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Molecular Recognition and Chemical Sensor Development at CCS

Ursula E. Spichiger-Keller*

Abstract: The Center for Chemical Sensors and Chemical Information Technology (CCS) at ETH-Zürich has devoted its activities to the development of highly selective devices that access chemical information about the composition of a specimen. In this article the most prominent projects and activities are summarized and the most relevant scientific results and research strategies are described. Apart from design and synthesis of novel ionophores for electrochemical sensors, research was focused on optical sensors, chromophores with specific characteristics as well as micro- and nanodevices. Generally the research focus shifted from using small organic molecules to the development of oligopeptides to solve analytical problems in biotechnology, food technology and bioassays. Many collaborations with industrial partners have been established, and profit and drawbacks of such collaborations are reconsidered at the end of the article. One so far unpublished feasibility study is described in more detail. In order to make commercial use of some of these developments, the spinoff companies SENSORIX and C-CIT AG were founded.

Keywords: Active proteomics · AFM-cantilevers · Array technology · Bioassays · CCS · Microsensors · Nanotechnology · Spinoff companies

1. Introduction

CCS is a self-supporting ETH group which belongs to the Department of Chemistry and Applied Biosciences and was founded in 1994 by U. Spichiger. The reader may ask the question: Why is an ETH group like CCS located at Technopark Zurich? Technopark Zurich offers an attractive open environment for industrial collaborations with easy but safe and well-managed access to research groups and excellent infrastructure in terms of seminar and conference rooms, restaurants, cash service, *etc.* The most relevant ***drawback***, is the distance to the 'Alma Mater', with the result that direct and fruitful exchange of scientific knowledge and the access to scientific instrumentation is hampered (for more information on the organization, working model and projects see [1] and homepage of CCS). Moreover, the situation at CCS is very similar to a private organization since the group shares the changes in strategy and in the economic situation of both partners, the enterprises and the funding authorities. The project-bound contracts and funding imply that only few projects can be followed up over years. This means that most projects are terminated after the contract deadline, and the projects may not be continued unless other funds can be raised.

*Correspondence: Prof. U.E. Spichiger-Keller
CCS, ETH-Technopark
Technoparkstr. 1
CH–8005 Zürich
Tel.: +41 01 445 12 31
Fax: +41 01 445 12 33
E-Mail: spichiger@chemsens.pharma.ethz.ch
http://www.chemsens.ethz.ch

Core competences are:

- The development of sensing devices and analytical procedures to provide qualitative or quantitative chemical information about target compounds present in complex specimens.
- Scientific investigations of novel molecular recognition schemes to be applied to and implemented in chemical sensors.

In collaboration with industrial partners, feasibility studies and proof of concepts are offered. At CCS, we look back on a long tradition of such collaborations originating from the experience of U. Spichiger collected in Clinical Chemistry at the University Hospital in Zürich and experience gained in the group of Prof. W. Simon at the Laboratory of Organic Chemistry at ETHZ. CCS holds a core position in an expanding international network.

The group has a ***size*** of 8 to 15 coworkers depending on the number of academic guests, Ph.D. and diploma students. The selection principle of staff is strictly in line with the strategies of the group and its high international reputation. In 1999 and 2002, two spinoff enterprises SENSORIX and C-CIT AG were founded in order for former students to mutate into entrepreneurs.

In the following, the projects running at CCS, the capabilities of the group, and projects to be reactivated are summarized.

2. Projects and Trends: Overview on Projects Which are Running at CCS or Should be Reactivated

Development Strategy

Besides the development of new materials that solve relevant analytical problems, strategies are focused on platform technologies which sustain and improve our scientific reputation, but also technologies which are predicted to have a high market potential in the future. If attractive to industrial partners, mature technologies are resumed and investigated further in view of a new application and new challenges. However, the product idea must be convincing and a solution to an important analytical problem.

The last domain of projects tackled at CCS are those with a high impact on the yield of 'true information' [2]. This type of project relies on instrumentation that provides highly accurate results and calculation programs that allow to refer the active molality measured by a sensor to molal concentrations. The ultimate goal is to provide reference specimens that show target values not only for wet chemical procedures but also for chemical sensors.

Overviews have been published in earlier volumes of CHIMIA (see [1][3][4]). The most relevant projects in terms of income were and are European projects such as the one with the synonym 'MICS' [5], which is part of the development of 'the electronic nose and the electronic tongue' [6], as well as directly funded projects such as a project for medical sensors in collaboration with Roche Diagnostics, Graz, which will be terminated by the end of June 2003, and with Robert Bosch GmbH in Stuttgart [4] for selective fire alarm sensors. Nevertheless we are particularly grateful for the many projects relevant in terms of scientific impact, projects which were and are granted by ETH (Swiss Federal Institute of Technology), CTI (Commission of Technology and Innovation) and SNF (Swiss National Science Foundation).

The entirety of these projects allowed us to develop many new chemical sensing schemes based on selective interactions between a host molecule and an analyte in order to yield specific chemical information (for more details please consult the homepage of CCS, *www.chemsens.ethz.ch*, or the author directly). In the following list the projects are grouped by well-known keywords:

Chemical Sensors and Microtechnology

The projects comprise: solid contact electrodes for magnesium-selective measurements (filed for patent application by Roche Diagnostics); optical microsensors that allow continuous monitoring of toxic gases in the range of picomolar concentrations [4], microsensors to monitor gases in medical applications [7], CMOS microsensors based on calorimetric, conductometric and resonance frequency modulations in collaboration with the group of Prof. H. Baltes, ETH Zürich [8][9]; opto-chemical microsensors based on integrated planar waveguides with high sensitivity for electrolytes and neutral analytes (GR-project no. P-012/02, funded by the Gebert-Ruef Foundation).

Optical Reflection Probes

Probes for monitoring amines, alcohols and aldehydes selectively and continuously in flow-through systems [10][11].

Biosensors and Bioassays

The projects comprise: screen-printed electrochemical biosensor microchips for D-glucose and L-glutamate monitoring (see Fig. 1) (KTI-project no. 6035.2/LoCoSens), and a bioassay for highly sensitive screening of the activity of serinproteases on integrated planar waveguides, a project running under the general term 'active proteomics' (GR-project no. P-012/02, funded by Gebert-Ruef Foundation).

Nanosciences

Sensors of nanoscopic size such as chemically modified AFM-cantilevers for tracing ion-channels selectively and destruction-free on biological tissues [12].

Development of Host Compounds and Chromoionophores/Indicator Dyes

CCS succeeded in the proof of concept for the development of peptides that react selectively with inorganic sulfate by a conformation change (helix formation, see Fig. 2) [13]. Thia-crown ethers synthesized with a clear strategy involving the capability to complex and extract heavy metal ions, especially lead and cadmium ions, were investigated electrochemically and in optodes [15]. Other projects included the modification of ruthenium(II)diimine complexes and investigations of their photo- and electrochemical stability [16]; systematic design and synthesis of Nile blue and meldola blue derivatives; investigation of the structure–activity relationship of these derivatives and of the chemical characteristics such as the attainable pK_a, photostability, lipophilicity and leaching (to be published);

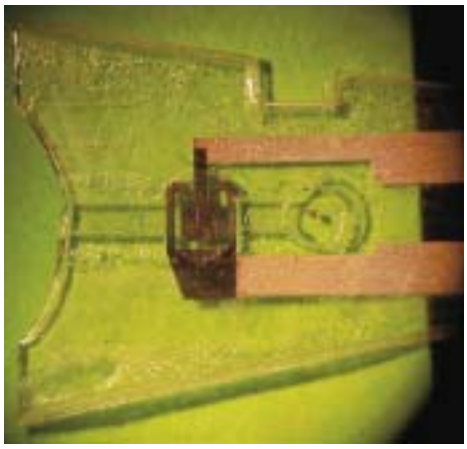

Fig. 1. Screen-printed electrochemical biosensor microchip for D-glucose and L-glutamate monitoring (disposable chip). The support by CCS was of benefit for the industrial partner in view of consultancy on how to print the biosensor paste and how to get the right consistency of the paste without inactivating the enzyme. Further, the wettability of the substrate materials and the dimensions of the capillary were crucial.

development of magnesium selective ionophores and upscaling of the synthesis of ETH 5504 and 5506 [2][17]; investigation of nucleophilic additions as reaction schemes for use in reversible sensors and for continuous monitoring [18][19]. This project is connected to the development of 'the electronic nose and electronic tongue' [5].

Collaborations with *Applied Universities* in Sitten and Wädenswil have proven to be attractive. Proof of concept studies involved cyanide detection in bitter almonds (B. Staufer, D. Marjanovic); HSO_3^- activity in wine (C. Dirren, L. della Torre); monitoring of magnesium, potassium and nitrate ions simultaneaously in fertilizer solutions for glass house cultures, including method comparison to standard procedures (S. Spichiger), *etc.*

Chemical Sensors to Identify 'Designer Drugs' by Chromoreactands

Trifluoroacetophenone derivatives were first investigated in solvent polymeric membranes by Behringer *et al.* in view of their use as anion-selective neutral ionophores [20]. Later nucleophilic additions were utilized to monitor alcohols of low molecular weight such as propanol, ethanol and methanol continuously or batchwise using optical sensors or optodes [22][23]. However these first investigations were made in UV light at 305 nm. A comparison between the reaction running at the active site of a 'zinc finger' enzyme such as the alcohol:NAD^+ oxidoreductase (ADH, EC. 1.1.1.1 [23]) showed surprising

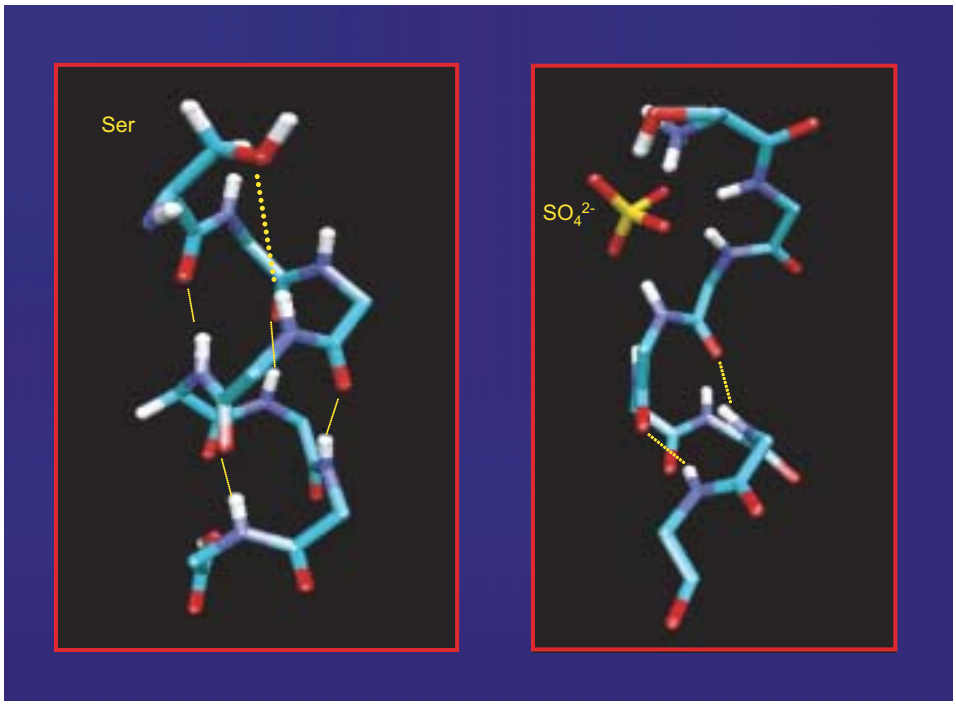

Fig. 2. Docking experiments with the purine nucleoside phosphorylase (PNP) mimicking peptide mutant 2 showing the amino acid sequence SGGLRLHLGLS (S, L-serine; G, glycine; L, L-leucin; R, L-arginine; H, L-histidine) [13]. The docking procedure using a Lamarckian genetic algorithm and randomly positioned inorganic primary sulfate induced to suggest a preferred clustering of the anion around the N-terminal half of the undecapeptide [14]. Salt bridges to the Ser1 N-terminus and/or the Arg5 side chain atoms as well as hydrogen-bonds to Gly2, Gly3 and Leu4 nitrogen atoms were suggested from the docking experiments. These results are in agreement with other NMR data and show that complexation of primary sulfate mainly involves backbone atoms of residues 2–5.

analogies [24]. At CCS, we continued to investigate these reaction principles and emerged with a range of new possibilities to design polymeric membrane sensors for neutral organic compounds as well as for humidity (H_2O). The absorbance maximum of the host compound was shifted to the visible range by synthetic approaches and the lipophilicity was increased in order to prevent leaching of the host compound. The results showed that not only the type of chemical reaction and the type of analyte influence the selectivity of the sensor but also the polarity of the membrane matrix, the pH of the sample and the pore size and polarity of the diffusion barrier (and reflector) which protects the membrane from direct contact to the specimen. Applying a light-reflecting barrier allowed the concentration of analytes to be determined using a transflection probe without any specimen interference [10]. All these parameters contributed to the selectivity of the sensor implemented into a flow channel of the analytical monitoring system.

In a next step, analytes with higher nucleophilicity than alcohols were investigated. Of main interest were aliphatic and aromatic amines as well as aldehydes [11] and ketones, where the reversed reaction scheme with the host presenting the amine function was tested. In the habilitation thesis by G. Mohr, the results of the investigations were summarized [18].

In addition, two different sensor designs were compared: an electrochemical approach based on α-cyclodextrins and calix[6]arene as host compounds, and an optical device based on a trifluoroacetophenone derivative (ETH^T 4001) in a semester work by C. Hinderling [25] and a diploma work by B. Wusk [26]. The characteristics of the two sensors were competitive. For the proof of concept or feasibility study, the optical sensor membrane was contacted with a number of drugs presenting a primary or secondary amine function. Drugs entered into the test were: ***rac. amphetamine sulfate and (S)-(+)-methamphetamine***, amantadine, procain hydrochloride, dapson, diphenhydramine hydrochloride, isoniazide, paracetamol. Other drugs were either not accessible on the market or were not soluble or not stable in aqueous solutions of pH 13. The solubility and the pK_a of compounds relative to the operating pH contribute to the selectivity of the analytical approach.

When exposing the optical sensors or sensing membranes to solutions of *rac*. amphetamine and (*S*)-(+)-methamphetamine, the same sensitivity was found for both target analytes. The detection limit was around 0.14 mM for a pathlength of approx. 5 μm polymer membrane in transmission photometry (see spectrum Fig. 3). The absorbance spectrum showed a maximum at 488 nm for ETH^T 4001 and a decreasing absorbance with increasing molality of the primary and secondary amine at pH 13 (see [28]). When the molarity of the amine solution was varied, an isosbestic point at 451 nm was generally observed for the two target analytes involved in this study. This indicates that the nucleophilic addition is the most prominent reaction involved and that the reaction is reversible if the concentration of the analytes is increased and decreased.

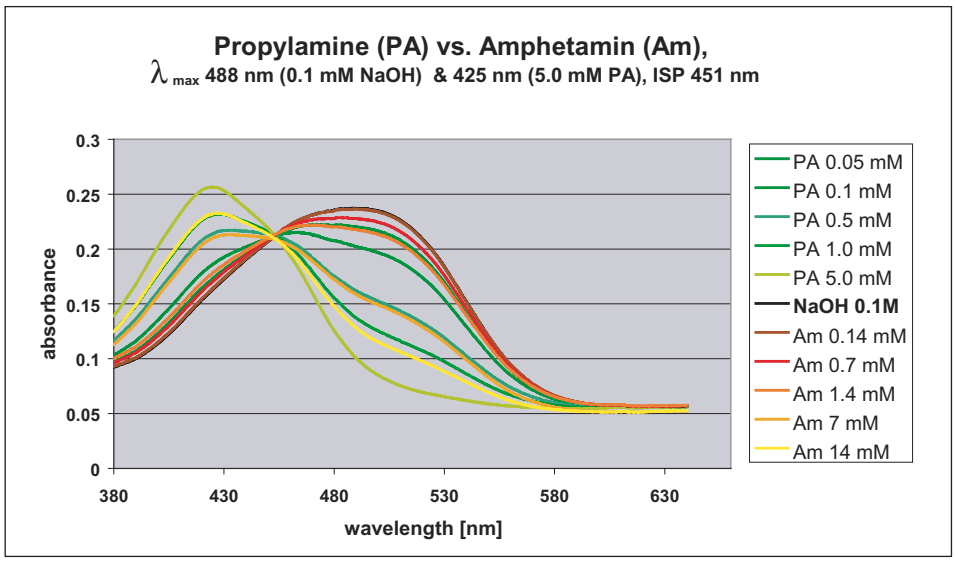

Fig. 3. Absorbance spectrum monitored in transmission through approx. 5 μm pathlength of polymer membrane [27]. Amphetamin molarity between 0.14 and 14 mM in 0.1 M sodium hydroxide. The reference compound is n-propylamine 0.05 up to 5 mM for lower limit absorbance of the hemiaminal and NaOH 0.1 M for maximum absorbance of the trifluoracetophenone derivative at 488 nm.

The ***sensing principle*** described above was shown to be particularly attractive when combined with calorimetric and mass-sensitive CMOS chips [9]. In both cases, the physical transducer responded with high sensitivity to small changes in concentration in the gas phase even for target compounds with low molecular mass. In order to reduce the risk of such a project, the extension of the mass change and the reaction heat were predicted based on theoretical models and compared with the sensitivity of the chips. In conclusion, the design of multiple sensing CMOS ***microchips*** and ***microarrays*** based on reactands for neutral analytes as host compounds and other materials described above are as feasible as the design of single use ***test strips*** with optical and/or electrochemical detection.

3. Transdisciplinarity

Owing to the organization model of CCS and its location at Technopark Zurich, 90% of the projects are running in collaboration with other research groups and industrial partners. In addition, most of the projects are running on a European or even international level. Therefore transdisciplinary understanding and agreements on transdisciplinary compromises are essential for the success of the projects. The ***major steps*** in these collaborations are: to agree on specifications; proof of concept and feasibility study; pricing and marketing plan; design and construction of a functional prototype; transfer of the technology to entrepreneurs; preparation of the supply chain and acquisition of materials; design and construction of the prototype; planning and realization of production. As the academic partner, CCS has been involved in nearly all steps of this process due to the profound knowledge about materials, their treatment and stability.

In our experience, both parties, the entrepreneurs as well as the academics, profit from collaborations between the research group and industry. The research group has the chance to acquire new materials, devices and chips, and gains support in instrumentation linked to real-life applications in industry. The industrial partner gets immediate support in terms of material knowledge for production and design, he has access to new technologies and academic know-how (literature and patent search, services generally) at a ***reasonable price*** and with a reasonable lag time. In order to ***reduce the risk*** of such projects, the academic party must be able to predict results based on fundamental scientific rules and theories. In this respect the quality of education of people involved is decisive. Complex projects have to be reduced by a stepwise process to tasks that can be fulfilled successfully.

In conclusion, communication talents, motivation and know-how are highly important for the success of the projects and depend mainly on the ***people involved*** and on clear instructions and engagement of the project managers. This means that people selected for transdisciplinary projects must be carefully chosen.

Acknowledgement

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