



BRAAVOO

biosensors for real time monitoring
of marine contaminants

MARINE BIOSENSORS WORKSHOP ABSTRACT BOOKLET

24-25 NOV. 2016, VILLARS-SUR-OLLON



BRAAVOO 1

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The BRAAVOO buoy during field testing in Ireland.
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Dear Participant

Welcome to Villars at the BRAAVOO Workshop *Biosensors for real time monitoring of marine contaminants*, and thank you for attending.

I am sure that we have excellent presentations and scientific exchanges. We are all fascinated by the idea that biosensors could one day be used for routine precise monitoring of contaminants. The FP7 BRAAVOO project was focused on three particular types of biosensors and I feel that we have been able to gain valuable experience in forcing ourselves to deploy the biosensors in automated instruments, as good as we could. Various other FP7 Oceans of Tomorrow projects had related objectives and I am excited that we have some of those projects being represented here, hoping that we can learn from each other and maybe build new partner constellations to advance the biosensor ideas further.

I wish you all a very pleasant and fruitful workshop, and hope that you will return with fond memories and lots of new ideas!

Kind regards

Jan Roelof van der Meer,
Scientific Coordinator BRAAVOO

**A WARM WELCOME
FROM BRAAVOO**

PROGRAMME

THURSDAY
24 NOVEMBER
2016

12.00	Arrival
14.00	Welcome
Session 1	
14.15	Shimshon Belkin – Molecular engineering of microbial bioreporters and their application to monitoring marine pollution
14.45	Fernando Rojo – Marine hydrocarbonoclastic bacteria as whole-cell biosensors for hydrocarbons
15.15	Jan van der Meer – Bacterial biosensor automation: concepts and experiences
15.45	Break
Session 2	
16.15	Laura Lechuga – Portable immunonanosensors as the next diagnostics generation for environmental protection
16.45	Hendrik von Hörsten – Label-free multichannel optical interrogator based on an integral field spectrometer for oceanic water monitoring in ENVIGUARD project
17.15	Jan Goetzen – Additive Manufacturing/ 3D Printing, from Microstructures to Microsystems
17.45	Blanca Chocarro Ruiz – Nanophotonic interferometric immunosensor for label-free and real-time monitoring of Irgarol 1051 in sea water
18.00	Clemence Roggo – Microfluidic chips to measure bacterial chemotaxis in a biosensory perspective
18.15	Diogo Tavares – Changing the specificity of Escherichia coli periplasmic binding protein RbsB from ribose towards new compounds
18.30	Dinner

20.30 Exhibition, beer and wine

PROGRAMME

FRIDAY
25 NOVEMBER
2016

Session 3	
8.30	Marie-Louise Tercier – Open and modular sensing solution for in situ simultaneous mapping of a range of chemical compounds coupled to master bio-physicochemical parameters.
9.00	Catherine Boccadoro – Microbial molecular sensors for the detection of oil contamination in the marine environment
9.30	John Wallace – Engineering Challenges for Marine Monitoring Biosensors – getting from benchtop to deployed
10.00	Break
Session 4	
10.30	Jonathan McQuillan – “Fish and Chips”: Emerging Capabilities in Marine Biosensing using Lab on a Chip
11.00	Maria Teresa Giardi – Novel biosensors based in microalga-paramecium symbiosis for improved marine monitoring
11.30	Vitali Maffenbeier – Chimeric bacterial bioreporter can be altered to access new target specificities
11.45	Ingunn A. Samdal – Control of antibody specificity through chemistry; use of synthetic haptens and novel derivatization strategies for production of antibodies to algal toxins
Session 5	
12.00	Cristina Bosch-Orea – Development of an indirect enzyme-linked immunosorbent assay for the detection of sulfonamides in seawater
12.30	Floris Falke – Optofluidic platform based on TriPLeX integrated interferometric biosensors in micro-arrays for label-free detection of analytes in food, water, environment, medicine and security.
13.00	Lunch
14.00	Departure

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MOLECULAR ENGINEERING OF MICROBIAL BIOREPORTERS AND THEIR APPLICATION TO MONITORING MARINE POLLUTION

Presented by
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The small size requirements, rapid responses and sensing versatility of bacterial-based whole-cell biosensors allow their integration into diverse types of devices, for laboratory as well as field applications, for environmental, pharmaceutical, security and industrial uses.

The relative ease by which molecular sensing and reporting elements can be fused together to generate dose-dependent quantifiable physical (luminescent, fluorescent, colorimetric, electrochemical) responses to pre-determined conditions allows the construction of diverse classes of sensors.

Over the last two decades we and others have employed this principle to design and construct microbial bioreporter strains for the sensitive detection of (a) specific chemicals of environmental concern (heavy met-

als, halogenated organics etc.) or (b) their deleterious biological effects on living systems (such as toxicity or genotoxicity). In many of these cases, additional molecular manipulations beyond the initial sensor-reporter fusion may be highly beneficial for enhancing the performance of the engineered sensor systems. Several of the approaches we have adopted over the years to achieve this aim will be highlighted, along with recent results on the adaptation of such sensors for pollution monitoring in the marine environment.

Whole-cell biosensors are useful and cost-effective systems for the in-situ monitoring of seawater for hydrocarbons derived from oil spills. Currently available biosensors for hydrocarbons show limitations derived from the low water-solubility of these hydrophobic compounds and by the high ionic strength of seawater. To tackle these problems, we tested the usefulness of the marine hydrocarbonoclastic bacterium *Alcanivorax borkumensis*, which is highly specialized in assimilating alkanes, as a host for a biosensor. Three plasmids were constructed allowing the expression of the green fluorescent protein (GFP) in response to C6-C10 n-alkanes, C8-C18 n-alkanes, or pristane, respectively. In *Escherichia coli*, the reporter plasmid responding to C6-C10 n-alkanes provided a fast response to pure alkanes (25-fold induction after two hours) as far as the alkanes were present in water samples of moderate ionic strength. In *A.*

borkumensis, this sensor efficiently responded to C6-C10 n-alkanes in seawater after 3-4 hours, rendering induction values of 18- to 50-fold, depending on the alkane considered. Under the conditions used, the detection threshold for octane of the *A. borkumensis* sensor was of 0.5 μM , four-fold better than that of the *E. coli* sensor. The *A. borkumensis* biosensor also rendered a better response to gasoline or crude oil in seawater.

A. borkumensis cells containing the reporter plasmid for C8-C18 n-alkanes, or that for pristane, required longer incubation times to render an induction signal (24-30 h). This is probably due to the high hydrophobicity of these hydrocarbons and to a relatively high basal expression of GFP in these two plasmids.

MARINE HYDROCARBONO- CLASTIC BACTERIA AS WHOLE-CELL BIOSENSORS FOR HYDROCARBONS

Presented by
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BACTERIAL BIOSENSOR AUTOMATION: CONCEPTS AND EXPERIENCES

Presented by
Jan Roelof van der Meer
University of Lausanne
Switzerland

Live bacterial cells may be used to measure the presence of recurrent chemicals in aqueous systems.

For this purpose they are engineered to produce an easily measurable “reporter” protein signal in response to encountering the chemical target. The principle of such whole cell living bioreporters has been demonstrated numerous times in laboratory settings, but there is little experience working with them in field situations. Here we present data from two field experiments using bacterial bioreporter assays to measure key target chemicals. In the first case we measured arsenic in groundwater samples, in the other we measured oil pollution at the North Sea.

Finally, we will present ongoing experiments and efforts to embed living cells in automated devices that could potentially be used for continuous monitoring of key pollutants.

The need to monitor and detect biological elements, related to human and environment health in a fast and reliable way, is one of the challenges faced by humanity at the dawn of the 21st century. Tests done nowadays in laboratories (as ELISA, PCRs, cell cultures, etc.) are slow (from several hours to days) and expensive.

Modern diagnostics is demanding novel analytical tools that could enable quick, accurate, sensitive, reliable and cost-effective results so that appropriate treatments can be implemented in time, leading to improved outcomes. Such portable point-of-care (POC) devices, able to deliver an instant diagnostics, could become a reality soon thanks to the last advances in nanobiosensors, lab-on-a-chip, wireless and portable technologies which promise to surpass the existing challenges, opening the door to a global diagnostics access.

The driving force of our research is to achieve such ultrasensitive platforms for POC label-free analysis using nanophotonic technologies and custom-designed biofunctionalization protocols, accomplishing the requirements of disposability and portability. We are using innovative designs of nanophotonic biosensors based silicon photonics technology (nanointerferometers) and full microfluidics lab-on-chip integration.

We have demonstrated the suitability of the photonic nanobiosensors for the detection, with extremely sensitivity and selectivity of marine pollutants, directly using untreated sea water.

PORTABLE IMMUNONANO- SENSORS AS THE NEXT DIAGNOSTICS GENERATION FOR ENVIRONMENTAL PROTECTION

Presented by
Laura Lechuga Gomez
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LABEL-FREE MULTICHANNEL OPTICAL INTERROGATOR BASED ON AN INTEGRAL FIELD SPECTROMETER FOR OCEANIC WATER MONITORING IN ENVIGUARD PROJECT

Presented by
Hendrik von Hörsten
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We experimentally demonstrated an optical interrogation device for measuring simultaneously the reflectance spectra of 12 label-free transducers made of resonant nano-pillars (RNP: Resonant Nano Pillars). The optical interrogation device is an integral field spectrometer fed by an array of 12 105/125 μm multimode optical fibers. Its bandwidth is 110 nm at 600 nm central wavelength and the pixel limited resolution is 70 pm. The device is driven by a Computer-on-Chip which performs image and data processing. The 12 spectra are automatically extracted and the resonant features (dip) of the RNPs are automatically fitted with a non-linear fitting algorithm in order to generate a 12 channels high precision sensogram with 0.5 seconds duty time.

The spectrometer precision was evaluated by monitoring the RNP dip central wavelength of 12 sensors as

a function of time using different refractive index solutions. A 3 σ precision better than 100 pm has been obtained without temperature control and without compensation with another channel. The latest results with chip temperature control and noise compensation using nearby channels will be presented during the workshop.

The multichannel spectrometer is part of the Chemical Detection Unit (CDU) of the ENVIGUARD project and aims at the detection of toxins and chemical pollutants (PCBs) in oceanic water. Its compact and low cost design makes it suitable for use in field or point-of-care applications.

microTEC's unique 3D printing technology RMPD® allows the production of microstructures and microsystems in sub micrometer resolution without tooling. Starting from a CAD model, the polymer based micro parts are generated by use of UV curable material and the photo polymerization process. In a parallel batch production process on substrates up to 14", microTEC is capable of serving its customers from prototyping to mass volume production. The possibility of integrating semiconductor parts in combination with the use of metal coatings from Physical Vapor Deposition (PVD) for electrical wiring in parallel, microTEC gives access to the market of 3D printed microsystems. This is applied, for instance, in the production of "optical sub assemblies" OSAs for optoelectronic data communication, and the advanced 3D printing technology has proven its capability for meeting the demand and target price for con-

sumer electronic applications. microTEC offers a full service package to translate product ideas into tangible solutions

In the BRAAVOO project microTEC's focus is on the miniaturization of lab size water analysis systems as well as their system integration.

Together with the partners' solutions for mobile miniature labs, disposable cartridges, and also chip packages, were developed.

ADDITIVE MANUFACTURING/ 3D PRINTING, FROM MICROSTRUCTURES TO MICROSYSTEMS

Presented by
Reiner Goetzen
microTEC Gesellschaft für
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NANOPHOTONIC INTERFEROMETRIC IMMUNOSENSOR FOR LABEL-FREE AND REAL-TIME MONITORING OF IRGAROL 1051 IN SEA WATER

Presented by
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Catalan Institute of
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Every year large quantities of wastes and pollutants are dumped in the sea contributing to a slow but constant degradation of the marine water quality. Conventional analytical methodologies are sensitive and selective tools for the sea quality control but they operate only at laboratory settings. Bringing the monitoring tools directly to the contaminated place can result in cost and time savings.

In the frame of the BRAAVOO project, we are developing a nanoimmunosensor module for the on-site analysis of sea pollutants. One of these pollutants is Irgarol 1051, a commonly used antifouling paint for marine vessels. Because of its biocidal effect, its presence in the marine environment has a negative impact on marine organisms like corals. We are developing a bimodal waveguide interferometer (BiMW) nano-immunosensor for real-time and label-free detection of

Irgarol 1051. The detection is based on a competitive inhibition format assay using a hapten with a similar structure to Irgarol, but with a carboxylic group which can be covalently attached to the sensor surface. Results show limits of detection of few ng/L and the stability of the sensor surface up to 30 measure-regeneration cycles with sea water samples. Each measure-regeneration cycle takes 15 minutes and the sample does not require any pre-treatment.

Acknowledgement – This work has been funded by the 7FP (EU, BRAAVOO Grant Agreement No 614010). ICN2 acknowledges support from the Severo Ochoa Program (MINECO, Grant SEV-2013-0295).

Chemotaxis is a behavior by motile bacteria to sense the environment and swim in the direction of or away from chemical compounds. In a uniform environment bacteria swim randomly to explore the maximum space but when they are in presence of a gradient of attractant, they bias their swimming direction toward the highest concentration of attractant. Chemotaxis is rapid, and could thus be exploitable for developing biosensors with quick response. In addition it is conserved among motile bacteria and some species show chemotaxis toward toxic compounds.

Here we pursue the design of microfluidic chips in which a gradient of attractant can be generated which enables measurement of bacterial chemotaxis. We demonstrate two different design principles. In the first, three parallel flow channels are produced, separated by 700 nm-shallow

filters, which allow the diffusion of small chemical molecules but prevent the passage of the bacterial cells. The chemical gradient forms perpendicular to the flow and cells are flown in the inner channel. The accumulation of cells on the side with the highest attractant concentration is observed by microscopy. We show the working principle both on *Escherichia coli* chemotaxis to serine and aspartate, as well as on *Cupriavidus necator* and the herbicide 2,4-dichlorophenoxyacetic acid.

To observe the chemotactic response of cells to a gradient even more directly, we fabricated a second device with valves permitting rapid opening and closing of channels. This allows to create a stable gradient and to bring in a package of cells, upon which individual trajectories can be followed.

MICROFLUIDIC CHIPS TO MEASURE BACTERIAL CHEMOTAXIS IN A BIOSENSORY PERSPECTIVE

Presented by
Clemence Roggo
University of Lausanne
Switzerland

CHANGING THE SPECIFICITY OF ESCHERICHIA COLI PERIPLASMIC RBSB FROM RIBOSE TOWARDS NEW COMPOUNDS

Presented by
Diogo Tavares
University of Lausanne
Switzerland

Bioreporter bacteria are genetically engineered to produce a measurable signal (reporter protein) in direct response to a specific chemical or physical agent present in their environment.

The design of bioreporter bacteria typically starts with known sensory/regulatory proteins, but it has been proposed that computational prediction of substrate binding interactions in a well-known receptor protein could lead to design of mutant proteins with novel target recognition specificities. Here a mutant library based on the ribose binding protein of *Escherichia coli* (RbsB) was produced with the goal to find mutations which would permit binding of ribose-similar molecules such as 1,3-cyclohexanediol and cyclohexanol. Rosetta simulation was used to predict critical amino acid residues and potential mutations in the RbsB binding pocket

which might permit binding of the two new target molecules. The result was a set of 6 million mutants containing one of 5 possible substitutions at each of 9 amino acid positions. The library was introduced into an *E. coli* expression strain, which carries a hybrid signaling pathway through which GFP is produced upon binding of the target molecule by the (mutant) RbsB. For better reproducibility, cells were grown as individual microcolonies in alginate beads which were screened by FACS for gain-of-function GFP expression in presence of 1,3-cyclohexanediol. Alternate rounds of screening and separation were performed to enrich potential responsive mutants but to eliminate constitutive GFP producers. Since FACS only allows screening of end-points of GFP expression, but not the induction itself, the enriched library is currently being screened by high-throughput microscopy.

Marine environments are highly vulnerable and influenced by a wide diversity of anthropogenic and natural substances and organisms that may have adverse effects on the ecosystem equilibrium, on living resources and, ultimately, on human health. Identification of relevant types of hazards at the appropriate temporal and spatial scale is crucial to detect their sources and origin, to understand the processes governing their magnitude and distribution, and to ultimately evaluate and manage their risks and consequences preventing economic losses. This can be addressed only by the development of innovative, compact, rugged, automated, sensor networks. Development of such tools is a challenging task as it requires many analytical and technical innovations.

The FP7-OCEAN 2013-SCHeMA project aims to contribute to meet this challenge by providing an open and modular

sensing solution for autonomous in situ high resolution mapping of a range of anthropogenic and natural chemical compounds (trace metals, nutrients, species relevant to the carbon cycle, VOCs, toxic algae species). To achieve this, SCHeMA activities focus on the development of: 1) an array of miniature sensor probes taking advantage of various innovative solutions; 2) ad-hoc ICT wireless networking solution; 3) dedicated web-based front-end system to insure easy interoperability with national and international marine observation systems (e.g. EMODnet, EuroGOOS, MyOCEAN, SeaDataNet). Field tests and inter-comparison with data obtained using established laboratory techniques are performed in parallel to these developments to allow evaluation and further optimization of the SCHeMA sensing devices.

This talk will present the progress highlights to date in the SCHeMA project.

OPEN AND MODULAR SENSING SOLUTION FOR IN SITU SIMULTANEOUS MAPPING OF A RANGE OF CHEMICAL COMPOUNDS

Presented by
Mary-Lou Tercier-Waeber
University of Geneva
Switzerland

MICROBIAL MOLECULAR SENSORS FOR THE DETECTION OF OIL CONTAMINATION IN THE MARINE ENVIRONMENT

Presented by
Catherine Boccadoro
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Oil pollution in the marine environment leads to immediate and long-term changes in microbial populations both on species and at the gene expression levels. Some species or groups of organisms that are initially undetectable in the environment can become more dominant following an exposure to hydrocarbons, and changes in the expression of many genes involved in the biodegradation can be detected. Markers of microbial species and genes can be developed and adapted for in situ marine monitoring platforms to detect hydrocarbon pollution or monitor early ecosystem changes in the marine environment. Target species and genes were identified by studying marine microbial communities in oil-contaminated coastal areas, as well as following oil spill events. Assays were developed during oil exposure mesocosm experiments in the laboratory, and these are now being refined in national and

EU projects. For example, in the H2020 European project JericoNext, oil-pollution microbial markers are being coupled to algal toxin sensors for their integration into marine coastal monitoring platforms.

The development of biosensor technology for use marine monitoring applications is exciting and offers great potential to expand our capabilities for contaminant detection. However, there are very significant challenges associated with making these instruments and the associated platforms to support them in a manner that can be sustained at sea. We have considered these challenges under four main headings which are:

- 1** Sustaining a microfluidic system in an environment with very significant suspended solids and aggressive biofouling,
- 2** Environmental control for key components of the biosensors including the preservation of bioreporter consumables and a controlled environment for sensing technology,

- 3** Coping with sustained and substantial physical stresses on the platforms and instruments and
- 4** Implementing a power system that is required to support a biosensors system.

We will describe these challenges in some detail and discuss how the BRAAVOO team have designed innovative solutions that make significant progress towards a sustainable, deployable system. We will present how these systems are implemented in the BRAAVOO autonomous robot boat and the BRAAVOO Data Buoy.

ENGINEERING CHALLENGES FOR MARINE MONITORING BIOSENSORS – GETTING FROM BENCHTOP TO DEPLOYED

Presented by
John Wallace
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“FISH AND CHIPS”: EMERGING CAPABILITIES IN MARINE BIOSENSING USING LAB ON A CHIP.

Presented by
Jonathan McQuillan
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United Kingdom

It is becoming increasingly important to monitor how the oceans respond to changing climatic and anthropogenic pressures, requiring the long-term, offshore deployment of oceanographic sensors. New innovations in engineering and biotechnology mean that complex bio-analytical methods, once restricted to the centralised, highly resourced laboratory, can now be implemented on portable or deployable, low power ocean observing platforms.

The Ocean Technology and Engineering Group (OTEG) at the National Oceanography Centre (NOC) are developing novel biological sensors (bio-sensors), currently at various Technology Readiness Levels (TRLs), offering new possibilities in marine metrology. Core OTEG sensor technologies, including microfluidic Lab on a Chip devices, and miniaturised low power electronics and optics, are being employed to develop new field-portable and deployable sys-

tems for detecting small molecule marine contaminants and harmful microorganisms in situ, obviating the need for a centralised lab, bulky equipment and trained personnel. These include aptamer-based sensors for the detection and quantification of polyaromatic hydrocarbons, and eco-genomic sensing platforms for the enumeration of faecal indicator organisms, harmful algal blooms and eDNA. This presentation will provide an overview of current OTEG sensing capabilities with emphasis on new bio-sensing platforms, and their applications for marine science.

Increasing pollution of marine environment requires the development of sensitive, cheap and adaptable early warning systems for on-site monitoring of chemical contaminants. Biosensors based on algae cells might constitute a promising alternative for environmental monitoring since they can provide rapid toxicity information in the case of pollution, while assessing the harmful effect of the contaminants on the ecosystem. In this study, an optical biosensor based on algal biomediators for monitoring of marine pollutants was developed. Various algae strains from Chlorophyceae, Trebouxiophyceae, Dinoflagellates, Diatoms and Eustigmatophyceae families were first evaluated in marine environmental conditions.

Further, the algae-protocista symbiotic association between *Chlorella vulgaris* and the ciliate *Tetrahymena pyriformis* was deeply studied as a potential biomediator for biosensor

development, showing enhanced resistance to the salinity of marine water when compared with free-living algae strains. The symbiosis was adapted to grow into microfluidic flow cells with integrated detectors for real-time detection of marine pollutants by fluorescence analysis of photosynthetic photosystem II. The fluorescence response was examined in the presence of three herbicides representative of different chemical families: atrazine, simazine and diuron alone or in combination. The limits of detection for simazine, atrazine and diuron were 1.35, 0.44 and 0.25 µg/L, respectively, with a linear range from 2.5 up to 100 µg/L. An integrated Paramecium-Chlorella symbiosis-based biosensor was successfully developed for real-time monitoring of marine water quality and evaluation of biotoxicity.

NOVEL BIOSENSORS BASED IN MICROALGA- PARAMECIUM SYMBIOSIS FOR IMPROVED MARINE MONITORING

Presented by
Maria Teresa Giardi
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CHIMERIC BACTERIAL BIOREPORTER CAN BE ALTERED TO ACCESS NEW TARGET SPECIFICITIES

Presented by
Vitali Maffienbeier
University of Lausanne
Switzerland

Two-component signaling systems interact with environmental stimuli upon which they trigger a cytoplasmic response. Their modular structure allows potential exchange of domains.

The first functional chimeric transmembrane receptor (TrzI) was constructed by Baumgartner et al. in 1994 by fusing the ligand binding (LB) and the Histidine kinases, Adenylate cyclases, Methylaccepting proteins and Phosphatases domain (HAMP) of the chemoreceptor Trg specific to Ribose with the response regulator domain (RR) of the osmosensor EnvZ.

Several other chimeras have been reported since but their functionality has not always been established. In order to be able to exploit the large natural diversity of bacterial chemoreceptors in simplified synthetic biosensor constructions, this work focuses on [I] understanding

the requirements for the fusion site enabling chimera functionality, [II] predicting ligand-binding in chimeras, and [III] constructing functional biosensors with new specificities.

A crucial step in developing any antibody-based assay for small-molecule analytes, regardless of whether it is an ELISA, radioimmunoassay or biosensor, is the availability of antibody with both appropriate specificity and sufficient affinity for the analyte(s) of interest. It is possible to control the antibody specificity by careful choice of linker and hapten chemistry. However, this aspect is often overlooked during the planning and preparation of immunogens. One of the keys to a successful outcome is a careful evaluation of which analytes one wishes to detect, or not to detect, with the assay, and then to design an immunogen that is likely to induce antibodies with the desired specificity.

When developing immunochemical assay methods, it is often desirable to produce antibodies which are either specific to a single analyte within a larger group, or generic antibodies that specifically recognise a whole class of

analytes. Successful use of (synthetic) haptens requires a good understanding of the chemistry and a very careful choice of substructure, appropriate linking chemistry and appropriate orientation of hapten on carrier proteins.

We will present examples of these strategies to produce antibodies against marine and freshwater algal toxins (e.g. domoic acid, azaspiric acids, microcystins, and the okadaic acid group of toxins), although the same principals can be applied to any small-molecule analyte. For some examples, collaboration with synthetic chemists was necessary, and/or novel derivatization strategies to provide the necessary conjugates for immunization and assay reagents.

Similar considerations can also be applied to other biosensor recognition elements.

CONTROL OF ANTIBODY SPECIFICITY THROUGH CHEMISTRY; USE OF SYNTHETIC HAPTENS AND NOVEL DERIVATIZATION STRATEGIES FOR PRODUCTION OF ANTIBODIES TO ALGAL TOXINS

Presented by
Ingunn A. Samdal
Norwegian Veterinary
Institute
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Norway

DEVELOPMENT OF AN INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF SULFONAMIDES IN SEAWATER

Presented by
Cristina Bosch-Orea
Institute of Environmental
Assessment and Water
Research, CSIC
Barcelona
Spain

The development of aquaculture activities has become an environmental threat related to antibiotics abuse. These compounds are very efficient to treat bacterial diseases in marine organisms and they are used in prophylactic therapy and for growth promotion, causing their propagation along the aquatic environment as well as along the food chain. Moreover, it has been demonstrated that antibiotics promote the bacterial acquired resistance even at low concentration levels.

Sulfonamides (SAs) are a large group of antibiotics widely used in aquaculture. A competitive indirect enzyme-linked immunosorbent assay (ELISA) has been developed to determine the presence of seven of the most common SAs in seawater. The obtained results will be used to develop a biosensor based on immunosensor system in the frame of the Sea-on-a-CHIP project.

The optimal concentrations of the immunoreagents were set by double checkerboard experiments, selecting those which absorbance values were near the unit. Concentrations were reached through successive serial dilutions in relation $\frac{1}{2}$ ranging from 1 $\mu\text{g}/\text{mL}$ of SA2-BSA and ranging from 1/1000 of As155. The optimal concentration was 0.25 $\mu\text{g}/\text{mL}$ for SA2-BSA and the dilution of 1/8000 for As155. The limit of detection (LOD) for the method (IC90) was $0.060 \pm 0.041 \mu\text{g}/\text{mL}$. IC50 was $1.051 \pm 0.384 \mu\text{g}/\text{L}$ ($4.182 \pm 1.712 \text{ nM}$).

The assay showed good performance for the most commonly used sulfamides antibiotics in veterinary medicine; the 7 compounds can be recognised as demonstrated the cross reactivity assays, while not significant matrix effects were observed using seawater.

The optofluidic platform consists of two parts: an optical detection with an extremely high sensitivity and an advanced microfluidic system based on innovative approach.

The core of the optofluidic platform is formed by highly sensitive planar waveguide interferometric sensors. In these waveguides, light propagates in single mode by internal reflection of light at the interface between the high (core) and low (cladding) refractive index (RI) of the transparent materials and along the boundary of the waveguide realized in chip technology. The so-called evanescent field sensing is well-known for its application in Mach-Zehnder interferometer based biosensors, in which the detection area is realized in the form local removal of the upper cladding in one of the two branches, allowing interaction of the evanescent field with the environment. A (small) change of the RI of

a biosensing layer as a result of specific capturing of analytes on the core surface, leads to a measurable shift in phase in the output of the combined branches of the interferometer.

The Mach Zehnder is located inside or close to the newly developed selector valve. This valve has an integrated microfluidic fused silica chip which contains all the relevant components for the assay with exception of the pumps. The platform has the ability for parallel fluidic pathways in order to allow different classes of assays simultaneously. The whole design is driven by the requirement that the dead volumes and total liquid volume are as small as possible.

OPTOFLUIDIC PLATFORM BASED ON TRIPLEX INTEGRATED INTERFEROMETRIC BIOSENSORS IN MICRO-ARRAYS FOR LABEL-FREE DETECTION OF ANALYTES IN FOOD, WATER, ENVIRONMENT, MEDICINE AND SECURITY

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The BRAAVOO buoy and vessel.

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