BIACORE 3000 – primed to perfection

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BIACORE 3000 represents the logical next step in the development of BIACORE systems for sophisticated binding studies, with better sensitivity, higher throughput, improved liquid flow properties and an easier software interface than previous systems in the series. In short, the best biomolecular interaction analysis system available today.

When biomolecular interaction analysis first became commercially available with the introduction of the BIAcore system in 1990, the door was opened on a new way of looking at molecular binding events. As Professor Marc Van Regenmortel expressed it at the first BIAsymposium in 1991, the technology "shows what the molecules do". The technology provided a general approach to some of the fundamental questions in molecular life science research:

- How specific is the binding between two molecules?
- How much of a given molecule is present and capable of binding to another?
- How fast does the binding proceed?
- How strong is the binding?

Today, eight years later, the scope of BIACORE analyses remains essentially unchanged, but the information detail provided by such analyses has increased enormously as both systems and experimental techniques have been continually refined. BIACORE 3000 is the latest system in the series that charts this development, and provides the most information and highest resolution available today. The technology, and more significantly the way it is implemented and used, has been primed to meet the demands of perfection made by users.

In comparison with its predecessors, BIACORE 3000 offers higher sensitivity, faster analyses, lower sample consumption and improved kinetic data for binding studies. In addition, so-called "BIACORE application wizards" are introduced in the software to guide the user through standard application types. This brings the accumulated expertise at Biacore directly to the user, in the immediate context of the current application where it is most needed.

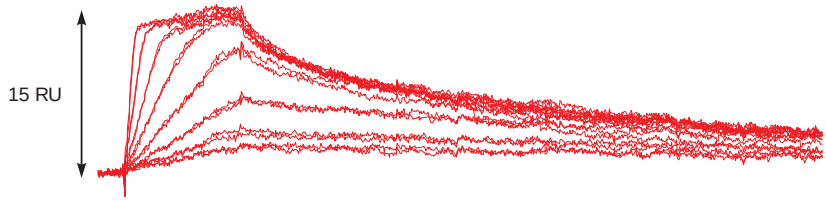


Figure 1a. Binding of thrombin inhibitor (MW 420 Da) to immobilized thrombin. Concentration range 0.39-50 nM.

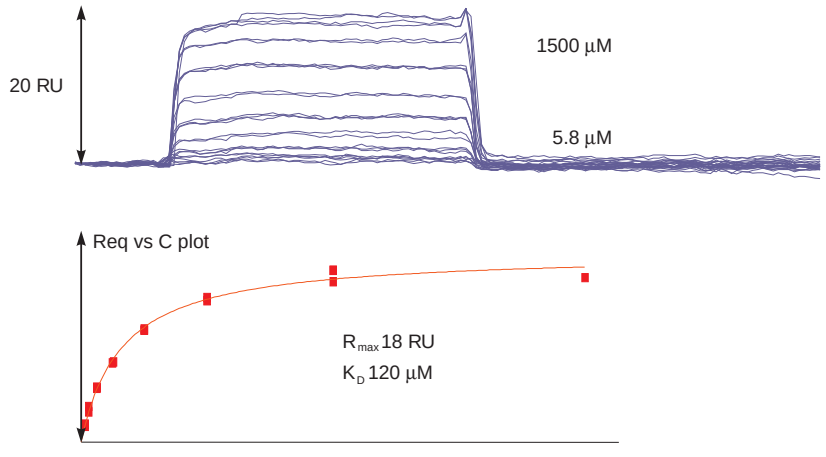


Figure 1b. Determination of the affinity constant for maltose (MW 360 Da) binding to immobilized anti-maltose antibody.

HIGHER SENSITIVITY

A new detection unit and improvements in the data acquisition procedures have been introduced, mainly to reduce instrument noise levels in BIACORE 3000. This lowers the threshold for a measurable response. In terms of technical signal-to-noise ratios the sensitivity has improved dramatically from the first commercial BIAcore system. At the same time, automatic subtraction of blank data from in-line reference flow cells increases confidence in low signal levels. The result is easier detection of small molecules and determination of weak interaction constants *(Figure 1)*.

The dynamic range of the detection unit in


Sensor Chip
SA
Sensor Chip
CM5
Certified Grade
Sensor Chip
HPA
Sensor Chip
C1
Sensor Chip
F1
Sensor Chip
B1
Sensor Chip
J1
PIONEER
BIACORE
How fast
How specific
How strong
How much

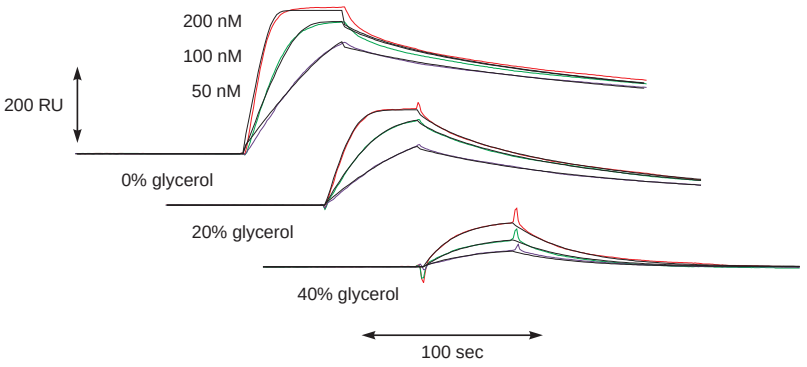


Figure 2. Hybridization of a 9-mer oligonucleotide in the presence of 0-40% glycerol.

BIACORE 3000 is extended by a factor of 2, increasing the maximum absolute response to 70,000 RU and enabling the study of interactions in the presence of solvent components with a high refractive index. This can be vital to investigations involving small molecules such as drug candidates which, because of their limited solubility, require addition of solvents like DMSO to the buffer. Signal-to-noise characteristics are essentially constant over the whole dynamic range, so that interactions involving small molecules can be measured with confidence in spite of the use of high refractive index solvents *(Figure 2)*.

Coupled with the higher sensitivity of BIACORE 3000, which opens the way for increasingly sophisticated analyses, is a requirement for more stringent procedures for sample handling. Recovery procedures in BIACORE 2000, using flow-through technology to collect material eluted from the sensor surface, are useful in many situations, but do not always meet the rigorous demands of applications like mass spectrometry and PCR where the recovery volume must be kept small and sample contamination must be kept to an absolute minimum. In response to this, an entirely new sample recovery procedure is introduced in BIACORE 3000. This is based on injection of a small volume (typically about 5 µl) of elution buffer sandwiched between air segments on to the sensor surface, followed by incubation at zero buffer flow for a defined time. The elution buffer is then recovered with essentially no dilution by reversing the flow. To further improve the recovery performance, a new wash procedure is added which washes the flow channels in the IFC but by-passes the flow cells with the sensor surface. The combined result of these innovations is that material can be eluted from the sensor surface in a very small volume of defined solution with practically no carry-over from previous runs or contamination from the running buffer used during the binding event.

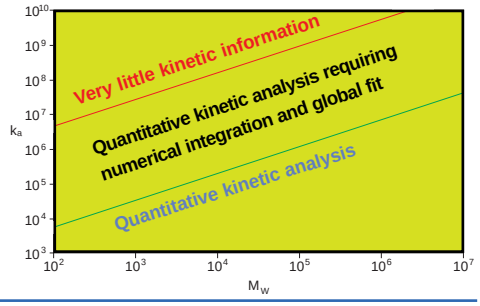


Figure 3. Theoretical limits for kinetic analysis as a function of k_a and molecular weight, based on maximum binding capacity of 10 RU and a flow rate of 30 µl/min.

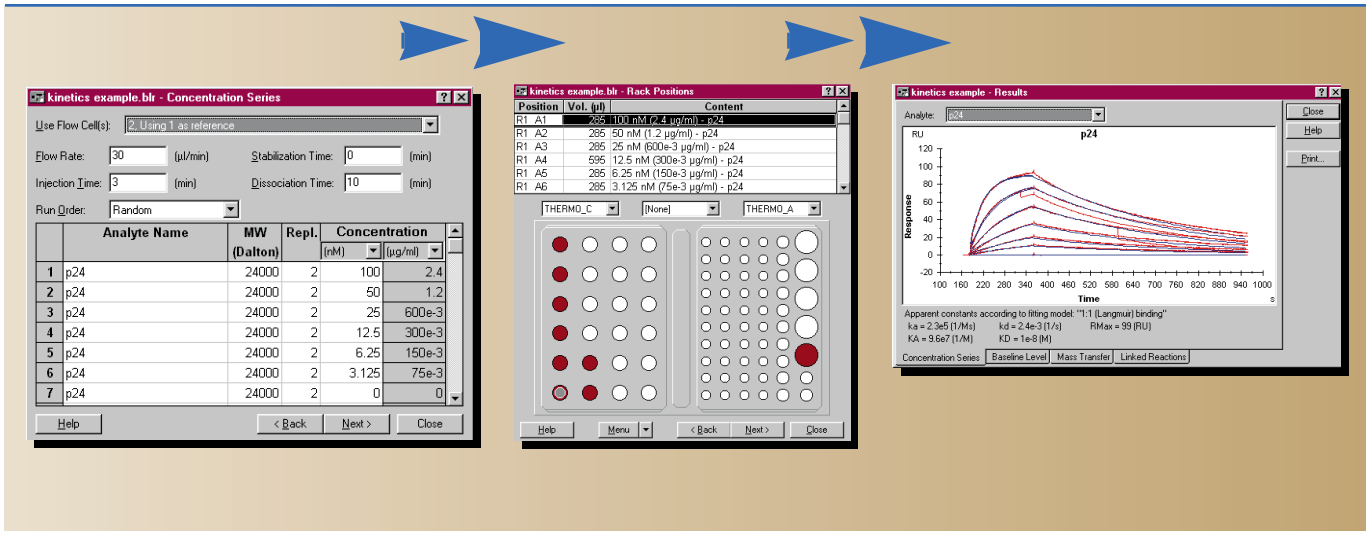
IMPROVED THROUGHPUT AND BETTER KINETICS

One of the most significant differences between BIACORE 3000 and its predecessors is in the design of the IFC, where the height of the flow cells has been reduced to less than half. This may sound trivial, but it has important consequences for both kinetic analysis and yes/no binding measurements in screening applications. The consequence of the lower flow cell height is improved mass transfer of analyte to the surface: the mass transfer coefficient for diffusion controlled transfer is inversely proportional to $(\text{height})^{2/3}$, so that reducing the height by a factor of 2 improves mass transfer by a factor of 1.6. In practical terms, this means that faster kinetics can be measured without interference from mass transfer processes *(Figure 3)*. It also means that in a mass transfer limited situation, the same response is achieved in a shorter time (with less sample), which can increase sample throughput in screening situations. Both gains are enhanced by the improved sensitivity, which allows confident work at lower relative response levels. Throughput in all automated analysis situations is also improved through streamlined wash routines between analysis cycles and by the introduction of a new reagent rack which allows all 192 wells in two microplates to be used for samples.

Some users may worry that the reduced flow cell height will lead to problems of clogging in the flow system when particulate samples such as crude extracts or whole cell suspensions are used. Fear of clogging in micro-flow systems is often quoted as an argument in favour of open cuvette systems, but users of BIACORE do not report problems of this kind even from analyses involving whole cells (see for example the article by Ravanat et al on page 30 of this issue). There is no reason to suspect that the lower flow cell height in BIACORE 3000 will create significant clogging problems.

EASIER SOFTWARE

The technical improvements in instrumentation in BIACORE 3000 are matched by introduction of new software features, designed both to simplify operation and improve analy-



Sample details | **Sample placement in autosampler racks.** | **Results from the kinetics analysis wizard**

Figure 4. Application wizards guide you from experimental design to results.

sis quality. Most of these features are also provided in the latest version of the control software for BIACORE 2000.

In-line reference subtraction, introduced with the two-channel system BIACORE X, is now implemented in BIACORE 2000 and 3000. This provides direct, real-time display of the blank-subtracted response, so that the progress of binding can be assessed with confidence immediately instead of relying on the alternatives of inspired guesswork or post-experimental data evaluation. Data quality is improved too, not least because the facility for in-line subtraction encourages the use of blank reference cells.

The new IFC in BIACORE 3000 permits the use of flow cells 3 and 4 as a pair, bypassing flow cells 1 and 2 and improving the cost-efficiency of chip utilization.

The dominant new feature in BIACORE control software however is the introduction of application wizards. An application wizard provides a step-by-step interactive guide to a particular kind of experiment from design all the way through to presentation and interpretation of results, with on-line help and feedback to give the best chance of success. The wizards themselves and the supporting information in the on-line help function build on experience of binding studies accumulated over the years at Biacore, giving both new and experienced users the benefit of the company's expertise. An example is illustrated in Figure 4.

Application wizards serve two purposes. They play a supportive role for new and inexperienced users, who are guided with a safe hand through the essential steps in an application. Many users in this category see the complexity of the system as a barrier to experimentation, and application wizards should help to lower that barrier. For the experienced user who feels more at home with the system, application wizards can provide a routine framework for analysis without the additional learning required for writing methods in the method definition language. For these users, application wizards provide a complement to manual or method-controlled operation, where somewhat restricted freedom in experimental design is compensated by an easy-to-use interactive framework for the analysis.

Currently, application wizards are provided for surface preparation on Sensor Chip CM5 and for analysis of binding kinetics, but the structure of the software allows new wizards to be added in a modular fashion. Among the wizards currently under development is support for general binding assays and customized interaction analysis.

MAKING THE MOST OF BIACORE 3000

In summary, BIACORE 3000 is the most advanced system in the BIACORE series, and represents the current state of the art in technology for affinity-based biosensors. With higher sensitivity, improved sample handling and better kinetic analysis facilities, the system will extend the range of applications that can be addressed by the technology to cover many of the small molecules such as cofactors and signalling substances in basic life science research and drug candidates in the pharmaceutical industry.

Parallel to refinement of the hardware and computer software involved in BIACORE systems is a development of approaches for dealing with the large amounts of high quality data that these systems can provide. As automated analysis becomes faster and more sophisticated, processing the data obtained becomes more and more of a bottleneck. The article on page 12 of this issue discusses some of the ways in which evaluation of binding data can be streamlined.