The volume of sequence information from the human genome project demands the development of a tool to rapidly scan DNA for mutations. Such a technology would also be valuable in cancer research as a tool to detect early mutation events. Today, single nucleotide polymorphisms (SNPs), are routinely detected using methods that rely largely on the separation of DNA fragments by gel electrophoresis or HPLC, neither of which are readily suited to high throughput analysis. This review discusses how Biacore's SPR technology may come to fill this role.
Kazuhiro Nakatani and colleagues at Kyoto University have used Biacore's SPR technology as an alternative means to single nucleotide polymorphism (SNP) analysis. They designed and synthesized a low molecular weight ligand that specifically binds double stranded DNA containing G:G mismatches arising from native C:G pairings and which has minimal reactivity with any of the other seven possible mismatch combinations (A:A, A:C, A:G, T:T, T:C, T:G or C:C) (1).

The ligand, naphthyridine, is modelled to recognize the characteristic open bulge that is created between DNA strands at the site of a G:G mismatch, at which no complementary base (cytosine) is present with which to form a hydrogen-bonded base pair. Naphthyridine is synthesized as a dimer and is therefore able to form simultaneous hydrogen bonds with guanine on both DNA strands.

Experiments showed that the melting temperature, at which half of the double stranded DNA in a sample dissociates into single chains and which is a measure of DNA stability, increased by 16.4°C in the presence of dimeric, but not monomeric naphthyridine, much higher than for native DNA or DNA of an otherwise identical composition containing other mismatches.

Naphthyridine contains an aminoalkyl chain and can therefore be covalently linked by amine coupling to carboxyl groups on a Biacore® CM5 chip. 27-mer double stranded DNA was passed over the prepared chips in a Biacore 2000 instrument and binding was seen only if the DNA contained G:G mismatches. DNA containing other SNPs or native DNA did not bind to the ligand (Figure 1). The sensitivity of the assay was shown by a linear increase in response using DNA concentrations from 125 nM to 1000 nM, a range that was optimised by changes in salt concentration. The intensity of response was a function of both DNA concentration and length.

Other non-gel based mismatch screening methods like melting curve analysis or MutS capture of mismatched fragments rely on detection systems such as labelling with a fluorescent dye or the use of labelled primers. Nakatani et al show that Biacore’s SPR technology is sensitive, specific, requires no primers or probes and can be adapted for high throughput analysis (2).

This is the first report in which Biacore’s SPR technology has been used for DNA mutation analysis and while the design and synthesis of ligands that are specific for other SNPs will be difficult, the principle of Nakatani’s work is a promising development in accelerating DNA mutation analysis.

References