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TRENDS

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# Real-time analysis on microarrays

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To obtain the maximum amount of information from the smallest amount of a sample is one of the major goals of analytical science. In molecular biology and all molecular-based life sciences there is also a broad demand for highly parallel analysis. Microarray technology is the answer to this demand. It enables massive parallel determination and multiple measurement of a variety of binding events to be performed simultaneously. It has, in addition, the advantage of requiring modest investment of labour and might, therefore, save much time and be automated easily. Microarrays usually consist of many microscopic spots each containing identical molecules, i.e. receptors, probes, or targets. The number of spots can vary from less than one hundred to several hundred thousand. The molecules are attached to a solid support which can be made from glass or a polymer. The primary task of a microarray experiment is to detect many binding events simultaneously.

Most applications use fluorescence as a label to detect the binding events. Before the experiment the sample must be labelled by means of a suitable fluorochrome. Binding is achieved in a separate incubation step and the final result is acquired after drying of the microarray. Modern microarray readers usually, therefore, acquire information about the fluorescence intensity at a given time of the binding process. Many applications have been reported during the past two years, mainly in the area of transcription analysis [1, 2, 3]. Most experiments compare two states of a cell type, under different conditions, to identify the relevant activated genes. Progress into more analytical and diagnostic applications is still slow, because quantitation of the results still is a problem and fabrication methods are not yet sufficiently reliable to enable production of larger series. The fluorescence intensity measured in microarray experiments represents the amount of bound, i.e. labelled, analyte; this depends on the concentration in the applied solution and on the affinity of the binding partners and the time allowed for binding. It is not usually possible to differentiate among these influencing factors in the usual arrangement. The physical situation of the microarray experiment can, moreover, withstand high selectivity when the association rate constant dominates the result, as has been outlined recently [4]. From biosensor technology we have learned to analyse complete binding processes on surface-immobilised receptors by combining optical or electronic signal transduction with microfluidics. To overcome the limitations of microarray technology developments are under way to facilitate measurement of binding kinetics in the microarray format. Homogeneous sample flow over the whole microarray is one technological problem that has recently been solved in our laboratory by use of computer-aided simulations. Finite element calculations were used to optimise the design of the flow cells [5].

The scientific community is still waiting for the successful expansion of label-free means of detection from biosensors to microarrays that is so easily envisaged by extrapolating biosensor principles such as surface plasmon resonance, quartz microbalance, or other transducing mechanisms [6]; it seems, however, to be rather difficult to transform these principles to larger surfaces. Progress in detection methods employing fluorescence has, on the other hand, been achieved by significant enhancement of the signal-to-noise ratio, by use of evanescent field excitation. Background was reduced by a factor of approximately eighty [7]. The same group has recently described a further step to even better *S/N* ratios – use of two-photon excitation in a planar wave-guide device [8].

Binding reactions are, however, only one type of biochemical process that might be observed in parallel and in real-time. Enzymatic reactions on immobilised substrates or templates might also be observed by use of a flow-through scanning device. As has been shown recently, single measurements with optical biosensors for a single type of substrate [9], parallel detection, and comparison of a variety of substrates *or templates* are now accessible in a single experiment. To demonstrate the power of the ap-

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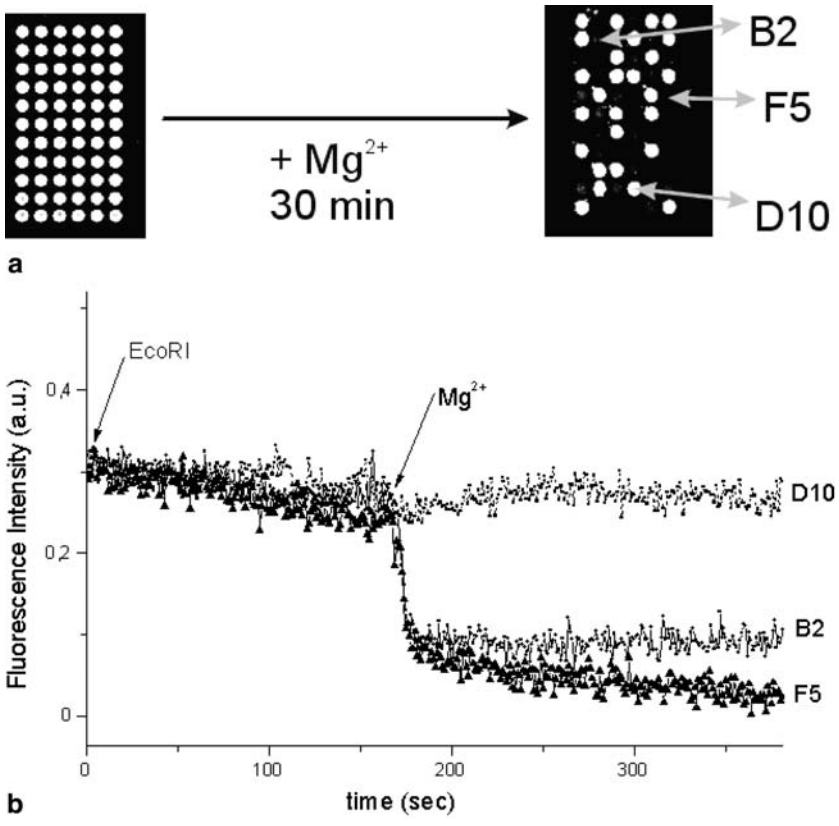


**Fig. 1** Real time measurement in a microarray. The figure shows the time course of hybridisation and enzyme activity in three spots out of an array of 66. (**a**) (*left*) Microphotograph of the array after hybridisation of labelled complementary strands, spot spacing 500 µm. (*right*) Microphotograph of the same array 30 min after addition of the cofactor ($Mg^{2+}$, 2 mmol $L^{-1}$) and enzymatic cleavage. In the spots where the fluorescence intensity decreased, the DNA with the recognition sequence of EcoRI has been immobilised (*B2*, *F5*). The DNA without the recognition sequence has been immobilised in the spots where the intensities remain unchanged at a high level (*D10*). (**b**) Decrease of the fluorescence intensity as a result of enzymatic cleavage is shown in real-time in spots where the DNA has been cleaved by the enzyme (*B2*, *F5*) compared with the spots where the DNA has not been cleaved (*D10*)

proach, we chose a restriction endonuclease. Oligonucleotides containing the restriction site, or a variation thereof, were immobilised on the microarray surface by covalent coupling. Glass slides were used in the format of the cover slips normally used for optical microscopy. After immobilisation the complementary DNA strand in solution attached to a fluorochrome binds to the immobilised DNA strand by hybridisation. The hybridisation process is performed by applying the three labelled oligonucleotides to the surface in a single solution. Because of the individual binding of the complementary strands this experimental step results in a labelled double-stranded oligonucleotide in each spot, as demonstrated in Fig. 1a (left), in which all the spots can be identified. The now doublestranded DNA fragment serves both as binding receptor for the enzyme and as substrate to be cleaved by the enzyme activity. The last step in this experiment, the action of the enzyme, can be controlled by addition of the cofactor $Mg^{2+}$. Binding of the enzyme alone does not generate any signal, because the enzyme makes no contribution to the fluorescence signal and the fluorochrome attached to the DNA is not affected by the binding. After addition of the cofactor the enzyme is switched on and release of the fluorochrome, i.e. dissociation of the cleaved DNA strand, is observed. This is demonstrated in Fig. 1a (right), where the spots in which the DNA is cleaved by the enzyme (e.g. spots B2, F5) are readily identified by the decrease in the fluorescence intensity. In the spots in which the DNA does not have this specific sequence (e.g. spot D10), the intensities remain unchanged, as expected.

In Fig. 1b the enzymatic reaction is observed in real-time. Initiation of the reaction is marked by the addition of the cofactor. Comparison of the three spots shows the dependence of the enzymatic reaction on the sequence. The intensity decreases for DNA with the recognition site (B2, F5) or does not change if the recognition site is absent (D10).

The method of real-time observation of a variety of spots in a microarray format might serve not only to obtain more information from the single spot in "classical" microarray applications, but might also be the platform for more complex analysis in the context of functional genomics. Parallel measurement of proteins in their active state will be the challenge in advanced genomic-based life sciences in the years to come.

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