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10th Anniversary of the Centre for Chemical Sensors and Chemical Information Technology (CCS) at ETH Zürich

Abstracts of the Scientific Conference on June 17/18, 2005, at Technopark Zürich

Introduction

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It was a pleasure to invite the international community of sensor and bioassay research for this conference marking the 10th anniversary of the Centre for Chemical Sensors and Chemical Information Technology (CCS). The 'Decennial Conference' united a small group of scientists with the highest reputations and was a showcase for excellent presentations. The Conference showed the splendid outcome of research projects supported by persistent academic and industrial interests. In addition, the papers showed the potential of risky innovative ideas realized on the basis of a profound knowledge in chemically tuned sensor technology. Anyone who missed the 'Decennial Conference' has missed a great chance to share the latest news in the development of chemical sensors and bioassays. The papers of this issue of CHIMIA and the abstracts provide an idea of the highlights.

CCS was established in March 1994, one year after the opening of Technopark Zurich. When we moved in, the laboratories at CCS were empty and Technopark hosted only a few brave tenant companies. The car park was much too large and, therefore 'a bone of contention' to the government of Zurich. Today, space is rare at CCS and at the Technopark. CCS operated as a self-supported institute by agreement with the Swiss Federal Institute of Technology (ETH).

Even though the situation was sometimes critical, a large number of partnerships and projects involving a large number of other research groups and companies enabled the development of innovative solutions; some of which have been protected by patent applications. In addition, CCS was able to outsource technologies to its spin-off companies SENSORIX and C-CIT AG founded in 1999 and 2002, respectively. In addition to stimulating collaborations with private enterprises, a good academic profile and curriculum was achieved. The following figures summarize some of the merits achieved at CCS:

- CCS has been involved in ten patent applications. One Trade Mark 'Lab in the Bag' (in words) was protected.
- Nine internal and ten external Ph.D. theses and one habilitation thesis were awarded. In total 85 people were offered a job, a position for a practicum, for a Ph.D. thesis, *etc*.
- The turnover within ten years climbed to a total of 7.2 mio CHF. Statistical data such as the average man-month salary were published at the conference. Only 4.3% of the turn-over was contributed by ETHZ whereas 21% were cash funds invested by Swiss research organizations and industrial partners. Overall, a share of >50% of the total turnover was contributed by organizations and private enterprises. Only a single EU project was acquired within the period between 1993 and 2004.
- CCS was the host to 16 post docs working in different fields such as synthesis, computing, pharmaceutical and analytical chemistry, biochemistry, protein and polymer chemistry. In addition, CCS trained twelve students from the department and 15 students from Applied Universities for diploma and semester projects, and hosted twelve international academic guests from seven different countries (Austria, Belgium, China (4), Japan, Lithuania, Spain (2), Switzerland and Ukraine).

With the Decennial Conference we gratefully acknowledge the many fruitful national and international contacts and collaborations, and the financial support of projects by the Swiss funding bodies especially by CTI (Commission of Technology and Innovation). Last but not least, doctoral students and post docs with a Master in Chemistry were the hard core during the first few years when CCS was under development. Thanks to their excellent background in natural sciences, their persistent and ambitious working style, the Centre expanded and was awarded an international reputation. Many thanks go to all these 'men of the hour'.

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Electrochemical Sensors in Medicine: New Solutions to Old Analytical Challenges

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Modern electrochemical sensor technology has had a dramatic impact in the design of portable clinical instruments capable of rapidly detecting the levels of important physiological species (*e.g.* O_2, pH, CO_2, glucose, electrolytes, *etc*.) in undiluted blood samples [1]. The development of essentially disposable sensors that can be mass produced in arrays at low cost was one hurdle that needed to be overcome to implement such technology in modern instrumentation. In the case of carbon dioxide measurements, this has been achieved using a novel planar, differential potentiometric membrane electrode design; an outgrowth of research on polymeric pH sensors originally initiated by the late Willi Simon and coworkers in Zurich in the early 1980s [2]. In this design, two planar polymeric pH electrodes are employed, and CO_2 is detected *via* a pH change in the very small volume of internal solution of the CO_2 sensing electrode. The second pH electrode has a highly buffered internal solution and serves as a pseudo reference electrode. Hence, by measuring the potential difference between the two pH electrodes, the effect of sample pH cancels, and only the pH change due to diffusion of CO_2 into the inner solution of the working electrode is detected.

Increasing the menu of analytes measurable in blood by electrochemical devices (to important drugs, proteases, *etc.*), and implementing catheter-style sensors for continuous, real-time *in vivo* monitoring of key species, has proven even more challenging. In this presentation, recent research advances in these two areas were highlighted. For example, new, non-equilibrium potentiometric poly-ion sensitive membrane electrodes now provide a simple means to quantitate therapeutic levels of various anticoagulant heparin drugs (including newer low molecular weight heparins) directly in whole blood samples [3–5]. These poly-ion sensors are prepared by doping polymer membranes with lipophilic ion-exchanger species that are capable of forming strong, cooperative ion-pair complexes with the analyte poly-ions in the organic membrane phase. Potentiometric response occurs as the poly-ions are extracted into the polymer membranes owing to the favourable thermodynamics of ion-pairing. These same poly-ion sensors can be used to devise novel assays for protease activities in blood samples based on use of polyionic substrates [6][7]. This approach enables the measurement of plasmin and plasminogen activators (*e.g.* clot-busting streptokinase, urokinase, tissue plasminogen activator) using simple potentiometric detection.

To enhance the analytical performance of intravascular electrochemical sensors (including also optical sensor designs), it will be shown that novel polymeric coatings that can release/generate low levels of nitric oxide (NO) can be employed to dramatically improve the blood biocompatibility and, hence, the analytical accuracy of such in-dwelling devices. Nitric oxide is a potent anti-platelet agent, and low levels of this species prevent platelet adhesion and activation on polymeric surfaces. Indeed, data will be presented showing that intravascular amperometric oxygen sensors prepared with NO release coatings (diazeniumdiolated polymers) exhibit significantly improved analytical performance compared to corresponding control sensors (without NO release), when implanted into the arteries of the same animals [8][9].

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'Attach of the Clones': Genetically Engineered Bioelectronics

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In amperometric biosensors, charge transfer between redox enzymes and electrodes was first accomplished by using free diffusing electron acceptors, such as oxygen, NAD^+ or low molecular weight compounds like ferrocene and its derivatives. Leading from this design principle, a more direct electrical communication between enzyme and electrode was reported where biomolecules are 'wired' on redox functionalised electrodes to facilitate charge transfer. Coincident with protein engineering technology is the possibility to use site directed mutagenesis (SDM) to create amino acid residues suitable for activation and 'wiring' to an electrode for an amperometric biosensor. This requires the amino acid attachment site for the redox 'wire' to be close to the redox prosthetic group(s), within a suitable distance (Marcus theory) for charge transfer. However, achieving this connectivity may not be a totally inconsequential action. Conformational change, required in some redox systems for electron transfer, may no longer be feasible with an anchored external redox agent, or the environment of the prosthetic group can be affected and result in a change in its redox potential from that carefully tuned cascade of redox groups in the protein, whose potential and conformation, controls efficient charge transfer or charge 'cul-de-sacs'.

This presentation will explored trimethylamine dehydrogenase, TMADH, as a model for a complex 'non-ideal' redox enzyme for biosensors and look at ways to achieve a more robust direct electrochemical recycling and external control of the prosthetic groups. Site directed wiring, so that the electron transport chain can be extended outside the protein without requiring diffusing electron transfer elements, is the first engineering step towards efficient 'designer' redox enzymes as electrochemical reagents [1][2]. The structure of the TMADH protein isolated from *Methylophilus methylotrophus* W3A1 has been determined to a resolution of 1.8 Å by X-ray crystallography and the gene encoding of the enzyme has been cloned and sequenced. The enzyme TMADH (EC 1.5.99.7) is an iron–sulfur flavoprotein which catalyses the oxidative demethylation of trimethylamine (TMA) to dimethylamine and formaldehyde. Upon substrate turnover, electrons are transferred

from the FMN binding site *via* the 4Fe–4S centre to external redox acceptors. The iron–sulfur complex engages in a one-electron redox mechanism, thus transferring electrons sequentially from the hydroquinone, FMNhq and semiquinone, FMNsq. For this cascade to operate successfully, the oxidation potential of the $[4Fe–4S]^{+/2+}$ has to remain lower than that of $FMN_{sq/hy}$ and within range of electron transfer to $FMN_{ox/sq}$. Furthermore, recent solution of the crystal structure of the TMADH complex with the natural electron transfer flavoprotein (2ETF) has shown a number of positions for the FAD group of ETF, but few are electron transfer competent. It follows therefore that covalent and non-covalent interactions close to either the FMN or 4Fe–4S sites in TMADH may upset charge transfer through and with the protein.

Nevertheless, protein engineering and site-specific chemical modification have been used to extend the electron pathway from the protein surface to wire with $[Fe(5\text{-}NH_2\text{-phen})_3]^{2+}$, the latter showing fast homogeneous electron exchange kinetics, ideal for 'wire' construction. The Y442C mutant was successfully attached to an electrode surface which had been chemically modified with the redox polymer, poly$[Fe(5\text{-}NH_2\text{-phen})_3]^{2+}$. This design enabled direct electrical communication between the enzyme and electrode. Using a partly oxidized polymer to limit the supply of oxidised electron acceptor, allowed the oxidation state of the enzyme to be controlled and electron flow, and thus substrate turnover, to be controlled. This pushes the enzyme through the so-called fast '0/2-cycle' to the '1/3-cycle' for the TMADH.

The observation that nature recruits a limited number of protein folds for building a variety of functions, together with advancing protein engineering techniques now offer a unique opportunity to alter active site interactions to create enzymes having catalytic activity towards substrates with no biological precedent. Entirely new activities have been engineered through rational design and assembly, so that in principle, the same methodology could be employed to tailor redox proteins for substrates of analytical interest, thus building on the biosensor family.

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Direct Electron Transfer between Heme Containing Enzymes and Electrodes as Basis for Third Generation Biosensors

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For practical applications in enzyme-based biosensors efficient direct electron transfer (ET) between the enzyme and the conducting material would facilitate the construction of simple biosensors with superior selectivity. For an increasing number of redox enzymes efficient direct ET reactions have been reported [1]. Most of these redox enzymes are metalloenzymes and many of them contain heme. Unfortunately, direct ET between the active site for most redox enzymes and electrodes is believed to be hindered due to kinetic restrictions originating from either too long a distance between the active site and the electrode surface or a non-favourable ET pathway. It could, however, be expected that direct ET at electrodes can be established if the enzyme is able to readily exchange electrons with other biological partner proteins. It is therefore very essential to obtain detailed structural information of the enzyme and of the electrode surface to develop methods for desired structural changes of both making efficient direct ET possible.

Plant peroxidases may serve as model enzymes in this respect. They are rather small heme containing enzymes with a molecular weight around 40 kDa and the 3D structure of several peroxidases is known today. In their wild type native form they are all glycosylated. Availability of recombinant horseradish peroxidase (HRP) allowed the effect of glycosylation to be compared [1]. In the case of native HRP adsorbed on graphite around 50% of the enzyme molecules are in direct ET contact, whereas for recombinant HRP the percentage drastically increases as does the heterogeneous ET rate constant (k_s). Additionally, several engineered recombinant forms of HRP ('outer mutants') with either a His tag attached to the N- or the C-terminus or surface-exposed cysteines have been studied [2]. These recombinant forms seem to be strongly adsorbed in an oriented fashion on Au-electrodes facilitating direct ET (increasing both the % of the adsorbed enzyme in direct ET contact as well as the k_s) compared with native and wild type recombinant HRP. Therewith, the kinetics of the electrochemical rereduction of the oxidised forms of HRP (compounds I and II) to the resting state was shown to be governed by a coupled electron-proton transfer [3]. Studies of the effect of pH and addition of proton donors to the contacting solution on k_s demonstrated drastic increase in efficiency of direct ET with increasing $[H^+]$. For the system with the best performance more than 90% of the adsorbed enzyme molecules are in direct ET contact with k_s-values close to 500 s^{-1} yielding a truly diffusion controlled response current for H_2O_2 in the lower concentration range (2 A M^{-1} cm^{-2}) with a detection limit around 10 nM [3][4]. As an extension of previous investigations of plant peroxidases, the electrochemistry of fungal peroxidases with lignin-olytic properties (lignin and manganese peroxidase) as well as various other heme containing redox enzymes (*e.g.* cellobiose [5], fructose dehydrogenase, theophylline oxidase, sulphite oxidase [6]) as well as complex II from the respiratory chain have been investigated when adsorbed on graphite or at thiol modified gold electrodes. The results of these investigations were shown and discussed.

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Chemically Selective Imaging with Scanning Tunnelling Microscopy Using Chemically Modified Tips

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The development of techniques to image objects at the molecular and atomic scale is one of the greatest challenges of nanotechnology. Scanning tunnelling microscopy (STM) and atomic force microscopy (AFM) belong to the most important analytical methods available for this purpose. Since their invention in the 1980s, these methods have revolutionized surface analysis because they allow the observation of surfaces with submolecular or even atomic resolution, not only in the vacuum but also in solution or the ambient atmosphere. STM and AFM not only provide ***molecular resolution*** images of periodic structures but truly represent samples at the level of ***individual molecules and atoms.***

STM has the advantage that true atomic resolution can be obtained much more readily than with AFM. This ***exceptional resolution*** results from the exponential dependence of the tunnelling current that flows between the STM tip and the sample. While AFM images reflect the topography of a sample, STM images are a function of the partial electron densities of sample surfaces. Unfortunately, a weakness of conventional scanning tunnelling microscopy is its ***limited capability for chemical recognition***, *i.e.* for the discrimination between different types of atoms or functional groups. Sometimes, the position of functional groups or heteroatoms in a STM image can be easily deduced from the partial electron densities at individual atoms or functional groups. For example, an image of a dialkyl sulfide or alkylthiol molecule adsorbed onto graphite easily reveals the position of the sulfur atom because the probability for electron tunnelling is much larger at the sulfur than at the methylene groups of the alkyl chains. However, more often chemically different groups cannot be distinguished from one another. For example, theoretical models for the description of STM images of alkanes and substituted alkanes adsorbed onto graphite predict that topographic effects dominate their STM images. Due of this limited chemical selectivity of STM, there are many submolecularly or atomically resolved STM images that have been interpreted only tentatively or not at all.

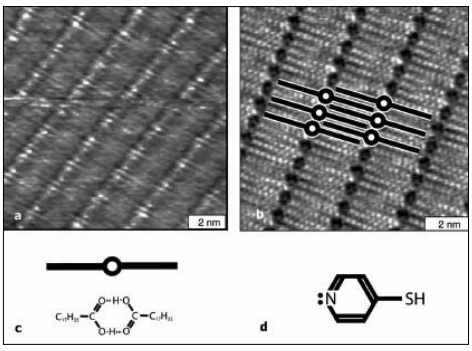

Fig. 1. (a) STM image of a monolayer of 1-octadecanoic acid on graphite, collected with a 4-mercaptopyridine modified tip. The terminal lone pair of pyridine on the tip interacts with the COOH hydrogen of the sample, resulting in an apparently raised region over COOH groups [2]. (b) Image collected with a bare gold tip. The molecules form dimers and lie flat on the surface, as depicted schematically. The COOH groups are aligned in dark rows. (c) Schematic representation of 1-octa-decanoic acid dimer. (d) Structure of 4-mercaptopyridine.

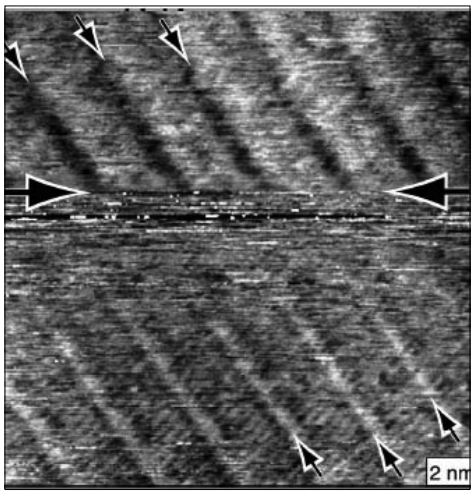

Fig. 2. STM image of a 1-octadecanoic acid monolayer on HOPG, showing establishment of contrast enhancement. Top: Not contrast-enhanced region. Large arrows: Tip conditioning with resultant instability. Lower half of image: Contrast enhancement (COOH stand out as raised regions; c.f. Fig. 1). Note: Resolution is lower than usual due to low scan rate required to limit instability region to small part of total image.

The problem of limited chemical selectivity can be overcome by allowing a ***STM tip to interact chemically with a sample*** (Fig. 1). Gold tips may be modified with self-assembled monolayers or polypyrrole for the ***selective recognition of functional groups*** that form hydrogen bonds. This method is also able to distinguish between different metal centres and between functional groups with ***different spatial orientations*** [1]. The chemical interaction between modified tips and the sample enhances electron transfer between the tip and sample, resulting in selective recognition of selected functional groups or atoms in a surface image.

In our initial work, only a limited number of tips showed this manner of contrast enhancement, and those that worked had a usable lifetime of just a few hours before contrast was lost. However, we recently devised a method of tip conditioning that allows for the *in situ* reestablishment of chemical contrast [2]. For example, while imaging a 1-octadecanoic acid monolayer on highly oriented pyrolytic graphite (HOPG) with a chemically modified tip, no contrast enhancement is initially observed. A voltage pulse of +1.9 V is applied for 15 s. The region of the image collected at this time appears unstable. The voltage is then returned to the negative imaging voltage, and chemical contrast is achieved (Fig. 2). The loss of enhanced contrast is thought to be due to the recession of SAM molecules away from the tip apex.

Despite the added versatility that tip conditioning brings, it cannot eliminate the occasional 'crashing' of tips into samples. Moreover, from a molecular point of view even the best metal tip has a relatively large diameter. This is not crucial when imaging relatively flat surfaces; for every good tip there will be one tip atom or molecule that sticks out a little bit further than all the others and is the site of electron tunnelling. However, more corrugated surfaces have crevices into which the metal tip cannot enter. Carbon nanotubes (CNTs) are a promising solution for both problems. We developed a method for the preparation of CNT STM tips based on the growth of CNTs on metal tips by chemical vapour deposition, followed by electrochemical deposition of ruthenium on the tip shaft. Tips that have undergone this preparation have been used to successfully image HOPG and monolayers of octadecanoic acid on HOPG.

We expect that the unique ability of STM to characterize samples in a range of environments (vacuum, liquids, gases), combined with the capability for chemical selectivity, will make this technique a general tool for nanosciences, improving our comprehension of nature at the molecular level.

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Functionalization of Cantilever Array Sensors

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Micromechanical cantilever arrays can be used as versatile chemical and biochemical sensors when coated with specific layers to absorb molecules that alter surface stress or add mass [1–4]. Reproducible coating procedures are essential to achieve reliable sensing. Two types of capillary devices have been developed to immerse in parallel eight cantilevers into different solutions. Moreover, we have explored ink jet technology for rapid printing of self-assembled monolayers, thiolated DNA and polymers onto selected cantilevers [5][6].

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Fibre-Optic Chemical Sensors with Luminescent Ru(II) Complexes: Photophysics and Photochemistry at Work

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The sensitivity, specificity, and versatility of the ***optical methods*** for chemical measurements have made spectroscopy one of the most popular techniques for ***environmental analysis***. Nevertheless, the very same attractive features have led (so far) in most cases to expensive instrumentation and complex procedures compared to, for instance, the handy electrochemical sensors. Fiber-optic chemical sensors (or '***optodes***') are bound to overcome such limitations provided they use cost-effective optoelectronic systems, versatile multiparameter monitors, and they can be shown to be specific, sensitive and robust enough to fulfil their analytical tasks.

Over the last 25 years hundreds of optical fibre sensors have been described for the analysis of chemical parameters but, surprisingly, only a very few of them have come to the market. The widely ***different*** spectroscopic ***properties*** of the ***many*** optical ***indicator*** dyes, the necessity of immobilizing them onto a ***suitable*** solid support, the lack of ***photochemical*** reactions with the required ***selectivity*** and ***sensitivity***, and the tough ambient conditions in ***environmental*** monitoring and ***industrial*** control, among other factors, are deemed responsible for such gaps. Competition of improved alternative sensors, the cost of sophisticated optodes and their field validation, as well as a frequent failure to recognize the end-user needs, help to understand the slow pace of transforming basic research in this area into industrial devices [1].

The design and synthesis of a ***homogeneous*** family of tailored ***luminescent*** indicators (coordination compounds of ***Ru(II)*** with polyazaheterocyclic chelating ligands) and the exploitation of selected sensing schemes in the author's laboratories represent attempts to fill the gap [2]. Selected examples of the molecularly engineered dyes and photochemistries were presented in detail at the meeting, such as those that have led to oxygen, temperature, pH, carbon dioxide, ammonia, biochemical oxygen demand (BOD), detergents, hydrocarbons, selected pesticides and humidity fibre-optic (bio)sensing using an 8-channel optoelectronic phase-sensitive fluorometer (Optosen®) for *in situ* environmental/industrial monitoring developed, manufactured and currently marketed by the Spanish company Grupo Interlab, S.A. (Fig.) [3].

Acknowledgements. In addition to Interlab, S.A. funding, additional support from the Spanish Ministry of Science and Technology (AMB, MAT and PROFIT Programmes) and the European Union (Brite-EuRam Programme) has been received.

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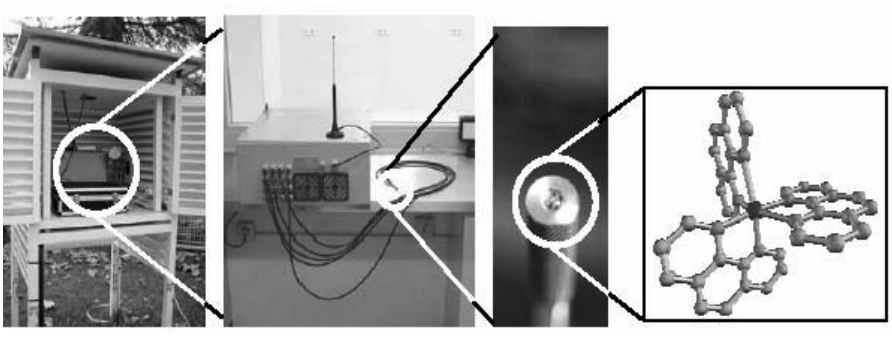
[3] The OPTOSEN® system is a customizable multichannel fibre-optic monitor specifically developed and marketed by INTERLAB Electronics and Control Engineering (Madrid, Spain; *www.interlab.es*) for environmental monitoring of chemical parameters and process control. LAP–UCM has developed the sensitive tips for this system under contract with Interlab.



Interlab's Optosen® fibre-optic unit with its luminescent sensitive heads developed at UCM for multiparametric environmental monitoring and industrial process control.

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Experiences/Development of an ETH/CCS Spin-Off

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C-CIT AG is a small company founded in January 2002. As it stems from CCS, C-CIT is entitled to call itself a spin-off of Zurich's Federal Institute of Technology (ETHZ). C-CIT AG is a kind of engineering firm that develops chemical and biochemical assays and sensors, measuring devices and on-line analysis systems. It has already launched one product, a sensor and instrument for detecting food oil oxidation, on the market. Two electronic boards and a continuous monitoring system can be ordered *via* C-CIT's homepage (*www.c-cit.ch*). Several other projects are in the pipeline. C-CIT maintains excellent links with research organizations, applied universities and marketing enterprises. It has especially good contacts with the University of Applied Science in Wädenswil, which has its core competence in biotechnology, food technology and agriculture, and helps C-CIT to gain access to real applications in these fields.

The chief executive manager of C-CIT (CEO) is Stefan Spichiger, Dipl. Ing. HTL in biotechnology. C-CIT ensures smooth technology transfers from CCS-ETHZ to C-CIT by taking over employees from CCS.

The development of the first product (a measuring instrument to assess the quality of frying oils) benefited greatly from the good reputation of CCS and its close connection with C-CIT. It serves as a kind of case-book example of how a spin-off can cooperate with a university institute.

What is this 'frying-oil instrument' and how did C-CIT AG get involved in this project? This instrument (Fig.) can be used by fast-food chains, large-scale catering establishments and other branches of the food industry to monitor the quality of different frying oils. It uses, as a measure of quality, the fraction of polar-material compounds in the oil. These compounds develop from long-chain fatty acids and other materials while the oil is heating up. The heating process may produce a bad (rancid) taste and unhealthy compounds.

Modular instrument measuring the capacity of oil. 1) User-friendly calibration and data storage unit with display. 2) Sensor unit with microstructured capacity sensor and gold surface.

The fraction of polar compounds in frying oil is determined by measuring the dielectric constant, which increases proportional to the increase in polar compounds. In principle, polar compounds orientate along the electric field lines between two electrodes where an alternating current of decisive frequency is applied. (Polar compounds respond to the frequency of an alternating electric field of decisive frequency and align with the electric field at a specific frequency). The relevant parameter which characterizes an oxidized oil is the capacity increasing with increasing fraction of polar moieties.

How did the project start? Northern Instruments (USA) developed an instrument named 'FOS' and applied for a patent in order to protect their know-how more than 30 years ago. A Swiss company imported the instruments and was commissioned to sell them to the Mc Donald's fast food chain, to State laboratories and industrial enterprises. The patent expired two years ago. The Swiss company still had instruments in stock, but replacement sensors were no longer available. The representatives in Switzerland started looking for a partner to produce the electrochemical sensors. As a first step, they contacted the ETHZ and were put in touch with CCS.

Since the project did not require significant scientific research, CCS asked C-CIT AG to take care of the project and to deal with the Swiss company. Within four months, C-CIT AG succeeded in developing a suitable sensor to replace those in the instruments already on the market. At the same time, they designed a new improved generation of instruments, which is currently being launched. The development costs were shared between the Swiss commissioning company and C-CIT AG. C-CIT AG was therefore able to obtain the right to develop the instrument for other applications independently.

The success of the project demonstrates: (1) the value of CCS's good reputation in the field of sensor research and development. Although CCS had never developed physical sensors, the customer contacted ETHZ and CCS. Logitech's CEO recently described a similar case when a marketing study was performed to find the most well-known keyboard and mouse producer. Although Logitech was not producing any keyboards at that time, customers still ranked it among the top three companies! (2) How good collaboration, a clear division of responsibilities and transparent strategies for both the spin-off company and the university group improve relationships between the parties involved and efficiency along ***the technology transfer chain***.

Optical Transcutaneous Blood Gas Measurement

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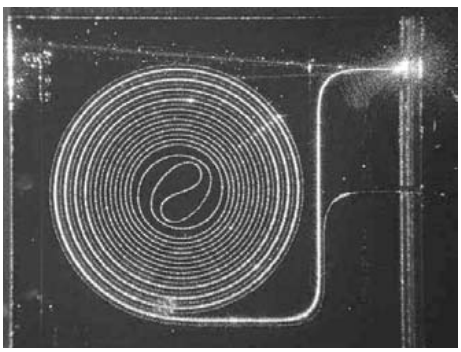
Continuous monitoring of arterial blood gas parameters is an integral part of monitoring critical ill patients, in particular in unstable respiratory or cardiopulmonary conditions. Transcutaneous monitoring of blood gases is the only non-invasive technique available today that allows continuous and simultaneous measurement of both oxygen and carbon dioxide partial pressures. The presently available systems use heated electrochemical sensors applied at the surface of the skin, which need to be frequently recalibrated and reprepared and are limited to the detection of few gases.

To overcome these drawbacks we have developed a new sensor technology using optical principles for gas sensing. We have chosen the measurement of CO_2 to establish the validity of this technology. CO_2 is measured by detecting the optical absorption in the evanescent wave at the surface of a transcutaneous sensor by using a modulation spectroscopy technique (patent pending). The sensor can be pre-calibrated at the factory and is not affected by drift.

We have built straight and non-crossing spiral waveguides based on silicon nitride technology in combination with laser diode modulation spectroscopy system [1][2]. The waveguide is shaped in a miniaturized spiral with a smaller than a 5 mm diameter area (Fig.). The curvature has to be reduced to a radius of 200 micrometer in the centre of the spiral. We have achieved low propagation loss of –0.4 dB/cm, over 50 cm of uncovered sensing length. Best performance with very low fibre-chip coupling loss



Miniature evanescent wave sensor, 6x6mm chip size, 16 cm waveguide length

of 3 dB leads to an overall insertion loss lower than 26 dB for 50 cm sensing length on a 6×10 mm chip size including pigtailed V-grooves.

The miniaturized non-crossing spiral waveguide has been applied for different CO_2 concentrations in nitrogen mixture showing a good correlation with gas absorption theory.

This validates the concept of applying evanescent wave to selectively sense gases diffusing throughout a medium, *e.g.* carbon dioxide diffusing throughout the human skin. It is foreseen to extrapolate this technology to other gases.

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Ion-Selective Electrodes Compared to Other Analytical Methods Applied in Aqua-Cultures

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The production of fish and plants in aqua-cultures could be developed as an additional income for farmers. To guarantee non-toxic levels of ammonium at a low water exchange rate, ammonium should be monitored continuously.

This would be an ideal application for ion-selective electrodes (ISE), because no other low-cost methods for real-time and completely continuous measurements are available. Because interfering ions such as potassium are contained in unknown and changing activities, the accuracy of the analytical data need to be checked and compared to other methods.

Therefore, ammonium in different types of water was measured using ion-selective electrodes, ion chromatography and the optical methods of Dr. Lange and of Reflectoquant by Merck, respectively [1]. The result was disappointing, no method was comparable to each other. The deviation of the ion-selective electrode could not be traced back to interfering ions such as potassium and sodium or to effects of temperature or pH.

Nevertheless, ion-selective electrodes are useful in distinguishing between upper (toxic) and lower (non-toxic) concentrations of ammonium (limit at 4 mg/k). This will enable the electrodes to be used as an alarm system. Further trials will need to prove these questions. The poster presented the conclusion of the corresponding experiments [2].

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Biosensor for Umami Detection in Tomato Paste

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Umami as an enjoyable taste has been known since the beginning of the last century. Kikunae Ikeda found that glutamic acid significantly contributes to the taste of food, L-glutamate (usually its monosodium salt) is known as a flavour enhancer in food, especially of seasonings, sauces, instant soups, and noodles, as well as seafood. Moreover, some kinds of vegetables such as corn and ripe tomato have quite a high amount of MSG (130–140 mg per 100 g of vegetable). However excessive intake of monosodium glutamate can cause some allergic reactions such as tension in stomach, headache, pain in neck, and shoulders. Therefore it is still relevant to control the amount of L-glutamate in food. Our aim was to monitor the L-glutamate concentration in different kinds of tomato pastes [1].

Tetrathiafulvalene and 7,7,8,8-tetracyanoquinodimethane (TTF-TCNQ) is a conductive organic complex salt – electron transfer agent often used in biosensors, because it is a non-toxic biologically friendly compound [2]. The biosensor was constructed from mediating paste, where TTF-TCNQ served as a mediator, which regenerates enzyme and transfers electrons to the electrode, and cross-linked L-Glutamate Oxidase (GLOD) from *Streptomycis* sp. on the surface of the paste electrode. It is well known that GLOD selectively oxidizes L-glutamate to 2-oxoglutarate releasing ammonia. The taste biosensor should comply with the goals of high selectivity (working at an applied potential <100 mV), sensitivity, negligible leaching of enzyme and mediator, response in the range between 0.25–2 mM of L-glutamate, and have a lifetime of at least 10 days (stored in buffer solution, at +4 °C). Since the pH of tomato paste is 3.3–4.5, it has to be diluted and buffered to pH 7, because GLOD is active only in neutral medium. The relative standard deviation of the amperometric glutamate detection using standard addition method in tomato pastes in imidazole buffer pH 7.0 was ≤3%.

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CCS Between Science and Industry

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It is difficult to make someone believe that a research group located at the Technopark in Zurich belongs to ETHZ and is involved in scientific research. CCS was and still is devoted to a dual mission, with two main but rather different goals: 1) According to its agreement with ETHZ, CCS is responsible for its own technology transfer and has to obtain financial support for each project through collaboration with private enterprises. 2) CCS belongs to the Department of Chemistry and Applied Biosciences and therefore has to ensure it meets the high standard in science required at ETHZ. The outcome is rated according to the number of publications, invited lectures, patents, and so on. The duality of its mission means that CCS has to fulfil both the scientific requirement to produce outstanding results and the commercial requirement to develop successful industrial projects that can be carried out by well-trained but inexperienced academics. Initially, both objectives were tackled with suboptimal efficiency.

Fortunately, in 2001, CCS had the contacts and the financial liquidity to have the Centre and its ongoing projects audited. The Swiss Technology and Consulting Group (STCG, Zurich) was engaged to carry out the investigations.

Each project was characterized and classified using the following ***criteria***:

- How well does it fit in with CCS's major goals (see above)?
- Does it draw on CCS's technological strengths and capabilities?
- Does it conform to CCS's profile (Centre of Excellence)?
- What is the market potential of the project?
- What benefits can customers expect from the project?
- Does the project involve a partner from industry?
- Does the project working on niche products?

Features like the outstanding training the project provides, the project's scientific reputation, its spin-off potential, and the level of financial support it received were also considered. The profile was not only technology-driven but, necessarily, also market-driven. The audit led to four ***classes of projects*** at CCS being identified:

1) Platform projects investigating and developing new, innovative technologies with a view to their analytical applications (optical sensors based on integrated planar waveguides [1]; Lab-in-the-Bag technology [2][3]);
2) Projects with high market potential involving new sensors and technologies (selective optical gas sensors with low detection limit [4][5], EU project Nr. BuI00.0344 ('MICS', advanced functional materials for the electronic tongue and electronic nose); artificial and genetically engineered enzymes [6][7]);
3) Projects with growing market potential, involving technologies mature enough for technology transfer and industrial applications (spin-off potential: Lab-in-the-Bag; biosensor chip, chemically modified AFM-cantilever [8][9]);
4) Unique projects providing publicity and enhancing CCS's reputation as a Centre of Excellence (installation of primary reference methods, active proteomics [1]).

Subsequently, three different ***strategies*** to be possibly pursued in future developments at CCS were defined: the first focused on the development of innovative platform technologies, the second on diversity and having considerable industrial impact, and the third on developments with high reputation and a special high-tech market potential. Founding two spin-off companies, Sensorix and C-CIT AG, enabled CCS to achieve enough freedom to focus on innovative bioanalytical projects and, additionally, to develop a novel strategy combining the features of strategy 1) and 3).

Since the Decennial Conference is focused primarily on scientific aspects, I have presented an overview of some of the developments at CCS and the main ideas behind them (for a list of all projects see *www.chemsens.ethz.ch*).

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