Akubio: label-free screening of molecular interactions using acoustic detection

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Akubio was formed in August 2001 to exploit highly sensitive acoustic detection technology discovered by scientists at the University of Cambridge, UK. The technology enables rapid, label-free determination of molecular interactions. Receptor–ligand interactions can be probed either in buffered solutions or in complex biological fluids, as the detection process is not adversely affected by non-specific binding of sample contaminants. Applications are being developed for the diagnosis of clinical infections, the screening of libraries for receptor binding, and the determination of molecular interaction affinities in general.

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▼ The analysis of molecular interactions is an integral part of the drug discovery process. Pharmaceutical companies spend many millions of dollars on screening compounds for receptor binding *in vitro* during the initial phase of the development of new drugs. The majority of this screening is carried out by interrogating a sample with some form of electromagnetic radiation: radio waves, microwaves, infra-red, visible light, ultraviolet light, x-rays or gamma radiation. The most widely used screens require some type of radio- 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. Fluorescent compounds are invariably hydrophobic and, in many screens, background binding is a significant problem, leading to false positives. Ideally, a screen should be universally applicable, label-free, sensitive, economic and rapid.

Quartz crystal resonators

Akubio has developed detection technology that does not use any form of electromagnetic radiation, but instead listens to the 'sound' made as molecular interactions are disrupted. To induce a molecular complex of moderate affinity to break apart requires very high accelerations, millions of times the force of gravity. This can be achieved with an acoustic wave device such as a quartz crystal resonator (Fig. 1). Quartz resonators are found in electronic devices, such as watches, computers and televisions, with over a billion units mass produced each year. They became of interest to physicists and chemists when it was demonstrated that there is a linear relationship between adsorbed mass and the resonant frequency of the crystal in air or in a vacuum [1]. Application to biological samples became possible when suitable oscillator circuits for operation in liquids were developed [2]. By monitoring the change in resonant frequency of the crystal, which occurs upon adsorption of mass to the surface, quartz crystal resonators can be used together with appropriate surface chemistry and fluidics to detect the adsorption of proteins, oligonucleotides, cells and other particles to surface-bound receptors [3]. This enables the label-free determination of interaction affinities and kinetics in real-time. However, the mass sensitivity is currently not sufficient to enable direct detection of small molecular weight compounds, and competitive inhibition assays are normally performed to overcome this limitation.

Improved sensitivity

Akubio is applying its proprietary technology to increase the sensitivity of traditional frequency change quartz crystal biosensors. It has recently

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Figure 1. A quartz crystal resonator that has been coated with gold, a chemical linker layer, and a protein receptor. The crystal holder makes electrical contact with the gold electrode that enables the quartz to be driven with an alternating current, thus inducing oscillation of the surface as a result of the piezoelectric effect.

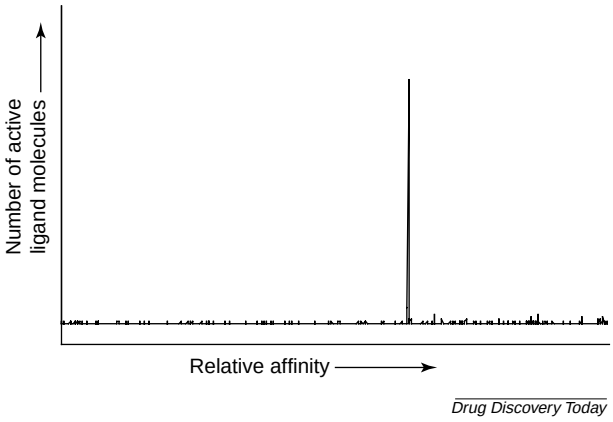

Figure 2. A typical acoustic detection experiment. A quartz disc is coated with a protein receptor that mediates specific attachment of a ligand bound to a nanoparticle (which in this case has a nanomolar affinity for the protein). The disc is transversely oscillated by applying an alternating voltage to gold electrodes on either side of the disc, inducing rupture of the bonds between the ligand and the receptor. The resultant burst of acoustic energy, or sound, is converted into an electrical signal. The position of the peak indicates the relative affinity (from mM to pM on the x-axis) and the height of the peak is proportional to the number of active ligand molecules present in the sample.

patented and published work in which the quartz crystal can be used in an ultra-high sensitivity mode [4]. 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 [5]. 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. 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 relative affinity for a surface-bound receptor (Fig. 2). The scanning process requires minimal sample preparation, works well in buffered solutions and in complex biological fluids such as serum, and takes only minutes to perform. 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 weight of only 0.5 fg. This corresponds to a mass sensitivity of 0.5 fg mm^{-2} ($<10^{-15}$ g mm^{-2}) [4]. In comparison, traditional frequency-change quartz crystal biosensors have a detection limit in the order of 1 ng mm^{-2} (10^{-9} g mm^{-2}), whilst the best surface plasmon resonance optical biosensors can detect a mass change of 1 pg mm^{-2} (10^{-12} g mm^{-2}). Neither of the latter techniques works well in complex fluids such as undiluted serum, as there is significant non-specific binding of materials such as serum proteins to the sensor surface. The Akubio technology can also combine the detection process 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 drug screening.

The company

The pioneering work behind Akubio's technology was carried out in the Chemistry and Pathology Departments at the University of Cambridge by a team including Matthew Cooper, Victor Ostanin, David Klenerman, Fedor Dultsev, Tony Minson and Chris Abell. The team soon realized that the applications of the technique were wide-ranging and Akubio has backing from investors that include the University of Cambridge, the UK Challenge Fund and Abingworth Management. Matthew

Cooper is leading Akubio as Founder and Chief Scientific Officer with scientific support from Tony Minson (head of Biological Sciences at the University of Cambridge and co-inventor of the Cantab DISC Vaccine), Chris Abell (reader at the University of Cambridge and co-founder of the crystallography and drug discovery company Astex Technologies) and David Klenerman (lecturer at the University of Cambridge and co-founder of the gene sequencing company Solexa). Stephen Bunting (Abingworth Chief Executive Officer), John Shields (Abingworth, ex-Cantab Pharmaceuticals) and Tim Rink (ex-Aurora Chief Executive Officer, Amylin and SmithKline Beecham) provide significant management expertise. The company has raised an initial £1.2 million in seed funding that will fund six full-time employees and associated consultants through to the end of 2002.

Future strategy

Akubio intends to retain control of core technology development and high-value applications, and partner or licence out other applications. The major challenges that lie ahead include the successful integration of the detection technology with fluidics, 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 patient point-of-care. Work is now being carried out to broaden the utility of the technique to include detection of viruses, bacteria, cells, proteins, oligonucleotides and small molecules. The company is currently in discussions with companies in the fields of drug discovery, proteomics, genomics, electronics and diagnostics. Akubio's acoustic detection technology thus has great potential as a new method for interrogating chemical and biological samples not only in drug discovery, but also in the fields of diagnosis and life sciences in general.

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