

Crude Sample Analysis Made Easy

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A new system enables biomolecular interaction analyses to be performed on impure samples – thereby saving on time, labour and costs.

Knowing the kinetic properties of biomolecules is increasingly important in drug research. Previously, it has been necessary to obtain purified molecules for such studies as impure samples pose challenges to non-specific binding and microfluidics. The ability to analyse impure samples would thus provide the benefits of saving time, labour and cost. At Attana, we have developed a system that makes this possible. In this article, we review the system and describe how it can be used in the screening of antibodies and determining their off-rates in serum containing hybridoma supernatants.

The instrument (the Attana 200) is a dual-channel biosensor for the automated analysis of biomolecular interactions. Incorporating quartz crystal microbalance (QCM) core technology, the system can be used to determine specificity, off-rates, kinetics, affinity, active concentrations and thermodynamics using crude samples. The QCM core technology enables not only

the study of biomolecules of varying species – such as proteins, nucleic acids and carbohydrates – but also of vastly different sizes, ranging from peptides to cells. This temperature-controlled, continuous flow system (see Figure 1) is being widely used both within industry and academia.



Figure 1: The Attana 200[®] system. Shown attached to a C-Fast pipetting robot for automation (Attana A200)

QCM TECHNOLOGY

In brief, by applying an alternating potential to a piezoelectric quartz crystal, the crystal can be controlled to oscillate at its resonance frequency. Different surface coatings offer the possibility to capture or immobilise molecules. As shown in Figure 2 (page 20), the technology can be used accordingly to study molecular interactions in real time.

In Figure 2, an antigen is initially immobilised on the sample surface, while an appropriate reference surface is constructed. As this adds mass, a new

Figure 2: Analysis of molecular interactions in real time. The figure shows the correlation between molecular interactions and sensorgram responses

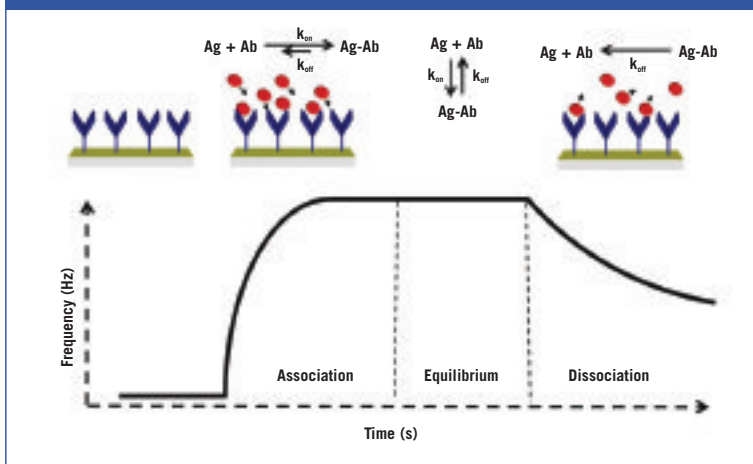
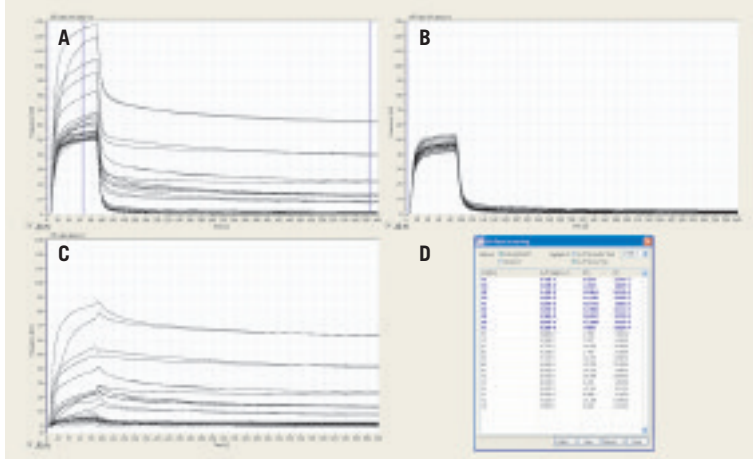


Figure 3: Screening and off-rate analysis of hybridoma supernatants. For all sensorgrams, the x-axis is time in seconds and the y-axis is response in hertz:

a) Supernatants interacting with antigen on the surface; b) Supernatants interacting with the reference surface; c) Reference corrected data used for off-rate analysis; d) Off-rates derived from the analysis displayed and selected in an easily comprehensible list



resonance frequency is registered. The antibody is then injected, and binding of the antibody to the surface-bound antigen increases the mass further, whereupon a new shift in the resonance frequency is registered. If only a measurement of affinity (K_D) is required, the interaction is allowed to reach equilibrium. To determine the off-rate constant (k_d), pure buffer is allowed to flow over the sensor surface, washing away unbound antibody. The surface is then regenerated leaving only immobilised antigen and is ready for injection of a new antibody.

SCREENING OF HYBRIDOMA SUPERNATANTS

Often, antibody-containing hybridoma supernatants have to be purified before affinity determination; this is time-consuming, labour-intensive and can encounter difficulties. To save time and labour, we have screened unpurified hybridoma supernatants containing 20 per cent serum directly against an antigen on the sensor surface (referred to as a direct approach) with good results using the Attana A200 system (an automated version of the Attana 200 system).

Hybridoma supernatants have previously been screened using a capture approach, where an antibody is first captured before the antigen is injected and the antibody-antigen interaction studied. The capture approach, however, consumes a large amount of sample and is time-consuming because of the extra step of capturing the antibody. The direct approach saves both time and consumption of sample, thereby decreasing the cost of expensive antigens while increasing throughput. The key requirement of the direct approach is that it has to be performed on surface chemistries that have very low, non-specific binding.

To address this need, we developed a low non-specific binding surface. A tagged antigen was immobilised on one surface and the tag only was immobilised on the reference surface. Hybridoma supernatants were then injected and screened directly against the antigen. The system's response on the antigen-coupled surface is shown in Figure 3a, and the response over the reference surface is shown in Figure 3b. Reference subtraction is then made to correct for bulk effects and drift (Figure

3c). Off-rate constants (k_{off}) were then directly determined from the resulting curves, and are displayed either in an easily comprehensible list that can be printed or exported to any of a number of formats (such as Excel), or as a report also containing graphical data of the interaction (Figure 3d). The relatively large dimensions of the fluidics in the systems reduce the incidence of problems such as clogging when running impure samples.

The Attana A200 system represents a new generation of biosensor systems designed with crude samples in mind in terms of aspects such as the fluidics and low non-specific binding surfaces. The system has been tested with a range of serums from different species used in the industry today. As shown in Figure 4, 20 per cent mouse serum (Figure 4a), 20 per cent rabbit serum (Figure 4b), 20 per cent foetal bovine serum (Figure 4c) and 20 per cent calf serum (Figure 4d) have been tested with good results. To further confirm this performance, applications using cell lysates in the system have also been tested using 100 per cent cell (see Figure 5a) and 100 per cent bacterial (Figure 5b) lysates.

REAL-TIME, DYNAMIC DATA

Using the Attana A200 can speed up the selection and characterisation of biomolecules. By enabling the measurement of real-time, dynamic data – rather than giving end-point values – the system provides a broader understanding of the interactions. The ability to view all phases of the interaction, continuously, gives information on specificity of binding, matrix effects and epitope competition. This is information that facilitates, for example, sandwich pair selection, enzyme-linked immunosorbent assay (ELISA) optimisation and study of non-specific binding, allowing selection to be made directly upon on- and off-rate constants, rather than just affinities. This eventually enables the system to offer higher resolution screening and the possibility for selection to be made directly on the basis of the off-rate. Additionally, the low non-specific binding surfaces expand the possibility to perform the analysis at an earlier stage, thereby saving the time and effort put into purifying and refining samples. Furthermore, the QCM technology requires no labelling of the biomolecules under study – something that is known to affect interaction studies.

As well as kinetic analysis, the system can be also used for quality control in manufacturing processes, assay

Figure 4: Non-specific binding from serum. Non-specific binding to naked surfaces probed using 20 per cent serum from different species: a) Calf serum; b) Foetal Bovine Serum; c) Rabbit serum; and d) Mouse serum

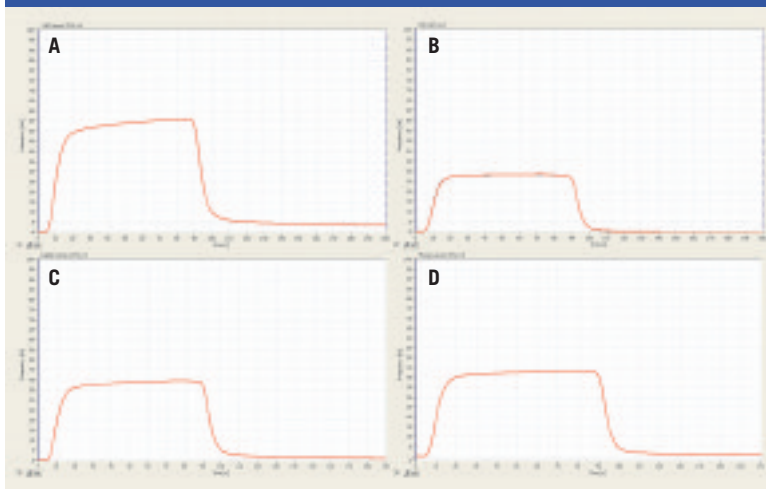
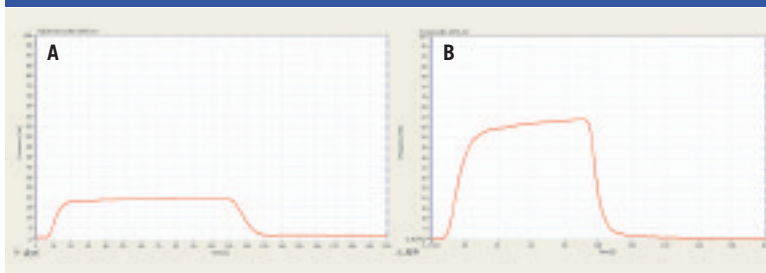


Figure 5: Non-specific binding from lysates. Non-specific binding to naked surfaces probed using 100 per cent lysates only, pre-treated by a fast centrifugation: a) Human cell line lysate; and b) *E Coli* lysate



development and troubleshooting in the *in vitro* diagnostics industry, and for helping to document and comply with regulatory requirements in the biopharmaceutical industry. The instrument also makes a strong contribution to the portfolio of technologies used in academia, with its versatility in studying proteins, nucleic acids and carbohydrates in purified as well as unpurified samples, adding crucial information about dynamics to the research and development programme.



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