Work Package 3: Biosensors

Optical biosensors for shellfish poisons, fungicides, antibiotics and growth promoter related biomarkers

Biosensors based on surface plasmon resonance (SPR) provide unique data on the interactions between proteins and their binding partners, including small molecules. The data are generated in real-time, without the use of fluorescent or radioactive labels. In the area of food safety, Biacore® Q, together with Qflex® kits, provide rapid and reliable solutions for the detection of veterinary drug residues, antibiotics and growth promoters.

Within BioCop, Xenosense Ltd. is developing three new kits for the detection of; 1) paralytic shellfish poisons (saxitoxins), in collaboration with WP5; 2) strobilurin fungicides, in collaboration with WP6; and 3) fluoroquinolone antibiotics, in collaboration with WP8.

Figure 1:
Reagents kits for the detection of Shellfish poisons, fungicides and antibiotics

The same biosensor technology will be used to develop a completely new instrument, dedicated to simultaneously quantify up to 16 biomarkers that are diagnostic for the effects of growth promoters. This is one of the new key-technologies, utilising a proteomics-based concept that focuses on measuring effect rather than on measuring traces of specific hormones.

This effect-oriented approach has a clear advantage in terms of throughput and has the ability to detect newly developed "designer steroids" and "hormone cocktails".

Expression levels of biomarkers, *i.e.*, proteins regulated in cattle upon treatment with growth promoters, are identified in WP2. Samples, such as plasma or tissue extracts, will be injected directly into the sensor and biomarkers will be captured using specific receptors developed in WP4.

Results from this biomarker assay will define the probability that a certain animal has been illegally treated with growth promoters. It is expected that each type of growth promoter will have a unique influence on the concentrations of the various biomarkers. This being the case, the biomarker fingerprint may identify the growth promoter to which the animal was exposed.

SPR/MS coupling

BioCop is developing coupled SPR biosensor / mass spectrometry (MS) systems to identify known and unknown bioactive substances that are identified as non-compliant in newly developed biosensor screens. The compounds in BioCop are low molecular weight drugs, toxins and contaminants.

Two types of interface between SPR-based screening assays and MS-based identification will be investigated. In the first, the sample is applied to a liquid chromatography column and the effluent split between two identical 96-well plates. One of these plates is subjected to the SPR assay for bioactivity, generating a "biogram", which is used to identify the relevant samples of the duplicate plate that require MS analysis.

A flow diagram of this is given in figure 2, for the identification of samples containing quinolone antibiotics. As an example, MS spectra of the fluoroquinolones norfloxacin and ciprofloxacin are displayed.

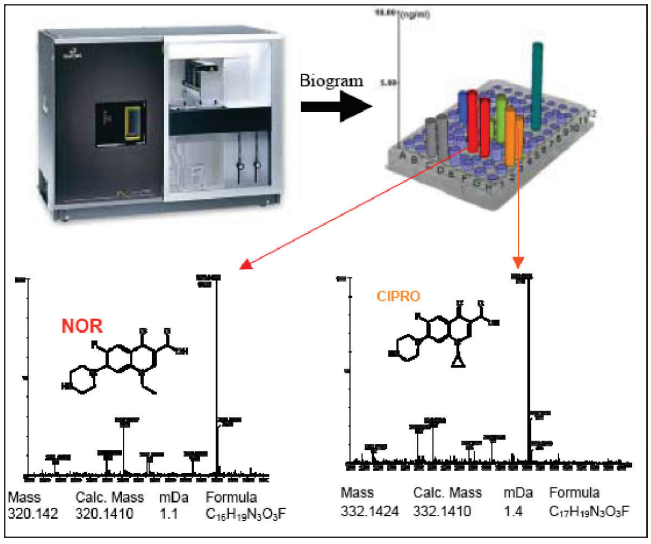

Figure 2: Biacore 3000 is used to generate a biogram that directs MS identification of fluoroquinolones to relevant sample fractions

In a second approach, the applicability of an automated serial SPR biosensor/ESI TOF-MS coupling will be explored to identify compounds captured on the sensor surface. The accurate mass capability of TOF-MS allows the identification of unknown substances. Chemical substance databases can be used to check whether the unknown substance has previously been identified as a product of metabolism, an un-marketed drug, or a synthetic intermediate in any patent or scientific paper. If not, a chemical structure of the unknown compound can be postulated using data from QTOF-MS/MS experiments.

Electrochemical sensors

Screen Printed Electrodes (figure 3) have been prepared (URTV) to detect trichothecenes. The advantages of these disposable devices are that: they produce rapid measurements; they are small in size and low in mass; they are cost-effective to produce. The PalmSens Electrochemical Instrument (figure 4) is also user-friendly, cost-effective and portable.

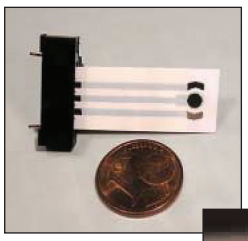

Figure 3 (left): Electrochemical sensors

Figure 4 (below): PalmSens electrochemical instrument

The first approach has been to optimise the analytical procedure for the detection of electroactive compounds coming from the chemical break-down (hydrolysis) of type-B trichothecenes. We will continue to optimise hydrolysis of these type-B trichothecenes and their detection using microwave hydrolysis (and possibly flow microwave hydrolysis) and the most advanced electrochemical techniques. The goal is to detect type-B trichothecenes in the 0.1-10ppm range.

For type-A trichothecenes a new approach is proposed, based on electrochemical immunosensors coupled with electrochemical instrumentation described here.

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