Sensors 2015

Bioanalytical Sensing Technologies

Conference with Posters and Exhibition

Programme

Organised by the Automation and Analytical Management Group
Royal Society of Chemistry

A one day meeting on
Tuesday 16th June 2015

At The Royal Society of Chemistry,
Burlington House,
Piccadilly, London W1J 0BA

Email: conference@aamg-rsc.org
Website: http://www.aamg-rsc.org

Bioanalytical Sensing Technologies

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Tuesday 16th June 2015
at The Royal Society of Chemistry, Burlington House, London

09:30 – 10:00 Registration and coffee

Session 1 Biosensor Technologies

Chair: **R. Narayanaswamy -** AAMG-RSC

10:00 Organic Field Effect Transistor Sensors using Peptide Recognition Elements
Mike Turner
University of Manchester, UK

10:30 A Platform Approach for Lableless Electrochemical Immunosensors
Seamus Higson
Cranfield University, UK

11:00 - 11:20 Tea / Coffee

11:20 Sensor Array for Ischemia Monitoring in Endoscopic Surgery
Josep Samitier
Director, IBEC, Barcelona

11:50 Diagnosis of Urological Malignancy using a GC Sensor: the Odoreader
Chris Probert
University of Liverpool, UK

12:20 Leaky Waveguide Biosensors
Nick Goddard
University of Manchester, UK

12:50 - 13:30 Lunch - Exhibition & Poster Session

Session 2 Applications and Informatics

Chair: **Alan Braithwaite -** AAMG-RSC

13:30 Recent Progress in Sensing Virus Infections
Vincent Emery
University of Surrey, UK

14:00 Small Molecule Sensing using Proteins as Specific Recognition Elements
Krishna Persaud
University of Manchester, UK

14:30 The Use of Breath and Headspace Analysis in Disease Diagnosis
Claire Turner
Open University, Cranfield, UK

15:00 - 15:20 Tea / Coffee

15:20 Pattern Recognition for Biological Sensing: Doing it Right
Conrad Bessant
Queen Mary University of London, UK

15:50 Sensors and Brokers: Collecting and Integrating Chemical and Environmental Data
Jeremy Frey
University of Southampton, UK

16:20 Concluding Remarks and End of Conference

ABSTRACTS

Organic Field Effect Transistor Sensors using Peptide Recognition Elements

Michael L Turner, Marion Wrackmeyer, Jonathan M. Behrendt, Barbara Urasinska-Wojcik, Daniel Tate, Debasmita Das, Ian Ingram

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ABSTRACT

Insects have the exceptional ability to detect trace amounts of chemical vapors. The odor sensing occurs in receptors of the antenna where small proteins, called odorant-binding proteins, recognize analytes in the environment. In the case of sensing explosives, short peptide sequences derived from the odorant binding proteins of bumblebees are able to selectively recognize and discriminate the important nitroaromatics, TNT and DNT.[1,2] This contribution describes the incorporation of selected short peptide sequences into organic semiconductor blends that are used to fabricate organic field effect transistors (OFETs) and electrolytically gated organic field effect transistors (EGOFETs). The charge mobility, source-drain current, and device threshold voltage were determined for devices exposed to vapors or solutions of TNT/DNT and controls. Evaluation of the multi-parametric response of the devices exposed to the analytes and controls showed that peptide recognition elements are able to effectively discriminate and quantify the nitro-aromatics TNT and DNT at sub-ppm concentrations as vapour and dissolved in water.

1. Z. Kuang *et al. ACS Nano* 2010, **4**, 452-458.
2. J. W. Jaworski *et al. Langmuir* 2008, **24**, 4938-4943.

A Platform Approach for Lableless Electrochemical Immunosensors

Séamus P J Higson

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ABSTRACT

The prospect of commercial lableless electrochemical sensors offers much promise for simplified and routine analysis across many sectors. A new platform approach will be presented for the development of impedimetric lableless electrochemical sensors based upon a micro-electrode array at screen printed carbon electrodes, which lend themselves to mass production at low unit cost. Sensors with lower limits of detection in the order of pg m^{-1} will, be described for, for example, neurone specific enolase [1], internalin B (a marker for Listeria) [2], and S-β100 (a marker for stroke) [3]; in this way sensors may in principle be developed for any analyte, provided a suitable antibody or antibody fragment may be harvested [4]. Chemistries for the site specific immobilisation of antibodies along with deposition methodologies will be discussed [5].

The approach may be further extended for the development of multi-analyte arrays for high throughput screening and multiplex analyses, which again may be interrogated electrochemically - in this instance by scanning electrochemical microscopy (SECM) based techniques [6-7].

References:

[1] A. C. Barton, F. Davis, S. P. J. Higson, 'Labeless Immunosensor Assay for the Stroke Marker Protein Neuron Specific Enolase Based Upon an AC Impedance Protocol', *Anal. Chem.,* **80**, (2008), 9411-9416.

[2] E. Tully, S.P.J Higson and R. O'Kennedy, 'The Development of a 'Labeless' Immunosensor for the Detection of *Listeria Monocytogenes* Cell Surface Protein, Internalin B', *Biosens. & Bioelec.*, **23** (6), (2008), 906-912.

[3] A.C. Barton, F. Davis and S.P.J. Higson, 'Labeless Immunosensor Assay for the Stroke Marker Protein S-100[β] Based Upon an AC Impedance Protocol', *Anal. Letts,* **43** (14), (2010), 2160-2170.

[4] R. L. Caygill, C.S. Hodges, J. L. Holmes, S.P.J. Higson, G.E. Blair, P.A. Millner, 'Novel Impedimetric Immunosensor for the Detection and Quantitation of Adenovirus Using Reduced Antibody Fragments Immobilized Onto a Conducting Copolymer Surface', *Biosens. & Biolec.,* **32***,* (2012), 104-110

[5] T. J. Holford, J. L. Holmes, S. D. Collyer, F. Davis, J. Laíz and S. P. J. Higson, 'Label-Free Impedimetric Immunosensors for Psoriasin - Increased Reproducibility and Sensitivity using an Automated Dispensing System', *Biosens. & Bioelec.,* **44**, (2013), 198-203

[6] J. L Holmes, F. Davis, S. D Collyer and S. P. J. Higson, 'A New Application of Scanning Electrochemical Microscopy for the Label-free Interrogation of Antibody-Antigen Interactions', *Anal. Chim. Acta*, **689** (2), (2011), 206-211

[7] W. S. Roberts, F. Davis, J. L. Holmes, S. D. Collyer, L. D. Larcombe, S. L. Morgan and S. P. J. Higson, 'Detection and imaging the expression of the trans-membrane protein CD44 in RT112 cells by use of enzyme-labeled antibodies and SECM', *Biosens. & Bioelec.,* **41**, (2013), 282-288.

Sensor Array for Ischemia Monitoring in Endoscopic Surgery

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ABSTRACT

Under ischemia conditions, tissue turns from aerobic respiration to anaerobic respiration because of oxygen shortage. Under anaerobic respiration, the glucose is broken down to pyruvic acid and converted to lactic acid. Also, the amount of ATP is less in anaerobic respiration leading to reduced certain metabolic actions such as ion pumping through the cell membrane. Once the ion pumps fail; the osmotic pressure moves the ions in correlation to internal and external ions concentration. Thus, potassium moves to extracellular fluid and in the contrary sodium moves to intracellular fluid attracting water into the cell and reducing the extracellular space. Another affected metabolic action is blood flow reduction using the removal of carbondioxide (CO_2), which causes an equilibrium shift which creates a pH decrease.

Ion concentration and pH modifications in the extracellular matrix can be detected with selective and specific ionophores inserted in ion selective sensors (ISE) matrix and measured electrochemically with potentiometry. Implantable electrochemical sensors, besides the inherent difficulties in miniaturization, the needs for stable and reliable solid state reference electrodes (RE) integrated with the sensors, makes more complicated its development. Few examples of ISE sensors are reported for ischemia detection in biological tissues under in vivo conditions.

In this work, an all-solid-state miniaturized and implantable array was developed to detect ischemia in vivo in gastro-intestinal tissue. This array contains two different all-solid-state ISE for monitoring the evolution of pH and potassium under ischemia conditions. The array also integrates miniaturized solid state RE required for the potentiometric measurement of the ions. This array was designed and fabricated to be inserted into the stomach by means of a gastroendoscope. Noninvasive monitoring of pH and potassium were performed inside the stomach in the pig model. This array was developed for surgical or endoscopic application for intra-operative or post-operative ischemia detection in anastomotic areas.

Diagnosis of Urological Malignancy using a GC Sensor: the Odoreader

Chris Probert and Raphael Aggio

University of Liverpool, UK

ABSTRACT

Bladder cancer is a common disease which could, potentially, be detected by screening
using biomarkers instead of expensive, repeated, invasive cystoscopy.

The Odoreader could be the solution to this problem. Headspace gases from clinical samples are separated by GC before interacting with a heated sensor the resistance of which changes in the presence of VOCs. We used the Odoreader to compare VOC profiles from patients with bladder cancer (n=24) and controls (n=74). Data were interpreted in three ways: linear DA defined 9 bins and correctly identified 100% of cancer and 95% of controls, after leave-one-out cross-validation the sensitivity and specificity was 96% and 93%, respectively [1]; partial least squares DA found 96% of cancers and 93% of controls [1]; wavelet based analysis had sensitivity of 87.2% and specificity 99.2%. Finally, Odoreader could be used to identify prostate cancer with 91% accuracy and to separate bladder cancer from prostate cancers with 93% accuracy.

Urological cancers can be identified with great accuracy, by the rapid analysis of gas from urine samples this user-friendly device could be used in screening and surveillance programmes, reducing patient discomfort and costs.

Reference:

1.Khalid T, de Lacy Costello B, Persad R, Ewen R, Johnson E, Probert C, Ratcliffe NM. (2013) A pilot study combining a GC-Sensor device with a statistical model for the identification of bladder cancer from urine headspace. PLoS One, 8(7).

Leaky Waveguide Biosensors

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ABSTRACT

Leaky waveguides (LW) are optical structures where light is not exclusively confined by total internal reflection (TIR). Symmetric slab LW have non-TIR interfaces at both the top and bottom of the slab waveguide, while asymmetric slab LW devices have one TIR and one non-TIR interface. Because light is not confined exclusively by TIR, we can relax the requirement that the waveguide must have a higher refractive index than the substrate and superstrate. In the simplest case, we can use Fresnel reflection to provide the non-TIR confinement at the substrate-waveguide interface and TIR at the waveguide-superstrate interface. This means that the waveguide can have a refractive index intermediate between those of the substrate and superstrate. To create a biosensor, we can use the aqueous sample (n ~ 1.333) as the superstrate and ordinary glass or some polymers (n ~ 1.5) as the substrate. This means that we can create a waveguide using materials having a refractive index between 1.333 and 1.5. Modelling these structures shows that the width of the waveguide resonance increases as the refractive index of the waveguide increases, so ideally to get the sharpest resonance, which is easiest to see small shifts caused by analyte binding, we should use a material with refractive index close to that of the sample. Cross-linked hydrogels such as agarose and chitosan work well, as do silica sol-gels.

To visualise the waveguide resonance, we can employ either an absorbing film between the substrate and waveguide, or dope the waveguide with a dye or fluorophore. The absorbing film can be either a metal such as titanium or a suitable dye such as titanyl phthalocyanine. It should be noted that the metal film is not used in the same way as in surface plasmon resonance sensors - LW and SPR sensors work in completely different ways.

We have used such structures as biosensors using immobilised antibodies, in multichannel flow cytometry to excite scattering and fluorescence and as a refractive index detector to monitor the progress of electrokinetic preconcentration.

Recent Progress in Sensing Virus Infections

Vincent Emery PhD FSB

University of Surrey

ABSTRACT

The need for rapid and sensitive detection of virus infections both human and animal has never been greater. The recent swine H1N1 pandemic and the Ebola outbreak in West Africa provide examples of why rapid point of care diagnostics are both needed but also how within the appropriate health care pathway they can contribute to community surveillance programmes. Biosensors offer multiple opportunities to be configured for the detection of viruses or the immune response against them. During the presentation I will summarise the types of biosensing approaches that can be used (electrochemical, optical, colorimetric, acoustic/gravitational). Using the examples of influenza virus and HIV I will illustrate how electrochemical and acoustic sensors have been deployed for detection. Focusing on work that we have undertaken with OJ-Bio I will illustrate how the combination of novel capture coatings combined with surface acoustic wave technology has allowed the development of a novel biosensor to detect HIV anti-gp41 and p24 antibodies. This has been facilitated through physical modeling of the surface characteristics to understand how the core chemistry and biophysical properties affect wave propagation and hence sensitivity. Many biosensor applications are demonstrated in artificial systems and so I will attempt to illustrate the performance characteristics of biosensors in real world setting of clinical samples.

Small Molecule Sensing using Proteins as Specific Recognition Elements

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ABSTRACT

The problems of detecting substances of abuse are increasing rapidly due to the increasing number of "designer" drugs arriving every day. Existing technologies are often not flexible enough to recognise these substances. Often, there is need for these substances to be to be detected in low concentrations, often in complex chemical backgrounds for security and customs applications. Existing technologies (mainly based on ion mobility spectrometry) often perform poorly, and reliance is placed on the dog's nose for detection of these substances. Odorant binding proteins have the potential to be used in biosensors that can detect a range of chemicals. *In silico* docking was carried out to determine the potential binding to an array of ligands with diverse structures. Investigation of the binding sites of *Anopheles gambiae* Odorant Binding Protein OBP1 docking target ligands, indicated that selected variants can be tailored to bind drugs of abuse. Variants of these proteins were expressed using molecular biology techniques giving an array of proteins that can detect substances such as atropine, cocaine, ∆9-tetrahydrocannabinol, 3,4-methylenedioxy-methamphetamine, heroin and ephedrine. These proteins have the potential to be deployed as arrays of recognition sites in biosensors for sensing drugs of abuse.

The Use of Breath and Headspace Analysis in Disease Diagnosis

Claire Turner
The Open University

ABSTRACT

The ancient Greeks noticed that the smell of someone's breath gave an indication about their state of health. They knew that if someone had the smell of rotten apples, then they had diabetes. A pungent aroma resulting from throwing sputum on a fire was a likely sign of phthisis, now known as tuberculosis. This means of disease diagnosis was then largely ignored until the 1970s when GC was used to identify volatile compounds in breath. At this point, the science of breath analysis was born.

Breath analysis has grown in interest since then, and in this presentation, its use in the diagnosis and monitoring of disease is described. There are many methodological difficulties involved with breath analysis which will be explored while describing a study to identify likely ranges of volatile compounds in the breath of healthy volunteers. These VCs may then be compared with those from people with a known disease condition, such as diabetes. Monitoring the breath of people with diabetes using a variety of techniques is described, and thus its potential for monitoring blood glucose is discussed.

Breath analysis is difficult without portable instrumentation, so the development of suitable portable or handheld instruments is described using different types of sensors. Development of these instruments must carefully consider the end user, so involving clinicians and nurses in their design is important.

An alternative to monitoring breath is the analysis of urine headspace. Urine is easy to obtain and store and thus offers some advantages over breath. Its potential for screening for or diagnosing disease is illustrated with the example of bladder cancer. The headspace of faecal samples is also used, especially when studying diseases of the gut, for example inflammatory bowel disease, colo rectal cancer or food intolerance. Examples of faecal headspace analysis in screening or diagnosing diseases are presented.

In fact, any body fluid or tissue may be analysed as volatile compounds are emitted from all of these. Understanding which VCs are localised and which are systemic is important, as it will dictate methodology and body fluid or tissue used. This is illustrated with examples.

Pattern Recognition for Biological Sensing: Doing it Right

Prof Conrad Bessant

Queen Mary University of London

ABSTRACT

Many modern bioanalytical sensing technologies are capable of producing rich multivariate data, capturing a wide array of information about the biochemical properties of the sample under study. Typical sensing applications involve using this multivariate data to classify samples into groups, for example to diagnose disease (i.e. classifying samples as healthy or diseased), to determine the origin of a food product, or to assess environmental safety.

This general process of determining class membership from multivariate data is often referred to as pattern recognition, machine learning, or multivariate classification. A wide range of powerful multivariate classification algorithms exists, including discriminant analysis, artificial neural networks, support vector machines and random forests.

Today, building a classification model using any of these methods is straightforward thanks to off-the-shelf software implementations and an abundance of computing power. However, ascertaining a truly representative indication of the classification accuracy for the intended application can be a challenge. Overly optimistic assessments of performance are commonplace, leading to classification models that appear to work well in a pilot study often failing when applied to data from a new set of samples. This damages the community's perception of not just the classification algorithms but of the bioanalytical sensing technologies themselves.

This talk will introduce the pitfalls associated with multivariate classification, and more importantly explain how these pitfalls can be overcome using techniques such as ensemble classifiers, bootstrap resampling and permutation testing. The *classyfire* R package will be introduced as a solution that makes all these techniques readily available to the bioanalytical sensing community.

Sensors and Brokers: Collecting and Integrating Chemical and Environmental Data

Jeremy G. Frey

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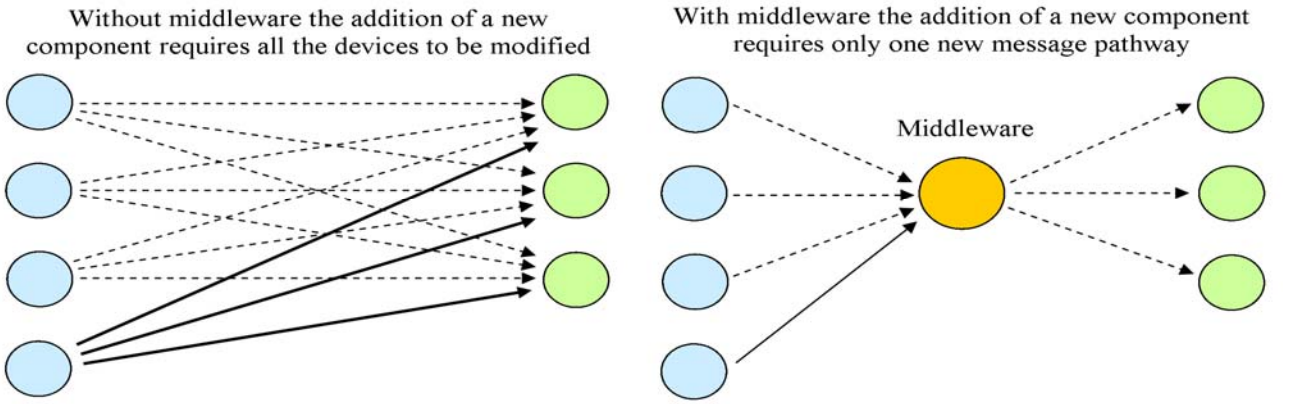
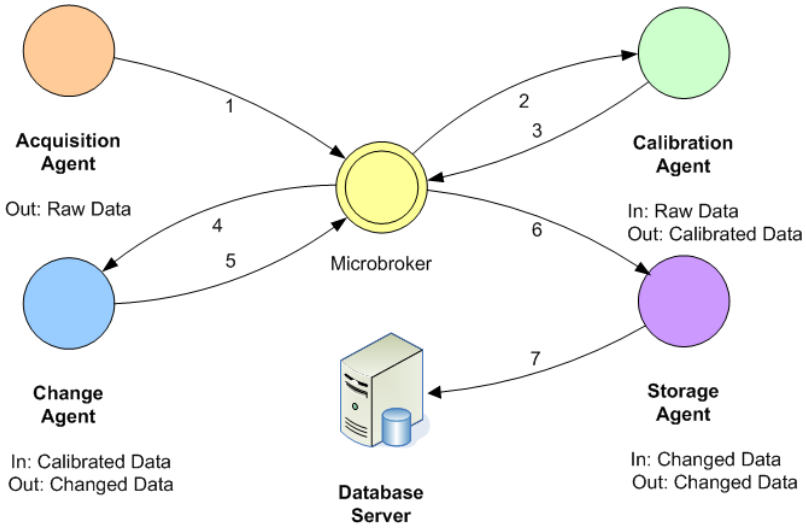
ABSTRACT

Initially as part of the CombeChem project, one of the UK e-Science programme pilot projects, we investigated ways in which monitoring of the laboratory environment could be undertaken simply and in a way that ensured that the access to the resulting data could be provided easily. The approaches we have followed have been based on using the publish/subscribe (Pub-Sub) model and associated data brokers. We have made use of the IBM MQTT lightweight protocol and corresponding web-based protocols to transfer the data from, and commands to, the sensors.



Over the last 10 years our work has evolved from the e-Science viewpoint to one that corresponds to the Internet of Things (IoT) framework of the Digital Economy in that we are looking for generic ways in which the sensor networks can be set up and interconnected without relying on bespoke and commercially specific solutions. To maintain maximum flexibility we champion the use of open standards and when possible open source software. One aspect of the IoT revolution is the increasing potential for machine to machine, so in our case sensor to sensor, communication. I will highlight some of the issues that may result from this delocalisation of control, and unrestrained traffic around the sensor networks.



These sensor networks should be considered as part of the laboratory infrastructure, and as such complement and provide content to the LIMS and ELN systems in use. We have investigated the way in which environmental sensor data can usefully be pushed or pulled into a digital research notebook, to provide context and provenance for experimental results employing automated upload of data. In this context I will look at the balance between the provision of easy to use services, such as energy monitoring systems designed to be used at home and the security and integrity of the data, for example the use of home IoT systems in conjunction with the IFTTT (If This Then That) web based services.

As part of my talk I will consider how successful we have been in enabling laboratory monitoring using an open and interoperable approach and how much bespoke interfacing is still required. I will discuss how this sensor network data is integrated with the experimental narrative and made available for dissemination and publication.

POSTER ABSTRACTS

Distance-Dependent Fluorescent Assay using Size Controlled Core-Shell Fluorescent Silica Nanoparticles

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POSTER ABSTRACT

Fŏrster resonance energy transfer is generally accepted to be limited to a maximal distance of 10 nm between two fluorophores as the efficiency of energy transfer is inversely proportional to the sixth power of the distance between donor and acceptor. Herein, we have developed a distance dependent fluorescent assay using size-controlled, fluorescent core-shell silica nanoparticles (SNPs). The fluorescent core consists of a rhodamine derivative fluorescent dye (TAMRA) covalently bound to the silica matrix. An onion-like structure was then built through addition of new layers of silica shells around the fluorescent core. In this study, we could add silica 'spacer' layers of controlled thickness before adding an outer silica layer containing a quencher (BHQ2®). Using this experimental approach, we could control the architecture of the core-shell SNPs and investigate the distance-dependent relationship between the fluorophore and quencher at the nanoscale. The separation distances between the fluorescent TAMRA-SNP core and the BHQ2® quencher bound to the SNP outer surface ranged from 35 nm to 175 nm. Fluorescence quenching rates were observed ranging from ~75% to ~25%, depending on the distance between fluorophore and quencher. These results indicate the possibility of using such controlled architecture core-shell nanoparticles in nanobiosensor applications where the distance between the fluorescent donor and its acceptor can be in excess of 10 times greater than the generally accepted Fŏrster limit.

Optical Fibre Long Period Grating Gas Sensor Modified with Metal Organic Framework Thin Films

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POSTER ABSTRACT

Fibre-optic sensing platforms based on long period gratings (LPGs) modified with appropriate functional coatings have been used to measure various range of measurands, where the key element of LPG based chemical sensor is the sensitive layer that captures analytes.

In this respect, metal organic frameworks (MOFs), because of their unique properties, offer an ideal platform for the development of the sensitive layer. They can be considered as crystalline materials with tuneable porosity, large internal surface area and organic functionality. The strong metal-oxygen-carbon bonds imbue the materials with high chemical and thermal stabilities.

An optical fibre long period grating (LPG) modified with a thin film of ZIF-8, a zeolitic immidazol framework material and a subgroup of metal organic framework family, was employed for the detection of organic vapours. ZIF-8 films were deposited onto the surface of the LPG using an in-situ crystallization technique.

The ZIF-8 film was characterized by scanning electron microscopy. The LPG was designed to operate at the phase matching turning point to provide the highest sensitivity. The sensing mechanism is based on the measurement of the change in the RI of the film induced by the penetration of the chemical molecules into the ZIF-8 pores. The responses of LPGs modified with 2 and 5 growth cycles of ZIF-8 to exposure to methanol, ethanol, 2-propanol and acetone were characterised. The sensitivity of the measurements to humidity as an interfering parameter was also investigated.

Development of Mutant Odorant Binding Proteins (OBPs) Biosensors for Security Applications: Detection of Explosives

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POSTER ABSTRACT

Odorant binding proteins are small soluble proteins found in the chemosensory systems of mammals and insects. Because of their conformational stability they are of interest as biorecognition elements for new biosensors. Using *in silico* mutagenesis and docking screening techniques the binding pocket of wild type (WT) mosquito *Anopheles gambiae* Odorant Binding Protein OBP1 (AgamOBP1) was modified to produce theoretical variants with enhanced affinities to selected target explosives. We then expressed selected AgamOBP1 mutants plus WT and determined their binding properties towards a selection of target explosives in solution using fluorescence based competitive binding assays. Some of these mutants indeed show very high affinity to the target analytes compared to WT protein. We have developed proteins with enhanced sensitivity to binding TNT; 2,4-DNT; 2,6-DNT; and NH_4NO_3. When OPBs were immobilised onto quartz crystal microbalances (QCM) it was found that they remain stable for many months and they sensitively detect target analytes in vapour phase. This opens a new approach to designing biosensors targeted to specific analytes.

Acknowledgements: The SNIFFER project was funded by the European Community (FP7/2007-2013) under grant agreement n°285203.

A Multisensory System to Control the Process Parameters of an Anaerobic Digestion Plant

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POSTER ABSTRACT

An Anaerobic Digestor (AD) was designed and built as part of an EU Project "ORION". The aim is to allow SMEs operating in the agro-food field to manage their organic wastes on site, decreasing treatment costs. AD involves different microorganisms cooperating to break down biomass, converting it mainly into hydrogen and methane. The concentration of the intermediate products, in particular volatile fatty acids and ammonia, together with the biomass chemical state (pH, ORP, ionic concentration…) are operational parameters that need to be monitored. Any variation in this equilibrium may result in decrease of AD conversion yield and eventually the death of the bacterial colonies.

A multisensory electronic system was developed to automatically monitor the liquid biomass being converted and the biogas being produced. A set of different gas sensors (electrochemical and infrared sensors) analyses the biogas to determine its methane, hydrogen, carbon dioxide and hydrogen sulphide composition. Aliquots of the reactor effluent are sampled and analysed by a set of ion selective electrode sensors to measure the concentration of Sodium, Calcium, Potassium and Ammonium ions together with its pH and ORP. An array of broad selectivity, low specificity conductive polymer sensors is used to estimate the concentrations of Acetic and Propionic Acid in the headspace in equilibrium with the effluent. These parameters are used to maintain optimum operation of the digestor and fault diagnosis.

Acknowledgements

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Sewage Biosensor to Identify Potential Population Biomarker for Monitoring of Public Health

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POSTER ABSTRACT

Wastewater-based epidemiology (WBE) has shown to be a powerful tool for the evaluation of human population's drugs use. This approach is based on the analysis of specific urinary biomarkers such as drug residues in wastewater after being excreted by humans at treatment plants, and this approach can also be utilized for the evaluation of public health by assessing disease biomarkers. Here we describe a new label-free electrochemical DNA (E-DNA) biosensor using a synthesized ferrocenyl (Fc) redox marker which intercalates to double-stranded DNA (dsDNA to detect human-specific mitochondrial DNA (mtDNA) associated with cancer biomarkers. The Fc intercalator binding to dsDNA was characterized by differential pulse voltammetry. This new biosensor was optimized to allow the detection of single base pair mismatched sequences, able to detect as low as 10 pM DNA, and covering a dynamic range from 10 pM to 100 nM. To validate the biosensor, DNA was extracted from wastewater and human-specific mtDNA was amplified with quantitative polymerase chain reaction (qPCR). The E-DNA biosensor was employed to detect mtDNA after PCR amplification. Experimental results show that the peak current from Fc intercalator oxidation depends on the amount of target mtDNA. The biosensor is able to detect mtDNA in PCR products even with a 100-time dilution, but with low non-specific adsorption. Our results demonstrate that human biomarkers are detectable in wastewater, and this may open the door to identify potential population biomarkers for the monitoring of public health using WBE.