Supernatant in, kinetics out

Knowing the kinetic properties of biomolecules is increasingly important. It has been necessary to obtain purified molecules because impure samples pose challenges to unspecific binding and microfluidics. However, the ability to analyze impure samples would have the benefit of cutting time, labor and cost. The Attana A100™ instrument presents this possibility, here exemplified with a screen of antibodies and determination of their kinetics in supernatants.

The Attana A100 instrument is a temperature-controlled, continuous-flow system for automated analysis of biomolecular interactions (**Fig. 1**). It can be used to determine active concentrations, novel ligands, affinities, kinetics and thermodynamics. The quartz crystal microbalance (QCM) core technology enables not only the study of biomolecules of varying species such as proteins, nucleic acids and carbohydrates but also of binding moieties of vastly different sizes, ranging from peptides to cells. The instrument is now used both within industry and academia, owing to its versatility, robustness and ease of use.

Principles of QCM and molecular interactions

In brief, by applying an alternating current potential to a piezoelectric quartz crystal, the crystal can be controlled to oscillate at its resonant frequency. Different surface coatings offer possibilities of capturing or immobilizing molecules. This technology can be used to study molecular interactions in real time (**Fig. 2**).

An antibody, for instance, may be initially immobilized on the surface of the crystal. As this adds mass, a new resonant frequency is registered. The antigen is injected next. Binding of the antigen to the surface-bound antibody increases the mass further, whereupon a new shift in the resonant 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 antigen. The surface is then regenerated, leaving only immobilized antibody, and the system is ready for injection of a new antigen.

Screening of hybridoma supernatants

Often antibody-containing hybridoma supernatants have to be purified before affinity determination. This is time-consuming and labor-intensive.

Cutting time and labor, we screened unpurified hybridoma supernatants directly against an antigen on the sensor surface with good results using the Attana A100 instrument.

However, a better approach is often to capture the antibodies directly from the hybridoma supernatant and then study exclusively the interaction with the antigen. Attana offers a range of capturing surfaces for antibodies and protein tags to reduce assay time and optimization. We captured antibodies from hybridoma supernatants and screened them directly against the antigen (**Fig. 3a**). After reference subtraction, on- and off-rate constants (k_a and k_d) were directly analyzed, and we selected antibodies for further study (**Fig. 3b**).

Figure 1 | The Attana A100, shown attached to a C-fast pipetting robot for automation.

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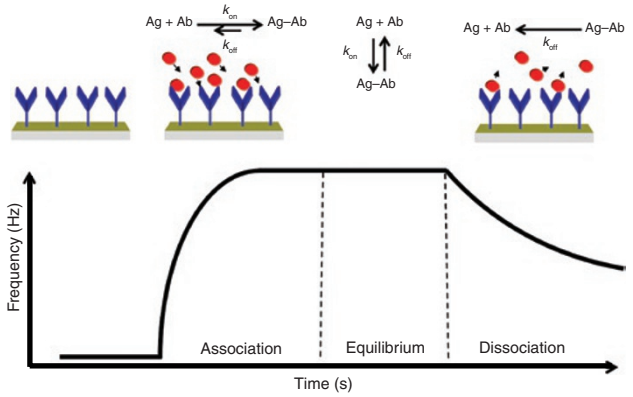

Figure 2 | Analysis of interactions in real time. The schematics show an antibody immobilized on the surface of a chip and an antigen injected as the analyte. The graph shows the instrument responses during the different phases of the interaction. The binding phases in the schematic are matched to the corresponding instrument responses below.

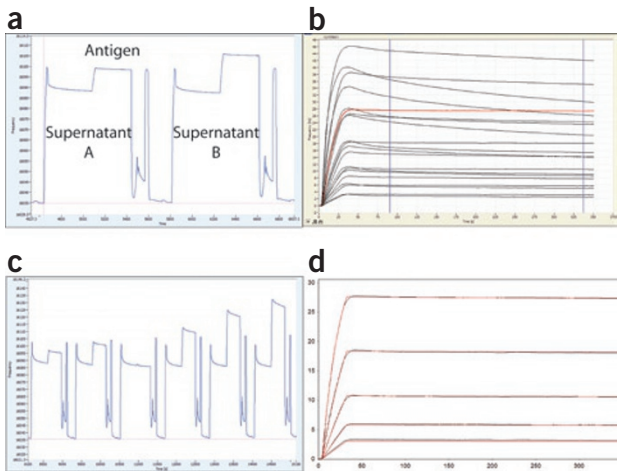

Figure 3 | Screening and kinetics analysis of hybridoma supernatants. (**a**) Instrument response as supernatants are sequentially captured and antigen is injected to probe the interaction. (**b**) Each curve is data for a different supernatant measured as in **a**. The curves are reference corrected, and off-rates are determined. (**c**) Analysis of one supernatant that is repeatedly captured followed by injections of antigen at increasing concentrations. (**d**) Global analysis of the data generated in **c** to establish on- and off-rate constants (k_a and k_d).

Detailed kinetics studies in hybridoma supernatants

In addition, Attana provides capabilities for detailed studies using the same surface. To determine detailed kinetics, we first captured hybridoma supernatant at a specific dilution and then injected the antigen. Then we regenerated the surface and, in subsequent cycles, captured the same hybridoma supernatant using the same dilution but varied the concentration of the antigen (**Fig. 3c**). We then reference-corrected the data and used a global analysis to verify the binding model and referencing and to determine the kinetics and affinity of the interaction in detail (**Fig. 3d**). As compared to competing biosensor technologies, the relatively large dimensions of the fluidics diminish problems with clogging.

Benefits and use

The Attana A100 instrument speeds up selection and characterization of biomolecules.

By enabling measurement of real-time, dynamic data, rather than just giving end-point values, the Attana A100 instrument helps to provide a broad understanding of the interaction. The ability to view all the 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) optimization and study of nonspecific binding. Providing on- and off-rate constants rather than just affinities, the Attana A100 instrument offers higher resolution and the possibility to select directly based on the off rate. Captured molecules may also be recovered for additional analysis. Also, the QCM technology requires no labeling of the biomolecules under study, something otherwise known to affect the interaction.

The stated benefits make the Attana A100 instrument not only a powerful tool for kinetic analysis but also for quality control in the manufacturing industry, assay development and troubleshooting in the *in vitro* diagnostics industry, and documentation and compliance with regulatory requirements in the biotherapeutics industry. In academia, the Attana instrument makes a strong contribution to the portfolio of technologies, with its versatility in studying proteins, nucleic acids and carbohydrates in purified as well as unpurified samples, adding information about dynamics.

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