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REVIEW

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Label-free screening of bio-molecular interactions

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Abstract The majority of techniques currently employed to interrogate a biomolecular interaction require some type of radio- or enzymatic- or fluorescent-labelling to report the binding event. However, there is an increasing awareness of novel techniques that do not require labelling of the ligand or the receptor, and that allow virtually any complex to be screened with minimal assay development. This review focuses on three major label-free screening platforms: surface plasmon resonance biosensors, acoustic biosensors, and calorimetric biosensors. Scientists in both academia and industry are using biosensors in areas that encompass almost all areas drug discovery, diagnostics, and the life sciences. The capabilities and advantages of each technique are compared and key applications involving small molecules, proteins, oligonucleotides, bacteriophage, viruses, bacteria, and cells are reviewed. The role of the interface between the biosensor surface (in the case of SPR and acoustic biosensors) and the chemical or biological systems to be studied is also covered with attention to the covalent and non-covalent coupling chemistries commonly employed.

Keywords Biosensor · Screening · Label-free · Review · Acoustic · Surface plasmon resonance · Quartz crystal microbalance · Calorimetry

Introduction

The screening of molecular interactions involving the determination of the affinity, activity, toxicity and predicted in vivo availability of drug candidates is an integral part of the drug discovery process. The majority of the pharmaceutical screens currently employed require some type of radio- or enzymatic- or fluorescent-labelling to report the binding of a ligand to its receptor. This labelling step imposes additional time and cost demands, and can in some cases interfere with the molecular interaction by occluding a binding site, leading to false negatives. Many reporter compounds are also hydrophobic, and in many screens background binding is a significant problem, leading to false positives. Most label-dependent screening platforms are based on the measurement of fluorescence (e.g. fluorescence resonance energy transfer or fluorescence polarization) or radioactivity (e.g. filter binding assays and scintillation proximity assays). These powerful technologies allow rapid determination of the affinities, and often the kinetics of a drug–receptor interaction with high sensitivity. Unfortunately, the dramatic increase in the number of compounds that can be screened using these technologies has not led to an increase in the number of new chemical entities released on the market by pharmaceutical and biotechnology companies. This article focuses on three label-free detection technologies that have the potential to deliver high quality, high information content screening to the pharmaceutical industry: optical biosensors, acoustic biosensors and micro-calorimetry. These relatively novel approaches do not allow screening of extremely large numbers of compounds (high throughput screening or HTS), however they do facilitate more critical evaluation of the quality of the data generated, particularly in regard to interaction specificity and ranking of affinities.

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Optical biosensors

Optical biosensors that exploit surface plasmon resonance, planar wave-guides and resonant mirrors, have been used to generate over 3000 scientific publications that encompass a wide range of disciplines in the life sciences. These include: ligand fishing [1, 2], apoptosis [3], bacteriology

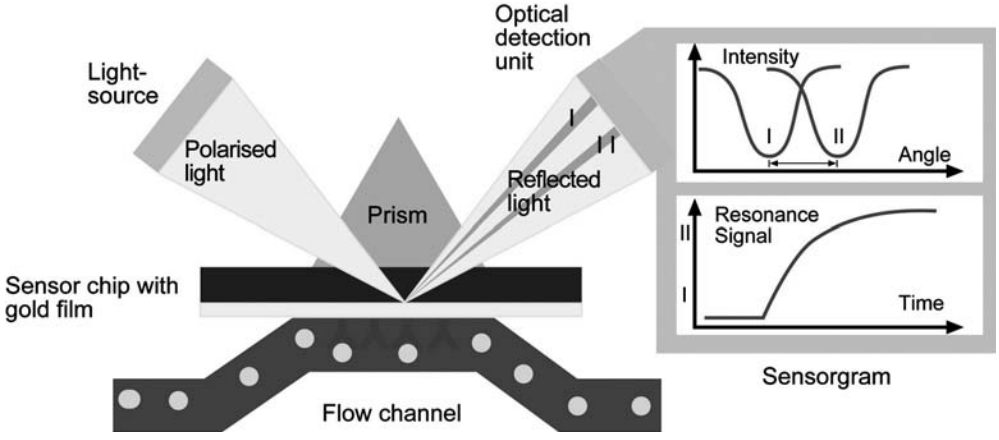


Fig. 1 Typical set-up for a surface plasmon resonance biosensor. Surface plasmon resonance (SPR) detects changes in the refractive index in the immediate vicinity of the surface layer of a sensor chip. SPR is observed as a sharp shadow in the reflected light from the surface at an angle dependent on the mass of material at the surface. The SPR angle shifts (from I to II in the diagram) when biomolecules bind to the surface and change the mass of the surface layer. This change in resonant angle can be monitored non-invasively in real time as a plot of resonance signal (proportional to mass change) versus time

[4, 5, 6], virology [7, 8, 9], epitope mapping [10, 11, 12], molecular engineering [13], cell biology [14, 15], cell adhesion [16, 17], signal transduction [18, 19], immune regulation [1], nucleotide–nucleotide [20, 21, 22] and nucleotide–protein [23, 24] binding, and enzyme mechanisms [25, 26]. Many of the commercially available optical biosensors exploit a surface-sensitive physical phenomenon called an evanescent wave. These sensors ultilize total internal reflection of light at a surface–solution interface to produce an electromagnetic field extending a short distance (hundreds of nanometers) into the solution. Several companies have exploited the surface-sensitivity of evanescent waves to produce label-free biosensors. At the time of press these included: Affinity Sensors (resonant mirror; www.affinity-sensors.com), Biacore (surface plasmon resonance; www.biacore.com), Farfield Sensors (dual waveguide interferometry; www.farfield-sensors.com), HTS Biosystems (grating-coupled surface plasmon resonance www.htsbiosystems.com), IBIS (surface plasmon resonance; www.ibis-spr.nl) and SRU Biosystems (colorimetric resonant reflection; www.srubiosystems.com).

Surface plasmon resonance, or SPR, is based on the transfer of light energy (photons) to a group of electrons on a metal surface [27]. Gold is the preferred metal as it is compatible with a number of linking chemistries and will not oxidize over time. Light is coupled into the surface by means of either a prism (Fig. 1) or a grating. This results in the propagation of charged density waves, called surface plasmons, along the metal surface. Plasmon propagation produces an electromagnetic field; the evanescent wave that is highly sensitive to changes in the dielectric constant of the adjacent medium. When the quantum energy of the photons and the plasmons are equal, there is an energy transfer. In the standard "Kretchman" SPR set up, the intensity of the reflected light is thus reduced as a result of this coupling, producing a shadow at a specific angle. As the evanescent wave has a short penetration depth, processes in the bulk solution have little influence on the angle of minimum reflectance (the SPR angle). This means that the conditions under which resonant coupling occur are dependent only on the optical properties of the region very close to the surface of the sensor. Hence, a change in the index of refraction at the surface of the sensor (due for example to drug binding) may be monitored as a shift in resonance angle observed when using monochromatic light (Fig. 1). This change can be monitored in real time to accurately measure:

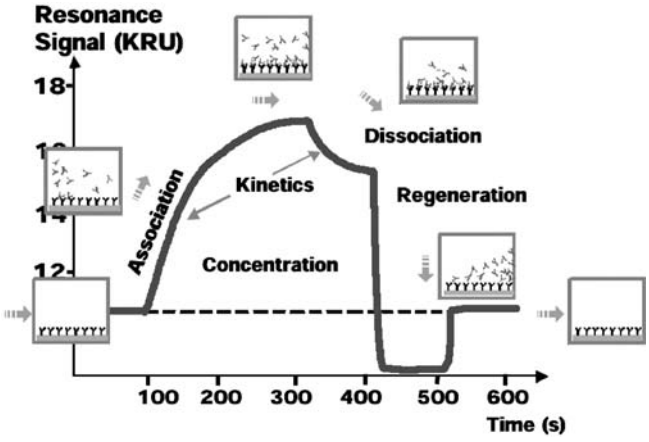


Fig. 2 A typical binding cycle observed with an optical biosensor. A receptor is immobilized on the sensor surface with appropriate coupling chemistry. At t=0 s, buffer is contacted with the receptor via a micro-fluidic flow cell or, in some commercial instruments, via a cuvette. At t=100 s a solution of analyte in the running buffer is passed over the receptor. As the analyte binds to the surface, the refractive index of the medium adjacent to the sensor surface increases, leading to an increase in the resonance signal. Analysis of this part of the binding curve gives the observed association rate (k_{obs}). If the concentration of the analyte is known, then the association rate constant of the interaction (k_{ass}) can be determined. At equilibrium, by definition, the amount of analyte associating and dissociating with the receptor is equal. The response level at equilibrium is related to the concentration of active analyte in the sample. At t=320 s the analyte solution is replaced by buffer and the receptor–analyte complex is allowed to dissociate. Analysis of these data gives the dissociation rate constant (k_{diss}) for the interaction. Many complexes in biology have considerable half-lives, thus a pulse of a regeneration solution (e.g. high salt, low pH etc.) is used at t=420 s to disrupt binding and regenerate the free receptor. The entire binding cycle is normally repeated several times at varying concentrations of analyte to generate a robust data set for global fitting to an appropriate binding algorithm. The affinity of the interaction can be calculated from the ratio of the rate constants (K_D=1/K_A=k_{diss}/k_{ass}) or by linear or non-linear fitting of the response at equilibrium vs. varying concentration of analyte. In addition to determining the interaction affinities and kinetics, thermodynamic analysis of a biomolecular interaction is also possible. This is done by applying van't Hoff's equations to the interaction affinities and kinetics of an interaction obtained at a variety of different temperatures [116, 117]

1. the amount of bound analyte,
2. its affinity for the receptor, and
3. the association and dissociation kinetics of the interaction (Fig. 2).

Alternatively, if polychromatic light is used, it is possible to measure the wavelength of the reflectance minimum at a fixed angle, which is seen as a change in colour of the reflected light.

Surface chemistry

The interface between the biosensor surface and the chemical or biological systems to be studied is a vital component of all surface-sensitive sensor systems. Receptors must be attached to some form of solid support whilst retaining their native conformation and binding activity. This attachment must be stable over the course of a binding assay and, in addition, sufficient binding sites must be presented to the solution phase to interact with the analyte. Most importantly, the support must be resistant to non-specific binding of the sample, which would mask the specific binding signal. Many coupling strategies utilize a chemical linker layer between the sensor base (e.g. the gold layer) and the biological component to achieve these ends. Functionalized alkane thiols [28] and alkoxy silanes [29] form stable layers on planar surfaces and act as ideal linkers. The alkyl termini of these molecules can be derivatized with ethylene glycol subunits to produce a protein-resistant planar surface [30], or can be mixed with molecules which possess suitable chemical reactivity for receptor capture (e.g. -epoxy, -carboxyl, -amino, -biotinyl,

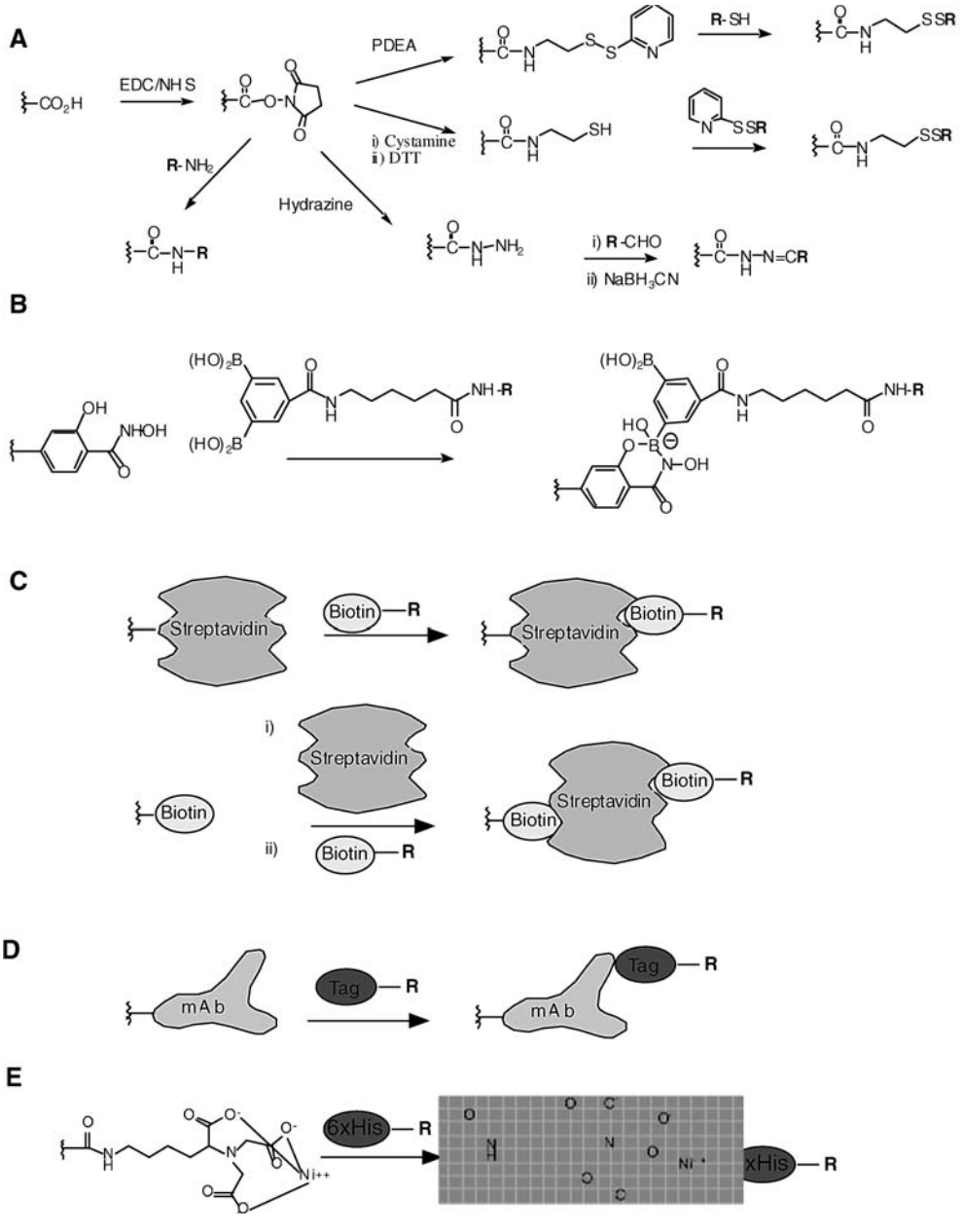


Fig. 3 Commonly employed coupling chemistries used to immobilize receptors for application with optical and acoustic biosensors

-nitrilotriacetic acid) [30, 31, 32]. The larger binding partner (e.g. the protein target) is normally immobilized on the surface, and the smaller binding partner (e.g. the drug) is allowed to bind to this surface from free solution. However, in some cases drug-like molecules have been attached directly to the chemical linker layer and receptors passed over the surface [33, 34].

The chemical linker layer can also be used as a substrate for attachment of a polymer coat or hydrogel, that renders the surface highly resistant to non-specific adsorption of proteins, nucleotides and drugs. The same polymer also provides a three-dimensional scaffold for receptor immobilization. The most widely employed biosensor polymer coat is carboxymethyl dextran [35], although other materials which produce a protein resistant hydrogel can also be used (e.g. hyaluronic acid, polyvinyl alcohol, poly(methyl methacrylate), sepharose, etc.). There are many strategies for either covalent or non-covalent attachment of receptors to either planar self-assembled surfaces or polymer coats (Fig. 3). Selection of the correct coupling chemistry requires careful consideration of:

1. the resultant orientation of receptor,
2. its local environment on the surface,
3. the stability of the linkage under the conditions used to regenerate the surface, and
4. possible effects of the coupling chemistry on components of the binding interaction.

Membrane proteins present their own unique challenges and are beyond the scope of this article.

Immobilization of a receptor to the sensor surface is of central importance to the design of a successful biosensor assay [36]. The coupling method must be efficient, must produce a highly stable association (to prevent signal drift) and must allow control of the amount of receptor immobilized. Amine coupling (e.g. to surface lysine residues or N-terminal residues on a protein receptor) will generally lead to a heterogeneous population of receptors with random orientation on the surface. However, if immobilization is performed at low pH, the amine terminus is likely to be much more reactive than the *gamma*-amino group of any lysine residues, in which case amine coupling can give rise to more ordered immobilization. Acidic receptors (pI<3.5) are difficult to immobilize by amine coupling, since the low pH required for electrostatic pre-concentration to the sensor surface protonates the primary amino groups and reduces the coupling efficiency. Further derivatization with sulfydryl-reactive reagents (e.g. pyridinyldithioethanamine (PDEA) or 3-(2-pyridinyldithio) propionic acid *N*-hydroxysuccinimide ester) (SPDP)) allows reaction with free surface thiols (e.g. Cys, Met) to form a reversible disulfide linkage. In a similar manner, stable thioether bonds may be formed using maleimide coupling reagents such as sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexanecarboxylate (Sulfo-SMCC) and *N*-(γ-maleimidobutyrloxy)sulfosuccinimide ester (GMBS). The surface may also be derivatized with cystamine to effect coupling with disulfide-activated receptor. Finally, treatment with hydrazine followed by a reductive amination enables coupling with aldehydes (Fig. 3a). The aldehyde groups may be native to the receptor or formed by mild oxidation of any *cis* diols present (Fig. 3a). These latter techniques have the advantage that they tend to produce a more homogenous population of oriented receptors on the surface [37, 38, 39].

Surfaces derivatized with salicylhydroxamic acid (SHA) can be used to produce reversible complexes with receptors that have been activated with phenyldiboronic acid (PDBA) [40] (Fig. 3b). Biotin or streptavidin presenting surfaces can be used to capture biotinylated receptors (Fig. 3c). The multiple biotin binding sites of streptavidin on each face of the molecule allow biotinylated ligands to be cross-linked by the streptavidin "double adaptor". This method is highly efficient and leads to very stable complexes, but is effectively irreversible. It is commonly employed to immobilize 5′-biotinylated oligonucleotides [20, 23, 41, 42]. Monoclonal antibodies can be covalently attached to a solid support via amine coupling as in a). Epitope tagged or fusion proteins can then be directly and reversibly coupled to the surface via the antibody–antigen interaction [36, 43, 44] (Fig. 3d). Commonly employed tags include GST, HSV, FLAG, 6×His, etc. Metal co-coordinating groups such as iminodiacetic acid (IDA) and nitrilotriacetic acid (NTA) have been widely employed for direct immobilization of 6×His and 10×His tagged receptors [45, 46, 47, 48]. The moderate affinity of the Chelate–Ni^{++}–Histidine ternary interaction means that there is sometimes considerable decay in the level of immobilized receptor. For this reason anti-6×His mAbs are often employed to effect stable, oriented immobilization of His-tagged receptors [49] (Fig. 3e).

Mass transport and bulk effects

Optical biosensors generally require a surface-immobilized receptor to function. It is thus important to consider the possibility of mass transport-limited binding between the analyte in bulk solution and the receptor on the sensor surface. This can occur when the binding of the analyte to the receptor is faster than the diffusion of the analyte from the bulk solution to the receptor at the surface. The same phenomenon also results in increased rebinding of the analyte in the dissociation phase, as the released analyte can rebind to free receptor before it diffuses into the bulk solution. The effect is most pronounced with very large analytes (which possess low diffusion rates) and with analytes that possess very fast association or dissociation rates (comparable to the diffusion rate). To minimize the effect, very low levels of receptor are immobilized and high flow rates are employed, which has the effect of reducing the surface-associated "unstirred" layer [36]. It is also possible to introduce a "mass transport" rate constant into fitting algorithms to ensure that the binding data is correctly analysed [50]. Results using a computer simulation have suggested that the carboxymethyl dextran hydrogel most commonly employed with optical biosensors could significantly retard the diffusion of analyte to its receptor at the

surface [51]. This supposition has subsequently been shown to be incorrect, as identical rate constants were obtained for analyte binding when a receptor was immobilized on either a carboxymethyl dextran hydrogel, or a planar self-assembled monolayer [36]. However, very large analytes (e.g. phage and vesicles) are significantly retarded by, or cannot penetrate, the hydrogel [52].

When using an optical biosensor, it is extremely important to include blank surface controls, and if possible, non-relevant receptor controls to correct for the effects of signal drift, non-specific binding and other bulk effects. The carboxymethyl dextran matrix normally employed for screening small molecules is a negatively charged hydrogel. In water and buffers the carboxymethyl dextran chains repel each other leading to expansion of the hydrogel and a change in mass distribution in the evanescent field near the surface. In addition, when slightly different amounts of a receptor are immobilized on different surfaces or spots, there are subtle differences in the amount of repulsion and the hydrogel can shrink or swell accordingly [36]. This does not normally affect assays carried out in biological buffers, as bulk refractive changes can be successfully corrected for by subtraction of a reference surface that contains no receptor, or a non-relevant control receptor. However, small molecular weight compounds are invariably prepared and stored as 1–10% DMSO solutions. When using DMSO solutions, the bulk effects arising from the variation in hydrogel void volume (i.e. that space not occupied by receptor) can mask the specific binding signal. It is possible to circumvent this problem by first creating a calibration curve using varying concentrations of DMSO in running buffer in the absence of the small molecule. This "normalizes" for the bulk refractive index changes on the different surfaces and high quality binding data can then be obtained [53].

Optical biosensor arrays

The analysis of many complex binding events requires multiplexed detection systems that can analyse many binding interactions simultaneously. Commercially available optical biosensors have been limited in their application to HTS and proteomics analysis by the low number of surfaces or spots that could be sensed simultaneously. A major breakthrough was achieved in 1988 by Rothenhausler and Knoll [54] when they demonstrated the simultaneous imaging of an entire surface using surface plasmon microscopy (SPM), which has very similar basic principles to SPR.

Following on from this pioneering work, several companies are now actively developing optical biosensor array platforms. Biacore has now developed a prototype SPR array that the company claims can simultaneously assay <50 spots, with no compromise on the high quality of information and high sensitivity possessed by the original four-spot system. HTS Biosystems employs an alternative approach to SPR, called grating-coupled SPR or GCSPR. In this case, the biosensor chip is made of plastic and can be moulded to include other features, such as channels, reaction chambers and fluid coupling ports. By moulding a fine grating onto the surface of a CD-ROM, a large number of addressable active sites could be created at very low cost. The CD-ROM could then be changed, as in a jukebox, allowing for low-technology automation and sensing. HTS Biosystems claims formats will also include single chips or 96- and 384-well microplate-compatible footprints with multiple high-density arrays. SRU Biosystems exploits a phenomenon called colorimetric resonant reflection using a surface that reflects a very narrow band of wavelengths when illuminated with normal white light. The sensor structure can be produced inexpensively in continuous sheets of plastic film and incorporated into various standard laboratory assay formats, such as 96, 384 and 1536 well microtitre plates, microarray slides, and fluid flow channels. SRU Biosystems is pursuing a novel approach for the detection of molecular interactions using a colorimetric resonant diffractive grating surface. A guided mode resonant phenomenon is used to produce an optical structure that, when illuminated with white light, is designed to reflect only a single wavelength. When molecules are attached to the surface, the reflected wavelength (colour) is shifted due to the change of the optical path of light that is coupled into the grating. By linking receptor molecules to the grating surface, complementary binding molecules can be resolved at the level of ~0.1 nm thickness of protein binding.

Biosensor arrays will enable a quantum improvement in assay throughput with a high level of flexibility in experimental design. Potential applications of multiplexed, label-free screening include cell proteome screening, high-throughput target identification, high-throughput screening of arrayed small molecules, arrayed antibodies and arrayed peptides, and finally high-throughput ADME/T receptor arrays for "on chip" drug profiling. In addition, the simultaneous interrogation of multiple reference sites brings with it a number of significant technical advantages. Multiple sites can be used to probe the different levels of signal shifts using repeated standards to improve the quality of the data. Sites can be designated as positive and negative controls for biology, chemistry or hardware. These in situ controls should reduce the need for more expensive engineering and production steps by normalizing for artefacts such as transducer inhomogeneity, uneven sample introduction and uneven temperature control.

Acoustic biosensors

Acoustic biosensors also allow the label-free detection of molecules and analysis of binding events. In general, they are based on quartz crystal resonators, which are found in electronic devices such as watches, computers and televisions, with over a billion units mass-produced each year (Fig. 4). They became of interest to physicists and chemists when it was demonstrated that there is a linear relationship between mass adsorbed to the surface and the resonant frequency of the crystal in air or a vacuum [55]. Application to biological samples became possible when suit-

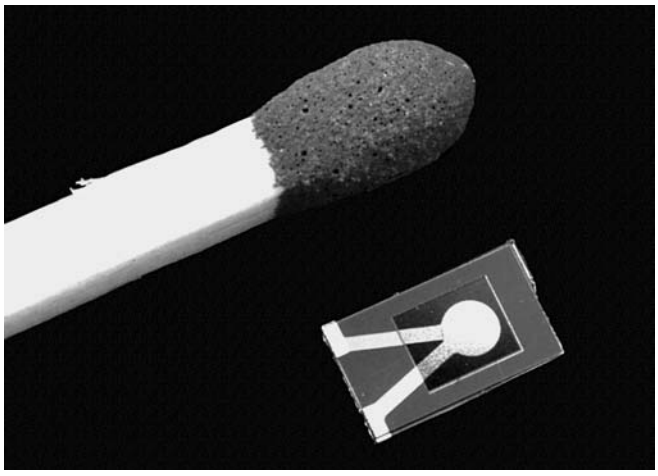
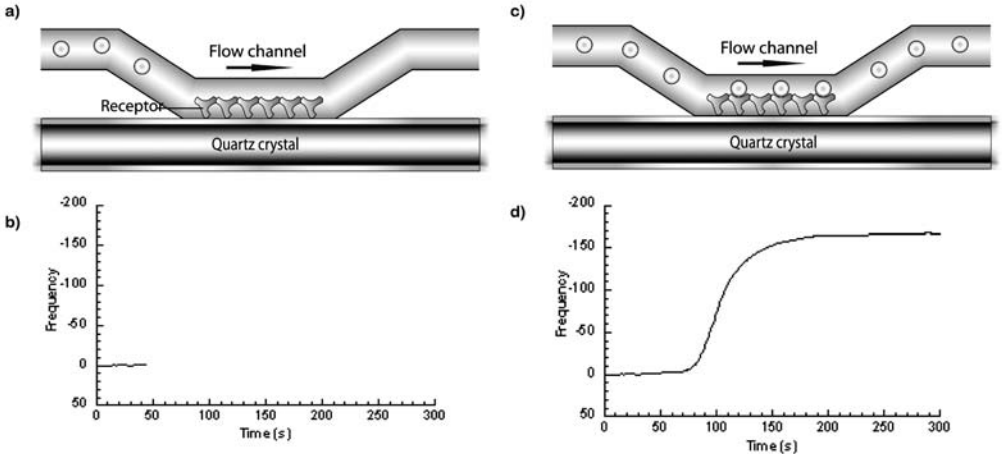


Fig. 4 A quartz crystal resonator coated with two gold electrodes that are then coated with a chemical linker layer and a receptor or cell. This device is then integrated into either a cuvette or a flow cell for delivery and removal of ligands, etc.

able oscillator circuits for operation in liquids were developed [56, 57]. By monitoring the change in resonant frequency and resistance that occurs upon adsorption of a ligand to the surface (Fig. 5), quartz crystal resonators can be used to characterize interactions with small molecules [58, 59, 60], peptides [61, 62, 63], proteins and immunoassay markers [64, 65, 66, 67, 68, 69, 70], oligonucleotides [71, 72], bacteriophage [73, 74, 75], viruses [76, 77, 78], bacteria [79, 80, 81, 82, 83, 84], and cells [85, 86, 87, 88, 89, 90, 91, 92, 93]. The technology can thus be applied to an extremely wide range of biological and chemical entities with a molecular weight range from less than 200 Daltons through to a entire cell. Real time monitoring of changes in the resonant properties of the crystal allows the label-free determination of interaction affinities and kinetics (Fig. 5), as is the case for SPR biosensors. However, acoustic sensors are more than just simple mass balances. Much more detailed information can be obtained about an interaction than is the case with SPR biosensors as the acoustic sensor response is sensitive not only to the mass of ligand bound, but also to changes in visco-elastic properties and charge of the receptor–ligand complex [94, 95].

In addition to monitoring the association and dissociation of molecules, a quartz crystal can be used in an ultra-high sensitivity mode by forcing bonds between a receptor and ligand to break and then "listening" to the sounds produced [96]. To induce a molecular complex of moderate affinity to break apart requires very high accelerations; millions of times the force of gravity. Quartz is a piezoelectric material, which means it deforms if an electric field is applied to it and, conversely, generates an electric field in response to mechanical stress [97]. As the magnitude of an applied electric field is increased, so the amplitude of oscillation increases, and hence there is increasing acceleration of particles adhered to the surface up to the level of 40 million times the acceleration due to gravity! This in turn results in an increasing force exerted by the surface on the particles, which ultimately causes rupture of the bonds attaching the particles to the surface. The quartz crystal can be used as a very sensitive microphone to detect the acoustic emission produced by bond rupture, which is then converted into an electrical signal. The signal indicates not only the presence of the particles but also the number of particles present and their affinity for a surface-bound receptor (Fig. 6). The process, developed by Akubio (www.akubio.com), and termed rupture event scanning (REVS™) requires minimal sample preparation, works well in buffered solutions and in complex biological fluids such as serum, and takes only minutes to perform [96, 98, 99]. The magnitude of acoustic emission, or "loudness" of the sound emitted, is proportional to the number of particles present over at least six orders of magnitude, right down to the level of a single particle with a

Fig. 5 (**a**) A quartz crystal resonator coated with a receptor integrated into a flow cell to which ligand can be added. (**b**) As liquid is passed over the surface the crystal resonates giving a signal in real time. (**c**) When ligand is added to the flow cell it binds to the receptor resulting in a change in the acoustic profile of the resonator leading to (**d**) a change in the resonant frequency. This changing signal is proportional to the amount of ligand bound to the receptor, which enables detection of a ligand and determination of the ligand–receptor interaction affinities and kinetics. It is also possible to measure the dampening of the resonator (the motional resistance), which gives information about the conformation of the receptor in real time



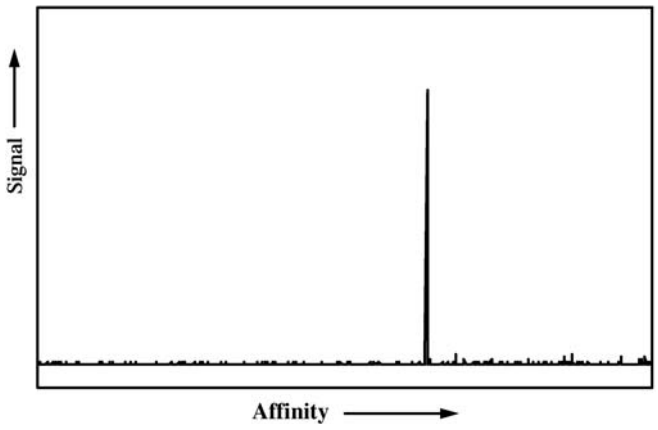


Fig. 6 A typical rupture event scanning result. A quartz disc decorated with a protein that mediates specific attachment of a ligand-coated nanoparticle (which in this case has a nanomolar affinity for the protein). The disc is transversely oscillated by applying an alternating voltage to gold electrodes on either side of the disc inducing rupture of the bonds between the ligand and the receptor. The resulting burst of acoustic energy, or sound, is converted into an electrical signal. The position of the peak indicates the relative affinity (from mmol L^{-1} to pmol L^{-1} on the x-axis) and the height of the peak is proportional to the number of active ligand molecules present in the sample

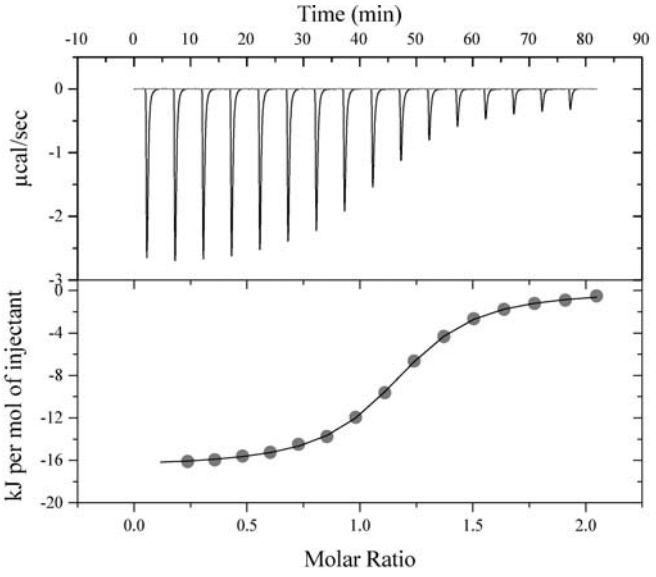


Fig. 7 A typical differential ITC binding isotherm for the binding of a ligand to a receptor. *Top*, raw data; *bottom*, binding isotherm created by plotting the integrated peaks against the molar ratio of ligand added to macromolecule present in the cell

weight of only 30 femtograms. This corresponds to a mass sensitivity of 30 fg mm^{-2} (3×10^{-14} g mm^{-2}) [96]. In comparison, the best surface plasmon resonance optical biosensors can detect a mass change of ~1 pg mm^{-2} (~10^{-12} g mm^{-2}). The latter technique is not directly applicable to complex fluids such as undiluted serum, as there is significant non-specific binding of materials such as serum proteins to the sensor surface. REVS detection can also be combined with a separation step, as low affinity binding interactions can be disrupted at low accelerations, thus leaving higher affinity interactions intact for subsequent desorption and decoding. The combination of sensitive detection, quantitation and separation provides a powerful and flexible platform for diagnostics, drug screening and life sciences research [100].

Calorimetry

Long viewed as the "gold standard" technique for characterizing the thermodynamics and stoichiometry of a molecular interaction, isothermal titration calorimetry (ITC) has been applied to many areas of pharmaceutical analysis. When substances bind to each other, heat is either generated or absorbed. Measurement of this heat allows determination of binding constants, reaction stoichiometry and the thermodynamic profile (enthalpy and entropy) of the interaction [101]. Unlike other some other methods, ITC does not require immobilization and/or modification of reactants, since heat of binding is a naturally occurring phenomenon. In an ITC assay, a ligand solution is titrated into a well-insulated, stirred cuvette containing a receptor kept at constant temperature. As heat is released or absorbed during a molecular interaction, a binding isotherm is obtained as a plot of the heat change versus the molar ratio of ligand to receptor (Fig. 7). Careful control experiments are needed to compensate for bulk effects such as the heat of dilution of the ligand and the receptor, and the heat of mixing [102, 103]. ITC has been routinely used to study many types of binding reactions [101, 103, 104, 105, 106] including, protein–protein [107], protein–membrane [108, 109, 110, 111], and drug–receptor [106, 112] interactions.

Similar in principle to ITC, differential scanning calorimetry (DSC) measures conformational changes in macromolecules and has been used in the elucidation of thermally induced structural transitions, estimation of formulation stability, and chemical half-life studies. Whereas in an ITC assay, the temperature of the sample is kept constant, in a DSC assay differences in heat generated in a sample and reference cell are measured as the temperature of the sample is either increased or decreased. The heat difference between the sample and the reference is related to the conformational energy of the receptor–ligand complex in the cuvette. By determining the different temperatures at which transitions (T_m) occur and the heat capacity (C_p) of these transitions, very detailed information can be gained regarding the dynamic structures of biological macromolecules. In addition, information on interaction reversibility may be obtained by cycling the temperature back and forth. DSC has been applied successfully to the study enzymes, receptors, growth factors, cell adhesion molecules and other targets. For detailed reviews see refs. [102, 108, 113, 114, 115].

Applications of DSC and ITC in the pharmaceutical industry have, until recently, been limited by the relatively

large amounts (>μmol L^{-1}) of receptor and ligand required and by the limited throughput available on commercial instrumentation. However, companies such as MicroCal (www.microcalorimetry.com), Thermometric (www.thermometric.com), Calorimetry Sciences Corp. (www.calorimetrysciences.com), and 3D Pharmaceuticals (www.3dp.com) are developing parallel, miniaturized thermal shift assays involving DSC, ITC or a combination of both. Vivactiss (www.Vivactiss.com) is developing a miniaturized microplate format DSC for higher throughput identification of novel active molecules.

Conclusion

There are an increasing number of commercially available instruments driving forward the development of novel sensors and receptor immobilization techniques that enable virtually any receptor–analyte complex to be screened. A label-free screening system imparts enormous flexibility to the process of assay design. Scientists in both academic life and industry are using biosensors in areas that encompass almost all areas of the chemical and biological sciences. The major challenges that lie ahead for label-free screening systems include the successful integration of the detection technology with microfluidics, chemistry, biology, signal processing, and data management. Quartz crystal resonators have been mass-produced for many years and are relatively inexpensive. As the detection system is entirely electronic, it is possible to multiplex the assay in a variety of ways and also to miniaturize the detector for direct detection in the field or at the patient point of care. These developments and the advent of SPR and waveguide arrays will accelerate acceptance of biosensors in new areas of drug discovery where high information content, rather than ultra-high throughput, is important. Calorimetry continues to provide the "gold standard" in the measurement of interaction thermodynamics and progress is being made to increase sample throughput and reduce sample consumption.

Label-free detection technology thus has great potential as a method for interrogating chemical and biological samples not only in drug discovery, but also in the fields of diagnosis, environmental monitoring, and the life sciences in general. The impact of optical, acoustic and calorimetric sensors should continue to grow dramatically over the next decade.

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