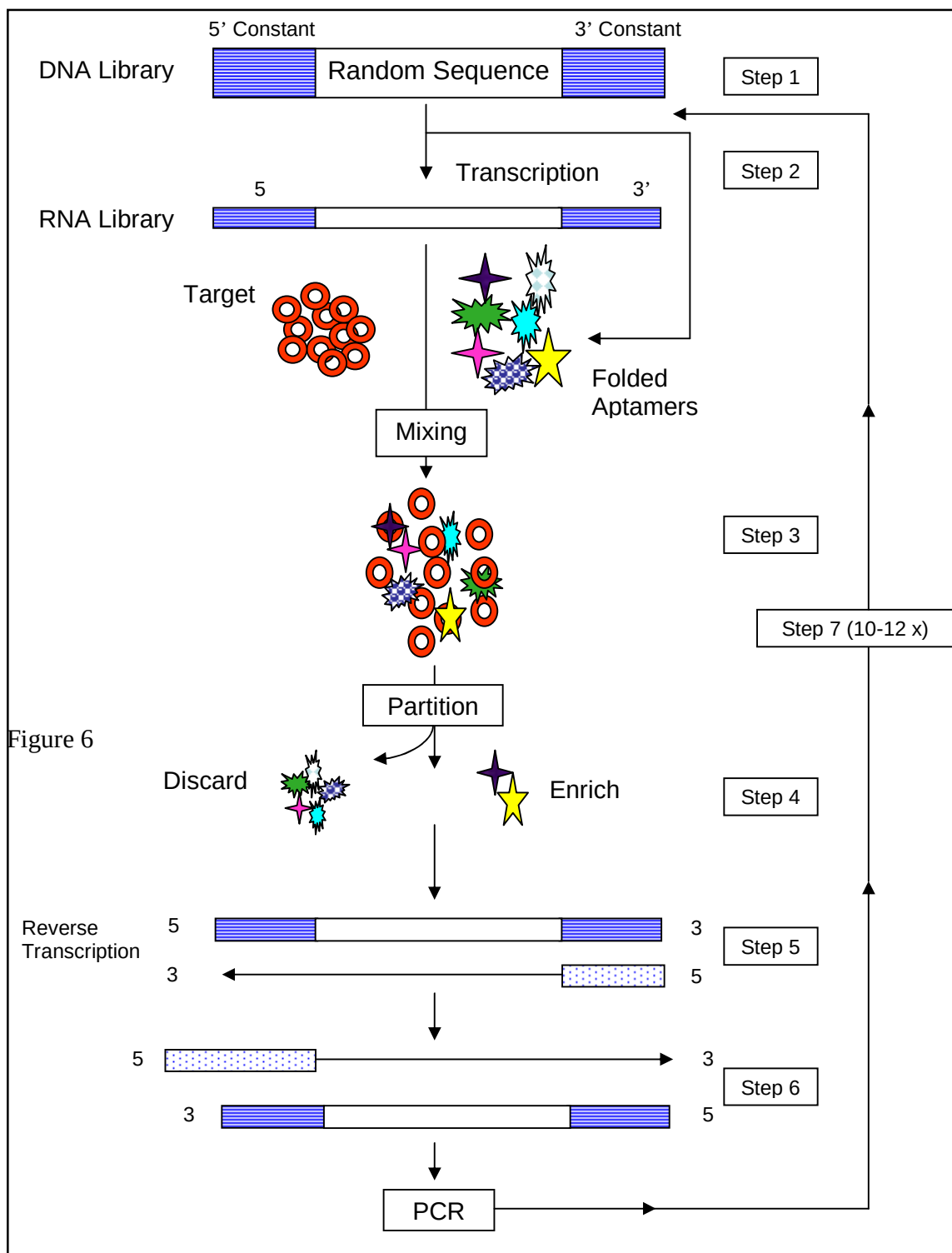


Figure 6

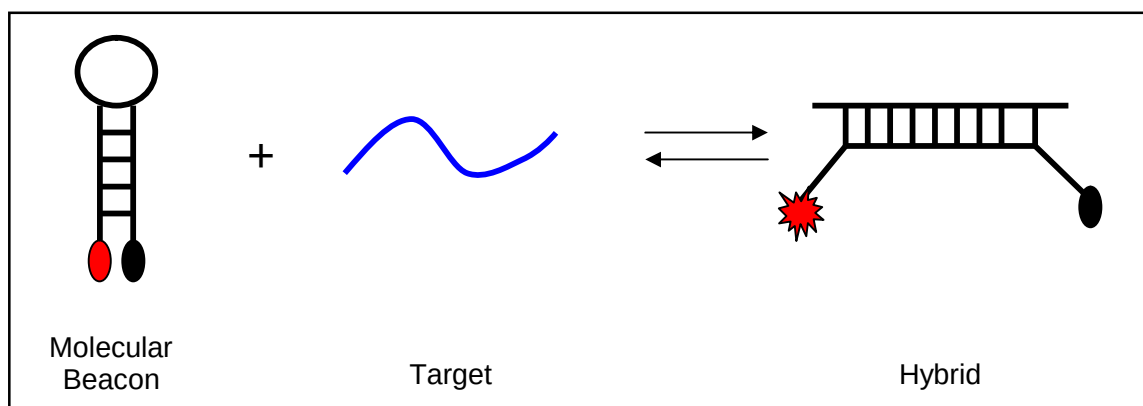


Figure 7

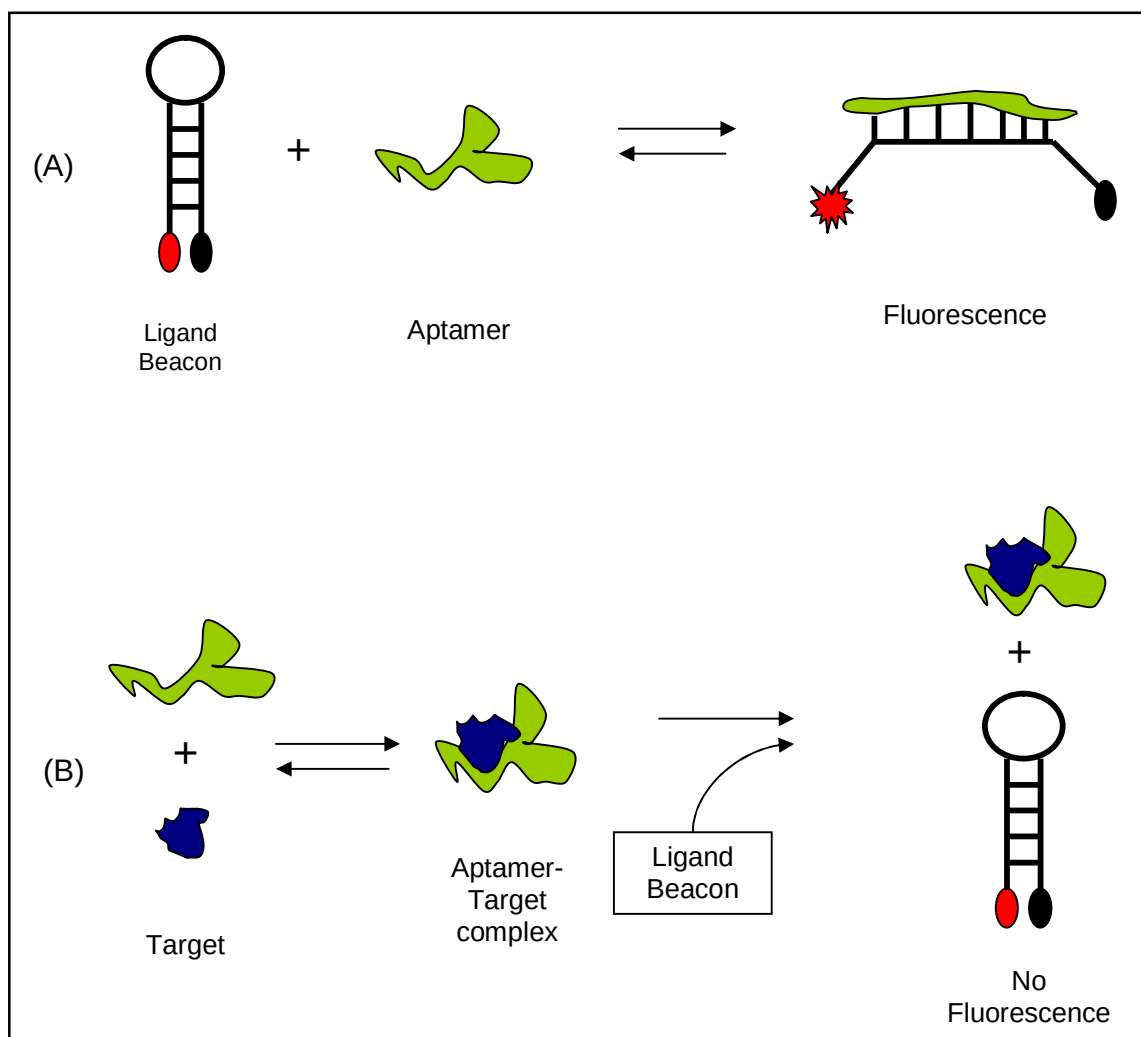


Figure 8

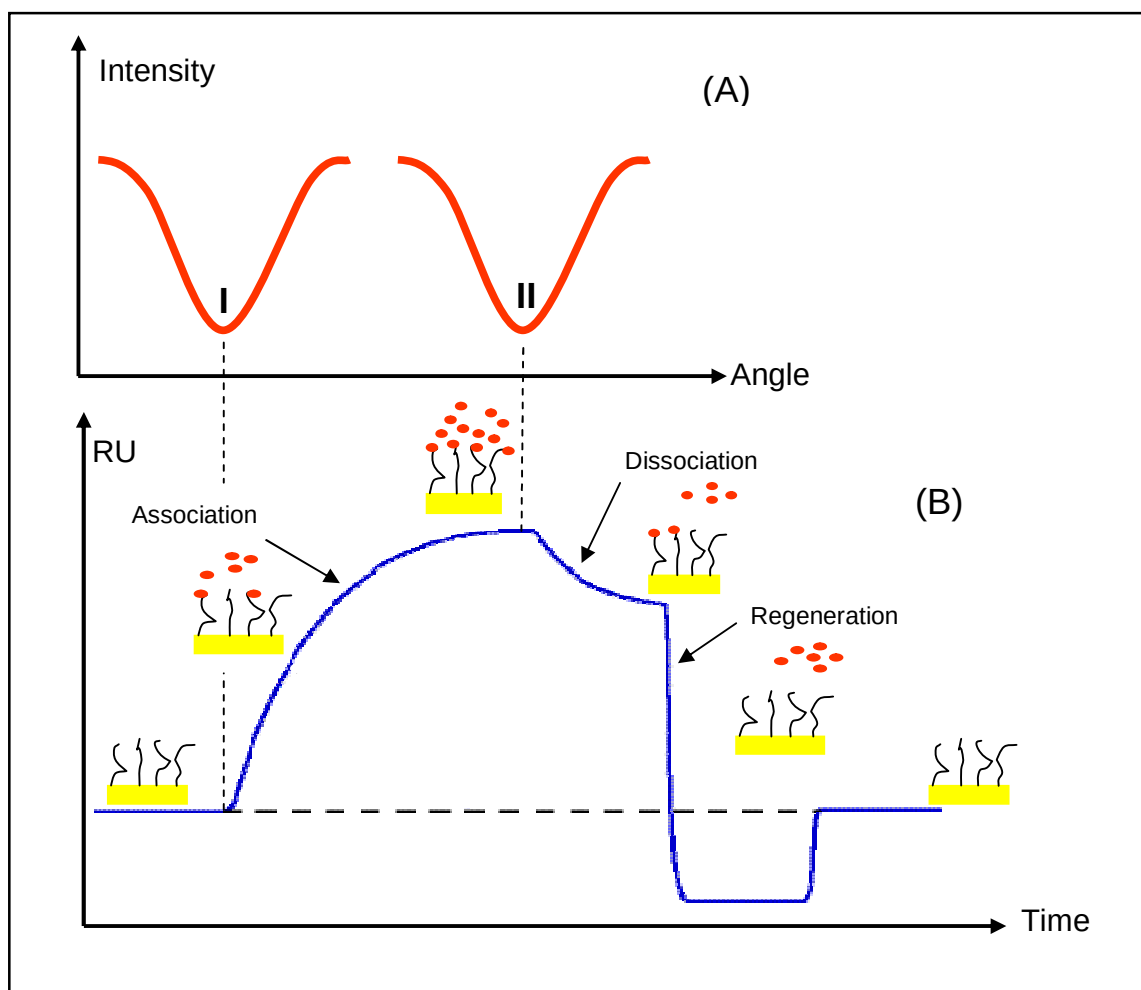


Figure 9

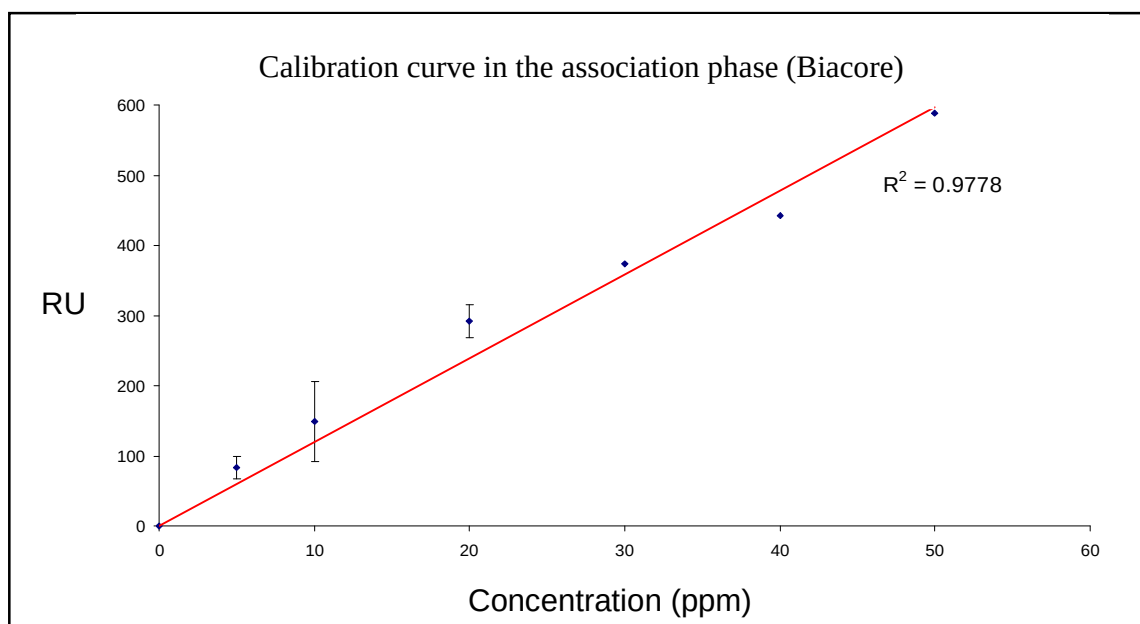


Figure 10

Figures

Figure 1 General structure of an immunoglobulin.

Figure 2 Structure of IgE (modified from 7). Note the various domains of the antibody.

Figure 3 The allergic cascade (modified from 7).

Figure 4 Immunosensor formats (modified from 20). (A) Direct competitive immunoassay. (B) Indirect competitive immunoassay. (C) Sandwich immunoassay.

Figure 5 Kretschmann configuration for SPR. See text for details.

Figure 6 The SELEX Protocol (modified from 53) (see text for details).

Figure 7 Functioning of a molecular beacon.

Figure 8 Functioning of a ligand beacon. (A) In the absence of the target analyte, a fluorescent signal is produced. (B) In the presence of the target analyte, no fluorescent signal is produced.

Figure 9 Sensogram from a typical SPR experiment (modified from 68). (A) Decrease in intensity of reflected light at resonance angles. (B) The shift in resonance angle (from position I to position II) is due to the binding of the analyte at the sensor surface.

Figure 10 Calibration curve in the association phase (Biacore). This curve was constructed for IgE binding in the association phase at concentrations from 5ppm to 50ppm. Standard deviations were calculated based on three samples at 5ppm, three at 10ppm and four at 20ppm, giving a CV_{ave} of 22%. Binding experiments at higher concentrations were only carried out once in order to conserve reagents.

Tables

Table 1 SPR biosensor applications (modified from 43).

Table 2 Advantages of aptamers over antibodies (modified from 51).

Table 1

Qualitative	Quantitative
Following molecular purification	Active concentration
Specificity	<i>Kinetics</i>
Epitope mapping	Equilibrium constants
Molecular assembly	<i>Thermodynamics</i>

<i>Ligand fishing</i>	Stoichiometry
<i>Small molecule screening</i>	<i>Mechanism</i>

Table 2

Antibodies	Aptamers
Requires the use of animals	No animals required
Limitations against non-immunogenic and toxic substances	Can be generated against non-immunogenic and toxic substances
Kinetic parameters cannot be modified on	Kinetic parameters can be modified on demand

demand	
Labelling can cause loss of affinity	Variety of reporter molecules can be attached without affecting binding properties
Not feasible to identify antibodies that interact with targets under non-physiological conditions	Selection conditions can be modified to select aptamers with particular properties
Problems with batch to batch variation	Little or no batch to batch variation
Limited shelf-life, temperature sensitive & susceptible to denaturation	Stable in long-term storage, can be transported at ambient temperature & can be regenerated after denaturation

Future Perspectives

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Biosensors comprise a biological or biologically derived sensing element intimately associated with or integrated within a physicochemical transducer. Over 1,500 papers are published each year describing the various permutations of sensing element and transducer [2]. These are applied mainly in medical diagnostics [3], environmental diagnostics [4], in the food industry [5] and for crime prevention and security [6]. The most significant impact of biosensors to date has been in the field of diabetes, where mediated amperometric biosensors account for over \$4 billion in sales. Until now, however, no device has come close to delivering a truly one-step procedure. In the immediate future we can expect to see new technology to deliver this objective. The development of suitably robust biosensors for many situations outside of glucose monitoring, has been hindered by several problems associated with the properties of biological material. The search for possible solutions to these problems has led to the development of biomimetic systems such as the electronic nose, which shows excellent practical potential for the detection of disease and infections [7]. An alternative approach has been to seek synthetic analogues of natural receptors and antibodies using supramolecular systems. If nature can produce nanomaterials with recognition and functional properties by evolution, molecular engineers should be able to accomplish comparable, but broader capabilities by design, guided by examples from living systems. One of the most promising areas of biomimetics is Molecularly Imprinted Polymers (MIPs) [8]. A key element here is the need for rational design [9]. The ability to construct highly stable sensing structures which are either small enough to be implanted in the body, distributed widely in the environment, configured as high density arrays or coupled to modern information systems, offers exciting new horizons in the information revolution by furnishing our information and telecommunication systems with sophisticated “senses”.

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